Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases

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Ninth Edition

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Mandell, Douglas, and Bennett's

Principles and Practice of Infectious Diseases

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Elsevier
1600 John F. Kennedy Blvd.
Ste 1600
Philadelphia, PA 19103-2899

MANDELL, DOUGLAS, AND BENNETT'S PRINCIPLES AND PRACTICE OF INFECTIOUS DISEASES, NINTH EDITION ISBN: 978-0-323-48255-4
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Library of Congress Control Number: 2019944671

https://t.me/MBS_MedicalBooksStore

Publishing Director: Dolores Meloni
Senior Content Strategist: Charlotta Kryhl
Senior Content Development Manager: Lucia Gunzel
Publishing Services Manager: Catherine Jackson
Book Production Specialist: Kristine Feeherty
Design Direction: Amy Buxton

Printed in Canada

Last digit is the print number: 9 8 7 6 5 4 3 2 1



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Introduction to Ectoparasitic Diseases
Lice (Pediculosis)
Scabies
Myiasis and Tungiasis
Mites, Including Chiggers
Ticks, Including Tick Paralysis

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Innate (General or Nonspecific) Host Defense Mechanisms

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Viral Hepatitis
Antiviral Drugs Against Hepatitis Viruses

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Ertapenem, Imipenem, Meropenem, Doripenem, and Aztreonam
Penicillins and β-Lactamase Inhibitors

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Maxwell Finland Professor of Medicine (Microbiology and Molecular Genetics), Harvard Medical School; Attending Physician, Beth Israel Deaconess Medical Center; Brigham and Women's Hospital, Boston, Massachusetts
The Common Cold
Antiviral Agents: General Principles
Zoonotic Paramyxoviruses: Nipah, Hendra, and Menangle Viruses
Astroviruses and Picobirnaviruses
Noroviruses and Sapoviruses (Caliciviruses)
Rhinovirus
Miscellaneous Antiviral Agents (Interferons, Tecovirimat, Imiquimod, Pocapavir, Pleconaril)
California Encephalitis, Hantavirus Pulmonary Syndrome, Hantavirus Hemorrhagic Fever With Renal Syndrome, and Bunyavirus Hemorrhagic Fevers

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Linezolid, Tedizolid, and Other Oxazolidinones

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Clostridioides difficile (*Formerly* Clostridium difficile) *Infection*

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Rotaviruses

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Rickettsia typhi *(Murine Typhus)*
Ehrlichia chaffeensis *(Human Monocytotropic Ehrlichiosis),* Anaplasma phagocytophilum *(Human Granulocytotropic Anaplasmosis), and Other Anaplasmataceae*

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Leprosy (Mycobacterium leprae)

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Bacillary Dysentery: Shigella *and Enteroinvasive* Escherichia coli

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Prevention of Infective Endocarditis

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Endophthalmitis
Introduction to Eye Infections
Periocular Infections
Infectious Causes of Uveitis

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Prevention of Infective Endocarditis

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Legionnaires' Disease and Pontiac Fever

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Candida *Species*

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Streptococcus agalactiae *(Group B Streptococci)*

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Acute Pneumonia

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Innate (General or Nonspecific) Host Defense Mechanisms

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Flaviviruses (Dengue, Yellow Fever, Japanese Encephalitis, West Nile Encephalitis, Usutu Encephalitis, St. Louis Encephalitis, Tick-Borne Encephalitis, Kyasanur Forest Disease, Alkhurma Hemorrhagic Fever, Zika)

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Chronic Fatigue Syndrome (Systemic Exertion Intolerance Disease)

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Respiratory Syncytial Virus

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*Brucellosis (*Brucella *Species)*

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Mucosal Immunity

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Malaria (Plasmodium *Species*)

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Tapeworms (Cestodes)

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Robert W. and Vivian K. Cahill Professor in Cancer Research, Emeritus, Stanford University School of Medicine, Stanford, California
A Molecular Perspective of Microbial Pathogenicity

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Human Metapneumovirus

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The Immunology of Human Immunodeficiency Virus Infection

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Bacillus *Species and Related Genera Other Than* Bacillus anthracis

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Staphylococcus epidermidis *and Other Coagulase-Negative Staphylococci*

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Vesicular Stomatitis Virus and Related Vesiculoviruses (Chandipura Virus)

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Mycobacterium tuberculosis

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Pharyngitis

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Rickettsia akari *(Rickettsialpox)*

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Endocarditis and Intravascular Infections

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Infections in Returning Travelers
Protection of Travelers

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Bacillus anthracis *(Anthrax)*

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Coccidioidomycosis (Coccidioides *Species*)

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Evaluation of the Patient With Suspected Immunodeficiency

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Human Immunodeficiency Viruses

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Human Immunodeficiency Virus Infection in Women

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Diseases Caused by Clostridium
Bacteroides, Prevotella, Porphyromonas, *and* Fusobacterium *Species (and Other Medically Important Anaerobic Gram-Negative Bacilli)*

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Blastomycosis

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Chlamydia pneumoniae

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Brain Abscess

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Marburg and Ebola Virus Hemorrhagic Fevers

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Babesia *Species*

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Topical Antibacterials

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Clostridioides difficile (*Formerly* Clostridium difficile) *Infection*

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Rubella Virus (German Measles)
Measles Virus (Rubeola)

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Infections in Asplenic Patients

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Microbial Keratitis

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Outpatient Parenteral Antimicrobial Therapy

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Bites

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Paracoccidioidomycosis

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Esophagitis

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Fusidic Acid

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Stenotrophomonas maltophilia *and* Burkholderia cepacia *Complex*

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Enterobacteriaceae

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Foodborne Disease

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Antimycobacterial Agents

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Diarrhea With Little or No Fever
Acute Dysentery Syndromes (Diarrhea With Fever)

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*Brucellosis (*Brucella *Species)*

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Leptospira *Species (Leptospirosis)*

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Mycobacterium tuberculosis

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Infections in Solid-Organ Transplant Recipients

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Microbial Keratitis

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Bordetella pertussis

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Chlamydia pneumoniae

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Entamoeba *Species, Including Amebic Colitis and Liver Abscess*

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Syndromes of Enteric Infection
Typhoid Fever, Paratyphoid Fever, and Typhoidal Fevers

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Coxiella burnetii *(Q Fever)*

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Approach to the Patient With Central Nervous System Infection
Acute Meningitis

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Hepatitis B Virus
Hepatitis Delta Virus

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Dermatophytosis (Ringworm) and Other Superficial Mycoses

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Infections Caused by Percutaneous Intravascular Devices

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Infections in Older Adults

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Human Genetics and Infection

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Immunization

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Antiretroviral Therapy for Human Immunodeficiency Virus Infection

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Acinetobacter *Species*

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Prevention of Infective Endocarditis

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Cell-Mediated Defense Against Infection

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Evaluation of the Patient With Suspected Immunodeficiency

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Endocarditis and Intravascular Infections

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Mycoplasma pneumoniae *and Atypical Pneumonia*

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Quinolones

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Health Care–Associated Urinary Tract Infections

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Chronic Meningitis

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Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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Urinary Tract Agents: Nitrofurantoin, Fosfomycin, and Methenamine
Relapsing Fever Caused by Borrelia *Species*

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Agents of Chromoblastomycosis
Agents of Mycetoma
Uncommon Fungi and Related Species

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Intestinal Nematodes (Roundworms)

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Bioterrorism: An Overview

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Campylobacter jejuni *and Related Species*

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Parainfluenza Viruses

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Nonsuppurative Poststreptococcal Sequelae: Rheumatic Fever and Glomerulonephritis

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Capnocytophaga

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Streptococcus pneumoniae

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Emerging and Reemerging Infectious Disease Threats

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Epstein-Barr Virus (Infectious Mononucleosis, Epstein-Barr Virus—Associated Malignant Disease, and Other Diseases)

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Listeria monocytogenes

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The Acutely Ill Patient With Fever and Rash

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Health Care–Acquired Hepatitis

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Anaerobic Infections: General Concepts

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Polymyxins (Polymyxin B and Colistin)

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Polymyxins (Polymyxin B and Colistin)

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Epstein-Barr Virus (Infectious Mononucleosis, Epstein-Barr Virus–Associated Malignant Disease, and Other Diseases)
Kaposi–Sarcoma-Associated Herpesvirus (Human Herpesvirus 8)

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Tissue Nematodes, Including Trichinellosis, Dracunculiasis, Filariasis, Loiasis, and Onchocerciasis

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Cyclospora cayetanensis, Cystoisospora belli, Sarcocystis *Species*, Balantidium coli, *and* Blastocystis *Species*

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Emerging and Reemerging Infectious Disease Threats

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Hepatitis A Virus

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Other Coryneform Bacteria, Arcanobacterium haemolyticum, *and Rhodococci*

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Tapeworms (Cestodes)

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Agents of African Trypanosomiasis (Sleeping Sickness)
Drugs for Protozoal Infections Other Than Malaria
Trypanosoma *Species (American Trypanosomiasis, Chagas Disease): Biology of Trypanosomes*

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Campylobacter jejuni *and Related Species*

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Blastomycosis

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Nosocomial Pneumonia

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Prosthetic Valve Endocarditis

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Myocarditis and Pericarditis

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Bartonella, *Including Cat-Scratch Disease*

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Chlamydia pneumoniae

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Anaerobic Cocci and Anaerobic Gram-Positive Nonsporulating Bacilli

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Agents of Mucormycosis and Entomophthoramycosis

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JC, BK, and Other Polyomaviruses: Progressive Multifocal Leukoencephalopathy (PML)
Neurologic Diseases Caused by Human Immunodeficiency Virus Type 1 and Opportunistic Infections

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Introduction to Protozoal Diseases

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Free-Living Amebae

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Toxoplasma gondii

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Transfusion- and Transplantation-Transmitted Infections

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Microbial Conjunctivitis

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Antibiotic Allergy

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Syndromes of Enteric Infection

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Outpatient Parenteral Antimicrobial Therapy

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Mycoplasma pneumoniae *and Atypical Pneumonia*

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Aminoglycosides

Alexander J. Lepak, MD
Assistant Professor, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin
Cephalosporins

Paul N. Levett, PhD, DSc
British Columbia Centre for Disease Control, Public Health Laboratory, Vancouver, British Columbia, Canada
Leptospira *Species (Leptospirosis)*

Donald P. Levine, MD
Professor Emeritus, Department of Medicine, Wayne State University, Detroit, Michigan
Infections in Injection Drug Users

Matthew E. Levison, MD
Professor of Public Health, Drexel University School of Public Health; Adjunct Professor of Medicine, Drexel University College of Medicine, Philadelphia, Pennsylvania
Peritonitis and Intraperitoneal Abscesses

Alexandra Levitt, PhD
Health Scientist, Special Advisor for Strategic Information Assessment to the Deputy Director for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
Emerging and Reemerging Infectious Disease Threats

Russell E. Lewis, PharmD
Associate Professor, Clinic of Infectious Diseases, Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy
Agents of Mucormycosis and Entomophthoramycosis

W. Conrad Liles, MD, PhD
Associate Chair and Professor of Medicine, University of Washington School of Medicine, Seattle, Washington
Immunomodulators

Aldo A.M. Lima, MD, PhD
Professor, Institute of Biomedicine, Federal University of Ceara, Fortaleza, Ceará, Brazil
Acute Dysentery Syndromes (Diarrhea With Fever)

Ajit P. Limaye, MD
Professor, Division of Allergy and Infectious Diseases, Director, Solid Organ Transplant Infectious Diseases Program, University of Washington School of Medicine, Seattle, Washington
Infections in Solid-Organ Transplant Recipients

Michail S. Lionakis, MD
Chief, Fungal Pathogenesis Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland
Candida *Species*
Cell-Mediated Defense Against Infection

W. Ian Lipkin, MD
Director, Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York
Zoonoses

Nathan Litman, MD
Professor of Pediatrics, Albert Einstein College of Medicine; Vice Chair, Clinical Affairs, Department of Pediatrics, Children's Hospital at Montefiore, Bronx, New York
Mumps Virus

Ruth Ann Luna, PhD
Director of Medical Metagenomics, Texas Children's Microbiome Center, Department of Pathology and Immunology, Baylor College of Medicine, Department of Pathology, Texas Children's Hospital, Houston, Texas
The Human Microbiome of Local Body Sites and Their Unique Biology

Joseph D. Lutgring, MD
Assistant Professor of Medicine, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia
Other Gram-Negative and Gram-Variable Bacilli

Conan MacDougall, PharmD, MAS
Professor of Clinical Pharmacy, Department of Clinical Pharmacy, University of California San Francisco School of Pharmacy, San Francisco, California
Antimicrobial Stewardship

Susan Maddocks, MBBS, PhD
Infectious Diseases Physician and Medical Microbiologist, Centre for Infectious Diseases and Microbiology, Westmead Hospital, Westmead; Institute of Clinical Pathology and Medical Research, New South Wales Health Pathology, Westmead; Clinical Senior Lecturer, Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia
Nocardia *Species*

Lawrence C. Madoff, MD
Professor of Medicine, University of Massachusetts Medical School; Director, Division of Epidemiology and Immunization, Massachusetts Department of Public Health, University of Massachusetts Memorial Medical Center, Division of Infectious Disease and Immunology, Worcester, Massachusetts
Appendicitis
Splenic Abscess
Infections of the Liver and Biliary System (Liver Abscess, Cholangitis, Cholecystitis)
Diverticulitis and Neutropenic Enterocolitis

Alan J. Magill, MD†
Director, Global Health Program, Bill & Melinda Gates Foundation, Seattle, Washington
Leishmania *Species: Visceral (Kala-Azar), Cutaneous, and Mucosal Leishmaniasis*

James H. Maguire, MD, MPH
Professor of Medicine, Harvard Medical School; Senior Physician, Division of Infectious Disease, Brigham and Women's Hospital, Boston, Massachusetts
Introduction to Helminth Infections
Trematodes (Schistosomes and Liver, Intestinal, and Lung Flukes)

Frank Maldarelli, MD, PhD
Head, Clinical Retrovirology Section, HIV Drug Resistance Program, National Cancer Institute -Frederick, National Institutes of Health, Frederick, Maryland
Diagnosis of Human Immunodeficiency Virus Infection

Lewis Markoff, MD
Laboratory Chief (Retired), Laboratory of Vector-Borne Virus Diseases, Center for Biologics Evaluation and Research, US Food and Drug Administration, Bethesda, Maryland
Alphaviruses (Chikungunya, Eastern Equine Encephalitis)

Jeanne M. Marrazzo, MD, MPH
Professor of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, Alabama
Neisseria gonorrhoeae *(Gonorrhea)*

Thomas J. Marrie, MD
Dean Emeritus, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada
Coxiella burnetii *(Q Fever)*

Thomas Marth, MD
Chief, Division of Internal Medicine, St. Elisabeth Krankenhaus, Lahnstein, Germany
Whipple Disease

David H. Martin, MD
Harry E. Dascomb, M.D., Professor of Medicine Emeritus, Department of Internal Medicine, Professor of Microbiology, Immunology, and Parasitology Emeritus, Louisiana State University Health Sciences Center, New Orleans, Louisiana
Genital Mycoplasmas: Mycoplasma genitalium, Mycoplasma hominis, *and* Ureaplasma *Species*

Gregory J. Martin, MD
Chief, Infectious Diseases - Tropical Medicine, Office of Medical Services, United States Department of State, Washington, DC
Bacillus anthracis *(Anthrax)*

†Deceased.

Francisco M. Marty, MD
Associate Professor of Medicine, Department of Medicine, Harvard Medical School; Division of Infectious Diseases, Brigham and Women's Hospital, Boston, Massachusetts
Cystic Fibrosis

Melanie Jane Maslow, MD
Chief, Infectious Diseases, VA New York Harbor Healthcare System; Professor of Medicine, Department of Internal Medicine, New York University School of Medicine, New York, New York
Rifamycins

Henry Masur, MD
Chief, Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland
Management of Opportunistic Infections Associated With Human Immunodeficiency Virus Infection

Alison Mawle, MD
Associate Director for Laboratory Science, Centers for Disease Control and Prevention, Atlanta, Georgia
Immunization

Kenneth H. Mayer, MD
Professor of Medicine, Harvard Medical School; Professor in Global Health and Population, Harvard T.C. Chan School of Public Health; Attending Physician, Beth Israel Deaconess Medical Center, Boston, Massachusetts
Sulfonamides and Trimethoprim; Trimethoprim-Sulfamethoxazole

James S. McCarthy, MD
Professor of Medicine, Department of Infectious Diseases Royal Brisbane and Womens Hospital; Senior Scientist, QIMR Berghofer Medical Research Institute, University of Queensland, Brisbane, Australia
Antimalarial Drugs
Drugs for Helminths
Drugs for Protozoal Infections Other Than Malaria

William McCormack, MD
Distinguished Teaching Professor of Medicine and of Obstetrics and Gynecology, Emeritus, Division of Infectious Diseases, Department of Medicine, SUNY Downstate Medical Center, Brooklyn, New York
Vulvovaginitis and Cervicitis

Catherine C. McGowan, MD
Associate Professor, Department of Medicine, Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee
Prostatitis, Epididymitis, and Orchitis

Kenneth McIntosh, MD
Professor of Pediatrics, Harvard Medical School; Adjunct Physician, Division of Infectious Diseases, Boston Children's Hospital, Boston, Massachusetts
Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)

Paul S. Mead, MD, MPH
Chief, Bacterial Disease Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado
Plague (Yersinia pestis)

Rojelio Mejia, MD
Assistant Professor of Infectious Diseases and Pediatrics, National School of Tropical Medicine, Baylor College of Medicine, Houston, Texas
Intestinal Nematodes (Roundworms)

Vijayashree Mekala, MD
University of Texas Medical Branch, Sugar Land, Texas
Rat-Bite Fever: Streptobacillus moniliformis *and* Spirillum minus

Nancy Messonnier, MD
Director, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
Emerging and Reemerging Infectious Disease Threats

Małgorzata Mikulska, MD
Division of Infectious Diseases, Department of Health Sciences (DISSAL), University of Genoa; IRCCS Ospedale Policlinico San Martino, Genoa, Italy
Prophylaxis and Empirical Therapy of Infection in Cancer Patients

Robert F. Miller, MB BS
Professor, Institute for Global Health, University College London, London, United Kingdom
Pneumocystis *Species*

Samuel I. Miller, MD
Professor of Medicine, Microbiology, and Genome Sciences, University of Washington School of Medicine, Seattle, Washington
Salmonella *Species*

William R. Miller, MD
Assistant Professor, Department of Internal Medicine, Division of Infectious Diseases, University of Texas Health Science Center at Houston, McGovern Medical School,
Houston, Texas
Enterococcus *Species,* Streptococcus gallolyticus *Group, and* Leuconostoc *Species*

Matthew Moffa, DO
Medical Director of Infection Prevention, West Penn Hospital, Division of Infectious Diseases, Allegheny Health Network, Pittsburgh, Pennsylvania
Tetracyclines, Glycylcyclines, and Chloramphenicol

Susan Moir, PhD
Chief, B-Cell Immunology Unit, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland
The Immunology of Human Immunodeficiency Virus Infection

José G. Montoya, MD
Professor of Medicine, Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California
Toxoplasma gondii

Shannon N. Moonah, MD, ScM
Assistant Professor of Medicine, Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia
Entamoeba *Species, Including Amebic Colitis and Liver Abscess*

Thomas A. Moore, MD
Clinical Professor of Medicine, University of Kansas School of Medicine-Wichita, Wichita, Kansas
Drugs for Helminths

Philippe Moreillon, MD, PhD
Emeritus Professor, Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland
Staphylococcus aureus *(Including Staphylococcal Toxic Shock Syndrome)*

Janet Morgan, BGS
Program Director, Vaccine Research Group, Beth Israel Deaconess Medical Center, Boston, Massachusetts
Antiviral Agents: General Principles

J. Glenn Morris, Jr., MD, MPH&TM
Director, Emerging Pathogens Institute, University of Florida; Professor of Medicine (Infectious Diseases), University of Florida College of Medicine, Gainesville, Florida
Human Illness Associated With Harmful Algal Blooms

Jose M. Munita, MD
Director, Millennium Initiative for Collaborative Research On Bacterial Resistance (MICROB-R); Associate Professor, Infectious Diseases, Clinica Alemana Universidad del Desarrollo, Santiago, Chile; Adjunct Assistant Professor, Infectious Diseases, Faculty, Center for Antimicrobial Resistance and Microbial Genomics, University of Texas Health Science Center, Houston, Texas
Daptomycin and Quinupristin-Dalfopristin

Edward L. Murphy, MD, MPH
Professor Emeritus, Departments of Laboratory Medicine and Epidemiology/Biostatistics, University of California San Francisco School of Medicine; Senior Investigator, Vitalant Research Institute, San Francisco, California
Human T-Cell Leukemia Viruses (HTLV-1, HTLV-2)

Timothy F. Murphy, MD
SUNY Distinguished Professor, Clinical and Translational Research Center, University at Buffalo, State University of New York, Buffalo, New York
Moraxella catarrhalis, Kingella, *and Other Gram-Negative Cocci*
Haemophilus *Species, Including* H. influenzae *and* H. ducreyi *(Chancroid)*

Barbara E. Murray, MD
J. Ralph Meadows Professor and Director, Division of Infectious Diseases, Department of Internal Medicine and Department of Microbiology and Molecular Genetics, University of Texas Medical School at Houston, Houston, Texas
Daptomycin and Quinupristin-Dalfopristin
Glycopeptides (Vancomycin and Teicoplanin) and Lipoglycopeptides (Telavancin, Oritavancin, and Dalbavancin)
Enterococcus *Species,* Streptococcus gallolyticus *Group, and* Leuconostoc *Species*

Clinton K. Murray, MD
United States Forces Korea, Command Surgeon, Camp Humphreys, Korea; Professor of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland
Burns

Daniel M. Musher, MD
Distinguished Service Professor of Medicine, Professor of Molecular Virology and Microbiology, Baylor College of Medicine, Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas
Streptococcus pneumoniae

Eleftherios Mylonakis, MD
Dean's Professor of Medical Science, Chief, Infectious Diseases Division, Alpert Medical School of Brown University Rhode Island Hospital, Providence, Rhode Island
Listeria monocytogenes

Jerod L. Nagel, PharmD
Clinical Specialist, Infectious Diseases, University of Michigan Health System, Ann Arbor, Michigan
Metronidazole

Susanna Naggie, MD, MHS
Associate Professor of Medicine, Duke University School of Medicine, Durham, North Carolina
Hepatitis C

Esteban C. Nannini, MD
Associate Professor, Division of Infectious Diseases, School of Medicine, Universidad Nacional de Rosario; Independent Researcher, National Council for Scientific and Technical Research (CONICET), Argentina
Glycopeptides (Vancomycin and Teicoplanin) and Lipoglycopeptides (Telavancin, Oritavancin, and Dalbavancin)

Theodore E. Nash, MD
Principal Investigator, Clinical Parasitology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland
Giardia lamblia
Visceral Larva Migrans and Other Uncommon Helminth Infections

William M. Nauseef, MD
Director, Iowa Inflammation Program; Professor of Medicine and Microbiology, Department of Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa; Iowa City Veterans Affairs Medical Center, Iowa City, Iowa
Granulocytic Phagocytes

Jennifer L. Nayak, MD
Associate Professor, Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Rochester School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, New York
Epiglottitis

Marguerite A. Neill, MD
Associate Professor of Medicine, Warren Alpert Medical School, Brown University, Providence, Rhode Island; Attending Physician, Division of Infectious Diseases, Memorial Hospital of Rhode Island, Pawtucket, Rhode Island
Other Pathogenic Vibrios

George E. Nelson, MD
Assistant Professor, Infectious Diseases, Vanderbilt University Medical Center, Nashville, Tennessee
Enterobacteriaceae

Joanna K. Nelson, MD
Clinical Assistant Professor, Infectious Disease and Geographic Medicine, Stanford University School of Medicine, Stanford, California
Bacterial Lung Abscess

Whitney J. Nesbitt, PharmD
Antimicrobial Stewardship Pharmacist, Pharmaceutical Services, Vanderbilt University Medical Center, Nashville, Tennessee
Macrolides and Clindamycin

M. Hong Nguyen, MD
University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
Antifungal Drugs: Echinocandins

Judith A. O'Donnell, MD
Professor of Clinical Medicine, Division of Infectious Diseases, Perelman School of Medicine at the University of Pennsylvania; Chief, Division of Infectious Diseases, Penn Presbyterian Medical Center; Hospital Epidemiologist and Director, Department of Infection Prevention & Control and Healthcare Epidemiology, Penn Presbyterian Medical Center, Philadelphia, Pennsylvania
Topical Antibacterials

Christopher A. Ohl, MD
Professor of Medicine, Section on Infectious Diseases, Wake Forest School of Medicine; Medical Director, Center for Antimicrobial Utilization, Stewardship, and Epidemiology, Wake Forest Baptist Health, Winston-Salem, North Carolina
Infectious Arthritis of Native Joints

Pablo C. Okhuysen, MD
Professor of Infectious Diseases, Infection Control and Employee Health, University of Texas MD Anderson Cancer Center; Adjunct Professor of Infectious Diseases, Baylor College of Medicine; Adjunct Professor of Epidemiology, Human Genetics and Environmental Health, University of Texas School of Public Health; Adjunct Professor of Infectious Diseases, McGovern Medical School at the University of Texas Health Science Center at Houston, Houston, Texas
Sporothrix schenckii
Bacillary Dysentery: Shigella *and Enteroinvasive* Escherichia coli

Andrew B. Onderdonk, PhD
Brigham and Women's Hospital, Microbiology Laboratory, Boston, Massachusetts
Diseases Caused by Clostridium
Bacteroides, Prevotella, Porphyromonas, *and* Fusobacterium *Species (and Other Medically Important Anaerobic Gram-Negative Bacilli)*

Steven M. Opal, MD
Professor of Medicine, Infectious Disease Division, The Alpert Medical School of Brown University; Co-Director, Ocean State Clinical Coordinating Center at Rhode Island Hospital, Providence, Rhode Island
Molecular Mechanisms of Antibiotic Resistance in Bacteria

Walter A. Orenstein, MD
Professor of Medicine, Pediatrics, Global Health, and Epidemiology, Emory University; Associate Director, Emory Vaccine Center, Atlanta, Georgia
Immunization

Douglas R. Osmon, MD
Professor of Medicine, Department of Infectious Diseases, Mayo Clinic, Rochester, Minnesota
Osteomyelitis

Michael N. Oxman, MD
Professor of Medicine and Pathology, University of California San Diego School of Medicine; Staff Physician (Infectious Diseases), Medicine Service, Veterans Affairs San Diego Healthcare System, San Diego, California
Myocarditis and Pericarditis

Slobodan Paessler, DVM, PhD
Associate Professor, Department of Pathology, Director, Galveston National Laboratory Preclinical Studies Core, Director, Animal Biosafety Level 3, Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, Texas
Lymphocytic Choriomeningitis Virus, Lassa Virus, and the South American Hemorrhagic Fevers (Arenaviruses)

Andrea V. Page, MSc, MD
Assistant Professor, Department of Medicine, University of Toronto; Staff Physician, Division of Infectious Diseases, Mount Sinai Hospital, Toronto, Ontario, Canada
Immunomodulators

Manjunath P. Pai, PharmD
Associate Professor, Department of Clinical Pharmacy, College of Pharmacy, University of Michigan, Ann Arbor, Michigan
Tables of Antiinfective Agent Pharmacology
Pharmacokinetics and Pharmacodynamics of Antiinfective Agents

Tara N. Palmore, MD
Chief, Hospital Epidemiology Service, Clinical Center, National Institutes of Health, Bethesda, Maryland
Infection Prevention and Control in the Health Care Setting

Raj Palraj, MBBS
Assistant Professor of Medicine, Mayo Clinic College of Medicine; Consultant, Infectious Diseases, Mayo Clinic, Rochester, Minnesota
Prosthetic Valve Endocarditis

Peter G. Pappas, MD
Professor of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, Alabama
Chronic Pneumonia

Daniel H. Paris, MD, PhD
Swiss Tropical and Public Health Institute, Basel, Switzerland; Faculty of Medicine, University of Basel, Switzerland; Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom
Orientia tsutsugamushi *(Scrub Typhus)*

Tom Parks, MD
Postdoctoral Clinical Research Fellow, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; Postdoctoral Clinical Research Fellow, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; Specialty Registrar in Infectious Diseases, Hospital for Tropical Diseases, University College London Hospitals, London, United Kingdom
Human Genetics and Infection

Julie Parsonnet, MD
George DeForest Barnett Professor of Medicine, Medicine and Health Research and Policy, Stanford University, Stanford, California
Bacterial Lung Abscess

Mark Parta, MD, MPHTM
Acting Chief, Infectious Diseases Consult Service, Warren Grant Magnuson Clinical Center, National Institutes of Health; Clinical Research Directorate, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Support to LCIM/ICMOB/NIAID (Transplant)
Pleural Effusion and Empyema

Mark S. Pasternack, MD
Associate Professor, Department of Pediatrics, Harvard Medical School; Chief, Pediatric Infectious Disease Unit, MassGeneral Hospital for Children, Massachusetts General Hospital, Boston, Massachusetts
Cellulitis, Necrotizing Fasciitis, and Subcutaneous Tissue Infections
Myositis and Myonecrosis
Lymphadenitis and Lymphangitis

Daniel M. Pastula, MD, MHS
Assistant Professor, Departments of Neurology, Medicine (Infectious Diseases), and Epidemiology, University of Colorado School of Medicine and Colorado School of Public Health, Aurora, Colorado
Coltiviruses (Colorado Tick Fever Virus) and Seadornaviruses

Robin Patel, MD
Elizabeth P. and Robert E. Allen Professor of Individualized Medicine, Professor of Medicine and Microbiology; Chair, Division of Clinical Microbiology; Director, Infectious Diseases Research Laboratory; Co-Director, Clinical Bacteriology Laboratory; Consultant, Divisions of Clinical Microbiology and Infectious Diseases; Mayo Clinic, Rochester, Minnesota
The Clinician and the Microbiology Laboratory: Test Ordering, Specimen Collection, and Result Interpretation

Thomas F. Patterson, MD
Professor, Department of Medicine/Infectious Diseases, The University of Texas Health Science Center, San Antonio, Texas
Aspergillus *Species*

Deborah Pavan-Langston, MD
Professor of Ophthalmology, Emerita, Harvard Medical School; Massachusetts Eye and Ear Infirmary, Boston, Massachusetts
Microbial Keratitis
Microbial Conjunctivitis

David A. Pegues, MD
Professor of Medicine, Division of Infectious Diseases, Perelman School of Medicine at the University of Pennsylvania; Medical Director, Healthcare Epidemiology, Infection Prevention and Control, Hospital of the University of Pennsylvania; Antimicrobial Management Program, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania
Salmonella *Species*

Stephen I. Pelton, MD
Professor of Pediatrics and Epidemiology, Pediatrics, Boston University Schools of Medicine and Public Health; Section of Pediatric Infectious Diseases, Pediatrics, Boston Medical Center, Boston, Massachusetts
Otitis Externa, Otitis Media, and Mastoiditis

Robert L. Penn, MD
Professor of Medicine, Infectious Diseases Section, Louisiana State University School of Medicine in Shreveport, Shreveport, Louisiana
Francisella tularensis *(Tularemia)*

John R. Perfect, MD
James B. Duke Professor of Medicine, Chief, Division of Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, North Carolina
Cryptococcosis (Cryptococcus neoformans *and* Cryptococcus gattii)

Ryan Perkins, MD
Clinical Fellow, Harvard Medical School, Division of Pulmonary Medicine, Boston Children's Hospital; Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Boston, Massachusetts
Cystic Fibrosis

Stanley Perlman, MD, PhD
Professor, Department of Microbiology and Immunology, and of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, Iowa
Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)

Brett W. Petersen, MD, MPH
Epidemiology Team Lead, Poxvirus and Rabies Branch Division of High Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
Orthopoxviruses: Vaccinia (Smallpox Vaccine), Variola (Smallpox), Monkeypox, and Cowpox
Other Poxviruses That Infect Humans: Parapoxviruses (Including Orf Virus), Molluscum Contagiosum, and Yatapoxviruses

William A. Petri, Jr., MD, PhD
Wade Hampton Frost Professor of Epidemiology, University of Virginia; Chief, Division of Infectious Disease and International Health, University of Virginia Health System, Charlottesville, Virginia
Introduction to Protozoal Diseases
Entamoeba *Species, Including Amebic Colitis and Liver Abscess*

Cathy A. Petti, MD
CEO, HealthSpring Global, Inc., Bradenton, Florida
Streptococcus anginosus *Group*

Jennifer A. Philips, MD, PhD
Division of Infectious Diseases, Department of Medicine, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri
Introduction to Bacteria and Bacterial Diseases

Julie V. Philley, MD
Associate Professor of Medicine, Chair, Department of Medicine, Division Chief, Pulmonary and Critical Care Medicine, University of Texas Health Science Center, Tyler, Texas
Antimycobacterial Agents

Michael Phillips, MD
Hospital Epidemiologist and Director of Infection Prevention and Control, NYU Langone Health; Clinical Associate Professor, Department of Medicine, Division of Infectious Diseases and Immunology, NYU School of Medicine, New York, New York
Acinetobacter *Species*

Larry K. Pickering, MD
Senior Advisor to the Director, National Center for Immunization and Respiratory Diseases; Executive Secretary, Advisory Committee on Immunization Practices, Centers for Disease Control and Prevention, Atlanta, Georgia
Immunization

Peter Piot, MD, PhD
Director and Professor of Global Health, London School of Hygiene and Tropical Medicine, London, United Kingdom
Global Perspectives on Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

Jason M. Pogue, PharmD
Clinical Pharmacist Specialist, Infectious Diseases, Sinai Grace Hospital, Detroit, Michigan
Polymyxins (Polymyxin B and Colistin)

Bruce Polsky, MD
Associate Dean, Faculty, Professor and Chairman, Department of Medicine, NYU Long Island School of Medicine and NYU Winthrop Hospital, Mineola, New York
Nutrition, Immunity, and Infection

Aurora Pop-Vicas, MD, MPH
Assistant Professor of Medicine, Infectious Disease Division, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin
Molecular Mechanisms of Antibiotic Resistance in Bacteria

Cynthia Portal-Celhay, MD, PhD
Assistant Professor of Medicine and Microbiology, Division of Infectious Diseases, New York University School of Medicine, New York, New York
Rifamycins

John H. Powers III, MD
Professor of Clinical Medicine, Department of Medicine, George Washington University School of Medicine, Washington, DC; Senior Medical Scientist, Division of Clinical Research, SAIC in support of National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland
Designing and Interpreting Clinical Studies in Infectious Diseases

Richard N. Price, MD
Professor, Global Health Division, Menzies School of Health Research and Charles Darwin University, Darwin, Northern Territory, Australia; Professor, Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom
Antimalarial Drugs

Yok-Ai Que, MD, PhD
Associate Professor, Faculty of Medicine, University of Bern and Senior Physician, Department of Intensive Care Medicine, Inselspital Bern University Hospital, Bern, Switzerland
Staphylococcus aureus *(Including Staphylococcal Toxic Shock Syndrome)*

Justin D. Radolf, MD
Professor, Departments of Medicine, Pediatrics, Immunology, Genetics and Genome Sciences and Molecular Biology and Biophysics, University of Connecticut School of Medicine, Farmington, Connecticut; Director of Research, Department of Medicine; Senior Scientific Advisor, Connecticut Children's Medical Center, Hartford, Connecticut
Syphilis (Treponema pallidum)

Sanjay Ram, MB, BS
Professor of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical Center, Worcester, Massachusetts
Complement and Deficiencies

Lalita Ramakrishnan, MD, PhD
Professor of Immunology and Infectious Diseases, University of Cambridge, Cambridge, United Kingdom
A Molecular Perspective of Microbial Pathogenicity

Didier Raoult, MD, PhD
IHU Meditérranée Infection, MEPHI, Aix Marseille University, Marseille, France
Introduction to Rickettsioses, Ehrlichioses, and Anaplasmoses
Rickettsia akari *(Rickettsialpox)*
Coxiella burnetii *(Q Fever)*

Jonathan I. Ravdin, MD
Milwaukee, Wisconsin
Introduction to Protozoal Diseases

Annette C. Reboli, MD
Dean, Professor of Medicine, Department Medicine, Cooper Medical School of Rowan University, Camden, New Jersey
Other Coryneform Bacteria, Arcanobacterium haemolyticum, *and Rhodococci*
Erysipelothrix rhusiopathiae

Henry Redel, MD
Clinical Instructor, Department of Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey
Nutrition, Immunity, and Infection

Marvin S. Reitz, Jr., PhD
Professor, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland
Human Immunodeficiency Viruses

David A. Relman, MD
Thomas C. and Joan M. Merigan Professor, Departments of Medicine and of Microbiology & Immunology, Stanford University School of Medicine, Stanford, California; Chief of Infectious Diseases, Veterans Affairs Palo Alto Health Care System, Palo Alto, California
A Molecular Perspective of Microbial Pathogenicity

Hilary E.L. Reno, MD, PhD
Assistant Professor, Medicine, Washington University in St. Louis, St. Louis, Missouri
Klebsiella granulomatis *(Donovanosis, Granuloma Inguinale)*

Ángela Restrepo-Moreno, MSc, PhD
Former Scientific Director, Senior Researcher, and Head, Medical and Experimental Mycology Unit, Corporacion para Investigaciones Biologicas, Medellín, Antioquia, Colombia
Paracoccidioidomycosis

John H. Rex, MD
Chief Medical Officer, F2G Limited, Eccles, Cheshire, United Kingdom; Adjunct Professor of Medicine, Infectious Diseases, McGovern Medical School at The University of Texas Health Science Center at Houston, Houston, Texas
Sporothrix schenckii

Elizabeth G. Rhee, MD
Director, Department of Clinical Pharmacology, Merck Research Laboratories, Kenilworth, NJ
Adenoviruses

Norbert J. Roberts, Jr., MD
Professor Emeritus, Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch, Galveston, Texas; Adjunct Professor, Department of Medicine, Division of Infectious Diseases and Immunology, New York University School of Medicine, New York, New York
Hyperbaric Oxygen

Andrej A. Romanovsky, MD, PhD
Professor, Thermoregulation and Systemic Inflammation Laboratory (FeverLab), St. Joseph's Hospital and Medical Center, Phoenix, Arizona
Temperature Regulation and the Pathogenesis of Fever

José R. Romero, MD
Horace C. Cabe Professor of Infectious Diseases, Department of Pediatrics, University of Arkansas for Medical Sciences; Director, Pediatric Infectious Diseases Section, Department of Pediatrics, Arkansas Children's Hospital; Director, Clinical Trials Research, Arkansas Children's Research Institute, Little Rock, Arkansas
Poliovirus
Parechoviruses
Coxsackieviruses, Echoviruses, and Numbered Enteroviruses (EV-A71, EVD-68, EVD-70)
Introduction to the Human Enteroviruses and Parechoviruses

Stacey R. Rose, MD
Assistant Professor, Department of Medicine, Section of Infectious Diseases; Assistant Dean of Clinical Curriculum, School of Medicine, Baylor College of Medicine, Houston, Texas
Bartonella, *Including Cat-Scratch Disease*

Ronald Rosenberg, ScD
Associate Director, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado
Emerging and Reemerging Infectious Disease Threats

Alan L. Rothman, MD
Research Professor, Cellular and Molecular Biology, The University of Rhode Island, Kingston, Rhode Island
Flaviviruses (Dengue, Yellow Fever, Japanese Encephalitis, West Nile Encephalitis, Usutu Encephalitis, St. Louis Encephalitis, Tick-Borne Encephalitis, Kyasanur Forest Disease, Alkhurma Hemorrhagic Fever, Zika)

Craig R. Roy, PhD
Professor of Microbial Pathogenesis, Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, Connecticut
Legionnaires' Disease and Pontiac Fever

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Classification of Streptococci

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Mediastinitis
Staphylococcus epidermidis *and Other Coagulase-Negative Staphylococci*

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Rabies (Rhabdoviruses)

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Typhoid Fever, Paratyphoid Fever, and Typhoidal Fevers
Vibrio cholerae

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Temperature Regulation and the Pathogenesis of Fever

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Syphilis (Treponema pallidum)

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Corynebacterium diphtheriae *(Diphtheria)*

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Hyperbaric Oxygen

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Myocarditis and Pericarditis

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Pulmonary Manifestations of Human Immunodeficiency Virus Infection

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Herpes Simplex Virus

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Psittacosis (*Due to* Chlamydia psittaci)

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Trichomonas vaginalis

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Lymphocytic Choriomeningitis Virus, Lassa Virus, and the South American Hemorrhagic Fevers (Arenaviruses)

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Neurologic Diseases Caused by Human Immunodeficiency Virus Type 1 and Opportunistic Infections

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Infections of the Liver and Biliary System (Liver Abscess, Cholangitis, Cholecystitis)
Diverticulitis and Neutropenic Enterocolitis

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Mycoplasma pneumoniae *and Atypical Pneumonia*

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Diagnosis of Human Immunodeficiency Virus Infection

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Infections in Solid-Organ Transplant Recipients

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Free-Living Amebae

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Pneumocystis *Species*

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Nocardia *Species*

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Osteomyelitis

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Lyme Disease (Lyme Borreliosis) Due to Borrelia burgdorferi

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Other Gram-Negative and Gram-Variable Bacilli

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Neisseria meningitidis

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Adenoviruses

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Mycobacterium tuberculosis

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Antifungal Agents: Amphotericin B

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Streptococcus pyogenes

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Klebsiella granulomatis *(Donovanosis, Granuloma Inguinale)*

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Quinolones

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Streptococcus anginosus *Group*

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Mycobacterium avium *Complex*

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Cyclospora cayetanensis, Cystoisospora belli, Sarcocystis *Species*, Balantidium coli, *and* Blastocystis *Species*

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Gastrointestinal, Hepatobiliary, and Pancreatic Manifestations of Human Immunodeficiency Virus Infection

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Surgical Site Infections and Antimicrobial Prophylaxis

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JC, BK, and Other Polyomaviruses: Progressive Multifocal Leukoencephalopathy (PML)

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Hepatitis Delta Virus

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Antifungal Drugs: Azole

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Innate (General or Nonspecific) Host Defense Mechanisms
Syphilis (Treponema pallidum)

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Influenza Viruses, Including Avian Influenza and Swine Influenza
Noroviruses and Sapoviruses (Caliciviruses)

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Approach to the Patient With Central Nervous System Infection
Brain Abscess
Subdural Empyema, Epidural Abscess, and Suppurative Intracranial Thrombophlebitis
Acute Meningitis
Cerebrospinal Fluid Shunt and Drain Infections

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Orthoreoviruses and Orbiviruses
Coltiviruses
Prions and Prion Disease of the Central Nervous System (Transmissible Neurodegenerative Diseases)

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Babesia *Species*

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Hepatitis A Virus

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The Human Microbiome of Local Body Sites and Their Unique Biology

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Parechoviruses

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Prophylaxis and Empirical Therapy of Infection in Cancer Patients

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Rickettsia rickettsii and Other Spotted Fever Group Rickettsiae (Rocky Mountain Spotted Fever and Other Spotted Fevers)
Rickettsia prowazekii *(Epidemic or Louse-Borne Typhus)*
Rickettsia typhi *(Murine Typhus)*
Ehrlichia chaffeensis *(Human Monocytotropic Ehrlichiosis),* Anaplasma phagocytophilum *(Human Granulocytotropic Anaplasmosis), and Other Anaplasmataceae*

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Antimycobacterial Agents
Infections Caused by Nontuberculous Mycobacteria Other Than Mycobacterium avium *Complex*

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The Acutely Ill Patient With Fever and Rash
Disinfection, Sterilization, and Control of Hospital Waste

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Cryptosporidiosis (Cryptosporidium *Species*)

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Mycobacterium avium *Complex*

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Drugs for Protozoal Infections Other Than Malaria

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Pasteurella *Species*

Preface to the 9th Edition

The field of infectious diseases continues its extraordinary expansion of knowledge. Now in its 9th edition, *Principles and Practice of Infectious Diseases* remains dedicated to a clear, complete, up-to-date, and—most importantly—authoritative presentation of the current information. In the last edition we included online updates to keep the text current, and we are planning for this in the 9th edition as well.

In the 9th edition and in clinical practice, previously rare or remote infectious diseases such as Zika, Ebola, and hepatitis E viral infections compete for attention with new drugs and diagnostic tests. Details and rationales are provided for new treatments for many infections, including hepatitis C, human immunodeficiency virus (HIV), tuberculosis, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Clostridioides (Clostridium) difficile,* as well as treatment options for increasingly antibiotic-resistant bacteria. Awareness of infections imported from overseas on food, travelers, exotic pets, and immigrants has become even more imperative as the world gets smaller. The complexities of managing infections in patients immunosuppressed by new drugs and by stem cell or organ transplantation requires extensive updating, as well as issues arising in patients with implanted mechanical hearts or prosthetic joints. Improved diagnostic tests for *C. difficile*, respiratory and enteric pathogens, *Tropheryma whipplei*, and many other organisms are now broadly available. In addition, there have been continuing advances in understanding of the human microbiome and in its relationships with both health and disease, and of molecular microbiology, pathogenesis, and host responses; all of these are addressed as well. As before, *Principles and Practice of Infectious Diseases* is divided into relevant sections that cover all of these areas and that are presented in an interrelated manner. Based on our custom, we focus on individual pathogens as well as on important clinical syndromes. This broadens the context to consider complex information in the setting of ill patients. We believe this provides tools for both the advanced practitioner and the beginner to understand and treat infectious diseases.

The authors who have been selected to write each of the individual chapters in the book are recognized experts in their fields, and, in turn, every chapter is carefully reviewed by all three editors to be placed into appropriate context and perspective. Thus, we anticipate that *Principles and Practices of Infectious Diseases* will be of interest and use to a wide audience of physicians, including infectious disease clinicians, internists, family practitioners, and HIV/AIDS specialists, as well as to health care providers in all other areas of medicine, public health experts, microbiologists, immunologists, hospital infection control specialists, and other medical scientists.

The editors and publisher of *Principles and Practice of Infectious Diseases* have gone to great effort to ensure that its content is highly accessible and current. The text, figures, and tables are readily available through Expert Consult, which is accessible through a powerful and easy-to-use search engine and is compatible with PC, Mac, most mobile devices, and eReaders. In addition, chapters have an introductory short summary, which is linked to individual content in each chapter. Individual chapters will also be updated on a regular basis to ensure that their content remains current. The appropriateness and significance of the updates will be emphasized by the authors and editors.

The 9th edition of *Principles and Practice and Infectious Diseases* represents the extraordinary efforts of many individuals. Foremost are the contributions of authors of the 323 individual chapters, who are dedicated to maintaining the tradition of an authoritative text that meets the highest standards of accuracy and integrity. Drs. Mark Parta, Yehuda Cohen, and Henry Redel served as assistant editors in the 8th edition and provide important assistance in the update program.

We are very grateful to Judy Webber, Janet Morgan, and Dr. Paola Frattaroli for the invaluable assistance that they have provided to us. We would also like to thank Lucia Gunzel, Taylor Ball, Lotta Kryhl, Dolores Meloni, and Kristine Feeherty at Elsevier for their overall support and efforts. And as always, this work would not have been possible without the encouragement, understanding, and—as needed—forbearance of our wives, Shirley Bennett, Kelly Dolin, and Maria Gloria Dominguez Bello.

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Basic Principles in the Diagnosis and Management of Infectious Diseases

I

A Microbial Pathogenesis

1 A Molecular Perspective of Microbial Pathogenicity

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Humans evolved on a planet dominated by microbes, which are mind-boggling in number and diversity, and thus have been intimately associated with them since the beginning. Host-associated microbes typically derive or provide benefits from this association and are thus called "commensals," which literally means "those that eat at the same table" (for definitions of classes of host-associated microbes, see Table 1.1). When they both give and receive benefits, the microbes are called "mutualists." Practically speaking, it is difficult to know whether a specific microbe is a commensal or a mutualist (or neither) because its role in the ecosystem may be subtle and its impact indirect via its relationships with other community members. In the environment, microorganisms live almost exclusively in complex communities with strong interactions among members, both cooperative and competitive, and dependencies as well as evidence of adaptation to their habitat. Not surprisingly, human commensals likewise live in complex communities; these communities are referred to as the human microbiota and, together with their genes, the human microbiome.[1,2] The number of microbial cells associated with the human body rivals the total number of human cells,[3] and the number of unique genes and gene functions associated with the human microbiome exceeds by at least 100-fold the number of unique human genes.

Host-microbiota associations are host-species specific. For example, the mouse gut microbiota is much more effective than the human or even the rat microbiota in driving differentiation of the murine immune system when used to colonize a germ-free mouse.[4] Variation in gut microbiota structure of terrestrial animals is only partly explained by host genetic relatedness; diet and gut anatomy, that is, whether fermentation takes place in the foregut or the hindgut, also explain some of this variation. More intriguing, the structure and function of human and other animal microbiotas exhibit distinct nonrandom patterns across body sites and, with time, across early life, weaning, puberty, and other life-stage transitions. The human microbiota confers a wide array of critical benefits upon its host, including nutrient and micronutrient (e.g., vitamin) availability and energy extraction from food; terminal postnatal differentiation of mucosal structures, such as the epithelial brush border and barrier function; immune system development; regulation of intermediary metabolism; processing of ingested chemicals; and "colonization resistance" against pathogens.[5] In turn, humans provide benefits to their microbiota, such as nutrients and growth factors, protected habitat, and the means for dispersal. It is important to note that this mutualistic relationship of the microbiota with the host does not necessarily mean that all individual members are also mutualists. Some may just be commensals, where they receive benefits from the host and are neither helpful nor harmful.

What then is a pathogenic microorganism? From an infectious diseases viewpoint, any microorganism that is capable of causing disease is a pathogen (see Table 1.1). Microbes that are pathogenic for humans are subsumed within the domains Bacteria and Eukarya but are restricted to the relatively few phyla that contain human-adapted members. Controversy surrounds the possible classification of some archaea as pathogens[6] (see later). As in previous editions, we will focus in this chapter on pathogenic bacteria, which are the best studied. The lessons gleaned from the study of the mechanisms by which bacteria cause disease are broadly generalizable to the less well-understood protozoa, helminths, and fungi. Viral pathogenesis mechanisms, many of which are understood in exquisite detail, are discussed in Chapter 131 and in the individual chapters on specific viruses. What is becoming increasingly clear is that there is considerable overlap in the pathogenic mechanisms of bacteria and viruses and in the host responses to them.

To be called a pathogen, a microorganism does not always have to cause disease; many common and serious infectious diseases in immunocompetent hosts are caused by organisms typically found within the human microbiota, competing with other indigenous microbes and for the most part adopting a commensal lifestyle (see Table 1.1). However, disease caused by these so-called commensal pathogens is almost certainly an accident because disease is not required for their evolutionary survival. In contrast, obligate pathogens depend on disease causation for transmission and thereby evolutionary survival (see Table 1.1), although they too can cause asymptomatic infection. A good example is *Mycobacterium tuberculosis*. The incubation period (i.e., the time from acquisition of the organism to overt disease) of tuberculosis (TB) is usually between weeks and months, although occasionally *M. tuberculosis* can cause asymptomatic infection for years.[7] Yet, *M. tuberculosis* is only transmitted through aerosol infection when diseased patients cough; asymptomatically infected individuals do not transmit infection. In

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.
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TABLE 1.1 Types of Microbes That Establish Relationships With Humans

Commensal	A microorganism that is a normal inhabitant of the human body. In commensal relationships, either the microbe or host derives benefit; neither is harmed. In mutualistic relationships, such as with *Lactobacillus crispatus*, both derive benefit.	*Faecalibacterium prausnitzii* *Ruminococcus bromii* *Bacteroides ovatus* *Akkermansia muciniphila* *Streptococcus sanguinis* *Lactobacillus crispatus*
Pathogen	A microorganism capable of causing disease. These include commensals and noncommensals. Operational classes of pathogen are defined in the rows below.	
Obligate pathogen	A microorganism that must produce disease to transmit and thereby survive evolutionarily. Obligate pathogens are not commensals, although they can produce asymptomatic infection.	*Mycobacterium tuberculosis* *Mycobacterium leprae* *Treponema pallidum* *Neisseria gonorrhoeae* *Shigella dysenteriae* *Salmonella* Typhi *Chlamydia trachomatis*
Commensal pathogen	A microorganism that is commonly found within the indigenous microbiota that can cause disease in normal hosts with some regularity. Commensals do not manifest as pathogens with equal frequency; *Bacteroides fragilis* and *Streptococcus anginosus* are occasional rather than regular pathogens, in contrast to the others on the list. Disease causation is not required for the commensal's survival and as such is an accident.	*Staphylococcus aureus* *Streptococcus pyogenes* *Streptococcus pneumoniae* *Neisseria meningitidis* *Haemophilus influenzae* *Helicobacter pylori* *B. fragilis* *S. anginosus*
Zoonotic pathogen	A microorganism that is a colonizer or pathogen in animals and that can be transmitted to humans either via an insect vector or via direct contact with the animal or its products. Disease causation in humans is accidental and not necessary for evolutionary survival.	*Yersinia pestis* *Francisella tularensis* *Borrelia burgdorferi* *Bacillus anthracis* *Brucella abortus* *Mycobacterium bovis* *Mycobacterium leprae* *Salmonella enterica* *Rickettsia spp.*
Environmental pathogen	A microorganism capable of causing disease that is transmitted to humans from an environmental source such as water or soil. Disease causation is accidental and not necessary for evolutionary survival.	*Clostridium tetani* *Clostridium botulinum* *Burkholderia pseudomallei* *Mycobacterium marinum* *Mycobacterium avium* *Pseudomonas aeruginosa* *Legionella pneumophila* *Vibrio cholerae*

the case of *Salmonella* Typhi another obligate pathogen, individuals can occasionally remain persistently, although asymptomatically infected after a bout of typhoid fever, and unlike the case of TB, these asymptomatically infected individuals can shed the organisms in their feces, as notoriously exemplified by "Typhoid Mary." However, the vast majority of transmission likely occurs through diseased patients; it is disease rather than asymptomatic shedding by a minority population that sustains the global burden of typhoid fever.

The remaining two classes of disease-causing microbes are zoonotic and environmental pathogens, where infection of humans originates from other animals and the environment, respectively (see Table 1.1). As with commensal pathogens, human disease from zoonotic and environmental pathogens is accidental and does not benefit the pathogen's survival. It is important to note that pathogens of all classes can cause very serious disease. Humanity's greatest infectious killers include not only tuberculosis, caused by an obligate pathogen, but also group A streptococcal disease, plague, and cholera, caused respectively by a commensal, a zoonotic, and an environmental pathogen. Thus countless millions have succumbed and continue to succumb to bacterial diseases that are of no benefit to the causative agent.

This classification of pathogens is not absolute because they continue to evolve and adapt at the same time as their hosts change in behavior and demographics. *Mycobacterium leprae* is a good example of a pathogen with dual pathogen class membership. A scourge of humankind for millennia, *M. leprae* was likely once a strictly obligate human pathogen (i.e., completely reliant on human-to-human transmission for its evolutionary survival). However, Hansen disease (leprosy) represents an instance of a "reverse zoonosis" on at least two different occasions. Humans infected red squirrels in the British Isles in medieval times, when there was likely close contact with squirrels owing to a squirrel fur trade, and because red squirrels were able to infect each other, they have leprosy to this day.[8] Then approximately 400 years ago, after *M. leprae* was brought into the new world through the slave trade, armadillos in the southeastern United States became infected, again probably through close human contact.[9] Leprosy is spreading among armadillos and from armadillos to humans and is now a recognized zoonotic disease in the United States.

Pathogens are not equally virulent (i.e., they do not have an equal probability of causing disease). For example, encapsulated pneumococci are more virulent than nonencapsulated pneumococci, and *Escherichia coli* strains that express Shiga-like toxins are more virulent than those that do not express these toxins. Thus it is useful to distinguish pathogens that regularly cause disease in some proportion of susceptible individuals with apparently intact defense systems ("primary pathogen") from others that cause disease only in immunocompromised individuals ("opportunistic pathogen"). A distinction, then, between a primary pathogen and opportunist is that the former has an *inherent* ability to breach the host barriers that ordinarily restrict other microbes, whereas the opportunist requires some underlying defect or alteration in the host's defenses, whether it be genetic, iatrogenic, ecologic (altered microbiota), or caused by underlying disease or trauma, to establish itself in a usually privileged host niche. However, the distinction is often not clear-cut because a primary pathogen is often opportunistic as well. *Streptococcus pneumoniae* can cause disease in apparently immunocompetent hosts, but individuals with asplenia or human immunodeficiency virus (HIV) infection are even more susceptible to it. *Neisseria meningitidis* is a dreaded primary pathogen to which individuals with terminal complement deficiencies are more likely to develop disease. *M. tuberculosis*, a major cause of disease and death in immunocompetent individuals, poses a higher risk for individuals with HIV infection.

The distinction between primary and opportunistic pathogen is actually even muddier, as illustrated by the case of *Pseudomonas aeruginosa* infections. *P. aeruginosa* is generally viewed as an opportunistic pathogen because it does not usually cause disease in individuals with intact host defense systems and is a more common cause of lethal pneumonia and bacteremia in neutropenic hosts. But even in normal hosts, *P. aeruginosa* can cause benign self-limited skin eruptions ("hot tub" folliculitis) in individuals exposed to contaminated water in hot tubs. Moreover, *P. aeruginosa* illustrates the point that pathogenicity can only be understood in the context of a specific host. In individuals with cystic fibrosis, *P. aeruginosa* produces a lung-destroying chronic bronchitis, but unlike the case with pneumonias in neutropenic hosts, the organism does not disseminate systemically, so overt bacteremia is not usually associated with the lung infection. In elderly patients with diabetes mellitus, *P. aeruginosa* can produce a completely different devastating disease—malignant (necrotizing) otitis externa, an invasive infection of the external auditory canal and bones of the skull base. In general the stereotypic patterns of infection by primary and opportunistic pathogens in distinct disorders of host defense provide useful clues for early diagnosis and treatment and about pathogenic mechanisms.

An emerging concept of microbial disease causation, with origins in the field of ecology, is the notion of "community as pathogen."[1] This notion is based on the idea that community members, incapable of causing disease on their own, together cause pathology through the kinds of cooperative interactions that are typical of all microbial communities, such as cross-feeding (one member secretes a factor that serves

as a nutrient for another member), syntrophy (see later), or cross-protection (one member secretes a factor that protects another member from a harmful environmental compound). Examples of such "pathogenic communities" have been studied in mouse models where microbial communities that arise only in mice with a dysregulated immune system are then capable of transmitting a form of ulcerative colitis to wild-type mice.[10] In humans "pathogenic communities" in the mouth are associated with chronic periodontitis.[11] Indeed, it is in the context of pathogenic communities that archaea have been implicated in human infectious disease causation.[11–13] For example, methanogens in the subgingival crevice may enhance the growth of fermentative, "nascent" pathogenic bacteria, and benefit themselves by consuming the hydrogen produced by the fermenters in a relationship called "syntrophy." Other hydrogen-consuming microbes, such as treponemes, may take the place of the archaeal methanogens in these communities. The concept of a pathogenic community poses special challenges for proofs of causation because the pathogenic "agent" is difficult to isolate, purify, and characterize, and relevant models of disease can be elusive. Dominant ideas of microbial disease causation (e.g., a single pathogenic agent in a susceptible host) may be too restrictive. Moreover, microbial diseases that require or support a consortium of microbes (e.g., intraabdominal abscess), pose challenges for pathogen identification.

Discussions about pathogenic communities have been grounded in traditional ecologic definitions of the term community that specify multiple interacting species with networked interspecies relationships. Yet, local populations of bacteria from the same species, even clonal diversified descendants of a single cell, can also be viewed as communities because of the seemingly cooperative behavior of diversified and heterogenous subpopulations. And this alternative view has provided important insights into the strategies, that is, "social behavior," of some pathogens.[14] For instance, clonal populations of pathogens can vary in their expression of genes. As one example, within a population of *Salmonella typhimurium* cells growing in axenic culture, there are subpopulations that express a virulence-associated specialized secretion system that facilitates invasion of intestinal epithelial cells. This preemptive expression of a virulence factor represents a form of "bet-hedging" to prepare the bacterium for a variety of different, changing local conditions and needs. Heterogeneity in gene expression is also seen in subpopulations of bacteria that have encountered different environmental conditions within the host and presumably responded accordingly. *Salmonella* attracts both macrophages and neutrophils to the intestinal mucosa; not surprising, bacteria phagocytosed by these two cell types express different genes even within the same inflamed tissue. Even extracellular bacteria close to each other might express distinct genes in response to local differences in oxygen tension or pH within an abscess.

Finally, populations of pathogens may display heterogeneity because of the emergence of "cheaters." Again, *S. typhimurium* provides a good example. Its specialized secretion system that facilitates invasion of intestinal epithelial cells also elicits a host inflammatory response that is favorable to itself and to a small select number of distant relatives (other members of *Enterobacteriaceae*) but not to the vast majority of commensal competitors. Because the secretion system is costly to make, cheaters arise that can benefit from the inflammation caused by their siblings without undergoing the cost of making the secretion system.[15] However, if cheaters become too numerous, then there will not be sufficient inflammation, and the entire population will be disadvantaged. Therefore there have evolved intrinsic measures to keep the number of cheaters in check, and in fact, bacteria are known to have "cheater detection" mechanisms!

ATTRIBUTES OF MICROBIAL PATHOGENS

Despite the difficulties in defining them, pathogens do share characteristic attributes (Table 1.2). All pathogens (other than commensal pathogens) must gain entry into the host in sufficient numbers to establish infection, either from another infected host, the environment, or an insect vector. All classes of pathogens must be able to establish themselves in a unique habitat; this typically occurs by breaching anatomic barriers to "go where other microbes dare not." Another important trait of a pathogen is replication within its host; disease production is usually dependent on this trait, as is transmission, an essential trait of obligate pathogens. These discrete steps are achieved by avoiding, circumventing, destroying, or even exploiting one or more essential host defenses. The degree to which a microbe can subvert to their advantage the cellular processes in a normal host not only distinguishes commensals from pathogens,[16,17] but also among commensals, organisms that have greater or less propensity to cause disease (see Table 1.1).

TABLE 1.2 Attributes Shared by Bacterial Pathogens

- Enter host. This can occur through the skin or any of the body's orifices. Commensal pathogens bypass this step as they are "already there."
- Cross anatomic barriers and/or breach other host defenses to establish themselves in a unique habitat and functional niche.
- Multiply within host.
- Exit from the host to infect new host. Only obligate pathogens need to do this.

Modified from Falkow S. I never met a microbe I didn't like. Nat Med. *2008;14:1053–1057.*

For the steps of pathogenesis to be executed, the microorganism must possess genetic properties, often complementary and coregulated, that promote its interaction with the human host. Commensal organisms also rely on their genetic properties to maintain their interactions with the host and with other community members. Indeed, the genetic traits of a given microorganism define the unique attributes that enable it to follow a common sequence of steps to establish colonization or disease.[18,19] Elegant molecular and genetic techniques have enabled the identification, isolation, and characterization of many of these genes and their products (see "Identification and Characterization of Virulence Genes"). We now also possess the complete genome sequences of virtually every major pathogenic bacterial species. This information provides important clues and insight into the potential of a microorganism for causing disease and facilitates new experimental strategies for understanding pathogens and commensals alike.[20,21]

These methods, information, and insights have led to the identification of *virulence factors*, the properties (e.g., gene products) that enable a microorganism to achieve its pathogenic potential through these steps; from a clinician's point of view, a virulence factor enhances the microbe's potential to cause overt pathology. The critical need for virulence determinants is obvious when one considers that the execution of the steps of pathogenesis (or, for that matter, colonization) in the face of a formidable array of host defense mechanisms is nontrivial. The availability of the host (e.g., human) genome sequence has significantly enhanced our understanding of the mechanisms of host defense and pathogen counterdefense,[22] while enabling multiple synergistic approaches for understanding virulence, including the identification of host susceptibility traits and genome-wide assessments of host response. It is becoming clear that pathogens possess specific determinants mediating virulence, distinct from those enabling general metabolic functions, that imbue them with a counterstrategy for each host defensive strategy.

The initial steps of entry and niche establishment require that the microorganism make contact with an appropriate host tissue that can serve as a jumping board to its eventual host niche. To accomplish this goal the infecting microbe may make use of motility (through *flagella*), chemotactic properties, and adhesive structures (or *adhesins,* such as *pili*) that mediate binding to specific eukaryotic cell receptors or to other microorganisms.[16,23] They must adapt, at least temporarily, to the particular nutrient environment in which they find themselves. They must resist host antimicrobial peptides and avoid phagocytosis and killing by patrolling innate immune cells of the host. They must contend with the indigenous microbiota that provides competition against establishment of the newcomer.

Because breaching barriers is generally an integral aspect of reaching their preferred site for replication, most pathogens have specific virulence determinants that enable them to do this. These barriers can be anatomic, cellular, or biochemical and may prevent entry by other microorganisms into what are ordinarily sterile tissue sites. Breaching these diverse types of barriers requires pathogens to elaborate toxins and enzymes that

destroy anatomic barriers while countering innate immune defenses by either avoiding phagocytosis, for instance, by means of an antiphagocytic capsule, or by simply killing phagocytes. Paradoxically, many intracellular pathogens (e.g., *Salmonella* and *Mycobacterium*), rather than breaching anatomic barriers, typically *use* phagocytes to ferry them across these barriers, and others (e.g., *Listeria*, *Rickettsia*, and *Shigella*), spread from one nonphagocytic cell to the next by co-opting the host cell actin assembly machinery.[24]

In most infectious diseases, save those few that involve a preformed toxin, the infecting organism must multiply to produce disease. This can be appreciated in clinical practice in terms of a characteristic incubation period spanning the time from exposure to the appearance of signs and symptoms of disease. The diversity of pathogen habitats—extracellular or intracellular, mucosal or submucosal, within the bloodstream or within another privileged anatomic site—has forced pathogens to evolve distinct biochemical tactics to achieve this goal. Intracellular pathogens have to ward off the defenses of the host cell, which in the case of professional phagocytes, such as macrophages and neutrophils, are geared toward killing microbes.

Finally, obligate pathogens have evolved diverse strategies to exit the host that serve to increase transmission to a new host. *Shigella dysenteriae* and *Neisseria gonorrhoeae* both elicit neutrophil-dominated mucosal inflammatory responses that lead to diarrhea and exudates, respectively, laden with organisms, that facilitate bacterial exit and transmission to new hosts either via the environment or directly. *M. tuberculosis* orchestrates the necrotic death of infected macrophages in the tuberculous granuloma, a process that enhances transmission.[25]

Microorganisms also use subtle biochemical mechanisms to avoid, subvert, or, as we now increasingly understand, manipulate host defenses. These strategies include the elaboration of immunoglobulin-specific proteases, iron sequestration mechanisms, coating themselves with host proteins to confuse the immune surveillance system or causing host cells to signal inappropriately, leading to dysregulation of host defenses or host cell death. Examples of these mechanisms include the production of immunoglobulin A1 protease by meningococci, the use of receptors for iron-saturated human transferrin and lactoferrin by gonococci, and the coating of *Treponema pallidum* with human soluble fibronectin. Antigenic variation and intracellular invasion are other common strategies used by successful pathogens to avoid immune-mediated elimination.[17,26] The broad principle is that for any host defense strategy, a successful pathogen must have evolved a counterstrategy.

Any discussion of virulence factors, and particularly their link to specific virulence functions, begs the question as to whether, how many, and which commensal organisms can also act as primary pathogens. The well-known virulence factors of commensal pathogens, many of which reside in the mucosa of the nasopharynx can be thought of as colonization factors run amok. These factors likely evolved to give the commensal a selective colonization advantage on mucosal surfaces rife with microbial competition. They might also help to maintain an equilibrium with host defenses. In support of this idea, vaccines against virulence factors often eradicate colonization along with disease. This is true for vaccines against bacterial capsules, for instance, those of *S. pneumoniae* and *N. meningitidis*, demonstrating that the capsules of these bacteria enable effective colonization.

Pathogenic bacteria have evolved sophisticated biochemical strategies to interfere with, or manipulate for their own benefit, the normal function(s) of host cells, but their "purpose" is not to "do in" their host! Rather, from a teleologic perspective, the diseases they cause are simply a by-product of the method and site chosen by (or thrust upon) them for replication and evolutionary persistence. In fact, disease per se is not a measure of microbial success—in evolutionary terms, a prevalent human commensal is just as successful as a prevalent human pathogen, such as *M. tuberculosis*, one of humanity's greatest killers. Although death of a host may promote transmission of some infections, it is more often detrimental to both parties involved. Therefore the rules of host-pathogen engagement, certainly for obligate pathogens, are generally designed to produce a tie: just enough pathogen multiplication and damage to the host to ensure its establishment within that host and transmission to a new host, but no more than is tolerated by the host. It is true that some of the most notorious infectious diseases (e.g., plague) occur predominantly in dramatic epidemic form; indeed, the so-called "emerging" infectious diseases reflect various aspects of imbalance in the relationships among host, pathogen, and environment.[27] However, most of these diseases are the result of accidental infection by zoonotic pathogens.[28] In most zoonotic diseases the rules of host-pathogen engagement are blurred, often to the detriment of both host and microbe, serving as an evolutionary dead end for both parties.

Finally, in framing the question "What is a pathogen?" it is important to consider that we yet do not know the true diversity and distribution of extant microorganisms capable of causing human disease. Previously unrecognized pathogens emerge with increasing frequency, and although most are zoonotic, the accelerated clip of pathogen discovery does highlight the uncertainty about how often, in what phylogenetic backgrounds, and through what mechanisms virulence for humans among microbes can arise. It is highly likely that some potential pathogens may not have had adequate contact with humans to have made themselves known yet.[29] Although pathogen detection and identification remain suboptimal, in part because of continuing dependence on cultivation methods and targeted species-specific assays that fail to detect novel pathogens,[30] it is also the case that pathogens-in-waiting are the beneficiaries of human activities that alter the climate and landscape, create crowded living conditions, and impede sanitation and other public health measures through strife and the withholding of needed resources.

EVOLUTION OF BACTERIAL PATHOGENICITY

Where do pathogens come from? The quest to understand how pathogenic bacteria cause disease dates back well more than a century. The notion that bacteria somehow "poison" host cells predates even the isolation of individual pathogens, a concept that was solidified with the demonstration in 1888 and 1890, respectively, that culture filtrates from *Corynebacterium diphtheriae* and *Clostridium tetani* were sufficient to cause their respective diseases in experimental animals. Since then, hundreds of bacterial toxins have been discovered and their mechanisms of action discerned. Other bacterial virulence factors (e.g., adhesins, capsules) have been identified as well, and a sophisticated understanding of their mechanisms achieved. But how did bacteria *become* pathogens, or in other words, how did they acquire these armaments? It turns out that virulence determinants such as toxins and adhesins, that distinguish pathogens from their nonpathogenic relatives, derive from specialized genes possessed by pathogens but absent in nonpathogens. These specialized genes reside on DNA that often is foreign to the bacteria, either as part of extrachromosomal plasmids, transposons ("jumping genes"), or bacterial viruses (bacteriophages) integrated into the bacterial chromosome (Table 1.3).

Virulence gene discovery (see "Identification and Characterization of Virulence Factors"), which was accomplished for decades by genetic and biochemical methods, has been greatly accelerated in recent years by the feasibility of large-scale whole-genome sequencing and genome-wide single nucleotide polymorphism analysis.[20] Since the first description of a complete genome sequence for a free-living organism, *Haemophilus influenzae,* in 1995,[31] more than 180,000 bacterial and archaeal complete genome sequences have been released to public databases (www.ncbi.nlm.nih.gov/genome/browse/). Comparative genome analyses suggest that the inheritance of pathogenic traits was not the result of slow adaptation to the host but rather a rapid acquisition of genes *en bloc* via mobile genetic elements (i.e., plasmids, transposons, phages). Consistent with their acquisition on mobile elements, these virulence-associated sequences are often bounded by repeated DNA segments, which are a signature of mobile DNAs. Moreover, inspection of genome sequences finds that these virulence determinants and their associated (residual) mobile elements often have a distinct genome nucleotide composition, suggesting that their ancestry derives from an unrelated microbe.

This duality of chromosomal nucleotide composition in pathogenic bacteria is most apparent in the context of *pathogenicity islands*, large blocks of genes that some pathogens have acquired through genetic transfer from other bacteria.[32] These islands comprise clusters of virulence-associated genes that encode specialized secretion systems

TABLE 1.3 Examples of Plasmid- and Phage-Encoded Virulence Determinants

ORGANISM	VIRULENCE FACTOR	BIOLOGIC FUNCTION
Plasmid Encoded		
Enterotoxigenic *Escherichia coli*	Heat-labile, heat-stable enterotoxins	Activation of adenylate/guanylate cyclase in the small bowel, which leads to diarrhea
	CFA/I and CFA/II	Adherence/colonization factors
Extraintestinal *E. coli*	Hemolysin	Cytotoxin
Shigella spp. and enteroinvasive *E. coli*	Gene products involved in invasion	Induces internalization by intestinal epithelial cells
Yersinia spp.	Adherence factors and gene products involved in invasion	Attachment/invasion
Bacillus anthracis	Edema factor, lethal factor, and protective antigen	Edema factor has adenylate cyclase activity; lethal factor is a metalloprotease that acts on host signaling molecules
Staphylococcus aureus	Exfoliative toxin	Causes toxic epidermal necrolysis
Clostridium tetani	Tetanus neurotoxin	Blocks the release of inhibitory neurotransmitter, which leads to muscle spasms
Phage Encoded		
Corynebacterium diphtheriae	Diphtheria toxin	Inhibition of eukaryotic protein synthesis
Streptococcus pyogenes	Erythrogenic toxin	Rash of scarlet fever
Clostridium botulinum	Botulism neurotoxin	Blocks synaptic acetylcholine release, which leads to flaccid paralysis
Enterohemorrhagic *E. coli*	Shiga-like toxin	Inhibition of eukaryotic protein synthesis
Vibrio cholerae	Cholera toxin	Stimulates adenylate cyclase in host cells

CFA, Colonization factor antigen.
Data from Elwell LP, Shipley PL. Plasmid-mediated factors associated with virulence of bacteria to animals. Annu Rev Microbiol. *1980;34:465–496; and Cheetham BR, Katz ME. A role for bacteriophages in the evolution and transfer of bacterial virulence determinants.* Mol Microbiol. *1995;18:201–208.*

and secreted effector molecules that provide the microbe with extraordinary properties to survive in a specific host, such as adhesins and proteins that regulate virulence gene expression (see "Regulation of Bacterial Pathogenicity" and "Close Encounters: Pathogens as Cell Biologists"). *S. typhimurium* is believed to have begun evolving as a pathogen from a common ancestor that it shares with *E. coli,* approximately 130 million years ago, through the sequential acquisition of at least two pathogenicity islands, one of which mediates internalization within host cells, and the other, survival and replication within an intracellular vacuole. Although genomic analyses provide us with fascinating stories about the evolution of pathogens, we still remain ignorant of the precise origins of these and other virulence-associated systems. They were probably acquired from a yet unknown ancient ancestor. Moreover, it seems likely that their acquisition by pathogens can be traced to their need for avoiding predation as more sophisticated organisms evolved, such as free-living amebae, nematodes, fungi, and a host of other tiny creatures that exploit microbes for food. Pathogenicity is an old and honorable bacterial trait!

Hence we can conclude that, in most cases, bacteria have evolved to become pathogens by *acquiring* genetic material encoding virulence determinants rather than by the gradual loss of genes. This is not to say that, over time, some pathogens do not dispense with genes that are no longer useful for their newly acquired pathogenic lifestyle. Indeed, gene loss or gene inactivation is often associated with the adaptation of a pathogen to a particular host. Continuing our genomic "stalking" of *Salmonella*, we find that *S. typhi,* the strictly human-adapted bacterium that causes typhoid fever, has acquired by horizontal gene transfer (HGT) a unique capsular polysaccharide, Vi, and a unique toxin not present in *S. typhimurium*.[33] Yet it has also lost or inactivated a large number of genes present in *S. typhimurium*.

Shigella and *Yersinia* provide other examples of evolution to pathogenicity through both acquisition and loss of genes. The different pathogenic *Shigella* spp. are believed to have arisen on several independent occasions from within different *E. coli* lineages, and in the case of *Shigella sonnei*, the emergence of the species occurred quite recently (i.e., only 400 years ago). The *Shigella* spp. arose through convergent evolution, with acquisition of a virulence plasmid carrying genes for invasion and manipulation of host cells and a bacteriophage carrying the Shiga toxin gene, along with loss of genes for flagella that were not only unnecessary in light of the new armaments that each species had acquired but even detrimental because the immunogenicity of flagella would provoke a host response that would promote elimination of the bacteria.[34]

The case of *Yersinia pestis* provides perhaps the most fantastic example of hand-in-hand gene acquisition and loss. It is estimated that *Y. pestis* evolved from the enteropathogenic *Yersinia pseudotuberculosis* only approximately 5000 years ago.[35] All pathogenic *Yersinia* spp. harbor a 70-kilobase virulence plasmid (pYV) needed for toxicity and to overcome host immune defenses, but there are two *Y. pestis*–specific plasmids that were more recently acquired by HGT. One encodes a plasminogen activator, a surface molecule that provides proteolytic, adhesive, and invasive functions and facilitates dissemination from an intradermal site of infection. The other plasmid encodes a capsular antigen that blocks phagocytosis and a toxin needed for survival in the flea. Thus this organism evolved to establish a distinct mammalian reservoir, ensure its transmission by a flea, and spread systemically in its preferred murine host, with obvious devastating effect in an accidental human host. In the process it rearranged its genome and inactivated genes that were required for its previous gastrointestinal life; these inactivated genes and rearrangements remain as evolutionary relics. That a microorganism can accomplish this remarkable feat of evolution in what is a blink of the eye in evolutionary terms, may be a cautionary lesson for what the future may hold for emerging pathogens.

In general, as bacteria evolve from free-living organisms with multiple habitats to obligate pathogens, host-restricted organisms, endosymbionts, or obligate intracellular organisms, their genomes become reduced in size, accumulate inactive or defective genes *(pseudogenes),* or both.[20,36] For example, the evolution of *Bordetella pertussis* as a host-specific, human-adapted pathogen from a *Bordetella bronchiseptica*–like ancestor has been accompanied by extensive gene loss and gene inactivation (3816 coding sequences vs. 5007 for *B. bronchiseptica*; 9.4% of coding sequences are pseudogenes vs. 0.4% for *B. bronchiseptica*).[37] In this case, a highly restricted host range (*B. pertussis* is a strictly human pathogen) has meant loss of genetic diversity. In contrast to *B. bronchiseptica*, which infects multiple animal hosts and can survive in the environment, *B. pertussis* varies little in gene content among different strains isolated over the past 50 years and across several continents.[38] However, more recent analyses of whole-genome sequence assemblies and gene order have revealed clone-specific genome structural rearrangements and have led to speculation that certain genome rearrangements may confer fitness benefits and differences in virulence.[39] *M. tuberculosis*, a human-adapted pathogen, has a significantly smaller genome than its soil-dwelling relative *Mycobacterium smegmatis*. *M. leprae*, the agent of leprosy, is so exquisitely host adapted that it cannot even be grown in axenic culture, and in accordance, its genome displays an extreme degree of gene decay. Overall, the primary evolutionary push to pathogenicity results from gene acquisition. More generally, gene acquisition is an effective strategy for microbial specialization and a means for haploid organisms to acquire new functions and maximize diversity while fulfilling their need to conserve essential functions. The gene loss that occurs alongside gene acquisition makes the organism more efficient in one environment yet

may make it more limited in others, *M. leprae* being an extreme example of evolving to a restricted niche.

One revelation from pathogen "genome gazing" is that the amount of acquired DNA associated with virulence and adaptation to a host habitat varies greatly between bacterial pathogens. In pathogenic *E. coli* strains this amount is substantial. For example, uropathogenic, enterohemorrhagic, and extraintestinal types of *E. coli* all display mosaic genome structure, with hundreds of distinct gene islands associated with each type, comprising as much as 40% of the overall gene content in each of these strains.[40] Each pathotype is as distinct from the others as each is from a nonpathogenic laboratory strain of *E. coli*. Conversely, no more than half of the combined gene set is common to all *E. coli* strains. From this and other similar findings arises the concept of the "pan-genome," or the complete set of genes for a species. *E. coli* has a relatively "open" pan-genome in that, with every new genome sequence, a new set of approximately 300 unique genes is discovered, suggesting ongoing evolution of this species by gene acquisition.[41] In contrast, many other pathogens, for instance, *Bacillus anthracis,* have a relatively closed pan-genome.

The sharing of genes among seemingly disparate microorganisms occupying the same niche should in principle provide these microbes with an endless number of combinations of genes for evolutionary experimentation, as it were, within a habitat such as the human intestinal tract.[42] However, a consistent finding from genomic analyses is that most natural populations of microorganisms, including pathogens, consist of only a small number of discrete clonal lineages.[43] This clonal population structure could suggest that the recombination rates of chromosomal genes between different strains of the same species and between different bacterial species are low; that is, only a few evolutionary experiments are attempted. Alternatively, it could imply that, although experimentation may occur aplenty, only a few experiments are "successful" so that emergence of a pathogen is relatively rare. In support of low recombination rates is the finding that even bacteria that possess naturally occurring genetic exchange mechanisms retain their individuality. The pneumococci are a good example of this apparent paradox; despite being naturally transformable and residing in the nasopharynx rich with other bacteria, they have retained a very distinct identity. Thus, despite the unmistakable gene shuffling within and between bacteria, we fail to see homogenization of bacterial species. Rather, bacteria have remained discrete and distinct taxonomic entities[44] because the bacterial chromosome has, in general, resisted rearrangement.

Finally, it is intriguing that most cases of serious disease are caused by only a few of the extant clones that constitute a pathogenic bacterial species. This is exemplified by meningococcal disease, where there is a clear predominance of a particular clone in large areas worldwide with only sporadic disease from other clones. In the case of the typhoid bacillus, there is only one major clone worldwide, although recent antibiotic resistance may be forcing diversity.[45] This is also true for *S. sonnei* and *B. pertussis*, both of which are found as one or a small group of closely related clonal types. Study of *E. coli* populations in the human intestinal tract indicates that only a small number of clonal lineages persist, whereas numerous unrelated cell lines appear and disappear.[43] *E. coli* urinary tract pathogens that cause symptomatic disease in humans may be even less genetically diverse than *E. coli* strains found in the intestinal microbiota or those that cause asymptomatic urinary tract colonization.[46] Perhaps the evolution of these *E. coli* strains to live in a more specialized epithelial niche results in constraints on recombination that preserve their added degree of specialization. This fitness for urinary tract colonization may well be a by-product for improved colonization of its "natural" intestinal niche. Indeed, in some individuals with recurrent urinary tract infections, there can be a simultaneous and identical shift in the dominant *E. coli* population of the bladder and distal gut between one episode and the next.[47] Yet, not all pathogenic bacterial species reveal this pattern of clonal organization. Two notable exceptions are *N. gonorrhoeae* and *Helicobacter pylori*, which appear to use chromosomal recombination quite extensively to increase their genetic diversity. In fact, because of strict human adaptation and extensive genomic diversity and drift, comparative analyses of *H. pylori* genome sequences have revealed important aspects of human migration and human population structure.[48]

REGULATION OF BACTERIAL PATHOGENICITY

If an organism possesses specialized gene products for its virulence, it must be able to use them when needed but not squander its metabolic energy producing them aimlessly. Moreover, indiscriminate expression when not required risks having the virulence determinant detected by host defenses and prematurely neutralized. In consequence, virulence factor expression must be tightly controlled, presenting an additional, yet essential complication of a pathogenic microbe's life.[49] Because the host presents an array of conditions strikingly distinct from those of the outside environment, a pathogen must turn on and off a large number of genes to change its behavior and accommodate its new environment. Because studying gene regulation in the laboratory cannot replicate the host environment, these laboratory findings may not truly represent microbial adaptation to the host; in some cases microbial gene expression can be studied using animal models or using snapshots of infection in humans.

Vibrio cholerae is an excellent example of the agility of gene expression in pathogens. *V. cholerae* is thought to persist in a "viable but nonculturable state" in brackish estuaries and other saline aquatic environments, often associated with the chitinous exoskeleton of various marine organisms.[50] Transition from this milieu to the contrasting environment of the human small intestinal lumen is accompanied by substantial genetic regulatory events, including increased expression of cholera toxin. Further "downstream," the massive increase in the number of vibrios in cholera stools may presage a hyperinfectious state and enhanced transmissibility.[51] The transcriptional profile of these organisms as they exit cholera patients is again different; it reflects the recent nutrient deprivation the pathogen has experienced in the colon and the down-modulation of toxin and chemotactic activity that are no longer needed.[51,52]

Despite its beguiling simplicity, the microbial cell possesses myriad means to rapidly detect, often simultaneously, changes in temperature, ionic conditions, oxygen concentration, pH, and metals such as calcium and iron. These signals often play a dual role; they signal the pathogen that it is in an environment that requires expression of certain virulence determinants, and they are essential for the precise mobilization of virulence determinants. For the gastric commensal pathogen *H. pylori*, and for intestinal pathogens that must traverse the stomach, pH may be a critical signal. The *H. pylori* response to low pH involves changes in transcript abundance for 7% of its genes and is associated with increased motility, perhaps as a means for penetrating the gastric mucous layer.[53] The response of certain pathogens to low iron conditions provides a fine example of how pathogens can turn adversity to their advantage. Iron is a critical component of many cell metabolic processes; therefore it is not surprising that animals have evolved to have high-affinity iron-binding and storage proteins that deprive microorganisms of access to this nutrient, especially at the mucosal surface. However, this strategy can backfire badly on the host. The production of many microbial toxins (e.g., diphtheria toxin) is induced under low iron conditions! Temperature is another obvious signal for microbes adapted to warm-blooded animals that may "come in from the cold." In fact, reversible regulation of the expression of virulence genes by temperature is a feature common to many pathogens, including enteropathogenic and uropathogenic *E. coli* (fimbriae and K-1 capsular antigen), *Shigella* spp. (invasiveness and Shiga toxin), and *Yersinia* spp. (virulence-associated determinants, including outer membrane proteins) (Table 1.4). Thermal regulation of these diverse virulence determinants is mediated by myriad mechanisms: changes in DNA topology, messenger RNA conformation, and protein conformation and stability.[54]

Another common mechanism for recognizing environmental signals and parlaying them into changes in gene expression involves the use of two-component regulatory systems that act on gene expression, usually at the transcriptional level.[55,56] Such systems make use of similar pairs of proteins; one protein of the pair spans the cytoplasmic membrane, contains a transmitter domain, and may act as a sensor of environmental stimuli, whereas the other is a cytoplasmic protein (response regulator) with a receiver domain that regulates responsive genes or proteins. Sensor proteins are often kinases that phosphorylate themselves at a conserved histidine residue. These high-energy intermediates then

TABLE 1.4 Examples of Bacterial Virulence Regulatory Systems

ORGANISM	REGULATORY GENE(S)	ENVIRONMENTAL STIMULI	REGULATED FUNCTIONS
Escherichia coli	*drdX* *fur*	Temperature Iron concentration	Pyelonephritis-associated pili Shiga-like toxin, siderophores
Bordetella pertussis	*bvgAS*	Temperature, ionic conditions, nicotinic acid	Pertussis toxin, filamentous hemagglutinin, adenylate cyclase, others
Vibrio cholerae	*toxR*	Temperature, osmolarity, pH, amino acids	Cholera toxin, pili, outer membrane proteins
Yersinia spp.	*lcr* loci *virF*	Temperature, calcium Temperature	Secretion of effector proteins Adherence, invasiveness
Shigella spp.	*virR*	Temperature	Invasiveness
Salmonella typhimurium	*pag*	pH	Virulence, macrophage survival
Staphylococcus aureus	*agr*	Cell density	α-, β-Hemolysins; toxic shock syndrome toxin 1, protein A

Data from Miller JF, Mekalanos JJ, Falkow S. Coordinate regulation and sensory transduction in the control of bacterial virulence. Science. *1989;243:916–922; and Mekalanos JJ. Environmental signals controlling the expression of virulence determinants in bacteria.* J Bacteriol. *1992;174:1–7.*

transfer their phosphate groups to a conserved aspartate residue within the receiver domain of the response regulator proteins. Competing dephosphorylases determine an overall phosphorylation state of these response regulators, hence their level of activity. Many of these regulators are DNA-binding proteins that regulate transcription of multiple gene targets. Systems of this type control, for example, the permeability properties of the *E. coli* cell envelope in response to osmotic stimuli (EnvZ/OmpR), toxin expression by enterotoxigenic strains of *Bacteroides fragilis* in the presence of colonic mucus (RprX/RprY), expression of numerous virulence factors in *Streptococcus pyogenes* (CovR/CovS), the switch from vegetative growth to sporulation by *Bacillus subtilis* (KinA/SpoOF, SpoOA), and even the ability of the soil bacterium *Agrobacterium tumefaciens* to induce tumors in susceptible plant cells in response to phenols found within plant wound exudates (VirA/VirG).

Pathogenic bacteria can also use small regulatory RNAs (sRNAs) to adapt to environmental stress. As an example, under conditions of low iron, oxidative stress, and membrane stress in the laboratory, *M. tuberculosis* produces an sRNA that inhibits expression of nonessential iron-containing proteins by binding to and compromising cognate mRNAs.[57] Under laboratory conditions, preexposure of *M. tuberculosis* to oxidative stress, followed by iron deprivation, hastens the iron-sparing response, suggesting that sRNAs allow pathogens to integrate multiple environmental signals and anticipate near-term challenges.

Pathogens have the ability to take their own census during infection. This phenomenon called "quorum sensing" is mediated through gene regulation, and it too is not unique to pathogenic bacteria; environmental bacteria keep track of their cell density and regulate their gene expression accordingly.[58] In pathogenic bacteria quorum sensing enables precise choreography of virulence factor production during the course of growth in a vigilant host. For example, in the early stages of a developing soft tissue abscess, *S. aureus* turns on antiphagocytic toxins just as the bacteria reach numbers sufficient to draw the attention of neutrophils.[59] *S. aureus* and other gram-positive bacteria use small peptides to sense cell density and regulate virulence gene expression. For many gram-negative bacteria, quorum sensing and cell-cell communication is achieved by secreting and responding to acylated homoserine lactones. *P. aeruginosa,* the agent of multiple diseases in compromised hosts (as discussed earlier) is activated to produce tissue-degrading enzymes by these autoinducing compounds when they reach sufficient concentration.[60] Quorum sensing is also inextricably linked to the formation of complex bacterial community structures on environmental surfaces; these "biofilms," which can form within the host on both endogenous tissues, such as heart valves, and implanted devices, may enable long-term persistence and resistance to host defenses and antibiotics. *V. cholerae* relies on quorum sensing not only to regulate biofilm formation on marine plankton but also to mediate release from these biofilms upon entry into a human host.[61] The use of quorum sensing for virulence may present therapeutic opportunities: quorum factors may serve as targets for novel therapeutic approaches.[58,62]

These major personality changes in the microbe as it shifts habitat from environmental denizen to host-associated pathogen require a significant "make-over," and it all must be tightly coordinated. The coordinated control of pathogenicity incorporates the important concept of a *regulon.* A regulon is a group of operons or individual genes controlled by a common regulator, usually a protein activator or repressor. This regulator may, in some cases, be the second component of a two-component system. A regulon provides a means by which many genes can respond in concert to a particular stimulus. At other times the same genes may respond independently to other signals. Global regulatory networks are a common feature of microbial virulence and basic microbial physiology (see Table 1.4). In many cases regulatory systems are essential for bacterial virulence. The complexity of virulence regulation in a single microbial pathogen is magnified by the coexistence of multiple interacting (cross-talking) systems and by regulons within regulons. *P. aeruginosa*, an organism with diverse environmental niches, contains genes for 55 sensors and 89 response regulators. In contrast, *H. pylori* contains genes for only 4 and 7, respectively, likely reflecting the more restricted environments it occupies.

Finally, pathogens use complex means of gene regulation not just to cope with host defenses but to evade them altogether. Some pathogens (e.g., various *Neisseria* spp. and *Borrelia* spp.) periodically vary prominent antigenic components of their surface and, by so doing, reduce the chance that the host will mount an adaptive immune response to them. Pili are essential for virulence of gonococci in the human host, probably as a result of their role in adherence to the mucosal target surface.[63,64] But pili, like many bacterial virulence determinants, also elicit specific local and systemic host antibody responses. Intermittent production of pili, as well as variation in pilus composition, are strategies used by gonococci to evade the host immune response. The molecular mechanisms behind these strategies are complex. In general terms phase and antigenic variation result from DNA rearrangements (gene conversion) that move pilin-related transcriptionally silent sequences scattered around the gonococcal chromosome to the expression site (*pilE* locus). Numerous different pilus types may be expressed by derivatives of a single *N. gonorrhoeae* strain.

Gene regulation also underlies the ability of *Borrelia* spp. to establish persistent infections in their mammalian hosts, despite humoral responses directed against antigenic proteins on their surface. Persistence by these pathogens depends upon their mechanisms for varying the expression of host-targeted surface proteins, so as to evade specific neutralizing antibodies. These *Borrelia* mechanisms were first elucidated for the relapsing fever agents, *Borrelia recurrentis* and *Borrelia hermsii*[65,66] but have more recently been characterized for the Lyme disease agent, *Borrelia burgdorferi*.[67] Recombination involving a gene conversion mechanism at the expression site of a surface-associated lipoprotein, VlsE, found on a linear plasmid in the pathogen, allows alternative gene copies from an adjacent tandem silent gene array to become expressed and their antigenically variable proteins to be substituted onto the spirochete surface. VlsE antigenic switching has been shown necessary for persistence of *B. burgdorferi* in mouse models of infection. Although

not yet fully understood at a mechanistic level, this phenomenon may serve as an important new target for adjunctive therapies in the quest to develop and deploy a Lyme disease vaccine. Among other microbial pathogens, DNA rearrangements account for flagellar protein variation in *Salmonella* spp.[68]

CLOSE ENCOUNTERS: PATHOGENS AS CELL BIOLOGISTS

Many bacterial pathogens depend on intimate interactions with host cells to execute their pathogenesis program. These interactions are accomplished because of their ability to hijack host cellular processes, often altering host cell membranes, to achieve any one of several distinct outcomes with respect to the host cell: attachment, phagocytosis, or the avoidance thereof. Attachment or close association with host cells is generally accomplished by pili or other adhesins through direct adherence or through binding to extracellular components. The enteropathogenic and enterohemorrhagic *E. coli,* EPEC and EHEC, respectively, usurp the cell's own machinery to do so. They use a specialized secretion system to form a structure containing reorganized actin that protrudes from the host epithelial cell surface, called a "pedestal" or pseudopod (Fig. 1.1). This pedestal facilitates intimate attachment of the bacterium to the host cell, mediated by the binding of the bacterial adhesin, intimin, to a receptor called Tir. Amazingly, Tir is also a bacterial product. The specialized secretion systems of these bacteria include the determinants required to assemble a supramolecular structure that spans the entire bacterial cell wall and resembles a hypodermic needle[69] that is used to secrete effector molecules directly across host cell membranes. Tir is secreted into the host cell through this "needle" together with other proteins that direct host cell phosphorylation of Tir by activating appropriate host signaling pathways. Tir becomes localized on the host cell membrane at the apical surface of the pedestal.[70] That such a complex series of events was evolutionarily selected to orchestrate this attachment structure is mind-boggling.

Because professional phagocytes—macrophages and neutrophils—are innate immune cells that are ready at hand to be rapidly recruited so as to engulf and kill bacteria, the virulence programs of most pathogens feature mechanisms to avoid phagocytosis by these cells. Capsules of gram-positive bacteria can inhibit their phagocytosis through a variety of mechanisms. Many gram-negative bacteria (e.g., *Yersinia*, *Pseudomonas*, *Vibrio*) use their specialized secretion systems to inject proteins into the host cell. These proteins disrupt the formation of polymeric actin complexes that are required for the forces and changes in membrane conformation that allow for phagocytosis.[71]



FIG. 1.1 Scanning electron micrograph depicting pseudopod, or "pedestal," formation by enteropathogenic *escherichia coli* (EPEC) as it interacts with the surface of an epithelial cell. This form of intimate adherence requires a bacterial adhesin, intimin; a receptor of bacterial origin, Tir, that is injected into the host cell; and a series of EPEC-initiated signaling events. Disruption of normal absorptive function results in diarrhea. Other bacterial pathogens are also capable of inducing pedestal formation on intestinal epithelial cells. *(From Rosenshine I, Ruschkowski S, Stein M, et al. A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation.* EMBO J. *1996;15:2613–2624. Courtesy B.B. Finlay.)*

At the same time, many bacterial pathogens thrive on an intracellular lifestyle for all or a significant portion of their life within the host. Intracellular pathogens must contend with multiple host defenses—reactive oxygen and nitrogen species, antimicrobial peptides, and acidification and hydrolytic enzymes of lysosomes and autophagosomes. In fact, intracellular residence may offer advantages. Pathogens can evade certain host defenses, such as complement and antibodies, and they can find access to otherwise restricted nutrients. Professional phagocytes are formidable would-be adversaries, as killing pathogens is one of their major functions. Yet many bacterial pathogens have evolved the means to enter, survive, multiply, and even persist within the very phagocytes designed to kill bacteria. Residence in phagocytes offers the additional advantage that these cells can transport pathogens across epithelial barriers.

Intracellular pathogens are found in all of the classes listed in Table 1.1. They can be obligate (e.g., *M. tuberculosis, S.* Typhi, *Chlamydia trachomatis*), zoonotic (e.g., *Brucella abortus*, *Rickettsia* spp.), or environmental (e.g., *Mycobacterium marinum* and *Legionella pneumophila*). Of note, commensal pathogens (see Table 1.1) appear to be missing from the known set of intracellular pathogens of humans, suggesting that avoidance of phagocytosis is a stringent requirement for a commensal to establish a niche.

How did pathogens become intracellular dwellers? The relationship of bacteria with eukaryotes is ancient; eukaryotic mitochondria are thought to be derived from a bacterial endosymbiont related to extant rickettsial species. Thus intracellular bacteria may have shaped the very essence of contemporary eukaryotes by giving them the capacity for aerobic respiration. But what about contemporary bacterial pathogens that parasitize professional phagocytes (most commonly, macrophages)? They may have been "trained" to live in macrophages through their ancient encounters with environmental amebae. For many pathogenic mycobacteria, their ability to survive in macrophages tracks completely with their ability to survive in amebae; moreover, pathogenic mycobacteria can grow in macrophages, whereas environmental, nonpathogenic species such as *M. smegmatis* cannot.[72–74] Further support for the idea that amebae provided the evolutionary training ground for intracellular growth in macrophages comes from the finding that mycobacterial virulence factors that promote their growth in macrophages also promote growth in amebae. Similarly, another intracellular human pathogen, *L. pneumophila*, an accidental human pathogen that can cause serious pneumonia, replicates in environmental amebae in the potable water sources responsible for human infection.

Once they are attached to host cells, pathogens use different tricks to enter these cells. Some gain entry through cellular receptors that are normally present, thus subverting their normal function. A pathogen can use multiple receptors to gain entry. For instance, *Chlamydia* can enter via the mannose receptor, the mannose-6-phosphate receptor, and the estrogen receptor, highlighting the stringent need for this obligate intracellular pathogen to become intracellular.[75] Pathogens can also modulate host signaling pathways to gain entry, by binding, for instance, cell surface integrins (e.g., *Yersinia* spp.) and tight-junction–apparatus cadherins (e.g., *Listeria monocytogenes).*[71] For macrophage entry, a pathogen needs a specific ligand to be phagocytosed; a coat of complement or antibody will get it internalized via complement or Fc receptors, respectively. However, many macrophage-adapted pathogens also possess "designer" entry mechanisms. Some pathogens, for instance, *Salmonella* and *Shigella*, can induce cytoskeletal rearrangements on the host cell surface that can then lead to their internalization through macropinocytosis, an endocytic pathway used by cells to internalize extracellular fluid via large endocytic vesicles. In these cases the cytoskeletal rearrangements are induced by specific bacterial proteins that are secreted into host cells upon surface contact. Thus, in general, contact of the pathogen with the host cell surface triggers a signaling cascade in both, indicative of a highly evolved process of coadaptation.[17,18] In accordance, some intracellular pathogens possess multiple proteins that contribute

to a coordinated sequence of cytoskeletal remodeling in the host cell so as to achieve their optimal intracellular niche.

Upon engulfment, bacteria, like other phagocytosed material, find themselves in a plasma membrane-bound compartment. When a nonpathogenic bacterium is internalized by a phagocyte, this compartment, or phagosome, interacts with the cell's endocytic machinery and is ultimately delivered to the lysosome for destruction. Therefore successful intracellular pathogens must have ways around this. Broadly speaking, intracellular pathogens resist destruction by one of two methods: They escape out of the vacuole to gain access to the cytosol as their habitat or they remain inside a vacuole while evading or tolerating the consequences. Access to the cytosol has the advantages of not only avoiding lysosomal degradation but also enabling efficient cell-to-cell spread, and it is a tactic used by diverse pathogens, such as *Listeria*, *Shigella*, and *Rickettsia* spp. *Listeria* uses specific proteins to break out of the initial phagocytic vacuole and then spread to adjoining cells by penetrating the double membrane formed by their apposition. Once in the cytoplasm, *Listeria* replicates and induces its own movement through a remarkable process of host cell actin polymerization and formation of microfilaments within a comet-like tail. *Shigella* also lyses the phagosomal vacuole and induces the formation of similar structures for the purpose of intracytoplasmic movement and cell-cell spread. In both cases bacterial and host factors involved in actin polymerization are distinct, reflecting convergent evolution.[71]

On the other hand, pathogens that remain intravacuolar, for instance, *Salmonella*, *Mycobacterium*, *Legionella,* and *Brucella*, create distinct replication niches in modified endosomal compartments. This is generally accomplished by disrupting normal phagosome maturation so as to live in specialized compartments that are permissive for survival and growth. Many different pathogens have evolved so as to create their own unique phagosome niches by intercepting or exploiting the function of small guanosine triphosphatases (GTPases) called Rabs (Ras-related proteins in brains), which are cellular membrane transport regulators. Some bacteria inhibit phagosome-lysosome fusion to avoid acidified conditions and hydrolytic enzymes or may tolerate compartments fused to lysosomes (*Coxiella burnetii* is an example of the latter). Many pathogens, for instance, mycobacteria, appear to use a two-pronged strategy with specific virulence determinants to both inhibit and tolerate phagosome fusion to lysosomes.[71,76] Finally, intracellular bacteria also have to contend with autophagy, a process through which cellular proteins, lipids, and organelles are targeted to lysosomes for degradation. Bacterial vacuoles can likewise be targeted for autophagic destruction, and most successful intracellular pathogens have diverse strategies to avoid autophagy, or, in some cases, even to exploit it for their growth.[71,77]

Intracellular pathogens can kill host cells from within, either as a means to modulate inflammation or to escape from the cell. A number of pathogens, including *Shigella*, *Salmonella*, *Yersinia,* and *Mycobacterium*, are capable of inducing death of macrophages. Although induction of cell death is a common strategy of many pathogens, each accomplishes this outcome through different mechanisms and with a different precise temporal program.[17] Moreover, the same bacterium can induce different types of cell death, depending on context. For instance, mycobacteria can induce apoptotic cell death through their specialized secretion system, ESX-1, and when tumor necrosis factor levels are dysregulated they can cause programmed necrosis of the macrophage with frank membrane rupture.[25] Each of these processes can affect the development and fate of the tuberculous granuloma. Initially, apoptotic death of an infected macrophage can contribute to new macrophage recruitment and thereby increase cellularity of the granuloma. Phagocytosis of the apoptotic macrophages by new macrophages can provide the mycobacteria with new cellular niches, thus serving to expand intracellular bacterial numbers.[25] Hence the granuloma, for 100 years assigned a central role in "walling off" *M. tuberculosis* infection, can also be a structure built by mycobacteria to promote their expansion and dissemination during early infection. Then with the advent of necrotic macrophage death, bacteria are released to the extracellular environment where they can grow further. Furthermore, necrotic granulomas lead to conditions for increased transmission of infection to new hosts.

IDENTIFICATION AND CHARACTERIZATION OF VIRULENCE GENES

The quest for the molecular basis of bacterial pathogenicity dates back more than 150 years to a time when medical microbiologists were trying to understand the basis of the then rampant toxin-mediated diseases diphtheria and tetanus. Characterization of microbial pathogenicity at the molecular level has traditionally begun with the identification of a virulence-associated phenotype. Such identification may come from clinical observation, epidemiologic investigation, or the use of a model system that reliably reproduces the microbial phenotype. The investigator then tries to identify microbial mutants that no longer have the phenotype. One way to do this is by targeting candidate genes (i.e., genes suspected on the basis of prior information) and then mutating them, often by substituting a mutant gene copy for the wild-type copy using homologous recombination. Nowadays, genome sequences can provide a powerful basis for identifying candidate virulence genes. An alternative agnostic approach is to create a "library" of bacterial mutants, often by using insertional genetic elements (e.g., transposons) as mutational agents and testing these mutants for the loss of the phenotype. Recent variations of this method include creating the library with individually tagged mutants so that after the pooled library is tested in a relevant model of pathogenesis, relevant mutants that failed to produce the phenotype can be more easily identified, a process called negative selection.[78,79] Genetic manipulation of microbes that have so far been genetically intractable (i.e., not amenable to homologous recombination or transposon mutagenesis), such as most fungi and many anaerobes, is increasingly feasible using CRISPR-Cas (clustered regularly interspaced short palindromic repeats–CRISPR associated) protein genome editing tools.[80]

A complementary approach to virulence gene identification comes from asking which bacterial genes are differentially expressed in a relevant pathogenesis model, compared with expression levels in the absence of host cells. These genes are prime candidates for virulence determinants and can then be mutated individually as above. *In vivo expression technology*[81] and *differential fluorescence induction*[82] are approaches based on this concept. Quantitative measurements of coding (gene) and noncoding transcripts, and comparisons of RNA abundance, are greatly facilitated by high-throughput random sequencing of complementary DNA with the generation of millions of expressed sequence tags that are then mapped back to genes and genomes with a method called RNAseq.[83] With RNAseq, gene-specific transcript counts are generated and then used as surrogate measurements for relative gene expression levels.

Through these approaches, genes, RNAs, and their products are incriminated by their relationship with a disease-associated process. Just as the original Henle-Koch postulates have provided a reference point for later revised criteria of microbial causality,[84] a molecular form of Koch's postulates[85] provides a guideline for an experimental approach to the molecular genetic basis of pathogenicity. These postulates continue to coevolve in conjunction with emerging insights into microbial virulence and rapidly improving experimental approaches and technologies. For example, alternative approaches for proof of causation are necessary for pathogens that cannot be isolated and for disease in which a "pathogenic community" is believed to be the cause.[1,86]

Identification of a virulence factor then moves the quest to a new level—to understand how it works. Comparisons of wild-type to mutant bacteria and studies of purified virulence factors, using combinations of biochemical, cell biologic, and immunologic techniques, have both provided insights, as have methods that integrate host responses. As discussed earlier, bacterial virulence factors typically act to counter specific host determinants. For instance, the *Salmonella SipB* gene (secreted by a specialized bacterial secretion system) induces host cell death through its interactions with a host protease called caspase-1. In accordance, in caspase-deficient mice, even wild-type bacteria are attenuated, behaving like the bacterial *SipB* mutant.[87] In a similar vein, methods for monitoring genome-wide host responses have helped to reveal virulence mechanisms.[88,89]

MOLECULAR MICROBIOLOGY AT THE BEDSIDE: PATHOGEN DETECTION, PATHOGEN DISCOVERY, AND GENOMIC PROFILING

As mechanisms of microbial pathogenicity are being revealed, pathogen detection, strain identification, drug resistance, and strain relatedness, as well as patient risk stratification and outcome prediction have all assumed increasing importance in the practice of clinical infectious diseases.[20] For instance, outbreak investigations and infection control both hinge on a precise identification of the etiologic agent. Genome sequences have been immensely beneficial in this regard; they provide a basis for sensitive and specific detection of pathogens and a means for establishing relationships among multiple isolates of the same species. Whole-genome sequencing sometimes provides the only clue that a group of cases are related, that is, that an outbreak of disease has occurred, as well as the relationships of the outbreak strain to other strains. As a result, seemingly unrelated cases occurring during an outbreak have been connected; similarly, geographically or temporally distinct outbreaks have been linked to the same pathogenic clone.[90] Molecular techniques have been used in other epidemiologic investigations to study transmission mechanisms and the role of avirulent microbial variants in the spread of disease. In contrast, traditional approaches, based on phenotypic and general metabolic features of isolates, often fail to indicate the true identity, relationships, and genetic diversity of and among strains.

Molecular, typically sequence-based methods have also revolutionized the search for previously uncharacterized microbial pathogens. There continue to be a vast and frustrating number of poorly explained cases of debilitating illness, including relatively common chronic inflammatory and "autoimmune" syndromes, such as inflammatory bowel disease, sarcoidosis, and various forms of arthritis, that share features with known infectious diseases but for which a microbial agent(s) (see prior discussion of "community as pathogen" earlier) has not been identified.[30,91,92] The principle behind these methods is reliance on molecular signatures to identify or classify a previously unrecognized pathogen; the most commonly used signature is genomic sequence, but other small molecules may prove useful. Phylogenetically reliable sequences, such as highly conserved regions of ribosomal RNA genes, are crucial to the characterization of agents whose sequences do not match exactly those of the agents currently known. These or any sequence can be recovered directly from affected (infected) tissues by amplifying or "capturing" them (by hybridization) from extracted nucleic acids or by random shotgun methods.[91] A critical next step is to assess whether or not the inferred agent has a role in causing the disease in question.[86] A number of organisms resistant to cultivation or propagation have been identified with non–culture-based methods, and cases are made for a role in disease causation.[93–95] It is possible, however, that many of the more easily detected bacterial agents have already been found. The large burden of still unexplained disease with features suggesting infection may be due to agents that have come and gone, agents that currently reside in sequestered anatomic sites in a relatively inactive state, or nonmicrobial causes.

Conceptual advances in our understanding of microbial virulence, revolutionary developments in our technical means, and emerging challenges from a rapidly changing environment around us suggest a number of future scenarios and goals. First, we should focus our efforts on the identification and characterization of pathogens directly from clinical specimens and infected hosts, using cultivation-independent approaches. Manipulation and genome-wide characterization of single bacterial cells is now entirely feasible.[96] Deep sequencing–based pathogen identification from clinical specimens is also a reality.[95,97] We should expect to be able to measure genome-wide microbial transcript abundance and metabolic activity directly from human specimens as well. Second, the composition and function of the indigenous microbial communities can be assessed using metagenomic and other community-wide postgenomic technologies.[98] By combining assessments of community and human response, we stand to gain new insights into the nature of chronic inflammatory disorders of skin and mucosa.[99] Third, we need to fully embrace the importance of host genetic variation in differential susceptibility to infection and subsequent disease.[100] Fourth, genomic and postgenomic technologies enable us to measure and interpret patterns of human gene and protein expression associated with the response to infectious disease; these patterns may serve as the basis for signatures, enabling early recognition and classification of patients on the basis of agent or future disease course.[30,101,102,103] As virulence factors for essential steps in pathogenesis are identified, it should be possible to interfere with their function. As they become better characterized, manipulation of global virulence regulatory systems may be used therapeutically to inhibit entire virulence programs. The result of these efforts should be a more informed and effective approach to the detection, treatment, and prevention of infectious diseases.

DEDICATION

Stanley Falkow, who passed away in May 2018, taught and inspired the other two authors, and many other scientists and clinicians, to appreciate and understand the life strategies of host-associated bacteria. His legendary contributions include the discoveries of the transmissible nature of antibiotic resistance, diverse mechanisms of bacterial pathogenesis, and the creation of a modern molecular version of Koch's postulates as a framework to understand microbial pathogenesis. The authors dedicate this chapter—whose underpinnings and content, like the field of bacterial pathogenesis, owe so much to Stanley—to his memory.

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The complete reference list is available online at Expert Consult.

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2 The Human Microbiome of Local Body Sites and Their Unique Biology

Kjersti Aagaard, Ruth Ann Luna, and James Versalovic

DEFINING THE HUMAN MICROBIOME

The human microbiota can be defined as all microorganisms (approximately 90 trillion bacteria, archaea, eukaryotic microbes, and viruses) residing in the human body; the human microbiome consists of the genes and gene products (RNA, proteins, metabolites) produced by resident microbial communities. The advent of high-throughput DNA and RNA sequencing technologies and computational methodologies has enabled scientists to systematically catalog the global set of microorganisms—cultured and uncultured—in a heretofore unparalleled manner. Different body habitats contain microbial communities and microbiomes that differ by microbial composition and function (metabolic modules and pathways). As a result, each body habitat is composed of characteristic bacterial species and other microbial taxa that are adapted to each body site. Differences in microbial composition yield differences in metabolic capacity and aggregate function of the human microbiome.

Traditional notions have been challenged, such as the ideas first put forth in Koch's postulates, whereby microbes were viewed as pathogens and as sole etiologic agents of infectious diseases. Such a "foe" view neglects our earliest sightings of oral and fecal microbes with Anton van Leeuwenhoek's microscopes, where it was observed that *animalcules* (microorganisms) reside in a symbiotic and likely mutually beneficial relationship with the host. We now appreciate that the microbial genome exceeds the human genome by at least 250-fold, and the cellular count of resident microbiota matches and slightly exceeds the human cell count.[1] Our concepts regarding the relative abundance and ubiquity of diverse human pathogens are growing more profoundly with advances in the science of the human microbiome. *Abundance* refers to the relative quantity of microbes within each individual or body site, whereas *ubiquity* refers to the presence of the same microbes in different individuals.

The Human Microbiome Project (HMP) documented the striking absence of canonical pathogens in healthy adults at 18 body sites.[2] Notable exceptions were the well-known pathogens *Staphylococcus aureus* and *Escherichia coli*. As an example, *E. coli* DNA was detected in 15% of individuals at 0.5% abundance and was detectable at any level in 61% of healthy adults. Canonical pathogens as defined by the National Institute of Allergy and Infectious Diseases[2] are generally absent from the human microbiome in healthy individuals, but opportunistic pathogens are widely distributed in healthy adults. A total of 59 opportunistic pathogens in the Pathosystems Resource Integration Center (PATRIC) database were detected in 242 healthy adults, and these species were shared in colonized individuals across multiple body sites. This finding contrasts with the relative habitat specificity of commensal species that lack evidence of pathogenicity. In summary, although canonical pathogens are rare in healthy individuals, opportunistic pathogens are relatively common in healthy individuals and explain why immunosuppression often results in opportunistic infections. Canonical pathogens, by contrast, must be transmitted to healthy individuals from other humans, animals, or the environment. Opportunistic pathogens may arise from within the indigenous microbiome, in addition to possible transmission from outside sources.

The Human Microbiome as a Complex Ecosystem Composed of Multiple Body Site Habitats and Niches

The HMP (funded by the US National Institutes of Health) and Metagenomics of the Human Intestinal Tract (MetaHIT; funded by the European Commission) initiatives established the first microbial gene catalogs of the human adult microbiota; the HMP effort spanned 15 body site niches in men and 18 in women.[1–4] Each primary body habitat in the healthy human microbiome contains a distinctive microbial community, when evaluated according to bacterial composition[2,3,5,6] (Fig. 2.1). Furthermore, the HMP reported that although no bacterial taxa were universally present among all body habitats and individuals, the relative distribution of several metabolic modules and pathways was surprisingly similar, with a greater degree of similarity observed within ethnic and racial groups.[2] On a population-wide scale, the greatest variation in both composition and function is observed when comparing one body niche to another. The next level of microbiome variation is observed when comparing composition and function between individuals of different health and disease states; geographic distribution; race, ethnicity, or both; and life stage. Relatively low-level variation is observed when comparing same body niches among similar groups of individuals in a relatively homogeneous population. In other words, our microbiomes are most distinct when comparing one body niche to another (i.e., gut to vagina, or oral to skin) and relatively less distinct when comparing among individuals (i.e., gut to gut). Expanded analysis of the original HMP cohort (HMP1 II) summarized strain-level variation from a comprehensive data set derived from 2355 metagenomes and 265 individuals.[99] Bacterial strain profiles were stable over time, with the identification of body site–specific subspecies clades. For example, *Haemophilus parainfluenzae* yields distinct subspecies clades in the oral cavity. The Bacteroidetes species contributed to personalized microbial composition of the intestine, compared with other body sites. Multicore metabolic pathways were identified as relatively human specific and included vitamin B_{12} biosynthesis as an example of a human microbiome–enriched pathway.

As a result, our rapidly evolving view of the human ecosystem augments the traditional view of a single pathogen being responsive for disease onset. Even if a single microbe is the etiologic agent of infection, the pathogenesis and pathophysiology of infection can be viewed within the context of the microbiome and human biology. We now appreciate that our human microbiome is a complex ecosystem, with distinct biologic niches. The resultant perspective for human health and disease shifts the focus to the global balance of our microbiota rather than the appearance of a specific infectious agent. As a result, a clear understanding of the role of microbial community structure in the host can facilitate a deeper understanding of infectious diseases and susceptibility to infections (Table 2.1). We are realizing the translational fruits of a broadened understanding of the human microbiome as metagenomic medicine makes strides in restoring health in highly morbid conditions (e.g., recurrent *Clostridioides difficile* [formerly *Clostridium difficile*] colitis).[7]

This chapter describes the current state of knowledge of the origin of the human microbiome and key features of human-associated microbial

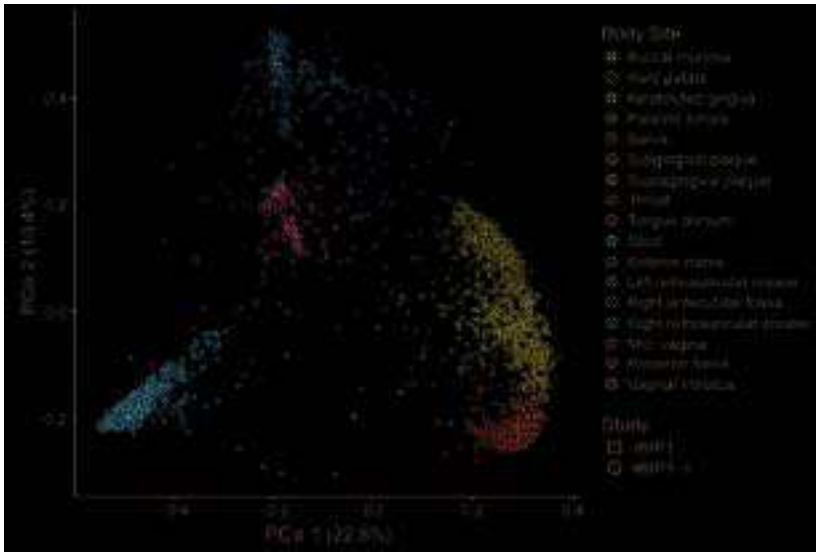

FIG. 2.1 The human microbiome is composed of distinct bacterial populations at different body sites. This principal components *(PCo)* analysis plot shows each distinct body site (indicated by distinct colors) and its microbial composition in healthy adults. Each colored circle in space represents an individual's microbiome as determined by 16S rRNA gene sequencing, and similar microbiomes are grouped more closely together in two-dimensional space. *HMP,* Human Microbiome Project. *(Modified from Lloyd-Price J, Mahurkar A, et al. Strains, functions and dynamics in the expanded Human Microbiome Project.* Nature. *2017;550:61–66.)*

communities in each primary body habitat. We render brief discussions regarding known determinants of the microbial structure of these niches and presumptive associations with several disease states (as examples).

From Whence and When Do Our Microbiomes Come?

It had long been thought that mammalian neonates were first exposed and colonized with microbiota during birth (intrapartum and parturition). However, multiple lines of evidence have converged to suggest that first exposure to microorganisms likely occurs in utero.[8–10,11–14,15–17,18] Although it is not clear whether this earliest microbial exposure results in true live colonization of the fetus or rather enables immune tolerance for later ex utero colonization of the neonate, it is evident that neonates are born with detectable microbes present, and they expand during early infancy to form relatively complex compositional and functional communities with the same body niche separation found in adults[8,14–17,19–36,37–42,43,44]

What are these lines of evidence supporting predelivery microbial exposure? They are numerous and come from not only DNA (i.e., metagenomic) level evidence, but also from cultivation and targeted bacterial species and strain analyses. First, the uterus and its endometrium is clearly not sterile, and an association between endometrial microbes and reproductive success has recently been suggested.[45–49,50,51–57,58,59] Second, the placenta of multiple mammalian species harbors a low-biomass, low-diversity microbiome that can be detected by metagenomics, immunohistochemistry, cultivation, or a combination and is distinguishable from potential "kit" or "DNA extraction buffer" contamination.[40–42,48,60–68,69,70,71–76,77–80,81] Although one group reported an inability to distinguish detection of taxa in the microbiome from "kit negative" and "environmental" controls, their analysis was limited to 16S rRNA gene-based taxa profiles based on V1V2 amplicon sequencing.[82] Moreover, shared taxa at a coarse level (i.e., above species or strain) does not establish contamination. Thus the preponderance of evidence available supports the presence of a low-biomass placental microbial community. Third, as noted previously, the neonate is not born sterile.[8,14–17,19–37,38–44] Fourth, exposures during pregnancy leave a lasting "footprint" on the offspring. Specifically, early factors potentially influencing the neonatal and infant microbiome include gestational age at delivery,[17] infant feeding patterns,[18,83] maternal high-fat diet intake throughout gestation and lactation,[9,19] antibiotic use,[84] and environmental exposures.[85,86] Fifth, there are mixed data concerning whether or not mode of delivery (cesarean versus vaginal) has a lasting impact on the structure and function of the neonatal and infant microbiome. Based on several recent studies, a meta-analysis, and expert committee opinions,[15,33,35,36,87,88–90,91–97,98] we and others support the conclusion that the long-term impact of mode of delivery on the composition and function of the human microbiome is likely minimal, modulated by multiple confounders and collinear factors, and largely limited to neonatal (<28 days after birth) and early infant life. Given the numerous and significant confounding factors in many studies comparing microbiota after cesarean and vaginal birth, it is presently difficult to state that the act of delivering via cesarean in and of itself confers dysbiosis to the offspring, let alone what species or strains might be responsible for disease risk later in life.

What then explains multiple studies suggesting an association between cesarean delivery and several microbiome-related health outcomes? In terms of the longitudinal establishment of the human microbiome, it was initially published and thought that the microbiomes in vaginally delivered versus cesarean-delivered infants yielded a modest difference at up to 6 months of age and appreciable differences years later.[99–101] However, more recent studies indicate that the human microbiome effectively "differentiates" at each body site by 6 to 8 weeks of age, and the effects of delivery mode largely subside by 2 months of age.[8,19] It was initially believed that neonates delivered by cesarean section have a characteristic deficiency of *Bifidobacterium* spp., whereas infants delivered vaginally have a predominance of *Bifidobacterium longum* and *Bifidobacterium catenulatum,* but these observations may be confounded by other factors such as maternal diet and breast-feeding.[33,85,89,99,102–106] In other words, both sets of observations can hold true. Although there may be an association between cesarean birth and several chronic, noncommunicable diseases (asthma, atopic allergies, obesity, type 2 diabetes mellitus), the act of the surgery is unlikely to change the microbiome community. Rather, the company that cesarean delivery keeps (such as underlying medical indication for the cesarean delivery and lower rates of exclusive human milk feeding) may be the primary factors. Thus, efforts aimed at reducing medical indications for cesarean and increasing exclusive human milk feeding may prove to be optimal.[87]

Is the capacity to influence our microbiome limited to early life? Clearly not. These same influential factors continue through adult life, with development and succession of the microbiome occurring during the human lifetime. Population-based studies have identified multiple factors that relate to observed variance in the composition, gene content, and function of the human microbiome. These factors include body habitat,[107,108] age,[109] environmental exposures (chemical and microbiologic), chronic disease,[110,111] genetics,[112] sex,[113] socioeconomic status,[20] geography,[109] and diet.[109,114] Although much has been made of the impact

TABLE 2.1 Explanation of Key Terms

TERM	DEFINITION
Biologic Terms	
Community structure	Used most commonly to refer to the taxonomic composition of a microbial community; can also refer to the spatiotemporal distribution of taxa
Diversity	A measure of the taxonomic distribution within a community, either in terms of distinct taxa or in terms of their evolutionary or phylogenetic distance
Dysbiosis	Abnormal distribution or quantity of microbes at a specific body site
FMT	Fecal microbiota transplantation; refers to the placement of donor fecal content into gastrointestinal tract of recipient
Germ-free	A host animal that carries no microorganisms
Gnotobiotic	A host animal that carries a defined set of microorganisms, either synthetically implanted or transferred from another host; often used to refer to model organisms with humanized microbiota
Metagenome	The total genomic DNA of all organisms within a community
Metagenomics	The study of uncultured microbial communities, typically relying on high-throughput experimental data and bioinformatic techniques
Metametabolome	The total metabolite pool (and possibly fluxes) of a community
Metaproteome	The total proteome of all organisms within a community
Metatranscriptome	The total transcribed RNA pool of all organisms within a community
Microbiome	The total microbial community and biomolecules within a defined environment
Microbiota	The total collection of microbial organisms within a community, typically used in reference to an animal host
Microflora	An older term used synonymously with "microbiota"
Ortholog	A homologous gene in two species distinguished only by a speciation event; in practice, used to denote any gene sufficiently homologous as to represent strong evidence for conserved biologic function
Prebiotic	A food substance metabolized by the microbiota so as to directly or indirectly benefit the host
Probiotic	A live microorganism consumed by the host with direct or indirect health benefits
16S rRNA	The transcribed form of the 16S ribosomal subunit gene, the smaller RNA component of the prokaryotic ribosome, used as the most common taxonomic marker for microbial communities
Analysis Terms	
Alpha diversity	Within-sample taxonomic diversity
Beta diversity	Between-sample taxonomic diversity
Binning	Assignment of sequences to taxonomic units
Chimera	An artificial DNA sequence generated during amplification, consisting of a combination of two (or more) true underlying sequences
Functional metagenomics	Computational or experimental analysis of a microbial community with respect to the biochemical and other biomolecular activities encoded by its composite genome
Gap filling	The process of imputing missing or inaccurate gene abundances in a set of pathways
OTU	Operational taxonomic unit; a cluster of organisms similar at the sequence level beyond some threshold (e.g., 95%) used in place of species, genus, and so on
WGS	Whole-genome shotgun; used to describe shotgun sequencing of individual organisms and, sometimes, microbial communities, although this is not completely accurate because no "whole genome" is typically involved
WMS	Whole-metagenome shotgun; used in reference to undirected metagenomic sequencing to distinguish it from sequencing directed toward specific taxonomic marker genes

of vaginal versus cesarean birth on the developing infant microbiome, the impact of mode of delivery on microbial composition and function remains unclear.

In addition, maternal factors, such as the indication for cesarean, use of preincision versus perioperative antibiotics, consumption of probiotics, maternal body mass index (BMI), and gestational diabetes status, are understudied likely modifiers.[11,99,101,115] These observations have been summarized in reviews by several investigative teams, and formed part of the basis for the American College of Obstetricians and Gynecologists (ACOG) Committee Opinion on "vaginal seeding".[19,36,87,116,117]

ORAL MICROBIOME

The oral microbiome is diverse and abundant. Although a significant amount of research has focused on the gut microbiome with respect to health and disease, there is a substantial body of work regarding the oral microbiome. The HMP demonstrated exquisite niche specificity within the oral microbiome, with distinct communities observed at the level of taxa and gene carriage patterns (Figs. 2.1 and 2.2). To put this into perspective, 1 mL of human saliva in a healthy adult contains approximately 100 million cells, which are discrete from the community of the surrounding oral microbiome. Several studies[2,3,118–121] have documented the unanticipated robustness of the oral microbiome. Microarray, early pyrosequencing, and culture methodologies estimated approximately 700 oral microbial phylotypes. However, dental plaque sampling pooled from 98 healthy adults was estimated to represent 22 phyla comprising 3621 and 6888 species-level phylotypes in the saliva and plaque, respectively.[118] The HMP estimated nearly 70 distinct genera in the same human specimen types.[2] The most abundant bacterial genera in healthy adults include *Actinomyces, Bacteroides, Prevotella, Streptococcus, Fusobacterium, Lautropia, Leptotrichia, Corynebacterium, Veillonella, Rothia, Capnocytophaga, Selenomonas,* and *Treponema,* and the TM7 lineage. In addition to the bacterial kingdom, *Methanobrevibacter*

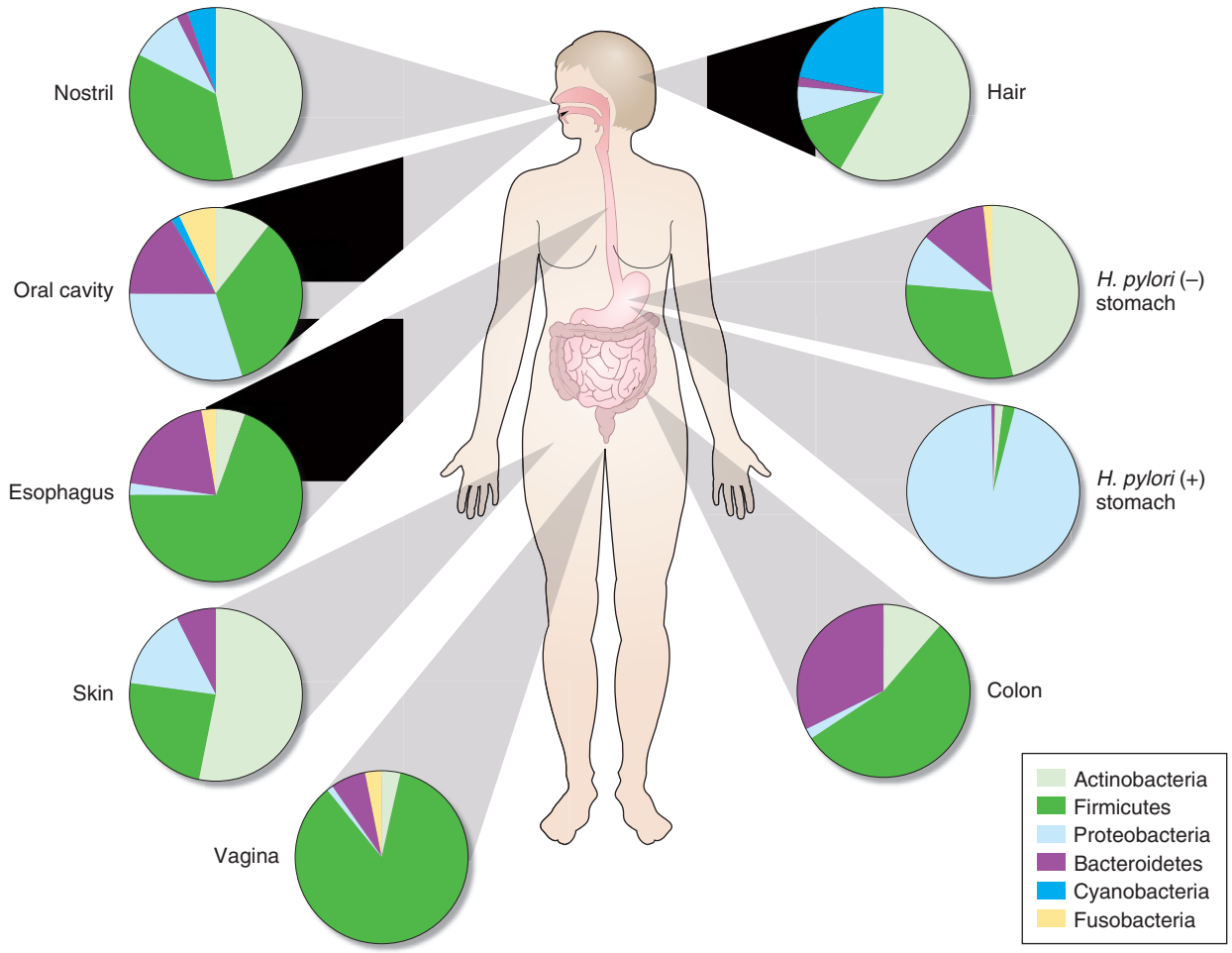

FIG. 2.2 Compositional differences in the microbiome by anatomic site. Metagenomic massively parallel sequencing approaches have demonstrated exquisite body site specificity, and higher level (e.g., phylum) taxonomic features display temporal (longitudinal) stability in individuals at specific anatomic sites. Represented in the figure are relative distributions (percentages) of taxa projected at the phylum level. *(Modified from Cho I, Blaser MJ. The human microbiome: at the interface of health and disease.* Nat Rev Genet. *2012;13:260–270.)*

spp. from the Archaea domain was also identified in the oral microbiome.

Interindividual variation in the microbiome is richly observed in the oral niche. For example, *Streptococcus* spp. dominate the oropharynx,[2,6] with exquisite strain-level genomic variation within microbial species and enriched for host-specific structural variants around genomic islands. Abundant *Streptococcus* phages were found to co-occur with many *Streptococcus* species in the oral cavity, contributing further to interindividual variation.[6] Although these differences are also observed in the gut and skin (leading to issues such as methicillin-resistant *S. aureus* [MRSA]), the oral microbiome is unique in its maintenance of closely adjacent subsites within the niche. The tonsil microbiome can be distinguished from the tongue, and the tongue from the palate. These differences are evident despite spatial proximity and constant contact between these sites.

Associations Between Oral Microbiota and Disease States

With maintenance of niche and subsite specificity in mind, it is not surprising that long-standing associations have been documented between oral health and disease manifestations in distal body sites. For example, periodontal disease is the most common infectious disease affecting the teeth. Left untreated or ineffectively treated, periodontitis is a known independent predictor of, and comorbid contributor to, preterm birth, cardiovascular disease, pulmonary disorders, diabetes, and obesity.[122] Additional strong correlations between the qualitative composition of the oral microbiota as a whole have been made with different disease states. The generation of the dental plaque biofilm that we experience daily has been well characterized.[118–121,122] A succession of early and late colonizing species, dominated early by *Streptococcus* spp., coat the dentin surface of the tooth. Once this early biofilm has been established, a series of highly coevolved oral bacterial and host interactions occur to likely layer bacteria on bacteria, which ultimately generates a large and diverse microbiota load on the tooth surface with a generally healthy periodontium (see Table 2.1). Novel structures of oral microbial communities have been characterized such as "hedgehogs" and "cauliflowers." Oral "hedgehogs" contain filamentous, anaerobic bacteria such as *Corynebacterium, Leptotrichia,* and *Fusobacterium* at the base, while aerobes such as *Streptococcus* and *Haemophilus* are found in the periphery.[123] The presence of *Streptococcus* in oral "hedgehogs" creates a lactate-rich, low-oxygen environment that is hospitable to *Fusobacterium* and *Leptotrichia*.[11] Perhaps these structures contribute to plaque-based oral pathogenesis by enabling oral pathogens to persist and proliferate in the oral cavity. The spatial distributions of microbes within human-associated communities are being unraveled, and highlight the nature of microbe-microbe interactions at body surfaces. A deeper appreciation of the microbial community structures and biofilms may lead to key insights in terms of pathology and treatment.

Periodontitis may be considered akin to bacterial vaginosis (BV) or inflammatory bowel disease (IBD) because it is not the singular absence or presence of a given species or subgenus that drives the oral gum inflammation. Rather, the complexity of the subgingival microbiota and biofilm establishment promote a model of a microbial community-associated disease. In one recent study using deep sequencing,

periodontitis was associated with a shift to populations enriched with gram-negative genera such as *Catonella, Haemophilus,* and *Tannerella*.[121] The microbiome-minded clinician scientist will consider the stability of oral microbiome composition as a hallmark of human health. One exception is infection by *Aggregatibacter actinomycetemcomitans* because this gram-negative rod appears to cause a highly aggressive periodontitis (localized aggressive periodontitis) in Africans with strong host tropism.[122] Other investigators have used whole-genome shotgun sequencing in smaller-scale studies to suggest a potential role for uncultured bacteria of the TM7 lineage in other forms of periodontitis, but broader health and disease associations have yet to be tested in a population-based cohort.

SKIN AND NASOPHARYNX

Because the integument (including skin, hair, and nails) that comprises the main body surface is in constant contact with the outside environment, the human skin consists of diverse sets of local habitats and niches for the human microbiome. The human skin comprises various ecosystems that differ markedly by relative differences in temperature, humidity, and glandular distribution. The human skin microbiome and the nature of the local environment can vary greatly depending on anatomic location. One report described bacterial compositional differences in 20 different sites on the human skin.[124] Recent studies have demonstrated that the skin microbiome differs among healthy individuals more than any other site.[2,3] Bacterial communities are composed largely of transient (superficial), autochthonous (deep surface), and adherent microbes lying adjacent to the epidermis, but they have also been detected in the subepidermal compartment including the dermis and dermal adipose tissue.[125]

Different factors contribute to variation of the human skin microbiome. These factors include host physiology (sex, age, site); environment (local climate, geographic location); immune system; host genotype; lifestyle (occupation, hygiene); and pathobiology (skin and systemic diseases).[107] Different regions of the human skin contain characteristic distributions of different types of glands. These glands produce oily substances such as sebum and other lipid, carbohydrate, and proteinaceous components that may serve as nutrients for the microbiome, and as inhibitors to particular classes of microbes. For example, sebaceous gland–rich regions include the head, shoulders, upper arms, and upper torso.[124,126] Eccrine glands are most abundant in the crown of the head, under the arms, and on the palmar surfaces of the hands. Apocrine glands are enriched around the eyes and ears, nipples, and genital regions. Relative humidity is another key factor affecting microbial composition of the skin. Areas rich in sebaceous glands are enriched for *Propionibacterium* spp. (now called *Cutibacterium* spp.), whereas moist and dry skin areas are enriched for *Corynebacterium* spp. and Betaproteobacteria, respectively (Fig. 2.3).[107,124]

Sebaceous sites contain microbial communities with the least species diversity, whereas dry skin sites house microbial communities with more compositional richness and evenness.[107]

The HMP provided the most comprehensive survey of the human skin microbiome in terms of the number of different male and female adult individuals.[2] A total of 242 individuals were fully analyzed at three body sites (anterior nares, antecubital fossa, retroauricular crease). This study confirmed that the skin microbiome is distinct from that of other body sites and is characterized by an intermediate degree of alpha diversity and richness per specimen. The phyla Actinobacteria, Firmicutes, and Verrucomicrobia were the dominant groups in the human skin,[127] in contrast with the predominance of Bacteroidetes, Firmicutes, and Proteobacteria in the human gut. Therefore even at the level of phyla composed of hundreds of different bacterial species, stark differences are evident in the skin compared with other body sites.[128] Results depend on technical considerations and different specimen types such as skin swabs, blade scrapings, and skin biopsy specimens.[129] Age is an important factor as evidenced by shifts in bacterial communities that occur during the sexual maturation process.[130] Corynebacteriaceae and Propionibacteriaceae predominated in Tanner 5 individuals compared with Tanner 1 children.

Specific groups of microbes may be conserved in the skin of healthy individuals, whereas interindividual variation may account for differences in relative abundances of microbes and differences in disease susceptibilities.[107] Different proportions of *Propionibacterium* spp. and Betaproteobacteria, for example, may be present on the backs or arms, respectively, in different individuals, but these bacterial groups are present in the majority of healthy individuals at these body sites. Representative bacterial genera in the human skin across sites include *Corynebacterium, Eubacterium, Propionibacterium, Staphylococcus,* and *Streptococcus*,[127] and the fungal *Malassezia* spp.[131] In the nares, *Corynebacterium* is the most common bacterial genus,[107] and persistent *S. aureus* colonization was found in the nares in 24% of healthy human subjects.[132] The nasal microbiota contains different proportions of staphylococci, with some individuals carrying mostly *S. aureus* and some individuals carrying mostly *Staphylococcus epidermidis.* The genus *Malassezia* is the predominant fungal genus of the human skin at multiple body sites, including the head, torso, arms, and legs,[127] except for sites on the foot.[131] Persistent effects of antifungal agents on skin fungi or "mycome" were observed in this study.[131]

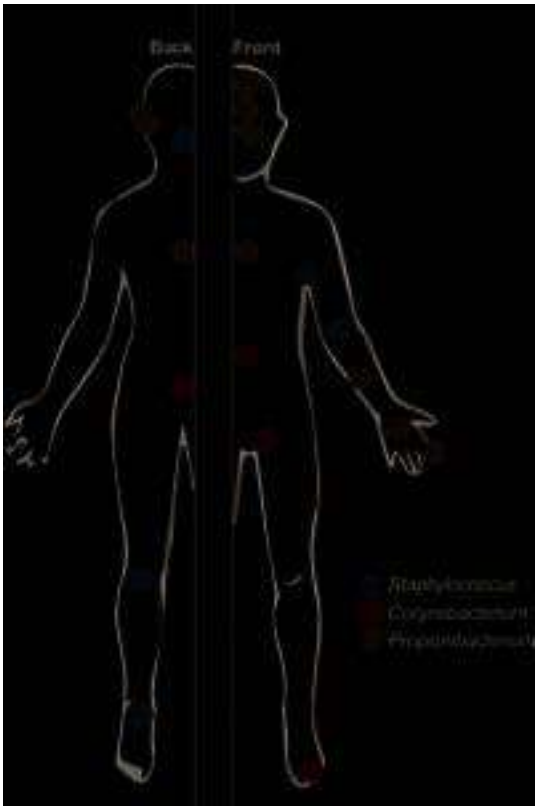

FIG. 2.3 Predominant microbes in specific skin sites. This schematic figure shows the predominant bacterial genera (by color) at each skin site on the human body. *(From Grice EA. The intersection of microbiome and host at the skin interface: genomic- and metagenomic-based insights.* Genome Res. *2015;25:1514–1520.)*

Relative distributions of commensal bacteria and opportunistic pathogens may help explain different patterns of cutaneous infections and systemic penetration of pathogens from skin surfaces. Hospitalized individuals with *S. aureus*–predominant nasal microbiomes were also more likely to carry MRSA associated with their hospitalizations.[133] In cases of atopic dermatitis, staphylococci including *S. aureus* and *S. epidermidis* populations appear to "bloom" and contribute to disease flares and relapse at specific skin sites.[134] With respect to acne, only *Cutibacterium acnes* (formerly *Propionibacterium acnes*) strains belonging to one of six ribotypes were associated with acne, whereas nonpathogenic *C. acnes* strains belonging to other ribotypes were not associated with disease.[135,136]

Bacterial composition of the human skin may be useful for forensic applications. One study described the utility of skin fingertip microbiome patterns for tracking the use of keyboards and perhaps other devices by specific individuals.[137] This study highlights the individualized nature

of the skin microbiome at specific sites and the fact that interindividual differences in bacterial composition may help explain relative disease susceptibilities.

AIRWAY AND PULMONARY MICROBIOME

Human lungs were once believed to be sterile, but innovative research and advances in DNA sequencing technology have provided evidence of microbial communities in upper and lower airways. The airway microbiome has now been characterized on the first day of life, with a predominance of Firmicutes and Proteobacteria noted.[138] When comparing extremely low-birth-weight (ELBW) preterm infants delivered vaginally with ELBW preterm infants delivered via cesarean section, no significant differences were seen in the communities isolated from tracheal aspirates.[139]

Specimens from different sites along the respiratory tract of six healthy adults were characterized to determine which specimen types were most appropriate for microbiome research.[140] Specimen types ranged from nasopharyngeal swabs to bronchoalveolar lavages (BALs). Microbial composition of lung communities appeared consistent among the various sampling sites, and diminished amounts of bacterial DNA content were isolated from deeper lung specimens. The microbiomes of the healthy, lower respiratory tract (BAL specimens) in healthy adults were composed mainly of Firmicutes and Bacteroidetes with a predominance of Veillonellaceae, Prevotellaceae, and Streptococcaceae. A subsequent study attempted to characterize the fungal communities in BAL specimens obtained from healthy individuals, and although few fungal sequences were obtained, common environmental organisms such as Davidiellaceae, *Cladosporium,* and *Aspergillus* were identified in low abundance.[141] More recently, protected specimen brushings and BAL specimens were obtained from eight healthy adults, and the findings suggested that bacterial communities detected in the lower airways were distinct from potential contaminants, with *Prevotella, Veillonella,* and *Streptococcus* as the most abundant genera in the lower airway microbiome.[142]

Staphylococcus carriage and subsequent infection in hospitalized patients was the topic of a separate study comparing nasal swabs in a healthy cohort and a cohort of hospitalized patients.[140] Although Actinobacteria (68%) and Firmicutes (27%) were the most common phyla in nasal swabs of healthy patients, a reversal in relative abundance was reported for the hospitalized cohort with Firmicutes (71%) and Actinobacteria (20%). This significant shift in community composition was attributed to an overall increase in the amount of *S. aureus* and *S. epidermidis,* and to reduced amounts of *P. acnes* in the hospitalized groups. As mentioned previously, disease states are generally associated with diminished bacterial diversity, but the contrary was found in a recent study in a subset of patients with pulmonary tuberculosis (TB). Sputum specimens collected from healthy individuals and patients with TB suggested greater bacterial diversity in the TB group compared with the healthy group.[143] At the phylum level, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria were detected as expected in the healthy group. The TB group indicated a decrease in Bacteroidetes and an increase in Firmicutes and Actinobacteria. Predominant genera were similar across both groups with the most common bacteria identified as *Streptococcus, Granulicatella, Actinomyces, Prevotella,* and *Veillonella.* Several genera were found only in the TB group, suggesting that the lungs of patients with TB may harbor unique bacterial species.

A comparison of children with persistent bacterial bronchitis (PBB) and healthy children revealed decreased bacterial diversity in the lungs of children with PBB versus healthy children.[144] Discrepancies were seen between the dominant organisms identified by sequencing and culture results, with only 15 of 24 patients (62.5%) producing a positive culture for the organism that was predominant in the pulmonary microbiome. *Haemophilus, Neisseria, Streptococcus,* and *Moraxella* were each shown to dominate microbial communities of patients with PBB.

Bacterial and viral pathogens have been implicated as possible causes of asthma and potential triggers of asthmatic episodes. In a study of healthy children and children with asthma, significant shifts in overall bacterial communities present in the respiratory tract were not detected at the phylum level, with both groups displaying the expected predominance of Bacteroidetes, Firmicutes, and Proteobacteria (the asthma group exhibited a slightly different order of prevalence: Firmicutes, Proteobacteria, and Bacteroidetes).[145] Interesting to note, although the healthy children were characterized by carrying *Prevotella, Streptococcus, Veillonella,* and *Fusobacterium,* the asthmatic group included increased relative abundance of *Haemophilus,* a pathogen *(Haemophilus influenzae)* previously implicated as a potential trigger of asthmatic episodes. In a more recent study focusing on severe asthma, multiple genera, most notably *Bacteroides*, *Faecalibacterium*, and *Roseburia*, were significantly increased in children with severe asthma compared with those without asthma.[146] Adding to the microbiome data in the asthma population is a compelling study from Ecuador, where treatment of respiratory illnesses differs greatly from the standard of care in the United States. Oropharyngeal swabs were obtained from both wheezing and healthy infants, and all patients had minimal exposure to antibiotics and no exposure to inhaled steroids.[147] The overall bacterial community in the study population consisted of Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Fusobacteria in order of predominance. The most common genera isolated were consistent with the findings of Hilty and colleagues,[145] with most bacteria identified as *Streptococcus, Veillonella, Atopobium,* and *Prevotella.* In the wheezing group, a greater frequency of *Neisseria, Prevotella, Corynebacterium, Staphylococcus, Actinomyces,* and *Haemophilus* was detected.[147]

The airways of patients with cystic fibrosis (CF) provide an ideal environment for bacterial proliferation leading to chronic and acute respiratory infections characteristic of the disease. In the general CF population, a diverse bacterial community has been found in respiratory specimens from younger patients with mild disease and good lung function, and decreased bacterial diversity has been found, as expected, in older patients with more severe disease and significant declines in lung function.[148–150] Microbiome-based studies have also confirmed that anaerobic bacteria are far more prevalent in patients with CF than indicated by routine culture detection. Cox and colleagues[148] conducted a PhyloChip-based study in both children and adults with CF in 2010. The results suggested that bacterial diversity in the pulmonary microbiomes of children with CF increased until approximately age 11 and continuously decreased during adulthood.[148] A core pulmonary microbiome has been suggested in CF, consisting of seven genera: *Pseudomonas, Streptococcus, Neisseria, Catonella, Porphyromonas, Prevotella,* and *Veillonella.*[151] *Pseudomonas aeruginosa* was the most prevalent bacterium identified in this adult cohort. Although limited data exist on fungal communities of the airways, *Candida, Aspergillus, Geotrichum,* and *Malassezia* spp. were commonly identified in the sputa of patients with CF.[152]

In a study of healthy subjects versus subjects with CF, the CF group was found to have an increased amount of Proteobacteria and Actinobacteria with a relative reduction in Bacteroidetes and complete loss of Fusobacteria.[149] Reduced bacterial diversity in the airways was confirmed in the CF specimens, with 17 different phyla identified in the healthy population compared with 10 different phyla identified in the CF population. Decreased bacterial diversity in patients with CF has repeatedly been associated with advanced disease, and increased amounts of *P. aeruginosa*.[150] In contrast, the relative abundance of *Streptococcus* spp. has been associated with increased bacterial diversity and relative clinical stability or lack of evidence of worsening of the disease phenotype in patients.[153] Longitudinal analysis confirmed the presence of airway microbial communities with decreasing diversity in children with CF and advancing age. The presence of bacterial pathogens was also associated with increased inflammation of the lower airways.[154]

Intubated patients are at risk for a variety of additional health complications including ventilator-associated pneumonia (VAP). Microbiome characterization of tracheal aspirates obtained from intubated and mechanically ventilated intensive care unit (ICU) patients showed that increased duration of mechanical ventilation and subsequent development of VAP were associated with decreased bacterial diversity.[155] Antibiotic administration was not shown to affect diversity in this cohort. A separate study compared subjects with respiratory failure requiring intubation and mechanical ventilation with healthy subjects undergoing bronchoscopy.[156] Bacterial diversity was reported in the ventilated subjects,

and diversity continued to decrease with extended time on the ventilator. Several microbiomes were dominated by a single microorganism, and pathogens identified via culture correlated with the most abundant DNA sequences in these cases. In two cases, specific pathogens were identified via sequencing and yielded clinically actionable information, in parallel with negative cultures. As another example of disease-specific microbial perturbations in the airways, alterations in the human airway microbiome may contribute to the development of lung disease (e.g., bronchopulmonary dysplasia).[140]

Connections among microbiomes in different body sites may help us understand patterns of human infections. An innovative study by Madan and colleagues[157] sought to characterize and compare gastrointestinal and respiratory tract specimens from infants with CF during the first 24 months of life. Overall, *Veillonella* and *Streptococcus* were most commonly identified among both specimen types. *Streptococcus, Veillonella,* and *Prevotella* were the most prevalent genera in the respiratory tract, and *Bacteroides, Bifidobacterium,* and *Veillonella* were the most prevalent genera in the gastrointestinal tract. In general, bacterial diversity increased over time, with more rapid diversification occurring in the developing respiratory tract. Interesting to note, gut colonization preceded subsequent respiratory colonization for several genera, including *Roseburia, Dorea, Sporacetigenium, Coprococcus, Blautia, Enterococcus,* and *Escherichia.* Aspiration may account for the spread of organisms from the gut to the airways, possibly resulting in infections in the compromised host.

GASTROINTESTINAL TRACT

Esophagus

The proximal esophagus and midesophagus are thought to mostly harbor transient bacteria and yeasts, and little is known about the nature of microbial communities at these locations. Microbes in these locations mostly originate from the oropharynx owing to swallowing or the stomach owing to reflux. Contributions of the microbiome to proximal esophagitis remains an open question. Relative susceptibilities to bacterial, fungal, and viral infections of the proximal and midesophagus may be affected by such transient microbial communities.

By contrast, the distal esophagus immediately cephalic to the gastroesophageal sphincter contains a moderately diverse microbiome. This esophageal region appears to harbor a collection of permanent residents that include bacteria, yeasts, and viruses in human patients. Older culture-based studies showed that gram-positive bacteria such as *Streptococcus* dominated the distal esophageal ecosystem. More recent studies pertaining to the HMP have generated comprehensive data sets based on 16S rRNA gene sequencing and whole-metagenome sequencing. In terms of global parameters, the distal esophageal microbiome is less rich and less diverse than that of the large intestine. The phenotypically normal distal esophagus contains a less complex microbiome composed largely of the phylum Firmicutes and dominated by the genus *Streptococcus.*[158,159]

In contrast with the large intestine, it appears that relative "overgrowth" and increased microbial diversity in the esophagus are associated with disease states. To explain these findings, we propose the microbial diversity setpoint hypothesis. This hypothesis states that increased diversity in regions that usually have lesser diversity is associated with disease and inflammation, and that reduced diversity in regions of greater diversity is associated with disease and inflammation. Increased bacterial species richness and diversity in the esophagus were associated with esophagitis and Barrett's esophagus.[159] The distal esophagus in patients with esophagitis or Barrett's esophagus contained a diverse microbiome dominated by gram-negative anaerobes and microaerobes,[159] and this population shift may explain the greater propensity toward inflammation in patients exposed to greater concentrations of endotoxin or lipopolysaccharides (LPSs) in the distal esophagus.[159] Qualitative aspects of bacterial composition, in addition to quantitative features, may contribute to host susceptibility to esophageal infections and esophagitis.

Stomach

Discovery of *Helicobacter pylori* in 1982 led to the widespread appreciation of bacterial colonization in the human stomach. Follow-up studies highlighted the differential abundance of *H. pylori* in different regions of the stomach, with the greatest concentration of bacteria generally present in the antrum. Recognition of the widespread prevalence of *H. pylori* in diverse human populations and evolutionary studies tracing patterns of human migration[160] emphasized its potential significance as a commensal bacterium that coevolved with man during thousands of years of human evolution. Blaser and colleagues[161] first proposed that *H. pylori* may represent an important commensal microbe in the human stomach, and its presence was protective against gastroesophageal reflux disease (GERD), Barrett's esophagus, and adenocarcinomas of the gastric cardia and distal esophagus. In summary, these discussions laid the foundation for concepts of the human microbiome and how antibiotics could increase chronic disease risk by extermination of valuable members of the human gastrointestinal microbiome.

More recent studies based on 16S rRNA gene sequencing demonstrated the presence of 128 bacterial phylotypes in human gastric biopsy specimens.[162] The dominant species was *H. pylori* in infected individuals, but this bacterial species did not affect the overall bacterial composition in a series of 19 subjects. The dominant phyla in the human stomach, such as Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria, overlap with those of the large intestine, but the phylum Fusobacteria seems to be differentially enriched in the stomach. An interesting finding was the presence of *Deinococcus*-like organisms in the stomach, undefined at the time of publication.

A more recent gastric microbiome study highlighted the ecologic importance of *H. pylori* and the presence of communities composed of different microbes.[163] Gram-positive bacteria such as *Streptococcus* and *Lactobacillus* spp. were found in the human stomach. When *H. pylori* is absent, the genus *Streptococcus* is the predominant genus in the gastric microbiome.[163] Differences in gastric bacterial communities, in the presence of *H. pylori*, may predispose patients to acute or chronic gastritis, intestinal metaplasia, and gastric adenocarcinoma.[164] The inability to maintain a sufficiently low luminal pH in conditions such as achlorhydria or proton pump inhibitor consumption has been associated with relative bacterial "overgrowth" and increased bacterial diversity associated with chronic disease states including gastric cancer.[165] In accordance with the microbial diversity setpoint hypothesis, the stomach is a region of limited bacterial diversity, so increased diversity would be predicted to be associated with inflammation and chronic disease. Regions with limited microbial diversity such as the esophagus, stomach, and proximal small intestine may also be more susceptible to pathogens including viruses that are able to survive and thrive in these ecosystems. Finally, the long-term effects on microbial gut composition after antibiotic therapy of *H. pylori* infection, including the diminution of Actinobacteria for weeks after treatment, highlight the potential risks of antimicrobial therapy.[166]

Intestine (Small and Large)

The biogeography of compartments in the small and large intestine affects bacterial composition and metabolic pathways present in the human microbiome. On the basis of older culture-based and more recent DNA sequencing–based studies, bacterial diversity gradually increases in a proximal-to-distal manner from the duodenum through the jejunum to the distal ileum and colon. The duodenum and jejunum can be considered areas of relatively limited microbial diversity, in contrast with the terminal ileum, which contains a rich and diverse microbiome similar to the proximal colon. Although substantial differences in microbial composition in different intestinal compartments are appreciated, detailed information is available only for the major compartments such as small intestine, large intestine (colon), and feces. Ileostomy specimens and small intestinal fluid specimens obtained with a nasoileal catheter provide glimpses into the composition and function of the small intestinal microbiome. The gram-positive Firmicutes phylum including genera such as *Streptococcus, Veillonella* (*Clostridium* cluster IX), and *Clostridium* (e.g., cluster XIVa) appear to dominate the small intestine,[167,168] in contrast with dominant bacterial groups in the colon. Other bacterial phyla such as Bacteroidetes and Proteobacteria (e.g., *E. coli* and other Gammaproteobacteria) were detected in relatively greater abundance in the distal small intestine. In active celiac disease, specific genera such as *Bacteroides, Clostridium,* and *Staphylococcus* are enriched in relative abundance.[158] Metagenomics and gene expression

profiling data show that dominant *Streptococcus* spp. express genes involved in carbohydrate metabolism and simple carbohydrate transport phosphotransferase systems (PTSs). Various intestinal bacterial species convert simple sugars into organic acids such as lactate, acetate, propionate, and butyrate, and ultimately affect the proliferation and virulence of various pathogens. For example, acetate produced by *Bifidobacterium* spp. suppresses the virulence of Shiga-like toxins produced by verotoxigenic *E. coli*.[169] The human gut virome contributes to the rapid evolution and microbiologic diversity in the intestine with implications for infectious diseases. Viruses such as the Microviridae (lytic phages) evolve rapidly in the intestine based on DNA substitution rates,[170] and viral populations undergo dramatic changes in the human gut during the first 2 years of life.[171] In terms of functional consequences, temperate phages that infect members of the Bacteroidetes phylum encode antibiotic resistance genes in the human intestinal microbiome.[172]

The large intestine (cecum; ascending, transverse, and descending colon; sigmoid and rectum) contains rich and highly diverse microbial communities with two predominant bacterial phyla in healthy individuals (Bacteroidetes and Firmicutes). The phylum Bacteroidetes is dominated by the genus *Bacteroides,* whereas the phylum Firmicutes contains diverse commensal microbes of genera such as *Clostridium, Faecalibacterium, Lactobacillus,* and *Ruminococcus*. The first week of life is highlighted by large-scale fluctuations in gut bacterial composition in neonates, and microbial succession patterns have been described in preterm infants with predictable increases in classes such as Gammaproteobacteria.[173,174] Multidrug-resistant enteric pathogens can become established in the neonatal gut microbiome and could predispose infants to difficult-to-treat enteric infections[17,173] By the end of the first 3 years of life, a relative equilibrium is reached with a diverse, adult-like gut microbiome.[109] Shifts in bacterial phyla, families, and genera occur throughout childhood. Genera such as *Bifidobacterium and Faecalibacterium* and microbial metabolic pathways such as vitamin B_{12} biosynthesis are enriched in healthy children versus healthy adults.[175,176] Major differences in gut microbial composition have been reported in different pediatric populations on three different continents.[177,178] In healthy adults, these phyla are less abundant with a proportionately greater predominance of the dominant phyla Bacteroidetes and Firmicutes. Notably, members of the genus *Bacteroides* have been associated with interindividual variation of the intestinal microbiome among healthy adults.[6] Genera such as *Bifidobacterium* gradually decline during adulthood. The Eldermet study[179] described shifts in the gut microbiome in elderly individuals, and bacterial composition depended on environmental factors such as type of residence (nursing homes versus community residence). Although the global importance of enterotypes remains controversial, bacterial DNA sequencing data from stool specimens indicated that healthy humans can be classified into three basic enterotypes (enriched for *Bacteroides, Prevotella,* or *Ruminococcus* in enterotypes 1, 2, and 3, respectively). The functional importance of these enterotypes and whether such microbiome "types" influence clinical outcomes remain unknown.

In regions of abundant microbial diversity such as the intestine, reduced diversity has been associated with increased disease susceptibility and disease relapse in the intestine. One example is the documented reduction in overall bacterial diversity in stool specimens from patients with recurrent *C. difficile* disease versus patients with single disease episodes.[180] IBD and necrotizing enterocolitis (NEC) are other disease phenotypes that have been associated with reductions in microbial richness and diversity in the intestine.[181] Reduced microbial diversity may contribute to diminished immunomodulatory functions by the microbiome and reduced resistance to intestinal pathogens, effectively predisposing the host to infectious enteritis or colitis.

Several well-established and potential pathogens belong to the enteric bacteria within the phylum Proteobacteria, a minority but prevalent phylum of the intestine. The class of Gammaproteobacteria includes pathogens belonging to the genera *Escherichia, Salmonella, Vibrio,* and *Yersinia*. Interesting to note, Gammaproteobacteria have been described in greater abundance in different disease conditions such as IBD, irritable bowel syndrome (IBS), and NEC.[182,183,184] Specific classes of bacteria such as Gammaproteobacteria may provoke inflammation by producing potent endotoxins or other molecules that exacerbate disease states. Acute or chronic disease states coupled with loss of integrity of the intestinal epithelial lining may predispose specific patients to colitis or abdominal infections.

Studies have largely depended on self-collected stool specimens, although numerous studies have documented findings in colonic biopsy specimens. Data from self-collected stool specimens appear to be a reasonably effective source of information about the distal intestinal microbiome, and these specimens have provided most of our current knowledge about the intestinal microbiome. Colonic biopsy specimens revealed the overlap in composition with stool, and differences in relative abundance and microheterogeneity present in different intestinal regions.[185] Largely on the basis of fecal data, intestinal bacterial composition, which represents the vast majority of microbial genetic content in the gut microbiome, is relatively stable in terms of composition and functional capacity within an individual. General surgical interventions, in addition to medications and diet, may profoundly alter the composition and function of the gut microbiome,[186,187] emphasizing that environment may be the dominant driver over host genetics when it comes to shaping the human microbiome.[188] A detailed short-term study examining the impact of diet on the microbiome confirmed that enterotypes were stable within a 10-day period even after major dietary changes such as introduction of high-fat, low-fiber or low-fat, high-fiber diets.[189] Although microbial composition may be relatively stable despite minor fluctuations within an individual, it appears that dietary changes including the introduction of probiotics may rapidly alter gene expression patterns in the gut microbiome. Data from mouse models[190] suggest that functional dynamism in terms of gene expression and microbial metabolomes may easily exceed the routine changes in intestinal microbial composition. Finally, resilience of the intestinal microbiome has been demonstrated by the nearly complete reconstitution of human gut bacteria within 4 weeks after cessation of oral antimicrobial therapy.[84] Such considerations of the relative dynamism and resilience may be important to consider in disease states with less diverse intestinal microbiomes, and these properties of the microbiome may be different in regions adjacent to the intestinal mucosa.

VAGINAL MICROBIOME

With use of traditional culture techniques and light microscopy, a preponderance of lactobacilli was first appreciated as comprising normal vaginal microbiota. In 1892, Gustav Döderlein described his discovery that the vagina was dominantly populated with *Lactobacillus* spp. Since that time, the notion that lactic acid– and hydrogen peroxide–producing lactobacilli are the keystone genera in a healthy vagina has led to the commonly accepted notion that *Lactobacillus* spp. stability and dominance are the hallmarks of a "healthy" vagina and are central to reproductive health. In the late 1800s, Menge and Kronig first described the isolation of anaerobes in addition to *Lactobacillus* from the vagina, often with a dearth of lactobacilli. In other cohorts of women, "abnormal microbes" were observed because of their association with a malodorous discharge, dominated by *Gardnerella vaginalis* and later ascribed as "bacterial vaginosis" (BV). A symbiotic relationship exists between the vaginal microbiota and each host that likely provides the host protection from colonization by harmful pathogens.[191] Molecular studies of the vaginal microbiome in healthy reproductive-aged women confirmed earlier observations demonstrating domination by *Lactobacillus* spp., producing lactic acid to lower the vaginal pH.[192] Species such as *Lactobacillus crispatus* and *Lactobacillus iners* are the most abundant vaginal bacterial species in the absence of BV, and *Eggerthella* and *Leptotrichia* species dominated the vaginal microbiome in the presence of BV.[193] Among smaller cohorts of equally healthy women, molecular interrogations have demonstrated that their vaginal microbiome is alternately dominated by a diverse array of anaerobic microorganisms.[192] Some studies hypothesize that the composition of these communities can be correlated with disturbance responses in the vagina.[192]

Bacterial Vaginosis: An Example of a Prevalent Pathobiont in the Vaginal Microbiome

The clinical diagnosis of BV is familiar to any medical student during training and includes vaginal secretions with a pH level greater than 4.5, a "fishy" odor best elicited by mixing vaginal secretions with 10% potassium hydroxide (KOH) solution, a milky white vaginal discharge,

and at least 20% prevalence of "clue cells" (vaginal epithelial cells coated with bacteria). These clinical criteria are often discussed in a modified form as Nugent scores, with a score of 7 to 10 being standardized as the diagnosis of BV and indicative of the absence of lactobacilli and a relative predominance of *G. vaginalis* and *Mobiluncus* spp. The Nugent score is rarely used by clinicians because reading the slides takes time and requires trained microscopists. Although there is much controversy regarding causation versus association, women with BV are at higher risk for pregnancy complications such as preterm birth, preterm premature rupture of the membranes, and postpartum endometritis.[194] Outside of pregnancy, BV is associated with an increased risk of acquisition of HIV, and women with BV and HIV have a greater risk of lifelong morbidity.[195–197] Although it is outside the scope of this chapter, it bears mentioning that treatment of asymptomatic BV is associated with an increased risk of preterm birth.[191,194,198] Although the pathophysiology mediating these observations is unclear, the microbiome-minded physician might choose to minimize clinical interventions that disrupt the complex vaginal ecosystem.

GROUP B STREPTOCOCCUS

Streptococcus agalactiae (colloquially referred to as GBS) is a gram-positive α-hemolytic bacterium that can cause invasive GBS disease in the early newborn (<6 days of age), characterized primarily by neonatal sepsis and pneumonia, or less frequently meningitis. In contrast to the early neonate, GBS rarely causes morbid disease in the pregnant women who carry it, but may occasionally be associated with urinary tract infections, amnionitis, endometritis, or sepsis or meningitis either during pregnancy or in the postpartum interval.[199] Therefore GBS may be considered to be a pathobiont member of the commensal gut and vaginal microbiomes, with GBS colonization of the vagina or rectum occurring in an estimated 10% to 30% of pregnant women. In an effort to eliminate neonatal mortality due to early invasive GBS disease, the current US standard for maternal GBS detection during pregnancy is universal screening by vaginal and rectal culture at 35 to 37 weeks gestation, or with preterm labor or preterm premature rupture of membranes.[200,201] Since 2011, US guidelines have provided a permissive statement for a limited role of nucleic acid amplification tests for intrapartum testing for GBS. The current US recommendation for a positive GBS culture test result (or with history of previous infant with GBS septicemia, positive maternal GBS bacteriuria during pregnancy) is intrapartum antibiotic prophylaxis, resulting in as many as 1 million U.S. women annually receiving multiple doses of penicillin or ampicillin, cefazolin, vancomycin, or clindamycin or erythromycin in labor.[199] However, other developed countries, including in the United Kingdom, with similar rates of asymptomatic maternal GBS colonization during pregnancy instead take a risk-based approach to GBS screening and treatment.[202] Irrespective of the method used to determine who receives GBS prophylaxis for the prevention of perinatal group B streptococcal disease, given a current case prevalence of invasive early newborn GBS disease of less than 0.4 cases per 1000 live births (and a prenational guidelines prevalence of 1.7 cases per 1000 live births[199]), thousands of women will be exposed to multiple antibiotic courses in order to prevent a single neonatal case. As expected, the current guidelines on either continent have had no effect on late-onset GBS disease (defined as occurring in neonates older than 6 days).

Although GBS colonization is not a risk for preterm birth per se, vertical transmission of GBS has long been independently associated with neonatal bacteremia and sepsis with worsened prognosis in the preterm (<37 weeks) neonate.[203] For several decades the standard of care in the United States was to treat women with at-risk pregnancies. As discussed previously, this approach has shifted in the last 2 decades. As a result, neonatal colonization has been dramatically altered. Ironically, the reduction of neonatal deaths from GBS septicemia has been partially offset by a proportionate increase in neonatal deaths due to infection by β-lactam-resistant *E. coli* in very-low-birth-weight (VLBW) and premature infants.[204] As a result, the overall rate of early-onset sepsis has not significantly changed, but the prevalence of resistant organisms has significantly risen.[204,205] This epidemiologic shift serves as a poignant reminder of the possible effects of broad population-based screening and treatment, and unintended consequences in affected cohorts.

THE HUMAN MICROBIOME DURING PREGNANCY

Despite several lines of evidence from many laboratories supporting the presence of a placental microbiome (as measured by metagenomic and other means) in both humans and other mammalian models,[a] there has been limited concern raised regarding the possibility of environmental contamination skewing these findings.[82,207] Ongoing studies in multiple laboratories should provide a clearer picture of the presence and functional role of placental microbes in fetal and neonatal colonization. At present, the relative contributory role of the placenta and maternal oral, vaginal, skin, and gut communities in the fetal, neonatal, and early infant microbiome remains imprecisely defined. What is evident is that neonates harbor colonizing bacteria at or very near the time of their birth. As discussed at the start of this chapter, it remains unknown whether this is due to true fetal colonization or fetal immune tolerance and ex utero colonization.

SUMMARY AND FUTURE DIRECTIONS

The human microbiome is composed of distinct microbial communities at different body sites, and these different body habitats provide niches for diverse bacterial species. Cellular elements of the microbiome may enhance immunity or prevent infections by canonical pathogens. Other microbes may serve as opportunists that typically colonize the human host without causing disease, but some of these organisms may cause infections in immunocompromised hosts. Bacterial strains, including but not limited to currently accepted probiotics, can stimulate immune responses and modulate inflammation, and antibiotic-mediated depletion of human-associated bacteria may result in immune dysregulation or infection-susceptible microbiomes. In the upcoming era of metagenomic medicine, infectious diseases must be considered in the context of the human microbiome and protective or pathogenic microbial communities. Human-associated microbes may serve as symbionts promoting interactions that are mutually beneficial or as benign commensals that simply colonize humans without bestowing any obvious benefits to the host. Future diagnostic tests may include components of the microbiome in disease evaluation, and decisions about antimicrobial therapy may rely on "typing" or profiling of the human microbiome in individual patients. The nature of the infection may dictate which body site is evaluated in terms of microbiome composition or function. Advances in understanding and management of infectious diseases will necessitate a deeper understanding of the microbiome context. The contributions or effects of microbial communities and metagenomes may have a large impact on infection susceptibility and disease pathogenesis.

[a]References 15, 41, 42, 52, 60, 62–64, 67, 68, 76, 78, 206.

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3 Prebiotics, Probiotics, and Synbiotics

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SHORT VIEW SUMMARY

Definitions
- Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."
- Prebiotics are nonviable substrates that are selectively used by host microorganisms and confer a health benefit to the host.
- Synbiotics are combinations of prebiotics and probiotics that are designed to have synergistic and/or additive effects benefiting the host. Probiotics are also marketed as food ingredients, dietary supplements, or "medical food."

Epidemiology
- Probiotics are used globally by millions of individuals.
- It is important to know that, for most marketed probiotic products, rigorous clinical trials to ascertain health benefits have not been done.

Microbiology
- Both bacterial (usually species of *Lactobacillus* or *Bifidobacterium*) and fungal (usually *Saccharomyces boulardii*) probiotics are available.
- Probiotics can be single organisms or contain several organisms, and numerous products are marketed.
- Studies have identified that at least 30% of probiotic products differ from their product labeling, with discrepancies between the stated and actual number of viable organisms, the concentration of the organisms, and the types of organisms in the product, among other concerns regarding quality and validity.
- Probiotics are not subject to minimal manufacturing standards with regulatory oversight.

Therapy and Prevention
- There are no US Food and Drug Administration–approved probiotics for disease prevention or therapeutic use.

Probiotics have been studied most extensively as therapy for acute infectious diarrhea and antibiotic-associated diarrhea and as prevention for *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea and necrotizing enterocolitis in preterm infants. However, probiotics are not standard-of-care for any condition.

Although certain probiotics are thought to be generally safe, current available data are considered insufficient to address the safety of probiotics with confidence. This is particularly true in vulnerable hosts, such as those at the extremes of age, critically ill, immunocompromised, or with existing hardware or catheters.

Probiotics are defined by the International Scientific Association for Probiotics and Prebiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[1] Neither fecal microbiota transplant (FMT) nor dead organisms or bioactive molecules produced by microbes are defined as probiotics. There are no US Food and Drug Administration (FDA)–approved probiotics for therapeutic use. Prebiotics are nonviable substrates that are selectively used by host microorganisms and confer a health benefit to the host.[1] This updated 2017 definition of prebiotics maintains that prebiotics are substances that benefit the host indirectly by influencing the growth of specific microbe or microbial communities, while also expressing the further considerations that prebiotics could be noncarbohydrates and can act outside the intestinal environment, such as in the skin or vagina.[1] Synbiotics are combinations of prebiotics and probiotics that are designed to have synergistic and/or additive effects benefiting the host.[2] Most investigations continue to focus on the evaluation of potential probiotics with more limited studies available for prebiotics and synbiotics. Both bacterial (usually species of *Lactobacillus* or *Bifidobacterium*) and fungal (usually *S. boulardii*) probiotics are the subjects of an extensive literature and increasing global use, by millions of users.[3,4] It is important to note that, for most of the marketed probiotic products, rigorous clinical trials to ascertain health benefits have not been done. Further, online sources appear biased toward therapeutic benefits, suggesting the importance of patient education by clinicians regarding probiotic effectiveness.[5]

In the English literature (www.ncbi.nlm.nih.gov/pubmed/) there have been more than 19,000 publications on probiotics dating to the 1950s. Approximately half of this literature is based on human studies. Probiotics, such as yogurts or fermented milks, are ancient, dating at least as far back as documented by Egyptian hieroglyphs and now have been aggressively marketed as food ingredients, dietary supplements, or "medical food"; probiotic infant formulas also are widely available.[6,7] All yogurt contains live cultures of lactase-producing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; those yogurt products further supplemented with additional live bacteria (e.g., *Bifidobacterium* spp., other *Lactobacillus* spp.) are considered probiotics.[8] In contrast, it has been debated whether yogurt in fact represents probiotics, although some data, for example, suggest yogurt, due to containing lactase-producing bacteria, improves tolerance to lactose with potential health benefit.[4] In contrast, kefir is a fermented milk drink dating to the late 1800s, now commercialized as Kefir, which is complex, containing multiple bacterial and yeast strains.[6,9]

ISSUES REGARDING THE COMPLEXITY AND VARIABILITY OF PROBIOTICS

Marketed probiotics are highly variable, with some products labeled to contain single microbes and others composed of multiple distinct microbes, such as VSL#3, a commercial product with eight strains of bacteria from the genera *Bifidobacterium*, *Lactobacillus,* and *Streptococcus*, or products containing multiple species of a single, usually bacterial, genus (e.g., *Lactobacillus casei*, *Lactobacillus rhamnosus*). Studies to verify the composition of marketed probiotic formulations, however, have found that there are commonly discrepancies (involving at least 30% of products) between the stated and actual number of viable organisms, the concentration of the organisms, and/or the types of organisms in the product compared with the product labeling.[3] In addition, some marketed probiotics are labeled with taxonomically incorrect or fictitious microbial names.[10] Thus uncertainty exists about the composition and reliable manufacturing practices for

TABLE 3.1 Concerns About Marketed Probiotic Formulations

Marketed with taxonomically incorrect or fictitious microbial names
Standards lacking to define the number of viable organisms in available probiotics, the shelf life of the products, or appropriate storage conditions to maintain probiotic viability
Lack of clear labeling of many probiotic products on dosing or toxicity
No US Food and Drug Administration or other oversight to provide minimal manufacturing standards for probiotics
Large number of different products labeled as probiotics without adequate scientific study to define the product efficacy, the biologic basis for proposed health benefit, and/or to demonstrate product safety

TABLE 3.2 Probiotics That Have Received GRAS (Generally Recognized as Safe) Status in the United States

Bacillus coagulans GBI 30, 6086 (activated, inactivated, and spores)[b]
B. coagulans strain Unique IS2 spores preparation
B. coagulans SANK 70258 spore preparation
B. coagulans SBC37-01, spore preparation
B. coagulans SNZ1969 spore preparation
Bacteroides xylanisolvens strain DSM23964
Bifidobacterium animalis subsp. *lactis* strain Bf-6
Bifidobacterium breve M-16
Bifidobacterium lactis strain Bb12 and *Streptococcus thermophilus* strain Th4
Bifidobacterium longum BB536
B. animalis subsp. *lactis* strains Bf-6, HN019, Bi-07, B1-04, and B420
Carnobacterium maltaromaticum strain CB1 (viable and heat treated[a])
Lactobacillus acidophilus La-14
L. acidophilus, Lactobacillus lactis, and *Pediococcus acidilactici*
L. acidophilus NCFM
Lactobacillus casei subsp. *rhamnosus* strain GG
L. casei strain Shirota
Lactobacillus fermentum strain CECT5716
Lactobacillus plantarum strain 299v
Lactobacillus reuteri strain DSM 17938
L. reuteri strain NCIMB 30242
Lactobacillus rhamnosus strain HN001
L. rhamnosus strain HN001 produced in a milk-based medium
Propionibacterium freudenreichii ET-3, heat killed[a]
Saccharomyces cerevisiae strain ML01, carrying a gene encoding the malolactic enzyme from *Oenococcus oeni* and a gene encoding malate permease from *Schizosaccharomyces pombe*
Saccharomyces cerevisiae strain P1Y0, a variant of *S. cerevisiae* parent strain UCD2034
Streptococcus salivarius K12

[a]The term "probiotic" is formally limited to live organisms.
[b]US Food and Drug Administration. GRAS noticies. https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices. Accessed February 21, 2018.

a large number of the currently available probiotic preparations (Table 3.1).[1,7]

Probiotics are not subject to minimal manufacturing standards with regulatory oversight, nor are scientifically sound studies demonstrating efficacy required when marketing a probiotic product.[3,7] Hence, for most available probiotic products, studies demonstrating that the probiotic confers a demonstrable health benefit are lacking, and even less information is available to define the mechanism(s) by which particular products promote human health in different clinical illnesses. In the United States probiotics may receive "GRAS" status ("generally recognized as safe") by the FDA, even if no efficacy data exist. GRAS substances are those "for which use in food has a proven track record of safety based either on a history of use before 1958 or on published scientific evidence and that need not be approved by the FDA before being used." Probiotics that currently possess GRAS status in the United States are listed in Table 3.2 (see also www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true). International guidelines encourage the assessment of probiotics in both food and nonfood formulations,[1,11,12] and, at least in the European Union, there is increasing regulatory oversight.

CLINICAL STUDIES OF PROBIOTICS

The available probiotic studies have many limitations and biases. Of importance, potential bias is an important consideration even in the context of the available randomized controlled trials (e.g., how randomization was conducted, blinding, missing data, analytical approach). Most often, studies of probiotics lack sufficient power to detect significant differences; this is one factor in interpreting the plethora of reported meta-analyses of probiotic use. Additional concerns include the variation in probiotic formulations, trial heterogeneity (e.g., adults, children, settings of studies), as well as in variability in the status of the conditions studied, or the treatments used (e.g., particular antibiotics administered to patients) that may impact results. One area of concern for health professionals is whether probiotic administration during or following a course of antibiotics improves the recovery from the antibiotic effects. To date, there is only limited information on the effect of antibiotic spectrum of antibacterial action and/or class on microbiota alterations and the subsequent impact on use of probiotics. Similarly, our understanding of how or if probiotics alter the intestinal microbiota, mucosal or luminal, is limited; recent data indicate substantive interindividual differences and even delays by probiotic administration in microbiota reconstitution after antibiotic exposure.[13,14]

At present, prebiotics, probiotics, and synbiotics have been studied in ≈300, 1700, and 130 randomized controlled clinical trials, respectively. Overall, the reported effects often differ among studies of similar topics. This is likely, in part, attributable to variable study design and rigor. Further, verification of probiotic content and viability is not a current standard used in reporting probiotic randomized controlled studies. For example, a review of 46 clinical trials of probiotic use in inflammatory bowel disease noted that only 23 reported studies were double-blind, randomized controlled trials and that, among the 46 reviewed trials, 32 used different probiotic products, 10 used different prebiotic products, and 4 used different synbiotics.[15] An additional concern is the study criteria leading to restricted enrollment in probiotic clinical trials. For example, a highly publicized randomized, double-blind, placebo-controlled trial, evaluating a probiotic *Lactobacillus* preparation to prevent antibiotic-associated diarrhea, enrolled 135 patients. The results suggested that the probiotic yielded benefit by significantly reducing both antibiotic-associated diarrhea and the number of patients who acquired *C. difficile*–induced diarrhea. However, only 8% of potentially eligible patients were enrolled in the study, limiting the ability to generalize the study results in clinical practice.[16]

Cochrane reviews use predefined criteria to provide a structured, collaborative, and multinational approach to evaluating interventions for the prevention and treatment of disease. Given the diversity of the probiotic literature, the Cochrane reviews of probiotic studies conducted in infectious diseases conditions are summarized in Table 3.3, providing an overview of the limited number of areas that have been evaluated by this rigorous approach. Among the infectious conditions evaluated (see Table 3.3), the studied probiotics may (1) shorten the duration and stool frequency of acute infectious diarrhea, (2) decrease antibiotic-associated diarrhea in children, (3) prevent *C. difficile*–associated diarrhea in children and adults, and (4) prevent necrotizing enterocolitis (NEC) in preterm infants. Among the other infectious conditions evaluated (see Table 3.3), datasets were small and variable, and most often results were noted to be derived from low-quality evidence.

Use of probiotics for acute gastroenteritis in children is the most common use of probiotics globally. However, two prospective, randomized, placebo-controlled, double-blind, multicenter trials of *Lactobacillus*-based probiotics, conducted in Canadian and United States pediatric emergency departments, observed no differences between children who received placebo ($N = 888$) or probiotic ($N = 882$) treatment in numerous outcome measures of gastroenteritis.[17,18] These well-done trials suggest that probiotic therapy should not be recommended for children with acute gastroenteritis, at least in Canada and the United States.

Data on use of probiotics for prevention of antibiotic-associated diarrhea (AAD) for children and adults are mixed, with moderate evidence (Cochrane analysis) supporting probiotic use in children and uncertainty in adults.[19–21] Of importance, a multicenter, randomized placebo-controlled trial in the United Kingdom (PLACIDE trial) did not identify benefit in reducing AAD or *C. difficile* diarrhea. However, disease incidence was low, and thus the trial, despite its size and excellent design, may have been underpowered.[20,22]

Prevention of *C. difficile* infection (CDI) is a key area that has remained a challenge in multiple health care settings. Cochrane analyses report that moderate evidence supports use of probiotics in prevention of primary CDI, whereas insufficient data exist on whether probiotics

may contribute to prevention of secondary CDI (i.e., prevention of recurrent CDI).[23] Multiple meta-analyses, inclusive of randomized controlled trials of differing numbers and design, support, with moderate-quality evidence, that probiotics prevent CDI in adults.[24–27] These analyses do not provide clarity on which probiotic formulation is optimal, but initial analyses suggest that multispecies probiotics may be more beneficial.[26] For one probiotic combination (*L. acidophilus*, *L. casei,* and *L. rhamnosus*; known as BioK Plus), randomized controlled trials, observational clinical data, and meta-analyses support usefulness,[23,28] whereas data, for example, for single probiotics (e.g., *L. rhamnosus* GG, *Saccharomyces boulardii*) are mixed.[23] Additional evidence suggests that probiotic administration is more likely to prevent CDI when the probiotic is given close to the first antibiotic dose,[24] when two or more antibiotics are prescribed,[26] and when CDI risk is >5%.[24,26,29]

Last, although not standard-of-care, studies support the use of probiotics to prevent NEC, even though the Cochrane analysis[30] reports that nosocomial sepsis was not reduced. A subsequent well-resourced, randomized, double-blind, placebo-controlled field trial of synbiotic (*Lactobacillus plantarum* plus the prebiotic fructooligosaccharide) administration to term or late-preterm infants in rural India reported significant reduction in combined sepsis and death (primary outcome) during the first 2 months of life.[31] The Data Safety and Monitoring Board terminated the study early because interim results convincingly favored the synbiotic preparation relative to placebo.[32] These results provide a basis for additional trials, even under challenging circumstances, to define the health benefits of probiotics.

PROPOSED MECHANISMS OF ACTION OF PROBIOTICS

Investigations are being conducted to define the mechanistic and biologic basis for the health benefit(s) of probiotics.[33] Although the mechanism(s) of action of most probiotics remain unexplored, it is generally presumed that the molecular mechanisms of probiotic activities are triggered by microbe–epithelial cell interactions at the site of probiotic application (e.g., gut, skin, vagina). Major mechanisms by which probiotics are thought to act include inhibiting bacterial growth (e.g., by bacteriocin secretion or by short-chain fatty-acid production that inhibits bacterial growth by lowering pH), suppressing expression of bacterial virulence factors, preventing colonization with pathogenic bacteria (i.e., colonization resistance), modulation of one or more of the mucosal and/or systemic immune responses, and/or improving gastrointestinal (GI) barrier integrity.[34,35] Experimental studies in vitro and in vivo are beginning to provide clues to how probiotics may act. Some data suggest that certain probiotics dampen nuclear factor kappa B activation and hence proinflammatory mucosal and/or systemic immune responses.[33,36–38] Other studies provide evidence that some probiotics augment antibody responses to immunization and/or infecting pathogens.[39–42] In some instances cell-free supernatants of studied probiotics similarly dampen inflammatory responses, suggesting that such probiotics may release cell-free antiinflammatory molecules.[36,43,44] Consistent with the idea that live organisms may not be required for probiotic-like activity, particular heat-killed probiotics or probiotic lysate formulations have yielded clinical improvement when used topically for atopic dermatitis or orally for diarrheal illnesses.[45–47] Such studies may open the way for development of specific health-promoting microbial proteins or metabolites. Although it has widely been presumed that probiotics, through mucosal adherence, displace pathogens and prevent their ability to colonize and initiate disease, experimental studies, in fact, have reported conflicting results on the ability of probiotics to displace pathogens from epithelial cells or the mucosa.[33,36,48] In human studies distinct strains of probiotics have shown differing capacities for colonization, as assessed by fecal studies.[49] Consistent with these

TABLE 3.3 Cochrane Database of Systematic Reviews: Efficacy of Probiotics in Infectious Diseases

GOAL OF PREVENTION AND TREATMENT	AUTHOR AND YEAR	NO. OF STUDIES INCLUDED IN ANALYSIS OF PROBIOTIC EFFICACY	MICROBES IN PROBIOTIC	CONCLUSION
Gastrointestinal Diseases				
Treatment of acute infectious diarrhea	Allen et al., 2011[79]	63 (7 adult, older children, or unclear age studies; 56 infant and young children studies)	Mostly *Lactobacillus casei* strain GG (13 studies), *Saccharomyces boulardii* (10 studies), and *Enterococcus* lactic acid bacteria (LAB) SF68 (5 studies)	Probiotics useful in shortening the duration of acute infectious diarrhea and decreasing stool frequency; more research needed to identify specific probiotic regimens in specific patient groups
Prevention of pediatric antibiotic-associated diarrhea	Goldenberg et al., 2015[21]	23 pediatric studies, age 0–18 yr (3938 participants)	*Bacillus* spp., *Bifidobacterium* spp., *Clostridioides butyricum*, *Lactobacilli* spp., *Leuconostoc cremoris*, *Saccharomyces* spp., *Streptococcus* spp., alone or in combination. 11 studies, single-strain probiotic; 12 studies used ≥2 probiotic strains	Moderate-quality evidence suggested a protective effect of probiotics in preventing antibiotic-associated diarrhea (number needed to treat = 10). Adverse events appeared to be rare.
Prevention of *C. difficile*–associated diarrhea in adults and children	Goldenberg et al., 2017[29]	39 studies (9955 participants)	Any probiotic strain or dose	Moderate-quality evidence suggests probiotics are effective for reducing risk of *C. difficile*–associated disease if baseline disease risk of disease exceeds 5%. However, fecal detection of *C. difficile* was not diminished.
Treatment of *C. difficile*–associated colitis in adults	Goldenberg et al., 2008[80]	4 (adult studies)	*Lactobacilli* spp., *S. boulardii*	Insufficient evidence; no evidence for probiotics by themselves; one out of the four studies showed benefit for adjunct usage
Prevention of necrotizing enterocolitis (NEC) in preterm infants	AlFaleh et al., 2014[30]	24 highly variable studies	*Lactobacilli* spp. alone or in combination with *Bifidobacterium* spp.	Probiotics reduced the risk of severe NEC and mortality in preterm infants without evidence of reduction in nosocomial sepsis
Methods of decreasing infection to improve outcomes after liver resection	Gurusamy et al., 2011[81]	2 (adult studies)	Synbiotic (containing 1×10^8 *B. breve* strain Yakult, 1×10^8 *L. casei* strain Shirota, and galactooligosaccharides [GOS])[a]; synbiotic (containing $\geq 4 \times 10^{10}$ *L. casei* strain Shirota, $\geq 1 \times 10^{10}$ *B. breve* strain Yakult, and GOS)[a]	Insufficient data in support of or against prebiotic or probiotic use to decrease postresection infections

TABLE 3.3 Cochrane Database of Systematic Reviews: Efficacy of Probiotics in Infectious Diseases—cont'd

GOAL OF PREVENTION AND TREATMENT	AUTHOR AND YEAR	NO. OF STUDIES INCLUDED IN ANALYSIS OF PROBIOTIC EFFICACY	MICROBES IN PROBIOTIC	CONCLUSION
Urinary Tract and Reproductive Health				
Prevention of urinary tract infections (UTIs) in adults and children	Schwenger et al., 2015[84]	9 studies (735 individuals, healthy or prior UTI)	Any probiotic strain, formulation, dose or frequency.	No significant benefit demonstrated but studies were small with poorly reported methods and high risk of bias
Prevention of urinary tract infections in people with neuropathic bladder	Toh et al., 2017[85]	3 studies (110 participants)	No study with oral probiotic; all studies used intravesical instillation of *E. coli* strains	No certain outcomes due to small studies with high risk of bias
Antimicrobials in bacterial vaginosis of nonpregnant women	Oduyebo et al., 2009[86]	3 (adult studies)	*Lactobacillus* spp.	Oral *Lactobacillus* augments the effects of metronidazole and is more effective than metronidazole when given intravaginally; need further studies of adverse events in *Lactobacillus* trials
Treatment of bacterial vaginosis	Senok et al., 2009[87]	4 (adult studies)	Any probiotic strain or dose	Insufficient evidence for or against the use of probiotics in treatment of bacterial vaginosis. Promising data for use of probiotics combined with metronidazole or estriol, although larger, standardized studies are needed
Prevention and treatment of vulvovaginal candidiasis in women with HIV infection	Ray et al., 2011[88]	1 (adult study)	*L. acidophilus*	No definitive evidence for or against the use of probiotics to *prevent* vulvovaginal candidiasis in HIV-infected women. No studies matching the predetermined inclusion criteria found for *treatment* of candidiasis with probiotics
Treatment of vulvovaginal candidiasis in nonpregnant women	Xie et al., 2017[89]	10 studies (1656 participants)	Any single- or multiple-species probiotic in any preparation type/dosage/route of administration. All studies used probiotics as adjuvants to antifungal drugs	Probiotics may increase rate of short-term clinical and mycologic cure but without evidence for longer-term clinical impact (low- and very low–quality evidence)
Respiratory Diseases				
Prevention of acute upper respiratory tract infections (URTI)	Hao et al., 2015[90]	12 studies (3720 participants, children, adults, all ages)	Any probiotic strain or dose	Probiotics were better than placebo in reducing the number of acute URTIs, mean duration of an episode of URTI, and cold-related school absence. However, the quality of the evidence was low or very low.
Prevention of ventilator-associated pneumonia	Bo et al., 2014[91]	8 studies (1083 participants)	*L. casei rhamnosus*; *L. plantarum*; synbiotic 2000FORTE; *Ergyphilus*; combination *Bifidobacterium longus*, *L. bulgaricus*, *S. thermophilus*	Decreased the incidence of VAP (low-quality evidence). Results were uncertain for mortality. Overall, data did not provide sufficient evidence to draw conclusions on the efficacy and safety of probiotics for the prevention of VAP in ICU patients.

HIV, human immunodeficiency virus; *ICU,* intensive care unit; *VAP,* ventilator-associated pneumonia.

observations, although *L. rhamnosus* GG (LGG) administration to 12 subjects (age 65–80 years) did not result in a detectable compositional microbiome change, functional transcriptional analyses suggested that LGG ingestion fostered an ecosystem change with new interactions among bacteria commonly regarded as health promoting.[50] Overall, debate exists over whether probiotics act via common or strain-specific mechanisms, with possibly both being important.[4] Data are clearly needed to understand the mechanisms by which specific probiotics act in specific diseases that will permit informed decisions by clinicians about the appropriate probiotic choice for use in differing clinical conditions.[51]

POTENTIAL ADVERSE EFFECTS OF PROBIOTIC THERAPY

Certain probiotics, particularly lactobacilli, lactococci, and *Bifidobacterium*, have long been proposed as generally safe based on their extensive use with likely daily ingestion by millions of people and limited reports of toxicity.[52–54] In fact, ingestion of *L. rhamnosis* GG is reported to have increased in Finland from 1 L to 6 L per person per year from 1990–2000, respectively. But fortunately, there was not an observed increase in *Lactobacillus* bacteremia.[54] Nevertheless, in general there is insufficient information on most marketed probiotic preparations to provide assurances regarding safety. This has been emphasized by a report by the Agency for Healthcare Research and Quality, concluding that current data are insufficient to address the safety of probiotics with confidence.[53] Further, a recent systematic review of 384 randomized trials of probiotics, prebiotics, and synbiotics conducted from 2015–18 identified that only 2% of trials adequately reported key safety components, and one-third of the trials gave no information on harms.[55]

Adverse events due to probiotics are generally considered in three categories: GI side effects, systemic infections, and deleterious metabolic activities.[54] Lateral gene transfer, especially of antibiotic resistance genes because antibiotics and probiotics are frequently taken together, and excessive immune stimulation have been raised as theoretical but, as yet, unsubstantiated concerns.[54] Mild GI side effects are most common but generally not concerning. In contrast, data raise concerns about the use of probiotics in vulnerable patient populations, particularly immunocompromised hosts; the severely ill (children and adults); premature newborns; those with serious comorbidities; patients with intravenous catheters, prosthetic material, or hardware; short bowel syndrome; abnormal cardiac valves; and/or the elderly, among others (Table 3.4).[52,54,56–60] In these hosts a variety of infectious complications have been reported, including bacteremia and fungemia, that have, for example, been occasionally fatal and/or led to endocarditis. For

TABLE 3.4 Populations Potentially at Risk of Harm From Probiotic Ingestion

Pregnant women
Premature neonates
Elderly individuals
Hospitalized children or adults
Immunocompromised patients (e.g., malnourished children; transplant recipients; treatment with immunosuppressive drugs, including corticosteroids; chemotherapy)
Structural heart disease (e.g., valve abnormality or replacement, history of endocarditis)
Potential for probiotic translocation across the bowel wall (e.g., active bowel leak, active colitis, neutropenia)
Critically ill children and adults
Critically ill adults
Liver transplant recipients
Patients with liver failure
Patients with HIV infection

HIV, Human immunodeficiency virus.
Modified from Doron S, Snydman DR. Risk and safety of probiotics. Clin Infect Dis. *2015;60(suppl 2):S129–S134.*

example, although *S. boulardii* (a subtype of *Saccharomyces cerevesiae,* or brewer's yeast) is an infrequent fungal bloodstream isolate, in one series 86% of *S. boulardii* fungemia episodes were identified in children or adults who ingested *S. boulardii* as a probiotic.[61] One clinical trial (PROPATRIA [Probiotics in Pancreatitis Trial] study), in particular, has raised concern about probiotic safety. In that randomized, double-blind, placebo-controlled trial designed to evaluate the effectiveness of a probiotic preparation (six different *Lactobacillus* or *Bifidobacterium* strains; total daily dose 10^{10} bacteria) on infectious complications of acute pancreatitis, there was increased mortality in the probiotic treatment group (16% in 152 patients treated with probiotics vs. 6% in the 144 patients treated with placebo; relative risk 2.53; 95% confidence intervals, 1.22 to 5.25) without any measurable impact on infectious complications.[62] The increased mortality, attributed to bowel ischemia, was significantly increased in the patients with acute pancreatitis who were treated with the probiotic. Although the mechanism(s) accounting for this striking imbalance in adverse outcomes are unknown, this trial is considered an example of deleterious metabolic effects due to probiotic use.

FECAL MICROBIOTA TRANSPLANT FOR INFECTIOUS CONDITIONS OTHER THAN *CLOSTRIDIOIDES DIFFICILE* INFECTION

The contribution of the enteric microbiome to health and disease is an expanding area of investigation. Human and murine studies of bacterial members of the microbiome, such as *Bacteroides thetaiotaomicron, Akkermansia muciniphila,* and *Faecalibacterium prausnitzii,* among others, have begun to provide insights into how common microbiome members may promote health or avert disease.[63–65] For example, *B. thetaiotaomicron* is believed to play an important role in nutrition through glycan foraging in the colonic lumen.[64,65] Conversely, microbiota disruption (also termed "dysbiosis") is believed to contribute to a wide spectrum of mucosal and systemic diseases, including obesity,[66] cardiovascular disease,[67] kwashiorkor,[68] and colon cancer,[69] among other conditions. The clinical success of FMT for therapy of CDI has spurred the study of FMT in other infectious and noninfectious conditions (e.g., metabolic syndrome, inflammatory bowel disease). Specifically, rising rates of antimicrobial resistant infections and the reported link between colonic microbiota alterations and higher rates of hematopoietic stem cell transplant (HSCT) graft-versus-host disease, bacteremia, disease relapse, and reduced survival[70–72] underpin recent clinical trials. In these trials, whether FMT can reverse colonization and disease by multidrug-resistant (MDR) bacteria and improve outcomes of HSCT are being assessed (clinicaltrials.gov; search FMT and MDR, FMT and hematologic neoplasms). Recent preliminary data reported from a clinical trial of autologous FMT versus no intervention among allo-HSCT patients suggests that autologous FMT can restore the fecal microbial diversity and composition present before antibiotic treatment.[73,73a] An alternative and emerging approach is identification of members of the microbiota that can restore colonization resistance within the colon and thus prevent colonization and infection by MDR pathogens.[74,75] These largely little-known microbiota members (e.g., *Blautia* spp., Clostridiales, bifidobacteria, *Eubacterium limosum*) are proposed as candidates for the development of next-generation probiotics.

SUMMARY AND FUTURE DIRECTIONS

The scientific definition of probiotics, "to enhance the health of the host," implies the requirement for documentation of health benefits in well-designed controlled clinical trials. At present, no marketed probiotics unequivocally meet this standard, with *L. rhamnosus* GG being the most extensively evaluated probiotic.[52,76] Furthermore, although the standard definition of probiotics refers to live organisms, the use of the term in the general population, and even within the scientific literature, is often more lax. Other unresolved issues include the impact of probiotic dosage and single- versus multiple-species composition on clinical efficacy. To date, no probiotic is approved by the FDA for any clinical use. Clinicians should discuss with patients the quality-control issues in probiotic manufacturing, the limitations of the data available on probiotic use in disease, and the potential adverse consequences of probiotic use. Defining the place of probiotics in medical care awaits improvements in the characterization of probiotics, their modes of action, and stringent reproducible studies to demonstrate benefit and safety in disease conditions. Debate remains on whether the safety and/or purported efficacy of one species or genus of probiotic can be generalized to other similar probiotics and on whether probiotics exhibit class-based versus strain- or species-specific disease activity. Based on available data, probiotic preparations should not be used in immunocompromised or critically ill populations, and caution should be exercised in individuals at the extremes of age and those with central venous catheters, disrupted mucosal barriers, short bowel syndrome, abnormal cardiac valves, prosthetic joints or valves, and other hardware or prosthetic materials. Available experimental data provide some provocative glimpses into how probiotics may act to foster human health. Beyond development of next-generation probiotics,[74,75] designer probiotics are disease-targeted recombinant probiotics engineered, for example, to absorb bacterial toxins, such as the Shiga toxins in the GI lumen,[77] or to secrete molecules that inhibit, for example, the virulence of *Vibrio cholerae*.[78] Similar to probiotic use and development, use of FMT in non–*C. difficile* infectious diseases awaits additional research on efficacy, mechanisms of action, and safety.

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B Host Defense Mechanisms

4 Innate (General or Nonspecific) Host Defense Mechanisms

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Humans are continually exposed to microorganisms in daily life. Although it is unclear the extent to which these frequent exposures impact immune function and homeostasis, we know that most of the time our armament of defense mechanisms effectively prevents disease. Host defense mechanisms against microbial invasion are a continuum that provide physical, chemical, and immune barriers operating over different time frames to prevent, contain, and eliminate pathogens. An invading microbe first encounters physical and chemical barriers—the skin, mucous membranes, the normal microbiome, and antimicrobial peptides or proteins. If microbes overcome these more immediate defenses, the next hurdle is to avoid detection by pattern recognition receptors (PRRs). PRRs bind to conserved structural motifs or molecular patterns unique to microorganisms, referred to as pathogen-associated molecular patterns (PAMPs), even though they are also present on nonpathogenic organisms.[1] Several PRR families have been described, including Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), and retinoic acid–inducible gene-I (RIG-I)–like receptors, that is, RLRs.[2] Once activated, signaling through these receptors initiates the appropriate immune responses to eliminate an invading pathogen. The initial host defense mechanisms that sense and engage the potential pathogens are collectively referred to as the *innate immune system*. It is an evolutionarily older defense mechanism that relies on a limited repertoire of inborn sensor proteins that can provide an immediate response to pathogens without genetic rearrangement.[3] The innate immune system must be breached for an infection to occur. Even when unable to prevent or clear infection, innate mechanisms play essential roles in limiting replication and directing the microbe-specific long-lasting immune memory of the adaptive immune system (Table 4.1). The focus of this chapter is on the earliest events in the host-pathogen interaction process, the general nonspecific interactions that mitigate invasion, and the processes for the detection and possible clearance of an invading organism by the host. If infection is established, how do the initial interactions create an environment for the adaptive immune system to respond? (See Chapters 5 to 7.)

Several areas of research are significantly changing our understanding of host-pathogen interactions. First, we now understand that skin and mucosal surfaces harbor commensal microbes that act in concert with the host to maintain a state of wellness.[4] Commensal microbiota not only play a role in preventing pathogen invasion via competition but also mediate critical aspects of the development, homeostasis, and function of both innate and adaptive immune cells.[5–7] Although many of the insights to date have come from animal models, it is clear that disruptions in the balance between host and microbiota have the potential to predispose the host to local or systemic disease (see Chapters 1 and 2).[8] Second, although we have long known that the human immune system is highly variable among individuals, we are only just beginning to understand the relative contributions that heritable and nonheritable influences play on immune responses and susceptibility to infection.[9] The susceptibility, morbidity, and mortality related to almost every pathogen and to infection in general are influenced by the host's genetic makeup. This was evident years ago when it was shown that if a child's parent died of an infectious disease, such as pneumonia, the child had an increased probability of dying from an infection (Fig. 4.1).[10] Genetic associations between increased susceptibility to diseases have been mapped to sequence polymorphisms and frank mutations in many aspects of the innate and adaptive immune pathways. Such genetic effects have been identified in TLR pathways,[11–15] complement,[16,17] cytokines and chemokines or their receptors,[18–20] human leukocyte antigen (HLA) alleles,[21,22] and cellular receptors.[22,23] Although heritable factors play a substantial role in human immune system variation, a recent study of healthy human twins revealed that nonheritable influences, such as environment and microbes, may have a much greater impact in shaping our immune system.[24] It is currently unclear the extent to which nonheritable influences shape innate immune pathways and responses, but recent studies suggest that aspects of innate immunity can be functionally reprogrammed through epigenetic mechanisms. It is also increasingly evident that the delineation between innate and adaptive immunity is more of a continuum that previously understood.

Many innate immune cells mediate important roles in the initiation and development of adaptive immunity, but there is also increasing evidence that some innate cells exhibit an increased response to secondary infections or vaccination, a phenomenon referred to as innate memory or trained immunity.[25] As opposed to the highly specific nature of adaptive immunity, these enhanced secondary responses by cells, such as monocytes, macrophages, and natural killer (NK) cells, can be against the same microorganism, but more commonly provide a level of nonspecific protection against other pathogens.[26,27] One of the most intriguing examples of heterologous protection is observed with the tuberculosis vaccine, bacillus Calmette-Guérin (BCG). Epidemiologic studies suggest that vaccination with BCG at birth significantly reduces childhood mortality caused by nonrelated infections,[28,29] but evidence for a causal link is lacking. Recently, a placebo-controlled trial showed that BCG vaccination protected against an experimental infection with an attenuated yellow fever virus vaccine strain and that epigenetic and functional changes in monocytes indicative of trained immunity correlated with protection.[30] Although not conclusive, this provides evidence that trained immunity mediates at least part of the heterologous protection observed with BCG vaccination. These and many other studies demonstrate that there is still much to be learned about the roles innate immunity plays in protecting us from invading pathogens.

PHYSICAL AND CHEMICAL BARRIERS TO THE ENTRY OF MICROORGANISMS INTO THE BODY

The skin and linings of the respiratory, urogenital, and gastrointestinal (GI) tracts each comprise thin layers of epithelial cells held together by tight junctions that form barriers against the external environment. The morphologic integrity of these surfaces is an effective first line of defense against invading pathogens, but epithelia function is more than just physical barriers. As mentioned earlier, epithelial surfaces contain communities of commensal microbes that compete with invading pathogens, make antimicrobial substances, and interact with host cells to influence immune responses. Epithelia are also specialized to defend against microbes they commonly encounter by producing a wide variety

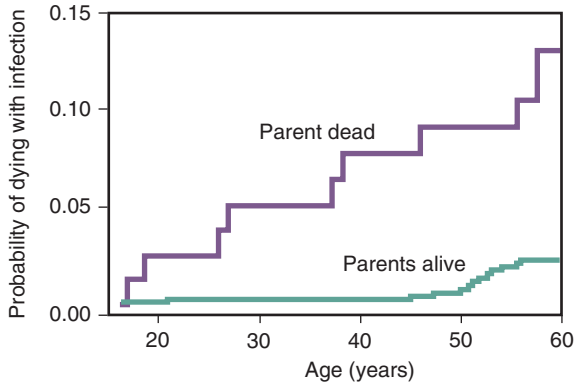

FIG. 4.1 Probability of dying from an infection before a given age for adoptees with at least one biologic parent who died before age 50 years (parent dead) of an infection versus adoptees whose biologic parents were alive at that age (parents alive). *(From Sorensen TIA, Neilson GG, Anderson PK, et al. Genetic and environmental influences on premature death in adult adoptees.* N Engl J Med. *1988;318:727–732.)*

TABLE 4.1 Features of Innate and Adaptive Immunity

	INNATE	ADAPTIVE
Components	Physical and chemical barriers Mucus AMPs Complement Innate cells (macrophages, DCs, granulocytes, NK cells)	Humoral immunity (B cells, antibodies) Cell-mediated immunity (T cells)
Receptors	Fixed in genome Rearrangement not necessary	Encoded in gene segments Rearrangement required
Recognition	Nonspecific or broadly specific molecular patterns	Antigen specific to a given pathogen
Response	Cytokines and chemokines Phagocytosis Complement action Activation of effector cells	Activation, proliferation, differentiation of antigen-specific B and T cells Secretion of antibodies Effector activity of T cells Generation of memory cells
Initiation	Many features constitutively present Immediate activation of effectors (minutes to hours)	Primary response delayed (days) Rapid recall on subsequent exposure
Duration	Can persist for days	Contraction after elimination of pathogen (weeks) Long-term memory (months/years)

AMPs, Antimicrobial proteins and peptides; *DCs,* dendritic cells; *NK,* natural killer. *Modified from Janeway CA, Medzhitov R. Innate immune recognition.* Annu Rev Immunol. *2002;20:197–216.*

of substances that inhibit microbial invasion or growth. Internal "mucosal" epithelial cells secrete mucus that can prevent microbes from adhering to their surface and can be expelled through mechanical flow. Epithelial cells also produce a diverse array of antimicrobial proteins and peptides (AMPs) and other immune mediators that can potently kill or inactivate invading microbes, often by targeting cell wall or cell membrane structures.[31]

The immune response to an invading microbe is largely dictated by the nature of the pathogen and the route of the infection. Efficient eradication of pathogens requires complex coordination between epithelial and innate immune cells at barrier surfaces, together with responding immune cells, locally and systemically.[32]

Skin

Human skin forms a complex physical and immunologic barrier to invasion by microbes. The epidermis is composed mostly of keratinocytes, which form a tight physical barrier and have a key role in detecting pathogens via a number of innate pathways. The epidermis also contains $CD8^+$ T cells and specialized dendritic cells (DCs), called Langerhans cells, that can sample microbes, migrate to draining lymph nodes, and prime the appropriate immune responses. The outermost stratum corneum layer of the epidermis supports a complex ecosystem of commensal microorganisms that are implicated in aspects of protection against pathogens, wound healing, and normal development of the immune system. The underlying dermis contains blood vessels, capillary beds, and draining lymphatics that eventually access lymph nodes. The dermis also harbors many cell types, including innate immune cells, such as macrophages, DCs, mast cells, innate lymphoid cells (ILCs), and resident $CD4^+$ and $CD8^+$ T cells.[33]

As noted, the skin possesses an array of antimicrobial properties that form a protective shield, including a battery of broad-spectrum defensive chemicals (principally peptides) able to kill or inactivate bacteria, fungi, viruses, and parasites. These AMPs respond to epithelial disruption to prevent the invasion of pathogenic organisms but also play important roles in maintaining the appropriate balance of the commensal microbiota.[34] Several AMPs in the skin, including β-defensins, the cathelicidin LL-37, and hexadecenoic acid, are depressed in atopic skin, a condition in which microbial suprainfection is common.[31]

The relative dryness, salinity, and mild acidity (the so-called acid mantle, pH ≈4.5–6) of skin, combined with normal skin microbiota, help make it an inhospitable environment for invading pathogens. Inflamed skin is more permeable and hence more hospitable to colonization. It has been speculated that oily skin may retard evaporation of water, resulting in increased numbers of colonizing microorganisms. Sebum, a lipid-rich coating that protects and lubricates hair and skin, has antimicrobial properties. However, bacterial species, including *Cutibacterium acnes*, hydrolyze triglycerides in sebum, releasing free fatty acids that ultimately promote colonization in sebaceous glands.[35] The continual desquamation of skin also aids in the elimination of microorganisms. Because few organisms can penetrate the skin, they usually gain access by some physical means, such as an arthropod vector, trauma, surgical incision, or intravenously placed catheter.

Mucous Membranes

Most pathogens enter the body through the mucosal surfaces of the respiratory, GI, and urogenital tracts. Mucus, formed by highly glycosylated proteins called mucins, which are often specific to the mucosal site, carries immune cells, antimicrobial factors, bacteria, nutrients, and waste. The continual secretion and elimination of mucus creates a flow that requires pathogens to move upstream and across epithelial surfaces. Together the physical and chemical properties of mucus create a robust barrier that is effective at immobilizing and inactivating pathogens before they contact epithelial surfaces, while also supporting commensal microbiota.[36] To subvert its function, many pathogens have developed strategies to penetrate, evade, degrade, or disrupt the production of mucus.[37]

Most mucosal epithelial cells possess the same peptide shield as those of skin.[31] However, body secretions, including saliva, cervical mucus, prostatic fluid, and tears, are endowed with unique antimicrobial properties. For example, lysozyme, one of the more potent antimicrobial substances produced in the intestine, mouth, and eye, is particularly effective against gram-positive bacteria because it enzymatically degrades peptidoglycan, a major component of bacterial cell walls.[38] However, many gram-positive and gram-negative bacteria evolved mechanisms to evade killing by lysozyme,[39] which in some cases (e.g., *Staphylococcus aureus*) may impact the balance of commensal bacteria at mucosal surfaces.[40]

Mucosal secretions contain significant levels of immunoglobulins that play important roles in the defense of these surfaces. Secretory immunoglobulin A (SIgA) is the most abundant antibody class in secretions, other than those in the genitourinary tract and lower respiratory tract, which have slightly higher levels of IgG.[41,42] Through binding to potential pathogens and toxins, SIgA can prevent their contact with the apical surface of epithelial cells and entrap them in mucus for subsequent clearance. SIgA is also capable of neutralizing pathogens or toxins that are internalized into epithelial cells.[43] In addition to

promoting the clearance of pathogens, SIgA coats the majority of commensal bacteria in the gut. This interaction facilitates targeting and sensing of commensals by tolerogenic DCs in Peyer patches of the small intestine,[44] shapes the composition of the microbiota, and contributes significantly to intestinal homeostasis.[45]

Respiratory Tract

Each breath we take brings with it the potential to inhale microorganisms that can cause respiratory disease if they are not eliminated quickly and reach the appropriate niche. To defend against this constant threat, the respiratory tract has a formidable array of antimicrobial defense mechanisms that must be compatible with the crucial function of gas exchange. Immune defenses are coordinated in relation to airway size, as effective defenses of the large airways, such as a thick mucus layer, can be harmful in smaller airways. Inhaled microbes must first penetrate and survive the aerodynamic filtration system of the upper airway and tracheobronchial tree. The airflow in these areas is turbulent, bringing large particles in contact with mucosal surfaces armed with antimicrobial defenses before they penetrate more deeply in the respiratory tract.[46]

The human trachea, bronchi, and bronchioles are lined by a highly ciliated epithelial surface that continuously propels mucus upward, leading to the mechanical clearance of any trapped pathogens that are subsequently ingested. This process (aided by coughing) is extremely efficient, leading to 90% of deposited material being cleared in less than 1 hour.[47] The airway epithelium contributes significantly to the resistance to infection through detection of PAMPs via PRRs and expression of inflammatory cytokines after infection. In addition, bronchial secretions contain antimicrobial substances able to kill or inactivate pathogens, including lysozyme, lactoferrin, β-defensins, and surfactant collectins.[48]

Once a microbe reaches the alveolus, physical expulsion becomes much less effective because, unlike the respiratory epithelium, the alveolar surface is not ciliated and lacks mucus, so it can facilitate gas exchange. As a result, alveolar epithelial cells rely on their intracellular defenses and stimulate the production of type I interferons (IFNs) to prevent the further spread of infection. Phagocytic cells such as alveolar macrophages (AMs), DCs, and neutrophils also play a more important protective role. Under homeostatic conditions, AMs phagocytose inhaled particles and degrade them without triggering inflammation or adaptive immune responses. During infection, AMs rapidly respond by activating complement (see Chapter 9), phagocytosis, producing inflammatory mediators, and recruiting neutrophils. These phagocytic cells are assisted in their defense by the collectin surfactants SP-A and SP-D, which bind to and opsonize diverse organisms, including gram-negative bacteria, viruses, and fungi.[41,49]

Like all immune defenses, these nonspecific mechanisms can be overcome by the introduction of large numbers of invading organisms (e.g., a contaminated respirator), particularly if the host is exposed for an extended period. Their effectiveness can also be decreased by environmental factors, air pollutants (e.g., cigarette smoke), mechanical respirators, concomitant infection, allergenic agents, and, in some cases, genetic defects (e.g., cystic fibrosis) and inhibitory factors of some pathogens.[46]

Gastrointestinal Tract

The GI tract must be able to sort through enormous antigenic exposure without harming the host. Although the oral mucosa has similarities with other mucosal sites, some of its immune mechanisms are unique. As a first line of defense, the oral epithelium, together with the underlying connective tissue of the lamina propria, provides resistance to the strong shear forces of mastication and helps prevent the penetration of microorganisms. Like the epidermis, the oral epithelium maintains its structural integrity and limits colonization by a process of continuous cell renewal to replace cells shed from the surface. Secretion and digestion of saliva limits the accumulation of unwanted microbes. Saliva also carries a range of host defense molecules, including mucins, lysozyme, AMPs, and SIgA and other immunoglobulins able to inactivate pathogens. Unique to the oral mucosa, the periodontal epithelium around teeth allows access of serum proteins and immune cells, providing a link between systemic and mucosal immunity.[50]

The acid pH of the stomach and the antibacterial effect of the pancreatic enzymes, bile, and intestinal secretions are effective, nonspecific, antimicrobial defense factors. The GI tract is also coated in mucus that has different properties in the stomach, small intestine, and colon. In the small intestine mucus limits the number of bacteria that can reach the epithelium and Peyer patches. In the large intestine the inner mucus layer remains relatively free of bacteria, whereas the outer mucus layer supports a subset of the commensal microbiota.[51] Paneth cells of the small intestine, located in the crypts of Lieberkühn, secrete AMPs such as β-defensins, lysozyme, REGIIIγ, and type II phospholipase A.[4,52,53] Most of these AMPs are localized in the mucus layer; thus, in addition to functioning as a physical barrier, mucus limits bacterial penetration by concentrating AMPs near the epithelial surface.[31] Mucus is continuously secreted from goblet cells and Paneth cells and moves distally with peristaltic waves, expelling potential pathogens and requiring bacteria to travel against mucus flow to reach the tissue surface.[54] Alteration of these physical and chemical parameters can lead to increased susceptibility of the host to infection and chronic disease. For example, evidence suggests that hypochlorhydric patients are more susceptible to pathogens such as *Vibrio cholerae* and *Salmonella,*[55,56] and mice deficient for the most abundant mucin, MUC2, spontaneously develop colitis and are predisposed to inflammation-induced colorectal cancers.[57,58] In addition, a number of disease states have been shown to be accompanied by the movement or translocation of gram-negative bacterial products across the epithelial barrier and into the circulation.[59] This is often accompanied by a loss of tight junctions between enterocytes or the loss of enterocytes altogether and, in some cases, may be tied to loss or impairment of Th17 cells (a subset of T cells), which stimulate mucin and AMP production, improve tight junction function, and increase IgA transport into the lumen.[60,61]

Specialized epithelial cells called microfold (M cells) and goblet cells sample luminal antigens and microorganisms for presentation to DCs and resident macrophages. Lymphocytes and other immune cells are found throughout the intestinal tract epithelium, but the majority are in the submucosal Peyer patches and lymphoid follicles (see Chapter 7).[62] The GI tract expresses PRRs on intestinal epithelial cells (IECs) and other cell types, but unlike other sites in the body where recognition of foreign microbes initiates highly inflammatory cascades, the abundance of commensals in the intestine requires an altered state of responsiveness.[63] Multiple studies demonstrate beneficial roles of commensal microbial TLR signals in gut function and homeostasis.[64,65] The ability to distinguish between commensal and pathogenic microbes is in part mediated by differential TLR signaling that occurs at apical versus basolateral surfaces of IECs. Although stimulation of basolateral TLR ligands leads to canonical activation of the transcription factor nuclear factor kappa B, apical exposure results in a net inhibitory effect through stabilization of its inhibitor, IκB. Of importance, the apical signal induces a state of tolerance to subsequent TLR stimulation, thus providing a means to differentially respond to commensal and pathogenic microbes.[66] It should be noted that although commensal microbial signals can be protective in the context of tissue damage or infection, they are implicated in a range of other diseases when homeostatic responses become dysregulated (see Chapters 1 and 2).[35,67]

Genitourinary Tract

Many immune mechanisms in the genital tract of both men and women bear similarity with other mucosal sites. Uniquely, the female reproductive tract (FRT) must be able to accept a semiallogeneic fetus while also conferring protection against pathogens. To achieve this balance, sex hormones coordinate aspects of epithelial, stromal, and immune cell number and function in each compartment throughout the menstrual cycle. Multilayered squamous epithelial cells cover the vagina and ectocervix, whereas single-layer columnar epithelial cells cover the endocervix, uterus, and fallopian tubes. The underlying stroma contains dynamic populations of immune cells; T cells are more abundant in the lower FRT, whereas granulocytes and NK cells are higher in the upper FRT. The lumen of the FRT is bathed in fluid that differs between the upper and lower tract and across the menstrual cycle. Mucus, which also varies with menstrual status, serves an important role in protecting epithelial cells from direct contact with pathogens. Cervicovaginal fluid

contains AMPs, including secretory leukocyte protease inhibitor (SLPI), human beta defensin 2 (HBD2), human neutrophil peptide 1 to 3 (HNP1–3), lysozyme, and lactoferrin. Despite the complex defense mechanisms of the FRT, accumulating evidence suggests that a window of vulnerability to infection may exist during the secretory phase of the menstrual cycle, which has programmed functions to facilitate fertilization.[68]

The human vaginal microbiota is dominated by four *Lactobacillus* spp. in most women of reproductive age, although studies show that there can be significant differences in vaginal community composition among women.[69] Under hormonal influence the vaginal epithelium contains increased amounts of glycogen that is depolymerized into simpler sugars. *Lactobacillus* spp. metabolize these sugars into lactic acid, lowering the pH of the vagina to create an environment that restricts growth of most invading organisms. The vaginal microbiota is implicated in preventing a number of urogenital diseases, including bacterial vaginosis, yeast infections, urinary tract infections (UTIs), and human immunodeficiency virus (HIV) infection.[69,70]

Urine, once considered sterile, is now known to contain commensal bacteria, although the contribution of these organisms to immune function remains to be determined.[71] The lower urinary tract is rinsed with urine four to eight times each day, eliminating potential pathogenic organisms, unless they are capable of firmly attaching to epithelial cells of the urinary tract (e.g., *Neisseria gonorrhoeae,* certain strains of *Escherichia coli*). Urinary retention or lack of complete bladder emptying impedes this flushing process. Urine is bactericidal for some strains of bacteria, mostly because of its pH, although factors such as hypertonicity, urea, and the presence of AMPs play a role. Uromodulin (also known as Tamm-Horsfall protein), a glycoprotein produced by the kidneys, is the most abundant protein in normal urine. In addition to protecting against kidney stones, it avidly binds uropathogenic *E. coli*, preventing these bacteria from gaining a foothold on the cellular lining of the urinary tract.[72]

The length of the male urethra (20 cm in an adult) also provides passive protection, and bacteria seldom gain access to the bladder in men unless introduced by instrumentation. The female urethra is much shorter (5 cm in an adult) and is traversed more readily by microorganisms, which is one reason why UTIs are much more common in women than in men.

The external surface of the nonerect penis is covered by keratinized squamous epithelium that is relatively resistant to infection unless the skin is broken or inflamed. In circumcised men, most or all of the foreskin epithelium is removed, leaving a similar dry keratinized surface also resistant to infection. In uncircumcised men the subpreputial epithelia covering the inner foreskin and glans/corona are mucosal surfaces that are more susceptible to HIV and possibly other viruses, such as herpes simplex virus (HSV) and human papillomavirus (HPV).[73]

Eye

Constant bathing of the eye by tears is an effective means of protection. Foreign substances are diluted continually and washed away via the tear ducts into the nasal cavity. Tears also contain large amounts of lysozyme, SIgA, lactoferrin, and lipocalin.[74]

INNATE IMMUNE SENSING OF INVADING PATHOGENS

PRRs are strategically located in many cell types and are localized within specific subcellular compartments to recognize and respond to invading infectious agents that breach immediate microbial defenses and physical barriers.[75] PRR families detect the full spectrum of molecules (proteins, carbohydrates, nucleic acids, and lipids in the form of PAMPs) associated with all types of pathogens, including viruses, bacteria, fungi, and protozoa. Bacterial and fungal PAMPs are often components of the cell wall, whereas because all viral components are synthesized in host cells, the main target of innate immune recognition is viral nucleic acids.[76]

Within this rapidly evolving field, several PRR families have been described. TLRs, the best-characterized PRR gene family, are expressed in both innate immune cells, such as macrophages and DCs, as well as in nonimmune cells, such as epithelial cells and fibroblasts.[77–79] There are 13 known mammalian TLRs, 10 of which are found in humans. TLRs are generally classified into two categories, depending on their cellular localization and PAMPs they recognize. Human plasma membrane-bound TLRs 1, 2, 4, 5, and 6 interact with microbial membrane components, whereas TLRs 3, 7, 8, and 9 are localized in intracellular vesicles, where they recognize viral and microbial nucleic acids.[75] TLR10 is the only human TLR member without known ligand specificity and function, although recent studies suggest that distinct from other TLRs, it functions to suppress inflammation.[80,81] PRR members of the CLR family, expressed as transmembrane or soluble proteins, bind a complex array of carbohydrates, and are essential for antifungal immunity.[82,83] Transmembrane CLRs, including dectin-1, dectin-2, mannose receptor, DC-SIGN (*d*endritic *c*ell *s*pecific *i*ntracellular adhesion molecule 3 *g*rabbing *n*on-integrin), and Mincle (macrophage inducible C-type lectin) induce intracellular signaling upon fungal recognition, whereas soluble CLRs, such as surfactant protein SP-A, SP-D, and mannose-binding lectin opsonize fungi and facilitate their recognition.[84] There are several known PRR families that detect viruses or bacteria within a cell. The NLR family, of which there are more than 20 members in humans, detects a range of microbial PAMPs in the cytosol.[85] Upon activation, NLRs recruit large signaling complexes that mediate inflammation, autophagy, or cell death.[86] The RLR family, including RIG-I, MDA5 (melanoma differentiation-associated protein 5), and LGP2 (laboratory of genetics and physiology 2), detects intracellular viral RNA.[87] In addition, an expanding repertoire of cytosolic sensors, including cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS), AIM2 (absent in melanoma 2), DAI (DNA-dependent activator of IFN-regulatory factors), DNA-PK (DNA-dependent protein kinase), IFI16 (IFN-inducible protein 16), and LRRFIP1 (leucine-rich repeat flightless-interacting protein 1) recognize and respond to cytosolic DNA and induce the production of type I IFNs.[88]

Some PRRs are also able to detect intracellular host-derived factors generated by cellular injury or tissue damage. Referred to as damage-associated molecular patterns (DAMPs), these factors can either be present before cell death or actively produced during cell death.[89] Although the original assertion that host molecules could alert the immune system and promote adaptive immune responses was controversial, specific DAMPs have now been shown to mediate inflammation via PRRs. For example, HMGB1 (high mobility group box 1) and heat shock proteins can stimulate TLR2 and TLR4.[90] Although accumulating evidence suggests that DAMP recognition can mediate aspects of innate and adaptive immunity, further research is required to better understand the extent to which DAMPs can fully substitute for PAMPs in initiating immune responses, or whether they more often operate in combination with PAMPS to elicit a specific immune response in the context of infection.

In addition to pattern recognition, the immune system has several strategies to recognize the absence of normal self-molecules that can result upon infection. The most studied example of missing self-recognition involves NK cells, the prototypical member of the ILC family (see Chapter 7). NK cells are cytotoxic innate lymphocytes that circulate through blood and tissues to defend against microbial infection and tumor progression. Patients and animals with altered NK function are more susceptible to recurrent HSV, HPV, and varicella virus infections.[91–93] The specificity and functions of NK cells are tightly regulated by a complex balance of activating and inhibitory receptors on the cell surface.[94] A major role of inhibitory receptors on NK cells is to detect class I major histocompatibility complex (MHC I) molecules, which are expressed on all nucleated cells but often downregulated during viral infection or cellular stress.[95,96] When NK cells encounter cells that have downregulated expression of MHC I inhibitory receptors (which normally prevent killing of healthy cells) they are released from inhibition, allowing the selective elimination of infected or stressed cells through the release of perforin and granzymes, as well as through engagement of target cell death receptors such as Fas.[97,98] Activated NK cells also produce proinflammatory cytokines that further stimulate innate and adaptive immune responses.[99]

Phagocytosis

Critical to the innate response are the cell types that seek and engage invading pathogens. As described earlier, both immune and nonimmune

cells at barrier surfaces express PRRs that sense and then trigger inflammatory responses. Next, specialized innate immune cells with another essential function, phagocytosis, are engaged. Phagocytosis is a complex mechanism by which relatively large particles (>0.5 μm), including altered self-particles (e.g., necrotic or apoptotic cells) or invading microbes, are engulfed within a plasma membrane envelope and then internalized into a large endocytic vesicle called a phagosome. The phagosome can then fuse with a lysosome(s) to form a phagolysosome that becomes acidified, and together with antimicrobial proteins and reactive superoxide and nitric oxide radicals usually kills the microbe.[100]

Although many cell types are capable of phagocytosis, several, including macrophages, monocytes, neutrophils, eosinophils, and DCs, are highly specialized for the process and hence termed "professional phagocytes."[101] Macrophages are the major phagocyte population in body tissues and have a diverse range of phenotypes and functions that in some cases are specialized for the location in which they reside.[102,103] Monocytes comprise ≈10% of nucleated cells circulating in blood, although significant pools exist in the spleen and lungs and can be mobilized when needed.[104,105] Neutrophils, a type of granulocyte that is the most abundant white blood cell in circulation (40%–80%), provide critical rapid defense against invading microbes. In response to inflammatory stimuli, neutrophils migrate into infected tissues, where they bind, engulf, and kill microbes, as well as release factors that inactivate extracellular microbes and prime immune responses (see Chapter 8).[106,107] There are two main functional types of DCs, both of which migrate into tissues and mediate important roles in shaping adaptive immune responses. Conventional DCs have an enhanced ability to sense inflammation, capture extracellular and cell-associated microbes, and then process them into peptides that can be presented on surface MHC molecules to induce an adaptive immune response.[108] Plasmacytoid DCs (pDCs), which circulate in blood and peripheral tissues, are best characterized for their ability to rapidly produce large amounts of type I IFNs in response to viral infection.[109,110] In addition to enhanced phagocytic activity, each of these cell types is armed with a multilayered array of antimicrobial mechanisms that inactivate pathogens, secrete cytokines, recruit immune cells, and prime adaptive immune responses.

Professional phagocytes variably express receptor systems that, together with PRRs, recognize microbes, particulate matter, and damaged host cells. Receptors that facilitate phagocytosis are classified as either opsonic or nonopsonic receptors. Opsonic receptors include Fc receptors that bind the conserved domain of IgG antibodies and complement receptors for complement-coated targets. Nonopsonic receptors involve the direct recognition of ligands on microbial surfaces. Examples include dectin-1, which recognizes β-glucans on fungal surfaces and members of the scavenger receptor family (SR-A, SR-A2, and MARCO [macrophage receptor with collagenous structure]) that bind to components of bacterial cell walls.[100] The initial contact of a target with a phagocytic cell results not only in the chemical sampling but also physical assessment of the target through the extension of pseudopodia, membrane ruffling, and the engagement of specific receptors. The physical and chemical properties of the target help instruct the nature of the subsequent response, which is also highly dependent on the state and function of the ingesting phagocyte.[111]

Many pathogens evolved mechanisms to interfere with different steps of the phagocytic process, but some, including *Mycobacterium tuberculosis* (Mtb), *Listeria monocytogenes,* and *Legionella pneumophila,* survive and replicate in phagocytes. These intracellular bacteria evolved unique strategies to perturb or evade phagosomal maturation and to withstand cellular antimicrobial factors.[112]

Autophagy

Autophagy ("self-eating") refers to a collection of processes that allow cells to digest long-lived cytosolic proteins, lipids, and organelles in autophagosomes that fuse with lysosomes. Although autophagy primarily serves as a survival mechanism in response to stress or starvation, it is now known to mediate important roles in homeostasis, development, and immunity. In concert with PRRs and other signaling pathways, autophagy can directly eliminate pathogens, regulate inflammation, secrete immune mediators, and promote adaptive immune responses through antigen presentation on MHC molecules.[113]

The importance of autophagy in immunity is demonstrated by the range of mechanisms used by microorganisms to prevent, counteract, or even exploit aspects of the process to promote their survival. For example, studies suggest that Mtb survival in macrophages is in part due to its ability to (1) inhibit the induction of autophagy, (2) disrupt autophagosome fusion with the lysosome, and (3) suppress autophagy-linked class II MHC antigen presentation.[114]

Much remains to be learned about how autophagy mediates innate and adaptive immune mechanisms that are also intrinsically linked to homeostasis. Polymorphisms in autophagy-associated genes are associated with increased risk of Crohn disease. Emerging data also implicates autophagy in other inflammatory disorders, metabolic conditions, neurodegenerative diseases, and cancers.[115] Given its many important roles, there is great interest in developing approaches to target the autophagy pathway for potential new therapies and antiinfectives.

The Inflammatory Response

The innate immune response is the assembly of signals arising from the range of PRR pathways triggered by the invading pathogen (Fig. 4.2). The nature and magnitude of the response depends not only on the class of the invading microbe but also on factors such as anatomic location, level of replication, virulence, and host-specific variables. Of importance, immune defenses are usually balanced to effectively eliminate pathogens while also minimizing damage produced by the immune system itself.[116]

When the physical and chemical barriers are breached and infectious agents enter tissues, a range of host factors and cell types are mobilized.[117] Although many of the initial response proteins are always present, it is their rapid quantitative increase that constitutes the inflammatory response. Upon sensing invading microbes or tissue damage, sentinel cells become activated and begin releasing small proteins called cytokines and chemokines (discussed later) and other mediators, such as vasoactive amines, prostaglandins, and products of complement activation. Together, these mediators induce a state of acute inflammation that increases blood flow and enables plasma proteins, and leukocytes (mostly neutrophils) to leave the circulation and accumulate at the site of infection. Once in the tissue, leukocytes become activated to destroy and remove invading microbes. If the infection is successfully eliminated, inflammation is resolved and the process of tissue repair proceeds. If the invading pathogen is not eliminated by the acute process, inflammatory signals shift to more actively engage macrophages and promote adaptive immune responses. If the combination of these responses is unable to clear a persistent pathogen, a state of chronic inflammation is established (e.g., HIV).

Cytokines and Chemotaxis

Cytokines are potent signaling proteins that orchestrate immune and inflammatory responses through communication between both immune and nonimmune cells. In response to stimuli, secreted cytokines alter the function of target cells through binding to specific cell surface receptors. Cytokines can act in an autocrine manner if the action is on the cell that secretes it, a paracrine manner if the action is on nearby cells, or an endocrine manner if the cytokine acts on distant cells.[118] Although many have both pleiotropic and overlapping functions, cytokines are known to mediate many important roles during the innate immune response and are crucial in the transition to adaptive immunity.

Detection of viral components leads to the rapid secretion of type I IFNs (IFN-α and IFN-β) and other proinflammatory cytokines. Type I IFNs can be secreted by most cell types, although pDCs, which preferentially express TLR7 and TLR9 and constitutively express IFN regulatory factor 7 (IRF7), produce the largest amounts of IFN-α/β during viral infection. Although best known for their potent ability to induce an antiviral state in both virally-infected and bystander cells, type I IFNs also have roles in defending against bacteria and other pathogens. The outcome of IFN-α/β signaling is highly context dependent. Engagement with the type I IFN receptor on target cells influences the transcription of IFN-stimulated genes dedicated to viral restriction and other cytokines, antibacterial effectors, proapoptotic and antiapoptotic molecules, and proteins involved in metabolic processes. Of importance, type I IFNs also significantly promote antigen presentation

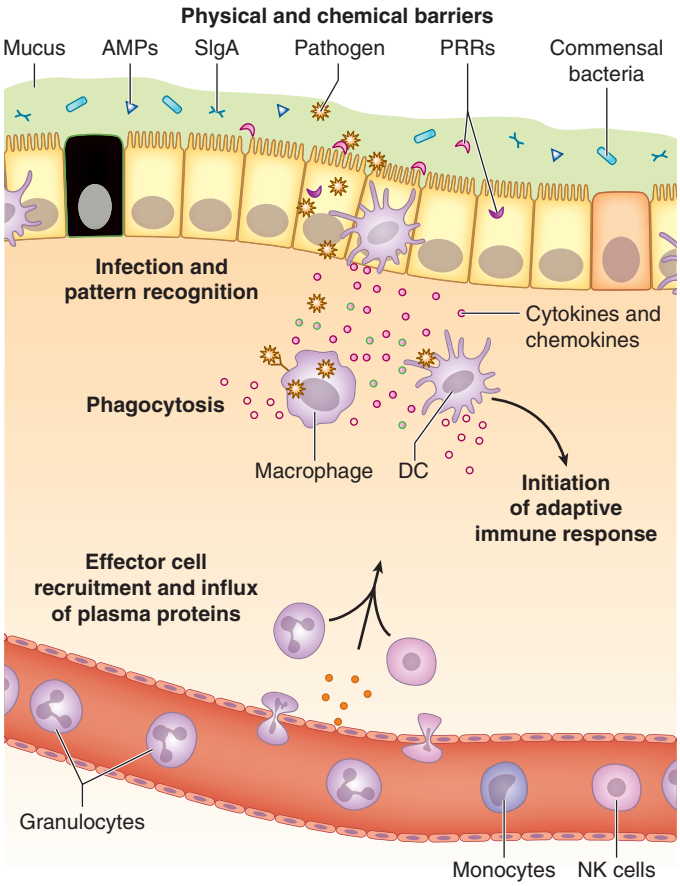

FIG. 4.2 Overview of innate immune defenses. Mucosal surfaces comprise epithelial cells interspersed with mucus-secreting cells and other cell types that produce antimicrobial factors and sample luminal antigens. Mucus carries antimicrobial peptides and proteins *(AMPs),* immunoglobulins such as secretory IgA *(SIgA),* immune cells, and other factors that can trap or inactivate pathogens before infection occurs. Commensal bacteria compete with invading pathogens, and stimulate innate immune responses. Pattern recognition receptors *(PRRs)* on the surface and within cells recognize and rapidly respond to invading pathogens by initiating signaling cascades that lead to the production of antimicrobial factors, cytokines, and chemokines. Granulocytes and macrophages ingest and kill pathogens and induce inflammation. Dendritic cells *(DCs)* produce cytokines that activate other immune cells and signal tissues to resist infection. The inflammatory response enables plasma proteins (e.g., complement) and leukocytes to leave circulation and accumulate at the site of infection. Once in tissues, they become activated to eliminate invading microbes and infected cells. If the pathogen is not eliminated, antigen-presenting cells, particularly DCs, transport antigen to lymphoid organs to promote adaptive immune responses. *NK,* Natural killer. *(Modified from Owen, JA, Punt J, Stranford S, Jones PP, Kuby J. Innate immunity. In: Owen JA, Punt J, Stranford SA, eds.* Kuby Immunology. *7th ed. New York: W.H. Freeman; 2013:142.)*

and NK-cell function, while modulating proinflammatory pathways and cytokine signaling. The net effect is the generation of adaptive responses and clearance of infection.[119]

Other proinflammatory cytokines important in the early immune response include tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6. Together, these powerful cytokines mediate central roles in stimulating natural immunity and the recruitment and activation of inflammatory cells. Cytokines, especially TNF and IL-1, increase the expression of adhesion molecules on endothelial cells and leukocytes that aid in the binding and transmigration of leukocytes into sites of inflammation. IL-1α, TNF, and IL-6 act on both the liver and central nervous system and are responsible for producing the fever and malaise that often comes with infection. In the liver these cytokines induce acute-phase response proteins, including C-reactive protein and other mediators, to promote elimination through complement activation and phagocytosis.[118]

Chemokines are a special family of cytokines that attract leukocytes (chemotaxis) to sites of pathogen invasion, although some also have direct antimicrobial activity and mediate roles in additional processes.[120] Defined by the number and arrangement of conserved cysteine residues, the majority of chemokines fall into two groups. Members of the CXC family, whose first two cysteines are separated by a single amino acid, stimulate the chemotaxis of neutrophils, monocytes, DCs, NK cells, B cells, and T cells. Members of the CC family, whose first two cysteines are adjacent, function primarily on monocytes, macrophages, and lymphocytes, with some activity on other cells types. Chemokines are produced by macrophages and other cells after recognition of invading microbes or in response to proinflammatory cytokines. The interactions of chemokines on target cell populations are extraordinarily complex. Although there appears to be a level of redundancy, with multiple chemokines signaling through the same receptor, differential expression of chemokine receptors and adhesion molecules provides the appropriate instructions for leukocyte trafficking.[121] It is then the coordinated action of chemokines, other proinflammatory cytokines, and vasoactive mediators that regulate the migration and activation of leukocytes in a stepwise process to appropriately respond to an infection.

PATHOGEN INTERFERENCE WITH INNATE IMMUNE RESPONSES

Essentially all successful pathogenic bacteria, viruses, fungi, and protozoa have evolved ways to evade or suppress innate immune responses to facilitate their establishment and replication in a host. Major points of pathogen interference parallel three major steps in innate immunity—PAMP recognition, intracellular signaling, and chemokine/cytokine expression triggered by the activated transcription factors.

Altering PAMP structure to avoid activation of PRRs is an important strategy in the establishment of infection for many pathogens and commensals. For example, *Helicobacter,*[122] *Coxiella,*[123] *Legionella,* and *Rhizobium* all have altered lipid A structures that are poorly recognized by TLR4.[124] Several fungal species, including *Candida* and *Pneumocystis*, alter their surface glycans during different stages of life to prevent recognition by CLRs, the predominant receptor family for sensing fungi.[125] Viruses such as the influenza A virus (IAV) and dengue virus replicate in membranous cellular compartments to avoid detection by intracellular RLRs, although others modify their viral genome (e.g., Crimean-Congo hemorrhagic fever virus) or shield viral RNA through binding of viral or host proteins (e.g., Ebolavirus and IAV).[126] In addition to avoiding activation of PRRs, bacteria have also developed multiple mechanisms to evade complement activation (see Chapter 9), including mimicry of complement regulatory proteins and expression of proteases or small proteins that can cleave or inhibit complement components.[127]

Many pathogens express PAMPs that can be sensed by PRRs but evolved strategies to evade intracellular signaling pathways. The most studied are viruses, some of which directly inhibit the activation of PRRs or target downstream signaling molecules to interfere with the expression of type I IFNs or the function of antiviral effector proteins. Inhibition can be mediated by virally encoded proteins that intervene in posttranslational modifications (e.g., phosphorylation) or by degrading key signaling components. The first example of viral inference with innate immunity was the blockage of the proteins responsible for the antiviral effects of IFNs.[128,129] It is now known that viruses such as human herpesvirus family members also encode microRNAs, which decrease the expression levels of specific mediators of TLR signaling. The result is avoidance of innate pathways, giving these viruses the upper hand.[130] Bacteria can also have profound effects on innate signaling pathways. For example, the anthrax toxin subunit lethal factor targets the mitogen-activated protein kinase (MAPK) kinase (MKK) for proteolytic degradation, which induces apoptosis of macrophages and DCs exposed to this toxin.[131]

A third major point of pathogen interference is on the initial inflammatory response. For example, poxviruses have devised two ways of interfering with the activation of IL-1β through the NLR pathway. First, infected cells secrete a caspase inhibitor that prevents the maturation of pro–IL-1β.[132] Second, the virus-infected cells also secrete a soluble IL-1β receptor, effectively reducing the serum concentration of this important cytokine.[133] Taken together, these adaptations demonstrate the diverse approaches that microbes take to successfully establish infection.

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5 Adaptive Immunity: Antibodies and Immunodeficiencies

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SHORT VIEW SUMMARY

- Antibodies are a principal mode of host defense against bacteria, fungi, parasites, viruses, and exotoxins prior to entry into host cells. Knowing the types and kinetics of antibody-mediated effector functions enables the clinician to better judge whether and when patients will recover from infections.
- Understanding the factors that initiate and perpetuate antibody production explains how vaccines can be optimized for protection against infectious agents and why it is particularly difficult to generate protection against pathogens whose surfaces are dominated by polysaccharide antigens.
- Defects in antibody production are the most common forms of inherited immunodeficiencies and are the only types that can be readily and effectively treated without resorting to bone marrow transplantation.
- Detection of a patient's antibody response to a pathogen is often the only means of diagnosing an infection. By further identifying the particular classes of specific antibody, the clinician can often ascertain whether the infection is ongoing or resolved.
- Specific antibodies, often in the form of monoclonal antibodies, are used as reagents to identify antigens, including infectious agents, in tissues. Some monoclonal antibodies are used for prevention and therapy of infectious diseases. An appreciation of the available assays, their strengths, and their potential pitfalls helps the clinician interpret test results.
- Infections can generate copious quantities of antigens that become incorporated into immune complexes with antibodies. Deposition of these immune complexes in blood vessel walls, renal glomeruli, or other vascularized beds causes inflammation that exacerbates the tissue injury caused by the infection.
- Depending on the structure of their Fc piece, antibodies can engage with either activating or inhibitory Fc receptors on cells and thereby enhance or downmodulate immune and inflammatory responses.
- Infections can promote the production of autoreactive antibodies, which, in turn, lead to autoimmune disease.

Antibodies are serum proteins that aid in the neutralization and clearance of pathogens or antigens. Antibodies are produced by B lymphocytes, or B cells. As a pre-B cell matures, it rearranges and selectively mutates the portion of its DNA that encodes the antigen-binding site in the antibody molecule. Each B-cell clone does this in a different way, leading to millions of B-cell clones, each producing antibodies with a slightly different configuration at the antigen-binding site. The opposite end of the antibody molecule remains constant, allowing it to interact with fixed (or invariant) elements of the immune system, such as neutrophils and monocytes that ingest and kill antibody-coated pathogens.

Antibodies were initially discovered in the 1890s through their ability to neutralize toxins. In the late 1800s, it was observed that animals immunized with bacterial toxins produced a circulating substance that could neutralize the toxin's activity. First called an *antitoxin,* this substance was later given the more general name *antibody.* In electrophoretic analyses of serum proteins, antibodies migrate in the third, or "gamma," globulin peak, which led to the alternative name *gamma globulins* or, finally, *immunoglobulins.*

IMMUNOGLOBULIN STRUCTURE

Basic Antibody Structure

Antibodies look a bit like lobsters, with the two claws serving as antigen-binding clefts (Fig. 5.1). The tail of the lobster/antibody interacts with receptors on cells of the immune system: neutrophils, monocytes, macrophages, B lymphocytes, dendritic cells, and, in certain cases, mast cells. The tail or carboxyl-terminal end of certain immunoglobulins also binds to specialized transport receptors that carry the antibody across epithelial barriers into secretions or across the placenta into the fetus. Near the insertion point of the claws into the lobster's body is a binding site for C1q, the first protein of the complement cascade, which helps to kill and clear pathogens and other antigens.

The basic antibody unit is composed of two identical light chains and two identical heavy chains. Each of the four polypeptide chains is made up of loops or domains of about 110 amino acids bridged by disulfide bonds. This structural motif is characteristic of the immunoglobulin superfamily that also includes some cell adhesion molecules, CD4, CD8, CD28, and members of the B7 family of costimulatory molecules. Light chains have two domains, and heavy chains have four or five domains. The loop at the amino ("lobster claw") end of the heavy and light chains is called the *V domain* because of its highly variable amino acid sequence. The other domains have relatively constant sequences and are called *C domains.* Each light chain is attached to its heavy-chain partner by disulfide bonds bringing together their V domains to form the antigen-binding site. The variation in amino acid sequence of the V domains is actually focused in three *hypervariable regions;* when the protein folds, the six hypervariable regions (three from the light chain and three from the heavy chain) form the walls of the antigen-binding cleft. The hypervariable regions are also called *complementarity-determining regions* (CDRs).

The two heavy chains are linked to each other by disulfide bonds to form the immunoglobulin molecule's lobster tail. There are five variations in heavy-chain constant domains, termed mu (μ), gamma (γ), alpha (α), epsilon (ε), and delta (δ). The *antibody classes* that they form are called immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin E (IgE), and immunoglobulin D (IgD), respectively. Antibody classes are also called *isotypes* because they were defined by the use of antibodies generated by immunizing phylogenetically distant animal species with human immunoglobulins. *Allotypes* are minor variations within an isotype that are found in some, but not all, humans and were discovered using antibodies generated by immunizing humans (or nonhuman primates) with immunoglobulin from other humans. *Idiotypes* are variations between antibodies that are otherwise of identical isotype and allotype. Idiotypic variations tend to be located in or near the antigen-binding site. For example, an IgG antibody specific for measles would have a different idiotype from an IgG antibody specific for mumps.

There are only two classes of light chains, kappa (κ) and lambda (λ), and they appear in all five immunoglobulin classes. On average,

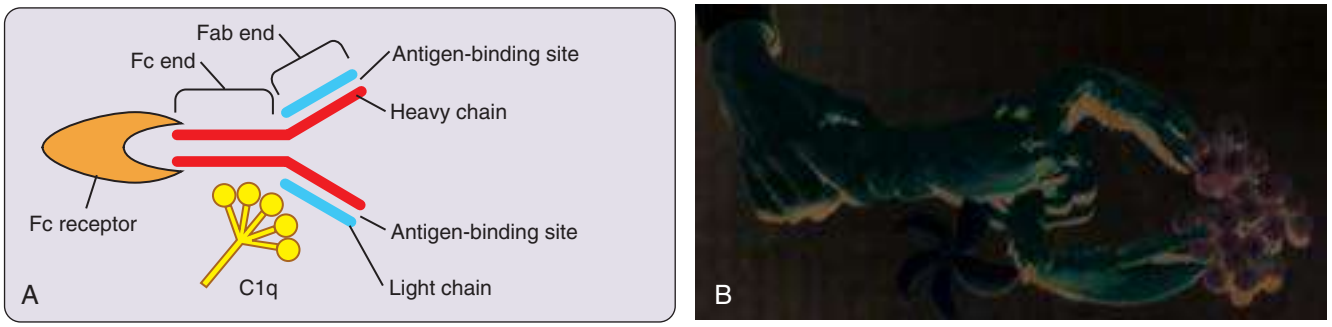

FIG. 5.1 Structure of antibodies. (A) Antibody molecules are composed of two heavy chains *(red lines)* and two light chains *(blue lines)* held together by disulfide bonds. The two heavy chains join to form a tail (Fc end), which can interact with Fc receptors on a variety of cells. The heavy and light chains each contribute to the Fab end. At the 5′ or amino-terminal end, these chains form two identical antigen-binding sites, much like two lobster claws (B). Near the hinge region of the antibody, there is a binding site for C1q, the first component of the complement cascade.

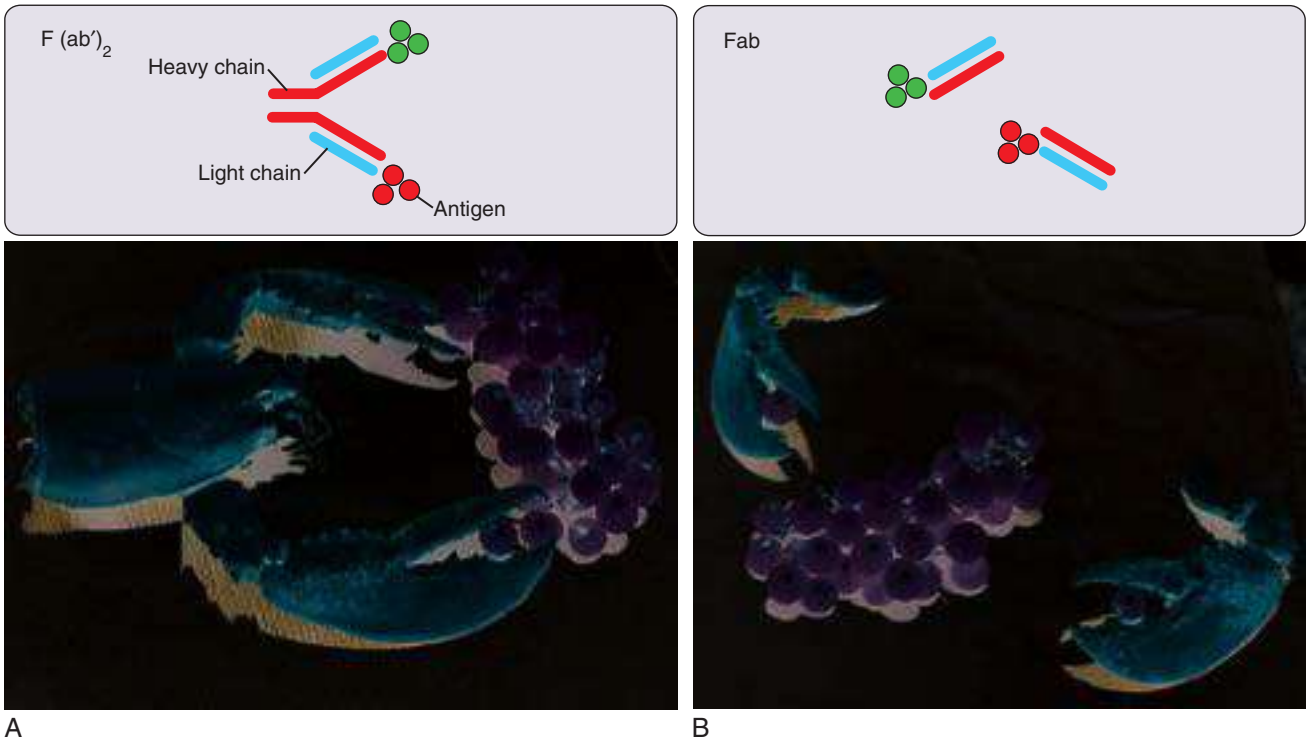

FIG. 5.2 Cleavage fragments of antibodies. (A) Papain digests the immunoglobulin molecule into an F(ab′)$_2$ fragment. This fragment is still dimeric, but it can no longer interact with Fc receptors on cells. Under reducing conditions, the disulfide bonds holding the two heavy chains together can be broken, leaving two monomeric Fab′ fragments (not shown). (B) Pepsin digests away all of the Fc piece, leaving two monomeric Fab pieces.

60% of antibody molecules use κ chains and 40% use λ chains. This information can be useful in the diagnosis of lymphomas. If virtually all of the B cells use the same light-chain class (i.e., all κ or all λ), it is likely that they arose by clonal expansion from a single malignant precursor.

F(ab′)$_2$, Fab, and Fc Pieces

Several regions of the immunoglobulin molecule have more specific names. The *Fab fragment* is the antigen-binding end, and the other end is the *Fc piece*. In the lobster analogy, the Fab region is the head and claws and the Fc region is the tail (see Fig. 5.1). All antibodies of a given isotype have the same Fc regions such that when Fc fragments were first generated by enzymatic cleavage, the identical molecules often crystallized—hence, the "c" designation. Cell receptors for the Fc pieces of antibodies are called FcRs. A Greek letter further indicates their isotype specificity; for example, an FcγR binds IgG and an FcεR binds IgE.

Papain cleaves our imaginary antibody-lobster at approximately midthorax, resulting in an Fc tail piece attached to a dimeric F(ab′)$_2$ piece (Fig. 5.2). Under reducing conditions, the disulfide linkage between the two heavy chains is broken, splitting the lobster/antibody in a sagittal direction to generate two monomeric Fab′ molecules. By way of contrast, pepsin digests the tail into tiny fragments, leaving just the two Fab monomers—claws with no lobster head (see Fig. 5.2).

Proteolytic fragments of antibodies are useful experimental reagents. Antibodies used to stain cells in immunohistochemical or immunofluorescent assays are often predigested into F(ab′)$_2$ fragments to eliminate antigen-nonspecific binding by FcRs found on many types of cells. Antibodies can be used as surrogate ligands to interact with cell surface receptors. To determine whether cross-linking of the cell surface receptor is required for signaling, the experimenter can compare the effect of dimeric intact or F(ab′)$_2$ fragments with the effect of Fab or Fab′ fragments that are monomeric and unable to cross link.

Antigen Binding, Affinity, and Avidity

Affinity refers to the strength of the interaction, which reflects the goodness-of-fit and energetics between the antigen-binding site and the antigen. Affinity is influenced by electrostatic, hydrogen-binding,

van der Waals, and hydrophobic interactions. *Avidity* measures the interaction of the intact antibody molecule and involves binding site affinity plus the additive effect of multiple antigen-binding sites. IgG, IgE, and IgD have two antigen-binding sites per antibody molecule. IgA can dimerize to generate four antigen-binding sites, and IgM is a pentamer with 10 antigen-binding sites. Because it is unlikely that all 10 antigen-binding sites will disengage simultaneously, IgM molecules can have relatively high avidity for a multivalent antigen even when the affinity of their antigen-binding site is relatively low.

The *epitope* is that portion of the antigen that fits into the antigen-binding cleft. The antigen-binding cleft can accommodate as many as 6 to 12 amino acids. *Linear epitopes* are composed of contiguous amino acids or oligosaccharides, whereas *conformational epitopes* are formed by protein and polysaccharide segments that are brought into close apposition by molecular folding. Peptide vaccines generate antibodies to linear epitopes. Denaturation or degradation can abolish native conformational epitopes while also generating novel conformational epitopes. A large antigen may have many epitopes and can react with multiple antibody molecules at the same time.

Immunoglobulin Classes

The concentrations of the five isotypes in serum vary widely, reflecting both different numbers of B cells producing each isotype and different intrinsic half-lives of the immunoglobulin classes. The isotype of an antibody dictates where in the body it is likely to be found and what types of effector functions it can mediate (Table 5.1).

Immunoglobulin M

IgM has the largest molecular weight of all of the isotypes, 900 kDa, which keeps it largely restricted to the intravascular compartment. It is composed of five immunoglobulin monomers whose μ chains are either covalently linked through disulfide bridges or held noncovalently by a joining, or "J," piece produced by the B cell. Steric hindrance typically allows only 5 of IgM's 10 antigen-binding sites to engage antigen simultaneously. Still, this multivalent binding ability allows IgM to provide effective defense despite its characteristic low affinity for antigen. IgM antibodies defend the host by blocking binding of pathogens to cells and by aggregating infectious agents to facilitate their clearance. IgM antibodies fix (activate) complement more efficiently than any other isotype. Monomeric IgM is displayed on the surface of B cells. This membrane IgM allows the B cell to detect encounters with cognate antigen and triggers its subsequent activation and proliferation.

Immunoglobulin G

IgG is the most plentiful isotype in the serum because of its high production rate (25 mg/kg/day) and its half-life of 23 days, which is 4 to 10 times longer than that of the other isotypes. As a 150-kDa monomer, IgG can move into the extracellular fluid so that less than half of the body content of IgG is in the circulation at any one time. IgG is the only isotype that is carried across the human placenta to the fetus. Beginning at 20 or 21 weeks of gestation,[1] maternal IgG crosses into the fetal circulation by means of a special placental transport receptor, FcRn.

There are four subclasses of the γ heavy chain: IgG1, IgG2, IgG3, and IgG4. They vary in amino acid composition and degree of glycosylation. IgG1 and IgG3 can fix complement, but IgG2 and IgG4 do not. Antibodies to proteins are largely IgG1 and IgG3. Antibodies to polysaccharides tend to be of the IgG2 subclass,[2] and individuals deficient in IgG2 may show an increased susceptibility to infections with encapsulated organisms.[3] Responses to helminths tend to be of the IgG4 class,[4] but there is no evidence that individuals deficient in IgG4 have a higher susceptibility to these organisms.[2]

TABLE 5.1 Characteristics of the Immunoglobulin Classes

	ISOTYPE			
CHARACTERISTIC	**IgM**	**IgG**	**IgA**	**IgE**
Half-life in serum (days)	10	21	6	2
Normal serum level in adults (mg/mL)	0.6–3.5	6.4–13.5	0.7–3.1	0.00004
Transported into secretions	±		+	
Crosses placenta to fetus		+		
Blocks binding of pathogens or toxins	+	+	+	
Opsonizes for phagocytosis via FcR		+		
Fixes complement via C1q	+	+		
Mediates ADCC		+		
Binds mast cells				+

ADCC, Antibody-dependent cellular cytotoxicity; *Ig,* immunoglobulin.
Modified from Stites DP, Stobo JD, Wells JV, eds. Basic and Clinical Immunology. *7th ed. Los Altos, CA: Appleton & Lange; 1991, with permission.*

Immunoglobulin A

Each day, humans produce about 66 mg of IgA per kilogram of body weight, which is about twice the quantity of IgG produced.[5] However, serum levels of IgA are relatively low, because most of the IgA is produced by submucosal plasma cells and immediately transported into secretions. There are two subclasses of IgA. IgA_1 is monomeric and found primarily in the serum. IgA2 can polymerize into multimers linked by the J piece and is transported into the secretions. Dimeric IgA, produced by submucosal B cells, binds to secretory component (SC) produced by epithelial cells. The IgA is endocytosed and carried through the cytoplasm of the epithelial cells. On the apical side, SC is cleaved, releasing the IgA into mucosal secretions. A fragment of the SC remains associated with the IgA molecule and protects it from cleavage by proteases in the secretions. IgM can also be carried across epithelium by this process.[6] Some pathogens express ligands that allow them to co-opt this transport system and use it to cross in the reverse direction into the subepithelium.[7] IgA-coated Epstein-Barr virus may gain entry into nasopharyngeal cells by this route.[8]

IgA defends mucosal surfaces against invading pathogens. IgA blocks the binding of bacteria, viruses, and toxins to cell receptors. IgA cross links pathogens and facilitates their clearance by ciliated epithelium. In the intestinal tract, IgA binds food antigens and prevents triggering of proinflammatory responses. IgA does not activate complement or bind to phagocytes. The relative inability of IgA to initiate inflammatory responses allows food antigens to be sequestered without deleterious consequences.[9] Failure of this process in IgA-deficient individuals may account for their increased frequency of allergic diseases.[10]

Immunoglobulin D

IgD is produced by all B cells during early stages of differentiation and is expressed on the cell membrane, where it has a key role in cell signaling. However, very little IgD is found in the serum, and IgD currently has no known effector role in host defense.[11]

Immunoglobulin E

High-affinity FcεRs scavenge IgE so quickly that its half-life in the circulation is only about 2 days, and very little is found in the serum. Once bound to mast cells, IgE persists for a long time, perhaps for the life span of the mast cell. Infused IgE can be detected on murine mast cells for up to 7 weeks.[12] IgE displayed on the mast cell surfaces mediates immediate hypersensitivity or allergic reactions. IgE appears to have a role in defense against parasitic infections. Mast cells are needed to clear intestinal helminthic infections,[13] and IgE-deficient mice, when infected, have higher burdens of *Schistosoma mansoni.*[14]

EFFECTOR FUNCTIONS MEDIATED BY ANTIBODIES

Historically, antibodies were considered as linker molecules or "transducers" to tag the pathogen and become a physical link between the pathogen and the killing mechanism, typically a leukocyte. As such, they were viewed as having no intrinsic antimicrobial function. However, in recent years several antibodies have been shown to have direct antimicrobial effects on bacteria and fungi.

Blocking or Neutralization

Invasion of host cells is a critical step in infectious processes, and a major protective function of antibodies is preventing the binding of viruses, toxins, or bacteria. The challenge for vaccine developers is to identify which microbial epitopes are integral to the pathogenic process and then devise vaccines that generate specific antibodies to block those interactions. Generation of protective immunity can be extremely difficult if, for instance, the key epitope is located deep within a cleft in the native protein and is inaccessible to antibodies. Steric hindrance is not the only way that antibodies can prevent infection. For example, picornavirus has multiple binding sites, but infection can be blocked by a single antibody. This suggests that antibody binding affects the charge characteristics or conformation[15] in the pathogen. Verifying that an antibody has "blocking" or "neutralizing" activity is key. Antibodies that bind to a pathogen but fail to effectively neutralize or block infection can paradoxically facilitate infection by allowing the pathogen to be taken into the cytoplasm through FcR or other receptors.[16] Such antibodies can also exacerbate the morbidity of an infection by triggering inflammatory processes without actually controlling the infection.[17]

Blocking adherence of bacteria and viruses to mucous membranes is probably the major defensive role of IgA in secretions. Viruses can also be neutralized by IgA within the cytoplasm of epithelial cells in the course of transepithelial transport.[18] Blocking is independent of the Fc piece; it can be accomplished by antibodies of any isotype and even antibodies from other species. This accounts for the efficacy of equine antitoxin in the early treatment of diseases such as tetanus.[19] However, nonhuman immunoglobulin is perceived as foreign by the immune system and triggers an antibody response. The complexes that form between horse immunoglobulin and human antibodies to horse immunoglobulin caused serum sickness, a condition with considerable morbidity and some mortality. Antisera produced in other animals are rarely used in the United States today except in cases such as snake antivenin, when it is not feasible to generate human hyperimmune sera. However, immune animal sera continue to be used in less resource-rich areas of the world for such purposes as prophylaxis against rabies.

Complement Activation

Complement is a series of serum proteins that augment or "complement" the action of antibodies by facilitating phagocytosis, attracting leukocytes, and directly lysing microbes (see Chapter 9). Antibodies interacting with and initiating the complement cascade are said to activate or "fix" complement.

IgG and IgM, but not IgG4, IgA, or IgE, have binding sites for C1q, the first protein in the classical complement cascade. To be activated, C1q must interact with at least two C1q binding sites. As a pentamer, IgM has five C1q binding sites, so only a single IgM molecule is needed to activate the cascade. For IgG, with only one C1q binding site per molecule, activation requires that C1q straddle at least two IgG molecules that are sufficiently close together. Because the C1q binding site is not accessible until the antibody binds to antigen, complement is not activated by soluble immunoglobulin in the circulation. Binding to antigen leads to a conformational change in the IgG molecule that increases the affinity of the C1q binding site 10,000-fold.[20]

When C1q is engaged by antibodies, it undergoes a conformational change of its own and activates the next member of the cascade. Eventually, C1r, C1s, C4, C2, C3, C5, C6, C7, C8, and C9 are activated, in that order. Complement provides immune defense by enhancing the uptake of C3b-coated pathogens, directly lysing target cells, and promoting the influx of immune effector cells. Antibodies augment the defensive efficacy of complement by greatly accelerating the rate at which complement is activated and by focusing the effect of complement onto the surface of the antibody-coated particle.

Opsonization

Neutrophils, monocytes, and macrophages are collectively referred to as *phagocytes* on the basis of their ability to ingest antigens. Phagocytes pull pathogens into phagosomes, where the organisms are killed with toxic agents, such as reactive oxygen species, nitric oxide, and enzymes. Phagocytes have pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) endogenous to many microbes (discussed later). Phagocytes also have receptors for the Fc end of IgG molecules (FcγRs) and for the C3b fragment of complement, and they use these to recognize and ingest IgG- or C3b-coated targets. Facilitation of phagocytosis is called *opsonization,* and IgG and C3b, in this role, are called *opsonins.* As an opsonin, IgG expands the repertoire of the immune system by enabling phagocytes to recognize pathogens, such as viruses, that do not express any PAMPs. C3b can bind spontaneously to the surface of microbes through the alternate pathway of complement activation. However, the accumulation of C3b on the surface of the pathogen is greatly accelerated when antibodies bind to the microbe first, fix C1q, and activate the complement cascade through the classical pathway.

Phagocytes can attach to C3b-coated targets by means of their C3b receptors. However, to complete the phagocytic process, the leukocyte needs to receive a second stimulus. This signal can come from the interaction of its FcR with the Fc of IgG bound to the pathogen, or it can come from C5a fragments generated by activated complement. Signaling through the FcγR also triggers an oxidative burst that increases the ability of the phagocyte to kill the organism it has just ingested. There are no FcμRs on phagocytes, so IgM cannot opsonize in this manner. However, a single molecule of IgM can activate complement through the classical pathway, leading to the deposition of many C3b molecules that can act as opsonins. IgA does not activate complement by the classical C1q pathway but can provide a site for deposition of C3b and thereby activate complement through the alternate pathway.[21]

There are two major families of receptors for IgG, type I and type II.[22] Type I Fcγ receptors include FcγRI, FcγRIIA, FcγRIIB, FcγRIIC, FcγRIIIA, and FcγRIIIB, and largely mediate effector functions of IgG such as opsonization and antibody-dependent cellular cytotoxicity (ADCC). Type II Fcγ receptors include DC-SIGN (*d*endritic *c*ell–specific *i*ntercellular adhesion molecule-3-grabbing *n*onintegrin) and CD23, and largely mediate antiinflammatory functions through induction of interleukin (IL)-33, suppressor monocytes, and helper T cell 2 (Th2) and T-regulatory (Treg) cells. Conformation of the Fc region of IgG is dictated by the composition of the Fc-associated glycan located in a cleft between the C_H2 regions of the two heavy chains.[22] Branching fucose moieties in the cleft induce an "open" configuration that promotes binding to type I FcR. Sialylization of the central N-linked glycans in the cleft promotes binding to type II FcγR. Fc glycoengineering can be used to modify the N-linked glycans on therapeutic antibody preparations to increase their desired efficacy in vivo. IgG1 and IgG3 interact effectively with FcγRs, but IgG2 and IgG4 do not.[22]

FcγRI has a high affinity for IgG, and it is the only FcγR that can bind monomeric IgG. FcγRI is constitutively expressed on monocytes and macrophages and can be induced on neutrophils, eosinophils, and basophils. FcγRII and FcγRIII have much lower affinity for IgG and only bind IgG in immune complexes or in aggregates. When engaged in immune complexes, IgG undergoes a conformational change that increases its affinity for the FcR. Furthermore, the additive effect of multiple FcRs interacting with the IgG molecules in the immune complex increases the overall avidity of the interaction. The restricted ability of FcγRII and FcγRIII to only bind to IgG in immune complexes ensures that they are only engaged during an ongoing immune response to antigen and not to monomeric IgG during steady state.

FcγRs can also be divided into activating versus inhibitory.[22] The activating FcRs are FcγRI, FcγRIIA, FcγRIIC, and FcγRIIIA, which have immunoreceptor tyrosine-based activation motifs (ITAMs) that provide docking sites for the Syk family of kinases, with subsequent activation of downstream pathways. Signaling through FcγRIIIA on natural killer (NK) cells and monocytes triggers ADCC and production of interferon-γ.[23] Antibodies with no neutralizing effect in vitro may still have protective activity in vivo through their ability to activate cells through their FcγRs.[24] There are also clinical correlations between expression of low-affinity variants of FcγRIIA and increased susceptibility to bacterial infections and sepsis. For example, homozygosity of the R131 variant of FcγRIIA, which has lower affinity for IgG2, is found more frequently in patients who develop sepsis as opposed to infected but nonseptic patients, and the H131 low-affinity variant is twice as prevalent in children who

develop sepsis with *Streptococcus pneumoniae,* as opposed to healthy blood donor controls.[25,26]

FcγRIIIB has no intracellular domain and can signal only through interaction with other receptors. The only FcγR that can antagonize the immunostimulatory signals of the other activating FcγRs is FcγRIIB with its immunoreceptor tyrosine-based inhibition motifs (ITIMs). For example, FcγRIIB is present on B cells, where it exerts a negative signal to downregulate antibody production when the formation of immune complexes indicates a relative excess of antibody.[27]

The ability of antibodies to engage activating and inhibitory receptors means that the antibody response in infection has the capacity for modulating the immune and inflammatory response. In this regard, the ability of an antibody response to be pro- or antiinflammatory will reflect a complex formula that includes the type of isotype made, the ability of the antibody to activate complement, the Fc receptor(s) engaged, and the type of antigen-antibody complexes that are made.

In addition to its role in transporting IgG across the placenta, FcRn has a pivotal role in extending the half-life of IgG in the circulation.[28] FcRn is found on the surface of macrophages and endothelial cells that pinocytose IgG. FcRn binds to IgG in the endosome, directs it away from the lysosomes, and instead recycles the IgG to the cell surface and back into the circulation.

There is an FcR for IgA (CD89) on monocytes, neutrophils, macrophages, and perhaps eosinophils, particularly those in the lungs.[29] There is no FcR for IgM, and antibodies of these classes cannot serve as direct opsonins for phagocytosis.

Antibody-Dependent Cellular Cytotoxicity

ADCC is carried out by monocyte-macrophages, NK cells, and neutrophils. ADCC allows these effector cells to kill targets, such as tumor cells and virally infected cells, that are too large to be ingested. Perforins, granzymes, and in some cases reactive oxygen intermediates are involved in this microbicidal activity. The role of the IgG antibody in ADCC is to bind to the FcγRIII or FcγRII on the NK cell or monocyte and identify the target cell to be killed. ADCC activity is a mechanism by which some nonneutralizing antibodies are able to protect against viral infections.

Direct Antibody-Mediated Antimicrobial Functions

Although antibodies have classically been considered as connector molecules between microbial antigens and effectors of the immune system such as phagocytes and complement, there is increasing evidence that some antibodies have direct antimicrobial activities. IgM and IgG are directly microbicidal to the outer surface protein of *Borrelia burgdorferi* in the absence of complement.[30] Antibodies to fungal mannoproteins are directly fungicidal against various fungi, including *Candida albicans.*[31] Although the mechanisms by which antibody can mediate direct microbicidal activity are not well understood, there is evidence that antibodies can induce metabolic changes in certain microbes. For example, antibody binding to the capsules of both the fungus *Cryptococcus neoformans*[32] and the bacterium *S. pneumoniae*[33] results in transcriptional changes that predispose the former to antifungal agents and activate an autolytic pathway in the latter.

Antibody-Mediated Catalysis

Many antibodies have catalytic-like motifs in their binding sites that allow them to hydrolyze antigens. Antibody-mediated catalytic activity has the potential to aid host defenses by cleaving microbial antigens that are involved in pathogenesis.[34] Consequently, some of these catalytic activities have been associated with resistance to infection. Specifically, an antibody that cleaves the urease made by *Helicobacter pylori* has been shown to reduce bacterial burden in experimental models of infection.[35] Similarly, other monoclonal antibodies have been shown to cleave viral and fungal antigens. Antibody-mediated catalysis is generally much slower than that observed with classical enzymes, but given that infectious processes take time, this activity could contribute to host defense by deactivating microbial components.

KINETICS OF ANTIBODY PRODUCTION AND DIAGNOSIS OF INFECTIONS

On first encounter with an antigen, the immune system generates a primary response that is detectable within 5 to 7 days (Fig. 5.3). This is the timing of the response in a laboratory setting with highly sensitive assays. The apparent kinetics of a patient's antibody response would be influenced by the sensitivity of the assay available to measure the antibodies such that a more sensitive assay would be able to detect a response earlier than a less sensitive assay. The primary antibody response consists of IgM antibodies with a relatively low affinity for antigen. Within several days to weeks, some antibody-producing B cells switch to making antibodies that have the same antigen specificity but of an IgG, IgA, or IgE isotype. To switch isotypes, B cells must receive signals from activated helper T cells that are typically specific for and activated by the same antigen as the B cells. Under the influence of T cells, some B cells also become memory cells.

On subsequent exposure to the same antigen, memory B cells rapidly divide and begin to produce large quantities of antibody within as little as 1 to 2 days. Faster kinetics and greater quantities of antibody are the hallmarks of a secondary antibody response. Secondary responses are also largely IgG, IgA, or IgE isotype, as opposed to IgM. The average affinity of antibodies in a secondary response is much higher than in a primary response. As the B cells divide in the lymph node germinal center, point mutations occur in the hypervariable region of the gene encoding the antigen-binding site. B-cell survival depends on continued antigen stimulation. B cells whose mutations increase their affinity have a competitive survival advantage. The net effect is to produce IgG antibodies with a progressively higher affinity for the immunizing antigen. As more and more antigen is incorporated in antigen-antibody complexes and metabolized, competition among B cells for antigen stimulation increases.

As the antigen stimulus becomes more scarce, this and other regulatory influences act to slow the production of antibody. Only a low level of residual production of IgG persists in response to antigen associated with follicular dendritic cells. However, quiescent memory B cells lie in wait for the next challenge with this same antigen. Should antigen reappear, these memory cells can rapidly activate, proliferate, and begin producing antibodies.

When the timing of antigen exposure is well defined, as with vaccines, it is easy to distinguish between the primary and secondary responses.

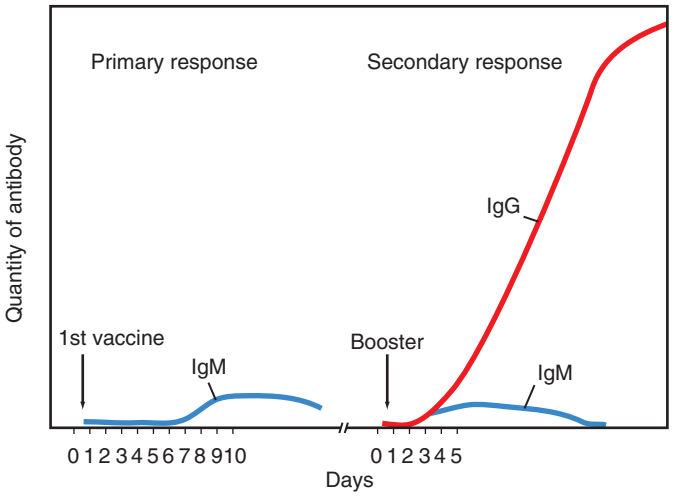

FIG. 5.3 Primary and secondary immune responses. A first exposure to antigen stimulates production of immunoglobulin M (IgM) antibodies (*blue line*), which become detectable within about a week. The antigen-specific B cells proliferate, and some become memory cells. On subsequent exposure to the same antigen, these memory cells are rapidly activated. Antibodies, mainly of the IgG class (*red line*), appear within about 2 days, and the quantity of antibody produced is much higher than that seen with the primary response. The affinity of the antibodies in the secondary response is also markedly higher than that seen in the primary response.

However, in the case of infection, antigen is released over a prolonged interval and there is no clear border between primary and secondary responses. So long as new clones of B cells emerging from the bone marrow are encountering antigen, IgM will be produced. Therefore the presence of IgM antibodies is indicative of active infection. By contrast, a response that is solely of the IgG isotype is considered to indicate a resolved infection. During the first few weeks of an infection, antigen-specific B cells proliferate and produce ever-increasing quantities of antibody. Therefore a rising titer of specific antibodies also indicates an active infection.

To compare titers, serum should be obtained at presentation (the acute serum) and again 1 or more weeks later (the convalescent sample). For maximum accuracy, the acute serum should be stored in a freezer and sent to the laboratory at the same time as the convalescent sample, so that the two samples will be assayed at the same time with identical reagents.

LABORATORY MEASUREMENT OF ANTIBODIES

Quantification of Total Immunoglobulin

Serum IgG, IgM, and IgA are readily quantified by most clinical laboratories. Age-specific reference tables must be used for children, because normal ranges for the various immunoglobulin classes are highly dependent on age. Two-thirds of IgG is of the IgG1 subclass, so a deficiency in this subclass would be apparent when total IgG is measured. However, patients who are missing IgG2, IgG3, or IgG4 could still have normal total IgG levels, and it may be appropriate to measure the subclasses individually.

By far the most common immunodeficiency is an absence of IgA, often in conjunction with a deficiency of one or more subclasses of IgG. Measurement of serum IgA is adequate to identify most IgA deficiencies. Deficiencies of the secretory piece, such that patients have serum IgA but no secretory IgA, is a very rare defect. Measurement of total IgE is not useful in the diagnosis of atopy or allergy, because serum levels may be normal or only mildly elevated in patients with clinically significant atopic disease (e.g., asthma, allergic rhinitis, urticaria). However, serum IgE may be strikingly elevated in parasitic infections.

Serum Protein Electrophoresis for Monoclonal Gammopathies

Antibody responses are typically polyclonal, meaning that many clones of B cells are stimulated, and the array of antibodies produced recognizes many different epitopes. These antibodies have diverse electrophoretic mobilities and produce a broad gamma globulin peak on protein electrophoresis. In multiple myeloma or other monoclonal gammopathies, a single B-cell clone proliferates in an unrestricted manner and produces large quantities of a single antibody type. This homogeneous product, referred to as a monoclonal antibody or M protein, appears as a spike with a single electrophoretic mobility.[36] Light chains from this clone may appear in the urine as Bence Jones proteins.

Measurement of Functional Antibody

When infectious agents cannot be cultured or otherwise specifically identified, diagnosis may depend on the ability to demonstrate that the patient is mounting a specific humoral response to the suspected pathogen. Common clinical examples would be hepatitis B, hepatitis A, Epstein-Barr virus, and cytomegalovirus, in which it is far easier to measure an ongoing antibody response than to culture the virus as a means of diagnosis. An ideal antibody test should be quantitative so that it can be used to look for rising titers indicative of active infection. If the test can also discriminate between IgG and IgM antibodies, then the presence of an IgM response can be used as indication of an active infection.

The most common format is a solid-phase immunosorbent assay. Antigen, such as an extract from the infectious agent, is immobilized on a plastic surface such as a microtiter plate or beads (Fig. 5.4A). The patient's serum is allowed to interact with the antigen, and nonspecific, unbound antibody is washed away. Then a detection reagent is added to measure the quantity of patient antibody bound to the antigen. The detection reagent is an antibody to human immunoglobulin that has been prepared by immunizing a sheep, rabbit, goat, or other animal. The greater the quantity of patient antibodies adherent to the antigen, the greater the amount of anti–human immunoglobulin that binds. Class-specific antiimmunoglobulin reagents can be prepared by immunizing the animal with a single isotype of human immunoglobulin (i.e., only IgG or only IgM) and removing antibodies that cross-react with light-chain antigens that would be present on other isotypes. Using these, it is possible to determine whether the patient's antibodies are IgM, IgG, IgA, or IgE. The anti–human immunoglobulin is typically purified and conjugated with a reagent to facilitate quantification. In an enzyme-linked immunosorbent assay (ELISA), the antiimmunoglobulin is conjugated with an enzyme, such as a peroxidase or a phosphatase, that converts a substrate to a colored or luminescent product. In a radioimmunoassay, the anti–human immunoglobulin is

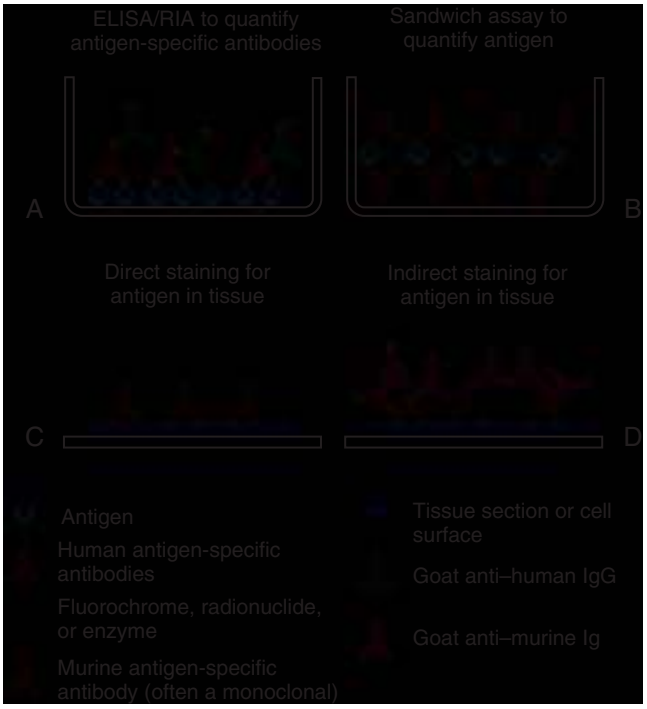

FIG. 5.4 Antibody-based assays. (A) In a solid-phase assay for antigen-specific antibodies, antigen is coated on a surface, typically a plastic well. The patient sample (e.g., serum) is incubated with the antigen, after which unbound antibodies are washed away. Patient antibodies bound to the antigen are detected with an antibody to human immunoglobulin that is produced by immunizing another species (in this case, a goat). The goat anti–human immunoglobulin *(Ig)* is conjugated with a moiety that allows it to be detected. This could be a radionuclide, a fluorochrome, or an enzyme that converts an added substrate to a colored or luminescent product. (B) A sandwich format can be used to measure antigen. A capture antibody is immobilized in the plastic well. The source of antigen is added, and then a second antigen-specific antibody is added to detect the bound antigen. This detection antibody is conjugated with a radionuclide, fluorochrome, or enzyme. The two antibody layers in the sandwich assay must recognize different epitopes on the antigen; otherwise, the first antibody would occupy all the epitopes and there would be none left for the second antibody to bind. (C) Direct staining for antigens in tissue or cells uses antigen-specific antibodies (often monoclonal antibodies) that have been purified and conjugated with a detection molecule such as a radionuclide, a fluorochrome, or an enzyme. (D) Indirect staining for antigens in tissues or cells uses an unconjugated antigen-specific antibody as the first reagent. This antibody is detected with the use of a second antibody (directed against the first antibody) that has been conjugated with a radionuclide, a fluorochrome, or an enzyme. The indirect technique often gives a higher signal than the direct technique, because multiple secondary antibodies (goat anti–mouse Ig in this case) can bind to one primary antibody (mouse monoclonal antibody in this case). However, the background staining for the indirect technique is also considerably higher than is seen with a primary or direct technique. *ELISA,* Enzyme-linked immunosorbent assay; *IgG,* immunoglobulin G; *RIA,* radioimmunoassay.

labeled with a radioisotope. In a fluorescence-linked immunosorbent assay (FLISA), the antiimmunoglobulin is conjugated with a fluorochrome. Ideally, the assay should include a standard curve prepared with purified specific antibody so that the results can be reported as actual concentrations, not optical densities or counts per minute.

A solid-phase immunosorbent assay can be isotype specific, sensitive, quantitative, and adapted for analysis of high numbers of samples. Its principal drawback is the potential for a false-positive result from two possible causes. Ideally, only antigen-specific antibodies from the patient's serum would bind to the immobilized antigen. However, nonspecific antibodies will also bind to the plastic well or bead containing the immobilized antigen (background binding). These nonspecific antibodies will be detected by the anti–human immunoglobulin. Background binding becomes a particular problem when a patient has abnormally high levels of serum immunoglobulin, as may occur in certain chronic infections such as malaria. For this reason, a background determination in which patient serum is added to wells without antigen should be included to estimate nonspecific uptake of immunoglobulin. The second cause of false-positive results involves the nature of the immobilized antigen. In many assays, the antigen is the pathogen or a relatively crude homogenate of the pathogen as a complex mixture of biologic products. A patient may coincidentally have antibodies that cross-react with an epitope present in the mixture. For this reason, positive results in solid-phase immunosorbent assays such as the human immunodeficiency virus (HIV) ELISA, which has been designed to be highly sensitive at the cost of being less specific, must be verified using the more specific Western blot.

Western Blot

A Western blot assay is a solid-phase immunosorbent assay in which the antigens are geographically distributed by molecular weight, so that it is possible to determine exactly which antigens are being recognized by the patient's antibodies. To accomplish this, the antigen mixture is first separated by molecular size using polyacrylamide gel electrophoresis and then transferred, or blotted, onto nitrocellulose paper. Patient serum is incubated with the paper strip, unbound antibodies are washed away, and bound antibodies are detected with anti–human immunoglobulin as in the ELISA. Because the antigens are distributed by molecular weight along the strip, the size—and therefore the likely identity—of the antigen recognized by the antibody can be identified.

The Western blot is useful for identifying false-positive reactions caused by cross-reactive antibodies that react with something unrelated in the antigen mixture. Recognition of just one antigen from a given pathogen could be attributed to chance cross-reactivity, but it is unlikely that a patient would have cross-reactive antibodies that recognize multiple antigens from a pathogen. It is more likely that such a patient has been exposed to or infected with the pathogen and has mounted a polyclonal antibody response. This is the basis for interpretation of the Western blot assay for HIV. Reactivity with only one HIV viral antigen is presumed to represent chance cross-reactivity or a very early HIV infection, before the patient has mounted a polyclonal response. Reactivity with two or more HIV antigens is interpreted as an immune response to an ongoing infection with HIV.

Agglutination and Complement Fixation

The ability of antibodies to cross link and aggregate antigens is the basis of the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA-TP). The microbes or their extracted antigens are coated onto particles such as latex beads or erythrocytes and then added to serum. Agglutination by specific antibodies is apparent on visual inspection. IgM antibodies can cross link two large particles, but IgG may be too small to bridge the distance. Therefore, anti–human IgG is often added in a second step as a "developing reagent" to agglutinate particles coated with specific patient IgG.

Agglutination cannot identify the isotype of the patient antibody. However, these assays are useful because they can be read within minutes and require only minimal laboratory resources. Results are typically reported as antibody titer, which is the maximum extent to which a sample can be diluted and still give a positive result. For example, a titer of 1:160 means that 1 part serum can be mixed with 159 parts buffer and still produce a positive reaction. Sera are typically tested in serial twofold dilutions, and titer differences between samples are not considered to be statistically different until there is a fourfold or greater difference in the titers of the two samples.

Complement-mediated lysis or complement fixation assays can be sensitive but technically challenging. In these assays, red blood cells are artificially coated with the desired antigen and then mixed with patient serum (as a source of antibodies). Fresh guinea pig serum (as a source of complement) is added. Patient antibodies that have bound to antigen on the red cells activate complement, and the red cells are lysed. The advantage of this assay is the ease of reading the macroscopically visible results. Limitations include the need for a source of biologically active complement that behaves in a consistent manner. Antigen-coated erythrocytes are unstable and cannot be stored for long periods. As would be predicted from the abilities of different immunoglobulin isotypes to activate complement, this format is excellent for measuring IgM, moderately effective for measuring IgG, and not useful for measuring IgA antibodies.

Immunofluorescence and Immunohistochemistry

A patient's specific antibodies can be identified using immunofluorescence and immunohistochemical techniques. The principle is the same as for the ELISA; the only difference is that the antigen is present in a histologic tissue section or immobilized on a slide. Examples of antigens used in this format include treponemes to look for antibodies to *T. pallidum* and virus-infected cells to look for virus-specific antibodies. Patient serum is incubated with the slide, unbound antibodies are washed away, and reactive antibodies are detected using a conjugated anti–human immunoglobulin, just as in the solid-phase immunosorbent assay. Antibodies conjugated with a fluorochrome are visualized with a fluorescence microscope. Immunohistochemistry studies use antibodies conjugated with an enzyme that deposits a precipitate onto the tissue wherever antigen is expressed. Use of an anti–human immunoglobulin that is selective for a particular immunoglobulin class makes it is easy to identify the isotype of the patient's antigen-specific antibodies.

Interpretation of immunohistochemistry and immunofluorescence findings is subjective, or at best semiquantitative, with results typically reported on a scale of 0 to 4+. Although antibodies cannot be formally quantified, an experienced laboratory technician may be able to estimate the relative quantity by determining the titer or the extent to which serum can be diluted and still give a positive test. As previously mentioned, samples should not be considered to be different unless their titers differ by at least two serial dilutions. Because assays are typically carried out with twofold dilutions, a significantly higher titer should be fourfold or greater. Appropriate controls must be included. At a minimum, a negative control should be run using the conjugated anti–human immunoglobulin alone (without addition of patient serum) to evaluate the extent to which the anti–human immunoglobulin reagent binds to the tissue. Background signal can often be reduced by using $F(ab')_2$ fragments of the anti–human immunoglobulin to prevent adsorption to tissues expressing FcR. Antibodies produced in goats or sheep are preferred, because they have a lower affinity for human FcR. It may also be appropriate to run a negative control using normal human serum in place of the patient's serum to evaluate nonspecific uptake of human immunoglobulin.

Enumeration of Antibody-Producing B Cells: The ELISPOT Assay

The enzyme-linked immunosorbent spot (ELISPOT) assay is designed to enumerate antibody-producing B cells. It can also be modified to enumerate cytokine-producing mononuclear leukocytes. To measure antibody production, B cells are allowed to settle onto a surface that has been coated with antibodies to the isotype of interest and are cultured in place for several hours. For example, to measure IgG production, the surface is coated with anti-IgG, which captures the IgG molecules as they are secreted by the B cells. The secreted and trapped B-cell products are identified by a second antiimmunoglobulin reagent that had been conjugated with a tracer, just as in the ELISA. After development, the spots are counted. Each spot represents a cell that secreted

the product of interest. To enumerate cytokine-producing cells, the surface is coated with anticytokine antibody, and the developing reagent is a second, tagged antibody to the same cytokine. A similar approach has been modified for use in the flow cytometer. Fluorochrome-tagged antigen can used to enumerate B cells with antigen-specific membrane immunoglobulin.[37] Alternatively, antigen-specific B cells can be identified as $CD19^+/CD20^-/CD27br/CD38br$ plasmablasts in the circulation after infection or vaccination.[38]

Assays to Measure Antigen

The assays described earlier can also be modified to detect antigen using antigen-specific antisera. Monoclonal antibodies are ideal reagents for these assays, because they are a reproducible source of antibodies with a well-characterized specificity. It must be recognized that these immunoassays measure antigen on the basis of its ability to interact with specific antibodies, not by its biologic activity. Unless the antibody measures a conformational epitope, it is very likely that the assay will detect both active and inactive (e.g., denatured) antigens. Soluble inhibitors may not interfere with an immunoassay but can block activity in vivo. For example, some cytokines have soluble inhibitors that block their biologic activity. An immunosorbent assay might indicate that a particular serum contained large quantities of cytokine when, in fact, because of the presence of inhibitor, very little cytokine *activity* was present.

Solid-phase immunosorbent assays typically use a sandwich format in which purified antibody to antigen is immobilized on a surface such as a plastic well and forms the bottom layer of the sandwich (see Fig. 5.4B). The patient sample containing antigen is allowed to react with the immobilized antibody. Then more antigen-specific antibody is added as the top of the sandwich. This second detection antibody is conjugated with a radiolabel, enzyme, or fluorochrome. When monoclonal antibodies are used, the capture and detection antibodies must recognize different epitopes on the antigen. Otherwise, the capture antibody will react with all of the available epitopes, leaving none for the second antibody.

Sometimes, diagnosis requires the identification of pathogens in the patient's tissue. Immunofluorescent and immunohistochemical techniques are used to locate antigens in patient tissues. For example, to look for virus, sections of patient tissues are probed with antibodies, often monoclonal antibodies, that are prepared by immunizing animals with viral proteins. The antigen-specific monoclonal antibodies can be directly conjugated with fluorochromes or enzymes and used in a so-called direct, or one-step, detection (see Fig. 5.4C).

Alternatively, the antigen-specific antibody such as a mouse monoclonal antibody is added first, followed by a conjugated anti-IgG prepared in another animal. This is a two-step or indirect assay (see Fig. 5.4D). Indirect assays often generate a stronger signal because several conjugated anti–mouse immunoglobulin antibodies can bind to each mouse immunoglobulin. However, this technique also has a higher background, and appropriate negative controls are critical for interpretation of the assay. Cells in suspension can be stained with multiple antibody specificities, each labeled with a different fluorochrome, and analyzed by flow cytometry. The flow cytometer enumerates the cells, measures the fluorescence associated with each, and discriminates among fluorochromes with different emission spectra. Control samples must be analyzed in parallel, using fluorochrome-conjugated monoclonal antibodies that are of the same isotype but are specific for irrelevant antigens, to measure background nonspecific uptake. To improve the signal-to-noise ratio and decrease nonspecific binding, FcRs on cells can be blocked with irrelevant IgG from another species and reagent antibodies can be prepared as $F(ab')_2$ fragments or both.

Immunofluorescence and immunohistochemistry can also be used to detect autoreactive antibodies that bind to antigens in autologous tissues. A direct assay is possible if the involved tissue can be sampled. Patient's autoantibodies bound to their own tissues are detected by the addition of conjugated anti–human immunoglobulin antibodies in a direct test. However, sometimes the involved tissue is not accessible, and the only option is to look for autoantibodies in the serum. This indirect technique involves incubating the patient's serum with tissue obtained from surgical samples or autopsies and using conjugated anti–human immunoglobulin to detect the patient antibodies that bind.

Measurement of Immune Complexes

Fixed tissue phagocytes in the liver and spleen are responsible for clearing complexes of antigen and antibody. However, if their capacity is exceeded, immune complexes circulate and deposit in tissues, where they activate complement and incite phagocytes to attempt to ingest the tissues to which the antibodies have bound. Diagnosis of immune complex–mediated diseases can be made by demonstrating deposits of immune complexes in the targeted tissues using immunofluorescent or immunohistochemical techniques. A second diagnostic strategy is to measure the quantity of complement components, particularly C3 and C4, in the serum, because these levels decrease when complement is activated by immune complexes. However, interpretation of the results can be difficult, because C3 is an acute-phase reactant and its levels increase during inflammatory conditions.

A third diagnostic strategy is to look for circulating immune complexes. Several approaches can be used.[39,40] Some assays depend on the ability of IgG or IgM antibodies to fix C1q when they are engaged with antigen. Serum can be incubated with immobilized antibodies to C1q to determine how much immunoglobulin coprecipitates with the C1q. Radiolabeled C1q can be added to the serum; after all the serum proteins in the size range of immune complexes have been precipitated, the amount of coprecipitating C1q is quantified. Another type of assay is based on the fact that circulating immune complexes contain bound C3b. C3b (and the attached complexes) are extracted from the serum using immobilized antibodies to C3b or the Raji cell line, which has an avid and abundant receptor for C3b. The quantity of human immunoglobulin that coprecipitates with the C3b is used as an estimate of the quantity of immune complexes present.

The various strategies for measuring immune complexes do not always give concordant results, which suggests that complexes may vary in size, composition, and biologic behavior among patients or at different stages of a disease process.

B-CELL MATURATION AND IMMUNOGLOBULIN PRODUCTION

There is not enough DNA in the human genome to encode each of the millions of antibody specificities that a person can produce when appropriately challenged. Rather than encode each antibody specificity in the germline sequence, B cells create antibody diversity by systematically mutating the genes that encode components of the antigen-binding cleft and selecting for those with the highest binding affinity. The DNA rearrangement process uses enzymes unique to lymphocytes, such as recombination-activating genes (*RAG1* and *RAG2*), as well as the full array of ubiquitous DNA repair enzymes common to all cells. B cells begin by producing IgM antibodies. With further antigen stimulation and signaling from T cells, progeny of these B cells switch to production of IgG, IgA, or IgE antibodies with the same antigen-binding site as the initial IgM.

DNA Rearrangement and Generation of Diverse Antigen-Binding Sites

The bone marrow produces about 10^9 pro-B cells per day. To become B cells, these precursors must undergo a specific series of gene rearrangements (Fig. 5.5 and Table 5.2). The first step is to rearrange the DNA to form a functional μ heavy chain. Four gene segments must be brought together to make a μ chain: the V or variable segment, the D or diversity segment, the J or joining segment, and the μ heavy chain gene. There are 50 functional gene segment choices for V, 27 choices for D, 6 choices for J, and 1 μ gene. Through random selection of these segments, 8100 ($50 \times 27 \times 6$) different IgM specificities can be generated by combinatorial diversity. One of the D gene segments is spliced to one of the J gene segments, and the resulting DJ gene product is then spliced to one of the V gene segments. Note the overlapping nomenclature: "V" is used to designate the V gene segment and also the V domains at the 5′ ends of the heavy and light chains.

The antigen-binding cleft is formed by V domains from the heavy and light chains. Each of these V domains has three hypervariable regions or CDRs. Two of the CDRs are encoded in the V gene. The third CDR is encoded by DNA that spans the splice junction between the V and J segments. Sloppiness or wobble in the splicing of these

genes inserts more variability onto the antigen-binding site through junctional diversity. DNA splicing requires cleavage, and the cut ends of the DNA are not immediately reattached to the next gene. Instead, the cut end is sealed back on itself to form a hairpin loop. Before splicing, the loop is reopened, but not necessarily at the same place. A shift in the exact site of the opening cut introduces further variability into the gene sequence. Additional nucleotides can be introduced to (or removed from) the cut end before it is spliced to the next gene to add even more diversity to the gene sequence.

These DNA rearrangements are a risky process for the B cell. Stop codons can be inadvertently introduced. Two of three times, the reading frame is shifted out of sequence and the gene no longer encodes a functional protein. Before it proceeds, the B cell must verify that the rearranged VDJ gene from the first gene encodes a functional μ chain gene. To test for functionality, the B cell attempts to pair the μ chain with a surrogate light chain and express the product on the cell membrane. It is not known how the B cell confirms that these two proteins are successfully displayed. However, if the criteria for success are met, the heavy chain gene on the other chromosome is prevented from rearranging by a process called *allelic exclusion*. If the μ chain does not display properly, the pro-B cell attempts to rearrange the other heavy chain allele.

Once the heavy chain rearrangement is successful, the pro-B cell divides, becomes a pre-B cell, and begins to rearrange the light chain (see Table 5.2). The rearrangement process for light chains is the same as for heavy chains, with the exception that light chain genes have only V, J, and constant region segments—they do not have a D segment. However, the odds of success are twofold better for light chains than for heavy chains. The κ chains are tried first; and if neither of the κ chain rearrangements is successful, the B cell can try to rearrange the λ chains. Once the light and heavy chains have been successfully rearranged to form functional membrane IgM, the cell officially becomes an immature B cell that is ready to encounter antigen (see Table 5.2).

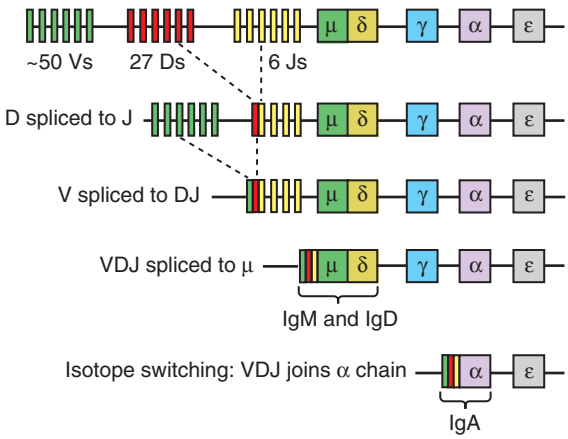

FIG. 5.5 DNA rearrangement. The scheme for rearranging heavy $CD4^+$ chain genes begins with splicing one of the D gene segments to one of the J gene segments. The DJ product is spliced to the V segment, and this is spliced to the μ chain gene. DNA encoding μ and δ chains are adjacent to each other, and the B cell can produce a single messenger RNA molecule spanning both. Through alternative splicing in posttranslational steps, the B cell produces both IgM and IgD, which are expressed on the cell's surface. The B cell also produces secreted IgM. After it receives signals from activated T cells, the B cell can switch from production of IgM to production of IgG, IgA, or IgE. The VDJ segment is spliced onto the new heavy chain genes, thereby preserving the antigen specificity of the antibodies produced.

Deletion of Autoreactive Clones

The next step is to eliminate B cells that are producing autoreactive antibodies that might injure the host. Immature B cells at this stage express only IgM on their surface and are located in the bone marrow. Antigens in this environment are likely to be self-antigens, or autoantigens. If a B cell's surface IgM binds to and is cross-linked by antigen (autoantigen), that B-cell clone is removed from the repertoire. B cells may be induced to undergo apoptosis in a process called *clonal deletion,* or they may become anergic or unresponsive to antigen. There is one potential rescue option for the autoreactive B cell. If sufficient levels of the enzyme RAG remain in the cytoplasm, the autoreactive B cells can try to rearrange a new light chain gene, which might result in a new IgM that no longer reacts with self-antigens. This process is called *receptor editing.*

Antigen Stimulation: First Signal

As the B cells leave the bone marrow, they begin to express both IgD and IgM on their membrane. To produce both isotypes concurrently, the B cell generates a long messenger RNA molecule with the rearranged VDJ region, the μ gene, and the δ gene. Through alternative splicing, the B cell can use this messenger RNA molecule to produce either IgM or IgD. This is a very different process from that used for isotype switching. The class switch from IgM to IgG, IgA, or IgE is irreversible because the B cell splices the VDJ sequence to a new heavy chain gene (γ, α, or ε) and discards the intervening DNA (see Fig. 5.5).

Production of IgD is an important threshold in the life of a B cell. Before this stage, cross-linking of surface IgM with antigen, which would have been an autoantigen from the bone marrow, led to inactivation or death. From this point forward, interaction with antigen has a stimulatory effect; moreover, the B cell's survival now depends on repeated stimulation by antigen. Naïve B cells leaving the bone marrow have about 1 week in which to locate their cognate antigen. Eighty percent of B cells fail to do so and die.[41]

Membrane IgM is the B-cell's (antigen) receptor, or BCR. When the BCR interacts with cognate antigen, membrane IgM reorganizes into lipid rafts in the B-cell membrane. The cytoplasmic tail of membrane IgM has no signaling capability on its own. Signaling is carried out by two other cytoplasmic proteins, Igα and Igβ, that associate with the IgM in the lipid raft. Like CD3 and the zeta (ζ) chain in T cells, Igα and Igβ have ITAMs. When IgM becomes cross-linked, these ITAMS are phosphorylated, which allows docking of the tyrosine kinase Syk. Syk is the B-cell equivalent of ZAP-70 in T cells. Syk and other tyrosine kinases activate the Ras–mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol-specific phospholipase C pathway, leading to increased cytosolic calcium and diacylglycerol. Ultimately, transcription factors such as nuclear factor of activated T cells (NFAT), nuclear factor kappa B (NF-κB), and activator protein 1 (AP-1) are produced.[42,43]

Coreceptors Amplify or Suppress Antigen Signaling

Antigen-mediated signaling can be greatly enhanced by the participation of several B-cell coreceptors. CD21, CD19, and CD81 form the B-cell

TABLE 5.2 Stages of B-Cell Development

CELL STAGE	PRO-B	PRE-B	IMMATURE B	MATURE B
DNA rearrangement for immunoglobulin	μ Chain being rearranged	Heavy chain complete; light chain being rearranged	Heavy and light chains complete	Isotype switch, somatic hypermutation in germinal center
Membrane immunoglobulin	None	μ Chain with surrogate light chain	IgM	IgM and IgD
Response to interaction with antigen	None	None	Receptor editing or deletion of autoreactive clones	Survival dependent on antigen stimulation

Ig, Immunoglobulin.

coreceptor complex. CD21, also known as complement receptor 2 (CR2), binds C3d. C3d is a degradation product of C3b, which accumulates on the surface of pathogens when complement is activated. While the pathogen antigens interact with membrane IgM, the C3d can cross link the CD21 on the B cells. This brings the cytoplasmic tail of CD19 into proximity with the BCR, where it can be phosphorylated by Syk and bound to Igα or Igβ. The phosphorylated CD19 ultimately activates phosphatidylinositol 3-kinase. Src family kinases linked to CD21 can also phosphorylate ITAMs in Igα and Igβ. The net effect of coreceptor involvement is to increase the concentration of signaling molecules. In a murine model, involvement of the coreceptor complex, modeled by cross-linking CD21 with antibodies, reduced the quantity of antigen needed to induce an immune response by 1000-fold.[44]

Other receptors, such as FcγRIIB, have an opposite effect and downregulate membrane IgM signaling. The cytoplasmic tail of FcγRIIB has an ITIM that inhibits the activity of ITAMs. ITIM activates a phosphatase that dephosphorylates the ITAM and interrupts the signaling pathway. As a result, when immune complexes containing antigen and IgG interact with the B cell, the enhancing signal delivered by the interaction of membrane IgM with antigen is countered by an inhibitory signal arising from the interaction of IgG with FcγRIIB.[27] This regulatory role for FcγRIIB was demonstrated by cross-linking membrane IgM with IgG anti-IgM as a surrogate for antigen. Intact IgG anti-IgM, which interacted with both membrane IgM and FcγRIIB, suppressed antibody responses. Treatment with $F(ab')_2$ fragments of the same anti-IgM, which could cross link membrane IgM but could not interact with FcR, stimulated antibody production.[45]

Second Signals and Interactions Between B Cells and T Cells

To switch to production of IgG, IgA, or IgE, B cells need to receive a signal from activated $CD4^+$ helper T cells (Fig. 5.6). Activated T cells express CD40 ligand (CD40L, or CD154), which binds to CD40 on B cells and activates the B cell. The critical role of CD40–CD40L interactions is best illustrated by the defects seen in individuals with a congenital deficiency of CD40L and a condition called *hyper-IgM syndrome*. Without CD40L, T cells cannot stimulate CD40 on B cells. B cells from affected patients produce greater than normal quantities of IgM but are unable to switch to production of other isotypes or to form germinal centers in lymph nodes. Key survival factors for B cells include B-lymphocyte stimulator (BLyS, also referred to as B-cell activating factor [BAFF]), IL-21, and APRIL (*a pro*liferation-*i*nducing *l*igand).[46]

Adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1, or CD54) and lymphocyte function–associated molecule 1 (LFA-1, or CD11a/CD18), stabilize the B cell and the T cell as a conjugate pair. The two cells may remain in contact for many hours as the T cell secretes cytokines in a polarized direction into the space between them. B cells can respond to numerous T-cell–derived cytokines, including IL-2, IL-4, IL-5, IL-6, and transforming growth factor-β (TGF-β). IL-10 and TGF-β drive the B cell to switch to the IgA isotype, whereas IL-6 induces it to become a high-rate IgA-producing plasma cell.[47] IL-4 and IL-5 promote switching to the IgE isotype.

After stimulation by the T cell, the B cell begins to divide every 6 to 7 hours and generates several thousand daughter cells. At this point, nature raises the stakes for the B cell. The B cell is doomed to die by apoptosis through a decrease in the level of BCL2 and an increase in Fas. The B cells can survive if they find antigen to interact with their surface immunoglobulin, which causes their level of $BCL\text{-}X_L$ to increase; if this does not occur, the B cell dies through Fas-mediated pathways of apoptosis. Moreover, the B cells cease expression of surface IgD and express only low quantities of IgM.

To enhance their ability to bind antigen, B cells undergo somatic hypermutation within the DNA segments coding for the antigen-binding clefts in an attempt to increase affinity for antigen. Both class switch recombination and somatic hypermutation are initiated by the same enzyme—activation-induced deaminase (AID). AID deaminates cytidine in single-stranded DNA to uracil, causing U:G mismatches that are processed as repairs, mutations, or DNA double-strand breaks.[48] B cells can introduce one mutation per 10^3 base pairs in this particular sequence,

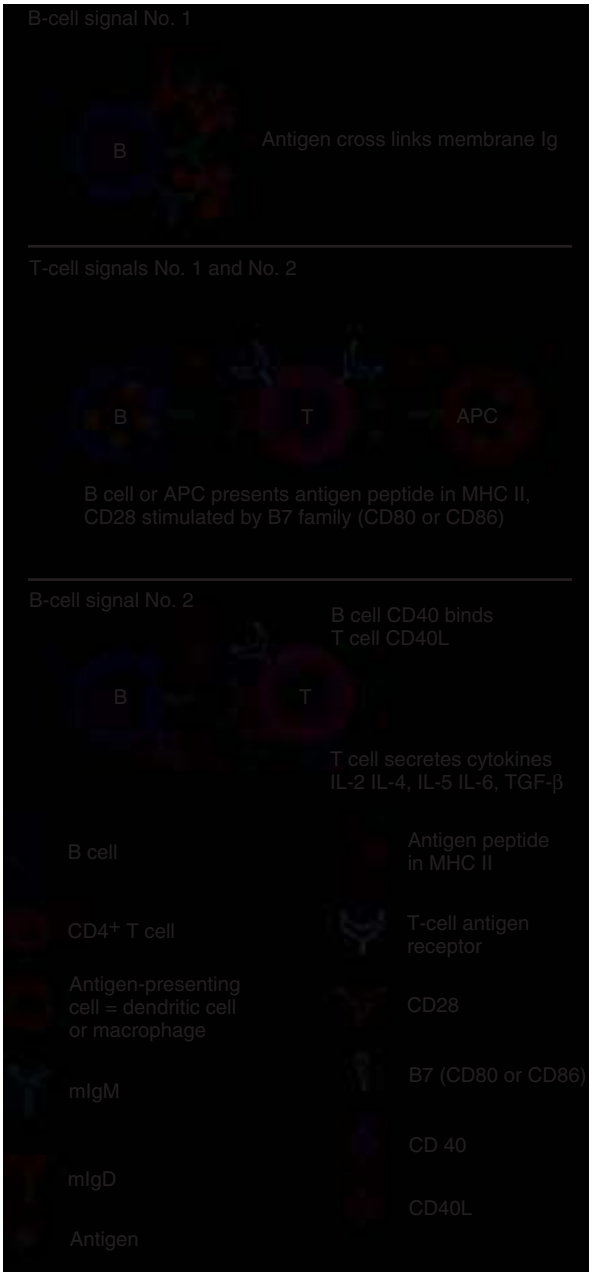

FIG. 5.6 B cells and T cells each need two signals. *Top,* The first signal for B cells comes when antigen cross links membrane immunoglobulin (Ig). If this occurs when the immature B cell is expressing only IgM, the signal is inhibitory. However, once the B cell is expressing both IgM and IgD, encounter with antigen is stimulatory. *Middle,* T cells are activated by encounter with antigen-presenting cells (APCs) such as dendritic cells. T cells need to recognize their cognate antigenic peptide expressed in a major histocompatibility complex (MHC) class II molecule, and they need a signal through their CD28 receptor. The ligand for CD28 is one of the members of the B7 family (CD80 or CD86). APCs upregulate their expression of B7 molecules when they encounter proinflammatory stimuli such as pathogen-associated molecular patterns on the antigen. This second APC signal confirms to the T cell that the antigen is "dangerous" and an immune response is needed. *Bottom,* To switch from IgM to other isotypes and to form memory cells, B cells need signals from activated T cells. These second signals include the interaction of CD40 on B cells with CD40 ligand (CD40L, or CD154) on activated T cells and various T cell–derived cytokines. *IL,* Interleukin; *MIgD,* membrane IgD; *MIgM,* membrane IgM; *TGF,* transforming growth factor.

which is 10 million–fold greater than the baseline mutation rate for somatic cells. Because continued stimulation by antigen is a prerequisite for survival, as antigen becomes scarce, B cells must compete by modifying their antigen-binding sites to generate a sequence with a higher affinity for antigen. Only those B cells making antibodies with the highest binding affinity will be able to compete for the critical survival signal provided by antigen. As a result, the average affinity of the antibody response becomes higher and higher.

Somatic hypermutation takes place within the follicular region of the lymph node or spleen in germinal centers.[49] B cells move through the basal light zone, competing for antigen expressed on follicular dendritic cells. Survivors become memory cells and plasma cell precursors.[50] Plasma cells can produce antibodies at a highly accelerated rate for a period of days, or perhaps even longer. Other B-cell progeny become memory cells and long-lived plasma cells that appear to survive for years.[51] Memory cells can sample antigen complexed with C3d and retained on the surface of follicular dendritic cells to maintain stimulation over prolonged period of time.

How B Cells Find and Activate T Cells

How does a B cell find the one-in-a-million T cell that is specific for a peptide from the same antigen? After B cells leave the marrow, they circulate through the bloodstream and pass through the lymph nodes or spleen, where they may encounter cognate antigens assembled from the periphery by dendritic cells. B cells that fail to find their matching antigen pass on to the next node. B cells that find a suitable antigen begin to express the chemokine receptor CCR7, which draws them toward chemokines produced by stromal cells in the T-cell zone.[52] Activated T follicular helper cells expressing CXCR5 are drawn, in turn, toward the chemokines produced in the B-cell zone.[53]

B cells can also increase their odds of contacting activated T cells specific for the same antigen by becoming antigen-presenting cells (APCs), which activate T cells. B cells ingest antigen and present relevant peptides to activate the very T cells they need. Unlike monocytes or dendritic cells, which pick up antigens on a random basis, B cells have an antigen-specific capture mechanism—their membrane IgM. Antigen captured by membrane IgM is brought into an endosomal compartment, degraded into peptides, and displayed at the cell surface in major histocompatibility complex (MHC) class II molecules to activate $CD4^+$ T cells. When membrane IgM is cross-linked by antigen, the B cell is activated to display even more MHC class II antigen, which further enhances its capacity to be an APC for $CD4^+$ T cells. Because the antigen was picked up with the B cell's antigen-specific membrane IgM, the B cell is presenting just the right peptides to engage T cells with the appropriate antigen specificity. The importance of B cells as antigen-specific APCs should not be underestimated. T-cell–mediated diseases can be ameliorated by the B-cell–specific monoclonal antibody rituximab, which appears to act by interfering with antigen presentation by B cells to T cells.[54] B cells also produce IL-10 and TGF-β and may be involved in the development of regulatory T cells.[55]

In addition to their ability to select the antigen-correct T cells, antigen-activated B cells can provide the necessary costimulatory molecules required to activate the T cell's CD28 receptor.[56] T cells require two signals to be activated. The first signal comes through their antigen receptor, and the second signal comes through CD28 and is typically provided by APCs: monocytes, macrophages, dendritic cells, or B cells. The two-signal requirement ensures that T cells do not mount an immune response to self-antigens. T cells have no way to discriminate pathogens from harmless antigens. If T cells were able to be activated with just one signal, they would respond to every peptide that fit their antigen receptors, and the host could be overwhelmed by inflammatory processes. It is the role of the APC to tell the T cell whether a particular antigen is "dangerous" and merits an immune response. APCs do this by expressing the B7 ligands whenever they encounter danger. There are two homologous members of the B7 family: B7-1 (CD80) and B7-2 (CD86). B7 molecules bind to CD28 and deliver the critical second signal that activates T cells and keeps them alive. T cells stimulated by a single signal (i.e., antigen without B7) undergo apoptosis.

How do the APCs identify danger? APCs identify pathogens through an assortment of receptors collectively called *pattern recognition receptors.* PRRs bind to PAMPs, such as lipopolysaccharides, peptidoglycans, lipoteichoic acids, mannans, bacterial DNA, and double-stranded RNA. When PRRs are engaged by PAMPs, the APC "recognizes" that this is a pathogen and begins to display the B7 costimulatory molecules. Cross-linking of membrane IgM, MHC class II molecules, or CD40 upregulates B7 molecules on B cells and transforms them into effective APCs.

Conjugated polysaccharide vaccines depend on the ability of B cells to serve as their own APCs to present antigen to T cells with a little bit of "bait-and-switch" (Fig. 5.7). Polysaccharides make poor vaccines, because they cannot stimulate the production of specific IgG antibodies. B cells producing antibodies to polysaccharides cannot make the switch from IgM to IgG and cannot generate memory cells, because there are no specific T cells to provide the necessary second signals. There are no polysaccharide-specific T cells because T cells are programmed to recognize peptides (i.e., protein fragments) displayed in MHC molecules. Polysaccharides do not generate peptides, so it is not possible to have polysaccharide-specific T cells. Conjugate vaccines are designed to "fool" T cells into providing help to polysaccharide-specific B cells. A conjugate vaccine links the polysaccharide antigen to a protein, such as tetanus toxoid, for which the host already has specific T cells. Polysaccharide-specific B cells internalize the conjugate using their polysaccharide-specific membrane IgM. The conjugate is degraded, and peptides from the conjugated tetanus toxoid are displayed in the B cell's MHC class II molecules. T cells specific for tetanus toxoid peptides pair up with this polysaccharide-specific B cell and provide the necessary help to drive isotype switching and formation of memory cells.

T-Cell–Independent Antigens

B cells typically require two signals to become efficient antibody-producing cells. The first signal comes from antigen, and the second signal usually comes from T cells. Some antigens can provide a type of second signal to the B cell without the participation of T cells. Examples of T-cell–independent antigens include the polysaccharides of *Haemophilus influenzae* type b, peptidoglycans, *Staphylococcus* protein A, and many viruses. T-cell–independent antigens typically have highly repetitive motifs and a flexible structure. Their repetitive structure and flexible backbone allow them to interact with multiple membrane IgM molecules that aggregate into a single focus and deliver a potent signal to the B cell. Many T-cell–independent antigens are pathogens that become coated with C3b, which degrades to C3d. C3d cross links CD21 on B cells, which shares a lipid raft with membrane IgM and further amplifies the signal to the B cell. B cells activated by T cell–independent antigens are still dependent on cytokines but may receive these from non–T-cell sources, such as macrophages. New data also suggest that neutrophils, stimulated by IL-10 from splenic sinusoidal endothelial cells, can also induce immunoglobulin class switching, somatic hypermutation, and antibody production by activating mantle zone B cells through a mechanism that involves the cytokine IL-21 as well as BAFF and APRIL, which are two Toll-like receptor–inducible B cell–stimulating factors related to the ligand for the T-cell molecule CD40.[57]

B cells in the marginal zone of secondary lymphoid tissue tend to express membrane IgM specific for T-independent antigens, particularly carbohydrate antigens. These B cells respond to bloodborne pathogens such as bacteria that are trapped by macrophages located around the marginal zone. Because they do not require the participation of T cells, marginal-zone B cells provide a pseudoinnate response to bacteremia by quickly releasing IgM antibodies.

Downregulation of Antibody Production

Once the antigen is cleared, antibody responses decline. There are several mechanisms that shut down an antibody response. Sequestration of antigen deprives B cells of the activating signal they require to survive.[58] Immune complexes formed between antigen and excess IgG antibody deliver inhibitory signals to the B cell by interacting with FcγRIIB, as described previously.[45] Anti-idiotypic antibodies spontaneously arise during an immune response and may have a role in both upregulating and downregulating immune responses.[59] Anti-idiotypic antibodies are autoantibodies that react with epitopes in or near the antigen-binding site of an antibody. Because they recognize epitopes unique to a particular

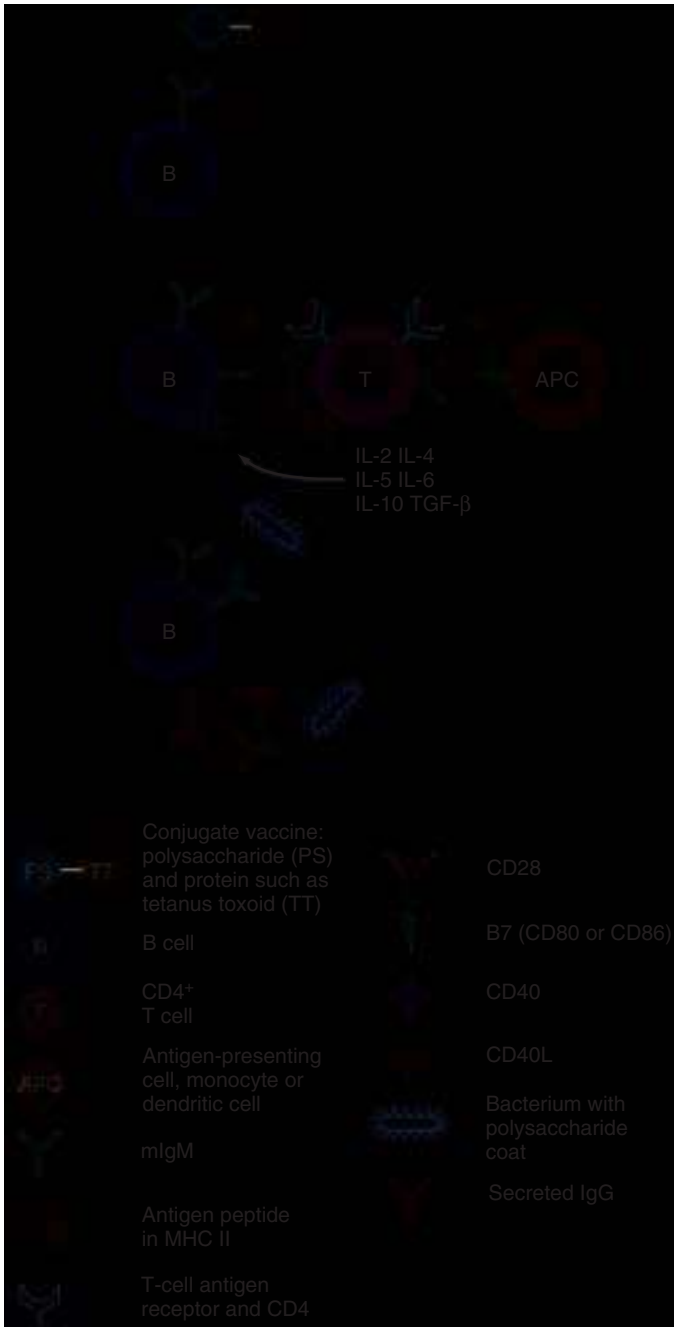

FIG. 5.7 Conjugate vaccines. The conjugate vaccine is designed to induce production of immunoglobulin G (IgG) antibodies to polysaccharide (PS) antigens, which would normally be unable to induce IgG antibodies. Polysaccharides do not induce IgG antibodies because antigen-presenting cells (APCs) cannot degrade them into the peptides that must be presented in major histocompatibility complex (MHC) class II molecules in order to stimulate antigen-specific T cells. The vaccine consists of the PS antigen linked to a protein antigen, such as tetanus toxoid (TT), to which the host has primed antigen-specific T cells. B cells that are able to make PS-specific antibodies ingest the vaccine through their PS-specific membrane IgM. The B cells degrade the vaccine molecule and present the TT peptides in their MHC class II molecules to a TT-specific activated $CD4^+$ T cell. The T cell can be activated by the B cell or by prior interaction with a dendritic cell displaying TT peptides. The activated TT-specific T cell provides PS-specific B cells (displaying peptides from TT) with the necessary help in the form of CD40–CD40L interactions and cytokines. Having received the necessary signals from a T cell, the PS-specific B cell can switch to production of IgG and can generate long-lived memory cells. *CD40L,* CD40 ligand; *IL,* interleukin; *mIgM,* membrane immunoglobulin M; *TGF,* transforming growth factor.

clone, they can modulate B cells in a highly selective manner. Anti-idiotypes interacting with membrane IgM, before the immature B cell expresses IgD, can suppress antibody production. Declining numbers of T cells also help temper the antibody response. With persistent activation, T cells begin to express both Fas and its ligand, FasL. Reciprocal interactions between activated T cells expressing both Fas and FasL lead to mutual apoptotic death.[60] Late in an immune response, activated T cells also begin to express cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 binds the B7 costimulatory molecules with a much higher affinity than does CD28.[61] Whereas CD28 sends a stimulatory signal, CTLA-4 sends an inhibitory signal to the T cell, so the net effect on the T cell is to suppress its activity. Finally, as antigen becomes scarce, fewer T cells and B cells receive the antigen signals necessary for continued activation and survival.

B1 Cells

The "usual" B cells discussed until now are called B2 cells to distinguish them from B1 cells. B1 cells are located primarily in the peritoneal and pleural spaces[62] and have a unique surface marker, CD5. B1 cells appear to regenerate continuously in the periphery rather than in the bone marrow. They produce only IgM, which suggests that they do not receive help from T cells. Their antigen-binding clefts are encoded directly by germline sequences without further modification, and the antigens they recognize tend to be microbial polysaccharides and effete or denatured host proteins. Antibodies produced by B1 cells may serve a housekeeping function by facilitating the clearance of cellular debris and denatured proteins.[63] B1 cells are thought to be the source of the many "natural" antibodies to microbial antigens, particularly those found on normal gut flora. These antibodies are present in low levels even in individuals who have not been deliberately immunized. Animals that have been raised in "germ-free" environments have low levels of circulating natural antibodies that react with a variety of commensal organisms, suggesting that B1 cells may be part of the endogenous immune system.

ANTIBODY-MEDIATED PATHOLOGY

Gell and Coombs Classification of Hypersensitivity Responses

Hypersensitivity reactions are immune responses that cause tissue injury and morbidity for the host. In some cases, an aberrant autoimmune response is specifically directed to host antigens and host cells. In many other cases, an exuberant immune response to an infectious agent injures host cells that are innocent bystanders. The Gell and Coombs classification divides hypersensitivity reactions into four types based on their underlying mechanism of action.[64] Types I, II, and III are mediated by antibodies and are discussed here. Although few diseases can be uniquely attributed to a single Gell and Coombs class, the classification scheme is a useful foundation for understanding pathogenetic mechanisms. It should also be noted that the term *hypersensitivity reaction* is often used, in a potentially paradoxical manner, to describe defense mechanisms that are actually beneficial to the host. For example, granulomas formed in response to tuberculous organisms help to contain the bacillus and protect the host, yet they are referred to as a type IV hypersensitivity mechanism.

Type I Hypersensitivity

Type I reactions involve mast cells. Mast cells contain preformed stores of histamine and heparin. They also produce leukotrienes, prostaglandins, cytokines such as tumor necrosis factor-α, and proteases. Local release of mast cell contents causes wheal-and-flare reactions, urticaria, and hives. Massive simultaneous degranulation of large numbers of mast cells throughout the body causes anaphylaxis, resulting in reduced blood pressure, a loss of fluids through permeable vessel walls, and constriction of smooth muscle. Mast cells can be degranulated by cross-linking of IgE antibodies held in FcεRs on the mast cell surface. This can be carried out by antigens with multiple repetitive epitopes or by antibodies to the IgE molecules or the FcεR. Opioids and contrast dyes can also degranulate mast cells without acting through IgE or the FcεR. As a result, the responses they trigger are referred to as *anaphylactoid* reactions. Fragments from the third and fifth complement proteins (C3a and C5a) can also degranulate mast cells, and these molecules are referred to as

anaphylatoxins. The clinician struggling to deal with IgE-mediated reactions to harmless substances such as penicillin or ragweed recalls with difficulty that type I responses have a beneficial role in the defense against intestinal parasites.

Type II Hypersensitivity

Type II reactions involve antibodies reacting with antigens on the surface of host cells. Cells decorated with antibodies may be lysed by activated complement or phagocytosed by neutrophils or monocyte-macrophages. Autoantibodies may recognize self-antigen on host cells. For example, autoimmune hemolytic anemia can be caused by antibodies to red blood cell antigens. Antibodies can also target external antigens that have bound to host cells. For example, patients with antibodies to penicillin can develop hemolytic anemia when penicillin binds to their red blood cells.

Origin of Autoreactive Antibodies

It may be helpful, at this point, to review the mechanisms that prevent the development of autoimmunity and how these processes can be circumvented. The immune system makes every effort to remove autoreactive B cells. Early in their development, B cells are induced to undergo apoptosis or to become unresponsive if they encounter cognate antigen. This reaction occurs during the window of vulnerability that persists while immature B cells express IgM and before they express IgD. Such B cells are likely to be situated in the bone marrow, or in early transition into the periphery, and any antigens they encounter are likely to be self-antigens. As B cells move into the periphery, where they are likely to encounter foreign antigen, they lose their IgD and interaction with antigen becomes an activating, life-prolonging stimulus. However, central induction of tolerance appears to be only partially effective, because virtually all people have circulating B cells that can be driven, in vitro, to produce antibodies reactive with autoantigens.

The nature of antigen signaling also appears to dictate whether a B cell will become responsive or anergic. Stimuli that tend to induce anergy include persistent stimuli, oligovalent antigens, low-affinity interactions, and immune complexes that can interact simultaneously with ITIM-containing FcγRIIB on the B cell. It is important to recognize that mature B cells in germinal centers undergo somatic hypermutation and generate new antigen-binding sites well after they are no longer vulnerable to the regulatory mechanisms that apply to pre-B cells in the bone marrow.

Control of B cells is imperfect, so much of the responsibility for preventing autoimmune reactions falls on the T cells.[65] T cells maturing in the thymus are carefully examined for their ability to bind to autologous antigens. A unique system allows thymic epithelium to express a variety of proteins normally found only in extrathymic tissues. The T cell is tested against a surprisingly wide repertoire of self-antigens while still within the thymus. Immature T cells that bind with high affinity to autologous antigens are deleted by a process called *negative selection*. After they leave the thymus, T cells cannot be activated by cognate antigen alone; they require a second signal transmitted through CD28, typically from APCs. T cells that receive a signal through their antigen receptor without an accompanying signal through CD28 are induced to undergo apoptosis. CD28 signaling is accomplished by B7 costimulatory molecules displayed on APCs. APCs upregulate the expression of B7 molecules when they recognize danger signals or PAMPs on a pathogen. APCs displaying peptides from host antigens typically do not express B7 molecules. However, in the course of an infection, an APC could display autologous peptides at a time when it has also been stimulated by proinflammatory cytokines to express B7 costimulatory molecules. Therefore during an infection or other inflammatory condition, T cells recognizing peptides from self-antigens may be activated and may, in turn, provide help to autoreactive B cells. This is one of the mechanisms postulated to lead to autoimmune responses.

Another possible mechanism leading to autoimmunity is molecular mimicry, which involves pathogen-derived antigens that closely resemble host antigens. Examples of molecular mimicry include cross-reactive epitopes found on the M protein of *Streptococcus pyogenes* and also on proteins in the myocardial sarcolemma,[66] and cross-reactive peptides found in both *Trypanosoma cruzi* and human neurons.[67] The first peptide recognized as a result of molecular mimicry may not trigger an autoimmune disease, but immune responses tend to expand to other epitopes on the same protein in a process called *epitope spreading*.[68] Epitope spreading is thought to occur when B cells producing antibodies to one epitope on a protein take up that protein through their membrane IgM and process it to antigenic peptides. The B cell displays those diverse peptides, in large numbers, along with costimulatory signals presumably induced by a coexistent inflammatory stimulus. This activated B cell, in its role as an APC, activates T cells that recognize other epitopes from the same protein. This expands the repertoire of CD4 cells that are activated and allows them to help a still wider array of B cells.[69] Eventually, this expanding repertoire can include autoreactive T cells and B cells whose specificity leads them to injure host tissues.

Type III Hypersensitivity

Type III hypersensitivity reactions are inflammatory responses triggered by soluble immune complexes that deposit in various tissues. Phagocytes try to ingest the immune complexes bound to tissues. Although tissues decorated with complexes are too large to be ingested, the phagocytes nonetheless try, and in the process they release injurious proteolytic enzymes and proinflammatory cytokines. Immune complexes can also activate complement, which deposits on the cell surface. Host cells usually protect themselves from complement attack with proteins that block formation of the membrane attack complex and accelerate the inactivation of complement components. However, when immune complexes activate complement through the classical pathway, the host may be unable to produce inactivator proteins at a sufficient rate. The site of type III hypersensitivity reactions depends entirely on where immune complexes deposit; the antigenic specificity of the antibodies is irrelevant. For example, complexes of antibodies and hepatitis antigens cause vasculitis when they deposit in blood vessel walls and glomerulonephritis when they deposit in the kidney.

Infectious diseases are commonly associated with type III hypersensitivity reactions because the infection generates a continuous source of antigen in large quantities that can be incorporated into immune complexes.[70] When antigen is in excess, each antibody can bind its own antigen, and the complexes are small. When antibody is in excess, antigen is covered with antibody, and complexes are still small. However, when antigen and antibody are present in near-equivalence, antibodies will cross link to contiguous antigen molecules and the ensuing lattice forms a large immune complex. Antibodies with low affinities release antigen quickly and tend to form small complexes regardless of their relative abundance.

Rheumatoid factors are antibodies that react with human (autologous) IgG and are a frequent component of immune complexes. These naturally occurring autoantibodies are found in almost all people. Sequences coding for many rheumatoid factors are present in the germline sequence and are expressed with little DNA rearrangement.[71,72] Their preservation in the germline DNA sequence suggests that these antibodies may have an immunoregulatory or housekeeping role.[73] Antiimmunoglobulins that react with epitopes in or near the antigen-binding site may emulate antigen signaling and have either positive or negative effects on antibody production by B cells.[74,75] They may also facilitate the clearance of effete immunoglobulin molecules.[76] In cases of chronic infection or inflammation, levels of rheumatoid factors can become quite elevated and contribute significantly to immune complexes that cause tissue injury. Cryoglobulinemia is a vasculitis triggered by complexes of rheumatoid factors and IgG that deposit preferentially in sites of reduced body temperature.

Hypergammaglobulinemia

Chronic infections such as malaria, endocarditis,[77] trypanosomiasis,[78] HIV,[79] and infections associated with cystic fibrosis[80] increase circulating immunoglobulin levels well above the normal range. The mechanism is thought to be bystander activation of B cells, because few of the antibodies produced are specific for antigens associated with the infectious agent. Epstein-Barr virus is also associated with elevated immunoglobulin levels, because the virus infects and activates a wide array of B cells, which then produce antibodies of diverse (polyclonal) specificities.[81]

Sera with very high immunoglobulin levels have higher levels of nonspecific binding in assays for specific antibodies. This high background can lead to a false-positive reading if the appropriate background subtractions are not done. Hypergammaglobulinemias are not associated with any defined pathologic process, with the exception of hyperimmunoglobulinemia D and the inherited periodic fever syndrome, in which overproduction of IgD may trigger chronic inflammation.[82]

IMMUNODEFICIENCIES

Immunoglobulin A Deficiency

Among the inherited immunodeficiencies, defects involving B cells are far more common than those involving T cells or phagocytes. The most common inherited immunodeficiency is selective IgA deficiency, which affects 1 in every 300 to 700 individuals. There are incompletely penetrant autosomal dominant and recessive modes of inheritance.

Many IgA-deficient people are relatively healthy and asymptomatic, and the true prevalence of this disease was unappreciated until serum IgA levels began to be measured systematically in blood donors. Symptoms may be minimized by compensatory transport of IgM into secretions.[6] A few IgA-deficient individuals develop recurrent sinopulmonary infections, atopy, autoimmune disorders, and malignancies. Their allergies are often directed toward dietary antigens, and it is postulated that the atopy occurs because they are unable to block absorption of environmental antigens from gastrointestinal surfaces. Individuals with the most morbidity often have a combined deficiency of both IgA and one or more of the IgG subclasses, particularly IgG2 or IgG4. Some of them go on to develop common variable immunodeficiency syndrome.

People with congenital IgA deficiency are potentially at risk for anaphylactic reactions to IgA in intravenously administered preparations of immunoglobulins or in unwashed packed red blood cells.[83] Immunoglobulin replacement does not benefit patients with IgA deficiency for two reasons. First, the preparations are IgG and do not contain IgA. Second, even if the replacement did contain IgA, intravenous IgA antibodies would not be transported into secretions. Only IgA2 antibodies produced in the submucosal lymphoid tissue are transported across epithelial barriers into the secretions.

Agammaglobulinemias

Children with inherited profound antibody deficiencies remain well for the first 6 to 9 months of life because of transplacentally acquired maternal IgG antibodies. The half-life of IgG is approximately 3 weeks, and it is not until 6 to 9 half-lives, or about 6 months, after birth that maternal IgG falls below protective levels. Thereafter, these children begin to develop infections such as sinusitis, otitis media, and pneumonias. *S. pneumoniae, H. influenzae,* meningococci, and *Mycoplasma* species are particularly common infectious agents.[84] By the time the underlying etiology is recognized and treated, these children may have already developed irreversible bronchiectasis. Intestinal infections with *Salmonella, Shigella, Campylobacter, Giardia,* and rotavirus are also common.[85,86] Rheumatologic symptoms occur in 10% to 30% of these children.[87] These children are prone to septic arthritis from common bacteria. They may also develop synovitis in the absence of infectious agents, and they are uniquely vulnerable to a chronic meningitis with enterovirus that often proves fatal.[88] Vaccination with inactivated agents is futile, because they cannot produce the desired antibodies. Live vaccines are to be avoided, because vaccine-induced poliomyelitis has occurred in patients with agammaglobulinemia.[89]

Many of the conditions that affect B-cell maturation and antibody production are X-linked. The most severe is X-linked agammaglobulinemia, which is caused by a defect in one of the cytoplasmic signal-transducing kinases in B cells. The defective protein is Bruton tyrosine kinase (Btk), and the condition is also called Bruton agammaglobulinemia. Without Btk, B cells are arrested at the pre-B stage and cannot develop into surface immunoglobulin–expressing cells. Many types of mutations of this gene have been described, but there is no apparent correlation between the specific mutation and the severity of the disease. Affected boys have less than 100 mg/dL of IgG and no serum IgM or IgA. B cells are virtually absent from bone marrow or periphery, but T cells are normal.

Hyper–Immunoglobulin M Syndrome

Hyper-IgM syndrome is usually X-linked, although there is a form that affects girls. These children have the same recurrent pyogenic infections as are seen in boys with X-linked agammaglobulinemia. Patients with hyper-IgM syndrome are also uniquely susceptible to *Pneumocystis* pneumonia. They have normal numbers of circulating B cells, low levels of IgG and IgA, and greater than normal levels of IgM. The defect is in their T cells, not B cells. They lack the CD40 ligand (CD154) that is typically expressed on the surfaces of activated T cells. Without this molecule, activated T cells cannot bind to CD40 on B cells and induce isotype switching. Therefore, the B cells continue to produce IgM but they do not switch to IgG or IgA and do not exhibit the affinity maturation that is characteristic of a secondary response. As might be predicted, the lymph nodes of these patients are populated with cells but have no organized germinal centers. Their susceptibility to *Pneumocystis* pneumonia may reflect the inability of their T cells to interact with CD40 on monocytes and activate their full microbicidal potential. These children are also prone to autoimmune hemolytic anemia, thrombocytopenic purpura, and recurrent neutropenia.[84]

Common Variable Immunodeficiency

The onset of common variable immunodeficiency is typically between 15 and 25 years of age,[90,91] which is strikingly later than with the congenital agammaglobulinemias. Although the late onset suggests an acquired etiology, there is a familial pattern that supports a genetic predisposition. These patients often, but not always, have normal numbers of B cells that express surface immunoglobulins but produce only very small quantities of circulating secreted immunoglobulin.[92] The underlying cause or causes are not yet well defined, but the defect may lie with the T cell.[93] Patients with common variable immunodeficiency are prone to malabsorption syndromes and autoimmune disorders. Some develop a sarcoid-like picture.[94] They are also at increased risk for gastrointestinal malignancies and lymphomas.[95] Relatives of patients with common variable immunodeficiency have a higher than normal incidence of IgA deficiency,[96] autoimmune disorders, and malignancies.[97]

Immunoglobulin G Subclass Deficiencies

Subclass deficiencies are an uncommon cause of susceptibility to infection. Isolated deficiency of IgG1 leads to significant morbidity, because this subclass dominates the IgG response; however, this condition is rare. IgG2 subclass deficiencies are often seen in combination with defects in IgG4, IgE, or IgA. Patients who are deficient in IgG2, IgG3, or IgG4 sometimes have recurrent bacterial infections,[98] but usually they do not.[99] If an IgG subclass deficiency is suspected, verification that the patient is deficient in the ability to produce functional antibody should be obtained before immunoglobulin replacement is proposed. This can be done by measuring the quantities of antibody before and after a booster vaccine for antigens such as diphtheria or tetanus.

Selective Immunodeficiencies

A few immunodeficiencies affect only the response to particular pathogens. Individuals with X-linked lymphoproliferative syndrome (Duncan syndrome) are unable to mount an adequate response to Epstein-Barr virus. Their B cells remain infected with the virus and continue to proliferate.

Another example of a pathogen-specific immunodeficiency is seen in people who are missing the VκA2 gene segment. This gene is often involved in the production of antibodies to *H. influenzae.* Presumably, the sequence of this V gene codes for an antigen-binding site that closely matches epitopes on the bacteria. Many members of the Navajo tribe are missing this V segment, and those who are without it are more prone to infections with *H. influenzae.*[100]

Combined T-Cell and B-Cell Defects

Children with severe combined immunodeficiency are unable to generate mature B cells or T cells. They are susceptible to all types of pathogens, including pyogenic bacteria, viruses, fungi, and opportunistic infections. Wiskott-Aldrich syndrome is an X-linked disease characterized by thrombocytopenia, severe eczematoid dermatitis, and deficient T- and B-cell responses. Patients have normal numbers of T and B cells, but a

defect in signal transduction impairs their ability to mount antigen-directed antibody responses. IgM and IgG levels are low, but IgE and IgA levels are high.[84] These children are vulnerable to infections with *S. pneumoniae* and *H. influenzae*, and may need immune globulin prophylaxis. Children with ataxia-telangiectasia have defects in DNA repair mechanisms that ultimately affect their immunoglobulin genes. Immunoglobulin replacement therapy should be given to children with demonstrable defects in their ability to generate antibodies to childhood vaccines.[84,101]

Malignancies

Antibody deficiencies may be seen in patients with chronic lymphocytic leukemia, multiple myeloma, or Waldenström macroglobulinemia.[102] These deficiencies may be caused by decreased numbers of B cells and suppression of normal antibody production by cellular elements of the tumor or their products.[103]

THERAPEUTIC USES OF ANTIBODIES

Passive Immunization

Passive immunization is the administration of immunoglobulin prepared from individuals known to have high levels of antibodies to the infectious agent in question. It can be used for an immunocompromised host if the ability to generate a sufficient immune response to vaccination is in doubt. For example, immunosuppressed allograft recipients may need cytomegalovirus immune serum after inadvertent exposure to this virus.

Passive vaccination is also used if a patient cannot make antibodies in sufficient time to protect against disease. For example, *Clostridium tetani* in a contaminated wound can produce lethal quantities of toxin long before an unvaccinated host could make neutralizing antibodies to the toxin. In such a case, the patient should receive tetanus immune globulin. The patient should also be vaccinated to protect against tetanus in the event of future injuries. Simultaneous administration of antibodies (hyperimmune serum) and antigen (toxoid vaccine) will not prevent the development of an adequate immune response. In fact, dendritic cells may store immune complexes and use them to stimulate B cells over long periods. The patient who has been previously vaccinated for tetanus should have some circulating antibodies to tetanus toxin, and a booster tetanus toxoid vaccine will trigger a rapid secondary response with production of large quantities of additional toxin-specific antibodies.

Another indication for passive vaccination is antivenin for snakebite. It is not practical, and indeed may be dangerous, to immunize human volunteers with the antigen. Because individuals who have become immune through natural exposure are rarely available, it is necessary to use antisera prepared from immunized animals. Antibodies from other species are adequate because their major role, in this case, is to block the binding of toxins to cell receptors. The limitation of nonhuman immunoglobulins is that they can trigger an immune response and a type III hypersensitivity reaction, particularly with repeat administration.

Intravenous Immune Globulin Replacement

Individuals with agammaglobulinemia or hypogammaglobulinemia need lifelong replacement with antibodies that can protect them against the diverse infectious agents they will encounter in ordinary life activities. Immune globulin replacement is unlikely to be necessary until IgG levels fall to less than 200 mg/dL. Even with low overall levels of IgG, some patients may still make adequate levels of specific antibodies. This can be readily assessed with the use of clinically available tests that measure response to vaccines such as tetanus toxoid, *H. influenzae* type b toxoid conjugate, or hepatitis B. Patients with agammaglobulinemia typically require 300 to 400 mg/kg of immune globulin every 3 to 4 weeks. Preparations specifically designed for intravenous administration are available. Intravenous immune globulin (IVIG) must be free of aggregated immunoglobulin, which can act as an immune complex and trigger type III hypersensitivity reactions. The life span of infused IgG is about 21 days. With repeated infusions at 3- to 4-week intervals, the trough levels slowly rise. The goal is to ensure that levels do not drop lower than 700 to 800 mg/dL. Infusions may be accompanied by fever, chills, myalgias, headache, and nausea, but these tend to become less frequent after repeated infusions.[101] Numerous IVIG preparations are currently available, and their use in primary humoral deficiencies has been reviewed by Schroeder and Dougherty.[104]

After its success was demonstrated in treating infectious diseases, clinicians began administering IVIG for inflammatory and autoimmune conditions whose cause was unknown but was speculated to be of infectious origin. IVIG is strikingly effective treatment of idiopathic thrombocytopenic purpura and Kawasaki disease. However, IVIG is currently being used to treat a wide range of hematologic, dermatologic, and neurologic disorders.[105] It is still not understood how IVIG exerts its immunomodulatory effects in these diseases. Indeed, they represent such a wide variety of pathogenetic processes that it is likely that IVIG may work through many different mechanisms.[105,106] IVIG should contain antibodies that neutralize bacteria, toxins, or superantigens that might be responsible for the disease. IVIG preparations are made from the plasma of at least 1000 and up to 100,000 donors. The array of antigen-binding sites thus represents essentially the entire human repertoire. Within this repertoire, there may be antiidiotypic antibodies that can downregulate pathologic autoimmune responses in certain patients.[107] IVIG infusions also appear to downregulate production of inflammatory cytokines, possibly because of cytokine-specific antibodies.[108,109] In cases in which antibody-coated host cells are attacked by the immune system, IVIG may slow the process by blockading the reticuloendothelial system. Because the half-life of IgG is influenced by the serum concentration, raising the serum level of IgG with IVIG may accelerate the clearance of autoantibodies. The doses of IVIG required for immunomodulation are four to five times higher than are used for replacement therapy in humoral immunodeficiency, which suggests that some of the effects of IVIG are not mediated through the traditional effector functions of antibodies.[105,106] The antiinflammatory activity of IVIG is related to its content of sialylated IgG molecules.[110] Sialylated IgG binds to type II FcγRs such as DC-SIGN and CD23, leading to the production of IL-33. This leads, in turn, to upregulation of the inhibitory FcγRIIB on macrophages, increasing their activation threshold, and suppressing inflammation.[110] The effect of IVIG may not even be due to antibodies at all but rather to other serum proteins that are present in trace quantities. For example, CD4, CD9, human leukocyte antigen molecules, and cytokines are all present in low levels.[111,112]

Monoclonal Antibodies

Uniform and reproducible preparations of antibodies are also used as therapeutic drugs in vivo and as reagents in vitro.[113] To produce monoclonal antibodies, an animal, typically a mouse or rat, is immunized with the desired antigen. B cells from the immunized animal are fused with malignant B cells that do not produce their own immunoglobulin, with the goal of producing a hybridoma that proliferates indefinitely and produces IgG at a high rate. The fused cells are distributed, one to a well, and allowed to proliferate. The supernatant is assayed for specific antibody, and clones producing high levels of desirable antibody are selected and propagated indefinitely. In recent years, the technology for making human monoclonal antibodies has improved tremendously such that it is possible to clone the variable regions from individual B cells into expression vectors that allow unlimited production of the desired immunoglobulin. Advances in technology combined with improved production practices have led to a biologics revolution in medicine as dozens of monoclonal antibodies have now been licensed for therapy of cancer and inflammatory diseases.[113]

Although this revolution has largely bypassed the treatment of infectious diseases for multiple reasons, including microbial antigen variability, the availability of conventional antimicrobial therapy and the fact that antibody therapies tend to be effective only when used early in infection has spawned great interest in developing antiinfective monoclonal antibodies. The first monoclonal antibody approved by the US Food and Drug Administration for prevention of infection was palivizumab for respiratory syncytial virus (RSV) in 1998.[114] There are now approved monoclonals for RSV, inhalational anthrax, *Clostridioides difficile* (formerly *Clostridium difficile*), and HIV-1. Clinical trials are underway for additional monoclonal antibodies for infections with RSV and HIV-1, as well as cytomegalovirus, hepatitis B and hepatitis C viruses, *Staphylococcus aureus, Pseudomonas aeruginosa,* herpes simplex

virus type 2, influenza A, Ebola virus, rabies, Shiga toxin–producing *Escherichia coli* hemolytic-uremic syndrome (STEC-HUS), and tuberculosis.[115,116] Promising monoclonals for immunotherapy of Zika virus infection are under development.[117] The challenges of producing antiinfective monoclonals have been well reviewed,[115,116,118] particularly the challenges presented by HIV-1.[119,120] Other approaches for monoclonal antibody treatment of HIV-1 include engineering bispecific antibodies that recognize HIV envelope proteins on the surface of infected cells and recruit effector cells.[121,122]

Although native immunoglobulins have significant therapeutic activity, there are measures that can be used to enhance their effectiveness, such as conjugating them to toxins or radionuclides.[123–125]

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The complete reference list is available online at Expert Consult.

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6 Cell-Mediated Defense Against Infection

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SHORT VIEW SUMMARY

- T lymphocytes represent the major constituent of cell-mediated defense against infection and express an antigen-specific and somatically recombined T-cell receptor.
- Phenotypically diverse T-cell subsets can be classified on the basis of T-cell receptor and coreceptor expression (i.e., CD4 and CD8), and undergo regulation by transcription factors to promote cytolytic, helper, regulatory, and memory functions.
- Antigen-specific T-cell memory can persist for many years in the absence of microbial antigen exposure and facilitate more rapid clearance of microbes upon secondary exposure.
- T-cell lymphopoiesis occurs primarily in the thymus and includes positive and negative selection steps to ensure T-cell binding to antigens presented by major histocompatibility complex (MHC) molecules and to exclude auto-reactive T-lymphocytes.
- All cells express MHC class I molecules, and MHC class I molecule–antigen complexes activate cytolytic CD8 T cells that play a central protective role against many viral pathogens. Antigen processing and loading on MHC class II molecules is a primary function of a heterogeneous group of antigen-presenting cells that activate CD4 T cells that play a central protective role against intracellular bacterial as well as fungal pathogens. Cooperation between CD4 and CD8 T-cell activity, and in broader terms between cell-mediated and humoral immunity, is a hallmark of immune responses against many infectious agents.
- The recognition of extracellular and intracellular pathogen-encoded molecular patterns by germline-encoded receptors links innate immune activation with cell-mediated defense against infection.
- Primary immunodeficiency disorders illustrate the functional role of molecular and cellular constituents in cell-mediated defense against infectious agents.

The principal function of the mammalian immune system is to combat infectious diseases. Immune responses to microbial pathogens are divided into those mediated by cells (i.e., cell-mediated immunity) and those mediated by antibodies (i.e., humoral immunity). Although innate and adaptive immune cells both contribute to cell-mediated immune responses, T lymphocytes represent the central player in this process by providing antigen specificity, by orchestrating antimicrobial activities of innate immune cells that generally lack antigen specificity, and by killing microbially infected host cells.

The induction of T-cell–mediated responses relies primarily on dendritic cells (DCs) that traffic from portals of microbial infection to peripheral lymphoid organs (e.g., lymph nodes) to present antigens to T cells and to provide contextual information (Fig. 6.1A).[1] T cells express T-cell receptors (TCRs) to detect pathogen-derived peptides presented on major histocompatibility complex (MHC) molecules. The somatically rearranged TCR complex confers antigen specificity to individual T lymphocytes. The primary encounter of naïve T cells with microbial antigen results in their activation, proliferation, and differentiation into effector T cells that migrate to the portal of infection (Fig. 6.1B), and exert T-cell effector functions to facilitate pathogen eradication (Fig. 6.1C). Effector T cells undergo contraction and long-term memory T cells emerge. Our understanding of T-cell responses to microbial pathogens has increased remarkably, as has our appreciation for the complexity of cell-mediated immune responses. This chapter summarizes current knowledge regarding T-cell responses to microbial pathogens.

T-CELL SUBSETS AND PHENOTYPIC DIVERSITY

Mammalian T lymphocytes are categorized on the basis of TCR and coreceptor expression. Most T lymphocytes implicated in antimicrobial defense express a heterodimeric TCR composed of an α- and a β-chain (αβ TCR).[2] These include heterogeneous populations of unconventional or "innate-like" T cells, such as invariant natural killer T (NKT) cells and mucosal-associated invariant T (MAIT) cells that typically express an αβ TCR with limited diversity (see "NKT Cells and T Cells With a Restricted αβ TCR Repertoire" later).[3,4] A small fraction of circulating human T lymphocytes, approximately 1% to 5% in humans, express a TCR composed of a γ- and a δ-chain and are referred to as γδ T cells (see "γδ T Cells" later).[5]

T lymphocytes are usually classified on the basis of CD4 or CD8 coreceptor expression since CD4 and CD8 T cells differ in terms of function, specificity, and role in antimicrobial defense. As a rule, CD4 T lymphocytes orchestrate antimicrobial defense but do not participate directly in microbial killing. The major strategy used by CD4 T cells to disable microbes is to secrete cytokines and chemokines that recruit leukocytes (e.g., monocytes and neutrophils) to and activate leukocytes (e.g., macrophages and B cells) at sites of infection. A subset of CD4 T cells, termed *regulatory T cells,* express the transcription factor forkhead box P3 (FOXP3) and can exert immune regulatory functions during microbial infections. The major strategy used by CD8 T cells is to secrete lytic proteins that destroy infected cells, depriving the pathogen of an environment that is suitable for replication and long-term survival. These functions, collectively called *T-lymphocyte effector functions,* are expressed selectively by different T-cell subsets, providing a diversity of antimicrobial mechanisms.[2,6,7]

CD4 T Cells

Naïve CD4 T cells circulate between the bloodstream and lymph nodes and undergo priming and clonal expansion upon encountering antigen-presenting cells (APCs) with cognate MHC class II–peptide complexes. Priming of naïve T cells in lymph nodes likely occurs in a stepwise fashion, with sequential interactions with distinct APCs (e.g., migratory DCs, lymph node–resident DCs) that each contribute to the phenotype of the effector CD4 T-cell response.[8] Following priming, CD4 T cells can differentiate into phenotypically distinct effector cells, a process that is determined by the inflammatory context induced by the microbe.[9] Classic studies of CD4 T-cell priming suggested that differentiation can give rise either to helper T cell 1 (Th1) cells that produce interferon-γ (IFN-γ) or to helper T cell 2 (Th2) cells that produce interleukin (IL)-4 (IL-4).[10] This model has been revised by studies conducted in the past

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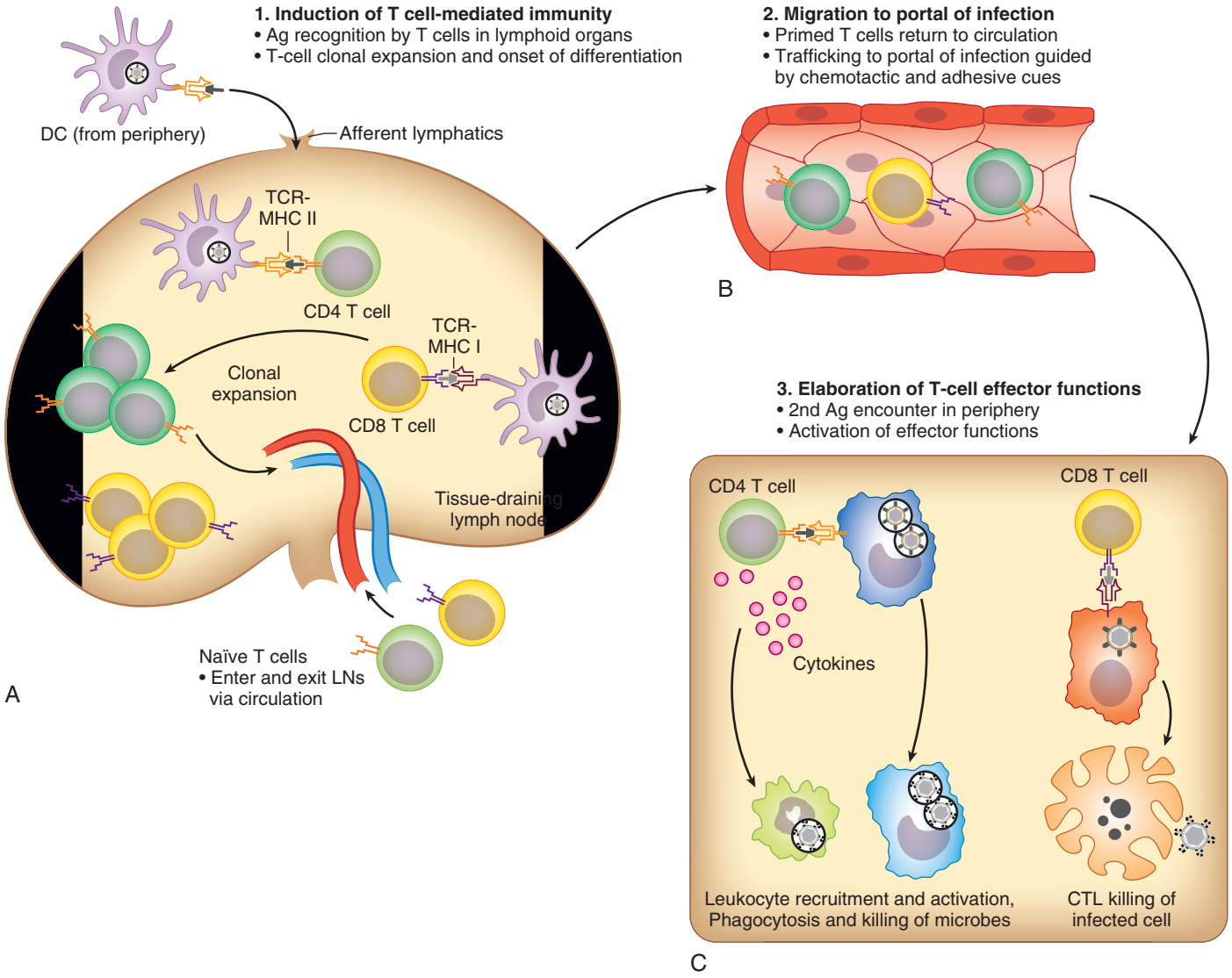

FIG. 6.1 Overview of T-cell activation and T-cell effector functions. (A) During the induction phase, naïve CD4 T cells and CD8 T cells enter the lymph node and recognize microbial-derived peptide antigens presented by dendritic cells (*DCs*) in the context of major histocompatibility complex *(MHC)* class II and MHC class I, respectively. The responding T cells undergo proliferation and begin the differentiation process into short-lived effector cells that enter the circulation. (B) T-cell trafficking follows chemokine- and integrin-mediated cues to reach the portal of infection. (C) Responding T cells elaborate effector functions, with CD4 T cells primarily recruiting and activating phagocytes to eradicate tissue-invasive microbes, and CD8 T cells directly killing infected host cells. *Ag,* Antigen; *CTL,* cytotoxic T lymphocyte; *LNs,* lymph nodes; *TCR,* T-cell receptor.

20 years that demonstrate a more diverse and flexible differentiation potential of naïve CD4 T cells.[9] Cytokines produced during infection promote the expression of distinct transcriptional regulators that determine which effector molecules will be expressed by responding CD4 T cells.

Distinct CD4 T-cell populations that respond to and control specific microbial pathogens have been well characterized based on the expression of transcriptional regulators and the secretion of specific cytokines (Fig. 6.2). *Epigenetic modifications,* including the chemical modification of histones and DNA (i.e., by acetylation, methylation, phosphorylation, ubiquination, and sumoylation) as well as the compaction and remodeling of nucleosomes and larger chromosomal segments, represent critical determinants to support CD4 T-cell lineage differentiation and specification. These mechanisms enable *cis*-regulatory elements that control helper T-cell lineage-specific genes (e.g., the *ifng* or *il4* gene) to bind to *trans*-acting factors (e.g., lineage-determining transcription factors), and regulate target genes.

Studies of in vivo CD4 T-cell priming, trafficking, and differentiation have been facilitated by the development of mice in which T-cell specificity is engineered by transgenic expression of defined TCRs. Adoptive transfer of T cells of defined specificity into normal recipient mice, followed by microbial challenge, has allowed investigators to determine the kinetics of CD4 T-cell activation and to measure trafficking from sites of T-cell priming to sites of infection. CD4 T-cell priming following respiratory challenge with influenza virus or the fungal pathogens *Aspergillus fumigatus* and *Blastomyces dermatiditis* is rapid and accompanied by differentiation into a Th1 phenotype.[11–13] Interestingly, complete differentiation of *Aspergillus*-specific CD4 T cells does not occur until CD4 T cells arrive in the lung. In contrast to these infections, CD4 T cells responding to *Mycobacterium tuberculosis* infection are not activated until at least 1 week postinfection[14,15] and do not reach the lung until 8 to 12 days postinfection. Adoptive transfer of *M. tuberculosis*–specific Th1 CD4 T cells prior to infection can provide substantial immunity, but not until the infection has progressed for roughly 1 week.[16]

Th1 T Cells

Naïve T cells are driven to differentiate into Th1 T cells by early exposure to IL-12 and IFN-γ at the time of T-cell priming. Th1 T cells secrete IFN-γ, a signature cytokine that activates macrophages and DCs and

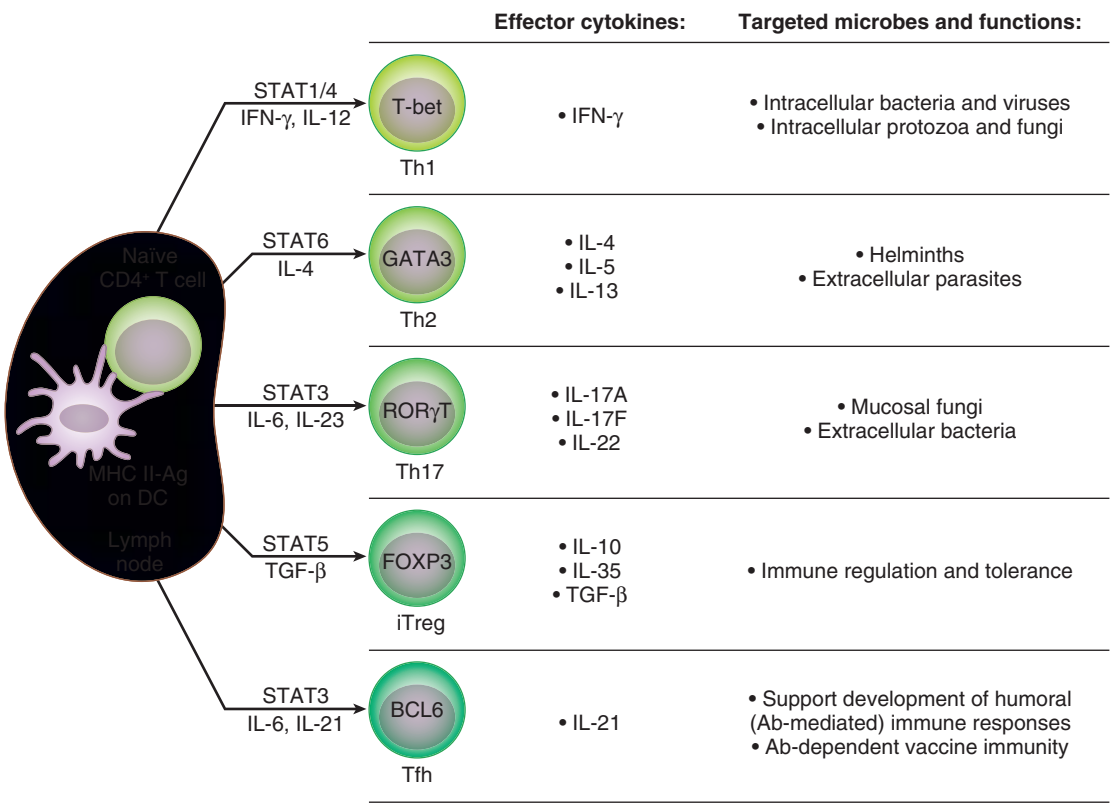

FIG. 6.2 Helper T-cell subsets. Naïve CD4 T cells proliferate and differentiate into distinct helper T-cell *(Th)* subsets following T-cell receptor (TCR) activation by cognate major histocompatibility complex *(MHC)* class II–peptide antigen *(Ag)* complexes during infectious and inflammatory states. Cytokine- and signal transducer and activator of transcription *(STAT)*–mediated signals drive Th differentiation into distinct Th effector subsets that are maintained by the expression of transcriptional activators *(T-bet, GATA3, RORγt, FOXP3)* or repressors *(BCL6)* and secrete distinct effector cytokines. Individual Th subsets demonstrate plasticity and may interconvert during infectious challenges to facilitate microbial eradication and reduce immune-mediated tissue injury. The major classes of pathogens that are targeted by individual Th subsets are indicated in the right column. *Ab,* Antibody; *BCL6,* B-cell lymphoma 6; *DC,* dendritic cell; *FOXP3,* forkhead box P3; *IFN-γ,* interferon-γ; *IL,* interleukin; *iTreg,* inducible T-regulatory cell; *LNs,* lymph nodes; *RORγt,* retineic acid receptor–related orphan nuclear receptor γt; *Tfh,* T follicular helper cell; *TGF-β,* transforming growth factor-β.

thereby enhances their ability to kill intracellular microbes and to present antigens to T lymphocytes. Th1 T cells can also secrete tumor necrosis factor (TNF), lymphotoxin, and IL-2, which contribute to antimicrobial defense as well.[9]

DCs and other myeloid cells that recognize pathogenic microbes via germline-encoded pattern recognition receptors often represent the earliest source of IL-12 during T-cell priming. Innate lymphoid cells (ILCs)—including NKT cells, unconventional T-cell populations, and neutrophils—in the setting of salmonellosis and toxoplasmosis can represent early sources of IFN-γ.[17,18] IFN-γ binds to the IFN-γ receptor on T cells, activates signaling via the signal transducer and activator of transcription (STAT) 1 (STAT1) pathway, and induces the expression of T-bet.[19] T-bet is a transcriptional regulator that promotes IFN-γ production by T cells and downregulates the expression of IL-4, a cytokine that promotes Th2 differentiation. IL-12 binds the IL-12 receptor heterodimer and signals via the STAT4 pathway, amplifying Th1 responses and enhancing IFN-γ production by responding Th1 cells.

Th1 T cells play an important role against infections by intracellular bacteria (e.g., *Salmonella typhimurium*, *M. tuberculosis*, and nontuberculous mycobacteria) and dimorphic fungi.[20] Clinical observations in humans with primary immunodeficiency disorders and experiments in mice link genetic defects in IL-12, IFN-γ, IL-12 receptor, IFN-γ receptor, or downstream signal transducers (e.g., STAT1) to susceptibility to these pathogens (Table 6.1).[21–25] Th1 CD4 T cells enhance defense against *Leishmania major* by stimulating IFN-γ–dependent macrophage killing of intracellular parasites.[26]

Th2 T Cells

Th2 cells express cytokines that influence B-cell differentiation and antibody production, eosinophil recruitment, and mucus production. The signature cytokines produced by Th2 cells are IL-4, IL-5, and IL-13, but Th2 cells can also produce IL-9, IL-10, IL-25, and amphiregulin.[27] Th2 responses are generated when naïve T cells are exposed to IL-4 at the time of T-cell priming. Following antigenic challenge, IL-4 can be produced by mast cells and basophils in the vicinity of T-cell priming[28,29] or, in the setting of low antigen concentrations, by responding T cells.[30] The IL-4 receptor transduces signals to naïve T cells via the STAT6 pathway to facilitate the expression of GATA-3, the master transcriptional regulator of Th2 differentiation.[31] This process can be enhanced by IL-4– and STAT6-independent GATA-3 activation, all of which drives the expression of additional downstream activators. For example, APCs that express the Notch ligand Delta can act on the Notch effector recombining binding protein suppressor of hairless (RBPJκ) to induce GATA-3 in the absence of STAT6 activation and to regulate *il4* gene transcription.[32]

Although Th2 cells are best known for causing or contributing to allergic diseases, Th2 cells contribute to defense against infections, particularly helminth infections of the gastrointestinal tract.[27] In this setting, eosinophil recruitment, immunoglobulin E (IgE) production, and mucus hypersecretion can enhance parasite expulsion in an IL-4– and IL-13–dependent manner, a notion that is supported by murine studies of *Nippostrongylus brasiliensis* infection.[33] The secretion of amphiregulin by Th2 cells can stimulate intestinal epithelial cell proliferation and expulsion of *Trichuris muris*, a nematode that infects mice.[34] Besides Th2 cells, tissue-resident and Th2 cytokine-secreting ILC, termed type 2 ILC, represent a significant source of IL-13 during the early stages of parasitic infection and promote expulsion.[27]

In humans, the type of disease associated with *Mycobacterium leprae* infection is tied to CD4 T-cell differentiation. Th1 differentiation is associated with tuberculoid leprosy, a paucibacillary infection in which IFN-γ–producing T cells enhance microbial killing. The induction of

TABLE 6.1 Clinical and Immunologic Characteristics of Primary Immunodeficiency Disorders That Result in Impaired Cellular Immune Responses

GENE (MODE OF INHERITANCE)	INFECTION SUSCEPTIBILITY AND NON-INFECTIOUS MANIFESTATIONS (WHERE APPLICABLE)	ASSOCIATED CELLULAR IMMUNE DEFECTS
Disorders of IL-12/IFN-γ Signaling (MSMD)		
IFNGR1 (AD or AR)	Intracellular bacterial, fungal, and mycobacterial infections	Defective IFN-γ responses by macrophages
IFNGR2 (AD or AR)	Intracellular bacterial, fungal, and mycobacterial infections	Defective IFN-γ responses by macrophages
IL12RB1 (AR or AD)	Intracellular bacterial, fungal, and mycobacterial infections	Defective IL-12–dependent IFN-γ production by Th1 cells
IL12B (AR)	Intracellular fungal and mycobacterial infections	Defective IL-12–dependent IFN-γ production by Th1 cells
STAT1 (AR)	Mycobacterial and viral infections, including herpes simplex encephalitis	Defective IFN-γ responses by macrophages
STAT1 (AD)	Mycobacterial, viral, and intracellular fungal infections; invasive mold infections; CMC; hypothyroidism; squamous cell carcinomas; autoimmunity	IFN-γ tachyphylaxis (intracellular infections); defective Th17 differentiation (CMC)
IRF8 (AD or AR)	Mycobacterial infections; CMC	Defective IL-12 responses or decreased DCs and monocytes (intracellular infections), or both; decreased Th17 cells (CMC)
ISG15 (AR)	Mycobacterial infections; intracranial calcifications	Defective IFN-γ production by Th1 cells
Anhidrotic ectodermal dysplasia with immune deficiency caused by mutations in: *NEMO/IKBKG* (X-linked)	Bacterial, mycobacterial and viral infections, including herpes simplex encephalitis; PJP; CMC; anhidrotic ectodermal dysplasia	Severe lymphopenia and decreased Th1 cells
RORC (AR)	Mycobacterial infections; CMC	Defective IFN-γ production by Th1 cells; defective Th17 differentiation (CMC)
GATA2 (AD)	Mycobacterial, viral, and invasive mold and intracellular fungal infections; myelodysplasia; lymphedema	Lymphopenia; decreased numbers of monocytes and DCs; neutrophil defects
Disorders of IL-17 Signaling		
IL17F (AD)	CMC; asthma	Defective IL-17F responses
IL17RA (AR)	CMC; skin staphylococcal infections; pulmonary bacterial infections; atopic dermatitis	Defective IL-17 responses
IL17RC (AR)	CMC	Defective IL-17 responses
ACT1 (AR)	CMC; skin staphylococcal infections; atopic dermatitis	Defective IL-17 responses
Job (hyper-IgE) syndrome caused by mutations in *STAT3* (AD)	Skin and pulmonary bacterial infections; CMC; invasive mold and intracellular fungal infections; eczema; skeletal abnormalities; aneurysms	Defective RORγt-dependent Th17 differentiation
DOCK8 (AR)	Skin viral infections; molluscum contagiosum; CMC; malignancies	Defective Th17 differentiation
STK4 (AR)	Bacterial and viral infections; CMC; lymphoproliferative disorder	Defective Th17 cell responses
APECED syndrome caused by mutations in *AIRE* (AR or AD)	CMC; endocrine and nonendocrine autoimmunity; ectodermal dystrophy	Autoantibodies against Th17 cytokines
Disorders of Pathogen Recognition Receptors and Associated Downstream Signaling		
MYD88 (AR)	Invasive pyogenic bacterial infections	Defective TLR/IL-1R signaling
IRAK4 (AR)	Invasive pyogenic bacterial infections	Defective TLR signaling
TLR3 (AD or AR)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
TRIF (AD or AR)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
UNC93B1 (AR)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
TBK1 (AD)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
TRAF3 (AD)	Herpes simplex encephalitis	Defective TLR3-dependent IFN-α/β responses
CLEC7A (AR)	Vaginal candidiasis; dermatophytosis	Defective Th17 responses
CARD9 (AR)	CNS candidiasis; CMC; deep-seated dermatophytosis; extrapulmonary aspergillosis; phaeohyphomycosis	Decreased numbers of Th17 cells; defective neutrophil recruitment and fungal killing
MALT1 (AR)	Bacterial and viral infections; CMC; bronchiectasis	Defective T-cell activation; hypogammaglobulinemia
BCL10 (AR)	Mycobacterial and viral infections; CMC	Lymphopenia; hypogammaglobulinemia
IFIH1 (AR)	Severe respiratory viral infections	Defective MDA5-dependent dsRNA sensing and downstream IFN-α/β responses

TABLE 6.1 Clinical and Immunologic Characteristics of Primary Immunodeficiency Disorders That Result in Impaired Cellular Immune Responses—cont'd

GENE (MODE OF INHERITANCE)	INFECTION SUSCEPTIBILITY AND NON-INFECTIOUS MANIFESTATIONS (WHERE APPLICABLE)	ASSOCIATED CELLULAR IMMUNE DEFECTS
Disorders of Lymphopenia or Lymphocyte Activation		
SCID caused by mutations in: *ADA, JAK3, IL7RA, RAG1, RAG2, ARTEMIS* (AR); *IL2RG* (X-linked)	Viral, bacterial, and invasive (PJP) and mucosal (CMC) fungal infections	Severe T, B, and/or NK lymphopenia
XLA caused by mutations in *BTK*	Bacterial infections	Significantly decreased or absent B cells; hypogammaglobulinemia
Classic or functional NK cell deficiency caused by mutations in: *MCM4, RTEL1, FCGR3A* (AR); *GATA2* (AD)	Severe viral infections; malignancies (GATA-2 additional features as above)	Absent NK cells or defective NK cell function
IL21R (AR)	Respiratory infections; cryptosporidiosis; CMC; PJP; liver fibrosis	Defective T-cell activation
CD40L (X-linked)	Bacterial, mycobacterial, fungal (PJP), and parasitic infections; inflammatory bowel disease	Defective T-cell activation
Bare lymphocyte syndrome type II caused by mutations in: *CIITA, RFX5, RFXANK, RFXAP* (AR)	Bacterial, mycobacterial, fungal, and viral infections; chronic diarrhea; failure to thrive	Absent MHC class II expression on mononuclear cells; defective T-cell activation
Disorders of the CD8 T Cell–MHC Class I Complex Interface		
Bare lymphocyte syndrome type I, caused by mutations in: *TAP1, TAP2* (AR)	Respiratory bacterial infections; bronchiectasis; skin ulcerative granulomas	Defective processing and presentation of MHC class I molecules
CD8A (AR)	Respiratory bacterial infections; bronchiectasis; skin ulcerative granulomas	Decreased or absent CD8 T cells
B2M (AR)	Respiratory bacterial infections; bronchiectasis; skin ulcerative granulomas	Defective processing and presentation of MHC class I molecules
ZAP70 (AR)	Bacterial, viral, and fungal (PJP) infections; failure to thrive	Decreased CD8 T cells; defective proliferation of CD4 T cells

AD, Autosomal dominant; *APECED,* autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy; *AR,* autosomal recessive; *CMC,* chronic mucocutaneous candidiasis; *DC,* dendritic cell; *dsRNA,* double-stranded RNA; *IFN,* interferon; *IgE,* immunoglobulin E; *IL,* interleukin; *IL-1R,* interleukin-1 receptor; *MDA5,* melanoma differentiation-associated gene 5; *MSMD,* mendelian susceptibility to mycobacterial disease; *NK,* natural killer; *PJP, Pneumocystis jirovecii* pneumonia; *SCID,* severe combined immunodeficiency disorder; *Th1,* helper T cell 1; *Th17,* helper T cell 17; *TLR,* Toll-like receptor; *XLA,* X-linked agammaglobulinemia.

type I interferon and IL-10 signaling in innate immune cells during leprosy can antagonize IFN-γ–dependent protection.[35] Th2 differentiation is associated with high tissue densities of *M. leprae* and more robust, but ineffective, antibody responses.[36,37]

Th17 T Cells

Helper T cell 17 (Th17) cells produce the signature cytokines IL-17A and IL-17F, often termed IL-17, since they can form homo- and heterodimers. Although Th17 T cells were initially characterized as the principal CD4 T-cell effectors that promote inflammatory diseases (e.g., rheumatoid arthritis, psoriasis, and multiple sclerosis),[38] the roles of Th17 T cells and IL-17–dependent signaling pathways in defense against microbial infection, particularly against extracellular bacteria and mucosal fungi, are now well established.

CD4 T-cell differentiation into Th17 cells requires early exposure to transforming growth factor-β (TGF-β) and IL-6 at the time of priming, IL-21–driven amplification, and IL-23–driven stabilization.[9,39–43] The combination of robust TCR stimulation and IL-6/TGF-β exposure induces IL-23 receptor expression and STAT3-dependent expression of the transcription factor retineic acid receptor–related orphan nuclear receptor γt (RORγt).[44] RORγt, together with RORα,[45] promotes IL-17A, IL-17F, IL-21, and IL-22 release. Th17 cell activation leads to the expression of chemokine receptors (i.e., CCR4, CCR6) that facilitate their migration to inflammatory lesions at mucosal sites, specifically the intestines, lung, and skin.[46]

Microbial factors that promote the differentiation of Th17 T cells include *M. tuberculosis*–derived complete Freund adjuvant, specifically trehalose dimycolate (i.e., cord factor) and peptidoglycan that synergize to recapitulate Th17-promoting adjuvant activity of mycobacteria.[47,48] The fungal cell wall components β-glucan and α-mannan trigger C-type lectin receptor signaling and stimulate caspase recruitment domain-containing protein 9 (CARD9)–dependent signals in DCs that enhance Th17 T-cell differentiation[49] (see "C-Type Lectin Receptors" later).

Th17- and IL-17–dependent functions in antimicrobial immunity are best understood at mucosal surfaces. IL-17–responsive epithelial cells produce chemokines that recruit proinflammatory cells, primarily neutrophils, to the site of infection, and release antimicrobial peptides for local control of extracellular pathogens.[50,51] Clearance of murine pulmonary infection with *Klebsiella pneumoniae* is dependent on cytokines secreted by Th17 T cells, including IL-17 and IL-22.[52] Patients with hyper-IgE syndrome, also known as Job syndrome, have impaired differentiation of Th17 T cells.[53] This defect results from mutations in STAT3, a signaling molecule that is essential for Th17 T-cell development, and accounts for a markedly increased risk of pulmonary infections, mucocutaneous candidiasis, and staphylococcal infections. Mendelian susceptibility to chronic mucocutaneous candidiasis and dermatophytosis maps to components of the IL-17A and IL-17F signaling pathway and underscores the importance of these Th17-derived cytokines against mucosal fungal infections (see Table 6.1).[20]

Th17-dependent and IL-17–dependent immune functions are not synonymous, since RORγt$^+$ type 3 ILCs, and unconventional and γδ T cells, can provide early sources of IL-17 and IL-22 prior to Th17 T-cell differentiation and trafficking to sites of infection.[54] In the intestine, colonization of germ-free mice with commensal segmented filamentous bacteria is sufficient to induce Th17 cells in the lamina propria.[55] In this model, tissue-resident type 3 ILCs trigger IL-22–dependent epithelial production of serum amyloid A proteins that in turn act on poised Th17 cells to amplify the production of effector cytokines.[56] These data suggest that intestinal commensal microbes can stimulate Th17-mediated mucosal protection against pathogenic microbes.

T-Regulatory Cells

T-regulatory cells (Tregs) can either emerge from the thymus as natural Tregs (nTregs), or they can be induced during T-cell priming of naïve T cells, in which case they are referred to as inducible Tregs (iTregs).[57] Tregs were first identified as a CD4$^+$CD25$^+$ T-cell subset in normal mice

and constituted roughly 10% of the CD4 T-cell population.[58] This T-cell population had the remarkable ability to prevent autoimmune inflammatory bowel disease when transferred into susceptible mice.

Similar to conventional CD4 and CD8 T lymphocytes, nTregs are selected in the thymus on epithelial cells.[59] iTregs, on the other hand, are induced in the periphery when naïve T cells are primed in the presence of TGF-β, which induces expression of FOXP3, a transcriptional regulator that induces iTreg differentiation.[60] nTregs emerging from the thymus also express FOXP3.[59] The human IPEX syndrome (*i*mmunodysregulation, *p*olyendocrinopathy, and *e*nteropathy that is *X*-linked) results from a mutation in the gene encoding FOXP3, and gives rise to a profoundly inflammatory disease characterized by the absence of Tregs.[61–63]

Both nTregs and iTregs contribute to and expand the TCR repertoire implicated in regulatory T-cell function, suppress the activation of autoimmune T lymphocytes, and mediate immune tolerance.[64] TGF-β and IL-10 have been implicated in this process, and, depending on the organ site, both can limit the development of inflammatory disease. In the large intestine, short-chain fatty acids (i.e., butyrate, propionate, and acetate) accumulate to concentrations of 50 to 100 mM in the colonic lumen due to the fermentation of dietary fiber by commensal anaerobic bacteria.[65] These microbial metabolites are sensed by G protein–coupled receptor signaling pathways (e.g., GPR43) that act to promote the formation of iTregs.[66]

The role of Tregs in defense against microbial infection is significant and varies based on the microbial pathogen and the type of inflammatory response, resulting in a positive or a negative role. For example, in murine infection with *L. major,* Tregs at the site of parasite inoculation prevent complete Th1-mediated microbial clearance, which enables long-term CD4 T-cell memory to be maintained.[67] Similarly, Tregs enable CD8 T-cell memory responses to West Nile virus in mice.[68]

In contrast, Treg depletion during herpes simplex virus and respiratory syncytial virus infections resulted in adverse outcomes as a result of disorganized early innate immune cell recruitment[69] or immunopathology linked to respiratory syncytial virus–specific CD8 T-cell responses.[70] During malaria infection, Tregs can restrict immune responses to create a lethal outcome, and Treg depletion enhances survival.[71] Similarly, Treg depletion in filarial infection models markedly enhances pathogen clearance.[72] During tuberculosis, pathogen-specific Tregs expand early during pulmonary infection and delay the arrival of effector CD4 and CD8 T cells in the lung,[73] yet undergo eventual elimination in response to IL-12–mediated signals.[74]

In humans, Tregs influence acute and chronic viral infections.[75] Although hepatitis A virus does not infect T cells, Tregs express the hepatitis A virus cellular receptor 1 (HAVCR1), and viral binding blocks its ability to recognize phosphatidylserine on apoptotic cells. At the peak of viral infection, patients lack Treg immunosuppressive function, yet clear the virus in the context of high IL-22 and low TGF-β levels that act in concert to limit hepatic injury.[76] Tregs may suppress the proliferation of acutely human immunodeficiency virus (HIV) –infected CD4 T cells, limiting HIV spread at early stages of disease.[77] In contrast, high Treg levels correlate with disease progression during chronic hepatitis B infection.[78] Similarly, high circulating Treg frequencies are found in patients with extrapulmonary tuberculosis.[75] In sum, although Tregs have a well-established role in preventing autoimmune diseases, their functional characteristics appear either beneficial or detrimental to infectious outcomes, depending on the specific microbial pathogen and the impact of their regulatory properties on inflammatory and memory responses.

Helper T-Follicular Cells

Helper T-follicular (Tfh) cells represent a distinct CD4 T-cell population that regulates antibody responses to T cell–dependent antigens in germinal centers, a specialized B-cell zone in secondary lymphoid organs.[79,80] Tfh cells produce IL-21 and promote immunoglobulin class switching and affinity maturation. Tfh cells are characterized by high expression of programmed cell death protein 1 (PD-1; CD279), inducible costimulator (ICOS), and the chemokine receptor CXCR5 for targeting to splenic B-cell zones. ICOS-dependent signals regulate the induction of a transcriptional repressor and Tfh master regulator, B-cell lymphoma 6 (BCL6).[80,81] Although not exclusive to Tfh cells, genetic defects in ICOS-1 lead to a marked reduction in circulating and germinal center Tfh cells and represent a rare monogenetic cause of common variable immunodeficiency.[82]

Plasticity of Helper T-Cell Subsets

The paradigm of distinct helper T-cell subsets has been critical in understanding CD4 T-cell–driven immunity to a wide range of microbial pathogens. However, the expression of master regulator genes and cytokine expression patterns is not as exclusive or enduring as initially proposed.[83] The process of switching CD4 helper T-cell effector functions is referred to as *trans*-differentiation. For example, IL-4–producing Th2 cells specific for murine lymphocytic choriomeningitis virus (LCMV) switch to IFN-γ production upon adoptive transfer and ensuing infection in mice.[84] During helminth infection, Th1 cells can convert into Th2 cells.[85] Th17 cells can be reprogrammed to secrete Th1 cytokines through IL-12 exposure.[86] In the resolution phase of inflammation, Th17 cells can convert into Tregs via TGF-β and aryl hydrocarbon receptor signaling.[87] These data are consistent with the notion that effector T cells remain responsive to external cues during the progression of microbial infections to optimize microbial eradication and to limit immunopathology.

CD8 T Cells

In contrast to CD4 T cells, which can differentiate into different subsets, naïve CD8 T cells typically differentiate into cytotoxic T lymphocytes (CTLs).[7] The principal effector function of these short-lived effector CD8 T cells is lysis of pathogen-infected cells, a process triggered by MHC class I–dependent presentation of microbial antigens. This function is particularly effective in defense against viral infections because lysis of infected host cells prevents further viral replication.[2] The most rapid cytolytic mechanism involves release of perforin granules by antigen-activated CD8 T cells. Perforin lyses the target cell membrane and enables granzymes, which are also released by cytolytic T cells, to enter the target cell and initiate the apoptotic pathway. In addition to perforin/granzyme–mediated lysis, CD8 T cells can mediate target cell death by expressing Fas ligand, which engages Fas on the target cell surface, resulting in Fas-mediated death. CD8 T-cell–mediated cytolysis generally targets the host cell and not the microbe. Human T cells produce a cytolytic protein called *granulysin* that can kill bacteria directly, including *M. tuberculosis* and *M. leprae*.[88,89] In most infections, pathogen-specific CD8 T cells secrete IFN-γ and TNF, which enhances macrophage- and neutrophil-mediated microbial killing. CD8 T cells also produce chemokines (e.g., CCL3-5) that contribute to the recruitment of inflammatory cells to sites of infection.

Studies in animal models have provided the clearest picture of pathogen-specific T cell responses. CD8 T cell responses are induced rapidly after viral (e.g., influenza, LCMV) and bacterial infections (e.g., *Listeria monocytogenes*). Pathogen-specific T cells are detectable 5 days after infection and expand rapidly to peak frequencies approximately 8 days after infection. Studies using adoptively transferred T cells of defined specificity have shown that antigen-specific T cells undergo rapid proliferation during this phase of the immune response, dividing at a rate of once every 6 hours.[90,91] In a matter of a few days, the frequency of antigen-specific T cells can increase from 1 in 100,000 CD8 T cells to 1 in 2 CD8 T cells in some viral infections.

During the early immune response, a rapid *expansion phase* of activated, antigen-specific CD8 T cells occurs (Fig. 6.3). The differentiation of quiescent naïve CD8 T cells to short-lived effector cells involves metabolic remodeling to a program of anabolic cell growth that depends on aerobic glycolysis, a process in which glucose is converted to lactate, to meet the demand for nucleic acid, protein, and lipid synthesis and to maintain cellular redox balance.[92] In addition, glycolytic intermediates activate the pentose phosphate pathway and serine, glycine one-carbon metabolism that in turn fuel nucleotide biosynthesis required for clonal expansion.[92,93] In contrast, quiescent naïve T cells primarily rely on pyruvate as metabolic fuel, generating energy via mitochondrial oxidative phosphorylation[92]; this process is also increased during their conversion to activated effector T cells.

During clonal expansion, cytokine receptors (e.g., the IL-7 receptor) that transmit homeostatic signals to naïve T cells are downregulated.

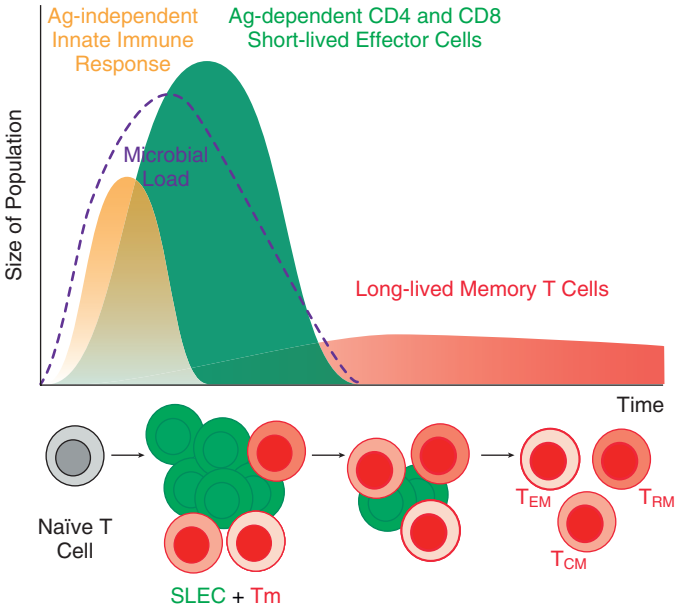

FIG. 6.3 Generation of T-cell memory. During a primary response to a microbe, the innate immune response provides rapid antigen-independent defense against microbial tissue invasion and growth. Antigen-dependent formation of CD4 T cell and CD8 T cell effectors occurs after several days and generally peaks within 1 to 2 weeks. Only 5% to 10% of short-lived effector cells (*SLEC*s) develop into long-lived memory T cell *(Tm)* populations that include central memory *(T_{CM}),* effector memory *(T_{EM}),* and resident memory *(T_{RM})* subsets. In the event of a secondary infection with the same microbe, memory T-cell populations give rise to short-lived effector cells more rapidly and in a higher magnitude than in the primary response. *Ag,* Antigen.

In contrast, the high-affinity IL-2 receptor is upregulated,[94] in part because IL-2, together with growth factors, promotes the metabolic switch to glycolysis by inducing the expression of nutrient transporters and activation of the mammalian target of rapamycin (mTOR) signaling complexes.[95] Upon initial stimulation, responding T cells are programmed and undergo proliferation and differentiation independent of further encounters with antigens or with the inflammation associated with infection.[96,97] During CD8 T cell expansion, antiapoptotic signaling pathways dominate, exemplified by glucocorticoid-induced TNF receptor–related protein (GITR) activity during acute influenza.[98]

As CD8 T cells complete the expansion phase, a small percentage (i.e., 5%–10%) survive and ultimately give rise to memory T-cell populations (see Fig. 6.3). Similar to the expansion phase, the *contraction phase* of CD8 T-cell responses is antigen and inflammation independent. During the contraction phase, effector CD8 T cells are prone to apoptosis because the proapoptotic receptor Fas is upregulated on the cell surface and the balance of the proapoptotic protein BCL-2–like protein 11 (BIM) and the antiapoptotic protein B-cell lymphoma 2 (BCL2) is shifted in favor of BIM-dependent contraction.[99]

NKT Cells and T Cells With a Restricted αβ TCR Repertoire

As discussed earlier, T-lymphocyte populations bear diverse TCRs that determine antigen binding specificity. A small subset of T lymphocytes, generally termed *NKT cells,* are heterogeneous and express a limited repertoire of TCRs that includes a single TCR α-chain (Vα24-Jα18 in humans) and a limited number of Vβ-chains. In addition to the TCR, these cells express natural killer (NK) cell markers and are either $CD4^+$ or double negative ($CD4^-/CD8^-$). NKT cells are of particular interest because they respond to bacterial, mycobacterial, and fungal lipid and glycolipid antigens presented on CD1 molecules, rather than MHC class I or II molecules.[100,101] Alternatively, NKT cell activation can occur in a cytokine-driven fashion, as has been observed in the context of viral infections,[102] or by sensing endogenous host lipids. While NKT cells primarily reside in peripheral tissues, they can also circulate in the blood. NKT cells may play a role in immune defense against *Borrelia burgdorferi, Streptococcus pneumoniae, Chlamydia* spp., tuberculosis, and viral diseases that include hepatitis B, HIV, influenza, and dengue.[103] NKT cells primarily secrete copious amounts of cytokines (e.g., IFN-γ, IL-4, granulocyte-macrophage colony-stimulating factor) and chemokines that activate or recruit, or both, NK cells, DCs, and macrophages to portals of infection.[104]

Additional populations of human T lymphocytes with a restricted TCR α-chain repertoire include MAIT cells that predominately reside in the intestine, express an invariant TCR α-chain (Vα7.2-Jα33 in humans) coupled to a limited TCR β-chain repertoire, and are activated by microbial riboflavin metabolites presented on MR1, an MHC class I–related molecule.[105,106] Circulating $CD4^+$ and $CD8^+$ MAIT cells are activated in humans following exposure to tuberculosis and postulated to play a role in clearance.[107] Human CD4 germline-encoded, mycolyl lipid–reactive (GEM) T cells express a limited TCR α-chain diversity and respond to *M. tuberculosis*–derived mycolic acids restricted by CD1b (see "CD1 and Antigen Presentation" later).[108]

TCR α-restricted lymphocytes, γδ T cells (see next section), and ILCs, including NK cells and lymphoid tissue inducer cells,[109] straddle the traditional boundaries between innate and adaptive immune cells. In general terms, all of these innate-like T-lymphocyte and innate lymphoid cell subsets produce cytokines that amplify and orchestrate antimicrobial defenses, primarily at barrier surfaces, and engage in tissue repair functions.

γδ T Cells

γδ T cells are predominately tissue-resident lymphocytes that are enriched in the intestinal mucosa and in epidermal layers of the skin. γδ T cells constitute a minor fraction (1%–5%) of T cells that circulate in the blood or are stationed in secondary lymphoid organs. γδ T cells encode a TCR that consists of a γ-chain and a δ-chain heterodimer; both chains undergo somatic V(D)J gene segment recombination. In specific subsets of γδ T cells, the γδ TCRs are invariant, or nearly invariant, though other γδ TCRs are quite diverse initially and later are selected by microbial infections.

Unlike αβ T cells that recognize discrete pathogen-specific epitopes (e.g., peptides presented in the context of the MHC), γδ T cells recognize peptide and nonpeptide ligands that vary in size, chemical composition, and structure.[5] Furthermore, the MHC-like Ib proteins (T10 and T22) can bind to the γδ TCRs absent of peptide antigens and activate a small subset (0.1%–1%) of murine γδ T cells. γδ T-cell antigens include surface and soluble proteins that are not obligately presented by the MHC or MHC complex-like molecules.[110] For example, *Clostridium tetani* tetanus toxoid, staphylococcal enterotoxin, and *L. monocytogenes* listeriolysin O all elicit γδ T-cell responses. γδ T-cell activation can also occur via lipid-derived mycobacterial antigens restricted by CD1 family members. Additional γδ T-cell ligands include phosphoantigens such as hydroxymethyl-but-2-enyl-pyrophosphate (HMBPP), an intermediate in bacterial and protozoan isoprenoid biosynthetic pathway, and isopentenyl pyrophosphate, an intermediate in the conserved mevalonate metabolic pathway found in bacteria and vertebrates and that is commonly activated by virus-infected cells. Heteromeric pairing interactions between butyrophilin family members, members of the B7 superfamily, are thought to facilitate phosphoantigen presentation to γδ T cells. In humans, butyrophilin family members are critical for the activation of circulating $Vγ9^+Vδ2^+$ cells.[111] Thus γδ T cells have the capacity to sense metabolic products that are associated with microbial infection.

During microbial infections, γδ T cells appear poised to amplify inflammatory responses and to exert cytolytic activity at portals of microbial invasion.[112] Following *Staphylococcus aureus* infection, murine epidermal $Vγ5^+$ γδ T cells produce IL-17 and facilitate neutrophil recruitment to resolve cutaneous bacterial lesions.[113] During tuberculosis, γδ T cells produce IL-17, TNF, and IFN-γ and contribute to disease containment. Beyond the release of proinflammatory cytokines, γδ T cells can produce bacteriostatic and lytic molecules such as granulysin and defensins (i.e., regenerating islet-derived protein 3α [REG3α] in humans) that contribute to their host defense functions.[5]

T-CELL MEMORY

Antigen-specific memory is a hallmark of the adaptive T-lymphocyte response.[114] T cells with specificity for a particular pathogen can persist in the host for many years after the pathogen has been eliminated, in the absence of continuous antigen exposure.

A single naïve CD8 T cell can give rise to both terminal effector and memory cells in a broad range of infections.[115,116] Thus memory fate decisions and formation occur independent of the priming antigen, the responding APC populations, and time of CD8 T-cell priming. Transcriptional responses that govern CD8 T-cell memory formation appear conserved in the context of LCMV and *Listeria monocytogenes* infections, despite differences in inflammatory responses elicited by viral and bacterial pathogens.[117] In the case of CD8 T cells, the size of the memory compartment appears to expand with immunologic experience.[118]

While naïve CD8 T cells depend on MHC- and IL-7–dependent stimuli for long-term survival, memory CD8 T cells survive and undergo homeostatic proliferation in an antigen-independent but cytokine-dependent manner, largely in response to IL-7 and IL-15.[119,120] Memory CD4 T-cell populations show a similar dependency on IL-7 and IL-15 signals,[121] indicating that in vivo maintenance of naïve and memory T cells is fundamentally different. Despite similarities in cytokine requirements for the maintenance of CD4 and CD8 memory T-cell populations, antiviral CD8 memory T-cell populations typically persist at relatively constant levels over time, while virus-specific CD4 memory T cells decrease in frequency.[122] Similarly, antigen persistence is required for long-term maintenance of *L. major*–specific CD4 memory T cells.[67] The concept of T-cell memory extends to the Treg CD4 T-cell subset, with the identification of long-term persistence of antigen-specific Treg cells with potent immunosuppressive properties.[123]

Several models of CD8 memory T-cell generation have been proposed.[124,125] First, researchers have proposed a concept of separate precursors for different memory cell populations based on signals that occur during thymic development. Most experimental evidence does not support this model because elegant adoptive transfer experiments of a single or of bar-coded naïve CD8 T cells yield heterogeneous effector and memory T-cell descendents.[115,116] Second, researchers have advanced the concept that signals (i.e., antigen, costimulatory molecules, and cytokines) encountered during the primary immune response stimulate the differentiation of naïve T cells first into effector T cells and then into memory T-cell populations.[126] In support of this model, loss of cytokine responsiveness via IL-21, IL-10, and STAT3 signaling disrupts the formation of virus-specific CD8 memory T cells, but not the formation of CD8 terminal effector differentiation.[127] Comparison of the TCR repertoire of effector and memory T cells suggests that these two populations share a common ancestry.[128] Memory T cells appear to have expressed genes encoding effector proteins, consistent with the model that memory T cells derive from effector T cells, though not all effector T cells have the equal potential to form memory T cells.[124]

Studies in mice have advanced several ideas to explain this form of T-cell diversification during primary immune responses. These include the formation of heterogeneous effector cell populations based on (1) the overall strength of three signals triggered by antigen, costimulation, and cytokines (signal-strength model), (2) the repetitive stimulation of naïve T cells by these three signals (decreasing potential model), and (3) asymmetrical cell division in which a single precursor T cell can divide into functionally distinct daughter cells with greater terminal effector or greater memory potential based on proximity to or distance from the APC.[124,125,129] This asymmetrical cell fate model predicts that daughter cells inherit immunologic synapse components in a variable manner and differ functionally in sensing the three signals. Asymmetrical cell division is relevant for the induction of Th1-like effector features versus CD4 T cell memory-like features during acute influenza.[130] Transcriptional studies have identified gene clusters whose expression correlates with the memory potential of responding CD4 and CD8 T cells.[117,131]

Detailed characterization of memory T lymphocytes reveals that they can differ with respect to expression of activation markers, homing properties, and effector capacities.[114,132,133] This discovery has led to the division of memory T cells into three major populations: central memory (T_{CM}), effector memory (T_{EM}), and resident memory (T_{RM}) T cells (see Fig. 6.3).

Human T_{CM} cells express high levels of CD45RO, CD62L, and CCR7 (i.e., a lymph node–homing chemokine receptor) and intermediate levels of CD44, do not express effector functions, and traffic preferentially to secondary lymphoid tissues (i.e., lymph nodes, splenic white pulp).[134] T_{CM} cells have a high proliferative capacity in recall responses, produce copious amounts of IL-2 upon activation, and provide superior protection in mice challenged with bacterial and viral pathogens compared to other memory CD8 T-cell populations.[135] Upon antigen encounter, T_{CM} cells give rise to heterogeneous progeny that are multipotent, capable of both terminal differentiation and self-renewal across serial adoptive transfers in mice,[136] and migrate to peripheral tissues to eliminate pathogens in concert with other memory T-cell subsets.[137]

Human T_{EM} cells express high levels of CD45RO and CD44 and low levels of CD62L and CCR7; they actively exert effector functions (e.g., cytokine synthesis and cytolytic activity), and traffic between peripheral tissues, the lymphatic system, secondary lymphoid tissues, and the circulation. The discovery that a subset of memory T cells can remain constrained in peripheral tissues following infection led to the characterization of human and murine T_{RM} cells. T_{RM} cells reside within the gut, skin, lung, genital tissues, and other mucosal tissues with minimal turnover[138–140] and are readily identified in the context of respiratory virus, herpes simplex virus (HSV), and parasitic infections.[141–143] T_{RM} cells respond to secondary pathogen encounters by releasing cytokines (e.g., TNF, IFN-γ, and lymphotoxin) that activate macrophages and NK cells and by exerting direct cytolytic activity. The distinct anatomic distribution of CD4 and CD8 memory T-cell subsets is maintained during the human life span.[144]

The generation and maintenance of T-cell memory is influenced by the chronicity of a microbial infection. Persistent infections result in CD8 T-cell populations that lose effector functions in a process referred to as *exhaustion,* a process that may be driven by sustained antigen presentation.[145] Exhausted CD8 T cells express PD-1 and cytotoxic T lymphocyte antigen-4 (CTLA-4; CD152), surface proteins that transmit an inhibitory signal to proliferating CD8 T cells.[146–148] Blocking PD-1 and CTLA-4 signaling (i.e., immune checkpoint blockade) with specific antibodies rescues exhausted T cells, enabling them to reexpress effector functions.[149] Immune checkpoint blockade therapy is a highly promising therapy for a number of human cancers (e.g., melanoma) in which regulatory mechanisms promote an exhaustion phenotype in tumor-infiltrating T lymphocytes. Monoclonal antibodies that target PD-1 and CTLA-4 do not appear to be associated with a significant increase in infectious complications in melanoma patients.[150] Early studies of patients with persistent HIV or hepatitis C virus infection demonstrate PD-1 expression on exhausted CD8 T cells, which is associated with disease progression.[151–153] In a mouse model of cryptococcosis, anti–PD-1 antibody therapy enhanced clearance of persistent lung infection.[154] The extent to which immune checkpoint blockade therapies may ameliorate chronic infections in humans remains to be explored.

T-CELL DEVELOPMENT AND LYMPHOID TISSUE ORGANIZATION

Thymic Selection of CD4 and CD8 T Cells

Mature CD4 and CD8 T cells are generated in the thymus,[155] though T-cell maturation can occur in children who underwent partial or complete surgical thymectomy in infancy, albeit at diminished levels.[156] In mice, extrathymic T-cell maturation can occur in the liver and in the intestine, though in the absence of thymopoiesis, T cells show a bias toward the γδ T-cell lineage and a relative paucity of αβ T cells.[157] Thymic selection has evolved to ensure that peripheral T cells are able to interact with self-MHC molecules, through the process of *positive selection,* but not to respond to self-peptides, through the process of *negative selection.* T-lymphocyte progenitors originate in the bone marrow and travel to the thymus, where they enter the cortex at the *double-negative* stage, defined by the absence of CD4 and CD8 coreceptors on the cell surface. In the thymic cortex, the TCR β-chain gene undergoes VDJ recombination, which, if successful, gives rise to the TCR β-chain protein. This gene recombination event is catalyzed by the *RAG1* and *RAG2* genes.[158] The TCR β-chain first complexes with the invariant

pre-TCR α-chain and travels to the cell surface.[159,160] If this process is successful, the endogenous α-chain locus undergoes VJ recombination and replaces the pre-TCR α-chain in subsequent TCR complexes. At this stage, the cell upregulates the expression of CD4 and CD8 on its cell surface and is referred to as a *double-positive thymocyte.* Positive selection occurs first through a process in which the double-positive thymocyte interacts with a cortical thymic epithelial cell (cTEC). The consensus model is that low-avidity interactions between the TCR on the double-positive thymocyte and MHC–self-peptide complexes on the cTEC drive cell proliferation and survival, whereas high-affinity interactions or no interaction result in programmed cell death, termed *death by neglect* if the double-positive thymocyte does not interact with the uniquely large repertoire of peptides presented by cTECs.[161] On completion of positive selection, the double-positive thymocyte down-regulates either CD4 or CD8, depending on whether positive selection occurred on MHC class II or MHC class I.

At this stage, thymocytes enter the thymic medulla, where they come into contact with medullary thymic epithelial cells, medullary DCs, and a small number of B cells that express a range of self-antigens. High-affinity interaction with self-antigens results in deletion of the single positive thymocyte, providing a second opportunity to eliminate potentially autoreactive T lymphocytes. Collectively, these interactions expose thymocytes to a complete representation of the proteins encoded in the genome.[162] Studies have identified a transcriptional regulator, the autoimmune regulator (AIRE), that promotes the ectopic expression of certain peripheral antigens in the thymus.[163,164] Individuals lacking AIRE develop severe multiorgan autoimmune disease (see Table 6.1).[165]

Upon completion of positive and negative selection, thymocytes exit the thymus in a process that is regulated by sphingosine-1-phosphate.[166,167] Thymic emigrants undergo postthymic maturation and become part of the peripheral T-cell compartment.[168] Naïve T cells generally express high levels of CD62L (also called L-selectin) and the chemokine receptor CCR7,[169] a combination of adhesion molecule and chemokine receptor that targets cells for the T-cell zone of lymph nodes and spleen. The diversity of T cells in the peripheral compartment is enormous. One estimate assumes that naïve human T cells express approximately 25 million distinct TCRs (i.e., clonotypes),[170] although technical and methodologic limitations impart a significant degree of uncertainty with regard to actual clonotype diversity.[171]

Thymic selection is crucial for the development of peripheral T lymphocytes. In patients with DiGeorge syndrome, caused by a deletion of a small region of chromosome 22, there is a congenital absence of thymic tissue and a corresponding absence of peripheral T lymphocytes.[172] Transplantation of thymic tissue into these patients corrects this problem.[173,174]

In humans, thymic function diminishes with aging. Young children have active thymic function and produce large numbers of naïve T lymphocytes, whereas older adults have a markedly smaller thymus that produces few new T lymphocytes.[175] However, adults in their 50s continue to have active thymic tissue and recent thymic emigrants in the bloodstream, suggesting the thymus continues to function despite its relatively small size.[176] Many endogenous and exogenous factors (e.g., glucocorticoids, chemotherapy) can injure thymic function. This issue is of particular importance in allogeneic hematopoietic stem cell transplantation, wherein long-term survival depends on reconstitution of the peripheral T-cell compartment. Studies that measure TCR excision circles, an indication of recent thymic emigration, and the kinetics of TCR repertoire reconstitution have shown that thymic function returns and TCR repertoire diversity increases in the posttransplantation setting.[177,178] Exogenous administration of IL-22 can enhance thymopoiesis by promoting the proliferation of thymic epithelial function and may restore thymic function following radiation- or chemotherapy-induced injury.[179]

Lymphoid Anatomy

Studies in humans and mice have provided a detailed picture of cell-mediated immunity and the cooperation between T-cell subsets that reside primarily in lymphoid tissues with those in peripheral and nonlymphoid sites.[180] In the classical view, T cell–mediated responses are predominately initiated in secondary lymphoid tissues (i.e., in lymph nodes and in the spleen). While lymph nodes survey an area of peripheral tissue defined by afferent lymphatic drainage, the spleen surveys the bloodstream for evidence of systemic infection. Following activation at these sites, activated lymphocytes traffic to nonlymphoid sites of infection. Because lymphoid tissues play this vital role in adaptive immunity, understanding lymph node and splenic anatomy is essential to understanding the comingling of antigen with its cognate immune cell.

Lymph Nodes

Lymph nodes serve as a nexus for lymphocyte interaction with APCs, overcoming the problem of bringing together rare, antigen-specific lymphocytes with small quantities of antigen.[181] Located at the interface between the blood and lymphatic systems, lymph nodes receive afferent lymphatic drainage from peripheral tissues as well as blood flow through arteries that enter the hilum of the node. The flow of cells, proteins, inflammatory mediators, and in some instances microbial pathogens to the lymph nodes (Fig. 6.4) from distant sites of infection via afferent lymphatic channels facilitates the activation of cell-mediated immunity.[181,182]

In the periphery, microbial antigens are taken up by migratory DCs that, after receiving signals through innate immune receptors, mature and travel in the afferent lymph fluid to a draining lymph node.[183] Inflammatory cytokines produced at the site of infection travel in lymph fluid to the draining lymph node concurrently.[184] Upon reaching the subcapsular space of the draining lymph node, the cellular and molecular immigrants follow different paths.

Afferent lymph is filtered slowly through the capsular sinus and the cortical sinus, and converges on the medullary sinus in the hilum before exiting via an efferent lymph vessel.[185] Lymph-borne DCs enter the subcapsular sinus, actively traverse an endothelial and fibroblastic layer of cells that forms the floor of the subcapsular sinus, and reach the lymph node cortex to access the collection of lymphocytes in the paracortical cords. Small protein molecules, such as cytokines and chemokines, and small microbe-derived molecules, do not traverse this barrier and fail to enter lymph node parenchyma.[186] Instead, these molecules are channeled into size-selective conduits that are both produced and enclosed by a tight network of fibroblastic reticular cells and contain an internal matrix consisting of collagen bundles. The collagen fibers provide structural support to the lymph node and define its architecture. Despite the enclosed nature of these conduits, leukocytes within the lymph node are able to sample soluble material from the lymphatic content. Lymphatic conduits associated with these collagen fibers provide connections between the subcapsular and cortical sinuses and the high endothelial venules (HEVs), enabling fluid flow and substrate transport from peripheral tissues via the subcapsular sinuses to the HEV, the site of cellular entry into the lymph node from the bloodstream.[181,185]

Endothelial cells in the HEVs can produce chemokines on their luminal surface and, since most fibroblastic, reticular cell–bounded conduits end in the HEV, intravascular T cells can respond to lymph-borne chemokines as well. Both processes trigger lymphocyte extravasation into the lymph node parenchyma.[186] To enter lymph nodes, lymphocytes undergo a four-stage process that involves rolling, activation, adhesion, and transmigration through the endothelium. On traversing the HEV, lymphocytes enter the perivenular channel, a narrow space bounded by fibroblastic reticular cells, that provides entry into the labyrinthine corridors of the paracortical cord. As lymphocytes move through this labyrinth side-by-side, they have ample opportunities to contact antigen-bearing DCs that have entered the cord from the subcapsular sinus.

Advances in live-imaging microscopy have enabled investigators to visualize interactions between T cells and DCs in lymph nodes over several hours and to monitor their trafficking patterns under homeostatic and inflammatory conditions.[181,187] T-cell movement within the paracortical cords occurs in a meandering fashion at a speed of approximately 10 to 12 μm/min. *Chemokinesis* is thought to be the underlying mechanism; the term refers to the ability of chemokines, in many instances bound on sessile DCs and stromal elements, to increase the general mobility of lymphocytes, analogous to a random walk.[181,188] T-cell chemokinesis enables sessile DCs to interact with an estimated 5000 T cells per hour.[189,190] This high frequency of DC–T cell interactions

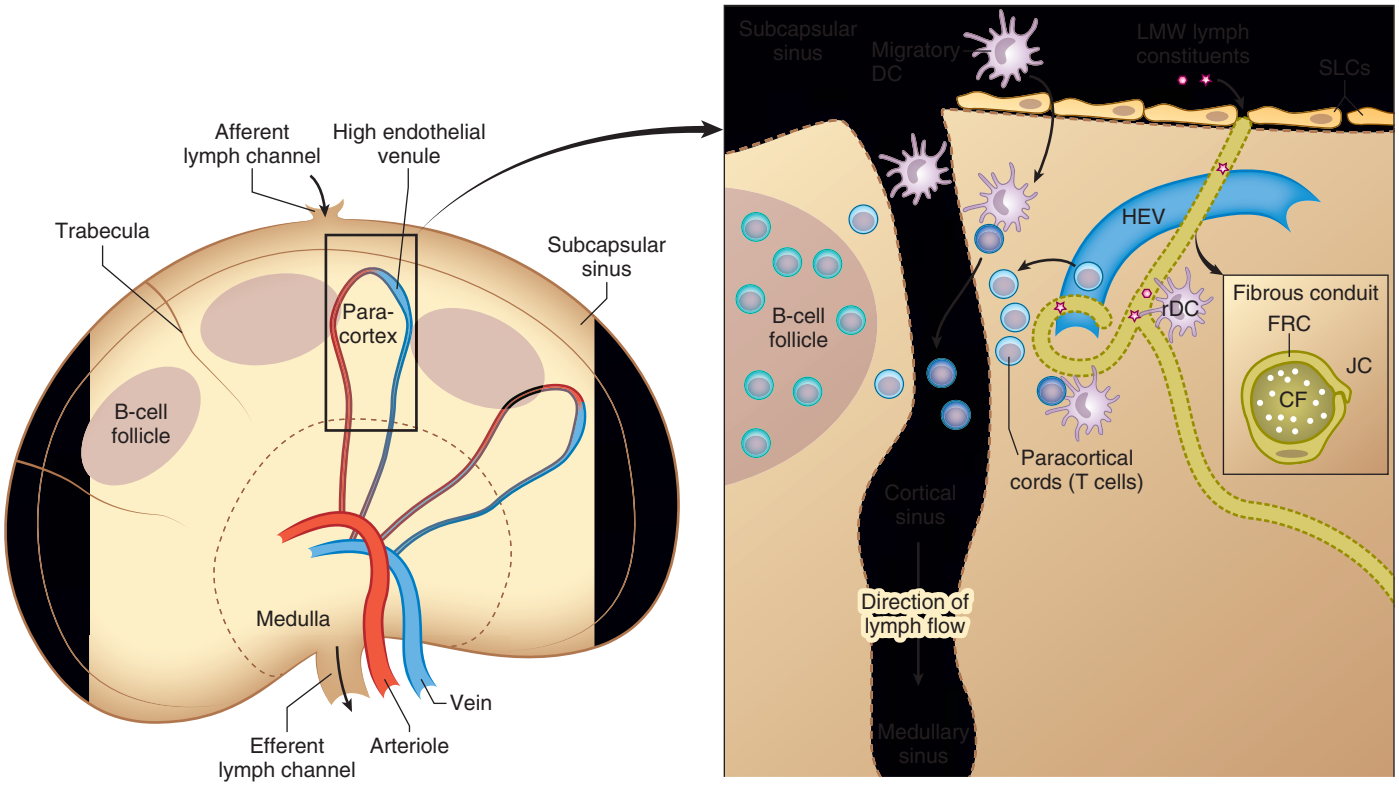

FIG. 6.4 Anatomy of lymph nodes. (A) Humans typically contain 500 to 600 bean-shaped lymph nodes that filter lymphatic fluid that is draining from peripheral tissues to the bloodstream via lymphatic vessels. Lymph nodes are encapsulated by dense, collagen-rich fibers that extend trabeculae into the lymph node substance. Afferent lymph fluid enters lymph nodes through channels that drain into subcapsular sinuses. Lymph fluid percolates through cortical and medullary sinuses before exiting the lymph node via efferent lymph channels. The outer region of the lymph node, termed the *cortex,* consists primarily of B cell–rich lymphoid follicles and T cell–rich paracortical aggregates. The inner region of the lymph node, termed the *medulla,* is far less cellular than the cortex, and this region contains coalescing lymph-filled sinuses. The blood supply to the lymph node enters and exits via the hilum. High endothelial venules represent the site of circulating leukocyte entry into the lymph node. Leukocytes exit the lymph node via the efferent lymphatic vessels and rejoin the circulation. (B) Lymph node microanatomy and network of conduits. This panel provides an outline of lymph node anatomy at a level that cannot be visualized by light microscopy, but that has been deduced by trafficking analyses of low-molecular-weight *(LMW)* markers that were experimentally introduced into lymphatic fluid and into blood that circulate into intact lymph nodes. Antigen-bearing migratory dendritic cells (*DC*s) can traverse the cellular lining of subcapsular and cortical sinuses to enter the paracortical cords to initiate antigen presentation to naïve T cells. Soluble inflammatory mediators and antigens are funneled into fibrous conduits that link lymphatic channels with high endothelial venules *(HEV)* and lymphoid follicles. Resident DCs can sample the antigenic content of fibrous conduits and present antigens to T cells that enter the paracortex through the HEV. Low-MW proteins, such as chemokines, can flow from the lymph node sinus through the conduit system to the HEV, where they can influence the recruitment of circulating lymphocytes. *CF,* Collagen fibers that provide structural support to the conduits and to the lymph node; *FRC,* fibroblastic reticular cells that surround the conduits; *JC,* junctional complexes that provide a tight barrier to the contents of the conduit; *rDC,* resident dendritic cells; *SLCs,* sinus-lining cells.

maximizes the likelihood that an individual T cell will encounter a DC that expresses a cognate peptide-loaded MHC.

Once a productive MHC-antigen-TCR interaction ensues, T cells remain in a complex stationary phase with the cognate DC for an estimate of 3 to 4 hours,[191] secrete IL-2 and IFN-γ, and then resume migratory behavior to enable additional interactions with DCs or T-cell subsets (e.g., CD4 T-cell help for CD8 T-cell activation), and ultimately lymph node exit. T cells exit the lymph node via the lymphatic sinuses and reenter the circulation via large lymphatic channels or the thoracic duct.[181]

The length of time that T lymphocytes remain in lymphoid tissues is regulated by a balance of retention signals, mediated primarily by chemokine receptor signaling via CCR7, and exit signals such as sphingosine-1-phosphate.[192] T-cell lymph node dwell times can be prolonged by expression of CD69, a C-type lectin that binds to and inhibits the activity of the sphingosine-1-phosphate receptor. CD69 expression is induced by inflammatory cues (e.g., type I interferon signaling) associated with microbial infections to increase the likelihood that a naïve T cell can productively interact with an antigen-bearing DC.[193]

Spleen

The spleen is a large secondary lymphoid organ that serves as an important filtration site to clear microbial pathogens from the bloodstream.[194] Although the spleen and lymph nodes share many T-cell, B-cell, and DC subsets and are similarly complex at the microanatomic level, they also differ in some important respects. The spleen is not a site for lymphatic drainage. Instead, antigens, pathogens, and circulating cells enter the spleen through the splenic artery that enters the hilum. The splenic artery branches into central arterioles (Fig. 6.5A), which course through the splenic cortex and are surrounded by lymphocytes in a structure called the *periarteriolar sheath.* T lymphocytes are most proximal to the central arteriole and are surrounded by aggregates of B lymphocytes in regions called *B-cell follicles* (Fig. 6.5B and 6.5C). The densely packed B and T lymphocytes constitute the splenic white pulp and are surrounded by the marginal zone (Fig. 6.5D), which separates white pulp from red pulp and contains marginal zone macrophages and marginal metallophilic macrophages. Both types of marginal zone macrophages have a role in trapping bloodborne particulate antigens.[195] Within the white pulp, $CD8\alpha^+$ conventional DCs localize to the periarteriolar sheath and are involved in tolerance to self-antigens and highly active in antigen cross-presentation[196] (discussed under "MHC Class I Antigen-Processing Pathway" later). In contrast, $CD8\alpha^-$ DCs reside primarily within the marginal zone and have a weaker cross-presentation ability and distinct cytokine responses to microbial stimuli compared to $CD8\alpha^+$ conventional DCs.[197]

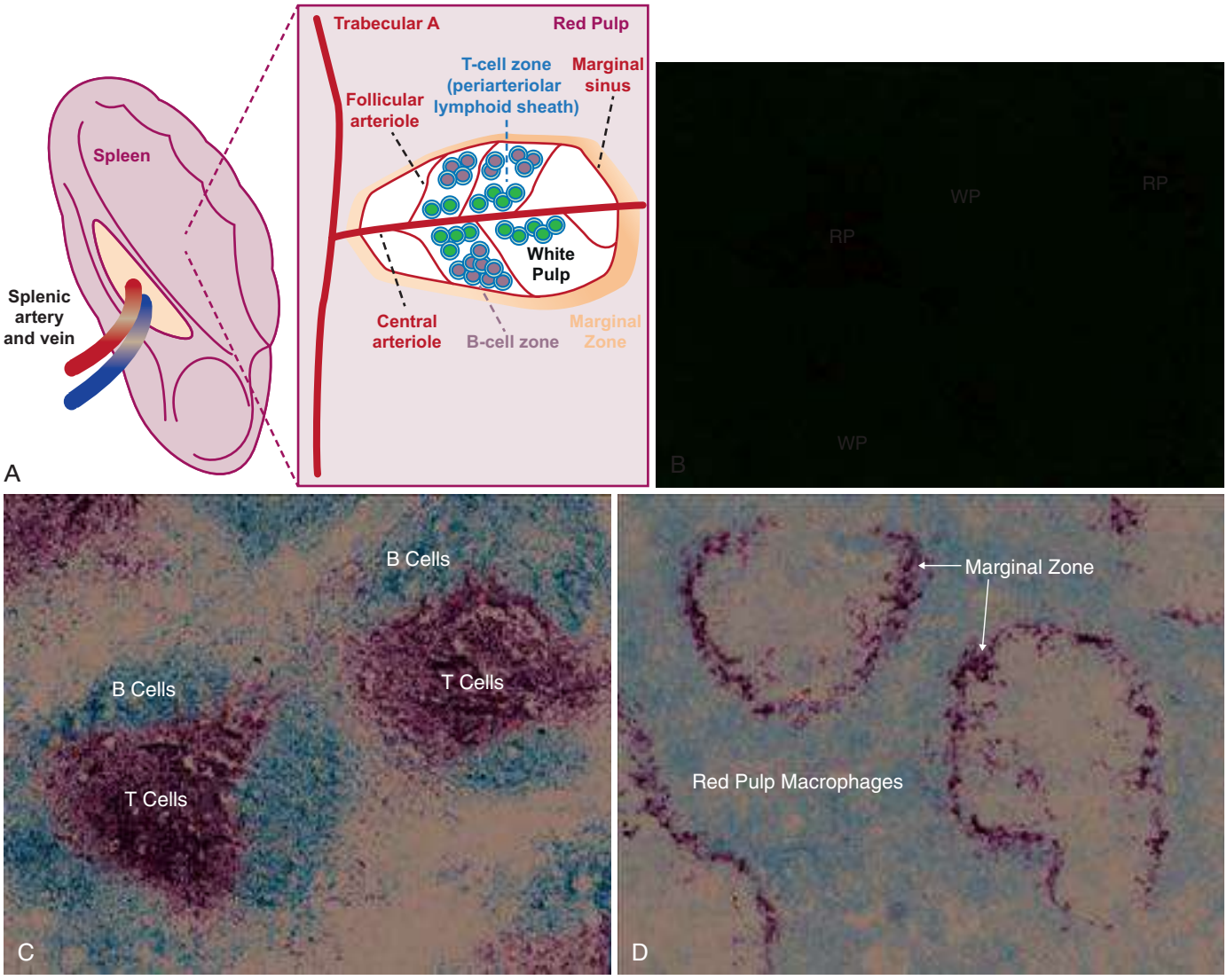

FIG. 6.5 Anatomy of the spleen. The spleen is a complex secondary lymphoid organ with compartmentalized cell populations. (A) Schematic diagram of spleen and a splenic arteriole that is surrounded by a periarteriolar lymphoid sheath (PALS) with a predominant T-cell population and a germinal follicle with a predominant B-cell population. The PALS and germinal follicles constitute the white pulp that is surrounded by a narrow marginal zone and the splenic red pulp. (B) Micrograph showing a hematoxylin-eosin–stained spleen section, demonstrating areas of white pulp *(WP)* separated by areas with more dispersed cell populations referred to as the red pulp *(RP).* (C) Micrograph showing a spleen section that has been stained with fluorescently labeled antibodies specific for B cells *(orange)* and T cells *(green),* demonstrating the distinct localization of B cells and T cells within the white pulp. (D) Micrograph showing staining for macrophages *(orange)* and the splenic marginal zone *(green),* demonstrating the density of macrophages and phagocytic cells in the red pulp and marginal zone.

The splenic red pulp is rich in macrophages (i.e., red pulp macrophages that clear senescent red blood cells) and contains many red blood cells, resulting from the percolating blood flow delivered by the termini of the splenic arterioles. The blood flow to the spleen predominantly terminates in the red pulp and the marginal zone. Most pathogens, on clearance from the bloodstream, first are localized to the marginal zone and red pulp of the spleen.[198] With respect to cellular trafficking into the spleen, the major entry point into the white pulp is from the marginal zone.[199] From here, activated APCs can enter the white pulp areas, most likely by traversing the marginal zone sinus and the metallophilic macrophage layer that forms a boundary between white pulp and marginal zone.

Access of protein antigens and other molecules into the splenic white pulp is highly restricted and, in many ways, similar to the system identified in lymph nodes. Specifically, fibroblastic reticular cells form small channels that surround collagen fibers that enter the T-cell and the B-cell zones of the spleen and deliver small molecules (i.e., generally <60 kDa) into the white pulp. The channels found in B-cell follicles bind chemokines associated with the recruitment of B cells, whereas the channels identified in T-cell zones bind chemokines associated with T-cell recruitment.

Mucosa-Associated Lymphoid Tissue

Mucosa-associated lymphoid tissue (MALT) is a network of lymphoid cell aggregates and tissue that is distributed in submucosal layers of the gastrointestinal, genital, respiratory, and urinary tracts, as well as in the eyes, skin, thyroid, breasts, tonsils, and salivary glands.[200] Cellular residents include T and B lymphocytes, as well as macrophages, all of which are positioned to trap and respond to antigens at mucosal portals of entry. Gut-associated lymphoid tissue (GALT), a MALT component, covers an estimated area of 300 m^2 in the human intestine. T lymphocytes are prevalent within the finger-like projections of the intestinal mucosa and can be found in the epithelial layer and in the underlying layer of connective tissue termed the *lamina propria.* GALT components include organized lymphoid tissues, such as Peyer's patches in the small intestine, and mesenteric lymph nodes.

T lymphocytes positioned in the epithelial layer at mucosal sites are a diverse population that, in aggregate, are called *intraepithelial lymphocytes*

(IELs).[180] Some IELs express the α- and β-chains of the coreceptor CD8 ($CD8\alpha^+\beta^+$), whereas others express exclusively α-chains ($CD8\alpha\alpha^+$) or no CD8 at all. With regard to TCR expression, γδ T cells are prominent cellular IEL constituents, as discussed earlier, though conventional CD8 T cells with αβ TCRs predominate in the intestinal lamina propria.[201,202] Nearly all IELs express adhesion molecules associated with an activated or memory phenotype and readily express effector functions, such as cytolytic activity and cytokine secretion.

Within the intestine, epithelial cells and DCs are postulated to present antigen to T lymphocytes.[203–205] DCs sample intestinal contents by transiently traversing the tight junctions of the intestinal epithelial cell layer.[206–208] After engulfing bacterial pathogens, such as *S. typhimurium,* DCs migrate to secondary lymphoid tissues to activate naïve T lymphocytes. However, a study proposed that CD8 effector memory cell precursors can be selected in the periphery by *thymus leukemia antigen,* an MHC class I molecule expressed by epithelial cells and DCs. In this model, thymus leukemia antigen preferentially binds to high-affinity $CD8\alpha\alpha^+$ or $\alpha\beta^+$ effector memory cell precursors and promotes affinity maturation of resident mucosal $CD8\alpha\beta^+$ T_{EM} cells for long-term persistence at mucosal sites of surveillance.[209]

Studies in mice have shown that intestinal infection with viral or bacterial pathogens induces robust expansion of pathogen-specific T lymphocytes in the small intestinal epithelium and lamina propria.[201,202,210] Systemic infection with viruses, such as vesicular stomatitis virus, or the intracellular bacterium *L. monocytogenes* also induces marked increases in the frequency of pathogen-specific CD8 T cells in the gut, suggesting that these T cells traffic to the intestine during systemic immune responses. The concept that immune responses to infectious pathogens result in the distribution of pathogen-specific T lymphocytes throughout the body is supported by two studies that measured whole-animal immunity.[211,212] In both studies, although T-cell priming occurred in secondary lymphoid tissues, after priming, antigen-specific T lymphocytes were found at higher frequencies in nonlymphoid tissues, such as liver, lamina propria, and adipose tissues.

T-CELL ACTIVATION

T-lymphocyte activation begins when the TCR binds to cognate MHC-peptide complexes, triggering a complex signaling cascade.[213,214] CD8 T cells bind to MHC class I complexes, while CD4 T cells bind to MHC class II complexes.

Beyond the first signal of MHC-TCR engagement, a second signal involves *costimulation,* and is mediated by the T-cell coreceptors CD28 and CD80/B7.1 and CD86/B7.2 on the APC. This interaction induces T-cell IL-2 production, which promotes their proliferation. Upon activation, T lymphocytes express CTLA-4 (CD152), which also binds CD80 and CD86, but instead of stimulating proliferation, this signaling molecule inhibits proliferation and acts as a brake on expansion. Studies have identified many other related B7 molecules that play a role in T-cell activation in the periphery.[215] One of these molecules, B7H2 (CD275), interacts with the inducible molecule ICOS on the surface of activated T cells and promotes their differentiation toward a Th2 phenotype.

Many TNF receptor superfamily members play a role in T-cell activation and differentiation.[216] Among these, CD40 and its agonist CD40L play a critical role in T-cell and B-cell immunity, since genetic defects in CD40 and CD40L underlie hyperimmunoglobulin M syndrome.[217] Affected individuals have defects in antibody isotype switching, accounting for high circulating immunoglobulin M levels, and are susceptible to infections with *Pneumocystis jirovecii,* indicating defective T-cell–mediated immunity (see Table 6.1). In mice deficient for CD40 or CD40L, T-cell activation and maintenance of memory T-cell populations are defective, accounting for increased susceptibility to some infections.[218,219] TNF receptor superfamily member 9 (CD137; 4-1BB) and TNF receptor superfamily member 4 (CD134; OX40) have also been implicated in the generation of pathogen-specific T-cell responses. OX40-dependent signals promote T-cell clonal expansion by inducing survivin, a protein that blocks T-cell apoptosis.[220]

The interface between T lymphocytes and APCs is a highly organized structure called the *immunologic synapse.*[221] This structure, also referred to as the supramolecular activation complex (SMAC), contains TCR and MHC contacts in the central portion and lymphocyte function–associated molecule 1 (CD18/CD11a; β_2/αL integrin) and intercellular adhesion molecule 1 (CD54) contacts in the periphery. The SMAC contains costimulatory (e.g., CD28) and signaling molecules as well. Synapse formation occurs when an antigen-specific T cell encounters an APC, enabling these cells to engage each other for several hours. The role of the SMAC in T-cell activation remains controversial, since T-cell signaling can precede synapse development.[222] Researchers have hypothesized that SMAC formation may play a more significant role in the response to infrequent peptide-MHC complexes or perhaps to lower affinity ligands for the T-cell receptor.[223]

Organization of the Major Histocompatibility Complex

The MHC, so named because its discovery resulted from studies of tissue transplant rejection, contains many of the genes associated with cell-mediated immune defenses.[224] The MHC complex encodes the α-chains of the MHC class I molecules human leukocyte antigen (HLA)-A, HLA-B, and HLA-C and the α- and β-chains of the MHC class II molecules HLA-DR, HLA-DP, and HLA-DQ, all of which are expressed in a co-dominant fashion.

MHC class I is expressed by all nucleated cells and platelets in jawed vertebrates, although the amount on the cell surface varies among cell types and under different inflammatory conditions.[225] The nucleotide-binding oligomerization domain (NOD)–like receptor (NLR) family member NLRC5 can regulate MHC class I expression at the transcriptional level, in response to viral and bacterial infections and to type I IFN and IFN-γ.[226] Consistent with this role, *nlrc5*-deficient mice exhibit a reduced CD8 T-cell response to systemic listeriosis, resulting in defective bacterial clearance.[227]

In contrast to MHC class I molecules, MHC class II expression is generally restricted to DCs, macrophages, B lymphocytes, innate lymphoid cells, and thymic epithelial cells under homeostatic conditions. However, MHC class II expression can be induced by various inflammatory signals, in particular IFN-γ. Regulation of MHC class II expression is mediated in part by the class II transactivator (CIITA) protein, a transcription factor that enhances expression of MHC class II molecules, the associated invariant chain, and other molecules associated with MHC class II antigen processing.[228] In humans, four promoters (i.e., pI–pIV) with different *cis*-regulatory elements regulate CIITA expression in specific cell types.[229] For example, pIV drives constitutive expression of MHC class II in thymic epithelial cells as well as IFN-γ-induced CIITA expression in endothelial cells, epithelial cells, fibroblasts, and astrocytes. Thus, during tissue inflammation, nonconventional myeloid cell populations that include neutrophils, basophils, and eosinophils, as well as certain epithelial, endothelial, and stromal cell populations can upregulate class II expression. Because most of these observations have been conducted in mice, it remains unclear whether these atypical class II–expressing cells contribute significantly to T cell activation and function in humans.[230]

Mutations in the *CIITA* gene give rise to bare lymphocyte syndrome type II, an important human primary immunodeficiency syndrome associated with profound immunosuppression, since circulating peripheral blood mononuclear cells do not express surface MHC class II molecules (see Table 6.1).[231] Other causes of bare lymphocyte syndrome type II include mutations in *RFX5, RFXANK,* and *RFXAP* (see Table 6.1).[232,233]

The MHC locus also contains genes associated with the antigen-processing pathways. These include genes that encode several proteasome subunits (low-molecular-mass polypeptides 2 and 7 [LMP-2, LMP-7] and multicatalytic endopeptidase complex–like 1 [MECL-1]), the peptide transporter associated with antigen processing 1 and 2 (TAP1 and TAP2) proteins, and the MHC class II processing–associated HLA-DM and HLA-DO proteins (Fig. 6.6).

A cardinal feature of the MHC is the polymorphism of some of its genes, particularly MHC class I and class II. For the MHC class I HLA-B gene, there are more than 2000 known alleles.[224] Although some of these alleles bind similar peptides, most, by virtue of their morphologically distinct peptide binding grooves, bind distinct families of peptides. An evolutionary force driving the diversity of MHC alleles comes from the microbial world and its ability to undergo antigenic variation.[234,235]

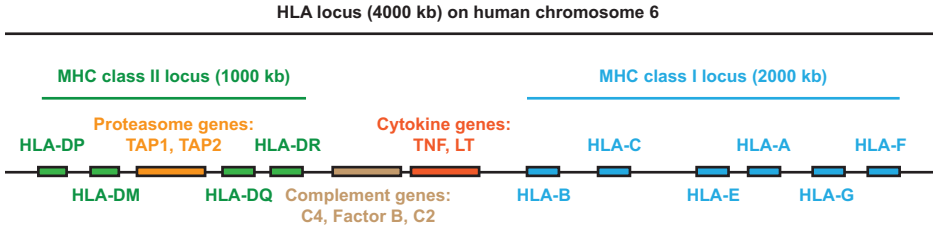

FIG. 6.6 Genes of the major histocompatibility complex *(MHC)* locus. Schematic map of the 4000-kb human MHC on chromosome 6, termed the *human leukocyte antigen (HLA) complex,* and major genes that encode proteins involved in antigen presentation, processing, and immune responses (drawing is not to scale). The MHC class II genes encode distinct α- and β-chains. *LT,* Lymphotoxin; *TAP,* transporter associated with antigen processing; *TNF,* tumor necrosis factor.

Human population studies in malaria-endemic regions demonstrated that certain HLA alleles are associated with a greater ability to survive malaria.[236] Conversely, malaria has a contravailing ability to alter protein sequences bound by HLA molecules, allowing parasite populations to evolve that are invisible to individuals with certain HLA molecules.[237] This escape strategy has been described for viral pathogens,[238,239] including HIV.[240]

The sequencing of the human genome, the advent of microarray-based, high-throughput genotyping technology, and the completion of the international HapMap project have facilitated numerous genome-wide association studies for human susceptibility to infectious agents.[23] Genome-wide association studies have identified significant associations of polymorphisms in MHC class I genes with HIV control, HIV viral load at set-point, and long-term nonprogression,[241] and in MHC class II genes with susceptibility to leprosy[242] and hepatitis B.[243]

The MHC complex also encodes proteins for MHC class I molecules that, unlike HLA-A, HLA-B, and HLA-C, are not highly polymorphic and generally highly conserved among individuals. These molecules also are called *MHC class Ib* molecules and in humans consist of HLA-E, HLA-F, and HLA-G (see Fig. 6.6). HLA-G is highly expressed in the placenta, regulates fetomaternal tolerance, and may protect cells from NK-cell–mediated lysis.[244] HLA-E binds the signal sequences of conventional MHC class I molecules, providing a readout of cellular synthesis of MHC class I molecules. HLA-E also binds to two NK-cell receptors, CD94/NKG2B and CD94/NKG2C, that inhibit NK-cell–mediated cytolysis.[245,246]

Antigen Presentation to T Lymphocytes via MHC Class I

Classic studies revealed that T lymphocytes recognize antigens in the context of MHC haplotype-matched APCs that degrade internalized pathogen-derived proteins and transport microbial antigens to the cell surface.[247,248] T cells respond to peptide fragments of pathogen-derived proteins, a process that can be recapitulated with corresponding short synthetic peptides.[249,250]

The precise nature of MHC involvement in antigen presentation became clear with the crystallization of HLA-A2, which showed a globular protein with a central groove that precisely accommodates a solitary peptide.[251,252] The identification of the TCR was the other key development that allowed a complete picture of the T-cell recognition process.[253] The TCR, a heterodimeric membrane-spanning receptor, provides diversity and specificity by a gene recombination process that is mechanistically similar to the generation of antibody diversity in B cells. The following sections describe the structure of MHC molecules and the process by which pathogen-derived peptides are presented to TCRs expressed by CD4 and CD8 T cells.[225]

MHC Class I Structure

MHC class I molecules present peptide antigens to CD8 T lymphocytes. MHC class I molecules are transmembrane proteins that consist of a single α-chain and associate with β_2-microglobulin for proper folding and trafficking to the cell surface. The characteristic structural features of MHC class I molecules are the α1, α2, and α3 domains. These create a globular protein in which a β-pleated sheet forms the floor of the peptide binding groove and two helical regions form the sides of the groove.[251,252] An important feature of the MHC class I groove is the restricted size of the peptide, typically 9 amino acids in length, that can be accommodated since the groove is closed at both ends[254] (Fig. 6.7).

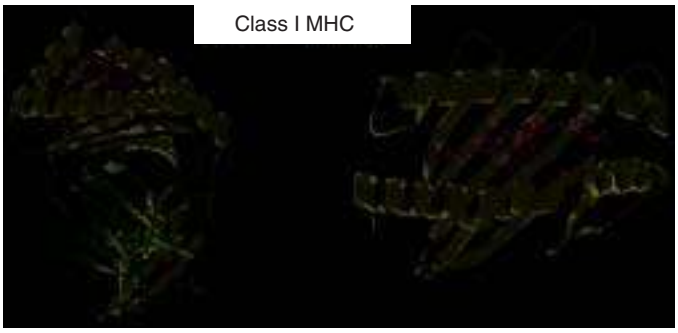

FIG. 6.7 Structure of class I major histocompatibility complex *(MHC)* with bound peptide. MHC class I molecules bind short antigenic peptides in a central groove formed by two α-helices of the heavy chain. The T-cell receptor binds the peptide and also associates with the MHC class I molecule in order to transmit an activation signal to the T lymphocyte. The depicted structures represent a murine H2-K^b MHC class I molecule *(purple)* that is binding a peptide derived from vesicular stomatitis virus *(green).* β_2-Microglobulin is shown in pink. The left panel shows a side view of the complex while the right panel shows a top view of the complex as it would be detected by a T-cell receptor on a CD8 T cell. *(Courtesy Dr. Chris Garcia, Stanford University, Palo Alto, CA.)*

Although binding of peptides and interaction with TCRs is mediated entirely by the MHC class I protein, stable surface expression of peptide–MHC class I complexes requires the association with β_2-microglobulin. In the absence of β_2-microglobulin, most MHC class I molecules fold improperly and are destroyed before leaving the endoplasmic reticulum (ER).[255]

MHC Class I Antigen-Processing Pathway

Antigens presented by MHC class I and class II molecules generally derive from different cellular compartments. The MHC class I antigen-processing pathway begins in the cytosol with the degradation of a protein that, in most cases, is an endogenous self-protein, or in the setting of infection, a microbial-encoded protein (Fig. 6.8).[225] In addition, exogenous proteins internalized into membrane-bound compartments by endocytosis, phagocytosis, or autophagy can enter the MHC class I processing pathway by a mechanism termed *cross-presentation.*[196]

In the cytosol, protein turnover and degradation is primarily mediated by the *ubiquitin-proteasome* pathway. Proteasomes are barrel-shaped multicomponent proteases that consist of four stacked rings with a heptameric structure; they hydrolyze cellular proteins in the cytosol. In activated cells or after exposure to IFN-γ, the proteasome composition can change, with some components replaced by subunits that enhance the generation of antigenic peptides and other components added to the ends of the barrel to alter the efficiency and specificity of protein degradation.[225] Proteasomes target endogenous and exogenous proteins for degradation by a variety of mechanisms. Misfolded proteins and

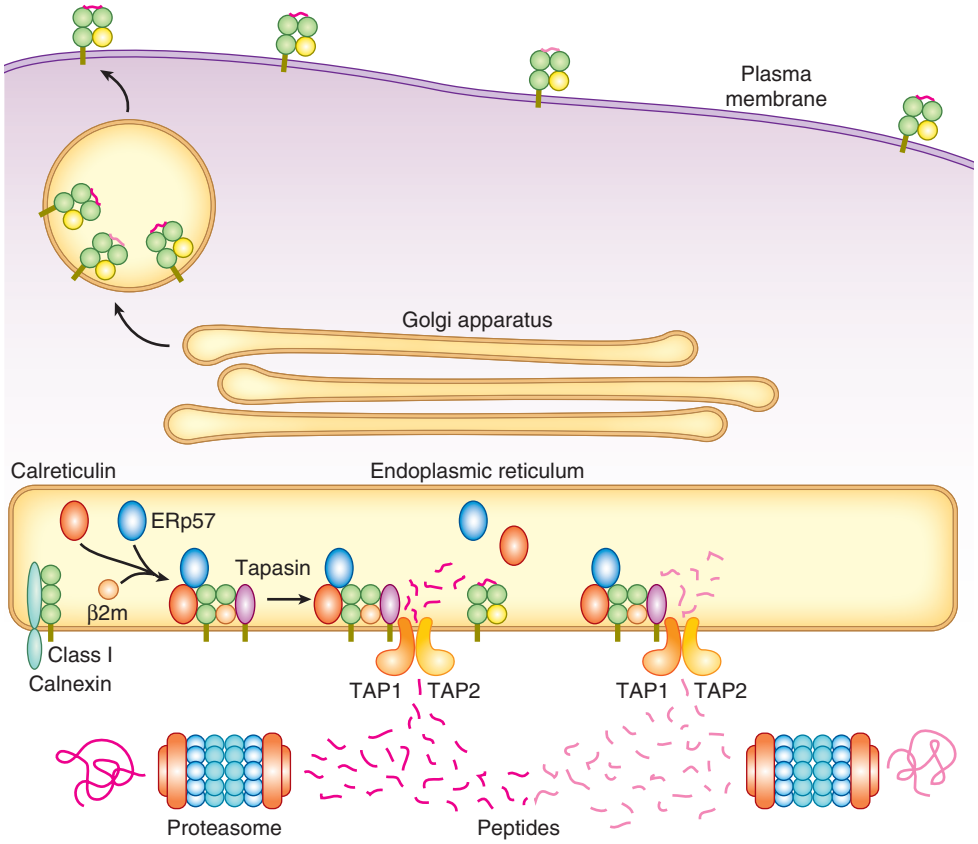

FIG. 6.8 The major histocompatibility complex (MHC) class I antigen-processing pathway. The MHC class I antigen-processing pathway begins in the cytoplasm with the degradation of proteins by proteasomes. Peptides are transported into the endoplasmic reticulum *(ER)* lumen, where MHC class I molecules bind and transport peptides to the cell surface. *β2m,* β_2-Macroglobulin; *ERp57,* endoplasmic reticulum protein 57; *TAP,* transporter associated with antigen processing. *(Courtesy Anne Ackerman and Peter Cresswell of Yale University, New Haven, CT.)*

defective ribosomal products, both of which fail to assume native conformation states, may reveal peptide sequences that are recognized by proteasomes, leading to their rapid degradation. Alternatively, many proteins targeted for rapid proteasomal degradation undergo conjugation to ubiquitin by enzymes that recognize the phosphorylation of specific amino acid residues.

In the setting of infection, it is unclear whether pathogen-derived polypeptides and proteins are degraded selectively and targeted for presentation by MHC class I molecules. Bacterial proteins in the cytosol are degraded rapidly because they express unique amino-terminal amino acids[256] or contain internal amino acid sequences that promote rapid degradation.[257] In most circumstances, however, pathogen-derived antigens probably are degraded nonselectively with endogenous proteins and must compete with far more prevalent endogenous peptides for a place in an MHC class I peptide-binding groove.

Proteasomes generate peptides that, by virtue of the length of the proteasome channel, are 9 to 12 amino acids in length. Proteasome-generated peptides are bound to the peptide transporter TAP. This heterodimeric, ATP-dependent transporter efficiently moves peptides from the cytosol into the lumen of the ER. TAP is the major peptide transporter involved in the generation of peptide–MHC class I complexes; mice with genetic deletions of TAP have markedly decreased levels of surface MHC class I and markedly diminished numbers of CD8 T cells.[258] TAP deficiency has been identified rarely in humans; it underlies bare lymphocyte syndrome type I, and is associated with markedly decreased numbers of circulating CD8 T cells and modest immunodeficiency (see Table 6.1).[259,260]

The TAP1 and TAP2 molecules each contain seven transmembrane regions and an ATP binding site and together transport peptides from the cytosol into the ER lumen.[261] Newly synthesized MHC class I molecules associate with TAP in the ER, and the recruitment of several other ER resident proteins and chaperones leads to the formation of the *peptide loading complex* (PLC). The PLC includes peptide-receptive MHC class I–/β_2-microglobulin complexes that are recruited by the chaperone calreticulin to the TAP channel that translocates antigenic precursor peptides into the ER. Tapasin and the thiol reductase ERp57 serve as an editing module to enable the ER resident protease ERAP (humans) or ERAAP (mice) to trim the peptide precursors before their final integration into the MHC class I groove.[262–264] The key role of β_2-microglobulin and of the PLC is to maintain the MHC class I binding groove in a conformation that favors the binding of high-affinity peptides. If the affinity of the peptide–MHC class I interaction is sufficiently high, the MHC class I–β_2-microglobulin–peptide complex is released from the PLC, allowing the complex to traffic via the Golgi complex to the cell surface. Low-affinity peptide–MHC class I interactions result in the reglucosylation of an *N*-linked glycan on the MHC class I heavy chain,[265] a reaction that redirects MHC class I molecules into the PLC for peptide exchange.

In the setting of microbial infection and inflammation, the MHC class I antigen-processing pathway is enhanced, allowing more efficient presentation of pathogen-derived peptides to CD8 T cells. IFN-γ, in particular, impacts the MHC class I pathway at multiple levels. First, IFN-γ enhances the transcription of many components of the MHC class I pathway, including MHC class I molecules, TAP, tapasin, and several components of the proteasome. Specifically, three subunits of the proteasome—LMP-2, LMP-7, and MECL—are induced and replace three subunits of the core proteasome complex.[225] IFN-γ induces additional accessory proteins (e.g., PA-28) that can increase the efficiency with which virus-derived, MHC class I–restricted epitopes are presented to CD8 T cells.[266]

Viral Interactions With the MHC Class I Antigen-Processing Pathway

CD8 T lymphocytes and the MHC class I antigen-processing pathway are involved principally in defense against viral infection. Viruses have evolved multiple strategies to evade MHC class I–dependent presentation, illustrating the importance of this process in antiviral defense. Broadly speaking, viruses can interfere with the peptide loading process, induce MHC class I–peptide complex retention in the ER, stimulate MHC class I–peptide complex degradation, and reroute MHC class I–peptide complex for concealment.[267]

To interfere with the peptide loading process, herpes simplex virus ICP47 blocks human TAP by plugging the peptide transport channel from the cytosolic side.[268,269] The human cytomegalovirus (CMV)–encoded protein US6 blocks TAP transport by obstructing the peptide transporter from the ER luminal side.[270,271] CMV also encodes a microRNA (i.e., miR-US4-1) that downregulates ERAP messenger RNA and prevents proteolytic processing of antigenic peptides in the PLC.[272]

The CMV US3 protein binds MHC class I molecules in the ER and prevents their trafficking to the cell surface.[273,274] A similar strategy is used by adenoviruses, which encode the type I membrane protein E3-19K.[275,276] This protein binds MHC class I molecules in the ER lumen and prevents their egress from the ER by expressing an ER retention motif on its cytoplasmic tail.

Another strategy for downregulating surface MHC class I retention is to displace ER-resident MHC class I molecules into the cytoplasm, where they are ubiquitinated rapidly by E3 ligases and targeted for proteasomal degradation. Human CMV encodes two proteins, US2 and US11, that mediate this process.[276,277] Remarkably, transport of proteins from the ER lumen back to the cytosol via the Sec61 translocon is a normal process that usually is restricted to misfolded or otherwise nonfunctional proteins. US2 and US11 seem to accelerate this process selectively for MHC class I molecules.

The Kaposi sarcoma herpesvirus also downregulates surface MHC class I expression, but by another mechanism. Two Kaposi sarcoma herpesvirus–encoded proteins, K3 and K5, function as ubiquitin ligases that selectively conjugate ubiquitin to the cytoplasmic tail of MHC class I and CD86/B7.2 molecules.[278,279] On ubiquitination, surface MHC class I molecules are internalized rapidly and targeted for lysosomal degradation. HIV also has evolved mechanisms to downregulate surface expression of MHC class I molecules.[280–282] In this case, the retrovirally encoded Nef protein selectively downregulates the expression of HLA-A and HLA-B molecules by associating with the clathrin adaptor complex.

A consequence of MHC class I downregulation is that affected cells become susceptible to NK cell–mediated lysis. NK cells express receptors that inhibit NK cell activation on contact with MHC class I molecules. To prevent NK cell–mediated lysis of virally infected cells, CMV encodes an MHC class I–like molecule, UL18,[283,284] that acts as a decoy for the NK cell inhibitory receptor LIR-1, providing yet another level of camouflage to the viral pathogen.

MHC Class I Cross-Presentation

The MHC class I antigen-processing pathway performs two principal functions. First, it presents antigens to naïve CD8 T cells in a manner that promotes their activation, proliferation, and differentiation. Second, it presents antigens to activated CD8 T cells as a signal of cellular infection. The first function is predominantly, if not exclusively, mediated by DCs.[285] Any MHC class I–expressing cell that becomes infected can perform the second function. The rules of antigen processing differ in these two circumstances. The conventional MHC class I antigen-processing pathway, as described in the preceding sections, applies to the second function.

Because they often are not infected directly, the major route for CD8 T-cell priming involves uptake of debris from infected cells by DCs, and re-presentation of pathogen-derived peptides[286] by an antigen-processing pathway that involves endocytosis and TAP-mediated transport of antigen into the ER.[287] The $CD8^+$ subset of DCs is particularly efficient at taking up and delivering exogenous antigens for cross-presentation by the MHC class I antigen-processing pathway. Antigen-containing phagosomes in DCs fuse with ER membranes, resulting in the recruitment of a retrotranslocation machinery that shuttles misfolded proteins or antigens from the phagosome lumen into the cytosol, where proteins are degraded by proteasomes and enter the conventional MHC class I processing pathway.[288]

A second route of cross-presentation involves a vacuolar pathway that does not require proteasomes or TAP. In this route, peptides are generated by cathepsin- or lysosomal protease–mediated hydrolysis in the endosome and loaded in situ on MHC class I complexes that traffic to the phagosome in a Sec22-dependent manner.[196,289]

Antigen Presentation to T Lymphocytes via MHC Class II

MHC Class II Structure

MHC class II molecules present peptide antigens to CD4 T lymphocytes. The folded MHC class II molecule consists of two transmembrane proteins, termed the α-chain and the β-chain. The α1 and β1 domains of each chain together form an open-ended peptide-binding groove.[290] The remaining α2 and β2 domains of each chain form an extracellular immunoglobulin–like domain. The open-endedness of the MHC class II groove accounts for the binding of substantially longer peptides than seen with MHC class I molecules.[291] Peptides bound by MHC class II molecules typically are longer than 10 amino acids, and occasionally are more than 20 amino acids in length. The structure of an MHC class II molecule binding an antigenic peptide is shown in Fig. 6.9.

MHC Class II Antigen-Processing Pathway

Although similarities to the MHC class I antigen-processing pathway exist, there are some key distinctions. First, most peptides presented by MHC class II molecules derive from extracellular proteins that have been endocytosed by MHC class II–expressing cells.[292] MHC class II molecules also present peptides from membrane or secretory proteins that are degraded in endosomal compartments during transport to the cell surface (Fig. 6.10). The MHC class II antigen-processing pathway has been implicated principally in the response to extracellular pathogens and pathogens that reside within vacuolar compartments, such as *S. typhimurium* and *M. tuberculosis*.

The first step in the MHC class II antigen-processing pathway is the translocation and assembly of MHC class α- and β-chains in the ER, a reaction mediated by a dedicated chaperone, the invariant chain. The α- and β-chains fold and use the invariant chain, a membrane-bound protein, as a substitute for a peptide. A portion of the invariant chain occupies the MHC class II groove as the complex exits the ER and traffics to the endosomal compartments. When endosomal compartments acidify, proteases (e.g., cathepsin B and D) are activated and hydrolyze the invariant chain except for the portion protected by the MHC class II groove.[293]

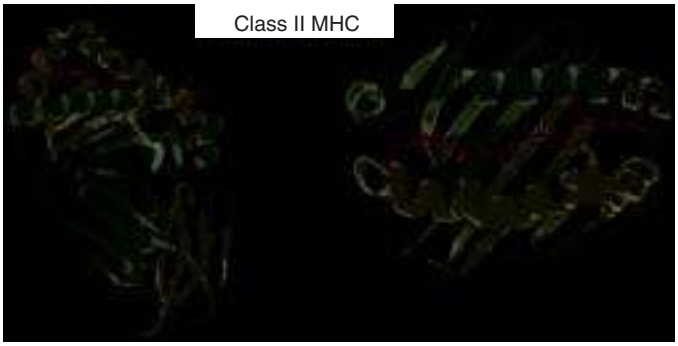

FIG. 6.9 Structure of class II major histocompatibility complex *(MHC)* with bound peptide. MHC class II molecules also bind antigenic peptides in a groove formed by the α- and β-chains. In contrast to peptides bound by MHC class I molecules, which are of defined length, the MHC class II structure accommodates peptides of various lengths. The MHC class II α- and β-chains are shown in pink and purple, while the antigenic peptide is shown in green. The left panel shows a side view of the complex while the right panel shows a top view of the complex as it would be detected by a T-cell receptor on a CD4 T cell. *(Courtesy Dr. Chris Garcia, Stanford University, Palo Alto, CA.)*

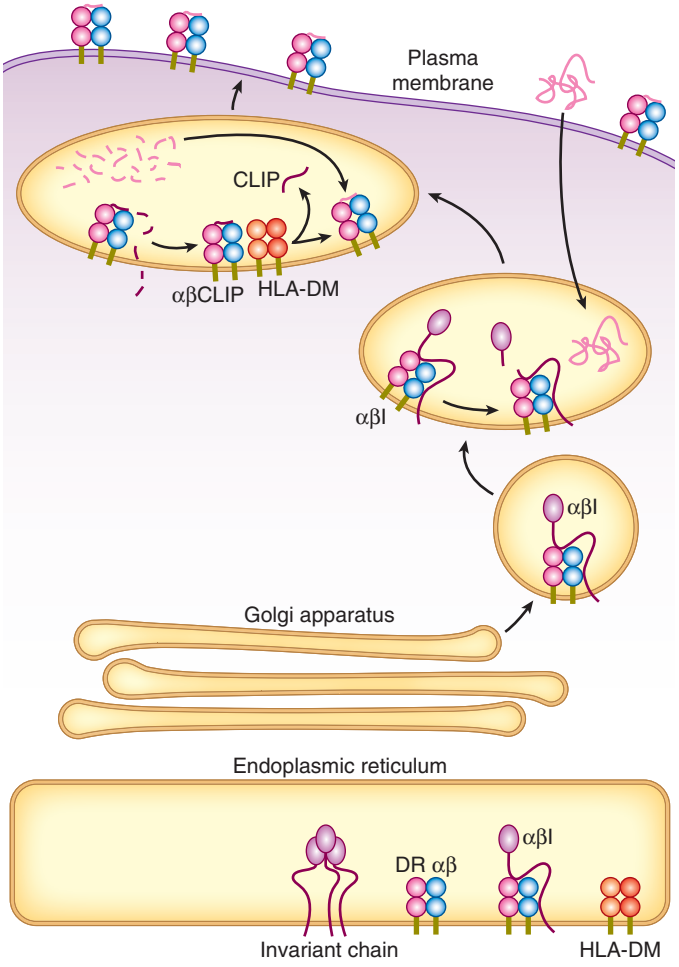

FIG. 6.10 The major histocompatibility complex (MHC) class II antigen-processing pathway. The MHC class II antigen-processing pathway presents peptides derived from extracellular proteins to $CD4^+$ T lymphocytes. Peptides are generated in an endosomal compartment. The peptide editor human leukocyte antigen DM *(HLA-DM)* promotes dissociation of the placeholder class II–associated invariant chain peptide *(CLIP)* from MHC class II molecules, allowing the exogenous peptide to associate with MHC class II. The MHC class II–peptide complexes are then transported to the cell surface. *(Courtesy Anne Ackerman and Peter Cresswell of Yale University, New Haven, CT.)*

In the acidified endosome, in a compartment called the *MCH class II–positive compartment (MIIC),* MHC class II molecules can interact with endocytosed antigens in the process of degradation.[294,295] An MHC class II–like molecule, HLA-DM, resides in MIICs and catalyzes the extraction of the invariant chain peptide fragment from the MHC class II molecule. Crystallographic studies reveal that HLA-DM stabilizes empty MHC class II proteins in a conformation that promotes the insertion of a high-affinity peptide.[296] In addition to proteases, a thiol reductase called *γ-interferon–inducible lysosomal thiol reductase (GILT)* is involved in the denaturation of some antigens before their degradation and presentation by MHC class II molecules.[297] GILT activates the major secreted virulence factor listeriolysin O, of the bacterial pathogen *L. monocytogenes,* providing an example of microbial exploitation of the MHC class II antigen-processing pathway.[298] Following peptide binding in the MIIC, MHC class II molecules traffic to the cell surface, where the MHC class II–peptide complex can be detected by CD4 T cells. MHC class II molecules are subsequently reinternalized, recycle to the endosomal compartments, and acquire new peptides before returning to the cell surface.

CD1 and Antigen Presentation

The CD1 family comprises antigen-presenting molecules that resemble MHC class I molecules in their general structure and association with β_2-microglobulin.[299] The human CD1 locus on chromosome 1 contains five distinct genes that encode for proteins designated CD1a through CD1e. In contrast to humans, mice lack CD1a, CD1b, and CD1c and have a duplicated CD1d gene. The extracellular portion of CD1 is composed of three domains that mediate antigen binding and form an antigen-binding groove that is structurally analogous to the peptide-binding groove of the MHC. The three-dimensional structure of mouse CD1[300] and human CD1 isoforms a through d[301] reveals a complicated network of hydrophobic channels that can accommodate diverse lipids of varying aliphatic chain length.[302]

The CD1 isoforms are enriched in different intracellular compartments, suggesting that each isoform of CD1 has evolved to survey the microbial antigens that appear in distinct parts of the endosomal-lysosomal network. All CD1 isoforms are found at the cell surface and internalized during endocytosis. CD1a is found predominantly in early endosomes, CD1c in late endosomes, and CD1b and CD1d in late endosomes and lysosomes. Specific amino acid residues in their short intracellular tails target CD1 isoforms to these various compartments by binding cytosolic adaptor molecules that mediate organelle trafficking.[303–305]

CD1b presents glycosylated and free mycolic acids and lipoarabinomannan, two major lipid and glycolipid components of the *M. tuberculosis* cell envelope. CD1-presented antigens have expanded to include other microbial glycolipids, including diacylglycerols from *Streptococcus pneumoniae*[306] and the fungal glycosphingolipid asperamide B.[302,307] Thus CD1 isoforms facilitate cell-mediated defense against infection by presenting a wide range of microbial lipidic antigens to CD1-restricted T cells.

INNATE IMMUNE RECOGNITION: SETTING THE STAGE FOR T-CELL RESPONSES

The adaptive immune system consists of B and T lymphocytes that express distinct, somatically recombined receptors with exquisite specificity, at the cost of low frequency. In contrast, the innate immune leukocytes (i.e., neutrophils, monocytes, macrophages, and DCs) express families of germline-encoded receptors that survey the cell surface, the cytosol, and endosomal or phagosomal compartments, and recognize a wide array of microbial-derived molecules (Fig. 6.11). It is clear from studies on the genetic basis of human susceptibility to infectious diseases and from studies in mice that innate immune responses to microbial molecules, by promoting the expression of costimulatory molecules and the secretion of cytokines, form the foundation for adaptive immune responses (see Table 6.1).

The molecular basis for antigen-specific responses derives from combinatorial receptors (i.e., αβ TCRs) that have almost infinitely diverse specificity. This system ensures that a great diversity of pathogenic antigens can be recognized, but, because pathogen-specific cells are infrequent, it requires time for expansion of these cells to numbers that are sufficient to combat the infection. In the hours after a pathogen breach of an anatomic barrier, the most rapid recognition events are mediated by cells bearing innate immune receptors. These receptors are not combinatorial, but recognize conserved structural elements in broad classes of microbial molecules that serve as a general signal of infection. This recognition event, rapid but relatively nonspecific, plays an essential role in the generation of pathogen-specific immunity through the generation of cytokines and chemokines that recruit APCs to the site of infection and shape the ensuing adaptive immune response. This section details the major known receptors of the innate immune system and links the function of these receptors to antigen-specific cellular immunity.

Molecular Recognition of Microbial Products

Several outstanding reviews provide a comprehensive overview on the molecular recognition of microbial products and its role on human susceptibility to infectious diseases.[308–314]

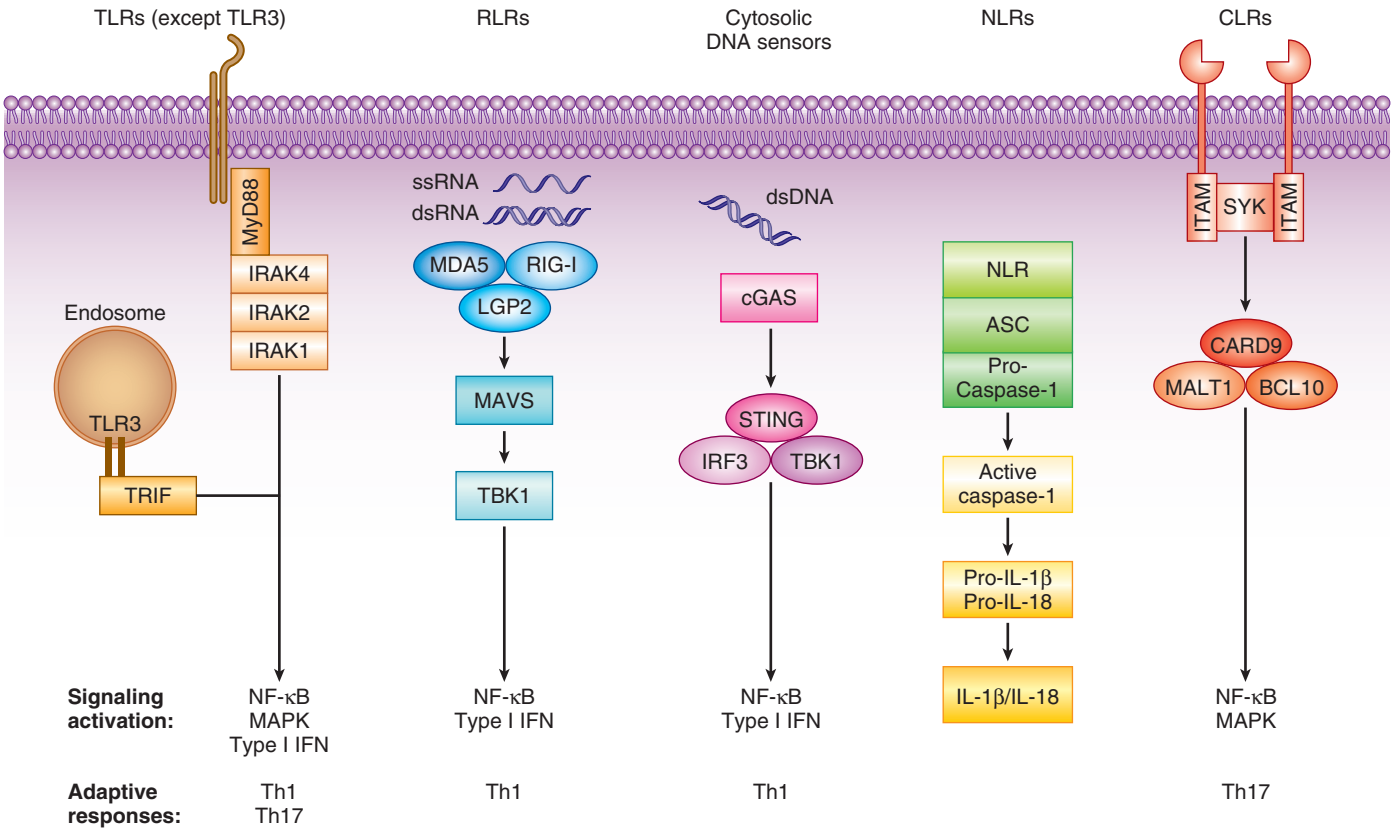

FIG. 6.11 Cell surface and intracellular classes of pattern recognition receptors (PRRs) and associated signaling pathways and adaptive responses. Toll-like receptors *(TLRs)* are present on the cell surface or in the endosome (TLR3, TLR7, TLR9). Most TLRs utilize the MyD88 protein, with the exception of TLR3, which utilizes TRIF, and TLR4, which utilizes both MyD88 and TRIF, as adaptor proteins for downstream signaling. TLR engagement induces NF-κB, MAPK, or type I IFN signaling, or a combination of these, and helper T cell Th1 or Th17 responses, depending on the pathogen. The RLR family of PRRs recognizes RNA ligands and consists of MDA5, RIG-I, and LGP2. RLRs signal through MAVS, an adaptor on the mitochondrial surface, and TBK1 to induce NF-κB and type I IFN signaling and Th1 responses. The cytosolic DNA sensor cGAS signals via the ER adaptor protein STING, which associates with TBK1 and IRF3 to induce NF-κB and type I IFN signaling and Th1 responses. NLRs are cytosolic PRRs, several of which are members of the inflammasome complex, which includes the adaptor protein ASC and pro-caspase 1. Inflammasome activation results in the activation of caspase 1, which leads to IL-1β and IL-18 release. CLRs are surface ITAM-bearing receptors that bind carbohydrates and signal via SYK and the CARD9–MALT1–BCL10 complex to induce NF-κB and MAPK signaling and Th17 responses. *ASC,* apoptosis-associated speck-like protein containing CARD; *BCL10,* B-cell lymphoma/leukemia 10; *CARD9,* caspase recruitment domain–containing protein 9; *cGAS,* cyclic GMP-AMP synthase; *CLR,* C-type lectin receptor; *dsRNA,* double-stranded RNA; *ER,* endoplasmic reticulum; *IFN,* interferon; *IL,* interleukin; *IRAK,* interleukin-1 receptor–associated kinase; *IRF3,* interferon regulatory factor 3; *ITAM,* immunoreceptor tyrosine-based activation motif; *LGP2,* laboratory of genetics and physiology 2; *MALT1,* mucosa-associated lymphoid tissue lymphoma translocation protein 1; *MAPK,* mitogen-activated protein kinase; *MAVS,* mitochondrial antiviral-signaling protein; *MDA5,* melanoma differentiation–associated gene 5; *MyD88,* myeloid differentiation primary response gene 88; *NF-κB,* nuclear factor kappa-light-chain-enhancer of activated B cells; *NLR,* nucleotide-binding oligomerization domain–like receptor; *RIG-I,* retinoic acid–inducible gene 1; *RLR,* RIG-I–like receptor; *ssRNA,* single-stranded RNA; *STING,* stimulator of interferon genes; *SYK,* spleen tyrosine kinase; *TBK1,* TANK-binding kinase 1; *TRIF,* TIR domain–containing adapter inducing interferon-β.

Toll-Like Receptors

Toll-like receptors (TLRs) are a family at least 10 distinct transmembrane proteins that mediate the recognition of extracellular (i.e., TLR1, TLR2, TLR4, TLR5, TLR6) and endosomal (i.e., TLR3, TLR7, TLR8, TLR9) microbial products. The TLR ectodomains contain leucine-rich repeats that bind to a broad range of microbial products, and an intracellular Toll–IL-1 (TIR) domain that activates downstream signal adaptors, transducers, and kinases, including the IL-1 receptor–associated kinase (IRAK) complex, typically resulting in the activation of nuclear factor kappa B (NF-κB), a transcription factor that promotes the expression of genes associated with immune defense.[308] TLR-mediated signals induce the secretion of proinflammatory cytokines, such as TNF and IL-12, and induce the maturation of DCs, enabling them to activate naïve, pathogen-specific T lymphocytes. In addition, stimulation of TLRs can directly stimulate antimicrobial effector mechanisms of the host cell, limiting pathogen replication until adaptive immune cells are recruited to the site of infection.[315]

Each TLR recognizes specific microbial products, and well-defined ligands include bacterial lipoproteins (TLR1, TLR2, TLR6), double-stranded RNA (TLR3), lipopolysaccharide (TLR4), the bacterial peptide flagellin (TLR5), single-stranded RNA (TLR7 and TLR8), and CpG motifs within DNA (TLR9). TLR heterodimerization can mediate novel antigen-binding functions and increase the microbial specificity of individual TLR family members. For example, TLR2 heterodimerizes with TLR1 or TLR6 to recognize triacylated or diacylated lipoproteins, respectively.[316] Structural studies have demonstrated how microbial ligands associate with different TLR proteins, revealing a common pattern of TLR dimerization induced by association with microbial ligands.[317,318]

With the exception of endosomal TLR3, which utilizes the alternative pathway adaptor molecule TIR domain–containing adapter inducing IFN-β (TRIF),[319] most TLRs signal through the adaptor protein myeloid differentiation primary response gene 88 (MyD88); TLR4 uses both MyD88 and TRIF. Children who have autosomal-recessive defects in MyD88-mediated signal transduction due to mutations in *MYD88* or *IRAK4,* two very rare primary immunodeficiencies, lack input from most TLRs and from IL-1 receptor family members and develop severe and life-threatening pyogenic bacterial infections, particularly invasive streptococcal, staphylococcal, and pseudomonal infections (see Table 6.1).[320,321] More commonly, polymorphisms in TLR coding sequences and regulatory elements can confer susceptibility to infectious diseases,[322] as illustrated by the association of a common TLR5 stop codon polymorphism with susceptibility to the flagellated bacterium *Legionella pneumophila.*[323]

Patients with deficiency in TLR3 itself or the ER protein UNC-93B, the adaptor TRIF, the TNF receptor–associated factor 3 (TRAF3), or the TRAF-associated NF-κB activator (TANK)–binding kinase 1 (TBK1), all of which are involved in TLR3 activation,[324–326] develop selective susceptibility to herpes simplex encephalitis (see Table 6.1). The distinct infection susceptibility pattern of MYD88/IRAK4 (bacterial) versus TLR3-pathway (herpetic) defects illustrates the pathogen specificity of and host tissue requirements[327] for individual TLRs in host defense. Elegant recent studies using induced pluripotent stem cell–derived neurons and oligodendrocytes from TLR3- and UNC-93B–deficient individuals revealed that impaired IFN-α/β–dependent intrinsic immunity to HSV-1 by these cells underlies the pathogenesis of herpes simplex encephalitis in children with TLR3 pathway deficiencies.[327]

NOD-Like Receptors, RIG-I–Like Receptors, the Cyclic GMP-AMP Synthase–Stimulator of Interferon Genes Sensing Pathway, and the Inflammasome

While TLRs predominantly respond to extracellular or to endosomal microbial ligands, NLR proteins detect microbial ligands in the cytosol.[309] Among the best characterized of these, NOD1 and NOD2 respond to fragments of bacterial peptidoglycan, while NLRC4 responds to bacterial flagellin in the cytosol.[328,329] NLRs activate NF-κB signaling, and assemble into multicomponent structures called inflammasomes that consist of an NLR, an adaptor protein, and caspase subunits.[330] Inflammasomes activate a host cell apoptotic pathway and the expression of IL-1 and IL-18 by activating caspase-1 or caspase-8.[164] Macrophage caspase-1 activity is also important for phagosomal maturation.[331] Thus detection of microbial molecules in the cytosol induces an inflammatory response that has consequences for adaptive Th1 and Th2 cell responses.[332]

The RIG-I–like receptors (RLRs) retinoic acid–inducible gene 1 (RIG-I), melanoma differentiation–associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) are members of a family of molecules that detect cytosolic nucleic acids from RNA viruses (e.g., measles virus, influenza virus, hepatitis C virus, and West Nile virus).[311] RLRs signal via the adaptor protein mitochondrial antiviral-signaling protein (MAVS, also known as IPS1/VISA/Cardif) on the mitochondrial surface and collaborate with other pattern recognition receptors to induce antiviral responses.[333–337] For example, inherited MDA5 deficiency is linked to recurrent rhinovirus, influenza, and respiratory syncytial virus infections (see Table 6.1).[338] Influenza and other viruses can subvert cytosolic detection systems by inhibiting RLR signal transduction and targeting components of this pathway for degradation.

Detection of cytosolic DNA, specifically to cyclic dinucleotides, occurs via receptors that signal through the endoplasmic reticulum adaptor molecule stimulator of interferon genes (STING).[339] A central regulator of cytosolic DNA sensing is cyclic GMP-AMP synthase (cGAS), an interferon-inducible nucleotidyl transferase that has broad antiviral activity against HSV-1, vaccinia virus, and Kaposi sarcoma–associated herpesvirus (see Fig. 6.11).[340–343] Notably, these and other viruses can evade cytosolic sensing by disrupting DNA binding to cGAS or interfering with downstream STING phosphorylation, or both.[313,314] RLR- and cGAS/STING-dependent signaling events, either alone or via their cross talk, turn on NF-κB and type I interferon signaling in both infected and bystander cells.[313,314] The role of cytosolic sensors of viral nucleic acids in shaping B- and T-cell responses remains less well defined, although emerging evidence implicates the cGAS-STING pathway in DC maturation, development of antigen-specific Th1 cells, and immunoglobulin G2c production.[344]

C-Type Lectin Receptors

C-type lectin receptors (CLRs) bind to carbohydrate moieties of endogenous and exogenous origin, trigger phagocytic responses, and activate innate immune cells, particularly of myeloid origin.[345,346] The binding specificity for some of these receptors has been elucidated and includes fungal, bacterial, mycobacterial, viral, and parasitic microorganisms.

Many CLRs signal via tyrosine-based motifs that are embedded in their intracellular domain or that are present in adaptor molecules that associate with CLRs. Other important components of this signaling pathway are spleen tyrosine kinase and CARD9, which forms a complex with mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) and B-cell lymphoma/leukemia 10 (BCL10) (see Fig. 6.11).[49] This pathway is central to the recognition of fungal β-glucans (via the CLR Dectin-1/Clec7a),[347] fungal mannans (via the CLRs Dectin-2/Clec4n in mice or Dectin-2/Clec6a in humans and Dectin-3/MCL/CLECSF8/Clec4d),[348,349] and ligands of *M. tuberculosis* (via Dectin-3),[350] *Malassezia* (via Dectin-2 and the CLR Mincle/Clec4e),[351] *B. dermatiditis* (via Dectin-2),[352] and *Fonsecaea pedrosoi* (via Mincle), the agent of chromoblastomycosis.[353] Dectin-1 and Dectin-2 ligation promotes macrophage and DC cytokine and chemokine release and favors the development of Th17 responses.[354]

Inborn errors of CLR-dependent signaling result in susceptibility to fungal infections (see Table 6.1). Biallelic mutations in *CLEC7A*, which encodes Dectin-1, are associated with chronic mucocutaneous candidiasis. CARD9 deficiency leads to greater fungal infection susceptibility to both mucosal and invasive mycoses with a unique predilection for central nervous system candidiasis, extrapulmonary aspergillosis, and subcutaneous and central nervous system phaeohyphomycoses. In these patients, mucosal fungal susceptibility correlates with impaired Th17 responses, whereas deep-seated fungal susceptibility is associated with impaired neutrophil recruitment to the site of infection.[355–358] In contrast to mutations in *CARD9* that result in fungal-specific infection susceptibility, deficiencies in its partners MALT1 or BCL10 cause mucosal candidiasis but also have far broader immune implications, and affected patients manifest with a severe combined immunodeficiency phenotype because these molecules are downstream components of T- and B-lymphocyte signaling cascades (see Table 6.1).[359]

Links Between Innate Immune Recognition and Adaptive Immune Responses

Antibody and T-cell responses to inoculation of protein antigens are minimal unless an innate inflammatory response is elicited concurrently. Thus adjuvants that induce innate inflammatory responses are coadministered with some vaccines.[360] Adjuvants can facilitate slow release of antigens at depot sites, promote the release of proinflammatory cytokines and chemokines, and facilitate the recruitment of APCs that mature by the induction of TLR, CLR, NLR, RLR, and inflammasome signaling pathways. This process enhances antigen processing and presentation capacity by the MHC class II pathway and facilitates the interactions with antigen-specific T and B lymphocytes to promote potent humoral and cellular responses that characterize vaccine immunity. In support of this model, early studies demonstrated that immunization with model antigens and Freund adjuvant led to impaired T-cell responses and abnormal isotype-specific antibody production in MyD88-deficient mice, suggesting that TLR recognition is essential for proper Th1 adjuvant effects.[361] DCs that directly engage in TLR signaling following stimulation with microbial products can promote the proliferation and differentiation of naïve T cells. In contrast, DCs that undergo indirect activation by exposure to cytokines elicited by TLR signaling only support T-cell proliferation, but not differentiation.[362,363]

More recent studies indicate that non-TLR signaling pathways can provide adjuvant effects and support T-cell priming and antibody generation. Alum, a commonly used adjuvant for human vaccines, enhances antibody production by stimulating the NLRP3 component of inflammasomes to produce IL-1 and IL-18.[364] The CLRs Mincle and Dectin-2 can drive adjuvant effects that result in the development of antigen-specific Th1 and Th17 cells following vaccination against mycobacterial or fungal pathogens.[352,365]

Dendritic Cells

Although other immune and nonimmune cells have antigen-presenting capacity, DCs are the major immune cell type that presents antigens to naïve T lymphocytes and promotes their proliferation and differentiation. In vivo elimination of DCs, as has been performed experimentally in mice, abrogates priming of naïve T cells that respond to microbial infections.[285] High surface levels of MHC class I and class II and the expression of an array of costimulatory molecules enable DCs to stimulate naïve T cells effectively. DCs are a highly complex and heterogeneous

population of cells that reside both in lymphoid and nonlymphoid tissues and share phenotypic and functional characteristics that distinguish these cells from other classes of myeloid cells.[366]

DCs derive from progressively committed pluripotent bone marrow precursors. In mice, a common DC precursor gives rise to circulating plasmacytoid DCs and pre-DCs. Plasmacytoid DCs express high levels of TLR7 and TLR9 and secrete very high levels of type I interferons on stimulation; these are critical to antiviral defense.[367,368] Pre-DCs give rise to CD8α$^+$ and CD11b$^+$ DC populations in lymphoid organs and to CD11b$^+$ and CD103$^+$ DCs in peripheral nonlymphoid tissues. A combination of transcriptomic and functional studies revealed that murine lymphoid tissue–resident CD8α$^+$ DCs and nonlymphoid tissue–resident CD103$^+$ DCs are the likely counterparts of human CD141$^+$ DCs, all of which are highly effective at antigen cross-presentation following viral, bacterial, and parasitic, but not fungal, infections.[369–374] Murine CD11b$^+$ DCs in non-lymphoid tissues are the likely homologue of human CD1c$^+$ DCs and mediate conserved mucosal Th17 responses across species.[375]

The role of DCs in antimicrobial defense extends beyond stimulation of T cells. In mice and humans, a common monocyte precursor gives rise to circulating monocytes that can differentiate into monocyte-derived DCs or macrophages at portals of infection.[376–382] Monocyte-derived DCs, often termed inflammatory DCs, express a range of TLRs, CLRs, and NLRs that induce their activation and result in their production of cytokines that can orchestrate helper T cell differentiation.[354,377,383,384] Furthermore, monocyte-derived DCs exert direct microbicidal activity at portals of infection, as has been demonstrated during pulmonary *A. fumigatus*, *B. dermatiditis*, and *K. pneumoniae* infection.[54,385–388]

DCs are also primarily infected in some settings. For example, CD11b$^+$ DCs and monocyte-derived DCs are major infected cell populations in murine tuberculosis and leishmaniasis.[377,389] In pulmonary aspergillosis and tuberculosis and in a vaccine model of blastomycosis, monocyte-derived DCs and CD11b$^+$ DCs played an important role in transporting microbial antigen to draining lymph nodes.[14,378,390,391] Trafficking of activated DCs into lymph nodes involves CCR7 upregulation on DCs, enabling them to respond to CCL19 and CCL21, which are expressed in the lymph node paracortex.[392] DC trafficking to draining lymph nodes has also been demonstrated following respiratory[393] and cutaneous viral infection,[394] as has trafficking from the marginal zone to the T-cell zone of the spleen following systemic bacterial infection.[395] With respect to systemic bacterial infections, DCs may play an important role in localizing bacteria to the spleen.[396,397]

In humans, autosomal-recessive or autosomal-dominant deficiency in interferon regulatory factor (IRF) 8, a transcription factor critical in the regulation of the myeloid lineage commitment, results in marked or selective depletion of the APC compartment, respectively (see Table 6.1). The recessive severe form of the disease results in complete absence of CD14$^+$ and CD16$^+$ monocytes and all DC subsets and manifests with mucosal candidiasis and nontuberculous mycobacterial infections.[398] The dominant milder form of the disease predisposes to nontuberculous mycobacterial infections and leads to selective absence of CD11c$^+$CD1c$^+$ DCs, which are major producers of IL-12 for development of protective Th1 responses against mycobacterial disease.[398]

MICROBIAL PATHOGENESIS AND THE CELLULAR IMMUNE SYSTEM

The diverse properties of microbial pathogens provide a challenge to the cellular immune system. Although the details of cellular immune responses to different pathogens vary substantially, some broad generalizations regarding cellular immune responses to microbial pathogens can be made. In most cases, the subcellular anatomic location of a pathogen (extracellular, endosomal/vacuolar, cytoplasmic) predicts the arm of the cellular immune response that is necessary to contain infection by that particular pathogen. In many cases, the most definitive information about the important arms of antimicrobial defense for a particular pathogen has come from inherited or acquired immunodeficiency states, in particular, defects in immune receptors or signaling molecules. In addition, because different classes of pathogens are associated with a particular type of cellular immune response, many pathogens have developed sophisticated molecular countermeasures to evade elimination.

Viral Infections

In general, viruses use the host cell for protein synthesis and thus viral proteins are ready substrates for the MHC class I antigen-processing pathway and CD8 T-cell activation. Due to intracellular viral replication, the cellular immune system eliminates viruses by cytolytic destruction of the infected host cell by virus-specific lymphocytes or by inhibition of viral replication in host cells.

There are several reports of congenital deficiency of CD8 T cells or MHC class I function caused by mutations in *CD8A, TAP1, TAP2, B2M,* or *ZAP70* (see Table 6.1)[259,260,399–401] These patients have reduced MHC class I surface expression or CD8 T cells, or both. In contrast to the canonical function presented for CD8 T cells in antiviral defense, these patients collectively do not manifest increased susceptibility to viral infections. Instead, they present with recurrent pulmonary bacterial infections and bronchiectasis in the presence or absence of prior pulmonary infections, and approximately 50% of them develop ulcerative granulomatous skin lesions infiltrated by activated NK cells.[402] These findings support the notion that NK cells may provide functional redundancy in the context of diminished CD8 T-cell–dependent cytolytic activity, yet cause tissue damage. Indeed, patients with inherited NK deficiency caused by mutations in *MCM4, GATA2, RTEL1,* or *FCGR3A* manifest with severe infections by herpesviruses, including HSV-1, varicella-zoster virus, CMV, and Epstein-Barr virus (EBV) (see Table 6.1).[403] Although congenital CD8 or MHC class I deficiency may be compensated by NK cells for effective viral surveillance, studies of MHC class I polymorphisms in HIV-infected patients support a role for CD8 T cells/MHC class I in the efficiency of viral infection control in humans.[241]

Some viruses become transcriptionally inactive after host cell infection (e.g., human herpesviruses) and represent a particular challenge for the cellular immune system. Although primary active infection with these viruses is controlled, herpesviruses establish clinical latency and can cause intermittent disease or disease in the setting of impaired immunity. Transcriptionally inactive proviral DNA is invisible to host T cells because viral proteins are not presented by MHC class I molecules and therefore these viruses cannot be detected by antigen-specific T lymphocytes. Although immunologic elimination of the infection is often impossible, immunologic control of intermittent reactivation is the rule.[404]

Beyond interfering with the MHC class I antigen-processing pathway, many viral pathogens interfere with cytokine signaling or chemokine-mediated cell recruitment. EBV contains a gene for an IL-10 homologue that binds the IL-10 receptor and induces immunosuppressive effects of this pleiotropic cytokine. Poxviruses express a high-affinity IL-18–binding protein that interferes with early IFN-γ–mediated inflammatory responses. Poxviruses and herpesviruses also produce virally encoded -C-, -CC-, and -CXC- chemokines or chemokine receptors, or a combination of these, that help evade the cellular immune response by one or more of three mechanisms: (1) disrupting the recruitment of monocytes, NK cells, and activated T cells to sites of viral infection, (2) promoting the recruitment of immune cells that support viral spread or latency, and (3) activating certain chemokine receptor axes that skew the cellular response from an antiviral Th1 state to a proviral Th2 state.[405]

Infections With Intracellular Bacteria and Fungi

Many important human pathogens have evolved mechanisms to escape antibody-, complement-, and neutrophil-mediated immune defense. One of the most effective escape mechanisms involves entering host cells, often phagocytic cells. In this circumstance, the host cell becomes a protective barrier from extracellular microbicidal defenses. Bacterial pathogens employ many strategies to exploit the interior of host cells to their benefit, by manipulating intracellular trafficking pathways or by targeting specific intracellular niches.[406,407] The challenge facing the cell-mediated immune system is to detect and eliminate these pathogens.

Phagosomal Pathogens

Multiple bacterial and fungal pathogens parasitize phagocytic cells, such as macrophages, and reside within the endosomal-phagosomal network.

These pathogens often are accessible to the MHC class II antigen presentation network and can colocalize with the antimicrobial effector molecules of macrophages that are delivered to the phagolysosome. Prototypical pathogens of this type include mycobacteria, endemic dimorphic fungi, and *Salmonella* spp. Control of these pathogens depends mostly on IL-12–dependent CD4 T-cell activation that results in IFN-γ release. In turn, IFN-γ activates macrophage effector mechanisms to kill pathogens through oxidative and nonoxidative mechanisms. The latter mechanisms, which remain poorly understood, are critical in humans since patients with chronic granulomatous disease who lack phagocytic oxidative burst are not susceptible to infections by these intracellular pathogens.[20] Mice deficient in IFN-γ are highly susceptible to intracellular pathogens, including *M. tuberculosis,* nontuberculous mycobacteria, *Histoplasma, Coccidioides, Cryptococcus, Toxoplasma,* and *Salmonella,* providing support for the importance of the Th1-mediated stimulation of antimicrobial killing of phagosomal pathogens.

In many parts of the world, infants receive bacillus Calmette-Guérin (BCG) vaccination. Although highly attenuated compared to its parental *M. bovis* strain, BCG can replicate within human hosts with impaired IL-12/IFN-γ–mediated immunity. This worldwide cohort of BCG-exposed infants has uncovered inherited defects in IL-12/IFN-γ–mediated immunity. The clinical syndrome is called *mendelian susceptibility to mycobacterial disease* (MSMD)[408] and includes patients with disseminated infection by BCG or other low-pathogenicity nontuberculous mycobacteria. These patients also show increased susceptibility to disseminated infections by endemic dimorphic fungi, *Cryptococcus* and *Salmonella*. Mutations that cause MSMD via impairing the IL-12/IFN-γ signaling axis involve the IFN-γ receptors 1 and 2, STAT1, IL-12p40 and its IL-12 receptor subunit β1, NF-κB essential modulator (NEMO), GATA2, IRF8, and interferon-stimulated gene 15 (ISG15) (see Table 6.1).[21,22,409–412] In agreement with these inherited defects, patients with neutralizing autoantibodies against IFN-γ exhibit an acquired immunodeficiency marked by disseminated infections with intracellular pathogens.[413] Similarly, the administration of TNF-α inhibitors results in susceptibility to disseminated mycobacterial disease and histoplasmosis.[414]

The data presented here collectively document the central role of the Th1 immunity and the CD4 T cell–macrophage cross talk in protection from and control of phagosomal pathogens. Notably, such pathogens have evolved countermeasures to dampen or subvert effective host immunity. For example, *M. tuberculosis* infection of macrophages renders these cells resistant to activation with IFN-γ.[415] *M. tuberculosis* also prevents acidification of vacuoles by excluding the proton–adenosine triphosphatase complex from the endosomes that it occupies. One possible outcome of diminished vacuolar acidification is decreased antigen degradation, resulting in diminished presentation of mycobacterial peptides by MHC class II molecules. Other pathogens, such as *Legionella pneumophila,* segregate themselves in an endosomal compartment that does not communicate with the MHC class II antigen-processing pathway.[416] Intracellular fungi such as *Cryptococcus, Coccidioides,* and *Histoplasma* can evade phagosomal destruction via generation of urease that creates a pH-neutral phagosomal environment, or by producing virulence factors that scavenge iron or counteract reactive oxygen species, or via lytic or nonlytic escape from the macrophage, or by a combination of these.[417]

Cytoplasmic Pathogens

Some bacterial pathogens have evolved a different intracellular survival strategy by escaping the phagocytic vacuole and replicating in the cytoplasm of host cells (e.g., *L. monocytogenes, Shigella flexneri,* and *Rickettsia* spp.).[407] These pathogens secrete proteins that are essential for virulence and that destroy the vacuolar membrane, providing direct access to the host cell cytosol. In terms of the cellular immune response, these pathogens are similar to viruses because defense against these agents predominantly depends on the MHC class I/CD8 T-cell axis. Because of their cytoplasmic location, the antimicrobial effector mechanisms of phagocytic cells cannot be localized spatially to the cytoplasmic site of infection, necessitating killing of the infected cell by cytolytic T cells to eliminate the infection. Extensive evidence from animal models of *L. monocytogenes* supports the role of CD8 T cells in protective immunity against cytoplasmic bacterial pathogens.

Infections With Extracellular Bacteria and Fungi

Bacteria that replicate extracellularly are accessible to antibody-mediated neutralization or killing by externalized microbicidal products of leukocytes. Defense against pyogenic bacteria (e.g., *S. aureus* and *S. pneumoniae*) depends on adequate humoral immunity and intact neutrophil function. To the extent that adequate specific and high-affinity antibody production depends on CD4 helper T cell function, patients with impaired CD4 T-cell function are susceptible to these pathogens. Th17 cells have been implicated in defense against *Klebsiella* infections in the mouse lung.[52,418] A role for CD8 T-cell responses in defense against extracellular bacteria has been postulated in anaerobic abscesses. In this setting, CD8 T cells recognize carbohydrate antigens of *Bacteroides fragilis.*

Host defense against extracellular fungi depends on IL-17 signaling at mucosal barrier surfaces. IL-17 signaling–deficient mice and humans with inherited mutations in *IL17F, IL17RA, IL17RC,* or *ACT1* develop chronic mucocutaneous candidiasis (see Table 6.1).[20,50,419] Patients with *IL17RA* and *ACT1* mutations also develop skin staphylococcal and pulmonary bacterial infections, underscoring the importance of this pathway in extracellular pathogen host defense at barrier sites.[420] Mechanistically, IL-17 produced by Th17 cells, γδ T cells, and type 3 ILCs promotes *Candida* control via the production of anti-*Candida* antimicrobial peptides by epithelial cells.[50] Consistent with the contribution of IL-17 signaling to mucosal antifungal host defense, patients with psoriasis treated with biologics that target the IL-17/IL-23 signaling axis are susceptible to mucosal candidiasis.[421,422]

Other inborn errors of IL-17 immunity that result in chronic mucocutaneous candidiasis include patients with Job syndrome due to *STAT3* mutations who have absent Th17 cells and are also susceptible to staphylococcal infections.[53] Mutations in *RORC, AIRE, CARD9, IRF8, STK4, DOCK8,* and *STAT1* (gain of function), as well as the various forms of severe combined immunodeficiency disorder, give rise to inborn errors of IL-17 immunity and are reviewed in detail elsewhere (see Table 6.1).[20]

PRIMER ON BASIC IMMUNOLOGIC TECHNIQUES

Rapid evolution of immunologic techniques has facilitated the increasingly sophisticated view of the mammalian immune system. Understanding current immunologic techniques is important for the practicing infectious diseases specialist for two reasons. First, these techniques form the basis on which we formulate our understanding of protective immunity. Second, immunologic techniques, such as flow cytometry, intracellular cytokine and MHC tetramer staining, and ELISPOT assays, increasingly are used in the clinical setting to evaluate immunologic function. We briefly review some of the more recently developed immunologic techniques.

Characterizing and Measuring Pathogen-Specific Immunity

Flow cytometry has transformed immunologic analysis by allowing rapid and efficient analysis of complex lymphocyte and nonlymphocyte cell populations. A flow cytometer analyzes single cells at a rate of up to 10^5 cells per second for the presence of typically 10 to 20 fluorescently labeled monoclonal antibodies, dyes, genetically encoded fluorescent proteins, and scatter parameters and allows each cell in a complex population to be scored for these cellular markers.[423] This powerful technique provides a detailed picture of mixed cell populations, such as lymph node, spleen, or peripheral blood cells (Fig. 6.12A-D). With the steady introduction of new monoclonal antibodies specific for novel surface or intracellular proteins, flow cytometry continues to uncover increasingly greater complexity among cell populations that previously were assumed to be homogeneous. A recurring theme in immunologic studies is the discovery that a cell subset, on the basis of a new marker, can be divided into two or three distinct cell populations. Flow cytometry is used routinely in the clinical arena for CD4 and CD8 T-cell quantitation in HIV-infected and in other immunocompromised patients.

Technical innovations that have affected cellular immunology studies involve the precise quantitation of antigen-specific T lymphocytes. Three

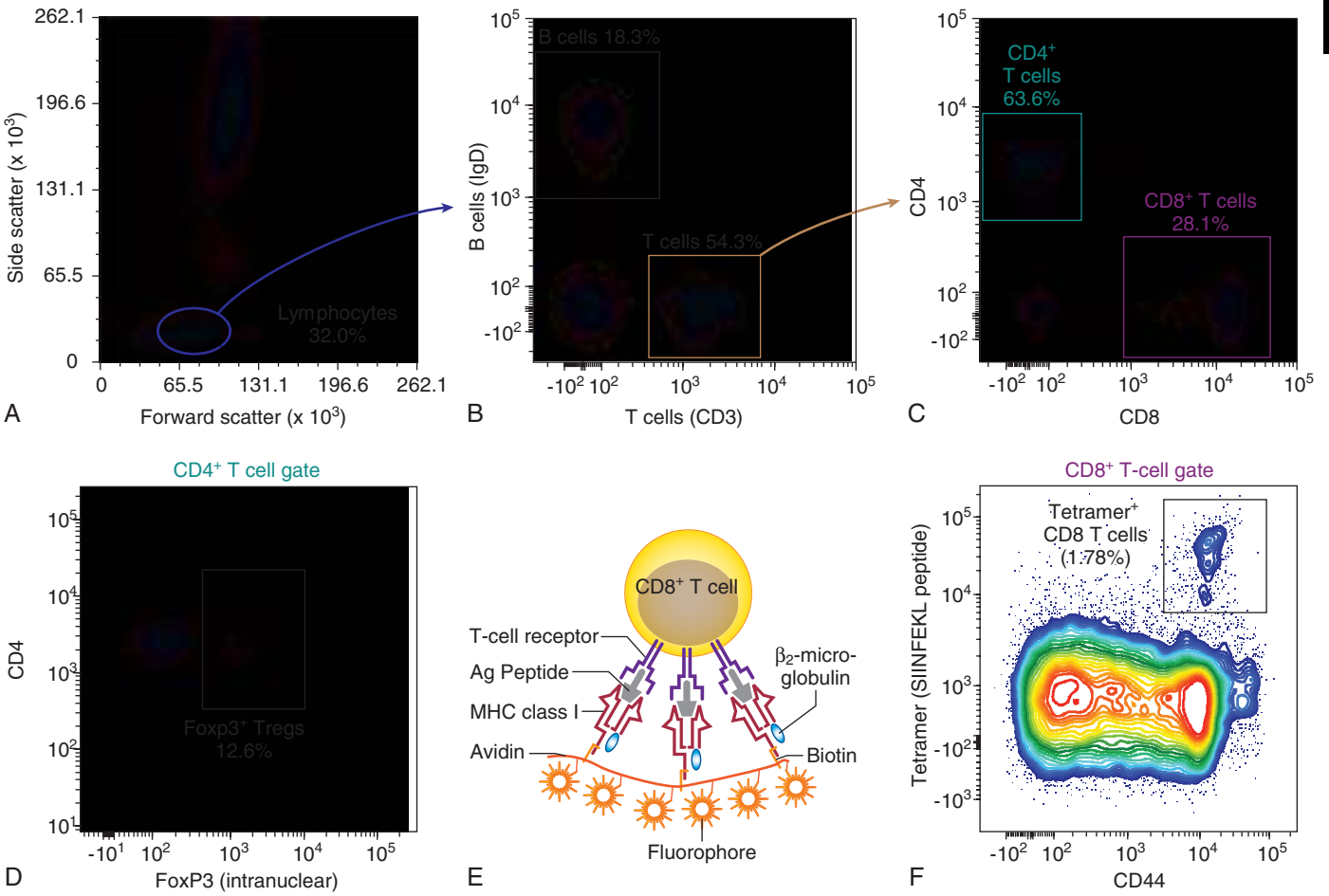

FIG. 6.12 Flow cytometry (A-D) and major histocompatibility complex *(MHC)* tetramer staining (E and F) can be used to identify and characterize distinct immune cell populations within a complex mixture of cells. (A) Plot of the forward scatter versus the side scatter of light that emanates from individual cells. These parameters group cells based on relative size and granularity. The region enclosed by a dark blue oval (gate) contains a cell population that is enriched for lymphocytes. The frequency of cells within the lymphocyte gate is indicated. (B) The cells within the lymphocyte gate are further divided into B cells (anti-IgD; vertical axis) and T cells (anti-CD3; horizontal axis) on the basis of staining with specific monoclonal antibodies that are coupled to different fluorochromes. The brown gate surrounds the B-cell population and the tan gate the T-cell population. (C) The gated T-cell population is analyzed for surface expression of CD4 (vertical axis) or CD8 (horizontal axis) using monoclonal antibodies that fluoresce at two additional wavelengths. (D) CD4 T cells are analyzed further for intranuclear Foxp3 expression by permeabilizing the nuclear membrane with a detergent and staining the cells with a fluorochrome-coupled anti-Foxp3 antibody. The CD4 T-cell subset in the *blue gate* represents Foxp3⁺ regulatory CD4 T cells. (E) Schematic showing a fluorochrome-coupled MHC tetramer that can simultaneously interact with multiple antigen-specific T-cell receptors. In most studies, MHC tetramers are generated by biotinylation of the carboxyl terminus of a soluble MHC molecule that contain a specific antigenic (Ag) peptide, followed by complex formation with a fluorescently labeled avidin molecule, which contains four biotin-binding sites. (F) MHC tetramer staining of CD8 T cells using a tetramer complexed with a SIINFEKL peptide. In this example, CD8 T cells are analyzed for CD44 staining as well. The black gate indicates the frequency of antigen-specific CD8 T cells that bind and recognize the MHC tetramer–peptide complex. *(Flow cytometry plots courtesy Dr. Rui Gardner, Memorial Sloan Kettering Cancer Center, New York, NY.)*

methods—enzyme-linked immunosorbent spot (ELISPOT) assays, intracellular cytokine staining, and MHC tetramer staining—have revolutionized the study of pathogen-specific T-cell responses. The ELISPOT assay is relatively simple and does not require a flow cytometer. ELISPOT assays provide accurate, quantitative data and can be performed with complex mixtures of cells, such as peripheral blood mononuclear cells or lymph node or spleen cells.[424] To perform this assay, complex mixtures of cells are stimulated with antigen on a membrane coated with a monoclonal antibody specific for a cytokine, such as IFN-γ, TNF, or IL-4. Antigen-specific T cells in the mixture, on stimulation, release cytokines that are captured by membrane-bound antibodies directly adjacent to the stimulated cell. Bound cytokines are detected with a secondary, enzyme-conjugated monoclonal antibody in a fashion identical to a standard sandwich enzyme-linked immunosorbent assay. Each stimulated cell leaves a "spot" on the membrane, and the number of spots is quantified.

Intracellular cytokine staining is similar to the ELISPOT assay in that complex cell populations are stimulated with an antigen, but in the presence of either Brefeldin A or monensin, drugs that inhibit cellular secretion of cytokines. During this incubation, cytokines are produced by antigen-specific T cells, but instead of being secreted, they accumulate within the cell. After stimulation, cells are fixed and permeabilized, then stained with cytokine-specific, fluorescently tagged antibodies. Permeabilization with a dilute detergent is necessary to provide antibody access to the accumulated intracellular cytokine. Stained cells are examined by flow cytometry, and antigen-specific T cells are identified and quantified on the basis of cytokine production. This technique can be modified to measure the levels of T-cell lineage–specific transcription factors in lymphocyte populations.[425] Although technically more demanding, intracellular cytokine staining is more informative than the ELISPOT assay.

Another direct method for quantifying antigen-specific T cells involves the use of MHC tetramers.[426] Because the interaction between TCRs and MHC-peptide complexes is of low affinity, attempts to identify antigen-specific T cells with soluble MHC-peptide complexes was not possible. Generation of tetrameric forms of MHC and peptide complexed

with a fluorophore readily enabled antigen-specific T cells to be stained and identified by flow cytometry (Fig. 6.12E and F). In addition, tetramer staining can be used to isolate viable, pathogen-specific T lymphocytes or NKT cells.[108,427] An advantage of MHC tetramer staining over either intracellular cytokine staining or ELISPOT assays is that T-cell or NKT-cell detection does not depend on cytokine production, which in turn depends on the T-cell phenotype.

In many cases, use of these quantitative assays radically revised prior estimates of pathogen-specific T-cell frequencies.[428–430] In some infections, such as primary EBV infection, the frequency of virus-specific $CD8^+$ T cells approaches 70%.[431,432] Although EBV is arguably an extreme example, in other infections, such as those caused by HIV, HSV, influenza virus, and *L. monocytogenes,* pathogen-specific T-cell frequencies are astonishingly large, generally ranging from 2% to 25%.[429,430,433,434]

These techniques enable researchers to combine measurements of pathogen-specific T-cell frequencies with T-cell functionality. An example of this application relates to understanding the expansion and effector cytokines produced by pathogen-specific T cells during immune reconstitution inflammatory syndrome (IRIS) in patients who receive highly active antiretroviral therapy for HIV. In longitudinal analyses, preexisiting CD4 cytokine-producing T cells that specifically targeted antigens of underlying coinfections (e.g., due to CMV, *Cryptococcus, Histoplasma, M. tuberculosis*) were increased in magnitude during IRIS flares. In contrast, T-cell responses against HIV or non-IRIS–associated infections were not enhanced during IRIS episodes or in non-IRIS patients, indicating that IRIS does not represent a general T-cell dysfunction, but rather a dysregulated CD4 T-cell response against a residual IRIS-associated opportunistic infection antigen.[435]

In clinical practice, the development of experimental procedures to generate pluripotent viral-specific T cells, even from third-party immune individuals with common HLA polymorphisms, has emerged as a major breakthrough in the management of allogeneic hematopoietic stem cell transplant recipients who are at high risk for developing life-threatening infections of CMV, EBV, and adenovirus.[436]

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The complete reference list is available online at Expert Consult.

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7 Mucosal Immunity

Peter B. Ernst and Hiroshi Kiyono

SHORT VIEW SUMMARY

SPECIALIZATION OF HOST RESPONSES IN MUCOSAL TISSUES
- Unique properties of mucosal immune responses
- The challenge
- Adaptation
- Impact of the microbiome

INDUCTION OF MUCOSAL RESPONSES
- Epithelial cells and immunophysiology
- Antigen-presenting cells
- Innate lymphoid cells
- Sensing
  - Microbiota
  - Metabolites
  - Discrimination between commensal and pathogens
- Adaptive immunity
  - Immunologic homeostasis in mucosal tissues
  - When mucosal responses go wrong
  - Mucosal response defines pathogenicity
  - Good bugs, bad bugs, and hygiene hypothesis
  - Mucosal immunization

ROLE OF MUCOSAL IMMUNITY BEYOND THE MUCOSA
- Role of the microbiota in homeostatic networks
- Mucosal immune responses discriminate between molecular signals that reflect a threat to the host versus signals that are benign or even enhance health.
- This balance between local mucosal immune responses and the microbiota appears to become established in infancy.
- The host-microbial interactions are shaped by millions of ligands in the local environment that are presented in an endocrine, paracrine, or autocrine manner.
- Although most of these ligands represent environmental antigens, they also include cytokines, chemokines, growth factors, integrins, metabolites, hormones, and neurotransmitters.
- The balance struck between mucosal immunity and local microbial communities is recognized for its impact on the health of tissues well beyond the site where the original encounter occurs.

SPECIALIZATION OF HOST RESPONSES IN MUCOSAL TISSUES

The Challenge

Mucosal tissues provide a barrier between the host and the microbial world, and these very delicate structures perform important physiologic functions as well. For example, to maximize air exchange in the respiratory tract or nutrient absorption in the gut, these tissues have numerous morphologic adaptations that expand their surface area. This vast surface is exposed to myriad environmental antigens including toxins, allergens, food antigens, and microbes. Furthermore, humans alter their habits and lifestyle, and these stimuli vary as humans change their location and diet and encounter new microbial challenges. Other challenges facing mucosal immune responses include the need to reach luminal "offshore" antigens and to protect against infection without compromising physiologic functions. Thus, immune and inflammatory cells in mucosal tissues adapt to changes in stimuli to acquire a pattern of differentiation that provides protection without disrupting adjacent tissues. Increasing evidence discussed in this chapter suggests this equilibrium is achieved by sensing the local microbial communities and stimulating local immune responses that contribute to homeostatic or pathologic responses.

The gastrointestinal tract provides an informative model to illustrate the unique properties of mucosal immunity. Although estimates vary, there are more bacteria, viruses, fungi, parasites, and archaea than nucleated host cells throughout the entire body, and the microbiome entails several hundred–fold more genes than the human genome. In response to this microbial burden, the gut has as many lymphocytes, in both diffuse (e.g., lamina propria) and organized (e.g., gut-associated lymphoid tissue) tissues, as found in the spleen. Moreover, the gut secretes antibody (e.g., secretory immunoglobulin A [SIgA]) that accounts for approximately 75% of the total amount of immunoglobulin produced by the entire body every day. Other mucosal tissues share similar challenges and require many comparable adaptations in their immune responses to accommodate the physiologic uniqueness of different tissues (e.g., upper and lower respiratory tracts, reproductive tissues, eye, middle ear).

Adaptation of Mucosal Immune Responses

The concept of a common mucosal immune system emerged based on the related interface separating mucosal tissues from luminal stimuli. Furthermore, the presence of polymeric immunoglobulin A (pIgA) is an adaptation found in most mucosal secretions. Humans differ from mice in that they have immunoglobulin A1 (IgA1) and IgA2 isotypes that are represented to varying degrees in different sites. As described in Chapter 5, the structure of IgA reflects its adaptation to the mucosal environment, as it is actively transported across the epithelium via the pIgA receptor, and the secreted complex of the two molecules comprises SIgA. IgA resists proteolysis as well as acid hydrolysis and serves to prevent the binding of pathogens or toxins without efficiently activating complement and its associated inflammatory effects. Furthermore, IgA antibodies have been shown to shape the symbiosis with commensal bacteria, as approximately 70% of these microbes are coated with IgA antibodies.[1,2] Paradoxically, IgA nephropathy is an IgA-mediated disease that is driven by the deposition of IgA1 that leads to the impairment of glomerular function.[3] Thus even with adapted systems, responses can go awry and contribute to disease in addition to protection.

After the discovery of IgA, Ogra and colleagues[4] demonstrated that oral immunization with a live, attenuated polio vaccine induced both IgG and IgA antibody responses in the serum and IgA in secretions, whereas systemic immunization with an inactivated virus had little effect on mucosal immunity. Evidence for the exchange of immunologic information among different mucosal tissues emerged from studies demonstrating that when antigens were administered orally, for example, antigen-specific SIgA was detected in other secretions such as tears. The detection of antibody in multiple tissues is attributable to the selective

circulation of antigen-specific B cells acquiring mucosal imprinting molecules (e.g., CCR9, CCR10, α4β7) allowing them to traffic from one mucosal tissue to another.[5]

Immunity includes many responses, some of which neutralize the intended target, but some may also mediate collateral damage to host tissues. In mucosal sites, the antigen burden requires a very delicate homeostatic balance. A major contribution to mucosal homeostasis is the acquired tolerance to antigens that persist in the lumen of either the digestive tract (oral tolerance)[6] or the airway.[7,8] This response allows the host to avoid adverse, immune-mediated diseases to environmental and nutritional antigens as well as to tolerate the microbiota that persist in the lumen. The ability of local host responses to adapt to changes in luminal content due to major changes in diet or microbial communities exemplifies the flexibility in immune regulation that is needed to prevent overly exuberant responses to benign, local antigens.

Scientists studying mucosal immune/inflammatory cells directly have gained an appreciation of the mucosal cell phenotype. One approach has employed lavage of the airway or reproductive tracts as a window to view the lineage and function of cells in these sites. Although many investigators have studied mucosal cells directly in the gastrointestinal tract, cells such as neutrophils are rarely isolated from these tissues due to technical challenges, whereas cells in the urogenital tract, salivary glands, and eye await more extensive characterization. As creative techniques to investigate these cells are developed, novel insights are expected to emerge that will be important to consider in a comprehensive understanding of mucosal immunity in different aerodigestive, reproductive, visual, and hearing tissues.

Although a multitude of factors shape mucosal immune responses, the selective accumulation of cells with a preferred phenotype for mucosal homeostasis occurs due to a limited array of biologic processes.[9] The selection of a preferred phenotype can be achieved by deletion of cells with an unwanted phenotype, selective homing of cells with the preferred phenotype to the mucosae, differentiation of cells toward the preferred phenotype, or expansion of the desired cells through proliferation (Fig. 7.1). With respect to proliferation, not all daughter cells are "identical twins." Proliferation in lymphocytes is often asymmetric; that is, the engagement of receptors in the immunologic synapse leads to the migration of receptors and their associated signaling molecules toward one pole. When cell division begins, the signaling molecules inherited by the daughter cells are asymmetrically distributed, and this necessarily changes the pattern of gene expression.[10–12] These processes contribute to the phenotype of antigen-presenting cells (APCs), mast cells, and T and B cells found in mucosal tissues. One can assume that in immune-mediated disease, the events change, and increased recruitment of immune/inflammatory cells differentiate toward a more responsive phenotype rather than acquiring an anergic tone.[13] Understanding the relative contribution of the signals and cell biology that contribute to immune cell phenotype in health or disease will enable the ability to promote mucosal health.

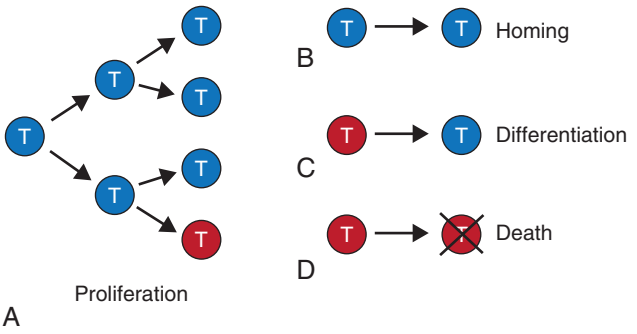

FIG. 7.1 Mechanisms contributing to selection of the immunologic phenotype in mucosal tissues. (A) The selection of a preferred phenotype (illustrated in blue) in mucosal tissues can be achieved by proliferation that expands the cells with the preferred traits. With respect to proliferation, not all daughter cells are "identical twins." Proliferation in lymphocytes is often asymmetric giving rise to daughter cells that are not always identical (red cell). (B–D) Accumulation of cells with the desired phenotype can also be achieved by homing of cells with the preferred phenotype to the mucosae (B), differentiation of cells toward the preferred phenotype (C), and deletion of cells with an unwanted phenotype (D). Targeting these pathways to direct the differentiation of an ideal mucosal immune response could be achieved with adjuvants or biologic response modifiers.

Microbial Communities and Development of Mucosal Immune Responses

The role of the microbial community in an individual's health continues to generate tremendous interest. Through endeavors such as the American Gut Project (www.americangut.org), vast amounts of data are accumulating about the factors that impact the intestinal microbiota and their potential impact on health. For example, it is becoming apparent that by approximately 3 years of age the microbiota takes on many of the characteristics of that in adults, and its composition may portend many aspects of wellness.[14] In general, the microbiota is influenced by factors such as the microbial composition of the mother, vaginal versus cesarean birth, breastfeeding versus formula feeding, diet, antibiotic exposures, genetics, aging, and immune status. These factors influence species diversity, which consists of the number of microbial species (richness), the genetic relationship between species (taxonomic diversity), and the relative abundance of one to another (evenness). The signature of diversity within an individual can impact immune cell phenotype or the metabolome, which often begins in mucosal sites but affects immunologic surveillance throughout the body. For example, animal models of autism are associated with a distinct intestinal microbiota that, in turn, can be offset by other, "protective" bacteria.[15]

Mucosal immune homeostasis reflects an equilibrium in which host responses and microbes influence each other and permit persistent infections.[16] As the immune system influences local immunity, microbes affect host responses. The loss of species diversity may be one phenomenon that accounts, at least partially, for the hygiene hypothesis (see "Good Bugs, Bad Bugs, and the Hygiene Hypothesis") that ascribes an increased risk of allergic and other immune-mediated diseases to the lack of diversity in childhood microbial communities. Using the middle of the 20th century as a key point in time, one can trace the increased use of antibiotics, convenience foods (processed foods, infant formula), increased rates of cesarean section versus vaginal birth, and an increasing emphasis on germaphobia as factors that have impacted diversity.[14,17,18] Furthermore, loss of diversity is passed between generations, resulting in a successive attenuation in microbial diversity[17–19] that may have an adverse effect on susceptibility to disease.

Although stool has been a convenient and informative source to sample, evidence points to the importance of microbes in specific tissues and sites including the mucosa (vs. the lumen) as well as even being embedded within lymphoid tissues. For example, the segmented filamentous bacteria found in the epithelial-mucus niche in mice can favor the development of Th17 responses that have also been associated with an exacerbation of immune-mediated disease.[20,21] The predominate juxtaposition of *Helicobacter pylori* to gastric epithelial cells and their impact on health may be another example of microbes within a limited niche.[22] Mucosal lymphoid tissue–resident bacteria (e.g., *Alcaligenes* spp.) are just beginning to be appreciated for their ability to shape mucosal immunity.[23] Bacteria in these sites have been shown to favor IgA production and induce protective interleukin-10 (IL-10)–producing responses that limit damage following mucosal provocation.[24,25] Most of these findings have been identified in mice, and it remains to be determined where such niches may be found in humans and how they could impact health.

INDUCTION OF MUCOSAL IMMUNE RESPONSES

Epithelial Cell Responses and Immunophysiology

Although single factors rarely explain complex biology, the interactions in mucosal immunity benefit from some insight into the individual elements. First, one should consider the interface between the host and its microbial milieu. In mucosal tissues, this is invariably the epithelial

barrier, its mucus coating, and a variety of secreted host factors (e.g., antimicrobial peptides, SIgA) that modify the microbial niche.[26–30] Mucosal epithelial cells provide a physical barrier that limits the translocation of microbes or their metabolites into the underlying layers. M cells are a specialized epithelial cell overlying the Peyer patches that serve as preferential sampling sites for the uptake of luminal antigens.[31] Many microorganisms are preferentially taken up by M cells.[32,33] Similar secondary lymphoid structures can also be found in the nasal-associated lymphoid tissue of the airway. Cells resembling M cells are scattered throughout the epithelium of the gastrointestinal tract[34] suggesting that their enhanced ability to sample is more widespread than the Peyer patches. In other studies, goblet cells have been shown to pass luminal antigens on to dendritic cells.[35]

Epithelial cells express receptors for many microbes, both pathogens and commensals, including human immunodeficiency virus (HIV),[36] respiratory syncytial virus,[37] *H. pylori,*[38–40] and *Salmonella* spp.[41] These interactions favor colonization and invasion that together lead to signaling events and the induction of mucosal immune responses.[27] Epithelial cells in the airway,[42–44] digestive tract,[45–47] and urogenital tract[48,49] respond to infection, toxins, and inflammation with the release of cytokines that not only alert the host of potential damage or danger but also focus the responses to the site of injury based on a gradient of chemokines and other factors.[50] Thus epithelial cells are able to sense a danger signal[51] and transduce responses that recruit and activate other immune or inflammatory cells.

Some epithelial cells are highly specialized to produce antimicrobial factors or to express pIgA receptor to mediate the transfer of pIgA into the lumen. The ability to transport IgA is shared by most epithelial cells and increased during inflammation. However, the secretion of antimicrobial factors tends to be more localized. For example, saliva contains secretory leukocyte protease inhibitor[52]; gastric parietal cells produce acid; and other epithelial cells produce a range of factors including lysozyme, lactoferrin, defensins,[28] and antibacterial lectins[26,53] that interfere directly with microbial growth or modify physiologic function to protect the host. One example of altered physiology in host defense is the role played by secretions to create a pressure gradient that impairs colonization in the deep regions of mucosal glands. Furthermore, highly adapted processes such as the mucociliary apparatus in the lungs aid in the elimination of environmental contaminants and microbes.

In the gut, motility partners with secretions to create a catharsis that contributes to the clearance of infections. This concept was illustrated in studies of immunity to nematodes. These organisms induce a robust Th2 response associated with the accumulation of IgE and mucosal mast cells.[54] Degranulation of mast cells releases mediators that contribute to epithelial cell secretion and an increase in motility, which, when impaired, decreases the clearance of several nematode species.[55–57] These physiologic responses are regulated by neuroendocrine cells and often triggered by microbial products[58,59] as well as cytokine responses that contribute collectively to mucosal immunity, as they facilitate the clearance of infections. Neuroendocrine factors also regulate immune cell function. In short the protective or pathogenic effects of mucosal immune and inflammatory cells cannot be separated from the other cells and mediators within the tissue. This raises the possibility that drugs impairing motility may counter integrated responses that normally help in clearance of some pathogens.

Antigen-Presenting Cells

The mucosal tissues have a full complement of macrophages and dendritic cells. These cells are found throughout the lamina propria; some are enriched in secondary lymphoid structures including the Peyer patches in the gut, similar subepithelial aggregates in the airway (e.g., nasopharyngeal-associated lymphoid tissue), or other tissues (e.g., tear duct–associated lymphoid tissue) as well as in the draining lymph nodes. It has been reported that some dendritic cells extend their dendrites into the lumen and sample antigens directly.[60,61] In addition to this occurring across the tight junction of absorptive epithelial cells,[60] some dendritic cells extend dendrites through M cell–specific transcellular pores for antigen sampling.[62] Phagocytes have also been shown to engulf apoptotic epithelial cells,[63–65] which, if infected, could carry microbes and create another mechanism of antigen sampling.[66,67]

It is increasingly clear that many factors within the mucosa select for unique APCs[68] capable of facilitating the accumulation of helper T (Th) cells that favor the induction of tolerance or mucosal IgA or both. Thus there is a feedforward as cells involved in the recognition and processing of antigen acquire a mucosal phenotype that, in turn, favors the expansion of Th cells that select for effector mechanisms such as IgA or mucosal mast cells that culminate in an ideal mucosal immune response. For example, initial engagement of nucleotide oligomerization domain 2 (NOD2) expressed by APCs with muramyl dipeptide administered intranasally stimulates the production of thymic stromal lymphopoietin and the induction of Th2 cells in the lung.[69] Similarly, initial stimulation of NOD2 in the gut or in the context of ocular toxoplasmosis[70] induces proinflammatory responses. However, repeated stimulation with NOD2 ligands renders APCs nonresponsive to ligands for NOD2 or Toll-like receptors (TLRs)[71,72] as evidenced by decreased production of IL-1β, IL-8, and tumor necrosis factor-α (TNF-α).[72] Mutations in the *NOD2* gene are associated with Crohn disease,[73] possibly by impairing the ability of APCs to adapt to these repeated signals from microbial ligands. Mouse models have shown that *NOD2* is important to stimulate homeostasis to protective bacteria, so mutations in this gene associated with immune-mediated disease could reflect a loss of homeostatic regulatory T (Treg) cell induction.[74]

Innate Lymphoid Cells

Complementing the transition from innate to adaptive responses is a population of innate lymphoid cells (ILCs). These cells are non-B, non-T cells with a lymphoid morphology.[75–77] Historically ILCs included natural killer (NK) cells and lymphoid tissue inducer cells. At the present time, it is clear that there are other subsets that are closely related to most, if not all, Th cell subsets, as they express similar transcription factors and patterns of cytokine production. To clarify this area, a new nomenclature has been applied in which ILCs are divided into subsets referred to as ILC1, ILC2, and ILC3.[78] ILC1 and ILC2 express transcription factors and cytokines that resemble Th1 and Th2 cells, respectively, whereas ILC3 includes cells resembling Th17 or Th22 cells as well as lymphoid tissue inducer cells (Fig. 7.2),[78] thus giving a functional homologue of Th cell subsets to innate immunity.

ILCs arise in the bone marrow from a common lymphoid precursor that distinguishes itself from T-cell and B-cell lineages by the expression of T cell factor-1. Subsequently a transcriptional program is engaged involving inhibitor of DNA binding 2 (Id2) that leads to ILC commitment and the subsequent transcription factor induction associated with the various ILC subsets.[79] Relatively little is known about the timing and mechanisms that lead to the tissue distribution of ILCs; however, they reside in the tissue constitutively and become activated by some of the cytokines released by APCs, epithelial cells, and T cells. In turn, ILCs express the transcription factors and cytokines that are appropriate to amplify the desired host response for the current antigenic signal. Given that adaptive immunity takes several days to be induced, ILCs complement the role played by macrophages and other innate cells in limiting infections as well as enhancing the milieu to select for the appropriate T-cell and B-cell responses that eventually confer adaptive immunity and memory.

Further evidence of the integration between ILCs and Th cells is found in the response of ILCs to Th-derived cytokines. For example, IL-17A or IL-22 from Th17 cells has been reported to activate NK cells and enhance immunity to fungi in mucosal sites such as the kidney[80] and oral cavity.[81] Other Th cell cytokines activate cells involved in acute/innate inflammatory response in different ways. For example, Th2 cells enhance the accumulation of mast cells, basophils, and eosinophils as well as increase IgE antibodies that enable IgE binding cells (e.g., mast cells and basophils) to recognize antigen and release their mediators that contribute to clearance of nematodes.[54]

Sensing Mucosal Microbiota

The importance of the microbiota in regulating host responses begins with the microbes having access to the host and the subsequent recognition of their molecular structures.[82–84] The sensing of microorganisms is mediated through pathogen-associated molecular patterns (PAMPs).[85] PAMPs are detected by pattern recognition receptors (PRRs) expressed

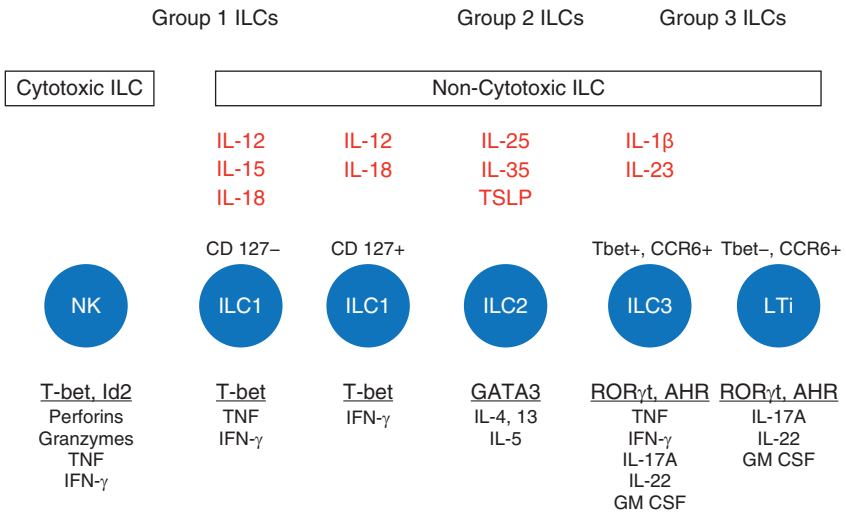

FIG. 7.2 Properties of innate lymphoid cells (ILCs). ILCs are believed to be derived from a common precursor and differentiate into their respective subsets based on the selectivity imparted by the milieu and their respective transcription factors. In response to specific cytokines (in red), transcription factors *(underlined)* are induced that control the selection of gene expression. In general, the pattern of transcription factor usage and cytokine gene induction in ILC1, ILC2, and ILC3 resemble the same control of differentiation observed in Th1, Th2, and Th17 cells. As reviewed elsewhere,[78] the stimuli of ILC1 include IL-12, IL-15, and IL-18; ILC2 is driven by IL-25, IL-33, and TSLP; and ILC3 require IL-1β and IL-23. ILC1 are often associated with cancer, immune-mediated diseases, and intracellular infections, typically viruses. ILC2 are more often seen in the context of nematode infections and allergic disorders. ILC3 are induced in response to different bacterial challenges in mucosal tissues and contribute to induction of secondary lymphoid tissue by the LTi subset. *AHR,* Aryl hydrocarbon receptor; *CCR6,* C-C chemokine receptor 6; *GATA3,* transcription factor binding to DNA sequence guanine-adenine-thymine-adenine; *GM CSF,* granulocyte macrophage colony-stimulating factor; *Id2,* inhibitor of DNA binding 2; *IFN-γ,* interferon-γ; *IL,* interleukin; *Lti,* lymphoid tissue inducer; *NK,* natural killer; *RORγt,* retinoic acid–related orphan receptor gamma; *T-bet,* T-box expressed in T cells; *TNF,* tumor necrosis factor; *TSLP,* thymic stromal lymphopoietin.

on the surface of several cell lineages. There are several classes of PRRs including TLRs[85]; NOD-like receptors (NLR) including the NOD family[86]; Rig-like helicases (RLH)[87]; Dectin-1 and Dectin-2[88,89]; and several proteins containing thrombospondin repeats such as thrombospondin 1,[90] mindin,[91] and brain angiogenesis inhibitor 1 (BAI1).[41] In addition to bacterial factors, tissue damage, including debris from cells dying of apoptosis or necrosis, provides an array of molecules referred to as alarmins or danger-associated molecular patterns (DAMPs).[92] Furthermore, the release of adenosine triphosphate (ATP) from dead cells in damaged tissue is important for additional phagocyte recruitment[93,94] and the regulation of inflammation that contributes to tissue repair.

Many PRRs engage their ligand on the cell surface, whereas others are activated in phagosomes or elsewhere in the intracellular space following translocation of the PAMPs. Soluble PAMPs in the blood such as endotoxin can activate surface receptors directly, although concentrations of these ligands rarely reach the thresholds needed to activate leukocytes ex vivo. Microbes, exemplified by *H. pylori,* remain primarily on the cell surface of (gastric) epithelial cells where surface PRRs may be engaged. Additional activation occurs subsequent to the translocation of bacterial effector molecules into the cytoplasm where peptidoglycan can induce IL-8 responses after sensing by NOD1.[95] Other organisms such as enteroinvasive *Escherichia coli* or various *Salmonella* spp. enter a cell by means of invasion as well as engulfment.[96] The internalization process creates phagosomes that can juxtapose PAMPs with PRRs within the phagosome where they activate their respective responses.

Following the engagement of PRRs, signaling pathways are stimulated leading to the activation of transcription factors and the production of various host response molecules. Perhaps best studied is the role of myeloid differentiation primary response protein (MyD88) as an integral part of the signaling for several TLRs.[97,98] The fact that several PRRs share a signaling pathway creates a synergy that may augment the potential stimulation from relatively small concentrations of individual PAMPs. Accumulating PAMPs within a phagosome would further enhance their concentration and potential for signaling.

Several distinct host responses are induced following sensing by PRRs. One of the first is the production of chemokines that recruit and activate other immune cells to the site of damage. Other responses include changes in the expression of accessory molecules on APCs that contribute to T-cell activation; oxidative burst; and production of reactive nitrogen species, various vasoactive mediators, or arachidonic acid metabolites.

An important function of innate cells is to clear microbes or damaged tissue by phagocytosis. Engulfment of sterile debris or benign microbes leads primarily to the production of antiinflammatory cytokines such as IL-10 or transforming growth factor-β1 (TGF-β1).[99,100] In contrast, clearance of pathogens usually leads to innate antimicrobial responses (e.g., reactive oxygen species) as well as production of cytokines that activate appropriate Th cell subsets. Another consequence of internalization of antigen into dendritic cells is presentation of antigen to $CD4^+$ or $CD8^+$ T cells leading to T-cell activation in a major histocompatibility complex (MHC) class II–dependent or MHC class I–dependent manner, respectively. Epithelial cells often express molecules associated with T-cell activation including molecules recognized by invariant NK-T cells[101] as well as T cells.[102,103] An interesting nonmicrobial example is the rapid uptake of gliadin by duodenal epithelial cells.[104] Subsequent to this uptake, gliadin is processed by tissue transglutaminase to create a peptide that fits well into the DQ2 peptide–binding or DQ8 peptide–binding pocket and leads to Th cell activation and celiac disease.[105] Microbial antigen uptake and processing by epithelial cells or APCs by similar or distinct mechanisms would enhance adaptive immunity to an organism.

Sensing Metabolites

PAMPs and DAMPs recognized by the host represent a fraction of the possible signals that shape mucosal immune responses. The broad collection of microbial and host metabolites constitutes a much larger pool of ligands that is just beginning to be appreciated. The value in understanding metabolomics can be illustrated with a few examples.

Several leukocyte lineages including dendritic cells and Th cells express a receptor that binds different ligands including polycyclic aromatic hydrocarbons, suggesting that environmental toxins modulate host responses.[106] The aryl hydrocarbon receptor is a ligand-activated transcription factor that regulates expression of many immune genes. In addition to toxins, natural ligands can be generated from dietary tryptophan by the initial enzymatic activity of indoleamine

2,3-deoxygenase to yield kynurenine. Kynurenine can accumulate in the placenta and induce Treg cells that may help prevent rejection of the semiallogeneic fetus.[107] Furthermore, catabolism of dietary tryptophan in the gut by bacteria or fungi to other aryl hydrocarbon receptor ligands can assist in the induction of IL-22, which binds to epithelial cells and stimulates the release of antimicrobial peptides.[108,109] Thus microbiota and the host compete for the same substrates to generate different metabolic products that may modify mucosal immune responses toward Treg or Th22 cells.[110]

Purine metabolism is another pathway in which bacteria and the host compete for the same substrate.[111] In brief the accumulation of ATP, from bacteria[112] or from dying cells,[93] can be metabolized to adenosine diphosphate, 5′-adenosine monophosphate (AMP), and then to adenosine.[111,113] ATP can be a proinflammatory signal that favors the accumulation of Th17 cells in the mucosa,[114] whereas the accumulation of adenosine depletes this stimulus and usually confers an antiinflammatory activity mediated by the A_{2A} adenosine receptor.[111] Although Treg cells can generate adenosine as a mediator of suppression, bacteria can also achieve this to suppress innate responses to favor colonization.[115]

Another example of the role for metabolism in regulating host responses is the conversion of vitamin A to retinoic acid by dendritic cells expressing retinol dehydrogenase.[116] This process plays an important role in conditioning leukocytes with a mucosal phenotype[117,118] and imparting the ability for these cells to home to mucosal tissues.[116,119] Furthermore, APCs exposed to retinoic acid assume the capacity to direct Th cell differentiation away from Th17 cells and toward Th2 and Treg cells[120,121] that subsequently increase IgA production along with anergic conditions that enable the survival of symbionts.[74,122,123] One can only imagine how new data emerging from studies of the microbiome will identify other metabolic reactions that contribute to the molecular environment shaping mucosal immune response and the microbial niche.

Discrimination Between a Commensal and a Pathogen

The crux of the matter for mucosal immune responses in health and disease is defined by the equilibrium of host-microbial interactions and knowing when and how to respond. For years, microbes have been described as being commensals or pathogens, even though these categories represent opposite ends of a spectrum that defines the interrelationship between the host and its microbial communities. However, many microbes exist as *amphibionts,* a term coined by Rosebury[124] to describe microbes that have a symbiotic relationship that can be either a benefit or a detriment to the host or microbe. As discussed later, this relationship is illustrated by organisms that can exist without harming the host, but in the context of a particular host response, they can assume a biologic role that is decidedly disadvantageous to the host.[19] The important point is that homeostasis is flexible and may be either plastic or elastic as host and microbes respond to their niche and that short-term processes may have long-term biologic significance.

Microbes produce hundreds of metabolites and molecules creating a cascade of stimuli beginning with binding to host epithelial cells; sometimes employing secretion systems that translocate bacterial effector molecules into host cells; invasion and/or engulfment; engaging numerous extracellular or intracellular pattern recognition receptors (such as TLRs, NODs, etc.); and the initiation of chemokine and cytokine production. This process, as described by Matzinger,[125] signals "danger," and the chemokines recruit and activate innate cells to phagocytose the offending microbe, amplify the host response, and induce appropriate effector mechanisms leading to the initiation of antigen-specific acquired immune response. This is illustrated by organisms such as *Salmonella* spp. that stimulate APCs to produce proinflammatory cytokines including IL-6, IL-12, or IL-23 that drive the differentiation of Th1 or Th17 cells.[126,127] Although these cells contribute to antimicrobial functions, host responses must be regulated carefully to avoid immune-mediated damage and to maintain the physiologic functions that are required to sustain life.

The conundrum of distinguishing pathogens from commensals emerges, as the latter can also express ligands for PRRs yet do not impart a strong danger signal.[71] There are several possible explanations for this paradox. It may be related to the burden of a particular microbial species; the age at which infection occurs or its duration; the structure of its PAMPs that may render them less proinflammatory; or possibly the absence of secretion systems, invasion mechanisms, or other virulence factors. The issue of duration may be best illustrated by organisms associated with gastric or colorectal cancer. In these cases the persistent gnawing of a low-grade oxidative stress associated with these infections chips away at the DNA until DNA damage leads to a key mutation. For example, mitochondrial DNA mutations have been associated with a loss of cytochrome-*c* oxidase activity that is directly correlated with age.[128] Time is essential for random mutations to accumulate in key genetic regions that impact cell growth.

At the other extreme, one may encounter pathogens that display an array of molecular patterns that allows them to send a different, perhaps "fresh" or more intense, set of signals that stimulates mucosal responses immediately. Furthermore, gene expression in the host can modulate transcription in microbes[129] resulting in changes in the metabolic profile and local immune responses that impact virulence and resonate throughout the body.[130–133]

Contrasting the inflammatory responses leading to a relative sterile immunity are infections that induce a degree of tolerance. For example, dendritic cells exposed to *H. pylori* favor the ability of these APCs to induce Treg cells[134,135] and contribute to persistent infection. Indeed, a pathogen such as *H. pylori* can coexist more or less peacefully for the host's entire life.[46,47] The molecular basis to explain this effect of *H. pylori* remains unclear but could include the modest inflammatory effect of its lipopolysaccharide[136] or any number of metabolic factors that remain to be defined. Adding to the complexity of this biology, *H. pylori* may even confer some advantages to health in the digestive tract[137] or other sites, as discussed later. Importantly, "sterile immunity" may be less advantageous to the host than a more mutually beneficial relationship.

Adaptive Immunity to Mucosal Stimulation

The transition from innate to adaptive immunity is based on the interaction among Th cells, APCs, and the molecular environment that drives Th cell differentiation. As illustrated in Fig. 7.3, the cytokine milieu induced following antigenic stimulation shapes the differentiation of Th cells. The combination of microbial factors, the conditioned responses of APCs that have adapted to their respective niche, and local cytokines (e.g., from ILCs or other T cells) all regulate Th cell differentiation. This is illustrated with nematode infections that induce thymic stromal lymphopoietin and IL-4 leading to the differentiation of naïve Th cells into Th2 cells.[138–140]

Th17 cells provide another important Th cell response in mucosal tissues. IL-17 production was first implicated in mucosal infections in studies of the pathogenesis of *H. pylori* in human gastric tissue.[141] Subsequently, Th17 cells, through the production of IL-17 and IL-22, have been shown to play an important role in immunity to mucosal pathogens[75] including *Citrobacter rodentium,*[142] *Klebsiella pneumoniae,*[143] *H. pylori,*[144] and *Candida albicans*.[81]

Most inflammatory responses are mixed, as cytokines capable of favoring different Th cell subsets are induced simultaneously. The plasticity of Th cells and the complexity of Th cell differentiation are attributable to the fact that multiple cytokines targeting a Th cell induce the same, complementary, or competitive signaling pathways. Whereas some responses can be uniform such as Th2 responses induced by nematodes[145] or Th1 responses induced by *Mycobacterium tuberculosis,*[146] other microbes induce mixed Th1/Th17 responses, and there are even reports of Th1 responses in Treg cells.[147] Moreover, IL-10–producing Treg cells expressing retinoic acid–related orphan receptor gamma (RORγt) are induced in the gut following infection.[121]

The Th cell–derived cytokines expand and differentiate $CD8^+$ cytotoxic T lymphocytes as well as B cells. In addition, these cytokines feedback on innate cells to increase the expression of cytokines produced by epithelial cells, ILCs, or APCs. For example, the production of interferon-γ, IL-2, and IL-15 is particularly effective at increasing cytotoxic activity mediated by NK cells, invariant NK-T cells, and cytotoxic T lymphocytes that would be effective at mediating immunity against intracellular pathogens including viruses and some invasive bacteria. For example, these responses would be induced most efficiently by Th1 cells in an environment in which the pathogen, or vaccine antigen, induced IL-1 and IL-12.

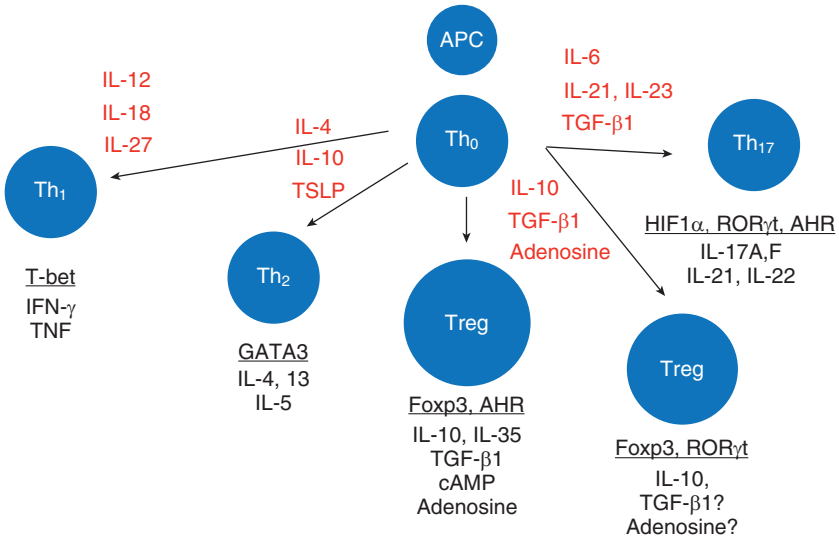

FIG. 7.3 Impact of T helper cell plasticity on immunologic function. Selection of Th cell subsets is driven by positive signals (in *red*) that favor the induction of specific transcription factors *(underlined)* and the genes they regulate. In addition, negative feedback provided by some of these cytokines favors the emergence of certain subsets. For example, production of IL-4 inhibits Th1 cells, whereas IFN-γ inhibits Th2 and Th17 cells. Based on the bias in cytokine production, these Th cell subsets mediate different functional activities. Th1 cells enhance cell-mediated immunity, which is well suited to protect against intracellular functions. Th2 cells enhance IgE and mast cell responses that are important for immunity against helminths. They also contribute to IgA production. Th17 responses are particularly important in antibacterial immunity and IgA regulation, whereas Treg cells not only favor IgA but also inhibit other responses and contribute to tolerance. Th17 cells induced by infection can also acquire Foxp3 and function as IL-10–producing Treg cells. However, when not appropriately regulated, these subsets contribute to disease, as evidenced by the association of Th1 and Th17 cells with gastritis and inflammatory bowel disease and the well-established role for Th2 cells in allergy. *AHR,* Aryl hydrocarbon receptor; *APC,* antigen-presenting cell; *cAMP,* cyclic adenosine monophosphate; *GATA3,* transcription factor binding to DNA sequence guanine-adenine-thymine-adenine; *HIF1α,* hypoxia inducible factor-1α; *IFN-γ,* interferon-γ; *IL,* interleukin; *RORγt,* retinoic acid–related orphan receptor gamma; *T-bet,* T-box expressed in T cells; *TGF-β1,* transforming growth factor-β1; *Th,* T helper; *TNF,* tumor necrosis factor; *Treg,* regulatory T; *TSLP,* thymic stromal lymphopoietin.

Mucosal B cells are noted for having undergone isotype switch from IgM-bearing cells to IgA-producing cells. Whereas IgA is usually the predominant antibody in mucosal tissues, other isotypes can be induced including various isotypes of IgG and IgE. IgG tends to be the predominant antibody isotype in the lower airway and reproductive tract. The process of isotype switch is mediated by different cytokines including IL-4, which favors IgE[148] and IgG1, as well as TGF-β1, B-cell–activating factor of the TNF family (BAFF), and A-proliferation–inducing ligand (APRIL), which direct switch to IgA.[149–151] One of the richest sources of TGF-β1 is the Treg cell subset.[123] Treg cells induce more IgA-producing cells than Th2 cells in addition to inhibiting proinflammatory cytokine responses. Treg cells can also differentiate into follicular Th cells in mouse Peyer patches for supporting IgA B-cell responses.[152] These multiple functions for current or former Treg cells illustrate the delicate balance that is required in mucosal immune homeostasis. Inhibition of host responses by Treg cells can favor persistent infection. Whereas some microbes induce host responses that eventually eliminate and prevent future infection, Treg cells may allow IgA antibodies that are sufficient to protect the host, while simultaneously limiting tissue damage that could emerge from excessive amounts of inflammation that may be induced by pathobionts.[123] Following the isotype switch to IgA, other Th cell–derived cytokines (e.g., IL-5, IL-6, IL-10) can expand the pool of IgA-producing cells[153] and coordinate an effective host response that remains under the control of Treg cells.

The production of IgE in the airway and digestive tract occurs in the context of nematode infections or allergies. Environmental triggers including microbial PAMPs can stimulate the production of cytokines that favor Th2 differentiation and IgE production.[69] Furthermore, rhinoviruses have also been implicated as a trigger that exacerbates atopy including food allergy as well as wheezing.[154] Thus careful regulation of mucosal immune responses is important for maintaining immunologic homeostasis to infections.

The innate-like mucosal-associated invariant T cells are a more recently described population of cells.[155] These cells reside in the mucosa and are unique in that they express T-cell receptors (usually Vα7.2) but with diversity limited to the recognition of nonpeptide antigens expressed by some microbes but not others. These antigens include modified vitamin B_2 metabolites derived from microbes and presented by an MHC class I–like molecule.[156] These MHC molecules are an evolutionarily conserved MHC-related protein 1 (MR1). It has been described that other metabolites create neoantigens by modifying the riboflavin metabolite. Some of these mucosal-associated invariant T cells have been shown to produce TNF-α in response to *Mycobacterium tuberculosis*–infected cells.[156] Although much remains to be studied, their significant presence in human tissues and blood compared with mice and their role in some bacterial and fungal infections, but not viral infections, suggest many advances will be reported in the near future.

Immunologic Homeostasis in Mucosal Tissues

Immune responses in mucosal tissues including the gut, airway, urogenital tract, and eye are regulated by $CD4^+$ Th cells, regulatory NK-T cells, $CD8^+$ T cells, and B cells. In response to benign antigens, which on their own do not deliver a loud danger signal, immune cells with a regulatory phenotype maintain immunologic homeostasis. Lineages with this function are represented unevenly in different tissues, but together they create a regulatory network to maintain equilibrium in mucosal immune reactivity.

Th cells are an important part of the adaptive immune system that contributes to the balance of immunity and immunologic restraint required for healthy mucosal tissues. A number of functionally distinct $CD4^+$ Th cell subsets have been elucidated including Treg cells, which mediate antiinflammatory activity. These cells are a topic of intense study because of their ability to control inflammatory responses and prevent autoimmune reactions.

Treg cells, or various Th cell subsets with regulatory function, inhibit immune and inflammatory responses through the production of IL-10 or TGF-β1 or both. In addition to these cytokines, interactions between surface cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) on Treg cells and receptor molecules on target cells limit immune responsiveness. Other data suggest that contact between Treg cells and effector T cells

stimulates the production of the antiinflammatory mediator IL-35.[157] A more intimate mechanism of intercellular contact was described by Bopp and coworkers,[158] who reported that the suppressive action of murine Treg cells depended on the transfer of cyclic AMP from Treg cells to responder T cells. Whereas pharmacologic manipulation may increase the levels of cyclic AMP available to mediate Treg cell function, there are biologically relevant stimuli that accomplish this. One example is adenosine.

There is now substantial evidence that adenosine is an important mediator of Treg cell function. For example, engagement of the A_{2A} adenosine receptor not only induces Treg cells[159] but is also required for optimal Treg cell function.[160,161] Subsequently, investigators reported that Treg cells had the ability to synthesize adenosine through the action of two ectoenzymes, CD39 (ectonucleoside triphosphate diphosphohydrolase-1 [NTPDase-1]) and CD73 (ecto-5′-nucleotidase). Many Treg cells in mucosal tissues express CD39 and CD73, which catalyze the transformation of ATP to adenosine.[162] ATP enhances dendritic cell maturation[163] and increases Th17 responses in the intestinal lamina propria.[114] As most Treg cells express CD39 and CD73, they not only generate adenosine that inhibits many responses including Th17 cells[164] but also degrade ATP and in so doing remove this proinflammatory stimulus from the immunologic synapse. As described earlier, ATP metabolism provides an intersection between the host and microbes that generate and use these products for their respective advantage.

The induction of Treg cells to infections has also been examined in the airway.[165,166] For example, infection with *Bordetella pertussis* stimulates the accumulation of Treg cells that can attenuate injury caused by immune and inflammatory cells induced by infection.[167] Mills et al. expanded this model to show that parenteral administration of an immunomodulatory antigen from *B. pertussis* protects against colitis induced by the adoptive transfer of $CD45RB^{high}$ Th cells.[168] This experiment shows that induction of one functional subset of Th cells by a specific microbial antigen can create a regulatory network that interferes with inflammation mediated by Th cells, even in other mucosal tissues. This notion is supported by the observations that Th2 cell responses induced by nematode infections attenuate disease mediated by Th1 or Th17 cells or both in the stomach[169] or intestine.[170,171] These reports provide evidence that immune deviation induced by mucosal infection deflects a potentially adverse host response to a more tolerable condition. These studies also demonstrate that new knowledge gleaned from research in one tissue or with one organism may have relevant applications in the management of other diseases in unrelated tissues.

When Mucosal Responses Go Wrong

Although mucosal immunity is intended to maintain immunologic homeostasis and health through protective responses, the host pays a price when inappropriate responses are stimulated by infection. The burst of cytokines, reactive oxygen or nitrogen species, and the release of enzymes or vasoactive mediators allow the infiltrating immune and inflammatory cells to contribute to disrupted tissue architecture and loss of function. In addition to tissue damage, these responses can cause pathologic amounts of catharsis, pain, or motility disorders including bronchoconstriction. Furthermore, the oxidative stress induced by chronic inflammation also increases the risk of various cancers.[172,173]

In some infections the onset of immune-mediated damage can begin with the acquisition of an infection that is sufficiently robust to break tolerance. For example, viral infections that induce prodigious amounts of IL-2 can break tolerance in autoreactive T cells leading to diabetes.[174] In the context of mucosal immunity, infection with one microbe not only activates host responses to this challenge but also simultaneously activates other T cells.[175] One well-known example of this is Guillain-Barré syndrome that is triggered by a mucosal infection with *Campylobacter jejuni*.[176,177] The microbe may express molecules that resemble the host (molecular mimicry). Thus if other significant immunologic events are occurring concurrently, the triggering infection may disrupt the generation or maintenance of tolerance contributing to allergies or immune-mediated diseases such as the induction of Guillain-Barré syndrome by *C. jejuni*.[177]

There are several signature changes in mucosal immunity in the context of chronic inflammation or immune-mediated disease. There is a predictable shift from the normal IgA responses to the accumulation of IgM and IgG as well as activated complement. These responses are observed in chronically inflamed tissues including the oral cavity with periodontal disease, the small intestine affected by celiac disease,[178] the stomach during infection with *H. pylori,*[179] and the intestines in response to inflammatory bowel disease (IBD).[180] Although many of the host responses are aimed at controlling these inflammatory conditions (e.g., through an increase in Treg cells and inhibitory cytokine production such as IL-10), the antigenic drive fueled by leaky epithelial cells and aberrant host responses prevents resolution of the inflammation. In addition, the recruitment of cells from the blood that lack the hyporesponsive mucosal phenotype[181] or disruption of proper differentiation toward this more anergic phenotype[182] or both lead to heightened responses not normally encountered in mucosal tissues. The contribution of the host response to the pathogenesis of immune-mediated disease triggered by an infection may be the price one pays for enhanced antimicrobial immunity.

Another concept that has emerged is related to the altered equilibrium that is associated with persistent or recurring inflammatory disease. Immune-mediated diseases can have a profound effect on microbial composition and vice versa. This has led to the concept of homeostatic scarring,[183] a process in which the equilibrium is perturbed and the simple treatment of a disease such as IBD with antiinflammatories may improve the inflammation but does little to restore homeostasis. Although it is not yet clear if the creation of a dysbiosis portends disease or if unrelated triggers lead to a proinflammatory microbiota, it increasingly appears that successful prevention or treatment of many diseases may require manipulation of the microbiota and some monitoring of mucosal immunity.

Mucosal Host Response Defines Pathogenicity

Much has been written about the role of microbial factors in disease pathogenesis[19]; however, it is also important to consider the contribution of local mucosal immune responses. Some of the most compelling evidence for immune-mediated damage following infection comes from genetic studies. First, with the emergence of genetically engineered animal models, it became clear that disruption of scores of genes that regulate immunity was sufficient to cause inflammation in the gastrointestinal tract, usually manifesting as colitis.[184–187] Of significance, disease is markedly attenuated or prevented by reducing the microbial burden with broad-spectrum antibiotics or raising animals in gnotobiotic conditions. These findings demonstrated that the normal, resident microbiota, often referred to as *commensals*, were sufficient to trigger disease. Second, more recent genome-wide studies of patient populations have shown that polymorphisms in genes encoding proteins that regulate host responses are associated with gastrointestinal diseases including gastric cancer associated with *H. pylori* infection,[188-191] Crohn disease, and ulcerative colitis.[192] These data have supported the concept that chronic, mucosal diseases such as IBD or gastric cancer are due to the inappropriate regulation of mucosal immune responses to microbial antigens in genetically susceptible hosts.[193]

Other reports implicate the host response in more acute, infectious diarrhea (Table 7.1). For example, challenging mice lacking stem cell factor with cholera toxin[194] or *Salmonella*[195] does not induce disease. Similarly, inhibiting the recruitment of leukocytes in chemokine receptor–deficient mice attenuates disease caused by *C. difficile* toxin.[196] By impeding the accumulation or activation of mast cells or neutrophils, these approaches remove a major source of mediators that stimulate epithelial cell secretion and diarrhea.

An aberrant host response does not provide a complete explanation for the pathogenesis of complex diseases, but immune responses contribute through their effects on colonization, translocation, tissue damage, and dissemination of infections. However, the interaction between the microbiota and the host is not always detrimental.

Good Bugs, Bad Bugs, and the Hygiene Hypothesis

The evolution of mucosal immunity was driven in part by the benefit of microbial-derived nutrients to the host and the need to keep microbial

TABLE 7.1 Role of Mucosal Immune Reponses in Microbial Pathogenesis

PATHOGEN	EVIDENCE FOR IMMUNOPATHOGENESIS
Vibrio cholerae	Absence of disease SCF-deficient mice[194]
Bacillus anthracis	Attenuation of disease in mice depleted of macrophages[251]
Bordetella pertussis	Clearance prevented by IL-10–producing Treg cells[167]
Clostridioides difficile (formerly *Clostridium difficile*)	Inhibiting leukocyte recruitment in chemokine-deficient mice prevents disease[196]
Salmonella spp.	Absence of disease in SCF-deficient mice[195]
Helicobacter pylori and gastric cancer	Class II MHC/HLA acts as receptor, and its expression is increased by cytokines[38] Tissue damage and epithelial cell apoptosis attributed to host response[252–257] Treg cells and antiinflammatory mediators control gastritis and contribute to persistence[258–263] Immunogenetic studies in humans implicate polymorphisms in genes that regulate host response (e.g., IL-1β, TNF-α, IL-10)[188,190]
Helicobacter hepaticus	Exacerbates disease in IL-10–deficient mice[264,265] Disease prevention associated with induction of IL-10–producing Treg cells[265]
Inflammatory bowel disease	Immunogenetic studies in humans implicating polymorphisms in genes that regulate host response to infections (e.g., NOD2, IL-17, TNF-α, IL-10)[192]

HLA, Human leukocyte antigen; *IL,* interleukin; *MHC,* major histocompatibility complex; *NOD2,* nucleotide oligomerization domain 2; *SCF,* stem cell factor; *TNF-α,* tumor necrosis factor-α; *Treg,* T regulatory.

communities under control. Increasingly the host response is recognized for its ability to shape microbial communities rather than sterilize them.[25,197–199] The notion that tissues should be sterile has never reflected reality, and attempts to scrub the mucosa to some artificial state of cleanliness are increasingly viewed as a contributor to many diseases. This notion has evolved from the hygiene hypothesis, which can be defined as the theory that children who are not exposed to robust microbial challenges have altered immune homeostasis and an increased risk for several immune-mediated diseases. Advocates of the hygiene hypothesis suggest that the symbiosis of a complex microbiota with the host response tends to establish a protective equilibrium. The cumulative effects of microbial virulence factors or metabolites, diet, environmental factors, and host genetics and epigenetics are all thought to impact mucosal immunologic homeostasis.

One clinical example that is often cited in support of the hygiene hypothesis is the continued increase in IBD in Western Europe and North America[200] and more recently in Asia.[201] Although many factors are associated with this trend, one hypothesis is that these Th1/Th17 predominant diseases occur less in countries that have a higher burden of infections, including nematodes. Indeed, helminth infection can attenuate IBD in humans[171] and animal models.[170] Other reports describe an antiinflammatory effect of nematodes on *H. pylori*–induced gastritis,[169] whereas probiotic bacteria[202–206] or fungal infections with *C. albicans* protect against colitis in animal models[207] by increasing IL-10 and decreasing IL-17A and IL-17F as well as TNF-α. In the case of *C. albicans,* the protection has been traced to its ability to induce indoleamine-2,3-dioxygenase production by dendritic cells that in turn affects the differentiation of Th cells.[208] Several other studies support the notion that PRRs and signaling through MyD88 are required to maintain mucosal homeostasis.[209,210]

Although diseases such as asthma and IBD occur less frequently in countries lacking the degree of hygiene found in Europe and North America, one cannot ignore the huge morbidity and mortality from mucosal infections in these populations that affect nutrition, growth, and cognitive development.[211] Ideally a limited microbial community that is more complex but lacks the most deleterious pathogens or virulence factors could achieve the potential benefit inferred by the hygiene hypothesis. *H. pylori* is one species that is widespread in countries cited as having lower rates of asthma or IBD. Epidemiologic studies suggest that *H. pylori* confers protection against esophageal cancers[137,212] or other infections including tuberculosis.[213] Müller et al. performed a series of studies in a murine model of asthma showing that neonatal infection with *H. pylori* induces Treg cells that inhibit gastritis and favor persistence but also attenuate airway disease.[134,135] Whereas one would have to weigh the risk of gastric cancer with the benefits of carrying *H. pylori,* it is possible that sufficiently attenuated strains will be generated that may be proven beneficial when included in a probiotic mix.

Additional evidence for the beneficial role of a complex microbiota comes from the use of fecal transplants as a strategy for preventing or attenuating diarrhea caused by *C. difficile*.[214] Furthermore, a natural shift in microbial communities has been implicated in recovery from cholera.[215] The current artificial approaches for "rePOOPulation"[216] lack the optimal specificity, assured freedom from pathogens, and the control of dose that are preferred in therapeutic approaches in medicine. Nonetheless, as the beneficial and deleterious microbial communities or metabolites/virulence factors continue to be identified, it may be possible to modify a host's microbiota for the benefit of the host's health through the use of prebiotics, probiotics, antibiotics, or even vaccines.

Mucosal Immunization

Given that many infections of significance to humans enter by a mucosal route, designing safe and effective mucosal vaccines remains a high priority to protect against infectious diseases including tuberculosis, HIV, various diarrheas, and influenza. Intact organisms, encountered naturally or as vaccine vectors, remain the most effective immunogens, as they entail multiple "danger" signals, some of which are not well understood. To make vaccines safe, scientists have opted for the reductionist approach; however, these approaches often lack the ligands that define tropism to a preferred inductive site and the requisite array of proinflammatory signals that determine if an antigen is ignored or acted on.

Effective mucosal immunization begins by providing the immunogen to a mucosal inductive site.[217] Traditionally, this has included administration by intranasal, sublingual, oral, and intravaginal routes, all of which have the goal of targeting mucosal tissues directly. In humans, one of the more successful mucosal vaccines is the oral polio vaccine. This is a live-attenuated virus vaccine and thus brings the advantage of replication and packaging that enhances the immunogenicity. Killed or subunit vaccines, particularly those delivered orally, are challenged by their relative lack of immunogenicity, as proteins are considered "food" more often than a danger signal. Other strategies to augment the immunogenicity have included the use of bacterial toxins as an adjuvant. Cholera toxin is profoundly immunogenic, although it carries significant side effects when administered orally. Mutated toxins, engineered as a fusion protein with the antigen of interest and possibly targeted to delivery by inhalation, could circumvent many unwanted side effects and yield a promising strategy in the future.[218] More recent efforts of fusion science in mucosal immunology, plant biology, and drug delivery engineering have provided an opportunity to advance strategies to develop effective antigen delivery vehicles targeting mucosal surfaces for the initiation of potent antigen-specific SIgA and serum IgG responses.[219,220] For example, a rice-based oral vaccine system has been shown to be effective for the induction of antigen-specific protective immunity against intestinal toxin produced by *Vibrio cholerae* and enterotoxigenic *E. coli*.[221,222] For nasal vaccination, the cationic form of pullulan-based nanogel is also effective for the induction of protective immunity against *Clostridium botulinum* type A neurotoxin and *Streptococcus pneumoniae*.[223]

Although the systemic route of immunization rarely induces protective mucosal immunity, exceptions exist; an example is the human papillomavirus vaccine.[224] Live virus vaccines, live vectors, or virus-like particles can be effective, as they are assumed to retain the tropism for mucosal tissues where they can replicate or be processed by APCs. This approach is believed to have the advantage of bringing a collection of signals that enhance immunogenicity and stimulate a response in an otherwise hyporesponsive environment—what Janeway eloquently

referred to as the "immunologist's dirty little secret."[225] It is theoretically possible that the strategic use of adjuvants or biologic response modifiers with systemic immunization may induce a phenotype that enables effector cells to populate mucosal tissues. For example, the role of retinoic acid in imparting the ability of APCs or lymphocytes to home to mucosal tissues could provide one strategy.[117] At the present time, no such adjuvants or biologic response modifiers that intentionally target mucosal trafficking are used routinely for this purpose in humans.

Considering the epidemiology of some transmissible diseases, there may be a strategic advantage to control infections through mucosal immunization of food-producing animals rather than attempt widespread immunization of humans. As a case in point, one would need a fraction of the vaccine preparations to control enterohemorrhagic *E. coli* (O157) by immunizing cattle rather than humans. By targeting the virulence factor Tir in these *E. coli,* infection in cattle is decreased,[226,227] and meat is thus rendered safer for human consumption without the need to extensively test another vaccine for use in humans.

Although some successes have been achieved in mucosal immunization, many challenges remain, including the pressing need for a vaccine against HIV and herpes simplex virus (HSV). When considering strategies for the successful induction of immunity, it is important to consider the known biology so that the immunogen gets delivered to the appropriate inductive site to stimulate a protective response in the tissue of interest. For HIV and HSV, the infection is spread naturally through mucosal routes of sexual contact including the rectum or female reproductive tract. However, very little is known, especially in humans, about which effector cell responses should be induced in these sites and how they can be induced. Based on other mucosal diseases and the vaccines that have been produced, one can predict that systemic immunization will be most effective at preventing infection if it can induce the required responses in these sites. Current efforts for preventing HIV and HSV infection at mucosal sites rely on a degree of blind luck to achieve immunity in the rectum or vagina given how little is known about the induction, regulation, effector mechanisms, and trafficking of immunologic cells in these sites. As evidenced by the parenterally administered polio vaccine, it is possible that systemic immunity may be sufficient to limit tissue injury and disease from a pathogen acquired through mucosal routes.

At the other end of the spectrum, mucosal immunization may be exploited as a strategy to decrease deleterious host responses through the induction of tolerance—through either immune deviation or active suppression. For example, if autoimmune diseases are driven by Th1/Th17 responses, the induction of Th2 cells may change the local homeostasis by dampening the Th1/Th17 cells. As described earlier (see "Good Bugs, Bad Bugs, and the Hygiene Hypothesis"), infection with nematodes has been shown to decrease gastritis[169] and colitis[170,171] in murine models as well as in humans with IBD.[228] Current efforts are focused on identifying specific molecules produced by the nematodes that could be incorporated into a mucosal delivery system. Other attempts to enhance tolerance to transplanted tissue or attenuate allergies or autoimmune diseases by the oral or inhaled delivery of the offending antigen have been contemplated, but limited success has been achieved in humans to date.

The field of mucosal immunology in disease prevention was advanced based on the importance of antibody production in breast milk. Ingestion of colostrum and breast milk is of great benefit to infants due to the presence of antibodies, glycans, and cytokines that protect the infant and condition the maturation of the infant's immune system.[229] Furthermore, the nutritional medium provided by breast milk (including oligosaccharides) creates a prebiotic effect that shapes microbial communities in the offspring.[230] Maternal antibodies are extremely relevant, as they reflect the microbial environment of the mother and hence that into which the infant is born. Consequently there is a great deal of interest in how specific antibody can be enriched by natural or artificial immunization. This has been pursued both in human medicine to decrease influenza and HIV transmission and in veterinary medicine to decrease zoonotic infections. The current successes in limiting *Coxiella burnetii* infection in ruminants has enhanced herd health by decreasing the pathogen burden and protected people working closely with these animals.[231] However, strategic immunization of mothers to protect infants from neonatal diarrheal or respiratory diseases has yet to be fully realized.

ROLE OF MUCOSAL IMMUNITY: BEYOND THE MUCOSA

In the gut, the microbial burden contributes directly to diarrhea, epithelial cell turnover,[232] and cancer[191,233,234] as well as to malnutrition, stunted growth, and impaired cognitive development.[211,235] However, this discussion would not be complete without pointing out the profound scope of bodily functions that are affected by interactions between the host and its microbial burden in mucosal tissues. Although it has been challenging to demonstrate that systemic antigen exposure modulates mucosal immunity, there is overwhelming evidence that mucosal exposure to antigens has profound effects on systemic responsiveness (e.g., through oral tolerance).[6,236,237] Another illustration is the emerging evidence that the host-microbial interactions in the gut modify diseases in systemic sites including hepatitis,[238] obesity,[238,239] diabetes,[240] and autoimmune diseases such as autoimmune encephalitis[241] and even memory[242] or behavior.[59,243] This ability to regulate disease or complex functions in multiple tissues adds a much broader relevance to the host-microbial interactions that constitute mucosal immunity.

One specific example of how bacteria in a mucosal site regulate host responses elsewhere was identified by the observation that mice from different vendors had sharp variations in the percentage of Th17 cells in the intestinal mucosa.[244] Subsequently, segmented filamentous bacteria (SFB) were identified as being sufficient to cause an expansion of Th17 cells.[21] Whereas mice can tolerate this expansion without obvious effects, on further provocation, the colonization with SFB and associated increase in Th17 cells exacerbated experimental autoimmune encephalitis.[241] These experiments yet again illustrate the outreach of the interactions between mucosal immune responses and the microbiota and the ambiguity in describing bacteria such as SFB as commensals when they may be better thought of as amphibionts.[19]

The current evidence does not provide a cogent explanation for these profound effects of the host-microbial interactions. It is safe to assume that to mediate systemic effects, molecules produced by mucosal microorganisms have to be "sensed" and tethered to changes in cell signaling, differentiation, and function that account for their effects—likely through immunophysiologic effects as much as pure immunologic responses. Increasingly, scientists are viewing the problem from a network perspective.[245] In a network, one can consider the entire microbiome—not merely from a taxonomic perspective but from metabolic and antigenic views.[246] Furthermore, the interaction of metabolites with other microbes as well as the host can be examined using a network analysis.[247] Similar rigor is required to understand the host genome, epigenome, transcriptome, and metabolome as well as environmental factors (including diet) and their collective impact on host responses and health.[248–250] These enormous data sets will require computational models that create a map of the biologic landscape that is defined by key intersections within the interactome.[245] F studies will subsequently be needed to validate the impact of these factors so that more strategic approaches can be used to improve health.

CONCLUSIONS

Understanding how diseases are caused or prevented by infection and the host response is in its infancy. The emerging evidence suggests that mucosal and systemic immune responses adapt throughout life in response to an array of dynamic changes in dietary, microbial, and other influences. Knowing the changes in metabolites that microbiota impart; how these are sensed, that is, in utero, in the neonatal period, and throughout life; and their effect on transcriptomes and function will enhance our understanding of the mechanisms by which microbes modulate disease. These complex interactions will require many advances in systems biology and computational modeling so that strategies to enhance immunity with vaccines or by improving health by manipulating microbial communities will be more effective. With more attention to the endless possibilities, technically difficult approaches will be overcome, and manipulation of the host-microbial interactions in the mucosa will become an important target for interventions that prevent or treat many diseases.

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8 Granulocytic Phagocytes

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Vertebrate host defense against microbes represents the integration of the innate and acquired immune systems, which together respond to a diverse array of infectious threats.[1,2] Innate (natural) immunity provides the host with the capacity to respond immediately to an infectious challenge, regardless of previous exposure to the specific invading agent, by using response elements encoded in germline genes. Elements of the innate system include phagocytic cells, polymorphonuclear leukocytes, mononuclear phagocytes, and circulating soluble proteins, including components of the complement system (see Chapters 4 and 9). This sensitive system for the recognition of structural elements that are inherently and uniquely microbial has functional analogues in the immune systems of a wide variety of multicellular organisms, including plants and insects. As such, innate immune elements comprise an evolutionarily ancient system that provides a rapid and sensitive surveillance mechanism to protect the host when challenged with any invading microorganism. However, on par with the importance of rapid recognition of and response to microbial threats is the need to resolve the inflammatory response and restore homeostasis. Granulocytes, the most numerous leukocytes in the peripheral circulation of humans, include neutrophils, eosinophils, and basophils. They represent the predominant cell type in the acute innate immune response and figure more broadly in the integration of innate and adaptive immunity.[2,3] Structurally, these cells share with one another a multilobed nucleus and the presence of numerous membrane-bound, characteristically staining cytoplasmic granules, but functionally they differ significantly.

NEUTROPHILS

Development

Neutrophils arise from pluripotent hematopoietic stem cells (HSCs) in the bone marrow through an orderly succession of phenotypically distinct cell types.[4,5] From the HSCs arise multipotential progenitors, a cell population with the capacity to differentiate into all hematopoietic lines but unable to multiply. The multipotential progenitors spawn common myeloid progenitors that serve as the source of precursors for the individual hematopoietic cell lines, including granulocyte/macrophage progenitors. The complex procession from HSC to granulocyte/macrophage progenitor and then to neutrophils is coordinated by specific transcription factors, including PU.1, CCAAT/enhancer-binding proteins (α, β, and ε), growth factor independent-1, and interferon regulatory factor 8.[5] Timely expression of such factors coordinates transcription of stage-specific genes that are responsible for the phenotypic and functional features that define myeloid intermediates along the differentiation pathway. In part, soluble proteins, such as interleukin-17 (IL-17), IL-23, and granulocyte colony-stimulating factor (G-CSF), modulate the relative levels of transcriptional factors within myeloid cells and thus influence the fate of the cell.[6] CSFs also alter the survival and direct the maturation and proliferation of myeloid cells. Each factor is named for the colony produced under its influence: GM-CSF, for granulocytes and macrophages; G-CSF, for granulocytes; M-CSF, for monocytes and macrophages; and multi-CSF (or IL-3), for a variety of colonies, including neutrophils, macrophages, eosinophils, megakaryocytes, and erythroid cells.

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

Emergency Granulopoiesis

In addition to the granulopoiesis essential to maintain steady-state levels of circulating neutrophils, the hematopoietic system has the capacity to mobilize additional functioning neutrophils in response to the increased demand imposed by infection.[7,8] Infection induces augmented production of cytokines, including G-CSF, GM-CSF, and IL-3, and these circulating proteins drive "emergency" granulopoiesis. IL-17, a cytokine produced by Th17 cells,[9] drives G-CSF production and promotes emergency granulopoiesis, as deduced from experimental models of chronic inflammation, but it does not contribute to homeostatic neutrophil production. G-CSF stimulates the production of granulocyte precursor cells, the proliferation of cells in the granulocyte lineage, and the survival of granulocyte precursors and neutrophils. Furthermore, G-CSF accelerates passage of granulocyte precursors through the bone marrow, thereby providing an immediate supply of young neutrophils into the circulation. Thus the control of granulocyte production can be modulated not only to maintain homeostatic levels of neutrophils as aged cells are cleared from the circulation but also to respond to increased demands created by infectious or other challenges.

Mitotic and Postmitotic Cells

The cell populations during steady-state granulocyte development in the bone marrow can be divided into three pools: a stem cell pool, a mitotic pool, and a postmitotic pool.[10] The stem cell pool includes undifferentiated HSCs, whereas the mitotic pool encompasses cells that proliferate and mature sequentially from myeloblasts into promyelocytes and myelocytes. Maturation is associated with the appearance of the cytoplasmic granules characteristic of neutrophils, eosinophils, and basophils.[11] The postmitotic phase of development includes metamyelocytes, band (or immature) neutrophils, and mature neutrophils, all cells held in reserve and ready for release.

Coincident with the appearance of morphologic changes, cells acquire the specific surface markers and functional properties of more mature cells.[12] For example, Fc receptors appear as the cells develop into promyelocytes, competence for phagocytosis arises in the early myelocyte stage, and complement receptors surface in the late myelocyte and metamyelocyte stages. Oxygen-dependent microbicidal activity appears in the early metamyelocyte stage, and cells in the late metamyelocyte–band stage demonstrate increased adhesiveness, cell motility, and chemotactic responses.[12] In addition, coordinated expression of genes encoding the granule proteins is synchronized with early stages of myeloid development, and normal granulocytic differentiation is intimately linked with expression of proteins localized in the specific granules.[12]

Morphologic and Structural Characteristics

The earliest histochemical studies of neutrophils classified the membrane-bound intracellular granules by their staining characteristics. Two populations of granules were distinguished based on staining with azure A: the positively staining azurophilic granules and the unstained specific granules. Sophisticated analyses of the composition of isolated neutrophil organelles have refined significantly our appreciation of the complexity and heterogeneity of neutrophil granules.[13,14] Such studies have provided novel insights into the biologic roles of the various proteins in the

matrix of the granule and have revealed functionally important proteins within the membranes of particular granule subsets.

Azurophilic Granules

At a first approximation, neutrophil granules can be categorized based on peroxidase staining. The peroxidase-positive granules are also known as *primary granules,* as they arise first in granulopoiesis, and as *azurophilic granules,* based on histochemical staining. Azurophilic granules contain myeloperoxidase (MPO)[15]; a variety of proteolytic enzymes, including the four serine proteases, cathepsin G, proteinase-3,[16] elastase,[17] and NSP4 (neutrophil serine protease 4)[18]; and the antimicrobial defensins[19] and bactericidal permeability-increasing protein (BPI) (Table 8.1).[20]

TABLE 8.1 Contents of Neutrophil Granules and Secretory Vesicles

GRANULE	MEMBRANE	MATRIX
Azurophilic (primary granule)	CD63,CD68, presenilin	MPO, elastase, cathepsin G, proteinase 3, defensins, BPI, lysozyme, sialidase, azurocidin, β-glucuronidase
Specific (secondary granule)	CD11b/CD18, CD66, CD67, gp91*phox*/p22*phox,* TNF receptor, SNAP-23, VAMP-2, stomatin	Collagenase, gelatinase, urokinase plasminogen activator, hCAP-18, NGAL, vitamin B_{12}–binding protein, lysozyme, lactoferrin, haptoglobin, pentraxin 3, prodefensin, SLPI, orosomucoid, heparanase, β_2-microglobulin, CRISP3
Gelatinase (tertiary granule)	CD11b/CD18, CD67, gp91*phox*/p22*phox,* MMP25, TNF receptor, SNAP-23, VAMP-2, Nramp1	Gelatinase, arginase 1, lysozyme, β_2-microglobulin, CRISP3
Secretory vesicles	CD11b/CD18, CD67, gp91*phox*/p22*phox,* MMP25, CD35, CD16, C1q receptor, CD14, fMLF receptor, SNAP-23, VAMP-2, Nramp1, alkaline phosphatase, DAF, CD10, CD13, CFTR	Plasma proteins

BPI, Bactericidal permeability-increasing protein; *CD,* cluster of differentiation; *CFTR,* cystic fibrosis transmembrane conductance regulator; *CRISP3,* cysteine-rich secretory protein 3; *DAF,* decay accelerating factor; *fMLF,* *N*-formyl-methionyl-leucyl-phenylalanine; *hCAP-18,* human cathelicidin protein-18; *MMP25,* matrix metalloproteinase 25; *MPO,* myeloperoxidase; *NGAL,* neutrophil gelatinase–associated lipocalin; *Nramp1,* natural resistance-associated macrophage protein; *SNAP-23,* synaptosomal-associated protein-23; *SLPI,* secretory leukocyte protease inhibitor; *TNF,* tumor necrosis factor; *VAMP-2,* vesicle-associated membrane protein-2.

From Borregaard N. Neutrophils, from marrow to microbes. Immunity. *2010;33:657–670; and Borregaard N, Sorensen OE, Theilgaard-Mönch K. Neutrophil granules: a library of innate immunity proteins.* Trends Immunol. *2007;28:340–345.*

Because of the acid hydrolase activity of the azurophilic granule contents, this compartment had been considered lysosomal in nature. However, azurophilic granules lack lysosome-associated membrane protein,[21] an identifying marker for lysosomes. Moreover, proteins such as MPO[22] and the defensins[23] segregate into the azurophilic granule independently of the mannose-6-phosphate receptor, a targeting system characteristic of lysosomal proteins. Taken together, these observations suggest that the azurophilic granule may be a specialized organelle that is distinctly different from conventional primary lysosomes.

Peroxidase-Negative Granules

The peroxidase-negative granules include *specific granules, gelatinase granules,* and *secretory vesicles.*[24] The contents of the specific and gelatinase granules overlap to a significant extent (see Table 8.1)[25] but differ from the contents of azurophilic granules and secretory vesicles. More striking, however, is the distribution of functionally important plasma membrane proteins in the membranes of peroxidase-negative granules.[26] These membranes contain flavocytochrome b_{558},[27,28] an essential component of reduced nicotinamide adenine dinucleotide phosphate (NADPH)–dependent oxidase (discussed later); receptors for chemotactic peptides[29]; extracellular matrix proteins[30]; cytokines[31]; opsonins[32]; and adhesion proteins.[33,34] The secretory vesicles are especially enriched for plasma membrane proteins[35] and can be rapidly recruited to fuse with the plasma membrane, amplifying the potential of the neutrophil to respond to stimulation. They therefore represent an intracellular reservoir of functionally important membrane proteins that can be quickly recruited to the cell surface during neutrophil activation. The existence of such compartments is ideally suited to the role of neutrophils as the major circulating cell in the innate immune system; a reservoir of readily accessible functional proteins allows a rapid response without the delays that would be incurred by requirements for new protein synthesis. The functional consequences of this compartmentalization of proteins in the matrix and in the membrane of granules are discussed later.

Nucleus and Actin Cytoskeleton

During granulocyte maturation, the nucleus becomes segmented (Fig. 8.1), and cytoskeletal elements, including microfilaments and microtubules, appear in the cytoplasm. A meshwork of microfilaments makes up the clear cortical veil that surrounds the cell and forms the lamellipodium of an advancing cell (see Fig. 8.1). These structures are polymers of actin, a protein representing 5% to 10% of the total cellular protein. Actin and its associated proteins constitute the contractile machinery necessary for cellular locomotion[36] and phagocytosis.[37] Actin monomers (G-actin), in the presence of actin-binding protein, polymerize to form cross-linked actin filaments (F-actin). Regulation of the length of the filaments and the degree of cross-linking provides the physicochemical dynamics of actin flux between the gel and sol states. Actin filaments are associated with the cytoskeleton or with the plasma membrane via membrane skeletal proteins.[38] Stimulation of the cell with chemotactic factors causes an abrupt increase in the amount of actin associated with the cytoskeleton[39] and a shift in microfilament organization from a parallel strand to a cross-hatched meshwork most evident at the leading edge of the directionally polarized cell.[40] Microtubules appear to be necessary

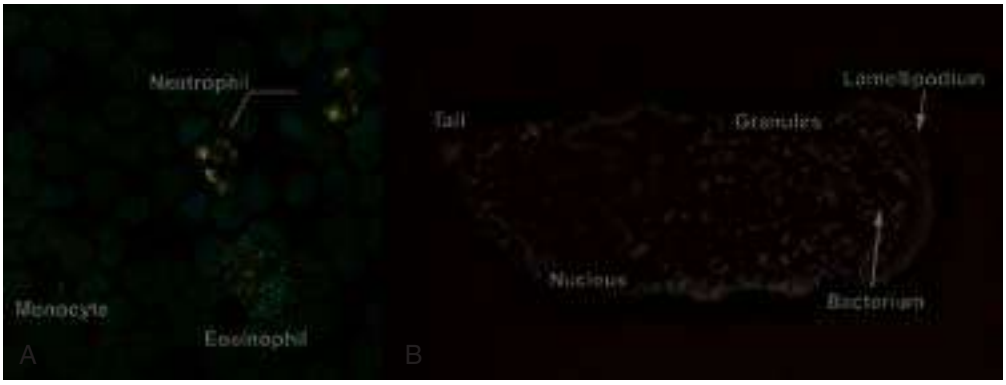

FIG. 8.1 Human neutrophils. Photomicrographs of phagocytes in whole blood (A) and a neutrophil as viewed by phase-contrast microscopy (B).

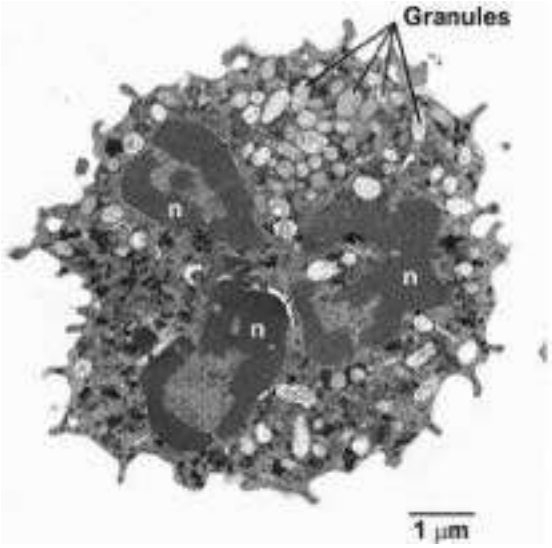

FIG. 8.2 Electron micrograph of a human neutrophil. Note the granules (as indicated) and glycogen particles *(small dark particles),* but few other prominent organelles. *n,* Nucleus (multilobed).

for the initial orientation of the cell in a chemotactic gradient; for the spatial organization of structures within the cell during locomotion; and for vesicle transport, degranulation, and the regulation of cell surface microviscosity during phagocytosis.

Organelles in the Cytoplasm

Mature neutrophils (Fig. 8.2; see Fig. 8.1) are characterized by a paucity of ribosomal material and mitochondria, in keeping with the relatively low levels of synthetic processes in these cells. However, studies over the past 3 decades have radically changed the image of neutrophils as biosynthetically inactive cells and have identified an array of proteins actively synthesized by neutrophils, both at rest and after stimulation. Among the proteins synthesized by neutrophils are major histocompatibility complex class I molecules,[41] complement receptors,[42] CXC chemokines, CC chemokines, proinflammatory and antiinflammatory cytokines, immunoregulatory molecules, colony-stimulating factors, tumor necrosis factor (TNF) superfamily members, and proteins important in angiogenesis and fibrogenesis.[3] As presented later, the transcriptional profile of recruited neutrophils includes genes directed toward wound healing.[43] Glycogen granules fill the cytoplasm and serve as a source of energy to support neutrophil activity.

Surface Receptors

As immune effector cells, neutrophils are equipped with surface receptors that sense extracellular signals and can recognize ligands to support the wide array of agonist-dependent activities within its functional repertoire. Surface receptors for immunoglobulin[44] and complement fragments[45] contribute to opsonin-dependent phagocytosis. Specific receptors on the plasma membrane initiate cell movement by recognizing interacting molecules on endothelial cells or extracellular matrix proteins, bacterially derived formylated proteins, chemotactic complement fragments C5a and C3a, platelet-activating factor, IL-8 and related chemokines, and leukotriene B_4 (LTB_4).[46–48,49,50] These receptors are homogeneously distributed over the surface of the resting cell but undergo an asymmetrical clustering at the front of the cell when it polarizes in response to a chemotactic stimulus. The distribution of receptors with different ligand specificities can be independently regulated, even though stimulation via these receptors may evoke similar functional responses.[51] Moreover, the various neutrophil functional activities exhibit differential requirements for receptor occupancy. For example, maximal degranulation requires brief receptor occupancy, whereas sustained oxidative responses depend on continuous ligand binding to the receptor.[52] Neutrophils also possess membrane receptors that signal to evade ("don't eat") or promote ("eat me") their own uptake by macrophages, a process known as efferocytosis (see later).[53]

Homeostasis of the Circulating Neutrophil Population

To maintain a stable number of circulating neutrophils, the production of new and functional cells must be balanced by clearance of cells that are aged and spent. The daily production of mature polymorphonuclear leukocytes in a healthy adult is remarkable, with approximately 10^9 cells/kg body weight entering the circulation from the bone marrow.[54] During acute infection or other inflammatory stresses, neutrophils are mobilized from the granulocyte marrow reserve, which is estimated to be $\approx 18.6 \times 10^9$ cells/kg (6.9×10^9 cells/kg are mature neutrophils).[54] Even in the presence of persistent stimulation, this reserve can be depleted only if there is nutritional deficiency or another disorder (e.g., ethanol abuse) that compromises mechanisms for augmenting delivery to meet demands. Augmented stem cell input, increased mitoses during the mitotic stage of development, use of a store of cells whose maturation had been inhibited (so-called hiatal cells), and shortening of the maturation time within the marrow all may occur.[10] Multiplication and differentiation of stem cells are stimulated by the CSF produced by peripheral blood monocytes, tissue macrophages, and stimulated lymphocytes.[54,55]

Distribution of Neutrophils

The total blood granulocyte pool ($\approx 7 \times 10^8$ cells/kg body weight) includes two compartments of similar size: intravascular circulating cells and marginating cells. The distribution of the marginated pool varies with the size and flow of the capillary bed in an individual organ. Liver, spleen, and bone marrow are included in this pool, but unsettled is the physiologic contribution of the pulmonary circulation.[10,56] Whereas experimental data based on intravascular transit time suggest that the lung is the predominant site of marginated neutrophils, studies using radionuclide imaging demonstrate relatively little neutrophil margination in normal human lungs. Overall, the contribution of the pulmonary circulation to the marginated pool of normal neutrophils in healthy humans remains unsettled.

A dynamic equilibrium exists between neutrophils in marginated and circulating compartments as cells marginate via transient endothelial interactions and then resume rapid flow, reflecting the balance between intercellular adherence and shear forces.[57–59] The intravascular half-life of circulating neutrophils is normally 6 to 8 hours, whereas their persistence in extravascular sites ranges from a few hours to several days. A report using in vivo labeling with ^2H_2O suggested that normal human neutrophils have a life span in circulation of 5.4 days,[60] more than 10-fold greater than previously thought. However, that conclusion has been challenged,[61,62] and alternative interpretations of the same data yield estimates that agree with the long-standing accepted value of 6 to 8 hours. Turnover is accelerated and half-life in circulation is shortened during infection, as neutrophils are recruited to sites of inflammation, where they ingest microbes and undergo accelerated apoptosis (see later). In fact, the redistribution of circulating neutrophils to sites of infection explains, in part, the failure of neutrophil blood counts to rise after granulocyte transfusions. Whereas granulocyte transfusions may promote resolution of acute infection, decrease the time to resolution of fever, and shorten the duration of antibiotic therapy, they do not typically increase peripheral white blood cell counts.[63,64]

Granulocytosis, a common feature of acute inflammation, is a consequence of certain physiologic and pharmacologic stimuli that typically redistribute neutrophils among the various granulocyte pools as well as increase cell production. For example, the acute administration of corticosteroids or endotoxin, perhaps mimicking pathophysiologic events that occur in severe infection, promotes granulocyte release from the marrow reserve. Sustained steroid administration produces granulocytosis primarily by decreasing neutrophil adherence and shifting cells from the marginating to the circulating pool. Similarly, exercise, stress, epinephrine, hypoxia, aspirin, and alcohol cause granulocytosis by mobilizing marginating cells.

In the setting of acute inflammation, spent and apoptotic neutrophils are ingested by macrophages by the regulated process of efferocytosis.[65] Neutrophils that are activated during their brief tour in circulation become senescent, a proapoptotic state characterized by increased surface expression of CXCR4 and decreased ability to perform antimicrobial

and proinflammatory functions.[66] Resident macrophages in liver, spleen, and likely bone marrow[56] routinely ingest and thereby clear senescent neutrophils from circulation in a manner that is immunologically silent—that is, without release of proinflammatory cytokines.

Inflammatory Response

Inflammation represents a remarkably integrated cascade of events involving both cellular and soluble factors that are precisely orchestrated spatially and temporally. As such, it is best conceptualized as a complex network of signals that modulate the responses of different cells and circulating molecules that, in turn, interact and are subject to a variety of regulatory checkpoints operating by local, systemic, and neural mechanisms.[3,67,68] Within the context of host response to invading microbes, the innate immune system sits poised to respond rapidly to perceived threats and in a stepwise fashion to recognize, contain, kill, and destroy potential pathogens. Because all successful biologic systems represent a balance among competing forces, the return of the host to homeostasis after an acute inflammatory response requires execution of a properly timed and appropriately proportioned antiinflammatory cascade. Thus the acute response of neutrophils requires a sensitive afferent limb to allow systemic recognition of a local threat at very low levels as well as an effector arm targeted against the noxious source to contain, kill, and degrade potential pathogens.[67] The coordinated response needs to occur before antiinflammatory events supervene to trigger neutrophil apoptosis and removal en route to resolution of the inflammatory reaction.[69] In addition, the fact that the noninflammatory homeostatic state is actively maintained by modulation of proinflammatory surveillance systems, rather than simply the absence of inflammatory stimuli, adds another layer of complexity and feedback signaling to an already intricate system.[68–70]

Circulating neutrophils are functionally heterogeneous, with most (80%), but not all, cells having the capacity to form immunoglobulin G (IgG) rosettes.[71] Because release from the bone marrow is not synchronized, this heterogeneity probably reflects in part maturational differences within a single cell line. More sophisticated analyses suggest that subsets of circulating neutrophils have distinct and important functional phenotypes.[72–78] In contrast to the heterogeneity of circulating neutrophils, those in tissue are relatively homogeneous, and more than 96% are capable of IgG rosette formation.[71] They contain fewer lysosomal granules and more glycogen than do their circulating counterparts because anaerobic glycolysis provides the energy for cell movement through the tissues.[79] The phenotypic differences between circulating and tissue neutrophils could reflect determinants required for neutrophils to immigrate into tissue, influences of transmigration per se, the impact of trophic elements in the tissue compartment, or other factors. In any case, the tissue neutrophil exhibits a phenotype different from that of the circulating, unstimulated neutrophil. For example, exudative neutrophils synthesize significantly more IL-8 and activate genes that collectively contribute to wound healing.[43,80] Furthermore, neutrophils exposed to concentrations of mediators that are too low to stimulate directly nevertheless prepare the cell for an enhanced response to a second, unrelated stimulus in a phenomenon known as *priming*.[81,82]

Priming

A broad array of proinflammatory mediators, including chemotactic factors, bacterial molecules, chemokines, cytokines, and certain lipids, can prime neutrophils, as can transmigration across the endothelium and migration into tissue. The primed state exists with respect to each of the major aspects of neutrophil function, persists for an extended period (longer than 20 minutes under experimental conditions in vitro) in relation to the response elicited by direct stimulation of the cell, and is reversible. It is not known whether priming agents share the same mechanism of action or if all the essential molecular events causing priming have been elucidated. Consistent with the diverse phenotypic features of primed neutrophils, partial assembly of the NADPH oxidase by phosphorylation and translocation of p47*phox,* secretory vesicle exocytosis and partial mobilization of specific granules to the plasma membrane resulting in increased surface expression of flavocytochrome b_{558} (see later), reorganization of the plasma membrane and distribution of receptors and signaling molecules into lipid rafts, modulation of intracellular signaling intermediates, and transcription of several gene families have been implicated in contributing to the more responsive state of the cell.[83–89] Most neutrophil priming agents also delay neutrophil apoptosis and thus prolong functional capacity, a phenomenon consistent with the potential for an enhanced proinflammatory response.[90]

Step 1: Neutrophil Recruitment

To combat invading microorganisms, neutrophils must emigrate from the circulation and into the extravascular tissue space, a process that reflects both the responses of neutrophils to the shear stress in the circulation and the summation of coordinated interactions of cells, specific receptors, and soluble mediators.[48,91,92] In fact, neutrophils in tissue, interacting with elements in the extracellular matrix, differ functionally from circulating neutrophils.[93–95] The process of neutrophil transmigration out of the circulation involves at least four discrete steps: rolling adhesion, integrin activation, firm adhesion, and transmigration (Fig. 8.3).[91] These



FIG. 8.3 Steps in the emigration of neutrophils from the vascular space. Neutrophils are depicted entering the marginating pool in a postcapillary venule, and the effector molecules participating in each event are indicated in gray text. Initial capture is mediated by interactions between neutrophil/leukocyte (L)-selectin and endothelial cell (E, P)-selectins and their respective carbohydrate ligands on the opposing cell surface (e.g., P-selectin glycoprotein ligand-1 *[PSGL-1]*), whereas integrins (e.g., very late antigen 4 *[VLA4]*) are responsible for firm adherence. Selectin-mediated signaling slows the rolling or tumbling neutrophils, partially countering the shear forces resulting from blood flow. Chemokines diffusing into the bloodstream from sites of microbial invasion in the tissues bind to specific receptors and activate the neutrophil, which is then arrested by integrin-dependent interactions. The activated neutrophil flattens against the endothelium and crawls along the luminal surface of the vessel wall. Platelet–endothelial cell adhesion molecule-1 *(PECAM-1)*, junctional adhesion molecules *(JAMs)*, and endothelial cell–selective adhesion molecule *(ESAM)* localized to interendothelial cell junctions interact with PECAM-1 and CD99 on the neutrophil surface to permit neutrophil migration between and through endothelial cells. Once in the tissues, the polarized neutrophils move up the mediator concentration gradient to reach the site of microbial invasion. See text for further details. *ICAM-1,* Intercellular adhesion molecule; *LFA-1,* leukocyte function–associated antigen; *MADCAM1,* mucosal addressin cell adhesion molecule 1; *PI(3)K,* phosphatidylinositol-3-phosphate kinase; *SRC,* sarcoma; *VAV1,* vav1 guanine nucleotide exchange factor; *VCAM1,* vascular cell adhesion molecule 1.

events are mediated in turn by four classes of adhesion proteins: selectins, integrins, immunoglobulin-like proteins, and mucin-like selectin ligands. In addition to neutrophils and endothelial cells, platelets figure prominently in the initiation of the inflammatory response, colocalizing with neutrophils and participating in P-selectin–dependent leukocyte binding.[92] The cooperation of several cell types and their secreted products culminates in events necessary to recruit circulating neutrophils to the site of inflammation, and the activation of autocrine and paracrine feedback loops modulates the extent of the host response.[91,92]

Selectin-Mediated Adhesion and Rolling

Through a process of repetitive ligand-receptor binding and release, marginating granulocytes become reversibly adherent to endothelial cells in the postcapillary venules and, under the influence of the physiologic shear forces of blood flow,[91,92] tumble or roll slowly along the vessel wall (see Fig. 8.3). The molecules mediating rolling adhesion are called *selectins,* to indicate that the amino-terminal lectin domain mediates their selective function and cellular expression. Homologous to C-type lectins, selectins require calcium for expression of binding activity. Individual members of the selectin protein family are named for the cell type on which they were originally identified (E, endothelia; L, lymphocytes; P, platelets). Selectins interact with sialylated Lewis X and A glycoproteins on the surface of interacting cells as well as sulfated and phosphorylated polysaccharides, such as heparin and mannose-6-phosphate. Individual selectins exhibit different but overlapping binding specificities, the bases for which largely remain to be determined.[96]

The presence of unique selectins on endothelial cells and neutrophils means that rolling adhesion can be modulated bidirectionally. For example, L-selectin is constitutively expressed on neutrophils and is shed after cell activation. In contrast, little or no E-selectin is present on resting endothelial cells, either in vitro or in vivo, but stimulation triggers inducible and transient E-selectin expression, which peaks within 4 hours after stimulation and dissipates over 24 hours.

β_2-Integrin–Mediated Cell-Cell Interaction

When neutrophils tumbling along the venule wall encounter inflammatory mediators and stimulated endothelial cells, adhesive interactions between the two cell types rapidly shift to a high-affinity state, reflecting activation of β_2-integrins.[91,92] The β_2-integrins, or leukocyte integrins, are members of a large family of heterodimeric molecules that mediate cell-cell and cell-matrix interactions.[91] The integrin protein family is subdivided on the basis of eight different β chains, any one of which can associate with multiple α chains to form a unique $\alpha\beta$ pair. Both the α and the β chains are transmembrane molecules with short cytoplasmic tails and large extracellular globular heads that interact to form the ligand-binding site. The three integrins on neutrophils express a common 95-kDa β_2 chain, CD18, but distinct α chains.[97] These molecular complexes are also referred to as leukocyte function–associated antigen-1 (LFA-1; $\alpha_L\beta_2$, CD11a/CD18), Mo-1 or Mac-1 ($\alpha_M\beta_2$, CD11b/CD18), and p150, 95 ($\alpha_X\beta_2$, CD11c/CD18).[91,97] CD11b/CD18 and CD11c/CD18 also function as receptors (CR3 and CR4, respectively) for the opsonic C3 fragments iC3b and C3d. Endothelial counterreceptors for the β_2-integrins include intercellular adhesion molecule-1 (ICAM-1) and ICAM-2. LFA-1 binds to both ICAM-1 and ICAM-2, whereas CD11b/CD18 and CD11c/CD18 bind only to ICAM-1 but at different sites from that for LFA-1. An additional LFA-1 counterreceptor, ICAM-3, is not present on endothelium but is expressed on all hematopoietic cells, where it may be involved in leukocyte-leukocyte interactions.[98,99] In addition to the β_2-integrins, neutrophils possess on their surface the leukocyte response integrin,[100] which, together with integrin-associated protein, modulates cellular responses, particularly responses induced by extracellular matrix proteins.[101–103] Although the precise details of the interactions of these various proteins are unknown, their importance is inferred from the observation that mice deficient in integrin-associated protein are unable to mount an inflammatory response after intraperitoneal challenge.[104]

To be functional, integrins require calcium, a specific membrane environment, and appropriate stimuli such as chemoattractant peptides, chemokines, or cytokines.[97] These stimuli appear to modulate integrin binding affinity by inducing conformational changes in the receptor, which, in turn, alter the interaction of the intracytoplasmic tails with each other and with the cytoskeleton.[97] Integrin activation results in increased surface number and avidity of β_2-integrins as well as clustering of receptors and cytoskeletal rearrangement. This conformational change–mediated process has been named "inside-out signaling."[91,97] By comparison, "outside-in signaling" refers to events that occur after ligand-induced integrin clustering and the change in receptor avidity (e.g., firm cell adherence and events leading to degranulation and neutrophil superoxide production).[91,97] Neutrophils change from spherical granulocytes with relatively little surface area involved in cell-cell contact to flattened, adherent cells with a broad surface area for cellular interactions (Fig. 8.4). Blood flow shear forces are no longer able to propel them forward along the vessel wall. This firm adhesion, the third step in transmigration, is mediated through interactions between β_2-integrins on neutrophils and ICAM-1 and ICAM-2 on endothelial cells.[91] Recent studies of neutrophil extravasation, using mice deficient in individual members of the β_2-integrin family, suggest that CD11a/CD18 may participate in neutrophil adhesion, whereas CD11b/CD18 supports crawling of neutrophils along the cells lining the vessel lumen.[105]

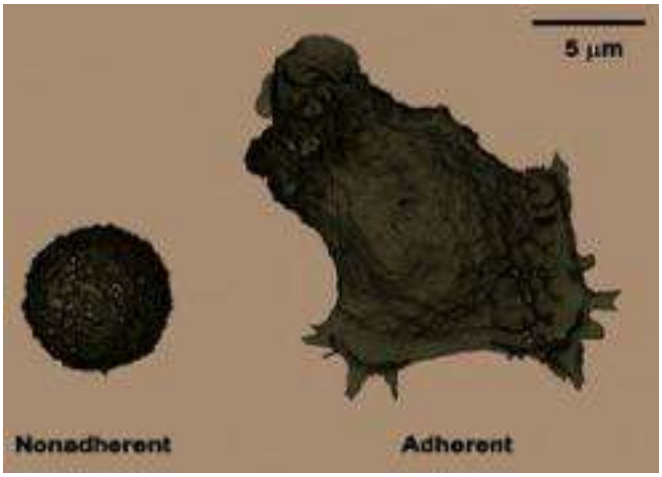

FIG. 8.4 Scanning electron micrographs of nonadherent and adherent neutrophils.

Tissue Migration

Transmigration, the final step in neutrophil immigration into tissue (see Fig. 8.3), involves multiple leukocyte and endothelial cell molecules, including platelet-endothelial cell adhesion molecule-1 (CD31), junctional adhesion molecules (e.g., JAM-A, JAM-B, JAM-C), endothelial cell selective adhesion molecule, ICAM-1 and ICAM-2, integrin-associated protein (CD47), CD11b/CD18, and CD99 (on neutrophils).[91,106] CD31 and CD47 are localized at the intercellular junctions between endothelial cells, whereas CD99 is expressed on neutrophils. JAMs are expressed on multiple cell types, including endothelial cells and neutrophils, and facilitate leukocyte adhesion to endothelial cells during transmigration.[107] Models reflecting current understanding dictate that transmigration typically occurs between endothelial cells, perhaps by neutrophil-dependent transient disorganization of adherens junctions at cell-cell interfaces.[91] However, there is accumulating evidence that neutrophil extravasation can occur by transcellular pathways.[91,108] Transcellular leukocyte movement involves formation of actin and vimentin-stabilized channels through which leukocytes migrate.[91]

Transmigration is rapid, occurring in less than 2 minutes after leukocyte-endothelial contact is established, and remarkably efficient in that neutrophils translocate without compromising the integrity of the endothelial monolayer. As neutrophils migrate through tissue, additional factors contribute to their ameboid movement. Complex signaling integrates actin-dependent cytoskeletal rearrangements, activity of small-molecular-weight guanosine triphosphate (GTP)ases, localized generation of specific phosphoinositides, and lipid mediators into complex feedback loops that promote responsive changes in cell shape coordinated in time and space.[92,109–115]

Although this paradigm applies to neutrophil extravasation from the vascular lumen into tissue, transmigration of neutrophils across epithelial barriers from tissue into visceral lumina, which is necessary in infections involving the gastrointestinal, genitourinary, and respiratory tracts, deviates in several ways from this theme.[116,117] Major insights into the mechanisms of neutrophil interaction with polarized epithelial cells have been derived from studies with human cell lines such as T84 and HT29.[118–120] Neutrophils can bind to either surface of these cells, and transmigration can occur in either direction. Evidence suggests that the mechanism for transmigration in each direction is distinct because immunomodulators such as lipoxin A_4[121] and interferon-γ (IFN-γ)[122] stimulate movement toward the basolateral surface but inhibit luminal migration. The interactions between epithelial cells and migrating neutrophils are remarkably coordinated, as evidenced by reciprocal secretion of adenosine and IL-6 by monolayers of T84 cells.[123] Furthermore, transmigration across intestinal epithelium triggers neutrophil release of elastase, which then disrupts the apical junctions in a very localized fashion, perhaps contributing to the loss of epithelial cell integrity during colitis.[124] Eventual characterization of regulated neutrophil movement across epithelium and the interactions between epithelial cells and migrating neutrophils will provide important insights into the pathophysiology of infectious diseases at such epithelial surfaces.

Production of Inflammatory Mediators

Neutrophil migration through tissue is likewise the consequence of a carefully regulated process involving the sequential release and compartmentalization of a wide variety of inflammatory mediators.[48,92] Early (0 to 5 hours) neutrophil influx into an area of induced injury appears predominantly to reflect the effects of IFN-γ, C5a, and LTB_4. IL-8 and IL-6 appear in a second wave of mediator activity (at 5 to 24 hours), and IL-1α, GM-CSF, and TNF-α appear in a third wave of activity (8 to 24 hours). Meanwhile, concentrations of IL-1, IL-2, and IL-4 remain unchanged. C5a, LTB_4, and IL-8 are potent neutrophil chemoattractants, as are hydroxyeicosatetraenoic acids and microbial oligopeptides analogous to *N*-formyl-methionyl-leucyl-phenylalanine (fMLF).

Tissue migration of neutrophils also reflects remarkable temporal regulation, well illustrated by the shift in the biosynthesis of lipid mediators during inflammation.[70] Proinflammatory molecules, such as leukotrienes and prostaglandins, are generated endogenously at inflammatory sites and stimulate neutrophil degranulation and chemotaxis. The arachidonate released by activated neutrophils is converted to LTB_4 by neutrophil 5'-lipoxygenase, whereas the arachidonate in the exudate likewise becomes a substrate for the 15'-lipoxygenase expressed by tissue macrophages recruited to the site. The latter reaction generates lipoxin A_4, which inhibits neutrophil activation in a receptor-dependent fashion and blocks inflammation. In addition, lipid-derived mediators known as resolvins and protectins, which are produced from eicosapentaenoic acid and docosahexaenoic acid, have strong antiinflammatory properties and participate in the resolution of acute inflammation (see later).[70] Taken together, the shift from the production of proinflammatory leukotrienes and prostaglandins early to antiinflammatory lipoxins, resolvins, and protectins late provides a mechanism for the sequential promotion of exudate formation, followed by resolution mediated by transcellular metabolism of lipid mediators generated in situ.[70]

Role of Chemokines in Neutrophil Recruitment

Among the soluble mediators that can recruit leukocytes, the chemokines represent a diverse and biologically important class of proteins. Chemokines are a family of structurally related, pluripotent proteins that trigger leukocyte activation, including adherence, chemotaxis, degranulation, and priming of the neutrophil oxidase; participate in angiogenesis; and figure prominently in the host response to infection.[3,48] Chemokines are classified into two major families, CXC and CC, distinguished by the presence or absence of an amino acid between the first two cysteines in the protein. Chemokines interact with specific receptors on target cells, although there is significant promiscuity and redundancy in the chemokine system, with some members exhibiting very limited ligand-receptor interaction and others binding to more than one receptor. All chemokines targeted for neutrophils are in the CXC family, including IL-8, which binds to the G protein–coupled receptors CXCR1 and CXCR2. Secreted by leukocytes, platelets, fibroblasts, epithelial cells, and activated endothelium, IL-8 triggers the full range of cellular responses in neutrophils, promoting cell migration, degranulation, priming of NADPH oxidase activity, and cell survival in tissue.

Chemotactic stimuli bind to high-affinity receptors on the leukocyte surface. Receptors for IL-8, fMLF, and C5a are members of a large family of proteins characterized by an external ligand-binding domain, seven membrane-spanning segments, and cytoplasmic regions that couple to G proteins.[125] In the presence of chemoattractant gradients across the cell as small as 0.1% to 1.0% (e.g., as the attractant diffuses from a focus of infection), ligand-linked receptors distribute asymmetrically and trigger the directed movement (chemotaxis) and net accumulation of neutrophils at sites of increasing concentrations of attractant. Chemoattractant signals effect cell movement by promoting changes in intracellular calcium, the polymerization state of actin, and a number of actin-binding and regulatory proteins of the cytoskeleton[126] as well as receptor-dependent interactions between leukocyte adherence molecules and the extracellular matrix.[48,92] Just as mystifying as the myriad signal transduction pathways that promote neutrophil movement is the complexity of how the migrating neutrophil deciphers and prioritizes competing signals that drive chemotaxis. For example, within the inflammatory site, the advancing neutrophil encounters host chemoattractants, such as IL-8 and LTB_4; bacterially derived factors, such as formylated peptides; and C5a, generated by the microbial activation of the complement cascade. An intracellular signaling hierarchy exists that favors neutrophil responses to targets derived from bacteria in preference to host chemokines.[127] The binding of chemoattractants to their receptors also primes neutrophils for the microbicidal response, which includes degranulation and the respiratory burst, although these responses generally require higher concentrations of the stimulus than chemotaxis does. For this reason, activation of these distal events is likely delayed until the cell reaches the infected tissue site.

Step 2: Phagocytosis

Phagocytosis is the intracellular uptake of particles greater than 0.5 μm by a mechanism independent of clathrin but dependent on the polymerization of actin.[128,129] After attachment to the cell surface (Fig. 8.5), the phagocytic particle is internalized, with subsequent phagosome maturation (described later) and eventual fusion with the intracellular granules to form a mature phagosome (analogous to a phagolysosome in macrophages) (Fig. 8.6; see Table 8.1).[130,131] Neutrophils may ingest some microorganisms in the absence of opsonins, as occurs with recognition of β-glucan on fungi by dectin-1 on the neutrophil surface.[132] However, most microorganisms must be opsonized for binding and ingestion by neutrophils to occur efficiently.

Opsonins

Specific IgG, complement, and mannose-binding lectin are the major opsonic factors promoting recognition and ingestion of most microorganisms by neutrophils, although mannose-binding lectin figures

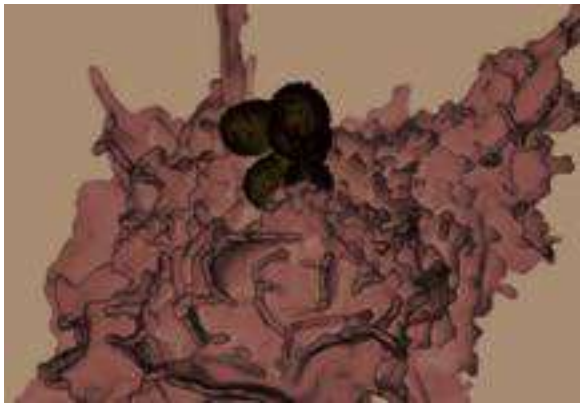

FIG. 8.5 Scanning electron micrograph of *Staphylococcus aureus* bound to the surface of a human neutrophil.



FIG. 8.6 Neutrophil phagocytosis and activation of microbicidal processes. See text for details. *CR,* Complement receptor; *FcR,* Fc receptor; *H_2O_2,* hydrogen peroxide; *HOCl,* hypochlorous acid; *MPO,* myeloperoxidase; *NADPH,* reduced nicotinamide adenine dinucleotide phosphate; *ROS,* reactive oxygen species.

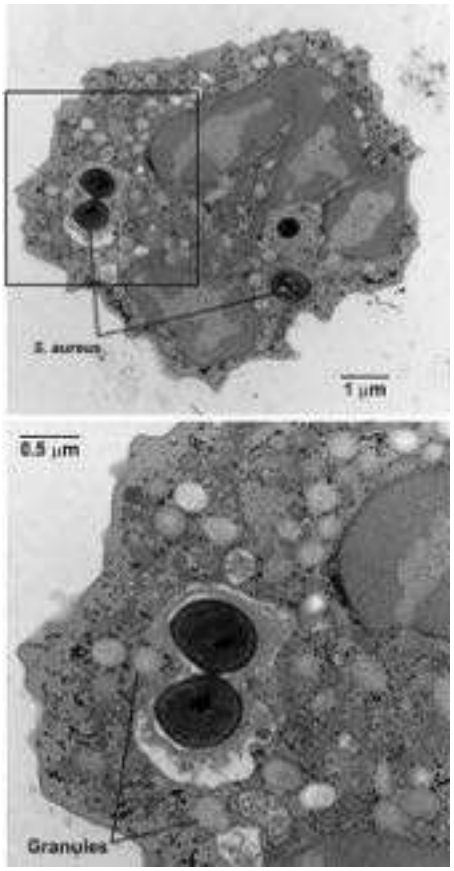

FIG. 8.7 Transmission electron micrograph of a neutrophil that has ingested *Staphylococcus aureus*. Bacteria are in phagocytic vacuoles formed by invagination of the external cell membrane. Degranulation into a phagocytic vacuole can be seen in the lower panel, which is a higher magnification image of the same neutrophil *(boxed area in top panel).*

predominantly in recognition of fungi rather than bacteria.[133] Antibody promotes phagocytic uptake by neutralizing antiphagocytic molecules on the bacterial surface, such as capsular polysaccharide; efficiently activating the classical pathway of complement, thereby promoting deposition of opsonic fragments of C3 on the bacterial surface; and mediating the interactions of organisms with the Fc receptor on the neutrophil membrane.[129] Activation of complement via either the classical or the alternative pathway leads to C3b and iC3b deposition on the microbial surface (see Chapter 9), and C1q deposition enhances Fc receptor–dependent ingestion.

Phagocytic Receptors

Distinct receptors for IgG (FcγRI through FcγRIII), but not other immunoglobulins, and for C3b (CR1) and iC3b (CR3) are present in the neutrophil membrane.[134,135] In addition to its iC3b-binding site, CR3 also bears a carbohydrate recognition domain that may bind to glycoproteins on the microbial surface. Data suggest that Fcγ receptors mediate phagocytosis via calcium-dependent pathways, whereas CR1 and CR3 use calcium-independent pathways.[136] FcγRII and FcγRIII are low-to-moderate affinity receptors that are constitutively expressed, whereas the high-affinity FcγRI is present only after cell stimulation (e.g., by IFN-γ).[137] Intracellular pools of receptors exist in the membranes of specific granules and secretory vesicles[14,130] and thereby provide a reservoir of functionally important membrane proteins that can be mobilized to the surface after exposure of the cell to a variety of inflammatory mediators. Such a structural organization affords neutrophils a mechanism to rapidly increase their capacity to recognize and respond to targets. In general, phagocytosis is most efficient when organisms are opsonized with both IgG and C3, thereby allowing cooperative interaction of the two types of receptors.

Ingestion

The sequential interaction between opsonic ligands distributed over the particle surface and their receptors on the phagocyte membrane culminates in ingestion of a surface-bound target (Fig. 8.7; see Fig. 8.6).[129,130] The sequential interaction of these opsonic ligands with their receptors in the phagocytic membrane initiates electrophysiologic changes in transmembrane potential, polymerization of actin microfilaments in the cytoplasm underlying the site of particle attachment, lipid signaling, and membrane remodeling, all of which result in circumferential flow of the cell membrane about the opsonized particle to create a phagosome (see Figs. 8.6 and 8.7).[129,138–140,141] The phagocytic capacity of neutrophils is remarkable: they can ingest more than nine yeast particles, each measuring 2 µm × 3 µm, despite having a diameter of only about 10 µm. They accomplish this impressive feat without generating new membrane or redistributing membrane from granules or endoplasmic reticulum, presumably by unfolding the membrane wrinkles that dominate the neutrophil surface (see Fig. 8.5).[142] The recruitment of membrane from the endoplasmic reticulum into nascent phagosomes, a potential mechanism in macrophages that has elicited significant controversy,[143–148] likely does not apply to neutrophils because of their relative paucity of endoplasmic reticulum.

Step 3: Fate of the Ingested Microbe

In addition to restricting access of ingested microbes to nutrient sources, internalization of organisms within phagosomes provides the host with an isolated compartment that can be made toxic both by the delivery of preformed cytotoxic agents and by the generation of reactive species de novo in the phagosome. Because of the small size of this specialized compartment (≈1.2 fL), extremely high concentrations of neutrophil-generated toxins are achieved, often in the micromolar range. Phagosome maturation, the development of phagosomes replete with a complete array of microbicidal agents, is a stepwise process whereby its contents and membrane composition are modified by sequential association with endosomal components.[37] Once internalization has begun, actin polymerization in the cytoplasm immediately adjacent to the nascent phagosome, actin-binding proteins are recruited to the periphagosomal space, and the phospholipid composition of the local membrane undergoes remodeling.[140,143,149–151] In an orderly fashion, cytosolic proteins associate with and disassociate from the phagosome sequentially, with eventual fusion of the phagosome with the neutrophil granules and generation of a mature phagosome. In

the mature phagosome, optimal microbicidal activity represents the coordinated generation of oxygen-derived species by activation of the NADPH-dependent oxidase and release of granule components.[152] Despite their shared lineage and overlapping functions, neutrophils and macrophages differ in many important ways, and the change in intraphagosomal pH that accompanies phagocytosis is a notable example. The intraphagosomal pH must be modified for optimal activation of some of the granule contents. To that end, activation recruits to the phagosomal membrane Na^+/H^+ exchangers, Na^+K^+-adenosine triphosphate (ATP) ases, and vacuolar-type proton ATPases from secretory vesicles and from primary and tertiary granules.[153,154] In contrast to the profound drop in phagosomal pH to 5.0 or less seen in macrophages, the phagosome in neutrophils is transiently alkaline (pH ≈ 7.8) and then drops to just below neutrality.[37,155–157,158,159] The consumption of protons by dismutation of superoxide anion ($O_2^{\bullet}$) to hydrogen peroxide (H_2O_2), the slowing of granule fusion, concurrent recruitment of vacuolar-type proton ATPases as degranulation proceeds, and altered phagosomal permeability to protons as reactive oxygen species are generated[157] all contribute to the blunted acidification of the neutrophil phagosome.[37]

Respiratory Burst

The oxidative or respiratory burst is mediated by a multicomponent enzyme complex in the plasma membrane and phagosomal membrane of stimulated neutrophils; oxidase activity is not present in resting, nonstimulated neutrophils.[160,161] An identical NADPH oxidase system exists in eosinophils, monocytes, and macrophages, with a hierarchy of relative activity of eosinophils > neutrophils > monocytes > macrophages. The phagocyte NADPH oxidase is a flavin adenine dinucleotide (FAD)–dependent electron transferase that shuttles electrons across the membrane, from cytosolic NADPH to molecular oxygen, to generate $O_2^{\bullet}$, the one-electron reduction product of oxygen, as the immediate product:

$$2O_2 + NADPH \rightarrow 2O_2^{\bullet} + H^+ + NADP^+ \qquad \text{(equation 1)}$$

The K_m for oxygen is low, ≈10 μM, thus supporting phagocyte oxidase activity at low oxygen tensions present in infected or damaged tissues.[162] The speed and capacity of electron transfer by the NADPH oxidase are remarkable; for example, more than 10^{10} electrons translocate within 5 minutes in response to fMLF.[163] Uncompensated, this magnitude of electron redistribution would depolarize the plasma or phagosomal membrane at greater than 200 mV within milliseconds and thereby terminate oxidase activity. However, the action of a voltage-gated proton channel compensates for more than 95% of the negative charge created by the translocation of electrons. Encoded by the gene *Hvn1,* the highly selective proton channel promotes the electrogenic activity of the phagocyte NADPH oxidase as well as calcium influx into and acid extrusion from cytoplasm, thereby protecting vulnerable systems from a low pH. Phagocytes from mice in which *Hvn1* is deleted exhibit reduced NADPH oxidase activity, defective antimicrobial action, and excess cytoplasmic acidification.[158]

Reactive Oxygen Species

Most of the superoxide formed readily undergoes dismutation to H_2O_2 and oxygen:

$$2O_2^{\bullet} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad \text{(equation 2)}$$

In the neutrophil phagosome, superoxide is converted quantitatively to H_2O_2.[164] This reaction occurs spontaneously with rapid kinetics at acidic pH, where a significant portion of the superoxide exists in its protonated form, the perhydroxy radical ($HO_2^{\bullet}$), because of the high rate constant for the reaction between $O_2^{\bullet}$ and the perhydroxy radical. MPO can act as a superoxide dismutase (SOD) by reacting with $O_2^{\bullet}$ to form compound III as an intermediate.[165] Although dismutation can be catalyzed by SOD, SOD is a cytoplasmic enzyme and absent from phagosomes. Because 1 mole of oxygen is regenerated for each mole of H_2O_2 formed, there is a net 1:1 stoichiometry between oxygen consumption and H_2O_2 formation, but a 2:1 relationship between $O_2^{\bullet}$ and H_2O_2 (Eqs. 1 and 2). Although the final product of the NADPH oxidase is H_2O_2, up to 72% of oxygen consumed by stimulated normal neutrophils can be recovered as hypochlorous acid (HOCl, or bleach), a consequence of the reactions of H_2O_2, MPO, and chloride (see later).[166] Although murine phagocytes generate nitric oxide (NO) that contributes to killing of microbes and tumors,[167] human neutrophils appear much less capable of generating reactive nitrogen species.[168,169] There is no nitration of fluorescein, a susceptible target, nor evidence for interactions between NO and $O_2^{\bullet}$ in phagosomes of human neutrophils.[170] Although it is possible that investigators have not applied the optimal in vitro conditions to support NO production by human neutrophils, the absence of agonist-dependent NO production may be another of the many differences between human and murine phagocytes.[171–173] To protect cytoplasmic contents from oxidative damage mediated by the reactive products of the NADPH oxidase that inadvertently leak from phagosomes, cytoplasmic SOD consumes $O_2^{\bullet}$, whereas both catalase and glutathione peroxidase catabolize H_2O_2, the latter in a fashion dependent on reduced glutathione. Taurine, present at a very high concentration (19 mM)[174] in neutrophils, may provide a sink for escaped HOCl, although it is also plausible that the product, taurine monochloramine, may modify susceptible targets in the cytoplasm. NADPH provides the reducing equivalents for the glutathione reductase–catalyzed regeneration of glutathione from glutathione disulfide. Consequently, NADPH levels must be maintained to support both the superoxide-forming oxidase and the glutathione cycle. The reduced pyridine nucleotide is regenerated by the activity of the hexose monophosphate shunt, which is enhanced 15-fold to 30-fold during phagocytosis.

NADPH Oxidase (Respiratory Burst Oxidase)

Dormant in resting neutrophils, the respiratory burst oxidase assembles and becomes active concomitant with cell stimulation, as during phagocytosis. The lag period between stimulus exposure and expression of NADPH oxidase activity varies from several seconds to a few minutes, depending on the agonist, and reflects the time required for assembly of the multiple components of the oxidase at the cytoplasmic face of the plasma membrane or phagosomal membrane. On the basis of studies both in intact neutrophils and from in vitro cell-free systems, the components of the NADPH oxidase include integral membrane proteins as well as soluble cytosolic proteins (Fig. 8.8).

Four proteins have been demonstrated to be essential in all settings for a functional phagocyte NADPH oxidase, with a fifth required for stable assembly on phagosomes.[160,161,175] Within the plasma membrane and membranes of specific granules and secretory vesicles is flavocytochrome b_{558}, so designated because of a characteristic 558-nm peak in its redox difference spectrum.[176–178] It is a heterodimer composed of large and small subunits, gp91*phox* and p22*phox* (where *phox* stands for *ph*agocyte *ox*idase), respectively, which are firmly but noncovalently associated with each other. In addition, the low-molecular-weight protein Rap1A frequently copurifies with flavocytochrome b_{558}, although the functional significance is unknown.[179] Flavocytochrome b_{558} contains two different types of redox centers, a FAD-binding domain and two inequivalent heme prosthetic groups,[180–182] and is the catalytic subunit of the oxidase, operating as an electron transferase. NADPH, the source of electrons driving the system, binds to a cytoplasmic domain in gp91*phox* and is oxidized by the transfer of two electrons to FAD, followed by two single-electron reductions of the heme groups ($Fe^{3+} \rightarrow Fe^{2+}$). The very low midpoint potential of the reduced hemes allows them to react directly with molecular oxygen, reoxidizing the iron moieties and forming two molecules of superoxide, $O_2^{\bullet}$. Because flavocytochrome b_{558} spans the membrane, electrons from the oxidation of cytoplasmic NADPH are shuttled to molecular oxygen outside the cell or inside the phagosome (in both cases, sites separated from cytoplasm by a membrane). Although flavocytochrome b_{558} operates as an electron transferase and mediates the catalytic functions of the respiratory burst oxidase, elements in two protein complexes that reside independently in the cytoplasm of resting neutrophils are required for enzyme activation and activity in vivo. One complex includes p47*phox,* p67*phox,* and p40*phox.*[183–192] p47*phox* possesses a very cationic carboxyl-terminal domain containing several serine residues that serve as substrates for agonist-dependent phosphorylation by various kinases, including protein kinase C, p21-activated kinase, and mitogen-activated protein kinases—extracellular signal-regulated kinase 1/2 and AKT, the individual kinases involved dictated by the specific agonist.[193] In the stimulated neutrophil,

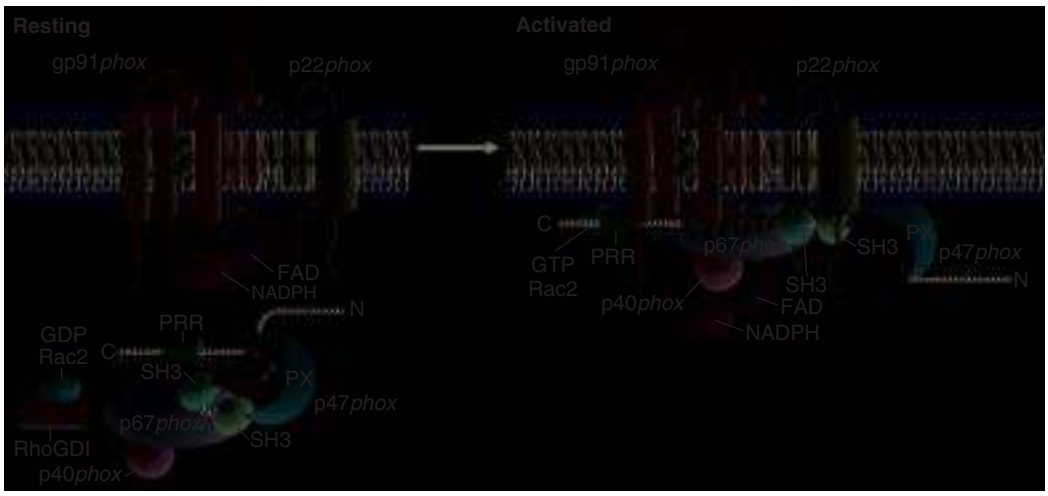

FIG. 8.8 Model of the agonist-dependent assembly of the neutrophil respiratory burst oxidase. Control of the activity of the reduced nicotinamide adenine dinucleotide phosphate *(NADPH)* oxidase reflects the spatial segregation of essential elements into two compartments of the cell. In resting neutrophils, flavocytochrome b_{558}, composed of gp91*phox* and p22*phox,* rests in the plasma membrane and membranes of secretory vesicles and specific granules. In contrast, inactive Rac2 bound to (Rho) guanosine diphosphate dissociation inhibitor *(RhoGDI)* as well as a complex of p47*phox,* p67*phox,* and p40*phox* resides in the cytoplasm. The latter complex depends on intermolecular interactions mediated by Src homology 3 domains *(SH3)* and proline-rich regions *(PRR)*. When the neutrophil is stimulated, several signal transduction events occur, including the phosphorylation of RhoGDI, which releases Rac2, with subsequent conversion from the guanosine diphosphate *(GDP)*–bound to the guanosine triphosphate *(GTP)*–bound state and association with the membrane. Concurrently, there is phosphorylation of multiple sites in the autoinhibitory region of p47*phox* (chain of small, light blue boxes). The latter event triggers a conformational change in p47*phox* that exposes otherwise cryptic sites on p47*phox* and p67*phox,* which subsequently support assembly of the oxidase at the membrane. *FAD,* Flavin adenine dinucleotide.

the partially phosphorylated protein translocates to the plasma membrane, where additional sites are phosphorylated.[194–198] As a consequence of conformational changes triggered by its phosphorylation, domains of p47*phox* that mediate interactions with specific phospholipids or cognate protein domains are exposed, thereby enabling association with flavocytochrome b_{558} at the plasma or phagosomal membrane to form the functional oxidase.[193,199–206]

Translocation of Cytosolic Components

Docking of cytoplasmic oxidase components at the plasma or phagosomal membrane depends on both protein-protein and protein-lipid interactions,[207] and phospholipid remodeling of neutrophil plasma membrane during phagocytosis serves as a critical step in oxidase organization.[208,209] Specific sites of gp91*phox* and p22*phox* have been implicated as mediating interactions with the cytosolic components.[175,210–217] p47*phox,* which lacks inherent enzymatic activity, serves as a docking platform or adaptor protein that organizes the functional oxidase at target membranes. Neutrophil stimulation also results in membrane translocation of p67*phox,* probably as a result of its association with p47*phox*; p67*phox* fails to translocate in the absence of p47*phox,* although p47*phox* is competent for translocation by itself.[197] p67*phox* possesses a domain that regulates NADPH reduction of FAD[218] and thus serves as an essential cofactor for the phagocyte oxidase. Enzymatic activity of p67*phox* requires its binding of Rac, a GTPase of the Rho protein family, to a domain in its N-terminus.[219] Both p47*phox* and p67*phox* contain two copies of a 50–amino acid domain related to a region of the Src oncoprotein, Src homology region 3 (SH3).[185,187] Proline-rich motifs similar to those that bind to SH3 domains in other proteins[220] are present in p47*phox,* p67*phox,* and p22*phox*. In general, SH3 domains are thought to mediate binding of proteins, expressing them to cytoskeletal and membrane elements. Both SH3 and proline-rich segments of the oxidase proteins are involved in the protein-protein interactions required for oxidase activation and function.[221,222] The net effect of phosphorylation and translocation of cytosolic proteins is the assembly on the membrane of the active oxidase complex, composed of the flavocytochrome b_{558} subunits, p47*phox,* p67*phox,* Rac2, p40*phox,* and perhaps other components.[179,223–228]

p40*phox*

p40*phox,* the third component of the complex with p47*phox* and p67*phox,* exhibits homology to p47*phox,*[190,229,230] including the presence of a PX or phox homology domain that associates with phosphoinositides in target membranes.[231–234,235,236] The PX domain of p40*phox* binds to phosphatidylinositol-3-phosphate (PI(3)P) that is generated on the cytoplasmic leaflet of phagosomal membranes by the action of class III PI(3)P kinases.[236–241] Defective oxidase activity in and oxidant-dependent killing by neutrophils from mice lacking p40*phox* suggested that p40*phox* played a specialized role in directing assembly of a functional oxidase.[233] However, the identification of a patient who lacks normal p40*phox* protein and whose neutrophils fail to generate oxidants in phagosomes or kill ingested *Staphylococcus aureus* normally demonstrated that class III PI(3)P kinase–mediated PI(3)P generation is essential for p40*phox* binding to phagosomes, a step critical to sustained oxidant generation and optimal microbicidal action in that compartment.[242] Disruption of the PI(3)P binding region by a single mutation (R105Q) in the PX domain of p40*phox* undermines stable oxidase assembly on phagosomes, although neutrophils have nearly normal oxidant production at the cell surface. Of note, the patient did not display signs and symptoms typical of chronic granulomatous disease (see Chapter 12) but rather presented with refractory inflammatory bowel disease. Genome-wide association studies have linked Crohn disease with *NCF4,* the gene encoding p40*phox,*[243,244] so elucidation of this association promises to provide new insights into links between innate immunity and inflammatory bowel disease.

Rac2

In addition to the ternary complex of p47*phox*-p67*phox*-p40*phox,* the low-molecular-weight GTP-binding protein Rac2 exists in its guanosine diphosphate–bound state coupled with the guanosine diphosphate dissociation inhibitor RhoGDI in the cytoplasm. Activation-dependent phosphorylation of RhoGDI results in a change in its conformation and the release of Rac2 from the complex, permitting Rac2GTP to bind to the target membrane and interact there with the flavocytochrome b_{558} and p67*phox*.[245–251] During neutrophil stimulation, Rac translocates to the plasma membrane, independent of the redistribution of p47*phox* and p67*phox*.[245–247]

In contrast to the major advances made in elucidating the biochemistry and cell biology of oxidase activation, relatively little is known about how activity of the NADPH oxidase is terminated. The cytoplasmic NADPH concentration is one limiting factor in the activity of the phagocyte oxidase,[252] and indirect evidence suggests that reactive products

of the oxidase, particularly those formed via the catalytic action of MPO, inactivate the enzyme,[253] but the precise mechanisms by which oxidase activity is regulated and terminated are not known.

Nonphagocyte Oxidases: The NOX Protein Family

The NADPH oxidase was thought to be an oxidant-generating system uniquely expressed by phagocytes and dedicated exclusively to antimicrobial action until the Lambeth group[254] cloned a homologue of gp91*phox* from a library of human colon complementary DNA; with that discovery, the NADPH oxidase (NOX) protein family was born.[161,238–251,252,253,254,255,256,257,258] The NOX family includes NOX1 through NOX2 (gp91*phox* is also known as NOX2) and two dual oxidases (DUOX1 and DUOX2), which are widely expressed throughout the plant and animal kingdoms.[256,259] Not only do most cells express a NOX protein, but many also possess multiple isoforms, each in a different subcellular compartment. Functions served by NOX proteins are as diverse as their tissue distribution is wide. In the context of host defense against infection, epithelial cells in the respiratory and gastrointestinal tracts express DUOX proteins, whose oxidant production mediates antimicrobial action, both directly against microbes and indirectly by disrupting microbe-driven signaling.[260–262] Furthermore, NOX1-dependent oxidants have been linked to epithelial cell repair of mucosal injury that accompanies inflammation.[263] The extent to which NOX-generated oxidant systems in epithelial cells contributes to mucosal immunity in humans awaits further delineation.

Degranulation

In parallel with activation of the respiratory burst oxidase, stimulated neutrophils release their granule contents either to the extracellular space or into the nascent phagosome (see Figs. 8.6 and 8.7).[264,265] Stimulated exocytosis, or degranulation, by neutrophils is a remarkable process, given that there are at least four distinct classes of membrane-bound vesicles (i.e., azurophilic granules, specific granules, gelatinase granules, and secretory vesicles) that are released and two potential target destinations for fusion events (i.e., plasma and phagosomal membrane). There is a hierarchy among these compartments with respect to the order in which each releases its contents after exposure to an agonist (i.e., secretory vesicles, tertiary granules, secondary granules, primary granules) and to the calcium requirements for exocytosis.[266,267]

Rearrangement of the actin-based cytoskeleton is a prerequisite for the release of all granule subtypes, with different processes occurring in specific sites in the cell for each granule type.[268] In the case of primary granules, there is a decrease in actin polymerization at the membrane, likely reflecting reorganization of the subplasmalemmal membrane cytoskeleton[38,269] and access to target membrane for granule membrane fusion.[270] Concurrently, actin polymerization occurs in the cytoplasm and promotes primary granule release.[270] The actin reorganization that accompanies primary granule release is regulated by Rac2, the same GTPase that is essential for activity of the NADPH oxidase (see earlier discussion).[270,271,272,273] Human neutrophils possess other low-molecular-weight GTPases, including members of the Rab family,[274] and Rab27a has been implicated in contributing to degranulation.[275,276] There is evidence that the Rab effector protein JFC/Slp1 and Munc 13-4 participate in regulation of exocytosis.[276,277]

As part of the cytoskeletal rearrangement that accompanies degranulation,[278,279] several actin-binding proteins are redistributed, including myristoylated alanine-rich C-kinase substrates (MARCKS).[280] A MARCKS-related peptide inhibits MPO release from stimulated neutrophils, suggesting that the N-terminal region of MARCKS contributes to a critical step in granule release.[281] Fusogenic proteins on the granules, including annexins,[282] synaptosomal-associated protein (SNAP)-23, syntaxin-4 and syntaxin-6, vesicle-associated membrane protein (VAMP)-1, VAMP -2, and VAMP-7, and likely other molecules, mediate association at the target membranes with specific receptors, including various target SNAP receptors (t-SNAREs).[283] Lipid modification probably contributes critically to fusion of the granule membrane with the plasma or phagosomal membrane, and such remodeling depends on the redistribution and activation of phospholipase D.[284,285] In addition, the activity of signaling proteins such as MEG2 requires specific membrane phosphoinositides generated locally.[286]

As discussed previously, the granules of neutrophils differ not only in the luminal contents but also in the proteins inserted in their membranes. Surface expressions of CD63, CD66, and CD35 are monitored experimentally as specific markers of plasma membrane fusion with primary granules, secondary granules, and secretory vesicles.[14] Primary, or azurophilic, granules fuse predominantly with the phagosome, thereby delivering microbicidal and hydrolytic proteins at high concentrations close to the ingested organisms. Specific granules and secretory vesicles fuse preferentially with the plasma membrane, releasing their contents extracellularly and bringing to the cell surface a variety of functionally important membrane proteins, including integrins, flavocytochrome b_{558}, and receptors for chemotactic agents and opsonins (see Table 8.1). Taken together, the process of degranulation affords concurrent recruitment of critical membrane proteins to the cell surface or phagosome and discharge of proteins that directly or indirectly contribute to the death and destruction of ingested microbes.

Step 4: Resolution of the Inflammatory Response

Briefly, resolution of inflammation occurs in a remarkably complicated yet orderly manner: locally produced CXC chemokines that elicit neutrophil migration switch to CC chemokines that enlist nonneutrophil leukocytes to migrate[287–289]; recruited neutrophils spontaneously, or in response to agonists (e.g., late after phagocytosis), undergo cell death; and macrophages and dendritic cells, both resident and recruited, ingest the apoptotic neutrophils in a nonphlogistic (noninflammatory) fashion and contribute to wound healing and reestablishment of the normal state. Ingestion of apoptotic neutrophils by macrophages or dendritic cells or both, known as efferocytosis, and subsequent removal from the inflammatory site are important for resolution of the acute inflammatory response.[53]

Apoptosis

Neutrophils have a relatively short half-life, approximately 6.5 hours, in the circulation,[290,291] and they live an additional 1 to 2 days after migrating into tissue, with senescent cells undergoing spontaneous apoptosis before being cleared by tissue macrophages.[53,292–295] Apoptotic neutrophils have markedly depressed functional capacity,[296–299] thereby preparing them for removal by tissue macrophages.[294] Collectively, the loss of proinflammatory potential in apoptotic neutrophils, their physical removal by macrophages, and the noninflammatory phenotype of macrophages that ingest apoptotic neutrophils[300] result in cell turnover in the inflammatory site that provokes little tissue damage. Nonsenescent neutrophils can become apoptotic in response to a variety of soluble agents[90,297,301–303] and after phagocytosis of bacteria.[304,305] The mechanisms underlying apoptosis in human neutrophils are complex; differ as a function of the agonist and cytokine context; and are, in some cases, modulated by pathogens.[306,307]

Neutrophils express members of the Bcl-2 family of apoptotic proteins, notably the proapoptotic proteins Bax, Bid, Bak, and Bad, but not the antiapoptotic Bcl-2.[307–309] Neutrophil apoptosis is caspase mediated, reflecting the involvement of mitochondria and complex crosstalk among several signaling pathways, including the caspases, reactive oxygen species, and mitogen-activated protein kinases.[307,310–312] Several molecules on the neutrophil surface have been implicated as participants in receptor-mediated uptake of apoptotic neutrophils by tissue macrophages, including phosphatidylserine exposed from the inner leaflet of the plasma membrane, CD47, CD31, calreticulin, proteinase 3, altered membrane carbohydrates, and oxidized membrane phospholipids.[53] Likewise, a variety of macrophage receptors have been proposed to mediate uptake, including a phosphatidylserine receptor, scavenger receptors, CD14, CD44, and the coordinated activity of CD36 and the integrin $\alpha_v\beta_3$.[53]

Proinflammatory Molecules Alter Apoptosis

Within the context of the acute inflammatory response, cytokines released at the site delay apoptosis, thereby extending the life span of neutrophils and permitting their participation in host defense.[90] However, the regulation of neutrophil apoptosis by cytokines such as TNF-α is complex; for instance, low concentrations of TNF-α delay neutrophil apoptosis, whereas high concentrations promote it.[303,313] Cytokines modulate proapoptotic and antiapoptotic pathways in a variety of ways, and

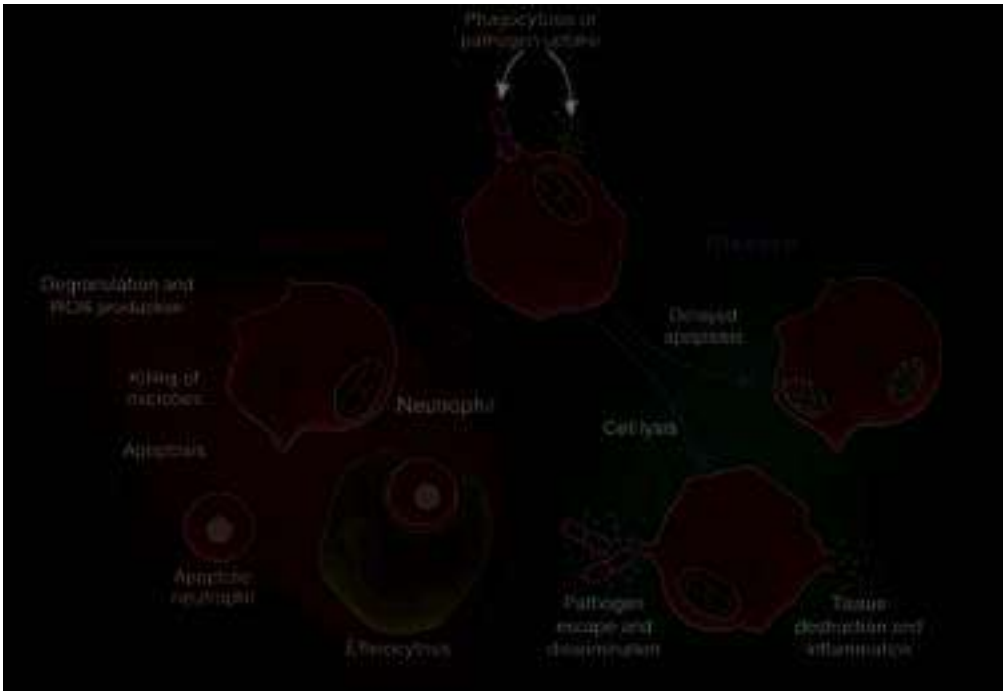

FIG. 8.9 Potential outcomes of the interaction of microbes with neutrophils. See text for details. *ROS,* Reactive oxygen species.

phagocytosis by neutrophils typically accelerates apoptosis—a phenomenon also known as phagocytosis-induced cell death, with an associated downregulation of their proinflammatory capacity (Fig. 8.9).[90,298,306,314,315] More recent studies also provide strong evidence that levels of neutrophils in circulation follow circadian cycles, and neutrophil clearance generates signals that modulate hematopoiesis.

Thus, the stimulated neutrophil at the peak of its response to an invading microbe initiates pathways leading to decreased proinflammatory activity and to apoptosis, en route to resolution of the acute phase of the inflammatory response.[299,306,316–322] Evidence supporting this interpretation comes from a consideration of the local consequences when accelerated apoptosis is not triggered by neutrophil stimulation. For example, neutrophils from individuals with chronic granulomatous disease (see "Neutrophils and Microbial Evasion of Host Defense")[316,323] exhibit delayed phagocytosis-induced apoptosis and produce less prostaglandin D_2, an antiinflammatory mediator, two deficiencies that may contribute to the chronic inflammation and granuloma formation that are hallmarks of this disease.

Microbial Pathogens Influence Neutrophil Fate

As a mode of cell death, apoptosis of neutrophils spares the host local tissue damage that might arise from necrosis and the attendant release of cytotoxic cellular contents. This aspect of neutrophil biology has been the focus of intense research over the past 15 years, and understanding of the process has progressed rapidly. Indeed, more recent studies on the cell biology of death and its evolving definitions based on morphologic, enzymatic, functional, and immunologic criteria[324] promise to provide important new insights into the overall economy of neutrophil homeostasis in the absence and presence of infection and other proinflammatory stimuli.

Inasmuch as neutrophil apoptosis is critical for the resolution of the inflammatory response, it is perhaps not surprising that some pathogens can alter this process to survive and thereby cause disease (see Fig. 8.9).[304,305] For example, *Anaplasma phagocytophilum,* the causative agent of human granulocytic anaplasmosis,[325] is internalized by receptor-mediated endocytosis rather than phagocytosis[326,327] and inhibits neutrophil apoptosis.[328] *A. phagocytophilum* is one of the few microbes that can replicate within neutrophils, and the ability of this pathogen to delay neutrophil apoptosis is important for its intracellular replication and ultimately for the organism to cause human infection.

Neutrophils as "Trojan Horses"

Apoptotic neutrophils may also serve as "Trojan horses," providing a vehicle for viable parasites, such as *Leishmania major,* to gain entry into recruited macrophages.[329] How widespread this phenomenon is and the extent to which it promotes infections are not known. *S. aureus* may use a variation of the Trojan horse phenomenon, in which neutrophils themselves serve as a vehicle to disseminate the pathogen.[330–332] Although *S. aureus* is ingested readily by neutrophils, some strains have significant capacity to survive after phagocytosis and eventually cause neutrophil lysis (see Fig. 8.9).[333–336] Lysis of *S. aureus*–containing neutrophils liberates previously ingested bacteria (which may then disseminate) and releases cytotoxic contents that can potentially cause undesired host tissue damage. The mechanism for neutrophil lysis after phagocytosis of *S. aureus* remains incompletely characterized, but neutrophils have some morphologic characteristics of apoptosis before lysis.[337]

Bioactive Lipid Mediators

Studies within the past 10 years have identified another family of biologic agents that contribute to the active resolution of the inflammatory response. Bioactive lipids, such as resolvins, protectins, and maresins derived from leukocytes at an inflammatory site, dampen the inflammatory reaction and promote its resolution.[69,70,338] Seen collectively, endogenous factors derived from cells recruited to the site of inflammation orchestrate the orderly recruitment of different types of inflammatory cells and their subsequent clearance. Under optimal circumstances, these endogenously generated lipids direct a controlled response that eliminates the infectious threat with minimal collateral injury and promptly restores the preinfectious healthy state.

Microbicidal Mechanisms

Intraphagosomal Killing of Microbes

Postphagocytic events deliver the products of degranulation and the respiratory burst to the phagocytic vacuole, a membrane-bound compartment in which an ingested microbe is exposed to high concentrations of toxic substances (see Fig. 8.6). Components of the antimicrobial response of neutrophils are often classified by their dependence, or lack thereof, on the products of the respiratory burst. Elegant studies defining the actions of these cytotoxic agents generally focus on their behavior in isolation or with only a subset of the phagosomal contents that would otherwise be present in vivo. However, intraphagosomal

events are much more dynamic and complex.[152,165,339,340] Reactive species present in the phagosome at any point in time act simultaneously on microbial targets and, in many cases, interact with each other as well. Oxidant-mediated modifications of surface structures on ingested bacteria may alter the susceptibility of bacterial targets to granule proteins, or granule protein interactions may change access or susceptibility of sites to oxidant attack. Individual granule proteins can synergize with each other or oxidants or both to exact damage not possible with any single agent. Some direct interactions among neutrophil products will benefit the host, as when the granule protein MPO catalyzes generation of HOCl by using NADPH oxidase–derived H_2O_2 as substrate.[166] Other interactions may be counterproductive, as when the MPO-generated HOCl oxidatively inactivates elastase[341] or cathepsin G.[342] Furthermore, antimicrobial collaborations in the phagosome are not limited to agents derived from neutrophils but include soluble factors internalized from the extracellular environs during phagocytosis. For example, group IIA phospholipase A_2, an acute-phase plasma protein with antimicrobial activity against gram-positive bacteria,[343–345] synergizes, at concentrations that alone are too low to be active, with products of the NADPH oxidase to exhibit potent antimicrobial and phospholipase activity within neutrophil phagosomes.[345] Adding to the complexity of intraphagosomal antimicrobial events is the inherent variability among ingested organisms. The microbial surface structure and composition vary among different organisms and within individual species differ with respect to growth phase. Consequently, surface components that are the immediate substrates for antimicrobial toxins will vary widely among and within species. Furthermore, phagocytosed microbes are not passive prey. The transcriptional responses of ingested organisms to the toxic phagosomal environment initiate microbial stress responses, structural modifications of the microbial surface, and release of secreted products, some of which serve as competing targets for host toxic agents. Overall, ingested organisms respond rapidly to intraphagosomal stress and thereby alter the array of potential targets for neutrophil attack. Given the combined number and complexity of host and microbial variables, it should not be surprising that no single neutrophil product, biochemical modification, or specific microbial target has been identified as being uniformly essential for the death and degradation of all types of organisms sequestered within the phagosome. The same collaborative and integrated approach that provides a flexible and generally effective host defense system also keeps understanding of the underlying mechanisms at bay. Nonetheless, with these challenges and limitations in mind, studies using a reductionist approach have provided substantial insights into the complexities of antimicrobial action within phagosomes.

Oxygen-Dependent Killing of Microbes

Optimal antimicrobial action against most microorganisms depends on the MPO-H_2O_2-chloride system.[166] First described by Klebanoff,[346,347] the system has three essential components uniquely present in neutrophils; in the presence of H_2O_2 (generated by the respiratory burst), MPO (released from primary granules) oxidizes chloride to generate the potent microbicide HOCl. MPO is unique among peroxidases in its capacity to oxidize chloride anion at physiologic pH (reviewed in Nauseef[348]). The resultant chemistry demands a steady flux of chloride ion into the phagosome. The relatively high chloride concentration in neutrophil cytoplasm provides the source for chloride, redistributed to phagosomes through membrane transporters, including cystic fibrosis transmembrane conductance regulator, to support HOCl generation and efficient antimicrobial action.[349–353] Although H_2O_2 itself has bactericidal properties, its microbicidal potency is enhanced approximately 50-fold in the presence of MPO. In the presence of suitable substrates, HOCl can generate chloramines, chlorine gas, chlorinated sterols, tyrosyl radicals, and nitric oxide–derived reactants that can extend the spectrum and duration of antimicrobial action.[166,339,354–356] However, reactivity of products of the MPO system lacks selectivity, and chlorinated bacterial and host proteins can be recovered from the phagosome of human neutrophils after microbial challenge.[357–359] However, the killing of ingested microbes correlates with the extent of chlorotyrosine generation on bacteria,[358] thereby linking the MPO-dependent modifications to antimicrobial action. The precise events responsible for the microbicidal activity of the MPO system are incompletely characterized[339]; candidate target sites in bacteria include components of the electron transport chain, iron-sulfur centers, penicillin-binding proteins, and sites in bacterial membranes that are necessary for initiation of chromosomal replication[360–363] as well as the generalized stress secondary to HOCl-mediated oxidative protein unfolding.[364] Oxidation of particular microbial targets may lead to the release of free iron, which can then participate in the formation of the highly reactive hydroxyl radical ($OH^{\bullet}$) and augmentation of the oxidant attack.[365] Studies have implicated the MPO-H_2O_2-halide system in tissue injury, in oxidation of lipids, and possibly in atherogenesis, extending the biologic importance of this system beyond its microbicidal activity.[366–369]

Metabolites of oxygen for which a role in neutrophil bactericidal activity has been suggested include H_2O_2, $O_2^{\bullet-}$, singlet oxygen, and hydroxyl radical. The fact that catalase, which degrades H_2O_2 into O_2 and H_2O, protects some bacteria from the bactericidal effects of neutrophils[370] supports a direct germicidal effect of H_2O_2. However, the permeability of the phagosomal membrane to H_2O_2 makes it unlikely that sufficient H_2O_2 accumulates within the lumen to support bacterial killing.[340] Superoxide by itself is thought to play little role in the killing of microorganisms but under appropriate conditions can react with other products of oxygen metabolism to generate hydroxyl radical and singlet oxygen. Evidence suggests that $O_2^{\bullet-}$ in the phagosome reacts directly with MPO in a catalytic cycle in which superoxide acts as a reductant and culminates in dismutation of superoxide to H_2O_2.[371] The bactericidal effect of these reactive oxidants may result from the initiation of a chain of oxidizing events in the bacterial cell wall.[339,372] Damage proceeds from outside to the inside of the target bacteria, with dysfunction or inactivation of critical functions localized to the bacterial inner membrane.[339] For example, the death of *Escherichia coli* parallels the oxidation of methionine residues in the inner membrane and cytoplasm but not in the outer membrane or periplasm of bacteria fed to human neutrophils.[373]

The hydroxyl radical is a potent bactericidal agent that can be formed by the direct reaction of superoxide with H_2O_2, a reaction that occurs slowly on its own but is catalyzed by the ferric ion.[374] Sensitive analytic systems indicate that activated neutrophils produce hydroxyl radicals by two different mechanisms, one dependent on the catalytic activity of MPO[375] and the other requiring transition metals in the Haber-Weiss reaction.[376] The relative contribution of each mechanism to the overall production of hydroxyl radicals in vivo depends on the availability of exogenous transition metals, usually iron. In the presence of supplemental iron, hydroxyl radical generation occurs through the Haber-Weiss reaction. However, lactoferrin and transferrin can interfere with this reaction by binding iron in a noncatalytic form. Therefore, under physiologic conditions, it appears that the small amounts of hydroxyl radical generated by stimulated neutrophils are derived from the MPO-dependent pathway.

Oxygen-Independent Killing of Microbes

Antimicrobial systems that operate in the absence of exogenous oxidants contribute greatly to overall innate immunity and neutrophil-dependent host defense,[377–379] as clearly demonstrated by the ability of neutrophils to kill certain organisms under anaerobic conditions, where the NADPH oxidase would not be functional. Agents contributing to oxygen-independent microbicidal activity include defensins, BPI, lactoferrin, lysozyme, peptidoglycan recognition proteins (PGRP), neutrophil gelatinase–associated lipocalin (NGAL), cathelicidins, and defensins. In many cases, these are highly charged cationic proteins that bind to negatively charged prokaryotic cell envelopes, thereby compromising the capacity of microbes to perform chemiosmotic work and maintain viability. BPI is a 59-kDa protein located in the primary granules of neutrophils. Its antimicrobial activity resides in a 25-kDa amino-terminal fragment.[380–384] In addition, BPI binds to lipopolysaccharide[385] and blocks the release of TNF-α elicited by bacteria.[382] Lactoferrin is an iron-binding protein found in secretions bathing mucosal membranes and in the specific granules of neutrophils.[386] Its bacteriostatic properties reflect an ability to deprive bacteria of the iron required for their growth, an effect eliminated by saturation of its iron-binding sites.[386] Lactoferrin plays a role in the alteration of the physicochemical properties of the neutrophil membrane that occurs during degranulation,[387] in the

modulation of hydroxyl radical production, in the regulation of granulopoiesis,[388] and in the modulation of complement function.[389]

Lysozyme, found mainly in the specific granules but also present in the primary granules, hydrolyzes the glycoside bond between *N*-acetylmuramic acid and *N*-acetylglucosamine, components of the peptidoglycan in bacterial cell walls. Although the bactericidal properties of lysozyme reflect this activity, peptide substitutions on the *N*-acetylmuramic acid residue in most bacteria make this bond inaccessible to lysozyme, thereby limiting its bacteriolytic properties. However, in the context of the complex environment of an inflammatory reaction, a bacterial cell wall already damaged by complement or granule proteins may allow access of lysozyme to its site of action.

Human PGRP-S binds avidly to the many forms of peptidoglycan on the surface of bacteria as dissimilar as *S. aureus* and *E. coli*, where it damages microbes by interfering with peptidoglycan biosynthesis and inducing conformational changes that prevent cross-linking of cell wall peptides.[390] Furthermore, PGRP-S acts synergistically with lysozyme to promote the lysis of *E. coli*.[391]

During investigation of gelatinase in specific granules, Kjeldsen and coworkers[392] discovered an associated protein (NGAL) that indirectly contributes to antimicrobial action by binding to catecholate-based bacterial siderophores, thereby interfering with iron acquisition by microbes. The contribution of NGAL to overall murine host defense against infection is illustrated by the failure of NGAL knockout mice to survive intraperitoneal challenge with *E. coli* that produced enterochelin, a catecholate-based siderophore.[393]

Specific granules also house precursor forms of a family of antimicrobial peptides collectively termed *cathelicidins* because of their sequence homology at their N-terminus with cathelin, an inhibitor of cathepsin.[394–396] The antimicrobial activity of cathelicidins resides in the cationic C-terminal portion of the protein, which is released from the holoprotein precursor during neutrophil degranulation. The spectrum of organisms susceptible to cathelicidins is broad, and the toxicity to target organisms is a consequence of disruption of the bacterial membrane. Among mammals there is significant variation in the number of cathelicidins expressed in individual species. Only one, human cathelicidin protein-18, which is processed to the antimicrobial peptide LL-37, has been identified in humans.[397] Of note, defensins, human cathelicidin protein-18, and NGAL are three antimicrobial proteins that are expressed in both keratinocytes and neutrophils, raising provocative questions about the synergistic interactions between recruited neutrophils and resident keratinocytes in contributing to tissue repair and wound healing at the site of cutaneous infection.[398,399]

Defensins are potent antimicrobial peptides in the primary granules of neutrophils,[377,400,401] along with prodefensins in specific granules,[402] and in epithelial cells of the gut and the genitourinary tract.[403,404] Defensins and closely related proteins are widely distributed in nature (e.g., the hemolymph of insects[405]) and probably represent an ancient approach to host defense. The precursor forms of the α-defensins present in azurophilic granules undergo proteolytic processing by the action of elastase and proteinase 3, two proteins in the same granule compartment, to yield active antimicrobial peptides.[406] In general, defensins are small molecules (3 to 4 kDa) rich in arginine and containing a characteristic disulfide motif. Elegant studies have defined many of the physical properties of purified defensins[407–409] and provide insight into their mechanism of action, which involves insertion into microbial membranes, resulting in the formation of pores that allow efflux of cytoplasmic components. The spectrum of organisms against which defensins are active is extremely broad, including gram-positive and gram-negative bacteria, fungi, and enveloped viruses.

Additional cationic proteins isolated from neutrophil primary granules[379] demonstrate preferential activity against specific bacterial species. These proteins include p15s,[410] azurocidin,[411] and indolicidin.[412] Understanding of the principles of antimicrobial activity of these proteins is incomplete at this time, but their mechanisms of action include both enzymatic and nonenzymatic components.

Extracellular Microbicidal Activity

Intraphagosomal killing of microorganisms is the primary method used by neutrophils to eliminate invading bacteria and fungi. However, studies indicate that nuclear DNA released from a small subset of neutrophils forms weblike or netlike structures known as neutrophil extracellular traps (NETs) that ensnare bacteria and fungi.[92,413,414–421] NETs are composed of decondensed nuclear DNA, histones, and azurophilic granule proteins such as MPO and elastase.[418] Although early studies indicated NETs immobilize and kill bacteria, more recent work suggests that NETs lack significant microbicidal activity.[422,423] Rather, these later data indicate that NETs may trap microbes and thereby prevent dissemination. Although the biologic relevance of NETs is the subject of controversy, three separate mechanisms for the formation of NETs have been proposed.

NETs were first proposed to form by a novel cytolytic cell death process eventually named *NETosis*.[419] During NETosis, nuclear and granule membranes lose integrity, and DNA mixes with the cytoplasm and granule proteins just before cell lysis. Subsequently, neutrophil elastase degrades histones, and the cell ruptures and releases decondensed DNA to form a NET decorated with antimicrobial proteins.[414,419] This process depends on reactive oxygen species produced by the NADPH oxidase.[414,419] Whether this process is truly distinct from traditional necrosis or cytolysis remains a topic of debate. It is also important to note that formation of NETs has been linked to host tissue damage and associated with specific human diseases,[415,424–426] findings consistent with processes that occur after typical neutrophil necrosis or lysis.

As an alternative to NETosis, reports have shown that a subset of live neutrophils form NETs by ejecting the nucleus, and the anuclear neutrophils remain functional and viable.[416,427] NETs formed by this mechanism appear functionally similar to NETs formed by the cytolytic process; they capture bacteria and prevent their dissemination. These intriguing findings were demonstrated in mouse bacterial infection models using live imaging techniques.[415,416] A third mechanism for formation of extracellular traps involves rapid "catapult-like" release of mitochondrial DNA from neutrophils[417] or eosinophils.[428] This mechanism of extracellular trap formation does not involve death of the phagocyte nor does it alter the life span of these cells.[417]

Although each proposed mechanism is unique and there is controversy related to extracellular traps formed by cytolysis or living cells, a feature common to the three mechanisms is the capture of microbes, which in animal models prevents microbial dissemination.

Neutrophils and Microbial Evasion of Host Defense

Ongoing investigation in microbial pathogenesis reflects the scientific advances in molecular microbiology and eukaryotic cell biology. In many ways, results from studies that apply these newer analytic approaches challenge our conventional understanding of microbial pathogenesis and the biologic meaning of colonization, commensalism, infection, and disease.[429] The application of gene microarrays to the study of host-microbe interactions has revealed the remarkably dynamic and interactive nature of both the invading microorganism and the responsive host cell. This area of research is rapidly evolving and encompasses infections with a variety of microbial species. The topic is too extensive to cover here in any detail. Instead, we provide a few selected examples of bacterial evasion of neutrophil function and refer the reader to more comprehensive reviews on specific topics.

Microbial Responses to Neutrophils

Phagocytosis elicits transcription of an array of neutrophil genes[299,430–433] and bacterial genes, including genes representing complex transcriptional responses that allow the organism to evade the attack by neutrophil-generated cytotoxins.[317,334,433–439] Such analyses demonstrate that the ingested microbe responds to its immediate environment in the phagosome rapidly and specifically. For example, among the many genes expressed by *E. coli* 7 minutes after being ingested by normal neutrophils are those regulated by *OxyR*, an oxygen-sensing transcription factor.[437] However, the same strain of *E. coli* does not express *OxyR*-regulated genes when ingested by chronic granulomatous disease neutrophils, which lack the capacity to generate reactive oxygen species to create oxidant stress within the phagosome.

Within the complex context of the interactive cell biology between host and microbe, it is clear that pathogens have evolved molecular

strategies for neutralizing one or more of the discrete steps in normal host defense.[335,438,440–442] To that end, the invading microorganism may exploit specific aspects of normal mammalian cell biology, including adhesive properties, signal transduction pathways, cytoskeletal rearrangements, and vacuolar trafficking.[440] In some cases, these properties are manifest only when microbes are in the appropriate host, demonstrating the exquisitely precise manner in which the invading microorganism has adapted to the context of the mammalian host.[443,444] *S. aureus* is perhaps an ideal example organism in this regard because it produces molecules that have the potential to inhibit virtually each step of the neutrophil response to invading microbes, including recruitment, phagocytosis, and bactericidal activity.[335,445]

Microbial Targeting of Neutrophil Surface Molecules

In some cases, bacteria adhere to surface proteins on target phagocytes, as with certain *Neisseria* species and CD66 on the neutrophil surface.[446] In other situations, bacterial proteins secreted into target cells may modify the host cell response, as is the case with the Yop proteins of *Yersinia*.[438] Once secreted into the host cell, the various members of the Yop family of proteins impair phagocytosis, induce apoptosis, paralyze cellular actin, and, in the case of the tyrosine phosphatase YopH, block Fc receptor–mediated activation of the respiratory burst oxidase.[447,448] In other settings, multiple mammalian cell types are targeted. For example, a glycoprotein of Ebola virus in its secreted form engages the neutrophil Fc receptor and inhibits cell activation and in its transmembrane form interacts with endothelial cells. In this way, the virus simultaneously inhibits the neutrophil-dependent early inflammatory response and induces endothelial cell damage, the clinical hallmark of Ebola virus infection.[449]

Survival of Microbes After Ingestion by Neutrophils

Certain microbes have evolved means to subvert or avoid host defenses and survive even after ingestion by neutrophils. For example, *S. aureus* produces many molecules that inhibit the function of neutrophil antimicrobial peptides and moderate the effects of reactive oxygen species.[335] During interaction with neutrophils, *Streptococcus pyogenes* upregulates a two-component signal transduction system that controls cell envelope biosynthesis and production of molecules that moderate the effects of reactive oxygen species.[436] In addition, M and M-like proteins of this pathogen promote survival after phagocytosis.[450] The activity or function of these molecules likely explains the ability of ingested *S. aureus* or *S. pyogenes* to survive long enough after phagocytosis to cause neutrophil lysis (see Fig. 8.9). Whereas microbes such as *S. aureus* survive because of high resistance to neutrophil microbicides, *A. phagocytophilum* and *Francisella tularensis* inhibit neutrophil superoxide generation and thereby avoid exposure to key microbicides.[451,452] Even with the limited understanding available, it is clear that there is as broad a range of microbial tactics for pathogenesis as there are host cell targets, and their elucidation will provide insights into the biology of both the host and the pathogen.

EOSINOPHILS

Eosinophils are bone marrow–derived, tissue-based granulocytes located subjacent to the skin and mucosal lining of the respiratory and gastrointestinal tracts (Fig. 8.10).[453,454] In these locations, they play a role in host defense against helminthic infections and figure prominently in the pathology of hypersensitivity diseases, such as asthma and certain dermatologic and gastrointestinal disorders.[453,454] In many ways, eosinophils have a functional repertoire that resembles that of neutrophils,[453,454] but there are several significant differences in the activities of these two types of granulocytes.[455] Recent studies have expanded recognition of the central role of eosinophils in modulation of inflammatory responses in selected situations.[453,454] The granules of eosinophils harbor not only cytotoxic contents but also pleiotropic inflammatory agents, including cytokines, chemokines, lipid mediators, and neuroactive substances.[453,454] Consequently, the biology of eosinophils extends beyond their contribution to host defense against helminths, and the elucidation of the scope of the role of eosinophils as immune cells promises to provide exciting insights into mechanisms for the integration of innate and adaptive immune systems.

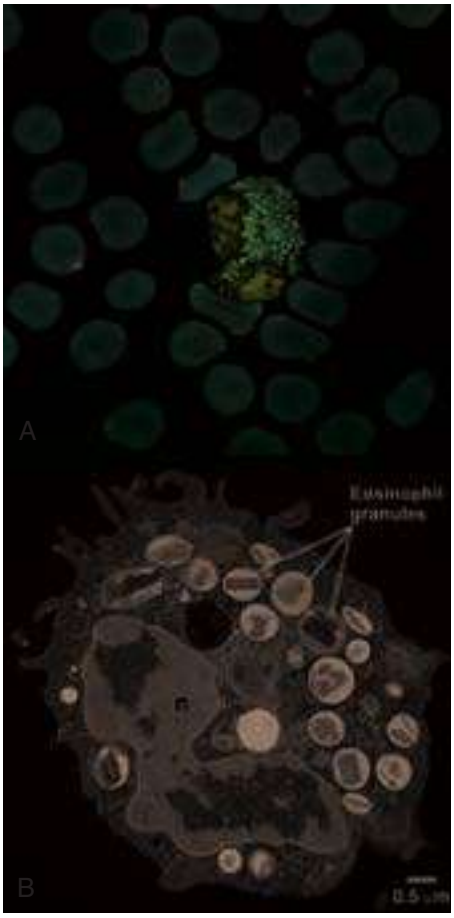

FIG. 8.10 Human eosinophils. Bright-field (A) and transmission electron (B) microscopy images of human eosinophils. Note the prominent granules with crystalloid cores. *n,* Nucleus.

Development and Characteristics

Eosinophils develop from bone marrow stem cells in 5 to 6 days.[456] Eosinophilopoiesis in humans appears to be uniquely dependent on IL-5, with IL-3 and GM-CSF also contributing to a lesser degree. IL-5 concentrations correlate with the appearance and magnitude of eosinophilia in vivo.[453,454] Eosinophil maturation is accompanied by the appearance of electron-dense primary granules, which appear during the promyelocyte stage of development, and large cytoplasmic granules known as crystalloid, specific, or secondary granules.[453,454,457–460] Primary granules contain Charcot-Leyden crystal protein (galactin 10) and lipid bodies.[453,460] Crystalloid granules are distinguished by their large size, an electron-dense crystalloid core containing major basic protein (MBP), and an electron-lucent matrix containing eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase (see Fig. 8.10).[454,457–459] Of note, these crystalloid granules also contain a wide range of chemokines (macrophage inhibitory protein-1α/CCL3, RANTES [regulated on activation, normal T cell expressed and secreted]/CCL5, eotaxin-1/CCL11, growth-regulated oncogene α/CXCL1, epithelial neutrophil-activating peptide-78/CXCL5), cytokines (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-16, IL-18, GM-CSF, IFN-γ, TNF-α, transforming growth factor-α, and transforming growth factor-β), enzymes (e.g., arylsulfatase B and collagenase), and growth factors (e.g., vascular endothelial growth factor and stem cell factor).[453,454,457,458,460]

Eosinophil Surface Receptors

Mature eosinophils can express an extensive repertoire of surface receptors—some constitutively expressed and others induced by stimuli—and thus have high immunomodulatory potential.[453] For

example, eosinophils express receptors for the Fc portion of IgA, IgD, IgM, IgG (FcγRII), and IgE (FcεRI and FcεRII), although the presence of IgE receptors on eosinophils is a subject of controversy.[460] Receptors for complement (CR1, CR3, CR4, ClqR, C3a, C5a), cytokines (e.g., receptors for IL-2, IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17, IL-23, IL-27, IL-31, IL-33, GM-CSF, IFN-γ, and TNF-α), platelet-activating factor, prostaglandins, and LTB_4 have also been reported to be present on eosinophils.[453,454] Eosinophils also express on their surface two members of the CD2 subfamily of receptors, CD48 and CD244. Engagement of CD48 elicits degranulation. Several inhibitory receptors on the eosinophil surface suppress their activation when engaged and thereby contribute to the immunomodulatory activities of eosinophils.

Eosinophil Granules

Eosinophils release their granules via several distinct mechanisms.[457,458] Eosinophils can undergo traditional granule exocytosis, a process similar to neutrophil degranulation, during which the entire contents of granules are released into the extracellular environment. This process may involve compound exocytosis, a variation of exocytosis whereby multiple granules fuse within the cell cytoplasm (thus forming a large, single granule) before exocytosis.[457,458] Eosinophil granule products can also be released by piecemeal degranulation, which is the primary mechanism for release of granule contents. During piecemeal degranulation, secretory vesicles or eosinophil sombrero vesicles transport selected molecules from crystalloid granules to the cell surface for secretion.[457,458] Release of granule contents can also occur by cytolysis,[457,458] and there is evidence that cell-free granules can be triggered to release selected molecules by specific agonists.[457,459,461]

Recruitment From Circulation

Circulating eosinophils can be separated into two populations based on buoyant density. Most eosinophils in healthy individuals are characterized as dense or normodense. Hypodense eosinophils are cells that have been activated. They express a greater number of functionally competent receptors, exhibit a higher resting level of oxidative metabolism, and predominate in the blood and tissues of persons with eosinophilia.

The intravascular half-life of eosinophils is approximately 2 hours. Eosinophil migration involves different adhesion molecules than neutrophil transmigration.[453,454,460,462,463] In addition to the β_2-integrins also expressed by neutrophils, eosinophils express β_1 and β_7 integrins[464] and display a form of P-selectin glycoprotein ligand-1 that binds more avidly to endothelial P-selectin than the form expressed by neutrophils.[460,465–467] That eosinophil transmigration is normal in leukocyte adhesion deficiency-1 provides evidence that β_2-integrins are not required for this process. Association with endothelium in the process of eosinophil trafficking appears to be mediated by IL-5, IL-4, IL-13, and chemokines and the coordinated expression and activation of integrins and their cognate binding partners.[453,468]

Role in Host Defense

Substantial evidence supports a role for eosinophils in immunity to helminthic parasites, as demonstrated by the greater worm burden and tissue damage in animals treated with antieosinophil serum and by the finding that the transfer of passive immunity requires the presence of these cells. This conclusion is buttressed by the demonstration of eosinophils on and around degenerating parasites in vivo and by the ability of eosinophils to kill these organisms in vitro.[460,469,470] It is noteworthy that many of the studies of the role of eosinophils in host defense against parasitic helminths have been performed in mouse infection models, and there are significant functional and morphologic differences between human and mouse eosinophils.[460]

Killing of parasites is related to exocytosis of eosinophil granule contents onto the parasite surface while it is in close apposition to the eosinophils.[453,460,471,472] The eosinophil peroxidase–H_2O_2–halide oxidation system plays a minor role in anthelmintic activity,[473] whereas the cationic granule proteins are responsible for the bulk of this activity. On a molar basis, eosinophil cationic protein exerts a more potent anthelmintic effect than MBP does, but the greater quantity of the latter in the eosinophil makes its contribution more significant. The anthelmintic effects of these proteins are also specific for different stages in the life cycle of the parasite. In addition to contributing to host defense against infections with helminths, eosinophils respond to RNA viruses, including respiratory syncytial virus,[460,474] perhaps through the ribonuclease activity of eosinophil cationic protein and eosinophil-derived neurotoxin.

Moderation of Type I Hypersensitivity Reactions

Recognition that eosinophil granules contain a number of substances capable of inactivating the chemical mediators of anaphylaxis has led to the suggestion that eosinophils may moderate the severity of type I hypersensitivity reactions.[453,475] In this scenario, stimulation of basophils and mast cells by the interaction of surface IgE with specific antigen results in the release of substances important in type I hypersensitivity reactions. These include vasoactive amines, slow-reacting substances of anaphylaxis (leukotrienes C, D, and E), platelet-activating factor, and eosinophil chemotactic factor of anaphylaxis (ECF-A). Histamine and ECF-A attract eosinophils to the site of antigen reaction with basophils and mast cells. ECF-A can also stimulate eosinophil degranulation, as can immune complexes that are phagocytosed by eosinophils. Histaminase secreted by the eosinophil may inactivate local histamine, and a substance present in eosinophils may inhibit further histamine secretion by basophils. Arylsulfatase and phospholipase present in the smaller eosinophil granules are capable of inactivating leukotrienes C, D, and E and platelet-activating factor. Therefore eosinophils may moderate immediate hypersensitivity reactions by inhibiting the release of mediators of the type I reaction as well as by destroying mediators that have already been released.[473]

Eosinophil-Mediated Tissue Injury

The association of eosinophilia of several weeks' duration with the development of endocardial lesions and the isolation of an eosinophil-derived neurotoxin capable of reproducing the neurologic picture observed in patients with cerebrospinal fluid eosinophilia strongly support roles for eosinophils in the pathogenesis of tissue injury in certain disorders, the most prominent of which is asthma. Substantial evidence indicates that eosinophil MBP is an important mediator of asthmatic tissue injury. For example, increased quantities of MBP are detectable in the bronchial washings from patients with asthma but not from bronchial washings from patients with other pulmonary disorders. Nanomolar concentrations of MBP, but not of other cationic proteins, cause exfoliation of epithelial cells, impaired ciliary function, net chloride secretion, and bronchial hyperreactivity. Immunofluorescent staining of bronchial epithelium in autopsy specimens from patients who died as a result of asthma revealed extensive deposition of MBP in the peribronchial areas and overlying regions of bronchial epithelial denudation. These findings were not observed in autopsy material obtained from patients who died of nonasthmatic pulmonary diseases. The importance of epithelial denudation lies in the resultant enhanced responsiveness of the underlying bronchial smooth muscle to contractile agonists, including acetylcholine and histamine, as well as to leukotriene C_4 produced by eosinophils.[476]

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The complete reference list is available online at Expert Consult.

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9 Complement and Deficiencies

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SHORT VIEW SUMMARY

- Complement comprises several soluble plasma proteins that are activated through three pathways, namely the classical, lectin, and alternative pathways. The system also consists of membrane-associated proteins that serve as receptors for activation products of complement or as inhibitors of complement.
- Complement plays a central role in innate immune defenses against invading pathogens. Activation of complement on microbes "marks" them with C3 fragments and generates anaphylatoxins such as C5a that facilitate opsonophagocytosis. Gram-negative bacteria can be killed after insertion of the membrane attack complex (C5b-9) pore.
- In addition to its function in combating infections, complement plays critical roles in several physiologic processes, including roles as a bridge between innate and adaptive immunity, in tissue regeneration and organogenesis, in disposal of immune complexes and apoptotic cells, and in metabolism.
- A fine balance exists between complement activation and its inhibition under physiologic conditions. Gain-of-function mutations of complement activators or loss-of-function mutations of complement inhibitors lead to excessive complement activation and damage of host tissues observed in conditions such as atypical hemolytic-uremic syndrome, C3 glomerulopathy, and age-related macular degeneration. Loss of glycophosphatidylinositol-anchored membrane proteins, including the complement inhibitors CD55 and CD59, results in paroxysmal nocturnal hemoglobinuria.
- Congenital deficiencies of complement protein are rare. Complete deficiencies of classical-pathway components predispose to autoimmune disorders and infections with *Haemophilus influenzae* and *Streptococcus pneumoniae*. Deficiencies of alternative or terminal complement components are associated with a several thousand–fold increase in the incidence of invasive meningococcal infections.
- Conditions associated with acquired deficiencies of complement—defective synthesis (e.g., severe liver disease), increased consumption (e.g., immune complex disease, vasculitis, C3 and C4 nephritic factors), or increased catabolism (nephrotic syndrome, protein-losing enteropathies)—are also associated with an increased risk of infection.
- Meningococcal infections in individuals with terminal complement pathway (C5 through C9) deficiencies often have a milder clinical course than in individuals with intact complement systems, which may be attributable to lower circulating endotoxin levels in the absence of membrane attack complex.
- Pharmacologic blockade of C5 (e.g., with eculizumab) is also associated with an approximately 2000-fold increase in the incidence of invasive meningococcal infection, often caused by nongroupable (unencapsulated) isolates against which capsular polysaccharide–based meningococcal vaccines are ineffective. Antibiotic prophylaxis should be considered in all persons on C5 inhibitor therapy. Clinicians should have a very low threshold for suspecting and treating meningococcal disease in this population.

Functional activity attributable to the complement system was first described in the late 1800s with the demonstration that fresh serum contained a heat-labile bactericidal factor called *alexin*.[1] Subsequently, it was shown that a heat-stable factor present in convalescent serum also contributed to bactericidal activity. At the turn of the century, Paul Ehrlich used the term *complement* to describe the heat-labile factor and *amboceptor* (antibody) to describe the heat-stable factor. With the 20th century came the recognition that complement was composed of more than one component, but it was not until 1941 that Louis Pillemer was able to separate functionally distinct components of the classical pathway from various serum fractions. In the early 1950s, Pillemer also described an antibody-independent mechanism for complement activation that he referred to as the *properdin pathway*.[1–3] However, the protein purification techniques of the time were unable to provide complement components of sufficient purity to convince others of the existence of this pathway.

With the 1960s and 1970s came the development of a mathematical model capable of describing the sequential activation of complement, and new techniques for the purification of individual complement components. The latter development led to the rediscovery of Pillemer's work, characterization of these proteins, and delineation of mechanisms that control their activity. The 1980s brought the recognition that the complement system also consists of membrane proteins—both receptors and inhibitors—that respectively mediate the cellular consequences of complement action and protect host cells from the detrimental effects of complement activation. With this advance, it was appreciated that complement functions optimally at the interface between the fluid phase and the cell surface.

Toward the end of the 20th century, an explosion in molecular biology research led to (1) cloning and structural characterization of all the complement proteins and an understanding of the molecular basis for their deficiency states, (2) characterization of a third pathway of complement activation, the lectin pathway, and (3) the use of genetically engineered mice to dissect the molecular details of complement function. Through this process, the complement system has grown to include more than 30 proteins (Table 9.1), and the diverse roles and implications of complement—as a bridge between innate and acquired immune systems, in the disposal of immune complexes and apoptotic cells, in metabolism, and in tissue regeneration and organogenesis—have been more clearly delineated.[4,5] Consequently, the view of the complement as merely augmenting host defense has been replaced by a more global one in which complement serves as one of the earliest pattern recognition systems differentiating self from nonself and provides a link between the innate and adaptive immune systems by virtue of its inflammatory and antiinflammatory actions.[4–6] The past decade has witnessed elucidation of solution and crystal structures of several complement proteins, either alone or in complex with their natural ligands, which has provided considerable insights into structure-function relationships.[7,8,9]

COMPLEMENT SYNTHESIS, CATABOLISM, AND DISTRIBUTION

Studies of cultured hepatocytes coupled with an examination of complement component polymorphisms in patients before and after orthotopic liver transplantation have established the liver as the major site of synthesis for most complement components.[10,11] The fractional catabolic

TABLE 9.1 Complement Plasma Proteins

COMPONENT	APPROXIMATE SERUM CONCENTRATION (μg/mL)	MOLECULAR WEIGHT	CHAIN STRUCTURE[a]	NO. OF GENETIC LOCI	CHROMOSOMAL ASSIGNMENT[b]
Classical Pathway					
C1q	70	410,000	(A, B, C) × 6	3 *(A, B, C)*	1p
C1r	34	170,000	Dimer of two identical chains	1	12p
C1s	31	85,000	Dimer of two identical chains	1	12p
C4	600	206,000	β-α-γ	2 *(C4A, C4B)*	6p
C2	25	117,000	One chain	1	6p
Mannose-Binding Lectin Pathway					
MBL	1–2	40,000	Homo-oligomers	1	10q
Ficolin-1 (M-ficolin)	0.04–0.1			1	9q
Ficolin-2 (L-ficolin)	3–4	34,000	Homo-oligomers	1	9q
Ficolin-3 (H-ficolin; Hakata antigen)	20	35,000	Homo-oligomers	1	1p
Collectin-11	Approximately 2			1	2p
MASPs	MASP-1, 4–30 MASP-2, 0.02–0.9 MASP-3, 2–10 MAp44, 1–3 MAp19, ND	74,000–94,000	A-B	1 per MASP	3q
Alternative Pathway					
D (adipsin)	1	24,000	One chain	1	ND
C3	1300	195,000	β-α	1	19q
B	200	95,000	One chain	1	6p
Membrane Attack Complex					
C5	80	180,000	β-α	1	9q
C6	60	128,000	One chain	1	5p
C7	55	97,000	One chain	1	5p
C8	65	150,000	Three nonidentical chains α-γ, β	3 *(A, B, G)*	α, β, 1p γ 9q
C9	60	79,000	One chain	1	5p
Control Proteins ***Positive Regulation***					
Properdin	25	220,000	Cyclic polymers of a single 57-kDa chain	1	Xp
Negative Regulation					
C1-INH	200	105,000	One chain	1	11q
C4BP	250	550,000	Seven identical chains	1	1q
Factor H	500	150,000	One chain	1	1q
Factor I	34	90,000	β-γ	1	4q
Anaphylatoxin inactivator (carboxypeptidase N)	35	310,000	Dimer of two nonidentical chains (H, L) × 2	ND	ND
S protein (vitronectin)	350	80,000	One chain	1	ND
SP-40,40 (clusterin)	50	80,000	α-β	1	8p

[a]For multichain components, parentheses indicate subunit structure; commas indicate noncovalent linkage of chains arising from separate genes; solid lines indicate covalent linkage of chains arising from posttranslational cleavage of a proenzyme molecule, with chains listed in order beginning at the amino terminus of the proenzyme molecule; a dash indicates covalent linkage of chains arising from separate genes.
[b]"p" indicates the short arm and "q" the long arm of the chromosome.
BP, Binding protein; *C1-INH,* C1 inhibitor; *H,* heavy chain; *L,* light chain; *MASP,* mannose-binding lectin–associated serine protease; *MBL,* mannose-binding lectin; *ND,* not determined.

rate for several complement components indicates that they are among the most rapidly metabolized of all plasma proteins.[12]

Fluctuations in the concentration of individual complement components largely reflect the fact that they are acute-phase reactants. Consequently, their synthesis can be modulated twofold to fivefold by a variety of immune mediators, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), interferon-γ (IFN-γ), and endotoxin.[13] In most cases, enhanced synthesis is mediated at the transcriptional level.

A variety of other cells also synthesize, store, and secrete a number of complement proteins. Most notable among these are neutrophils,

monocytes, macrophages, and adipocytes, but microglia, astrocytes, fibroblasts, and endothelial cells are also important sites of local complement production.[14] Complement synthesis by monocytes can be modulated by IFN-γ, endotoxin, IL-1, and TNF. Local synthesis is an important aspect of complement-mediated host defense, as evidenced by the observation that monocytes and macrophages can synthesize sufficient amounts of complement to promote opsonization, ingestion, and killing of bacteria.[13]

In healthy people, most complement is found in plasma. Concentrations of complement proteins in normal mucosal secretions are approximately 5% to 10% of serum levels, and in normal spinal fluid they are even lower, perhaps 1% or less. In the presence of local inflammation, complement concentrations in mucosal secretions and in cerebrospinal fluid increase, most likely as a result of altered vascular permeability barriers but also as a consequence of enhanced synthesis and secretion by local mononuclear cells.

Serum complement activity is reduced in preterm infants in proportion to the magnitude of their immaturity. In contrast, complement levels in healthy full-term infants range from 60% to 100% of those in healthy adults. Despite these almost normal levels, defective complement activation in either the classical or the alternative pathway has been noted in as many as 40% of such infants.[15]

COMPLEMENT ACTIVATION

Overview: C3, the Linchpin of the Complement System

The importance of C3 in the complement cascade is evident from its position at the convergence of the classical and alternative pathways, its role in activating and amplifying alternative-pathway activation, the multitude of functional activities associated with its various cleavage products, the fact that it is a major point of regulation of complement activity (Fig. 9.1), and the fact that its concentration in plasma (1.6 mg/mL) exceeds by 2- to 10-fold the concentration of all other complement components (see Table 9.1).[16]

C3 cleavage and its stable, covalent linkage to target surfaces is the critical outcome of complement activation. The crystal structure of C3 reveals that the molecule is organized into 13 domains (Fig. 9.2).[17] The reactive thioester moiety, which is required for covalent attachment to target surfaces, is protected from hydrolysis in native C3. Proteolytic cleavage of the C3a fragment from C3 is accompanied by a remarkable conformational change, where the thioester moves approximately 85 Å from its position in native C3, becomes fully exposed, and can form a covalent bond.[18] The calculated half-life of the reactive thioester after initial cleavage of C3 is less than 100 μsec[19,20]; failure to form a covalent bond within this period results in hydrolysis (reaction with an H_2O molecule), and the C3b remains in solution. The short half-life of the nascent thioester ensures that C3b is deposited on targets proximate to the site of complement activation.

C3 cleavage can occur through three general pathways of complement activation: the classical, lectin-binding, and alternative pathways. The product of each of these pathways is the formation of specific enzyme complexes (C3 convertases: C4b2a and C3bBb) capable of amplifying C3 cleavage and initiating formation of the membrane attack complex (MAC) (see Fig. 9.1).[6]

Pattern recognition molecules with properties ranging from rather broad specificity (e.g., mannose-binding lectin [MBL]) to multispecificity (e.g., properdin, immunoglobulin M [IgM]) to high specificity (IgG) trigger complement activation on binding to their respective targets. A common structural feature of MBL, properdin, IgM, and C1q is the presence of multiple binding pods, some with specificity for different targets. Engagement of these pods promotes stable binding, efficient initiation of the complement activation sequence, and expression of the many complement-mediated pathophysiologic effects early in the course of disease.[21]

Elegant studies using knockout mice established clearly that, in the absence of specific IgG antibody, complement activation via natural IgM and the classical pathway is responsible for initial C3 deposition and marking of most cells in a target population. Subsequent amplification via the alternative pathway is responsible for increasing the amount of C3 on the particle.[22]

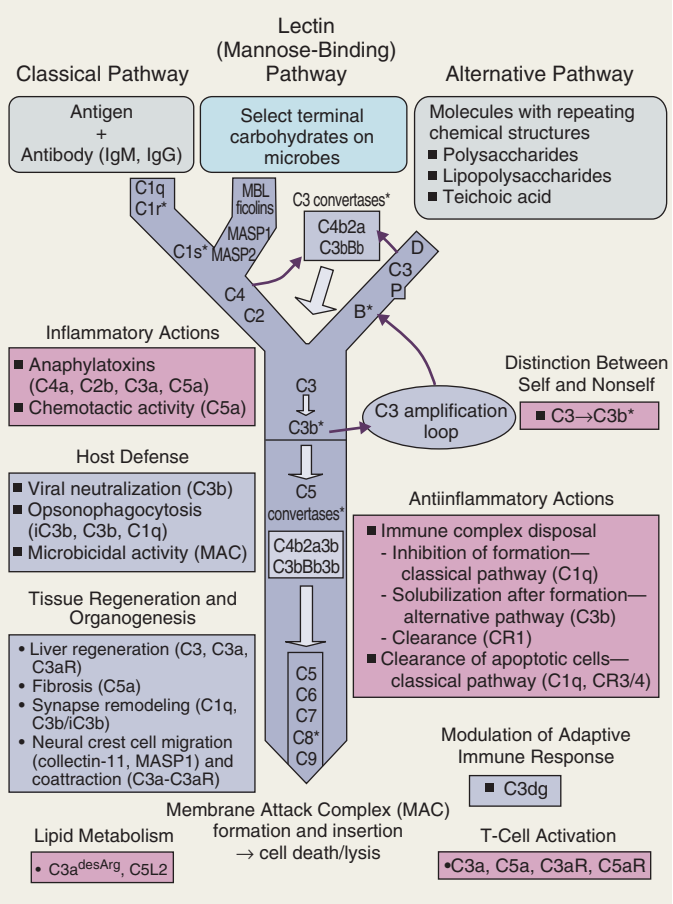

FIG. 9.1 The complement cascade. Within each pathway, the components are arranged in order of their activation and aligned opposite their functional and structural analogues in the other pathways. Rounded boxes relate to activation of the pathways; squared boxes reflect complement functions; asterisks indicate sites of downregulation of complement activity (see Table 9.2). *B,* Factor B; *C,* complement; *CR1,* complement receptor 1; *D,* factor D; *Ig,* immunoglobulin; *MAC,* membrane attack complex; *MASP,* MBL-associated serine protease; *MBL,* mannose-binding lectin; *P,* properdin.

In most instances, the recognition molecule and complement act synergistically. For example, specific antibody or MBL binding leads to more rapid and efficient complement activation and serves to direct complement deposition to nearby sites on the surface of an invading pathogen. Opsonization of infectious agents with both antibody and complement leads to more efficient ingestion and killing of these microbes than opsonization with either substance alone.[23]

Generation of the Classical-Pathway C3 Convertase

The classical pathway can be activated by multiple mechanisms, including specific antibody; MBL; ficolins; collectins; select members of the pentraxin family of proteins, such as C-reactive protein (CRP), serum amyloid P component (SAP), and pentraxin 3 (PTX3); and proteases extrinsic to the complement system per se. In the case of specific antibody, activation occurs through the formation of an immune complex as a consequence of antigen recognition by immunoglobulin, C1 binding, and sequential enzymatic activation of downstream complement components. Amino-acid and glycosylation differences in the C_H2 and C_H3 antibody regions contribute to the different complement-activating potentials among the antibody classes and IgG subclasses (IgG3 > IgG1 > IgG2; IgG4 does not activate complement).[24,25]

C1 is a trimolecular complex containing one molecule of C1q and two molecules each of C1r and C1s. C1q consists of a central core with six radiating, collagen-like fibrillar strands that terminate in globular heads that contain the antibody-binding sites. Although C1q can bind

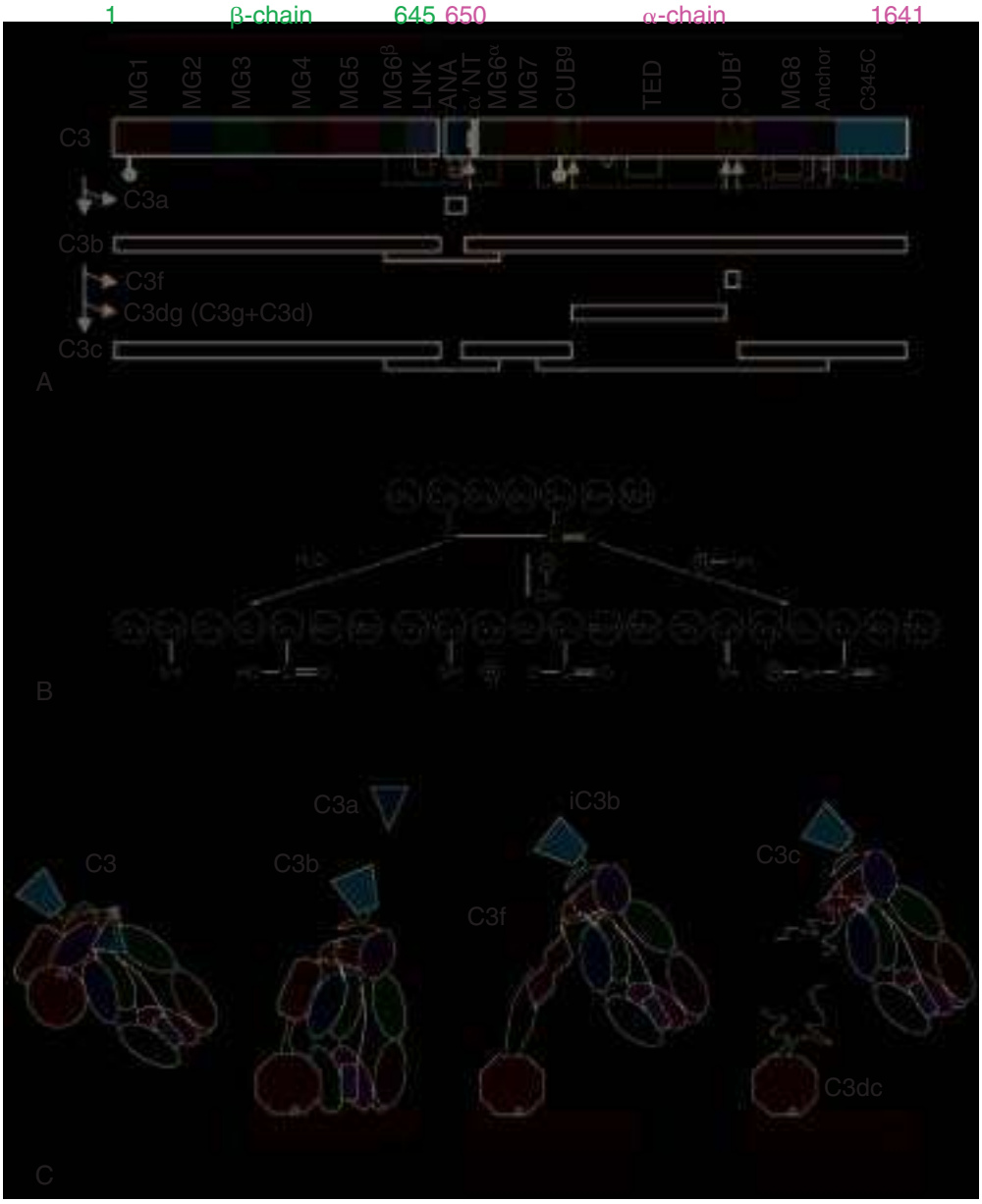

FIG. 9.2 C3 structure, activation, and fate of the internal thioester bond. (A) During activation, C3a is released from the amino terminus of the α-chain of C3. The exposed internal thioester bond becomes accessible to nucleophilic attack and can react with water or available hydroxyl or amine groups on cell surfaces (B). Analogous reactions occur with C4. Together, these reactions involving C3 and C4 are responsible for covalently linking complement deposition to the cell surface. C3 is organized into 13 domains (colors of domains in the lower panel correspond to colors of the amino-acid stretches of the α- and β-chains in A). *Arrows* indicate physiologic cleavage sites. The location of the thioester bond is indicated by the inverted white triangle. Sites of N-linked glycosylation are shown by the "inverted lollipop" symbol. Locations of disulfide bridges are also shown. Activation of C3 is accompanied by an approximately 85-Å displacement of the thioester domain, and the resulting C3b molecule can form covalent bonds with targets (C). Cleavage of C3b to iC3b also results in conformational changes that contribute to ligand specificity. *ANA,* Anaphylatoxin; *Asn,* asparagine; *CUB,* complement C1r/C1s, Uegf, Bmp1; *Cys,* cysteine; *Glu,* glutamic acid; *Gly,* glycine; *LNK,* linker; *Met,* methionine; *MG,* macroglobulin; *TED,* thioester-containing domain. *(From Janssen BJ, Huizinga EG, Raaijmakers HC, et al. Structure of complement component C3 provide insights into the function and evolution of immunity.* Nature. *2005;437:505–511; Janssen BJ, Christodoulidou A, McCarthy A, et al. Structure of C3b reveals conformational changes that underlie complement activity.* Nature. *2006;444:213–216; and Gordon DL, Hostetter MK. Complement and host defense against microorganisms.* Pathology. *1986;18:365–375.* www.tandf.co.uk/journals. *Accessed February 2009.)*

directly to negatively charged surfaces and in doing so exert functionally important effects (e.g., elimination of apoptotic cells),[26] C1q binding is classically initiated as a consequence of the recognition of antigen by antibody. In the case of IgM, functionally important C1q binding occurs after the change in configuration that accompanies binding of a single IgM molecule to multiple sites on the target particle. Elegant cryoelectron tomography studies have revealed that surface-bound antibodies are oriented as hexamers formed through noncovalent Fc:Fc interactions.[27] Fc hexamers permit engagement of each of the six globular heads of C1q in the C1 complex by Fc, which triggers classical-pathway activation. These findings explain why IgG in solution does not activate complement. Because of this topographic stipulation, many IgG molecules must be bound to a target particle to ensure sufficient density for hexamer formation. Functionally, this requirement means that complement activation by IgG is less efficient than that by IgM.[24,28] IgG variants with an enhanced ability to form hexamers could improve the therapeutic activity of antibodies that rely on complement-dependent cytotoxicity for their function.[27,29]

C1 binding by antibody results in a change in the structural configuration of the C1q molecule such that the C1r and C1s tetramer contained within the radiating pods of C1q becomes autocatalytically active. This structural alteration may involve the release of C1 inhibitor (C1-INH), which binds reversibly to proenzyme C1. Expression of enzymatic activity by C1r and C1s represents the initial activation and amplification step in the classical pathway. Many molecules of substrate are cleaved by a given enzyme complex, resulting in the fixation of subsequent complement components in the cascade in close proximity to the antibody-binding site. Therefore, antibody serves not only to activate complement in a kinetically efficient manner but also to deposit it nearby on the target surface, which includes the antibody itself (see later discussion).

Activated C1s cleaves a 9-kDa fragment, C4a, from the amino terminus of the α-chain of C4. This results in exposure of an internal thioester bond that links the sulfhydryl (SH) group of a cysteine residue with the carboxyl-terminal group of glutamic acid. This bond is subject to nucleophilic attack by hydroxyl or amino groups, leading to the formation of covalent ester or amide linkages.[30] Through this reaction, along with the analogous one involving C3 (see Fig. 9.2), the complement system acquires a chemically stable association with target surfaces. Because of gene duplication, two slightly different C4 genes exist—*C4A* and *C4B*. The product of the *C4A* gene preferentially forms amide bonds with target surfaces and is hemolytically less active than the product of the *C4B* gene, which preferentially forms ester bonds.[31] Consequently, C4A binds more effectively to proteins (e.g., antigen-antibody complexes) than does C4B.[31,32] The molecular basis for this difference is associated with an aspartic acid residue in the C4A molecule and a histidine residue in the C4B molecule, both of which influence the susceptibility of the thioester bond to nucleophilic attack by reactive groups on the target surface. This difference may play a role in the clinical presentation of patients with inherited deficiencies of C4A and C4B molecules.[32]

Activated C1s also cleaves C2 to produce a small fragment, C2b, which is released into the environment, and a larger fragment, C2a, which binds to C4b on the surface of the target particle. This complex, *C4b2a*, is the classical-pathway C3 convertase (see Fig. 9.1).

Although recognition of antigen by antibody is the historically preeminent initiator of classical-pathway activation, studies have confirmed a vital clinical role for complement activation via the lectin pathway (see Fig. 9.1). To date, five lectin molecules that can bind to a variety of terminal monosaccharides and initiate complement activation have been described. These include the collectins (collagen-containing C-type [calcium-dependent] lectins) MBL and collectin 11,[33] and ficolin-1, ficolin-2, and ficolin-3 (also called M-, L-, and H-ficolin, respectively).[34] Ficolins contain a fibrinogen-like domain combined with a collagen-like domain and therefore are not classified as collectins.

The recognition molecules of the lectin pathway are trimers that comprise three identical polypeptide subunits, each terminating in a calcium-dependent carbohydrate recognition domain. These trimers are organized into higher-order oligomers that resemble a "bouquet." MBL is structurally and functionally homologous to C1q.[35] Like C1q, it exists in serum as a complex with serine proteases, termed MBL-associated serum proteases, or MASPs. Four such molecules—MASP-1, MASP-2, MASP-3, and MAp19—are the product of two genes arising from a common ancestor shared with C1r and C1s.[36] MASP-2 plays a particular role in cleaving C4 and C2 and generating the classical-pathway C3 convertase, as described earlier.[35,36] An individual with a nonsense mutation in *MASP1* (and therefore lacking both MASP-1 and MASP-3) had a nonfunctional lectin pathway.[37] Reconstitution with MASP-1 resulted in MASP-2 cleavage and full restoration of lectin-pathway activity. MASP-1 and MASP-2 were cocomplexed with MBL, supporting a model in which MASP-1 transactivates MASP-2, analogous to C1r and C1s activation.

The carbohydrate recognition domains on MBL bind to a variety of terminal monosaccharides, including mannose, *N*-acetyl-mannosamine, *N*-acetyl-D-glucosamine, fucose, and glucose. Collectin-11 binds preferentially to L-fucose and D-mannose. The ficolins all appear to bind preferentially to acetylated sugars such as *N*-acetyl-D-glucosamine. In addition, M-ficolin binds to *N*-acetyl-D-galactosamine and select sialoglycans, such as those present in the capsule of *Streptococcus agalactiae*. Ligands reported for L-ficolin include β-(1→3)-D-glucan, *N*-acetylneuraminic acid, lipoteichoic acid, CRP, fibrinogen, DNA, and certain corticosteroids, whereas H-ficolin binds to fucose. These sugars frequently decorate microbial surfaces but rarely appear as the terminal unit on oligosaccharides or glycoconjugates on human cells. This fact has immense implications for host defense because it provides a mechanism for differentiating nonself from self and for rapidly activating the complement cascade. In these respects, the lectins share several critical features with IgM or "natural antibody": Both are polyreactive, bind to surface carbohydrates, require binding of just a single molecule to effect complement activation, and in contrast to IgG, do not require clonal expansion of a specific population of lymphocytes before recognition of antigen.[6,35]

Extrinsic proteases, such as Hageman factor (factor XII in the clotting cascade), can also activate the classical pathway.[21,38] Gain-of-function mutations of factor XII are seen in about one-third of individuals with type 3 hereditary angioedema.[39] The role of other proteases in activating downstream complement components (e.g., activation of C5 by thrombin) has been reported,[40] and their clinical significance is currently being clarified.

Generation of the Alternative-Pathway C3 Convertase

Just as several new mechanisms for generation of the classical-pathway C3 convertase have come to light, data have pointed to two mechanisms for generation of the alternative-pathway C3 convertase: the standard fluid-phase C3 "tickover" model and the newly described properdin-directed model. Several unique features characterize alternative-pathway complement activation: (1) antibody is not required, although it can facilitate the activation process; (2) activation proceeds both in the fluid phase and on cell surfaces; and (3) a component of the activation process, C3b, is also a product of the reaction, resulting in a positive feedback loop that amplifies the activation process. Consequently, C3b deposition resulting from C3 cleavage by either alternative-pathway or classical-pathway C3 convertase can initiate the alternative-pathway amplification loop (see Fig. 9.1).[30,41]

The "Tickover" Model

C3, the critical reactant of the alternative pathway, contains an internal thioester bond within its α-chain.[42] This internal thioester bond undergoes spontaneous low-rate hydrolysis to form C3(H_2O) (see Fig. 9.2). For a brief moment before its inactivation by the control proteins, C3(H_2O) can form a complex with factor B. A series of subsequent reactions yields a fluid-phase C3 convertase, C3(H_2O)Bb, that can cleave more C3 to generate metastable C3b capable of forming covalent ester or amide linkages with appropriate chemical constituents on the surfaces of nearby cells. C3(H_2O) formed in plasma may constitute an important source of intracellular C3 stores that serves to maintain T-cell homeostasis and other cellular functions,[43,44,45] as discussed later. Surface-bound C3b can bind additional factor B, which in turn can be cleaved by factor D to produce *C3bBb,* the alternative-pathway convertase (see Fig. 9.1). This convertase is inherently labile, with a half-life of approximately 90 seconds. Properdin binding to C3bBb stabilizes the complex and prolongs its half-life 5- to 10-fold,[46] thereby providing reaction conditions sufficient for further C3 cleavage and signaling initiation of the amplification phase of alternative-pathway activation.

Although antibody is not required for activation of the alternative pathway, it acts synergistically with properdin to facilitate the activation process.[47] Facilitation depends on the Fab portion of the antibody molecule rather than the Fc fragment responsible for classical-pathway activation.[47,48] The molecular basis for facilitation is uncertain but may depend on the identity of the carbohydrate moieties present on IgG. The hydroxyl groups in these moieties can serve as sites for ester bond formation with C3.[48] Moreover, the alternative-pathway C3 convertase generated on IgG is relatively resistant to inactivation by regulatory proteins.[48–50]

The Properdin-Directed Model

The properdin-directed model, articulated by Hourcade and coworkers, is exciting for several reasons, not the least of which is that it provides direct support for Pillemer's original postulate for the existence of an

alternative pathway of complement activation, which he called the properdin pathway.[51–53]

Properdin is a positively charged molecule made up of identical subunits, each of which is composed of six globular thrombospondin type 1 repeats that associate to form dimers, trimers, and tetramers. These multimers can bind directly to a variety of cell surface molecules, particularly sulfated glycosaminoglycans (GAGs), such as heparan sulfate proteoglycans and chondroitin sulfate E. Properdin is synthesized by immune cells, especially those of phagocytic origin. Discharge of properdin from these cells increases its local concentration and could focus alternative-pathway activation on specific targets. Properdin bound directly to target cells may serve as a preferential platform for binding of fluid-phase–generated C3b. Subsequent binding of factor B and its cleavage generate the already stabilized alternative-pathway C3 convertase, *C3bBb.*[51,53] Purified properdin has a propensity to aggregate with freeze-thawing or prolonged storage, which may result in artifactual binding to biologic surfaces. Thus, experimental data with unfractionated properdin should be interpreted with caution; at this time, the role of properdin as an initiator of the alternative pathway remains controversial.[54,55]

Assembly of the Membrane Attack Complex

Of the various forms of C3, *only* C3b can perpetuate complement activation. C3b binding to the C3 convertases generates new complexes, the C5 convertases (C4bC2aC3b*n* and C3bBbC3b*n*),[56] which are responsible for cleaving C5 and initiating assembly of the MAC. C5 is the structural homologue of C4 and C3, except that its α-chain does not contain an internal thioester bond. Analogous to C4 and C3, activation of C5 proceeds via cleavage of an 11.2-kDa fragment, C5a, from the amino terminus of its α-chain. The remaining C5b binds noncovalently to the surface of the target particle.[57] The remaining terminal complement components—C6, C7, C8β, C8α-γ, and C9—share a high level of structural organization at both DNA and protein levels.[58,59] Unlike the early components of the classical and alternative pathways, these proteins lack enzymatic activity but as a group are characterized by their amphipathic properties. They circulate in plasma in hydrophilic form, undergoing hydrophobic transformation on binding to the nascent MAC.

Assembly of the MAC begins when binding of C5b to hydrophobic sites on the cell surface exposes binding sites for C6 and C7, leading to the formation of a stable trimolecular complex, C5b-7. Incorporation of C7 results in hydrophilic-to-amphiphilic transitioning of the assembling complex and promotes direct insertion into cell membranes. Subsequently, C8 binds to C5b at a site on its β-chain.[60] In the final step, C8 initiates polymerization of C9 through a binding site on C8α-γ.[61] A current model of this process suggests that the function of C5b-8 is to create a discontinuity in the membrane lipid bilayer, thereby establishing an environment for the stepwise unfolding, insertion, and polymerization of monomeric C9.[62] In its completely assembled state, the MAC consists of a single molecule each of C5b, C6, C7, and C8 and multiple (1–18) molecules of C9, with an internal pore diameter of about 110 Å.[57,63]

Fully inserted and polymerized C9 has a tubular shape and the properties of an integral membrane protein. Previously thought to form a closed ring structure similar to pores formed by perforin and other cholesterol-dependent cytolysins, electron cryomicroscopy studies have shown that the MAC adopts a "split-washer" configuration because C6 and the final C9 molecules do not make extensive contact.[63] The inner aspect of this tubular structure is hydrophilic and allows the passage of water and ions, whereas the outer surface is hydrophobic and causes varying degrees of membrane disorganization during insertion. Both of these effects contribute to the microbicidal and cytolytic properties of the MAC.[57,62]

REGULATION OF COMPLEMENT ACTIVATION

A major feature of the complement cascade is its controlled production of an inflammatory reaction that is sufficient to enhance host defense and the immune response, yet not so potent as to lead to host injury. Upregulation of this process is achieved by the inherent property of enzymes to turn over multiple substrate molecules rapidly and by stabilization of enzyme complexes (e.g., by properdin). Downregulation is achieved in a temporal fashion through the short half-lives of the enzymatic complexes and the anaphylatoxins, and in a spatial manner through direction of complement activation to the target surface (e.g., by antibody, MBL, properdin). Modulation of the potentially injurious effects of indiscriminate complement activation is achieved by specific regulatory proteins that act at three major levels: activation (C1), effector initiation (C3), and cytolysis (MAC). Unique disease entities that result from deficiencies of these control proteins are testimony to the importance of complement regulation.

Regulation of C1 Activation

C1 esterase inhibitor (i.e., C1-INH) binds reversibly to pro-C1, thereby preventing its spontaneous activation.[28] Binding of C1q to antibody subverts this control by causing dissociation of C1-INH from pro-C1 and allowing autocatalytic cleavage to proceed. At some point after C1 activation, C1-INH binds covalently to the active sites on C1r and C1s, inactivating their catalytic function and dissociating them from C1q. C1-INH neither prevents nor inhibits initial activation; rather, its roles are to prevent amplification of fluid-phase C1 activation and to limit excessive activation on the target cell. Complete C1 inactivation requires the binding of four molecules of C1-INH, one per catalytic site. In contrast to its binding to pro-C1, C1-INH binding to C1r and C1s is irreversible; it prevents cleavage of C4 and thereby controls the initial amplification step of classical-pathway activation.[28,64] C1-INH also limits the activity of MASP-2 and several proteases of the coagulation-anticoagulation system, including factor XI, factor XII, plasma kallikrein, plasmin, and tissue plasminogen activator.[65]

Regulation of the C3 Convertases

The classical-pathway and alternative-pathway C3 convertases are functionally analogous molecules (see Fig. 9.1). Control of their activity occurs via three basic mechanisms that use functionally identical or shared regulator proteins (Table 9.2)[30,41]:

1. *Spontaneous decay:* Both convertases (C4b2a and C3bBb) are inherently labile and undergo spontaneous decay, with the loss of C2a or Bb from their respective complexes.
2. *Accelerated decay:* Spontaneous decay can be accelerated by C4b-binding protein (C4BP) and especially by factor H. These regulatory proteins compete with C2a and Bb for binding sites on C4b and C3b. In doing so, they inhibit new convertase formation and enhance the rate of dissociation of already formed convertases.
3. *Facilitated inactivation:* Exposed C4b and C3b molecules are highly susceptible to enzymatic cleavage by factor I. C4BP and factor H serve as cofactors to promote factor I–mediated cleavage and production of inactivated C4b (iC4b) and C3b (iC3b), respectively. Inactivation eliminates the ability of these molecules to re-form the C3 convertases.[66,67] Under typical circumstances, the functional half-life of C3b is just 90 seconds, whereas its cleavage product, iC3b, has a half-life of approximately 35 minutes.

Several additional points have emerged from the many studies on C3 convertase regulation. First, control of C3 convertase activity is expressed both in the fluid phase and on host cell surfaces. C4BP and factor H modulate convertase activity in both locations, whereas membrane-bound proteins (complement receptor 1 [CR1], membrane cofactor protein [MCP], and decay-accelerating factor [DAF]) primarily control convertase activity on cell surfaces. Second, all control proteins either accelerate the decay of the C3 convertases (DAF) or promote factor I–mediated cleavage of C4b or C3b (MCP), or both (CR1, C4BP, factor H). Third, C4BP and factor H, unlike their membrane-bound counterparts, exhibit relative specificity for the classical-pathway and alternative-pathway C3 convertases, respectively.[64,67] Of these two serum proteins, factor H plays the dominant regulatory role.

These regulatory molecules contain a structural motif called *short consensus repeats (SCRs).*[68] SCRs are tandem repeats of approximately 60 amino acids that share a conserved consensus sequence. The number of repeats varies considerably among the control proteins, from a low

TABLE 9.2 Plasma and Membrane Proteins That Regulate or Mediate Complement Activity

LOCATION AND PROTEIN	SPECIFICITY	FUNCTIONS
Plasma		
C1-INH	C1r, C1s	Binds to and inactivates C1r and C1s in the C1 complex
C4BP	C4b	Inhibits assembly and accelerates decay of C4b2a; cofactor for C4b cleavage by factor I
Factor H	C3b	Inhibits assembly and accelerates decay of C3bBb; cofactor for C3b cleavage by factor I
Factor I	C4b, C3b	Proteolytic inactivation of C4b and C3b
Properdin	C3bBb	Stabilizes alternative-pathway C3 convertase
S protein (vitronectin), SP-40,40 (clusterin)	C5b-7	Binds fluid-phase C5b-7; prevents attachment of alternative C5b-7 and C5b-9 to membranes
Carboxypeptidase N	C4a, C3a, C5a	Inactivates these anaphylatoxins by removal of carboxyl-terminal arginine
Cell Membranes		
CR1 (CD35)	C3b, C4b, iC3b	Inhibits assembly and accelerates decay of C3 convertases; binds immune complexes to erythrocytes; phagocytosis
Membrane cofactor protein (CD46)	C3b, C4b	Cofactor for cleavage of C4b/C3b by factor I
Decay-accelerating factor (CD55)	C4b2a, C3bBb	Promotes decay of C3 convertases
CR2 (CD21)	C3d, C3dg	Phagocytosis; modulates B-cell responses; Epstein-Barr virus receptor
CR3 (CD11b/CD18)	iC3b	Phagocytosis
CR4 (CD11c/CD18)	C3dg, C3d	Phagocytosis
CD59	C8 in C5b-8	Binds to C8; inhibits polymerization of C9
C3a/C4aR	C3a, C4a	Vasodilation
C5aR	C5a, $C5a^{desArg}$	Chemotaxis, cell activation, cytokine secretion
C1qR	C1q	Phagocytosis
CRIg	C3b, iC3b	Phagocytosis; soluble form inhibits C3-containing convertases

BP, Binding protein; *CR,* complement receptor; *i,* inactive; *INH,* inhibitor.

of 3 in MCP and DAF to a high of 59 in C4BP. SCRs constitute the binding domains for C3b and other molecules. Two to four SCRs are typically required to form a complete binding site, but the number of SCRs and which ones in a sequence form a specific binding site differ among the proteins and their binding ligands.[68,69]

As noted, control of C3 convertase activity, and in particular amplification of alternative-pathway convertase activity, is expressed both in the fluid phase and on cell surfaces. Convertase control under these two circumstances is achieved through differential interaction among several C3b-binding sites and polyanion-binding sites on factor H. Fluid-phase regulation proceeds rapidly and depends on the interaction of C3b with SCRs 1 through 4 at the amino terminus of factor H. In contrast, regulation of convertase activity on cell surfaces depends on SCRs 16 through 20 at the carboxyl terminus of factor H. These SCRs bind both cell surface polyanions (e.g., sialic acid, GAGs, heparan sulfate) and C3b. The simultaneous recognition of surface polyanions and C3b by the same factor H molecule greatly increases the affinity of factor H for C3b, thereby enhancing convertase control. These findings are particularly relevant to our understanding of the pathophysiology of the hemolytic-uremic syndrome and factor H deficiency (see later discussion).[70,71,72]

In summary, the C3 convertases represent the major site of both complement amplification and complement regulation. The membranes of host cells contain both specific proteins that act to downregulate the C3 convertases and polyanions that enhance the affinity of fluid-phase factor H for surface-bound C3b and promote its regulatory activity. The chemical microenvironment on microbial surfaces is also a major determinant of the outcome of the competition between C3b and C4BP with C4b in formation of the classical-pathway convertase, and between Bb and factor H with C3b in formation of the alternative-pathway convertase. Nonpathogenic microbes typically possess an activating surface environment, whereas pathogenic microbes usually manifest a nonactivating environment (see later discussion).[73]

Regulation of the Membrane Attack Complex

MAC assembly is controlled in two ways—by proteins that bind to the C5b-7 complex and by proteins that inhibit C9 incorporation and polymerization within the MAC. Nascent C5b-7 molecules have the potential to insert into any cell membrane and are not restricted to the surface on which complement is activated. By binding to this trimolecular complex, S protein (vitronectin) and clusterin abrogate the ability of C5b-7 to insert into cell membranes and consequently its hemolytic potential.

Although several proteins inhibit C9 incorporation and polymerization within the MAC, by far the most potent is CD59. CD59 is very widely distributed. Its presence as a membrane protein probably accounts for homologous restriction,[74] a phenomenon whereby cells are not lysed by complement from the same species but can be (although are not always) lysed by complement from a different species. Although it is clear that CD59 binds to C9, the site of binding and the mechanism by which it prevents C9 incorporation and polymerization within the MAC are unclear.[66]

Nucleated eukaryotic cells are resistant to complement-mediated cytolysis, even in the face of a nonhomologous complement source. Resistance is associated with the capacity of the cell to maintain high synthetic rates of membrane lipids and the ability to shed MAC from the cell surface.[75,76] Insertion of the MAC in eukaryotic cell membranes is accompanied by a rapid influx of calcium, generation of multiple signals, and stimulation of arachidonic acid metabolism.[77,78] These events probably promote normal physiologic functions and contribute to host cell injury.

Basis for Discriminating Between Host and Microbial Cell Surfaces

The potential of C4 and C3 to form covalent bonds with reactive groups on cell surfaces makes them inherently incapable of distinguishing between host and microbial cells. It follows that the basis for discrimination between self and nonself must depend on other factors.[73] One of these elements is the presence of complement regulatory proteins in the membranes of host cells but not on the cells of microbial organisms.[73] Another element is the chemical composition of the cell surface. Because covalent bond formation is nondiscriminatory, the basis for discrimination must lie in the capacity for chemical differences on the cell surface to affect the outcome of the competition between factor B and factor H for the binding site on C3b. Typically, this is accomplished by modulation of the affinity of factor H, not that of factor B, for C3b. Enhanced affinity for and binding of factor H favors C3 convertase decay and diminishes activation of the alternative pathway and its amplification on the cell surface. Decreased affinity and binding of factor H results in the opposite effect (see earlier discussion). Cells in the former category (e.g., "self") are nonactivators, whereas those in the latter category (e.g., "nonself") are activators with respect to the alternative pathway. C3b bound to the surface of a nonactivating particle binds factor H with about 100-fold greater affinity than does C3b bound to an activator particle. Consequently, factor B binding and subsequent amplification of complement activation are favored on the latter particle.[30,41,70–72]

Chemical constituents in cell membranes that contribute to a microenvironment that enhances factor H binding include polyanions such as sialic acid and sulfated acid mucopolysaccharides (e.g., heparan sulfate). These molecules, which are present on most human cells, bind to the anion-binding sites on factor H and enhance its affinity for C3b, thereby contributing to the nonactivator status of host cells.[70–72,79,80] Structural data confirm that simultaneous binding of surface-bound C3b and GAGs to factor H domains 19 and 20, respectively, enhances the avidity of factor H binding.[81,82] Avid factor H binding increases the rate of C3 convertase decay over the rate of its formation, thus rendering the surface a "nonactivator." In contrast, loss of GAGs reduces the affinity of factor H and favors the rate of C3 convertase formation over its decay, thereby enhancing complement activation and effector functions.

COMPLEMENT RECEPTORS

Complement receptors have been described primarily on peripheral blood cells, including erythrocytes, neutrophils, monocytes, macrophages, B and T lymphocytes, eosinophils, mast cells, and platelets. They fall into two broad categories: (1) those that bind diffusible complement fragments released during activation of the complement cascade and (2) those that bind complement components deposited on cell surfaces such that the component serves as a bifunctional ligand, or bridge, linking the target cell to the receptor (see Table 9.2).

Receptors in the first category mediate many of the clinical manifestations of the inflammatory response in that they bind C4a, C3a, and C5a, the complement-derived inflammatory mediators. Of these, the high-affinity C5a receptor (CD88) has been best studied. This G protein–coupled receptor is present on neutrophils, monocytes, and macrophages, and its perturbation causes migration (chemotaxis) of these cells in the direction of increasing C5a concentration. Studies have furnished interesting examples of bidirectional crosstalk between engagement of macrophage Fcγ receptors (FcγRs) and C5a receptors (C5aRs).[83] Engagement of FcγR initiates C5 release; C5a generated by cleavage of this C5 binds to its receptor on phagocytes and stimulates increased synthesis and expression of FcγRs that possess an immunoreceptor tyrosine-based activation motif (ITAM), thereby resulting in enhanced efficiency of macrophage function.[23] On the other hand, engagement of FcγRIIb, which is the only FcγR that possesses an immunoreceptor tyrosine-based inhibition motif (ITIM),[84] inhibits signaling by C5aR. A second receptor for C5a called C5L2 (GPR77) is a G protein–independent receptor. The functions of C5L2 remain to be fully elucidated,[85] but some studies have suggested that it serves as a decoy receptor with regulatory functions. Experimental evidence has confirmed the presence of receptors for C3a on B lymphocytes, guinea pig ileum, vascular endothelium, adipocytes, and mast cells.[86] Several studies in animal models have begun to elucidate the complex role of anaphylatoxins and their receptors in the outcome of polymicrobial sepsis,[87] airway hypersensitivity,[88] modulation of signaling through Toll-like receptors (TLRs),[89] and in T-cell expansion and differentiation,[90–94] often with conflicting results.

The second category of receptors includes C1qR, CR1, CR2, CR3, CR4, and CRIg (*c*omplement *r*eceptor of the *i*mmuno*g*lobulin superfamily). C1qR is a carbohydrate-rich protein expressed on phagocytic cells and lymphocytes that modulates phagocytosis, cytokine release, cytotoxicity, and interactions with endothelial cells. Functional ligands in addition to C1q that are recognized by C1qR include MBL, surfactant protein A, and conglutinin, all of which exhibit structural homology with C1q.[95]

Receptors for the cleavage products of C3 and C4 (CR1, CR2, CR3, and CR4) have been studied more extensively. CRIg is the newest member of the complement receptor family.[96] Although they recognize closely related ligands, each of these receptors is structurally distinct and exhibits a unique pattern of distribution across peripheral blood cells or tissue macrophages. A portion of these receptors are linked to the cellular cytoskeleton, an association that is important for signal transduction.[95,97,98]

CR1, the C3b/C4b receptor, is present on erythrocytes, neutrophils, monocytes, B cells, subpopulations of T cells, follicular dendritic cells (DCs), and glomerular podocytes. Four polymorphic variants differ in size (190–280 kDa) and in the number of C3b/C4b-binding sites.[99] The number of CR1 molecules per cell is determined genetically but varies with cell type and disease activity. CR1 mediates immune complex binding and clearance, promotes ingestion of C3b/C4b-bearing particles, modulates certain lymphocyte functions,[95,97] and carries certain blood group antigens.[100]

CR3 and CR4 are members of the integrin family of heterodimeric proteins.[101] They recognize iC3b as their major binding ligand. CR3 also binds to C3b and C3dg and bears a lectin-like domain that recognizes specific carbohydrates on microbial surfaces. The three–amino-acid sequence arginine-glycine-aspartic acid (Arg-Gly-Asp, also known as the RGD motif), which is present in C3 and other ligands, represents an important binding motif for CR3.[102] Together, CR3 and CR4, particularly the former, recognize the various combinations of C3b, iC3b, and C3dg present on the surfaces of microbial cells and play a major role in their elimination by all types of phagocytic cells.[103] In addition, CR3 plays an important role in adherence-related functions of neutrophils (see Chapter 8).

CR2 is present on B lymphocytes and follicular DCs and serves to recognize C3d and C3dg. The association of CR2 and CD19 in the B-cell membrane constitutes an important mechanism for B-cell activation.[104] CR2 acts to target C3dg-bearing particles or immune complexes to lymphocyte-rich areas in the spleen and lymph nodes, thereby driving antigen activation of these cells and promoting long-term immunologic memory.

CRIg was first identified on Kupffer cells (resident macrophages in the liver) and binds to C3b and iC3b.[96] The Kupffer cells in the liver of CRIg knockout mice, compared with wild-type mice, were impaired in their ability to clear C3-opsonized pathogens, such as *Listeria monocytogenes* and *Staphylococcus aureus,* from the circulation. Although CRIg knockout mice had lower bacterial burdens in the liver, significantly higher bacterial loads were seen in the bloodstream and other organs, such as the spleen and lungs, which accounted for the increased mortality compared with wild-type mice. On binding to C3b present in alternative-pathway C3 and C5 convertases, soluble CRIg prevents the association and cleavage of C3 and C5, respectively, thereby functioning as an inhibitor of the alternative pathway.[105,106]

FAMILIES OF COMPLEMENT PROTEINS

The complement cascade represented in Fig. 9.1 emphasizes features shared by the three pathways with respect to activation and regulation. Complement components can also be grouped into several different protein families[107]:

- The serine protease family (C1r, C1s, MASP-1 through MASP-3, MAP19, C2, factor D, factor B, and factor I).
- Disulfide-linked, multichained molecules with homology to an ancestral protein that contained an internal thioester bond (C4, C3, and C5).
- Proteins that are the products of class III major histocompatibility complex (MHC) genes located on chromosome 6 (C2, factor B, C4A, and C4B).
- Proteins that bind C3 and C4 fragments and belong to a closely clustered supergene family located on the long arm of chromosome 1 (C4BP, factor H, DAF, MCP, CR1, and CR2); these proteins share a common SCR motif (see earlier discussion) with other complement components that bind to C3 and C4 (e.g., C2, factor B) and with some other complement and noncomplement proteins that do not bind these two components.[68,69]
- Proteins that share homology with the low-density lipoprotein (LDL) receptor (C6, C7, C8α, C8β, and C9); the large number of disulfide bonds in these cysteine-rich molecules is thought to convey a tertiary structure that facilitates the hydrophilic-hydrophobic transition that occurs when they interact with lipid membranes during assembly of the MAC.[58,59]

The MHC III genes are located between the class I and class II loci on the short arm of chromosome 6[107] and merit discussion. The genetic material in this region appears to have undergone two duplication events, resulting on the one hand in the structurally and functionally related proteins C2 and factor B, and on the other in the C4 and 21-hydroxylase

A and B variants.[107] Recombinant events in this region of the chromosome tend to be suppressed, leading to the usual inheritance of the entire region intact from each parent.[108] The polymorphic variants of the complement components encoded by these genes in a given individual are referred to as *complotypes.*[109] The association of specific complotypes with specific products of the MHC I and II genes probably contributes to the association of specific complotypes with certain disease states (e.g., systemic lupus erythematosus [SLE]).

COMPLEMENT-MEDIATED FUNCTIONS

Complement plays major roles in the distinction between self and nonself, development of an inflammatory response, elimination of microbial pathogens, modulation of the adaptive immune response, limitation of the potential for an injurious inflammatory response (through the disposal of immune complexes and apoptotic cells), metabolism, angiogenesis, tissue regeneration, and organogenesis (see Fig. 9.1).[4,6]

Whether initial formation of a C3 convertase is followed by its enhanced decay or by its amplification is the event that distinguishes self from nonself, respectively, as described earlier.[6]

Small, diffusible peptide fragments released from C4, C3, C5, and probably C2 during their activation initiate and modulate the inflammatory response.[86] Collectively, C4a, C3a, and C5a are referred to as *anaphylatoxins,* and together they stimulate histamine release from mast cells (C3a), promote vascular dilation (C3a, C4a), increase endothelial permeability (C3a), and stimulate neutrophil responses (C5a). In addition to these proinflammatory activities, C3a acts via its receptor on B cells to downregulate cytokine synthesis and antibody production.[110] Carboxypeptidase N–mediated removal of the carboxyl-terminal arginine from the anaphylatoxins abrogates their functional activity by preventing their interaction with specific receptors.[104]

Elimination of Pathogens

Complement activation promotes the elimination of microorganisms in conjunction with phagocytic cells by opsonophagocytosis or, in the case of certain gram-negative pathogens, by direct bactericidal attack. Complement-mediated opsonophagocytosis promotes uptake via complement receptors, predominantly CR1 and CR3, that recognize C3b and iC3b, as described previously (see Table 9.2). In the case of bacteria, opsonization with C3b or iC3b, especially in conjunction with IgG, promotes ingestion of the organism and triggers the microbicidal mechanisms of phagocytic cells (see Chapter 8). Ingestion appears to be more efficient when the organism is opsonized with iC3b than with C3b.[111] Complete activation of the complement cascade, with assembly of the MAC and its effective insertion into cell membranes, results in the death and eventual lysis of the cell. Death and lysis are independent events, and in the case of prokaryotes, evidence suggests that a metabolic response is required by the organism before the lethal effects of the MAC can be expressed.[112] For some organisms, assembly of the MAC through C8 is sufficient for killing,[113] but in all cases the incorporation of C9 accelerates this process. Complement-mediated virucidal activity has also been well described and in some cases seems to require deposition of only the early components of the classical pathway.[114]

Modulation of Adaptive Immune Responses

Substantial data indicate that C3 modulates the adaptive immune response.[115–117] This evidence includes (1) the absolute requirement for C3 binding to effect antigen localization within splenic and lymphoid germinal centers; (2) the presence of complement receptors, especially CR2, on B lymphocytes, follicular DCs, and other antigen-presenting cells; (3) impaired antibody responses in animals or humans who lack one of the complement components (C1, C2, C4, C3) required for classical-pathway C3 convertase formation and restoration of the immune response by replacement of the missing component; and (4) the association of these deficiencies in humans with depressed concentrations of IgG4 and IgG2.[118,119] In general, studies have demonstrated that soluble C3 fragments (especially C3a) inhibit adaptive immune responses, whereas C3 fragments (especially C3d) covalently linked to target particles enhance these responses. Complement activation via the classical pathway and engagement of CD21/CD35 receptors promote differentiation of naïve B cells and elimination of self-reactive cells.[115] Ligation of C3d to its receptor, CR2, leads to its association with CD19 in the B-cell membrane and constitutes an important signal for the activation of these cells.[104] Antigen-bound C3d acts as an immune adjuvant, lowering the stimulation threshold necessary for B-cell activation. This adjuvant role is particularly critical in enhancing the response to antigens with a low affinity for the B-cell receptor.[120–122] In addition to its adjuvant role, C3d facilitates isotype switching, anamnestic responses after secondary antigenic exposure, and B-cell survival and long-term immunologic memory. Similarly, C3 fragments promote the expansion of $CD8^+$ T cells after viral infection, and costimulation of CD3 and CD46 (MCP) promotes development of regulatory T cells (Tregs). Thus, complement activation contributes to the development of both B-cell and T-cell acquired immune responses.[115,116,123]

Local production and activation of complement and signaling through the anaphylatoxin receptors can determine the outcome of T-cell responses.[93] Engagement of TLRs on DCs results in secretion of alternative-pathway components and upregulation of C3aR and C5aR. C3a and C5a act on their cognate receptors on the DCs and induce secretion of IL-6, IL-12, or IL-23. Stimulation of CD28 on T cells induces expression of C3aR and C5aR; engagement of the latter by the anaphylatoxins generated by DCs induces IL-12R expression and a series of signaling events that result in IFN-γ and IL-2 production. The interleukins secreted by the DCs then determine whether responses are skewed toward Th1 or Th17. In the absence of activation of DCs through pattern recognition receptors, local complement production ceases and the lack of signaling through C3aR and C5aR is associated with increased production of transforming growth factor-β (TGF-β) and induction of suppressive $Foxp3^+$ Treg cells.[94] During this process, upregulation of C5L2 sequesters any local C5a, ensuring absence of C5aR stimulation. These events depend on the production of complement locally, not on systemically circulating complement.

Some experts believe that an ancient form of C3—part of a primitive complement system in simple multicellular pathogens such as members of the phylum Porifera (sponges)[124,125]—functioned intracellularly to regulate metabolic processes and also to fight intracellular pathogens. The more familiar extracellular functions of C3 may have evolved as organisms became more complex and developed circulatory systems, when C3 was secreted into the vasculature. However, the intracellular functions of complement likely have been retained over the course of evolution. As discussed later, intracellular C3 fragments play a role in cellular survival and metabolic reprogramming of T cells. In addition to T cells, intracellular complement may function in most cells of the body.

A recently elucidated novel recycling pathway describes a source for intracellular C3. $C3(H_2O)$, the hydrolytic product of native C3, is taken up by cells and constitutes an important source of intracellular C3.[43] About 80% of $C3(H_2O)$ is recycled back to the extracellular space.[43] A fraction of intracellular $C3(H_2O)$ is cleaved by lysosomal cathepsin L to generate C3a and C3b. C3a binds to C3aR on lysosomes and activates the metabolic-checkpoint kinase, mammalian target of rapamycin (mTOR), at a low level that permits survival of T cells.[126] Activation of the T-cell antigen receptor (TCR) and costimulation through the coreceptor CD28 results in redistribution of C3aR and cathepsin L from their intracellular locations in the resting state to the cell surface. Cleavage of C3 at the cell surface generates C3a and C3b, which signal through C3aR and CD46, respectively, resulting in upregulation of growth factor receptors and secretion of cytokines characteristic of Th1 (such as IFN-γ) and Th17 (such as TNF and IL-17) responses, but not Th2 cytokines (IL-4 and IL-5). Expansion of Th1 cells is accompanied by high local levels of IL-2; signaling via IL-2R and CD46 induces IL-10 production and initiates contraction of the Th1 cell population.

Metabolic reprogramming determines the fate of T-cell differentiation. Activation of $CD4^+$ T cells results in autocrine activation of C3aR and CD46 by C3a and C3b, respectively, and sustained activation of mTOR complex (mTORC1), which stimulates glycolysis and oxidative phosphorylation and induction of a Th1 response.[127] Accordingly, individuals with CD46 deficiency show impaired production of IFN-γ but intact Th2 cytokine responses.[125] Proteolytic cleavage of intracellular C5 generates C5a, which activates C5aR1, and results in generation of reactive oxygen species, which activates the NLRP3 inflammasome. NLRP3 activation cleaves pro-IL-1β to active IL-1β, which in an autocrine fashion promotes differentiation to Th1 cells.[128]

Clearance of Immune Complexes and Apoptotic Cells

The incorporation of complement in immune complexes enhances clearance and helps to minimize the potential for tissue damage.[129,130] This process includes inhibition of immune complex precipitation, solubilization of immune complexes, and clearance of C3b-bearing immune complexes via the CR1 receptor. Under conditions of antibody excess or antibody-antigen equivalence, the probability that both antigen-binding sites on a single antibody will bind to epitopes on a single antigen and the probability that multiple antibody molecules will bind to a given molecule of antigen are increased. This situation promotes antibody–antibody interactions via Fc fragments and subsequent immune complex precipitation.[129] C1q binding to the Fc portion of antibody inhibits Fc–Fc interactions and leads to covalent binding of C3b to the immune complex. Subsequent recruitment of the alternative pathway via the C3b amplification loop promotes further C3b deposition within the immune complex lattice, thereby reducing the forces that hold the lattice together and causing separation (solubilization) of smaller complexes from the lattice network. Thus, classical-pathway activation inhibits immune complex precipitation, whereas the alternative pathway promotes immune complex solubilization.[129,130] However, in the context of disease pathogenesis, it must be stressed that complement is 10 times more efficient in inhibiting immune complex precipitation than in solubilizing precipitated complexes. This property probably contributes greatly to the close association of classical-pathway component deficiencies with immune complex disease formation (e.g., SLE).

In healthy people, most immune complexes bearing C3b are bound to cells bearing C3b receptors (CR1). The number of these receptors per cell varies from a low of 950 for erythrocytes to a high of 57,000 for neutrophils.[131] However, because there are 1000 times more erythrocytes than leukocytes, 95% of the CR1 receptors in the peripheral circulation are located on erythrocytes. Consequently, immune complexes bearing C3b are 500 to 1000 times more likely to be cleared from the circulation by erythrocytes than by leukocytes.[131] These complexes are removed from the erythrocyte, along with the CR1, during passage through the liver and the spleen. This extraction probably involves fixed macrophages that line the sinusoids of these organs.[132]

Recent studies have extended our understanding of the antiinflammatory role played by complement in promoting the clearance of apoptotic cells. Under steady-state conditions, the billions of host cells that die every day are eliminated with minimum induction of an inflammatory or immune response. Despite the number of cells involved and completion of the apoptotic cycle over a period of several hours, few apoptotic cells are identified in tissues or in the circulation. Rapid complement-dependent phagocytic removal of apoptotic cells by macrophages appears to account for this apparent paradox. During apoptosis, the cell membrane bulges to form blebs that contain macromolecular complexes of proteins and nucleic acids, a finding that may be a significant contributing factor in the development of SLE (see later discussion). The exposed surface of the bleb contains several unique phospholipids that have been translocated from the inner to the outer leaflet of the lipid bilayer of normal cell membranes. Some of these phospholipids, especially phosphatidylserine, bind C1q directly to activate the classical pathway. In addition, data have suggested that the phospholipid-binding proteins annexin 2 and annexin 5 may also serve as ligands for C1q on apoptotic cells.[133] An analogous process occurs on ischemic cells on reperfusion; exposed GAGs promote properdin-directed alternative-pathway activation.[53] Unlike viable eukaryotic cells, apoptotic cells and ischemic cells "permit" both C3 convertase formation and amplification. These cells can then be eliminated via C1qR, CR3, and CR4 on mononuclear cells, fixed macrophages, and DCs. The importance of CR3 in clearing apoptotic cells is underscored by the observation that a variant allele (R77H) in CD11b (α-chain of CR3) that impairs phagocytosis is one of the strongest risk factors for SLE.[134–137] Elimination in this fashion minimizes the inflammatory potential of injured cells.[138,139]

Complement and Metabolism

One of the characteristics of the metabolic syndrome is increased inflammation, including activation of the complement system.[140] Insulin resistance and abdominal obesity are associated with higher serum concentrations of C3. Adipocytes are the main source for factor D (also known as adipsin) and also synthesize C3 and factor B. Local activation of the alternative pathway results in production of C3a that is rapidly converted to $C3a^{desArg}$. $C3a^{desArg}$ (also known as acylation-stimulating protein [ASP])[141] stimulates triglyceride synthesis in fat cells by increasing the activity of diacylglycerol acyltransferase. C5L2 also appears to play a role in lipid clearance by $C3a^{desArg}$, although it is not clear whether C5L2 and $C3a^{desArg}$ interact directly.

Complement and Cancer

Complement plays complex and multifaceted roles in the pathogenesis of cancer, both protective and pathogenic.[142,143] The conventional view has been that complement plays a (protective) role in immune surveillance against cancer cells by promoting antibody-dependent cellular cytotoxicity (ADCC) and lysis of cells by the MAC. Many tumor cells, however, express high amounts of membrane inhibitors of complement, such as CD46, DAF, and CD59, and in addition can recruit soluble complement inhibitors, such as factor H and factor H–like protein 1 (FHL-1; an alternatively spliced variant of factor H), to their surface and thus resist direct lysis by complement.[144] Complement also promotes angiogenesis and may therefore contribute to tumor growth.[145] C3a and C5a stimulate the secretion of vascular endothelial growth factor (VEGF) and facilitate neovascularization. This effect contributes to the choroidal neovascularization seen in age-related macular degeneration (AMD; see later) and forms the basis for the use of VEGF inhibitors in the treatment of this disease. The ability of complement to degrade extracellular matrix may increase tumor invasion and migration. C5a generated by the classical pathway in the tumor microenvironment enhanced the growth of cervical cancers in mice. C5a attracts myeloid-derived neutrophil- and monocyte-like suppressor cells to the tumor, which generate reactive oxygen and nitrogen species that interfere with the ability of T cells to respond to tumor antigens.

Complement in Tissue Regeneration and Organogenesis

Complement proteins can also modulate diverse developmental processes, such as cell survival, growth, and differentiation in various tissues.[146] For example, C3 and C5 have been implicated as mediators of lens and limb regeneration in lower vertebrates.[147] Impaired liver regeneration was observed in both C3 and C5 knockout mice after partial hepatectomy; infusion of C3a and C5a into these mice restored hepatic regeneration. Enhanced signaling through the canonical Wnt pathway is associated with aging-associated decline in tissue regeneration. Aging mice have higher serum levels of C1q; C1q binds to Frizzled receptors, whereas C1s cleaves the Wnt coreceptor low-density lipoprotein receptor–related protein 6 (LRP6), which activates the Wnt pathway and impairs skeletal muscle regeneration.[148]

Studies in mice have provided evidence for a critical role of the classical pathway in synaptic remodeling.[149] C1q expression is upregulated when neurons are exposed to immature astrocytes, which results in deposition of C3 fragments and elimination by macrophages or microglia. Mice deficient in C1q or C3 could not eliminate unwanted synapses. Neuronal pentraxins that bear 20% to 30% structural homology to the immune pentraxins PTX3 and CRP were cited as possible C1q binding molecules in this process. Complement activation may contribute to neuronal pathologic conditions in adults. A strong genetic link exists between C4A alleles associated with high levels of C4A expression and schizophrenia. Increased complement activation associated with higher C4 levels may result in synapse elimination in the prefrontal cortex, which is characteristically seen in the brains of individuals with schizophrenia.[150] Studies in mice suggest C1q-dependent complement activation initiated by soluble β amyloid oligomers, accompanied by microglial pruning of complement-coated synapses, may contribute to the pathology of Alzheimer disease.[151] Studies in *Xenopus* embryos revealed a role for the C3a-C3aR interaction in neural crest migration. A role for the lectin pathway, specifically collectin 11 and MASP-1, in neural crest migration and craniofacial development is discussed later.

The context in which complement activation occurs is important. Although complement may have salutary effects in development and some aspects of tissue regeneration, complement activation contributes

to ischemia-reperfusion injuries related to acute myocardial infarction, stroke, and organ transplantation, and to the pathologic features of diseases such as hepatic, pulmonary, and renal fibrosis, Alzheimer disease, Parkinson disease, and multiple sclerosis.

MICROBIAL INTERACTIONS WITH THE COMPLEMENT SYSTEM

The demonstration by Roantree and Rantz[152] that gram-negative bacteria isolated from blood were almost always resistant to complement-mediated killing, whereas two-thirds of those isolated from mucosal surfaces were serum sensitive, was one of the first findings to suggest an important clinical role for complement-mediated bactericidal activity in host defense. This suggestion was borne out by subsequent studies of people with complement deficiencies (see later discussion) and by elucidation of the strategies and the extremes to which microorganisms go to escape host defense mechanisms. In the case of complement, these strategies parallel those used by host cells to circumvent injury during the inflammatory response—that is, they are focused on decreasing complement activation, accelerating convertase decay, and inhibiting the formation or insertion of the MAC.[153] In many instances, the microbial proteins responsible for these effects share molecular, structural, immunologic, and functional homology with their human counterparts.

As noted earlier, sialic acid is a well-characterized modulator of alternative-pathway activity, the action of which is expressed through enhanced factor H binding. Sialylation of the lipooligosaccharides (LOSs; lipopolysaccharide [LPS] molecules that lack O-antigenic repeats) of the pathogenic Neisseriaceae, *Haemophilus influenzae* (both typeable and nontypeable), and *Campylobacter jejuni* may enhance interactions of factor H with surface-bound C3 fragments,[154] or directly with the bacterial surface as reported with *Neisseria gonorrhoeae.*[155] Sialic acid is also a prominent chemical constituent of capsular polysaccharides on type 3 group B streptococci, K1 *Escherichia coli,* and groups B and C meningococci.[73,80] Consequently, the capsules of these organisms are nonactivators of the alternative pathway and are poor stimuli for antibody production. In this context, it is noteworthy that K1 *E. coli*, group B streptococci, and group B meningococci are prominent causes of neonatal and infant sepsis and meningitis. The frequent absence in these young patients of specific antibody to activate the classical pathway, coupled with bacterial sialic acid–mediated inhibition of alternative-pathway activity, may provide the ideal clinical setting for infection with these organisms.

Elegant experiments correlating virulence with LPS composition and complement activation in three isogenic *Salmonella typhimurium* variants, which differed only in the chemical structure of their LPS side chains, demonstrated the importance of limited complement deposition on the surfaces of bacteria. The greatest rate of C3 consumption and extent of C3b deposition were initiated by the least virulent strains. Subsequent experiments demonstrated that discrete differences in O-antigen structure were expressed at the level of alternative-pathway amplification, as manifested in the greater affinity of factor B for C3b on the surface of the least virulent compared with the most virulent strains. In contrast, the affinity of factor H for C3b was the same in all strains.[156,157]

Appropriation of complement regulatory proteins is a strategy used by a wide range of microbial pathogens, as has been demonstrated for many pathogenic bacteria, viruses, fungi, and worms.[158–160] These organisms express any of a number of complement regulator–acquiring surface proteins (CRASPs) on their surfaces. A chief binding target of these proteins is factor H, although the site to which factor H binds varies slightly among the CRASPs.[161] M protein in group A streptococci functions as a CRASP. Its α-helical coiled-coil repeats promote binding, not just of factor H but also of C4BP and MCP. The fact that group A streptococci bind multiple proteins to regulate complement at the level of C3 attests to the critical importance of C3 activity for survival of the organism, and likewise for host defense against the organism. Through these interactions, M protein not only limits complement deposition on the streptococcal surface but also promotes adhesion to keratinocytes.[161,162] Meningococci bind factor H in a human-specific manner through two surface molecules, factor H–binding protein (fHbp) and neisserial surface protein A (NspA).[163–165] fHbp is a key antigen in two licensed vaccine preparations against group B meningococcal disease (Bexsero [GlaxoSmithKline {Philadelphia, PA}] and Trumenba [Pfizer {New York, NY}]).[166]

Other organisms use related strategies. The envelope of type 1 herpes simplex virus contains a virus-specific protein, gC-1, that interferes with properdin-dependent stabilization of the alternative-pathway C3 convertase, thereby limiting complement-mediated effects. Deletion mutants lacking gC-1 are exquisitely sensitive to complement-mediated lysis. Natural mutants have not been isolated with any frequency in surveys of clinical specimens, attesting to the importance of this protein and perhaps this mechanism in the pathogenesis of infection. Vaccinia virus bears a C4BP structural and functional homologue that accelerates the decay of the classical-pathway convertase.[153,167,168]

A variation on this theme occurs in serum-sensitive gonococci isolated from patients with symptomatic local genital disease. These organisms possess a sialyl transferase but lack the ability to synthesize cytidine monophospho-*N*-acetyl neuraminic acid (CMP-NANA). Consequently, they are incapable of endogenous sialylation of their LOS; rather, they appropriate host CMP-NANA for this purpose. Exogenous sialylation confers serum resistance to these gonococci by reducing the binding of bactericidal antibody[169] and by enhancing binding of factor H (see earlier); it also reduces phagocytic uptake and may alter C3 cleavage and intracellular survival. *Trypanosoma cruzi* accomplishes the same effect by means of a *trans*-sialidase that removes terminal sialic acid residues from host glycoconjugates and transfers them to acceptor molecules on the parasite's surface.[170]

The striking metamorphosis undergone by protozoa during transformation from insect-infective to human-infective forms is accompanied by the acquisition of resistance to complement-dependent killing. This phenomenon has been studied most extensively in *T. cruzi,* in which surface proteins have been identified that block assembly and promote decay of the alternative-pathway C3 convertase.[153,170] These proteins function in a manner identical to human CR1 and DAF (see Table 9.2). *Ixodes* tick saliva contains a protein, Salp15, that, when bound to *Borrelia burgdorferi,* inhibits MAC assembly and prevents complement-mediated lysis of the organism.[171] Gametes of *Plasmodium falciparum* bind to human factor H through a surface protein called PfGAP50, which protects them from complement-mediated lysis in the mosquito midgut.[172]

The growth of DNA sequence data banks and investigations into molecular pathogenesis have served to focus attention on virus-complement interactions and the mechanisms by which these organisms elude complement-mediated attack. Studies of the human immunodeficiency virus (HIV) are particularly illustrative. During viral replication, the virus is assembled and released from the infected cell by budding, a process that incorporates host cell membrane proteins into the viral envelope. Host cell DAF and CD59 incorporated into the viral envelope in this manner function efficiently to limit amplified complement deposition on HIV and its subsequent lysis. In addition, the HIV-specific envelope proteins gp120 and gp41 both contain factor H–binding domains, which, in the case of the latter protein, demonstrate significant homology with C3. Factor H passively absorbed from serum and secretions serves to further limit complement deposition on the virus.[167,173]

Other organisms owe their serum resistance to functional homologues of CD59, the protein that interferes with MAC assembly on host cell membranes. For example, the galactose-specific adhesin of *Entamoeba histolytica* functions in this manner and also shares DNA sequence homology and antigenic cross-reactivity with CD59.[174] Plasmids in *S. typhimurium* and *Yersinia enterocolitica* contain the *rck* and *ail* genes, respectively, which encode products of a family of virulence-associated outer membrane proteins. By preventing C9 polymerization, these proteins function similarly to CD59 and mediate serum resistance.[175,176] Ail has recently been shown to recruit factor H and C4BP, whereas Rck binds to factor H, providing evidence that a single protein can block complement using multiple mechanisms.[177–181] Akin to protein Ail, select gonococcal porin molecules can bind both factor H and C4BP, thereby regulating the alternative and classical/lectin pathways, respectively.[182,183]

Bacterial capsular polysaccharides modulate the effects of complement deposition on the organism and interaction of the organism with the host (see earlier discussion). In the absence of specific antibody, the

capsule, by masking C3 deposited on subcapsular structures, blocks C3 interaction with complement receptors on phagocytic cells. These effects contribute to the antiphagocytic properties of capsular polysaccharides. In addition, capsular polysaccharides and outer membrane blebs are shed during organism growth and complement attack. Shedding serves to divert complement attack from the intact organism to these shed complexes. The ability of capsular-specific antibody to reverse these effects is testimony to the importance of antibody in redirecting complement deposition to a relevant site on the surface of the organism.[184]

Gonococci isolated from patients with disseminated gonococcal infection are resistant to the bactericidal activity of normal human serum.[185] The serum resistance of these strains is multifactorial. In the absence of bactericidal antibody, the MAC is assembled on the organism's surface but fails to insert properly into the outer membrane.[186] MAC insertion and killing occur normally in the presence of anti-LOS IgG found in the convalescent serum of some patients with this infection.[187] However, some sera also contain IgG specific for gonococcal outer membrane protein 3.[188] This antibody competes with bactericidal antibody for binding sites on the surface of the organism, thereby blocking its bactericidal effect. Although the blocking antibody promotes complement deposition on the organism, it apparently does so at sites that do not lead to killing of the organism.[189] Blocking antibody also appears to account for the resistance of meningococci to killing by the serum of some adults who acquire this infection.[190,191–193] Antibodies that block killing of *Pseudomonas aeruginosa* and *Brucella abortus* have also been described.[194–196] A study in HIV-infected adults in Africa showed that antibodies directed against the LPS of nontyphoidal *Salmonella* blocked killing by otherwise bactericidal antibodies directed against outer membrane proteins.[197] Thus, blocking antibodies may represent a fairly ubiquitous yet relatively underappreciated mechanism of complement evasion by bacteria. These findings illustrate the influence of the composition of the outer membrane of gram-negative bacteria in determining sensitivity to complement-mediated killing and the importance to the host of specific antibody in overcoming the resistance of these organisms to killing.[198]

Redundancy in complement evasion mechanisms by pathogens appears to be the rule, rather than the exception. This is well illustrated by the following examples with *S. aureus*.[199] Staphylococcal protein A (SpA) and staphylococcal binder of immunoglobulins (Sbi) engage IgG on the bacterial surface in a manner that precludes activation of the classical pathway. Staphylokinase cleaves IgG and releases it from the bacterial surface. Several small molecules secreted by *S. aureus* interfere with complement activation at various steps. Staphylococcal complement inhibitor (SCIN) and its homologues SCIN-B and SCIN-C bind to the classical- and alternative-pathway C3 convertases and block their function. Extracellular fibrinogen-binding protein (Efb) and extracellular complement-binding protein (Ecb; also called Efb homologous protein or Ehb) also target and block the activity of the C3b-containing convertases (C3bBbC3b and C4bC2aC3b). The chemotaxis inhibitory protein of *S. aureus* (CHIPS) binds to the C5aR and formyl peptide receptor (FPR) on neutrophils and inhibits chemotaxis.

Appreciation has been growing for the number of intracellular pathogens that use complement receptors to gain entry into cells.[200,201] Organisms vary as to whether entry in this fashion initiates an appropriate signal transduction response and whether it is sufficient to establish effective intracellular infection. For example, gp350 on Epstein-Barr virus serves as a ligand for CR2 to initiate viral entry into B cells. The resulting cellular transformation probably contributes to the polyclonal gammopathy observed early in infectious mononucleosis.[200,201] In addition to its factor H–binding domain, HIV gp120 contains several C3b-binding regions. Cells harboring latent HIV can be activated by the uptake of additional HIV or other particles via CR3. Interactions via this receptor can induce the cellular transcription factor, nuclear factor kappa B (NF-κB), which in turn binds to promoter regions in the virus to stimulate generation of progeny virus.[6,35] The pathogenic mycobacteria (*Mycobacterium tuberculosis, Mycobacterium leprae,* and *Mycobacterium avium*) can bind to (preformed) C2a, which results in the formation of a C3 convertase on the bacterial surface. Subsequent C3 cleavage and C3b deposition facilitate bacterial uptake by macrophages through CR1.[202] C3 fragment deposition mediated by natural IgM facilitates uptake of *Francisella tularensis* by human neutrophils through CR1 and CR3, whereas uptake by macrophages occurs through engagement of CR3 and CR4.[203]

Over the past decade, our concept of extracellular organisms has been modified by the recognition that many of these organisms can enter and survive inside epithelial cells. Both the meningococcus and the gonococcus, like the measles virus, use CD46 (see Table 9.2) to gain entry to such cells, activating intracellular signaling cascades that are important for their infectivity.[204,205] Primary human cervical epithelial cells synthesize all components of the alternative pathway and also express CR3.[206] Gonococcal pilin and porin can interact with the I-domain of CR3, which, in cooperation with iC3b deposited on bacteria, facilitates gonococcal invasion into epithelia.[207]

COMPLEMENT DEFICIENCY STATES

Incidence

Complement deficiency states can be acquired or inherited. Acquired deficiency can occur acutely, as part of an abrupt insult such as infection, or in conjunction with chronic rheumatologic or autoimmune disease. The frequency of inherited complement deficiencies in the general population is about 0.03%. Because these states are rare, the usefulness of screening tests is greatest in populations that bear the clinical correlates of abnormal complement inheritance—that is, persons with rheumatologic disease or recurrent bacterial infection.[208–210] The frequency of complement deficiencies reported among people with these disorders is affected by both methodologic and biologic factors.[211] The most important methodologic variables are sample size and degree of ascertainment. The most important biologic considerations are the ethnic makeup of the population and the incidence of the target disease in that population.

One such study, using immunologic and functional assays, detected a single individual with homozygous C2 deficiency among 545 patients with rheumatologic disease.[211] This frequency (0.2%) is approximately 10-fold greater than that in the general population. Studies using DNA typing methodologies have found the frequency of homozygous C2 deficiency in whites with SLE to be about 1.7%.[212] These studies provide clear support for the association of complement deficiency states with certain rheumatologic disorders, particularly SLE.[213]

Reports of an association between systemic meningococcal and gonococcal infections and inherited deficiency of C5, C6, C7, or C8 led to several studies of the prevalence of such deficiencies among patients with these infections. These studies found that as few as 0 (<2%) of 47 and as many as 8 (50%) of 16 individuals presenting with a first episode of documented meningococcal disease had a complement deficiency.[209,211] Analysis of these studies demonstrated an inverse relationship between the prevalence of complement deficiency among people with meningococcal disease and the incidence of the disease in the general population—that is, a high prevalence of complement deficiency was found in populations with hypoendemic disease and a low prevalence in populations with epidemic disease (Fig. 9.3). This finding suggests that the overall prevalence of complement deficiencies is relatively constant (0.03%–0.11%) but that among populations in which the level of protective antibody is low and meningococcal disease is epidemic, a greater number of healthy than complement-deficient persons will be infected because the number of healthy persons who lack specific antibody is significantly greater than the number of those who are complement deficient. As the level of immunity in the population increases, the incidence of meningococcal disease falls; however, because the prevalence of complement deficiency in the general population is relatively stable, the frequency of infectious states among those individuals with meningococcal disease increases—that is, complement deficiency becomes a proportionately greater determinant of the risk of infection.[211] The best estimate of inherited complement deficiency states among patients with *endemic* neisserial disease (approximately 10 cases per 1 million population; see Fig. 9.3) is about 5% to 10%. However, the likelihood of complement deficiency is increased dramatically (to 31%) among patients who have had more than one episode of meningococcal infection or who have a family history of meningococcal disease.

Although the number of infections caused by *Streptococcus pneumoniae* and *H. influenzae* appears to be increased in patients with

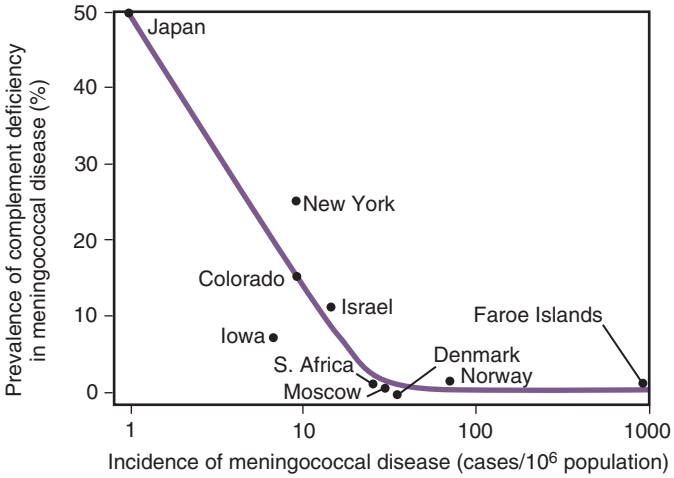

FIG. 9.3 Relationship between the prevalence of complement deficiency and the incidence of meningococcal disease. *(From Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies.* Clin Microbiol Rev. *1991;4:359–395.)*

complement deficiencies, the prevalence of complement deficiency among persons with systemic infection caused by these organisms does not appear to be markedly different from that in the population at large.[214] The basis for this apparent paradox, especially for *H. influenzae,* which shares many features with meningococci, is unknown.

General Aspects of the Molecular Basis for Complement Deficiencies

As with most other inherited conditions, the basis for complement deficiency states exhibits substantial heterogeneity, especially among people who represent different ethnic or racial ancestries. Within a defined ethnic or racial population, the probability that a single molecular defect will predominate is substantially increased. A corollary of this principle is that the same functional deficiency occurring among persons of different backgrounds is likely to exhibit molecular heterogeneity. The latter generalization is useful in the selection of patients whose deficiencies, when characterized at the molecular level, are likely to yield new information. Current studies are focusing increasingly on the contribution of various component polymorphisms to the pathophysiology of different disease states.

Classical-Pathway Deficiencies

Clinical Aspects

Immune Disorders

The association of immune disorders, in particular SLE, with complement deficiency states is most evident in persons lacking C1, C4, C2, or C3 (Table 9.3). Even though these inherited deficiencies are present in a small minority of patients with SLE, their association with this disorder is convincingly underscored by the very high penetrance of the disease in these patients. "The strength of the association of complement deficiency with SLE itself and with the severity of the disease is inversely correlated with the position of the deficient protein in the activation sequence of the classical pathway."[6] Uncertainty exists as to whether both homozygous and heterozygous complement deficiencies, or only the former states, constitute a risk factor for SLE. Early studies that used immunologic and functional assays suggested an association in both homozygous- and heterozygous-deficient persons. More recent studies using DNA typing techniques strongly suggested that, at least in C1q and C2 deficiency, the association exists only for the homozygous state.[213]

Assessment of this issue in patients with partial C4 deficiency is complicated by the existence of separate *C4* genes, *C4A* and *C4B.* As a result, complete C4 deficiency (i.e., the absence of the products of all four C4 genetic loci) is extremely rare. Conversely, heterozygous C4 deficiency is very common, occurring in approximately 25% of the general population.[215,216] In addition, as a result of linkage disequilibrium, the C2 and C4 null genes occur as part of distinct, extended haplotypes. These haplotypes include MHC I, MHC II, complement, and TNF genes, all of which are candidate disease susceptibility genes. The specific haplotype associated with C2 deficiency occurs in about 93% of affected persons. In the case of C4 deficiency, multivariate analysis of HLA-DR and C4 genotypes has confirmed an independent contribution of C4AQ0 and DR alleles to the development of SLE. The C4B null gene *(C4BQ0)* was not associated with SLE.[217–219] The chemical preference of the internal thioester in C4A to form amide bonds and to react with immune complexes may contribute to the effect that the C4A null gene has on the development of SLE.[31]

Pathophysiologic roles for complement in systemic lupus erythematosus. The pathophysiologic basis for the association between these classical-pathway deficiencies and SLE is incompletely understood, but our emerging understanding of the role of complement in the clearance of apoptotic cells (see earlier discussion) has provided additional insight and an increasingly compelling logic for this association.[138,139] Despite the significant insights afforded by studies of these individuals and of genetically engineered complement-deficient mice, it remains clear that predisposition to SLE is genetically multifactorial.[220]

The key clinical features of SLE in complement-deficient persons include early age at onset (median, 7 years), increased severity (often with impressive photosensitivity), central nervous system involvement, and increased frequency of glomerulonephritis.[221] A striking hierarchy of decreasing penetrance of SLE is seen among classical-pathway deficiencies: 93%, 57%, 57%, 75%, and 10% for C1q, C1r, C1s, C4, and C2 deficiencies, respectively. This hierarchic relationship is also generally evident in the severity of SLE and in the frequency and type of autoantibodies present in the sera of these patients. SLE is more severe in patients with C1q, C1r, C1s, or C4 deficiency.[219,221–223] In persons with C2 deficiency, the severity of SLE appears to be similar to that in the general population, and in C3-deficient persons SLE may be less severe.[214,219,223]

Additional evidence of the importance of complement deficiency as a predisposing factor for SLE comes from studies of the occurrence of SLE in some patients with acquired hypocomplementemia. Among these findings, C1-INH deficiency is especially interesting because the absence of this regulatory protein is associated with uncontrolled consumption of the classical-pathway complement components, which is particularly severe and prolonged in a small number of patients. It appears that it is these patients who are at risk for development of SLE.[221]

The unique functional roles played by the classical pathway, impairment of which seems to play a role in the development of SLE, are elimination of apoptotic cells and clearance of immune complexes.

As noted earlier, membrane blebs on apoptotic cells express phosphatidylserine, annexin 2, and annexin 5, to which C1q can bind, thereby activating the classical pathway and promoting opsonophagocytic elimination by complement receptors (e.g., through cC1qR and its signaling partner, CD91) on macrophages and mononuclear cells. Engulfment of apoptotic cells via these receptors appears not to initiate a prominent inflammatory response, in part because apoptotic cells also bind to complement inhibitors such as C4BP and factor H.[138,139] Sunlight is very effective in inducing apoptosis of dermal cells,[26] an observation that probably helps explain photosensitivity in SLE, and the positive lupus band that is demonstrated in skin biopsy specimens of people with SLE, regardless of disease activity. The inability of macrophages to clear apoptotic cells effectively is associated with an increased display of apoptotic cell debris on the surfaces of DCs, where it may be presented to autoreactive lymphocytes as an early step in the development of autoantibodies and autoreactive T cells. Particularly intriguing is the observation that membrane blebs on apoptotic cells contain the very macromolecular complexes of ribonucleic proteins to which SLE autoantibodies are directed and that the relevant epitopes on these complexes become exposed during incorporation into the blebs.[6,138,139]

Approximately one-third of all patients with SLE develop autoantibodies to C1q that lead to secondary C1q deficiency. In contrast to primary C1q deficiency in which the deficiency is strongly linked to the development of SLE, secondary deficiency in SLE is a result of the disease

TABLE 9.3 Complement Deficiency States

COMPONENT	NO. OF REPORTED PATIENTS (APPROXIMATE)	MODE OF INHERITANCE	FUNCTIONAL DEFECTS	DISEASE ASSOCIATIONS
Classical Pathway				
C1qrs C4	40 26	ACD ACD	Impaired immune complex handling, delayed C′ activation, impaired immune response	CVD, 48%; infection (encapsulated bacteria), 22%; both, 18%; healthy, 12%
C2	100	ACD		
Mannose-Binding Lectin Pathway				
MBL	Many	ACD	Impaired complement activation	Pyogenic infection; meningococcal disease
MASP-2	9	ACD	Defective association with MBL	Ulcerative colitis, CVD, pneumococcal disease
Ficolin-3	4		Impaired activation of the lectin pathway	Recurrent lower respiratory infections (*Haemophilus influenzae* and *Pseudomonas aeruginosa*), recurrent digital warts, bilateral cerebral abscess (nonhemolytic streptococcus)
Collectin-11 MASP-1	10 9		Impaired activation of the lectin pathway Impaired activation of the lectin pathway	3MC syndrome (craniofacial-ulnar-renal syndrome); facial dysmorphism, cleft lip/palate, craniosynostosis, learning disability; genital, limb, and vesicorenal anomalies
Alternative Pathway				
D P	4 70	ACD XL	Impaired C′ activation in absence of specific antibody	Infection (meningococcal), 74%; healthy, 26%
Junction of Classical and Alternative Pathways				
C3	19	ACD	Impaired immune complex handling, opsonophagocytosis; impaired granulocytosis, chemotaxis, immune response, and absent SBA	CVD, 79%; recurrent infection (encapsulated bacteria), 71%
Terminal Components				
C5 C6 C7 C8	40 80 80 75	ACD ACD ACD ACD	Impaired chemotaxis; absent SBA Absent SBA Absent SBA Absent SBA	Infection (*Neisseria,* primarily meningococcal), 58%; CVD, 4%; both, 1%; healthy, 25%
C9	165	ACD	Impaired SBA	Healthy, 91%; infection, 9%
Plasma Proteins Regulating C′ Activation				
C1-INH	Many	AD; Acq	Uncontrolled generation of an inflammatory mediator on C′ activation	Hereditary angioedema
Factor H	25	ACD	Uncontrolled alternative-pathway activation→low C3	CVD, 40%; CVD plus infection (encapsulated bacteria), 40%; MPGN; healthy, 20%
Factor I	30	ACD	Uncontrolled alternative-pathway activation→low C3	Infection (encapsulated bacteria), 100%
Membrane Proteins Regulating C′ Activation				
Membrane cofactor protein (CD46)	9	ACD/AD	Uncontrolled alternative-pathway amplification on host cells	aHUS
Decay-accelerating factor (CD55) Homologous restriction factor (CD59)	Many	Acq	Impaired regulation of C3b and C8 deposited on host red blood cells, polymorphonuclear neutrophils, platelets→cell lysis	Paroxysmal nocturnal hemoglobinuria
CR3	>20	ACD	Impaired PMN adhesive functions (i.e., margination), chemotaxis, iC3b-mediated opsonophagocytosis	Infection (*Staphylococcus aureus, Pseudomonas* spp.), 100%

TABLE 9.3 Complement Deficiency States—cont'd

COMPONENT	NO. OF REPORTED PATIENTS (APPROXIMATE)	MODE OF INHERITANCE	FUNCTIONAL DEFECTS	DISEASE ASSOCIATIONS
Autoantibodies				
C3 nephritic factor	>59	Acq	Stabilizes alternative-pathway C3 convertase→low C3	MPGN, 41%; PLD, 25%; infection (encapsulated bacteria), 16%; MPGN plus PLD, 10%; PLD plus infection, 5%; MPGN plus PLD plus infection, 3%; MPGN plus infection, 2%
C4 nephritic factor		Acq	Stabilizes classical-pathway C3 convertase→low C3	Glomerulonephritis, 50%; CVD, 50%

ACD, Autosomal codominant; *Acq,* acquired; *AD,* autosomal dominant; *aHUS,* atypical hemolytic-uremic syndrome; *C',* complement; *C1-INH,* C1 inhibitor; *CVD,* collagen vascular disease; *iC3b,* inactive C3b; *MASP,* mannose-binding lectin–associated serine protease; *MBL,* mannose-binding lectin; *MPGN,* membranoproliferative glomerulonephritis; *PLD,* partial lipodystrophy; *PMN,* polymorphonuclear neutrophils; *SBA,* serum bactericidal activity; *XL,* X-linked.
Data from Ross SC, Densen P. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. Medicine (Baltimore). *1984;63:243–273; and Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies.* Clin Microbiol Rev. *1991;4:359–395.*

process itself. Nonetheless, autoantibody to C1q impairs complement consumption via the classical pathway, potentially leading to further impairment in the clearance of apoptotic cells, which in turn may reinforce the SLE disease process.[223]

Patients with high concentrations of antibody to C1q may also develop hypocomplementemic urticarial vasculitis. Clinically, these patients are often young females who present with chronic urticaria and associated leukocytoclastic vasculitis. Although called urticaria, the rash is nonpruritic and persistent. They may also develop angioedema, airway obstruction, glomerulonephritis, arthralgia, and neuropathy. Their laboratory findings are characterized by profound hypocomplementemia (as measured by total hemolytic complement [CH_{50}]) and marked depression of C1q, C4, and C2 concentrations, and a moderate reduction in C3.[223] Normal levels of C1 inhibitor distinguish this disease from hereditary angioedema. These individuals characteristically lack serologic markers for SLE, such as antinuclear antibodies or anti–double-stranded DNA (dsDNA) antibodies. Histologically, the skin lesions show perivasculitis or a leukocytoclastic vasculitis.

Approximately one-third of patients with SLE develop antibodies to phospholipids, again presumably as a result of the exposure of these molecules in apoptotic cell blebs. These patients may present with recurrent thrombotic events or spontaneous abortion—the clinical hallmarks of the antiphospholipid syndrome. Fetal wastage in these patients has been shown to be causally related to both placental infarction and massive complement consumption in the fetus. The factors that determine which patients with SLE develop which autoantibodies (e.g., anti-C1q, antiphospholipids) are unknown.[223,224]

In addition to altered apoptotic cell elimination, complement-deficient patients with SLE display several distinct abnormalities in immune complex clearance that have been documented by elegant in vivo experiments.[225–228] These abnormalities include (1) increased size of immune complexes, consistent with the key role of C1q in inhibiting complex formation (see earlier discussion); (2) decreased numbers of CR1 on erythrocytes, consistent with their increased removal in association with immune complexes during passage through the spleen[229]; (3) an increased proportion of immune complexes circulating unbound in plasma rather than bound to red blood cells, consistent with both less C3 bound to the complexes and fewer CR1 on erythrocytes; and (4) altered rate and pattern of immune complex clearance by the liver and spleen—a greater number of complexes being removed more rapidly by the liver, with a portion of these being released back into the circulation after a short delay.[131,132] The altered clearance reflects the greater proportion of complexes circulating in an unbound state and their uptake by IgG receptors on hepatic macrophages. A portion of these receptors exhibit relatively low affinity for IgG and release the complexes over time. In contrast, splenic removal of immune complexes is almost totally C3 and CR1 dependent. Each of these abnormalities, with the exception of CR1 number, is reversed by replacement of the missing complement component,[230] and reduction in the number of circulating immune complexes as a consequence of effective treatment for SLE is associated with an increase in the number of CR1 on circulating erythrocytes. Studies in genetically engineered mice suggest that tissue injury reflects a greater role for events consequent to the engagement of immune globulin receptors and also for the phlogistic activities of complement anaphylatoxins, especially C5a.[223]

Infectious Diseases

The relatively low frequency of infection (20%) in persons with a deficiency of C1, C4, or C2, compared with other component deficiencies (see Table 9.3), has been attributed to the presence of an intact alternative pathway in these patients. Bacterial infection, when it occurs, is usually caused by encapsulated bacteria, especially *S. pneumoniae,* and may be recurrent. The most common sites of infection are the sinopulmonary tree, meninges, and blood.[208,209]

Molecular Aspects

C1q is the product of three separate genes (*A, B,* and *C;* see Table 9.1). Mutations in each of these genes have been associated with C1q deficiency. A predominant mutation has not yet emerged from the few patients whose defect has been characterized, nor is there any apparent difference in the clinical picture found among persons with these various defects.[231]

C2 deficiency is probably the most common of all the total complement component deficiencies. It occurs predominantly in white persons of northern European extraction and is inherited in association with a distinct haplotype. The molecular basis for this defect in more than 90% of the cases (type I C2 deficiency) is a 28–base-pair gene deletion that causes skipping of exon 6 during messenger RNA (mRNA) splicing. Exon skipping in turn results in the generation of a premature stop codon and the synthesis of a nonfunctional protein.[232,233] The remainder of cases (type II C2 deficiency) result from a point mutation that encodes a dysfunctional polypeptide that is retained intracellularly.

The predominant molecular basis for C4A and C4B deficiencies involves large deletions that encompass both the respective C4 and associated 21-hydroxylase genes.[217] A 2–base-pair insertion in exon 29 of the *C4A* gene has been reported in association with the haplotype HLA-B60 DR6.[234]

Lectin-Pathway Deficiencies

Deficiency of Mannose-Binding Protein

Clinical Aspects

In 1976, a group of children with recurrent infection and failure to thrive were described; their serum failed to opsonize *Saccharomyces cerevisiae.* This defect was subsequently found in 5% to 7% of the general population. In 1989, MBL deficiency was identified in a substantial proportion of these patients.[235] Complete deficiency of MBL has not been reported; genetic variations that result in low protein levels (see later) are reported as MBL deficiency in the literature. Although the association with infection has been best documented in children, it spans the entire age range and has been confirmed in multiple ethnic

populations. One study that used genetic techniques found MBL deficiency in 42% of 345 children admitted with infection to one hospital, compared with 24% of 272 children admitted to the same hospital for other reasons. The prevalence of an abnormal *MBL* gene among infected children was almost twice that among uninfected children (23% vs. 13%), and homozygous deficiency was strikingly prevalent (3%) in the entire group of hospitalized children. In this study, affected patients with MBL deficiency presented with a wide variety of infections caused by a broad range of organisms. Common diagnoses included sinopulmonary infection (31%), meningococcal disease (13%), and fever of unknown origin (10%). The basis for the incomplete penetrance of infection among affected individuals with MBL deficiency is unknown but most likely reflects polymorphisms in other genes, in addition to environmental effects.[236,237]

Molecular Aspects

MBL is encoded by a single gene that contains four exons. The first exon encodes the signal peptide and the collagen-like region, the second exon encodes the remainder of the collagen region, the third encodes the "neck" region, and the fourth encodes the carbohydrate recognition domain. The three known MBL deficiencies are caused by mutations clustered in the first exon. Each of these point mutations results in an amino-acid substitution that interferes with oligomerization of three single chains to form the mature protein, and each is associated with reduced serum concentrations of MBL.[6,36]

In addition to mutations in the coding portion of the gene, three polymorphic sites are found in the promoter region of the gene: H/L, X/Y, and P/Q. Four of the total possible polymorphic combinations (LXP, LYP, LYQ, and HYP) account for most of the observed promoter haplotypes. These polymorphisms affect transcription of the gene through alterations in the binding of transcriptional factors, with LXP being associated with the lowest MBL serum concentration and HYP with the highest. These polymorphisms also exist in linkage disequilibrium with the three structural mutations in exon 1 to create haplotypes that differ markedly in their frequency among various populations. Therefore, MBL serum concentrations reflect the aggregate effects of promoter polymorphisms, structural gene mutations, and interaction between these two factors. Low MBL concentrations are associated with an increased risk of pyogenic infection, as noted earlier; however, they may protect against mycobacterial infection. Conversely, high MBL serum concentrations may increase the risk of mycobacterial infection.[6,36]

Deficiency of Ficolin-3

A case of complete ficolin-3 was reported in a 32-year-old man with a history of repeated lower respiratory tract infections resulting in bronchiectasis, recurrent digital warts, and bilateral frontal cerebral abscesses caused by nonhemolytic streptococci. The defect was a deletion at position 1637 of the *FCN3* gene (*FCN3+1637delC*) that resulted in a missense mutation. About 1.1% of healthy whites are heterozygous for this mutation in *FCN3* and do not appear to be at an increased risk of infections.[238] Ficolin-3 deficiency appears to be associated with necrotizing enterocolitis (NEC) among preterm infants.[239] A case of total ficolin-3 deficiency was reported in a preterm infant with group B streptococcal infection.[240]

Deficiencies of Collectin 11 (CL-K1) and MASP-1

Clinical Aspects

Deficiencies of both proteins are considered together because of their association with the 3MC syndrome, a term used to unify four overlapping rare autosomal recessive disorders: Mingarelli, Malpuech, Michels, and Carnevale syndromes. The 3MC syndrome is characterized by developmental abnormalities, including characteristic facial dysmorphism (high-arched eyebrows, ptosis, asymmetrical skull as a result of cranial synostosis, cleft lip and/or palate, and downturned mouth); learning disability; and genital, limb, and vesicorenal anomalies. Elegant gene-knockdown studies in zebrafish embryos showed that collectin 11 and MASP-1 both served to guide migration of neural crest cells during development.[241] The reported cases of 3MC syndrome have not been associated with an increased risk of infections. The serum of a 9-year-old girl with a nonsense mutation in *MASP1* and with clinical features consistent with the 3MC syndrome showed a nonfunctional lectin pathway; an episode of severe urinary tract infection was the only infection reported in this patient.[37] In contrast to this MASP-1–deficient individual who had a functional alternative pathway, *Masp1* knockout mice could not cleave pro–factor D to factor D and therefore lacked alternative-pathway function.[242] The reasons for the apparent differences in the outcomes of MASP-1 deficiency in mice and humans are unclear but could be the result of additional or alternative mechanisms of pro–factor D cleavage in humans.

Molecular Aspects

All individuals with clinical features of the 3MC syndrome have homozygous mutations of either *COLEC11* or *MASP1*. *COLEC11* mutations include deletions of exons 1 through 3, frameshift mutations in exon 2 (encodes the N-terminal domain) or exon 6 (neck domain), or missense mutations in exon 8 (carbohydrate recognition domain). The *MASP1* defects were all missense mutations in regions encoding for the serine protease domain. One family harbored a missense mutation in a *MASP1* exon that uniquely splices to *MASP3* (2059G→A, leading to 687G→R).

Deficiency of Mannose-Binding Protein–Associated Serine Protease 2 (MASP-2)

A single patient with MASP-2 deficiency has been described. This patient manifested ulcerative colitis at age 13, erythema multiforme bullosum at age 29, and three episodes of severe pneumococcal infection at 28 to 30 years of age. Severe hypocomplementemia and antibodies to C1q were documented at 35 years of age, along with low concentrations of MASP-2 and defective association between MBL and MASP-2. MASP deficiency in this patient was shown to be caused by a point mutation that resulted in the replacement of an aspartic acid residue with glycine. Recombinant MASP-2 protein bearing this amino-acid substitution displayed altered binding to MBL that mirrored what was observed in the patient's serum.[243]

Alternative-Pathway Deficiencies

Clinical Aspects

Inherited deficiencies of components of the alternative pathway appear to be less common than those of other complement proteins. To date, no individuals with homozygous factor B deficiency have been identified (see Table 9.3). In the presence of specific antibody, persons with alternative-pathway defects activate the classical and lectin pathways normally, but in the absence of specific antibody, a defect in the alternative pathway can lead to a significant impairment in complement activation and serum bactericidal activity. Infection in such persons might be expected to have dire consequences, a prediction supported by the poor outcome in patients with properdin deficiency, three quarters of whom develop meningococcal infection. Such infection is frequently characterized by a fulminant course and a high mortality rate (Table 9.4). Similarly, factor D deficiency is also associated with severe invasive meningococcal infection.[244,245]

Molecular Aspects

Three properdin-deficient variants have been described: type 1, characterized by extremely low concentrations (<0.1 μg/mL) of properdin and absent properdin function[246,247]; type 2, characterized by a low concentration (approximately 2 μg/mL) of antigenically detectable but functionally altered properdin; and type 3, characterized by a normal concentration (approximately 25 μg/mL) of antigenically detectable properdin but absent function.[248–250] Type 1 deficiency stems from various mutations, all of which result in premature stop-codon formation and the production of truncated proteins, which are presumably neither functional nor secreted.[251] Type 2 deficiency arises from mutations leading to amino-acid substitutions that may affect molecular charge. The altered molecules are secreted normally but appear to have an accelerated rate of extracellular catabolism.[251] Type 3 deficiency results from an amino-acid substitution that affects neither the secretion of the molecule nor its extracellular catabolism. Instead, folding of the molecule seems to be altered, and

TABLE 9.4 Comparison of Meningococcal Disease in Persons Deficient Versus Nondeficient in Late Complement Components or Properdin

CHARACTERISTICS	NONDEFICIENT	DEFICIENT IN C5, C6, C7, OR C8	DEFICIENT IN C9	PROPERDIN DEFICIENT[a]
No. of homozygotes	—	250	165	54–70
No. with meningococcal disease	—	146	15	25–37
Frequency of infection (%)	0.0072	58	9.1	46–53
Median age at first episode (yr)	3	17	16	14–11.5
Recurrence rate (%)	0.34	44	0	2–1.4
Relapse rate (%)	0.6	7.9	0	0
Mortality per 100 episodes (%)	19	1.5	0	12–51.4
Infecting serogroup				
No. of isolates	3184	67	2	16
B (%)	50	19.4	50	18.7
Y (%)	4.4	32.8	0	37.5

[a]If a range is given, the first number refers to documented cases, and the second number refers to documented plus probable and possible cases.
From Densen P. Human complement deficiency states and infection. In: Whaley K, Loos M, Weiler JM, eds. Complement in Health and Disease. *Dordrecht, The Netherlands: Kluwer Academic Publishers; 1993:173–197.*

this appears to lead indirectly to a decreased affinity of properdin for C3b.[252]

Mutations in *CFD* (gene encoding factor D) include (1) mutation of the codon for Ser42 that resulted in a premature stop codon in a Dutch family[245] and (2) two simultaneous homozygous mutations (Val213Gly and Cys214Arg) in a Turkish family, which resulted in undetectable factor D levels.[253]

C3 Deficiency

Clinical Aspects

Primary C3 deficiency is uncommon (see Table 9.3). As might be expected from the central position of C3 in the complement cascade and its key functions, virtually all persons with this defect are seriously ill.[208,209] Approximately three-quarters develop SLE or a related rheumatologic syndrome. Moreover, the inability to use either the classical or the alternative pathway results in a multitude of severe defects in host defense, including impairments in opsonization, immune response, and neutrophil chemotaxis and an inability to generate serum bactericidal activity. Consequently, severe and recurrent pneumococcal, *H. influenzae,* and meningococcal infections involving the sinopulmonary tree, meninges, and bloodstream are common, occurring in about 70% of such patients.[208,209]

Secondary C3 deficiency is observed in patients with an inherited absence of either factor H or factor I because of unregulated alternative-pathway activation leading to C3 consumption and low serum C3 concentrations (<10%). Autoantibodies to C3 (C3 nephritic factor, or C3 nef) or to factor H also lead to low C3 concentrations via a similar mechanism. These patients exhibit varying degrees of susceptibility to infection, especially to meningococcal disease, as a consequence of impaired host defense mechanisms. In addition, some patients with C3 nef develop partial lipodystrophy, a disorder characterized by the gradual onset of bilateral and symmetrical loss of subcutaneous fat from the face, neck, upper extremities, thorax, and abdomen, in a "cephalocaudal" sequence, and which spares the lower extremities (see Table 9.3).[254] C3 nef is seen in more than 80% of cases of partial lipodystrophy.[255] Complement activation may target adipose tissue because fat cells are a major reservoir of factor D (also known as adipsin), the activating enzyme of the alternative pathway. It is worth noting that about 30% of cases of partial lipodystrophy are associated with membranoproliferative glomerulonephritis type II (MPGN II) (discussed later), further supporting a common link in their pathogenesis.

Molecular Aspects

Primary C3 deficiency is uncommon, but cases have been recognized in ethnically diverse populations. The molecular basis for this deficiency arises from two different splicing defects: one causing a substantial deletion and the other a single base-pair change that leads to a defect in C3 secretion.[256]

Late Complement Component Deficiencies

Clinical Aspects

People who have a deficiency of one of the terminal complement components exhibit a striking susceptibility to systemic neisserial infections, especially meningococcal disease. Despite the chemotactic defect associated with C5 deficiency, the clinical manifestations of meningococcal disease in people with this defect and in those with other terminal-component deficiencies are remarkably similar.[211] Therefore the basis for the occurrence of meningococcal disease in these persons appears to be their inability to assemble the MAC and express complement-dependent bactericidal activity.

This conclusion was supported by detailed population-based epidemiologic studies that demonstrated an approximate 5000-fold increased risk of meningococcal disease in C7-deficient compared with complement-sufficient Japanese patients. In contrast, C9-deficient Japanese patients experienced an approximate 700-fold increase in risk.[257] The increased risk of meningococcal disease in persons deficient in C5, C6, C7, or C8, compared with those deficient in C9, is consistent with in vitro studies demonstrating that C9-deficient sera can kill meningococci, albeit at a slower rate.[113] This dose-response relationship, coupled with the fact that the structural genes that encode these proteins are located on multiple chromosomes, provides strong evidence for a cause-and-effect relationship between the absence of complement-dependent bactericidal activity and the increased susceptibility of these patients to meningococcal disease.

Meningococcal Disease in Complement Deficiency

Meningococcal disease is the single most common infection sustained by persons with complement deficiency, accounting for 75% to 85% of etiologically identified infections.[208,209] Although meningococcal disease has been reported in people with a deficiency of any of the plasma complement proteins, it is most common in those with a deficiency of properdin, C5, C6, C7, or C8, of whom 50% to 60% experience at least one episode of infection during their lifetime. This striking association confirms the importance of the complement system in host defense against meningococci.

Meningococcal disease in patients with these complement deficiencies exhibits several unique features that help to distinguish it from that in complement-sufficient persons (see Table 9.4). These features provide important clinical clues that should suggest the underlying presence of a deficiency and the need to screen for a complement deficiency state. These features are unlikely to result solely from ascertainment bias

for the following reasons. First, they have been confirmed in multiple studies in varied populations around the world. Second, each feature has been borne out by investigations of complement-deficient families after exclusion of the proband from the analysis. Third, at least in the case of the late complement component deficiencies, family studies have failed to reveal undiagnosed infection or unexplained or premature death.

Data compiled from the literature and from a detailed population-based study suggest that these complement deficiency states increase the risk of meningococcal disease 5000- to 10,000-fold. In the general population, the median age at onset of meningococcal infection is 3 years, and 56% of infections occur before 5 years of age; in contrast, the median age of first infection in complement-deficient patients is 17 years, and only 10% of infections occur before 5 years of age. Therefore, most deficient persons pass through the age of life when the deficiency might be expected to increase maximally their susceptibility to meningococcal disease without evidence of that susceptibility. The basis for this observation is unknown, but it likely relates to the relatively greater importance of specific antibody in protection against this infection. As specific antibody develops during childhood as a result of asymptomatic carriage, the contribution of complement deficiency states as a risk factor for systemic meningococcal disease increases relative to the contribution of antibody. Consequently, more cases of complement deficiency are detected among older individuals with meningococcal disease.

Meningococcal disease in these patients is caused by uncommon serogroups—particularly groups Y, W135, and X—more often than in persons without complement deficiencies.[208,209,258] Likewise, the prevalence of complement deficiencies is increased among patients with meningococcal disease caused by these serogroups.[259] The physiologic basis for this observation is not known with certainty, but factors that may be important include (1) the critical requirement for anticapsular antibody for prevention of disease in deficient compared with nondeficient persons, (2) better elimination of group B isolates by phagocytic cells in the absence of capsular antibody,[260] and (3) the propensity for uncommon meningococcal serogroup organisms to cause disease in older persons.[261]

Recurrent meningococcal disease, defined as a new infection that occurs more than 1 month after a previous episode, occurs in about 40% to 45% of persons deficient in C5, C6, C7, or C8. This recurrence rate is approximately 100 to 150 times greater than that in the general population. Results of a statistical analysis of the number of patients with a specified number of episodes of meningococcal disease were consistent with the interpretation that the risk of meningococcal disease in complement-deficient persons is independent of previous infection.[209] Previous disease did not reduce the risk of subsequent meningococcal infection in these patients; the estimated probability of each infection was 0.39.[209] A similar analysis, using a slightly different statistical approach, led to an identical conclusion and produced an estimated probability of infection of 0.6.[262,263] The latter analysis also demonstrated that the interval between infections (4–5 years) did not differ, again suggesting that the risk of subsequent meningococcal infection was not reduced by previous episodes of disease. The explanation for the failure of previous infection to reduce the risk of subsequent episodes of meningococcal disease in these complement-deficient persons appears to lie in their critical dependence on capsular antibodies for protection and the fact that infection constitutes a relatively poor stimulus for production of these antibodies. These antibodies are highly efficient in promoting opsonophagocytic elimination of meningococci. In contrast, antibodies to subcapsular antigens, although bactericidal and protective in the nondeficient host, are relatively poor opsonins and afford little protection in complement-deficient patients who lack the effector proteins necessary for the expression of bactericidal activity.[264]

Relapse of meningococcal disease, defined as infection with the same serogroup occurring less than 1 month after the initial infection, is observed in 7.6% of patients with meningococcal disease and who are deficient in C5 through C8, as reported in the literature. This frequency, which is approximately 10 times greater than in the general population, suggests that meningococci may be sequestered intracellularly, where they are relatively protected from antibiotics.[209]

One of the most striking aspects of meningococcal disease in persons with late complement component deficiencies is that, despite a several thousand–fold increase in the risk of infection, they experience a 5- to 10-fold reduction in the chance of dying from the disease, compared with healthy persons.[209] Therefore the same defect that predisposes to infection appears to provide protection from the lethal consequences of the disease. This remarkable observation suggests that the host's exuberant response to the organism is as much responsible for the clinical manifestations and outcome as is the organism itself. This deduction is supported by the report of Brandtzaeg and coworkers[265] of a close correlation between the extent of complement activation and mortality in meningococcal disease and suggests that the latter is in part dependent on the assembly of the MAC.

The basis for the lower mortality rate of meningococcal disease in persons who are deficient in late complement components is unknown, but variables that may be relevant include milder disease,[262] the possibility that fewer organisms are required to initiate infection, and the ability to better tolerate a given endotoxin load with less host cell injury.

The possibility that fewer organisms may be required to establish systemic meningococcal disease in deficient than in nondeficient persons is attractive, but data that address this point are conspicuously absent in the literature. Such an effect would account for the increased number of infections and also for the milder disease and the decreased case-fatality rate, in that mortality is directly related to the number of organisms in the bloodstream.[266] A reduction in organism load might translate into a lower concentration of circulating endotoxin and less systemic inflammation. Alternatively, because insertion of the MAC into the outer membrane of gram-negative organisms results in release of free endotoxin, the inability of persons with late complement component deficiencies to assemble the MAC may be associated with a reduction in the quantity of circulating endotoxin for a given load of organisms. This reduction, in turn, might lessen ongoing complement activation and decrease secretion of various cytokines linked to the development of septic shock in meningococcal disease.[265,267,268] Finally, insertion of the MAC into host cell membranes might occur in vivo as a consequence of exuberant complement activation in the vicinity of innocent bystander cells or as a consequence of endotoxin binding to these cells and subsequent complement activation on their surfaces. Such an event might stimulate adverse cellular responses. For example, MAC insertion activates leukocytes, stimulating release of a plethora of potentially noxious mediators[269] and increased expression of procoagulant molecules on endothelial cells.[270] Interruption of these processes in the patient with a late complement component deficiency would result in an improved ability to tolerate a given load of organisms and endotoxin.

Molecular Aspects

The basis for C5 deficiency is molecularly heterogeneous, with multiple but different defects in whites and African Americans. In one study, all the African-American patients were compound heterozygotes who possessed nonsense mutations in exons 1 and 36.[271] Of 109 patients diagnosed with meningococcal disease in the Western Cape region of South Africa, 3 (2.8%) were homozygous for the A252T mutation in the *C5* gene and displayed very low (1%–2% of normal) circulating C5 levels; all three individuals were of native African descent.[272]

Worldwide, complete C6 deficiency is molecularly diffuse. This deficiency is particularly common among native Africans and individuals of mixed ethnicities from the Western Cape in South Africa.[273] Several distinct types of C6 deficiency have been described, including complete deficiency and two different subtotal deficiencies (SDs), one of which occurs in association with a C7 SD. The structural genes for C6 and C7 are tightly linked on chromosome 5 (see Table 9.1). Combined C6/C7 SD states represent novel combinations of the *C6* SD allele with a *C7* SD allele and various *C6* and *C7* null alleles. These partial deficiencies typically come to clinical attention when some initiating event generates production of the C5b6 complex, which in turn converts the partial deficiency into a total one. Persons in whom the two SD alleles are shared in combination with a *C6* null allele are functionally *C6* SD. Their serum contains a near-normal concentration of C7, despite the presence of the *C7* SD allele, because the markedly reduced C6 activity is insufficient to lead to consumption of the existing C7. Complement consumption converts the subtotal state to complete C6 deficiency. Similarly, persons in whom the *C6* SD allele is paired with two different

C7 SD alleles are functionally C7 deficient, despite the expression, albeit reduced, of functional C7, because the C5b6 complex consumes the C7.[274,275]

Three genes (see Table 9.1) encode the C8 molecule, but defects have been reported in only the *A* and *B* genes. C8β deficiency has been reported predominantly in whites, especially those of Russian descent. Ninety-five percent of all the C8β null alleles characterized to date have been caused by cytosine to thymine (C→T) transitions, including 85% in exon 9 and 10% in other locations. It is unclear why C→T transitions should be such a common underlying mechanism for this deficiency.[276]

C9 deficiency is particularly common in the Japanese population. In this population, a C→T transition in exon 4 that converts an arginine codon to a stop codon is the dominant mutation associated with this deficiency.[277]

Deficiencies of Complement Regulatory Proteins

Hereditary Angioedema: C1 Inhibitor Deficiency

People who lack C1-INH present with a distinctive clinical picture historically referred to as hereditary angioneurotic edema (HAE).[278] The hereditary form of this disease was recognized more than 100 years ago, and an acquired variant was identified more recently as a distinct entity. HAE is an autosomal dominant disorder, but about 20% of newly identified patients lack a positive family history and reflect spontaneous mutations. Type 1 HAE accounts for 75% to 85% of cases and is characterized by the presence of low (5%–30%) levels of normal C1-INH protein arising from the intact allele. Type 2 HAE is characterized by the presence of normal to increased levels of antigenic C1-INH, representing a mixture of functional and dysfunctional gene products.[64,278,279] In a recently described third form of HAE, the serum from affected individuals contains normal amounts of functional C1-INH. Most of these patients have a gain-of-function mutation in the coagulation factor XII (Hageman factor) gene that may lead to enhanced bradykinin generation during contact activation (see later discussion).[279]

The acquired forms of this disorder (AAE) are considerably less common. Historically, two variants have been recognized; one occurs in association with B-lymphocyte disorders, and the other because of the presence of an autoantibody to C1-INH. Studies have suggested that this distinction may be inaccurate and that autoantibodies may account for both types of AAE. Antibody binding does not interfere with cleavage of C1-INH by C1s, but rather prevents the formation of a covalent linkage between the enzyme and the cleaved inhibitor. This alteration effectively converts C1-INH from an inhibitor to a substrate and permits C1s action to continue unchecked. This leads to complement consumption in the fluid phase and associated low levels of C1s, C1r, C4, and C2 that are the hallmark of the disease.[280,281]

Because HAE is inherited as an autosomal dominant trait, the serum from all of the patients contains some normally functioning C1-INH.[64] In contrast, persons with the acquired variants have markedly reduced or absent functional C1-INH activity in their serum. As a consequence of this basic difference, the serum from patients with HAE contains normal amounts of C1 and C1q but reduced levels of C4 and C2, whereas serum from those with AAE contains strikingly reduced amounts of C1, C1q, C4, and C2.[64,139,279]

The health of patients with HAE or AAE is punctuated by attacks of nonpitting, nonpruritic, and nonpainful edema of the extremities, face, or larynx. Angioedema of the larynx is the most severe complication of the disorder and is a common cause of death in these patients. The gastrointestinal tract may also be affected, and such attacks manifest as episodes of acute, crampy abdominal pain frequently associated with nausea, vomiting, and occasionally diarrhea. In HAE, attacks typically begin in childhood, increase in frequency and worsen in severity during adolescence, increase during menstruation, are markedly reduced during pregnancy, and diminish gradually in the fifth and sixth decades of life. A typical attack lasts 2 to 3 days.[64,279]

The mechanism whereby C1-INH deficiency produces the clinical syndrome of angioedema is incompletely understood. Evidence supports a role for both complement-derived and contact system mediators. Intradermal injection of activated C1s leads to nonpainful, nonpruritic swelling in both humans and guinea pigs. This response does not occur if activated C1 is injected into C2-deficient humans or guinea pigs but is observed after injection into C3-deficient patients.[282] These data provide support for a C2-derived anaphylatoxin in the clinical picture of angioedema. However, in addition to its role as the sole inhibitor of C1 esterase, C1-INH is an important inhibitor, if not the major inhibitor, of factor XII and kallikrein. Plasma from patients with HAE exhibits an impaired ability to inactivate these mediators. Detailed studies of a particular family with dysfunctional C1-INH demonstrated that the molecule was defective in its ability to inhibit C1r and C1s but retained full ability to complex and inhibit kallikrein and factor XIIa. None of the 10 family members whose sera possessed this dysfunctional C1-INH had ever experienced an attack of angioedema.[283] These data support an important role for the contact system in the clinical picture of HAE. Together, these separate lines of inquiry suggest that symptoms probably result from the interaction of several factors within these cascade systems.[279]

As expected from the various C1-INH protein phenotypes, the genetic basis for HAE is heterogeneous. A substantial proportion of type 1 HAE defects are associated with mutations within short, interspersed nucleotide elements called *Alu* clusters. These mutations cause a variety of rearrangements that lead to deletions or duplications within the gene, impaired transcription, and reduced levels of specific mRNA and plasma concentrations of C1-INH. In contrast, type 2 HAE, which is associated with normal concentrations of a dysfunctional protein, is typically caused by point mutations. These mutations usually affect the arginine at the reactive center of the molecule or amino acids in its immediate vicinity. On occasion, mutations affect C1-INH glycosylation. In all instances, the mutation leads to synthesis of a protein with an altered ability to react with its substrates. The resulting altered catabolism is responsible for the normal or elevated concentration of plasma C1-INH.[284,285]

The treatment of HAE, especially of acute life-threatening attacks, is being revolutionized by the availability of purified C1-INH, which, in controlled trials, has initiated the resolution of attacks within 30 to 60 minutes after intravenous administration. Purified C1-INH has also been used prophylactically to successfully reduce the probability of an attack in deficient patients undergoing medical procedures likely to precipitate an acute attack of HAE. Because patients with HAE synthesize some normal C1-INH (see earlier discussion), they do not develop antibodies to purified C1-INH after its administration. Use of purified C1-INH has rapidly replaced the administration of fresh-frozen plasma for treatment of and prophylaxis against acute attacks of HAE; however, it has not yet been approved for use in the United States. Administration of purified C1-INH as long-term therapy for patients with HAE has not yet been reported. Treatment with impeded androgens, despite their significant side effects, to increase the biosynthesis of C1-INH continues to be the mainstay of long-term management of the hereditary form of this disease.[64,278,279,286]

Factor H Deficiency

Factor H is the most abundant complement control protein and the third most abundant component overall (see Table 9.1).[161] As described earlier, factor H is a multifunctional, multiligand-binding protein whose binding specificity is determined by SCRs. SCRs 1 through 4 predominantly mediate binding to fluid-phase C3b and regulation of the alternative-pathway C3 convertase. SCRs 16 through 20 mediate binding to polyanions and C3b on cell surfaces and regulate convertase activity at those sites. Although these properties were determined experimentally, the delineation of their relevance to the pathophysiology of MPGN II, hemolytic-uremic syndrome (HUS), and AMD has underscored the critical importance of controlling C3 convertase formation, both in the fluid phase and on host cells, and the crucial role that factor H plays in this process. Strikingly different clinical consequences result depending on whether factor H is missing (quantitative defect: MPGN II) or exhibits altered function (qualitative defects: atypical HUS [aHUS], AMD).[70–72] The association of different disease states with abnormalities in individual segments of the factor H molecule provides convincing proof of the principle that specific domains within the molecule differentially contribute to its overall function.

Clinical Aspects: Membranoproliferative Glomerulonephritis Type II

Quantitative factor H deficiency is an autosomal recessive disorder characterized by low or absent factor H concentrations and low concentrations of C3. As described earlier, the secondary consumption of C3 that occurs in these individuals is associated with increased risk of infection. A number of these patients also develop MPGN II at an early age. This disorder is characterized by the deposition of substantial quantities of C3 within the glomerular basement membrane, associated membrane thickening, and impaired function. Because MPGN II is not a feature of patients with primary C3 deficiency, C3 deposition appears to be a critical pathologic event. The importance of C3 deposition in this pathophysiologic process is supported by the development of histologically typical MPGN II in factor H–deficient pigs and its prevention by infusion of factor H.[287,288] Most of these patients have mutations that result in truncated factor H molecules, the secretion of which is severely impaired.[72]

Clinical Aspects: Atypical Hemolytic-Uremic Syndrome

HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Most cases occur in young people who present with fever and Shiga toxin–positive diarrhea caused by *E. coli* O157:H7 or other Shiga toxin–producing strains, for instance, O104:H4. A small percentage of HUS cases occur in patients without diarrhea (aHUS), and some of these exhibit familial clustering consistent with an autosomal dominant pattern of inheritance.[72] Investigation of these patients has led to the identification of persons who have normal levels of complement and factor H but whose factor H bears mutations that affect its function. A striking majority of these mutations are clustered in SCR 20, which is exposed in the native molecule and mediates binding to cell-surface polyanions and C3b. Factor H from these individuals is fully capable of regulating C3 convertase formation and expressing cofactor activity in the fluid phase, consistent with the functional integrity of SCRs 1 through 4, but it binds poorly to C3b- or heparin-coated surfaces and to cultured endothelial cells. Consequently, factor H from these individuals is unable to regulate C3 convertases on cell surfaces.[70–72,289,290]

A family of SCR-containing proteins that bear varying degrees of homology to factor H, called factor H–related proteins (FHRs), have been identified over the past 2 decades. The genes coding for these proteins, in the order *CFHR3, CFHR1, CFHR4, CFHR2,* and *CFHR5,* are located 3′ to *CFH* (the gene encoding factor H). The FHRs are believed to modulate complement regulation by factor H through their interactions with surface-bound C3 fragments. CFH-CFHR1 (substitution of the three C-terminal SCRs of factor H with those of CFHR1) and CFH-CFHR3 (substitution of factor H domain 20 with CFHR3) hybrid molecules that arise from recombinational events result in loss of complement regulation at cell surfaces and the development of aHUS. Approximately 10% of all cases of aHUS are associated with function-impairing autoantibodies against factor H, and such individuals commonly harbor deletions of *CFHR1* and/or *CFHR3.* The aforementioned are only select examples of the genetic rearrangements and mutations in the CFH/CFHR locus that lead to the development of aHUS. The molecular basis for complement dysregulation that occurs as a result of these complex defects is only beginning to be unraveled.[291]

Further support for the importance of impaired regulation of the alternative-pathway C3 convertase on cell surfaces in the pathogenesis of the aHUS stems from reports of individuals who have mutations in the gene encoding MCP (also called CD46).[292,293] MCP/CD46 is a membrane homologue of factor H. Its expression is particularly robust on glomerular capillary endothelial cells. aHUS has also been reported in a few individuals with a mutation in factor I. Mutations in factor B and C3 have also been reported in aHUS, but in contrast to the loss-of-function mutations in factor H, MCP, and factor I, these result in gain of function.[70,290]

The renal histopathologic appearance in patients with Shiga toxin–induced HUS is indistinguishable from that in patients with aHUS. From the pathogenetic standpoint, these patients differ only in that the precipitating event is known in "typical" but not in "atypical" HUS. Because only about 15% of patients with Shiga toxin–associated diarrhea develop HUS, these observations suggest that minor variations in the expression levels or functional activity of any of the aforementioned proteins may constitute the substrate for development of HUS in the setting of specific vascular injury (e.g., that induced by Shiga toxin).[132] Renal failure is a significant consequence for patients with factor H–associated MGPN II or HUS. Therapeutic renal transplantation in such individuals is best reserved for those whose pathogenesis of disease is related to membrane complement regulatory proteins. Transplantation corrects the causative defect in such patients. In contrast, in patients whose disease is related to abnormalities in the plasma complement proteins, the defect will persist after transplantation, and renal disease will recur, often rapidly, in the transplanted kidney.

Clinical Aspects: Age-Related Macular Degeneration

Histologic studies and experimental observations have demonstrated that complement is a significant component of the drusen deposits that characterize AMD.[294] Linkage studies have demonstrated a strong association between AMD and polymorphisms in factor H (SCR 7), factor B, and C2.[295–299] For factor H, these studies established either a predisposing (402His) or a protective effect (402Tyr), depending on the specific polymorphism. Together, polymorphisms in factors H and B, and C2, are present in about 75% of patients with AMD, a remarkable association for a complex disease.[300] SCR 7 is an anion-binding site within factor H that exhibits relative binding specificity for heparin, CRP, DNA, and streptococcal M protein.

Factor H 402His showed lower affinity for streptococcal M proteins than factor H bearing Tyr at position 402, which promoted C3b deposition and phagocytosis of group A streptococci. Intriguingly, 402His homozygosity was less frequent in patients with a history of erysipelas or recurrent tonsillitis than in control subjects.[301] As such, it differs from the SCR 16-20 anion-binding site associated with aHUS, which demonstrates relative specificity for GAGs, heparin, and sialic acid.[70,71] Studies in mice have shown that factor H binds to malondialdehyde (MDA), a lipid peroxidation product that accumulates in AMD. Binding of factor H to MDA-modified proteins blocks their uptake by macrophages and reduces proinflammatory responses. In contrast, reduced binding of the 402His (predisposing) polymorphism to MDA-modified proteins results in relatively unimpeded uptake of these proteins by macrophages and a higher inflammatory response, thereby providing a molecular basis for the role of factor H in the pathogenesis of AMD.[302]

CD59 Deficiency: Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon syndrome that typically occurs in adults between the ages of 30 and 50 years. Classically, affected persons present with bouts of intravascular hemolysis that are worse at night and last for several days to weeks. The events precipitating hemolysis are usually inapparent. In contrast, the more common presentation, which occurs in about half of patients, is one of chronic hemolysis. Patients may have back pain, cramping abdominal pain, and headaches. Although the major clinical features of the disease relate to intravascular hemolysis, the full syndrome includes a propensity for venous thrombosis and diminished hematopoiesis. The thrombosis is characteristic but unusual in that it typically involves major intraabdominal and hepatic veins and is often precipitated by surgery.[229]

The basic problem in patients with PNH is an increased susceptibility of their erythrocytes to hemolysis. The peripheral blood of these patients contains varying proportions of three populations of erythrocytes. PNH type 1 red cells are normal, whereas type 2 cells exhibit a 3- to 6-fold increased sensitivity to complement-mediated lysis, and type 3 cells have a 15- to 25-fold increased sensitivity. The severity of the clinical picture correlates best with the proportion of PNH type 3 cells present in the peripheral circulation.[303]

PNH erythrocytes lack more than 20 different surface proteins. The presence of these molecules in normal amounts on endothelial cells in persons with PNH suggests the clonal origin of this disorder within bone marrow precursor cells. The key feature shared by these proteins

is their linkage to the cell membrane through a carboxyl-terminal glycosylphosphatidylinositol (GPI) anchor.[304,305] PNH arises as a result of a defect in the first step of the synthesis of the GPI anchor responsible for linking the missing surface proteins to the cell membrane. This step is catalyzed by an enzyme that transfers activated *N*-acetyl glucose to the phosphatidylinositol acceptor.[305] This enzyme is the product of the *PIGA* gene, and multiple mutations in this gene have been reported in association with PNH. Unlike most defects that affect synthetic pathways, these genetic abnormalities are expressed in a dominant fashion in progeny cells. This unusual characteristic arises because the *PIGA* gene is located on the X chromosome, and the somatic mutations in this gene arise after inactivation of one of the X chromosomes.[304]

Two of the proteins that are missing from the membranes of PNH cells are the complement regulatory proteins DAF and CD59.[306] The inability to regulate complement deposition on PNH cells explains the underlying pathophysiology of this disorder. People who have an inherited defect that affects expression of only DAF on their erythrocyte membranes do not exhibit the PNH phenotype, but those lacking solely CD59 do manifest the phenotype.[307,308] Therefore the absence of the CD59 molecule accounts directly for the increased susceptibility of PNH cells to intravascular hemolysis.

In the absence of CD59, many MACs are inserted into the platelet membrane, causing vesiculation and providing sites at which prothrombinase is generated, with resultant thrombin formation. Free hemoglobin released during hemolysis scavenges and reduces concentrations of nitric oxide (NO). Low NO concentrations, in turn, increase the susceptibility of platelets to complement-mediated activation.[309] These alterations may contribute to the susceptibility of patients with PNH to thrombosis. In addition, the absence of the GPI-linked urokinase receptor from PNH cells may render clots more resistant to dissolution, although neither of these abnormalities explains the propensity for intraabdominal thrombus formation. Similarly, the absence of the GPI-linked FcγRIII receptor from phagocytic cells may contribute to the slightly increased susceptibility of these patients to infection. Studies on the diminished hematopoiesis that occurs in patients with PNH have suggested that PNH cells do not possess a proliferative advantage within the marrow. However, in an abnormal marrow in which normal cells are at a survival disadvantage, PNH cells appear to be resistant to abnormal influences and emerge as the predominant cell type. The absence of a GPI-linked receptor (e.g., for a growth factor) has been postulated as the basis for this effect. Whether the factors that contribute to the development of an abnormal marrow environment are the same as those giving rise to the somatic mutation responsible for PNH is unknown.[304,305]

The development of a humanized monoclonal antibody to C5 (eculizumab) has provided highly specific, effective, and safe therapy for patients with PNH. A randomized, double-blind, placebo-controlled, multicenter trial clearly demonstrated a reduction in the degree of hemolysis and transfusion requirements for treated patients.[310,311] A subsequent report also documented a decrease in the frequency of thrombotic events.[312]

Patients receiving eculizumab have little or no functional complement activity for the first 2 to 3 weeks after administration of each dose. Like individuals with a deficiency of one of the terminal complement components, they demonstrate increased susceptibility to meningococcal infection (see earlier discussion). Therefore patients with PNH should receive a conjugate, tetravalent meningococcal vaccine several weeks before administration of the first dose of eculizumab. Conversely, intercedent inflammatory diseases may stimulate increased C5 biosynthesis, necessitating a shortening of the monoclonal antibody dosing interval or an increase in the dose at a fixed interval.

COMPLEMENT IN DISEASE STATES

The increasing availability of genetically engineered mice coupled with modern molecular techniques is leading to a progressively sophisticated understanding of the role of complement in various types of inflammation and in the pathogenesis of tissue injury and repair. Mediators released during complement activation may play a role in the development of symptoms or in the outcome of these disorders. Evidence that supports this suggestion includes the observations that (1) the extent of complement activation often parallels disease activity, (2) complement is deposited at the site of tissue injury, and (3) in animal models of these disorders, specific manipulation of complement activation modulates the course of disease. The role of complement has been studied most extensively in infectious diseases, rheumatologic disorders, and glomerulonephritis characterized by obvious inflammation. However, it has become increasingly clear that complement activation and mediator generation play important roles in such diverse entities as atherosclerosis,[313] restenosis, postperfusion injury,[314] demyelination disorders,[315] a variety of dermatoses,[316] and hyperacute graft rejection in xenogeneic transplantation.[317] Damage in these disorders is complement dependent—that is, it can be prevented by complement depletion or by infusion of proteins that regulate complement activation, such as soluble CR1 and CD46.[318] In the case of xenotransplantation, rejection can be prevented through the development of transgenic animals whose organs express the genes for human complement regulatory molecules.[319]

Infectious Diseases

Complement activation during infection is particularly impressive in diseases such as dengue fever, bacterial endocarditis, and sepsis, in which the organisms or their products react with antibodies to form circulating immune complexes and initiate complement consumption. It is especially striking in meningococcal disease and less so in other forms of gram-negative sepsis and septic shock. Studies have convincingly demonstrated a protective role for complement in endotoxic shock. Genetically engineered C3- and C4-deficient mice did not clear endotoxin as efficiently, had higher levels of TNF and IL-1, and had much higher mortality rates than did wild-type mice. C1-INH and fibrinogen levels were also lower in the deficient mice. Reconstitution of C1-INH significantly reduced mortality and restored fibrinogen concentrations to normal, despite the persistent deficiency of C3 or C4. Together, these findings indicate that endotoxin-containing immune complexes initiate complement consumption via the classical pathway and that failure to incorporate C3 into the immune complexes leads to deficient endotoxin clearance, ongoing complement consumption, and C1-INH depletion. The absence of C1-INH permits contact system activation, as manifested by fibrinogen consumption (a potential counterpart of disseminated intravascular coagulation in humans). The fact that C1-INH replacement protected the deficient animals and restored fibrinogen levels to normal strongly implicates the direct involvement of the contact system in endotoxic shock and death.[320] Later experiments demonstrated that the immune complexes initiating this sequence of events were composed of natural IgM antibody with specificity for the endotoxin O antigen.[321] In this respect, these results are reminiscent of those implicating natural antibody and complement consumption via the classical pathway in reperfusion injury.[314] A critical role for LPS in mediating hemodynamic instability and cytokine release was confirmed with use of meningococci in a porcine model of sepsis. In this model, an LPS-deficient mutant also caused "sepsis-like" physiology, although 10- to 20-fold higher challenge inocula were required, suggesting that molecules other than LPS could contribute to morbidity in sepsis in gram-negative bacteremia.[322]

Studies have implicated C5a as a key mediator in the development of septic shock and the acute respiratory distress syndrome in humans,[323] in a monkey model of gram-negative shock,[324] and in the cecal ligation puncture (CLP) model of sepsis in the rat. Of the two animal models, CLP most closely mimics sepsis in humans; affected rats, left untreated, die in 4 to 5 days.[325] Investigators who have used this model have demonstrated substantial C5a bound to neutrophils, which in turn exhibit a markedly suppressed oxidative burst. Treatment of the animals with anti-C5a antibody at the time of CLP reduces rat mortality by 50%, prevents neutrophil dysfunction, and is associated with a marked reduction in bacterial counts in the blood, liver, and spleen.[325] These studies also show that C5aR expression is upregulated in the lung, liver, kidney, and heart early in the course of sepsis and that blockade of this receptor with specific antibody not only improves survival but also reduces the serum concentrations of IL-6 and TNF-α.[326] Immune suppression is a newly recognized hallmark of sepsis in humans, evidenced by the depletion of lymphocytes in the white pulp of the spleen and by peripheral lymphocytopenia that is temporally associated with the multiorgan failure syndrome.[327] In the CLP model, immunodysregulation occurs as a result of caspase-dependent, NF-κB–independent apoptosis

of T lymphocytes in the context of decreased expression of Bcl-X_L. Lymphocyte apoptosis can be blocked by anti-C5a administration.[328] Whether this apoptosis-sparing effect is the result of direct inhibition of events downstream from the ligation of T-cell lymphocyte C5aR or a consequence of decreased levels of cytokines (e.g., IL-6) is unknown.[329]

Complement, in conjunction with the reticuloendothelial system, plays a critical role in the removal of encapsulated bacteria from the bloodstream.[330] Delineation of the contribution of these variables to the clearance process, which was accomplished in an animal model of pneumococcal bacteremia, demonstrated that the more virulent the organism, the greater the role of the spleen in performing this clearance function.[330] Complement depletion led to a significant decrease in the number of pneumococci needed to kill 50% of the animals, demonstrating an important role for complement in the clearance function. Investigations into the role of each of the specific pathways have yielded equivocal results. In one study, clearance of pneumococci was similar in healthy and in C4-deficient animals, indicating that complement activation and fixation to bacteria via the alternative pathway were particularly relevant in this process. Moreover, the presence of immune antibody shifted the burden of clearance from the spleen to the liver, and this effect was absolutely dependent on a functional alternative-complement pathway.[331] Increased mortality in both C1q and factor B knockout mice after intranasal inoculation of *S. pneumoniae* in another study provided evidence for a role of both the classical and alternative pathways in organism clearance.[22] More recently, mice deficient in ficolin, MASP-2, or SAP exhibited increased susceptibility to select pneumococcal isolates.[332–334] Thus the strain of bacteria, challenge dose, and route of inoculation used are all important considerations in drawing conclusions from data in experimental animal models.

Rheumatologic Disorders

Substantial clinical and experimental evidence links complement deficiency syndromes and complement activation to a variety of rheumatologic diseases, most notably SLE (see earlier discussion). Additional support for this relation is the finding that pharmacologic agents (e.g., hydralazine, isoniazid) associated with medication-induced SLE inactivate C4 by nucleophilic attack on its internal thioester and formation of amide bonds.[335] Evidence that complement activation may be associated with manifestations of the disease and with tissue injury includes the demonstration of C3 and immune complex deposition at the dermal-epidermal junction in cutaneous lesions from patients with SLE or discoid lupus erythematosus. Similar immunohistochemical alterations have been demonstrated in biopsy specimens of healthy skin from the same patients. However, the finding of MAC in areas of affected but not unaffected skin from these patients strengthens the hypothesis that complement activation may partially mediate tissue injury in these disorders.[315]

Incorporation of C3 into immune complexes promotes their binding to C3b receptors (CR1) on erythrocytes, as noted earlier. Erythrocyte CR1 is removed along with immune complexes during passage through the liver and spleen. The number of erythrocyte CR1 molecules is reduced in people with disorders such as SLE, which are characterized by circulating immune complexes.[229] The degree of CR1 reduction correlates well with disease activity and with the extent of complement activation. The decrease in CR1, coupled with the inability of circulating erythrocytes to resynthesize them, further exacerbates the defect in immune complex clearance, thereby promoting their deposition in the tissues, with resultant damage to the host. The number of erythrocyte CR1 molecules returns to the genetically prescribed level after the disease is controlled and erythrocyte numbers have returned to normal.

Renal Disorders

Complement deposition in renal diseases that are associated with immune disorders is related to the deposition of immune complexes within the kidney,[336–338] whereas complement deposition in the absence of immune complexes is postulated to occur through activation of the alternative pathway.[339] In a rat model of chronic tubulointerstitial disease, loss of renal mass and function was correlated with increased ammonia production and systemic acidosis. Under these conditions, peritubular deposition of C3 and the MAC was readily demonstrated. Deposition of these components and evidence of tubulointerstitial inflammation were markedly decreased in diseased animals treated with sodium bicarbonate. These and other findings indicate that ammonia attacks the C3 internal thioester to form amidated C3. Amidated C3 serves to activate the alternative-complement pathway in the fluid phase, leads to C3 and C5b-9 deposition in the tissue, and elicits an inflammatory response and tissue injury.[339,340]

Experiments using C6-sufficient and C6-deficient rabbits and the infusion of C8-deficient serum into rats clearly demonstrated that the development of proteinuria in membranous glomerulonephritis depends on the assembly and deposition of a complete MAC on the glomerular epithelial cells. A substantial portion of this injury results from MAC-mediated stimulation of prostaglandin and thromboxane synthesis because development of proteinuria was reduced by treatment with indomethacin, an inhibitor of cyclooxygenase.[341]

Many patients with chronic renal disease ultimately require hemodialysis. Exposure of plasma to first-use filter membranes during dialysis results in complement activation.[342] Anaphylatoxins released during this process (e.g., C5a) have been associated in a concentration-dependent and temporal fashion with the onset of respiratory distress in some dialysis patients.[323,342] This association is believed to relate in part to C5a-dependent neutrophil aggregation and stimulation and the formation of microemboli and their deposition in the lung (see Chapter 8).[323] Strategies to reduce complement activation either at the level of C3 or blockade of C5aR through use of small-molecule inhibitors (e.g., analogues of the C3 inhibitor compstatin) are currently being developed and may represent viable therapeutic strategies to reduce undesired complement activation during hemodialysis.

EVALUATION AND TREATMENT OF COMPLEMENT DISORDERS

Evaluation

Evaluation of the complement system is indicated when the diagnosis of a complement deficiency state is being considered or when specific measures of complement proteins are being used to assess disease activity or response to therapy. As pointed out earlier, several clinical clues should lead the clinician to suspect a complement deficiency state.[208,209] Foremost among these is a medical or family history of recurrent systemic infection caused by encapsulated bacteria, especially meningococci. A family history of fulminant meningococcal disease occurring in males in alternate generations should suggest the possibility of X-linked properdin deficiency. Meningococcal disease occurring in persons older than 10 years, especially when caused by non–group B meningococci, warrants evaluation of the complement system because 5% to 10% of these patients have a complement deficiency state, even in the absence of recurrent disease. Children with a history of recurrent respiratory infections beyond the first few years of life should prompt investigations of the classical and lectin pathways (MBL and ficolins) and an evaluation of IgG subclasses. Likewise, a history of SLE in family members or the occurrence of atypical features of SLE should also suggest the need for evaluation of the complement system. Specific syndromes, including angioedema, partial lipodystrophy, aHUS, AMD, and PNH, are other indications for assessment of complement status.

Any of a number of specific inherited complement deficiencies can produce one of the typical clinical syndromes associated with these disorders; hence, it is important that the initial test be one that measures the function of the *entire* complement cascade. The most common of these tests is the standard CH_{50}, which measures the function of the classical- and terminal-complement pathways. If possible, an initial assessment should also include an analogous test, the AH_{50}, which measures the function of the alternative- and terminal-complement pathways. Many hospital laboratories do not perform the latter test, so it may be necessary to contact a research or commercial laboratory with specific expertise in complement measurement. A negative or extremely low result in either of these two assays warrants further diagnostic evaluation.

The combined results of the tests of classical- and alternative-pathway functions dictate which additional tests need to be performed. If both the classical- and alternative-pathway CH_{50} values are extremely low, the defect must lie in one of the components shared by both pathways:

C3 through C9 (see Fig. 9.1). If the alternative pathway is normal but the classical pathway is not, the deficient component must be C1, C2, or C4. Conversely, a normal classical but defective alternative pathway suggests a defect in factor D, factor B, or properdin. Diagnosis of these specific defects can frequently be accomplished by the use of immunochemical methods to demonstrate an absence of the relevant antigen. However, several complement deficiency states involve absent function in the presence of normal amounts of antigenic protein. In addition, low levels of C3 can be encountered in factor H or factor I deficiency states. Therefore, confirmation of the diagnosis of a specific component deficiency should be documented with specific functional assays for the protein under consideration and by demonstration that replacement of the missing component restores both specific and total complement activity. Such assays require the expertise of a laboratory that specializes in complement function.

It should be noted that diseases such as aHUS and AMD represent dysregulation of complement and therefore may not be detected by most of the standard complement assays described earlier. Sequencing of one or more target complement genes may need to be carried out to detect mutations (or polymorphisms) associated with the disease. Advances in sequencing technologies, coupled with decreasing costs of sequencing, have prompted efforts to determine the "complotype" of individuals suspected to have disorders characterized by complement dysregulation. Polymorphisms that are associated with either gain or loss of function of individual complement proteins could interact and have a profound effect on overall activity of a pathway.[343] As an example, disease penetrance, severity, or the response to treatment, including the outcome of renal transplantation, depends on the gene(s) affected, as elucidated in a recent study of 745 individuals with aHUS.[344] However, interpretation of such data is not straightforward and requires the expertise of specialized laboratories. A better understanding of the fine balance of complement activation and inhibition may elucidate subtle differences in complement activation among individuals, that is, factors dictating susceptibility to various infections, in addition to the infections classically associated with complete deficiencies of complement components described earlier.

Acquired complement deficiencies can complicate the interpretation of complement disorders. Most acquired complement deficiency states occur in the setting of acute or chronic inflammatory disease and involve activation of the entire complement cascade. As a result, specific component testing typically demonstrates low levels of multiple complement proteins. In contrast, specific component testing in individuals with an inherited complement disorder (other than a defect in one of the complement regulatory proteins) typically demonstrates a low concentration of a single protein.[345] An important corollary of these general principles is that complement assessment can be optimized if testing is performed after the patient has recovered from any acute illness or after the disease has been treated successfully.

Treatment

Therapeutic issues related to specific complement deficiencies have already been described. Two general issues that arise frequently during formulation of a therapeutic approach for patients with complement deficiency states are replacement of the missing protein and prevention of infection.

At the present time, although advances in knowledge of the molecular basis of the various complement deficiency states are beginning to yield alternative means of therapy, replacement of deficient components usually requires the infusion of fresh-frozen plasma. This approach has been successfully used in therapy for acute attacks of angioedema (see earlier discussion),[64,278,279] in restoration of C3 levels toward normal in patients with C3 deficiency, and in treatment of a C2-deficient patient with SLE unresponsive to conventional therapy.[346,347] It has also been recommended as a possible therapeutic option for patients with aHUS or dysfunctional factor H. This approach has several potential limitations, First, the half-life of most complement proteins in vivo is short,[12] although a notable exception occurs in patients with low C3 levels caused by factor I deficiency. In these patients, replacement therapy restores factor I activity, thereby markedly reducing the accelerated breakdown of C3 that is observed in this disorder.[348] Second, replacement of a genetically absent protein may stimulate the production of antibody to the missing component, thereby limiting the value of subsequent therapy. This consideration is of limited concern in persons with autosomally inherited disorders such as hereditary angioedema, whose serum contains some normal protein, and in those with other complement deficiency disorders characterized by the presence of antigenically normal amounts of a dysfunctional protein (e.g., qualitative factor H deficiency). Third, the relative frequency of infection in most of these patients must be balanced against the potential risk of acquisition of any of a variety of bloodborne infections during plasma infusion, especially because alternative modes of therapy are often available. Whether the short-term infusion of fresh-frozen plasma might be beneficial in the treatment of life-threatening infection, especially in properdin-deficient patients, remains untested. Recombinant human MBL is currently being considered for use clinically and may address some of the limitations of fresh-frozen plasma.

The central role of complement activation in the pathogenesis of aHUS prompted the use of eculizumab (a humanized anti-C5 monoclonal antibody) in this condition. Amelioration of symptoms resulted in approval of eculizumab for use in aHUS in Europe and the United States in 2011. Eculizumab may also be useful in the prevention and treatment of recurrences of aHUS after renal transplantation.[349] Individuals with dense deposit disease (MPGN II) who have evidence of increased systemic complement activation (high C5b-9 levels) may also benefit from C5 blockade.[350–352] The clinical course of Shiga toxin–associated HUS was also attenuated after the use of eculizumab in three children.[353]

In an effort to develop a small-molecule inhibitor of complement at the level of C3, a 13-mer cyclic peptide called compstatin, which binds to the C3 and C3b and inhibits the action of C3 convertases on C3, was identified.[354,355] Although the long-term systemic use of C3 convertase–inhibiting compounds could predispose individuals to serious infections, short-term or tissue-specific inhibition of complement could be beneficial. As an example, intraocular instillation of a compstatin analogue has shown promise in the treatment of AMD in preclinical studies.[355,356] Despite strong data linking complement activation with dry AMD, intraocular administration of the Fab fragment of a factor D function-blocking monoclonal antibody (lampalizumab), did not halt progression of geographic atrophy in a phase III clinical trial. These data highlight the complex nature of disease pathology; addressing the predisposing or inciting event at later stages of the disease may not halt or reverse symptoms.

Prevention of infection in complement-deficient patients is best achieved through vaccination. All deficient persons should be vaccinated with a tetravalent conjugate meningococcal vaccine (Menactra [Sanofi Pasteur, Swiftwater, PA] or Menveo [GlaxoSmithKline]). Two recombinant protein vaccines (Bexsero and Trumenba) for group B meningococci are available in the United States and may be considered in complement deficiency, including eculizumab-treated patients. These vaccines are directed against meningococcal membrane proteins and therefore may also protect against nongroupable (unencapsulated) strains that have been associated with invasive disease in eculizumab recipients (see later). Antibody titers wane rapidly during the first year after vaccination, and no recommendations for revaccination are available (see Chapter 211). Those with classical-pathway deficiencies should also receive the polyvalent pneumococcal and conjugated *H. influenzae* vaccines. Given the low cost and high potential benefit of these vaccines, all three should probably be administered to any individual with complement deficiency. Conjugate vaccines, such as that for *H. influenzae,* which initiate a T-cell–dependent response, stimulate the production of higher antibody concentrations and their longer persistence, and induce immunologic memory. The elicitation of T-cell help by such vaccines circumvents the qualitative defect in antibody production observed in these patients[357]; therefore these vaccines are preferred if they are available.

Successful vaccination leads to the production of anticapsular antibodies that promote use of the classical pathway in patients with an alternative-pathway defect and facilitate alternative-pathway use in patients who lack one of the classical-pathway components.[247] In such patients, these antibodies may promote bactericidal activity and microbial elimination by enhancing opsonophagocytosis.

Neither clinical nor in vitro studies have explored the potential for vaccination to help protect C3-deficient persons from infection.

The theoretical basis for this approach lies in the ability of antibody alone to facilitate phagocytic elimination of organisms, albeit at a reduced rate of killing. This property is most relevant to the clearance of organisms from the bloodstream via the reticuloendothelial system, in which the structural architecture and lining of the sinusoids with tissue macrophages contribute greatly to surface phagocytosis. In view of the suboptimal response to protein and polysaccharide antigens in C1-, C2-, C4-, and C3-deficient humans and animals, documentation of the patient's response to vaccination with these antigens seems prudent.

Although anticapsular antibody cannot enhance serum bactericidal activity in persons with a deficiency of one of the terminal complement proteins, it promotes opsonization and killing of these organisms by phagocytic cells.[260] In vitro studies of prevaccination and postvaccination sera have documented the ability of such patients to respond to the tetravalent meningococcal vaccine and the likelihood that their response will facilitate phagocytic killing of the corresponding meningococci.[264,358,359] In addition, clinical investigations have demonstrated that vaccination reduces the frequency of infection from 0.15 to 0.04 episodes per year and prolongs the interval between infections from 3.6 to more than 6 years.[360,361] These studies, like those in other groups of complement-deficient individuals, attest to the usefulness of vaccination as an immunologic means to circumvent the major clinical manifestations of these deficiencies.

Of the terminal complement deficiencies, C5 deficiency presents a unique problem. Whole blood from a C5-deficient patient failed to kill a group B isolate of *Neisseria meningitidis* even in the presence of high titers of specific antibody that supported killing when pure C5 was added back.[362] The rate of meningococcal disease in eculizumab recipients despite meningococcal vaccination was 342 per 100,000 person years,[363] which represents an approximately 2000-fold higher incidence of disease than in the general population. Of the 16 reported cases of invasive meningococcal disease in eculizumab recipients between 2008 and 2016, 11 were caused by nongroupable (unencapsulated) strains, 4 were caused by group Y strains, and the identity of one isolate was not determined. A group B meningococcal vaccine (Bexsero), which targets protein antigens and was expected to provide protection against nongroupable isolates, was administered to 3 individuals. The addition of eculizumab to whole blood from 12 immunized individuals abrogated killing of meningococci in every instance, illustrating again the importance of C5 for opsonophagocytic efficacy of antimeningococcal vaccine antibody.[364] In contrast, C7 blockade permitted killing of meningococci by whole blood in a strain-dependent manner.[364] Lack of C5a generation differentiates C5 deficiency (and pharmacologic inhibition of C5 with eculizumab) from deficiencies of C6 through C9. Despite loss of C5a and the resulting impairment of neutrophil recruitment and function, there is no evidence to suggest that the clinical course of invasive meningococcal disease in C5-deficient persons differs from disease severity in other late complement component deficiencies. As discussed earlier, lack of MAC formation, a common feature of all terminal complement deficiencies, limits endotoxin release and may be the overriding factor that contributes to milder disease in these individuals. In light of these observations, meningococcal vaccines may not confer the expected protection in individuals on long-term treatment with C5 inhibitors such as eculizumab, necessitating constant and careful evaluation for invasive disease.

An alternative strategy for the prevention of meningococcal disease is the use of prophylactic antibiotics[365] or the administration of antibiotics for preemptive treatment at the onset of symptoms. The use of prophylactic antibiotics significantly reduces the frequency of infection in C6-deficient persons and has its greatest use in populations in which group B disease is highly prevalent.[366] Such a strategy may merit consideration in persons with C3 or C5 deficiency (either congenital or acquired), in whom vaccination may not provide maximal protection for reasons discussed earlier. It is unclear whether antibiotic prophylaxis should be lifelong or whether the development of antibiotic resistance will limit its efficacy.

ACKNOWLEDGMENT

The author and editors recognize and thank Dr. Peter Densen, who prepared this chapter and its antecedents for eight editions over 40 years, before turning it over completely to the next generation. We appreciate his career as a physician and scientist, and his work educating his colleagues all over the world about innate immunity and complement. Such dedication is at the bedrock of civilization, allowing knowledge to be transferred forward for posterity.

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10 Human Genetics and Infection

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SHORT VIEW SUMMARY

- This chapter summarizes the evidence for host genetic susceptibility to infection.
- Rapid technologic advances have brought about increasing use of high-throughput genome-wide approaches including microarray genotyping of 0.5 to 1 million polymorphisms, "whole-exome" sequencing of the 30 million bases of the protein-coding genome, and "whole-genome" sequencing of the 3 billion bases of the entire human genome.
- Genome-wide association studies using microarray genotyping technology have become increasingly dominant and continue to provide new insight into genetic susceptibility to infection. These studies use very stringent statistical thresholds to declare associations, meaning that the reported signals are substantially more robust and reproducible than in the candidate gene era.
- Substantial evidence has now accumulated to link variation in HbS, *ABO,* and *G6PD* to susceptibility to *Plasmodium falciparum* malaria. However, robust reappraisal of variants linked to malaria susceptibility in the candidate gene era has brought numerous previously reported associations into question, emphasizing the need for cautious interpretation of candidate gene literature more broadly.
- The HLA locus plays an important role in a number of infectious diseases, ranging from viral infection, including human immunodeficiency virus (HIV) and hepatitis B virus; bacterial diseases, including enteric fever, tuberculosis, and leprosy; and protozoal diseases, including visceral leishmaniasis.
- Evidence is also accumulating that host variation elsewhere in the genome affects susceptibility to a variety of infectious diseases. The growing list of robust non-HLA loci now includes, for example, *CFB* and *CD40* for persistence of hepatitis B virus, and *WT1* and *ASAP1* for tuberculosis, in addition to emerging findings including, for example, *STAT4* for nontyphoidal *Salmonella* bacteremia.
- The importance of robust study design is underscored by the recent finding that beyond the 32-bp deletion in *CCR5,* most of the previously reported associations with susceptibility to, as opposed to control of, HIV infection can be attributed to frailty bias because cohorts comprising prevalent cases of HIV are enriched for variants associated with prolonged disease-free survival.
- Increasing sample sizes and improved technologies have brought into question many other previously reported susceptibility signals, including the widely known associations between variation in the *MBL* locus and susceptibility to a variety of traits including sepsis.
- Overall studies investigating host genetic susceptibility continue to tell us much about the molecular basis of the host response to infection, providing important insights that can guide drug and vaccine development.

Genetic variation in the host has a substantial influence on the course of infectious diseases caused by many microorganisms. Such interactions have been well studied in humans because the pathogens and the host genome are well characterized. In recent years, the methods and techniques available for analyzing human genetic variation have advanced rapidly, leading to the identification of a large number of genes associated with altered susceptibility to infectious pathogens. Entire human genome sequences and millions of mapped single nucleotide polymorphisms (SNPs) provide a powerful resource for molecular analysis of differential susceptibility. Although many genes have already been associated with susceptibility to various diseases, it is likely that these represent only a small fraction of all the relevant genes. Indeed, susceptibility to most infectious diseases in humans is likely to be highly polygenic, and the identification of susceptibility and resistance genes is providing new insights into disease pathogenesis and resistance mechanisms. Conversely, it appears increasingly likely that a substantial proportion of the functional variation in the human genome has evolved to facilitate defense against infectious pathogens, leading to the observed polygenic variation in susceptibility among individuals and different human populations.

MAGNITUDE OF THE HOST GENETIC EFFECT

There are various types of evidence that demonstrate a significant role for host genetics in variable susceptibility to infectious disease. For some infections, such as leprosy, disease has long been known to cluster in families,[1] but it is often difficult to assess the relative importance of proximity to an index case and shared genes. Nonetheless, the increased risk of disease in a sibling compared with the general population is a useful measure of the extent of the genetic component in a multifactorial disease and appears to be of the order of 1.5 to 5 for several infectious diseases.[2]

Apparent interpopulation and racial differences in susceptibility have also been noted.[3,4] Particularly striking is the apparently increased susceptibility to viral infections and tuberculosis (TB) in some previously unexposed populations.[5] In the Qu'Appelle Indians of Saskatchewan, after some decades of exposure to TB and resulting high mortality, the incidence of disease dropped 50-fold, likely reflecting a very strong selective pressure against TB susceptibility loci.[6] The use of malaria therapy for syphilis in nonimmune individuals[7] and the accidental vaccination of some children in Lübeck, Germany, with *Mycobacterium tuberculosis* rather than the Calmette-Guérin bacillus provided direct evidence of variable susceptibility to these pathogens.[7a]

In addition to these observational data, studies of adoptees and of twins have provided more direct quantification of the importance of host genetics in susceptibility to infectious diseases. In a study of more than 900 Scandinavian adoptees, the early death of a biologic, but not an adoptive, parent from an infectious disease was associated with an almost sixfold increase in the risk of an infectious cause of death in the adoptee, consistent with a substantial role for host genetics.[8] Also, several twin studies have found higher concordance rates among monozygotic than among dizygotic twin pairs, who share, respectively, 100% and, on average, 50% of their genes. The diseases studied include TB, poliomyelitis, leprosy, persistent hepatitis B virus (HBV) infection, *Helicobacter pylori* infection, and malaria (Table 10.1).[9–14] In general, it has been easier to demonstrate a significant role for host genetic factors in infections in which only a proportion of those infected develop disease, in chronic rather than in acute infections, and in the severity of infectious disease rather than susceptibility to infection itself.

TABLE 10.1 Twin Studies of Selected Infectious Disease Phenotypes

PHENOTYPE	POPULATION	CONCORDANCE MZ	CONCORDANCE DZ
Tuberculosis	Germany United States United Kingdom	65 62 32	25 18 14
Poliomyelitis	United States	36	6
Leprosy	India	52	22
Hepatitis B	Taiwan	35	4
Helicobacter pylori infection	Sweden	81	63

[a]All showed significantly higher concordance in monozygotic (MZ) than in dizygotic (DZ) twin pairs.

TABLE 10.2 Examples of Genes That Confer Resistance to Malaria

GENE NAME (SYMBOL)	VARIANT	*PLASMODIUM* SPECIES
β-Globin *(HBB)*	Sickle, thalassemias	*P. falciparum*
α-Globin *(HBA)*	Thalassemias	*P. falciparum*
Erythrocyte band 3 *(SLC4A1)*	27-bp deletion	*P. falciparum, P. vivax*
Duffy chemokine receptor *(DARC)*	Promoter variant	*P. vivax*
Glycophorin C *(GYPC)*	Exon 3 deletion	*P. falciparum*
Glucose-6-phosphate dehydrogenase *(G6PD)*	Deficiency variants	*P. falciparum*
ABO glycosyltransferases *(ABO)*	Blood group O	*P. falciparum*

Approaches

Several different and usually complementary approaches have been taken to identifying genes involved in susceptibility to infectious diseases. Historically, a widely adopted strategy was the assessment of candidate genes in case-control association studies. Here, the frequencies of common variants (with a frequency >1%, defined as polymorphisms) of a gene with a suspected role in resistance are compared in individuals with and without the disease. However, the degree of replication between reported association studies of candidate genes is often disappointingly low, in part reflecting limitations in study design. In particular, such studies often have inadequate sample sizes and fail to perform statistical correction for multiple testing of different polymorphisms. Despite these shortcomings, a number of robust genetic associations with infectious disease susceptibility have been successfully identified with the candidate gene approach.

Another largely historical approach was to search for genetic linkage to, rather than association with, an infectious disease in family studies. Identification of a chromosomal region genetically linked to susceptibility indicates that there is a susceptibility gene (or genes) somewhere in that region. Limitations of the linkage approach have been the difficulty in recruiting multicase families and a lack of study power. Such family-based linkage approaches were successfully used, however, to map and identify genes underlying rare monogenic susceptibility phenotypes such as susceptibility to weakly pathogenic mycobacteria.[15,16]

More recently, the availability of millions of SNPs mapped onto the human genome sequence has allowed the development of microarrays of typically 0.5 to 1 million SNPs, allowing markers across the entire human genome to be studied for possible disease association in a single experiment. This approach, known as a genome-wide association study (GWAS), uses tagging SNPs to identify markers that may be causally associated with susceptibility. A major advantage of the genome-wide approach is that, unlike the use of candidate genes, it is not dependent on existing biologic hypotheses and so has the potential to uncover novel, previously unsuspected susceptibility genes and pathways. A drawback is that very large sample sizes (typically comprising thousands of individuals) are needed to provide statistical power to detect disease associations after correction for the large number of polymorphisms tested. Collecting such large sample sizes poses a major challenge in many infectious diseases, particularly diseases with acute presentations in resource-poor countries. Despite this, the application of GWAS to selected infectious diseases has been successful in both replicating genes implicated from candidate and linkage approaches and identifying novel susceptibility genes.[17]

Finally, recent advances in sequencing technology have permitted the selective sequencing of complete coding regions of the genome (the "whole exome"), and indeed the entire genome, in individual humans. Because coding regions comprise only 1% of the human genome, whole-exome sequencing is an efficient strategy for the identification of rare variants underlying disease. Although this approach will not reveal causal variants in noncoding regions, it is widely presumed that the majority of large-effect variants will be located in coding regions. Whole-exome sequencing has been successfully used to identify rare single-gene variants underlying extreme phenotypes of infectious disease, primarily monogenic primary immunodeficiencies. Rare mutations in genes leading to primary immunodeficiency states are described in Chapter 12.

DISEASES

Malaria

Historically, more genes have been implicated in the differential susceptibility to malaria than to any other infectious disease of humans or other animals (Table 10.2). This in part reflects the very strong selection of malaria on pre–reproductive-age human mortality, the early success in identifying the relevance of the sickle hemoglobin (HbS) polymorphism, and the geographic distribution of some malaria resistance alleles.[18] Early evidence of differential susceptibility to malaria in nonimmune subjects came from studies of the use of malaria therapy in the management of syphilis.[7] Marked differences in susceptibility to the same dose and strain of malaria parasite were observed among individuals. Numerous studies of HbS heterozygotes and glucose-6-phosphate dehydrogenase (G6PD) deficiency provided evidence of their protective relevance against *Plasmodium falciparum* malaria.[19,20]

Based on the distribution of thalassemia in the Mediterranean, Haldane[21] proposed that certain hemoglobin gene variants might have reached high frequencies in malarious regions by providing resistance to this disease. The protective efficacy of HbS in heterozygotes against *P. falciparum* malaria was discovered a few years later.[18] The greatest protection is afforded against death and severe life-threatening malaria, with somewhat less protection against uncomplicated disease and least protection against becoming infected. This pattern of greater protection against severe disease than infection appears to apply for many resistance genes.

The α- and β-thalassemias are extremely common disorders of hemoglobin synthesis that lead to imbalanced globin chain production. The mild forms of thalassemia are among the most prevalent single-gene disorders. Both α- and β-thalassemias have been shown to afford some protection against *P. falciparum* malaria, in keeping with their geographic distribution, but the mechanism of protection remains unknown.[22–24] There is no detectable impairment of parasite growth in vitro, and the protection afforded appears less marked than for HbS. Hemoglobin C (HbC),[25,26] which is common in parts of West Africa, and HbE,[27,28] which is widely distributed in Southeast Asia, are also protective. Interestingly, there appears to be an epistatic interaction between the protective HbAS and α-thalassemia genotypes such that they cancel out each other's protective effect against malaria; this might explain why very mild forms of α-thalassemia have not reached higher frequencies in sub-Saharan Africa.[29]

Early work on the human leukocyte antigen (HLA) locus found both class I and class II alleles associated with malaria susceptibility in Africa in large case-control studies.[30,31] However, there is evidence that the particular alleles showing such associations differ among populations. Interpopulation heterogeneity can result from many causes, but a

prominent one in malaria is likely to be the marked polymorphism of immunodominant malaria antigens. Indeed, HLA has been found to influence the strain of malaria parasite associated with clinical malaria, and complex interactions among parasite strains may lead to further variability in HLA associations.[32] Overall it seems likely that the antigenic specificities of predominant immune protective mechanisms against malaria vary geographically with transmission patterns.

Malaria parasites vary in their capacity to form rosettes with uninfected erythrocytes and to sequester in capillary beds, and both of these phenotypes have been implicated in increased malaria severity.[33,34] Later work therefore investigated the relationship between candidate genes and malaria susceptibility, including the polymorphic host receptors involved in parasite sequestration such as *CD36* and *ICAM1* (intercellular adhesion molecule 1).[35–37] However, a reappraisal of reported malaria resistance loci by the Malaria Genomic Epidemiology Network (MalariaGEN) found only a weak protective signal for *CD36* and no relationship between *ICAM1* variants and susceptibility.[38] Moreover, this study, based on data from 11,890 cases and 17,441 controls from 12 locations in Africa, Asia, and Oceania, found that 22 of the 27 previously reported associations were not replicated, underscoring the importance of interpreting the candidate gene literature with caution.

One important locus that did replicate was the *ABO* gene, which encodes the glycosyltransferase enzyme that determines ABO blood groups. Individuals with blood group O had a reduced risk of both cerebral malaria and severe malarial anemia.[38] Consistency across these two distinct clinical phenotypes was also observed for HbS and HbC, which implies a general pathogenic mechanism, contrasting with the strong heterogeneity observed at other malaria susceptibility loci including the *G6PD* gene.[38] A number of variants around *G6PD* cause deficiency of the G6PD enzyme in an X-linked fashion, which in certain situations predisposes to hemolytic anemia.[39] Acute hemolysis can be triggered by a variety of drugs, some infections, and ingestion of fava beans; male G6PD-deficient infants may have neonatal jaundice. More than 100 mutations of *G6PD* have been described through molecular analysis, and a small group of uncommon variants is associated with chronic hemolytic anemia in the absence of any environmental agents. Most *G6PD* variants are associated with lesser degrees of enzyme deficiency and are found at higher frequency. As with the hemoglobinopathies, the geographic distribution of G6PD deficiency in the "malaria belt" suggests its selective advantage.[6] In several locations, populations with historical exposure to malaria had significantly higher frequencies of G6PD deficiency than related populations that had not been exposed to malarial selection. In the MalariaGEN study, the major form of G6PD deficiency in Africa (G6PD+202T) appeared to have independent effects on cerebral malaria and severe malarial anemia, with a strikingly neutral effect on severe malaria overall.[38] Moreover, amalgamating the effects of multiple *G6PD* variants by using a World Health Organization (WHO) classification system revealed that other variants at the locus also contribute to malaria susceptibility such that increasing G6PD deficiency is associated with decreased risk of cerebral malaria and increased risk of severe malaria anemia.[40] Such results raise the possibility that the high rates of G6PD polymorphism seen in human populations may in part be due to an evolutionary trade-off between different outcomes of *P. falciparum* infection.[40]

Four large GWASs of severe malaria have been reported to date. The first replicated the association at HbS but only after sequencing the region in a subset of samples using a technique called genetic imputation to infer the HbS genotypes in the remainder of the collection.[41] Undertaken in The Gambia, this important study illustrated some of the challenges of studying genetic susceptibility in non-European populations, including greater population structure due to differences in the ancestry of cases and controls, and smaller haplotype blocks—especially in African populations—which mean causal variants are less likely to be tagged by the SNPs included on genotyping arrays. A second GWAS again confirmed the protective effects of HbS and of blood group O but also identified two novel associations including the *ATP2B4* (ATPase plasma membrane Ca^{2+} transporting 4) gene encoding the primary calcium pump of erythrocytes,[42] a finding that has since been replicated.[40,43] Two subsequent publications from the MalariaGEN Consortium have now brought the sample size up to 25,000 individuals and identified a novel resistance locus close to a cluster of genes encoding glycophorins, which are the receptors for erythrocyte invasion by *P. falciparum*.[43,44] Fascinating to note, this signal overlaps a region of genome in which genetic variation has been maintained by balancing selection identified by analysis of haplotype sharing between humans and chimpanzees.[44] Later, sequencing of African individuals from 10 ethnic groups revealed that a previously unknown and complex copy number variant that underlies a distinct blood group antigen known as Dantu is associated with protection against severe malaria.[45]

Finally, although most genes that influence susceptibility to infectious disease probably have relatively small effects, it is noteworthy that a few malaria resistance genes are exceptions to this rule. In particular, heterozygosity for HbS provides about 90% reduction in the risk of severe malaria.[30] In addition, the protection from *Plasmodium vivax* afforded by the Duffy-negative (CD234, chemokine receptor) blood group is essentially complete because this parasite is almost always unable to invade Duffy-negative erythrocytes.[46]

Mycobacterial Diseases

Genetic susceptibility studies of mycobacterial diseases have been relatively common for several reasons. Familial clustering of leprosy and TB has long been recognized, and leprosy was regarded by some as a genetic disorder before *Mycobacterium leprae* was identified.[1] An accident in Lübeck, Germany, in which children immunized with *M. tuberculosis* rather than Calmette-Guérin bacillus experienced a range of clinical manifestations from asymptomatic to severe infection and death, provided early evidence for variable susceptibility to TB.[47] This was substantiated by several large twin studies that found higher concordance rates among monozygotic compared with dizygotic twin pairs (see Table 10.1),[9] although a reanalysis of the most recent of these studies found less clear evidence of a genetic effect.[48] A large twin study of leprosy in India also reported higher concordance rates in monozygotic twins but was inconclusive on the question of genetic susceptibility to leprosy type.[11] Observations on the introduction of TB to some populations previously free of the infection suggested that the decline in frequency of the disease over time might in part reflect some natural selection for resistance genes.[6] Candidate gene approaches to TB and leprosy susceptibility implicated a number of genes (Table 10.3), including the HLA region, *SLC11A1* (solute carrier family 11 member 1; previously known as natural resistance–associated macrophage protein-1 gene [*NRAMP1*]), the signaling adaptor protein *TIRAP* (Toll–interleukin-1 [IL-1] receptor [TIR] domain containing adaptor protein; as known as MyD88 adaptor-like [Mal]) and the C-type lectin *CD209,* although the level of replication between studies has been generally low.[48–55] Despite the availability of large multicase families, genome-wide linkage studies in TB had only limited success,[56,57] whereas such studies in leprosy provided a number of promising findings including a signal overlapping the *MRC1* (mannose receptor C-type 1; previously known as the human mannose receptor) gene.[58] In addition, a gene-centric approach identified a coding variant in the *TLR1* (Toll-like receptor 1) gene that was associated with a reduced risk of leprosy, a finding that is notable because of the substantial differences in frequency among populations, perhaps due to natural selection.[59]

More recently, there have been several GWASs of TB and leprosy of varying sample size. The first GWAS of TB found a susceptibility locus within a gene-poor region on chromosome 18q11.2. The signal was driven by a variant that is common in Africa but rarer in other regions, and although the causative variant remains unknown, a regulatory effect on a gene elsewhere on the chromosome is likely.[60] A second GWAS identified a TB resistance locus on chromosome 11p13 in an intergenic region downstream of the *WT1* (Wilms tumor 1) gene.[61] This protein has been shown to interact with the vitamin D receptor, which was previously implicated in TB pathogenesis. A further GWAS of TB in over 10,000 individuals of Russian ancestry not only replicated the *WT1* locus but also identified a further signal overlapping the *ASAP1* (ArfGAP with SH3 domain, ankyrin repeat and PH domain 1) gene, for which there was signal in the earlier African datasets.[62] These variants are believed to alter expression of *ASAP1* in dendritic cells, which may impair their migration to lymph nodes during the early stages of infection. Two additional GWASs undertaken in Europeans and Han Chinese

TABLE 10.3 Examples of Susceptibility and Resistance Genes Implicated in Bacterial Diseases and Related Phenotypes

GENE SYMBOL	DISEASE	METHOD	EFFECT
HLA-DR/HLA-DQ	Leprosy	GWAS/candidate	Susceptibility
TLR1	Leprosy	Gene-centric	Susceptibility
NOD2	Leprosy	GWAS	Susceptibility
TNFSF15	Leprosy	GWAS	Susceptibility
RIPK2	Leprosy	GWAS	Susceptibility
HLA-DR/HLA-DQ	Tuberculosis	GWAS/candidate	Susceptibility
ASAP1	Tuberculosis	GWAS	Susceptibility
HLA-DR	Enteric fever	GWAS	Resistance
ABO glycosyltransferases	Cholera	Candidate	Susceptibility
MBL	Pneumococcal disease	Candidate	Susceptibility
PTPN22	Pneumococcal disease	Candidate	Susceptibility
Mal/TIRAP	Pneumococcal disease, bacteremia, tuberculosis	Candidate	Resistance
CFH-CFHR3	Meningococcal disease	GWAS/candidate	Resistance

ASAP1, ArfGAP with SH3 domain, ankyrin repeat and PH domain 1*; CFH,* complement factor H; *CFHR3,* CFH-related protein 3; *GWAS,* genome-wide association study; *HLA,* human leukocyte antigen; *IL23R,* interleukin-23 receptor; *Mal,* myeloid differentiation primary response gene 88 (MyD88) adaptor-like protein; *MBL,* mannose-binding lectin; *NOD2,* nucleotide-binding oligomerization domain containing 2; *PTPN22,* protein tyrosine phosphatase nonreceptor, type 22; *TIRAP,* Toll-interleukin-1 receptor (TIR) domain adaptor protein; *TLR1,* Toll-like receptor 1 gene; *TNFSF15,* tumor necrosis factor (ligand) superfamily member 15.

TABLE 10.4 Examples of Susceptibility and Resistance Genes Implicated in Viral Diseases

GENE SYMBOL	DISEASE	METHOD	EFFECT
CCR5	HIV infection/progression	GWAS/candidate	Resistance
CCR2	HIV viral control	GWAS/candidate	Resistance
HLA-B, HLA-C	HIV progression	GWAS/candidate	Resistance
HLA-DP	HBV persistence	GWAS/candidate	Resistance
IL28B	HCV persistence	GWAS/candidate	Resistance
MICB	Dengue shock syndrome	GWAS	Susceptibility
PLCE1	Dengue shock syndrome	GWAS	Susceptibility
IFITM3	Influenza A	Candidate	Susceptibility
FUT2	Norovirus disease	Candidate	Resistance

CCR, Chemokine receptor; *FUT2,* fucosyltransferase 2; GWAS, genome-wide association study; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen; *IFITM3*, interferon induced transmembrane protein 3; *IL28B,* interleukin-28B; *MICB,* major histocompatibility complex class I polypeptide–related sequence B; *PLCE1,* phospholipase C, epsilon 1.

confirmed the association between TB and the HLA class II region,[63,64] and introduced additional putative susceptibility loci including a region on chromosome 1 where genes of potential relevance to TB pathogenesis included *MFN2* (mitofusin 2) and *TNFRSF8* (TNF receptor superfamily member 8).[64] Finally, given the challenges involved in diagnosing TB, an alternative approach to investigating susceptibility to TB is to study high-risk individuals who do not develop disease. Illustrating this, a GWAS of human immunodeficiency virus (HIV)–positive individuals in Uganda and Tanzania identified two SNP haplotypes including a missense *IL9* (interleukin-9) variant that protected against tuberculin skin test positivity during follow-up.[65]

The first GWAS of leprosy susceptibility reported robust associations with polymorphisms in the loci *HLA-DR/HLA-DQ, RIPK2* (receptor-interacting serine-threonine kinase 2), *TNFSF15* (tumor necrosis factor [TNF] superfamily member 15), *LACC1* (laccase domain containing 1; previously known as *C13orf3*), *CCDC122* (coiled-coil domain containing 122), and *NOD2* (nucleotide-binding oligomerization domain containing 2), and a weak association with *LRRK2* (leucine-rich repeat kinase 2).[66] Current understanding of the functions of *C13orf31/LACC1* and *CCDC122* remains incomplete, but, interesting to note, the other associated loci *RIPK2, TNFSF15,* and *NOD2* and *TLR1* all encode proteins known to be involved in the innate immune response. A second GWAS of leprosy identified two more susceptibility genes, *IL23R* (interleukin-23 receptor) and *RAB32* (RAB-32, member *RAS* oncogene family).[67] Two further expansions of these analyses also set in Chinese Han populations have increased the total to 22 susceptibility loci.[68,69] The latest of these studies both fine-mapped the signal in HLA to the *DRB1**15:01 allele and estimated the heritability from the GWAS data at 0.199, such that genome-wide significant associations explain approximately 13.5% of leprosy risk.[69]

Genome-wide approaches have been highly successful in the study of leprosy, perhaps reflecting the relatively conserved nature of this pathogen, and these findings may further understanding of the biology of other diseases in addition to leprosy. For example, there is a striking overlap between genes underlying susceptibility to leprosy and Crohn's disease: *NOD2, TNFSF15, LRRK2, C13orf31/LACC1, CCDC122,* and *IL23R* are all associated with Crohn's disease,[68] raising the intriguing possibility of a shared immunologic basis for these two conditions.

Viral Diseases

Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

The role of human genetic variation in susceptibility to HIV infection and progression of acquired immunodeficiency syndrome (AIDS) has been extensively investigated. This work followed the early observation that a small minority of individuals remain HIV seronegative despite repeated exposure from infected sexual partners.[70] Some of these resistant sex workers have immunologic evidence of exposure to the virus. There also is clear evidence that individuals vary in the rate of disease progression to AIDS once infected, and a small number of genes have now been found to influence this rate (Table 10.4).

A large number of studies of HLA type in relation to rate of disease progression have been reported. Although there are marked differences among studies, some alleles have now been associated with susceptibility or resistance in more than one population. Variation in the HLA class I region appears consistently more important than in the HLA class II region, with *HLA-B* being the most important gene in the class I locus.[71] *HLA-B**35 and the HLA-A1-B8-DR3 haplotype have been associated with more rapid disease progression in several studies.[72–74] Similarly, *HLA-B**27 and *HLA-B**57 are associated with a lower rate of progression.[75,76] Particular combinations of HLA class I and II alleles and variants of the transporter associated with antigen-processing genes have also been implicated.[77] Evidence of linkage of the major histocompatibility complex (MHC) locus to the rate of decline of CD4-positive T cells in patients with AIDS provides further support for the relevance of polymorphism in this region.[78]

It is possible to cluster HLA class I types into so-called supertypes based on the types of peptides bound by particular molecules; analysis of supertypes also shows convincing association with rate of disease progression.[79] The HLA type of the host appears to influence the diversity of HIV sequences that emerges during an infection, indicating that HLA variation can directly influence virus evolution.[80] Genome-wide association studies found evidence that loci other than *HLA* genes within the MHC may affect the rate of disease progression.[81,82] A further large GWAS of untreated HIV-1 controllers and progressors identified more than 300 significantly associated polymorphisms, all located in the MHC.[83] This study extended previous HLA associations to identify *HLA-B**57:01, *HLA-B**27:05, and *HLA-B**14 as protective and *HLA-C**57

as associated with progression to AIDS. Genes encoding the killer cell immunoglobulin-like receptors (KIR), which modulate natural killer cell activity and interact physically with HLA class I molecules, may also interact genetically or epistatically so that KIR gene variants modulate the risk associated with an HLA type.[84]

The discovery of the role of chemokine receptors as coreceptors with CD4 for viral entry into macrophages and lymphocytes has given rise to numerous studies of genetic variants of these receptors and their ligands. The *CCR5* (C-C motif chemokine receptor 5) gene associations with resistance to infection and slower disease progression are well established,[85] and variants in the *CCR2* (C-C motif chemokine receptor 2) gene have also been associated with altered disease progression.[86] A 32-bp deletion in the CCR5 chemokine receptor (*CCR5*Δ32) is found at allele frequencies of up to 10% in European and derived populations.[87] This variant is rare or absent in other populations.[88] CCR5 is the coreceptor for macrophage-tropic strains of HIV-1 involved in viral transmission. Heterozygotes for *CCR5*Δ32 progress more slowly to AIDS once infected but are not at reduced risk of HIV infection.[85] In contrast, homozygotes for this variant have very substantial resistance to HIV infection, and only a few infected homozygotes have been identified. Important to note, the effects of these genetic variants helped support successful pharmaceutical programs to develop a new class of anti-HIV drug, entry inhibitors, that block the interaction of HIV with these coreceptors. Finally, the high prevalence of *CCR5*Δ32 among northern Europeans is intriguing. Initial analysis of flanking molecular markers suggested that this deletion is found on a rare background haplotype and suggested that it arose less than 3000 years ago.[89] Because this implied that the variant allele had probably been subjected to positive selection, there was much speculation about the possibility that this process had been driven by an infectious disease such as bubonic plague or smallpox; however, subsequent work suggests that the allele may in fact be older than previously thought, with substantially weaker evidence of selection such that the deletion may have been a neutral variant present in European populations prior to arrival of HIV.[90]

Following much interest in identifying additional HIV susceptibility loci, a large collaboration compiled data from 25 cohorts, studies, and institutions including matched HIV-uninfected controls.[91] Crucially, this GWAS found the previous links between HLA and susceptibility can be attributed to frailty bias because cohorts comprising prevalent cases of HIV are enriched for variants associated with prolonged disease-free survival (i.e., long-term nonprogressors). Remarkably, beyond *CCR5*Δ32 homozygosity, this study found no convincing evidence to support earlier associations with HIV susceptibility; the investigators concluded that genetic influences on HIV acquisition either are rare or have small effect sizes.[91]

Persistent Hepatitis

The ability or inability to clear HBV is one of the most striking immunogenetic dichotomies in medicine, with 1% to 12% of infected individuals becoming chronic carriers. Factors such as age of acquisition significantly affect the likelihood of developing chronic carriage, although evidence suggests that host genetic influences also play a role. One relatively small twin study in Taiwan provided evidence that susceptibility to HBV chronic carriage, but not to HBV infection itself, is genetically determined.[12] After early candidate gene studies implicated HLA class I and II genes in HBV clearance and chronic infection, the role for variants in both *HLA-DP* and *HLA-DQ* in the HLA class II region in protection against chronic HBV infection was clearly established by GWASs.[92,93] The precise mechanism remains unclear, although it may reflect a specific effect on viral clearance after antigen presentation. Subsequent GWASs have expanded the list of associated genes to include *CFB* (complement factor B), which lies within the HLA region, in addition to *CD40* located elsewhere.[94–96] Important to note, several other associations reported in these studies were replicated in a further GWAS with a more stringent design in which cases were compared with controls shown serologically to have cleared the virus (based on negative hepatitis B surface antigen (HBsAg) and positive anti-HBs and anti-HBc) rather than unselected members of the general population.[97] In addition to linking the HLA signal to specific alleles by imputation, this study also identified a novel susceptibility locus that appears to function as an expression quantitative trait locus for the *INTS10* (integrator complex subunit 10) gene. Although this gene had not previously been implicated in viral disease, functional studies provided support for a role in HBV pathogenesis including viral clearance through interferon regulatory factor 3 (IRF3).[97]

Spontaneous clearance of hepatitis C virus (HCV) has also been studied with both candidate gene and GWAS approaches, and clear associations have been described with polymorphisms close to the *IFNL3* (interferon lambda 3; also known as *IL28B*) gene in European and African populations.[98,99] Of interest, the same *IFNL3* polymorphism is also associated with response to IFN-α treatment of HCV.[98,100–103] *IFNL3* encodes IFN-λ3, which binds to a different receptor complex than IFN-α but activates the same Janus kinase (JAK) signal transducer and activator of transcription (STAT) antiviral pathway.[104]

Finally, studies involving combined analysis of host and pathogen genome are scarce. However, a study of HCV integrated GWAS data from the host with whole-genome sequencing of the virus and demonstrated that polymorphisms in both *ILNL4* and *HLA* are associated with sequence polymorphisms in the HCV genome. Moreover, remarkably, this study demonstrated that interaction between *ILNL4* genotypes and a specific amino-acid residue in the HCV NS5A protein determined viral load.[105]

Other Viral Diseases

Host genetic variants underlying other viral diseases have also been identified. Dengue has been subject to study by GWAS; polymorphisms within the MHC region and at *PLCE1* (phospholipase C, epsilon 1) on chromosome 10 were identified in association with susceptibility to dengue shock syndrome in a study of Vietnamese children.[106] The gene responsible for the MHC association signal has not yet been fully defined, although the strongest signal appears to originate from the gene *MICB* (MHC class I polypeptide-related sequence B), which encodes an inducible activating ligand that is upregulated in human dengue infection[107] and activates antiviral immunity by natural killer and $CD8^+$ T cells.[108] Further study is required to robustly identify the exact gene underlying this disease association and to explore its role in the pathogenesis of dengue shock syndrome.

Studies of the development of severe influenza A in humans have found association with polymorphism in the gene encoding IFITM3 (interferon-inducible transmembrane protein), an antiviral protein that restricts viral entry into host cells.[109,110] Two different *IFITM3* polymorphisms have been described in association with risk of severe influenza, although these findings have not been replicated across all cohorts.[111] Use of whole-exome sequencing has identified genetic deficiency of *IRF7* (interferon regulatory factor 7) with impaired amplification of type I and III interferon in a single, previously healthy child who developed severe influenza.[112] In a similar approach, mutations in genes encoding RNA polymerase III subunits underlying acute severe varicella zoster infection in four otherwise healthy children have been reported.[113] However, the contribution of such individually rare mutations to influenza and varicella susceptibility at the population level is currently unknown.

Other Infectious Disease Phenotypes

Host susceptibility to a variety of other infectious disease phenotypes have been studied. These range from disease caused by specific pathogens to composite traits such as bacteremia or sepsis, which may be caused be a range of pathogens. However, although an increasing number of susceptibility loci that confer susceptibility to specific pathogens have been identified, it would appear that variants affecting such composite traits either are rare or have small effect sizes, which is perhaps a consequence of their diverse etiologies. For example, a GWAS of susceptibility to bacteremia among African children identified a novel susceptibility locus overlapping a lincRNA on chromosome 7 that was associated with susceptibility specifically to pneumococcal bacteremia[114] and a *STAT4* (signal transducer and activator of transcription 4) variant in association with nontyphoidal salmonella,[115] whereas only the previously reported HbS polymorphism was associated with susceptibility to bacteremia overall.[114] Along similar lines, there are no current GWAS reports of susceptibility loci for sepsis despite substantive efforts to understand this major cause of premature death. Two studies of survival

among patients with sepsis[116,117] reported promising findings including intronic variants in the *FER* (FER tyrosine kinase) gene, encoding a non–receptor tyrosine kinase, that almost halved the risk of death in sepsis due to pneumonia.[116] Independent replication of these findings is still needed, but the *FER* pathway represents a potential therapeutic target.

Various other forms of invasive bacterial infection have been studied, including meningococcal disease. On the basis of this, a key role for complement in immune defense against *Neisseria meningitides* is now well established, polymorphisms in the *CFH* (complement factor H) gene having been robustly associated with susceptibility to meningococcal disease through candidate gene approaches and GWASs.[118,119] Rare mutations in complement components are also well described in association with recurrent meningococcal disease.[120] In addition, a GWAS of susceptibility to enteric fever set in Vietnam and Nepal found the *HLA-DRB1**04:05 allele conferred a nearly fivefold greater resistance against disease than the minor allele.[121] This result is important because it represents one of relatively few links between HLA and bacterial disease in the GWAS era.

Other work has focused on identifying susceptibility genes for neglected tropical diseases including schistosomiasis, visceral leishmaniasis, and rheumatic heart disease. For example, a family linkage study of *Schistosoma mansoni* worm burden in Brazil found evidence of linkage to a region of the long arm of chromosome 5.[122] This region may also be relevant in Senegalese families[123] and encodes genes for numerous cytokines, including *IL4, IL9,* and *IL13*. The same region has been genetically linked to various manifestations of atopy and asthma, consistent with the speculation that a gene selected for resistance to helminthic infections might predispose to asthma or atopy. Furthermore, a GWAS for susceptibility to visceral leishmaniasis in patients from India and Brazil found a robust association with polymorphisms in the *HLA-DRB1*/*HLA-DQA1* class II region.[124] The functional polymorphism within *HLA* has not yet been defined, and the extensive linkage disequilibrium across this region makes precise identification of causative variants challenging.

There has also been interest in delineating susceptibility to rheumatic heart disease, which is triggered by group A *Streptococcus*. The first GWAS, set in the South Pacific, identified a susceptibility locus in the immunoglobulin heavy chain locus fine-mapped to a haplotype of coding variants overlapping the *IGHV4-61* (immunoglobulin heavy variable 4-61) gene.[125] This finding is of interest because it represents the first time the immunoglobulin heavy chain locus, which recombines during B-cell maturation, has been linked to susceptibility to disease in the GWAS era. Another GWAS in Aboriginal Australians confirmed the association with the HLA class II region identified in the candidate gene era.[126] Once again, independent replication and larger studies are needed to confirm these findings.

Finally, studies of new-variant Creutzfeldt-Jakob disease (CJD) attributed to infection with the bovine spongiform encephalopathy agent,[127,128] sporadic CJD,[129] iatrogenic CJD,[130] and kuru[131,132] have all shown strong associations with variation in the *PRNP* (human prion protein) gene. Common variation in *PRNP* genotype appears to have a striking effect on susceptibility to disease; homozygotes for either of the amino acids methionine or valine, commonly found at position 129 in *PRNP,* were markedly more susceptible to disease than heterozygotes.[130,133] The maintenance of this genetic variant over long periods of human evolution has been interpreted as evidence for widespread cannibalism in some early human populations.

SPECIFIC SUSCEPTIBILITY AND RESISTANCE GENES

For several human genes, there is compelling evidence that one or more genetic variants affect susceptibility to infectious pathogens (see Tables 10.2 to 10.4). Specific genes and pathways of interest are discussed in this section.

Blood Groups

ABO blood group associations were investigated in a large number of infectious diseases in early studies. The most striking blood group association is of the Duffy blood group with susceptibility to *P. vivax* malaria. This parasite uses the Duffy blood group antigen as the receptor to invade erythrocytes.[134] The Duffy blood group antigen is a promiscuous chemokine receptor (CD234). Most sub-Saharan Africans are Duffy blood group negative because of homozygosity for a mutation in the promoter of this gene and are completely resistant to almost all *P. vivax* infections. Such individuals also express the Duffy antigen on some other tissues because the promoter mutation that is in the recognition site for an erythroid-specific enhancer is tissue specific.[135] It is unclear whether the Duffy genotypes prevented *P. vivax* from ever entering Africa or whether an earlier and more virulent form of this parasitic infection might have selected the variant in Africa. In addition, as described earlier, a large study of Africans provided compelling evidence that blood group O associates with reduced risk of severe *P. falciparum* malaria.[40] Moreover, a complex copy number variant, involving the replacement of the *GYPB* (glycophorin B) gene with two copies of a novel *GYPA-B* hybrid, corresponding to a serologically distinct blood group antigen known as Dantu, has been associated with a 40% reduction of risk of severe malaria.[45]

Other traits linked to blood groups include cholera; blood group O has been associated with increased severity of cholera symptoms in several studies.[136,137] Moreover, the ability to secrete blood group substances (such as secretor histo-blood group antigens [HBGAs]) into saliva and at other mucosal surfaces is genetically determined. Most individuals are secretors, but about 20% of most populations are nonsecretors because of mutation in the *FUT2* (fucosyltransferase 2) gene.[138] In relatively small studies, nonsecretion was suggested to be associated with susceptibility to some bacterial and fungal infections and with resistance to certain common viral infections.[139,140] Nonsecretor status is clearly associated with susceptibility to recurrent urinary tract infection,[141] and a possible mechanism for this has been proposed.[142] Nonsecretor status was found to protect completely against infection with Norwalk virus in volunteer challenge studies[143] and is associated with substantially reduced risk in the general population.[144] More recent studies have reported symptomatic norovirus infection in nonsecretors, and in vitro binding studies have demonstrated that binding of noroviruses to HBGA is strain specific, with some norovirus strains evolving different HBGA-binding targets in an attempt to evade genetically determined host resistance.[145] However, overall, apart from associations with norovirus and urinary tract infections, there is a need for larger studies to show compelling evidence of association.

Human Leukocyte Antigens

The pivotal position of HLA in the initiation and regulation of immune responses, together with their well-documented polymorphism, has led to numerous studies of their influence on infectious disease susceptibility.

After early evidence from candidate gene and linkage approaches, GWAS of infectious disease have robustly confirmed the key role for HLA in susceptibility to many of these conditions.[17] Evidence that particular HLA types are associated with altered susceptibility to infectious disease supports the view that the remarkable diversity of HLA types has been generated and maintained through natural selection by infectious pathogens. The relatively modest magnitude of the reported associations, compared with some HLA associations with autoimmune disease, is in keeping with this possibility. Small selective effects can, over time, markedly change allele frequencies. The observation that cellular immune responses are restricted by HLA molecules suggested an attractive mechanism whereby heterozygosity for HLA type might be evolutionarily advantageous.[146] Heterozygotes should be able to recognize more peptide epitopes in a foreign pathogen than homozygotes, permitting a more protective immune response. For example, a protective effect of heterozygosity has been observed for HLA class II antigens with regard to clearance of HBV,[147] for HLA class I antigens in HIV disease progression,[74] and in human T-cell lymphotropic virus type 1 (HTLV-1) proviral load.[148]

Another feature of HLA associations with infectious disease is that associations often vary geographically. In some cases, this may result from geographic strain variation in the infectious pathogen, and an association between HLA type and the strain of parasite causing infection has been reported in malaria.[32] More detailed analysis of the mechanisms

of identified associations should further explain this population diversity and provide insights into the immune mechanisms of protection and pathogenesis.

Mannose-Binding Lectin

Mannose-binding lectin (MBL) is a serum protein that plays a role in innate immunity. It is a collagenous lectin with at least two important roles in host defense.[149] It binds to sugars, particularly *N*-acetylglucosamine and mannose, on the surface of microorganisms and facilitates their opsonization by macrophages. It also activates complement by means of two MBL-associated serine proteases. Inactivating mutations of the *MBL2* gene are quite prevalent, with frequencies of up to 40% in various populations. Three single amino-acid changes are found at codons 52, 54, and 57, each of which leads to a substantial reduction in MBL concentration in heterozygotes. Homozygotes and compound heterozygotes for these variants have absent or extremely low MBL levels in serum; variation in the promoter of the gene has less marked functional effects.[150]

The potential role of MBL deficiency in susceptibility to human infection has been extensively investigated. Early case reports and small-scale studies suggested that MBL deficiency might predispose to a wide variety of infectious phenotypes,[151] but most subsequent studies of individual diseases, including meningococcal disease, malaria, TB, persistent HBV infection, and sepsis have failed to show clear associations.[152–154] An exception is that homozygotes for *MBL* codon changes appear to be more susceptible to invasive pneumococcal disease (IPD), with a 2.5-fold increase in risk.[155,156] A large, population-based longitudinal study of adults failed to show an association between MBL deficiency and excess mortality or morbidity,[157] and the degree to which MBL deficiency influences susceptibility to infectious disease remains controversial.

Toll-Like Receptor Pathway Genes

With increasing definition of the importance of the TLRs, NOD-like receptors (NLRs), and retinoic acid–inducible gene (RIG)-like helicases (RLHs) and their signaling pathways in detecting pathogens and triggering and amplifying innate immune responses,[158,159] analyses of genetic variation in these pathways have increased. Both rare mutations and common polymorphisms have been reported in genes encoding TLR pathway members in association with different infectious disease phenotypes (Fig. 10.1). Rare mutations within four genes in the TLR pathway have been described in individuals with primary immunodeficiencies characterized by episodes of IPD: *IKBKG* (inhibitor of nuclear factor kappa B [NF-κB] kinase subunit gamma; also known as NF-κB essential modulator [*NEMO*]), *NFKBIA* (NF-κB inhibitor alpha), *IRAK4* (IL-1 receptor associated kinase 4), and *MYD88* (myeloid differentiation primary response 88).[160] Mutations in *NEMO* and *NFKBIA* interrupt not only the TLR pathway but also multiple other pathways that converge on NF-κB, and the resulting immunodeficiency conditions are characteristically severe and with a broad spectrum, including encapsulated bacteria, atypical mycobacteria, fungi, and viruses.[160] In contrast, mutations in *IRAK4* and *MYD88* interrupt only TLR and IL-1 receptor signaling and lead to a narrow-spectrum immunodeficiency characterized by childhood susceptibility to pyogenic encapsulated bacteria, particularly recurrent IPD.[160] Such rare mutations appear to have little impact on susceptibility to pneumococcal disease at the population level in adults, however.[161] Other rare TLR pathway mutations have been reported in association with susceptibility to severe viral disease in otherwise immunocompetent children—for example, mutations in TLR3 signaling genes and herpes simplex virus 1 (HSV-1) encephalitis (HSE), and IRF7 deficiency and severe influenza.[112,162]

Coding polymorphisms are observed relatively frequently in some of the TLRs, with functional consequences. A TLR1 coding variant has been implicated in leprosy[59,163]; coding changes in TLR4 in malaria[164]; and a change in the flagellin receptor TLR5 in *Legionella* pneumonia.[165] All of these implicated changes have functional consequences for receptor signaling. Polymorphisms in *NFKBIA* and the related genes *NFKBIZ* (NF-κB inhibitor zeta) and *TONSL* (Tonsoku-like, DNA repair protein; also known as *NFKBIL2*), which encode IκBs, have also been found to be associated with risk of IPD.[166–168] A common polymorphism in the Mal/TIRAP adaptor protein that binds to TLR2 and TLR4 appears to render the variant nonfunctional, and heterozygotes were found to be less frequent in series of patients with bacteremia, IPD, TB, and malaria.[54] Healthy volunteer individuals with the protective heterozygote Mal/TIRAP genotype generate intermediate levels of inflammatory cytokine signaling after intravenous challenge with lipopolysaccharide, a gram-negative bacterial cell wall component that is recognized by TLR4.[169] Taken together, these findings suggest that both common and rare genetic variation in this key signaling pathway may affect the risk of multiple major infectious diseases and, furthermore, that extremes of TLR signaling may be detrimental.

EVOLUTIONARY PERSPECTIVE

From an overview of the information currently available, it seems likely that susceptibility to most infectious diseases will prove to be highly polygenic. The contrary view—that there may be a few major single genes for many infectious diseases—has been suggested by complex segregation analysis of multicase families[170] and may be incorrect,[171] despite the occurrence of well-documented, diverse, but very rare monogenic phenotypes.[172] The existence of multiple genes affecting infectious disease susceptibility probably simply reflects the major role that infectious pathogens have played in shaping variation in the human genome through natural selection. Indeed, it has been found that genes playing a role in host defense against infectious pathogens evolve at a higher rate than any other class of genes.[173] Also, initial analysis of the distribution of variants associated with infectious diseases between exons and regulatory regions of genes indicated that relevant variants are more often found in exons for infectious than for noninfectious disease.[174] This likely reflects the impact of selection for resistance amplifying the frequencies of many exonic mutations and has led to increasing use of whole-exome sequencing in the field. Natural selection for resistance to infectious pathogens may also explain why the observed effects of most individual genes are relatively modest in magnitude. In the absence of a counterbalancing selective force, alleles that markedly increased or decreased the risk of a major infectious disease would have been quickly eliminated or selected to very high frequency, abolishing polymorphism. Polygenic susceptibility has also been found in extensive analyses of the genetic basis of susceptibility to autoimmune diseases in both humans and mice.[175]

Given the pressure for fixation of selectively advantageous variants, one of the major questions in evolutionary biology has related to the mechanisms that maintain substantial genetic diversity in populations. Some aspects of this question are particularly well addressed in human populations, in which the host genome and the infectious pathogens have been characterized in most detail. Heterozygote advantage is an attractive mechanism by which two alleles may be maintained in a population, and it is classically exemplified by the HbS polymorphism and resistance to malaria. However, this appears to be a relatively unusual means of maintaining genetic diversity, and other mechanisms, such as frequency-dependent selection and fluctuations in selection, may be more generally important.

Another relevant factor is likely to be variation in the genome of infectious pathogens and in the microbiome. Increasing attention is being paid to specific interactions between variants of the host and the pathogen. Particular HLA types have been associated with disease caused by specific serotypes of human papillomavirus,[176] HLA type may also influence the strain of *P. falciparum* causing malaria,[32] and HIV may evolve away from prevalent HLA class I types.[80] Furthermore, immunologic mechanisms have been identified that may underlie interaction between competing strains of microorganisms.[32] There may be exquisite specificity in some of these host-pathogen interactions, leading to coevolution of genetic variation in host and pathogen. This implies that individual susceptibility to disease is the result of a variety of host and pathogen genetic factors that have been tempered by a constellation of environmental variables. This dynamic evolutionary perspective implies that the genes affecting susceptibility to an infectious disease may show significant interpopulation heterogeneity resulting from geographic variation in the pathogen genome, in the environment, and in the frequencies of interacting genes in the host, a prediction well supported by available data on malaria susceptibility.

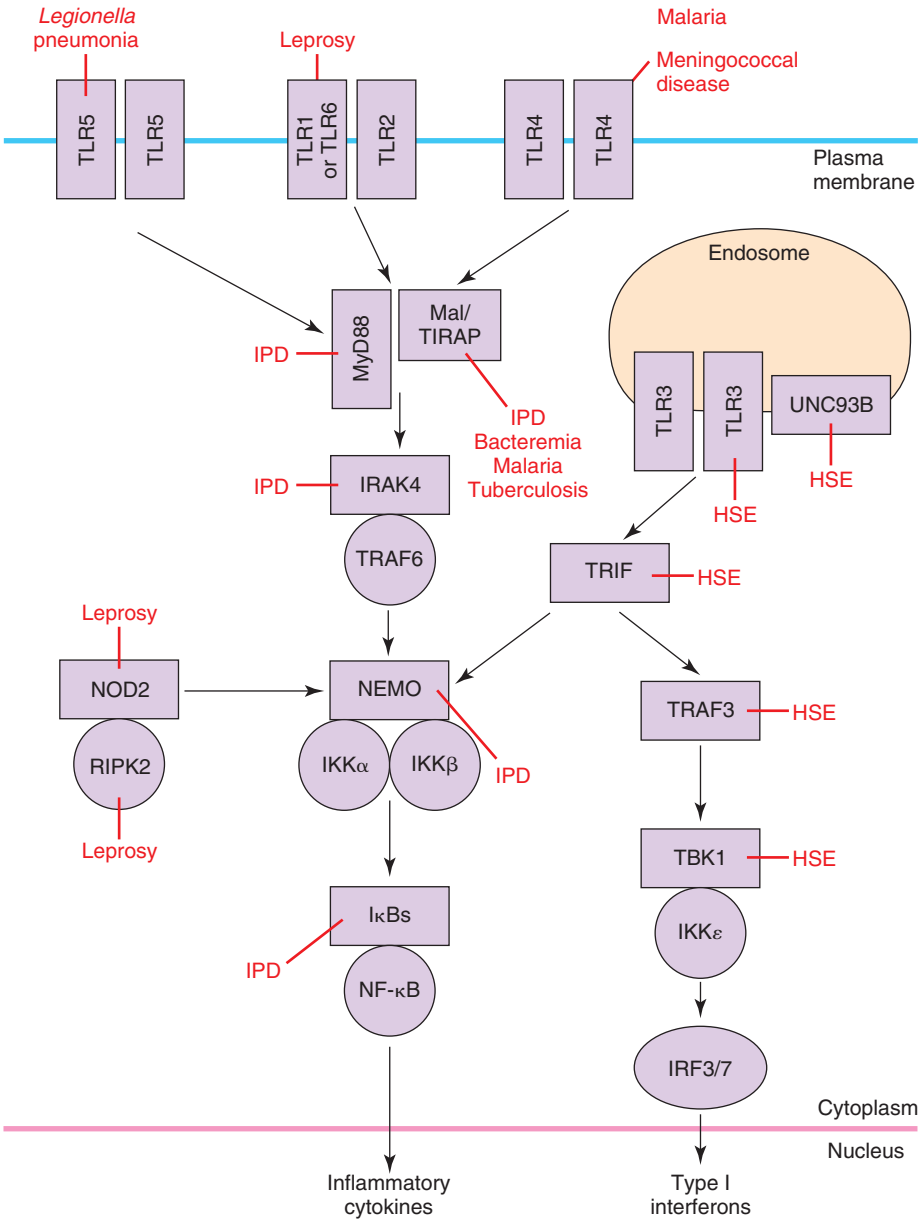

FIG. 10.1 Toll-like receptor signaling and infectious disease. Examples of pathway components for which genetic variation is associated with specific human infectious diseases are highlighted. *HSE,* Herpes simplex virus encephalitis; *IκBs,* inhibitors of NF-κB activation; *IKKα, β, ε,* inhibitory kappa (alpha, beta, epsilon) kinase; *IPD,* invasive pneumococcal disease; *IRAK4,* interleukin-1 receptor-associated kinase 4; *IRF3/7,* interferon regulatory factor 3/7; *Mal,* myeloid differentiation primary response gene 88 *(MyD88)* adaptor-like protein; *NEMO,* NF-κB essential modulator; *NF-κB,* nuclear factor kappa B; *NOD2,* nucleotide-binding oligomerization domain containing 2; *RIPK2,* receptor-interacting serine-threonine (protein) kinase 2; *TBK1,* TANK (TRAF-associated NF-κB activator)–binding kinase 1; *TIRAP,* Toll–interleukin-1 receptor (TIR) domain adaptor protein; *TLR,* Toll-like receptor; *TRAF,* tumor necrosis factor receptor-associated factor; *TRIF,* Tir-domain adaptor inducing interferon-β; *UNC93B, unc-93* homologue. *(Modified from Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease.* Nat Rev Genet. *2012;13:175–188.)*

APPLICATIONS

Most of the apparent genetic component to any infectious disease cannot be accounted for by the polymorphisms and associations identified to date. The increased use of GWAS and more recently whole-exome sequencing has, however, greatly enhanced the power of genomic analyses. There are several potential advantages for the use of modern molecular genetics to understand genetic susceptibility more fully. An obvious application is in risk prediction, which might influence behavior, the use of prophylactic antimicrobials, or immunization or travel patterns. In the future, it may be possible to offer a genetic profiling test to estimate individual susceptibility to particular pathogens. This may be particularly relevant to individually rare or even unique, highly penetrant genetic variants that may be important contributors to risk of developing severe infectious diseases.[177]

Another application is in the understanding of particular pathways used in host resistance to infection. For example, the HLA-B53 association with resistance to malaria[30] supported a protective role for $CD8^+$ T cells in this disease, encouraging efforts to develop vaccines that elicit this immune response.[178]

A third application will be in the identification of molecules and pathways as targets for pharmacologic intervention. Demonstration of the almost complete resistance to HIV infection of homozygotes for a deletion in the *CCR5* gene underpinned successful attempts to develop pharmacologic blockers of this viral coreceptor. In another example, a whole-exome sequencing approach to patients with recurrent respiratory infection and bronchiectasis has identified underlying mutations that increase phosphoinositide 3-kinase delta (PI3Kδ) activity[179]; isoform-selective PI3Kδ inhibitors have been developed for the treatment of

hematologic malignancy and may also prove effective in this immunodeficiency.[180]

Finally, an increased understanding of the genetic basis of infectious disease may uncover genetically distinct subgroups of patients that are clinically indistinguishable but may respond differentially to specific therapies. The potential of genomics to identify "molecular subphenotypes" may have particular application in the use of immunomodulatory agents to treat excessive inflammation in the setting of infectious diseases—for example, in sepsis or severe forms of community-acquired pneumonia or meningitis. Clinical trials in these areas have been largely disappointing, which may reflect the grouping together of patients with unidentified, distinct inflammatory response profiles; tailoring specific immunomodulatory therapy to individual patients on the basis of their genetically determined inflammatory phenotype may add a new level of precision to clinical trials and pave the way for personalized medicine.

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11 Nutrition, Immunity, and Infection

Henry Redel and Bruce Polsky

Although the effects of malnutrition have been recorded for centuries, the mechanistic links between nutrition, immunity, and resistance to infection have been defined only over the past several decades.[1,2,3] The relationship between nutrition and immunity is most striking in the developing world, where protein-energy malnutrition (PEM) occurs. In both developed and developing countries, however, specific micronutrient deficiencies are relatively common, even where PEM syndromes are rare. Both PEM and micronutrient deficiencies are associated with immune dysfunction and infection risk. Malnutrition not only affects immune function but also virulence of infectious agents, progression of chronic infections such as mycobacteria infections and human immunodeficiency virus (HIV), and transcriptional regulation of inflammatory genes that may determine the outcome of infections.[1,2]

Overnutrition—obesity—has emerged as a major public health threat, particularly in the United States in the past 2 decades as noted in reports from the Centers for Disease Control and Prevention (www.cdc.gov/obesity/data/index.html). According to the World Health Organization (WHO), obesity and overweight are evolving issues in developing countries, where some populations, usually in urban areas, are experiencing rapidly increasing numbers of overweight people, while other populations, usually in rural areas, continue to experience widespread undernutrition.[4] Obesity, typically defined as a body mass index (BMI) of 30 or higher, has a complex association with infection risk and outcome, including increasing risk for developing diabetes. More recent developments are highlighted in the following sections.

This chapter explores the relationships among nutritional factors, host immunity, and the virulence of pathogens and their ties to the management of infectious diseases.

EPIDEMIOLOGY OF MALNUTRITION

The WHO estimates that 462 million people were underweight and more than 1.9 billion adults were overweight or obese in 2015.[5] More than 30% of residents in developing nations are affected by hunger and malnutrition, with PEM cited as the primary cause of immunodeficiency worldwide. Among children younger than 5 years of age, five infectious diseases—acute respiratory infections, diarrhea, malaria, measles, and HIV/acquired immunodeficiency syndrome (AIDS)—account for more than 50% of all deaths, and about half of those deaths are due to malnutrition.[2,6,7] In developed nations, at-risk populations for malnutrition, primarily micronutrient malnutrition rather than PEM, include children, elderly adults, pregnant women, people experiencing homelessness, people who abuse alcohol, patients affected by acute and chronic illnesses including HIV/AIDS and end-stage renal disease, and people with dietary restrictions such as patients with anorexia nervosa or bulimia and consumers of vegan diets.

Malnutrition can be broadly defined as a decrease in nutrient reserve; under this definition, undernutrition is prevalent even in the United States, affecting up to 15% of ambulatory outpatients, 25% to 60% of patients in long-term care, and 35% to 65% of hospitalized patients.[8] A major reason for the wide range of rates cited is that many definitions for malnutrition exist. Several biochemical markers of nutritional status can provide a general indication of nutritional reserve[9] including visceral proteins (albumin, prealbumin, and transferrin), but such low levels of visceral protein are not specific and may have other causes including hepatic or renal disease and increased capillary permeability. Global indicators of cell-mediated immune function are commonly used to assess malnutrition and can be estimated by total lymphocyte count and delayed-type hypersensitivity testing with a series of common antigens. Compromise of cell-mediated immunity due to malnutrition is suggested by a total lymphocyte count less than 1000/mm^3 or by lack of skin induration greater than 5 mm above glycerin control at 48 hours to recall antigens such as mumps, unless another cause of lymphocyte dysfunction is present.[9] However, these tests should be interpreted cautiously during an acute illness because cell-mediated immunity may be depressed in the absence of malnutrition.

The Institute of Medicine of the National Academy of Sciences and the Food and Nutrition Board provide dietary reference intakes including recommended dietary allowances for overall protein, calories, and specific nutrients including age-specific, sex-specific, and condition-specific recommendations.[10] The dietary reference intakes and recommended dietary allowances should guide clinicians in their assessments of patients' nutritional status and in their plans to address macronutrient malnutrition as well as micronutrient deficiencies.

MALNUTRITION AND IMMUNE FUNCTION

PEM describes nutritional macrodeficiency syndromes including marasmus (deficiency of calories), kwashiorkor (deficiency of protein), nutritional dwarfism in children, and wasting syndromes in adults. Primary PEM, caused by inadequate nutrient intake, typically affects children and elderly adults, and effects of brief PEM are typically reversible with nutritional therapy. However, primary PEM can result in serious and irreversible changes in organ function and growth. Secondary PEM results from illnesses, injuries, or treatments that alter appetite, digestion, absorption, or metabolism and has similar effects as primary PEM. Although the nutritional deficits of patients with PEM due to gastrointestinal tract dysfunction often can be restored to normal if adequate nutritional support is provided by dietary supplements, enteral tube feeding, or parenteral nutrition, wasting disorders such as cancer or AIDS are characterized by involuntary weight loss, often despite increased caloric intake. In wasting diseases, alterations in metabolism result in greater loss of muscle tissue than would be expected from reductions of caloric intake alone, and muscle mass is not restored by nutritional supplementation unless the underlying inflammatory disease is treated. Weight gain that occurs as a result of nutritional support in patients with these syndromes usually represents increases in fat mass and body water without significant change in lean tissue.

PEM has been associated with a number of impairments in immune responses.[1,2,11,12] Documented abnormalities of innate immunity include reduced production of cytokines, reduced phagocytosis, interruptions in the integrity of physical barriers and diminished quality of mucus, and reductions in complement components (especially C3 and C5). Alterations in adaptive immunity include reduced or delayed cutaneous hypersensitivity to recall and new antigens and reductions in $CD4^+$ and $CD8^+$ T-cell subsets, $CD4^+/CD8^+$ ratio, lymphocyte proliferative capacity, immunoglobulin (Ig) G (IgG), and secretory IgA. In addition, malnourished children and elderly adults demonstrate elevated baseline biomarkers of inflammation such as interleukin (IL)-6.

SPECIFIC NUTRIENTS AND THEIR ROLES IN IMMUNITY

A complete review of nutrients and their roles in immune function is beyond the scope of this chapter. Several outstanding reviews are available, and the reader is referred to these publications for additional

information.[11–13,14,15] A very brief review of major micronutrients and their roles in immunity follows.

Fat-Soluble Vitamins

Vitamin A

Vitamin A is a subclass of the retinoic acids, a family of lipid-soluble compounds that includes retinols, β-carotenes, and other carotenoids. Retinol, or preformed vitamin A, is the most biologically active form; it is found primarily in animal food sources or can be synthesized from carotenoids. The important role of vitamin A in immune system function is well established.[16] Vitamin A deficiency can affect host immunity through direct actions on immune cell function and through indirect effects on epithelial cell differentiation and, consequently, host barrier defenses. Vitamin A deficiency results in reduced mitogen-stimulated T-cell proliferation and antigen-specific IgA and IgG production, impairs the ability of $CD4^+$ T lymphocytes to stimulate Th2 responses (B-cell antigen-specific IgG_1 responses), and limits the ability of neutrophils to phagocytose bacterial pathogens.[16,17]

Vitamin A supplementation has been examined in a number of randomized, double-blind, placebo-controlled trials of undernourished and malnourished children in developing nations. Antibody-mediated responses are impaired in individuals with vitamin A deficiency; in some (but not all) studies, supplementation has improved antibody titer responses to measles vaccine, sustained gut integrity, and lowered the incidence of respiratory tract infections.[18] A Cochrane Review found that oral vitamin A supplementation in children in developing countries reduced overall mortality (relative risk [RR], 0.76; 95% confidence interval [CI], 0.69–0.83) and diarrheal-associated mortality (RR, 0.72; 95% CI, 0.57–0.91). Furthermore, supplementation reduced the incidence of diarrhea (RR, 0.85; 95% CI, 0.82–0.87) and measles (RR, 0.50; 95% CI, 0.37–0.67) and reduced the prevalence of vision problems in children 6 months to 5 years of age.[19]

In light of these trials and others demonstrating beneficial effects of vitamin A supplementation on immune function and its efficacy in preventing infection, the WHO recommends that vitamin A supplementation be provided to young children and mothers residing in developing nations even in the absence of signs or symptoms of deficiency. Implementation of this program has been one of the great WHO success stories.

Although studies suggest an association between vitamin A deficiency and increased mortality, disease progression, and vertical transmission of HIV, vitamin A supplementation has not been shown to improve outcomes in HIV-infected populations.[20] Similarly, vitamin A supplementation does not improve outcomes in patients with active tuberculosis. Therefore, caution should be exercised in applying recommendations to other populations, especially populations at much lower risk for vitamin A deficiency. Excessive intake of vitamin A can produce acute toxic manifestations (headache, vomiting, stupor, and papilledema). Chronic toxicity is associated with weight loss, nausea, and vomiting; dryness of the mucosa of the lips; bone and joint pain; hyperostosis; and hepatomegaly with parenchymal damage and fibrosis. In the 1990s two large trials evaluating the role of β-carotene in lung cancer prevention observed an increased risk for lung cancer in subjects (male smokers) receiving β-carotene.[21,22] The reasons for this finding of increased risk remain unclear, emphasizing the need for further study on the role of retinoids in human health and immunity before widespread supplementation with retinoid precursors can be recommended in well-nourished populations.

Vitamin D

Vitamin D deficiency is remarkably common even in developed nations, particularly in areas with reduced exposure to sunlight or reduced dairy intake or both. However, as with other vitamins, the prevalence depends on which metabolite is measured (25-hydroxyvitamin D or 1,25-dihydroxyvitamin D) and the cutoff value used to identify deficiency. Most population surveys use serum 25-hydroxyvitamin D levels because they are easy to measure and widely available, but the definition of deficiency depends on the outcome measured, and no standard is uniformly accepted.[23] Regardless of the standard used, women more than men, older adults more than young adults and children, and people of races other than white are more likely to be deficient.[24] Older institutionalized adults and people with comorbidities that reduce their sunlight exposure or oral intake of fortified dairy products are also at greatly increased risk.

The role of vitamin D (1,25-dihydroxycholecalciferol) in immune response has been recognized for decades but has received increased attention more recently as the importance and ubiquity of vitamin D receptor expression have been shown and its role in host defenses against mycobacterial disease has been better defined. Vitamin D suppresses many adaptive immune responses (T-cell proliferation, antibody production) and some innate immune responses (dendritic cell costimulation and cytokine secretion). In contrast, vitamin D is essential for proper function of other aspects of the innate immune system, particularly in macrophage-mediated defenses.[15] This appears to be principally important in Toll-like receptor–mediated defenses against intracellular pathogens such as *Mycobacterium tuberculosis*.[25–27]

Individuals with low 25-hydroxyvitamin D levels appear to be more susceptible to tuberculosis and at higher risk for progression from infection to disease.[28,29] However, vitamin D supplementation trials have not demonstrated an improvement in tuberculosis-related mortality or sputum smear conversion rates.[30–32] Additional data suggest that vitamin D deficiency is associated with impaired wound healing and predisposes to periodontal disease[15,26] and upper respiratory tract infection,[33] although no trials to date document benefits of supplementation. Ongoing studies of vitamin D supplementation for the reduction of infection-related, cancer-related, and cardiovascular disease–related morbidity and mortality should provide additional insight into the complex role of vitamin D in human disease.

Vitamin E

Eight closely related fat-soluble compounds (four tocopherols and four tocotrienols) all exhibit vitamin E biologic activity, but α-tocopherol is the most active form, is abundant in many foods, and is most widely available as a supplement. Vitamin E is one of a group of antioxidants that scavenge free radicals formed in redox reactions throughout the body.[34] Vitamin E activity is complemented by that of selenium, which, as a constituent of glutathione peroxidase, also metabolizes peroxides before they cause membrane damage.

Hypovitaminosis E resulting from a deficient diet is uncommon in the developed world and occurs almost exclusively in association with severe fat malabsorption, in low-birth-weight infants, and in patients with rare genetic disorders such as abetalipoproteinemia. Vitamin E supplementation has multiple immunologic effects including enhanced T-cell proliferation, perhaps mediated by suppressed production of prostaglandin E_2, a T-cell–suppressive compound, and enhanced delayed-type hypersensitivity responses.[13,14,34] Clinical effects of vitamin E supplementation have been studied mainly in elderly subjects (see "Older Adults"). There has been extensive research on the biology of immune senescence and the effects of vitamin E on age-related immune dysfunction.[34] Although beneficial effects of vitamin E supplementation have been suggested in a variety of noninfectious diseases such as macular degeneration and hepatic steatosis, the findings of increased risk for prostate cancer in healthy men receiving vitamin E supplementation will impact future intervention trials with vitamin E.[35]

Water-Soluble Vitamins

Vitamin C

Vitamin C (ascorbic acid) can augment a number of biochemical reduction reactions involving iron and copper and acts as an enzymatic cofactor and antioxidant in physiologically important processes including fatty acid transport, collagen synthesis, and neurotransmitter formation.[13,14] In humans, vitamin C deficiency is manifested as scurvy, which is due to impaired collagen synthesis, with signs including capillary fragility, bleeding gums, delayed wound healing, and impaired bone formation. Animal studies and a limited number of human studies have suggested an immunomodulatory role for ascorbic acid, with increased resistance to viral illness and some anticarcinogenic effects, perhaps via reduced T-cell apoptosis.[13,14]

Many trials have been performed to determine whether vitamin C may be useful to prevent or treat the common cold. A comprehensive review

concluded that vitamin C supplementation did not reduce the incidence of common colds but did produce a consistent reduction in the duration of cold symptoms. A reduction in symptom days in adults (8%; 95% CI, 3%–12%) and in children (14%; 95% CI, 7%–21%) was summarized in a Cochrane review.[36] In contrast to most other dietary antioxidants, vitamin C appears to be safe even at high levels of consumption.

Trials have sought to assess the role of vitamin C in sepsis. A retrospective before-after study of 47 control subjects and 47 patients examined intensive care unit (ICU) data 7 months before and 7 months after a protocol that administered a combination of vitamin C, hydrocortisone, and thiamine during severe sepsis and septic shock. The treatment arm was associated with a lower hospital mortality (8.5% vs. 40.4%; $P < .001$).[37] Although the results are promising, randomized controlled studies are currently being performed to assess whether these findings can be confirmed before this protocol can be incorporated into the care of patients with sepsis.

Vitamin B_6

Vitamin B_6 (pyridoxine) plays an essential role in nucleic acid and protein synthetic pathways. Not surprisingly, it appears to be essential for optimal lymphocyte function, maturation and growth, antibody production, cytokine production, and natural killer (NK) cell activity.[13,14] Increased consumption of vitamin B_6 enhanced lymphocyte proliferation and cytokine production.

Folate

Folate deficiency is associated with reduced numbers of circulating lymphocytes, poor lymphocyte proliferation, impaired Th1 immunity including delayed-type hypersensitivity responses, and impaired NK-cell activity. Supplementation studies with immune response end points are few, but supplementation has improved age-related declines in NK-cell function.[13]

Vitamin B_{12}

Similar to folate, vitamin B_{12} is essential to cell reproduction including lymphocyte proliferation. Vitamin B_{12} deficiency is associated with reduced production of antibody to pneumococcal polysaccharide; however, whether repletion of vitamin B_{12} reverses this defect has not been addressed, and no causal relationship has been established.

Trace Metals

Zinc

Zinc is a dietary trace mineral that plays a critical role in the structure of cell membranes and in the function of cells of the immune system. Zinc is required for the activity of hundreds of enzymes associated with carbohydrate and energy metabolism, protein synthesis and degradation, nucleic acid synthesis, heme biosynthesis, and carbon dioxide transport.[13]

Zinc deficiency occurs most commonly in association with starvation, PEM, and malabsorption syndromes. In the developed world, zinc deficiency is seen primarily in children and elderly adults, although it is estimated that a larger proportion of North Americans may be at risk. Zinc deficiency has been documented in association with conditions of relative immunocompromise including pregnancy, alcoholism, kidney disease, burns, inflammatory bowel disease, and HIV infection.[13] Clinical manifestations of zinc deficiency include growth impairment, delayed sexual maturation, hypogonadism, impotence, oligospermia, alopecia, dysgeusia, night blindness, impaired wound healing, skin abnormalities, and impaired immunity.

Clinical trials have examined the role of zinc in immune system modulation during infection and other illnesses. For children living in developing nations, zinc supplementation limited growth stunting and reduced the duration and intensity of diarrheal illness, acute lower respiratory tract infections, and pneumonia.[38] Children receiving zinc supplements had higher $CD3^+$ and $CD4^+$ lymphocyte counts and higher $CD4^+/CD8^+$ ratios in peripheral blood and improved cell-mediated immunity compared with control subjects. Zinc supplementation also reduced the incidence of clinical disease caused by *Plasmodium falciparum*.[38] In patients with sickle cell disease, zinc supplementation increased IL-2 production and decreased microbiologically confirmed infections and hospitalizations.[39]

A number of studies have evaluated the role of zinc in protecting against the common cold. Postulated mechanisms include zinc-mediated interference with rhinoviral protein cleavage and assembly of viral particles and protection of plasma membranes against lysis by cytotoxic agents such as microbial toxins and complement; some of these effects may be due to correction of subclinical zinc deficiency. It has also been suggested that the common cold symptoms sneezing and nasal discharge may be reduced in intensity by elevations in intranasal zinc salts through production of a "chemical clamp" on trigeminal and facial nerve endings.[40] A meta-analysis of eight published randomized trials found no clear benefit for the use of zinc gluconate lozenges in the treatment of the common cold.[41] An individual patient data meta-analysis of three randomized placebo-controlled trials of zinc acetate at doses of at least 75 mg/day showed earlier recovery from cold symptoms: 70% versus 27% recovery by day 5.[42] One possible mechanism that could account for the discordant results is that acetate has lower affinity for zinc than gluconate, leading to a higher available concentration of the zinc cation. Data are mixed/inconclusive on the efficacy of zinc to prevent otitis media or as adjunctive treatment for pneumonia in children.[43,44] The benefit of zinc acetate lozenges, 75 mg/day, in treating the common cold appears to be significant, given the lack of proven alternative therapies and the minimal side-effect profile. As such, zinc acetate may be considered to have a role in the early treatment of cold symptoms.

Selenium

Selenium is essential for the function of selenium-dependent proteins, which play critical roles in the redox regulation of key enzymes, transcription factors, and receptors. Beyond its role as an antioxidant, selenium may have additional immune properties that contribute to the maintenance of normal immune function.[13] Selenium is ubiquitous in the soil and enters the diet through both plant and animal sources. Dietary intake varies depending on geographic region. Overt selenium deficiency is rare and is limited to certain regions of China. However, the effects of relative selenium deficiency on disease susceptibility and disease progression remain only partially characterized and are a subject of intense ongoing studies of both hosts and pathogen (see "Host Nutritional Status and Pathogen Virulence").

Selenium deficiency has been shown to decrease the production of free radicals and killing by neutrophils, IL-2 receptor affinity and expression on T cells, T-cell proliferation and differentiation, and lymphocyte cytotoxicity. In vitro, selenium deficiency results in enhanced neutrophil adherence to endothelial cells, an early event in the inflammatory response. In both mice and humans, supplementation with selenium has been shown to increase lymphocyte proliferative responses, IL-2 receptor expression, and macrophage and cytolytic T-lymphocyte–dependent tumor cytotoxicity. Even at plasma selenium levels associated with normal dietary intake in the United States, supplementation with 200 μg of selenium per day has considerable immunoenhancing effects, although an upper limit is likely because megadose therapy may be associated with reduced immunity.[45] There appears to be risk in selenium supplementation in terms of immune function in patients without selenium deficiency, so widespread supplementation should be avoided.

Iron

Iron deficiency is the most common trace element deficiency worldwide. It is estimated to affect 20% to 50% of the world's population including infants, children, and women of childbearing age in tropical regions.[46] The effects of iron deficiency are seen in multiple systems of the human body including the immune system.[13] In animal and human studies, iron deficiency has been associated with impairments in cell-mediated immunity, reductions in neutrophil activity with decreased myeloperoxidase activity and bactericidal activity, and diminished NK-cell activity.[13,47] Iron deficiency has been shown to impair lymphocyte and neutrophil functions in children, although no resultant increase in susceptibility to infection has been described. Whereas iron deficiency and infection often coexist in developing nations, cause-and-effect relationships have not been established in most cases. The exceptions to this include heavy infestations of hookworms leading to subsequent gastrointestinal blood loss and *Helicobacter pylori* likely causing decreased absorption and in some cases occult gastrointestinal blood loss.[48]

Many of the immune abnormalities associated with iron deficiency appear to be reversible with iron replacement, but this has been difficult to demonstrate in human studies. Studies in laboratory animals have demonstrated reversible, deleterious effects of iron deficiency on measures of functional immunity,[47] even in mildly iron-deficient animals.

Most clinicians routinely replace iron in documented iron deficiency to avoid anemia and associated morbidities. However, controversy exists regarding possible deleterious effects of iron supplementation in some settings. Many microorganisms require trace elements such as iron and zinc for survival and replication in the host and may increase in pathogenicity with supplementation. Iron deficiency appears to protect against severe malaria,[49,50] and oral iron supplementation has been associated with increased infection rates.[51,52] Furthermore, parenteral iron supplementation has been shown in human and animal studies to be harmful when administered during infection,[47] and certain pathogens such as *Yersinia enterocolitica* and *Vibrio vulnificus* thrive in the setting of iron and iron-chelating agents.[53] Therefore administration of iron, particularly intravenous iron, or iron-chelating agents such as deferoxamine should be delayed in subjects with active infection.

Fatty Acids

Three major groups of dietary fatty acids—oleic acid, linoleic acid, and linolenic acid—serve as precursors for the biosynthesis of polyunsaturated fatty acids (PUFAs). Metabolic competition exists among these groups of fatty acids, and modification of dietary fatty acid intake can lead to alterations in the fatty acid composition of tissue lipids and changes in cellular responses.

PUFAs including arachidonic acid and eicosapentaenoic acid (EPA) can be enzymatically converted to eicosanoids. Extensive data suggest a strong modulatory role for fatty acids in various cellular responses including inflammation and immune function,[54] and there is growing evidence that they also act as second messengers or regulators of signal-transducing molecules.[54] Among the fatty acids, omega-3 (ω-3) PUFAs possess the most potent immunomodulatory activities; ω-3 PUFAs found concentrated in fish oil, EPA, and docosahexaenoic acid are more biologically potent than α-linolenic acid.

A number of clinical trials have assessed the benefits of dietary supplementation with fish oils in several inflammatory and autoimmune diseases in humans including rheumatoid arthritis, Crohn disease, ulcerative colitis, psoriasis, lupus erythematosus, and multiple sclerosis. Animal and clinical studies of acute respiratory distress syndrome and sepsis suggest that a high-fat diet containing EPA (fish oil), γ-linolenic acid (borage oil), and antioxidants can improve lung microvascular permeability, oxygenation, and cardiopulmonary function; reduce proinflammatory eicosanoid synthesis and lung inflammation; and improve survival (see "Surgical and Critically Ill Patients").[55,56]

OVERNUTRITION: OBESITY AND INFECTIOUS DISEASES

Obesity, defined as a BMI greater than 30, is epidemic in the United States and rapidly increasing globally (www.who.int/mediacentre/factsheets/fs311/en/index.html). The association of obesity with diabetes, cardiovascular disease, osteoarthritis, and many other chronic illnesses is well known, but the impact of obesity on infection and immunity is a relatively new field. Infection risk and outcomes for many syndromes are influenced by obesity but not in a uniform direction (Table 11.1). For example, it has been well documented that obesity was a major risk factor for adverse outcomes in hospitalized subjects during the 2009 pandemic H1N1 influenza outbreak. Additionally, obesity is a risk factor for surgical site infection, prosthetic joint infection, and hospital-acquired infections. However, and perhaps surprisingly, there is a strong and consistent association with *better* clinical outcomes in obese patients with community-acquired pneumonia compared with their nonobese counterparts.[57] Several excellent reviews have outlined the impact of obesity on infectious disease acquisition and outcome as well as postulated immune changes that may contribute to these clinical findings.[58,59,60]

The immune mechanisms affected by obesity are broad, involve all aspects of the immune response, and in general upregulate inflammatory responses while diminishing most cellular responses, but there are notable exceptions.[61,62,63,64] In humans, obesity has been associated with an increased inflammatory milieu that has been hypothesized to increase the production of short-term memory T-cell and B-cell responses but impaired memory responses (Fig. 11.1). This is consistent with the finding that obesity does not impair initial vaccine responses to influenza and other antigens but shortens the duration of protective immunity.[65,66,67,68]

At least in the case of 2009 H1N1 influenza, overexuberant inflammatory responses may have been a major driving factor in adverse outcomes and were likely mediated by the high leptin levels in obesity. Increased mortality in obese mice, similar to that seen in obese humans, is markedly attenuated by administration of antileptin antibodies.[69]

SPECIAL POPULATIONS: CLINICAL TRIALS OF NUTRITIONAL SUPPLEMENTATION TO REDUCE INFECTION RISK

To illustrate the general issues discussed to this point, three clinical populations of specific interest are highlighted: surgical and critically ill patients, patients with HIV/AIDS, and older adults. These populations are frequently encountered by infectious disease specialists, and research in these three groups has been of particularly high quality, with well-designed epidemiologic and interventional studies.

Surgical and Critically Ill Patients

Total Parenteral Nutrition Versus Enteral Nutrition[a]

Enteral and parenteral nutrition have been compared in a number of conditions in critically ill patients in randomized controlled trials; these have confirmed the utility of using enteral nutrition whenever possible. Furthermore, in acute pancreatitis, enteral nutrition was associated with reduced risks for death, multiple organ failure, systemic infection, and local septic complications as well as reduced length of stay.[70] For severe pancreatitis, the reduction in mortality for enteral versus parenteral nutrition was greater than 80% (RR, 0.18; 95% CI, 0.06–0.58).[70] Thus the adage of "if the gut works, use it" appears applicable across the breadth of critical care patient populations, and enteral nutrition should be used whenever possible. Total parenteral nutrition can be useful in patients for whom enteral nutrition cannot be applied. Data from an international survey of nearly 3000 critically ill patients suggested a strong inverse relationship between 60-day mortality and ventilator-free days with total daily calorie intake, particularly in patients with a BMI less than 25 or greater than 35, and this was true even in the 25% of patients who received total parenteral nutrition alone or in combination with enteral nutrition to achieve caloric goals.[71] Randomized controlled studies in both adult and pediatric critically ill patients show that delayed initiation of parenteral nutrition does not change overall mortality and leads to decreased rates of infection.[72,73]

Immunonutrition

Although the mechanism through which nutritional supplementation exerts positive effects on critically ill patients is uncertain, the reduced infection risk with enteral feeding is hypothesized to be related to the promotion of intestinal mucosal integrity, blunting of proinflammatory responses, and enhanced wound healing.[74,75,76] This hypothesis has given rise to the concept of "immunonutrition"—specific formulations to replace micronutrients that frequently become deficient in acute inflammatory states and to provide antiinflammatory nutrients that reduce infection risk.[74] Studies using glutamine, arginine, *N*-acetylcysteine, branched-chain amino acids, nucleotides, ω-3 PUFAs, antioxidant vitamins and trace elements, taurine, and various mixtures of these compounds as supplements have been investigated. Guidelines published by the Society of Critical Care Medicine and American Society for Parenteral and Enteral Nutrition in 2016 make a number of recommendations regarding immunonutrition. The guidelines recommend routine use of an immune-modulating formula (containing both arginine

[a]An outstanding reference with exhaustive and well-organized topical reviews of the concepts discussed in this section can be found at http://criticalcarenutrition.com/index.php?option=com_content&view=category&layout=blog&id=21&Itemid=10.

TABLE 11.1 Risk for Acquiring Infections and/or Adverse Outcome in Obese vs. Nonobese Patients

INFECTION/ SYNDROME	RISK FOR ACQUISITION[a]	RISK FOR ADVERSE OUTCOME[a]	COMMENT	REFERENCES
Respiratory Viruses				
Influenza (H1N1 2009 pandemic)	±	≫	Obesity clearly increases risk for morbidity and mortality in hospitalized patients with H1N1 2009 strain, but the limited data regarding acquisition do not suggest a big increase in risk. Elevated leptin, impaired CD4 and CD8 T-cell responses, and poor TLR-based responses in obese subjects may play a pathogenic role.	Multiple; reviewed in 61, 62, and 63
Influenza (non-H1N1)	±	>	Weaker database than for H1N1, but some data suggest hospitalization due to influenza more likely in obese patients, but obese patients not more likely to acquire influenza. Adequacy of vaccine response depends on outcome measured (seroprotection/ seroconversion [which is not impaired] vs. duration of antibody rise, memory cell production [which is impaired]).	65, 66, 67, 68
Respiratory syncytial virus	NR	> (pediatric data only)		132
Community-Acquired Bacterial Pneumonia				
	±	≪	Risk for acquisition data greatly confounded by associated comorbidities; once these are adequately controlled, there is little/no association of obesity with acquisition of pneumonia. There is a surprising and consistent *reduction* in mortality in obese patients vs. nonobese patients.	Risk: 133 (reviewed in 62) Outcomes: 134–138
Bacteremia and Sepsis				
	>	> (bacteremia) < (sepsis)	Bacteremia *without sepsis* on presentation is associated with increased mortality in obese patients in small studies. Although obese subjects have a greater risk for sepsis, when presenting with *sepsis, severe sepsis,* and *septic shock,* they fare *better* than nonobese subjects.	139–142
Surgical Site Infections				
	≫	NR	Consistent increase across general, colorectal, spinal, joint replacement, cesarean section, and other surgeries	Multiple; reviewed in 143–146
Bone and Joint Infections				
Osteomyelitis	NR	NR	Clearly associated with DM, but no data about obesity risk alone when appropriately controlling other comorbidities	
Septic arthritis	NR	NR		
Prosthetic joint infection	≫	NR	May be due to increased surgical time, underdosing of prophylactic antibiotics, etc.	58, 147–151
Urinary Tract Infection				
	>	NR	More prominent effect of obesity in male patients than in female patients	59, 60
Hospital-Acquired Infections[b]				
	≫	>	Worse outcomes in obese patients include longer length of stay and ventilator duration in addition to adverse clinical outcomes (e.g., mortality)	152–156
***Helicobacter pylori* Infection**				
	≫	>	Multiple studies demonstrate increased prevalence of *H. pylori* in obese individuals perhaps due to lack of *H. pylori* altering ghrelin expression and predisposing to obesity, rather than obesity predisposing to *H. pylori* infection	157–159
HIV Infection				
	NR	>	Greatly increased obesity prevalence, particularly among women with HIV; weight gain is substantial after initiating ART; obesity associated with increased risk for DM, cardiovascular disease, frailty, and multimorbidity	160–164

[a]≫, substantially greater risk; >, greater risk; ±, little difference; <, less risk; ≪, substantially less risk; NR, not reported.
[b]Consists of hospital-acquired bacteremia, catheter-related infection, pneumonia, urinary tract infection, and *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.
DM, Diabetes mellitus; *ART,* antiretroviral therapy; *HIV,* Human immunodeficiency virus; *TLR,* Toll-like receptor.
Modified from data from references 165–167.

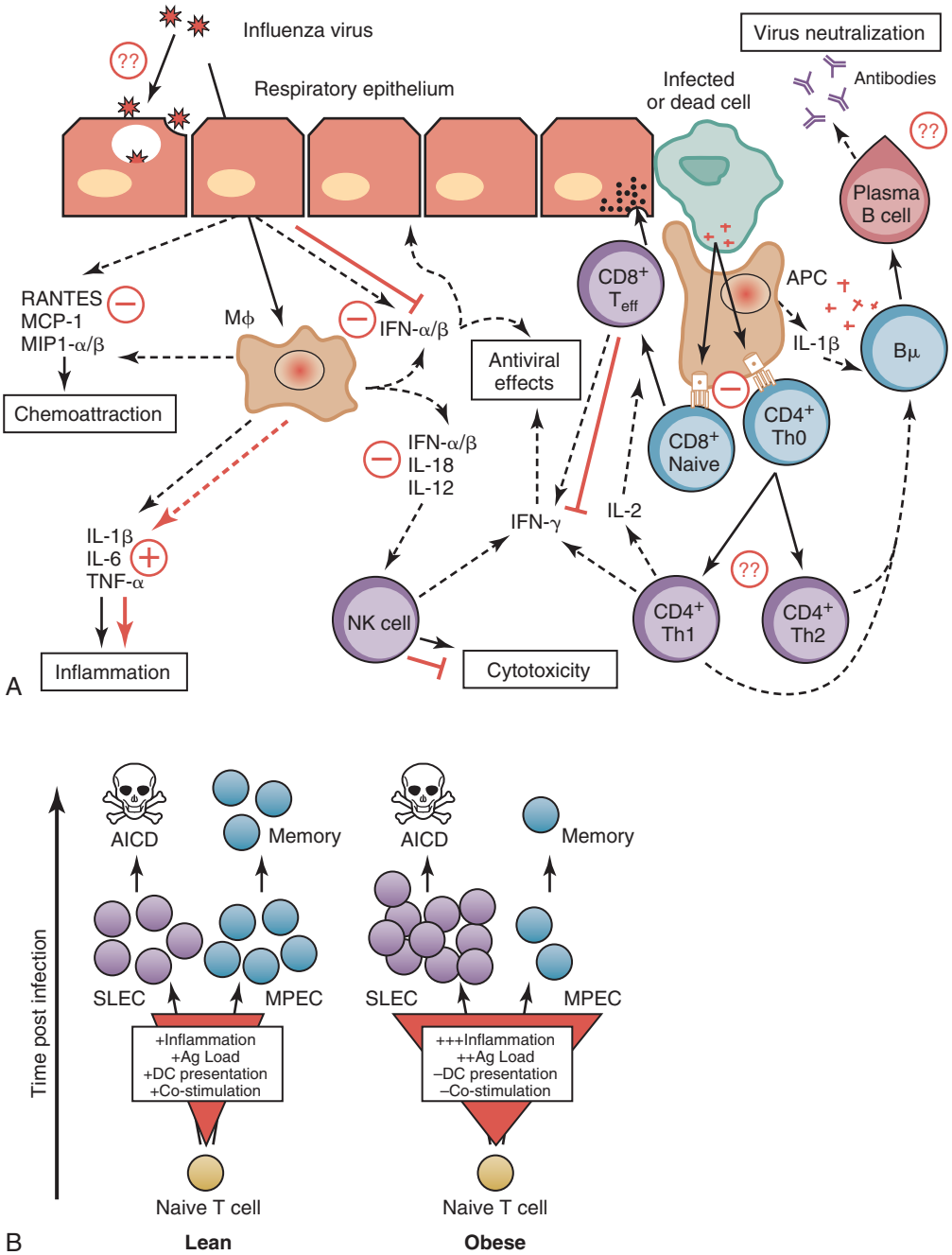

FIG. 11.1 Hypothesized alterations in immune responses to influenza in obese hosts. (A) Influenza infection and response processes modified by obesity are shown in red. (B) Net result of obesity is elevated levels of short-lived effector cells (*SLEC*) but impaired long-lived memory cells. *AICD,* Activation-induced cell death; *APC,* antigen-presenting cell; *DC,* dendritic cell; *IFN,* interferon; *IL,* interleukin; *MCP,* monocyte chemotactic protein; *MPEC,* memory precursor effector cells; *NK,* natural killer; *RANTES,* regulated on activation, normal T cell expressed and secreted; *TNF,* tumor necrosis factor. *(From Karlsson EA, Beck MA. The burden of obesity on infectious diseases.* Exp Biol Med (Maywood). *2010;235:1412–1424.)*

and fish oils) in patients with severe trauma and postoperative surgical ICU patients requiring enteral nutrition therapy and recommend the use of either arginine-containing immune-modulating formulations or EPA/docosahexaenoic acid supplement with standard enteral formula in patients with traumatic brain injury. The guidelines further recommend against adding supplemental enteral or parenteral glutamine routinely in critically ill patients and that immune-modulating formulas not be used routinely in patients with severe sepsis.[77]

A Cochrane review of 19 randomized controlled trials (with 1099 participants) assessing the effects of immunotherapy in postoperative outcomes in head and neck cancers found there were no differences in mortality or risk of infection, but there was a decreased risk in fistula formation in the immunotherapy arm (5.4% vs. 11.3% absolute risk).[78]

A multicenter randomized controlled trial comprising 301 patients on assisted ventilation in the ICU (including medical, surgical, and trauma patients) assessed the use of high-protein feeds with or without immunomodulators (glutamine, ω-3 fatty acids, and antioxidants) and found no difference in infection rates, but noted an increased 6-month mortality in the immunomodulator arm for the subgroup of medical patients (54% vs. 35%, $P = .04$).[79] These findings along with others showing trends toward increased 6-month mortality with glutamine supplementation[80] without clinical benefit[79,80,81] indicate serious safety concerns for medical patients receiving immunomodulating nutritional supplements that include glutamine. These findings have led to the update in guideline recommendations that had previously recommended glutamine supplementation for all critically ill patients.[82]

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

Recognition of malnutrition and principles of nutritional management are important aspects of the primary care of patients with HIV infection.[83,84] Severe malnutrition was one of the first recognized manifestations of advanced disease in HIV-infected patients, initially described as "slim disease" in early reports from Africa.[85] Wasting syndrome was later defined by the Centers for Disease Control and Prevention as a body weight loss equal to or greater than 10% with associated fatigue, fever, and diarrhea unexplained by another cause; however, any weight loss of more than 5% is associated with accelerated disease progression, impaired functional status, and increased mortality.[86] Early in the HIV epidemic, studies reported wasting in up to 20% of patients at the time of AIDS diagnosis in the United States. Despite advances in the management and treatment of HIV infection, wasting syndrome and other forms of malnutrition remain highly prevalent in HIV-infected patients.[87] Wasting is associated with increased mortality and remains a significant prognostic factor in advanced HIV disease.

The cause of AIDS-associated wasting is multifactorial.[88,89] Wasting has been associated with decreased oral intake, malabsorption syndromes, endocrine dysfunction, and increased cytokine production. Reductions in food intake may be caused by disease-associated or drug-associated anorexia, central nervous system dysfunction, dysphagia, and odynophagia. Absorption may be impaired by infectious or drug-associated intestinal inflammation, dysfunction, and diarrhea. Endocrine abnormalities include alterations in thyroid and adrenal function and fluctuations in growth hormone levels. Production of proinflammatory cytokines leads to accelerated metabolic degradation of essential micronutrients, further compromising the HIV-infected host. The use of nutritional therapy throughout the course of HIV infection and with increased intensity during symptomatic infection can slow and perhaps reverse the compounding effects of nutrition-associated immunodeficiency.

PEM is the most common form of malnutrition seen in HIV disease worldwide, but alterations in the stores of fat-soluble and water-soluble vitamins and trace elements are also seen. Vitamin A deficiency has been associated with the progression of HIV disease, development of secondary infections, increased HIV-associated mortality, and increased maternal-fetal transmission.[90–92] Deficiencies of water-soluble vitamins appear to occur less frequently than deficiencies of fat-soluble vitamins, and only cobalamin (vitamin B_{12}) deficiency is associated with HIV disease progression.[93] Of the trace elements, deficiencies in iron, zinc, and selenium have been described. Zinc levels decline as HIV disease progresses, and zinc supplementation in HIV infection has been shown to improve immune responses.[94]

Selenium deficiency occurs more commonly in HIV-infected patients, as documented by low plasma and red blood cell levels of selenium, diminished activity of glutathione peroxidase, and low selenium levels in cardiac muscle of patients with AIDS. Plasma selenium concentrations may be reduced by 50% in patients with AIDS, with lesser reductions seen in patients receiving antiretroviral therapy.[95,96] The mechanism of selenium depletion in HIV-infected patients is poorly understood and is probably multifactorial. Declines in selenium levels have been documented even in the earliest stages of HIV infection,[97] and accelerated progression of HIV disease has been described in patients with selenium deficiency.[98]

Selenium supplementation trials in patients with HIV infection improved markers of oxidative stress, improved $CD4^+$ T-cell counts, and, in pregnant women in Tanzania, reduced mortality associated with diarrheal illness.[99] Based on the consistency of the literature, we recommend zinc and selenium supplementation in patients with HIV and deficiencies of these micronutrients.

Strategies to Combat Weight Loss and Wasting Associated With Human Immunodeficiency Virus

Appetite stimulants, hormone replacement therapy, and anticytokine therapies—all with or without exercise—have been used in small clinical trials to combat wasting syndrome in HIV-infected patients with variable success. Treatment with anabolic steroids, particularly intramuscular testosterone in testosterone-deficient men, may offer sustainable gains in muscle mass and improvements in mood and quality of life. A full examination of these strategies is beyond the scope of this review, but specific nutritional approaches are evaluated in the following paragraphs.

Macronutrient Supplementation

Macronutrient (calorie and protein) supplementation was evaluated in a Cochrane Collaboration Review by Grobler and colleagues.[100] Fourteen trials comprising 1996 HIV-positive participants including 245 children met the stringent criteria for inclusion; excluded were studies of more than one nutritional intervention, studies involving pregnant women, and studies examining total parenteral nutrition versus enteral nutrition. Total caloric and total protein intakes were enhanced by supplementation of 600 to 960 kcal/day, but body weight, fat mass, fat-free mass, $CD4^+$ count, and HIV viral load were unchanged.

As pointed out in the Cochrane Review, most studies were small, and combining studies is difficult given the varying formulations used. A meta-analysis showed that in studies in which underweight participants were targeted an increase in weight, BMI, and $CD4^+$ counts was detected with supplementation, whereas studies that recruited subjects regardless of baseline nutritional status did not show differences in these parameters.[101] Very little data exist in older age groups or in HIV-infected patients with lipodystrophy or obesity, in whom nutritional supplementation could result in increased rates of diabetes or cardiovascular disease. Patients with HIV who are underweight should be treated with macronutrient supplementation to improve their BMI and $CD4^+$ counts as an aid to improving their immune function.

Micronutrient Supplementation

Micronutrient deficiency is even more common than macronutrient deficiency in the developing world, and there is strong epidemiologic evidence linking micronutrient deficiencies to adverse outcomes in many infectious diseases including HIV infection. A Cochrane Systematic Review evaluating micronutrient supplementation in HIV-infected children and adults was published in 2005 and then updated in 2010 and again in 2017.[99] An important feature of this meta-analysis is that most of the studies were conducted in resource-poor countries where malnutrition is very common. The analysis included 33 studies involving 10,325 HIV-positive participants. Most participants came from multiple micronutrient supplementation studies. The nutrients studied were vitamin A, vitamin D, vitamin E, folinic acid, zinc, and selenium.

In essence, micronutrient supplementation had very little effect on morbidity, mortality, or surrogate markers of immune function in adults. We agree with the authors who note that trials have not revealed consistent clinically important benefits with routine multiple micronutrient supplementation in people living with HIV, but that should not lead to denial of supplementation of patients with micronutrient deficiencies.

Two important subgroups of HIV-positive patients are pregnant women and children. Nutritional indicators of adverse outcomes in pregnancy for HIV-infected women in Africa include a BMI of less than 21.8, a hemoglobin concentration of less than 8.5 g/dL, and weight loss or excessive weight gain during pregnancy.[102] One relatively large (N >1000) randomized trial of multivitamin/mineral supplementation in pregnant or lactating Tanzanian women demonstrated benefit for both mothers and infants.[92,103,104] Women experienced less AIDS-related progression of disease and mortality, fewer adverse pregnancy outcomes, and less diarrheal morbidity. Infants of mothers with impaired immunologic and nutritional parameters suffered less early childhood mortality if they were born to women in the multivitamin-supplemented group. A study from Malawi in iron-deficient children with HIV found that children given iron supplementation had improved hemoglobin and $CD4^+$ counts but had increased incidence of malaria.[105] Clinicians giving iron supplementation to children in malaria-endemic regions should consider these findings and encourage enhanced malaria prophylaxis including interventions such as bednets.

Older Adults

Older adults represent a population at significant risk for malnutrition and its related health problems. Malnutrition and decreased oral intake in older adults are often multifactorial. In the inpatient setting, "nothing

by mouth" orders, inability to self-feed, and increased caloric needs head the list. In the outpatient setting, depression, medications, dental or swallowing problems, and social issues (e.g., choices between food and medicine) are paramount. Studies using anthropometric measures and laboratory values estimate 40% to 60% of hospitalized older adults are malnourished.[106,107] A prospective study of non–terminally ill elderly patients discharged from an acute care hospital found a BMI less than or equal to 20 kg/m^2 to be associated with a markedly increased risk for death within 1 year.[108] Among long-term and subacute care residents, the prevalence of malnutrition is 15% to 72%,[109,110] and malnutrition is even prevalent in community-dwelling seniors (Table 11.2). Micronutrient deficiencies are also prevalent among older adults, with 10% to 30% having subnormal levels of some vitamins or minerals (see Table 11.2). A prospective study in France of 252 patients older than 70 years of age who were admitted for rehabilitation found that lower baseline energy intake and lower albumin, zinc, selenium, and vitamin C levels were risk factors for developing health care–associated infections. These associations were not significantly changed by adjusting for T-cell subset values.[110]

Nutritional Supplements in Older Adults: Effects on Immunity and Clinical Outcomes

Although malnutrition in elderly persons is clearly associated with impaired immunity and poor clinical outcomes, nutritional supplementation has not definitively been shown to reverse this trend. Many studies examined only immune response variables rather than clinical end points, and these have been reviewed.[14,111,112] Studies specifically addressing clinical end points are emphasized in the following paragraphs for the most well-studied entities: multivitamin/mineral supplements, vitamin E, and zinc.

Multivitamin and Trace Mineral Supplements

Studies of multivitamin/mineral supplementation for the prevention of infection that have been performed in both outpatient healthy elderly persons and long-term care residents are summarized in Table 11.3.[113–118] Overall, it has been difficult to show any clear benefit from multivitamin/mineral supplementation in community-dwelling older adults. In long-term care residents, a series of studies hints that trace mineral (zinc

TABLE 11.2 Prevalence of Nutritional Deficiencies in Older Adults Residing in Various Settings

NUTRIENT	LONG-TERM CARE OR HOSPITALIZED (% DEFICIENT)	COMMUNITY-DWELLING (% DEFICIENT)
Protein/calories	20–50	1–16
Vitamin A	1–2	ND
Vitamin B_{12}	ND	20
Vitamin D	50–85	50
Vitamin E	1–3	1
Zinc	30–50	10

[a]Data are pooled from multiple studies. See references 106, 107, 109, 115, 118–120, 168–171.
ND, No data.

TABLE 11.3 Randomized, Placebo-Controlled Multivitamin/Mineral Supplementation Trials in Older Adults

REFERENCE (Year)	NO. AND POPULATION STUDIED	STUDY DURATION	RANDOMIZED GROUPS	OUTCOME MEASURED	COMMENT
Girodon et al.[113] (1997)	81 NH residents	24 months	1. Daily MVI 2. Daily Zn^{2+}, Se 3. Daily MVI, Zn^{2+}, Se 4. Placebo	MD-confirmed respiratory or urogenital infection–related mortality	No mortality difference; lower infection with TM[a] or TM[a] + MVI, but not with MVI alone
Girodon et al.[114] (1999)	725 NH residents	24 months	1. Daily MVI 2. Daily Zn^{2+}, Se 3. Daily MVI, Zn^{2+}, Se 4. Placebo	MD-confirmed respiratory or urogenital infection–related mortality	No mortality difference; borderline reduction in infection (P = .06) and improved influenza vaccine responses with TM, but not with MVI alone
Graat et al.[115] (2002)	652 community-dwelling older adults	15 months	1. Daily MVI, Zn^{2+}, Se 2. Daily MVI, Zn^{2+}, Se, vitamin E 3. Vitamin E 4. Placebo	Self-reported respiratory tract infection	No difference in incidence of infection; significantly worse symptom severity in vitamin E recipients
Barringer et al.[116] (2003)	130 community-dwelling older adults (33 were >65 y; others had DM)	12 months	1. Daily MVI, Zn^{2+}, Se 2. Placebo	Self-reported infection confirmed by MD	Lower incidence of infection overall (P < .001) and in subset with type 2 DM (P < .001), but not in elderly subset (P > .2)
Avenell et al.[117] (2005)	910 community-dwelling older adults	12 months	1. Daily MVI, Fe, I, Zn^{2+}, Mn 2. Placebo	No primary care MD visits for infection, self-reported infection, quality of life	No significant difference in any parameter measured
Liu et al.[118] (2007)	763 NH residents	18 months	1. Daily MVI, Ca, Mg, Fe, I, Cu, Zn^{2+}, Se 2. Placebo	Infection control practitioner surveillance for all infections, hospitalizations, and antibiotic use	No significant difference except in post hoc analysis: after excluding subjects with dementia, infection risk was reduced in remaining NH residents (RR, 0.81; 95% CI, 0.66–0.99).

[a]Zinc and selenium supplementation.
Ca, Calcium; *CI,* confidence interval; *Cu,* copper; *DM,* diabetes mellitus; *Fe,* iron; *I,* iodine; *MD,* medical doctor; *Mg,* magnesium; *Mn,* manganese; *MVI,* multivitamin; *NH,* nursing home; *RR,* relative risk; *Se,* selenium; *TM,* trace minerals; *Zn^{2+},* zinc.

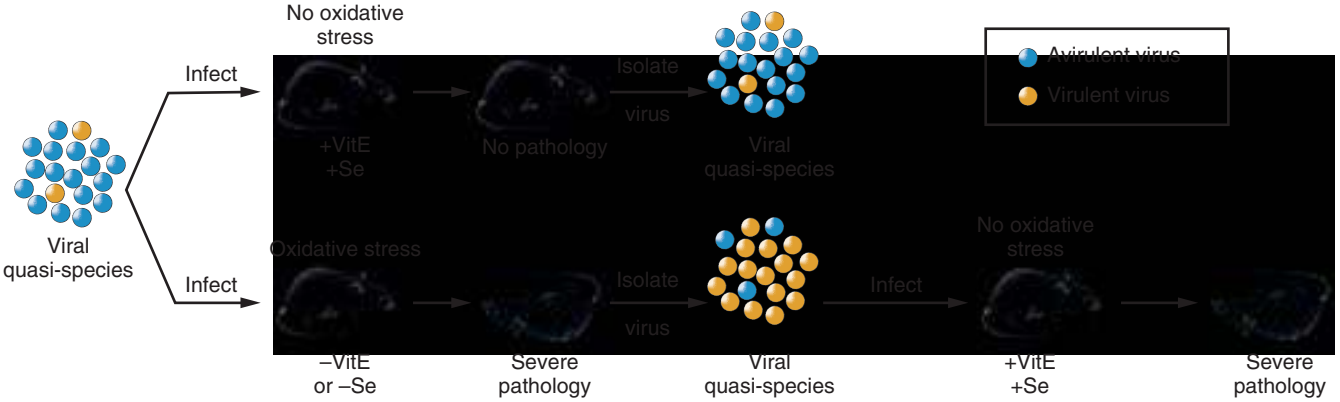

FIG. 11.2 Effect of malnutrition on virulence of a viral pathogen. Replication of relatively nonvirulent strains of a virus within a malnourished host (e.g., mouse with selenium [*Se*] deficiency) leads to hypermutation within the viral genome, resulting in the emergence of more virulent strains. Once the hypervirulent quasispecies emerge, they cause more serious illness even in nutritionally replete hosts. *VitE,* Vitamin E. *(From Beck MA, Handy J, Levander OA. Host nutritional status: the neglected virulence factor.* Trends Microbiol. *2004;12:417–423.)*

and selenium) supplementation may be more effective than multivitamins for reducing the incidence of respiratory tract infection, but there is little effect on urinary tract infection or pressure ulcers.[113,114] Furthermore, patients without dementia may benefit most from supplementation, based on a post hoc analysis of one study.[118]

Vitamin E

Vitamin E has been shown to enhance both humoral and cell-mediated immune responses in elderly individuals. Supplementation with vitamin E at 200 mg/day or 800 mg/day in healthy older adults improved delayed-type hypersensitivity responses and immunization responses to hepatitis B, but not to pneumococcal polysaccharide or tetanus.[34] However, in another study, the severity of symptoms due to infection was significantly *worse* in the vitamin E–supplemented group, with greater total illness duration and number of symptoms and more frequent fever and activity restriction (compared with subjects receiving placebo),[115] although it is possible that these findings represent enhanced immune responses in the vitamin E recipients. One additional study in long-term care residents showed varied benefit from vitamin E supplementation, depending on the outcome measured.[119] There was no difference in the number of overall days of respiratory tract infection, the primary end point of the study, but a lower proportion of subjects in the vitamin E group experienced one or more respiratory tract infections (RR, 0.88; 95% CI, 0.76–1.0), and the effect appeared to be most prominent for upper (vs. lower) respiratory tract infections. There was no effect on the amount of antibiotic use. A subanalysis of this study implicated low serum zinc levels as a risk factor for pneumonia in these subjects,[120] suggesting that trace minerals should be a focus of subsequent studies. Furthermore, there may be interactions between genotypes at specific loci (e.g., IL-10), sex, and vitamin E on risk for respiratory tract infection.[121] The same study suggested that specific single nucleotide polymorphisms of IL-1, IL-10, and interferon-γ have lower risk for respiratory infection regardless of vitamin E supplementation. There are also complex interactions of vitamin E supplementation in male smokers and risk for upper respiratory tract infections, pneumonia, or tuberculosis depending on duration and severity of prior smoking, activity level, and other vitamin supplementation.[122–125] Much work remains to determine people who may benefit or not from vitamin E supplementation for reducing infection risk.

It is clear that high doses of vitamin E should be avoided for most older adults. A meta-analysis of vitamin E supplementation trials demonstrated increased mortality for older adults receiving daily doses of 400 IU or greater.[126]

Zinc

As noted earlier, zinc has a role in immune function. Zinc deficiency is more common in elderly adults.[127] Zinc supplementation was included in all the clinical trials of vitamin E or multivitamin supplementation to prevent infection in older adults cited previously. Zinc supplementation was studied as the primary micronutrient provided to institutionalized elderly adults in an additional study,[128] and trends found were lower risk for overall infection, fever, and upper respiratory tract infections. An important insight regarding zinc and infection risk was noted in a reanalysis of a prior trial based on end-of-trial serum zinc level.[120] In that study, the incidence of pneumonia, duration of pneumonia, and use of antibiotics for pneumonia all were dramatically reduced in subjects with an end-of-trial serum zinc level of more than 70 µg/dL, whereas baseline serum zinc level was not associated with any of these outcomes. This indicates that the ability to increase serum zinc levels—perhaps due to unmeasured factors such as absorptive capacity, comorbidity, or zinc excretion—is a strong indicator of pneumonia risk, but the role of the zinc replacement itself remains to be defined.

HOST NUTRITIONAL STATUS AND PATHOGEN VIRULENCE

An important link of infection and malnutrition may not lie within the host's impaired response but instead within the pathogen itself, which may be altered by specific conditions present in the nutritionally impaired host. In selenium-deficient mice, infection with either coxsackievirus B or influenza virus led to the rapid development of mutations in the viral genome; in the case of influenza, mutations occurred specifically in the M1 and M2 matrix proteins. These mutations altered the virulence of influenza, increasing the severity of illness in subsequent hosts even if those hosts were adequately nourished (Fig. 11.2).[129,130] It appears that the redox status of the host is the key factor governing this process. In follow-up studies, Gay et al.[131] demonstrated that advanced age, characterized by a limited capacity to buffer oxidative stress (similar to selenium deficiency), could also induce the accelerated mutation rate and virulence change in coxsackievirus.

Although animal data support the model of enhanced mutation rates and augmented virulence shown in Fig. 11.2, there are sparse data in humans. There have been limited investigations of mutation rates in subjects with marginal selenium status.[129] Selenium supplementation was provided to a small group of human subjects before, during, and after administration of live oral poliovirus vaccine, and stool samples were collected to determine the mutation rate in the selenium-supplemented and control groups. The mutation rate of the oral poliovirus vaccine strain was significantly higher in the group that did not receive selenium supplementation, supporting the animal findings previously outlined. This observation led to the theory that hosts who are nutritionally deficient (e.g., selenium deficient) or who are oxidatively stressed (e.g., aged adults[131]) may provide an environment that induces a high mutation rate in infecting pathogens, leading to enhanced virulence (see Fig. 11.2).

ACKNOWLEDGMENTS

We acknowledge the contributions of Caryn Gee Morse and Kevin P. High, the authors of this chapter in previous editions of *Principles and Practice of Infectious Diseases*, whose basic chapter structure has been retained and updated for the 9th edition.

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The complete reference list is available online at Expert Consult.

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12 Evaluation of the Patient With Suspected Immunodeficiency

Steven M. Holland and John I. Gallin[a]

SHORT VIEW SUMMARY

Overview
- Infection is often the first and potentially the deadliest manifestation of congenital or acquired immunodeficiency.
- Infections involving common pathogens can be more prolonged, recurrent, or severe than in normals, or may be due to opportunistic organisms.
- A history of frequent infections and/or an unusual pathogen or site of infection should be a trigger for clinicians to consider that an immunodeficiency syndrome may be present; which may be either congenital or acquired.

Etiology
- The genetic disorders underlying the most common congenital problems have been gradually uncovered, providing strong tools for proper classification and leading to clinical approaches (see Table 12.2).

Infecting Agents
- The spectrum of infections associated with genetic immunodeficiencies is broad, including herpes simplex encephalitis, staphylococcal abscesses, *Burkholderia* bacteremia, mycobacterial osteomyelitis, severe human papillomavirus infections, disseminated coccidioidomycosis, and *Aspergillus* pneumonia, in addition to severe cytomegalovirus and *Pneumocystis,* among many others.
- However, particular infecting agents often are associated with specific immunodeficiency conditions (see Table 12.1), providing immediate clues about the probable basis of the illness.

Other Manifestations
- Immunodeficiency is also commonly associated with autoimmunity, reflecting the failure to protect against autoreactivity and invasion.

Diagnostic Approach
- Based on the type of clinical presentation, particular diagnostic tests can be used to determine the particular immunodeficiency present.
- A small battery of screening tests, emphasizing complete blood count, immunoglobulin levels and complement, and neutrophil function, will enable detection of most common disorders (see Table 12.3). Based on those findings and the clinical presentation, more specific functional assays can be undertaken (see Table 12.4).

Therapy and Prevention
- The clinical presentation, results of diagnostic testing, and characterization of the genetic lesion will dictate the therapeutic approach, and in some cases a preventive strategy.

The most common causes of immunodeficiency are iatrogenic and result from the widespread use of therapies that modulate the immune system, either by design or incidentally. With the expanding recognition, characterization, and—in an increasing number of cases—correction of immune abnormalities, making the correct diagnosis is of critical importance. Identification and cloning of disease-related genes has now made precise antenatal diagnosis and genetic counseling a reality. With successful stem cell transplantation and the emerging field of gene therapy, it is essential to use a sensible, problem-oriented approach to the patient suspected of having an immunodeficiency. What follows are some general principles involved in the consideration of whether a patient may have an immunodeficiency and how to proceed with a diagnostic evaluation before, or as an adjunct to, referral or discussion with a specialist.

INDEX OF SUSPICION

Concern about the immune status of a patient is usually raised on the basis of the frequency or severity of infections or the finding of an unusual infectious agent. Table 12.1 lists some infectious organisms and the affected limb of host defense implied by their isolation. Clearly, not every isolation of a herpesvirus or *Staphylococcus* implies an immunodeficiency in a specific patient. However, in the setting of abnormally frequent or severe infections or failure to thrive, isolation of these organisms from patients should make one consider possible underlying diagnoses. In contrast, identification of *Pneumocystis, Mycobacterium, Burkholderia cepacia, Aspergillus,* or *Nocardia* from a patient without a known immunodeficiency is sufficient grounds for pursuing the probability of an underlying defect.

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

Recurrent hematogenous neisserial infections indicate deficiencies in the late components of complement.[1] *Pneumocystis (carinii) jirovecii* pneumonia indicates T-cell abnormalities.[2] *B. cepacia* bacteremia strongly suggests chronic granulomatous disease.[3] Some specific immunodeficiencies are listed in Table 12.2, along with the gene defects, if known, and some pertinent findings. Recognition and appreciation of the genetic bases of these disorders have been critical to the development of therapy for them and are fundamental to the curative approaches now available.

INITIAL EVALUATION

The screening approach to a patient with suspected immunodeficiency is listed in Table 12.3. Careful attention to historical detail is critical. Age at onset of the illness is helpful: hyperimmunoglobulin E–recurrent infection syndrome (HIES or Job syndrome) often has an onset within the first days to weeks of life, whereas antibody deficiency states appear only after several months of life, when maternal immunoglobulin (Ig) levels have fallen.[4] Failure to thrive and diarrhea are important points in favor of a substantial problem but are not specific in terms of etiology. Birth history should include the condition and time of separation of the umbilical stump because stump separation is abnormally delayed in leukocyte adhesion deficiency type 1 (LAD1).[5] The past medical history should note the administration of vaccines, especially for measles, mumps, and rubella, and Calmette-Guérin bacillus (BCG), difficulties with which are suggestive of dysfunctional T-cell or monocyte immunity. A dental history can be quite informative because patients with abnormalities of phagocytic defense often have gingivitis with periodontal disease[6] (Fig. 12.1); patients with Job syndrome usually have prolonged retention of the primary teeth,[7] and patients with nuclear factor kappa B (NF-κB) essential modulator (NEMO) deficiency often have conical or widely spaced teeth. Specific questioning regarding parental consanguinity is critical, even if uncomfortable.

TABLE 12.1 Selected Pathogens Associated With Immunodeficiency Diseases

PATHOGEN	HISTORY	HOST DEFENSE AFFECTED	CLINICAL EXAMPLES
Pneumocystis (carinii) jirovecii, Cryptococcus neoformans, herpesviruses	Disseminated infections, opportunistic infections, persistent viral infections	T cells	Severe combined immunodeficiency, acquired immunodeficiency syndrome
Haemophilus influenzae, Streptococcus pneumoniae, Giardia lamblia, Campylobacter spp., enteroviruses	Recurrent respiratory infections with encapsulated organisms, chronic diarrhea, aseptic meningitis	B cells	Common variable immunodeficiency, X-linked agammaglobulinemia
Staphylococcus aureus, Burkholderia cepacia, Serratia marcescens, Aspergillus spp., *Nocardia* spp.	Gingivitis, aphthous ulcers, recurrent pyogenic infections, delayed umbilical stump separation	Phagocytes	Chronic granulomatous disease, Chédiak-Higashi syndrome, leukocyte adhesion deficiency
Nontuberculous mycobacteria, BCG, *Salmonella, Histoplasma capsulatum, Coccidioides immitis*	Disseminated infections, multifocal osteomyelitis	Monocytes	Interferon-γ/IL-12 pathway defects, STAT1, NEMO, GATA2
S. pneumoniae, Neisseria meningitidis, S. aureus, herpes simplex virus	Recurrent meningitis, bacteremia, lack of fever	TLR pathway	MyD88, IRAK-4 defects, TLR defects
Neisseria spp.	Recurrent bacteremia, recurrent meningitis	Complement	Late complement component deficiency
S. aureus, H. influenzae, S. pneumoniae, Candida albicans	Eczema, kyphoscoliosis, pathologic fractures, pulmonary and cutaneous infections mucocutaneous candidiasis	T cells, phagocytes	Hyperimmunoglobulin E recurrent infections (Job) syndrome

BCG, Calmette-Guérin bacillus; *GATA2,* a hematopoietic transcription factor; *IL-12,* interleukin-12; *IRAK-4,* interleukin-1 receptor–associated kinase 4; *MyD88,* myeloid differentiation (primary response) protein 88; *NEMO,* nuclear factor kappa B essential modulator; *STAT1,* signal transducer and activator of transcription 1; *TLR,* Toll-like receptor.

TABLE 12.2 Congenital Immunodeficiencies

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
T Cells						
X-linked SCID	Interleukin (IL)-2 receptor gamma common chain	Xq13-21.1	X	T-cell proliferation, antibody production	Lymphopenia, hypogammaglobulinemia	2, 16
Adenosine deaminase (ADA) deficiency	Adenosine deaminase	20q13-ter	AR	T-cell functions, antibody production	Absent ADA activity, lymphopenia, hypogammaglobulinemia	14
Purine nucleoside phosphorylase (PNP) deficiency	Purine nucleoside phosphorylase	14q13.1	AR	T-cell functions	Absent PNP activity, low CD3 cells, increased NK cells, low uric acid	15
Defective major histocompatibility complex (MHC) molecules	RF-X	19q13	AR	Cell-mediated immunity	B cells normal, Ig normal or low, absent MHC molecules	24
IL-2 deficiency	Nuclear factor–activated T cells (NFAT)	?	AR	Cell-mediated immunity, antibody production	Lymphopenia, hypogammaglobulinemia	20–23
Reticular dysgenesis	Adenylate kinase 2	1p34	AR	Leukocyte mitochondrial metabolism	Pancytopenia and deafness	11–13
DiGeorge syndrome, velocardiofacial syndrome CATCH 22	TBX-1 and contiguous gene products	22q11.21-q11.23	AD	Anomalous development of third and fourth pharyngeal pouches	Thymic aplasia, parathyroid aplasia, cardiac anomalies, abnormal facies	8, 26
Ataxia-telangiectasia	Ataxia-telangiectasia mutated (ATM)	11q22.3	AR	DNA repair, T cells	Low IgA, low CD3 and CD4 cells, malignancies	27
Wiskott-Aldrich syndrome	Wiskott-Aldrich syndrome protein (WASP)	Xq11-11.3	X	T cells and platelets	Eczema, thrombocytopenia, low platelet volume, low IgM, high IgA, IgE	28–33
B Cells						
X-linked agammaglobulinemia	B-cell progenitor kinase (BTK)	Xq22	X	B cells	Very low antibody levels	2, 34, 35

Continued

TABLE 12.2 Congenital Immunodeficiencies—cont'd

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
Immunodeficiencies with hyper-IgM	CD40 ligand (CD40L, gp39)	Xq26	X	B cells, T cells, monocytes	High IgM, low IgG, IgA, poor T-cell responses	36, 37
	NF-κB essential modulator (NEMO)	Xq28	X	Neutrophils	High or normal IgM, low IgG, broad spectrum of infections	38
	CD40	20q12	AR	Monocytes	High or normal IgM, low IgG, poor T-cell function	39
	Activation-induced cytidine deaminase (AID)	12p13	AR	B cells	Lymph nodes present, normal-to-high IgM, low IgG	40
X-linked lymphoproliferative syndrome (Duncan syndrome)	SLAM-associated protein (SAP)	Xq25	X	EBV response	Low EBNA antibody, uncontrolled cellular activation	42–44
Common variable immunodeficiency	ICOS and others	2q33 and others	AR	Antibody synthesis T-cell function	Low IgG, poor antibody response, low IgA common	45, 142
IgA deficiency	IgA	?6p21.3	AR	IgA	Associated with other immunodeficiencies	45, 46
Phagocytes						
Chronic granulomatous disease (CGD)				Bacterial and fungal killing defective in all forms of CGD	Infections with catalase-positive microbes, granulomas, and reduced superoxide generation	76–78, 143
X-linked CGD	gp91*phox*	Xp21.1	X 70% of CGD			
Autosomal recessive CGD	p22*phox*	16q24	AR <5% of CGD	—	—	
	p47*phox*	7q11.23	AR 25% of CGD	—	—	
	p67*phox*	1q25	AR <5% of CGD	—	—	
	p40*phox*	22q12.3	AR <1% of CGD	Less severe superoxide impairment	Predominantly colitis	
	EROS	17q25.3	AR <1% of CGD	Less severe superoxide impairment	Predominantly colitis	
Severe chronic neutropenia and cyclic neutropenia	Neutrophil elastase (ELANE)	19p13	AD	Neutropenia	Cyclic hematopoiesis, cycle ≈21 days; some cases of severe chronic neutropenia	49–54
	Growth factor independent 1 (GFI1)	1p22	AD	Neutropenia	Severe neutropenia	51
	HAX-1	1q21.3	AR	Neutropenia	Kostmann syndrome, severe chronic neutropenia	53
MonoMAC; dendritic cell, myeloid, and NK-cell lymphopenia (DCML); Emberger syndrome; familial acute leukemia/myelodysplasia	GATA2	3q17	AD haploinsufficiency	Monocytopenia, B and NK lymphopenia, macrophage dysfunction	Warts, dysplastic megakaryocytes, hypoplastic bone marrow, cytopenias, pulmonary alveolar proteinosis	124
Warts, hypogammaglobulinemia infections, and myelokathexis (WHIM)	CXCR4	2q21	AD	Impaired chemokine receptor activity	Impaired neutrophil exit from the marrow (myelokathexis), low IgG, warts	144
Chédiak-Higashi syndrome	Lysosomal transport protein (LYST)	1q43	AR	Chemotactic defect neutropenia	Giant granules in neutrophils, oculocutaneous albinism	57–59
Leukocyte adhesion deficiency type 1	CD18 (ITGB2)	21q22.3	AR	Absent leukocyte integrins	Chronic leukocytosis, delayed umbilical cord separation, recurrent infections	5, 61, 63, 64
Leukocyte adhesion deficiency type 2	Sialyl-Lewis X (due to mutations in GDP-fucose transporter-1 [FUCT1])	11p11.2	AR	E-selectin ligand, fucose metabolism	Short stature, mental retardation, Bombay blood type	64, 65

TABLE 12.2 Congenital Immunodeficiencies—cont'd

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
Leukocyte adhesion deficiency type 3	KINDLIN-3 (FERMT3)	11q12	AR	Signaling from integrin ligation	Bleeding diathesis, leukocytosis	66
Neutrophil-specific granule deficiency	CCAAT enhancer binding protein (C/EBPε)	3q21	AR	Neutrophil granule products	Absent neutrophil-specific granules, absent defensins (primary granules)	67–72
Myeloid differentiation primary response gene 88	MyD88	3p22	AR	Signal transduction through the Toll-like receptors, the IL-1 receptor, and others	Severe pyogenic infections in childhood	98
IL-1 receptor–associated kinase-4 deficiency	IRAK-4	12q12	AR	Signal transduction through the Toll-like receptors, the IL-1 receptor, and others	Severe pyogenic infections in childhood	97
Toll-like receptor 3	TLR3	4q35	AR	TLR3 signaling	Recurrent HSV encephalitis	99
Myeloperoxidase deficiency	Myeloperoxidase (MPO)	17q21-q23	AR	Catalysis of superoxide to hydrogen peroxide	Absent MPO, usually unassociated with infections	73
Interferon-γ (IFN-γ) receptor 1 deficiency	IFN-γR1	6q23-q24	AR AD	Absence of IFN-γ binding Overaccumulation of defective receptor	Recurrent nontuberculous mycobacterial and *Salmonella* infections Nontuberculous mycobacterial osteomyelitis	102–104 106, 107
IFN-γ receptor 2 deficiency	IFN-γR2	21q22.1-q22.2	AR	Absence of IFN-γ signaling	Recurrent nontuberculous mycobacteria	105
IL-12 receptor β1 deficiency	IL-12Rβ1	19p13.1	AR	Absence of IL-12 signaling	Recurrent nontuberculous mycobacterial and *Salmonella* infections	109, 110, 112
IL-12p40 deficiency (IL-12p40)	IL-12p40	5q31	AR	Lack of IL-12 leading to low production of IFN-γ	Recurrent nontuberculous mycobacterial and *Salmonella* infections	111
Signal transducer and activator of transcription 1 (STAT1)	STAT1	2q32.2	AD AR AD	Reduced IFN-γ receptor signaling Absent IFN-γ and IFN-α/β receptor signaling Gain of function; reduced IL-17 producing T cells	Severe disseminated nontuberculous mycobacteria and BCG Disseminated fatal mycobacterial and viral infections Chronic mucocutaneous candidiasis; disseminated histoplasmosis or coccidioidomycosis	114 113 115–118
Hyper-IgE–recurrent infection syndrome (Job syndrome)	STAT3	17q21	AD	Reduced STAT3-dependent signaling (IL-6, IL-17)	Extremely high IgE, eczema, facial, dental and bony abnormalities, pneumatocele formations	7, 119, 121–123
Complement ***Classical Pathway***						
C1q deficiency C1r deficiency C1s deficiency C2 deficiency C3 deficiency C4A deficiency C4B deficiency C5 deficiency C6 deficiency C7 deficiency C8 deficiency C9 deficiency	C1q C1r C1s C2 C3 C4A C4B C5 C6 C7 C8 C9	1p36.3 12p13 12p13 6p21.3 19p13.3 6p21.3 6p21.3 9q34.1 5p13 5p13 1p32 5p13	AR AR AR AR AR AR AR AR AR AR AR AR	Antibody-dependent complement lysis is depressed in all forms of classical complement component deficiencies.	Low CH_{50} is seen with all forms of classical complement component deficiency. Individual components are very low or absent. Autoimmune disease is common in early-component deficiencies (C1-C4). Bacteremia and meningitis are common in all types of complement deficiencies.	1

Continued

TABLE 12.2 Congenital Immunodeficiencies—cont'd

CLINICAL DISEASE	AFFECTED GENE PRODUCT[a]	CHROMOSOMAL LOCATION	INHERITANCE	FUNCTIONAL DEFECT	IMPORTANT FINDINGS	REFERENCES
Alternative Pathway						
Properdin deficiency	Properdin	Xp11.4-23	X	Antibody-independent complement lysis is depressed in alternative complement component deficiencies	More severe susceptibility to infection than classical component deficiencies	
Factor H deficiency	Factor H	1q32	AR			
Factor I deficiency	Factor I	4q25	AR			

[a]The affected gene product is not always the gene in which the lesion has occurred. The genetic lesion may disable a regulatory gene required for expression or function of the affected gene product.

AD, Autosomal dominant; *AR,* autosomal recessive; *BCG,* Calmette-Guérin bacillus; *CATCH 22,* cardiac defects, abnormal facial features, thymic hypoplasia, cleft palate, and hypocalcemia; CH_{50}, total hemolytic classical complement; *EBNA,* Epstein-Barr virus nuclear antigen; *EBV,* Epstein-Barr virus; *ELANE,* elastase neutrophil expressed; *GATA2,* a hematopoietic transcription factor; *GDP,* guanosine diphosphate; *HAX-1,* HCLS-1 (hematopoietic cell-specific Lyn substrate 1)–associated protein X-1; *HSV,* herpes simplex virus; *ICOS,* inducible costimulator; *Ig,* immunoglobulin; *IRAK-4,* interleukin-1 receptor–associated kinase 4; *MonoMAC,* monocytopenia and mycobacterial infections; *MyD88,* myeloid differentiation (primary response) protein 88; *NF-κB,* nuclear factor kappa B; *NK,* natural killer; *RF,* regulatory factor; *SCID,* severe combined immunodeficiency; *SLAM,* signaling lymphocyte activation molecule; *STAT1,* signal transducer and activator of transcription 1; *TLR3,* Toll-like receptor 3; *X,* X-linked inheritance.

TABLE 12.3 Screening Evaluations for Immune Defects

History

Medications and treatments
Relatedness of parents, umbilical stump separation, age of onset, dental history
Frequency, severity, distribution, types of infections
Vaccination history, especially handling of live vaccines and antibody response to vaccination
Causative infectious agents

Physical

Weight and height
Hair: sheen, pigmentation
Abdomen: organomegaly
Skin: dystrophic scars, telangiectasis, eczema, warts
Oropharynx: thrush, ulcers, gingivitis, secondary tooth eruption
Facies: nasal size, eye slant, philtrum
Skeleton: kyphoscoliosis, fractures

Routine Laboratory Values
Complete Blood Count

Differential: lymphopenia, neutropenia, monocytopenia
Peripheral smear: giant granules, specific (secondary) granules
Platelet count: thrombocytopenia
Erythrocyte sedimentation rate: usually elevated in infection

Chemistries

Serum calcium
Serum uric acid
Liver functions

Immunoglobulins

IgA, IgM, IgG, IgE
Isohemagglutinins
Antibody titers (tetanus, pneumococcus, etc.)

Complement

Total hemolytic complement (classical CH_{50}, alternative AH_{50})

Radiography

Plain chest films: kyphoscoliosis, pneumatoceles, scarring



FIG. 12.1 Severe periodontal disease in leukocyte adhesion deficiency type 1 as shown by gum recession.

Physical examination can yield findings diagnostic or highly suggestive of lesions in specific arms of the immune system. Facial anomalies, including hypertelorism, shortened philtrum, and down-slanting palpebral fissures, are encountered in DiGeorge syndrome,[8] whereas characteristic facies with a broad nose and a triangular mandible are seen in Job syndrome.[7,9] Hair with a silvery sheen and irregular melanin distribution is seen in Chédiak-Higashi syndrome.[10] Cutaneous signs of immune defects include telangiectasia over the bulbar conjunctivae and skin in ataxia-telangiectasia, the severe eczema that accompanies Job syndrome, or the dystrophic scarring seen in LAD1.

The initial laboratory examination should consist of a complete blood count with differential, platelet count, examination of the peripheral blood smear, and erythrocyte sedimentation rate. Chédiak-Higashi syndrome and neutrophil-specific granule deficiency can be detected on peripheral smear, whereas severe neutropenias and Wiskott-Aldrich syndrome can be largely excluded by normal neutrophil or platelet counts, respectively. An Ig profile with total IgA, IgM, and IgG levels will help detect cases of IgA deficiency and hypogammaglobulinemia. In cases in which eczema is a prominent feature or Job syndrome is suspected, measurement of IgE levels is indicated. The humoral immune system is functionally interrogated by preimmunization and postimmunization antibody levels. Testing of total hemolytic complement (CH_{50}) gives a quick assessment of the functional integrity of the classical component of the complement cascade from C1 through the membrane attack complex (C5 to C9), whereas the alternative pathway hemolytic complement (AH_{50}) tests the alternative pathway of complement activation. Plain radiographs of the chest can demonstrate pulmonary scarring, pneumatoceles, and parenchymal destruction, often encountered in phagocyte defects and Job syndrome. Scoliosis, osteoporosis, rib and long bone fractures, and their sequelae are frequently seen in Job syndrome.[7,9]

LYMPHOCYTE IMMUNE DEFECTS

T Cells and Cell-Mediated Immunity

The initial manifestations of congenital T-cell defects usually, but not always, occur within the first few months of life and include severe mucocutaneous candidiasis, recurrent persistent respiratory infections, diarrhea, and failure to thrive. A broad spectrum of onset and severity is seen. Reticular dysgenesis (adenylate kinase 2 deficiency) appears within the first weeks of life and is characterized by pancytopenia, infection, deafness, and early demise.[11–13] The lymphocyte enzymopathies (adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency) tend to occur after several months of life, when lymphocyte counts begin to fall because of accumulation of the toxic metabolites deoxyadenosine triphosphate and deoxyguanosine triphosphate, respectively.[14,15] The occurrence of *P. (carinii) jirovecii* pneumonia, disseminated BCG infection after vaccination, persistent poliovirus infection after oral polio vaccination, or persistent respiratory virus infection should initiate consideration of a defect in lymphocyte function.[16] Graft-versus-host disease, either acquired from in utero transfer of maternal lymphocytes or through transfusion of unirradiated blood, may be the underlying cause of cutaneous eruptions, transaminase elevations, or malabsorption and diarrhea and is a strong indicator of defective T-cell immunity.[16]

In many forms of T-cell abnormality, B-cell function is also compromised, and both cell-mediated and humoral deficiencies occur, a state referred to as severe combined immunodeficiency (SCID).[16] This syndrome tends to be recognized after maternal antibody levels wane and recurrent bacterial infections begin. The finding of low Ig levels in association with marked lymphopenia in the appropriate setting should lead to the consideration of severe combined immunodeficiency.[2,16]

The most direct and simplest assessment of T-cell immune status is the determination of lymphocyte number, which is obtained in the screening differential count. Circulating lymphocyte numbers range from about 7000 cells/μL in infants to 4000 cells/μL in children and about 2000 cells/μL in adolescents and adults through old age.[17] Of these lymphocytes, roughly half are T cells, with the remainder split between B cells and natural killer (NK) cells. Severe quantitative abnormalities of lymphocytes are relatively uncommon; low absolute lymphocyte numbers are encountered in the great majority of cases of SCID, and in several other congenital immunodeficiencies (see Table 12.2). The lymphocyte number is now detected as part of newborn screening through the analysis of T-cell receptor excision circles (TRECs)—small, stable DNA circles that reflect thymic output and are detectable with use of 50 μL of dried blood on the Guthrie card.[18] In almost all of the United States, newborns are screened and low TRECs are confirmed with flow cytometry; those with very low T-cell numbers are referred for evaluation in the first days of life, before severe infections begin. Acquired immunodeficiency syndrome (AIDS); high plasma corticosteroid levels (iatrogenic or endogenous); obstructed lymphatic circulation (e.g., intestinal lymphangiectasia or protein-losing enteropathy); severe systemic illness (e.g., carcinomatosis, miliary tuberculosis); systemic lupus erythematosus; sarcoid, cytotoxic, or immunosuppressive therapy; and severe right-sided congestive heart failure can also cause lymphocytopenia. These latter causes are much more common than the congenital ones.

T-lymphocyte function is initially and most easily assayed in vivo through testing of type IV cell-mediated immunity or delayed-type hypersensitivity (DTH) (Table 12.4). DTH is elicited by the intradermal injection of an antigen to which the subject has been exposed. Antigen-specific $CD4^+$ T cells are recruited, and in turn, these T cells recruit macrophages, with resulting interstitial fibrin deposition and induration. Erythema and edema are seen early after the injection but are not indicative of DTH. Frank induration, which is the best marker of effective DTH, is best appreciated between 24 and 48 hours and then gradually falls off.

In the screening evaluation for immune defects, it is important to consider the patient's experiences, such as immunizations, previous infections, and regional exposures, in the selection of antigens. It is useful to test several different antigens simultaneously for the determination of T-cell responsiveness, such as mumps, *Candida, Trichophyton,* streptococcal antigens, and tetanus. The demonstration of intact DTH confirms the presence of functional $CD4^+$ T cells and excludes most of the severe congenital defects in cell-mediated immunity. DTH can also be evaluated in vitro, as antigen-specific T-cell proliferation or cytokine response. Of importance, DTH can be preserved until relatively late into human immunodeficiency virus (HIV) infection. Therefore the presence of DTH should not be considered evidence against HIV infection per se. Selective anergy to the antigens of the infecting organism has been observed in active visceral leishmaniasis, lepromatous leprosy, and active tuberculosis. These specific defects typically reverse after successful treatment of the underlying infection. Complete anergy to a battery of antigens is a relatively nonspecific finding in terms of etiology, insofar as the differential diagnosis includes all the entities that cause lymphopenia. However, anergy is an indication for further evaluation of cell-mediated immunity.

TABLE 12.4 Directed Tests of Immune Function

T Cells
FACS (lymphocyte subsets, cytokine receptors)
Delayed-type hypersensitivity: mumps, *Candida,* tetanus, *Trichophyton*
Lymphocyte enzymes (adenosine deaminase and purine nucleoside phosphorylase)
Cytokine production and response
In vitro proliferation: stimulation with antigen, lectin, antibody, allogeneic cells, protein kinase C stimulants, and calcium ionophores
B Cells
FACS
Antigen challenge, recall or new
In vitro antibody production
Phagocytes
FACS for leukocyte adhesion molecules
Dihydrorhodamine oxidation, nitroblue tetrazolium reduction
Superoxide generation, hydrogen peroxide generation
Adherence
Staphylococcal killing
Chemotaxis
Phagocytosis
Complement
Assay of individual complement components, functional or quantitative

FACS, Fluorescence-activated cell sorting.

Fluorescence-activated cell sorting (FACS) analysis allows rapid enumeration and characterization of lymphocyte, monocyte, and neutrophil subsets.[19] Lymphocyte subset determination, specifically for $CD4^+$ T cells, is standard in the management of HIV infection, where it provides guideposts for initiation and monitoring of antiviral therapy. With the recent identification and cloning of many immunodeficiency-related genes and the development of specific antibodies, FACS is able to confirm or exclude specific immunodeficiencies.

Evaluation of T-cell function in vitro requires laboratory personnel skilled in the isolation, preparation, and stimulation of peripheral blood mononuclear cells. Stimulation is typically done on unseparated peripheral blood mononuclear cells and therefore represents the product of both lymphocyte and monocyte contributions. Proliferation is usually determined as some measure of lymphocyte division after stimulation with cell membrane–binding lectins (e.g., phytohemagglutinin [PHA], concanavalin A [ConA], pokeweed mitogen [PWM]), direct stimulants of cellular signaling pathways that bypass the need for membrane components (phorbol myristate acetate and the calcium ionophore ionomycin), antigens (purified protein derivative [PPD], *Candida,* tetanus), cytokines (interleukin [IL]-2), or allogeneic cells (mixed lymphocyte reaction).[19] Significantly low proliferation in vitro can result from lymphocytopenia; absent or impaired cell surface receptors, such as the CD3 or IL-2 receptor complexes[20]; antigen-specific anergy; IL-2 deficiencies[21–23]; or major histocompatibility complex abnormalities.[24]

At least four important $CD4^+$ T-cell subsets are distinguished: Th1 cells produce interferon-γ (IFN-γ) and IL-2 and are the predominant cells in the DTH response; Th2 cells produce IL-4, IL-5, IL-10, and IL-13 and regulate the differentiation of B cells and eosinophils; Th17 cells produce IL-17 and IL-22 and regulate neutrophil number and

epithelial responses to infection, including *Candida* and epithelial inflammation; and T regulatory cells (Tregs) control and regulate T-cell and B-cell responses and are essential for keeping T-cell responses in line. Defects in Tregs lead to aberrant inflammation, such as the immunodysregulation-polyendocrinopathy-enteropathy–X-linked (IPEX) syndrome. All of these cell types also regulate one another, opening new areas for immune defects and therapeutic manipulations of their relationship. Cytokine levels in the supernatants of stimulated and unstimulated cells can be readily determined and may demonstrate states of either deficiency[21–23] or excess.

DiGeorge syndrome is caused by anomalous development of the third and fourth pharyngeal pouches, with agenesis of the thymus and parathyroids and subsequent immunodeficiency and hypocalcemia. Neonatal tetany is an expected manifestation.[8] The defect in DiGeorge syndrome is due to interstitial deletions in chromosome 21, including mutations in the *TBX-1* gene.[25,26] Ataxia-telangiectasia is associated with low IgA and low $CD3^+$ and $CD4^+$ T-cell levels, progressive ataxia, and oculocutaneous telangiectasia. These patients usually experience recurrent bacterial respiratory infections and are at increased risk of malignancy because of abnormalities in DNA repair.[2,27] Wiskott-Aldrich syndrome is characterized by eczema, elevated IgE, thrombocytopenia with small platelets, and recurrent opportunistic infections resulting from mutations in the Wiskott-Aldrich syndrome protein (WASP).[28–31] Mutations in WASP also have been found to cause isolated X-linked thrombocytopenia[32] and X-linked neutropenia.[33]

B Cells and Humoral Immunity

Almost 70 years have elapsed since the first clear description of an immunodeficiency syndrome, X-linked agammaglobulinemia (XLA), by Colonel Bruton.[34] The clinical features of isolated Ig defects are distinct from those of T-cell or combined defects. The age at onset for congenital deficiencies is between 6 months and 2 years, and the initial infections are predominantly with encapsulated bacteria. The thymus gland is present and apparently normal in XLA, but peripheral lymphoid tissues, such as tonsils and lymph nodes, are typically absent.[35] B cells and plasma cells in the circulation and the periphery are rare despite normal numbers of pre–B cells in the bone marrow. These patients have elevated T-cell numbers, normal T-cell subsets, and intact T-cell functions. Although they have significant problems with bacterial infections, patients with XLA also have severe difficulty with persistent, disseminated echovirus infections, especially in the central nervous system. IgG, IgA, and IgM levels are extremely low, as are isohemagglutinins. No antibody to either new or previously administered antigens is detectable. In contrast, patients with any of the syndromes that can give rise to one of the hyperimmunoglobulin M syndromes (the X-linked deficiency of CD40 ligand [CD40L or CD154][36,37]; X-linked ectodermal dysplasia with immunodeficiency [NEMO][38]; autosomal recessive CD40 deficiency[39]; autosomal recessive, activation-induced cytidine deaminase deficiency[40]; phosphatidylinositol 3-kinase catalytic delta [PI3KCD][41]; and others) may have preserved lymphoid tissue mass, hepatosplenomegaly, normal numbers of B cells and T cells, and variable neutropenia. Autoimmune phenomena such as Coombs-positive hemolytic anemia and thrombocytopenia are common. IgM is often normal to elevated, and IgG and IgA are usually quite low; isohemagglutinins may be elevated.[35] The X-linked lymphoproliferative disorder—XLP or Duncan syndrome—occurs in males, typically after infection with Epstein-Barr virus (EBV), and can cause problems ranging from aplastic anemia to hypogammaglobulinemia to a full-blown immunodeficiency syndrome, including hemophagocytic lymphohistiocytosis (HLH), which can be fatal.[42] The causative gene product associates with the signaling lymphocyte–activation molecule (SLAM) and is called the SLAM-associated protein (SAP), which is critical for T-cell signaling.[43,44]

The initial test for the integrity of the humoral arm of immunity is determination of levels of isohemagglutinins (see later, this section) and levels of IgG, IgA, and IgM. Normal Ig levels are relatively low in infancy and childhood and increase with age. If Ig levels are appropriate, XLA can be excluded. Low Ig levels may be seen in the first year of life in the transient hypogammaglobulinemia of infancy.[2,35] However, these infants usually have detectable isohemagglutinins and can mount antibody responses to new antigenic challenges. Despite family histories notable for relatives with immunodeficiencies, patients with transient hypogammaglobulinemia of infancy tend to normalize their Ig levels over the first 2 years of life. In contrast, depressed levels of Ig in an adult with recurrent bacterial sinopulmonary infections raise the possibility of common variable immunodeficiency (CVID).[45] CVID is a heterogeneous group of diseases that share the features of hypogammaglobulinemia and an increased susceptibility to chronic enteric infections with *Giardia lamblia, Campylobacter,* and disseminated echovirus infections, in addition to sinopulmonary bacterial infections. Patients with CVID often have low isohemagglutinin levels and abnormal DTH and fail to make antibody to new antigens. IgG subclass analysis may show selective defects in IgG1 and IgG3 or in IgG2 and IgG4, in addition to defects in IgA.[45] However, the value of checking IgG subclasses is debated.[46]

Isohemagglutinins are IgM antibodies directed against blood group A and B antigens, which occur in all healthy people, except those with blood group AB. By the age of 3 years, 98% of patients with blood groups A, B, or O have isohemagglutinins with a titer of at least 1:16. Isohemagglutinin levels are determined in blood banks as a prerequisite to transfusion. Challenge with antigen is probably the simplest and most effective method for determining the functional integrity of the humoral immune system. Polysaccharide pneumococcal vaccinations examine the response to polysaccharide antigen, whereas tetanus challenge is more specific for peptide responses. Serum titers should be checked before and 3 to 4 weeks after immunization. Use of a recall antigen allows testing of anamnestic responses. Novel or rare antigens, such as bacteriophage ΦX174, keyhole limpet hemocyanin, or rabies vaccine, make it possible to test antibody responses even during Ig administration.[47]

PHAGOCYTE IMMUNE DEFECTS: NEUTROPHILS

The clinical findings of patients with neutrophil disorders often share common features: gingivitis, periodontal disease, and oral ulceration.[6] Cutaneous infections with *Staphylococcus aureus* are recurrent and can be severe. In neutrophil disorders characterized by inadequate inflammation (neutropenia, LAD1, Chédiak-Higashi syndrome, neutrophil-specific granule deficiency), infections can extend locally and subcutaneously with little reaction until marked destruction has taken place. Visceral and, especially, sinopulmonary involvement helps distinguish neutrophil defects from other syndromes in the differential diagnosis. Hepatic abscess is a frequent manifestation of chronic granulomatous disease (CGD) and is most often caused by *S. aureus,* an organism rarely encountered in the liver in patients with normal neutrophils.

Neutropenia

A neutrophil count of less than 500 cells/μL carries a profound risk of bacterial and fungal infection.[48] Although this principle was first extensively documented and is still most frequently displayed in patients undergoing combination chemotherapy, its importance has been confirmed in patients with genetic disorders that affect neutrophil number. Cyclic neutropenia is an autosomal dominant disease characterized by relatively regular 21-day oscillations in the levels of blood neutrophils, monocytes, eosinophils, lymphocytes, platelets, and reticulocytes.[49] The defects are usually the result of mutations in the elastase neutrophil expressed *(ELANE)* gene.[50] Severe chronic neutropenia (SCN) can also be caused by mutations in *ELANE* and in several other genes, most notably *HAX-1* (HCLS-1 [hematopoietic cell-specific Lyn substrate 1]–associated protein X-1).[51] Patients are usually first seen in childhood and have recurrent episodes of fever, malaise, mucosal ulcers, and occasionally, life-threatening infections associated with periods of profound neutropenia (<200/μL).[49] Neutrophil number is the defect, but function is normal. The diagnosis is suspected in children with recurrent stomatitis, gingivitis, cutaneous infections, lymphadenopathy, and fever and should be entertained in patients with intermittent neutropenia, especially if a periodicity of about 21 days can be documented on serial blood draws (the mechanisms behind the periodicity are unknown). Molecular diagnostics should be pursued to confirm the specific gene involved. In SCN (also known as Kostmann syndrome[52]), neutrophil counts are consistently low from birth and without periodicity.[53] Of

interest, some cases of SCN are also caused by mutations in *ELANE.*[54] Neutropenic syndromes are typically treated with recombinant granulocyte colony-stimulating factor; myeloid malignancies are increased in these populations.[55] Adult-onset cases have also been described, with an associated clonal proliferation of large granular lymphocytes ($CD3^+$/$CD8^+$/$CD57^+$).[56]

Chédiak-Higashi Syndrome

Chédiak-Higashi syndrome is a rare autosomal recessive disorder caused by defects in the lysosomal transport protein LYST, encoded by the *CHS1* gene.[57,58] It is characterized by recurrent bacterial infections, partial oculocutaneous albinism, photophobia, nystagmus, and peripheral neuropathy.[10] Many patients die in childhood from infection. An aggressive "lymphoproliferative" phase, with diffuse organ infiltration and death, develops in about half of the patients who survive into adolescence. Patients who live into adulthood may develop a severe peripheral neuropathy.[10] Microscopic examination reveals giant abnormal granules in neutrophils, melanocytes, hair, Schwann cells, the central nervous system, and other granule-containing cells.[10] In neutrophils, the granules are formed mainly by fusion of azurophilic or primary granules to each other and, to a lesser extent, by fusion to specific or secondary granules.[59]

Features of Chédiak-Higashi syndrome include central and peripheral nervous system involvement with peripheral neuropathy, myopathy, autonomic dysfunction, and leptomeningeal involvement.[10] Low intelligence has been noted in some series.[10] The lymphoproliferative "accelerated phase" is prevented by bone marrow transplantation, whereas the neuropathic features are not.[60] The phenotypic diagnosis of Chédiak-Higashi syndrome is easily made by inspection of the peripheral smear for giant lysosomes or microscopic examination of hair for characteristic melanin clumps.

Leukocyte Adhesion Deficiency

Leukocyte adhesion to endothelium and other leukocytes is mediated by several sets of molecules, among which are the integrins and the selectins. Defects in either of these two intercellular adhesion pathways can lead to overlapping clinical phenotypes. LAD type 1 is a rare autosomal recessive disorder involving one set of the leukocyte integrins—the molecules required for leukocyte adherence to endothelium, other leukocytes, and bacteria.[5,61] Deficiency of the integrin component CD18 leads to a corresponding deficiency of the complexes with which it partners—leukocyte function–associated antigen 1 (LFA-1), cell surface glycoprotein Mac-1, and p150,95, resulting in abnormalities of cellular adhesion. The absence of a marginated pool of neutrophils leads to chronic leukocytosis. Patients with LAD1 are prone to both recurrent infections and dysregulated inflammation. The infections are due to failure of neutrophil response. However, the failure of neutrophils to penetrate the tissues leads to increased macrophage expression of IL-23 and its downstream effector, IL-17, leading to exacerbated local inflammation.[62]

LAD1 defects can be severe or moderate, depending on the degree of CD18 deficiency.[5] Severe deficiency (<0.5% of normal protein expression) is characterized by delayed umbilical stump separation, umbilical stump infection, persistent leukocytosis in the absence of active infection (>15,000/μL), and severe destructive periodontitis with associated loss of dentition and alveolar bone, which is IL-17 mediated. Septicemia and recurrent infections of the skin, upper and lower airways, bowel, and perirectal area are common and usually due to *S. aureus* or gram-negative rods, most notably *Pseudomonas* species. Infections tend to be necrotizing and ulcerative but demonstrate almost no tissue neutrophils at histopathologic evaluation. Patients with moderate deficiency (3%–30% of normal expression) tend to be diagnosed later in life, have normal umbilical stump separation, and have fewer life-threatening infections. Leukocytosis is still the rule, as are delayed wound healing and periodontal disease. Although patients with a moderate form of the disease are less ill and tend to live past childhood, deaths from infection have been reported in young adults.[5]

The severe form shows grossly defective granulocyte and mononuclear cell mobilization in vivo and diminished neutrophil migration in response to the bacterial chemoattractant f-Met-Leu-Phe in vitro, despite normal numbers of receptors.[5] Histologic sections of infected tissues show mononuclear cells but very few neutrophils. Granulocyte adherence to glass, plastic, nylon, wool, and other LAD granulocytes is greatly reduced and not stimulated by exposure to f-Met-Leu-Phe or phorbol myristate acetate. The absence of CD18 also leads to the absence of the inactivated C3b (iC3b) receptor CR3. Therefore, complement-mediated phagocytosis is severely impaired, whereas IgG-mediated phagocytosis is normal. Although viral infections are not usually special problems in LAD, antibody-dependent cellular cytotoxicity by patient cells is also diminished. The diagnosis is established by eliciting a thorough history, with special attention directed to consanguinity, evidence of depressed inflammation in the neonatal period, delayed umbilical stump separation, and recurrent infections. A dental history is helpful inasmuch as most of these patients have severe problems with gingivitis, periodontal disease, tooth loss, and alveolar bone erosion. Wounds often heal abnormally, with dystrophic, paper-thin scars remaining. The diagnosis is confirmed when FACS shows reduction or absence of the leukocyte adhesion molecule CD18 or its partners CD11a, CD11b, and CD11c; and sequence abnormality in the gene encoding CD18, *ITGB2.*

In LAD2, neutrophil adherence to endothelial cells is defective because of absence of the sialylated Lewis X antigen (CD15s) on the neutrophil surface, which is the binding site for E-selectin.[63,64] The patients have neutrophilia, recurrent pulmonary, periodontal, and cutaneous infections, abnormal chemotaxis, mental retardation, short stature, distinctive facies, and the Bombay (hh) blood phenotype. The defect is autosomal recessive and caused by mutations in guanosine diphosphate (GDP)-fucose transporter 1 gene *SLC35C1.*[63,65] This disease is also known as congenital disorder of glycosylation IIc (CDG IIc). LAD3 is characterized by recurrent infections, impaired neutrophil migration, and platelet dysfunction caused by mutation in the gene *KIND3,* which links surface ligation of receptors to cellular signaling.[66]

Neutrophil-Specific Granule Deficiency

Neutrophil-specific (secondary) granule deficiency is a rare autosomal recessive heterogeneous disease characterized by a profound reduction or absence of neutrophil-specific granules and their contents.[67] Associated abnormalities in the few patients reported include bilobed or trilobed neutrophil nuclei, absence of some neutrophil primary (azurophil) granule proteins, mononuclear eosinophils without eosinophilic granules, and dysfunction of platelet α-granules. The neutrophil-specific granule protein lactoferrin is diminished or absent in these patients' neutrophils, whereas production by lacrimal glands is normal.[67–69] Neutrophil-specific granule deficiency results from mutation in the transcriptional regulator *C/EBPε*, which controls early neutrophil granule biogenesis.[70–72] However, cases without *C/EBPε* mutation suggest that there may be several genes that can lead to the same defect.

Myeloperoxidase Deficiency

Myeloperoxidase (MPO, also called verdoperoxidase), the heme-binding protein that makes pus green, catalyzes the conversion of hydrogen peroxide to hypochlorous acid (bleach). MPO deficiency, the most common neutrophil disorder, affects about 1 in 2000 persons but is quite silent in most cases. Neutrophil function is affected by MPO deficiency in a variety of ways. The respiratory burst in MPO-deficient neutrophils is prolonged and, as a result, exaggerated amounts of hydrogen peroxide are produced.[73] Phagocytosis is normal to increased in MPO-deficient neutrophils, whereas bactericidal activity is somewhat slower than normal. Killing of *Aspergillus* conidia by MPO-deficient neutrophils is retarded, whereas the combination of MPO-deficient neutrophils with CGD neutrophils (see "Chronic Granulomatous Disease"), which are unable to generate hydrogen peroxide but do produce MPO, results in normal killing of *Aspergillus* conidia.[74] Pathologic sequelae of MPO deficiency are brought out only in the presence of other impairments of host defense, such as diabetes mellitus. A very few MPO-deficient diabetic patients have had severe yeast infections. Results of the dihydrorhodamine oxidation assay, which is used for CGD diagnosis, can be falsely abnormal in MPO deficiency.[75]

Chronic Granulomatous Disease

CGD is caused by deficient function of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is responsible for the

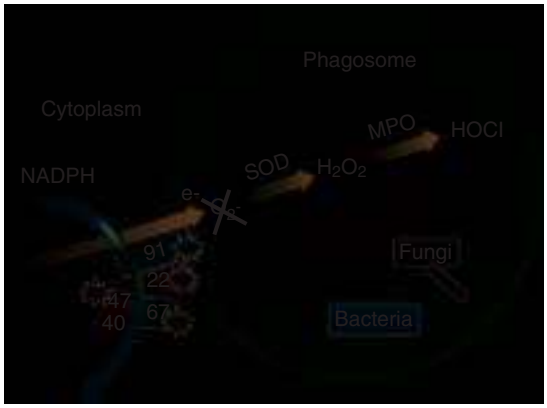

FIG. 12.2 The phagocyte respiratory burst. The neutrophil oxidative burst is mediated by the structural components of the reduced nicotinamide adenine dinucleotide phosphate *(NADPH)* oxidase: the membrane-bound components gp91*phox* and p22*phox* and the cytosolic factors p47*phox,* p67*phox,* and p40*phox.* In the setting of cellular activation, these factors coalesce on the phagosome membrane and catalyze the transfer of an electron from NADPH to molecular oxygen. This, in turn, creates superoxide, which is converted to hydrogen peroxide (H_2O_2) and then to hypochlorous acid (*HOCl,* bleach). The genetic absence of any of these structural components causes chronic granulomatous disease. *MPO,* Myeloperoxidase; *$NADP^+$*, oxidized nicotinamide adenine dinucleotide phosphate; *SOD,* superoxide dismutase.

respiratory burst and the generation of phagocyte superoxide, hydrogen peroxide, and hypochlorous acid (Fig. 12.2). Five distinct gene defects converge on this point in phagocytic cell oxidative metabolism, and their defects lead to recurrent life-threatening infections with bacteria and fungi and dysregulated inflammation.[76] The frequency is greater than 1 in 200,000 persons.[77] Clinically, CGD is quite variable; the time of onset may occur from infancy to late adulthood, although it is diagnosed in most patients while they are toddlers and young children. However, CGD is diagnosed in a significant number of patients later in life.[76–78] Children with CGD tend to be short and small for their age, even in the absence of overt infections, but often achieve the height predicted by their parents' height.[79]

Pulmonary, cutaneous, lymphatic, and hepatic infections are frequent. Osteomyelitis, perianal abscess, and genitourinary obstruction are also common.[76–78] The microbiologic features of infections associated with CGD are remarkable for their relative specificity: *S. aureus, B. cepacia* complex, *Serratia marcescens, Nocardia* spp., and *Aspergillus* spp. account for the overwhelming majority of infections in North America and Europe (Fig. 12.3). Where exposure is common, BCG, tuberculosis, and *Salmonella* infections are also prominent. As in other neutrophil abnormalities, the most common offender in CGD is *S. aureus.* Whereas the typical liver abscess in an immunologically normal patient involves enteric organisms and is liquid and easily drained, the liver abscesses encountered in patients with CGD are dense, caseous, and staphylococcal (Fig. 12.4). In the absence of antibiotic prophylaxis, lung, skin, and bone infections are also usually staphylococcal. However, *Aspergillus* spp. and some of the rarer fungi, such as *Exophiala dermatitidis*[80] and *Paecilomyces* spp.,[81] are encountered in CGD. Infections with *Nocardia* spp., *Chromobacterium violaceum, S. marcescens,* and *B. cepacia* are seen frequently in patients with CGD and strongly suggest the diagnosis.[3,76–78,82] Bony involvement can occur by direct extension in the case of *Aspergillus* or hematogenously, as in the case of *Staphylococcus* and *Nocardia.*[83] Infection with *Aspergillus nidulans* is especially aggressive and requires more intensive therapy than does infection with *Aspergillus fumigatus.*[84] Antibiotic prophylaxis has altered the frequency of infections in CGD and has reduced the frequency of staphylococcal infections in particular.[3,85] Therefore, infections outside the liver or lymph nodes occurring in CGD patients who have been taking antibacterial prophylaxis should not be presumed to be staphylococcal. The rate of fungal infections in CGD is lower than that for bacterial infections and has apparently



FIG. 12.3 Multifocal asymptomatic *Aspergillus fumigatus* pneumonia in a patient with chronic granulomatous disease.

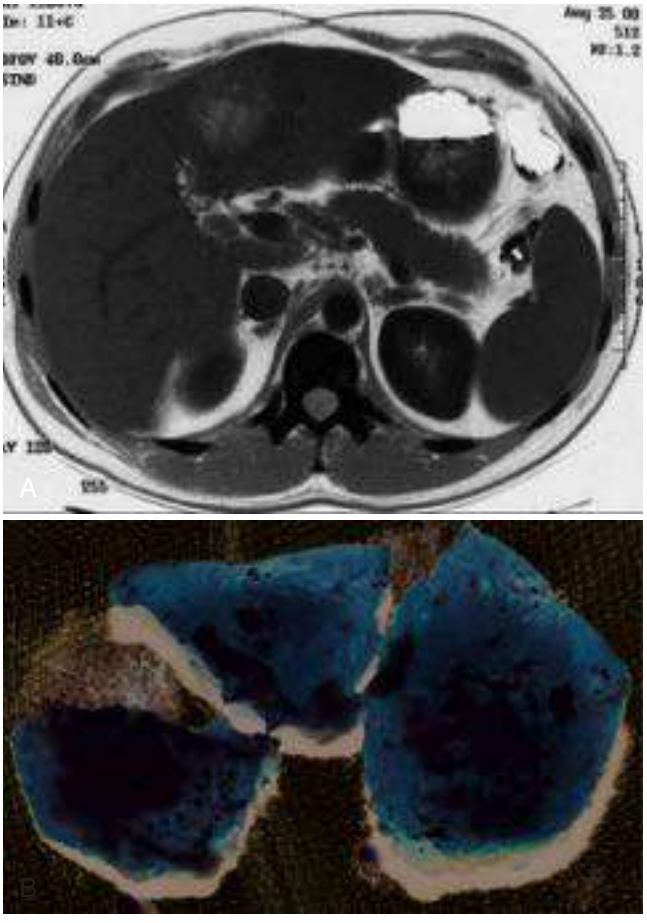

FIG. 12.4 Staphylococcal liver abscess in a patient with chronic granulomatous disease. (A) It is large, diffuse, and multiloculated. (B) The excised abscess shows a dense fibrocaseous mass that is typically not amenable to catheter drainage and requires surgical removal.

not changed in the setting of prophylactic antibiotics.[85] Itraconazole prophylaxis has reduced the frequency and severity of fungal infections in patients with CGD.[86]

The granulomas that occur in CGD are presumed to originate from an inflammatory response to infectious or irritative foci (e.g., sutures) that fails to eradicate the infection or irritation. This may be because hydrogen peroxide, a moiety missing in CGD phagocytes, is involved in the degradation of inflammatory mediators, such as leukotrienes and complement factors.[87,88] This persistent inflammatory reaction may

lead to exuberant and often obstructive granulomatous lesions. The gastrointestinal and genitourinary tracts are frequently involved by granulomatous lesions in CGD, sometimes as the site of the initial complaint, sometimes asymptomatically. Malabsorption, abundant lipid-laden histiocytes in bowel biopsy specimens, and pigmented histiocytes and granulomas in bowel biopsy material are sometimes difficult to distinguish from Crohn disease.[89] Esophageal, jejunal, ileal, cecal, rectal, and perirectal involvement occurs in greater than 40% of patients with CGD, affecting patients with X-linked more than autosomal disorders.[90] Gastric outlet obstruction is an especially common manifestation and may be the initial feature of CGD. In a comprehensive review of genitourinary manifestations, Walther and colleagues[91] found that 38% of patients with CGD had some kind of urologic event, including bladder granulomas, ureteral obstruction, and urinary tract infection. The diagnosis of CGD is made by assays of superoxide or hydrogen peroxide production, such as dihydrorhodamine oxidation or nitroblue tetrazolium reduction. Mothers of boys with the X-linked form of CGD are typically carriers of a defective X chromosome. Therefore, by lyonization, a certain proportion of the mother's cells will fail to produce superoxide and thus will give a characteristic mosaic pattern at testing, so that by performing a functional test on the mother's blood, one can determine whether a boy has the X-linked form of CGD. X-linked carriers of CGD have an increased incidence of discoid lupus.[92]

IFN-γ is a cytokine with neutrophil- and monocyte-macrophage–stimulating properties that has reduced the frequency and severity of infections in patients with CGD.[93] Although granulocyte transfusions supply only a small portion of the body's normal neutrophil output in a day, they may be effective in helping to clear severe infections. Transfused granulocytes produce superoxide, which can be used by the intact MPO system in CGD neutrophils, thereby allowing the biochemical defect to be bypassed. The granulomatous complications of CGD pose a special problem in management. Although these patients are already somewhat immunocompromised, the judicious use of corticosteroids in conjunction with antibiotics has been successful in opening and maintaining the patency of hollow viscera in patients with CGD.[90] Furthermore, adjuvant steroid therapy coupled with antibiotic therapy for staphylococcal liver abscess obviates surgery and has shown better overall survival than standard surgical therapy.[94,95]

DEFECTS AFFECTING PHAGOCYTE SIGNALING

Among the critical ways through which myeloid, lymphoid, and other cells sense pathogens are preformed receptors on the cell surface or in vesicles. The Toll-like receptors (TLRs) are important mediators of this sensing that signal through intracellular molecules, including myeloid differentiation (primary response) protein 88 (MyD88), IL-1 receptor–associated kinase (IRAK), and NF-κB[96] (Fig. 12.5). Defects in any of the genes along this innate immune pathway lead to severe infections. Mutations in the *IRAK4* or *MyD88* gene lead to severe, recurrent pyogenic infections in infancy and childhood that improve with age, perhaps because of acquired immunity.[97] Mutations in the gene *IKBKG* (NF-κB essential modulator, NEMO) often have a profound defect that encompasses pyogenic in addition to mycobacterial, viral, and *Pneumocystis* infections, depending on the specific mutation.[98] Many of these patients also have mild-to-moderate forms of ectodermal dysplasia, reflecting the fact that morphogenic pathways also signal through NF-κB.[38] Mutations in specific TLRs confer highly pathogen-specific susceptibility, such as TLR3 and herpes simplex virus (HSV) encephalitis[99] and TLR4 and meningococcemia.[100]

PHAGOCYTE AND LYMPHOCYTE DEFECTS AFFECTING MONONUCLEAR CELLS

Although tuberculosis is highly virulent in humans and infects more than one-third of the globe's population, infections with nontuberculous mycobacteria (NTM) are much less common; when these infections are disseminated, they bespeak substantial immunocompromise. If HIV infection and iatrogenic causes are excluded, one is left with patients with intrinsic immune defects, some of which have recently been characterized at a genetic level.

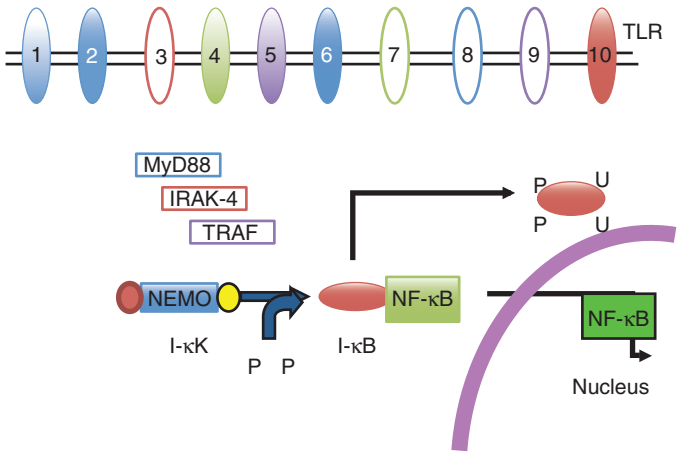

FIG. 12.5 Toll-like receptor *(TLR)* signaling. The TLRs signal through a variety of molecules, mostly culminating in nuclear factor kappa B *(NF-κB)* activation. NF-κB is held latent in the cytoplasm by inhibitor of kappa B kinase *(I-κB),* and it is released only after I-κB is phosphorylated *(P)* and ubiquitinated *(U).* Mutations have been identified in myeloid differentiation (primary response) protein 88 *(MyD88),* interleukin-1 receptor–associated kinase 4 *(IRAK-4),* and NF-κB essential modulator *(NEMO),* which predispose to severe bacterial infections, predominantly with encapsulated bacteria. Mutations in NEMO are also highly associated with mycobacterial infections and *Pneumocystis.* TLRs signal in response to bacterial, fungal, and viral products. Mutations in TLR3 are associated with recurrent herpes simplex virus encephalitis. Mutations in TLR4 are associated with meningococcemia. Polymorphisms in other TLRs have been shown to affect infection susceptibility to tuberculosis, *Legionella,* and other agents. Shaded TLRs are surface displayed. Open TLRs are maintained in intracellular vesicles. *TRAF,* Tumor necrosis factor receptor–associated factor.

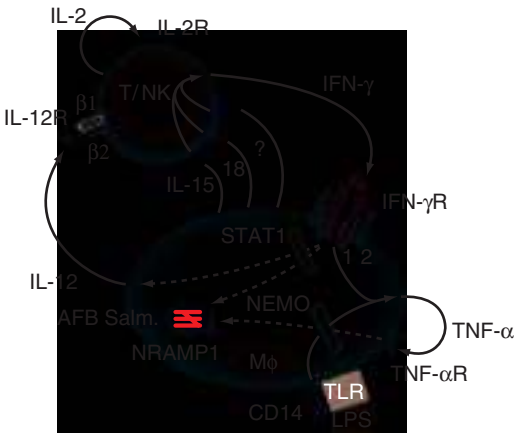

FIG. 12.6 The interacting cytokine and receptor pathways that regulate the resistance to, and killing of, mycobacteria and *Salmonella.* Lesions resulting in severe disseminated mycobacterial disease have been described in the interferon-γ *(IFN-γ)* receptors, signal transducer and activator of transcription 1 *(STAT1),* interleukin *(IL)*-12p40, IL-12 receptor *(R)* β1, and the signal transducing molecule nuclear factor kappa B essential modulator *(NEMO). AFB,* Acid-fast bacilli; *CD14,* cluster of differentiation antigen 14; *LPS,* lipopolysaccharide; *MΦ,* macrophage; *NK,* natural killer cell; *NRAMP1,* natural resistance–associated macrophage protein 1; *Salm., Salmonella; T,* T-cell; *TNF-α(R),* tumor necrosis factor-α (receptor).

IFN-γ is produced by lymphocytes and acts through its cognate receptor on monocyte-macrophages to facilitate the killing of intracellular pathogens, such as mycobacteria, *Salmonella,* and dimorphic yeasts (Fig. 12.6).[101] Therefore, it is expected and observed that severe, disseminated infections with NTM, such as *Mycobacterium avium* complex, develop in humans with defects in either the ligand binding (IFN-γ receptor 1) or the signal transducing (IFN-γ receptor 2) chains of the IFN-γ receptor.[102–105] These infections often have their onset in childhood, and if BCG is given

to these infants, it often disseminates. Patients with complete defects of the IFN-γ receptors have autosomal recessive defects, characteristically fail to form granulomas, and have very high mortality from their *M. avium* complex infections. They are also susceptible to recurrent disseminated infections with the intracellular pathogen *Salmonella*. Patients with partial defects in IFN-γ receptor 1 have an intermediate phenotype with severe but curable mycobacterial infections and retain the ability to form granulomas.[106] The autosomal dominant form of IFN-γ receptor 1 deficiency is the most common; patients present in childhood and later, typically with treatable nontuberculous mycobacterial infections involving bone. In these patients, a nonfunctional IFN-γ receptor 1 overaccumulates on the cell surface because of a common mutation in the intracellular domain that impairs receptor recycling.[107,108] The diagnosis of complete recessive (receptor absent) or partial dominant (defective receptor increased) IFN-γ receptor 1 deficiency is most easily accomplished by examination of the cell surface for IFN-γ receptor 1 expression by flow cytometry. However, demonstration of protein-positive IFN-γ receptor 1 deficiency and all cases of IFN-γ receptor 2 deficiency must still be performed by molecular assays.

IL-12 is a monocyte-macrophage product that acts on lymphocytes to drive the production of IFN-γ. As expected, defects in IL-12 signaling resulting from receptor (IL-12Rβ1, IL-12Rβ2)[109] or ligand (IL-12p40)[110] defects result in disseminated infections with NTM and *Salmonella*. However, because patients with IL-12 defects have some residual IFN-γ production, granuloma formation is preserved, and these patients tend to be less severely affected than those with complete IFN-γ receptor defects.[111,112] Treatment of IL-12– or IL-12 receptor–deficient patients with disseminated infections can be greatly aided by the use of IFN-γ. The diagnosis of IL-12 receptor deficiency currently requires functional or molecular assays.

Signal transducer and activator of transcription 1 (STAT1) mediates most signals from the IFN-γ and IFN-α receptors. Three discrete kinds of STAT1 defects occur: recessive complete loss of function, dominant negative loss of function, and dominant gain of function. Severe complete recessive STAT1 defects ablate both IFN-γ and IFN-α signaling, leading to fatal mycobacterial and viral infections.[113] In contrast, autosomal dominant mutations in STAT1 affect primarily IFN-γ receptor signaling, resulting in treatable disseminated nontuberculous mycobacterial infections in childhood.[114] Surprisingly, dominant gain-of-function mutations lead to accentuated STAT1 signaling, resulting in impaired formation of IL-17–producing T cells in some cases, which leads to chronic mucocutaneous candidiasis.[115,116] Other complications of the gain of function mutations include disseminated histoplasmosis, coccidioidomycosis, progressive multifocal leukoencephalopathy,[117] and IPEX-like syndrome.[118] This latter set of infections and complications may in part reflect IFN-γ excess.[117]

Hyperimmunoglobulin E–Recurrent Infection Syndrome (Job Syndrome)

Job syndrome is a rare disorder characterized by recurrent infections (typically of the lower respiratory system and skin), eczema, extremely elevated levels of IgE, and eosinophilia resulting from dominant negative mutations in the *STAT3* gene.[119,120] Most patients have facial abnormalities, including a prominent, protruding, triangular mandible and a broad, somewhat bulbous nose.[4,7,9] Failure of primary teeth to fall out is common and results in a frequent need for dental extractions to allow eruption of normal secondary teeth.[7,121] Moderate scoliosis develops in most patients. Many also have abnormalities of bone formation and metabolism, osteopenia, fractures, and craniosynostosis.[7,9,119,120] Job syndrome occurs spontaneously in all racial and ethnic groups and is transmitted as an autosomal dominant trait.[4,7,119,120]

Patients are usually noted within the first days to months of life to have severe eczema, mucocutaneous candidiasis, and cutaneous, sinus, or pulmonary infections, predominantly with *S. aureus* or *Haemophilus influenzae*. Pneumatoceles are often noted by adolescence, and these, in turn, provide a hospitable site for subsequent infections with *Aspergillus* and *Pseudomonas aeruginosa* (Fig. 12.7). Otitis media and externa are relatively common, as are intertriginous infections and breast abscesses. Infections occur less frequently in bones and joints and very infrequently in the liver, kidneys, and gastrointestinal tract. Documented sepsis is rare. Recurrent "cold" abscesses of the skin are commonly due to staphylococci. The inflammatory response in these patients is impaired, in part because of impaired IL-6 signaling and diminished circulating IL-17–producing T cells.[122] Therefore, STAT1 gain-of-function and STAT3 loss-of-function mutations overlap in having defects of IL-17–producing T cells, and thus mucocutaneous candidiasis, but differ because of STAT3's extensive role in somatic development. Pathogens recovered from patients with Job syndrome include *S. aureus, H. influenzae, Aspergillus* spp., *P. aeruginosa, Streptococcus pneumoniae,* group A streptococci, *P. (carinii) jirovecii*, *Cryptococcus neoformans, Histoplasma capsulatum*, and *Candida albicans*.[4,7,9,119]

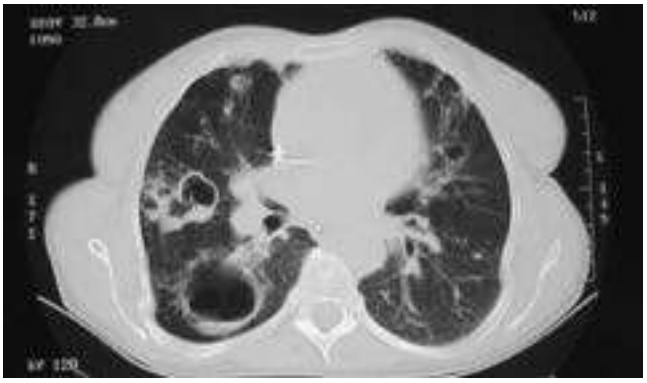

FIG. 12.7 Multifocal postinflammatory pneumatoceles in a patient with hyperimmunoglobulin E–recurrent infection (Job) syndrome.

The syndrome is defined by marked elevations of IgE (>2000 IU/mL), with levels of greater than 50,000 IU/mL reported.[4,7,9] Levels may start out elevated in cord blood and then climb through infancy and childhood, but they may also decline into the normal range over time.[7] Therefore, authentic cases do at times lack elevated IgE. Chronic leukopenia with borderline neutropenia has been observed in several patients.[9] Mild-to-moderate eosinophilia is the rule, although exceptions do occur.[7] No correlation between IgE levels and the degree of eosinophilia or clinical disease has been made.[7,9] The diagnosis should be suspected in the setting of combined immunologic and somatic features, including extremely elevated IgE, eczema, recurrent sinopulmonary infections, recurrent skin abscesses, failure of primary dental deciduation, scoliosis, characteristic facies, and a positive family history. Protein-positive heterozygous mutations in *STAT3* correlate well with clinical scores.[119,123]

GATA2 Deficiency

Haploinsufficiency of the primitive hematopoietic transcription factor GATA2 underlies several clinical syndromes, including monocytopenia and mycobacterial infections (MonoMAC); dendritic cell, monocyte, B, and NK lymphoid (DCML) deficiency; familial myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML); and Emberger syndrome (primary lymphedema with MDS).[124–129] These syndromes encompass immunodeficiency, bone marrow failure, leukemia, pulmonary alveolar proteinosis (PAP), and lymphedema. *GATA2* mutations also account for significant percentages of pediatric neutropenia, idiopathic CD4 lymphocytopenia, and aplastic anemia.[130,131] The age at presentation ranges from early childhood into late adulthood, for reasons that are still unclear.

Peripheral blood counts are normal when patients are asymptomatic but decline as complications mount. Profound B and NK lymphocytopenia and monocytopenia are strongly suggestive of GATA2 deficiency and should prompt genetic testing.

NK dysfunction and cytopenia underlie the susceptibility to human papillomavirus, cytomegalovirus, and varicella-zoster virus. Systemic IFN-α improves NK-cell cytotoxicity in vitro and may be beneficial for patients with refractory human papillomavirus.[132] EBV-positive mesenchymal tumors, as seen in advanced HIV/AIDS, indicate a profound host defect that may also be NK-cell mediated. Likewise, the deficit

and dysfunction of monocytes and macrophages may underlie susceptibility to intramacrophagic pathogens, including NTM and *H. capsulatum*. GATA2 also regulates alveolar macrophage phagocytosis, dysfunction of which may underlie PAP.

Complement-Mediated Immunity

Deficiencies in complement components clinically manifest as recurrent systemic bacterial infections. Bacteremia and meningitis are common in all the complement deficiencies.[1] Pneumonia is common in the early classical pathway (C1, C4, C2) and alternative pathway (factors I and H, properdin, C3) defects. Late-component defects (C5 to C9) are associated with recurrent *Neisseria* bacteremia and meningitis. Surprisingly, the bacteremia associated with late-component defects occurs at a much later age (average, 17 years) than does meningococcal bacteremia in the healthy population. Although these patients also have much higher rates of relapse and reinfection than do healthy people, their mortality from the infection is lower than normal. Patients with deficiencies of the early components of complement (C1, C4, C2, and C3) tend to have considerably higher rates of collagen-vascular disease, such as systemic and discoid lupus erythematosus, than do either healthy populations or patients with late-component defects.

Except for properdin deficiency, which is X-linked, complement deficiency states are inherited as autosomal recessive disorders. Heterozygotes have 50% of normal levels, whereas homozygous-defective persons tend to have very low levels, if any, of the affected component. Screening for the presence of classical complement deficiencies is best accomplished with use of the test for total hemolytic complement (the CH_{50} assay). This test examines the integrity of the classical pathway of the complement system by determining the ability of complement in patient serum to lyse antibody-coated sheep erythrocytes in vitro. Cell lysis leads to the release of hemoglobin, which can be determined spectrophotometrically. Specific classical complement component defects can be detected with a modified CH_{50} assay that uses purified proteins and selectively omits the one to be assayed so that patient serum must supply the missing factor.[19] Direct determinations of immunologically reactive protein, including members of the alternative pathway, can be performed with enzyme-linked immunosorbent assays or diffusion assays. These types of direct assay systems do not offer functional data but can help quantify apparent functional defects. The alternative complement pathway is similarly screened using the AH_{50} assay.

Acquired Immunodeficiencies

The most common immunodeficiencies are acquired after birth and are not clearly traceable to an immune genetic basis. Like other immunodeficiencies, they are best approached through a thorough history and physical examination to search for associated findings and to guide diagnostic testing. Special attention to the infecting organisms can point to underlying abnormalities in host defense. AIDS is caused by HIV, which induces progressive $CD4^+$ T-cell depletion.[133] A syndrome that manifests with opportunistic infections, as AIDS does, but is unassociated with HIV infection is idiopathic $CD4^+$ lymphopenia.[134] The diagnosis is made by excluding all other known causes of immunodeficiency, including HIV, and determining that the $CD4^+$ T-cell count is 300/μL or less. Certain malignancies, particularly hematopoietic and lymphoid malignancies, result in immune dysfunction by causing a deficiency in immune effector cells or dysregulation of such activities as antibody synthesis, and they are associated with severe or opportunistic infections. Drug therapy can be complicated by rare or idiosyncratic reactions, such as aplastic anemia with chloramphenicol or drug-induced neutropenia.[135] During chelation of iron overload with deferoxamine, the available circulating iron makes the intravascular environment more hospitable for certain bacteria, notably *Yersinia enterocolitica*.[136–138] Splenectomy, especially after trauma, predisposes to overwhelming infection with encapsulated organisms, such as *S. pneumoniae* and *Capnocytophaga canimorsus* (previously biogroup DF-2 [dysgonic fermenter 2]),[139] and parasites, such as *Babesia microti* and *Plasmodium* spp.[140] Severe thermal injury is associated with selective degranulation of neutrophil-specific granules, decreased chemotaxis, and profound susceptibility to infection.[141]

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The complete reference list is available online at Expert Consult.

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13 Applied Epidemiology for the Infectious Diseases Physician

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WHAT IS EPIDEMIOLOGY?

The word *epidemiology* originates from the Greek *epi-* (meaning "surrounding"), *demos* (meaning "people"), and *logos* (meaning "the study of"). Thus epidemiology literally refers to the study of population occurrences. We now define epidemiology as *the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems*.[1] Historically, the discipline of epidemiology reaches back in time over 2500 years and includes contributions from thinkers such as Hippocrates, John Graunt, James Lind, and William Farr. However, John Snow, an English anesthesiologist, is widely considered the "father of field epidemiology." His famous study identified a single water pump on Broad Street as the cause of a cholera epidemic in the Golden Square of London. In 1854, twenty years before the invention of the microscope, Snow mapped the distribution of households with cholera cases during the epidemic and recognized that the cases were clustered around a particular water pump, the Broad Street pump. To confirm his suspicion that contaminated water from this pump was causing the cholera epidemic, Snow removed the handle of the pump, and the outbreak ended.[2]

The field of epidemiology has expanded from the investigation of infectious disease outbreaks, to the study of injuries and violence, and to the examination of any exposure that influences health outcomes, including molecular and genetic factors.[2] Epidemiology is used in many ways, such as for evaluating a population's health, informing individual decisions, and establishing causality.[2] Using rigorous statistical methods, epidemiology can answer complex questions, such as whether risk patterns correlate with exogenous factors or whether a public health program modifies risk.[2] For infectious diseases clinicians, the epidemiologic factors associated with a case or cluster of cases can be used to great benefit, potentially decreasing the time to diagnosis, limiting the need for extensive testing, and tailoring the use of broad-spectrum antibiotics. Epidemiologic data may also be used to inform policies or guidelines that affect groups or individuals, such as choosing which populations to vaccinate, setting speed limits on a highway, or determining how many times a week to exercise.[2]

WHAT CAN EPIDEMIOLOGY TELL THE INFECTIOUS DISEASE CLINICIAN?

Epidemiology asks the clinician to think of the patient's community and exposures, as well as the individual patient. Is my patient's respiratory infection part of an outbreak of influenza in the community and, if so, what can be done to identify and control it? Interaction with the local health department or hospital infection control staff in this instance might have a broader impact than treating a single patient but will also require understanding how epidemiologic questions are formulated and answered.

THE EPIDEMIOLOGIC TRIAD MODEL: AGENT, HOST, AND ENVIRONMENT

To better inform clinical and public health decisions, epidemiologists (and clinicians) try to find causal links between exposures and health outcomes. If this is an influenza outbreak, how is it being spread, and how severe are the illnesses? The epidemiologic triad, or triangle, is one of the simplest models used to describe disease causation.[2] In this model disease arises from the interaction between three components: an external "agent," a susceptible "host," and an "environment" that supports the transmission of the agent from its source to the host (Fig. 13.1).[2] By tradition the agent represents an infectious microbe, such as a virus, bacterium, or parasite. However, this component of the model does not sufficiently describe all diseases; some can be chronic processes with multiple contributing causal factors. Host susceptibility can depend on an individual's genetic makeup, immune status, and host defenses, such as skin integrity, gastric acidity, ciliary function, and other factors. Host immunity, acquired immunodeficiency, and principles of immunization are discussed in Chapters 4 to 6, 118, and 316. Environmental factors that promote agent transmission to a susceptible host include the presence of animals and other reservoirs of infectious agents, vector populations, crowding, poor sanitation, climate, and geography.[2] A detailed understanding of the epidemiology, pathogenesis, and ecology of a disease is required to construct optimal epidemiologic triads (agent, host, and environment).

THE SUFFICIENT-COMPONENT CAUSE MODEL OF EPIDEMIOLOGY

A second causation model that considers the multiple factors that contribute to disease is called the "sufficient-component cause model."[3] In this model each disease pathway has "component causes" or factors that contribute to disease. Different combinations of component causes are considered "sufficient causes" of disease when they complete the causal pathway. A component cause that is present in every combination of factors in the causal pathway is considered a "necessary cause" because the pathway cannot be complete without it. For example, human papilloma virus (HPV) infection is a necessary cause of cervical cancer because persons must be infected with this virus to develop HPV-associated cervical cancer. However, HPV infection is not a sufficient cause of cervical cancer because not all individuals infected with HPV develop cervical cancer.

OTHER METHODS TO CHARACTERIZE INFECTION TRANSMISSION

Multiple classifications exist to describe modes of disease transmission or the ways in which an agent leaves its natural environmental reservoir to infect a susceptible host. One classification distinguishes "direct" from "indirect" transmission.[2] Direct transmission includes direct contact and droplet spread (short-range aerosols produced by sneezing, coughing, or talking), whereas indirect transmission includes airborne, vehicle-borne, and vector-borne (mechanical or biologic) spread.[2]

DESCRIPTIVE EPIDEMIOLOGY

Descriptive epidemiology is used to characterize events in terms of person, place, and time and might answer the following questions: What health risks exist for this population? Who is getting influenza this winter?

Who are the population members at risk? Is the influenza strain circulating in nursing homes, hospitals, or schools? Which geographic locations are associated with increased risk? How has the level of risk changed over time? Case reports and case series are types of descriptive studies conducted in clinical settings.[4] Case reports describe the clinical features of a disease, along with demographic and historical details relevant to its presentation. Case series include the presentation, clinical features, and other significant facts pertaining to multiple patients with similar health conditions. Case reports (and series) often represent the first or novel presentations of a condition and can be very valuable from both a clinical and an epidemiologic perspective. For example, ribavirin was recently shown to be effective in a series of patients with chronic hepatitis E, not an original indication.[5] Descriptive epidemiology may also assist in recognition of an emerging disease or in generating hypotheses about causal factors and factors that increase the risk of disease.[2]

ANALYTIC EPIDEMIOLOGY

When one would like to more definitely establish a relationship between an exposure and a disease, analytic epidemiology is required. Hypotheses about the relationship are normally formulated, and a study design is selected based on the available data, the research question, ethical considerations, feasibility, and issues of validity and efficiency. Analytic epidemiologic studies fall into two categories: experimental and observational[2] (Table 13.1). An observational study has no intervention but attempts to link the exposure to the disease passively. In an experimental study the exposure is actively managed and the outcome measured prospectively. Precision, validity, and bias should all be considered when designing an epidemiologic study but are covered elsewhere.[2]

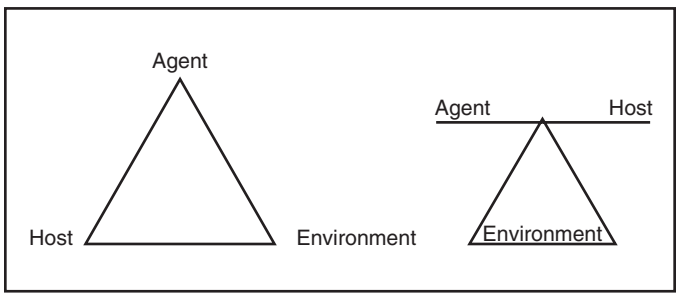

FIG. 13.1 **Epidemiologic triangle (triad).**

EXPERIMENTAL STUDIES

In an experimental study an investigator assigns similar groups of individuals (clinical trial) or communities (community trial) to different exposures or interventions and then follows the groups over time to ascertain the effects of the exposure.[2] A classic example of a clinical trial is a vaccine trial, where one group of individuals is assigned to receive an immunization to prevent a particular disease and another group of individuals to receive a placebo. There are numerous examples of vaccine trials, including the definitive demonstration of the effectiveness of influenza immunization in children.[6] After a defined period of time, the investigator monitors each participant to diagnose any occurrence of the disease in question, the investigator compares the rates of infection between the vaccine and placebo groups to determine if the vaccine successfully protected recipients from the disease. In a community trial, groups of individuals undergo randomization to receive an intervention, and investigators ascertain community health outcomes after a period of time relevant to the intervention and health outcome under investigation. Community trials are ideal for evaluating broad-based interventions, such as case management algorithms and health-related policies. An example of a community trial is testing the utility of long-lasting insecticide-treated bed net distribution on malaria incidence in endemic areas.[7]

OBSERVATIONAL STUDIES

Cohort Studies

The most common types of observational study designs are cohort and case-control studies. Cohort studies are conceptually similar to experimental studies. The investigator evaluates the exposure status of participants and then follows them over time to evaluate the incidence of one or more health outcomes in each exposure group. If a health outcome is significantly greater in the exposed group relative to the unexposed group, then the exposure is considered to be associated with the disease.

TABLE 13.1 Epidemiologic Study Design Strengths and Weaknesses

STUDY DESIGN	ADVANTAGES	DISADVANTAGES	METHOD	MEASURE OF ASSOCIATION
Ecologic	Very inexpensive Very fast Hypothesis generation	Unit of study is the population Risk of ecologic fallacy No temporal relationship Cannot control for confounders	Evaluates population-level occurrences	Prevalence ratio
Cross-sectional	Very inexpensive Very fast Individualized data Hypothesis generation Can control confounders	No temporal relationship Not good for rare diseases Not good for diseases of short duration	Evaluates two factors for all persons in a population	Prevalence ratio, prevalence odds ratio
Case-control	Inexpensive Fast Good for rare diseases Can assess multiple exposures	Cannot directly measure risk Can only evaluate one outcome Can introduce bias through control selection Prone to recall bias Can be hard to demonstrate temporality	Sampling conducted with respect to outcome status, and exposure evaluated retrospectively	Odds ratio
Cohort	Establish temporal relationships Individualized data Can control confounders Assesses multiple exposures Assesses multiple outcomes Can calculate incidence Can nest other studies within the cohort study	Expensive Time consuming (prospective) Not good for rare diseases Requires high follow-up rates	Sampling conducted with respect to exposure status, and outcome evaluated prospectively or retrospectively	Relative risk
Clinical trial	Controls bias via randomization Controls confounders via selection criteria Can assess multiple exposures (depending on number of arms) Can assess multiple outcomes	Expensive Ethical concerns for certain exposures of interest Participants may not represent the general population Must consider differences in adherence	Randomization of participants to exposure, then outcome evaluated prospectively	Relative risk

Cohort studies differ from experimental studies in that the investigator does not assign individuals to an exposure group; rather, the exposure group is determined passively. Cohort studies can be performed prospectively or retrospectively. In a prospective study the exposure status is established in advance, and the participant is followed over time to determine the occurrence of the outcome of interest. In a retrospective study both the exposures and the outcomes have already occurred at different points in time. The investigator collects the retrospective data and compares the rates of the outcome in the exposed and unexposed groups. A recent example of a well-designed cohort study described the frequency and distribution of pregnancy outcomes after Zika virus infection.[8]

Case-Control Studies

In contrast, case-control studies evaluate a single health outcome in relation to one or more exposures. For a case-control study, subjects with the health outcome of interest (e.g., became infected) are enrolled as well as control subjects without the health outcome of interest (e.g., did not become infected). The exposure histories of both sets of subjects are then compared, with the control group providing an estimated baseline or expected amount of exposure for the population of interest. If exposures are high among subjects with the health outcome of interest relative to the exposures expected based on evaluation of the control group, then the outcome is determined to be associated with that exposure. A recent case-control study from Peru demonstrated a link between latent tuberculosis infection and acute myocardial infarction.[9]

Cross-Sectional and Ecologic Studies

Cross-sectional and ecologic studies are also observational study designs. Cross-sectional studies examine the relationship between an exposure and an outcome among individuals in a defined population at a single point in time, like a snapshot. An example is determining the prevalence of syphilis in a population of prisoners in Brazil to decide if an intervention is required.[10] Ecologic studies evaluate associations using population-level exposures and outcome rates, rather than using individuals as the unit of analysis. Both cross-sectional and ecologic study designs have important limitations that make them less scientifically rigorous than other study designs.[11,12] Most important, they do not establish temporal sequences between exposures and outcomes, which limits the ability to determine causal inferences. In ecologic studies the lack of individual-level information contributes to what is known as the "ecologic fallacy," meaning that individual-level relationships between exposures and outcomes cannot be inferred from population-level associations.[12,13] In other words, the individuals with the exposure may not be the same individuals who developed the disease, even though a particular population has high rates of the exposure and outcome.[12]

DETERMINING THE APPROPRIATE EPIDEMIOLOGIC STUDY DESIGN

When determining which study design is most appropriate, it is important to consider the strengths and weaknesses of each (see Table 13.1). Experimental studies are ideal for evaluating an intervention for a particular outcome with a high degree of validity, such as when an intervention is expected to have a small effect or when a highly controlled setting with randomized participants is needed to minimize bias and confounding.[12] Only randomization, preferably blinded, can reduce the effect of unidentified factors or confounders that can potentially bias the outcome. The disadvantages of experimental studies include high cost and ethical considerations related to exposing participants to certain risks. Observational studies can be used to evaluate a wider range of exposures, including preventions, treatments, and causes of disease. The primary limitation of observational studies is the inability to control for confounding factors. Cohort studies are useful for investigating rare exposures, multiple outcomes, and outcomes for which little is known about exposure. Compared with other study designs, case-control studies generally take less time, require fewer resources, and can provide information about multiple exposures when little is known about the etiology of a particular health outcome.[12] In addition, case-control studies are more efficient than cohort studies for studying rare diseases and diseases with long incubation or latency times because they do not require long-term prospective follow-up.[12] Case-control studies are also more efficient when the population of interest is dynamic or when the study requires expensive or hard-to-obtain exposure data.[12] Although caution must be exercised with temporal inference, cross-sectional studies are useful because they can be performed in a short time period and yield highly generalizable results when based on large samples from the general population. Likewise, ecologic studies are rapid, inexpensive, and simple, and they can provide useful information about the context in which populations live.[13]

BASIC BIOSTATISTICS

A basic understanding of biostatistics is required to interpret medical literature or to plan appropriate epidemiologic studies. This section will cover measures of disease frequency, measures of association, and significance of association only. For more extensive reading on biostatistics, please refer to the classic references 2, 12, and 14. For the use of biostatistics in clinical trials see Chapter 52.

Measures of Disease Frequency

Measuring the frequency of disease in a population is the first step in characterizing the distribution and determinants of disease in populations.[12] To conceptualize disease occurrence, it is important to consider the size of the underlying population, the number of persons affected, and the length of time that the population is observed.

Ratios, Proportions, and Rates

These are the three types of calculations used to describe disease occurrence. A ratio is one number divided by another number. For example, the maternal mortality ratio is a ratio of two unrelated numbers, the number of maternal deaths per 100,000 live births. Proportions, known as fractions or percentages, also consist of one number divided by another, but the numerator must always be a subset of the denominator. Measures of disease occurrence are often written as rates. A rate also consists of one number divided by another number, but the denominator always includes time. The attack rate of a disease represents the proportion of individuals who developed illness divided by the total number of individuals exposed to a pathogen. For example, annual incidence rates for Lyme disease are calculated by state per 100,000 persons.[15]

Incidence

The two most basic measures of disease frequency are incidence and prevalence. Incidence is the number of new cases of disease that occur in an "at-risk" population over a specified period of time. Incidence takes into account how many persons are at risk in a particular population. For example, women are not at risk for prostatitis. Thus the incidence of prostatitis in a population would not include women in the denominator. The time period during which a population is followed is also important for incidence calculations. Incidence rate refers to the number of new cases of disease that occur during person-time observation, where time is included in the denominator. Incidence rate calculations include the amount of time that each individual contributes to the denominator before being diagnosed with the disease of interest. Cumulative incidence refers to the number of at risk individuals who become diseased during a specified period of time. It can be expressed as a proportion of the at-risk population and therefore ranges from 0% to 100%, or 0 to 1.[12]

Prevalence

Although incidence measures the frequency of the development of new disease, prevalence measures the frequency of existing disease in a population. Prevalence is defined as the number of persons with disease in a population divided by the total number of persons in that population. Unlike incidence, the prevalence numerator includes all persons with the disease, regardless of when it developed, and the prevalence denominator includes everyone in the population, whether or not the individuals are at risk. There are two different prevalence measures: point prevalence and period prevalence. Point prevalence refers to the proportion of the population with the disease at a specific point in time, such as a particular date. Period prevalence refers to the proportion of

		Outcome?		
		Yes	No	TOTAL
Exposed?	Yes	a	b	a+b
	No	c	d	c+d
		a+c	b+d	a+b+c+d

FIG. 13.2 Construction of a standard two-by-two table.[14]

the population with the disease over a specified period of time, such as a calendar year. Period prevalence includes the number of persons with disease present at the start of the time period, in addition to the number of persons who developed the disease during the specified period.[2] Prevalence depends on the rate at which new cases of disease develop (incidence rate), in addition to the duration of illness. The duration of disease begins when a person acquires the disease, and it ends when a person is cured or dies.

Measures of Association

Measures of association, or measures of effect, quantify the strength of the statistical association between exposures and health outcomes of interest. The respective measures of association for each type of study are shown in Table 13.1.

Two-by-Two Tables

To understand how to calculate measures of association, it is important to understand the basic construct of a two-by-two table. In many epidemiologic studies exposure and outcome data are dichotomous (e.g., "yes" or "no"). Thus the relationship between exposure and outcome can be cross-tabulated in a two-by-two table, which has two categories of exposure and two categories of outcome (Fig. 13.2[14]). By convention, outcome status is placed in columns, and exposure status is placed in rows. The four cells of the two-by-two table are labeled a, b, c, and d, and each letter refers to the number of persons with the outcome indicated by the column heading and the exposure status indicated by the row heading.

$$\text{Risk ratio (relative risk)} = \text{Risk}_{\text{exposed}} / \text{Risk}_{\text{unexposed}} = (a/[a+b])/(c/[c+d])$$

Risk ratio (RR) describes the excess risk of a health outcome in exposed persons compared with unexposed persons, who represent the background or expected risk. Mathematically, the RR is expressed as the risk in the exposed group divided by the risk in the unexposed group. A risk ratio greater than 1.0 indicates that risk is greater in the exposed group when compared with the unexposed group, whereas RR less than 1.0 indicates that the risk in the exposed group is less than in the unexposed group. This happens when the exposure protects against a health outcome, such as an immunization that protects against an infectious disease.[2]

$$\text{Odds ratio (cross-product ratio, relative odds)} = ad/bc.$$

Odds ratios (ORs) are measures of association used in case-control studies to approximate RR. The OR is also called the "cross-product ratio" because it is obtained by calculating the cross products of the cells in a two-by-two table, with "a" × "d" in the numerator and "b" × "c" in the denominator. In case-control studies, epidemiologists enroll a group of "cases" with the outcome and a comparable group of "controls" without the outcome. The number of individuals in each group is determined a priori, and the size of the populations from which the cases and controls arise is often unknown. This lack of population denominator data prevents direct calculation of the RR. However, the OR approximates the RR when the outcome of interest is rare.

Prevalence Ratio and Prevalence Odds Ratio

Cross-sectional studies usually measure the prevalence of a health outcome, rather than its incidence. Because prevalence is a function of both incidence and duration of illness, measures of association based on prevalent cases reflect the effect of the exposure on both incidence and duration, or survival. The prevalence ratio and the prevalence OR, respectively, are the measures of association that correspond to the RR and the OR in a cross-sectional study. These ratios are calculated using the same formulas but recognizing that the data in the two-by-two table is prevalence data.[2]

STATISTICAL SIGNIFICANCE

P Values

To make hypotheses about causal relationships between exposures and outcomes, three alternative explanations for study results should be considered: bias, confounding, and random error. Bias is a systematic error in study design, measurement, or analysis that leads to a false association between the exposure and outcome. Confounding is a systematic mixing of effects between the exposure, outcome, and a third variable called a confounder, unmeasured or unrecognized, which distorts the measure of association between the exposure and outcome. Random error arises from chance and leads to a false association between the exposure and outcome that is uncontrollable and appears not to have an assignable cause. Random error can occur from mistakes in measurement or sampling variability that occurs by randomly selecting an unrepresentative population sample purely by chance.

Hypothesis testing is a method of evaluating random error in an epidemiologic study. The default assumption, or "null hypothesis," is that there is no relationship between exposure and the outcome. For example, eating raspberries this July did not increase the chances of getting cyclosporiasis. The compatibility between the data observed in the study with the null hypothesis is evaluated using a statistical test. Computing the test statistic yields a *P* value, which represents the probability of obtaining the observed or more extreme results by chance alone. The *P* value ranges from 0.0 to 1.0, with a small value indicating that the null hypothesis may need to be rejected. By convention, a *P* value cutoff of 0.05 is traditionally used to determine whether to reject the null hypothesis, which assumes that a 5% or lower chance of random error is acceptable when asserting that there is a true relationship between exposure and outcome. This cutoff is also called the alpha or significance level.

Confidence Intervals

Another way to quantify random error in studies is to estimate the confidence interval (CI). CIs quantify the variability around a point estimate. The width is determined by random error, in addition to an arbitrary certainty factor, usually set at 95%, although some studies use 90% or 99%. Using a 95% CI as an example, the strict interpretation is that 95 of 100 CIs would contain the true measure of association if a study were repeated 100 times to obtain 100 point estimates with 100 CIs. Wider CIs mean that there is a larger amount of random error relative to narrower CIs. In general, sample size affects CI width, and wider CIs indicate that a relatively smaller sample created the point estimate, whereas narrower CIs generally indicate point estimates created by larger samples. CIs can also be used to determine statistical significance. For example, if the null value for a measure of association (example: RR of 1.0) falls within the 95% CI, then the result is by convention not statistically significant. Some epidemiologists prefer using CIs to *P* values when determining statistical significance because CIs are expressed in the same units as the measure of association and contain the point estimate, whereas the point estimate cannot be estimated by examining a *P* value. Nevertheless, similar to *P* values, CIs do not account for bias and confounding, nor can they confirm that a given correlation is causal.

DISEASE SURVEILLANCE

Surveillance for infectious diseases is one of the most critical functions of a public health system, from the clinic or hospital level all the way to the national and global level. Surveillance can take many forms but generally entails the continuous and systematic collection and analysis

of data, and the subsequent reporting of any significant findings to effect change. There are many valid indications for implementing surveillance, including measuring baseline levels of disease, identifying hypotheses to conduct further research, monitoring the effectiveness of interventions, and detecting outbreaks. Perhaps most relevant for infectious diseases clinicians are the recognition and initial management of outbreaks, because it is often physicians who first recognize an increase in cases. A brief discussion on outbreak management is included in the following text.

Classification of Surveillance

Surveillance can be either active or passive and may collect data on incident cases, prevalent cases, or both. Active surveillance is usually more sensitive for discovering an outbreak but is more costly and labor intensive. Passive surveillance is often less sensitive but is also less costly and thus can be deployed on a broader scale.

Surveillance systems in the United States often follow a hierarchical reporting scheme, with local clinics, hospitals, or laboratories tabulating cases and reporting them to the local public health authorities. The collated data is then passed to the state-level public health officials, who in turn report to the Centers for Disease Control and Prevention (CDC). The CDC reports to World Health Organization (WHO) through the US government focal point, if necessary, about outbreaks of potential global significance. Reports of outbreaks are updated by the CDC on the Epidemic Information Exchange (Epi-X), a secure web-based reporting system (https://emergency.cdc.gov/epix/index.asp) limited to public health officials, as well as in the Morbidity and Mortality Weekly Report (MMWR), which is available to the public electronically at http://www.cdc.gov/mmwr/ and in hard copy. An international treaty, the International Health Regulations (IHR [2005]) governs what type of outbreak is reportable and to whom and under what time requirements it must be reported.[16] Each signatory country is responsible for controlling disease within its own borders. The IHR (2005) is designed to allow less-developed countries to request assistance from WHO and its response mechanism, the Global Outbreak Alert and Response Network (GOARN). The GOARN and WHO Outbreak News websites provide public information about current outbreaks of potential global concern.[17]

Some sentinel surveillance systems attempt to identify specific diseases using representative sites. For example, FoodNet is part of CDC's Emerging Infections Program and conducts surveillance for enteric pathogens in 10 state health departments in collaboration with the US Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) and the US Food and Drug Administration (FDA).[18] The 10 sites cover approximately 48 million persons (≈15% of the US population) and include Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, and Tennessee, and selected counties in California, Colorado, and New York.[18] This system has identified many outbreaks of gastrointestinal infections, such as the multistate outbreak of *Salmonella* Virchow in 2015–16.[19]

Disease surveillance is also conducted on a global scale. Perhaps the best example of this is the Global Influenza Surveillance and Response System (GISRS), which is a unique, laboratory-based global surveillance network to monitor seasonal and emerging influenza viruses to mitigate pandemic influenza spread.[20] The GISRS network, established in 1952, consists of 142 National Influenza Centers, 6 WHO Collaborating Centers, and 4 Essential Regulatory Laboratories. Influenza isolates are systematically collected throughout the year and compared with previously circulating strains to predict which strain is likely to be predominant in the following season. Different compositions of influenza vaccine (containing influenza A H3N2, A H1N1, and B components) can be selected for Northern and Southern Hemisphere seasons.[21]

Technology is revolutionizing disease surveillance, especially in the field of infectious diseases. Classic tools, such as the outbreak line list and population serosurveys, remain important for understanding and controlling disease, but increasingly the power of "big data" is being leveraged to more rapidly and precisely address the challenges posed by infectious diseases. Syndromic surveillance aims to identify unusual or novel diseases by monitoring for general clinical syndromes, such as febrile pneumonia. In response to the intentional anthrax outbreak of 2001, electronic disease (syndromic) surveillance was intensified in the United States with the goal of rapidly identifying any clusters of illness that were compatible with a biowarfare attack. The Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE) is used by federal (CDC, the National Syndromic Surveillance platform), state, and local public health entities to monitor novel and emerging infectious diseases, chronic illnesses, and other public health conditions of interest, such as mental and behavioral health disorders and environmental exposures.[22]

Novel methods of disease surveillance are continuously being developed and refined. Some reporting systems, such as ProMED mail, have been functioning since the 1990s and rely on a network of health care professionals and other interested parties (more than 70,000 subscribers in 185 countries) to report outbreaks around the world.[23] This email- and web-based system covers outbreaks affecting humans, animals, and plants, and it is moderated by a panel of public health experts who screen each report before release. Digital disease detection or surveillance is a new technology that relies on collating and analyzing diverse streams of data that are increasingly available in machine-readable format. The prototypical digital disease detection platform is HealthMap (http://www.healthmap.org/en), which harnesses the broad reach of the Internet by searching "online" news outlets and applying automated logic to sort "real" outbreaks from rumor.[24] The CDC and the Infectious Diseases Society of America collaborate on the Emerging Infections Network (https://ein.idsociety.org/), an email-based list-serv that connects practicing infectious diseases physicians from the United States and some other areas around the world to conduct surveillance for emerging infectious diseases.[25] The GeoSentinel Surveillance System (http://www.istm.org/geosentinel) is likewise a worldwide network of travel medicine clinics that reports illnesses among travelers.[26]

Machine learning and related deep-learning computer algorithms are synthesizing and analyzing unapproachably large data sets that surveillance systems can collect. This allows aberration detection within surveillance data streams and may even lead to improved prediction of epidemiologic trends or forecasting of disease events.[27] Better-quality data also allow more precise geospatial representation of disease, increasing the efficiency of identification, response, and control of outbreaks.[28]

The astute and vigilant clinician arguably remains the most important factor in outbreak surveillance. The first case of severe acute respiratory syndrome (SARS) was identified by a physician in Hanoi, Vietnam, who noticed an unusually severe, atypical pneumonia.[29] The first cases of acquired immunodeficiency syndrome (AIDS) were identified by a clinician who noted rare infections among a group of homosexual men.[30] Also, West Nile virus was discovered by an observant clinician in New York City, following an independent veterinary investigation reporting a die-off of birds.[31] It is important for clinicians to understand that as individual providers they may be seeing the proverbial "tip of the iceberg." Only by reporting these cases can an outbreak be appreciated as cases are examined in aggregate.

DISEASE PREVENTION

Prevention includes a wide array of interventions aimed at promoting health by reducing risks that lead to poor health outcomes or disease. *Primary, secondary,* and *tertiary* prevention are three terms that describe the points in the disease process targeted by an intervention.[2] Primary prevention aims to prevent the occurrence of disease. This is done by preventing exposure to hazards that cause poor health outcomes, that is, changing unhealthy or unsafe behaviors that lead to poor health outcomes and increasing resistance to poor health outcomes if exposure occurs. Examples of primary prevention include legislative measures that control health hazards, education about healthy behaviors, and immunization against diseases.[2] Secondary prevention aims to minimize the impact of a poor health outcome that has already occurred. This can be done by identifying and treating a health condition as soon as possible to prevent its progression, implementing strategies to prevent recurrence, and modifying activities to help persons return to their original state of health. Examples of secondary prevention include screening tests that identify diseases during their earliest stages, such as hepatitis C screening. Tertiary prevention aims to lessen the impact of a poor health outcome with lasting effects, such as chronic disease or injury leading to disability. This can be done by managing long-term

ailments to lessen disability, improve quality of life, and lengthen life expectancy. Early antiretroviral therapy for human immunodeficiency virus (HIV) infection is an example of tertiary prevention.

DISEASE CONTROL: QUARANTINE AND ISOLATION

There are many strategies to mitigate the spread of infections, and those relevant to hospital infection control are given in Chapter 298. Some strategies targeting public health intervention, namely quarantine and isolation, and a short primer on outbreak investigation are discussed as follows.

Quarantine (separating and restricting movement of individuals *exposed* to a communicable disease) and isolation (separating and restricting movement of individuals *suspected or confirmed* to be infected with communicable disease) are public health strategies used to prevent exposure of the public to individuals or populations infected with a communicable disease. In an increasingly connected world, public health officials must interact with multiple entities and many levels of government to balance public health and humanitarian efforts with economic priorities, such as international travel and trade. In the 2014 West African Ebola epidemic, for example, the CDC partnered with WHO, the International Organization for Migration, nongovernmental organizations, and domestic entities, such as the US Department of Homeland Security and state and local health departments, to prevent international transmission of Ebola while minimizing disruption of economic activities.[32] Domestically, in addition to communicating with travelers via travel notices and airport messaging, CDC teams provided training and equipped US entry-point personnel to conduct screening using body-heat sensing devices and questionnaires to identify symptomatic and potentially exposed travelers arriving in the United States. If a potentially exposed individual was identified through screening, the domestic quarantine strategy relied on measures such as active self-monitoring, controlled movement, and daily communication with public health officials (for high-risk individuals, such as health care workers) during the 21-day Ebola incubation period, with the aim of applying the least restrictive measures necessary to prevent potential transmission.[32]

OUTBREAK INVESTIGATION PRIMER

Outbreaks of infectious disease can arise anywhere in the world and pose a threat to local and global populations without respect for political borders, geographic separation, or cultural differences. Many current conditions facilitate this potential for rapid spread of infectious diseases, such as the globalization of the food supply, overuse of antibiotics, the growth of megacities with severe crowding, our increasing proximity to animals and vectors of disease, and even climate change.[33]

There have been numerous recent outbreaks that have seized the public's attention. The SARS epidemic of 2003 and the recent resurgence of measles in North America and Europe were watershed events and hastened the strengthening of the IHR. These were followed by multiple outbreaks of imported foodborne gastroenteritis caused by *Cyclospora* and *Salmonella,* as well as by MERS, Nipah virus, pandemic H1N1 influenza, and, most recently, Ebola virus disease and Zika virus syndrome.[33,34] The scope and impact of these outbreaks underscore the importance of maintaining an informed network of clinicians who understand the dynamics of an outbreak and how to investigate one. This section describes the key concepts involved in an outbreak investigation, discusses basic transmission dynamics, and provides a simple guide for conducting an outbreak investigation.

An outbreak is an increase beyond expectation in the number of cases of a disease or condition, occurring in a specific population in a defined geographic location and period of time.[14] The cases are epidemiologically related, although this linkage is often not initially evident and may only be discovered after thorough investigation. For example, in the 1981 multistate outbreak of *Salmonella muenchen*, the epidemiologic link was discovered only when case patients reported smoking marijuana more frequently than control patients. This rare *Salmonella* species was then isolated from marijuana samples found in the homes of patients with the illness.[35]

Outbreaks very often have an infectious origin, although some can be due to noninfectious agents, such as food intoxication or even hysteria. It is often difficult to define how many cases beyond expectation constitutes an outbreak, but even one case can indicate an outbreak if the disease has been eradicated (smallpox), eliminated (poliomyelitis in Europe), or is novel to humans (highly pathogenic H5N1 avian influenza in the Americas).

In the absence of timely control measures, outbreaks can spread and lead to epidemics or even pandemics. Epidemics are conceptually identical to outbreaks but are more widely disseminated in time and space, such as the cholera epidemic in London during the mid-1800s and SARS in Southeast Asia in 2003. Pandemics spread globally and may persist through months, years, or decades. Examples of pandemics include such historic scourges as bubonic plague in medieval days and influenza in 1918-19, as well as AIDS or the influenza A/H1N1 pandemic of 2009 more recently.

Many infectious diseases are endemic in certain settings and occur routinely. Malaria, dengue and enteropathogens that cause diarrheal disease in the tropics are all examples. Endemnicity does not preclude the occurrence of outbreaks, however, that may occur during point-source foodborne outbreaks when large pools of susceptible individuals are exposed at a single time. *Vibrio parahemolyticus* and Norovirus, for example, are endemic causes of sporadic gastroenteritis but can also lead to outbreaks when contaminated seafood is eaten raw.[36,37] Often, all that is required for an outbreak to occur is a sufficiently large, naïve population that is exposed to an infectious inoculum of the agent.

OUTBREAK EPIDEMIOLOGY

General

The methodology for conducting outbreak investigations and the study designs and methods used have been well described in the literature.[2,14,38–41] Reviews of published outbreaks suggest that foodborne transmission accounts for nearly half of all reported outbreaks. Most efforts to improve the standardized reporting of outbreaks have been focused on either foodborne disease or nosocomial infections.[42,43] The CDC collects and collates data on outbreaks in the United States and maintains a dashboard that includes visualizations and statistics through 2016. Outbreak data can be explored at the National Outbreak Reporting System, which is found at https://wwwn.cdc.gov/norsdashboard/.[44] A current list of ongoing outbreaks, both domestic and significant international, can be found at https://www.cdc.gov/outbreaks/index.html.

KEY OUTBREAK PRINCIPLES

Incubation Period

Incubation period is the time elapsed from exposure to a certain infectious agent until the development of symptoms and is often expressed as a range. In an outbreak investigation the incubation period is a key parameter that may help to discern between multiple potential etiologic causes. For example, symptoms of foodborne staphylococcal intoxication often appear 30 minutes to 6 hours after exposure, whereas the incubation period for salmonellosis is 6 to 72 hours. So, abrupt gastroenteric illness in multiple subjects who recently shared a common meal is less likely to be caused by an agent with a longer incubation period like *Salmonella*. However, it should not be mistakenly assumed that infectious agents with lengthy incubation periods cannot cause outbreaks because transmission of *Mycobacterium tuberculosis* during air travel has been demonstrated on several occasions.[45] Cases from outbreaks caused by agents with long incubation periods will typically appear over extended time periods, making their common origin less obvious. The routine application of genetic sequencing of pathogens may assist in defining the relatedness of cases that are separated by time and space.[46]

Epidemic Curves

Epidemic curves are histograms depicting the number of cases of a disease during the duration of an outbreak or epidemic.[14] When properly prepared, epidemic curves are key epidemiologic tools in the outbreak investigation that may aid in determining the transmission mode of the outbreak, the incubation period and possible period of exposure. The main mode of transmission can often be identified from epidemic curves (point source, person-to-person, or continued common source),

although on occasion two of these modes are seen at different stages during a single outbreak. The crucial step in preparing an epidemic curve is choosing a time interval unit that corresponds to a fraction of the median incubation period of the suspected agent (usually between ⅓ to ¼ of the incubation period) so that the epidemic curve demonstrates the details of the outbreak and clearly identifies the onset, peak, and tail of the outbreak.[2,14]

Transmission Modes

Point source outbreaks can occur after the exposure of a group of people to an agent during a single, short period of time. Cases often present in a single group during a short time period, corresponding to the range of the incubation period, unless there is secondary transmission. The epidemic curve demonstrates an abrupt onset and gradual descent with a single peak. Food and beverages are common point sources, and single-source outbreaks very often present as gastroenteric illness (Fig. 13.3).[47]

Person-to-person, or propagated, outbreaks are characterized by the presence of two or more clusters of cases in time separated by approximately one median of the incubation period, thus suggesting secondary transmission. The distance between clusters of cases usually becomes less clear as the outbreak progresses because the ranges of the incubation periods tend to blend into each other. Respiratory transmission is the most efficient mechanism of person-to-person transmission, the most classic example being the influenza virus. Measles, adenovirus, and pneumonic plague may also be transmitted in this manner. (Fig. 13.4)[48]

In continuous-source outbreaks, exposure to the agent can occur over an extended period. Therefore cases appear over longer periods of time, often substantially beyond the range of the incubation period. Cases sometimes present in several clusters, but the timing between clusters does not necessarily correspond closely to the median incubation period. Vector-borne and zoonotic infections are typical of this transmission pattern, but waterborne, respiratory, and nosocomial outbreaks have occasionally been described as continuous-source outbreaks as well. (Fig. 13.5)[49]

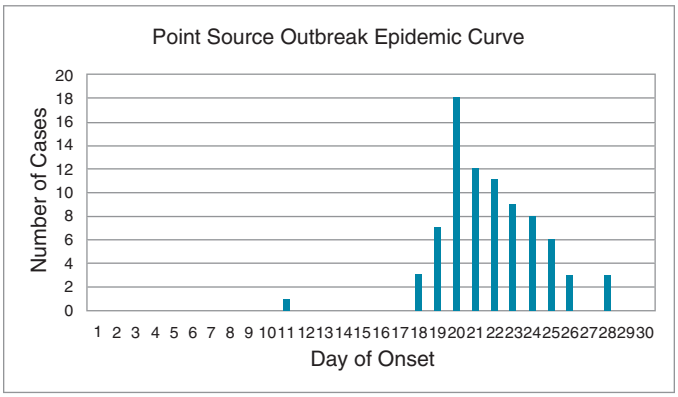

FIG. 13.3 Epidemic curve: single-point source outbreak.

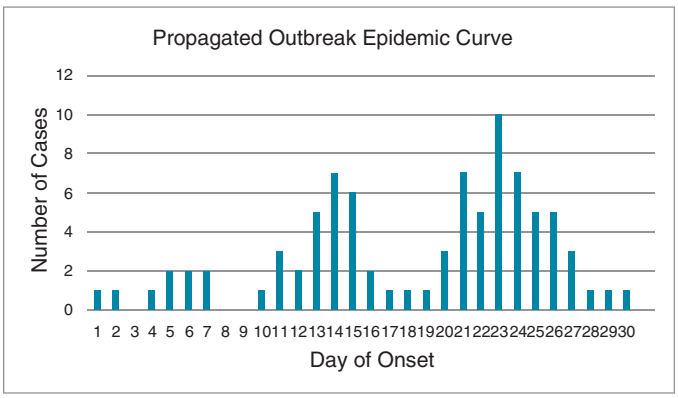

FIG. 13.4 Epidemic curve: propagated (person-to-person) transmission.

Chains of Transmission

Exploring the connection between initial waves of cases and those occurring later often reveals important interactions that can occur during propagated outbreaks with person-to-person transmission or a continuous common source. Clearly identified transmission waves can be observed, such as those related to cultural practices such as burial traditions in Africa[49] or the transmission of Nipah virus in a Bangladeshi community (Fig. 13.6).[48] Transmission chains can also provide hints about the incubation period of the agent, although these may be difficult to tease out of the data.

New molecular diagnostic tools are revolutionizing the study of outbreaks, especially in elucidating chains of transmission where the cases are separated by time and distance. Whole-genome sequencing of pathogens requires less time, money, and equipment than ever before in the United States and in many high-income settings.[46] Next-generation sequencing platforms are increasingly available in the field, as is the ability to interpret the massive volumes of data inherent to this technology. Genetic sequencing can be used to precisely determine transmission chains, relatedness of pathogens to known standards, and even characteristics such as antimicrobial resistance and virulence, and are extremely valuable tools for outbreak management.[46]

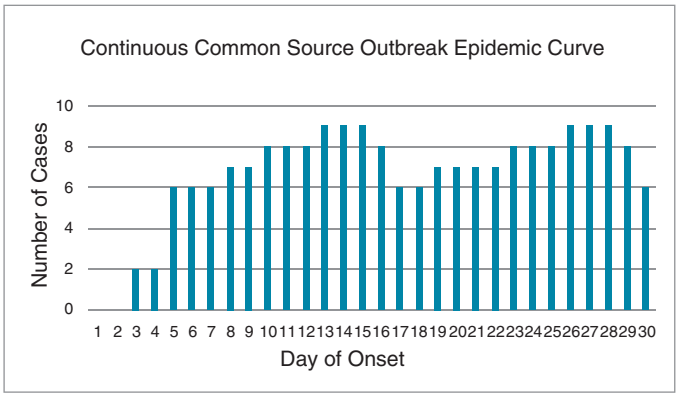

FIG. 13.5 Epidemic curve: continuous common source.

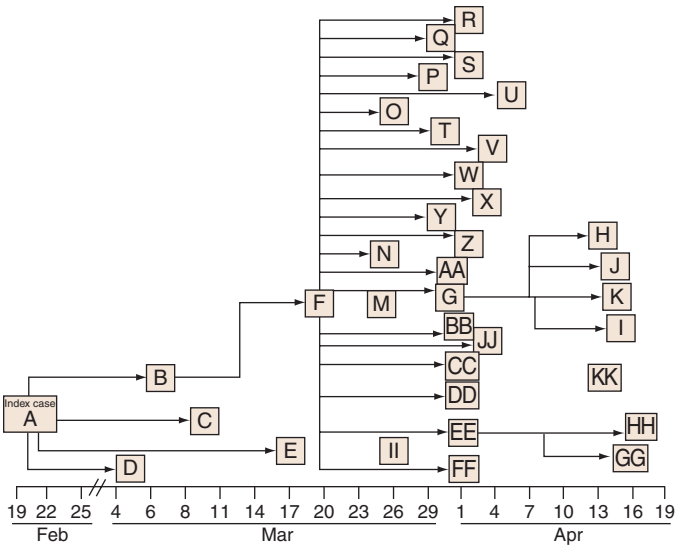

FIG. 13.6 Person-to-person chain of transmission of Nipah virus in a Bangladesh village with dates of onset of illness in 2004. Letters designate individual patients. Patients KK and II had no known contacts with any ill patients before onset of illness.

TYPES OF OUTBREAKS

Foodborne Outbreaks

Surveillance of foodborne outbreaks is routinely conducted in the United States and Europe but in fewer low-income countries where one might expect more frequent foodborne illness. The most recent comprehensive data are published by the CDC from 1998 through 2016. In the United States there were 19,991 outbreaks affecting 388,238 persons that led to 15,631 hospitalizations and 365 deaths.[50] For reference, this represents almost half of the total outbreaks[41] throughout this period in the United States. An etiologic agent is identified in approximately half of the foodborne outbreaks, and 91% of them were caused by an infectious agent, mainly viruses (54%) or bacteria (36%). Nearly all outbreak-causing foodborne viruses were Norovirus, and 52% of the bacterial causes were *Salmonella* spp.[51]

The primary factor leading to foodborne outbreaks is temperature mismanagement while preparing, cooking, or storing food. If food workers were involved in the food preparation, the outbreak was often associated with a single, nongloved worker who handled the implicated food or fecally contaminated foods that were improperly refrigerated.[52]

Nosocomial Outbreaks

Outbreaks in hospital settings often benefit from better infrastructure for diagnosis and investigation. There are a wide variety of pathogens that can lead to outbreaks in hospitals, from respiratory pathogens such as influenza or coronoviruses, to diarrheal pathogens such as *Clostridioides difficile* (formerly *Clostridium difficile*), to bloodstream infections such as multidrug-resistant gram-negative bacilli. Extensive work on infection control has led to the publication of the Outbreak Reports and Intervention Studies of Nosocomial Infection (ORION) guidelines for reporting nosocomial outbreaks.[44] An analysis of an open-access database of nosocomial outbreaks (www.outbreak-database.com) demonstrated that outbreaks occurred mainly in hospitals (83%) and more often in intensive care units (47%).[53] Surgical, neonatology, and internal medicine services accounted for similar proportions of the outbreaks. A source was identified in only 63% of outbreaks, often an index patient (40%), contaminated equipment or devices (21%), or the environment (19%). The mode of transmission was unidentified in 28% of outbreaks, and in the remainder it was primarily contact (60%). The three most frequent pathogens were *Staphylococci* (15%), *Pseudomonas* (8%), and *Klebsiella* (7%). Most of the staphylococci outbreaks (71%) were caused by methicillin-resistant *S. aureus*, and 24% of the *Klebsiella* outbreaks were due to extended spectrum β-lactamase–producing bacteria. Many smaller nosocomial outbreaks are likely excluded from this database due to publication bias in the literature, and most of the published cases come from high-income countries.[53]

Respiratory and Other Person-to-Person Outbreaks

The most typical outbreaks of respiratory illness are those caused by respiratory viruses with short incubation periods and potential for rapid dissemination in a population, such as influenza or adenovirus.[54] Viruses transmitted by droplet nuclei, such as measles and mumps, follow similar transmission patterns, although their incubation periods are often somewhat longer. Tuberculosis, on the other hand, is less frequently observed in outbreaks, probably because of its very lengthy incubation period and the consequent difficulty in establishing a clear epidemiologic link between index and secondary cases. On occasion, this link can be established, such as when there is a common airline flight or workplace.[51] Coronoviruses (CoV) such as SARS-CoV and, more recently, Middle East respiratory syndrome (MERS-CoV) have recently emerged as respiratory pathogens with epidemic potential. They are originally zoonotic in nature but can be transmitted person-person and in health care settings.[55]

Sexually Transmitted Outbreaks

Sexually transmitted diseases are typically less efficiently transmitted because an intimate relationship must be established. HIV has taught us that sexual transmission may be inefficient but was certainly sufficient to establish a global pandemic. The Internet and enhanced connectivity through virtual social networks has been cited as one mechanism that increases the probability of contact between cases and naïve, susceptible individuals.[56]

Zoonotic and Vector-Borne Outbreaks

Increased interaction between humans, animals, and their environment have led to a relentless surge of emerging and reemerging infections, most of them zoonotic or vector-borne in nature (see Chapter 317). No single common transmission pattern can describe these outbreaks because of their diversity. Point source exposures are often observed, such as in an outbreak of leptospirosis after a freshwater exposure during a military exercise in Guam.[57] Continuous common source exposures are also observed, especially for vector-borne diseases, such as dengue, malaria, or Zika virus.[58] Finally, the Ebola epidemic in West Africa in 2015–16 contributed much to the understanding of outbreak management, from the science of transmission, to the many social implications of infection control, to the mundane challenges of waste management.[59]

OUTBREAK INVESTIGATION AND RESPONSE

Conducting an investigation may help us to understand the mode of transmission of the disease, identify the etiologic agent and who may be at risk of infection, and ultimately prevent additional cases and reduce the overall morbidity and/or mortality rates. Conducting an outbreak investigation may also allow us to evaluate the sensitivity and specificity of a surveillance system, evaluate or implement intervention strategies (i.e., vaccination, social distancing, or removal of a point source) and contribute to the epidemiology and scientific knowledge of the disease.

A systematic, step-by-step approach to conducting an outbreak investigation is imperative for identifying the source of the outbreak and for controlling and preventing additional cases. This systematic approach can be divided into 13 distinct steps (Table 13.2), but these are not rigid in their order, and several steps are often accomplished simultaneously.[2,14]

1. Preparation for an outbreak should involve prestaging of standardized sample collection materials, personal protective equipment, and most important, the presence of trained personnel. It is also important to think about permissions for conducting the study, whether it is the Institutional Review Board (IRB) of the hospital or CDC, or jurisdictional approval if you are traveling to another state or country.
2. Conducting an outbreak investigation can be resource intensive. Before initiating the investigation, it is imperative to first determine whether or not an outbreak (or pandemic) is actually occurring. Several data sources may be available to help determine if the number of observed cases exceeds that of the expected baseline number (i.e., notifiable disease registries, death registries, hospital discharge summaries, etc.). It is important to recognize that new or improved diagnostic tests, a new or enhanced surveillance system, or simply increased awareness of a disease may artificially indicate that an outbreak is occurring.

TABLE 13.2 13 Steps to Conduct a Successful Outbreak Investigation

Prepare for field work.
Establish the existence of an outbreak.
Verify the diagnosis.
Construct a working case definition.
Find cases systematically and record information.
Perform descriptive epidemiology.
Develop hypotheses.
Evaluate hypotheses epidemiologically.
As necessary, reconsider, refine, and reevaluate hypotheses.
Compare and reconcile with laboratory and/or environmental studies.
Implement control and prevention measures.
Initiate or maintain surveillance.
Communicate findings.

3. Verifying the diagnosis often goes hand-in-hand with confirming the existence of an outbreak. It may be necessary to collect additional biologic samples and, if possible, request specialized diagnostic procedures or have the results confirmed at a secondary reference laboratory. However, in the event of an outbreak of a new pathogen, it may be necessary to rely on clinical diagnosis alone, as was the case in the early stages of the SARS epidemic. Furthermore, it is highly recommended to interview patients to gather additional clinical and epidemiologic features.
4. Enumerating the number of cases during an outbreak is only possible once a standard case definition has been established. This can be one of the most difficult and contentious components of an outbreak investigation. The case definition is generally based on clinical features, such as sudden onset of fever higher than 38°C, cough or sore throat, difficulty breathing, and so forth. The case definition is almost always restricted by person (i.e., children younger than years, no history of yellow fever vaccine), place (i.e., patients in a specific wing of a hospital, attendees of a county fair), and time (i.e., persons with illness onset within the previous 24 hours). The initial case definition is often quite broad, to capture all possible cases; however, as the investigation proceeds it generally becomes more refined and divided into subcategories, such as suspect (i.e., fever only), probable (i.e., fever with cough and shortness of breath, death with history of fever and cough), and confirmed (i.e., fever and cough with laboratory confirmation of influenza virus H5N1 infection).
5. Counting and tracking cases is most easily achieved by constructing a line listing of cases. Data in the line listing should include information such as symptom onset date, patient identification data, clinical data, demographic data, laboratory data, and some risk factor or epidemiologic data. (Fig. 13.7)
6. The next step, and often most revealing, is descriptive analysis of the epidemiologic data. The line listing is converted to visual depiction of the data, referred to as the epidemic curve (as described earlier), which is graphed with time on the x-axis (typically symptom onset data) and number of cases on the y-axis (Figs. 13.3 to 13.5). The epidemic curve can give clues about trend (i.e., person-to-person transmission or point/common source exposure), size of outbreak, and incubation period. The data can also be displayed in map form, revealing potential information such as common source exposure or clustering of cases. Global Positioning System (GPS)/Global Information Systems (GIS) are tools now commonly used in modern epidemiologic outbreak investigations.
7. The next step, albeit a process that likely begins from notification of the first case, is to begin to develop hypotheses about the cause of illness and/or source of infection. In general, conducting the initial descriptive epidemiologic analysis of the data will give us clues to better refine our hypotheses. Furthermore, it is important that the investigator speaks with an adequate representative sample of the initial patients to understand potential links between cases. It is important to remember that the working hypotheses must be testable with statistical methods (i.e., chi-square analysis, logistic regression, etc.).
8. After careful consideration and development of our initial hypotheses, we must evaluate these hypotheses with analytic methods. Two methodologies are typically used for outbreak investigations: cohort studies or case-control studies. These are described earlier under the "Analytic Epidemiology" section.
9. Once the hypotheses have been tested using conventional analytic epidemiologic methods, it may be necessary to refine the hypotheses by conducting additional studies (i.e., laboratory or environmental testing) to further support the conclusions. For example, identifying *Cyclospora cayetanensis* in the raspberry filling of a wedding cake would greatly increase your epidemiologic evidence that eating cake at the wedding was significantly associated with developing diarrheal illness.[60] If no conclusive results were initially revealed, a new hypothesis may be proposed.
10. The CDC recommends an explicit step to reconcile epidemiologic findings with the available laboratory and environmental data that have been collected. This is an important step but is often done in parallel with the hypothesis testing.
11. The primary purpose of conducting an outbreak investigation is to not only determine the source of the infection but also simultaneously control the spread of the outbreak and prevent additional cases. For example, during an outbreak of yellow fever it may be necessary to vaccinate any at-risk populations, apply insecticide, and educate the community regarding ways to reduce mosquito breeding sites. Planning for this step is often done in an empirical manner at first and refined as better data are accumulated.
12. As noted earlier, disease surveillance is one of the key components of a public health system and often is responsible for identification of an outbreak. It is important to remember to continue or even enhance surveillance during an outbreak, to assure that cases are not missed and the control measures that have been implemented are effective.
13. Finally, it is imperative to communicate all findings. Unfortunately, this last step is often overlooked. The results of the study should be presented to the stakeholders (i.e., hospital staff, local health authorities, scientific community) so that others can learn from the investigation and recommendations.

Case #	Patient Initials	Onset Date	Date of Death	Gender	Age	Case Status	Symptoms			Laboratory Values			Epidemiologic Data	
							Fever	Hematemesis	Bloody Diarrhea	Decreased Platelets	Ebola IgM	Ebola PCR	Contact With Other Cases	Bushmeat Hunter
1	JM	21-Aug	28-Aug	Male	39	C	+	+	+	+	+	+	–	+
2	DB	21-Aug	26-Aug	Male	39	C	+	+	+	+	+	+	+	+
3	WL	22-Aug	30-Aug	Male	40	C	+	–	–	–	–	+	+	–
4	VG	22-Aug	30-Aug	Male	27	P	+	+	–	–	NS	NS	+	–
5	JS	23-Aug	28-Aug	Male	42	C	+	+	+	+	+	+	+	–
6	KM	24-Aug		Female	38	S	+	+	–	–	TP	TP	–	–
7	MB	24-Aug		Female	40	S	+	–	–	–	TP	TP	–	–
8	BG	24-Aug		Male	25	S	+	+	+	–	TP	TP	–	–
9	HR	25-Aug	30-Aug	Male	28	C	+	+	+	+	+	+	+	+
10	MR	25-Aug		Female	26	S	+	–	–	–	TP	TP	–	–

NS No sample
TP Test pending
C Confirmed
P Probable
S Suspect

FIG. 13.7 **Example line list.**

Although infectious diseases do not necessarily respect or recognize boundaries and jurisdictions of cities, states, and countries, it is important to remember that local and state health departments, as well as national and international authorities have specific roles and responsibilities. An investigation of a nosocomial outbreak limited to an individual hospital or a diarrheal disease outbreak at a single daycare center would require coordination at the local health department level only. However, if it is determined in the course of the outbreak investigation that the source of the nosocomial outbreak was in multiple states (e.g., from contaminated blood products), the investigation would require coordination with other states and likely national authorities (i.e., CDC) to coordinate the investigation and response across borders. Similarly, if an outbreak was occurring in multiple countries (a pandemic such as SARS or influenza), then coordination would need to occur via WHO and the GOARN.[16,17]

Consequences of an Outbreak

Outbreaks of infectious disease have the potential to be sensational and may at times lead to significant public relations difficulties. The economic, social, or political disruptions that can result when the public learns about an outbreak may be a significant disincentive to reporting it in a timely fashion and may even lead, in some cases, to lack of cooperation with the outbreak investigation. This may occur at a local level, such as when a restaurant does not desire to be associated with an outbreak of foodborne disease for fear of losing customers, or on an international level, when a country does not report an epidemic for fear of decreased tourism or limited exportation of goods.[61] These sensitivities must be considered when investigating an outbreak, dealing with the media, and releasing public statements, with an effort to minimize sensationalism, but impart the correct information and secure the public's health.

The primary goal of any outbreak investigation is to control the disease within the affected population and to prevent the disease from spreading to other individuals or populations. Outbreak investigations are one of the most exciting aspects of epidemiology and are rewarding when they result in a tangible, positive outcome. Outbreak investigations can also be extremely stressful, such as when they are conducted under severe political and/or economic pressure and results are demanded on a short timeline. In these days of frequent pandemic threats, and with the reemergence of numerous diseases, careful preparation and a clear understanding of disease outbreaks is more essential than ever for timely and adequate disease control.

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The complete reference list is available online at Expert Consult.

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14 Emerging and Reemerging Infectious Disease Threats

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SHORT VIEW SUMMARY

- Infectious disease threats (Table 14.1)—such as the acquired immunodeficiency syndrome (AIDS) pandemic, the severe acute respiratory syndrome (SARS) outbreak, the Ebola outbreak in West Africa, the Zika epidemic in the Americas, and major Ebola outbreaks in West Africa and the Democratic Republic of the Congo (DRC)—shock the world and spur renewed efforts to improve capacity to detect and control emerging and reemerging diseases.
- Some infectious diseases can cause or contribute to chronic diseases (Table 14.2), and some human genetic variants may influence disease susceptibility or severity (Table 14.3).
- This chapter identifies factors that favor disease emergence and spread, considers lessons learned from major outbreaks, and reviews infectious threats of current concern, including:
  - **Antimicrobial Resistance.** Drug-resistant infections are a major danger to global health and well-being. Urgent threats include strains of gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae, that are resistant to all available antibiotics. Other serious threats include multidrug-resistant tuberculosis, methicillin-resistant *Staphylococcus aureus,* and *Candida auris,* a multidrug-resistant fungus that causes health care–associated infections.
  - **Acute Respiratory Tract Infections.** Lower respiratory tract infections are the leading cause of acute incident disease and communicable disease mortality worldwide. Clinically important respiratory pathogens first recognized in the 21st century include human metapneumovirus, SARS coronavirus, human bocavirus, Middle Eastern respiratory syndrome coronavirus, and pandemic influenza A(H1N1). Public health authorities continue to monitor novel influenza A viruses with pandemic potential, such as avian influenza A(H5N1) and A(H7N9) viruses (Figs. 14.1 and 14.3).
  - **Enteric Diseases.** Diarrheal diseases are the second leading cause of acute incident diseases and communicable disease mortality worldwide. Pathogens that cause enteric disease are transmitted through food, water, and person-to-person contacts, as well as through environmental or zoonotic exposures. Enteric diseases of current concern include infections caused by noroviruses, by *Clostridioides difficile* (formerly *Clostridium difficile*), and by multidrug-resistant strains of *Campylobacter* and *Salmonella.* Other public health issues related to enteric diseases include the reemergence of cholera, which caused a major outbreak in Haiti in 2010 following an earthquake, and the identification of new food vehicles for disease transmission during investigations of multistate foodborne outbreaks in the United States (Table 14.4).
  - **Vector-Borne Diseases.** Vector-borne pathogens are transmitted by blood-feeding arthropods, typically insects and ticks. Mosquito-borne examples include viruses carried by *Aedes* mosquitoes that cause dengue, Zika, yellow fever, and chikungunya diseases, as well as the protozoan parasites carried by *Anopheles* mosquitoes that cause malaria. Tick-borne diseases of public health concern include Lyme disease, caused predominately by the bacterium *Borrelia burgdorferi,* the most common vector-borne disease in the United States; Rocky Mountain spotted fever, caused by *Rickettsia rickettsii;* and the recently discovered Heartland virus and Bourbon virus diseases.
  - **Ebola and Marburg Hemorrhagic Fevers.** Ebola and Marburg viruses—whose natural reservoir is thought to be fruit bats—cause rare, severe, and often-fatal diseases in humans and nonhuman primates. Since 2000, outbreaks of Ebola or Marburg virus have occurred multiple times in central and East Africa, in the Democratic Republic of the Congo, in Sudan, and in Uganda. The first Ebola outbreak in West Africa—and the largest outbreak of Ebola to date, involving major urban settings—began in Guinea in 2014 and spread to neighboring Sierra Leone and Liberia, causing more than 11,000 deaths. Secondary outbreaks occurred in Nigeria, Senegal, Mali, and the United States, illustrating in a profound way that infectious diseases do not recognize borders. During 2018–2019, the second largest Ebola outbreak ever reported occurred in the DRC.
- Global health initiatives to address emerging and reemerging diseases include the Global Alliance for Vaccines and Immunization (Gavi); the Global Fund to Fight AIDS, Tuberculosis and Malaria; the World Health Organization International Health Regulations of 2005; the Global Health Program of the Bill and Melinda Gates Foundation; the US President's Emergency Plan for AIDS Relief (PEPFAR); and the World Health Organization Global Action Plan on Antimicrobial Resistance (Table 14.5).

The rapid pace of microbial evolution enables pathogens to overcome human defenses, exploit human behaviors, and elude control efforts across a highly connected world.[1,2] Though the incidence of endemic diseases that are major killers of children and young adults has declined significantly since the 1990s—with fewer deaths due to respiratory tract and diarrheal diseases,[3,4] vaccine-preventable diseases,[5] and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis, and malaria[6–10]—new threats continue to emerge and spread. The dangers and uncertainties of emerging pathogens are epitomized by the 2014–16 epidemic of Ebola in West Africa and the 2015–17 epidemic of Zika in the Americas, which presented major challenges to global health,[11–14] as had severe acute respiratory syndrome (SARS) in 2003 and HIV/AIDS in the 1980s and 1990s.

As discussed in this chapter, emerging and reemerging infectious diseases of current concern include infections with drug-resistant pathogens, acute respiratory tract infections (see also Chapter 67), enteric diseases, vector-borne diseases (see also Chapter 296 [ticks]), and viral hemorrhagic fevers (see Chapter 164). Many emerging threats are zoonotic in origin, having "jumped" from animals to humans while continuing to be maintained in animal reservoirs.[2,15,16] Zoonotic pathogens (see also Chapter 317) are responsible for severe

human infections with avian influenza A(H7N9) virus in China and for recurring epidemics of Middle East respiratory syndrome (MERS) in Saudi Arabia; plague in Madagascar; and monkeypox in the Central African Republic, the Democratic Republic of the Congo, and Nigeria (Table 14.1). The recent Zika outbreak in the Americas—caused by a mosquito-borne virus—has focused attention on pathogens that can cross the placenta during pregnancy and cause birth defects[17,18] or congenital illness,[19–21] or both. Perinatal transmission issues of current concern in the United States (in addition to Zika virus) involve hepatitis C virus[20] and *Treponema pallidum* (the spirochetes that cause syphilis[21]).

Although vaccines have eliminated rubella in the Americas and eradicated smallpox worldwide, vaccine-preventable diseases such as measles can reemerge even in the setting of high-functioning immunization programs. In many parts of the world, weak primary health care systems and limited access to the most vulnerable populations result in many children being unimmunized. Most resurgences of vaccine-preventable diseases, such as the recurrent yellow fever outbreaks in South America and Africa, stem from low immunization coverage, but both reduced vaccine acceptance in some affluent countries and waning vaccine immunity in the setting of high vaccination coverage have emerged as threats to protecting communities from vaccine-preventable diseases.

INFECTIOUS CAUSES OF CHRONIC DISEASES

Several infectious organisms—including viruses, bacteria, parasites, and prions—have been linked to the development of chronic diseases such as cancer, arthritis, cirrhosis, gastric or peptic ulcers, and neurologic disorders[22] (Table 14.2). These diseases may be due to inflammation

TABLE 14.1 Examples of the Outbreaks, Pathogen Discoveries, and Other Notable Infectious Disease Events in a Recent Decade (2008–2017)

2008	Ebola-like outbreak in Zambia due to a previously unknown virus: Lujo hemorrhagic fever virus, an arenavirus related to Lassa fever virus, which is associated with rodents.
2008	Isolation in Australia of a new virus (transplant-associated arenavirus related to lymphocytic choriomeningitis virus) after three recipients of liver or kidney transplants from a single donor developed febrile illness and died.
2008	Increasing outbreaks and international spread of carbapenem-resistant Enterobacteriaceae, and first detection of New Delhi metallo-β-lactamase 1, a genetic element that can confer such resistance.
2009	Outbreak of severe fever with thrombocytopenia syndrome (SFTS) in China caused by a novel phlebovirus (the SFTS virus).
2009	Discovery of two novel tick-borne pathogens in the United States: the Heartland phlebovirus in Missouri and a pathogenic *Ehrlichia* species in Wisconsin and Minnesota.
2009–2010	Influenza pandemic caused by a new influenza virus strain, influenza A(H1N1)pdm09.
2009–2010	Locally transmitted dengue in Florida, representing the first cases acquired in the continental United States outside the Texas-Mexico border region since 1945.
2009 on	Emergence and global spread of *Candida auris,* the cause of multidrug-resistant health care–associated infections.
2010	Outbreak of cholera in Haiti.
2010 on	Rise in US cases of infection with hepatitis C virus (HCV)—including cases of congenital HCV—associated with the US opioid epidemic.
2011	Outbreak of Shiga toxin–producing *Escherichia coli* O104:H4 (STEC O104:H4) infections in Germany.
2011	Identification of a strain of gonorrhea (H041) resistant to all available antibiotics.
2011–2012	Influenza cases in the United States traced to a variant swine-origin influenza A(H3N2) virus carried by pigs exhibited at agricultural fairs.
2011–2014	Outbreaks of valley fever (coccidioidomycosis) in California.
2012	Ebola hemorrhagic fever outbreaks in Uganda and the Democratic Republic of the Congo and outbreak of Marburg hemorrhagic fever in Uganda.
2012	Outbreak of hantavirus pulmonary syndrome in Yosemite National Park, California.
2012	A novel rhabdovirus (Bas-Congo virus) identified by whole-genome sequencing as the cause of an outbreak of acute hemorrhagic fever in the Democratic Republic of the Congo.
2012–2013	Multistate outbreak of fungal meningitis in the United States caused by tainted steroid injections.
2012 on	Outbreaks of severe respiratory disease in Middle East countries and South Korea caused by a novel coronavirus (MERS-CoV) that belongs to the same viral species as the SARS coronavirus.
2013 on	Human infections with avian influenza A(H7N9) virus—an influenza virus strain with pandemic potential—reported in China.
2013 on	First cases of chikungunya reported in the Americas—on the Caribbean island of Saint Martin—with subsequent spread through North, Central, and South America.
2014	Marburg hemorrhagic fever outbreak in Uganda.
2014	Bourbon virus, the first human thogotovirus discovered in the Western Hemisphere, isolated from a resident of Bourbon County, Kansas, who died of the infection. Thogotoviruses are enveloped RNA viruses transmitted by ticks.
2014–2015	Outbreak of legionnaires' disease associated with the water system crisis in Flint, Michigan.
2014–2016	Outbreak of Ebola hemorrhagic fever in rural Guinea that spread to the capital cities of Guinea, Liberia, and Sierra Leone, causing a major global health crisis.
2014–2017	Outbreaks of bubonic and pneumonic plague in Madagascar.
2014 on	India was certified as polio-free in 2014. In the same year, however, WHO declared the international spread of poliovirus in other nations a Public Health Emergency of International Concern (PHEIC) under the International Health Regulations and issued Temporary Recommendations to prevent further spread.
2015	Outbreak of meningococcal meningitis in Niger caused by *Neisseria meningitides* serogroup C, the first detection of a large outbreak caused by this serogroup.
2015	Multistate measles outbreak in the United States likely started from an infected traveler who visited Disneyland.

Continued

TABLE 14.1 Examples of the Outbreaks, Pathogen Discoveries, and Other Notable Infectious Disease Events in a Recent Decade (2008–2017)—cont'd

2015	Community outbreak of HIV infection in a small town in Indiana, associated with injection drug use and the US opioid epidemic.
2015–2016	Lassa hemorrhagic fever outbreak in Nigeria.
2015–2016	Outbreak of yellow fever in Angola and the Democratic Republic of the Congo.
2015–2017	Outbreak of Zika virus disease in the Americas associated with birth defects.
2015–2017	Recurring outbreaks of monkeypox in the Central African Republic, the Republic of the Congo, and Nigeria.
2015–2017	Outbreaks of yellow fever in Brazil, including sporadic cases outside São Paulo and Rio de Janeiro.
2015 on	Emergence and global spread of the mobilized colistin resistance (*mcr-1*) gene, which confers resistance to colistin, a last-resort antibiotic for the treatment of gram-negative bacterial infections.
2016	Multistate outbreak of salmonellosis in the United States associated with exposure to pet turtles.
2016	Multistate outbreak of infections caused by *Elizabethkingia anophelis*—a bacteria commonly found in soil, river water, and reservoirs—in the United States.
2016–2017	Outbreak of cholera in Yemen, exacerbated by famine and war.
2017	Outbreaks of Marburg hemorrhagic fever in Uganda and Kenya and an outbreak of Ebola hemorrhagic fever in the Democratic Republic of the Congo.
2017	Outbreak of leptospirosis in Puerto Rico in the aftermath of Hurricane Maria.
2017	Multistate US outbreaks of Seoul virus infection associated with exposure to pet rats and *Campylobacter* infection associated with exposure to pet store puppies.
2017	Brucella RB51 infections in Texas and New Jersey, associated with drinking raw milk.
2017	Multistate foodborne outbreaks in the United States caused by contamination of papayas with *Salmonella;* of soft raw cheese with *Listeria;* and of soy nut butter with Shiga toxin–producing *Escherichia coli* O157:H7.

TABLE 14.2 Examples of Infectious Agents That Cause or Contribute to Chronic Diseases

PATHOGEN	CHRONIC CONDITION CAUSED/ EXACERBATED BY THIS PATHOGEN
Bacteria	
Borrelia burgdorferi	Antibiotic-refractory arthritis
Helicobacter pylori	Peptic ulcer disease Gastric carcinoma Mucosa-associated lymphoid tissue lymphoma
Viruses	
Epstein-Barr virus	Nasopharyngeal carcinoma (undifferentiated) Burkitt lymphoma Posttransplant lymphoproliferative disease B-cell lymphoma
Hepatitis B and C viruses	Cirrhosis Hepatocellular carcinoma
Human herpesvirus 8 (HHV-8)	Kaposi sarcoma
Human immunodeficiency virus	Lymphoma HIV-related neurocognitive disorders and peripheral neuropathy
Human papillomavirus	Cervical carcinoma Anogenital and oropharyngeal cancers
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell leukemia/lymphoma
Parasites	
Liver flukes	Cholangiocarcinoma
Schistosoma haematobium	Bladder cancer
Prions	
Variant CJD prion	Degenerative brain disorder

CJD, Creutzfeldt-Jakob disease; *HIV,* human immunodeficiency virus.

caused by chronic infection (e.g., ulcers caused by *Helicobacter pylori* infection[23]) or triggered by an immune response to infection (e.g., Guillain-Barré syndrome following *Campylobacter jejuni* infection[24]). It has also been suggested that neonatal exposure to infection might predispose a person to chronic illness in later life.[25] Some infectious organisms also have adverse impacts on reproductive health. Infertility is associated with sexually transmitted diseases that cause pelvic inflammatory disease[26] and with neglected tropical diseases, which can also cause maternal death and poor birth outcomes.[27]

Confirmation of causative relationships between viruses and specific chronic diseases led to development of the first two vaccines against cancer: hepatitis B vaccine, which prevents cancers of the liver,[28] and human papillomavirus vaccine, which prevents cervical cancer, as highlighted in the National Cancer Institute's *Cancer Moonshot Blue Ribbon Panel Report*.[28a] Researchers are evaluating many other potential linkages between chronic conditions and infectious pathogens (for examples, see references 29–36), as well as genetic factors that influence infectious disease susceptibility and disease progression (Table 14.3).

FACTORS THAT FAVOR DISEASE SPREAD

Increased air travel has facilitated the international spread of new threats and the resurgence of old ones, including measles and drug-resistant forms of tuberculosis. Other important factors include increased urbanization, globalization of the food supply, environmental changes that enlarge the habitats of disease vectors, population movements due to war or famine, and changes in human demographics and behaviors. In the United States, the complexity of interactions between microbes and humans is illustrated by a community outbreak of HIV infection[37] and an increased incidence of hepatitis C[38] associated with injection drug use and the US opioid epidemic. Disease spread can also be accelerated by breakdowns in public health infrastructures caused by natural disasters[39] or inadequate maintenance of water and sewage systems.[40]

The impact of urbanization and travel on disease spread is illustrated by the Ebola outbreak in West Africa. Previously reported Ebola outbreaks occurred in remote areas and were limited in size and geographic scope.[40a] During the West Africa outbreak, however, the Ebola virus spread from a rural village in Guinea to the densely populated capital cities of Guinea, Liberia, and Sierra Leone. Subsequent travel by ill persons led to cases of Ebola virus disease in Lagos, Nigeria (a megacity of 16 million people)[41]; Bamako, Mali[42]; Dakar, Senegal[43]; and Dallas, Texas.[44] In addition, a small number of infected health care workers from Spain, Italy, the United Kingdom, and the United States were medically evacuated from West Africa to their home countries, and a hospital nurse in Madrid contracted Ebola virus disease from an infected missionary

TABLE 14.3 Examples of Human Genetic Variants That Influence Disease Susceptibility or Severity

GENETIC VARIANT	EFFECT ON DISEASE
Globin gene variant alleles (e.g., sickle globin and α- and β-thalassemias)	Reduced susceptibility to malaria
Duffy null blood group	Complete resistance to *Plasmodium vivax* malaria
Blood group O	Reduced severity of *Plasmodium falciparum* malaria Increased susceptibility to cholera
Chemokine receptor CCR5 delta-32 allele	Reduced susceptibility to HIV-1 infection Reduced progression of HIV-1 infection Increased severity of West Nile virus infection
HLA B*5701 allele	Reduced progression of HIV-1 infection Hypersensitivity to abacavir
Interferon lambda-4 variant alleles	Reduced clearance of hepatitis C virus
Toll-like receptor 4 variant alleles	Increased susceptibility to gram-negative bacterial infections and septic shock
SLC11A1 (NRAMP1) variant alleles	Increased progression of tuberculosis
Interleukin 10 variant alleles	Increased susceptibility to leprosy
Apolipoprotein L-1 variant alleles	Reduced susceptibility to African sleeping sickness caused by *Trypanosoma brucei rhodesiense*

[a]A discussion of "how the history of past pandemics is written in our genomes" may be found at Pittman KJ, Glover LC, Wang L, Ko DC. The legacy of past pandemics: common human mutations that protect against infectious disease. *PLoS Pathog.* 2016;12:e1005680.
HIV, Human immunodeficiency virus.

evacuated from Sierra Leone.[45] Intensive public health efforts contained disease spread in each country, and additional cross-border spread was prevented due to enhanced disease surveillance by neighboring countries[46] and at airports.[47] The West African Ebola outbreak highlighted the need for sustained vigilance and international collaboration to ensure early detection and response to new threats.

ANTIMICROBIAL RESISTANCE

While the introduction of antibiotics in the early to mid-20th century remains one of the most significant health achievements to date, antimicrobial resistance is currently regarded as one of the greatest threats to global health and well-being.[48–54] The widespread availability and indiscriminate use of antimicrobial agents among humans and animals, along with a globalized society in which emerging resistant strains can quickly spread worldwide, has created an environment of increased antibiotic exposure that has intensified selective pressure, boosting the inherent capacity of microbes to develop and acquire resistance genes. As a result, treatment of drug-resistant bacterial infections often requires the use of less effective, more expensive, and often more toxic drugs. Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die as a direct result of these infections.[55] Moreover, the problem of antimicrobial resistance—which extends beyond bacteria and includes viral (e.g., HIV, influenza), fungal (e.g., *Candida, Aspergillus*), and parasitic (e.g., malaria) infections—threatens our economic future.[54] A 2017 analysis by the World Bank states that, by 2050, drug-resistant infections could cause global economic damage on par with the 2008 financial crisis.[54]

The evolving nature of microbes defines antimicrobial resistance. Resistance genes have been found in humans and animals in areas with limited to no antibiotic exposure, in bacterial DNA frozen in the Arctic for 30,000 years, and in bacteria from underground caves isolated for 4 million years, some of which were resistant to synthetic antibiotics developed in the 20th century.[56–58] After introduction of each new class of antibiotics has come the emergence of resistant strains—due to natural selection of bacteria with mutations that allow them to survive and live on to reproduce—followed by their rapid global spread, facilitated by international travel and trade. As early as the 1930s, sulfonamide-resistant strains of *Streptococcus pyogenes* were noted in military hospitals, and numerous bacterial strains, including *Staphylococcus aureus,* were found to be resistant to penicillin shortly after its introduction and use in the 1940s.[59,60] In 1961, just 2 years after the introduction of methicillin, methicillin-resistant *S. aureus* (MRSA) strains emerged in British hospitals,[61,62] and strains exhibiting intermediate- or high-level resistance to vancomycin have been identified since 1996.[63–66] Today, MRSA is associated with significant morbidity and mortality, spurring research on alternative treatment strategies.[67,68]

In the United States the most urgent antimicrobial resistance threats, as identified by the Centers for Disease Control and Prevention (CDC) in a 2013 report,[55] include infections with *Clostridioides difficile* (formerly *Clostridium difficile*), drug-resistant *Neisseria gonorrhoeae,* and strains of gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae (CRE), which have become resistant to all available antibiotics. Severe, life-threatening *C. difficile* infections are usually associated with recent antibiotic use and often occur among currently or recently hospitalized patients (see "Enteric Diseases" later). Strains of *N. gonorrhoeae* with decreased or no susceptibility to third-generation cephalosporins have threatened a return to potentially untreatable gonorrhea and prompted changes in US treatment guidelines that aim to prolong the effectiveness of these drugs.[69–71] CRE, which are primarily transmitted in health care settings, can cause severe disease and high mortality rates.[72,73] Patients who require prolonged hospitalization and critically ill patients exposed to invasive medical devices (e.g., ventilators and central venous catheters) are at particular risk.

Resistance to carbapenem antibiotics is mediated through the production of carbapenemases (enzymes that inactivate carbapenems), including *Klebsiella pneumoniae* carbapenemase (KPC), first identified in 2001 in the United States,[74] and New Delhi metallo-β-lactamase (NDM), first identified in 2008 in India.[75] The genes encoding KPC and NDM (carried on plasmids) have spread from *K. pneumoniae* to other gram-negative bacteria, including *Escherichia coli, Klebsiella* species, and *Enterobacter* species. More recently, emergence of the mobilized colistin resistance gene *mcr-1* and its *mcr-2* and *mcr-3* variants—genes that cause resistance to colistin, a last-resort antibiotic used to treat resistant infections—has been reported.[76–78] The *mcr* gene is particularly worrisome because it is also found on plasmids and so can transfer to other bacteria, including CRE.[78a]

Other serious threats (both domestically and globally) include multidrug-resistant tuberculosis; MRSA infections; drug-resistant forms of enteric pathogens such as *Salmonella* and *Campylobacter* (see "Enteric Diseases" later); and a newly emerged multidrug-resistant fungus, *Candida auris,* that causes health care–associated infections (HAIs) and is difficult to identify with standard laboratory methods.[79] Over the past 2 decades, highly drug-resistant strains of *Mycobacterium tuberculosis* have emerged worldwide,[80–83] including multidrug-resistant tuberculosis (defined as tuberculosis that is resistant to isoniazid and rifampin, the two most effective first-line tuberculosis drugs) and extensively drug-resistant tuberculosis (defined as multidrug-resistant tuberculosis that is also resistant to any fluoroquinolone drug and at least one of three second-line injectable drugs: amikacin, kanamycin, or capreomycin). An estimated one-third of the world's population is infected with *M. tuberculosis,* and each year approximately 9 million people develop tubercular disease and there are 2 million tuberculosis-related deaths.[83a,83b] These infections present serious public health challenges and raise the specter of virtually untreatable endemic and epidemic tuberculosis.[83c,83d]

The increasing use of antibiotics in both humans and animals, the spread of HAIs between health care settings and into communities, and a virtual standstill in antibiotic development have escalated bacterial antibiotic resistance to crisis levels and garnered the attention of public health, clinical, and policy leaders worldwide.[54] In 2015, the World Health Assembly endorsed a global action plan to address antibiotic resistance,[83e] and in September 2016 global leaders at the United Nations General Assembly in New York committed to a common effort to fight antimicrobial resistance.[83f] This was only the fourth time in the history of the United Nations that a health topic was discussed by the General

Assembly, the earlier ones being HIV, noncommunicable diseases, and the Ebola virus.

In the United States, intensified efforts to address antimicrobial resistance are described in the *National Action Plan for Combating Antibiotic-Resistant Bacteria*, issued in 2015 by the CDC.[83g] Implementation of the National Action Plan has included establishment by the CDC and the US Food and Drug Administration (FDA) of the Antimicrobial Resistance Isolate Bank, which offers panels of resistant bacteria to researchers who develop diagnostic devices and new antibiotic agents,[83h] and the Antibiotic Resistance Laboratory Network, whose seven regional laboratories help states and localities detect and track existing and emerging types of antibiotic resistance.[83i] Progress has also been made in reducing several types of HAIs—many of which are drug resistant—through improved detection, focused infection prevention and control, better antimicrobial stewardship, and coordination of prevention efforts by health care facilities located in the same city or region.[83j,83k] In the United States, HAIs are reported and tracked by the Centers for Medicare and Medicaid Services and through reporting mandates in many states, using the CDC's National Healthcare Safety Network. This secure, Internet-based surveillance system collects data from more than 12,000 facilities in all 50 states on hospital-acquired infections and related issues, including the incidence or prevalence of multidrug-resistant organisms, health care personnel safety and vaccination, and the occurrence of transfusion-related adverse events.

Reviews of antimicrobial stewardship programs in both large and small hospitals have documented their effectiveness and cost-effectiveness.[84–89] Because poor antibiotic prescribing harms patients—and because antibiotic prescribing practices vary widely and errors are common[90]—education of patients and providers on the importance of and health benefits from responsible use of antibiotics remains paramount.[90a] Critical to the improvements in HAIs and to better addressing antibiotic resistance is innovative research into better approaches and tools to detect, control, and prevent these infections.

Use of antibiotics as growth promoters in healthy food-producing animals has also been a significant concern. Globally, the amount of antibiotics used in food-producing animals surpasses the amount of antibiotics used to treat human disease.[55,91] The human health implications of this use are increasingly being recognized.[92,93] Linkages have been found between livestock-associated MRSA and multidrug-resistant *S. aureus* carriage among industry workers and between *E. coli* isolates from retail meat (chicken) and extraintestinal pathogenic *E. coli* urinary tract infections in humans.[94,95] Several nations have taken steps to address the nontherapeutic use of antibiotics in food-producing animals, beginning with Sweden in 1986 and extending to the European Union in 2006, with bans on agricultural growth promoters.[96] The FDA strategy to promote the judicious use of antibiotics important in treating humans involves working with industry to end use of antibiotics to promote growth in food-producing animals.[97] The FDA guidance recommends that medically important antibiotics be used only to address animal health needs and only under veterinary oversight.[97] Another area of concern that is being increasingly recognized and that needs better understanding is the contribution of antimicrobial resistance in the environment related to contamination from human and animal sewage, antimicrobial manufacturing, and use of antimicrobials as pesticides.[98–100]

For antimicrobial drug development, the outlook remains grim, spurring innovative investments to accelerate research and stimulate the pipeline. Despite the continued rise in antibiotic-resistant pathogens, the development of new antibiotics has dramatically slowed.[101,102] Particularly troubling, there have been no new classes of drugs to treat gram-negative bacteria in 4 decades.[85] With the discovery of new antimicrobial agents more scientifically challenging compared with earlier years, unfavorable profit margins from short-course therapies, and other disincentives, many pharmaceutical companies have abandoned the market. Steps taken to counteract these effects are imperative.[103,104] Efforts include the Food and Drug Administration Safety and Innovation Act,[105] passed into law in 2012, which seeks to increase the development of and patient access to new antimicrobial agents, and the CARB-X initiative, a partnership between the US Department of Health and Human Services' National Institutes of Health and Biomedical Advanced Research and Development Authority (BARDA), the Wellcome Trust, and Boston University, which provides competitive funds to foster research for the development of drugs to address the most urgent resistance threats.[105a]

More recent efforts have focused on the use of new technologies and host targets to better understand and reduce antimicrobial resistance.[106–108] Whole-genome sequencing (WGS) techniques can track bacterial transmission from person to person and better define and help understand transmission linkages in outbreaks of resistant HAIs.[109–113] As these technologies continue to advance, rapid, point-of-care diagnostic tests could be used to quickly identify resistant infections and target treatment. Efforts to prevent and control infectious diseases by altering the host-microbe interaction as opposed to targeting microbes (e.g., through use of fecal transplants to treat *C. difficile* or through other approaches to sustain a healthy gut microbiome) also offer tremendous promise for reducing antimicrobial resistance.

Vaccines represent the optimal solution to addressing infections and antimicrobial resistance, and research and development of new vaccines is an urgent need. Effective immunization programs have stopped emergence of resistant strains and precluded the need for new antimicrobial agents for multiple infectious diseases, allowing focus to be shifted to diseases for which drug resistance remains a major global health threat. A recent example of this impact includes the pneumococcal conjugate vaccine. Over the past several years, use of pneumococcal conjugate vaccines has significantly reduced the rate of disease.[114–119] Researchers continue to monitor evolving serotypes to detect the emergence of any resistant strains that are not targeted by the vaccine.[114,118,120]

ACUTE RESPIRATORY TRACT INFECTIONS

Infections involving the respiratory tract represent one of the most dynamic areas for emerging and reemerging diseases, producing dramatic examples such as the first recognized outbreaks of legionellosis in the 1970s and hantavirus pulmonary syndrome in the 1990s. Legionellosis has been on the rise in the United States since 2000, with 6000 cases reported in 2015; while some of this increase is attributable to increased use of diagnostic testing and better reporting, this also likely reflects a true increase in the frequency of disease. Examples in the 21st century include newly recognized pathogens such as human metapneumovirus (hMPV, first identified in 2001[121]), the coronavirus associated with SARS (identified in 2003[122]) human bocavirus (identified in 2005[123]), pandemic influenza A(H1N1) (identified in 2009[124]), and Middle Eastern novel coronavirus associated with severe respiratory illness (identified in 2012).[125] Other newly recognized respiratory viruses include two additional coronaviruses (NL63 and HKU1), novel human polyomaviruses KI and WU, rhinovirus groups C, and parechoviruses, although in some instances the role of these agents as pathogens is still being clarified.[126] More virulent strains of known respiratory pathogens have also emerged. Examples of this phenomenon include human disease associated with avian influenza viruses, especially highly pathogenic avian influenza A(H5N1) and avian influenza A(H7N9),[127,128] and extensively drug-resistant tuberculosis[129] (see "Antimicrobial Resistance" earlier).

Acute respiratory tract infection constitutes a broad category of diseases that include infections of both the upper and lower respiratory tracts, such as acute pharyngitis, epiglottitis, bronchitis, pneumonia, and influenza. Although upper respiratory tract infections are capable of causing severe illness, virtually all (98%) respiratory disease–related deaths are a consequence of infection of the lower respiratory tract, especially pneumonia. In 2015, lower respiratory tract infections caused 2.74 million deaths, making them the leading infectious cause of death and the fifth-leading cause of death globally.[3]

Among children less than 5 years of age, at least 704,000 deaths were attributed to lower respiratory tract infections, representing 12.1% of deaths in this age group.[130] The poorest countries in sub-Saharan Africa and South Asia have the largest burden, with three of every four deaths from respiratory infections occurring in these two regions. Globally, 42% of all deaths from lower respiratory tract infections (mostly due to pneumonia) occur in children younger than 5 years of age. The four most common etiologies were *Streptococcus pneumoniae*, *Haemophilus influenzae,* respiratory syncytial virus, and influenza. Between 2005 and 2015, deaths due to lower respiratory tract infections decreased

36.9% worldwide in children under 5 years old, although incidence of infection has decreased at a slower rate. In many regions, the burden has increased in people older than age 65 and because of growing and aging populations, global mortality due to lower respiratory tract infections has remained relatively stable.[130] In the United States, influenza and pneumonia are the leading cause of infectious disease–related mortality and the eighth most frequent cause of death.[131]

Human Metapneumovirus

In 2001, hMPV was first reported as a cause of acute respiratory tract infections when the virus was isolated from specimens collected over a 20-year period from hospitalized children in the Netherlands with undiagnosed upper and lower respiratory tract illnesses.[121] Subsequent serologic and virologic analyses of banked specimens have demonstrated evidence of hMPV infection as far back as the 1950s, and genetic studies suggest the virus is considerably older.[121,132] Thus this virus may have been in circulation for some time before diagnostic tools were available to detect it. A paramyxovirus, hMPV is closely related to respiratory syncytial virus, another member of the Paramyxovirinae subfamily of Paramyxoviridae.[132,133] Genetic analyses have demonstrated two major hMPV types, designated groups A and B, with two subtypes (1 and 2) within each group; subtype variants have also been reported.[132]

hMPV causes illness similar to other major viral respiratory pathogens with a winter/spring predominance, and occurs globally in both high- and low-income countries, although incidence varies by year.[133] By 5 years of age virtually all children demonstrate evidence of prior hMPV infection.[134] Clinically significant illness and severe disease most commonly occur in children younger than 2 years of age, but hMPV-related illness can occur throughout childhood.[135,136] The virus (either alone or with copathogens) has been identified in 2% to 20% of children with acute respiratory tract infections, and in 3% to 7% of children hospitalized with acute respiratory tract infections or fever.[132,135] In contrast, hMPV is rarely (<1%) identified in children or adults who are not ill and when found is usually present in much lower titers than when detected during illness.[132,135,137] A multiyear, multicenter prospective study suggested that hMPV produces 20,000 hospitalizations, 263,000 emergency department visits, and 1 million outpatient visits annually among children younger than 5 years of age in the United States.[137] Because disease is also found in older children and in adults, these numbers represent an unknown proportion of the overall burden of illness due to hMPV.[133]

hMPV is one of the leading causes of bronchiolitis in infants. In children, most severe hMPV disease manifests as pneumonia or bronchiolitis and a substantial proportion of hospitalized children have underlying medical conditions, particularly asthma.[135–138] The virus has also been associated with childhood upper respiratory tract infections (up to 10% in some series) as well as acute otitis media.[133]

Most infections with hMPV in adults are mild, but severe disease can occur, especially in the elderly and in persons with chronic pulmonary disease or congestive heart failure.[139,140] This virus has been estimated to involve 3% to 7% of acute respiratory tract infections and 4.5% of acute respiratory tract infection hospitalizations in adults.[140] Outbreaks associated with hMPV have been reported in both children and adults, especially among long-term institutionalized elderly, with case-fatality rates as high as 33%.[140–142] As with respiratory syncytial virus, hMPV can produce severe and recurrent disease in immunocompromised hosts, including transplant recipients, those with hematologic malignancies, and those with HIV infection.[133,143] Ribavirin and immune globulin have been used to treat severe disease but have not been systematically assessed; efforts are underway to develop candidate vaccines against hMPV.[132,144]

Human Coronaviruses

Although coronaviruses were once considered to be pathogens most typically associated with the common cold, their public health significance has changed considerably in recent years.[145] The 2003 outbreak of SARS is widely considered to be a major emerging infectious disease event of the early 21st century, with profound public health, economic, sociologic, and political ramifications.[146] The expanded research and monitoring of coronaviruses that resulted from the SARS episode led to the recognition of two additional human coronaviruses (NL63 and HKU1) associated with upper respiratory tract infections.[145–148] In 2012, a novel coronavirus associated with severe and fatal pulmonary disease was identified among patients in the Middle East or patients linked to the Middle East.[125] A number of studies suggest that the human coronaviruses appear to be zoonotic in origin, with bats being especially important reservoirs.[149] Because bats harbor many coronaviruses, there is the potential for future pathogenic coronaviruses to emerge from this reservoir.

SARS was recognized in February 2003 when an explosive outbreak of adult respiratory distress syndrome was carried globally by more than a dozen individuals who were all guests at a Hong Kong hotel over a single weekend while a physician from adjacent Guangdong Province with fatal respiratory illness was also present.[150–152] Before traveling to Hong Kong, the source physician had been caring for patients with a similar illness, with 305 cases of undiagnosed respiratory diseases occurring in Guangdong Province since the previous November. A global consortium of laboratories rapidly identified the causative agent as SARS coronavirus (SARS-CoV),[122] a previously unrecognized betacoronavirus. The outbreak, which involved substantial transmission in health care settings, was contained within 4 months of recognition, primarily through the employment of standard public health measures such as droplet and contact precautions in hospitals, community isolation, and quarantine.[150–152] In the interim, a total of 8096 (confirmed and probable) cases of SARS were recorded in 29 countries on five continents, with 774 (9.6%) fatalities[153]; however, 98% of the cases occurred in just five locations, with only 8 cases in the United States. No cases of SARS have been recognized anywhere in the world since 2004.

A novel human coronavirus, initially referred to as HCoV-EMC for the Erasmus Medical Center in the Netherlands and now known as MERS coronavirus (MERS-CoV), was first identified in July 2012 in a 60-year-old man from Saudi Arabia with fatal lower respiratory tract infection.[125] The earliest recognized cases were retrospectively identified in 2 persons in Jordan who were part of an 11-person hospital cluster of respiratory illnesses in April 2012.[154] Between September 2012 and March 31, 2019, a total of 2399 laboratory-confirmed cases have been reported internationally, with at least 827 deaths (a case-fatality proportion of 34.5%[155]).

The median age of MERS-CoV patients is 52 and 66% are male; most index case patients have reported at least one chronic comorbid condition.[156] Cases have been reported from 27 countries, all with a direct or indirect link to locations in and around the Arabian peninsula; the majority of cases have been reported from Saudi Arabia (82%). Illness has been characterized by severe respiratory distress and pneumonia often requiring mechanical ventilation, and some cases have been accompanied by renal insufficiency.[157,158] Diffuse alveolar damage without evidence of extrapulmonary MERS-CoV antigen detection was reported in one well-characterized analysis of autopsy tissue specimens.[159] In May 2014, the CDC confirmed two cases of MERS in the United States, one in Indiana and the other in Florida. Both cases were health care providers who had lived and worked in Saudi Arabia, where they are believed to have been infected. Both had traveled to the United States, and both were hospitalized in the United States and later discharged after fully recovering.[160] No other patients in the United States have ever tested positive for MERS-CoV infection, while more than 1020 people have tested negative, as of December 2017.[154] Person-to-person transmission of MERS-CoV has been well documented, with at least 100 spatiotemporal clusters reported in household or health care settings; however, there is no clear evidence of sustained community transmission. The largest outbreak outside of the Arabia peninsula occurred in the Republic of Korea in 2015, with 186 cases and 36 deaths; 184 of the patients were infected nosocomially.[161] Since December 2016, 31% of 199 MERS-CoV cases reported to WHO were associated with transmission in a health care facility, including 40 cases among health care workers due to insufficient compliance with infection control measures and exacerbated by delayed identification of suspected MERS patients.[156] Asymptomatic infection due to MER-CoV has been documented but the role of asymptomatic or mildly symptomatic illness in transmission is unclear. Clinical trials of MERS-CoV candidate vaccines and therapeutics are in progress.[162]

A zoonotic origin of MERS-CoV was initially suggested by genetic analyses of this novel coronavirus showing that it is the first lineage C

betacoronavirus infecting humans and has a close relationship to betacoronaviruses HKU4 and HKU5 found in bats, including insectivorous bat species present in the Middle East.[163–165] A virus with 100% sequence homology to the MERS-CoV isolated from the 2012 Saudi Arabian index case was found in an Egyptian tomb bat (*Taphozous perforatus*) trapped in the vicinity of that patient's home.[166] Transmission has also been linked to dromedary camels.[167,168] Antibodies to MERS-CoV have been detected in archived samples from camels that date back to 1983, also implicating dromedary camels as a natural reservoir.[169] Moreover, a study of 348 primary human MERS cases reported that 55% had dromedary camel exposures (e.g., direct or close contact with respiratory droplets or saliva, potentially to unpasteurized camel milk).[169a] These findings suggest that bats and dromedary camels play an important role in the ecology of MERS-CoV and could be the reservoirs for this new coronavirus. The virus targets a unique receptor and appears capable of infecting multiple mammalian cell lines, suggesting the potential for a zoonotic intermediary, and serologic evidence of infection with MERS-CoV has been reported in mammalian species.[170–172]

Human Bocaviruses

Human bocavirus, a member of the Parvoviridae virus family, is a newly described cause of lower respiratory tract and gastrointestinal infections, primarily in children. This small, nonenveloped virus with a single-stranded DNA genome is one of only two known parvovirus pathogens, the other being parvovirus B19.[173] Human bocavirus was first detected in 2005 in Sweden when investigators used molecular virus screening methods by taking cell-free, filtered supernatants of stored respiratory specimens looking for virus-sized particles and then performing random polymerase chain reaction (PCR) amplification of the genetic material.[123] Among the sequences identified was a previously unrecognized parvovirus most closely related to animal viruses of the genus *Bocavirus*. Since the original identification of bocavirus DNA in respiratory specimens, three additional bocaviruses have been found in stool specimens; these agents are now referred to as human bocavirus (HBoV) 1 to 4. HBoV1 causes predominantly respiratory disease while HBoV2, HBoV3, and HBoV4 are associated with gastrointestinal illness.[174]

Human bocaviruses have been challenging to study because of the lack of in vitro or animal models. Although primary bocavirus infection is associated with respiratory illness, HBoV1 has also been detected in asymptomatic children, raising concerns about causality.[174] Moreover, as often as 83% of the time, bocavirus is present with other coinfecting respiratory pathogens.[173] However, in most studies HBoV1 has been found more commonly in children with acute respiratory disease than asymptomatic children; copy numbers of the virus are higher in monoinfections than coinfections and are usually low in asymptomatic children. High titers of HBoV in respiratory secretions have also been associated with clinically recognized illness. Children ages 6 to 24 months are most commonly infected; serologic studies show that almost all children have evidence of previous HBoV1 infection by 6 years of age.[174,175] Human bocavirus has been reported worldwide, although infection rates vary widely and illness is most common in winter. Infection can produce either upper or lower respiratory tract infection and in children is often accompanied by wheezing.[174,176] DNA has been detected in serum and urine of children with acute infections, suggesting systemic infections can occur.[174]

Emerging Influenza Viruses

Influenza viruses are one of the most challenging infectious diseases for clinicians, researchers, and public health and agricultural officials. The viruses are constantly changing, spread rapidly in populations, and can cause significant illness. Of the four main types of influenza viruses—A, B, C, and D—only influenza A viruses circulate in multiple animal and human reservoirs, sometimes spilling over to cause illness in other species and adapting and exchanging genes to generate new influenza viruses with potential to cause pandemics.

Influenza viruses live on the edge of chaos. The genome of influenza is relatively small and replication of these negative-sense, single-stranded RNA viruses is error prone, leading to numerous defective viruses.[177,178] Although the high error rate can be a disadvantage, it can also be an advantage, allowing a few viable escape mutants to acquire new successful adaptations for evading host immunity and continuing the spread of infection. Influenza viruses have a segmented genome—eight separate genes coding for one or more proteins necessary for constructing a functional influenza virus. Two of the eight genes provide the instructions for building two important surface glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA). Influenza virus nomenclature categorizes influenza A viruses into subtypes on the basis of the antigenic characteristics of these two surface proteins (e.g., H1N1, H7N9, H3N2, H5N1, etc.).

The HA and NA proteins are critical for virus infection and transmission. The HA protein binds to glycan receptors on respiratory and other cells, making it possible for the virus to enter the host cell and begin the process of replication. The glycan receptors on host cells are glycan chains that are tethered to the host cell at one end and have sialic acid residues at the free end; HA proteins attach to the sialic acid.[179] After replication, as the viruses are emerging on the host cell surface, the NA protein cleaves the receptors, allowing the newly formed viruses to be released. The HA protein is constantly under selective immune pressure, from either prior influenza infection or vaccination. Under this pressure, the virus must change enough to evade host antibodies that might prevent attachment of the HA to receptors on the host cells and, at the same time, not change too much and risk losing the ability to attach to host receptors. These ongoing, incremental virus adaptations are described as *antigenic drift*. This characteristic of influenza viruses allows them to find ways to evade host immunity, returning each year to infect the same population repeatedly.

Reservoirs of Influenza A

Influenza A viruses have three main reservoirs: birds, humans, and swine; however, transmission is also maintained in dogs, horses, bats, and other mammals.[180,181] The circulation of influenza A viruses in these reservoirs is essentially separate but, under certain conditions, infection can be transmitted from one species to another. Over the last 100 years, influenza A viruses circulating among humans have been limited to H1N1, H2N2, and H3N2 subtypes. The avian reservoir, mostly in migratory waterfowl, is the most diverse, supporting multiple subtypes of influenza A viruses in various combinations of HA and NA proteins (H1–H16, and N1–N9).[182] The flyways of many of these waterfowl allow influenza A viruses to travel great, intercontinental distances, introducing new viruses to naïve populations.

Domestic poultry, such as chickens, ducks, and geese, also maintain circulation of influenza A viruses. The impact of influenza on domestic poultry can be significant due to infection with highly pathogenic avian influenza (HPAI) A viruses.[183] HPAI A viruses are avian influenza A viruses of subtypes H5 or H7 (e.g., H5N1 or H7N7), which have HA proteins containing a multibasic cleavage site at the base of the protein. This protein signature is associated with increased replication of the virus and systemic spread with multiorgan failure, presenting as higher-severity illness in domestic poultry and sometimes in infected mammals. Poultry flocks infected with HPAI A viruses can manifest disease rapidly, often with 100% fatality of the flock. Because of the impact on trade and protein security, most governments require immediate reporting of HPAI outbreaks and mandate culling of all associated poultry to prevent any further spread.[184]

The swine reservoir of influenza A viruses maintains a more limited set of subtypes, generally H1 to H3 and N1 and N2. Compared with humans and birds, swine have a much greater ability to be infected with either bird or human influenza A viruses due to the presence of a more diverse set of receptors in the swine respiratory tract.[185] Influenza viruses attach to host cell receptors through binding of the HA protein to sialic acid residues. Avian influenza A viruses have a strong affinity for receptors with an α(2,3)-linked sialic acid, which are more abundant on bird respiratory and enteric epithelial cells. Human influenza A viruses prefer receptors with an α(2,6)-linked sialic acid, which are more abundant in the upper respiratory tract of humans. Swine, however, have both type of receptors, allowing infection with either bird or human influenza A viruses. Because of this, swine have been called the "mixing vessel" for influenza A viruses.[185] Avian influenza A viruses, infecting a pig, have the opportunity to adapt to binding the more human-like α(2,6) receptors. More importantly, pigs can be coinfected with avian,

swine, and human influenza A viruses, allowing the viruses to exchange genes and form new hybrid influenza viruses referred to as *reassortants*. This significant adaptive change in influenza A viruses when a human is infected is referred to as *antigenic shift*, and represents a fitness advantage that may allow the virus to transmit efficiently in humans, potentially emerging to cause a global pandemic.

Influenza A and Pandemics

In the last 100 years, there have been four antigenic shift events leading to reassortant influenza A viruses that caused pandemics in humans.[186] Each of these viruses had genes of either avian or swine origin, or both. The most devastating of these pandemics was the 1918 H1N1 "Spanish" pandemic, which caused at least 50 million deaths globally. The pandemic emerged during World War I and impacted young adults disproportionately, ultimately killing more soldiers than died in combat.[187] Subsequent pandemics in 1957 ("Asian" H2N2 pandemic) and 1968 ("Hong Kong" H3N2 pandemic) had less severe impacts, each causing approximately 1 million deaths globally.[186] The fourth pandemic in the last 100 years emerged in 2009 and was caused by an H1N1 influenza A virus with HA genes derived from the 1918 H1N1 pandemic virus.[188] Sometime in the 1930s, the H1N1 virus circulating in humans entered the swine population and underwent multiple gene reassortments, including incorporation of avian, human, and other swine influenza A virus genes. Most likely, this virus moved back and forth between humans and swine, becoming increasingly adapted to humans[189,190] and eventually achieved sustained and efficient human-to-human transmission, thus igniting the pandemic in 2009.[188]

It is very difficult to know when and where the next influenza pandemic virus will emerge; however, it is certain that one will occur. Early detection of these viruses is a critical component of pandemic preparedness and response. Addressing this need, global laboratory surveillance for influenza viruses circulating in humans is in place through the WHO's Global Influenza Surveillance and Response System (GISRS).[191] One hundred and forty-four National Influenza Center (NIC) laboratories in GISRS have access to standardized molecular testing reagents provided by the CDC to test respiratory specimens year-round. Influenza-positive specimens are sent to one of six WHO Collaborating Centers (Atlanta, London, Beijing, Tokyo, Melbourne, and Memphis) responsible for characterizing influenza viruses and selecting vaccine viruses. NICs can detect known circulating human influenza viruses, but are also able to detect "novel" influenza A virus infections (i.e., infections in humans caused by influenza A viruses circulating in birds, swine, or other animals). Since 2005, human influenza A virus infections caused by a new subtype is a condition for which WHO requires notification under the International Health Regulations (IHR)[192] (see "Conclusion: Controlling the Threats" later).

Novel Influenza A Viruses

The number of reported novel influenza A virus infections has been increasing significantly since the first reported case in 1959 (Fig. 14.1).[180,193,194] Increased surveillance and improved diagnostic tests have clearly added to the increase; however, other factors are contributing. The expanding number of pig and poultry operations, including live animal markets, that are located in or near densely populated areas provides new opportunities for human exposure to avian influenza A viruses. In addition, international air travel now makes it possible to travel to almost any place on the globe within the incubation period of influenza virus infection. Fig. 14.2 shows the considerable increase in the populations of pigs, poultry, people, and passengers since 1960. This convergence of humans, animals, and transportation may be an important reason for the significant rise in human cases due to two novel influenza A viruses of recent concern: H5N1 and H7N9.

Influenza A(H5N1)

In May 1997, a young child in Hong Kong developed acute febrile respiratory illness and died of multiorgan failure. HPAI A(H5N1) virus was isolated from a tracheal aspirate specimen.[195] This was the first known fatal case of avian influenza A virus infection. Overall, 18 virologically confirmed A(H5N1) virus infections with 6 deaths were reported in Hong Kong during 1997.[196] Seroepidemiologic studies suggested that poultry-to-human A(H5N1) virus transmission occurred among exposed poultry workers,[197] and limited human-to-human transmission may have occurred in a household[198] and through nosocomial transmission.[199] Temporary closure of live poultry markets, depopulation of 1.5 million chickens, and stopping importation of live poultry from mainland China ended the outbreak.[196] In early 2003, severe and fatal A(H5N1) virus infections were identified in family members from Hong Kong who visited southern China.[200] This was the beginning of the reemergence of human infections associated with the spread of HPAI A(H5N1) viruses among poultry to more than 60 countries. Each year, sporadic human infections have been reported in some countries, typically during colder temperature months. Although human infections have generally declined since 2006, a surge in A(H5N1) virus infections occurred in Egypt during 2014 and 2015.[201] The clinical spectrum ranges from asymptomatic infection to fulminant pneumonia, acute respiratory distress syndrome (ARDS), and multiorgan failure.[128] Close or direct exposure to sick or dead poultry or visiting a live poultry market are the main risk factors for A(H5N1) virus infection,[202,203] but limited, nonsustained human-to-human transmission has been reported among blood-related family members in multiple countries.[204,205] Prevention and control measures in poultry include surveillance for outbreaks, depopulation, quarantine, and use of H5 vaccines, but HPAI A(H5N1) viruses continue to circulate and evolve among poultry in some countries. From November 2003 to March 2018, 860 human infections with HPAI A(H5N1) virus, with greater than 50% mortality, in 16 countries were reported to WHO.[206]

Influenza A(H5N6)

Human infections with HPAI A(H5N6) virus resulting in severe pneumonia, respiratory failure, and death were first reported in China in 2014.[207,208] Although one case of mild clinical illness with A(H5N6) virus infection was reported, almost all hospitalized patients have required

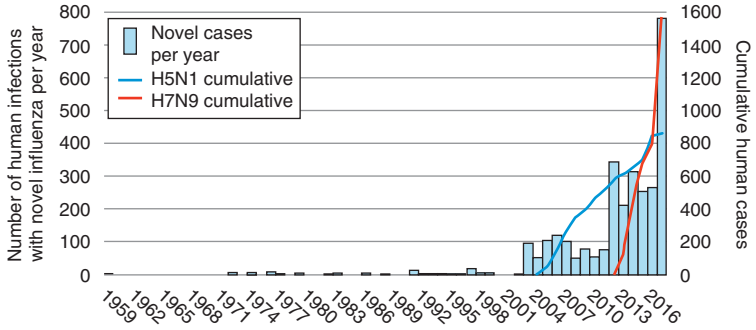

FIG. 14.1 Increasing number of human cases of novel influenza A virus infection, 1959 to 2017. Cumulative cases of H5N1 and H7N9, as reported by the World Health Organization. Graph includes avian H4, H5, H6, H7, H9, and H10; and swine H1 and H3 (not pH1N1). *(Data from Freidl GS, Meijer A, de Bruin E, et al. Influenza at the animal-human interface: a review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1).* Euro Surveill. *2014;19:pii-20793.)*

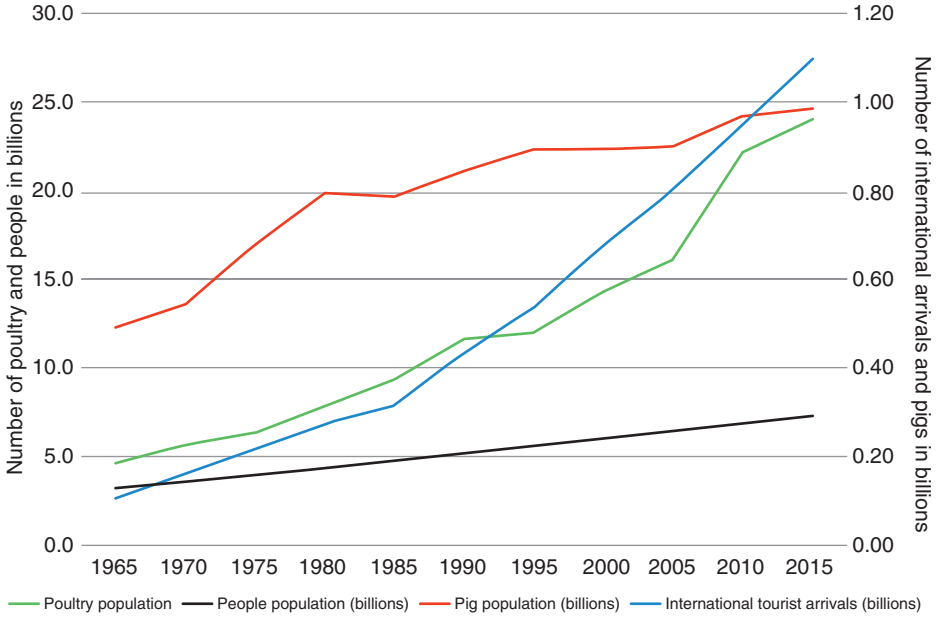

FIG. 14.2 Poultry, pigs, people, and travel trends. *(Data from Areppim AG. International tourist arrivals.* http://stats.areppim.com/stats/stats_ita.htm*; United Nations Department of Economic and Social Affairs. World population prospects: the 2017 revision.* https://www.un.org/development/desa/publications/world-population-prospects-the-2017-revision.html*; and United Nations Food and Agriculture Organization. Data, agriculture.* http://www.fao.org/faostat/en/#data.*)*

invasive mechanical ventilation, with high mortality.[209] As of November 2018, 22 sporadic human cases of A(H5N6) virus infection with high mortality were reported in China.[210] HPAI A(H5N6) viruses that have infected humans are reassortant viruses that are related genetically to HPAI A(H5N1) viruses circulating among poultry in China. HPAI A(H5N6) virus caused severe pneumonia in experimentally infected ferrets, but did not transmit via droplets.[211] HPAI A(H5N6) viruses, including genetically related as well as unrelated reassortant viruses, have been identified in wild birds and in poultry in multiple countries and regions of the world.[212–216] Other related HPAI viruses of H5 subtypes, such as H5N2, H5N5, and H5N8 (often referred to as "H5NX") viruses, have emerged through genetic reassortment and have been detected in wild birds or have caused poultry outbreaks in North America, Europe, and Asia.[217–219] As of April 2019, no human infections with HPAI A(H5) viruses other than H5N1 and H5N6 viruses have been reported.

Influenza A(H7N9)

In the early spring of 2013, the first three human infections with A(H7N9) virus were identified in eastern China.[127] All three infected persons died; this was the first time that a low-pathogenicity avian influenza (LPAI) A virus had caused fatal outcomes. This Asian-lineage A(H7N9) virus was a reassortant virus with the H7 and N9 genes derived from other avian influenza A viruses and six genes derived from A(H9N2) viruses circulating among poultry. During the spring of 2013, most A(H7N9) virus infections were sporadic and occurred in older men with a recent history of exposure to poultry in urban areas of eastern China.[220] The main risk factor identified for A(H7N9) virus infection was visiting a live poultry market.[221] Most reported A(H7N9) virus infections have resulted in severe pneumonia, and infected persons were admitted to intensive care units with complications such as respiratory failure, ARDS, and multiorgan failure.[222] Clinical management has included antiviral treatment with an NA inhibitor and supportive care. NA inhibitor resistance has emerged during antiviral treatment.[223,224] Some cases of asymptomatic or mild illness with A(H7N9) virus infection were identified, and some studies estimated that many mild cases occurred but were not detected, suggesting a wide clinical spectrum of infection.[225–227] Closure of live poultry markets in large urban areas was effective in reducing human A(H7N9) virus infections.[228,229] However, as A(H7N9) viruses spread among poultry into rural areas, exposure to backyard poultry was identified as a risk factor for human infection.[230]

Annual epidemics of sporadic human A(H7N9) virus infections have continued to occur in China, with a surge in infections during 2016 and 2017, prompting pandemic concerns.[231–233] This huge increase in human infections was attributed to geographic spread of A(H7N9) viruses among poultry throughout China and ongoing viral evolution into multiple lineages.[234] During 2016, HPAI A(H7N9) viruses emerged to cause poultry outbreaks in several areas of China with associated severe and fatal human infections in 2017.[234] Limited, nonsustained human-to-human A(H7N9) virus transmission has occurred in small clusters of cases among both blood relatives and unrelated persons, but no increase in human-to-human transmission was identified during 2013 to 2017.[235] Human infections with A(H7N9) virus have been exported from China to Malaysia,[236] Taiwan,[237] and Canada.[238] During 2017 and 2018, very few human A(H7N9) virus infections were reported. The reasons for the dramatic reduction in human cases are unknown, although multiple factors may have contributed, including widespread H5/H7 vaccination of commercial sector poultry in China. As of April 2018, 1567 human infections with A(H7N9) virus and 615 deaths (39%), all infected in China, had been reported to WHO.[239]

Other Influenza A Virus Subtypes

Sporadic human infections with HPAI A(H7) viruses other than A(H7N9) have resulted in a wide range of disease severity. Multiorgan failure with fatal outcome occurred in one case of HPAI A(H7N7) virus infection in the Netherlands during widespread poultry outbreaks in 2003.[240] However, most human infections with HPAI A(H7N7) virus have resulted in mild upper respiratory tract illness or conjunctivitis.[241] Conjunctivitis has been reported with HPAI A(H7N3) virus infection in Mexico.[242] A wide range of illness has also been reported for LPAI A virus infections. Conjunctivitis has been reported from infections with LPAI viruses A(H7N2) in the United Kingdom,[243] A(H7N3) in Canada and the United Kingdom,[244,245] A(H7N7) in the United Kingdom,[246] and A(H10N7) in Australia.[247] Upper respiratory tract illness has occurred from infections with LPAI viruses A(H6N1) in Taiwan,[248] A(H7N2) in the United States and the United Kingdom,[243,249] A(H7N3) in Canada and the United Kingdom,[244,245] A(H9N2) in China, Hong Kong, Bangladesh, and Egypt,[250–252] and A(H10N7) in Australia.[247] Lower respiratory tract illness has been reported from infections with LPAI viruses A(H7N2) in the United States, Hong Kong, and China,[249–253] A(H7N4) in China,[254] A(H9N2) in Hong Kong and China,[253,255] and A(H10N8) in China.[256]

In addition to LPAI A(H7N9), fatal outcomes have been reported in China with A(H9N2)[257] and A(H10N8)[256] virus infections. Cat-to-human transmission of an LPAI A (H7N2) virus resulting in upper respiratory tract illness was reported in the United States.[258] Experimental infection of a seal with a closely related LPAI A(H7N7) virus resulted in seal-to-human transmission with resultant conjunctivitis in the United States.[259]

Variant Influenza A Infections From Swine

Sporadic human infections with multiple subtypes of influenza A viruses circulating among pigs have been reported worldwide for many years. Swine influenza A viruses that have infected humans are referred to as "variant viruses" and denoted with "v" after the subtype. Most reported variant virus infections have occurred in children. Most infections with variant viruses (H1N1v, H1N2v, H3N2v) have resulted in mild upper respiratory tract illness (H1N1v, H1N2v, H3N2v),[260–263] conjunctivitis (H1N1v, H3N2v),[262,263] or gastrointestinal symptoms (H3N2v).[263] However, hospitalizations, including one death, were reported for 5.2% of H3N2v virus infections during a multistate outbreak associated with exposures to pigs at agricultural fairs in 2012 in the United States.[263] Critical illness, including respiratory failure and ARDS, with H1N1v virus infection have been reported in an adult man in Italy[264] and a child in the Netherlands.[265] Fatal outcomes of other variant virus infections have been reported.[261] Most variant virus infections likely result from sporadic swine-to-human transmission, but limited, nonsustained human-to-human transmission has been reported.[261,263,266]

Influenza Risk Assessment

Given the diversity and significant increase in human cases due to avian- and swine-origin influenza A viruses, public health officials have developed tools to assess the pandemic potential of these viruses.[267,268] One of these tools, the CDC's Influenza Risk Assessment Tool (IRAT),[267] uses 10 scientific criteria covering an influenza A virus' characteristics (e.g., receptor binding tropism, genetic variation, antiviral drug resistance markers, transmissibility and pathogenesis in animal models), epidemiology, ecology, and the disease it causes in humans. These data are used to assess potential pandemic risk based on two different scenarios: (1) the likelihood that the virus will emerge to transmit efficiently in humans and cause a pandemic, and (2) the disease severity impact the virus would cause in humans if it were to emerge. Fig. 14.3 provides examples of IRAT scores for selected novel influenza A viruses, indicating that A(H7N9) viruses currently have the highest risk scores among those viruses evaluated. The IRAT and other risk assessment tools provide frameworks for assisting public health officials in determining the appropriate response to an emerging novel influenza A virus.

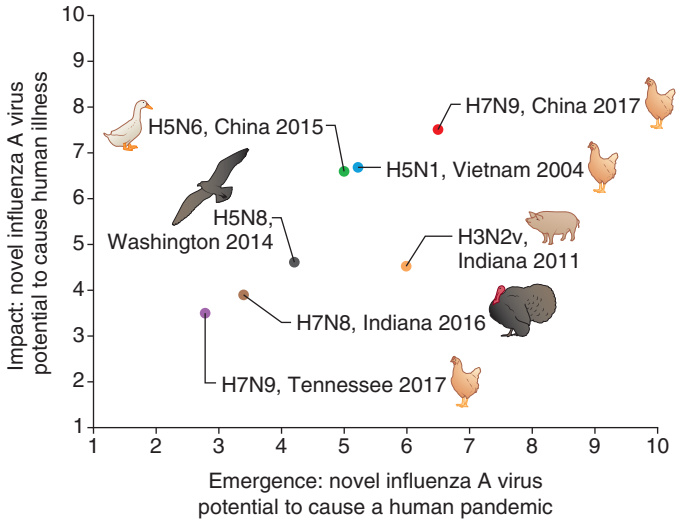

FIG. 14.3 Influenza Risk Assessment Tool. Average risk scores with selected viruses.

In conclusion, influenza A viruses maintain reservoirs of circulation in humans, birds, swine, and other animals. The high error rate of virus replication, coupled with the ability to exchange gene segments among avian, swine, and human viruses, allows the virus to continue evading host immunity, to spill over and cause disease across species, and to periodically introduce pandemic viruses into the human population. Maintaining surveillance in humans, birds, and swine is critical to identifying, preventing, and controlling emerging novel influenza A viruses with pandemic potential.

ENTERIC DISEASES

Enteric diseases encompass all the attributes of emerging and reemerging infectious diseases, and their emergence and spread is influenced by microbial, human, and environmental factors, as discussed previously. Enteric pathogens are constantly changing in their ability to survive in their reservoir environments and in their ability to infect and harm the human host. New variants are discovered with increasing frequency, due in part to advances in technologies such as WGS that are applied to large numbers of pathogen isolates and that can identify changes in genetic virulence factors. In addition to increased international travel and mass production (including huge farms and dairies), increased international trade in food is expanding the number and types of foods that may serve as vehicles for foodborne diseases. For example, in the United States, imports of fruit and nuts more than doubled and imports of fish and seafood increased over 60% during 1998 to 2007.[269] Moreover, individual food items may contain ingredients from multiple countries.

Other factors that facilitate the spread of enteric diseases include wars, social disruption, and associated population migrations, which often result in the breakdown of basic public health and health care services, leading to reemergence of ancient scourges such as cholera, with devastating consequences. Weather events can also increase human exposures to enteric pathogens, especially with the dramatic destruction and flooding from hurricanes and typhoons associated with waterborne diseases. Less obvious are the gradual environmental effects of changes in climate. These effects were evident in an outbreak of *Vibrio parahaemoliticus* infections due to contaminated oysters in 2004.[270] Typically, *V. parahaemoliticus* contamination of shellfish is associated with warm water harvest areas, but in this case the oysters were harvested from Prince William Sound in Alaska. Investigators determined that the water temperatures in the sound reached record high temperatures above 15°C at the time of harvest, the threshold above which *Vibrio* sp. tend to proliferate. This outbreak extended the northernmost documented source of vibriosis by more than1000 km. An enteric pathogen has also been associated with intent to harm, another factor contributing to reemergence of infectious diseases. Prior to the anthrax-laden letters responsible for the bioterrorism event in 2001, the only bioterrorism event in the United States was due to *Salmonella* contamination of salad bars in restaurants in Oregon in 1984.[271] Members of a Rajneesh cult had intentionally contaminated the salad with *Salmonella,* resulting in over 750 cases of salmonellosis in the community, in an attempted to disrupt local elections.

The WHO Global Burden of Disease Study for 2015 ranked diarrheal diseases as the second leading cause of acute incident diseases (2.4 billion)[272] and communicable disease mortality (1.3 million deaths) worldwide, behind lower respiratory infections.[130] This study noted a number of important characteristics of the global diarrheal disease burden. As a sign of progress, the death rate from diarrheal disease decreased 36% between 2005 and 2015. Although diarrheal disease affects persons of all age groups and in all geographic locations, the greatest burden of death falls on children younger than 5 years, accounting for 499,000 deaths. In 2015, rotavirus enteritis was the leading cause of death due to diarrheal diseases (15.2%) among all ages, followed by shigellosis (12.5%) and salmonellosis (6.9%). Rotavirus enteritis was also the leading cause of death due to diarrheal diseases (29.3%) among children younger than 5 years, followed by cryptosporidiosis (12.1%) and shigellosis (11%). Among all ages and for children younger than 5 years, the population attributable fractions for deaths due to diarrheal diseases decreased most for rotavirus enteritis from 2005 to 2015 (40.7% and 43.6% decreases, respectively), likely due to the increasing use of rotavirus vaccines. *C. difficile* infection was a major cause of mortality

due to diarrheal diseases in high-income countries and was the only cause for which the attributable fraction increased from 2005 to 2015 (40.1%).

Routes of Transmission for Enteric Pathogens

The pathogens that cause enteric diseases are transmitted primarily by three routes—foodborne, waterborne, and person-to-person—though other environmental and zoonotic exposures also play a role in transmission. For example, *Clostridium perfringens* and *Bacillus cereus* are transmitted almost exclusively through food, *Shigella* species and norovirus are transmitted primarily from person to person, and *Giardia* species are principally waterborne. Many enteric pathogens can be transmitted by more than one route, including simultaneously. The relative importance of these transmission patterns varies by pathogen and setting; however, systematic studies to determine the population attributable fractions for different transmission routes are difficult to perform. Foodborne transmission has been studied most rigorously. Estimates in the United States suggest that 48 million episodes of foodborne illness occur annually, resulting in approximately 128,000 hospitalizations and approximately 3000 fatalities,[273,274] and that endemic waterborne disease results in 4.3 to 32.8 million cases of acute gastrointestinal illness annually.[275]

The interaction among foodborne pathogens, their environments, and human behaviors are especially complex and in constant flux. Some foodborne pathogens, such as *Campylobacter,* Shiga toxin–producing *E. coli* (STEC), and nontyphoidal *Salmonella,* have animal or environmental reservoirs, and humans are often incidental hosts, acquiring the pathogen after eating foods or ingredients contaminated from those reservoirs somewhere along the chain of production, slaughter, and processing. Other pathogens, such as norovirus or *Shigella,* have a primary human reservoir and cause foodborne illness when an infected human contaminates foods. In addition, our food supply is changing as more food is imported from distant lands, food processing becomes more centralized and industrialized, and consumer tastes and cooking practices evolve. Food animals are raised in close quarters and are slaughtered and processed with ever-greater efficiency. Fresh fruits and vegetables are available year round, often shipped from distant countries. Processed foods such as peanut butter[276] and flour[277] have caused large outbreaks when food safety measures were insufficient to prevent microbial contamination. In the kitchen, microwaving is replacing traditional cooking, which means that the heating that kills microbes is less thorough and more difficult to monitor. Consumers may desire local foods and foods eaten with minimal cooking as well as convenience. In the United States, many states permit the sale of raw unpasteurized milk, despite the raw milk–associated outbreaks that occur more frequently in those states.[278]

These changes in food production and human behavior result in regular emergence of new food vehicles for foodborne diseases (Table 14.4). For example, the increased popularity of packaged fresh leafy vegetables has been associated with numerous outbreaks due to STEC and *Salmonella.* In 1996 a large outbreak of *E. coli* O157:H7 infections linked to packaged fresh spinach shook the produce industry in the United States. Investigators identified 225 cases in 27 states; 39 (14%) case-patients developed hemolytic-uremic syndrome and 5 died.[279] An environmental investigation of source spinach-growing fields in California identified multiple potential sources of *E. coli* O157:H7 contamination from surrounding water sources and animals.[280] Two different STEC serotypes were isolated from spinach during the investigation, including Orough:NM and O146:H21, which further illustrated the multiple sources of environmental contamination. Also in 1996, thousands of cases of cyclosporiasis due to *Cyclospora cayetanensis* occurred in the United States and Canada among persons who consumed fresh raspberries imported from Guatemala.[281] Before this episode, only small numbers of cases, primarily associated with travel to developing countries,[282] had been reported in North America.

Among other modes of transmission, the United States has documented a significant increase in outbreaks due to *Cryptosporidium* contamination of recreational water venues.[283] Different types of water venues have become popular and are associated with multiple outbreaks, including splash pads with water spouts and recirculating water. Since first identified in 1988, recreational water–associated outbreaks due to *Cryptosporidium,* a choline-tolerant parasite, increased from one outbreak in 1988 to 37 outbreaks with 890 cases in 2011 and 2012.

Enteric diseases due to zoonotic transmission have been associated with a wide variety of pathogens and animals. In the United States, the proportion of illnesses from seven pathogens due to animal contact was estimated to be 14%, representing approximately 450,000 illnesses annually.[284] *Campylobacter* had the largest proportion of illnesses attributed to animal contact (17%), followed by *Cryptosporidium* (16%) and *Salmonella* (11%). In a change in human behavior that may be specific to the United States, raising poultry in urban backyard poultry flocks has become more popular.[285] Poultry sold for backyard flocks are produced by approximately 20 hatcheries in the United States, which sell more than 50 million chicks annually. Live poultry–associated *Salmonella* outbreaks increased substantially from 17 outbreaks from 1990 to 2005 to 36 outbreaks during 2006 to 2014.

TABLE 14.4 New Food Vehicles Identified in US Multistate Infectious Disease Outbreaks Since 2006

1. Bagged spinach
2. Carrot juice
3. Peanut butter
4. Broccoli powder on snack food
5. Dog food
6. Pot pies/frozen meals
7. Canned hot dog chili sauce
8. Fresh hot chili peppers
9. Black pepper
10. Tahini sesame paste
11. Raw cookie dough
12. Aquatic water frogs
13. Fresh papaya
14. Frozen mamay fruit pulp
15. Bologna
16. In-shell hazelnuts
17. Pine nuts
18. Par-cooked, broiled chicken livers
19. Scraped tuna
20. Cashew cheese
21. Sugar cane juice
22. Sprouted chia seeds
23. Almond butter
24. Caramel apples
25. Sprouted nut butters
26. Dried mushrooms (in truffle oil puree)
27. Pistachios
28. Wheat flour
29. Powdered meal supplements
30. Soy nut butter

International Spread: Cholera in the Western Hemisphere

The movement of pathogens has had major repercussions in the reemergence of enteric diseases. The introduction of *Vibrio cholerae* O1 into the Western Hemisphere has caused two major epidemics in the past 30 years. Cholera had not been recognized in South America during the 20th century, but in January 1991 cases were identified in coastal areas of Peru.[286] Within weeks, thousands of cases were occurring, and cholera quickly became the most commonly diagnosed cause of diarrheal illness in many parts of Peru.[287,288] During the next 3 years, the disease spread throughout mainland South and Central America, significantly altering the distribution patterns of etiologic agents of diarrheal disease. Epidemic cholera in Latin America has since been essentially eliminated with major improvements in safe water sources.

In October 2010, 9 months after a severe earthquake, a cholera epidemic was identified in Haiti that spread within 2 months to the entire country and across the border to the Dominican Republic.[289,290] Molecular epidemiologic studies have indicated that the *V. cholerae* strain is very similar to South Asian strains,[291] and epidemiologic investigations revealed that initial cases occurred downstream from a

camp where United Nations peacekeepers from Nepal were stationed.[292,293] The source of introduction of the epidemic strain was a politically sensitive topic.[294] Although the incidence has been reduced, cholera transmission persists. Cases in Haiti and the Dominican Republic accounted for greater than 30% of the global cases reported to WHO in 2016.[295]

Evolution of Enteric Pathogens

Changes in pathogens have been recognized as a factor of emergence in a number of events in recent years. A dramatic example occurred in 2011 when a large outbreak of STEC O104:H4 stuck Germany, associated with fenugreek sprouts.[296,297] This pathogen had a newly recognized combination of virulence factors. In addition to producing a virulent subtype of toxin (Shiga toxin 2) encoded on a prophage, it contained genes associated with enteroaggregative *E. coli* and a plasmid-encoded extended-spectrum β-lactamase gene.[298] The combination of efficient attachment to intestinal epithelial cells and a toxin particularly toxic to endovascular cells in one *E. coli* strain resulted in a devastating outbreak. The contaminated seeds used for sprouting originated in Egypt[299] and were distributed widely, resulting in over 4000 illnesses, 800 cases of hemolytic-uremic syndrome, and 50 deaths in Germany and 15 other countries.[297]

Multiple drug resistance in bacterial enteric pathogens has emerged in the United States and internationally. The problem is most severe in foodborne pathogens of animal origin, primarily in *Campylobacter* and *Salmonella* strains.[300] A multidrug-resistant strain of *Salmonella typhimurium* known as definitive type 104 (DT104) emerged in the United States and Europe in the 1990s.[301,302] Patients infected with multiply-resistant strains are more likely to be hospitalized.[303] Fluoroquinolone-resistant strains of *C. jejuni* infections have also emerged; some infections are associated with foreign travel while others are acquired domestically and have been associated with poultry consumption.[304] In a study of resistance in over 1100 *Shigella* isolates in the CDC's Foodborne Diseases Active Surveillance Network (FoodNet) sites from 2008 to 2010, 74% were resistant to ampicillin, 36% to trimethoprim-sulfamethoxazole, 28% to tetracycline, and 0.5% to ciprofloxacin; 5% of isolates were resistant to five or more antimicrobials.[305] Resistance was more common in persons with a history of recent foreign travel. A foodborne outbreak in Los Angeles in 2012 was caused by *Shigella sonnei* with decreased susceptibility to azithromycin, the first such outbreak identified in the United States.[306]

Another example is the emergence of a new strain of multidrug-resistant *S. typhimurium* (multilocus sequence type ST313) in sub-Saharan Africa. The strain causes invasive nontyphoidal disease, sometimes accompanied by diarrhea, primarily in patients with HIV infection, malaria, and malnutrition.[307,308] Application of WGS-based phylogenetic methods has identified two closely related, highly clustered lineages estimated to have evolved independently approximately 52 and 35 years ago. Lineage II strains have replaced lineage I strains, perhaps because of their acquisition of chloramphenicol resistance.[305]

The incidence and severity of *C. difficile*–associated disease has increased dramatically in adults and children in the United States, Canada, Europe, and Australia.[309–312] Moreover, while *C. difficile*–associated disease used to be primarily health care associated, community-associated cases are on the rise. In the United States during 2011 in Emerging Infections Program sites the overall adjusted incidence per 100,000 persons was 147 (95% confidence interval [CI], 129–165).[313] Sixty-six percent of *C. difficile* infections were health care associated, resulting in an incidence of 95 (95% CI, 86–105); 24% had hospital onset of illness. Community-associated *C. difficile* infections accounted for 46% of cases, resulting in an incidence of 52 (95% CI, 43–61). Persons over 65 years old had by far the greatest incidence at 628 (95% CI, 567–687). The death rate was higher for health care–associated disease (8.9; 95% CI, 5–13) than for community-associated disease (0.7; 95% CI, 0.4–0.9). Disease due to a strain of restriction endonuclease analysis group BI, pulsed-field gel electrophoresis type NAP1, and PCR ribotype 027 (BI/NAP1/027) is responsible for much of this increase, including many nosocomial outbreaks.[314–316] Two distinct epidemic lineages, both of which are resistant to fluoroquinolone antibiotics, have recently been identified.[311] The FQR1 lineage, first seen in 2001, is associated with North American outbreaks and sporadic infections in South Korea and Switzerland. The FQR2 lineage originated in 2003 in North America but subsequently spread more widely, causing hospital outbreaks in the United Kingdom, continental Europe, and Australia.[317] Since 2005, another *C. difficile* strain (PCR ribotype 078) has emerged in the Netherlands and in the United Kingdom.[318] This strain also appears to be hypervirulent and is more likely to affect younger persons and to be associated with community-onset disease. Isolates of *C. difficile* also have been obtained from retail ground meat samples,[319] prompting the need for evaluation of the potential role of foodborne transmission in community-associated cases. Recent experiences with fecal microbiota transplantation have suggested an important role for this approach in the management of patients with severe *C. difficile* infections.[320,321]

In addition to changes in toxin production in emerging *C. difficile* strains, investigators have observed changes in other clostridial toxins. A purported new *Clostridium botulinum* toxin type was found in 2013, designated toxin type H at the time.[322] *C. botulinum* has been known to produce seven botulinum toxin types (A–G), and noninfant botulism is treated with Botulism Antitoxin Heptavalent (Emergent BioSolutions Canada), an equine-based product of polyclonal antibodies that treats all known serotypes of botulinum toxin. *C. botulinum* and its associated toxins are considered select agents due to their potential use as biological terrorism agents,[323] thus a new toxin type that could escape the action of available antitoxins was a security concern. The identification of a new botulinum toxin type stirred controversy concerning information that should or should not be shared about the new toxin based on the potential dangerous misuse of the information for nefarious purposes.[324] Additional investigation found the new toxin to have a hybrid structure similar to the structures of toxins A and F. Furthermore, available type A antitoxin was able to neutralize the toxin, deescalating the security concern.[325]

Noroviruses

Noroviruses are transmitted not only from person-to-person but also by food, water, and contact with contaminated environmental surfaces.[326–328] These viruses have become an important cause of diarrheal illness outbreaks among cruise ship passengers, hospitalized patients, nursing home residents, college students, restaurant patrons, and military personnel, reflecting their high infectivity and low infectious dose (<20 viral particles).[329] A systematic literature review published in 2014 indicated that norovirus disease is associated with 18% (95% CI, 17–20) of all cases of acute gastroenteritis.[330] Prevalence was similar across age groups, but higher among cases of acute gastroenteritis in community and outpatient settings (24% and 20%, respectively) compared with inpatient settings (17%). Prevalence was also higher in developed countries (20%) compared to high-mortality developing countries (14%), suggesting a more prominent role for other pathogens in developing countries. Noroviruses are the leading cause of epidemic gastroenteritis in the United States.[331,332] Following the introduction and use of rotavirus vaccines, noroviruses have also become the leading cause of medically attended acute gastroenteritis in US children under 5 years of age; these viruses were found in 21% of children seeking medical care for acute gastroenteritis in 2009 and 2010.[333] In March 2012, a new GII.4 norovirus strain named GII.4 Sydney was identified in Australia and subsequently spread to the United States, the United Kingdom, and a number of other countries.[334] GII.4 norovirus outbreaks have been associated with higher rates of hospitalization and mortality compared with outbreaks caused by other genotypes.[335] New variants of the GII.4 genotype have emerged every 2 to 4 years over the last 2 decades, likely driven by escape from population immunity.[336] These genotype variants represent an additional mechanism of enteric pathogen change resulting in emergence of novel strains as a result of antigenic shifts.

Future Trends

The observation of changes in virulence factors and antigens such as in *E. coli, Clostridium* species, and norovirus, as described previously, provides an increased appreciation of the extent to which pathogens may change with acquisition, rearrangement, or mutation of associated genes. Indeed, the genes responsible for virulence may be encoded on plasmids or other transmissible genetic elements and thus may recombine

to produce pathogens with enhanced virulence, which may emerge rapidly in varied populations and places.

Looking to the future, we can expect to identify the emergence of more pathogens and pathogen subtypes, including pathogens with a differing and expanded repertoire of antibiotic resistance determinants. Clinical laboratories are increasingly adopting syndromic panel-based diagnostic tests that can rapidly detect a wide range of bacterial, viral, and parasitic enteric pathogens and yield more positive results than conventional testing methods, resulting in an increase in pathogen-specific diagnosis among enteric illnesses.[337] The routine and systematic application of next-generation DNA sequencing technology to the surveillance and investigation of listeriosis in the United States has resulted in the detection and successful investigation of more and smaller listeriosis clusters.[338] WGS analyses allow the high-resolution characterization of pathogens, including genetic determinants of virulence, antibiotic resistance, antigenic shifts, and a host of other factors related to changes in pathogen behavior. Large publicly available DNA sequence databases enable research for the discovery of new pathogen virulence factors and antibiotic resistance determinants.

However, these new technologies also present challenges. The adoption of syndrome panel-based diagnostic tests that can detect multiple pathogens increases the chance of identifying a noninfecting transient pathogen, a minor pathogen, or multiple pathogens, which can confuse diagnosis. In a particularly ironic twist, the use of these diagnostic panels threatens the ability to conduct DNA sequence-based pathogen surveillance and investigation.[339] This is because these PCR-based diagnostic panels detect potential pathogens directly from sample material and do not result in cultured isolates. Next-generation DNA sequence technologies require cultured isolates for analyses. The logical path to resolve this challenge is likely the convergence of diagnostic and DNA sequence technologies with metagenomics.[340] The technical ability to sequence and analyze any or all DNA contained in a clinical sample is in its infancy but shows great promise for clinical, public health, and research applications and may lead to the next paradigm shift in microbiology.

VECTOR-BORNE DISEASES

Vector-borne diseases are caused by pathogens transmitted by blood-feeding arthropods, typically insects and ticks. In all cases the pathogen is amplified in the vector and in some—*Plasmodium* and *Borrelia* are examples—it is transformed. Vector-borne pathogens include representatives from most major categories of disease-causing agents—viruses, protozoa, bacteria, and helminths—and are responsible for some of the world's most destructive epidemics. Malaria, plague, yellow fever, typhus, and dengue have been known since antiquity but recent years have brought new threats from once obscure or unknown pathogens, such as the mosquito-borne chikungunya and Zika viruses, and tick-borne agents. Vector-borne pathogens are predominately zoonoses, and vectors frequently bridge the gap between humans and animal reservoirs that rarely come into direct contact, an especially important contribution to pathogen emergence. Sometimes humans remain dead-end victims, as happens with West Nile virus and Lyme disease, but for others, such as dengue and Zika viruses, the pathogens have gained the ability to be vectored between humans. Some human *Plasmodium* species that made the transition from animals to exclusively humans eons ago still retain the capacity for infecting wild, nonhuman primates.

There are, so far, only two highly efficacious vaccines in widespread use against vector-borne pathogens, those against yellow fever and Japanese encephalitis viruses. This puts the burden of preventing and controlling the large number of other vector-borne diseases on eliminating or reducing contact with the vectors. This can be done by denying vector access to humans, as with bed nets or repellents, or by eliminating the vector, using insecticide or altering habitat ("source reduction"). An advantage of vector control is that elimination of a single vector species can often eliminate transmission of more than one pathogen. The mosquito *Aedes aegypti,* for example, transmits dengue, yellow fever, chikungunya, and Zika viruses; most malaria vectors, such as *Anopheles gambiae,* can transmit all species of human *Plasmodium.* A second advantage is that insecticides and many other control tools will work against a variety of vectors species. But despite early successes,[341] vector control, which typically requires a large, well-organized work force and access to property, has often proved to be unsustainable. The indiscriminate use of insecticides—both in public health and agricultural applications—has resulted in widespread vector resistance while few new active agents are coming to market.[342] Development of methods of biologic control, such as release of sterile male mosquitoes or replacement of wild populations of mosquitoes with those that are pathogen resistant, are being actively pursued.[343]

The *Aedes*-Transmitted Viruses: Dengue, Zika, Yellow Fever, and Chikungunya

The small black and white mosquito, *A. aegypti,* evolved to cohabit with humans. It lays its eggs in water-filled artifacts common in and around dwellings, often rests in houses, and tends to bite during daylight. Although most common in the tropics, its range increasingly extends into temperate zones; a cognate species, *Aedes albopictus,* is even more accommodating of cool climate and has been responsible for epidemics of chikungunya virus in Italy[344] and France.[345] The close proximity of *A. aegypti* to humans makes it a potent vector of four of the most consequential arthropod-borne viruses ("arboviruses"), all of which have caused major epidemics since 2013.

Dengue

Dengue is the most important mosquito-borne viral disease in the world, estimated to infect nearly 400 million people annually.[346] Epidemics are common: more than 80,000 cases were reported from Sri Lanka in 2017, a year in which there were also major outbreaks in Vietnam, Burkina Faso, and Cote d'Ivoire. Dengue fever is caused by four related but antigenically distinct flavivirus species (DEN-1, -2, -3, and -4), sometimes referred to as serotypes. They are single-stranded, positive-sense RNA viruses that possibly jumped from simians to humans during historical times; sylvatic transmission still occurs in parts of West Africa and Southeast Asia.[347] There has been a dramatic increase in reported cases of dengue disease since 2000, especially in Latin America and Africa.[348] The reasons for worldwide increase are likely a combination of increased global movement of people and goods, warming climate, insecticide resistance, and, especially, urbanization. Infection with any of the four dengue species confers lifelong immunity to only that species, and the most severe forms of disease occur in those subsequently infected by another species. While most infections are mild, and often go unreported, severe dengue (dengue hemorrhagic fever and dengue shock syndrome) is life threatening if not properly managed.[349]

The decades-long quest for an efficacious vaccine against all four dengue species has been complicated by concerns about the association of severe disease with secondary heterotrophic infection, and by the lack of an animal model that could be used to establish correlates of protection. The first vaccine to successfully complete clinical trials, Sanofi-Pasteur's Dengvaxia, appeared commercially in 2015, but its limited efficacy against DEN-2 and DEN-4 has been further complicated by a relatively high rate of postvaccination hospitalization in children who had been seronegative at the time of vaccination.[350,351] At least four other tetravalent vaccines are in clinical trials, including live-attenuated, subunit, DNA, and purified inactivated candidates.[350,351]

Zika

Zika virus is also a flavivirus of apparent simian origin. Discovered in 1947 in a sentinel macaque during yellow fever investigations in Uganda, Zika infections were rarely reported in humans until an epidemic in Micronesia in 2007. It subsequently moved south and eastward through the Pacific before reaching Brazil by 2014.[352] Its epidemic spread through the naïve populations of the tropical Americas during 2015 to 2017 dramatically illustrates the dangers inherent in the emergence of a pathogen. Before Brazil, Zika virus was characterized as causing mild, inconsequential disease in no more than about 20% of those infected. Its symptoms might not be distinguished from mild dengue: fever, rash, and myalgia, although with significantly higher occurrence of conjunctivitis. Although details of its pathogenesis are still being investigated, it is clear that Zika virus infection of pregnant women, especially during the first trimester, was responsible for an epidemic of congenital brain abnormalities, most obviously manifested as microcephaly. No other

arbovirus is yet known to cause birth defects, although several are neurotropic, and none has before been found, as Zika virus has been, to be sexually transmitted.[353] Virus isolates from Latin America are the Asian lineage, which had not been previously observed to cause birth defects, although a retrospective review of records from French Polynesia suggests an association between Zika virus infection and microcephaly was overlooked earlier.[354] If Zika virus is widely endemic in Africa and Asia, as recent investigations in Indonesia indicate,[355] it is possible many females reaching puberty there are naturally immune. Much attention is being devoted to improving serologic tests capable of reliably differentiating a history of Zika infection from dengue[356] and to developing a vaccine.[357]

Yellow Fever

Since 1937 hundreds of millions of people at risk from yellow fever virus have been immunized with a highly efficacious live-attenuated vaccine (17D and its derivatives). Nevertheless, yellow fever, the eponymous *Flavivirus,* continues to cause large deadly epidemics in South America and Africa. The virus is transmitted in both a sylvatic cycle among monkeys by forest dwelling *Aedes* mosquitoes and in an urban cycle among humans, predominately by *A. aegypti.* The cycles are linked when urban or semiurban dwellers are infected while working in the forest. In late 2015, a major urban epidemic began near Lusaka, Angola, later spreading to western regions of the Democratic Republic of the Congo (DRC).[358] Nearly 7500 suspected cases were reported, with fatality rates among 962 laboratory-confirmed cases of 14% to 21%. At least 11 cases were carried to China by migratory workers. Although China, like all of Asia, is yellow fever free, *A. aegypti* are common; fortunately there was no secondary transmission. A major epidemic of sylvatic yellow fever began in Brazil in late 2016,[359] affecting southeastern regions where vaccination rates were low. Up to June 2018, there had been more than 2000 confirmed cases with a fatality rate of approximately 30%. Urban transmission did not happen, possibly because of emergency mass distribution of more than 40 million vaccine doses. Together with about 30 million doses sent to West Africa by WHO, vaccine stockpiles became so depleted that efficacious fractional administration with 20% of the standard dose was successfully implemented in both Brazil and the DRC.[360]

Chikungunya

Unlike dengue, Zika, and yellow fever viruses, chikungunya is in the family Togaviridae, but like them it is a single-stranded, positive-sense RNA virus transmitted by *Aedes.* First described from Tanzania in 1953, a pandemic began in coastal northeastern Kenya in 2004 and by 2013 had spread through the Indian Ocean to South Asia, Southeast Asia, the Pacific, and the Caribbean.[361] By 2015 it had spread through much of Latin America. The disease is notable for the high proportion of infections developing symptoms, in particular painful arthralgia of the small joints; fatalities are rare. RNA viruses have high potential for mutation, and sometime early in the pandemic a single nucleotide substitution occurred that replaced an alanine with valine at position 226 in the viral envelope protein, E1.[362] This seemingly insignificant change increased the ability of one chikungunya lineage to infect *A. albopictus,* which extends farther into temperate zones than does *A. aegypti*. Sporadic outbreaks of chikungunya in Italy[344] and France[345] since 2007 were driven by *A. albopictus* transmission.

Plasmodium vivax, Plasmodium knowlesi, and Zoonotic Malaria

The primate malarias are transmitted by a wide range of *Anopheles* mosquito species. Although predominantly diseases of the tropics today, *Plasmodium* was endemic in North America and Europe well into the 20th century. Major public health initiatives in Africa and Asia have substantially reduced disease from *Plasmodium falciparum,* but these advances are countered by rising drug resistance, increased awareness of pernicious *P. vivax* disease, and the emergence of zoonotic malaria.[363]

There is not yet an efficacious, licensed malaria vaccine. Therapy for *P. falciparum* infections, and increasingly for *P. vivax,* has depended on artemisinin-based combination therapies. The recent rise of *P. falciparum* resistance in Southeast Asia to artemisinins and some partner compounds threatens elimination campaigns and complicates treatment.[364] For reasons that are poorly understood, an area of southwestern Cambodia has generated most examples of *P. falciparum* resistance, beginning with chloroquine in 1968 and followed by mefloquine, tafenoquine, fansidar, and, in 2016, artemisinin.[365] Rising resistance of the *Anopheles* vectors to insecticides, including the pyrethroids used in treated bed nets, also hamper control[366]; while there have been advances in engineering mosquitoes incapable of transmitting malaria, designing means to drive modified genes into wild populations remains a challenge.[343]

P. vivax is the most cosmopolitan human malaria and was long considered relatively benign compared to *P. falciparum* malaria. There is mounting evidence that it does, in fact, cause serious disease and death.[367,368] Complications of *P. vivax* infection, which might elicit greater inflammatory response than does *P. falciparum,* seem especially to afflict children with comorbidities, common in malaria-endemic regions.[369] The extent of *P. vivax* endemicity also continues to be reappraised. It had been thought that the invasion of reticulocytes by *P. vivax* merozoites was exclusively mediated by the Duffy antigen/chemokine receptor (DARC), which is absent in most sub-Saharan populations.[370,371] Since the discovery that *P. vivax* was being naturally transmitted to DARC-negative people in Kenya,[372] there have been mounting reports of *P. vivax* both in Africa and in the African diaspora.[373,374] The existence of alternate erythrocyte invasion pathways could affect development of *P. vivax* blood stage vaccine candidates designed to block the *P. vivax* merozoite's access to DARC.[375]

Until recently it was dogmatic that naturally acquired human malaria was caused by four species: *P. falciparum, P. vivax, Plasmodium malariae,* and *Plasmodium ovale.* It is now known that in Southeast Asia humans are not only being infected with the macaque malaria, *P. knowlesi,* but that in some places it is the predominant cause of clinical malaria.[376] It is not yet clear whether human cases all originate in monkeys or if human-to-human transmission is also occurring.[377] The importance of *P. knowlesi* was long overlooked because in the microscopic examination of thick, dehemoglobinized blood films, *P. knowlesi* is easily mistaken as *P. malariae* or *P. vivax.* Whereas *P. knowlesi* is a distinct simian malaria that has jumped to humans, in Brazil it appears that *Plasmodium simium,* which infects howler and capuchin monkeys and is increasingly reported infecting humans, was originally human *P. vivax.*[378] Similarly, *Plasmodium brasilianum,* a parasite of New World monkeys that has been found infecting people in Venezuela, has a genome nearly identical to *P. malariae.*[379] The increasing use of sophisticated genomic investigative methods in epidemiology are likely to further qualify our knowledge about human malaria. If zoonotic transmission of malaria is more common than presumed, it could complicate efforts to eliminate the disease in some places.

Tick-borne Pathogens

The epidemic potential of tick-borne pathogens is constrained by biology: unlike mosquito-borne pathogens, humans are always dead-end hosts and tick dispersion is more limited than for mosquitoes. But the persistence of tick-borne pathogens in the environment and the increasing rate of emergence make them major public health concerns. Lyme disease (*Borrelia burgdorferi*) is the most common vector-borne disease in the United States.[380] Recent analyses have estimated the incidence of Lyme borreliosis to be greater than 300,000 cases per year in the United States, 8- to 10-fold higher than reported cases.[381,382] Despite underreporting, it is also clear that the geographic range of the vector, the black-legged tick (*Ixodes scapularis*), has been steadily expanding from its core in New England westward to the Great Lakes, northward to southern Canada, and southward to the Mid-Atlantic states.[383] The black-legged tick is also the vector of two recently described relapsing fever agents—*Borrelia miyamotoi* and *Borrelia mayonii*—related to the Lyme spirochete. *B. miyamotoi* was first described in Japan in 1995[384] and in the United States in 2013[385] but is now known to occur in Europe as well,[386] suggesting it is not a recent introduction. *B. mayonii,* first reported in 2016 from a group of Lyme disease–negative patients, is characterized by an unusually high spirochete density in the blood.[387] Bourbon virus, the first human thogotovirus discovered in the Western Hemisphere,

was isolated from a postmortem specimen in Kansas in 2014.[388] It was initially suspected to be Heartland virus, a tick-borne phlebovirus virus discovered in Missouri in 2009.[389] Both viruses have been identified in *Amblyomma americanum,* the lone star tick, common in wooded and scrub areas in much of the eastern and midwestern regions of the United States, where it can also transmit species of *Ehrlichia.*

The range of Rocky Mountain spotted fever (*Rickettsia rickettsiae*) extends through much of North America, where the most common vectors are *Dermacentor variabilis,* the American dog tick, and *D. andersoni,* the Rocky Mountain wood tick. Recently, however, especially pernicious foci have been found on Native American lands in Arizona, where *Rhipicephalus sanguineus,* which feeds on both humans and dogs, is the vector. The close association between people and their dogs is the route of transmission, and a program to collar dogs with insecticide bands significantly reduced incidence.[390] There is increasing evidence that a significant proportion of reported *R. rickettsiae* cases are in fact *Rickettsia parkerii,* which presents with nearly identical symptoms and responds equally well to doxycycline, but has a much lower mortality when untreated.[391]

EBOLA AND MARBURG HEMORRHAGIC FEVERS

Ebola and Marburg viruses cause rare, severe, and often-fatal diseases in humans and nonhuman primates (monkeys, gorillas, and chimpanzees). Symptoms include fever, headache, joint and muscle aches, sore throat, and weakness, followed by diarrhea, vomiting, and stomach pain. Some patients also exhibit a rash, red eyes, hiccups, and internal and external bleeding. There is no licensed vaccine or standard treatment for Ebola or Marburg, and supportive therapy with fluid and electrolytes replacement, maintaining oxygen status and blood pressure, and providing treatment for any complicating infections remain the primary treatment.[392,393] Hemorrhagic fever outbreaks typically result from a single or small number of spillover events from the virus reservoir with subsequent chains of human-to-human transmission.[394] Prior to the 2014–16 outbreak of Ebola in West Africa, it was thought that transmission in health care settings could be prevented by adherence to basic infection control practices.[395] Experience gained during the outbreak response, including experience with "super-spreading" situations such as burial ceremonies, led to the adoption of more stringent control measures regarding use of personal protective equipment and disposal of potentially infectious items.[396]

The causative agents of Ebola and Marburg hemorrhagic fevers are filoviruses, belonging to the Filoviridae. These viruses are associated with fruit bats, which may be their natural animal reservoirs.[397–400] Marburg virus was first detected in 1967, when 31 cases (7 fatal) occurred in Germany and Yugoslavia among laboratory workers handling tissues from African green monkeys.[401] Eight years later, in 1975, a traveler returning from Rhodesia (now Zimbabwe) died in a hospital in Johannesburg, South Africa; his traveling companion and a nurse subsequently became ill, although both survived.[402] During the 1980s, two cases of Marburg hemorrhagic fever were reported in visitors to Kitum Cave in Mount Elgon National Park, Kenya.[403,404]

Ebola virus was first detected in 1976 as the cause of outbreaks with high fatality rates in Zaire (now the DRC)[405] and the Sudan.[406] The Ebola species associated with the 1976 outbreaks are Ebola virus and Sudan virus (formerly Ebola-Zaire and Ebola-Sudan). Other Ebola species that cause human disease include Tai Forest virus (isolated in 1994 when a scientist became ill after conducting an autopsy on a chimpanzee from the Tai Forest in Cote D'Ivoire)[407] and Bundibugyo virus (isolated in 2007 during an outbreak in Uganda).[408] A case of Sudan virus infection was reported in the United Kingdom in 1976 in a laboratory worker infected via the accidental stick of a contaminated needle,[409] and a case of Ebola virus infection was reported in South Africa in 1996 in a physician who had traveled to Johannesburg after treating Ebola virus–infected patients in Gabon (the site of three Ebola outbreaks during the 1990s). The physician survived, but a nurse who took care of him became infected and died.[410] A fifth species of Ebola was identified in Reston, Virginia, as the cause of severe illness and death in Philippine monkeys imported by research facilities in the United States in 1989 and 1990[411,412] and Italy in 1992.[413] In 2008, Reston virus was detected in pigs on two farms in the Philippines.[414] Six workers from the pig farm and from a slaughterhouse developed antibodies but did not become ill.

Since 2000, outbreaks of Ebola have recurred multiple times in central and East Africa, in the DRC, in Sudan, and in Uganda,[414a] and Marburg outbreaks have occurred in the DRC, Angola, and Uganda.[414b] The first Ebola outbreak in West Africa—and the largest outbreak of Ebola to date—began in Guinea and spread to neighboring Sierra Leone and Liberia, caused by the Ebola virus.[415,416] By the time it was controlled in the spring of 2016, it had caused more than 28,000 cases and 11,000 deaths in these three countries.[417] It was the first time Ebola transmission occurred in major urban settings. Secondary outbreaks occurred in Nigeria, Senegal, Mali, and the United States, and infected health care workers from Spain, Italy, the United Kingdom, and the United States were evacuated to their home countries (see also "Factors That Favor Disease Spread" previously). All of the secondary outbreaks were contained, and the epidemic virus did not spread outside of the affected countries in West Africa. The case-fatality proportion for patients managed in biocontainment units in Europe and the United States was 18.5% (versus 40%–70% in West Africa), highlighting the importance of critical care and advanced organ support.[418] The index case of the secondary outbreak in the United States was a hospitalized traveler from Liberia whose illness was initially misdiagnosed[44]; lessons learned from that experience led to improved local awareness and better understanding of infection control needs in hospitals.[392,393,396]

In addition to devastating health effects, Liberia, Sierra Leone, and Guinea experienced significant losses in gross domestic product.[419] An unprecedented international response was mounted to help control the outbreak, and trials were set up to evaluate promising therapeutics and vaccines, which advanced our toolbox to deal with Ebola. Helpful data on safety and immunogenicity of one of two vaccines was generated, but did not yield definitive efficacy results because the outbreak was waning.[420–423] With 17,000 Ebola survivors from the 2014–16 outbreak, long-term complications of joint and vision problems were observed.[424] It was also learned that the virus can persist in some body fluids, including semen, indicating potential risk of sexual transmission.[425–427]

Public health capacity building in West Africa to address viral hemorrhagic fevers, both during and after the Ebola outbreak, has included a focus on emergency response, laboratory capacity, disease surveillance, and workforce development.[428–431] Sustained efforts are needed to improve regional disease surveillance and to develop and test additional tools for prevention, early diagnosis, and treatment; clinical investigation during outbreaks is critical to evaluate vaccines and therapeutics and optimize clinical care and outcomes. Ecologic investigations of Ebola and Marburg viruses are also needed to better understand the natural reservoirs of these viruses and how they emerge and spread.

In May 2018, an Ebola vaccine developed during the outbreak in West Africa was deployed under a research protocol to help control an outbreak in Central Africa—in the DRC—that by May 2019 had infected 1600 people and claimed 1069 lives.[431a] Efforts to control that outbreak were hampered by violence and civil strife that limited responders' ability to provide treatment and immunization to affected communities.

CONCLUSION: CONTROLLING THE THREATS

Major infectious threats such as the AIDS pandemic, the 2003 SARS outbreak, and (more recently) the West Africa Ebola outbreak and the Zika virus epidemic in the Americas shock the world and spur renewed efforts to improve capacity to detect and control emerging threats. Efforts directed at emerging diseases must work in concert with initiatives to reduce high-burden infections that account for the majority of disease and disability caused by microbial agents. Each emergent event teaches particular lessons: HIV's emergence was long and insidious; Ebola's was explosive and logistically complex; Zika's dangers were unappreciated too long. In all cases, however, the most critical advantage would have been early detection of the initial cases of disease.

During the 1990s, the global devastation caused by AIDS, which spread undetected for many years, led nations around the world to address emerging diseases as a major diplomatic issue. In summit

TABLE 14.5 Global Health Initiatives to Address Emerging Infectious Diseases

The emergence and international spread of HIV/AIDS in the 1980s and 1990s led to the establishment of global health initiatives that address infectious diseases of international concern. They include:

1990s

- The Joint United Nations Program on HIV/AIDS (UNAIDS; http://www.unaids.org/)
- RBM Partnership to End Malaria (https://endmalaria.org/)
- Stop TB Partnership (http://www.stoptb.org/)
- International Partnership Against AIDS in Africa (IPAA; https://www.un.org/ga/aids/ungassfactsheets/html/fsipaa_en.htm)

2000s

- United Nations Development Programme (UNDP) Millennium Development Goals (2000–2015), including Goal 6: Combat HIV/AIDS, malaria, and other diseases (https://www.un.org/millenniumgoals/)
- Global Alliance for Vaccines and Immunization (Gavi; https://www.gavi.org/)
- Global Health Program, Bill and Melinda Gates Foundation (https://www.gatesfoundation.org/)
- Global Fund to Fight AIDS, Tuberculosis and Malaria (https://www.theglobalfund.org/en)
- International Health Regulations (IHR 2005; https://www.who.int/topics/international_health_regulations/en/)
- United States President's Emergency Plan for AIDS Relief (PEPFAR; https://www.pepfar.gov/)
- U.S. President's Malaria Initiative (PMI; https://www.pmi.gov/)
- One Health Initiative[a] (https://www.onehealthinitiative.com/)
- Emerging Pandemic Threats Program, USAID (https://www.usaid.gov/news-information/fact-sheets/emerging-pandemic-threats-program)

2010s

- Global Health Security Agenda (GHSA) partnership (https://www.ghsagenda.org/)
- UNDP Sustainable Development Goals (2016–2030), including Goal 3: Health (https://www.who.int/topics/sustainable-development-goals/targets/en/)
- Coalition for Epidemic Preparedness Innovations (CEPI; https://cepi.net/)
- U.S. National Strategy for Combating Antibiotic-Resistant Bacteria (CARB; https://www.cdc.gov/drugresistance/us-activities/national-strategy.html) issued in 2014, followed in 2015 by:
  - Publication of a US National Action Plan for Combating Antibiotic-Resistant Bacteria (https://www.cdc.gov/drugresistance/us-activities/national-action-plan.html)
  - Establishment of a Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB; https://www.cdc.gov/drugresistance/us-activities/paccarb.html)
- World Health Assembly endorsed a Global Action Plan on Antimicrobial Resistance in 2015 (https://www.who.int/antimicrobial-resistance/global-action-plan/en/)
- Meeting of global leaders at the United Nations General Assembly in New York in September 2016 to commit to fighting antimicrobial resistance together (https://www.who.int/antimicrobial-resistance/events/UNGA-meeting-amr-sept2016/en/)

[a]The One Health Initiative began as the One Health Initiative Team (2006–2015), which established a One Health Initiative website in 2008 (http://www.onehealthinitiative.com/news.php?query=History+of+the+One+Health+Initiative+team+(April+2006+through+September+2015)+and+the+One+Health+Initiative+website+since+October+1,+2008).

meetings in 1998 and 2000, the leaders of the Group of Eight industrialized nations pledged support for improving global surveillance for emerging threats, as well as for reducing deaths from "diseases of poverty" and vaccine-preventable diseases.[432] Global health initiatives such as the Bill and Melinda Gates Foundation, the Global Alliance for Vaccines and Immunization (now called Gavi, the Vaccine Alliance), and the Global Fund to Fight AIDS, Tuberculosis and Malaria were created to advance these goals (Table 14.5).

In the aftermath of the 2003 SARS outbreak, which spread overnight from Hong Kong to Canada via airplane travelers, the global public health community finalized the 2005 International Health Regulations (IHR 2005) as an international treaty that requires identification, reporting, and control of emerging threats.[432a] IHR 2005 requires member nations to report all Public Health Emergencies of International Concern (PHEICs) to the WHO, including not only outbreaks of infectious diseases but all biological, chemical, or radionuclear threats and natural disasters. IHR 2005 also requires prompt reporting of a single case of smallpox; poliomyelitis due to wild-type poliovirus; SARS; and human influenza caused by a new subtype. Although its primary goal is to improve national capacities to control threats at their source, IHR 2005 also encourages participation in international systems such as the WHO Global Outbreak Alert and Response Network (GOARN), which provides technical assistance during outbreak investigations, and the GISRS,[191] which tracks influenza viruses with pandemic potential (see "Influenza A and Pandemics" earlier). In 2014, government and nongovernment donors established the Global Health Security Agenda (GHSA) partnership, which provides guidance and training to help countries meet IHR requirements (https://www.ghsagenda.org/).

Containing and stopping the Ebola hemorrhagic fever epidemic in West Africa, which resembled historic outbreaks of pneumonic plague in its rapid person-to-person spread and high fatality rate, was especially challenging in a globalized world. Stopping transmission required close and complicated coordination among affected countries and their neighbors, donors, and international nongovernmental organizations. In the United States, lessons learned about coordination and continuity of outbreak operations informed the creation of the CDC Global Rapid Response Team (GRRT), which can be rapidly mobilized for extended missions and works in close partnership with GOARN.[433] A commitment was also made to help build public health capacity in high-risk countries, including the affected countries in West Africa.[434]

The outbreak also showed the importance of having plans and funds in place to develop and validate vaccines and identify the best available treatments while an outbreak is in progress.[420,435] The experience in West Africa led to calls for a global vaccine development fund[436] and for the creation of public-private research partnerships like the Coalition for Epidemic Preparedness Innovations (CEPI; Table 14.5), which aims "to stop future epidemics by developing new vaccines for a safer world" (https://cepi.net/). Efforts are also underway to develop universal influenza vaccines—vaccines that protect against all or most influenza viruses—as well as vaccines with enhanced immune protection and faster production in case of a pandemic.[437]

Other lessons include the need for greater innovation and investment to address vector-borne diseases such as Zika[380] (i.e., through improved vector control methods and enhanced expertise in entomology) and zoonotic diseases such as SARS and Ebola (i.e., through One Health approaches to monitoring infectious diseases in humans and animals).[438] Like the MERS epidemic, the SARS and Ebola epidemics were initiated by zoonotic pathogens that had gained capacity for human-to-human transmission, as had happened with HIV. Rapid detection of epizootic outbreaks in animals can forestall or mitigate human spillover, as has been demonstrated for influenza[439] and plague.[440] However, for obscure and emerging pathogens the surest warnings come from sensitive human surveillance, which is often poor in the places most receptive to emergence.[441]

In the future, technical advances in bioinformatics and next-generation sequencing will improve pathogen discovery[442] and eventually allow for earlier and more definitive identification of the causative agents of outbreaks, whether due to emerging pathogens, drug-resistant pathogens, or acts of bioterrorism. Public health laboratories in the United States are transitioning to rapid molecular methods with technical assistance from the CDC's Advanced Molecular Detection initiative.[442] As part of this transition, PulseNet and PulseNet International laboratories are using WGS to speed detection of outbreaks of foodborne diseases by linking cases of enteric disease.[443,444] Eventually diagnostic laboratories will likely further transition to metagenomic methods in which genetic material is recovered directly from clinical samples, without prior isolation (as needed for WGS), and analyzed by computer algorithms that rapidly identify pathogen sequences.

Further advances in data science that facilitate data-sharing and visualization of complex data will enable earlier recognition of emerging problems and better coordination of response efforts, especially in remote areas. Early warning systems such as the Program for Monitoring Emerging Diseases (ProMED-mail; https://www.promedmail.org/),[445] the Public Health Agency of Canada's Global Public Health Intelligence Network (GPHIN)[446], and HealthMap (https://www.healthmap.org/en/) are collecting, categorizing, and disseminating real-time outbreak

information from formal and informal sources. Internet and social media networks are increasingly being used for rapid exchange of health information and for broadening public health partnerships among nontraditional partners such as law enforcement, the media, and members of the public at local, national, and global levels.

Public health research in areas such as metagenomics[340,447,448] and mathematical modeling of infectious disease dynamics[449] are also moving us toward faster detection and control of emerging threats. Other promising areas include studies that improve understanding of host-pathogen interactions (e.g., microbiome research[450]) and develop tools for treatment and prevention (e.g., new antibiotics, environmentally safe pesticides, and strategies that use genetic manipulation to alter the population dynamics of disease-carrying mosquitoes[451]).

Combining modern innovations in disease surveillance and control with proven measures to reduce the burden of infectious diseases and advance global health equity can have a major impact on global health.

ACKNOWLEDGMENTS

Many thanks to Robin Moseley for coordinating the writing of this chapter, and to Marta Gwinn for her help in drafting Table 14.3 (Examples of Human Genetic Variants That Influence Disease Susceptibility or Severity).

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The complete reference list is available online at Expert Consult.

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15 Bioterrorism: An Overview

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Exposures to biological agents, such as microbes and toxins, are usually unintentional. Notably, these agents can be used as weapons to cause intentional harm to humans, animals, or plants as well as to contaminate the environment. Unlike nuclear, radiologic, chemical, and explosive threat agents, the diseases and conditions caused by biological agents as well as their treatment could be encountered during the practice of medicine. Similarly, the surveillance of and response to these agents is within the purview of many public health systems. This feature of encountering these biological agents in practice highlights the importance of understanding which of the biological agents are associated with the greatest health consequences (Table 15.1) both for the individual and for the public, when to suspect their presence, how to diagnose them, and what epidemiologic clues may suggest that the case(s) identified may have been caused intentionally.[1]

The anthrax letter attacks that occurred in the United Sates in 2001 highlighted the need for improvements in diagnosis, detection, treatment, preparedness, and response by clinicians, health care systems, and public health professionals.[2] Subsequently, guidelines for the medical management of anthrax and other high-consequence biological agents, in deliberate use settings, have continued to improve, reflecting our increased understanding of these pathogens (Table 15.2).

DEFINING BIOLOGICAL WARFARE, BIOTERRORISM, AND BIOCRIMES

Some confusion exists over how to categorize incidents associated with the intentional use of biological agents (pathogens and toxins) to cause harm. Although there is no universally accepted definition of these three terms, the underlying motivation and objectives for intentional use of these biological agents has been suggested as a way to distinguish biological warfare, bioterrorism, and biocrimes. Descriptions of each may help guide clinicians and public health professionals in determining when interactions with law enforcement personnel may be anticipated.

Bioterrorism is commonly viewed as an action by a non–state actor to achieve a political, ideological, or religious goal.[3] Implied in the word *bioterrorism* is the desire to terrorize as much or more than causing casualties. A biocrime, on the other hand, occurs when a biological agent is used by a person or group against an individual or small group, often for revenge or extortion, in the absence of an underlying ideology; the perpetrator(s) are often believed to have underlying psychopathology. Biological warfare occurs when a state actor uses a biological agent as part of its armamentarium in waging war. Importantly, biological agents have been used intentionally in all three settings—bioterrorism, biocrime, and biological warfare.

HISTORY OF BIOLOGICAL WEAPONS DEVELOPMENT AND USE IN WARFARE AND AGAINST INSURGENCIES

Proven biological weapons use by countries (or state actors) to wage warfare against other states as well as internally against insurgents has been documented since the 18th century and has been purported to have occurred prior to the "germ theory." However, there is no reliable evidence that previously alleged use of biological agents in war occurred in the ancient world or in medieval times, including use of *Yersinia pestis,* although plague outbreaks were identified during periods of conflict.[4] The scale of programs and manner of use of biological weapons varied over time. Increasing sophistication of state-run programs was noted beginning in World War I (WWI) through termination of offensive programs by most countries in the 20th century.

Pre–World War I Biological Weapons Development and Use

The first well-documented use of biological weapons occurred in 1763 when the British used contaminated blankets and clothing in an attempt to infect Delaware tribe Native Americans with smallpox[5]; there is no evidence of a successful outcome.[6] A similar attempt to use fomites to cause disease deliberately was documented during the American Civil War when a Confederate physician attempted to spread yellow fever (before its vector-borne transmission was known) to populations living in Union cities through the introduction of contaminated clothing and bedding.[7] It was not until the latter half of the 19th century that high-quality microscopes of good optical quality, underpinning the new science of bacteriology, and the work of Louis Pasteur and Robert Koch made modern biological warfare possible.

Development and Use of Biological Weapons During World War I

During WWI, Germany had the first documented state-run biological weapons program (Table 15.3). It began in 1915, with documented use of anthrax against animals being shipped for use by Allied forces. France had the second documented biological weapons program (see Table 15.3).[8] In 1925, following WWI, the Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases and of Bacteriological Methods of Warfare (also known as the Geneva Protocol),[9] was drawn up and signed under the auspices of the League of Nations and entered into force as a treaty in 1928. Although the Geneva Protocol, which had been officially agreed to by 137 State Parties as of March 15, 2013,[10] banned the use of biological weapons, it did not proscribe the research, production, or possession of biological weapons, and many of the State Parties to the treaty reserved the right to retaliate in kind should they or their allies be attacked. In addition, no provision was made for verification, and compliance was voluntary. A number of countries agreeing to the Geneva Protocol began or continued biological weapons programs after signing the treaty, including Canada, France, Germany, Italy, Japan, Poland, the United Kingdom, and the Soviet Union (see Table 15.3). The United States did not ratify the Geneva Protocol until 1975, after it had ended its biological weapons program.[8]

Development and Use of Biological Weapons During World War II

Seven countries had biological weapons programs during World War II (WWII): Canada, France, Hungary, Italy, Japan, the Soviet Union, the United Kingdom, and the United States (see Table 15.3). Among these seven countries, only Japan waged biological warfare during WWII. Its biological weapons program—organized at its Unit 731 and based in Harbin, China—used multiple biological agents and employed more than 3000 scientists plus smaller units at a number of other sites in China, which were under Japanese occupation. More than 10,000

[a]The views expressed in this chapter do not necessarily represent the views of the United States government.

TABLE 15.1 Bioterrorism Agents/Diseases Posing a Risk to National Security, by Priority Category

Category Designation	A	B	C
Priority/Consequence	1	2	3
Definition	• Easily disseminated *or* easily spread person-to-person AND • High case fatality rate and potential serious public health impact AND • May cause social disruption or panic AND • Requires deliberate and special action for public health preparedness	• Moderately easy to disseminate AND • Low case fatality rate but moderate morbidity rate AND • Requires enhanced disease surveillance and specific enhancements to the CDC's diagnostic capacity	• Could be engineered for widespread dissemination due to: • Availability • Ease of production and dissemination AND • High case fatality rate and morbidity rate potential
Disease Agent	Anthrax (*Bacillus anthracis*) Botulism (*Clostridium botulinum* toxin) Plague (*Yersinia pestis*) Smallpox (*Variola major*) Tularemia (*Francisella tularensis*) Viral hemorrhagic fevers: Filoviruses (Ebola virus, Marburg virus) Arenaviruses (Lassa virus, Machupo virus)	Brucellosis (*Brucella* species) Unknown pathogenesis in humans (*Clostridium perfringens* types B or D epsilon toxin[a]) Foodborne safety threats: *Salmonella* species *Escherichia coli* O157:H7 *Shigella* species Glanders (*Burkholderia mallei*) Melioidosis (*Burkholderia pseudomallei*) Psittacosis (*Chlamydia psittaci*) Q fever (*Coxiella burnetii*) Ricin intoxication/poisoning[b] (*Ricinus communis* toxin [from castor beans]) SEB intoxication/poisoning[c] (*Staphylococcus aureus* enterotoxin B) Typhus fever (*Rickettsia prowazekii)* Viral encephalitis (Alphaviruses [e.g., EEE, VEE, WEE]) Water safety threats: *Vibrio cholera* *Cryptosporidium parvum*	Engineered pathogens or emerging pathogens that could be engineered and could cause various diseases/syndromes: Nipah virus Hantavirus species

[a]Produces fatal enterotoxemia in ruminant animals but no data to support human disease. Concern for humans is theoretical based on animal data, particularly if exposed to aerosolized toxin (see reference 43).
[b]Ricin intoxication-related manifestations are dependent upon the route of exposure: inhalation vs. injection vs. ingestion vs. skin vs. eye (see reference 42).
[c]SEB intoxication-related manifestations are dependent upon dose and route of exposure, which have an impact on disease expression (see reference 44).
CDC, Centers for Disease Control and Prevention; *EEE,* eastern equine encephalitis; *SEB,* staphylococcal enterotoxin B; *VEE,* Venezuelan equine encephalitis; *WEE,* western equine encephalitis.
Modified from Centers for Disease Control and Prevention. Emergency preparedness and response: bioterrorism agents/diseases; 2017. https://emergency.cdc.gov/agent/agentlist-category.asp. Accessed November 15, 2017.

prisoners died as a result of experimental infections or execution after experimentation.[11] Significant adverse impact among the civilian populations of at least 11 cities in China was recorded following Japanese attacks using various delivery mechanisms to deliver *Bacillus anthracis, Vibrio cholera, Shigella* spp., *Salmonella* spp., and *Y. pestis.*[11] Additionally, during the war, Polish resistance fighters used biological agents as weapons for assassination.[4]

Biological Weapons in the Post–World War II Period Through the End of the Cold War

Revelations of the successful use of biological weapons by the Japanese during WWII led several countries to expand their programs and others to create them; 11 countries had a biological weapons program at some time following WWII (see Table 15.3). In the post-WWII period, three new programs in Israel,[12] Rhodesia,[13] and South Africa[14] used biological agents on a small scale, with limited, if any, proven consequences. Use by the governments of Rhodesia and South Africa were internally directed against insurgents seeking regime change.[13,14]

In the post-WWII period, the only large biological weapons programs were found in the Soviet Union and the United States; the former is estimated to have been 10 times the size of the latter and much larger than both the Iraqi (1000 times the size) and South African (10,000 times the size) programs.[15] The US program was terminated in 1969; it is unknown if Russia has a current biological weapons program.

Offensive Biological Weapons Programs in the United States

Although the biological weapons program operated by the Soviet Union was the largest in the world, the US offensive biological weapons program and toxin weapons program, before termination by President Richard Nixon in 1969[16] and 1970,[17] respectively, were the most scientifically sophisticated. Human, animal, and plant pathogens were studied; human disease–causing pathogens assessed were *B. anthracis,* botulinum toxins, *Francisella tularensis, Brucella suis, Coxiella burnetii,* staphylococcal enterotoxin B, and Venezuelan equine encephalitis. Technical advances were made that permitted large-scale fermentation and storage of biological warfare agents as well as optimal aerosol dissemination techniques, although no new discoveries regarding the physics of aerosol particles were made by the US program.[18] Rather, the focus was on identifying optimal battlefield dissemination techniques to create extensive clouds of high concentration using small amounts of a biological agent.[18]

In an effort to learn more about how to protect against these agents under "field conditions," the US Department of Defense conducted a series of classified biological warfare and vulnerability tests from 1962 through 1973.[19] Both land-based and ship-based tests were conducted under the name Project 112/SHAD.[19] Humans, plants, and animals were all potentially exposed to biological agents, chemical agents, biological stimulants, decontaminants, or tracers; approximately 6000 service members were possibly exposed, but no long-term exposure health effects were identified.[19,20]

TABLE 15.2 Management Guidelines and Recommendations for the Management of Disease Caused by High-Consequence Bioterrorism Threat Agents

CONDITION	MANAGEMENT GUIDELINE OR RECOMMENDATION	YEAR PUBLISHED	ON-LINE LINK
Anthrax[b,c,d,e]	Clinical Framework and Medical Countermeasure Use During an Anthrax Mass-Casualty Incident	2015	https://www.cdc.gov/mmwr/pdf/rr/rr6404.pdf
Botulism	Botulism: Information for Health Professionals	2017	https://www.cdc.gov/botulism/health-professional.html
Ebola virus disease	Ebola Virus Disease (EVD) Information for Clinicians in US Healthcare Settings	2016	https://www.cdc.gov/vhf/ebola/healthcare-us/preparing/clinicians.html
Glanders (and melioidosis)	Workshop on Treatment of and Postexposure Prophylaxis for *Burkolderia pseudomallei* and *B. mallei* Infection, 2010	2012	https://wwwnc.cdc.gov/eid/article/18/12/12-0638_article
Plague	Plague: Resources for Clinicians	2015	https://www.cdc.gov/plague/healthcare/clinicians.html
Smallpox[f]	Clinical Guidance for Smallpox Vaccine Use in a Postevent Vaccination Program	2015	https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6402a1.htm?s_cid=rr6402a1_w

[a]Guidelines and recommendations of the Centers for Disease Control and Prevention. Health care provider bioterrorism training modules and video webcasts can be found at https://emergency.cdc.gov/bioterrorism/training.asp.
[b]Mass casualty: Bower WA, Hendricks K, Pillai S, et al. Clinical framework and medical countermeasure use during an anthrax mass-casualty incident. *MMWR Recomm Rep.* 2015;64(No. RR-4):1–22.
[c]Anthrax prevention and treatment in adults: Hendricks KA, Wright ME, Shadomy SV, et al. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. *Emerg Infect Dis.* 2014;20:e130687.
[d]Pediatric anthrax guideline: Bradley JS, Peacock G, Krug SE, et al. Pediatric anthrax clinical management. *Pediatrics.* 2014;133:e1411–e1436.
[e]Considerations in pregnant women: Meaney-Delman D, Zotti ME, Creanga AA, et al. Special considerations for prophylaxis for and treatment of anthrax in pregnant and postpartum women. *Emerg Infect Dis.* 2014;20:e130611.
[f]Smallpox vaccine use guideline: Petersen BW, Damon IK, Pertowski CA, et al. Clinical guidance for smallpox vaccine use in a postevent vaccination program. *MMWR Recomm Rep.* 2015;64(No. RR-2):1–26.

TABLE 15.3 Known Biological Weapons Programs, by Region, Country, and Functioning Program Dates

REGION	COUNTRY	FUNCTIONING PROGRAM DATES
Africa (sub-Saharan)	Rhodesia[a]	1977
	South Africa	1981–1993
The Americas	Canada	1940–1958
	United States of America	1941–1971
Asia	Japan	1934–1945
Europe	France	1915–1916 1922–1926 1934–1940 1947–1966 or 1967
	Germany	1915–1918
	Italy	1934–1940 or 1943
	Poland	Unknown[b]
	Soviet Union[c]	1918–1991[c]
	United Kingdom	1940–1957
Middle East[d]	Egypt	1960s–?[e]
	Iraq	1974–1978 1981–1991
	Israel	1948–?[e]
	Syria	1970s–?[e]

[a]Name changed to Zimbabwe with independence.
[b]Polish scientists fleeing German takeover in World War II admitted to destroying program elements.
[c]Does not apply to current-day Russia.
[d]Includes Iran, Asia Minor, Mesopotamia, the Levant, the Arabian Peninsula, and Egypt (15 countries and Palestine).
[e]Probable continuing program; other probable biological weapons programs exist in China, Iran, and North Korea.
Data from Carus WS. A Short History of Biological Warfare: From Pre-History to the 21st Century. *Center for the Study of Weapons of Mass Destruction, Occasional Paper, No. 12. Washington, DC: National Defense University Press; 2017.*

LIMITING USE OF BIOLOGICAL WEAPONS: THE BIOLOGICAL WEAPONS CONVENTION

Over time there was increasing international concern that the Geneva Protocol did not ban the research, production, or possession of biological weapons and lacked the means to verify adherence by signatory countries. In 1969, draft proposals for a new protocol were submitted to the Committee on Disarmament of the United Nations (UN).[21] Then in 1972, the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological and Toxin Weapons and on their Destruction—commonly referred to as the Biological Weapons Convention (BWC)—was opened for signature.[22] State Parties to the BWC—which entered into force in 1975—are obligated to not develop, produce, stockpile, or otherwise acquire or retain microbial or other biological agents or toxins of types and in quantities that have no justification for prophylactic, protective, or other peaceful purposes. They may not use any means of delivery of such agents or toxins for hostile purposes and must take necessary measures to prohibit or prevent such activities in their territories. Further, State Parties should destroy or divert to peaceful purposes all agents, toxins, weapons, equipment, and means of delivery and not transfer to any recipient or in any way assist, encourage, or induce to manufacture or otherwise acquire biological agents, toxins, weapons, equipment, or means of delivery. A total of 196 State Parties may become members of the BWC. As of September 2017, 179 State Parties have acceded to or ratified the treaty.[23] The BWC does not explicitly address non–state actors, such as terrorists or criminals.

Monitoring and Compliance Under the Biological Weapons Convention

There is no formal verification protocol to monitor compliance. Accordingly, verifying adherence to and compliance with the BWC by State Parties has proven challenging.

Civilian Research in the Soviet Union

The Soviet Union became a State Party to the BWC in 1975. Little is known of the country's biological weapons efforts from the end of WWII to the early 1970s. In 1973, a large, science-based research and development program to address offensive issues related to biological agents,

called Biopreparat, was created in the civilian sector, complementary to a less sophisticated program in the Ministry of Defense.[24,25] In the late 1980s and early 1990s serious concerns arose regarding the biological weapons capability of the Soviet Union,[21] heightened by information provided by defectors and revelations by program scientists after the fall of the Soviet Union describing a biological weapons program that was far more extensive and sophisticated than anyone had imagined at the end of the Cold War.[26,27] Biopreparat was a system of 18 nominally civilian research laboratories employing approximately 30,000 staff prior to the fall of the Soviet Union; research was undertaken on biological agents, often at the request of the Ministry of Defense.[28] One of the larger and more sophisticated of these laboratory centers, the State Research Center of Virology and Biotechnology (VECTOR), continues to function in a role stated to be similar to that of the US Centers for Disease Control and Prevention (CDC).

Military Activities in the Soviet Union

Another facility of concern was the Soviet Union's principal production center for smallpox virus located near Moscow, at Sergiev Posad. It was reportedly able to produce upwards of 20 tons of smallpox virus annually, primarily for delivery via intercontinental ballistic missile as a strategic weapon.[27] Currently, the site houses the Russian Federation's Ministry of Defense Microbiology Scientific Research Institute, a laboratory research complex known to maintain a national collection of dangerous pathogens, including Ebola, Marburg, and Lassa viruses.[29] In 1992, Russian officials confirmed the existence of a biological weapons program it had inherited from the Soviet Union and committed to dismantling it.[30] The dissolution of the Soviet Union in 1991 and the halting of the inherited Soviet offensive biological weapons program by Russian President Yeltsin in 1992 resulted in profound reductions in Biopreparat funding and personnel, raising concerns that former biological weapons scientists may sell their expertise to states or groups seeking such knowledge.[31] In 2000 it was estimated that approximately 15,000 Biopreparat scientists remained employed within the system, which could pose a proliferation risk.[32]

Iraq's Biological Weapons Program

Another example that illustrates the challenges of verifying compliance to the BWC is the case of Iraq, which signed the BWC in 1972. After the first Gulf War (Operation Desert Storm) in 1991, UN Security Council Resolution 687 established, among other things, the UN Special Commission (UNSCOM) to carry out on-site inspections of Iraq's biological and chemical weapons and missile capabilities and to oversee their destruction or removal, or procedures to render them harmless.[33] In April 1991, under its initial declaration (as required under UN Resolution 687), Iraq declared that it did not have a biological weapons program and accepted Resolution 687; in August 1991 it declared to the first Biological Weapons Inspection Team that it had conducted only "biological research activities for defensive military purposes."[34] It was not until July 1995, after 4 years of UNSCOM investigations and "in the light of irrefutable evidence," that Iraq admitted for the first time that it had an offensive biological weapons program.[34] Notably, Iraq initially denied creating weapons for use, but a defector with responsibility for Iraq's program, General Hussein Kamel, revealed to UNSCOM the full extent of the Iraqi program upon arrival in Jordan in August 1995. Subsequently, Iraq admitted to "a far more extensive biological warfare programme" than previously admitted, including weaponization.[34]

HISTORY OF BIOLOGICAL WEAPONS USE TO COMMIT ACTS OF BIOTERRORISM

Non–state actors, including individuals and groups (e.g., terrorist groups, criminal networks), are not explicitly covered under the BWC. They present a unique, complex, and growing challenge with respect to the development and use of biological weapons. During the 20th century, confirmed or threatened use of biological weapons against humans was more common by criminals than by terrorists. However, use by both types of these non–state actors increased over this 100-year period.[3] Additionally, in the first years of the 21st century, one confirmed use of a biological agent, with human health outcomes, in an act of bioterrorism occurred in the United States.[2] Multiple subsequent deliberate hoaxes using nonpathogens followed this latter bioterrorism incident, and all have been classified as crimes.[35]

During the 20th century there were 15 confirmed incidents during which a biological weapon was used by non–state actors with resulting adverse human health effects.[3] Six events (40%) occurred in the final 2 decades of the century, one of which was due to bioterrorism, compared with an average of one incident in each of the preceding 8 decades. The biological agents were delivered in food in nine (60%) of the confirmed intentional incidents, five (33%) by injection, and one by an unknown delivery mechanism; all but one of the perpetrators was trained or practicing in a health-related field (Table 15.4). There was only one event associated with the use of a highest consequence pathogen, referred to as a CDC Category A agent (see Table 15.1); nine involved use of a CDC Category B agent, and five were not on the CDC "ABC" list of bioterrorism agents.

20th-Century Bioterrorism Against Humans: The Rajneeshee Cult

The only confirmed terrorist use of a biological agent against humans in the 20th century occurred in 1984.[37] In 1990, a second alleged case was perpetrated by a Japanese cult, the Aum Shinrikyo, but without documentary evidence of use.[3] In the 1984 incident, members of a religious cult, the Rajneeshees, in an effort to influence a local county election, aimed to incapacitate opposition voters by deliberately contaminating salad bars located along a stretch of an Oregon interstate highway with *Salmonella typhimurium* (now *Salmonella enterica* subsp. *enterica* serovar Typhimurium), a CDC Category B agent (see Table 15.1 and Table 15.3). The pathogen was legally obtained by the cult's health care system and prepared by a cult member, who was a health care provider who also had laboratory skills. Although there were no deaths among the 751 persons who became ill, 45 were hospitalized.[37] Importantly, the public health investigation of this incident in 1984 initially failed to determine how the salad bars became contaminated. It was not until 1 year after the outbreak that dissension among the perpetrators led law enforcement officials to discover that the contamination was deliberate with the ultimate goal of disrupting the local election.[3] This episode illustrates the difficulty in differentiating an ordinary foodborne outbreak from a small-scale biological weapons attack conducted by non–state actors.

21st-Century Bioterrorism Against Humans: The Anthrax Letters

Since the commencement of the 21st century, there has only been one successfully executed bioterrorism incident. Notably, some experts classify it as a crime rather than an act of terrorism due to assessed motivation by the alleged perpetrator. In late September and October 2001, multiple letters containing *B. anthracis* spores were sent through the US mail, targeting members of Congress and the media, resulting in 22 cases of anthrax—11 inhalational and 11 cutaneous.[2] Five of the victims died. A key concept is the health care provider as a first responder. Physicians provided the initial evaluation that led to diagnosis and needed treatment. Public health professionals responded to the situation with the outbreak investigative skills needed. When a deliberate etiology of the outbreak was suspected, law enforcement expertise was required to identify the perpetrator(s) and to stop further attacks, if possible.

Over the subsequent 8 years, law enforcement carried out the so-called Amerithrax Investigation.[38] This was a complex, expensive, and lengthy investigation conducted mainly by the Department of Justice's Federal Bureau of Investigation (FBI) and the US Postal Inspection Service. Although a number of persons initially were investigated as possible perpetrators, there were two major persons of interest, both with work experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID).[38] In 2002, the first major suspect, a physician, was subjected to an intensive and wide-ranging investigation but was not indicted when it was determined that there was no documented evidence and insufficient circumstantial evidence to support arrest.[38] The investigation subsequently focused on an anthrax vaccine expert who had worked at USAMRIID since late 1980—a civilian with a doctorate in microbiology—as a person of interest; however, in 2008

TABLE 15.4 Incidents of Confirmed Deliberate Use of Biological Agents to Commit a Crime or as an Act of Bioterrorism, With Resulting Human Morbidity and Mortality, 1900-2017

YEAR	LOCATION	TYPE[a]	MOTIVE	BIOLOGICAL AGENT	MODE OF DELIVERY	CDC CATEGORY[b]	PERPETRATOR[c]
20th Century[d]							
1910	Russia	C	Financial gain	Diphtheria toxin	Injection	NA	Physician
1912	France	C	Financial gain	*Salmonella typhi*	Food	B	Pharmacist
1913	Germany	C	Financial gain	*S. typhi*	Food	B	Not an HCP
1916	USA	C	Financial gain	Several including *S. typhi*	Unclear	B	Dentist
1933	India	C	Financial gain	*Yersinia pestis*	Injection	A	Physician
1936	Japan	C	Revenge	*S. typhi*	Food	B	Physician
1939	Japan	C	Revenge	*S. typhi*; *S. paratyphi*	Food	B	Physician
1964	Japan	C	Revenge	*S. typhi*; *Shigella* spp.	Food	B	Physician
1970	Canada	C	Revenge	*Ascaris suum*	Food	NA	Parasitologist[e]
1984	USA	B	Political	*S. typhimurium*	Food	B	Nurse[f]
1992	USA	C	Revenge	HIV	Injection	NA	Phlebotomist
1993	Netherlands	C	Revenge	HIV	Injection	NA	Unknown
1994	USA	C	Revenge	HIV	Injection	NA	Physician
1995	USA	C	Revenge	Ricin	Food	B	Physician
1996	USA	C	Unknown	*Shigella* spp.	Food	B	Laboratory technician
21st Century[g]							
2001	USA	B[h] vs. C[h]	Political vs. – Revenge – Career preservation	*Bacillus anthracis*	Aerosol (primary) Cutaneous (secondary)	A	Research microbiologist and anthrax vaccine expert

[a]Type of deliberate use: B, bioterrorism; C, crime.
[b]CDC Category of Bioterrorism Biological Agents, from highest consequence (Category A) to lowest consequence (Category C) if used in an act of bioterrorism (see Table 15.1).
[c]Perpetrator: Healthcare-related training or experience.
[d]Twentieth century data from Carus W. *Bioterrorism and Biocrimes: The Illicit Use of Biological Agents since 1900 (Revised).* Washington, DC: Center for Counterproliferation Research, National Defense University; 2001. http://www.fas.org/irp/threat/cbw/carus.pdf. Accessed July 18, 2017.
[e]Graduate student in parasitology.
[f]Family nurse practitioner with additional laboratory skills.
[g]Twenty-first century incident data from United States Department of Justice. Amerithrax investigative summary: released pursuant to the Freedom of Information Act. Washington, DC: US Department of Justice; 2010. http://www.justice.gov/archive/amerithranx/docs/amx-investigative-summary.pdf. Accessed September 15, 2017; and Expert Behavioral Analysis Panel. The Amerithrax case: report of the Expert Behavioral Analysis Panel. Vienna, VA: Research Strategies Network; 2011. http://web.archive.org/web/20110708081506/http://www.dcd.uscourts.gov/dcd/sites/dcd/files/unsealedDoc031011.pdf. Accessed September 20, 2017.
[h]Post facto analysis of unsealed mental health provider notes and documents suggest motives that are more akin to criminal activity. However, statements made to colleagues suggested political or ideological motivation. (See reference 36.)
HCP, Health care professional; *HIV,* human immunodeficiency virus.

he committed suicide before he could be arrested, indicted, and tried.[38] Following this alleged perpetrator's death, the FBI requested a review by the National Research Council of the investigative forensic methods; the panel concluded that there was insufficient scientific evidence to definitively conclude that the anthrax spores came from this microbiologist's laboratory.[39] However, circumstantial evidence collected during the Amerithrax Investigation and the findings of a court-ordered comprehensive postmortem behavioral analysis expert panel[36] led the FBI to conclude that the late microbiologist acted alone and planned and executed the 2001 anthrax attack.[40] The case was then closed and no additional suspects were sought.

Limiting Development and Use of Biological Weapons by Terrorists

In response to increasing concerns regarding the risk that non–state actors might acquire and use biological, chemical, and nuclear weapons and the fact that the BWC does not explicitly address non–state actors, UN Security Council Resolution 1540 was appended to Chapter VII of the UN Charter by unanimous vote on April 28, 2004.[41] Chapter VII sets out the UN Security Council's powers to maintain peace. Resolution 1540 adds the requirement that all member states develop laws with regulatory enforcement measures aimed at preventing the creation, proliferation, delivery, and spread of chemical, biological, and nuclear weapons by non–state actors. The desired intended outcome is to reduce the threat of non–state actors gaining access to and disseminating these weapons. The objectives of this Resolution were reiterated and the mandate was extended under UN Security Council Resolutions 1673, 1810, and 1977, with the Resolution 1540 Committee mandate extended to the year 2021 to ensure full implementation of the original resolution through capacity building and technical assistance.[41]

CLASSIFICATIONS OF BIOLOGICAL AGENTS OF CONCERN

Three systems are used for identifying biological agents that would have potential for highly adverse outcomes for humans, animals, or plants. The first, commonly used by clinicians and public health professionals, is the CDC Category A, B, and C agents (the CDC "ABC" list; see Table 15.1), which helps to prepare for, diagnose/detect, and treat/respond to national security threats posed by biological agents. The

second system, a derivative of the first, is the National Institute of Allergy and Infectious Diseases (NIAID) Category A, B, and C Priority Pathogens.[42] In addition to the CDC "ABC" list, the NIAID list adds pathogens of public health interest that are not necessarily viewed as agents of or diseases likely to be caused by an act of bioterrorism. The list was created as an intramural and extramural program management tool in keeping with the NIAID biodefense and emerging infection missions. The third list of biological agents, the Select Agents and Toxins List, are those that are monitored by the mandated Federal Select Agent Program. The scrutinized agents may be possessed or transferred by nonclinical locations, such as research laboratories, and have the potential to pose a severe threat to the public, animal, or plant health if released. This is a regulatory program with established regulations; program personnel inspect locations in possession of these agents, set compliance standards, and conduct investigations when noncompliance is suspected. The CDC "ABC" list is not the same as the Select Agents list,[43] although many agents are on both lists. The US Department of Health and Human Services (HHS) contribution to the list was formulated based on the following criteria for each agent or toxin: (1) its effect on human health following exposure; (2) its degree of communicability and the route of transmission; (3) the availability and effectiveness of medical countermeasures (MCMs) to treat or prevent it; and (4) other criteria such as needs of vulnerable populations, including children.[44] The Tier 1 select agents on the list are very similar to the CDC Category A agents because these are considered to have the highest potential adverse associated consequences following exposure.

Formulation of the CDC List of Category A, B, and C Agents/Diseases of Bioterrorism

The CDC "ABC" list is the classification system most commonly used by healthcare providers and public health professionals to identify potential bioterrorism threats (see Table 15.1). It reflects the 1999 initial assessment undertaken by a multidisciplinary group of experts and the Working Group on Civilian Biodefense consensus panel.[45] There were two critical inputs to this process. One was a threat assessment to evaluate the likelihood that a particular intentional bioterrorism event will occur given estimated capabilities and intentions of the potential perpetrator(s).[46] This process is fraught with uncertainties.[47] Therefore the second element, a risk assessment, was added to complement the threat assessment. The risk assessment is used to estimate the probability of adverse public health impacts from each biological agent under specified exposure conditions, to identify the production capabilities needed, and to examine person-to-person transmissibility, the estimated public level of concern/panic should there be a bioterrorism event involving each pathogen, and if special preparation is needed, and at what level, in advance of a bioterrorism event to minimize casualties.[48] These assessments are revisited on a regular basis.

Category A agents are those with the highest priority, specifically defined as those that (1) are most likely to have the highest consequence to individuals and populations due to their ease of dissemination or ease of person-to-person transmission, (2) may result in high mortality rates and have the potential for a major public health impact, (3) may cause associated public panic, and (4) require special actions to attain public health preparedness.[45] These highest adverse consequence conditions, the Category A diseases (see Table 15.1), include anthrax, botulism, plague, smallpox, tularemia, and viral hemorrhagic fevers, which are rarely, if ever, seen by clinicians and public health professionals.

The Category B agents are considered to have lower associated adverse health outcomes. Some of the agents, particularly food safety threats (see Table 15.1), are commonly seen by health care providers in routine practice and by public health practitioners undertaking commonly occurring foodborne outbreak investigations. The Category C agents (see Table 15.1) capture the concern of intentional engineering of existing pathogens to render them more virulent and then disseminating them to cause deliberate harm. This category also captures the possibility that a newly emerging infection or condition could be used in nefarious ways.

The CDC and NIAID Biodefense and Priority Pathogen Lists in Perspective

The impact of those endemic and epidemic emerging infectious diseases not found on the CDC "ABC" list but found on the NIAID list is orders of magnitude greater than what was noted with the deliberate use of biological weapons. A proposed impact measure helps to put the outcomes of biological weapons use in perspective. Using this impact typing system, deliberately caused bioterrorism incidents and biocrimes have fallen into the lowest impact tier, Individual level (type A), affecting less than 1000 persons.[49] Interestingly, since the end of WWII the use of biological weapons by state actors also has been in this lowest impact tier.[12-14] This is in contrast to past planned usage by state-sponsored offensive biological weapons programs targeting outcomes at the Town level (type B), affecting between 1000 to up to 1 million persons, or at the Country level (type C), affecting 1 million to under 1 billion.[49] At the Global level (type D), there is a potential for 1 billion or more deaths—unlikely to be found outside of science fiction novels.

Dual Use Research of Concern, Gain-of-Function Research, and Synthetic Biology

A subtext to the three categorization schema discussed previously is the concept of research conducted on one of the listed pathogens, ostensibly for peaceful purposes, which may be directed at future nefarious use with broad adverse consequences to health and safety—so-called dual use research of concern (DURC).[50] Relatedly, research that inadvertently or deliberately seeks to enhance virulence of existing less pathogenic organisms—gain-of-function (GOF) research—is a concern.[51] This latter concern has engendered extensive debate on the impact of curtailing such research when used to gain insights that may be critical for the development of improved MCMs. Following a pause in US government funding for GOF research involving influenza, severe acute respiratory syndrome, and Middle East respiratory syndrome viruses, which was initiated in October 2014, the ban was lifted on December 19, 2017 after a multidisciplinary review was completed and a policy guidance issued.[52]

In addition to DURC and GOF research is the concern regarding the use of synthetic biology to create modified or new biological agents to deliberately cause harm. The definition of synthetic biology is often debated but generally is thought to encompass either the redesign of existing natural biological systems or the design and creation of new biological systems or parts for useful purposes.[53] This burgeoning and relatively new field has coupled advances in computing power with our ability to quickly synthesize complete genomes and edit genes through the use of new and improved techniques, such as clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9).[54] Although considered an academic field of study, the tools of synthetic biology can be moved from academia into other sectors to develop needed useful products. The power of synthetic biology is exemplified in the creation and then production of synthetic artemisinin for treatment of *Plasmodium falciparum* following shortage of the naturally grown *Artemisia annua* plant in Asia.[55,56] However, this same suite of powerful tools could be misused by large and small enterprises with requisite knowledge, skills, and resources to create a biological weapon without first acquiring a pathogen or toxin.[57]

ADDRESSING THE HIGHEST PRIORITY BIOLOGICAL AGENTS

Using the results of the mandated biannual Biological Terrorism Risk Assessment[58] conducted by the US Department of Homeland Security, coupled with evaluations undertaken by the US government's interagency Public Health Emergency Medical Countermeasures Enterprise (PHEMCE), potential national security threats needing a high-priority for MCM development are identified.[59] The focus of MCM development efforts are to mitigate the health outcomes through the use of vaccines, other biologic products, drugs, diagnostic tests, and personal protective equipment. All CDC Category A biological agents are targets for MCM development by PHEMCE, as well as multidrug-resistant *B. anthracis*, found on the Select Agent list, and *Rickettsia prowazekii*, *Burkholderia mallei*, and *B. pseudomallei*, found on the CDC Category B list (see

Table 15.1). Pandemic influenza (see Chapter 165) is also found on this list of PHEMCE high-priority agents but is not discussed here.

Approval and Use of Medical Countermeasures for Priority Biological Agents

The infrequent occurrence of most of the priority agents means that there may not be MCMs that are approved for use by the US Food and Drug Administration (FDA). Despite being targets for product development, many of these MCMs are unapproved (i.e., in development and under Investigational New Drug [IND] status). The terrorist attacks in 2001, particularly the anthrax letter attacks, highlighted the need for regulatory mechanisms to authorize the use of unapproved MCMs with the potential for preventing or treating biological agents, if needed, in large populations during emergencies. Separately, the Animal Rule (also known as the Animal Efficacy Rule) was established as a regulatory pathway for the development of MCMs whose efficacy could not be readily studied in human clinical trials.[60,61] Not surprisingly, the first use of these two statutory remedies was for products to prevent or treat anthrax.[62] Similar approaches for streamlining the development and use of investigational products in an emergency exist in other countries.

Emergency Use Authorization

During a public health emergency potentially involving a large number of persons—including during an outbreak of disease due to a biological agent, such as one following a bioterrorist attack—needed MCMs may not have completed their development pathway to approval by the FDA. In such a public health emergency, if the diagnosis and treatment of large numbers of people requires use of a large number of investigational MCMs, applying the regulatory requirements for use under IND status could lead to delayed care with possible heightened morbidity and mortality. In anticipation of such conditions, a regulatory mechanism was created that could be applied not only for bioterrorism but also for other serious or life-threatening diseases or conditions resulting from chemical, radiologic, or nuclear threats.

The Project Biosheld Act of 2004 (Public Law [P.L.] 108-276)[61] added provisions to the Food, Drug, and Cosmetic Act to create statutory remedies, with subsequent amendments to the law made by the Pandemic and All-Hazards Preparedness Reauthorization Act (PAHPRA) of 2013 (P.L. 113-5)[63] and then in 2016 by the 21st Century Cures Act (P.L. 114-255).[64] An emergency use authorization (EUA) allows use of certain unapproved medical products or certain unapproved use of approved medical products, in a time-limited manner, when certain conditions are met (Fig. 15.1), including that there is no other readily available and existing regulatory mechanism. The authority to issue an EUA for a product is delegated by the Secretary of HHS to the Commissioner of the FDA. There are five statutory requirements, all of which must be met for an EUA to be issued, but even if met, the Commissioner of the FDA may opt not to use the authority (see Fig. 15.1). First, the Secretary of HHS must declare that there is a emergency or threat that would permit EUA use. Second, the threat in the Secretary's declaration must be capable of causing a serious or life-threatening disease or condition. Third, there must be evidence of product effectiveness for its intended use. Fourth, a risk-benefit analysis favors likely benefit over risk. Fifth, there are no existing adequate alternatives among available FDA-approved products.

The steps in the process are exemplified by the first EUA issued in 2005 for the use of anthrax vaccine adsorbed (AVA), a vaccine licensed, at the time, for prevention of cutaneous anthrax but not approved for prevention of inhalational anthrax. The first step was the issuance, on December 10, 2004, of the required determination by the Deputy Secretary of Defense that there was a significant potential for a military emergency involving a heightened risk to US military forces of an attack with anthrax.[65] The Secretary of HHS then issued, on January 14, 2005, the required declaration that circumstances existed to warrant an EUA; the EUA was also issued on that day and was terminated on February 1, 2006.[66]

The Animal Rule Use for Approval of Drugs, Vaccines, and Biologics

In the wake of the anthrax letter attacks, the 107th Congress passed the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (P.L. 107-188) instructing the Secretary of HHS to complete the final rulemaking, started in 1999, to permit the use of animals for approval of MCMs, under rule-specified conditions.[67,68] This act allowed the use of animals to approve an MCM that is targeted for use to prevent or ameliorate serious or life-threatening conditions caused by exposure to substances, including biological agents, when human efficacy studies are not ethical or field trials are not feasible. The final rule, known as the Animal Efficacy Rule or the Animal Rule, was added to the Code

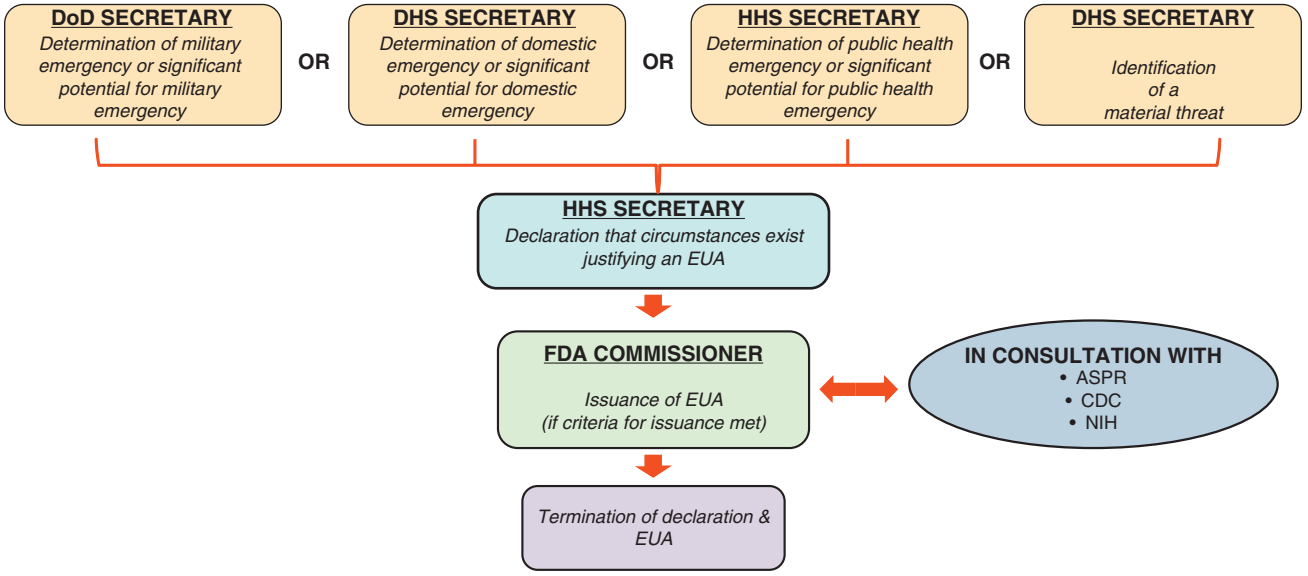

FIG. 15.1 Summary of the process for an Emergency Use Authorization *(EUA)* issuance. *ASPR,* Office of the Assistant Secretary for Preparedness and Response; *CDC,* Centers for Disease Control and Prevention; *DHS,* US Department of Homeland Security; *DoD,* US Department of Defense; *FDA,* US Food and Drug Administration; *HHS,* US Health and Human Services; *NIH,* National Institutes of Health. *(Modified from US Food and Drug Administration. Summary of process for EUA issuance; 2018.* https://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm411445.htm.*)*

TABLE 15.5 Criteria for Approval of Medical Countermeasures Under the Animal Rule and Approved High-Consequence Biological Agents, 2002–2018

Effective Evidence Criteria for Use of the Animal Rule[a]

CRITERION	DESCRIPTION
1	Reasonably well-understood pathophysiological mechanism of toxicity or the biological agent and its prevention or substantial reduction by the MCM submitted for licensure or approval.
AND	
2	Effect of the MCM is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized model for predicting the response in humans.
AND	
3	Animal study end point is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity.
AND	
4	Data or information on the kinetics and pharmacokinetics of the product or other relevant data or information, in animals AND humans, allows selection of an effective dose in humans.

Approved High-Consequence Biological Agent MCM[b]

CLASS OF MCM	MCM	PATHOGEN TARGETED	SPECIFIC INDICATIONS	DATE OF APPROVAL
Antibiotics				
	Levofloxacin	*Yersina pestis*	ID and SD Rx and PEP[c]	April 22, 2012
	Ciprofloxacin	*Yersinia pestis*	ID and SD Rx and PEP[c]	February 2, 2015
	Moxifloxacin	*Yersinia pestis*	ID and SD Rx and PEP[c]	May 8, 2015
Nonvaccine Biologics				
	Raxibacumab (Abthrax®)	*Bacillus anthracis*	ID Rx and PEP + Abx	December 12, 2012
	Botulinum antitoxin, heptavalent (A, B, C, D, E, F, G), equine	*Clostridium botulinum* toxin, types A–G	Symptomatic botulism following documented or suspected exposure to types A–G	March 22, 2013
	Anthrax immune globulin intravenous, human (Anthrasil®)	*Bacillus anthracis*	ID Rx + Abx	March 25, 2015
	Obiltoxaximab (Anthim®)	*Bacillus anthracis*	ID Rx[d] + Abx PEP[e]	March 21, 2016
Vaccines				
	Anthrax vaccine adsorbed	*Bacillus anthracis*	PEP + Abx	November 23, 2015
Antivirals				
	Tecovirimat	Variola virus (smallpox virus)	Treatment[f]	June 13, 2018

[a]See Code of Federal Regulations: 21 CFR 314.610a for drugs and 21 CFR 601.9 for vaccines and other biologic products.
[b]Approved for use in adult and pediatric patients except where indicated.
[c]Moxifloxacin approved only for use in adults.
[d]Does not cross the blood-brain barrier and does not prevent or treat meningitis.
[e]Only when alternative therapies are not available or not appropriate for use.
[f]Oral treatment only; children weighing 13 kg or more and adults.
Abx, Antibacterial drugs; *ID,* inhalational disease; *MCMs*, medical countermeasures; *PEP,* prevention after exposure; *Rx,* treatment; *SD,* septicemic disease.
Modified from US Food and Drug Administration. CDER drug and biologic animal rule approvals as of July 17, 2018. https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/NDAandBLAApprovalReports/UCM578137.pdf. Accessed July 25, 2018.

of Federal Regulations on May 31, 2002 and became effective on July 1, 2002,[69] with multiple subsequent revisions, the latest in April 2017.[70] The initial published draft guidance for use of the Animal Rule was updated and published in final form by the FDA on October 28, 2015.[71] To date, four new MCMs and four new uses of previously approved MCMs have been approved under the Animal Rule to prevent or treat high-priority biological agents—the first in 2012 (Table 15.5).

CLINICAL ASPECTS OF HIGH-CONSEQUENCE BIOTERRORISM PATHOGENS

Variola (Smallpox) Virus

Smallpox, a disease caused by variola virus (see Chapter 132), was declared eradicated by the World Health Organization (WHO) in 1980.[72] Concerns have been raised about the risk of a bioterrorism attack using this virus because of the possibility that all remaining stocks of smallpox virus were not destroyed following declared eradication. The VECTOR laboratory in Novosibirsk Region in the Russian Federation and the CDC in Atlanta, Georgia in the United States are the only two declared repositories designated by WHO to maintain stocks of the smallpox virus. Both institutions continue to do research on smallpox, albeit under the close scrutiny of the WHO Advisory Committee on Variola Virus Research.[73] It is difficult to ascertain whether other, undisclosed, repositories exist. The 2014 discovery of vials containing smallpox on the research campus of the National Institutes of Health highlights this reality,[74] along with the potential use of synthetic biologic techniques to recreate this pathogen.[75] Although the risk is unknown, and is thought to be very low, a release of smallpox today could result in a public health catastrophe.

It is estimated that a single virus particle in contact with the mucosa could initiate infection.[76] The virus is relatively stable as an aerosol and can be stored in a lyophilized form for many years. If an aerosol were deliberately released, the initial cases would likely present with a hemorrhagic pulmonary form of smallpox due to the high exposure dose. This hemorrhagic form of disease was a rare presentation during the days of endemic smallpox. Typical smallpox lesions are deep-seated vesicles or pustules in the same stage of development. In the past, endemic smallpox had an associated mortality rate of 30% of those infected.

A clinical guideline for the use of smallpox vaccine in the postevent setting has been published by the CDC (see Table 15.2). The Strategic

National Stockpile holds a large stockpile of licensed live, attenuated smallpox vaccine (ACAM2000®) and a smaller stockpile of a second investigational live, attenuated virus vaccine. A third vaccine, nonreplicating modified vaccinia Ankara, which was never evaluated in the field when smallpox was an endemic disease, is available as an investigational MCM for use in certain immunocompromised persons. Most countries have little or no vaccine, and worldwide production capacity is minimal.

Additionally, there is an antiviral treatment, tecovirimat (ST-246), now approved for use in the treatment of smallpox (see Table 15.5).[77]

Bacillus anthracis

A CDC Category A agent, *Bacillus anthracis* (see Chapter 207) usually causes anthrax in nonhuman warm-blooded animals, primarily ruminant herbivores, and is enzootic in most parts of the world. In the United States it is enzootic most commonly in Texas, North Dakota, and Nevada. Humans in contact with infected animals are at risk of developing anthrax, primarily as a cutaneous infection. In the United States up to 2 cases of human anthrax, usually cutaneous, are reported annually, commonly among persons in contact with infected, unvaccinated livestock.[78] Occasional cases of inhalational or gastrointestinal diseases have been reported among persons handling untreated, spore-contaminated animal hides or drums made from these hides.[78] It is not transmitted person-to-person. Additionally, *Bacillus cereus* Biovar *anthracis,* which has caused animal anthrax in sub-Saharan Africa, is classified as a Tier 1 select agent.[79,80]

As previously noted, *B. anthracis* was one of the principal biological weapons in the arsenal of former state-run biological weapons programs, including in the former Soviet Union and in Iraq (see Table 15.3). Non–state actors have also pursued anthrax for weapons purposes. For example, the Japanese Aum Shinrikyo cult's attempt to develop an anthrax weapon went undetected for 5 years until the cult's sarin gas attack in the Tokyo subway system in 1995 attracted the attention of authorities.[3] In the years prior to the US military engagement in Afghanistan in 2001, the terrorist group Al-Qaeda pursued establishing a biological weapons capability that included *B. anthracis*.[81]

The organism's ubiquitous nature and long-lived spore form highlight its availability and, when coupled with the minimal technical skills and permissive growth requirements needed to grow this organism, underscore why it may be explored for use by terrorists. Additionally, methods to induce multidrug resistance in the organism have been published in the peer-reviewed scientific literature.[82,83] Whether such strains maintain virulence is unknown. If used intentionally, an aerosol delivery route of exposure, as seen with the anthrax letter attacks, would likely be the most efficient and the most deadly.[84]

There are multiple FDA-approved MCMs to prevent and treat infection, including AVA for use prior to exposure; AVA is commonly given to large animal veterinarians, for example, who care for ruminants. AVA is also recommended in combination with antibiotics for postexposure treatment for persons who are without symptoms of disease.[85] There are now three licensed biologic treatments in the United States. The first is anthrax immune globulin for intravenous infusion (Anthrasil®), approved in 2015 (see Table 15.5). It is manufactured from the plasma of persons vaccinated against anthrax who have mounted documented antibody that neutralizes anthrax toxins produced by the organism.[86,87] The product is indicated for treatment of persons with inhalational anthrax and is used in combination with antibacterial drugs. The second product is raxibacumab (Abthrax®), a monoclonal antibody that binds domain IV of the anthrax protective antigen,[86,88] which was approved in 2012 (see Table 15.5). The third product is obiltoxaximab (Anthim®), also a monoclonal antibody and approved in 2016. All are given intravenously to treat inhalational anthrax in combination with antibacterial drugs. These MCMs were tested in humans for safety but efficacy was demonstrated in animals using the Animal Rule,[71] which permits licensure of a product based upon animal efficacy studies when a human clinical trial would be unethical. The CDC has produced a guideline for the clinical management and use of MCMs during an anthrax mass casualty incident (see Table 15.2).

Clostridium botulinum Toxin

Botulism is a neurologic intoxication caused by one of the seven botulinum toxins (designated type A to type G) produced by *Clostridium botulinum* (see Chapter 245), a CDC Category A bioterrorism agent. Like *B. anthracis,* the microorganism is ubiquitous in soil in its spore form, which can remain dormant for 30 years or more.[89] The toxins, produced by the vegetative form of the organism, are the most potent poison known and are associated with high case fatality if untreated. In the United States approximately 200 cases of botulism are reported annually.[78] Most cases in the United States occur following ingestion of food containing the preformed toxin. Other forms include wound botulism or intestinal botulism, in which the spore undergoes in situ germination to produce toxin that is then systemically absorbed.[89] Notably, very dilute amounts of type A and type B toxin have therapeutic and cosmetic purposes.[90] Importantly, botulism following inhalation of aerosol purified toxin has occurred in the research laboratory setting following minute exposure doses.[91]

Botulinum toxins were one of the principal weapons in the arsenal of the former Soviet Union and are known to have been produced as a weapon by Iraq.[92] One terrorist group, the Aum Shinrikyo, tried unsuccessfully to produce and disseminate botulinum toxin.[93] *Clostridium botulinum* static and fermenter-based growth requirements for toxin production are well known and have been perfected over the years, particularly for the production of therapeutic and cosmetic products, making the production technology readily available.[94] Deliberate aerosol dissemination would create an initial diagnostic dilemma because standard serologic and body fluid detection methods often give confusing results.[95] Furthermore, deliberate use of preformed botulism toxin by contamination of food or by aerosol release could cause a large number of casualties that could cripple the health care delivery system if the number of casualties exceeds the intensive care and ventilator support capacity available for treatment.[96]

Yersinia pestis

Plague is caused by *Yersinia pestis* (see Chapter 229), a CDC Category A bioterrorism agent, and is both endemic in the United States[78] and periodically epidemic in various parts of the world. It is usually a zoonotic infection of humans.[97] Untreated bubonic plague can lead to septicemia that is associated with pneumonia and subsequent aerosol transmission to others manifesting as pneumonic plague.[98] Although uncommon, infected dogs can transmit to humans via the respiratory route.[99] The person-to-person transmissibility of pneumonic plague made *Y. pestis* a candidate for development and use in state-sponsored biological weapons programs.[98] Primary pneumonic plague would result from an aerosol exposure and lead to a rapidly progressive and lethal infection absent immediate medical intervention; this form of plague is transmissible to others.

There is no longer an FDA-licensed vaccine for the prevention of plague. An inactivated *Y. pestis* vaccine, plague vaccine USP, was licensed in the United States on October 5, 1994, but production discontinued in 1999.[100] A recombinant F1-V fusion protein plague vaccine completed a phase IIa clinical trial in healthy adults in 2012.[101] It received Orphan Drug designation for the prevention of plague (*Y. pestis*) by the FDA on November 30, 2016.[102] The vaccine is intended for administration for persons at high risk for exposure to aerosolized *Y. pestis;* it is anticipated that future licensure could follow if efficacy is demonstrated in clinical studies or through the Animal Rule. There is no known stockpile of this vaccine.

There are now three FDA-approved bactericidal antibiotics indicated for use to treat inhalational plague or those persons exposed to the pathogen but not yet ill—levofloxacin, ciprofloxacin, and moxifloxacin (see Table 15.5). Streptomycin, not widely available in the United States, has long been an approved parenteral treatment for plague. Gentamicin, although not FDA approved for the treatment of plague, has been used off-label to treat plague, usually in combination with other drugs.[103,104] Like a large aerosol release of botulinum toxin, a large *Y. pestis* release could overwhelm critical care resources needed for immediate management of pneumonic disease, although for a shorter period than that anticipated for botulism.

Francisella tularensis

Tularemia is caused by the bacterium *Francisella tularensis* (see Chapter 227), a CDC Category A bioterrorism agent (see Table 15.1) found in many northern hemisphere countries, including the United States (all states except Hawaii).[78] There are six clinical forms of this zoonotic

infection depending upon exposure route.[105] Unintentional routes of infection include direct contact with animals, ingestion of contaminated food or water, arthropod bites, and inhalation of infected aerosols. It is a hardy organism capable of surviving for weeks in the environment.[106] Between 2005 and 2015 there were between 100 and 300 reported cases of tularemia annually, usually in the south central United States, the Pacific Northwest, and parts of Massachusetts.[78]

Francisella tularensis was developed into an aerosol biological weapon by several countries in the past, including the United States while it was operating its offensive biological weapons program. There are several reasons for selection of this pathogen. First, inhalation of as few as 10 organisms can cause disease—and may lead to serious illness and death.[105] Second, given its widespread occurrence in nature, it is theoretically easy to obtain. Third, it can be grown on common nonselective media that are not difficult to obtain. If used as a bioweapon in the future, it is likely to be introduced by the aerosol route. Importantly, it is not transmitted person-to-person, even the respiratory form of infection.

A live tularemia vaccine strain brought to the United States from the Soviet Union in 1942 was used to develop a vaccine in the United States, designated as a live vaccine strain (LVS).[107] The vaccine has been used as an IND product for prevention of tularemia in laboratory workers, primarily at Department of Defense laboratories. Existing and new lots of the vaccine were tested in a phase II, multicenter, double-blind, randomized comparison safety and immunogenicity trial in 228 subjects ending in 2012; no differences between the vaccines were identified.[108] No further development plan has been identified.

Existing approved antibacterial agents have been recommended for the treatment of inhalational tularemia.[109] However, none of these products has an on-label indication for this specific pathogen or form of disease.

Rickettsia prowazekii

Louse-borne or epidemic typhus is caused by *Rickettsia prowazekii* (see Chapter 189), a bacterium carried and transmitted by body lice. It is a CDC Category B agent (see Table 15.1) and a Tier 1 select agent.[43,44] Unintentionally occurring disease is typically associated with war, famine, and other poor causes of unhygienic conditions associated with human lice infestation. Transmission is from person to person via lice. Additionally, in societally disrupted settings, the environment can be contaminated with dried louse feces carrying the microorganism, leading to aerosolized bacteria in louse fecal dust.[110] The median infectious dose is fewer than 10 organisms.[111]

In 1940, the Japanese Unit 731 biological weapons program conducted tests over a 5-month period, spreading 70 kg of *R. prowazekii* by an unclear dissemination method and precipitating a typhus epidemic in Ning Bo, a Chinese seaside resort town.[112] This pathogen was also reported by a defector to have been one of the first biological weapons developed during the 1930s in the Soviet Union.[26]

The concern is the intentional delivery of this biological agent by the aerosol route at a dose much higher than that seen under natural conditions, which could alter the natural history, expression, and fatality associated with the disease. There is no licensed *R. prowazekii* vaccine in the United States. Treatment with doxycycline is usually effective.[111]

Burkholderia mallei

Glanders is caused by infection with the bacterium *Burkholderia mallei* (see Chapter 221). It is CDC Category B agent (see Table 15.1) and a Tier 1 select agent. *Burkholderia mallei* causes an infection primarily of solipeds, found in lower resource settings. The last case in the United States was in 1942 in a horse.[113] Transmission from solipeds to humans is uncommon, even in the setting of close contact animals, likely because the usual exposure settings are associated with dosages too low to cause disease.[114] Notably, when it did occur in the preantibiotic era, over 90% of human cases were fatal. Usual modes of acquisition involve infectious animal material coming in contact with nonintact skin or mucosal surfaces. Unintentional disease is rarely fatal with prompt and appropriate antibiotic treatment. Rare cases have occurred following laboratory accidents, most often by the aerosol route, when a higher infecting dose is delivered.[114]

Importantly, *B. mallei* has been used as a biological weapon. It was the first biological weapon used by the Germans during WWI by infecting livestock and horses with the agent before they could be shipped to the Allies for use by cavalry members.[115] During WWII, this pathogen was part of the Japanese Unit 731 biological weapons program, which included infecting prisoners with *B. mallei* and performing autopsies on 21 fatal cases.[112] The concern today is that *B. mallei* could be deliberately released as a high-dose aerosol, causing a large number of rapidly evolving pulmonary and septicemic cases, with many initial deaths due to delays in diagnosis and treatment. Prolonged therapy to prevent relapse is needed. There is no preventive licensed vaccine available to prevent disease.[116]

Burkholderia pseudomallei

Melioidosis is caused by *Burkholderia pseudomallei* (see Chapter 221),[116] a CDC Category B agent (see Table 15.1) and a Tier 1 select agent.[43,44] Unlike its zoonotic cousin *B. mallei*, which causes glanders, *B. pseudomallei* is a saprophytic bacterium found in soil and water in tropical and semitropical areas of the world, particularly Southeast Asia and northern Australia. In endemic areas, the routes of transmission are primarily by inoculation of abraded areas of nonintact skin, percutaneous, by ingestion, and by inhalation in persons with direct soil contact. Inhalational transmission was supported epidemiologically when significantly different rates of acute pulmonary melioidosis were noted among US military helicopter winch operators in Vietnam compared with other troops.[117] Person-to-person transmission rarely occurs, even in severe pulmonary disease.

The disease is associated with a high mortality due to the speed with which septicemia develops, particularly in immunocompromised hosts—especially those with comorbidities in whom metabolic acidosis is present—and the inherent resistance of the bacteria to several classes of antibiotics.[118] Prolonged courses of antibiotics are required to treat melioidosis, with the risk of recurrence following treatment.[116] As with glanders, prolonged antimicrobial therapy is required to treat it and to prevent its relapse.

There is no evidence that *B. pseudomallei* has been included in any past known state-sponsored biological weapons program. However, the organism can survive for months to years in endemic areas, particularly in waterlogged, heavy clay soils following monsoons, typhoons, and hurricanes.[117] The organism persists in biofilms, in drinking water with generally used chlorine levels, and within amoebic cysts.[117]

Viral Hemorrhagic Fever Viruses

Several important viral hemorrhagic fever viruses are classified as CDC Category A agents (see Table 15.1). These include the Filoviridae Ebola virus and Marburg virus (see Chapter 164) and the Arenaviridae Lassa virus and Machupo virus (see Chapter 167). These viruses usually have high associated morbidity and mortality.[119] None of these pathogens is known to have been used in the past as a biological warfare agent (see Table 15.3) or to commit an act of terrorism or a biocrime (see Table 15.4). However, concerns exist that the Russian Federation may have developed and stockpiled one or more of these agents as biological weapons.[29-31,32]

The large West African Ebola virus disease outbreak, extending from late 2013 (initially undetected) through early 2015,[120] served to hasten development of MCMs for this pathogen and to update management guidelines (see Table 15.2). Health care workers, including burial attendants, had the highest case fatality rate.[121] There are now investigational MCMs for use following exposure, including investigational vaccines, one of which has been shown to be efficacious in a postexposure setting when used within 6 days of exposure; specific monoclonal antibody combinations; and small-molecule antiviral agents.[122] Aerosolization of Ebola virus or Marburg virus with subsequent inhalation of virus, a concern should viral hemorrhagic fever virus be used deliberately, has not been proven to be a mode of transmission.

BIOTERRORISM INCIDENTS

Clinical Provider Preparedness and Response

The early detection of a bioterrorism attack is critical to the most efficient and effective response by health care and public health systems, directed at minimizing morbidity and mortality. Health care providers are on the detection front line.[123] It is anticipated that primary care providers,

emergency medicine physicians, and infectious disease specialists will continue to be key to the earliest identification of a suspect event through their application of epidemiologic principles while evaluating the acute illness presentations of their patients.[1]

Many of the CDC Category A high-consequence pathogens are endemic in parts of the United States but at a low background level. Therefore most providers have little experience with these conditions, even when they are naturally occurring. Preevent basic knowledge of the pathogens and their usual presentations is important in helping to trigger a clinical index of suspicion that a bioterrorism even may be occurring (Table 15.6). Health care providers can complete bioterrorism training modules and video webcasts to enhance knowledge in this area as well as reviewing biological agent—specific clinical management guidances (see Table 15.2).

A suspicion that a presenting infection or intoxication may be deliberately caused is often triggered by being alert for patterns of illness and diagnostic clues. For example, if a healthy young person presents with a relatively acute onset of shortness of breath and a widened mediastinum on a chest radiograph and reports recently attending a summertime event at a local arena with three friends who also are experiencing a similar but less severe illness, this should alert the clinician to the presence of possible deliberately caused inhalational anthrax. This brief patient-presented history provides both diagnostic clues and suggests a clustering of illness. Other alerting features include the occurrence of a relatively severe, acute illness in a previously healthy person, and a report of illness in others who attended the same event, suggesting a possible point source.

In addition to diagnostic testing and treatment initiation for this and other possible conditions, contacting local public health authorities immediately along with the health care system leadership, if employed in this setting, is important. Sharing this information as quickly as possible will ensure that the next steps in detection, investigation, response, and public outreach are undertaken in a timely manner. The frontline clinician is generally not expected to inform law enforcement. This generally falls to public health authorities, especially should the diagnosis be confirmed.

Supplementing Health Care Provider Resources

In the event of a large bioterrorism event, there may be insufficient providers to care for patients. When supplementation of clinical response efforts is needed, the National Disaster Medical System (NDMS) and its Disaster Medical Assistance Teams (DMATs)[124] and the Medical Reserve Corps (MRC)[125] can be activated.

The NDMS, a system coordinated by the HHS, acts to temporarily supplement state and local medical care needs after a disaster of any kind. The NDMS can provide personnel, supplies, and equipment at the site or at definitive care sites in unaffected areas, and can provide patient care movement. DMATs are local units activated for 2-week deployments with sufficient supplies and equipment to be self-sustaining for at least 72 hours before resupply is necessary.[124] In the event of a national disaster, DMATs may be moved from their local area, at which time they are made federal employees with medical credentials recognized in all states and protected under the Federal Tort Claims Act[126] against any malpractice claim. Predisaster employment is protected under the Uniformed Services Employment and Reemployment Rights Act.

The MRC was created in 2002 as community-based and locally organized groups of health care volunteers who donate their time and expertise to prepare for and respond to existing emergency medical and public health resources when needed. There are more than 300 units in the United States. Units have been active, for example, in providing services after hurricanes. The Office of the Surgeon General acts as a clearinghouse for information and best practices in establishing and maintaining MRC units. Liability protection for individual MRC practitioners is determined by each state.

TABLE 15.6 Recognizing a Possible Illness Caused by Exposure to a Biological Weapon

- Have a high index of suspicion.
- Be alert for patterns of illness and diagnostic clues.
- Think like an epidemiologist—consider the patient within a population context. **Ask yourself several questions:**
  - Does the patient have an unusually high fever? Unique features of a respiratory tract or gastrointestinal infection?
  - Does the patient have symptoms or signs or a confirmed condition not commonly seen in your location of practice, and the patient has not traveled to an endemic location?
  - Is the illness appearing in an unusual age group or as part of a cluster of cases?
  - Is there a simultaneous human outbreak of the condition with an epizootic in local animals?
  - Is there an unusual temporal or geographic clustering suggesting a point source exposure?

If you think what you are seeing is unusual, call your Health Department immediately.

Supplementing MCMs With the Strategic National Stockpile

In fiscal year 1999, the CDC received $51 million for the development of an emergency stockpile of MCMs, then called the National Pharmaceutical Stockpile, primarily antibiotics for anthrax and a vaccine for smallpox. Now named the Strategic National Stockpile, this is an important part of the response armamentarium after a bioterrorism incident.[127] The Strategic National Stockpile contains antibiotics, antitoxins, vaccines, life-support medications, and medical supplies that can be used to supplement state and local resources during a large-scale public health emergency. Within 12 hours of a request, a Push-Package containing an initial supplemental cache of MCMs and supplies can be at the targeted destination. These packages have been prepositioned in strategically located secure warehouses to facilitate prompt delivery. If additional support is necessary, a vendor-managed inventory is called on to deliver ongoing needed MCMs and supplies. Distribution is coordinated in concert with the receiving jurisdiction.

Health Care Facility Preparedness and Response

Persons with severe illness following deliberate exposure to a biological agent may likely present first to a hospital emergency room or be directly admitted to a hospital by their primary care provider. Preparedness for addressing public health emergencies, with resulting stress on the care systems, is critically important for a successful response.[128]

Through its Hospital Preparedness Program, the HHS aims to assist in the preparation of the health care delivery system through the development of health care coalitions.[129] Specific performance measures in four capability areas for hospital readiness to respond to a public health emergency, including a bioterrorism attack, have been created. These targets include creating a sustainable health care coalition among health care facilities, emergency medical services, emergency management organizations, and public health agencies for planning, training, exercising, and managing resources; coordinating health care and medical response approaches and teams; ensuring continuity of services even in the setting of a disrupted health care delivery system; and determining how to maintain surge response in the setting of a public health emergency. Although all hospitals do not need to have the capacity to completely manage one or more patients needing care in a highly contained area due to infection with a highly communicable high-consequence pathogen, each facility does need to know how to properly respond to and safely transfer such a patient.

Responding to Ebola virus provides an example of a tiered approach to manage future patients with possible or confirmed Ebola virus disease.[130] The majority of hospitals in the United States are designated as Frontline Health Care Facilities. These first-tier facilities function to quickly identify and isolate a patient with possible Ebola virus infection, notify infection control and state and local health officials, and have enough personal protective equipment for at least 12-24 hours of patient care. The second tier consists of 55 designated Ebola Assessment Hospitals that can isolate the patient, perform immediate laboratory studies, and coordinate Ebola testing with the state or the CDC,[130] and are able to care for the patient for up to 5 days with enough personal protective

equipment on hand. The final tier consists of Ebola Treatment Centers, which can care for the patient throughout the duration of illness. There is now a regional Ebola treatment network in the United States consisting of 10 facilities with so-called biocontainment units that have the capacity to receive in transfer and admit or directly admit patients with known or suspect Ebola virus or other high-consequence pathogen infections.[130]

Clinical laboratories within health facilities are considered as Sentinel Laboratories in the Laboratory Response Network (LRN) for bioterrorism.[131] There are approximately 25,000 private and commercial laboratories in the United States at this level. Most are hospital based or part of clinical institutions. The key role of these laboratories is early detection of biological threat agents, application of "rule-out" algorithms, and referral of needed samples to the next level of the LRN, one of about 155 reference laboratories.[132]

Hospital epidemiology and infection control (HEIC) plays a critical role in the overall management of a potentially transmissible high-consequence pathogen in the health care setting. Within the hospital setting HEIC functions to manage any facility outbreak related to the identified pathogen; ensure that standards are in place and followed for the protection of health care workers, volunteers, and patients; and control anxiety/concerns of families, employees, and unaffected persons within the health system.[133]

Public Health Preparedness and Response

Communication

A major challenge to public health officials is the institution of necessary measures to avoid panic in the face of an epidemic of a traditionally feared disease.[134,135] Reviews of past epidemics indicate that the most essential factor is effective leadership and competent, frequent, and open communication with the public, the press, professionals, and others concerned in dealing with the epidemic. This is an area that is too often neglected. The 2001 anthrax outbreak illustrated the problems resulting from inadequate lines of communication.[136] Health departments at all levels were overwhelmed by requests for information from the public, from health professionals, and especially from the media. None had experienced an epidemic threat such as this previously and none was prepared.[136] Frequent, authoritative, up-to-date reports through the media to the public proved absolutely vital, but it took time before a pattern for these became established.[136] The need for communication between and among professionals was clear, and this is now being addressed in part by the national Health Alert Network, which is financed by federal preparedness funds.[137]

Another important lesson learned from the anthrax letter attack response was the need for command centers to coordinate and direct operations and to facilitate the flow of information; these took time to become established and to begin to function well. Sophisticated centers are now in place in the office of the Secretary of HHS, in the CDC, and in many states and cities; they are now staffed on a 24-hour-per-day, 7-day-per-week schedule. The creation of such emergency operations centers during any public health crisis worldwide is now a part of the Global Health Security Agenda to meet the requirements of the WHO International Health Regulations of 2005.[138-140] Information and educational materials have been prepared with respect to Category A diseases and are available throughout the health system.[141] At federal, state, and local levels, exercises are being conducted to test response systems to determine how well they are actually functioning.[142]

A second factor in muting the likelihood of panic is to do everything possible to keep the normal day-to-day activities of citizens and the city as minimally disrupted as possible. Public officials at all levels have often been prone to want to invoke quarantine measures, whether to close airports or other parts of the transportation network or to forbid entry into or departure from cities or other large areas. This was the situation in all countries that reported cases of severe acute respiratory syndrome in 2003.[143] Experience has shown that quarantine measures are seldom effective and, in fact, often lead to more serious problems as many people seek to flee an area or deny the presence of possible cases in family or friends, thus precluding appropriate containment measures.[144]

Epidemiology

The epidemiologic challenges associated with responding to a bioterrorism attack are uniquely different from those associated with responding to an explosion or to the release of a chemical agent. The effects of the latter two events are readily apparent, allowing early approximations to be made as to the geographic extent of the problem and the number and nature of casualties to be expected. Needed response efforts can thus be gauged and initiated immediately. When a microbe or toxin is used, however, the incubation period of the biological agents means there is an inherent delay from the time a covert attack is launched until the realization that an attack has occurred—most likely by the identification of a cluster of sick patients or a single person with a condition associated with suspect clinical clues (see Table 15.6). The varying incubation periods of the disease inevitably mean a delay in gauging the magnitude and scope of the attack and deploying appropriate response efforts on the basis of the epidemiology of the ensuing outbreak. Simultaneous attacks with an aerosolized biological agent in several locations, for example, could generate a large, complex geographic distribution of cases, thereby delaying the timely development of an epidemic curve used as a tool to assist in characterizing the event. In addition, exposure to a large inoculum of aerosolized biological agents could result in atypical disease presentations and clinical courses (e.g., shorter incubation periods, compressed and severe disease course), thus creating both clinical and epidemiologic conundrums.

On the basis of experience gained during the anthrax letter attacks, it is to be expected that there will be widespread apprehension, fear, and concern about the possibility of further cases—from the spread of a contagious agent and/or from sequential attacks. Many people may live in fear that they or their families will be the next victims. Experience shows that this inevitably complicates event investigation and response efforts.[145-148] For example, in the 2001 anthrax attack it was not until more than 2 weeks after the initial anthrax-laden letters were sent through the US Postal Service that the index case was diagnosed and reported.[2,136] By that time nine cases of anthrax had actually occurred (two inhalational and seven cutaneous). As health and law enforcement authorities subsequently worked to determine what had happened and to implement appropriate response measures, additional anthrax-laden letters were sent (3 weeks after the initial letters), resulting in an additional 13 cases of anthrax (9 inhalational and 4 cutaneous). There was widespread heightened concern among various segments of not only the US population but citizens in other areas of the world as well, who were experiencing a large number of hoaxes with envelopes containing powders—all of which required investigation by public health and law enforcement officials.[136]

Laboratory Preparedness and Response

A network of National, Reference, and Sentinel Laboratories defines the Laboratory Response Network, which was established by the HHS at the CDC in 1999.[132] The LRN includes a component that specifically addresses biological terrorism in collaboration with the Association of Public Health Laboratories and the FBI. It defines a tiered system of laboratories for the identification and verification of biological agents. The Sentinel Laboratories, previously described, are part of the health care system and function in the Biosafety Level 1 (BLS-1) or BLS-2 environment. The American Society of Microbiology works closely with the CDC and Sentinel Laboratories to provide needed protocols and training for laboratorians. The Sentinel Laboratories refer questionable samples to the second tier of approximately 150 Reference Laboratories, which form the backbone of the LRN's public health laboratory arm and function as high as BSL-3 for further identification and investigation. This tier includes state and local public health, military, veterinary, agriculture, food, and water testing laboratories. Additionally, certain countries, such as Australia, Canada, the United Kingdom, Mexico, and South Korea, have their own Reference Laboratories and are part of the LRN. If needed, final confirmation of a threat agent is done at one of the National Laboratories capable of functioning at BSL-4 that is also part of the LRN. The CDC and USAMRIID, for example, have BSL-4 capacity.

Surveillance

In the United States, governments (federal, state, local, tribal, and territorial), health care systems, and private sector companies, such as those

involved in the preparation and sale of foodstuffs, have taken steps to improve surveillance to detect unusual outbreaks and cases of infectious diseases as quickly as possible. There are federal, state, and local human, animal, and plant disease surveillance systems that identify specific pathogens. In recent years, improved surveillance systems at the international, national, and local levels—including an improved network of public health laboratories—have enabled the detection of outbreaks of novel infectious diseases with an exceedingly small number of cases.[132]

A multicomponent, interagency biosurveillance initiative was created and funded beginning in fiscal year 2004 to fill the gap in surveillance and to provide early warning of a potential terrorist attack or infectious disease outbreak. This effort was further enhanced in 2012 with the first-ever National Strategy for Biosurveillance.[149] Because there may be delays in final identification of pathogens, syndromic surveillance systems[150,151] have been utilized and integrated to provide for timely communication across multiple sectors reaching from the community to the national level.

The National Syndromic Surveillance Program (NSSP), coordinated by the CDC, serves as a community-based and community-driven effort for data collection.[152] The effort utilizes clinical data collected from hospitals, pharmacies, emergency departments, and urgent care facilities and contextual data collected from schools, poison control centers, environmental data, and large events. Collected data are placed on BioSense, a cloud-based platform, and then public health agencies at all levels, associations, and other partners can utilize these data for improving situational awareness and monitoring specific conditions and patterns of illness that indicate a new or different event requiring rapid assessment and follow-up. Statistical tools are used to detect, monitor, and characterize unusual events for further investigation.

BioSense, which was mandated by Congress in 2002, has evolved to become an essential part of the NSSP.[153] It has undergone iterative improvements from its initial CDC-centric and bioterrorism-only focus from 2002 to 2009, to a more technology-focused and stakeholder-driven effort from 2010 to 2013. It is now serving multiple stakeholders at all levels and is capable of quickly identifying unusual events that may represent a new intentional or unintentional threat for further investigation.

ACKNOWLEDGMENT

The authors dedicate this work to Donald A. Henderson (1928–2016)—our mentor, colleague, and friend—who coauthored previous versions of this chapter with us.

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D Clinical Microbiology

16 The Clinician and the Microbiology Laboratory: Test Ordering, Specimen Collection, and Result Interpretation

Robin Patel

SHORT VIEW SUMMARY

BACKGROUND
- Optimal care for infected patients requires collaboration between clinicians and the clinical microbiology laboratory.
- Challenges may include distancing of diagnostic laboratories from health care providers, outsourcing of tests, financial and time constraints on the laboratory and clinicians, and ongoing rapid changes in diagnostic microbiology.
- This chapter provides guidelines to the clinician for making best use of the microbiology laboratory.

CLINICIAN'S RESPONSIBILITY TO THE MICROBIOLOGY LABORATORY
- Alert laboratory to specimens that may pose a hazard to laboratory personnel.
- Obtain appropriate specimens and order appropriate tests.
- Prioritize tests if specimen amounts are limited.
- Advise laboratory about selection of antimicrobial agents for susceptibility testing, as appropriate.
- Maintain open communication for:
  - Specimens needing specialized testing
  - Need to add new tests to or remove obsolete old tests from test menu

MICROBIOLOGY LABORATORY RESPONSIBILITIES TO CLINICIANS
- Maintain a system that provides an appropriate test menu, easily interpretable results, and rapid clinical alerts regarding new significant test results.
- Provide information about performance, clinical utility, turnaround time, and cost of tests.
- Maintain open communication about policy, test menu, and reporting changes.
- Collaborate with infection prevention and control and antimicrobial stewardship programs
  - Provide periodic data on local antimicrobial susceptibility results.
  - Maintain data needed by hospital epidemiology and antimicrobial stewardship groups.

Infectious diseases clinicians are highly reliant on diagnostic microbiology testing and should work in concert with clinical microbiologists to maximize the value and impact of clinical microbiology testing. This includes working with laboratory staff to ensure that the ideal menu of testing is offered, that testing is appropriately ordered (i.e., neither overused nor underused), and that important results are correctly and expeditiously acted on. These activities ensure quality patient care, guide appropriate interventions, and help control cost and appropriate use of antimicrobial agents. In some cases, laboratory testing may be performed off-site or in reference laboratories. Off-site testing potentially increases the time between specimen collection and laboratory processing, possibly compromising specimen integrity if not carefully managed, and may result in delays in the availability of critical test results. Conversely, point-of-care infectious diseases diagnostics are increasingly available,[1] substantially decreasing test turnaround time. These changes make it increasingly important for infectious diseases clinicians and clinical microbiologists to work together. A number of expectations regarding the responsibilities of the infectious diseases clinician should be recognized, as delineated in Table 16.1.

The infectious diseases clinician should be knowledgeable about the test menu and ought to work with the laboratory director to ensure that the test menu is up-to-date and that test turnaround time is sufficient to satisfy patient care needs. Selection of tests on the menu and availability of infrequently offered tests should be determined after discussions with key members of the medical staff and should be revised as patient care needs change. New commercial tests become available on a regular basis; infectious diseases clinicians should be involved in decision making regarding the merits or lack thereof in bringing in individual new tests. Infrequent, expensive, or sophisticated testing may need to be performed in a reference laboratory; this can be associated with significant cost. The infectious diseases clinician should work with diagnosticians to ensure that the send-out menu and method for vetting send-out tests is appropriate. The laboratory should provide the medical staff with a menu of the offered tests, indications and performance characteristics for each test, and turnaround times for test results. Information about appropriate collection of specimens including timing and technique of specimen collection and appropriate transportation of the specimens to the laboratory should be specified. This information should be readily available in the hospital computerized information system and ideally integrated with electronic ordering of tests. Poorly collected specimens may yield recovery of colonizing or commensal organisms and may be rejected by the laboratory.

The complexity of laboratory testing for infectious diseases has increased in recent years and is expected to continue to do so. As such, it is increasingly important to ideally display laboratory tests in the electronic medical record and implement and maintain clinical decision support systems vis-à-vis laboratory testing. Such clinical decision support systems may, for example, limit repeat ordering of some tests, limit the numbers of specimens that can be submitted for a specific test, or restrict test ordering to certain types of practitioners. Decisions about what is appropriate in this regard should be made by the clinical microbiologist and the infectious diseases clinician working together. Test utilization is becoming increasingly important and is the domain of both the clinical microbiologist and the infectious diseases clinician. For example, these two can work together to reduce ordering of urine cultures in asymptomatic patients or, conversely, to ensure that *Legionella* urinary antigen is ordered in patients with community-acquired pneumonia who need this type of testing. The computer system should

TABLE 16.1 Responsibilities of Infectious Diseases Clinician Vis-à-Vis Laboratory Testing

- Maintain knowledge of the laboratory test menu
- Assist the laboratory director with display of laboratory tests in the electronic medical record (ordering and resulting) and in implementing and maintaining clinical decision support systems vis-à-vis laboratory testing
- Assist in defining which antimicrobial agents should be routinely tested for individual bacteria, mycobacteria, and fungi as well as when routine additional susceptibility testing is needed and the nature thereof
- Alert the laboratory when an organism that could be hazardous to laboratory personnel is suspected
- Prioritize test requests when limited quantities of specimen will be or have been collected
- Establish open communication with the laboratory director when testing needs are not satisfied by the available test menu (e.g., specific testing is unavailable)
- Establish open communication with the laboratory director when special handling or testing of a specimen (e.g., testing for a particularly fastidious pathogenic organism) is required or questions about appropriate laboratory testing arise

TABLE 16.2 Organisms About Which the Laboratory Should Be Notified if Suspected

Avian influenza
Bacillus anthracis
Brucella spp.
Burkholderia mallei
Burkholderia pseudomallei
Clostridium botulinum
Coccidioides immitis/Coccidioides posadasii
Coxiella burnetii
Francisella tularensis
Hemorrhagic fever viruses (e.g., Ebola, Marburg, Chapare, Crimean-Congo, Guanarito, Hanta, Junin, Kyasanur Forest disease, Lassa fever, Lujo, Machupo, Omsk hemorrhagic fever, Sabia)
Measles
Middle East respiratory syndrome–associated coronavirus, severe acute respiratory syndrome–associated coronavirus
Nipah virus, Hendra virus
Smallpox
Yersinia pestis

document receipt of specimens in the laboratory, provide a record of testing in progress, and report preliminary and final results. Each institution should establish and communicate which results constitute critical results and therefore require immediate telephone notification. Although telephone communication provides the optimal and most reliable means to communicate information, it can be disruptive to the health care provider and the laboratory alike and so must be targeted and focused; other methods of communication such as text messaging, paging, and electronic messaging can be used to connect with health care providers in other contexts.[2]

The laboratory must ensure that its operation meets all current regulatory requirements including initial verification and ongoing validation of procedures that are used and training and competency of staff. Participation in proficiency testing, an effective program of quality control, and the use of established quality assurance benchmarks help ensure accuracy of the laboratory test results. Infectious diseases clinicians also play a role in maintaining quality of laboratory testing. If important results are observed that do not make clinical sense, especially if an important and unexpected false-positive result is suspected, the laboratory director should be notified. A system of short-term storage of select specimens should be established to facilitate additional testing if required; infectious diseases clinicians should work in concert with laboratories to select specimen types appropriate for this.

When unusual organisms that are challenging to isolate (e.g., *Corynebacterium diphtheriae*) are suspected, the laboratory should be notified. Even with non–organism-specific cultures, the laboratory should be notified if a specific organism is suspected; this facilitates optimized testing. Infectious diseases clinicians should understand testing from a specimen standpoint (i.e., what tests can be performed on specific specimen types and how these specimens should be collected), from a microorganism standpoint (i.e., what are the best tests to diagnose infection with a specific microorganism), and from a syndromic standpoint (i.e., what are the best tests for a patient with a specific syndrome [e.g., acute gastroenteritis, meningitis, community-acquired pneumonia, hospital-acquired pneumonia, ventilator-associated pneumonia]).

Infectious diseases clinicians should be prepared to prioritize test requests when only a limited quantity of specimen can be or has been collected. In this situation, discussions with the laboratory director can facilitate optimization of the testing process (e.g., need for routine stains, selection of media, testing conditions to optimize recovery of both bacteria and fungi using a limited number of media). There should be a professional discussion with the laboratory director when testing needs are not satisfied by the available test menu (e.g., specific testing is unavailable or an available test is not offered because it has not been validated for a specific specimen type). Communication with the laboratory director is also important when clinical testing needs are not satisfied by test turnaround time or when a laboratory policy causes patient care problems. With the explosion in rapid diagnostic testing, there is increasing need for clinical microbiologists to work together with infectious diseases clinicians to communicate results to the clinical practice in real time on a patient-by-patient basis to ensure that such results are appropriately acted on. Likewise, an anticipated explosion in deep sequencing-based microbial diagnostics will be best addressed by infectious diseases clinicians working closely with diagnosticians.

MICROORGANISMS HAZARDOUS TO LABORATORY WORKERS

Infectious diseases clinicians should alert the laboratory when an organism that could be hazardous to laboratory personnel is suspected (Table 16.2). This notification should cover all specimens from implicated patients that could pose a danger to laboratory staff. For example, isolation of *Brucella* species in culture can be hazardous to laboratory staff if cultures are not handled in a biological safety cabinet. This organism grows in routine blood culture bottles and on other culture media such that if brucellosis is suspected, all cultures from the patient in question should be carefully handled. Similarly, as *Coccidioides immitis* and *Coccidioides posadasii* grow readily on bacteriology media, bacteriology cultures from a patient with suspected coccidioidomycosis need to be specially handled. Infectious diseases clinicians should work with their institutions and laboratories to determine how high-consequence infectious diseases such as Ebola virus should be handled from the minute a patient with a suspected infection enters the system. This involves handling the patient and his or her specimens and includes how specimens should be collected, transported, and tested and whether any restrictions should be placed on laboratory testing to maximize patient outcome while protecting clinical and laboratory staff. Infectious diseases clinicians may be called on to help in managing laboratory exposures to hazardous agents and therefore should be familiar with appropriate postexposure management strategies.

TAXONOMY

A challenge that confronts the microbiologist and clinician alike is the continuous revision of the taxonomic nomenclature, particularly for bacteria and fungi. Much of the taxonomic reorganization now under way is driven by our increased ability to classify organisms using DNA sequencing. Moreover, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (see "Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry") in addition to gene and genome sequencing has enhanced the ability of laboratories to identify uncommon bacterial and fungal species on a routine basis. The advantage of more detailed identification is better understanding of the relationship between microorganisms and disease. The disadvantage is the practical challenge of remembering a rapidly expanding list of new names and the difficulty this presents for performing searches of the medical literature. In addition, if clinicians are not familiar with a new name and do not spend the time to research its significance, they may fail to recognize its clinical implications. An up-to-date list

of validly published names of bacteria is accessible at https://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date.html. The International Committee on Taxonomy of Viruses publishes a similar database for viruses (www.ictvonline.org). The *Journal of Clinical Microbiology* published taxonomy updates for parasites and mycobacteria in 2017.[3,4] A useful reference for fungal nomenclature is the MycoBank Database (http://www.mycobank.org/).

INFECTION PREVENTION AND CONTROL

The clinical microbiology laboratory plays an important role in infection prevention and control; infection prevention and control staff and the laboratory should establish a strong and collaborative relationship. Together, they should agree on what the clinical microbiology laboratory staff should notify infection prevention and control about and how that notification should occur. Given the rapid evolution of antimicrobial-resistant organisms and evolving diagnostic tests, there should be a regular review of these practices. Changes in laboratory testing, reporting, or informatics that may impact infection prevention and control should be appropriately handled. The type of isolation that should be used if patients are hospitalized (or ideally systems to automatically make this happen) based on detection of specific microorganisms and resistance factors should be defined. The clinical microbiologist and infection prevention and control specialist should work together to determine what, if any, additional or specialized testing is to be done for infection prevention and control and how billing of such testing should be handled. An example of specialized testing is screening for carbapenemase genes. A list should be put together of key clinical isolates that should be retained for potential future typing to determine relatedness and define potential nosocomial transmission. Clinical microbiology laboratory staff have the unique perspective of seeing all the microorganisms isolated from patients and knowing about all organisms detected in patients throughout the institution and therefore may be the first to recognize a small cluster of unique organisms. However, they are not necessarily systematically looking for this. Infection prevention and control staff also have a unique perspective that involves the ability to assess the likelihood that an infection in an individual patient is nosocomial and a systematic approach to looking for nosocomial transmission. Working in concert, clinical microbiology laboratory staff and infection prevention and control staff play an important role in guiding a potential outbreak investigation.

BACTERIAL, MYCOBACTERIAL, AND FUNGAL IDENTIFICATION

Bacteria, mycobacteria, and fungi were traditionally identified by assessing colony morphology, odor, and hemolytic patterns in addition to results of staining, agglutination tests, and manually generated biochemical tests. Today biochemical profiles are often determined by assessing reactions of individual organisms with substrates using commercial systems. Proteomic analysis using MALDI-TOF mass spectrometry (see following section) enables identification of bacteria and fungi in minutes and is used in many clinical microbiology laboratories. Nucleic acid amplification and, in many cases, sequencing technologies are also used for organism identification. Metabolomic analyses, which may enable identification during growth, are currently in their early stages.

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

MALDI-TOF mass spectrometry is the state-of-the art identification system for bacteria, mycobacteria, and fungi.[5] MALDI stands for matrix that assists in desorption and ionization of highly abundant bacterial and fungal proteins through energy from a laser (Fig. 16.1). Material from a bacterial or fungal colony is placed on a spot on a MALDI-TOF mass spectrometry target plate (a reusable or disposable plate with test spots). Spots are overlain with matrix (or first mixed with a formic acid solution, which is dried, and then overlain with matrix) and dried, and the target plate is placed into a mass spectrometer. The matrix (e.g., α-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile and 2.5% trifluoroacetic acid) isolates microbial molecules from one another, protecting them from fragmentation and enabling their desorption by laser energy; most of the laser energy is absorbed by the matrix, changing it to an ionized state. As a result of random collision in the gas phase, charge is transferred from matrix to microbial molecules; ionized microbial molecules are then accelerated through a positively charged electrostatic field into a time of flight (TOF) tube. Inside the tube, which is under vacuum, ions travel toward an ion detector, with small analytes traveling the fastest, followed by increasingly larger analytes; a mass spectrum is produced, representing the number of ions of a given mass impacting the detector over time. Highly abundant (mainly ribosomal) proteins produce the mass spectrum. Although they are not separately characterized, together they provide a profile unique to specific types of organisms, with peaks specific to species and genera. Computer software compares the produced mass spectrum with a databank of reference spectra, generating a list of the most closely related organisms with numeric rankings. Depending on how high the value (percent or score) of the topmost match is (and bearing in mind the next best matches), the organism is identified at the family, complex, group, genus, species, or subspecies level. The main commercial MALDI-TOF mass spectrometry systems for clinical microbiology testing are from Bruker Daltonics Inc. (Billerica, MA) and bioMérieux Inc. (Durham, NC).

ANTIBACTERIAL SUSCEPTIBILITY TESTING

The gold standard method for susceptibility testing is phenotypic susceptibility testing, which involves growing the organism with and without specific antimicrobial concentrations and comparing results. The traditional measurement of susceptibility is the minimal inhibitory concentration (MIC)—the lowest concentration of the antimicrobial agent that inhibits the growth of the organism. Methods used to determine MICs are standardized and comprise use of uniform organism amounts and growth conditions including media, incubation conditions, and incubation durations. To measure the MIC, the organism is grown in multiple concentrations of an antimicrobial agent spanning a clinically significant range. Dilutions can be prepared in wells of a microtiter well plate (or alternatively tested in test tubes or incorporated into agar plates) and, by convention, are doubled using a base of 1 μg/mL, for example, 0.5, 1, 2, 4, 8, 16, and so on. A standardized inoculum of the isolate is added, and after incubation for a defined time, wells are examined for turbidity produced by microbial growth. The first well in which visible growth is absent (e.g., indicated by clear broth) is the MIC of that organism. The MIC obtained is converted to susceptible, intermediate, susceptible dose dependent, or resistant categories by referring to an interpretative table from the US Food and Drug Administration (FDA), a standards development organization such as the Clinical and Laboratory Standards Institute, or the US or European Committees on Antimicrobial Susceptibility Testing. The 2016 US 21st Century Cures Act has created a system to accelerate the recognition of antimicrobial susceptibility test interpretive criteria or breakpoints. Antibacterial susceptibility test interpretive criteria can be found at https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm575163.htm. Antifungal susceptibility test interpretive criteria can be found at https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm575166.htm. Use of the term *susceptible* indicates that the MIC is at a concentration achievable in blood or other appropriate body fluid using typically suggested doses. The term *resistant,* the opposite of *susceptible,* denotes that the MIC is not exceeded by normally attainable levels. As in all biologic systems, the MIC of some organisms lies between susceptible and resistant levels. Borderline results may be referred to as intermediate or susceptible dose dependent due to technical variability or to indicate that the antimicrobial agent may still be used but at increased doses. Urine levels of some antimicrobial agents may be so high as to enable their use to treat urinary tract infections in the face of high MICs. Hence for some antimicrobial agents, different interpretations of *susceptible* may apply to urinary versus nonurinary isolates. Likewise, for central nervous system infections, there may be separate interpretations. Susceptibility testing may be automated using commercial systems in which organisms are incubated with multiple antimicrobial agents in specialized modules that are read automatically at regular intervals.

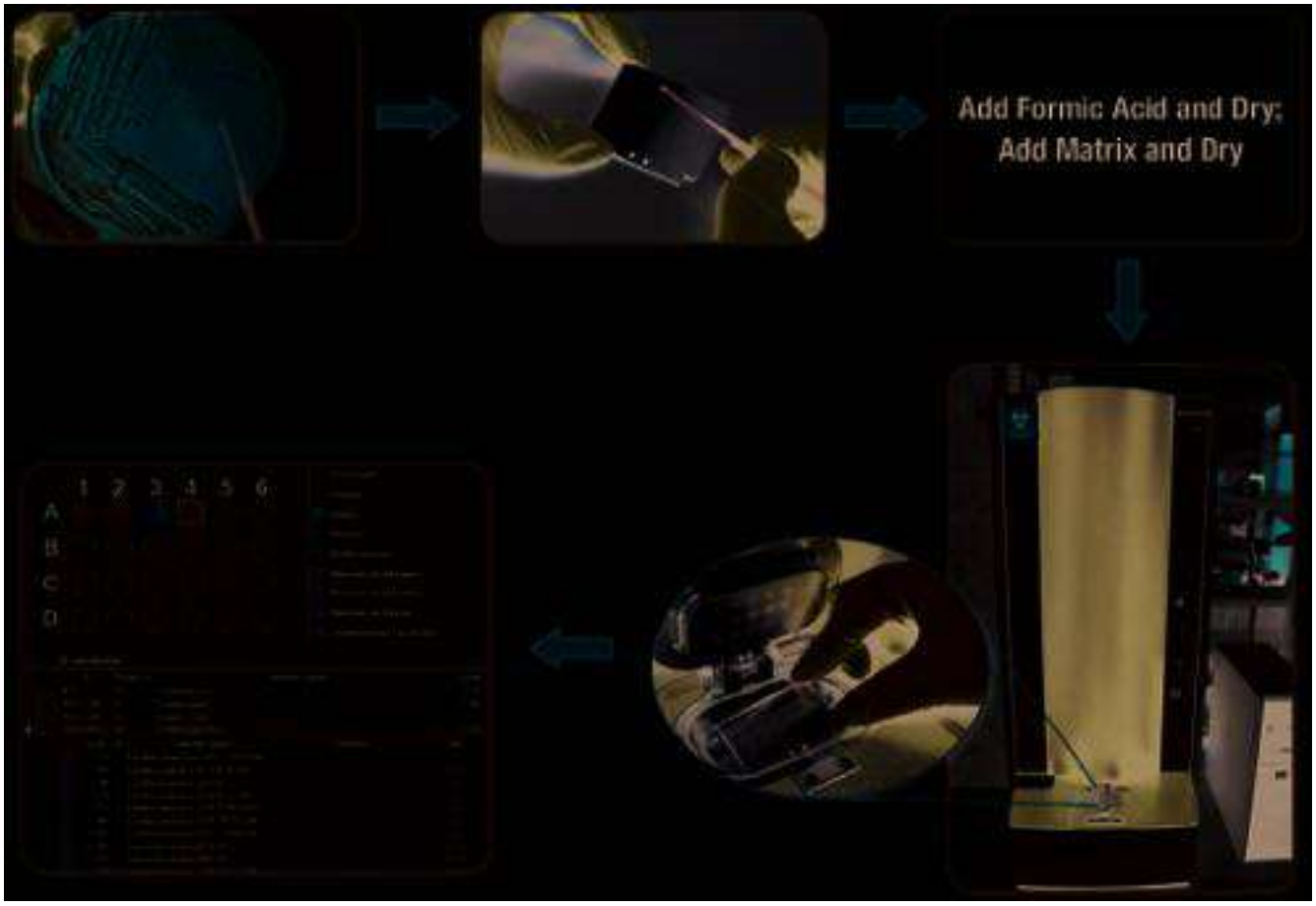

FIG. 16.1 Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. A colony is transferred from a culture plate to a MALDI-TOF mass spectrometry target plate. One or many isolates may be tested at a time, each on a different spot on the MALDI-TOF mass spectrometry target plate. In this example, cells are treated with formic acid on the target plate and allowed to dry. The spot is overlain with matrix and dried. The plate is placed in the ionization chamber of the mass spectrometer. A mass spectrum is generated and compared against a database of spectra by the software, resulting in identification of the microorganism. *(Modified from and used with permission of Mayo Foundation for Medical Education and Research. All rights reserved.)*

With disk diffusion testing, the inoculum is seeded onto the surface of an agar plate, to which disks containing defined amounts of antimicrobials are applied. While the plates are incubating, antimicrobial agents diffuse into the medium, producing a circular gradient around the disk. After incubation, the diameter of the zone of growth inhibition around the disks is used as an indirect measurement of the MIC of the organism. Such testing is influenced by the growth rate of the microorganism, how well the antimicrobic diffuses into the medium, and other technical factors. The diameter of the zone of inhibition obtained is converted to susceptible, intermediate, susceptible dose dependent or resistant categories by referring to an interpretative table. An additional diffusion procedure uses a strip that contains a gradient concentration of an antimicrobic, which produces elliptical zones of inhibition that can be directly correlated with MICs.

Standards development organizations may reset breakpoints over time. New breakpoints should be implemented in a timely manner. Changes in susceptibility testing should be promptly announced by the laboratory to clinicians to avoid interpretive errors. Delays in the adoption of new breakpoints by laboratories may occur; sometimes the manufacturers of automated platforms represent a barrier to implementation of the most current breakpoints. Clinicians should be aware of whether or not their laboratory is using updated breakpoints.

Molecular methods are increasingly applied to antimicrobial susceptibility testing. In some cases, resistance mechanisms for specific organism-antimicrobial agent combinations are so specific as to enable molecular susceptibility testing (e.g., *mecA/mecC* detection for diagnosis of methicillin resistance in *Staphylococcus aureus*). In other cases, molecular mechanisms of resistance are complex, precluding accurate molecular susceptibility testing (e.g., ceftriaxone resistance in *Klebsiella pneumoniae*).

ANTIMICROBIAL STEWARDSHIP

As with infection prevention and control, the microbiology laboratory plays an important role in antimicrobial stewardship; the laboratory director should play an active role in the institution's antimicrobial stewardship program and work collaboratively with antimicrobial pharmacists, if they are present, and infectious diseases clinicians involved in antimicrobial stewardship. In many countries, especially outside of the United States, clinical microbiologists undertake clinical roles outside the laboratory, especially if they are also trained in infectious diseases. Such dual training has certain advantages vis-à-vis antimicrobial stewardship, but either way, clinical microbiologists can play an important role in antimicrobial stewardship. Examples of activities that clinical microbiologists and stewardship groups can undertake in partnership include putting together antibiograms or cumulative antimicrobial susceptibility reports, providing enhanced culture and susceptibility reports, offering guidance in the preanalytic phase, making available rapid diagnostic tests, educating providers, and creating alert and surveillance systems.[2]

Laboratories must publish (preferably online or in an application software ["app"]) the antimicrobial susceptibility patterns for common

bacteria and fungi tested in their laboratory in the recent past (antibiograms or cumulative antimicrobial susceptibility reports) at least annually.[6] The Clinical and Laboratory Standards Institute publishes guidelines for the analysis and presentation of cumulative susceptibility tests.[6] Only diagnostic (i.e., not surveillance) isolates and only routinely tested agents should be included. For *Streptococcus pneumoniae,* data should be reported using both meningitis and nonmeningitis breakpoints, and for *S. aureus,* data should be reported for all isolates and methicillin-resistant *S. aureus*. The antibiogram can be used to guide empirical therapy while culture results are pending, inform and update local guidelines for empirical treatment of common infection syndromes, update procedural or surgical prophylaxis recommendations, inform antimicrobial formulary selection, survey local resistance patterns, identify targets for antimicrobial stewardship interventions and best practices, and provide a background for new drug susceptibility testing.[2] The antibiogram and communication of its results should be reviewed by infection prevention and control staff, pharmacy staff, and the antimicrobial stewardship team of the institution before release to identify and correct errors that might lead to antimicrobial misuse.[2] The antibiogram can be put together only if there are at least 30 isolates in the particular category being addressed. If the number is adequate and there are important differences, data may be stratified by service, unit, resistance mechanism, or specimen type. Combination therapy susceptibilities can be considered and may be useful to guide empirical double therapy when culture results are pending and resistance may be present (e.g., defining which two antimicrobial agents best cover *Pseudomonas aeruginosa*).[2] With the adoption of electronic medical records, antibiograms may be prepared without the participation of the microbiologist, in some cases by systems themselves. Although the information provided can be accurate, errors in interpretation and reporting may lead to the conveyance of misinformation to prescribers, so the clinical microbiologist should remain involved at some level.[2]

The laboratory director should work with infectious diseases clinicians, pharmacy staff, and the antimicrobial stewardship team at their institution to develop guidelines for performing and reporting antimicrobial susceptibility results for bacteria, mycobacteria, and fungi. The primary agents tested and reported should be determined based on the patient population served, local resistance patterns, the hospital formulary, and cost. It should also be determined when additional susceptibility testing should be routinely performed and reported (e.g., when certain resistance patterns are observed). There is a relationship between the antibiotics listed in the clinical report and antibiotic prescription. Cascade or selective reporting (e.g., not reporting carbapenem susceptibility when a microorganism is susceptible to drugs with a narrower spectrum) can be used to promote the judicious use of antimicrobials.[7]

Infectious diseases physicians should work with their laboratories to routinely add reporting comments when specific organisms or resistances are detected in particular specimen types, including advising specific treatment, interpreting results, and suggesting infectious diseases consultations. For example, for staphylococci for which rifampin has been tested and found to be susceptible, a comment such as "Rifampin should not be used as monotherapy" could be added. Automatically added messages are preferred, as their addition is less likely to be overlooked by laboratory technologists than messages added manually.

SPECIMEN SELECTION, COLLECTION, TRANSPORTATION, AND INITIAL PROCESSING

Selection of the appropriate specimens and testing for the detection of an organism responsible for a patient's disease is the ultimate objective. If a specific pathogen is suspected, selection of the appropriate specimen for the specific diagnostic test is relatively straightforward (Table 16.3); however, the differential diagnosis is typically not limited, and a variety of pathogens are usually considered as possibilities. This increases the types of specimens that must be collected and the number of tests ordered. Although this may not pose a problem if the quantity of specimen is sufficient (e.g., blood, urine, stool, sputum), this can present a challenge if specimen quantity is limited or a specimen is difficult to obtain (e.g., tissue biopsy). Thus the clinician must carefully consider potential pathogens and the diagnostic tests that should be ordered. The clinician also must ensure that specimens representative of the site of infection are collected in the appropriate container and transported to the laboratory in a timely fashion. In addition to delaying results, delays while specimens remain in the patient care areas may adversely affect diagnostic testing results.

There are several points to consider regarding specimen quality. Specimens must be accurately labeled. Inadequately labeled specimens as well as specimens of poor quality should be rejected. Commensal microbiota must be avoided when collecting specimens from body sites (e.g., respiratory tract, wounds) that have normal microbiota that can contaminate an inappropriately collected specimen and complicate interpretation. Swabs are appropriate for throat, nares, nasopharyngeal, and some ocular specimens but are not substitutes for tissues, aspirates, or fluids when these are collectable (e.g., at surgery) because swabs hold exceptionally small volumes of specimen. Flocked swabs are more effective than Dacron, rayon, and cotton swabs because their flocked nature allows for efficient release of contents for assessment. Clinicians should not request that the laboratory report "everything that grows" from specimens harboring commensal microbiota or large numbers of species, as this can provide irrelevant information and result in inaccurate diagnosis and inappropriate therapy and is time consuming and costly. Antimicrobial susceptibility testing should be done only on clinically significant isolates and not on all organisms recovered in culture.

Selection of appropriate specimens depends on multiple factors. If the diagnostic test is isolation of the organism in culture, the specimen must contain viable organisms; care should be taken to avoid organisms that may either suppress or overgrow the pathogen or confound the interpretation of the culture. Ideally, specimens for culture (and even for non–culture-based testing based on microbial detection) should be collected before administration of antimicrobial agents. An adequate volume of specimen should be collected to maximize recovery of the pathogen, and an adequate number of specimens should be collected if the organism is transiently present, present in low quantities, or unevenly distributed in clinical specimens. Microscopy is a rapid but typically insensitive method for detection of pathogens. If the quantity of specimen is limited, the value of microscopy must be balanced against the need for other diagnostic tests. In some situations, microbial stains are of such limited value (e.g., diagnosis of prosthetic joint infection) as to not be recommended, whereas in others, microscopy is the primary diagnostic test (e.g., examination of blood smears for malaria). Selection of appropriate specimens for detection of microbial antigens is determined by the suspected pathogen. For example, cerebrospinal fluid (CSF) is appropriate for diagnosis of cryptococcal meningitis, but both CSF and urine may be collected for diagnosis of pneumococcal meningitis. Likewise, the optimal specimens for diagnosis of *Legionella* pneumonia are respiratory secretions for nucleic acid amplification tests (NAATs) and culture and urine for antigen testing. Serologic tests are useful if the collection of blood is properly timed to coincide with the peak level of antibodies or to demonstrate a significant rise in antibodies but may be affected by antibodies elicited by vaccination, prior exposure to the same or similar organisms, or cross-reacting antibodies. Because the value of serologic testing varies for individual pathogens, a pathogen-specific and patient-specific approach is needed.

Transportation of specimens to the microbiology laboratory must be done in a timely fashion and particularly so for urgent requests. Efforts to minimize transit time (e.g., use of pneumatic tube systems) should be made. Generally transport times should be a maximum of 2 hours. Prolonged transportation times can result in loss of microbial viability, compromising sensitivity, or paradoxically, overgrowth of organisms, compromising specificity (e.g., urine cultures). Transit times should be monitored systematically as part of a diagnostic quality assurance program. If there are unanticipated delays, they should be indicated in the clinical report. It is also important to time collection and transportation of specimens to ensure they arrive in the laboratory before established cutoff times for performance of batched tests, especially if such batched tests are infrequently performed. If there are regular delays because of test batching, timing of specimen collection or test

Text continued on p. 202

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens

MICROORGANISM[a]	DETECTION METHOD: Microscopy	Culture	Antigen Detection	NAAT	Antibody Detection	COMMENTS
Adenovirus		±		√		NAAT preferred for active infection
Anaplasma phagocytophilum, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia muris	±			√	√	NAAT preferred for active infection
Aspergillus spp.	√	√	√	±		
Babesia spp.	√			√	√	NAAT or microscopy preferred for active infection
Bacillus anthracis	√	√		±		Notify laboratory for safety reasons and to ensure laboratory knows to look for this organism
Bartonella spp.	±			±	√	Culture insensitive
BK virus	±			√		NAAT preferred for active infection and monitoring response to therapy
Blastomyces spp.	√	√	√	±	√	
Bordetella pertussis		±		√	±	NAAT preferred for active infection
Borrelia burgdorferi sensu lato complex (*B. burgdorferi* [US], *B. garinii* [Europe, Asia], *B. afzelii* [Europe, Asia], *B. mayonii* [US])		±		±	√	
Borrelia miyamotoi				√	√	
Brucella spp.	±	√		±	√	Notify laboratory for safety reasons
Burkholderia pseudomallei	±	√			±	Notify laboratory for safety reasons
Candida spp.	√	√		√		
Chlamydia psittaci				±	√	
Chlamydia trachomatis				√		
Clostridium botulinum	±	√	√			Notify laboratory to ensure appropriate testing is performed
Clostridioides difficile (formerly *Clostridium difficile*)		±	√	√		See text
Clostridium tetani	±	√				Diagnosis primarily clinical; notify laboratory to ensure appropriate testing is performed
Coccidioides immitis/Coccidioides posadasii	√	√	√	±	√	Notify laboratory for safety reasons
Coronaviruses		±		√		
Corynebacterium diphtheriae		√				
Coxiella burnetii				±	√	Notify laboratory for safety reasons; serology test of choice; acute Q fever: fourfold increased titer to phase II antigen or IgM titer ≥50 and IgG titer ≥200; chronic Q fever: IgG titer ≥800 to phase I antigen
Cryptococcus neoformans/ Cryptococcus gattii	√	√	√	±		CSF cryptococcal antigen test preferred for cryptococcal meningitis; fungal stain and culture should also be ordered; NAATs not as sensitive as antigen tests for monitoring CSF on therapy
Cryptosporidium spp.	±		√	√		
Cyclospora cayetanensis	√			√		
Cytomegalovirus	±	±	±	√	√	Quantitative NAATs of plasma used for immunocompromised hosts; antibody detection used to assess immune status and for workup of infection in immunocompetent hosts
Dengue			√	√	√	Detection of dengue virus RNA, nonstructural protein 1 antigen, or anti–dengue virus IgM suggestive of acute infection with dengue virus
Dermatophytes (*Epidermophyton* spp., *Trichophyton* spp., *Microsporum* spp.)	√	√				Submit skin scrapings, hair follicles, nail scrapings, tissue biopsy specimen
Ebola				√		Notify laboratory for safety reasons; viral culture should not be performed on patients with suspected Ebola virus disease

Continued

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens[a]—cont'd

	DETECTION METHOD					
MICROORGANISM[a]	**Microscopy**	**Culture**	**Antigen Detection**	**NAAT**	**Antibody Detection**	**COMMENTS**
Entamoeba histolytica	√		√	√	√	Antibody detection useful only for invasive/disseminated disease (e.g., amebic liver abscess)
Enterobius vermicularis	√					Submit paddle or cellophane tape after sampling perianal area
Enterovirus/parechovirus		±		√	±	NAAT preferred for acute diagnosis
Epstein-Barr virus				√	√	Quantitative NAATs of blood used for immunocompromised hosts; antibody detection used to assess immune status and for workup of infection in immunocompetent hosts
Erysipelothrix rhusiopathiae	±	√				
Francisella tularensis		√			±	Notify laboratory for safety reasons and to ensure appropriate media are inoculated for culture
Free-living amebas (*Acanthamoeba* spp., *Balamuthia mandrillaris, Naegleria fowleri*)	√	√		√		
Giardia spp.	±		√	√		
Haemophilus ducreyi	±	√				Selective and differential medium needed for culture
Helicobacter pylori	√	±	√	±		See text; urea breath test also recommended
Hepatitis A, B, C, D, and E			√	√	√	See Chapter 117
Herpes simplex virus	±	±		√	±	NAAT preferred for diagnosis of acute infection; antibody detection useful to assess immune status
Histoplasma capsulatum	√	√	√	±	√	
Human herpesvirus 6				√	√	NAAT preferred for diagnosis of acute infection
Human immunodeficiency virus		±	√	√	√	See Chapter 120
Human metapneumovirus				√		
Human papillomavirus	√			√		
Influenza		±	√	√		NAAT preferred for diagnosis of acute infection; negative result by rapid antigen testing should be confirmed by NAAT or viral culture
Intestinal nematodes (*Ascaris lumbricoides, Strongyloides stercoralis, Trichuris trichiura,* hookworms)	√	[b]			[b]	
JC virus				√	±	NAAT preferred for diagnosis of acute infection
Kingella kingae	±	√		√		NAAT particularly useful in young children with osteoarticular infection
Klebsiella granulomatis	√			±		Microscopy (Giemsa stain, not Gram stain) test of choice; does not grow on conventional media
La Crosse virus					√	
Legionella spp.		√	√	√	±	Urine antigen tests sensitive (>80%) for *Legionella pneumophila* serogroup 1. *Legionella* culture and NAATs preferred (sensitive and specific, although NAATs are more sensitive than culture); antibody detection less sensitive, as antibodies may develop slowly and IgM may persist for ≥1 year
Leishmania spp.	√	√		√	√	Serology useful for visceral leishmaniasis; species identification (used to guide treatment of American cutaneous leishmaniasis) requires culture or NAAT or both
Leptospira spp.		±		±	√	Culture requires specialized techniques with organisms isolated in blood during first week of illness and urine thereafter; microscopy (darkfield) insensitive and requires experienced observer; serology most frequently performed test; microagglutination titer ≥200 or seroconversion diagnostic; serology is insensitive in early stages of disease, and seroconversion may be delayed
Listeria monocytogenes	√	√		±		
Lymphocytic choriomeningitis virus				±	√	

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens[a]—cont'd

MICROORGANISM[a]	DETECTION METHOD					COMMENTS
	Microscopy	Culture	Antigen Detection	NAAT	Antibody Detection	
Malassezia spp.	√	√				Notify laboratory when suspected; some species require addition of lipids to culture medium for growth
Measles				√	√	
Mucorales	√	√		±		Ask laboratory to avoid grinding tissue if suspected
Mumps				√	√	
Mycobacterium, nontuberculous (other than species below)	√	√				Microscopy and culture most commonly performed; molecular probes used to identify cultured isolates
Mycobacterium genavense	√	√				Notify laboratory when suspected; requires addition of mycobactin J to medium for ideal growth in culture
Mycobacterium haemophilum	√	√				Notify laboratory when suspected; requires addition of X-factor to medium and lower temperature (30°C) for ideal growth in culture
Mycobacterium marinum	√	√				Notify laboratory when suspected; requires lower temperature (30°C) for ideal growth in culture
Mycobacterium tuberculosis	√	√		√		See text; microscopy, culture, and NAATs most commonly performed; interferon-γ release assays and tuberculin skin tests provide evidence of infection (latent or active) but may be negative with active infection
Mycobacterium ulcerans	√	√		√		Notify laboratory when suspected; requires lower temperature (30°C) for ideal growth in culture
Mycoplasma pneumoniae				√	±	NAAT preferred; seroconversion may be delayed; cold agglutinin testing not recommended
Neisseria gonorrhoeae	±	±		√		See text; NAATs preferred; microscopy and culture less sensitive compared with NAATs; culture used if an isolate is needed for susceptibility testing
Neisseria meningitidis	√	√		±		
Nocardia spp.	√	√		±		Grow on mycobacterial and fungal cultures (and sometimes on bacterial media such as that used for *Legionella* culture)
Norovirus			√	√		
Parainfluenza		√		√		
Parvovirus B19	±			√	√	
Plasmodium spp.	√		±	±		Confirm antigen detection test results (positive and negative) by microscopy or NAAT when possible
Pneumocystis jirovecii	√		√	√		Does not grow in culture; β-D-glucan test may be positive
Powassan/deer tick virus				√	√	
Rabies virus	√		√	√	√	
Relapsing fever *Borrelia* spp. (*B. hermsii* [western US], *B. parkeri* [western US], *B. turicatae* [southwestern US], *B. mazzottii* [southern US], *B. recurrentis* [sub-Saharan Africa])	√	±		±	±	Darkfield microscopy or Wright, Giemsa, or Diff-Quik stains of peripheral thin or thick blood smears (or both) recommended; may be seen in direct wet preparation of blood in some cases
Respiratory syncytial virus		±	√	√		
Rhodococcus equi	√	√				
Rubella					√	Antibody detection useful to assess immune status
Sapovirus				√		
Scabies/lice	√					Macroscopic and microscopic visualization
Sporothrix schenckii complex	√	√			√	
St. Louis encephalitis virus					√	
Streptococcus pneumoniae	√	√	√	±		Microscopy and culture most commonly used tests; urine antigen tests can be helpful

Continued

TABLE 16.3 Methods for Detection of Selected Microorganisms in Clinical Specimens[a]—cont'd

MICROORGANISM[a]	DETECTION METHOD: Microscopy	Culture	Antigen Detection	NAAT	Antibody Detection	COMMENTS
Streptococcus pyogenes (group A)	√	√	√	√	±	Antigen detection methods (backed up by culture, if negative) and NAATs used to test throat swabs; antibody detection used for diagnosis of poststreptococcal diseases
Taenia solium (neurocysticercosis)	±				√	
Tick-borne encephalitis virus					√	Detection of specific IgM in either blood or CSF
Toxocara spp.					√	Larvae rarely seen in histopathologic sections of biopsies of infected tissues (e.g., liver)
Toxoplasma gondii	±			±	√	
Treponema pallidum	±			±	√	See text
Trichinella spiralis	±				√	
Tropheryma whipplei	√			√		
Trypanosoma cruzi	√				√	
Trypanosomiasis, African (African sleeping sickness; *Trypanosoma brucei gambiense* [West African], *T. b. rhodesiense* [East African])	√				√	
Varicella-zoster virus	±	√		√	±	NAAT preferred for diagnosis of acute infection; antibody detection useful to assess immune status
Vibrio spp.		√		±		
West Nile virus				√	√	
Yersinia pestis	√	√	±	±		
Zika				√	√	

[a]Bacteria and fungi not specifically listed are most commonly detected by microscopy and culture. For esoteric organisms not listed, contact the local laboratory.
[b]Antibody detection and stool agar culture may be useful for *S. stercoralis*.
CSF, Cerebrospinal fluid; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *NAAT,* nucleic acid amplification test.

performance should be addressed. Table 16.4 summarizes normally accepted guidelines for specimen collection and transportation of common specimen types for microbiology testing. Not all situations are covered; for special instances, clinicians should contact their laboratory to discuss the best procedures for ensuring optimal handling of specimens.

SPECIFIC SPECIMEN GUIDELINES AND INITIAL LABORATORY PROCESSING

Blood Cultures

Blood cultured for bacteria and *Candida* species is one of the most essential tests performed in the clinical microbiology laboratory. Because relatively few bacteria and *Candida* species are present in the blood of patients with bacteremia or candidemia, a sufficient volume of blood should be cultured. Current blood culture systems include commercial purpose-specific blood culture bottles that are incubated on specifically configured blood culture instruments that are fully automated from the moment blood culture bottles are loaded onto the instruments until the bottles signal positive. These systems monitor blood culture bottles for microbial growth every 10 to 24 minutes. A variety of blood culture bottle types are available for these systems, including aerobic and anaerobic bottles, bottles configured for children, and fungal and mycobacterial bottles. Resin-supplemented bottles are designed to bind and remove antibiotics and therefore improve recovery of bacteria in patients who have been treated with antibiotics.[8] Blood cultures are routinely incubated for 5 days, although most are positive within 48 hours. Incubation beyond 5 days is seldom required, although it may be useful for recovering *Cutibacterium acnes* (e.g., in cases of *C. acnes* endocarditis). Bacteria that historically required special blood culture procedures such as HACEK bacteria (*Haemophilus* spp. *[H. parainfluenzae], Aggregatibacter* spp. *[A. actinomycetemcomitans, A. aphrophilus], Cardiobacterium* spp. *[C. hominis, Cardiobacterium valvarum]/Eikenella corrodens,* and *Kingella* spp.), *Abiotrophia* and *Granulicatella* species, and *Brucella* species are readily isolated using today's routine blood cultures. *Brucella* species will usually grow within a standard 5-day incubation period, although some experts recommend holding blood cultures for *Brucella* species for 7 days.[9] *Bartonella* species do not grow well in current routine blood culture systems; bartonellosis is best diagnosed serologically or using NAATs. Likewise, *Coxiella burnetii* is best diagnosed serologically or with a NAAT.

Specially designed fungal and mycobacterial blood culture bottles are available. A lysis-centrifugation system such as the ISOLATOR system (Abbott/Alere, Waltham, MA) with prolonged incubation can be used for recovery of dimorphic fungi, molds, mycobacteria, and *Nocardia* species (*Nocardia* species may also grow in routine blood culture bottles). Although lysis-centrifugation offers advantages for these organisms, it is labor intensive and has high rates of contamination; therefore it should not be used for routine blood cultures. *Candida* species typically grow in standard blood culture bottles.

The most important factor that influences recovery of organisms in blood cultures is the volume of blood collected; typically the number of organisms in the blood of a septic patient is low (an average of less than 1 organism/mL).[10] The current recommendation for adult patients is that 20 to 30 mL of blood be collected and distributed into two to three blood culture bottles per draw and that two draws are routinely performed. Routine use of a blood culture from a single draw is not acceptable. To maximize yield, six bottles, collected across two to three sets, should be collected. Although the concept of spacing blood culture draws (i.e., to detect continuous bacteremia) has been commonly practiced, there is little evidence supporting the need for this; separation of blood culture draws over time should not be the standard for routine blood culture draws. For endocarditis diagnosis, three sets of blood cultures, with each set including one aerobic and one anaerobic bottle, may be collected; alternatively, two sets may be collected, with two aerobic bottles and one anaerobic bottle per set (i.e., a total of six blood culture bottles).[11] Repeat blood cultures are generally not useful in patients with recent negative blood cultures. For neonates and children,

TABLE 16.4 Collection and Transportation of Specimens for Microbiologic Testing

SPECIMEN	COLLECTION GUIDELINES	OTHER COMMENTS
Abscess		
	Aspirate pus or fluid in anaerobic transport vial; clean surface of closed abscess with 70% alcohol; collect specimens at margins of abscess	Aspirates in anaerobic transport tubes acceptable for aerobic and anaerobic bacterial, fungal, and mycobacterial cultures; separate vessel, without media, should be used for NAATs, if desired
Blood for Culture		
Routine aerobic and anaerobic bacteria and *Candida* spp.	Disinfect bottle tops with 70% isopropyl alcohol; disinfect phlebotomy site with alcohol, followed by tincture of iodine, chlorine pexoide, or chlorhexidine; allow disinfectants to dry Collect 20–30 mL in adults for each blood culture; divide blood into two or three blood culture bottles, consisting of one to two aerobic bottles and an anaerobic bottle; perform two blood culture draws routinely for adults	Blood culture bottles should be loaded onto blood culture instruments within 2 hours of collection Blood cultures should be collected before administering antibiotics; resins designed to inactivate antibiotics may be helpful for patients who have received antibiotics, but administration of antibiotics should be avoided, if possible, before blood culture collection
Mycobacteria, and fungi	Use special fungal/mycobacterial blood culture bottles	*Candida* spp. will grow in routine blood culture bottles
Blood for Microscopic Examination		
Plasmodium spp., *Anaplasma* spp., *Ehrlichia* spp., *Borrelia* spp. causing relapsing fever	Prepare thick and thin smears from fingerstick or EDTA blood	Transport rapidly; NAATs more sensitive than direct smears for *Anaplasma* and *Ehrlichia* spp.
Intravascular Catheter Tip		
	Remove aseptically, cut at least 2-inch segment from tip, place segment in sterile container	Transport rapidly (prevents drying out) Culture of Foley and drainage catheters not recommended
Ear		
Inner	For intact eardrum, clean ear canal with soap solution or 70% alcohol, then aspirate by syringe For ruptured eardrum, collect drainage with swab	If specimen collected by aspiration, inoculate media at time of collection or immediately transport syringe to laboratory
Outer	Remove crusted debris and firmly scrape or swab outer ear canal	
Eye		
Conjunctiva	Roll swab over conjunctiva; stains should be prepared and media inoculated at time of collection	Number of stains prepared and media inoculated limited because of small amount of material collected
Cornea	Use sterile spatula to scrape surface of cornea; stains should be prepared and media inoculated at time of collection	Number of stains prepared and media inoculated limited because of small amount of material collected; anesthetics may inhibit growth of some bacteria
Vitreous and anterior chamber fluids	Because limited amounts of fluid can be collected by aspiration, fluid should be transported immediately to laboratory in properly enclosed collection syringe, or stains should be prepared and media inoculated at time of collection	Number of stains prepared and media inoculated limited because of small amount of material collected
Stool		
	Collect in sterile container and deliver immediately to laboratory or collect and transport in Cary-Blair medium depending on local laboratory policies and tests ordered	Notify laboratory if specific pathogen is suspected (e.g., *Vibrio* spp., *Aeromonas* spp.).Testing for bacterial pathogens other than *Clostridioides difficile* (formerly *Clostridium difficile*) not recommended for patients hospitalized for more than 3 days
Body Fluids (Other Than Blood, Urine)		
Pleural, pericardial, peritoneal fluids	Collect sufficient volume for all tests ordered, including potential future "add-on" tests	If sufficient material can be collected, inoculate aerobic and anaerobic blood culture bottles for bacterial and candidal cultures; separate vessel needed for other tests (e.g., Gram stain, NAATs)
Synovial fluid	Collect sufficient volume for all tests ordered, including potential future "add-on" tests	If sufficient material can be collected, inoculate aerobic and anaerobic blood culture bottles for bacterial and candidal cultures; separate vessel needed for other tests (e.g., Gram stain, NAATs)
Cerebrospinal fluid	Collect sufficient volume for all tests ordered, including potential future "add-on" tests	Do not inoculate blood culture bottles
Genital Tract (Commonly Submitted Specimens)		
Cervical, urethral	Remove mucus and secretions from cervical os; using fresh swab, sample endocervical canal Collect material expressed from urethra on sterile swab or use thin urethral swab to sample urethra	Specimens collected for culture or stains should be inoculated onto media at collection or immediately transported to laboratory; do not allow swab to dry If specimen being submitted for a NAAT, use specific collection device designed for the system
Vaginal	Obtain secretions from vagina with swab	
Nails and Hair		
	Clip affected areas; transport to laboratory in dry, sterile container	Culture for yeasts and dermatophytes

Continued

TABLE 16.4 Collection and Transportation of Specimens for Microbiologic Testing—cont'd

SPECIMEN	COLLECTION GUIDELINES	OTHER COMMENTS
Respiratory, Upper		
Anterior nares	Insert swab 1–2 cm into anterior nares and rotate against nasal mucosa	Generally used to detect carriage of *Staphylococcus aureus*
Nasopharynx	Nasopharyngeal washings and swabs used for respiratory viruses, *Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae,* and *Mycoplasma pneumoniae*	
Paranasal sinuses	Ideally collect by aspiration (see text); if bacteria suspected, submit in anaerobic transport system	Aerobic and anaerobic bacterial cultures should be performed if bacterial etiology suspected; fungal stains and culture should be performed if fungal infection suspected
Throat or pharynx	Swab posterior pharynx, avoiding saliva, for detection of *Streptococcus pyogenes*	Notify laboratory if an organism other than *S. pyogenes* suspected and testing for it desired
Respiratory, Lower		
Bronchoalveolar lavage fluid	Large volume of fluid may be collected; transport in sterile container	
Bronchial brushing or biopsy	Transport specimen in sterile container	
Sputum, expectorated	Have patient rinse mouth with water to get rid of food particles or saliva; instruct patient to cough deeply and expectorate secretions from lower airways; collect and transport in sterile container	Presence of epithelial cells indicative of contamination with oral flora; contaminated specimen unacceptable for routine bacterial culture but may be processed for mycobacterial and fungal culture
Sputum, induced	Induced with sterile saline using nebulizer	
Endotracheal aspirate or tracheal secretions	Acceptable for same tests as expectorated sputum but not screened for acceptability	Contamination with oral bacteria common; specimen processed for bacteria despite presence of epithelial cells
Tissues and Biopsies		
	Keep specimen moist and transport rapidly to laboratory; transport in anaerobic transport vial if testing for anaerobic bacteria desired; do not use bacteriostatic saline or formalin; biopsies suitable specimens for aerobic and anaerobic bacterial, fungal, and mycobacterial cultures, depending on site of biopsy	Specify type of tissue and anatomic location from which it was collected
Urine		
Midstream	Instruct women to wash hands, hold labia apart, discard first portion of voided urine, and collect midstream portion in sterile container Instruct men to wash hands, retract foreskin, discard first portion of voided urine, and collect midstream portion in sterile container	Cleansing before voiding does not consistently improve specimen quality; if patient is unable to provide proper specimen, cleansing and supervised collection may be helpful Collect first-voided urine for urethral pathogens (e.g., *Chlamydia trachomatis, Neisseria gonorrhoeae*) Urine specimens should be cultured within 2 hours of collection if stored at room temperature or within 24 hours of collection if refrigerated; if refrigeration is not an option, addition of boric acid to prevent overgrowth of contaminating organisms may be considered *Actinotignum schaalii* is a uropathogen that typically grows in 48 hours (or under anaerobic conditions) with 5% carbon dioxide
Catheterized	If patient has an indwelling Foley catheter, it should ideally be removed and urine collected either by midstream voided or repeat catheterization Transport as for midstream specimen	
Suprapubic aspirate	Transport as for midstream specimen	Only urine specimen acceptable for anaerobic culture; expel air bubbles from syringe and inject into anaerobe culture vial

EDTA, Ethylenediaminetetraacetic acid; *NAAT,* nucleic acid amplification test.
Data from Miller JM, Binnicker MJ, Campbell S, et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis. *2018;67:e1–e94.*

proportionately smaller volumes of blood are collected; guidelines regarding recommended volumes of blood for culture in children have been published.[12]

Peripheral venipuncture is the preferred approach for obtaining blood for culture because intravascular catheter collection may be associated with a higher likelihood of contamination. Contaminants, typically originating from the skin, are common in blood cultures, typically in approximately 1.5%, but up to 3% is acceptable. Ideally suspected contaminants (e.g., single blood cultures positive for coagulase-negative *Staphylococcus* spp.) should be rapidly but minimally identified and reported with a comment indicating that they are likely contaminants, such as the following: "Possible blood culture contaminant (unless isolated from more than one blood culture draw or clinical case suggests pathogenicity). No antibiotic treatment is indicated for blood culture contaminants." To minimize the risk of contamination, scrupulous care should be taken in skin disinfection before venipuncture. Following scrubbing of the skin with isopropyl alcohol, the skin is prepared with tincture of iodine, chlorine peroxide, or chlorhexidine gluconate before collecting blood cultures.[13,14] Chlorhexidine gluconate is not recommended in infants younger than 2 months of age. The septum of the culture bottle or tube should be wiped with 70% isopropyl alcohol and allowed to air dry before puncture. Products that allow diversion and

discard of the first few milliliters of blood that are most likely to contain skin contaminants (e.g., as a result of containing a skin plug) can decrease contamination rates[15] but add cost to blood cultures.[16]

Blood can be collected with a needle and syringe or directly into the culture bottles with a collection system. If a needle and syringe are used, the needle need not be changed between collection and inoculation of the blood culture system because this poses an unnecessary risk of needlestick injury and does not reduce contamination rates.[17] In patients with central venous catheters in whom catheter-associated bacteremia is suspected, one blood culture should be collected via venipuncture and another through the catheter. If the catheter has multiple lumina, blood cultures should be collected through each of the lumina if trying to diagnose catheter-associated bacteremia. Blood cultures collected through a contaminated line lumen have a higher inoculum than cultures collected from a peripheral vein; therefore cultures drawn through the contaminated line lumen will be positive before those drawn peripherally. If both blood cultures grow the same organism and the blood culture drawn from the line lumen is positive more than 2 hours before the blood culture drawn by peripheral venipuncture, there is a high likelihood of catheter-associated bloodstream infection.[18]

A number of techniques can be used to rapidly test positive blood culture bottles to determine the organisms present and, in some cases, their susceptibility. Direct testing of positive blood culture bottles by MALDI-TOF mass spectrometry after short-term subculture plate incubation (i.e., 2–6 hours) of high-inoculum subcultures on solid media has been adopted by many laboratories as a cost-effective approach to rapid organism identification. (Methods for testing positive blood culture bottles directly using MALDI-TOF mass spectrometry have also been described.) In addition to assays that detect small numbers of organisms and resistance genes, there are multiplex assays approved by the FDA that simultaneously detect a panel of microorganisms as well as select resistance genes directly from positive blood culture bottles; examples include the FilmArray Blood Culture Identification panel (BioFire Diagnostics LLC, Salt Lake City, UT) and the Nanosphere Verigene Gram-Positive and Gram-Negative Blood Culture tests (Luminex, Northbrook, IL).[19] The Accelerate Pheno system (Accelerate Diagnostics, Inc., Tucson, AZ) is an automated system that uses gel electrofiltration and fluorescence in situ hybridization for identification of a limited number of bacteria and yeast directly from positive blood cultures. It is the first assay approved by the FDA to offer rapid phenotypic susceptibility testing directly from positive blood cultures. The system provides MIC values by analyzing bacterial growth in the presence of antibiotics using automated microscopy and time lapse imaging.[20] Given the costs of the multiplex molecular assays, numerous studies have assessed their economic and clinical effects. Although these studies generally demonstrate a decrease in time to optimization of antimicrobial therapy, there have been inconclusive results in terms of impact on mortality and lengths of stay. Institution-specific variables, such as distinct local resistance rates and patient populations, and the availability of antimicrobial stewardship programs probably influence the clinical impact of rapid molecular assays for testing positive blood culture bottles. These panels have the most impact when results are reported as quickly as possible and properly acted on by providers caring for patients. Antibiotic deescalation is ideally accomplished in the setting of delivery of results to an expert in antimicrobial stewardship (e.g., infectious diseases pharmacist, infectious diseases physician, doctoral-level clinical microbiologist) who can then deliver individualized and rapid direction to providers caring for patients.[21]

Intravenous Catheter Tips

The semiquantitative method of Maki et al.,[22] performed by rolling the tip across a blood-agar plate, is the most common method for culturing catheter tips. The detection of 15 or more colonies is indicative of a catheter infection. Adjustments of the Maki method have been described, as have approaches that use vortexing, sonication, flushing, or brushing of the catheter tip, but are not routinely performed.

Cerebrospinal Fluid

Three or four tubes of CSF are usually submitted for laboratory studies. The first has the most potential for contamination with skin flora and therefore should not be sent to the microbiology laboratory for direct smears, culture, or NAATs. The volume of CSF submitted should be sufficient for all tests ordered, as well as for those tests that may be added on at a later time; the total volume needed can be calculated by consulting the laboratory's test catalog. Mycobacterial and fungal cultures in particular ideally require larger volumes of CSF for ideal sensitivity. When the specimen volume is less than necessary for numerous test requests, prioritization of testing must be provided to the laboratory.

The most common causes of acute meningitis/encephalitis today are enteroviruses, herpes simplex virus (HSV), and bacteria (*S. pneumoniae* and *Neisseria meningitidis*). CSF NAATs for enterovirus and HSV types 1 and 2 are the tests of choice for central nervous system infection caused by these organisms. CSF NAATs for parechoviruses and varicella-zoster virus may be considered under relevant clinical conditions. If possible, specimens for bacterial culture should be obtained before antimicrobial therapy initiation, but antimicrobial therapy should not be delayed to collect CSF. CSF Gram stains should be performed on cytocentrifuged specimens, with positive results called to the patient care area immediately. Gram stain is generally positive in patients with bacterial meningitis (infection with *Listeria monocytogenes* is an exception); however, prior exposure to antibiotics can rapidly eliminate organisms in CSF or alter their Gram stain properties. Aside from the possible exception of *S. pneumoniae,* use of direct antigen tests for bacteria in CSF is not recommended. Antigen tests for *Cryptococcus neoformans* and *Cryptococcus gattii* are, however, sensitive and specific. Cultures for the classic causes of bacterial meningitis (e.g., *Streptococcus agalactiae, S. pneumoniae, N. meningitidis, L. monocytogenes*) are generally positive within 1 to 2 days; however, their recovery in culture can be decreased by prior exposure to antibiotics. The cryptococcal antigen test has replaced India ink staining for rapid diagnosis of cryptococcal meningitis. For central nervous system infection, this test is more sensitive when performed on CSF rather than serum.

Organisms that cause chronic meningitis include *Mycobacterium tuberculosis,* fungi, and spirochetes. Because the sensitivity of NAATs for *M. tuberculosis* in CSF may be poor, acid-fast bacillus (AFB) smear and mycobacterial cultures, performed on large volumes (≥5 mL) of CSF, should be requested in addition to NAATs if tuberculous meningitis is suspected.[23] For diagnosis of central nervous system infection caused by *Histoplasma capsulatum, C. immitis* and *C. posadasii, Blastomyces* spp., or *Sporothrix schenckii* complex, CSF fungal stains and culture should be supplemented with serologic testing (with concomitant serum testing), urine antigen testing (for *H. capsulatum, C. immitis* and *C. posadasii, Blastomyces* spp.), and, where available, NAATs performed on CSF. Serologic testing is the test of choice for *Treponema pallidum* (neurosyphilis) and *Borrelia burgdorferi* (neuroborreliosis).

Infection with a number of other central nervous system infection pathogens including bacteria (e.g., agents of ehrlichiosis and anaplasmosis, *Bartonella* spp., *C. burnetii, Leptospira* spp., *Mycoplasma pneumoniae, Nocardia* spp., *Rickettsia* spp., *Tropheryma whipplei*), viruses (e.g., adenovirus, cytomegalovirus, human herpesvirus 6, influenza virus, human immunodeficiency virus, lymphocytic choriomeningitis virus, measles virus, mumps virus, rabies virus, West Nile virus, Zika virus, California virus, Eastern equine encephalitis virus, St. Louis encephalitis virus, Western equine encephalitis virus, Powassan virus, and Jamestown Canyon virus, as well as other arboviruses), parasites (e.g., *Acanthamoeba* spp., *Balamuthia mandrillaris, Baylisascaris procyonis, Naegleria fowleri, Trypanosoma brucei, Toxoplasma gondii*), and prions can be assessed by testing CSF. Details regarding testing for each of these can be found in individual chapters of this book and the Infectious Diseases Society of America guidelines.[12] In shunt-associated or postneurosurgical infections or focal infections of the brain parenchyma, the differential diagnosis is broadened; in this case, CSF should be cultured for aerobic bacteria as well as anaerobic bacteria, and a broader list of fungi should be considered in the differential diagnosis.

A multiplex polymerase chain reaction panel for testing CSF (FilmArray Meningitis/Encephalitis panel; BioFire Diagnostics LLC) has been approved by the FDA. The panel includes 14 targets (*Escherichia coli* K1, *H. influenzae, L. monocytogenes, N. meningitidis, S. agalactiae, S.*

pneumoniae, cytomegalovirus, enterovirus, HSV types 1 and 2, human herpesvirus 6, human parechovirus, varicella-zoster virus, and *C. neoformans* and *C. gattii*) and generates results in approximately 1 hour.[24] Sanger sequencing-based (e.g., 16S ribosomal RNA gene) and shotgun metagenomics approaches are also being applied. Studies are needed to define the impact on treatment decisions, cost-effectiveness, and, ultimately, patient outcomes of these types of tests.

Peritoneal, Pleural, and Pericardial Fluids

Peritoneal, pleural, and pericardial fluids may be cultured in aerobic and anaerobic blood culture bottles if the presence of multiple organisms is reasonably uncertain (e.g., spontaneous bacterial peritonitis). If inoculated into blood culture bottles at the bedside, additional fluid in a sterile container should be submitted for any desired tests other than bacterial culture (e.g., Gram stain). Culture in blood culture bottles is not ideal for polymicrobial specimens, as this strategy may not accurately assess the variety of organisms present because some organisms may be obscured by overgrowth of other more rapidly growing organisms. Negative cultures in the presence of other indicators of infection should prompt an assessment for fastidious or slow-growing organisms (e.g., *M. tuberculosis,* fungi, *Neisseria gonorrhoeae*). As with CSF, fungal and mycobacterial cultures of peritoneal, pleural, and pericardial fluids are best performed with large specimen volumes. The microbiology laboratory should be contacted if *N. gonorrhoeae* is under consideration because special culture media or NAAT, or both, may be appropriate. Swabs are inappropriate for testing peritoneal, pleural, and pericardial fluids.

Bone and Joint Specimens

Processing synovial fluid is similar to processing peritoneal, pleural, and pericardial fluids except that a smaller volume of fluid is generally available, and infection is most commonly caused by a single organism. As with peritoneal, pleural, and pericardial fluids, swabs are inappropriate for testing synovial fluid. Synovial fluid is ideally cultured in aerobic and anaerobic blood culture bottles. Additional fluid in a sterile container should be submitted for any desired tests other than bacterial culture (e.g., Gram stain). Gram stain is recommended for native joint septic arthritis. In young children with septic arthritis in whom *Kingella kingae* is being considered, NAAT is recommended because this organism does not always grow in culture. In patients with an arthroplasty at the site from which the synovial fluid is aspirated, some organisms otherwise considered contaminants may be pathogens (e.g., coagulase-negative staphylococci such as *Staphylococcus epidermidis*). *C. acnes,* which can cause prosthetic joint infection, may require up to 2 weeks for isolation.

For diagnosis of osteomyelitis, as much specimen as possible should be submitted for aerobic and anaerobic cultures and (except in the case of prosthetic joint infection) Gram stain; specimens may include pieces of intact bone and scrapings, shavings, or excised or aspirated necrotic material. Swabs and cultures of sinus tracts are generally not recommended because recovered organisms, aside from *S. aureus,* do not correlate with organisms found in deep cultures. In patients with diabetic foot osteomyelitis, specimens for aerobic and anaerobic culture of bone can be obtained by open débridement, needle puncture, or transcutaneous biopsy. Readers are referred to an Infectious Diseases Society of America guideline on the diagnosis of diabetic foot infections.[25]

For vertebral osteomyelitis, disk space infection, and spondylodiskitis, aerobic and anaerobic bacterial and candidal blood cultures are recommended; *Brucella* blood cultures and serologic tests should be obtained in patients in areas endemic for brucellosis, fungal blood cultures should be obtained in patients with relevant epidemiologic or host risk factors, and a purified protein derivative test or interferon-γ release assay should be obtained in patients at risk for tuberculosis. Patients suspected to have native vertebral osteomyelitis based on clinical, imaging, and laboratory studies with *S. aureus, Staphylococcus lugdunensis,* or *Brucella* bloodstream infection or, in an endemic setting with a positive *Brucella* serology, do not need further testing. For all others, imaging-guided aspiration or biopsy of a disk space or vertebral endplate with the specimens submitted for Gram stain and aerobic and anaerobic culture and, if adequate tissue can be obtained, histopathology, is recommended. If results are negative or inconclusive (e.g., *Corynebacterium* spp. is isolated), a second imaging-guided aspiration biopsy, percutaneous endoscopic diskectomy and drainage procedure, or open excisional biopsy should be considered to collect additional specimens for repeat and additional testing. Readers are referred to an Infectious Diseases Society of America guideline on the diagnosis of native vertebral osteomyelitis in adults.[26]

In patients with potential prosthetic joint infection, multiple tissue specimens should be submitted for aerobic and anaerobic cultures—four if using conventional plate and broth cultures and three if culturing tissues in aerobic and anaerobic blood culture bottles, a strategy that increases sensitivity.[27,28] Two or more intraoperative cultures or a combination of preoperative aspiration and intraoperative cultures that yield the same organism are considered definitive evidence of prosthetic joint infection. Single positive tissue or synovial fluid cultures, especially for organisms that may be contaminants (e.g., *S. epidermidis, C. acnes*), should not be considered definitive evidence of prosthetic joint infection. Tissue Gram stain is not recommended for prosthetic joint infection diagnosis. Isolation of *C. acnes* may require culture incubation times of 14 days. If the arthroplasty is resected, the implant components may be vortexed and sonicated and the resultant sonication fluid semiquantitatively cultured to sample biofilms on its surface.[29] As mycobacteria and fungi are rare in this setting, they should not be routinely sought.

Skin and Soft Tissue Specimens

Skin and soft tissue specimens sent to the laboratory include tissue biopsies, aspirates or swabs of abscesses, wound swabs, material obtained by surgical débridement, and drainage samples. The usefulness of processing these specimens by microscopy and culture is limited by site of the lesion. Lesions that communicate directly with the skin or mucosal surfaces are commonly contaminated with mixed populations of organisms. For meaningful test results, surgically obtained tissue samples or aspirates of closed abscesses, pus, or fluid are preferred. When anaerobic bacteria are expected, specimens should be placed into an anaerobic transport container and delivered promptly to the laboratory. Swabs, including swabs of superficial skin ulcers, from the skin surface of a sinus tract or from open abscesses commonly yield mixed flora and often do not reflect organisms of real infectious significance; they should be avoided.

Wound infections and abscesses are caused by many different organisms including aerobic and anaerobic bacteria, mycobacteria, and fungi. It is important for the clinician and the laboratory to recognize that certain organisms are often associated with particular types of wounds or abscesses. An infected animal bite may yield *Pasteurella multocida* or *Capnocytophaga* species; a posttraumatic hand infection may yield *S. aureus, Mycobacterium marinum,* or *S. schenckii* complex depending on the source of trauma; a postoperative wound infection may yield *P. aeruginosa* or *Acinetobacter baumannii,* among others. Fungal, mycobacterial, and anaerobic cultures must be specifically requested if clinically indicated. When *Francisella tularensis* or *Brucella* species are sought, the laboratory should be notified so that cultures can be set up appropriately and isolated organisms can be safely handled. Bartonellosis is best diagnosed by histopathology, serology, and, if needed, NAAT. Providing the laboratory with the type or location of the wound, abscess, or tissue is frequently useful because it may hasten recognition of specific pathogens known to be associated with a particular type of infection or anatomic site (e.g., cat bite, brain abscess).

If sufficient specimen is available, microscopy should be performed to obtain a preliminary indication of the infecting organism. Additionally, if antimicrobial therapy has been initiated, direct smear may be the single available guide to the cause because growth may be inhibited. Impression smears can be made by gentle pressing of a freshly cut surface of the tissue onto a slide, which can then be stained for bacteria, fungi, or mycobacteria. Direct examination of specimens by microscopy can provide preliminary information about the quality of the specimen (e.g., many epithelial cells in a wound specimen indicates skin contamination), guide empirical therapy, and direct culture strategies. For example, if organisms resembling *Nocardia* species or mycobacteria are observed in a Gram-stained specimen submitted for routine culture, additional processing to recover these organisms can be performed.

Organisms observed by microscopy should be correlated with culture results. Discrepancies may help identify an organism that would not otherwise be detected. For example, faintly staining gram-negative bacilli that do not grow on routine aerobic plates may suggest the possibility of an anaerobic organism, such as *Bacteroides* species.

Direct Gram smears of known quantities of specimen can be used to give an assessment of organism load. Quantitative wound, burn eschar, and tissue biopsy specimen cultures have been used to help predict the likelihood of wound sepsis, burn infection, or the success of skin grafting. They require weighing and careful preparation of the specimen for serial dilutions to determine colony counts. Because quantitative cultures are time consuming and labor intensive, not all laboratories perform these assays. The availability of quantitative wound cultures should be checked by consultation with the laboratory before such tests are ordered.

Respiratory Tract Specimens

Respiratory tract specimens can be divided into two major categories, upper and lower respiratory specimens. Common upper respiratory tract specimens include throat swabs and nasopharyngeal swabs or washings. Common lower respiratory tract specimens include expectorated and induced sputum; endotracheal aspirate (tracheal secretions); and bronchoalveolar lavage (BAL), brushing, and biopsy. Fine-needle lung aspirate and open lung biopsy specimens may also be collected.

Throat swabs submitted from patients with bacterial pharyngitis are typically processed for *Streptococcus pyogenes* only unless a specific request is submitted to look for other agents. Although other bacteria including *Streptococcus dysgalactiae, Arcanobacterium haemolyticum, Fusobacterium necrophorum*, *N. gonorrhoeae, C. diphtheriae,* and *M. pneumoniae* can cause pharyngitis, they are not routinely tested for and must be specifically requested if they are sought. *N. gonorrhoeae* and *M. pneumoniae* are best detected using NAATs. If *C. diphtheriae* is being considered, a specialized culture (which uses selective and differential media) should be specifically requested. If culture for *C. diphtheriae* is positive, toxin testing of the isolate is required to demonstrate production of diphtheria toxin. NAATs and direct antigen tests including point-of-care tests are used for rapid detection of *S. pyogenes* in throat swabs. Some direct antigen tests are not sensitive. The Infectious Diseases Society of America and the American Heart Association recommend performing cultures for children and adolescents with negative rapid antigen detection tests but do not recommend reflex cultures for adults with negative rapid antigen detection tests results, given the lower incidence of *S. pyogenes* pharyngitis and rheumatic fever in this population.[30,31] However, evidence suggests that culture of antigen-negative throat swabs may be indicated for adults as well as children and adolescents, as rapid antigen detection tests fail to detect *S. pyogenes* pharyngitis in a significant number of adults.[32] Antimicrobial susceptibility testing is not routinely done on *S. pyogenes,* as it is universally susceptible to penicillin, although it is not universally susceptible to macrolides and azalides. Although respiratory viruses are common causes of pharyngitis, it is usually of limited usefulness to define a specific etiology in patients with pharyngitis due to respiratory viruses because there is usually no pathogen-directed treatment required for these agents.

Detection of viruses in respiratory samples has changed with the introduction of FDA-approved multiplex NAATs that detect 17 or more respiratory viruses.[19] These panels have largely replaced culture-based and antigen-based tests for viral detection in respiratory samples. The preferred sample is a swab passed through each nostril all the way to the back of the nasopharynx and placed into viral transport medium. Once in the laboratory, test time is 1 to 8 hours, depending on platform. Some laboratories have validated testing on lower respiratory samples (e.g., BAL fluid). The specific organisms included vary and include adenoviruses, coronaviruses, human bocavirus, human metapneumovirus, influenza A virus (including subtypes H1, H3, and 2009 H1N1), influenza B virus, parainfluenza virus 1–4, and respiratory syncytial virus (including A and B); bacteria including *Chlamydia pneumoniae, M. pneumoniae, Bordetella pertussis, Bordetella parapertussis* and *Bordetella bronchiseptica,* and *Bordetella holmesii* are included in some panels. NAAT performed on a posterior nasopharyngeal swab or aspirate is the recommended test of choice for *B. pertussis* and *B. parapertussis* detection. Although *B. pertussis* can be cultured on specialized media, culture is less sensitive than NAATs.

Aspirates of the middle ear are occasionally submitted. In patients with otorrhea or myringotomy tubes, the ear canal can be cleaned and drainage collected with mini-tip swabs.

Aspirates of paranasal sinuses may be submitted for diagnosis of sinusitis; nasopharyngeal swabs or washings should not be submitted for this indication. Endoscopically collected swab cultures may recover bacterial pathogens but infrequently detect the causative fungi. In maxillary sinusitis, antral puncture with sinus aspiration and, in adults, swabs of material draining from the middle meatus collected under endoscopic guidance are suitable specimens. To establish a fungal etiology, an endoscopic sinus aspirate is suggested. Surgical procedures are required to sample sphenoid, frontal, or ethmoid sinuses.

Some laboratories screen high-risk patients for methicillin-resistant *S. aureus* or *S. aureus* carriage using anterior nares swabs. A variety of selective, differential media have been developed for this purpose, as have NAATs. (Additional sites that may be sampled for assessing *S. aureus* carriage include the axillae, groins, stool, and throat.)

Expectorated and induced sputa and tracheal aspirates (tracheal secretions) are the most common specimens submitted for diagnosis of lower respiratory tract infections. Although these are readily obtained, results can be difficult to assess because microorganisms colonizing the upper airways including those that can be pathogenic in the lower respiratory tract can contaminate the specimen. Although it is difficult to avoid contamination of induced sputa and tracheal aspirates, this can be reduced in expectorated sputa by instructing the patient to cough deeply and expectorate secretions from their lower chest directly into a clean container. A single coughed specimen is sufficient for bacterial culture. Laboratories screen expectorated specimens submitted for bacterial culture and reject them if they are contaminated with oral secretions, as identified by the presence of epithelial cells. Although not ideal, contaminated sputum specimens can be processed for *Legionella* species, *Nocardia* species, *Mycobacterium* species, and mold cultures because selective media are used to suppress growth of contaminants.

Noninvasive sampling of the respiratory tract is recommended for hospital-acquired and ventilator-associated pneumonia.[33] In nonventilated patients, appropriate specimens include expectorated sputum, induced sputum, or, in an uncooperative patient, nasotracheal aspirates; in ventilated patients, endotracheal aspirates are appropriate.[33] Cultures of endotracheal aspirates, although likely to contain the true pathogen, may yield more mixtures of species of bacteria, including colonizing bacteria, than specimens obtained bronchoscopically; this can lead to needless antibiotic therapy. Quantitative assessment of invasively obtained samples such as BAL and protected specimen brush specimens may be performed.[33] Bacterial quantities above a threshold (dependent on timing of antimicrobial therapy) are diagnostic of pneumonia, whereas those below that threshold are more consistent with colonization. Generally accepted thresholds are 10^6 CFU/mL for endotracheal aspirates, 10^4 CFU/mL for BAL fluid, and 10^3 CFU/mL for protected specimen brush samples. Quantitative cultures require a large amount of specialized laboratory work and are not endorsed by current guidelines.[12] Isolation of respiratory pathogens in biopsy specimens or fine-needle aspirates of the lungs is almost always significant.[33] Likewise, regardless of the collection method or number of organisms present, some pathogens should always be considered significant (e.g., *Legionella* spp., *Nocardia* spp., *M. tuberculosis, H. capsulatum, Blastomyces* species, *C. immitis* and *C. posadasii, C. neoformans* and *C. gattii*). Multiplex NAATs that detect multiple bacterial species and resistance genes as well as viruses have been approved by the FDA for testing sputum, tracheal aspirates, and BAL fluid. Infections with some respiratory pathogens (e.g., *S. pneumoniae, Legionella* spp.) may be diagnosed by detecting specific antigens in urine; these tests should be performed in addition to culture. Microscopy (e.g., Gram stain, acid-fast stain, calcofluor white stain) can provide a rapid diagnosis if positive but is insensitive, so alternative test methods should also be used. The use of a specific NAAT is becoming the approach of choice for some pathogens and an important supplementary test for others. NAATs are particularly important when a specific organism is considered in

the differential diagnosis (e.g., *M. tuberculosis, Pneumocystis jirovecii, M. pneumoniae, Legionella* spp.).

The microbiology of pulmonary infections in patients with cystic fibrosis is different from that in patients without cystic fibrosis. Specialized culture techniques for cystic fibrosis respiratory specimens should be used; these techniques help recover pathogens in the context of the presence of mucoid *P. aeruginosa* strains. Mycobacterial and fungal stains and cultures may also be appropriate.

Pulmonary infections in immunocompromised hosts have a broader differential diagnosis than infections in nonimmunocompromised hosts and may be associated with severe morbidity and mortality. As such, when rapid and noninvasive tests are not revealing, bronchoscopy with BAL with or without transbronchial biopsy is usually performed. Microbiology laboratories should collaborate with infectious diseases clinicians and pulmonologists to develop an algorithm for processing samples that comprises testing for all major categories of pathogens. At my institution, for example, an immunocompromised host BAL panel is offered that includes cell count and differential, cytology, viral NAATs (influenza, respiratory syncytial virus, adenovirus), galactomannan, Gram stain, bacterial culture, fungal stain and culture, *Nocardia* stain, mycobacterial stain and culture, viral culture, *Legionella* culture and NAAT, and *Pneumocystis* NAAT. Cytology or histopathology or both can also be helpful (e.g., to identify pulmonary hemorrhage), as can serum and BAL galactomannan and (1–3)-β-D-glucan tests. Transthoracic needle aspiration, computed tomography–guided biopsy, and open lung biopsy may be considered if less invasive diagnostic tests are unrevealing.

Urine

Urine, including voided midstream and catheterized urine, is the most common specimen processed in the clinical microbiology laboratory. Reliable results are substantially impacted by appropriateness of testing, as it is impossible to differentiate asymptomatic bacteriuria from urinary tract infection based on microbiology results alone. With a few exceptions (e.g., pregnant women and patients about to undergo urologic surgery), performance of urine cultures on asymptomatic patients is not recommended. Reliable results are affected by specimen quality and transportation to the laboratory. Culture contamination is an issue because the urethra is colonized with large numbers of bacteria (e.g., *Lactobacillus* spp., streptococci), introduction of which into the specimen during collection can compromise the specimen. Similarly, contamination of the specimen with vaginal or fecal bacteria can compromise results. The impact of contaminated urine cultures is amplified if urine is not promptly transported to the laboratory. Because urine is so easily contaminated, urine specimens for bacterial culture should be collected with attention to curbing contamination from perineal and superficial mucosal microbiota. Specific, detailed instructions should be provided to patients at the time of urine collection to minimize bacterial contamination. This includes discarding the first portion of the voided specimen, which could be contaminated with the urethral flora (i.e., collection of midstream urine). Although some studies suggest that skin cleansing in preparation for collection of midstream urine specimens may be of little benefit, many laboratories find that culture of specimens obtained without skin cleansing regularly contain mixed flora and accordingly yield high numbers of one or more potential pathogens, rendering interpretation of results challenging.[12] Urine collected in a sterile container and transported at room temperature should be plated for culture within 2 hours of collection or, if this is not possible, transferred to tubes with agents (e.g., boric acid) that inhibit bacterial overgrowth or refrigerated. An exception to the preference for collection of midstream urine is collection of urine for diagnosis of urethritis (e.g., *N. gonorrhoeae, Chlamydia trachomatis*), as a first-voided urine is preferred in this situation to sample the urethra. Suprapubic aspirates, primarily performed on infants or other patients in whom assessment of midstream urine is difficult or in whom an anaerobic infection is suspected, as well as specimens obtained by cystoscopy or by other invasive procedures should be clearly labeled as such, as all growth should be identified and reported.

Rapid screening techniques for urinary tract infection include direct Gram stain and a variety of commercially available products, such as dipsticks and fluorescence-based flow cytometry. A positive Gram stain is equivalent to greater than or equal to 10^5 CFU/mL. Because urine specimens from infected patients may have quantities of bacteria less than 10^5 CFU/mL, Gram staining is a relatively insensitive screening method for these specimens (i.e., patients can have negative Gram stains but positive cultures). The presence of mixed bacterial types or a moderate amount of squamous epithelial cells suggests contamination with genital flora. In-and-out catheterization of a correctly prepared patient usually offers a less contaminated specimen than a midstream specimen. Laboratories should routinely perform susceptibility testing on potential pathogens present in significant numbers.

Specimens from urinary catheters in place for more than a few hours often have colonizing flora due to biofilm formation on the catheter surface; accordingly, positive urine cultures collected from these catheters may not represent urinary tract infection, and culture from indwelling catheters is strongly discouraged. If urine cultures are needed in catheterized patients, the catheter should be removed and midstream urine collected, or a new catheter should be placed and urine collected from the sampling port of the newly inserted device. Cultures of Foley catheter tips are of no clinical value. Collection from urinary diversions such as ileal loops is also not suggested because of the tendency of these locations to be chronically colonized. Chronic nephrostomy collections and bagged urine collections are of dubious value for culture.

Yeasts, particularly *Candida* species, may be isolated from routine midstream urine cultures; their quantitation is not useful in assessing clinical significance. Recovery of *Candida* species even in high numbers is not infrequent from patients who do not actually have yeast urinary tract infection. Mycobacterial urine cultures (typically looking for *M. tuberculosis*) in addition to urine cultures for *C. neoformans* and *C. gattii, Blastomyces* species, *H. capsulatum,* and *C. immitis* and *C. posadasii* require large volumes of first-voided urine (typically at least 20 mL) and use of specialized media.

For acute bacterial prostatitis, a midstream urine culture with or without culture of expressed prostate secretions is recommended; for chronic bacterial prostatitis, a midstream urine culture with culture of expressed prostatic secretions is recommended.[12] The traditional Meares-Stamey four glass specimen involving collection of first void urine, midstream urine, expressed prostate secretions, and post–prostate massage urine is positive if there is a 10-fold higher bacterial count in the expressed prostate secretions than the midstream urine.[34] A two-specimen variant involving only the midstream and the expressed prostate secretions specimens is easier to use and is recommended. Clinicians should be aware that prostatic massage in a patient with acute bacterial prostatitis may precipitate bacteremia. For epididymitis testing, clinicians are referred to a recently published guideline.[12]

Detection of adenovirus in cases of cystitis is usually done by NAAT. BK virus nephropathy is best diagnosed by renal biopsy; however, plasma BK virus viral load testing may be used as a screening test for BK virus nephropathy, prompting renal biopsy if the viral load is >4 log copies/mL. Alternatively, urine testing for decoy cells or BK virus using a quantitative NAAT can be used as a screening test, and if positive, may be followed by BK viral load testing of plasma, which has a higher specificity.

Stool

Acute diarrhea can have a variety of etiologies including bacteria, viruses, and parasites in addition to noninfectious causes. Not all patients with acute diarrhea need testing, as most cases are self-limited and do not require specific treatment. The reader is referred to recent recommendations regarding who should be tested and with what tests.[19,35] Recommendations for minimizing unnecessary stool cultures include rejection of routine bacterial stool cultures in patients who have been hospitalized for 3 days or longer. Likewise, testing for *Clostridioides difficile* (formerly *Clostridium difficile*) is usually appropriate only on diarrheal stools, defined as specimens that are loose enough to take the shape of the container. Most laboratories have guidelines in place that limit repeat testing for enteric pathogens within specified time intervals (e.g., maximum weekly testing for *C. difficile*).[36,37] Repeat testing of patients previously positive for *C. difficile* as a "test of cure" is not appropriate.

Laboratories should have a routine in place to evaluate for common bacterial agents of acute gastroenteritis, which should include at a minimum *Campylobacter, Salmonella,* and *Shigella* species in addition to Shiga toxin–producing *E. coli* (typically detected using NAAT or antigen test). Laboratory test catalogs and reports should indicate which of the enteric pathogens would be detected with a routine stool culture because this may vary from institution to institution. Comprehensive workup of fecal specimens requires a variety of selective, differential culture media as well as microscopy, antigen tests, and NAATs. Enrichment broths to detect small numbers of organisms may or may not be used. Rectal swabs provide an insufficient quantity of specimen and are discouraged. Stool should be immediately sent to the laboratory or mixed in Cary-Blair transport medium after the specimen is collected. Laboratories should be notified if pathogens are suspected that are not included in routine stool cultures (e.g., *Vibrio cholerae*) because selective culture methods or the use of antigen tests or NAATs may be required.

Numerous methods may be employed for the diagnosis of *C. difficile*–associated diarrhea. Toxigenic culture and cytotoxin assay are sensitive but time consuming and labor intensive. Enzyme immunoassay and immunochromatographic methods for toxin detection have lower sensitivities but are faster; assays that detect both toxin A and toxin B are ideal. Glutamate dehydrogenase antigen assays are sensitive but have poor specificity. NAATs for *C. difficile* are as sensitive as toxigenic culture. To minimize turnaround time, mitigate costs, and improve accuracy of diagnosis of clinically significant *C. difficile*–associated diarrhea, some laboratories employ an algorithm that uses glutamate dehydrogenase as a rapid screening test followed, if results are positive, by toxin A and B detection or NAAT or both.

There are currently multiple FDA-approved multiplex NAATs that simultaneously detect five or more stool pathogens (and several more that detect smaller numbers of stool pathogens); examples include the Luminex xTAG Gastrointestinal Pathogen panel (Luminex GPP; Luminex), the BioFire FilmArray Gastrointestinal Panel (BioFire GI Panel; BioFire Diagnostics LLC), and the Verigene Enteric Pathogens Nucleic Acid Test (Verigene EP; Luminex). The Luminex GPP detects *Campylobacter* species, *C. difficile, E. coli* O157, enterotoxigenic *E. coli,* Shiga toxin–producing *E. coli, Salmonella* species, *Shigella* species, *V. cholerae,* adenovirus 40/41, norovirus, rotavirus A, *Cryptosporidium* species, *Entamoeba histolytica,* and *Giardia* species in approximately 5 hours. The BioFire GI Panel can identify 22 targets in approximately 1 hour, and targets include all of those on the Luminex GPP plus *Vibrio* species, *Yersinia enterocolitica,* enteropathogenic *E. coli,* enteroaggregative *E. coli, Plesiomonas shigelloides,* astrovirus, sapovirus, and *Cyclospora cayetanensis*; *Shigella* species and enteroinvasive *E. coli* are targeted instead of *Shigella* species. The Verigene EP targets *Campylobacter* group, *Salmonella* species, *Shigella* species, *Vibrio* group, *Y. enterocolitica,* Shiga toxin–producing *E. coli,* norovirus, and rotavirus and runs on the Nanosphere Verigene system, yielding results in <2 hours. These panels are FDA approved on the basis of accurate detection and identification of the pathogen. Determination of clinical significance of results is the responsibility of the clinician. In the absence of evidence of a new infection, follow-up testing should be limited; these panels should not be used as a test of cure.[38] They are expensive and should be used with a test utilization strategy in place.[19] Conventional methods are required to detect pathogens not covered by the panels (e.g., *Aeromonas* spp.) and provide antimicrobial susceptibility information when required. To provide isolates for susceptibility testing, clinical laboratories using multiplex panels may contemplate culturing for the organisms identified molecularly (i.e., reflexive culture), a strategy that can also be adopted when public health laboratories need isolates.[39,40] The ideal panel of organisms to be included has not been well defined.

Some institutions screen selected patients for fecal carriage of multidrug-resistant organisms such as vancomycin-resistant enterococci or carbapenemase-producing gram-negative bacilli. Selective media or NAATs targeting resistance genes of interest (e.g., *vanA, vanB,* bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48}$, bla_{VIM}, bla_{IMP}) can be used to test rectal swabs or stool for this purpose. Perianal swabs for *S. pyogenes* cultures are appropriate for diagnosis of perianal cellulitis, which most commonly occurs in children.

Vaginitis and Vaginosis Diagnostics

Vaginitis with discharge can be caused by *Candida* species, bacterial vaginosis, or *Trichomonas vaginalis*. Swabs of vaginal discharge are typically collected. Vaginal candidiasis is best diagnosed by a fungal smear showing many budding yeasts and pseudohyphae. Although candidal culture can be performed, growth of *Candida* species is not necessarily diagnostic of candidal infection because many asymptomatic women harbor *Candida* species.

For bacterial vaginosis, a graded Gram stain of vaginal discharge is recommended. The healthy vagina has a predominance of lactobacilli, whereas the flora of a patient with bacterial vaginosis is dominated by coccobacilli, reflecting an increase in growth of *Gardnerella vaginalis* and other anaerobes. Nugent criteria can be used to quantify or grade bacteria via Gram stain of vaginal samples. Nugent criteria evaluate *Lactobacillus* species, *Bacteroides* species and *Gardnerella* species, and *Mobiluncus* species by Gram stain, grading each on a scale of 1 to 4 (1+ is <1 cell per field, 2+ is 1–4 cells per field, 3+ is 5–30 cells per field, and 4+ is >30 cells per field). *Lactobacillus* species and *Bacteroides* species and *Gardnerella* species are then given scores between 0 and 4, with *Mobiluncus* species receiving scores of 0 and 2. Total scores are calculated and used as follows: 0 to 3, consistent with normal bacterial vaginal flora; 4 to 6, altered vaginal flora not consistent with bacterial vaginosis (this frequently represents a transitional stage); and 7–10, consistent with bacterial vaginosis. As an alternative to the Nugent criteria, Hay/Ison criteria can be used.[41] Vaginal discharge with a pH greater than 4.5, a fishy amine odor after addition of potassium hydroxide, and the presence of clue cells on saline wet mount are also used to support a diagnosis of bacterial vaginosis. *G. vaginalis* cultures are not recommended. Tests that assess the vaginal microbiome using NAATs have a similar performance to the graded Gram stain.

For *T. vaginalis* vaginitis, vaginal pH is also typically >4.5. Visualization of motile trichomonads allows a diagnosis but is not as sensitive as NAATs. Other approaches including culture, DNA hybridization probe detection, and rapid antigen testing are also not as sensitive as NAATs. Notably, NAATs for trichomoniasis can be performed on a number of specimen types including vaginal, endocervical, urethral, rectal, or pharyngeal swabs and urine or liquid-based cytology specimens.

Genital Lesions and Syphilis Diagnostics

Genital lesions are a challenge to diagnose. Patients with genital lesions should be assessed with a serologic test for syphilis as well as diagnostic tests for HSV and, where chancroid is prevalent, *Haemophilus ducreyi*.

Darkfield examination for motile spirochetes and NAAT are useful for diagnosing primary syphilis but are unavailable in the most laboratories. *Treponema pallidum* cannot be cultured in the routine laboratory. Accordingly, testing for syphilis is most commonly performed by serology. Traditional testing has consisted of initial screening with a nontreponemal test (e.g., rapid plasma reagin [RPR]), followed by testing of reactive specimens with a treponemal test (e.g., *T. pallidum* particle agglutination). Alternatively, a reverse testing sequence can be used beginning with a specific treponemal test such as an enzyme immunoassay or chemiluminescence immunoassay and then retesting reactive results with a nontreponemal test such as RPR to confirm the diagnosis. Screening with a treponemal test can identify persons previously positive, treated, or partially treated for syphilis as well as yield false-positive results in patients with low likelihood of infection. If the follow-up confirmation test (RPR) is negative, the laboratory should perform a different treponemal specific test (e.g., fluorescent treponemal antibody absorption).

For suspected cases of HSV genital lesions, NAATs are recommended, as viral culture or direct fluorescent antibody testing is less sensitive. A swab can be rubbed over the surface of the lesion, even a closed vesicle, for NAATs. Alternatively, a vesicle may be aspirated or unroofed and the liquid material swabbed. NAAT should test for HSV types 1 and 2. Consideration may also be given to additionally testing for varicella-zoster virus if the clinical picture is suggestive of this possibility.

Chancroid, caused by *Haemophilus ducreyi;* lymphogranuloma venereum caused by *C. trachomatis* serovars L1, L2, or L3; and granuloma inguinale (donovanosis) caused by *Klebsiella granulomatis* are uncommon causes of genital ulcers in the United States and are typically diagnosed

based on clinical presentation, identification of risk factors, and exclusion of the more common causes of genital lesions, syphilis and HSV. Chancroid may be identified by Gram stain and specialized culture. The laboratory must be notified if this organism is suspected. NAATs for *C. trachomatis* detect, but do not call out, lymphogranuloma venereum serovars; none are approved by the FDA for genital ulcer sites. *K. granulomatis* does not grow on conventional media. Diagnosis is made by observing the organism in tissue with the Giemsa stain.

Testing for human papillomavirus is beyond the scope of this chapter and is discussed in Chapter 143. The diagnosis of genital warts is normally through visual inspection; biopsy may be performed if there is any question about the diagnosis. High-risk human papillomavirus testing of genital warts is not recommended. Likewise, molluscum contagiosum is typically a clinical diagnosis; biopsy may be performed if there is any question about the diagnosis.

Urethritis and Cervicitis Diagnostics

For laboratory diagnosis of *C. trachomatis* and *N. gonorrhoeae,* NAATs are preferred because of their sensitivity and specificity and ability to be performed on urine. Urine or vaginal specimens (either collected by the provider or self-collected) in women and urine specimens in men are preferred specimen types. Other specimen types include endocervical, urethral, rectal, pharyngeal, and conjunctival swabs. Not all of these are approved by the FDA, so clinicians should check with their laboratories to determine whether these sources will be tested. Antibacterial resistance in *N. gonorrhoeae* is an emerging challenge that may necessitate culture for antimicrobial susceptibility testing. *N. gonorrhoeae* is labile; therefore, if culture is performed, the specimen should be sent promptly to the laboratory in appropriate transport media (not swabs) or plated at the point of care.

Mycoplasma genitalium causes urethritis in men and women and cervicitis and pelvic inflammatory disease in women. Antimicrobial resistance is a challenge. An NAAT is the test of choice for diagnosis of *M. genitalium*. *T. vaginalis* may cause urethritis or cervicitis; diagnostics are discussed in "Vaginitis and Vaginosis Diagnostics."

Helicobacter pylori Diagnostics

Noninvasive tests for *Helicobacter pylori* include stool antigen and urea breath tests.[42] Serology is not recommended. If endoscopy is performed, biopsy can be performed for histopathology or rapid urease testing or both in addition to specialized culture to recover an isolate for antimicrobial susceptibility testing, which is particularly germane to patients who fail to respond to therapy, given *H. pylori* antimicrobial resistance.[43]

Mycobacterium tuberculosis Diagnostics

Latent tuberculosis can be diagnosed using an interferon-γ release assay or a tuberculin skin test (TST). The former is recommended over the latter in individuals who have a history of bacillus Calmette-Guérin vaccination or are unlikely to return to have their TST read. Although both interferon-γ release assay and TST provide evidence for infection with *M. tuberculosis,* they cannot distinguish active from latent tuberculosis.

To diagnose active tuberculosis, AFB smear microscopy and mycobacterial cultures are recommended for patients suspected to have pulmonary tuberculosis. False-positive microscopy results (due to infections with nontuberculous mycobacteria) and false-negative results occur. Testing of three respiratory specimens each of 5 to 10 mL using AFB smear and mycobacterial culture is recommended. Concentrated respiratory specimens and fluorescence microscopy are preferred for AFB smears.

An NAAT is also recommended on the initial respiratory specimen from patients suspected to have pulmonary tuberculosis. In AFB smear–positive patients, a negative NAAT makes tuberculosis disease unlikely. In AFB smear–negative patients with an intermediate to high level of suspicion for disease, a positive NAAT is presumptive evidence of tuberculosis, but a negative result cannot be used to exclude pulmonary tuberculosis. It is recommended that rapid molecular drug susceptibility testing for rifampin with or without isoniazid be performed in individuals with risk factors for resistance. Collection of induced sputum rather than BAL fluid is recommended for adults with suspected pulmonary tuberculosis who are unable to expectorate sputum, with collection of BAL fluid recommended if collection of induced sputum proves unsuccessful. Postbronchoscopy sputum specimens can be collected for AFB smears and mycobacterial cultures. Testing for extrapulmonary disease is well outlined in the American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines on diagnosis of tuberculosis in adults and children.[44]

ACKNOWLEDGMENTS

Tables have been adapted from the previous edition, which was authored by Patrick R. Murray. I thank my colleagues Drs. Elitza S. Theel, Bobbi S. Pritt, Nancy L. Wengenack, and Matthew J. Binnicker for their thoughtful input into Table 16.4. Content is based, in part, on Miller et al.[12]

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E Antiinfective Therapy

17 Principles of Antiinfective Therapy

Brad Spellberg

SHORT VIEW SUMMARY

History
- Antimicrobial agents revolutionized the practice of medicine, resulting in dramatic reductions in death from a variety of diseases. As a result, death from infections in the United States declined from approximately 280 per 100,000 population in 1936 (presulfa) to approximately 60 per 100,000 population by 1950 (postpenicillin), with virtually no further decline since then.

Societal Trust
- Antibiotic efficacy is lost with each use of the drugs due to antibiotic resistance.
- Antibiotic resistance is transmissible from person to person such that each individual's use of an antibiotic affects the ability of everyone else in society to have the drugs remain effective for their own use. Thus antibiotics are a shared societal trust.
- No one has a right to misuse these drugs because one person's misuse hurts everyone else in society. Infectious diseases practitioners have added responsibility for stewarding these drugs to maintain their effectiveness for society.

Principles of Antimicrobial Use
- Ten fundamental principles underpin the use of antibiotics to optimize cure rates while minimizing unnecessary/inappropriate use. Medicine is complex, and individual clinical scenarios may result in the need to deviate from such principles.
- Principle 1: Proper selection of empirical antibiotics starts with an accurate differential diagnosis that includes likely bacterial infection.
- Principle 2: Even in a patient with an obvious bacterial infection, treat only when therapy will alter the patient's clinical course.
- Principle 3: Empirically target the microbes that cause the diseases identified in the differential diagnosis.
- Principle 4: In critically ill patients there should be a lower threshold for the use of empirical therapy.
- Principle 5: Host factors affect the breadth of empirical therapy by altering the probabilities of the likely agents of infection.
- Principle 6: Pharmacokinetic-pharmacodynamic principles can influence optimal treatment.
- Principle 7: Clinicians should deescalate antibiotic therapy based on microbiology results and clinical and biomarker responses.
- Principle 8: If therapy is not working, consider source control and alternative diagnoses before blaming resistance and broadening therapy.
- Principle 9: Distinguish new infection from the failure of initial therapy.
- Principle 10: The duration of therapy should be evidence based, with a bias toward a short course whenever possible.

Controversial Principles:
- The distinction between bacteriostatic versus bactericidal is an in vitro construct. Clinical trial data do not support an intrinsic clinical efficacy difference between static and cidal antimicrobial agents.
- Combination therapy may be beneficial in specific clinical circumstances. However, its use is not supported by clinical data in many circumstances, and its use may result in increased toxicity, disruption of the host microbiome, and/or selection for antimicrobial resistance.

THE ANTIBIOTIC MIRACLE: WHAT WE ARE FIGHTING FOR

A Brief History of "Antibiotics"

From the earliest history of human civilization, we have sought "magic bullets" to cure diseases.[1] The ancient Greeks used natural substances, including aromatic extracts from woods, such as myrrh, as well as wine, honey, and flakes of copper and aluminum minerals to prevent or treat wound infections.[2] Ancient Chinese and Egyptian physicians used moldy tofu and bread, respectively, to treat skin infections.[3] These ancient treatments likely did have some, albeit limited, clinical benefit because modern science demonstrates that each of these substances possess antimicrobial effects.[1,2]

The crude nature of these ancient therapeutic approaches would not be advanced upon until the 20th century. Dr. Paul Ehrlich won the Nobel Prize in 1908 for his studies of the chemistry of antibody-antigen interactions. He reasoned that because chemistry governed the benefits of immune serum, it should be possible to recapitulate those benefits by creating or discovering small molecules that could similarly chemically interact with microbes.[4] He coined the term *chemotherapy* to describe the use of small molecules to treat diseases and principally infectious diseases.[4]

Ehrlich's efforts led to his discovery of the first antiinfective small molecule, arsphenamine (Salvarsan), a derivative of arsenic, which he found could successfully treat syphilis.[5] The drug became an enormous commercial success, which quickly faded when it was realized that the drug had potentially fatal side effects. Nevertheless, the sophisticated chemical library screen Ehrlich designed and conducted to discover arsphenamine became a blueprint for subsequent drug discovery that ultimately led to identification of many other antibiotics and other drugs as well.

The word *antibiosis*—meaning *against life*—was coined in 1889 by the French scientist Jean Paul Vuillemin.[1,6] He used the term to refer to the process of "one creature destroying the life of another to sustain its own."[6] Half a century later, in 1942, future Nobel laureate Dr. Selman Waksman built on this definition, coining the term *antibiotic* to mean "a chemical substance, of microbial origin, that…[inhibits] the growth or the metabolic activities of bacteria and other microorganisms."[6] Thus the term *antibiotic* technically refers to a natural substance produced by a living organism to inhibit the growth of another. However, in common parlance (and in the remainder of this chapter) the term is often used more liberally to encompass both natural and synthetic antimicrobial substances because at the clinical level the distinction is not important.

Alexander Fleming discovered penicillin-containing mold by-products in 1928. However, penicillin's first clinical use as a purified substance was not until 1940. Thus the first safe and effective, specific antibiotics (inclusive of synthetic drugs) to be clinically deployed were the synthetic sulfa agents discovered by Dr. Gerhard Domagk and his team in 1931.[1,7] Domagk's team developed sulfa drugs by chemical modification of industrial red dyes originally intended for clothing. The first sulfa drug, prontosil rubrum, had miraculous curative effects in mice with lethal bacterial infections.[7] By late 1932 news of the miracle had spread, and local physicians began to treat patients with sulfa drugs before the animal studies were even published.[7]

On May 17, 1933 a colleague of Domagk used a new sulfa drug to cure a 10-month-old boy with what had been considered an inevitably fatal bloodstream infection caused by *Staphylococcus aureus*.[7] This child was the first person in history ever to be cured of a lethal bacterial infection using a safe and effective antibiotic.

As powerful as sulfa drugs were at saving lives, they had limited or no efficacy against many types of infections. Purified penicillin, when it came along, was far more effective. The first patient ever treated with purified penicillin was a British police constable with severe staphylococcal facial cellulitis.[8] He improved dramatically but then died after the small supply of penicillin ran out. On March 14, 1942, Mrs. Ann Sheafe Miller became the first patient in the United States to have her life saved by penicillin.[9] She was dying from bacteremic, postpartum streptococcal sepsis that had failed to respond to sulfa drugs. Her treating physician procured penicillin, which resulted in such a dramatic eradication of the virtually invariably fatal infection that one of Mrs. Miller's consultants was overhead muttering to himself, "black magic!" while reviewing her chart.[9] Indeed, so powerful was penicillin at reducing death from infections that within 3 years of its widespread availability in the United States experts were already speculating on the end of bacterial infections as a significant clinical problem.[10]

The success of sulfa drugs and penicillin at curing disease inspired a generation of scientists, resulting in the discovery of the other major antibiotic classes in the 1940s and 1950s. All told, more than 150 antibiotics of various classes have been discovered and developed since 1935. And all have quickly suffered from the clinical emergence of resistant bacteria, which eroded the miraculous gains. It is the clinical use and protection of these antibacterial agents, as well as antifungal, antiviral, and antiparasitic agents, that concern us for the remainder of this chapter and the succeeding chapters on antiinfective therapy.

The Power of Antibiotics: What We Stand to Lose Due to Resistance

Antibiotics have unquestionably revolutionized the practice of medicine. According to a reliable eyewitness, the most common substance prescribed by physicians in Boston before sulfa drugs became available was bourbon; prescriptions were written in Latin script so it seemed impressive to patients and made them feel reassured.[11] Indeed, before sulfa drugs, placebos were used frequently in medicine because few truly effective therapies were available. The sudden availability of effective antibacterial therapy transformed the practice of medicine from one in which the primary focus was prognostication to one in which cure became expected.[11]

The massive declines in death enabled by antibiotics ran across all types of invasive bacterial infections (Table 17.1). For example, the mortality rate of cellulitis fell from an extraordinary 11% in the preantibiotic era to approximately 0.3% with the availability of penicillin.[12] Thus in the preantibiotic era, the mortality of cellulitis approximated that of myocardial infarction, and the reduction in death resulting from treating cellulitis with antibiotics (>10% absolute reduction in death) is far greater than that resulting from treating myocardial infarction with aspirin or thrombolytics (3% absolute reduction in death).[13]

At a population level the death rate from infectious diseases (ID) in the United States fell from approximately 280 per 100,000 in 1936 (the year before sulfonamides were available in the United States), to approximately 200 per 100,000 in 1945 (the year before penicillin became widely available), to approximately 60 per 100,000 by the early 1950s.[14] Despite the advent of modern intensive care unit (ICU) methodologies, and all other medical technologies over the past half century, there has been virtually no further decline in the death rate from infections in the United States since the penicillin era began,[14] underscoring how powerful the transformation of medical practice was.

Beyond saving the lives of infected patients, the enormous efficacy of antibacterial agents enabled conduct of complicated and deeply invasive surgery; aggressive chemotherapy for treatment of cancer; fundamental elements of critical care, such as central venous catheter placement and mechanical ventilation; care for premature neonates; and solid and liquid organ transplantation. None of these medical advances would be feasible without effective antibacterial agents to deal with the infections that result as a side effect of the advances themselves.

In short, the power of antibacterial therapy resulted in a revolution in the practice of medicine, transforming it from a primarily diagnostic-focused field to a therapeutic, interventional profession. As one eyewitness to the miracle of the emergence of antibiotic therapy wrote, antibiotics "place in the hands of a barefoot, nonliterate villager more real power to affect the outcome of a…critically ill [patient]…than could have been exerted by the most highly trained urban physician [from the preantibiotic era]."[15]

THE SOCIETAL TRUST OF ANTIBIOTICS MUST UNDERPIN PRINCIPLES OF USE

In many parts of the world antibiotics are available over the counter and without a prescription, which contributes to marked overuse and misuse of the drugs by patients who feel ill but do not have bacterial infections. Unfortunately, even in countries where antibiotics are available only by prescription, providers overuse and misuse the drugs for a variety of complex psychosocial reasons.[16,17]

Indeed, antibiotic overprescription is an example of the "tragedy of the commons."[16] The tragedy of the commons was originally described by Garrett Harding in 1968.[18] His example of this complex psychosocial phenomenon was the illegal overgrazing of public, shared lands in feudal Europe ("the commons") by livestock owned by private citizens. The tragedy of the commons results when individuals undertake an action that they perceive to be in their own self-interest but which causes harm to society at large. When such an action is undertaken rarely, the harm to society is not noticeable. When it happens tens of millions of times per year, as with inappropriate antibiotic prescriptions, the collective harm to society is catastrophic.[16]

TABLE 17.1 Antibiotic-Mediated Mortality Reductions for Specific Infections

DISEASE	PREANTIBIOTIC MORTALITY RATE	ANTIBIOTIC MORTALITY RATE	CHANGE IN MORTALITY
Community-acquired pneumonia	~35%	~10%	–25%
Nosocomial pneumonia	~60%	~30%	–30%
Bacterial endocarditis	~100%	~25%	–75%
Gram-negative bacteremia	~70%	~10%	–60%
Bacterial meningitis	>80%	<20%	–60%
Cellulitis	~11%	<0.5%	–10%

[a]By comparison, treatment of myocardial infarction with aspirin or streptokinase reduces death by 3%.
Data from references 12 and 139–153.

Given the power of antibiotics to save lives and the constant erosion of that power through use, one of the most important functions of the ID specialist (whether physician or pharmacist) is to serve as an expert in the use and protection of antimicrobial agents, fighting against the tragedy of the commons. Any licensed provider can prescribe an antibiotic; it takes an expert to know when not to prescribe and when to stop those already prescribed.

Genetic analysis indicates that antibiotic resistance mechanisms were developed by microbes as early as 2 to 2.5 billion years ago.[19] Microbes have thus been waging war among themselves with these weapons for 20 million times longer than humans have known of their existence.[17] We may presume that in 2 billion years of evolutionary warfare, microbes have invented poisons to disrupt every biochemical pathway that can be disrupted and, out of necessity, resistance mechanisms to protect all of those pathways.

The widespread existence in nature of antibiotic resistance mechanisms is underscored by a study in which microbes were cultured from the walls of a cave system that had never been explored by humans before and had been isolated from the surface of the planet for 4 million years.[20] Among the microbes cultured, resistance was encountered to every class of modern antibiotics. Resistance was encountered not just to natural substances (e.g., penicillin) but to synthetic drugs that did not exist on planet Earth until the 1960s–80s (e.g., fluoroquinolones, linezolid, daptomycin).

This remarkable finding underscores a critical—and perhaps poorly understood—point about antibiotic resistance. Resistance does not newly develop when we begin to use antibiotics clinically. Rather, there are already, widespread in nature, resistance mechanisms to drugs we have not yet invented. Our clinical use of antibiotics applies selective pressure that results in the increasing frequency of preexisting antibiotic-resistance mechanisms that were already in nature at low levels before our use of antibiotics.

Antibiotics Are a Societal Trust

As early as 1945 the man who discovered penicillin, Alexander Fleming, began warning the public that doctors and patients were abusing the miracle drug by using it to treat diseases not caused by bacteria and that resistance was being bred out as a result.[21] In his Nobel Prize lecture, he admonished that those who so abused penicillin would be "morally responsible" for the deaths of patients killed by resistant bacteria that could no longer be treated with it. This clarion call for antibiotic stewardship, more than 70 years ago, went unheeded.

As a result, resistance caught up to the antibiotic armamentarium by the late 1950s and early 1960s, sending industry back to their chemical libraries and natural substance screens. A roundtable of leading ID experts concluded as early as 1965 that there was a critical need for new antibiotics to "overcome the problems of resistance, to use against gram-negative bacillary infections, more active and less toxic ones for fungal infections, better ones for mycobacteria."[22] A half century later, after having been bailed out of the resistance problem by a flurry of new compounds discovered from the 1970s to 1980s, we are again having the same conversation with little institutional memory of what preceded us.

What history teaches us, therefore, is that in only 80 years of experience with compounds that microbes have kept successfully active for 2 billion years, we have already on several occasions nearly burned through the precious limited resource that is active antibiotic therapy. We will never win a war against microbes, which outnumber us by a factor of 10^{22} outweigh us by 100 million times, can replicate 500,000 times faster than we can, and have been doing this for 10,000 times longer than our species has existed.[23] Resistance will always be with us. It is the natural result of use of antibiotics. These facts place an extraordinary responsibility upon society, and upon ID experts as stewards in society, to protect antibiotics from abuse and misuse.

Antibiotics are a societal trust, different from all other classes of drugs (and indeed virtually any other technology).[24] Every individual's use of an antibiotic negatively affects the ability of everyone else in society to have the antibiotic available as an effective therapy. Thus the burden is on those trained in our specialty to help teach both our medical colleagues and the lay public how precious these drugs are and how they are to be properly deployed. We are the stewards of their incredible power to save lives and reduce morbidity.

FUNDAMENTAL PRINCIPLES OF ANTIBIOTIC THERAPY

Given the critical importance of antibiotics as a lifesaving medical intervention, fundamental principles should underpin their use. The practice of medicine is complex, and clinical scenarios can of course result in the need to deviate from individual principles in specific circumstances and with specific justification. Nevertheless, incorporation of fundamental principles of appropriate use of antibiotics into practice can help improve proper antibiotic use.

Ten fundamental, sequential principles guiding appropriate antimicrobial use are described here. These principles build on one another, from the initial decision regarding whether antibiotics are needed or not, to selection of which antibiotics to use empirically and then definitively, and ultimately to deciding for how long to prescribe them.

1. Proper Selection of Empirical Antibiotics Starts With an Accurate Differential Diagnosis That Includes Likely Bacterial Infection

Antibiotics are usually started empirically, meaning without knowing precisely which bacteria are infecting a patient, or even if the patient actually has a bacterial infection. Thus the first principle of appropriate use of antibiotics depends on establishing a rational and accurate differential diagnosis for the patient's current illness. Antibiotics should be given only if the differential diagnosis includes likely invasive bacterial infections. Although it may seem obvious that antibiotics should be given only to patients who have bacterial infections, the failure to adhere to this first principle is one of the most common causes of unnecessary antibiotic use.

Many viral infections and noninfectious diseases mimic the signs and symptoms of bacterial infections. Overestimating the probability of bacterial infections, or the harm that may come by not treating them (such as for upper respiratory tract infections), is a potent driver of inaccurate differential diagnoses, leading to inappropriate antibiotic prescriptions. Furthermore, providers frequently underestimate the harm of antimicrobial prescriptions—one in five patients given antibiotic prescriptions are harmed by them due to adverse events or superinfections by resistant pathogens or *Clostridioides difficile* (formerly *Clostridium difficile*).[25] This combination of fear from uncertainty of the diagnosis and lack of appreciation of how dangerous antibiotics can be is a particularly important driver of inappropriate prescriptions for upper respiratory tract illnesses in outpatient settings. Novel psychological approaches to combat the fear driving such prescriptions including audit and feedback and the gentle nudge of public commitment,[16,24,26,27] have been demonstrated in large clinical trials to markedly reduce inappropriate prescriptions in this setting.

Another common driver of inappropriate prescription results from mistaking positive cultures as evidence of infection. The presence of a positive culture in the absence of signs or symptoms of infection should not reflexively trigger antibiotic prescription. In most cases, absent signs or symptoms of infection, positive cultures represent colonization or contamination and should not trigger treatment.

Cultures from nonsterile sites are a particular problem. Surface swabs from skin or wounds, or cultures taken from respiratory (even via bronchoalveolar lavage), urinary, or gastrointestinal tracts all sample the microbiome. Growth of organisms from such cultures does not indicate infection in asymptomatic patients. Even if patients have symptoms, one cannot necessarily discern the microbial etiology of infection by growing organisms from nonsterile sites, which have polymicrobial microbiomes. There often is a tendency to apply therapy for every strain of bacteria grown, even though most of the organisms encountered are typically not pathogenic, and among those with pathogenic potential, it can be impossible to distinguish bystanders from etiologic pathogen(s).

Absent signs or symptoms of clinical disease, generally antibiotics should not be administered irrespective of culture results from skin, wound swabs, respiratory secretions, or urine.

2. Even in a Patient With a Bacterial Infection, Treat Only When Therapy Will Alter the Patient's Clinical Course

The administration of antibiotics should not be a reflexive response to infection, but rather should be incorporated into an overall, rational therapeutic plan for the patient. Patients who lack bacterial infections cannot have their clinical course improved by antibiotics (as discussed in Principle 1). But even patients who do have bacterial infections still may not warrant antibiotic therapy.

One classic example of this scenario is an osteomyelitis in chronically exposed bone. In this case, the driver of antibiotic efficacy for the osteomyelitis will be whether there is a feasible plan for long-term flapping of the wound so that the bone does not remain exposed.[28] It is not rational to attempt to cure the osteomyelitis with antibiotics absent such a plan. A brief course of antibiotics may be warranted if there is a superimposed acute cellulitis in the tissue around the osteomyelitis, but the course should not be prolonged to attempt cure of the underlying osteomyelitis.[28] All that will be achieved by administering prolonged antibiotics to such a patient is selection for antibiotic resistance and exposure to the risk of side effects from the antibiotics, without the possibility of cure of the bone infection. Once the wound is flapped, a curative course can be considered.

Other examples of irrational use of antimicrobial therapy in patients with confirmed infections include the use of antibiotics to treat infection in a terminally ill patient who will die shortly due to their underlying disease irrespective of the antibiotics, or administration of antiretroviral therapy to a patient with acquired immunodeficiency syndrome who is not yet ready to be adherent with the medications. The former scenario raises complex medical ethics questions. Should the wishes of a patient or their family be adhered to regarding administering antibiotics even at the cost of harming other people in society by driving antibiotic resistance? It is important to include consideration of the harm to society caused by the prescription in decision making, as it may outweigh the limited benefit of antimicrobial therapy in such cases. In the latter scenario, administering antiretroviral agents will cause resistance to develop, converting a treatable infection into a nontreatable one. Thus giving antimicrobial agents (inclusive of antivirals) to such patients will not be of benefit and can cause harm. Such use fails the core of the Hippocratic oath: *primum non nocere.*

3. Empirically Target Microbes That Cause the Diseases in the Differential Diagnosis

Once antiinfective therapy is determined to be necessary based on a rational differential diagnosis, and appropriate based on its likely ability to change the course of the patient's outcome, the proper agents must be selected. In general, preferred antimicrobial regimens are those previously established by clinical investigations to be safe and effective for the target disease(s). However, clinicians frequently encounter patients who would not have met enrollment criteria for comparative studies. Furthermore, multiple regimens may have been established to be safe and effective for the target disease(s). In either case the spectrum of activity is an important consideration when selecting which antimicrobial regimen to use.

The spectrum of activity of the empirical antiinfective agents prescribed should be only as broad as necessary to cover the likely microbial flora that cause the diseases in the differential diagnosis.[24] Use of overly broad therapies to treat pathogens unlikely to be encountered is inappropriate and will select for resistance while providing no benefit to the patient.

As a basic principle, nosocomial infections are often caused by more resistant pathogens, and hence require coverage for *Pseudomonas* or other nonfermenting gram-negative bacilli. Conversely, generally speaking community-onset infections are not caused by such bacteria, and empirical antibiotic coverage for community infections should not routinely include such coverage.

The issue of spectrum of activity raises another critical distinction between antibiotics and other drugs. For most drugs the diseases for which they were found to be safe and effective treatments in clinical trials define the relevant treatment population after approval. For antibiotics the issues are more complex.[17,24] Even if a drug with antipseudomonal activity is safe and effective for the disease in question, if the disease is typically caused by streptococci or other less resistant pathogens (e.g., community-onset skin infections or pneumonia), the first-line use of the agent with antipseudomonal coverage is inappropriate despite its safety and efficacy, and irrespective of US Food and Drug Administration indication.[24] Thus safety and efficacy are not sufficient, per se, to define appropriate use of an antibiotic. The spectrum of activity targeted to likely microbial etiology must be considered.

4. A Lower Threshold for Empirical Therapy Should Be Used in Critically Ill Patients

The margin for error is an important consideration in selecting empirical antimicrobial therapy. A patient who is hemodynamically stable and complains only of a fever and malaise may be able to wait without starting any empirical therapy while the workup proceeds. If therapy is to be started in such a patient due to a high pretest probability of bacterial infection, it can be focused on pathogens highly likely to be encountered.

In contrast, a hypotensive patient who is being treated with pressors in the ICU cannot wait. Multiple studies have found that delays of even as short as an hour in initiation of effective antibiotic therapy in such patients markedly increases the risk of progression to septic shock and death.[29–35] Thus the threshold for triggering initiation of empirical therapy should be lower in unstable patients.

The breadth of therapy in critically ill patients can also be wider, but therapy should still focus on the pathogens most likely to be encountered. For example, community-acquired pneumonia, intra-abdominal, skin, and urinary infections typically are not caused by *Pseudomonas* outside very specific circumstances (e.g., patient with cystic fibrosis or with history of bronchiectasis, chronic dialysis patient, or patient with indwelling catheters or recent surgery). Thus even in a critically ill patient with community-onset infections, antipseudomonal therapy generally should not be included outside such circumstances.

Hemodynamically compromised patients should have antibiotics infusing as soon as possible. It is important to emphasize that just ordering the antibiotic is not enough; its infusion should be begun immediately in such patients. Follow-on doses should be given on schedule, as a delay in the second dose has also been associated with higher mortality.[36]

When providers order multiple antibiotics to be administered to a critically ill patient, it may be useful to indicate on order sets or protocols the "anchor" antibiotic that is to be infused first. Delineating the anchor antibiotic streamlines the process so that the pharmacy and nursing unit are able rapidly to deliver the most time-sensitive therapy.

5. Host Factors Affect Breadth of Empirical Therapy by Altering Likely Microbial Causes of Infection

The primary impact that an immunocompromised state should have on selecting antimicrobial therapy is to broaden the differential diagnosis for pathogens likely to be encountered. Unusual bacterial etiologies, such as *Listeria* and *Nocardia*, viral and fungal etiologies, and even parasitic etiologies may need to be considered and therefore treated empirically in a patient who is immunocompromised. Generally speaking, viral and bacterial infections occur acutely in immunocompromised hosts (e.g., after hours to days), and fungal and parasitic infections occur subacutely or chronically (e.g., after weeks to months).

Different forms of immune compromise predispose to different types of pathogens. For example, neutropenic patients are at high risk for acute pyogenic bacterial pathogens and subsequently invasive mycoses. In contrast, patients with defects in T cells are at higher risk for chronic infections caused by atypical pathogens, such as *Pneumocystis* pneumonia, *Cryptococcus* meningitis, tuberculosis (TB), and so forth, as well as pneumococcal pneumonia. Patients with defects in humoral immunity (e.g., from congenital B-cell defects, acquired B-cell defects such as from lupus or treatment with rituximab, antibody-wasting diseases such as nephrotic syndrome) acquire infections caused by encapsulated

bacterial pathogens (e.g., pneumococci, *Neisseria meningitidis*, *Haemophilus influenzae*). A thorough understanding of immune mechanisms and how they defend against specific pathogens is important to enable proper prioritization of microbes likely to be encountered, and hence empirical antimicrobial therapy, in immunocompromised patients.

However, it is not true that immunocompromised patients are intrinsically more likely to be infected by antibiotic-resistant bacterial pathogens. The immune system does not dictate the nature of genetic resistance mechanisms present in bacteria. Rather, environmental and prior antimicrobial exposures do. There is no evidence that immunocompromised patients who acquire their infections in the community, and without having recently been exposed to health care environments (e.g., hemodialysis) or antimicrobial agents, are likely to be infected with more resistant pathogens than immunocompetent patients. Thus the breadth of coverage for highly antibiotic-resistant bacterial pathogens should be dictated by environmental and recent antimicrobial exposure rather than immune status.

For some types of lethal pyogenic infections, blunting the immune system is actually required to enhance efficacy of antimicrobial agents. Morbidity and mortality from many infections are driven as much by the host inflammatory response to the organism as to the bacterial density, and administration of antimicrobial therapy can result in sudden lysis of microbes, releasing highly inflammatory antigens into systemic circulation. The classically described example of this phenomenon is the Jarisch-Herxheimer reaction of syphilis.[37] Similarly, patients with high-grade bacteremia caused by gram-negative bacilli may suddenly decompensate shortly after antimicrobial therapy is begun, and organisms begin to lyse and release endotoxin into the bloodstream.[37] Other proven examples where a sudden lysis of pathogens exacerbates morbidity and mortality of infection, and administration of adjunctive corticosteroids on top of antimicrobial therapy improves morbidity and mortality, are patients with *Pneumocystis* pneumonia, pneumococcal meningitis, or tuberculous meningitis.[38–40]

The differential diagnosis of infection should drive the breadth of antimicrobial coverage. If the differential includes unusual pathogens or nonbacterial pathogens, which is more likely in an immunocompromised patient, then therapy should indeed be broader. However, if the differential diagnosis is clearly focused on acute pyogenic bacterial infections, antibacterial therapy generally does not need to be broader in spectrum in an immunocompromised host than in an immunocompetent host. Highly resistant pathogens generally do not need to be targeted with therapy in immunocompromised hosts who acquire their infections in the community and without other risk factors for developing antibiotic-resistant infections.

6. Pharmacokinetic-Pharmacodynamic Principles Can Influence Optimal Treatment

Having considered clinical trial data supporting specific antimicrobial use, the requisite spectrum of activity based on the differential diagnosis, its likely microbial etiologies, and the patient's clinical stability and risks for antibiotic-resistant pathogens, pharmacologic principles should be considered. Infections in the bloodstream may be treated preferably with antibiotics that achieve higher blood levels. Antibiotics with large volumes of distribution, which penetrate widely into tissues but have lower blood levels, may be less desirable for treatment of bacteremia. Such antibiotics include macrolides, tetracyclines, and rifamycins. Because many antibiotics penetrate into the central nervous system (CNS) poorly, higher than standard doses may be required to optimize achievable drug levels for such infections (e.g., ceftriaxone 2 g intravenous [IV] every 12 hours in lieu of 1 g IV once per day). In contrast, many drugs penetrate well into the kidney and urine. Among those with the highest urinary levels are aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole (TMP-SMX), and β-lactam antibiotics. Fluoroquinolones and TMP-SMX also have excellent prostatic penetration. Hence such antibiotics may be preferred for prostate infections.

For many years medical lore suggested that IV therapy was required to treat chronic bone infections. However, multiple antibiotics achieve levels in bone adequate to treat osteomyelitis when administered orally, including TMP-SMX, clindamycin, linezolid, rifampin, metronidazole, and so forth.[41] Validating the pharmacology data, multiple randomized controlled clinical trials have found that oral therapy with TMP-SMX (especially with rifampin), clindamycin, or fluoroquinolones are as effective as IV therapy for the treatment of osteomyelitis and are associated with fewer severe side effects due to the sparing of prolonged IV catheterization.[41–43]

In critically ill patients oral antimicrobial therapy may be less desirable for two reasons. First, there are concerns about the absorption of drugs from the intestines in critically ill patients, who may be experiencing diminished blood flow to the bowel. Second, all drugs will achieve peak levels faster when administered intravenously, which may be important in a critically ill patient.

Different antibiotics kill via different pharmacodynamic principles.[44–47] Some antibiotics, such as β-lactams, are time-dependent killers, and the time spent above the minimum inhibitory concentration (MIC) is a much better predictor of efficacy than how high the antibiotic concentration is above the MIC. For such drugs it is rational to prolong the infusion, which results in greater time spent above the MIC, while smoothing out the peak and troughs of the drug concentrations.[48–50] Indeed, clinical data validate that prolonged infusions of β-lactams may result in superior clinical outcomes,[51,52] and such strategies are increasingly used clinically. It is important to emphasize, however, that lower peak drug levels will be achieved with prolonged infusion. As such, if the organism being treated has a high MIC, prolonging the infusion may diminish efficacy by precluding achievement of antibiotic concentrations above the MIC. For example, at carbapenem MIC greater than 16 μg/mL, prolonging the infusion of the carbapenem will preclude achieving concentration of the drug above the MIC, which will result in diminished bacterial killing.[49] Hence intermittent dosing to achieve peak levels above the MIC may be preferred for isolates with higher MICs and particularly those in the intermediate-to-resistant range.

Some antibiotics have nonlinear dosing pharmacokinetics, and optimal dosing can result in superior drug levels. For example, increasing the dose of levofloxacin from 500 to 750 mg once per day (a 50% increase) enables a 100% increase in achieved serum concentration.[53] Similarly, because its biliary transport becomes saturated at a dose of approximately 450 mg, dosing rifampin at 600 mg once per day results in far superior 24-hour area-under-the-curve serum concentrations than dosing 300 mg twice per day.[54,55]

Finally, it is important to ensure that the antibiotic will retain antimicrobial activity at the site of infection. Although most antibiotics are active in most tissue environments, there are exceptions. For example, low oxygen tension and low pH environments, such as those found in dense abscesses, can inactivate aminoglycoside efficacy by blocking their uptake.[56] Hence aminoglycosides are not desirable agents to treat abscesses.[57] A unique situation was discovered with daptomycin during its pivotal clinical trial for the treatment of community-acquired pneumonia. Daptomycin was found to be inferior in efficacy to the comparator,[58] and a subsequent study discovered that pulmonary surfactant inactivates daptomycin.[59] Thus daptomycin should not be used to treat alveolar pneumonia. These are specific examples of tissue microenvironments altering antimicrobial effectiveness. In most cases, if adequate drug levels can be achieved at the site of infection, effective antimicrobial action will be achieved.

7. Deescalate Antibiotic Therapy Based on Microbiology Results and Clinical and Biomarker Responses

Having started empirical antimicrobial therapy in the absence of knowledge of an etiologic pathogen, the therapy should then be tailored based on several factors. The first is subsequent microbial identification or not. The Gram stain result or identification of the pathogen's species is often sufficient to enable "deescalation" of antibiotics, even without the results of susceptibility testing. For example, for patients growing gram-negative bacilli in blood culture bottles, empirical vancomycin can be stopped. Conversely, for a patient growing gram-positive cocci in the blood, empirical antibiotics targeting gram-negative bacilli can be stopped. Similarly, for patients with community-acquired pneumonia who grow *Streptococcus pneumoniae*, the atypical coverage can be stopped

and β-lactam therapy continued. Once susceptibility results are available, therapy should be tailored/narrowed to match the susceptibility profile.

Even when microbiology results are negative, the absence of positive cultures can be informative to deescalation. For example, empirical vancomycin coverage is often included for patients with sepsis of unknown etiology or nosocomial pneumonia to cover for methicillin-resistant *S. aureus*. *S. aureus* grows very well in standard cultures. If after 24 to 48 hours there is no growth of gram-positive cocci in clusters, empirical vancomycin typically can be discontinued.

The other factor that must be considered is the clinical response to therapy. Any time antibacterial therapy is initiated, the treating clinician should know a priori which parameters to follow to determine if the therapy is effective. This is a particularly important principle when therapy is empirical and the etiologic pathogen(s) is not known or its sensitivities are not known. Antibacterial therapy active against a target pathogen is remarkably effective. Signs, symptoms, and biomarkers of most types of infections should improve dramatically within 1 to 2 days after the initiation of active therapy. The key is to determine which signs, symptoms, or biomarkers of infection are present at baseline, and to use changes in those biomarkers to guide subsequent therapeutic decisions.

Fever and leukocytosis are among the oldest clinical biomarkers used by clinicians to determine response to antimicrobial therapy. Both are frequently used, and resolution of both can be extremely informative that the infection is being adequately treated with the selected regimen. A more recently studied biomarker for enabling early cessation (or avoidance entirely) of antibacterial therapy is procalcitonin. Dozens of randomized controlled trials have investigated the impact of making the procalcitonin result available to treating providers or not. These studies have evaluated the ability of procalcitonin to help avoid antibiotic prescriptions for patients without sepsis or to help with earlier cessation of antibiotics in sicker patients with sepsis. Studies have been conducted in a variety of care settings, including the emergency department, inpatient wards, and ICUs.

Recent meta-analyses of 26 such trials found that incorporation of procalcitonin into therapeutic decision making resulted in a >25% reduction in the duration of antibiotic therapy, significant reduction in antibiotic-related adverse events, and not only no worse outcomes but actually significantly improved mortality in the face of shorter courses of antibiotic therapy.[60,61] In practice, ID experts need to help steward use of the procalcitonin, as there may be a tendency for providers to ignore negative procalcitonin results as being spurious but use a positive procalcitonin to justify antibiotic use. If stewarded properly and incorporated as an evidence-based biomarker, procalcitonin can be a useful tool for antibiotic stewardship teams to provide reassurance to clinicians that it is safe to stop antibiotics early in sick patients or potentially avoid antibiotics entirely for less sick patients.

Patients responding to initial empirical therapy with improvements in signs, symptoms, or biomarkers of infection can be continued on that therapy until microbial information becomes available to enable tailoring therapy. For patients who are responding to therapy but never have a microbial etiology identified—common for lung, abdominal, or skin infections—the empirical therapy can be continued to complete a defined course of therapy (more on duration of therapy later). Note that in this case it is still reasonable to deescalate empirical therapy by stopping agents targeting highly resistant pathogens if they have not grown in cultures (e.g., stop empirical vancomycin if methicillin-resistant *S. aureus* does not grow).

However, if the patient is not responding to therapy within 1 or 2 days, it may be necessary to consider alternative therapeutic or diagnostic interventions even in the absence of knowledge of the microbial etiology of infection.

8. If Therapy Is Not Working, Consider Source Control and Alternative Diagnoses Before Blaming Resistance and Broadening Therapy

If an antimicrobial regimen has not resulted in improvement in fever curve, white blood cell count, purulent secretions, signs of inflammation (rubor, tumor, dolor, calor), procalcitonin level, and so forth, within a day or two, the treatment decision may need to be reconsidered. Although the treating clinician's initial tendency is typically to blame "antibiotic resistance" and seek to broaden antimicrobial therapy, two alternative causes should be considered before reflexively broadening therapy.

One possibility is that adequate source control has not been obtained. Adequacy of source control, such as removing drainable pus and removing foreign materials or other niduses of infection, is the cornerstone of successful management of infections.[62–64] Failure of source control independently increases treatment failure and mortality irrespective of antimicrobial therapy.[62,65–75] Adequacy of source control must always be considered when patients do not appear to be responding to antimicrobial therapy. Investigation for source control may require imaging to establish whether drainage has been achieved or if there is a nidus of infection that is obscure externally and is responsible for treatment failure (e.g., occult subcutaneous abscess in a patient failing cellulitis therapy, new abscess formation during treatment of an intraabdominal infection, empyema in a patient with community-acquired pneumonia, failure to remove a central line in a bacteremic or fungemic patient). Broadening therapy will not result in clinical response in the absence of adequate source control.

A second explanation for lack of adequate response to therapy is incorrectly attributing the patient's clinical condition to a bacterial infection. Thus, for patients who are not responding to antimicrobial therapy, in addition to assessing source control, the differential diagnosis should be reconsidered. Common infections, such as community-acquired pneumonia and cellulitis, have signs and symptoms that can be strikingly similar to noninfectious ailments. Indeed, patients are commonly misdiagnosed with cellulitis and instead have a variety of inflammatory skin disorders ranging from venous stasis to contact dermatitis/allergic reactions to necrobiosis lipoidica diabeticorum.[76,77] Similarly, bacterial pneumonia can be clinically mimicked by pulmonary edema, pulmonary embolism, reactive airways disease, aspiration pneumonitis, viral pneumonia, and so forth.

Other frequently encountered noninfectious causes of fever not responding to antibiotics include drug fever (which may in fact be caused by the antibiotic, an unfortunate tautology that can lead to prolonged and futile antibiotic courses), malignancy, and rheumatologic disorders that mimic infections. Thus failure of therapy should prompt a reevaluation to ensure that the patient really does have a bacterial infection and not a noninfectious mimicker or a viral infection.

For patients who do not appear to be responding to antimicrobial therapy within the first day or two, a full reevaluation of source control and the differential diagnosis should precede reflexive changes to broaden therapy based on fears of antibiotic resistance or concerns for infections caused by organisms of another kingdom (e.g., fungi).

9. Distinguish New Infection From Failure of Initial Therapy

Patients may suffer from multiple, serial infections while in health care settings. Thus a patient who has evidence of clinical response to initial therapy, with resolution of signs and symptoms of infection, and who then begins to spike fevers anew, should be evaluated differently than a patient who never responds to initial therapy (the latter issue is the focus of Principle 8). New onset of infectious signs, symptoms, and biomarkers after resolution of prior infection should raise the concern of a new infection rather than persistence of the original infection.

Rarely, recrudescence of signs and symptoms may reflect emergence of antibiotic resistance on therapy from the initial pathogen—this may be seen more with specific bacterial pathogens, such as *Acinetobacter*, than with others.[78] In any event an initial apparent response to infection followed days or weeks later by new onset of infectious signs or symptoms should prompt a complete reevaluation of the patient for a new infection, including reculturing and imaging if necessary. In such patients it is generally reasonable to broaden therapy to cover highly resistant pathogens. Such patients have been treated with prior, recent courses of antibiotics, and have a higher risk of being infected by antibiotic-resistant pathogens.

When changing antibacterial therapies due to breakthrough infection or lack of response to initial therapy, it is generally advisable to change one antibiotic at a time. Such a change enables determination of which new drug is likely the cause of any improvement in signs, symptoms, and/or biomarkers of infection. Therapy then can be tailored/narrowed

TABLE 17.2 Short-Course Therapy Is Equally Effective as Longer Therapy for Many Infections

DISEASE	SHORT COURSE STUDIED (DAYS)	LONG COURSE STUDIED (DAYS)	OUTCOME
Chronic bronchitis and obstructive pulmonary disease, acute exacerbations[a]	≤5	≥7	Equivalent
Intraabdominal infection	4	10	Equivalent
Neutropenic fever	Until afebrile and stable	Until afebrile, stable, and nonneutropenic	Equivalent
Osteomyelitis, chronic	42	84	Equivalent
Pneumonia, community-acquired	3–5	7–14	Equivalent
Pneumonia, nosocomial (including ventilator-associated)	≤8	10–15	Equivalent
Pyelonephritis	5–7	10–14	Equivalent
Skin infections (cellulitis, major abscesses, wound infections)	5–6	10–14	Equivalent
Sinusitis, acute bacterial[a]	5	10	Equivalent

[a]Most patients with these diseases do not require antibiotics at all. Nevertheless, if they are prescribed, there is no advantage to prescribing longer courses.
Data from references 79 and 154.

based on that knowledge. However, in patients who are critically ill, changing multiple antibiotics at one time may be necessary, especially for patients who are hemodynamically unstable.

10. The Duration of Therapy Should Be Evidence Based and Short Course Whenever Possible

Antibiotics should be administered only for as long as necessary to optimize cure rates. Unfortunately, even in the era of evidence-based medicine, the duration of most courses of antibiotic therapy can be traced back to Constantine the Great, who decreed in 321 CE that the week would consist of 7 days.[79] This approximately 1700-year-old decree remains the primary driver for many antibiotic courses, which are proffered in 7-day increments.

Fortunately, in recent years numerous clinical trials have been conducted comparing long- to short-course antibiotic therapy, with the short courses often not based on 7-day increments. In every such trial ever conducted for patients with specific types of infections (Table 17.2), short-course therapy was found to be equally effective as longer-course therapy, and in several studies with diminished selection for antibiotic resistance.[79]

Notable exceptions to short-course therapy include otitis media in children younger than 2 years[80] and penicillin—but not cephalosporin—therapy for streptococcal pharyngitis.[81,82] In both of these cases, cure rates were lower for short-course antibiotic therapy. The reasons for the failure of the short-course therapy for these specific disease/demographics are not entirely clear. The primary purpose of antibacterial therapy in the treatment of streptococcal pharyngitis is the prevention of rheumatic fever, which requires eradication of the organism. Despite diminished rate of clinical cure at end of therapy with short-course penicillin, rheumatic fever rates were not higher in these studies. However, the rate of rheumatic fever is low, and the studies were not powered for this end point.

Shorter courses of antibiotics reduce the risk of side effects from the antibiotics and may result in decreased impact on the microbiome, avoiding superinfections and possibly other metabolic consequences of microbiome disruption that are under investigation.[83–85] Thus it is no longer necessary—and indeed no longer appropriate—to choose durations of antibiotics for many infections based on 7-day increments. Wherever possible, treatment durations should be short courses, based on trial data showing equivalent efficacy to longer courses (see Table 17.2).

CONTROVERSIAL PRINCIPLES OF ANTIBIOTIC THERAPY

The Greatly Misunderstood Dogma of Bactericidal Versus Bacteriostatic

One of the most pervasive dogmas in the field of ID has been that antimicrobial agents that are "cidal" are more effective than those that are "static." Although it seems intuitive that antibiotics that more rapidly kill bacteria should be more clinically effective, clinical data do not support this assertion. Furthermore, there are a variety of misunderstandings around the meanings of cidal and static.

Static and cidal are relative in vitro terms not based on linkage to any predictive ability of the outcome of infections in vivo. Contrary to common belief, static antibiotics *do* kill bacteria, they just require a higher concentration to achieve specific thresholds of bacterial reduction. The formal definition of a cidal antibiotic is one for which the minimum bactericidal concentration (MBC) of the drug is ≤4-fold above the MIC.[86] The MBC is the concentration of the drug that results in a 1000-fold reduction in bacterial density at 24 hours of in vitro growth in specific media, at a specific temperature, and a specific carbon dioxide concentration. The MIC is the concentration of the antibiotic that inhibits visible growth at 24 hours.

Thus antibiotics that achieve a >1000-fold reduction in bacterial density at a concentration that is 8-fold above its MIC, or 500-fold reduction in bacterial density at 4-fold above its MIC, are considered static despite clearly killing bacteria. All currently available antibiotics that are considered static do kill bacteria in vitro, just with the MBC-to-MIC ratio greater than would be for cidal agents.

Given that these definitions are based on in vitro conventions and not based on specific clinical principles, perhaps it is not surprising that there is no clinical evidence of benefit of cidal agents over static agents. A systematic literature review identified 56 randomized controlled trials published since 1985 that compared the efficacy of static versus cidal antibiotics head to head for patients with invasive bacterial infection.[87] Forty-nine of the trials found no significant difference in efficacy between static versus cidal antibiotics, including for highly lethal infections of critically ill patients, such as typhoid fever, severe pneumonia, severe sepsis, and so forth.

The exceptions that did find a clinical efficacy difference between static and cidal agents disprove the rule that cidal agents are intrinsically more effective. In fact, six trials found the static agent linezolid to be superior in efficacy to cidal agents (vancomycin or cephalosporins).[87] In contrast, only one trial found a cidal antibiotic superior in efficacy to a static agent. That trial compared tigecycline versus imipenem for the treatment of ventilator-associated pneumonia and found that tigecycline was inferior.[88] However, subsequent pharmacologic analysis determined that the tigecycline dose used in the trial was too low, resulting in inadequate drug levels compared with the susceptibility of bacteria causing the infections.[89] When a subsequent trial was conducted with double the dose of tigecycline, tigecycline was similar in efficacy to imipenem for the same disease.[90]

Similar conclusions are drawn from studies in which rapidly cidal antimicrobial strategies have been compared with agents that are more slowly cidal. Daptomycin is one of the most rapidly bactericidal agents known, yet it was not superior in efficacy to the slowly cidal agent vancomycin for the treatment of staphylococcal bacteremia or right-sided endocarditis.[91]

Similarly, addition of aminoglycosides to β-lactam therapy results in a synergistic, marked improvement in the rapidity of kill of staphylococci and more rapid clearance of bacteria from the blood in patients.[92–94] However, meta-analysis of several studies comparing β-lactam monotherapy with combination therapy with β-lactam and aminoglycosides found no difference in clinical cure or mortality, whereas nephrotoxicity was worse.[94] Thus there is no clear, overall clinical benefit apparent from the increased rate of bacterial kill. Nevertheless, addition of aminoglycosides for several days—keeping the aminoglycoside duration short to minimize nephrotoxicity—may be a rational approach for patients with refractory staphylococcal bacteremia.

The last frontier of the static versus cidal debate lies in the territory of bacterial endocarditis. In the 1950s Finland found that static agents, including a variety of tetracyclines and macrolides, resulted in poor outcomes when used to treat endocarditis.[95,96] Similarly, a rabbit study and several case series suggested excess treatment failure rates when clindamycin was used to treat aortic valve staphylococcal endocarditis.[97] These studies led to the belief that static agents generally are inferior as therapeutic agents for endocarditis.

However, there are a number of problems with such assertions. Tetracyclines and macrolides achieve very low blood concentrations. Thus, based just on pharmacologic principles (low blood concentrations), having nothing to do with how rapidly they kill microbes, these agents would not be anticipated to be desirable agents to use to treat high-grade bloodstream infections. Furthermore, clindamycin is static for anaerobic bacteria but is actually cidal for staphylococci.[98] Thus, if clindamycin performs poorly in treating staphylococcal endocarditis, it is actually a negative mark against a cidal agent, not a static agent.

In contrast, although never studied in a randomized controlled trial, the static agent linezolid, which has favorable bloodstream pharmacokinetics, has been found to result in high cure rates for bacterial endocarditis in a number of published case series.[99–101] Finally, the rapidly cidal antibiotic daptomycin has been described to result in worrisome failure rates in some patients with left-sided endocarditis.[102]

Overall, there is no evidence that cidal antibiotics are intrinsically more clinically effective than static antibiotics. The majority of studies comparing static and cidal agents head to head for the treatment of infections have found no difference in clinical outcomes or mortality. When differences have been found in such head-to-head studies, they have usually favored the static agent as being clinically superior to the cidal agent. When static agents have been found inferior, the explanation appears to be more likely inadequate dosing and/or achievable levels at the site of infection, and not rapidity of kill of the microbe. Thus pharmacokinetic-pharmacodynamic principles and achievement of adequate levels of the drug at the site of infection appear to be more important parameters than static versus cidal properties for predicting clinical antimicrobial efficacy.

Combination Therapy: The Good, the Bad, and the Ugly

Whether or not combination antibiotic therapy is appropriate is highly dependent on the intended purpose. There are general circumstances where combination therapy, if judiciously applied, is rational and appropriate (the "good"). There are also settings in which combination therapy is wasteful or toxic (the "bad"). Finally, there are settings in which there may be theoretical but unproven advantages of combination therapy, leaving the clinician in a quandary as to whether combination therapy should be used or not (the "ugly").

The Good

There are three general settings, elaborated as follows, in which combination antimicrobial therapy is known to be advantageous. The first is in the empirical setting, when treating an infection for which the potential etiologic microbial species are sufficiently broad that one agent is unlikely to cover all of them. The second is for preventing the emergence of resistance in specific clinical settings. The third is a rare circumstance in which two active agents are known to result in superior clinical outcomes compared with a single active agent against a susceptible organism.

The Good #1: Ensuring Adequate Empirical Coverage

Many types of serious infections may require more than one antimicrobial agent to be started empirically to ensure coverage of the potential etiologic pathogens. For example, when treating community-acquired pneumonia, a second agent (macrolide or doxycycline) is added to cover atypical organisms that the backbone β-lactam antibiotic will not cover. If a microbial etiology is identified, the empirical therapy can then be narrowed to targeted, definitive therapy.

Intraabdominal infections are polymicrobial in nature, caused by both aerobic and anaerobic bacteria in the gut. Agents that are superior for killing the aerobes and facultative anaerobes (e.g., cephalosporins) may not have adequate activity to cover the obligate anaerobes as well as metronidazole. Thus combination therapy is appropriate for the treatment of intraabdominal infections, although there are some single agents that when used are broad enough to provide adequate coverage as monotherapy (e.g., piperacillin-tazobactam, carbapenems).

For empirical therapy, the breadth of coverage warranted depends on the local resistance patterns among the likely pathogens. For routine nosocomial pneumonia coverage in a patient not exposed to prior antibiotics, monotherapy with a third- or fourth-generation cephalosporin, piperacillin-tazobactam, or meropenem is likely to cover most gram-negative bacterial pathogens encountered. Use of more than one agent should not be necessary in most situations. In contrast, in a specific ICU or ward where infections caused by organisms resistant to these β-lactam agents are regularly encountered, it may be reasonable to start two empirical antibiotics to try to ensure at least one active agent is being administered. This practice may be referred to colloquially as the use of "double gram-negative coverage." Dual-agent empirical therapy also may be reasonable to start in a patient with a history of recent (e.g., within 90 days) exposure to broad-spectrum antibiotics, or known colonization by resistant pathogens.

If empirical multiple-agent therapy is warranted for the possibility of encountering resistant gram-negative bacilli, it is important to consider the potential overlap in the coverage. An often used pairing is a cephalosporin (e.g., ceftazidime or cefepime) plus a fluoroquinolone. Unfortunately, bacterial strains resistant to a third- or fourth-generation cephalosporins are often resistant to fluoroquinolones as well. Thus very little may be gained by adding a fluoroquinolone to an advanced-generation cephalosporin. When using empirical double coverage for the purpose of trying to assure that at least one agent is active, the local antibiogram should guide selection of the second agent. Generally speaking, an aminoglycoside may offer less overlapping coverage to β-lactams than fluoroquinolones and hence increase the likelihood that at least one active agent is being administered.

When multiple empirical agents are initiated, the goal should be to deescalate therapy by stopping all but one of the empirical agents once a microbial etiology is known. Unfortunately, in practice clinicians often continue multiple-agent empirical therapy even after a microbe is identified and therapy could be deescalated to target that pathogen. This continuation may occur because clinicians forget that multiple antibiotics are being administered or because they do not wish to "rock the boat" if a patient is improving on the initial therapy. In either case the role of the ID expert is to prompt deescalation. Continuation of multiple agents in this setting exposes the patient to side effects and both the patient and society to selection for antibiotic resistance from the unneeded additional agents.

One specific scenario in which the treating clinician may make a conscious choice to continue two active agents after an organism is identified is in the treatment of *Pseudomonas aeruginosa*. As discussed further, this practice is generally unnecessary and inappropriate.

The Good #2: Preventing Resistance Emergence

TB is an unusual infection. The etiologic organism has no environmental reservoir, is found only in mammals (and especially humans), grows exceedingly slowly, and spends much of its time in a nonreplicating persister state in vivo in the infected host, making it invisible to most antibiotics. It also can achieve very high bacterial densities (particularly in cavitary disease) such that spontaneous mutations can lead to emergence of resistance on therapy. Thus combination therapy is required for

active TB, not only to ensure initial therapy is adequate but also to ensure resistance does not emerge on therapy. Indeed, active TB is the classic bacterial example for which use of combination therapy prevents the emergence of resistance on therapy, which would result in clinical failure.

The other major category of disease for which combination therapy is required to prevent the emergence of resistance on therapy is viral infections. Infections caused by the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are the classic paradigms for combination therapy to prevent emergence of resistance. Resistance is catastrophic in these settings, as they convert a treatable (HIV) and curable (HCV) infection into potentially fatal illnesses.

However, as will be discussed further, whether or not combination therapy prevents the emergence of resistance among typical, pyogenic bacterial infections remains unclear. TB, HIV, and HCV are examples of infections for which the pathogens are not normal microbiota, have no environmental reservoir, and have very high rates of emergence of resistance while on therapy. These factors fundamentally distinguish TB, HIV, and HCV from typical pyogenic bacterial infections. As will be discussed further, it is not safe to extrapolate the appropriate use of combination chemotherapy for these three infections as a model for the use of combination regimens to prevention of emergence of antibiotic resistance among typical bacterial pathogens.

The Good #3: Improving Cure

There are a select few bacterial infections for which use of combination therapy with more than one active agent against susceptible bacteria is likely to result in superior clinical cure rates than use of a single active agent. For bacteria these diseases can be separated into one of two categories: slow-growing infections often caused by intracellular pathogens or rapidly growing infections associated with exotoxin-mediated tissue destruction. Combination therapy is more commonly needed to optimize cure for complex eukaryotic pathogens, particularly those with multiple phases of their life cycle.

Slow-growing infections/nonreplicating persisters. One type of bacterial infections for which combination therapy may result in superior outcomes are those in which nonreplicating persister organisms are fundamental to the pathogenesis of the infections. Nonreplicating persister bacteria shut down metabolic pathways, making them invisible to most antibiotics because they are not expressing the metabolic targets of the antibiotics.[103–105] TB is the classic example. Rifampin and pyrazinamide are capable of killing nonreplicating persister bacteria more effectively than other agents. As a result, the addition of rifampin and pyrazinamide allowed shortening courses of therapies from years to 6 months for active pulmonary TB.[106,107] Thus combination therapy for TB illustrates all three potential advantages: (1) ensuring initial empirical therapy is active, (2) preventing the emergence of resistance, and (3) improving clinical cure with a shorter duration of therapy.

Similarly, chronic osteomyelitis has a relatively high rate of relapse after an initial course of therapy. Multiple randomized controlled clinical trials and observational studies have found that addition of rifampin to backbone antibiotic therapy decreases the rate of late relapses of bone and prosthetic joint infections.[41,108]

Another example where combination therapy results in superior outcomes is the treatment of endocarditis caused by refractory, slow-growing bacteria, including *Enterococcus* spp. or penicillin-resistant streptococci. Addition to penicillin/ampicillin of gentamicin, or more recently ceftriaxone, results in superior cure and survival rates for such infections.[109]

Combination therapy also results in superior cure rates for the slow-growing, intracellular pathogen *Brucella*, for which single therapy is not able to effectively kill the pathogen.[110] Another atypical, intracellular pathogen for which combination therapy results in superior outcomes is *Coxiella burnetii*. Addition of hydroxychloroquine to antibiotic therapy improves cure from *Coxiella* infection by a specific physiologic mechanism.[111] Hydroxychloroquine alkalinizes the phagolysosome that *Coxiella* hides within, making the organism more susceptible to antibacterial therapy that it would otherwise resist. Without this adjunctive therapy, the pH in the phagolysosomal becomes highly acidic, which neutralizes the activity of the antibiotics that would otherwise be effective to kill the pathogen.

Exotoxin-mediated necrotizing infections. The opposite spectrum of bacterial diseases for which combination therapy may improve cure are exotoxin-mediated diseases that are extremely rapid, destructive, and aggressive. Necrotizing fasciitis caused by exotoxin-producing streptococci, or *Clostridium* tissue necrosis, are examples. The theoretical advantage of adding clindamycin or linezolid as a second agent for treating such infections is that these antibiotics act by terminating protein synthesis inside the bacteria. As such, they can decrease the production of the exotoxin causing tissue degeneration, even while the bacteria are being killed. Adding protein synthesis inhibitors (clindamycin or linezolid) to backbone antibacterial therapy has indeed been shown to result in marked improvement in survival in preclinical models of necrotizing fasciitis and in several retrospective studies in patients as well.[112–115]

However, neither infections caused by slow-growing atypical bacteria or exotoxin-producing bacteria are reflective of the pathogenesis of most typical bacterial infections. As discussed later, it is not necessarily safe to extrapolate the salutary effects of combination therapy for these diseases to typical, pyogenic bacterial infections.

Eukaryotic infections. In contrast to bacteria, there are multiple examples of infections caused by eukaryotic pathogens where combination therapy is beneficial. Cryptococcal meningitis is an example of a fungal infection for which a randomized controlled trial has demonstrated that amphotericin plus 5-fluorocytosine resulted in superior clinical outcome compared with amphotericin alone.[116,117]

Dual therapy for protozoal parasites is necessary in some cases because the two agents kill organisms in different phases of the parasitic life cycle. For example, primaquine is added to backbone therapy for *Plasmodium vivax* or *Plasmodium ovale* infection because it kills hepatic-phase hypnozoites that are not killed by other agents and cause late relapse if not treated. Similarly, acute amebic colitis can be treated with metronidazole but must be followed by a luminicidal agent (e.g., iodoquinol or paromomycin) that kills encysted organisms in the bowel lumen that are metabolically inactive and not killed by the metronidazole.

Another example of combination therapy for parasitic infections is the addition of doxycycline to antiparasitic therapy for nematode infections. Pathogenic nematodes may harbor commensal bacteria, such as *Wolbachia*, which are essential for the parasite's viability or fertility. Treatment with antibacterial agents, including tetracyclines or possibly rifampin, has been shown in some studies to enhance the ability of microfilaricidal agents (e.g., ivermectin or albendazole) to kill adult worms, reducing parasite burden.[118,119]

A final example of dual therapy for parasitic infections is neurocysticercosis. In a randomized controlled trial, combination albendazole plus praziquantel therapy resulted in superior outcomes in patients with more than two active lesions of neurocysticercosis.[120,121] The combination of albendazole and praziquantel results in dual mechanism killing of the parasite. There is also a pharmacokinetic interaction resulting in higher drug levels when the combination is used,[122] akin to use of ritonavir to boot protease inhibitor levels for the treatment of HIV. This enhancement in drug levels may improve penetration of the cysticidal agents into the CNS and the cysts within the CNS, which reflect sequestered sites within a sequestered site.

"The Bad": Redundant Definitive Therapy for Typical Bacterial Infections

In contrast to the earlier unusual infections, there are very little data in favor of using dual active agents as definitive therapy for acute, pyogenic bacterial infections. During most acute pyogenic bacterial infections, the organisms are in planktonic growth, not in multiple phases of a life cycle. There are no commensal organisms within the bacteria to kill, and pharmacology and killing activity of effective antibiotics are good. Thus there is no clear rationale for dual therapy.

The best studied organism in this regard is *P. aeruginosa*. There have been dozens of clinical investigations, including randomized controlled trials, seeking to determine if dual definitive therapy results in superior cure rates for infections caused by *P. aeruginosa* compared with monotherapy. Yet decades and dozens of studies later, there remains no

compelling evidence that dual therapy results in superior outcomes.[123] Similarly, two recent randomized controlled trials found no evidence of benefit of combination therapy for carbapenem-resistant *Acinetobacter* infections.[124,125]

Randomized controlled trials and meta-analyses of combination therapy for severe infections/sepsis have also found no advantage of dual active therapy compared with monotherapy.[123,126,127] However, dual therapy is more likely to result in drug toxicity and harm and will cause selection for resistance to two drugs instead of one in the microbiome and environment. Hence, outside of very specific infections, such as those described earlier, evidence does not currently exist to support the routine use of dual active therapy for most acute bacterial infections.

"The Ugly": Imperfect Data and Controversy

Controversy Over Superior Outcomes With Combination Therapy for Some Infections

The one acute pyogenic infection for which available data suggests dual active therapy may result in superior clinical outcomes is bacteremia due to *Klebsiella pneumoniae*. However, the data supporting this conclusion are of poor quality and are mixed. In two retrospective case series of patients with *Klebsiella* bacteremia, dual therapy (β-lactam plus aminoglycoside) resulted in far superior clinical response and survival compared with monotherapy.[128,129] Similarly, in more recent retrospective series, patients with bacteremia due to carbapenem-resistant *K. pneumoniae* (CRKP) had higher survival rates when treated with a variety of combination regimens compared with monotherapy, even when the monotherapy was active against the etiologic pathogen in vitro.[130–132] However, these data are retrospective, and the monotherapy for CRKP was colistin or tigecycline, which are known to be inferior agents. Furthermore, other case series have not found improved outcomes of *Klebsiella* bacteremia treated with dual versus monotherapy when adjusting for disease severity.[133,134] Thus the data are mixed as to the benefit of dual therapy for *Klebsiella* bacteremia, and a definitive trial would be required to settle the question.

The major impetus for combination therapy in recent years has been in the world of fungal infections, outcomes of which are traditionally worse with antimicrobial therapy than typical bacterial infections. Aside from cryptococcal meningitis, randomized controlled trials are lacking demonstrating superiority of combination therapy for invasive fungal infections. For candidemia, the combination of fluconazole plus amphotericin was compared with fluconazole alone in a randomized controlled trial.[135] The study found no significant difference in clinical cure or mortality between combination therapy or monotherapy, although patients with an intermediate severity of illness may have had benefit from combination therapy in a hypothesis-generating, post hoc analysis.[135]

Invasive aspergillosis has also been subjected to study of combination therapy. Bolstered by preclinical data and several retrospective studies suggesting benefit of combination therapy, Marr and colleagues[136] randomized patients with probable or proven invasive aspergillosis in the setting of hematologic malignancy to treatment with voriconazole plus placebo versus voriconazole plus anidulafungin.[136] The study found a trend to improved cure and survival in the combination therapy arm ($P = .08$). In a post hoc analysis of patients who had a positive galactomannan, and hence confirmed invasive aspergillosis, the survival benefit of combination therapy achieved statistical significance ($P = .04$). Doubters of combination therapy have chosen to interpret the study as negative and discouraged use of combination therapy. Supporters of combination therapy point to the near statistical miss on the primary end point, the well-diverged Kaplan-Meier survival curves, and the statistical significance in the galactomannan-positive population, which is enriched for true invasive aspergillosis. Furthermore, there was no apparent toxicity detriment of the combination regimen. Thus the study can be interpreted either way. What is clear is that any advantage of dual therapy for invasive aspergillosis is incremental rather than transformational.

Controversy Over Preventing Emergence of Resistance

Another area of confusion for combination therapy, alluded to earlier, is the potential to reduce emergence of resistance among typical, pyogenic bacterial infections. Some experts have advocated for this approach based on in vitro modeling data and preclinical animal studies, and extrapolating from infections such as TB.[137,138] It is clear that bacterial growth in the presence of two antibiotics in vitro can suppress the emergence of resistant subpopulations. Thus it may be that combination therapy can reduce the emergence of resistance among bacteria at the site of infection.

However, what remains unclear is the impact of combination therapy on the microbiome and on the environment. It is conceivable that treating with two antibiotics to prevent resistance among typical bacterial pathogens would amount to a pyrrhic victory, reducing resistance at the site of infection in the short term but resulting in selection for resistance in the microbiome and environment to two antibiotics rather than one, triggering loss of efficacy for both drugs in the long run. As mentioned, this problem does not exist for infections such as TB, HIV, or HCV because these organisms are not part of the microbiome and have no environmental reservoir. Clinical data are not available to address this issue at the current time, and the question remains controversial.

CONCLUSIONS

Antibiotics are miracle drugs that fundamentally altered the practice of medicine. Yet their incredible power to cure and heal is fleeting. The more we use them, the faster we lose them. And the transmissibility of resistance results in a shared risk across society. When any one of us uses an antibiotic, it affects the future ability of that drug to be available as an effective treatment for everyone else. No one has a right to waste this shared societal trust.

One of the founding fathers of antiinfective chemotherapy, Dr. Walsh McDermott, wrote: "It is not too much to state that the introduction of [antibiotics] has represented a force for change in the 20th century of the same general kind as James Watt's modification of the steam engine did in the 18th. The crossing of the historic watershed could be felt at the time. One day we could not save lives, or hardly any lives; on the very next day we could do so across a wide spectrum of diseases. This was an awesome acquisition of power."[15]

Experts with training in ID bear the burden of being at the front lines in this struggle to heal and cure, while preserving this awesome power and preventing its disruption of our ancestral microbiome in the process.

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18 Molecular Mechanisms of Antibiotic Resistance in Bacteria

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MOLECULAR GENETICS OF ANTIBIOTIC RESISTANCE

Genetic variability is essential for microbial evolution to occur. The fitness of a microorganism depends on its capacity to adapt to changing environmental conditions.[1] Antimicrobial agents exert strong selective pressures on bacterial populations, favoring organisms that can resist them.[1,2] Genetic variability may occur by a variety of mechanisms. Point mutations may occur at a nucleotide base pair (bp), which is referred to as *microevolutionary change.* These mutations can alter enzyme substrate specificity or the target binding site of an antimicrobial agent, interfering with its activity. Point mutations at crucial locations on "old" β-lactamase genes (e.g., genes for Temoneira-1 [TEM-1], sulfhydryl variable-1 [SHV-1]) are primarily responsible for the remarkable array of newly recognized extended-spectrum β-lactamases (ESBLs).[3,4]

A second level of genomic variability in bacteria is referred to as a *macroevolutionary change* and results in whole-scale rearrangements of multiple nucleotide sequences as a single event. These rearrangements may include inversions, duplications, insertions, deletions, or the transposition of segments of DNA from one location of a bacterial chromosome or plasmid to another location in the genome. These large-scale alterations of the bacterial genome are frequently generated by specialized genetic elements such as *integrons, transposons,* or *insertion sequences,* which have the capacity to move and insert independently throughout the bacterial genome.[2]

A third level of genetic variability in bacteria is created by the acquisition of large segments of foreign DNA carried by resistance (R) plasmids, bacteriophages, naked sequences of DNA, or specialized transposable genetic elements known as *integrative and conjugative elements* (ICE) from other bacteria.[2] These events are termed lateral or horizontal gene transfer and are now appreciated to be a frequent event, especially for naturally competent bacteria (e.g., pneumococci and gonococci) that can take up exogenous DNA from the environment. Large-scale transfer of resistance genes for different classes of antibiotics as a single event also occurs among enteric bacteria within the gut microbiota by conjugal transfer of large R plasmids. Whole-scale acquisition of many antibiotic-resistance genes at the same time is referred to as "evolution by quantum leaps,"[5] and provides a major advantage for bacteria residing in patients receiving multiple courses of different classes of antibiotics.

Inheritance of foreign DNA further contributes to bacterial genetic variability and its capacity to respond to selection pressures imposed by antimicrobial agents.[3] These mechanisms endow bacteria with the seemingly unlimited capacity to develop resistance to any antimicrobial agent (Fig. 18.1). Examples of plasmid-mediated carbapenemase-producing *Klebsiella pneumoniae*,[6] vancomycin-resistant *Staphylococcus aureus* (VRSA) and daptomycin-resistant *S. aureus,*[7,8] multidrug-resistant *Escherichia coli* plasmids now with polymyxin/colistin resistance genes,[9] and transferable quinolone resistance in enterobacteria[10] attest to the capacity of microorganisms to adapt to environmental stresses such as antibiotic exposure. When an antibiotic-resistance gene evolves, this gene can spread between bacteria by transformation, transduction, conjugation, or transposition. Favored clones of bacteria may proliferate in the microbiota of patients who receive antibiotics.[11]

Antibiotic-resistance genes existed well before the introduction of antimicrobials for treatment for human infections and can be found within bacterial genomes preserved in Arctic region permafrost samples untouched by human hands for over 30,000 years.[11] Antibiotics are often synthesized by antibiotic-producing bacteria when they are about to enter the late static growth phase and are about to become dormant or sporulate. Antibiotic-producing bacteria are resistant to the antibiotics they produce. Therefore, antibiotics left in the microenvironment by dormant bacteria are avoided as potentially toxic to competitors yet serve as a ready source of carbon (food) for the next generation of the antibiotic-producing bacterial strain once the growth phase begins again. Environmental levels of multiple classes of antimicrobial agents are now so common in soil and water samples that multiple bacterial genera have strains that subsist entirely on antibiotics as their sole carbon source.[12] These bacteria express remarkably high levels of multiple antibiotic resistance to a wide array of antibiotic classes. Aquatic environments are particularly rich with bacterial populations replete with antibiotic-resistance genes.[12] Such environmental bacterial strains likely provide potential human pathogens with a source of novel antibiotic-resistance genes.[12,13] Selection pressures placed on microbial populations by antibiotics favor the expansion of strains that have the capacity to resist the inhibitory effects of antibiotics.

These resistant populations proliferate and spread antibiotic-resistance genes vertically to subsequent generations and horizontally to susceptible strains of related bacteria, or even between species or different genera.[14] Although some antibiotic-resistance genes place a metabolic "burden" on bacteria, many microorganisms evolved strategies to limit this cost by repressing gene expression when not needed or by phase variation. This allows favorable, but sometimes "costly," antibiotic-resistance genes to be held in reserve in the absence of antibiotic selection pressure yet express their resistance potential on reexposure to antibiotics.[15]

Continuous exposure to foreign DNA within microbial communities is so commonplace that many bacteria have evolved systems to defend their genomes from exogenous DNA, phages, and plasmid insertions. This is accomplished through at least two mechanisms: (1) species- and strain-specific DNA modifying enzymes (e.g., methylation of selected sequences of host DNA into specific patterns) and restriction enzymes that survey cellular host DNA and degrade foreign DNA that lacks appropriate DNA modification sequences; and (2) a type of adaptive defense system against foreign DNA known as CRISPR (clustered regularly interspaced short palindromic repeats).[16] CRISPRs are detectable in nearly 50% of all bacterial genomes, and this genetic element protects their genomes from attack by foreign DNA during transformation, phage invasion, or plasmid insertion. This same CRISPR-associated caspase 9 system in bacteria has become an indispensable genetic tool for gene insertion and editing by molecular biologists doing animal and human genetic studies.[17]

The mechanism of protection in bacteria is mediated by insertion of small sequences of the mobilized invading DNA between palindromic repeats within the CRISPR. On reexposure to similar DNA sequences from phage or invading bacteria, the existing sequence within the CRISPR is transcribed into a small RNA (known as crRNA) that associates with CRISPR-associated nucleases and prevents integration of the targeted foreign DNA.

Maintaining the fidelity of the host genome, while permitting limited variation by microevolutionary and macroevolutionary changes, allows pathogens to strike a balance between genomic stability and plasticity in rapidly changing microenvironments. Recent evidence in the enterococci indicates that deletion of CRISPR elements is inversely related to multiple antibiotic-resistance development. CRISPR-deficient strains have been selected for, and are particularly well-adapted for,

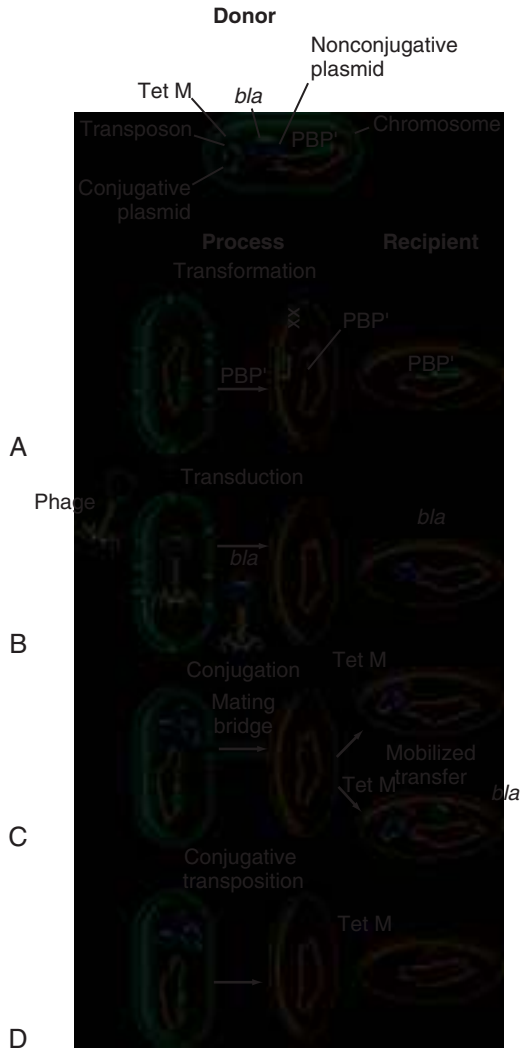

FIG. 18.1 Examples of recombination events and molecular spread of antibiotic-resistance genes. The donor organism depicted here has three antibiotic-resistance genes: the first on the chromosome, designated as *PBP,* a low-affinity penicillin-binding protein; the second (a β-lactamase gene labeled *bla*) on a small nonconjugative plasmid; and the third (*Tet M,* a tetracycline resistance determinant) on a transposon residing on a large self-conjugative plasmid. (A) Genetic exchange may occur by transformation (naked DNA transfer for dying bacteria to a competent recipient). This generally results in transfer of homologous genes located on the chromosome by recombination enzymes (RecA). (B) Transduction also may transfer antibiotic-resistance genes (usually from small plasmids) by imprecise packaging of nucleic acids by transducing bacteriophages. (C) Conjugation is an efficient method of gene transfer, requiring physical contact between donor and recipient. Self-transferable plasmids mediate direct contact by forming a mating bridge between cells. Smaller nonconjugative plasmids might be mobilized in this mating process and be transported into the recipient. (D) Transposons are specialized sequences of DNA that possess their own recombination enzymes (transposases), allowing transposition ("hopping") from one location to another, independent of the recombination enzymes of the host (RecA independent). They may transpose to nonhomologous sequences of DNA and spread antibiotic-resistance genes to multiple plasmids or genomic locations throughout the host. Some transposons possess the ability to move directly from a donor to a recipient, independent of other gene transfer events (conjugative transposons or integrative and conjugative elements).

health care–associated infections. These strains have significantly larger genomes because of insertion of large sequences of DNA, including genes that mediate multiple antibiotic resistance.[18]

Plasmids

Extrachromosomal elements were present in bacteria before the advent of antibiotics.[11,14] The introduction of antibiotics into clinical medicine in the 20th century created selection pressures, however, that favor the dissemination of resistance genes via mobile genetic elements.[2,3,13] Plasmids are particularly well adapted to serve as agents of genetic exchange and resistance-gene dissemination.[1,3] Plasmids are autonomously replicating genetic elements that generally consist of covalently closed, circular, double-stranded DNA molecules ranging from less than 10 kilobase pairs (kbp) to more than 400 kb. They are extremely common in bacteria.[14] Although multiple copies of a specific plasmid, or multiple different plasmids, or both may be found in a single bacterial cell, closely related plasmids often cannot coexist in the same cell. This observation led to a classification scheme of plasmids based on incompatibility (Inc) groups.[1,4]

Plasmids may determine a wide range of functions besides antibiotic resistance, including virulence and metabolic capacities. All plasmids possess an origin of replication for DNA polymerase to bind and replicate plasmid DNA. Plasmids must also retain a set of genes that facilitate their stable maintenance in host bacteria. The transfer of plasmid DNA between bacterial species is a complex process, and the genes needed for transfer (*tra* genes) make conjugative plasmids larger than nonconjugative ones. Some small plasmids may be able to transfer to other bacteria via the use of the conjugation apparatus provided by coresident conjugative plasmids or even conjugative transposons. Many plasmid-encoded functions enable bacterial strains to persist in the environment by resisting noxious agents, such as heavy metals. Mercury released from dental fillings may increase the number of antibiotic-resistant bacteria in the mouth.[19] Compounds such as hexachlorophene and quaternary ammonium compounds are used as topical bacteriostatic agents, and plasmid-mediated resistance to these agents has increased significantly.[2] Recently, mathematical modeling and long-term gene transfer experiments within complex, multispecies environments such as the gut microbiome have confirmed that R plasmids serve another critical role in retention of resistance genes once antibiotic therapy is discontinued.[20] R plasmid horizontal transfer by conjugation occurs at a surprisingly high rate, allowing R plasmids to transfer and continuously "infect" a sufficient proportion of antibiotic-susceptible host bacteria to maintain the resistance genes within bacterial genomes even after the antibiotic has been discontinued. This explains the problem of lingering persistence of resistance genes in patients, even after good antibiotic stewardship programs are working to curtail unnecessary antibiotic use.[20] This chapter primarily focuses on the molecular aspects of antibiotic resistance. Readers interested in practical recommendations to limit the spread of antibiotic-resistance genes in clinical settings are referred to Chapters 14, 51, and 298.

Transposable Genetic Elements

Transposons can translocate as a unit from one area of the bacterial chromosome to another or between the chromosome and plasmid or bacteriophage DNA. Transposable genetic elements possess a specialized system of recombination that is independent of the generalized recombination system that classically permits recombination of largely homologous sequences of DNA by crossover events (the *recA* system of bacteria). The *recA*-independent recombination system ("transposase") of transposable elements usually occurs in a random fashion between nonhomologous sequences of DNA and results in whole-scale modifications of large sequences of DNA as a single event (Fig. 18.2).[2,5]

There are two types of transposable genetic elements, called *transposons* and *insertion sequences,* which have similar characteristics. Bacterial chromosomes are replete with transposable elements.[2,5] These mobile sequences probably play an important physiologic role in genetic variation and evolution in prokaryotic organisms. Transposons differ from insertion sequences in that they encode functional genes that mediate a recognizable phenotypic characteristic, such as an antibiotic-resistance marker. Either element can translocate as an independent

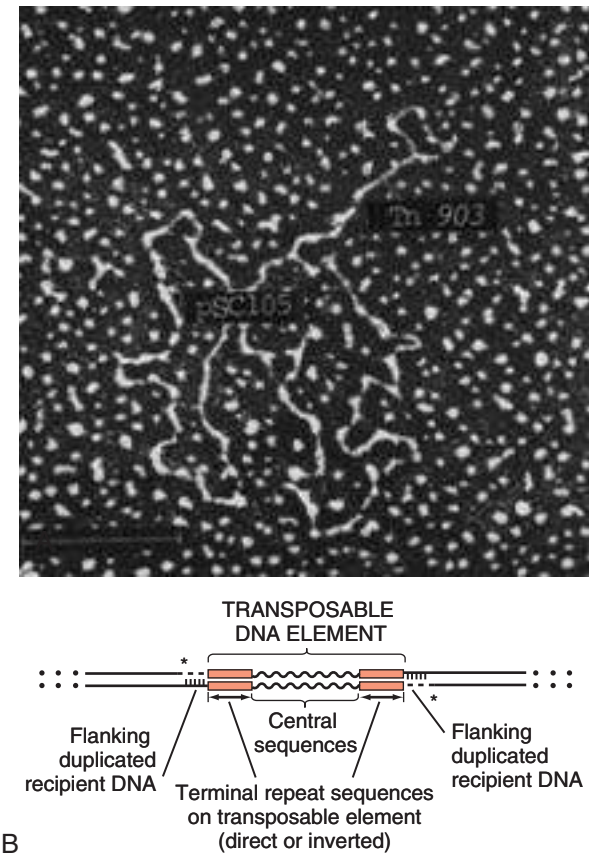

FIG. 18.2 (A) Characteristic appearance of a transposon with electron microscopy showing the stem-loop configuration. The kanamycin resistance transposon Tn*903* is inserted into a small plasmid (pSC105). After denaturation, intrastrand annealing of the complementary 1000-bp, inverted repeat, terminal sequences of the transposon form the stem structure. The kanamycin-resistance gene and the genes necessary for transposition are located in the central loop structure. (B) Structure of a transposable element inserted into a recipient DNA sequence. The transposon (*rectangles* and *wavy lines*) consists of a central sequence containing the phenotypic marker gene(s) (antibiotic-resistance gene) and the "transposase" genes. The terminal-repeat sequences of the transposon flank the central sequences on both sides. Insertion of the transposon results in single-strand, staggered cuts in the recipient DNA *(asterisks).* Subsequent gap-filling DNA synthesis and ligation results in duplication of a short sequence of recipient DNA at either end of the transposon.

unit. Both elements are usually flanked on either end by short identical sequences of DNA in reverse order *(inverted repeats).* These inverted-repeat DNA termini are essential to the transposition process. Transposons (Tn) and insertion sequences (IS) are incapable of autonomous self-replication and must exist on a replicon, such as the chromosome, bacteriophage, or plasmid, to be replicated and maintained in a bacterial population. Some transposons have the capability to move from one bacterium to another without being fixed within a plasmid or bacteriophage. These elements are referred to as *conjugative* transposons, or integrative and conjugative elements (ICE). The ubiquitous transposable element Tn*916* and its derivatives are examples of conjugative transposons and have been found primarily in aerobic and anaerobic gram-positive organisms, although they can also exist in gram-negative bacteria.[2,21]

Transposition usually results in the localized replication of the transposable element from the original donor sequence of DNA and the insertion of a copy of the transposable element into the recipient sequence of DNA (replicative transposition).[1,2] Transposition, like point mutation, is a continuous, ongoing process in bacterial populations. An example of this phenomenon is the spread of a tetracycline-resistance transposon among *Neisseria gonorrhoeae, Mycoplasma hominis,* and *Ureaplasma urealyticum.*[22,23]

An important variant of transposition is "one-ended" transposition, wherein only one end of the transposon is responsible for asymmetrical replication. This type of element is highly efficient in mobilizing resistance genes adjacent to its insertion site. These elements play a prominent role, along with ICE and integrons, in the evolution of large regions of the chromosome where multiple resistance genes accumulate into one set of resistance gene cassettes known as resistance genomic islands.[2] Some of these genomic islands are enormous in size and scope. For example, the AbaR1 genomic island of *Acinetobacter baumannii* is 86 kb long and contains 46 different antimicrobial resistance genes to a wide swath of antimicrobials, antiseptics, and heavy metals.[24] Once genomic resistance islands form, they often persist and gain new genes over time. These islands serve as convenient depots for insertions of new DNA without the risk of insertion into a critical metabolic or structural gene of the bacterial genome.

The "fitness cost" of acquiring new resistance genes is minimized by deposition of genes in genomic resistance islands.[2] However, the metabolic cost of retaining all these accessory, antibiotic-resistance genes by host bacteria remains. The extra metabolic burden is worth it only in the presence of repeated environmental antibiotic exposures, as might exist in hospitals and special care areas, such as the critical care unit, where antibiotic selection pressures favor multidrug-resistant organisms. R plasmids containing antibiotic-resistance genes can be shed by bacteria replicating for prolonged periods in antibiotic-free environments, despite persistence mechanisms employed by many types of R plasmids.[20]

Transposons contribute to the evolution of R plasmids that contain multiple antibiotic-resistance determinants.[21] The spread of carbapenem resistance from metallo-β-lactamases (MBLs) among the Enterobacteriaceae in some European hospitals was facilitated by transposition of resistance genes on integrons to separate plasmids from different bacterial genera of gram-negative bacilli.[25] High-level vancomycin resistance *(vanA)* in enterococci is mediated by a composite transposon that encodes a series of genes needed to express vancomycin resistance.[26] Single transposons may also encode multiple antibiotic-resistance determinants within their inverted-repeat termini.[2]

Although lateral gene transfer is usually more frequent between closely related bacterial strains, genetic exchange of antibiotic-resistance genes does occur between bacteria of widely disparate species and even different genera.[27] Identical aminoglycoside-resistance genes occur in streptococci and *Campylobacter*,[28] and enterococci apparently have acquired aminoglycoside[29] and β-lactam[30] resistance from staphylococci. Given the highly variable environmental selection pressures created by the clinical, agricultural, and industrial use of antibiotics and the plasticity of bacterial genomes, the ongoing evolution of multiresistant species seems inevitable.[31,32]

DNA Integration Elements

The structural genes that mediate antibiotic resistance often are closely linked and may exist in tandem along the bacterial chromosome or plasmid. Genetic analysis of sequences of DNA adjacent to antibiotic-resistance genes has revealed that unique integration units often exist near promoter sites.[32]

These integration elements, called *integrons,*[33] function as recombination "hot spots" for site-specific recombination events between largely nonhomologous sequences of DNA. Integrons facilitate the lateral transfer and integration of antibiotic-resistance genes from mobile gene cassettes. The integron provides its own unique integrase function[33] that facilitates *recA*-independent recombination and a specialized attachment and integration site consisting of a variable length (57–141 bp) but often a 59-bp spacer sequence of highly conserved DNA. This 59-bp element is preserved at the 3′ end of inserted antibiotic-resistance genes.[34,35]

Although these integration elements differ structurally and functionally from transposons,[34] they seem to be widespread in bacterial populations and play an important role in the dissemination of antibiotic-resistance genes.[2] Integrons do not transpose independently as a specific unit structure from one sequence of DNA to another. This capability of autonomous movement of large sequences of DNA is reserved primarily for transposons, insertion sequence elements, and bacteriophages. Integrons can become flanked, however, by transposable elements and become mobilized as an integrated structure into an existing transposon.

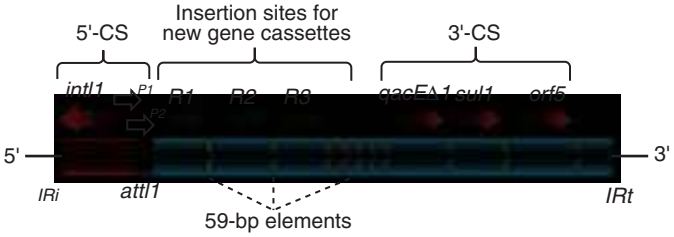

FIG. 18.3 Organization of a hypothetical class I integron. The 5′ conserved sequence (5′-CS) contains a site-specific integrase *(intI1)*; an attachment site *(attI1),* which functions as a receptor for new gene cassettes; and two potential promoter sites (*P1* and *P2*). The promoter is the initiation site for the transcription of the multiple, potential, antibiotic gene cassettes (labeled *R1, R2, R3*) that are inserted downstream from the promoter. Repeated, variable-length, but usually 59-bp, elements flank the central antibiotic-resistance gene cassettes. The conserved 3′ end of the integron (3′-CS) usually consists of a gene for resistance to quaternary ammonium compounds, a sulfonamide-resistance gene, and another open reading frame *(orf5).* The outer boundaries of the integron structure are flanked by a 25-bp inverted repeat sequence (noted as *IRi* and *IRt*).

The principal role of integrons is to provide a convenient insertion site for antibiotic-resistance genes from foreign DNA sources.

Five classes of integrons that encode antibiotic-resistance genes have been recognized, with type 1 integrons being the most common in pathogenic microorganisms.[36] A schematic representation of a class 1 integron is shown in Fig. 18.3. Integrons also serve as expression cassettes for antibiotic-resistance genes in that an efficient promoter site is provided in close proximity to the 5′ end of the newly inserted DNA sequence. The frequency of transcription of integrated cassettes of antibiotic-resistance genes depends on the proximity of the gene to the promoter at the 5′ upstream end of the integron. The level of expression of a resistance gene diminishes as the distance between the promoter and the specific antibiotic-resistance gene cassette increases.[31,32] Numerous clusters of different antibiotic-resistance genes have been identified that have evolved through specific insertions into common integrons. Integrons have been found to possess five or more antibiotic-resistance genes lined up in a tandem sequence along a single, functioning integron.[36]

Complex integrons have recently been described that are typified by a common open reading frame *(orf 513)* linked to the 3′ end of a typical integron, followed by a series of inserted genes (often ESBL) after a duplication of the 3′ end of the integron. This common 3′ end of type 1 integrons encodes genes for resistance to quaternary ammonium compounds *(qacE1),* sulfonamide resistance *(sul1),* and an open reading frame of unknown function *(orf5).* These complex integrons can be mobilized and spread by adjacent insertion sequences to disseminate among bacterial populations.[35,37,38]

MOLECULAR MECHANISMS OF ANTIBIOTIC RESISTANCE

At least eight distinctive mechanisms of antibiotic resistance have been described in bacteria (Table 18.1). Examples of each of these mechanisms are described in the following paragraphs.

Enzymatic Inhibition of Antimicrobial Activity

β-Lactamases

Resistance to β-lactam antibiotics occurs primarily through production of β-lactamases, enzymes that inactivate these antibiotics by splitting the amide bond of the β-lactam ring. They have likely coevolved with bacteria as mechanisms of resistance against natural antibiotics over time.[39,40] The selective pressure exerted by the widespread use of antimicrobial therapy in modern medicine may have accelerated their development and spread.[41–43]

β-Lactamases are encoded either by chromosomal genes or by transferable genes located on plasmids and transposons. In addition, β-lactamase genes *(bla)* frequently reside on integrons, which often carry multiple resistance determinants. If mobilized by transposable

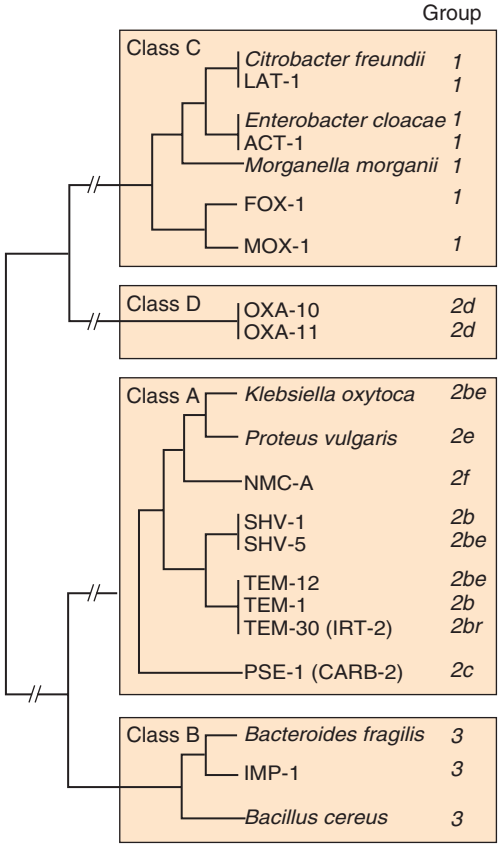

FIG. 18.4 Correlation between amino-acid sequences (Ambler classes) and functional properties of β-lactamases (Bush-Jacoby-Medeiros groups). *ACT-1,* AmpC type-1; *CARB-2,* carbenicillin-2; *FOX-1,* cefoxitin-1; *IMP-1,* active on imipenem; *IRT-2,* inhibitor-resistant TEM-2; *LAT-1,* latamoxef; *MOX-1,* moxicillin-1; *NMC-A,* not metalloenzyme carbapenemase-A; *OXA,* oxacillin; *PSE-1, Pseudomonas*-specific enzyme; *SHV,* sulfhydryl variable; *TEM,* Temoneira. *(Modified from Philippon A, Dusart J, Doris B, Frère JM. The diversity, structure and regulation of* β*-lactamases.* Cell Mol Life Sci. *1998; 54:341-346.)*

elements, integrons can facilitate further dissemination of multidrug resistance among different bacterial species.[38]

β-Lactamases can be classified according to their amino-acid structure into four molecular classes, A through D (Table 18.2), as first suggested by Ambler and later modified and expanded in the Bush-Jacoby-Medeiros classification system (Table 18.3).[39,40] Class A, C, and D β-lactamases hydrolyze the β-lactam ring through a serine residue at their active site, whereas class B enzymes are MBLs that use zinc $(Zn)^{2+}$ to break the amide bond (Fig. 18.4).

The first β-lactamase was described as a "penicillinase" capable of hydrolyzing penicillin in *E. coli* in 1940, about the same time as the first clinical use of penicillin was reported in the literature.[41] The next years witnessed the rapid spread of plasmid-encoded penicillin resistance among the majority of *S. aureus* clinical isolates.[42] Among gram-negative organisms, the rise in ampicillin resistance in the 1960s was ascribed to the emergence of TEM-1, a plasmid-encoded β-lactamase named after a Greek patient, Temoniera, in whom the first isolate was recovered.[43] The family of TEM β-lactamases was disseminated worldwide through various Enterobacteriaceae, in addition to *Pseudomonas aeruginosa, Haemophilus influenzae,* and *N. gonorrhoeae*.[44] Similarly, both chromosomally encoded and plasmid-mediated SHV-type β-lactamases, with a molecular structure related to TEM enzymes, became widely prevalent among *E. coli* and *K. pneumoniae* isolates.

Extended-Spectrum β-Lactamases

The pharmaceutical industry's development of third-generation cephalosporins, initially stable to the action of TEM- and SHV-type

TABLE 18.1 Eight Major Mechanisms of Resistance by Antimicrobial Class

	β-LACTAM	AMINO-GLYCOSIDE	CHLORAM-PHENICOL	MACROLIDE	SULFONAMIDE	TETRACYCLINE	TRIMETHOPRIM	QUINOLONE	GLYCOPEPTIDE	LINOSAMIDE; STREPTOGRAMIN	RIFAMPIN
Enzymatic inactivation	+++	+++	+++	+ (gram-negative)	–	–	–	+	–	–	–
Decreased permeability	+ (gram-negative)	+ (gram-negative)	+ (gram-negative)	++ (gram-negative)	–	+ (gram-negative)	+ (gram-negative)	+ (gram-negative)	++ (gram-negative)	+ (gram-negative)	–
Efflux	+	+	+	++	–	+++	–	+	–	–	–
Alteration of target site	++	++	–	+++	++	+ *(Helicobacter pylori)*	+++	+++	+++	+++	+++
Protection of target site		–	–	–	–	++	–	+	–	–	–
Overproduction of target	–	–	–	–	++	–	++	–	+	–	–
Bypass of inhibited process	–	–	–	–	+	–	+	–	–	–	–
Bind up antibiotic	–	–	–	–	–	–	–	–	++	–	–

+++, Most common mechanism; ++, common; + less common.

TABLE 18.2 Ambler Classification of β-Lactamases

CLASS	ACTIVE SITE	ENZYME TYPE	SUBSTRATES	EXAMPLE
A	Serine	Penicillinases:		
		Broad-spectrum	Benzylpenicillin, aminopenicillins, carboxypenicillins, ureidopenicillins, narrow-spectrum cephalosporins	PC1 in *Staphylococcus aureus* TEM-1, SHV-1 in *Escherichia coli, Klebsiella pneumoniae,* other gram-negative bacteria
		Extended-spectrum (β-lactamase)	Substrates of broad-spectrum plus oxymino-β-lactams (cefotaxime, ceftazidime, ceftriaxone) and aztreonam	In Enterobacteriaceae: TEM-derived, SHV-derived, CTX-M–derived; PER-1, VEB-1, VEB-2, GES-1, GES-2, IBC-2 in *Pseudomonas aeruginosa*
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	KPC-1, KPC-2, KPC-3 in *K. pneumoniae;* NMC/IMI, SME family
B	Metallo-β-lactamases (Zn^{2+})	Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	NDM-1 in Enterobacteriaceae, IMP, VIM, GIM, SPM, SIM lineages in *P. aeruginosa, Acinetobacter* spp.
C	Serine	Cephalosporinases	Substrates of extended-spectrum plus cephamycins	AmpC-type enzymes in Enterobacteriaceae, *Acinetobacter* spp.
D	Serine	Oxacillinases:		
		Broad-spectrum	Aminopenicillins, ureidopenicillin, cloxacillin, methicillin, oxacillin, and some narrow-spectrum cephalosporins	OXA-family in *P. aeruginosa*
		Extended-spectrum	Substrates of broad-spectrum plus oxymino-β-lactams and monobactams	OXA-derived in *P. aeruginosa*
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	OXA-derived in *Acinetobacter* spp.

AmpC, Ampicillin C; *CTX-M,* cefotaxime-M; *GES,* Guyana extended-spectrum β-lactamase; *GIM,* German imipenemase; *IBC,* integron-born cephalosporinase; *IMI,* imipenem hydrolyzing; *IMP,* imipenem; *KPC, K. pneumoniae* carbapenemase; *NDM,* New Delhi metallo-β-lactamase; *NMC,* not metalloenzyme carbapenemase; *OXA,* oxacillin; *PC1,* penicillin 1; PER, *Pseudomonas* extended resistance; *SHV,* sulfhydryl variable; *SIM,* Seoul imipenemase; *SME, Serratia marcescens* extended-spectrum β-lactamase; *SPM,* Sao Paulo metallo-β-lactamase; *TEM,* Temoneira; *VEB,* Vietnam extended-spectrum β-lactamase; *VIM,* Verona integron-encoded metallo-β-lactamase.

TABLE 18.3 Bush-Jacoby-Medeiros Functional Classification Scheme for β-Lactamases

GROUP	ENZYME TYPE	INHIBITION BY CLAVULANATE	MOLECULAR CLASS	NO. OF ENZYMES	EXAMPLES[a]
1	Cephalosporinase	No	C	57	*Enterobacter cloacae* P99 (C), MIR-1 (P)
2a	Penicillinase	Yes	A	20	*Bacillus cereus* I, *Staphylococcus aureus* (B)
2b	Broad-spectrum	Yes	A	16	SHV-1 (B), TEM-1 (P)
2be	Extended-spectrum	Yes	A	81	*Klebsiella oxytoca* K1 (C), TEM-3 (P), SHV-2 (P)
2br[b]	Inhibitor-resistant	Diminished	A	13	TEM-30 (IRT-2) (P)
2c	Carbenicillinase	Yes	A	15	AER-1 (C), PSE-1 (P), CARB-3 (P)
2d	Cloxacillinase	Yes	D or A	21	*Streptomyces cacaoi* (C), OXA-1 (P)
2e	Cephalosporinase	Yes	A	19	*Proteus vulgaris* (C), FEC-1 (P)
2f[b]	Carbapenemase	Yes	A	3	IMI-1 (C), NMC-A (C), KPC (P), Sme-1 (C)
3	Carbapenemase	No	B	15	*Stenotrophomonas maltophilia* L1 (C), NDM-1 (P), IMP-1 (P)
4	Penicillinase	No		7	*Burkholderia cepacia* (C), SAR-2 (P)

[a]*B,* Both; *C,* chromosomal; *P,* plasmid.
[b]New groups; derived from Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother.* 1995; 39:1211–1233.
AER, Aeromonas; *CARB,* carbenicillin; *FEC,* isolated from feces; *IMI,* imipenem hydrolyzing; *IMP,* imipenem; *IRT,* inhibitor-resistant TEM; *KPC, K. pneumoniae* carbapenemase; *MIR,* Miriam Hospital; *NDM,* New Delhi metallo-β-lactamase; *NMC-A,* not metalloenzyme carbapenemase-A; *OXA,* oxacillin; *PSE, Pseudomonas*-specific enzyme; *SAR,* southern Africa–related enzyme; *SHV,* sulfhydryl variable; *TEM,* Temoneira.

β-lactamases, was soon followed by the emergence and global spread of ESBL, capable of hydrolyzing monobactam and broad-spectrum cephalosporins.[3,43–45] In addition, increasing reports of carbapenemase emergence and spread have raised concern over the currently limited antimicrobial arsenal against infections with multidrug-resistant gram-negative bacteria.[46,47]

TEM-derived β-lactamases. TEM-1 is the most common β-lactamase in gram-negative bacteria, and it can hydrolyze penicillins and narrow-spectrum cephalosporins in Enterobacteriaceae, *N. gonorrhoeae,* and *H. influenzae.*[3] The extended-spectrum of activity of TEM-derived ESBLs occurs through changes in a single or a few amino acids that change the configuration of the enzyme at its active site, making it more accessible to the bulky R1 oxymino side chains of third-generation cephalosporins (cefotaxime, cefpodoxime, ceftazidime, ceftriaxone), and monobactam (aztreonam).[3,45,47] The first TEM-derived ESBL, TEM-3, was reported in 1988.[48] There are now more than 220 TEM-derived ESBLs (see Lahey Clinic [Burlington, MA] website, www.lahey.org/Studies/temtable.asp). These enzymes are found primarily in *E. coli* and *K. pneumoniae* isolates but also in other Enterobacteriaceae, such as *Enterobacter aerogenes, Morganella morganii, Proteus* spp., and *Salmonella* spp. The majority of TEM-derived ESBLs remain susceptible to inhibition by clavulanic acid, although inhibitor-resistant TEM variants have also been described.[49]

SHV-derived β-lactamases. The SHV-1 β-lactamase has a biochemical structure similar to that of TEM-1 (68% of amino acids are shared[48–50]), and its ESBL derivatives are also produced by point mutations (one or more amino-acid substitutions) at its active site. SHV-type β-lactamases are found primarily in *K. pneumoniae* strains. A three-dimensional image of TEM-type and SHV-type ESBLs is provided in Fig. 18.5.

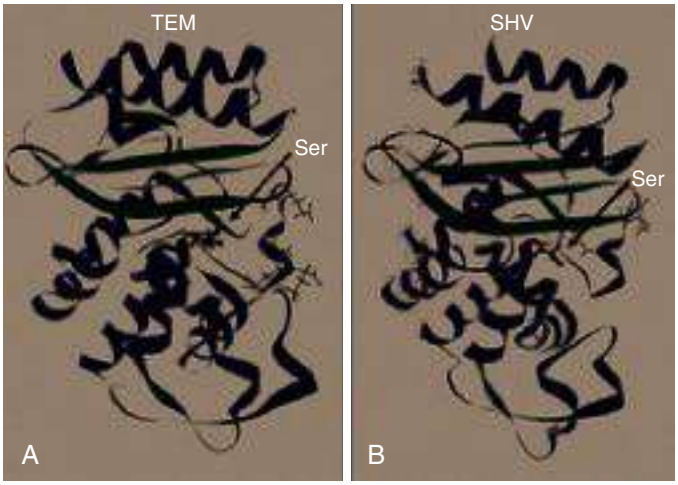

FIG. 18.5 Ribbon diagrams of TEM β-lactamases (A), and SHV β-lactamases (B). α-Helices are shown in yellow, β-strands in pink, and turns in gray. The *(Ser)* serine residue (marked by *white arrows*) involved in the hydrolysis of the β-lactam antibiotic ring is shown in ball-and-stick mode in the active site at the center of each molecule. The surrounding atoms, shown in stick mode, represent various sites of amino-acid substitutions (point mutations) that yield an extended-spectrum β-lactamase phenotype. *SHV,* Sulfhydryl variable; *TEM,* Temoneira. *(Modified from Jacoby GA, Munoz-Price SL. The new beta-lactamases.* N Engl J Med. *2005; 352:380–391.)*

CTX-M–derived extended spectrum β-lactamases. Cefotaxime-M (CTX-M) β-lactamases are not evolutionarily related to SHV and TEM families because they are thought to have been acquired by plasmids from the chromosomal ampicillin C (AmpC) enzymes of *Kluyvera* spp., environmental gram-negative rods of low pathogenic potential.[51] In general, the CTX-M family hydrolyzes cefotaxime and ceftriaxone better than ceftazidime, and they are inhibited more by tazobactam than by clavulanic acid,[50] although point mutations leading to increased activity against ceftazidime can occur. CTX-M enzymes have disseminated rapidly and are now among the most prevalent ESBLs worldwide. Recent reports of community-acquired bloodstream infections with multiresistant, CTX-M *E. coli* isolates from Spain and Israel have raised significant public health concerns.[52,53] The ST131 (O25:H4) clone associated with the CTX-M-15 enzymes has emerged as an important multidrug-resistant pathogen and may have been responsible for the majority of infections with multidrug-resistant *E. coli* infections in Europe and the United States since 2007.[54]

OXA-derived β-lactamases. Oxacillin (OXA)-type β-lactamases are also plasmid derived and hydrolyze oxacillin and its derivatives very effectively; they are poorly inhibited by clavulanic acid.[42,47] OXA-derived ESBLs have been described mainly in *P. aeruginosa,* in which they confer high-level resistance to oxymino-β-lactams.[47]

AmpC β-lactamase enzymes. AmpC β-lactamases are primarily chromosomal enzymes that confer resistance to penicillins, narrow-spectrum cephalosporins, oxymino-β-lactams, and cephamycins and are not susceptible to β-lactamase inhibitors such as clavulanic acid (molecular class C, functional group 1).[55] Cefepime and aztreonam are usually poor substrates, although modulation by point mutations at the R2 loop of the active site have been responsible for variants with increased ability to hydrolyze cefepime. AmpC production in gram-negative bacilli is normally repressed. However, a transient increase in production (10- to 100-fold) can occur in the presence of β-lactam antibiotics in the following species that possess inducible AmpC enzymes: *Enterobacter, Citrobacter freundii, Serratia, M. morganii, Providencia,* and *P. aeruginosa.*[55] AmpC β-lactamase production returns to low levels again after antibiotic exposure is discontinued, unless spontaneous mutations occur in the *ampD* locus of the gene, leading to permanent hyperproduction (derepression) in these species. Third-generation cephalosporin use in *Enterobacter* spp. infections can therefore select for the overgrowth of these stably derepressed mutants, leading to the emergence of antibiotic resistance during treatment.[56]

More than 20 plasmid-mediated AmpC enzymes, derived from chromosomally encoded genes in Enterobacteriaceae or *Aeromonas* spp., have been described in *E. coli, K. pneumoniae, Salmonella enterica,* and *Proteus mirabilis.* β-Lactam resistance attributable to this system appears to be increasing and confers a resistance phenotype similar to that of *Enterobacter* spp.[44]

Carbapenemases. Carbapenemases confer the largest antibiotic-resistance spectrum because they can hydrolyze not only carbapenems but also broad-spectrum penicillins, oxymino-cephalosporins, and cephamycins. The *K. pneumoniae* carbapenemase (KPC) enzymes are currently the most important class A serine carbapenemases. Since initially reported from *K. pneumoniae* isolates in several northeastern US outbreaks,[57] KPCs have been found worldwide in multiple other gram-negative species, such as *E. coli, Citrobacter, Enterobacter, Salmonella, Serratia*, and *P. aeruginosa.*[6]

Class B MBLs use a Zn^{2+} cation for hydrolysis of the β-lactam ring; are susceptible to ion chelators, such as ethylenediaminetetraacetic acid (EDTA); and are resistant to clavulanic acid, tazobactam, and sulbactam. They confer resistance to all β-lactam antibiotics except monobactams. Chromosomally encoded MBLs are primarily found in environmental isolates of *Aeromonas, Chryseobacterium,* and *Stenotrophomonas* spp. and are of usually low pathogenic potential.[58] Most clinically important MBLs belong to five different families (imipenem [IMP], Verona integron-encoded metallo-β-lactamase [VIM], Sao Paulo metallo-β-lactamase [SPM], German imipenemase [GIM], and Seoul imipenemase [SIM]), typically transmitted by mobile gene elements inserted into integrons and spread through *P. aeruginosa, Acinetobacter,* other gram-negative nonfermenters, and enteric bacterial pathogens.[24]

The New Delhi metallo-β-lactamase–1 (NDM-1) has received the most attention recently. Originally described in a *K. pneumoniae* isolate from India in 2008, NDM-1 enzymes have since been reported in the United States, the United Kingdom, and a number of other countries, primarily in connection with travel to India or Pakistan.[59,60] These enzymes confer resistance to all β-lactams except aztreonam. However, most MBLs reside on mobile gene cassettes inserted into integrons that harbor additional antibiotic-resistance genes to other antimicrobial classes; this multidrug resistance can be transferred to other species via transposons and plasmids, severely limiting therapeutic options in serious infections.[61] Recent meta-genomic analyses have indicated that the majority of MBLs probably originate from *Shewanella* spp. and that the genes for many more, as yet uncharacterized, metallo-enzymes are present in environmental and commensal bacterial species.[62]

Finally, class D carbapenemases have been described among four subfamilies of OXA-type β-lactamases (OXA-23, OXA-24, OXA-58, and OXA-146), primarily in *A. baumannii.* The intrinsically weaker carbapenemase activity is augmented by coupling β-lactamase production with an additional resistance mechanism, such as decreased membrane permeability or increased active efflux.[63]

Gram-Positive Bacterial β-Lactamases

Among gram-positive bacteria, staphylococci are the major pathogens that produce β-lactamase. Staphylococcal β-lactamases preferentially hydrolyze penicillins. Most are inducible and are excreted extracellularly.[3] The genes that determine staphylococcal β-lactamases usually are carried on small plasmids or transposons. Larger plasmids encoding β-lactamase and other resistances also exist and can transfer by conjugation, not only between strains of *S. aureus* but also between *S. aureus* and *Staphylococcus epidermidis.*[64]

Enterococci produce a plasmid-determined β-lactamase that seems to be of staphylococcal origin.[65] Since the appearance of the first strain in Texas in 1981, β-lactamase–producing enterococci have been found throughout the United States and in South America.[66] The genes often coexist with genes that determine high-level resistance to gentamicin and may occur on transposons and on plasmids. These transposons are like staphylococcal β-lactamase transposons and may have been derived from them.[67]

Anaerobic Bacteria

β-Lactamases also contribute to the resistance of anaerobic bacteria to β-lactam antibiotics.[68,69] The β-lactamases of *Fusobacteria* and *Clostridia*

spp. are principally penicillinases.[70] The β-lactamases produced by *Bacteroides fragilis* are predominantly cephalosporinases, some of which have been found to hydrolyze cefoxitin and imipenem and may be transferable.[71] Most of the cephalosporinases are inhibited by clavulanate, sulbactam, or tazobactam. Some isolates of *Bacteroides* spp. produce carbapenemases, metalloenzymes inhibited by EDTA but not clavulanate, which confer resistance to imipenem.

Contribution of β-Lactamases to β-Lactam Antibiotic Resistance

The level of antibiotic resistance mediated by a particular β-lactamase in a population of bacteria is determined by at least five variables. The efficiency of the β-lactamase in hydrolyzing an antibiotic depends on (1) its rate of hydrolysis and (2) its affinity for the antibiotic. Other variables are (3) the amount of β-lactamase produced by the bacterial cell, (4) the susceptibility of the target protein (penicillin-binding protein [PBP]) to the antibiotic, and (5) the rate of diffusion of the antibiotic into the periplasm of the cell.

Within the bacterial cell, β-lactamases contribute to antibiotic resistance in several ways. The simplest model is that of penicillinase-producing staphylococci, in which the bacteria, on exposure to penicillin, begin to produce β-lactamase, which they excrete extracellularly. Two events then take place concurrently: (1) penicillin lyses bacteria and (2) β-lactamase hydrolyzes penicillin. If viable bacterial cells remain after the level of penicillin has declined to less than the minimal inhibitory concentration (MIC), regrowth of bacteria occurs.[40]

Another model is exemplified by gram-negative bacteria, which (1) produce a β-lactamase that remains trapped in the periplasmic space and (2) have no barrier to antibiotic penetration. An example is *H. influenzae* strains that produce the TEM-1 β-lactamase.[72] In this model and the first one discussed, a marked inoculum effect occurs in that the MIC for a large inoculum (10^6 colony-forming units [CFUs] per milliliter) may be 1000-fold greater than that for a small inoculum (10^2 CFUs/mL). Lysis of the organism by ampicillin releases the trapped β-lactamase into the microenvironment, providing partial protection to adjacent bacteria residing in the same location. If a high inoculum exists before ampicillin exposure occurs, the release of all the periplasmic β-lactamase enzymes in a confined space might be sufficient to protect some of the remaining viable bacteria of the original population of microorganisms. However, the low level of resistance of single cells makes it possible for ampicillin to cure some infections caused by β-lactamase–producing strains of *H. influenzae* when the initial inoculum of infecting bacteria is low.

Another model is exemplified by ampicillin resistance of *E. coli* strains that produce the TEM-1 β-lactamase. These bacteria have a barrier to entry of β-lactam molecules (the outer membrane), and they produce a β-lactamase that remains localized to the periplasmic space. In this model, the kinetics are more complicated. The enzyme is situated strategically between the barrier to antibiotic penetration (outer membrane) and the antibiotic targets (PBPs on the cytoplasmic membrane). In this position, the enzyme can destroy antibiotic molecules sequentially as they make their way through the barrier, analogous to a sharpshooter with abundant ammunition who aims at targets passing through a single entry point. Consequently, high levels of resistance occur with single bacterial cells, in contrast to the previous example.[40]

Variations on this model occur when the amount of β-lactamase produced increases with exposure to a β-lactam (induction), as occurs in *Enterobacter* and *Pseudomonas* spp. High levels of β-lactamase are produced only after a period of exposure to the inducing antibiotic, and resistance may be expressed late. When *Enterobacter* strains are exposed to two β-lactam antibiotics, one of which is a potent inducer (e.g., cefamandole), antagonism between the two antibiotics may result.[70]

Table 18.4 lists mechanisms of resistance to β-lactam antibiotics. Often these mechanisms work in concert and may accumulate in a single patient. An example is a 19-month-old child with aplastic anemia who over 3 months had nine blood isolates of *E. coli,* all derived from a common ancestor, despite multiple courses of antibiotics, including ceftazidime.[72] The first isolate produced a TEM-1 β-lactamase but was susceptible to ceftazidime (MIC, 0.25 μg/mL). A subsequent isolate became resistant (MIC of ceftazidime, 32 μg/mL) by acquiring a new plasmid-determined β-lactamase (SHV-1) linked to an efficient promoter and turning off production of an outer membrane porin. An even higher level of resistance (MIC of ceftazidime ≥128 μg/mL) occurred when the SHV-1 β-lactamase mutated to form the ESBL SHV-8, which hydrolyzes ceftazidime much more rapidly. By turning off porin production to slow the rate of entry of ceftazidime into the periplasmic space and producing an extended-spectrum, ceftazidime-inactivating β-lactamase, the infecting *E. coli* used two mechanisms synergistically to achieve a high level of resistance to ceftazidime.[73]

Aminoglycoside Resistance–Modifying Enzymes

Among aerobic bacteria, aminoglycoside resistance is most commonly due to enzymatic inactivation through aminoglycoside-modifying enzymes. These may be coded by genes on plasmids or chromosomes. Several aminoglycoside-modifying enzymes have been shown to be carried on transposons.[74]

Aminoglycoside-modifying enzymes confer antibiotic resistance through three general reactions: *N*-acetylation, *O*-nucleotidylation, and *O*-phosphorylation. For each of these general reactions, there are several different enzymes that attack a specific amino or hydroxyl group. The nomenclature for these enzymes lists the molecular site where the modification occurs after the type of enzymatic activity. An aminoglycoside acetyltransferase (AAC) that acts at the 3′ site is designated AAC(3′) (Table 18.5). There may be more than one enzyme that catalyzes the same reaction, however, and Roman numerals may be necessary (e.g., AAC[3′]-IV).

Enzymatic aminoglycoside resistance is achieved by modification of the antibiotic in the process of transport across the cytoplasmic membrane.[74] Resistance to a particular aminoglycoside is a function of two different rates—that of drug uptake versus that of drug inactivation. An important factor in determining the level of resistance is the affinity of the modifying enzyme for the antibiotic. If an enzyme has a high affinity for the specific aminoglycoside, drug inactivation can occur at very low concentrations of the enzyme.

The differences in the worldwide distribution of aminoglycoside-modifying enzymes may be partially a function of antibiotic selection pressures and may have had profound implications on the choice of antibiotics used at specific medical centers. Aminoglycoside phosphotransferase (APH)(3′) and APH(3″) are distributed widely among gram-positive and gram-negative species worldwide and have led to decreased use of kanamycin and streptomycin. The gene for aminoglycoside nucleotidyltransferase (ANT)(2″) has been associated with multiple nosocomial outbreaks in the 1990s across the United States. The gene

TABLE 18.4 Mechanisms of Resistance to β-Lactam Antibiotics

I. Alter target site (PBP)
  A. Decrease affinity of PBP for β-lactam antibiotic
    1. Modify existing PBP
      a. Create mosaic PBP
        Insert nucleotides obtained from neighboring bacteria (e.g., penicillin-resistant *Streptococcus pneumoniae*)
        Mutate structural gene of PBP(s) (e.g., ampicillin-resistant β-lactamase–negative *Haemophilus influenzae*)
    2. Import new PBP, e.g., mecA in methicillin-resistant *Staphylococcus aureus*
II. Destroy β-lactam antibiotic
  A. Increase production of β-lactamase
    1. Acquire more efficient promoter
      a. Mutate existing promoter
      b. Import new one
    2. Deregulate control of β-lactamase production
      a. Mutate regulator genes (e.g., *ampD* in "stably derepressed" *Enterobacter cloacae*)
  B. Modify structure of resident β-lactamase
    1. Mutate its structural gene (e.g., extended-spectrum β-lactamases in *Klebsiella pneumoniae*)
  C. Import new β-lactamase(s) with different spectrum of activity
III. Decrease concentration of β-lactam antibiotic inside cell
  A. Restrict its entry (loss of porins)
  B. Pump it out (efflux mechanisms)

PBP, Penicillin-binding protein.

TABLE 18.5 Aminoglycoside-Modifying Enzymes

ENZYMES	USUAL ANTIBIOTICS MODIFIED	COMMON GENERA
Phosphorylation		
APH(2″)	K, T, G	SA, SR
APH(3′)-I	K	E, PS, SA, SR
APH(3′)-III	K, ±A	E, PS, SA, SR
Acetylation		
AAC(2′)	G	PR
AAC(3′)-I	±T, G	E, PS
AAC(3′)-III, -IV, or -V	K, T, G	E, PS
AAC(6′)	K, T, (A)	E, PS, SA
Adenylation		
ANT(2″)	K, T, G	E, PS
ANT(4′)	K, T, A	SA
Bifunctional Enzymes		
AAC(6′)APH(2″)	G, Ar	SA, Ent
AAC(6′)-Ib cr	G, K, T, FQ*	E

A, Amikacin; *AAC,* aminoglycoside acetyltransferase; *ANT,* aminoglycoside nucleotidyltransferase; *APH,* aminoglycoside phosphotransferase; *Ar,* arbekacin; *cr,* ciprofloxacin resistance; *E,* Enterobacteriaceae; *Ent,* enterococci, **FQ,* fluoroquinolone (acetylates the piperazine ring in some fluoroquinolones), *G,* gentamicin; *K,* kanamycin; *PR, Providencia-Proteus*; *PS,* pseudomonads; *SA,* staphylococci; *SR,* streptococci; *T,* tobramycin.

TABLE 18.6 Mechanisms of Tetracycline Resistance

RESISTANCE MECHANISM	COMMON TET DETERMINANTS	COMMON BACTERIAL SPECIES
Drug efflux	Tet A-L, P[a], V, Y, Z, *otrB, tcr*(3), Tet 30	Enterobacteriaceae, *Pseudomonas, Streptomyces, Staphylococcus, Streptococcus* spp.
Ribosomal protection	Tet M, O, P[a], Q, S, T, W, *otrA*	Gram-positive and gram-negative anaerobes, *Neisseria, Haemophilus, Enterococcus. Staphylococcus, Streptococcus* spp.
Enzymatic inactivation	Tet X, Tet 34, Tet 37	*Bacteroides, Vibrio* spp.
Unknown mechanism	Tet U, *otrC*	*Mycobacterium, Enterococcus* spp.
Altered ribosomal target	—	*Helicobacter pylori*

[a]Tet P has two different genes mediating different mechanisms of resistance.

for aminoglycoside acetyltransferase AAC(6′)-I has been found to be more prevalent in enteric bacteria and in staphylococci in East Asia.[75] The AAC(3′) group of enzymes have been responsible for outbreaks of antibiotic resistance in South America, Western Europe, and the United States. Although each outbreak of aminoglycoside-resistant Enterobacteriaceae has its own pattern, the most typical manner of spread has been the appearance of a plasmid-carrying, aminoglycoside-resistant strain of *K. pneumoniae,* usually carrying the *ANT(2″)* gene, with subsequent dissemination to other strains of the species and further spread later to other species and genera of Enterobacteriaceae.[76]

Major increases in plasmid-mediated aminoglycoside resistance have been noted among enterococci,[77] initially in the developing world, but increasingly in the United States and Europe. Their clinical impact is exacerbated by the frequent cotransmission of β-lactamases, resulting in a loss of synergy when combination therapy is used for serious enterococcal infections. *S. aureus* and *S. epidermidis* have become increasingly resistant to aminoglycosides because of the interspecies and intraspecies dissemination of plasmid-mediated, aminoglycoside-modifying enzymes.[77]

The two most interesting developments in aminoglycoside-modifying enzymes have been the discovery of bifunctional enzymes. The first example is the AAC(6′) APH(2″) enzyme, which has two functioning active sites, one for acetylation and the other for phosphorylation of aminoglycosides. This bifunctional enzyme probably arose from a fusion event of the genes for these two enzymes. This enzyme is now widespread in staphylococci and enterococci, frequently residing on a common transposon Tn*4001* found on the chromosome and on transferable plasmids. The gene *aac(6′) aph (2″)* accounts for most of the high-level gentamicin and arbekacin resistance observed in methicillin-resistant *S. aureus* (MRSA) and enterococcal isolates in many countries worldwide.[78,79]

Recently, another major discovery with regard to aminoglycoside-modifying enzymes involves evidence of variant enzymes that can modify the structure of an entirely different class of antimicrobial agent. The first bifunctional enzyme that can modify aminoglycosides and a fluoroquinolone (ciprofloxacin) was described in 2006.[80] This enzyme, designated as AAC(6′)-Ib-cr, not only acetylates kanamycin, gentamicin, and tobramycin, but also acetylates the piperazinyl side group of ciprofloxacin. This acetylated quinolone is fourfold less active than the parent compound and may lead to clinically significant resistance in enteric bacteria, particularly in strains that possess other mechanisms for diminished quinolone activity. This enzyme has two important mutations in the gene for the basic AAC(6′) enzyme (W102R and D179Y) and 12 unique base pairs at its 5′ end that are essential to alter substrate specificity and allow ciprofloxacin acetylation function for this enzyme. The widespread use of ciprofloxacin and other fluoroquinolones over the past 2 decades has changed the selection pressures on bacterial populations, promoting development of quinolone resistance.[80]

Chloramphenicol Acetyltransferase

Resistance to chloramphenicol in gram-positive and gram-negative organisms is mediated primarily by the inactivating enzyme *chloramphenicol acetyltransferase.* This is an intracellular enzyme that inactivates the drug by 3-*O*-acetylation[81] and is encoded by plasmid-borne or chromosomal genes. Despite homology at the active site of this enzyme, there is considerable diversity between chloramphenicol acetyltransferase enzymes isolated from gram-positive and gram-negative organisms.[82]

Macrolide-, Lincosamide-, Streptogramin-Inactivating Enzymes

Although resistance to erythromycin and other macrolides is frequently the result of alteration in the ribosomal target site or efflux pumps, several substrate-inactivating enzymes have been characterized.[83] Erythromycin esterases have been isolated from *E. coli* that hydrolyze the lactone ring of the antibiotic and result in its inactivation. This is a plasmid-mediated resistance determinant that is constitutively produced and results in high-level resistance to erythromycin (MIC >2000 μg/mL).[84] This type of high-level macrolide-resistance gene is often linked with the carbapenemase-producing, NDM-1–bearing plasmids.[59] These resistance genes limit the usefulness of oral erythromycin or other macrolides in reducing the aerobic gram-negative flora of the intestinal tract before gastrointestinal surgical procedures. Other plasmid-mediated resistance genes generate specific inactivating enzymes in *Staphylococcus haemolyticus* and *S. aureus* that adenylate[85] lincosamides or acetylate[86] or hydrolyze[87] streptogramins.

Tetracycline Inactivation

A tetracycline-inactivating enzyme called TetX has been described rarely in *Bacteroides* spp.[88] Tetracycline resistance is principally mediated by other mechanisms, including efflux and ribosomal protection (Table 18.6). However, environmental bacteria frequently express enzymes

called tetracycline destructases, and it is possible that such enzymes might find their way into clinically relevant pathogens in the future.[89]

Decreased Permeability of Bacterial Membranes

Outer Membrane Permeability

Alexander Fleming recognized early in the history of antibiotic development that penicillin is effective against gram-positive bacteria but not against gram-negative bacilli.[90] This difference in susceptibility to penicillin is due in large part to the presence of the outer membrane in gram-negative bacilli, a thick lipopolysaccharide layer that acts as a barrier to the penetration of many antibiotics into the cell.[91] Situated outside the peptidoglycan cell wall of gram-negative bacteria, this outer membrane is absent in gram-positive bacteria. The lipopolysaccharide is made up of tightly bound hydrocarbon molecules that impede the entry of hydrophobic antibiotics, such as nafcillin or erythromycin. Agents that disrupt the integrity of the lipopolysaccharide layer, such as polymyxin, or mutations that lead to the production of defective lipopolysaccharides, result in increased permeability of hydrophobic antibiotics.[92]

The passage of hydrophilic antibiotics through this outer membrane is facilitated by the presence of porins, proteins that are arranged to form water-filled diffusion channels through which antibiotics may traverse.[93] Bacteria usually produce many porins; approximately 10^5 porin molecules are present in a single cell of *E. coli*. Bacteria can regulate the relative number of different porins in response to the osmolarity of the surrounding media. In hyperosmolar media, *E. coli* may repress production of the larger porins (OmpF) while continuing to express smaller ones (OmpC).[94]

The rate of diffusion of antibiotics through this outer membrane is a function not only of the numbers and properties of the porin channels but also of the physicochemical characteristics of the antibiotic. In general, the larger the antibiotic molecule, the more negatively charged, and the greater the degree of hydrophobicity, the less likely that it will penetrate through the outer membrane.[95] Small hydrophilic molecules with a zwitterionic charge, such as imipenem, are highly permeable. Conversely, larger highly charged molecules, such as carbenicillin, are much less permeable.

Mutations resulting in the loss of specific porins can occur in clinical isolates and determine increased resistance to β-lactam antibiotics. Resistance to aminoglycosides and carbapenems emerging during therapy has been associated with a lack of production of outer membrane proteins. For example, emergence of imipenem resistance during therapy, observed in up to 25% of *P. aeruginosa* infections, has been ascribed to mutational loss of its OprD protein (also known as the D2 porin).[96]

Resistance to nalidixic acid and other quinolones has been associated with alterations of outer membrane proteins in *Serratia marcescens* and *P. aeruginosa*.[97,98,99] Single-step, high-level mutational resistance to nalidixic acid by aerobic gram-negative bacilli occurs with a 10^{-7} frequency, whereas only low-level resistance to the newer quinolones (<10 × MIC) usually is obtained with a single-step selection of less than 10^{-9}.[100] Plasmid-mediated chloramphenicol resistance resulting from decreased cell membrane permeability has been shown in *E. coli*.[101]

Inner Membrane Permeability

The rate of entry of aminoglycoside molecules into bacterial cells is a function of their binding to a usually nonsaturable anionic transporter, whereupon they retain their positive charge and subsequently are "pulled" across the cytoplasmic membrane by the internal negative charge of the cell.[102] This process requires energy and a threshold minimal level of internal negative charge of the cell that has to be present before significant transport occurs *(proton motive force)*.[103] The level of the internal charge that is required may depend on the actual aminoglycoside concentration at a given time. The energy generation or the proton motive force that is required for substrate transport into the cell may be altered in mutants resistant to aminoglycosides.

These aminoglycoside-resistant isolates can develop during the course of long-term aminoglycoside therapy.[101] These isolates usually have a "small colony" phenotype resulting from their reduced rate of growth. They may be unstable and revert to a sensitive phenotype in the absence of selective aminoglycoside pressure. The clinical significance of these isolates is not clear. They may retain some virulence[104] and rarely may cause fatal bacteremia.[105] Because oxidative metabolism is essential for aminoglycoside uptake action and cell growth and development, *Pseudomonas* mutants have been found that have been deficient in specific cytochromes. Resistant mutants with defective electron transport systems have been described in *E. coli, S. aureus,* and *Salmonella* spp.

Promotion of Antibiotic Efflux

Tetracyclines

Active efflux of antimicrobial agents is recognized increasingly as a common mechanism of resistance in many clinically relevant pathogens. Some strains of *E. coli, Shigella* spp., and other enteric organisms express a membrane transporter system that leads to multidrug resistance by drug efflux.[106] Many of these are multicomponent, regulated, energy-dependent transporter systems that promote the active efflux of multiple classes of antibiotics. Specific efflux pumps also exist that promote the egress of single classes of antimicrobial agents.

The major mechanism of resistance to tetracyclines found in enteric gram-negative organisms results from the decreased accumulation of tetracycline (see Table 18.6). This reduced uptake is an energy-dependent process that is related to the generation of an inner membrane protein produced by the tetracycline-resistance determinant (designated as Tet) or resistance gene products for oxytetracycline derivatives (Otr). The primary mechanism for the decreased accumulation of tetracycline is through the active efflux of the antibiotic across the cell membrane.[107] Decreased uptake of tetracycline from the extracellular environment also accounts for decreased accumulation of tetracycline inside resistant cells. These resistance determinants may be found on the chromosome or plasmids and frequently are found on transposable genetic elements. Tetracycline-resistance genes are generally inducible by subinhibitory concentrations of tetracycline. There are now over 40 recognized tetracycline-resistance determinants, most of which mediate drug efflux.[107,108] The determinants have been designated in the past by letters (e.g., Tet A, Tet B). Because there are now more determinants than letters in the English alphabet, new *tet* genes now are designated by numbers.[106] Tetracycline efflux pumps primarily affect first-generation tetracyclines, but have little activity against newer glycylcyclines (tigecycline) or the aminomethylcyclines such as omadacycline.[109]

Macrolides and Streptogramins

In some strains of *Streptococcus pneumoniae, Streptococcus pyogenes, S. aureus,* and *S. epidermidis,* an active efflux mechanism causes resistance to macrolides, streptogramins, and azalides.[110] This efflux mechanism is mediated by the *mef* (for *m*acrolide *ef*flux) genes in streptococci and *msr* (for *m*acrolide *s*treptogramin *r*esistance) genes in staphylococci.[111] A similar efflux system, encoded by a gene called *mreA* (for *m*acrolide *r*esistance *e*fflux), has been described in group B streptococci.[112] This mechanism of resistance is prevalent in community-acquired infections,[113] and dissemination of these resistance genes among important bacterial pathogens is a considerable threat to the usefulness of macrolide antibiotics (Table 18.7).

β-Lactams

Active efflux mechanisms also may contribute to the full expression of β-lactam resistance in *P. aeruginosa*. Multidrug efflux pumps in the inner and outer membrane of *P. aeruginosa* act in concert with periplasmic β-lactamases and membrane permeability blockers to protect the bacterium from β-lactam agents.[114]

Fluoroquinolones

Active efflux of fluoroquinolones has been detected in enteric bacteria[115] and staphylococci.[113] This efflux may be related to a multiple antibiotic-resistance transporter[113] (i.e., NorA) or a specific quinolone efflux pump (i.e., EmrAB, AcrAB, or plasmid-mediated *qepA* gene efflux system).[116] This mechanism of limiting access of high levels of fluoroquinolones works in concert with other mechanisms (point mutations of DNA

TABLE 18.7 Resistance Mechanisms Against the Macrolides, Lincosamides, and Streptogramins

BACTERIAL SPECIES	GENE DESIGNATION	PHENOTYPE	RESISTANCE MECHANISM	RESISTANCE PATTERN			
				14- OR 15-MEMBERED RING	16-MEMBERED RING	CLINDAMYCIN	STREPTOGRAMIN B
Streptococci, Enterococci	*erm (A, B)*	MLS_B—inducible	Ribosomal methylation	(s) I or R	(s) I or R	(s) I or R	(s) I or R
	erm (A, B)	MLS_B—constitutive	Ribosomal methylation	R	R	R	R
	mef (A or E)	M	Efflux	I or R	S	S	S
	L4/L22 mut	M	Ribosomal mutation	R	R	S	S
	inu (B)	L	Inactivation	S	S	S-I	S
Staphylococci	*erm (A, C)*	MLS_B—inducible	Ribosomal methylation	R	(s)	(s)	(s)
	erm (A, C)	MLS_B—constitutive	Ribosomal methylation	R	R	R	R
	msr (A or B)	MS_B	Efflux	R	S	S	R
	vgb, vgbB	S_B	Inactivation	S	S	S	R
	ere (A or B)	M	Inactivation	R	R	S	S
	inu (A)	L	Inactivation	S	S	S-I	S

Fourteen- or 15-membered ring structures: erythromycin, clarithromycin, azithromycin. Sixteen-membered ring structures: spiramycin.
I, Intermediate susceptibility; *L,* lincosamides; *M,* macrolides; *MLS_B,* macrolides, lincosamides, and streptogramin B; *R,* resistant; *(s),* appears susceptible in vitro but may select resistant clones in vivo; *S,* sensitive.

gyrases, gyrase protection, permeability barriers, and acetylation) for full expression of quinolone resistance.[117]

Altered Target Sites

Alteration of Ribosomal Target Sites

Macrolides, Lincosamides, Streptogramins

Resistance to a wide variety of antimicrobial agents, including tetracyclines, macrolides, lincosamides, streptogramins, and the aminoglycosides, may result from alteration of ribosomal binding sites. Failure of the antibiotic to bind to its target site or sites on the ribosome disrupts its ability to inhibit protein synthesis and cell growth. For macrolides, lincosamides, and streptogramin B (MLS_B), this is the principal mechanism of multiple-agent resistance among aerobic and anaerobic gram-positive organisms.[111] Resistance is mediated by the products of the *erm* (erythromycin ribosome methylation) gene, the variety of methylase enzymes (MLS_B, determinant) that dimethylate adenine residues on the 23S ribosomal RNA (rRNA) of the 50S subunit of the prokaryotic ribosome, disrupting the binding of MLS_B to the ribosome (see Table 18.7). Different classes of this resistance determinant may be located on plasmids or on the bacterial chromosome.

MLS_B resistance resulting from ribosomal methylation has been described in many species, including *S. aureus, Streptococcus sanguinis, B. fragilis,* and *Clostridium perfringens.* MLS_B resistance may be constitutive or inducible by either older macrolides (e.g., erythromycin) or newer azalides. In *S. pneumoniae*, resistance is encoded by the *erm(B)* gene, which is responsible for the methylation of loop V of the 23S ribosomal subunit.[118] In addition, point mutations in ribosomal proteins L4 and L22 of the 50S subunit have also been described that render *S. pneumoniae* resistant to macrolides.[119]

Inducible resistance in streptococci is generated by a variety of lincosamides and macrolides, resulting in cross-resistance to the MLS_B antibiotics. In staphylococci, only 14 or 15 numbered macrolides induce MLS_B methylation, and the organisms express resistance to macrolides only.

Tetracyclines

Tetracycline resistance may be mediated by a variety of mechanisms, the most common of which are efflux mechanisms and ribosomal protection mechanisms (see Table 18.6).[120] An additional, unusual mechanism of tetracycline resistance by altering the target site of action has been found in *Helicobacter pylori.*[121] This organism can possess a mutation in its 16S rRNA that limits tetracycline binding to its target site at the 30S subunit of the bacterial ribosome.

Aminoglycosides

Resistance to aminoglycosides is also mediated at the ribosomal level. In Enterobacteriaceae and nonfermenting gram-negative bacteria, methylation of the 16S rRNA (the site where aminoglycosides bind and inhibit protein synthesis) by enzymes usually carried on plasmids is mediated by at least seven different genes (*armA, rmtA, rmtB, rmtC, rmtD, rmtE,* and *npmA*).[122] This is now recognized as a major mechanism of resistance to all parenteral aminoglycosides, which appears to be spreading globally.[123,124] Mutations of the S12 protein of the 30S subunit have been shown to interfere with binding streptomycin to the ribosome. Ribosomal resistance to streptomycin may be a significant cause of streptomycin resistance among enterococcal isolates.[125] Ribosomal resistance to the 2-deoxystreptamine aminoglycosides (gentamicin, tobramycin, and amikacin) seems to be uncommon and may require multiple mutations in that these aminoglycosides seem to bind to several sites on the 30S and 50S subunits of the prokaryotic ribosome. Ribosomal resistance often is associated with decreased intracellular accumulation of the drug.[126]

Ketolides

Ketolides have seen limited use clinically, and yet clinical isolates of *S. pneumoniae* resistant to telithromycin have already been reported. Resistance is due to constitutive expression of the *erm* gene, mutations in domains II and V of the 23S rRNA binding sites, and mutations within ribosomal proteins L4 and L22.[127]

Oxazolidinones

Resistance to linezolid, the main oxazolidinone currently in clinical use, has been described in gram-positive isolates (*S. aureus, Enterococcus faecium, Enterococcus faecalis, S. epidermidis*) as a result of point mutations within the genes encoding the 23S rRNA of the 50S ribosomal subunit, the antibiotic's main binding site.[128] Specifically, translocation of peptidyl–transfer RNA (tRNA) from the A site to the P site during translation from bacterial ribosomes is inhibited through guanine (G) to uridine (U) substitutions at position 2576 or similar binding sites in the peptidyl transferase region of 23S rRNA.[129] This remains the most common mechanism of linezolid resistance among clinical isolates. Resistance development has been observed during prolonged linezolid therapy in vancomycin-resistant enterococci (VRE) bacteremia,[130] and a number of other pathogens, including coagulase-negative staphylococci and *S. aureus.* Another mechanism of resistance, mediated by the *cfr* gene, is the result of methylation of adenosine at position 2503 of 23S rRNA by a methyl transferase reaction.[131]

Alteration of Cell Wall Precursor Targets: Glycopeptides

Enterococci

Glycopeptide antibiotics (vancomycin, teicoplanin) bind to D-alanyl-D-alanine dipeptide at the termini of peptidoglycan precursor stem peptides, preventing their incorporation into the cell wall and stopping further peptidoglycan synthesis. In enterococci, high-level acquired vancomycin resistance develops by alteration of these cell wall precursor targets through an amino-acid substitution that changes their end to a D-alanyl-D-lactate depsipeptide. The gene responsible for this change, *vanA,* encodes a protein that synthesizes these modified peptidoglycan precursors, greatly reducing vancomycin binding affinity.[132,133]

Resistance of enterococci to vancomycin has been classified as phenotypes A through N based on genotype, type of target site alterations, and levels of resistance to vancomycin or teicoplanin (Table 18.8).[134,135] Strains of *E. faecium* and *E. faecalis* with high-level resistance to vancomycin and teicoplanin have class A resistance, which can transfer by conjugation from *E. faecium* to other gram-positive bacteria,[136] including *E. faecalis, S. pyogenes, Streptococcus sanguis,* and *Listeria monocytogenes*. Strains of *E. faecium* and *E. faecalis* with class B resistance have levels of resistance to vancomycin that range from high (MIC, 1024 µg/mL) to low (MIC, 4 µg/mL) and are susceptible to teicoplanin. The genes determining the VanB phenotype are self-transferable by conjugation to other *Enterococcus* strains[137,138] and have also been observed in anaerobic species, presumably through genetic transfer within the human bowel.

All isolates of *Enterococcus gallinarum, Enterococcus casseliflavus,* and *Enterococcus flavescens* possess intrinsic low-level resistance to vancomycin and are susceptible to teicoplanin (class C phenotype). The resistance is mediated by chromosomal genes known as $vanC_1$, $vanC_2$, or $vanC_3$, which encode the synthesis of alternative dipeptides, D-alanine-D-serine, where vancomycin and other glycopeptide antibiotics, such as teicoplanin, bind to D-alanine-D-alanine (D-ala-D-ala) dipeptide, which is present at the termini of the stem peptide in peptidoglycan precursors.

Strains of *E. faecium* and *E. faecalis* with class B resistance have levels of resistance to vancomycin that range from high (MIC, 1024 µg/mL) to low (MIC, 4 µg/mL) and are susceptible to teicoplanin. Vancomycin, but not teicoplanin, can induce resistance to vancomycin and teicoplanin in these strains. The genes determining the VanB phenotype are self-transferable by conjugation to other *Enterococcus* strains.[137,138]

All isolates of *E. gallinarum, E. casseliflavus,* and *E. flavescens* possess low-level resistance to vancomycin and are susceptible to teicoplanin (class C phenotype). The resistance is mediated by chromosomal genes known as $vanC_1$, $vanC_2$, or $vanC_3$. The *vanC* gene complex gives rise to resistance to vancomycin by synthesis of an alternative dipeptide, D-alanine-D-serine, in which a serine replaces the terminal alanine. Other variant genes known as *vanE* and *vanG* have been found in enterococcal species that also mediate various levels of glycopeptide resistance[134] (see Table 18.8).

Staphylococci

Since 1987, reports from the United States and Japan have documented outbreaks of vancomycin-intermediate *S. epidermidis,*[139] *S. haemolyticus,*[140] and *S. aureus* (VISA).[141] The first high-level VRSA isolate in the United States was recovered in 2002 from a diabetic patient on chronic hemodialysis harboring both *S. aureus* and VRE in a foot ulcer.[142] DNA sequencing revealed identical *vanA* genes in both isolates, and further molecular analysis revealed plasmid-mediated transfer of resistance through the Tn*1546* genetic element encoding the *vanA* gene from the *enterococcus* (VRE) strain into the vancomycin-susceptible MRSA strain, rendering it vancomycin resistant (VRSA).[143]

VISA arose likely during prolonged exposure to vancomycin, often at suboptimal concentrations, and are the result of multiple mutations in the genes controlling cell wall biosynthesis. Phenotypically, VISA clones express unusually thick peptidoglycan cell walls that are not completely cross-linked.[144] The cell wall in some strains of VISA contains nonamidated glutamine precursors that provide an increased number of false binding sites to vancomycin.[145–147] The vancomycin molecules are absorbed to these excess binding sites, preventing the antibiotic from reaching its target, and allowing peptidoglycan synthesis in the cytoplasmic membrane to continue uninhibited.[143]

Alteration of Target Enzymes

β-Lactams

β-Lactam antibiotics inhibit bacteria by binding covalently to PBPs in the cytoplasmic membrane. These target proteins catalyze the synthesis of the peptidoglycan that forms the cell wall of bacteria.[148] Alterations of PBPs can lead to β-lactam antibiotic resistance.

In gram-positive bacteria, resistance to β-lactam antibiotics may be associated either with a decrease in the affinity of the PBP for the antibiotic[149] or with a change in the amount of PBP produced by the bacterium.[150] Multiple mechanisms seem to be present in some clinical isolates. Penicillin-resistant strains of *S. pneumoniae* isolated in South Africa have shown several changes in PBPs (i.e., decreased affinity of some PBPs, loss of others, and appearance of PBPs not present in the more susceptible cells).[150] The genes that encode these PBPs are mosaics, composed of segments from susceptible pneumococci and segments from resistant commensal streptococci.[151] In *S. aureus*[152,153] and *E. faecium,*[154] additional PBPs may be inducible (i.e., their production is stimulated by exposure of the microorganism to the β-lactam antibiotic). These inducible PBPs have a lower affinity for β-lactam antibiotics, making them less susceptible to inhibition by low concentrations of the drug.

MRSA Resistance

In *S. aureus*, methicillin resistance is conferred by the expression of the *mecA* gene, which encodes PBP2A, a protein with low affinity for β-lactam antibiotics, conferring resistance to methicillin, nafcillin, oxacillin, and cephalosporins. The *mecA* gene is the structural component of the *mec* gene cassette and is inserted into the larger staphylococcal cassette

TABLE 18.8 Vancomycin Resistance in Enterococci and Staphylococci

	A	B	C	D	E	G	L	M	N
Vanco (µg/mL) (MIC)	64 to >500	4 to >500	2–32	64–128	16	12–16	8	>256	8
Teico (µg/mL) (MIC)	16 to >500	0.5–2	0.5–2	4–64	0.5	0.5	0.5	64 to >256	0.5
Expression	Inducible	Inducible	Constitutive, inducible	Constitutive	Inducible	Inducible	Inducible	Inducible	Inducible
Genetic location	P, C	P, C	C	C	C	C	C	P, C	P, C
Target alteration	D-ala-D-lac	D-ala-D-lac	D-ala-D-ser	D-ala-D-lac	D-ala-D-ser	D-ala-D-ser	D-ala-D-ser	D-ala-D-lac	D-ala-D-ser
Common species	*Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus*	*E. faecalis, E. faecium*	*Enterococcus gallinarum* (C-1) *Enterococcus casseliflavus* (C-2) *Enterococcus flavescens* (C-3)	*E. faecium*	*E. faecalis*	*E. faecalis*	*E faecalis*	*E faecium*	*E faecium*

C, Chromosome; *D-ala,* D-alanine; *D-lac,* D-lactate; *D-ser,* D-serine; *MIC,* minimal inhibitory concentration; *P,* plasmid; *Teico,* teicoplanin; *Vanco,* vancomycin.

chromosome mec (SCCmec), which appears to have been acquired through horizontal transfer from a coagulase-negative *Staphylococcus* species.[154,155] At least five different SCCmec types of various genetic sequences and size have been described: types I to III, associated with health care–associated MRSA strains, tend to be larger and multidrug resistant; types IV and V, associated with community-acquired MRSA strains, tend to be smaller in size and more susceptible to antibiotics other than β-lactams.

Expression of the methicillin-resistance gene is controlled by two regulatory components of the *mec* gene: *mecR1-mecI,* and the β-lactamase genes *blaI, blaRI,* and *blaZ,* which can downregulate *mecA* transcription. Although the *mecA* gene is present in all MRSA isolates, the phenotypic expression of methicillin resistance is more variable. For example, *S. aureus* isolates grown at 32°C, rather than at 37°C, are more likely to express methicillin resistance.[155] In addition, the expression of methicillin resistance seems to be modified also by auxiliary genes, such as *fem* and *aux,* which are present in the staphylococcal chromosome and affect various steps in peptidoglycan synthesis.[156]

The PBPs of β-lactamase–negative, penicillin-resistant strains of *N. gonorrhoeae, Neisseria meningitidis*, and *H. influenzae* have shown reduced penicillin-binding affinity.[157–159] Their PBPs seem to be encoded by hybrid genes containing segments of DNA scavenged from resistant strains of related species, similar to penicillin-resistant pneumococci. Mutations leading to a loss of outer membrane proteins also may be associated with the acquisition of penicillin resistance in non–penicillinase-producing strains of *N. meningitidis*, suggesting that altered permeability also contributes to resistance.[160] Progressive loss of β-lactam activity through multiple mechanisms in *N. gonorrhoeae,* coupled with its remarkable capacity to acquire other resistance genes by transformation and plasmid transfer, can give rise to pan-resistant, untreatable gonorrhea.[161]

Permeability changes and decreased affinity of PBPs are mechanisms found jointly in clinical isolates of *P. aeruginosa*[162] and in non–β-lactamase-producing strains of *H. influenzae.*[163] Multiple mutations may be necessary to develop this type of resistance.

Quinolones

DNA gyrase (also called *bacterial topoisomerase II*) is necessary for the supercoiling of chromosomal DNA in bacteria to have efficient cell division.[164] Another related enzyme, topoisomerase IV, also is required for segregation of bacterial genomes into two daughter cells during cell division. These enzymes consist of two A subunits encoded by the *gyrA* gene and two B subunits encoded by the *gyrB* gene (or *parC* and *parE* for topoisomerase IV). Although spontaneous mutation in the *gyrA* locus is the most common cause of resistance to multiple fluoroquinolones in enteric bacteria, B-subunit alterations also may affect resistance to these drugs. Quinolone resistance may also occur from a combination of decreased cell wall permeability, efflux, or enzyme protection mechanisms.[10,113]

DNA gyrase is the primary site of action in gram-negative bacteria, whereas topoisomerase IV is the principal target of quinolones in gram-positive bacteria, including *S. aureus*. Mutations in a variety of chromosomal loci have been described that resulted in altered DNA gyrases resistant to nalidixic acid and the newer fluoroquinolones in Enterobacteriaceae and *P. aeruginosa.*[165] Many of these mutations involve the substitution of single amino acids at the quinolone-resistance determining region (QRDR, located between amino acids 67 and 106 in the gyrase A subunit) that is involved in the generation of the DNA gyrase–bacterial DNA complex.[166] Clinical isolates of *C. freundii* in Japan have been found to be highly resistant to the newer quinolones via alterations in the DNA gyrase.[167]

Plasmid-mediated quinolone resistance has been found in various Enterobacteriaceae and is conferred by *qnr*-encoded proteins that bind to the DNA gyrase antibiotic target and protect it from quinolone action. Although fluoroquinolone resistance associated with plasmid-borne *qnr* genes is low-level resistance, these genes are usually linked to other antibiotic-resistance determinants carried on the same mobile element and have been associated with clinical phenotypes of multidrug resistance.[9,113,168,169] Another plasmid-derived quinolone-resistance determinant, encoded by the *aac(6′)-Ib-cr* gene and derived by mutation of a plasmid-contained aminoglycoside-modifying enzyme, appears widely disseminated among *E. coli* isolates in the United States, mediating low-level ciprofloxacin resistance.[78,113]

Sulfonamides

There are two common genes that mediate resistance to sulfa drugs in pathogenic bacteria: *sul1* and *sul2.* These genes give rise to altered forms of the target enzyme for sulfonamide, dihydropteroate synthase (DHPS).[170] This enzyme is essential for folic acid synthesis in susceptible bacteria. The altered DHPS enzymes mediated by the sulfonamide-resistance genes no longer bind to sulfa, yet continue to synthesize dihydropteroate from para-aminobenzoic acid substrate. The ubiquitous *sul1* gene is part of the class 1 integron family, giving rise to widespread resistance to sulfonamides.[32,170]

Trimethoprim

Trimethoprim is a potent inhibitor of bacterial dihydrofolate reductase (DHFR). Many altered DHFR enzymes with loss of inhibition by trimethoprim have been described from genes found primarily on R plasmids. These altered DHFR genes are widespread in gram-negative bacteria and are found in staphylococci (*dfrA* gene).[171,172]

Protection of Target Site

Tetracyclines

Tetracycline resistance also may occur through a mechanism that interferes with the ability of tetracycline to bind to the ribosome. Resistance genes such as *tetM* and others (see Table 18.6) protect the ribosome from tetracycline action. The Tet M determinant is dispersed widely in gram-positive organisms in addition to *Mycoplasma, Ureaplasma, Campylobacter,* and *Neisseria* spp.[22,23] The *tetM* gene generates a protein with elongation factor–like activity that stabilizes ribosomal-transfer RNA interactions in the presence of tetracycline molecules.

Fluoroquinolones

The newly recognized plasmid-mediated, antibiotic-resistance gene mediating quinolone resistance seems to function as a target protection system.[10] The resistance mechanism seems to protect DNA gyrase from binding to quinolones, allowing the bacterium to resist quinolone inhibitory effects. When this low-level resistance determinant is expressed in concert with other quinolone-resistance genes, such as DNA gyrase mutations or efflux pumps, clinical failures with the use of fluoroquinolones can result.[113]

Overproduction of Target

Sulfonamides and Trimethoprim

Sulfonamides compete with para-aminobenzoic acid to bind the enzyme DHPS and halt the generation of pteridines and nucleic acids. Sulfonamide resistance may be mediated in some bacteria by the overproduction of the synthetic enzyme DHPS. The gene responsible for DHPS is *folP,* and strains of bacteria that produce excess DHPS can overwhelm sulfa inhibition.[170] Trimethoprim resistance may occur in a similar fashion, by making excess amounts of DHFR from the bacterial chromosomal gene *folA.*[172]

Bypass of Antibiotic Inhibition

Another mechanism for acquiring resistance to specific antibiotics is by the development of auxotrophs, which have growth factor requirements different from those of the wild-type strain. These mutants require substrates that normally are synthesized by the target enzymes, and if the substrates are present in the environment, the organisms can grow despite inhibition of the synthetic enzyme. Enterococci can be folate auxotrophs, requiring environmental acquisition of folic acid for growth. They become intrinsically resistant to the folic acid inhibitors (sulfa drugs or trimethoprim) in the process. In addition, bacteria with mutations in the enzyme thymidylate synthetase can retain viability but become "thymine dependent." They require exogenous supplies of thymidine to synthesize thymidylate via salvage pathways and are highly resistant to sulfa drugs and trimethoprim.[173]

A compilation of the most frequent mechanisms of resistance used by common bacterial pathogens to inhibit the actions of antibiotics is provided in Table 18.9. Multiple mechanisms are increasingly in operation

at the same time within individual bacterial cells. The problem of multiple antibiotic-resistance expression is considered in the following sections.

Resistance to Newer, Older, and Miscellaneous Agents

The major mechanisms of bacterial resistance to newer agents or older agents, such as polymyxin B and colistin, which have become the subjects of renewed interest in response to progressive antibiotic resistance, are summarized in Table 18.10.

Oxazolidinones

Linezolid resistance mechanisms are primarily related to alterations in the 23S rRNA-binding sites that inhibit linezolid actions on bacterial translation.[174] Efflux mechanisms may also contribute to reduced activity of linezolid in some bacterial species.[175]

The lipopeptide *daptomycin* is now widely used for treatment of MRSA and glycopeptide-resistant *S. aureus* (GRSA) infections. The drug induces permeability changes and loss of intracellular potassium in susceptible gram-positive bacteria. Resistance is often associated with the abnormally thick cell wall characteristic of VISA strains.[176,177] Accumulation of mutations, especially with the gene *mprF* (encoding lysylphosphatidylglycerol synthetase) indicates the alterations in potential cell membrane binding sites that account for reduced daptomycin activity.[178] In enterococci, mutations in the gene *LiaF*, involved in the bacterial cell envelope response to antibiotics and antimicrobial peptides, and the *gaped* gene, which generates an enzyme likely involved in the cell membrane phospholipid metabolism, have been suggested as mechanisms of daptomycin resistance.[179,180]

Tigecycline is a glycylcycline antibiotic with the mechanism of action of tetracyclines but with remarkable resistance to many of the standard resistance mechanisms. Evidence indicates that resistance is associated with some unusual efflux pumps expressed in some multiresistant gram-negative bacilli.[181,182] Another novel tetracycline-like agent known as omadacycline appears thus far to be resistant to the effects of multiple tetracycline resistance mechanisms.[109] It will be interesting to determine

TABLE 18.9 Resistance Mechanisms Found in Common Bacterial Pathogens

PATHOGEN	RESISTANCE PHENOTYPE		MAJOR RESISTANCE MECHANISM
Streptococcus pneumoniae	β-Lactams		Alteration of target enzymes (PBPs)
	Macrolides, lincosamides, streptogramin B		Alteration of ribosomal target sites (methylation of adenine residue in domain V of 23S rRNA—*ermB*); efflux *(mefE)*
	Tetracycline		Protection of ribosomal target site *(tetM)*
	Trimethoprim and sulfonamides		Alteration of target enzymes (dihydrofolate reductase–trimethoprim; dihydropteroate synthase—*sul1, sul2* in sulfonamines)
	Fluoroquinolones		Alteration of target enzymes (DNA gyrase—*gyrA* mutations; topoisomerase IV—*parC* mutations)
Staphylococcus aureus	β-Lactams	Penicillin	Enzymatic inhibition (penicillinase production)
		Methicillin, oxacillin, nafcillin, and cephalosporins (MRSA)	Alteration of target enzyme—PBP2A (*mecA)*
	Glycopeptide	GISA	Alteration of cell wall precursor targets (thickened cell wall binds drug, preventing it from reaching its target)
		GRSA	Alteration of cell wall precursor targets (plasmid-mediated transfer of *vanA* genes from VRE, resulting in D-ala-D-lac peptidoglycan precursors)
Enterococci	β-Lactams (ampicillin)		Alteration of target enzymes (PBP5 in *Enterococcus faecium*); enzymatic inhibition–rare (penicillinase in *Enterococcus faecalis*)
	Aminoglycosides		Altered ribosomal target site mutations, enzymatic inhibition (high-level resistance: aminoglycoside-modifying enzymes)
	Vancomycin		Alteration of cell wall precursor targets (high-level resistance: VanA, VanB, VanD phenotypes; low-level resistance: VanC, VanE, VanG phenotypes)
	Linezolid		Alteration of ribosomal target sites (G2576U mutation in domain V of 23S rRNA)
	Quinupristin-dalfopristin		Enzymatic inhibition; efflux; target modification *(E. faecium)*
Neisseria gonorrhoeae	Penicillins		PPNG: enzymatic inhibition (plasmid-acquired penicillinase); CRNG: altered target enzymes (PBPs)
	Fluoroquinolones		Alteration of target enzymes (DNA gyrase; topoisomerase IV); efflux (MtrR-CDE efflux system)
	Tetracycline		Protection of ribosomal target (*tetM* gene)
	Macrolides		Efflux; alteration in ribosomal targets (C2611T mutation in domain V of the 23S rRNA)
	MDR		Efflux (MtrR-CDE system: penicillin, tetracycline, macrolides)

Continued

TABLE 18.9 Resistance Mechanisms Found in Common Bacterial Pathogens—cont'd

PATHOGEN	RESISTANCE PHENOTYPE	MAJOR RESISTANCE MECHANISM
Pseudomonas aeruginosa	β-Lactams	Enzymatic inhibition (AmpC cephalosporinases, extended-spectrum β-lactamases, metallo-β-lactamases); active efflux (MexAB); reduced outer membrane permeability (loss of OprD channel)
	Aminoglycosides	Enzymatic inhibition (aminoglycoside-modifying enzymes); efflux (MexXY); alteration of ribosomal targets (ribosomal methylation)
	Fluoroquinolones	Efflux (MexAB, CD, EF, XY, GH, VW); alteration of target enzymes (DNA gyrase mutations—*gyrA*)
	MDR	Overexpression of the MexA-MexB-OprM active efflux system (resistance to quinolones, tetracyclines, and trimethoprim)
Acinetobacter baumannii	β-Lactams	Enzymatic inhibition (AmpC cephalosporinases, plasmid-acquired β-lactamases of the TEM, SHV, CTX-M, PER, VEB families, metallo-β-lactamases of the IMP, VIM, SIM families, and OXA-type serine carbapenemases); alteration of target enzymes (PBPs); reduced outer membrane permeability; efflux pumps
	Aminoglycosides	Enzymatic inhibition (aminoglycoside-modifying enzymes); efflux pumps
	Quinolones	Efflux pumps
	Tigecycline	Efflux pumps
Stenotrophomonas maltophilia	β-Lactams	Impermeable outer membrane Enzymatic inhibition (inducible metallo-β-lactamases L1, L2)
	TMP-SMX	Alteration in sulfonamide target enzymes (*sul1*, *sul2* genes—associated with plasmids or class 1 integrons)
	Fluoroquinolones	Alteration of target enzymes (DNA gyrase mutations); efflux pumps
	MDR	MDR efflux pump (*smeDEF* confers resistance to tetracycline, erythromycin, chloramphenicol, norfloxacin, ofloxacin)
Klebsiella pneumoniae	β-Lactams	Enzymatic inhibition (constitutive expression of penicillinases; extended-spectrum β-lactamases; KPC, NDM-1 carbapenemases); decreased outer membrane permeability
	Fluoroquinolones	Alteration of target enzymes (DNA gyrase mutations—*gyrA*); efflux; protection of target site (plasmid-mediated *qnr* genes)
	Aminoglycosides	Enzymatic inhibition (aminoglycoside-modifying enzymes); alteration of ribosomal targets (ribosomal methylation)
Bacteroides spp.	β-Lactams	Enzymatic inhibition (chromosomally encoded CepA cephalosporinases; metallo-β-lactamases); efflux (homologues of RND-pumps); alteration in drug targets (PBPs)
	Macrolides, lincosamides, streptogramin B	Alteration of ribosomal targets
	Tetracycline	Protection of ribosomal target *(tetQ)*; efflux
	Quinolones	Alteration of target enzymes (DNA gyrase mutations—*gyr A*); efflux

AmpC, Ampicillin C; *CRNG,* chromosomally resistant *N. gonorrhoeae*; *CTX-M,* cefotaxime-M; *GISA,* glycopeptide intermediate *S. aureus*; *GRSA,* glycopeptide-resistant *S. aureus*; *IMP,* imipenem; *KPC, K. pneumoniae* carbapenemase; *MDR,* multidrug resistance; *MRSA,* methicillin-resistant *S. aureus; MtrR,* multiple transferable resistance; *NDM,* New Delhi metallo-β-lactamase; *PBP,* penicillin-binding protein; *PER, Pseudomonas* extended resistance; *PPNG,* penicillinase-producing *N. gonorrhoeae*; RND, resistance-nodulation-cell division; *rRNA,* ribosomal RNA; *SHV,* sulfhydryl variable; *SIM,* Seoul imipenemase; *TEM,* Temoneira; *TMP-SMX,* trimethoprim-sulfamethoxazole; *VEB,* Vietnam extended-spectrum β-lactamase; *VIM,* Verona integron-encoded metallo-β-lactamase; *VRE,* vancomycin-resistant enterococci.

TABLE 18.10 Resistance Mechanisms of Newer, Older, and Other Antimicrobial Agents

	POLYMYXIN	FOSFOMYCIN	DAPTOMYCIN	LINEZOLID	QUINUPRISTIN-DALFOPRISTIN	METRONIDAZOLE	TIGECYCLINE
Enzymatic inactivation	–	–	–	–	++	–	–
Decreased permeability	–	++	+ (gram-negative)	+ (gram-negative)	–	–	+
Efflux	+	-	–	+	+	–	++
Alteration of target site	+++	+	++	++	+	–	–
Protection of target site	–	-	+	–	–	–	–
Overproduction of target	–	-	–	–	–	–	–
Bypass of inhibited process	–	-	–	–	–	++	–
Bind up antibiotic	++	+	–	–	–	–	–

+++, Most common mechanism; ++, common; + less common.

the types of new resistance mechanisms that arise among pathogens in response to this aminomethylcycline agent.

A novel aminoglycoside agent, plazomicin, is now approved for urinary tract infections in 2018 and has improved activity compared with older aminoglycosides through resistance to many of the existing aminoglycoside-modifying enzymes.[183,184] This new agent has a substitution of a hydroxyl-aminobutyric acid at position 1 and a hydroxyethyl substitution at position 6′ of the aminoglycoside core structure, rendering them less susceptible to many common aminoglycoside-modifying enzymes. Some difficult-to-treat pathogens, such as highly antibiotic-resistant strains of *Acinetobacter* and *Pseudomonas* spp., might still be susceptible to plazomicin.

Quinupristin-dalfopristin is a combination of synergistic streptogramins that inhibit protein synthesis by binding to the 23S rRNA in the 50S ribosomal subunit. Resistance in gram-positive bacteria can develop through enzymatic modification (*vatD* and *vatE* genes encoding for acetyltransferases that inactivate dalfopristin), active efflux, and target alteration.[185]

Polymyxins are cationic peptide antibiotics that alter the permeability of the outer membrane of gram-negative bacteria. The use of polymyxin B or colistin (a prodrug, also called polymyxin E) has been rekindled, given the limited number of therapeutic options available to treat multidrug-resistant bacilli. Resistance has been attributable to porin mutations or binding of the agent to the polysaccharide capsule,[186] alterations in the affinity of binding to the outer membrane lipid target of polymyxin B (the *pmr* operon),[187] or increased expression of the efflux pump (the *metAB oprM* gene).[188] Recently, a new mechanism of resistance has appeared that is mediated by a plasmid-borne phosphoethanolamine transferase.[9] Addition of this phosphate moiety to the lipid A β-1,6 diglucosamine backbone of lipopolysaccharide blocks binding access of the polymyxins to their target site within the outer membrane of gram-negative bacteria. Three different mobile colistin resistance genes, designated as *mcr-1* through *mcr-3,* are now known to be spreading in enteric bacteria.[9]

Fosfomycin

Fosfomycin is a cell wall–inhibitor antibiotic that binds to an enzyme essential in the initial step in peptidoglycan biosynthesis (MurA). It has activity against both gram-positive and gram-negative bacteria. Fosfomycin resistance occurs through several mechanisms: (1) decreased permeability through reduced antibiotic uptake by impaired fosfomycin transporters; (2) modification of the antibiotic target (MurA); and (3) antibiotic modification by one of three different fosfomycin resistance proteins—FosA, FosB, or FosX—which are responsible for opening of the oxirane ring of the fosfomycin antibiotic, rendering it ineffective.[189]

Metronidazole

Metronidazole resistance is related to loss of activity of reduced nicotinamide adenine dinucleotide (NADPH) nitroreductase via mutations of the synthetic gene *rdxA*. This enzyme activity is essential to convert metronidazole to its active metabolite.[190] These mechanisms are summarized in Table 18.10.

MULTIDRUG-RESISTANCE MECHANISMS AMONG BACTERIA

Bacteria can express more than one mechanism of antibiotic resistance, leading to phenotypes of multidrug resistance, or even pan-resistance. For example, molecular analysis of *P. aeruginosa* isolates from a nosocomial outbreak in Belgium revealed the convergence of several strategies for antibiotic resistance: (1) overexpression of AmpC chromosomal β-lactamases, conferring resistance to multiple β-lactam antibiotics; (2) mutational loss of OprD porin, conferring resistance to imipenem; and (3) upregulation of the MexXY efflux system (a member of the resistance-nodulation-cell division [RND] family), which exports fluoroquinolones, tetracycline, aminoglycosides, and antipseudomonal β-lactam agents.[191]

In general, multiple antibiotic resistance in gram-negative bacteria often starts with the relatively limited outer membrane permeability to many antibiotic agents, coupled with the overexpression of multidrug-resistance efflux pumps, which can export multiple unrelated antibiotics.[192] In addition, by reducing the intracellular concentration of the antimicrobial below the MIC required for bacterial killing, efflux mechanisms may allow bacterial survival for longer periods of time, facilitating the accumulation of new antibiotic-resistance mutations (i.e., those encoding topoisomerase IV or DNA gyrase targets, rendering fluoroquinolones ineffective).[193]

The clinically important multidrug-resistance efflux pumps belong to several different families: (1) the RND family, (2) the major facilitator superfamily (MFS), (3) the staphylococcal multiresistance (SMR) family, and (4) the multidrug and toxic compound extrusion (MATE) family. Such efflux pumps are widespread among prokaryotes (Fig. 18.6) and are responsible for the export of toxic substances, allowing survival in a noxious environment, such as the biliary system for enteric bacteria.[194] They may also play a role in mediating bacterial adherence to host tissues and exporting virulence determinants, as has been described for *P. aeruginosa*.[195]

Bacteria can also acquire multidrug resistance through sequential transfer of multiple resistance determinants located on mobile genetic elements. For example, conjugative transposons such as Tn*916,* conferring resistance to tetracycline and chloramphenicol, can easily disseminate between bacterial species.[196] Transposons often coexist with other genetic

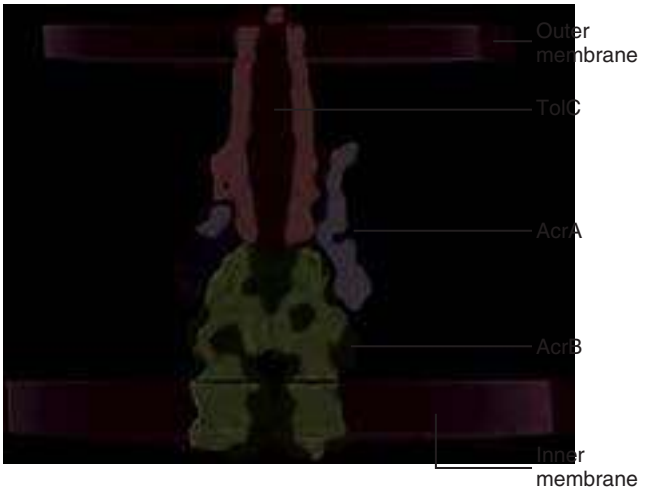

FIG. 18.6 Proposed structural model for the multidrug-resistance AcrAB-TolC efflux pump in *Escherichia coli*. The AcrAB-TolC efflux system is the most important resistance-nodulation-cell division (RND) transporter in *E. coli* and is composed of three interconnected elements: (1) the transmembrane AcrB transporter, which protrudes from the inner membrane into the periplasm; (2) the outer membrane channel TolC, which crosses from the periplasm through the outer membrane, providing the exit route for substrates into the extracellular medium; and (3) the AcrA periplasmic accessory protein, which stabilizes the complex. The pump recognizes a wide variety of substrates, including hydrophobic organic solvents and lipids, in addition to anionic, cationic, and zwitterionic antimicrobials, yielding a multidrug-resistant phenotype. *(Modified from Lomovoskaya O, Zgurskaya HI, Totrov M, et al. Waltzing transporters and "the dance macabre" between humans and bacteria.* Nat Rev Drug Discov. *2007;6:56–65.)*

elements, such as plasmids that may carry additional antibiotic-resistant determinants. For example, analysis of a plasmid encoding the *bla-CTX-15* gene responsible for resistance to extended-spectrum cephalosporins in an *E. coli* outbreak in Toronto, Canada, revealed a large multidrug-resistance region, encoding multiple transposons, and numerous other resistance genes, such as *bla(OXA-1), bla(TEM-1), tetA,* and aminoglycoside-resistance genes *aac(6′)-Ib* and *aac(3′)-II.*[195] The ability of bacteria to capture multiple antibiotic-resistance genes into existing integrons, with their seemingly endless recombination potential, is astonishing.[197,198]

CONTROL OF ANTIBIOTIC RESISTANCE

Although the emergence of antibiotic-resistant bacteria generally has been correlated with the rise and fall of specific antibiotic use in clinical practice, the chain of causality is not always clear-cut. Bacterial strains contain complex aggregations of genes that may be linked together. The use of one antibiotic may select for the emergence of resistance to another. Mobile genetic elements and rapidly evolving integron cassettes with multiple antibiotic-resistance genes endow bacteria with a remarkable capacity to resist antibiotics.[198] Although the development of antibiotic resistance may be inevitable, the rate at which it develops might be mitigated by the rational use of antibiotics and antibiotic stewardship programs.

The ability to track antibiotic-resistance genes with molecular techniques has enhanced the ability to track the spread of antibiotic resistance. With the appropriate computerized surveillance, a hospital laboratory may be able to rapidly detect the emergence of a new type of resistance or the presence of a new microbial strain within a specific unit or patient population. Techniques such as rapid microbial genomics and the increasing availability of rapid genomic sequencing can now be correlated with the phenotypic measures determined by the clinical microbiology surveillance system. Use of molecular techniques greatly augments surveillance data because large data sets may obscure subtle changes ("mini-epidemics") that may be more amenable to the institution of stringent infection-control measures.[195,197–199] Clinical methods to prevent the spread of antibiotic-resistance genes are further discussed in detail in Chapters 51 and 298.

Some bacterial strains have the ability to hypermutate in stressful environments, increasing the risk of acquisition of resistance mutations.[200,201] Because prokaryotic organisms all contribute to a common "gene pool," the total complement of favorable genes mediating antibiotic resistance (the metagenomic resistome) may disseminate among bacterial populations. Reports of the rapidly spreading NDM-type carbapenemases suggest that common, invasive, microbial pathogens may become refractory to any chemotherapeutic agent in the future.[196–198,202] Rational antibiotic-usage policies suggest the curtailment of the unnecessary use of antibiotics in situations such as animal husbandry. The causal link between the use of antibiotics for animal growth promotion and their augmentation of the resistance in human pathogens acquired from food is now reasonably well established.[203–205]

Unfortunately, the paucity of new antimicrobials in development and the rapid spread of multidrug-resistant pathogens, especially among gram-negative bacilli, have resulted in limited therapeutic options in severe infections. Resistance can develop even during therapy with seemingly adequate doses of antibacterial agents to which the pathogen appears to be susceptible with standard sensitivity testing. Three types of subclones existing within a large population of bacteria can survive a single therapeutic dose of a bactericidal antibiotic. These subpopulations include (1) bacterial persisters (antibiotic sensitive when growing but refractory when metabolically dormant); (2) rare, relatively resistant subpopulations within large populations; and (3) mutator strains (high baseline mutation rate clones), which can all be selected for, and lead to, in vivo acquisition of resistance during or after antibiotic therapy.[206] These small subpopulations are present in insignificant numbers ($<10^{-8}$) and are readily eliminated by host antimicrobial defenses under normal circumstances. Their very existence goes unnoticed during antibacterial treatment for most infectious diseases. However, these relatively resistant subpopulations can survive initial low doses of antibiotics, regrow, and become a source of in vivo development of resistance during treatment of infections with large microbial loads, infections adherent to foreign bodies or nonviable tissues, and in the absence of adequate host defenses (e.g., large populations of pathogens in undrained abscesses, infected joint prostheses, severe immunocompromised states).

Dosing strategies that follow pharmacokinetic and pharmacodynamic principles, and dosing regimens that consider mutant protection concentration (MPC) rather than just the MIC, have been suggested as another component of antibiotic stewardship programs. However, the MPC can be as much as 10- to 20-fold higher than the MIC for many classes of antibiotics.[207–210] Larger concentrations of antibiotics can eliminate resistant subpopulations that express one or two resistance mutations, allowing them to persist at concentrations just above the MIC. These surviving subpopulations are selected for during antibacterial treatment. If they can accumulate additional resistance capacity through hypermutation or acquisition of genes from neighboring bacteria, clinical failures with in vivo development of resistance might result. If the initial dose of antibiotic is high enough to eradicate even these resistant subpopulations (above the MPC), treatment can succeed and avoid this problem. Regrettably, the MPC is not easily calculated in most clinical laboratories, and the MPCs for some antibacterials might be difficult to achieve without toxicity. The higher dosing calculations could lead to greater unintended collateral damage to the patient's microbiome.[211] These pharmacokinetic and pharmacodynamic principles are best fit with fluoroquinolone use and might not extend to other antibiotic classes. They work best when resistance is mediated by stepwise increasing resistance from nonlinked, chromosomal, constitutive mutations such as point mutations to common DNA gyrase genes.[208] Mycobacterial resistance predictions using MPC as an in vitro guide might be another situation in which MPC might be predictive of in vivo resistance from nonlinked chromosomal resistance genes.[210]

Early recognition and treatment before large bacterial loads accumulate, short courses of adequate doses of antibiotics to limit resistance development, and restriction of antimicrobial agents to only patients who actually need them are all important in the prevention of antibiotic resistance. In addition, as antibiotics' deleterious effects on the microbiome become increasingly recognized, the quest for developing newer

antimicrobial therapeutic approaches that minimize both antimicrobial resistance potential and alterations in the host's normal flora is imperative.[211]

Antibiotics are a precious commodity, and we should do what we can to preserve the activity of antimicrobials to treat human infections. Dosing regimens should be chosen for both clinical efficacy and for the prevention of resistance development. Academic national and international organizations such as the United Nations, the World Health Organization, and the World Alliance Against Antibiotic Resistance (WAAAR) are now advocating for the commonsense measures to limit progressive antibiotic resistance in clinical and nonclinical use. Research priorities include (1) promotion of research into new antibiotics; (2) development of antibiotic companion agents (e.g., antibiofilm agents, immune adjuvants, antibacterial vaccines, antiexotoxin and antivirulence agents, immunotherapies); (3) limitation or termination of the nonmedical use of antibiotics for agricultural purposes; (4) consideration of the use of curing agents to delete R plasmids from bacteria[20]; and (5) introduction of nonantibiotic therapies to treat bacterial infections.[211,212,213] The best hope for the future is in the development of a greater understanding of how antimicrobial resistance spreads, intelligent use and development of improved bacterial vaccines, antibiotic stewardship, and the implementation of effective infection-control strategies.[212]

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The complete reference list is available online at Expert Consult.

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19 Pharmacokinetics and Pharmacodynamics of Antiinfective Agents

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SHORT VIEW SUMMARY

- Maximizing the safety and efficacy of antiinfective agents requires application of quantitative systems pharmacology using pharmacokinetics and pharmacodynamics (PK-PD) to ensure that the right dosage regimen is selected.
- This chapter explains the general PK principles that govern the processes by which drugs enter and leave the body through absorption, distribution, metabolism, and elimination.
- The shapes of these concentration-time profiles impact the PD activity of antiinfectives and support selection of alternative regimens (reduced frequency, longer infusions) to reduce toxicity or optimize efficacy.
- Illustrative examples of the clinical translational potential of PK-PD from in vitro to in vivo, initial clinical trial dose selection, and dose refinement once clinical knowledge is gained are provided to demonstrate the applicability of this approach to deliver precision medicine.

The right drug against the right pathogen must be administered at the right dose to be safe and effective. Pharmacokinetics (PK) and pharmacodynamics (PD) systems analyses provide the mathematical framework within pharmacology for the design of precision antiinfective dosing regimens.[1] *Pharmacology* is the knowledge base of a compound concerning its history, source, physical and chemical properties, compounding, biochemical and physiologic effects, mechanisms of action and resistance, absorption, distribution, metabolism, excretion, and therapeutic and other uses.[1]

Pharmacokinetics (PK) describes the process by which a drug enters and leaves the body based on absorption, distribution, metabolism, and excretion to define systemic exposure.[2] *Pharmacodynamics* (PD) describes the biochemical and physiologic response of the drug and its mechanism of action.[2] PK-PD analyses are integrated to define the exposure response, where the response can be a measure of safety, efficacy, or emergence of resistance. Optimal dose regimens are now defined by modeling and simulation by balancing the probability of efficacy relative to safety thresholds.[1,2] Antiinfective PK-PD is unique in pharmacology in that this relationship includes the effects of the drug on the infective pathogen and the host as well as collateral effects on the microbiome.[3,4] Because antiinfective agents affect nonmammalian target sites, the doses of these agents tend to be an order of magnitude higher than other pharmacologic classes.[5] Another challenge involves reductions in potency of antiinfective agents over time due to emergence of resistance.[5] This chapter focuses on the clinical translational potential of PK-PD from in vitro to in vivo, initial clinical trial dose selection, and dose refinement once clinical knowledge is gained. The numerous terms and corresponding abbreviations used to describe specific PK-PD parameters in this chapter are summarized in Table 19.1.

PHARMACOKINETICS

Antimicrobials are administered by the oral, intravenous, and intramuscular routes with systemic exposure quantified using serum or plasma concentrations. Measurement of tissue concentrations requires invasive or semiinvasive techniques that result in estimation using drug physiochemical properties such as protein binding. Physiology-based PK models are increasingly used in drug development to predict drug biodistribution but are difficult to validate clinically. In some instances, samples such as epithelial lining fluid, cerebrospinal fluid, and synovial fluid may be obtained in humans, but in general our understanding of tissue and body compartment drug biodistribution relies on preclinical data.[6] *Physiology-based PK models* are increasingly being used to estimate tissue drug distribution and rely on drug physiochemical properties and on species-related physiologic data (Fig. 19.1). If a drug is administered intravenously, the rate of systemic entry is defined by the rate of drug infusion (R_0). Likewise, if a drug is administered orally or by some other extravascular route, the rate of entry into systemic circulation is defined by the absorption rate constant (k_a). In this second scenario, the extent of absorption may not be complete, and so the ratio of the systemic exposure profile by oral (or extravascular) absorption to that of intravenous administration defines the bioavailability (F). The rate of drug transfer between systemic circulation to tissues and organs depends on numerous factors including (1) molecular size and charge, (2) protein binding, (3) influx and efflux transporters, and (4) cardiac output. Most drugs undergo biotransformation through various metabolic processes to support elimination primarily via the kidneys and liver into the feces. As stated, the stepwise process of drug entry and departure is regulated by complex physiologic processes that are simplified and visually represented by the plasma or serum concentration-time curve. The shape of this concentration-time curve can be modified by altering the rates of drug entry, distribution, metabolic transformation, and elimination through intrinsic or extrinsic factors.[7] As shown in Fig. 19.2, administration of a 500-mg dose of an antiinfective agent by oral and intravenous routes can lead to distinctively different profiles. In the case of intravenous administration, the rate of infusion clearly can increase or decrease the time that a concentration is above a threshold value, such as the minimal inhibitory concentration for 90% of isolates (MIC_{90}). More detailed explanations of these pharmacokinetic processes are provided as follows.

Absorption

Absorption describes the movement of drug from an extravascular space to an intravascular space.[1] For antiinfective agents, the two most common modes of drug administration that require absorption are the oral and intramuscular routes. The amount of drug that reaches the systemic circulation is expressed as a percentage of the total amount that could have been absorbed. This percentage is defined as the drug's absolute or relative *bioavailability. Absolute bioavailability* means that the amount of drug absorbed by the extravascular route of administration has been compared with the intravenous route, whereas *relative bioavailability* means that two different extravascularly administered dosage forms have been compared.[1]

A major process variable in oral drug absorption is the solubility and permeability of the compound in the gastrointestinal tract. Most drugs in development tend to be insoluble, requiring significant efforts related to formulation development. The Biopharmaceutics Classification System

TABLE 19.1 Quick Reference Pharmacologic Abbreviations and Their Definitions

TYPE OF TERM	ABBREVIATION	DEFINITION
Pharmacokinetics		
Absorption	F	Bioavailability; absolute bioavailability
	K_a	Absorption rate constant
Distribution	V_d	Volume of distribution
	V_d/F	Apparent volume of distribution
	V_{ss}	Volume of distribution at steady state
	V_{ss}/F	Apparent volume of distribution at steady state
	CL_D	Distributional clearance
	CL_D/F	Apparent distributional clearance
Metabolism	Km	Drug concentration at which the rate that an enzyme system can metabolize a drug is half of Vm (Michaelis-Menten type of metabolism [saturable metabolism])
	Vm	Maximal metabolic capacity (Michaelis-Menten type of metabolism [saturable metabolism])
	CYP	Cytochrome P-450 enzyme systems
Elimination	CL_r	Renal clearance
	CL_{nr}	Nonrenal clearance
	CL_{nr}/F	Nonrenal oral clearance
	CL_T	Total clearance
	CL_T/F	Total oral clearance
	$t_{1/2}$	Half-life
Pharmacodynamics		
	MIC_{90}	Minimal inhibitory concentration for 90% of isolates
	EC_{50}	Effective concentration for 50% of all isolates
	MPC	Mutant prevention concentration
	MSW	Mutant selection window
	IC_{50}	Inhibitory concentration for 50% of isolates
	C_{max}/MIC	Peak antimicrobial serum concentration to MIC ratio (concentration-dependent killers)
	AUC/MIC	24-h area under the serum antimicrobial concentration-time curve to MIC ratio
	AUIC	24-h area under the inhibitory curve
	t	Half-life
	T > MIC	Time that serum antimicrobial concentrations are above the organism's MIC (time-dependent killers)
	SBT	Serum bactericidal titer (concentration)
	IQ	Inhibitory quotient ratio of trough serum concentration to IC_{50}
	PAE	Postantibiotic effect

(BCS) is currently used to categorize drugs into four groups based on high solubility, low solubility, high permeability, and low permeability. Ciprofloxacin serves as a model BCS class IV compound (low solubility/low permeability) that is poorly absorbed but can be transformed to a BCS class I compound (high solubility/high permeability) through medicinal chemistry. Oral absorption can be saturable or nonsaturable with factors such as degradation in the gut by acid or proteolysis gut metabolism and first-pass liver drug metabolism by enzymes and influx and efflux transporters influencing the rate and extent of absorption. Other factors that can impair absorption and bioavailability are drug interactions with other compounds or food that may bind the drug or reduce solubility.[8] The response of certain antiinfective agents is linked to the peak concentration (C_{max}) or the total exposure (area under the concentration-time curve [AUC]), which can be adversely impacted by reduced rate of absorption even if the extent is not.[2] As shown in Fig. 19.2, a more rapid rate of intravenous administration leads to higher C_{max} values. Alternatively, the response of microbes to some antiinfective agents is linked to maintenance of concentrations above a threshold concentration such as the MIC.[3,4] In this setting the rate of infusion can be extended (see Fig. 19.2) or a controlled-release oral formulation can be used to target steady concentrations above a threshold.

Distribution

The shape of the concentration-time curve is modeled most commonly using a proportionality constant known as the *volume of distribution (V_d)* and is termed the *apparent volume of distribution (V_d/F)* when the drug is administered via the extravascular route.[9] The V_d is not a real or physiologic volume, but rather a value that relates drug concentration in the system to the amount of drug present in that system. This system can be defined as a single compartment (i.e., V_{d1}) or as multiple compartments (i.e., V_{d1}, V_{d2}, … V_{dn}) to mathematically fit the shape of the concentration-time curve. Factors that alter the physiologic distribution of drug into tissue include lipophilicity, partition coefficient of the drug between different types of tissues, blood flow to tissues, pH, and binding affinity to plasma proteins relative to tissue components.[1] However, actual measurement of concentrations in these tissues or interstitial fluids is necessary to confirm site-specific distribution and cannot be easily estimated by V_d.[9]

Drug transporters play a role in defining the net drug concentration at the site of infection through influx and efflux transporters.[10] Transporter function can be influenced by genetic and environmental factors and thus varies from person to person. Drugs binding to serum proteins have a major influence on V_d. Acidic drugs tend to bind to albumin and typically have lower V_d values because they are retained in the plasma compartment. Basic drugs tend to bind to α_1-acid glycoprotein and are retained within tissues leading to larger V_d estimates. Protein binding is an important consideration for antimicrobial agents because unbound drug is available to exert antimicrobial activity, and in vitro methods used to assess potency through the MIC evaluate unbound drug.[11] Changes in the unbound fraction of drug may be caused by

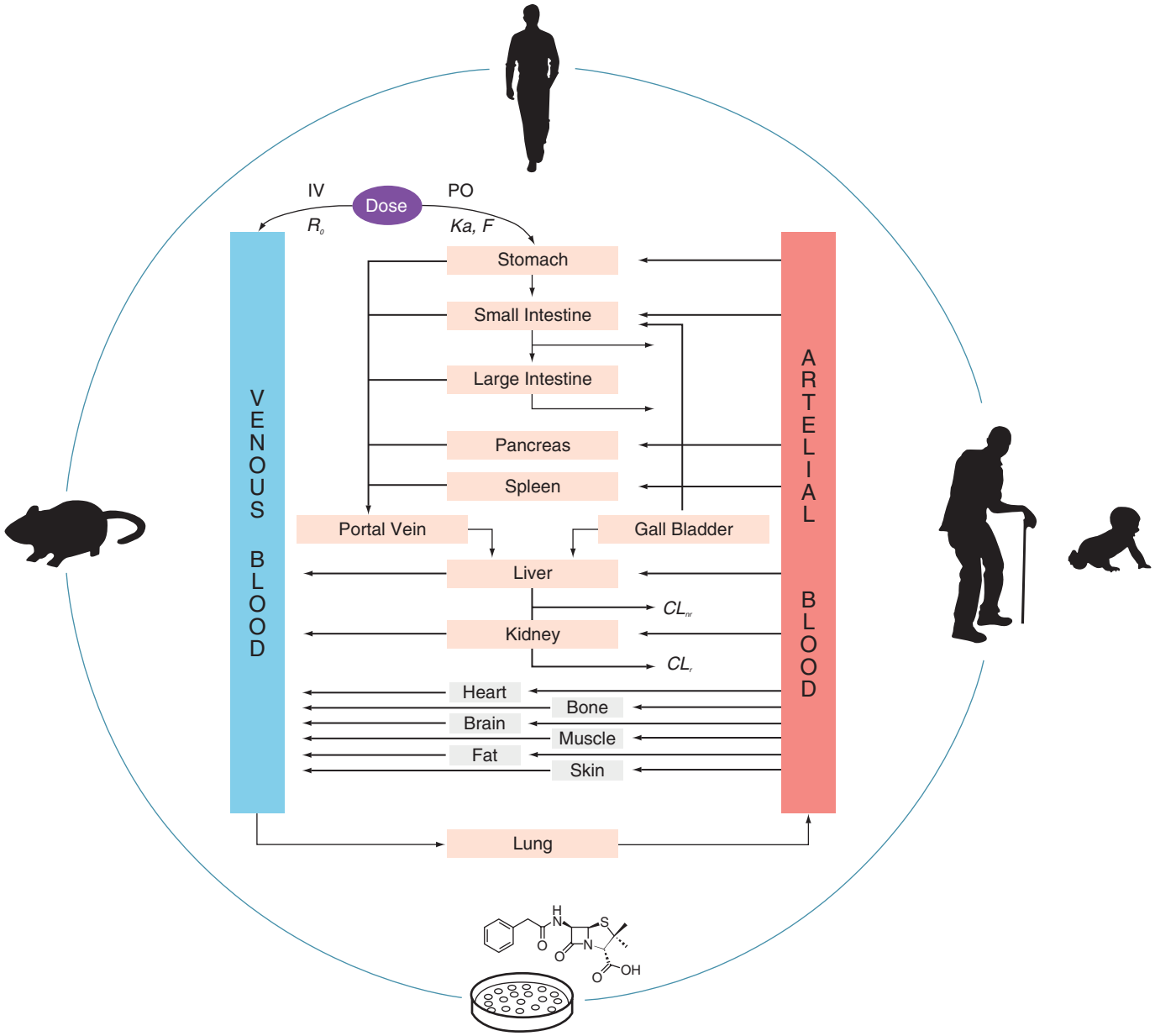

FIG. 19.1 Overview of a physiology-based pharmacokinetic model to predict plasma and tissue drug distribution in preclinical species to first in man and special populations using physiologic, microbiologic, physiochemical, and pharmacodynamics data. CL_r, Renal clearance; CL_{nr}, nonrenal clearance, *F*, bioavailability; *IV*, intravenous; K_a, absorption rate constant; *PO*, oral, R_0, rate of drug infusion.

displacement from other drugs, changes in serum protein concentrations, or accumulation of endogenous substances such as free fatty acids.[2] Although changes in protein binding may alter PK of an antimicrobial agent, substantial changes in PD would be unlikely.[12]

Metabolism and Biotransformation

Drugs undergo oxidative, reductive, and conjugation-based metabolism through enzymes primarily in the liver but in other tissues as well and by the gut microbiome. Drug metabolism is determined by genetics and environmental (external) factors.[13] Similar to transporters, some drug-metabolizing enzymes (DMEs) show genetic polymorphism, meaning that at least 1% of the population exhibits different DME activity (increased activity, decreased activity, or no activity) than the rest of the population. In some instances even for enzymes for which genetic polymorphism has not been shown, a large range in the rate and extent of drug metabolism can be found.[14] Environmental factors are many but include concurrent drugs, underlying diseases (both infectious and noninfectious), nutrients, herbal preparations and supplements, nutritional status, pregnancy, and sex (although for antiinfective agents sex differences have not been reported in general).

Drug metabolism reactions are classified as either phase I or phase II reactions.[2,8] *Phase I reactions* can transform a substrate into an active or inactive metabolite and in some cases into a more toxic substrate. Phase I reactions generally are under the control of the *cytochrome P-450 (CYP) system.* CYP enzymes are heme-containing proteins that are located in the endoplasmic reticulum of a variety of cell types, most abundantly in the liver. CYP enzymes are controlled by a superfamily of genes that are classified into families according to their amino acid sequences. Each family is divided further into subfamilies. The term *CYP3A4* designates a mammalian enzyme (CYP) family 3, subfamily A, gene 4. To date, many drugs that are metabolized by phase I enzymes have been shown to be metabolized by five primary CYP enzymes. In decreasing order of importance for drug metabolism, they are CYP3A, CYP2D6, CYP2C, CYP1A2, and CYP2E1. Although a complete discussion of the CYP system is beyond the scope of this chapter, many of the newer antiinfective agents, particularly antiretroviral agents, can induce,

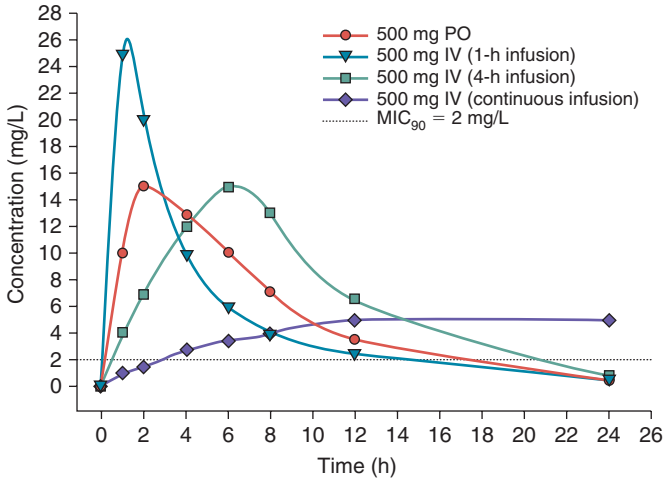

FIG. 19.2 Simulated serum concentration-time profile based on administration of a single antiinfective dose by oral *(PO)* and intravenous *(IV)* routes, with infusion at varying rates. *MIC_{90},* Minimal inhibitory concentration for 90% of isolates.

activate, or inhibit CYP enzymes, and in many cases they are substrates for CYP enzymes and are affected by changes in CYP activity.

CYP enzymes are affected by many factors that stimulate or inhibit their ability to metabolize drugs. Genetic factors have been shown to result in a phenomenon called *polymorphism.* Simply put, polymorphism means that individuals vary in their genetically determined ability to metabolize CYP substrate. For some CYP enzymes, such as CYP2D6, distinct poor, intermediate, extensive, and ultrarapid metabolic patterns exist in a population; in a white population, 4% to 6% are poor metabolizers, and the rest fall into the other metabolic groups, with the majority being extensive metabolizers. CYPs such as CYP2C9, CYP2C19, CYP2A6, and CYP2B6 also show genetic polymorphism. These CYPs are important in drug metabolism. For other CYPs, such as CYP3A, genetic polymorphism has been described; however, the significance of this remains confusing. These phenomena have important implications for antiinfective agents, for which efficacy against infecting organisms and toxicity to the host are determined by the PK of the agent and its resultant PD effect.

Clinically, drug, food, disease, and herbal effects on the CYP system may translate into inhibition, activation, or induction of metabolism. Inhibition of CYP activity occurs through reduction of enzyme production, inactivation, or competition for CYP substrate. Generally individuals with increased enzyme activity exhibit a greater inhibition of the CYP system with an inhibiting agent than individuals with less activity. Enzyme inhibition may result in increased PD effect, with the potential not only for greater efficacy but also for greater toxicity. This inhibitory process may be used in the clinical setting advantageously. Ritonavir has been used to decrease the activity of CYP3A isozymes in the gut, allowing greater absorption of other protease inhibitors (PIs) such as tipranavir and darunavir and reducing the overall cost of therapy. Cobicistat, an analogue of ritonavir, was specifically developed as a PK enhancer to boost oral absorption of antiretrovirals.[15] Induction of CYP increases production of the DME and a resultant increase in the ability to metabolize specific compounds. An example is the induction by rifampin of CYP3A with a subsequent increase in the metabolism of PIs. Many inducers of CYP enzymes also induce phase II conjugation reactions and transporters. Activation increases DME activity but to a much lesser extent (approximately 65% less) than enzyme induction.

Phase II reactions, which also show genetic polymorphism, involve conjugation of the parent compound with larger molecules, which increases the polarity of the parent molecule and permits excretion. Although phase II reactions generally lead to inactivation of the parent compound, occasionally conjugation increases the potency of the parent compound or results in the formation of another biologically active compound. When the conjugated compounds are secreted into the intestine, enzymatic cleavage may occur with release and reabsorption of the active parent compound, a phenomenon called *enterohepatic recirculation.*[1] As mentioned, the liver is not the only place in the body where metabolism occurs. Metabolism and detoxification of foreign substances can occur in most other organ systems.

Elimination

The AUC over a specific time period is proportional to the dose administered (for drugs that follow linear PK) and inversely related to *total drug clearance (CL_t).*[7] CL_t reflects the unit volume of a system that is cleared of drug per unit time (e.g., L/h). The physiologic drug clearance process is driven by elimination of the biotransformed or unchanged drug. This elimination of drugs is further categorized as renal and nonrenal clearance. *Renal clearance (CL_r)* describes the volume per unit time that the body eliminates a substance via the kidneys, through various mechanisms including glomerular filtration, tubular secretion (an energy-dependent process), and tubular reabsorption. It is important to realize that almost all xenobiotics are freely filtered through the glomerulus, but in most instances they undergo tubular reabsorption. Tubular secretion is a transporter-mediated process, and dose-dependent PK can be shown for substances that undergo tubular secretion as their primary route of elimination (e.g., piperacillin-tazobactam). *Nonrenal clearance (CL_{nr} or CL_{nr}/F)* describes the sum of clearance pathways that do not involve the kidneys.[2] These mechanisms may involve the biliary tree (e.g., ceftriaxone) or the intestine (e.g., azithromycin). Furthermore, the composition and enzymatic activity of the intestinal microbiota affect whether deconjugation occurs that impacts the rates of excretion and reabsorption. Other, uncommon mechanisms can be used such as elimination of alcohol through the skin and lungs (respiration) and ionization, DNA chelation, and inactivation of aminoglycosides by the sputum in patients with cystic fibrosis with elimination through expectoration.[16] Extracorporeal elimination through procedures such as dialysis (hemodialysis or peritoneal dialysis) also can be construed as a form of nonrenal elimination.[17] As expected, significant interindividual variability exists in the PK of drugs. *Population PK analyses* are used to identify and quantify sources of interindividual variability of PK parameters to better define doses in the broad population as well as special populations.

PHARMACODYNAMICS

Antiinfective PD is a science that is used to integrate PK information and in vitro measures of drug potency with effect.[4] This effect can be measured in vitro and in vivo (animal models) as the rate and extent of microbial death/growth inhibition or emergence of resistance.[18] Alternatively, this effect can be defined clinically by a measure of biologic response such as survival, time to clinical response, probability of clinical response, and so on.[7] As expected, PK-PD systems analysis currently represents a stepwise hierarchical process that integrates in vitro and in vivo data followed by clinical validation.[19]

Antimicrobial Potency

An antiinfective agent may inhibit growth and replication ("-static") or cause bacterial cell death ("-cidal"). A factor that affects whether a drug is bacteriostatic or bactericidal is the concentration at the site of action, but this may not imply differences in clinical efficacy.[20] Antimicrobial agents may be bacteriostatic at low concentrations but bactericidal at high concentrations. These bacteriostatic and bactericidal concentrations have been used to quantitate the activity of an agent against an organism. Approaches to measure this activity have broadly included use of agar-based dilution and broth macrodilution and microdilution systems.[21] Agar-based dilution systems lead to measurement of activity as a zone of inhibition. Broth dilution systems lead to measurement of an MIC that is based on a doubling-dilution ($\log_2$) scale (e.g., 0.5, 1, 2, 4 mg/L).[21] Agar-based methods can also incorporate susceptibility testing using an E-Test strip that creates antimicrobial gradients to quantify the MIC on an arithmetic scale (e.g., 0.5, 0.75, 1, 1.5, 2 mg/L). The *MIC for 90% of all surveyed isolates* of a bacterial species (MIC_{90}) and the *inhibitory or effective concentration for 50% of all surveyed isolates* of a strain of virus (IC_{50} or EC_{50}) are conventionally used to describe drug activity against pathogens. The minimal bactericidal concentration

provides information on the lowest concentration at or above the MIC required to kill a microorganism. Although these in vitro parameters are helpful epidemiologically, they represent fixed values that do not reflect the dynamic in vivo process such as (1) the time course of activity or the potential for persistent antiinfective effect after the concentration at the site has decreased below the MIC or minimal bactericidal concentration, (2) the interaction of the immune system with the drug, and (3) exposures necessary to prevent the development of resistance or organism mutation. Importantly, these parameters reflect specific drug-organism in vitro measurements that cannot reflect the combination drug use profile as empirical therapy and for documented polymicrobial infections.[21]

Although antiinfective agents can be used individually, in many instances they are used together. *Synergism* is defined as activity of two or more antiinfective agents given together that is greater than the sum of activity had the agents been given separately. *Additivity* (also known as *indifference*) is defined as activity of two or more agents together that equals the sum of activity of each agent. *Antagonism* is defined as activity of two or more antiinfective agents given together that is lower than the activity of the most active agent given separately. Combinations of agents are used to enhance efficacy and rate and extent of organism killing or to reduce the development of resistance but can have conflicting results.[22]

Pharmacodynamics Indices

PD combines PK parameters and microbiology parameters to describe drug effect in relation to some measure of exposure. These PK measures of exposure include the maximal concentration (C_{max}; peak), minimal concentration (C_{min}; trough) or AUC integrated over a specified time period (AUC_{0-t}) or infinity (AUC_{0-inf}). Alternatively the duration of time that the concentration exceeds a threshold value can be correlated to an event related to efficacy, safety, or emergence of resistance. In the case of antiinfective agents, three PK-PD indices based on serum or plasma concentrations have been associated with efficacy and include C_{max}/MIC, AUC/MIC, or T > MIC.[19] As shown in Fig. 19.3, measurement and classification of the relevant C_{max} are not straightforward. For an intravenously administered agent, C_{max} occurs at the end of the infusion, whereas this is more variable for an orally administered agent but typically between 1 and 4 hours. The rise and fall in drug concentrations are often evaluated through blood sampling and most often have two phases. The first phase follows a more rapid initial decline referred to as the *distribution phase,* followed by a reduction in the slope referred to as the *elimination phase.* This second phase in the concentration-time profile often coincides with the concentration profile expected in the interstitial space of tissues with the scale of this profile dependent on the degree of plasma protein binding. From this perspective, C_{max} and C_{min} are single point estimates but are rarely measured at the exact time of their occurrence, which requires a monoexponential or biexponential function to translate.[23] This information can also be modeled and integrated over time to generate an overall exposure or AUC that is easier to translate between serum and other tissue compartments.[7] Thus the effect of many antimicrobial agents often correlates to the AUC/MIC index because AUC (mg · h/L) represents both concentration (mg/L) and time-dependent (h) components.[24] Antimicrobial agents deemed to be concentration dependent with a good correlation to C_{max}/MIC also have some degree of correlation to AUC/MIC.[24] The same phenomenon is true for antimicrobial agents deemed to manifest time-dependent PK-PD.[24] For antimicrobial agents that are deemed to be time dependent (good correlation to T > MIC), a good correlation with AUC/MIC is also observed if this agent has persistent sub-MIC effects.[18] For antiinfective agents, such as antiviral and some antiretroviral agents that are activated intracellularly, serum and plasma PK are often not reflective of the site of cellular and subcellular activity. These agents are also retained intracellularly for longer periods of time than reflected by the intravascular concentration-time profile.

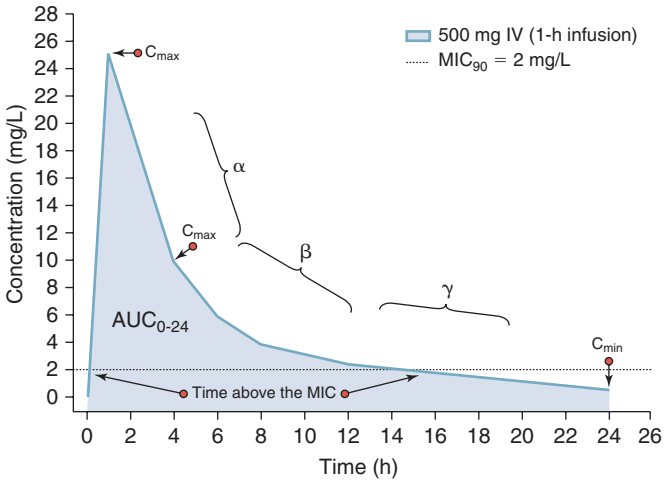

FIG. 19.3 Common antibiotic pharmacokinetics and minimal inhibitory concentration *(MIC)* pharmacodynamics relationships based on an antiinfective agent (e.g., an aminoglycoside) with a triphasic serum concentration-time profile. *AUC_{0-24},* Area under the curve for first 24 hours after time 0; *C_{max},* maximum concentration; *MIC_{90},* minimum inhibitory concentration for 90% of isolates.

Methodology for Study of Pharmacodynamics Effects of Antiinfective Agents

In Vitro Models

The most widely accepted model used to study in vitro PK-PD effects of antiinfective agents is the "hollow fiber model" system.[25,26] The system uses a cartridge that is composed of thousands of hollow porous fibers that are sealed at each end so that growth media that enter one end go through the inside of the fibers to the opposite end. Microorganisms or virally infected cells are inoculated on the outside of the fibers and multiply in the space between the fibers known as the extracapillary space. In this system, antiinfective agents, nutrients, and metabolic waste can cross the fibers but the larger microorganisms cannot cross through the pores. As a result, the microorganisms can be exposed to predetermined dynamic or static concentrations of antiinfective agents under conditions that can simulate the expected PK in humans.[25,26] The extracapillary compartment can be sampled via a port to quantify the microorganism load and drug concentrations. Although these models offer control over bacterial inoculum and drug concentration-time profiles that mimic clinical cases, they do not currently assess the effects of the immune system on organism killing or growth inhibition. They assess the relationship of free drug concentrations to effect, assisting in the development of relationships of protein-bound drug in humans. However, these in vitro models result in relatively high organism loads and lack host immune function and so have the theoretical potential of predicting higher effective doses than would be defined by immune intact animal models. More recent applications have included the evaluation of antiinfective combinations against multidrug-resistant gram-negative pathogens. This approach streamlines experimental designs and aids evaluation in clinical trials in a more efficient manner. This is a valuable approach to improve on existing agents given the limited number of available antiinfective agents against multidrug-resistant pathogens[26] and the important collateral effects of antibiotic overdosing.

Animal Models

Animal models have used a variety of species, often with the animals rendered neutropenic before infection. Craig and others[27–29] showed that the presence of neutrophils may affect antibacterial activity with fluoroquinolones, penicillin, clindamycin, and doxycycline. Animal infectious disease models have been developed to mimic human infections. Animal models allow for frequent sampling of blood and tissue and allow a broad dosage range to be investigated along with a wide range of organism inocula, allowing investigators to study the effects of variation in a single parameter at a time. Problems with animal models include a lack of standardization of inocula size (often large inocula are required to produce infection). The faster rate of drug elimination in small mammals compared with humans often leads to the use of dosing regimens that may match human AUC values but

may not replicate human concentration-time profiles. Immunocompetent animals have been used to attempt to develop more realistic guidelines for PK-PD targets in infected patients, many of whom are not neutropenic. Despite these limitations, animal models can yield necessary data to aid dose translation from preclinical to early stage clinical studies.

Clinical Trials

Preclinical and early clinical (phase I and II) PK-PD relationships are often being applied to justify dose selection for the two phase III clinical trials that are necessary to gain regulatory approval to market a drug.[18,19] This dose selection often includes testing of a single fixed dosage (e.g., 500 mg intravenously once daily) or weight-based dosage (e.g., 6 mg/kg intravenously once daily) regimen. After completion of the first phase III trial, PK-PD analyses may validate or contradict the pre–phase III study assumptions. The high cost of phase III trials limits significant modification of the study design to rectify and retest assumptions about dose selection.[19] Hence most human trials[29–38,39,40] that have defined PK-PD relationships have been based on the retrospective review of postmarketing drugs or post hoc subgroup analyses of prospectively collected data.[41–44] Little, if any, prospective data have been generated with either dosage adjustment during therapy or comparison of different dosing regimens to attain different exposures during treatment (concentration-response trials).[45] These trials have used three measures of assessment to relate to antimicrobial PK-PD: (1) clinical outcome (cure/fail or improved); (2) eradication of bacteria from the site of infection or reduction in virus concentration (viral load) in blood or other sites, or both; and (3) improvement in surrogate markers of infection such as temperature or leukocyte count. Many trials have not reported free drug PD indices. Because free (unbound) drug is considered active, correction for protein binding is important for highly bound drugs. Few human trials have focused on relationships of drug exposure to toxicity or on the development of resistance. Clinicians often fail to appreciate that exposure response is a fundamental paradigm in pharmacology, and so for every drug an opportunity exists to individualize the dosage regimen and not rely on a "one size fits all" dosage regimen.[7] However, the impracticality of this approach and limited clinical experience to generate confidence in a safety threshold has limited the application of these principles to a handful of antiinfective agents. Lack of availability of a commercial assay, limited knowledge of interlaboratory reproducibility, and clinical interpretation of systemic concentrations delay the process by which clinicians can apply these principles in practice. Despite these limitations, well-designed proof-of-concept studies are emerging in the literature and align with the desire to deliver precision antiinfective therapies.[46]

Concentration-Dependent Killing Agents

Concentration-dependent killing agents (e.g., fluoroquinolones, aminoglycosides, macrolides, azalides, ketolides, metronidazole, daptomycin, and oritavancin) exert their positive effect on bacteria when their concentrations are well above the MIC of the organism.[18] When the ratio of the concentration at the site of infection to the MIC is increased further, greater killing occurs. This concept is illustrated in Fig. 19.4 for tobramycin and ciprofloxacin against *Pseudomonas aeruginosa*.[47] In addition, some of these agents exhibit postantibiotic effect (PAE) (see "Postantibiotic Effect" later). Growth inhibition continues for a variable period after the concentration at the site of the bacteria has decreased below the MIC for the antimicrobial agent. In vivo, the C_{max}/MIC ratio has been shown to be the clinical correlate used as the PD predictor for outcome of concentration-dependent killing agents. In clinical trials, the AUC/MIC ratio also has been correlated with improved outcome.[33–44] This finding is not surprising because C_{max} and AUC increase in proportion to the administered dose and consequently are correlated.[18] For drugs such as fluoroquinolones, different goals for AUC/MIC ratios are required for gram-positive pathogens compared with gram-negative pathogens.[47–48]

Time-Dependent Killing Agents

Time-dependent killing agents include penicillins, cephalosporins, aztreonam, vancomycin (for which AUC/MIC is predictive), carbapenems, macrolides, linezolid, tigecycline, doxycycline, and clindamycin.[18] For agents active against gram-negative bacteria, the rate of kill is maximized when concentrations at the site of the bacterial infection are typically four times higher than the MIC of the organism; this is shown for ticarcillin against *P. aeruginosa* in Fig. 19.4.[3]

The amount of time that the concentration needs to be above the MIC has been the subject of debate.[19] A report using animal studies with *Streptococcus pneumoniae* in which treatment was performed with penicillins or cephalosporins showed that when T > MIC was 20% or less of the dosing interval, mortality was 100%. In contrast, a mortality rate of 0% to 10% occurred when serum concentrations were above the MIC for longer than 40% to 50% of the dosing interval.[19,49]

Evaluation of the influence of PK/PD on bacterial resistance has best been characterized with fluoroquinolones and aminoglycosides and is being explored for other agents. Blaser and colleagues[49] examined the C_{max}/MIC ratio for enoxacin and netilmicin against various gram-negative organisms. Regrowth of organisms occurred in all cultures when enoxacin or netilmicin attained ratios lower than 8. On redosing of these antibiotics after bacterial regrowth, no killing was seen because of the development of resistance. Marchbanks and associates[50] using

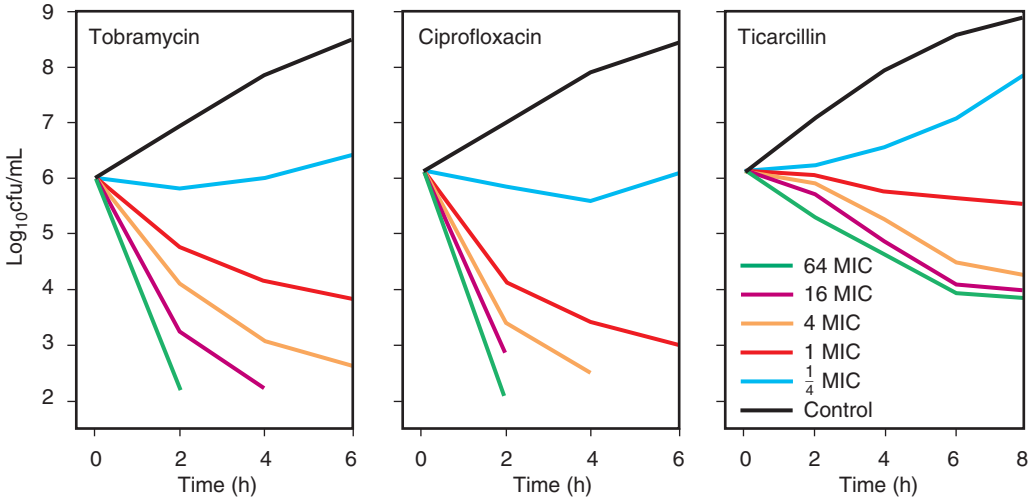

FIG. 19.4 Time-kill curves for *Pseudomonas aeruginosa* ATCC 27853 with exposure to tobramycin, ciprofloxacin, and ticarcillin at concentrations from one-fourth to 64 times the minimal inhibitory concentration *(MIC).* *(From Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: a review.* Scand J Infect Dis. *1991;74:63–70.)*

ciprofloxacin noted the development of resistant *P. aeruginosa* when the organism was exposed to a C_{max}/MIC ratio of 6 compared with no resistance when the C_{max}/MIC ratio was 12, even though both regimens showed adequate rates of bacterial killing. Additional studies have confirmed that the high initial concentrations do impact emergence of resistance when testing ciprofloxacin against *P. aeruginosa.*[51] Recent hollow-fiber model studies have also identified PK-PD–resistance emergence relationships between linezolid and *Mycobacterium tuberculosis,* which would be very difficult to characterize in vivo.[52] A disadvantage of these investigations, however, is that they do not account for the role of the immune system in "cleaning up" small numbers of resistant bacteria before they can become pathogenic.

Postantibiotic Effect

During in vitro testing of antimicrobial agents, there may be a delay before microorganisms recover and reenter a log-growth period.[47] This phenomenon is termed the *postantibiotic effect (PAE).*[3,47] The exact duration of the PAE is both species and drug dependent. Aminoglycosides and fluoroquinolones produce in vitro PAEs against gram-negative bacilli of 2 to 6 hours. β-Lactam antibiotics (except for imipenem) produce little or no PAE against gram-negative organisms under identical experimental conditions but generally induce 2-hour PAEs against gram-positive organisms.[18] Other factors that affect the in vitro PAE include combinations of antimicrobial agents, antimicrobial concentration, duration of antimicrobial exposure, and pH. Potential factors that also may affect PAE include size of inoculum, type of growth medium, and bacterial growth phase.[18]

Studies in animal models have verified that PAE is not an artifact of in vitro testing. Investigational animal models that have been studied include a neutropenic mouse thigh model, a rabbit meningitis model, a rat endocarditis model, and a guinea pig pneumonia model. These studies showed that an in vivo PAE exists against gram-negative organisms for aminoglycosides, fluoroquinolones, erythromycin, clindamycin, and tetracycline, but not for β-lactams. As in the in vitro studies, β-lactam agents produce abbreviated PAEs against gram-positive organisms.

A definitive common mechanism to explain the PAE has been elusive for years. Nonspecific binding, nonlethal damage, antibiotic persistence in the periplasmic space, and antibiotic binding kinetics all have been postulated as potential mechanisms. Srimani and colleagues[53] elegantly demonstrated that drug detoxification within individual cells after the drug is eliminated from the extracellular system can explain this phenomenon among antibiotics with variable mechanisms of action. The presence or absence of a PAE has been used to alter antimicrobial dosing schedules. Theoretically an agent with a long PAE can be dosed less frequently than an antimicrobial agent lacking a PAE. Alternatively an agent with little or no PAE may be most effective if it is given as a continuous infusion so that the serum concentration always exceeds the MIC. Dosing strategies such as these are theoretical and require clinical investigation in human studies of sufficient size before implementation into clinical practice.

Applied Clinical Pharmacokinetics and Pharmacodynamics

The exposure-response relationship predictive of effect and safety may not be complete when an antiinfective agent is first marketed. Over the past 40 years, the principles outlined earlier have been applied to improve the clinical management of patients through design of alternative drug dose regimens that take advantage of the exposure-response relationship.[18] These strategies have broadly included the use of higher-dose extended-interval dosing and continuous or extended infusions for antimicrobial agents with concentration-dependent and time-dependent PK-PD characteristics, respectively. Infected patients are in a dynamic physiologic state that is a corollary to "shooting at a moving target" with antiinfective dose selection.[19] Conceptually, use of more intensive dosing regimens at treatment initiation followed by dose titration with clinical improvement or worsening would be ideal. Testing this approach requires more complex covariate-adjusted response-adaptive designs of antiinfective agents with a companion biomarker of response to tease out true differences between regimens.[54] In the interim, examples of specific dose regimen designs that have taken advantage of the presumed PK-PD profile are provided as follows. Therapeutic drug monitoring (TDM) to improve the dosing of certain agents is also described to illustrate that the dosing of an antiinfective agent can evolve with increasing clinical experience.

Higher-Dose Extended-Interval Dosing

This dosing strategy has primarily been used to optimize the PK-PD profile of concentration-dependent antimicrobial agents such as tobramycin, levofloxacin, daptomycin, oritavancin, dalbavancin, and time-dependent antimicrobial agents such as azithromycin.[39,42,55–58] High-dose extended-interval aminoglycoside dosing serves as the model for validation of this approach. The original regulatory approved doses of gentamicin and tobramycin were 1 mg/kg three times daily, but these agents are now clinically administered as 5 to 7 mg/kg once daily in patients with good kidney function.[59] A tobramycin dose of 10 mg/kg once daily is recommended in patients with cystic fibrosis who are being managed for an acute pulmonary exacerbation secondary to *P. aeruginosa.*[59] The objective of the tobramycin regimen of 5 to 10 mg/kg once daily is to achieve a serum concentration of 16 to 20 mg/L 1.5 to 2 hours after a half-hour infusion (postdistribution phase). We seek to achieve a serum C_{max}/MIC ratio of 8 to 10, based on an MIC_{90} of tobramycin against *P. aeruginosa* of 2 mg/L because this PK-PD index has been correlated to predict clinical success.[33] The half-life of tobramycin in most patients with good kidney function is 2 to 4 hours. As a consequence, the serum tobramycin concentrations are expected to be less than 2 mg/L for 6 to 14 hours of the 24-hour dosing regimen. This sustained effect of tobramycin (despite long sub-MIC exposures) in the clinical setting is based on in vitro and animal model demonstrations of the PAE and sub-MIC effects of this agent.[53] However, aminoglycosides are rarely administered as monotherapy (except for urinary tract infections), so definitive clinical proof of this concept is unavailable. Meta-analyses also have not clearly shown this dosing concept to be superior to the individualized daily dosing approach, with the resultant dosing being one or multiple times a day depending on the individualized patient PK parameters.[58] Well-designed clinical trials in patients with cystic fibrosis have not shown a clear efficacy benefit but have suggested a lower potential for nephrotoxicity with this agent.[60] Despite this lack of clear clinical benefit, the emergence of multidrug-resistant gram-negative pathogens, convenience of this dosing strategy, and theorized benefits continue to support adoption of this aminoglycoside dosing strategy.[60]

Although the basis of this dosing strategy evolved with the aminoglycosides, the most successful clinical application of these principles has actually occurred with the use of levofloxacin, azithromycin, daptomycin, and oritavancin.[42,55,56,58] These dose regimens are also being tested with antimicrobial agents in clinical development. However, the motivation for this dosing strategy is distinct for each of these agents. The clinical outcomes associated with the use of 750 mg of levofloxacin once daily for 5 days are similar to outcomes associated with the use of 500 mg once daily for 10 days for the treatment of community-acquired pneumonia.[61] Similarly, azithromycin has been approved as a 5-day treatment course for community-acquired pneumonia based on a loading dose of 500 mg on the first day followed by 250 mg once daily for 4 days.[55] In both cases, high epithelial lining fluid and alveolar macrophage concentrations serve as the pharmacologic basis of these shorter-course regimens.[6] In the case of azithromycin, shorter 3-day courses or even single-dose administrations have been approved for certain clinical indications.[54] The prolonged intracellular retention of azithromycin and immunomodulatory effects of this agent (not predicted by in vitro studies) has been credited to support this dose design.

The application of higher doses at treatment initiation has more recently been referred to as front-loaded regimens, based on similar theories to suppress tumor growth.[62] The efficacy and safety of the new glycopeptide oritavancin with a front-loaded regimen compared with daily dose administration has been tested in a phase II study.[63] A single oritavancin dose regimen (1200 mg) was shown to have a similar safety and efficacy profile as daily administration (200 mg for 3–7 days) for complicated skin and soft tissue infections. Based on supportive phase II trial data, use of a higher dose of rifampin (15 mg/kg/day) versus the current dose of rifampin (10 mg/kg/day) was studied in patients

(≥15 years of age) with tuberculous meningitis.[64] The probability of survival was not improved with this intensified regimen.[64] Peloquin and colleagues[65] studied higher rifampin doses (20 mg/kg/day) in patients with tuberculosis and provided the pharmacologic basis to evaluate higher doses in future studies. Similarly, use of a high-dose twice-yearly combination of albendazole (800 mg) and ivermectin (400 µg/kg) was superior to standard-dose albendazole (400 mg) and ivermectin (150 µg/kg) at suppressing *Wuchereria bancrofti* microfilaremia.[66] Microfilaremia was detectable in 57% and 28% of patients with the standard dose at 12 and 24 months after treatment but was undetectable in all patients treated with the high-dose regimen. These studies suggest the potential either to maintain clinical effect with a more convenient dosing strategy or to actually improve clinical outcomes without an increase in the risk for adverse reactions.

Continuous-Infusion and Extended-Infusion Regimens

The effects of intermittent, extended, and continuous infusion on the serum concentration time profile are illustrated in Fig. 19.5 with a 3 g/day dose of an antimicrobial agent, assuming a one-compartment system with a CL = 9.3 L/h, V_{d1} = 36.3 L, and an elimination half-life of 5.4 hours. As illustrated by this simulation, concentrations above a threshold concentration of 8 mg/L are maintained for the longest period of time with the use of an initial combination of a short infusion (loading dose) followed by a continuous infusion. As a consequence, concentrations can be maintained above this threshold with a 2 g/day regimen compared with a 3 g/day regimen, that is, a 30% lower total daily dose. The concentrations in serum with a continuous infusion regimen with or without a loading dose will converge; however, use of a continuous infusion without a loading dose will lead to a delay in the time that the concentration exceeds a threshold (Fig. 19.6). Martinez and associates[18] reviewed this topic and highlighted the importance of achieving effective concentrations at treatment initiation when the organism load is expected to be at its highest.

A systematic review and meta-analysis of extended and continuous infusion of piperacillin-tazobactam and carbapenems based on non-randomized studies suggested that these dosing approaches may be associated with a lower risk for mortality compared with shorter intermittent infusions.[67] Well-designed multicenter studies have yielded varying results when evaluating the impact of longer infusion duration on the outcome of β-lactam–based therapies in the intensive care setting.[68,69] A meta-analysis of prolonged-infusion piperacillin-tazobactam studies suggested a 1.46-fold lower odds of mortality with prolonged infusions compared with intermittent infusion of this agent.[70] These data support the overwhelming in vitro and animal model data that concentrations above a threshold improve activity of β-lactams. However, surveys suggest that translation or acceptance of this dosing paradigm occurs in less than 20% of institutions in the United States and abroad.[71–73]

Dose-Refinement Considerations

Selection of a specific antiinfective dose regimen relies on the assumption that a dose-response relationship exists in support of this regimen. The dose regimen that is approved for clinical use represents the population central tendency estimates, which maximizes the probability of clinical effect. This regimen is validated internally in a subset of the general population that is evaluated through clinical trials. Hence external validity of this dose regimen for most antiinfective agents occurs after it is marketed; that is, we may study hundreds of patients for drug approval but ultimately use the drug in millions of patients. Furthermore, the optimal dose of an antiinfective agent for every patient subgroup (e.g., pregnant patients, patients receiving dialysis, pediatric patients) is not well known when it is first marketed. Therefore a system to aid dose selection or refinement in these subpopulations of patients is a common expectation and a constant clinical challenge. TDM was developed to meet this specific challenge but has not been embraced universally because it may not be apparent during drug development that a specific therapeutic range or exposure exists. Again, the evaluation of a relatively small and carefully selected patient population before market approval limits the discovery of a therapeutic exposure range.

The clearest example of this point was documented with the triazole antifungal agents, owing to high interpatient variability in drug absorption and metabolism. Itraconazole, voriconazole, and posaconazole are three triazoles that are used to manage invasive fungal infections that have now been shown to require TDM to optimize outcomes.[74] In the case of itraconazole and posaconazole, unpredictable oral absorption represents the primary reason that TDM is necessary.[74] Voriconazole is metabolized in part via cytochrome P-450 2C19 (CYP2C19) isoenzymes that are encoded by a gene that is known to be polymorphic with ethnic-based variation among individuals. Common coadministered drugs such as omeprazole can also inhibit this isoenzyme system. Therefore the dose-exposure profile of voriconazole is highly unpredictable in the clinical milieu of ethnic diversity and potential drug-drug interactions.[74] The most recent practice guidelines for the diagnosis and management of aspergillosis include clinical scenarios in which TDM of voriconazole, itraconazole, and posaconazole is justifiable.[75]

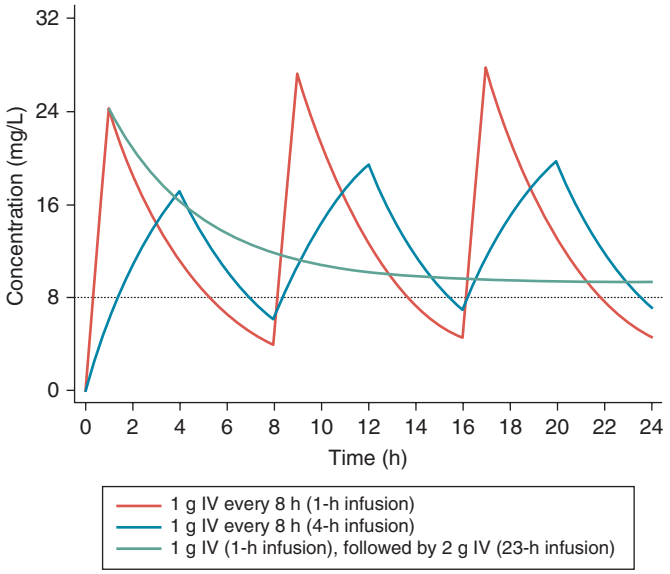

FIG. 19.5 Simulated serum concentration-time profile of an antimicrobial agent (3 g/day) administered as a 1-h, 4-h, and continuous infusion (with initial short infusion dose) with a reference concentration threshold of 8 mg/L. *IV,* Intravenous.

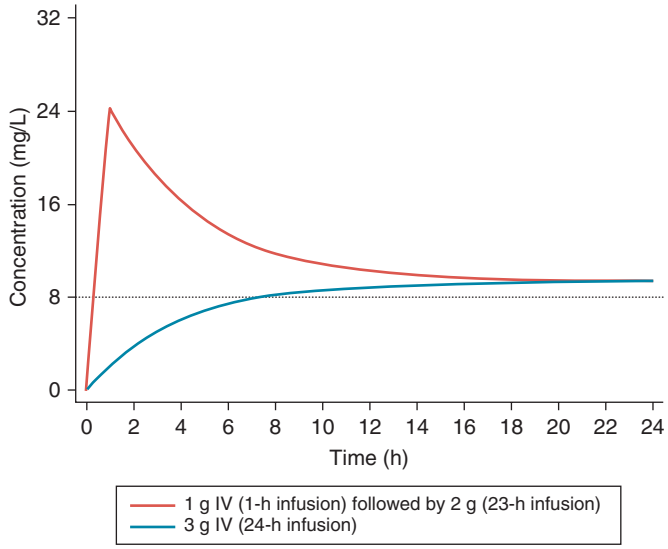

FIG. 19.6 Simulated serum concentration-time profile of an antimicrobial agent (3 g/day) administered as continuous infusion, with and without initial short infusion dose, with a reference concentration threshold of 8 mg/L. *IV,* Intravenous.

These observations with the triazole antifungal agents have also been made with antituberculosis agents.[76] Use of standard dose regimens of isoniazid may not be reasonable in all populations given the known polymorphisms in enzymes that influence its metabolism.[76] Limited access to commercially available assays prevents practical application of dose refinement that is necessary to calculate the correct dose.[77]

In practice, TDM of the aminoglycosides and vancomycin is most common, but definitions of the target exposure ranges associated with effect and toxicity have changed over time.[78,79] As with any measure, it is important to recognize that a distribution surrounds a presumed central tendency target exposure value and that this target may not be fixed or linearly translatable by MIC. With tobramycin as an example, an AUC_{24} of 75 mg · h/L and 192 mg · h/L may be sufficient for pathogens with an MIC of 0.5 to 1 mg/L and 2 to 4 mg/L based on in vitro models and mathematical simulation.[78] Let us assume that a vancomycin AUC/MIC target greater than 400 h^{-1} is associated with a higher probability of effect against a pathogen with a modal MIC_{90} of 1 mg/L. A 2-g daily dose achieves a median AUC of 400 mg · h/L in a population and so represents the average population dose. An average patient is treated with 2 g/day empirically but then is determined to be infected with methicillin-resistant *Staphylococcus aureus* (MRSA) in the bloodstream that has a vancomycin MIC of 0.5 mg/L (48 hours into the admission). Given this scenario, should we reduce the dose in half to achieve an AUC of 200 mg · h/L? The answer is most likely to be no. Alternatively if the MIC was 2 mg/L, should we double the daily dose? The answer in this scenario may be yes, but we should also expect the risk for toxicity to increase. As shown, current application of these principles at a population level is quite helpful for dose selection, but simple linear translation may be spurious, especially when factoring in uncertainty associated with MIC measurement.[80] We expect the current practice of vancomycin TDM to evolve from the simple measurement and dose adjustment that is based on trough concentrations to one that considers AUC estimation using two serum samples.[81,82] Similarly, we may discover over time that TDM is necessary with other agents used to treat MRSA such as linezolid and daptomycin that have high interpatient variability in PK in certain subpopulations such as critically ill patients. Alternatively, empirical combination antiinfective treatment as a strategy to curb antiinfective resistance against pathogens that we currently manage with monotherapy (e.g., MRSA) may be adopted over time and require our reevaluation of the necessary exposure targets and doses.

Antiretroviral Pharmacodynamics

In contrast to the above-discussed antimicrobials, antiretrovirals are unique in their site of action, which is most commonly inside or on the surface of the mammalian cell. Toxicity can result from interference with the host cell's physiology as seen with the early antiretroviral agents commonly associated with adverse events including diarrhea and mitochondrial toxicity. Yet as with other antimicrobials, failure to achieve effective concentrations can result in rapid development of drug-resistant mutations and subsequent treatment failure. Thus using dosing strategies that achieve a concentration profile between effective and toxic thresholds in each individual patient is of utmost importance. Fig. 19.7 illustrates these key concepts of the exposure-response relationship and the therapeutic window. One strategy to manage this balance is to treat with combinations of antiretrovirals that affect different points in the viral life cycle. This strategy harnesses the pharmacologic principles of additivity and synergy, where the effect of the drugs given in combination is either equal to (additive) or above (synergistic) what would be expected given the potency of each individual agent.[83] Thus combination dosing can lower the concentrations required for viral suppression thereby widening the therapeutic window.

At the present time there are more than 36 antiretrovirals approved by the US Food and Drug Administration within five therapeutic classes including six single tablet regimens consisting of three or more agents.[84] For therapeutic classes with a low genetic barrier, a single point mutation can confer resistance to most agents within that class; thus treatment failure can greatly limit subsequent treatment options. Successive antiretroviral regimens also do not perform well for as long a duration as the initial regimen, and increased mortality risk has been associated with multiple regimen failures.[85,86] Therefore optimizing the first regimen

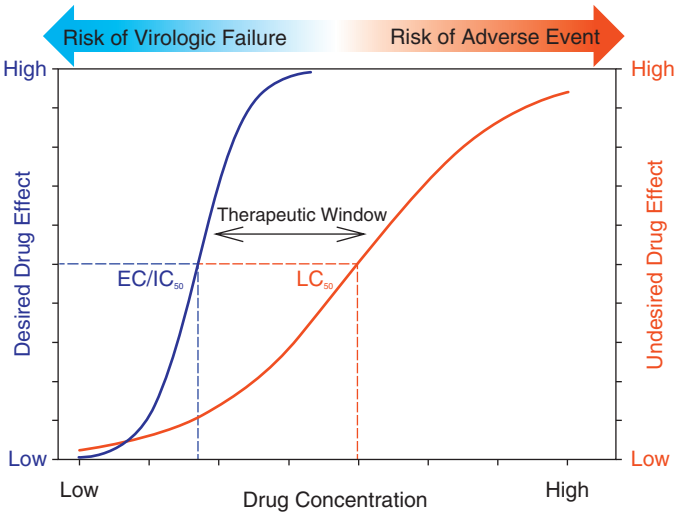

FIG. 19.7 Hypothetical exposure vs. desired (blue) and undesired (red) response curves for a drug with a narrow therapeutic window. The 50% effective concentration/inhibitory concentration *(EC/IC$_{50}$)* is marked by blue dashed reference lines. The 50% toxic concentration *(LC$_{50}$)* is marked by red dashed reference lines.

for success is crucial. Early-phase clinical trials frequently attempt to correlate PK parameters (i.e., indices) such as C_{max}, C_{min}, and AUC to outcome, usually measured by changes in plasma human immunodeficiency virus (HIV) RNA or $CD4^+$ T lymphocytes. However, variability in drug exposure within and between patients can be an important factor in interpreting these data. Thus a comprehensive understanding of these PK-PD relationships and of exposure variability is important for making clinical decisions that maximize the likelihood of treatment success for every patient.

Defined Exposure (PK)-Response (PD) Relationships by Class

Protease inhibitors. PK-PD relationships have been well studied for commonly prescribed PIs. For atazanavir, AUC[87] but not C_{min}[88] significantly correlates with antiviral activity as well as hyperbilirubinemia, a common, atazanavir-specific adverse event.[87,89,90] The latter relationship is likely due to reduced activity in the metabolizing enzyme, UGT1A1, within a subset of patients carrying the genetic polymorphism, UGT1A1rs887829.[89] Between-study discrepancies have been noted for darunavir, where early reports described a significant PK-PD relationship[91] for twice-daily dosing, which was not substantiated in later studies for either once-daily or twice-daily dosing.[92,93] A potential explanation of this discordance is that high interpatient (between patients) and intrapatient (within the patient) variability in darunavir plasma concentrations obscures this relationship; also, high intrapatient variability has been previously associated with failure to achieve virologic suppression.[94] Although lopinavir/ritonavir C_{min} is an important predictor of response in antiretroviral treatment–experienced patients,[95,96] it is not correlated with response in antiretroviral-naïve patients.[97] This finding demonstrates the importance of viral resistance in influencing PK-PD relationships (see later discussion).

Nonnucleoside reverse transcriptase inhibitors. C_{min} is correlated with virologic response to nevirapine.[98] Historically, efavirenz plasma concentrations between 1000 ng/mL and 4000 ng/mL have been targeted to minimize the risk of virologic failure and central nervous system toxicity.[99] However, more recent reports exploring efavirenz combined with modern nucleoside reverse transcriptase inhibitors (NRTIs) suggest a C_{12h} cutoff between 0.47 ng/mL and 0.76 ng/mL as a more sensitive and specific efficacy threshold.[100,101] In a phase IIb study, no relationship was observed between rilpivirine exposure and virologic response rates.[102] All doses in this study produced plasma concentrations above the protein-adjusted EC_{50} and were likely at the plateau of the exposure-response curve. For etravirine, no relationship was observed between AUC or C_{min} and virologic response in treatment-naïve patients taking

400 mg once daily.[103] However, in treatment-experienced patients who are taking 200 mg twice daily, multiple studies describe C_{min} as a significant predictor of virologic response with a proposed target of >300 ng/mL.[92,104] These results suggest that C_{min} measurements may be useful for prediction of nonnucleoside reverse transcriptase inhibitor (NNRTI) responses.

Integrase strand transfer inhibitors. The role of C_{min} as a marker of efficacy of integrase strand transfer inhibitors may be less clear. In a comprehensive PK-PD analysis, dolutegravir C_{min} best predicted plasma viral load reduction on day 11 of monotherapy. The in vivo EC_{50} of 36 ng/mL was also identified as the potential C_{min} target.[105] Plasma PK-PD relationships correlating C_{min} with antiviral activity have also been described for once-daily dosing with elvitegravir and raltegravir.[106,107] However, this relationship is not observed for raltegravir with 400 mg twice-daily dosing[107,108] or with the new 1200-mg once-daily formulation,[109] both of which achieve C_{min} values that are greater than twofold to sixfold higher (543 nM and 113 nM, respectively) than the IC_{50} (31 nM) and the C_{min} of the 800-mg once-daily dose referred to previously (40 nM). Thus this absence is likely attributed to achieving exposures at the plateau of the exposure-response curve.

Entry inhibitors. The CCR5 inhibitor maraviroc also demonstrates a clear PK-PD relationship where AUC is predictive of response.[110]

Nucleoside reverse transcriptase inhibitors. Defining PK-PD relationships for NRTIs is more difficult because these drugs are intracellularly phosphorylated to their active diphosphate and triphosphate metabolites. Multiple rate-limiting phosphorylation steps, cellular membrane efflux transporter activity, and differential phosphorylation rates in activated versus quiescent $CD4^+$ cells[111-113] result in plasma parent drug concentrations that do not consistently correlate with metabolite concentrations.[114] However, a 50% increase in zidovudine triphosphate concentrations and a 33% increase in lamivudine triphosphate concentrations have been positively correlated with the rate of HIV-1 RNA decline after starting therapy.[114–116] For tenofovir, disoproxil fumarate, and emtricitabine, the plasma PK-PD relationship plateaus beyond the treatment doses of 300 mg and 200 mg once daily,[117–119] suggesting saturation of cellular phosphorylation processes. Therefore the active metabolite C_{min} associated with these doses (approximately 84 fmol/million cells and 4000 fmol/million cells, respectively) represent potential therapeutic targets. High plasma tenofovir concentrations have also been associated with increased risk of nephrotoxicity.[120,121] The novel prodrug formulation, tenofovir alafenamide, reduces this risk by maximizing cellular uptake, thereby minimizing plasma tenofovir exposure.[122] This case illustrates the use of a targeted-prodrug approach to alter biodistribution and PK-PD of a compound (tenofovir) to improve efficacy and safety of the compound.

Antiretroviral Therapeutic Drug Monitoring

With established PK-PD relationships, antiretroviral TDM can be considered. Some antiretroviral drugs (particularly PIs and raltegravir) can have significant intraindividual PK variability owing to food effects and other environmental influences (e.g., prescription and nonprescription medications, nutraceuticals) on DMEs and transporters.[123] This makes interpreting single drug concentrations challenging. Yet TDM for efficacy and toxicity can be warranted in certain clinical circumstances where virologic response may be unpredictable or antiretroviral options are limited. Current guidelines recommend TDM for pregnant patients who have risk factors associated with virologic failure (such as not achieving viral suppression during early stages of pregnancy); patients with pathophysiologic conditions that alter drug PK such as gastrointestinal, hepatic, or renal dysfunction; antiretroviral treatment–experienced patients; patients with clinically significant drug or food interactions; patients with concentration-dependent drug toxicities; patients with alternative dosing regimens or antiretroviral combinations; and treatment-adherent patients with lack of virologic response.[84] Based on convenience, trough concentration cutoffs for efficacy (and occasionally toxicity) have been offered for PIs and NNRTIs—mostly assigned by expert consensus[84]; the review by van Luin and colleagues[124] provides a consolidated list of these targets. An inherent limitation to using these concentration targets to guide clinical decision making is the inability to account for additive or synergistic drug interactions within the specific antiretroviral regimen being interrogated. Even so, TDM-guided therapy has been shown to be effective in certain clinical settings.[125,126]

Alternative PK-PD Indices for Antiretroviral Therapy

Viral heterogeneity and drug resistance can impact the PK-PD relationship. Various resistance mutations can make a virus less sensitive to a drug, either completely (e.g., K103N mutation against nevirapine or efavirenz) or in an escalating fashion (e.g., accumulating mutations can confer increasing resistance to PIs). Therefore relating drug concentrations to the susceptibility of an individual patient's viral isolate has been explored. First described by Ellner and Neu,[127] the inhibitory quotient (IQ) integrates drug exposure (defined as total or protein-unbound AUC, C_{max}, or C_{min}) and viral susceptibility (expressed as the in vitro IC_{50}, IC_{90}, IC_{95}, or IC_{99}, with or without the presence of plasma proteins). The IQ is most commonly calculated as the ratio of the drug concentration at the end of the dosing interval (C_{min}) to the in vitro IC_{50}: C_{min}/IC_{50}. Several derivatives (nIQ, vIQ, and gIQ) have also been proposed to account for confounding factors in the PK-PD relationship, such as the presence of multiple mutations and the effect of protein binding.[128] The nIQ (normalized inhibitory quotient) was developed to eliminate protein binding confounding and is the ratio of C_{min} to the fold change in antiretroviral susceptibility (using virtual phenotype) related to a fixed ratio of the population mean antiretroviral C_{min} to the cutoff for resistance; for example, nIQ = (C_{min}/fold change in IC_{50}) ÷ (population C_{min}/fold change resistance cutoff).[129] The vIQ (virtual inhibitory quotient) is defined as the ratio of C_{min} to the IC_{50} of wild-type virus multiplied by the virtual phenotype (a calculated fold decrease in susceptibility mathematically derived from the individual patient viral genotype and matched to a genotype-phenotype database): C_{min}/IC_{50} · virtual phenotype. The gIQ (genotype inhibitory quotient) is calculated as the ratio of C_{min} to the number of clinically important PI mutations. Of all of these measures that incorporate PK and viral susceptibility into a PD target, the gIQ has found the most utility in clinical study.[128,130,131] The IQ and its derivatives, rather than individual PK parameters, may more strongly predict antiviral efficacy for darunavir, lopinavir/ritonavir, and etravirine in highly treatment-experienced patients.[91,95,96,132,104] There are, however, a number of limitations of using the IQ to predict virologic response. There is no current standardization for calculating or selecting the IQ or its derivatives for prediction of virologic response among clinical trials evaluating the IQ. Therefore collating information from the available literature is difficult. Also, there is no consensus on which IQ derivative achieves the best predictive power, and each derivative is accompanied by its own unique set of considerations. Regardless, since 2010 with more potent and easy-to-use antiretroviral therapy available, there has been less practical application of these techniques in clinical care.

Finally, Shen and associates[133] investigated mathematical approaches to characterizing antiretroviral PD and relative potency. The median effect model of dose response evaluates the slope of a dose-response curve using the proportion of affected virus, proportion of unaffected virus, drug concentration, and IC_{50}. In this model, slope is characteristic of the drug class, whereby antiretroviral drug classes with steeper slopes (e.g., PIs) require lower concentration/IC_{50} ratios for virologic suppression. However, this model does not take into account the multifactorial influences of antiretroviral PD durability such as tolerability, drug interaction potential, and genetic barriers to resistance. Additionally, some inconsistencies have been noted in the model. For example, although the model assigns raltegravir a comparatively low slope of 1.1 ± 0.05, clinically, raltegravir demonstrates rapid, potent, and sustained virologic suppression, suggesting that these models need more refinement to explain more of the variability in the model prediction.

Pharmacodynamics for Other Antiviral Drugs

PK-PD relationships have also been established in the treatment of other viral infections including hepatitis C virus (HCV), cytomegalovirus (CMV), and herpes simplex virus (HSV). Since the first direct-acting agents (DAA) were approved for HCV treatment in 2011, 10 more agents with improved potency have joined the market. Virologic suppression of these first DAAs, boceprevir and telaprevir, was highly dependent on C_{min}. This relationship was demonstrated in the phase II

telaprevir trial,[134] in which higher rates of sustained virologic response (SVR) were observed for a lower total daily dose divided three times daily compared with a higher total daily dose divided twice daily. With the improved potency and combination dosing of newer DAAs, PK-PD relationships have been more difficult to define. Specifically, multiple studies have failed to describe a predictable relationship for sofosbuvir-ledipasvir PK estimates and SVR.[135] Likewise, after correcting for covariates, a PK-PD analysis of paritaprevir, ombitasvir, ribavirin, and dasabuvir found that only ombitasvir AUC was predictive of SVR.[136] For paritaprevir, increased AUC was associated with increased probability of experiencing a grade 3 adverse event.[137]

Foscarnet, a polymerase and reverse-transcriptase inhibitor used to treat CMV, exhibits both a PK-PD and PK-toxicity relationship that strongly correlates AUC with the outcomes of increased days to progression of CMV retinitis and increased nephrotoxicity risk.[138] TDM is not well validated for foscarnet but has been successfully employed in patients receiving hemodialysis.[139] In this report, dose adjustments were made to achieve C_{max} concentrations of 500 to 800 μM between the proposed efficacy (CMV IC_{50} = 100–300 μM) and toxicity (>1000 μM) thresholds.

Finally, the interplay between PK and PD was illustrated in the observation that intravaginal administration of 1% tenofovir gel decreased risk for HSV type 2 (HSV-2) in the CAPRISA004 trial,[140] which aimed to evaluate the gel for HIV prevention. This finding was unexpected because oral dosing in women was known to achieve genital tract tenofovir concentrations of approximately 70 ng/mL (well below the estimated HSV-2 EC_{50} of 14,000–19,000 ng/mL).[141,142] However, topical administration resulted in genital tract concentrations that were approximately 3 logs higher than oral dosing. A subsequent PK-PD analysis and clinical trial (CAPRISA004) demonstrated that tenofovir 1% gel protected against HSV-2 acquisition with decreased risk among women achieving genital tract concentrations ≥10,000 ng/mL.[143] These findings illustrate the importance of a comprehensive understanding of the PK-PD relationship of antiretroviral and antiviral agents in the development of new drugs and new clinical applications for old drugs.

CONCLUSIONS

Optimal dose selection of an antiinfective agent for an individual patient is an indispensable goal of clinical practice. The study of the interrelationship between drug exposure and response through PK-PD analyses is now an established component of antiinfective drug development to meet this goal. This domain of pharmacology has developed to follow a pathway that integrates information from in vitro, in vivo, clinical, and in silico experiments to define a dosing regimen that increases the probability of effect and reduces the probability of toxicity in a population. However, various nonpharmacologic factors can influence efficacy and safety-related outcomes in individuals. These unmeasured or immeasurable factors can confound our assessment of the "true" exposure-response relationship. Clinical use of an agent in populations underrepresented in early studies leads to an identification of pharmacologic and nonpharmacologic factors that influence outcome. Thus our understanding of the specific antiinfective exposure-response relationship evolves with the clinical use of an agent. Continued innovations in genomic, assay, and computer software capabilities will foster individualized antiinfective dose selection. Importantly, discovery of the complex interactions between the metagenome and antiinfective agents may help to explain some of the interindividual variability in PK-PD and perhaps help direct antiinfective therapy more precisely.

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20 Penicillins and β-Lactamase Inhibitors

Yohei Doi

SHORT VIEW SUMMARY

PENICILLIN G

Usual Adult Dose: 12 to 24 Million Units/Day Intravenously (IV) in Equally Divided Doses Every 4 to 6 Hours
- Renal and hepatic failure: decrease dose in renal failure
- Cerebrospinal fluid (CSF) penetration: poor
- Adverse effects: hypersensitivity reaction, hyperkalemia (potassium salt), hypokalemia (sodium salt)

OXACILLIN

Usual Adult Dose: 1 to 2 g Every 4 Hours IV
- Renal and hepatic failure: no adjustment
- CSF penetration: poor
- Adverse effects: hypersensitivity reaction, interstitial nephritis

NAFCILLIN

Usual Adult Dose: 1 to 2 g Every 4 Hours IV
- Renal and hepatic failure: no adjustment
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, interstitial nephritis, hypokalemia

AMPICILLIN

Usual Adult Dose: 2 g Every 4 to 6 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction

AMPICILLIN-SULBACTAM

Usual Adult Dose: 1.5 to 3 g Every 6 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, diarrhea

AMOXICILLIN

Usual Adult Dose: 500 mg Every 8 Hours Orally or 1 g Every 12 Hours Orally
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction

AMOXICILLIN-CLAVULANATE

Usual Adult Dose: 500 mg Every 8 Hours Orally or 875 mg Every 12 Hours Orally
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, diarrhea

PIPERACILLIN

Usual Adult Dose: 3 to 4 g Every 4 to 6 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction

PIPERACILLIN-TAZOBACTAM

Usual Adult Dose: 2.25 to 4.5 g Every 6 to 8 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, diarrhea

CEFTOLOZANE-TAZOBACTAM

Usual Adult Dose: 1.5 to 3 g Every 8 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: not available
- Adverse effects: hypersensitivity reaction

CEFTAZIDIME-AVIBACTAM

Usual Adult Dose: 2.5 g Every 8 Hours IV Infused Over 2 Hours
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: not available
- Adverse effects: hypersensitivity reaction

MEROPENEM-VABORBACTAM

Usual Adult Dose: 4 g Every 8 Hours IV Infused Over 3 Hours
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: not available
- Adverse effects: hypersensitivity reaction

PENICILLINS

Penicillin was discovered by Alexander Fleming from *Penicillium notatum* (now *Penicillium chrysogenum*) in 1928.[1] The work of Florey, Chain, and associates isolated penicillin and made possible the commercial production of penicillin G.[2] By the middle of the 1940s, penicillin G was available for general use in the United States, thus initiating the modern antibiotic era.

Chemistry

The basic structure of penicillins is a nucleus consisting of a thiazolidine ring, the β-lactam ring, and a side chain (Fig. 20.1). The core ring structures, particularly the β-lactam ring, are essential for antibacterial activity. The side chain determines in large part the antibacterial spectrum and pharmacologic properties of each particular penicillin.

Emergence of β-lactamase–producing organisms, particularly *Staphylococcus aureus,* prompted development of compounds resistant to hydrolysis by β-lactamases and the search for agents more active than penicillin G against gram-negative species. The isolation of the penicillin nucleus, 6-amino-penicillanic acid, from a precursor-depleted fermentation of *P. chrysogenum* made possible the production and testing of numerous semisynthetic penicillins, including methicillin, active against β-lactamase–producing *S. aureus;* ampicillin, active against selected gram-negative bacilli; and carbenicillin, active against *Pseudomonas aeruginosa.* Since then, numerous agents with different pharmacologic and antimicrobial properties have been developed.

Mechanism of Action

The antibacterial activity of penicillin, such as that for all β-lactam antibiotics, is triggered by its inhibition of bacterial cell wall synthesis. Although the precise mechanism by which penicillin kills bacterial cells is not known, stimulation of the production of deleterious hydroxyl radicals that irreversibly damage the cell appears to be a final common pathway of bactericidal, but not bacteriostatic, antibiotics, including the penicillins.[3]

The cell wall of both gram-positive and gram-negative bacteria is composed of peptidoglycan, which allows cells to contain and resist

high osmotic pressure.[4] The cell wall of gram-positive bacteria is a substantial layer 50 to 100 molecules in thickness, whereas in gram-negative bacteria it is only one or two molecules thick. An outer membrane lipopolysaccharide layer, not found in gram-positive bacteria, is present in gram-negative species. The basic subunit of the peptidoglycan component is a disaccharide monomer of *N*-acetylglucosamine (NAG, or GlcNAc) and *N*-acetylmuramic acid (NAM, or MurNAc) pentapeptide (Fig. 20.2). The cytoplasmic enzymatic reactions that generate cell wall precursors, the disaccharide monomer subunit translocation across the cytoplasmic membrane, and the transglycosylase reaction that links the subunit to the peptidoglycan polymer are not sensitive to penicillin.[5] Penicillin inhibits enzymes that catalyze the final step in bacterial cell wall assembly, which is the formation of the cross-links that bridge peptidoglycan, giving it its structural integrity.

Peptidoglycan is composed of long β-linked polysaccharide chains of NAG and NAM pentapeptides whose synthesis is catalyzed by a series of Mur enzymes. Each pentapeptide consists of amino-acid residues alternating between L- and D-stereoisomers and terminating in D-alanyl-D-alanine (see Fig. 20.2). After translocation from the cytoplasm to the exterior of the membrane, a stem peptide of variable length and composition is attached to the third amino acid of this pentapeptide. Pentapeptides are then joined with stem peptides to form a cross-link between polysaccharide chains. This reaction is catalyzed by a transpeptidase that forms an amide bond between the terminal free amine group of a stem peptide and a penultimate D-alanine of a pentapeptide, displacing the terminal D-alanine in the process. This transpeptidation reaction is sensitive to inhibition by penicillin. There are distinct transpeptidases that provide for anchoring of new peptidoglycan to old, that cross link special structures, and that direct formation of the cell wall septum. Although there are penicillin-sensitive reactions, such as those catalyzed by carboxypeptidases, these reactions do not seem to be essential.

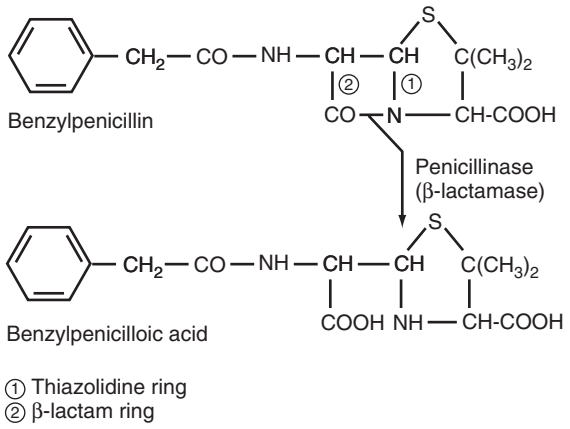

FIG. 20.1 Structure of penicillin and site of β-lactamase attack.

The penicillin-sensitive reactions are catalyzed by a family of closely related proteins, called penicillin-binding proteins (PBPs).[6] Bacteria produce four types of PBPs, which structurally resemble and likely are derived from serine proteases. High-molecular-weight PBPs (i.e., >50 kilodaltons [kDa]) and low-molecular-weight PBPs catalyze transpeptidation and carboxypeptidation reactions of cell wall assembly, respectively. Class A high-molecular-weight PBPs are bifunctional enzymes that have transpeptidase and transglycosylase domains that cooperatively interact during cell wall synthesis.[7] Signal-transducing membrane proteins bind β-lactams and generate a transmembrane signal leading to induction of certain β-lactamases and PBP2a, which mediates staphylococcal methicillin resistance.[8,9] β-Lactamases are PBPs that catalyze hydrolysis of the β-lactam ring. Except for β-lactamases, which may be either secreted or membrane associated, PBPs are membrane bound. PBPs are inhibited by β-lactam antibiotics through covalent binding of the active-site serine residue. Because the essential functions for survival of the cell generally reside with high-molecular-weight PBPs, it is binding to and inhibition of these PBPs that mediates the antibacterial activity of β-lactam antibiotics.

PBPs vary both in amounts present and in the physiologic functions they serve during cell wall assembly. They differ in their affinities for binding β-lactam antibiotics, which explains at least in part why β-lactam antibiotics differ in their antibacterial properties and spectrum of activity. Spratt's studies in *Escherichia coli* were the first to elucidate the different functions of PBPs.[10] Inhibition of PBP1b, which has transpeptidase activity, or a substitute enzyme 1a, results in cell lysis.[11] PBP1 is speculated to be important for cell elongation. Inhibition of PBP2 results in formation of round cells that eventually lyse, indicating that it has a role in cell elongation and in determining the rod size and shape in *E. coli*.[12] Inhibition of PBP3, also a transpeptidase, produces long, filamentous cells, indicating that it is important for the ordered process of cross-wall formation and cell division.[13] Low-molecular-weight PBPs are carboxypeptidases, which play a role in the maintenance of cell shape and septum formation.[7,14]

β-Lactam antibiotics produce their lethal effect on bacteria by inactivation of multiple PBPs simultaneously, but inhibition of cell wall synthesis by itself is not necessarily lethal. For example, cells that are not growing and cells that are osmotically protected survive the presence of penicillin. Unopposed action of autolysins occurring when PBPs are inhibited by β-lactam antibiotics may contribute to the antibacterial effect in some organisms. Cell lysis, although it certainly is lethal and often accompanies cell wall inhibition, is also not required for cell death. The lethal effect in both gram-positive and gram-negative organisms appears to be cell cycle dependent, with inhibition of PBPs leading to disruption of a crucial event probably at the time of cell division. This disturbed morphogenesis is hypothesized to initiate cell death.[3,15]

Resistance Mechanisms

Four mechanisms account for clinically significant bacterial resistance to penicillins and for other β-lactam antibiotics as well: (1) destruction of antibiotic by β-lactamase, (2) failure of antibiotic to penetrate the

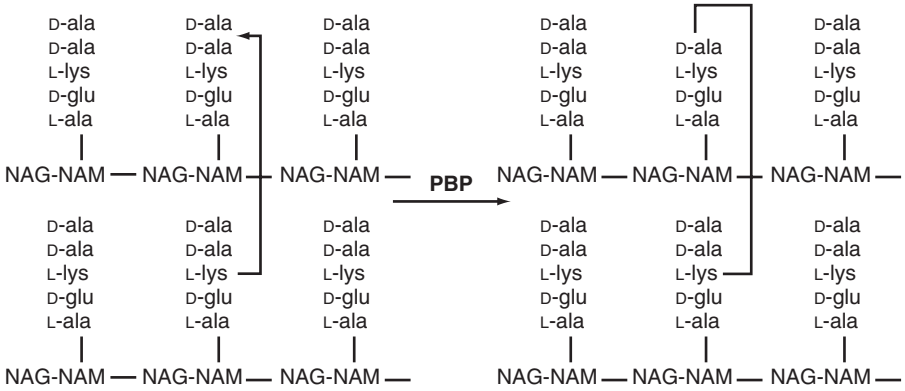

FIG. 20.2 Penicillin-binding protein *(PBP)* transpeptidation reaction that cross links bacterial cell wall. The structure for *Staphylococcus aureus* is shown. *NAG, N*-acetylglucosamine; *NAM, N*-acetylmuramic acid.

outer membrane of gram-negative bacteria to reach PBP targets, (3) efflux of drug across the outer membrane of gram-negative bacteria, and (4) low-affinity binding of antibiotic to target PBPs. β-Lactamase destruction of antibiotic is the most common mechanism of resistance, which in gram-negative bacteria, in general, and in *Pseudomonas aeruginosa,* in particular, is often accompanied by reduced permeability and augmented efflux.[16] β-Lactamases covalently react with the β-lactam ring, rapidly hydrolyze it, and destroy activity of the drug.

β-Lactamases can be categorized into one of four classes, Ambler classes A through D, based on amino-acid sequence similarity and molecular structure (Table 20.1).[17] Classes A, C, and D β-lactamases contain penicillin-binding motifs and are PBPs. They differ from other PBPs in that they typically are smaller, around 35 kDa versus greater than 50 kDa, and they are not cell wall–synthetic enzymes. They react with penicillin through the same series of reactions as other PBPs. There is initial, reversible binding and formation of the Michaelis-Menten complex, followed by acylation of the active-site serine and then followed by hydrolysis of the acyl intermediate in a deacylation reaction regenerating the active enzyme. Biochemically, the main distinction between cell wall–synthetic PBPs and β-lactamases is the rate of deacylation: The deacylation rate of penicillin-bound cell wall synthetic PBP is relatively slow, amounting to irreversible inhibition of its activity, whereas the deacylation rate of β-lactamase usually is orders of magnitude faster, rapidly hydrolyzing and turning over β-lactam molecules. Class B β-lactamases, although they also hydrolyze the β-lactam ring, structurally are unrelated to PBPs. They are zinc-dependent enzymes that use a different series of reactions to open the β-lactam ring and are inhibited by metal-chelating agents.

β-Lactamases that are most often clinically significant are class A or class C. Class A enzymes can be produced chromosomally or via a plasmid and typically hydrolyze penicillins preferentially, but some also have cephalosporinase or carbapenemase activity.[18] They are inhibited by β-lactamase inhibitors, such as clavulanic acid. Point mutations can render the enzyme resistant to inhibitors or extend the spectrum of activity to include third-generation cephalosporins and aztreonam, so-called extended-spectrum β-lactamases (ESBLs). Class C β-lactamases preferentially hydrolyze cephalosporins and are not inhibited by clavulanic acid. These β-lactamases usually are encoded on the chromosome and are inducible, although they may also be plasmid encoded and produced constitutively. Class B enzymes are intrinsically produced by some lactose-nonfermenting gram-negative species, such as *Stenotrophomonas maltophilia*, but it is the plasmid-mediated class B β-lactamases that have the broadest spectrum and are able to hydrolyze all β-lactams except aztreonam.

The outer membrane of gram-negative organisms is an important barrier to drug penetration and an important component of resistance.[19] β-Lactamases of gram-negative bacteria are located in the periplasmic space between the inner cytoplasmic membrane and outer lipopolysaccharide membrane and have been selected to be strategically concentrated to protect target PBPs from exposure to active β-lactam antibiotics. Small polar molecules (e.g., glucose, essential nutrients, β-lactam antibiotics) cross this barrier through protein channels called porins. Porins constrain entry of molecules into the cell according to size, structure, and charge. β-Lactam antibiotics that satisfy the entry requirements can traverse porin channels to the periplasmic space and bind to target PBPs. Absence or deletion of a critical porin, usually in the presence of a β-lactamase activity, can result in resistance.[20]

The third mechanism of resistance is efflux; the drug that enters the periplasmic space is pumped back across the outer membrane.[20,21] Efflux may operate independently of other mechanisms but more often exclusion of antibiotic by porins, destruction of antibiotic by β-lactamases, or both contribute to resistance by limiting periplasmic antibiotic concentration. Species differences in porins, pumps, β-lactamases, and target PBPs determine whether the organism is susceptible or resistant to a particular β-lactam.

The fourth general type of resistance mechanism involves production of a PBP that has low affinity for binding of β-lactam antibiotic.[22] The transpeptidase and transglycosylase activities of PBPs are essential in cell wall synthesis and assembly, and low affinity of PBPs toward β-lactam antibiotics allows the bacteria to circumvent their actions. This may be the result of mutations in PBP genes that lower binding affinity, as in penicillin-resistant pneumococci or *Neisseria* spp., or may be due to the presence of an extra, low-affinity PBP, such as PBP5 produced by *Enterococcus faecium* or PBP2a produced by methicillin-resistant staphylococci. Solution of crystal structures of low-affinity PBPs has identified critical molecular features and interactions that are responsible for resistance.[23,24] In the case of PBP2a, low-affinity binding of β-lactam antibiotic is mediated by structural changes that result in energetically unfavorable interactions between antibiotic and protein so that the active-site serine is inactivated not at all or too slowly to effectively block cell wall synthesis and bacterial growth.

TABLE 20.1 Classification of β-Lactamases

AMBLER MOLECULAR CLASS	MAJOR SUBTYPES[a]	PREFERRED SUBSTRATES	INHIBITOR[b]	MAIN GENETIC LOCALIZATION	REPRESENTATIVE ENZYME(S)
A	Gram-positive β-lactamase 2a	Penicillins	Clavulanic acid	Chromosome or plasmid	PC1
	Gram-negative β-lactamase 2b	Penicillins, early cephalosporins	Clavulanic acid	Plasmid or chromosomal	TEM-1, SHV-1
	Extended-spectrum β-lactamase 2be	Penicillins, extended-spectrum cephalosporins, aztreonam	Clavulanic acid	Plasmid	TEM-24, SHV-12, CTX-M-15
	Inhibitor-resistant TEM β-lactamase 2br	Penicillins	Clavulanic acid[c]	Plasmid	TEM-30, SHV-10
	Carbenicillin-hydrolyzing β-lactamase 2c	Carbenicillin	Clavulanic acid[c]	Plasmid	PSE-1, CARB-3
	Cephalosporin-hydrolyzing β-lactamase 2e	Extended-spectrum cephalosporins	Clavulanic acid	Chromosome	CepA
	Carbapenem-hydrolyzing β-lactamase 2f	Carbapenems	Avibactam[d]	Chromosome or plasmid	KPC-2, SME-1
B	Metallo-β-lactamase 3a	All β-lactams except monobactam	EDTA, divalent cation chelators	Chromosome or plasmid	IMP-1, VIM-2, NDM-1
C	AmpC-type β-lactamase 1	Cephalosporins	Cloxacillin, avibactam	Chromosome or plasmid	AmpC, CMY-2
D	Oxacillin-hydrolyzing β-lactamase 2d	Oxacillin	Clavulanic acid[c]	Chromosome or plasmid	OXA-1, OXA-10
	Extended-spectrum β-lactamase 2de	Extended-spectrum cephalosporins	Clavulanic acid[c]	Plasmid	OXA-11, OXA-15
	Carbapenem-hydrolyzing β-lactamase 2df	Carbapenems	None	Plasmid	OXA-23, OXA-40, OXA-48

[a]The updated Bush-Jacoby group[3] is indicated.
[b]Tazobactam and sulbactam have activities similar to those of clavulanic acid.
[c]Indicates relatively weaker inhibition.
[d]Avibactam generally inhibits β-lactamases inhibited by clavulanic acid.
EDTA, Ethylenediaminetetraacetic acid.

Classification

Penicillins can be conveniently divided into five classes on the basis of antibacterial activity with considerable overlap among the classes: (1) natural penicillins—penicillin G and penicillin V; (2) penicillinase-resistant penicillins—methicillin, nafcillin, and isoxazolyl penicillins; (3) aminopenicillins—ampicillin and amoxicillin; (4) carboxypenicillins—carbenicillin and ticarcillin; and (5) acyl ureidopenicillins—azlocillin, mezlocillin, and piperacillin. The carboxypenicillins and ureidopenicillins are also referred to as antipseudomonal penicillins. Differences within a class are principally pharmacologic, although one compound in a class may be more active than another.

The susceptibility profiles of various species of microorganisms are shown in Tables 20.2 to 20.4. Natural penicillins are most active against non–β-lactamase–producing gram-positive bacteria, anaerobes, and selected gram-negative cocci, such as *Neisseria* spp. Gram-positive bacteria inhibited by penicillin G tend to be more susceptible to penicillin G than to semisynthetic penicillins.[25] Penicillin V (used orally) can be substituted for penicillin G, except against gram-negative species, because it is less active than penicillin G against *Neisseria* and *Haemophilus* spp. Semisynthetic penicillinase-resistant penicillins are the drugs of choice only for penicillin-resistant *S. aureus* and *Staphylococcus epidermidis*. They are also active against streptococci but not against enterococci. Aminopenicillins possess the same spectrum as penicillin G, plus they are more active against gram-negative cocci and Enterobacteriaceae that do not produce β-lactamase.[26] Carboxypenicillins and ureidopenicillins have activity against some ampicillin-resistant aerobic gram-negative bacteria, such as *P. aeruginosa.* Carboxypenicillins are less active than the ureidopenicillins against streptococci and *Haemophilus* spp. Anaerobic gram-positive species are susceptible to the penicillins. Gram-negative anaerobic bacteria are susceptible to most penicillins, with the exception of isolates of *Bacteroides fragilis*, other *Bacteroides* spp., and some *Prevotella* spp., which produce chromosomal class A β-lactamase, and are inhibited by high levels of penicillin G or the semisynthetic antipseudomonal agent piperacillin.[27]

Pharmacologic Properties

Penicillins differ markedly in their oral absorption (Table 20.5). Acid-labile compounds, penicillin G, methicillin, and antipseudomonal penicillins are poorly absorbed. Acid-stable compounds can have major differences in oral absorption. Semisynthetic penicillins, except nafcillin, are well absorbed. Ampicillin is only partially absorbed (30%–55%), and food decreases absorption, whereas amoxicillin has higher oral bioavailability (74%–92%) that is not affected by food.[28,29]

Orally absorbed penicillins yield peak concentrations 1 to 2 hours after ingestion. Peak serum levels are delayed after ingestion with food, and peak levels also are lower, except for amoxicillin. Penicillin G procaine and benzathine penicillin G, repository forms of penicillin G, are absorbed more slowly from intramuscular (IM) sites than are the crystalline salts. Lidocaine can also be used as a diluent for IM injection of antipseudomonal penicillins.

Penicillins are bound to serum proteins in varying degrees, ranging from 17% for the aminopenicillins to 97% for dicloxacillin (see Table

TABLE 20.2 Usual Minimal Inhibitory Concentrations for Penicillins Against Cocci (μg/mL)

ORGANISM	PENICILLIN G	PENICILLIN V	AMPICILLIN, AMOXICILLIN	OXACILLIN[a]	PIPERACILLIN
Streptococcus pneumoniae[b]	0.03	0.03	0.03	0.13	0.05
Streptococcus pyogenes	0.015	0.015	0.03	0.13	0.2
Streptococcus agalactiae	0.06	0.03	0.12	0.13	0.2
Viridans group streptococci	0.06	0.12	0.12	0.5	0.25
Enterococcus faecalis	2	4	1	16	4
Enterococcus faecium	>16	>16	8	>16	>16
Peptostreptococcus spp.	0.13	0.13	0.13	2	0.5
Staphylococcus aureus[c]	0.03	0.03	0.12	0.13	0.8
Staphylococcus epidermidis[c]	0.015	0.03	0.03	0.13	0.8
Neisseria gonorrhoeae[d]	0.015	0.03	0.2	0.4	0.03
Neisseria meningitidis	0.03	0.12	0.12	0.1	0.01

[a]Oxacillin is representative of all antistaphylococcal penicillins; not active against methicillin-resistant strains.
[b]Penicillin-susceptible strains only; 10% or more of strains are penicillin-resistant in the United States (see www.cdc.gov/abcs).
[c]Penicillin and methicillin-susceptible strains only; most strains are resistant.
[d]Penicillin-susceptible strains only; 10% or more of strains are penicillin-resistant in the United States (see www.cdc.gov/std/).
Data from references 132–151.

TABLE 20.3 Activity of Penicillins Against Selected Bacilli and Anaerobic Organisms

	MEAN MINIMAL INHIBITORY CONCENTRATION (μg/mL)			
ORGANISM	Penicillin G	Ampicillin, Amoxicillin	Oxacillin[a]	Piperacillin
Clostridium perfringens	0.5	0.1	0.25	0.25
Corynebacterium diphtheriae	0.1	0.2	3.1	
Listeria monocytogenes	0.25	0.5	4	2
Haemophilus influenzae[b]	1	0.25	32	0.1
Prevotella melaninogenica	0.1	0.1	0.5	0.25
Fusobacterium nucleatum	0.1	0.1	0.13	0.06
Bacteroides fragilis	32	16	64	20

[a]Oxacillin is representative of all antistaphylococcal penicillins.
[b]β-Lactamase–producing strains occur and are resistant to the penicillins.
Data from references 133, 134, 141, 143, and 152–160.

TABLE 20.4 Activity of Penicillins Against Enterobacteriaceae, *Acinetobacter* spp., and *Pseudomonas aeruginosa*

	MEAN MINIMAL INHIBITORY CONCENTRATION (μg/mL)			
ORGANISM	**Penicillin G**	**Ampicillin, Amoxicillin**	**Oxacillin[b]**	**Piperacillin**
Escherichia coli	200	>200	200	32
Klebsiella spp.	50	50	>200	16
Enterobacter spp.	>200	>200	—	4
Citrobacter freundii	—	100	—	6
Serratia marcescens	>200	>200	—	4
Morganella morganii	>200	>200	>200	2
Proteus mirabilis	3.1	6.3	>200	1
Proteus vulgaris	400	>200	>200	4
Providencia spp.	50	>200	>200	4
Salmonella enterica	12.5	6.3	>200	4
Shigella spp.	25	6.3	>200	2
Acinetobacter spp.	100	50	>200	25
Pseudomonas aeruginosa	>200	>200	>200	32

[a]Some of the data are historic and may not reflect current activity.
[b]Oxacillin is representative of all antistaphylococcal penicillins.
Data from references 133, 138, and 161–172.

TABLE 20.5 Pharmacokinetic Properties of Penicillins

				PEAK SERUM LEVELS				SERUM $T_{1/2}$ (h)	
	ORAL ABSORPTION (%)	**FOOD DECREASES ABSORPTION**	**PROTEIN BINDING (%)**	**Total Drug (μg/mL)**	**Free Drug (μg/mL)**	**PERCENTAGE OF DOSE METABOLIZED (%)**	**PERCENTAGE EXCRETED IN URINE UNCHANGED (%)**	**Normal (CrCl > 90 mL/min)**	**With Renal Failure (CrCl < 10 mL/min)**
Penicillin G	15–30	Yes	60	1.5–2.7[a]	0.6–1.0	19	15–30	0.7	6–10
Penicillin V	60	No	80	3–5[a]	0.8	56	26	0.52–0.78	
Oxacillin	33	Yes	90	5.6[a]	0.39	49	17	0.4–0.7	0.5–1
Cloxacillin	49	Yes	93	7–14[a]	0.6	22	38	0.5	0.8
Dicloxacillin	37	Yes	96	15–18[a]	0.6	10	33	0.8	1–1.5
Flucloxacillin	44	Yes	96	11–20[a]	0.6	10	41	0.8	2.9
Nafcillin	Variable, low	Yes	90	4.3[b]			9 (PO) –31 (IM)	0.55–1	1.2
Ampicillin	33–54	Yes	20	2–6[a]	3.2	21	26–43	1–1.3	8–20
Amoxicillin	74–80	No	20	7–8[a]	6	28	50–64	1–1.3	16
Piperacillin	Nil		48	58.3[c]			67.5	0.867	3.33

[a]Peak level after 500-mg dose taken fasting.
[b]Peak level after 500-mg intramuscular injection.
[c]Peak level after 2-g intravenous infusion.
CrCl, Creatinine clearance; *IM,* intramuscular; *PO,* orally.
Data from references 173–181.

20.5). The major protein to which they bind is albumin.[25] Only unbound drug exerts antibacterial activity. However, protein binding is a reversible process, and it is possible for bound penicillin to be released and then to kill bacteria in tissue or in the bloodstream. The major mechanism by which most of these drugs are removed from the body is by excretion as intact molecules via the kidney. Penicillins are metabolized to a minor degree.[30] Even minor differences in metabolism can result in clinically significant differences in half-life in the presence of renal failure. Biliary excretion of penicillins does occur, but it probably is important only for nafcillin and the antipseudomonal penicillins.

Penicillins are cleared by glomerular filtration, and they also are rapidly secreted into urine by renal tubular cells, and hence they have a short half-life, ranging from less than 30 minutes for penicillin to 70 minutes for carbenicillin. The ability of the renal tubular cells to secrete penicillin varies with the agents, but up to 4 g of penicillin G per hour can be excreted. Secretion can be blocked by probenecid, an inhibitor of organic acid secretion by tubular cells, which prolongs the serum half-life of all the penicillins.[31] Probenecid may also increase free penicillin concentration by competing for binding sites on albumin. Renal excretion of all penicillins in neonates is markedly less than in older children because tubular function is not fully developed at birth. Therefore dosing of penicillins must be modified when these drugs are given to neonates or low-birth-weight infants.

Reduction in renal function must be taken into account in the administration of certain penicillins. For creatinine clearance (CrCl) greater than 10 to 20 mL/min, only minor adjustments in the dosage

TABLE 20.6 Antibiotic Dosage Change in Renal Disease and After Dialysis

	DOSE CHANGE IN RENAL FAILURE		
AGENT	**Creatinine Clearance 30–50 mL/min**	**Creatinine Clearance 10–29 mL/min**	**DOSAGE FOR HEMODIALYSIS**
Penicillin G	NC	1–2 × 10^6 units/6 h	0.5–2 × 10^6 units/6 h; dose after HD
Penicillin V	NC	250 mg/6 h	250 mg/6 h; dose after HD
Ampicillin	NC	0.5–2 g/12 h	1 g/12–24 h; dose after HD
Amoxicillin	NC	500 mg/12 h	500 mg/12-24 h; dose after HD
Cloxacillin	NC	NC	NC
Dicloxacillin	NC	NC	NC
Flucloxacillin	NC	NC	NC
Nafcillin	NC	NC	NC
Oxacillin	NC	NC	NC
Piperacillin	NC	3 g/8–12 h	3 g/12 h; 1 g after HD

HD, Hemodialysis; *NC,* no change in dosing.

of other penicillins are required. In the presence of anuria, reduction is necessary in the total daily dose of the natural penicillins, of many of the penicillinase-resistant penicillins, and of the aminopenicillins (Table 20.6).

Peritoneal dialysis removes variable amounts of the penicillins. After hemodialysis, which yields a CrCl of 10 to 15 mL/min, and during continuous venovenous hemofiltration, with a 25 mL/min CrCl, the dose of penicillin G, ampicillin, amoxicillin, and piperacillin must be adjusted, but the dose of nafcillin or the isoxazolyl penicillins need not be adjusted.

Penicillins are well distributed to most tissues, including lung, liver, kidney, muscle, and placenta.[32] The levels of penicillins in bones are variable.[33] The levels of penicillins in abscesses and middle ear, pleural, peritoneal, and synovial fluids are sufficient to inhibit most susceptible bacteria.[32,34] Most penicillins are relatively insoluble in lipid and penetrate cells poorly. Distribution of all the penicillins to eye, brain, cerebrospinal fluid (CSF), or prostate is insufficient in the absence of inflammation.[32] Inflammation alters normal barriers, permitting entry of penicillins, which achieve concentrations in CSF that range between 5% to 10% for penicillin G and 13% to 14% with ampicillin.[35] Penicillins with low rates of protein binding reach levels in fetal serum equivalent to levels in maternal serum 30 to 60 minutes after injection. In contrast, the highly protein-bound semisynthetic penicillins achieve low concentrations in both amniotic fluid and fetal serum.[36]

Urinary concentrations of penicillins are high except for nafcillin, even in the presence of moderately reduced renal function, but in people with rates of CrCl less than 10 mL/min the urinary levels may not exceed those in the blood. Cortical and medullary concentrations of penicillins exceed serum levels.[37]

Most penicillins are actively secreted unchanged into the bile, yielding biliary concentrations well in excess of those in serum. The biliary levels of penicillin G and ampicillin are at least 10 times those in the serum, and the levels of nafcillin in bile are as high as 100 times the simultaneous serum level.[38] In the presence of common duct obstruction the levels of penicillins in bile are markedly reduced.[39] Because the biliary transport system is a saturable one, with drug doses producing very high serum levels, the biliary levels are not significantly increased over those measured at lower serum levels. Reabsorption and reexcretion is minimal.[40]

Untoward Reactions

The most important adverse effects of the penicillins are hypersensitivity reactions, which range in severity from rash to anaphylaxis[41] (Table 20.7) (see Chapter 23). Penicillins can act as haptens to combine with proteins contaminating the solution or with human proteins after the penicillin has been administered to humans. Penicilloyl and penicillanic acid derivatives are the major determinants of penicillin allergy. The penicilloyl determinant, which is produced through opening of the β-lactam ring, thereby allowing amide linkage to body proteins (Fig. 20.3), is the most important antigenic component. Penicillanic acid and its derivatives are produced when reconstituted penicillins break down in solution from acidity or temperature elevation. Minor determinants of allergy are benzylpenicillin itself and sodium benzyl penicilloate, which can act either as sensitizing agents or on their own elicit an allergic reaction. Both major and minor determinants may be involved in anaphylactic reactions, as well as in urticarial reactions. These reactions are mediated by immunoglobulin E (IgE) antibody. A person who has been sensitized by the hapten-carrier complex can have a reaction to penicillin alone or to penicillin that has formed dimers or polymers in solution. Although a history of penicillin allergy is quite common, less than 2% will have an allergic reaction if challenged, but the risk is higher for those with history of an immediate hypersensitivity reaction or a positive skin test.[42]

Serum sickness may occur with penicillins, but it is exceedingly uncommon (<0.01%).[43] The illness is characterized by fever, urticaria, joint pains, and angioneurotic edema. Exfoliative dermatitis and Stevens-Johnson syndrome are rare forms of allergic reactions to penicillins. The morbilliform eruptions that develop after penicillin therapy probably are due to IgM antibodies to the benzyl penicilloyl hapten and to the minor determinants. In many patients these rashes disappear, even if the penicillin is continued, owing to the production of IgG-blocking antibody. There is a risk, however, that the rash could progress to generalized desquamation. If a hypersensitivity reaction does occur, epinephrine given IM or intravenously (IV) usually aborts the reaction. Antihistamines and corticosteroids have not been shown to be of benefit.

Another allergic reaction to penicillins is that of allergic vasculitis with the development of cutaneous and visceral lesions similar to that found with polyarteritis nodosa.[44] This reaction is also extremely uncommon (<0.01%).[45]

Hematologic toxicity is rare, although neutropenia has been encountered with the use of all types of penicillins, particularly when large doses are used.[46] The mechanisms of the neutropenia are unknown, and white blood cell counts return to normal rapidly if the offending agent is discontinued. Sometimes a lower dose of drug can be used without production of neutropenia. Coombs-positive hemolytic anemia occurs rarely.[47] All penicillins at high concentrations bind to the adenosine diphosphate receptor site in platelets, preventing normal platelet aggregation.[48] Clinically significant bleeding may occur more commonly with penicillins than cephalosporins.[49]

Renal toxicity from penicillins is variable, ranging from allergic angiitis to interstitial nephritis.[50] Interstitial nephritis is seen with all penicillins and was particularly common with methicillin, which is no longer available for use. The clinical syndrome is one of fever, macular rash, eosinophilia, proteinuria, eosinophiluria, and hematuria. Initially the reaction is one of nonoliguric renal failure with a decrease in CrCl and a rise in serum urea nitrogen and serum creatinine concentrations. This reaction can progress to anuria and renal failure. Biopsy specimens

TABLE 20.7 Adverse Reactions to Penicillins

TYPE OF REACTION	OCCURS MOST FREQUENTLY WITH	FREQUENCY (%)
Allergic		
IgE antibody Anaphylaxis Early urticarial (<72 h)	Penicillin G	0.015–0.04
Cytotoxic antibody Hemolytic anemia	Penicillin G	Rare
Antigen-antibody complex disease	Penicillin G	Rare
Serum sickness		
Delayed hypersensitivity contact dermatitis	Ampicillin, amoxicillin	2–5
Idiopathic	Ampicillin, amoxicillin	2–5
Rash		
Fever		
Late-onset urticaria		
Gastrointestinal		
Diarrhea	Ampicillin, amoxicillin	3–11
Clostridioides difficile (formerly *Clostridium difficile*)–associated colitis	Ampicillin	Rare
Hematologic		
Hemolytic anemia	Penicillin G	Rare
Neutropenia	Penicillin G, nafcillin, oxacillin	10–17[a]
Platelet dysfunction	Piperacillin	43–73
Hepatic		
Elevated serum aspartate aminotransferase level	Oxacillin, flucloxacillin	0.01–22
Electrolyte Disturbance		
Hypokalemia	Nafcillin, oxacillin	Rare
Acute hyperkalemia	Penicillin G	Rare
Neurologic		
Seizures	Penicillin G	Rare
Bizarre sensations (Hoigné syndrome)	Procaine penicillin	Rare
Renal		
Interstitial nephritis	Any penicillin	Variable

[a]With prolonged therapy.
IgE, Immunoglobulin E.
Data from references ***49***, ***56***, *182–190.*

of the kidney show an interstitial infiltrate of mononuclear and eosinophilic cells with tubular damage but no glomerular lesions. Discontinuation of the penicillin results in the return of renal function to normal in most cases.

Administration of massive doses of any penicillin may result in hypokalemia, owing to the large dose of nonreabsorbable anion presented to the distal renal tubules, which alters hydrogen ion excretion and secondarily results in potassium loss. Among the penicillinase-resistant penicillins, nafcillin may carry higher risk of hypokalemia than oxacillin.[51]

Central nervous system (CNS) toxicity in the form of myoclonic seizures can follow the administration of massive doses of penicillin G (40–100 million units/day).[52] If there is reduced renal function, the drugs accumulate, and this form of toxicity becomes more likely, even with lower doses.[53] Direct instillation of small doses of oxacillin or nafcillin into the ventricles at the time of surgery for placement of atrioventricular shunts has not resulted in seizures, but direct application of penicillin to the cortex provokes seizure activity.

Gastrointestinal (GI) disturbances may follow the use of any of the oral forms but are most pronounced with the amoxicillin-clavulanate combination.[54] *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea has followed the use of penicillins, but the risk appears to be lower than with cephalosporins.[55] All penicillins used at high doses for prolonged periods alter the normal microbiota, with resulting colonization with resistant gram-negative bacilli or with fungi such as *Candida.* Abnormalities in liver function test results, such as elevation of the alkaline phosphatase and aminotransferase levels, have been reported, most often after the use of oxacillin and flucloxacillin.[56] Human leukocyte antigen (HLA)-B*5701 genotype has been identified as a major determinant of this reaction by flucloxacillin.[57] Major hepatic injury is uncommon.

Clinical Use

Some uses of penicillins are shown in Table 20.8. Penicillin G remains the primary agent for treatment of infections due to *Streptococcus pyogenes,* penicillin-susceptible strains of *Streptococcus pneumoniae,* and penicillin-susceptible strains of *S. aureus.* IV penicillin G remains the treatment of choice for pneumococcal and meningococcal meningitis, streptococcal endocarditis, and neurosyphilis. None of the semisynthetic penicillins or agents in other classes has been shown to be more effective. For *Enterococcus faecalis,* the most potent activity is observed with ampicillin. Penicillin-susceptible strains of *S. pneumoniae* are inhibited at concentrations less than 0.1 μg of penicillin per milliliter. Other penicillins also are highly active, although minimal inhibitory concentrations (MICs) usually exceed that of penicillin G. This hierarchy of activity of the penicillins is maintained against penicillin-resistant strains of *S. pneumoniae,* albeit at higher MICs. Penicillin, ampicillin, and amoxicillin are the most active compounds, with MICs rarely exceeding 4 μg/mL.[58] Except in pneumococcal meningitis, for which clinical failures with penicillin are well documented, infections caused by pneumococci with MICs of up to 1 μg/mL appear to respond to penicillin G, provided a sufficiently high dose is used.[59] For serious pneumococcal infections caused by penicillin-resistant strains with MICs greater than 1 μg/mL, particularly in immunocompromised patients, vancomycin or another non–β-lactam antibiotic may be preferred over a penicillin or other β-lactam antibiotic based on susceptibility.[60] Penicillin should be used alone to treat pneumococcal meningitis only if the isolate is fully penicillin susceptible.[61]

Virtually all *Neisseria meningitidis* strains are susceptible to penicillin G. *Neisseria gonorrhoeae* strains frequently are resistant to penicillin, which is no longer recommended for treatment of gonorrhea. Penicillin G is the drug of choice for syphilis at all stages. Puerperal infections due to anaerobic streptococci or group B streptococci *(Streptococcus agalactiae),* as well as genital clostridial infections, are treated with penicillin G. Infections produced by anaerobic oral microbiota, including gram-positive and gram-negative cocci and actinomycetes, can be treated with penicillin G, although penicillin-resistant strains of *Prevotella melaninogenica* that produce β-lactamase are encountered.[62]

The penicillinase-resistant penicillins are indicated solely for the treatment of infections caused by methicillin-susceptible strains of staphylococci, for which they are the agents of choice. They also can be used to treat viridans streptococci, *S. pyogenes* and other hemolytic streptococci, penicillin-susceptible strains of *S. pneumoniae,* anaerobic gram-positive cocci, and anaerobic gram-positive bacilli, but penicillin is more active against these organisms and is the preferred drug. These compounds are inactive against *Listeria monocytogenes, Enterococcus* spp., and methicillin-resistant strains of staphylococci. The basis of methicillin resistance in staphylococci is the production of PBP2a, which has low affinity for binding methicillin. They are also active against gram-negative cocci, including *Neisseria* spp.

Aminopenicillins are indicated for treatment of upper respiratory tract infections, lower respiratory tract infections, bacterial gastroenteritis (ampicillin only), bacterial endocarditis, meningitis, and urinary tract infections (UTIs) caused by susceptible (i.e., non–β-lactamase–producing) organisms. Amoxicillin, because of its excellent bioavailability, is the preferred agent for oral administration in most situations; it is well tolerated even in high doses up to 4 g/day (80 to 90 mg/kg/day in

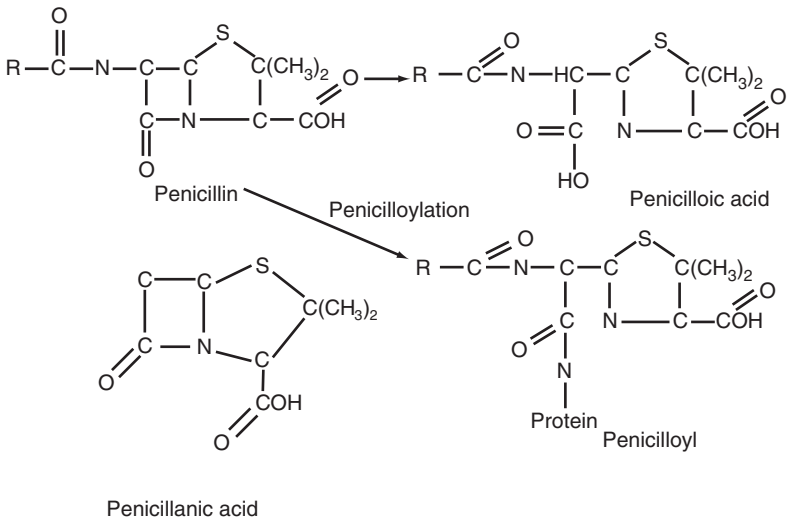

FIG. 20.3 **Mechanisms for formation of antigens from penicillins.**

children).[63] Amoxicillin is also recommended as one component of several triple-drug combination regimens for eradication of *Helicobacter pylori*.[64]

The antipseudomonal penicillins are indicated for the treatment of infections caused by resistant gram-negative bacilli, especially *P. aeruginosa*. The ureidopenicillins, particularly piperacillin, are active against many strains of *Klebsiella* spp., *Enterobacter* spp., *Serratia marcescens,* and *Providencia* spp. and can be used to treat infections caused by these organisms if their susceptibility is confirmed. Piperacillin is often given in combination with tazobactam because of the prevalence of β-lactamase–producing strains. However, the activity of piperacillin against many of the gram-negative species, such as *P. aeruginosa, Enterobacter* spp., *Citrobacter freundii, S. marcescens,* and *Providencia* spp., is usually not improved by tazobactam because these organisms typically produce a class C β-lactamase, which is not inhibited well by tazobactam, and piperacillin can be used without tazobactam for infections caused by these organisms.

Prophylactic Use

Penicillins have been used in a number of situations for prevention of infection. The oral administration of 250 mg of penicillin V every 12 hours or IM injections of 1.2 million units of penicillin G benzathine given once every 3 to 4 weeks are effective in prevention of recurrences of rheumatic fever. Outbreaks of streptococcal infection due to *S. pyogenes* have been aborted by the use of oral penicillin G or V given twice daily for 5 days, by single injections of procaine penicillin daily, or by administration of benzathine penicillin.

Intrapartum prophylaxis with penicillin is recommended at the time of labor or rupture of membranes to prevent early-onset *S. agalactiae* disease in the infant for pregnant women who are colonized with this organism. The recommended dosing regimen of penicillin G is 5 million units IV, followed by 2.5 to 3 million units IV every 4 hours.[65]

Ampicillin or amoxicillin has been administered orally to asplenic children or to children with agammaglobulinemia to prevent infections caused by *Haemophilus influenzae* and *S. pneumoniae*. Amoxicillin is recommended as a single 2-g (adults) or 50-mg/kg (children) oral dose for prophylaxis of bacterial endocarditis among those with the highest risk of adverse outcome.[66] A recent double-blind, randomized, controlled trial in the United Kingdom and Ireland showed that penicillin prophylaxis decreased the risk of recurrent lower extremity cellulitis in subjects with two or more prior episodes of cellulitis.[67] Penicillin prophylaxis has not been of benefit in the prevention of meningococcal infection, bacterial infection after viral respiratory tract infection, or pneumonia after coma, shock, or congestive heart failure.

PROPERTIES OF INDIVIDUAL PENICILLINS

Dosage guidelines for the penicillins are shown in Tables 20.9 and 20.10.

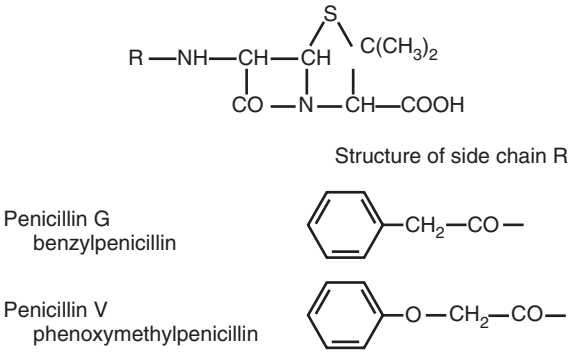

FIG. 20.4 **The structures of penicillin G and penicillin V.**

Natural Penicillins

Penicillin G

Penicillin G, or benzylpenicillin (Fig. 20.4), is available as salts for oral and parenteral administration and as repository salts for IM injection. Because penicillin G is unstable in acid, penicillin V or amoxicillin should be used for oral administration.

Crystalline penicillin G in aqueous solution has been used IM, subcutaneously, IV, and intrathecally. Given IM as an aqueous solution, penicillin G is very rapidly cleared from the body, and it may be preferable to use a repository form. It is available as sterile dry powder in vials containing 5 or 20 million units per vial. Each million unit of penicillin G contains 1.7 mEq of sodium or potassium.

Repository penicillins provide tissue depots from which the drug is absorbed over hours in the case of penicillin G procaine or over days in the case of benzathine penicillin G. Repository penicillins are only for IM use and cannot be used IV or subcutaneously. Penicillin G procaine is a mixture of equal-molar parts of procaine and penicillin G. The use of this suspension delays the peak of activity to 2 to 4 hours after injection and provides serum and tissue levels for 24 hours.[68] Doubling the dose of penicillin G procaine given at a single injection site does not double the serum level. To increase the peak level, it is necessary to use two body sites, as is done in the treatment of gonorrhea, for example, with 2.4 million units of penicillin G procaine given in each buttock.

Benzathine penicillin G is a repository form of penicillin G that combines penicillin G and dibenzylethylenediamine.[69] It provides detectable serum levels for 3 to 4 weeks, depending on the size of the dose.[68] Although benzathine penicillin G is the treatment of choice for syphilis, concentrations of penicillin G in the CSF after use of benzathine penicillin G may be inadequate to treat neurosyphilis, thus IV penicillin G is preferred.[70]

TABLE 20.8 Antimicrobial Spectrum of Penicillins

INFECTING ORGANISM	PENICILLIN OF CHOICE	ALTERNATIVE ACCEPTABLE PENICILLIN	FREQUENCY OF RESISTANCE TO PENICILLINS (%)
Gram-Positive Cocci			
Streptococcus pneumoniae	G	Amoxicillin	10–20
Streptococcus pyogenes	G	V	None
Streptococcus agalactiae	G	Ampicillin	None[a]
Viridans streptococci	G		5–15
Streptococcus bovis	G		Rare
Enterococcus faecalis	Ampicillin	Piperacillin	Rare
Staphylococcus aureus (non–penicillinase-producing)	G	Penicillin-resistant	Rare[b]
S. aureus (penicillinase-producing)	Penicillin-resistant		50–60[c]
S. aureus (methicillin-resistant)	None	None	100
S. epidermidis	Penicillin-resistant		80[c]
S. epidermidis (methicillin-resistant)	None	None	100
Gram-Negative Cocci			
Neisseria meningitidis	G	Ampicillin	Rare
Neisseria gonorrhoeae	G	Ampicillin	5–20
Gram-Positive Bacilli			
Bacillus anthracis	G		None
Corynebacterium diphtheriae	G		None
Listeria monocytogenes	Ampicillin	G	None
Anaerobic Species			
Peptostreptococcus spp.	G	Ampicillin	5
Actinomyces spp.	G	V	None
Prevotella spp.	G	Piperacillin	80
Fusobacterium spp.	G	Ampicillin	10
Bacteroides fragilis	None		100
Clostridium spp.	G	Ampicillin	Rare
Gram-Negative Bacilli			
Haemophilus influenzae	Ampicillin		30
Escherichia coli	Ampicillin		50–60
Proteus mirabilis	Ampicillin		25–30
Salmonella enterica serovar Typhi	Ampicillin		5–10
Salmonella enterica, non-Typhi	Ampicillin		10–20
Klebsiella spp.	Piperacillin		50
Enterobacter spp.	Piperacillin		50–60
Citrobacter spp.	Piperacillin		40
Proteus, indole-positive spp.	Piperacillin		25
Serratia marcescens	Piperacillin		40
Morganella morganii	Piperacillin		20
Pseudomonas aeruginosa	Piperacillin		15–30
Acinetobacter baumannii	Piperacillin		>70
Providencia spp.	Piperacillin		30
Stenotrophomonas maltophilia	None		85–90
Other Organisms Infrequently Encountered			
Erysipelothrix spp.	G	Ampicillin	None
Pasteurella multocida	G	Ampicillin	Rare
Streptobacillus moniliformis	G		None
Spirillum minus	G		None
Treponema pallidum	G	Ampicillin	None

[a]Reduced susceptibility has been observed on rare occasions.
[b]Most non–penicillinase-producing strains are methicillin susceptible.
[c]Approximate frequency of methicillin-resistant strains among penicillinase-producing strains.

TABLE 20.9 Dosage of Penicillins

COMPOUND	DOSAGE		
	Oral	Intramuscular	Intravenous[a]
Penicillin G			Adult: 2–4 million units q4h Pediatric: 300,000 units/kg/day, 6 divided doses
Procaine		Adult: 600,000–1 million units daily Pediatric: 50,000 units/kg daily	
Benzathine		Adult: 1.2–2.4 million units qwk Pediatric: 50,000 units/kg qwk	
Penicillin V	Adult: 125–500 mg qid Pediatric: 25–75 mg/kg/day, 3–4 divided doses		
Ampicillin	Adult: 500 mg qid Pediatric: 50–100 mg/kg/day, 4 divided doses	Adult: 2 g q4–6h Pediatric: 300 mg/kg/day, 4 divided doses	Adult: 2 g q4–6h Pediatric: 300 mg/kg/day, 4 divided doses
Amoxicillin	Adult: 0.5–1 g tid Pediatric: 25–90 mg/kg/day, 2–3 divided doses		
Cloxacillin	Adult: 250–500 mg qid Pediatric: 50–100 mg/kg/day, 4 divided doses		
Dicloxacillin	Adult: 125–500 mg qid Pediatric: 12.5–100 mg/kg/day, 4 divided doses		
Flucloxacillin	Adult: 250–500 mg qid Pediatric: 62.5–125 mg qid	Adult: 250 mg q6h Pediatric: 125 mg q6h	Adult: 2 g q6h Pediatric: 100–200 mg/kg/day, 4 divided doses
Nafcillin		Adult: 0.5–1 g q4–6h Pediatric: 50–100 mg/kg/day, 4 divided doses	Adult: 1–2 g q4h Pediatric: 200 mg/kg/day, 4 divided doses
Oxacillin		Adult: 1 g q4–6h Pediatric: 100 mg/kg/day, 4 divided doses	Adult: 1–2 g q4h Pediatric: 200 mg/kg/day, 4 divided doses
Piperacillin		Adult: 2 g q6–12h Pediatric: 100–125 mg/kg/day, 2–4 divided doses	Adult: 3–4 g q4–6h Pediatric: 200–300 mg/kg/day, 4–6 divided doses

[a]Intravenous doses used for more severe infections, including meningitis and endocarditis.
qid, Four times daily; *qwk,* once per week; *tid,* three times daily.

TABLE 20.10 Dosage of Antibiotics in Neonates

AGENT	INFANTS <1 WK OLD		INFANTS 1 WK TO 1 MO OLD	
	Dosage (per kg/day)	Interval Between Doses (h)	Dosage (per kg/day)	Interval Between Doses (h)
Penicillin G	100,000 units	12	150,000 units	8
Ampicillin (intravenous)	150 mg	8	200 mg	6
Nafcillin	75 mg	8–12	100–150 mg	6–8
Oxacillin	75 mg	8–12	150–200 mg	6–8

Penicillin V

Phenoxymethyl penicillin, or penicillin V (see Fig. 20.4), is available only for oral use as a sodium or a potassium salt in suspension or tablets in doses of 125, 250, and 500 mg. The usual dosage for children is 25 to 50 mg/kg/day and for adults is 1 to 4 g/day in three or four divided doses. The potassium salt produces higher blood levels than the other salts. Serum levels are from two to five times those obtained with oral penicillin G. Blood levels after 500 mg administered to an adult are equivalent to the levels achieved with 600,000 units of penicillin G procaine administered IM. Penicillin V can be substituted for penicillin G in most situations in which it is reasonable to treat an infection by the oral route. However, penicillin V is less active than penicillin G against *Haemophilus* and *Neisseria* spp.

Penicillinase-Resistant Penicillins

The antibacterial spectra of all penicillinase-resistant penicillins are identical (Fig. 20.5). They are active against methicillin-susceptible strains of staphylococci; penicillin-susceptible strains of streptococci, including *S. pneumoniae;* and most anaerobic gram-positive cocci. None are active against methicillin-resistant staphylococci, high-level penicillin-resistant streptococci, enterococci, *L. monocytogenes,* aerobic gram-negative cocci or bacilli, or anaerobic gram-negative bacteria.

Methicillin

Methicillin (2,6-dimethoxyphenylpenicillin) was the first of several penicillinase-resistant penicillins developed. Methicillin is the least active of the penicillinase-resistant penicillins by weight; it is acid labile and therefore can be administered only parenterally; and it is more likely to cause interstitial nephritis. For these reasons, methicillin is no longer available for clinical use.

Nafcillin

Nafcillin (2-ethoxy-1-naphthylpenicillin) has more intrinsic activity than methicillin against susceptible organisms. It is highly protein bound. Oral absorption of nafcillin is erratic, and levels after IM injection are low; therefore the only practical route of administration is IV. The

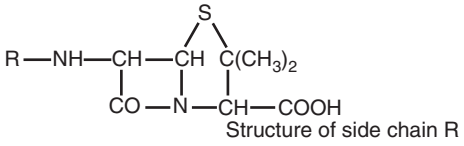

Structure of side chain R

Methicillin
2,6-dimethoxyphenylpenicillin

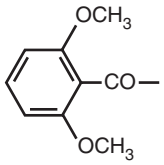

Nafcillin
2-ethoxy-1-naphthylpenicillin

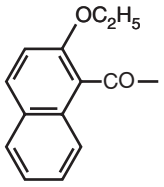

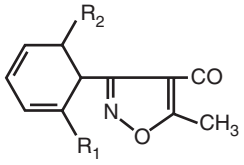

	R_2	R_1
Oxacillin 3-phenyl-5-methyl-4-isoxazolylpenicillin	H	H
Cloxacillin 3-(2-chlorophenyl)-5-methyl-4-isoxazolylpenicillin	Cl	H
Dicloxacillin 3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolylpenicillin	Cl	Cl
Flucloxacillin 3-(2-chloro-6-fluorophenyl)-5-methyl-4-isoxazolylpenicillin	Cl	F

FIG. 20.5 **Antistaphylococcal penicillins.**

antibiotic is primarily excreted by the liver and to a lesser extent by the kidney. Serum levels are elevated, and the half-life is prolonged by probenecid. The usual dosage of nafcillin is 6 to 12 g/day, depending on the severity of the infection, and 100 to 200 mg/kg/day for children.

Isoxazolyl Penicillins

The isoxazolyl penicillins (oxacillin, cloxacillin, dicloxacillin, and flucloxacillin) are absorbed after oral administration, but absorption is adversely affected by food. Dicloxacillin and flucloxacillin yield the highest total drug serum concentrations, but because of differences in protein binding, free-drug concentrations are similar for all three compounds (see Table 20.5). After IV infusion of 1 g over 15 minutes, serum levels are approximately 25 μg/mL 1 hour later and less than 1 μg/mL at 6 hours. The isoxazolyl penicillins undergo some metabolism but are excreted primarily by the kidney, with slight biliary excretion. Oxacillin undergoes more rapid degradation in the body than does cloxacillin or dicloxacillin.

Oxacillin sodium for injection may be given IM or IV. The adult dosage is 6 to 12 g/day, and for children it is 100 to 200 mg/kg/day given every 4 to 6 hours. Oxacillin may be associated with a higher incidence of hepatotoxicity and rash than nafcillin or other antistaphylococcal agents.[56]

Cloxacillin sodium is available in the United States only as an oral solution (125 mg/5 mL) or capsules of 250 and 500 mg. The dosage for children is 50 to 100 mg/kg/day given as four equal doses, and the dosage for adults is 1 to 2 g/day given as four equal doses.

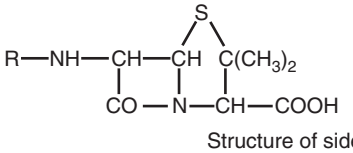

Structure of side chain R

Ampicillin
D(-) α-aminobenzylpenicillin

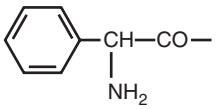

Amoxicillin
D(-) α-amino-p-hydroxybenzylpenicillin

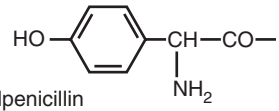

FIG. 20.6 **Aminopenicillins.**

Dicloxacillin sodium is available as a suspension (62.5 mg/5 mL) and as capsules of 125 and 250 mg. The dosage for children weighing less than 40 kg is 12.5 to 100 mg/kg/day given as four doses. For adults a dosage of 125 to 500 mg every 6 hours can be given, depending on the severity of the infection.

Flucloxacillin and dicloxacillin are similar in pharmacokinetics, activity, and indications.[71] It is not available in the United States but is widely used in Europe and other countries. Flucloxacillin is available as a sodium salt in an oral suspension (125 mg/5 mL or 500 mg/5 mL), capsules (250 and 500 mg), and in powder form for reconstitution for parenteral administration.

Aminopenicillins

The antibacterial activities of all aminopenicillins are similar (Fig. 20.6). They are susceptible to β-lactamases. For practical purposes the activity of aminopenicillins is virtually identical to that of penicillin G against penicillin-susceptible organisms, except that aminopenicillins are slightly more active against enterococci. Non–β-lactamase–producing strains of *H. influenzae* and *Haemophilus parainfluenzae* are susceptible. Strains of *E. coli, Shigella sonnei,* and *Salmonella* spp., including many strains of *Salmonella enterica* serovar Typhi, once uniformly susceptible to aminopenicillins, often are resistant due to β-lactamase production. Species that intrinsically produce β-lactamase, such as *Enterobacter* spp., *Serratia* spp., *Acinetobacter* spp., *Pseudomonas* spp., and indole-positive *Proteus,* are resistant to aminopenicillins.

Ampicillin

Ampicillin is moderately well absorbed after oral administration, but peak levels are delayed and lowered if it is ingested with food. Peak blood levels of 3 μg/mL occur 1 to 2 hours after ingestion of 500 mg; levels peak later in diabetic patients with neurologic disease and in patients with renal failure. After IM injection of 0.5 g, peak levels of 10 μg are achieved at 1 hour. The elimination half-life is approximately 80 minutes. Probenecid increases the magnitude of peak levels and the area under the concentration-time curve (AUC). Ampicillin is well distributed to body compartments and after parenteral administration achieves therapeutic concentrations in cerebrospinal, pleural, joint, and peritoneal fluids in the presence of inflammation. The drug undergoes enterohepatic circulation, and significant levels appear in bile and stool. Urinary levels are high even in the presence of markedly reduced renal function. Peritoneal dialysis is ineffective in removing the drug, but hemodialysis removes approximately 35% in a 4-hour period. The half-life of ampicillin is about 3 hours during continuous venovenous hemofiltration.

Ampicillin is available for oral use as the sodium salt as 250-mg or 500-mg capsules and oral suspensions of 125 or 250 mg/5 mL. The sodium salt can be used for either IM or IV administration. For most indications, oral ampicillin has been abandoned in favor of oral amoxicillin because of the greater bioavailability of the latter. Ampicillin is effective for upper and lower respiratory tract infections caused by *S. pneumoniae,* β-hemolytic streptococci, and non–β-lactamase–producing

strains of *H. influenzae*. It is effective in the treatment of meningitis caused by group B streptococci, *L. monocytogenes, N. meningitidis,* and penicillin-susceptible strains of *S. pneumoniae*. Although formerly useful in treating UTIs caused by *E. coli* and gastroenteritis caused by *S. enterica* or *Shigella* spp., owing to the high prevalence of β-lactamase–producing strains, ampicillin should not be used until susceptibility has been documented.

Dosage varies with the age of the patient, the status of renal function, and the severity of the disease. For children older than 1 month the oral dosage is 50 to 100 mg/kg/day in four divided doses; the IM or IV dosage is 100 to 300 mg/kg/day in four or six doses. For adults the oral dosage is 2 to 4 g/day given in divided doses every 6 hours. For severe infection the parenteral dosage is 6 to 12 g/day given in divided doses every 4 hours.

Amoxicillin

Amoxicillin differs from ampicillin only in the presence of hydroxyl group in the *para* position of the benzene side chain. Its in vitro activity is identical to that of ampicillin. It is significantly better absorbed when given by mouth compared with ampicillin, and for this reason it is preferred for most indications. Peak blood levels are from 2 to 2.5 times those achieved with a similar dose of ampicillin, and food does not decrease absorption. Oral amoxicillin produces blood levels similar to those produced by IM administration. The elimination half-life averages 80 minutes in adults with normal renal function. Urinary excretion of amoxicillin is greater than that of ampicillin, and tissue distribution is similar to that of ampicillin. Parenteral amoxicillin, which is available in Europe but not in the United States, is pharmacologically identical to parenterally administered ampicillin.

Clinical studies with amoxicillin have been extensive; this antibiotic has been used in the treatment of otitis media, bronchitis, pneumonia, typhoid, gonorrhea, and UTIs. High-dose amoxicillin (80–90 mg/kg/day) is first-line therapy for otitis media in children because it covers penicillin-resistant pneumococci.[72] Amoxicillin 1 g three times daily is recommended in the oral therapy for community-acquired pneumonia.[60] Amoxicillin can achieve concentrations that exceed MICs effective for nonmeningeal infections caused by penicillin-resistant strains of *S. pneumoniae*.[73,74] Amoxicillin is less effective than ampicillin for the treatment of shigellosis.[75]

Side effects of amoxicillin are similar to those seen with ampicillin, although diarrhea may be less common than with ampicillin. The usual dosage for children is 20 to 40 mg/kg/day and as high as 90 mg/kg/day given in two or three divided doses every 8 hours; for adults the dosage is 0.5 to 1 g every 8 to 12 hours, although it has been used in doses up to 1 g every 4 hours.

Carboxypenicillins

Ticarcillin, carbenicillin, and indanyl carbenicillin (Fig. 20.7)—α-carboxy esters of carbenicillin used for oral administration—are no longer used because of the large doses required, the greater potential for toxicity, and the availability of more potent alternatives.

Ureidopenicillins

Ureidopenicillins (see Fig. 20.7) all have similar spectra of activity and pharmacologic properties. Azlocillin and mezlocillin are no longer on the market, having been replaced in clinical practice by piperacillin-tazobactam, and are not discussed further.

Piperacillin is similar to ampicillin in activity against gram-positive species. It has excellent activity against streptococcal species and against *Neisseria, Haemophilus,* and many members of the family Enterobacteriaceae. It also has excellent activity against anaerobic species of both cocci and bacilli. It inhibits 60% to 90% of *P. aeruginosa* strains at concentrations less than 16 μg/mL. Like ampicillin, it is hydrolyzed by class A β-lactamases. Although it is hydrolyzed by class C β-lactamases produced by *P. aeruginosa* and *Enterobacter* spp., it is not an inducer, which accounts for its activity against the majority of strains of these and related species. Emergence of resistance to piperacillin is often due to selection of mutants that constitutively express high levels of class C β-lactamase.[76] Piperacillin acts synergistically against most *P. aeruginosa* strains and against some of the Enterobacteriaceae species when it is combined with aminoglycosides[77,78]; the antibacterial activity is either synergistic or additive when combined with a fluoroquinolone.[79,80]

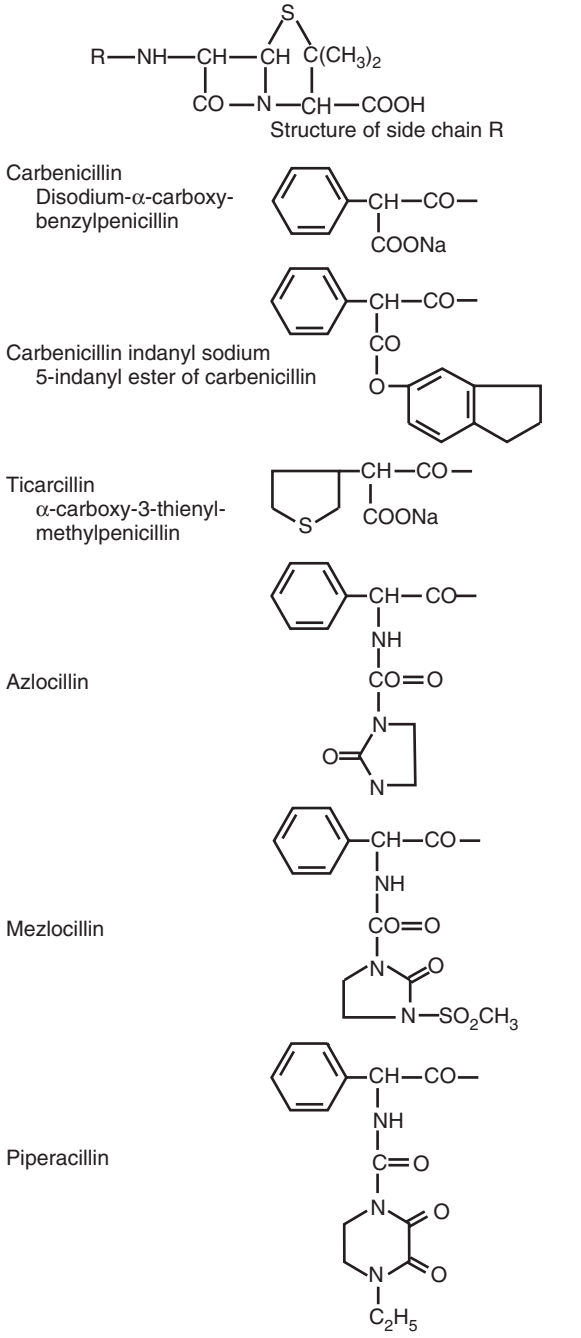

FIG. 20.7 **Penicillins active against gram-negative bacteria.**

IV bolus administration of 4 g produces peak serum drug levels above 300 μg/mL. Piperacillin's kinetics are dose dependent. It accumulates in renal failure to a lesser degree than does carbenicillin, and its half-life is only 4 to 6 hours at CrCls less than 10 mL/min. It is removed through hemodialysis and should be dosed after dialysis. The half-life with continuous venovenous hemofiltration is approximately 5 hours, and 4 g administered twice daily is recommended.[81]

Piperacillin has shown adverse reactions similar to those for the other penicillins noted earlier. After prolonged administration at high doses, neutropenia has been reported. Alteration of bleeding time and hypokalemia occur infrequently. Clinical studies have shown that it is a useful agent in the treatment of a variety of infections.[26] It is administered to adults in daily doses of 12 to 18 g. It is almost always used

as a piperacillin-tazobactam combination to extend its activity against class A β-lactamase–producing strains.

β-LACTAMASE INHIBITORS AND INHIBITOR COMBINATIONS

β-Lactamase inhibitors are grouped into β-lactam compounds (e.g., clavulanic acid, sulbactam, and tazobactam) and non–β-lactam compounds (e.g., avibactam, vaborbactam). Five β-lactamase inhibitors are currently in clinical use: clavulanic acid, sulbactam, tazobactam, and avibactam and vaborbactam. The former three are potent inhibitors of class A β-lactamases, with a notable exception of the *Klebsiella pneumoniae* carbapenemase (KPC) β-lactamase. The latter two are inhibitors of class A β-lactamases, including KPC β-lactamase, and also class C (AmpC) β-lactamase. When combined with β-lactam antibiotics that are substrates for these β-lactamases and therefore inactive against bacteria producing them, the inhibitors prevent hydrolysis of the antibiotics, thereby restoring their activity (Tables 20.11 and 20.12). Although competitive inhibition is seen, the β-lactam β-lactamase inhibitors—clavulanic acid, sulbactam, and tazobactam—primarily act as suicide inhibitors that form a stable intermediate, rendering the enzyme inactive. Avibactam and vaborbactam differ from β-lactam β-lactamase inhibitors in that they are reversible inhibitors with unique non-β-lactam structures.

Each inhibitor is available only as a fixed-combination preparation with an active β-lactam antibiotic as the companion agent. There are minor differences in potency, activity, and pharmacology among the β-lactam β-lactamase inhibitors, but it is the companion β-lactam antibiotic that primarily determines antibacterial spectrum of activity. β-Lactam β-lactamase inhibitors are effective only against Ambler class A β-lactamases (i.e., penicillinases), which often are plasmid encoded. Class A β-lactamases are produced by *S. aureus, H. influenzae, Moraxella catarrhalis, Bacteroides* spp. (the β-lactamases are chromosomal in the latter two species), and Enterobacteriaceae, which produce the Temoniera (TEM) and sulfhydryl-variable (SHV) β-lactamases. Clavulanic acid and piperacillin also have some inhibitory activity in vitro against extended-spectrum β-lactamases (ESBLs), including the emerging cefotaxime/ceftazidime-hydrolyzing (CTX-M) type, which are class A β-lactamases. However, reports of failure with β-lactam β-lactamase inhibitors in combination with a penicillin (i.e., amoxicillin, ampicillin, or piperacillin) for treatment of bacteremia and other systemic infections caused by ESBL-producing organisms suggest that activity in vivo may not be predictable. KPC-type β-lactamase, also belonging to class A, is only minimally inhibited by β-lactam β-lactamase inhibitors. Ambler class C β-lactamases (i.e., AmpC cephalosporinases) of *Serratia* spp., *C. freundii, Enterobacter* spp., *P. aeruginosa,* and some other Enterobacteriaceae species, which typically are chromosomally encoded, are not effectively inhibited by these β-lactam β-lactamase inhibitors either. In contrast, avibactam, a non–β-lactam diazabicyclo[3.2.1]octanone β-lactamase inhibitor, and vaborbactam, a non–β-lactam cyclic boronic acid β-lactamase inhibitor, inhibit both class A β-lactamases, including ESBLs and KPC-type β-lactamase, and class C β-lactamases. In addition, avibactam, but not vaborbactam, inhibits oxacillinase (OXA)-48, which is a class D β-lactamase capable of hydrolyzing carbapenems in Enterobacteriaceae. None of the currently approved β-lactamase inhibitors inhibits class B metallo-β-lactamases (MBLs), which are structurally unrelated to the other β-lactamase classes in that they are not PBPs but are derived from metalloproteases.

Clavulanic Acid

The β-lactamase inhibitor clavulanic acid (Fig. 20.8) was originally identified in cultures of *Streptomyces clavuligerus.*[82] Clavulanic acid is

TABLE 20.11 Usual Minimal Inhibitory Concentrations (μg/mL) of Penicillin–β-Lactamase Inhibitor Combinations

ORGANISM	AMOXICILLIN, AMPICILLIN	AMOXICILLIN-CLAVULANATE[a]	AMPICILLIN-SULBACTAM[b]	PIPERACILLIN	PIPERACILLIN-TAZOBACTAM[c]
Staphylococcus aureus (MSSA)	16	1	1	8	1
S. aureus (MRSA)	>16	16	8	>16	>16
Haemophilus influenzae[d]	>16	0.5	0.25	16	0.06
Moraxella catarrhalis	2	0.06	0.06	0.5	0.015
Escherichia coli	>16	4	2	2	2
Klebsiella pneumoniae	>16	2	4	8	4
Proteus mirabilis	1	1	1	0.5	0.5
Proteus vulgaris	>16	4	8	1	0.5
Bacteroides fragilis	>16	0.5	2	8	2
Enterobacter, Citrobacter, Serratia spp.	>16	8–>16	2–>16	2–8	1–8
Pseudomonas aeruginosa	>16	>16	>16	4	4

[a]Contains amoxicillin and clavulanic acid in a 2:1 to 16:1 ratio.
[b]Contains amoxicillin or ampicillin and clavulanic acid or sulbactam in a 2:1 ratio.
[c]Contains piperacillin and tazobactam in an 8:1 ratio.
[d]β-Lactamase–producing strains.
MRSA, Methicillin-resistant *S. aureus; MSSA,* methicillin-susceptible *S. aureus.*
Data from references 170 and 191–201.

TABLE 20.12 Properties of β-Lactamase Inhibitors

	CLAVULANIC ACID	SULBACTAM	TAZOBACTAM	AVIBACTAM	VABORBACTAM
Class	β-Lactam	β-Lactam	β-Lactam	Diazabicyclooctane	Boronic acid
Spectrum of inhibition	Class A, including some ESBLs	Class A	Class A, including some ESBLs	Class A, including KPC and ESBLs Class C Class D, including OXA-48	Class A, including KPC and ESBLs Class C
Partner β-lactams	Amoxicillin	Ampicillin, cefoperazone	Piperacillin, ceftolozane	Ceftazidime	Meropenem

ESBLs, Extended-spectrum β-lactams; *KPC, Klebsiella pneumoniae* carbapenemase; *OXA,* oxazolidinone.

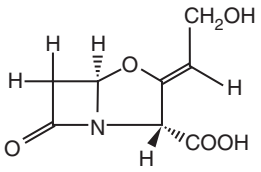

FIG. 20.8 **Clavulanic acid.**

formulated in combination with amoxicillin, which is available for oral administration only in the United States, and is available as a parenteral preparation in many other countries.

Pharmacology

Clavulanic acid is moderately well absorbed from the GI tract. Peak serum concentration of 3 μg/mL in adults occurs 40 to 120 minutes after ingestion of 125 mg.[83] Combining clavulanic acid with amoxicillin does not alter significantly the pharmacologic parameters of either drug. The absorption of clavulanic acid is largely unaffected by the simultaneous administration of food, milk, H2 receptor antagonists, or aluminum hydroxide–containing antacids.[84] After IV infusion of clavulanic acid combined with amoxicillin, the drug is distributed rapidly, producing peak serum concentrations of approximately 8 μg/mL after a 100-mg IV dose. The serum half-life of clavulanic acid is about 1 hour. No accumulation occurs until CrCl is less than 10 mL/min. Dose adjustment is made on the basis of the desired dose for amoxicillin. Clavulanic acid is degraded in vivo, with metabolites being excreted via lung, feces, and urine; only 20% to 60% appears unchanged in urine 6 hours after an oral dose. Urinary levels after a 125-mg dose of clavulanic acid are between 20 and 30 μg/mL 4 to 6 hours after dosing.[85]

Clavulanic acid produces therapeutic levels in bile, middle ear fluid, and tonsillar tissue. It crosses the placenta and may be found in the cord blood of neonates and in the amniotic fluid, but no clavulanic acid can be detected in breast milk.[86] Clavulanic acid does not penetrate noninflamed meninges. In patients with meningitis CSF levels are in the range of 0.25 μg/mL.[87] Clavulanic acid concentrations of less than 1 μg/mL are achieved in sputum after the oral administration of amoxicillin-clavulanate.[88] There is rapid penetration of clavulanic acid into peritoneal fluid, with an AUC ratio comparable to serum.[89]

Adverse Reactions

No major adverse reactions to the use of clavulanic acid combined with amoxicillin have been reported. Delayed hepatotoxicity may occur, which usually follows a benign course.[90] The incidence of skin reactions has been similar to that seen when penicillin is used alone. Diarrhea is the most common side effect.[54] Nausea also accompanies use of these doses. In accordance, the oral dose of clavulanic acid is recommended not to exceed 125 mg two or three times a day.

Amoxicillin-Clavulanate

Amoxicillin-clavulanate (Augmentin) has proved useful as therapy for acute otitis media in children.[91,92] It also has been used to treat sinusitis or pneumonia caused by susceptible β-lactamase–producing or non–β-lactamase–producing bacteria. It is particularly useful in treating polymicrobial infections in which β-lactamase–producing organisms may be present, including bite wounds of human or animal origin and diabetic foot infections.[93] Skin structure infections caused by streptococci and staphylococci have responded to amoxicillin-clavulanate with results comparable to those achieved with oral antistaphylococcal agents and oral cephalosporins.

The agent is formulated as tablets containing 250, 500, or 875 mg of amoxicillin combined with 125 mg of clavulanic acid and also as a sustained-release formulation of 1000 mg of amoxicillin combined with 62.5 mg of clavulanic acid. The usual dose is 250 mg of amoxicillin every 8 hours to 875 mg every 12 hours by mouth. The 500-/125-mg and 875-/125-mg dosage forms are effective as twice-daily regimens, which are associated with less diarrhea.[94] In children the dose is 20 to 45 mg/kg/day in two or three divided doses; a variety of oral suspensions and chewable tablets are available.

The extended-release formulation is administered as a dose of 1000/62.5 mg twice daily. This dosage is effective for treatment of bacterial sinusitis and community-acquired pneumonia, including infections caused by penicillin-resistant strains of *S. pneumoniae.* Infectious Diseases Society of America guidelines include amoxicillin-clavulanate extended-release 2000 mg/125 mg twice daily in combination with a macrolide as an alternative to fluoroquinolones for empirical, outpatient therapy for community-acquired pneumonia in patients with medical comorbidities.[60]

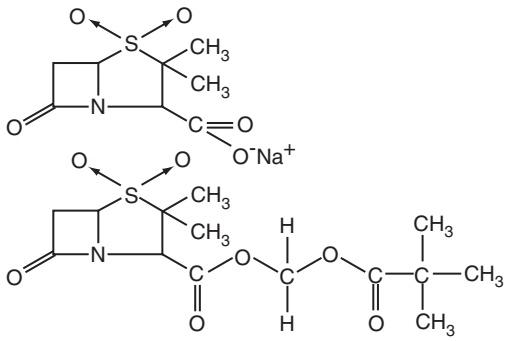

FIG. 20.9 **Sulbactam *(top)* and sulbactam oral ester *(bottom).***

Sulbactam

Sulbactam (Fig. 20.9) is a 6-desaminopenicillin sulfone. It is a somewhat broader spectrum β-lactamase inhibitor than clavulanic acid but less potent. Sulbactam is available in the United States only in combination with ampicillin (Unasyn), in a ratio of 0.5 g of sulbactam to 1 g of ampicillin as a parenteral formulation for IV administration. It is also available in combination with cefoperazone (Sulperazon) in a 1 : 1 ratio as a parenteral formulation in certain countries and used primarily for treatment of *Acinetobacter* infections.[95]

Pharmacology

Sulbactam has pharmacokinetics in humans similar to those of ampicillin.[96] The average peak serum level after IV infusion of 1 g is 60 μg/mL. The serum half-life is 1 hour. Sulbactam is excreted by the kidney and has a urinary recovery rate of 70% to 80% of a dose.[97] Biliary excretion is minimal, and metabolism is less than 25%. Renal excretion is blocked by probenecid. The half-life is not altered significantly until the CrCl decreases to less than 30 mL/min. The half-life is 9.2 hours at clearances of 5 to 15 mL/min and 20 hours in anuria. Dosage adjustment is required for CrCl less than 50 mL/min and is based on the ampicillin component. Concentrations of sulbactam in intestinal fluid and peritoneal secretions are comparable to levels in serum. Penetration of sulbactam into inflamed meninges is low and variable, with levels of less than 2% to 32% of the concentrations in the serum.[97]

Adverse Reactions

Clinical studies of the combination of sulbactam plus ampicillin have revealed no major hematologic, renal, hepatic, or CNS reactions.[98] Diarrhea has not been a major problem after IV use. Skin reactions are similar to those found for ampicillin, and there is occasional elevation of aminotransferase levels.

Clinical Use

Ampicillin-sulbactam has a spectrum of antibacterial activity that is similar to that of amoxicillin-clavulanate. It has been used in the treatment of mixed bacterial infections, such as intraabdominal infections, obstetric and gynecologic infections, and soft tissue and bone infections.[99] Sulbactam alone has modest activity in vitro against strains of *Acinetobacter baumannii.*[100] Because it is available only as a combination, its clinical utility independent of the companion β-lactam for treatment of infections caused by *A. baumannii* cannot be determined. One retrospective study

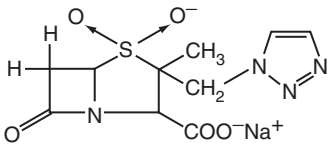

FIG. 20.10 Tazobactam.

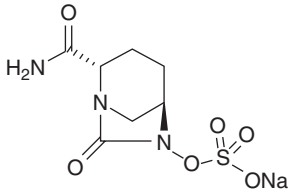

FIG. 20.11 Avibactam.

found that ampicillin-sulbactam was more efficacious than polymyxins for treatment of infections caused by carbapenem-resistant *A. baumannii* strains,[101] and its addition to a combination regimen may have a role in the treatment of pandrug-resistant *A. baumannii* infection.[102]

Tazobactam

Tazobactam is a penicillanic acid sulfone β-lactamase inhibitor with a structure similar to that of sulbactam (Fig. 20.10).[103] Its spectrum of β-lactamase inhibition is similar to that of sulbactam, but its potency is more like that of clavulanic acid. It is available for parenteral administration in combination with piperacillin in an 8:1 ratio (Zosyn) and in combination with ceftolozane in a 2:1 ratio (Zerbaxa).

Pharmacology

Mean peak serum concentration after a 30-minute IV infusion of 500 mg of tazobactam in combination with piperacillin is about 24 μg/mL in healthy subjects.[104] Plasma elimination half-life is approximately 1 hour. Tazobactam is cleared primarily renally, and dosage interval should be extended for CrCls of less than 40 mL/min. Combining tazobactam with piperacillin reduces clearance of tazobactam, but the clearance of piperacillin is not affected. Clearances of piperacillin and tazobactam are similar in subjects with normal renal function. Peak serum concentrations are approximately 50% higher in patients with end-stage renal disease.[105] The half-life of tazobactam is approximately 7 hours in patients with end-stage renal disease. These differences in pharmacokinetics of piperacillin and tazobactam do not require adjustment of the dose of tazobactam independent of piperacillin; the dose is adjusted based on the pharmacokinetics of piperacillin.

Tissue levels of tazobactam reflect a percentage penetration that is similar to that of piperacillin for each tissue type. Tazobactam penetrates inflamed meninges. CSF concentrations of piperacillin and tazobactam were 16% and 32% of simultaneous serum concentrations in a rabbit meningitis model.[106]

Adverse Reactions

Limited clinical data do not indicate any new or unusual toxicity unique to tazobactam. Diarrhea and skin reactions are common.[107]

Piperacillin-Tazobactam

Clinical Use

Clinical studies of piperacillin-tazobactam have been conducted mainly in adults; experience with this combination for treatment of serious infections in children indicates that it is safe and effective.[108] In clinical trials the efficacy of piperacillin-tazobactam has been equivalent and occasionally superior to similarly broad-spectrum comparator drugs (e.g., carbapenems, third-generation cephalosporins) for treatment of pneumonia, skin and soft tissue infections, intraabdominal infections, polymicrobial infections, and febrile neutropenia in combination with an aminoglycoside.[109] The usual adult dose is 12 g of piperacillin/1.5 g of tazobactam administered at a dose of 3.375 g (3 g piperacillin, 0.375 g of tazobactam) every 6 hours, or 4.5 g every 6 to 8 hours for CrCl greater than 40 mL/min.

ESBL-producing strains of *E. coli* and *Klebsiella* spp. often are susceptible to piperacillin-tazobactam in vitro, but there has been some reluctance to use this agent because of the possibility of treatment failure when β-lactam antibiotics other than carbapenems are used to treat infections caused by ESBL-producing strains. Whether bacteremia caused by ESBL-producing organisms can be treated as efficaciously with piperacillin-tazobactam as with carbapenems remains uncertain. However, a recently completed randomized trial comparing piperacillin-tazobactam and meropenem as definitive therapy for bacteremia due to ceftriaxone-resistant *Klebsiella* spp. and *E. coli* showed significantly higher mortality rates among patients randomized to receive piperacillin-tazobactam[110,111]; meropenem monotherapy performed better.

Ceftolozane-Tazobactam

Ceftolozane is an antipseudomonal cephalosporin with improved stability against AmpC compared with ceftazidime. Ceftolozane-tazobactam is active against the majority of *P. aeruginosa* strains, including multidrug-resistant ones. It is active against approximately 60% of carbapenem-nonsusceptible *P. aeruginosa* strains when using the US Food and Drug Administration (FDA)-approved susceptibility breakpoint of 4 μg/mL for this species.[112] The combination is also active against most Enterobacteriaceae clinical strains. It is active against about 90% of ESBL-producing *E. coli* when applying the approved susceptibility breakpoint of 2 μg/mL for this family. Ceftolozane-tazobactam is not active against carbapenem-resistant *K. pneumoniae*, including those producing KPC-type carbapenemase or MBL. Its activity against lactose-nonfermenting organisms other than *P. aeruginosa* is limited.[113]

Ceftolozane-tazobactam has been approved for clinical use based on two phase III randomized trials: one for complicated UTIs and the other for complicated intraabdominal infections. In the former trial, in which the majority of patients had pyelonephritis, the efficacy of ceftolozane-tazobactam was noninferior to levofloxacin.[114] In the latter trial the efficacy of ceftolozane-tazobactam in combination with metronidazole was found to be noninferior to meropenem.[115]

Postmarketing clinical experience suggests that ceftolozane-tazobactam may be a reasonable option for serious infections, such as pneumonia, due to multidrug-resistant *P. aeruginosa*, but detectable bacterial resistance at baseline may be associated with treatment failure, and treatment-emergent resistance also has been noted.[116,117] Susceptibility testing is therefore recommended when treatment with this agent is considered.

Avibactam

Avibactam (Fig. 20.11) is a bridged diazabicyclo[3.2.1]octanone non–β-lactam β-lactamase inhibitor with a structure that is distinct from the conventional β-lactam β-lactamase inhibitors.[118] Its spectrum of β-lactamase inhibition includes class A β-lactamases, including ESBLs and KPCs, class C β-lactamases, and some class D β-lactamases, notably OXA-48 carbapenemase. It does not inhibit MBLs.[119] It is available for parenteral administration in combination with ceftazidime in a 4:1 ratio of ceftazidime to avibactam (Avycaz, Zavicefta). The FDA-approved susceptibility breakpoint is 8/4 μg/mL for both Enterobacteriaceae and *P. aeruginosa*.

Pharmacology

At steady state, mean peak serum concentration after a 2-hour IV infusion of 500 mg of avibactam in combination with ceftazidime is about 15 μg/mL in healthy subjects.[120] Plasma elimination half-life is approximately 2.7 hours. Most avibactam is excreted unchanged in the urine, and dosage should be reduced for CrCls of less than 50 mL/min. About 55% of avibactam is removed during a 4-hour hemodialysis session, which suggests that a dose should be given after dialysis for patients on intermittent hemodialysis.

Adverse Reactions

The most common adverse reactions observed in the registrational clinical trials were vomiting, nausea, constipation, and anxiety, which were similar to those observed among the comparator patients.

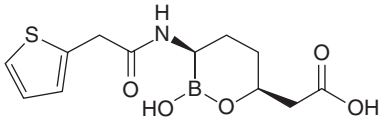

FIG. 20.12 Vaborbactam.

Clinical Use

The addition of avibactam to ceftazidime extends the spectrum to include Enterobacteriaceae that produce ESBL, AmpC, and KPC by reducing the ceftazidime MICs by 16- to greater than 1024-fold. MIC_{90} values for ESBL-producing *E. coli* and KPC-producing *K. pneumoniae* are 0.25 and 1 μg/mL, respectively.[120] Potentiation of ceftazidime activity toward *P. aeruginosa* is more modest, with MIC_{90} values for meropenem and ceftazidime nonsusceptible strains at 16 μg/mL.

Phase III randomized clinical trials have been conducted for adult patients with complicated UTIs, including acute pyelonephritis; complicated intraabdominal infections; and hospital-acquired bacterial pneumonia/ventilator-associated bacterial pneumonia in which ceftazidime-avibactam (in combination with metronidazole for complicated intraabdominal infections) showed comparable safety and efficacy with doripenem, meropenem, and meropenem, respectively.[121,122] Another phase III study comparing it with best available therapy for these types of infections caused by ceftazidime-resistant gram-negative bacteria has shown comparable overall favorable clinical outcome.[123] Since its approval for these indications, ceftazidime-avibactam has been mostly used for the treatment of infections caused by KPC-producing Enterobacteriaceae. Emergent postmarketing observation data suggest that the use of ceftazidime-avibactam–based therapy is associated with lower mortality than the use of colistin-based therapy in the treatment of infections due to carbapenem-resistant Enterobacteriaceae.[124] However, treatment-emergent resistance to avibactam has also been reported.[125]

Vaborbactam

Vaborbactam (Fig. 20.12) is a cyclic boronic acid non–β-lactam β-lactamase inhibitor with activity against class A β-lactamases, including ESBLs, KPC carbapenemase, and class C β-lactamases.[126] It does not inhibit MBLs or class D carbapenemases, including OXA-48. It is available for parenteral administration in combination with meropenem in a 1:1 ratio (2 g each) of meropenem to vaborbactam (Vabomere). The FDA-approved susceptibility breakpoint is 4/8 μg/mL using a fixed concentration of vaborbactam (8 μg/mL).

Pharmacology

The steady-state maximum plasma concentration after a 3-hour infusion of a 2-g dose of vaborbactam is about 40 μg/mL with AUC_{0-t} of 145 μg·h/mL in healthy individuals.[127] Plasma elimination half-life is approximately 1.7 hours. Renal clearance constitutes 80% to 90% of the total clearance of vaborbactam. In accordance, dosage is reduced for CrCls of less than 50 mL/min. A dose should be given after dialysis for patients on intermittent hemodialysis as 53% of vaborbactam is removed during hemodialysis.[128]

Adverse Reactions

The most common adverse reactions occurring in ≥3% of patients in the clinical trials were headache, phlebitis or infusion-site reactions, and diarrhea (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209776lbl.pdf).

Clinical Use

Meropenem-vaborbactam exhibits excellent activity against KPC-producing Enterobacteriaceae with MIC_{50} and MIC_{90} of 0.12 μg/mL and 0.5 μg/mL, respectively, compared with >32 μg/mL for both with meropenem alone.[129] On the other hand, its activity against OXA-48–producing Enterobacteriaceae and MBL-producing Enterobacteriaceae is similar to that of meropenem alone, with $MIC_{50/90}$ of 16/>32 μg/mL for OXA-48 producers and 32/>32 μg/mL for MBL producers. Likewise, there is no appreciable potentiation of meropenem activity against *P. aeruginosa*, *A. baumannii*, and *S. maltophilia*.

A phase III clinical trial has been completed for complicated UTIs and acute pyelonephritis, where patients were randomized to receive meropenem-vaborbactam or piperacillin-tazobactam.[130] The overall treatment success rate at the end of IV therapy was higher with meropenem-vaborbactam, and therapy was well tolerated. Another phase III trial was conducted for infections due to carbapenem-resistant Enterobacteriaceae, where the comparator was best available therapy at the enrolling sites. This trial was suspended early when monotherapy with meropenem-vaborbactam was shown to be associated with statistically higher clinical cure rates and lower treatment-emergent adverse event rates.[131]

Given the spectrum of vaborbactam, most clinical use of meropenem-vaborbactam will likely be in the treatment of infection confirmed as due to KPC-producing Enterobacteriaceae, with better results than have been obtained in the past for colistin- or tigecycline-based regimens.

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21 Cephalosporins

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Although the discovery of the cephalosporin antibiotic class was reported in 1945, it took almost 2 decades for this class to achieve clinical utility. Giuseppe Brotzu is widely credited for discovery of the broad-spectrum inhibitory effects of sewage outflow in Sardinia, Italy.[1] Professor Brotzu subsequently isolated the mold *Cephalosporin acremonium* (now *Acremonium chrysogenum*) and demonstrated antimicrobial activity of culture filtrates against both gram-positive and gram-negative bacteria. He also demonstrated the in vivo activity of these culture filtrates in animal infection models and in several patients.

A decade after the initial discovery, the cephalosporin substances were isolated and identified as fermentation products of the mold.[2] Investigators at Oxford, including Florey and Abraham, systematically studied the physical, chemical, and structural characteristics of cephalosporins, as they had for the penicillin class a decade earlier. Three substances—cephalosporin P, N, and C—were identified. Each of the products possessed antimicrobial activity, but only cephalosporin C demonstrated activity against both gram-negative and gram-positive bacteria. In addition, it had advantageous stability in the presence of acid and penicillinases.[2] Cephalosporin C became the foundation of subsequent drug development.

The first cephalosporin pharmaceutical, cephalothin, was introduced for clinical use in 1964. There are more than 20 cephalosporin antibiotics in use today. The cephalosporin class is among the most widely prescribed antimicrobial classes because of its broad spectrum of activity, low toxicity, ease of administration, and favorable pharmacokinetic profile.

CHEMISTRY

Most of the available cephalosporins are semisynthetic derivatives of cephalosporin C. The basic structure of the cephem nucleus includes a β-lactam ring fused to a six-member sulfur-containing dihydrothiazine ring (Fig. 21.1). The cephem nucleus is chemically distinct from the penicillin nucleus, which contains a five-member thiazolidine ring. Basic structure numbering of the cephalosporin ring system begins within the dihydrothiazine ring at the sulfur moiety. The starting material used as the nucleus for current cephalosporin development is 7-aminocephalosporanic acid (7-ACA). Attempts to alter the physiochemical and biologic properties of the cephalosporins by chemical side chain modifications were based on successes with similar structural changes at the 6-aminopenicillanic acid side chain of penicillin.[3] Chemical modifications of the basic cephem structure by substitution of constituents at positions C1, C3, and C7 led to the various cephalosporin compounds in use today.[4,5] Alterations in positions C7 and C3 are also commonly referred to as R1 and R2, respectively. In general, changes at R1 affect the microbial spectrum of activity. These modifications often have an impact on the stability of the compound to enzymatic destruction by β-lactamases or on its affinity for the drug target (i.e., penicillin-binding proteins [PBPs]). Modifications at R2 often alter the pharmacology of the compound. For example, changes in the R2 constituent may influence the ability of the compound to reach certain infection sites, such as the central nervous system, or may prolong the elimination half-life of the drug. An exception to this rule is enhancement of oral absorption by the substitution of an aminobenzyl group in the C7 (R1) position.[5] Cephalexin, cephradine, cefaclor, cefprozil, and loracarbef all have this structure or a closely related one (Figs. 21.2 and 21.3). However, absorption of later-generation cephalosporins is enhanced by the production of ester formulations. Axetil, proxetil, or pivoxil esters of cefuroxime, cefpodoxime, and cefditoren are examples.

The predominant changes at R1 (position C7) include the substitution of the hydrogen with a methoxy group or the addition of an acyl side chain.[5] The R1 methoxy substitution led to the development of the cephamycin group of compounds, including cefoxitin, cefmetazole, and cefotetan (see Fig. 21.3). This alteration enhanced resistance to β-lactamase produced by gram-negative anaerobic and aerobic bacteria.[5,6] However, these compounds have lower affinity for the PBP target in gram-positive bacteria.[7]

Most of the chemical modifications in cephalosporin development that have resulted in changes in microbiologic spectrum are alterations at the α-carbon of the acyl side chain.[5] Many modifications of the acyl side chain have been undertaken. These changes have ranged from the relatively simple addition of a hydroxyl group to the addition of large synthetic moieties. Each of the acyl side chain alterations has led to enhanced gram-negative potency because of improved β-lactamase stability. The first compounds resulting from addition of a thienyl ring or a tetrazole structure at R1 included the first-generation cephalosporins—cephalothin, cephaloridine, and cefazolin (see Fig. 21.2). The addition of a hydroxyl group at the α-carbon led to the second-generation cephalosporin cefamandole. The second-generation cephalosporin cefuroxime resulted from the addition of a methoxyimino group in the α-position, along with a furyl ring at the β-acyl side chain. Addition of a 2-aminothiazol group to the C7 β-acyl side chain and a methoxyimino group to the α-carbon led to many of the third- and fourth-generation cephalosporins (Figs. 21.4 and 21.5).[8,9] Cefotaxime, ceftizoxime, ceftriaxone, cefepime, cefpirome, and cefpodoxime all have a similar structure at the C7 position. Ceftazidime and ceftolozane differ from these drugs in that the methoxyimino group is replaced with a dimethylacetic acid moiety attached to the imino group.[8,10] This alteration enhances activity against *Pseudomonas aeruginosa* but reduces activity against staphylococci. Two other modifications that have resulted in compounds with increased activity against *P. aeruginosa* are the addition of a ureido-2,3-dioxopiperazine group or a carboxyl group on the α-carbon, producing cefoperazone and moxalactam, respectively.[5] These changes are similar to those of piperacillin and carbenicillin.

Numerous modifications at R2 (the C3 position) have also played a significant role in the development of the current cephalosporins. An acetoxy side chain is present in cephalothin, cephapirin, and cefotaxime.[5] Cephalosporins with this structure can be metabolized in both serum and liver to a less active desacetyl derivative. Such drugs also tend to have a short half-life. A chloride substitution at R2 enhanced the gram-negative spectrum of activity and led to the development of cefaclor, an early second-generation cephalosporin. The unique pharmacology of ceftriaxone results from an R2 modification. Substitution of a heterocyclic thiomethyl group at the C3 position increases biliary secretion and remarkably prolongs the elimination half-life of the compound because of high protein binding.[5,11] The addition of a positively charged quaternary ammonium moiety in the C3 position contributed to the development of the fourth-generation cephalosporins cefepime and cefpirome.[9] The chemical modification produces a zwitterion, which enhances the ability of the compound to penetrate the outer membrane of gram-negative organisms. The inclusion of additional amino groups to this ammonium moiety further enhances activity against *P. aeruginosa*.[10]

More recently, cephalosporins with enhanced activity against methicillin-resistant *Staphylococcus aureus* (MRSA) have been developed (Fig. 21.6). A variety of structural alterations at the C3 and C7 positions have increased the drug's stability to β-lactamase inactivation and enhanced tight binding to the altered PBP2A'.[12,13] Because some of these compounds require more lipophilicity at the C3 position for activity, prodrugs have been required to enhance aqueous solubility.[14,15]

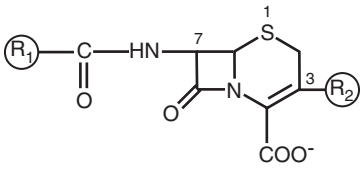

FIG. 21.1 Basic cephalosporin nucleus.

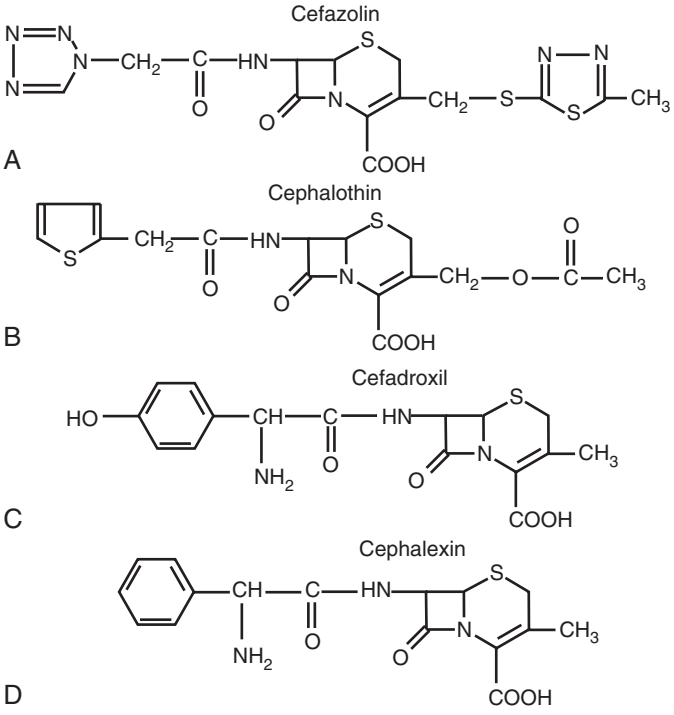

FIG. 21.2 First-generation cephalosporins. (A) Cefazolin. (B) Cephalothin. (C) Cefadroxil. (D) Cephalexin.

Ceftolozane (Fig. 21.7) is a 7-aminothiadiazole structure that is structurally similar to ceftazidime but contains a large pyrazole constituent at R2. This larger R2 moiety particularly inhibits AmpC β-lactamases, but also ensures that ceftolozane activity is unaffected by porin (OprD) loss and efflux pump activity.[16,17] These combined effects and high affinity for PBP1B, PBP1C, and PBP3 result in enhanced in vitro potency, especially against *P. aeruginosa,* compared with other agents.[18,19]

The newest members of the cephalosporin class are the siderophore cephalosporins; however, they have yet to be available for clinical use. Iron is a critical element for bacterial survival but is limited because it is tightly bound to mammalian proteins. In response, bacteria upregulate production of siderophores, which scavenge iron from the environment for bacterial use. Structurally, siderophore cephalosporins contain the traditional cephalosporin structure with the addition of a cathecol moiety at R2 that binds iron, after which it is taken up by the bacterial cell by iron transport systems in a manner that has been described as the "Trojan horse" effect.[20] The delivery of the cephalosporin into the cell allows its cellular action to take place. Important to note, siderophore cephalosporins have demonstrated in vitro activity against drug-resistant gram-negative rods.[21-25] Cefiderocol is the furthest siderophore cephalosporin in the developmental pipeline, which contains the same R1 sidechain as ceftazidime and an R2 catechol moiety (Fig. 21.8).

Not all modifications have led to desired effects. The placement of a thiomethyl tetrazole ring (methylthiotetrazole [MTT]) at the R2 position enhanced antibacterial activity but also resulted in two important adverse effects that have limited use of these compounds.[26,27] Cefamandole, cefotetan, cefoperazone, and moxalactam (an oxycephem) contain this MTT side chain, which is responsible for coagulation abnormalities related to antagonism of vitamin K action. This side chain is also responsible for the disulfiram-like properties of these compounds.

CLASSIFICATION

There are several microbiologic and pharmacologic differences that could serve as a basis for classification among the drugs in the cephalosporin class. The most widely accepted classification includes five divisions, or generations, based loosely on the microbial spectrum of activity (Table 21.1). The first-generation cephalosporins exhibit activity focused primarily on gram-positive bacteria. The second-generation drugs have enhanced activity against gram-negative bacilli but maintain varying degrees of activity against gram-positive cocci. The cephamycin group is included in the second-generation classification. The cephamycins are noted for their additional activity against gram-negative anaerobic bacteria, such as *Bacteroides* spp. The third-generation cephalosporins have markedly increased potency against gram-negative bacilli; however, for some compounds in this group, activity against gram-positive cocci is reduced. Among the third-generation group, a few compounds, such as ceftazidime and ceftolozane, are considered separately for activity against *P. aeruginosa.* The fourth-generation drugs have the widest

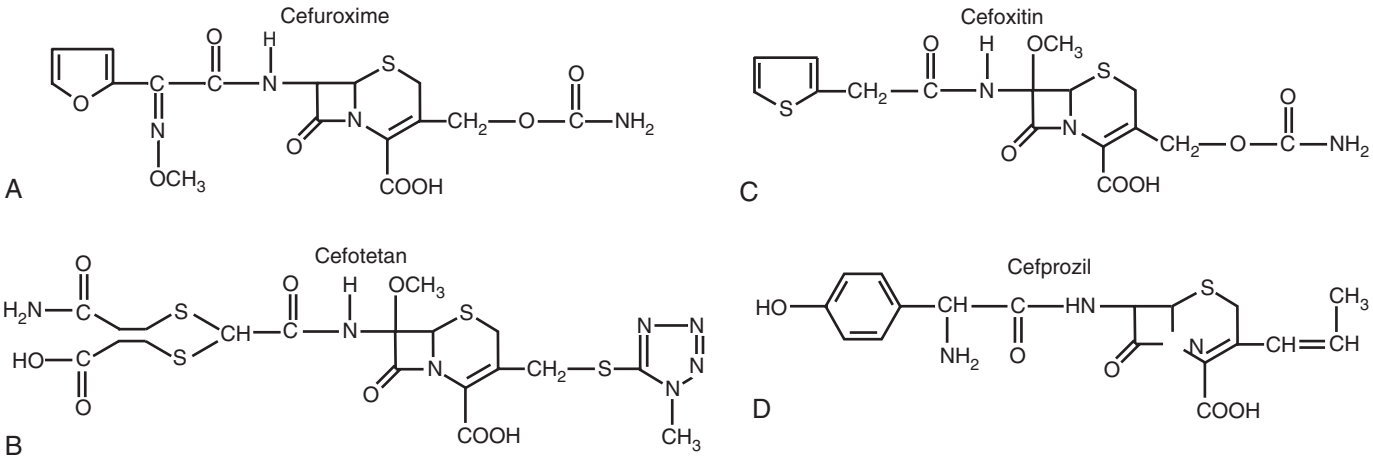

FIG. 21.3 Second-generation cephalosporins. (A) Cefuroxime. (B) Cefotetan. (C) Cefoxitin. (D) Cefprozil.

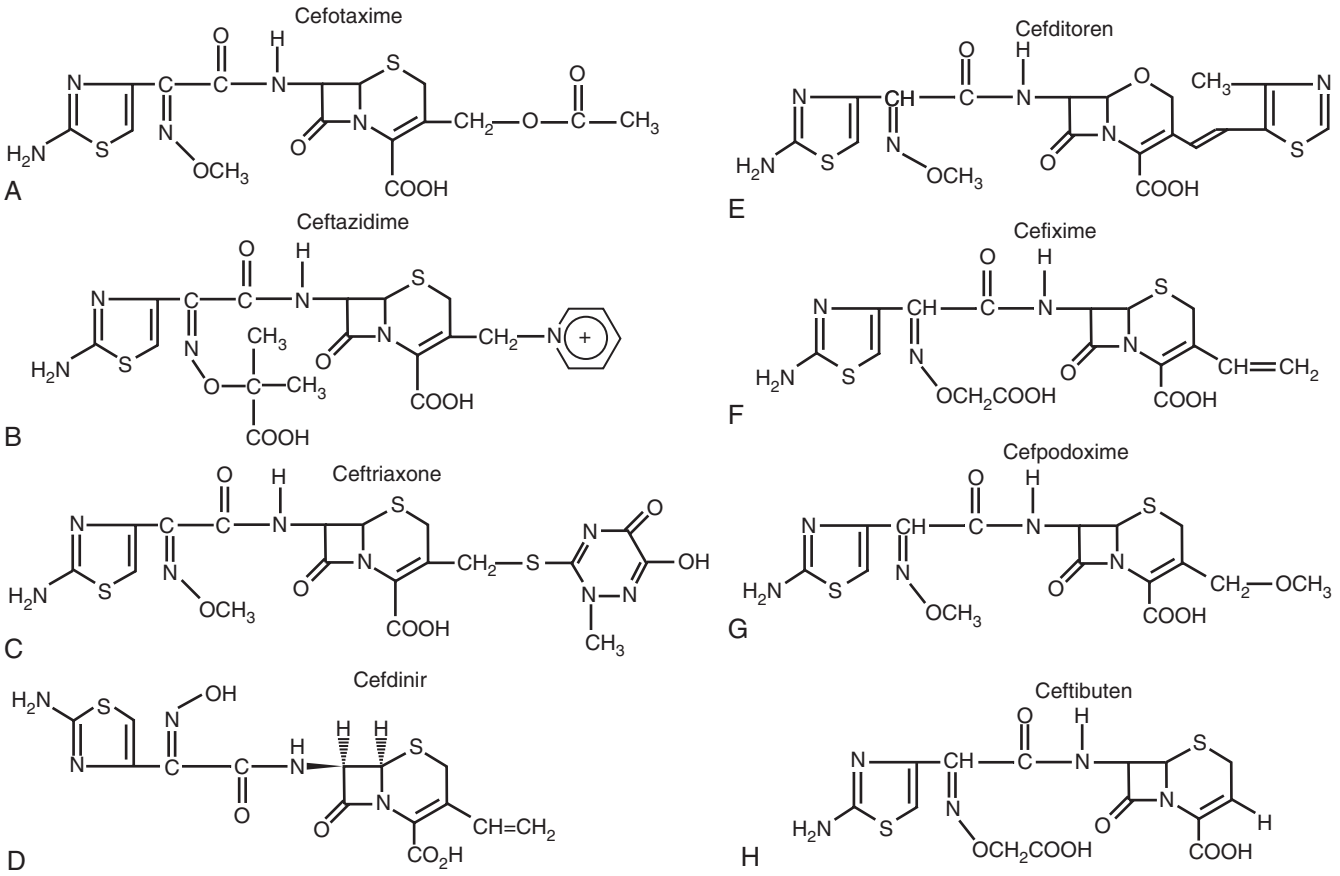

FIG. 21.4 Third-generation cephalosporins. (A) Cefotaxime. (B) Ceftazidime. (C) Ceftriaxone. (D) Cefdinir. (E) Cefditoren. (F) Cefixime. (G) Cefpodoxime. (H) Ceftibuten.

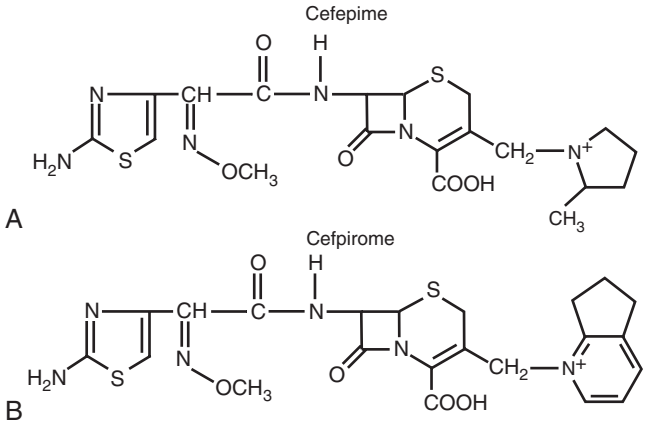

FIG. 21.5 Fourth-generation cephalosporins. (A) Cefepime. (B) Cefpirome.

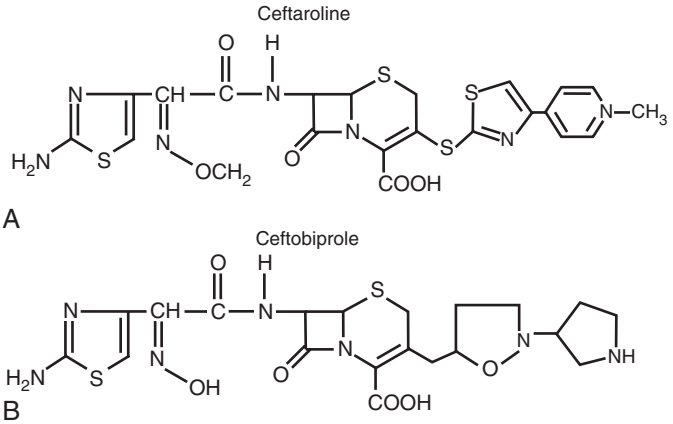

FIG. 21.6 Methicillin-resistant *Staphylococcus aureus*–active cephalosporins. (A) Ceftaroline. (B) Ceftobiprole.

spectrum of activity of the five groups. These drugs, such as cefepime and cefpirome, have activity against most gram-negative bacilli, including *P. aeruginosa,* and maintain their potency against gram-positive cocci. The third- and fourth-generation drugs combined are also called the extended-spectrum cephalosporins. The fifth group is referred to as the MRSA-active cephalosporins and currently includes ceftaroline and ceftobiprole. In addition to this unique activity against MRSA among the cephalosporins, these drugs also have enhanced activity against *Streptococcus pneumoniae* and *Enterococcus faecalis.* Their activity against gram-negative bacilli is similar to that of the third-generation cephalosporins. Ceftobiprole also has activity against *P. aeruginosa.*

More recently, a few cephalosporins susceptible to inactivation by extended-spectrum β-lactamases (ESBLs) have been combined with β-lactamase inhibitors (BLIs) to expand their activity against gram-negative bacilli. Ceftazidime is combined with avibactam, whereas ceftolozane is combined with tazobactam. The aforementioned siderophore cephalosporin and continued development of novel cephalosporin-BLI combinations with enhanced activity against drug-resistant gram-negative organisms exhibit mechanistic and spectrum novelties leading to distinct cephalosporin categories (see Table 21.1).

MECHANISM OF ACTION

The mechanism of antibacterial activity of cephalosporins is similar to that of other β-lactam drugs. Bacterial growth is inhibited by interference with the synthesis of the cell wall. The primary target of these compounds within the cell wall is the peptidoglycan cross-linkage structure.[28]

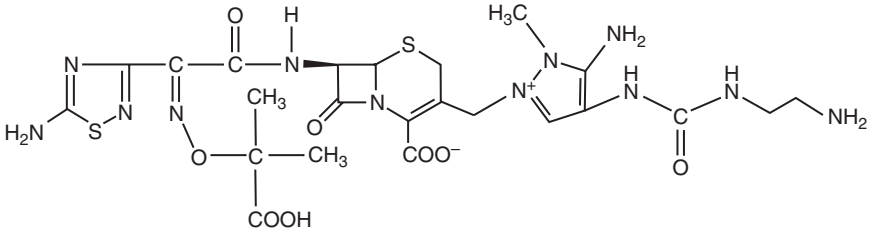

FIG. 21.7 Ceftolozane.

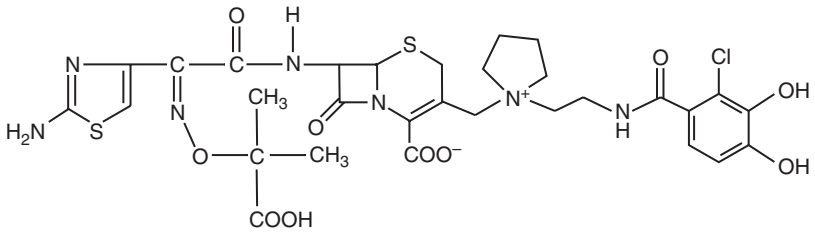

FIG. 21.8 Siderophore cephalosporin cefiderocol.

Peptidoglycans are polysaccharide chains consisting of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues. The polysaccharide chains are cross-linked at the pentapeptide side chain of the *N*-acetylmuramic acid residues to form a netlike structure. These structures are inserted into the cytoplasmic membrane from the cytoplasm by the action of a group of enzymes that includes transpeptidases, carboxypeptidases, and endopeptidases. The lactam ring provides for penicillins and cephalosporins a conformation similar to the terminal D-alanine-D-alanine of the pentapeptide.[29] These antibiotics bind covalently to these enzymes, in particular to the transpeptidases, resulting in loss of enzyme activity.[28] The enzyme drug targets are referred to as penicillin-binding proteins (PBPs).[30,31]

The location of the PBPs relative to the extracellular space differs between gram-positive and gram-negative bacteria. The peptidoglycan of gram-positive bacteria is located on the outer surface of the cell. Conversely, a complex lipopolysaccharide (LPS) structure is located on the outermost surface of gram-negative bacteria such that a cephalosporin must first penetrate or diffuse across the LPS membrane to reach the PBPs. The PBP targets within bacteria vary by size and affinity for different β-lactam antibiotics. It is generally agreed the high-molecular-weight PBPs (PBP1A, PBP1B, PBP2, and PBP3) are the most critical for cellular function, and inhibiting more than one high-molecular-weight PBP can provide superior antimicrobial action.[32,33] At low concentrations cephalosporins preferentially bind to PBP3 in gram-negative bacilli, resulting in filament formation with septae.[33,34] At higher concentrations, many cephalosporins also bind to PBP1A, PBP1B, or both, resulting in spheroplast formation and rapid lysis.[33] Cephalosporins with enhanced gram-positive activity (e.g., cefazolin) likely owe their effects to high-affinity binding of both PBP2 and PBP3.[33] Finally, as mentioned previously, the MRSA-active cephalosporins maintain their activity by means of binding to the altered penicillin binding protein PBP2A′ (mecA gene product), which confers methicillin resistance.

In general, cephalosporins are considered to be bactericidal drugs. The rate of killing of bacteria by cephalosporins exhibits minimal dependence on the concentration of the antibiotic.[35] Maximal bacterial killing is observed at concentrations four times the minimal inhibitory concentration (MIC). Cephalosporins produce persistent suppression of bacterial growth (i.e., the postantibiotic effect) of several hours' duration with gram-positive bacteria, but they induce very short or no postantibiotic effects with gram-negative bacilli.[36,37] The duration of time during which drug concentrations exceed the MIC is the major determinant of the antibacterial activity of the cephalosporins.[37,38,39] Net stasis efficacy of cephalosporins against gram-positive pathogens, such as *S. aureus* or *S. pneumoniae,* is noted at free-drug concentrations exceeding the MIC (T>MIC) for 25% to 40% of the dosing interval. Conversely, stasis activity for cephalosporins against most gram-negative organisms, including *P. aeruginosa,* occurs at free-drug concentrations T>MIC of 35% to 45% of the dosing interval, and maximal effects at about 60% of the dosing interval.

SPECTRUM OF ACTIVITY

The cephalosporins are active against a wide variety of aerobic and anaerobic bacteria (Tables 21.2 and 21.3).[a] Most drugs are active against streptococci and staphylococci, which is expected given their similar structure to penicillins. However, there are important differences in potency among cephalosporin agents noted for penicillin-resistant pneumococci. Ceftaroline and ceftobiprole have the greatest potency against these organisms, followed by cefditoren, ceftriaxone, cefotaxime, cefepime, and cefpirome.[70,74] A few members have less activity against methicillin-susceptible staphylococci, including the cephamycins, ceftazidime, cefixime, and ceftibuten. Extensive surveillance susceptibility data are lacking for newer cephalosporin-BLI combinations in terms of gram-positive coverage, but it is likely that the same activity exists for these (ceftazidime-avibactam and ceftolozane-tazobactam) as is noted for ceftazidime, given the structural similarity. Although MRSAs are resistant to all the earlier cephalosporins, the new MRSA-active cephalosporins ceftaroline and ceftobiprole exhibit low MICs of 0.25 to 2 μg/mL.[70,74] Enterococci have also consistently been resistant to the cephalosporins, with most MICs greater than 32 μg/mL. However, the new MRSA-active cephalosporins have much lower MICs for ampicillin-susceptible strains. These have ranged from 0.12 to 4 μg/mL for both drugs.[70,74]

The first-generation cephalosporins are not very active against *Haemophilus influenzae* or *Moraxella catarrhalis.* The second-generation drugs are about fourfold more potent against these respiratory pathogens. The third-generation cephalosporins have the lowest MICs for *H. influenzae* and *M. catarrhalis*—10 to 100 times lower than those of the second-generation drugs. The first-generation cephalosporins also are not as active against *Neisseria* spp. as the second-, third-, and fourth-generation drugs.

Although all of the cephalosporins are considered to be active against *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis,* the potency of the third- and fourth-generation drugs and the MRSA-active cephalosporins is 10- to 100-fold greater than that of the first- and second-generation cephalosporins. The increased potency of the later-generation

[a]References 16, 18, 40, 41-48, 49, 50-62, 63, 64, 65-73, 74, 75-82, 83, 84-87, 88, 89, 90, 91, 92, 93, 94, 95.

TABLE 21.1 Classification of Parenteral and Oral Cephalosporins

1ST GENERATION	2ND GENERATION	CEPHAMYCINS	3RD GENERATION	4TH GENERATION	5TH GENERATION (MRSA-ACTIVE CEPHALOSPORINS)	CEPHALOSPORIN–β-LACTAMASE INHIBITOR COMBINATIONS	SIDEROPHORE CEPHALOSPORINS
Parenteral Cephalosporins							
Cefazolin (Ancef, Kefzol) Cephalothin (Keflin, Seffin)[a] Cephapirin (Cefadyl)[a] Cephradine (Velosef)[a]	Cefamandole (Mandol)[a] Cefonicid (Monocid)[a] Cefuroxime (Kefurox, Zinacef)	Cefmetazole (Zefazone)[a] Cefotetan (Cefotan) Cefoxitin (Mefoxin)	Cefoperazone (Cefobid)[a] Cefotaxime (Claforan) Ceftazidime (Fortaz) Ceftizoxime (Cefizox)[a] Ceftriaxone (Rocephin) Moxalactam[a]	Cefepime (Maxipime) Cefpirome (Cefrom)[a]	Ceftaroline (Teflaro) Ceftibiprole (Zeftera)[a]	Ceftolozane-tazobactam Ceftazidime-avibactam	Cefiderocol
Oral Cephalosporins							
Cefadroxil (Duricef, Ultracef) Cephalexin (Keflex, Biocef, Keftab) Cephradine (Velosef)[a]	Cefaclor (Ceclor)[a] Cefprozil (Cefzil) Cefuroxime-axetil (Ceftin) Loracarbef (Lorabid)[a]		Cefdinir (Omnicef) Cefditoren (Spectracef) Cefixime (Suprax) Cefpodoxine (Vantin) Ceftibuten (Cedax)				

[a]No longer marketed in the United States.
MRSA, Methicillin-resistant *Staphylococcus aureus.*

TABLE 21.2 In vitro Activity of Cephalosporins Against Selected Gram-Positive Cocci, *Haemophilus Influenzae, Moraxella Catarrhalis,* and *Neisseria* Species

	STREPTOCOCCUS PNEUMONIAE (PSSP)	*S. PNEUMONIAE* (PRSP)	*STREPTOCOCCUS PYOGENES*	*STREPTOCOCCUS AGALACTIAE*	VIRIDANS STREPTOCOCCI GROUP	*STAPHYLOCOCCUS AUREUS* (MSSA)	*S. AUREUS* (MRSA)	*STAPHYLOCOCCUS EPIDERMIDIS*	*HAEMOPHILUS INFLUENAZAE*	*MORAXELLA CATARRHALIS*	*NEISSERIA MENINGITIDIS*	*NEISSERIA GONORRHOEAE*
First Generation												
Cefazolin	0.5/4	32/>32	0.12/0.12	0.12/0.12	0.12/0.12	0.5/2	—	0.5/>32	4/16	2/4	—	16/32
Cephalothin	0.12/0.25	8/16	0.05/0.10	0.12/0.5	0.25/0.50	0.12/0.5	—	0.5/32	4/8	4/8	0.25/0.5	8/32
Cefadroxil (O)	2/4	>32	0.12/0.25	0.25/2	—	2/8	—	4/>32	16/>32	2/4	—	8/64
Cephalexin (O)	1/2	>32	0.25/2	0.5/4	—	2/4	—	1/>32	8/16	2/8	2/2	4/16
Cephradine (O)	2/4	>32	0.25/2	0.5/2	—	1/4	—	4/>32	4/16	2/4	—	8/16
Second Generation												
Cefamandole	0.12/0.5	8/>32	0.12/0.12	0.12/0.5	0.12/4	1/1	—	0.5/>32	2/8	1/4	0.12/0.12	0.25/4
Cefonicid	0.5/1	4/>32	0.12/0.12	0.12/2	0.12/8	1/2	—	2/>32	0.5/1	1/4	0.12/2	0.06/0.5
Cefuroxime	0.12/0.25	4/>32	0.12/0.12	0.12/0.12	0.12/0.5	1/2	—	0.5/>32	1/2	0.5/2	0.12/2	0.015/0.25
Cefaclor (O)	0.5/1	16/>32	0.06/0.5	0.5/2	—	1/8	—	1/>32	2/32	0.5/2	0.06/0.25	0.25/16
Cefprozil (O)	0.12/0.5	8/>32	0.03/0.12	0.06/0.25	—	0.5/2	—	0.25/32	2/16	1/8	—	0.12/4
Loracarbef (O)	0.5/2	>32	0.5/2	0.5/2	—	1/4	—	4/>32	1/4	0.5/4	0.12/0.25	0.5/4
Cephamycins												
Cefmetazole	2/16	>32	0.5/0.5	2/2	2/4	4/16	—	8/>32	1/4	0.12/0.5	0.12/0.12	0.25/4
Cefotetan	8/16	>32	2/4	4/8	2/8	8/16	—	32/>32	1/2	0.12/2	0.12/0.25	0.25/2
Cefoxitin	2/4	32/>32	1/2	2/2	4/16	4/8	—	2/>32	1/4	0.25/0.5	0.12/0.25	0.25/4
Third Generation												
Cefoperazone	0.06/0.12	4/16	0.12/0.12	0.12/0.25	0.5/1	2/4	—	2/>32	0.015/0.25	0.12/2	0.12/0.5	0.03/0.06
Cefotaxime	0.015/0.06	0.5/2	0.015/0.015	0.03/0.25	0.06/0.25	2/2	—	4/>32	0.008/0.015	0.5/1	0.004/0.008	0.004/0.008
Ceftazidime	0.25/1	16/>32	0.12/0.25	0.25/0.5	1/2	8/32	—	8/>32	0.06/0.12	0.03/0.5	0.015/0.06	0.03/0.06
Ceftizoxime	0.25/1	16/32	0.015/0.015	0.12/0.12	0.25/2	4/8	—	4/>32	0.015/0.03	0.03/0.5	0.008/0.03	0.008/0.015
Ceftriaxone	0.03/0.06	0.5/2	0.015/0.03	0.03/0.06	0.06/0.25	2/4	—	4/>32	0.008/0.015	0.25/0.5	0.008/0.015	0.002/0.004
Moxalactam	1/1	—	1/2	—	—	8/16	—	8/>32	0.03/0.12	0.03/0.12	0.008/0.06	0.015/0.06
Cefdinir (O)	0.06/0.12	2/8	0.015/0.03	0.03/0.06	—	0.25/0.5	—	0.25/>32	0.12/0.5	0.06/0.25	0.06/0.25	0.008/0.06
Cefditoren (O)	0.015/0.03	0.5/2	0.008/0.015	0.06/1	—	0.25/1	—	0.25/>32	0.008/0.015	0.25/1	<0.06/0.06	0.004/0.06
Cefixime (O)	0.25/1	32/>32	0.06/0.25	0.12/0.25	—	16/>32	—	16/>32	0.015/0.12	0.03/0.5	<0.06/0.06	0.015/0.06
Cefpodoxime (O)	0.015/0.06	2/>32	0.06/0.12	0.03/0.12	—	2/4	—	2/>32	0.015/0.12	1/2	<0.06/0.06	0.06/0.06
Ceftibuten (O)	4/8	>32	0.5/2	4/16	—	16/>32	—	16/>32	0.06/0.12	2/4	0.06/0.25	0.015/0.5
Fourth Generation												
Cefepime	0.06/0.12	0.5/2	0.015/0.12	0.05/0.05	0.016/0.03	2/4	—	2/>32	0.06/0.12	1/4	0.03/0.06	0.03/0.06
Cepirome	0.03/0.12	0.5/2	0.008/0.06	0.06/0.06	0.06/0.25	1/2	—	1/>32	0.06/0.12	0.5/2	0.06/0.06	.015/0.12
Fifth Generation												
Ceftaroline	≤0.016/≤0.016	0.12/0.25	≤0.016/≤0.016	≤0.016/≤0.016	≤0.016/0.03	0.25/0.25	0.5/1	0.25/1	1/4	0.06/0.12	0.002/0.004	0.008/0.03
Ceftobiprole	≤0.06/≤0.06	0.25/0.5	≤0.06/≤0.06	0.06/0.12	≤0.06/0.25	0.25/0.5	0.5/2	0.12/2	0.5/4	0.12/0.5	0.002/0.004	0.03/0.06

Minimal inhibitory concentration (MIC) for 50% and 90% of strains in μg/mL.
MRSA, Methicillin-resistant *Staphylococcus aureus; MSSA,* methicillin-susceptible *S. aureus; O,* oral; *PSRP,* penicillin-resistant *Streptococcus pneumoniae; PSSP,* penicillin-susceptible *S. pneumoniae.*

TABLE 21.3 In vitro Activity of Cephalosporins Against Selected Aerobic and Anaerobic Gram-Negative Bacilli

	ESCHERICHIA COLI	*KLEBSIELLA PNEUMONIAE*	*PROTEUS MIRABILIS*	*ENTEROBACTER AEROGENES*	*ENTEROBACTER CLOACAE*	*CITROBACTER FREUNDII*	*SERRATIA* SPP.	*PSEUDOMONAS AERUGINOSA*	*MORGANELLA* SPP.	*BACTEROIDES FRAGILIS*	*SALMONELLA* SPP.	*SHIGELLA* SPP.
First Generation												
Cefazolin	2/16	2/>16	4/16	>32	>32	>32	>32	>32	>32	>32	2/4	2/8
Cephalothin	4/8	1/16	8/16	>32	>32	>32	>32	>32	>32	>32	2/4	4/8
Cefadroxil (O)	4/>16	8/>16	16/>32	>32	>32	>32	>32	>32	>32	>32	8/>16	4/16
Cephalexin (O)	8/>16	8/32	16/>32	>32	>32	>32	>32	>32	>32	>32	4/16	8/>16
Cephradine (O)	4/>16	4/>16	16/>32	>32	>32	>32	>32	>32	>32	>32	4/>16	8/>16
Second Generation												
Cefamandole	1/2	1/8	1/2	4/>32	2/>32	2/>32	16/>32	>32	4/>32	32/>32	0.5/4	0.5/2
Cefonicid	2/8	2/8	1/2	4/>32	8/>32	8/>32	>32	>32	16/>32	32/>32	2/8	2/8
Cefuroxime	2/8	2/16	2/4	8/>32	8/>32	8/>32	>32	>32	32/>32	8/>32	4/8	2/4
Cefaclor (O)	2/>16	2/32	2/4	>32	>32	>32	>32	>32	>32	>32	2/8	4/16
Cefprozil (O)	2/8	1/>32	2/2	>32	>32	16/>32	16/>32	>32	16/> 32	>32	2/8	4/16
Loracarbef (O)	1/>16	0.5/8	0.5/2	16/>32	16/>32	4/>32	>32	>32	32/>32	>32	0.5/8	0.25/8
Cephamycins												
Cefmetazole	0.5/1	1/2	2/4	>32	>32	>32	16/>32	>32	4/8	8/>32	0.5/2	1/2
Cefotetan	0.12/0.5	0.12/0.5	0.12/0.5	32/>32	8/>32	0.5/>32	1/8	>32	2/4	8/>32	0.12/0.12	0.12/0.5
Cefoxitin	2/8	2/8	2/4	>32	>32	>32	16/>32	>32	8/16	8/32	2/4	2/4
Third Generation												
Cefoperazone	0.12/8	0.25/8	0.5/1	0.25/8	0.25/8	0.5/32	2/8	4/>32	1/8	32/>32	0.5/4	0.25/1
Cefotaxime	0.06/0.25	0.06/0.25	0.06/0.25	0.12/16	0.25/32	0.25/>32	0.25/2	16/>32	0.25/4	8/>32	0.06/0.12	0.06/0.25
Ceftazidime	0.06/0.25	0.25/1	0.06/0.5	0.25/32	0.25/32	0.5/>32	0.25/2	2/16	0.12/0.5	>32	0.12/0.5	0.06/0.25
Ceftizoxime	0.03/0.12	0.03/0.12	0.008/0.015	0.06/16	0.06/16	0.25/>32	0.12/0.5	32/>32	0.25/2	16/>32	0.015/0.25	0.008/0.25
Ceftriaxone	0.06/0.12	0.06/0.25	0.008/0.03	0.25/16	0.25/>32	0.12/>32	0.25/4	32/>32	0.008/0.25	8/>32	0.06/0.25	0.03/0.12
Moxalactam	0.12/0.25	0.12/0.25	0.25/0.5	0.25/16	0.5/8	0.25/8	0.25/4	32/>32	0.25/0.5	2/32	0.12/0.25	0.12/0.25
Cefdinir (O)	0.12/0.25	0.06/0.25	0.06/0.12	0.5/>32	0.5/>32	0.25/>32	4/32	16/>32	4/16	16/ >32	0.12/0.25	0.25/0.5
Cefditoren (O)	0.25/0.5	0.25/1	0.12/1	0.5/>32	1/>32	1/>32	2/32	>32	—	4/>32	0.25/0/5	0.25/0.5
Cefixime (O)	0.12/0.25	0.03/0.12	0.008/0.03	0.5/>32	0.12/>32	2/>32	2/>32	>32	2/32	16/>32	0.06/0.25	0.25/0.5
Cefpodoxime (O)	0.25/1	0.5/2	0.06/0.12	2/>32	4/>32	2/>32	1/8	>32	2/>32	16/>32	0.5/1	0.12/0.25
Ceftibuten (O)	0.12/0.25	0.06/0.25	0.015/0.03	1/32	2/>32	1/>32	0.5/8	>32	0.25/8	16/>32	0.06/0.25	0.06/0.25
Fourth Generation												
Cefepime	0.03/0.06	0.03/0.25	0.06/0.12	0.06/0.5	0.06/2	0.06/2	0.12/0.5	2/16	0.03/0.12	>32	0.06/0.12	0.03/0.06
Cefpirome	0.06/0.12	0.06/0.25	0.06/0.12	0.06/0.5	0.12/4	0.03/2	0.25/2	2/16	0.03/0.12	32/>32	0.06/0.25	0.06/0.12
Fifth Generation												
Ceftaroline	0.06/0.5	0.06/0.25	0.06/4	0.12/8	0.12/1	0.5/8	0.06/16	16/128	0.06/2	8/>32	0.12/0.25	0.03/0.12
Ceftobiprole	0.06/0.12	0.06/>8	0.03/0.12	0.03/>32	0.12/>32	0.12/4	0.06/2	4/16	—	16/>64	0.03/0.06	0.03/0.06
Cephalosporin–β-Lactamase Inhibitor												
Ceftolozane-tazobactam	0.12/0.5 (all) 0.5/4 (ESBL)	0.25/8 (all) 0.5/64 (ESBL) ≥32/≥32 (KPC)	0.25/0.5 (all) 1/8 (ESBL)	0.25/8	0.25/8	0.25/8	0.5/1	0.5/2 (all) 4/>32 (MDR)	—	1/4	—	—
Ceftazidime-avibactam	0.06/0.12 (all) 0.12/0.25 (ESBL)	0.12/0.5 (all) 0.25/1 (ESBL) 0.25/1 (KPC)	0.06/0.12	0.25/0.5	0.25/1	0.25/0.5	0.25/0.5	2/8 (all) 4/32 (MDR)	0.06/0.12	4/32	0.25/0.5	—

Minimal inhibitory concentration (MIC) for 50% and 90% of strains in μg/mL.
ESBL, Extended-spectrum β-lactamase producing organisms; *KPC, Klebsiella pneumoniae* carbapenemase–producing organisms; *MDR,* multidrug-resistance; *O,* oral.

drugs extends to strains of *Enterobacter, Serratia, Citrobacter,* and *Morganella* spp., which are usually resistant to the first- and second-generation drugs. Several cephalosporins, such as ceftazidime, ceftolozane, cefoperazone, cefepime, cefpirome, and ceftobiprole, are active against many strains of *P. aeruginosa.* The addition of β-lactamase inhibitors (e.g., avibactam and tazobactam) significantly increases the activity of cephalosporin-BLI combination drugs (e.g., ceftazidime-avibactam and ceftolozane-tazobactam) against drug-resistant gram-negative rods.

The third- and fourth-generation cephalosporins also exhibit enhanced potency against strains of *Salmonella* and *Shigella.* Many cephalosporins are active against penicillin-susceptible gram-positive anaerobes, such as peptostreptococci.[96,97] Among the cephalosporins, the best activity against *Bacteroides fragilis* is with the cephamycins and the cephalosporin-BLI combinations. Many of the drugs are active against spirochetes, including the agents of Lyme disease and syphilis.[98,99] As a group, the cephalosporins have very poor activity against *Chlamydia, Mycoplasma,* and *Listeria* spp.[100-102]

MECHANISMS OF RESISTANCE

Four general mechanisms can result in resistance to cephalosporin antibiotics: (1) antibiotic destruction by hydrolyzing β-lactamase enzymes, (2) reduced penetration of the antibiotic through the LPS membrane to the PBP target, (3) enhanced efflux of the drug from the periplasmic space, and (4) alteration in the PBP target, resulting in reduced binding affinity. Most often, resistance in a bacterial population is caused by a single mechanism; however, an increasing percentage of organisms, particularly Enterobacteriaceae and *P. aeruginosa,* exhibit multiple mechanisms.[103,104,105,106,107]

Production of β-lactamase enzymes that hydrolyze the β-lactam ring is a common resistance mechanism for many gram-negative bacteria. Although all gram-negative bacilli produce β-lactamase enzymes, the type and amount of enzyme vary among organisms. These enzymes are located in the periplasmic space between the outer and inner membranes that comprise the gram-negative cell wall. The penetration of drugs across the outer membrane is through water-filled channels formed by various membrane proteins, termed *porins.* Movement through porin channels depends on the size, shape, charge, and hydrophilic properties of the compound. Drugs that are able to penetrate the outer membrane via porins are then subject to degradation by β-lactamase enzymes before being able to reach the PBP target. The net antimicrobial activity of cephalosporins against gram-negative bacilli is dependent on both the rate of penetration across the outer membrane (i.e., rate of movement through porins) and the stability of the drug to the various hydrolyzing β-lactamases. The concentration of a drug with a slow rate of penetration is low relative to the amount of β-lactamase within the periplasmic space. In this case, if there are sufficiently active β-lactamase enzymes present, the result would be near-complete antibiotic inactivation. Conversely, a drug can penetrate so rapidly that even if active β-lactamase enzymes exist, the antibiotic can remain active because the enzymatic capacity for degradation is overwhelmed. For example, the zwitterion of cefepime enhances movement across the membrane, resulting in very high concentrations in the periplasmic space and a relative net resistance to drug hydrolysis.[108]

The number of unique β-lactamases is markedly increasing, with more than 1000 distinct enzymes; even a single amino-acid difference from one enzyme to another may affect phenotype. These proteases may be genetically encoded chromosomally or extrachromosomally (i.e., mobile elements). Stable derepression of a chromosomal mutation is a common genotypic scenario. This is observed predominantly in *Enterobacter* spp., *Serratia* spp., *Citrobacter freundii,* and *P. aeruginosa.*[109] Transmissible plasmids are the largest and most common reason for resistance in Enterobacteriaceae, especially for *E. coli* and *K. pneumoniae.* Multiple classification schemes have been devised; the simplest is the molecular or Ambler classification (see Chapter 18 for further details).[110] Examples of the most relevant β-lactamase enzymes resulting in cephalosporin resistance are discussed here.

Class A β-lactamases TEM (Temoneira) and SHV (sulfhydryl variable) confer resistance most commonly to penicillins and earlier classes of cephalosporins. However, newer enzyme variants have arisen as the result of amino-acid substitutions related to point mutations in the common β-lactamase genes (i.e., TEM and SHV). These are capable of inactivating many third- and fourth-generation cephalosporins.[111,112] These enzymes have been observed most commonly in *K. pneumoniae* and *E. coli* and are referred to as extended-spectrum β-lactamases (ESBLs). The new MRSA-active cephalosporins are also susceptible to inactivation by ESBLs.[113,114] More recently, the cefotaxime/ceftazidime-hydrolyzing (CTX-M) family of β-lactamases, derived from the chromosomal enzyme of *Kluyvera* spp., have spread into *K. pneumoniae* and *E. coli.*[115] CTX-M enzymes commonly confer resistance to cephalosporins including extended-spectrum cephalosporins, such as ceftazidime and cefepime. Indeed, CTX-M variants have been responsible for the massive explosion of ESLB-positive clinical isolates of *E. coli* throughout the world.[116,117] Finally, the first enzyme member responsible for carbapenem resistance in Enterobacteriaceae (now commonly termed CRE) was the KPC (*Klebsiella pneumoniae* carbapenemase) variant, which breaks down nearly all traditional β-lactam therapies including cephalosporins and carbapenems.[118,119] As discussed later, there have been successful efforts to combat class A β-lactamases that confer extended-spectrum β-lactam resistance (i.e., CTX-M) and carbapenem resistance (i.e., KPC) with newer cephalosporin therapies.

Class B β-lactamases are termed the *metallo-β-lactamases* owing to the dependence of zinc ions for activity rather than serine found in the other classes. VIM (Verona integron-encoded metallo-β-lactamase), NDM (New Delhi metallo-β-lactamase), and IMP (imipenemase) are examples and are all members that produce the carbapenem-resistant phenotype similar to KPC enzymes. Cephalosporins are rendered inactive, and unfortunately, novel cephalosporin antimicrobials remain inactive against the metallo-β-lactamases.

The prototypical class C β-lactamases are the AmpC enzymes, which are potent hydrolyzing enzymes for many cephalosporins.[120] The AmpC cephalosporinase is capable of inactivating almost all current cephalosporins, including the cephamycins. Emergence of this type of resistance is frequent when infections resulting from these organisms are treated only with broad-spectrum cephalosporins.[121,122] Cefepime, cefpirome, and ceftobiprole are less susceptible to inactivation by AmpC β-lactamases.[113,123,124]

Class D β-lactamases are termed OXA for their oxacillinase enzymatic activity. There are many OXA subgroups with varied phenotypic effects. A number of them, for example OXA-11 and OXA-15, hydrolyze cephalosporins including extended-spectrum cephalosporins, and resistance to numerous β-lactam drugs including carbapenems has been observed in organisms carrying OXA-48.[106,110]

Novel members of the cephalosporin class have been developed to combat β-lactamase resistance mechanisms. Ceftazidime-avibactam was developed specifically for activity against class A, C, and some class D enzymes.[91] The retained activity against these organisms is exclusively due to the presence of avibactam, a novel BLI developed in recent years. The result of this combination is an effective option for many of the most concerning epidemic types of β-lactamase-producing Enterobacteriaceae, which includes those that have the CTX-M or KPC enzymes. Although this is encouraging, not surprisingly, soon after release reports of resistance to ceftazidime-avibactam emerged.[125,126] Ceftolozane-tazobactam is similarly active against many class A and C enzymes, primarily though tazobactam activity.[16] The combination is active against many ESBL organisms, most importantly the CTX-M family, and maintains high stability in the face of AmpC β-lactamases, but unlike ceftazidime-avibactam, it does not have appreciable activity against KPC producers or class D enzymes (e.g., OXA). Finally, siderophore cephalosporins have demonstrated potent activity against class A, B, C, and D β-lactamases including carbapenemase enzymes such as KPC, NDM, IMP, VIM, and OXA.[127]

It is unlikely that deletion or mutation of porin proteins causes primary resistance to cephalosporins. However, such changes can alter the relationship between the concentrations of drug and β-lactamase in the periplasmic space, resulting in much more hydrolysis of the cephalosporin. For example, strains of *K. pneumoniae* containing ESBLs have been shown to be resistant to cephamycins because of the lack of an outer membrane porin protein.[128] Porin-deficient strains are especially high in *Enterobacter aerogenes.*[129] The endogenous AcrAB multidrug efflux system in *E. coli* affects the potency of penicillins but has little

effect on the activity of cephalosporins.[130] However, a major contribution to resistance in *P. aeruginosa* has been associated with porin-efflux pump systems, such as the MexAB-OprM efflux pump for ceftazidime and the MexXY-OprM efflux pump for cefepime and ceftobiprole.[131-134] The differences among these organisms in the impact of somewhat similar pumps are probably due to the markedly higher outer membrane permeability in *E. coli* compared with *P. aeruginosa*.[135]

Most cephalosporins, with the exception of cephaloridine, are poorly hydrolyzed by staphylococcal penicillinases. Thus, enhanced activity against many gram-positive organisms is noted for the early-generation cephalosporins in addition to some third- and fourth-generation cephalosporins such as ceftriaxone and cefepime. This differential activity of the cephalosporin class to gram-positive and select gram-negative pathogens is primarily due to differences in affinity to PBP2. Resistance to cephalosporins is therefore due almost entirely to alteration of the PBP2 target—for example, cephalosporin-resistant *S. pneumoniae,* MRSA, *H. influenzae,* and some *Neisseria gonorrhoeae* strains.

PHARMACOLOGIC PROPERTIES

The pharmacologic properties of selected cephalosporins are listed in Table 21.4.[b] Cephalosporins are polar, water-soluble compounds. Within each of the first-, second-, and third-generation classifications, there are both oral and parenteral formulations. The fourth- and fifth-generation compounds are available for parenteral use only. The parenteral formulations are available for both intravenous and intramuscular administration. All of the parenteral formulations, with the exception of cephradine, are stable in solution at room temperature for 24 hours or longer. Drug stability at room temperature facilitates use of these compounds for home intravenous therapy, including continuous infusions. Many of the parenteral compounds can also be administered by the intraperitoneal route for treatment of peritoneal infections associated with continuous ambulatory peritoneal dialysis.[169] Formulations of the oral cephalosporins are available as tablets, capsules, or suspensions.

In contrast to many other β-lactams, oral preparations of the cephalosporins are stable in the acid milieu of the upper gastrointestinal tract. Cephalosporins can be actively absorbed if their structure mediates transport by the dipeptide and tripeptide transport systems in the brush border membrane of the small intestine.[170] Cephalexin, cephradine, cefadroxil, cefaclor, cefprozil, and loracarbef have an aminobenzyl group or a similar group in the C7 position and have high oral bioavailability (80% to 95%). Ceftibuten, cefixime, and cefdinir have other groups in the C7 position and exhibit more variable bioavailability after oral administration. Absorption by the dipeptide and tripeptide transport systems appears to be both pH and calcium dependent.[170] Drugs that have low oral bioavailability can be esterified to enhance absorption; the ester prodrug is hydrolyzed after absorption in the intestinal epithelial cells. The esters commonly used include axetil, proxetil, and pivoxil formulations.[171] Ester prodrug formulations exist for cefuroxime, cefditoren, and cefpodoxime. Absorption of the ester is still not complete; in fact, the percent oral bioavailability of ester formulations is lower than that of most nonesterified compounds. Absorption of the ester formulations is enhanced by concomitant food intake[171] because food within the stomach delays gastric emptying and prolongs contact with the mucosal surface.

Distribution of cephalosporins within the body is governed by the lipid solubility of the drug and the extent of protein binding. β-Lactams bind almost exclusively to albumin. The extent of protein binding can vary from less than 10% to as much as 98%.[160] Because only unbound drug can pass through capillary pores into interstitial fluid or across cell membranes into intracellular fluid, avidly bound compounds tend to exhibit high serum concentrations and low tissue concentrations. In general, the cephalosporins are largely confined to the extracellular compartment. Drug concentrations in subcutaneous blisters, a model for extracellular drug penetration, are similar to those found in serum.[157,172] Techniques measuring extracellular drug concentrations in human tissues (e.g., microdialysis) have demonstrated that concentrations of unbound drug in interstitial fluid are also similar to those in serum.[173,174] The cephalosporins have relatively poor intracellular concentrations such that tissue homogenates, which mix intracellular and extracellular fluid, always provide concentrations that are lower than those in serum because of dilution by the larger intracellular volume.[172] This group of compounds does not achieve intracellular concentrations adequate to treat most intracellular pathogens (e.g., *Legionella* spp.).[175]

In the absence of infection, drug concentrations in the cerebrospinal fluid (CSF) and in the vitreous humor are low. None of the oral cephalosporins achieve therapeutic concentrations in the CSF. Penetration of most parenteral drugs from the first- and second-generation groups is similarly poor. Parenteral cefuroxime is an exception, and this drug also has the lowest MICs for common meningeal pathogens among the first- and second-generation cephalosporins.[160] The parenteral third- and fourth-generation drugs, such as ceftriaxone, cefotaxime, ceftazidime, and cefepime, achieve concentrations that would allow treatment of central nervous system infections.[161-166] The presence of an active transport system that transports many cephalosporins from the CSF back to serum contributes to the low drug levels in the CSF observed with many of the earlier-generation drugs. The transport protein involved in this system is similar to the protein involved in renal secretion of β-lactam antibiotics.[176] Ceftriaxone, cefotaxime, ceftazidime, and cefepime exhibit minimal renal tubular secretion and are poor substrates for the choroid plexus pump, contributing to higher CSF concentrations. Probenecid is a competitive substrate for this pump and can produce higher concentrations with drugs that are effluxed by this transport system.[177] Infection results in higher CSF levels because inflammation can enhance penetration and interfere with efflux by active transport.

Very few drugs from the cephalosporin class are extensively metabolized. The three exceptions are cefotaxime, cephalothin, and cephapirin, which undergo deacetylation of the acetoxymethyl side chain in the liver.[178,179] The metabolic desacetyl products still possess modest microbiologic activity. The elimination half-life of desacetylcefotaxime is significantly longer than that of the parent compound, allowing less frequent administration of this cephalosporin.[179] The remaining drugs in the cephalosporin class are excreted from the body unchanged.

Most cephalosporins are eliminated by the kidney, with half-lives of 1 to 2 hours. The major mechanism for renal excretion of many compounds is tubular secretion. This active transport process is largely unaffected by protein binding.[160] Probenecid inhibits this organic acid transport system and can prolong the half-life of these compounds. For several compounds, glomerular filtration is more important, and protein binding can significantly prolong their elimination half-life.[180] For some drugs, the elimination half-life is 3 to 8 hours, allowing for 12- and 24-hour dosing intervals. A few compounds with high protein binding and high molecular weights, such as ceftriaxone and cefoperazone, are eliminated to a large extent by the biliary route.[181] Between 50% and 70% of the active parent compound may be recovered in the bile and eventually in the feces.

The maximal daily doses of agents eliminated primarily by the kidney must be reduced for patients with renal impairment. Most often, this adjustment includes both a reduction in dose level and a lengthening of the dosing interval. Recommended dose adjustments for various degrees of renal impairment and for patients receiving dialysis are presented in Table 21.5 for the currently available cephalosporins.[182,183] For drugs eliminated by the biliary system, such as ceftriaxone, dose adjustments are unnecessary unless concomitant severe hepatic insufficiency and renal insufficiency are present.[182] Most of the cephalosporins excreted by the renal route are eliminated by hemodialysis. Between 20% and 50% of the parent compound is removed after a usual dialysis session. It is recommended that the drug be given again after hemodialysis. On the other hand, few cephalosporins are removed to any significant extent (<10%) by peritoneal dialysis, and additional dosing is not recommended after a peritoneal dialysis session. Continuous venous hemofiltration (CVH) is frequently used in critically ill patients. Compounds eliminated by the kidneys and by hemodialysis are also removed by CVH. Most often, the efficiency of drug removal is thought to be similar to a creatinine clearance of 10 to 30 mL/min, and appropriate dosing modification is recommended.[184]

[b]References 15, 16, 79, 91, 136-138, 139, 140-145, 146, 147-159, 160, 161-168.

TABLE 21.4 Pharmacokinetics of Cephalosporins

CEPHALOSPORIN	USUAL ADULT DOSING REGIMEN	PEAK SERUM CONCENTRATION (μg/mL)	HALF-LIFE (h)	SERUM PROTEIN BINDING (%)	ROUTE OF EXCRETION	CSF CONCENTRATION RANGE (μg/mL)	CSF PENETRATION (%)	STABILITY AT ROOM TEMPERATURE (h) OR ORAL BIOAVAILABILITY (%)
First Generation								
Cefazolin	0.5–1 g q8h	193 (1 g)	1.9	74–86	R (65%–100%)	<0.7	0–4	24
Cephalothin	0.5–2 g q4–6h	64 (1 g)	0.5–1.0	50–80	R (50%–70%)	0.16–0.31	1	24
Cephapirin	0.5–2 g q4–6h	70 (1 g)	0.6	50–60	R (60%–85%)	NS	NS	24
Cephradine	0.5–1 g q6h	50 (1 g)	0.7	8–17	R (75%–100%)	NS	NS	2–10
Second Generation								
Cefamandole	0.5–2 g q6h	88 (1 g)	0.7–1.3	50–78	R (80%)	0.35–7.4	0–8.6	24
Cefonicid	0.5–1 g q24h	221 (1 g)	4.4	98	R (95%)	NS	NS	24
Cefuroxime	0.75–1.5 g q8h	39 (1 g)	1.2–1.8	33–50	R (70%–100%)	0.35–22.5	11.6–13.7	24
Cephamycins								
Cefmetazole	2 g q8h	143 (2 g)	1.3–1.8	68	R (75%–85%)	NS	NS	24
Cefotetan	1–2 g q12h	158 (2 g)	3.5	76–90	R (80%)	1.1–4.8	0.8–3.6	24
Cefoxitin	1–2 g q6h	110 (1 g)	0.8–1	41–79	R (90%)	1.2–22	0.8–35	24
Third Generation								
Cefoperazone	1–3 g q8–12h	153 (1 g)	1.6–2.1	90	H (80%) R (20%)	<0.8–119	2.5–5.9	12
Cefotaxime	1–2 g q12h	102 (1 g)	1–1.2	35–40	R (50%–80%)	1–83	4–55	24
Ceftazidime	1–2 g q8–12h	107 (1 g)	1–2	17	R (80%–90%)	1.4–30	14–45	24
Ceftizoxime	1–2 g q6–12h	113 (1 g)	1.4–1.7	31	R (70%–100%)	<0.5–29	3–22.6	24
Ceftriaxone	1–2 g q12–24h	145 (1 g)	6.4	85–95	R (50%) H (40%)	2–20	1.5–7	72
Moxalactam	1–2 g q8h	70 (1 g)	2.2	50	R (67%–88%)	0.8–39	12–69	24
Fourth Generation								
Cefepime	1–2 g q12h	79 (1 g)	2	16–19	R (85%)	5.7	11.8	24
Cefpirome	1–2 g q12h	80 (1 g)	2	10	R (90%)	0.8–4.2	5–67	24
Fifth Generation (MRSA Active)								
Ceftaroline	0.6 g q8–12h	21 (0.6 g)	2.6	7–19	R (85%)	NS	NS	24
Ceftobiprole	0.5 g q8–12h	34 (0.5 g)	2.85	17	R (82%)	NS	NS	24
Cephalosporin-BLI Combinations								
Ceftolozane-tazobactam	1 g/0.5 g q8h	75/18	3.1/1.0	16–21/30	R (95%/80%)	NS	NS	24
Ceftazidime-avibactam	2 g/0.5 g q8h	90/15	2.8/2.7	10/8	R (80%–90%/97%)	NS	NS	12
Oral—First Generation								
Cefadroxil	0.5–1 g q12h	15 (0.5 g)	1.3–1.6	20	R (90%)	NA	NA	80%
Cephalexin	0.5–1 g q6h	5.8 (250 mg)	0.5–1.2	6–15	R (80%–100%)	NA	NA	90%
Cephradine	0.5–1 g q6h	15 (0.5 g)	1–2	10–20	R (80%–90%)	NA	NA	95%
Oral—Second Generation								
Cefaclor	250–500 mg q8h 375 mg q12h	6 (250 mg) 19.2 (400 mg)	0.5–1	25–50	R (50%–80%)	NA	NA	50%–90% FE
Cefprozil	500 mg q12h	9.3 (500 mg)	1.3	35–45	R (61%)	NA	NA	95%
Cefuroxime (axetil)	250–500 mg q12h	4.6 (250 mg)	1.2	33–50	R (66%–100%)	NA	NA	52%–68% FE
Loracarbef	200–400 mg q12h	8 (200 mg)	1.0	25	R (87%)	NA	NA	90%
Oral—Third Generation								
Cefdinir	300 mg q12h	2.9	1.5–1.7	60–73	R (18%)	NA	NA	25%
Cefditoren (pivoxil)	200–400 mg q12h	2.5 (200 mg)	0.8–1.6	88	R (16%–22%)	NA	NA	17% FE
Cefixime	200–400 mg q12–24h	2.8 (200 mg) –4.5 (400 mg)	3–4	65–70	R (50%) H (5%)	NA	NA	40%–50%
Cefpodoxime (proxetil)	200 mg q12h	2.2 (200 mg)	2.2–2.7	18–40	R (29%–33%)	NA	NA	50%–80% FE
Ceftibuten	400 mg q24h	15 (400 mg)	2.4	65–77	R (57%)	NA	NA	75%–90%

BLI, β-Lactamase inhibitor; *CSF,* cerebrospinal fluid; *FE,* food effect; *H,* hepatic; *MRSA,* methicillin-resistant *Staphylococcus aureus*; *NA,* not applicable because oral cephalosporins are not indicated for CSF-related infections; *NS,* not studied in robust human pharmacokinetic studies and/or limited to case reports without pharmacokinetic analyses; *R,* renal.

ADVERSE REACTIONS AND TOXICITIES

The safety profile of the cephalosporins as a class is generally favorable. The incidence of specific adverse reactions for these compounds is relatively similar among drugs within the class, with few exceptions (Table 21.6). As with other β-lactam drugs, hypersensitivity reactions are the most common adverse effect associated with cephalosporin therapy.[185,186] The frequency of hypersensitivity reactions to cephalosporins is less than that for penicillins. Various cutaneous rashes, often associated with eosinophilia and occasionally with fever, occur in 1% to 7% of patients receiving these drugs.[187] More severe hypersensitivity reactions, such as serum sickness, anaphylaxis, or angioedema, occur very infrequently. These immunoglobulin E (IgE)-mediated reactions are estimated to occur in fewer than 1 in 100,000 patients.[188] However, there have been reports of a strong association between serum sickness in children and use of cefaclor.[189]

Cross-reactivity among drugs from the cephalosporin class and other β-lactams has been extensively investigated.[190] Important to note, the risk appears to depend on the similarity of side chains on the molecule to those on penicillins or other cephalosporins.[191] For example, in patients with allergy to amoxicillin, cross-reactivity was observed in 38% of those receiving cefadroxil, which has a similar side chain, and in none of those receiving cefamandole, which has a different side chain.[192] Recent estimates suggest a cross-reaction frequency of 1% or less.[193,194,195] Cross-reactivity with second-, third- and fourth-generation drugs has been very low.[190,193,196]

Use of the penicillin skin test to predict cephalosporin reactions is unreliable. In a study of almost 100 individuals who were given a cephalosporin and had a history of reaction to the penicillin determinants used in skin testing, only 1 patient had a reaction.[197] The practical clinical decision to use a cephalosporin in a patient with a prior history of reaction to either a penicillin or another cephalosporin should be guided by the severity of the prior reaction and the cephalosporin to be used. In patients with prior nonsevere, non–IgE-mediated reactions to other β-lactams, use of a cephalosporin with a different side chain than the prior agent is considered safe.[190,191,196] Although the risk of a similar reaction is slightly increased, the reactions are rarely severe. However, in the setting of a prior severe IgE-mediated reaction with another β-lactam compound, use of a cephalosporin is discouraged.

Immunologically mediated reactions to cephalosporins may manifest as hematologic or renal toxicities. Eosinophilia is the most commonly reported laboratory abnormality.[198] Overt hematologic toxicity—such as anemia, leukopenia (e.g., neutropenia), or thrombocytopenia—with cephalosporin use is uncommon.[199] Hemolytic anemia due to cephalosporin use, in particular ceftriaxone, has been reported but is rare.[200-202] Although the results of Coombs laboratory tests are reported to be positive in a significant percentage of patients receiving many of the cephalosporins, these patients most often do not have hemolytic anemia[203] because this is a false-positive reaction related to cross-reactivity that occurs in up to 3% of treated patients. Neutropenia is a potential complication of high-dose or prolonged use of β-lactams including cephalosporins; however, the effects are limited to the antibiotic exposure period, and cell counts recover after discontinuation.[204-207] Acute interstitial nephritis (AIN) is a well-described form of kidney injury secondary to β-lactam use. Drug-induced AIN due to cephalosporins commonly manifests as an abrupt decline in renal function; urine sediment with white cells, red cells, and white cell casts; proteinuria; and eosinophiluria.[208] Classic symptoms attributed to AIN including skin rash, eosinophilia, and fever are not common, and many patients are asymptomatic; the mainstay of treatment is withdrawal of the offending agent. Direct cytotoxicity to renal tubular cells can occur, particularly with an older cephalosporin cephaloridine, but this is quite rare.[209,210]

Nonimmunologic hematologic toxicities have been reported with a similarly low frequency. Bleeding abnormalities have been related to two mechanisms. Impaired adenosine diphosphate–induced platelet aggregation has been reported with moxalactam[211] but not with other cephalosporins. Another coagulopathy is specifically associated with the MTT side chain present on cefamandole, cefotetan, cefoperazone, and moxalactam.[212] The MTT side chain can dissociate from the parent cephalosporin and act as a competitive inhibitor of the vitamin K–dependent carboxylase responsible for converting clotting factors II, VII, IX, and X to active forms.[213] In addition, other cephalosporin side chains may interact with warfarin and increase inhibition of vitamin K 2,3-epoxide reductase, which converts vitamin K to its active form.[214,215] These reactions can lead to prolongation of the prothrombin time and clinically significant bleeding. Patients with poor nutritional status, advanced age, or recent surgery on the gastrointestinal tract are at increased risk for clinically significant bleeding.[216-218] In addition, patients with renal failure may be at increased risk for bleeding because of the accumulation of the side chain.[219] Vitamin K administration rapidly reverses the abnormality in 24 to 36 hours.

The MTT side chain can also produce a disulfiram-like reaction with ethanol ingestion that may persist for several days after antibiotic administration.[27,220] The disulfiram reaction manifests with flushing, tachycardia, headache, sweating, nausea, vomiting, hypotension, confusion, or blurred vision. The reaction is caused by a block in alcohol metabolism at the acetaldehyde step, which results in the accumulation of acetaldehyde and subsequent symptoms.

A variety of adverse reactions within the gastrointestinal tract have been reported with variable frequency. Diarrhea is the most commonly reported side effect, with rates ranging from 1% to 20%.[187] Upper gastrointestinal symptoms occur much less frequently. Mild and transient hepatic toxicity has been reported with most compounds from the class and manifests as twofold to fourfold elevations in transaminase levels in up to 7% of patients.[221] Obstructive biliary toxicity has also been reported with ceftriaxone.[222,223] The high biliary ceftriaxone concentrations cause crystallization of a ceftriaxone-calcium salt and the clinical syndrome of biliary pseudolithiasis. This biliary abnormality has been reported most often in children who were receiving high doses of ceftriaxone and in patients with preexisting biliary abnormalities.[224] The syndrome is reversible and in most reports has cleared within 10 to 60 days after discontinuation of the drug. It was recommended that ceftriaxone and calcium-containing products not be mixed in vials or infusion lines for therapy in neonates younger than 28 days because of the risk of precipitation in their plasma. This warning was later retracted because other cephalosporins had a similar low precipitation risk.[225]

Adverse reactions in the nervous system are uncommon and are similar in nature to those reported with other β-lactams.[226,227] The main mechanism of neurotoxicity is inhibition of γ-aminobutyric acid A.[227] Encephalopathy and seizures have been reported primarily in patients with renal insufficiency who were receiving high doses of these drugs, in particular with cefepime use.[226-231] Decreased protein binding and inhibition of the choroid plexus pump occur with uremia and may contribute to enhanced toxicity in patients with renal impairment. Local phlebitis reactions related to intravenous administration of the parenteral compounds have been reported with variable frequency, ranging from 1% to 5%.[232] On the other hand, pain associated with intramuscular administration is common with all of the parenteral compounds.[233] The local discomfort can be reduced by the use of 1% lidocaine in diluent. With the exception of once-daily intramuscular ceftriaxone and cefepime, most parenteral cephalosporins are administered by the intravenous route.

The cephalosporins have not been studied extensively in pregnancy. All drugs within the cephalosporin group are placed in pregnancy category B.[234] All of the compounds are secreted to a small degree into breast milk, but as a class they are considered safe for use in this situation.

In addition to the false-positive Coombs test result, laboratory abnormalities in urine glucose and serum creatinine have been reported with certain cephalosporins. False-positive results on glucosuria tests performed with the copper reduction technique (Clinitest) have been reported with cefaclor, cefadroxil, cefamandole, cefonicid, cefotaxime, cefoxitin, and ceftazidime.[235] Similarly, false increases in serum creatinine were reported in patients receiving cefoxitin or cephalothin in laboratories using the Jaffe technique.[236]

Because the cephalosporins have broad-spectrum activity, superinfection or overgrowth of *Candida* in the gastrointestinal and vaginal tracts can occur.[237,238] Similarly, overgrowth of *Clostridioides difficile* (formerly *Clostridium difficile*) in the gastrointestinal tract with diarrhea

TABLE 21.5 Dosing Adjustments of Commonly Used Cephalosporins in Adult Patients With Renal Insufficiency

CEPHALOSPORIN	USUAL ADULT DOSING REGIMEN[a]	DOSING REGIMEN WITH RENAL INSUFFICIENCY				DOSING REGIMEN WITH DIALYSIS	
		GFR 51–90 mL/min	GFR 31–50 mL/min	GFR 11–30 mL/min	GFR ≤10 mL/min	INTERMITTENT HEMODIALYSIS	CAPD
Intravenous: First Generation							
Cefazolin	1–2 g q8h	NC	NC	1–2 g q12h	1 g q24h	0.5–1 g q24h[b] or 2 g 3 times weekly[b]	0.5 g q12h
Intravenous: Second Generation							
Cefuroxime	0.75–1.5 g q8h	NC	NC	0.75–1.5 g q12h	0.75–1.5 g q24h	0.75–1.5 g q24h[b]	0.75–1.5 g q24h
Intravenous: Cephamycin							
Cefoxitin	2 g q6h	NC	2 g q8h	2 g q12h	1 g q12–24h	0.5–1 g q24–48h and supplemental dose after each dialysis (usually 1 g)	1 g q24h
Intravenous: Third Generation							
Cefotaxime	2 g q6–8h	NC	2 g q8–12h	2 g q8–12h	2 g q24h	1–2 g q24h[b]	1 g q24h
Ceftazidime	2 g q8h	NC	1 g q12h	1 g q24h	0.5 g q24h	1 g q24h[b]	0.5 g q24h
Ceftriaxone	1–2 g q24h	NC	NC	NC	NC	NC	NC
Intravenous: Fourth Generation							
Cefepime	2 g q8–12h	NC	2 g q12–24h	1–2 g q24h	0.5–1 g q24h	1 g q24h[b]	2 g q48h
Intravenous: Fifth Generation (MRSA Active)							
Ceftaroline	0.6 g q12h	NC	0.4 g q12h	0.3 g q12h	0.2 g q12h	0.2 g q12h[b]	0.2 g q12h
Ceftobiprole	0.5 g q8h	NC	0.5 g q12h	0.25 g q12h	0.25 g q12h	0.25 g q12h[b]	0.25 g q12h
Intravenous: Cephalosporin–β-Lactamase Inhibitor Combinations							
Ceftolozane-tazobactam	1.5 g q8h	NC	0.75 g q8h	0.375 g q8h	NA	0.75 g load, then 0.15 g q8h[b]	NA
Ceftazidime-avibactam	2.5 g q8h	NC	1.25 g q8h	0.94 g q12h	0.94 g q24h	0.94 g q24[b]	NA
Oral: First Generation							
Cefadroxil	500 mg q12h	NC	NC	500 mg q24h	500 mg q36h	500 mg 3 times weekly[b]	500 mg q24h
Cephalexin	500 mg q6h	NC	500 mg q12h	250 mg q8–12h	250 mg q24h	500 mg q24h[b]	500 mg q24h
Oral: Second Generation							
Cefprozil	500 mg q12h	NC	NC	250 mg q12h	250 mg q12h	500 mg q24h[b]	250 mg q12h
Cefuroxime (axetil)	500 mg q12h	NC	NC	500 mg q24h	250 mg q24h	250 mg q24h and supplemental dose after each dialysis (usually 250 mg)	250 mg q24h
Oral: Third Generation							
Cefdinir	300 mg q12h	NC	NC	300 mg q24h	300 mg q24h	300 mg 3 times weekly[b]	300 mg q24h
Cefditoren	400 mg q12h	NC	200 mg q12h	200 mg q24h	200 mg q24h	NA	NA
Cefixime	400 mg q12–24h	NC	300 mg q24h	200 mg q24h	200 mg q24h	300 mg q24h[b]	200 mg q24h
Cefpodoxime	200-400 mg q12h	NC	NC	200–400 mg q24h	200 mg q24h	200–400 mg 3 times weekly[b]	200 mg q24h
Ceftibuten	400 mg q24h	NC	200 mg q24h	100 mg q24h	100 mg q24h	400 mg 3 times weekly[b]	100 mg q24h

[a]For intravenous administration, the dosing regimen is based on the standard infusion time recommended in package insert.
[b]Doses given on the day of dialysis should be given after the dialysis session.
CAPD, Continuous ambulatory peritoneal dialysis; *GFR,* glomerular filtration rate; *MRSA,* methicillin-resistant *Staphylococcus aureus*; *NA,* not applicable or no data; *NC,* no change.

TABLE 21.6 Potential Adverse Effects of Cephalosporins

EFFECT TYPE	SPECIFIC EFFECT	FREQUENCY (%)
Hypersensitivity	Rash	1–3
	Urticaria	<1
	Serum sickness	<1
	Anaphylaxis	0.01
Gastrointestinal	Diarrhea	1–19
	Nausea and vomiting	1–6
	Transient transaminase elevation	1–7
	Biliary sludge	20–46[a]
Hematologic	Eosinophilia	1–10
	Neutropenia	<1
	Thrombocytopenia	<1–3
	Hypoprothrombinemia	<1
	Impaired platelet aggregation	<1
	Hemolytic anemia	<1
Renal	Interstitial nephritis	<1–5
Central nervous system	Seizures	<1
	Encephalopathy	<1
False-positive laboratory results	Coombs positive	3
	Glucosuria	Rare
	Serum creatinine	Rare
Other	Drug fever	Rare
	Disulfiram-like reaction[b]	Rare
	Superinfection	Rare
	Phlebitis	Rare

[a]Ceftriaxone.
[b]Cephalosporins with thiomethyl tetrazole ring side chain.

and colitis has been associated with increased use of broad-spectrum cephalosporins.[239,240,241]

MAJOR CLINICAL USES OF CEPHALOSPORIN ANTIBIOTICS

The usual dosing regimens for adults and children for the various cephalosporins by generation are listed in Table 21.7. The daily doses recommended to treat serious infections are also listed. It should be noted that prolonged-infusion and continuous-infusion β-lactam therapy has become common at many institutions. The rationale behind this is the optimization of the time that drug concentrations remain above MIC (T>MIC) for the dosing period.[37,38,39] In providing a slow, prolonged infusion rate, drug concentrations can remain above the MIC for a longer period of time, especially for drugs with relatively short half-life in which dosing intervals are either every 6 hours or every 8 hours. This dosing strategy can increase the chances of pharmacokinetic and pharmacodynamic target attainment while potentially decreasing the total daily dose necessary.[242] Important to note, outcomes in general have been either equivalent or improved with a prolonged-infusion dosing strategy in comparison with standard infusion rates, and this has been particularly useful for cefepime.

First-Generation Cephalosporins

The first-generation cephalosporins available in the United States are cefazolin, cephalexin, and cefadroxil. The first-generation cephalosporins have been extensively used as alternatives to penicillin for susceptible staphylococcal and streptococcal infections. Most commonly, these include skin and soft tissue infections and more severe infections such as streptococcal and methicillin-susceptible *S. aureus* bacteremia. Cefazolin is not metabolized and is eliminated more by glomerular filtration than by tubular excretion.[180] Its moderate protein binding slows the glomerular filtration of the drug, resulting in a half-life of 1.5 to 2 hours, which allows dosing every 8 hours and every 12 hours. With coadministration of probenecid, cefazolin has been effective in skin and soft tissue infections with once-daily dosing.[243] Cefazolin is still recommended in penicillin-allergic patients for more serious staphylococcal infections, such as endocarditis, even though the drug is more readily hydrolyzed by staphylococcal β-lactamase than other first-generation cephalosporins.[244,245] Cefazolin is recommended as the prophylactic antibiotic of choice for foreign-body implantation and for many clean and clean-contaminated surgical procedures in which there is a high risk of infection.[246] These include cardiac and vascular surgery, insertion of orthopedic devices, head and neck surgery that crosses the oropharyngeal mucosal barrier, vaginal and abdominal hysterectomy, high-risk cesarean section, and high-risk gastroduodenal and biliary tract procedures. Because of its poor activity against *Bacteroides* spp., cefazolin alone is not recommended for intraabdominal procedures that involve the intestine.

The oral first-generation cephalosporins, cephalexin and cefadroxil, have very high oral bioavailability. Cefadroxil has a slightly longer half-life than cephalexin, which allows twice-daily dosing instead of the usual four times a day.[137] These drugs provide appropriate outpatient therapy for many skin and soft tissue infections. However, they are not effective for animal bites and scratches involving *Pasteurella multocida*.[247] The drugs are quite active against *Streptococcus pyogenes* and provide effective therapy in streptococcal pharyngitis.[248,249] They have poor activity against penicillin-resistant pneumococci, *H. influenzae,* and *M. catarrhalis* and are not recommended for sinusitis, otitis media, or lower respiratory tract infections. The drugs are effective against some Enterobacteriaceae and because of renal excretion are effective in uncomplicated urinary tract infections; however, they are less effective than trimethoprim-sulfamethoxazole or fluoroquinolones.[250]

Second-Generation Cephalosporins

The second-generation cephalosporins currently available in the United States include two true cephalosporins, cefprozil and cefuroxime, and one cephamycin, cefoxitin. The two groups of drugs have different spectra of antimicrobial activity and different clinical uses. Because of their activity against *S. pneumoniae, H. influenzae,* and *M. catarrhalis,* the true second-generation cephalosporins, such as cefuroxime, have been used extensively for treatment of various respiratory tract infections in hospitalized patients.[251] Cefuroxime can also be used to treat meningitis caused by penicillin-susceptible pneumococci, *H. influenzae,* or *Neisseria meningitidis*.[252] However, its use has been largely supplanted by third-generation cephalosporins, which result in faster eradication of bacteria from the CSF.[253] Cefuroxime was in the past one of the recommended agents for empirical therapy of community-acquired pneumonia (CAP) in hospitalized patients.[254] Although cefuroxime has good activity against penicillin-susceptible and penicillin-intermediate strains of *S. pneumoniae,* its activity against most penicillin-resistant strains is suboptimal. In an observational study of 844 patients with pneumococcal bacteremia, mostly resulting from pneumonia, resistance to cefuroxime was associated with significantly greater mortality.[255] The latest recommendations on CAP list ceftriaxone or cefotaxime for initial empirical therapy.[256] Cefuroxime provides effective therapy for other serious infections caused by susceptible pathogens, including skin and soft tissue infections, epiglottitis, complicated sinusitis, and gynecologic infections.[257,258] Cefuroxime is also an important cephalosporin for surgical prophylaxis for many of the same surgical procedures for which cefazolin is recommended.[246] Cefuroxime should not be considered for empirical therapy of nosocomial pneumonia or other nosocomial infections because of its poor activity against most strains of *Enterobacter, Citrobacter, Serratia, Morganella,* and *P. aeruginosa.*

The cephamycins have inferior activity against staphylococci but enhanced antibacterial activity against certain Enterobacteriaceae and especially for their activity against anaerobic bacteria such as *B. fragilis.* Given this, cephamycins are important adjuncts to surgical prophylaxis for colorectal procedures and appendectomies.[246] For elective colorectal surgery, cefoxitin is commonly administered even when an oral bowel preparation with erythromycin and neomycin is used. Cephamycins are also used for the treatment of intraabdominal, pelvic, and gynecologic infections; infected decubitus ulcers; diabetic foot infections; and mixed aerobic-anaerobic soft tissue infections.[259,260,261,262] They also demonstrate good in vitro activity against ESBL-producing strains of *E. coli* and *K. pneumoniae*; however, their reliability in treating infections caused by ESBL-producing strains has not been proven. It is important to note though that as many as 15% to 20% of *B. fragilis* strains are resistant to the various cephamycins, and drugs with better anaerobic activity

TABLE 21.7 Dosing Regimens for Cephalosporins in Adults and Children

	ADULT		CHILDREN
CEPHALOSPORIN	**USUAL DOSE**	**SEVERE DISEASE**	**USUAL DOSE**
First Generation			
Cefazolin	0.5–1 g q8-12h	2 g q6–8h	12.5–33 mg/kg q6–8h
Second Generation			
Cefuroxime	0.75–1.5 g q8h	1.5 g q8h	12.5–60 mg/kg q6–8h
Cephamycins			
Cefotetan	1–2 g q12h	2-3 g q12h	Not recommended
Cefoxitin	1–2 g q6h	2 g q4–6h	20–25 mg/kg q4–6h
Third Generation			
Cefotaxime	1 g q8–12h	2 g q4–8h	25–30 mg/kg q4–6h
Ceftazidime	1 g q8–12h	2 g q8h	30–50 mg/kg q8h
Ceftriaxone	1 g q24h	2 g q12–24h	50–100 mg/kg q24h
Fourth Generation			
Cefepime	1 g q12h	2 g q8–12h	50 mg/kg q8h
Cefpirome	1 g q12h	2 g q12h	Not recommended
Fifth Generation (MRSA Active)			
Ceftaroline	0.6 g q12h	0.6 g q8h	Not recommended
Ceftobiprole	0.5 g q12h	0.5 g q8h	Not recommended
Cephalosporin–β-Lactamase Inhibitor Combination			
Ceftolozane-tazobactam	1.5 g q8h	1.5 g q8h[b]	No safety or efficacy data, ongoing trials
Ceftazidime-avibactam	2.5 g q8h	2.5 g q8h	No safety or efficacy data, ongoing trials
Oral: First Generation			
Cephalexin	250–500 mg qid	1 g qid	6.25–25 mg/kg qid
Cefadroxil	500 mg bid	1 g bid	15 mg/kg bid
Oral: Second Generation			
Cefprozil	250–500 mg bid	500 mg bid	7.5–15 mg/kg bid
Cefuroxime (axetil)	250–500 mg bid	500 mg bid	10–15 mg/kg bid
Oral: Third Generation			
Cefdinir	300 mg bid or 600 mg qd	300 mg bid or 600 mg qd	7 mg/kg bid or 14 mg/kg qd
Ceditoren	200–400 mg bid	400 mg bid	Not recommended
Cefixime	200 mg bid or 400 mg qd	400 mg bid	4 mg/kg bid or 8 mg/kg qd
Cefpodoxime	200–400 mg bid	400 mg bid	5 mg/kg bid
Ceftibuten	400 mg qd	400 mg qd	9 mg/kg qd

[a]These dosing regimens are for standard infusion rates.
[b]Studies for higher doses including 3 g q8h are currently ongoing for ceftolozane-tazobactam.
MRSA, Methicillin-resistant *Staphylococcus aureus.*

should be used for empirical therapy of serious *Bacteroides* infections.[263,264] All of the cephamycins are active against *N. gonorrhoeae,* including penicillin-resistant strains. However, recommended therapy for infections with this organism is with ceftriaxone, which is effective as a single dose.[265] However, resistance of *N. gonorrhoeae* to ceftriaxone is increasing worldwide.[266] Cefoxitin in combination with doxycycline provides effective therapy for pelvic inflammatory disease.[267] Cefoxitin is also a useful addition to combination regimens for certain atypical mycobacterial infections, such as those caused by *Mycobacterium abscessus.*

The oral second-generation cephalosporins, including cefuroxime axetil and cefprozil, are effective for treatment of a variety of mild-to-moderate community-acquired infections. Double-tap studies in acute otitis media and acute maxillary sinusitis demonstrated that bacterial eradication was related to the drug's ability to produce serum concentrations exceeding the MIC of the infecting pathogen for 40% to 50% of the dosing interval.[268,269] In this regard, cefuroxime axetil and cefprozil are the best agents for *S. pneumoniae* and are effective against penicillin-susceptible isolates and most penicillin-intermediate strains. None of the oral second-generation cephalosporins provides optimal therapy for penicillin-resistant pneumococci. For *H. influenzae* and *M. catarrhalis,* cefuroxime axetil would be a better choice than cefprozil. Clinical and bacteriologic outcomes have demonstrated that 5- to 10-day courses of therapy with the second-generation cephalosporins are equivalent to or more effective than 10 days of therapy with penicillin V for the treatment of group A β-hemolytic streptococcal pharyngitis.[270,271] Cefuroxime axetil is also a recommended alternative to doxycycline and penicillin for treatment of early Lyme disease.[272]

Third-Generation Cephalosporins

Third-generation cephalosporins available in the United States are cefdinir, cefditoren, cefixime, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, and ceftriaxone. The third-generation cephalosporins are major drugs for the treatment of many important infections because of their high antibacterial potency, wide spectrum of activity, low potential

for toxicity, and favorable pharmacokinetics (e.g., enhanced drug concentrations in the CSF). They have been especially useful in infections resulting from gram-negative bacilli that are resistant to other β-lactam antibiotics. However, their superior activity against the Enterobacteriaceae is being challenged by the increasing frequency of organisms with β-lactamase–mediated resistance. New AmpC β-lactamases, ESBLs, and carbapenemases, which inactivate third-generation cephalosporins, present a distinct threat to the continued utility of these agents.

Cefotaxime, ceftriaxone, and ceftazidime are the major parenteral third-generation cephalosporins in clinical use for the treatment of nosocomial infections caused by susceptible gram-negative bacilli. Cefotaxime and ceftriaxone are also two of the most potent cephalosporins against penicillin-resistant pneumococci. Because of its high protein binding, ceftriaxone has the longest half-life and is usually administered once daily. Ceftazidime is administered two or three times daily, and effective dosing of cefotaxime, which has the shortest half-life, has varied from every 4 hours to twice daily. Ceftazidime is usually reserved for infections that are likely to involve *P. aeruginosa.*

Monotherapy with cefotaxime or ceftriaxone has provided effective treatment for a variety of nosocomial infections caused by susceptible gram-negative bacilli, including complicated skin and soft tissue infections, prosthetic joint infections, pneumonia, complicated urinary tract infections, and intraabdominal infections such as peritonitis.[273-276,277] However, cephalosporin monotherapy for infections caused primarily by *Enterobacter, Citrobacter, Serratia, Morganella, Proteus,* and *Providencia* spp. can be complicated by the emergence of stably derepressed resistant mutants.[120,278,279] Combination antimicrobial therapy may be beneficial in reducing this emergence of resistance, which results from increased chromosomal β-lactamase production. Organisms containing ESBLs have also been observed to result in failed therapy with third-generation cephalosporins, even when the organism was judged susceptible in laboratory tests.[280] The carbapenems are the recommended drugs for these ESBL-producing strains.

Cefotaxime and ceftriaxone have provided effective therapy for meningitis caused by a variety of different bacteria.[281-283,284] They are the drugs of choice for meningitis caused by *H. influenzae* and various Enterobacteriaceae.[285] Cefotaxime and ceftriaxone also provide effective therapy for meningitis caused by *N. meningitidis* and against pneumococci that have MICs of 1.0 μg/mL or less. Organisms with higher MICs have resulted in failed monotherapy with these cephalosporins. Therefore, empirical therapy with cefotaxime or ceftriaxone is combined with vancomycin (with or without rifampin) until the laboratory determines the susceptibility of the pneumococcal isolate.[285] If the organism is susceptible to cefotaxime or ceftriaxone, the vancomycin (and rifampin) can be discontinued. Treatment of meningitis requires maximal doses of these cephalosporins, such as 2 g every 12 hours in adults and 50 mg/kg twice daily or 100 mg/kg once daily in children for ceftriaxone, and 2 g every 4 to 6 hours in adults or 100 to 150 mg/kg every 6 to 8 hours in children for cefotaxime.

Cefotaxime and ceftriaxone continue to be active against most bacteria producing CAP. In a large observational study of pneumococcal bacteremia, resistance to cefotaxime and ceftriaxone was not associated with higher mortality.[255] Cefotaxime and ceftriaxone were also effective in treating patients with nonmeningeal pneumococcal infections, mostly pneumonia, caused by strains with MICs as high as 2 μg/mL.[286] The Clinical and Laboratory Standards Institute (CLSI) has created higher susceptibility and resistance breakpoints for pneumococci causing nonmeningeal infections than for *S. pneumoniae* causing meningitis.[287] As a result of these changes, cefotaxime and ceftriaxone are considered active against most penicillin-resistant pneumococci and are recommended, in combination with a macrolide, for empirical therapy for CAP requiring hospitalization.[256] Single intramuscular doses of ceftriaxone are also highly effective in eradicating *H. influenzae* and penicillin-susceptible strains of *S. pneumoniae* from middle ear fluid.[288] However, three daily doses of ceftriaxone were required in one study to eradicate penicillin-resistant pneumococci.[289]

The oral third-generation cephalosporins, which include cefdinir, cefditoren pivoxil, cefixime, cefpodoxime proxetil, and ceftibuten, are approved for oral therapy of mild-to-moderate respiratory infections, such as otitis media, sinusitis, and acute exacerbations of chronic bronchitis. These drugs have very potent activity against *H. influenzae,* but their activity against pneumococci is more variable.[290-292] Cefdinir, cefditoren, and cefpodoxime have activity similar to that of cefuroxime or cefprozil and are active against penicillin-susceptible and most penicillin-intermediate strains of *S. pneumoniae*. Cefixime is active only against penicillin-susceptible strains, and ceftibuten is marginal even for penicillin-susceptible pneumococci. Short courses of most of these drugs have also provided equivalent rates of eradication in group A β-hemolytic streptococcal pharyngitis.[293-295] Their increased potency over other oral cephalosporins for *E. coli, K. pneumoniae,* and *Enterobacter, Citrobacter,* and *Serratia* spp. enhances their utility for treatment of complicated urinary tract infections.[296]

Ceftazidime is the third-generation cephalosporin used for serious infections in which *P. aeruginosa* is documented or highly likely. It is one of the recommended drugs, either alone or in combination with an aminoglycoside, for initial empirical management of febrile neutropenia.[297] However, ESBLs and AmpC β-lactamases have reduced the utility of ceftazidime for monotherapy, and therefore cefepime has largely supplanted its use for empiric therapy in neutropenic fever. Ceftazidime has been effective for the treatment of acute exacerbations of chronic pulmonary infections in patients with cystic fibrosis.[298] The drug penetrates into CSF and is one of two cephalosporins as treatment of choice for meningitis caused by *P. aeruginosa.*[299]

Third-generation cephalosporins have also become established therapy for a variety of specific infections. Ceftriaxone 250 to 500 mg intramuscularly is highly active against *N. gonorrhoeae,* including penicillin- and quinolone-resistant strains. It is the drug of choice for all forms of gonococcal infection and is used in combination with a single oral dose of azithromycin.[265] A single oral dose of cefixime is also highly effective for uncomplicated gonococcal infections of the cervix, urethra, and rectum.[265,300,301] However, because of increasing resistance, it is no longer recommended as first-line therapy.[302] Single-dose intramuscular ceftriaxone is recommended therapy for chancroid.[265] Ceftriaxone and cefotaxime are recommended therapy for treatment of early Lyme disease in patients with neurologic involvement or third-degree atrioventricular heart block.[303] These drugs are also recommended in Lyme disease patients with both arthritis and objective evidence of neurologic disease and for those with late neurologic disease affecting the central or peripheral nervous system. Ceftriaxone is considered as alternative therapy in penicillin-allergic patients with syphilis.[265,304] Ceftriaxone is a recommended alternative therapy for typhoid fever and for severe infections caused by *Shigella* spp. or by non-*typhi* spp. of *Salmonella*.[305] Third-generation cephalosporins have provided effective therapy for focal *Salmonella* infections, brain abscess caused by gram-negative bacilli, and endocarditis caused by fastidious gram-negative coccobacilli.[306,307] Single doses of ceftriaxone are highly effective in eradicating nasopharyngeal carriage of *N. meningitidis.*[308,309] The long half-life of ceftriaxone, which allows for once-daily dosing, has enhanced its use in the outpatient setting for both streptococcal and staphylococcal infections. Ceftriaxone has been effective for the outpatient treatment of staphylococcal and streptococcal skin and soft tissue infections, including osteomyelitis and prosthetic joint infection.[277,310,311] The drug is also effective as monotherapy for the outpatient treatment of nonenterococcal streptococcal endocarditis.[312]

Fourth-Generation Cephalosporins

Cefepime is the only fourth-generation cephalosporin available in the United States. The fourth-generation cephalosporins have the widest spectrum of all the cephalosporins. They have enhanced activity against certain gram-negative bacilli, such as *Enterobacter, Citrobacter,* and *Serratia* spp. These drugs are zwitterions, which cross the outer membrane of gram-negative bacilli more rapidly than other cephalosporins. They are also less susceptible to inactivation by AmpC β-lactamases. As a result, about 75% to 80% of the Enterobacteriaceae resistant to third-generation cephalosporins are susceptible to the fourth-generation drugs, and they are the treatment of choice for serious infections caused by AmpC inducible-resistant strains.[313,314] They are active against *P. aeruginosa* and, unlike ceftazidime, maintain good potency against gram-positive cocci, most notably methicillin-susceptible *S. aureus, S. pneumoniae* (including most penicillin-resistant strains), and other streptococci.[54,315-318] Only two fourth-generation agents have been

developed so far. Cefepime has a slightly longer half-life than ceftazidime and is usually administered twice daily, although 8-hour dosing is recommended for documented *P. aeruginosa* infections. Cefpirome, which is not available in the United States, has a pharmacokinetic profile very similar to that of cefepime.

At some hospitals, the fourth-generation agents have largely replaced third-generation cephalosporins for treatment of serious gram-negative bacillary infections. They have proved to be effective in a variety of serious gram-negative infections, such as bacteremia, pneumonia, skin and soft tissue infections, prosthetic joint infections, and complicated urinary tract infections.[313,314,320,321,322] With continuous infusion of cefepime, optimal efficacy was observed when serum concentrations were at least fourfold greater than the MIC.[323] Comparative trials with ceftazidime or other agents have generally demonstrated equivalent efficacy.[324-327] Cefepime therapy for ESBL-producing organisms with maintained cefepime susceptible MICs is controversial.[328] Both clinical failures and successes have been noted against these organisms, and studies have highlighted the importance of achieving pharmacodynamic target (i.e., appropriate time above MIC) and inoculum effect or severity of infection as important parameters.[328] Cefepime is recommended as monotherapy or in combination for initial empirical therapy in febrile neutropenic patients.[297] The drug is one of the recommended agents for the empirical treatment of severe CAP, for health care–associated pneumonia, and for ventilator-associated pneumonia, especially when *P. aeruginosa* or resistant Enterobacteriaceae are suspected.[254,329] The drug's activity against pneumococci is similar to that of ceftriaxone in patients with CAP requiring hospitalization.[330,331] The drug penetrates well into the CSF and produces outcomes similar to those of cefotaxime in acute bacterial meningitis.[332,333] However, it is not approved for treatment of meningitis in the United States. Resistance is an increasing issue for cefepime, and many of the new ESBL and carbapenemase enzymes (usually class A or class B) can inactivate these fourth-generation drugs.[124]

A meta-analysis of cefepime versus other β-lactam drugs in the treatment of febrile neutropenia and other serious infections reported that all-cause mortality with cefepime was higher than with other cephalosporins or with a β-lactam–BLI combination.[335,336] An additional report suggested that the difference in mortality in patients with febrile neutropenia was caused by greater progression of underlying disease in the cefepime arm.[337] A meta-analysis by the Center for Drug Evaluation of the US Food and Drug Administration (FDA) using a larger database of both published and unpublished comparative studies did not find a significant increase in mortality in cefepime-treated patients compared with those treated with other antibacterials.[338]

Fifth-Generation Cephalosporins (MRSA-Active Cephalosporins)

The MRSA-active cephalosporins have unique activity compared with other cephalosporins. They have excellent activity again MRSA and against ampicillin-susceptible strains of *E. faecalis.*[70,73] They also exhibit potent activity against pneumococci with multiple mutations in genes encoding PBP1A, PBP2B, and PBP2X. MIC_{90} values that were as high as 4 and 8 μg/mL to the most active third-generation cephalosporins were fourfold to eightfold lower with the MRSA-active cephalosporins.[339,340] On the other hand, the activity of these drugs against gram-negative bacilli is similar to that of many of the third-generation cephalosporins. The first approved drug in this group (in a few countries, but not the United States) was ceftobiprole medocaril, the prodrug for ceftobiprole. The US-approved MRSA-active cephalosporin is ceftaroline fosamil, the prodrug for ceftaroline. Ceftobiprole is more resistant than ceftaroline to inactivation by AmpC β-lactamase[113] and has activity against strains of *P. aeruginosa* that is similar to that of ceftazidime. However, the maximal doses of ceftobiprole studied so far are fourfold lower than those of ceftazidime.

The MRSA-active cephalosporins have exhibited comparable efficacy to vancomycin plus a broad-spectrum β-lactam in patients with complicated skin and skin-structure infections.[341,342,343] The results with ceftobiprole and ceftaroline were similar to those obtained with vancomycin in the large number of patients with MRSA infections. Studies with ceftaroline and ceftobiprole have shown efficacy in hospitalized patients with CAP similar to that of comparators.[344-346] Ceftobiprole also has shown similar efficacy in hospital-acquired pneumonia (excluding ventilator-associated pneumonia) to that of ceftazidime plus linezolid.[347] Ceftaroline has been effective in patients with soft tissue infections complicated by MRSA bacteremia and in other small studies of complicated bacteremia with or without endocarditis.[348,349,350] Ceftaroline also increases the membrane binding of daptomycin against daptomycin-nonsusceptible MRSA, resulting in enhanced killing and improved clinical outcome.[351,352] Little is known about the CSF penetration of ceftobiprole and ceftaroline in humans or whether the enhanced activity of these drugs against pneumococci will be useful in treating meningitis. These drugs may also become useful agents for treating severe enterococcal infections in penicillin-allergic patients.[353]

Cephalosporin and β-Lactamase Inhibitor Combinations

Efforts have been focused on combining cephalosporins with BLIs to combat increasing drug resistance. Ceftolozane-tazobactam and ceftazidime-avibactam are the first successful agents to make it to clinical use thus far.

In the case of ceftazidime-avibactam, the novel BLI avibactam targets class A, class C, and some class D enzymes and completely restores full susceptibility (MIC ≤1 mg/L) for most strains producing ESBLs, AmpCs, or both.[91,354] For *P. aeruginosa,* avibactam reduces the MIC_{90} fourfold and produces MICs less than or equal to 8 mg/L for 60% of multidrug-resistant strains.[355] Avibactam has minimal effect on ceftazidime MICs for *Acinetobacter baumanii* and most anaerobic bacteria.[91,356] Clinically, the combination of avibactam and ceftazidime has resulted in comparable efficacy to carbapenems in complicated urinary tract infections and in complicated intraabdominal infections.[357-359] Small uncontrolled studies have demonstrated encouraging outcomes for patients with CRE[360,361,362,363]; however, reports of development of resistance to ceftazidime-avibactam have emerged.[125,126]

Tazobactam is the BLI that is being combined with ceftolozane, a new cephalosporin with enhanced activity against strains of *P. aeruginosa.* The MICs of ceftolozane against *P. aeruginosa* are 4- to 16-fold lower than ceftazidime.[364,365] The drug also appears to have earlier in vitro killing and more rapid in vivo killing than ceftazidime.[366,367] Although tazobactam has a minimal effect on ceftolozane's activity against *P. aeruginosa,* it does enhance its activity against other gram-negative bacilli producing primarily class A and class C β-lactamases.[16] At a concentration of ceftolozane and tazobactam of 8 and 4 mg/L, respectively, 70% to 76% of *Enterobacter* strains that are AmpC hyperproducers or producing ESBLs are susceptible to the drug combination.[368] Thus, the combination is active against many ESBL organisms, most importantly the CTX-M family. Its main difference from ceftazidime-avibactam is that it does not have appreciable activity against KPC producers and is therefore not effective against most CRE organisms. Clinically, ceftolozane-tazobactam has been approved for complicated urinary tract infections and, along with metronidazole, for complicated intraabdominal infections based on large randomized studies.[369,370,371] These studies also demonstrated high efficacy for those isolates that were ESBL positive.[372] Retrospective studies examining outcome for patients infected with multidrug-resistant *P. aeruginosa*, including carbapenem-resistance, treated with ceftolozane/tazobactam are encouraging, but prospective, randomized controlled trials are needed.[373,374] As with ceftazidime-avibactam, resistance to ceftolozane-tazobactam has been documented during therapy in a small number of cases.[374,375]

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The complete reference list is available online at Expert Consult.

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22 Ertapenem, Imipenem, Meropenem, Doripenem, and Aztreonam

Yohei Doi

SHORT VIEW SUMMARY

ERTAPENEM
- Usual adult dose: 1 g daily intravenously
- Renal and hepatic failure: decrease dose in renal failure
- Cerebrospinal fluid penetration: no human data
- Adverse effects: diarrhea, nausea, infused vein complication
- Contraindications: prior allergy
- Drug-drug interactions: avoid probenecid, valproic acid

IMIPENEM
- Usual adult dose: 0.5 g every 6 hours or 1 g every 8 hours intravenously
- Renal and hepatic failure: decrease dose in renal failure
- Cerebrospinal fluid penetration: moderate
- Adverse effects: seizure, diarrhea, nausea, phlebitis
- Contraindications: prior allergy
- Drug-drug interactions: avoid probenecid, valproic acid, ganciclovir

MEROPENEM
- Usual adult dose: 0.5 to 1 g every 8 hours intravenously
- Renal and hepatic failure: decrease dose in renal failure
- Cerebrospinal fluid penetration: moderate
- Adverse effects: diarrhea, nausea/vomiting, headache, inflammation of injection site
- Contraindications: prior allergy
- Drug-drug interactions: avoid probenecid, valproic acid

DORIPENEM
- Usual adult dose: 0.5 g every 8 hours intravenously
- Renal and hepatic failure: decrease dose in renal failure
- Cerebrospinal fluid penetration: moderate
- Adverse effects: diarrhea, headache, nausea, rash, phlebitis
- Contraindications: prior allergy
- Drug-drug interactions: avoid probenecid, valproic acid

CARBAPENEMS

Four carbapenems—imipenem, meropenem, ertapenem, and doripenem—are approved for clinical use in the United States. In addition, panipenem is available in Japan and China, tebipenem in Japan, and biapenem in Japan, China, and Thailand. Carbapenems are active against a broad range of gram-positive and gram-negative aerobic and anaerobic bacteria due to their efficient penetration through the bacterial outer membrane, their high affinity for multiple penicillin-binding proteins (PBPs), and their stability against most β-lactamases, including class A extended-spectrum β-lactamases (ESBLs) and class C β-lactamases (AmpCs).

Chemistry

Carbapenems are derivatives of thienamycin, an antibiotic produced by the soil organism *Streptomyces cattleya.* They differ from penicillins by a carbon atom replacing the sulfur at position 1 and a double bond between C2 and C3 in the five-membered thiazolidine ring (Fig. 22.1). The *trans*-1α-hydroxyethyl side chain in the *trans*-configuration at C6 confers the excellent β-lactamase stability, which is associated with the broad spectrum of activity of carbapenems. Thienamycin was chemically too unstable, which prompted the development of its *N*-formimidoyl derivative imipenem. However, imipenem is degraded in vivo by mammalian renal dehydropeptidase (DHP-I) and must be coadministered with cilastatin, a selective antagonist of this enzyme. Meropenem, ertapenem, and doripenem differ from imipenem by having a 1β-methyl, 2-thiopyrrolidinyl substituent at C2. The 1β-methyl constituent is believed to provide stability to DHP-I, which allows it to be administered without a DHP-I inhibitor, unlike imipenem.

Mechanism of Action

Carbapenems inhibit cell wall synthesis by binding to most high-molecular-weight PBPs. They traverse the outer membrane of gram-negative bacteria through specific outer membrane proteins (OMPs) to reach the periplasmic space. The most significant OMP is OprD in *Pseudomonas aeruginosa.* Although variations exist depending on the specific agent, carbapenems preferentially bind to PBPs 1a, 1b, 2, and 4 and to a lesser extent PBP3, which is the primary target of aminopenicillins and cephalosporins. This low affinity with PBP3 is thought to be responsible for the formation of sphere forms without the production of long filaments on bacterial lysis. The affinity of carbapenems to multiple PBPs of various bacteria contributes to the broad spectrum of activity of these agents.

Resistance

Resistance to carbapenems is mediated by one or a combination of the following mechanisms: (1) production of β-lactamase that hydrolyzes carbapenems; (2) diminished permeability due to impaired expression of certain OMPs; (3) efflux of drug across the outer membrane; and (4) production of an altered or low-affinity target, which is more relevant in gram-positive bacteria. In gram-negative bacteria, although a single mechanism may not be sufficient to cause a clinically relevant degree of resistance, frank resistance occurs through an interplay involving β-lactamase production, impaired permeability, and enhanced efflux.

Carbapenems are readily hydrolyzed by Ambler class B β-lactamases, which are zinc-dependent metalloenzymes.[1] Some lactose-nonfermenting species, including *Stenotrophomonas maltophilia* and *Elizabethkingia meningoseptica,* are intrinsically resistant to carbapenems due to class B β-lactamase production. In addition, acquired class B β-lactamases, such as Verona integron-encoded metallo-β-lactamase (VIM) and New Delhi metallo-β-lactamase (NDM), confer resistance in a wide array of gram-negative bacteria. Several Ambler class A β-lactamases hydrolyze carbapenems. In particular, *Klebsiella pneumoniae*–type carbapenemases (KPCs) have emerged as an important carbapenem resistance determinant in gram-negative bacteria worldwide, mostly in *K. pneumoniae.*[2] Ambler class D oxacillinase β-lactamases (OXAs) that hydrolyze carbapenems are also found frequently in *Acinetobacter baumannii* and are emerging in *K. pneumoniae* in certain geographic areas.[2]

Downregulated production or absence of porin protein OprD is responsible for resistance to carbapenems in *P. aeruginosa* when combined with background production of AmpC β-lactamase.[3,4] Imipenem is the preferred substrate of OprD and is affected most, whereas the effect of the lack of OprD on resistance is less pronounced for meropenem and

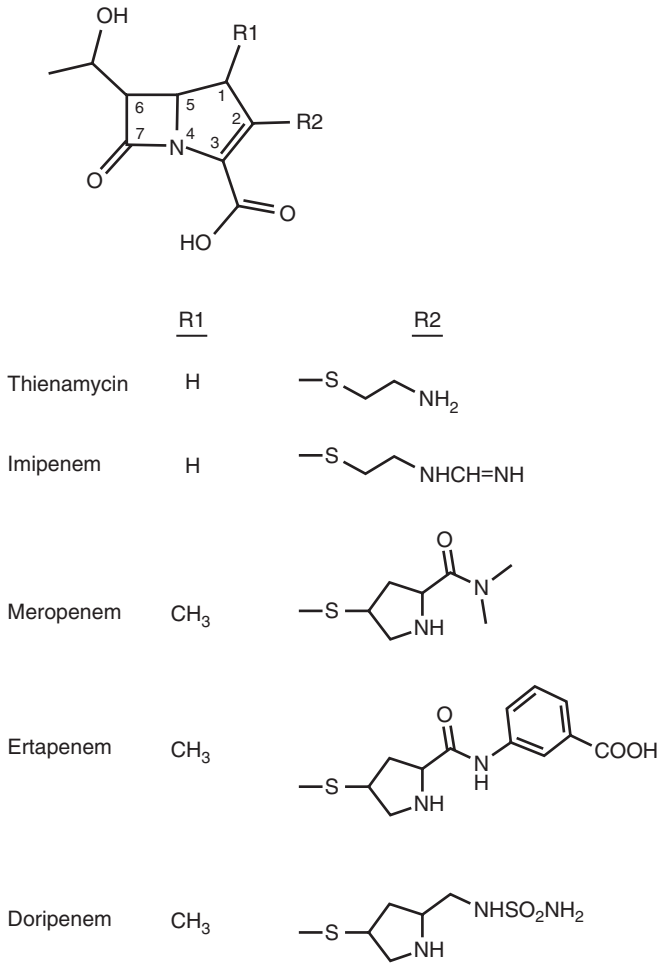

FIG. 22.1 Core structure and substituents for carbapenem antibiotics. They differ from penicillins by a carbon atom replacing the sulfur at position 1 and a double bond between C2 and C3 in the five-membered thiazolidine ring. The *trans*-1α-hydroxyethyl side chain in the *trans*-configuration at C6 confers the β-lactamase stability.[28]

doripenem.[5,6] Likewise, decreased expression of OmpK35 and OmpK36 for *K. pneumoniae* and OmpC and OmpF for *Enterobacter* spp. has been associated with carbapenem resistance.[5,7–9]

Meropenem and doripenem, but not imipenem, are substrates of the tripartite multidrug efflux system MexA-MexB-OprM in *P. aeruginosa*.[10,11] Here, MexB is the cytoplasmic protein, OprM is the outer membrane component forming channels, and MexA is the membrane fusion protein linking the two membrane proteins. Upregulation of this efflux system augments resistance to meropenem and doripenem.

Production of a low-affinity PBP may mediate β-lactam class resistance, including carbapenem resistance in gram-positive bacteria. Examples include PBP2a in oxacillin-resistant staphylococci[12] and PBP5 in *Enterococcus faecium*.[13] Approximately 50% of *Staphylococcus aureus* clinical strains are resistant to oxacillin and by extension to carbapenems, whereas 90% of *E. faecium* clinical strains are resistant to ampicillin and by extension to carbapenems due to low-affinity PBPs.[14,15]

CARBAPENEM-RESISTANT ENTEROBACTERIACEAE

Increasing incidence of carbapenem-resistant Enterobacteriaceae, including those producing NDM-1 and KPC, has aroused concern about erosion of utility for this important class of antibacterial agents.[2] Equally concerning is the difficulty encountered in detecting carbapenem-resistant Enterobacteriaceae in the routine diagnostic laboratory. In 2011, the Clinical and Laboratory Standards Institute decreased the carbapenem breakpoints of Enterobacteriaceae fourfold for imipenem, meropenem, and ertapenem.[16] This change was made to accommodate carbapenem resistance mechanisms that were remaining undetected at the higher 2010 breakpoints. Automated susceptibility testing systems do contain low drug concentrations to account for the lowered breakpoints, but manufacturers are not permitted to change the interpretive criteria on their instrument without approval by the US Food and Drug Administration (FDA) of the revised system. Before implementing new interpretive criteria onto FDA-approved instruments, microbiology laboratories are required to conduct rigorous in-house validation. The revised breakpoints negate the need to do phenotypic carbapenemase detection tests (e.g., Modified Hodge Test, modified Carbapenem Inactivation Method, Carba NP test) unless warranted by epidemiologic and infection control purposes. Carbapenem resistance in Enterobacteriaceae by current standards may be missed if susceptibility testing is only done on automated systems for which the laboratory did not implement the updated breakpoints.

Antibacterial Activity

The carbapenems have similar antibacterial spectra.[17,18] All have excellent in vitro activity against gram-positive cocci in general (Table 22.1). For *Streptococcus pneumoniae,* the minimal inhibitory concentrations (MICs) are less than 0.03 μg/mL for penicillin-susceptible strains and around 0.5 μg/mL for penicillin-resistant strains, which are far exceeded by plasma levels. β-Hemolytic streptococci are exquisitely susceptible to carbapenems. Oxacillin-susceptible strains of *S. aureus* and coagulase-negative staphylococci are inhibited at carbapenem concentrations of less than 0.5 μg/mL, but oxacillin-resistant strains are highly resistant to currently available carbapenems. *Enterococcus faecalis* is typically susceptible to imipenem, with MICs of 2 μg/mL or less, but is more resistant to ertapenem, meropenem, and doripenem. None of the carbapenems is active against *E. faecium*.

Neisseria gonorrhoeae and *Neisseria meningitidis* are highly susceptible to carbapenems, with MICs typically less than 0.1 μg/mL. Ceftriaxone-resistant *N. gonorrhoeae* remains fully susceptible to ertapenem.[19] MICs of *Haemophilus influenzae* are 0.1 μg/mL or less for ertapenem, meropenem, and doripenem and 1 μg/mL or less for imipenem, including β-lactamase–producing strains. Most Enterobacteriaceae are inhibited by imipenem at concentrations of 1 μg/mL or less, whereas *Morganella* and *Proteus* strains have slightly higher MIC values. Corresponding MICs of ertapenem, meropenem, and doripenem are typically 0.1 μg/mL or less, including for strains producing ESBLs. Carbapenemase-producing *K. pneumoniae* strains are typically resistant to all carbapenems, with MICs of 8 μg/mL or greater. Carbapenemase-producing *Escherichia coli* are inhibited at lower carbapenem concentrations, between 1 and 4 μg/mL.

Doripenem is the most active carbapenem against *P. aeruginosa,* including strains hyperproducing AmpC β-lactamase, with typical MICs of 0.5 μg/mL or less compared with 1 μg/mL for meropenem and 2 μg/mL for imipenem. Ertapenem has no activity against *P. aeruginosa*. Carbapenem-susceptible strains of *A. baumannii* are inhibited by less than 1 μg/mL of imipenem, meropenem, and doripenem, but resistance to carbapenems is increasingly reported in this species. Ertapenem is not active against *A. baumannii.* All *S. maltophilia* and most *Burkholderia cepacia* strains are resistant to carbapenems.

Carbapenems are highly active against most obligately anaerobic species, including anaerobic gram-positive cocci, *Bacteroides fragilis,* non-*fragilis* species of *Bacteroides, Clostridium* spp. with the exception of *Clostridioides difficile* (formerly *Clostridium difficile*) *Fusobacterium* spp., *Prevotella* spp., *Porphyromonas* spp., and other species with MICs of 1 μg/mL or less.

Nocardia spp. are inhibited by carbapenems, but *Nocardia farcinica* and *Nocardia otitidiscaviarum* may be resistant.[20] *Actinomyces* spp. are susceptible to carbapenems.[21] Susceptibility of rapidly growing *Mycobacterium* spp. to imipenem is variable.[22] *Mycobacterium tuberculosis* and *Mycobacterium avium* are considered resistant to carbapenems, but in vivo efficacy of imipenem has been proposed.[23]

Pharmacology

Ertapenem, imipenem, meropenem, and doripenem are formulated as parenteral agents because they are absorbed poorly after oral ingestion. Imipenem, meropenem, and doripenem are pharmacologically similar. A 30- to 60-minute infusion of 500 mg of any of these agents in healthy

TABLE 22.1 Comparative Activity of Ertapenem, Imipenem, Meropenem, and Doripenem Against Selected Aerobic and Anaerobic Bacteria

	MIC_{90} (μg/mL)				
ORGANISM	**Ertapenem**	**Imipenem**	**Meropenem**	**Doripenem**	**REFERENCES**
Gram-Positive Organisms					
Staphylococcus aureus, oxacillin susceptible	0.25	0.12	0.12	0.06	52–54, 55–56
Staphylococcus aureus, oxacillin resistant	>16	>16	>16	>16	52, 55, 57
Coagulase-negative staphylococci, oxacillin susceptible	0.25	0.12	0.12	0.03	56, 58
Coagulase-negative staphylococci, oxacillin resistant	>16	>16	>16	>16	55, 59
Streptococcus pneumoniae	0.06–0.5	0.06–0.25	0.06–1	0.06–0.5	53, 55, 56
β-Hemolytic streptococci	0.06	0.06	0.06	0.06	53, 55, 56
Viridans group streptococci	0.12	0.03	0.03	0.06	53, 55, 56
Enterococcus faecalis	8	1	8	4	53, 55
Enterococcus faecium	>8	>8	>8	>8	53, 55, 56
Bacillus anthracis	—[b]	0.12	0.05	—[b]	60, 61
Gram-Negative Organisms					
Listeria monocytogenes	0.25	0.06	0.12	0.12	18, 62, 63
Haemophilus influenzae	0.03	0.25	0.06	0.12	55–56, 57, 64
Moraxella catarrhalis	0.03	0.25	0.03	0.03	56, 57
Neisseria gonorrhoeae	0.06	0.25	0.03	0.05	19, 65, 66
Neisseria meningitidis	0.03	0.03	0.03	0.06	18, 65, 67
Escherichia coli	0.06	0.5	0.03	0.03	52, 53, 55, 56
Escherichia coli, ESBL producing	0.06	0.5	0.06	0.06	52, 55, 56
Salmonella spp.	0.06	≤0.5	0.03	0.06	56
Shigella spp.	0.06	≤0.5	0.03	0.06	56
Klebsiella pneumoniae[c]	0.12	0.5	0.12	0.12	52, 53, 55
Klebsiella oxytoca	0.06	0.5	0.12	0.12	55
Enterobacter cloacae	0.06	0.5	0.12	0.12	52, 54, 64
Enterobacter aerogenes	0.06	0.5	0.12	0.12	55, 64
Morganella morganii	0.06	8	0.12	0.5	63, 68, 69
Citrobacter spp.	0.06	0.5	0.12	0.12	55, 56, 70
Serratia marcescens	0.06	0.5	0.12	0.12	55, 64
Proteus mirabilis	0.06	1	0.12	0.12	55, 56
Aeromonas spp.	0.25	0.5	0.12	0.5	56
Pseudomonas aeruginosa[c]	>8	1–>8	0.5–>8	0.5–8	53–54, 55–56
Acinetobacter baumannii[c]	4–>8	0.5–>8	0.5–>8	0.25–>8	55, 56, 71
Stenotrophomonas maltophilia	>8	>8	>8	>8	55, 56
Burkholderia cepacia	>8	>8	4	8	56
Anaerobic Organisms					
Peptostreptococcus spp.	0.125	0.25	0.125	0.125	53, 72
Fusobacterium spp.	0.03	0.12	0.03	0.03	73
Bacteroides fragilis	0.5	0.5	0.25	0.5	72
Clostridium perfringens	0.06	0.5	0.06	0.06	72
Clostridioides difficile (formerly *Clostridium difficile*)	4	2	2	2	72, 74

[a]Values less than 0.03 μg/mL are rounded up to 0.03 μg/mL.
[b]No specific data available.
[c]Susceptibility varies widely on the basis of local epidemiology.
ESBL, Extended-spectrum β-lactamase; *MIC_{90},* minimal inhibitory concentration for 90% of isolates.

persons produces mean peak serum concentrations of approximately 25 μg/mL and areas under the plasma concentration-time curve (AUCs) of 30 to 45 μg•h/mL.[24–26] A 1-g dose of ertapenem administered intravenously produces a peak serum concentration of approximately 150 μg/mL and an AUC of greater than 500 μg•h/mL.[27] The plasma half-life is 1 hour for imipenem, meropenem, and doripenem and 4 hours for ertapenem. The longer half-life of ertapenem is due to extensive protein binding (>90%) compared with imipenem (20%), meropenem (2%), and doripenem (8%) and permits once-daily dosing. Imipenem is typically administered every 6 hours, and meropenem and doripenem are given every 8 hours. All carbapenems undergo extensive renal elimination and thus require dosage adjustment in patients with reduced renal function, but not in patients with impaired liver function. Between 30% and 50% of ertapenem, imipenem, meropenem, and doripenem is removed by hemodialysis. Between 25% and 50% of imipenem, meropenem, and doripenem is removed during continuous venovenous hemofiltration or hemodiafiltration.

Imipenem is subject to degradation by DHP-I located in the brush border of renal tubules; thus, it is coformulated with the selective competitive DHP-I antagonist cilastatin.[24] Ertapenem, meropenem, and doripenem do not require a DHP-I antagonist because they are not substrates of this enzyme.

Carbapenems are well distributed to various body compartments and penetrate well into most tissues.[28]

As β-lactam agents, the most important pharmacodynamic parameter predicting bacteriologic and clinical efficacy of carbapenems is the time of free plasma drug concentration exceeding the MIC (*f*T > MIC) of the infecting organism.[29] For bacteriostatic and bactericidal activity in vivo, *f*Ts greater than MICs of 20% and 40% of the dosing interval, respectively, are required.[29]

Adverse Reactions

Carbapenems are generally well tolerated. There seems to be no particular propensity for them to cause major adverse effects, *C. difficile*–associated colitis, coagulation abnormalities, nephrotoxicity, or hepatotoxicity. The most common adverse events possibly, probably, or definitely related to the carbapenems are nausea, vomiting, diarrhea, rash, headache, and phlebitis, occurring in 1% to 3% of patients. All carbapenems have been associated with seizures, believed to be related to their structural similarity with γ-aminobutyric acid and antagonism at the receptor site.[30] Although the overall incidence is low, the risk is elevated in patients with renal failure and neurologic comorbidities. Seizures are more common with imipenem (1%–2%) than ertapenem, meropenem, and doripenem (0.1%–0.3%). Drug interactions are uncommon, though the combination of valproic acid and carbapenems leads to grossly subtherapeutic valproic acid levels.[31]

The incidence of imipenem and meropenem hypersensitivity has been estimated to be less than 3% in the general population.[32] Carbapenems have been considered potentially cross-allergenic with penicillins. The occurrence of hypersensitivity reactions to a carbapenem has ranged between 0% and 11% of patients with documented or self-reported history of penicillin allergy.[33–35] Most of the reactions observed were maculopapular rash. However, studies using skin testing suggest that patients with a positive skin test to penicillins for immunoglobulin E–mediated (i.e., immediate) hypersensitivity rarely have a positive skin test to carbapenems (<1%), and those with a negative skin test tolerate graded challenge doses of imipenem or meropenem.[36–38] Therefore administration of a carbapenem is considered safe in patients with a history of penicillin immediate hypersensitivity if their skin test is negative for that carbapenem. In the absence of skin testing, it appears prudent to administer carbapenem in graded doses until extended safety data on up-front administration of full doses to patients with a history of penicillin immediate hypersensitivity become available.

Clinical Use

Carbapenems display broad-spectrum activity covering gram-positive, gram-negative, and anaerobic bacteria and thus are useful for treatment of a wide variety of moderate to severe infections, including bacteremia, hospital-acquired pneumonia, intraabdominal infections, complicated urinary tract infections, bone and soft tissue infections, and obstetric and gynecologic infections. Meropenem is the only carbapenem approved by the FDA for treatment of bacterial meningitis. Imipenem should be avoided because of its propensity to cause seizures.

Carbapenems are generally active against cephalosporin-resistant Enterobacteriaceae producing ESBLs and AmpC β-lactamases but not those producing KPC β-lactamases. Recently developed β-lactamase inhibitors capable of inhibiting KPC β-lactamase as well as ESBLs and AmpC β-lactamases can be combined with a carbapenem to restore its activity against KPC β-lactamase–producing strains. One such combination (meropenem-vaborbactam) has been approved for clinical use in the United States[39] (also see Chapter 20). Ertapenem has only limited activity against enterococci and lactose-nonfermenting gram-negative species, including *P. aeruginosa.* Carbapenems other than ertapenem are drugs of choice for treatment of infections caused by multidrug-resistant strains of *A. baumannii* that remain susceptible to carbapenems; however, resistance to carbapenems is rapidly increasing.[40]

Imipenem, meropenem, and doripenem are therapeutically equivalent and interchangeable in most clinical situations, with imipenem being slightly more active against gram-positive organisms (especially *E. faecalis*) than meropenem and doripenem, and meropenem and doripenem being slightly more active against gram-negative organisms than imipenem.[28] In *P. aeruginosa,* however, predominant mechanisms of resistance can be different for imipenem (most affected by downregulation of OprD but not substrate for multidrug efflux pumps) and meropenem and doripenem (substrates of MexAB-OprM efflux pump but less affected by downregulation of OprD). Consequently, resistance to imipenem is not always predictive of resistance to meropenem or doripenem, and vice versa.[41] Doripenem is the most active carbapenem against *P. aeruginosa* in vitro. Imipenem, meropenem, and doripenem are all appropriate for use in the treatment of hospital-acquired infections because of their antipseudomonal activity. However, doripenem carries an increased risk of death and lower cure rates compared with imipenem when used to treat patients with ventilator-associated pneumonia, which is not an approved indication. *S. maltophilia, E. meningoseptica,* and *Chryseobacterium indologenes* are intrinsically resistant to all carbapenems because of chromosomal production of metallo-β-lactamase.

The recommended adult dose of imipenem for patients with creatinine clearance of greater than 50 mL/min is 250 to 500 mg every 6 hours or 1 g every 8 hours intravenously. The pediatric dose is 15 to 25 mg/kg every 6 hours. The recommended adult dose of meropenem for patients with creatinine clearance greater than 50 mL/min is 500 mg to 1 g every 8 hours. For treatment of severe infections, doses of up to 6 g/day have been used safely. The pediatric dose is 10, 20, or 40 mg/kg every 8 hours, with the highest dose indicated for treatment of meningitis. The recommended adult dose of doripenem for patients with creatinine clearance greater than 50 mL/min is 500 mg every 8 hours. The pediatric dose has not been established for doripenem. The doses should be adjusted according to the creatinine clearance for patients with renal impairment.

Ertapenem differs from other carbapenems in two important respects: it has a long half-life permitting once-daily dosing, and it has relatively poor activity against *P. aeruginosa* and *A. baumannii.* Like all carbapenems, ertapenem has excellent antianaerobic activity and thus is especially useful in a single daily dosage regimen for polymicrobial infections. Ertapenem is active against ESBLs and AmpC-producing Enterobacteriaceae and can be used for infections caused by these organisms. The recommended adult dose of ertapenem for patients with creatinine clearance greater than 30 mL/min is 1 g daily. The pediatric dose is 15 mg/kg twice daily.

MONOBACTAMS

Monobactams are monocyclic β-lactam agents characterized by the presence of a 2-oxoazetidine-1-sulfonic acid moiety. Aztreonam is the only monobactam currently approved by the FDA. Aztreonam is a synthetic monocyclic β-lactam, the core structure of which was originally isolated from *Chromobacterium violaceum* (Fig. 22.2). It has high affinity for PBP3 of gram-negative bacteria, causing their filamentation, bacterial lysis, and death.[42] It has a broad spectrum of activity against gram-negative bacteria but has no activity against gram-positive or anaerobic bacteria.[43] Aztreonam readily penetrates the outer membrane of gram-negative

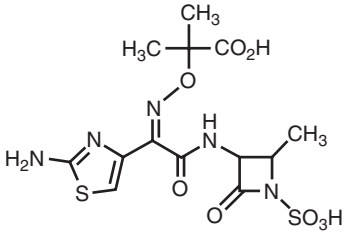

FIG. 22.2 The structure of aztreonam. The core structure is monocyclic, unlike penicillins and cephalosporins, which are bicyclic. The 1-sulfonic acid group activates the β-lactam, whereas the 4α-methyl group provides stability against β-lactamases. The aminothiazole oxime moiety on the acyl side chain is responsible for the activity against aerobic gram-negative bacteria.[77]

bacteria. It is resistant to hydrolysis by all class B β-lactamases and most class A and D β-lactamases but is hydrolyzed by KPC β-lactamases, ESBLs, and also AmpC β-lactamases when they are produced in large amounts.

Aztreonam inhibits most Enterobacteriaceae at concentrations less than 0.5 μg/mL (Table 22.2). Some *P. aeruginosa, Enterobacter cloacae,* and *Citrobacter freundii* strains are resistant. Most *P. aeruginosa* strains are inhibited by less than 16 μg/mL of aztreonam. Most *B. cepacia, S. maltophilia,* and *A. baumannii* strains are resistant.

Aztreonam is not absorbed from the gastrointestinal tract. A 1-g intravenous dose of aztreonam produces serum concentrations of approximately 50 μg/mL at 1 hour.[44] Aztreonam is 56% protein bound and distributes well in tissues and fluids throughout the body. Mean cerebrospinal fluid concentration in the presence of inflamed meninges after a single 2-g dose is 2 to 3 μg/mL; higher concentrations can be achieved after multiple doses.[45]

Aztreonam is renally excreted, with 60% to 65% recovered in urine.[44] In adults with normal renal and hepatic function, the elimination half-life is 1.7 hours.[44] In neonates 7 days old, the half-life of aztreonam is approximately 6 hours.[46] The rate of serum clearance of aztreonam is linearly related to the rate of urinary creatinine clearance. The half-life of aztreonam is approximately 6 hours at creatinine clearances of 10 mL/min or less. The half-life of aztreonam is slightly prolonged in patients with hepatic impairment; dose adjustment is not necessary in patients with chronic hepatic disease if renal function is not impaired. Aztreonam is cleared by continuous venovenous hemofiltration, hemodialysis, and peritoneal dialysis. Standard hemodialysis removes about half of a 1-g dose given just before dialysis.[47]

Aztreonam is well tolerated. Local reactions, including phlebitis, occur in less than 2% of patients. Diarrhea, nausea, vomiting, and rash can occur in about 1% of patients.[48] Cross-reactivity with penicillins and cephalosporins is extremely rare, even in patients with immunologically proven hypersensitivity to other β-lactams,[49] though caution is still recommended in the setting of repeated exposure to aztreonam.[50]

TABLE 22.2 Activity of Aztreonam Against Selected Aerobic Bacteria

ORGANISM	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	REFERENCES
Haemophilus influenzae	0.06	0.12	75
Moraxella catarrhalis	0.12	0.25	75
Neisseria gonorrhoeae	0.125	0.25	76
Neisseria meningitidis	≤0.06	≤0.06	77
Escherichia coli	≤0.12	0.25	78
Escherichia coli, ESBL producing	4	>16	78
Salmonella spp.	≤0.12	0.25	78
Shigella spp.	≤0.12	≤0.12	78
Klebsiella spp.	≤0.12	0.5	78
Klebsiella spp., ESBL producing	>16	>16	78
Enterobacter spp.	≤0.12	>16	78
Citrobacter spp.	≤0.12	>16	78
Serratia spp.	≤0.12	0.5	78
Proteus mirabilis	≤0.12	≤0.12	78
Aeromonas spp.	≤0.12	0.25	56
Pseudomonas aeruginosa	8	>16	56
Acinetobacter spp.	>16	>16	56
Stenotrophomonas maltophilia	>16	>16	56
Burkholderia cepacia	16	>16	56

ESBL, Extended-spectrum β-lactamase; *MIC_{50},* minimal inhibitory concentration for 50% of isolates; *MIC_{90},* minimal inhibitory concentration for 90% of isolates.

Aztreonam is rarely used alone empirically because its spectrum of activity is limited entirely to aerobic gram-negative bacteria. Aztreonam has been used safely and effectively in conjunction with agents that have gram-positive and anaerobic activity. Its greatest utility is for definitive treatment of infections caused by aerobic gram-negative bacteria, which are susceptible to aztreonam, in a patient who has severe allergy to penicillin or other β-lactams. It may also have a role in combination therapy of infections caused by metallo-β-lactamase–producing gram-negative bacteria, although these strains often produce other β-lactamases that hydrolyze aztreonam.[51] The usual dose is 1 to 2 g every 6 to 8 hours intravenously or intramuscularly, with a daily dose for serious infection of up to 6 g. The pediatric dose is 30 mg/kg every 6 to 8 hours.

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The complete reference list is available online at Expert Consult.

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23 Antibiotic Allergy

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The majority of adverse drug events are not immunologically mediated allergic reactions but rather what are categorized as type A drug reactions, referring to predictable, dose-dependent events secondary to the pharmacologic action of the drug. Only 10% to 15% of reactions are defined as type B, which are seemingly random and unrelated to pharmacologic effects and include the development of drug hypersensitivity and other idiosyncratic reactions. Antibiotics are the most important cause of allergic drug reactions for infectious disease specialists. Maculopapular exanthems and urticaria account for most of these, but more severe reactions may also occur.

Correct labeling of an adverse drug reaction as a pharmacologic side effect of the drug versus a true immunologic event is essential for prevention of erroneous allergy labels. Improving the accuracy of antibiotic allergy reporting is required in order to reduce the impact of antibiotic allergy labels.

PATHOPHYSIOLOGY

According to the Gell and Coombs classification, there are four main pathophysiologic mechanisms that result in allergic drug reactions. Immunoglobulin E (IgE)-mediated immediate reactions (type 1) are relatively common and may manifest as symptoms of urticaria and angioedema or anaphylaxis. Cytotoxic reactions (type 2) are exemplified by drug-induced hemolytic anemia or thrombocytopenia and are a relatively uncommon reaction from antimicrobial agents. Immune complex reactions (type 3) are serum sickness–like reactions with rashes, fever, and arthralgias and typically occur several days after initiation of the culprit drug. Finally, delayed-type hypersensitivity reactions (type 4), induced by T-cells, represent the most common manifestation of antimicrobial drug reactions as exemplified by the maculopapular exanthem.

Four additional subclassifications have been introduced for type IV reactions that highlight the heterogeneous T-cell response and clinical variability in these delayed drug reactions.[1,2] Reactions of the type IVa subclassification are Th1-mediated reactions involving macrophage activation and occur as an exanthem; type IVb reactions are Th2 mediated with eosinophil-rich exanthems or bullous lesions; type IVc reactions are cytotoxic reactions with bullous exanthems or pustular reactions; and type IVd reactions are neutrophil-rich pustular reactions, such as acute generalized exanthematous pustulosis (AGEP).

Although this hypersensitivity classification system is still used today in the management of drug hypersensitivity, many drug reactions do not neatly fit into this system. Pseudoallergic reactions cause reactions similar to IgE-mediated reactions but are due to IgE-independent mast cell activation. Red man syndrome from vancomycin is an example. Recently, the human G protein–coupled receptor MRGPRX2 has been identified as a mast cell specific receptor critical for pseudoallergic drug reactions to drugs such as ciprofloxacin.[3] Delayed severe cutaneous adverse drug reactions (SCARs) have unclear mechanisms that may involve classic features of delayed-type hypersensitivity and also other mechanisms.

Approach to a Patient With an Antibiotic Allergy

The accurate diagnosis of drug allergy is important not only to prevent serious reactions secondary to further exposure but also to avoid the unnecessary restriction of a drug to which the patient may not truly be allergic.

The clinical history is integral in evaluating the likelihood of a drug allergy, because only a small percentage of patients with a reported reaction have a history compatible with actual hypersensitivity. Even in the presence of a consistent history, the proportion of true reactors is low.

Key historical features to seek out include a detailed description of the nature and timing of the reaction, and concomitant ingestion of other medications. A clinical history of anaphylactic shock has the highest predictive value for IgE-mediated allergy, with a fourfold increase in risk.[4] In addition, taking a thorough medical history for underlying risk factors (e.g., viral infections, prior drug reactions, atopy) is crucial for evaluating antibiotic allergy.

Information to Be Obtained When Taking a History of Antibiotic Allergy

Concerns regarding the reaction include:

- Timing of the reaction in relation to drug administration
- Symptoms and evolution of the reaction
- Description of cutaneous symptoms (e.g., maculopapular, urticarial, bullous)
- Involvement of mucosal surfaces or internal organs
- Treatment administered, response, and duration of reaction
- History of prior exposure to the implicated agent
- Other medications ingested at the time of the reaction
- Whether the medication or similar medications were taken (and tolerated) thereafter
- Whether there are potential confounders (e.g., underlying viral or bacterial infections)
- History of other drug reactions and allergies (many patients with multiple drug intolerance syndrome do not have true drug allergies)
- Whether the patient has experienced recurrent, similar reactions without known exposures (e.g., chronic urticaria)
- The likelihood of future need of the medication

CLINICAL MANIFESTATIONS

The clinical spectrum of antibiotic allergy is extremely heterogeneous. There are two broad entities based on timing and pathophysiologic mechanism.

Immediate (Immunoglobulin E–Mediated or Pseudoallergic) Drug Reactions

Immediate IgE-mediated reactions result from the interaction of drug antigens with preformed drug-specific IgE antibodies bound to mast cells or basophils, with the consequent release of preformed mediators (histamine, proteases, and chemotactic factors) and newly generated mediators (prostaglandins, leukotrienes, and platelet-activating factor). These reactions usually occur within 1 hour of drug administration and clinically manifest as urticaria, angioedema, rhinitis, bronchospasm, or anaphylaxis. IgE-mediated reactions require prior sensitization to the drug or structurally related drugs, typically from prior exposure. Pseudoallergic reactions may also result in immediate reactions, often with first exposure to the drug. These reactions are due to nonspecific (IgE-independent) activation of mast cells and can cause reactions clinically indistinguishable from IgE-mediated reactions. The mast cell receptor MRGPRX2 has been identified as an important receptor in

pseudoallergic reactions to several drugs.[3] It is also possible for drug-specific IgG to activate complement, which results in acute rashes and non–IgE-mediated mast cell activation.

Nonimmediate Drug Reactions

Nonimmediate drug reactions have been further subdivided as accelerated (predominantly urticaria) or late reactions. By definition, they occur more than 1 hour and within 7 days after the last drug administration. Although drug reactions can involve multiple organs, cutaneous reactions are the most common. There are numerous cutaneous manifestations of drug reactions, which include maculopapular exanthems, bullous lesions, and pustules, to name a few. Severe cutaneous adverse reactions (SCARs), including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), may include internal organ involvement. It should be kept in mind that a significant proportion of maculopapular or urticarial reactions labeled as drug reactions are secondary to the underlying infection itself, without any contribution by the suspected agent.[5] In other situations, the incriminated antibiotic may cause allergic manifestations only in the presence of a specific underlying predisposing factor (e.g., ampicillin-induced rash in Epstein-Barr infection; increased risk for sulfonamide-related rashes in people infected with human immunodeficiency virus [HIV] and CD4 lymphopenia).

Drug Rash With Eosinophilia and Systemic Symptoms

Drug rash with eosinophilia and systemic symptoms (DRESS), also known as drug (induced) hypersensitivity syndrome (DHS or DiHS), is a systemic reaction that is distinguished from other antimicrobial reactions by a delayed appearance after a 2- to 10-week exposure to several antibiotics, including sulfonamides and vancomycin. Clinical characteristics include rash, fever, lymph node swelling, hepatitis, or involvement of other organs. Facial swelling is a common feature and may be confused with IgE-mediated angioedema; some patients have signs of a capillary leak syndrome. Interesting to note, patients may develop disease flares after discontinuation of the culprit drug, which may result in other medications being falsely labeled as causing a drug reaction. In addition, the course is characterized by relapses that have been attributed to the reactivation of latent viral infection, in particular human herpesvirus 6.[6] DRESS reactions are also atypical in that clinical manifestations may worsen despite drug withdrawal, and symptoms may persist for months and in some cases require immunosuppressive therapies.

DIAGNOSIS AND MANAGEMENT OF ANTIBIOTIC ALLERGY

Many antibiotic-associated adverse drug reactions are unlikely to be true allergies that preclude drug therapy. Although the quantification of drug allergy risk through history is essential, this alone is grossly insufficient in clinical decision making. However, investigation is often hampered by lack of reliable immunologic tests, which are available for only a few drugs. Skin tests for most antimicrobial agents lack high negative predictive values. Therefore patients may be tested for drug tolerance via a drug challenge, or a desensitization procedure may be performed to induce temporary drug tolerance.

Tests for Immunoglobulin E–Mediated Immediate Reactions

Skin Testing: Prick and Intradermal Tests

The reagents used in skin testing are seldom standardized (except for penicillins, as detailed later); and in the majority of cases, the predictive value of skin testing remains indeterminate. Its use for various antibiotics is described in the sections on individual antibiotics.

In vitro Tests

Serum-specific IgE assays have been used for evaluating immediate reactions to β-lactams (mainly penicillins), with a relatively low sensitivity (~50%) compared with skin testing and a specificity approaching 90%.[7] The role of the flow cytometric basophil activation test (BAT) in the diagnostic evaluation of immediate reactions is still being investigated. The BAT detects the upregulation of activation markers CD63 and CD203c on the surface of basophils after incubation with the implicated drug. Multiple studies with BATs have been performed for β-lactams and fluoroquinolones and have found such testing to be highly specific but with low sensitivity. Commercially available assays have not undergone rigorous testing and are generally not recommended for use in making management decisions.

Drug Challenge (Drug Provocation Tests)

A drug challenge is generally accepted as the gold standard to establish tolerance to a drug. For clinical purposes, drug challenges are recommended when a true drug allergy is deemed unlikely based on the history and available diagnostic tests. In most cases, when the pretest probability is determined to be low for a drug allergy, drug challenges allow drug hypersensitivity to be excluded in a large percentage of patients.[8,9] Pretest probability is dependent on the nature of the reaction and the date of onset. The probability of true IgE-mediated allergy is high (20%–50%) in the setting of antibiotic-associated anaphylaxis within 3 months.[10] On the other hand, the risk of immediate hypersensitivity decreases to 1% to 2% with a distant (more than 10 years) history of benign symptoms. Unlike desensitization, challenge protocols are not designed to alter the immune response to a drug and merely confirm the presence or absence of sensitization. If the clinical question is whether a patient will tolerate a drug, a drug challenge is the appropriate procedure. Most drug challenges for immediate reactions are performed in a graded fashion with escalated dosing every 30 to 60 minutes. A common protocol is to start with $\frac{1}{10}$ of the therapeutic dose, followed by the final dose. Multistep challenges do not contribute further safety to the procedure.[11] If a patient tolerates the challenge, he or she may receive a therapeutic course of the drug. If the entire therapeutic course is tolerated without reaction, it is reasonable to remove the drug allergy label from the patient's chart.

For delayed reactions such as maculopapular exanthems and delayed-appearing urticaria, drug challenges are recommended when a true drug allergy is deemed unlikely based on the history and available diagnostic tests.[9,12] Protocols vary for delayed drug challenges. Some protocols are performed over days to weeks until a therapeutic dose is achieved. Similar to immediate drug challenges, a dose escalation starting with $\frac{1}{100}$ or $\frac{1}{10}$ of the final dose is performed, but the interval of dose escalation may be 2 to 3 days or a week, depending on the time interval between the drug intake and the index reaction. Another approach uses the immediate drug challenge approach, reaching a therapeutic dose in 1 to 2 hours. For more acute indications of antimicrobial agents, this latter approach may be more practical. Some experts suggest that multiday challenges do not appear to be any safer or more useful than single-dose challenges.[13] It is essential to update the patient's medical records and ensure that the antibiotic allergy label is removed after an uneventful challenge.

Contraindications to drug challenges include non–cutaneous-based reactions (e.g., hepatitis, cytopenias, and pneumonitis), serum-sickness reactions, drug-induced vasculitis, bullous eruptions, and SCARs, which include SJS and TEN, DRESS, and AGEP reactions. Although anaphylaxis is rare, equipment to treat anaphylactic reactions should be readily available, including epinephrine.

Skin and in vitro Tests for Nonimmediate Reactions

There is currently no established gold standard for the diagnosis of a delayed-type allergy.

Skin Testing: Delayed Intradermal and Patch Tests

Both delayed reading intradermal testing and patch testing have been used as in vivo methods for diagnosis of nonimmediate allergy, with various levels of sensitivity and specificity.[12,14]

Intradermal testing should not be performed in patients with SCARs owing to the risk of reactivation and an increased risk of systemic events. A multicenter study confirmed that patch tests are safe in patients with SCARs and have the highest sensitivity for DRESS (32%–80%) and AGEP reactions (58%–64%).[15] The negative predictive value for most drugs has not been well established. Thus a positive test result may suggest delayed hypersensitivity, but a negative test result does not exclude a drug allergy. According to European guidelines, patch testing

with suspect antibiotics should be used as first line of investigation in severe delayed reactions.[16] In the United States, few centers use drug patch testing in the evaluation of SCAR.

In vitro Tests

The lymphocyte transformation test (LTT), the lymphocyte activation test (LAT) evaluating for CD69 expression through flow cytometry, and enzyme-linked immunospot (ELISPOT) assays have been used to assess drug-specific, cytokine-producing T-cell activity in vitro.[17,18] Performance characteristics of these tests vary by laboratory, and they are mainly used for research purposes. Commercially available assays have not undergone rigorous testing and are generally not recommended for use in making management decisions.

Procedures to Induce Temporary Drug Tolerance

Drug Desensitization

In drug-allergic patients for whom no therapeutic alternative exists, a procedure to induce temporary drug tolerance can be considered.[19] These procedures are usually referred to as drug desensitizations. In contrast to drug challenges, which are merely a diagnostic test for tolerance to a drug, desensitization procedures actively induce tolerance through mechanisms that are still unclear but may involve internalization of high-affinity IgE receptors.[20] Desensitization procedures cause a state of temporary tolerance and allow a patient to receive an uninterrupted therapeutic course of an antimicrobial agent. It is critical to recognize that this "desensitized" state of drug tolerance is transient and that after cessation of the drug the patient's prior hypersensitive state returns. If the patient requires the drug again, a desensitization procedure will be required before each therapeutic course.

Desensitization protocols have been established for several different antibiotics in the event of documented (e.g., positive drug skin test result) or presumed IgE-mediated sensitization. Most protocols begin with a dilute concentration of drug (e.g., $\frac{1}{10,000}$ of dose), and the dose is typically doubled every 15 minutes until a full therapeutic dose is reached. Drug desensitizations for antibiotics are generally well tolerated, and anaphylactic reactions are rare. Antibiotic desensitizations should be conducted by personnel familiar with the procedure, equipment should be available to treat anaphylaxis, and close monitoring should be maintained. Owing to the requirement for close monitoring and frequent dose adjustments, desensitization procedures are often performed in an intensive care unit, although this is not a strict requirement. Desensitizations can be performed intravenously or orally. If feasible, an oral route is preferred because it may be a safer route of administration. The same aforementioned contraindications for drug challenges apply to drug desensitizations.

"Treating Through" Antibiotic-Associated Exanthems

"Treating through" refers to unchanged continuation of the antibiotic therapy despite the occurrence of maculopapular exanthem when the benefits of antibiotic therapy outweigh the risks. There are a very small number of reports describing this strategy and it must be conducted by an experienced allergist, with close monitoring of cutaneous symptoms and laboratory parameters.[21]

DRUG ALLERGY TO SPECIFIC ANTIMICROBIAL AGENTS

β-Lactams

β-Lactams represent the main cause of both immediate and nonimmediate allergic drug reactions. The four main groups share in common a four-membered β-lactam ring; and if this ring is fused to a thiazolidine ring, the β-lactam is classified as a penicillin. This includes piperacillin and the antistaphylococcal penicillins. The thiazolidine ring is replaced by a dihydrothiazine ring in the cephalosporin nucleus. In addition, penicillins have only one side chain (R1 group at 6-position), whereas cephalosporins have two side chains (R1 and R2 at the 3- and 7-positions, respectively). Monobactams contain a monocyclic ring structure, whereas carbapenems have a bicyclic nucleus composed of a β-lactam ring with an associated five-membered ring.

β-Lactams act as haptens and require conjugation to a carrier molecule to be recognized as a sensitizing molecule and to elicit an allergic response.

Penicillins

Epidemiology

The prevalence of self-reported penicillin allergy is high, at 10% to 15% among hospitalized patients. Because the diagnosis is often not substantiated by further testing, the use of suboptimal antibiotics contributes to worsening antibiotic resistance and increased health care costs. Recent literature suggests that up to 98% of patients with a history of penicillin allergy have negative findings on investigation.[22] This extreme discrepancy is secondary to the previously discussed factors of inaccurate history and compounding infectious causes that elicit similar reactions. Furthermore, IgE-mediated sensitivity to penicillins has been documented to wane over time, to less than 20% at 10 years after the reaction.[23] In a cohort of β-lactam allergic children (confirmed with drug provocation testing [DPT]), systematic follow-up DPT demonstrated tolerance at a mean of 3.5 years.[24]

Addressing the label of penicillin allergy has emerged as a significant public health risk. Several observational studies have reported clinical and economic outcomes associated with penicillin allergy, mostly focusing on inpatient admissions. In one institution, patients with reported penicillin allergy had a 51% increased risk of developing surgical site infections, primarily related to the substitution of non–β-lactam prophylactic antibiotics for cephalosporins.[25] King and colleagues calculated average savings of $297 per patient with a switch from a non–β-lactam antibiotic to a β-lactam antibiotic.[26] Macy and Contreras estimated indirect costs from associated complications in a study of 51,582 inpatients with a label of penicillin allergy, including increased use of non–β-lactam antibiotics, increased methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), and longer average hospital stay (0.59 days).[27] The authors presented calculations regarding potential cost savings with penicillin skin testing of $64.6 million by shortening the hospital stay by 0.59 days per patient (assuming negative skin test result in 95% of patients). This cost analysis was based on a single patient encounter, and the authors postulated that the cost throughout a patient's life would likely yield much larger differences.

Evaluation of Penicillin Allergy as Part of Antimicrobial Stewardship

Because penicillins are often the drug of choice for many infections, use of the label of penicillin allergy leads to more broad-spectrum antibiotic use, which leads to increased morbidity. The vast majority of patients with a label of penicillin allergy are not allergic; therefore addressing reported penicillin allergy has become an important part of antimicrobial stewardship. Recent position statements from the American Academy of Allergy, Asthma, and Immunology endorse that penicillin allergy testing should be done routinely in patients with self-reported penicillin allergy.[28] This will be discussed later in greater detail.

Pathophysiology

Two major mechanisms have been implicated in allergic reactions to β-lactams: IgE-dependent responses and T-cell–mediated reactions. In the event of the former, rapid and stable cleavage of the β-lactam ring results in the generation of defined epitopes that act as haptens, as described earlier. The major determinant derived from the β-lactam ring is known as benzylpenicilloyl (accounting for 95% of haptenated penicillin), and there are also minor determinants (of which only penicillin G is commercially available in the United States).[29] Furthermore, the R1 side chain is recognized as the antigenic determinant in the case of some reactions involving aminopenicillins, and these patients are selectively allergic to amoxicillin or ampicillin but tolerate other agents. This selective aminopenicillin allergy is commonly reported in European studies but is rarely reported in the United States. In addition, some patients react only to clavulanic acid, thereby tolerating amoxicillin but reacting to amoxicillin-clavulanate. Again, there are limited data on this phenomenon, and all are from southern Europe.

Clinical Manifestations

Penicillin allergy may manifest either as anamnestic responses to IgE formation or as delayed T-cell–mediated reactions. Maculopapular exanthems occur at a frequency of 2% to 3% during treatment, but SCARs may also develop. Amoxicillin is the most commonly used β-lactam in North America and Europe, and the most frequent drug involved in immediate and in nonimmediate reactions.

Diagnosis

Penicillin skin testing is an excellent diagnostic tool. The clinical history is known to be an unreliable predictor of skin test results.[30] The negative predictive value for skin testing with commercially available reagents (benzylpenicilloyl polylysine [PRE-PEN] and penicillin G) is approximately 97%. On the other hand, the positive predictive value of penicillin skin testing is unknown owing to ethical concerns regarding challenging patients who have positive reactions. Most practices follow a negative penicillin skin test result with an open challenge to amoxicillin, which increases the negative predictive value of the evaluation to almost 100%. Amoxicillin is commonly used to challenge after negative penicillin skin test results because it addresses the core β-lactam of penicillin and also side chain–specific reactions, which may not be detected through skin testing with penicillin itself. Although mainly indicated for immediate reactions, penicillin skin testing is often performed in patients with a history of delayed reactions because histories are often inaccurate and exclusion of the presence of penicillin IgE is important. It is typically recommended that penicillin skin testing be performed at least 6 weeks after a reaction (especially if anaphylactic) owing to the potential for false-negative skin test results. This recommendation is more theoretical and not evidence based. It is important to note that penicillin skin testing has no value in SJS and TEN, DRESS, and other noncutaneous organ-based reactions.

Studies have demonstrated uneventful direct oral challenges without skin testing in patients with a history of non–life-threatening reactions, particularly in the case of drug exanthems.[31] Furthermore, fatal anaphylaxis to penicillin is rare, with the majority of reported cases occurring in patients without a prior allergic reaction. Hence, a graded-dose challenge is often appropriate and usually well tolerated in these situations.[32] Recently, more data have been emerging regarding the safety of direct oral challenges without a preliminary negative skin test result in cases of low-risk phenotypes and nonanaphylactic symptoms, such as in patients with benign delayed-onset nonurticarial rashes. Studies in children and adults with histories predominantly of delayed reactions to penicillin have confirmed the safety of this approach.[33,34] This approach requires a skilled allergist and a reliable historian, and will need further confirmation in larger studies before it can become the new standard of care. A clinically significant delayed hypersensitivity to amoxicillin will typically manifest within 5 days of a single-dose oral amoxicillin challenge.[35]

Conversely, in vitro testing for anti-penicillin IgE vastly exaggerates sensitivity. Because anti-penicillin IgE has a poor correlation with clinically significant symptoms, commercially available serologic tests have little clinical usefulness.[36]

Desensitization

Patients with positive penicillin skin testing results should receive alternative agents, but if there are no alternative antibiotics indicated, desensitization procedures may be used. The procedure is typically successful, although 30% of patients tend to develop minor cutaneous reactions such as urticaria.

Cephalosporins

Pathophysiology

Cephalosporins form a heterogeneous group with five generations available in terms of antibiotic spectrum. Allergic reactions to cephalosporins may occur because of sensitization to antigenic determinants shared with penicillin or to unique cephalosporin haptens. In contrast to penicillins, cephalosporins undergo extensive fragmentation at the dihydrothiazine ring, with the consequent formation of a large number of degradation products, which has hindered the understanding of its haptenic determinants. However, the R1 group side chain is believed to contribute to most of the antigenicity. Consequently, IgE-mediated cephalosporin hypersensitivity is not necessarily a class hypersensitivity but rather is based on R1 side chain similarity.[37]

Clinical Manifestations

The majority of allergic reactions to cephalosporins are delayed rashes, but IgE-mediated urticaria and anaphylaxis can also occur.

Diagnosis

Evaluation of immediate reactions to cephalosporins may include skin testing with the suspected compound, along with other cephalosporins and penicillin determinants. However, even though skin tests with cephalosporins have been evaluated in various studies, they are not as well validated as with penicillin. In particular, the negative and positive predictive values are not fully established.[38] Testing with nonirritating concentrations (2 mg/mL) has been used to identify IgE-mediated allergy, although the exact sensitivity (ranging from 30.7% to 72% in various studies) and specificity of this skin test have not been determined.[39,40] In patients with a cephalosporin allergy, skin testing to structurally different cephalosporins appears to have good negative predictive value in indicating tolerance to other cephalosporins.[36] The role of skin testing is even less well defined with a history of delayed reactions to cephalosporins. Although intradermal and patch testing have been used, overall rates of sensitization are extremely low (~5%). In one study, most delayed skin manifestations attributed to cephalosporin treatment did not demonstrate positive skin test results, and subsequent challenges failed to reproduce the symptoms.[39] Thus, like patients with histories of penicillin allergy, many patients with histories of cephalosporin allergy appear to tolerate cephalosporins. Drug challenges are also appropriate to evaluate cephalosporin-allergic patients when the likelihood of true allergy is low.

Desensitization

Desensitization to cephalosporins may be considered for documented or presumed IgE-mediated reactions. Successful desensitization to cefotaxime and ceftazidime has been reported without major side effects.

Carbapenems

Current carbapenems available in the United States include imipenem-cilastatin, meropenem, doripenem, and ertapenem, and the incidence of associated hypersensitivity is estimated to be less than 3%.

Skin testing has not been well studied in carbapenem allergy, and thus the negative predictive value is unknown. Successful desensitization regimens to both imipenem and meropenem have been described, mostly as case reports, wherein carbapenems were the only antibiotic indicated.

Monobactams

Aztreonam is generally less immunogenic than other β-lactams because reactive haptenic breakdown products are less likely to be formed. It is considered a useful therapeutic alternative to patients with sensitivity to other β-lactams. Aztreonam does not cross react with other β-lactams except for ceftazidime, with which it shares an identical R-group side chain.

Cross-Reactivity Among β-Lactams

There has been a definite paradigm shift in the use of cephalosporins among the penicillin-allergic population. It is now recognized that cross-reactivity is not equal among all β-lactams, with most recent studies describing much lower rates of potential cross-reactivity.[16] Cross-reactivity between cephalosporins and penicillins is especially rare for cell-mediated reactions.[41] In most instances, positive in vitro testing results suggestive of potential cross-reactivity between penicillins or cephalosporins do not translate into clinical reactions.[42] In addition, penicillin allergy predisposes to a threefold higher risk for reactions to structurally unrelated antibiotics, and a concomitant cephalosporin allergy does not necessarily indicate cross-reactivity.

For patients with histories of nonsevere reactions to penicillin, the likelihood of reacting to cephalosporins is approximately 0.2%, based on retrospective evaluations of patients with reported allergy.[43,44] If

patients with a history of penicillin allergy are found to have negative skin test results, the risk for reacting to a cephalosporin is negligible. Studies involving patients with a history of penicillin allergy and positive penicillin skin testing results who subsequently received cephalosporins have found an overall reaction rate of 2%. Cross-reactivity between penicillins and cephalosporins can be predicted based on the presence of similar R1 side chains, such as occurs between ampicillin/amoxicillin and cephalexin/cefadroxil, in the range of 10% to 38% (Table 23.1).[45–48] Although most patients tolerate the corresponding cephalosporin, anaphylactic reactions have been noted. For these and other higher-risk patients, graded cephalosporin challenges can be performed to ensure tolerance.[44,49] Subjects with an IgE-mediated hypersensitivity to penicillin can be treated with cephalosporins with dissimilar side chains.[50] Third-generation cephalosporins can be used in patients with a history of nonimmediate or non–life-threatening allergy to penicillin. Fig. 23.1 outlines a suggested approach to patients with penicillin allergy in need of other β-lactams.

Conversely, in patients evaluated after an immediate allergic reaction to cephalosporins, the rates of positive results of testing for reactions to penicillin determinants have been found to be 4.8% to 25.5% in various series.[51–53] However, the studies with higher rates of sensitization were not confirmed in most patients by subsequent clinical challenges. If penicillin skin testing is not available, graded challenges may be performed starting with $\frac{1}{10}$ of the therapeutic dose. Only 1% to 3% of cephalosporin-allergic patients have positive results of skin tests for carbapenems or aztreonam.[51] Among the different kinds of cephalosporins themselves, the R1 side chain is an important antigenic determinant, and cross-reactivity must be considered in terms of structural similarity.[40,53] Of note, important cross-reactivity has been detected among ceftriaxone, cefotaxime, and cefepime, which share an identical side chain at the R1 position.[53–55] Recent work by Romano and colleagues demonstrated that patients with cephalosporin allergy commonly tolerated a different cephalosporin of varied R1/R2 side chain.[37]

Fig. 23.2 outlines a suggested approach to patients with cephalosporin allergy who are in need of other β-lactams.

The first study to determine the potential for cross-reactivity between carbapenems and penicillins based on skin testing estimated a frequency of approximately 50% in penicillin skin test–positive patients.[56] Subsequent clinical studies of penicillin-allergic patients have evidenced progressively lower rates, 0% to 11% for imipenem and meropenem,[57–59] in patients with histories of penicillin allergies. A systematic review found a 2.4% to 4.3% rate of hypersensitivity response to carbapenems in patients with penicillin IgE-mediated allergies.[60] The lowest rates (0%) have been noted in prospective studies of penicillin skin test–positive subjects (all carbapenem skin test negative) who were challenged with carbapenems in clinical studies. Higher incidences (6%–11%) of reactions have been reported in retrospective studies of patients with reported

TABLE 23.1 β-Lactams With Common Side Chains

Five Groups of β-Lactams With Common R1 Side Chains

COMMON AMINOBENZYL GROUP	COMMON AMINOBENZYL GROUP	COMMON METHYLENE GROUP
Amoxicillin Cefadroxil Cefatrizine Cefprozil	Ampicillin Cephalexin Cefaclor Cephradine Cephaloglycin Loracarbef	Benzyl penicillin Cephalothin
COMMON METHOXYIMINO GROUP	**COMMON AMINOTHIAZOLE GROUP**	
Ceftriaxone Cefuroxime Cefotaxime Cefepime	Ceftazidime Aztreonam	

Six Groups of β-Lactams With Common R2 Side Chains

Cephalexin Cefadroxil Cephradine	Cefotaxime Cephalothin Cephaloglycin	Cefuroxime Cefoxitin
Cefotetan Cefamandole Cefmetazole Cefpiramide	Cefaclor Loracarbef	Ceftibuten Ceftizoxime

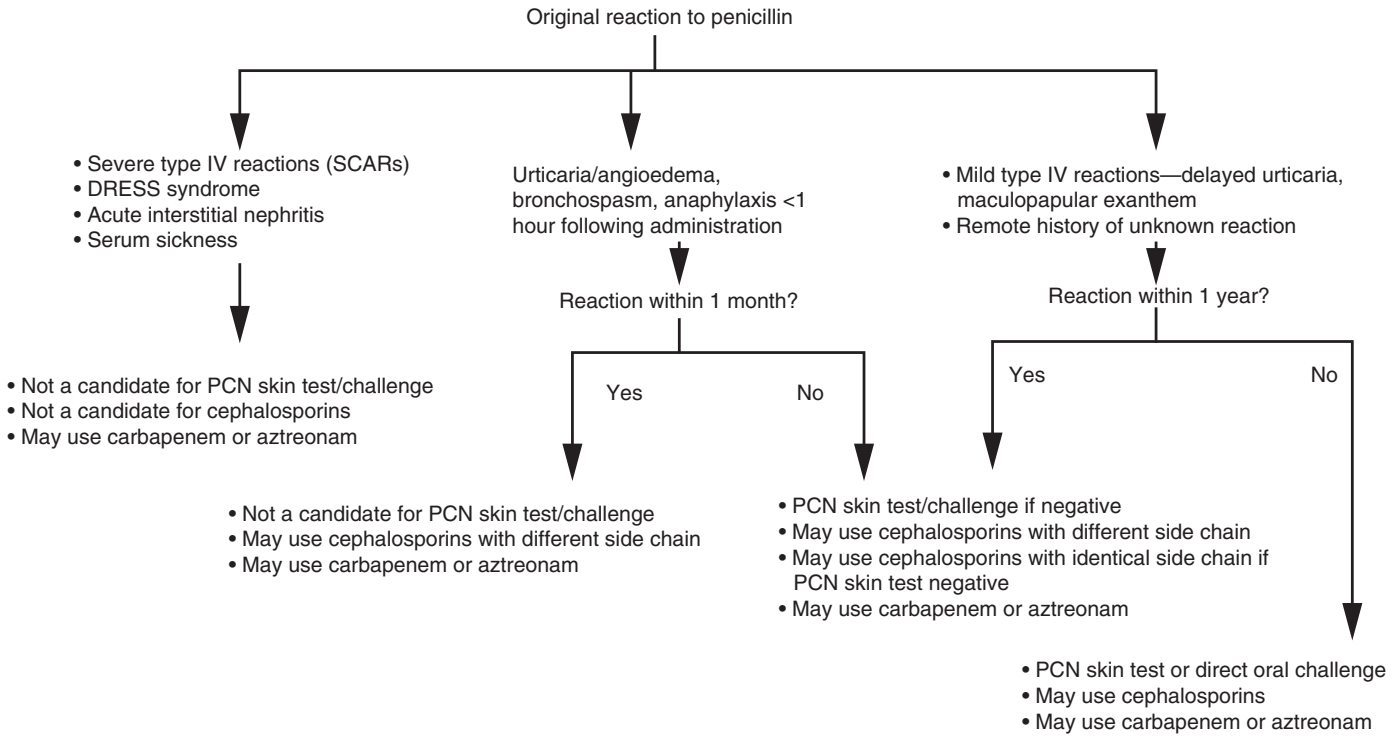

FIG. 23.1 Approach to the use of β-lactams in patients with a history of penicillin allergy. *DRESS,* Drug rash with eosinophilia and systemic symptoms; *PCN,* penicillin; *SCAR,* severe cutaneous adverse reaction.

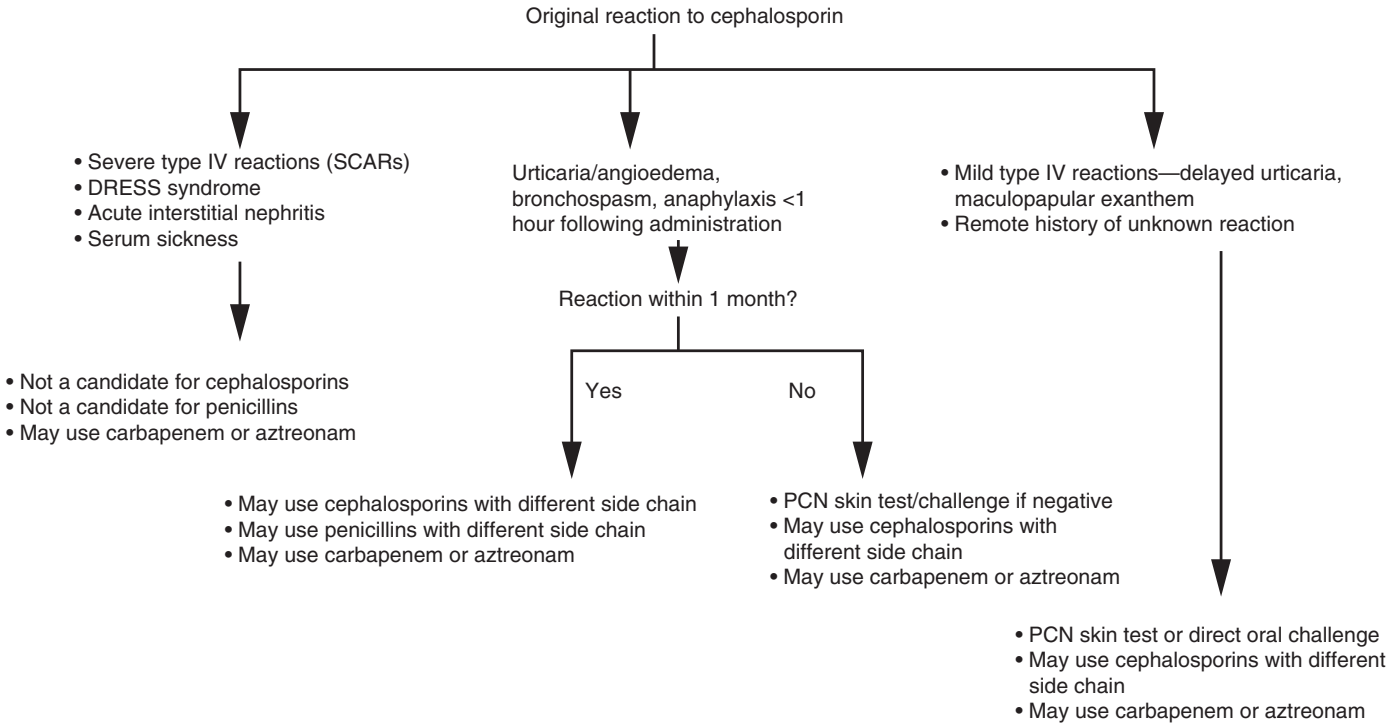

FIG. 23.2 Approach to the use of β-lactams in patients with a history of cephalosporin allergy. *DRESS,* Drug rash with eosinophilia and systemic symptoms; *PCN,* penicillin; *SCAR,* severe cutaneous adverse reaction.

penicillin allergy who received carbapenems. This discrepancy in rates may be due to potential confounding factors in the retrospective reports. Carbapenems may be safely administered in patients with negative results of skin tests for both penicillins and carbapenems. A typical approach for penicillin-allergic patients (when penicillin skin tests are unavailable or the results are positive) is to administer $\frac{1}{10}$ of the carbapenem, and, if tolerated, administer the rest of the therapeutic dose 1 hour later.

The tolerability of aztreonam in populations with IgE-mediated hypersensitivity to β-lactams has been well established,[61] except with a history of reactions to ceftazidime by reason of identical side chains.

In conclusion, in most instances there is minimal evidence for clinical cross-reactivity among various classes of β-lactams. The highest reported rate is in skin test–positive cephalosporin-allergic patients, in whom 25% have positive penicillin skin test results. It should be noted that this cross-sensitization at skin testing does not always translate into true clinical reactivity on subsequent challenge.

Sulfonamides

Pathophysiology

Sulfonamide antibiotics contain an SO_2-NH_2 group that is metabolized to reactive, haptenic nitroso compounds in the liver. IgE-mediated reactions are infrequent, and most cases are due to T-cell–mediated reactions.

Clinical Manifestations

Although maculopapular exanthems are the most common form of drug allergy with sulfonamides, they are an established cause of more serious reactions, such as SJS and TEN or DRESS. In fact, trimethoprim-sulfamethoxazole (TMP-SMX) was implicated in 66.6% of cutaneous drug reactions requiring hospitalization in one study.[62] Of note, TMP-SMX has also been observed to have a much higher reaction rate in the HIV-infected population, mostly in the form of exanthems.[63]

Diagnosis

Skin testing with sulfonamides lacks good predictive value. As with other drugs, graded challenges can be performed if the likelihood is low for true allergy.[64] Most experts believe that testing for sulfonamide allergy should be approached with caution because of the well-known propensity of these drugs to cause SCARs.

Treatment

In nonimmediate reactions with mild rashes, especially in HIV-positive individuals, the antibiotic can be continued ("treating through") or readministered after a gradually increasing dosing protocol, with good success rates (54%–100%).[65] Such reactions are not IgE mediated, but the term *desensitization* has been used to describe the various protocols to induce tolerance to TMP-SMX. Furthermore, these so-called desensitization procedures may or may not truly induce drug tolerance and do require monitoring for more serious systemic involvement. Other studies have shown similar rates of success in achieving a therapeutic dose by simply rechallenging patients versus using a "desensitization" procedure.[66,67] A 6- or 14-step graded administration over 1 day in patients without HIV was successful in 89% to 98% of patients, who were able to start therapy with TMP-SMX.[68] Whether a simple rechallenge in these non–HIV-positive patients would be similarly effective has not been studied.

Vancomycin

One of the most common adverse reactions to vancomycin is the infusion-related red man syndrome, which is secondary to direct mast cell activation and histamine release. It is related to the rate of infusion and typically manifests as pruritus and erythematous flushing, particularly of the face and neck. In some cases, urticaria, angioedema, and even hypotension may also occur but may not always be IgE mediated. These pseudoallergic reactions can be prevented by slowing the rate of infusion and premedicating with H1 blockers. Concomitant opiates can also contribute to these pseudoallergic reactions. Desensitization protocols have been described for both IgE-mediated and non–IgE-mediated hypersensitivity reactions. Linear IgA bullous dermatosis (LABD) is the most commonly identified hypersensitivity reaction to vancomycin; it is an autoimmune, subepidermal vesiculobullous reaction typically associated with the use of vancomycin[69] that resolves with drug withdrawal. Mucosal lesions may be confused with SJS. Case reports of DRESS syndrome and acute interstitial nephritis (AIN) have also been published.[70]

Fluoroquinolones

Because of the increased recent use of quinolones, a history of quinolone allergy is becoming more frequent. Immediate hypersensitivity to quinolones has been estimated to range from 0.4% to 2%, with delayed exanthems being a more common reaction.[71] Quinolones are capable of causing non–IgE-mediated histamine release through MRGPRX2, and this mechanism is thought to participate in pseudoallergic reactions, which cannot be demonstrated through skin testing. Moxifloxacin has been shown to increase the odds of immediate hypersensitivity by fourfold compared with other quinolones.[72] Skin testing has not been validated to provide useful information regarding this nonspecific histamine release, which is induced at even minimal concentrations. BAT has been studied for in vitro diagnosis, and ciprofloxacin shows a higher sensitivity than moxifloxacin, even when moxifloxacin is the culprit drug. Drug challenges remain the cornerstone of diagnosis. The necessity for DPT is evident from the fact that only 7% to 32% of patients with suspected immediate and delayed reactions to quinolone who underwent DPT could be confirmed as allergic.[73,74] There are reports of cross-sensitization among the different quinolones, but the true rate of this apparent cross-reactivity is unclear. Limited data suggest that levofloxacin may be the most reasonable alternative.[75]

Macrolides

A history of allergic reactions to macrolides is rare. These reactions develop in 0.4% to 3% of the treated population. Macrolide hypersensitivity is clearly overestimated but rarely reproduced with oral challenge. On the basis of DPT results in the literature, 90% of these patients are unnecessarily avoiding these antibiotics.[76,77] Although skin or in vitro testing may be an adjunctive strategy to verify tolerance, these methods have little value as diagnostic tools. Drug challenge is the most important diagnostic tool in management and should be considered based on a favorable risk-benefit assessment.[78]

Aminoglycosides

Although topical aminoglycoside antibiotics are common causes of allergic contact dermatitis, systemic hypersensitivity is unusual and descriptions have mostly been limited to isolated reports of anaphylaxis to various compounds such as gentamicin and tobramycin.[79,80]

ANTIBIOTIC ALLERGY AND ANTIMICROBIAL STEWARDSHIP

Unverified penicillin allergy in hospitalized patients has several negative impacts on antibiotic optimization, including increased treatment costs, antibiotic resistance, and a heightened risk of *Clostridioides difficile* (formerly *Clostridium difficile*) infection and other adverse effects. There has been a recent surge in reports dealing with the important topic of antibiotic allergy labeling and delabeling, with implications for future care and outcomes. Penicillin allergy testing is now recommended as part of an effective antibiotic stewardship program. Many hospitals are developing dedicated multidisciplinary antibiotic allergy delabeling teams, involving the expertise of pharmacists, allergists and immunologists, and infectious disease physicians, and using computer-derived algorithms to stratify patients.[81,82] A decrease in the use of alternative antibiotic classes has been noted in parallel with penicillin allergy delabeling. However, treatment algorithms vary across health care systems, and this current divergence is not conducive to a standardized approach. The allergy and infectious disease communities need a consensus guideline for the evaluation of all patients with suspected penicillin allergy.

MULTIPLE DRUG INTOLERANCE SYNDROME

Multiple drug intolerance syndrome has been defined to include patients with reactions to three or more drugs that are not structurally or pharmacologically related. In studies of these patients, confirmation of true allergy is often lacking, and reactions are driven by anxiety (rather than IgE-mediated reactions).[83,84] Many patients may have subjective symptoms of their drug reactions. Evaluation of these patients typically requires blind, placebo-controlled drug challenges.

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24 Fusidic Acid

Jason Trubiano and M. Lindsay Grayson

SHORT VIEW SUMMARY

- Fusidic acid is an antimicrobial largely used for oxacillin-resistant *Staphylococcus aureus* (methicillin-resistant *S. aureus* [MRSA]), although drug susceptibility is much broader (see Table 24.1). Drug resistance arises rapidly in MRSA when fusidic acid is used alone.
- Nearly complete absorption from the gastrointestinal tract and few side effects or drug interactions (except with statins) have made fusidic acid useful in soft tissue infections, in combination with another active agent such as rifampin (see Table 24.2).
- Metabolism is largely by the liver, and no useful amount of bioactive drug is in the urine.
- Fusidic acid inhibits hepatic pathways of statin metabolism, which may lead to rhabdomyolysis.
- Topical preparations for the skin and conjunctiva have been useful, although they have been linked with widespread emergence of resistance.
- Fusidic acid (CEM-102) is not available in the United States; as of this writing it is undergoing phase III clinical trials.

STRUCTURE AND MECHANISM OF ACTION

Fusidic acid (in the United States, CEM-102) is an antibiotic derived from the fungus *Fusidium coccineum* and the only commercially available antibiotic from the fusidane group. It has a steroid structure, without steroid activity and with chemical similarities to cephalosporin P1. (Fig. 24.1) The sodium salt of fusidic acid (sodium fusidate) is used clinically and was developed in Denmark by Leo Laboratories and introduced into practice in 1962, primarily as an antistaphylococcal therapy.[1–3]

Fusidic acid is a bacteriostatic antibiotic, with bactericidal properties at higher concentrations. Its mode of action is via inhibiting bacterial protein synthesis[4]—primarily by blocking the translocation of peptidyl transfer RNA to the growing peptide chain (elongation phase) via impeding the action of the elongation factor G (EF-G) on the ribosome. Subsequent reductions in surface proteins render organisms such as staphylococci more susceptible to phagocytosis.[5]

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of fusidic acid is summarized in Table 24.1. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) provides clinical breakpoints (see Table 24.1), with a minimal inhibitory concentration (MIC) breakpoint of less than or equal to 1 µg/mL as susceptible for *Staphylococcus aureus* (99.7% for methicillin-resistant *S. aureus* [MRSA] strains; 99.3%–99.9% for multidrug-resistant *S. aureus* phenotypes).[6] A number of authors have described MICs and zone diameters for fusidic acid against staphylococci, assigning less than or equal to 0.5 µg/mL as susceptible and greater than or equal to 2 µg/mL as resistant (≥21 mm susceptible, ≤17 mm resistant for zone diameters).[7–9] Thus, routine susceptibility testing methods appear to provide comparable accurate results for fusidic acid against *S. aureus.*[10]

Overall, fusidic acid has a narrow spectrum with activity against gram-positive bacteria such as staphylococci, including MRSA and coagulase-negative staphylococci; *Clostridium* spp., including *C. tetani, C. perfringens,* and *C. difficile*; *Corynebacterium* spp.; *Peptostreptococcus* spp.; and most anaerobes except *Fusobacterium* spp. It has some activity against streptococci[11] and limited activity against enterococci. Gram-negative organisms are generally resistant, except for *Neisseria* spp., *Legionella pneumophila,* and some anaerobic gram-negative organisms.[11,12] A study found reasonable in vitro activity against multidrug-resistant strains of *Neisseria gonorrhoeae,* and *Chlamydia trachomatis,* raising the possibility of fusidic acid as a treatment option for associated sexually transmitted diseases, although clinical studies are lacking.[13]

Despite having some in vitro activity against *Mycobacterium tuberculosis*[11] and *Plasmodium falciparum,*[14] this has no clinical relevance. Limited activity against *Mycobacterium leprae*[15,16] and *Coxiella burnetii*[17] has been noted, but therapeutic efficacy requires exploration.

RESISTANCE

The mechanisms of resistance to fusidic acid have been reviewed.[18,19] Although fusidic acid resistance was recognized during drug development,[20] only modest rates of resistance were noted globally until the past 10 years or so.[18,21] Subsequently, resistance emergence has been largely linked to the increased use of fusidic acid monotherapy (especially in topical ointments).[6,18] Such isolates display enhanced "fitness" and are often clonally linked.[22,23] Although there is no known cross-resistance between fusidic acid and other antibiotic classes, the barriers to developing resistance are low because only a single point mutation is required.[24,25]

Information regarding fusidic acid resistance is largely based on research associated with staphylococci. Overall, the mean rate of fusidic acid resistance is approximately 5% in this species. It is higher in regions with increased topical fusidic acid use (Europe > United States/Australia),[26–30] especially among burn patients treated with chronic monotherapy.[21] Rates of fusidic acid resistance among *S. aureus* strains are up to 78% in dermatology patients exposed to prior topical fusidic acid monotherapy,[29] with outpatient topical fusidic acid prescribing associated with high rates of fusidic acid–resistant methicillin-susceptible *S. aureus.*[18,31] Previously, in the United States, where fusidic acid use has been limited largely to clinical trials, the rate of resistance (MIC ≥2 µg/mL) among *S. aureus* strains was 0.3%, but for coagulase-negative staphylococci it was 7.2%.[26] An assessment of over 2000 isolates in the United States demonstrated that fusidic acid inhibited 99.8% of *S. aureus* isolates at ≤1 µg/mL.[32]

Traditional resistance to fusidic acid is mediated via various mutations in the *fusA* gene that encodes EF-G, a guanosine triphosphatase that binds the ribosome and catalyzes the final step of peptide chain elongation.[33–35] Fusidic acid binds EF-G, preventing release from the ribosome and ongoing protein synthesis. At least five other resistance genes have been identified *(fusB-F).* The genes *fusB, fusC, fusD,* and *fusF* are thought to encode proteins that protect EF-G,[22,36–38] whereas *fusE* encodes a secondary fusidic acid binding region, *rplF* (ribosomal protein L6).[39] *fusB* and *fusC* are elements that can be chromosomal or plasmid mediated (plasmid puB101). A combination of *fus* resistance mutations does not appear to translate into increased resistance.[22] Fusidic acid–resistant *S. aureus* generally has detectable resistance mechanisms (*fusA, fusB, fusC,* and *fusE*),[6] particularly if MIC values are greater than or equal to 4 µg/

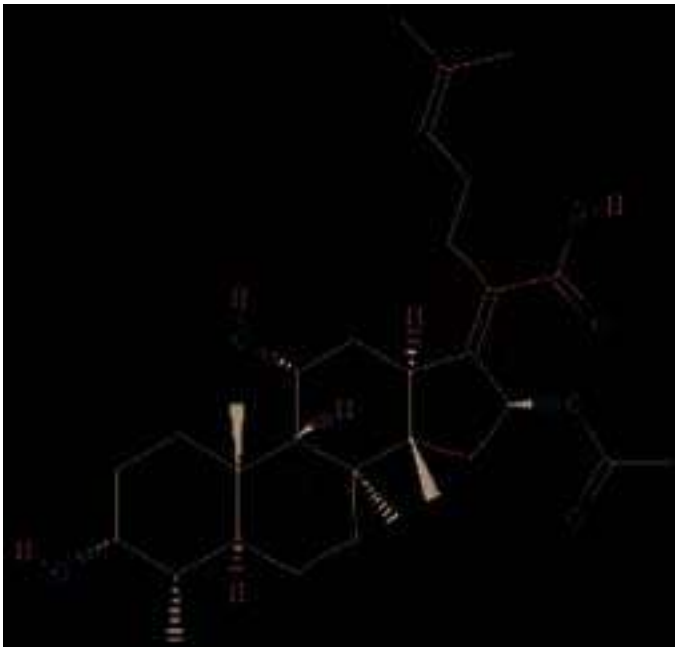

FIG. 24.1 **Chemical structure of fusidic acid.**

mL. Throughout Europe and Scandinavia, chromosomal *fusB* resistance mutants have been associated with epidemic clonal outbreaks.[22,23,40,41] In New Zealand, the emergence of a fusidic acid–resistant MRSA clone harboring the *fusC* gene was associated with increased topical fusidic acid use nationally.[42–44] Fusidic acid resistance has also been noted to occur in non–multidrug-resistant strains of community-associated MRSA.[45,46] Resistance due to *fusC* has also been reported in other staphylococcal species, including *S. hominis* subsp. *hominis, S. epidermidis, S. haemolyticus,* and *S. capitis* subsp. *ureolyticus.*[44] Meanwhile, *fusA* has also been described in *Clostridium* spp., *Bacillus* spp., and *Salmonella enterica.*[47] In some lesser susceptible β-hemolytic streptococci strains, the resistance mechanism remains unknown, despite apparent clinical treatment response in soft tissue skin infections involving streptococci.[2]

Gram-negative bacilli appear to be inherently resistant to fusidic acid owing to an inability of the drug to penetrate the cell wall, although in cell wall–free *Escherichia coli* systems, fusidic acid inhibits protein synthesis.[48] Other mechanisms of fusidic acid resistance include binding and sequestering of fusidic acid by the type 1 chloramphenicol acetyltransferase found in Enterobacteriaceae, deacetylation by an esterase produced in *Streptomyces* species,[21] and efflux by the AcrAB efflux system in *E. coli*.[49]

ADMINISTRATION AND DOSING

The modes of administration and dosing are shown in Table 24.2. Fusidic acid can be administered as an oral, an intravenous, or a topical formulation and has been used in antibiotic-impregnated gauze, tulle, or wicks.

TABLE 24.1 In vitro Activity of Fusidic Acid Against Common Pathogens Using EUCAST Criteria

ACTIVITY	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	MIC (RANGE) (μg/mL)	MIC BREAKPOINT (μg/mL)[a]	REGIONS OF EMERGING RESISTANCE AND COMMENTS
Gram Positive					
MSSA	0.06[11] 0.06[87] 0.12[6]	0.06[11] 0.125[87] 0.25[6]	0.06–0.12[11] <0.015–0.124[87] —	1 1 1	Limited *Staphylococcus aureus* resistance in United States (0.35%), Australia, and Canada (7%)[6,26] Highest rates in Greece (52.5%), Ireland (19.9%), United Kingdom (11.8%). 1%–3% *S. aureus* resistance in Germany, Israel, Italy, Poland, Spain, and Sweden[26]
MRSA	0.06[11] 0.125[93]	0.06[11] 4[93]	0.03–8[11] 0.03–8[93]	1 1	New Zealand[42]
hVISA	—	—	0.03–8[93]	1	
CoNS	0.12[11]	0.25[11]	0.03–8[11]	1	Overall CoNS resistance (2.5%) > *S. aureus*[7] CoNS resistance highest in Ireland (50%), France (49.4%), Switzerland (47.4%), and Belgium (42.9%) isolates[26]
Staphylococcus epidermidis	0. 2[11] 0.25[7]	0.25[11] 0.5[7]	0.03–8[11] <0.12–4[7]	1 1	
Staphylococcus saprophyticus	4[7] 4[8]	4[7] 4[8]	0.12–4[7] 2–8[8]	ND ND	*S. saprophyticus* intrinsically resistant (*fusD* mutation)[22]
Enterococcus spp.[b]	25[6]	25[6]	12->8[6]	IE	
Enterococcus faecalis	4[7] 25[94]	8[7] 25[94]	1–32[7] 3.12–25[94]	ND ND	*E. faecalis* resistant to fusidic acid, 99.3% in one study[93]
VRE	4[95]	4[95]	2–16[95]	ND	
Corynebacterium jeikeium	0.03[96]	0.06[96]	ND	ND	No data available for *Corynebacterium diphtheriae*
Micrococcus luteus	—	—	0.25-0.5	ND	
Streptococcus pneumoniae	8[94] 2[96]	8[94] 4[96]	2-8[94] ND	ND ECOFF < 32[96]	
Streptococcus agalactiae	16[6] 8[94]	16[6] 8[94]	16[6] 4-8[94]	32[6] ND	Despite increased MIC values, β-hemolytic streptococci have been clinically responsive to fusidic acid therapy[2]
Streptococcus pyogenes	2[96] 4[6]	4[96] 8[6]	ND 1-8[6]	IE ECOFF < 16[96]	
Gram Negative[c]					
Bacteroides fragilis	2[97]	2[97]	ND	ND	
Enterobacteriaceae	>100[11]	—	—	IE	Aerobic gram-negative bacilli inherently resistant
Haemophilus spp.	—	—	8–32	ND	

Continued

TABLE 24.1 In vitro Activity of Fusidic Acid Against Common Pathogens Using EUCAST Criteria—cont'd

ACTIVITY	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	MIC (RANGE) (μg/mL)	MIC BREAKPOINT (μg/mL)[a]	REGIONS OF EMERGING RESISTANCE AND COMMENTS
Legionella spp.	—	—	—	IE	No available data despite case report evidence of treatment success
Moraxella catarrhalis	0.12[94]	0.12[94]	0.06–0.12[94]	ND	
Neisseria gonorrhoeae	0.6[98]	2[98]	0.25–2[98]	ND	
Neisseria meningitidis	0.03[99] ≤0.015[86]	0.12[99] ≤0.015[86]	0.015–0.5[99] ≤0.015–0.06[86,99]	ND ND	No reports of global resistance
Prevotella melaninogenica	0.5[97] —	0.5[97] —	<0.25-0.5[97] 0.6-0.12[86,94]	ND	
Anaerobic Gram Positive					
Clostridioides difficile (formerly *Clostridium difficile*)	2[100]	2[100]	0.5–64[100]	ND ECOFF: <2[100]	Fusidic acid not used widely for *C. difficile* owing to rapid inducible resistance due to *fusA* resistance mechanisms
Clostridium perfringens	0.12[101]	0.5[101]	≤0.06–1[101]	ND	
Fusobacterium necrophorum	16[101]	32[101]	16–32[101]	ND	
Peptostreptococcus spp.	0.25[101]	0.5[101]	<0.06–2[101]	ND	
Cutibacterium acnes (formerly *Propionibacterium acnes*)	0.25[101]	1[101]	<0.06–2[101]	ND	
Other					
Actinomyces israelii	6.3[102]	12.5[102]	6.3–25[102]	ND	
Coxiella burnetii	0.5[101]	1[101]	<0.06–2[101]	ND	
Mycobacterium leprae	—	—	—	IE	
Mycobacterium tuberculosis	8[103]	16[103]	4–32[103]	IE	
Nocardia asteroides	3.12	6.25	0.78–6.25	ND	

[a]When available, EUCAST breakpoints were reported; the disk content used for EUCAST was 10 μg.
[b]*Enterococcus* spp. included *E. faecium, E. faecalis,* and 82 other enterococcal species.
[c]Gram-negative bacteria predominately found to have fusidic acid MIC values greater than 32 μg/mL.
CoNS, Coagulase-negative *Staphylococcus; ECOFF,* common epidemiologic cutoff values; *EUCAST,* European Committee on Antimicrobial Susceptibility Testing; *hVISA,* heterogeneous vancomycin intermediate *S. aureus*; *IE,* insufficient evidence that species is a good fusidic acid target; *MIC,* minimum inhibitory concentration; *MRSA,* methicillin-resistant *S. aureus; MSSA,* methicillin-sensitive *S. aureus; ND,* no data; *VRE,* vancomycin-resistant *Enterococcus.*

TABLE 24.2 Dosage and Mode of Administration for Fusidic Acid Therapy

ROUTE	FORMULATION	DOSAGE	FREQUENCY
Oral[a]	Film-coated tablets (fusidate sodium)	500 mg[b]	BID-TID[c]
Intravenous[d]	Fusidate sodium	500 mg (per vial)	BID-TID[c,e]
Topical[f] Cream Ointment Gel	 2% fusidic acid 2% fusidate sodium[g] 2% fusidate sodium	 Small quantity topically	 BID
Ophthalmology	1% viscous eye drops fusidic acid	Topical to conjunctival sac	BID

[a]Available in film-coated tablets or pediatric oral suspension (fusidic acid hemihydrate), recommended with food.
[b]Front-loaded dosing regimens can be used; see "Pharmacokinetics and Pharmacodynamics" section.
[c]BID dosing generally for skin and soft tissue infections. For serious infections, TID dosing recommended.
[d]Dilute with normal saline for administration. Intravenous rarely used clinically owing to excellent oral bioavailability.
[e]Infuse over 2 hours or longer.
[f]In some countries combined with steroid formulation (with hydrocortisone acetate or betamethasone 17-valerate).
[g]Ointment-impregnated sterile gauze squares available.
Modified from references 2,21,51–53,57,104.

Where appropriate, oral administration with film-coated tablets (sodium fusidate) is preferred over intravenous formulations owing to its excellent bioavailability. Bioavailability and tolerability have improved significantly since the advent of film-coated tablets, compared with previously marketed capsules and enteric-coated tablets.[50] Dosing regimens that include a loading dose have gained interest because of possible reductions in emergence of resistant subpopulations and faster attainment of steady state.[51,52]

The routine dose for adults with normal renal and hepatic function (oral, 500 mg three times daily; intravenous, 20 mg/kg/day in two to three divided doses[1,50,53]) does not need to be altered in renal impairment, with routine dosing for hemodialysis, continuous ambulatory peritoneal dialysis (CAPD), and continuous renal replacement therapy (CRRT). However, fusidic acid should be avoided in acute liver disease or severe preexisting liver disease. In chronic liver disease, low albumin levels and reduced protein binding are offset by reduced glucuronidation and excretion. In acute liver disease, in which albumin levels are often normal, higher bilirubin concentrations compete with fusidic acid for the limited glucuronidation mechanism, without being offset by the increased elimination of free fusidic acid usually associated with hypoalbuminemia.[54]

Routine doses can be used in the elderly, unless gastrointestinal intolerance (e.g., nausea) is encountered, in which case a dose reduction from 500 mg three times daily to 500 mg twice daily may be considered. Pediatric patients should receive three divided doses as follows: age 1 year or younger, 50 mg/kg/day; 1 to 5 years, 250 mg three times daily; and 6 to 12 years, 500 mg three times daily.[1]

PHARMACOKINETICS AND PHARMACODYNAMICS

Fusidic acid is highly (95%–97%) protein bound,[1] and oral bioavailability is excellent (91%) with newer film-coated tablets introduced since the 1980s. Older capsule formulations were associated with 69% bioavailability.[1,50,53,55] Thus, it should be noted that older pharmacokinetic studies, which used the capsule formulations and reported intersubject variation, may not be totally representative of the pharmacokinetics of the newer film-coated tablet formulation.[50]

Peak serum levels occur at 2 hours (range, 24–52 µg/mL after a 500-mg dose)[1,53] via both oral and intravenous administration.[56] Food consumption reduces the rate of oral drug absorption and serum levels but not overall absorption.[53,57] The drug is metabolized via the liver, with no bioactive drug in urine.[56,58] A small amount is metabolized and excreted in bile. Fecal excretion is minimal,[58] although there is good intraluminal gut activity against *C. difficile* (see "Clinical Uses" later). The serum half-life ranges from 10 to 16 hours independent of administration mode.[59] Mean serum levels after a 96-hour, 500-mg three-times-daily regimen are 71 µg/mL, with a maximum of 123 µg/mL recorded.[56] Steady state for a 500-mg, twice-daily regimen is achieved in 3 weeks[52] compared with 1 day with regimens that include a loading dose (e.g., up to 1650 mg twice daily on day 1 and 500 mg twice daily thereafter).[52,59,60] In one study that used a 1650-mg loading dose followed by high-dose maintenance therapy (825 mg twice daily), the day 1 and day 8 trough levels were 146 µg/mL and 204 µg/mL, respectively.[59]

Fusidic acid is detectable in phagosomes, has good intracellular activity (40%–100% of extracellular), and performs better at reduced pH.[61] It achieves good tissue, bone, and abscess levels but has limited penetration into normal meninges (Table 24.3) and can accumulate with repeated infusions.[53]

Coadministration of fusidic acid with rifampin may affect the pharmacokinetics of rifampin, potentially increasing available rifampin levels, although data are currently limited.[62] Notably, in a US clinical study of rifampin plus fusidic acid for the treatment of prosthetic joint infections, fusidic acid levels were substantially lower than expected, so the trial was terminated early.[63] However, these findings are inconsistent with decades of successful combination therapy for these and similar prosthetic infections in Europe and Australia; hence, further studies are required in order to investigate this recent observation.[1]

ADVERSE REACTIONS

Key potential adverse reactions are shown in Table 24.4. Overall, fusidic acid use has limited safety concerns, even among patients requiring treatment for extended durations.[64–66] Mild gastrointestinal symptoms have been reported with oral use, and reduced toxicity has been noted with newer formulations. Case reports of hematologic toxicities, including sideroblastic anemia and neutropenia, exist.[67–69] No clear renal toxicities exist, and anaphylaxis is rare.[70] Recent phase II studies purposed for US licensing suggest a similar safety profile for fusidic acid as for linezolid for short-term therapy,[60] with limited adverse events occurring in patients treated for longer than 11 days.[65]

Fusidic acid should be avoided in newborns because its strong protein binding may displace bilirubin and be associated with bilirubin encephalopathy.

Although fusidic acid is generally associated with few drug interactions, rhabdomyolysis secondary to concurrent fusidic acid and statin

TABLE 24.3 Penetration of Fusidic Acid Therapy Into Various Tissues

REGION	PENETRATION	COMMENTS
Adipose tissue	Good	Levels above MIC achieved following oral FA 500 mg TID[105]
Blood	Good	Highly protein bound (95%–97%) with peak serum levels up to 64 times *Staphylococcus* MIC[1]
CSF and CNS	Poor	Limited data to support use owing to poor levels in un-inflamed meninges Animal modeling suggests infiltration in inflamed meninges; however, antagonism may occur with β-lactam therapy[106,107]
Bile and biliary system	Good	Good FA levels noted within bile fluid[50]
Bone and joint	Good	Good penetration into synovial tissue (28%–78% of serum)[108–110] and bone (16%–78% of serum).[50] Likely to be improved since the introduction of film-coated formulation in 1980s with increased bioavailability
Soft tissue and muscle	Good	Topical FA penetrates normal, damaged, and avascular skin[111] Oral therapy achieves high levels in blister fluid at BID dosing, burn crusts, and interstitial dermal edema[53,112,113]
Heart tissue and mediastinum	Good	Levels 12- to 20-fold higher than staphylococcal MIC have been isolated from cardiac tissue after FA administration[114,115]
Ocular tissue	Good	Topical FA resulted in corneal tissue levels well above MIC at BID dosing,[116,117] yet poor aqueous humor levels with oral dosing[50]
Placenta	Good	Known to cross the placenta TGA pregnancy category C; use with caution, insufficient data[118]
Prostate	Unknown	No clear in vitro or in vivo evidence for use in prostatitis
Pus	Good	Good levels, slightly lower than that achieved in serum (83% of serum)[50]
Respiratory secretions	Poor	Low FA levels in sputum from cystic fibrosis patients (6%–8% serum)[119]

CNS, Central nervous system; *CSF,* cerebral spinal fluid; *FA,* fusidic acid; *MIC,* minimal inhibitory concentration; *TGA,* therapeutic drug administration.

TABLE 24.4 Adverse Reactions Associated With Fusidic Acid

ADVERSE EVENT	FREQUENCY	COMMENTS
Allergic reactions	U	Allergic responses, including urticaria and atopic dermatitis, are infrequently reported (4.6% of recorded events).[120] No cross-reactivity occurs with β-lactam allergies. Anaphylaxis is rare.
Gastrointestinal and hepatic	C	Gastrointestinal side effects constitute the majority of recorded adverse events associated with fusidic acid (30%–58%). Nausea, vomiting, and diarrhea can occur with oral administration; reduction to twice daily can ameliorate these effects. Up to 30% of patients may have raised bilirubin, usually transient and more frequent with higher doses.[59] The noted rise in bilirubin occurs without fulminant hepatotoxicity and is due to inhibition of bile transport as in Dubin-Johnson syndrome.[a,121,122]
Hematologic	U	Infrequent; immune thrombocytopenic purpura occurs rarely and cytopenias are noted in 7.4% of events.
Neurologic	U	Infrequent; 3.3% of reported events.
Phlebitis	U	Infrequent; generally associated with older IV preparations.
Rash	U	Infrequent; maculopapular rash most common.
Rhabdomyolysis	U	Uncommon, but potentially serious—due to drug interaction with concurrent statin use.[b]

[a]More severe if intravenous dose given too rapidly or if dosing is excessive.[24]
[b]Rosuvastatin, atorvastatin, and simvastatin known interactions (see text for references).
C, Common; *U,* uncommon.

therapy has now been widely reported[71–77] In a recent review, rhabdomyolysis was generally recognized 20 to 30 days after commencement of drug coadministration, usually occurred in patients in their late 60s, and was associated with a 24% to 28% fatality rate.[78] Three pathways (breast cancer resistance protein [BCRP], organic anion transporting polypeptide 1B1 [OATP1B1], and cytochrome P-450 3A isozyme [CYP3A]) are inhibited in liver microsomes, resulting in elevated statin levels.[79,80] Thus, careful monitoring of such patients is required, and in some cases temporary cessation of statin administration should be considered during fusidic acid therapy.

CLINICAL USES

Fusidic acid has predominately been used in countries outside the United States in combination therapy regimens for treatment of staphylococcal infections.[81] Monotherapy is associated with resistance rates of 5.1% compared with 0.8% seen in combination therapy.[18,21,82] Widespread monotherapy use corresponds with high rates of fusidic acid–resistant *S. aureus* isolates and treatment failures.[27,30,83–85]

Various antibiotics have been used in combination with fusidic acid therapy, including rifampin, novobiocin, and β-lactams, although rifampin plus fusidic acid regimens are most common.[18,64,66] Despite discrepancies in historical synergy studies, modern in vitro studies demonstrate improved fusidic acid activity when combined with rifampin and, to a lesser extent, with other antimicrobial combinations (levofloxacin, oxacillin, ceftriaxone, vancomycin, ciprofloxacin, gentamicin).[86] Nonetheless, combination therapy is recommended not primarily for synergy but rather to prevent emergence of resistance.[87] Fusidic acid resistance is low in countries in which combination therapy is used, despite decades of availability.[26,88] The emergence of broadly resistant gram-negative organisms, such as carbapenem-resistant *Acinetobacter baumannii,* have led to reports of in vitro activity when fusidic acid is combined with colistin.[89–91]

The current clinical indications for fusidic acid use are shown in Table 24.5. Primary indications include staphylococcal infections involving skin and soft tissue, bone, and joints. After a period of effective control with intravenous antibiotic therapy, oral fusidic acid in combination with another orally active agent (e.g., rifampin) may provide a suitable treatment option for such infections. Other orally available alternatives with possible activity against *S. aureus* may include linezolid, tetracyclines, and trimethoprim-sulfamethoxazole, but the safety profile of fusidic acid has advantages in many clinical situations, especially when prolonged therapy may be required.[6] Topical fusidic acid therapy has been used for noninfective skin conditions, including eczema; a randomized double-blind placebo-controlled trial demonstrated limited effectiveness,[92] and therefore its use should be discouraged.

TABLE 24.5 Clinical Indications and Recommended Dosage for Fusidic Acid Therapy

INFECTION SITE OR PATHOGEN	DOSING	COMMENTS[a]
Common Indications		
Skin and soft tissue	Systemic therapy 500 mg BID-TID oral	Antistaphylococcal therapy with concurrent streptococci activity; 81%–96% cure rate.[123] FA 500 mg BID achieves blister levels of 79 μg/mL; above staphylococci and streptococci MICs.[2] Similar cure rates with FA compared with flucloxacillin therapy.[1,65] Similar efficacy, tolerance, and end-of-treatment resistance when loading dose FA regimens compared with linezolid therapy for soft tissue infections.[60] A randomized controlled trial of FA with rifampin versus standard of care was terminated early owing to concerns of low FA levels[63] in the combination therapy arm (n = 6); the impacts of reduced dosage and implications for clinical efficacy are uncertain (these findings appear inconsistent with other reports—see text for details).
	Topical	Effective therapy for superficial staphylococcal soft tissue infections,[18] with equal or greater efficacy to oral FA for limited impetigo.[124] Review of overall effectiveness reported to be 82%–100%.[123] FA successful for impetigo treatment, with 2% therapy as effective as mupirocin[125] or retapamulin.[126] Major concerns regarding emerging resistance with topical use; increased FA use associated with the emergence of *Staphylococcus aureus*–resistant clones and impetigo outbreaks across Europe.[18,29,31] Limited end-of-treatment FA resistance after <14 days of topical therapy noted in some populations.[27,123]
Bone and joint	500 mg TID oral[b]	FA not recommended for monotherapy owing to high failure rates.[127] Successful combination therapy for osteomyelitis, septic arthritis, and prosthetic joint infections, primarily involving *S. aureus.*[18] FA commonly used with β-lactam[128] or rifampin.[64,66,81] Successful FA-rifampin therapy for *S. aureus* prosthetic joint infections with prosthetic retention and débridement.[64] Seventy-seven percent 24-month infection-free survival for FA-rifampin therapy in prosthetic joint infections with prosthetic retention; better outcomes in non-MRSA and with longer therapy.[66]
Other Reported Uses		
Endocarditis and bacteremia	500 mg TID IV or oral	For staphylococcal bacteremia, FA is not effective as staphylococci monotherapy and is recommended for use only in combination therapy,[129] generally with rifampin.[18,81] FA–β-lactam or glycopeptide therapy of staphylococci endocarditis is used in some countries, despite limited published evidence.[130] Evidence of in vitro antagonism with penicillin G is of uncertain clinical significance.[c]
Clostridioides difficile (formerly *Clostridium difficile*)	250–500 mg TID oral	FA not superior to standard therapies (metronidazole and vancomycin) in randomized trials. Concerns over higher relapse rates[131,132] and rapid emergence of resistance during therapy.[133,134]
Cystic fibrosis–associated respiratory infections	500 mg BID-TID oral	FA–β-lactam used in *S. aureus* infections in cystic fibrosis patients.[135,136] Prolonged treatment reduced rates of MRSA infections.[137] FA-rifampin–based decolonization regimens have been used in MRSA-colonized patients.[138]
Leprosy	500–750 mg daily	Weak bactericidal antileprosy agent with clinical improvement noted only in case studies.[16] Possible future role in combination *Mycobacterium leprae* therapy. Topical nasal FA reduced smear positivity; usefulness in reducing infectiousness unknown.[139]
Meningitis	Generally not recommended	No conclusive supportive data for FA or FA-β-lactam therapy from in vivo/in vitro animal studies or single retrospective review of efficacy (11 of 104 cases treated with FA).[107,140] If FA required, consider monitoring CSF levels.
Conjunctivitis	1% BID topical drops	Similar efficacy compared with first-line antibiotics in randomized controlled trials.[117,141,142] Similar effectiveness as placebo raises concerns over therapy for conjunctivitis in general.[143]

TABLE 24.5 Clinical Indications and Recommended Dosage for Fusidic Acid Therapy—cont'd

INFECTION SITE OR PATHOGEN	DOSING	COMMENTS[a]
Neisseria spp.	Uncertain	Rising fluoroquinolone resistance may result in FA use in future postexposure *Neisseria meningitidis* prophylaxis.[99] In vitro susceptibility reported for resistant *Neisseria gonorrhoeae;* also in vitro activity against *Chlamydia trachomatis.*[13,86]
Legionella spp.	Uncertain	Addition of FA achieved cure in single case of Legionella pulmonary abscess in renal transplant patient.[144]
Anaerobic infections due to *Bacteroides fragilis*	Uncertain	Effective high-dose therapy (1 g TID) in case series of five patients.[12]
Staphylococcal decolonization	Uncertain	Topical or oral FA therapy does not eradicate *S. aureus.*[82] FA-rifampin therapy was effective at MSSA/MRSA eradication.[137,138]
Surgical prophylaxis	Topical and oral therapy	Not recommended. Lower infection rate vs. placebo in neurosurgical patients given single-dose FA monotherapy[106] and in catheter line prophylaxis[145] without evaluation of impact on resistance. No difference in peritonitis rates in patients with continuous ambulatory peritoneal dialysis[141] or postoperative orthopedic infections when used as prophylaxis.[146] Effective preoperative prophylaxis before ocular surgery with fusidic acid drops (1%).[147,148]

[a]Oral single-agent therapy is not recommended for any clinical indication due to observed rapid emergence of resistance.
[b]In most cases, IV antibiotic (β-lactam or glycopeptide) therapy was used initially and then changed to ongoing oral fusidic acid.
[c]Evidence of failure of flucloxacillin and fusidic acid therapy for staphylococcal endocarditis.[1,130]
FA, Fusidic acid; *MIC,* minimal inhibitory concentration; *MRSA,* methicillin-resistant *S. aureus*; *MSSA,* methicillin-susceptible *S. aureus.*

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The complete reference list is available online at Expert Consult.

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25 Aminoglycosides

James E. Leggett

SHORT VIEW SUMMARY

- Usual intravenous adult dose: 7 mg/kg every 24 hours for gentamicin, netilmicin, and tobramycin; 15 mg/kg for plazomicin; and 20 mg/kg every 24 hours for amikacin
- Usual dose for synergy against gram-positive infections: 3 mg/kg/day gentamicin every 8, 12, or 24 hours
- Renal failure: 60% dose reduction
- Therapeutic drug monitoring: not always necessary for brief administration (<5 to 6 days); trough concentrations should be unmeasurable; typically, two serum concentrations in postdistribution phase (≥1 hour after administration, separated by ≥1.5 half-lives)
- Cerebrospinal fluid penetration: low
- Common adverse effects: nephrotoxicity, ototoxicity
- Contraindications: hypersensitivity
- Drug-drug interactions: increased nephrotoxicity risk with concomitant amphotericin B, clindamycin, foscarnet, furosemide, intravenous radiocontrast agents, and vancomycin
- Indications: atypical mycobacterial infections, brucellosis, cholangitis, cystic fibrosis, diverticulitis, endocarditis, endophthalmitis, meningitis, pelvic inflammatory disease, plague, health care–associated pneumonia, synergy for gram-positive infections, tularemia, urinary tract infections

Aminoglycoside antibiotics have been used since the 1940s, when systematic screening of soil actinomycetes for the elaboration of antimicrobial substances yielded streptomycin. Produced by a species of *Streptomyces,* it was the first antibiotic in the aminoglycoside family (Table 25.1)[1] to be derived, directly or indirectly, from *Streptomyces* spp. (aminoglycosides with names ending in *-mycin*) or from *Micromonospora* spp. (aminoglycosides with names ending in *-micin*). Neomycin, kanamycin, and gentamicin are fermentation products with two or three chemical constituents. Amikacin, netilmicin, and others are semisynthetic derivatives of the natural product. All aminoglycosides share similar physical, chemical, and pharmacologic properties.

Aminoglycosides demonstrate concentration-dependent killing and prolonged postantibiotic effects against susceptible organisms.[2,3] Several members of the aminoglycoside family have predictable in vitro activity against *Pseudomonas aeruginosa* and most other aerobic gram-negative bacilli. Some aminoglycosides have useful activity against mycobacteria; one, paromomycin, has been used to treat selected colonic protozoan pathogens, and a related antibiotic, spectinomycin, has been used to treat infections with *Neisseria gonorrhoeae*. The aminoglycoside antimicrobial activity may be additive to or synergistic with that of β-lactams against infection by aerobic gram-negative bacilli or aerobic gram-positive cocci. Although most studies have failed to demonstrate improved outcomes in patients treated with antibiotic combinations compared with monotherapy,[4,5] patients with septic shock receiving combinations including aminoglycosides showed reduced mortality in a trial and observational study.[6,7] The prevalence of aminoglycoside resistance has remained low,[8] and emergence of bacterial resistance during therapy has been rare. Mean aminoglycoside use in US academic centers declined by 41% from 2002 to 2009, with concomitant significant and opposite change in rates of resistance for some organisms and no change for others.[8]

The members of the aminoglycoside family share the potential for nephrotoxicity, ototoxicity, and, rarely, neuromuscular blockade. Clinically relevant toxicity may be decreasing as shorter drug courses are used. Allergic reactions are rare. Because of patent expirations, the cost of many aminoglycosides is low. The efficacy of the aminoglycosides and resistance problems with newer drugs presage a continued need. More recent reviews have reported that aminoglycoside use is again increasing because of emerging gram-negative resistance to other available drugs.[9–13]

CHEMISTRY

All aminoglycosides have an essential six-membered ring with amino group substituents, hence the name *aminocyclitol*.[1] The descriptor *aminoglycoside* results from the glycosidic bonds between the aminocyclitol and two or more amino-containing or non–amino-containing sugars. Spectinomycin differs in that it has an aminocyclitol ring but no amino sugars and no glycosidic bonds. The central aminocyclitol for streptomycin is streptidine, whereas for all other current aminoglycosides, it is 2-deoxystreptamine. The standard numbering convention is illustrated in Fig. 25.1. The aminocyclitol ring is numbered counterclockwise, and the linked sugar molecules are numbered clockwise. Fig. 25.1 also illustrates the structural basis of the aminoglycoside subgroups. Neomycin and paromomycin are derived from *Streptomyces* spp. and link to cyclic sugars at positions 4 and 5 of 2-deoxystreptamine. Among the commonly used aminoglycosides, neomycin contains the largest number of free amino groups (six). The neomycin family comprises framycetin, neomycin, and paromomycin. All are too toxic for parenteral use. Framycetin is available outside of the United States.

Kanamycin, tobramycin, amikacin, arbekacin, and dibekacin comprise the kanamycin family (Table 25.2). All derive from *Streptomyces* spp. and link to cyclic sugars at positions 4 and 6 of 2-deoxystreptamine. Tobramycin is 3′-deoxykanamycin B. Amikacin is kanamycin A with the semisynthetic addition of 2-hydroxy-4-aminobutyric acid to the amino group at position 1 of the aminocyclitol. Arbekacin and dibekacin are available for clinical use outside of the United States.

Gentamicin is a mixture of C_1, C_{1a}, and the enantiomers C_2 and C_{2a} elaborated by *Micromonospora* spp. with glycosidic linkages at positions 4 and 6. Sisomicin is the dihydro analogue of gentamicin C_{1a}, and netilmicin is derived from sisomicin by the addition of an ethyl group to the amino group at position 1 of the aminocyclitol. Plazomicin is a semisynthetic derivative of sisomicin with the addition of a hydroxy-amino-butyric acid group at the N1 position and a hydroxyethyl substituent at the 6′ amine position. Isepamicin is not approved for use in the United States.

Aminoglycosides are highly soluble in water and insoluble in organic solvents.[14] The latter property correlates with the limited ability of aminoglycosides to cross lipid-containing cellular membranes. Aminoglycosides have a molecular size in the range of 445 to 600 Da.[14] The molecular structure is unchanged by freezing, by heating to 100°C for up to 4 hours, or by alterations in solution pH ranging from 3.0 to 12 over several hours.[15–17] The pK_a values of the individual amino groups can be determined by nuclear magnetic resonance spectroscopy.[18] The overall pK_a for gentamicin is about pH 8.4.[14] Hence, at pH 7.4, the aminoglycosides have a very high positive charge and are cationic.

The overall positive charge contributes to both antimicrobial activity and toxicity. Antibacterial activity is enhanced in media with an alkaline

TABLE 25.1 Family of Aminoglycosides in Clinical Use

GENERIC NAME	PROPRIETARY NAME	SOURCE	YEAR REPORTED	CHEMISTRY
Streptomycin	None	*Streptomyces griseus*	1944	Unique central aminocyclitol ring
Neomycin	Mycifradin, Neobiotic	*Streptomyces fradiae*	1949	Roughly equal proportions of neomycin B and C
Kanamycin	Kantrex	*Streptomyces kanamyceticus*	1957	Mixture of 95% kanamycin A and 5% kanamycin B
Paromomycin	Humatin	*Streptomyces fradiae*	1959	Part of neomycin family
Spectinomycin	Trobicin	*Streptomyces spectabilis*	1961	Chemically distinct but closely related to aminoglycosides
Gentamicin	Garamycin	*Micromonospora purpurea and Micromonospora echinospora*	1963	Roughly equal proportions of gentamicin C_1, C_{1a}, and enantiomers C_2 and C_{2a}
Tobramycin	Nebcin	*Streptomyces tenebrarius*	1967	Natural 3′-deoxy derivative of kanamycin B
Sisomicin[a]	Siseptin	*Micromonospora inyoensis*	1970	Dehydro analogue of gentamicin C1a
Dibekacin[a,b]		*Streptomyces kanamyceticus*	1971	Dideoxy derivative of kanamycin B
Amikacin[b]	Amikin	*Streptomyces kanamyceticus*	1972	Semisynthetic derivative of kanamycin A
Netilmicin[b]	Netromycin	*Micromonospora inyoensis*	1975	*N*-Ethyl derivative of sisomicin
Isepamicin[a,b]		*Micromonospora purpurea*	1978	*I-N-S*-α-Hydroxy B amino propionyl derivative of gentamicin B
Plazomicin	Zemdri	*Micromonospora inyoensis*	2018	Semisynthetic derivative of sisomicin

[a]Approved for human use in countries other than the United States.
[b]Semisynthetic aminoglycosides.
Modified from Wright GD, Berghuis AM, Mobashery S. Aminoglycoside antibiotics: structures, functions, and resistance. In: Rosen BP, Mobashery S, eds. Resolving the Antibiotic Paradox: Progress in Understanding Drug Resistance and Development of New Antibiotics. *New York: Plenum; 1998.*

TABLE 25.2 Chemical Families, Names, and Worldwide Availability

FAMILY	MEMBERS	EXAMPLE TRADE NAME	AVAILABLE IN UNITED STATES	AVAILABLE OUTSIDE UNITED STATES
Streptomycin	Streptomycin	Streptomycin	Yes	Yes
Kanamycin	Amikacin	Amikin	Yes	Yes
	Arbekacin	Habekacin	No	Yes
	Dibekacin	Dikacine	No	Yes
	Kanamycin	Kantrex	Yes	Yes
	Tobramycin	Nebcin	Yes	Yes
Gentamicin	Gentamicin (C_1, C_{1a}, C_2, C_{2a})	Garamycin	Yes	Yes
	Isepamicin	Isepacine	No	Yes
	Netilmicin	Netromycin	No	Yes
	Sisomicin	Sisogen	No	Yes
	Plazomicin[a]	Zemdri	Yes	Yes
Neomycin	Framycetin	Soframycin	No	Yes
	Neomycin	Mycifradin, Neobiotic	Yes	Yes
	Paromomycin	Humatin	Yes	Yes
Spectinomycin[b]	Spectinomycin	Trobicin	No	Yes

[a]Approved by the US Food and Drug Administration but not available in United States.
[b]An aminocyclitol, no glycosidic bonds.

pH and reduced in media with an acidic pH.[17] The positively charged aminoglycosides bind to the negatively charged RNA backbone, cell wall lipopolysaccharide (LPS), cell membrane phospholipids, and other anionic molecules.[19]

Cationic aminoglycosides interact chemically with β-lactam antibiotics.[20–22] The reaction results in a nucleophilic opening of the β-lactam ring, with acylation of an amino group of the aminoglycoside and mutual loss of antibacterial activity. In vitro, gentamicin and tobramycin are inactivated with greater ease than netilmicin, amikacin, or isepamicin. The antipseudomonal penicillins (e.g., piperacillin) are the β-lactams most susceptible to the reaction. The reaction requires several hours in vitro, so the clinical importance is limited. In general, penicillins and aminoglycosides should not be mixed in the same solution before infusion, although a recent product reformulation does allow piperacillin-tazobactam coadministration with gentamicin at specific doses.[23] If patients with renal failure are given an aminoglycoside and an antipseudomonal penicillin concomitantly, there is a 10% to 20% reduction in the serum aminoglycoside concentration compared with the levels observed when each drug is administered alone.[24]

MECHANISMS OF ACTION

Both electrostatic interactions between positively charged aminoglycoside amino groups and negatively charged RNA phosphate groups, as well as hydrogen bonds between multiple amino and hydroxyl groups of both, contribute to production of a tightly bound complex that derails translation.[25] Deoxystreptamine rings I and II, conserved and sequence specific among most aminoglycosides, are essential for binding to the ribosomal decoding acceptance (A) site. Aminoglycoside antibiotics preferentially bind with high avidity to a region of highly conserved nucleotides at the A site[26–28] in the 16S reverse transfer RNA portion of the messenger RNA (mRNA) decoding region of the 30S subunit of prokaryotic ribosomes.[25,29] Aminoglycoside binding induces a conformational change in three adenine residues that reduces the fidelity of normal mRNA translation and translocation (Fig. 25.2),[29,30] leading to

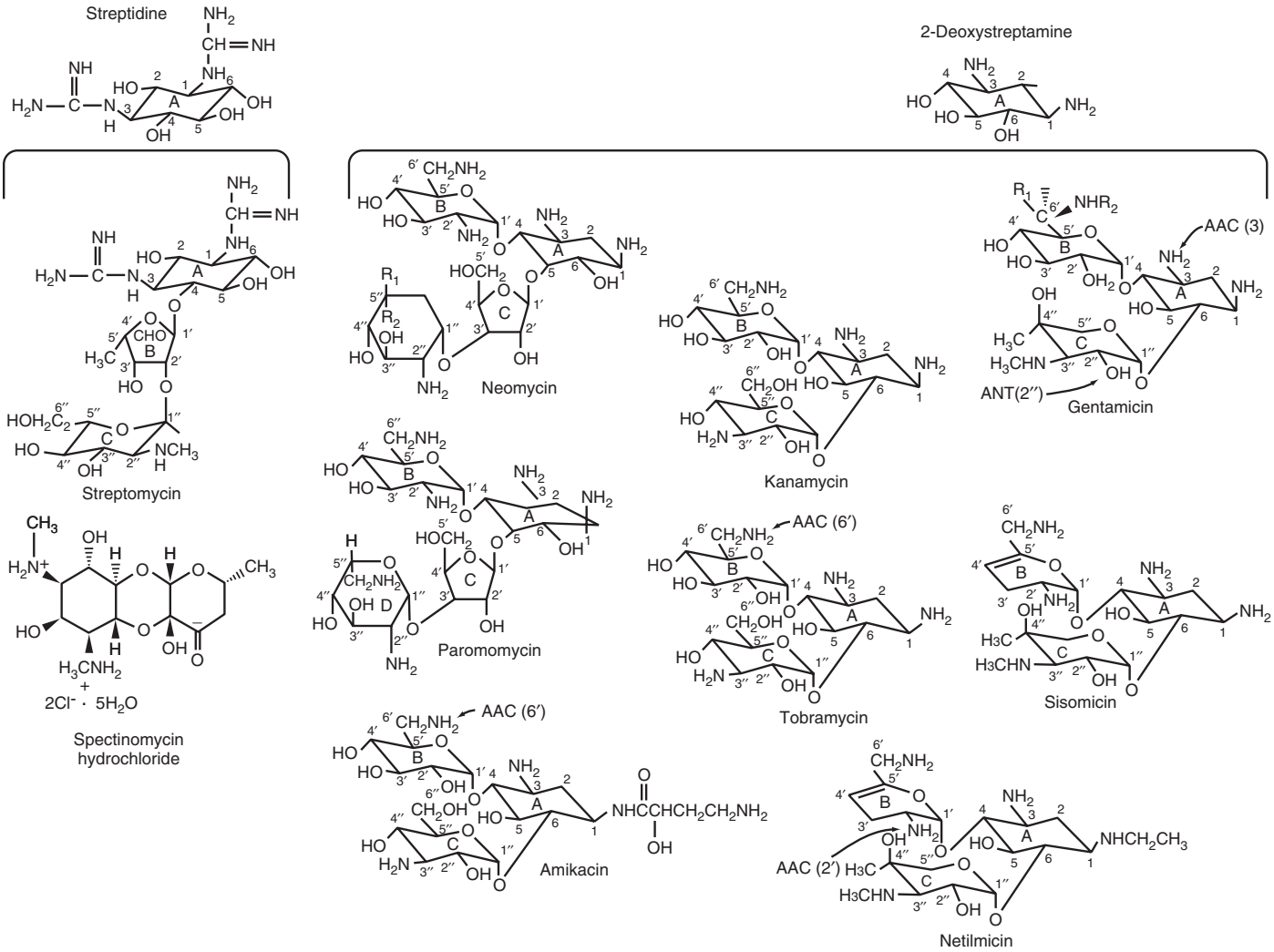

FIG. 25.1 Chemical structure of the aminoglycosides and spectinomycin. Neomycin contains approximately equal amounts of neomycin B (R_1 = H; R_2 = CH_2NH_2) and neomycin C (R_1 = CH_2NH_2; R_2 = H). Kanamycin is principally kanamycin A, as shown. Gentamicin is gentamicin C complex with roughly equal amounts of C_1 (R_1 = R_2 = CH_3), C_{1a} (R_1 = R_2 = H), and C_2 (R_1 = CH_3; R_2 = H). The sites of action of four inactivating enzymes are shown including three acetyltransferases—Aac(3′), Aac(2′), and Aac(6′)—and one adenyltransferase, Ant(2″).

the accumulation of truncated or nonfunctional proteins in bacteria. The respective nucleotide sequence differences between bacterial and human ribosomal subunits leads to lower affinity of aminoglycosides for the latter. In eukaryotic cytosolic ribosomes, adenosine is replaced by a guanosine within the A site, with a concomitant reduction in binding of aminoglycosides. The double-adenosine nucleotides in prokaryotic ribosomes create an internal bulge and a larger groove that allows access to the ribosomal binding site.[31] Avidity of binding varies with the aminoglycoside, depending on the number of amino groups and their state of protonation.[18]

Permeability of the polycationic aminoglycosides is enhanced in aerobic bacteria that use membrane-bound electron transporters. Initial electrostatic binding of aminoglycosides to the cell surface is followed by two energy-dependent uptake phases and binding to ribosomes.[32–34] In gram-negative bacteria, the cationic aminoglycosides rapidly bind to negatively charged residues in the LPS, polar heads of phospholipids, and anionic outer membrane proteins.[35–38] By competitively displacing cell wall magnesium ion (Mg^{2+}) and calcium ion (Ca^{2+}) bridges that normally link adjacent LPS molecules,[39,40] aminoglycosides result in a rearrangement of LPS, with subsequent bleeding of the outer membrane, formation of transient holes in the cell wall, and disruption of normal permeability function of the cell wall.[41]

After initial binding, aminoglycosides are transported across the bacterial cytoplasmic membrane by a slow energy-dependent phase, EDP-I, that transports the drug into the cytosol and then bind to the ribosome in a subsequent rapid energy-dependent phase, EDP-II.[32–34,38,42,43] The onset of bacterial killing is coincident with the transition from EDP-I to EDP-II.[33,34] Most bacterial cells manifest lethal injury after only 25% of the maximum EDP-II uptake.[43] The higher the external concentration of aminoglycoside, the quicker the intracellular drug concentration reaches a level necessary to trigger EDP-II uptake, which forecasts the death of the organism.[44]

Binding of aminoglycosides to prokaryotic ribosomes is a prerequisite for the drugs' antimicrobial activity. However, the exact mechanisms of bactericidal activity remain unknown.[1,25] The binding to ribosomes is reversible, which usually results in a bacteriostatic rather than a bactericidal effect.[1] Ribosomal binding results in a measurable decrease in protein synthesis as a result of misreading of mRNA. Although bactericidal drugs stimulate hydroxyl radical formation in bacteria as a function of metabolism-related depletion of reduced nicotinamide adenine dinucleotide, destabilization of iron-sulfur clusters, and stimulation of the Fenton reaction,[45] killing by antibiotics appears to be unrelated to reactive oxygen species.[46,47]

The mechanisms of the intracellular accumulation of high concentrations of drug and of cell death are unclear. High intracellular concentrations may result from aminoglycoside closure of voltage-gated channels, with subsequent trapping of drug.[48] Accumulation of large concentrations of aminoglycoside inside bacterial cells results in binding

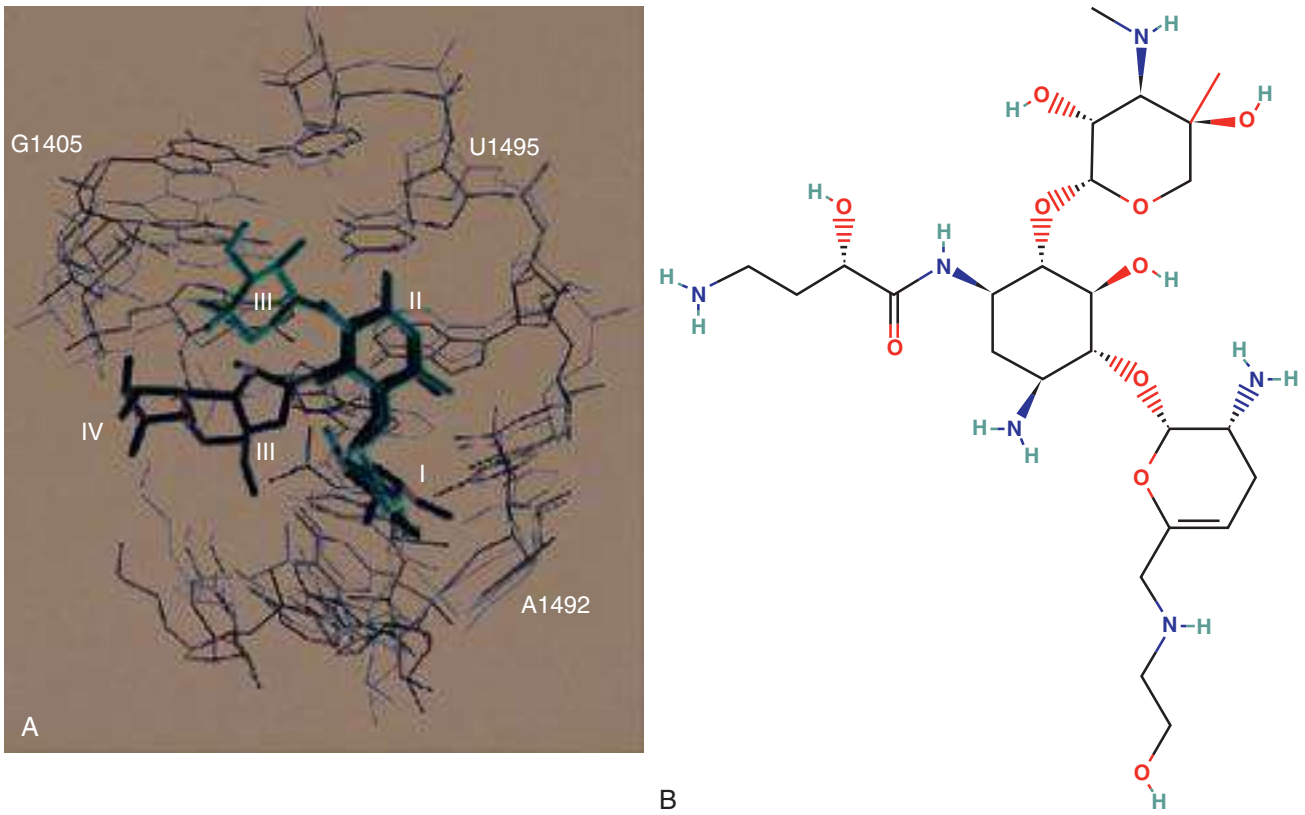

FIG. 25.2 (A) Three-dimensional structures of the aminoglycoside antibiotics paromomycin *(yellow)* and gentamicin C_{1a} *(red)* bound to different sections of the *Escherichia coli* 16S ribosomal RNA (shown in *light tan* and *dark tan*, respectively). Rings I and II of the two drugs, which are required for antibiotic activity, bind in the same mode in both complexes; the additional rings (III and IV in paromomycin and III in gentamicin C_{1a}), which are less important for drug activity, make diverse contacts with the RNA. (B) Structure of the aminoglycoside antibiotic plazomicin, newly approved by the US Food and Drug Administration. *(A courtesy Joseph D. Puglisi, Director, Stanford Magnetic Resonance Laboratory, Stanford University School of Medicine. B from Plazomicin. PubChem: Open Chemistry Database.* https://pubchem.ncbi.nlm.nih.gov/compound/Plazomicin#section=2D-Structure.*)*

to ribosomes with mistranslation of mRNA yielding abnormal proteins, loss of cell membrane integrity with efflux of intracellular ions, and inhibition of DNA replication, in addition to stimulating hydroxyl radical formation.[1,45]

The aminoglycosides have a variety of other biologic activities that are the subjects of ongoing study. Approximately 1800 genetic diseases (e.g., cystic fibrosis [CF], Duchenne muscular dystrophy, Hurler syndrome, nephrogenic diabetes insipidus) arise, in part, from base pair insertions, deletions, or substitutions that produce premature stop codons, resulting in production of incorrectly truncated, nonfunctional proteins.[49] Aminoglycosides can suppress premature stop codons and restore physiologically active amounts of functional protein.[50–58] The mechanism of the suppression of the stop codons is under study; uracil and cytosine nucleotides are major determinants of optimal gentamicin-induced readthrough.[59]

MECHANISMS OF RESISTANCE

Bacterial resistance to aminoglycosides results from some combination of the following three mechanisms: (1) reduction of intracellular aminoglycoside accumulation resulting from bacterial membrane alterations that reduce uptake or active efflux systems or both; (2) decreased aminoglycoside binding by mutation or methylation of the 16S ribosomal RNA (rRNA) binding site; and (3) enzymatic deactivation of aminoglycosides by *N*-acetylation, *O*-nucleotidation, or *O*-phosphorylation.[60–67] Aminoglycosides also induce bacterial biofilm formation, with associated bacterial cell wall surface adhesiveness. Biofilm formation is a concern in the treatment of chronic infections, especially infections with foreign bodies.[68] In addition, genomic studies in *Pseudomonas aeruginosa* have suggested other novel mechanisms of resistance.[69,70] As aminoglycosides require an active electron transport chain to generate an electrical potential difference across the membrane, anaerobic bacteria are intrinsically resistant to aminoglycosides.

Low-level aminoglycoside resistance attributed to impaired cell wall permeability may be the result of drug efflux mechanisms. Multidrug (including aminoglycoside) efflux pumps include adenosine triphosphate–dependent active pumps.[25] Constitutive expression results in intrinsic low-level resistance; overexpression of these transporters may result from regulatory gene mutations or increased antibiotic substrate concentration. Examples include the resistance nodulation cell division–type superfamily in *P. aeruginosa* and the major facilitator superfamily in *Escherichia coli*.[25,65] Activation of the MexXY efflux pump also appears to explain adaptive resistance, defined as transient resistance to aminoglycosides that follows the rapid, early, concentration-dependent killing of susceptible bacteria.[36,62] The refractory state lasts beyond the postantibiotic effective period into the time of regrowth. Adaptive resistance has been documented in vitro in animal models and in patients with CF,[63,71,72] where the predominant identified mechanism of aminoglycoside resistance is efflux by the MexXY-OprM system.[25,73,74] Exposure of susceptible bacteria to aminoglycosides can select a second type of drug resistance. A subpopulation results from small colony variants with deficient energy-dependent uptake of aminoglycosides and may result in clinical treatment failure.[75]

For most bacteria, the least common mechanism involves alteration of the 16S ribosomal target of aminoglycosides. However, the best known example is resistance of *Mycobacterium tuberculosis* to streptomycin as a result of point mutations in ribosomal protein S12 and in the 16S rRNA. Resistance of *Mycobacterium abscessus* and *Mycobacterium chelonae* to amikacin is the result of a 16S rRNA point mutation. Methylating enzymes that modify the 16S rRNA and decrease drug-binding affinity, mostly located on transposons within transferable plasmids, have been described in a growing number of aminoglycoside-resistant clinical isolates worldwide.[67,76–81]

The most common cause of aminoglycoside resistance is deactivation by specific enzymes derived from bacterial genes originally encoding enzymes involved in normal cellular metabolism of both gram-positive and gram-negative bacterial pathogens.[1,25] Examples of sites of enzyme activity are shown in Fig. 25.1. Enzyme modification results in loss of antibacterial activity.

Three covalent modifications of aminoglycosides are recognized: *N*-acetyltransferases (AAC), *O*-nucleotidyltransferases (ANT), and *O*-phosphotransferases (APH).[1,82] The enzymatically modified drugs bind poorly to ribosomes and result in high levels of resistance. In addition, the aminoglycoside may bind directly to a modifying enzyme in lieu of the ribosomal target.[83]

Specific enzymes are categorized by a nomenclature published in 1993.[1,84] Each enzyme is described by its class (AAC, ANT, or APH), a number in parentheses signifying the location of modification of the drug, and a Roman numeral indicating a unique aminoglycoside resistance profile. Distinct genes resulting in identical resistance phenotypes are indicated by a lowercase letter after the Roman numeral. For example, Aac(6′)-Ia describes an acetylating enzyme that modifies aminoglycosides at the 6′ position (see Fig. 25.1); the resistance profile is the same as that of Aac(6′)-Ib, but the enzyme protein is unique. A summary of modifying enzymes and their profile, source, and phenotype is available elsewhere.[1]

Modifying enzyme genes are spread by plasmids or transposons, or both. Some are chromosomal. The plasmid-transposon genes can result in rapid spread of drug-resistant phenotypes both within and between bacterial species. The resistance genes in gram-negative pathogens are diverse. In gram-positive pathogens, resistance is limited to Aph(3′)-IIIa, Ant(6′), and a unique bifunctional enzyme known as Aac(6′)-Aph(2″). In gram-negative organisms, a complex pattern of *aac(6′)*-I genes, combined with *aac(3)* and *ant(2″)* and others, is observed. The presence of the enzyme results in high-level resistance of gram-positive cocci to all aminoglycosides except streptomycin. Similar genes have been described in amikacin-resistant gram-negative bacterial clinical isolates.[85,86]

All enterococci have intrinsic resistance to aminoglycosides, with minimal inhibitory concentrations (MICs) ranging from 4 to 256 μg/mL.[87] The resistance is attributed to the facultative anaerobic metabolism of enterococci, which reduces the transmembrane potential and thereby limits drug intake. Concomitant exposure of enterococci to a cell wall–active drug such as ampicillin or vancomycin facilitates access of aminoglycosides to their ribosomal target site and classic synergistic bactericidal activity.

Acquisition of genes that encode aminoglycoside-modifying enzymes leads to high-level aminoglycoside resistance and loss of synergistic activity with penicillins or vancomycin. At least nine genes have been described that mediate resistance to aminoglycoside synergism in enterococci.[88] The most important is the bifunctional gene *aac(6″)-Ie–aph(2″)-Ia,* which encodes the bifunctional enzyme Aac(6′)-Ie–Aph(2″)-Ia. A combination of resistance genes can result in failure of synergism with all aminoglycosides available in the United States. Arbekacin, a derivative of dibekacin, available only in Japan, has shown promising results in the presence of a variety of modifying enzymes,[88] as has plazomicin.

The current threshold for detection of high-level resistance to gentamicin in vitro is an MIC of 2000 μg/mL or greater[89]; for resistance to streptomycin, it is an MIC of 500 μg/mL or greater. There are species differences in susceptibility to aminoglycosides. The MIC of tobramycin against *Enterococcus faecium* ranges from 62 to 1000 μg/mL; there is no synergism with a cell wall–active drug. The difference is caused by an enzyme that modifies tobramycin, but not gentamicin.[90]

Surveillance of the in vitro susceptibility of Enterobacteriaceae and nonfermentative gram-negative bacilli demonstrates increasing resistance to aminoglycosides as well as other classes of antibacterial drugs. Because the multiclass resistance genetic elements are on mobile units such as transposons or plasmids, their continued spread is anticipated.[91] One or more of the mechanisms of aminoglycoside resistance (e.g., enzymatic, target methylation, efflux pumps) are present in multidrug-resistant gram-negative bacilli. As a result of rapidly changing patterns of resistance, only general statements are possible regarding the anticipated in vitro and in vivo spectrum of antibacterial activity of aminoglycosides.[92–98]

ANTIMICROBIAL ACTIVITY

Although marked regional and individual hospital differences exist for in vitro susceptibility patterns, most aerobic and facultative gram-negative bacilli, including Enterobacteriaceae, *P. aeruginosa*, and *Acinetobacter* spp. in the United States, remain susceptible to gentamicin, tobramycin, and amikacin.[8] Plazomicin is less active against lactose-nonfermenting gram-negative bacteria than against Enterobacteriaceae.[99] High rates (50%–60%) of cephalosporin and fluoroquinolone resistance are noted in aminoglycoside-nonsusceptible isolates.[13,67] *Yersinia pestis* is inhibited by streptomycin, and *Francisella tularensis* is inhibited by both streptomycin and gentamicin. The aminoglycosides show no inhibitory activity against *Stenotrophomonas maltophilia* or *Burkholderia (Pseudomonas) cepacia*. Among gram-positive aerobic bacteria, methicillin-sensitive *Staphylococcus aureus* is susceptible, and methicillin-resistant *S. aureus* isolates are resistant. All streptococci, including *Streptococcus pneumoniae*, are resistant. Because the aminoglycosides require aerobic metabolism to exert an antibacterial effect, it is not surprising that all the anaerobic bacteria are resistant to aminoglycosides.

There are some minor differences in relative degrees of in vitro potency of aminoglycosides. For strains of *S. aureus,* gentamicin and netilmicin show the most potency. The MIC or minimal bactericidal concentration of gentamicin against *Serratia* spp. is consistently twofold lower than that of the other aminoglycosides, and the MIC or minimal bactericidal concentration of tobramycin against *P. aeruginosa* is also consistently twofold lower than that of the other aminoglycosides. This increased potency increases both the peak/MIC and 24-hour area under the curve (AUC)/MIC ratios. Although tobramycin is more active in animal models of pneumonia, to date, no clinical efficacy data have been presented that parallel these in vitro differences. Even though amikacin MICs are generally higher against Enterobacteriaceae than gentamicin, amikacin remains active against most (>80%) strains as well as many *P. aeruginosa* strains that have acquired both gentamicin and tobramycin resistance.[17,100–102] Plazomicin is generally not inactivated by common aminoglycoside-modifying enzymes; however, 16S RNA methyltransferases render it and all aminoglycosides inactive. 16S RNA methyltransferases are currently found in <0.1% of Enterobacteriaceae in the United States.[67,103]

Aminoglycosides have in vitro activity against *Haemophilus* spp. and *Legionella* spp. but are not used clinically for infections with these organisms. Legionellae are intracellular pathogens, and the intracellular antimicrobial activity of the aminoglycosides is hampered by their low concentration in the acidic lysosomal compartment.[104] Nonetheless, aminoglycosides are used successfully in the treatment of other intracellular infections such as brucellosis, chronic forms of bartonellosis, tuberculosis, tularemia, and yersiniosis.[104–106] Aminoglycosides in combination with other drugs have been used successfully to treat infections with staphylococci, streptococci, enterococci, *Listeria,* and mycobacteria. Susceptibility of mycobacteria varies with the species. Streptomycin is the most potent aminoglycoside in vitro against *M. tuberculosis,* whereas amikacin is the most active against *Mycobacterium avium-intracellulare* complex.

The aminoglycoside paromomycin is too toxic for parenteral administration. Because the drug is not absorbed from the intestinal tract, it can be used safely as an alternative therapy for infection caused by *Entamoeba histolytica* as well as ulcerative *Leishmania major*.[105,107] Spectinomycin was an alternative agent used intramuscularly in the treatment of *N. gonorrhoeae,*[105] but it is no longer distributed in the United States.

Urine is known to partially inhibit the activity of aminoglycosides against urinary tract pathogens. Inhibition is believed to result from the low pH and high osmolality caused by the high salt and glucose concentrations. In addition, present data support the hypothesis that betaines, normally found in urine, permit the expression of increased aminoglycoside resistance.[108]

Three characteristics describe aminoglycoside antibacterial activity: concentration-dependent killing, the presence of a postantibiotic effect (PAE), and synergism with other drugs.[109] Aminoglycosides are rapidly bactericidal, and their rate of bacterial killing increases as the antibiotic concentration is increased, regardless of the inoculum.[3] Exposure of both gram-positive and gram-negative organisms to less frequent, higher

doses with in vitro pharmacokinetic models resulted in more and faster killing initially, followed by regrowth of resistant mutants and very similar 24-hour results, compared with more frequent dosing regimens.[110,111] Further studies indicated that emergence of resistance could be prevented with peak/MIC ratios greater than 8.[112]

In thigh infections of neutropenic mice, the rate of killing increased with increasing mg/kg doses over the first few hours, whereas regrowth was suppressed for several hours despite aminoglycoside levels less than the MIC.[113] The duration of this postexposure growth suppression, or PAE, also increases with higher doses.[114,115]

The PAE represents suppression of bacterial growth after short antimicrobial exposure.[114,115] In vitro, the tobramycin-induced PAE against *E. coli* correlated with inhibition of protein synthesis, but not of DNA or RNA synthesis.[116] An aminoglycoside PAE can be demonstrated after incubation with *S. aureus* but not after contact with *S. pneumoniae*.[114] Inoculum size, oxygenation, and pH of the medium affect the duration of in vitro PAE.[114,115,117] For the aerobic or facultative gram-negative rods tested, combinations with β-lactams other than imipenem result in the same PAE as that of the aminoglycoside alone.[114,118] Rifampin was associated with synergistic enhancement of the PAE induced in *P. aeruginosa* by tobramycin.[115,119]

The in vivo PAE of aminoglycosides has been studied in at least five animal models.[113,114,120] In neutropenic mouse thighs infected with 15 clinical isolates of Enterobacteriaceae, the in vivo PAE after gentamicin therapy varied from 1.4 to 6.9 hours. In the same model infected with *P. aeruginosa,* increasing the dose of tobramycin fivefold increased the PAE from 2.2 to 7.3 hours.[113] The in vivo PAE is prolonged further in renally impaired or nonneutropenic mice and larger animals.[121]

Aminoglycoside combinations have been widely studied in vitro and for many years have been used in combination with other antibiotics to enhance bacterial killing and improve clinical efficacy. Synergy, a more than additive effect, has been shown in multiple time-kill curve experiments between an aminoglycoside and a cell wall–active antimicrobial (e.g., penicillin, cephalosporin, monobactam, carbapenem, glycopeptide).[2,109,122] The mechanism of aminoglycoside synergistic activity may not be the same for all target organisms. Enhanced aminoglycoside uptake in the presence of a cell wall–active drug has been demonstrated with enterococci, viridans streptococci, *S. aureus,* and *P. aeruginosa*.[109] No combination of aminoglycoside and cell wall–active drug is indicated as effective for methicillin-resistant *S. aureus*. In vitro synergism against *P. aeruginosa* and Enterobacteriaceae[109,123] does not appear to translate into all clinical situations.[124] Of equal importance, the bactericidal activity of aminoglycosides can be antagonized by bacteriostatic agents such as chloramphenicol and tetracycline.[109] The mechanism is unclear. Possibilities include inhibition of the energy-dependent uptake of aminoglycosides and interference with movement of the ribosome along mRNA. Provocative modeling work suggests new innovative combinations may likewise prove synergistic.[125]

Aminoglycoside combinations have been evaluated in animal models of endocarditis, meningitis, pneumonia, peritonitis-bacteremia, pyelonephritis, osteomyelitis, myositis (mouse thigh), and subcutaneous infection with or without a foreign body, as well as additional conditions.[109,115,126–135] In general, if the organism is susceptible to both the aminoglycoside and the companion drug, antibacterial activity is enhanced; virtually all studies showing effectiveness of combined therapy used a cell wall–active drug (β-lactam or glycopeptide) with the aminoglycoside.

An aminoglycoside as part of combination therapy may prevent or delay the emergence of bacteria resistant to either the aminoglycoside or the companion drug. In a series of studies, aminoglycosides were shown to reduce, but not to fully prevent, the emergence of quinolone-resistant strains of Enterobacteriaceae or *P. aeruginosa* in a murine model of peritonitis.[136–138] The concomitant use of an active β-lactam appears to prevent the emergence of gentamicin-resistant subpopulations in neutropenic animals.[139]

Treatment of infected animals with gentamicin markedly reduced mortality but had virtually no influence on the incidence of abscess formation.[140–142] Treatment with clindamycin or metronidazole reduced the incidence of abscess formation but had no effect on peritonitis-bacteremia or lethality.[143] Combination therapy reduced both acute mortality and late abscess formation despite conditions adversely affecting aminoglycoside activity such as low pH, at low oxygen tension, and in the presence of drug-binding purulent debris.[17,19,144]

Data from infected animals and analysis of clinical trial data support the correlation between high peak levels, increased dose, and antibacterial efficacy.[131,145–148] Dose fractionation studies reduce the interdependence among the various pharmacokinetic parameters in small rodents with rapid aminoglycoside clearance. In these models, the therapeutic efficacy of aminoglycosides correlates with the peak serum concentration and the area under the concentration versus time curve (i.e., AUC) over time.[131,132] A compilation of clinical trials involving aminoglycosides for gram-negative bacillary infections has shown that peak/MIC and 24-hour AUC/MIC ratios predicted outcome.[147,149,150] Clinical efficacy increased from 55% for a peak/MIC ratio less than or equal to 2 to 90% for a ratio greater than 8 when four different aminoglycosides all were administered by identical traditional every-8-hour dosing regimens. Efficacy improved from 47% for tobramycin AUC/MIC ratios less than 110 to 80% for ratios greater than 110. Both peak greater than or equal to 10 and AUC greater than 150 correlated with rapidity of fever and leukocytosis resolution in gram-negative pneumonia. These favorable results applied only to organisms with MICs less than or equal to 0.5 μg/mL. Current maximal dosing regimens of 7 mg/kg routinely achieve peak/MIC ratios greater than or equal to 10 for bacteria with MICs less than or equal to 1 μg/mL, but Clinical and Laboratory Standards Institute susceptibility breakpoints are 4 μg/mL. Synergistic activity with β-lactams may allow adequate coverage for those increasing numbers of organisms with MICs 2 to 4 μg/mL.[151,152]

More than 55 clinical trials, including more than 30 prospective randomized trials and 9 formal meta-analyses, have compared aminoglycoside once-daily administration with traditional 8- or 12-hour administration, showing equivalency or superiority of once-daily regimens. In largely prospective fashion, more than 6500 mostly nonneutropenic adult patients have received amikacin, gentamicin, netilmicin, or tobramycin for periods of 7 to 14 days.[153–199] Comparable antimicrobial efficacy and toxicity were recorded for a broad range of infections, including pneumonia, intraabdominal infections, gram-negative sepsis, febrile neutropenia, pelvic inflammatory disease, and urinary tract infections. Populations studied included elderly patients,[169,182] febrile neutropenic patients,[a] critically ill patients,[156,164,182,198] and patients with variable levels of renal insufficiency.[162,182,193,195]

Five meta-analyses have shown statistically significantly improved clinical outcomes, and three have shown significantly lower nephrotoxicity, when aminoglycosides were dosed once daily. Likewise, equivalence or a trend toward lower ototoxicity was shown with once-daily administration. Among trials identifying a time to onset of toxicity, once-daily dosing delayed nephrotoxicity compared with 8-hour dosing until total duration approached 10 to 14 days, when the incidence became similar in both groups. Two trials comparing once-daily with twice-daily dosing of amikacin, gentamicin, and tobramycin showed that the incidence of nephrotoxicity was lower with once-daily dosing when using short courses of therapy and was delayed more for an equivalent total amount of standard therapy drug.[200,201]

Once-daily dosing regimens have similar or slightly better clinical efficacy than twice-daily or three times–daily regimens. Plazomicin has been developed solely for a once-daily dosing regimen. If current recommendations limiting duration to only 5 to 6 days[200–202] are followed, once-daily dosing can also delay the onset of nephrotoxicity. For treatment regimens lasting longer than 5 to 6 days, aminoglycoside dosing by individualized pharmacokinetic modeling may result in less nephrotoxicity.[203] Consideration should also be given to morning administration of aminoglycosides to minimize nephrotoxicity, when food intake and higher urinary pH in diurnally active humans may reduce renal toxicity.[204]

An overview[194] of the large, published, extensively analyzed experience in patients indicates that once-daily aminoglycoside administration

- Is as efficacious as the traditional multiple-dose method (see later discussion of exceptions).
- May lower, but not eliminate, the risk of drug-induced nephrotoxicity and ototoxicity.

[a]References 155, 170, 177, 182, 193, 197.

- Is simpler (less human error–prone), less time-consuming, and more cost-effective than multiple-dose regimens.
- Is not universally advocated in patients with enterococcal endocarditis.[205,206]
- Needs further study in selected populations of patients, such as pregnant women, patients with CF (see "Cystic Fibrosis"), patients with meningitis caused by aerobic gram-negative bacilli, and patients with osteomyelitis
- Does not worsen neuromuscular function even in critically ill, ventilated patients; nonetheless, rapid intravenous infusion should be avoided.[207]

At the present time, once-daily aminoglycoside administration is as efficacious as traditional multiple-dose regimens and may reduce the risk of nephrotoxicity, but further study in selected populations is needed.

CLINICAL PHARMACOLOGY

Aminoglycosides are generally administered intravenously over a 30- to 60-minute period. However, outpatient slow push infusion over 3 to 5 minutes has been safely administered in more than 5000 patients over 15 years in one center.[208] Aminoglycoside given intramuscularly is absorbed completely, with maximal serum levels achieved 30 to 120 minutes after administration.[209] Absorption may be delayed in elderly patients and patients with compromised renal function, hypotension, or impaired tissue perfusion. Aminoglycosides are minimally absorbed from the gastrointestinal tract.[210] Nonetheless, instances of deafness have resulted from administration of oral neomycin to patients with hepatic encephalopathy and impaired renal function.[211] Also, increased absorption in the presence of concomitant inflammatory bowel disease is of theoretical concern. In contrast, patients with acquired immunodeficiency syndrome and severe cryptosporidiosis have ingested large amounts of paromomycin over protracted periods without evidence of toxicity. Other exposures may lead to systemic toxicity. Topical application of aminoglycoside on inflamed skin leads to no or minimal absorption. However, patients with extensive burns or other severe dermal injury may absorb a drug and be at risk for toxicity.[212] Aminoglycosides can be instilled into the pleural space or the peritoneal cavity; absorption is rapid, with resultant serum concentrations proportionate to the concentration of drug instilled. The use of aminoglycosides in abdominal irrigation solutions is not recommended because rapid absorption with subsequent neuromuscular blockade has been reported.[213] In contrast, aminoglycosides have been administered as a bladder irrigant, as an aerosol, and by direct instillation into the lumbar sac or lateral ventricles without evidence of detectable concentrations in the blood.[214–216] Inhalation has also been used, especially in patients with CF.[217–223]

As anticipated for drugs with a low level of protein binding (approximately 10%–20%) and a high level of solubility in water, the aminoglycosides are distributed freely in the vascular space and relatively freely in the interstitial spaces of most tissues.[224] Significant interpatient variability in aminoglycoside volume of distribution has been demonstrated, as well as intrapatient variation during therapy.[225,226] In the absence of disease or infection, the volume of distribution mirrors that of the extracellular fluid compartment—0.2 to 0.3 L/kg.[227] The volume of distribution increases in edematous states including ascites, in patients with burns, in patients with CF, and in some severe infections when conditions may vary from hour to hour in critically ill patients.[228] The volume of distribution increases less than expected or actually decreases in obese individuals.[229] Because of their size, polycationic charge, and lipid insolubility, aminoglycosides cross biologic membranes poorly, with the exception of renal tubular cells and perhaps inner ear cells that appear to have an inherent transport mechanism. The cells of the renal proximal convoluted tubule can concentrate aminoglycosides to levels that exceed those of plasma or interstitial fluid.[230]

Adequate antibiotic concentrations are achieved in most body fluids.[231] Aminoglycosides enter synovial fluid easily, with subsequent concentrations only slightly less than simultaneous serum concentrations.[232] Parenteral aminoglycoside administration results in low concentrations of active drug in bronchial secretions.[216] Penetration into epithelial lining fluid ranges from 32% to 54% of serum concentrations.[233,234] Much higher concentrations can be achieved by aerosol administration.[218] Diffusion is slower in bile, feces, prostatic fluid, and amniotic fluid.[235–237]

Aminoglycosides traverse the blood–cerebrospinal fluid and blood-brain barriers poorly[238] but do cross the placenta and achieve fetal serum concentrations 21% to 37% of maternal concentrations.[237] Intraventricular administration results in high concentrations in both ventricular and spinal fluid.[238–241] Therefore the intraventricular route is recommended for meningitis caused by aerobic gram-negative bacilli in adults in the rare cases in which this therapy is necessary. However, in newborns, intraventricular aminoglycoside is no more effective and perhaps more toxic than the drug given intravenously. Aminoglycoside penetration into the tissues of the eye is poor; neither systemic nor subconjunctival administration in single doses produces reliable levels in the vitreous humor of humans.[242–245] Direct intravitreal injection is recommended for the treatment of endophthalmitis.

Urine concentrations of aminoglycosides exceed peak plasma levels 25- to 100-fold within 1 hour after drug administration.[246,247] Because of renal tubular cell absorption and subsequent release, urine concentrations remain above therapeutic levels for several days after a single dose, even in severe renal impairment. After termination of a multiple-dose regimen, urine levels remain above therapeutic levels for days, with a terminal half-life of 48 to 200 hours.[247–249]

Aminoglycosides are primarily eliminated unchanged by the kidney via glomerular filtration. Less than 1% is eliminated in the feces, and 1% is eliminated in saliva.[250] Aminoglycosides can be removed by dialysis, leading to unpredictable clearance depending on the specific modality and dialysis membrane capacity.[251] With normal renal function in adults, more than 90% of an administered dose is recovered in urine unchanged during the first 24 hours.[250,252] The remainder is slowly recycled to the tubular lumen, with a tissue half-life of 30 to 700 hours,[252,253] creating a multicompartmental elimination profile.[254,255]

The pharmacokinetics of aminoglycosides in clinical use are very similar, even though interpatient variability is broad and associated with body weight, body composition, and renal function as noted earlier. Typical expected aminoglycoside pharmacokinetic parameter values are summarized in Table 25.3. Peak serum concentrations of gentamicin, netilmicin, and tobramycin after a dose of 7 mg/kg infused over 30 minutes range from 15 to 20 μg/mL and yield an AUC of 70 to 100 mg · h/L. An amikacin dose of 15 mg/kg similarly infused produces peak levels of 41 to 49 μg/mL and an AUC of 110 to 145 mg · h/L.[2] In patients with renal impairment, peak concentrations are higher, and AUCs are larger. Suggested initial dosing regimens for typical aminoglycosides in clinical use are listed in Table 25.4. One caveat to proper interpretation of these published nomogram concentrations is that the "peaks" were likely obtained before the end of distribution, whereas pharmacodynamic data use actual postdistribution peaks. Blind use of a 7-mg/kg dose may not achieve peaks related to expected pharmacodynamic outcomes.[256] Disease states that result in significant loss of muscle mass are associated with low serum creatinine values. In such patients the Cockcroft-Gault formula may seriously overestimate the glomerular clearance of aminoglycosides.[257] For this reason, it has been suggested that the minimum value for subcutaneous administration used in the formula be 0.8 μg/mL.[258] However, a study in elderly patients showed that such a practice significantly underestimated creatinine clearance (CrCl) and recommended that the practice should be avoided.[259] The US Food and Drug Administration (FDA), several decades before pharmacodynamic research into dose optimization, approved gentamicin and tobramycin for up to 1.7 mg/kg per dose, three doses per day, for a total daily dose of 5.1 mg/kg.[260] For netilmicin the total daily dose is 6.0 mg/kg, and for amikacin total daily dose is 15 mg/kg. In one clinical study that included a high percentage of critically ill patients the peak serum gentamicin concentration after the first dose of 5.1 mg/kg was less than 16 μg/mL in 48% of the patients.[260] Therefore in critically ill patients, it is reasonable to give an initial dose of 7.0 mg/kg.[198] Extended-interval dosing is the norm today, used in 75% of all acute care hospitals.[261] No controlled studies have compared 5 mg/kg/day versus 7 mg/kg/day of gentamicin or tobramycin, but clinical experience with 7 mg/kg/day is substantial.[192,193] With the exception of patients with CF, the clinical use of single doses of amikacin or netilmicin exceeding the licensed total daily dose has not been reported. To date, FDA-approved labeling on therapeutic drug monitoring kits for aminoglycosides suggests "normal ranges" of serum concentrations are achievable with traditional dosing of three

times daily rather than the higher, more effective serum concentrations now achievable with extended-interval dosing.

Various dialysis modalities clear differing amounts of aminoglycosides, roughly one-half of circulating aminoglycoside per hemodialysis period, depending on the characteristics of the dialysis membrane, duration of dialysis, the patient's blood pressure during dialysis, and other variables. Depending on variables related to both the patient and the filter, continuous hemofiltration results in the equivalent of a CrCl of 10 to 50 mL/minute.[262,263] For patients undergoing continuous ambulatory peritoneal dialysis who have a systemic infection and are receiving an intravenous dose of aminoglycoside every 2 to 3 days, it is necessary to give small daily intravenous supplements to replace the drug lost in the dialysate or dosed based on serum concentrations (www.pdiconnect.com/content/30/4/393.full.pdf+html). For patients on hemodialysis, a traditional dose is given every 48 to 72 hours, and on the day of hemodialysis an additional one-half of the full dose is given (if the traditional dose was administered prior to dialysis) after dialysis in order to replace drug that was removed by the dialysis or simply dosed after dialysis. Because of individual variability, serum levels should be measured.[264–266]

TABLE 25.3 Typical Pharmacokinetic Parameters for Amikacin, Gentamicin, Netilmicin, and Tobramycin in Adults With Normal Renal Function

PARAMETER	MEAN (± SED) OR RANGE
Clearance, mL/min/kg	
Creatinine ≤1.5	1.33 (0.61)
Creatinine >1.5	0.53 (0.35)
CrCl ≥100	1.51 (0.63)
Volume of distribution, L/kg	
Dehydration	0.07–0.15
Euvolemic	0.15–0.25
Expanded ECF	0.35–0.70
Half-life, h	
Creatinine <1.5	0.15–15
CrCl ≥100	0.5–7.6
Age <30 y	0.5–3
Age >30 y	1.5–15
Urinary excretion	85%–95%

CrCl, Creatinine clearance; *ECF,* extracellular fluid; *SED,* standard error of the difference.
Modified from Schentag JJ, Meagher AK, Jelliffe RW. Aminoglycosides. In: Burton ME, Shaw LM, Schentag JJ, et al, eds. Applied Pharmacokinetics and Pharmacodynamics: Principles of Therapeutic Drug Monitoring. *Philadelphia: Lippincott Williams & Wilkins; 2006.*

Dosing for morbidly obese patients is generally based on excess body weight multiplied by 0.4 plus ideal body weight. More recent work indicates that lean body weight permits simplified aminoglycoside dosing across all weight strata, with clearance best predicted by the Chronic Kidney Disease Epidemiology Collaboration equation.[267–269] However, the best clinical descriptor of renal function for pharmacokinetic modeling in elderly patients is the Cockcroft-Gault–based model.[270] Monitoring early peak concentration (and another to estimate AUC) may assist in optimizing aminoglycoside efficacy.[9,151] It is unclear whether every patient administered an aminoglycoside requires an individual pharmacokinetic evaluation,[271] but individualized dosing is essential in a critically ill patient with altered volume of distribution of drug and unstable renal function. Use of new Bayesian analysis in routine clinical care holds promise.[272,273] Overall, individualized dosing results in greater efficacy and reduced toxicity compared with the nomogram approach.[259,270,274]

Typically, two concentrations in the postdistribution phase (>1 hour after administration, separated by >1.5 half-lives) are used. Trough levels should be below the limit of detection.

With the exception of the aminocyclitol spectinomycin, aminoglycoside antibiotics share the potential for causing injury to the renal proximal convoluted tubules, damage to the cochlea or vestibular apparatus or both, and neuromuscular blockade (Table 25.5). The inherent toxicity and relative toxic potential of the aminoglycosides correlate with their positive electrical charge at physiologic pH.[14]

Also important are untoward effects that are encountered rarely. Hypersensitivity reactions and drug fever are uncommon, and the aminoglycosides do not provoke inflammation. Hence phlebitis at intravenous infusion sites is rare; intramuscular injection sites do not become painful; instillation into the pleural space, abdominal cavity, or cerebrospinal fluid causes no irritation; and incorporation of an aminoglycoside into methyl methacrylate prosthetic joint cement is well tolerated over protracted periods. The aminoglycosides are not hepatotoxic, do not induce photosensitivity, and have no identified adverse influence on hematopoiesis or the coagulation cascade.

Nephrotoxicity

Experimental Nephrotoxicity

The precise mechanisms of aminoglycoside-induced injury to renal proximal tubular cells remain incompletely understood. There are as yet unidentified genetic factors and major differences in susceptibility to nephrotoxicity between animal species and between inbred strains of a specific animal.[275]

Aminoglycosides initially bind to an endocytotic receptor, megalin,[276,277] important in the endocytotic uptake of proteins in the renal proximal tubule.[276–278] In megalin receptor knockout mice, there

TABLE 25.4 Suggested Dosing Regimens for Adults

ESTIMATED CREATININE CLEARANCE (mL/min)	DOSE (mg/kg)			DOSING INTERVAL (h)
	Gentamicin, Netilmicin, Tobramycin	Plazomicin	Amikacin	
100	7	15	20	24
90	7	15	20	24
80	7	15	20	24
70	5	15	15	24
60	5	15	15	24
50	4	10	12	24
40	4	10	12	24
30	5	10	15	48
20	4	10	12	48
10	3	10	10	48
<10	2.5	N/A	7.5	48

N/A, Not available.
Modified from Gilbert DN, Bennett WM. Use of antimicrobial agents in renal failure. Infect Dis Clin North Am. *1989;3:517–531.*

TABLE 25.5 Estimated Frequency of Serious Clinical Adverse Reactions After Administration of Aminoglycoside Antibiotics

ADVERSE REACTION	ESTIMATED USUAL FREQUENCY (%)	REFERENCES
Nephrotoxicity	5–15	252–262
Ototoxicity	2–14	
Cochlear	2–10	284–286
Vestibular	3–14	284,312,313
Neuromuscular blockade	Exceedingly rare	145,325

TABLE 25.6 Factors That Increase Risk for Aminoglycoside Nephrotoxicity

- Patient-related factors
  - Older patient
  - Preexisting renal disease
  - Female[214]
  - Male[260]
- Volume depletion, hypotension
- Hepatic dysfunction
- Aminoglycoside factors
  - Recent aminoglycoside therapy
  - Larger doses
  - Treatment for ≥3 days
  - Drug choice (e.g., gentamicin)[255]
  - Frequent dosing interval[128,268]
- Concomitant drugs
  - Vancomycin[277–279]
  - Amphotericin B
  - Furosemide
  - Clindamycin
  - Piperacillin
  - Cephalosporins?[263,274–276]
  - Methoxyflurane[282]
  - Foscarnet
- Intravenous radiocontrast agents

Data from references 215, 216, 253, and 260 and references cited in table.

was no renal uptake of aminoglycosides.[279] After binding to the anionic megalin in clathrin-coated pits,[276,280] the cationic aminoglycoside is transferred to endosomes, and the megalin is returned to the apical plasma membrane. A portion of drug-containing endosomes fuses with lysosomes, where the aminoglycosides inhibit lysosomal phospholipases, resulting in lysosomal whorl-like membrane changes that, because of their morphologic appearance, have been termed *myeloid bodies*.[276] Some aminoglycoside-containing endosomes move rapidly to the Golgi apparatus.[281] The time frame correlates with the observed rapid decrease in cellular protein synthesis after aminoglycoside exposure.[282–285]

In cells that internalize aminoglycosides, cellular necrosis occurs gradually, during which time a variety of abnormalities are demonstrable,[286–297] leading to activation of the apoptotic pathway with subsequent necrosis of the cells of the proximal tubules.[297] Initially, animals manifest nonoliguric renal failure.[298] After several days, there is a fall in glomerular filtration. The cells of the proximal tubule can regenerate with return of glomerular filtration. Regeneration occurs even if there is continued administration of the aminoglycoside.[299] In animals the regenerated tubular cells have a reduced capacity to take up aminoglycosides.

A hierarchy of nephrotoxic potential exists among the aminoglycosides.[300–302] Neomycin is the most toxic aminoglycoside,[303] and streptomycin is the least nephrotoxic, perhaps because the drug does not accumulate in the renal cortex.

Aminoglycoside uptake into the proximal renal tubular cell is saturable at clinically relevant concentrations, governed by Michaelis-Menten kinetics.[276] Thus for a given total daily dose of a specific aminoglycoside the magnitude of toxicity is greatest when the dose is divided into multiple small increments and least when it is given as a single daily dose.[301,304,305] In addition, age, sex, volume status, pH, and electrolyte balance all have been examined, with older age, dehydration, lower urinary pH, and electrolyte depletion associated with increased toxicity.[b] The impact of experimental liver disease or diabetes is unclear, but accumulated clinical data indicate that concomitant liver disease is a risk factor for nephrotoxicity.[318–322] Various drugs influence the severity of experimental aminoglycoside nephrotoxicity.[323–331] Vancomycin and the related drug teicoplanin amplify experimental aminoglycoside nephrotoxicity.[323–331] Extended-spectrum penicillins reduce the risk of kidney injury.[326,327] Polymers of aspartic acid dramatically reduce renal tubular injury despite the accumulation of very high renal concentrations of aminoglycoside.[332–341] Animal experiments with an aminoglycoside plus cephalothin, cefazolin, or cefamandole indicated either no effect or an attenuation of nephrotoxicity compared with an aminoglycoside plus placebo.[342] Fosfomycin may reduce aminoglycoside experimental nephrotoxicity by inhibiting gentamicin-induced lipid peroxidation.[343] In general, ascribing causation to a single factor in a multivariate interaction resulting in toxicity is not a statistically valid approach. In a clinical study, no identifiable risk factor alone or in combination reliably predicted nephrotoxicity.[344]

Clinical Nephrotoxicity

One factor in the nephrotoxicity of aminoglycosides is that they accumulate in the kidney, accounting for 40% of the total drug in the body,[255] and nearly 85% of this is located in the renal cortex. The reported incidence of nephrotoxicity ranges from 0% to 50%, with most reports in the 5% to 15% range (see Table 25.5).[345–352] The variability results from differences in the definition of nephrotoxicity, the frequency of testing, and the particular tests used to measure renal function and the clinical setting in which the drugs are administered. Minimal clinical differences were seen in a survey of clinical trials involving approximately 10,000 patients between 1975 and 1982.[345] Frequencies of nephrotoxicity averaged 14.0% for gentamicin, 12.9% for tobramycin, 9.4% for amikacin, and 8.7% for netilmicin. In prospective, randomized studies with definitions of nephrotoxicity that reflect a substantive decrement in the glomerular filtration rate (GFR) in seriously ill patients, the reported incidence varied between 5% and 10% of patients' courses.[347–352]

In studies of the etiology of acute renal failure, medication-induced renal injury is reported as a major cause. In an analysis of more than 2000 hospitalized patients, almost 100 experienced renal insufficiency, and seven episodes were attributed to aminoglycoside therapy.[353] In general the aminoglycoside-induced decrement in the GFR is small. Most patients have a nonoliguric fall in CrCl; progression to dialysis-dependent oliguric-anuric renal failure is rare. As in animal models, the tubular injury is reversible, and in a few patients, recovery of renal function has been documented despite continued administration of the aminoglycoside.[354]

Reported clinical risk factors for aminoglycoside nephrotoxicity can be grouped as being related to the patient, to the aminoglycoside, or to the influence of the selected concomitant drug (Table 25.6).[c] Factors identified in clinical trials include older age, preexisting renal or liver disease, shock, larger volume of distribution, location in intensive care unit, pneumonia, rapidly fatal prognosis, leukemia, longer duration of therapy, and concomitant vancomycin or other nephrotoxin. Female sex was identified as a risk factor in one study but was not confirmed in others.[308,345,350–352] Reported clinical studies with cephalosporins did not include an aminoglycoside-only group of patients, so it is not possible to ascertain the effect of cephalosporins on the risk of nephrotoxicity. The correlation of increased risk of toxicity with age and with preexisting renal disease may be misleading. It is unclear whether a risk exists when the dosing regimen is adjusted for a preexisting decrease in the GFR, and this was not a risk factor in several studies.[345,346,350] Hypotensive patients, especially patients with septic shock or sepsis syndrome, have an increased incidence of renal insufficiency. The role of aminoglycosides is unclear in that infection-induced low perfusion pressures, consumptive coagulopathy, cytokine-mediated endothelial damage, and other factors may be etiologic in the fall in GFR.[355,356] Liver disease was identified as

[b]References 66, 68–73, 75–90, 92–98, 100–102, 104–198, 200–204, 208–222, 224–272, 274–291, 306–317.

[c]References 308, 309, 345, 346, 350, 357, 358.

a risk factor in the retrospective analysis of two large clinical trials and was then validated in two additional prospective trials.[320,357]

Clinical trial data support the concept that several days of therapy are needed to cause nephrotoxicity of clinical consequence. In an observational study of patients treated with combination therapy, including an aminoglycoside for infective endocarditis, there was a 0.5% decrease per day in the estimated endogenous CrCl.[358] In contrast, accidental massive overdosage of 1 day or less has not resulted in acute tubular necrosis.[359,360]

The influence of concomitant drugs is difficult to interpret in patients with serious or complex disease states who are receiving many drugs. Nonetheless, most studies suggest an increased risk of a fall in GFR when other nephrotoxic drugs are administered concomitantly with aminoglycosides.[d] Three prospective studies, one of which was a double-blind study, found the combination of cephalothin plus aminoglycoside more nephrotoxic than the combination of a penicillin derivative plus an aminoglycoside.[361–363] Subsequent multiple logistic regression risk factor analysis identified a variety of cephalosporins as risk factors. These results are consistent with ceftazidime enhancement of gentamicin enzymuria in healthy volunteers.[364] Two studies evaluated concomitant vancomycin administration; one analysis included a control group that received only an aminoglycoside.[365,366] Both studies indicated that vancomycin was a risk factor. In febrile neutropenic patients administered gentamicin or tobramycin plus carbenicillin or ticarcillin, the reported incidence of nephrotoxicity was 2% to 6% compared with 10% to 15% or higher when the aminoglycoside was combined with other β-lactam antibiotics.[361,362,367] A risk factor analysis found an increased risk with concomitant piperacillin but not with carbenicillin or ticarcillin. The authors speculated that the lower sodium content of piperacillin might explain the difference.[327]

Ototoxicity

Aminoglycoside antibiotics can cause cochlear and vestibular damage in experimental animals and humans.[372] Streptomycin-induced hearing loss and dizziness were included in the first clinical report of the drug's efficacy.[373] Overt ototoxicity was noted in 2% to 10% of patients in early clinical experience.[374,375] Ototoxicity is of particular concern because it is usually irreversible and can appear after the end of treatment; moreover, repeated exposure engenders cumulative risk.[376] A patient may suffer only cochlear damage or only vestibular damage, but in general the predominant lesion is vestibular.[377] Rarely, both organs are injured. It is unusual to have both ototoxicity and nephrotoxicity in the same patient.

Cochlear Toxicity

Few recipients of aminoglycoside therapy complain of hearing loss, and yet the reported incidence is as high as 62% when asymptomatic high-frequency audiograms are performed repeatedly.[378] The overall incidence has been reported to be between 3% and 14%.[379]

Normal sound perception extends to frequencies of 20 kHz; perception of human speech requires sound detection in the range of 0.3 to 3 kHz. A loss of hearing threshold of 25 to 30 dB is necessary before the patient is aware of the deficit. A commonly used definition for drug-induced ototoxicity is an increase in auditory threshold of 15 dB or greater at any of two or more frequencies.[376]

Controlled data on cochlear toxicity are sparse. In a series of prospective clinical studies that examined the efficacy and toxicity of gentamicin, tobramycin, and amikacin in combination with β-lactam antibiotics, 22% of the aminoglycoside recipients had documented audiometric toxicity compared with 7% of cefotaxime-treated patients.[349] In a different study of 53 subjects administered gentamicin, tobramycin, or amikacin for at least 4 days, loss was unilateral in 55% and bilateral in 45% of the patients, and hearing loss was initially detected after a mean of 9 days of therapy.[379]

Aminoglycosides penetrate into endolymph and cochlear and vestibular tissue[380] cells either by endocytotic uptake or by mechano-electrical transduction channels[381] and lysosomal accumulation after transstrial trafficking.[382–384] In experimental animals, aminoglycosides can be detected in inner ear fluid within 3 hours of administration, but cochlear damage requires 3 weeks of daily injections.[376,385,386] The precise mechanism of cochlear toxicity has eluded detection.[387–389] As in renal tubular cells, there is evidence for aminoglycoside-induced apoptotic cell death in cells of both the organ of Corti and the vestibular apparatus.[390] Hair cell loss has been considered irreversible. However, animal studies in nonmammals and mammals have documented potential regeneration.[391–393]

The greatest risk for cochlear toxicity may be genetic predisposition.[394] In numerous reports, deafness has developed in family members of multiple pedigrees after treatment with an aminoglycoside and has been associated with five or more different mutations in the mitochondrial 12S rRNA gene,[395–404] which may result in greater binding affinity. No genetic predisposition to aminoglycoside vestibular or renal toxicity has been identified.

Human toxicity is related to the dose and duration of aminoglycoside therapy.[376] Ototoxicity is related to the AUC of concentrations in cochlear endolymph, itself correlated with AUC in serum.[405] Thus the same total daily dose will result in the same incidence of ototoxicity, independent of the frequency of dosing.[186,187,189,372] Measurable differences in the risk of cochlear toxicity between gentamicin, tobramycin, amikacin, and netilmicin are minimal and less than some chemotherapeutic agents.[379,406–416] Prolonged therapy beyond 10 days, renal or hepatic impairment, and prior exposure to aminoglycosides are major risk factors.[413–415,417–419]

Concomitant loop diuretics, vancomycin, and loud ambient noise increase the risk of cochlear toxicity.[376,410–412] The use of aspirin reduced the incidence of ototoxicity from 13% to 3%.[406,420]

Vestibular Toxicity

The target of drug-related vestibular toxicity is the type I hair cell of the summit of the ampullary cristae.[421] The true incidence of vestibular toxicity in ill patients is virtually impossible to determine. Because vestibular injury is bilateral and initially symmetrical, it can be compensated by visual and proprioceptive cues, so patients can sustain considerable injury before the appearance of symptoms or clinical findings. Suspicion is raised at the bedside by complaints of nausea, vomiting, and imbalance.[377,422] Visual blurring with head movement (i.e., oscillopsia) may occur. Symptoms are exacerbated in the dark, when the eyes are closed, with moving or uneven surfaces, and in other situations that block compensatory pathways. Nystagmus may be evident. Systematic surveillance of patients with electronystagmography is seldom performed; in one clinical study using electronystagmographic surveillance, abnormalities were demonstrated in 4% to 6% of patients receiving gentamicin or amikacin.[351,352] There are no data that compare, in a controlled fashion, the toxic potential of the commonly prescribed aminoglycosides.

Vestibular injury can be unilateral or bilateral and mild or severe.[377,422] Functional recovery, even with bilateral damage, was reported to occur in up to 53% of patients at 10 days to 9 months after cessation of drug exposure.[422–425] In addition to visual and proprioceptive compensation, recovery may be due to hair cell regeneration, as demonstrated in animal models.[406,426,427] The relevance of the latter to humans is unclear.

Vestibular hair cells are purposely damaged by gentamicin as therapy for Meniere disease that fails to respond to conservative measures.[428,429] Injection into the middle ear allows gentamicin to pass through the round window membrane, penetrate the labyrinth, and destroy hair cells. A single injection is reported to effect good control of vertigo in 75% of patients, with minimal sensorineural hearing loss.[430]

Neuromuscular Blockade

Neuromuscular blockade after aminoglycoside administration is a rare but serious and potentially lethal adverse effect. Neuromuscular blockade has been described in patients administered neomycin, streptomycin, kanamycin, tobramycin, gentamicin, amikacin, or netilmicin.[213,431] In general, blockade has occurred in clinical situations in which a disease state or a concomitant drug interferes with neuromuscular transmission.[431–437] Drug exposure may have been a result of intraperitoneal, intravenous, intramuscular, intrapleural, oral, topical, or retroperitoneal administration.[213,431] Aminoglycoside therapy in ventilated patients or others in intensive care has not generally been associated with adverse effects.[207,438,439] Blockade

[d]References 308, 309, 345, 346, 350, 368–371.

results from inhibition of the presynaptic release of acetylcholine as well as blockage of postsynaptic receptor sites of acetylcholine.[440–443]

CLINICAL INDICATIONS

The aminoglycosides are effective in empirical treatment of infections caused by or suspected to be caused by aerobic gram-negative bacilli including *P. aeruginosa*. Aminoglycosides have in vitro activity against *S. aureus,* but resistant small colony variants may appear within 24 hours unless a concomitant antistaphylococcal β-lactam or vancomycin is administered.[444] Activity against *Enterococcus* spp. requires a concomitant active penicillin or vancomycin. Aminoglycosides have no practical activity against pneumococci or anaerobic organisms. For reasons of anticipated spectrum of activity or to achieve an additive or synergistic effect, aminoglycosides are often combined with a β-lactam antibiotic, vancomycin, or a drug active against anaerobic bacteria.

The efficacy of empirical aminoglycoside therapy has been documented in published symposia describing the results of clinical trials that served as the basis for licensure and subsequent trials that compared one aminoglycoside with another or with a β-lactam.[e] In febrile neutropenic patients, a high failure rate was experienced after monotherapy with an aminoglycoside; therefore in such patients the aminoglycosides are administered in combination with a β-lactam antibiotic active against aerobic gram-negative bacilli.[367] Guidelines on the treatment of the febrile neutropenic patient suggest avoidance of aminoglycosides, if possible; instead, empirical monotherapy with a carbapenem or broad-spectrum β-lactam is used.[445]

Given that optimal peak/MIC and AUC/MIC pharmacodynamic ratios are obtained only for organisms with MICs of 0.5 μg/mL or less with conventional doses of gentamicin, netilmicin, and tobramycin (2 μg for amikacin) and MICs of 1.0 μg/mL with doses of 7 mg/kg,[9] it is not surprising that several reviews have demonstrated inferior clinical efficacy of aminoglycoside monotherapy in severe gram-negative infections compared with therapy with β-lactams and fluoroquinolones.[367,459] A review emphasized individualized dosing based on pharmacodynamics to optimize care of critically ill patients.[460] Although aminoglycosides have been used in combination with β-lactams and fluoroquinolones to enhance killing and improve clinical efficacy, most studies have failed to demonstrate better outcomes with combinations compared with monotherapy.[461]

Bacteremia

In contrast to most older studies[5,462] and a more recent retrospective review of *P. aeruginosa* bacteremia,[124] other more recent studies demonstrate improved outcome in patients with septic shock and gram-negative bacillary bacteremia treated with a combination of aminoglycoside plus β-lactam including carbapenem-resistant pathogens.[6,12,463] Combination therapy provided a greater degree of initial appropriate therapy than β-lactam monotherapy, broader coverage than fluoroquinolones, and improved outcomes even in neutropenic patients.[464,465] Reduced endotoxin release seen with aminoglycoside therapy[355,356] may contribute to the diminished early mortality observed with combination therapy in patients with septic shock. Combination therapy in selected cases is endorsed in the most recent international guidelines for management of severe sepsis.[466] Short-course therapy was not associated with increased nephrotoxicity.[466]

A detailed analysis and a meta-analysis of use of aminoglycosides in the treatment of bacterial endocarditis have been published.[205,206,467,468] The use of aminoglycosides in combination with a β-lactam or vancomycin may be beneficial for patients with streptococcal or enterococcal endocarditis. The standard dosage to achieve a synergistic effect is 1 mg/kg intravenously every 8 hours; newer regimens have used 12- and 24-hour intervals.[469] The risk of nephrotoxicity increased in parallel with the duration of therapy.[358] For viridans streptococcal endocarditis, three times–daily, twice-daily, and once-daily regimens appear to be of equal efficacy in reported clinical trials.[205,206,447,467–470]

The long-held practice of administering combination β-lactams plus aminoglycosides for staphylococcal bacteremia and endocarditis has been challenged by some investigators,[471,472] who recommended against the addition of low-dose gentamicin (1 mg/kg every 8 hours) because of its added nephrotoxicity. Others have instead emphasized the suboptimal dosing strategy used[473] or emphasized the significant earlier (2 days vs. 4 days) defervescence.[474] Aminoglycoside combinations remain the standard of care for enterococcal bacteremia and endocarditis, although dual β-lactam regimens are being used as alternatives.[475,476]

Pneumonia

Aminoglycoside use in pneumonia is typically reserved for hospital-acquired or hospital-associated gram-negative bacillary respiratory tract infections, including infections seen in patients on ventilators or with CF. Combination therapy with a β-lactam yields superior results[477] compared with aminoglycosides alone, without generally improving outcome over β-lactam monotherapy for Enterobacteriaceae.[478] The role of combination therapy for *P. aeruginosa* pneumonia is still unclear, with one meta-analysis suggesting significant mortality benefit,[478] but another large study showing no additional benefit as long as the isolate was susceptible to more than one agent.[124]

Aerosolized aminoglycosides, initially used to treat CF exacerbations,[217–222] have shown promise in chronic bronchiectatic infections[479,480] and ventilator-associated pneumonias. Most of the infections studied have involved *P. aeruginosa* and the inhaled aminoglycoside used in conjunction with a systemic β-lactam. Aerosolized aminoglycosides were associated with improved clinical and microbiologic cure rates, with less nephrotoxicity.[481–483]

Intraabdominal Infections

Current guidelines on empirical therapy for intraabdominal infections of moderate or high severity do not recommend the combination of an aminoglycoside with metronidazole.[449] This judgment is based on both the toxic potential of the aminoglycosides and the availability of equally efficacious regimens. Two meta-analyses of more than 5000 patients (including more than 3000 enrolled in randomized controlled trials) demonstrated clinical inferiority of aminoglycoside therapy (usually as clindamycin-gentamicin combination) to its comparator, β-lactam, for intraabdominal infections. Nephrotoxicity was seen more often with aminoglycoside therapy, but overall toxicity was equivalent, as were all-cause mortality and mortality attributable to infection.[484] Alternative first-choice regimens include a β-lactam–β-lactamase inhibitor combination (e.g., piperacillin-tazobactam) plus a fluoroquinolone or monotherapy with a carbapenem.

Urinary Tract Infections

A systemic review and meta-analysis of randomized controlled trials in nearly 2500 patients enrolled in 26 trials showed that aminoglycoside monotherapy was equally effective as comparators in terms of all-cause mortality and treatment failure.[446] Few trials enrolled patients with sepsis. A higher rate of bacteriologic failure and nephrotoxicity was observed with aminoglycosides. The duration of therapy was not recorded in the report, so it is unclear if 5 to 7 days of therapy would provide equivalent clinical efficacy to longer courses, given the presence of therapeutic aminoglycoside levels for most pathogens in urine for 72 hours or longer after a single dose. Intravesicular gentamicin has been investigated, with anecdotal success, for recurrent urinary tract infections in intermittently catheterized patients.[485]

Cystic Fibrosis

Patients with CF demonstrate altered aminoglycoside pharmacokinetics requiring much larger doses of drug to achieve therapeutic serum levels.[486,487] The frequency of nephrotoxicity in patients with CF is less than that in patients without CF.[488,489] The prevalence of hearing loss after aminoglycoside therapy in adult patients (18–37 years of age) with CF is 17%, which is roughly the same as the rate in patients without CF. However, the risk per course of treatment is 2% in patients with CF compared with 7.5% in patients without CF.[490] It is unclear how CF might protect against aminoglycoside cochlear toxicity.

The Cochrane Database of Systematic Reviews provides updated, periodic critical evaluation of the use of aminoglycosides and other antiinfectives in the treatment of pneumonia in patients with CF. Examples include single versus combination intravenous antibiotic

[e]References 4, 5, 347, 374, 375, 445–458.

therapy, nebulized antipseudomonal antibiotics, once-daily aminoglycoside therapy, and elective versus symptomatic antibiotic therapy.[221,491–495]

In 10 placebo-controlled trials of nebulized antipseudomonal antibiotics that included 758 patients,[221] patients receiving treatment had a 12% increase in forced expiratory volume in 1 second and a reduced odds ratio (0.69; 95% confidence interval, 0.5–0.96) of need for hospitalization. Over time, the incidence of aminoglycoside-resistant *P. aeruginosa* was greater in the treatment group, although one study demonstrated safety and tolerability after 8 weeks.[223]

Within 2 hours after the dose, sputum concentrations fall to approximately 14% of the levels found at 10 minutes after inhalation.[220] Absorption into serum is low. The average serum drug concentration 1 hour after inhalation was 1.0 μg/mL (range, 0.2–3.0 μg/mL).[222] Ototoxicity has not been reported, but transient tinnitus occurred in a few individuals during clinical trials. Nephrotoxicity has not been observed. Aerosol therapy presents advantages of higher local and less systemic exposure, self-administration at home, and improvement in lung function with a reduced burden of *P. aeruginosa*.

PROPHYLAXIS

Clinical practice guidelines for antimicrobial prophylaxis in surgery[496] suggest gentamicin or tobramycin, 5 mg/kg given intravenously, as an alternative in patients with β-lactam allergy in genitourinary and gastrointestinal procedures. For patients with valvular heart disease, prophylaxis is no longer recommended solely to prevent endocarditis. For patients with a known or possible enterococcal urinary tract infection, it is reasonable to include drugs with antienterococcal activity in the perioperative regimen for gastrointestinal or genitourinary procedures.[497]

The risk of infection after elective colorectal procedures was significantly reduced by mechanical cleansing of the bowel plus oral administration of, usually, neomycin and erythromycin or metronidazole in addition to standard intravenous antibiotic prophylaxis in controlled trials.[496,498] A controlled trial demonstrated that an oral selective digestive decontamination containing gentamicin effectively eradicated carbapenem-resistant *Klebsiella pneumoniae* gastrointestinal carriage[499] and may be used in nosocomial outbreaks.[500] However, the safety and efficacy of topical gentamicin in cardiac surgery have not been clearly established.[496,501] Multiple tunneled-catheter antibiotic-lock studies including a prospective randomized trial[502] have demonstrated reduced catheter-related infections, but emergence of resistant pathogens remains a concern.[503]

Spectinomycin and Gonorrhea

Spectinomycin has been not available in the United States since 2006 but may be available in other countries. It was previously recommended for pregnant women, patients in areas with a high prevalence of fluoroquinolone resistance, and men who have sex with men. It showed 98% efficacy in uncomplicated urogenital and anogenital gonococcal infections but only 52% efficacy in gonococcal infections involving the pharynx, where it does not reach therapeutic concentrations.[452] It is not effective in the treatment of infections with *Treponema pallidum* or *Chlamydia trachomatis*. The drug is neither nephrotoxic nor ototoxic. It is an alternative therapy for patients who are allergic to β-lactams and for patients who are infected with resistant strains of gonococci. No intravenous form of the drug is available. Alternatively, gentamicin has been used to treat urogenital gonococcal infection.[504]

Aminoglycosides in Orthopedic Surgery

Antibiotic-impregnated cement is used with increasing frequency in primary hip and knee arthroplasties as well as revision procedures of infected total joint arthroplasties,[496] for which the FDA has approved premixed aminoglycoside in bone cement products. Incorporation of larger amounts of antibiotic, usually gentamicin or tobramycin, allows release of higher drug concentrations but may adversely affect mechanical properties. Concentrations and properties vary among producers.[505–510] Persistence of bacterial growth as adherent biofilms remains a potential problem,[444,509,511] and nephrotoxicity has been reported.[512] To date, prophylactic use in primary joint arthroplasty appears to be favorable, but a multicenter evaluation failed to show reduction in the risk of infection,[513] with an increased risk of nephrotoxicity.[514] A review of 20 mostly uncontrolled reports of antibiotic-containing spacers did not allow evaluation of whether such adjunctive therapy provided additional benefits to systemic antibiotic therapy.[515] Prospective trials in aminoglycoside-containing beads for established osteomyelitis and prosthetic joint–associated infections are needed.[516]

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The complete reference list is available online at Expert Consult.

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26 Tetracyclines, Glycylcyclines, and Chloramphenicol

Matthew Moffa and Itzhak Brook

SHORT VIEW SUMMARY

DOXYCYCLINE
- The usual adult dose is 100 mg PO or IV every 12 hours.
- For patients with renal or hepatic failure, no dose adjustment is required.
- Cerebrospinal fluid (CSF) penetration is low.
- Common adverse effects include gastrointestinal upset, photosensitivity, rash, *Candida* vaginitis, and dental staining in children.
- Doxycycline is contraindicated in children and in pregnant or breastfeeding patients.
- There are no important drug-drug interactions.
- Indications are as follows:
  - Used to treat rickettsioses, scrub typhus, ehrlichiosis, anaplasmosis, psittacosis, actinomycosis, *Mycoplasma pneumoniae, Chlamydia pneumoniae,* Lyme, syphilis, rat bite fever, *Chlamydia trachomatis* (including cervicitis, urethritis, lymphogranuloma venereum, and trachoma), Whipple disease, community-associated methicillin-resistant *Staphylococcus aureus*
  - Used in combination for brucellosis, tularemia, malaria, *Helicobacter pylori,* some rapid-growing mycobacteria, some filaria *(Wolbachia), Vibrio vulnificus*
  - Used for prophylaxis against leptospirosis, malaria, infections caused by tick bite (Lyme), and bioterrorism agents
  - Exposure to anthrax, tularemia, plague, Q fever, or brucellosis

TETRACYCLINE
- The usual adult dose is 250 to 500 mg PO every 6 hours.
- In patients with renal failure, reduce the dose.
- Contraindications, adverse effects, and indications are the same as for doxycycline.

MINOCYCLINE
- The usual adult dose is 200 mg PO or IV, then 100 mg every 12 hours.
- In patients with renal or hepatic failure, no dose adjustment is necessary.
- CSF penetration is low.
- Common adverse effects are similar to those with doxycycline, but with more vertigo.
- With regard to indications, doxycycline usually preferred, although minocycline is preferred for nocardiosis.

TIGECYCLINE (A GLYCYLCYCLINE)
- The usual adult dose is a loading dose of 100 mg, then 50 mg IV every 12 hours.
- In patients with renal failure, no dose adjustment is required.
- In patients with severe hepatic failure (Child-Pugh C), the dose is 100 mg loading, then 25 mg every 12 hours.
- CSF penetration is low.
- Common adverse effects are similar to those with doxycycline, except that nausea and vomiting are more frequent. Other effects include anorexia, dry mouth, and dysgeusia. There is a small increase in mortality across all clinical trials; the US Food and Drug Administration (FDA) has issued a boxed warning.
- Indications include complicated skin and skin structure infections, complicated intraabdominal infections, community-acquired bacterial pneumonia, and off-label use in combination with other drugs for infections with susceptible multidrug-resistant, gram-negative bacilli.

CHLORAMPHENICOL
- The usual adult dose is 50 mg/kg/day, divided into 6-hour doses. Chloramphenicol is available only in intravenous formulation in the United States.
- In patients with renal failure, no dose adjustment is required. Decrease dose or avoid in patients with hepatic failure.
- CSF penetration is moderate.
- Adverse effects include neutropenia, aplastic anemia, optic neuritis, gray baby syndrome (circulatory collapse in infants), and rash.
- There are no indications for use; this agent has been replaced by safer drugs.

TETRACYCLINES

Historical Overview and Classification

Tetracyclines have been an important class of broad-spectrum antibiotics since the discovery of chlortetracycline in 1948 by mycologist Benjamin M. Duggar.[1] They are bacteriostatic with a wide range of activity, including gram-positive bacteria, gram-negative bacteria, intracellular organisms, and protozoan parasites. Duggar derived chlortetracycline from *Streptomyces aureofaciens,* a golden-yellow bacterium found in soil. In 1950, oxytetracycline was isolated from *Streptomyces rimosus.* Tetracycline was later prepared by the catalytic dehalogenation of chlortetracycline in 1953 at Lederle Laboratories, and was independently derived from oxytetracycline at Pfizer Laboratories during that same time period.[2,3] Doxycycline is a semisynthetic derivative of oxytetracycline and became available in 1967. Minocycline, also derived semisynthetically, was derived in 1972.

Shortly after the discovery of tetracyclines, resistance developed, in large part because of their extensive clinical and nonclinical uses, including as growth promoters in animal feeds.[4,5] This widespread use has selected for a large number of resistant determinants collectively termed the *tetracycline resistome.*[6] This resistance led to a period of time when tetracyclines were replaced by newer antibiotics, such as the fluoroquinolones in the 1970s and 1980s. Like every other antibiotic, the fluoroquinolones would also fall to the selective pressure of antimicrobial resistance, leading to the development of newer, semisynthetic tetracyclines called *glycylcyclines.* In 2005, tigecycline became the first glycylcycline approved by the US Food and Drug Administration (FDA) for treatment of complicated skin and soft tissue infections and complicated intraabdominal infections (cIAIs). Later, in 2009, it was approved for use in community-acquired pneumonia (CAP).

Tetracyclines as a class are commonly divided according to two distinct classification methods: duration of action or year of discovery (Table 26.1, Fig. 26.1). Short-acting tetracyclines include the first-generation oxytetracycline and tetracycline. Intermediate-acting tetracyclines include another first-generation member, demeclocycline. Of note, demeclocycline is rarely used for infections. Its main side effect is nephrogenic diabetes insipidus. Thus it has found its niche in treatment of hyponatremia in the setting of the syndrome of inappropriate antidiuretic hormone secretion, first reported in the 1970s.[7] Long-acting tetracyclines include the second-generation agents doxycycline and minocycline and the third-generation glycylcycline tigecycline.

Structure and Mechanism of Action

Tetracyclines all share a four-benzene ring as their core structure, with a hydronaphthacene nucleus (see Fig. 26.1). Variations in gastrointestinal

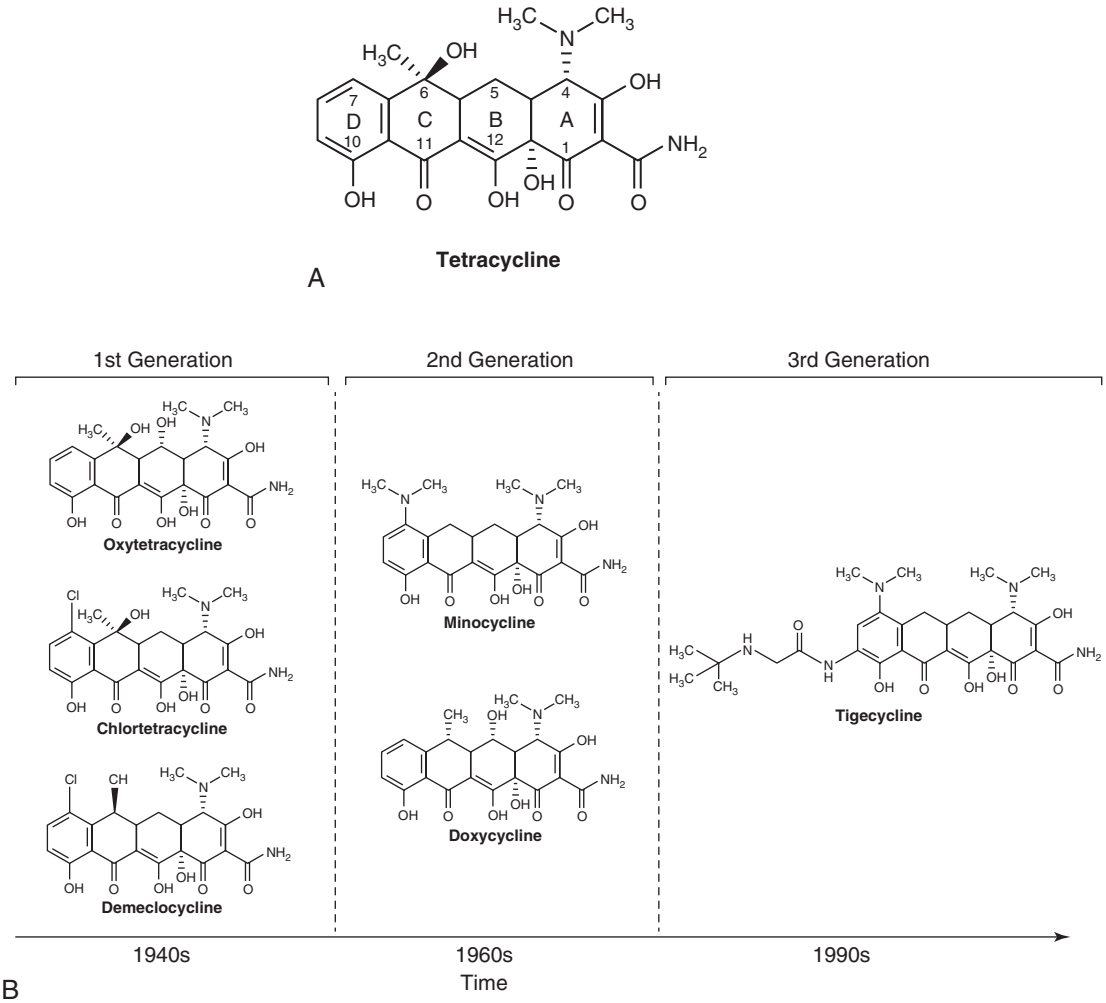

FIG. 26.1 **Chemical structures of various tetracycline antibiotics.** (A) Structure of tetracycline. (B) Advances in new-generation tetracycline development over a period of time. *(From Thaker M, Spanogiannopoulos P, Wright GD. The tetracycline resistome.* Cell Mol Life Sci. *2010;67:419–431.)*

TABLE 26.1 Tetracycline Formulations Currently Available in the United States

GENERIC NAME	MAJOR BRAND NAME (COMPANY)	PREPARATION	USUAL ADULT ORAL DOSE	NOTES
Short-Acting				
Oxytetracycline	Terramycin (Pfizer)	Capsules: 125, 250 mg Syrup: 125 mg/5 mL	500 mg q6h	No longer widely used
Tetracycline HCl	—	Capsules: 250, 500 mg Syrup: 125 mg/5 mL	500 mg q6h	
Intermediate-Acting				
Demeclocycline HCl	Declomycin (Wyeth)	Tablets: 150, 300 mg	150 mg q6h or 300 mg q12h	Used for syndrome of inappropriate antidiuretic hormone secretion
Long-Acting[b]				
Doxycycline	Vibramycin (Pfizer)	Capsules (hyclate): 50, 100 mg Tablets: 50, 100 mg Syrup (calcium): 50 mg/5 mL Syrup (monohydrate): 25 mg/5 mL	100 mg q12h	
Minocycline	Minocin (Wyeth) Solodyn ER tablets (Medicis)	Capsules and tablets: 50, 100 mg Suspension: 50 mg/5 mL Extended-release tablets 45, 90, 135 mg	200 mg, then 100 mg q12h 1 mg/kg/day	
Long-Acting, Third-Generation				
Tigecycline	Tygacil (Wyeth)	Vial: 50 mg lyophilized (reconstituted to 10 mg/mL)	100 mg, then 50 mg q12h	IV

[a]Other brands are available for some of the analogues.
[b]Long-acting agents exist in intravenous preparations and can be given at the same doses recommended for oral therapy (doxycycline 100-mg vial, 200-mg vial; minocycline, 100-mg vial).

absorption, affinity for multivalent cations, protein binding, and antimicrobial activity can be achieved with substitutions at carbons 5, 6, and 7 of the four-ring, six-membered carbocyclic structure, leading to variations in pharmacokinetic properties.[3]

Tetracyclines work by inhibiting bacterial protein synthesis. This is accomplished primarily by reversibly binding to the 30S ribosomal subunit of the bacteria. This inhibits the enzyme binding of aminoacyl-tRNA to the adjacent ribosomal acceptor site, which in turn prevents peptide chain elongation and inhibits protein synthesis.[2] Because the binding of tetracyclines to the 30S ribosomal subunit is reversible, it is postulated that this is the explanation as to why they exhibit bacteriostatic properties. For tetracyclines to get to the 30S ribosomal subunit, they need to be able to penetrate cell walls, which is accomplished through passive diffusion. With gram-negative organisms, tetracyclines become positively charged cation complexes, presumably with magnesium. They then use OmpF and OmpC porin channels to cross the outer membrane. After entering the periplasmic space, tetracycline dissociates, resulting in an accumulation of uncharged tetracycline. With gram-positive organisms, tetracyclines penetrate through the inner cytoplasmic membrane via an active transport system that depends on the ΔpH.[3,8] Tetracyclines also reduce bacterial pathogenicity by suppressing adhesion of bacteria to human cells. This is likely achieved through inhibition of the synthesis of a specific protein in the bacterial cell surface.[9]

Doxycycline displays additional protein synthesis inhibition in mitochondria through binding of the 70S ribosomes.[10] This enables it to exhibit activity against various protozoa because they contain mitochondria. Doxycycline also targets parasites via the apicoplast ribosomal subunits in *Plasmodium falciparum.* This occurs late in the malarial cell cycle, resulting in the slow antimalarial effect of doxycycline.[10,11]

Pharmacology

Administration and Dosing

Tetracycline

Tetracycline HCl comes in 250-mg and 500-mg capsules and in 125 mg/5 mL syrup. The usual adult oral dose is 250 mg every 6 hours or 500 mg every 6 hours for more serious infections. Larger doses do not provide additional benefit, and the excess drug is excreted in feces. Intravenous preparations of tetracycline are no longer used owing to their potential hepatotoxicity. Acute fatty liver has occurred with large intravenous doses, particularly during pregnancy. Tetracycline is labeled category D and is to be avoided in pregnancy. It should also be avoided in children, particularly those younger than 8 years during the period of tooth development in order to avoid permanent discoloration. Children are also at risk for retardation of bone development.

With renal impairment, tetracycline should be avoided because it can cause further deterioration of kidney function and the drug tends to accumulate in the serum.[12] Tetracycline is slowly removed by hemodialysis, but not very well by peritoneal dialysis.[13] Caution is advised in administering tetracycline in patients with hepatic impairment because this may result in liver damage.

Doxycycline

Doxycycline comes in various dose forms, but most typically in both 50-mg and 100-mg capsules and tablets. It is also available as 25 mg/5 mL and 50 mg/5 mL syrup. The usual adult dose is 100 mg every 12 hours and should be taken with at least 100 mL of water. For malaria chemoprophylaxis, the adult dose is 100 mg daily. Occasionally an intravenous preparation of doxycycline is required for certain cases of rickettsial infections, ehrlichiosis, or severe psittacosis. The usual adult dose is 200 mg followed by 100 mg every 12 hours. The dose should be given over 30 to 60 minutes and should be dissolved in 500 to 1000 mL of glucose or saline.[10] Like tetracycline, doxycycline is also pregnancy category D and should be avoided in children. If the benefits of doxycycline outweigh the risks in children, the pediatric dose is 2.2 mg/kg every 12 hours.

Unlike tetracycline, doxycycline is safe to use with renal impairment. Urinary excretion is reduced in renal impairment, but doxycycline does not accumulate in the serum because its gastrointestinal excretion increases.[14] During hemodialysis, only about 10% of the drug is removed.[15] With hepatic impairment, there are few pharmacokinetic data. Unlike tetracycline and minocycline, doxycycline does not appear to cause hepatitis.[16]

Minocycline

Like tetracycline and doxycycline, minocycline is primarily administered orally. It comes in 50-mg, 75-mg, and 100-mg capsules and tablets, and a 50 mg/5 mL suspension. There are also 45-mg, 90-mg, and 135-mg extended-release tablets. The usual adult dose is 200 mg followed by 100 mg every 12 hours. The dose is the same if minocycline is given intravenously. Each dose should be given over 30 to 60 minutes and must be dissolved in 500 to 1000 mL of glucose or saline.[17] Like tetracycline and doxycycline, minocycline is pregnancy category D and should also be avoided in children. If minocycline needs to be given to children, the pediatric dose is 4 mg/kg followed by 2 mg/kg every 12 hours.

With impaired renal function, various studies have shown contrasting results. Minocycline does not accumulate in the serum in patients with renal failure, and excretion in such patients is not significantly reduced.[18,19] This is because only a small amount of drug is eliminated in the urine. Other reports have found that minocycline can exacerbate preexisting renal insufficiency and can possess a prolonged serum half-life, which is directly related to the severity of any renal insufficiency.[20,21] With hemodialysis and peritoneal dialysis, minocycline pharmacokinetics are not significantly altered.[18,22] In cirrhotic patients, no changes for minocycline are observed.[22]

Absorption and Bioavailability

The absorption of the tetracyclines occurs primarily in the stomach and proximal small bowel. Tetracycline has a bioavailability of 77% to 88%, and maximum serum concentrations are reached 2 to 4 hours after standard doses with a serum half-life of 7 hours.[2,23] Doxycycline is almost completely absorbed in the duodenum and has a prolonged serum half-life of 12 to 16 hours, with peak serum levels usually achieved 2 to 3 hours after administration.[10] Minocycline is almost completely absorbed in the stomach, duodenum, and jejunum. It peaks in serum after 2 hours and also has a prolonged half-life of 16 hours after the first dose, and up to 21 hours after repeated doses.[17,20,24]

Doxycycline and minocycline absorption are not significantly altered by the administration of food, with levels decreased by less than 20%.[25,26] In contrast, tetracycline absorption decreases by approximately 50% when administered with food.[26] Multivalent cations (such as aluminum, calcium, iron, and magnesium) chelate with the tetracyclines, resulting in a decrease in drug absorption by 50% to 90%. An interval of 3 hours between the ingestion of tetracyclines and cations prevents this interaction.[27]

Drug Distribution

The tetracyclines penetrate well into various body fluids and tissues. Protein binding varies among drugs. Doxycycline is the highest protein-bound drug, estimated between 82% and 93%, followed by minocycline at 70% to 80% and tetracycline at 24% to 65%.[3,13,17,28] Tissue and fluid penetration differs on the basis of how lipid soluble each compound is. Doxycycline has been reported to be 5 times as lipophilic as tetracycline, and minocycline is 10 times more lipophilic than tetracycline.[29,30]

Serum concentrations of tetracycline rise slowly after oral administration, and C_{max}, depending on the dose, is generally in the range of 1 to 5 μg/mL.[28] Tetracycline penetrates readily into pleural, ascitic, and synovial fluids and placental-cord serum.[2] In maxillary sinus secretions, tetracycline concentrations can nearly equal serum concentrations with repeated administration.[31] However, tetracycline can be detected in only low concentrations in saliva and tears. Sputum levels of tetracycline in the range of 0.4 to 2.6 μg/mL have been detected after oral administration of 250 mg every 8 hours.[32] Cerebrospinal fluid (CSF) levels of tetracycline are about 10% of the simultaneous serum concentration.[2] Tetracycline penetrates readily into breast milk but chelates with milk, thus lowering its bioavailability.[33]

The serum C_{max} of doxycycline peaks at around 1.7 to 2 μg/mL after oral administration of 100 mg.[28] With oral administration of 200 mg,

this increases to 2.6 to 5.9 μg/mL. Doxycycline concentrates in the bile at 10 to 25 times greater levels than in the serum.[34] In thoracic duct lymph and peritoneal fluid, doxycycline concentrations are about 75% of serum levels.[35] In the prostate, doxycycline concentrations are up to 60% of serum levels.[36] After an intravenous dose of 200 mg was given to patients with pleurisy, pleural fluid levels of doxycycline were 25% of serum levels after 2 hours.[37] Blister fluid model studies have shown that interstitial fluid concentrations of doxycycline are 54% of serum levels.[38] Lower levels of doxycycline have been noted in sputum (8%–25%), and in bone (2%–6% orthopedic, 86% mandible), skin, subcutaneous fat, and tendon tissue.[39–42] In the CSF, doxycycline penetration has been studied in both neuroborreliosis and neurosyphilis. In patients with Lyme borreliosis treated with doxycycline 200 mg orally every 12 hours, CSF penetration was noted to be 15% with a concentration of 1.1 μg/mL. At a dose of 100 mg orally every 12 hours, the CSF concentration was only 0.6 μg/mL, suggesting that treatment should be with the higher dose.[43] In five patients with latent syphilis or neurosyphilis receiving 200 mg orally every 12 hours, doxycycline CSF penetration was 26% with a concentration of 1.3 μg/mL, suggesting doxycycline as an alternative to penicillin.[44] Like tetracycline, doxycycline penetrates readily into breast milk, with levels of up to 40% of plasma levels.[33] Because it is less bound to calcium than the other tetracyclines, an infant being breastfed is at greater risk of side effects.

After a single 200-mg dose, minocycline achieves a C_{max} of 3 to 3.6 μg/mL.[28] Because minocycline is the most lipophilic of the tetracyclines, it penetrates the most readily into tissues and bacterial cells.[45] The highest concentrations are found in the thyroid, lung, gastrointestinal tract, liver, gallbladder, and bile. Other tissues with concentrations higher then serum levels are the prostate, uterus, ovaries, fallopian tubes, breast, skin, tonsils, maxillary sinuses, and eyes.[17,22,30,46,47] Minocycline penetrates into CSF better than the other tetracyclines but is able to achieve only low levels.[18,22,30] Minocycline can gain higher concentrations in saliva than other tetracyclines, likely explaining why it is effective at eliminating meningococcal carriage.[48] As with tetracycline, bioavailability in breast milk is low because of chelation with milk.[33]

Drug Elimination

The tetracyclines are each eliminated slightly differently. Tetracycline is eliminated primarily in the kidneys, and about 30% to 60% of an oral dose is excreted in the urine.[2,3] The drug accumulates with renal insufficiency, which is why it should be avoided in such patients. About 20% to 60% is eliminated in feces.[2] With doxycycline, usually less than 30% of an oral dose is renally excreted.[3,49,50] However, unlike tetracycline, fecal excretion is increased in the setting of renal impairment and prevents accumulations of the drug. As much as 90% of doxycycline can be excreted in feces. Minocycline is extensively metabolized in the liver to produce at least six inactive metabolites. Only about 4% to 19% is eliminated by the kidneys, and 20% to 34% is excreted in the feces.[19,22,30,51]

Antimicrobial Activity

The tetracyclines as a class have a wide spectrum of antimicrobial activity. Similar susceptibility patterns are displayed among the nonglycylcycline members. They have activity against many aerobic and anaerobic gram-positive and gram-negative bacteria, in addition to intracellular organisms and protozoan parasites. Because they are bacteriostatic and many organisms have developed resistance over the years, they have been replaced by other antibiotics as first-line therapy in the majority of cases. When interpreting minimal inhibitory concentration (MIC) results, tetracycline is generally used as the class representative. However, there may be times when specific testing for minocycline or doxycycline is warranted because some organisms may still be susceptible even when resistant or with intermediate resistance to tetracycline.

Most bacterial organisms, including Enterobacteriaceae, non–lactose-fermenting gram-negative bacilli, *Staphylococcus* spp., *Enterococcus* spp., and anaerobes, are considered susceptible to tetracycline when the MIC is 4 μg/mL or less. At 8 μg/mL, tetracycline is considered intermediately active. Resistance is defined as 16 μg/mL or greater. *Haemophilus* spp., β-hemolytic streptococci, and viridans-group streptococci have lower MIC breakpoints, with susceptible, intermediate, and resistant set at 2 μg/mL or less, 4 μg/mL, and 8 μg/mL or more, respectively. Even lower is *Neisseria gonorrhoeae,* with susceptible, intermediate, and resistant set at 0.25 μg/mL, 0.5 to 1 μg/mL, and 2 μg/mL or more, respectively. *Streptococcus pneumoniae* has susceptible, intermediate, and resistant breakpoints of 1 μg/mL or less, 2 μg/mL, and 4 μg/mL or more for tetracycline, and 0.25 μg/mL or less, 0.5 μg/mL, and 1 μg/mL or more for doxycycline, respectively. The susceptibility breakpoint of minocycline for *Neisseria meningitidis* is 2 μg/mL or less.[52]

Gram-Positive Bacteria

Tetracyclines possess broad activity against gram-positive organisms, including community-acquired *Staphylococcus aureus* and *S. pneumoniae.* Data from the SENTRY Antimicrobial Surveillance Program from 1997 through 1999 evaluated the susceptibilities of 15,439 patients infected with *S. aureus* and 6350 infected with coagulase-negative staphylococci (CoNS).[53] Samples were taken from various parts of the globe. In the United States, 94.2% of methicillin-susceptible *S. aureus* (MSSA) strains and 83.7% of methicillin-resistant *S. aureus* (MRSA) strains were susceptible to tetracycline. Results were similar in isolates from Canada, whereas those obtained from Latin America, Europe, and the Western Pacific had much lower MRSA susceptibilities to tetracycline, 17.8% to 41.4%. In the United States, CoNS were susceptible to tetracycline in 82.9% of the oxacillin-sensitive strains and 79.6% of the oxacillin-resistant strains. These findings were similar to those in the other regions.[53] More recent studies have also shown that the majority (80%) of MRSA isolates were susceptible to doxycycline and minocycline.[54]

Reports of *S. pneumoniae* resistance to doxycycline range geographically from 2% to more than 20%, limiting the use of this drug for severe pneumococcal infections.[10,55–56,57] Resistance may be more than 60% in penicillin-resistant strains.[57] Data on β-hemolytic streptococci are lacking. Swedish studies have shown doxycycline resistance rates of 15% for *Streptococcus pyogenes* and 68% for group B streptococcal isolates.[58,59] Among *Enterococcus* spp., surveillance data from 1997 through 1999 observed resistance rates approaching 50% in North America, 60% in Europe, and 60% to 70% in the Asia-Pacific region.[60] *Actinomyces israelii* has shown susceptibility to tetracycline, and *Listeria monocytogenes* and *Bacillus anthracis* to both tetracycline and doxycycline.[61–64]

Gram-Negative Bacteria

As a class, tetracyclines also have a broad range of activity against gram-negative organisms, although they are infrequently used against Enterobacteriaceae. Doxycycline has shown good activity against *Yersinia pestis* and has more activity than tetracycline.[65,66] Among *Campylobacter jejuni* and *Campylobacter coli* strains, isolates that have resistance to ciprofloxacin tend to also be resistant to doxycycline.[67] Tetracyclines are useful in the treatment of *Vibrio* spp. in a wide range of clinical conditions from food-borne gastroenteritis to necrotizing skin and soft tissue infections.[68–70]

In a Spanish study with *Acinetobacter baumannii,* doxycycline had activity against only 30% to 40% of strains tested, whereas minocycline was active against 70% and tigecycline was active against 76%.[71] Doxycycline has excellent activity against *Burkholderia pseudomallei* with susceptibility rates above 96%, along with low rates of resistance developing during treatment.[72] *Stenotrophomonas maltophilia* is highly susceptible to doxycycline,[69,73] which also exhibits activity against *Aeromonas hydrophila*.[70,74] In a global collection of clinical isolates (*A. baumannii, S. maltophilia,* and *Burkholderia cepacia*), minocycline demonstrated the highest activity of the tetracyclines.[75] Anaerobes in the gastrointestinal tract, including the *Bacteroides fragilis* group, have shown susceptibility to doxycycline, but with higher MIC breakpoints.[76] *Brucella* spp. and *Bartonella* spp. are also highly susceptible to doxycycline, with low MIC values.[77,78]

Atypical Bacteria

Among the *Mycoplasma* spp., tetracyclines are highly active against *Mycoplasma pneumoniae*. *Mycoplasma hominis* is usually susceptible, whereas *Mycoplasma genitalium* is often doxycycline resistant.[79–81] *Legionella pneumophila* is susceptible to doxycycline in vitro, but this is dependent on the inoculum size because *Legionella* is intermediately susceptible to doxycycline with larger inocula.[82] *Chlamydia pneumoniae* and *Chlamydia psittaci* have also been shown to be susceptible to

doxycycline.[83,84,85] *Chlamydia trachomatis* is typically susceptible to the tetracyclines, although susceptibility testing is not standardized.[84,86]

Spirochetes and Rickettsiae

The tetracyclines play a prominent role in the treatment of spirochetes. Doxycycline is the mainstay treatment of Lyme disease caused by *Borrelia burgdorferi.*[87] In a recent study looking at doxycycline, tetracycline, and tigecycline activity against *B. burdorferi,* tigecycline had the lowest MIC_{90} (≤0.016 mg/L) versus 0.25 mg/L for both doxycycline and tetracycline.[88] For *Leptospira* spp., doxycycline has also been a mainstay of treatment.[89] Tetracycline has been shown to have activity against *Treponema pallidum.*[90] Among *Rickettsia* spp., tetracyclines display activity against the spotted fever group, scrub typhus, and epidemic typhus. Doxycycline has been shown to be the most active antimicrobial agent.[91,92] Doxycycline has also shown to be highly active against *Anaplasma phagocytophilum, Ehrlichia chaffeensis,* and *Ehrlichia canis.*[93]

Mycobacteria and Nocardia

Among mycobacteria, tetracyclines have activity among different species. Against the rapidly growing bacteria, doxycycline has been reported to have activity against 41% to 56% of *Mycobacterium fortuitum* strains. Less activity is seen against *Mycobacterium chelonae* (8%–26%) and *Mycobacterium abscessus* (4%–8%).[94–96] Similar results are seen with minocycline, although minocycline appears to be more active against *M. chelonae* and *M. fortuitum*. Minocycline also appears to have more activity against *Mycobacterium marinum* than doxycycline and has activity against *Mycobacterium kansasii* as opposed to doxycycline.[96–98] All of the tetracyclines show poor in vitro activity against *Mycobacterium avium* complex.[96] More recently, interest in using doxycycline against *Mycobacterium tuberculosis* has developed. A Russian study that evaluated second-line agents with activity against multidrug-resistant (MDR) *M. tuberculosis* found doxycycline to be active against 92.6% of the 68 isolates tested.[99] Doxycycline has also been shown to decrease matrix metalloproteinase activity in a cellular model and to suppress mycobacterial growth in vitro and in guinea pigs.[100]

Among the *Nocardia asteroides* complex, minocycline appears to have the greatest activity against these and a large number of *Nocardia farcinica* isolates.[10,101] Minocycline has also been shown to be more active than tigecycline, with an MIC_{90} of 2 μg/mL.[102]

Parasites

Tetracyclines are effective but slow-acting antimalarial drugs. They have been shown to specifically block expression of the apicoplast genome in cultured *P. falciparum,* resulting in the distribution of nonfunctional apicoplasts into daughter merozoites.[11] The tetracyclines have also been shown to have activity against *Toxoplasma gondii, Giardia lamblia, Trichomonas vaginalis, Leishmania major,* and *Entamoeba histolytica.*[17,103–105]

Clinical Uses

General

Tetracyclines previously were widely used for an array of respiratory, gastrointestinal, and genitourinary tract infections. The widespread increase in resistance along with the availability of newer, more bactericidal drugs has decreased the number of diseases for which these agents are considered to be the drugs of choice. For treatment of most diseases, tetracycline and doxycycline are interchangeable. Often doxycycline is the preferred agent because it can be given just twice a day, has fewer gastrointestinal side effects, and can be taken with food or milk. For a full table of therapeutic indications for tetracyclines, see Table 26.2.

Respiratory Tract Infections

According to the 2007 CAP practice guidelines of the Infectious Diseases Society of America, doxycycline was given a weak level III recommendation as empirical monotherapy in the outpatient setting in

TABLE 26.2 Therapeutic Indications for Tetracyclines

MAJOR INDICATIONS	SECOND-LINE TREATMENT	PROPHYLAXIS
Anaplasma (formerly *Ehrlichia*) *phagocytophilum* infection	*Acinetobacter baumannii* infections (sensitive strains)	Anthrax *(Bacillus anthracis)*
Anthrax inhalation, cutaneous postexposure	Acne	Colonic surgery
Bacillary angiomatosis in patients with human immunodeficiency virus infection (*Bartonella henselae* and *Bartonella quintana*)	*Actinomyces israelii* when penicillin is contraindicated	Lyme disease, after tick bite
Balantidium coli infection (infectious colitis)	Acute intestinal amebiasis (as adjunct therapy)	Malaria
Bartonellosis: Oroya fever and verruga peruana *(Bartonella bacilliformis)*	*Burkholderia mallei* (glanders) infections (only in combination with other drugs)	Plague *(Yersinia pestis)*
Brucellosis (plus rifampin, streptomycin, or gentamicin)	*Burkholderia pseudomallei* (melioidosis; only in combination with other drugs)	Traveler's diarrhea
Cat-scratch fever *(B. henselae)*	*Campylobacter fetus* infections	Treatment of asymptomatic carriers of *Neisseria meningitidis* to eliminate meningococci from the nasopharynx (minocycline)
Cervicitis due to *Chlamydia trachomatis*	*Clostridium* infections when penicillin is contraindicated	Tularemia *(Francisella tularensis)*
Cholera	*Dientamoeba fragilis*	
Community-acquired pneumonia	*Eikenella corrodens* infection	
Ehrlichia chaffeensis infection	Filariasis	
Granuloma inguinale *(Klebsiella granulomatis)*	Leptospirosis if penicillin contraindicated	
Inclusion conjunctivitis *(C. trachomatis)*	*Mycobacterium leprae* (only minocycline is active)	
Lyme disease *(Borrelia burgdorferi)*	*Nocardia* (minocycline)	
Lymphogranuloma venereum *(C. trachomatis)*	*Pasteurella multocida*	
Mycobacterial infections caused by *Mycobacterium marinum* and some isolates of *Mycobacterium fortuitum* and *Mycobacterium chelonae*	Rat-bite fever *(Spirillum minus, Streptobacillus moniliformis)*	
Nongonococcal urethritis	Syphilis, primary and secondary (patients with penicillin allergy; *Treponema pallidum*)	
Pelvic inflammatory disease	Vincent angina	
Peptic ulcer (with other agents; *Helicobacter pylori*)	Whipple disease *(Tropheryma whipplei)*	
Periodontitis	Yaws caused by *Treponema pertenue* when penicillin is contraindicated	
Pneumonia caused by *Chlamydia pneumoniae*		
Psittacosis and ornithosis *(Chlamydia psittaci)*		
Q fever *(Coxiella burnetii)*		
Relapsing fever, both louse- and tick-borne		
Rickettsial infections (spotted fever group and rickettsialpox)		
Scrub typhus *(Orientia tsutsugamushi)*		
Trachoma *(C. trachomatis)*		
Trench fever *(B. quintana)*		
Urethral infections caused by *C. trachomatis* or *Ureaplasma urealyticum*		
Vibrio vulnificus infection		

previously healthy patients with no risk factors for drug-resistant *S. pneumoniae* (DRSP) infection.[106] It may also be used as an alternative to a macrolide in combination with a β-lactam antibiotic for use in outpatients with comorbidities or who are at risk for DRSP, and for those treated in inpatient non–intensive care unit settings.[106] Doxycycline has good coverage of the atypical pathogens encountered, such as *M. pneumoniae, L. pneumophila*, *C. pneumoniae,* and *C. psittaci.* Increasing rates of resistance with *S. pneumoniae* and *Haemophilus influenzae* limit doxycycline's use as monotherapy. Higher doses of doxycycline, 200 mg every 12 hours for 72 hours followed by standard 100 mg every 12 hours, have been used to treat moderate-to-severe legionellosis.[107,108] Tetracycline has been shown to be as effective as erythromycin for treatment of *M. pneumoniae.*[109] The drug of choice for psittacosis caused by *C. psittaci* has been shown to be doxycycline; intravenous therapy may be required.[110–112]

Gastrointestinal Tract Infections

Tetracycline has been extensively studied in the treatment of cholera, a *Vibrio cholerae* infection. Oral tetracycline has been shown to be effective in decreasing the amount and duration of diarrhea from cholera, and in eradicating the pathogen from stool.[113] It had been useful for prophylaxis of close contacts during an outbreak.[114] Later studies showed that a single dose of 1 g of tetracycline was effective in treating cholera.[115,116] Doxycycline has been shown to be as effective as tetracycline with a 4-day course of each drug, although bacterial elimination took slightly longer.[117] However, tetracycline resistance is now common throughout the world, and thus these drugs are no longer considered appropriate therapy unless susceptibility has been demonstrated.[118]

Second-line therapy for *Helicobacter pylori* infection typically includes a quadruple drug-based regimen with tetracycline 500 mg every 6 hours along with metronidazole, bismuth salt, and a proton pump inhibitor for 7 to 14 days. This combination has led to eradication rates close to 100%.[119] When comparing triple- versus quadruple-based regimens, a meta-analysis showed equal efficacy when used as first-line therapy, with similar compliance and side-effect profiles.[120] Doxycycline-containing regimens with amoxicillin, bismuth salt, and omeprazole for 1 week have been studied as salvage regimens with a reported 91% eradication rate.[121]

Doxycycline at a dose of 100 mg daily for 3 weeks has been shown to be effective in preventing traveler's diarrhea in regions with doxycycline-susceptible enterotoxigenic strains of *Escherichia coli* (ETEC).[122] Newer data suggest that the addition of doxycycline to ceftriaxone offers protection against the development of *Clostridioides difficile* (formerly *Clostridium difficile*) infection (CDI). In a study of 2305 patients, the incidence of CDI was 1.67 cases per 10,000 patient-days in those who received doxycycline along with ceftriaxone, compared with 8.11 per 10,000 patient-days in those who did not receive doxycycline along with ceftriaxone. For each day of doxycycline receipt, the rate of CDI was 27% lower than in patients who did not receive doxycycline.[123] This apparent protective effect of doxycycline was also observed in a large case-control study from 1999 to 2005 that evaluated multiple antibiotics.[124]

Genitourinary Tract Infections

Doxycycline is a mainstay of treatment for nongonococcal urethritis. Along with a single 1-g dose of azithromycin, doxycycline 100 mg orally twice daily for 7 days is recommended for treatment of uncomplicated *C. trachomatis* genital infection in the Centers for Disease Control and Prevention's 2015 sexually transmitted diseases treatment guidelines.[125] Cure rates with doxycycline have been reported to be 98% as compared with 97% for azithromycin, with similar tolerability noted.[126] A more recent study of a closed population receiving directly observed treatment for urogenital chlamydia infection found that the efficacy of azithromycin was 97%, and the efficacy of doxycycline was 100%. Azithromycin did not establish noninferiority.[127] Even when suboptimal compliance with doxycycline is documented, cure rates have been noted to remain high at 94%.[128] Other causes of nongonococcal urethritis, such as *Ureaplasma urealyticum* and *M. hominis,* are responsive to the tetracyclines but with less consistency. Resistant strains of *U. urealyticum* have been reported.[129] *M. genitalium* has been shown to be a persistent and recurring cause of urethritis in those treated with doxycycline.[130,131] Doxycycline is no longer recommended for the treatment of gonorrhea owing to increasing *N. gonorrhoeae* resistance.[132,133] In a randomized controlled pilot study, daily doxycycline taken prophylactically was associated with a decreased incidence of *N. gonorrhoeae, C. trachomatis,* or syphilis incident infections among a core group of high-risk human immunodeficiency virus (HIV)–infected men who have sex with men.[134]

The tetracyclines are active against the sexually transmitted infection lymphogranuloma venereum caused by *C. trachomatis* serovars L1, L2, and L3.[135] Clinical manifestations include inguinal lymphadenopathy and proctitis. Recommended therapy is doxycycline 100 mg orally twice daily for 21 days.[125] Granuloma inguinale (donovanosis) caused by *Klebsiella granulomatis* causes painless genital ulcerative disease and regional lymphadenopathy. It is rarely found in the United States and is endemic in certain parts of India, Papua New Guinea, central Australia, and southern Africa. Recommended therapy is doxycycline 100 mg orally twice daily for at least 3 weeks and until all lesions have completely healed.[125] Because of *Haemophilus ducreyi* resistance, doxycycline is not recommended for chancroid.[125]

In pelvic inflammatory disease (PID), a combination of initial intravenous cefotetan or cefoxitin with oral doxycycline 100 mg every 12 hours for 14 days is recommended. For outpatient treatment of mild-to-moderate PID, an intramuscular cephalosporin combined with 14 days of doxycycline with or without metronidazole is recommended.[125] When inpatient versus outpatient therapy in mild-to-moderate PID were compared, there were no differences in pregnancy rates or recurrence of PID.[136]

Spirochetal Infections

The tetracyclines are often used to treat illnesses caused by spirochetal infections. Doxycycline (100 mg orally twice daily for 10 to 21 days), along with amoxicillin or cefuroxime, is recommended for treatment of adult patients with early localized or early disseminated Lyme disease, caused by *B. burgdorferi,* associated with erythema migrans in the absence of specific neurologic manifestations or advanced atrioventricular heart block.[137] This is an A1 recommendation by the Infectious Diseases Society of America. In areas where human granulocytic anaplasmosis (HGA) is prevalent, doxycycline is preferred because it will treat both Lyme disease and HGA. Lyme arthritis should be treated with a longer course of 1 month.[138] Long-term doxycycline therapy has not been shown to have additional beneficial effects on health-related quality of life in patients with persistent symptoms attributed to Lyme disease.[139] A randomized controlled study has shown that a single 200-mg dose of doxycycline is effective in preventing clinical Lyme disease in patients with an *Ixodes scapularis* tick bite if given within 72 hours.[140] Although the recommended treatment of neuroborreliosis is parenteral agents, doxycycline 200 mg orally twice daily appears reasonable and an alternative for patients with a significant penicillin allergy.[141] A single oral 100-mg dose of doxycycline or a 500-mg dose of tetracycline is effective in treating louse-borne relapsing fever caused by *Borrelia recurrentis.*[142]

Doxycycline can also be used in patients with a significant penicillin allergy with treponemal infections. In primary or secondary syphilis caused by *T. pallidum,* doxycycline 200 mg orally once daily for 14 to 28 days may be appropriate.[143] A regimen of 100 mg orally twice daily for 14 days in early syphilis was shown to be just as effective as penicillin in treating early syphilis.[144] In HIV-infected individuals, doxycycline also appears to be effective in early syphilis, with similar serologic response to that in patients given penicillin.[145] In latent syphilis and neurosyphilis, five patients treated with doxycycline 200 mg orally twice daily for 21 days showed good CSF drug levels of doxycycline.[44] Small case reports of HIV-infected individuals with neurosyphilis treated successfully with oral doxycycline 200 mg twice daily for 28 days have been documented but would need further studying.[146] Minocycline was studied in three penicillin-allergic patients with neurosyphilis, at a dose of 100 mg twice daily for 14 consecutive days per month for 9 months. Clinical improvement was noted within 1 month and there were no significant side effects.[147] Nonvenereal treponemal infections, such as yaws (caused by *T. pallidum* subspecies *pertenue*), bejel or endemic syphilis (caused by *T. pallidum* subspecies *endemicum*), and pinta (caused

by *Treponema carateum*), can also be treated with tetracyclines as an alternative to penicillin.[148]

Doxycycline was shown to be as effective as penicillin or cefotaxime in treating severe leptospirosis in a randomized trial in northern Thailand. There was no difference in mortality or time to defervescence among the different treatment groups.[149] It has also been used as chemoprophylaxis against leptospirosis in endemic areas, with varying results. A systematic review found that the regular use of weekly 200 mg oral doxycycline increases the odds for nausea and vomiting with unclear benefit in reducing *Leptospira* seroconversion or clinical consequences of infection.[150]

Malaria Treatment and Chemoprophylaxis

Doxycycline is used for both treatment and chemoprophylaxis of malaria. Both *P. falciparum* and *Plasmodium vivax* are susceptible to doxycycline. Along with atovaquone-proguanil and artemisinin compounds, the combination of quinine and doxycycline (or tetracycline) is recommended for treatment of chloroquine-resistant *P. falciparum* strains.[151] Doxycycline is less often used to treat *P. vivax* infections owing to its slower rate of parasitic clearance and limited activity in the liver stages compared with other regimens.[152]

As for chemoprophylaxis, doxycycline has been shown to be as effective as mefloquine in regions with chloroquine-resistant *P. falciparum.* In two studies, doxycycline was protective in 99% to 100% of participants, although mefloquine was better tolerated in one study.[153,154] Other studies have shown that malaria developed in those taking doxycycline prophylaxis who ended therapy shortly after leaving an endemic area, likely a result of the fact that doxycycline has limited effect on preerythrocytic liver stages of malaria.[155–158] On the basis of these studies, it is recommended that doxycycline be taken for 4 weeks after returning from an endemic area.[159]

Other Infections

Doxycycline is the preferred agent for treating rickettsial infections such as Rocky Mountain spotted fever (RMSF), Mediterranean spotted fever, epidemic louse-borne typhus, murine typhus, scrub typhus, and tick typhus.[92,160–163] Regimens are usually between 5 and 15 days long.[92] However, shorter courses of doxycycline may also be appropriate. A randomized controlled trial of 43 patients with Mediterranean spotted fever showed that a 2-day course of doxycycline or ciprofloxacin was effective, and all participants were cured. Those treated with doxycycline produced more rapid defervescence.[161]

Brucellosis is typically treated with a 6-week course of doxycycline 100 mg orally twice daily with either streptomycin 15 mg/kg intramuscularly daily for the first 2 to 3 weeks or rifampin 600 to 900 mg daily for 6 weeks. This has been the long-standing recommendation of the World Health Organization and is still recommended as first-line therapy for brucellosis without serious complications.[164] However, the regimen of doxycycline with streptomycin has been shown to be superior with lower rates of relapse.[165] In a randomized controlled study looking at the combination of intramuscular gentamicin 5 mg/kg daily for 7 days with doxycycline versus standard doxycycline-streptomycin therapy, both arms were equivalent.[166] The addition of levofloxacin to the combination of doxycycline and rifampin for acute or subacute brucellosis may increase its efficacy in terms of lowering relapse rates within 6 months after stopping therapy, but not in terms of the rates of therapeutic failure after 6 weeks of therapy.[167] Complications of brucellosis, such as spondylitis, need to be treated for at least 12 weeks.[168]

Q fever, caused by *Coxiella burnetii,* ranges from a self-limiting febrile illness to difficult-to-treat endocarditis. Treatment with a tetracycline must begin within 2 days of symptom onset to become effective.[169] Doxycycline has been shown to reduce the duration of fever slightly better than tetracycline.[170] Recommended therapy in acute disease is doxycycline 100 mg orally twice daily for 14 days.[171] Patients with Q fever meningoencephalitis may respond to a 21-day course of doxycycline.[172] Patients with preexisting valvular heart disease who contract acute Q fever are at risk of developing endocarditis, reportedly at a rate of 39%.[173] Therapy with doxycycline and hydroxychloroquine for 1 to 15 months has been shown to reduce the risk of endocarditis. This observation has led the authors to recommend a 12-month course of dual therapy in such at-risk individuals.[173] Doxycycline 100 mg twice daily with hydroxychloroquine 200 mg three times a day is also typically used in treatment of Q fever endocarditis. This regimen was shown to result in fewer relapses than in patients treated with doxycycline combined with ofloxacin. In the doxycycline with hydroxychloroquine group, relapse occurred in two patients who were treated for 12 months, whereas no relapse occurred in patients who were treated for at least 18 months.[174]

Melioidosis, a *B. pseudomallei* infection, is usually treated initially with intravenous ceftazidime. After the induction phase, maintenance treatment is given for 12 to 20 weeks with a multidrug regimen. In an open-label, randomized study looking at chloramphenicol, doxycycline, and trimethoprim-sulfamethoxazole (TMP-SMX) versus doxycycline and TMP-SMX, the latter regimen was just as effective and better tolerated than the traditional four-drug group.[175] However, when the traditional four-drug regimen was compared with doxycycline monotherapy, doxycycline was inferior.[176]

Doxycycline has also been studied in diseases with limited therapeutic options. In a randomized study of 231 patients with dengue hemorrhagic fever, the group treated with 7 days of doxycycline had a 46% lower mortality compared with standard supportive care alone. Administration of doxycycline resulted in a significant decrease in levels of tumor necrosis factor and interleukin 6.[177] No effective treatment has yet been found for Creutzfeldt-Jakob disease (CJD). Data regarding the efficacy of doxycycline for CJD have been conflicting, but further study may be warranted.[178,179]

In the era of multidrug-resistant (MDR) gram-negative infections, minocycline has garnered recent interest owing to the limited treatment options for these organisms. Successful outcomes have been reported for the treatment of MDR *Acinetobacter,* carbapenem-resistant *Klebsiella pneumoniae,* and *S. maltophilia*.[180–182]

Acne Vulgaris

Acne vulgaris is a common adolescent disease usually treated with topical keratolytics for mild disease. Both doxycycline and minocycline are used for more severe disease as a way of treating patients with *Cutibacterium (Propionibacterium) acnes*–infected sebaceous glands in hair follicles. Antibiotics are usually prescribed for an average of 6 months and should be given for at least 2 months before consideration is given to switching antimicrobials because of poor therapeutic response.[183] In a randomized placebo-controlled study, minocycline 1 mg/kg daily over 12 weeks significantly reduced the number of inflammatory lesions compared with placebo.[184] This usage had raised concern for tetracycline resistance in the patient and in the population.

Antiinflammatory Uses

The tetracyclines have also drawn interest for their antiinflammatory properties. In a randomized study of 66 patients with early seropositive rheumatoid arthritis, initial therapy with methotrexate (MTX) plus doxycycline was superior to treatment with MTX alone. The therapeutic responses to low-dose (20 mg twice daily) and high-dose doxycycline (100 mg twice daily) were similar, suggesting that the antimetalloproteinase effects of doxycycline were more important than the antibacterial effects.[185] Minocycline has also been shown to be safe and effective for patients with mild-to-moderate rheumatoid arthritis, or Takayasu arteritis.[186–188] Data regarding the efficacy of minocycline for multiple sclerosis are conflicting.[189,190]

Bioterrorism Prophylaxis

Doxycycline is active against potential bioterrorism agents, including *B. anthracis, Y. pestis, Francisella tularensis, C. burnetii,* and *Brucella* spp. Compared with the fluoroquinolones, doxycycline is much less expensive and appears to have similar efficacy in most scenarios on the basis of clinical case studies. As a result, doxycycline should be considered as a first-line antibiotic in the event of a bioterrorism attack.[191]

Mechanism of Resistance

Resistance to the tetracyclines developed soon after their clinical introduction. The first reported case was in 1953 in an isolate of *Shigella dysenteriae.*[192] Tetracycline-resistant isolates can currently be found among a wide range of organisms. Increased prevalence of tetracycline

resistance was documented among Enterobacteriaceae, *Staphylococcus, Streptococcus,* and *Bacteroides* species by the 1970s, and in *N. gonorrhoeae* by the mid-1980s.[8] On the other hand, obligate intercellular pathogens, such as *Chlamydia* and *Rickettsia* species, have not been demonstrated to have significant tetracycline resistance.[193]

Resistance has developed among many different bacterial species as a result of horizontal exchange of resistance genes. There have been 33 distinct tetracycline-resistant genes identified, called *tet* genes, and three oxytetracycline resistant genes identified, called *otr* genes. These are found primarily on mobile units such as plasmids and transposons.[8,193,194]

There are various mechanisms of resistance to the tetracyclines. In the clinical setting, resistance via active efflux pumps and the production of ribosomal protection proteins (RPPs) are the principal mechanisms. Resistance can also develop via enzymatic degradation, decreased drug permeability, and target mutation.[6]

The tetracycline efflux system contains 26 different classes of efflux pumps that are responsible for the export of tetracycline from the cell. The efflux occurs because of genes encoding proteins that are members of the major facilitator superfamily (MFS) group of integral membrane transporters.[195] The efflux pumps are integral membrane proteins that span the lipid bilayer of the inner cell membrane 12 to 14 times.[6] This results in the export of tetracycline from the cell through an energy-dependent process. The end effect is a reduction of intracellular concentration of tetracycline, resulting in ribosomal protection of the bacteria. These pumps can be found in both gram-positive and gram-negative bacteria and typically confer resistance to early-generation tetracyclines but not to doxycycline and minocycline.

RPPs protect the ribosomes from the action of tetracycline. Eleven different types of RPPs have been identified, spanning both gram-positive and gram-negative bacteria. *Tet*(O) and *tet*(M) are the most prevalent and widely studied of the RPPs.[6] The RPPs work by weakening the interaction of tetracycline with the ribosome, preventing tetracycline from binding.[196] Thus, protein synthesis continues on tetracycline release. This mechanism generates resistance to both first- and second-generation tetracyclines but not to the newer third-generation glycylcyclines. Tigecycline is able to effectively exhibit antibacterial activity against *tet*(M) RPPs, likely because of a stronger affinity to its ribosomal target.[197]

Newer methods of tetracycline resistance have been identified through mosaic derivatives of known tetracycline resistance genes and are products of the RPP group of resistance determinants. At least one part of the gene shares more than 80% homology with a known tetracycline resistance gene; the other part shares homology with another known or new determinant.[6] Perhaps the best described example is from a ribosomal protection gene *tet*(32) isolated from *Clostridium* strain K10. This gene shares 60% identity with *tet*(O).[198]

A less common method of tetracycline resistance is a result of drug inactivation by tetracycline inactivators. In the entire tetracycline resistome, only three genes have been associated with tetracycline enzyme inactivation: *tet*(X), *tet*(34), and *tet*(37).[6] The first discovered was *tet*(X) in 1989 from a strain of *B. fragilis* that conferred resistance to *E. coli* when cells with the gene were grown aerobically.[199] The gene *tet*(X) inactivates tetracycline by selectively adding a hydroxyl group to the C-11a position of the antibiotic, which causes an unstable compound that undergoes nonenzymatic decomposition to a black polymer.[200] *Tet*(X) confers resistance to first- and second-generation tetracyclines and to tigecycline. *Tet*(X) uses nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of magnesium and converts tigecycline to 11a-hydroxytigecycline, which has a weakened ability to inhibit protein translation.[201]

Less common methods of tetracycline resistance include decreased drug permeability and target mutation. Tetracyclines are able to pass through the lipopolysaccharide-containing outer membrane of gram-negative bacteria by forming a magnesium complex followed by passage via porin channels. *E. coli* is able to overexpress the global activator protein MarA. This both induces the MDR efflux pump AcrAB and downregulates synthesis of the porin channel OmpF. The net effect reduces tetracycline uptake and accumulation in the cell.[202] Target modification has been shown in mutations of the 16S-rRNA of *H. pylori.* Studies have shown substitutions of nucleotides at positions 926, 927, and 928 in tetracycline-resistant isolates of *H. pylori*.[203,204] These substitutions can have a cumulative effect on the level of tetracycline resistance.

Adverse Reactions

General

As a class, the tetracyclines are generally well tolerated. A systematic review of studies between 1966 and 2003 summarized the adverse events (AEs) of doxycycline and minocycline. A total of 130 and 333 AEs were noted in published case reports of doxycycline and minocycline, respectively. In 24 doxycycline clinical trials ($n = 3833$) and 11 minocycline trials ($n = 788$), the ranges in incidence of AEs were 0% to 61% and 11.7% to 83.3%, respectively. The most common AEs encountered with doxycycline were gastrointestinal. Central nervous system and gastrointestinal AEs were most common with minocycline. The incidence of AEs with either drug is very low, but doxycycline had fewer reported AEs.[205]

In a more recent French review, adverse drug reactions (ADRs) of the tetracyclines were reviewed in studies performed between 1985 and 2007. Minocycline-associated ADRs were more serious and were reported more frequently than for the other tetracyclines. Minocycline and doxycycline ADR patterns differed. With doxycycline, gastrointestinal disorders (especially esophageal lesions) predominated. Intracranial hypertension and hepatic disorders were primarily reported with minocycline. Autoimmune disorders, drug reaction with eosinophilia and systemic symptoms, and other hypersensitivity reactions were also more frequent with minocycline. These findings led to the recommendation that minocycline should no longer be considered first-line therapy for inflammatory skin disorders, especially acne.[206]

Gastrointestinal Side Effects

As a class, tetracyclines commonly cause nausea, vomiting, diarrhea, heartburn, and epigastric pain. As stated earlier, gastrointestinal side effects are clearly the most common type of side effect of doxycycline, including pill esophagitis. Prolonged retention of the capsule in the esophagus, along with the high acidity of doxycycline in solution, is the likely cause of esophageal ulceration, and patients should drink at least 100 mL of water while standing upright for at least 90 seconds.[207,208] Patients with esophageal strictures should not take doxycycline. Diarrhea may occur with doxycycline, but there appears to be a reduced risk of CDI.[123,124] Minocycline also commonly causes nausea, but vomiting, diarrhea, and esophageal ulcerations occur much less frequently.[205,209]

Photosensitivity and Hyperpigmentation

A photosensitive rash commonly occurs in sun-exposed areas of patients taking tetracyclines.[205,206] This is caused by radiant-exposure–dependent inhibition of cellular incorporation of thymidine.[210] This reaction occurs as a result of drug accumulation in the skin and is phototoxic. This may occur shortly after exposure to the sun and can persist for a few days after the drug is discontinued. Severe cases may be associated with edema, papules, vesiculations, and onycholysis.[211]

Hyperpigmentation of various body parts has been well described with the tetracyclines, particularly minocycline.[205] Different types of cutaneous pigmentation have been described. It can cause a blue-black discoloration that occurs at sites of prior inflammation or scarring, a blue-black or gray localized macular pigmentation on normal arm skin, and a muddy-brown pigmentation predominantly on sun-exposed areas.[212,213] Blue-black discoloration of gums secondary to bone pigmentation that is visible through nonpigmented oral mucosa has been described with long-term use of minocycline and appears to be permanent.[214–216] Asymptomatic black pigmentation of the thyroid associated with minocycline has also been reported.[217,218]

Teeth and Bone

Tetracyclines deposit in teeth and bones as a result of chelation of tetracycline with calcium. The teeth can become stained owing to the formation of tetracycline-calcium orthophosphate complexes that darken with sun exposure.[219,220] This phenomenon is mostly cosmetic, but it may also cause demineralization and enamel hypoplasia leading to tooth decay.[221] Enamel hypoplasia has been most commonly described

in premature infants.[222] Doxycycline has a lower potential for teeth and bone deposition than the other tetracyclines because of its lower avidity for calcium. The degree of tooth discoloration is dependent on the total amount of tetracycline product administered.[223] Children receiving tetracycline early in life tend to have deposition in their deciduous teeth. This can also occur in developing fetuses when their mother receives tetracycline during pregnancy, particularly after the 25th week of gestation.[224] Tetracycline has also been noted to inhibit bone growth in infants receiving the drug. Premature infants displayed a 40% reduction of normal fibula skeletal growth after receiving tetracycline.[225] Fortunately, this appears to be reversible after drug discontinuation.

Hepatotoxicity

Even though hepatotoxicity is rarely associated with tetracycline use, it is potentially fatal. Hepatotoxicity has been well described with high-dose intravenous preparations of tetracycline.[226] This led to the withdrawal of intravenous tetracycline from the US market. Rarely, acute symptomatic hepatitis requiring hospitalization may develop with prolonged use of oral tetracycline over a period of 10 days.[227] Doxycycline does not appear to have the same side-effect profile and is rarely associated with significant liver toxicity.[16,228]

Nephrotoxicity

In the setting of impaired renal function, the tetracyclines can exacerbate renal malfunction by inhibiting protein synthesis. This causes a catabolic effect on amino-acid metabolism, leading to azotemia, hyperphosphatemia, and acidosis.[229] Expired tetracycline has been shown to produce a reversible Fanconi-like syndrome with renal tubular acidosis, likely due to citric acid, which accelerates the deterioration of stored tetracycline.[230] Current formulations of tetracycline have been modified so that they do not contain citric acid. Doxycycline is considered safe to use in the setting of renal impairment; it does not accumulate in the serum because its gastrointestinal excretion increases.[14] The side effect of demeclocycline, which causes nephrogenic diabetes insipidus, is used to treat the chronic syndrome of inappropriate antidiuretic hormone secretion.[7]

Neurotoxicity

Central nervous system effects have been described the most with minocycline. Minocycline has been noted to cause reversible dizziness, vertigo, tinnitus, and lack of concentration. Vestibular side effects have been reported to be higher in women (70.4%) than in men and are usually seen by the third day.[209] Pseudotumor cerebri, or idiopathic intracranial hypertension, has been noted with prolonged use of minocycline, and with tetracycline and doxycycline.[231–235] Symptoms may occur within 2 weeks to 6 months of therapy. Withdrawal of the offending antibiotic and treatment for increased intracranial pressure should lead to resolution of the pseudotumor cerebri syndrome, but visual field loss may persist.

Hypersensitivity Reactions

Hypersensitivity reactions are uncommon but appear to occur more frequently with minocycline.[206] They can include urticaria, facial edema, drug-induced autoimmune disease, and rarely anaphylaxis. Minocycline has been associated with drug-induced autoimmune disorders, the most common being drug-induced lupus.[236] Rare cases of anaphylaxis have been reported with administration of tetracycline to a penicillin-allergic patient.[237] Stevens-Johnson syndrome due to doxycycline is also rare but has been reported.[238] Cross-allergic reaction occurs between different drugs in this class. A patient with a hypersensitivity reaction to one antibiotic should be considered hypersensitive to all tetracyclines.

Teratogenicity

The tetracyclines are able to cross the placenta into the fetus. In a review of 18,515 pregnancies with congenital abnormalities, 0.3% were exposed to doxycycline. This was slightly higher than 0.19% of the 32,804 pregnancies without congenital abnormalities that were exposed to doxycycline. The conclusion of the study was that doxycycline poses little, if any, teratogenic risk to a fetus and there should be no contraindication to its use in pregnancy.[239] A more recent study of 1795 pregnancies exposed to doxycycline showed that there was no increase in risk of fetal abnormalities.[240] The tetracyclines are still generally avoided in pregnancy owing to their potential to stain teeth and cause enamel hypoplasia.

Drug and Food Interactions

Significant interactions between tetracyclines and other drugs or food are summarized in Table 26.3.

GLYCYLCYCLINES

Tigecycline

Glycylcyclines are a novel class of antibacterial drugs that have been developed to combat the emergence of resistant organisms. Tigecycline is the first member of the class to be approved by the FDA, in June 2005. It was originally approved for the treatment of complicated skin and skin structure infections (cSSSIs) and complicated intraabdominal infections (cIAIs) based on noninferiority studies.[241,242] In March 2009, tigecycline was approved for the treatment of community-acquired bacterial pneumonia based on comparable cure rates in patients treated with levofloxacin.[243,244] Tigecycline is considered bacteriostatic and is active in vitro against a broad range of resistant aerobic and anaerobic bacteria. The FDA issued a warning regarding an increased risk of death with tigecycline compared with other antibiotics used to treat similar infections based on the pooled analysis of 13 clinical trials.[245] In 2013 the FDA approved a new boxed warning about this risk of death and warned health care professionals to reserve tigecycline for use in situations in which alternative treatments are not suitable.[246] In general, the deaths appear related to worsening or complications of the infection or underlying comorbidities. Study-level and patient-level analyses have identified that patients in the hospital-acquired pneumonia (HAP) trial, particularly those with ventilator-associated pneumonia (VAP) with baseline bacteremia, were at a higher risk of clinical failure and mortality.[247]

Structure and Mechanism of Action

Tigecycline is a semisynthetic derivative of minocycline. It has an *N*-alkyl-glycylamido moiety attached to the 9-position of minocycline.[248] (See Fig. 26.1 for molecular structure.) The design is based on the premise that a peptide substituent would enhance ribosomal permeation and binding. The 9-glycyl substitution results in steric hindrance that enables tigecycline to overcome the two major types of tetracycline resistance: tetracycline-specific efflux pumps and ribosomal protection.[249]

Tigecycline's antibacterial mechanism of action is similar to that of older tetracyclines. It works by inhibiting bacterial protein synthesis by binding to bacterial 30S ribosomal subunits, ultimately blocking entry of aminoacyl transfer RNA molecules into the A site of the ribosome. This prevents amino acids from incorporating into elongating peptide chains, thus inhibiting protein synthesis.[249,250] Like other tetracyclines, tigecycline is considered bacteriostatic because its interaction with the ribosome is reversible.[8] Because glycylcyclines bind five times more strongly to the ribosome than tetracyclines, they are able to overcome the ribosomal protection mechanism of the tetracycline resistome.[251,252]

Pharmacology

Administration and Dosing

Tigecycline is available only in intravenous formulation owing to its poor oral absorption. The recommended regimen is an initial dose of 100 mg, followed by 50 mg every 12 hours. Intravenous infusions should be administered over approximately 30 to 60 minutes. According to the package insert, the recommended duration of treatment for cSSSIs or for cIAIs is 5 to 14 days. The recommended duration for treatment of community-acquired bacterial pneumonia is 7 to 14 days. It has not been studied in children younger than 18.[253]

In patients with renal impairment, no dose adjustment is necessary. In a pooled pharmacokinetic evaluation of tigecycline in healthy adult volunteers and in those with renal impairment, tigecycline had mostly linear pharmacokinetics across all dose ranges. Tigecycline is also not significantly removed by hemodialysis.[254]

TABLE 26.3 Significant Food-Drug and Drug-Drug Interactions With Tetracyclines

INTERACTING AGENT	EFFECT	COMMENTS
Food	**Tetracycline** Food may reduce absorption by 50% **Doxycycline** Absorption may be reduced by up to 20% when taken with food or milk **Minocycline** Absorption may be reduced by up to 20% when taken with food or milk	 Bioavailability is 60%–80% when taken on an empty stomach Food effect not clinically significant Food effect not clinically significant
Divalent or trivalent cations: aluminum, calcium, magnesium, iron, zinc	Significant reduction of all tetracycline absorption	Should not be administered concurrently with foods or drugs containing divalent or trivalent cations (i.e., antacids, sucralfate, didanosine, multivitamins)
Kaolin and pectin	Significant reduction of tetracycline absorption	
Bismuth subsalicylate	Significant reduction of tetracycline absorption	Separate administration of tetracycline from divalent or trivalent cations by 2 hours
Sodium bicarbonate	May decrease absorption of tetracycline	
Cimetidine	Decreased tetracycline absorption	Effect not clinically significant
Carbamazepine, phenytoin, barbiturates	Decreased half-life of doxycycline	Increases hepatic metabolism
Chronic ethanol ingestion	Decreased half-life of doxycycline but not tetracycline	Possible mechanism: induction of hepatic microsomal enzymes
Methoxyflurane or fluorinated anesthetic	Nephrotoxicity when administered with tetracycline agents	
Diuretics	Increased blood urea nitrogen	Volume depletion may increase the nephrotoxic effects of tetracycline by unknown mechanisms
Oral anticoagulants	Increased risk of bleeding	Tetracyclines may impair utilization of prothrombin and may decrease vitamin K production by intestinal bacteria; tigecycline decreases warfarin clearance, although a study found no effect in healthy volunteers
Oral contraceptives	Reduced levels when administered with tetracyclines	Women should use an additional (mechanical) form of birth control Possible mechanism: reduction in bacterial hydrolysis of conjugated estrogen in the intestine
Antiinfectives	May reduce antimicrobial activity of aminoglycosides and penicillins	Rare reports of in vitro antagonism Some clinicians recommend that the drugs not be used concomitantly

In patients with mild-to-moderate hepatic impairment (Child-Pugh A and B), no dose adjustment is necessary. In patients with severe hepatic impairment (Child-Pugh C), the recommended regimen is an initial dose of 100 mg, followed by a reduced maintenance dose of 25 mg every 12 hours. These patients should be treated with caution and monitored closely. In a study looking at various stages of liver dysfunction, patients with moderate liver disease had systemic clearance reduced by 25%, and the half-life was increased by 23%. In patients with severe liver disease, systemic clearance was decreased by 55% and the half-life was increased by 43%.[255]

There do not appear to be any significant differences in tigecycline pharmacokinetic parameters on the basis of age, sex, or weight. Therefore, no dose adjustment appears necessary.[256]

Absorption and Bioavailability

Tigecycline has limited oral bioavailability; hence, it is available only in intravenous preparations. Food does not significantly alter pharmacokinetics and may reduce the nausea and vomiting seen as a side effect. The half-life of tigecycline ranges from 37 to 67 hours.[257]

Drug Distribution

In vitro plasma protein binding of tigecycline is 71% to 89%, and the volume of distribution is 7 to 10 L/kg, indicating extensive distribution into the tissues.[257] In a 100-mg single-dose study of patients undergoing elective surgery or a medical procedure for tissue extraction, various tissue concentrations were measured. The respective mean and median concentration values of tigecycline in serum immediately after the end of a 100-mg infusion were 1.94 and 1.32 mg/L. Subsequent serum concentrations of tigecycline gradually declined to mean values of 0.22, 0.19, 0.11, and 0.07 mg/L at approximately 4, 8, 12, and 24 hours, respectively. Tissue penetration, expressed as the ratio of area under the curve $(AUC)_{0-24h}$ in tissue or body fluid to serum, was 537 for bile, 23 for gallbladder, 2.6 for colon, 2.0 for lung, 0.41 for bone, 0.31 for synovial fluid, and 0.11 for CSF.[258] In a separate study of subjects without infections undergoing elective orthopedic procedures, multiple doses of tigecycline were shown to result in bone-to-serum ratios of approximately 4.77, showing penetration into bone.[259] After standard dosing to 33 healthy volunteers, the tigecycline AUC_{0-12h} in alveolar cells was 78-fold higher than the AUC_{0-12h} in the serum. In epithelial lining fluid, the AUC_{0-12h} was about 32% higher than in the serum.[253] In the urine, after a 100-mg dose of tigecycline, urinary concentrations were about 0.3 μg/mL.[260] Clinical trials in urinary tract infections were abandoned on precompletion review because there was limited urinary recovery of active drug.[261] Like the earlier-generation tetracyclines, tigecycline displays a time-dependent killing pattern and exhibits a considerable postantibiotic effect.[262]

Drug Elimination

The primary route of elimination for tigecycline is biliary excretion of unchanged tigecycline and its metabolites. Using radiolabeled carbon 14 (^{14}C)-tigecycline, studies have shown elimination primarily by the liver via biliary excretion of unchanged tigecycline in the feces (59%). Glucuronidation and renal excretion of unchanged tigecycline are secondary routes. Thirty-two percent of tigecycline is eliminated in the urine.[257,263]

Antimicrobial Activity

Tigecycline has antibacterial activity against a wide variety of aerobic and anaerobic bacteria. For reference to MIC cutoff values for various clinically relevant species, see Table 26.4.

Gram-Positive Bacteria

Tigecycline is active against many *Staphylococcus, Enterococcus,* and *Streptococcus* species, including organisms resistant to many other

TABLE 26.4 Overview of the MIC_{50} and MIC_{90} Ranges, and the Wild-Type Cutoff Values for Various Species That Are Clinically Relevant for Tigecycline Use

	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	RANGE (μg/mL)	EUCAST WILD-TYPE CUTOFF
Gram-Positive Bacteria				
Staphylococcus aureus, methicillin-resistant	0.12–0.25	0.25–0.5	0.06–1	0.25
S. aureus, methicillin-sensitive	0.12	0.12–0.5	≤0.06–1	0.25
Staphylococcus epidermidis, methicillin resistant	0.12–0.25	0.25–0.5	≤0.06–2	0.5
S. epidermidis, methicillin-sensitive	0.12–0.25	0.25–0.5	0.06–2	0.5
Enterococcus faecalis, vancomycin susceptible	≤0.06–0.12	0.12–0.25	≤0.06–2	0.25
Enterococcus faecium, vancomycin susceptible	≤0.06	≤0.06–0.25	≤0.06–2	0.25
E. faecium, vancomycin resistant	≤0.06	≤0.06–0.12		0.25
Streptococcus pyogenes	0.06	0.06–0.12	0.06–0.5	0.125
Streptococcus agalactiae	0.03–0.06	0.12–0.25	0.03–1	0.25
Streptococcus pneumoniae	≤0.03–0.12	0.06–0.5	≤0.06–1	0.125
Gram-Negative Bacteria				
Enterobacteriaceae	0.5	1	0.04–16	—
Enterobacter spp.	0.5	1–2	0.06–8	—
Enterobacter cloacae	0.5–1	1–2	0.5–16	2
Enterobacter aerogenes	0.5–1	1–2	0.06–16	2
Escherichia coli	0.12–0.25	0.25–0.5	0.03–4	1
Haemophilus influenza	0.12–0.5	0.25–1	≤0.008–2	1
Klebsiella spp.	0.5	1–2	0.5–8	2 *(K. pneumoniae)*
Klebsiella pneumoniae ESBL+	1	2	0.12–8	—
Moraxella catarrhalis	0.06–0.12	0.12–0.25	≤0.03–0.5	0.125
Morganella morganii	2	4	1–8	8
Proteus spp.	4	4–8	1–16	4/8
Serratia spp.	1–2	1–4	0.5–16	4
Stenotrophomonas maltophilia	0.38–1	1.5–4	0.25–8	4
Pseudomonas aeruginosa	8–16	16–≥32	0.12–64	64
Acinetobacter spp.	0.5–3	1–6	0.03–8	—
Anaerobic Bacteria				
Bacteroides fragilis group	0.5–1	0.5–8	0.06–32	—
Clostridioides difficile (formerly *Clostridium difficile*)	0.06–0.12	0.06–0.25	≤0.06–2	—
Clostridium perfringens	0.12	1	0.06–2	—
Fusobacterium spp.	0.03–0.12	0.06–0.12	≤0.015–0.25	—
Peptostreptococcus spp.	0.06	0.06–0.125	0.06–0.12	

ESBL, Extended-spectrum β-lactamase; *EUCAST,* European Committee on Antimicrobial Susceptibility Testing; *MIC,* minimal inhibitory concentration.
From Nabuurs-Franssen M, Mouton JW. Tigecycline. In: Grayson ML, ed. Kucers' The Use of Antibiotics. 6th ed. Boca Raton, FL: CRC Press; 2010:881–892.

classes of antibiotics. Among *S. aureus,* tigecycline has shown potent activity among both methicillin-sensitive and methicillin-resistant strains.[264–266] Tigecycline has also been shown to be highly active against community-acquired MRSA strains as seen in a study of 1989 isolates with a susceptibility rate of 98.2%.[267] In a European survey of common skin and skin structure pathogens, all MRSA isolates were susceptible to tigecycline.[268] *Staphylococcus epidermidis* has also been shown to be susceptible to tigecycline, including strains that are erythromycin resistant.[269]

Tigecycline is also highly active against *Enterococcus* spp., including strains resistant to vancomycin. In a study of 97 vancomycin-resistant isolates, tigecycline was equally active against all VanA, VanB, and VanC phenotypes of glycopeptide resistance of enterococci.[270] In the Tigecycline Evaluation and Surveillance Trial (TEST) program, which took place from 2004 to 2009 and looked at various antimicrobials against *Enterococcus faecalis* and *Enterococcus faecium,* all 2068 isolates of *E. faecalis* were susceptible to tigecycline in various European countries. Only 2 out of 893 (0.2%) of *E. faecium* isolates were resistant to tigecycline.[271] In a Korean study, 4 out of 2005 (0.2%) of vancomycin-resistant enterococci (VRE) isolates were resistant to tigecycline.[272]

Streptococci have also been shown to be highly susceptible to tigecycline, including bloodstream isolates of viridans-group streptococcal species.[273] Tigecycline has also shown excellent activity against β-hemolytic streptococci.[274] Tigecycline was twofold to fourfold more active than comparator agents against all 435 isolates of *S. pneumoniae* studied in the global TEST program, including those that were penicillin resistant and penicillin intermediate. Against the penicillin-susceptible *S. pneumoniae* isolates, tigecycline was less active than β-lactam agents.[275] In another study, tigecycline was noted to be bactericidal against 11 of 12 strains of *S. pneumoniae.*[276]

Tigecycline has also been shown to be highly active against *L. monocytogenes* and coryneform bacteria.[277]

Gram-Negative Bacteria

Tigecycline has good activity against most Enterobacteriaceae with the exception of most strains of *Proteus* spp., *Providencia* spp., and *Morganella* spp.[274,278,279] Enterobacteriaceae that have developed resistance to tetracycline remain susceptible to tigecycline because it remains stable and largely unaffected by the commonly occurring efflux and ribosomal protection resistance mechanisms. In a large global study, tetracycline-resistant populations demonstrated only modest decreases in potency to tigecycline, which appeared to be species dependent (up to twofold only for *E. coli*, *Salmonella* spp., *Shigella* spp., and *Pantoea agglomerans* and up to fourfold for *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp.[280] Other resistance mechanisms, such as extended-spectrum β-lactamase (ESBL) production, AmpC hyperproducers, carbapenemase-producing Enterobacteriaceae, serine- and metallo-β-lactamases, fluoroquinolone resistance, fluoroquinolone/ampicillin resistance phenotype, fluoroquinolone/TMP-SMX resistance phenotype, and isolates with MDR phenotypes, do not have an influence on the MIC value of tigecycline.[280–284]

Tigecycline has good activity against most nonfermenting gram-negative bacilli with the notable exception of *Pseudomonas aeruginosa*. It remains one of the most active agents against *Acinetobacter* spp.[275,278,285] Although most studies have noted a high level of *Acinetobacter* spp. susceptibility to tigecycline, one study in Israel noted that of 82 isolates, 66% were resistant, 12% were intermediate, and just 22% were susceptible to tigecycline.[286] In the United States, most antimicrobial agents have shown reduced susceptibility to *A. baumannii* over recent years, including tigecycline.[285] *S. maltophilia* has been shown to be highly susceptible to tigecycline in vitro. In a large global survey of 1586 isolates, 95.5% were susceptible to tigecycline, which was second only to TMP-SMX (96%).[287]

In another global study, 1220 strains of *H. influenzae,* 495 strains of *Moraxella catarrhalis,* and 17 strains of *N. meningitidis* were all susceptible to tigecycline.[288] *Brucella melitensis* is highly susceptible to tigecycline both in vitro and in blood and bone marrow cultures of adult patients with acute brucellosis.[289–291] Tigecycline has reduced activity against *B. cepacia*.[252]

Anaerobic Bacteria

Tigecycline is active against most gram-positive and gram-negative anaerobes. In vitro activities of tigecycline were tested against 831 isolates of the *B. fragilis* group representing all of the species within the group. Tigecycline was more active than clindamycin, minocycline, and cefoxitin and less active than imipenem or piperacillin-tazobactam against all isolates of the *B. fragilis* group.[292] In the European TEST program looking at *Bacteroides, Prevotella, Anaerococcus, Clostridium, Finegoldia,* and *Peptostreptococcus,* the lowest MIC values were noted for meropenem and tigecycline. Of note, tigecycline showed the lowest MIC_{90} against *C. difficile* (0.25 mg/L).[293]

Atypical Bacteria

Tigecycline is highly active against *C. pneumoniae, C. trachomatis, M. pneumoniae,* and *M. hominis*.[84,294] It is less active against *U. urealyticum* than tetracycline, with an MIC_{90} of 8 μg/mL.[294] In a guinea pig model, tigecycline was shown to be as effective as erythromycin against *L. pneumophila,* but the drug was inactivated by test medium that confounded susceptibility data. Tigecycline prevented death in the study, but treated guinea pigs were noted to have residual bacterial concentrations in the lungs, suggesting that tigecycline may not be the drug of choice for severe legionnaires' disease, and prolonged therapy would be required.[295]

Mycobacterium and *Nocardia*

The rapidly growing mycobacteria (RGM) appear to be highly sensitive to tigecycline. In two separate studies, every RGM isolate, mostly consisting of *M. fortuitum, M. abscessus,* and *M. chelonae,* was susceptible to tigecycline.[96,296] All slowly growing nontuberculous mycobacteria, including *M. avium* complex, *Mycobacterium lentiflavum, M. marinum,* and *M. kansasii,* are resistant to tigecycline.[96] The in vitro activity against *M. tuberculosis* appears poor.[297] Tigecycline has modest activity against *Nocardia* spp., with an MIC_{90} of 4 μg/mL. By comparison, minocycline has an MIC_{90} of 2 μg/mL.[102]

Clinical Uses

Tigecycline is currently FDA approved for three indications: treatment of cSSSIs, cIAIs, and community-acquired bacterial pneumonia. It is also used off label for many other disease processes.

Skin and Skin Structure Infections

Several clinical studies have compared tigecycline versus a combination of vancomycin and aztreonam for up to 14 days for the treatment of skin and skin structure infections. Similar results have been seen across studies that have shown tigecycline to be noninferior. Among the clinical modified intention-to-treat populations, tests of cure were between 73.3% and 84.3% for tigecycline and between 66.6% and 86.9% for the vancomycin-aztreonam group. Tests of cure for the clinically evaluable populations were 78.6% to 89.8% and 75.8% to 95%, respectively. Increased nausea and vomiting was consistently seen in the tigecycline group, whereas rash and elevated transaminases were consistently seen in the vancomycin-aztreonam group.[241,298–301] In the largest study, no differences were noted in clinical cure rates in patients who were bacteremic (86% vs. 87.5%). Tigecycline had equal efficacy in patients with monomicrobial and polymicrobial infections.[241]

A phase III, randomized, double-blind trial was conducted in subjects with diabetic foot infections with and without osteomyelitis comparing a 150-mg once-daily regimen of tigecycline versus ertapenem with or without vancomycin. For the clinically evaluable population at test-of-cure assessment, 77.5% of tigecycline-treated participants and 82.5% of ertapenem-treated participants with or without vancomycin were cured. Corresponding rates for the clinical modified intention-to-treat population were 71.4% and 77.9%, respectively. Tigecycline did not meet the criteria for noninferiority. Clinical cure rates in the substudy were low (<36%) for a subset of patients with osteomyelitis treated with tigecycline. Higher rates of nausea and vomiting with higher discontinuation rates were observed for tigecycline in this trial than in other phase III studies.[302]

Intraabdominal Infections

Tigecycline is an attractive option to treat cIAIs because its broad spectrum of activity is favorable against a potentially polymicrobial infection. In two large studies of cIAIs, tigecycline was compared with imipenem-cilastatin for 5 to 14 days of treatment. Complicated appendicitis was the most common infection, followed by complicated cholecystitis. Acute Physiology and Chronic Health Evaluation (APACHE) II scores were similar, and less than 4% of patients had scores over 15. Clinical cure rates were 86.1% for tigecycline and 86.2% for imipenem-cilastatin, meeting noninferiority criteria. There was no difference in cure rates between the two therapies for monomicrobial versus polymicrobial infections. Clinical cure rates for bacteremia were also similar (82% vs. 80%, respectively). The most common organisms were *E. coli*, *Streptococcus anginosus* group, and *B. fragilis*. Only vancomycin-susceptible isolates were included. Eradication rates were similar for ESBL-producing bacteria. Nausea and vomiting were more frequent in the tigecycline group.[242,303] In a more recent Chinese study comparing the same two therapies for up to 2 weeks, 86.5% (45/52) of tigecycline and 97.9% (47/48) of imipenem-cilastatin–treated patients were cured at the test-of-cure assessment (12–37 days after therapy).[304]

Two other studies have compared tigecycline with a combination of ceftriaxone plus metronidazole for cIAIs. Clinical cure rates were between 70.4% and 81.8% in the tigecycline group and 74.3% and 79.4% in the ceftriaxone-metronidazole group, again displaying noninferiority.[305,306]

Respiratory Tract Infections

Tigecycline has been approved for the treatment of community-acquired bacterial pneumonia. It has been compared with levofloxacin in multiple studies with treatment durations between 7 and 14 days and Fine Pneumonia Severity Index scores typically from II to IV. Among the clinical modified intention-to-treat populations, test-of-cure results were between 78.7% and 84.4% for tigecycline and between 77.8% and 82.1% for the levofloxacin group. Test-of-cure results for the clinically evaluable populations were 88.9% and 90.6% and 85.3% and 87.2%, respectively.

All trials met noninferiority criteria for tigecycline. As with other clinical trials, more patients tended to have nausea and vomiting in the tigecycline groups.[243,244,307,308]

Tigecycline has also been looked at for treating VAP and HAP. In a retrospective review of 117 patients with VAP treated with tigecycline, 74 (63%) were determined by the physician to have clinical success. About half of the patients had a microbiologic diagnosis, and of those the most common organisms were *Acinetobacter* spp. (69%) and MRSA (22%). Eighty-four percent of the cases with *Acinetobacter* spp. were carbapenem resistant. Global mortality proportion was 33%.[309] In a phase III randomized controlled trial involving 945 patients, tigecycline was compared with imipenem-cilastatin for the treatment of HAP. Cure rates were 67.9% for tigecycline and 78.2% for imipenem-cilastatin in the clinically evaluable group and 62.7% and 67.6% in the clinical modified intention-to-treat group, respectively. A statistical interaction occurred between VAP and non-VAP subgroups, with significantly lower cure rates in tigecycline-treated VAP patients compared with imipenem; in non-VAP patients, tigecycline was noninferior to imipenem. Overall mortality did not differ between the tigecycline (14.1%) and imipenem-cilastatin regimens (12.2%), although more deaths occurred in VAP patients treated with tigecycline than imipenem-cilastin.[310] In a subsequent phase II study, the safety and efficacy of two higher doses of tigecycline (150 mg followed by 75 mg every 12 hours or 200 mg followed by 100 mg every 12 hours) were compared with imipenem-cilastatin in participants with HAP. In the clinically evaluable population, the rate of clinical cure with tigecycline 100 mg (17/20, 85.0%) was numerically higher than with tigecycline 75 mg (16/23, 69.6%) and imipenem-cilastatin (18/24, 75.0%). No new safety signals with the high-dose tigecycline were identified.[311] Further studies with this higher-dose regimen are needed.

Other Uses

Tigecycline is also used for many off-label indications. In an early-use registry of 113 patients, 22% received tigecycline for approved indications and 78% for off-label indications (56% with scientific support and 22% with limited or without any scientific support).[312]

Although there are no randomized prospective trials looking at the use of tigecycline for bacteremia, secondary bacteremia in prior clinical trials has been analyzed. Pooled data from subjects enrolled for treatment of cSSSI, cIAI, or CAP presenting with bacteremia from seven double-blind and one open-label trial of tigecycline compared with vancomycin-aztreonam, imipenem-cilastatin, levofloxacin, vancomycin, or linezolid were analyzed. A total of 170 subjects were identified, and clinical cure rates were 81.3% and 78.5% for tigecycline and the comparator, which did not achieve significance. Nine patients treated with tigecycline were noted to have persistent bacteremia, as compared with one in the comparator group.[313] Tigecycline, in a solution with *N*-acetylcysteine and heparin, was evaluated as a catheter-lock solution for catheter salvage in patients with hemodialysis catheter-associated bacteremia. Eighteen case patients received the catheter-lock solution for 14 days plus systemic antibiotic therapy. Treatment was successful for 15 (83%) of the 18 case patients within 90 days of follow-up.[314]

In critically ill patients who have developed bacterial infections with multidrug resistance, tigecycline remains an attractive option owing to its ability to maintain in vitro activity. A phase III, open-label, noncomparator study evaluated tigecycline in patients with selected serious infections caused by resistant gram-negative bacteria, patients with treatment failures who had received prior antimicrobial therapy, or patients unable to tolerate other appropriate antimicrobials. Infections included cSSSI, cIAI, CAP, HAP, VAP, and bacteremia (including catheter-associated bacteremia). Clinical cure rate was 72.2%, and the microbiologic eradication rate was 66.7%.[315] Another phase III study compared the safety and efficacy of tigecycline with vancomycin or linezolid in hospitalized patients with MRSA or VRE infections. Infections included cSSSI, cIAI, pneumonia, and bacteremia. For MRSA infection, clinical cure rates in the microbiologically evaluable population ($n = 117$) were 81.4% (70/86 patients) with tigecycline and 83.9% (26/31 patients) with vancomycin. Most infections were cSSSIs. There were only 15 patients enrolled with VRE, and the authors were not able to draw any conclusions.[316] In a report of 110 cancer patients who developed serious infections, 64% were noted to have a clinical response when treated with tigecycline (92% were treated in combination with an antipseudomonal agent). Interesting to note, more patients with bacteremia than pneumonia had clinical response (79% vs. 51%). Mortality rates of those with pneumonia were 44%, compared with 16% with bacteremia.[317] The combination of piperacillin-tazobactam and tigecycline was found to be more effective than piperacillin-tazobactam alone in febrile, neutropenic hematologic patients with cancer in an epidemiologic setting characterized by a high rate of MDR microorganisms.[318] In a prospective, observational study of adults with extensively drug-resistant *A. baumannii* bacteremia, increased 14-day mortality was observed with the combination of colistin-tigecycline compared with a colistin-carbapenem regimen. The excess mortality may have been restricted to the subgroup with higher tigecycline MICs (>2 mg/L).[319]

Clinical trials that have looked at use of tigecycline for urinary tract infections were abandoned at precompletion review because there was limited urinary recovery of active drug.[261] In a retrospective cohort study of carbapenem-resistant *K. pneumoniae* bacteriuria, tigecycline, polymyxin B, and an aminoglycoside were compared. The microbiologic clearance rate was 88% in the aminoglycoside cohort ($n = 41$), 64% in the polymyxin B cohort ($n = 25$), 43% in the tigecycline cohort ($n = 21$), and 36% in an untreated cohort.[320] Success with tigecycline for treating MDR *K. pneumoniae* and *A. baumannii* urinary tract infection or urosepsis has been reported with use of "high-dose" tigecycline, with initial doses between 200 and 400 mg and subsequent maintenance doses of 100 to 200 mg every 24 hours.[260]

Interest in using tigecycline for *C. difficile* infections (CDIs) has arisen owing to its in vitro activity and high stool concentrations. Currently, no clinical trials have evaluated tigecycline for the treatment of CDI. There have been multiple case reports of use of tigecycline (alone or in combination with other CDI therapies) for the treatment of CDI, with most having a successful outcome.[321–323] In a retrospective case-control study of severe CDI, 18 patients who received tigecycline (along with other CDI therapies) were compared with 26 similarly ill patients who did not receive tigecycline. There was no difference with regard to CDI survival, rates of colectomy, or relapse rates.[324]

Limited data exist regarding use of tigecycline for bone and joint infections. In the previously mentioned phase III study in diabetic foot infections with and without osteomyelitis, clinical cure rates in the substudy were low (<36%) for a subset of tigecycline-treated patients with osteomyelitis.[302] In a retrospective study of 15 patients with culture-negative pyogenic vertebral osteomyelitis, tigecycline was given as second-line empirical therapy. One patient dropped out because of severe nausea and vomiting. The other 14 patients were deemed to have had sustained clinical success.[325] Tigecycline was also evaluated in a retrospective case series of 19 patients with osteomyelitis. Five patients ceased therapy because of AEs. A sixth patient dropped out for unknown reasons. Eleven patients met the primary efficacy end point of clinical success.[326]

Treatment of infections with RGM can be difficult. The safety and efficacy of a tigecycline-containing regimen for salvage treatment of 52 patients with *M. abscessus* and *M. chelonae* was performed. Included patients had both lung and extrapulmonary infections. Tigecycline given for at least 1 month as part of a multidrug regimen resulted in improvement in over 60% of patients, including those with underlying cystic fibrosis, despite failure of prior antibiotic therapy. AEs were reported in over 90% of cases, the most common being nausea and vomiting.[327]

Mechanism of Resistance

Tigecycline remains active against bacteria that contain efflux-mediated resistance to tetracyclines. This is likely a result of the efflux pump's ineffectiveness in transporting tigeclycine out of the cell, or from the inability of tigecycline to induce efflux proteins.[328,329] There is also currently no evidence of gene mutations coding for RPPs for the *tet* family (*tet* M, O, S) for reduced susceptibility to tigecycline.

The most common intrinsic tigecycline resistance observed in *Proteus mirabilis* and *Morganella morganii* is drug recognition and upregulation by the AcrAB and MexAB-OprM efflux pumps, which typically confer MDR phenotypes.[330,331] Overexpression of these pumps is also associated with the development of resistance to other gram-negative bacteria.

P. aeruginosa is intrinsically resistant to tigecycline as a result of the MexXY multidrug efflux pump.[332]

The overexpression of mepA, a novel MATE family efflux pump, may contribute to decreased activity of tigecycline against *S. aureus*.[333]

Adverse Reactions

Gastrointestinal Side Effects

As with older tetracyclines, the most commonly reported adverse reactions to tigecycline are gastrointestinal related. As seen in multiple phase III clinical trials, nausea (26%) and vomiting (18%) were the most reported side effects.[241,242,253,298,334] The majority of these symptoms (95%) were mild to moderate and occurred after 1 to 2 days of therapy. The side effects are dose related; 100 mg was the highest dose tolerated in fasting healthy participants, and 200 mg was the highest dose tolerated with food.[257] Less-reported side effects are anorexia, dry mouth, dysgeusia, and loose stool.[281]

Hepatotoxicity and Pancreatitis

Transient elevations in transaminases and alkaline phosphatase levels have been seen.[335] However, in phase III clinical trials, the incidence of increased liver function test results was significantly lower with tigecycline than with comparator drugs.[241,243] Case reports of mild tigecycline-induced acute pancreatitis have also been reported.[336]

Other Side Effects

Other side effects reported from clinical trials that have an incidence between 2% and 7% include (in descending order) infection, headache, anemia, hypoproteinemia, asthenia, phlebitis, elevated blood urea nitrogen (BUN), abnormal healing, dizziness, skin rash, pneumonia, and abscess formation.[253]

Increased Mortality

In 2010 the FDA issued a warning of an increased risk of death with tigecycline compared with other antibiotics used to treat similar infections on the basis of pooled analysis of 13 clinical trials.[245] In those studies, death occurred in 4% (150/3788) of patients receiving tigecycline and 3% (110/3646) of patients receiving comparator drugs. Based on a random effects model by trial weight, an adjusted risk difference of all-cause mortality was 0.6% (95% confidence interval, 0.1–1.2) between tigecycline- and comparator-treated patients. Deaths were generally the result of worsening infection, complications of infections, or underlying comorbidities. Among indications, the mortality difference was not statistically significant. However, mortality in tigecycline-treated patients was numerically greater in every infection and considerably greater in VAP (19.1% vs. 12.3%). Study-level and patient-level analyses have identified that patients in the HAP trial, particularly those with VAP with baseline bacteremia, were at a higher risk of clinical failure and mortality.[247]

In 2013 the FDA approved a new boxed warning about this risk of death and warned health care professionals to reserve tigecycline for use in situations in which alternative treatments are not suitable. After the 2010 warning was issued, data were analyzed from 10 clinical trials conducted only for FDA-approved uses (cSSSI, cIAI, community-acquired bacterial pneumonia), including trials conducted after the drug was approved. This analysis showed a higher risk of death among patients receiving tigecycline compared with other antibacterial drugs: 2.5% (66/2640) versus 1.8% (48/2628), respectively. The adjusted risk difference for death was 0.6% with corresponding 95% confidence interval (0.0–1.2).[246]

Several meta-analyses prompted the FDA to look into the efficacy and safety of tigecycline. Depending on what clinical trials (published and unpublished) were included, results varied.[337–339]

Drug Interactions

In vitro studies in human liver microsomes have indicated that tigecycline is not a substrate, inhibitor, or inducer of common cytochrome P450 enzymes; therefore such pharmacokinetic drug interactions are unlikely.[340] However, concomitant administration of tigecycline and warfarin in healthy participants resulted in a decrease in clearance of *R*-warfarin and *S*-warfarin by 40% and 23%, an increase in C_{max} by 38% and 43%, and an increase in AUC by 68% and 29%, respectively.[253] No significant effects of tigecycline on digoxin pharmacokinetics and pharmacodynamics were noted in a study of 20 healthy men.[341]

NEW DRUG APPROVALS: ERAVACYCLINE AND OMADACYCLINE

Two new tetracycline derivatives were approved by the FDA in late 2018. Eravacycline is a fluorocycline antibiotic and is FDA approved for complicated intraabdominal infections in adults.[341a] Omadacycline is an aminomethylcycline and is FDA approved for the treatment of acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP).[341b] Both drugs carry the same package insert label, which is to recommend that these two drugs should be used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria. This precaution is to reduce the development of drug-resistant bacteria and maintain the effectiveness of eravacycline and omadacycline and other antibacterial drugs.[341c] Eravacycline also has a label precaution that it is not indicated for the treatment of complicated urinary tract infections. Omadacycline has an additional label precaution about the mortality imbalance in patients with CABP. In the CABP trial, a mortality rate of 2.1% was observed in omadacycline-treated patients compared to 0.8% in moxifloxacin-treated patients ($P = .14$).[341b] The cause of the mortality imbalance has not been established. Providers should closely monitor clinical response to therapy in CABP patients, particularly in those at higher risk for mortality.

Both drugs have broad in vitro activity against many gram-positive, gram-negative, atypical, and anaerobic bacteria, including activity against MRSA, VRE, penicillin-resistant *Streptococcus pneumoniae*, *A. baumannii*, and Enterobacteriaceae expressing extended-spectrum β-lactamases. Similar to tigecycline, neither agent performs well against *Pseudomonas* spp.

Eravacycline recommend dosing is 1 mg/kg by intravenous infusion every 12 hours. No dose adjustment is required in patients with renal impairment. In patients with severe hepatic impairment (Child Pugh C), the dose should be reduced starting on day 2 to 1 mg/kg every 24 hours. With concomitant use of a strong CYP3A inducer, the dose should be increased to 1.5 mg/kg every 12 hours. Omadacycline recommended dosing for ABSSSI and CABP is 200 mg by intravenous infusion on day 1 (given once or divided into two 100-mg doses), followed by 100 mg intravenously daily or 300 mg orally daily. For ABSSSI treatment, loading doses can be done with 450 mg orally once daily on days 1 and 2, followed by 300 mg orally daily. No dose adjustment is recommended in patients with renal or hepatic impairment. Both drugs have similar label warnings for patients who are on anticoagulant therapy that they may require downward adjustments of anticoagulant dosing while on these agents. Breastfeeding is not recommended while on either agent. Both drugs carry warning labels for permanent tooth discoloration and enamel hypoplasia, and reversible inhibition of bone growth if used during the second and third trimesters of pregnancy and during childhood up to age 8. Similar to other tetracyclines, omadacycline absorption is impaired by antacids containing aluminum, calcium, or magnesium; bismuth subsalicylate; and iron containing preparations.

CHLORAMPHENICOL

Chloramphenicol is a broad-spectrum antibiotic that has been in clinical use since 1949. It was developed from the soil organism *Streptomyces venezuelae*.[342] It remains an inexpensive drug that is active against many gram-positive, gram-negative, anaerobic, and atypical organisms. Because of the risk of aplastic anemia, chloramphenicol is no longer the drug of choice for any specific infection.[343,344] Its use in the developed world is limited to life-threatening infections without safer alternatives. However, it is still commonly used in many parts of the developing world.

Structure and Mechanism of Action

The structure of chloramphenicol is shown in Fig. 26.2. It has a paranitrobenzene ring attached to a propanediol group with a dichloracetamide side chain. Thiamphenicol, which is an analogue of chloramphenicol, is available in Europe and Japan, but not in the United States.

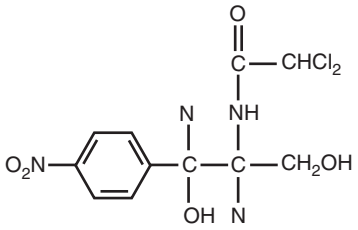

FIG. 26.2 **Chemical structure of chloramphenicol.**

Chloramphenicol inhibits protein synthesis by reversibly binding to the peptidyl transferase cavity of the 50S subunit of the bacterial 70S ribosome. This prevents the aminoacyl-tRNA from binding to the ribosome, thus terminating polypeptide chain synthesis.[345,346] Once chloramphenicol is removed, protein synthesis is able to resume if drug exposure was brief enough.[347] This mechanism results in bacteriostatic activity against most organisms. However, chloramphenicol is bactericidal at clinically achievable concentrations against some meningeal organisms such as *S. pneumoniae, N. meningitidis,* and *H. influenzae.*[348] It has been postulated that the dose-related bone marrow suppression of chloramphenicol is caused by its inhibition of protein synthesis in mammalian mitochondria, which contain 70S ribosomes.[349]

Pharmacology

Administration and Dosage

Chloramphenicol is available in multiple formulations. Oral preparations are 250-mg capsules and a 150-mg/mL palmitate solution. In the United States the oral preparations have not been manufactured since 1991. Intravenous preparations come in 1-g vials of sodium succinate powder for reconstitution. Infusions in adults are generally administered over a period of 30 minutes. There is an otic topical formulation that is a 0.5% otic solution, which is not available in the United States. There are a few different ophthalmic preparations: 0.5% solution, 1% ointment, and 25 mg/vial powder for ophthalmic solution. Only the ointment is available in the United States.

The recommended dose for newborn infants younger than 2 weeks old is 25 mg/kg/day divided in 6-hour intervals. Full-term infants older than 2 weeks can receive 50 mg/kg/day divided in 6-hour intervals. In older children and adults, the dose is 50 mg/kg/day divided in 6-hour intervals. For severe infections, such as meningitis, doses up to 100 mg/kg/day in 6-hour intervals may be used. The maximum daily intake should not exceed 4 g total.[344] Routine monitoring of serum concentrations should be performed, particularly in newborns and those with hepatic dysfunction. Peak serum levels between 15 and 25 mg/L have been suggested for children.[350,351] Others have suggested 10 to 20 mg/L and that levels higher than 25 mg/L are associated with toxicity.[352] If the white blood cell count decreases below 2500/μL, discontinuation of the drug should strongly be considered.

In patients with impaired renal function, usual doses may be administered because active chloramphenicol does not accumulate in the serum.[13] Inactive chloramphenicol metabolites do accumulate in the serum, but this has not been associated with toxicity.[353] Increased clearance of chloramphenicol occurs during dialysis, but this may be important only in patients with hepatic dysfunction. It is recommended that the normal maintenance dose of the drug be administered after dialysis to avoid the possibility of increased clearance.[354] Peritoneal dialysis does not alter the half-life of active chloramphenicol; thus the dose does not need adjustment.[355] Limited data exist for dosing during continuous renal replacement therapy.

Total body clearance of chloramphenicol is reduced in patients with liver dysfunction because the drug is metabolized by the liver.[356] Serum levels should be monitored closely.

Absorption and Bioavailability

Oral chloramphenicol capsules are approximately 80% bioavailable as they are rapidly absorbed from the intestinal tract. Serum levels with the oral palmitate solution are lower than with the capsule form. The palmitate ester is biologically inactive and is absorbed after being hydrolyzed by pancreatic lipases in the upper intestinal tract to free chloramphenicol. Even so, the oral palmitate ester can achieve higher serum levels as compared with the intravenous succinate form.[353] The rate of hydrolysis of the succinate by hepatic enzymes determines the concentration of active chloramphenicol, which is why oral preparations can be more bioavailable. Approximately 44% to 60% of the drug is bound to protein in the serum.[356] After a 1-g dose in adults, peak serum levels occur after 2 hours, with levels of 10 to 13 mg/L.[357] The half-life is 1.6 to 3.3 hours, and therapeutic levels may persist 6 to 8 hours after administration.[358]

Chloramphenicol succinate given intravenously has a wide variability of half-lives in infants. In adults, the serum half-life is approximately 1.2 hours, with an elimination half-life of approximately 4 hours.[353] Intramuscular injection is not recommended owing to variable hydrolysis and/or delayed absorption, leading to unpredictable serum concentrations.[359]

Drug Distribution

Chloramphenicol is highly lipid soluble and has good penetration into many body tissues, penetrates well into pleural and ascitic fluids, and also crosses the placenta and can be found in breast milk. It does not usually achieve adequate concentrations in the bile.[344] In bacterial peritonitis, drug levels in ascitic fluid can exceed half of the serum concentration.[360] Chloramphenicol is also able to achieve high levels in synovial fluid.[361] Even without inflamed meninges, chloramphenicol readily crosses the blood-brain barrier. CSF concentration may be 50% or more of the serum concentration, a higher proportion than with any other antibiotic.[362] Also, unlike most antibiotics, chloramphenicol can penetrate well into many parts of the eye.[363] It has also been shown to be concentrated in alveolar macrophages and polymorphonuclear leukocytes.[364]

Drug Elimination

Chloramphenicol is primarily metabolized by the liver, where it undergoes conjugation with glucuronic acid. The major metabolite is the inactive chloramphenicol glucuronide. This, along with other minor metabolites, is then eliminated through the urine (accounting for about 75%–90% of drug elimination). About 5% to 15% of the unchanged, active drug is eliminated through glomerular filtration. Less than 3% is eliminated through bile. Less than 1% is eliminated in feces.[358]

Antimicrobial Activity

Chloramphenicol has a broad spectrum of activity against many gram-positive and gram-negative bacteria, anaerobes, rickettsiae, chlamydiae, and mycoplasmas. As stated previously, chloramphenicol is generally bacteriostatic but may be bactericidal against meningeal organisms, especially at higher concentrations. For most organisms, susceptibility is defined as having an MIC value of 8 mg/L or less. Exceptions are *S. pneumoniae* (≤4 mg/L) and *H. influenzae* (≤2 mg/L).[365]

Gram-Positive Bacteria

Chloramphenicol has in vitro activity against streptococci, staphylococci, and enterococci. Resistance varies in many different geographic regions. In surveillance studies from the late 1990s, *S. pneumoniae* had higher rates of resistance in the Western Pacific Region and South Africa (17.1%), compared with Europe (12.7%), the United States (10.6%), Canada (4.5%), and Latin America (4.3%).[366,367] Chloramphenicol resistance is typically higher among penicillin-intermediate and penicillin-resistant strains.

In a North American surveillance study, chloramphenicol had activity against 81.6% of all *S. aureus* isolates tested.[368] Another North American study also reported high susceptibility rates to both methicillin-sensitive (96%) and methicillin-resistant (81%) strains.[369] Chloramphenicol has also been demonstrated to have activity against vancomycin-intermediate and vancomycin-resistant strains.[370,371]

Against *Enterococcus* spp., chloramphenicol susceptibility rates in North America have been shown to be 87%.[372] In another study of 886 VRE isolates, investigators reported that 28.6% of 56 *E. faecalis* isolates from North America were chloramphenicol resistant, compared with 7.1% of 14 isolates from Europe. This was in contrast to *E. faecium* isolates. In North America, 0.5% of 776 isolates were resistant, compared with 15% of 40 isolates from Europe, the latter a result of clonal occurrences.[373]

Certain gram-positive bacilli are sensitive to chloramphenicol. *Corynebacterium diphtheriae, L. monocytogenes*, and *B. anthracis* are almost always susceptible, whereas *Corynebacterium jeikeium* and *Nocardia* spp. are usually resistant.[344,374,375]

Gram-Negative Bacteria

Chloramphenicol has variable activity against gram-negative organisms. It is highly active against community-acquired organisms, such as *H. influenzae* and *M. catarrhalis*.[366] *N. meningitidis* and *N. gonorrhoeae* are also both highly susceptible.[376,377]

Activity against *E. coli* is variable worldwide. A surveillance study demonstrated a wide range of chloramphenicol-resistant isolates, from 8% in Curaçao to 82% in Ghana.[378] Another study in the United Kingdom showed that rates of resistance declined from 20.2% in 1991 to 7.9% in 2004.[379] Chloramphenicol generally has good activity against some other members of the Enterobacteriaceae, such as *P. mirabilis, Salmonella* spp., *Shigella* spp., and *Yersinia* spp., whereas others, such as *Klebsiella* spp., *Serratia* spp., *Morganella* spp., and *Enterobacter* spp., are usually more resistant.[380–383] The combination of chloramphenicol with polymyxin B was found to be synergistic in vitro against New Delhi metallo-β-lactamase (NDM)-producing MDR *K. pneumoniae*.[384]

P. aeruginosa is generally resistant to chloramphenicol by means of an active efflux pump.[385] *Acinetobacter* spp. are also generally resistant, whereas *S. maltophilia* is usually susceptible.[386,387] Chloramphenicol has activity against *B. pseudomallei,* whereas *B. cepacia* is usually resistant owing to decreased drug permeability.[388,389]

Anaerobic Bacteria

Most gram-positive and gram-negative anaerobic bacteria are susceptible to chloramphenicol. Anaerobic gram-positive cocci, such as *Peptostreptococcus* spp., are all susceptible at achievable concentrations. Anaerobic gram-positive bacilli, such as *Clostridium, Lactobacillus,* and *Propionibacterium* spp., are also readily susceptible.[390] However, resistant strains of *Clostridium perfringens* and *C. difficile* have been isolated.[391,392] Against gram-negative anaerobic bacteria, chloramphenicol is one of the most active antimicrobial agents. *Bacteroides* spp., *Fusobacterium* spp., and *Prevotella* spp. are all highly susceptible.[390] A US study of 5225 *B. fragilis* group isolates demonstrated that every single one was susceptible to chloramphenicol.[393]

Other Organisms

Chloramphenicol has activity against chlamydiae, mycoplasma, rickettsiae, *T. pallidum, C. burnetii,* and leptospirae.[344,394–396] It is not active against mycobacteria and protozoa.

Clinical Uses

In the developed world, chloramphenicol is generally reserved for serious infections with limited alternative antimicrobial choices because of its well-known toxicities. In the developing world, it is still commonly used because it may be the only broad-spectrum agent available. It also has the advantage of an oral formulation and is available without a prescription in many parts of the world.

Bacterial Meningitis and Brain Abscess

Chloramphenicol remains a useful drug for the treatment of bacterial meningitis in countries that do not have access to third-generation cephalosporins. The use of single-dose intramuscular injections of oily chloramphenicol has been suggested as a nationwide antibiotic of choice for meningitis epidemics.[397] Conclusions from a meta-analysis determined that in circumstances in which ceftriaxone or cefotaxime is not available or affordable, an ampicillin-chloramphenicol combination may be used as an alternative because the two treatment groups did not have important clinical differences.[398] In a Nigerian study of childhood bacterial meningitis, similar results were seen in children treated with chloramphenicol as compared with those given cephalosporins.[399] Another study during a meningococcal meningitis epidemic in sub-Saharan Africa demonstrated that single-dose intramuscular ceftriaxone had clinical outcomes at 72 hours that were comparable to those of single-dose intramuscular oily chloramphenicol and was actually cheaper.[400] Unfortunately, poor outcome was noted in areas with high rates of chloramphenicol-resistant *H. influenzae* type b. This was seen in children treated in Papua New Guinea, where resistance rates were 20%.[401] Areas with penicillin-resistant pneumococcal meningitis also have poor outcomes. In one study in South Africa, 20 of 25 children with chloramphenicol-susceptible, penicillin-resistant strains had poor outcomes, including death.[402]

Chloramphenicol had been used extensively for the treatment of brain abscesses until the availability of safer agents. It may still have a role in treating this infection, particularly in patients with severe penicillin allergies.[403] A case report of a patient with a culture-negative brain abscess in whom multiple antibiotic regimens had failed had dramatic clinical and radiographic improvement after 7 days of chloramphenicol therapy.[404]

Salmonella Infections

Chloramphenicol was once the drug of choice for typhoid fever.[405] It has been largely replaced by the more efficacious and safer fluoroquinolones and ceftriaxone.[406–408] In areas previously burdened with chloramphenicol-resistant *Salmonella* Typhi, sensitive strains are reappearing.[409] Usual treatment for typhoid fever is a 2-week course.[410] Clinical failure rates are rather low (estimated 4.8%), but relapse rates approach 5.6%.[411]

Treatment of acute *Salmonella* gastroenteritis with chloramphenicol is not indicated and usually prolongs fecal excretion after clinical recovery.[412,413] There does not appear to be any benefit with antibiotics in general with mild disease. Infections due to *Salmonella enterica* serotype Choleraesuis and *S. enterica* serotype Typhimurium can be treated with chloramphenicol, but resistance rates as high as 83% have been reported.[414] Third-generation cephalosporins seem to be the drug of choice.

Other Infections

There are various other potential uses of chloramphenicol. It may be the drug of choice in rickettsial infections, such as RMSF, in pregnant women, or in patients allergic to tetracyclines.[415] Overall, it is inferior to the tetracyclines for the treatment of RMSF.[416] It may be useful when it is clinically difficult to distinguish between RMSF and meningococcemia. Chloramphenicol is also effective therapy for Mediterranean spotted fever, tick typhus, epidemic louse-borne typhus, murine typhus, and scrub typhus.[92]

Renewed interest was previously seen in chloramphenicol for treatment of serious VRE infections, including bacteremia.[417,418] However, this enthusiasm has since subsided with the availability of newer agents, such as daptomycin, linezolid, quinupristin-dalfopristin, and tigecycline. Other serious infections may find roles for chloramphenicol, such as a case report of successful treatment in combination with ciprofloxacin for prosthetic valve endocarditis due to *S. maltophilia*.[34,419]

Chloramphenicol has fallen out of favor as inclusion in multidrug therapy for the maintenance treatment of melioidosis, caused by *B. pseudomallei.* In an open-label, randomized study looking at chloramphenicol, doxycycline, and TMP-SMX versus doxycycline and TMP-SMX, the latter group was just as effective and better tolerated than the traditional four-drug group.[175]

Topical chloramphenicol can be used for treatment of bacterial conjunctivitis, and it has excellent intraorbital penetration.[420] It was also found to be superior to topical povidone-iodine in preventing neonatal chlamydial conjunctivitis in a trachoma-endemic area of Mexico.[421] However, two randomized trials have trivialized the early use of topical chloramphenicol. In one trial, 326 children with infective conjunctivitis (250 cases were bacterial) were randomized to chloramphenicol or placebo. There was no significant difference in clinical cure rates at day 7 or with further episodes of conjunctivitis. AEs were rare in both groups.[422] Another trial of 307 patients with infective conjunctivitis compared early topical chloramphenicol, delayed treatment, and no treatment. Delaying antibiotics was found to reduce antibiotic use, resulted in similar duration and severity of symptoms as immediate prescribing, and reduced reattendance for eye infections.[423]

Because of its activity against tularemia, plague, and anthrax, chloramphenicol has been suggested by the Working Group on Civilian Biodefense as an effective alternative agent in the setting of biologic warfare.[424–426]

Mechanism of Resistance

Resistance to chloramphenicol occurs by various mechanisms. The most common is by enzymatic acetylation and inactivation by chloramphenicol acetyltransferases (CATs).[427] Most CAT genes are located on mobile genetic elements, can be distributed to other bacteria, and often confer resistance to other classes of antimicrobial drugs.[428,429] The plasmid-mediated spread of these resistant genes was the cause of the global decrease in *Salmonella* Typhi susceptibility.[430]

Other mechanisms of resistance occur by decreasing membrane permeability, which prevents uptake of chloramphenicol. Some gram-negative bacteria prevent drug penetration by a change in their outer membrane proteins.[431] This was demonstrated to be plasmid mediated in *P. aeruginosa* and chromosomally mediated in *H. influenzae*.[432,433]

Transmembrane efflux pumps have also been shown to confer resistance to both gram-positive and gram-negative organisms.[434,435] Lastly, alteration of a 50S ribosomal subunit was found to reduce chloramphenicol binding in *Bacillus subtilis*.[436]

Adverse Reactions

Hematologic Toxicity

Bone Marrow Suppression

The most significant toxic effect of chloramphenicol is its effect on the bone marrow. There are two types of adverse effects. The first is the more common reversible bone marrow suppression that is a direct pharmacologic effect of the antibiotic and results from inhibition of mitochondrial synthesis. It has been postulated that chloramphenicol does this by binding to the 70S ribosomes in mammalian mitochondria, and by suppressing the activity of ferrochelatase. Ferrochelatase is an enzyme that normally catalyzes hemoglobin synthesis in the mitochondria of bone marrow erythroid cells.[349] As a result, any combination of reticulocytopenia, anemia, leukopenia, or thrombocytopenia may occur. Serum iron levels may also increase in association with a reduced uptake of radioactive iron by the red blood cells, indicating diminished hemoglobin synthesis. Within the bone marrow, there is vacuolization of the erythroid and myeloid precursors. These findings are common, are dose related, and occur during therapy.[437] They are more likely to occur in patients receiving at least 4 g/day or in patients with serum levels over 25 mg/L. The effect is reversible once chloramphenicol is discontinued.[438]

Hemolytic anemia has also been described in patients with glucose-6-phosphate dehydrogenase deficiency who have been treated with chloramphenicol.[439]

Aplastic Anemia

The other hematologic toxicity is the rare, but often fatal, aplastic anemia. This is the reason why chloramphenicol use has been widely replaced by other antibiotics. In an epidemiologic US study, aplastic anemia was estimated to occur in 1 in 24,500 to 1 in 40,800 courses of treatment. It was felt that fatal aplastic anemia appeared to be 13 times more frequent after the use of chloramphenicol than in the general population. It was also not related to dosage.[440] Most cases occur after the completion of therapy, and only about 20% occur during active treatment.[362] Mortality from aplastic anemia has been estimated to be higher than 50%.[441]

The mechanism appears to be different from the previously described dose-related bone marrow suppression and is not completely understood. It is believed to occur as a result of the metabolism of chloramphenicol to toxic nitro derivatives, which subsequently damage hematopoietic stem cell DNA.[442–444] Thiamphenicol, which has not been reported to cause aplastic anemia, does not have a p-NO_2 group. There also appears to be a possible host factor. Aplastic anemia has been reported in identical twins, suggesting a genetic predisposition.[445] A study involving mice demonstrated that mice given chloramphenicol after treatment with busulfan had a progressive decline in pluripotential stem cells as opposed to control mice. This suggests that aplastic anemia might occur in patients with unrecognized preexisting residual bone marrow damage, either genetic or acquired.[446]

It was previously thought that only the oral administration of chloramphenicol was associated with aplastic anemia.[447] This belief changed in the late 1970s after reports of aplastic anemia associated with parenteral administration started surfacing.[448–451] Whether or not chloramphenicol eyedrops cause aplastic anemia has been a topic of debate. In a British study, estimates of serious hematologic toxicity were reported to be 3 in 442,543.[452] Another study from Spain found that the risk of aplastic anemia could not be totally excluded but was less than 1 per million treatment courses.[453] In a review of 426 cases of aplastic anemia, no patient was found to have used chloramphenicol eyedrops.[454]

Chloramphenicol as a cause of childhood leukemia has also been a concern, but reports have been conflicting. A Chinese population-based case-control interview study of 309 childhood leukemia cases and 618 age- and sex-matched control subjects showed a significant dose-response relationship between chloramphenicol and the risk for acute leukemia.[455] However, another study from the same area failed to demonstrate a similar association.[456] Interesting to note, another study from the United States actually found that the odds ratio for total leukemia was decreased among patients who took chloramphenicol.[457]

Gray Baby Syndrome

The gray baby syndrome is a type of circulatory collapse that can occur in premature and newborn infants and is associated with excessively high serum levels of chloramphenicol.[458] It is characterized by an ashen-gray color, abdominal distention, vomiting, flaccidity, cyanosis, circulatory collapse, and death. It usually starts 2 to 9 days after treatment is started. The syndrome is a result of chloramphenicol impairing myocardial contractility by directly interfering with myocardial tissue respiration and oxidative phosphorylation.[459,460] It is believed to occur more often in neonates owing to their diminished ability to conjugate chloramphenicol and to excrete the active form in the urine. There have also been reports in small children and adults who have had accidental overdoses of the drug.[461] The syndrome is generally associated with serum levels of chloramphenicol greater than 50 mg/L and may occur with unexplained metabolic acidosis.[462] To accelerate drug removal, exchange transfusion and charcoal hemoperfusion have been used.[463,464]

Optic Neuritis and Neurologic Side Effects

Optic atrophy and blindness have been described in patients receiving prolonged chloramphenicol therapy.[465] Symptoms typically tend to be reversible, although permanent vision loss may occur. Fundal changes may not necessarily be seen. Supplementation with B vitamins has been used for treatment.[466] Other neurologic manifestations of chloramphenicol use include peripheral neuritis, headache, ophthalmoplegia, depression, and confusion.

Other Reactions

Gastrointestinal adverse reactions, including nausea, vomiting, diarrhea, glossitis, and stomatitis, may occur but are usually not a significant problem. Hypersensitivity reactions, including rashes, drug fever, and anaphylaxis, are rare. Jarisch-Herxheimer reactions have been observed during therapy for syphilis, brucellosis, and typhoid fever.[344] With prolonged oral administration, chloramphenicol may induce bleeding. This may be a result of either bone marrow suppression or reduction in intestinal flora with consequential inhibition of vitamin K synthesis.[467] Chloramphenicol has also been associated with acute porphyria attacks and should be avoided in patients with porphyria.[468] If given during active immunization, chloramphenicol may interfere with the development of immunity.[469]

Drug Interactions

Significant interactions between chloramphenicol and other drugs are listed in Table 26.5. Particular attention needs to be paid to monitoring serum drug levels of chloramphenicol when other agents metabolized by the liver are used concurrently. Chloramphenicol inhibits the activity of several liver enzymes, including CYP2C9 and CYP3A4. Chloramphenicol may prolong the half-life of tolbutamide, chlorpropamide, phenytoin, cyclophosphamide, and warfarin.[470] Concurrent administration of phenytoin and chloramphenicol may result in potentially toxic serum chloramphenicol levels.[471] Rifampin and phenobarbital have been observed to increase the total body clearance of chloramphenicol, thus decreasing the serum concentration. This is felt

TABLE 26.5 Important Drug-Drug Interactions With Chloramphenicol

OBJECT DRUG	EFFECT
Cimetidine	Possible additive or synergistic bone marrow–suppressant effects with concomitant administration Two case reports of fatal aplastic anemia
Cyanocobalamin	Can diminish the therapeutic effect of cyanocobalamin The expected response for the treatment of anemia may be opposed
Cyclophosphamide	Reduced effectiveness of cyclophosphamide due to decreased metabolism to active cyclophosphamide metabolite by chloramphenicol
Cyclosporine	Increased concentration of cyclosporine due to inhibition of metabolism by chloramphenicol Increased risk of renal dysfunction, cholestasis, paresthesias
Phenobarbital	Reduced serum concentration of chloramphenicol by 30%–40% Increased serum concentration of phenobarbital by up to 50%
Phenytoin (and fosphenytoin)	Can increase or decrease chloramphenicol serum concentration Chloramphenicol can inhibit the metabolism of phenytoin, resulting in increased serum concentration of phenytoin
Rifampin, rifabutin	Decreased serum concentrations of chloramphenicol due to induction of chloramphenicol metabolism
Sulfonylureas	May decrease the metabolism of sulfonylureas
Tacrolimus	Increased blood concentrations of tacrolimus due to decreased metabolism of chloramphenicol
Typhoid vaccine	May diminish the therapeutic effect of the live-attenuated Ty21a strain of typhoid vaccine

to occur by the induction of hepatic microsomal enzymes, and serum concentrations should be monitored when these drugs are administered concurrently.[472]

Chloramphenicol, which is primarily a bacteriostatic agent, may antagonize the bactericidal activity of certain penicillins, cephalosporins, fluoroquinolones, and aminoglycosides in vitro.[473–475] The clinical significance may be minimal in most instances. However, caution should be implemented in the event of such combinations for infections requiring bactericidal activity for efficacy, such as meningitis. Chloramphenicol may also delay the response of various anemias to supplementation with iron, folic acid, and vitamin B_{12}.[470]

ACKNOWLEDGMENT

The authors wish to thank Drs. Mirella Salvatore and Burt Meyers for the use of a figure and several tables from a previous edition of this chapter.

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The complete reference list is available online at Expert Consult.

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27 Rifamycins

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SHORT VIEW SUMMARY

OVERVIEW

- The rifamycin class of antibiotics acts through inhibition of RNA synthesis by high-affinity binding to the DNA-dependent RNA polymerase of prokaryotes. Spontaneous resistance limits use as monotherapy. The rifamycins differ in their pharmacokinetics and adverse reactions, but most have significant drug interactions, especially with CYP3A4. In addition to the traditional uses of rifamycins in mycobacterial and staphylococcal infections, there are both newer indications for rifampin and unique rifamycins, such as rifaximin, which are used to treat gastrointestinal infections and disorders. The indications and doses of the specific drugs are detailed as follows.

RIFAMPIN

- *Mycobacterium tuberculosis* infection: 600 mg daily (10 mg/kg)
- *Mycobacterium kansasii* and *Mycobacterium leprae* infection: 600 mg daily
- Staphylococcal prosthetic valve endocarditis: 300 mg three times daily
- Staphylococcal prosthetic joint infections: 300 to 450 mg twice daily or 600 mg daily
- Methicillin-resistant *Staphylococcus aureus* osteomyelitis in combination with TMP/SMX, doxycycline, minocycline, clindamycin of a fluoroquinolone based on susceptibilities: 600 to 900 mg daily (single dose or divided in two)
- Multidrug-resistant gram-negative bacilli: 10 mg/kg intravenously every 12 hours
- *Bartonella henselae* infection: 300 mg twice daily with doxycycline for complicated disease
- Brucellosis: 600 to 900 mg daily with doxycycline (second-line therapy)
- Chemoprophylaxis for *Neisseria meningitidis:* 600 mg twice daily for 2 days
- Chemoprophylaxis for infection with *Haemophilus influenzae* type b: 600 mg daily for 4 days

RIFABUTIN

- *Mycobacterium avium* complex infection: 300 mg daily; 450 mg daily when used with efavirenz and 150 mg daily when used with human immunodeficiency virus (HIV) protease inhibitors
- *M. avium* complex prophylaxis: 300 mg daily (azithromycin is preferred)
- *M. tuberculosis:* 300 mg daily; for HIV-infected patients on protease inhibitors: 150 mg daily; if on efavirenz, 450 mg daily; decrease dose to 150 mg daily for creatinine clearance less than 30 mL/min

RIFAPENTINE

- Tuberculosis (active): 600 mg (10 mg/kg) twice weekly for induction phase and once weekly for maintenance; for latent disease: 900 mg weekly with isoniazid for 12 weeks

RIFAXIMIN

- Hepatic encephalopathy: 400 mg every 8 hours; prevention: 550 mg twice daily
- Recurrent *Clostridioides difficile* (formerly *Clostridium difficile*) infection: 400 mg twice daily
- Traveler's diarrhea: 200 mg three times daily for 3 days
- Irritable bowel syndrome: 550 mg three times daily for 14 days

The rifamycins were isolated by Sensi and colleagues at the Dow-Lepetit Research Laboratories in Milan in 1959 as a mixture of five substances from fermentation cultures of the organism now classified as *Amycolatopsis mediterranei,* in the Actinomycetaceae family. The nickname for the drug, "Rififi," was taken from the title of a French crime movie popular at that time and became the root of the name given to this class, the rifamycins.[1] Rifamycin SV, the first rifamycin used clinically in 1963, was replaced by rifampicin (rifampin) in 1968 due to its improved bioavailability and greater activity against gram-positive and gram-negative bacteria and, especially, *Mycobacterium tuberculosis*. Rifampin is increasingly used as an adjunct for foreign-body infections associated with biofilm production, in combination with other antibiotics for infections due to multidrug-resistant (MDR) gram-negative bacilli and for infections caused by intracellular pathogens.[2] The newer rifamycins have fewer drug interactions and improved pharmacokinetics, resulting in shorter treatment courses for tuberculosis (TB). Some of the newer rifamycins have an expanded spectrum of activity and unique pharmacokinetics, and are used to treat *Clostridioides difficile* (formerly *Clostridium difficile*) infection, hepatic encephalopathy, traveler's diarrhea, and inflammatory bowel diseases. Novel investigational rifamycins and new delivery systems are in development.

STRUCTURE AND MECHANISM OF ACTION

The rifamycins belong to the ansamycin family (from the Latin *ansa,* meaning "handle") of antimicrobial agents because of their basket-like structure containing an aliphatic chain connecting two ends of a naphthoquinone core. Rifampin is the 3-(4-methyl-1-piperazinyl)-iminomethyl derivative of rifamycin SV. The structures of the four rifamycins currently approved in the United States—rifampin, rifabutin, rifapentine, and rifaximin—are shown in Fig. 27.1.

The antibacterial action of the rifamycins results from high-affinity binding to the DNA-dependent RNA polymerase of prokaryotes (RNAP) and inhibition of RNA synthesis. RNAP is a complex enzyme with an $\alpha_2\beta\beta'\omega$-subunit structure. The structure of the *Thermus aquaticus* core RNAP in complex with rifampin, determined at 3.3-Å resolution, showed that rifampin binds to a site on the β-subunit deep within the DNA/RNA channel, in a position that physically hinders the growing oligonucleotide chain after the first- or second-chain elongation step.[3] The structure, combined with biochemical results, led to a mechanistic model for inhibition of RNAP in which rifamycins sterically block synthesis of an RNA product longer than three nucleotides ("steric-occlusion model"). Artsimovich and associates[4] proposed a different mechanism for inhibition of RNAP by rifamycins that operates in addition to, or instead of, the steric-occlusion mechanism. Their model proposes that rifamycins also inhibit transcription at earlier steps through an allosteric signal that disfavors binding of Mg^{2+} ion in the active site, slowing down catalysis and facilitating dissociation of short RNA transcripts. The allosteric model remains controversial because others have shown that rifamycins do not affect the affinity of binding of Mg^{3+} to the RNAP active center.[5]

MECHANISMS OF RESISTANCE

Rifampin possesses highly effective bactericidal action against *M. tuberculosis* and is used as a first-line regimen against this agent. However, many exposed bacteria develop resistance to rifampin at a rate of 10^{-8} to 10^{-9} per bacterium per cell division, which is a potential source of transmissible resistance that selects for resistance in the microbiome.

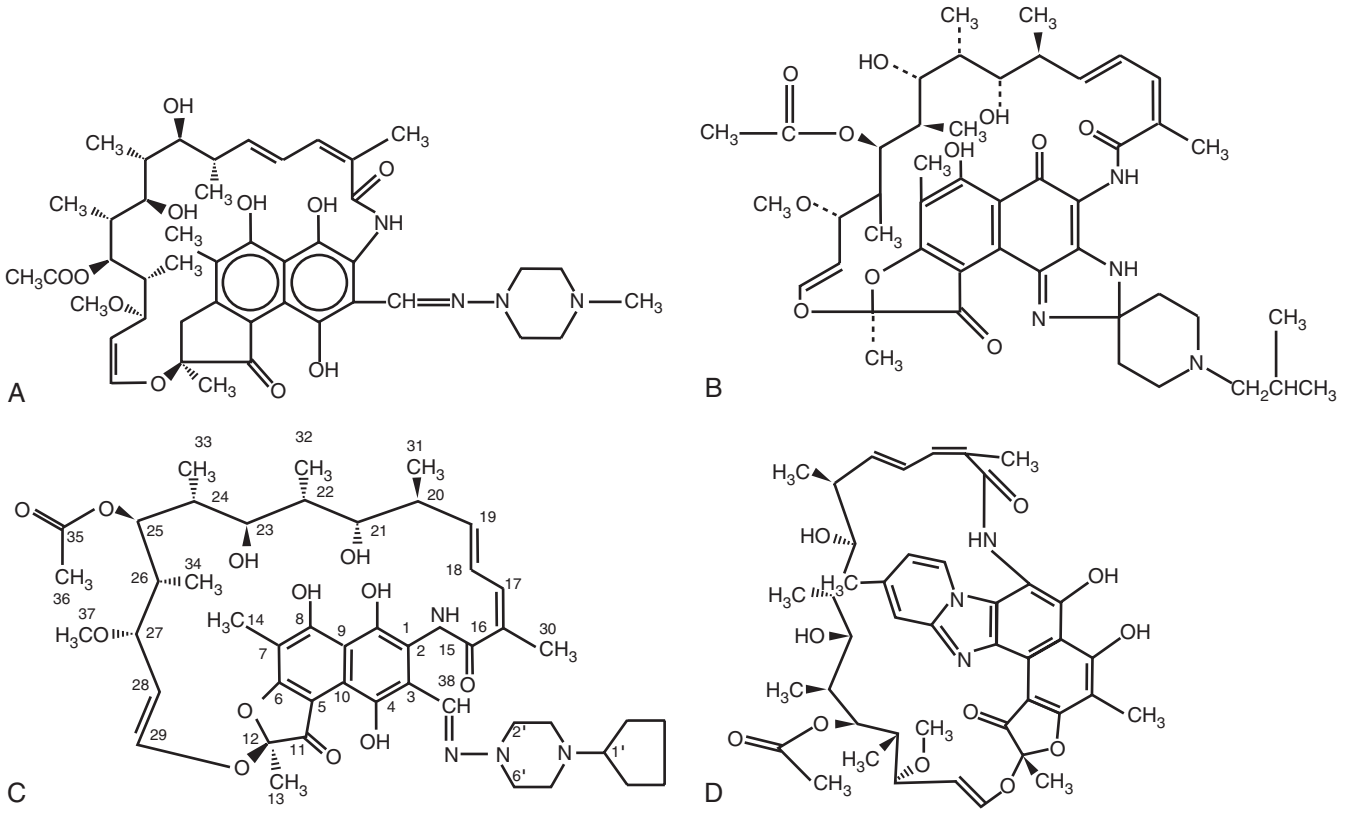

FIG. 27.1 Structures of the four major rifamycins. (A) Rifampin. (B) Rifabutin. (C) Rifapentine. (D) Rifaximin.

Mutations conferring rifampin resistance are confined almost exclusively to the *rpoB* gene in most organisms and result in a decreased affinity of the RNAP to the antibiotic. Greater than 95% of *M. tuberculosis* clinical strains resistant to rifampin harbor a mutation in an 81–base pair region of the *rpoB* gene known as the rifampin resistance determining region (RRDR), which includes codons 507 to 533 and encodes 27 amino acids.[6] Of importance, most of the strains have alterations at codon 526 that result in amino-acid replacements or have missense mutations in codon 531. Studies with patient isolates have confirmed and extended these findings.[7,8] Studies designed to determine the relationship between *rpoB* structural changes and rifampin minimal inhibitory concentrations (MICs) have found a strong correlation. In particular, amino-acid substitutions at positions 526 and 531 conferred high-level resistance to rifampin, rifabutin, and rifapentine.[9] The molecular mechanism in the 4% of the rifampin-resistant *M. tuberculosis* isolates that lack RRDR changes is unknown.

Alternative mechanisms of rifampin resistance also have been observed. *Pseudomonas fluorescens* has impaired cellular uptake of rifampin. Antibiotic modification occurs in strains of *Mycobacterium smegmatis* that carry the *arr* gene, causing ribosylation of rifampin and decreased susceptibility. An *arr2* gene, similar to that in *M. smegmatis,* has been identified on a plasmid in clinical isolates of *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and Enterobacteriaceae. Finally, the enzyme from some species, such as *Nocardia,* is intrinsically less susceptible to rifampin inhibition because of metabolic changes at the critical hydrogen-binding sites.[10]

Delineation of the molecular mechanisms of rifamycin resistance has resulted in the development and application of several polymerase chain reaction (PCR)-based strategies designed to rapidly detect mutations associated with rifampin resistance. One of the latest assays, Xpert MTB/RIF (Cepheid; Sunnyvale, CA), a heminested PCR, detects *M. tuberculosis* and rifampin resistance by amplifying five overlapping probes complementary to the RRDR of the *rpoB* gene and subsequently probes this region for mutations associated with rifampin resistance.[11,12] Compared with conventional diagnosis methods, the Xpert MTB/RIF assay can detect *M. tuberculosis* and rifampin resistance in one sputum sample within 2 hours, is nearly fully automated, and requires minimal biosafety measures. The Xpert MTB/RIF assay has been validated by several studies as a highly sensitive and specific detection test for the diagnosis of pulmonary tuberculosis and rifampin resistance.[13] Recent evidence supports the use of this assay in nonrespiratory clinical samples as well,[14] and the Xpert MTB/RIF assay is now recommended by World Health Organization (WHO) for the diagnosis of extrapulmonary tuberculosis and rifampin resistance.[15]

Other molecular assays to detect rifampin resistance are commercially available. The Genotype MTBDRplus assay (Hain Lifescience; Nehren, Germany)[16] and the INNO-LiPA Rif assay (Innogenetics; Gent, Belgium) have been validated and are currently in use in several countries.[17] In a systematic review, all three commercial tests performed well when detecting drug resistance in clinical samples, although the Xpert MTB/RIF assay had the highest sensitivity and specificity and appeared to be most cost-effective.[18]

Although presence of mutations in the RRDR is highly associated with rifampin resistance, false rifampin resistance detected by Xpert MTB/RIF has been reported.[19] Therefore the package insert of the Xpert MTB/RIF assay states that detection of rifampin resistance must have results confirmed by a reference laboratory. Given that the prevalence of rifampin resistance is low in the United States, the Centers for Disease Control and Prevention (CDC) recommends that a positive result indicating a mutation in the *rpoB* gene should be confirmed by rapid DNA sequencing and growth-based drug susceptibility testing.[20] Cases with discrepant genotypic (resistant) and phenotypic (susceptible) rifampin susceptibility results account for at least 10% of all rifampin-resistant diagnoses obtained with molecular tests. Some of the discrepant strains harbor "low-level" *rpoB* mutations that confer MICs above the level determined for fully susceptible strains but below the critical concentration of 1 μg/mL currently used in standardized culture-based susceptibility tests.[21] The complexity of distinguishing false-positive from true-positive rifampin-resistant results underscores the need for new guidelines to inform therapeutic decision making.

More novel diagnostic tests, such as TB intradermal reaction,[22] pyrosequencing assays,[23] whole-genome sequencing,[24] and next-generation

TABLE 27.1 Comparative Pharmacokinetics of the Rifamycins in Healthy Adults

DRUG	BIOAVAILABILITY (%)	t_{max} (hr)	C_{max} (μg/mL)	$t_{1/2}$ (hr)	PROTEIN BINDING (%)
Rifampin	68	1.5–2	8–20	2–5	80
Rifabutin	20	2.5–4	0.2–0.9	32–67	72–85[a]
Rifapentine	70 with food	4.8–6.6	8–30	14–18	98
Rifaximin	<0.4	0.8–1	3.4 ± 1.6 ng/mL	1.8–4.8	62–67.5

[a]From Blaschke TF, Skinner MH. The clinical pharmacokinetics of rifabutin. *Clin Infect Dis.* 1996;22(suppl 1):S15–S21.
C_{max}, Peak serum concentration; *ng*, nanograms; t_{max}, time to C_{max}; $t_{1/2}$, serum half-life.
Modified from Burman WJ, Gallicano K, Peloquin C. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin Pharmacokinet. *2001;40:327–341.*

sequencing[25,26] are being configured in a low-cost test platform to provide rapid diagnosis and drug susceptibility information for tuberculosis in the developing world and in countries dealing with a dual burden of human immunodeficiency virus (HIV) infection and MDR tuberculosis, where the need is acute.

SHARED PROPERTIES OF THE RIFAMYCINS

The rifamycins are highly lipophilic compounds that share a common chemical structure but differ in their pharmacokinetic properties,[27] summarized in Table 27.1. The systemic rifamycins—rifampin, rifabutin, and rifapentine—have long postantibiotic effects against *M. tuberculosis* of 68 to75 hours.[28] These drugs are metabolized in hepatocytes and intestinal microsomes to deacetylated, hydroxylated, and formyl derivatives.[27] The parent drug and the active 25-*O*-deacetyl metabolite are found in serum, excreted in bile, and eliminated in feces. Repeated administration of rifampin and rifabutin results in autoinduction by gut or hepatic metabolism and increasing biliary secretion, resulting in a decrease in the area under the concentration-time curve (AUC) of both drugs and a decrease in the half-life of rifampin.

The major rifamycin adverse reactions are divided by daily versus intermittent administration. The rifamycins have similar side-effect profiles, except for rifabutin, which is discussed later. Despite the adverse reactions, the overall incidence of drug discontinuation was 1.9% in a large retrospective study, with discontinuation in over half the cases judged to be unnecessary.[29]

Drug-drug interactions are common with the rifamycins. The HIV integrase inhibitors and the direct-acting antivirals (DAAs) for treatment of hepatitis C (HCV) are recent additions to the interaction list. Coadministration of the rifamycins may lead to a decrease in the plasma concentrations of the integrase inhibitor and HCV DAAs. An excellent review of rifamycin drug interactions and dosing recommendations was done by Baciewicz and colleagues.[30] The 2016 antiretroviral interactions can be found in the latest US Department of Health and Human Services Guidelines.[31]

Gastrointestinal (GI) symptoms from the rifamycins are nausea, vomiting, diarrhea, and abdominal pain. Headache, fever, and pruritus can be seen. Rash may occur early and resolve without discontinuation of the drug. More severe reactions include urticarial and diffuse rash, which may be accompanied by systemic symptoms. A lupus-like syndrome with detection of antinuclear antibodies has been reported with rifampin and rifabutin, often attributed to cytochrome P (CYP) drug interactions, and usually resolves after discontinuation of the drug.[32]

RIFAMPIN

Pharmacokinetics

Rifampin is dosed daily as 600 mg in adults and 10 to 20 mg/kg in children. The drug undergoes rapid and complete absorption, improved if taken on an empty stomach. Rifampin is the only rifamycin available in an intravenous formulation. It is widely distributed to most body tissues and fluids, including the cerebrospinal fluid (CSF), where doses of 20 mg/kg in children achieve mean CSF levels of 2 μg/mL.[33] Concentrations in bone are similar to or exceed serum concentrations,[34] and the drug crosses the placenta and enters breast milk. Rifampin undergoes an enterohepatic circulation and deacetylation with rapid elimination in bile. Urinary excretion is 13% to 24% with no dose adjustment necessary in renal insufficiency. Dose adjustment is recommended with severe hepatic insufficiency. The rifampin substrate is red, and the high lipophilic properties and wide distribution often turn body fluids, such as urine, tears, sweat, and feces, and contact lenses per se, red-orange.

Rifampin has the greatest effect on induction of hepatic and intestinal enzymes, especially CYP3A. Induction is responsible for the majority of rifampin drug interactions. The maximal effect occurs approximately 1 week after initiation of therapy. The serum concentration of rifampin is rarely affected because the metabolism is not mediated by CYP. Most drug interactions result from induction of metabolic enzymes, especially CYP3A4, in the liver and small intestine. Rifampin and the other rifamycins have numerous interactions, outlined in Table 27.2, that usually result in a decrease in the AUC or maximum drug concentration (C_{max}) of the concomitant drug, requiring dose adjustments. Rifampin also induces CYP1A2, CYP2C, and CYP2D6 and some drug transporter proteins, including P-glycoprotein, a transmembrane protein that acts as a cellular efflux pump. The HIV protease inhibitors are especially problematic when used with rifampin, with significant decreases in AUC.

Adverse Reactions

Allergic reactions to rifampin range from 0.01% to 0.26% and often occur in patients previously treated or with intermittent dosing. Type I hypersensitivity reactions, mediated by immunoglobulin (Ig)E antibodies, can occur with symptoms ranging from angioedema, bronchospasm, and urticaria to shock. Anaphylaxis can occur within minutes of the dose or be associated with prodromes, such as rash, from prior or intermittent dosing. Patients with HIV infection had poorer outcomes, and antirifampin serum IgE antibodies were detected.[35]

The rifampin-associated flulike syndrome consists of fever, chills, malaise, myalgias, and headache several hours after dosing and usually resolves within several hours. Flulike symptoms are most commonly seen with intermittent regimens, although HIV-infected patients experience a higher incidence of flulike symptoms with higher daily dosages, which may be due to a direct toxic effect of the drug in this population.[35] The incidence of flulike syndrome escalates with higher doses (900–1200 mg) given weekly and can be as high as 54%.[35] The flulike syndrome usually develops after 3 months of therapy and is rarely associated with thrombocytopenia, hemolysis, and renal failure. The etiology is believed due to circulating IgG or IgM immune complexes that fix complement on endothelial cells or bind on the surface of cytokine-producing cells.[35] After a change to weekly dosing or intermittent therapy, a more robust immune response may be seen on rechallenge. Switching to daily rifampin therapy usually results in disappearance of all symptoms. Thrombocytopenia and hemolysis are also likely immune mediated with formation of antigen-antibody complexes that bind to erythrocytes and platelets. Recent reports highlight newly observed adverse reactions, including recurrent disseminated intravascular coagulation, with intermittent and continuous dosing.[36] Acute renal failure from rifampin usually occurs with intermittent dosing and presents as acute interstitial nephritis with tubular necrosis. Less common presentations are rapidly progressive glomerulonephritis and light-chain proteinuria with polyclonal κ and λ chains. Most cases are secondary to adhesion of antirifampin antibodies to the renal tubular epithelium with subsequent complement binding. Recovery is usually complete after stopping the drug.[37] Hemolysis and thrombocytopenia may occur in conjunction with renal toxicity.

TABLE 27.2 Major Rifamycin Drug Interactions

Anticoagulants
Warfarin
Clopidogrel
Dabigatran
Rivaroxaban
Apixaban

Antimicrobial Agents
Atovaquone
Azoles
Caspofungin
CCR5 antagonists[a]
Chloramphenicol
Clarithromycin
Cobicistat
Dapsone
Doxycycline
HCV direct-acting antivirals[d]
HIV integrase inhibitors[a]
HIV NNRTIs[b]
HIV protease inhibitors[c]
Linezolid
Maraviroc
Mefloquine
Moxifloxacin
Praziquantel
Tenofovir alafenamide

Cardiovascular Drugs
β-Blockers
Calcium channel blockers
Digoxin
Disopyramide
Endothelin receptor antagonists and agonists[f]
Losartan
Quinidine
Tocainide

Immunosuppressive Agents
Cyclosporine
Mycophenolate mofetil
Sirolimus
Tacrolimus

Endocrine Agents
Biguanide
Glucocorticoids
HMG-CoA reductase inhibitors
Levothyroxine
Oral contraceptives
Sulfonylureas
Tamoxifen

Neuropsychiatric and Neurologic Agents
Benzodiazepines
Bupropion
Citalopram
Clozapine
Haloperidol
Lamotrigine
Nortriptyline
Phenytoin
Risperidone

Opiates/Analgesics
Buprenorphine[e]
Cyclooxygenase inhibitors
Fentanyl transdermal
Ketamine
Methadone
Oxycodone
Propofol

Other Drugs
Cabozantinib
Cediranib
Deferasirox
Imatinib
Mirodenafil
Omeprazole
Roflumilast
Theophylline
Vandetanib

[a]Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in adults and adolescents living with HIV. Department of Health and Human Services; 2016. http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf (tables 18, 19a, 19b, 19d, and 19e).
[b]Efavirenz, nevirapine, etravirine, rilpivirine.
[c]Rifabutin preferred for use with HIV protease inhibitors in decreased doses.
[d]Includes N53/4A protease inhibitors, nucleoside and nucleotide NS5B inhibitors, N5SA inhibitors, and nonnucleoside NS5B polymerase inhibitors.
[e]Reported with rifampin only.
[f]Includes bosentan, ambrisentan, and atrasentan.
HCV, Hepatitis C virus; *HIV,* human immunodeficiency virus; *HMG-CoA,* 3-hydroxy-3-methylglutaryl coenzyme A; *NNRTIs,* nonnucleoside reverse-transcriptase inhibitors.
Modified with permission from Baciewicz AM, Chrisman CR, Finch CK, et al. Update on rifampin, rifabutin, and rifapentine drug interactions. Curr Med Res Opin. *2013;29:1–12.*

Rifampin can cause changes in liver function, but serious injury is usually confined to patients with underlying liver disease due to alcohol, viral hepatitis, and other hepatotoxins, such as isoniazid, which often is coprescribed. Long-term therapy is associated with minor, transient elevations in serum aminotransferase levels in 10% of patients. Liver injury from rifampin is primarily cholestatic. In most patients serum bilirubin levels increase during the first few days of therapy and normalize spontaneously. Within several weeks of starting rifampin in patients with underlying liver disease, there may be a significant increase in direct and total bilirubin without evidence of liver injury. The bilirubin elevation is attributed to inhibition of bilirubin excretion and may be related to genetic defects in multidrug-resistance protein 2 (MRP2), the major bilirubin glucuronide transporter in hepatocytes. There are many reports of hepatitis and jaundice in patients with tuberculosis treated with rifampin, isoniazid, and pyrazinamide. However, the actual incidence of hepatotoxicity from rifampin is 1.1%.[38] When rifampin is combined with isoniazid, there is an increased incidence of hepatotoxicity. One explanation is an additive hepatotoxic effect; however, a synergistic effect is favored. The induction of isoniazid hydrolase by rifampin is believed to lead to increased production of hydrazine, which can be activated by rifampin-induced CYP to hepatotoxins. This is seen more frequently in slow acetylators with the *N*-acetyltransferase 2 genotype.[39] The recommendation is to discontinue all antituberculous medications if hepatotoxicity occurs. If the patient is severely ill, an option is to start three new medications (often a fluoroquinolone, streptomycin, and ethambutol) until liver function normalizes, at which time the original drugs are introduced one at a time, usually starting with rifampin.[40]

Severe hepatitis has been reported in 1.2% to 13% of patients treated with rifampin and pyrazinamide for latent tuberculosis infection, leading to updated recommendations regarding use and monitoring of this combination.[41] A single nucleotide polymorphism in the gene encoding the organic anion transporting polypeptide 1B1, which plays a role in transporting bile acids and rifampin, has recently been identified with the *15 haplotype, a predisposing factor to rifampin-induced liver injury.[42]

Antimicrobial Activity

Although commonly associated with treatment of tuberculosis, the rifamycins exhibit broad antibacterial activity. Rifampin is most potent against gram-positive bacteria, including *Staphylococcus aureus,* coagulase-negative staphylococci, *Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus viridans, C. difficile,* and *Listeria monocytogenes.* Gram-negative bacterial coverage includes *Haemophilus influenzae, Neisseria meningitidis,* and *Helicobacter pylori.*[43] Rifampin has synergistic activity with other agents against MDR bacteria, especially *P. aeruginosa* and *Acinetobacter baumannii.* In addition to potent activity against *M. tuberculosis,* rifamycins have activity against nontuberculous mycobacteria, including *M. avium-intracellulare* complex (MAC), *M. kansasii,* and *M. marinum.*[44] Rifampin has activity against intracellular pathogens, including *Chlamydia, Legionella, Brucella,* and *Bartonella* spp.[45,46] The MICs of rifampin for representative organisms are shown in Table 27.3.

RIFABUTIN

Pharmacokinetics

Rifabutin is a spiropiperidyl rifamycin analogue with a slower and larger volume of distribution compared with rifampin and a long terminal half-life, permitting once-daily dosing. Food does not affect the AUC but increases the peak concentration. Rifabutin is more lipid soluble than rifampin and extensively distributed throughout the body, with a CSF concentration one half that of serum after a 450-mg dose.[47] The extensive redistribution of rifabutin in tissues leads to minimum plasma concentrations of 10% to 15% of the peak 24 hours after an oral dose. The two active metabolites are 25-*O*-desacetyl rifabutin and 31-OH-rifabutin. Fifty-three percent is excreted in urine as the primary active C-25 desacetyl metabolite, and dose reduction is recommended for a creatinine clearance less than 30 mL/min.[48] Rifabutin should be administered with caution in patients with severe hepatic disease. The ability to induce metabolic enzymes, especially CYP3A4, is least with rifabutin,[27] and cytochrome P450 (CYP) 3A is the major isozyme involved in the hydroxylation of rifabutin.[27] When used with HIV protease inhibitors, this leads to an increase in the rifabutin level, and decreased doses are required.[27] Rifabutin concentrations can be significantly increased, with the potential for complex bidirectional interactions with concomitant administration of other medications, particularly in patients taking antiretrovirals. Major interactions are seen with fluconazole, ketoconazole, clarithromycin, and the HIV protease and integrase inhibitors.[31]

Adverse Reactions

Rifabutin has a unique toxicity profile, including uveitis, leukopenia, and polyarthralgias. In a study of elderly patients with pulmonary MAC treated with 600 mg of rifabutin with ethambutol, streptomycin, and a macrolide, adverse events were seen in 77%, requiring dose adjustment in over half.[49] The most common event was leukopenia, followed by GI symptoms, abnormal liver enzymes, a diffuse polyarthralgia syndrome in 19%, and anterior uveitis in 8%. Uveitis was seen in patients receiving clarithromycin and is likely secondary to a drug interaction that raises rifabutin serum concentrations. Uveitis may mimic an infectious endophthalmitis with the presence of a hypopyon and has been reported in both HIV-infected and noninfected patients with pulmonary MAC infection with the 600-mg dose. Cases typically occur after several months of therapy, predominantly involve the anterior chamber, and

TABLE 27.3 Antimicrobial Activity of Rifampin

SPECIES	MIC[a] RANGE (μg/mL)	MIC_{90} (μg/mL)	MIC_{50} (μg/mL)
Staphylococcus aureus	0.008–0.015	0.015	0.015
Staphylococcus epidermidis	0.004–0.015	0.015	0.015
Group A streptococci	0.03–0.12	0.12	0.12
Group B streptococci	0.25–1	1	1
Streptococcus pneumoniae	0.06–32	0.12	0.12
Streptococcus viridans	0.03–8	0.06	0.12
Enterococcus faecalis	1–8	2	8
Haemophilus influenzae	0.5–64	1	1
Neisseria meningitidis	0.015–1	0.03	0.5
Listeria monocytogenes	≤0.12–0.25	≤0.12	0.25
Escherichia coli	8–16	8	16
Klebsiella pneumoniae	16–32	32	32
Proteus mirabilis	4–8	4	8
Enterobacter cloacae	16–64	64	64
Acinetobacter spp.	4–16	8	8
Clostridioides difficile (formerly *Clostridium difficile*)	0.10–>100	1.56	>100
Pseudomonas aeruginosa	32–>64	32	64
Mycobacterium tuberculosis	0.06–0.25	0.25	0.25
Mycobacterium avium-intracellulare	0.78–>100	6.25	50
Mycobacterium kansasii	0.025–3.13	0.2	3.13
Mycobacterium marinum	0.1–0.39	0.2	0.39
Mycobacterium fortuitum	12.5–100	50	100
Mycobacterium scrofulaceum	0.1–6.25	0.78	6.25
Legionella spp.	0.001–0.5	≤0.25[b]	0.125
Bartonella spp.	0.03–0.06	0.12	0.25
Chlamydia spp.	0.0075–0.03	0.015	0.031
Brucella spp.	0.5–1	2	4
Helicobacter pylori	0.125–0.75	0.125	0.125

[a]MIC ≤2 μg/mL is considered susceptible.
[b]Geometric mean MIC.
MIC, Minimal inhibitory concentration; *MIC_{50},* minimal inhibitory concentration for 50% of isolates; *MIC_{90},* minimal inhibitory concentration for 90% of isolates.

respond to corticosteroids and cycloplegics. The etiology is believed to be an immunologic reaction to cell wall proteins of dead organisms, although a direct toxic effect of rifabutin has been suggested.[50] Uveitis has been seen in HIV-infected patients receiving 300-mg dosing for prophylaxis as late as 14 months after initiation,[51] often in combination with fluconazole or azithromycin or both. The flulike syndrome has been reported with intermittent therapy. Hepatitis is more likely from concomitant use of hepatotoxins.

Antimicrobial Activity

Rifabutin is active against *M. tuberculosis* and *M. avium complex*.

RIFAPENTINE

Pharmacokinetics

Rifapentine, a cyclopentyl rifamycin, is a more potent and longer-acting rifamycin.[52] Food increases the absorption, and a high-fat meal results in a 50% increase in serum concentration and is recommended to be taken with a meal. Rifapentine achieves high intracellular concentrations, exceeding that of rifampin. CSF concentrations are undetectable.[52] Less than 10% of rifapentine is excreted in urine unchanged.[27] Rifapentine does not induce its own metabolism. The primary route of metabolism, mediated by an esterase enzyme, is nonoxidative to the 25-desacetyl metabolite. Rifapentine also induces CYP3A4 and CYP2C and can interact with the same drugs as rifampin. The degree of interaction correlates with the dose and frequency of administration. Rifapentine is not recommended for use in combination with the HIV protease inhibitors and may be associated with decreased absorption.[27]

Adverse Effects

Rifapentine is better tolerated than rifampin, likely due to higher protein binding. Once-weekly dosing has a lower incidence of the flulike syndrome[52]; however, cases with hemolysis and renal failure have been reported. The incidence of hepatotoxicity is similar to rifampin. Hyperuricemia has been reported with rifapentine in up to 21.3% of patients.[52]

Antimicrobial Activity

Rifapentine is active against *M. tuberculosis* only.

RIFAXIMIN

Pharmacokinetics

Rifaximin has an additional ring linking the C-3 and C-4 positions. Absorption after an oral dose is minimal secondary to low intestinal permeability and water solubility. Less than 0.01% of the dose is detectable in plasma, and 97% is recovered unchanged in the stool. Fecal concentrations were 8000 μg/mL in one study.[53] The AUC increases 10 to 20 times, and the C_{max} increases 6 to 10 times in patients with cirrhosis.[54] Rifaximin can induce cytochrome P450 3A4 but has no significant drug interactions secondary to extremely low systemic concentrations. Although caution is recommended when using rifaximin in patients with Child-Pugh class C cirrhosis, no dosage adjustments are required.

Adverse Effects

Rifaximin is associated with few adverse effects. An unusual case of rifaximin-associated neutropenia was described in a patient receiving 1200 mg daily with severe ulcerative colitis and a transjugular intrahepatic portosystemic shunt, suggesting increased absorption and decreased metabolism.[55]

Antimicrobial Activity

Rifaximin is bactericidal against enterotoxigenic and enteroaggregative strains of *E. coli, Salmonella, Shigella,* and *Campylobacter* and *C. difficile*. Antiprotozoal activity includes *Cryptosporidium parvum* and *Blastocystis hominis*.[56] Use in inflammatory bowel diseases, hepatic encephalopathy, and effects on the microbiome are discussed as follows.

IMMUNE-MODULATING EFFECTS OF RIFAMPIN

In addition to bactericidal activity, rifampin has immune-modulating properties. An in vitro study showed inhibition of interleukin (IL)-1β and tumor necrosis factor-α (TNF-α), with increases in IL-6 and IL-10 when rifampin was added to stimulated human monocytes.[57] Incubation of rifampin with proinflammatory cytokines in alveolar cells and human epithelial liver cells (HepG2) led to increased production of nitric oxide and increases in IL-8, interferon-γ (IFN-γ), and IL-β1.[58] Cytoprotective effects of rifampin have also been observed. Pretreatment of human lung carcinoma cells with rifampin before infection with *A. baumannii* significantly decreased the rate of cell death by reducing oxidative stress and release of TNF-α and IL-6, in the absence of bactericidal activity.[59] Pretreatment with rifampin in animal models of *S. pneumoniae* meningitis decreased neuronal loss by decreasing inflammation and free-radical production in CSF.[60,61] Rifampin also decreased TNF-α production and inhibited macrophage apoptosis in *M. tuberculosis* infection.[62] In vivo, patients with tuberculosis who mounted a higher IFN-γ response after 2 months of therapy had higher $AUC_{0–8}$ values to rifampin, which predicted a disease-free outcome. It is unclear whether the higher rifampin concentrations led to a stronger IFN-γ response, but the authors suggest that IFN-γ may be a useful surrogate marker for predicting outcome in tuberculosis.[63]

RIFAMYCINS FOR THE TREATMENT OF TUBERCULOSIS

The treatment of active tuberculosis requires combination chemotherapy to avoid the selection of naturally occurring drug-resistant mutants.

Since the introduction of rifampin 4 decades ago, the rifamycins have been cornerstone agents in the treatment of tuberculosis, particularly when it was shown that rifampin in combination therapy reduced the overall treatment time from 18 to 9 months.[64] In addition, it has been shown that infections caused by rifampin-resistant strains of *M. tuberculosis* require a longer duration of treatment and are associated with higher rates of treatment failure than those caused by drug-susceptible strains.[64]

The current rifamycins used in the treatment of tuberculosis include rifampin, rifapentine, and rifabutin. Rifampin has better penetration into cavitary lung lesions, it has a long track record of safety, it is inexpensive and globally available, and thus it is currently the first-line rifamycin drug for treatment of tuberculosis.[65] Rifampin is bactericidal against *M. tuberculosis*, and among the first-line agents against tuberculosis has the most potent sterilizing activity, which is defined as the ability of a drug to kill persistent and/or metabolically dormant organisms. This may be in part due to its rapid onset of action.[66] International guidelines recommend a maximum daily dose of 600 mg (10 mg/kg) for daily and intermittent treatment. However, these recommendations were based on historical pharmacokinetic,[67,68] toxicity,[69,70] and cost arguments[71] and have largely been refuted by more recent scientific data.[72] Data from in vitro systems and animal models provide compelling evidence that rifampin's activity against *M. tuberculosis* is concentration dependent and correlates best with the quotient of the AUC and the MIC.[72] Using the mouse model, Jayaram and coworkers[73] found that the 10 mg/kg dose of rifampin used in humans falls at the low end of a steep and tall dose-response curve, indicating that increasing doses may produce log linear increases in bactericidal activity. These data are supported by small-scale studies that showed an early bactericidal activity using high doses (20 mg/kg) of rifampin.[74,75] However, studies of early bactericidal activity reflect bactericidal and not necessarily sterilizing activity. Therefore more studies are needed to evaluate the potential of a higher dose of rifampin to further reduce treatment duration to less than 6 months. Evidence of this potential is provided by the study of Kreis and associates,[76] who evaluated a 3-month regimen with daily rifampin (1200 mg), isoniazid (900 mg), and streptomycin (1 g) and achieved near-complete sputum culture negativity after 90 days, without additional toxicity. Unfortunately, this study had several limitations. First, without pyrazinamide, the recurrence rate was 11.4% during the first year after treatment. Second, there was no direct comparison of daily administration of the current standard dose (600 mg) against daily administration of higher doses. In a recent trial 2 weeks of rifampin up to 35 mg/kg were safe and well tolerated.[77] This observation was confirmed by another study showing that a dose of 35 mg/kg rifampin was safe, reduced the time to culture conversion, and could be a promising component of future, shorter regimens.[78]

The primary toxicities attributed to rifampin are discussed in detail in the section on adverse reactions and are usually associated with intermittent dosing and not expected to occur more frequently with increased daily dosing. Transaminitis and increased bilirubin levels after starting treatment do not appear dose related,[79] and hepatoxicity usually occurs with preexisting liver disease or with use of concomitant hepatotoxins.[80] Systemic side effects, such as the "flulike syndrome," are related to intermittent treatment rather than to the dose of rifampin.[81] Furthermore, rifampin has been used at higher doses for nonmycobacterial infections, such as brucellosis,[82] staphylococcal infections,[83] and cutaneous leishmaniasis,[84] without reports of increased adverse effects, supporting the idea that the majority of rifampin's adverse effects are idiosyncratic and not dose related.

A recent study has suggested that high-dose rifampin may play a role in infections caused by low-level resistant strains[85] and may help prevent the emergence of drug resistance.[86] Recent data also suggest that regimens containing higher doses of rifampin are safe and could be associated with a survival benefit in patients with severe disease.[87]

In summary, higher doses of rifampin may help reduce the duration of treatment of tuberculosis without a significant increase in adverse events. However, more extensive, dose-ranging, tolerability, and extended bactericidal activity studies of high doses of rifampin are still undergoing, and their results should be expected soon.

Rifapentine, with its more potent in vitro activity (MIC, 0.06 vs. 0.25 μg/mL)[88] and longer serum half-life (11–18 hours vs. 2–4 hours) than rifampin,[27,89] was developed with the goal to provide an option for once-weekly therapy for tuberculosis.[90] The approved US Food and Drug Administration (FDA) dose for the treatment of active tuberculosis is 600 mg administered once or twice weekly in combination with other antimycobacterial drugs. However, it has seen little clinical use because once-weekly rifapentine-isoniazid during the continuation phase of treatment was associated with greater drug-susceptible relapse among HIV-negative patients with lung cavitation[91] and a significant incidence of acquired rifamycin monoresistance among HIV-positive patients. Based on this study, rifapentine intermittent therapy should not be used for treatment of tuberculosis in HIV-infected patients[92] A recent randomized study compared standard continuation phase therapy with regimens containing rifapentine and moxifloxacin in place of isoniazid and found similar rates of efficacy and no acquired drug resistance; however, larger studies are needed to assess the risk of rifamycin resistance.[93]

Recent work using the mouse model of antituberculosis chemotherapy has shown that regimens containing rifapentine administered 5 days per week can achieve durable cure without relapse after 3 months. Their results also indicated that rifapentine is four times more potent than rifampin in the murine model.[94] These encouraging results prompted a randomized, multicenter, phase II clinical trial conducted by the Tuberculosis Trials Consortium (TBTC study 29) to determine the safety, tolerability, and antimicrobial activity of a regimen in which rifampin 10 mg/kg was replaced by rifapentine 10 mg/kg administered 5 days per week during the first 8 weeks (intensive phase) of combination treatment of pulmonary tuberculosis.[95] Their results showed that the rifapentine regimen was safe but not significantly more active than a standard rifampin regimen by the surrogate end point of culture status at completion of the intensive phase. However, one limitation of this study was administration without food, which has been shown to increase the C_{max} of rifampin[96] but decreases rifapentine bioavailability.[97]

The optimal rifapentine dose and schedule for treating tuberculosis in humans have not been established, but the mouse model suggests that the bactericidal and sterilizing activity of rifapentine increases virtually without plateau up to doses of 160 mg/kg and that daily dosing improves outcomes. Dooley and colleagues[98] conducted a rifapentine dose-escalation study in healthy adults and showed that daily rifapentine doses of up to 20 mg/kg administered with food were safe and produced rifapentine exposures exceeding those in mice receiving 10 mg/kg. Their results provided safety and tolerability data to support the need of future clinical trials of rifapentine at high daily doses for treatment of tuberculosis. A recent trial showed that high rifapentine exposures were associated with high levels of sputum sterilization at completion of intensive phase, suggesting that regimens that deliver high rifapentine exposures can shorten duration of treatment to less than 6 months.[99]

Rifabutin has a much longer half-life than rifampin (35 hours vs. 2–4 hours). In clinical trials, the efficacy of rifabutin administered daily or intermittently has been shown to be similar to that of rifampin.[100,101] Rifabutin's primary advantage in the treatment of tuberculosis is its reduced induction of hepatic metabolism (cytochrome P450 system)[102] and its usefulness in patients with tuberculosis and HIV coinfection who are also receiving antiretroviral therapy (ART). Rifampin markedly decreases serum concentrations of the HIV type 1 (HIV-1) protease inhibitors[103] and has substantial effects on concentrations of nonnucleoside reverse-transcriptase inhibitors (NNRTIs).[104] Therefore rifabutin-based therapy was recommended by the CDC as a way of avoiding serious drug-drug interactions and allowing use of rifamycins during treatment of tuberculosis in HIV-coinfected patients.[105] However, Benator and colleagues[91] raised the question of whether the use of rifabutin was a cause of acquired rifampin resistance in HIV-infected patients who had low $CD4^+$ T-lymphocyte counts. Additional studies suggest that low concentrations of the rifamycin component related to intermittent dosing, rather than the specific agent, determine the risk for acquired rifamycin resistance.[106] Recently, Naiker and colleagues[107] evaluated the pharmacokinetics of different doses of rifabutin in African HIV-infected patients with tuberculosis and on lopinavir/ritonavir-based ART regimen. They demonstrated that a daily dose of 150 mg of rifabutin in

combination with lopinavir/ritonavir safely maintained rifabutin plasma concentrations.[107]

The most recent guidelines for the treatment of active tuberculosis in HIV-coinfected patients recommend that, despite pharmacokinetic drug interactions, a rifamycin should be included in regimens for patients receiving antiretroviral agents, with dosage adjustment if necessary. If a protease inhibitor–based regimen is used, rifabutin is the preferred rifamycin, but the dose should be decreased from 300 mg daily to 150 mg daily if given with unboosted protease inhibitor or to 150 mg every other day if given with ritonavir. If efavirenz-based regimen is used, the rifabutin dose should be increased from 300 mg to 450 mg daily, and the dose of efavirenz should remain unchanged.[31] However, rifampin is the rifamycin of choice for patients taking efavirenz-based ART, because efavirenz reduces the concentration of coadministered rifabutin.

Recent data examined the tolerability of rifabutin when coadministered with other medications affected by rifampin's induction of cytochrome P450, such as methadone and coumadin, and in patients who previously had a rifampin-related adverse effect. Their results showed that rifabutin is well tolerated and has limited the need for dosing adjustments of other medications. Their results also suggest that rifabutin could be considered as an alternative agent in patients who develop rifampin-related liver injury but that rifabutin should be used with caution in patients with other types of underlying liver disease.[108] Data from tuberculosis-infected transplant patients receiving tacrolimus or cyclosporine also suggest that rifabutin should be used in this population, instead of rifampin, to avoid pharmacologic interactions and achieve a lower risk for acute graft rejection.[109]

RIFAMYCINS FOR TREATMENT OF LATENT TUBERCULOSIS INFECTION

The current standard regimen for the treatment of latent *M. tuberculosis* infection (LTBI) is 9 months of daily isoniazid. Shorter LTBI regimens based on other regimens are of growing interest, given concerns about isoniazid-related hepatoxicity, poor adherence attributed to long-duration isoniazid regimens, and the growing proportion of tuberculosis cases that are resistant to isoniazid in foreign-born persons.

Early experiments in a mouse model demonstrated the potential efficacy of three short-course regimens that included rifampin, with or without companion medications. The results suggested that 2 months of therapy with rifampin with pyrazinamide or 3 months of therapy with rifampin alone were more effective than 6 months of therapy with isoniazid alone.[110] This study prompted a number of clinical trials with three different rifampin-containing regimens. A 2-month regimen of rifampin and pyrazinamide was shown to be as effective as isoniazid.[111,112] Unfortunately, this regimen has been largely abandoned, owing to severe liver toxicity.[113] Results from several randomized trials evaluating the combination of isoniazid and rifampin given for 3 to 4 months have shown an equivalent completion rate, toxicity, and effectiveness as 6 to 9 months of isoniazid.[114,115] A 4-month daily rifampin regimen has been recommended by the American Thoracic Society (ATS) as an alternative regimen in the treatment of LTBI after few studies have consistently found significantly better completion rates and significantly lower serious adverse effects, particularly hepatotoxicity, when compared with 9 months of isoniazid.[116]

With the use of animal models, it was shown that once-weekly isoniazid plus rifapentine combination therapy for 18 weeks was an effective preventive regimen with sterilizing potency and bacillary load reduction comparable with daily isoniazid therapy for 18 weeks.[117,118] These promising results were followed by a large-scale randomized clinical trial that was recently completed and published. Sterling and colleagues[119] showed that directly observed, once-weekly therapy with rifapentine plus isoniazid for 3 months was as effective as self-administered daily isoniazid for 9 months, with higher completion rates and lower rates of severe adverse events. This regimen has now been recommended as an equal alternative to the 9-month isoniazid regimen for otherwise healthy patients aged 12 years or older with LTBI.[120] A recent systematic review compared the efficacy and completion rate of different LTBI drug regimens: INH plus rifapentine once weekly for 12 weeks, 3 to 4 months of daily INH and rifampin, 6 and 9 months of daily INH, and 4 months of rifampin alone. The review showed no significant difference between the different groups in efficacy, but regimens of 3 to 4 months duration were more likely to be completed than longer regimens.[121]

RIFAMYCINS FOR NONTUBERCULOUS MYCOBACTERIAL INFECTIONS

Mycobacterium leprae

Rifampin is rapidly bactericidal against *M. leprae,* with bacteriologic success within 3 weeks of starting therapy. Even a single high dose was efficacious in a mouse footpad model.[122] Rifampin should never be used as monotherapy because of rapid development of resistance. Although not FDA approved for leprosy, rifampin is recommended by WHO for every manifestation of disease. Recommendations are a combination therapy of rifampin with dapsone for paucibacillary disease or rifampin with dapsone and clofazimine for multibacillary disease. The recommended duration of therapy is 6 months for patients with paucibacillary disease and 12 months for those with multibacillary disease, although longer treatment might be required in some patients with a high bacterial burden at diagnosis to prevent relapse.[123] Of concern is the emergence of rifampin-resistant strains of *M. leprae* from northern and eastern India, discovered in new cases of leprosy. DNA sequencing showed mutations at previously reported sites in addition to new codon positions, including Ala411 and Gln442, in the *rpoB* gene. The latter mutation leads to upregulation in the messenger RNA expression of the *rpoB* gene, which may lead to overexpression of this gene and other associated genes.[124,125] There is an urgent need to continue monitoring for rifampin resistance in these areas and include new drugs in treatment regimens. The use of rifamycins as chemoprophylaxis against leprosy has been suggested. The overall incidence of leprosy among contacts of patients with newly diagnosed disease can be reduced by a single dose of rifampin. Whether to use this approach more widely is under discussion, and a longer observation time is necessary to show whether the effect of rifampin prophylaxis will be sustained over long periods of time.[126]

Mycobacterium avium-intracellulare Complex

A variety of compounds and their combinations have been reported to be effective against MAC, the rifamycins (particularly rifampin and rifabutin) among them.[127] Medical treatment of MAC pulmonary disease has yielded inconsistent results and limited cure rates. Major limitations for effective therapy include the lower bactericidal activity of the rifamycins against MAC than against *M. tuberculosis,*[88] the absence of correlation between in vitro susceptibility for rifampin, clinical response for MAC disease, and the markedly decreased immunologic status of the host.[128] The limited success of current treatment regimens is also caused by an incomplete understanding of the relationships between the doses of the drugs used, their pharmacokinetics, and the eventual in vivo treatment outcome. The interactions between rifamycins and macrolides are likely most important, because these drugs are cornerstones of recommended treatment regimens for MAC lung disease. The ATS and the Infectious Diseases Society of America (IDSA) currently recommend for most patients with nodular/bronchiectatic disease a three-times-weekly regimen of clarithromycin (1000 mg) or azithromycin (500 mg), rifampin (600 mg), and ethambutol (25 mg/kg). For patients with fibrocavitary MAC lung disease or severe nodular/bronchiectatic disease, a daily regimen of clarithromycin (500–1000 mg) or azithromycin (250 mg), rifampin (600 mg) or rifabutin (150–300 mg), and ethambutol (15 mg/kg), with consideration of three times per week amikacin or streptomycin early in therapy, is recommended.[129] A recent study showed that currently recommended regimens for MAC lung disease yield important pharmacologic interactions and low concentrations of key drugs.[130] Trials of new drugs and new strategies are needed.

Disseminated MAC infection occurs largely in patients with HIV infection, especially with $CD4^+$ cells at less than 50 cells/mm.[4] The role of rifamycins in the treatment of disseminated MAC disease is unclear. If a rifamycin is used as a second-line agent, most experts would use rifabutin, which is more potent than rifampin against MAC, has a postantibiotic effect of 26 hours at the MIC, and has activity against some strains that are resistant to rifampin. Rifabutin accumulates within

phagocytes to a greater extent compared with rifampin[131] and is easier to use with most antiretroviral agents, especially protease inhibitors. A study of 160 patients with the acquired immunodeficiency syndrome (AIDS) and MAC bacteremia compared triple therapy with rifabutin, clarithromycin, and ethambutol to double therapy with clarithromycin with rifabutin or ethambutol. After 12 weeks, the microbiologic response was the same, but patients in the triple-therapy group had increased survival and fewer relapses.[132] Based on this study, some experts recommend the addition of rifabutin as a third drug for disseminated MAC infection in AIDS, especially with a high mycobacterial burden, but with careful monitoring for drug-drug interactions.[133] There is no role for rifabutin therapy in the prophylaxis of MAC due to the added toxicity seen when added to a macrolide.

Mycobacterium kansasii

There have been no randomized trials of treatment of disease caused by *M. kansasii.* However, there are several retrospective and prospective studies of various treatment regimens. Strains of *M. kansasii* often are susceptible to rifampin. With rifampin-containing multidrug regimens, sputum conversion within the first 4 months of treatment was achieved in 100% of the cases, and treatment failures and long-term relapses occur in approximately 1% of the cases when treated for at least 12 months.[134,135] The ATS and IDSA currently recommend a regimen for treating pulmonary *M. kansasii* disease of rifampin (600 mg/day), isoniazid (300 mg/day), and ethambutol (15 mg/kg/day) for a duration that includes 12 months of negative sputum cultures.[129] However, in a recent study a 12-month course of treatment, although effective in most cases, did not cure all patients with *M. kansasii* pulmonary disease. For older patients with debilitating conditions, treatment for longer periods guided by periodic sputum cultures is advisable.[136]

Other Nontuberculous Mycobacteria

The rifamycins show in vitro activity against several other nontuberculous mycobacteria, including *M. marinum, M. xenopi, M. scrofulaceum, M. haemophilum, M. terrae* complex, *M. malmoense, M. szulgai,* and *M. gordonae.* Rifampin is included in the multidrug therapy for infections caused by these nontuberculous mycobacteria. Rapidly growing mycobacteria, such as *M. fortuitum* and *M. chelonae,* are naturally resistant to rifamycins.[43]

RIFAMYCINS FOR NONTUBERCULOUS MYCOBACTERIAL INFECTIONS

Staphylococcal Infections

Rifampin is a logical treatment choice for staphylococcal infections because of its potent bactericidal activity in vitro against both *S. aureus* and coagulase-negative staphylococci (CoNS). The ability of rifampin to sterilize phagocytes containing bacteria was demonstrated by Mandell and colleagues[137] in the 1970s. Rifampin monotherapy was short lived, due to the rapid emergence of resistance, especially with high bacterial burdens seen in abscesses and endocarditis, resulting in combination regimens containing rifampin.

In vitro studies, including time kill and checkerboard studies, were inconsistent when rifampin was added to β-lactams, vancomycin, quinolones, linezolid, and daptomycin and did not correlate with in vivo results. When added to bactericidal antibiotics there was decreased or unchanged killing, whereas if added to a bacteriostatic antibiotic there was some increased bactericidal activity.[3] The rabbit model of *S. aureus* endocarditis[138] provided the first correlation to human infection. The combination of penicillin with rifampin was less effective than penicillin with gentamicin and led to emergence of rifampin-resistant strains.[138] In the mouse model of methicillin-sensitive *S. aureus* (MSSA) endocarditis, rifampin with nafcillin resulted in an improved survival rate of 77% without selection of resistance.[139] Animal studies of methicillin-resistant *S. aureus* (MRSA) bacteremia and endocarditis yielded variable results. Only daptomycin and linezolid were effective in animal models of MSSA and MRSA endocarditis in reducing colony-forming units in vegetations and septic pulmonary emboli, respectively.[140] In the 1960s there were reports of successful outcomes when rifampin was used in combination for patients with MSSA and MRSA bacteremia and native valve endocarditis (NVE).[3] Early retrospective data demonstrated emergence of resistance and treatment failure, leading to the conclusion that addition of rifampin to treat *S. aureus* bacteremia without surgery and/or drainage did not improve outcomes and selected for resistance.[3,141] A clinical study of complicated *S. aureus* infections, including NVE, comparing oxacillin or vancomycin with rifampin, showed no clinical difference and emergence of resistance.[142] Based on multiple studies, the IDSA Clinical Practice Guidelines for MRSA infections and the 2015 American Heart Association guidelines recommend against the addition of rifampin for the treatment of staphylococcal NVE.[143] In a study that addressed the role of rifampin therapy in patients with surgically treated staphylococcal endocarditis, patients given adjunctive rifampin for 3 or more days did not have a reoperation-free or survival benefit, suggesting that surgery significantly reduces or eliminates biofilm formation. Based on this study the authors do not recommend rifampin use in surgically treated staphylococcal endocarditis.[144]

A multicenter retrospective study of 357 patients with MSSA bacteremia associated with a deep focus of infection (including endocarditis, pneumonia, septic arthritis, osteomyelitis [OM], abscess, and foreign-body infection) demonstrated that rifampin therapy started within 7 days and continued for at least 14 days from the onset of bacteremia reduced the risk of a fatal outcome by one-third. The positive prognostic impact of adjunctive rifampin in this study merits additional investigation.[145] Rifampin in combination with other antimicrobials has been studied in *S. epidermidis* NVE. A rabbit model study of methicillin-resistant *S. epidermidis* endocarditis found increased sterilization rates with rifampin-gentamicin-vancomycin and ciprofloxacin plus rifampin.[146] Human data, however, do not support the routine use of rifampin in NVE caused by CoNS. One exception is *Staphylococcus lugdunensis,* a virulent biofilm-producing species that causes fulminant NVE, pacemaker lead endocarditis, and bone and joint infections, and is associated with a high mortality.[147] Combination therapy may be indicated for bactericidal effects, and rifampin has been used successfully in *S. lugdunensis* infections.[147]

Staphylococcal Biofilms: Foreign-Body Infections and the Role of Rifampin

The ability of staphylococci to produce biofilm significantly impacts chronicity of infections, particularly those with indwelling devices. Biofilm is a community of bacterial cells encased within a polymeric matrix produced by the organisms that adhere to a living or foreign body.[148] Within biofilms organisms exist in distinct populations, in different stages of growth, and exhibit heterogeneous gene expression. Antimicrobial resistance occurs by decreased penetration and tolerance induced by the anoxic environment and nutrient depletion.[149] Rifampin in vitro is effective against staphylococci in various stages of growth, including metabolically dormant bacteria, penetrates *S. epidermidis* biofilm, and can prevent biofilm formation within minutes. The lipid solubility of rifampin, activity within the acidic environment within some biofilms, and accumulation within neutrophils facilitate the activity of rifampin,[150] which is commonly used in combination regimens when biofilms are known or presumed to be present.

In vitro the addition of rifampin to *S. epidermidis* and MRSA biofilm infections improved the susceptibility to other antibiotics and decreased the adherence of bacteria to the foreign material.[151] Rifampin with linezolid and daptomycin in animal models of foreign-body infection showed higher activity against planktonic bacteria when rifampin was used.[152,153] When used with daptomycin, tigecycline, and linezolid against MRSA biofilms in catheter lock solutions, rifampin combinations were better at eradicating biofilm compared with vancomycin and linezolid alone.[154] New delivery systems of rifampin, including polymethylmethacrylate beads[155] and lipid-based nanoparticles,[156] show promise in vitro in reducing biomass-established biofilms and decreasing viability of intracellular bacteria.

In vivo studies and case reports have used combinations of rifampin with vancomycin, β-lactams, and aminoglycosides for the treatment of *S. epidermidis* prosthetic valve endocarditis (PVE). An early retrospective study of 23 patients with methicillin-resistant *S. epidermidis* PVE found a cure rate in 87% of patients treated with vancomycin with rifampin (900–1200 mg/day) or with rifampin and an aminoglycoside. Only 38%

of those receiving a β-lactam with rifampin, with or without an aminoglycoside, were cured. The lower response in the patients treated with β-lactams was attributed to heteroresistance within the *S. epidermidis* population.[157] This study and multiple case reports led to inclusion of rifampin as part of a triple therapeutic regimen for CoNS PVE with vancomycin, rifampin, and gentamicin for 2 weeks, followed by vancomycin and rifampin for at least 4 weeks. These recommendations were expanded to include *S. aureus* PVE, for which vancomycin plus rifampin (300 mg every 8 hours) for at least 6 weeks, with gentamicin for the first 2 weeks, is recommended.[158]

Vancomycin-Intermediate and Vancomycin-Resistant *Staphylococcus aureus* and Rifampin

Since the emergence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) strains, alternative antibiotics have been studied in vitro. Rifampin was active against 92% of VRSA isolates but only 51% of VISA isolates in one study.[159] A possible explanation may be found in another study that identified five *rpoB* mutations in 71% of VISA strains.[160] Among these strains, 55% were resistant to rifampin, supporting a possible link between resistance to vancomycin and rifampin exposure in *S. aureus.* In 29% of VISA strains there was no *rpoB* mutation, suggesting a different resistance mechanism. When rifampin-resistant mutants from nine clinical MRSA isolates were tested, more than 95% had decreased vancomycin susceptibilities. The *rpoB* mutations were observed in 31% of rifampin-susceptible VISA strains, raising the possibility that selection of resistance in these strains occurred by vancomycin and/or other antibiotics.[160]

Prosthetic Joint Infections

Most prosthetic joint infections (PJI) are caused by staphylococci and are a leading cause of morbidity. PJI usually require explantation of the device, prolonged antibiotic therapy, and reimplantation of a new device for cure. Rifampin has been shown to penetrate biofilm on the surface of the hardware. For patients who are not candidates for a two-stage procedure, débridement, antibiotics, and implant retention (DAIR) is used. Clinical outcomes vary with different species. In a study of 163 patients with staphylococcal orthopedic implant infections and different procedures, cure was achieved in 57% of MRSA, 72% of MSSA, and 82% of CoNS infections. Rifampin was used in combination with other antibiotics in 45% of MRSA, 55% of MSSA, and 64% of CoNS infections and did not influence cure. Infection with MRSA and an infected arthroplasty were factors inversely associated with cure.[161]

A 3- to 6-month regimen of rifampin with ciprofloxacin was evaluated in patients with PJI after débridement and a 2-week course of parenteral antibiotics. All patients in the rifampin-containing group who completed the study were cured.[162] The efficacy of a rifampin-based regimen with DAIR in staphylococcal PJI, according to practice guidelines with an oral regimen of rifampin (900 mg/day) and levofloxacin (750 mg/day), was compared with a historical cohort with and without rifampin. Patients treated according to guidelines in the prospective cohort had a lower failure rate (7%) compared with the historical rifampin and nonrifampin groups (32% and 37%, respectively).[163] In a retrospective series, 77% of patients with PJI due to MRSA treated with rifampin and fusidic acid were infection free at 2 years.[164] Treatment failure was associated with MRSA infection, a single or four or more débridements, and fewer than 90 days of antibiotics. The incidence of rifampin resistance was 7%. The IDSA recommendations for MRSA PJI with DAIR are parenteral therapy combined with rifampin for 2 weeks followed by a fluoroquinolone with rifampin for 3 months for hip prostheses and for 6 months for knee prostheses.[158] A small study of staphylococcal PJI managed with DAIR randomized patients to an 8-week course of levofloxacin plus rifampin versus the IDSA-recommended courses. The short course was noninferior to standard courses with hip prostheses, with inconclusive results for knee prostheses.[165] Additional studies are needed before shorter courses can be recommended. *Propionibacterium acnes* produces biofilm and causes PJI and other foreign-body infections, including ventriculoperitoneal shunts and PVE. In a guinea pig model of *P. acnes* biofilm, rifampin showed the highest activity in biofilm (MIC, 0.007 μg/mL). Rifampin with daptomycin had the highest in vivo success rate of 63% without detection of resistance.[166] In a review of 50 patients with *P. acnes* PJI treated with DAIR or one- or two-stage exchange arthroplasty or both, 35 patients received long-term parenteral and oral therapy with rifampin with either cefazolin or clindamycin. The overall cure rate was 92%; analysis by antibiotic regimen was not reported.[167]

Osteomyelitis

Chronic staphylococcal OM is associated with multiple relapses, repeated débridements, and long treatment courses. In vitro models demonstrate the ability of *S. aureus* to invade osteoblasts. The bacteria remain active for lengthy periods (up to decades) and recruit phagocytes, resulting in cytokine expression that contributes to chronic inflammation.[168] Therapeutic levels of rifampin are measurable 12 hours after administration in cancellous bone,[34] and rifampin eradicates slow-growing staphylococci at the chronic phase of OM. In animal models of chronic staphylococcal OM, rifampin is synergistic with other antibiotics.[169,170] In humans débridement followed by parenteral and oral antibiotics, including β-lactams, linezolid, clindamycin, and trimethoprim-sulfamethoxazole (TMP-SMX), is recommended for staphylococcal bone and joint infections.[158,171,172] In a retrospective study of 100 patients with staphylococcal deep sternal wound infection, a rifampin-containing regimen was the only factor associated with a decrease in failure rate.[173] For MRSA OM, many experts recommend the addition of an extended course of an oral rifampin-based combination with either TMP-SMX, a quinolone, clindamycin, and either doxycycline or minocycline, based on susceptibilities.[158] In a recent review of chronic OM, the authors recommend adding rifampin for all patients who can tolerate it.[174] In a murine model of *S. aureus* implant-associated OM, rifampin with vancomycin or linezolid resulted in a greater reduction in bacterial load. However, even rifampin-containing regimens were ineffective in eradicating infections when biofilms form on the implant surface.[175]

Central Nervous System Infections

Rifampin has good central nervous system (CNS) penetration and activity against many meningeal pathogens. Rifampin has been added to vancomycin for cephalosporin-resistant strains of *S. pneumoniae* and when dexamethasone is used with vancomycin.[176,177] A recent study showed that pretreatment with one dose of rifampin 30 minutes before ceftriaxone reduced CSF inflammatory markers in children with bacterial meningitis. The rates of neurologic sequelae were reduced at 3 months; the difference was not statistically significant, possibly due to the small sample size.[178] This observation may be clinically important, and thus confirmatory studies are needed. For MRSA meningitis, some experts recommend addition of rifampin. Rifampin with vancomycin is also recommended for the treatment of staphylococcal CNS shunt infections, especially when the shunt cannot be removed.[176,179]

Infections Caused by Other Bacteria

Streptococcus pneumoniae

Two studies of penicillin-resistant *S. pneumoniae* (PRSP) meningitis in the rabbit model found ceftriaxone with rifampin to be equivalent to ceftriaxone plus vancomycin.[180,181] Other animal studies showed poorer outcomes with rifampin. With the increasing incidence of PRSP, the IDSA recommends adding rifampin to a third-generation cephalosporin if resistance is documented and the isolate is rifampin sensitive or if there is an expected delay in clinical and/or bacteriologic response.[176] Emergence of rifampin-resistant isolates has been documented.[182]

Enterococci

Rifampin may be an adjunct therapy for enterococcal infections. When *Enterococcus faecalis* biofilm from hip and knee PJI was grown in microtiter wells or on beads of bone cement treated with multiple antibiotics alone and in combination with rifampin, ciprofloxacin or linezolid with rifampin were most effective in reducing the bacterial count.[181] Rifampin resistance developed when used alone and with ampicillin.[183] Rifampin and tigecycline were effective in a rat ureteral stent model of *Enterococcus faecium* in preventing infection.[184] Another effective combination in an *E. faecalis* mouse wound model is tigecycline with rifampin or daptomycin.[185] Rifampin with either vancomycin or

tigecycline was synergistic and improved survival in a *Galleria mellonella* (wax moth) infection model against strains of vancomycin-resistant *E. faecium*.[186] For serious urinary tract infections caused by MDR *Enterococcus,* aminoglycosides or rifampin are suggested as adjunct therapy.[187]

Legionella

Retrospective studies of rifampin, with a macrolide or quinolone for severe *Legionella pneumophila* pneumonia, showed the combination with erythromycin or pefloxacin to be superior to erythromycin alone. The quinolone alone and in combination with rifampin was the most active regimen.[188] The combination of clarithromycin with rifampin was evaluated in 32 patients with no additional benefit in the combination group and a longer length of stay, possibly due to adverse reactions and drug interactions from rifampin.[189] A literature review concluded that rifampin should be considered only for patients with severe *Legionella* infection or serious comorbid conditions. Caution is advised for drug interactions and other adverse events.[190]

Rhodococcus

Rhodococcus equi, typically a veterinary pathogen, can cause opportunistic infections in immunocompromised patients. Rifampin achieves high intracellular concentrations and is bactericidal against this organism. A combination of two to three antibiotics, including rifampin, vancomycin, a macrolide, quinolones, minocycline, and carbapenems for 6 to 9 months is recommended.[191,192]

Multidrug-Resistant Gram-Negative Bacilli

Rifampin is used in combination with other antibiotics to treat MDR strains of gram-negative bacteria, especially *A. baumannii*, *P. aeruginosa,* and *K. pneumoniae.* With the emergence of carbapenem-resistant (CR) strains, many in vitro studies use rifampin and polymyxin B (PMB) or colistin in combination. This is especially relevant with heteroresistant *A. baumannii,* where in vitro use of colistin with rifampin inhibited growth of colistin-resistant subpopulations and lowered the concentration required to block all single-step mutations.[193,194] In a time-kill study, rifampin and PMB demonstrated bactericidal killing in 42% of CR *A. baumannii* isolates and was the most effective combination tested.[195] An in vitro study of 20 CR isolates of *P. aeruginosa, A. baumannii, K. pneumoniae,* and *E. coli* found bactericidal activity in 90% of isolates using combinations of PMB, doripenem, and rifampin.[196] Rifampin in combination with sulbactam, colistin, and tigecycline, against 25 extensively drug-resistant *A. baumannii* strains, leads to good in vitro activity.[197] In an immunosuppressed mouse model of pneumonia using CR *A. baumannii* isolates with different resistance mechanisms, rifampin-based regimens (with imipenem, colistin, and tigecycline) were effective in clearing bacteremia and improved survival in all strains, but the antibacterial effects of rifampin differed according to the resistance mechanism.[198] Colistin with rifampin was active in vitro against eight strains of CR *K. pneumoniae* producing the New Delhi metallo-β-lactamase,[199] and imipenem with rifampin was effective in vitro against clinical isolates of MDR *P. aeruginosa* with a porin frameshift mutation.[200] A recent review and meta-analysis concluded that colistin and rifampin have significant in vitro synergy in MDR strains of *A. baumannii*.[201] The use of silver nanoparticles (AgNPs) with antibiotics has been studied for CR *A. baumannii* infections. An in vitro and in vivo study, using a mouse peritonitis model, found synergy with AgNPs with either rifampin or PMB against extremely drug-resistant *A. baumannii* patient isolates.[202] If safety in humans is established, this is a potential option for CR *A. baumannii* infections.

Human studies of MDR *A. baumannii* with rifampin and colistin show inconsistent results. This combination was used in 29 patients with pneumonia and bacteremia in isolates susceptible to colistin. Clinical and microbiologic responses were observed in 76% of patients, with an infection-related mortality rate of 21%.[203] A subsequent randomized study of colistin with rifampin versus colistin alone for serious infections caused by extensively drug-resistant *A. baumannii* from patients in intensive care units (ICUs) found that the 30-day mortality was not reduced by addition of rifampin (43% in both groups). Incidence of hepatotoxicity was higher in the rifampin group, but this group had more frequent eradication of the organism from the primary source.[204] A prospective study of colistin versus colistin with rifampin in patients with *A. baumannii* ventilator-associated pneumonia showed a shorter time to microbiologic clearance, more rapid radiologic improvement, and improved mortality in the combination group; however, the results were not statistically significant.[205] In summary, there is insufficient data to support the routine use of rifampin and a polymyxin in patients with serious infections caused by CR gram-negative bacilli, especially *A. baumannii.*

Brucella

The most widely used combination for treatment of brucellosis is doxycycline with either an aminoglycoside or rifampin. Other regimens include rifampin with a quinolone or triple combinations. In a meta-analysis doxycycline and streptomycin had lower relapse and higher response rates than rifampin and doxycycline.[206,207] However, parenteral therapy is problematic in areas of the world where brucellosis is endemic, and quinolones with rifampin were better tolerated than doxycycline-containing regimens in another review.[206] A recent study of patients with brucellosis in Egypt compared a 6-week regimen of doxycycline plus rifampin with a triple regimen of levofloxacin, doxycycline, and rifampin. The clinical response at 6 weeks was nearly equal, but the relapse rate at 6 months was significantly higher with dual therapy (22.6%) versus triple therapy (9.3%). Adverse events were higher with triple therapy but not statistically significant.[208] Larger studies are needed before recommending triple therapy. In addition, in vitro susceptibility testing of 355 *Brucella* spp. with rifampin in Egypt revealed a 19% rate of resistance in 2000 compared with 7% in 1999.[209] The significance of increasing resistance for treatment is unclear at this time. For laboratory and environmental exposures the recommended prophylaxis is a 3-week course of doxycycline and rifampin.[210]

Bartonella

Rifampin has potent in vitro activity against *Bartonella henselae* (MIC, 0.03–0.06 μg/mL), the agent of cat-scratch disease. Azithromycin is first-line therapy for extensive lymphadenopathy; doxycycline with rifampin is an alternative.[211] For complicated disease (retinitis, encephalitis, and hepatosplenic), doxycycline and rifampin is recommended in adults for 4 to 6 weeks and rifampin with azithromycin or TMP-SMZ for children.[211] *B. henselae* also causes endocarditis and bacillary angiomatosis in HIV-infected persons. Bacillary angiomatosis is usually treated with long courses of erythromycin or doxycycline. However, combination therapy with rifampin and either erythromycin or doxycycline is recommended for immunocompromised patients with life-threatening *Bartonella* infection, and doxycycline with rifampin is preferred for CNS disease.[211] *Bartonella quintana,* the etiologic agent of trench fever, is usually treated with doxycycline. However, in areas of the world such as Thailand, where doxycycline is less active, rifampin is more effective.[212]

Fungal Infections

Based on early evidence of synergistic activity of amphotericin B (AMB) with rifampin against fungi, Medoff[213] demonstrated synergy in vitro with this combination against *Histoplasma capsulatum, Aspergillus* spp., and *Saccharomyces cerevisiae.* In a mouse model, combination therapy with AMB and rifampin was more effective against *H. capsulatum, Blastomyces dermatitidis,* and *Aspergillus* spp. An in vitro study on killing of *Candida* biofilms using AMB alone or in combination with either rifampin or clarithromycin found a synergistic effect of rifampin and AMB in 66.6% of *C. parapsilosis,* 42.8% of *C. albicans,* and 33.3% of *C. glabrata* biofilms.[214] In vitro data show synergy with rifampin and AMB in 68.7% of *Fusarium* spp.[215] Rifampin with AMB significantly enhanced the activity of AMB alone in vitro against 95 clinical isolates of *Fusarium solani* and *Aspergillus flavus* spp. complex from patients with keratomycosis.[216] There are few clinical reports, with insufficient data to support the use of rifampin in combination with antifungal agents.

SECOND-LINE INDICATIONS FOR RIFAMYCINS

Rifabutin has been successful as "rescue therapy" for *H. pylori* infection. A combination of rifabutin with amoxicillin and a proton-pump inhibitor for 10 days used after failure of standard quadruple therapy was successful

and well tolerated in 50% to 66.6% of patients in two studies.[217,218] Q fever endocarditis is usually treated with doxycycline in combination with hydroxychloroquine. Doxycycline with rifampin (900 mg/day) was effective in some case reports, and erythromycin with rifampin was successful in chronic Q fever of pregnancy.[219] Doxycycline is first-line treatment for scrub typhus, but in areas where doxycycline resistance is present, rifampin is an alternative.[220] Doxycycline is also the treatment of choice for human granulocytic ehrlichiosis, but rifampin is successful in treating young children.[221] Rifampin at doses of 300 to 600 mg/day reduces pruritus associated with cholestasis, possibly by induction of microsomal enzymes that promote glucuronidation of toxic bile salts. A review of 12 trials documented a significant decrease in pruritus.[222]

Chemoprophylaxis

Antibiotics for chemoprophylaxis of *N. meningitidis* contacts include rifampin, ciprofloxacin, minocycline, and ceftriaxone. A 2-day course of rifampin (600 mg twice daily) is effective in eradicating the bacteria from 75% to 95% of carriers; however, adverse drug reactions, drug interactions, and selection of resistant isolates have limited use to young children. An extensive review found ceftriaxone to be more effective than rifampin after 1 to 4 weeks of follow-up. Rifampin was effective for up to 4 weeks after treatment, but resistant isolates were seen, raising concern that use of rifampin during an outbreak may lead to the circulation of resistant strains. Use of ciprofloxacin, ceftriaxone, or penicillin should be considered.[223]

The *H. influenzae* type b conjugate vaccines have significantly decreased the incidence of invasive disease in children. Chemoprophylaxis is recommended for close contacts of a child with invasive *H. influenzae* type b infection using rifampin at doses of 20 mg/kg in children and 600 mg in adults daily for 4 days, based on earlier studies showing elimination of oropharyngeal carriage of *H. influenzae* type b in 97% of household or daycare contacts.[224,225]

Another area of infection prophylaxis is the rifampin-minocycline–impregnated catheter, which has been demonstrated to be effective in decreasing catheter-related bacteremia.[226] Two studies in pediatric burn patients[227] and in immunocompromised patients with transplants, cancer, or on dialysis[228] concluded that these catheters are effective in reducing the incidence of catheter-associated bacteremia. The CATCH trial, comparing minocycline-rifampin–impregnated central venous catheters, heparin impregnated catheters, and standard catheters in children admitted to pediatric ICUs, found that the antibiotic-impregnated catheter reduced the risk of bloodstream infection by 57% compared with standard catheters and 58% compared with heparin-impregnated catheters.[229]

For decolonization of MRSA carriers, mupirocin ointment and chlorhexidine are effective in 60% of patients. Oral therapy is recommended if topical regimens fail, which is seen with mupirocin resistant isolates. Rifampin for 5 to 10 days in combination with another agent is recommended if the strain is susceptible and other regimens are ineffective.[158]

NOVEL AND FUTURE INDICATIONS FOR RIFAMPIN

Rifampin has been successful in the treatment of cholestasis of pregnancy, in combination with ursodeoxycholic acid, in women unresponsive to ursodeoxycholic acid alone.[230] The mechanism is thought to be due to enhancement of bile acid detoxification, bilirubin conjugation, and bilirubin excretion. A 6- to 9-month course of rifampin was effective treatment in 30 patients with idiopathic granulomatous lobular mastitis, a chronic inflammatory condition of unclear etiology, associated with masses, abscesses, and sinus tracts.[231] A recent study confirmed the effectiveness of an 8-week course of rifampin with streptomycin in curing Buruli ulcer, caused by *Mycobacterium ulcerans*, in Ghana.[232]

Two innovative studies of rifampin are for suppression of osteolysis after joint replacement and the prevention of neurodegenerative diseases. Using in vitro data and a mouse calvarial osteolysis model, rifampin inhibited titanium-induced osteolysis and osteoclastogenesis in vivo and reduced expression of osteoclast-specific markers and osteoclast formation through regulation of receptor activator of nuclear factor-κB ligand signaling.[233] Using a transgenic mouse model of Alzheimer disease, rifampin for 1 month reduced accumulation of amyloid-β oligomers, tau hyperphosphorylation, synapse loss, and microglial activation. The 1-mg/day dose improved memory similar to the nontransgenic littermates.[234] Future studies are needed to validate a role for rifampin in prevention of periimplant osteolysis, diseases with excessive osteoclast activity, and in neurodegenerative diseases.

RIFAXIMIN: A SELECTIVE GASTROINTESTINAL RIFAMYCIN

Rifaximin is a unique rifamycin with negligible systemic absorption, enhanced fecal concentrations, decreased systemic toxicity, and few drug interactions. Rifaximin is bactericidal against many enteric pathogens, including enterotoxigenic and enteroaggregative *E. coli, Salmonella, Shigella,* and *Campylobacter*,[56] and is active against *C. difficile,* although high-level resistance has been reported.[235] Rifaximin has antiprotozoal activity, including *Cryptosporidium parvum* and *Blastocystis hominis.*[56] In addition to traditional single-pathogen–based antibiotic activity, rifaximin affects the microbiome of the GI tract. Rifaximin can downregulate inflammation by inhibiting activation of nuclear factor-κB and reducing expression of the proinflammatory cytokines IL-1 and TNF-α.[236] Rifaximin may also modulate inflammatory and immune responses in gut epithelial cells.[237] Clinical trials of traveler's diarrhea demonstrate that rifaximin (400 mg twice daily) was similar to traditional antimicrobial agents in decreasing time to symptom resolution.[56] Rifaximin was 72% effective in chemoprophylaxis of traveler's diarrhea in Mexico, where most cases are caused by *E. coli.*[238] At subinhibitory concentrations, rifaximin reduced expression of bacterial enterotoxins and surface adhesion intestinal-binding factors of enterotoxigenic *E. coli.*[239] However, the fluoroquinolones are preferred for chemoprophylaxis due to an effectiveness of over 90% and activity against most invasive intestinal pathogens.[240] Rifaximin (550 mg twice daily) is approved for the treatment of hepatic encephalopathy. In one study rifaximin use led to fewer breakthrough episodes of hepatic encephalopathy and significantly reduced the risk for hospitalization compared with placebo.[241] The reduction of microbes that degrade nitrogenous compounds results in decreased ammonia levels and was more than or equally effective as lactulose and other agents and better tolerated.[242]

Small trials of rifaximin for *C. difficile* infection have been published, although rifaximin may best be used for recurrent *C. difficile* infection, with a response rate as high as 83% in one study.[243] A 2-week course of rifaximin is often used after a 6-week course of vancomycin for recurrent disease. There is concern about development of resistance, however. Using the rifampin Etest, one study found 14 of 80 clinical isolates had an MIC greater than 32 μg/mL. Of these 14 isolates, 9 (64%) belonged to the BI/NAP1/027 group.[244] In small bowel overgrowth, rifaximin may play a role in microbial decontamination.[227] Rifaximin is FDA approved for treatment of irritable bowel disease without constipation. Patients reported a modest improvement in bloating, abdominal pain, and stool consistency. Rifaximin may be beneficial in inflammatory bowel diseases, including ulcerative colitis, Crohn disease, and pouchitis as a steroid-sparing regimen,[235] possibly due to the effect on both the microbiome and the (subsequent) antiinflammatory effect. Studies of rifaximin alone or in combination with other therapies have demonstrated improved rates of remission and/or clinical improvement in Crohn disease, ulcerative colitis, and pouchitis.[245] There may be a role for rifaximin in the treatment of diverticular disease when used 7 days per month along with traditional therapies.[246] Caution is warranted for such empirical therapies because rifampin-resistant strains of *S. aureus* and CoNS were found in the perineum, hands, and upper extremities after rifaximin therapy that persisted up to 9 weeks after discontinuation, consistent with other studies demonstrating rifaximin-resistant *E. coli* in the intestinal tract.[247,248] In a recent study a delayed-release formulation of rifaximin had a protective effect in reducing the number and severity of small bowel mucosal lesions and large erosions and/or ulcers when used with a nonsteroidal anti-inflammatory drug. This effect is believed to be secondary to a shift in the microbiota away from proinflammatory gram-negative bacteria.[249]

RIFALAZIL

Rifalazil is a rifamycin that has an MIC 64-fold higher than rifampin against many *M. tuberculosis* isolates, has a half-life greater than 100

hours, achieves high intracellular concentrations, and has no CYP interactions.[250] Rifalazil has potent in vitro bactericidal activity against *Chlamydia* spp., with an MIC_{90} of 0.00025 μg/mL against *C. trachomatis,*[251] and is active against all stages of the life cycle. Initial development was limited by a flulike syndrome and leukopenia in phase I trials. A phase II trial in pulmonary tuberculosis with once-weekly dosing with isoniazid for 2 weeks, followed by 6 months of standard therapy, was better tolerated, but a higher incidence of adverse events was observed in the rifalazil arm.[252] A phase IIB study in men with nongonococcal urethritis showed superiority of 25 mg of rifalazil versus 1 g of azithromycin.[253] A phase II study of 25 mg of rifalazil versus 1 g of azithromycin in women with uncomplicated genital *C. trachomatis* infection demonstrated a cure rate of 85% in the rifalazil group versus 92% in the azithromycin group, and lower adverse reactions in the rifalazil group, but was unable to demonstrate noninferiority to azithromycin.[254] Rifalazil development in the United States was terminated in 2013 due to safety concerns.

RIFAMYCINS IN DEVELOPMENT

New rifamycins include aerosolized formulations, rifamycin-loaded scaffolds for prevention of orthopedic prosthetic infections, and compounds that circumvent development of resistance. Advantages of aerosol formulations are decreased dosing, improved compliance, decreased adverse events, and improved pharmacodynamics, because particles attain high intracellular concentrations in pulmonary macrophages. Two investigational compounds are a dry powder formulation with rifampin-derived microcrystals coated with polymers[255] and liposomes coated with chitosan-xanthan gum.[256] A new inhaled formulation of rifampin with colistin, aimed at delivering both drugs directly to the respiratory tract, was found to have synergistic activity, high aerosolization efficiency, and moisture protection, offering a less toxic formulation for resistant gram-negative respiratory infection.[257] Synthetic bone scaffolds constructed by electrospinning nanofiber poly(caprolactone) with concentrations of 10% and 20% rifampin when inoculated with *S. epidermidis* and *P. aeruginosa* completely inhibited bacterial growth and prevented biofilm formation within the scaffolds through the first 6 hours.[258]

Rifamycin SV MMX (RIF-MMX) is a semisynthetic derivative of rifamycin SV in a nonabsorbable modified-release multimatrix structure that delivers the active ingredient directly into the colon.[259] The bioavailability is less than 0.1%, and close to 90% of the dose is recovered in feces. RIF-MMX is under investigation for treatment of traveler's diarrhea, *C. difficile* infection, diverticulitis, inflammatory bowel diseases, and hepatic encephalopathy. A 3-day course of RIF-MMX versus placebo was well tolerated and effective in achieving clinical cure within 120 hours in patients with traveler's diarrhea (81.4% of RIF-MMX vs. 56.9% of placebo). There was decreased susceptibility in bacterial isolates remaining after therapy, but this was not associated with diminished efficacy, suggesting an additional nonbacterial mechanism of action.[260]

Studies of rifampin-loaded nanoparticles showed enhanced delivery of rifampin in vitro into the intracellular compartment of polymorphonuclear leukocytes[261] and targeted delivery to alveolar macrophages in vitro and in a rat model.[262] Nanoparticles carrying rifamycins are promising new delivery systems for the treatment of tuberculosis, *S. aureus,* and other intracellular bacterial infections.

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The complete reference list is available online at Expert Consult.

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28 Metronidazole

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SHORT VIEW SUMMARY

Description

- Metronidazole is a nitroimidazole drug that is widely prescribed as a first-line agent for various anaerobic and parasitic infections and is recommended as an alternative agent (in patients unable to take oral vancomycin or fidaxomicin) for treatment of *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.

Spectrum

- There is minimal resistance to *Bacteroides* and other gastrointestinal anaerobes. However, emerging resistance is noted with *Gardnerella vaginalis*, *Helicobacter pylori*, and *C. difficile*, and metronidazole lacks activity against a number of non–spore-forming, gram-positive anaerobic bacteria including *Actinomyces, Bifidobacterium, Lactobacillus, Propionibacterium*,[1,2,3] and *Cutibacterium acnes* (formerly *Propionibacterium acnes*).

Clinical Pharmacology

- Metronidazole is available in many formulations; it has excellent bioavailability and excellent tissue penetration, including penetration into the central nervous system (CNS).

Toxicity

- Most patients tolerate metronidazole, but common adverse effects include nausea, diarrhea, dry mouth, metallic taste, candidal vaginitis, and stomatitis. Serious adverse effects include dizziness, headache, confusion, vertigo, insomnia, Stevens-Johnson syndrome, pancreatitis, ophthalmologic toxicity (myopia and blurred vision), ototoxicity, bullous pemphigoid, and hemolytic-uremic syndrome.

HISTORY

Metronidazole was first synthesized in the 1950s when the pharmaceutical company Rhône-Poulenc was searching for an effective antitrichomonal drug for the treatment of vaginal trichomoniasis.[4] Initially, a crude extract of a *Streptomyces* bacterium was found to kill *Trichomonas vaginalis,* and the active component was determined to be azomycin, a previously characterized nitroimidazole antibiotic.[5] Metronidazole was a synthetic analogue of azomycin that was both more active against *T. vaginalis* and less toxic.[4,6] It was initially called 8823 R.P.[4] This discovery soon led to its successful use in human clinical studies of trichomoniasis.[7,8] The presumed antibacterial activity of metronidazole was discovered incidentally in 1962 by Shinn, who reported improvement in ulcerative gingivitis in a patient treated for a concomitant *T. vaginalis* infection, a result he confirmed in a series of patients with ulcerative stomatitis.[9]

MECHANISM OF ACTION

Metronidazole and other nitroimidazoles (e.g., nimorazole, ornidazole, ronidazole, secnidazole, tinidazole) are inert, and their spectrum of antimicrobial activity is determined by the capacity of susceptible organisms to activate the drugs once they enter the cell via passive diffusion. The structures of metronidazole and related compounds are illustrated in Fig. 28.1. Their bactericidal and parasiticidal activities are rapid and proportional to the concentration of the activated drugs within the target cell.[10] The members of this class of antibiotics are therefore best considered as prodrugs, which are activated through a reduction step to form highly reactive products that interact with intracellular targets (see later).[11] Metronidazole's mechanism of action describes that of the other nitroimidazoles.

The nitroimidazoles share a heterocyclic structure consisting of an imidazole-based nucleus with a nitro group, NO_2, in position 5. There are four major steps involved in the mechanism of action of metronidazole that result in the intracellular formation of critical redox intermediate metabolites.[12] In the first two steps, the drug enters cells by passive diffusion and an electron is transferred to the nitro group of metronidazole, resulting in the production of a short-lived nitroso free radical, which is cytotoxic and can interact with cellular DNA.[1] This process of activating the prodrug creates a concentration gradient that augments the increased uptake of the drug by the organism, further increasing its antimicrobial effect. The third step in metronidazole's action relates to the cytotoxic effect of the reduced product because the activated metronidazole compound can inhibit DNA synthesis and induce DNA damage via oxidation, resulting in single-strand and double-strand breaks.[1] Thus, metronidazole induces DNA degradation and cell death.[1] Finally, there is the release of inactive end products of the drug.[13]

The microbial selectivity of metronidazole reflects the inability of aerobic bacteria to activate the prodrug because they lack the necessary electron transport proteins with sufficient negative redox potential.[1] However, in susceptible anaerobic bacteria, the redox potential of components of the electron transport chain is sufficiently negative to reduce the nitro group of metronidazole. The drug is activated in anaerobic bacteria when it receives an electron from ferredoxin or flavodoxin, which are themselves reduced by iron-sulfur proteins called pyruvate:ferredoxin oxidoreductases (PFORs).[14] The exact electron donors involved in nitroimidazole reduction vary depending on the organism.[1] In the microaerophile *Helicobacter pylori,* for example, a separate mechanism appears to be involved in metronidazole susceptibility, involving a two-electron transfer step mediated by an oxygen-insensitive nitroreductase (RdxA).[1] Several microaerophilic protists (*Giardia lamblia, Entamoeba histolytica,* and *T. vaginalis*) have bacteria-like enzymes (nitroreductases) capable of activating metronidazole.[15]

SPECTRUM OF ACTIVITY

Metronidazole and related nitroimidazoles are active against a variety of anaerobic bacteria, in addition to microaerophilic bacteria and protozoa. Resistance, as detailed later, has been increasingly detected in certain organisms, although this may not be identified easily because sensitivity testing for anaerobes is not performed routinely. However, the emergence of resistance suggests that ongoing surveillance is important.[1]

Many gram-negative anaerobes are susceptible to metronidazole.[16,17] As a rule, members of the genera *Bacteroides* and *Parabacteroides* are susceptible to metronidazole, with resistance generally detected in fewer than 5% of isolates.[18,19,20,21,22,23] Higher rates of resistance have been reported among *Bacteroides* isolates in South Africa.[24] *Desulfovibrio* species are also highly susceptible,[25] and clinically relevant members of the *Fusobacterium, Porphyromonas, Prevotella,* and *Bilophila* genera are usually sensitive.[16,26] However, nonsusceptible *Prevotella* strains have been described.[16] Metronidazole resistance has been reported in oral

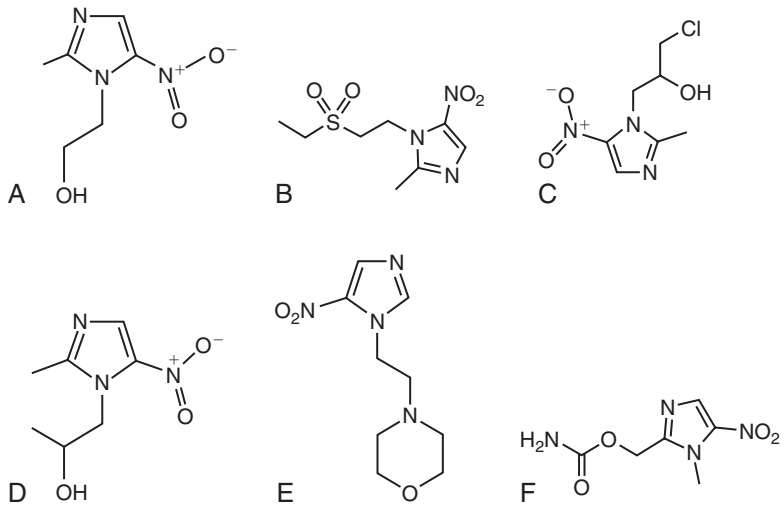

FIG. 28.1 Structures of nitroimidazoles. (A) Metronidazole. (B) Tinidazole. (C) Ornidazole. (D) Secnidazole. (E) Nimorazole. (F) Ronidazole.

isolates of *Porphyromonas gingivalis.*[27] Reduced susceptibility has also been noted in isolates of *Sutterella*[16] and in the gram-negative coccus *Veillonella.*[17,28] The bacterial vaginitis–associated bacteria of the genus *Mobiluncus* are usually not susceptible to metronidazole.[29,30]

Facultative anaerobes have variable susceptibility to metronidazole and in general are not empirically treated with this agent. *Aggregatibacter actinomycetemcomitans* is occasionally susceptible, but resistance is sufficiently common that empirical metronidazole should not be used for these infections.[27,31] Another oral, facultative anaerobe, *Eikenella corrodens,* is generally resistant to the nitroimidazoles.[32] The CO_2-requiring members of the genus *Capnocytophaga* are generally resistant to metronidazole.[33] Although *Campylobacter jejuni* and *Campylobacter coli* isolates may be susceptible in vitro to metronidazole, the drug is not recommended for therapy against these pathogens.[34] *Gardnerella vaginalis,* which is associated with bacterial vaginosis, is variable in its sensitivity, with nearly 30% of isolates in one series demonstrating resistance, suggesting that resistance should be considered in cases of treatment failure.[35] A survey from India documented that nearly 70% of *G. vaginalis* strains were metronidazole resistant, but the cases included metronidazole-exposed women exhibiting recurrent infection.[36] The clinical relevance of in vitro resistance of *Gardnerella* to metronidazole is difficult to interpret because a hydroxy metabolite of metronidazole is actually more active against *G. vaginalis* than the parent compound.[37,38]

H. pylori is a facultative anaerobe that was initially sensitive to metronidazole but has increasingly developed clinically important resistance. Globally, the prevalence of resistance to metronidazole in *H. pylori* is as high as 41.6% to 99.5% depending on the setting (including the United States) and the method of testing.[39,40] Treatment failure for *H. pylori* was a significant risk factor for harboring a metronidazole-resistant isolate.[41]

Among the gram-positive anaerobes, the clostridia remain quite susceptible to metronidazole.[2] However, reduced susceptibility to metronidazole has been increasingly noted among strains of *Clostridioides difficile* (formerly *Clostridium difficile*).[42,43,44,45] In Europe and Israel, resistance to metronidazole has been noted in 13.3% and 18% of *C. difficile* isolates, respectively.[45] Notably, the Etest overestimates metronidazole susceptibility in *C. difficile* isolates, compared with the more involved agar incorporation–based methods for determining minimal inhibitory concentrations (MICs).[42,44] Whether the clinical response to metronidazole is affected by reduced in vitro susceptibility remains to be determined. However, this deserves closer attention in light of the highly variable concentrations of metronidazole in the stools of treated patients[46,47] and increasing reports of treatment failure and recurrence of *C. difficile* infection (CDI) in metronidazole-treated patients.[48]

An important hole in the anaerobic spectrum of metronidazole is found in its lack of activity against a number of non–spore-forming, gram-positive anaerobic bacteria that possess intrinsic resistance to the drug. These include isolates of *Actinomyces, Bifidobacterium, Lactobacillus,* and *Propionibacterium.*[1,2,3] *Cutibacterium acnes* (formerly *Propionibacterium acnes*) is highly resistant.[2,17,49] Metronidazole should not be routinely used to treat infections with these organisms unless susceptibility is confirmed. In contrast, the genus *Eubacterium* is generally sensitive to metronidazole in vitro.[2]

The nitroimidazoles possess good activity against several protozoa. Apart from *T. vaginalis,* metronidazole has activity against *Giardia* (syn. *G. duodenalis, G. lamblia, G. intestinalis*) and *E. histolytica.* Resistance is uncommon in *Giardia,* and clinical efficacy is generally greater than 90%, but in vitro testing has revealed reduced susceptibility to metronidazole in clinical isolates, causing concern.[50,51] Nitroimidazoles exhibit in vitro activity against *Dientamoeba fragilis,* which is a trichomonad known to cause gastroenteritis.[52]

Apart from its antimicrobial actions, metronidazole exhibits immunosuppressive and antiinflammatory actions[53] and has been used effectively in the treatment of rosacea,[54] although the extent to which this relates to metronidazole's antibacterial properties is unclear.

EFFECTS ON THE HUMAN MICROBIOME

Metronidazole, like many antimicrobials, affects the human microbiome. However, because this has mostly been examined in subjects exposed to metronidazole in combination with other antimicrobials, it is difficult to fully understand the impact of metronidazole itself.[1,55] Although traditionally thought of as a narrow-spectrum agent, metronidazole has a broadly antianaerobic activity, which could significantly shift microbial populations rich in anaerobes, such as the gastrointestinal tract. Although early culture-based studies of the impact of metronidazole monotherapy on bacterial communities in the human gastrointestinal tract described little impact of the drug on microbial populations,[47,56] some investigators reported a suppression of anaerobes and a relative increase in the abundance of certain aerobic bacteria (*Escherichia coli* and fecal streptococci).[57] The reasons for the relatively low impact on normal gut microbes are not well understood but may relate to the pharmacology of metronidazole, which achieves low concentrations in the feces of healthy adults.[46,58] Substitution of similar (but metronidazole-resistant) bacteria could also occur in the setting of metronidazole therapy, which could be missed with use of standard culture-based techniques. At present there are few studies of metronidazole's impact on the gastrointestinal microbiome that have used culture-independent techniques such as DNA pyrosequencing, apart from studies involving antibiotic combinations.[55] This is an area in need of new research.

Nucleic acid sequencing methods have been applied to understand the impact of metronidazole on the microbiome of the female

reproductive tract, particularly in the context of bacterial vaginosis. Topical metronidazole has been evaluated for its impact on the vaginal microbiome in women with bacterial vaginosis.[59–61] Use of metronidazole for 5 to 7 days consistently reduced the diversity of bacterial communities in these studies of bacterial vaginosis, compared with no treatment, and for many women it restored a more normal, *Lactobacillus*-dominated mucosal microbiome.[59–61]

PHARMACOLOGY

Metronidazole is commercially available in a variety of formulations: oral capsules and tablets (immediate and extended release); intravenous solution; topical gels, creams, and lotions; and vaginal gels.[10,62] Although oral metronidazole suspension is not commercially available, it is commonly compounded in pharmacies by crushing immediate-release tablets and mixing in a 1:1 ratio with an aqueous suspending solution and buffered oral syrup.[63] The dose and duration of treatment are dependent on the specific product and indication (Table 28.1). An intravenous loading dose of 15 mg/kg, followed by 7.5 mg/kg every 6 to 8 hours, is recommended in the package insert, with a maximum daily dose limit of 4 g.[10] A fixed dose of 500 mg given intravenously every 8 hours maintains concentrations above typical MICs for *Bacteroides* species and is effective for treatment of intraabdominal infections.[64,65,66] An infusion time of 1 hour is traditionally recommended, but 20- to 30-minute infusions have been used.[67] Given the long half-life and concentration-dependent activity, high-dose metronidazole, administered as 1 to 1.5 g every 24 hours, may be a safe and effective alternative to 500 mg every 6 to 8 hours.[68,69] The typical duration of oral or intravenous metronidazole courses ranges from 1 to 10 days depending on the indication and patient condition. Longer durations may be prescribed, but caution should be exercised with durations greater than 1 month owing to increased risk of peripheral neuropathy and central nervous system (CNS) adverse effects, which is likely due to the effects of metronidazole on mitochondrial function.[70,71,72]

Oral metronidazole is rapidly and almost completely absorbed, with bioavailability approaching 100%.[10,62] When rectally administered, metronidazole is also well absorbed, with reported bioavailability of 59% to 94%; topical and vaginal metronidazole achieve detectable systemic concentrations with bioavailability ranging from 2% to 25%.[10,62,73] Administration of oral metronidazole with food is encouraged to minimize gastrointestinal adverse effects and does not affect bioavailability but may delay the time to peak serum concentrations. Peak serum concentrations range from 12 to 40 μg/mL and occur 1 to 2 hours after oral administration and approximately 3 hours after rectal administration.[10,62]

Metronidazole is a lipophilic molecule with low protein binding and a moderate-to-large volume of distribution, allowing extensive distribution into various tissues (Table 28.2).[10,62] Penetration into inflamed cerebrospinal fluid, epithelial lining fluid, saliva, and bile is excellent and concentrations are similar to those in serum.[10,62,74] Patients with noninflamed meninges still achieve therapeutic concentrations of approximately 43% in serum.[75] In addition, penetration into abscesses, appendix tissue, peritoneal fluid, and pancreatic tissue is very good, ranging from 2.3 to 7.2 μg/mL.[10,62,76] However, patients with obstructive cholecystitis have negligible amounts of drug detected in the bile.[10,62] Metronidazole crosses the placental barrier and penetrates into breast milk and may be teratogenic during the first trimester (see "Precautions").[77] Stool concentrations during *C. difficile* colitis are highest at the beginning of infection and taper as inflammation subsides and stool becomes formed, but concentrations generally remain well above reported MICs.[46] This effect of higher stool concentrations when diarrhea is present is also noted during flares of Crohn disease.[47]

Metronidazole undergoes oxidation as the primary step in eliminating the drug from the body, and 6% to 18% of active unchanged drug is found in the urine.[10,62,78] Oxidation, glucuronidation, and metabolism by cytochrome P-450 system yield five major metabolites, including 1-2 hydroxyethyl-2-hydroxy-methyl-5-nitroimidazole (hydroxy metabolite), which maintains antimicrobial activity; 2-methyl-5-nitroimidazole-1-acetic acid (acetic acid metabolite) is another major metabolite but has no antimicrobial activity.[10,62,78] All metabolites are extensively excreted in the feces or urine and undergo enterohepatic circulation. The enterohepatic circulation and metronidazole pharmacokinetic properties are altered with renal dysfunction, hepatic dysfunction, and severity of diarrhea.[10] The half-life of metronidazole is approximately 8 hours in healthy patients and 18 to 20 hours with end-stage hepatic failure.[79] Patients with moderate-to-severe hepatic diseases should receive a 50% dose reduction.[10] In addition, patients with end-stage renal disease (creatinine clearance <10 mL/min) will have slightly longer half-life of metronidazole and significantly impaired clearance and accumulation of the hydroxy and acetic metabolites.[10,80–86] Metronidazole and metabolites are removed through conventional, continuous, and peritoneal hemodialysis. During a 4-hour conventional hemodialysis session, 45% of drug is removed, and patients should receive a supplemental dose after dialysis. Patients receiving continuous renal replacement therapy also experience significant removal of metronidazole and its metabolites and do not require dose adjustment.[79] Peritoneal dialysis removes approximately 10% of drug.[87] Patients on peritoneal dialysis and those with creatinine clearance less than 10 mL/min not receiving conventional or continuous hemodialysis will have accumulation of active metabolites.[79,80,81,83,84,86] Renal dose adjustment is currently not recommended because the ramifications of metabolite accumulation are not well understood, but caution should be used in long-term therapy.[62] Finally, preterm infants 32 weeks' gestational age or younger will have impaired clearance and may require dose adjustment depending on chronologic age.[88,89]

ADVERSE EFFECTS, CONTRAINDICATIONS, AND PRECAUTIONS

Contraindications

Metronidazole is associated with carcinogenic activity in rats and mice. It should be avoided during the first trimester of pregnancy and used during the second and third trimesters only if clearly necessary.[10,62] There are case reports of fetal malformation occurring with metronidazole exposure during pregnancy, but three large studies failed to demonstrate a link of fetal malformations compared with the general population.[77,90,91] Metronidazole is also excreted in breast milk[92] and has been shown to achieve infant plasma concentrations approximately one-fifth of those observed in the mother's plasma.[93] It has been recommended that nursing be withheld during metronidazole therapy for 12 to 24 hours after oral single doses.[93]

Whenever possible, metronidazole should be avoided during lactation because of its effects on the developing microbiota. Infants may have increased risk of diarrhea, but there is a lack of evidence linking breast milk with carcinogenic effectors or developmental disorders.[93]

Precautions

Patients should avoid metronidazole if there is a history of hypersensitivity with metronidazole, parabens, or nitroimidazole agents or intake of alcohol within 3 days of therapy and/or concomitant use of disulfiram within 2 weeks of metronidazole therapy. Disulfiram-like reactions with alcohol can occur with all routes of administration, including topical and vaginal administration.[10,62] Caution should be exercised when prescribing metronidazole in patients with peripheral neuropathy, hepatic disease, history of seizures, or a history of antibiotic-associated vaginal candidiasis.[10,62] In addition, patients currently taking metronidazole who develop aseptic meningitis, conjunctivitis, edema, seizure, local skin lesions, and peripheral neuropathy should discontinue therapy until drug-related adverse effects can be excluded.[10,62]

Adverse Effects

Metronidazole is generally well tolerated. The most common adverse effects are dose dependent, mild, and reversible. Nausea, diarrhea, dry mouth, metallic taste, candidal vaginitis, and stomatitis occur in 2% to 10% of patients.[10,62] Serious CNS adverse effects (ataxia, encephalopathy, dysarthria, seizure, aseptic meningitis, and peripheral neuropathy) have been reported most commonly with prolonged therapy but are reversible.[77,94] Caution should be used when prescribing metronidazole in patients with seizure history. Other mild CNS effects have been reported, including dizziness, headache, confusion, vertigo, and insomnia. In addition, rare and serious adverse effects associated with metronidazole

TABLE 28.1 Major Preparations and Indications for Metronidazole: Administration and Dosage

PRODUCT	DOSAGE FORM	STRENGTHS	INDICATIONS	DOSE AND ADMINISTRATION
Metronidazole tablet (Flagyl) Metronidazole capsule (Flagyl)	Tablet Capsule	250 mg 500 mg 375 mg	Symptomatic trichomoniasis, asymptomatic trichomoniasis, treatment of asymptomatic consorts, amebiasis, anaerobic bacterial infections, intraabdominal infections, skin and skin suture infections, gynecologic infections, bacterial septicemia, bone and joint infections, CNS infections, lower respiratory tract infections, endocarditis	**Adults** *Acute intestinal amebiasis:* 750 mg tid for 5–10 days *Amebic liver abscess:* 500 or 750 mg tid for 5–10 days *Anaerobic bacteria:* 7.5 mg/kg every 6 h for 7–10 days (may be longer) *Trichomoniasis:* 250 mg tid daily for 7 days 375 mg (capsule) bid for 7 days 2 g single dose or 1 g bid for 1 day **Children** 35–50 mg/kg daily divided into 3 doses for 10 days
Metronidazole extended-release tablet (Flagyl ER)	Extended-release tablet	750 mg[a]	Bacterial vaginosis	**Adults** 750 mg once daily for 7 days
Metronidazole intravenous solution (Metro)	Intravenous solution	500 mg/100 mL (0.74% NaCl) 5 mg/mL (0.74% NaCl)	Anaerobic infections, intraabdominal infections, skin and skin structure infections, gynecologic infections, bacterial septicemia, bone and joint infections, CNS infections, lower respiratory tract infections, endocarditis, prophylaxis	**Adults** *Anaerobic infections:* Loading dose of 15 mg/kg IV over 1 h Maintenance dose: 7.5 mg/kg IV over 1 h every 6 h; usual duration 7–10 days *Colorectal surgery prophylaxis:* Initial: 15 mg/kg IV over 30–60 min about 1 h before surgery Maintenance: 7.5 mg/kg IV over 20–60 min at 6 and 12 h after initial dose
Metronidazole gel (MetroGel-Vaginal, Vandazole)	Vaginal gel	0.75%	Bacterial vaginosis	**Adults** *Bacterial vaginosis:* 1 applicatorful (approximately 5 g containing metronidazole 37.5 mg) intravaginally once or twice daily for 5 days. For once-a-day dosing, administer at bedtime
Metronidazole cream (MetroCream, Rosadan—0.75%) (Noritate—1.0%) Metronidazole gel (Metrogel—1.0%) (Rosadan—0.75%) Metronidazole lotion (MetroLotion) Metronidazole Kit (Rosadan)	Cream Gel Lotion Kit	0.75% 1.0%[a] 0.75% 1.0%[a] 0.75% Metronidazole 0.75% cream + wash[a]	Rosacea	**Adults** *1% strength:* apply a thin film once daily *0.75% strength:* apply a thin film twice daily
Tinidazole (Tindamax)	Tablet	250 mg 500 mg	Trichomoniasis, amebiasis, anaerobic bacterial vaginosis, intraabdominal surgical prophylaxis, giardiasis, and *Helicobacter pylori* infection, nongonococcal urethritis	**Adults** *Acute intestinal amebiasis:* 2 g qd for 3 days *Amebic liver abscess:* 2 g qd for 3–5 days *Trichomoniasis:* 2 g one-time dose *Giardiasis:* 2 g one-time dose *Bacterial vaginosis:* 2 g qd for 2 days **Children** *Acute intestinal amebiasis:* 50 mg/kg qd for 3 days *Amebic liver abscess:* 50 mg/kg qd for 3–5 days *Giardiasis:* 50 mg/kg one-time dose
Secnidazole	Tablet	1000 mg		**Adults** *Acute intestinal amebiasis:* 2 g one-time dose *Trichomoniasis:* 2 g one-time dose *Giardiasis:* 2 g one-time dose *Bacterial vaginosis:* 2 g one-time dose **Children** *Acute intestinal amebiasis:* 30 mg/kg one-time dose *Giardiasis:* 30 mg/kg one-time dose
Ornidazole	Tablet	500 mg		**Adults** *Acute intestinal amebiasis:* 1.5 g qd for 3 days *Bacterial vaginosis:* 1.5 g qd for 3 days **Children** *Acute intestinal amebiasis:* 25 mg/kg qd for 5–10 days *Giardiasis:* 40–50 mg/kg one-time dose

[a]No generic available.
CNS, Central nervous system.

therapy include Stevens-Johnson syndrome, pancreatitis, ophthalmologic toxicity (myopia and blurred vision), ototoxicity, bullous pemphigoid, and hemolytic-uremic syndrome.[10,62,95]

MECHANISMS OF RESISTANCE

Resistance to metronidazole (and other nitroimidazoles) in strict anaerobes remains unusual, and not all mechanisms that reduce susceptibility to the nitroimidazoles have been characterized.[1] On the basis of critical steps in its mechanism of action, several models of drug resistance have been proposed, including reduced antibiotic uptake, active drug efflux, reduced drug activation (e.g., by decreased expression of activating nitroreductase enzymes), drug inactivation (e.g., *nim*-encoded nitroimidazole reductase), and altered DNA repair.[1]

Metronidazole resistance (MIC ≥32 μg/mL) among *Bacteroides* strains is uncommon, generally occurring in less than 5% of isolates.[17,18,23] Although many mechanisms of resistance have been induced in vitro,[96] the best-characterized mechanism in clinical isolates is encoded by the

TABLE 28.2 Pharmacokinetic and Pharmacologic Properties of Metronidazole

PHARMACOLOGIC OR PHARMACOKINETIC FACTOR	RESULT	COMMENTS
Absorption		
Oral	98%–100%	
Rectal	59%–94%	
Vaginal cream	20%	
Vaginal gel	56%	
Topical	2%	
Time to peak		
Oral	1–2 h	
Rectal	3 h	
Topical	8–12 h	
Peak serum concentrations		
Intravenous	25 and 18 μg/mL	After 15 mg/kg load and 7.5 mg/kg every 6 h
Oral	6, 12, 21.4, and 40 μg/mL	After single dose of 250 mg, 500 mg, 750 mg, and 2000 mg
Rectal	18.5 μg/mL	After 500-mg dose
Topical	27.5 μg/mL	After application of 1% cream
Volume of distribution		
Adults	0.55 L/kg	
Neonates	0.54–0.81 L/kg	
Tissue and fluid penetration		
CSF (inflamed meninges)	Approximates serum concentration	
CSF (noninflamed meninges)	45% of serum concentration	
Bile	Approximates serum concentration	
Epithelial lining fluid	Approximates serum concentration	
Saliva	Approximates serum concentration	
Abscess	Variable, but high concentration	
Peritoneal fluid	High concentrations: 7.2–14.2 μg/mL	
Pancreatic tissue	High concentration: 5.1–8.5 μg/mL	
Metabolism		
Oxidation	Primary mechanism of elimination	
Glucuronidation	Secondary mechanism of elimination	
Cytochrome P450	Secondary mechanism of elimination	
Excretion		
Unchanged drug	6%–18%	
Metabolites	60%–80%	
Hemodialysis	Removes 25%–45% over 4 h	
Peritoneal dialysis	Removes 10% over 7.5 h	
Protein binding	<20%	
Pregnancy	Avoid in first trimester Category B	
Lactation	Avoid	Significant penetration into breast milk

CSF, Cerebrospinal fluid.

nim (5-nitroimidazole reductase) genes *(nimA–H* and *nimJ).*[97,98] The *nim* genes induce the reduction of the nitrate residue of metronidazole (and related compounds) into an inert amino derivate without any toxicity for the bacterial chromosome.[18] These genes may occur in all *Bacteroides* species and are located either on plasmids or on the chromosome.[21] In a recent survey of 640 *Bacteroides* isolates obtained from across Europe, 21 strains (3.3%) had MIC values greater than or equal to 4 μg/mL. Notably, of only three isolates (two *Bacteroides fragilis* strains and one *Bacteroides thetaiotaomicron* isolate) harboring *nim* genes, one was resistant to metronidazole.[21] The two metronidazole-susceptible *B. fragilis* isolates had chromosomal *nimA* and *nimC* genes, whereas a *nimE* gene was identified on a plasmid in the resistant *B. thetaiotaomicron* strain.[21] It was speculated that a small number of *nim*-negative, but metronidazole-resistant, *Bacteroides* strains identified in this study might have used diverse resistance strategies, including reduced uptake, nitroreductase and/or PFOR activities, increased lactate dehydrogenase activity, or mutations that alter the carbohydrate utilization affecting the redox state.[21] Studies have suggested that alterations in the *recA* gene encoding the RecA protein involved in DNA damage repair might be a resistance strategy in *Bacteroides.*[99]

Resistance and heteroresistance of *C. difficile* have been reported for metronidazole, and the mechanisms are unclear but appear to be multifactorial, including the assay and testing condition.[100] A recent review noted that metronidazole resistance in *C. difficile* likely reflects several mechanisms, including exclusion by biofilms and alterations in yet to be defined metabolic pathways, such as those involving the activity of nitroreductases, iron uptake, and DNA repair.[45] The high prevalence of metronidazole resistance among *H. pylori* strains is concerning and appears to be due primarily to decreased activation of the drug, which would also be expected to affect its accumulation within the bacterium. Mutations in the oxygen-insensitive reduced nicotinamide adenine dinucleotide phosphate (NADPH) nitroreductase RdxA or the NADPH-flavin-oxidoreductase FrxA confer resistance to metronidazole in *H. pylori.*[101] Mutations in *rdxA* and *frxA* are frequently associated with metronidazole resistance, but next-generation sequencing studies have revealed that a gene, *rpsU,* might also play a role in *H. pylori* resistance.[102] *H. pylori* might use other strategies to protect itself against nitroimidazole-induced damage, but the relative importance of such mechanisms in vivo remains speculative.[103,104]

Resistance to metronidazole has emerged in protozoal pathogens. Trichomonal resistance was suspected on the basis of clinical treatment failure as early as 1960[105] and was documented in vitro in 1962 in treatment-refractory clinical isolates,[106] but prevalence has generally remained below 10%.[107] A survey from six US cities tested 538 *T. vaginalis* isolates for nitroimidazole resistance (aerobic minimal lethal concentration [MLC] >50 μg/mL) and found that 23 (4.3%) exhibited low-level in vitro metronidazole resistance (MLC, 50–100 μg/mL).[107] However, there were no isolates identified with moderate- to high-level nitroimidazole resistance.[107] An unsettling report from New Guinea revealed that 17.4% of *T. vaginalis* strains (4 out of 23 isolates) exhibited metronidazole resistance under aerobic conditions (MIC >200 μM), but the number of isolates tested was relatively small and the organisms were sensitive under anaerobic conditions.[108]

The mechanisms of resistance in *T. vaginalis* appear to differ between laboratory-generated nitroimidazole resistance and those found in clinical isolates.[109] Laboratory-induced resistance manifests in anaerobic conditions and results from a loss of the aforementioned drug-activating pathways that reduce the inert prodrug metronidazole to active metabolites.[109] Clinical resistance, on the other hand, typically occurs under aerobic conditions owing to actions of oxygen itself.[109] For example, a study of resistance in clinical strains of *T. vaginalis* found that flavin reductase activity was downregulated, or even absent, in metronidazole-resistant strains.[109] It was postulated that because flavin reductase can reduce oxygen to hydrogen peroxide, its downregulation might impair oxygen scavenging.[109] This would result in resistance because oxygen interferes with the activation of nitroimidazoles by either inhibiting drug-activating pathways or by reoxidizing a critical, toxic, nitroradical anion intermediate, also resulting in reduced metronidazole uptake.[109] A genome sequencing–based approach of *T. vaginalis* identified 72 single nucleotide polymorphisms (SNPs) associated with metronidazole resistance, and a comparison of SNPs within several laboratory-derived resistant lines revealed an overlap with the clinically resistant isolates.[110] Although several SNPs occurred in genes for which no function has yet been assigned, many involved functionally characterized genes implicated in metronidazole resistance (e.g., *PFOR*). Common changes in the expression of genes involved in drug activation (e.g., flavin reductase), accumulation (e.g., multidrug resistance pump), and detoxification (e.g., nitroreductase) were observed.[110] Metronidazole should still be considered first-line therapy for *T. vaginalis,* but resistance should be suspected in patients who do not respond to therapy.

As noted, in *Giardia,* clinical resistance occurs in approximately 20% of cases.[51] Microbiologic resistance to metronidazole is complex but appears to be due to a lack of activation of the prodrug to the active nitroso free radical.[111] Resistance to metronidazole has traditionally been explained by a loss of ferredoxin and PFOR activities.[112] However, recent studies have suggested that some metronidazole-resistant *G. lamblia* strains have normal activity levels of these redox proteins, and metronidazole can be activated by a flavin adenine dinucleotide (FAD)–dependent *G. lamblia* thioredoxin reductase.[111] Although not entirely clear, drug resistance in those isolates appeared to be related to lower availability of reduced FAD.[111] Resistance to metronidazole in amebas has been associated with an increase in iron-containing superoxide dismutase, without a significant decrease of the PFOR activity.[112]

CLINICAL USES

Parasitic Infections

Trichomonas

Metronidazole was developed for its use as an antitrichomonal agent.[6] The nitroimidazoles remain the most important pharmaceutical class for these infections, and tinidazole appears to be equivalent or superior to metronidazole in this regard.[113] The emergence of nitroimidazole resistance (see earlier) and treatment failures is problematic because alternative therapies are not reliably curative.[113] Metronidazole appears to be safe for use in pregnant women with *T. vaginalis* infections.[114] Although trichomoniasis is associated with adverse pregnancy outcomes, including low birth weight, premature membrane rupture, and preterm birth, it is not clear that treatment alters the incidence of such complications.[115] Symptomatic pregnant women, regardless of pregnancy stage, should be tested and considered for treatment with single-dose (2 g) oral metronidazole.[115]

Dientamoeba

Symptomatic gastrointestinal *D. fragilis* infections of adults and children have been treated successfully with metronidazole.[116,117] A parallel, placebo-controlled, double-blind trial of metronidazole in children with chronic symptoms attributed to *D. fragilis* failed to find a benefit of this antimicrobial.[118] The nitroimidazoles are among the most potent agents against this parasite in vitro.[52]

Entamoeba

Amebic infections caused by *E. histolytica* are treated with metronidazole, depending on whether the infection is luminal within the intestinal tract or invasive, such as occurs with hepatic abscess.[119] Metronidazole is the most commonly used medication for amebic colitis, although tinidazole was reported to be better tolerated and more efficacious.[119,120] The treatment of amebic liver abscess includes metronidazole (or tinidazole) with or without aspiration.[119] Treatment for colitis or hepatic abscess with a tissue amebicide such as metronidazole should be followed by an agent active against luminal amebas, such as paromomycin.[119]

Giardia

Giardiasis is primarily treated with nitroimidazole drugs such as metronidazole, tinidazole, and nitazoxanide[119] Tinidazole and nitazoxanide are recommend first-line options, are indicated for patients older than 3, and can be given as a single-dose regimen and a 3-day regimen, respectively.[121] Metronidazole is also an option for treatment of *Giardia* but should be prescribed for 5 to 7 days. There are limited data evaluating tinidazole in patients younger than 3 years, and nitazoxanide in patients younger than 1 year. It might be prudent to use metronidazole, because there are established safety and efficacy data in patients younger than 1 year. Refractory or relapsed *Giardia* infection could be the result of resistance, secondary downregulation of nitroreductase 1 (NTR1), and upregulation of (NTR2); use of alternative classes of antiparasitic agents, such as albendazole, mebendazole, or paromomycin (possibly in combination with a nitroimidazole agent), should be considered.[122]

Anaerobic Infections

In light of metronidazole's potent bactericidal activity against anaerobes and its favorable pharmacodynamics profile (distributing throughout the body, including the CNS and into abscess cavities), it is effective for the management of myriad anaerobic infections.[1,10] Metronidazole is commonly used to treat anaerobic infections of the abdomen, CNS infections (including meningitis and brain abscess), gynecologic infections, bacteremia, endocarditis, bone and joint infections, respiratory tract infections, skin and skin structure infections, oral and dental infections, and tetanus.[1,10,123]

The role of metronidazole in the management of lung abscesses is unclear.[124] Small clinical studies have demonstrated striking clinical

failures of metronidazole monotherapy in the management of anaerobic lung abscess,[125,126] including a comparative trial with clindamycin that was halted prematurely because of poor response in the metronidazole arm. Metronidazole failed in patients with lung abscess or necrotizing pneumonia.[126] It has been postulated that the lack of response in lung abscess therapy reflects metronidazole's lack of activity against aerobic and microaerophilic streptococci, and the inclusion of a β-lactam could surmount this challenge.[124]

The emergence of antimicrobial resistance has created new difficulties in treating previously susceptible infections, as described earlier. However, metronidazole is not effective in the treatment of actinomycosis and infections with *C. acnes* owing to intrinsic resistance.[127,128] These exceptions to metronidazole use should be kept in mind.

Metronidazole, tinidazole, and clindamycin are each approved for use to treat bacterial vaginosis.[129] Both metronidazole and clindamycin are approved for either oral or topical application.[129] Head-to-head trials have demonstrated equal efficacy of oral and vaginal clindamycin and metronidazole, although in the majority of studies clindamycin tended to have fewer adverse effects, with oral metronidazole primarily causing a disturbing metallic taste and gastrointestinal upset.[129] Metronidazole appears to be safe for use to treat bacterial vaginosis in pregnancy, although this has not reduced preterm births, a complication associated with bacterial vaginosis.[129,130]

Clostridioides difficile Infection

Metronidazole was once the first-line drug of choice for the treatment of CDI, but increasing reports of treatment failures, recurrent disease after treatment, and inferior performance compared with oral vancomycin in some clinical trials have led to changes to metronidazole's place in CDI therapy.[131,132] A meta-analysis found that vancomycin was superior to metronidazole for the initial treatment of acute, severe CDI but not different for mild-to-moderate disease, and recurrence rates did not differ between the regimens.[133] A large retrospective study of more than 10,000 initial CDI cases conducted through the US Veterans Affairs Healthcare System compared metronidazole and vancomycin and found no difference in initial response to therapy or recurrent infection, but patients with more severe CDI had lower 30-day mortality if treated with vancomycin.[134] In addition, a Cochrane review of clinical trial data concluded that oral vancomycin or fidaxomicin is superior to metronidazole for the treatment of CDI.[135] The emergence of metronidazole resistance in *C. difficile* might also play a role in its diminishing usefulness as a first-line agent.[45,136] In 2017 guidelines for CDI in adults from the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America, oral metronidazole is recommended as a first-line agent for mild-to-moderate CDI.[131] Vancomycin is recommended as first-line therapy for severe infection.[131] More recent guidelines are similar with regard to metronidazole's role in CDI management.[137] Metronidazole resistance in *C. difficile* isolates remains uncommon but a concern for the future (see earlier).

Helicobacter pylori Infection

Despite increasing antimicrobial resistance in *H. pylori*, metronidazole remains an important agent for use against infection with this organism.[138] It is recommended as part of a combination three- or four-drug approach to treatment, and metronidazole should be used as part of the regimen whenever possible, even in the presence of resistance.[138]

Other Therapeutic Uses

Metronidazole has been used to treat a number of (apparently) noninfectious diseases. For example, metronidazole has been applied in the treatment of inflammatory bowel disease, with the best evidence for benefit in the settings of perianal Crohn's disease and ulcerative colitis–associated pouchitis.[139] A number of dermatologic disorders have been treated successfully with topical metronidazole, including rosacea and acne vulgaris.[10] In neoplastic diseases, metronidazole has been used in high doses as a radiosensitizing agent.[140]

Prophylactic Use

Metronidazole has held an important place in surgical prophylaxis, particularly for procedures involving mucosal organs colonized by anaerobes, such as the gastrointestinal tract and female reproductive tract. According to surgical prophylaxis guidelines,[141] metronidazole (usually in combination with other antimicrobials) is a first-line recommended agent for the prevention of infection in appendectomy for uncomplicated appendicitis, obstructed small intestinal surgery, colorectal surgery, clean contaminated head and neck cancer surgery, and clean contaminated urologic surgery (extensively reviewed by Bratzler and colleagues[141]). However, the indications for surgical prophylaxis with metronidazole given in Chapter 313 are much more limited. Metronidazole is also recommended as an alternative agent for β-lactam–allergic or intolerant patients for many surgical indications that carry risk for anaerobic infection, in combination with other antimicrobials.[141]

In obstetric and gynecologic procedures, metronidazole has been recommended for preoperative prophylaxis. For example, data suggest that metronidazole reduces infectious complications of surgical abortion.[142] It has been recommended for manual removal of the placenta after parturition and for repair of third- and fourth-degree vaginal tears.[143] It has also been recommended in patients undergoing hysterectomy, hysterosalpingography, or hysteroscopy and for chromotubation in patients with dilated tubes or a history of pelvic inflammatory disease or tubal damage.[143]

DRUG INTERACTIONS AND INTERFERENCE WITH LABORATORY TESTS

The major interactions between metronidazole and other pharmaceuticals or food are listed in Table 28.3. An important and serious interaction exists between warfarin and metronidazole; metronidazole increases the blood levels and hypothrombotic effects of warfarin through inhibition of enzymes responsible for oxygenation of *S*-warfarin.[144] Preemptive

TABLE 28.3 Drug-Drug and Drug-Food Interactions

INTERACTING AGENT	RESULT	COMMENTS
Alcohol	Disulfiram reaction	Symptoms include vomiting, tachycardia, palpitations, or nausea Possible acute psychosis or confusion in severe cases
Amiodarone	May increase amiodarone levels	May increase risk for torsades de pointes and ventricular tachycardia
Amprenavir oral solution	Disulfiram reaction	Propylene glycol in oral solution may cause disulfiram reaction
Busulfan	May increase busulfan levels	Avoid metronidazole administration if possible
Carbamazepine	May increase carbamazepine levels	May increase risk for dizziness, diplopia, nausea
Cimetidine	May increase metronidazole levels	
Cyclosporine	May increase cyclosporine levels	Monitor levels and adjust accordingly
Lithium	May increase lithium levels	Monitor levels and adjust accordingly
Phenytoin	May increase phenytoin levels	Monitor levels and adjust accordingly
Rifampin	May decrease metronidazole levels	
Tacrolimus	May increase tacrolimus levels	Monitor levels and adjust accordingly
Warfarin	May increase warfarin levels	Monitor levels and adjust accordingly Empirical dose adjustment may be considered depending on anticoagulation indication and international normalized ratio

dose reduction of warfarin and close monitoring of prothrombin activity have been recommended if the two drugs require concomitant administration.[145] An uncommonly reported interaction suggests that metronidazole reduces the clearance of the alkylating chemotherapy agent busulfan, increasing the levels of the latter drug.[146] Metronidazole therapy should be avoided with concomitant busulfan whenever possible.

Several case reports have proposed that metronidazole use can increase the systemic concentration of concomitant CYP3A substrates, including amiodarone, carbamazepine, quinidine, tacrolimus, and cyclosporine.[147] However, in vitro studies have not consistently supported CYP3A inhibition as a mechanism of metronidazole drug interaction.[147] Empirical dose adjustments are not required for potential CYP3A interactions, but increased monitoring and patient education are prudent.

Although it is not commonly thought of as a drug that poses a risk for inducing QT interval prolongation or arrhythmias, reports have linked metronidazole to long QT and torsades de pointes.[148,149] In most cases, this appeared to be due to impairment by metronidazole of the cytochrome P-450 metabolism of other agents that were responsible for lengthening the QT interval.[148] Anecdotal reports suggest that metronidazole itself can prolong the QT interval, but this is likely rare.[149]

Metronidazole is largely believed to cause a disulfiram-like effect on ethanol metabolism, leading to symptoms such as severe nausea and vomiting.[150] Adverse effects similar to disulfiram-like reactions have been reported with topical metronidazole administration, including vaginal administration, so ethanol should be avoided during therapy.[150] However, there is controversy about the actual risk for and nature of ethanol-metronidazole interactions.[150,151] Although disulfiram inhibits hepatic aldehyde dehydrogenase, resulting in the accumulation of blood acetaldehyde concentrations after ethanol consumption, metronidazole has not been demonstrated to share this ability.[150] Thus, although metronidazole is associated with disulfiram-like symptoms when administered with ethanol, the mechanism is poorly defined and the incidence is not well understood.[150]

OTHER NITROIMIDAZOLE ANTIMICROBIALS

Tinidazole, secnidazole, and ornidazole are other members of the 5-nitroimidazole class. Trindiazole, which has been widely prescribed in Europe and developing countries, was approved for used in the United States in 2004. All agents in the class exhibit similar mechanism of action, spectrum of activity, toxicity, and adverse effects.[152] However, the distinguishing feature among agents is the half-life and need for less-frequent administration compared with metronidazole.[152] The half-lives for tinidazole, secnidazole, and ornidazole are 10 to 15 hours, 17 to 28.8 hours, and 11 to 14 hours, respectively, which allows for a once-daily dose (see Table 28.1).[152] These agents offer a potential advantage over metronidazole, as a single-dose option for the treatment of intestinal amebiasis, giardiasis, and bacterial vaginosis. However, metronidazole should be considered the drug of choice for life-threatening anaerobic infections because there are limited data evaluating the efficacy and safety of other nitroimidazole agents.[153,154]

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The complete reference list is available online at Expert Consult.

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29 Macrolides and Clindamycin

Whitney J. Nesbitt and David M. Aronoff

SHORT VIEW SUMMARY

MACROLIDES

Erythromycin

- Usual doses
  - Oral: base—250 to 500 mg every 6 to 12 hours; maximum: 4 g daily
  - Ethylsuccinate: 400 to 800 mg every 6 to 12 hours; maximum: 4 g daily
  - IV: lactobionate—15 to 20 mg/kg/day divided every 6 hours, *or* 500 mg to 1 g every 6 hours, *or* given as a continuous infusion over 24 hours; maximum: 4 g daily
- Renal or hepatic failure
  - In renal failure, slightly dialyzable (5%–20%); supplemental dose is not necessary in hemodialysis or peritoneal dialysis or in continuous arteriovenous or venovenous hemofiltration
  - No dose adjustment in hepatic failure
- Cerebrospinal fluid (CSF) penetration
  - CSF-to–blood level ratio: normal meninges—2% to 13%; inflamed meninges—7% to 25%
- Adverse effects
  - Cardiovascular corrected QT (QTc) interval prolongation, torsades de pointes, gastrointestinal (GI) upset, pruritis, erythema multiforme, cholestatic jaundice
- Contraindications
  - Caution for patients with underlying heart disease or for those taking other drugs that may prolong the QT interval regarding potential for inducing fatal ventricular arrhythmias
  - Hypersensitivity to erythromycin, any macrolide antibiotics, or any component of the formulation
  - Concomitant use with cisapride, ergotamine, terfenadine, lovastatin, or simvastatin
- Drug-drug interactions
  - Major inhibitor of cytochrome P-450 isoenzyme 3A (CYP3A4)
- Indications
  - *Bartonella* spp. infections, chancroid, granuloma inguinale, legionnaires' disease, lymphogranuloma venereum, nongonococcal urethritis, pertussis

Azithromycin

- Usual dose
  - 500 mg on day 1, followed by 250 mg once daily on days 2 to 5
- Renal or hepatic failure
  - No dosage adjustment required; use with caution in patients with severe renal impairment (glomerular filtration rate <10 mL/min)
- CSF penetration
  - Poor central nervous system (CNS) penetration
- Adverse effects
  - Cardiovascular (QTc) prolongation, torsades de pointes, GI upset, pruritis, erythema multiforme, vaginitis, cholestatic jaundice
- Contraindications
  - Caution for patients with underlying heart disease or for those taking other drugs that may prolong the QT interval regarding potential for inducing fatal ventricular arrhythmias
  - Hypersensitivity to azithromycin, any macrolide antibiotics, or any component of the formulation
  - History of cholestatic jaundice/hepatic dysfunction associated with prior azithromycin use
- Drug-drug interactions
  - Amiodarone and other QTc-prolonging agents, quinine
- Indications
  - Treatment of acute otitis media; pharyngitis/tonsillitis; community-acquired pneumonia; pelvic inflammatory disease; genital ulcer disease (in men) due to *Haemophilus ducreyi* (chancroid); acute bacterial exacerbations of chronic obstructive pulmonary disease; acute bacterial sinusitis; prevention of *Mycobacterium avium* complex (alone or in combination with rifabutin) in patients with advanced human immunodeficiency virus (HIV) infection; treatment of disseminated *M. avium* complex (in combination with ethambutol) in patients with advanced HIV infection; skin and skin structure infections (uncomplicated) due to *Staphylococcus aureus, Streptococcus pyogenes,* or *Streptococcus agalactiae;* urethritis and cervicitis due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae*

Clarithromycin

- Usual dose
  - Oral: 250 to 500 mg every 12 hours or 1000 mg (two 500-mg extended-release tablets) once daily for 7 to 14 days
- Renal or hepatic failure
  - Creatinine clearance <30 mL/min: decrease clarithromycin dose by 50%
  - Hemodialysis: administer after hemodialysis session is completed; in hepatic failure, no dosing adjustment is necessary as long as renal function is normal
- CSF penetration
  - Poor CNS penetration
- Adverse effects
  - Cardiovascular (QTc) prolongation, torsades de pointes, hypersensitivity reactions
- Contraindications
  - Caution for patients with underlying heart disease or for those taking other drugs that may prolong the QT interval regarding potential for inducing fatal ventricular arrhythmias
  - Hypersensitivity to azithromycin, any macrolide antibiotics, or any component of the formulation
  - History of cholestatic jaundice/hepatic dysfunction associated with prior azithromycin use
- Drug-drug interactions
  - Colchicine toxicity, major inhibitor of CYP3A4
- Indications
  - Pharyngitis/tonsillitis; acute maxillary sinusitis; acute exacerbation of chronic bronchitis; community-acquired pneumonia; uncomplicated skin/skin structure infections due to susceptible *S. aureus, S. pyogenes;* disseminated mycobacterial infections due to *M. avium* or *Mycobacterium intracellulare;* prevention of disseminated mycobacterial infections due to *M. avium* complex disease (e.g., patients with advanced HIV infection); duodenal ulcer disease due to *Helicobacter pylori* in regimens with other drugs, including amoxicillin and lansoprazole or omeprazole

KETOLIDES

- No longer manufactured

CLINDAMYCIN

- Usual doses
  - Oral: 150 to 450 mg/dose every 6 to 8 hours; maximum dose: 1800 mg daily
  - IM, IV: 1.2 to 2.7 g/day in 2 to 4 divided doses; maximum dose: 4800 mg daily
- Renal or hepatic failure
  - Renal failure: no adjustment required
- CSF penetration
  - Poor
- Adverse effects
  - Diarrhea, abdominal pain, vaginitis

SHORT VIEW SUMMARY—cont'd

- Contraindications
  - Hypersensitivity to clindamycin, lincomycin, or any component of the formulation
- Drug-drug interactions
  - Lincosamide antibiotics may diminish the therapeutic effects of erythromycin
- Indications
  - Treatment of susceptible bacterial infections, mainly those caused by anaerobes, streptococci, pneumococci, and staphylococci; pelvic inflammatory disease (IV)

The macrolide antibiotics (erythromycin, azithromycin, and clarithromycin) and the lincosamide antibiotics (lincomycin and clindamycin) are chemically unrelated but possess many similar biologic properties in terms of mechanisms of action and resistance, antimicrobial activity, and clinical pharmacology. Erythromycin, the macrolide in longest use, is sometimes useful as an alternative to penicillin G and other antibiotics. Azithromycin and clarithromycin have some advantages over erythromycin related to their antimicrobial activity, pharmacokinetics, fewer gastrointestinal side effects, and effectiveness in certain infections. Although more expensive, they have largely replaced erythromycin in clinical use. Azithromycin is noteworthy among the macrolides for fewer drug interactions and antiinflammatory activities. Dirithromycin, a semisynthetic derivative of erythromycin, is not available in the United States, is not widely used in Europe, but is prescribed in China; it is not included in this chapter but reviews of its use are available.[1,2] All of the macrolide antibiotics in clinical use have the potential for inducing cardiac arrhythmias. Telithromycin, the first member of the ketolide class, exhibited increased activity against many bacterial strains that were resistant to macrolides. It was associated with rare, yet severe hepatic toxicity that substantially limited its clinical usefulness, and manufacturing has therefore been discontinued. Clindamycin has been restricted in use by its association with *Clostridioides difficile* (formerly *Clostridium difficile*) colitis; it remains mostly as an alternative-choice antibiotic in the treatment of several infections, especially certain anaerobic infections. Lincomycin is now mainly of historic interest.

ERYTHROMYCIN

Derivation, Chemistry, and Preparations

Erythromycin was derived in 1952 from a strain of *Saccharopolyspora erythraea* (originally named *Streptomyces erythreus*) obtained from soil from the Philippines. It consists of a mixture of antibiotics in which erythromycin A is the active component. The structure (Fig. 29.1) of erythromycin A consists of a 14-member macrocyclic lactone ring—therefore the class name *macrolide*—attached to two sugar moieties, desosamine and L-cladinose. Erythromycin base is poorly soluble in water, has a pKa of 8.8, is rapidly inactivated by gastric acid, and is often inconsistently absorbed after oral administration. Pharmaceutical preparations for oral use have been made with an aim to diminish destruction by gastric acid and to promote better absorption. Oral preparations available include (1) enteric-coated tablets, enteric-coated pellets in capsules for delayed release, and "film"-coated tablets of the base; (2) the stearate salt (formed in association with the amino group on desosamine), available as film-coated tablets; and (3) the ethylsuccinate ester (formed with the hydroxyl group on desosamine), available in tablet, chewable, and liquid forms. The water-soluble salt of erythromycin prepared for intravenous use is erythromycin lactobionate. Erythromycin lactobionate is not given intramuscularly due to injection site pain. Erythromycin base is also available in 0.5%, 2%, and 3% topical solutions, gels, and creams for treatment of acne vulgaris and in an ophthalmic ointment for treatment of bacterial conjunctivitis and prevention of neonatal gonococcal and chlamydial conjunctivitis.

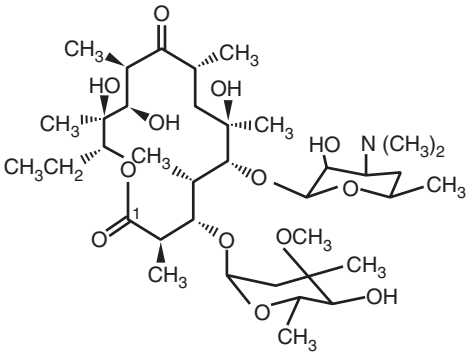

FIG. 29.1 **Erythromycin base.**

Mechanisms of Action

Erythromycin inhibits RNA-dependent protein synthesis at the step of chain elongation in susceptible organisms. X-ray crystallography studies indicate that several functional groups on erythromycin A bind to sequences on domain V of the 23S ribosomal RNA (rRNA) that is a component of the 50S subunit of the bacterial ribosome.[3,4] That binding site is near the peptidyl transferase center, and peptide chain elongation is thereby prevented by blocking of the polypeptide exit tunnel.[3–5] As a result, peptidyl transfer RNA (peptidyl-tRNA) is dissociated from the ribosome.[3,4] Data provided by macrolide-resistant mutants indicate that there are also important interactions between macrolides and specific ribosomal proteins or bases of the 50S subunit near the peptidyl transferase center.[5,6]

Studies in *Escherichia coli* and *Staphylococcus aureus* have demonstrated that erythromycin also inhibits the formation of the 50S ribosomal subunit.[3,5,7] In some bacteria, erythromycin interferes with the ribosomal binding of other macrolides, lincomycin, and chloramphenicol, suggesting common or overlapping binding sites for these antibiotics.

Mechanisms of Resistance

Decreased Microbial Entry or Export of Drug

Enterobacteriaceae exhibit decreased permeability of the outer cell envelope to macrolides.[8–10] This intrinsic resistance is also exhibited by *Pseudomonas* spp. and *Acinetobacter* spp. In addition, chromosomally encoded efflux pumps of several families can provide macrolide and, in some cases, lincosamide and streptogramin resistance.[6] A different efflux system for erythromycin and other 14- and 15-member macrolides (but not 16-member macrolides, lincosamides, or analogues of streptogramin B), called the *M phenotype,* has been elucidated in erythromycin-resistant strains of *Streptococcus pyogenes, Streptococcus pneumoniae,* group C streptococci, and enterococcal species. This efflux system is encoded by the *mef*(A) gene, is carried on a transposable element, and consists of transmembrane domains across the cytoplasmic membrane.[3] The M phenotype resistance is expressed by *S. pneumoniae* at moderate levels with erythromycin minimal inhibitory concentrations (MICs) of 1 to 64 μg/mL.[11]

Target Site Alterations

Mutations in genes for 50S ribosomal proteins or bases of critical domains of the 23S rRNA receptor site confer resistance to erythromycin and sometimes to other macrolides (M), lincosamides (L), and streptogramin type B (S_B); in some but not all strains, this is associated with a decreased binding affinity for erythromycin in *S. pneumoniae, Helicobacter pylori, Mycobacterium avium, Bacillus subtilis, S. pyogenes, Campylobacter* spp., *Mycoplasma pneumoniae, E. coli,* and *S. aureus.*[3,5,6,12–16] This pattern of resistance, referred to as the MLS_B phenotype, is mediated by the *erm* (erythromycin ribosome methylation) genes on plasmids or transposons on chromosomes, which are self-transferable. There are many identified classes of *erm* genes, the most important of which are the *ermA, ermB, ermC,* and *ermF* classes. The resistance results from decreased binding of the antibiotics to their overlapping targets on the ribosome. It is the most widespread mechanism of resistance to the macrolides and the lincosamides and can be exhibited by strains of *S. aureus* (particularly having *ermA* or *ermC* genes), *S. pyogenes* (with *ermA* or *ermB* genes), *S. pneumoniae, Enterococcus* spp., *Corynebacterium*

diphtheriae, Campylobacter spp., *Bacteroides fragilis* (*ermF* gene), *Clostridium perfringens, Listeria* spp., *M. pneumoniae,* and *Legionella* spp. The MLS_B resistance phenotype may be constitutive or inducible by subinhibitory concentrations of erythromycin or other macrolides that bring about induction of the methylating enzyme. Lincosamides are not inducers of this system. When of the inducible type, macrolide resistance is manifest, but clindamycin susceptibility is often found in vitro; however, in that latter case, resistance will often emerge in vivo by selection of mutants that are constitutively producing a methylating enzyme, especially in infections with high bacterial density.[14] Strains of *S. pyogenes* with the *ermB* gene are constitutive producers of methylase and therefore are usually resistant to the macrolides and clindamycin.[17] Strains of *S. pyogenes* with the *ermA* gene are usually inducible producers of methylase that consistently exhibit macrolide resistance and both macrolide and clindamycin resistance when the strain is exposed to those antibiotics.[14,17]

Drug Inactivation

Enzymatic inactivation of erythromycin and some other 14-, 15-, and 16-member ring macrolides by phosphotransferases has been described in strains of *S. aureus, E. coli,* and *Nocardia* spp. and is encoded by genes designated *mph*(A), *mph*(B), and *mph*(C).[6] Esterase genes (*ere*[A] and *ere*[B]) on plasmids encode for the hydrolysis of the macrocyclic lactone of erythromycin and have been found in strains of *E. coli, Klebsiella* spp., *Citrobacter* spp., *Proteus* spp., *Enterobacter* spp., and rare strains of *S. aureus.*[6]

Polymerase chain reaction methods that allow for relatively rapid detection of the different mechanisms of macrolide resistance among clinical isolates (genotypic testing) have been developed.[18] A given bacterial strain may possess more than one type of macrolide resistance mechanism, resulting in complex resistant phenotypes.

Antimicrobial Activity

The antimicrobial activity of erythromycin is broad in spectrum, being exhibited against gram-positive and gram-negative bacteria, including actinomycetes and mycobacteria, as well as against treponemes, mycoplasmas, *Chlamydia,* and rickettsiae. Depending on drug concentration, bacterial species, phase of growth, and density of the inoculum, erythromycin is essentially bacteriostatic. Bacterial killing is favored by higher antibiotic concentrations, higher pH, lower bacterial density, and rapid growth.[19] The activity of erythromycin, which is a weak base, increases markedly with increasing pH over the range of 5.5 to 8.5 for both gram-positive and gram-negative bacteria,[20,21] possibly reflecting increased entry into the bacterial cell of the un-ionized drug that is more plentiful at the higher pH.

The in vitro susceptibilities of potential pathogens to erythromycin are listed in Table 29.1.[6,16,22–33] Erythromycin shows high activity against the majority of pneumococci and group A streptococci isolated in the United States; however, resistant clinical isolates have been increasingly encountered worldwide, especially in *S. pneumoniae* and particularly among penicillin-resistant group A streptococci.[22,34–37] In the United States (1998–2011), the prevalence of erythromycin resistance among *S. pneumoniae* strains was approximately 45% by 2011.[38] In another study of *S. pneumoniae* strains from the United States, approximately 37% of strains with intermediate resistance to penicillin (MIC, 0.12–1.0 μg/mL) and 69% of strains showing high-level resistance to penicillin (MIC, >2.0 μg/mL) were resistant to erythromycin.[39] *S. pneumoniae* strains demonstrate complete cross-resistance among the macrolides,[40,41] but cross-resistance extending from the macrolides to clindamycin is variable, depending on whether the resistance mechanism is of the MLS_B or M phenotype.[41,42] The M phenotype had been the predominant one for *S. pneumoniae* in the United States, accounting for 83% of isolates and usually associated with low levels of erythromycin resistance, which typically does not extend to clindamycin.[35,43] Recent surveillance data from the United States, however, demonstrate a decrease in prevalence of the M phenotype and an increase in prevalence of clones with both MLS_B and M phenotypes.[44,45] The MLS_B phenotype is the predominant one in most of Europe[46] and Asia[47] for *S. pneumoniae,* and it is associated with a high level of erythromycin resistance (MIC, 128 to >1024 μg/mL)[43] and generally with clindamycin resistance.

Worldwide, erythromycin resistance in *S. pyogenes* is demonstrated in approximately 7% to 39% of isolates,[48–50] with lower rates of macrolide resistance of 5% to almost 7% reported in the United States.[17,51,52] As with *S. pneumoniae,* there is cross-resistance among the macrolides in *S. pyogenes;* however, resistance does not extend to clindamycin if resistance is due to the M phenotype.[50,53] A survey of 1885 clinical strains isolated in 2002 to 2003 from 45 medical centers in the United States showed that almost 7% were macrolide resistant, with 56% of them demonstrating the MLS_B phenotype and 44% having the M phenotype.[17]

Resistance to erythromycin by *S. aureus* may be selected by its use in hospitals.[54] Most methicillin-resistant strains and many methicillin-sensitive clinical isolates are now resistant to this agent.[55,56] Analyses of staphylococcal isolates from pediatric and adult patients revealed that across all ages, strains that were methicillin sensitive were on average 35% resistant to erythromycin, whereas strains that were methicillin resistant were 88% resistant.[57] In addition, there is a potential for the emergence, during treatment in an individual patient, of erythromycin resistance by *S. aureus.*[58,59] These strains may demonstrate the emergence of high-level resistance to erythromycin alone, or they may show cross-resistance to other macrolides and to lincomycin and clindamycin[14] due to the inducible type of MLS_B resistance, described earlier.

The viridans group of streptococci has traditionally been considered generally susceptible to erythromycin. However, macrolide resistance may be increasing in some areas, with 57.8% susceptible in North America, 62.7% in Asia and the Pacific, and 73.7% in Europe.[48,60]

Other susceptible gram-positive organisms include the majority of strains of *Listeria monocytogenes* and *C. diphtheriae.*[61,62] Appreciable in vitro activity has been demonstrated against *Nocardia asteroides* when combined with ampicillin.[63]

Erythromycin displays activity against a wide range of gram-positive anaerobes, including *Actinomyces israelii,*[64] *Peptostreptococcus* spp., *Propionibacterium, Lactobacillus, Eubacterium, Bifidobacterium,* and many strains of *Peptococcus.*[65] *Clostridium tetani* and *C. perfringens* are generally susceptible[66]; however, many strains of *C. perfringens* may be only moderately sensitive[67] due to erythromycin target-site alterations.[68] Resistance among *C. difficile* strains has increased over time, with resistance rates ranging from 27% to 63%[69,70] primarily due to the increase in prevalence of certain ribotypes, such as polymerase chain reaction ribotype 001.[70]

With gram-negative bacteria, erythromycin displays activity against *Bordetella pertussis,* for which reports of resistance are rare,[71] and *Moraxella catarrhalis*[72]; moderate activity against *Neisseria meningitidis*[73] and *Neisseria gonorrhoeae*[74,75]; and relatively poor activity against *Haemophilus influenzae.*[74] Enterobacteriaceae are resistant to erythromycin.[76] However, erythromycin activity against gram-negative organisms is affected by pH: as the pH rises to 8.5, so does erythromycin's activity against organisms such as *E. coli* and *Klebsiella pneumoniae.*[21] In 2004, only 0.3% of *Campylobacter jejuni* isolates tested in the United States were resistant (MIC, ≥32 μg/mL) to erythromycin.[77]

Breakpoint interpretive criteria for macrolide activity against anaerobic bacteria has not been established, but erythromycin generally has moderate activity against some species of gram-negative anaerobes, such as *Prevotella* and *Porphyromonas,* but *B. fragilis* strains are usually resistant.[78] Erythromycin also demonstrates clinically useful activity against such diverse organisms as *Legionella pneumophila,*[30] *M. pneumoniae,*[30] *Ureaplasma urealyticum,*[79] *Chlamydia trachomatis,*[28] and *Chlamydia pneumoniae.*[80] Extracellular and intracellular *L. pneumophila* strains show substantial susceptibility to erythromycin.[30,81] Erythromycin is about 30 times more potent against *M. pneumoniae* than is levofloxacin[30] and 50 times more potent than tetracycline.[82] Macrolide-resistant variants of *M. pneumoniae* range in incidence between 2% and 26% in European countries,[83,84] 13.2% in the United States,[85] and up to 90% in some areas of Japan and China.[86,87]

Atypical mycobacteria are erythromycin sensitive; however, both clarithromycin and azithromycin are more active than erythromycin against mycobacteria.[88] *Mycobacterium tuberculosis* is resistant to erythromycin.[89]

Clinical Pharmacology

The peak serum levels obtained after single doses of various erythromycin preparations are given in Table 29.2.[90–92] Erythromycin base is subject

TABLE 29.1 In vitro Susceptibilities to Erythromycin, Azithromycin, and Clarithromycin

	ERYTHROMYCIN		AZITHROMYCIN		CLARITHROMYCIN	
ORGANISM	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
Streptococcus pneumoniae						
Penicillin-susceptible (MIC ≤0.06 μg/mL)	0.03	1.0	0.125	1.0	0.016	0.25
Penicillin-intermediate (MIC = 0.12–1.0 μg/mL)	0.03	>64.0	0.125	>64.0	0.03	>64.0
Penicillin-resistant (MIC ≥2.0 μg/mL)	1.0	>64.0	1.0	>64.0	0.5	>64.0
Streptococcus pyogenes	≤0.06	0.06	0.12	0.25	0.03	0.06
Streptococcus agalactiae	0.06	8	0.06	0.12	0.03	0.06
Viridans streptococci	0.12	>128	2.0	8.0	0.5	8
Enterococcus spp.						
Vancomycin-sensitive	1	2	>8	>8	0.5	1
Vancomycin-resistant	>128	>128	>8	>8	>128	>128
Staphylococcus aureus						
Methicillin-sensitive	0.25	>128	—	—	0.06	>128
Methicillin-resistant	>128	>128	>128	>128	>128	>128
Staphylococcus epidermidis	32	>128	16	128	16	>128
Corynebacterium diphtheriae	0.015	0.026	0.044	0.058	0.006	0.008
Listeria monocytogenes	0.125	0.25	1	1	0.06	0.125
Moraxella catarrhalis	≤0.25	≤0.25	≤0.06	0.06	≤0.25	≤0.25
Haemophilus influenzae	4	8	1	2	8	16
Bordetella pertussis	0.03	0.06	0.03	0.06	0.06	0.06
Neisseria gonorrhoeae	0.5	2	0.12	0.25	0.25	1
Neisseria meningitidis	1	1	0.5	1	0.12	0.5
Campylobacter jejuni	0.5–2	1–4	0.25	0.12–0.5	0.5–2	1–8
Helicobacter pylori	0.12	0.25	0.25	0.5	0.008	0.015
Mycoplasma pneumoniae	≤0.015	≤0.015	≤0.015	0.015	≤0.015	≤0.015
Chlamydia trachomatis	—	≤0.25[b]	—	0.25[b]	—	≤0.015[b]
Chlamydia pneumoniae	0.125	0.25	0.125	0.25	NA	0.03
Legionella pneumophila	0.125	0.5	0.12	0.5	0.032	0.046
Bacteroides fragilis	32	>32	>32	>32	4	8
Peptococcus, Peptostreptococcus	2	16	1	>64	1	4
Clostridium perfringens	2	2	4	4	0.125	0.125
Propionibacterium spp.	≤0.06	0.5	0.125	2	≤0.06	≤0.06
Mycobacterium avium complex	—	≥64	8[c]	—	2[c]	—

[a] *MIC_{50} (MIC_{90})*, Minimal inhibitory concentration for 50% (90%) of isolates (μg/mL); values are ranges reported in referenced publications.[6,16,22–32,61,67,74,201,388–390]
[b] Reported as MIC_{100}.
[c] Reported as median MIC.

TABLE 29.2 Serum Levels of Erythromycin in Adults

			PEAK SERUM LEVELS	
PREPARATION	**DOSE (mg)**	**ROUTE**	**Hours After Dose**	**Concentration (μg/mL)**
Base	250	Oral	4	0.3–1.0[a]
	500		4	0.3–1.9
Stearate	250 (fasting)	Oral	3	0.2–1.3
	500 (fasting)		3	0.4–1.8
	500 (after food)		3	0.1–0.4[b]
Ethylsuccinate	500	Oral	0.5–2.5	1.5[c] (0.6[d])
Estolate	250	Oral	2–4	1.4–1.7
	500		3.5–4	4.2[c] (1.1[d])
Lactobionate	200	Intravenous	Immediately	3–4
	500		1	9.9

[a] Somewhat higher levels reported with some enteric-coated preparations after repeated doses.[62]
[b] One study demonstrated higher levels (to 2.8 μg/mL) with dose taken during a meal.
[c] Total drug (inactive ester and free base).
[d] Free base.

to destruction by gastric acid, and preparations of the base have been made with an acid-resistant coating to delay dissolution of the drug until it reaches the small bowel. The esters and ester salts of erythromycin used in the liquid suspension are more acid stable, form a stable suspension in water, and are tasteless. Erythromycin base (absorbed intact), stearate (absorbed as the base), and ethylsuccinate (absorbed both as the intact ester and as the free base after hydrolysis in the intestine) are usually absorbed more completely in the fasting state, although one study demonstrated increased absorption of a stearate preparation when it was taken with a meal.[93] After absorption, about 45% of the ethylsuccinate preparation is present in the serum as the inactive ester and about 55% as the active base.

Average serum levels achieved under fasting conditions with these preparations are similar; however, results with the base may be erratic. Erythromycin base has become available in a capsule containing enteric-coated granules; this preparation is promoted as giving more uniform absorption,[93,94] but some enteric-coated tablets may provide similar blood levels.[95] The level of base achieved is similar to that achieved by the other oral preparations taken in comparable doses in the fasting state. The clinical significance of the much less active esterified form of the drug that is present in serum in appreciable concentration is controversial. It would seem that in treatment of infections of only moderate severity by organisms highly sensitive to erythromycin, differences in therapeutic results using the various oral preparations would be insignificant. Limited clinical comparisons confirm that suspicion.[96]

Intravenous preparations of erythromycin achieve appreciably higher serum levels and should be used to treat serious infections requiring erythromycin.

Erythromycin is distributed through total body water.[97] Values given for protein binding vary from 40% to 90%. The drug persists in tissues longer than in the blood. The ratios of tissue or body fluid concentrations to simultaneous serum concentrations (usually at peak) are as follows: aqueous humor, 0.3; ascites, 0.4; bile, 28; middle ear exudate in otitis media, 0.3 to 0.7; pleural fluid, 0.7; prostatic fluid, 0.4; cerebrospinal fluid (CSF) without meningitis, 0 to 0.02, and with meningitis, 0.05 to 0.1; infected maxillary paranasal sinus, 0.4 to 0.8; and tonsil, 0.3. Concentrations achieved in the middle ear in otitis media are adequate to treat pneumococcal and group A streptococcal infections involving sensitive strains of these species but are not adequate to consistently eradicate *H. influenzae*.[98,99] High concentrations of erythromycin are achieved in alveolar macrophages[100] and polymorphonuclear leukocytes[101] compared with those in extracellular fluid.

Erythromycin does not enter the CSF in the setting of normal (uninflamed) meninges.[102] However, concentrations of erythromycin achieved in the CSF of patients with meningitis suggest that large parenteral doses may be effective against highly susceptible organisms such as *S. pneumoniae*.[103] Limited data from patients with septic arthritis suggest poor penetration of synovial fluid. Erythromycin is transferred across the placenta; fetal serum concentrations are about 2% of those in maternal serum, but higher concentrations accumulate in fetal tissue and amniotic fluid.[104] The drug is excreted in breast milk with a ratio of 0.41 between maternal milk and plasma levels, but it is considered safe if the infant is of an age to receive erythromycin directly.[105]

Up to 4.5% of an oral dose and 15% of a parenteral dose of erythromycin are recoverable in the urine. Urine concentrations after oral doses are often high but quite variable. Erythromycin is concentrated by the liver and excreted into the bile in high concentrations; however, only about 1.5% of the dose of the base and 0.2% of the ester can be recovered from bile in the first 8 hours, and some of this is reabsorbed from the intestine.[106] After an oral dose, large concentrations of the antibiotic are found in feces, probably representing ingested drug that was never absorbed as well as some that was excreted in bile. A large proportion of absorbed drug cannot be accounted for by urinary or biliary excretion or by tissue binding and may be inactivated in the liver by demethylation.[107]

The normal serum half-life of erythromycin is 1.4 hours, and appreciable serum levels are maintained for 6 hours. In anuric patients, the half-life is prolonged to about 5 hours, but dosage reduction in patients with renal failure is generally not necessary.[108,109] Erythromycin is not removed by peritoneal dialysis or hemodialysis.

Adverse Reactions

Although frequently used specifically for its motility effects, gastrointestinal adverse effects (30%) are the most common adverse effects caused by erythromycin. Symptoms including abdominal pain (16%), nausea and vomiting (14%), and diarrhea occur more commonly in children and young adults than in older persons and may be associated with either intravenous or oral administration.[110] Pseudomembranous colitis caused by overgrowth of toxin-producing *C. difficile* occurs rarely with the use of erythromycin.[111,112]

Thrombophlebitis with intravenous use can be decreased by appropriate dilution of the dose in at least 250 mL of solution and by avoidance of rapid infusions. Infusion should take place over 45 to 60 minutes.

Allergic reactions including skin rash, fever, and eosinophilia are rare. Severe reactions such as Stevens-Johnson syndrome have been reported.[113] Cholestatic hepatitis occurs rarely,[114] and chiefly in adults.[115] The syndrome typically begins after 20 days of therapy, but more rapidly in those previously treated, and consists of nausea, vomiting, and abdominal pain followed by jaundice, fever, and abnormal liver function tests consistent with cholestatic hepatitis. These findings are sometimes accompanied by rash, leukocytosis, and eosinophilia. The abnormalities usually clear within days to a few weeks after the drug is stopped but may return rapidly on rechallenge. However, hepatocyte toxicity induced by the drug or its metabolites, as well as allergy to altered hepatocyte components, may be contributory.[116] Reversible hepatotoxicity, including jaundice, has occurred with the stearate salt and with the ethylsuccinate ester of erythromycin.[117]

Ototoxicity has been reported rarely in association with the use of large intravenous doses of erythromycin lactobionate or large doses of oral erythromycin.[118,119] This may occur more commonly in older adults, in patients with hepatic or renal insufficiency, with receipt of higher doses, and with concurrent use of ototoxic medications.[120,121] Symptoms are usually reversible; however, irreversible tinnitus and hearing loss have been reported.[113,122]

Mitochondrial toxicities have also been reported with the use of macrolides. When simulating concentrations of locally applied antibiotics in a cell culture model of primary human osteoblasts, erythromycin inhibited mitochondrial energetics, namely, proliferation and metabolic activity, of the osteoblasts, but to a lesser degree than azithromycin.[123] Mitochondria isolated from rat cardiomyocytes have also been affected by exposure to macrolide antibiotics. The resulting induced cascade of reactive oxygen species formation, mitochondrial membrane permeability, mitochondrial swell, and cytochrome *c* release within the cardiomyocytes with exposure to azithromycin, clarithromycin, and erythromycin has been proposed as a starting point for cardiotoxicities, including arrhythmias, QT prolongation, and torsades de pointes.[124]

Polymorphic ventricular tachycardia (torsades de pointes) with QT prolongation has been reported in association with treatment with intravenous and oral erythromycin.[125–127] The drug has been shown to affect repolarization in the isolated heart and to block electrical current in guinea pig ventricular myocytes in a way consistent with the observed arrhythmia.[128] The possibility for interaction with potential-lengthening drugs (classes IA and III antiarrhythmics) and for increased risk in the presence of electrolyte abnormality or prolonged QT interval should be kept in mind. Other major contributing factors to macrolide-associated torsades de pointes include the coadministration of cytochrome P-450 isoenzyme 3A4 (CYP3A4) inhibitors (resulting in increased drug exposure). One study reported that coadministration of CYP3A4 inhibitors and erythromycin led to a fivefold greater risk of cardiac sudden death.[127] (See further discussion of the potential for macrolide-induced arrhythmias and sudden death in the "Adverse Effects" section under "Azithromycin and Clarithromycin".)

Superinfection, especially of the gastrointestinal tract or vagina, with *Candida* species or gram-negative bacilli may occur, as with other antibiotics.

Infantile hypertrophic pyloric stenosis has been epidemiologically linked to early exposure to erythromycin in children. It has been hypothesized that erythromycin interacts with motilin receptors, inducing

strong gastric and pyloric contractions leading to pyloric hypertrophy.[129,130] There is no substantive evidence of a risk associated with prenatal exposure.[131] Erythromycin is classified as pregnancy category B.

Drug Interactions

Incompatibility during administration between intravenous preparations of erythromycin and other drugs has been reported; the latter include vitamin B complex and vitamin C, cephalothin, tetracycline, chloramphenicol, colistin, and heparin.

Erythromycin may produce interactions with other drugs by interfering with their hepatic metabolism through the CYP3A subclass of the cytochrome P-450 enzyme system.[132,133] The resulting increased drug levels may result in serious toxicity (Table 29.3).[134] For example, elevations of midazolam serum concentrations have led to unconsciousness. The reverse of that process, in which drugs that elevate levels of erythromycin may promote its proarrhythmic effects, has been discussed earlier (see the "Adverse Reactions" section under "Erythromycin").

Erythromycin can increase the bioavailability of digoxin, possibly by interfering with its inactivation by gut flora.[132] Erythromycin may inhibit the assay organism used in some determinations of serum folic acid. Sequential use of erythromycin and clindamycin should be avoided when possible because of the potential for the development of cross-resistance or dissociated resistance.

Uses of Erythromycin

Erythromycin has few indications for use as the drug of choice due to the availability of other macrolides with improved pharmacokinetic profiles, in addition to fewer side effects and drug interactions (Table 29.4).[6,75,109,135–146]

Although erythromycin continues to be useful in the treatment of community-acquired respiratory infections, its utility has become limited to an alternative to penicillin G for group A β-hemolytic streptococci

TABLE 29.3 Potentially Clinically Significant Drug Interactions Produced by Macrolides

DRUG	INTERACTIONS
Erythromycin	Alfentanil, alprazolam, amiodarone, bromocriptine, buspirone, carbamazepine, cilostazol, cisapride, clomipramine plus risperidone, clozapine, colchicine, cyclosporine, diazepam, digoxin, disopyramide, dofetilide, ergot alkaloids, felodipine, lidocaine, levofloxacin, loratadine, lovastatin, methylprednisolone, midazolam, moxifloxacin, phenytoin, pimozide, quinidine, repaglinide, rifabutin, rifampin, ropivacaine, saquinavir, sertraline, sildenafil, simvastatin, sirolimus, sotalol, tacrolimus, terfenadine, theophylline, triazolam, valproate, verapamil, warfarin
Clarithromycin	Alprazolam, amiodarone, amprenavir, atazanavir, atorvastatin, carbamazepine, cisapride, colchicine, cyclosporine, darunavir, diazepam, digoxin, disopyramide, disulfiram, dofetilide, efavirenz, ergot alkaloids, fluoxetine, itraconazole, lidocaine, loratadine, lovastatin, methylprednisolone, midazolam, phenytoin, pimozide, quinidine, repaglinide, rifabutin, rifampin, ritonavir, saquinavir, sildenafil, simvastatin, sirolimus, tacrolimus, terfenadine, theophylline, verapamil, warfarin, zidovudine
Azithromycin	Cyclosporine (one case), digoxin, phenytoin, rifampin, tacrolimus, warfarin

[a]Interactions generally lead to increased levels of the listed drugs caused by interaction of the macrolide with cytochrome P-450 hepatic metabolism. Exceptions are digoxin, in which the raised levels are caused by interference with digoxin inactivation by gut flora, and zidovudine, in which serum concentrations may be decreased by unknown mechanisms. Rifampin and rifabutin may lower the levels of clarithromycin, and clarithromycin may raise the levels of rifampin and rifabutin. Ritonavir may raise clarithromycin levels. Clarithromycin may produce modest reduction of zidovudine levels.
Modified from Kim RB and the Editors of the Medical Letter. The Medical Letter Handbook of Adverse Drug Interactions. *New Rochelle, NY: The Medical Letter on Drugs and Therapeutics; 2003.*

TABLE 29.4 Major Indications for Use of Macrolides

INFECTIONS IN WHICH MACROLIDES ARE THE DRUGS OF CHOICE

INFECTION	MACROLIDE	ADULT DOSAGES[a]
Bartonella henselae (cat-scratch disease bacillus)	Azithromycin Clarithromycin[b]	500 mg PO on day 1, then 250 mg PO on days 2–5 500 mg PO bid for 7–10 d
Bartonella henselae or *B. quintana* (bacillary angiomatosis, trench fever)	Erythromycin Azithromycin	0.5 g qid PO for 12 wk 500 mg qd PO for 4–6 wk (patients with endocarditis should receive treatment for 4–6 mo)
Bordetella pertussis	Erythromycin Azithromycin Clarithromycin[b]	0.5 g qid PO for 14 d 500 mg PO on day 1, then 250 mg PO on days 2–5 500 mg PO bid for 7 d
Campylobacter jejuni[c]	Azithromycin Erythromycin	500 mg daily PO for 3–7 d 250 mg qid PO for 5–7 d
Chlamydia pneumoniae (TWAR strain)	Azithromycin Clarithromycin[b] Erythromycin	500 mg qd PO/IV for 1–2 d, then 500 mg PO qd to complete 7–10 d[d] 250–500 mg bid PO for 7–10 d 0.5 g tid–qid PO for 7–10 d
Chlamydia trachomatis (inclusion conjunctivitis)[e]	Erythromycin Azithromycin	Erythromycin base or ethylsuccinate 50 mg/kg/d PO divided into 4 doses daily for 14 d Single dose of 20 mg/kg up to a maximum dose of 1 g
Chlamydia trachomatis (pneumonia)[e]	Erythromycin	Erythromycin base or ethylsuccinate 50 mg/kg/d PO divided into 4 doses daily for 14 d
Chlamydia trachomatis (trachoma)	Azithromycin	1 g PO, single dose
Chlamydia trachomatis (urethritis or cervicitis)	Azithromycin	1 g PO, single dose
Diphtheria[f]	Infection: erythromycin Carrier: erythromycin	125–500 mg qid PO for 14 d 250 mg qid PO for 7–10 d
Haemophilus ducreyi (chancroid)	Azithromycin	1 g, single dose
Helicobacter pylori	Clarithromycin[b] (+amoxicillin, *or* metronidazole + proton pump inhibitor)	500 mg bid PO for 10–14 d
Legionella spp. pneumonia	Azithromycin ± rifampin (or a fluoroquinolone ± rifampin)	1 g IV or PO on day 1, then 500 mg qd for 7–10 d total duration

TABLE 29.4 Major Indications for Use of Macrolides—cont'd

INFECTIONS IN WHICH MACROLIDES ARE THE DRUGS OF CHOICE		
INFECTION	**MACROLIDE**	**ADULT DOSAGES[a]**
Mycobacterium avium complex disseminated disease	Clarithromycin[b] (+ethambutol ± rifabutin) Azithromycin (+ethambutol ± rifabutin)	500 mg PO bid for variable periods[g] 500–600 mg qd PO for variable periods[g]
Mycobacterium avium complex prophylaxis	Azithromycin Clarithromycin[b]	1200 mg once weekly PO until initiation of effective antiretroviral therapy 500 mg bid PO until initiation of effective antiretroviral therapy
Mycobacterium avium complex pulmonary infiltrative disease	Clarithromycin[b] (+ethambutol ± rifabutin) Azithromycin (+ethambutol ± rifabutin)	500 mg bid PO for 1 yr after sputum cultures are negative 500–600 mg qd PO for 1 yr after sputum cultures are negative
Mycobacterium fortuitum/chelonae complex	Clarithromycin[b] (+amikacin)	500 mg bid PO for 4–6 mo
Mycoplasma pneumoniae	Azithromycin Clarithromycin[b] Erythromycin	500 mg PO qd for 5–10 d 250 mg PO bid for 14 d 0.5 g tid–qid PO for 14–21 d
Nongonococcal urethritis in men (*C. trachomatis* or *Ureaplasma urealyticum*)	Azithromycin	1 g PO, single dose
INFECTIONS IN WHICH MACROLIDES ARE AN IMPORTANT ALTERNATIVE DRUG		
INFECTION	**MACROLIDES AND ADULT DOSAGES[a]**	**DRUG OF CHOICE**
Groups A, C, G streptococcal infection	Erythromycin 250–500 mg qid PO[h] Azithromycin 500 mg PO on day 1, then 250 mg PO on days 2–5 Clarithromycin[b] 250 mg bid PO[h]	Penicillin G or V
Streptococcus pneumoniae infection	Erythromycin 250–500 mg qid PO[i] Azithromycin 500 mg qd[i] Clarithromycin[b] 250–500 mg bid[i]	Penicillin G, ceftriaxone, or cefotaxime
Moraxella catarrhalis	Azithromycin 500 mg PO on day 1, then 250 mg PO on days 2–5 Erythromycin 250–500 mg qid PO Clarithromycin[b] 250–500 mg bid PO	Cefuroxime; a fluoroquinolone
Haemophilus influenzae (upper respiratory infection and bronchitis)	Azithromycin 500 mg PO on day 1, then 250 mg PO on days 2–5 Clarithromycin[b] 250–500 mg bid PO	Trimethoprim-sulfamethoxazole
Shigella	Azithromycin 500 mg PO on day 1, then 250 mg PO on days 2–5	Fluoroquinolone
Prevention of infection after colorectal surgery	1 g PO each of neomycin and erythromycin base at 1, 2, and 11 PM on the day before 8 AM surgery (combined with vigorous purgation over second day before surgery)	Cefoxitin or cefotetan
Rheumatic fever prophylaxis	Erythromycin 250 mg bid PO	Penicillin G
Anthrax	Erythromycin 500 mg qid PO for 10 d[j]	Ciprofloxacin, doxycycline
Lymphogranuloma venereum	Erythromycin 500 mg qid PO for 21 d	A tetracycline
Acne vulgaris	Erythromycin 250 mg qid PO or topical preparation	A tetracycline PO and a number of topical drugs
Borrelia burgdorferi (Lyme disease)	Azithromycin 500 mg PO for 7–10 d[k]	Doxycycline, amoxicillin, cefuroxime axetil PO[k]
Babesia microti	Azithromycin 500 mg on day 1 and 250 mg on days 2–7 + atovaquone 750 mg q12h	Clindamycin + quinine

[a]Intravenous therapy should be used in serious illness or when oral therapy is not possible or reliable.
[b]Not recommended for use in pregnancy.
[c]In some areas, such as in Thailand, macrolide- and fluoroquinolone-resistant strains have become common.
[d]Mild-to-moderate severity: azithromycin 500 mg PO on day 1, then 250 mg PO on days 2–5.
[e]Diseases of infants.
[f]Antitoxin is essential primary therapy for disease.
[g]May be discontinued after >1 yr with *M. avium* complex treatment, when CD4 cell count >100 cells/mm^3 for 3–6 mo on highly active antiretroviral therapy (HAART), and patient is asymptomatic.
[h]Treatment should be continued for 10 days for group A.
[i]Resistance to macrolides is increasing and is particularly frequent in penicillin-resistant strains.
[j]Therapy may need to be continued for prolonged periods until vaccination is completed in those infected by the pulmonary route.
[k]For treatment of erythema migrans, uncomplicated facial nerve palsy, mild cardiac disease, and arthritis, oral therapy is satisfactory. For other neurologic or more serious cardiac disease, intravenous therapy with ceftriaxone, cefotaxime, or penicillin G is recommended.

in the setting of penicillin hypersensitivity. Use is further limited in areas in which the incidence of penicillin-resistant pneumococci is high, and thus resistance to erythromycin is common.[44,147] Erythromycin is also effective for the treatment of *M. catarrhalis*. Erythromycin is not consistently effective in treatment of infections caused by *H. influenzae*.[98,99] Treatment of *M. pneumoniae* infection with erythromycin, as with tetracycline, shortens the clinical course of the infection, even if started late in the course of illness; radiologic clearing of pulmonary lesions occurs earlier with erythromycin.[148,149] Clinical experience and studies in vitro and in guinea pigs suggest that erythromycin is effective in treating pneumonia caused by *L. pneumophila* or *Legionella micdadei*[150]; however, its use has been superseded by the newer macrolides, azithromycin and clarithromycin.[151] The US Food and Drug Administration has approved azithromycin and levofloxacin for the treatment of

legionellosis, and they are now considered preferable to erythromycin, especially when given orally.[140,152]

Early treatment of pertussis with erythromycin is associated with clinical improvement, a rapid clearance of *B. pertussis* from the nasopharynx, and a reduction in secondary transmission in households. Erythromycin is also recommended for postexposure prophylaxis of pertussis.[153,154] Treatment of infants with erythromycin for pneumonia or conjunctivitis caused by *C. trachomatis* is approximately 80% effective, although a second course of antimicrobial therapy may be required.[155] Because of reports of an association between oral erythromycin and infantile hypertrophic pyloric stenosis, monitoring for its signs and symptoms should be implemented in treated infants who are younger than 6 weeks of age.[130]

Erythromycin treatment of patients with gastroenteritis caused by *C. jejuni* hastens the eradication of the organism from the feces but does not appear to alter the clinical course of uncomplicated infection when therapy begins 4 days or more after the onset of symptoms.[156] However, earlier treatment of young children with acute dysentery associated with *C. jejuni* has been shown to shorten the course of diarrhea and fecal excretion of susceptible organisms.[157,158]

Erythromycin base given orally together with neomycin on the day before colorectal surgery and combined with vigorous purgation is about as effective as parenteral cephalosporin administration just before surgery in decreasing the incidence of septic complications.[159] No advantage has been demonstrated for the use of a combination of oral and intravenous antibiotics.[160] In the presence of bowel obstruction or when there is need for emergency surgery, the parenteral antibiotic regimen should be used.[109]

Erythromycin given orally for up to 3 months remains the drug of choice in treating certain visceral or angioproliferative *Bartonella* infections (bacillary angiomatosis and bacillary peliosis hepatis in immunocompromised patients), but might be difficult to tolerate.[109,161,162] Relapses have been described, especially of lesions in bone and skin and when antibiotics are given for a shorter duration (<3 months), particularly in severely immunosuppressed patients.[163]

A comparative study involving a small number of children with cholera, who were all treated with rehydration solutions, showed that erythromycin or trimethoprim-sulfamethoxazole was effective and superior to treatment without an antimicrobial agent.[164] Erythromycin may be used as an alternative antibiotic in the treatment of anthrax. In view of the availability of more effective alternative drugs, erythromycin should not be used alone in the treatment of deep-seated staphylococcal infections because of the potential for the emergence of resistant strains during therapy.[59,165] Erythromycin may occasionally be useful in treating urinary tract infections caused by gram-negative bacilli that might otherwise require the use of more toxic agents.[166] Urine pH must generally be raised to 8.0 or above to achieve effective activity at urinary concentrations against the gram-negative bacilli.

Erythromycin and other 14-member ring macrolides have a gastrointestinal motility–stimulating effect. In this regard, erythromycin acts as a motilin receptor agonist in the gut and gallbladder.[167] Erythromycin lactobionate 3 mg/kg IV every 8 hours has been effective for the treatment of gastroparesis in hospitalized diabetic patients.[168] When given orally, erythromycin may improve gastric emptying for several weeks but is often associated with tachyphylaxis due to downregulation of the motilin receptor when given for longer than 4 weeks.[169] These prokinetic effects have also been studied for the treatment of postvagotomy gastroparesis,[170] gastroparesis in critically ill patients receiving mechanical ventilation,[171] and intestinal dysmotility in young infants.[172]

There has been interest in the antiinflammatory activities of low-dose erythromycin (600 mg/day) and other macrolides, first suggested due to the beneficial effect of erythromycin treatment of patients with diffuse panbronchiolitis.[173,174] Those activities include interference with oxidant production by neutrophils (in which the cladinose moiety of erythromycin was found to be the key structure[175]), acceleration of neutrophil apoptosis, suppression of the release of proinflammatory cytokines, and promotion of the release of nitric oxide from endothelial cells.[175,176] At the molecular level, macrolides appear to modulate inflammation in some cells, such as human bronchial epithelial cells, by inhibiting transcription factors,[177,178] which regulate the expression of interleukin-8, among others, a chemokine that acts as a major recruiter of neutrophils in chronic airway disease.[179] The antiinflammatory protective effects of erythromycin appear to be a slow process as demonstrated by the requirement of at least a 28-day pretreatment with erythromycin to suppress an inflammatory response in zymosan-induced peritonitis in rats.[180] The 14-member ring macrolides have been effective in animal models in preventing the acute exacerbation of interstitial pneumonia and acute lung injury, such as after the use of bleomycin.[23,181] In vitro studies have demonstrated that erythromycin and other macrolides at subinhibitory concentrations reduce the adherence of various pathogenic bacterial species, such as *Pseudomonas aeruginosa,* to host cells.[182]

AZITHROMYCIN AND CLARITHROMYCIN

Azithromycin and clarithromycin were developed to improve the qualities of erythromycin. They have better oral absorption, longer half-lives, fewer gastrointestinal side effects, and a greater antimicrobial spectrum of activity than erythromycin.

Derivation, Chemistry, and Preparations

Azithromycin is derived from erythromycin, differing in having a methyl-substituted nitrogen in its 15-member lactone ring (Fig. 29.2). It is therefore an azalide antibiotic. Clarithromycin, having a 14-member ring structure, is produced by modifying position C6 of the lactone ring of erythromycin to possess a methoxy group (Fig. 29.3). These changes increase the stability of these compounds in gastric acid, improving absorption by the oral route.[183]

Azithromycin is available in capsules for oral use as azithromycin dihydrate equivalent to 250 mg of azithromycin; in film-coated tablets of 250 mg, 500 mg, and 600 mg; as a powder for oral suspension (1 g/packet, 100 mg/5 mL, 200 mg/5 mL, and 2000 mg/60 mL as a single dose); and as IV powder for solution (500 mg). The 2000-mg/60-mL formulation of azithromycin is incorporated into sustained-release microspheres that release the drug slowly, allowing for most of the drug to be released into the lower gastrointestinal tract, thereby potentially

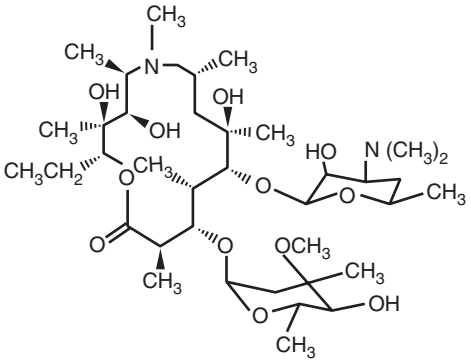

FIG. 29.2 **Azithromycin base.**

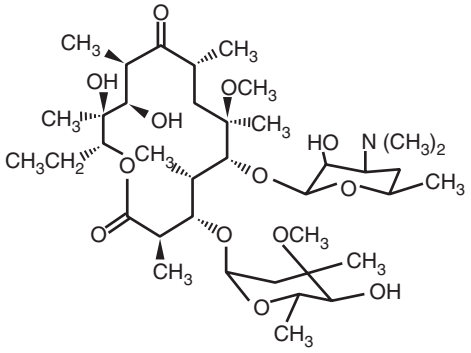

FIG. 29.3 **Clarithromycin base.**

reducing gastrointestinal side effects, and allowing for a higher dose to be administered as a single dose.

Clarithromycin is provided in 250- or 500-mg film-coated tablets, extended-release 500-mg tablets, and as granules for oral suspension (125 mg/5 mL and 250 mg/5 mL). Clarithromycin 500 mg is also available for intravenous administration in some countries.

Mechanisms of Action and Resistance

Limited studies suggest that azithromycin, clarithromycin, and erythromycin bind to the same receptor on the bacterial 50S ribosomal subunit and inhibit RNA-dependent protein synthesis by the same mechanism.[4,184] Azithromycin has greater activity than the 14-member macrolides erythromycin and clarithromycin against gram-negative bacteria (especially for *M. catarrhalis* and *H. influenzae*) and therefore appears to better penetrate the outer envelope of those organisms.[185] Like other macrolides, azithromycin and clarithromycin are generally considered to be bacteriostatic agents; however, bactericidal activity is demonstrated in vitro against such species as *S. pyogenes, S. pneumoniae,* and *H. influenzae.*[184,185] In vitro activity of the newer macrolides increases with rising pH, as with erythromycin.

Mechanisms of resistance to azithromycin or clarithromycin are the same as or similar to those for erythromycin.[3,5,6] There is complete cross-resistance among erythromycin, azithromycin, and clarithromycin for gram-positive organisms showing resistance to erythromycin by the MLS_B phenotype because the methylation mechanism already described operates for all of the 14- and 15-member macrolides.[15] Cross-resistance among the 14- and 15-member macrolides is also characteristic of the M phenotype and is the result of an efflux system for the drugs. Macrolide-resistant strains of *H. pylori* with point mutations in the 23S rRNA genes are increasing and are thought to be due to prior macrolide exposure.[186,187] Further studies with such strains suggest that horizontal transfer of the mutated gene can occur.[188] Similar point mutations in the 23S rRNA gene associated with macrolide resistance in *M. avium* complex have been selected in patients undergoing clarithromycin or azithromycin monotherapy for disseminated infections.[189]

Antimicrobial Activity

Although the most widely prescribed antibiotic in the United States, azithromycin is about two- to fourfold less active than erythromycin against *S. pneumoniae* and *S. pyogenes.*[183,184,190] Clarithromycin is highly active against gram-positive bacteria, being two- to fourfold more active than erythromycin against most streptococci, including *S. pneumoniae* and *S. pyogenes,* and methicillin-sensitive *S. aureus.*[34,183,184] Streptococci and staphylococci that are resistant to erythromycin are resistant to clarithromycin and azithromycin.[40,41,184,191] Selection for penicillin and multidrug-resistant *S. pneumoniae* appears to be associated with macrolide, as well as cephalosporin, use.[190] Additional information regarding the emergence of macrolide resistance in clinical isolates of *S. pneumoniae* and *S. pyogenes* was discussed earlier under "Erythromycin". Most methicillin-resistant staphylococci are resistant to the newer macrolides.[55,56] An active metabolite of clarithromycin, 14-hydroxyclarithromycin, has slightly greater activity than the parent compound against *S. aureus* and *S. pneumoniae* and is additive in vitro to the activity of clarithromycin.[6,191]

The activity of clarithromycin against many gram-negative bacteria is similar to that of erythromycin,[6,184] although it is slightly more active against *M. catarrhalis.* 14-Hydroxyclarithromycin also has slightly greater activity for *M. catarrhalis* and *H. influenzae* than the parent compound.[191] Azithromycin is more active than erythromycin or clarithromycin against gram-negative bacteria, especially against *H. influenzae* and *M. catarrhalis,* likely because of its higher penetration into the bacteria due to higher lipophilicity.[183,192,193] The greater activity of azithromycin against the Enterobacteriaceae is of questionable clinical significance. However, it is of interest that prolonged incubation of *P. aeruginosa* strains with macrolides at clinically achievable concentrations is associated with decreased viability and diminished protein synthesis.[194] Azithromycin is the most potent in that regard. Furthermore, macrolides have been shown to inhibit the expression of quorum sensing of *P. aeruginosa,* thereby downregulating quorum sensing–regulated genes that encode virulence factors such as adhesion molecules and those that bring about cytotoxicity and inflammation.[195] Azithromycin is more active than erythromycin against *N. meningitidis* and *N. gonorrheae;* however, azithromycin resistance among *N. gonorrheae* isolates has increased up to 4% and 7.9% as of 2014 in the United States and European countries.[196,197]

Azithromycin and clarithromycin have equal or slightly better in vitro activities than erythromycin against *L. pneumophila.*[30,81] All three macrolides generally have good activity against *M. pneumoniae* and *C. pneumoniae,*[30,79,191] although *M. pneumoniae* macrolide-resistant isolates have been detected sporadically in recent years, especially in Japan, as discussed earlier in the "Antimicrobial Activity" section under "Erythromycin". Azithromycin and clarithromycin have significantly greater activity than erythromycin against *C. trachomatis* and *U. urealyticum*[28,79] and somewhat greater activity against *Borrelia burgdorferi.*[184,185] The small in vitro differences in potency among these macrolides may not have any clinical significance with regard to efficacy.[198] Both azithromycin and clarithromycin also have significant and approximately equal activity against *Toxoplasma gondii* in tissue culture systems.[199]

The macrolides show little activity against *M. tuberculosis.*[200] In contrast, clarithromycin shows substantial activity against *Mycobacterium leprae* and is superior in this respect to erythromycin and azithromycin.[191,200] Clarithromycin and azithromycin have appreciable activities against *M. avium* complex. Clarithromycin is about fourfold more active than azithromycin against this organism in vitro[201] and is somewhat more active in slowing its replication in infected human macrophages.[202]

Azithromycin and clarithromycin demonstrate activity against *Mycobacterium abscessus* complex species; however, this organism produces an inducible 23S rRNA methylase encoded by *erm*(41), which promotes macrolide resistance. Overall, clarithromycin induces greater *erm*(41) expression leading to higher macrolide resistance compared to azithromycin, but levels of inducible clarithromycin resistance are distinguishable among the subspecies of *M. abscessus.*[203] *M. abscessus* subspecies *bolletii* demonstrate inducible resistance, as do *M. abscessus* subspecies *abscessus,* if the *erm*(41) sequevar T28 is present. Conversely, *erm*(41) sequevar C28 is associated with clarithromycin susceptibility among *M. abscessus* subspecies *abscessus. M. abscessus* subspecies *massiliense* may also harbor *erm*(41) but remain susceptible to clarithromycin.[204,205]

Clinical Pharmacology

The oral bioavailability of azithromycin after a single 500-mg dose is 37%.[206] Food increases the maximum serum concentration by approximately 50%,[207] but absorption is unaffected by magnesium- or aluminum-containing antacids.[208] The maximum serum concentration achieved after a single 500-mg oral dose was 0.41 μg/mL within 2 to 4 hours.[184] The maximum concentration and 24-hour area under the curve after a single 2-g dose of the sustained-release formulation are two and three times higher than those achieved with a total treatment dose of 1.5 g of conventional, immediate-release azithromycin.[209]

Protein binding of azithromycin in serum varies between 7% and 50% depending on the drug concentration.[184] Azithromycin is widely distributed in tissues, and for most tissues the drug concentration exceeds that in serum by 10- to 100-fold,[206] particularly in sputum and lung. Very high concentrations were found in alveolar macrophages and neutrophils.[210] The extensive tissue uptake of azithromycin has been attributed to cell uptake of this basic compound into relatively acidic lysosomes because of ionic trapping.[210] Very low concentrations were noted in CSF in patients without meningitis and in the aqueous humor of the uninflamed eye.[211] However, appreciable concentrations of azithromycin have been detected in the brains of patients undergoing resections of brain tumors after they received 500 mg orally.[211] The average half-life in many tissues is between 2 and 4 days,[206] so it is estimated that significant antibacterial activity against many pathogens persists in tissue for at least 5 days after a 5-day course of treatment.[206] The average terminal half-life is 68 hours, consistent with a slow release of drug from tissues, followed by elimination from the vascular compartment. About 6% of an oral dose appears as unchanged drug in the urine within 1 week of administration, and another small proportion is metabolized to inactive compounds, particularly by demethylation.[206] Most of the drug that is absorbed remains unmetabolized and is probably

eliminated in feces by way of biliary excretion and possibly transintestinal elimination.[206] Biliary concentrations of azithromycin are higher than in the serum, and most of the drug in the bile is unchanged.[206] There are no data available on dose adjustments required with severe renal or hepatic failure.

Clarithromycin is well absorbed after oral administration, with or without food, and is approximately 55% bioavailable.[184] Mean peak serum concentrations at steady state with oral doses of 250 and 500 mg every 12 hours are 1 and 2 to 3 μg/mL, respectively. The elimination half-lives for those two regimens are 3 to 4 and 5 to 7 hours, respectively. Clarithromycin is appreciably metabolized in the liver by oxidation and hydrolysis to a number of compounds, accounting for a recovery of 78%. The major metabolite, 14-hydroxyclarithromycin, has antibacterial activity and accounts for 20% of the metabolites.[184] With the 250-mg and 500-mg oral doses given every 12 hours, about 20% to 30%, respectively, of the drug is excreted into the urine unchanged and 10% to 15% as the hydroxy metabolite. At higher doses, there is some nonlinearity of half-life, apparently because of saturation of metabolic mechanisms with a higher proportion of unchanged drug eliminated in the urine.[184] About 65% to 70% of the drug is bound to protein in the serum. With renal insufficiency involving creatinine clearances of less than 30 mL/min, there is a marked increase in the half-life of clarithromycin.[191] Dose adjustment is suggested in patients with severe renal failure, including recommendations for a 500-mg loading dose, followed by 250 mg once or twice daily depending on the type of infection.[191] No dosage adjustment is recommended at present for severe hepatic dysfunction.[191]

Clarithromycin is widely distributed and penetrates well into various tissues, generally exceeding peak maximum serum levels by severalfold.[191] Concentrations of clarithromycin and its 14-hydroxy metabolite in middle ear fluids of children with acute otitis media exceeded the plasma concentrations by approximately ninefold and fourfold, respectively, 12 hours after the sixth dose when the drug was given every 12 hours.[212] Concentrations generally exceed the MIC of most strains of middle ear pathogens, except for highly penicillin-resistant *S. pneumoniae*. Data indicate minimal penetration of clarithromycin and its 14-hydroxy metabolite into CSF in patients without meningitis.[213] The levels achieved were below the MIC for the usual pathogens associated with bacterial meningitis and were 1% to 2% of the corresponding plasma levels. Clarithromycin, like the other macrolides, penetrates well into phagocytic cells.[214]

Adverse Reactions

Adverse reactions to clarithromycin and azithromycin at the usual doses have been rare.[183,184,215] The most common complaints are gastrointestinal (diarrhea, nausea, abdominal pain), and discontinuance of therapy is rarely required. Azithromycin has also been shown to have a profound effect on the gastrointestinal microbiome. When compared to household contacts who did not receive antibiotics, patients who received either azithromycin or amoxicillin experienced substantial decreases in microbiota diversity that were sustained throughout the 6-month study period.[216] Studies in mice show long-term effects on the microbiome from macrolides, especially in young animals,[217,218] and studies in schoolchildren in Finland confirm the more profound and prolonged perturbation compared to β-lactam antibiotics.[219]

Acute psychosis or "mania" has been noted in a few patients receiving clarithromycin.[125,126] High doses of clarithromycin in animals have been associated with teratogenic effects, and therefore that drug is not recommended for use in pregnancy.[109] Azithromycin is classified as pregnancy category B.

Abnormalities in liver function are occasionally encountered in patients treated with these drugs, and reversible cholestatic hepatitis has been reported with azithromycin.[220] With the high doses of these drugs used in the treatment of *M. avium* complex, tinnitus, dizziness, and reversible hearing loss have been reported.[221,222] Rarely, severe allergic reactions have occurred with the use of azithromycin.

The risk of macrolide-associated torsades de pointes (a polymorphic ventricular tachycardia) has been associated with increasing age, female sex, chronic use, and concomitant drug use, especially with cisapride.[223,224] Chronic exposure to azithromycin in an animal model promoted intracellular sodium loading, which could lead to proarrythmia.[224] A retrospective study reported that patients taking a 5-day course of azithromycin had a 2.88 increased risk of cardiovascular death compared with those taking no antibiotics, with risk being most pronounced among patients at high risk for cardiovascular disease.[225] Additionally, in an observational case series of patients who received treatment for *H. pylori* with either clarithromycin or amoxicillin, there was an increased risk of myocardial infarction, arrhythmia, and cardiac mortality during or shortly after clarithromycin therapy. However, clarithromycin use was not associated with cardiovascular risks long term.[226] After following patients for a year or longer in a large randomized, multicenter study an increased number of deaths were observed among patients with underlying coronary artery disease who received 2 weeks of clarithromycin.[227] Conversely, a recent retrospective cohort study found that macrolide antibiotics were not associated with a higher 30-day risk of ventricular arrhythmia or all-cause mortality compared to nonmacrolide antibiotics.[228] Although these conflicting observations are of concern, definitive association of increased cardiovascular-related death and exposure to azithromycin or clarithromycin cannot be based on a retrospective cohort design, given residual confounding that may have influenced the outcome.[229] However, as a result of that study, the US Food and Drug Administration has required changes in the labeling of azithromycin warning about the risk of QT interval prolongation and cardiac arrhythmias, as well as changes in the labeling of clarithromycin warning about the increased risk of death among patients with underlying heart disease even in the setting of short courses of therapy based on the study by Jespersen and coauthors.[227,230] It now seems prudent not to use a macrolide antibiotic together with another drug known to prolong the QT interval and to exercise caution in the use of a macrolide antibiotic, even alone, in patients with underlying heart disease.

Drug Interactions

Azithromycin minimally inhibits CYP3A4 and therefore appears to be the safest macrolide derivative from a drug interaction perspective.[231]

Clarithromycin has been reported to be associated with increased concentrations of several drugs that undergo hepatic metabolism by the CYP3A subclass of the cytochrome P-450 enzyme system (see Table 29.3).[134] As with erythromycin, these interactions can lead to serious toxicity. It is not yet clear whether the clarithromycin interactions can occur with all the drugs that interact with erythromycin, but a conservative clinical approach would be to consider that potential. These interactions with the hepatic metabolism of other drugs have not been documented with azithromycin, which does not appear to induce or bind and inactivate the cytochrome P-450 enzymes, probably because of its different azalide structure.[133,232]

Clarithromycin may decrease the serum concentration of zidovudine by unknown mechanisms when the two drugs are taken at the same time.[233] However, in another study with a somewhat different design, there was no significant alteration of zidovudine bioavailability in volunteers with acquired immunodeficiency syndrome (AIDS) who took the two drugs 2 hours apart.[234]

Clarithromycin, like erythromycin, may occasionally lead to digoxin toxicity, possibly by diminishing the bacterial metabolism of digoxin in the gut.[235]

Uses of Clarithromycin and Azithromycin

Clarithromycin and azithromycin have several indications for use as the drug of choice and some important applications as an alternative drug (see Table 29.4).[6,75,109,135–146] Clarithromycin and azithromycin were as effective as other commonly used antimicrobial agents when employed in randomized multicenter trials for the treatment of pharyngitis, sinusitis, community-acquired pneumonia (including *M. pneumoniae* and *C. pneumoniae* pneumonia), and skin infections.[147,183,184,236] Clarithromycin and azithromycin have been considered alternatives to penicillin in treating group A β-hemolytic streptococcal pharyngitis in patients with penicillin allergies.[237] Treatment with a 10- to 14-day course of either clarithromycin (250 mg twice daily) or penicillin VK (250 mg every 6 hours) is equally effective.[184,238] Azithromycin administered for 5 days (500 mg on day 1, followed by 250 mg daily for 4 days) is effective in

eradicating group A streptococci from the pharynx,[239] although shorter courses of 3-day treatment with azithromycin were associated with lower levels of bacteriologic eradication when compared with a 10-day course of penicillin V.[240] One study demonstrated that a 10-day course of treatment with clarithromycin (250 mg twice daily) led to more bacteriologic eradication compared with a 5-day course with azithromycin (500 mg on day 1, followed by 250 mg daily).[241] Clinical studies have demonstrated equal or superior tolerability as well as clinical and bacterial efficacy of azithromycin (12 mg/kg, once daily for 5 days) compared with a 10-day therapy with penicillin V in the treatment of pediatric streptococcal pharyngitis.[242,243] However, the effectiveness of short-course azithromycin in preventing acute rheumatic fever is unknown.[244] Moreover, with the variable prevalence of macrolide resistance by group A streptococci, the effectiveness of these agents in treating such infections when the susceptibility of the organism is unknown should be questioned.

Azithromycin administered for 5 days or clarithromycin for 7 to 10 days for the treatment of acute otitis media in children has been effective,[245,246] but use is limited due to increasing macrolide resistance amongst *S. pneumoniae* isolates, and cases of meningitis have been reported during oral therapy.[247]

Guidelines available for the treatment of adult outpatients with community-acquired pneumonia include the use of a macrolide alone as a first-line agent in patients with no significant medical problems and without the recent use of antimicrobial agents or as combination therapy with a β-lactam or respiratory fluoroquinolone in patients with comorbidities such as chronic heart, lung, or liver disease; diabetes; or immunosuppressing conditions.[147] The aforementioned guideline is now further complicated for patients with heart disease by the concern for potentiating cardiac arrhythmias with either a macrolide or a fluoroquinolone. All three macrolides have good activity against most pathogens that commonly cause community-acquired pneumonia, including many strains of *S. pneumoniae* and almost all strains of *M. pneumoniae, C. pneumoniae, Legionella* species, and *Moraxella catarrhalis* (see Table 29.1).[34] However, the rising prevalence, already discussed, of macrolide resistance by strains of *S. pneumoniae,* especially those that are penicillin resistant, requires caution in the use of any of the macrolides as a sole agent when that organism may be causing pneumonia. That concern would be greatest for older adults or persons with underlying medical conditions. A study by Martinez and colleagues[248] suggested lower mortality benefit with combination therapy including a macrolide in community-acquired pneumonia associated with *S. pneumoniae,* including penicillin-susceptible strains, although sample size was small and the study was retrospective in design. Macrolides, with their ability to concentrate intracellularly, are effective against intracellular pathogens, including *C. pneumoniae, Legionella* species, and *Coxiella burnetii* (the agent of Q fever).[249] Treatment with macrolides of lower respiratory tract infection with *M. pneumoniae* and *C. pneumoniae* in children shortens the clinical course of infection.[250] In the treatment of patients with nonpneumococcal pneumonia, erythromycin and azithromycin were equally effective (76% and 79%, respectively) in achieving clinical and radiologic resolution.[198] The longer half-life of azithromycin allows a shorter duration of therapy, and in one retrospective study, a 3-day course of azithromycin (daily dose, 500 mg) was as effective as a 5-day course (500 mg in a single dose the first day, followed by 250 mg daily for 4 days) in the treatment of atypical pneumonia.[251]

The generally recommended empirical therapy for community-acquired pneumonia requiring hospitalization includes the combination of a macrolide and a β-lactam,[147] which was associated with briefer hospital stays and a lower mortality rate than treatment with a cephalosporin alone.[252,253] In addition, two retrospective studies found that in cases of bacteremic pneumococcal pneumonia, dual antimicrobial therapy, including a macrolide, reduced mortality.[254,255]

B. pertussis is susceptible in vitro to azithromycin and clarithromycin. Furthermore, data from a multicenter randomized trial in North America demonstrated that azithromycin was as effective as erythromycin, better tolerated, and associated with improved compliance.[256] The Centers for Disease Control and Prevention (CDC) recommends that azithromycin should be used for all neonates younger than 1 month and erythromycin, clarithromycin, or azithromycin are noted to be acceptable for the treatment of pertussis in persons age 1 month or older.[257] Resistance to azithromycin has been rarely reported, but limited studies are available on its clinical effectiveness.[258] In a study involving a small number of children with pertussis in Japan, a 5-day course of azithromycin and a 7-day course of clarithromycin were as effective in eradicating *B. pertussis* from cultures 1 week after treatment as in historical control subjects who had been treated with erythromycin for 2 weeks.[137] Until more data from clinical studies evaluating clarithromycin and azithromycin are available, the American Academy of Pediatrics recommends erythromycin as the antimicrobial agent of choice for treatment of and prophylaxis against pertussis and clarithromycin (15 mg/kg/day orally in two divided doses, with a maximum of 1 g/day, for 7 days) and azithromycin (10 mg/kg/day orally in one dose, with a maximum of 500 mg/day, for 5 days) as alternatives for patients who cannot tolerate erythromycin.[259]

Clarithromycin (500 mg orally twice daily) or azithromycin (500–600 mg orally once daily) in addition to ethambutol with or without rifabutin are now considered the drugs of choice in the treatment of disseminated *M. avium* complex infections in patients with AIDS.[109] Relatively few mutations are associated with macrolide resistance in mycobacteria, and all are in genes encoding the peptidyl transferase region of the 23S rRNA.[260] Therefore the use of macrolides alone is often associated with clinical relapse and the emergence of macrolide-resistant organisms.[261] In AIDS patients with $CD4^+$ T-lymphocyte counts lower than 100 cells/mm^3, prophylaxis of disseminated *M. avium* complex infection with clarithromycin (500 mg orally twice daily) or azithromycin (1200 mg orally once weekly) is effective.[262,263] Clarithromycin prophylaxis of this condition has been demonstrated to increase survival.[262] Discontinuation of a macrolide-based prophylaxis for *M. avium* complex infection may be possible in patients who have been given prophylaxis for at least 1 year and whose $CD4^+$ counts have increased to greater than 100 cells/μL on highly active antiretroviral therapy.[264] In patients who develop disseminated infection despite prophylaxis, macrolide-resistant isolates are frequently found.[262]

A single oral dose of azithromycin (1-g single dose oral dose) is a highly effective treatment for trachoma and has become the treatment of choice for *C. trachomatis*.[109,265] In fact, in a recent study conducted in trachoma endemic areas, such as sub-Saharan Africa, it was hypothesized that biannual mass distributions of azithromycin to children ages 1 to 59 months would reduce mortality. While limitations of the study and the downstream effects of mass distribution of antibiotics should be considered, it is interesting to note that childhood mortality was 13.5% lower (P <.001) among children in communities who received azithromycin than in those who received placebo.[266] A single oral 1-g dose of azithromycin has been as effective as a 1-week course of doxycycline in the treatment of *C. trachomatis* urethritis and cervicitis[141] and acute nongonococcal urethritis (caused by *C. trachomatis* or *U. urealyticum*) in men.[267] Azithromycin (1-g single oral dose) is as effective as ceftriaxone (250-mg intramuscular dose) in treating chancroid and is considered first-line treatment.[155] A single 2-g dose of azithromycin is not recommended as treatment for *N. gonorrhoeae* infection because of gastrointestinal side effects, expense, and concern for the induction of resistance.[268]

Clarithromycin (500 mg orally twice daily) combined with amoxicillin (1 g twice daily) or metronidazole (500 mg two to three times daily) and a proton pump inhibitor for 14 days is recommended as a first-line therapy for the treatment of *H. pylori* in areas where clarithromycin resistance for this pathogen is <15% and in patients without a history of macrolide exposure.[269] Failure is often associated with primary resistance by *H. pylori* strains to one of these antimicrobials[270] or with the emergence of secondary resistance, which occurs more frequently if only one antimicrobial agent is used or if two are used in the presence of primary resistance to one of them.[271,272] Sequential therapy involving 5 days of use of a proton pump inhibitor together with amoxicillin, followed by another 5 days of the proton pump inhibitor plus clarithromycin and a nitroimidazole, is associated with high cure rates (approximately 90%)[270] and is a suggested first-line therapy.[269]

Azithromycin is effective in the treatment of *Campylobacter* enteritis, but in some areas macrolide-resistant strains are common.[138,273] Because of its lower toxicity, azithromycin should be used instead of erythromycin

when therapy is indicated and cost not a major factor. Azithromycin treatment for 5 days was found to be as effective as ciprofloxacin in the treatment of shigellosis in a randomized controlled trial and can be considered an alternative therapy for that condition, especially for children and pregnant women.[109,143]

Clarithromycin has been used effectively as the central drug or as an alternative in a variety of mycobacterial infections, including *M. tuberculosis, M. avium* complex, *Mycobacterium chelonae,*[274] *Mycobacterium fortuitum, Mycobacterium genavense,* and *Mycobacterium kansasii.*[109] In deep-seated infections and in immunocompromised patients with these infections, combination therapy with more than one active agent is suggested to decrease the chance for the emergence of resistant strains.[275] Limited data suggest the effectiveness of clarithromycin in treating leprosy, for which it can serve as an alternative drug.[109,276]

Clarithromycin (500 mg twice daily for 14–21 days) or azithromycin (500 mg for 7–10 days) is an alternative treatment of early Lyme disease[277,278]; however, it is less effective than doxycycline, amoxicillin,[278] and cefuroxime.[279] In some situations, such as allergy to β-lactam antibiotics in pregnant women or allergy to both β-lactam and tetracycline antibiotics, azithromycin may be an effective alternative treatment for Lyme disease. However, present guidelines do not recommend macrolides as first-line therapy for Lyme disease.[279]

Azithromycin (when given with atovaquone) is as effective as clindamycin and quinine for the treatment of *Babesia microti* infections, a frequent coinfecting agent with *B. burgdorferi.*[146] Although clindamycin plus quinine remains the preferred treatment regimen in severe cases of babesiosis, atovaquone plus azithromycin is an effective regimen for non–life-threatening babesiosis in immunocompetent adult patients.[146,279]

Azithromycin has been shown to have antimalarial activity similar to that of doxycycline in animal models.[280] Two human volunteer studies suggested that azithromycin has potential in the prevention of chloroquine-resistant *Plasmodium falciparum* infection.[280,281] Two field trials on the use of azithromycin as a single agent for malaria prophylaxis demonstrated only moderate efficacy (70%–90%) for *P. falciparum* compared with high efficacy for *Plasmodium vivax.*[282,283] Azithromycin in combination with other antimalarial agents, such as chloroquine, quinine, or sulfadoxine-pyrimethamine in pregnant women, produces synergistic interactions in vitro and may be effective for the treatment of uncomplicated *P. falciparum* malaria in adults.[284–286]

There has been great interest in the potential role of chronic infections (especially those caused by *C. pneumoniae*), and more recently in the role of inflammation per se, in potentiating atherosclerosis and vascular thromboses, including coronary artery disease.[287] In this regard, azithromycin has been investigated because of its activity against *C. pneumoniae* and its ability to accumulate in high concentrations in atherosclerotic plaques.[288] One study in a small number of men who had survived myocardial infarction, had substantial *C. pneumoniae antibody* titers, and were given azithromycin (500 mg daily for 3 or 6 days) demonstrated a significant decrease in subsequent adverse cardiovascular events compared with a similar group of men who were not treated with azithromycin.[289] However, clinical trials have not shown a benefit of macrolide use in preventing recurrent events in patients with acute coronary syndromes.[290,291] Assuming a real effect of the macrolide on such cardiac events, it is uncertain whether the result is related to its antimicrobial activity, its antiinflammatory activity on atheromata or thrombogenesis (as discussed earlier in the "Antimicrobial Activity" section under "Erythromycin"), both of these activities, or other unknown effects. A large randomized, placebo-controlled trial of stable patients with previous myocardial infarction and immunoglobulin G antibodies to *C. pneumoniae* treated with azithromycin for 12 weeks and followed for a median of 14 months did not demonstrate a significant reduction of clinical sequelae of coronary heart disease.[292] A randomized, placebo-controlled trial of clarithromycin 500 mg once daily for 2 weeks in several thousand Danish patients with stable coronary artery disease followed over 3 years showed no benefit of treatment and a significant increase in cardiovascular mortality in those receiving clarithromycin (5.1% vs. 3.5%, $P = .01$).[227] Potential reasons for the increase in cardiovascular mortality found in those who received clarithromycin include a nonspecific random effect, unknown inequalities in risk between the two randomized groups, and proarrhythmic and drug interaction effects of clarithromycin (though cardiovascular mortality did not differ during the first month of follow-up).

Efficacy of azithromycin has been evaluated for treatment of patients with cystic fibrosis (CF). Four randomized, placebo-controlled trials comparing placebo with azithromycin administered for a period of 3 to 12 months found considerable improvement of lung function as assessed by increase of forced expiratory volume in 1 second from baseline, and reduced risk of bacterial exacerbations among patients receiving azithromycin.[293–296] The use of daily azithromycin at a dose of 250 mg once daily for 1 year was found to decrease exacerbations among patients at high risk for acute exacerbations of chronic obstructive pulmonary disease (COPD). However, patients were also more likely to have hearing loss and harbor macrolide-resistant organisms.[297] Beneficial effects demonstrated in CF, COPD, and ventilator-associated pneumonia[298,299] are likely related not only to the bacterial growth-inhibiting effects of the macrolide but also to the general antiinflammatory activities of the macrolides, the downregulating effects of the drug on the production of bacterial virulence factors of *P. aeruginosa* (already discussed), or both.[300] The use of azithromycin chronically in patients with COPD requires prior screening for QT interval prolongation and hearing loss and should not be used in those needing the administration of other drugs that are known to prolong the QT interval. Caution is also advised for such use in those with underlying heart disease.[225] When azithromycin is used chronically for those with COPD, it seems likely that administration three time per week rather than daily may be sufficient, given the long half-life of the drug in macrophages. Another caution comes from a recent study reporting that long-term use of azithromycin by adults with CF was associated with the development of infection with nontuberculous mycobacteria, particularly multidrug-resistant *Mycobacterium abscessus.* A suggested possible mechanism was that azithromycin can block autophagosomal clearance by preventing lysosomal acidification, thereby attenuating killing of intracellular mycobacteria.[301]

The potentially useful antiinflammatory actions of the macrolides (aside from their antimicrobial activity) were discussed earlier under "Erythromycin."

KETOLIDES

Ketolides are a class of semisynthetic agents derived from erythromycin A that have increased acid stability and increased antibacterial potency against many bacteria resistant to macrolides. They are unable to induce the MLS_B methylase type of resistance. Telithromycin (HMR 3647, or Ketek), which was approved for clinical use in the United States in April 2004 and had been in use in Europe since 2001 and in some Latin American countries, was the first member of this new class. However, its potential (although rare) for serious, even fatal, hepatotoxicity was recognized in 2006, which severely and appropriately limited its use, and the drug has now been removed from the market in the United States.

LINCOMYCIN AND CLINDAMYCIN

Derivation, Chemistry, and Preparations

Lincomycin was isolated in 1962 from an organism, *Streptomyces lincolnensis,* obtained from soil near Lincoln, Nebraska. Many of its biologic properties are similar to those of erythromycin, but it is chemically unrelated, consisting of an amino acid linked to an amino sugar (Fig. 29.4). Chemical modification provided clindamycin (7-chloro-7-deoxylincomycin) (see Fig. 29.4) with increased antibacterial potency and absorption after oral administration.[302] Because there are no therapeutic advantages for lincomycin over clindamycin, the discussion here concentrates on the latter, although both are still marketed as pharmaceuticals. Both are weak bases that are readily water soluble when provided as salts.

Although lincomycin (Lincocin) is available in the United States as the hydrochloride salt in solution (300 mg/mL) for parenteral use, it is rarely used. Clindamycin is prepared as the hydrochloride salt of the base in 75-, 150-, and 300-mg capsules and of the palmitate ester as a powder for pediatric suspension (75 mg/5 mL). It is supplied as the phosphate ester for IM use (150 mg/mL) and for IV use

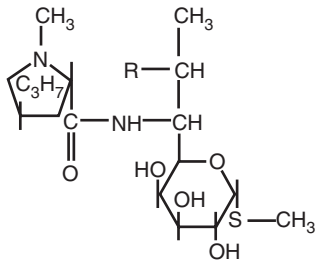

trans-L-4-n propylhygrinic acid

FIG. 29.4 The lincosamide antibiotics. In lincomycin, R = OH; in clindamycin, R = Cl.

(150 mg/mL, 300 mg/mL, 600 mg/mL, 900 mg/mL). It is also available in topical solution, gel, lotion, foam, and pad (all at 1%) for the treatment of acne vulgaris and in a concentration of 2% in a vaginal cream and as a vaginal suppository (100 mg) for the treatment of bacterial vaginosis. A relatively new lincosamide, pirlimycin, is a *cis*-4-ethyl-L-picecolic acid amide of clindamycin that is exclusively approved for veterinary applications.[303]

Mechanism of Action

The lincosamide antibiotics have, in susceptible organisms, the same or overlapping 50S ribosomal binding sites as those for the macrolides and chloramphenicol, and they may compete with these drugs for binding.[3] Protein synthesis is inhibited primarily in early chain elongation by interference with the transpeptidation reaction,[303] possibly by blocking of the P (peptidyl donor) site. Like the macrolides, the lincosamide antibiotics may also stimulate the dissociation of peptidyl-tRNA from ribosomes.

Mechanisms of Resistance

There are several mechanisms of resistance to the lincosamide antibiotics. First, and most important, is the alteration in the 23S rRNA of the 50S ribosomal subunit by methylation of adenine,[3,304] which has been discussed earlier in the "Target Site Alterations" section under "Erythromycin". It is usually plasmid mediated and provides the MLS_B type of resistance, which includes that exhibited by some strains of *S. aureus*, *S. pyogenes,* and *B. fragilis* to clindamycin. This type of resistance in *S. aureus* is encoded by the *ermA* or *ermC* genes and, when of the macrolide-inducible variety, is characterized by a positive erythromycin-clindamycin "D test"—a double-disk diffusion test in which the zone of inhibition around the clindamycin disk is blunted on the side facing the erythromycin disk.[305] The MLS_B resistance mechanism in staphylococci more frequently manifests clindamycin resistance when the strain possesses the *ermA* gene as compared with the *ermC* gene.[306] With that type of resistance in *S. aureus,* exposure to clindamycin (which is not an inducer) in vitro or in vivo may result in clindamycin resistance due to selection of preexisting constitutive *erm* mutants, especially when the organism is at high inoculum.[306,307] An additional methyltransferase enzyme, cfr, was found to cause multidrug resistance in *S. aureus* to chloramphenicol, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (PhLOPSA) through the methylation of the 23S ribosomal RNA nucleotide.[308–310] Second mutations in the bacterial ribosomal RNA may confer resistance to clindamycin as demonstrated in some strains of *Mycobacterium smegmatis.*[311] Third, alteration in particular 50S ribosomal proteins of the receptor site confers resistance to erythromycin and often to the lincosamides[3]; this mechanism was previously discussed for erythromycin. Fourth, resistance is conferred through inactivation of lincomycin and clindamycin by a few isolates of staphylococci (including *S. aureus*) and *Bacteroides* spp. that possess a plasmid-mediated 3-lincomycin 4-clindamycin *O*-nucleotidyltransferase that catalyzes the nucleotidylation of the hydroxyl group in position 4 of clindamycin.[8,312,313] This adenylation of the lincosamides is associated with high-level resistance to lincomycin, but clindamycin resistance may not be detected by routine methods. The adenylation of clindamycin is associated with impaired bactericidal activity and decreased activity at high inoculum levels. The *lnu* genes (formerly known as *linA* and *linA´*), include *lnu(A), lnu(B), lnu(C),* and others, which can be chromosomal or plasmid-carried.[303] Plasmids carrying *lnu(A)* are found in *S. aureus* and coagulase-negative staphylococci, and *lnu(B)* has been identified in staphylococci, enterococci, and *Erysipelothrix,* while *lnu(C)* was first found in *Streptococcus agalactiae.*[303]

Finally, Enterobacteriaceae, *Pseudomonas* spp., and *Acinetobacter* spp. are intrinsically resistant to clindamycin, apparently because of poor permeability of the cellular outer envelope to the drug.[8]

TABLE 29.5 In vitro Susceptibilities to Clindamycin

ORGANISM	MINIMAL INHIBITORY CONCENTRATION (μg/mL) Range	MIC_{50}
Streptococcus pneumoniae	≤0.25–≥128	≤0.25
Streptococcus pyogenes	≤0.06–>64	≤0.06
Viridans-group streptococci	≤0.06–>256	≤0.06–0.12
Enterococcus	0.12–>8	>8
Staphylococcus aureus	≤0.06–>8	0.25
Staphylococcus epidermidis	<0.12–>8	0.12
Clostridium perfringens	≤0.06–>4	1
Neisseria gonorrhoeae	0.01–6.3	3.1
Haemophilus influenzae	0.4–50	12.5
Bacteroides fragilis group	0.03–>128	1–3
Bacteroides melaninogenicus	≤0.1–1	<0.1
Bacteroides thetaiotaomicron	0.06–>128	1.7–4
Fusobacterium spp.	≤0.008–>16	0.25
Peptococcus spp.	≤0.1–>100	≤0.5
Peptostreptococcus spp.	≤0.06–>32	0.125
Prevotella sp.	0.03–>128	0.12
Mycoplasma pneumoniae	1.6–3.1	3.1

MIC_{50}, Minimal inhibitory concentration for 50% of isolates (μg/mL).

Antimicrobial Activity

In vitro susceptibilities to clindamycin are given in Table 29.5.[a] Clindamycin is more potent than lincomycin but similar in potency to erythromycin against staphylococci, pneumococci, *S. pyogenes,* and streptococci of the viridans group when strains are sensitive to both. However, although erythromycin demonstrates at least moderate activity against *Enterococcus, H. influenzae,* and *N. meningitidis,* clindamycin is generally inactive against these organisms at clinically achievable concentrations. In contrast, clindamycin has shown significantly greater activity than erythromycin against most clinically significant anaerobic bacteria, particularly *B. fragilis.*[78] Clindamycin is not active against *Nocardia* species, most aerobic gram-negative bacilli, or mycobacteria.

Clindamycin used to be one of the most active antibiotics available against *B. fragilis;* however, resistance is increasing to high levels (from approximately 30% to 60%).[318] With other agents available that are more reliably active (metronidazole, piperacillin-tazobactam, and carbapenems), empirical therapy with clindamycin for an anaerobic infection should be started with caution.[319,320] Estimates of resistance to clindamycin by anaerobes also include nearly 20% to 25% of clostridial species other than *C. perfringens,*[321,322] 15% to 20% of peptostreptococci,[323] 10% of *Fusobacterium* species,[323] and 30% to 40% of *Prevotella* strains.[321–323] All the Enterobacteriaceae are resistant to clindamycin.

[a]References 48, 50, 55, 67, 314–317.

Clindamycin provides high activity against pneumococci and group A streptococci; however, clinical isolates showing resistance to clindamycin and erythromycin have been increasingly reported from different areas, as already discussed under "Erythromycin". The majority of strains of *S. pneumoniae* remain clindamycin-susceptible, with resistance rates approaching 10% in some areas of the United States.[324] In the United States, most surveys conducted on macrolide-resistant *S. pneumoniae* and *S. pyogenes* strains isolated through the 1990s found them to be of the M phenotype, which does not show cross-resistance to clindamycin.[41] However, a survey of 1885 clinical strains of *S. pyogenes* isolated in 2002 and 2003 from 45 medical centers in the United States showed that almost 7% were macrolide resistant and 0.5% were clindamycin resistant, with 56% of the macrolide-resistant strains demonstrating the MLS_B phenotype (almost all of the inducible type) and 44% having the M phenotype.[16] Emergence of resistance might be expected during clindamycin treatment of macrolide-resistant *S. pyogenes,* initially showing clindamycin susceptibility, when the strain possesses the MLS_B-inducible phenotype. It is therefore suggested that the D test be performed on macrolide-resistant *S. pyogenes* to determine clindamycin susceptibility.[16] A recent study of *S. pyogenes* isolates from the United States predicted 12.7% resistance to erythromycin and clindamycin based on whole-genome sequencing.[325]

The antibacterial activity of clindamycin against *S. pyogenes* displays several characteristics that have the potential to be clinically advantageous and are less prominently demonstrated or absent in the penicillin family. In contrast to β-lactam drugs, clindamycin acts on stationary growth–phase bacteria, suppressing the production of bacterial proteins such as *S. pyogenes* superantigens,[326] supported by an observational clinical study finding that clindamycin treatment improved survival of patients with streptococcal toxic shock syndrome.[327] In vitro studies suggest that subinhibitory concentrations of clindamycin paradoxically induce virulence mechanisms in *S. pyogenes*, suggesting that therapeutic dosing is important.[326]

Group B *Streptococcus* (*S. agalactiae*) used to be predictably sensitive to clindamycin but that is no longer the case, with nearly one-third of strains resistant to clindamycin.[328] Both constitutive and inducible resistance are important issues in group B *Streptococcus*, which is now on the CDC's list of antibiotic-resistant threat agents.[328] Clindamycin continues to have substantial activity against methicillin-sensitive *S. aureus* but against fewer strains that are methicillin resistant.[329] Cross-resistance of *S. aureus* between lincomycin and clindamycin is complete. The MICs of clindamycin and erythromycin in vitro are generally similar for *S. aureus* strains that are sensitive to both agents; however, resistance can be selected in vitro by serial subculture in the presence of subinhibitory concentrations of either drug, and it occurs slowly for clindamycin and more rapidly for erythromycin.[302,330] In contrast, strains that are sensitive to clindamycin and resistant to erythromycin can be rapidly selected for clindamycin resistance by serial subculture on clindamycin. Consistent with these in vitro observations, the emergence of clindamycin-resistant *S. aureus* has been noted in clindamycin-treated patients, in particular when the organisms had demonstrated erythromycin resistance at the onset of treatment (i.e., the dissociated resistance of Garrod [inducible MLS_B]).[302,330] The bacterial strains possessing the inducible MLS_B resistance mechanism can often be detected in the laboratory with the D test, as discussed earlier. That test should be performed when clindamycin treatment is being considered for the treatment of *S. aureus* or *S. pyogenes* infections when the bacterial strain shows macrolide resistance. A positive D test suggests that emergence of resistance to clindamycin will often occur if that drug is used in treatment. A survey of nasal strains of *S. aureus* isolated in 2003 and 2004 showed that, among 237 that were methicillin sensitive, 2% were resistant to clindamycin with only a constitutive MLS_B phenotype, and 22.4% were resistant when including both inducible and constitutive MLS_B phenotype strains.[331] In the same study, among 134 strains that were methicillin resistant, 33.6% were resistant to clindamycin with only a constitutive MLS_B phenotype, and 62.4% were resistant when including both inducible and constitutive MLS_B phenotype strains. When methicillin-resistant *S. aureus* strains are of the community-associated variety, clindamycin resistance has been less common than among hospital-associated methicillin-resistant strains[332]; however, the proportion of the community-associated strains that are clindamycin resistant, often of the MLS_B inducible phenotype, has been increasing.[333] The antibacterial activity of lincomycin and clindamycin has been shown, in limited in vitro studies, to be bactericidal for *S. pneumoniae, S. pyogenes,* and *S. aureus.* Its killing activity is similar to that of erythromycin and therefore probably varies with the concentration, bacterial species, and inoculum. It is more slowly bactericidal for *S. aureus* than are the penicillins,[334] and it is inconsistently bactericidal for *B. fragilis.*[335] Studies in vitro have demonstrated that clindamycin is capable of suppressing the production of some *S. aureus* extracellular toxin proteins.[336]

Clindamycin has substantial in vitro activity against *T. gondii* in infected human fibroblasts.[337] Clindamycin and its three major metabolites are inhibitory against *P. falciparum.*[338]

Clinical Pharmacology

Peak serum levels achieved after oral administration of clindamycin occur earlier and are at least twice as high as those of lincomycin. Absorption of clindamycin is about 90% and is slightly delayed, but not decreased, by ingestion of food, whereas that of lincomycin is markedly decreased.[302] Mean peak serum concentrations of clindamycin in adults after single oral doses of 150 and 300 mg occur at 1 hour and are 2.5 and 3.6 μg/mL, respectively; at 6 hours, they are 0.7 and 1.1 μg/mL, respectively. The esters clindamycin palmitate in suspension for oral use and clindamycin phosphate for parenteral use are absorbed as the inactive ester and are rapidly hydrolyzed in the blood to the active base. After IM administration, which causes little pain, mean peak serum levels are reached in 3 hours and are about 6 μg/mL after a 300-mg dose and 9 μg/mL after a 600-mg dose; at 12 hours, they are 0.7 and 0.9 μg/mL, respectively.[339] In adult healthy volunteers, immediately after 20- to 45-minute intravenous infusions of 600, 900, or 1200 mg of clindamycin phosphate, serum levels of base are 10, 11, and 14 μg/mL, respectively. Higher levels after intravenous infusion have been reported in infected patients under treatment.[340] Dose regimens of IV clindamycin using 900 mg every 8 hours or 600 mg every 6 hours are considered acceptable.[341]

Limited studies have demonstrated good penetration of most tissues by the lincosamides, except for clinically insignificant entry of clindamycin into the CSF, even with meningitis.[342] Thus clindamycin should not be used to treat infections of the central nervous system. The concentration in bone compared with serum is particularly high.[343] Clindamycin administered to pregnant women readily passes the placental barrier and enters fetal blood and tissues.[104] Clindamycin is actively transported into polymorphonuclear leukocytes and macrophages[344] and is present in relatively high concentrations, compared with peak serum levels, in experimental abscesses.[345]

The normal half-life of clindamycin is 2.4 hours. Most of the absorbed drug is metabolized, probably by the liver, to products with variable antibacterial activity, including *N*-demethyl-clindamycin (more active than the parent compound) and clindamycin sulfoxide (less active), which have been detected in bile and urine but not in serum.[339] High bioactivity is found in bile, mostly as the *N*-demethyl metabolite; this represents a minor route of excretion and accounts for the activity assayed in feces after parenteral administration.[339,346] Clindamycin activity in feces persists for at least 5 days after 48 hours of parenteral administration and is associated with a major reduction in the population of sensitive bacteria in the colon that lasts for up to 14 days.[347] Clindamycin concentration in bile is markedly diminished or absent when the common bile duct is obstructed.[348] High clindamycin bioactivity, also mostly in the *N*-demethyl form, is found in the urine and persists for up to 4 days after a single dose, suggesting slow release from tissues.[340] Accurate data on the proportion of absorbed clindamycin that is excreted in the urine are not available because of the variable activity of the metabolites and their unknown proportions in urine.

The half-life of clindamycin is increased from 2.4 to about 6 hours in patients with severe renal failure, and peak blood levels after parenteral administration are about twice those in healthy people.[349] If modified at all, parenteral doses should be halved in such patients. Some prolongation of clindamycin activity in serum is noted in patients with severe liver disease.[350] Appreciable dose modification should be made when a patient exhibits concomitant severe renal and hepatic disease. Neither

hemodialysis nor peritoneal dialysis removes significant amounts of clindamycin.

Adverse Reactions

In a report from the CDC of emergency department visits in 2004 to 2006 for antibiotic-associated adverse events, those associated with clindamycin use were among the highest, with 18.5 emergency department visits per 10,000 outpatient prescription visits.[351] Cutaneous reactions are common adverse responses to clindamycin, including delayed maculopapular eruptions, urticaria, erythema multiforme, fixed drug eruptions, drug rash with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome, toxic epidermal necrolysis, and acute generalized exanthematous pustulosis.[352] Recent data from China suggest a genetic predisposition to cutaneous adverse events in persons with an HLA-B*51:01 genotype.[352]

Diarrhea occurs in up to 20% of clindamycin-treated patients and is more common with oral administration. However, the major toxicity of lincomycin and clindamycin that now appreciably limits their use is the occurrence of pseudomembranous colitis caused by toxins secreted by *C. difficile* that overgrows in the presence of these antibiotics.[353] In the early reports of that infection, it was noted in 0.01% to 10% of clindamycin-treated patients.[354] The infection may occur in association with the administration of other antibiotics or occasionally without a history of recent antibiotic use, and it has become more frequent and severe.[355] Antibiotics other than clindamycin—particularly cephalosporins and the fluoroquinolones, which have less potency than clindamycin to change the normal balance of intestinal microbiota and thereby allow the overgrowth of *C. difficile*—have become more frequently involved as inducers of the infection because they are now used more frequently than clindamycin.[356,357] *C. difficile* infection has been reported after the use of clindamycin vaginal cream in a patient being treated for bacterial vaginosis.[358]

Minor reversible elevation of transaminase levels, unassociated with other evidence of liver abnormality, has commonly been observed in patients receiving clindamycin, especially by the parenteral route. Some of these may have been false-positive reactions associated with colorimetric rather than specific enzymatic measurements.[302] However, rare cases of frank hepatotoxicity, including jaundice associated with hepatocellular damage, have been observed.[359]

Isolated cases of reversible neutropenia, thrombocytopenia, and agranulocytosis associated with lincomycin or clindamycin therapy have been reported; their relation to the administration of the antibiotic was uncertain.

Hypotension and electrocardiographic changes have occasionally been reported. Cardiopulmonary arrest has occurred rarely, when large intravenous doses of lincomycin were given rapidly. These effects have not been reported with clindamycin.

Local irritative reactions are rare with these drugs. Intramuscular or intravenous administration is generally well tolerated.

Drug Interactions

Clindamycin may block neuromuscular transmission and may enhance the action of other blocking agents.[134] Clindamycin may decrease the effect of cyclosporine.[134] Clindamycin phosphate in solution is physically incompatible with ampicillin, diphenylhydantoin, barbiturates, aminophylline, calcium gluconate, and magnesium sulfate.

Uses of Clindamycin

The higher activity and absorption properties of clindamycin compared with lincomycin, along with no greater potential for toxicity, favor the former in all indications for use of these antibiotics. The lincosamides have been used in a variety of infections, often with good effect; however, appreciation of the potential for serious or even fatal toxicity with pseudomembranous colitis and the availability of safer alternative antibiotics should now generally limit the use of clindamycin to a few indications as an alternative-choice antibiotic.[109]

Clindamycin is largely used for gram-positive or anaerobic bacterial infections of the soft tissues, or both, though increasing resistance among pathogens has limited its utility.[360] While clindamycin has been known as an alternative choice for the treatment of infections outside of the central nervous system that are likely to involve *B. fragilis* or other penicillin-resistant anaerobic bacteria, it has lost favor as an empirical agent for polymicrobial intraabdominal infections.[361,362]

Clindamycin may offer some advantage over penicillin G in the treatment of anaerobic bronchopulmonary infections[363]; in addition, it may serve as an alternative in patients who are allergic to penicillin. In a prospective, randomized study of 39 patients with community-acquired putrid lung abscess, clindamycin was more effective than penicillin in the time until eradication of fever and fetid sputum and in the "overall response" to treatment.[364] The study involved small numbers of patients and had some flaws in the analysis[365]; however, the superiority of clindamycin for some patients was demonstrated and may relate to observations that 15% to 25% of anaerobic pulmonary infections involve β-lactamase–producing strains of *B. fragilis, B. melaninogenicus, Prevotella ruminicola,* and *B. ureolyticus,* which are resistant to penicillin.[365] Another similar study also demonstrated a higher failure rate with penicillin than with clindamycin and attributed it to penicillin-resistant anaerobes.[366] That study was problematic in that penicillin oral therapy was used to complete the course of treatment of some patients in the penicillin group. Nevertheless, clindamycin may be preferable for treatment of this condition,[367] particularly in seriously ill patients and in those who have responded poorly to penicillin.

Clindamycin is useful as an alternative to penicillin in treatment of infections with *C. perfringens,* which is generally susceptible to this drug.[321] Clindamycin was more effective than penicillin in reducing mortality in a mouse model of *C. perfringens* gas gangrene and in vitro in suppressing the α-toxin activity produced by that organism.[368,369]

Depending upon susceptibilities, clindamycin can be useful in the treatment of staphylococcal infections, especially when therapy is appropriate by the oral route for a methicillin-resistant strain and when there is a history of β-lactam allergy for a methicillin-sensitive strain. However, the more limited bactericidal rate with clindamycin for staphylococci compared with that of the β-lactams, the real potential for the emergence of clindamycin-resistant strains in treated patients, and the substantial potency of clindamycin for sometimes inducing *C. difficile* colitis are disadvantages. The problem of emergence of clindamycin resistance, as discussed earlier and noted especially, but not only with erythromycin-resistant strains, appreciably limits its effectiveness as therapy for deep-seated, high-bacterial-density staphylococcal infections, particularly endocarditis.[370] The use of the D test (discussed earlier) is recommended to detect the likelihood of emergence of resistance to clindamycin when a strain is resistant to erythromycin. In general, vancomycin, daptomycin (not for pneumonia), or linezolid for methicillin-resistant strains or β-lactams for methicillin-sensitive strains are better choices for treatment of staphylococcal infections. Although high concentrations of clindamycin are achieved in bone, an advantage of clindamycin for the treatment of osteomyelitis has not been established.[343]

The topical clindamycin/benzoyl peroxide gel is more effective in the treatment of acne vulgaris than topical clindamycin alone.[371] However, pseudomembranous colitis associated with the use of topical clindamycin has been reported.[372] In the treatment of bacterial vaginosis, clindamycin vaginal cream (2% for 3–7 days) appears to be similar in efficacy and in the incidence of side effects to oral metronidazole (both used for 7 days).[373] Alternative dosing regimens, including oral clindamycin (300 mg twice daily for 7 days) or intravaginal clindamycin ovules (100 mg once daily for 3 days), can also be used for this condition. A case report of *C. difficile* colitis associated with clindamycin vaginal cream has already been noted, but this appears to be exceedingly rare.[358] Bacterial vaginosis is a risk factor for preterm delivery, and studies of clindamycin treatment for bacterial vaginosis have shown mixed results with respect to preventing preterm labor. Thus opinions differ as to the capacity of clindamycin to prevent preterm birth in women being treated for bacterial vaginosis.[374,375]

Clindamycin is an effective alternative regimen in treating animals experimentally infected with toxoplasma and when combined with pyrimethamine (and leucovorin) in treating patients with toxoplasmosis of the central nervous system.[376] This is a preferred alternative regimen for patients with cerebral toxoplasmosis who cannot tolerate sulfadiazine or do not respond to first-line therapy.[377] This combination, however,

does not prevent *Pneumocystis jirovecii* infection; therefore additional prophylaxis must be administered when it is used.

Clindamycin in combination with primaquine is an effective and well-tolerated regimen for the treatment of mild and moderately severe *P. jirovecii* pneumonia in patients with AIDS. In a comparative trial, this combination showed an efficacy similar to that of trimethoprim with sulfamethoxazole or trimethoprim with dapsone.[378] An analysis of 82 AIDS cases with *P. jirovecii* pneumonia from Copenhagen, London, and Milan, which had previously been reported in observational studies and had initial treatment regimens that were switched to other regimens after at least 5 days because of poor clinical response, showed that in 22 cases in which the switch was made to clindamycin with primaquine the response rate was 73% and was comparable with that for trimethoprim-sulfamethoxazole (68%) and superior to that for intravenous pentamidine (44%).[379]

Clindamycin in combination with quinine is effective in the treatment of falciparum malaria.[109] That regimen used for 4 days was found to be superior to quinine used alone for 7 days in a randomized trial in Gabonese children with severe disease.[380] The same combination has also been reported to be useful in the treatment of babesiosis.[109,381] A randomized controlled trial conducted in sub-Saharan Africa involving 100 Gabonese children with uncomplicated *P. falciparum* malaria showed that a 3-day oral regimen of artesunate with clindamycin was highly successful and at least comparable in efficacy and safety with a 3-day oral regimen of quinine with clindamycin.[382]

The coexistence of β-lactamase–producing *S. aureus* or *Bacteroides* species and group A streptococci may be associated with the failure of penicillin to eradicate the latter, resulting in recurrent tonsillitis. Limited evidence suggests that recurrence rates may be lowered when clindamycin is used.[383] In another study, patients with group A streptococcal pharyngitis that recurred after 10 days of treatment with phenoxymethyl penicillin were randomly allocated to re-treatment for 10 days with phenoxymethyl penicillin or clindamycin.[384] Group A streptococci were not recovered from any of the patients receiving clindamycin, but the same (or similar) strain was cultured from 64% of those re-treated with the penicillin. However, widespread use of clindamycin for this common problem is likely to lead to a substantial number of cases of *C. difficile* colitis, as well as selection for clindamycin-resistant strains of group A streptococci.

Although penicillin has been the traditional drug of choice for the treatment of group A streptococcal infections, clindamycin must be considered as potentially more effective in serious soft tissue infections, on the basis of data, already discussed, from the treatment of experimental infections in mice and the effectiveness of that agent compared with penicillin in decreasing the in vitro production of several of the virulence factors of the pathogen. Data from retrospective clinical studies do support such an advantage for clindamycin[385]; however, because some strains of *S. pyogenes* may be resistant to clindamycin, that drug should be used *in combination* with penicillin for the empirical treatment of life-threatening group A streptococcal infections, until sensitivity data are available.[386] Limited in vitro data suggest that the addition of penicillin to clindamycin does not antagonize the bactericidal effects of the latter.[387]

The dosage of clindamycin for adults depends on the site and severity of infection and the condition of the patient. Oral doses are usually 150 to 450 mg every 6 hours, and parenteral doses, given every 6 to 12 hours, usually total 600 to 2700 mg/day, occasionally higher.

ACKNOWLEDGMENTS

The authors thank Drs. Neal Steigbigel and Sumathi Sivapalasingum for their dedicated work on this chapter in prior editions of PPID.

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30 Glycopeptides (Vancomycin and Teicoplanin) and Lipoglycopeptides (Telavancin, Oritavancin, and Dalbavancin)

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SHORT VIEW SUMMARY

VANCOMYCIN

- Vancomycin inhibits late stages of cell wall synthesis in dividing gram-positive microorganisms by interacting with the D-Ala-D-Ala termini of peptidoglycan precursors.
- *Staphylococcus aureus* is a major target for vancomycin; strains with decreased susceptibility to vancomycin include vancomycin-intermediate *S. aureus* (VISA), which display minimal inhibitory concentrations (MICs) between 4 and 8 μg/mL, and vancomycin-resistant *S. aureus* (VRSA) isolates harboring the enterococcal *vanA* gene cluster with even higher MICs. The precursors of VISA, heteroresistant VISA (hVISA) strains, exhibit MICs within the susceptible range (≤2 μg/mL).
- Vancomycin-resistant enterococci (VRE), usually *Enterococcus faecium*, are found worldwide, with the VanA and VanB phenotypes accounting for the majority of these isolates.
- In adults with normal renal function, the average dose is 15 to 20 mg/kg every 8 to 12 hours. For many infections, 15 mg/kg every 12 hours is adequate (obtaining trough levels ≤15 μg/mL). Even though trough levels of 15 to 20 μg/mL have been suggested for serious infections caused by methicillin-resistant *S. aureus* (MRSA), the optimal vancomycin concentration for efficacy and avoidance of toxicity is still a matter of debate; a loading dose of 25 to 30 mg/kg achieves therapeutic levels sooner. The use of area under the concentration-time curve estimation for optimal vancomycin dosing may be a better parameter to follow than trough levels. Dosing should be modified in patients with renal failure, and adjustment based on blood levels is still recommended.
- Red neck or red man syndrome can be seen during vancomycin administration and is related to a rapid infusion and/or a large dose. The risk for vancomycin-induced nephrotoxicity increases with trough levels ≥15 μg/mL, concomitant use of nephrotoxic agents, and duration of therapy. Ototoxicity, vertigo, and tinnitus, as well as neutropenia and thrombocytopenia, are rarely reported.
- Vancomycin is still the drug of choice for treatment of severe skin infections and osteomyelitis and probably also for treatment of bacteremia and endocarditis caused by MRSA; many clinicians still use it as the first-line agent for MRSA pneumonia and as an alternative agent for enterococcal endocarditis and for endocarditis caused by other gram-positive bacteria. Vancomycin is used for suspected or proven penicillin-resistant pneumococcal meningitis (in combination with cefotaxime or ceftriaxone) and for cerebrospinal fluid shunt–related infections caused by methicillin-resistant staphylococci.
- Oral vancomycin is used for severe *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.

TEICOPLANIN

- Teicoplanin is available in many countries in Europe, Asia, and South America but not in the United States. The spectrum of antimicrobial activity overlaps with that of vancomycin.
- It is administered once daily by intravenous (IV) bolus or by the intramuscular route. After an IV loading dose of 6 mg/kg every 12 hours for three doses, the maintenance dose follows with 400 mg (6 mg/kg) every 24 hours. For more serious infections the loading dose should be 800 mg (up to 12 mg/kg) every 12 hours three times and then every 24 hours.
- Teicoplanin appears less nephrotoxic than vancomycin, and the most common side effects are rash and drug-related fever. The red neck syndrome is uncommon.
- In countries where both antibiotics are available, teicoplanin is infrequently used in place of vancomycin, although it could be considered for enterococcal infections or to continue the outpatient treatment of certain MRSA infections.

TELAVANCIN

- Telavancin is the first commercially available agent among lipoglycopeptides, a group of semisynthetic derivatives of glycopeptides that is approved in the United States for acute bacterial skin and skin structure infections (ABSSSI) due to gram-positive pathogens and nosocomial pneumonia caused by susceptible *S. aureus* when other alternatives are not suitable.
- Telavancin inhibits peptidoglycan synthesis as do glycopeptides and produces concentration-dependent alterations of the cell membrane.
- The in vitro spectrum of activity includes *S. aureus,* coagulase-negative staphylococci, and vancomycin-susceptible *Enterococcus faecalis* and *Enterococcus faecium* strains. Higher concentrations are needed to suppress growth of VanA-type VRE in vitro. Telavancin seems to have good activity in vitro and in vivo against VISA strains.
- The approved dose is 10 mg/kg/day, which should be reduced to 7.5 mg/kg/day and to 10 mg/kg every 48 hours in those with a creatinine clearance of 30 to 50 mL/min and 10 to 30 mL/min, respectively.
- In the ABSSSI and the hospital-acquired pneumonia trials, the rate of creatinine increase was higher in patients receiving telavancin than those treated with vancomycin. Besides this reversible renal impairment, other potential side effects are infusion-related reactions, and minor increases in the QTc.

DALBAVANCIN

- Dalbavancin is approved for use in adults with ABSSSI caused by various susceptible gram-positive organisms in United States and Europe.
- Dalbavancin shows in vitro activity against all gram-positive pathogens except those intrinsically resistant to glycopeptides and those exhibiting high-level resistance to

SHORT VIEW SUMMARY—cont'd

vancomycin, mainly mediated by the *vanA* gene cluster.
- The terminal half-life is 8 to 9 days, with a high volume of distribution and protein binding of 93%.
- The current approved dosing is a single administration of 1500 mg or 1000 mg, followed by 500 mg 1 week later for adults with ABSSSI.
- Patients on hemodialysis can receive the regular dose, and those not on hemodialysis with a creatinine clearance <30 mL/min should receive 1125 mg as a single dose or 750 mg on day 1 and 375 mg on day 8 in the two-dose regimen. No need for dose adjustment is required in patients with hepatic insufficiency.
- Adverse reactions include nausea, diarrhea, pruritus, mild elevations of alanine aminotransferase (ALT), and infusion-related reactions.
- A two-dose regimen of dalbavancin is currently under study in an open-label trial of pediatric patients with ABSSSI.

ORITAVANCIN
- Oritavancin is the third lipoglycopeptide available and is approved for use in adults with ABSSSI caused by susceptible gram-positive organisms in United States and Europe.
- Oritavancin is a derivative of the lipoglycopeptide chloroeremomycin, similar to vancomycin, and acts by inhibiting transglycosylation, transpeptidation, and disrupting the cell membrane.
- Oritavancin is active in vitro against staphylococci, streptococci, and enterococci, including VRE.
- The approved dose is one infusion of 1200 mg over 3 hours; the drug is widely distributed, has high protein binding (85%–90%), and a prolonged terminal half-life (≈240 hours), allowing the one-time dosing strategy.
- Headache, nausea, vomiting, diarrhea, infusions reactions such as flushing and pruritus, and mild ALT elevation were among the observed side effects.
- Oritavancin has a potential for drug-drug interaction through the inhibition of several cytochrome P450 enzymes. In addition, because oritavancin causes false elevation of activated partial thromboplastin time for up to 5 days, the use of IV unfractionated heparin sodium is contraindicated in this period.
- Patients with moderate liver or renal impairment do not require dose adjustment.

GLYCOPEPTIDES

Vancomycin

Vancomycin, the first glycopeptide antibiotic developed for clinical use, was isolated from *Amycolatopsis orientalis* (known previously as *Streptomyces orientalis* and later as *Nocardia orientalis*), found in a soil sample from Borneo in the mid-1950s. In 1958 vancomycin was introduced into clinical practice as an agent active against penicillin-resistant *Staphylococcus aureus*. However, a few years later the use of vancomycin was relegated to patients allergic to β-lactam antibiotics because of the availability of new penicillinase-resistant β-lactams, methicillin and cephalothin, and the high rate of toxicity observed with the initial vancomycin formulation. Indeed, early lots of vancomycin (compound 05865) were called "Mississippi mud" owing to the color provided by their large quantity of impurities, but later manufacturing procedures markedly improved purification. Since the 1980s a steady rise in vancomycin use has occurred in the United States, from 2000 kg/yr in 1984 to 11,200 kg/yr in 1996,[1] a trend that also occurred in most European countries[2] and has likely contributed to the rise of strains exhibiting decreased glycopeptide susceptibility. Of note, an important proportion (between 40% and 70%) of the inpatient and outpatient use of vancomycin has been considered to be inappropriate.[3,4]

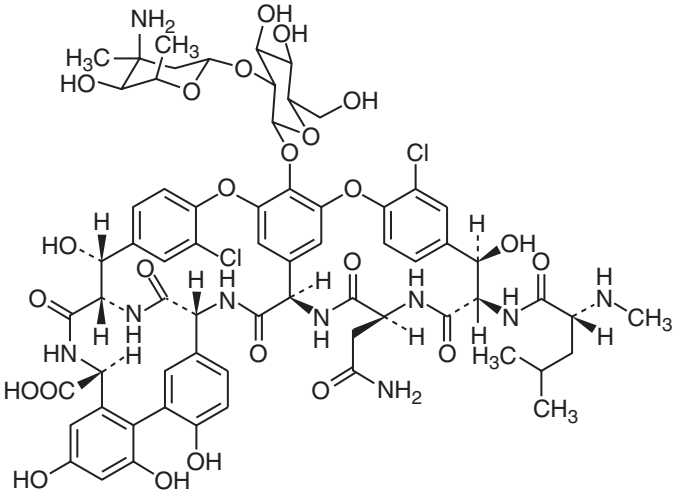

FIG. 30.1 **Chemical structure of vancomycin.**

Structure and Mechanism of Action

Vancomycin is a complex tricyclic glycopeptide that consists of a seven-membered peptide chain forming the tricyclic structure (Fig. 30.1) and an attached disaccharide composed of the amino sugar vancosamine and glucose. The molecular weight is 1485.73 dalton (Da), much higher than that of other antimicrobial agents except teicoplanin, daptomycin, and lipoglycopeptides.

The primary effect of glycopeptides is inhibition of late stages of cell wall synthesis in dividing bacteria. The target of glycopeptides is the nascent peptidoglycan precursor units (lipid II) as they emerge from the bacterial cytoplasm. Vancomycin and other glycopeptides form complexes with the two carboxyl-terminal D-alanine residues of peptidoglycan precursors, and molecular modeling studies indicate that the acyl-D-alanyl-D-alanine moiety is held firmly by the antibiotic via five hydrogen bonds. Binding of the antibiotic at this step blocks the incorporation of disaccharide pentapeptide subunits into the nascent peptidoglycan by transglycosylation and also likely inhibits transpeptidation.

Antimicrobial Activity

Vancomycin has broad activity against gram-positive microorganisms. Staphylococci are normally susceptible to vancomycin, with minimal inhibitory concentrations (MICs) ≤2 μg/mL and minimal bactericidal concentrations (MBCs) within twofold of the MIC; heteroresistance of *S. aureus* to vancomycin (the hVISA phenotype) is discussed later. Vancomycin remains active against most *Enterococcus faecalis* and a variable percent of *Enterococcus faecium* isolates but is not bactericidal even against susceptible isolates, with MBCs more than 32 times the MICs; however, as with other cell wall agents, the addition of an aminoglycoside (if the strain is not highly aminoglycoside resistant) increases the bactericidal activity. All strains of *Streptococcus pneumoniae* and *Streptococcus pyogenes* are susceptible to vancomycin, as are virtually all *Streptococcus agalactiae* and group C and group G streptococci, although rare isolates of streptococci (e.g., *Streptococcus gallolyticus*) have acquired *vanB* genes. Vancomycin also shows good in vitro activity against *Granulicatella* spp. and *Abiotrophia defectiva* (formerly classified as nutritionally variant streptococci).

Listeria monocytogenes is susceptible to vancomycin, but strains with high MBCs have been reported. Vancomycin also displays good in vitro activity against *Bacillus anthracis* isolates, *Bacillus cereus,* and other *Bacillus* spp., with MICs of 2 μg/mL or less. Against *Corynebacterium* spp., including *Corynebacterium jeikeium,* vancomycin has good activity and is the drug of choice for serious infections caused by these organisms until susceptibilities are known. *Rhodococcus equi* is also susceptible to vancomycin. The typical susceptibility to vancomycin of *Lactobacillus acidophilus* helps differentiate this organism from other *Lactobacillus*

spp., which are intrinsically vancomycin resistant. *Leuconostoc* spp., *Pediococcus* spp., and *Erysipelothrix rhusiopathiae* are also intrinsically resistant to glycopeptides.

Among the gram-positive anaerobes, *Peptostreptococcus* spp., *Actinomyces* spp., *Propionibacterium* spp., and *Finegoldia magna* are usually susceptible to vancomycin, as are most *Clostridium* spp., including *Clostridioides difficile* (formerly *Clostridium difficile*), except strains of *Clostridium ramosum* (MIC in 90% of isolates [MIC_{90}], 8 μg/mL) and *Clostridium innocuum* (MIC_{90}, 16 μg/mL). Vancomycin displays no in vitro activity against most gram-negative organisms, except for some nongonococcal *Neisseria* spp.

Mechanisms of Resistance

Development of resistance to vancomycin by mutations was predicted to be a rare occurrence in the clinical setting because the MIC of vancomycin against staphylococcal isolates increased only modestly after serial passages in the presence of the drug, compared with 100,000-fold when penicillin was used. Although higher MICs and MBCs of vancomycin are noted when using high inocula (10^7 colony-forming units [CFUs]/mL) of *Staphylococcus epidermidis* isolates,[5] it was not until the mid-1980s that the first clinical isolates of *S. epidermidis* with reduced susceptibility to glycopeptides were described. Several years later vancomycin-resistant enterococci (VRE; MIC ≥32 μg/mL) isolates were reported in Europe and subsequently the rest of the world. In 1997 the first clinical isolate of *S. aureus* with diminished susceptibility to vancomycin (strain Mu50) was described from Japan.[6] This strain displayed a vancomycin MIC of 8 μg/mL, which is in the range of intermediate susceptibility (4–8 μg/mL) per current Clinical and Laboratory Standards Institute (CLSI) breakpoints[7] and thus was referred to as vancomycin-intermediate *S. aureus* (VISA) or glycopeptide-intermediate *S. aureus* (GISA). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has deleted the intermediate classification for *S. aureus,* and strains with MICs >2 μg/mL are considered vancomycin resistant (www.eucast.org/clinical_breakpoints). The initial VISA strain report was followed by others from various countries. Precursors of these VISA isolates are isolates that harbor subpopulations of cells that are able to grow in the presence of higher concentrations of vancomycin (designated heteroresistant VISA, hVISA, or hGISA). More recently, highly vancomycin-resistant *S. aureus* (VRSA) strains with much higher MICs (ranging from 32 μg/mL–024 μg/mL[7]) that harbor the enterococcal *vanA* gene cluster have been described, mostly from the United States, including a community-associated strain of methicillin-resistant *S. aureus* (MRSA).

Enterococci

Among enterococci, nine types of glycopeptide resistance have been described (VanA, VanB, VanC, VanD, VanE, VanG, VanL, VanM, and VanN), which are named based on their specific ligase that catalyzes the binding of the last two amino acids or substitutes of peptidoglycan precursors (e.g., D-Ala-D-Lac or D-Ala-D-Ser ligases). Related gene clusters have been found in nonpathogenic organisms: *vanF,* in *Paenibacillus* (formerly *Bacillus*) *popilliae* strains (a biopesticide used in the United States to suppress Japanese beetle population) and *vanJ* and *vanK* in the non–glycopeptide-producing actinomycete *Streptomyces coelicolor.* The common end point for vancomycin resistance is the formation of peptidoglycan precursors with decreased affinity for glycopeptides, resulting in decreased inhibition of peptidoglycan synthesis. Peptidoglycan precursors ending in the depsipeptide D-alanyl-D-lactate are produced by VanA-, VanB-, and VanD- and VanM-type strains, whereas VanC, VanE, VanL, and VanN isolates produce precursors terminating in D-alanyl-D-serine, instead of the normally occurring D-alanyl-D-alanine. The *vanA* gene cluster is often found on Tn*1546* transposon or related genetic elements that are usually carried on plasmids and occasionally on the host chromosome; *vanA*-carrying Tn*1546* also has been found in clinical isolates of *S. aureus* (VRSA strains).

Glycopeptide resistance in enterococci is classified as either intrinsic (as a species characteristic) or acquired. The former is a characteristic of the motile species *Enterococcus gallinarum* and *Enterococcus casseliflavus/flavescens,* members of which all carry the naturally occurring *vanC-1,* and *vanC-2/vanC-3* genes, respectively, as part of their core genome. These enterococci show variable MICs of vancomycin, with many falling in the susceptible range, and clinical failures have been reported after vancomycin use. In general, the isolation of these species does not require strict infection control isolation procedures, unless they are highly resistant, suggesting the added presence of potentially transferable *vanA* or *vanB* genes.

Acquired glycopeptide resistance is found most often in *E. faecium,* followed by *E. faecalis* (≈80% of *E. faecium* and ≈5% of *E. faecalis* strains in the United States are vancomycin resistant), and is much less common in other enterococcal species. VanA and VanB account for the vast majority of glycopeptide resistance, with the former more frequently found. VanA isolates (and the recently described VanM) show high MICs of vancomycin and teicoplanin, whereas VanB strains often have lower vancomycin MICs and, typically, are susceptible to teicoplanin. VanD strains have moderate-level resistance to both glycopeptides, whereas VanC, VanE, VanG, VanL, and VanN isolates display low-level resistance to vancomycin and susceptibility to teicoplanin.

Expression of the *vanA* gene cluster is regulated by a membrane-associated sensor kinase (VanS) that likely senses changes in the cell envelope and activates the cytoplasmic response regulator (VanR), which triggers transcription of the resistance as well as the regulatory genes. Similarly, the *vanB* gene cluster has $VanS_B$ and $VanR_B$; the VanB sensor kinase ($VanS_B$) does not appear to recognize teicoplanin.

Recent work has revealed two very distinct clades of *E. faecium* that differ significantly in both their core and accessory genomes and appear to have diverged from each other long before the modern antimicrobial era.[8] The clade with most human clinical and outbreak strains also contains animal isolates and shows higher MICs of ampicillin. The community-associated clade consists primarily of non–hospital-associated human isolates with MICs of ampicillin ≤2 μg/mL. Remarkably, hospital-associated isolates carry not only a variety of antimicrobial-resistance genes but also more putative virulence genes, such as *esp*, *ebpA* and *hyl*, encoding an adhesin, a gene involved in the synthesis of pili and a glycosyl hydrolase, respectively, which likely contribute to a survival advantage in the hospital environment. Some particular clonal clusters (CC2 and CC9) of *E. faecalis* have also been reported to predominate in clinical specimens from hospitalized subjects.

The epidemiology and the beginning of VRE spread in Europe and the United States have notable differences. In Europe the glycopeptide avoparcin was frequently fed to animals as a growth enhancer, apparently selecting for vancomycin resistance in commensal strains found in the intestinal microbiota of animals. The contamination of food from animals, such as poultry products, presumably led to the VRE colonization seen in many healthy individuals from European countries. In the United States, on the other hand, glycopeptides were never approved for animal feed use, and VRE carriage was not detected (except the endogenously resistant species *E. gallinarum* and *E. casseliflavus*) outside the health care setting, at least early on. The widespread use of vancomycin in the hospital setting is likely one of the culprits, along with the widespread broad-spectrum cephalosporin use, for the rapid selection and proliferation of VRE within this environment. The proportion of *E. faecium* among enterococcal isolates from health care–associated infections is significant because this species has accounted for 25% of all enterococci, as reported by the National Healthcare Safety Network of the Centers for Disease Control and Prevention (CDC).[9] About 80% of *E. faecium* strains are vancomycin resistant, and 90% of them are also ampicillin resistant.

In the last 1 to 2 decades, the evolution of *E. faecium* in the European Union has followed the earlier trend in the United States. Namely, there has been spread of a major hospital-adapted *E. faecium* subcluster (largely CC17), which typically shows higher levels of resistance to ampicillin than non-CC17 strains, with subsequent acquisition of vancomycin resistance by these ampicillin-resistant strains. Among European Union countries, the overall mean percentage of vancomycin resistance among *E. faecium* isolates causing invasive infections showed a slight increase from 9% in 2013 to 11.8% in 2016 albeit with considerable variability, that is, four European countries had rates of vancomycin resistance between 5% and 10%, seven between 10% and 25%, and seven >25% but <50% (European Antimicrobial Resistance Surveillance System, www.ecdc.europa.eu 2017 report).

Another phenomenon described in *E. faecalis* and *E. faecium* is the existence of strains that can only sustain growth in the presence of vancomycin, so-called vancomycin-dependent enterococci (VDE), or when supplemented with the dipeptide D-alanyl-D-alanine. VDE strains have an inactive D-alanine-D-alanine ligase (and thus do not produce D-alanyl-D-alanine) but, with vancomycin exposure, are able to synthesize cell wall using the D-alanine-D-lactate ligase produced from their *vanA* or *vanB* gene cluster. When vancomycin is not present, these precursors are not produced and the organism cannot grow. Dependence on vancomycin has been reported after prolonged exposure and appears to be related to mutations in the gene *ddl,* leading to loss of the cell's endogenous D-alanine-D-alanine ligase and loss of peptidoglycan synthesis. VDE strains can revert to vancomycin-independent (and resistant) growth, either by reverting the mutation in the *ddl* gene or by constitutively expressing their *van* genes, which leads to D-alanine-D-lactase ligase activity and peptidoglycan synthesis is restored. VDE strains seem to be extremely rare and treatment involves stopping vancomycin and following closely because the isolates revert readily to a nondependent phenotype.

Staphylococcus aureus

Heteroresistant vancomycin-intermediate *Staphylococcus aureus* (hVISA)/VISA. As mentioned earlier, the different phenotypes of decreased susceptibility in *S. aureus* isolates include VISA/hVISA and VRSA. Because the disk diffusion method only detects strains with very high vancomycin MICs (i.e., VRSA) and fails to detect VISA isolates, MIC determinations by agar or broth dilution or by Etest (bioMérieux, Marcy l'Étoile, France) (a gradient diffusion method using antibiotic impregnated strips) are recommended for vancomycin susceptibility. In 2006, and to improve the correlation of the in vitro susceptibility assessment with clinical response, the CLSI decreased the vancomycin MIC breakpoints for *S. aureus* to be susceptible at ≤2 μg/mL, intermediate at 4–8 μg/mL, and resistant at ≥16 μg/mL. It should be noted that VISA isolates also display decreased susceptibility to teicoplanin (MIC ≥8 μg/mL)[10] and that *S. aureus* strains with decreased susceptibility to teicoplanin, while remaining susceptible to vancomycin, were reported before the emergence of VISA isolates. The combination of vancomycin plus β-lactams (e.g., nafcillin, cefazolin, and ceftaroline) showed synergistic activity against hVISA and VISA isolates with an in vitro infection model,[11,12] but conflicting data were found in different animal models using oxacillin[13] and ceftobiprole.[14] *S. aureus* isolates exhibiting vancomycin MICs within the susceptible range might still have another form of decreased susceptibility to vancomycin, a phenomenon called heteroresistance. hVISA are considered precursors of VISA isolates having the same but less pronounced changes associated with decreased susceptibility to vancomycin. It is recognized that hVISA strains are more often encountered in clinical practice than VISA isolates. The exact prevalence of these isolates has been difficult to establish largely because the only reliable manner to detect hVISA isolates is by population analysis (see later), a technique that is too cumbersome for clinical laboratories. Geographic variability in the hVISA prevalence is seen, which may be related to clonal spread in certain institutions.

A systematic literature review of 91 published studies found an overall increase of hVISA and VISA strains among thousands of MRSA tested—from 4.7% and 2% before 2006 to 7% and 7.9% between 2010 and 2014, respectively.[15] In this report the prevalence of hVISA/VISA strains was more common in Asia than in Europe/America and in blood culture samples than from other clinical specimen; MRSA isolates within the sequence types (ST) 239 and ST5 and those carrying staphylococcal cassette chromosome (SCC)*mec*II and III were the most prevalent among VISA strains. Increasing rates of hVISA are usually seen among isolates with higher vancomycin MIC; one study found a rate of 10.5% in those MRSA strains with an MIC of 2 μg/mL and only 0.1% in those with an MIC of 1 μg/mL determined by Etest.[16]

Mechanisms of decreased susceptibility to vancomycin. The exact mechanism and genetic basis underlying the decreased susceptibility to vancomycin of VISA isolates remains a subject of active investigation, but none of these strains carries the vancomycin-resistance genes found in enterococci. A common feature of VISA isolates appears to be the presence of a thickened cell wall; indeed, it has been postulated that the D-alanyl-D-alanine–ending precursors of thickened cell walls may trap vancomycin molecules in outer layers of the peptidoglycan, allowing newly emerged peptidoglycan precursors to be used for transglycosylation and transpeptidation reactions (close to the cytoplasmic membrane).[10] Other common phenotypic characteristics of the VISA strains include an increased cell wall turnover, decreased autolytic activity, an increased positive cell wall charge (presumably responsible also for decreased susceptibility to daptomycin), and reduced accessory gene regulator (Agr) functionality. The hVISA phenotype appears to be a precursor of VISAs and has been associated with mutations in a two-component regulatory system (TCS, designated glycopeptide-resistance–associated sensor/regulator [GraSR]) that controls several genes involved in cell wall homeostasis, including the susceptibility of *S. aureus* to lysozyme and cationic antimicrobial peptides. Further sequential mutations in other TCSs have been shown to be responsible for full expression of the VISA phenotype.

One of these TCSs is vancomycin-resistance–associated sensor/regulator (VraSR), consisting of the histidine-kinase VraS and the response regulator VraR that are involved in the regulation of transcription of genes encoding proteins and enzymes participating in cell wall turnover (designated cell wall regulon, ≈30 genes) and cell envelope stress response.[17] Another TCS associated with full expression of the VISA phenotype is WalKR (also designated YycFG; WalK is the histidine kinase, and WalR is the response regulator), which appears to contribute to decreased autolytic activity.[18,19] Mutations in these TCSs are associated with increased vancomycin MICs and cell wall thickness and with significant reduction in autolytic activity and biofilm formation.[19] Of note, alteration of some of these two-component regulatory systems is also associated with decreased susceptibility to daptomycin, suggesting a common pathway of resistance to cell envelope–acting antimicrobial agents.

The *agr* gene operon in *S. aureus* encodes a global regulatory system that coordinates several virulence pathways. The loss of *agr* functionality (*agr* genotype II) predominates among VISA strains and has been linked to a survival advantage in the presence of vancomycin and to vancomycin treatment failure.[20–22] Because downregulation of expression of the *dhl* gene (encoding delta-hemolysin) was seen in both hVISA and VISA isolates, the delta-hemolysin assay has been proposed as a biomarker for reduced vancomycin susceptibility.[23]

Laboratory detection of hVISA strains. Heteroresistance means that only some of cells in a culture, sometimes as low as 1 in 100,000 bacteria, are able to grow at a higher concentration of vancomycin (>2 μg/mL) on vancomycin-containing agar. When testing these isolates at a standard inoculum (5×10^4 per well with broth microdilution MIC), this subpopulation of cells will not be detected, and the vancomycin MIC will fall within the susceptibility range most of the time. Therefore conventional susceptibility tests would not identify heteroresistance until a much higher fraction of resistant cells is present; population analysis profile (PAP; determination of the number of surviving cells at increasing antibiotic concentrations) is required for detection. This test was later modified by calculating the area under the time-concentration curve (AUC) of the original PAP result and comparing it with the PAP of the reference hVISA strain Mu3; a PAP/AUC ratio <0.9, from 0.9 to 1.3, and >1.3 defines a strain as vancomycin-susceptible *S. aureus* (VSSA), hVISA, and VISA, respectively.[24] As mentioned earlier, this test is not suitable for clinical practice; thus several screening methods have been studied for routine laboratory conditions to detect these strains using a higher inoculum, prolonged incubation, or more nutritious agar. In routine laboratory conditions the "macro" Etest method (MET) may be used to identify hVISA with a fair degree of accuracy. The method uses a higher inoculum (equivalent to a 2 McFarland standard) that is streaked onto the surface of a brain-heart infusion agar to which vancomycin and teicoplanin Etest strips are applied; readings take place at 24 and 48 hours and are considered positive if vancomycin and teicoplanin MICs are ≥8 μg/mL or the teicoplanin MIC is ≥12 μg/mL.[25] Another method for hVISA screening uses a double-strip Etest combining vancomycin and teicoplanin with a nutritional supplement (Etest GRD [bioMérieux, Marcy l'Étoile, France]), a Mueller-Hinton 5% blood agar with standard inoculum, and a 24- and 48-hour reading; an MIC of vancomycin or teicoplanin ≥8 μg/mL by one of these methods for a

strain that tested as susceptible for standard methods defines an hVISA strain.[26] Both the MET and Etest GRD methods may be used to identify hVISA strains with a fair degree of accuracy, although some differences in sensitivity and specificity with the population analysis exist.[25,27] The vancomycin and teicoplanin MIC determined by broth macrodilution method (10^5 CFU in 1 mL) or agar testing when read at 48 hours could detect a significantly higher number of strains classified as hVISA and VISA than the standard microdilution method; the higher number of bacteria used in the broth macrodilution method likely explains these findings.[28] Several other screening tests have been developed with variable sensitivity, but the potential clinical impact in the management of patients with deep-seated *S. aureus* infections is unclear.

Clinical impact of strains with increased vancomycin minimal inhibitory concentration. Several studies have reported a rise in the vancomycin MICs over time, a phenomenon also known as vancomycin "MIC creep," although the use of this terminology is still controversial. The increase in vancomycin MICs might be explained by replacement of local MRSA clones with strains with higher vancomycin MICs rather than by a vancomycin "creep" per se within an MRSA clone.[29] In fact, this vancomycin MIC creep phenomenon can be seen in centers where vancomycin is frequently prescribed and/or in the setting of a specific clone predominance with high vancomycin MIC; however, there does not appear to be increased resistance overall in *S. aureus* around the world.

There is evidence that VISA isolates (MICs, 4–8 μg/mL) are associated with therapeutic failure. In addition, poorer clinical outcomes have been associated with hVISA isolates compared with non-hVISA isolates among patients treated with vancomycin in many but not in all clinical studies. A decrease in the virulence properties of hVISA isolates has been suggested by some studies that reported lower rates of invasive infections and diminished risk for septic shock compared with VSSA strains.[30,31]

It is also important to note that a poor response to vancomycin has been documented in an experimental endocarditis model using hVISA strains and that decreased in vitro killing by vancomycin (likely to be seen with hVISA isolates) has been significantly associated with worse clinical outcome,[32,33] although time-kill experiments cannot be performed by most laboratories.

Several initial retrospective[32–35] and prospective[36,37] studies and meta-analysis[38] have reported unsatisfactory response to vancomycin in the treatment of invasive MRSA infections, including bacteremia caused by strains with vancomycin MICs >1.5 μg/mL. However, another meta-analysis[39] and some recent prospective studies[40,41] have failed to confirm such association. Part of the dilemma likely arises from the inherent variability in the results of MIC testing, with the CLSI criteria considering a result accurate to ± one twofold dilution. Moreover, vancomycin MICs measured by Etest and by some automated methods are generally one-half to one dilution higher and one to two dilutions lower than the gold standard broth microdilution method, respectively.[42] Thus the exact meaning of a high vancomycin MIC (but within the susceptible range) among invasive *S. aureus* isolates is still a matter of debate. Current Infectious Diseases Society of America (IDSA) practice guidelines for the treatment of MRSA infections suggest that, for strains with vancomycin MIC ≤2 μg/mL, a decision to continue vancomycin treatment should depend more on clinical response than on the MIC value.[43]

Vancomycin-resistant *Staphylococcus aureus*. Another mechanism of decreased susceptibility to vancomycin defined as "true" vancomycin resistance (MIC >16 μg/mL) was reported in 2002, primarily in the United States (mainly in the state of Michigan) with 14 VRSA clinical isolates reported to date.[44,45] These isolates display a median vancomycin MIC of 512 μg/mL, with a range of 32 to 1024 μg/mL. Most patients from whom VRSA were isolated had chronic underlying diseases, prior or current MRSA and VRE colonization or infection, and extensive exposure to vancomycin. All the strains had acquired the enterococcal *vanA* gene, and most of them belonged to the MRSA lineage USA100 containing SCC*mec* type II within clonal cluster 5.[44] However, a community-associated ST8 strain of VRSA was reported in Brazil,[46] with acquisition of the *vanA* gene cluster also by methicillin-susceptible *S. aureus* (MSSA).[47] Molecular studies of the transfer of the *vanA* cluster from enterococci to these MRSA isolates can result in replication of the actual enterococcal *vanA* plasmid in the new staphylococcal host after plasmid transfer by conjugation (less common) or transposition of the Tn*1546* element to a staphylococcal plasmid with subsequent loss of the enterococcal plasmid.[47,48] Infection control measures, with perhaps decreased transmissibility or fitness, appear to have controlled the spread of these isolates. VRSA strains have been reported, albeit very rarely, from other parts of the world, such as India, Iran, Portugal, Guatemala, and Brazil. Because cocolonization with MRSA and VRE strains is not a rare event and the population of *S. aureus* that can acquire the *vanA*-containing enterococcal plasmid is very widespread, it is likely that reports of VRSA strains will continue in the future.

Coagulase-Negative Staphylococci

Studies in the 1980s found high MICs of teicoplanin, sometimes within the resistance range, among methicillin-resistant *Staphylococcus haemolyticus.* Overall, MICs of teicoplanin against coagulase-negative staphylococci show a wide range, occasionally higher than the CLSI resistance breakpoint (MIC ≥32 μg/mL). For this group of organisms, MICs of vancomycin are generally less variable and within the susceptible range (susceptible at ≤4 μg/mL, intermediate at 8–16 μg/mL, and resistant at ≥32 μg/mL by CLSI, and susceptible at ≤4 μg/mL and resistant at >4 μg/mL by EUCAST). Overall, reduced vancomycin susceptibility due to heterogeneous resistance to glycopeptides has been reported between 7% and 18% of the studied coagulase-negative staphylococci isolates, most of which were associated with glycopeptide exposure.[49] As described with *S. aureus,* the mechanism for reduced glycopeptide susceptibility among coagulase-negative strains appears to be related to changes in cell wall homeostasis leading to thickened cell walls.

Other Gram-Positive Bacteria

Vancomycin-resistant pneumococci have not been reported, although some series have found reduced bactericidal activity of vancomycin, that is, tolerance, in up to 8.7% of isolates.[50,51] The first *S. pneumoniae* isolate showing this phenotype was isolated from a patient with meningitis and named the "Tupelo" strain. Tolerant strains appear to be more commonly resistant to other antibiotics and have been recovered as colonizers or causing invasive disease with similar frequency.[50] The clinical implications of this phenomenon are difficult to assess; an apparent vancomycin (with cefotaxime) therapeutic failure for pneumococcal meningitis caused by a vancomycin-tolerant strain has been reported and, in a retrospective analysis, vancomycin-tolerant isolates causing meningitis were associated with worse clinical outcome.[50] The mechanism for tolerance is not well understood but may involve a deficiency in LytA (a cell-wall hydrolase with major autolytic function) activity or changes in the CiaRH system, which has an established lysis protection role. Exposure of *S. pneumoniae* to vancomycin induces the transcription of a four-gene operon named *ptv* (phenotypic tolerance to vancomycin), which might act by modulating cell membrane properties.[52]

Although less often associated with human disease, the genera *Leuconostoc* and *Pediococcus,* and certain *Lactobacillus* spp. (*L. rhamnosus, L. casei,* and *L. plantarum*) are intrinsically resistance to glycopeptides. The mechanism of resistance also involves production of peptidoglycan precursors that terminate in D-alanyl-D-lactate. The D-alanyl-D-lactate ligase of these organisms, however, is only remotely related to the VanA or VanB ligase found in VRE strains. Another gram-positive organism, *E. rhusiopathiae,* is also typically vancomycin resistant.

Clinical Pharmacodynamics and Pharmacokinetics

A considerable number of studies have found that the bactericidal activity of vancomycin is concentration independent once a concentration of four to five times the MIC for the organism is reached. Finding the pharmacodynamic parameter able to predict vancomycin treatment success has not been straightforward,[53] but it seems that the 24-hour AUC/MIC ratio is the best predictor of efficacy in clinical studies.[54,55] For example, in patients with MRSA pneumonia, higher rates of clinical success and more rapid bacterial eradication were associated with achievement of an AUC_{24}/MIC ratio ≥400.[55] Of note, no relationship

between percentage of time higher than the MIC and response was found in this study.[55]

For the correct interpretation of the data related to studies addressing the pharmacodynamics of vancomycin, the following considerations should be taken into account: (1) Because vancomycin susceptibility is determined by methods that significantly differ from the standard broth microdilution method (e.g., vancomycin MICs by Etest are onefold or 0.5–1.5 $\log_2$ dilution higher than broth microdilution MICs, whereas automated methods such as Sensititre [Thermo Scientific/TREK Diagnostic Systems; Oakwood, OH] and Vitek-2 [BioMérieux, Durham, NC], tend to underestimate the MICs value), small variations in the MIC will represent significant changes in the AUC/MIC ratio; (2) trough level is used as a surrogate marker for AUC because the latter is not calculated in clinical practice; (3) in patients with serious infections, especially those caused by MRSA, vancomycin levels may be used to modify the dose to attain the target serum level; (4) maximal optimization of the vancomycin dosing does not seem to be required for less serious MRSA infections, such as most acute bacterial skin and skin structure infections (ABSSSI), for which dosing based on renal function and actual patient weight is likely to be adequate; (5) measurement of the peak value is not recommended; and (6) low trough vancomycin levels (<10 μg/mL) have been associated with development of hVISA isolates.

For serious infections caused by MRSA, such as endocarditis, bacteremia, arthritis, osteomyelitis, meningitis, pneumonia, and severe ABSSSI, the consensus from the IDSA, the American Society of Health-System Pharmacists, and the Society of Infectious Diseases Pharmacists published in 2009 recommends trough vancomycin concentration between 15 and 20 μg/mL.[56] In those receiving vancomycin as a continuous infusion, the recommended target plateau concentration has been 20 to 25 μg/mL. The range of recommended trough serum levels between 15 and 20 μg/mL has been correlated with a 24-hour AUC (±standard deviation) of 418 ±152,[46] which will achieve a vancomycin AUC/MIC ratio ≥400 if the MIC of the infecting strain is ≤1 μg/mL. To attain these levels, the vancomycin dose is usually 15 to 20 mg/kg every 8 to 12 hours based on actual body weight in patients with normal renal function. Indeed, a recent prospective study found that dosing vancomycin based on AUC estimation rather than on trough concentration was associated with lower rates of nephrotoxicity, shorter duration of therapy, and lower overall vancomycin exposure.[57]

The need to target an AUC/MIC ratio ≥400 was initially based on clinical studies in patients with MRSA lower respiratory tract infections reporting higher rates of clinical success and more rapid bacterial eradication in those patients with achievement of an AUC/MIC ratio ≥400, where vancomycin MICs were determined by broth microdilution.[55] In a single-center and retrospective study of 320 patients with MRSA bacteremia treated with vancomycin, the group of patients that did not reach an AUC/MIC ratio of at least 421 was associated with a significantly higher failure rate than those that did achieve this cutoff (61% vs. 49%, $P = .038$) (MIC measured by broth microdilution).[58] More recently, a group of researchers reported a retrospective and then a prospective study of patients with MRSA bacteremia treated with vancomycin, demonstrating a significant association between a low AUC/MIC ratio and treatment failure; in both studies and for broth microdilution and E-tests susceptibility tests, the AUC/MIC cut-off values were between 392 and 430.[59,60] In accordance, a systemic review of published studies, including 916 patients with different types of MRSA infections, found that a high AUC/MIC ratio was significantly associated with lower clinical failure and mortality; the authors proposed an AUC/MIC ratio of 400 as a reasonable target (MIC measured by either broth microdilution or by E-test).[61]

Even though vancomycin AUC is not measured in clinical practice, it can be estimated with the use of several computer programs having patient information such as the trough vancomycin level at steady state, the estimated volume of distribution, and renal function, among others. It has been shown that a significant proportion of patients can achieve an AUC/MIC ratio ≥400 with a trough concentration <15 μg/mL; therefore the target of at least 15 μg/mL may overexpose patients to vancomycin and its related nephrotoxicity risk.[62] In this regard, a Monte Carlo simulation model suggested that targeting a vancomycin trough level >15 μg/mL may not be necessary for MRSA strains with an MIC of 0.5 μg/mL.[63] On the other hand, and as previously shown[64] for strains with vancomycin MICs of 2 μg/mL, the probability of achieving the target AUC/MIC ratio is 57% and 15% for vancomycin dosing of 2 g and 1 g every 12 hours, respectively,[63] implying that many of these patients would be given vancomycin doses ≥4 g/day to achieve the AUC/MIC target for severe MRSA infections.

Analyzing the pharmacodynamic AUC_{24}/MIC parameter for vancomycin efficacy in infections caused by enterococci, a retrospective single-center study found that among 57 patients with enterococcal bacteremia (32 *E. faecium*, 21 *E. faecalis*, and 4 other enterococci) treated with vancomycin, the 30-day mortality rate was significantly lower in those with an AUC/MIC ratio (MIC measured by Etest) ≥389.[65]

Of interest, a recent retrospective study showed that *S. aureus* isolates tolerant to vancomycin (defined as an MBC/MIC ratio ≥32 by broth microdilution) were significantly associated with vancomycin failure in patients with *S. aureus* bacteremia. This association was even maintained in patients with MSSA bacteremia treated with β-lactams, suggesting some fundamental difference in the organisms' response to these antibiotics.[66]

Besides the management of serious staphylococcal infections, other special circumstances where it seems prudent to measure vancomycin concentrations include, for the most part, patients concomitantly receiving another nephrotoxic agent, especially aminoglycosides; patients receiving high-dose vancomycin (e.g., very obese patients); those with rapidly changing renal function; and subjects undergoing hemodialysis, especially if high-flux membranes are used. Other situations include the measurement of cerebrospinal fluid (CSF) levels in patients receiving vancomycin for central nervous system (CNS) infection, whether by intrathecal, intraventricular, or intravenous (IV) routes (see later); vancomycin administration in neonates; and in extremely ill patients or in the presence of possible therapeutic failure, to ensure adequate drug presence.

The in vitro postantibiotic effect of vancomycin against staphylococci and enterococci has been described mostly as of short duration. As with β-lactams (i.e., nafcillin), the in vitro bactericidal activity of vancomycin is reduced when a high inoculum of *S. aureus* is used. The activity of vancomycin is affected, at least to some degree, by the presence of biofilm, which is often seen in the setting of medical device–related infection.

A study comparing innovator vancomycin product with vancomycin generics raised concern that generic vancomycin was less potent, possibly due to higher amounts of crystalline degradation products (CDP-1).[67] However, the US Food and Drug Administration (FDA) laboratories tested six commercially available vancomycin generic products and found minimal amounts of CDP-1,[68] and no statistically significant differences were observed between these product and the innovator molecule in an endocarditis animal model.[69]

Distribution

Vancomycin pharmacokinetics in adults is best described by a two- or three-compartment model. After a single IV dose of 0.5 g and 1 g in normal volunteers, vancomycin concentrations achieved in serum at 2 hours are about 10 μg/mL and 25 μg/mL, respectively, which decrease to 2 μg/mL by 6 to 8 hours after 0.5 g and by 12 hours after 1 g.[70] The drug shows a short distribution phase of about 7 minutes and then an intermediate phase of serum decline (half-life of 30–90 minutes). This is followed by a highly variable elimination phase of 3 to 11 hours (averaging 6 hours) in subjects with normal renal function; in this phase the vancomycin concentration is inversely affected by the creatinine clearance. The volume of distribution of vancomycin at steady state ranges from 390 to 970 mL/kg in studies including adults, children, and infants; and the percentage protein binding in serum varies between 30% and 55%.

Trough vancomycin serum level should be obtained at steady state, usually before the fourth dose, although steady state may occur earlier if a loading dose is used. When obtaining a trough vancomycin level, it is important to collect the sample within 30 minutes before administration because drawing too early is a common cause of elevated vancomycin levels, which can lead to inappropriate dosing change.

Penetration of vancomycin into the CSF is minimal in the absence of meningeal inflammation,[71] which usually results in higher vancomycin passage into the CSF in patients with meningitis than in those with ventriculitis. In adults with ventriculitis CSF penetration ranges from 5% to 10% after IV administration, probably resulting in subtherapeutic levels; for this reason, it is important to send CSF for determination of vancomycin levels when using it to treat CSF infections. In children with meningitis the vancomycin concentration in CSF has ranged from 14% to 28% (mean, 21%) of that in serum after a vancomycin dose of 60 mg/kg/day in conjunction with dexamethasone; this concentration is considered adequate and predictable. Dexamethasone, through reduction of meningeal inflammation, may decrease vancomycin CSF penetration, which was associated with delayed CSF sterilization in experimental meningitis, although with higher doses, therapeutic CSF levels were achieved. Low vancomycin CSF levels have been associated with clinical failures in adults with pneumococcal meningitis, although it has been difficult to establish a clear correlation between vancomycin CSF concentration and cure.[72] To overcome the relatively poor vancomycin CSF penetration, high-dose vancomycin administered in continuous infusion (50–60 mg/kg/day after a loading dose of 15 mg/kg) has been evaluated in adults with meningitis, resulting in a CSF penetration rate of a mean of 30%[73] to 48%.[74]

Animal studies have documented high concentrations of vancomycin in kidney, liver, and spleen of rats, but data on concentrations in the equivalent human organs are limited. A relatively good concentration was found in kidney, liver, aorta, lung, heart tissue, and in abscess fluid in a patient after several vancomycin doses, and the concentration of vancomycin in soft tissue appears to be lower in diabetic than nondiabetic patients. Vancomycin concentrations are generally adequate to treat susceptible organisms in fluids from the pericardial, ascitic, pleural, and synovial fluids/spaces.[71] Concentrations in heart valve, subcutaneous tissue, and muscle were found to be 52%, 29%, and 27% of the concomitant serum level, respectively, 6 hours after a single vancomycin dose.[75] In patients undergoing vascular surgery receiving continuous vancomycin infusion after a loading dose, serum/tissue concentration ratios of 0.22 and 0.50 for fat and vessel wall, respectively, were reported.[76] These results appear to support adequate vancomycin penetration into vascular tissue but probably not into fat, at least with the mentioned dosing. Heterogeneous diffusion of vancomycin into vegetations has been demonstrated in experimental endocarditis models.

Studies addressing vancomycin concentrations in lung tissue found significant variability, depending mainly on the sample used and the presence or not of inflamed lung tissue. Moreover, indirect measurements of vancomycin concentrations have been used for distal airways and alveoli. Twenty percent to 30% of the serum concentration has been reported in lung tissue. Others have reported its penetration into the epithelial lining fluid as approximately 16% that of concomitant vancomycin serum levels. In another study vancomycin serum trough levels >20 μg/mL were required to have detectable concentration in the epithelial lining fluid in patients with MRSA pneumonia. In contrast, the vancomycin epithelial lining fluid concentrations were 50% of those obtained in serum in 10 healthy volunteers after a 1-g infusion; however, a high degree of variability was found among the pharmacokinetic results.[77] Patients with higher concentrations of albumin in the bronchoalveolar lavage had higher concentrations of vancomycin, probably linked to inflammation.

Although vancomycin appears to penetrate into bile, it is not concentrated there. Vancomycin, like many other antimicrobial agents, penetrates very poorly into the aqueous humor of the eye. Human studies evaluating the concentration of vancomycin in infected and uninfected bone have reported highly variable results. A mean concentration of vancomycin in the sternum of 10.4 μg/g 60 minutes after administration of 15 mg/kg has been documented in subjects undergoing cardiac surgery.[78] In another study the penetration of vancomycin appeared to be satisfactory and suboptimal into the cancellous and the cortical bone, respectively.[79] Of note, this study also reported higher vancomycin bone concentrations in association with increased local inflammatory markers, likely secondary to bone inflammatory conditions. More recently, adults undergoing total knee replacement receiving vancomycin as prophylaxis were evaluated, with solid tissue concentrations measured by microdialysis chips; a delayed and low concentration of vancomycin in bone was found, with lower levels in the cortical than in the cancellous bone, with a time to reach 2 μg/mL of 110 and 27 minutes, respectively.[80] Nonetheless, it has been difficult to find a correlation between antibiotic bone concentrations and clinical outcome.

Transplacental passage of vancomycin during the second trimester of pregnancy and at time of delivery has been documented, and the concentration of vancomycin in breast milk 4 hours after IV infusion was 12.7 μg/mL. This could lead to a potential infant oral dose of 1.9 mg/kg/day.

Excretion

Vancomycin is primarily excreted unchanged via the kidneys by glomerular filtration, with no direct evidence of tubular secretion or resorption.[71] A linear correlation between creatinine clearance and vancomycin levels was recognized early on in patients with varying degrees of renal dysfunction. Some investigators report a lower rate of vancomycin clearance in patients with hepatic dysfunction, and variable levels of vancomycin have been found in stool samples of patients on IV vancomycin only after 5 days of therapy, although vancomycin does not appear to have enterohepatic circulation. However, nonrenal clearance does not appear to account for >5% of the total drug clearance, and therefore dose adjustment in patients with hepatic dysfunction alone is unlikely to be necessary.

Administration

Vancomycin is given intravenously for the treatment of systemic infections caused by susceptible organisms. In certain circumstances vancomycin can be administered through oral, intraperitoneal, intrathecal or intraventricular, and intraocular routes, but intramuscular injection is not recommended because it causes severe local pain. Vancomycin is poorly absorbed when administered orally, yielding high fecal concentrations (1406 ± 1164 μg/g of feces) using doses of 125 mg every 6 hours; in the presence of diffuse colonic inflammation and renal insufficiency, detectable levels of vancomycin may be obtained in serum, but monitoring drug levels does not seem necessary.

For IV administration, vancomycin is generally diluted in 100 to 250 mL of 5% dextrose or 0.9% saline solution with a concentration ≤5 mg/mL and infused at a rate not exceeding 15 mg/min (i.e., 0.5 g and 1 g over 30 and 60 minutes, respectively) to minimize the occurrence of infusion-related toxicities. Antihistamines may be used to reduce the incidence of red man (or red neck) syndrome, characterized by an acute onset of an erythematous rash affecting the upper trunk and neck during or at the end of an infusion, which, when seen, is usually associated with rapid infusion or a high vancomycin dose. The usual recommended IV dose in adults with normal renal function is 30 mg/kg/day divided into two or four doses (typically, 500 mg every 6 hours or 1 g every 12 hours). Based on pharmacodynamics studies, the need for higher trough levels (between 15 and 20 μg/mL) in the setting of severe MRSA infections has been reflected in a dosing recommendation of 15 to 20 mg/kg every 8 to 12 hours; this dose should be based on actual body weight, not exceeding 2 g per dose (Table 30.1). Continuous infusion at a dose of 30 mg/kg/day after a loading dose of 15 mg/kg also has also been used. This mode of administration appeared to be associated with lower rates of nephrotoxicity but not with increased efficacy[81]; prospective randomized studies are needed.

A few studies have evaluated the use of a loading dose of 25 to 30 mg/kg (at an infusion rate of 500 mg/h) or of 2 g, particularly in suspected severe MRSA disease, such as endocarditis, meningitis, pneumonia, or sepsis, to achieve higher trough concentrations earlier in therapy,[82,83] which has been associated with better initial outcome.[34,64] Prospective studies to address this therapeutic issue would be needed to make firmer recommendation on clinical criteria but is a logical pharmacologic approach.

Because obese patients were found to have increased volumes of distribution, higher renal clearance, and probably lower levels of free vancomycin in serum,[84] these individuals should receive vancomycin based on their actual total body weight instead of their ideal weight. To avoid very high peak values, a more frequent dosing schedule should be considered, and serum concentration should be obtained routinely

TABLE 30.1 Route of Administration, Recommended Dosages, and Infusions of Vancomycin, Teicoplanin, Telavancin, Dalbavancin, and Oritavancin

DRUG	ROUTE OF ADMINISTRATION	RECOMMENDED DOSAGE (ADULTS)	INFUSION	COMMENTS
Vancomycin	Intravenous	The average dose is 15–20 mg/kg q8–12h. If continuous infusion is indicated, the daily dose is 30 mg/kg after a loading dose of 15 mg/kg.	Should use a concentration of ≤5 mg/mL and a rate of <15 mg/min (i.e., 1 g over 60 min)	For many infections, 15 mg/kg q12h is adequate. For severe MRSA infections, a loading dose (see text) should be considered and, although still debated, current recommendations for monitoring include a trough between 15–20 μg/mL or an AUC/MIC ratio of ≥400.
	Oral	125–500 mg q6h		Severe *Clostridioides difficile* (formerly *Clostridium difficile*) colitis
Teicoplanin	Intravenous or intramuscular	Loading dose: 400 mg (6 mg/kg) q12h for 3 doses Maintenance: 400 mg q24h	Can be administered in bolus	For serious infections: 800 mg (up to 12 mg/kg) q12h for 3 times and then q24h
	Oral	100–400 mg q12h		Severe *C. difficile* colitis
Telavancin	Intravenous	10 mg/kg/day	1-hour infusion	Supplied as a 25-mg or 750-mg vial to be reconstituted with 15 mL and 45 mL, respectively, for a concentration of about 15 mg/mL
Dalbavancin	Intravenous	Twice-weekly regimen: 1000 mg then 500 mg 1 week later. One-time dosing: 1500 mg	30-min infusion	Supplied in 500-mg vials to be diluted in 5% dextrose for a final concentration of 1–5 mg/mL
Oritavancin	Intravenous	1200-mg single dose	3 hours	Supplied in 400-mg vials to be diluted (only in 5% dextrose) to a concentration of 1.2 mg/mL

AUC/MIC, Area under the curve/minimal inhibitory concentration; *MRSA,* methicillin-resistant *Staphylococcus aureus.*

when high doses are used. Significantly higher vancomycin clearance has been shown in burn patients, suggesting also the need for higher and more frequent doses and monitoring of serum levels in this group of patients. Even though a significant decrease of serum vancomycin levels during cardiopulmonary bypass has been documented, concentrations were maintained within the therapeutic range after a 15-mg/kg dose of this drug administered intravenously 1 hour before skin incision. No dosage adjustment appears to be necessary during pregnancy.

The dose of IV vancomycin for children with non-CNS infections should be 10 mg/kg every 6 hours, and, for infections involving the CNS and other serious infections, it should be 15 mg/kg every 6 hours.[85] In children age 1 month to 12 years with normal renal function, an increase in the prescribing dose from 45 mg/kg/day divided every 8 hours to 60 mg/kg/day divided every 6 hours was correlated with higher initial trough levels. This decreased the percentage of patients with trough levels <5 μg/mL from 38% to 17%.[86] However, a retrospective study found that about 60% of children (median age, 6 years) receiving empirical treatment with vancomycin attained subtherapeutic level (<15 μg/mL) even when high doses such as 20 mg/kg every 6 to 8 hours were used, probably secondary to increased creatinine clearance in this population. In particular, age younger than 12 years was significantly associated with initial low trough levels (<10 μg/mL) compared with those older than 12 years, even after adjustment for creatinine clearance.[87]

Neonates and young infants appear to have a lower vancomycin clearance rate. For neonates younger than 1 week, the recommended dose is 15 mg/kg every 12 hours; and for those between age 1 week and 30 days, it is 15 mg/kg every 8 hours.[85]

In premature infants longer intervals may be required because the clearance of vancomycin correlates with postconceptional age.[88] The classic dosing schedule in premature neonates has been to dose vancomycin 15 mg/kg every 12 or 18 hours, and every 8 or 12 hours when the postconceptional age is 29 weeks or younger and 30 to 44 weeks, respectively. However, because this dosing guideline aimed for a trough concentration between 5 and 10 μg/mL, and in one study 30% of neonates had serum concentrations <5 μg/mL, higher doses have been suggested.[89] The percentage of neonates (average gestational age, 28.2 weeks) achieving a trough of 10 to 20 μg/mL has been only 25% after empirical vancomycin treatment in the intensive care unit (ICU); among those who achieved the therapeutic goal, the median daily dose was 30 mg/kg.[90] It is also recommended to closely monitor serum concentrations of vancomycin in these patients.

After intraperitoneal administration of vancomycin in patients on continuous ambulatory peritoneal dialysis (CAPD), the concentrations of vancomycin were still 7 ± 1.2 μg/mL and 3.6 ± 1.1 μg/mL for serum and dialysate, respectively, 7 days after the intraperitoneal administration of 30 mg/kg (in 2 L of dialysate, with 6 hours' retention/settling).[91] An intermittent dosing schedule (one exchange daily) targeting a serum trough level >15 μg/mL has been recommended using a intraperitoneal vancomycin dose of 15 to 30 mg/kg in long dwell (at least 6 hours) every 5 to 7 days (patients on automated peritoneal dialysis may require additional doses); the dosing interval will depend on patients' residual renal function and peritoneum permeability.[92] A continuous (in each dialysate exchange) dosing regimen (loading dose of 30 mg/kg, followed by a maintenance dose of 1.5 mg/kg/bag), has also proven to be efficacious in this setting.[92] Conversely, the peritoneal dialysate concentration of vancomycin after IV administration alone does not reliably provide adequate levels to treat peritonitis caused by susceptible organisms.[71] The pharmacokinetics of vancomycin in children undergoing CAPD appear to be similar to those described in adults.

Because of concern that CSF levels of vancomycin are inadequate to treat ventriculitis (which can be assessed by sending CSF fluid for level determination), even with active infection, intrathecal or intraventricular administration of vancomycin should be considered, particularly when vancomycin is used as monotherapy. The recommended initial intraventricular dose for the treatment of ventriculitis or shunt-related infections is 5 mg, 10 mg, and 15 to 20 mg in patients with slit, normal-sized, and enlarged ventricles, respectively.[93,94] Immunocompromised patients and those with difficult-to-treat infections might benefit from using the higher dosage of 20 mg/day. Considering the CSF volume in infants, the intraventricular dose should be reduced by about 60% in this population. The recommended dosing frequency of vancomycin depends on the CSF drainage volume; with daily CSF volume drainage of >100 mL, between 50 mL and 100 mL, and <50 mL, the doses should be given every 24, 48, and 72 hours, respectively. Nevertheless, further adjustments based on the CSF levels should be made because of unpredictable kinetics of this drug in the CSF. The recommended optimal vancomycin trough concentration (24 hours after last

administration) should be high enough to exceed about 10 to 20 times the microorganism MIC, usually representing target concentrations between 10 and 20 μg/mL[95] However, it has been difficult to clearly find a correlation between levels, toxicity, and efficacy with the usual ventricular dosage of 5 to 20 mg/day[72] For administration of vancomycin into the CSF, the drug may be diluted in sterile 0.9% saline solution to a volume of 1 to 10 mL, at a concentration of 2.5, 5, or 10 mg/mL solution, depending on the dose.[94]

The use of IV vancomycin in patients with postoperative endophthalmitis results in subtherapeutic concentrations in the vitreous. However, after intraocular administration of 1 mg of vancomycin, vitreous concentrations are in the therapeutic range for at least 3 days.

Vancomycin for oral administration is formulated in capsules and oral solution and can be given by mouth or, as needed in case of ileus or toxic megacolon, by nasogastric tube and even via rectal tube (intracolonic administration) or ileostomy. The recommended oral dose for pseudomembranous colitis in adults ranges from 125 mg to 500 mg every 6 hours, depending on the severity of the colitis. In children the usual dose is 40 mg/kg/day (not exceeding 2 g/day) divided in three or four doses. For intracolonic administration, 500 mg of vancomycin dissolved in 1 to 2 L of 0.9% saline solution has been infused as a retention enema several times (two to six) a day.

Dosing in Renal Insufficiency

Dosing nomograms have been developed to reflect the more recent recommendation of targeting higher vancomycin trough concentration (between 15 and 20 μg/mL) for severe staphylococcal infections.[43,56] Despite the bedside calculation of the vancomycin dosing, individualized drug monitoring based on a patient's serum levels is required owing to the relatively high interindividual variability in patients with any degree of renal failure.

The recommended daily dose for patients with renal failure not on hemodialysis with a creatinine clearance between 60 to 89 mL/min, 30 and 59 mL/min, and 15 and 29 mL/min are 20 to 30 mg/kg, 10 to 20 mg/kg, and 7 to 10 mg/kg respectively. For those with <15 mL/min, the dose should be 10 mg/kg every 48 hours.[96]

The prediction of vancomycin pharmacokinetics in patients undergoing hemodialysis is difficult; actual body weight, timing of vancomycin administration, residual kidney function, and type of dialysis membranes are among the variables that affect this prediction.[97] The use of a loading dose of 20 to 25 mg/kg, followed by a fixed 500-mg dose during or after high-flux dialysis membranes, has been a commonly used dosing regimen.[98] However, only 28% of the patients were within the target trough vancomycin range of 15 to 20 μg/mL using this dosage. Another dosing schedule for patients on dialysis included a loading dose of 20 mg/kg, followed by 1 g of maintenance during the last hour of dialysis, which obtained a good mean trough of 19 ± 6.6 μg/mL, but 38% of the patients had levels >20 μg/mL.[99] More recently, also including patients on high-flux hemodialysis and using a Monte Carlo simulation method, a new protocol for vancomycin dosing was developed, considering a subject's weight: a 1-g loading dose, 500-mg maintenance (given during the last hour of each dialysis session) dose for patients weighing <70 kg; 1.25 g, followed by 750 mg for those weighing 70 to 100 kg; and 1.5 g, followed by 1 g for those weighing >100 kg; this dosing regimen was prospectively validated to achieve therapeutic serum troughs, with a mean of 17.3 ± 4.0 mg/L, and almost 90% of the maintenance troughs between 10 and 22 μg/mL.[100] The older low-flow membranes have lower ultrafiltration coefficients than high-flux membranes; after a loading dose (15–20 mg/kg), the dose to attain the vancomycin serum target is 15 mg/kg every 3 to 5 days and 7 mg/kg in the last hour of each hemodialysis session with low-flow cellulose acetate and polysulfone membranes, respectively.[101] In addition, trough levels before each dialysis session should be obtained to guide therapy.[97] Because CAPD can decrease the elimination half-life of vancomycin, a modest increase in the IV dose appears necessary in these patients.

It should also be considered that the administration of vancomycin during the last hour of dialysis renders lower serum levels than when administered after dialysis.

The pharmacokinetics of vancomycin in patients managed with intermittent hemodialysis is significantly different from those receiving continuous renal replacement therapy (CRRT). Clearance of vancomycin in patients with acute renal failure undergoing CRRT, such as continuous venovenous hemofiltration (CVVH) or continuous venovenous hemodialysis (CVVHD), is significantly increased. This clearance depends on operational factors, such as the ultrafiltration flow rate and the type of hemofilter. Based on the study of 24 critically ill patients undergoing CVVHD and posterior Monte Carlo simulation, the recommended initial dosing regimen of vancomycin for a target attainment between 15 and 20 μg/mL was 15 mg/kg every 24 hours, after the loading dose.[102,103] However, attainment of desired target concentrations was low, suggesting the importance of therapeutic drug monitoring for further adequacy of vancomycin dosing. Because about 20% of the vancomycin dose is removed during a 12-hour period of CVVH, the recommended maintenance dose of vancomycin is 500 to 750 mg every 12 hours, targeting a steady-state trough concentration of 15 to 20 μg/mL.[104] More recently, vancomycin, in continuous infusion administered based on a nomogram that included the CVVH intensity, achieved therapeutic levels (15–25 μg/mL) in >80% of 52 critically ill adults.[105] Drug removal appears to be higher when continuous venovenous hemodiafiltration (CVVHDF) (≈50%) is used, requiring doses of 750 mg every 12 hours, although drug accumulation has been described. Measurements of vancomycin levels are generally needed for patients on any form of CRRT.

Adverse Reactions

One of the first reports of the clinical use of vancomycin included six patients who developed severe ototoxicity.[106] Most of them were receiving 1 to 2 g of vancomycin per day despite renal insufficiency; when vancomycin levels were measured, values between 80 and 100 μg/mL were observed.[106] After 50 years of clinical use of vancomycin, with more purified material and because of the relatively few cases of confirmed vancomycin-related ototoxicity reported and existing data from animal studies showing no evidence of vancomycin-induced hearing damage, many authors concluded that vancomycin-related ototoxicity was a rare reaction. However, a retrospective study evaluating patients receiving higher doses of vancomycin (mean trough level, 19 μg/mL) reported an overall occurrence of worsening audiogram of 12%, a rate that was higher in subjects older than 53 years.[107] When ototoxicity develops, it generally appears to be reversible after drug discontinuation. Vertigo and tinnitus are also rarely reported during vancomycin therapy and may precede hearing loss. Prospective studies addressing the true rate of vancomycin-related ototoxicity in the era of higher vancomycin target serum levels are needed.

Nephrotoxicity associated with vancomycin has been reported since the beginning of its clinical use, thought to be related, at least in part, to impurities in the early preparations. This nephrotoxicity is usually defined as an increase in serum creatinine concentration of 0.5 mg/dL or a ≥50% increase from the baseline serum creatinine level. The mechanism underlying vancomycin-related nephrotoxicity appears to be related to its oxidative effects on cells of the proximal renal tubule, leading to renal tubular ischemia.[108] Vancomycin, in an animal model, appeared to induce apoptosis in proximal tubular cells via mitochondrial production of reactive oxygen species with peroxidation of the mitochondrial phospholipid cardiolipin.[109]

Initial studies noted renal toxicity in animals when high doses of vancomycin were administered. However, and despite its prior reputation as a nephrotoxic agent, vancomycin use alone has been associated with a relatively low rate of renal dysfunction in clinical studies that avoided the inclusion of confounding factors. Most prospectively designed studies report an incidence of renal function impairment between 0% and 12%, which appeared associated with vancomycin trough levels ≥15 μg/mL,[34] concomitant use of nephrotoxic agents, and duration of vancomycin therapy.[34,110] A more recent meta-analysis found that vancomycin troughs concentrations >15 μg/mL and treatment duration more than 7 days were associated with nephrotoxicity (odds ratio, 2.67; 95% confidence interval [CI], 1.95-3.65).[111] Most of these studies found a relationship between trough levels and renal toxicity, with rates usually between 15% and 30% when trough levels were 15 to 20 μg/mL.[112] A prospective multicenter study conducted between 2008 and 2010 found nephrotoxicity rates of 29.6% and 8.9% in patients with vancomycin serum trough

concentrations of ≥15 µg/mL and <15 µg/mL, respectively.[113] Lodise and associates[114] had previously reported a significantly higher rate of nephrotoxicity in patients receiving 4 g/day or more of vancomycin versus those receiving <4 g/day (34.6% and 6.7%, respectively). In the analysis of the relationship between vancomycin levels and nephrotoxicity, it should also be considered that levels may rise as result of a decreased renal clearance.

Other factors associated with increased vancomycin-related nephrotoxicity are weight ≥101.4 kg (224 lb), an estimated creatinine clearance ≤86.6 mL/min, critically ill patients,[114] and concomitant administration of other nephrotoxic agents, especially aminoglycosides.[115] The time to the onset of vancomycin-related nephrotoxicity has been between 4.3 and 17 days of therapy initiation.[111]

Even though vancomycin-associated nephrotoxicity is usually reversible, patients with nephrotoxicity tend to have worse outcomes with prolonged hospital stays, higher health care costs, and even higher mortality than those patients that did not developed nephrotoxicity.[112] Of note, use of a vancomycin loading dose has not been related to renal toxicity.

The coadministration of vancomycin (or an antistaphylococcal penicillin) plus gentamicin even at low dose (and for only the first 4 days of therapy) for *S. aureus* native valve endocarditis and bacteremia in a prospective randomized trial was associated with a significant decrease in creatinine clearance (≈25%) compared with those treated with daptomycin alone (≈8%).[116] An increased rate of acute kidney injury was reported with the association of vancomycin and piperacillin-tazobactam, both in adults and children.[117] The augmented risk of this association maintained its significance when compared not only with monotherapy with vancomycin or piperacillin-tazobactam but also to the combination of vancomycin plus cefepime or a carbapenem.[118] Rarely, acute interstitial nephritis has been associated with vancomycin use.

The overall risk for nephrotoxicity appears lower in the pediatric population than in the adult one. A rate of 14% has been noted in a retrospective study including patients from age 1 week to 19 years.[119] Trough levels >15 µg/mL, concomitant use of furosemide, and critically ill patients were the factors associated with decreased renal function.[119] Premature infants appear to have an even lower risk for developing vancomycin-related nephrotoxicity.

Several studies have assessed the nephrotoxicity associated with the mode of vancomycin administration as continuous infusion versus intermittent infusion. Different meta-analysis found that, overall, continuous infusion of vancomycin was associated with a lower risk for nephrotoxicity compared with intermittent infusion with no effect on treatment failure or mortality rates.[81,120] Specific biologic markers in blood, such as neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, insulin-like growth factor-binding protein 7, and tissue inhibitor of metalloproteinases-2, among others, for early detection of vancomycin-induced renal damage have been developed and may become a useful tool for patients on vancomycin and at risk for nephrotoxicity.[108,121]

Infusion-related reactions are the most common side effects seen with vancomycin. A rapid onset of an erythematous rash or pruritus affecting the head, face, neck, and upper trunk, with or without associated angioedema and hypotension (anaphylactoid reaction), commonly known as red neck or red man syndrome, has been reported during the infusion of vancomycin with variable frequency, ranging from 3.4% to 14%. Vancomycin may cause histamine release from degranulation of cutaneous mast cells. A retrospective study done in children observed a red man syndrome incidence of 14%; factors associated with it were vancomycin concentration and dose, white ethnicity, age older than 2 years, and antecedent use of antihistamines; a single nucleotide polymorphism in the diamine oxidase gene may be associated with predisposition to this reaction.[122]

Severe hypotension and even cardiac arrest have also been reported during vancomycin infusion. The mechanism for these effects is probably related to histamine release from basophils and mast cells. These reactions usually subside soon after stopping the infusion without other measures. The incidence of these side effects can be reduced by decreasing the infusion rate or the concentration and by using antihistamines (histamine type 1 receptor antagonists). Local reactions, such as phlebitis, have been reported in 3% to 14% of patients receiving vancomycin.

Neutropenia is also observed with vancomycin with a frequency of 1% to 2%, although this increases to 12% to 13% in patients with long-term vancomycin therapy. Neutropenia usually resolves after discontinuation of the drug, and it is recommended to monitor the leukocyte count weekly in patients receiving vancomycin for more than 1 week. A case of agranulocytosis was described on rechallenge in a patient with prior vancomycin-induced neutropenia. Neutropenia can be a glycopeptide class effect because cross-reactivity with teicoplanin has been described.

Thrombocytopenia associated with vancomycin use is very rarely reported, although it may be unrecognized. Vancomycin-dependent, platelet-reactive antibodies have been found in a high proportion of patients with suspected vancomycin-induced thrombocytopenia, suggesting an immune-mediated platelet destruction.[123] Some cases can be associated with significant bleeding and, rarely, a significant drop in the platelet count can be seen within 24 hours after initiation of vancomycin administration.[123]

Presumptive vancomycin-induced maculopapular or erythematous rash and drug-related fever were noted in 3% and 2% of patients receiving vancomycin, respectively.

Vancomycin has been associated with a series of immune-mediated reactions, or hypersensitivity reactions that include maculopapular rash, drug rash eosinophilia and systemic symptoms (DRESS) syndrome, linear immunoglobulin A bullous dermatosis, and Stevens-Johnson syndrome/toxic epidermal necrolysis. The majority are nonimmediate reactions.[124] These immune-mediated reactions associated with vancomycin may show cross-reactivity with teicoplanin.

Despite the clinical efficacy of oral vancomycin for treating *C. difficile*–related diarrhea, cases of *C. difficile* colitis attributed to the use of IV vancomycin have been reported. Even though mild increases of transaminases have been reported in some patients receiving vancomycin, this antibiotic is not considered a drug with associated risk for severe liver injury.[125]

Intraventricular administration of vancomycin is regarded as safe, although an episode of reversible decreased consciousness and two cases of CSF eosinophilia have been reported. Intraperitoneal administration of vancomycin is rarely associated with chemical peritonitis.

Scarce published data exist on the use of vancomycin during pregnancy, and it is classified as pregnancy category C. Vancomycin was noted to show minimal maternal-fetus transplacental passage in an ex vivo human placental perfusion model, but it reaches therapeutic levels in fetal circulation in the setting of overt amnionitis. Infants born to mothers who received a course of vancomycin during the second or the third trimester of pregnancy had no nephrotoxicity or sensorineural hearing loss. However, because no reports exist on vancomycin use during the first trimester, it is unknown if this drug produces fetal harm. This drug should be used only in situations when it is needed, considering the maternal benefit and the possible fetal risk. Vancomycin is excreted in human milk, resulting in potential exposure of infants to this agent, with an expected substantial effect on the intestinal microbiota.

Drug Interactions

Precipitation has been noted when a highly concentrated solution of vancomycin was mixed with ceftazidime, and the use of different syringes for the intraocular administration of these two antibiotics for the treatment of endophthalmitis is recommended. Vancomycin has also been reported to be incompatible in IV solutions with other compounds, including chloramphenicol, methicillin, corticosteroids, aminophylline, barbiturates, thiazides, phenytoin, sodium bicarbonate, and sulfisoxazole. Precipitation with decreased activity of vancomycin was reported when infused together with heparin, although others were unable to detect an effect of heparin on vancomycin stability or activity. Anion-exchange resins, such as cholestyramine, can bind to vancomycin, decreasing the activity of vancomycin in the gut lumen when orally administered. In neonates with patent ductus arteriosus the use of indomethacin and ibuprofen has been associated with a 40% and 28% decreased clearance of vancomycin, respectively.

Clinical Uses

Skin and Soft Tissue Infections

During the past decade or so, vancomycin has been included as the comparator in numerous studies of ABSSSI caused by gram-positive bacteria when new agents with activity against MRSA were evaluated. None of these trials showed significant differences in the primary clinical outcome, although post hoc analysis done in some of these studies displayed some differences favoring the new drug. Thus, and because of its long experience and low cost, when an IV antibiotic is required, vancomycin is still considered the drug of choice for ABSSSI caused by MRSA.[43,126]

Bacteremia and Endocarditis

Although recently debated,[64,127] vancomycin is still the first treatment option for bacteremia, endocarditis, and other serious infections caused by methicillin-resistant staphylococci; the same indication applies for those infections caused by methicillin-susceptible strains in subjects with a history of significant allergic reactions to β-lactams who cannot be desensitized. Failures have been reported with this glycopeptide in the treatment of staphylococcal endocarditis, and its effectiveness has been questioned based on the high rate of unsatisfactory response among injection drug users with *S. aureus* endocarditis, the slow response (median duration of bacteremia, 7 days) in patients with MRSA endocarditis, and the higher failure rate for right-sided MSSA endocarditis when compared with cloxacillin. Because of studies showing worse outcomes in patients with MSSA bacteremia, vancomycin should not be used purely for its dosing convenience or in patients with MSSA endocarditis with a suspicious history of immediate hypersensitivity reaction to β-lactam antibiotics for whom skin testing for penicillin allergy should be performed and patients desensitized, as needed.

The addition of gentamicin to vancomycin was previously suggested for the treatment of MRSA native valve endocarditis.[128] However, owing to the lack of clinical studies showing improved outcome with this combination and the increase in nephrotoxicity with vancomycin plus aminoglycosides (which may be higher than previously appreciated),[129] the risks of adding gentamicin appear to exceed the presumed benefits.[130] The addition of rifampin to vancomycin in the treatment of native valve endocarditis caused by *S. aureus* was successful in a few reports and in an animal model, but not in a randomized clinical trial, done in an era before the documentation of emergence of hVISA. A retrospective study of patients with native valve *S. aureus* endocarditis, some of whom received rifampin, warned about the potential risks associated with the addition of rifampin, such as emergence of rifampin resistance, hepatotoxicity, and drug interactions. Even more, a recent randomized trial showed that adjunctive rifampin to standard therapy did not affect the *S. aureus* bacteremia–related mortality.[131]

Different studies have reported a median of 7 to 9 days of bacteremia in patients with MRSA endocarditis treated with vancomycin. The decision as to when a patient treated with vancomycin for a serious MRSA infection is a clinical failure is a matter of debate. Several factors might be involved in this determination, such as the patient's overall clinical response, the presence of adequate vancomycin levels and of eradicable foci of infection, and the vancomycin MIC of the infecting strain. Although some have promoted a move away from vancomycin use when the infecting organism's vancomycin MIC value is 1.5 μg/mL per Etest measurement, this needs to be carefully interpreted because of the limitations and variability of the available susceptibility methods, such as Etest, automated, and broth microdilution.[32,132] Even with MRSA strains having vancomycin MICs <2 μg/mL, clinical and microbiologic response should be closely monitored; if response is not considered adequate despite removal of foci of infection or foreign-body device, then another treatment might be indicated regardless of the vancomycin MIC of the infecting strain.[43] In any situation an alternative treatment to vancomycin is recommended for MRSA isolates with MIC >2 μg/mL (VISA strains).[43]

The combination of vancomycin with β-lactams has demonstrated enhanced in vitro activity against *S. aureus,* including MRSA, hVISA, and VISA isolates. Retrospective studies appear to show an advantage of this combination over vancomycin monotherapy in patients with MRSA bacteremia,[133,134] and a prospective randomized trial of vancomycin plus flucoxacillin (for the first 7 days) versus vancomycin monotherapy also demonstrated a shorter duration of MRSA bacteremia (mean of 1.94 versus 3 days in the combination and monotherapy group, respectively) with no differences in rates of mortality, metastatic infection, and nephrotoxicity.[135]

Studies from the 1990s have shown that vancomycin was ineffective for most infections caused by MRSA strains with a vancomycin MIC ≥4 μg/mL[136] However, there has been much debate on the clinical impact of infections caused by MRSA (and MSSA) strains with high vancomycin MICs but within the current susceptibility range (MIC ≤2 μg/mL). A meta-analysis published in 2012 found that infections caused by MRSA isolates with an MIC ≥1.5 μg/mL were associated with increased mortality[38]; however, another meta-analysis including 8291 episodes of *S. aureus* bacteremia did not find any relationship with an MIC ≥1.5 μg/mL and mortality.[39] A more recent study also failed to find an association between high MIC (≥1.5 μg/mL) and 30-day mortality among 1027 episodes of invasive *S. aureus* infections.[41] These discrepancies might be in part related to the innate variation among the tests used to determine the vancomycin MIC (i.e., broth microdilution, Etest, automated methods) and to the several other clinical, and even genetic, factors that might also impact the clinical outcome of patients with severe infections caused by MRSA and MSSA. Whether there is a relationship between clinical outcome and vancomycin MIC in patients with MSSA bacteremia/endocarditis treated with β-lactams is also unclear. Several retrospective and prospective studies looking for a possible effect have found an association between high vancomycin MIC (≥1.5 μg/mL) and clinical outcome, although others have not.[36,137] Thus clinical indicators rather than the vancomycin MIC per se should be the most important factor to guide therapeutic decisions in regard to vancomycin use.

For the treatment of enterococcal endocarditis, vancomycin may be considered if the infecting strain is highly ampicillin resistant (typically an *E. faecium* isolate) or the patient is truly allergic to β-lactam antibiotics (preferably confirmed by skin test) and cannot be desensitized. In these instances vancomycin could be combined with gentamicin or streptomycin to achieve bactericidal activity (if the organism does not display high-level resistance to the aminoglycoside).[138]

For native valve endocarditis caused by viridans streptococci, *Granulicatella* spp. and *Abiotrophia defectiva* (both formerly classified within the group of nutritionally variant streptococci), *Gemella* spp., and *S. bovis* (now *S. gallolyticus*), a 4-week course of vancomycin has been recommended for patients unable to tolerate β-lactams.[128,139] In the rare case of oral streptococci highly resistant to penicillin (MIC ≥4 μg/mL), vancomycin is the first therapeutic option.

For prosthetic valve endocarditis due to methicillin-resistant staphylococci, vancomycin in combination with rifampin for 6 weeks, together with gentamicin for the first 2 weeks (if the strain is susceptible), is the recommended regimen. This combination was derived from patients with *S. epidermidis* prosthetic valve endocarditis and has also been recommended for MRSA prosthetic valve endocarditis despite lack of proven clinical benefit,[128] because of the poor prognosis associated with this condition; also for the latter reason, surgical treatment should be undertaken if at all possible.

Vancomycin also plays an important role in the treatment of endocarditis caused by diphtheroids, including *Corynebacterium jeikeium,* which typically occurs in patients with prosthetic valves. The addition of rifampin has been suggested, although with a paucity of clinical evidence. Vancomycin has also been effective in a few reported cases of penicillin- and cephalosporin-resistant *S. pneumoniae* endocarditis and in the experimental model of endocarditis caused by this organism. Empirical treatment of central venous catheter–related infections should include a glycopeptide antibiotic because methicillin-resistant coagulase-negative staphylococci, MRSA, or other gram-positive organisms are frequently found, and clinical trials have shown the effectiveness of vancomycin in this setting. Please refer also to Chapter 80.

Meningitis and Ventriculitis

Vancomycin in combination with cefotaxime or ceftriaxone is the treatment of choice for empirical therapy of patients with suspected or proven pneumococcal meningitis until susceptibility data are available, in areas where infections caused by penicillin-resistant *S. pneumoniae*

have been documented[140] or where the prevalence of isolates not susceptible to ceftriaxone is >3% in adult and 9% in children.[141] If pneumococcal meningitis is confirmed and the isolate shows an MIC of ceftriaxone/cefotaxime ≥1 μg/mL (considered nonsusceptible for meningeal isolates by the CLSI), the administration of the combination of vancomycin and ceftriaxone or cefotaxime should continue.[142] In children rifampin may be added to this regimen if the *S. pneumoniae* isolate shows an MIC of ceftriaxone/cefotaxime of >2 μg/mL.[140] In adults the addition of rifampin, if susceptible, to the regimen appears justified in cases caused by ceftriaxone-nonsusceptible *S. pneumoniae* strains when dexamethasone is also administered[142]; the clinical benefit of this approach has not been evaluated, but rifampin increased the activity of ceftriaxone and vancomycin in an experimental model using a highly ceftriaxone-resistant *S. pneumoniae* strain. Vancomycin as the only antimicrobial agent for which concomitant administration of dexamethasone has been associated with a high failure rate in adults with pneumococcal meningitis. Higher vancomycin doses appear to overcome the negative effect of dexamethasone on the vancomycin CSF concentration.

Vancomycin has a major role in the treatment of infections related to CSF shunts, the most common cause being *S. epidermidis.* IV with or without intraventricular vancomycin administration (see "Administration" for dosing suggestions), together with shunt hardware removal, followed by external drainage and placement of a new shunt after confirmed CSF sterility, appears to be the most appropriate treatment modality. Determination of vancomycin levels in the CSF is recommended to ensure adequate concentrations. The addition of rifampin to the regimen should be contemplated for susceptible organisms when bacterial eradication is not achieved with vancomycin alone. Vancomycin is also recommended for the empirical treatment of postsurgical meningitis.[142]

Owing to the poor prognosis and low therapeutic response to standard doses of vancomycin in patients with postsurgical MRSA meningitis, high-dose vancomycin (15–20 mg/kg every 8–12 hours) targeting a trough concentration of 25 to 30 μg/mL has been recommended.[43] Another option, especially in nonresponding cases, is to administer vancomycin by continuous infusion, also at a high dose (50–60 mg/kg/day after a loading dose of 15 mg/kg)[74]; the toxicity of both options is likely greater. Rifampin could be added to vancomycin despite the lack of clinical data supporting the combination, and intrathecal administration of vancomycin may also be another therapeutic approach.[142] In rare but severe cases of community-acquired meningitis caused by MRSA, the addition of rifampin or trimethoprim-sulfamethoxazole (TMP-SMX) to vancomycin has been used.[143] For more details in the management of meningitis, see Chapters 86, 87, and 92.

Pneumonia

Vancomycin has been considered the drug of choice for MRSA pneumonia, but high failure rates associated with its use have been consistently reported, which may be related to the fact that many of these patients are critically ill and, at least in part, to underdosing this agent, including lack of a loading dose. Indeed, clinical and bacteriologic success rates have been associated with higher AUC/MIC values (≥400) in patients with MRSA pneumonia.[55] In two randomized, double-blind trials of patients with nosocomial pneumonia, no significant differences in the response rate were observed in the vancomycin group compared with the linezolid group.[144,145] However, when a post hoc analysis of a subset of patients with MRSA ventilator-associated pneumonia was done, linezolid displayed significantly better cure rates. In a more recent randomized multicenter clinical trial, linezolid showed significantly better cure rates than vancomycin (15 mg/kg every 12 hours, with adjustment based on trough levels) in patients with nosocomial pneumonia caused by MRSA (57.6% vs. 46.6%, respectively; 95% CI, 0.5% to 21.6%; $P = .042$).[146] The group of patients treated with linezolid also experienced a significantly higher rate of eradication or presumed eradication at the end of the study. However, this trial had various shortcomings, including the fact that fewer than half of the patients had achieved the target trough level (15 μg/mL) on day 3, there was no vancomycin loading dose, more patients receiving vancomycin had concomitant bacteremia and kidney disease, about half of the patients received vancomycin for fewer than 10 days, and there was no difference in the 60-day mortality. Considering these caveats, linezolid is a good choice, if not better, for the treatment of documented MRSA nosocomial pneumonia than is standard vancomycin (without loading) dosing, particularly in patients at high risk for nephrotoxicity. The addition of rifampin to vancomycin appeared to improve the outcomes of ventilator-associated pneumonia caused by MRSA, compared with vancomycin alone, in a small randomized open-label trial[147]; this finding should be confirmed in other prospective studies.

More recently, vancomycin was the comparator arm in two large randomized clinical trials of hospital-acquired pneumonia caused by gram-positive pathogens in which telavancin was noninferior to vancomycin.[148] Another agent, such as TMP-SMX, was found superior to vancomycin, with significant lower clinical failure and 30-day mortality rate in a small, retrospective, case-control, and single-center study of patients with MRSA pneumonia[149]; prospective studies are needed.

Osteomyelitis

Vancomycin is the agent of choice for osteomyelitis caused by methicillin-resistant staphylococci, and it is an alternative for methicillin-susceptible strains in patients with intolerance or with allergic reactions to β-lactams agents.[43] Among serious MRSA infections, osteomyelitis is among those with highest relapse and failure rates.[150] Failure rates between 35% and 46% have been reported in retrospective studies of patients with osteomyelitis treated with vancomycin.[150,151] In accordance, animal models have shown poor results using vancomycin for the treatment of experimental MRSA osteomyelitis, whereas the coadministration of rifampin has been consistently associated with improved response. The duration of treatment of osteomyelitis has not been clearly defined; at least 8 weeks of therapy has been suggested for MRSA osteomyelitis, although the decision about the length of IV versus oral (or oral step-down after an IV course) has to be individualized.[43] Complete surgical débridement is critical for a successful outcome. Vancomycin has been used successfully in conjunction with rifampin as in some studies of prosthetic joint infection.

Pseudomembranous Colitis

Oral vancomycin was used historically for the treatment of pseudomembranous colitis caused by *C. difficile* and pseudomembranous enterocolitis caused by *S. aureus,* now a rare disease. Oral metronidazole and oral vancomycin, administered for 7 to 10 days, were found to have similar failure and relapse rates in the treatment of *C. difficile* colitis and, because of concern about selection of VRE, this drug was recommended for the treatment of *C. difficile* colitis only when there is no clinical response or intolerance to metronidazole or when the infected woman is pregnant.[152] However, since the emergence of an epidemic toxin-hyperproducing strain in North America and Europe (named BI/NAP1/027) associated with more severe disease, higher rates of metronidazole failures have been reported. Studies comparing oral metronidazole with oral vancomycin showed no differences in the outcome for mild disease but, for severe *C. difficile* colitis, the response rate was 76% and 97% for metronidazole and vancomycin, respectively ($P = .02$).[153] Therefore treatment with vancomycin should be considered in patients with severe *C. difficile* disease, defined as those having two or more of the following: age older than 65 years, fever, leukocyte count ≥15,000 cells/mm,[3] serum creatinine concentration increase ≥50% from baseline, and serum albumin concentration <2.5 mg/dL, or the presence of pseudomembranous colitis at endoscopy or hospitalization in an ICU.[153] Until 2011, when fidaxomicin was approved, oral vancomycin was the only FDA-licensed drug for the treatment of *C. difficile* colitis. It can be administered as a capsule formulation or by directly taking the IV form. Because of the poor concentration in stools after IV administration, vancomycin should not be used solely via this route. In cases of severe disease or in the presence of ileus or toxic megacolon, or both, IV metronidazole plus high doses of oral vancomycin (500 mg every 6 hours)[154] and intracolonic administration of this glycopeptide antibiotic[154] have been helpful, although, in some cases colectomy has been needed. Fecal transplantation has been successful in various reports, including for severe and/or relapsing disease. In two randomized trials the new macrocyclic nonabsorbable antibiotic fidaxomicin showed similar efficacy with fewer recurrence rates than oral vancomycin for

the treatment of *C. difficile* colitis.[155] Vancomycin, as well as fidaxomicin, is also an option for secondary recurrences in a tapered or pulsed regimen.[156]

Please also see Chapter 243 for further discussion on treatment options.

Febrile Neutropenia

The use of vancomycin in febrile neutropenic patients has been a controversial issue for a number of years. No differences in morbidity and mortality have been detected with the use of vancomycin as part of the initial regimen, even if a gram-positive organism was initially isolated or in persistently febrile patients despite the use of piperacillin-tazobactam for 48 to 60 hours. The inclusion of vancomycin in the initial empirical treatment of patients with febrile neutropenia is recommended in certain clinical situations, such as presumptive serious catheter-related infection, prior colonization with resistant microorganisms (i.e., penicillin- and cephalosporin-resistant *S. pneumoniae* and viridans streptococci or MRSA), positive blood culture for a gram-positive microorganism, skin or soft tissue infection of any site, radiologically documented pneumonia, or evidence of hemodynamic instability.[157] Others advocate empirical initial use of vancomycin in the presence of severe mucositis and prior prophylaxis with quinolones, which could be associated with severe penicillin-tolerant viridans streptococci infections. However, carbapenems, cefepime, and piperacillin-tazobactam are considered effective monotherapy regimens in this situation. Empirical vancomycin, if used, should be stopped after 2 days if initial workup did not reveal a gram-positive organism resistant to the patient's antimicrobial regimen. See also Chapter 306.

Prophylaxis

Vancomycin is an alternative choice for prophylaxis against endocarditis in subjects with cardiac conditions considered at risk for endocarditis and who are allergic to ampicillin.[158] Vancomycin is also recommended as a prophylactic agent for β-lactam–allergic patients undergoing cardiovascular surgery or orthopedic procedures with hardware placement and for surgical procedures requiring prophylaxis in centers with a high prevalence of MRSA, although not all studies have shown the effectiveness of this approach.[159] In the setting of a cluster of infections by methicillin-resistant staphylococci at the institutional level, a switch from β-lactams to vancomycin, or the addition of vancomycin, as surgical prophylaxis may be recommended.[159]

A retrospective study of patients undergoing primary joint arthroplasty showed the addition of vancomycin to cefazolin did not decrease the surgical site infection rate.[160] And indeed, it was associated with significantly higher incidence of acute kidney injury than the cefazolin alone group.[161]

If vancomycin is chosen for prophylaxis of endocarditis or surgical site infections, the infusion should start within 120 minutes of the beginning of the procedure. Each hospital should develop institutional guidelines on the use of vancomycin for the prevention of surgical site infections.[159] The CDC guidelines have recommended the use of vancomycin for the prevention of perinatal group B streptococcal disease in the case of penicillin-allergic women at high risk for β-lactam anaphylaxis, in whom a streptococci isolate was resistant (or with inducible resistance) to clindamycin or with an unknown susceptibility pattern.[162]

The use of local vancomycin powder in adults undergoing spine surgery appears to be effective to prevent deep and superficial surgical site infections[163]; however, most of these data come from retrospective studies, and the form and dose of each application have not been clearly established. The presumptive benefit of this approach appears to be most appreciated in high-risk patients or in centers with high postsurgical infection rates. In those spine surgeries where vancomycin powder has been applied and surgical site infection still occurs, a predominance of gram-negative bacilli has been reported.[164]

Even though no major side effects have been associated with this route of vancomycin administration, in vitro damage of dural cell damage has been induced by vancomycin in a concentration-dependent manner.[165] More prospective studies are necessary in this field before a more concise recommendation can be made. For further details, see Chapter 313.

Other Uses

Vancomycin is active against the majority of bacteria that cause post-traumatic and postoperative endophthalmitis and is the recommended agent for empirical therapy for intraocular gram-positive organisms in this disease. Animal models of endophthalmitis have also demonstrated the usefulness of vancomycin in this setting. Administration of intraperitoneal vancomycin as an intermittent or continuous dosing schedule is recommended in patients undergoing CAPD with bacterial peritonitis.[92] For uncomplicated peritonitis caused by coagulase-negative staphylococci, the duration of treatment is usually 2 weeks; for enterococci and *S. aureus,* a 3-week course appears necessary. Many of the peritonitis cases caused by *S. aureus* are associated with exit site infection that required peritoneal catheter removal to resolve the infectious process.

In uncomplicated intraluminal bacteremia associated with a tunneled central venous catheter or implantable devices, especially those caused by coagulase-negative staphylococci, the use of antibiotic lock therapy appears to improve the rate of catheter salvage when added to the standard IV treatment. A lock solution containing vancomycin at a final concentration of 5 mg/mL plus normal saline or 50 to 100 units of heparin has been recommended.[166] The lock solution should have an indwelling time of no more than 48 hours, and the duration of the lock therapy is usually 2 weeks. If the isolated pathogen is *S. aureus,* because high failure rates have been described with antibiotic lock therapy, the catheter should be removed. The use of prophylactic antibiotic lock solution has also been recommended for the prevention of long-term central venous catheter infection for patients with recurrent catheter-related bacteremia and, in oncology patients, for those at high risk for severe infections or where the baseline central catheter infection rate is increased.[167] Prevention of central venous catheter infections in patients undergoing hemodialysis with antibiotic lock solutions also appears to be effective, although use of other agents may be more common.

Teicoplanin

Teicoplanin (formerly known as teichomycin A_2) was obtained from *Actinoplanes teichomyceticus* recovered from a soil sample in India in 1978. This glycopeptide antibiotic is currently available in many countries in Europe, Asia, and South America but not in the United States. The FDA has not approved it, probably because of no clear benefit over vancomycin (Fig. 30.2). Teicoplanin is actually a mixture of related glycopeptide analogues with a basic structure characterized by a linear heptapeptide, the distinct carbohydrates D-mannose and D-glycosamine, and an acyl residue that carries various fatty acids, which define the members of the teicoplanin complex. Teicoplanin has a molecular weight estimated as 1900 Da.

Antimicrobial Activity and Resistance

Teicoplanin inhibits cell wall synthesis by a mechanism similar to that described for vancomycin, although some differences in activity exist. For instance, MICs of teicoplanin against coagulase-negative staphylococci tend to be more variable. In one survey, 21% and 1% of coagulase-negative staphylococci and *S. aureus* strains, respectively, from bacteremic patients were nonsusceptible to teicoplanin based on the EUCAST breakpoint (MIC >4 μg/mL).[168] A more recent study done in France found that 33% of the coagulase-negative staphylococci isolates were nonsusceptible to teicoplanin (MIC >4 μg/mL).[169] High teicoplanin MICs appear to be more frequent among *S. haemolyticus* than other staphylococcal species.[170] On the other hand, the MICs of teicoplanin for *Enterococcus* spp., *S. pneumoniae, S. gallolyticus* (formerly *S. bovis*), viridans-group streptococci, and other streptococci are usually lower than those of vancomycin. The in vitro activity of teicoplanin seems to be similar to that reported with vancomycin against *L. monocytogenes, Corynebacterium* spp. (including *C. jeikeium*), and gram-positive anaerobes, such as *Clostridium* spp. (including *C. difficile*), *Peptostreptococcus* spp., *Actinomyces* spp., and *Propionibacterium* spp. No international consensus has been established for teicoplanin susceptibility breakpoints for *S. aureus,* but they have been set as ≤2 μg/mL and ≤8 μg/mL by the EUCAST and CLSI, respectively (www.eucast.org/clinical_breakpoints). The same breakpoints have been provided by both groups for *Enterococcus* spp. For coagulase-negative staphylococci, these values have been set as ≤4 μg/mL by the EUCAST and ≤8 μg/mL by the CLSI.

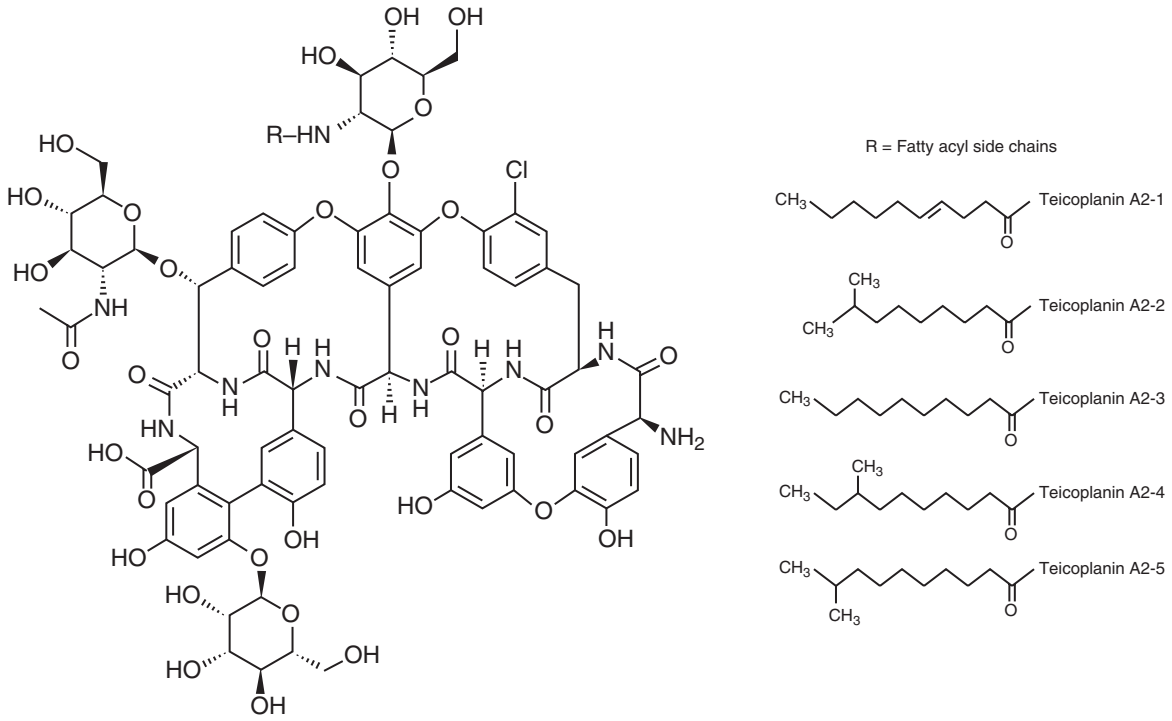

FIG. 30.2 **Chemical structure of teicoplanin.**

Strains of *S. aureus* susceptible to vancomycin but displaying higher MICs of teicoplanin were reported before the description of VISA isolates, and high MICs of teicoplanin were subsequently found in VISA isolates as well[10] (also called GISA). Almost all VISA strains are "cross-resistant" to teicoplanin. However, some teicoplanin-heteroresistant *S. aureus* strains may test susceptible to vancomycin, and specific cell wall changes may affect teicoplanin more so than vancomycin. Greater MIC increases for teicoplanin compared with vancomycin were found by inactivation of the *tcaA* gene in GISA strains, which encodes a transmembrane protein presumably associated with cell wall metabolism. As expected, decreased activity of teicoplanin and selection of subpopulations with higher glycopeptide MICs were observed in animal models infected with VISA strains. Because disk tests and some automated systems do not reliably recognize MRSA strains with decreased susceptibility to teicoplanin, Etest and agar dilution methods are recommended when needed. As described with vancomycin, MICs determined by Etest tend to be higher than those determined by broth microdilution. Most of the VRSA isolates carrying the enterococcal *vanA* gene cluster described in the United States also showed decreased susceptibility to teicoplanin (≥16 μg/mL).

In general, strains that produce peptidoglycan precursors ending in D-Ala-D-Lac, encoded by the *vanABDM* gene clusters, are likely to be resistant to teicoplanin (VanA, VanB, VanD, and VanM). However, VanB-type strains are usually susceptible in vitro because teicoplanin does not induce expression of the gene cluster. However, mutations that result in constitutive expression of the *vanB* operon can occur in vivo and lead to resistance. Thus, even if MICs are within the susceptible range, teicoplanin should probably not be used to treat infections caused by VRE strains due to the risk for development of resistance while on therapy. Other enterococci that synthesize peptidoglycan precursors ending in D-Ala-D-Ser (VanC, VanE, VanG, and VanN) show low teicoplanin MICs.

Clinical Pharmacokinetics

Teicoplanin has favorable pharmacokinetic properties that permit administration by IV bolus or intramuscular route. As with vancomycin, this agent is not significantly absorbed when administered orally. After an IV dose of 6 mg/kg, the mean peak (at 2 hours) and trough (at 24 hours) concentrations of teicoplanin in serum are 111.8 μg/mL and 4 μg/mL, respectively. At steady state, teicoplanin mean trough concentrations are 14 μg/mL after IV administration of 6 mg/kg/day and 23 μg/mL after 12 mg/kg/day. It appears that teicoplanin trough concentrations ≥10 μg/mL are required for clinical success in the majority of infections by susceptible organisms, although for serious staphylococcal infections (i.e., endocarditis), trough levels >20 μg/mL are recommended.[171]

The distribution of teicoplanin is best described by a three-compartment kinetic model, and its volume of distribution at steady state ranges from 800 to 1600 mL/kg.[172] Teicoplanin is approximately 90% bound to serum proteins (albumin) and highly bound in tissues, which may explain its low clearance and long half-life, which has ranged from 83 to 168 hours.[172] Animal studies have reported better bone concentrations with teicoplanin than with vancomycin after equivalent IV infusion. However, in patients with osteomyelitis, vancomycin achieved slightly higher levels in cortical and cancellous bone than teicoplanin.[79] The concentration of teicoplanin in cortical and cancellous bone, which has more abundant vascular supply, was 12% (mean, 2 μg/mL) and 49% (mean, 7.5 μg/mL) of concomitant plasma concentration at steady state (with a daily dose of 10 mg/kg), respectively.[79] Penetration into the heart; pericardium; mediastinal tissue; and synovial, pleural, peritoneal, and pericardial fluid is also considered adequate. The concentration of teicoplanin in the epithelial lining fluid at steady state was about 30% (mean concentration, 4.9 μg/mL) of the corresponding trough serum level in 13 patients with ventilator-associated pneumonia treated with 12 mg/kg/day.[173]

In experimental endocarditis teicoplanin appears to be concentrated only at the periphery of the vegetation. After IV infusion, significant concentrations of teicoplanin are generally not achieved in vitreous samples nor in the CSF, even in the presence of meningitis. A high concentration of teicoplanin in feces is achieved after oral administration of 100 mg.

Teicoplanin is almost entirely eliminated by renal mechanisms and, even though this agent used to be regarded as a nondialyzable drug, hemodialysis using high-flux membranes, CVVHD, CVVH, and CVVHDF remove significant quantities. Teicoplanin levels seem not to be significantly modified in subjects undergoing cardiopulmonary bypass surgery. Teicoplanin should not be used during pregnancy (pregnancy category B) or lactation unless the potential benefits outweigh the possible risks.

The oral dose of teicoplanin for the treatment of *C. difficile* pseudomembranous colitis ranges from 100 to 400 mg twice daily for 10 days. The parenteral teicoplanin dose depends on the patient's age and renal function, the suspected or known microorganism, and site of infection. Because of its long half-life, a loading dose of teicoplanin is required to achieve earlier optimal steady-state serum levels. For most infections the regimen recommended is 400 mg (6 mg/kg) every 12 hours for three doses and then once daily, which attains a trough level >10 μg/mL by the fourth day of treatment. The loading dose should be given to all patients regardless of the patient's creatinine clearance. Then, in adults with normal renal function, 400 mg (6 mg/kg) every 24 hours is the usual maintenance dose. A higher dose, 800 mg (up to 12 mg/kg) every 12 hours for three doses and then every 24 hours to target a trough concentration of more than 20 μg/mL, is recommended for more difficult infections, such as septic arthritis, osteomyelitis, and endocarditis, although some have recommended a trough >30 μg/mL for the latter[174] (see Table 30.1). However, teicoplanin trough levels of 28 μg/mL or greater have been associated with hepatotoxicity.[175]

Even though the recommended high trough levels of teicoplanin appear associated with better clinical outcome, reaching the target concentration is challenging. A recent retrospective study found that only 32% of patients with MRSA infections treated with teicoplanin achieved a desired trough ≥15 μg/mL on day 3 or 4 of therapy. Emphasizing the effect of the initial dosing, the trough target was readily achieved in those receiving a higher loading dose (1600 mg given as 800 mg on day 1 and on day 2) and in those with a creatinine clearance <56 mL/min receiving a lower loading dose (1200 mg, 800 mg/day on day 1).[176] Even more, an enhanced teicoplanin dosing regimen was evaluated in a prospective study of patients with a creatinine clearance <60 mL/min (10 mg/kg twice on the first day, followed by 10 mg/kg/day and 6.7 mg/kg/day on the second and third day, if the creatinine clearance was between 40 and 60 mL/min and <40 mL/min, respectively). The median minimum concentration (C_{min}) on day 4 and the proportion of patients with the target C_{min} between 15 and 30 μg/mL in the enhanced loading group versus those in the conventional regimen group were 18.1 μg/mL and 12.1 μg/mL, and 20.4% and 7.1% ($P < .001$), respectively.[177] In the same study, among 106 patients with MRSA infection, a significantly higher clinical success rate was observed in those achieving a C_{min} >15 μg/mL.

The recommended doses for neonates is 16 mg/kg initially, followed by 8 mg/kg daily and, for children older than 2 months, 10 mg/kg every 12 hours for three doses and then every 24 hours.[172] In the presence of renal insufficiency, teicoplanin dose adjustment is required only after the end of the loading doses. In subjects with creatinine clearance between 30 and 80 mL/min., the daily dose should be halved or given every 48 hours and, in those with more severe renal failure or on hemodialysis with conventional membranes, the daily dose should be one-third or be administered every 72 hours.[172,178] Others have suggested the administration of 10 mg/kg every 48 to 72 hours in patients on hemodialysis, which was consistently associated with trough levels >10 μg/mL.[179]

Teicoplanin is significantly removed by CVVH and CVVHDF but with high variability, depending on the operating conditions of the renal replacement therapy used and the patient's serum albumin concentration. A reasonable proposed dosing regimen to generate a trough teicoplanin level of 10 to 20 μg/mL is 6 mg/kg every 12 hours for three or four doses, followed by 3 to 6 mg/kg/day[180]; others have suggested higher daily doses (600–1800 mg/day), targeting 15 to 25 μg/mL in patients undergoing CVVH.[181] However, because teicoplanin serum levels in this setting can be affected by the serum albumin concentration, the presence of residual renal function, and the volume ultrafiltration rate, therapeutic drug monitoring is recommended in critically ill patients undergoing CRRT.[180]

Peritoneal administration of teicoplanin results in serum concentrations similar to those achieved by IV concentration; however, after IV administration, penetration of teicoplanin into the peritoneal dialysate does not achieve local therapeutic levels. Teicoplanin has been administered in doses of 20 mg/L in each bag for the first week, in alternate bags during the second week, and only in the overnight dwell bag in the third week. Another approach is to dose 20 mg/L in each exchange (four times daily) for 10 days or for 5 days after clearing of bacteria from the dialysate. Others have successfully used intermittent intraperitoneal dosage of teicoplanin (15 mg/kg every 7 days) in children with CAPD peritonitis.

Monitoring of teicoplanin serum levels is not generally needed with doses <12 mg/kg/day. IV drug abusers with endocarditis have a higher clearance rate of teicoplanin, thus suggesting a need for serum level measurements in this population. It has been suggested that a trough level should be drawn to ensure teicoplanin concentrations in serum of at least 20 μg/mL and even >30 μg/mL in patients with bone and joint infections and endocarditis, respectively,[171,178] especially if teicoplanin is administered as monotherapy. Other clinical scenarios in which measurement of teicoplanin serum levels might be appropriate include patients not responding to treatment, patients with severe burns, and patients with rapidly changing renal function or on CRRT.

As with vancomycin, some discrepancies exist on the clinical impact of teicoplanin MIC in patients with MRSA bacteremia because a teicoplanin MIC >1.5 μg/mL by Etest was associated with worse outcome in one retrospective study but not in others, including 101 and 270 patients treated with teicoplanin (6 mg/kg/day after loading dose), respectively.[182,183] Of interest, the in vitro combination of teicoplanin with various cephalosporins could decrease the teicoplanin MIC to ≤2 μg/mL of several hVISA and VISA isolates.[184]

Adverse Events

Teicoplanin is generally regarded as a safe drug. Rates of adverse events and nephrotoxicity have been reported more frequently in individuals receiving vancomycin than those receiving teicoplanin. Teicoplanin is nephrotoxic in animals, although at much higher doses than those used in humans. A lower rate of nephrotoxicity was reported with teicoplanin combined with aminoglycosides or with amphotericin B, compared with vancomycin combined with these agents.[185] The most common side effects associated with teicoplanin are maculopapular or erythematous rash and drug-related fever in about 7% and 6% of the patients, respectively.[186] These events are more frequent in patients receiving doses >12 mg/kg/day. Cases of allergic cross-reactions between vancomycin and teicoplanin have been reported, but vancomycin-allergic patients also have been successfully treated with teicoplanin. For example, in one study, cross-reaction in patients with vancomycin-induced fever or rash or both was seen in about 10%, whereas 50% of patients with vancomycin-related neutropenia developed neutropenia while on teicoplanin.[187] Compared with vancomycin, a meta-analysis showed that teicoplanin was associated with a lower rate of total adverse events, nephrotoxicity, and red man syndrome than vancomycin.[188]

The anaphylactoid reaction that has been described with vancomycin IV administration (known as red man or red neck syndrome) is extremely uncommon with the infusion of teicoplanin. Ototoxicity related to teicoplanin is also rare. Thrombocytopenia can occur at a rate similar to that found with vancomycin use and also appears to be more frequent at higher doses. Other hematologic effects, such as neutropenia and eosinophilia, are infrequently reported.

Clinical Uses

A failure rate of >50% in severe staphylococcal infections was found in initial studies using a low dose of teicoplanin (3 mg/kg/day). Even at higher doses (6 mg/kg/day and 10 mg/kg/day), teicoplanin was associated with a significantly lower response rates compared with vancomycin in patients with endocarditis or intravascular infection caused by *S. aureus*. Teicoplanin trough levels <20 μg/mL have been correlated with treatment failure.[171] Because a high failure rate was observed in patients with MSSA bacteremia treated with teicoplanin at 400 mg/day, if this agent is used for the treatment of *S. aureus* endocarditis or other serious staphylococcal infection, a high dose (probably 12 mg/kg/day) should be considered. For less serious infections, teicoplanin at standard doses appears as efficacious as vancomycin, as shown by a meta-analysis including 24 randomized clinical trials in which no difference in terms of 30-day mortality was found.[188] Also, for patients with hospital-acquired MRSA bacteremia (mostly catheter-related infections), teicoplanin showed similar outcomes to vancomycin in an observational prospective study.[189]

Teicoplanin might be an alternative in the treatment of native valve endocarditis caused by viridans streptococci and enterococci, in doses

of 600 mg/day (or 6 mg/kg/day). Of interest, against *E. faecalis*, several patients were cured with teicoplanin monotherapy. In an experimental model of enterococcal endocarditis, teicoplanin was shown to be as effective as ampicillin and more effective than vancomycin. In this model the addition of an aminoglycoside to teicoplanin resulted in enhanced efficacy.

Teicoplanin has been shown to be effective in the treatment of susceptible organisms causing skin and soft tissue infections, lower respiratory tract infections, and catheter-related infections. Teicoplanin was equivalent to vancomycin in a study of neutropenic patients with persistent fever. Initial studies have shown similar response and relapse rates in patients with *C. difficile* infection who received oral teicoplanin, metronidazole, or vancomycin; however, a recent prospective, nonrandomized, observational study found a significantly lower rate of clinical failure and recurrence among 107 patients treated with oral teicoplanin versus 180 treated with oral vancomycin (100 mg and 200 mg every 12 hours and 125 mg and 500 mg every 6 hours, for noncomplicated and complicated infection, respectively).[190]

Intraperitoneal administration of teicoplanin has been successfully used for the treatment of CAPD-related peritonitis. Teicoplanin does not penetrate into the CSF; a small case series of CSF shunt-related infections treated with intraventricular teicoplanin have been reported, using doses of 10 to 20 mg every 24 to 48 hours. Teicoplanin (400 mg IV dose at the time of anesthesia induction) is as effective as first-generation cephalosporins in the prevention of hip or knee implant-related infections, although it was not as effective as standard of care in patients undergoing cardiac and prosthetic vascular surgeries. In a retrospective study of 1896 patients undergoing hip or knee arthroplasty, the addition of one dose of teicoplanin (800 mg) to cefuroxime was able to significantly decrease the rate of surgical site infections at this center.[191] Teicoplanin, like vancomycin, is recommended as an option for prophylaxis of infective endocarditis in patients allergic to penicillin.

Overall, it appears that in countries where both antibiotics are available, teicoplanin is infrequently used in place of vancomycin, at least in the acute phase of the infection under treatment. The main reasons for this approach are that teicoplanin appears to have overall lower response rates when compared with vancomycin in patients with severe MRSA infections. As such, high doses (12 mg/kg/day) are recommended for this type of infection, leading to possible toxicities and that teicoplanin is expensive.

In some situations teicoplanin could be considered for mild-to-moderate enterococcal infections or, as outpatient therapy, to continue the treatment of certain MRSA infections (e.g., osteoarticular infections) after vancomycin, once the patient has improved. In other instances teicoplanin could be chosen as a prophylactic antibiotic for selected surgical procedures, such as cardiovascular surgery in centers with a high prevalence of MRSA. Teicoplainin may also be useful as outpatient therapy for some streptococcal infections (e.g., viridans) that require prolonged therapy when there is documented β-lactam allergy.

LIPOGLYCOPEPTIDES

Lipoglycopeptides are semisynthetic derivatives of naturally occurring glycopeptides produced by changes of glycopeptide molecules. These three new agents—ritavancin, dalbavancin, and telavancin—contain lipophilic side chains, which appears to increase the ability to kill bacteria by also binding to the cell membrane, producing important disruptions in the physicochemical properties of this cell component, leading to cell death. Furthermore, the lipophilic chain confers a longer half-life and converts these agents (with the exception of dalbavancin) into concentration-dependent bactericidal antibiotics.[192] Lipoglycopeptides display similar spectrum of activity to that of glycopeptides, but their additional chemical modifications seem to increase their potency. Of importance, the hydrophobicity of these compounds has made it difficult to standardize susceptibility testing because the drugs diffuse poorly in agar media and tend to bind to plastic surfaces. These properties led to important modifications in the method for MIC determination with the addition of the nonionic surfactant polysorbate-80 at a final concentration of 0.002%.[193] Indeed, using this methodology, isolates with MICs higher than the breakpoint for susceptibility for each lipoglycopeptide have been extremely rare, although both in vitro and in vivo selection of resistant strains has been observed.[194,195]

Telavancin

Structure and Mechanism of Action

Telavancin (Vibativ; Theravance Biopharma, South San Francisco, CA) was the first of the lipoglycopeptides to be available on the market and is approved in the United States and Canada for the treatment of adults with ABSSSI due to gram-positive pathogens. In addition, telavancin has an indication in the United States and Europe for hospital-acquired pneumonia, including ventilator-associated pneumonia caused or believed to be caused by MRSA when other options are not suitable (Fig. 30.3).

Telavancin, a derivative of vancomycin, is produced by alkylation of the vancosamine nitrogen with a hydrophobic (decyl-aminoethyl) side chain and a hydrophilic (phosphonomethyl aminomethyl) group linked on the 4′ position of amino acid 7 of the cyclic peptidic core.[192] The mechanism of action of telavancin is similar to that of glycopeptides and involves binding to peptidoglycan precursors (D-alanine-D-alanine

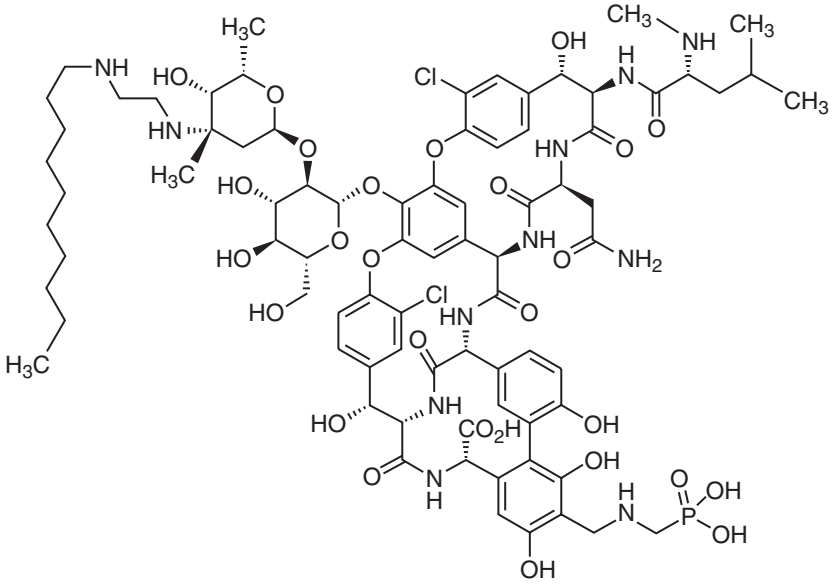

FIG. 30.3 **Chemical structure of telavancin.**

termini) at the outside of the bacterial cell, as they emerge from the cytoplasm. Of interest, the resulting inhibition of transglycosylase activity achieved by telavancin is about 10-fold greater on a molar basis than that observed with vancomycin.[196] However, a second mechanism of action is likely to play an important role in the bactericidal activity of the drug.[196a–196c] This mechanism is appears to be related to the rapid and concentration-dependent disruption of bacterial cell membrane homeostasis (e.g., *S. aureus*).[196] The alterations in membrane physiology have been associated with leakage of important ions such as potassium, alteration of adenosine triphosphate turnover, increased cell permeability, and eventually, cell death. Even though the interaction between telavancin and lipid II seems crucial for membrane depolarization, the nature of such interaction with this peptidoglycan precursor is unknown.[197]

Antimicrobial Activity and Resistance

As mentioned earlier, potent, concentration-dependent bactericidal activity has been described with telavancin,[196b] which also occurs in stationary phase of growth. The increased in vitro activity of telavancin observed since the inclusion of polysorbate-80 in its dilution MIC testing led to changes in the susceptibility breakpoints. Current breakpoints are as follows: *S. aureus*, *Streptococcus pyogenes*, and *Streptococcus agalactiae* are all ≤0.12 μg/mL; *Streptococcus anginosus* group, ≤0.06 μg/mL; and *Enterococcus faecalis* (vancomycin-susceptible strains), ≤0.25 μg/mL. EUCAST only has breakpoints available for *S. aureus* (MIC ≤0.125 μg/mL) and does not provide breakpoints for streptococci or enterococci due to insufficient data available (www.eucast.org/clinical_breakpoints).

The MIC_{90} of telavancin for *S. aureus* and coagulase-negative staphylococci isolates (independent of methicillin susceptibility) is 0.06 μg/mL.[198] Telavancin is very potent against *S. pneumoniae* with all isolates inhibited at concentrations ≤0.03 μg/mL and displays comparable activity to that of penicillin against β-hemolytic streptococci, including *S. pyogenes* and *S. agalactiae*.[198] Overall, telavancin MICs are onefold to twofold higher for hVISA and VISA isolates, and those with higher telavancin MICs also display higher vancomycin MICs. Against VRSA strains, telavancin exhibits decreased bactericidal activity, which is likely due to the change in composition of peptidoglycan precursors in these strains (D-alanine-D-lactate instead of D-alanine-D-alanine).[198a] Telavancin displays good in vitro bactericidal activity against MSSA, MRSA, and VISA strains with MBCs within twofold of the MIC.[196b] Telavancin has also been reported to retain bactericidal activity against daptomycin-nonsusceptible *S. aureus* isolates, including those that also exhibit decreased susceptibility to vancomycin.[198b]

In a recent study with more than 10,000 strains from a worldwide collection of gram-positive cocci, telavancin inhibited all *S. aureus* at a concentration ≤0.125 μg/mL.[199] For *S. aureus* isolates with a vancomycin MIC of 2 μg/mL, the MICs of telavancin were 0.06 μg/mL (lower than the susceptibility breakpoint), but these MICs were higher than those observed for isolates with vancomycin MIC <2 μg/mL (0.03 μg/mL).[199] In the same study the MIC_{90} of telavancin for coagulase-negative staphylococci was 0.06 μg/mL; for *E. faecalis,* 0.12 μg/mL; for vancomycin-susceptible *E. faecium* (VSE), 0.03 μg/mL; for *S. pneumoniae,* ≤0.015 μg/mL; and for viridans-group streptococci and β-hemolytic streptococci, 0.03 μg/mL.

A variety of gram-positive anaerobes are susceptible to telavancin concentrations <2 μg/mL,[200] including *Actinomyces* spp., *C. difficile* (and other *Clostridium* spp.), *Eubacterium* group, *Lactobacillus* spp., *Propionibacterium* spp., *Peptostreptococcus* spp., and *Corynebacterium* spp. *L. monocytogenes* and *B. anthracis* are also highly susceptible to telavancin. Organisms that produce peptidoglycan precursors ending in D-Ala-D-Lac, including vancomycin-resistant enterococci, *Pediococcus, Leuconostoc,* and *Lactobacillus* are considered nonsusceptible, with telavancin MICs ≥2 μg/mL.[200] Selection of telavancin resistance in vitro among gram-positive isolates has been difficult to achieve, and clinical resistance has not been fully characterized. A recent patient appeared to have developed in vivo resistance to telavancin while on treatment with this compound for mediastinitis and persistent bacteremia caused by an MRSA/hVISA strain in the setting of a left ventricular assist device infection. Of note, the telavancin MIC increased to 1.5 μg/mL with concomitant increases in vancomycin and daptomycin MICs.[194]

Clinical Pharmacodynamics and Pharmacokinetics

Telavancin is supplied as a 250-mg or 750-mg single vial of a lyophilized powder that should be reconstituted with 15 mL and 45 mL, respectively, of 5% dextrose, sterile water, or 0.9% sodium chloride, leading to a final concentration of 15 mg/mL. The approved IV dose of telavancin is 10 mg/kg daily, which should be administered as a 1-hour infusion (see Table 30.1).[200a] This dosing schedule has resulted in peak plasma concentrations of 93.6 ± 14.2 μg/mL and an AUC of 666 ± 107 μg•hr/mL. The elimination half-life of telavancin ranges from 6.1 to 9.1 hours, and serum levels of telavancin are linear and predictable with minimal accumulation in patients with normal renal function. The percentage of drug bound to protein is relatively high (90%),[192,200b] and the tissue distribution of telavancin is similar to that of vancomycin.[192] The concentration of telavancin in blister fluid was 40% that of serum.[200c] In healthy adults telavancin concentrations in the epithelial lining fluid displayed a mean peak of 3.7 μg/mL and a trough of 1 μg/mL, 8 hours and 24 hours after infusion, respectively.[200d] The concentration in alveolar macrophages was 45 μg/mL and 42 μg/mL at 12-hour and 24-hour time points, respectively. Using population pharmacokinetic modeling, it has been reported that telavancin concentrations achieved in lung tissue are higher than the MICs for MRSA isolates for the majority of the time interval between two doses.[200e]

Dose adjustment of telavancin is required in the presence of renal dysfunction because clearance of the drug occurs via the kidneys. Thus the telavancin dose should be reduced to 7.5 mg/kg/day and 10 mg/kg every 48 hours in patients with creatinine clearances 30 to 50 mL/min and 10 to 30 mL/min, respectively.[200f] These recommendations were validated using a population pharmacokinetic model derived from 749 subjects enrolled in clinical trials.[200g] Insufficient information has been collected to make dosing recommendations in patients with creatinine clearance <10 mL/min, including those receiving hemodialysis. The European Medicines Agency (EMA) has advised against its use in patients with acute renal failure and with severe renal impairment.[201] Telavancin is coformulated with hydroxypropyl-β-cyclodextrin to improve its solubility. The latter compound might accumulate in patients with renal dysfunction. Of note, no drug adjustment is required for subjects with mild and moderate (Child-Pugh class B) hepatic impairment, and no significant drug interactions are expected to occur with telavancin through hepatic metabolism.

Efficacy of Telavancin in Animal Models

The efficacy of telavancin has been evaluated in various animal models. In the neutropenic murine thigh infection and subcutaneous infection models, telavancin showed significant concentration-dependent decreases in the bacterial titers of *S. aureus* and compared favorably with vancomycin and linezolid (MRSA) and nafcillin (MSSA).[200b] In a rabbit model of *S. aureus* endocarditis, telavancin yielded a significantly greater reduction in CFUs per gram of vegetation than vancomycin against two MRSA strains.[201a] In the same model, telavancin decreased bacterial counts in vegetations to a greater degree than vancomycin against two VISA isolates, although the difference was not statistically significant.[201b] In an experimental animal model of MRSA pneumonia, telavancin and vancomycin showed similar efficacies against MRSA strains with vancomycin MICs <2 μg/mL, but telavancin produced significantly greater reduction than vancomycin in bacterial counts in lung tissue in three out of four VISA isolates tested.[201c] In the rabbit model of meningitis caused by a penicillin-resistant *S. pneumoniae* strain (telavancin MIC, 0.06 μg/mL), telavancin exhibited a statistically significant reduction of CFUs in CSF compared with ceftriaxone plus vancomycin despite penetrating only 2% into CSF with inflamed meninges.

Adverse Reactions

Initial studies of telavancin infusions in healthy volunteers showed minor increases in the QTc interval; however, in advanced-phase clinical trials no significant differences in QTc interval were found compared with vancomycin.[148] Nonetheless, telavancin should be used with caution in patients taking medications that are known to cause QTc prolongation and avoided in patients with known long QTc interval, uncompensated heart failure, and severe left ventricular hypertrophy because these

patients were excluded from the clinical trials. A pooled analysis of all side effects in the phase II and phase III ABSSSI and hospital-acquired pneumonia trials showed that nausea, vomiting, taste disturbance, chills, and creatinine elevation were significantly more frequent in the telavancin group than in the comparator arm of the trials.[201d] The most common adverse events that led to discontinuation of telavancin were nausea, vomiting, and renal dysfunction.[148,202] Nephrotoxicity was reported to be greater in patients with concomitant medications that might affect kidney function (e.g., diuretics, nonsteroidal antiinflammatory drugs, angiotensin-converting enzyme inhibitors) and in those older than 65 years. The rate of significant creatinine increase (>50% increase from baseline and a maximum value >1.5 mg/dL) was significantly higher in patients receiving telavancin than those treated with vancomycin, both in the ABSSSI trials (6% vs. 2%, respectively)[202] and in the hospital-acquired pneumonia trials (16% vs. 10%, respectively).[148] In a retrospective analysis of the use of telavancin postmarketing, creatinine increase was found in 33% of the patients after a median of 9 days of treatment, although these patients had different comorbidities, were receiving other potentially nephrotoxic agents, and telavancin was prescribed for nonapproved indications.[202a] The renal toxicity, the most worrisome telavancin side effect, appears to be reversible, but cautious monitoring is required. Of interest, a recent updated meta-analysis of the published clinical data available from the telavancin trials showed no significant difference in clinical and microbiologic outcomes compared with other therapies. However, patients receiving telavancin had significantly higher rates of serum creatinine increases and hypokalemia.[203] Moreover, in the trials of hospital-associated pneumonia, lower survival rates (all-cause mortality at day 28) were observed among patients treated with telavancin than those receiving vancomycin when the patient had baseline moderate-to-severe renal impairment (creatinine clearance ≤50 mL/min) (59% and 70%, respectively; difference 11%; 95% CI, −19.9% to −1.3%).[204]

Telavancin has been associated with laboratory interference in quantification of urine protein (when tested by reagent strip and dye methods), prothrombin time (PT), activated partial thromboplastin time (aPTT), activated clotting time, and factor Xa coagulation tests. Because these effects appear to be minimal at drug trough, it is recommended to draw blood for these tests before the next dose of telavancin. As a derivative of vancomycin, telavancin has been associated with infusion-related reactions, such as red man syndrome–like reaction, flushing, pruritus, and rash. Slowing the infusion rate tends to mitigate these reactions.

There are no available data on the use of telavancin in pregnant women, lactating mothers, or pediatric patients. Telavancin is considered pregnancy drug class C and should be avoided in pregnant women unless the potential benefits outweigh the fetal risks. Increased rates of limb and digit malformations were seen in three animal species exposed to telavancin. As many other antibiotics, diarrhea caused by *C. difficile* occurred in patients receiving telavancin.

Clinical Uses

Skin and Soft Tissue Infections

Telavancin is currently approved for the treatment of ABSSSI caused by gram-positive pathogens. Two phase II studies and a large phase III study with telavancin in patients with ABSSSI have been published.[202,204a,204b] The first phase II study used a daily dose of 7.5 mg/kg, but thereafter the dose was 10 mg/kg/day. MRSA was the most common isolated pathogen. The cure rates for telavancin and vancomycin were 88.3% and 87.1% and 90.6% and 86.4% in the clinically evaluable patient group and those infected with MRSA, respectively.[202] A meta-analysis of the ABSSSI clinical trials found that, if the infected organism was MRSA, the microbiologic eradication rate was more favorable in those receiving telavancin than those treated with vancomycin.[201d] However, among patients with renal insufficiency (creatinine clearance <50 mL/min), decreased efficacy was seen in the telavancin group.

Hospital-Acquired Pneumonia

Telavancin was noninferior to vancomycin (1 g every 12 hours) in two large randomized trials of hospital-acquired pneumonia caused by gram-positive pathogens.[148] Ninety percent of the patients in the microbiologic evaluable population had an *S. aureus* strain isolated from respiratory samples, of which >55% were MRSA. The observed cure rates for telavancin- and vancomycin-treated patients were 82.4% and 80.7%, respectively. Telavancin performed better than vancomycin in those with monomicrobial *S. aureus* pneumonia and in those infected with MRSA, although the difference only reached statistical significance for monomicrobial hospital-acquired pneumonia caused by *S. aureus* isolates, with a vancomycin MIC ≥1 μg/mL.[205]

Other Clinical Uses

Telavancin was tested in a phase II randomized, double-blind trial of uncomplicated *S. aureus* bacteremia versus vancomycin or anti–staphylococcal penicillins, showing no differences in efficacy at 84 days of follow-up.[206]

Postmarketing use of telavancin has included 14 patients with refractory MRSA bacteremia (most of whom had endocarditis), 8 of whom were successfully treated.[207] Other reports of success include a case of pacemaker lead–related infective endocarditis due to a VISA/non–daptomycin-susceptible strain,[208] a few cases of MRSA osteomyelitis,[209] and prosthetic joint infections.[210]

Dalbavancin

The in vitro activity of dalbavancin was initially assessed in the late 1990s. Clinical development was pursued thereafter based on its antibacterial activity and favorable pharmacokinetic behavior. In 2014 in the United States and in 2015 in the European Union, dalbavancin was approved for treatment of adults with ABSSSI, initially as a two-weekly IV regimen of 1000 mg, then 500 mg 1 week later and more recently as a single 1500-mg dose regimen.

Structure and Mechanism of Action

Dalbavancin (Dalvance/Xydalba; Allergan; Dublin, Ireland) is a semisynthetic lipoglycopeptide derived from the semisynthetic derivative of the teicoplanin-like glycopeptide A40926, with a half-life of about 8.5 days and that allows once-weekly dosing. It is synthesized from a fermentation product of *Nonomuraea* spp., and comprises five related active components.

Dalbavancin, as other lipoglycopeptides, forms a stable complex with the C-terminal D-alanyl-D-alanine of the pentapeptide in the nascent cell wall peptidoglycan; dalbavancin's lipophilic side chain enhances its affinity to the target site. However, dalbavancin appears to adopt a closed conformation upon ligand binding, an interaction with cell wall precursors called noncooperative, which does not contribute to its own activity.[211,212] This differs from vancomycin and the other lipoglycopeptides, telavancin and oritavancin.

Antimicrobial Activity and Resistance

Dalbavancin shows in vitro activity against almost all significant gram-positive bacteria except those intrinsically resistant to glycopeptides, such as some *Lactobacillus* spp. and those having the VanA phenotype of vancomycin-resistance. The current FDA breakpoints for susceptibility of dalbavancin are ≤0.25 μg/mL for *S. aureus* (including MRSA), *S. pyogenes*, *S. agalactiae*, *S. dysgalactiae*, viridans group streptococci (*S. anginosus* group only), and *E. faecalis* (vancomycin-susceptible isolates only). The breakpoint for these microorganisms determined by the EUCAST is one dilution lower (≤0.125 μg/mL).

Against susceptible species, dalbavancin displays significantly greater potency than vancomycin. Despite this, the in vitro activity of dalbavancin has been characterized as slowly bactericidal against *S. aureus* and *S. pyogenes*,[213] similar to the effect of vancomycin. Against hVISA isolates, dalbavancin displayed bacteriostatic activity.[214] For VRE, dalbavancin lacks useful activity against VanA-type strains and appears to be active against VanB type (presumably due to lack of induction of resistance, similar to teicoplanin). However, mutants that express VanB resistance constitutively are readily selected during drug exposure (as seen with teicoplanin). Dalbavancin is also active in vitro against the VanC-type enterococci (*E. gallinarum* and *E. casseliflavus*). The dalbavancin MIC_{90} for VRE type A is >4 μg/mL and for VanB and VanC is between ≤0.06 μg/mL and 2 μg/mL.[215]

Against more than 60,000 *S. aureus* isolates collected from the United States and Europe in a surveillance program over a decade (2002–12), dalbavancin demonstrated activity with MIC_{90} of 0.06 g/mL against strains

including those nonsusceptible to daptomycin, linezolid, and tigecycline.[216] Analyzing strains from pediatric patients with skin and skin structure infections in the United States (2014–15), dalbavancin also displayed similar in vitro activity against *S. aureus* (MRSA and MSSA strains) (MIC_{90}, 0.06 μg/mL), coagulase negative staphylococci (MIC_{90}, 0.06 μg/mL), and β-hemolytic streptococci (MIC_{90}, 0.03 μg/mL).[217]

Dalbavancin shows good in vitro activity against many anaerobic gram-positive bacteria, including most *Clostridia* spp. (except *Clostridium clostridioforme*), *Propionibacterium* spp., *Peptostreptococcus* spp., *Eubacterium* spp., and *Actinomyces* spp.; some species of *Lactobacillus* displayed MICs >32 μg/mL.[218] Dalbavancin is also active against other gram-positive organisms, such as *Bacillus* spp., *Corynebacterium* spp., and *Micrococcus* spp. Selection of dalbavancin nonsusceptible variants from fully susceptible isolates in vitro has not been successful; however, a recent case report described the emergence of a dalbavancin-nonsusceptible VISA strain isolated from a patient who had received dalbavancin and vancomycin treatment for a catheter-related MRSA bacteremia.[195]

Clinical Pharmacodynamics and Pharmacokinetics

The current approved doses of dalbavancin in patients with normal renal function (creatinine clearance ≥30 mL/min) or on regular hemodialysis include a single administration of 1500 mg or 1000 mg, followed by 500 mg 1 week later, infused in 30 minutes for adults with ABSSSI. When IV dalbavancin was given as 1000 mg on day 1, followed by 500 mg weekly for 7 weeks in healthy volunteers, there was no accumulation, and mean serum concentrations >30 μg/mL from day 8 to 50 were found. The mean peak serum concentration (C_{max}) after a dose of 1000 and 1500 mg is 287 μg/mL and 423 μg/mL, respectively; the terminal half-life of dalbavancin is 8 to 9 days, with a high volume of distribution (between 9 and 15 L), suggesting extravascular distribution. The plasma protein binding of dalbavancin is approximately 93%, mainly to albumin. A mean of 45% is excreted in urine (33% unchanged and 12% as the metabolite hydroxyl-dalbavancin) and 20% in feces.

The penetration of dalbavancin in skin blister fluid is ≈60%, with a mean concentration ≈30 μg/mL by day 7, following a dose of 1000 mg.[219] The cortical bone, synovial tissue, skin, and plasma concentrations at 2 weeks after a single dose of 1000 mg of dalbavancin were 4.1 μg/g, 15.9 μg/g, 13.8 μg/g, and 15.3 μg/mL, respectively.[220] Based on a population pharmacokinetic modeling, two 1500-mg IV infusions given 1 week apart have been proposed for 6 to 8 weeks as a potential treatment of *S. aureus* osteomyelitis because this would provide free dalbavancin exposure higher than the *S. aureus* MIC_{90} for the treatment period.[220]

Even though dalbavancin is not approved for use in children, a model based on plasma concentrations from 43 pediatric patients (3 months–11 years of age), to simulate one-dose and two-dose adult regimens, respectively, yielded the following eventual dosing recommendations: from 6 to <18 years of age: 18 mg/kg (1500 mg maximum), and 12 mg/kg (up to 1000 mg) on day 1 and 6 mg/kg (500 mg maximum) on day 8; and from 3 months to <6 years of age: 22.5 mg/kg (up to 1500 mg), and 15 mg/kg (1000 mg maximum) and 7.5 mg/kg (500 mg maximum) on day 8.[221] The pharmacokinetic parameters of dalbavancin was similar to those described in adults among 10 healthy adolescents (between 12 and 17 years of age) receiving 1000 mg (or 15 mg/kg if the participant weighed <60 kg) infused in 30 minutes.[222]

The pharmacodynamic parameter that best describes the activity of dalbavancin is the 24-hour AUC/MIC. The efficacy of this compound was dose dependent in animal models against *S. aureus* and pneumococci; for the latter the drug exposure for similar efficacy was much lower than for *S. aureus*. The estimated target 24-hour AUC/MIC for *S. aureus* was between 100 and 300.[223]

Dalbavancin clearance is affected in patients with severe renal impairment.[224] Therefore doses should be adjusted in patients not on hemodialysis with a creatinine clearance <30 mL/min; in this case, the dose of dalbavancin should be 1125 mg as a single dose or 750 mg on day 1, followed by 375 mg on day 8 in the two-dose regimen. Patients on hemodialysis can receive the regular dose without considering the timing of dialysis. No need for dose adjustment is required in patients with hepatic insufficiency, even though drug exposure has been 27% to 36% lower in those with severe liver impairment.[224]

Dalbavancin is supplied in 500-mg powder vials that should be reconstituted with 25 mL sterile water or 5% dextrose, to be further diluted in 5% dextrose to a final dalbavancin concentration of 1 to 5 mg/mL. The time from reconstitution to administration should be <48 hours and can be stored either at 2°C to 8°C or at 20°C to 25°C. The IV infusion time is 30 minutes (more rapid infusions can cause red man syndrome–type of reactions), and it should not be coadministered with other medications or saline-containing infusions; lines should be cleared with 5% dextrose solution before and after use.[225]

Adverse Reactions

In phase III clinical trials that compared the two-dose regimen of dalbavancin (1000 mg on day 1 and 500 mg on day 8) with vancomycin, followed by oral linezolid, the most common adverse events in the dalbavancin arm were nausea (2.5%), diarrhea (0.8%), and pruritus (0.6%); these latter two were significantly less frequent than in the comparator arm. Reactions associated with infusion were seen in 1.4% of the patients in the dalbavancin group, although the majority of them occurred during the placebo infusions required in the trial.[226]

Hypersensitivity and skin reactions have been described, and cross-reactions might occur in patients with prior history of glycopeptide allergy. *C. difficile*–associated diarrhea has been described in patients receiving dalbavancin. Increases in alanine transaminase (ALT) levels have been reported in 0.8% of dalbavancin-treated patients compared with 0.2% of those in the comparator arm, although most of these patients had hepatic underlying conditions; these increased ALT levels were reversible. Dalbavancin in single doses of 1000 mg or 1500 mg showed no significant effect on heart rate, PR, QRS, and QTc intervals in a thorough study including 200 volunteers.[227]

Dalbavancin is classified as pregnancy category C because delayed fetal maturation was observed in rats receiving high doses of this compound, and there are no data about pregnant women. The excretion of dalbavancin in human milk has not been addressed, and the risk of drug-drug interactions appears minimal because it does not interfere with CYP450 isoenzymes or P-glycoprotein.

Clinical Uses

Dalbavancin showed similar outcomes as the comparator arm in phase II trials[228] of adults with ABSSSI. These studies were followed by phase III double-blind randomized trials that confirmed the noninferiority of dalbavancin administered as 1000 mg infused on day 1, followed by 500 mg vancomycin on day 8, with the option of oral linezolid on day 3. These studies led to the approval by the FDA and EMA of dalbavancin for ABSSSI caused by various susceptible organisms. More recently a 1500-mg single-dose of dalbavancin infused in 30 minutes showed similar outcome to the two-dose regimen in adults with ABSSSI, with a similar rate of adverse events,[229] leading to the FDA and EMA approval of this new dosing. A two-dose regimen of dalbavancin is currently under study in a phase III open-label and randomized trial (identifier NCT02814916) of pediatric patients (3 months–17 years of age) with ABSSSI compared with standard of care. Dalbavancin, administered as two 1500-mg weekly doses, showed good clinical outcomes and no serious related adverse events in a recently published, open-label, phase II clinical trial, of patients with chronic osteomyelitis caused by gram-positives.[229a] Of concern, a recent failure of dalbavancin has been reported in a pregnant patient with MRSA right-sided endocarditis treated with this drug, having relapsed with a VISA and lipoglycopeptide nonsusceptible isolate.[230] More clinical data on the potential use of dalbavancin in staphylococci bacteremia and/or endocarditis and osteomyelitis would be particularly interesting, considering its intrinsic antibacterial activity and its favorable pharmacokinetic profile that allows weekly administration.

Oritavancin

Oritavancin was selected for clinical development in 1994 based on its in vitro activity and its advantageous dosing profile; however, its development was slow due to ownership transference among pharmaceutical companies and the presence of an in vitro phenomenon that overestimated the MICs. Oritavancin finally was granted approval for use in adults with ABSSSI caused by susceptible gram-positive organisms in the United States and Europe in 2014 and 2015, respectively.

Structure and Mechanism of Action

Oritavancin (Orbactiv) is a semisynthetic derivative of the lipoglycopeptide chloroeremomycin structurally similar to vancomycin, with the addition of an aminated sugar (4-epi-vancosamine) and a hydrophobic side chain, responsible for the amphipathic property of the drug and the membrane anchoring characteristic, also documented for telavancin. Oritavancin has three known mechanisms of action, including inhibiting transglycosylation, inhibiting transpeptidation, and disrupting the cell membrane.[231] First, like vancomycin, oritavancin binds to the D-alanyl-D-alanine peptidoglycan termini of lipid II, inhibiting the glycan chain extension (transglycosylation). Second, probably through its lipophilic side chain, oritavancin also interacts at another level of the pentapeptide terminus of lipid II (the pentaglycyl bridge and the D-iso-glutamine residue in position 2),[232] allowing a significant inhibition of the transpeptidase activity; this might explain its activity against vancomycin-resistant organisms. And third, oritavancin was shown to produce membrane depolarization and permeabilization, which appears independent of cellular growth and division.

Antimicrobial Activity and Resistance

Oritavancin has in vitro activity against staphylococci (including MRSA), streptococci, and enterococci (including VRE).[233,234] As with other lipoglycopeptides, the CLSI recommends performing oritavancin MIC testing using 0.002% polysorbate-80, to inhibit the adherence of the compound to test tube walls. The oritavancin CLSI and EUCAST breakpoints for susceptibility against staphylococci, streptococci, and VSE (only CLSI) are ≤0.12 μg/mL, ≤0.25 μg/mL, and ≤0.12 μg/mL, respectively.

Oritavancin has low MIC_{90} values against vancomycin-susceptible and VanB-mediated vancomycin-resistant strains, whereas the MICs for VanA strains tend to be slightly higher. For example, oritavancin MIC_{90} against enterococcal isolates from Europe and the United States, collected between 2011 and 2013, were 0.06 μg/mL and 0.12 μg/mL for vancomycin-susceptible *E. faecalis* and VanA *E. faecium*, respectively.[235] In addition, oritavancin exhibited in vitro bactericidal activity for both VRE and VSE in time-kill experiments; however, an increased concentration was required for bactericidal activity against VRE.[236,237]

Oritavancin also showed good in vitro activity against 1008 *S. aureus* clinical isolates from patients with invasive infections in the United States between 2013 and 2014, with an MIC_{90} of 0.06 μg/mL.[238] Oritavancin also displays in vitro activity against *C. difficile*, even more potent than that of vancomycin against most of the isolates.

In vitro studies including time-kill curves have shown a synergistic activity of oritavancin when combined with cefazolin and with nafcillin against MRSA strains, possibly secondary to the seesaw effect in which the glycopeptide (and lipopeptide) MICs are inversely proportional to the β-lactam MICs. The combination of oritavancin with ceftaroline was also synergistic against MRSA and was the most effective combination against the MRSA, daptomycin-nonsusceptible MRSA, and hVISA isolates tested. In the same study the synergistic effect of the combination of oritavancin with ampicillin, ertapenem, and ceftaroline against vancomycin-resistant *E. faecalis* and *E. faecium* isolates was limited and strain dependent.[239]

Clinical Pharmacokinetics and Pharmacodynamics

Oritavancin is administered as a single dose of 1200 mg over 3 hours, after which an average peak of 138 mg/L in serum is obtained.[240] Oritavancin is widely distributed, fitting a dose-linear, three-compartment model with a high volume of distribution of almost 100 L and a slow release from tissue absorption sites. This increased volume of distribution, together with high protein binding (85%–90%), may explain, in part, the prolonged terminal half-life of about 240 hours, supporting the one-time dosing strategy for ABSSSI.[240] Oritavancin accumulates intracellularly in the liver, kidneys, spleen, lymphoid tissue, and lungs, from where it is subsequently released; only trace amounts of the administered dose are found in urine and feces. An adequate penetration of oritavancin into skin blister fluid of approximately 19% of the plasma concentration has been reported.[241]

Oritavancin displays a rapid bactericidal and concentration-dependent activity against a wide variety of gram-positive organisms. In the neutropenic murine thigh infection model using *S. aureus* isolates, the activity is best predicted by the C_{max}:MIC ratio and the AUC/MIC ratio.[242] It has also been observed that a greater bactericidal effect is seen when a single full dose of oritavancin is given than when it is administered in fractioned doses. Oritavancin also exhibits concentration-dependent bactericidal activity against stationary phase and biofilm culture of *S. aureus* in vitro.[243]

Oritavancin is supplied in 400-mg vials that should be reconstituted and further diluted in dextrose 5% (1000 mL) to a concentration of 1.2 mg/mL to be infused over 3 hours. This compound is not compatible with normal saline and should only be diluted in dextrose 5%.

Adverse Reactions and Drug Interactions

Among the ABSSSI phase III trials,[244,245] the most common side effects were headache, nausea, vomiting, and diarrhea; rates of discontinuation were not different from the comparator arm. Transient and mild ALT elevations were also observed. Infusions reactions, such as flushing and pruritus, may occur but less frequently than with vancomycin and also resolve after slowing the infusion rate. Hypersensitivity reactions were unusual (<1.5%), but some evidence suggests they may develop more frequently in those patients with prior reactions to glycopeptides. In clinical trials the median duration of the hypersensitivity reactions was 2.4 days. Oritavancin is classified as pregnancy category C, and its use in nursing mothers has not been studied.

Oritavancin, unlike other lipoglycopeptides, has a potential for drug-drug interaction through the inhibition of several cytochrome P450 enzymes. In this regard, a recent study showed no effect of oritavancin on S-warfarin C_{max} or AUC, although patients receiving these drugs concomitantly should still be observed for possible bleeding.[246] In addition, as with telavancin, oritavancin can affect laboratory phospholipid-dependent coagulation tests, such as PT/international normalized ratio (INR) and aPTT, whereas anti-FXa assay and thrombin time remain unaffected. The duration of the interference with these tests depends on the commercial reagents used. The maximum time to resolution of these test abnormalities has been 12 hours for PT/INR and 120 hours for aPTT; hence the use of IV unfractionated heparin sodium is contraindicated for 5 days after the administration of oritavancin. Surprisingly, about 30% of the healthy subjects receiving oritavancin experienced increased levels of D dimer that returned to normal in up to 72 hours.[247] In patients with mild to moderate liver or renal impairment, adjustment of oritavancin dosage is not required, although its use in those with severe hepatic or renal insufficiency has not been evaluated.

Clinical Uses

Oritavancin showed evidence of efficacy in a phase II trial of patients with complicated skin and soft tissue infections[248] and in another study of patients with *S. aureus* bacteremia administered in four different daily doses.[249] In two identical phase III randomized trials of adult patients with ABSSSI, oritavancin, as a single-dose of 1200 mg, was noninferior to 7 to 10 days of vancomycin.[244,245] These trials enrolled 1987 patients, and the primary end point was cessation (or reduction) of spread of baseline skin lesion, absence of fever, and no need for other antibiotics after 48 to 72 hours of therapy initiation. In both trials MRSA was found in ≈20% of all patients and in ≈0% of those with a positive culture. With these results the FDA approved this agent in 2014 for the treatment of adult patients with ABSSSI caused by susceptible isolates of the following gram-positive microorganisms: *S. aureus* (including MRSA), *S. pyogenes, S. agalactiae, S. dysgalactiae, S. anginosus* group, and *E. faecalis* (vancomycin-susceptible isolates only). Oritavancin is not currently approved in the pediatric population, although it is being evaluated in an ABSSSI clinical trial of children age 3 months to 18 years (clinicaltrials.gov identifier NCT02134301). Based on its significant in vitro activity and its ease of administration, studies of oritavancin for infections such as bacteremia, endocarditis, and osteomyelitis caused by susceptible gram-positive organisms, including VRE, MRSA, VISA, hVISA, and VRSA, seem warranted.

CONCLUSIONS

Among the drugs reviewed in this chapter, vancomycin is still the most important option for severe skin infections requiring hospitalization and the usual choice for parenteral therapy for osteomyelitis, bacteremia, and endocarditis caused by MRSA. This view reflects the long experience with and confidence in its use, along with its relatively low cost; however, the recommendation of targeting higher serum levels in severe disease has reduced the therapeutic window. Telavancin has been approved for the treatment of ABSSI and hospital-acquired pneumonia caused by gram-positive pathogens when other agents are unsuitable; however, its current use is probably jeopardized in view of the lower efficacy observed in patients with moderate-to-severe renal failure and its higher rate of creatinine increase than the comparator in the ABSSI and hospital-acquired pneumonia trials. Due to its favorable pharmacokinetic properties, dalbavancin and oritavancin appear as attractive agents for the treatment of ABSSI caused by gram-positive pathogens, allowing the administration of only a single dose. This strategy might be particularly useful to avoid hospitalization of a significant proportion of patients with ABSSI; however, the cost of these compounds might be a limitation for more extensive use. Further efficacy studies on drug-resistant gram-positive infections are expected.

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The complete reference list is available online at Expert Consult.

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31 Daptomycin and Quinupristin-Dalfopristin

Jose M. Munita, Barbara E. Murray, and Cesar A. Arias

SHORT VIEW SUMMARY

DAPTOMYCIN

- Daptomycin (Cubicin) is a high-molecular-weight cyclic lipopeptide antibiotic that targets the cell membrane of gram-positive bacteria in a calcium-dependent manner.
- The antimicrobial activity closely overlaps that of glycopeptides. Daptomycin retains activity against most organisms with decreased susceptibility to glycopeptides, although high minimal inhibitory concentration (MICs) of daptomycin have been observed in some vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates.
- Currently, daptomycin is approved by the US Food and Drug Administration (FDA) for treatment of acute bacterial skin and skin structure infection (ABSSSI) caused by gram-positive cocci and for *S. aureus* bacteremia, including right-sided endocarditis.
- Daptomycin is administered intravenously, once daily; the approved doses are 4 and 6 mg/kg/day for ABSSSI and for *S. aureus* bacteremia and right-sided endocarditis, respectively. However, some experts recommend higher doses (8–12 mg/kg/day) for serious infections, particularly those caused by vancomycin-resistant enterococci (VRE). Dosage adjustment is required if the creatinine clearance is less than 30 mL/min.
- Reversible muscle toxicity is the main adverse event. Less frequent adverse events are paresthesias, peripheral neuropathies, and eosinophilic pneumonia.
- Because of the high risk for development of resistance and the "seesaw effect" (increased susceptibility to β-lactams when organisms become nonsusceptible to daptomycin), combinations of daptomycin and β-lactams are potential options for recalcitrant infections.

QUINUPRISTIN-DALFOPRISTIN

- Quinupristin-dalfopristin (Synercid) contains a streptogramin B (quinupristin) and a streptogramin A (dalfopristin) component in a 30:70 ratio. Streptogramins act synergistically within the 50S ribosomal subunit of the 70S unit in the elongation stage of protein synthesis.
- This combination is active against most gram-positive organisms (except *Enterococcus faecalis*) and a few gram-negative organisms.
- Variable rates of resistance among *Enterococcus faecium* isolates have been reported. *Staphylococcus* spp. strains with high MICs of quinupristin-dalfopristin have been rare.
- The intravenous dose is 7.5 mg/kg every 12 hours for ABSSSI caused by *S. aureus* and *Streptococcus pyogenes*. Dosage adjustment is not required in renal failure, but a lower dose may be considered with hepatic disease. Quinupristin-dalfopristin may increase the levels of drug metabolized through the cytochrome P-450 3A4 isoenzyme system.
- Irritation at the venous site is common when the drug is administered through peripheral veins. Arthralgias and myalgias may lead to drug discontinuation.
- The FDA approval of quinupristin-dalfopristin for VRE was withdrawn after the initial trials failed to prove clinical benefit; however, anecdotal cases of success, usually in combination with other agent(s), for serious VRE infections suggest that it still may have a role in selected patients.
- Because of its potential adverse events, the need for a central venous catheter for administration, and issues with efficacy and resistance, it is not often used in clinical practice.

DAPTOMYCIN

Daptomycin is a high-molecular-weight (1620.67 Da) cyclic 13-member lipopeptide antibiotic produced by *Streptomyces roseosporus* that was discovered in the early 1980s (Fig. 31.1). In 1991, despite clinical trials that showed some efficacy, skeletal muscle toxicity was observed with twice-daily doses in phase II trials, which led to discontinuation of clinical studies. The drug was subsequently "resurrected" in the form of once-daily dosing, which reduced the muscle toxicity. Daptomycin was approved for use in 2003 in the United States and in 2006 in Europe.

Mechanism of Action

The exact mechanism for the antimicrobial activity of daptomycin is not fully understood. This agent targets the cell membrane of gram-positive organisms in a calcium-dependent manner, becoming a de facto cationic antimicrobial peptide.[1] Insights into the mechanism of action of daptomycin suggest that the antibiotic binds to the cell membrane preferentially at the level of the division septum.[2] The interaction of the antibiotic with the bacterial cell membrane produces important distortions in the architecture of the membrane that likely result in displacement of inner membrane proteins essential for cell wall synthesis and cell division.[3] Moreover, an important step in the mechanism of daptomycin-mediated disruption of the cell membrane is the oligomerization of antibiotic molecules within the cell membrane, a step that seems to depend on the presence of the phospholipid phosphatidylglycerol, a negatively charged phospholipid.[4] As a final result, daptomycin produces irreversible alteration of the cell envelope structure and physiology, leading to loss of ions such as potassium that eventually results in cell death by mechanisms that have yet to be fully elucidated.[1] Interesting to note, staphylococci and enterococci that develop nonsusceptibility to daptomycin are able to withstand the antibiotic-mediated alteration of the cell membrane physiology, supporting the fact that the membrane is the primary target. In addition, daptomycin exerts its bactericidal effect against *Staphylococcus aureus* without significant cell lysis, which may explain the decreased release of proinflammatory mediators from infected macrophages compared with oxacillin and vancomycin.

Antimicrobial Activity

The spectrum of antimicrobial activity of daptomycin closely overlaps that of glycopeptides. Because the in vitro activity of this drug is dependent on the presence of calcium in the medium, testing for susceptibility to daptomycin needs to be performed with dilution methods using calcium-adjusted Mueller-Hinton broth medium (calcium concentration of 50 μg/mL); the use of the Kirby-Bauer disk diffusion method is inaccurate and is not recommended. Calcium-supplemented gradient diffusion strips (Etest [bioMérieux, Marcy l'Etoile, France]) for use on agar are reliable, although discrepancies with broth macrodilution occur and, most important, poor interlaboratory reproducibility

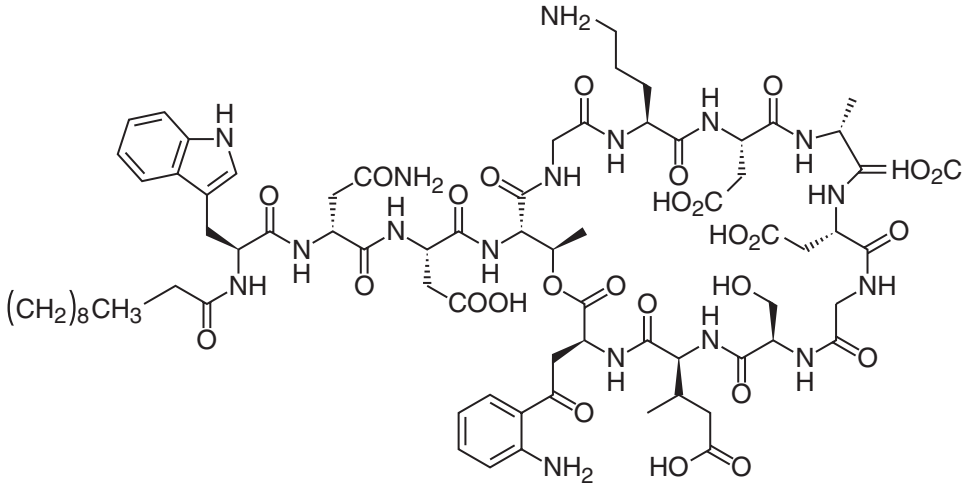

FIG. 31.1 **Chemical structure of daptomycin.**

is common. Automated and semiautomated systems for susceptibility have been developed and are also available.[5]

The US Food and Drug Administration (FDA)–, Clinical and Laboratory Standards Institute (CLSI)–, and European Committee on Antimicrobial Susceptibility Testing (EUCAST)–approved breakpoints for daptomycin with use of calcium-supplemented Mueller-Hinton broth are less than or equal to 1 μg/mL (susceptible) for staphylococci and streptococci. In the case of *Enterococcus* spp., data are less clear and, therefore, wider interagency variation exists. The FDA established a susceptibility breakpoint only for *Enterococcus faecalis* (≤4 μg/mL). CLSI has recently changed the breakpoints for enterococci. Indeed, daptomycin-susceptible enterococci are now considered to have an MIC ≤1 μg/mL. A susceptible dose-dependent category (doses of 8–12 mg/kg of daptomycin) was introduced for isolates with MICs of 2 to 4 μg/mL. Finally, an MIC ≥8 μg/mL is now considered fully resistant. It is important to note that the breakpoints were established using broth microdilution as the preferred testing method and not Etest. EUCAST has not established breakpoints for daptomycin.

Important to note, daptomycin shows rapid, concentration-dependent bactericidal activity in vitro against staphylococci, pneumococci, *E. faecalis,* and *Enterococcus faecium,*[6] including methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), and vancomycin-resistant enterococci (VRE) isolates.[6,7] Daptomycin also shows good in vitro activity against gram-positive anaerobes such as *Peptostreptococcus* spp. (MIC_{90}, 1 μg/mL), *Clostridium perfringens* (MIC_{90}, 0.5 μg/mL), and *Clostridioides difficile* (formerly *Clostridium difficile*) (MIC_{90}, 1 μg/mL); some other clostridial species require higher concentrations for inhibition. It should be noted, however, that there are no established breakpoints for daptomycin susceptibility for anaerobes. The activity of daptomycin against different species of *Actinomyces* is variable (MIC_{90}s, 4–32 μg/mL), and some *Lactobacillus* spp. appear to be less susceptible to daptomycin, whereas most *Propionibacterium* spp. and the vancomycin-resistant species *Leuconostoc* and *Pediococcus* are inhibited by higher concentrations of 2 μg/mL.

A correlation between increased vancomycin MICs and daptomycin nonsusceptibility has been observed in *S. aureus,* with a high proportion of VISA strains (80% of isolates with vancomycin MIC of 4 μg/mL) reported as nonsusceptible to daptomycin (MIC >1 μg/mL)[8]; this observation has not been confirmed in other studies.[9] Unlike many other antibacterial agents, daptomycin at high concentrations maintains its bactericidal activity against *S. aureus* even in stationary phase cultures,[10] a phenomenon that could be related to its effect on the cell membrane. Daptomycin appears to maintain its activity against *Staphylococcus epidermidis* and *S. aureus* strains embedded in biofilm, perhaps explaining its reported efficacy against these structures and as antibiotic lock therapy in an animal model of staphylococcal central venous catheter infection.

Resistance

Development of resistance to daptomycin in vitro is uncommon, although strains with decreased susceptibility were obtained after serial passage.[11] Only 0.7% and 0.04% of 10,000 clinical *S. aureus* strains in one large survey had an MIC ≥1 μg/mL and ≥2 μg/mL, respectively.[9] These results were later confirmed in a more recent study evaluating more than 36,000 isolates from Europe and the United States.[12] Nevertheless, increased daptomycin MICs of 2 μg/mL or greater were observed in 6% of patients participating in a large *S. aureus* bacteremia and an endocarditis clinical trial.[13] The majority of these strains were associated with microbiologic failure and, in most cases, patients had an undrained focus of infection.[13] A much higher rate of development of nonsusceptibility to daptomycin was described among 10 patients with persistent *S. aureus* bacteremia who were switched from vancomycin to an approved dose of daptomycin (4–6 mg/kg/day).[14] *S. aureus* strains with higher daptomycin MICs displayed several phenotypic changes at the level of the cell membrane, including enhanced membrane fluidity, increased net positive surface charge, resistance to depolarization and/or permeabilization, a reduced amount of phosphatidylglycerol, increased pigment production, and decreased daptomycin surface binding. All of the aforementioned features correlated with reduction of daptomycin-induced depolarization.[15]

Mutations in several genes have been implicated in the development of daptomycin nonsusceptibility in *S. aureus.* Among the most studied are mutations in (1) *mprF,* a gene that encodes a lysyl-phosphatidylglycerol (LPG) synthase with flippase activity that contributes to increased cell membrane positive charge by adding more LPG (a positively charged phospholipid) to the outer leaflet of the cell membrane[16]; (2) genes encoding two-component regulatory systems, specifically *yycFG (walKR)* and *vraSR,* which appear to participate in the regulation of cell envelope homeostasis and stress response[17]; (3) the *dlt* cluster, which encodes the enzymatic machinery necessary for the D-alanylation of cell wall teichoic acids, contributing to increased cell surface charge[18]; (4) *rpoB* and *rpoC,* encoding the subunits of RNA polymerase; and (5) *pgsA* and *cls,* which encode enzymes involved in cell membrane phospholipid metabolism (phosphatidylglycerol synthase and cardiolipin synthase, respectively), that modify cell membrane composition preventing daptomycin binding.[19] In addition, development of daptomycin nonsusceptibility has also been associated with cross-resistance to endogenous antimicrobial peptides, including thrombin-induced platelet microbicidal proteins and human neutrophil-derived defensin 1.[20] Finally, evidence suggests that some *S. aureus* strains have the ability to inactivate daptomycin by releasing membrane phospholipids (particularly phosphatidylglycerol) that bind and inactivate daptomycin; however, the clinical relevance of this phenomenon remains to be elucidated.[21]

Although the majority of enterococci remain apparently "susceptible" to daptomycin in vitro, the emergence of daptomycin nonsusceptibility seems to be more frequent in these organisms than in staphylococci. Indeed, several case reports have documented development of resistance to daptomycin (MICs ranging from 6 to >32 μg/mL) during treatment of enterococcal infections caused by *E. faecalis* and *E. faecium*. Moreover, the presence of daptomycin resistance in the absence of exposure to the antibiotic has also been described.[22] Insights into the mechanism of daptomycin resistance have been provided.[23–25] Although some phenotypic changes in the cell envelope and cell membrane appear to be similar to those described in staphylococci, the genetic pathways seem to be different. The initial crucial event in the development of daptomycin resistance in enterococci involves changes in the LiaFSR system (a homologue of VraTSR of *S. aureus*), a three-component regulatory system that is predicted to orchestrate the cell envelope response to antibiotics and antimicrobial peptides. Indeed, deletion of *liaR,* the putative transcriptional regulator of the system, was able to completely restore daptomycin susceptibility in *E. faecium* and *E. faecalis*, independent of the genetic background.[26,27] Moreover, a mutation in the first gene of the system, *liaF,* encoding a putative transmembrane protein, was sufficient to decrease daptomycin susceptibility (although not above the clinical breakpoint) and abolish the in vitro bactericidal activity of the antibiotic.[25] An increase in daptomycin MICs above the breakpoint is usually associated with additional mutations in genes encoding enzymes involved in cell membrane phospholipid metabolism (e.g., *gdpD* and *cls*) that affect cell membrane homeostasis.[25] Of note, a marked decrease in cell membrane phosphatidylglycerol content, important for daptomycin oligomerization within the cell membrane (see earlier discussion), has been documented in daptomycin-resistant *E. faecalis* and *E. faecium* recovered from patients during therapy with daptomycin[28] and in other daptomycin-resistant gram positive organisms.[29]

The events leading to increased daptomycin nonsusceptibility in both staphylococci and enterococci can result in increased susceptibility to certain β-lactams (the "seesaw effect").[30] Indeed, daptomycin plus oxacillin significantly decreased colony-forming units per gram of vegetation when compared with daptomycin alone in experimental endocarditis caused by *S. aureus* with daptomycin-nonsusceptible strains (MICs of 2 and 4 μg/mL).[30] The combination of daptomycin with other β-lactams (nafcillin, imipenem, amoxicillin-clavulanate, cefotaxime, ceftriaxone, ertapenem, and ceftaroline) has been associated with increased in vitro and in vivo activity against daptomycin-nonsusceptible MRSA strains. Although the mechanisms underlying this synergy are not fully understood, it has been postulated that β-lactams decrease the net cell membrane positive charge in daptomycin-nonsusceptible strains, favoring daptomycin binding to the cell membrane.[31] In addition, recent evidence in *S. aureus* suggests that daptomycin nonsusceptibility is associated with mislocalization of penicillin-binding proteins (PBPs) and changes in proteins involved in the maturation of important PBPs (e.g., PBP2A), providing a mechanistic link for the hypersusceptibility to β-lactams observed in these strains.[32] Moreover, in vitro and in vivo studies have shown that the addition of β-lactams to daptomycin prevents or delays the emergence of daptomycin nonsusceptibility against *E. faecalis, E. faecium, Streptococcus mitis,* and *Streptococcus oralis* isolates.[33,34]

Some streptococci (e.g. *S. mitis*) are capable of developing rapid and sustainable daptomycin resistance on exposure to the drug, both in vivo and in vitro.[35,36] The mechanism has been attributed to loss of function mutations in *cds* encoding a phosphatidate cytidylyltransferase, an enzyme that is required for the synthesis of phosphatidylglycerol and cardiolipin in cell membranes.[37] Similarly, there are several reports of daptomycin resistance arising in vivo and in vitro in *Corynebacterium* spp., most of them related to patients developing endovascular or device-related infections due to *Corynebacterium striatum*.[38,39,40]

Clinical Pharmacokinetics and Pharmacodynamics

Distribution and Elimination

The pharmacokinetics of once-daily intravenous daptomycin are linear up to doses of 12 mg/kg with minor accumulation.[41] The mean daptomycin peak serum concentrations in healthy volunteers are approximately 55, 86, 116, 130, and 165 μg/mL after a single intravenous dose (infused over 30 minutes) of 4, 6, 8, 10, and 12 mg/kg, respectively.[41,42] The daptomycin area under the concentration-time curve (AUC_{0-24h}) at steady state is in the range of 500 and 750 μg•h/mL for once-daily doses of 4 and 6 mg/kg, respectively, and 850 μg•h/mL for a dose of 8 mg/kg/day.[41,42] Daptomycin exhibits a long terminal half-life (7.3–9.6 hours) and small volume of distribution (92–117 mL/kg), suggesting distribution mainly into plasma and interstitial fluid.[41,42]

Daptomycin has significant affinity for plasma proteins in humans (90%–93% protein bound)[41,42] but lower affinity for tissue proteins and is eliminated primarily through renal excretion, largely as unchanged drug. Little daptomycin crosses the uninflamed blood-brain barrier (about 2%), although it was effective for the treatment of *Streptococcus pneumoniae* in a rabbit meningitis model of central nervous system (CNS) infection (penetration rate of approximately 6%).[43] A similar percentage of CNS penetration (5%) was reported in a clinical case of methicillin-susceptible *S. aureus* (MSSA) meningitis treated with daptomycin.[44] Mean daptomycin concentrations in inflammatory skin blisters of 68% of those in plasma have been observed in healthy volunteers after a dose of 4 mg/kg/day.[45] In an experimental endocarditis model, daptomycin achieved concentrations in cardiac vegetations that were about half those in serum and with homogeneous distribution within the vegetations. Important to note, the antimicrobial activity of daptomycin is abolished by the interaction with pulmonary surfactant, resulting in failure to reduce bacterial burden in a mouse model of bronchoalveolar pneumonia caused by *S. pneumoniae*. On the other hand, daptomycin was effective in an animal model of *S. aureus* hematogenous pneumonia and inhalation anthrax. Poor penetration of daptomycin into uninfected and infected bone has been shown in a rabbit model of osteomyelitis. However, bone maximal concentration (C_{max}) of daptomycin in metatarsal bones of a group of patients with diabetic foot infection was 4.7 μg/mL.[46]

Pharmacodynamics

The in vivo parameters that best correlated with efficacy in an animal model of infection with *S. aureus* and *S. pneumoniae* were the peak/MIC and the 24-hour AUC/MIC ratios.[47] In the same model of infection, daptomycin unbound (free drug) peak concentrations of 2.5 to 7 and 7 to 25 times the MIC were required to produce a bacteriostatic and bactericidal effect, respectively.[47] Clinical studies have not been able to confirm a specific AUC/MIC cutoff value to predict clinical efficacy. Daptomycin was shown to produce an in vitro postantibiotic effect with a mean of 2.5 and 1.7 hours against staphylococci and pneumococci, respectively.[48] The in vivo postantibiotic effect in a neutropenic murine thigh infection model was 5 hours against *S. aureus* and 10.8 hours against *S. pneumoniae*.[47] Against *E. faecalis,* daptomycin exerted a dose-dependent postantibiotic effect that was longer than that noted with vancomycin (0.6–6.7 hours and 0.5–1.0 hours, respectively).[49]

Drug Dosage and Administration

Daptomycin is administered intravenously, diluted in 0.9% sodium chloride, once daily (by injection over a 2-minute period or infused in 30 minutes); the drug is not compatible with dextrose-containing solutions. The approved doses are 4 and 6 mg/kg/day for acute bacterial skin and skin structure infection (ABSSSI) and *S. aureus* bacteremia and right-sided endocarditis, respectively, although some experts recommend a higher dose of 8 to 12 mg/kg/day (Table 31.1). In patients on hemodialysis, the dose should be given after the completion of the hemodialysis. Dosage adjustment is required in subjects with a creatinine clearance of less than 30 mL/min, including patients on hemodialysis and on peritoneal dialysis. In these patients, according to the underlying infection, the dose prescribed should be given every 48 hours. This proposed regimen yielded adequate serum levels even in 3-day interdialysis periods in one study[50] but not in another.[51] In patients under extended intermittent dialysis (starting 8 hours after daptomycin infusion), a daily dose of 6 mg/kg appears to be needed.[52] There is still no uniform recommendation as to how to administer daptomycin to patients on continuous renal replacement therapy (CRRT). In a small number of critically ill patients undergoing continuous venovenous hemodialysis (CVVHD), daptomycin 8 mg/kg administered every 48

TABLE 31.1 Route of Administration, Recommended Dosages, and Infusions of Daptomycin and Quinupristin-Dalfopristin

DRUG	ROUTE OF ADMINISTRATION	RECOMMENDED DOSAGE (ADULTS)	INFUSION	COMMENTS
Daptomycin	Intravenous	4 mg/kg/day for ABSSSI; 6 mg/kg/day for bacteremia and right-sided endocarditis (FDA approved)	2-min injection or 30-min infusion	*For severe infections:* higher doses (8–12 mg/kg/day) are recommended by some experts
Quinupristin-dalfopristin	Intravenous (frequent need for a central venous access)	7.5 mg/kg q12h for ABSSSI	1-h infusion	Infrequently used owing to side effects

ABSSSI, Acute bacterial skin and skin structure infection.

hours resulted in higher peak and lower trough concentrations than the regimen of 4 mg/kg every 24 hours.[53] Patients treated with continuous venovenous hemodiafiltration (CVVHDF) appear to have higher clearance of daptomycin than those undergoing CVVHD.[54] Because daptomycin given as 6 mg/kg/day led to drug accumulation in a small series of patients undergoing CVVHDF, a regimen of 8 mg/kg every 48 hours has been suggested in this setting, although, owing to high variability in serum levels and to data suggesting that administration every 48 hours could result in less-than-optimal drug exposure, performing drug monitoring would be useful if feasible.[55,56] Daptomycin-related muscle toxicity should be monitored more frequently than once weekly in patients with renal insufficiency.

Daptomycin dosage does not require adjustment in patients with moderate hepatic impairment (Child-Pugh class B). Subjects with obesity have higher C_{max} and AUC concentration than nonobese patients but still within the safety range, indicating that dosage adjustment is not required for this population. Even though daptomycin has not been approved for use in children, a favorable outcome with no attributable adverse events was accomplished in most of 15 children (median age, 6.5 years) with invasive staphylococcal infection, the majority of whom had persistent community-acquired MRSA bacteremia.[57] A multicenter randomized trial was recruiting pediatric patients (2–17 years old) with complicated skin and soft tissue infections to receive daptomycin given at 5, 7, and 9 mg/kg/day for ages 12 to 17 years, 7 to 11 years, and 2 to 6 years, respectively (see NCT 00711802 at ClinicalTrials.gov), although it is was on hold at the time of this writing. Animal studies have failed to detect abnormalities or harm to the fetus, but there are insufficient clinical data to support the use of daptomycin during pregnancy in humans (teratogenic effect: pregnancy category B). Because it is unknown if this drug is excreted in human milk, daptomycin should be administered in these circumstances only when the potential benefits outweigh risks.

Adverse Reactions and Drug Interactions

Daptomycin has been generally well tolerated in preclinical and clinical studies; the proportion of patients who discontinued the drug because of an adverse event has been similar in the daptomycin arm to that in study participants receiving the comparator antibiotic. Of initial concern was the description of daptomycin-related skeletal muscle toxicity in early trials when the drug was administered at 4 mg/kg every 12 hours. Subsequent animal studies showed that this reversible toxicity was related more to the frequency than to the total daily dose of the drug. Analysis of daptomycin-related muscular toxicity disclosed a microscopic degenerative-regenerative process of the myofibers associated with increased serum creatine phosphokinase (CPK) levels with no muscle cell lysis or fibrosis and no electrophysiologic changes. Of note, cardiac muscle cells do not appear to be affected by daptomycin. The mechanism of skeletal muscle damage has not been delineated yet, although it has been associated with direct cell membrane toxicity to the muscle cell. Moreover, a good correlation with the daptomycin minimal concentration (C_{min}) in serum and time of exposure appears to exist. In clinical trials using the 4 mg/kg/day dose, no significant differences were observed in the level of serum CPK during treatment between those patients receiving daptomycin and those receiving standard of care (2.1% vs. 1.4%, respectively).[58] When daptomycin was administered at 6 mg/kg/day in an *S. aureus* bacteremia and endocarditis phase III clinical trial,[13] a higher rate of CPK elevation was observed in the group of patients receiving daptomycin than those in the standard-of-care group receiving other antibiotics (6.7% vs. 0.9%, respectively); however, daptomycin was discontinued owing to CPK elevation in only 2.5% of the patients, and CPK serum level returned to normal range during or after daptomycin therapy in the majority of the patients in whom this adverse event occurred.[13] After analysis of data from this trial, a significantly higher number of patients (50%) with C_{min} values of greater than or equal to 24.3 mg/L developed CPK elevation than those with levels below this value (2.9%).[59] A retrospective case series study of 61 patients receiving daptomycin for various indications at a mean dose of 8 mg/kg/day for 25 days reported a rate of symptomatic CPK elevation of 4.9%.[60] Overall, symptoms associated with muscle toxicity typically appear after at least 7 days of therapy and resolve about 3 days after daptomycin is discontinued. Daptomycin should be discontinued in patients with unexplained signs and symptoms of myopathy together with increased CPK serum level to more than 1000 units/L and in those without muscle pain but with CPK levels above 2000 units/L (10 times the normal upper limit).

In clinical trials using daptomycin at 6 mg/kg/day, the frequency of gastrointestinal symptoms was not different from that observed in the comparative arm; however, at this dosage, symptoms related to the peripheral nervous system such as paresthesias, dysesthesias, and peripheral neuropathies were significantly more common in participants in the daptomycin arm of the study than in those on standard therapy (9% vs. 2%, respectively); these events were mild to moderate in severity, and most resolved during treatment.[13] Daptomycin use was associated in a single patient with acute renal failure and hepatotoxicity without CPK increase or rhabdomyolysis.

Daptomycin did not affect the QTc interval in human studies and, in the bacteremia trial, the rate of nephrotoxicity was lower in those on daptomycin than in those in the comparator arm of the study, which included gentamicin for the first few days.[13] Interesting to note, daptomycin has been reported to exert a protective role on the aminoglycoside-induced renal toxicity by counteracting the inhibition of the phospholipase activity induced by these drugs, especially gentamicin.

Confirmed cases of acute eosinophilic pneumonia associated with the use of daptomycin have been described.[61] This potentially serious event of unclear mechanism is rare, usually occurs after 10 days of therapy, tends to affect older patients, and is associated with fever, diffuse pulmonary infiltrates, hypoxemia, and a high percentage of eosinophils in bronchoalveolar lavage (eosinophilia might be absent) or eosinophilic pneumonia at lung biopsy. A high degree of clinical suspicion is essential because discontinuation of daptomycin is followed by resolution of the clinical picture. Corticosteroids have been used for treatment of this condition in some cases, and chronic corticosteroid dependence has been reported.

Because daptomycin is not metabolized through the cytochrome P-450 system, no interactions are expected with drugs metabolized in this system. CPK levels should be monitored more frequently when daptomycin is coadministered with other drugs carrying potential muscle toxicity, such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, although retrospective studies found no increase in rates of muscular toxicity when patients received daptomycin plus these drugs. Daptomycin may cause a false increase in the prothrombin time depending on the reagents and assay used to measure it; if this

occurs, blood should be drawn at the lowest serum daptomycin concentration for prothrombin time assessment.

Clinical Uses

Skin and Soft Tissue Infections

Two randomized phase III, evaluator-blinded trials for the treatment of ABSSSI at a dose of 4 mg/kg every 24 hours showed efficacy comparable to that of conventional therapy (vancomycin or antistaphylococcal semisynthetic penicillins), leading to FDA approval for use in the treatment of infections caused by the following susceptible gram-positive cocci: *E. faecalis* (vancomycin-susceptible isolates only), *S. aureus* (including methicillin-resistant isolates), *Streptococcus agalactiae, Streptococcus dysgalactiae* subsp. *equisimilis,* and *Streptococcus pyogenes.* A subset analysis of patients with infected diabetic foot ulcers enrolled in the two phase III studies of ABSSSI showed comparable results in terms of clinical and microbiologic outcome in the two study arms.[62] Daptomycin could be considered an option in patients with ABSSSIs with prior failure or intolerance to glycopeptides and in ABSSSIs caused by MRSA with higher vancomycin MICs.

Staphylococcus aureus Bacteremia and Right-Sided Endocarditis

Daptomycin has also been granted FDA approval for *S. aureus* bloodstream infection, including right-sided endocarditis, based on a multicenter, open-label, randomized trial comparing daptomycin at 6 mg/kg/day with standard of care (penicillinase-resistant penicillins or vancomycin, each with gentamicin 1 mg/kg every 8 hours for the first 4 days).[13] About half of the patients had complicated bacteremia (defined by positive blood cultures for at least 2 days up to study day 5, evidence of spread of infection, or infected prosthesis material not removed within 4 days), and less than 10% had left-sided endocarditis; MRSA accounted for 38% of the isolated strains. Daptomycin was not inferior to standard therapy, with similar success rates across all final diagnoses; and no significant differences were observed when patients with MSSA and MRSA infection were analyzed separately. The median time to clear the MSSA or MRSA bacteremia was similar in both treatment groups: 4 and 8 days for daptomycin and 3 and 9 days for standard therapy, respectively. Persisting or relapsing *S. aureus* infection occurred in 16% (19 of 120) of patients receiving daptomycin and in 10% of those in the standard therapy group (11 of 115, with 9 receiving vancomycin and 2 patients on an antistaphylococcal penicillin); most of these patients did not receive adequate surgical drainage for a deep-seated infection.[13] Among those patients with microbiologic failure, strains with increased MICs to greater than or equal to 2 μg/mL were found in 6 of 19 patients receiving daptomycin and in 4 of 9 receiving vancomycin.[13] Low success rates in both arms of the study were found in the few patients with left-sided infective endocarditis, owing, at least in part, to the very rigorous definition of cure. Similar outcomes in subgroups of patients with different degrees of renal impairment (excluded from the *S. aureus* bacteremia-endocarditis trial) were observed in a retrospective postmarketing study evaluating daptomycin for the treatment of bacteremia caused by gram-positive organisms (mainly MRSA, VRE, and coagulase-negative staphylococci). A clinical cure rate of 83% among 92 patients with *S. aureus* endocarditis was noted in a retrospective report on the European postmarketing use of daptomycin. Most of these patients were treated with a dose of 6 mg/kg, but in only 36% as monotherapy.[63] Similar cure rates were found for patients with endocarditis caused by coagulase-negative staphylococci.

In a large retrospective study comparing the use of a standard dose (6 mg/kg/day; $n = 233$) versus higher doses (≥7 mg/kg/day; $n = 138$) of daptomycin in patients with MRSA bloodstream infections, patients receiving higher doses were found to have a significantly lower propensity score-matched 30-day mortality (8.6% vs. 18.6%, respectively; hazard ratio, 0.31; 95% confidence interval [CI], 0.1–0.94). Of note, further analyses suggested that the benefit of higher daptomycin doses was particularly relevant in patients with higher predicted probabilities of 30-day mortality.[64] Similar results regarding the benefit of higher daptomycin doses (≥8 mg/kg/day) were reported in another retrospective study that included 250 patients with a variety of invasive infections caused by MRSA and VRE, in which daptomycin was associated with a good clinical response rate (84%), a low frequency of adverse events (1.2%), and a 5.2% rate of development of nonsusceptible strains (mainly in patients with prior extensive exposure to vancomycin).[65]

Several retrospective studies have compared the use of daptomycin versus vancomycin for the treatment of MRSA bacteremia caused by strains with vancomycin MICs greater than 1 μg/mL. Overall, these retrospective data suggest that patients receiving daptomycin tend to have better clinical outcomes; however, despite reported apparent benefits associated with daptomycin use, prospective data are needed before a universal recommendation of early treatment with high-dose daptomycin is made for infections caused by such strains.[66–68]

The combination of daptomycin with other agents against staphylococci has been explored in in vitro and in vivo models. The bactericidal activity of daptomycin against *S. aureus* was enhanced by the addition of gentamicin in various in vitro models. The interaction between daptomycin and rifampin in vitro has been strain dependent, and neither synergism nor antagonism has been reported consistently. However, when this combination was evaluated in an experimental MRSA endocarditis model, antagonism between these two antibiotics was reported.[69] High-dose daptomycin (10 mg/kg/day) and combination with gentamicin or rifampin prevented the development of daptomycin-nonsusceptible isolates that appeared when daptomycin at 6 mg/kg/day was used in the simulated vegetations model using *S. aureus* strains. Another approach includes the coadministration of a β-lactam plus daptomycin to take advantage of the previously mentioned seesaw effect. In vivo studies have also shown that this strategy prevented the emergence of isolates with high daptomycin MICs as compared with daptomycin monotherapy.[70] Six patients with persistent non–catheter-related MRSA bacteremia were successfully treated with daptomycin (8–10 mg/kg/day) plus nafcillin (or oxacillin in one case) after the failure of multiple regimens including vancomycin and daptomycin (6–8 mg/kg/day) monotherapy.[31] In addition, there are reports of patients with infective endocarditis due to daptomycin-nonsusceptible MRSA (one case of which was also ceftaroline resistant and vancomycin intermediate) and persistent bacteremia that were successfully treated with the addition of ceftaroline to daptomycin[71,72]; this combination also restored in vitro activity of daptomycin against this strain.[71]

Therefore, based on the concentration-dependent activity of daptomycin and the reported risk of development of resistance during treatment of deep-seated staphylococcal infections, clinicians often use high-dose daptomycin (e.g., 8–10 mg/kg/day), especially in patients in whom vancomycin therapy has previously failed. Other approaches considered by some experts consist of combination therapy with high-dose daptomycin with β-lactams (including ceftaroline) or gentamicin (if the infecting strain is susceptible) or trimethoprim-sulfamethoxazole, which demonstrated a significant increase in the in vitro activity of daptomycin against *S. aureus.* Any of these novel approaches deserves further clinical evaluation.

Osteoarticular Infections Caused by Staphylococci

Daptomycin displayed efficacy similar to that of vancomycin in animal models of chronic MRSA osteomyelitis. Daptomycin has not been approved for this indication, but many studies addressing its efficacy have been published. In humans, a retrospective analysis of daptomycin for chronic osteomyelitis caused by gram-positive bacteria, mostly MRSA, showed an overall success rate of 82% (cure, 63%; improvement, 19%) at a median follow-up of 76 days (range, 1–547 days).[73] About half of the patients had received another antibiotic, and, important, a significantly higher success rate was observed in those patients who received an initial dose higher than 4 mg/kg/day.[73] Post hoc analysis of 32 patients from the *S. aureus* bacteremia-endocarditis study who also had osteoarticular infections (mostly septic arthritis and vertebral osteomyelitis) showed similar success rates to those in the comparator arm of the study.[74] Daptomycin at 6 and 8 mg/kg/day was compared with standard of care (vancomycin, teicoplanin, or semisynthetic penicillin) in a phase II randomized trial including 49 patients with prosthetic joint infections caused by *S. aureus* who underwent two-stage revision arthroplasty. Although higher clinical success rates were observed in the daptomycin arm of the trial (58% and 61% for the 6 and 8 mg/kg/day dosage groups,

respectively) compared with the pooled comparator arm (38%), the results did not reach statistical significance.[75] Because daptomycin does not reach high levels in bone tissue and resistance might emerge during therapy, high-dose daptomycin (i.e., 8–10 mg/kg/day) and the administration of other agents with good bone penetration are suggested by some investigators. In this regard, the addition of rifampin to daptomycin in an experimental model of MRSA osteomyelitis in rabbits was associated with increased efficacy and reduced incidence of development of nonsusceptibility to either drug.[76] Daptomycin also appeared to be efficacious for the treatment of septic arthritis caused by *S. aureus,* as shown in a retrospective study[77] in which 41% and 50% of the treated patients were considered cured or improved, respectively. The median dose of daptomycin in this study was 5 mg/kg/day (range, 3–6.3 mg/kg/day), the drug was administered for a median of 22 days (range, 3–52 days), and in two-thirds of the patients another antibiotic (most commonly rifampin) was concomitantly given.[77]

Enterococcal Infections

Daptomycin and comparator agents showed similar efficacy for the treatment of ABSSSI in which vancomycin-susceptible *E. faecalis* was isolated in the randomized clinical trials mentioned earlier.[58] Infections caused by VRE were excluded in this study because vancomycin was used in the comparator arm and there were insufficient vancomycin-resistant *E. faecium* isolates to assess efficacy. Daptomycin and comparator agents (vancomycin, teicoplanin, and amoxicillin) displayed similar activity for a variety of enterococcal strains in an experimental endocarditis model. Daptomycin (low dose) activity appeared to be enhanced by the addition of gentamicin against enterococci, both in vitro and in animal models. In humans, the use of daptomycin for the treatment of VRE infections has been reported, mainly in retrospective analysis, and with variable success rates. Around 45% of patients with VRE *(E. faecium)* bacteremia in neutropenic or nonneutropenic patients were considered to have achieved clinical cure, as were about 90% of those with catheter- and non–catheter-related bacteremia in a postmarketing study.[78] Two retrospective studies including patients with VRE bacteremia reported no difference in microbiologic and clinical cure and in mortality rates between daptomycin- and linezolid-treated patients.[79,80] In one of these studies, those receiving daptomycin experienced a higher rate of recurrence, but significantly more patients in this group had hematologic malignancies or were liver transplant recipients. A retrospective study from a national cohort of Veterans Affairs (VA) patients with VRE bacteremia found that treatment with linezolid was associated with higher 30-day all-cause mortality and microbiologic failure rates than daptomycin.[80] A larger subsequent retrospective study including 2630 patients found similar results, with better 30-day survival, in patients receiving daptomycin compared with linezolid. Of note, subgroup analyses suggested that this association was mainly driven by patients with infective endocarditis.[81]

Despite these data, several reports have shown daptomycin failure in the treatment of VRE infections, mainly in cases of bacteremia and endocarditis, with emergence of resistant strains during daptomycin therapy. Important to note, data are accumulating supporting the use of higher daptomycin doses, the potential relevance of the daptomycin MICs displayed by the infecting enterococcal isolates, and the advantage provided by the association with a β-lactam (see previous discussion). Indeed, a retrospective study comparing different dosage strategies in patients with vancomycin-resistant enterococcal bacteremia treated with daptomycin (three groups: standard, medium, and high doses [6, 8, and 10 mg/kg/day, respectively]) found improved overall survival, 30-day mortality, and microbiologic clearance with medium and high doses; no increase in adverse events was observed.[82] Moreover, Chuang and colleagues performed a prospective observational study and found that patients receiving doses of 9 mg/kg/day or higher had the lowest 14-day mortality, with adjusted odds ratios of 10.57 (P = .003) and 5.01 (P = .03) as compared with doses of <7 and 7 to 9 mg/kg/day, respectively.[83]

Three retrospective studies have evaluated the potential impact of the daptomycin MIC in the outcomes of patients treated with daptomycin for severe vancomycin-resistant enterococcal infections. The first study found that subjects infected with isolates that displayed an MIC close to the established breakpoint (3–4 μg/mL) had a higher risk of microbiologic failure than isolates whose MIC was ≤2 μg/mL.[84] The second study included 262 patients and found similar results in terms of efficacy of daptomycin when MICs were considered, but, interesting to note, the daptomycin efficacy was similar (88% vs. 79% for MIC ≤2 and 3–4 μg/mL, respectively; P = .41) when investigators stratified for the use of concomitant β-lactam therapy.[85] In contrast, a third study, a small single-center report of 42 patients with hematologic malignancies and stem cell transplant recipients, did not find any statistically significant differences when stratifying by daptomycin MIC of the initial infecting isolate.[86] Therefore, owing to the high protein binding of daptomycin, the relative decreased susceptibility displayed by enterococci compared with staphylococci (reflected in the recent change of breakpoints), and the possibility of development of resistance during therapy, a high-dose regimen of daptomycin (e.g., 10–12 mg/kg/day) in combination with another active agent might be considered for severe enterococcal infections.[22] High-dose daptomycin has been successfully used with concomitant administration of rifampin and gentamicin, gentamicin and ampicillin, fosfomycin, and tigecycline for selected cases of VRE endocarditis. This approach may enhance daptomycin activity and/or avoid the emergence of mutant strains with decreased susceptibility to this agent. More recently, high-dose daptomycin plus ampicillin appeared to be beneficial in a patient with ampicillin-resistant VRE *(E. faecium)* native aortic valve endocarditis refractory to 7 days of daptomycin (standard dose) and linezolid.[87] In vitro analysis of this strain revealed increased bactericidal activity of daptomycin in the presence of ampicillin; interesting to note, the activity of some innate cationic antimicrobial peptides was also augmented by ampicillin. The use of the combination of ampicillin and daptomycin (despite ampicillin resistance) for enterococcal infections deserves further clinical study. Other combinations that have been shown to be efficacious in vitro in selected strains include daptomycin plus ceftaroline or ertapenem, although clinical data to support these combinations are still lacking.

Other Clinical Uses

Even though daptomycin has been successfully used in some cases of staphylococcal meningitis, including postsurgical meningitis and ventriculitis, daptomycin has been administered with another active agent in most of these reports.[88] The poor CNS penetration of daptomycin (about 5% with inflamed meninges)[44] and a reported daptomycin failure in a neutropenic patient with MRSA meningitis precludes the use of this antibiotic for CNS infections as monotherapy. Intraventricular administration of daptomycin has been communicated in a few reports of patients with ventriculitis due to *E. faecium, E. faecalis,* and methicillin-resistant coagulase-negative staphylococci, all of them with an intravenous drug used concomitantly.[89,90] A suggested potential benefit of the use of daptomycin in pneumococcal meningitis might be derived from the lower propensity of daptomycin to induce inflammatory changes compared with ceftriaxone in an experimental model.[91] Because of the inhibitory interaction between daptomycin and pulmonary surfactant described earlier, daptomycin (4 mg/kg/day) was inferior against the comparator drug (ceftriaxone, 2 g/day) in a clinical trial of community-acquired pneumonia.[92] Therefore, daptomycin should not be administered for the treatment of pulmonary infections unless the lung process is secondary to hematogenous spread of infection (mainly *S. aureus*).[92]

STREPTOGRAMINS

Quinupristin-Dalfopristin

The group of antibiotics named "streptogramins" is composed of different compounds, including mikamycin, virginiamycin, pristinamycin, and quinupristin-dalfopristin (Fig. 31.2). Each of these streptogramin antibiotics contains two macrocyclic lactone peptolide components referred to as streptogramin A and streptogramin B. The former are polyunsaturated cyclic peptolides, and the latter are cyclic hexadepsipeptides. Virginiamycin, a secreted product from *Streptomyces virginiae,* includes virginiamycin M (a streptogramin A) and virginiamycin S (a streptogramin B) and has been used mainly in animals as a growth promoter. Pristinamycin, a naturally occurring mixture produced by *Streptomyces pristinaespiralis,* also contains a streptogramin A (pristinamycin II_A) and

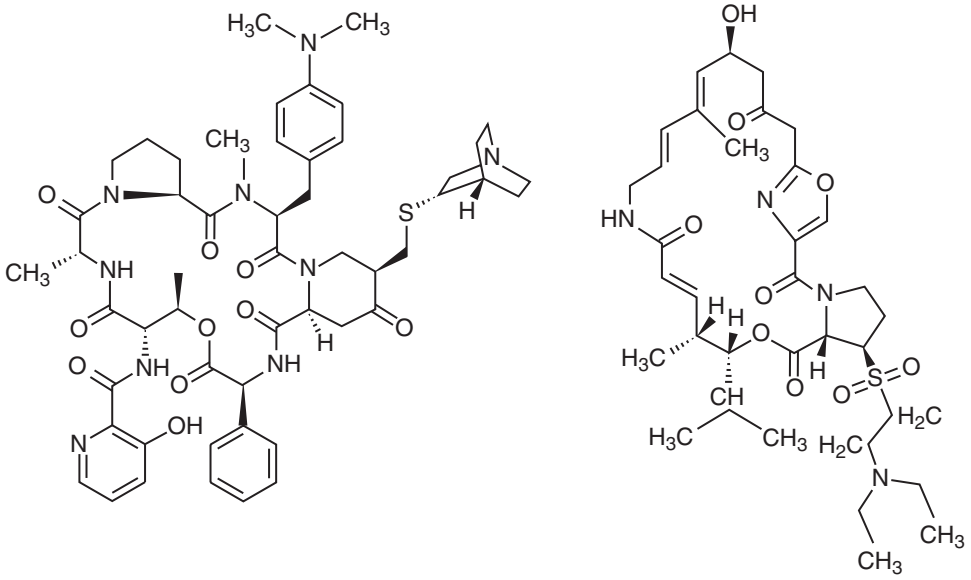

FIG. 31.2 **Chemical structures of quinupristin and dalfopristin.**

a streptogramin B (pristinamycin I_A) component, and has been used orally in some European countries, mainly France, for the treatment of skin and soft tissue infections caused by streptococci and staphylococci. Quinupristin-dalfopristin (Synercid, formerly RP 59500) is a water-soluble combination in a 30:70 ratio suitable for intravenous administration. Quinupristin, a derivative of pristinamycin I_A, is the streptogramin B constituent, and dalfopristin, a derivative of pristinamycin II_B, is the streptogramin A component.

Mechanism of Action

Streptogramins exert their action within the 50S ribosomal subunit of the 70S unit in the second phase, or elongation stage, of protein synthesis. Type A streptogramins (e.g., dalfopristin) appear to bind to the free arms of the peptidyl transferase site in the 50S ribosomal subunit, blocking the addition of new amino acids from the aminoacyl-tRNA molecule to the growing peptide chain, and thus inhibiting the earliest process of elongation. Quinupristin (a streptogramin B compound), like macrolides, works at a later phase of protein synthesis, preventing further peptide elongation and causing release of incomplete peptide chains. In the absence of specific resistance mechanisms, the affinity of quinupristin for the 50S ribosomal subunit is considerably enhanced by a conformational change produced within it by the binding of dalfopristin, explaining the synergistic antimicrobial activity observed between the two streptogramin components. The irreversibility of the complex formed results in bactericidal activity against most susceptible organisms. The blockage of different steps of the protein synthesis pathway may also help explain this synergistic activity.

Antimicrobial Activity

Quinupristin-dalfopristin is active against most gram-positive organisms (with the notable exception of *E. faecalis*) and some gram-negative organisms. In vitro, each compound separately displays bacteriostatic activity, and the bactericidal activity that can be observed with the combination may be diminished if resistance to one or both components is present. Against *E. faecium* isolates, quinupristin-dalfopristin generally displays MICs of less than or equal to 1 μg/mL, including against vancomycin- and erythromycin-resistant isolates, but is generally bacteriostatic in the presence of erythromycin resistance—for example, *ermB. E. faecalis* strains are inherently resistant to quinupristin-dalfopristin,[93] although some exceptions exist. The in vitro activity of quinupristin-dalfopristin against other enterococcal species is variable.

The MIC_{90} of quinupristin-dalfopristin against *S. aureus* and *S. epidermidis* is less than or equal to 1 μg/mL, regardless of the methicillin, vancomycin, erythromycin, or clindamycin resistance pattern. The MIC_{90} of *S. pyogenes, S. agalactiae,* group C and group G streptococci, *S. pneumoniae,* and viridans-group streptococci is less than or equal to 1 μg/mL. *Corynebacterium* spp., including *Corynebacterium jeikeium,* and *Listeria monocytogenes* are also susceptible, as are *Bacillus* spp., *Leuconostoc* spp., *Lactobacillus* spp., *Pediococcus* spp., and *Erysipelothrix rhusiopathiae.* Low MICs of quinupristin-dalfopristin against a variety of gram-positive anaerobes and some gram-negative organisms (e.g., *Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrhoeae,* and *Neisseria meningitidis*) have been reported, but in vivo studies have not been performed. Quinupristin-dalfopristin displays no activity against Enterobacteriaceae, *Pseudomonas aeruginosa,* or *Acinetobacter* spp.

Resistance

Because quinupristin and dalfopristin are chemically distinct compounds and their specific target sites within the 50S ribosomal subunit are different, the mechanism(s) leading to resistance to these components are specific for each one. The three general types of resistance to these compounds are (1) conformational changes in the target site decreasing the binding affinity of the drug (quinupristin), (2) enzymatic inactivation (quinupristin and dalfopristin), and (3) active transport of the compound(s) out of the cell (quinupristin and dalfopristin).

The species *E. faecalis* is naturally resistant to quinupristin-dalfopristin owing to the presence of an apparently intrinsic gene, named *lsa,* which encodes a homologue of an adenosine triphosphate (ATP)–binding protein that mediates resistance to lincosamides and type A streptogramins. *E. faecium* human clinical isolates with a quinupristin-dalfopristin MIC greater than or equal to 4 μg/mL have been infrequently reported. However, among VRE *(E. faecium)* strains, resistance to quinupristin-dalfopristin appears more common, especially in Europe. Indeed, in 2003, resistance to quinupristin-dalfopristin was found in 0.6% of these isolates from the United States and in 10% of those from Europe.[94] More recently, 30% of 60 clinical VRE *E. faecium* (VanA or VanB) strains from France isolated between 2006 and 2008 were resistant to quinupristin-dalfopristin (MIC_{90} of 4 μg/mL).[95] Higher rates of resistance are found among *E. faecium* strains isolated from animals in some European countries, probably related to the use of virginiamycin in animal feeds. *Staphylococcus* spp. and *S. pyogenes* strains with high MICs of quinupristin-dalfopristin have been rare.

The most common mechanism of resistance to streptogramin B in gram-positive cocci is through modification of the ribosomal target site, known as MLS_B (conferring resistance to *m*acrolides, *l*incosamides, and *s*treptogramin *B*), which does not affect dalfopristin; this phenotype is

encoded by various *erm* (erythromycin ribosomal methylation) genes. The *erm* genes encode methylases that add one or two methyl groups to a specific adenine residue (A2058) in the 23S ribosomal RNA within the 50S ribosomal subunit, resulting in reduced binding affinity of MLS_B antibiotics for their specific target. The *erm* genes are often located on transposons and can reside on the chromosome or on plasmids. These genes can be constitutively expressed or inducible and are found in many species, including staphylococci, streptococci, enterococci, *Clostridium* spp., and *Bacillus* spp., among others. In staphylococci, the ribosomal binding of 14-membered (e.g., erythromycin and clarithromycin) and 15-membered (e.g., azithromycin) ring macrolides causes a conformational change in the mRNA upstream of the specific *erm* gene (e.g., *ermC*), which then unblocks Erm translation and results in synthesis of the Erm methylase at an increased efficiency.[96] The genes conferring MLS_B resistance typically found in staphylococci are *ermA, ermB, ermC,* and *ermY.* Staphylococcal strains with inducible MLS_B ($iMLS_B$) resistance are resistant to inducer macrolides (e.g., erythromycin) and susceptible to noninducer macrolides, lincosamides (clindamycin), and streptogramin B (quinupristin). However, deletions, duplications, and multiple- or single-point mutations in the *erm* regulatory region can lead to constitutive expression—that is, constant synthesis of the Erm methylase, and resistance to all the MLS_B antibiotics, including clindamycin and quinupristin. Constitutive mutants can be selected from $iMLS_B$ strains in vitro, and clinical failures with clindamycin have been reported during treatment of such staphylococcal strains.[97] Among nosocomial MRSA isolates, constitutive expression of MLS_B ($cMLS_B$) resistance is much more common than $iMLS_B$ type, which on the other hand is more frequent in MSSA isolates and also among recent isolates from community-onset MRSA infections. Despite causing resistance to quinupristin, the presence of the $cMLS_B$ phenotype alone has no effect on the MICs of quinupristin-dalfopristin against staphylococci but decreases the bactericidal activity in vitro and in animal models. Increased MICs of quinupristin or of clindamycin appear to be a useful tool for screening for the decreased bactericidal activity of quinupristin-dalfopristin.

In enterococci, MLS_B resistance is most frequently related to the presence of *ermB* and is often $iMLS_B$ type. However, unlike staphylococci, all the MLS_B antibiotics can act as inducers of the MLS_B resistance system; therefore, resistance is expected to all MLS_B antibiotics whether MLS_B resistance is inducible or constitutively expressed. In the experimental endocarditis model, quinupristin-dalfopristin was less efficacious when the infecting *E. faecium* strain had the $iMLS_B$ resistance phenotype (vs. an MLS_B-lacking strain), although different degrees of diffusion of the two streptogramins into the vegetation may partially explain these results. The MICs of quinupristin-dalfopristin against *S. pneumoniae, S. pyogenes, S. agalactiae,* and viridans-group streptococci appear not to be affected by the presence of MLS_B resistance. The *cfr* gene, typically associated with linezolid resistance through ribosome methylation in staphylococci and enterococci, has also been shown to decrease the susceptibility to streptogramin A.

Hydrolysis of streptogramin B compounds is another mechanism of resistance to these agents. The *vgb* genes encoding a streptogramin-inactivating enzyme (lyase) have been identified in staphylococci and, rarely, in *E. faecium.* Efflux systems coding for ATP-binding transporters such as *msrA* and *msrB* in staphylococci and *msrC* in *E. faecium* can pump macrolides and streptogramin B compounds out of the cell. Streptogramin A compounds can be inactivated by acetyltransferases, which are encoded by the genes *vatA, vatB,* and *vatC* in staphylococci and by *vatD* and *vatE* in *E. faecium,* and are subjected to the action of efflux pumps encoded by the *vgaA* and *vgaB* genes in staphylococci. High MICs of quinupristin-dalfopristin generally require the presence of more than one gene conferring resistance to the individual components. Organisms with decreased susceptibility (*E. faecium* more so than *S. aureus* isolates) have been recovered from subjects in whom therapy has failed, most likely owing to a mutational mechanism.

Clinical Pharmacodynamics and Pharmacokinetics

Quinupristin-dalfopristin displays a postantibiotic effect against many gram-positive organisms with a mean of 2.8 hours for pneumococci and 4.7 hours for staphylococci, although it is shorter for isolates with $cMLS_B$ expression. For *E. faecium* isolates, the postantibiotic effect averages 2.6 and 8.5 hours for vancomycin-resistant and vancomycin-susceptible strains, respectively. The AUC/MIC ratio appears to be the pharmacodynamic parameter that best predicts response to quinupristin-dalfopristin.

Distribution and Elimination

The mean peak concentrations of quinupristin and dalfopristin after a single intravenous infusion of 7.5 mg/kg in healthy volunteers were 2.3 μg/mL and 6.1 μg/mL, respectively.[98] The terminal half-lives of both compounds are relatively short, ranging from 0.7 to 1.2 hours. The volume of distribution at steady state was reported as 460 to 540 mL/kg and 240 to 300 mL/kg of quinupristin and dalfopristin, respectively. Quinupristin displayed protein binding of 55% to 78%, and dalfopristin, 11% to 26%.[98] Quinupristin-dalfopristin is excreted mainly in the feces and only partially in the urine; it does not reach therapeutic levels in cerebrospinal fluid (CSF) and appears not to cross the placenta. Excretion into breast milk was detected in lactating rats, although no data exist in women. Both streptogramins are metabolized in the liver, resulting in metabolites with antimicrobial activity. Studies in monkeys found high levels of drug in bile and in gallbladder, liver, and kidney tissues after infusion of radiolabeled quinupristin-dalfopristin. Quinupristin diffuses homogeneously within infected vegetation, whereas dalfopristin concentrates only in the periphery, which may hinder clinical efficacy. Quinupristin-dalfopristin penetrates poorly into the peritoneal fluid of patients undergoing continuous ambulatory peritoneal dialysis (CAPD).

Administration and Dosing

The recommended intravenous dosing for quinupristin-dalfopristin is 7.5 mg/kg every 12 hours for ABSSSI, respectively (see Table 31.1). The same doses have been safely used in pediatric patients. Reconstituted drug should be diluted in 5% dextrose (not in saline) to a concentration of about 2 mg/mL and then infused over 60 minutes. If significant venous irritation is noted, which it often is, the solution can be further diluted or the drug administered through a central line, the preferred route. Dosage adjustment appears not to be necessary in patients with renal failure whether or not they are receiving hemodialysis or are undergoing CAPD. The lack of data in subjects undergoing CRRT precludes making dosage recommendations in this setting. A lower dose of quinupristin-dalfopristin may be considered in patients with hepatic disease, although further studies are needed.

Adverse Events and Drug Interactions

More than 30% of patients experience irritation at the venous site when quinupristin-dalfopristin is administered through peripheral veins.[99,100] Arthralgias (9.1%) and myalgias (6.6%), sometimes severe and typically with normal CPK levels, are the side effects that most commonly lead to drug discontinuation; these can be severe, resolving after cessation of therapy.[99,101] Risk factors associated with the development of these side effects are having chronic liver disease, having elevated bilirubin concentrations at baseline, being a liver transplant recipient, and concomitantly using cyclosporine or mycophenolate. Less common adverse events associated with quinupristin-dalfopristin are nausea, vomiting, diarrhea, rash, pruritus, headache, and asthenia[99,102]; laboratory abnormalities are mostly increased liver enzymes and total and conjugated bilirubin levels, which appear to be secondary to competition for excretion between bilirubin and this agent.[99,102] These laboratory abnormalities are rarely severe enough to cause discontinuation of this drug. Quinupristin-dalfopristin has not been associated with significant teratogenic abnormalities in animal studies, but there are no data on the use of this antibiotic in pregnant women (pregnancy category B) or in nursing woman, for whom this drug should be used only in situations in which there are no other therapeutic alternatives.

Quinupristin-dalfopristin produces significant inhibition of the cytochrome P-450 3A4 isoenzyme system, resulting in increased levels of drugs that are metabolized through it; a few examples include diazepam, verapamil, HMG-CoA reductase inhibitors, most of the human immunodeficiency virus type 1 protease inhibitors, vinca

alkaloids, cyclosporine, tacrolimus, methylprednisolone, quinidine, lidocaine, and disopyramide.[102] Drugs that are metabolized through the cytochrome P-450 3A4 isoenzyme system that can produce prolongation of the QTc interval should not be coadministered with quinupristin-dalfopristin.

Clinical Uses

Quinupristin-dalfopristin is currently approved in the United States for the treatment of skin and skin structure infections caused by MSSA or *S. pyogenes,* although this combination is seldom used. Quinupristin-dalfopristin was the first compound approved by the FDA for the treatment of VRE *(E. faecium)* infections; however, lack of consistent clinical benefit in adequately designed comparative trials led to withdrawal of this indication from its label. In open-label trials, quinupristin-dalfopristin showed a response rate of 75% to 86% in individuals with urinary tract infections, catheter-related bacteremia, and bone and joint infections caused by vancomycin-resistant *E. faecium* isolates.[99,100] Slightly lower response rates were attained in individuals with skin and skin structure infections, intraabdominal infections, and bacteremia of unknown origin; only about 25% of patients with endocarditis had a successful outcome.[99,100] In another study including mostly liver transplant recipients with vancomycin-resistant *E. faecium* infections at diverse sites, an initial favorable response was seen in approximately 80% of the patients, although 4 of 23 patients later experienced bacteriologic and clinical relapse.[103] In a prospective randomized study in cancer patients with vancomycin-resistant *E. faecium* infections, a clinical response rate of 43% was seen with quinupristin-dalfopristin, which was comparable to that of linezolid. A few cases of vancomycin-resistant *E. faecium* endocarditis have been successfully treated with quinupristin-dalfopristin in combination with rifampin and doxycycline or with high-dose ampicillin. Some patients with shunt-related meningitis caused by vancomycin-resistant *E. faecium* have been successfully treated with the addition of intraventricular quinupristin-dalfopristin[104]; based on pharmacokinetics of quinupristin-dalfopristin in the CSF, a dose of 2 mg or greater appears to be the appropriate intraventricular daily dose.[104] Intravenous plus intraperitoneal quinupristin-dalfopristin (25 mg/L in alternate dialysate bags) has been effective in a few cases of vancomycin-resistant *E. faecium* CAPD-related peritonitis.

Clinical success rates of about 70% were observed for skin and skin structure infections and bone and joint infections when quinupristin-dalfopristin was evaluated for the treatment of patients with MRSA infections who were intolerant of or were not responding to other therapies.[101] Lower response rates (about 50%) were seen in patients with endocarditis, and neither of two bacteriologically evaluable patients responded favorably.[101] A randomized trial including patients with nosocomially acquired pneumonia (aztreonam was given for empiric gram-negative therapy) in which *S. aureus* was the most common isolated organism found a clinical success rate of 56% for quinupristin-dalfopristin and 58% for vancomycin,[105] suggesting that quinupristin-dalfopristin may be an alternative for the treatment of staphylococcal nosocomial pneumonia, although it is rarely used for this indication.

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32 Polymyxins (Polymyxin B and Colistin)

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SHORT VIEW SUMMARY

- The polymyxins are old antibiotics available as polymyxin B and colistin, the latter of which is formulated as its inactive prodrug, colistimethate sodium. They are mainly used intravenously.
- Because of their toxicity (especially nephrotoxicity, with rates ranging from 30% to 60%), they should be reserved for use only as drugs of last resort when dealing with multidrug-resistant bacterial infections.
- These agents act electrostatically by disrupting cell membranes of gram-negative bacilli, and are bactericidal.
- They are highly active against problematic resistant gram-negative aerobic bacilli, namely *Pseudomonas aeruginosa, Acinetobacter baumannii,* and Enterobacteriaceae, although resistance is increasing, and in some settings can be commonplace.
- Resistance is usually mediated via a reduction in the negative charge of the outer membrane; concerningly, plasmid-mediated resistance genes have recently become disseminated.
- Colistimethate sodium (CMS) must be hydrolyzed to colistin in the bloodstream to be active, and this is a slow and inefficient process leading to unpredictable pharmacokinetics.
- Polymyxin B is given as its active form and thus has more reliable and predictable pharmacokinetics.
- The serum concentrations of polymyxins that are required to achieve antibacterial effect overlap with those associated with nephrotoxicity.
- Colistin and polymyxin B undergo extensive tubular reabsorption in the kidney and are eliminated by nonrenal mechanisms. CMS is renally cleared. This leads to the need to renally dose adjust CMS, but not polymyxin B.
- CMS in the urine will hydrolyze to active colistin, making it the preferred polymyxin for urinary tract infections.
- Because of more predictable pharmacokinetics and perhaps less nephrotoxicity, polymyxin B should be preferred over CMS where both are available, with the exception of urinary tract infections.

The polymyxins are among the oldest antibiotics, discovered in 1947. They were used parenterally from 1962 until anti-*Pseudomonas* aminoglycosides (e.g., gentamicin) came into common use after the middle-to-late 1960s.

The polymyxins fell into disuse by 1980 because of their nephrotoxicity and subsequently were reserved mainly for topical and oral use.[1,2] However, the emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* carbapenem-resistant Enterobacteriaceae (CRE), and other gram-negative bacilli resistant to all other antimicrobial agents has resulted in an increasing need for their use as injectable agents. The two parenteral polymyxins that have been used are polymyxin B and polymyxin E (colistin, formulated as colistimethate sodium [CMS], which is also named colistin methanesulfonate sodium). There are no solid head-to-head studies comparing the effectiveness of CMS and polymyxin B, but comparative toxicity data have begun to emerge. The only direct efficacy comparison was a small retrospective review, which showed similar rates of cure.[3] Unfortunately, this analysis was limited by small numbers, suboptimal dosing, and concomitant antimicrobial exposures. Recent investigations into the comparative toxicity of the polymyxins have suggested that polymyxin B might be associated with lower rates of nephrotoxicity.[4] In recent years, series of patients treated with CMS have been reported in the literature much more frequently than series of patients treated with polymyxin B. However, due to an emerging and growing understanding of the favorable pharmacokinetic parameters and potential safety advantage of polymyxin B,[4,5,6,7] there has been a recent "shift" to preferential use of polymyxin B in areas where both agents are available (such as the United States).

STRUCTURE, SOURCE, AND AVAILABLE PREPARATIONS

The polymyxins are cyclic cationic polypeptide detergents with molecular weights of 1000 Da or greater. Polymyxin B (Fig. 32.1) is derived from products of strains of *Bacillus polymyxa,* and colistin (polymyxin E; Fig. 32.2) is derived from products of *Bacillus colistinus.* The sulfomethylated formulation of colistin, CMS, is an inactive prodrug that must be hydrolyzed to be active as an antibiotic.[8] Hydrolysis occurs at body temperature and within in vitro testing systems. Polymyxin B is available in a parenteral preparation as the active moiety that can be given intramuscularly and intravenously. Polymyxin B is also available for topical use.

Colistin has been available as colistin sulfate for use topically and orally, and as CMS formulations for intramuscular (IM) and intravenous (IV) use. CMS has also been used for inhalation therapy, and CMS and polymyxin B have been administered intrathecally or intraventricularly. CMS and polymyxin B are both available in the United States, whereas other countries typically only have one agent available; colistin sulfate is only available topically.

In the United Kingdom and Europe, CMS and polymyxin B are dosed based on international units (IU), with the rationale that the various preparations have different weights and this can cause confusion (see "Pharmacokinetics" later).

MECHANISM OF ACTION

The polymyxins are surface-active amphipathic agents containing both lipophilic and lipophobic groups. They penetrate into the outer cell membranes of gram-negative bacteria, interact electrostatically with phospholipids in the membranes, and quickly disrupt the membranes via competitive displacement of divalent cations. They also bind to the lipid A portion of cell wall lipopolysaccharide (LPS) and, in animal studies, they block many of the biologic effects of this endotoxin.[9] Resistance in gram-negative bacteria is usually related to modifications of LPS, such as the addition of ethanolamine or aminoarabinose, which lowers the overall charge of the LPS, effectively inhibiting the initial interaction between the positively charged peptides of the polymyxin and the negatively charged LPS.[2] In addition, complete loss of LPS in *A. baumannii* has been reported as a mechanism of bacterial resistance.[10] In 2016, researchers in China identified the first known plasmid-mediated mechanism of colistin resistance, MCR-1,[11] and this has since been

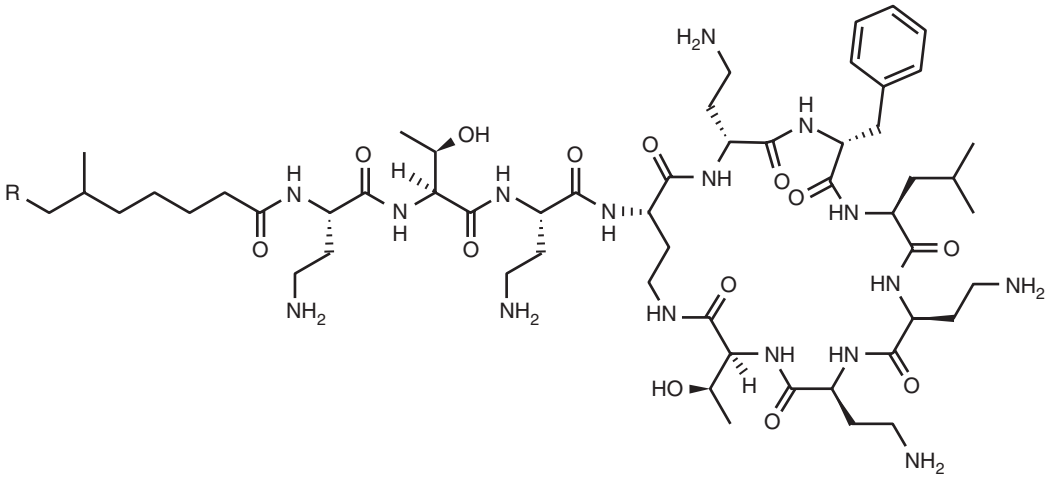

FIG. 32.1 Polymyxin B.

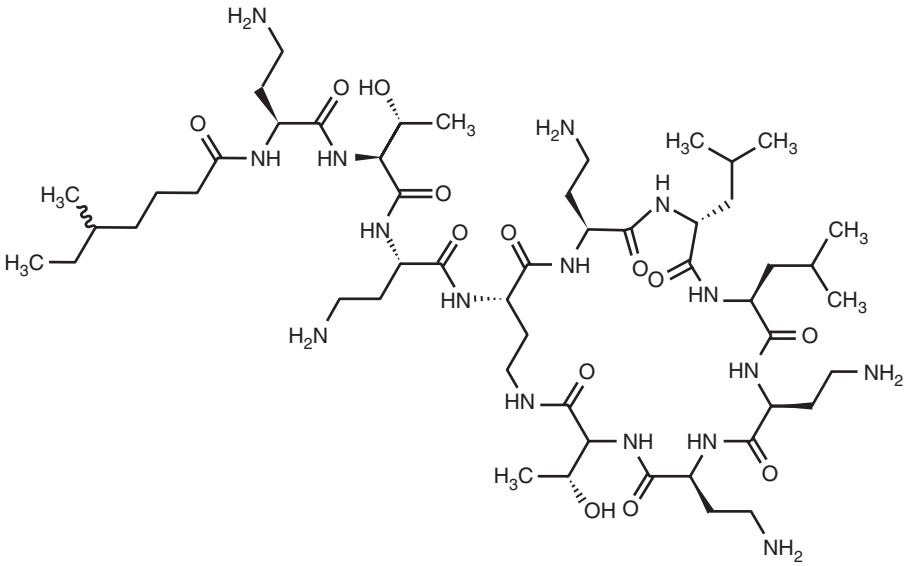

FIG. 32.2 Colistin.

isolated from clinical specimens worldwide. Two other plasmids mediating colistin resistance, MCR-2 and MCR-3, have also been described.

ANTIMICROBIAL ACTIVITY

The polymyxins are active against a broad array of gram-negative aerobic bacilli, with the notable exception of *Proteus* species, most of which are highly resistant. They also have poor activity against *Providencia, Burkholderia, Serratia, Moraxella, Helicobacter, Campylobacter, Vibrio, Brucella, Aeromonas, Morganella,* and *Edwardsiella* species.[2] Gram-positive organisms, gram-negative cocci, and most anaerobes are resistant. The antibacterial activity of the polymyxins is decreased by the presence of divalent cations such as calcium and magnesium. The polymyxins have retained activity against many MDR gram-negative bacilli, such as *P. aeruginosa* and *A. baumannii,* and CRE, with a minimal inhibitory concentration for 90% of isolates (MIC_{90}) often less than or equal to 2 μg/mL. Importantly, however, an increasing number of publications are reporting polymyxin resistance rates exceeding 10%, and, perhaps most alarming, are reports from regions where CRE are epidemic, where more than half of the CRE isolates tested are polymyxin resistant.[12] Furthermore, heteroresistance is common in polymyxin-susceptible strains of these organisms.[13–15] There is complete cross-resistance between different polymyxins.

There is a minor lack of uniformity between susceptibility breakpoints of the Clinical and Laboratory Standards Institute (CLSI) and those of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). CLSI has a susceptibility breakpoint for colistin of less than or equal to 2 μg/mL for *P. aeruginosa* and *A. baumannii* but only an epidemiologic cutoff for Enterobacteriaceae (also ≤2 μg/mL). This is in slight contrast to EUCAST, which has clinical breakpoints for colistin susceptibility to each of the aforementioned pathogens of 2 μg/mL. There are no formal breakpoints for polymyxin B, and often clinical laboratories do not have the capability to test polymyxin B susceptibility. This often leads to colistin minimal inhibitory concentrations (MICs) and breakpoints serving as surrogates for polymyxin B. However, data are lacking to support this strategy. Furthermore, there are significant concerns regarding the appropriateness of these breakpoints in light of recently published pharmacokinetic/pharmacodynamic (PK/PD) data (see next two sections).

PHARMACOKINETICS

Neither colistin nor polymyxin B is absorbed when given orally, and oral usage is reserved for selective gut decontamination or decolonization strategies. Distribution of polymyxins to the cerebrospinal fluid, biliary tract, pleural fluid, and joint fluid is poor. The polymyxins are readily dialyzed. Serum levels of the polymyxins are very low after inhalation therapy in cystic fibrosis patients.[16] However, one analysis reported detectable systemic levels in patients receiving CMS at 5 million IU by inhalation every 8 hours.[17]

Pharmacokinetic parameters of intravenous CMS and polymyxin B from the "old" literature are poorly described, and this poor description

TABLE 32.1 Package Insert Dosing Recommendations for Polymyxins

POLYMYXIN PREPARATION	DOSING RECOMMENDATIONS			
	CREATININE CLEARANCE			
	≥ 50 mL/min	30–49 mL/min	10–29 mL/min	<10 mL/min
EMA CMS package insert (based on IU of CMS)	9 MIU (300 mg CBA)/d divided in 2–3 doses	5.5–7.5 MIU (182–248 mg CBA)/d divided in 2 doses	4.5–5.5 MIU (149–182 mg CBA)/d divided in 2 doses	3.5 MIU (116 mg CBA)/d divided in 2 doses
		PLASMA CREATININE		
		0.7–1.2 mg/100 mL	1.3–1.5 mg/100 mL	1.6–2.5 mg/100 mL	2.6–4.0 mg/100 mL
US CMS package insert (based on mg/kg IBW of CBA)[a]	Urea clearance (% of normal)	80–100	40–70	25–40	10–25
	Unit dose of CMS (in mg of CBA)	100–150	75–115	66–150	100–150
	Total daily dose	300 mg divided in 2–4 doses	150–230 mg divided in 2 doses	133–150 mg in 1 or 2 doses	100 mg q36h
	Approximate daily dose,	5 mg/kg/d[b]	2.5–3.8 mg/kg/d	2.5 mg/kg/d	1.5 mg/kg/d
	Normal Dose	CrCl 20–50 mL/min	CrCl 10–20 mL/min	CrCl <10 mL/min *or* Hemodialysis	
Polymyxin B[c] package insert	1.5–2.5 mg/kg/d divided q12h	This amount should be reduced from 15,000 units/kg downward for individuals with kidney impairment.			

[a]Colistin: 1 million IU = 33 mg CBA.
[b]Package insert gives a range of 2.5–5 mg/kg, but the package insert table only lists 5 mg/kg/d.
[c]Polymyxin B: 1 mg = 10,000 IU.
CBA, Colistin base activity; *CMS,* colistimethate sodium; *IBW,* ideal body weight; *IU,* international units; *M,* million.

is one of the major reasons that current package insert dosing regimens are difficult to understand, and, in many cases, inappropriate and inadequate. Modern-day pharmacokinetic data have begun to unravel the mystery surrounding colistin and to a lesser degree polymyxin B pharmacokinetics, and important differences have emerged. Colistin has much more complex pharmacokinetics due to the fact that it is administered in the form of its inactive prodrug methanesulfonate (CMS). It is important to realize that CMS is considered to have no antimicrobial activity until it is hydrolyzed to colistin. The competing elimination of CMS via both hydrolysis to active colistin and renal clearance, which are described in detail next, leads to an extremely complicated and inefficient pharmacokinetic profile for colistin. As is shown next, polymyxin B offers the advantage of much simpler pharmacokinetics due to administration as its active moiety.

Colistimethate Sodium

CMS package insert dosing recommendations differ significantly with regard to which preparation is selected and, with the exception of the recently modified European Medicines Agency (EMA) package insert, are based on levels obtained by nonspecific microbiologic assays of biologic fluids. These assays were unable to differentiate the inactive prodrug, CMS, from the active moiety, colistin. Recent evidence has shown that only approximately 20% of the sum of CMS and colistin in serum is active colistin.[18]

When comparing recommended doses of CMS from different manufacturers, it is important to know that 1 million international units (MIU) equals 80 mg of CMS. It is also essential to understand that although the US product comes in vials containing approximately 360 mg of CMS, the vials are labeled as containing 150 mg "colistin base activity" (CBA), which is equal to approximately 4.5 MIU. In the literature, because of the US labeling, 1 MIU has been considered as equal to approximately 33 mg of CBA. However, the term *colistin base activity* has been challenged as inaccurate because there is no directly available "colistin base" in the vials, but the term is used in this chapter because so much of the literature uses it.[19] The recommended US package insert dosage of CMS for patients with normal renal function is 2.5 to 5 mg/kg of ideal body weight of CBA each day, not to exceed 300 mg CBA daily. Although the package insert limits the daily dose to 300 mg CBA, this limit is often ignored in patients greater than 60 kg in whom doses greater than 300 mg CBA are required to give 5 mg/kg of CBA. In terms of IU, this recommended dosage equates to about 4.5 to 9 MIU/day in a 60-kg person and about 5.3 to 10.6 MIU/day in a 70-kg person, if the 300-mg (9-MIU) limit is ignored. The EMA has updated its package insert based on recent pharmacokinetic evidence. In addition to recommending a 9-MIU (300-mg CBA) loading dose in critically ill patients (rationale described later), they recommend a daily maintenance dose of 9 MIU (300 mg CBA) divided two to three times a day in patients with normal renal function, with a suggestion that doses up to 12 MIU (about 400 mg CBA) might be needed in patients with "good renal function."[20] There are virtually no data available pertaining to dose adjustments in obese patients, and optimal dosing to use in this patient population remains undefined.

Recommendations in the package insert for the dose adjustment of the US CMS product in renal insufficiency are based on plasma creatinine, whereas recommendations for the European product are based on creatinine clearance (Table 32.1).

The controversy and confusion with regard to dosing with renal insufficiency has taken on a new dynamic over the past decade because pharmacokinetic analyses have demonstrated that polymyxin elimination is complex. Although colistin, CMS, and polymyxin B all are filtered by the glomeruli, both colistin and polymyxin B undergo extensive tubular reabsorption, have limited renal elimination in the urine, and are largely eliminated by poorly described nonrenal mechanisms.[5,14,21] On the contrary, CMS does not undergo tubular reabsorption and is primarily eliminated in urine.[14]

Two pharmacokinetic analyses with IV CMS in critically ill patients have enhanced understanding of the pharmacokinetics of CMS.[18,22] Plachouras and colleagues[22] demonstrated that with a CMS dose of 3 MIU (~100 mg CBA) every 8 hours, the predicted peak serum concentration (C_{max}) of active colistin occurred approximately 7 hours after each dose and was only 0.6 μg/mL with the initial dose; the half-life of colistin was 14.4 hours, and at steady state (i.e., after 4–5 half-lives), the C_{max} was 2.3 μg/mL. Therefore a loading dose of 9 to 12 MIU (300–400 mg CBA) was recommended to ensure more rapid attainment of therapeutic concentrations. This finding was in marked contrast to all previous package insert dosing recommendations. The second, much larger analysis by Garonzik and colleagues[18] included patients with renal insufficiency. In their analysis, patients received a wide range of daily doses (median CBA dose, 200 mg/day; range, 75–415 mg/day). The average steady-state concentration of colistin was similar in this analysis (median, 2.36 μg/mL), but there was a wide range of the average steady-state colistin concentrations (0.48–9.38 μg/mL). The higher concentrations were

primarily seen in patients with lower creatinine clearance. However, at all levels of creatinine clearance, there was wide interpatient variability of the serum colistin concentrations obtained, varying up to 10-fold in some instances. The authors demonstrated that the half-life in plasma of CMS increased from a median of 4.6 hours in patients with creatinine clearances >70 mL/min to a median of 11 hours in patients with renal insufficiency, whereas the colistin half-life ranged from a median of 9 hours in those with creatinine clearances >70 mL/min to a median of 13 hours in those with creatinine clearances <10 mL/min. The increase in CMS half-life was not surprising because CMS is eliminated via the kidneys. It was unexpected that the colistin half-life also increased in renal insufficiency because the drug is not eliminated in the urine due to extensive tubular reabsorption. The proposed mechanism for the increase in colistin half-life relates to decreased CMS removal secondary to renal insufficiency. Increased CMS concentrations lead to increased hydrolysis and conversion to active colistin. In total, these findings support the need for dose adjustments based on renal function. The authors also confirmed the need for a loading dose and suggested 5 mg/kg of CBA, with a maximum dose of 300 mg CBA.

It is important to note that while renal dose adjustments are warranted for CMS due to the reasons just outlined, the package insert recommendations for how to accomplish this differ substantially between countries. Compared to the EMA package insert, the US package insert significantly underperforms with regard to its ability to achieve target concentrations due to overreductions in dose when creatinine clearances are <30 mL/min. This underdosing effect is shown in a recent publication in which the authors demonstrate that using the US package insert dosing recommendations in patients with creatinine clearances <30 mL/min would attain the target steady-state concentrations of 2 µg/mL or greater only 10% to 30% of the time.[6]

In early 2017 these same investigators developed a dosing algorithm based on the final results of a National Institutes of Health–funded study.[7] These recommendations are largely in line with the EMA package insert doses and were derived to provide an 80% likelihood of achieving a colistin average steady-state concentration of 2 µg/mL. This target concentration is based on pharmacodynamic data from the mouse thigh model (see "Pharmacodynamics" later) and current susceptibility breakpoints, as well as toxicodynamic data associating average steady-state concentrations of higher than 2 µg/mL with an increased incidence and severity of nephrotoxicity.[23,24] While the target concentration of 2 µg/mL was determined by considering both safety and efficacy, it remains unclear whether this target is optimal (and whether EUCAST and CLSI breakpoints are optimal). It is also important for clinicians to be aware that when patients are described as having "good renal function," often considered to be creatinine clearances ≥80 mL/min, *all* dosing algorithms underperform with regard to attaining the target concentration.[6] This is likely due to enhanced clearance of the prodrug CMS prior to conversion to the active moiety colistin, resulting in subsequent subtherapeutic concentrations of colistin.

Polymyxin B

One milligram of polymyxin B equals 10,000 IU. Although dosing for polymyxin B is simpler and is the same in the United States and elsewhere, no pharmacokinetic rationale for the dosing recommendation is provided in the current package insert, and the dosing recommendation appears to be empirical in nature. The recommended IM dose is 2.5 to 3 mg/kg/day (25,000–30,000 IU/kg/day) in divided doses every 4 to 6 hours, but because IM polymyxin injections cause severe pain, the drug should not be used IM. The recommended IV dose is 1.5 to 2.5 mg/kg/day (15,000–25,000 IU/kg/day) by continuous IV infusion or in divided doses every 12 hours, infused over a period of 60 to 90 minutes.[25] Importantly, however, given the upper range of 3 mg/kg for IM dosing, some experts recommend 3 mg/kg as the upper limit of IV polymyxin B doses. While our knowledge remains limited, recent data have helped to clarify the pharmacokinetics of polymyxin B.[5,21] An analysis of eight critically ill patients given doses of 0.5 to 1.5 mg/kg in 60-minute IV infusions reported peak serum levels ranging from 2.4 to 13.9 µg/mL at the end of the infusion.[21] In a larger study of 24 critically ill patients, dosing by total body weight was found to be superior to dosing by ideal body weight, and the authors suggested that a loading dose would be required to provide an adequate area under the curve (AUC) exposure on day 1.[5] In that study, the half-life of polymyxin B in plasma was 11.9 hours. Based on modeling from these data, average predicted steady-state concentrations with polymyxin B using 2.5- to 3-mg/kg/day dosing would be 3.0 and 3.6 µg/mL, respectively.[16]

The current polymyxin B package insert has no specific recommendations for dose adjustment, and merely states that the dose "should be reduced from 15,000 units/kg/day downward for individuals with kidney impairment."[25] As mentioned earlier, polymyxin B undergoes extensive tubular reabsorption and is not eliminated in urine. The fact that only a small portion is recovered unchanged in urine suggests that, despite package insert recommendations and current practices, dose adjustments in renal insufficiency are not warranted.[5,21] Clinical data support not requiring renal dose adjustments, because doses <1.3 mg/kg/day were associated with increased mortality from infection; the majority of these lower doses were due to unnecessary adjustments in patients with renal insufficiency.[26]

PHARMACODYNAMICS

In vitro, the polymyxins are rapidly bactericidal in a concentration-dependent manner. There is a postantibiotic effect for *P. aeruginosa* but not for *A. baumannii* or *Klebsiella pneumoniae*.[27,28] There is rapid in vitro regrowth of *A. baumannii* and *K. pneumoniae* because of heteroresistance; regrowth also occurs with *P. aeruginosa* but is delayed.[2,13,27,28] The AUC/MIC ratio appears to be the pharmacodynamic parameter that is best associated with bactericidal activity. Data from a neutropenic mouse thigh model suggest that free AUC/MIC exposures of approximately 7 to 17 are associated with 1 to 2 log kill in both *A. baumannii* and *P. aeruginosa*.[29] These data serve as an important foundation for the recommendation that the target average steady-state concentration should be 2 µg/mL. Once protein binding of approximately 50% is taken into account, the average AUC/MIC ratio needed for bactericidal activity is approximately 24 (free AUC/MIC of 12 is a total AUC/MIC of 24 when 50% protein binding is considered). Given the current susceptibility breakpoint of 2 µg/mL, this would suggest the need for a total daily AUC ($AUC_{(0\text{-}24)}$) of 48 mg•h/L (48/2 = 24), which equates to an average steady-state concentration of 2 µg/mL. It is important to note, however, that data from mouse lung infection models are much less encouraging and suggest the need for much higher AUC targets for treatment of *P. aeruginosa* pneumonia. Perhaps even more concerning, even at the highest tolerable dose, bacteriostasis was not obtained in the mouse lung model for *A. baumannii* pneumonia. In addition, concerns exist for the ability to safely achieve higher concentrations, and whether these dosing recommendations and clinical breakpoints are appropriate remains unsettled. For these reasons, it has been suggested that polymyxins be used in combination with another active or synergistic drug.

TOXICITY

Hypersensitivity is unusual. There is dose-related nephrotoxicity, which is often, but not always, reversible after discontinuing the drug. Rates of nephrotoxicity vary greatly in the literature, in part because different definitions of nephrotoxicity are used and because critically ill patient populations are often studied. Modern analyses report rates of nephrotoxicity, as defined by the RIFLE criteria (*r*isk, *i*njury, *f*ailure, *l*oss, *e*nd-stage renal disease), in the 30% to 60% range for both agents.[30–32] Recent evidence has suggested that colistin might have higher rates of nephrotoxicity than polymyxin B.[4] There is also dose-related reversible neurotoxicity manifested by neuromuscular blockade, which can result in muscle weakness and even apnea. Neuromuscular blockade may occur with drug overdosage and in patients with renal insufficiency or those who are receiving curariform drugs, although this is rare. With respiratory paralysis, support of respiratory function is required until the effects of the polymyxin wear off. Intravenous calcium may help reverse apnea. Aminoglycosides may potentiate the neurotoxic effects.

Paresthesias around the lips, tongue, and extremities, as well as peripheral neuropathy and other neurotoxic side effects, are not uncommon.[1] Recent reports have suggested an association between polymyxin B and skin hyperpigmentation.[33,34] Intrathecal and intraventricular administration have generally been well tolerated.

TABLE 32.2 Authors' Dosing Recommendations for Polymyxins Based on Newer PK/PD Information

POLYMYXIN PREPARATION	LOADING DOSE	DOSING RECOMMENDATIONS			
		CrCl ≥50 mL/min	CrCl 30–49 mL/min	CrCl 10–29 mL/min	CrCl <10 mL/min *or* Hemodialysis
		Start 8 hr After Loading Dose	Start 12 hr After Loading Dose	Start 12 hr After Loading Dose	Start 24 hr After Loading Dose
CMS (in mg/kg IBW of CBA): daily dose can exceed 300 mg CBA[a]	5 mg/kg × 1 dose (maximum 300 mg CBA)	5 mg/kg/d divided q8h	3.5 mg/kg/d divided q12h	2.5 mg/kg/d divided q12h	1.5 mg/kg q24h[b]
		Start 12 hr After Loading Dose			
Polymyxin B[c] (in mg/kg ABW)	2.5 mg/kg × 1 dose	2.5 mg/kg/d divided in two equal daily doses[d]			

[a]Colistin: 1 million international units (MIU) = 33 mg CBA.
[b]Colistin and colistimethate are efficiently removed via hemodialysis; therefore doses should be given after dialysis on dialysis days.
[c]Polymyxin B: 1 mg = 10,000 IU.
[d]At the time of publication, the best evidence suggests that renal dose adjustments are unnecessary. Therefore the authors recommend not reducing the dose in the setting of renal insufficiency.[5,21]
ABW, Actual body weight; *CBA,* colistin base activity; *CrCl,* creatinine clearance; *CMS,* colistimethate sodium; *IBW,* ideal body weight; *PK/PD,* pharmacokinetic/pharmacodynamic.

CLINICAL USE

Package insert recommendations for the use of CMS and polymyxin B are listed in Table 32.1. Our recommendations, based on the newer PK/PD information, are found in Table 32.2. For CMS, we find it reasonable for US practitioners to follow the EMA package insert dosing recommendation because it is driven by recent PK/PD data. However, if a clinician prefers to use weight-based dosing, we suggest the renal dosing recommendations found in the Table 32.2. These were extrapolated from the pharmacokinetic data on which the most recent EMA dosing recommendations were based and should provide similar exposures for patients with advanced as well as moderate renal dysfunction. For CMS, 30-minute infusions are commonly used. Assuming normal renal function, we recommend that after giving the loading dose, there should be an every-8-hour administration schedule of 5 mg/kg/day CBA, as detailed in Table 32.2. The EMA is the only label that has pediatric dose recommendations and suggests 75,000 to 150,000 IU/kg/day (2.5–5 mg/kg CBA/day) for patients ≤40 kg.

For polymyxin B, based on recent pharmacokinetic data,[5] we recommend the upper end of the package insert dosing recommendation (2.5 mg/kg/day divided every 12 hours, infused over 60 minutes). In addition, we recommend a loading dose (2.5 mg/kg × 1) and suggest not making any dose reductions based on renal insufficiency.

For treatment of gram-negative bacillary meningitis, polymyxin B has been given intrathecally in doses of 5 to 10 mg/day for the initial 3 days of therapy and then every other day. CMS has been given via the intrathecal and intraventricular routes. The Infectious Diseases Society of America recommends that a CBA dose of 10 mg/day be used via the intraventricular route to treat gram-negative bacillary meningitis.[35] The EMA package insert recommends 125,000 IU/day (~4 mg CBA/day) for intraventricular use. Oral colistin sulfate and polymyxin B have been used for intestinal decontamination. The oral preparation of colistin is not available in the United States.

Inhalation therapy with aerosolized CMS has been used with varying results to treat colonization or infection of the bronchial system in patients with cystic fibrosis, especially with MDR *P. aeruginosa*, often in conjunction with systemic therapy.[36,37] The recommended dose of aerosolized CMS in the EMA is 1 to 2 MIU (33–66 mg CBA) three times daily for all patients ≥2 years of age and 0.5 to 1 MIU (17–33 mg CBA) twice daily for those less than 2 years of age. Although there are no formal US recommendations, clinicians often empirically use a CBA dose of 75 to 150 mg every 12 hours. Recent data suggest that some systemic absorption can occur with inhaled CMS. Nebulization therapy may cause bronchoconstriction; to minimize the risk of bronchoconstriction, CMS should not be premixed until immediately before therapy.[38]

CMS and polymyxin B have been used parenterally to treat systemic infections caused by MDR gram-negative bacilli, often ventilator-associated pneumonia.[36,37,39,40] In general, rates of response to therapy have been similar in patients receiving a polymyxin, compared with patients receiving piperacillin, imipenem, and ciprofloxacin for the treatment of pneumonia caused by *P. aeruginosa*.[36] However, there are conflicting data suggesting inferior outcomes of both polymyxin agents when compared with other antimicrobial classes.[41–43] Use of suboptimal dosing strategies has likely contributed to the poor performance of the polymyxins in some of these studies. Emerging data with novel β-lactam–β-lactamase inhibitor combinations and new aminoglycosides suggest that dose-optimized CMS is clinically inferior to these newer therapies.[44,45] Superinfection with colistin-resistant strains of *Serratia marcescens* and *Stenotrophomonas maltophilia* has been reported.[46]

Because of high toxicity rates that limit the ability to safely achieve PK/PD targets, a still undefined optimal dosing strategy, and questionable current breakpoints for susceptibility, parenteral CMS and polymyxin B should be reserved for use when no other less toxic or potentially more effective drug is available. For serious infections, whenever possible, another agent, preferably one that demonstrates in vitro activity or synergism with polymyxins, should be added to the polymyxin. Because of the more reliable pharmacokinetics and perhaps less toxicity, polymyxin B should be preferred over CMS where it is available, with the exception of urinary tract infection, for which CMS is preferred.

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The complete reference list is available online at Expert Consult.

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33 Linezolid, Tedizolid, and Other Oxazolidinones

Heather L. Cox and Gerald R. Donowitz

SHORT VIEW SUMMARY

- Linezolid and tedizolid are the first two oxazolidinones approved for clinical use by US Food and Drug Administration (FDA).

Linezolid

- FDA-approved for use in adults and children for the following infections caused by susceptible bacteria:
  - Infection with vancomycin-resistant *Enterococcus faecium*
  - Community-acquired and nosocomial pneumonia
  - Uncomplicated skin and skin structure infections
  - Complicated skin and skin structure infections including diabetic foot infections (without osteomyelitis)
- *Unlabeled use:* alternative/adjunctive therapy of mycobacterial and *Nocardia* infections
- *Usual oral or intravenous adult dose:* 600 mg every 12 hours with no dosage adjustment for renal or hepatic dysfunction
- *Drug interactions:* serotonergic and adrenergic agents due to monoamine oxidase inhibition
- *Serious adverse effects:* hematologic toxicity (most commonly thrombocytopenia), lactic acidosis, peripheral and optic neuropathy
- *Resistance:* uncommon but most often associated with 23S RNA G2576T point mutation or acquisition of *cfr* ribosomal RNA methyltransferase; clinically related to previous or prolonged drug exposure or horizontal spread

Tedizolid

- FDA-approved for acute bacterial skin and skin structure infections in adults caused by susceptible isolates of gram-positive microorganisms including *Staphylococcus aureus* (including methicillin-susceptible and methicillin-resistant strains), *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus anginosus* group, and *Enterococcus faecalis*
- *Usual oral or intravenous adult dose:* 200 mg daily
- *Drug interactions:* serotonergic and adrenergic agents due to monoamine oxidase inhibition possible, but less likely than with linezolid
- *Serious adverse effects:* similar to linezolid but less likely
- *Resistance:* mechanisms of resistance are similar to those of linezolid, but resistance occurs less commonly; may demonstrate activity against linezolid-resistant strains of *S. aureus,* coagulase-negative staphylococci, and enterococci with the *cfr* gene

The oxazolidinones are a class of antimicrobial agents prepared completely by organic synthesis. In 1978, a patent was issued to E. I. du Pont de Nemours and Company for a series of 5-(halomethyl)-3-aryl-2-oxazolidinones that had antimicrobial activity against plant pathogens. Further manipulation of the molecule led to the development of linezolid, which displayed activity against human pathogens.[1] A number of oxazolidinones are being investigated. Only linezolid (Zyvox) and tedizolid (Sivextro) are approved by the US Food and Drug Administration (FDA) for clinical use.

CHEMICAL STRUCTURE

The basic molecular structure of the oxazolidinones is shown in Fig. 33.1A.[2] The structure-function relationships of these compounds have been reviewed recently.[3,4,5] Manipulation of the A-ring at the C5 position and manipulation of the N-aryl B ring are necessary elements of oxazolidinone antibacterial activity. Fluorination of the B-ring further increases activity. In linezolid (Fig. 33.1B), the acetamide moiety at the C5 position of the A-ring adds to the overall activity of the drug. Tedizolid (Fig. 33.1C) instead contains a hydroxymethyl group at the same position, which serves to preserve activity against linezolid-resistant organisms carrying the *cfr* gene. The favorable minimal inhibitory concentration (MIC) profile of tedizolid is associated with the pyridine (C-ring) and tetrazole (D-ring) moieties. The unique chemical structure of oxazolidinones renders cross-resistance with β-lactams, vancomycin, quinupristin-dalfopristin, and daptomycin unlikely.

MECHANISM OF ACTION

The oxazolidinones are inhibitors of protein synthesis and are usually bacteriostatic, although in some models, bactericidal activity against some organisms has been observed. The mechanism of action is believed to be unique, involving inhibition of the earliest steps of bacterial protein synthesis.[6] These agents bind to the V-domain of the 23S RNA component of the 50S ribosomal subunit. Interaction of oxazolidinones and the A-site of the peptidyl transferase center blocks peptide elongation.[2,7] Binding is competitively inhibited by chloramphenicol and lincomycin, which suggests either shared or overlapping binding sites.[6]

ANTIMICROBIAL ACTIVITY

General Considerations

In vitro assays have established the MICs of linezolid and tedizolid against a variety of organisms. In general, comparisons have shown tedizolid to be two to eight times more potent.[2,3,4,5,8] It is important to recognize that this may not reflect improved clinical activity. Efficacy of both oxazolidinones seems most directly related to the drug concentration area under the curve (AUC)/MIC ratio. Although tedizolid does in fact have lower MICs for most organisms tested, linezolid has higher AUC values, making the AUC/MIC ratio for these drugs similar.[4] Only direct clinical comparisons will be able to determine if one oxazolidinone is actually more effective than another in a specific clinical situation.

Activity Against Gram-Positive Organisms

Both linezolid and tedizolid demonstrate consistent activity against most clinically important gram-positive organisms, such as *Staphylococcus aureus* (methicillin-susceptible and methicillin-resistant strains and vancomycin-intermediate and vancomycin-resistant strains); coagulase-negative staphylococci; *Enterococcus faecalis* and *Enterococcus faecium* (vancomycin-susceptible and vancomycin-resistant strains); and streptococci, including penicillin-resistant *Streptococcus pneumoniae* (Table 33.1).[2,4,8,9,10,11,12,13] Tedizolid has consistently lower MICs for these organisms.

A variety of other gram-positive organisms may be susceptible to linezolid or tedizolid, although fewer isolates have been evaluated. These include *Corynebacterium* spp., *Listeria monocytogenes, Bacillus* spp., *Micrococcus* spp., *Erysipelothrix rhusiopathiae, Leuconostoc* spp., *Rhodococcus equi,* and *Pediococcus* spp.[8,14]

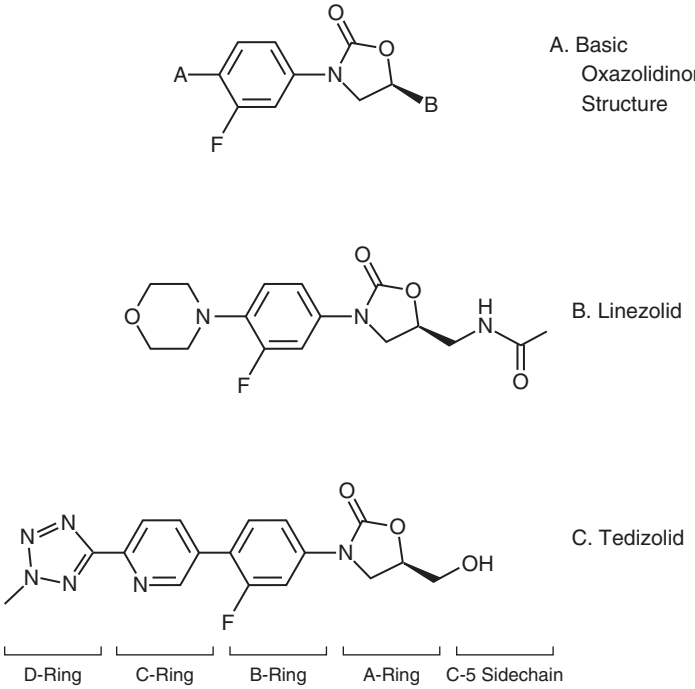

FIG. 33.1 (A) Basic molecular structure of oxazolidinone antibiotics. (B) Structure of linezolid. (C) Structure of tedizolid. *(Modified from Rybak JM, Roberts K. Tedizolid phosphate: a next-generation oxazolidinone.* Infect Dis Ther. *2015;4:1–14.)*

TABLE 33.1 In vitro Susceptibility of Common Aerobic Gram-Positive Organisms to Linezolid and Tedizolid[a]

	LINEZOLID		TEDIZOLID	
ORGANISM	**MIC_{90} (μg/mL)**	**Susceptible (% of Strains)**	**MIC_{90} (μg/mL)**	**Susceptible (% of Strains)**[c]
Staphylococcus aureus				
Oxacillin-susceptible	1–2	100	0.25–0.5	99.9
Oxacillin-resistant	1–2	99.9	0.25–0.5	99.6
Coagulase-negative staphylococci				
Oxacillin-susceptible	0.5–2	99.4	0.25–0.5	—
Oxacillin-resistant	0.5–2	99.1	0.5	—
β-Hemolytic streptococci	1	100	0.25	100
Streptococcus pneumoniae	1	100	0.25	—
Viridans group and other streptococci	1	100	0.25	—
Enterococcus faecalis	2	100	0.5	99.4
Enterococcus faecium				
Vancomycin-susceptible	2	100	0.5	—
Vancomycin-resistant	2	98.5	0.5	—

[a]Data from references 4, 8, 12, and 13.
[b]Clinical and Laboratory Standards Institute susceptibility breakpoints for *S. aureus* are ≤4 μg/mL for linezolid and ≤0.5 μg/mL for tedizolid; for enterococci, ≤2 μg/mL for linezolid and ≤0.5 μg/mL for tedizolid (*E. faecalis* only); for β-hemolytic streptococci, ≤2 μg/mL for linezolid and ≤0.5 μg/mL for tedizolid (*Streptococcus pyogenes* and *Streptococcus agalactiae* only).
[c]Dash indicates no determined breakpoints for susceptibility.
MIC_{90}, Minimal concentration at which 90% of strains are inhibited.

Activity Against Higher Order Bacteria

Linezolid has in vitro activity against a wide number of *Nocardia* spp. with MIC_{90} (minimal inhibitory concentration for 90% of isolates) values of 1 to 4 μg/mL.[15] Virtually all strains tested have proved susceptible. Tedizolid has demonstrated MIC_{90} values several-fold lower than those of linezolid for a number of species with the exception of *Nocardia nova* complex and *Nocardia brasiliensis*, where values were comparable.[16] Limited data suggest that some actinomycete species may be susceptible to linezolid and tedizolid.

Activity Against *Mycobacterium* spp.

The oxazolidinones have demonstrated activity against *Mycobacterium tuberculosis* and a variety of nontuberculous mycobacteria, such as *Mycobacterium avium* complex and *Mycobacterium abscessus* complex, including *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*.[6,17–20] Tedizolid appears to be more potent for the strains tested, but as with other in vitro analysis, the clinical significance of these findings is unclear.[20] With regard to *M. tuberculosis,* the MIC_{90} of linezolid is on the order of 1 to 2 μg/mL with a review showing lower values of

0.125 to 0.5 µg/mL for susceptible, multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains.[6,17–19,21] Limited data using an intracellular model to assess antituberculous activity have demonstrated that tedizolid is effective against a nonresistant strain.[22]

Activity Against Other Organisms

Most aerobic gram-negative organisms have had no demonstrated susceptibility to oxazolidinones, although not all have been studied. Assessment of potential respiratory tract pathogens has shown that both agents provide incomplete coverage for *Haemophilus influenzae* and *Moraxella catarrhalis*.[3,6,23]

Both oxazolidinones possess activity against gram-positive and gram-negative anaerobic organisms including *Clostridium* spp. and *Bacteroides* spp.[8,10,24] In general, gram-positive anaerobes appear more susceptible, with tedizolid demonstrating the same level of in vitro superiority over linezolid as it does with aerobic organisms. The clinical utility of these agents in infections with these organisms is unclear.

RESISTANCE

Resistance to linezolid was reported before the drug was released in the United States among patients treated for vancomycin-resistant enterococci infections as part of the Linezolid Compassionate Use Program.[25] Despite having been marketed in the United States since 2000, resistance to linezolid remains less than 1%.[12] Resistant strains of staphylococci and enterococci have been reported with prior exposure to the drug and long durations of therapy noted as common predisposing factors.[26,27] Resistance in the absence of linezolid exposure has been described particularly in cases of horizontal transmission or in institutions with high linezolid use.[28–32] Although unusual, outbreaks of infection due to linezolid-resistant bacteria are well described.[27–30] Tedizolid resistance does not appear to be as frequent or as inducible as linezolid resistance. Having been released in 2014, extensive longitudinal data on overall resistance rates of tedizolid are lacking.[4,33] However, over a 5-year period from 2009 to 2013, tedizolid inhibited 99.7% of 11,231 gram-positive isolates from the United States and Europe with MICs ≤0.5 µg/mL.[13]

The most frequent mechanism of resistance involves chromosomal mutations to the oxazolidinone binding site on the 23S ribosomal RNA domain V region. Because bacteria have numerous copies of this gene, multiple mutations are necessary for clinical resistance. Additional binding site modifications resulting from mutations in genes encoding ribosomal proteins L3 and L4 have contributed to resistance in staphylococci, *S. pneumoniae,* and *Clostridium perfringens,* among other pathogens.[34–36]

Non–mutation-related resistance has also been identified among strains of bacteria expressing the acquired *cfr* (chloramphenicol-florfenicol resistance) ribosomal RNA methyltransferase that confers resistance to phenicols, lincosamides, pleuromutilins, and streptogramin A antibacterial agents in addition to the oxazolidinones.[37] The molecular structure of tedizolid allows it to retain antimicrobial activity against some isolates of linezolid-resistant *S. aureus,* coagulase-negative staphylococci, and enterococci carrying the *cfr* gene. However, tedizolid resistance may occur when the *cfr* gene is present in combination with chromosomal mutations associated with linezolid resistance.[4] More recently, a new plasmid-mediated resistance gene, *optrA,* has been identified as a source of linezolid and tedizolid resistance in isolates of *E. faecalis* and *E. faecium*.[38] The gene appears to encode an adenosine triphosphate–binding cassette, giving it a different substrate than the other mechanisms of drug resistance. Initially identified in China, it remains unclear how common this resistance gene is worldwide, which bacterial species may contain it, and how important it is clinically.

PHARMACOLOGY

Linezolid

The approved dose of linezolid for adults and adolescents is 600 mg intravenously or orally every 12 hours for serious infections, with a dose of 400 mg every 12 hours for adults with uncomplicated skin and soft tissue infections. Absorption after ingestion is rapid, with peak serum levels occurring after 1 to 2 hours and bioavailability approaching 100%. The most common dosing regimen of 600 mg intravenously or orally twice daily generates peak serum levels of 15.1 µg/mL and 21.2 µg/mL, respectively.[39] These concentrations represent data from small numbers of healthy volunteers and likely do not represent significant differences between formulations.

Linezolid is 31% bound to plasma proteins. It readily distributes into well-perfused tissues, and penetration into various body sites has been documented in small numbers of patients.[39] Trough cerebrospinal fluid concentrations in patients after neurosurgery and in a patient with meningitis ranged from 1.46 to 7.0 µg/mL with cerebrospinal fluid/plasma ratios greater than 1.[40,41] Peak cerebrospinal fluid concentrations of 3.12 to 12.5 µg/mL have been documented in patients with meningitis.[41,42] Concentrations adequate to treat most relevant pathogens are achievable in pulmonary epithelial lining fluid, alveolar cells, skin, and soft tissue. Bone and joint tissue penetration is more variable.[43]

Linezolid is metabolized by oxidation and appears to interact minimally with cytochrome P-450 enzymes. Urinary excretion accounts for approximately 85% of drug elimination, with 30% as unchanged parent drug. Fecal excretion of its two inactive carboxylic acid metabolites accounts for most of the drug remaining.[39,43] The elimination half-life in adults ranges from 3 to 7 hours.[43] No dose adjustment has been suggested for patients with renal or hepatic insufficiency, but as linezolid and its metabolites are removed by dialysis, administration after hemodialysis is suggested.[39] Similarly, continuous renal replacement therapies also remove linezolid, but no routine change in dosage has been definitively recommended.[44] A prospective, observational study of 30 critically ill patients, some of whom required continuous renal replacement therapies or extracorporeal lung assistance, documented wide variability in serum concentrations that could predispose to clinical failure or toxicity. Dose optimization via therapeutic drug monitoring was proposed.[45] Results from a retrospective analysis of linezolid use in hospitalized patients also recommended therapeutic drug monitoring to optimize therapy, where creatinine clearances of ≤40 mL/min and ≥100 mL/min were associated with overexposure and underexposure, respectively, in multivariate analysis.[46]

Pharmacokinetic and pharmacodynamic parameters most predictive of efficacy are the time above MIC (T > MIC) and AUC/MIC ratio.[43] A study of intermittent versus continuous infusion of linezolid in 18 critically ill patients showed that a continuous infusion was more likely to achieve proposed targets of T > MIC exceeding 85% and AUC/MIC values between 80 and 120. However, the study was not designed to determine clinical efficacy.[43,47]

Linezolid shows some potential for drug-drug interactions. When administered to healthy volunteers in combination with rifampin, a 32% decrease in its AUC was observed.[48] Levothyroxine has resulted in reduced linezolid concentrations as well.[49] Conversely, the addition of clarithromycin produced a more than threefold increase in the linezolid AUC in a patient receiving treatment for XDR *M. tuberculosis*.[50] Some authors have proposed that these effects may be explained by linezolid acting as a P-glycoprotein substrate.[50,51] Linezolid overexposure has also been documented with concomitant administration of amlodipine, amiodarone, and omeprazole, whereas an increase in the international normalized ratio was observed among 6 patients receiving warfarin following cardiac surgery.[52,53] Drug-drug interactions with serotonergic and adrenergic agents are discussed later in "Untoward Reactions."

Tedizolid

Tedizolid phosphate (TR-701) is a prodrug converted in vivo by plasma phosphatases to active tedizolid (TR-700) following oral and intravenous administration. The phosphate group enhances aqueous solubility, whereas an absolute bioavailability of 91% permits no dosage adjustments between formulations. Peak serum levels following 200 mg oral and intravenous doses are 1.8 to 2.4 µg/mL and 2.6 to 3.5 µg/mL, respectively. Its half-life of approximately 12 hours supports once-daily dosing, and the oral product can be taken without regard to meals. Plasma protein binding is 70% to 90%, whereas adipose and skeletal muscle tissue penetration results in exposure similar to free drug in plasma. Relative pulmonary distribution in healthy volunteers was higher with AUC ratios of penetration into epithelial lining fluid and alveolar macrophages versus plasma of 40 and 20, respectively. Tedizolid is primarily metabolized by the liver and eliminated in feces as an inactive sulfate conjugate with only 18% excreted unchanged in urine. Pharmacokinetic parameters

are similar for patients with hepatic impairment, renal insufficiency (including patients on hemodialysis), and in the setting of obesity (body mass index ≥30 kg/m^2).[3,54,55] Thus no dosage adjustment has been deemed necessary for any of these special populations.

The free AUC/MIC ratio most closely predicted tedizolid efficacy in murine models of infection. However, markedly decreased antistaphylococcal activity was observed compared with linezolid in granulocytopenic mice,[56] emphasizing that its safety and efficacy in the setting of neutropenia have not been established. Higher tedizolid exposure for achievement of similar effects in a murine model of pneumococcal lung infection was also required in the absence of granulocytes, although the difference was much less pronounced.[57]

Tedizolid does not appreciably interact with cytochrome P-450 enzymes but is known to inhibit intestinal breast cancer resistance protein (BCRP) and increase serum concentrations of orally administered BCRP substrates. For example, coadministration of tedizolid increased the maximum concentration of the BCRP substrate, rosuvastatin, by 55%, and its AUC by 70%.[54] Drug interaction potential with serotonergic and adrenergic agents is discussed later in the chapter.

CLINICAL USE

Linezolid

Linezolid was approved by the FDA in April 2000 for adults and children with a variety of clinical infections involving susceptible gram-positive organisms. Current FDA-approved indications include (1) nosocomial pneumonia caused by methicillin-resistant (MRSA) or methicillin-susceptible (MSSA) *S. aureus* or *S. pneumoniae;* (2) community-acquired pneumonia caused by *S. pneumoniae* including cases with concurrent bacteremia or MSSA; (3) complicated skin and skin structure infections including diabetic foot infections without concomitant osteomyelitis caused by *S. aureus* (MRSA or MSSA), *Streptococcus pyogenes,* or *Streptococcus agalactiae;* (4) uncomplicated skin and skin structure infections caused by MSSA or *S. pyogenes;* and (5) vancomycin-resistant *E. faecium* infections including those with concurrent bacteremia.[39]

Tedizolid

Tedizolid has been approved for clinical use since 2014 for use in adults with skin and skin structure infections caused by susceptible organisms including MRSA and MSSA, *S. pyogenes, S. agalactiae*, *Streptococcus anginosus* group, and *E. faecalis*.[54] Therapy with linezolid and tedizolid for infections caused by specific organisms is described in the following paragraphs.

Staphylococcus aureus Including Methicillin-Resistant *S. aureus*

Linezolid

Skin and skin structure infections are the most common staphylococcal infections for which the oxazolidinones are used. MRSA has received the most attention because effective antibiotic choices are more limited.

Linezolid has been shown to be as effective or superior to other antibiotics in this patient group. In a Cochrane review of 9 of 33 trials comparing linezolid versus vancomycin for skin and soft tissue infections, linezolid was shown to produce superior clinical and microbiologic cure rates as well as shorter hospital stays and lower overall cost.[58] Subgroup analysis revealed that this superiority was true for both MRSA and MSSA but was observed only in adults and not in patients <18 years of age. Overall, the studies analyzed were thought to be of poor methodologic quality with potential for publication bias, suggesting that further investigation is needed to more clearly establish the superiority of any given regimen over another.[58] Adverse effects may also be more common with linezolid.[14]

Another major area of interest for linezolid use has been for treatment of nosocomial pneumonia involving MRSA. In pooled data from two randomized, double-blind studies, linezolid in combination with aztreonam proved equivalent to vancomycin plus aztreonam for treatment of nosocomial pneumonia in which S. *aureus* (both MSSA and MRSA) and *S. pneumoniae* were the most commonly isolated gram-positive pathogens.[59] Subgroup analysis revealed that linezolid was associated with significantly higher cure rates and improved survival when considering only patients treated for MRSA infection. The subgroup analysis and therefore the results of this study have been questioned.[60]

When the same authors conducted a prospective, double-blind, multicenter study of linezolid versus vancomycin, they again found that linezolid was superior. However, as more patients randomly assigned to vancomycin were mechanically ventilated and had MRSA bacteremia, and the median vancomycin serum trough concentrations on day 3 were suboptimal, questions regarding the validity of the findings were again raised.[61]

In the most recent of several meta-analyses comparing linezolid with vancomycin, one looking specifically at MRSA nosocomial pneumonia and including nine randomized controlled studies, no differences in clinical or microbiological efficacy or all-cause mortality were noted.[62] There were no significant differences in adverse events including gastrointestinal toxicity and thrombocytopenia between the groups studied. The only significant difference in toxicity between groups was the higher incidence of nephrotoxicity in the vancomycin-treated patients, a finding that was noted previously.[61] Although none of the studies has been viewed as definitive, this analysis seems to be the most complete at the present time.

Although levels of evidence vary, guidelines for the treatment of MRSA infection in adults and children recommend linezolid as initial or alternative therapy for complicated skin and soft tissue infections, pneumonia, bone and joint infections, and central nervous system infections and for select patients with persistent bacteremia, vancomycin treatment failure, or both.[63] Several studies have suggested that linezolid may be effective salvage therapy in patients with MRSA bacteremia who fail to respond to vancomycin; however, this use has not been clearly established.[64,65] Linezolid use in endovascular infections with MRSA including endocarditis has yielded inconsistent results, and guidelines have not listed linezolid as suggested therapy for MRSA endocarditis.[66,67]

Tedizolid

Major clinical use of tedizolid thus far has been in skin and soft tissue infection. Two trials have compared tedizolid with linezolid; only oral formulations were used in one trial, whereas in the other, intravenous formulations could have been used before changing to the same oral product.[68,69] Tedizolid 200 mg once daily was used for 6 days and compared with linezolid 600 mg twice daily for 10 days. *S. aureus* was the most commonly isolated organism, with MRSA isolated in 27% to 42% of infections. In both trials, tedizolid was noninferior to linezolid using an initial assessment of response at 48 to 72 hours and subsequent assessments at the end of therapy. Although the overall incidence of drug-related toxicity was similar, gastrointestinal adverse effects (nausea, vomiting, diarrhea) were less common in tedizolid-treated patients. Thrombocytopenia was uncommon in both groups but was less frequent with tedizolid. Pooled data from both studies were analyzed and led to similar findings.[70]

Tedizolid has also been compared with other agents used in skin and skin structure infections. In a network meta-analysis of 15 randomized clinical trials of skin and skin structure infections, the clinical response to tedizolid was superior to that of vancomycin but comparable to other agents including linezolid, tigecycline, ceftaroline, teicoplanin, daptomycin, and telavancin.[71] Drug-related adverse events did not differ between any of the agents reviewed. The heterogeneity of the studies and differences in proportion of MRSA infections in each all lead to a limited interpretation as to the comparable efficacy of tedizolid versus the other agents. A phase III randomized controlled trial of tedizolid versus linezolid for treatment of adults with gram-positive hospital-acquired or ventilator-associated pneumonia is underway.[72]

Coagulase-Negative Staphylococci

Case reports and case series have described cure after linezolid therapy for bone and joint infection, meningitis, ventriculoperitoneal shunt infections, and endocarditis caused by coagulase-negative staphylococci, often in the setting of prosthetic material[73–76]; there are inadequate data to recommend linezolid routinely for initial treatment. Although small numbers of coagulase-negative staphylococcal infections have been treated with tedizolid, no statements can be made regarding its overall clinical utility.

Vancomycin-Resistant Enterococci

Linezolid has been shown to be effective for treatment of infection with vancomycin-resistant enterococci. Successful treatment of bacteremia,[77] endocarditis,[73] peritoneal dialysis–related infections,[78] osteomyelitis,[75] endophthalmitis,[79] ventriculitis,[76] meningitis,[41,42,76,80] intraabdominal infections,[81] and urinary tract infections[81] has been reported, although failures have occurred.[81–83] Clinical data are insufficient to denote superiority among available agents. Linezolid has been compared with daptomycin, the other drug commonly used for these infections. Although several meta-analyses have reported linezolid superiority, limitations of the studies included have hindered the conclusions that could be drawn.[84–86] A retrospective cohort study carried out within the US Department of Veterans Affairs health care system found contradictory results with daptomycin used at even relatively low doses, showing higher clinical success rates and lower 30-day mortality.[87] At the present time, antibiotic selection for these infections should be individualized and based on considerations such as in vitro susceptibility of the organism, antecedent antibiotic exposure, source of infection, comorbidities, and potential for drug-related toxicity. With regard to linezolid use for vancomycin-resistant enterococcal endocarditis, despite its being bacteriostatic, there have been cases of treatment success and failure. Linezolid is recommended for therapy, as is daptomycin, for endocarditis caused by enterococci (usually *E. faecium*) resistant to penicillin, aminoglycosides, and vancomycin. The strength of the recommendation is limited by small numbers of patients and should not be viewed as a well-established guideline.[66]

Tedizolid

The in vitro characteristics and pharmacodynamic properties of tedizolid suggest that it would be useful for vancomycin-resistant enterococci infections including some linezolid-resistant strains. Only case reports and abstracts are described, so its overall efficacy is yet to be determined.[88,89]

Streptococci Including *Streptococcus pneumoniae*

Linezolid

The efficacy of linezolid against *S. pneumoniae* has been demonstrated in open-label trials involving community-acquired pneumonia in hospitalized adults and children and in a controlled multicenter study.[59] Despite approval of linezolid for treatment of pneumococcal community-acquired pneumonia, it has been noted that limited activity against *Haemophilus influenzae* and inconsistent activity against *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* should preclude its use as first-line empirical therapy.[6]

Data describing the efficacy of linezolid for pneumococcal central nervous system infection are limited to case reports and case series and largely involve its use in combination with ceftriaxone for penicillin-nonsusceptible isolates. In a single case, a postsurgical cerebral abscess caused by penicillin-susceptible *S. pneumoniae* was treated successfully with linezolid.[76] Linezolid has also been used successfully for treatment of skin and soft tissue infections caused by streptococci including *S. pyogenes* and *S. agalactiae*.[14]

Tedizolid

Streptococcal infection involving skin and skin structures has been successfully treated with tedizolid in the ESTABLISH-1 and ESTABLISH-2 trials. Although *S. aureus* including MRSA was isolated most commonly, *S. pyogenes, S. anginosus* group organisms, *S. agalactiae,* and *Gemella morbillorum* were isolated and treated successfully.[68,69] Response rates to tedizolid were lower in patients with infections with *S. anginosus* group organisms compared with linezolid (70% vs. 89%), although numbers were insufficient to determine statistical significance.[70] The role of tedizolid for other streptococcal infections including those caused by *S. pneumoniae* has not yet been established.

Mycobacterium Including *Mycobacterium tuberculosis*

Linezolid

Linezolid demonstrates in vitro bacteriostatic activity against *M. tuberculosis,* including MDR and XDR strains. In vitro studies of linezolid with first-line and second-line antituberculous agents usually reveal additive rather than synergistic interactions.[90,91] In small numbers of patients with infection caused by MDR and XDR *M. tuberculosis,* the addition of linezolid to failing regimens led to sterilization of sputum after 4 to 6 months of therapy in 89% of patients, with sputum remaining negative 1 year after therapy in 71%.[92,93] Systematic reviews and meta-analysis of the use of linezolid in patients with MDR and XDR tuberculosis have reported similar success rates. Adverse events with linezolid, especially marrow toxicity and neuropathies, are commonly reported due to the need for extended use, leaving unanswered questions as to the best dosing regimen and means of toxicity monitoring in these patients.[94,95]

Tedizolid

Although in vitro activity of tedizolid is similar to that of linezolid and its safety profile suggests it may be used with fewer adverse reactions, published clinical data in patients infected with *M. tuberculosis* are lacking.

Nocardia spp.

Linezolid

Small case series and case reports have demonstrated the efficacy of linezolid for treatment of *Nocardia* infections including central nervous system disease in immunosuppressed and immunocompetent hosts.[96–99] It has been used both as monotherapy and in combination regimens. Adverse events including cytopenia are commonly reported especially with long-term therapy and the usual dosing regimen. Lower doses have been successfully used with the implication that adverse events may be avoided by less drug exposure.[100]

Tedizolid

There are few data regarding use of tedizolid in patients with *Nocardia* infections. It has been used successfully in central nervous system disease, but its overall utility in these infections and its efficacy compared with linezolid require further testing.[101]

UNTOWARD REACTIONS

Both linezolid and tedizolid are relatively well tolerated, with headache, diarrhea, nausea, and vomiting predominating in larger clinical trials.[39] Serious but less common adverse events are discussed next.

Hematologic Toxicity

Reversible myelosuppression, including pure red blood cell aplasia, pancytopenia, and especially thrombocytopenia, has been clearly documented and related to linezolid use.[102] Thrombocytopenia is most common, and although the mechanism is not definitively established, both a drug-induced immune-mediated phenomenon and inhibition of platelet progenitor cells have been suggested.[103,104] Adults completing phase III clinical trials developed thrombocytopenia in 2.4% of cases (range, 0.3%–10%), but the risk is increased with prolonged durations of therapy and renal insufficiency.[39,43,105] Furthermore, delayed neutrophil recovery and worsening thrombocytopenia have been documented in patients with baseline marrow suppression.[106,107] Anemia appears to be caused by suppression of normal erythropoiesis, similar to the marrow effect of chloramphenicol.[104] Weekly monitoring of hematologic parameters is therefore recommended, particularly for therapeutic durations exceeding 2 weeks.[39] Thrombocytopenia occurred in 6.4% of patients treated with tedizolid compared with 12.6% receiving linezolid in a phase III study.[54] No hematologic toxicity was observed among healthy volunteers given tedizolid for 21 days.[108] Correction of linezolid-associated myelotoxicity following a change to tedizolid has also been described.[109]

Monoamine Oxidase Inhibition

Linezolid is a reversible, nonselective monoamine oxidase inhibitor and has been associated with the development of serotonin syndrome (fever, agitation, mental status changes, tremors) in patients receiving concurrent serotonergic agents.[39,110] Small increases in systolic blood pressure have been documented in patients receiving tyramine concurrently with linezolid, leading to recommendations for dietary restriction. Similarly, blood pressure monitoring is recommended for patients taking

adrenergic agents such as pseudoephedrine and phenylpropanolamine.[39] Tedizolid exhibits weak, reversible monoamine oxidase inhibition as well. Although patients receiving concurrent serotonergic agents were excluded from phase II and III study, neither human nor animal model investigation revealed adverse hypertensive or serotonergic effects at therapeutic dosing.[43]

Adverse Effects Associated With Mitochondrial Toxicity

Disruption of mitochondrial protein synthesis has been viewed as the explanation for linezolid-induced neuropathies and lactic acidosis, both of which are described in more detail subsequently.[110–112] Case reports of suspected linezolid-associated rhabdomyolysis and drug-induced liver injury have also raised the possibility of mitochondrial toxicity as an etiology.[113,114] Although tedizolid is thought to be a more potent mitochondrial protein synthesis inhibitor, it has been proposed that less free drug exposure and a period of mitochondrial recovery during each dosing interval may decrease the risk of associated toxicity.[115] More study is needed to confirm these findings in humans.

Neuropathy

Linezolid-induced neuropathies are well documented.[110] Peripheral neuropathy may begin with dysesthesias in the hands and is poorly reversible. Optic neuropathy causes gradual onset of blurring and can lead to permanent loss of useful visual acuity if the drug is not discontinued; when detected early, visual loss has generally been reversible. Peripheral neuropathy and optic nerve disorders in a phase III study of patients receiving tedizolid for 6 days or linezolid for 10 days occurred with similar frequency.[54] When healthy volunteers received tedizolid for 21 days, no evidence of neuropathy was observed.[117]

Lactic Acidosis

Lactic acidosis, including fatal cases, has been reported most commonly during prolonged durations of linezolid therapy but can develop within the first week.[110,118] Prompt recognition and drug discontinuation are critical. Age, renal insufficiency, and drug interactions associated with linezolid overexposure increase the risk. The possibility of genetic predisposition has also been suggested.[116] No postmarketing cases of tedizolid-associated lactic acidosis have been reported to date.

Miscellaneous Untoward Reactions

In patients treated with linezolid for intravascular catheter-related infections, increased mortality was observed in the setting of gram-negative infection, mixed gram-positive and gram-negative infections, or no clear infection at the time of therapy.[113] The poor gram-negative activity of linezolid should limit its use as a single agent in clinical situations that may involve these organisms. Increased mortality with no clear infection remains to be explained.

Other notable yet rare postmarketing reports of adverse effects with linezolid include posterior reversible leukoencephalopathy syndrome, seizures, tooth and tongue discoloration, black hairy tongue (lingua villosa nigra), and hypoglycemia among diabetic patients receiving insulin or oral hypoglycemic agents.[39,114,119,120]

LINEZOLID VERSUS TEDIZOLID

At the present time, tedizolid is approved by the FDA only for therapy of skin and skin structure infections. As its efficacy appears similar, the convenience of once-daily dosing for a shorter duration of therapy and a reduced average wholesale price (for a 6-day course of tedizolid vs. a 10-day course of linezolid) may precipitate increased clinical use in this setting. Although an economic advantage may dissipate when durations of therapy are equivalent, tedizolid could offer an improved adverse effect profile when prolonged administration is required. However, its role in comparison with linezolid for other infections has not yet been established due to a paucity of clinical experience.

OTHER OXAZOLIDINONES

Cadazolid (formerly ACT-179811) is a nonabsorbable quinolonyl-oxazolidinone active against *Clostridioides difficile* (formerly *Clostridium difficile*), including hypervirulent epidemic strains as well as strains resistant to fluoroquinolones and linezolid. A phase II study of patients with *C. difficile* infection found similar clinical cure rates and tolerability when comparing three dosages with oral vancomycin as an active reference.[121] A phase III multicenter, randomized, double-blind study of a 250-mg oral suspension given twice daily versus oral vancomycin has since been performed.[122] Compounds completing phase II study include radezolid (RX-1741) for mild-to-moderate community-acquired pneumonia and uncomplicated skin and skin structure infections and sutezolid (PNU-100480) for treatment of infection with *M. tuberculosis*.[123–125]

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34 Sulfonamides and Trimethoprim; Trimethoprim-Sulfamethoxazole

Stephen H. Zinner and Kenneth H. Mayer

SHORT VIEW SUMMARY

- Among the earliest antimicrobial compounds in clinical use, sulfonamides still remain useful for the treatment of urinary tract infections and infections caused by *Nocardia* species, atypical mycobacteria, and *Toxoplasma gondii*.
- The most common adverse reactions to sulfonamide include leukopenia, thrombocytopenia, acute hemolytic anemia, nausea, vomiting, rash, and fever.
- Serious but less common adverse reactions include agranulocytosis, aplastic anemia, acute tubular necrosis, interstitial nephritis, drug-induced lupus, toxic epidermal necrolysis, and Stevens-Johnson syndrome. The risk of kernicterus precludes the use of sulfonamides in late pregnancy.
- Today, sulfonamides are most frequently used with trimethoprim as a fixed-dose combination of 160 mg/800 mg of trimethoprim-sulfamethoxazole (TMP-SMX).
- TMP-SMX is useful in the treatment of *Stenotrophomonas maltophilia, Pneumocystis jiroveci, Listeria monocytogenes, Cystoisospora belli,* leprosy (as part of combination therapy), urinary tract infections, prostatitis, acute exacerbations of chronic bronchitis, methicillin-resistant staphylococcal skin and soft tissue infections, and malarial infections caused by susceptible organisms.
- TMP-SMX is indicated for the prophylaxis of *P. jiroveci* in immunocompromised patients, including those with human immunodeficiency virus (HIV) infection.
- The adverse effects of TMP-SMX are similar to those seen with sulfonamides alone and may be more frequent in HIV-infected patients.
- Iclaprim is an investigational diaminopyrimidine with enhanced activity against gram-positive cocci, including trimethoprim-resistant strains.

The modern era of antimicrobial chemotherapy began in 1932 with the first reports by Domagk of the protective activity of sulfachrysoidine (Prontosil) against murine streptococcal infections. This drug was developed initially by the German dye industry and had been available commercially since the early 20th century. Sulfachrysoidine exerted its antibacterial activity through the release in vivo of para-aminobenzenesulfonamide (sulfanilamide). This was the first antibacterial agent used in the United States, in July 1935, in an unsuccessful attempt to treat a 10-year-old girl late in the course of meningitis and sepsis caused by *Haemophilus influenzae*.[1] During the late 1930s, the basic sulfanilamide compound was modified to remove unpleasant side effects and expand its spectrum of activity, resulting in compounds with distinctive properties—for example, compounds that are concentrated in the kidney for the treatment of urinary infections or nonabsorbable compounds for increased activity within the gastrointestinal tract.

Trimethoprim is a 2,4-diaminopyrimidine that inhibits dihydrofolate reductase (DHFR), resulting in interference in folic acid and pyrimidine synthesis in the bacterial cell. Trimethoprim was one of several such compounds synthesized and studied by Hitchings and coworkers in the 1950s and 1960s. The use of trimethoprim as a potentiator of sulfonamide activity was introduced by Bushby and Hitchings[2] in 1968. In the subsequent decade, the combination of trimethoprim-sulfamethoxazole (TMP-SMX) was introduced clinically and gained a role in chemotherapy for many infectious diseases. TMP-SMX, available in a fixed drug combination, shows true antibacterial synergism against a wide variety of organisms.

SULFONAMIDES

Structure

The clinically useful sulfonamides are derived from sulfanilamide, which is similar in structure to para-aminobenzoic acid (PABA), a compound required by bacteria for folic acid synthesis (Fig. 34.1). A free amino group at the 4-carbon position is associated with enhanced activity. Increased activity caused by increased PABA inhibition is associated with substitutions at the sulfonyl radical (SO_2), which is attached to the 1-carbon, as seen with sulfadiazine, sulfisoxazole, and sulfamethoxazole, all of which are more active than the parent compound, sulfanilamide. The nature of these substitutions determines other pharmacologic properties of the drug, such as absorption, solubility, and gastrointestinal tolerance. Substitutions at the 4-amino group result in decreased absorption from the gastrointestinal tract (e.g., phthalylsulfathiazole).

Derivation and Nomenclature

Since the introduction of sulfonamides into clinical medicine, dozens of compounds have been used. Relatively few survive today, however. The various compounds can be classified as (1) short-acting or medium-acting sulfonamides, (2) long-acting sulfonamides, (3) sulfonamides limited to the gastrointestinal tract, or (4) topical sulfonamides. Many branded sulfonamide preparations have been discontinued or replaced by generic products.

Short-Acting or Medium-Acting Sulfonamides

Sulfisoxazole (*United States Pharmacopeia* [USP]), sulphafurazole *(British Pharmacopeia), and* *N*′-(3,4-dimethyl-5-isoxazolyl) sulfanilamide are available to treat urinary infections. Sulfamethoxazole USP, *N*′-(5-methyl-3-isoxazolyl) sulfanilamide, is less soluble than sulfisoxazole and yields higher blood levels. It is the sulfonamide most frequently combined with trimethoprim. Sulfadiazine USP (*N*′-2-pyrimidinylsulfanilamide) is highly active, attains high blood and cerebrospinal fluid levels, and is associated with low protein binding and lower solubility than the previously mentioned drugs.

Short-acting sulfonamides also are available in several combinations. Sulfisoxazole and sulfamethoxazole have been combined with phenazopyridine, a urinary analgesic.

Long-Acting Sulfonamides

Sulfamethoxypyridazine (*N*′-[6-methoxy-3-pyridazinyl] sulfanilamide) and sulfameter (*N*′-[5-methoxy-2-pyrimidinyl] sulfanilamide) are no longer available for single-daily-dose therapy because they were associated with hypersensitivity reactions such as Stevens-Johnson syndrome. Neither sulfadimethoxine nor any other long-acting sulfonamide, other than sulfadoxine, is currently available in the United States.

Sulfadoxine, originally known as *sulformethoxine* (*N*′-[5,6-dimethoxy-4-pyrimidyl] sulfanilamide), is a very-long-acting sulfonamide that, combined with pyrimethamine, was formerly available in the United States. Sulfadoxine has a half-life of 100 to 230 hours and reaches a

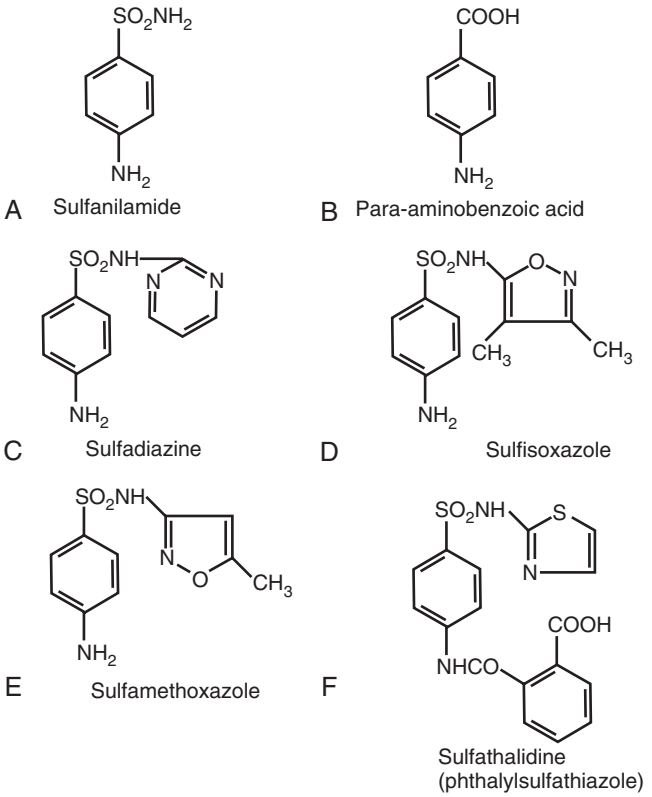

FIG. 34.1 Sulfonamides. (A–F) Structural formulas of selected sulfonamides.

peak serum level of 51 to 76 μg/mL 2.5 to 6 hours after an oral dose of 500 mg. Although resistance is noted, this combination is still used in malaria prophylaxis during pregnancy in some countries.[3]

Sulfonamides Limited to the Gastrointestinal Tract

Sulfaguanidine (*N*′-[diaminomethylene] sulfanilamide), sulfasuxidine (succinylsulfathiazole [4′-{2-thiazolylsulfamoyl}] succinanilic acid), and sulfathalidine (phthalylsulfathiazole [4′-{2-thiazolylsulfamoyl}] phthalanilic acid) are relatively poorly absorbed from the gastrointestinal tract. They have been used in the past to suppress the susceptible bowel flora before surgery. Salicylazosulfapyridine (sulfasalazine, Azulfidine) is a sulfonamide derivative used to treat ulcerative colitis. This drug is absorbed in its parent form as sulfapyridine, and significant blood levels of this compound are measurable.

Topical Sulfonamides

Mafenide acetate (para-aminomethylbenzene sulfonamide) is available for use in the topical treatment of burns. Its use has been limited, however, by metabolic acidosis caused by carbonic anhydrase inhibition. Silver sulfadiazine has fewer side effects and is used for burns,[4] although other silver compounds are being introduced.[5] In these formulations, the sulfonamide acts primarily as a vehicle for release of silver ions that exert an antibacterial effect. Outbreaks of silver-resistant infections in burn units ultimately may limit its usefulness.[6] Various combinations of other sulfonamides are available as vaginal creams or suppositories. A variety of ophthalmic ointments and solutions of sulfacetamide sodium USP (a highly soluble sulfonamide) are available for use in the treatment of conjunctivitis caused by susceptible bacteria and as adjunctive therapy for trachoma. Sulfacetamide is also used as an antiinflammatory and antimicrobial agent in the treatment of acne, rosacea, and seborrheic dermatitis.

Mechanisms of Action

Although a wide variety of chemical modifications of the sulfonamides have been synthesized, all basically share the same mechanism of action. The sulfonamides are bacteriostatic in that they inhibit bacterial growth by interfering with microbial folic acid synthesis. More specifically, sulfonamides inhibit competitively the incorporation of PABA into tetrahydropteroic acid,[7] and they may be incorporated into dihydropteroate.[8] Sulfonamides may have a higher affinity for the microbial enzyme tetrahydropteroic acid synthetase than the natural substrate PABA. Richmond[9] suggested that sulfonamides may act on bacterial repressor genes or by feedback inhibition to decrease formation of new enzymes. The ultimate result of decreased folic acid synthesis is a decrease in bacterial nucleotides, with subsequent inhibition of bacterial growth.

Antimicrobial Activity in vitro

Sulfonamides exhibit in vitro inhibitory activity against a broad spectrum of gram-positive and gram-negative bacteria; *Mycobacterium, Actinomyces, Chlamydia, Plasmodium,* and *Toxoplasma* species; and some fungi. Sulfamethoxazole is also active against *Acanthamoeba* strains.[10] The in vitro antimicrobial sensitivity of sulfonamides is influenced strongly by the size of the inoculum and the composition of the test medium. High concentrations of PABA and thymidine inhibit sulfonamide activity.

Antimicrobial Resistance

Resistance to sulfonamides is widespread and increasingly common in community-acquired and nosocomial strains of bacteria, including streptococci, staphylococci, Enterobacteriaceae, *Neisseria* spp., and *Pseudomonas* spp. Cross-resistance among different sulfonamides is common. In recent decades, sulfonamide resistance in Enterobacteriaceae and gram-positive bacteria has increased substantially.[11,12,13]

Organisms may develop point mutations in the *folP* gene, which encodes a structural change in dihydropteroate synthetase that produces an enzyme with lowered affinity for sulfonamide. The PABA binding site may be altered by F28L/T and P64S mutations in *Escherichia coli.* PABA overproduction has been implicated in resistant strains of *Neisseria gonorrhoeae* and *Staphylococcus aureus.*[14] Resistance also may be mediated by integrons that carry *sul1, sul2,* and *sul3* genes, which code for the production of drug-resistant enzymes, such as dihydropteroate synthetase, or by plasmids that decrease bacterial cell permeability to sulfonamides.[15] Plasmid transfer can occur in the gastrointestinal tract in vivo and has been seen with multiple species of Enterobacteriaceae.[16]

Transformational exchanges of dihydropteroate synthetase among *Neisseria* spp. raise the specter of wider dissemination of sulfonamide resistance by chromosomal and plasmid genes. More than one resistance mechanism may be operating simultaneously.[17]

Plasmid-mediated sulfonamide resistance in diverse species has increased greatly in recent years, often in conjunction with trimethoprim resistance. The majority of *Salmonella* isolates from human or animal origin are resistant to sulfonamides, often in conjunction with resistance to other antibiotic classes. The presence of the *sul2* gene is responsible for high-level sulfonamide resistance among *Shigella* isolates in Latin America. A US survey showed that 32% of 1118 *Shigella* isolates were sulfonamide resistant, as are more than 75% of isolates in China.[12,18] The increase in sulfonamide-resistant *Haemophilus ducreyi* in Asia and Africa has been associated with a plasmid related to those found in Enterobacteriaceae.[19] Bacterial resistance to sulfonamides has increased among human immunodeficiency virus (HIV)–infected patients during the era of increased use of TMP-SMX prophylaxis.

Pharmacology

Routes of Administration

Sulfonamides are usually administered orally. Sulfacetamide is available as a topical cream, ointment, or lotion and in ophthalmic preparations; silver sulfadiazine and mafenide acetate are applied topically in burn patients and are associated with significant percutaneous absorption of sulfonamide. Vaginal preparations are available for topical application.

Absorption

Most of the short-acting and medium-acting sulfonamides are absorbed rapidly and almost completely in the nonionized state from the small intestine and stomach. Compounds with *N*-1 substitutions are absorbed poorly, as are more acidic compounds (e.g., phthalylsulfathiazole; see

TABLE 34.1 Blood Levels, Cerebrospinal Fluid Levels, Plasma Half-Life, and Protein Binding of Some Sulfonamides

DRUG	PEAK BLOOD LEVEL[a] (mg/mL)	LEVEL IN CSF (%)	PLASMA HALF-LIFE (hr)	PROTEIN BINDING (%)
Sulfadiazine	30–60	40–80	17	45
Sulfisoxazole	40–50	30–50	5–6	92
Sulfamethoxazole	80–100	25–30	11	70
Sulfadoxine	50–75	20–30	100–230	80–98

[a]Approximate free sulfonamide level after a 2-g oral dose.
CSF, Cerebrospinal fluid.

Fig. 34.1F). Topical sulfonamides are absorbed and may be detectable in blood.

Distribution

The sulfonamides are generally well distributed throughout the body, entering the cerebrospinal fluid and synovial, pleural, and peritoneal fluids with concentrations approaching 80% of serum levels. Blood and tissue levels are related to the degree of protein binding (Table 34.1) and lipid solubility. Sulfonamides administered in pregnancy readily cross the placenta and are present in the fetal blood and amniotic fluid.

Metabolism and Excretion

Acetylation and glucuronidation occur in the liver, and free and metabolized drug appears in the urine. Glomerular filtration is probably a route of excretion, although partial reabsorption and active tubular secretion are also involved, especially at low creatinine clearance rates. Urinary excretion is more rapid for sulfonamides with low pK_a values (e.g., sulfamethizole, sulfisoxazole), and alkalinization of the urine increases excretion by this route. Plasma half-lives vary widely; they are related inversely to lipid solubility and directly to pK_a values but are not related clearly to the degree of protein binding. Small amounts of sulfonamides are found in bile, human milk, prostatic secretions, saliva, and tears.[20]

Sulfamethoxazole is primarily metabolized by the CYP2C9 hepatic enzyme system, but also by CYP3A4, and the substrate inhibits the activity of CYP2C9.[21] Excretion may be decreased in older patients, especially those with decreased creatinine clearance. Sulfadoxine half-life and area under the concentration-time curve (AUC) are reduced and clearance is increased during pregnancy.[22]

Protein Binding and Blood or Tissue Levels

Sulfonamides are bound variably and not irreversibly to plasma albumin, and the bound drug is inactive (see Table 34.1). Levels obtainable in cerebrospinal fluid and other body fluids are related inversely to the degree of protein binding. The amount of free drug in plasma is related directly to the pK_a.

Toxicity and Adverse Reactions

Sulfonamides can cause nausea, vomiting, diarrhea, rash, fever, headache, depression, jaundice, hepatic necrosis, drug-induced lupus,[23] and a serum sickness–like syndrome. Sulfadiazine used in excessively high doses is associated with crystalluria and tubular deposits of sulfonamide crystals. These complications can be minimized by maintenance of high urine flow and alkalinization of the urine. Tubular necrosis, interstitial nephritis, and necrotizing angiitis may be associated rarely with sulfonamide sensitivity.

More serious adverse reactions caused by sulfonamides may include acute hemolytic anemia sometimes related to a deficiency in erythrocyte glucose-6-phosphate dehydrogenase (G6PD), aplastic anemia, agranulocytosis, thrombocytopenia, and leukopenia.

Sulfonamides administered during the last month of pregnancy compete for bilirubin-binding sites on plasma albumin and may increase fetal blood levels of unconjugated bilirubin, increasing the risk of kernicterus. Also, because of the immature fetal acetyltransferase system, blood levels of free sulfonamide may be increased, further adversely affecting the risk of kernicterus.

Significant hypersensitivity reactions can occur with sulfonamides administered via any route. The most important of these reactions are erythema nodosum, erythema multiforme (including Stevens-Johnson syndrome), fixed-drug eruption, vasculitis similar to periarteritis nodosa, and anaphylaxis. One report suggested that cutaneous reactions, including toxic epidermal necrolysis, may be related to an inherited constitutional defect in detoxification of metabolites.[24] Locally applied sulfonamides (e.g., to skin) may be associated with any of these adverse reactions.

Many HIV-infected patients who have adverse reactions to sulfa drugs can be desensitized by gradual dose escalation or may tolerate rechallenges without severe adverse reactions.[25] Patients who are not desensitized successfully to sulfa drugs have tolerated the changing of their regimen to dapsone, with or without pyrimethamine.

Drug Interactions

Sulfonamides may displace from albumin-binding sites drugs such as warfarin, increasing the effective activity of the displaced drug. Anticoagulant dosage should be reduced during sulfonamide therapy. Sulfonamides also displace methotrexate from its bound protein, increasing methotrexate toxicity. An increased hypoglycemic effect of chlorpropamide and tolbutamide may occur during sulfonamide therapy, possibly because of the same mechanism or structural similarities. Sulfonamides may compete for binding sites with some anesthetic agents such as thiopental, and reduced barbiturate doses might be necessary. Sulfonamides may potentiate the action of some thiazide diuretics, phenytoin, and uricosuric agents. Conversely, sulfonamides themselves can be displaced from binding sites by indomethacin, phenylbutazone, salicylates, probenecid, and sulfinpyrazone, resulting in increased sulfonamide activity. Cyclosporine levels may be reduced by sulfonamides. Oral contraceptive failure during sulfonamide therapy has been noted rarely.[26]

The activity of sulfonamides may be decreased by procaine and other local anesthetics derived from PABA. Methenamine compounds should not be used with sulfonamides because of the formation of insoluble urinary precipitates. Sulfonamides may decrease protein-bound iodine and iodine 131 (^{131}I) uptake and may produce false-positive Benedict test results for urine glucose and false-positive sulfosalicylic acid test results for urine proteins.

Major Clinical Uses

Sulfonamides have been used frequently in the treatment of acute urinary tract infections. Increasing resistance has diminished their effectiveness. Because of widespread resistance of uropathogenic *E. coli,* the choice of therapy should be based on community prevalence of susceptible infecting organisms (see Chapter 72).[27]

Sulfonamides are effective for the treatment of infections caused by *Nocardia asteroides* and other *Nocardia* species, although resistance may be increasing.[28] Therapy must include 4 to 6 g or more daily after a loading dose of 4 g and should be continued for 4 to 6 months or longer, if necessary (see Chapter 253). Sulfonamides may be useful in combination with antimycobacterial drugs for the management of infections caused by some atypical mycobacteria (see Chapter 252).

Sulfonamides have been used to treat toxoplasmosis in patients with or without acquired immunodeficiency syndrome (AIDS) and chloroquine-sensitive or chloroquine-resistant *Plasmodium falciparum* malaria (with pyrimethamine). Studies have found that sulfadoxine-pyrimethamine

malaria prophylaxis is well tolerated in pregnant women,[3] but increased resistance has been reported. The optimal treatment for toxoplasmic encephalitis is the combination of pyrimethamine plus sulfadiazine or, for patients intolerant to sulfonamides, pyrimethamine plus clindamycin. In most studies, both regimens seem equally efficacious.[29] The pyrimethamine-sulfadiazine regimen begins with a 4- to 8-week induction course of pyrimethamine (100–200 mg once daily for 1 day, followed by 50–75 mg once daily) plus sulfadiazine (1–2 g four times daily). Leucovorin (5–50 mg once daily) is administered to prevent pyrimethamine-associated folinic acid deficiency. Sulfadiazine may be given twice daily (2 g), even during induction therapy.[30] Sulfonamide desensitization has been effective for patients with cerebral toxoplasmosis and allergies to sulfonamides. High-dose TMP-SMX (see later) is an effective alternate for AIDS-related cerebral toxoplasmosis, although pyrimethamine-sulfadiazine has been considered preferable.[29] Melioidosis, dermatitis herpetiformis, lymphogranuloma venereum, and chancroid have responded to sulfonamides. Nongonococcal urethritis caused by *Chlamydia,* but not caused by *Ureaplasma urealyticum,* responded to sulfonamide therapy (see Chapters 180 and 184). Sulfasalazine is used in the treatment of inflammatory bowel diseases.[31] Currently, sulfonamides are used most frequently in combination with trimethoprim (see later). Sulfamethoxazole at 800 mg dosing might have a future role in the treatment of multidrug-resistant (MDR) tuberculosis.[32]

TRIMETHOPRIM

Structure and Derivation

Trimethoprim is a 2,4-diamino-5-(3′,4′,5′-trimethoxybenzyl) pyrimidine (Fig. 34.2). This drug was synthesized by Bushby and Hitchings[2] as a DHFR inhibitor thought to potentiate the activity of sulfonamides by sequential inhibition of folic acid synthesis. In the United States, trimethoprim is available as a single agent and in combination with sulfamethoxazole (co-trimoxazole; see later). Trimethoprim has antibacterial activity of its own.

Mechanism of Action

Trimethoprim owes its activity to powerful inhibition of bacterial DHFR, which is the next enzymatic step after folic acid synthesis is blocked by sulfonamides. Trimethoprim is 50,000 to 100,000 times more active against bacterial DHFR than against the human enzyme. Trimethoprim interferes with the conversion of dihydrofolate to tetrahydrofolate, the precursor of folinic acid and ultimately of purine and DNA synthesis (Fig. 34.3). The sequential blockage of the same biosynthetic pathway by sulfonamides and trimethoprim results in a high degree of synergistic activity against a wide spectrum of microorganisms. Humans do not synthesize folic acid but require it in their diet, and human purine synthesis is not affected significantly by the enzyme inhibition of trimethoprim.[33]

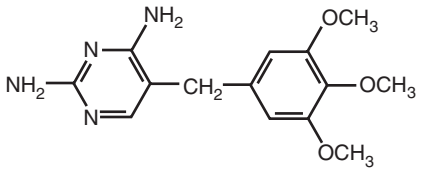

(2,4-diamino-5-[3′,4′,5′-trimethoxybenzyl] pyrimidine)

FIG. 34.2 Chemical structure of trimethoprim.

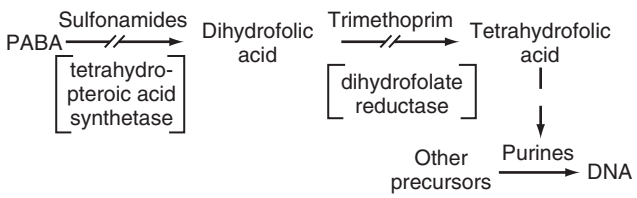

FIG. 34.3 Action of sulfonamides and trimethoprim on the metabolic pathway of bacterial folic acid synthesis. *DNA,* Deoxyribonucleic acid; *PABA,* para-aminobenzoic acid.

Antimicrobial Activity

Trimethoprim is active in vitro against many gram-positive cocci and most gram-negative rods, except for *Pseudomonas aeruginosa* and *Bacteroides* spp. *Treponema pallidum, Mycobacterium tuberculosis, Mycoplasma* spp., and most anaerobes are resistant. Thymidine inhibits the in vitro activity of trimethoprim, but the addition of thymidine phosphorylase or 5% lysed horse blood, which releases the phosphorylase to Mueller-Hinton medium or other sensitivity media, removes this inhibition. The minimal inhibitory concentration (MIC) varies considerably with the medium used. Trimethoprim alone has good in vitro activity against *H. influenzae*; a survey has shown that 76% of 978 strains remain susceptible, but higher resistance rates are reported elsewhere.[34] *Streptococcus pyogenes* MICs have been reported at less than or equal to 2 μg/mL,[35] but several transferable genes mediating resistance to trimethoprim are increasingly common and limit usefulness against these organisms.[36]

Resistance to Trimethoprim

Clinical resistance to trimethoprim is increasing and may be due to selection of bacterial cells with altered permeability, loss of drug-binding capacity, overproduction of or alterations in DHFR, or a combination of these factors. A decrease in the ratio of strains resistant to sulfamethoxazole and trimethoprim compared with strains resistant only to trimethoprim may reflect an increase in independent trimethoprim resistance, which might be a useful monitoring parameter in hospitals.[37]

Clinically, the most important mechanism is plasmid-mediated DHFRs that are resistant to trimethoprim.[38] Distinctive DHFRs mediated by *dfr* genes have been described in Enterobacteriaceae, *P. aeruginosa, H. influenzae, Streptococcus pneumoniae, S. aureus,* and *Campylobacter* spp. that may be chromosomally resistant to high trimethoprim concentrations. These resistant enzymes often may be plasmid mediated and disseminated by highly mobile transposons (e.g., Tn7) with wide host species ranges. Outbreaks caused by trimethoprim-resistant conjugative plasmids have been noted in multiple centers in Europe, Asia, and the Americas.[39] Many of the outbreaks occurred in immunocompromised hosts, with organisms manifesting resistance to multiple other antibiotic groups. DHFR variants in *P. jiroveci* pneumonia isolates may be associated with resistance to TMP-SMX.[40]

Variable local increases in trimethoprim resistance, particularly among Enterobacteriaceae, have been reported. Recent surveys have found trimethoprim resistance steadily increasing among *E. coli* uropathogens.[41] Whether the clinical use of trimethoprim alone in some countries has resulted in increasing resistance to TMP-SMX is unclear.

Pharmacology

Routes of Administration

Trimethoprim is available as 100-mg tablets for oral use. Trimethoprim is absorbed readily and almost completely from the gastrointestinal tract. Peak serum levels appear 1 to 4 hours after ingestion of 100 mg and approach 1 μg/mL. The coadministration of sulfamethoxazole does not affect the rate of absorption or serum levels of trimethoprim.

Distribution

Trimethoprim is distributed widely in tissues and may appear in kidney, lung, and sputum in higher concentrations than in plasma and in bile, saliva, human breast milk, and seminal fluid.[42] Trimethoprim is also found in prostatic fluid at two to three times the serum concentration, but lower levels may be present in patients with chronic prostatitis. Cerebrospinal fluid concentrations are about 40% of serum levels.

Metabolism and Excretion

Approximately 60% to 80% of an administered dose of trimethoprim is excreted in the urine via tubular secretion within 24 hours. The remainder of the drug is excreted by the kidney in one of four oxide or hydroxyl derivatives. The urinary metabolites are bacteriologically inactive.[43] Trimethoprim is also a major substrate of CYP2C9 and a minor substrate of CYP3A4. Its metabolism inhibits CYP2C8 and CYP2C9.[21] Trimethoprim is also excreted in the bile. The serum half-life ranges from 9 to 11 hours in healthy subjects and is prolonged in patients

with renal insufficiency. In contrast to sulfamethoxazole, the excretion rate of trimethoprim is increased with acidification of the urine, and serum protein binding (65%–70%) does not decrease significantly with increasing degrees of uremia. Urine concentrations in healthy subjects (60–1000 μg/mL) are usually greater than the MIC of many urinary pathogens.

Clinical Use

The primary use of trimethoprim alone is in the prevention of recurrent uncomplicated urinary tract infections in women with structurally normal anatomy.[44] Trimethoprim has been successful in the treatment of acute urinary tract infections at a dose of 100 mg twice daily,[45] but increasing resistance threatens its usefulness.[38]

TRIMETHOPRIM-SULFAMETHOXAZOLE

Mechanism of Action and Antimicrobial Activity

Potentiation of the action of trimethoprim is seen in combination with sulfamethoxazole in a ratio of 1:5 for oral use (trimethoprim, 80 mg; sulfamethoxazole, 400 mg). Double-strength and quarter-strength pediatric tablets are available, as is an oral suspension containing 40 mg of trimethoprim and 200 mg of sulfamethoxazole per 5 mL. Intravenous (IV) trimethoprim (16 mg/mL) plus sulfamethoxazole (80 mg/mL) is available. When administered intravenously, 10 mL or 160 mg of trimethoprim (with 800 mg of sulfamethoxazole) produces a peak serum trimethoprim concentration of 3.4 μg/mL in 1 hour. After repeated doses, the peak trimethoprim concentration may approach 9 μg/mL.[43] Similar peak levels may be reached with oral therapy, but at 2 to 4 hours after administration.[46]

The combination of trimethoprim and sulfamethoxazole (TMP-SMX, co-trimoxazole) is active in vitro against many isolates of *S. aureus,* including many community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains, but because of local variations, susceptibility testing should be performed. Dissemination of trimethoprim resistance in MRSA via the *dfrG* gene from Africa to Europe has been reported.[47] In vitro TMP-SMX activity is reported for *S. pyogenes, S. pneumoniae* (although resistance is increasing in patients receiving TMP-SMX prophylaxis[48]), *Moraxella catarrhalis, E. coli, Proteus mirabilis, Burkholderia cepacia, Burkholderia pseudomallei,*[49] *Yersinia enterocolitica* (but resistance has been reported), *N. gonorrhoeae* (resistance rates higher than 20% reported[50]), and *Stenotrophomonas maltophilia.* TMP-SMX–resistant strains of *S. maltophilia,* often mediated by one of several circulating integrons, are increasing and may occur in severely ill patients with serious morbidity.[51,52] Of pneumococci carried in the nasopharynx of children treated for malaria with a sulfonamide or TMP-SMX, 50% were resistant to TMP-SMX.[53] A study from Finland has documented an increasing prevalence of TMP-SMX resistance in *S. pneumoniae, M. catarrhalis,* and *H. influenzae* and found that regional use of TMP-SMX was associated with increasing resistance among pneumococci.[54] Variable TMP-SMX resistance has been reported in penicillin-susceptible and penicillin-nonsusceptible pneumococci.[55] A large Finnish study of more than 20,000 *S. pneumoniae* isolates correlated TMP-SMX resistance with increasing use.[56] A more recent study among HIV-infected South African patients with invasive pneumococcal infections reported that TMP-SMX prophylaxis was associated with pneumococcal nonsusceptibility to TMP-SMX, in addition to rifampin and penicillin.[48] The Etest might not be reliable for the determination of TMP-SMX–resistant pneumococci. A wide range of TMP-SMX susceptibilities among *Salmonella* and *Shigella* spp. has been reported; for example, 80% of *Shigella* isolates from Shanghai were resistant,[18] as were 90% in Bangladesh.[57]

Variable bactericidal effects have been noted when enterococci are tested against TMP-SMX, but TMP-SMX is not clinically useful. However, one report suggested that standard in vitro testing might not predict clinical outcome and might underestimate the effectiveness of trimethoprim alone in enterococcal urinary tract infections.[58] The susceptibility of Enterobacteriaceae may vary greatly among locations and within the same location from year to year because of the spread of trimethoprim-resistant plasmids and transposons. Almost all strains of *P. aeruginosa* are resistant in vitro to TMP-SMX.

Trimethoprim combined with sulfamethoxazole or dapsone has been effective in the treatment of *Pneumocystis jiroveci* pneumonia in immunocompromised patients (see later). *Listeria monocytogenes, M. catarrhalis,* and atypical mycobacteria have been shown to be susceptible to TMP-SMX, but variable rates of resistance are reported.

The optimal ratio for in vitro synergism of TMP-SMX in combination is 1:20, but this ratio does not always apply in vivo. The synergism seen depends on the sensitivity of the organism to each drug.

Trimethoprim-Sulfamethoxazole Resistance

Concomitant trimethoprim and sulfonamide resistance may limit the usefulness of TMP-SMX against some strains of MRSA and *Staphylococcus epidermidis.* The most commonly circulating CA-MRSA clone (USA300) often has been susceptible to TMP-SMX,[59] but increasing levels of TMP-SMX resistance have been reported in European isolates.[60] In HIV-infected patients, TMP-SMX use has been associated with TMP-SMX resistance among *S. aureus* and Enterobacteriaceae,[61] with recent increases in resistance documented in respiratory and urinary isolates.[62] Until recently, there was limited evidence that TMP-SMX selects for resistance to other antibiotic classes.[63] However, more recent studies have suggested that multiple drug resistance is common now in *E. coli* isolated from the urinary tract, with TMP-SMX resistance rates approaching 25% in the United States.[64] *E. coli* carrying extended-spectrum β-lactamases may have concomitant high rates of resistance to TMP-SMX (65% in one study[65]), which are often plasmid mediated. Patients with *E. coli* urinary tract infections caused by the 025b/ST131 clone are less likely to respond to TMP-SMX, and the related MDR ST131-H30R1 and ST1131-H30Rx strains are becoming more widely disseminated.[66,67]

Permeability changes may occur in the bacterial cell and result in resistance to trimethoprim and sulfonamides. Thymine-requiring auxotrophs may also account for clinically significant resistance to both drugs. These mutants lack thymidylate synthetase and are probably less virulent than sensitive strains.[38] Many *Shigella* spp. are resistant to TMP-SMX, mediated by several different integrons.[57,68,69] TMP-SMX resistance is increasing among many *Salmonella* spp., with international travel potentiating spread.[70] Except for strains originating in Southeast Asia, many *Salmonella* Typhi strains retain susceptibility.[71] However, multiclass plasmid-mediated resistance, including TMP-SMX resistance, has been reported in *Salmonella enterica.* A widely disseminated integron has been associated with high levels of TMP-SMX resistance in multiple species of Enterobacteriaceae.[72] Resistance is also increasing in *Acinetobacter baumannii* strains.[73] *P. jiroveci* may also develop sulfonamide and trimethoprim resistant mutations in the course of therapy of immunocompromised HIV-infected patients, resulting in treatment failure.[74]

Metabolism and Excretion

TMP-SMX can be given in the usual doses to patients with a creatinine clearance of 30 mL/min or higher. One half of the usual daily dose can be given to patients with a creatinine clearance of 15 to 30 mL/min, but TMP-SMX is not recommended for use in patients with a creatinine clearance lower than 15 mL/min.[75] Trimethoprim and nonacetylated sulfamethoxazole are removed by hemodialysis. Patients needing long-term peritoneal dialysis can be given the equivalent of one double-strength TMP-SMX tablet every 48 hours.

Toxicity and Side Effects

The toxic and undesired effects of TMP-SMX include all the effects discussed previously for sulfonamides. Nausea, vomiting, diarrhea, anorexia, and hypersensitivity reactions are the most common.[76]

Dermatologic Reactions

Rash and other adverse reactions, including hypouricemia (after high-dose therapy)[77] and Sweet syndrome (acute febrile neutrophilic dermatosis), have been noted frequently in patients with AIDS, in whom adverse reactions to TMP-SMX appear more frequently than in other patients.[78] The pathophysiology of sulfonamide hypersensitivity associated with HIV infection is multifactorial and probably relates to a number of metabolic, immunologic, host-related, and viral factors.[79–81] HIV-associated T-cell dysfunction[79,80] and major histocompatibility complex

(MHC)–dependent drug presentation[81] likely contribute to these effects. Toxic epidermal necrolysis and Stevens-Johnson syndrome may occur in 1 to 2 per 1000 treated individuals.[82] Patients have been desensitized successfully with the use of oral regimens.[29] Other dermatologic manifestations, including exanthems, fixed drug eruptions, baboon syndrome, erythrodermic psoriasis, and nail loss, have been reported. Drug rash with eosinophilia and systemic symptoms (DRESS syndrome) also may occur.[83]

Hematologic Reactions

In addition, impaired folate use may be seen in humans with prolonged administration. This usually manifests as a megaloblastic marrow, with hypersegmented polymorphonuclear leukocytes. Methemoglobinemia (neonatal) and leukopenia, thrombocytopenia, and granulocytopenia or agranulocytosis may be seen with fatal results.[76,84] Hypoprothrombinemia and drug-induced thrombocytopenia have been reported.[76] The administration of folinic acid usually prevents or effectively treats the antifolate effects of trimethoprim, and trimethoprim's antibacterial efficacy is not impaired, except possibly against enterococci.

Renal and Electrolyte Abnormalities

Renal dysfunction may occur in patients with preexisting renal disease, but this is reversible with dose reduction. Hyponatremia, hypernatremia, and hyperkalemia have been noted, especially after high doses and in patients with renal insufficiency.[76] The incidence of TMP-SMX–induced hyperkalemia is increased in older patients, especially those treated with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and spironolactone, but not with β-blockers.[76,85] When used in combination with an angiotensin-converting enzyme inhibitor in older patients, TMP-SMX was associated with an elevated risk of sudden death compared with amoxicillin and other antibiotics.[86,87] Interstitial nephritis attributable to TMP-SMX has been described, especially in transplant recipients[88] and patients with AIDS. Lactic acidosis may occur, and although renal tubular acidosis is uncommon,[89] it has been reported in children undergoing treatment for acute lymphocytic leukemia.

Gastroenterologic and Hepatic Effects

Pseudomembranous colitis has been described with TMP-SMX but is uncommon, possibly because the effect of the drugs on the predominantly anaerobic colonic microbiota is low. Drug-induced, dose-dependent cholestasis and hepatocellular injury, especially severe in African Americans and in HIV-infected patients, have been reported,[90,91,92] and prolonged cholestasis with pruritus may occur rarely. Pancreatitis[93] and fulminant hepatic failure have also been reported, especially in liver transplant patients.[94] The incidence of hepatotoxicity may be decreased in patients receiving concomitant fluconazole because it can decrease tissue TMP-SMX levels.

Neurologic and Psychiatric Effects

TMP-SMX–induced meningoencephalitis and aseptic meningitis have been described.[76] Although other central nervous system effects are seen primarily in HIV-infected patients, tremor and gait disturbances have been reported.[76] Acute psychosis and hallucinations associated with TMP-SMX treatment have been reported.[95,96]

Miscellaneous Adverse Effects

Drug-induced hypoglycemia may occur, especially in combination with sulfonylureas,[76,97] and rhabdomyolysis has been described in an HIV-infected patient.[98] Acute parotitis may occur.[99] Anterior uveitis and retinal hemorrhage have been described with trimethoprim alone and with TMP-SMX, as have reversible myopia and angle-closure glaucoma. In patients with AIDS, anaphylactoid reactions and transient diffuse pulmonary infiltrates with hypotension have been described after reexposure to TMP-SMX. TMP-SMX–induced acute fibrinous organizing pneumonia has been described.

Antiphospholipid antibody syndrome may occur.[100] TMP-SMX is associated with an increased risk of fetal abnormalities,[101] low birth weight, and preterm delivery.[102] Anti-ribonucleoprotein (RNP) antibodies or genetic polymorphisms may predict TMP-SMX hypersensitivity and adverse events.[103,104]

TABLE 34.2 Clinically Relevant Drug Interactions With Trimethoprim (TMP) and Sulfamethoxazole (SMX)

Drugs That May Increase TMP-SMX Levels
Carbamazepine
Phenobarbital
Phenytoin
Rifampin
Rifapentine
Secobarbital
Drugs Used With TMP-SMX That May Result in Toxicity
Amantadine (delirium)
Angiotensin-converting enzyme inhibitors (hyperkalemia)
Angiotensin receptor antagonists (hyperkalemia)
Cyclosporine (nephrotoxicity)
Methotrexate (anemia)
Pyrimethamine (anemia)
Sulfonylureas (hypoglycemia)
Warfarin (hypoprothrombinemia and bleeding)
Drugs With Which TMP-SMX May Increase Drug Levels
Amiodarone
Bosentan
Dapsone
Fluoxetine
Glimepiride
Glipizide
Losartan
Montelukast
Nateglinide
Paclitaxel
Phenytoin
Pioglitazone
Repaglinide
Rifampin
Rosiglitazone
Warfarin
Zafirlukast

Modified from Wen X, Wang JS, Backman JT, et al. Trimethoprim and sulfamethoxazole are selective inhibitors of CYP2C8 and CYP2C9, respectively. Drug Metab Dispos. *2002;30:631–635.*

Drug Interactions

Drug-drug interactions occur; the major interactions are summarized in Table 34.2.[21] TMP-SMX interacts with warfarin through the CYP450, CYP2C9 system, increasing bleeding risk.[105] Active levels of phenytoin may be increased markedly by TMP-SMX, especially in elderly patients.[106] Also, concomitant administration of TMP-SMX and methotrexate results in decreased renal clearance of free methotrexate, and severe or even fatal pancytopenia may result.[107] Reversible inhibition of tubular creatinine excretion may be caused by trimethoprim in the presence of cyclosporine,[21] but TMP-SMX did not affect sirolimus pharmacokinetics in a study of renal transplant recipients.[108] Digoxin levels may be increased, and sulfonylureas may be potentiated with resulting hypoglycemia. Serum rifampin levels may increase during concomitant therapy with TMP-SMX.[21] Older patients treated with concomitant TMP-SMX and spironolactone are at increased risk for sudden death.[109] Although TMP-SMX may inhibit the metabolism of some antiretroviral drugs, including lamivudine and saquinavir, the effect is minimal and does not require adjustment of antiretroviral drug dosage.

Trimethoprim Plus Other Antimicrobial Agents

Other sulfonamides, such as sulfamoxole, sulfadiazine, sulfadimidine, and sulfametrole, have been combined with trimethoprim, but clinical experience is limited. Although combinations of trimethoprim with other agents, such as rifampin, polymyxin, amikacin, and metronidazole, have been studied, extensive clinical experience with these combinations is lacking. A randomized controlled trial of HIV-infected patients with mild-to-moderate *P. jiroveci* pneumonia found that trimethoprim-dapsone was comparable with TMP-SMX in terms of overall activity and tolerability.[110] Because it is the least expensive and most carefully studied

regimen, TMP-SMX continues to be first-line therapy for HIV-associated *P. jiroveci* pneumonia.

Clinical Uses of Trimethoprim-Sulfamethoxazole

The increasing worldwide rates of TMP-SMX resistance have seriously limited the clinical usefulness of co-trimoxazole and its components.

Urinary Tract Infections

TMP-SMX is useful in the treatment of recurrent or chronic urinary tract infections caused by susceptible organisms. The combination is effective for acute pyelonephritis and cystitis (see Chapter 72), although either antibiotic alone could be appropriate for susceptible isolates. Recent reports have cautioned against their routine use if rates of resistance approach 20%, as they currently do in several populations.[41,65,111] Because trimethoprim accumulates in prostatic secretions, TMP-SMX is often effective in bacterial prostatitis and in orchitis and epididymitis caused by susceptible bacteria, and is frequently used after transurethral prostate biopsy.

The usual adult dosage for the treatment of acute prostate or urinary infection is two tablets every 12 hours or one double-strength tablet every 12 hours. The pediatric dose for urinary tract infection is 150 to 185 mg/m^2 for trimethoprim and 750 to 925 mg/m^2 for sulfamethoxazole daily in two divided doses. Single-dose therapy with one or two double-strength tablets may be effective in some women with uncomplicated lower urinary tract infection, but longer-term therapy is usually necessary in patients with complicated urinary tract infections (see Chapter 72).

TMP-SMX has been shown to be useful in the long-term suppressive therapy of adults and children with chronic or recurrent urinary infections, and extremely low doses (one-half to one tablet at bedtime or every other night) may still be effective. This approach has been useful in preventing recurrent urinary tract infections in children with vesicoureteral reflux, although long-term outcomes were no different with or without prophylaxis.[112] A recent study found that although children receiving TMP-SMX prophylaxis to prevent recurrent urinary tract infections were more likely to develop resistant isolates, their rates of recurrence did not differ from the rates in those who did not receive prophylaxis.[113] Postcoital prophylactic TMP-SMX may reduce recurrent urinary tract infections related to intercourse. Trimethoprim achieves effective concentrations in the vaginal secretions, and it might exert a protective effect on reducing the number of recurrent infections despite the fact that TMP-SMX–resistant organisms may be present in the vaginal and stool flora.[114] As mentioned earlier, trimethoprim alone is effective therapy for uncomplicated and recurrent urinary tract infections in women. Usual doses are 100 to 200 mg twice daily, and nightly doses of 100 mg may be effective suppressive therapy. Increasing rates of trimethoprim and TMP-SMX resistance should minimize the widespread empirical use of these drugs, unless bacterial susceptibility is known.[27,114]

Respiratory Tract Infections

TMP-SMX is effective in the treatment of acute bronchitis and pneumonitis caused by sensitive organisms, although it is not the treatment of choice for any single organism. Previously, TMP-SMX was as effective as tetracyclines in the reduction of acute exacerbations in patients with chronic bronchitis, but more recent studies have show equivalent results with TMP-SMX compared with ciprofloxacin.[115]

TMP-SMX was effective for the treatment of otitis media, otitis externa, and sinusitis. However, a later report strongly suggested that TMP-SMX no longer be used as first-line therapy for acute otitis media, especially when resistant respiratory tract pathogens predominate.[116] Ampicillin-resistant strains of *H. influenzae* and *M. catarrhalis* might or might not be susceptible.

Gastrointestinal Infections

Although antibiotics per se prolong the carrier state in acute gastroenteritis caused by *Salmonella* spp., TMP-SMX was effective in eliminating chronic *Salmonella* carriage, including carriage of *Salmonella* Typhi, especially in patients older than 2 years. Typhoid fever was also treated successfully with this combination, although the development of resistant strains has been reported. TMP-SMX may be effective in shigellosis, but resistance is high.[12,18] TMP-SMX also was effective in treating diarrhea caused by enteropathogenic *E. coli* and in the treatment and prophylaxis of traveler's diarrhea with or without loperamide, but increased resistance precludes its empirical use. The combination may be a useful adjunct to fluids in the treatment of cholera, but Huovinen[38] has suggested that with the exception of this possible second-line or third-line use in cholera, TMP-SMX no longer is indicated for *Salmonella, Shigella, Campylobacter,* or enterotoxigenic *E. coli* infections. Comparable efficacy with a fluoroquinolone has been reported for the prevention of spontaneous bacterial peritonitis in cirrhotics.[117]

Skin, Soft Tissue, and Bone Infections

With the increasing incidence of community-acquired methicillin-resistant staphylococcal soft tissue infections,[118] antimicrobial susceptibility testing of clinical isolates has become important. Several community-based series have documented that the vast majority of isolates from the most commonly disseminated USA300 clone are susceptible to TMP-SMX.[47,119] Several studies have suggested that oral TMP-SMX in combination with prompt incision and drainage of fluctuant lesions can result in rapid resolution of infections that, if untreated, can result in bacteremia, pyomyositis, and other severe complications.[120] Two recent studies showed that TMP-SMX (or clindamycin) results in improved outcomes after incision and drainage for uncomplicated skin or subcutaneous abscesses due to MRSA.[121,122] TMP-SMX may be useful in cellulitis, impetigo,[123] acne vulgaris,[124] and head lice (with topical permethrin).[125] TMP-SMX plus rifampin may be as effective as linezolid in chronic MRSA osteomyelitis, and oral TMP-SMX was reported to be effective in the treatment of acute osteomyelitis in children.[126]

Sexually Transmitted Diseases

Historically, TMP-SMX had been effective in the treatment of uncomplicated gonorrhea when used in several dosage regimens (e.g., two tablets orally twice daily for 5 days, four tablets for 2 days, and a single dose of eight tablets). For pharyngeal gonorrhea, especially that caused by penicillinase-producing *N. gonorrhoeae,* nine tablets daily for 5 days was recommended previously, but these regimens no longer are included in the Centers for Disease Control and Prevention guidelines for treatment of sexually transmitted infections. TMP-SMX is ineffective for syphilis. As a result of increased resistance, TMP-SMX is no longer indicated for the treatment of gonorrhea, chancroid, or *Chlamydia trachomatis* infections.

Recent reports have documented clusters of CA-MRSA among men who have sex with men (MSM).[59] A review of personal practices has suggested that increased transmission of MRSA in MSM occurs among those with increased numbers of sexual partners, particularly if they engage in practices that abrade skin or mucous membranes. Almost all isolates from these clusters were multidrug resistant, but most were susceptible to TMP-SMX.[47]

Other Infections

TMP-SMX remains useful against brucellosis (long-term therapy for 6 weeks), but resistance is increasing. Nonetheless, a publication from Turkey reported that only 1 of 42 bacteremic isolates was resistant to TMP-SMX.[127] In combination with rifampin and a tetracycline, TMP-SMX was successful in treating *Brucella* endocarditis.[128] Sulfonamides and TMP-SMX are useful in nocardiosis, including *Nocardia* keratitis, but resistance may occur.[129] Prolonged oral TMP-SMX after 10 to 14 days of IV broad-spectrum β-lactam therapy has been successful in many cases of melioidosis, but optimal dosing should be weight based[130]; TMP-SMX alone may be as effective as in combination with doxycycline.[131] TMP-SMX may be successful in *B. cepacia* bacteremia. Trimethoprim and sulfonamides have been used successfully in Whipple disease, but genetically mediated *Tropheryma whipplei* resistance can be acquired during therapy, resulting in treatment failure.[132] Optimal outcomes were obtained with 2 weeks of IV ceftriaxone followed by 3 months of oral TMP-SMX.[133] TMP-SMX alone may result in relapse[134] and is not as effective as doxycycline plus hydroxychloroquine. TMP-SMX is used in granulomatosis with polyangiitis (GPA) alone or as adjunctive therapy and may reduce relapses.[135] TMP-SMX can be used to treat

infections caused by *Mycobacterium chelonae, Mycobacterium fortuitum,* and *Cyclospora cayetanensis*.

IV TMP-SMX has been useful in treating gram-negative rod bacteremia and staphylococcal bacteremia and endocarditis, although other agents are preferred, given the increasing resistance of Enterobacteriaceae[136] and staphylococci globally. A study showed comparable efficacy for TMP-SMX or vancomycin in the treatment of MRSA bacteremia.[137] TMP-SMX plus vancomycin has shown in vitro synergistic activity when tested against vancomycin-intermediate *S. aureus*, and TMP-SMX plus ceftaroline may be useful in MRSA bacteremia and endocarditis.[138] A TMP-SMX lock solution may prevent bloodstream infections in patients with hemodialysis catheters.

TMP-SMX has been recommended for the treatment of *S. maltophilia* bacteremia; most isolates are susceptible in vitro.[139,140,141] Outcomes have been good in patients with *S. maltophilia* lower respiratory infections who were treated with TMP-SMX. TMP-SMX plus ticarcillin-clavulanate has been used to treat *S. maltophilia* infections in patients with cancer.[142]

Meningitis caused by susceptible organisms may be treated successfully, but other agents are usually preferred. The combination is effective in meningitis caused by *L. monocytogenes,*[143] and TMP-SMX prophylaxis may prevent listeriosis in transplant recipients.[144] *Mycobacterium kansasii, Mycobacterium marinum,* and *Mycobacterium scrofulaceum* are inhibited in vitro by TMP-SMX, and several clinical successes have been reported, often in combination with other agents.[145] TMP-SMX is used in combination with isoniazid and rifampin to treat leprosy.[146]

TMP-SMX has been used in the treatment of susceptible *P. falciparum* infections, although this combination is not active against MDR strains.[147] Sulfadoxine-pyrimethamine with or without azithromycin is effective antimalarial therapy in pregnant women at delivery.[148] HIV-infected patients with *Cystoisospora belli* enteritis have had clinical responses after receiving TMP-SMX; however, relapse after treatment is common, necessitating long-term suppressive therapy.[29,149] TMP-SMX has been shown to decrease the duration of oocyst excretion in children with *C. cayetanensis* infection[149] and is more effective than ciprofloxacin in HIV-infected adults with cyclosporiasis or isosporiasis. TMP-SMX has no activity against other related coccidial parasites, such as *Cryptosporidium parvum*.

TMP-SMX is active against *Toxoplasma gondii* in vitro and has been used clinically but has not been considered superior to first-line therapy with pyrimethamine-sulfadiazine; however, a meta-analysis has suggested that clinical response and medication toxicity rates are comparable between the two regimens.[150] Also, a study has suggested comparable activity in ocular toxoplasmosis,[151] and TMP-SMX may be taken by the oral or intravitreous route; TMP-SMX plus azithromycin is also effective.[152] TMP-SMX prophylaxis in HIV-infected patients protects against toxoplasmosis and may reduce the incidence of bacterial infections.[29] TMP-SMX plus spiramycin may reduce *Toxoplasma* transmission from mother to child.[153] TMP-SMX has excellent in vitro activity against *Acanthamoeba,*[10] and two cases of *Acanthamoeba* meningitis were treated successfully with combination therapy that included TMP-SMX.[154] TMP-SMX is recommended in the management of Q fever in pregnant women[155] and is as effective as a macrolide in the eradication of nasopharyngeal *Bordetella pertussis*.[156]

Pneumocystis jiroveci Infections in HIV-Infected Patients

TMP-SMX has been highly efficacious in the treatment of *P. jiroveci* pneumonia in immunocompromised HIV-infected patients and is considered first-line therapy.[157] TMP-SMX therapy for HIV-infected patients with *P. jiroveci* pneumonia had been associated with a better safety and toxicity profile than trimetrexate or pentamidine and a higher survival rate than atovaquone (see also Chapter 269).[157] Other alternative agents include trimethoprim-dapsone or clindamycin and primaquine. Patients with AIDS frequently respond to therapy but have a higher incidence of adverse reactions, particularly neutropenia and rash.[29,76] Severe hyponatremia and hyperkalemia have also been described. TMP-SMX may be administered orally (two double-strength tablets every 8 hours) or intravenously (5 mg/kg of trimethoprim plus 25 mg/kg of sulfamethoxazole every 8 hours) for 3 weeks. Patients were more likely to manifest a hypersensitivity reaction while receiving TMP-SMX if they had higher $CD4^+$ T-lymphocyte counts and were treated for longer than 2 weeks, suggesting a role for intact cell-mediated immunity in the pathogenesis of these reactions. A multicenter study suggested that lower doses of TMP-SMX (<15 mg/kg of TMP per day) for *P. jiroveci* pneumonia may be comparably effective to full dosing and may result in lower levels of toxicity; another study found comparable therapeutic responses when TMP-SMX doses were decreased during the course of therapy.[158,159] Trimethoprim has also been used successfully with dapsone for treatment of mild-to-moderate *P. jiroveci* pneumonia.[110] The development of TMP-SMX resistance mutations has been associated with *P. jiroveci* treatment failures.[74] The clinical significance of *P. jiroveci* mutations has been debated; at this time there is no indication to monitor *P. jiroveci* resistance mutations prospectively.

TMP-SMX has been used successfully for primary and secondary chemoprophylaxis of *P. jiroveci* pneumonia,[29] is highly cost-effective, and is generally selected as the first-line agent, but its long-term use may be limited by toxicities such as rash or leukopenia.[76] WHO now recommends that all HIV-infected patients with a $CD4^+$ count of 350 cells/mm^3 or lower, thrush, or prior *P. jiroveci* pneumonia receive prophylaxis with TMP-SMX unless they are sulfa allergic, and that in settings with "a high burden of infectious disease," prophylaxis should be considered irrespective of CD4 count.[160] A prospective study has shown that a double-strength tablet taken every other day had comparable efficacy to daily TMP-SMX, with fewer side effects. AIDS patients receiving *P. jiroveci* prophylaxis with TMP-SMX may have fewer serious bacterial infections than patients who did not receive prophylaxis. A study from Africa showed that extending TMP-SMX prophylaxis in children with HIV decreases rates of hospitalization with malarial and nonmalarial infections,[161] consistent with the findings of other studies that HIV-infected and uninfected children receiving TMP-SMX prophylaxis had decreased rates of malaria.[162] Another study found decreased rates of *Loa loa* in patients receiving TMP-SMX prophylaxis, suggesting a possible anthelmintic effect, because the effect was not mediated by antiretroviral use.[163] TMP-SMX is comparable in tolerability and efficacy to second-line regimens, is much less expensive, and is associated with improved survival. Current guidelines suggest discontinuing primary prophylaxis for patients who have a sustained $CD4^+$ count higher than 200 cells/mm^3 for at least 3 months after the initiation of highly active antiretroviral therapy.[29] *P. jiroveci* pneumonia prophylaxis with TMP-SMX confers cross-protection against toxoplasmosis.[29] TMP-SMX is the recommended prophylactic agent for toxoplasmosis for all patients with a $CD4^+$ count lower than 100 cells/mm^3 and a previous episode of toxoplasmic encephalitis. Dapsone is an effective alternative in patients with TMP-SMX allergy. TMP-SMX prophylaxis has been lifesaving when used in immunosuppressed AIDS patients in sub-Saharan Africa and is now the standard of care.[164] TMP-SMX prophylaxis is moderately protective against malaria in HIV-exposed infants and HIV-infected children,[165] and in African children in areas with high levels of antifolate resistance.[166] HIV-infected patients who discontinue TMP-SMX prophylaxis are at increased risk of malaria and diarrhea.[167,168] TMP-SMX may be used in patients with paracoccidioidomycosis, but is not as effective as itraconazole.[169]

Trimethoprim-Sulfamethoxazole Use in Other Immunocompromised Patients

Previous evidence for a striking reduction in gram-negative rod bacteremia in neutropenic patients treated prophylactically with TMP-SMX is controversial.[170] In a more recent meta-analysis, TMP-SMX was effective in reducing gram-positive bacteremia in these patients.[171] Oral prophylaxis with TMP-SMX may decrease the incidence of serious bacterial infections in patients with multiple myeloma. Concerns have been raised about the routine use of TMP-SMX prophylaxis in neutropenic patients because of increasing streptococcal resistance.[172] TMP-SMX remains the preferred *P. jiroveci* prophylactic regimen for patients receiving chemotherapy for hematologic malignancies and for stem cell transplant recipients.[173] Effective prophylactic use of TMP-SMX in chronic granulomatous disease has been reported.[174] Twice-weekly TMP-SMX provides effective *Pneumocystis* prophylaxis for leukemic children.[175] Half-strength (200/40 mg) daily TMP-SMX was found to be optimal for *P. jiroveci* prophylaxis in patients with rheumatologic diseases[176]

TMP-SMX has been successfully used with caspofungin to treat severe *P. jiroveci* pneumonia in renal transplant patients.[177] The addition of an echinocandin to TMP-SMX for severe *P. jiroveci* treatment and prophylaxis in heart transplant recipients was associated with decreased mortality in a small, nonrandomized study.[178]

Trimethoprim Use in Pregnancy

The teratogenicity of trimethoprim in humans has not been fully defined, but this drug has not been recommended for use in pregnancy. One study showed an increased risk of neural tube defects, oral clefts, and cardiovascular defects in infants whose mothers received DHFR inhibitors, including trimethoprim, early in pregnancy.[179] However, a subsequent meta-analysis of 24 studies found that TMP-SMX use in HIV-infected pregnant women was generally well tolerated, and in that setting benefits to the mother outweighed potential risks.[101] Trimethoprim is well tolerated in pediatric patients.

ICLAPRIM

Iclaprim is an investigational new diaminopyrimidine, 5-[(2-cyclopropyl-7,8-dimethoxy-2h-chromen-5-yl)methyl]pyrimidine-2,4-diamine. It exists as a racemic mixture of enantiomers with equal inhibitory activity against bacterial DHFRs. The mechanism of action is similar to that of trimethoprim in selectively blocking DHFR and thymidylate biosynthesis, but it has 8- to 32-fold more activity and therefore might be active against trimethoprim-resistant bacteria. Iclaprim showed synergism with sulfonamides but not with other agents against gram-positive or gram-negative organisms.[180]

Iclaprim has in vitro bactericidal activity against many gram-positive cocci including *S. pneumoniae* (MIC_{90} = 0.06–0.25 μg/mL for penicillin-susceptible strains, 2–8 μg/mL for penicillin-intermediate strains, 2–16 μg/mL for penicillin-resistant strains), *S. pyogenes* (MIC_{90} = 0.03–0.06 μg/mL), *Streptococcus agalactiae* (MIC_{90} = 0.25–0.5 μg/mL), *Enterococcus faecalis* (MIC_{90} = 0.06–>32 μg/mL, *Enterococcus faecium* (MIC_{90} = 0.6–0.32 μg/mL), and *S. aureus,* including methicillin-susceptible, methicillin-resistant, vancomycin-intermediate, and trimethoprim-resistant strains. The MIC_{90}s for methicillin-susceptible *S. aureus* (MSSA) range from 0.06 to 0.12 μg/mL, and for MRSA from 0.06 to 0.5 μg/mL.[181] Iclaprim appeared active against two vancomycin-resistant strains of *S. aureus*, but MIC_{90}s against coagulase-negative staphylococci are too high for reliable clinical activity. MIC_{90}s for iclaprim against *N. gonorrhoeae* are 8 μg/mL for penicillin-susceptible and penicillin-resistant strains and 8 μg/mL for ciprofloxacin-resistant strains.[182]

Oral administration results in 40% bioavailability. The IV preparation at a dose of 1.6 mg/kg produces plasma, extracellular lung fluid, and alveolar macrophage levels (0.6 mg/L, 12.6 mg/L, and 24.5 mg/L, respectively) that exceed the MICs for penicillin-susceptible and penicillin-resistant pneumococci for up to 7 and 4 hours, respectively.[183] These levels exceeded the MIC_{90} for MRSA by up to 7 hours. The terminal half-life is about 2 hours, and the drug's hepatic metabolites are excreted in the urine. Although an earlier trial revealed cardiotoxicity, especially QTc prolongation, and did not meet acceptable clinical results, reevaluation at a lower fixed dose of 80 mg infused over 2 hours at 12 hourly intervals has been used in clinical trials. In a phase II study in hospital-acquired pneumonia due to gram-positive pathogens, iclaprim was comparable to vancomycin.[184] In a phase III randomized controlled trial in acute bacterial skin and soft tissue infections caused by gram-positive organisms, compared with vancomycin, IV iclaprim showed noninferiority at an early time point and acceptable safety.[185]

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The complete reference list is available online at Expert Consult.

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35 Quinolones

David C. Hooper and Jacob Strahilevitz

SHORT VIEW SUMMARY

- Usual adult dose
  - Norfloxacin: oral (PO) 400 mg every 12 hours
  - Ciprofloxacin: PO 250 to 750 mg every 12 hours; intravenous (IV) 200 to 400 mg every 12 hours
  - Ofloxacin: PO or IV 200 to 400 mg every 12 hours
  - Levofloxacin: PO or IV 250 to 750 mg daily; 240-mg nebulizer solution (available outside United States)
  - Moxifloxacin: PO or IV 400 mg daily
  - Gemifloxacin: PO 320 mg daily
  - Delafloxacin: PO 450 mg every 12 hours; IV 300 mg every 12 hours
- Renal and hepatic failure: decrease dose in renal failure for all except moxifloxacin.
- Cerebrospinal fluid penetration: low
- Adverse effects
  - Common: gastrointestinal upset, central nervous system stimulation
  - Less common: seizures, tendinitis and tendon rupture, *Clostridioides difficile* (formerly *Clostridium difficile*) disease, dysglycemia, exacerbations of myasthenia gravis, peripheral neuropathy
- Contraindications: prior quinolone allergy, prior neuropathy
- Drug-drug interactions
  - Do not take oral formulations with aluminum-, calcium-, magnesium-, or iron-containing compounds.
  - Avoid other agents that prolong the QT interval (particularly with moxifloxacin).
  - Avoid concomitant use of tizanidine.
  - Variable interactions occur with warfarin; monitor international normalized ratio.
- Indications (US Food and Drug Administration approved)
  - Norfloxacin: used to treat uncomplicated and complicated urinary tract infections and prostatitis. Can be used for urethral or cervical gonorrhea *only* if infecting isolates are known to be susceptible.
  - Ciprofloxacin: used to treat complicated and uncomplicated urinary tract infections, chronic bacterial prostatitis, uncomplicated cervical and urethral gonorrhea (*only* if infecting isolates are known to be susceptible), complicated intraabdominal infections, bacterial diarrhea, typhoid fever, acute bacterial sinusitis, lower respiratory tract infections (when *not* caused by *Streptococcus pneumoniae*), inhalational anthrax, skin and skin structure infections, and bone and joint infections.
  - Ofloxacin: used to treat complicated and uncomplicated urinary tract infections, bacterial prostatitis, uncomplicated cervical and urethral gonorrhea (*only* if infecting isolates are known to be susceptible), nongonococcal urethritis and cervicitis caused by *Chlamydia trachomatis,* acute pelvic inflammatory disease, acute bacterial exacerbations of chronic bronchitis, community-acquired pneumonia, and uncomplicated skin and skin structure infections.
  - Levofloxacin: used to treat complicated and uncomplicated urinary tract infections, acute pyelonephritis, chronic bacterial prostatitis, acute bacterial exacerbations of chronic bronchitis, community-acquired pneumonia, hospital-acquired pneumonia, inhalational anthrax, acute bacterial sinusitis, and complicated and uncomplicated skin and skin structure infections. Nebulizer solution used to treat chronic pulmonary infections due to *Pseudomonas aeruginosa* infections in patients with cystic fibrosis (outside United States).
  - Moxifloxacin: used to treat community-acquired pneumonia, acute bacterial exacerbation of chronic bronchitis, acute bacterial sinusitis, and alternative agent in multidrug-resistant tuberculosis (off-label use).
  - Gemifloxacin: used to treat community-acquired pneumonia and acute bacterial exacerbations of chronic bronchitis.
  - Delafloxacin: used to treat acute bacterial skin and skin structure infections.

The first member of the quinolone class of antimicrobial agents, nalidixic acid, is a 1,8-naphthyridine structure that was identified by Lesher and associates in 1962 as a byproduct of chloroquine synthesis. Oxolinic acid and cinoxacin were also developed in the 1970s, but it was the identification in the 1980s of the fluorine- and piperazinyl-substituted derivatives with substantially greater potency and expanded spectrum that enabled expansion of this class of compounds. A broad spectrum of activity, good oral absorption, and generally good overall tolerability have resulted in extensive clinical use of the newer fluoroquinolones. Several quinolones, however, including temafloxacin, sparfloxacin, grepafloxacin, trovafloxacin, and gatifloxacin, were removed from clinical use after approval because of toxicities, which were uncommon but severe in some cases. This chapter focuses on those quinolones that are currently in clinical use.

CHEMICAL STRUCTURES

All current quinolone derivatives in clinical use have a dual ring structure with a nitrogen at position 1, a carbonyl group at position 4, and a carboxyl group attached to the carbon at the 3 position of the first ring (Fig. 35.1). Several different dual ring structures—cinnoline (nitrogens at positions 1 and 2), pyridopyrimidine (nitrogens at positions 1, 6, and 8), and 2-pyridone (a dual ring structure with the nitrogen located at the junction of the two rings)—have been developed, but quinolones, which themselves have a carbon at position 8 in the second ring, and naphthyridines, which contain a nitrogen at position 8, have been most widely successful. Both quinolones and naphthyridines, however, are commonly referred to as quinolones.

Nalidixic acid is a 1,8-naphthyridine with 1-ethyl and 7-methyl substituents (see Fig. 35.1). Oxolinic acid (quinolone ring; see Fig. 35.1) and cinoxacin (cinnoline ring; not shown in Fig. 35.1) also have 1-ethyl substitutions, as well as a dioxolo ring bridging positions 6 and 7. Potency is greatly improved by the addition of a fluorine at position 6, and potency against gram-negative bacteria is further enhanced by the addition of a piperazinyl (norfloxacin, enoxacin, ciprofloxacin), methyl-piperazinyl (pefloxacin, ofloxacin, lomefloxacin, fleroxacin, temafloxacin, levofloxacin, grepafloxacin, gatifloxacin), or dimethyl-piperazinyl (sparfloxacin) substituent at position 7.[1] Methyl substituents on the piperazine ring generally result in improved oral bioavailability. These structural features are common to most of the newer quinolone derivatives now in clinical use. Pyrrolidinyl (tosufloxacin, clinafloxacin, gemifloxacin) or dual ring substituents (trovafloxacin, moxifloxacin, sitafloxacin) at position 7 enhance activity against gram-positive bacteria. Delafloxacin

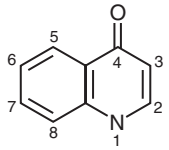

Quinolone Core Structure

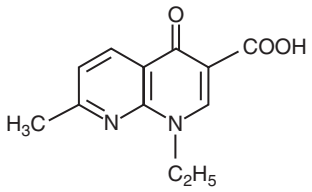

Nalidixic Acid

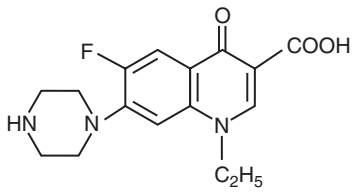

Norfloxacin

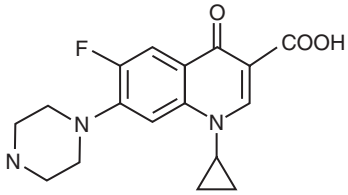

Ciprofloxacin

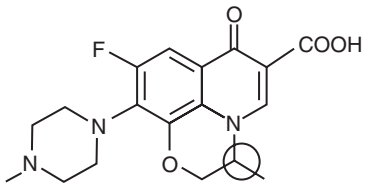

Levofloxacin

Ofloxacin

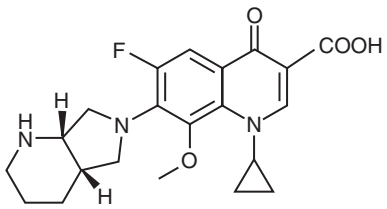

Moxifloxacin

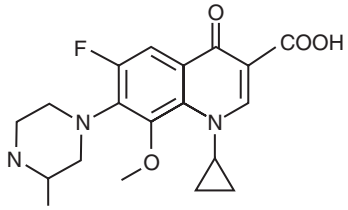

Gatifloxacin

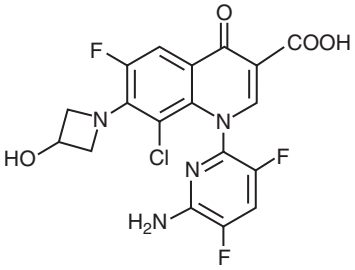

Delafloxacin

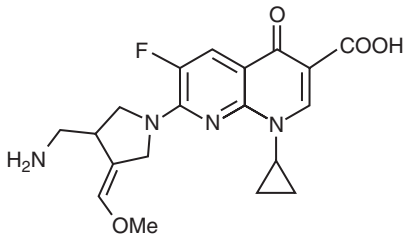

Gemifloxacin

FIG. 35.1 Structures of selected quinolones in clinical use or under development. The circle in the levofloxacin structure indicates the asymmetric carbon resulting in the stereoisomers that make up the racemic mixture in ofloxacin. Levofloxacin is the more active of the two stereoisomers (levofloxacin and ciprofloxacin) of ofloxacin.

is distinct because of its anionic 3-hydroxy-1-azetidinyl substituent at position 7, which contributes to its increased potency against gram-positive bacteria and increased activity at low pH. A number of compounds (sparfloxacin, gatifloxacin, moxifloxacin, gemifloxacin) use the 1-cyclopropyl group, which enhances potency, particularly against gram-negative bacteria, and was originally identified for ciprofloxacin. The 1-difluorophenyl group found in temafloxacin, tosufloxacin, and trovafloxacin and the 1-6-amino-3,5-difluoro-2-pyridinyl found in delafloxacin add potency against gram-positive bacteria. An additional ring structure bridging positions 1 and 8 is found in ofloxacin and levofloxacin; the additional ring of ofloxacin contains an asymmetric carbon, resulting in stereoisomeric forms. Ofloxacin is a racemic mixture of the two stereoisomers, and levofloxacin is the more potent of the two and predictably is twice as potent as ofloxacin in vitro. At position 5, replacement of the hydrogen by an amino group (sparfloxacin) or a methyl group (grepafloxacin) results in some enhancement of activity against gram-positive bacteria. At position 8, addition of a halide (chlorine-clinafloxacin, fluorine-sparfloxacin, and sitafloxacin) or a methoxy group (gatifloxacin, moxifloxacin) enhances activity against anaerobic bacteria. Halides at position 8 increase the risk of phototoxicity, but methoxy groups at this position reduce risks of phototoxicity, even relative to compounds with a hydrogen at position 8.

Coming full circle in structural modifications, "desfluoro" quinolones (garenoxacin) have been identified with excellent and similar potency

to their fluorinated counterparts but with possible reductions in joint toxicities.[2–4]

MECHANISM OF ACTION

The quinolones rapidly inhibit bacterial DNA synthesis, an event that is followed by rapid bacterial cell death. The molecular events that underlie these actions are understood in part, but details remain to be defined.[5]

Quinolones inhibit the enzymatic activities of two members of the topoisomerase class of enzymes—DNA gyrase and topoisomerase IV—and promote the cleavage of DNA in these enzyme-DNA complexes. DNA gyrase, which was the first-recognized target of quinolones, is an essential bacterial enzyme composed of two A and two B subunits, products of the *gyrA* and *gyrB* genes, respectively.[6,7] DNA gyrase uniquely catalyzes the introduction of negative superhelical twists into closed covalently circular chromosomal and plasmid DNA within the bacterial cell. The superhelical state of intracellular DNA is regulated by the actions of DNA gyrase and topoisomerase I, which removes DNA superhelical twists but is not inhibited by quinolones. DNA superhelicity affects the initiation of DNA replication and transcription of many genes. DNA gyrase is also responsible for removing positive superhelical twists that accumulate ahead of the DNA replication fork. These activities result from the enzyme's coordinated breaking of both strands of duplex DNA, passage of another segment of DNA through the break, and resealing of the break, a mechanism that defines type II topoisomerases.

Quinolones also inhibit the activities of topoisomerase IV, another type II topoisomerase that is composed of two subunits encoded by the *parC* and *parE* genes. Topoisomerase IV and DNA gyrase are structurally related; *parC* is homologous to *gyrA*, and *parE* is homologous to *gyrB*.[8] Topoisomerase IV functions to resolve (decatenate) interlinked (catenated) daughter DNA molecules that result from replication of circular DNA, to allow their segregation into daughter cells. Thus DNA gyrase and topoisomerase IV have distinct essential roles in bacterial DNA replication.[9] Gyrase can mediate the functions of topoisomerase IV (albeit less efficiently as a decatenase), but topoisomerase IV is unable to introduce negative supercoils into DNA, a function that is unique to DNA gyrase. A few species of human pathogens (e.g., *Mycobacterium tuberculosis*, *Treponema pallidum*) lack topoisomerase IV, and in the case of *M. tuberculosis,* gyrase appears to serve the decatenation function of topoisomerase IV, in addition to its own functions.[10]

Quinolones inhibit enzyme function by blocking the resealing of the DNA double-stranded break, but in addition this process stabilizes a catalytic intermediate covalent complex of enzyme and DNA that serves as a barrier to movement of the DNA replication fork[11] or transcription complexes[12] and can be converted to permanent double-stranded DNA breaks,[13] thereby functioning as topoisomerase poisons and contributing to bactericidal activity.[14] Quinolones have been shown to bind specifically to the complex of DNA gyrase and DNA rather than to DNA gyrase alone.[15,16] Single *gyrA* or *gyrB* mutants of *Escherichia coli* can produce quinolone resistance in bacteria, resistance to gyrase inhibition, and gyrase-DNA complexes with reduced quinolone binding (see "Mechanisms of Acquired Bacterial Resistance").[16] In contrast to the information for *E. coli,* which is also similar to that for other gram-negative bacteria studied, for *Staphylococcus aureus* and *Streptococcus pneumoniae* interactions of quinolones with topoisomerase IV have been shown to determine antibacterial activity by the identification of *parC* (initially named *grlA* in *S. aureus*) and *parE* (*grlB* in *S. aureus*) single mutants that have reduced quinolone activity.[17] Based on studies of mutants of this type, a general pattern has emerged. For most gram-negative bacteria, DNA gyrase is the primary quinolone target, and for many gram-positive bacteria, topoisomerase IV is the primary target, with gyrase being the secondary target. There are exceptions, however, that depend on the quinolone studied.[18,19] These patterns appear to result from the relative sensitivities of these two topoisomerases to a given quinolone, with the more sensitive of the two enzymes in a particular bacterial species defining the primary target of a particular quinolone.[20] Some quinolones under development appear to have similar potencies against both DNA gyrase and topoisomerase IV in some bacterial species.[21,22]

Quinolone inhibition of bacterial DNA replication and bacterial killing may be dissociated under some conditions, suggesting that events in addition to the initial interaction of quinolones with the topoisomerase-DNA complex may be required for cell killing.[23] In particular, inhibitors of RNA and protein synthesis reduce the bactericidal activity of some quinolones but do not affect their ability to inhibit bacterial DNA synthesis.[24] Thus inhibition of bacterial DNA synthesis per se is not sufficient to account for bacterial killing, and possibly, newly synthesized gene products may also be necessary. This effect may account for the observations that at high concentrations of quinolones, which also secondarily inhibit protein synthesis, cell killing is reduced.[5,23]

Quinolones, β-lactams, and aminoglycosides have all been shown to generate the production of hydroxyl radicals through a proposed complex series of events involving stimulation of oxidation of reduced nicotinamide adenine dinucleotide via electron transport, which generates superoxide radicals that then damage intracellular thiol-bound iron clusters, releasing ferrous iron for oxidation by the Fenton reaction. Production of hydroxyl radicals can damage cellular macromolecules and contribute to bacterial cell death, thus suggesting one component of a common pathway of bacterial lethality for different classes of bactericidal antibiotics.[25]

Eukaryotic cells also contain topoisomerases, and eukaryotic topoisomerase II, which is a homodimeric enzyme that is a member of the type IIA class of topoisomerases, such as DNA gyrase and topoisomerase IV, has a domain structure and limited primary amino-acid sequence homology similar to the bacterial enzymes.[26–28] Current antibacterial quinolones in clinical use have only minimal activity against mammalian topoisomerase II,[29] but other quinolone structures, containing a 7-hydroxyphenyl substituent or an isothiazolo ring bridging positions 2 and 3, have been shown to have substantially enhanced potency against the mammalian enzyme.[27]

MECHANISMS OF ACQUIRED BACTERIAL RESISTANCE

Bacteria acquire resistance to quinolones from spontaneously occurring mutations in chromosomal genes that either alter the target enzymes, DNA gyrase, and topoisomerase IV, or alter drug permeation across the bacterial cell membranes.[30,31] Recently, several plasmid-mediated quinolone resistance mechanisms have been identified in clinical isolates of Enterobacteriaceae. These horizontally acquired genes are generally not sufficient alone to confer clinical resistance to fluoroquinolones but enable survival under drug exposure and facilitate selection of chromosomal mutations.[32] The products of the plasmid-encoded *qnr* genes have been shown to protect DNA gyrase and topoisomerase IV from quinolone action.[33,34] A modification of a common plasmid-encoded aminoglycoside acetylating enzyme, Aac(6′)-Ib-cr, mediates quinolone resistance through acetylation of the nitrogen on the piperazinyl substituent at position 7 of ciprofloxacin and norfloxacin.[35] Genes encoding efflux pumps, such as QepA[36,37] and OqxAB,[38] that include quinolones in their substrate profiles have also been found on plasmids, albeit infrequently to date.

Resistant chromosomal mutants may be selected in the laboratory by plating bacteria on drug-containing agar. The frequency of occurrence of spontaneous mutants differs with the selecting drug concentration and the drug. For gram-negative bacteria selected with the newer fluoroquinolones, frequencies range, in general, from 10^{-6} or higher at twofold above the minimal inhibitory concentration (MIC) to undetectable ($<10^{-10}$) at 16- to 32-fold above the MIC. With a similar selection with nalidixic acid, mutants are detected more frequently when selected at a similar factor greater than the MIC because single mutations can cause a higher level of increase in resistance (>30-fold) relative to ciprofloxacin (eightfold) and other fluoroquinolones. This difference results in part because the magnitude of the increase in resistance conferred by a single-target mutation is modified by the interaction of drug with the second target enzyme. For example, for nalidixic acid, which has little activity against topoisomerase IV, a common mutation in the GyrA subunit of DNA gyrase causes a 30-fold increase in the MIC. In contrast, for ciprofloxacin, which has activity against topoisomerase IV (albeit less than its activity against gyrase), the same *gyrA* gene mutation causes only an eightfold increase in the MIC, despite

the fact that both drugs exhibit similar loss of activity against purified DNA gyrase reconstituted with the resistant GyrA subunit.[39] Thus quinolone interaction with a second target enzyme puts a ceiling on the magnitude of the increase in resistance caused by mutation in the first enzyme target. For some quinolones that have similar potency against both target enzymes, frequencies of selection of first-step mutants may be particularly low because single-target mutations produce little or no increment in resistance.[40] Thus, for such quinolones, mutations in both targets are needed to produce substantial increments in the MIC. Serial passage of bacteria with increasing concentrations of quinolones selects mutants with high levels of resistance resulting from the additive effects of multiple mutations involving both enzyme targets.[41]

Alterations in the A subunit of DNA gyrase that cause quinolone resistance have been defined in a substantial number of clinical and laboratory isolates of *E. coli.* These alterations are clustered between amino acids 67 and 106 in the amino terminus of the A protein near the active site of the enzyme (tyrosine-122).[31] In particular, changes in serine-83 (to leucine or tryptophan) are most common and cause the largest increment in resistance, as well as reduced binding of drug to the gyrase-DNA complex in vitro. Leucine-83 causes a 128-fold increase in resistance to nalidixic acid but lesser increases in resistance to the newer fluoroquinolones (16- to 32-fold), thus likely accounting for the greater ease of selection of resistant mutants with nalidixic acid. Similar changes in the A subunit have been associated with resistance in many species of gram-negative bacteria. Single amino-acid changes in the midportion of the gyrase B protein have also been found to cause lower levels of resistance to nalidixic acid and fluoroquinolones.[31,42]

Resistance mutations in the *parC* gene of topoisomerase IV in *S. aureus* and *S. pneumoniae* have been most commonly found at position 80, in which a wild-type serine (homologous to serine-83 of DNA gyrase) is replaced by phenylalanine or tyrosine.[17,43] These mutations cause eightfold increases in resistance to several fluoroquinolones. Resistance mutations have also been found less commonly in the *parE* gene, often in positions similar to those of resistance mutations in *gyrB*.[31]

Stepwise increasing resistance occurs by sequential mutations in the *gyrA* (or *gyrB*) and *parC* (or *parE*) genes, with the first target mutation occurring in a gene for the more sensitive target enzyme. In the most highly resistant clinical strains of both gram-positive and gram-negative bacteria, one or more mutations in both *gyrA* and *parC* have been found commonly. Some species, including *M. tuberculosis, Helicobacter pylori,* and *T. pallidum,* appear to lack genes for topoisomerase IV.[31] Thus target resistance may occur more readily in the absence of a second drug target to limit the effects of resistance mutations in gyrase.

The routes of quinolone permeation across bacterial cell membranes are not fully defined, but the hydrophilic quinolones appear to diffuse across the gram-negative bacterial outer membrane through porin channels. In *E. coli* and *Pseudomonas aeruginosa,* resistance mutations in genes that affect expression of outer membrane proteins have been described.[44,45] In both cases, resistance cannot be explained by reduced diffusion alone, and reduced drug accumulation in some mutants is energy dependent, being abolished by agents that collapse the proton gradient across the membrane. In *E. coli,* resistance of multiple antibiotic resistance mutants, which exhibit reduced porin channels, is dependent on the AcrAB efflux pump, which is linked to the TolC outer membrane protein.[46,47] Overexpression of AcrAB has also been associated with quinolone resistance in clinical isolates.[48] In *P. aeruginosa,* resistance has been shown to be caused by increased expression of one of several sets of three genes that encode an efflux pump in the inner membrane, a periplasm-spanning membrane fusion protein, and a linked outer membrane protein (e.g., MexAB-OprM, composed of the MexA membrane fusion protein, MexB inner membrane efflux pump, and OprM outer membrane protein).[31,44,49] Such increased expression of one or more pump complexes is found commonly along with *gyrA* mutations in quinolone-resistant clinical isolates of *P. aeruginosa.*[50] AcrAB and MexAB are members of the resistance-nodulation-division (RND) family, which are common in gram-negative bacteria. Other RND pumps that confer quinolone resistance include MexCD-OprJ, MexEF-OprN, and MexXY-OprM (*P. aeruginosa*),[51] AcrAB homologs (*Salmonella* spp.[52] and *Enterobacter aerogenes*[53]), CmeABC (*Campylobacter jejuni*), OqxAB-TolC (*Klebsiella pneumoniae*),[54] AdeABC and AdeFGH (*Acinetobacter baumannii*), and SmeDEF (*Stenotrophomonas maltophilia*). Resistance in many mutants of this type is pleiotropic, with additional low levels of resistance to tetracycline, chloramphenicol, some β-lactams, and other antibiotics because of the broad substrate profiles of most such pumps; hence the term *multidrug resistance* is often applied to these efflux pumps.[44,55]

In gram-positive bacteria, which lack an outer membrane, overexpression of endogenous efflux pumps has also been shown to cause low-level quinolone resistance. The *S. aureus norA* gene encodes a native membrane protein that pumps hydrophilic quinolones, driven by the proton gradient across the cell membrane,[56] and overexpression of NorA, because of a mutation in the *norA* promoter region or in other regulators, causes resistance to norfloxacin, ciprofloxacin, and levofloxacin, in order of decreasing magnitude of the effect on MICs.[57] Some quinolones, such as moxifloxacin, are not affected by NorA overexpression. Other related pumps, such as NorB and NorC, can cause resistance to these quinolones as well.[58,59] In *S. pneumoniae,* PmrA, a pump with a structure similar to NorA, can also contribute to reduced quinolone susceptibility.[60] Reserpine, an inhibitor of several efflux pumps in gram-positive bacteria, improves MICs of some quinolones in clinical isolates of *S. pneumoniae* and viridans streptococci.[61] Other efflux transporters of the major facilitator superfamily, like NorA, NorB, and NorC, that can contribute to quinolone resistance in *S. aureus* include MdeA (norfloxacin, ciprofloxacin),[62] SdrM (norfloxacin),[63] QacB(III) (norfloxacin, ciprofloxacin),[64] and LmrS (gatifloxacin),[65] and in *Listeria monocytogenes* Lde.[66] Members of other pump families have also been shown to confer quinolone resistance, including MepA[67] in *S. aureus,* FepA in *L. monocytogenes,* PatAB[68] in *S. pneumoniae,* and SatAB[69] in *Streptococcus suis.* For reviews of clinically relevant chromosomally encoded multidrug resistance efflux pumps and their regulation, see the reviews by Piddock[55] and by Hooper and Jacoby.[30]

Regulation of expression of many pumps is complex, and overexpression has in many cases been shown to be due to mutations in specific regulators or networks of regulators.[30] Notably, expression can be selectively increased in certain infection environments and can contribute to fitness in those environments in the absence of antibiotics. In such cases, physiologic overexpression of drug resistance pumps in an infection environment could contribute to a reduced response to the affected antimicrobial, as well as discordance between determinations of bacterial susceptibility in vitro and responses to antimicrobials in vivo.

Plasmid-mediated quinolone resistance, long thought not to occur, was first identified and verified in multidrug-resistant, clinical isolates of *K. pneumoniae* from Alabama.[70] Used as donors, these isolates transferred plasmids by conjugation to a recipient laboratory *E. coli* with selection for resistance to β-lactams. Unexpectedly, the recipients acquired low-level resistance to quinolones as well. The plasmid-encoded gene responsible for quinolone resistance, *qnr,* was located on class I integrons flanked by other resistance genes, which can transfer multidrug resistance en bloc with the plasmid.[33] The gene *qnr* encodes a protein of the pentapeptide repeat family that is able to protect purified DNA gyrase and topoisomerase IV from quinolone action. How this protection occurs at the molecular level has not yet been defined. The plasmid-borne *qnr* genes currently comprise seven families—*qnrA, qnrB, qnrS, qnrC, qnrD, qnrE,* and *qnrVC*—and include multiple alleles.[71,72] The *qnr* genes have already been detected worldwide and are found in 1% to 7% of tested Enterobacteriaceae, predominantly in strains of *Enterobacter* spp., *K. pneumoniae,* and *E. coli.* Although resistance mediated by *qnr* genes alone is usually low level, *qnr* plasmids are usually found in strains of Enterobacteriaceae with additional chromosomal resistance mutations, and the presence of *qnr* has been shown to increase the frequency of selection of these mutations, presumably by reducing the quinolone therapeutic index.[73]

Resistance mediated by a fluoroquinolone-modifying enzyme has also been reported. A variant of the gene encoding aminoglycoside acetyltransferase Aac(6′)-Ib was able to reduce the activity of ciprofloxacin by *N*-acetylation at the amino nitrogen on its piperazinyl substituent.[35] The increase in the MIC conferred by Aac(6′)-Ib-cr was smaller than that conferred by Qnr proteins, and it was selective only for ciprofloxacin and norfloxacin, as predicted by their chemical structure. Other quinolones lacking an unsubstituted piperazinyl nitrogen were unaffected

(see Fig. 35.1). Although the increase in the MIC of ciprofloxacin and norfloxacin was modest (threefold to fourfold), the effect on mutant prevention concentration was marked and facilitated selection of resistant clones of wild-type *E. coli* at 1.6 µg/mL, a level approximating the peak serum concentration of free ciprofloxacin during therapy.[35] The gene *aac(6′)-Ib-cr*, like the *qnr* genes, is now common worldwide and also associated with multidrug resistance.

Recently a second plasmid-encoded quinolone-modifying enzyme was reported.[74] The gene *crpP* was found on a plasmid in a clinical isolate of *P. aeruginosa* and when cloned in *E. coli* conferred a selective eightfold increase in resistance to ciprofloxacin but not other quinolones. Purified CrpP phosphorylated the carboxyl group of ciprofloxacin in an ATP-dependent fashion, and the phosphorylated ciprofloxacin was followed by subsequent degradation steps. The extent to which *crpP* is found in clinical isolates and its contribution to ciprofloxacin resistance in *P. aeruginosa* remains uncertain.

Two plasmid-mediated quinolone efflux pumps have also been found: OqxAB, which confers resistance to the antibiotic olaquindox (a quinoxaline derivative that is used in agriculture as a veterinary growth promoter) as well as an increased MIC to nalidixic acid and ciprofloxacin, and QepA, which mediates increased resistance to several antibiotics, including ciprofloxacin and erythromycin.[75,76]

ANTIMICROBIAL ACTIVITY

Current quinolones are most active against aerobic gram-negative bacilli, particularly members of the family Enterobacteriaceae and *Haemophilus* spp., and against gram-negative cocci, such as *Neisseria* spp. and *Moraxella (Branhamella) catarrhalis* (Table 35.1).[77–80] Relative to nalidixic acid, the fluoroquinolones also have additional activity against gram-negative bacilli, such as *P. aeruginosa* (see Table 35.1), and against staphylococci (Table 35.2). Ciprofloxacin remains the most potent marketed fluoroquinolone against gram-negative bacteria, and it and levofloxacin in the United States and sitafloxacin in Japan are the only available quinolones with sufficient potency for use against susceptible strains of *P. aeruginosa.* Resistance may emerge easily, however, when these quinolones are used alone for treatment of serious pseudomonal infections. For norfloxacin, ciprofloxacin, and ofloxacin, activity against streptococci and many anaerobes is limited (Table 35.3; see also Table 35.2). Agents released subsequently in the United States—levofloxacin, gatifloxacin (marketed as an ophthalmic solution), moxifloxacin, gemifloxacin, and delafloxacin—however, have greater potency against these organisms, with gemifloxacin and delafloxacin being especially potent against *S. pneumoniae* and *S. aureus,* respectively. For the fluoroquinolones that are used for treatment of infections outside the urinary tract, the MICs listed in Table 35.1 should be interpreted in relation to peak drug concentrations in serum, which range from 1.1 to 6.4 µg/mL (with usual dosing), and in relation to drug concentrations in urine that are manyfold higher for most quinolones, except those that are largely excreted by nonrenal mechanisms (see "Pharmacology"). For highly susceptible organisms, MICs may be 10- to 30-fold below achievable serum concentrations. Oral ciprofloxacin and levofloxacin are recommended for prophylaxis of anthrax, with potential similar efficacy and toxicity to doxycycline. For treatment of severe anthrax disease, parenteral fluoroquinolones are favored over doxycycline.[81] It should be noted that quinolone resistance can be selected for in *Bacillus anthracis.*[82] Thus efficacy may not be predicted in the unfortunate case of use of *B. anthracis* as an agent of bioterrorism. Among the currently available fluoroquinolones with activity against *S. aureus,* only delafloxacin exhibits a low probability for selection of resistant mutants and is thus recommended for skin and soft tissue infections.

Fluoroquinolones also have activity against mycobacteria (see Table 35.3).[77,83] Ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin are active against *M. tuberculosis, Mycobacterium fortuitum, Mycobacterium kansasii,* and some strains of *Mycobacterium chelonae* but, except for moxifloxacin, have only fair or poor activity against *Mycobacterium avium-intracellulare* complex. Ofloxacin and pefloxacin have activity against *Mycobacterium leprae* in animal models. Inclusion of fluoroquinolones added to the bactericidal activity but failed to contribute to shortening the duration of treatment for pulmonary tuberculosis,[84,85] or improve survival in tuberculous meningitis.[86,87]

Other bacteria are also inhibited by quinolones in vitro (see Table 35.1).[88] Ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, moxifloxacin, gemifloxacin, and delafloxacin all have activity against the agents of atypical pneumonias, including *Legionella pneumophila, Mycoplasma pneumoniae,* and *Chlamydia pneumoniae,* and against genital pathogens, such as *Chlamydia trachomatis, Ureaplasma urealyticum,* and *Mycoplasma hominis. Treponema pallidum* is resistant to ofloxacin in animal models, and no other quinolone has been shown to have activity against this spirochete. Moxifloxacin, delafloxacin, and sitafloxacin among available quinolones have increased potency against anaerobes.

Activity in vitro is reduced in the presence of urine but generally not in the presence of serum. Activity is also reduced at pH values below 7 and in the presence of magnesium concentrations at 8 to 16 mM. Both of these factors often contribute to the reduced quinolone activity observed in the presence of urine. Unlike other fluoroquinolones, delafloxacin is weakly acidic, thus providing enhanced antibacterial potency and reducing the MICs of pathogens in environments with lower pH. Low pH and elevated concentrations of magnesium are associated with reduced drug accumulation in *E. coli.*[78]

Minimal bactericidal concentrations of quinolones are usually within twofold to fourfold of the MIC, and the magnitude of bacterial killing increases with further increases in drug concentration, reaching a maximum at about 30-fold above the MIC. Above this maximal killing concentration, paradoxical reductions in killing are observed and are associated with additional inhibition of protein synthesis by high concentrations of quinolones.[5,89]

The postantibiotic effect (PAE) is the period of time required for bacteria surviving a brief exposure to an antimicrobial agent to resume growth. Although PAE may be relevant in estimating the proper interval for drug dosing, its clinical importance, which may be greatest in patients with compromised host defenses, has not been proved. For quinolones, the duration of PAE has been in the range of 1 to 2 hours and tends to increase with increasing drug concentrations and length of drug exposure.

Combinations of quinolones with other antimicrobial agents have been extensively studied, and interactions with β-lactams and aminoglycosides, as measured by fractional inhibitory or bactericidal concentrations or time-kill curve studies, have generally been found to be indifferent or additive.[77] Synergistic interactions were found in a minority of strains, although for *P. aeruginosa* in some studies, synergy was seen in a substantial minority (30%–50%) of strains, including those already lacking susceptibility to one or both drugs.[90–92] Antagonistic interactions of quinolones with other antimicrobial agents have been rare. Rifampin reduced the bactericidal activity of ciprofloxacin against *S. aureus* and of moxifloxacin against *M. tuberculosis* in some studies.[93,94]

PHARMACOLOGY

Absorption

The quinolones are well absorbed from the upper gastrointestinal tract, with bioavailability exceeding 50% for all compounds and approaching 100% for several (Table 35.4).[95,96] Peak concentrations in serum are usually attained within 1 to 3 hours of administering a dose. Neither food nor achlorhydria substantially affects the extent of quinolone absorption, but food may delay the time to reach peak drug concentrations in serum.[97,98] Enteral feedings given orally, however, may reduce absorption.[99] Absorption is good when ciprofloxacin is given by nasogastric or jejunostomy tube[100] but may be decreased by concurrent enteral feedings given through these tubes.[99]

Peak fluoroquinolone concentrations in serum, after a 200-mg to 500-mg dose, range from 1.4 to 1.5 µg/mL for gemifloxacin and norfloxacin to 5.7 and 7.45 µg/mL for levofloxacin and delafloxacin, respectively (see Table 35.4). A 1-g dose of nalidixic acid produces concentrations of 20 to 50 µg/mL of serum. Drug binding to serum proteins (see Table 35.4) is generally low (30%–50%) but is higher for gemifloxacin (55%–72%) and delafloxacin (84%).

Distribution in Tissues

The volumes of distribution of quinolones are high and in most cases, with the exception of delafloxacin, exceed the volume of total body water (see Table 35.4), indicating accumulation in some tissues.

Text continued on p. 436

TABLE 35.1 Activity of Selected Quinolones Against Selected Gram-Negative, Mycoplasmal, and Chlamydial Pathogens in vitro

	REPRESENTATIVE MIC_{90} (range) (μg/mL)[a,b]									
ORGANISM	**Nalidixic Acid**	**Norfloxacin**	**Pefloxacin**	**Ciprofloxacin**	**Ofloxacin**	**Levofloxacin**	**Gatifloxacin**	**Moxifloxacin**	**Gemifloxacin**	**Delafloxacin**
Acinetobacter spp.	(32–256)	(8–64)	(1–8)	0.25–2 (0.25–>128)	0.25–>8	0.05–32	0.03–8	>0.25–16	4–32 (0.008–32)	>4 (0.015–>4)
Aeromonas spp.	0.5	0.03	0.03	0.008–≤0.06	0.03–≤0.06	≤0.015–0.03	0.03	0.03	0.03	—
Burkholderia cepacia	16	8 (8–50)	—	(2–>256)	4–25	128 (2–256)	128 (2–256)	128 (1–256)	32 (2–64)	16 (0.25–16)
Campylobacter jejuni	8 (4–64)	(0.25–2)	0.5	0.03–64	0.25–32	0.12–32	0.25–4	0.06–0.13	—	—
Chlamydia pneumoniae	—	—	—	2	1	0.5–1	0.12	0.06–1	0.25	0.125
Chlamydia trachomatis	—	≥16	—	0.5–2	2 (0.25–4)	0.25–0.5	0.06	0.06 (0.015–0.12)	—	
Citrobacter spp.	8	0.5 (<0.25–50)	0.4–1	0.06–0.25	0.25–1	0.5 (0.12–4)	0.25–2	0.25–2	2 (0.004–16)	2 (0.008–>4)
Enterobacter aerogenes	8	0.5 (0.2–2)	0.25	0.5 (0.03–>16)	0.25 (0.1–1)	0.5 (0.06–16)	1 (1–16)	2 (0.25–>16)	0.25 (0.008–2)	0.5–2 (0.02–>16)
Enterobacter cloacae	8	0.5 (<0.25–2)	0.5	0.25 (0.025–2)	1 (0.12–1)	0.5 (0.05–2)	0.5 (0.06–1)	1 (0.06–2)	0.25–1 (0.008–16)	0.5–2 (0.02–>16)
Escherichia coli	4 (1–8)	0.12 (0.016–0.5)	0.12–0.25	0.25 (0.004–>128)	0.25 (0.032–0.25)	0.5 (0.016–32)	0.25 (0.004–8)	0.25 (0.016–32)	0.016–0.03 (0.004–32)	4 (0.004–16)
Haemophilus influenzae	0.5–4	0.06	0.06	≤0.004–0.032	0.016–0.12	0.008–0.064	0.004–0.032	0.008–0.125	≤0.004–0.032	≤0.004–0.25
Klebsiella pneumoniae	8–16	0.5 (0.2–2)	2 (0.5–2)	0.5 (0.008–>64)	2 (0.03–>64)	0.5 (0.03–>64)	1 (0.016–>64)	1 (0.03–>64)	0.25 (0.06–>32)	(0.03–>4)
Legionella spp.	1	(0.2–2)	—	0.016–0.06	0.03–0.12	0.016–0.03	0.03	0.06	0.003–0.03	0.12
Moraxella catarrhalis	2	0.4	0.25	≤0.015–0.12	0.06–0.12	≤0.03–≤0.5	≤0.03–0.05	≤0.015–0.12	≤0.004–0.015	(0.004–0.06)

Continued

TABLE 35.1 Activity of Selected Quinolones Against Selected Gram-Negative, Mycoplasmal, and Chlamydial Pathogens in vitro—cont'd

	REPRESENTATIVE MIC_{90} (range) (μg/mL)[a,b]									
ORGANISM	**Nalidixic Acid**	**Norfloxacin**	**Pefloxacin**	**Ciprofloxacin**	**Ofloxacin**	**Levofloxacin**	**Gatifloxacin**	**Moxifloxacin**	**Gemifloxacin**	**Delafloxacin**
Morganella morganii	8	0.12 (<0.06–25)	(0.25–4)	0.06 (0.015–1)	0.25 (0.12–0.25)	0.12 (0.06–1)	0.5 (0.25–2)	0.5 (0.13–4)	0.12 (0.016–8)	0.25 (0.06–0.25)
Mycoplasma hominis	>256	8–16	4	0.5–4	0.5 (0.25–4)	0.25 (0.03–2)	0.12 (0.03–0.25)	0.06 (0.03–0.25)	0.06 (0.015–0.12)	0.016 (0.008–0.016)
Mycoplasma pneumoniae	—	12	4	0.5–4	1	0.5–2.5	0.13–0.5	0.12–0.3	0.25	0.5 (0.06–0.5)
Neisseria gonorrhoeae	1	0.06	0.06	0.001–2	0.03–2	≤0.008–2	0.004–0.025	0.015–1	—	0.125 (0.001–0.25)
Neisseria meningitidis	0.5	0.03	0.03	0.004–0.008	0.03	≤0.008–0.016	≤0.008	≤0.008–0.016	0.002	0.001
Proteus mirabilis	8	0.1 (0.064–0.5)	0.25	0.12 (0.008–>4)	0.25 (0.064–0.5)	0.12 (0.032–>4)	0.25 (0.032–>4)	0.5 (0.064–16)	0.12–0.5 (0.032–8)	(0.064–2)
Proteus vulgaris	8	0.1 (0.12–0.5)	0.25	0.06 (0.008–0.12)	0.5 (0.032–0.5)	0.12 (0.016–0.25)	0.39 (0.032–0.5)	1 (0.032–1)	0.12 (0.032–0.5)	—
Providencia rettgeri	16	2 (0.25–3.1)	0.5	0.5 (0.025–4)	2–4	1 (0.1–4)	0.5	1 (0.5–2)	—	>1–4
Providencia stuartii	32	2 (<0.25–2)	4	4–8 (0.12–>16)	>8 (1–>16)	4 (0.25–>16)	0.5	1 (0.5–2)	0.25–16 (0.015–1)	>1–4
Pseudomonas aeruginosa	16	2 (2–16)	2	0.032–128	0.125–>128	0.125–128	>4–32	0.125–>128	4–8 (0.032–256)	0.25–>4
Salmonella spp.	2–4	≤0.06 (≤0.06–0.25)	0.12	0.01–0.25	0.12–0.5	0.03–0.25	0.06–0.25	0.12–0.25	0.015–0.12	(0.004–8)
Serratia marcescens	≥100	1 (0.025–50)	1 (1–8)	2 (0.5–12.5)	4 (1–25)	2 (0.25–8)	4 (2–12.5)	4 (0.5–8)	1–2 (0.008–4)	—
Shigella spp.	8	≤0.06–0.12	0.25	0.008–≤0.06	0.06–0.12	0.016–0.03	0.016–0.03	0.03–0.06	≤0.015–0.25	
Stenotrophomonas maltophilia	16	2 (2–25)	4	>2–32	8–64	2–32	2–32	1–128	4 (0.016–16)	(0.12–16)
Yersinia enterocolitica	2	≤0.12	0.25	0.016–0.06	0.12–0.25	0.03–0.06	0.06	0.06–0.12	0.015–0.03	

[a] MIC_{90}, Minimal inhibitory concentration for 90% of strains.
[b] Resistance has been increasing in many hospital pathogens over time. Therefore it is important to consult individual antibiograms, because resistance can vary among institutions.
Data from references 77, 78, 80, 88, and 546–552.

TABLE 35.2 Activity of Selected Quinolones Against Selected Gram-Positive Bacteria in vitro

ORGANISM	REPRESENTATIVE MIC_{90} (range) (μg/mL)[a]									
	Nalidixic Acid	Norfloxacin	Pefloxacin	Ciprofloxacin	Ofloxacin	Levofloxacin	Gatifloxacin	Moxifloxacin	Gemifloxacin	Delafloxacin
Staphylococcus aureus—methicillin susceptible	100	2 (1–4)	0.5 (0.1–2)	0.5 (0.03–2)	0.5 (0.25–1)	0.25 (0.25–0.5)	0.12 (0.10–0.25)	0.12 (0.06–0.25)	0.06 (0.03–0.06)	0.008 (0.002–4)
Staphylococcus aureus—methicillin resistant	—	—	—	≥32 (25–128)	32 (62.5–50)	16 (8–>32)	16 (4–>32)	4 (2–16)	8 (1–8)	0.5 (0.004–4)
Coagulase-negative staphylococci—methicillin susceptible	100	2 (0.4–>4)	1 (0.5–4)	2 (0.25–16)	0.5	1 (0.25–2)	(0.25–4)	0.13 (0.12–1)	0.3 (0.015–0.03)	0.5 (0.002–1)
Coagulase-negative staphylococci—methicillin resistant	—	—	—	>16 (0.39–64)	32 (>8–32)	(0.39–16)	(0.25–8)	4 (0.13–8)	2 (0.25–2)	(0.12–2)
Streptococcus pneumoniae	>128	16 (4–16)	12 (8–16)	2 (1–8)	2 (1–8)	1 (1–2)	0.5 (0.25–1)	0.25 (0.06–0.5)	0.06 (0.03–0.06)	0.015 (0.004–0.12)
Streptococcus pyogenes	>100	4 (2–16)	8 (8–16)	2 (0.5–3.1)	2 (1–4)	1 (0.5–2)	0.5 (0.39–0.5)	0.25 (0.12–0.25)	0.06 (0.015–0.06)	0.015 (0.001–0.03)
Streptococcus agalactiae	>128	16 (4–16)	32	2 (0.5–2)	4 (1–4)	1	0.5	0.5 (0.12–0.5)	0.12 (0.03–0.25)	0.015 (0.001–0.5)
Streptococcus spp.	>64	16 (4–32)	>12.5	4 (1–8)	4 (2–8)	2 (1–2)	0.5 (0.5–1)	0.25 (0.25–2)	0.12	0.03 (0.004–2)
Enterococcus faecalis	>64	8 (4–32)	4–8	(1–128)	(2–32)	(2–50)	(1–>4)	(0.5–16)	2 (2–4)	1 (0.004–2)
Enterococcus faecium	>64	≥12.5	—	(2–>128)	(4–100)	(2–64)	(3–>32)	(4–≥32)	8	(0.008–>4)
Listeria monocytogenes	>64	8 (4–16)	6–8	1 (0.5–4)	4 (2–4)	1 (1–2)	0.5	0.5	0.25 (0.12–0.25)	0.12 (0.06–0.12)
Corynebacterium spp.	—	4 (4–>128)	8 (8–>128)	1 (0.05–128)	1 (0.5–64)	(2–>16)	>4	2	(0.5–16)	—
Bacillus spp.	—	1	—	0.25 (0.06–1)	0.5	0.25 (0.06–2)	0.25	—	—	—
Nocardia spp.	>128	64	64	(1.4–>25)	(2.6–>25)	(0.12–>32)	—	(0.03–32)	(0.03–32)	—

[a]MIC_{90}, Minimal inhibitory concentration for 90% of strains.
Data from references 77, 78, 547, 548, and 552–555.

TABLE 35.3 Activity of Selected Quinolones Against Selected Anaerobic Bacteria and Mycobacteria in vitro

	REPRESENTATIVE MIC_{90} (μg/mL)[a]									
ORGANISM	**Nalidixic Acid**	**Norfloxacin**	**Pefloxacin**	**Ciprofloxacin**	**Ofloxacin**	**Levofloxacin**	**Gatifloxacin**	**Moxifloxacin**	**Gemifloxacin**	**Delafloxacin**
Bacteroides fragilis	512	>128	16	4–64	2–12.5	2–>16	0.25–8	0.5–8	0.5–4	0.12
Bacteroides spp.	512	128	—	16–>64	2–32	4–16	2–8	8	—	
Fusobacterium spp.	256	16	32	2–4	2–16	0.39	—	2	0.5	0.015
Clostridium spp.	256	2	1	1–16	1–8	0.12–4	—	—	—	
Clostridium perfringens	64	8	8	0.5–1.56	0.5–8	0.39	0.39–1	1	0.12	0.008
Clostridioides difficile (formerly *Clostridium difficile*)	>128	128	64	6.25–12.5	12.5–16	6.25–128	1.56–2	2–16	2	(0.015–2)
Anaerobic gram-positive cocci	256–512	16–64	16	2–6.25	2–8	0.32–4	2	0.5–2	0.125–0.5	0.03
Mycobacterium tuberculosis	—	8	8	1	0.8–1.3	0.25–1 (<0.032–16)	0.12–0.5	0.125–0.5 (<0.032–4)	8	0.78–6.25
Mycobacterium avium complex	—	≥16	>64	16	10–100	0.5–64	0.5–32	0.5–16	—	25
Mycobacterium chelonae	—	>16	>64	8	>20	4–128	4–64	8–64	—	
Mycobacterium fortuitum	—	2	2	0.3	1–3.2	0.06–2	0.03–0.25	0.06–0.5	—	
Mycobacterium kansasii	—	8	4	8	1–3.2	0.25	—	—	—	

[a]MIC_{90}, Minimal inhibitory concentration for 90% of strains.
Data from references 77, 78, and 556–559.

TABLE 35.4 Pharmacokinetics of Selected Quinolones

PHARMACOKINETIC PARAMETER	NORFLOXACIN	PEFLOXACIN	CIPROFLOXACIN	OFLOXACIN	LEVOFLOXACIN	MOXIFLOXACIN	GEMIFLOXACIN	DELAFLOXACIN
Dose (mg) PO	400	400	500	400	500	400	320	450
C_{max} (μg/mL) PO	1.5	3.2	2.4	4.6	5.7	4.3	1.4	7.45
Dose (mg) IV	—	400	400	400	500	400	—	300
C_{max} (μg/mL) IV	—	5.8	3.4–6.7	5.5	5.7	4.5	—	9.29
Serum protein binding (%)			30	30	24–52	39–52	55–73	84
Half-life (h)	3.3	11	4	4–5	6–8	9.5	7	4–8.5
Bioavailability (%)	(50)	>95	70	>95	99	86–100	71	59
V_D (L)	—	112	231	102	102	122	280	30–48
Cl_r (mL/min)	234	20	358	195	116	30	193	109
Renal excretion (%)	27	—	40	73	77	20	36	35–45

C_{max}, Peak serum concentration; *Cl_r*, renal clearance; *IV*, intravenous; *PO*, oral; *V_D*, volume of distribution.

Concentrations in prostate tissue, stool, bile, lung, and neutrophils and macrophages usually exceed serum concentrations (Table 35.5). Concentrations in urine and kidney tissue are high for quinolones with a major renal route of elimination, particularly so for levofloxacin and substantially less for moxifloxacin, which has a major route of nonrenal elimination. Concentrations of quinolones in saliva, prostatic fluid, bone, and cerebrospinal fluid (CSF) are usually lower than drug concentrations in serum. Active transport systems appear to be involved in reducing concentrations of levofloxacin in CSF.[101] Fluoroquinolones vary in terms of penetration into the CSF. Their penetration into the CSF in the absence of meningeal inflammation is, however, much higher than that of β-lactam antibiotics.[102] In patients with tuberculous meningitis, CSF penetration (AUC_{CSF}/AUC_{plasma} ratio) of levofloxacin was similar to that of moxifloxacin and greater than that of ciprofloxacin.[103,104] Penetration into ascitic fluid in patients with liver failure has been found for ofloxacin (120%).[105] Penetration into human breast milk has also been documented for ciprofloxacin and ofloxacin.[106]

Elimination

The terminal half-lives of elimination from serum range from 3 hours for norfloxacin and ciprofloxacin to 12 hours for moxifloxacin, allowing twice- or once-daily dosing (Table 35.6; see also Table 35.4). The principal routes of elimination differ among quinolones. Ofloxacin, levofloxacin, and sitafloxacin are eliminated predominantly by the kidneys, and nalidixic acid and moxifloxacin are eliminated predominantly by nonrenal pathways. Most other quinolones have mixed excretion by both renal and nonrenal routes.

Renal clearances of norfloxacin, ciprofloxacin, ofloxacin, sitafloxacin, and levofloxacin exceed glomerular filtration rates, indicating net tubular secretion. In support of tubular secretion, renal clearances of norfloxacin and ciprofloxacin are reduced by probenecid, but drug accumulation does not occur. In contrast, the renal clearance of pefloxacin is below or equal to the glomerular filtration rate, suggesting net tubular reabsorption.[107]

TABLE 35.5 Body Tissues, Fluids, and Cells in Which Quinolone Concentrations Exceed Quinolone Concentrations in Serum

SITE	FOLD INCREMENT
Prostate tissue	0.9–2.3
Feces	100–1000
Bile	2–20
Lung tissue	1.6–6
Macrophages and neutrophils	2–>100

Hepatic metabolism accounts for the majority of the elimination of moxifloxacin and nalidixic acid. In the latter case, however, active metabolites contribute to antibacterial effects. The hydroxynalidixic derivative of nalidixic acid is more active than its parent compound. The metabolites and inactive glucuronide conjugate to the 3-carboxyl group are excreted in the urine. Conversion of norfloxacin, ciprofloxacin, and delafloxacin to less active metabolites accounts for 10% to 20% of elimination. There is minimal hepatic biotransformation (<10%) of ofloxacin and levofloxacin. Hepatic metabolism and biliary excretion are the principal routes of elimination of moxifloxacin (>60% of dose; 38% sulfoconjugation, 14% glucuronide conjugation). In addition to glucuronide conjugates and desmethylpiperazinyl derivatives, other metabolites of quinolones that have been identified have had predominantly alterations of the piperazine ring, including *N*-oxide, *N*-sulfo, *N*-formyl, and desethylene derivatives.[95,107]

Transintestinal secretion has been identified after intravenous (IV) administration of ciprofloxacin and accounts for about 10% to 15% of drug excretion,[107] and this effect may be mediated by P-glycoprotein and other intestinal transporters.[95]

Dosage Adjustments in Renal and Hepatic Insufficiency

As expected from differences in the routes of excretion, increases in drug half-life in the presence of severe renal insufficiency are greatest for ofloxacin and levofloxacin (fourfold to fivefold) and least for moxifloxacin (no change), with other quinolones exhibiting intermediate effects (about twofold). To prevent excessive drug accumulation, dosage reduction (increase in the dose interval from 12 to 24 hours or halving the daily dose for those quinolones normally given once daily; see Table 35.6) is indicated at creatinine clearances below 50 mL/min for ofloxacin and levofloxacin, below 40 mL/min for gemifloxacin, and below 30 mL/min for norfloxacin, ciprofloxacin, and delafloxacin. For delafloxacin, dose reduction is advised for the intravenous formulation because of concern over potential toxicities associated with accumulation of the intravenous vehicle, sulfobutylether-β-cyclodextrin. No dosage reduction is indicated for nalidixic acid and moxifloxacin. Clearance by hemodialysis is low (<14% of plasma clearance) for norfloxacin, ciprofloxacin, ofloxacin, levofloxacin, and moxifloxacin.[108] Similarly, peritoneal dialysis contributes little to the clearance of ciprofloxacin and ofloxacin.[109] Continuous venovenous hemofiltration in patients with severe renal failure constitutes 16% to 70% clearance of levofloxacin, 6% to 37% clearance of ciprofloxacin,[110,111] and about 9% clearance of moxifloxacin.[112] In patients on continuous venovenous hemofiltration, levofloxacin is dosed at 250 mg/day and ciprofloxacin at 400 mg/day. No dose adjustment is needed for moxifloxacin.

Fewer data are available on the effects of hepatic insufficiency on quinolone half-lives, but there is no need for dosage adjustments of norfloxacin, ciprofloxacin, ofloxacin, moxifloxacin, and delafloxacin.[113]

TABLE 35.6 Dosing of Quinolones in Patients With Normal and Reduced Renal Function

	NORMAL RENAL FUNCTION		RENAL FAILURE WITH GFR (mL/min)		
QUINOLONE	Oral	Intravenous	10–50	<10	REMOVAL BY DIALYSIS
Norfloxacin	400 mg q12h	—	1×dose q24h	1×dose q24h	No (H, P)
Pefloxacin	400 mg q12h	400 mg q12h	No change	No change	No (H)
Ciprofloxacin	250–750 mg q12h	200–400 mg q12h	1×dose q18h	1×dose q24h	No (H, P)
Ofloxacin	200–400 mg q12h	200–400 mg q12h	1×dose q24h	½ dose q24h	No (H, P)
Levofloxacin	250–750 mg q24h	250–750 mg q24h	½ dose q24h	½ dose q48h	No (H, P)
Moxifloxacin	400 mg q24h	400 mg q24h	No change	No change	No (H, P)
Gemifloxacin	320 mg q24h	—	½ dose q24h	½ dose q24h	20%–30% (H)
Delafloxacin	450 mg q12h	300 mg q12h	Below 30 mL/min switch to oral therapy	Not recommended	19% (H)[a]

[a]Intravenous vehicle (sulfobutylether-β-cyclodextrin) is also removed by hemodialysis.
GFR, Glomerular filtration rate; *H,* hemodialysis; *P,* peritoneal dialysis.

Changes in renal function that accompany severe liver disease may, however, affect ciprofloxacin and ofloxacin elimination to a lesser extent.

Interactions With Other Drugs

When coadministered by mouth with aluminum-, magnesium-, or, to a lesser extent, calcium-containing antacids, quinolones have markedly reduced oral bioavailability, presumably because of the formation of cation-quinolone complexes that are poorly absorbed.[114,115] Sucralfate, which contains large amounts of aluminum ions, also reduces absorption of quinolones. Tablets should be taken at least 2 hours before or 2 to 6 hours after these drugs. In general, histamine type 2 receptor antagonists and proton pump inhibitors do not have important effects on absorption of quinolones. Concurrent administration of quinolones with iron sulfate, multivitamin-mineral regimens containing zinc, and the buffered formulation of dideoxyinosine has also been reported to reduce quinolone absorption.[116] Nutritional supplements given by nasogastric tube may reduce the absorption of quinolones given concurrently by the same route, probably because these supplements also contain multivalent cations such as iron and zinc. Concomitant administration of morphine decreases maximal serum concentrations of oral ciprofloxacin by 35% to 50%.

For intravenous formulations of ciprofloxacin, precipitates have been reported when these quinolones were infused through the same intravenous tubing with aminophylline, amoxicillin with and without clavulanate, or flucloxacillin. Separate infusions are indicated.

Quinolones vary in the extent to which they impair the elimination of the methylxanthines theophylline and caffeine. The effects appear to result from inhibition by some quinolones of hepatic cytochrome P-450 isozyme 1A2 (CYP1A2),[117] which is involved in theophylline and caffeine metabolism. With ciprofloxacin, a 30% reduction in clearance and 20% to 90% increases in serum concentrations of theophylline are observed, but norfloxacin, ofloxacin, levofloxacin, moxifloxacin, and gemifloxacin had little or no effect (2%–11% increases in serum concentrations of theophylline).[115,118,119] In patients receiving ciprofloxacin in combination with theophylline, serum levels of theophylline should be monitored and reductions in the dose of theophylline considered. No such adjustments should be needed in patients receiving theophylline concurrently with other fluoroquinolones. Concurrent administration of ciprofloxacin at the usual daily dose (500 mg twice daily) with caffeine resulted in a higher peak serum concentration (C_{max}) and cumulative 24-hour serum concentration of ciprofloxacin in females relative to males. This effect, however, disappeared when parameters were corrected for body weight.[120]

Tizanidine, clozapine, and methadone are also metabolized by CYP1A2. Concomitant administration of ciprofloxacin and tizanidine may increase the central nervous system and systemic hypotensive effects of the latter.[121] Low doses of ciprofloxacin have increased serum levels of clozapine, and one patient was reported to have developed symptoms of methadone overdose while taking ciprofloxacin.[122] Thus the effects of ciprofloxacin on tizanidine, clozapine, and methadone (and possibly other drugs affected by CYP1A2, such as haloperidol, mexiletine, cimetidine, and paroxetine) should be monitored similar to those with methylxanthines.

Another hepatic P-450 isozyme, CYP3A4, is affected by many classes of antimicrobials, which can be inhibitors, inducers, or substrates of this metabolism enzyme. Such antimicrobials include macrolides, streptogramins, rifampin, azoles, and a variety of antiretroviral agents but not quinolones. Thus the occasional case reports of apparent interactions of quinolones with other drugs interacting with CYP3A4 are difficult to assess for their importance or predictive value. Such case reports include those associating ciprofloxacin with increased cyclosporine levels and nephrotoxicity or increased levels of diazepam.[115]

Moxifloxacin is coadministered with rifampicin in the treatment of mycobacterial and other bacterial infections. Increased activity of the sulfate conjugation pathway of moxifloxacin metabolism by rifampicin has been shown to reduce moxifloxacin levels, potentially associated with reduced efficacy, and may warrant an increase of moxifloxacin dosage.[123,124] In direct studies of interactions of warfarin with quinolones, no effects on coagulation tests were seen generally, and in those cases in which a drug interaction was seen (ciprofloxacin), there was an increase in the relatively inactive *R*-enantiomer of warfarin, which is metabolized by the P-450 isozyme CYP2C9, and there was no effect on the active *S*-enantiomer or prothrombin times.[125] Case reports of patients who developed bleeding while on warfarin and ciprofloxacin have appeared, however, suggesting that in uncommon special settings, quinolone promotion of an anticoagulant effect of warfarin may occur, possibly caused by other concomitant therapies, by effects on microbial flora that synthesize vitamin K in the intestine, or by changes in patients' food intake, or promoted by the underlying disease conditions or genetic predispositions, which would not be reflected in data collected in controlled clinical studies. Limited case series suggest that treatment with moxifloxacin, which has enhanced activity against anaerobic bacteria compared with either ciprofloxacin or levofloxacin, may prolong the prothrombin time earlier than the other quinolones.[126] A case-control study of continuous warfarin users older than 65 years identified an association between exposure to quinolones and an increased risk of bleeding, albeit lower than that with other antibiotics.[127] As a precaution, prothrombin times should be rechecked in patients on warfarin after initiation of quinolones or other antimicrobial agents. When mixed with heparin as a lock solution used for the treatment of catheter-related bloodstream infection, the maximum concentration of ciprofloxacin is limited because of precipitation at higher concentrations.[128]

Disturbances of glucose metabolism have been rarely reported with quinolones, but there have been case reports of elevated glyburide levels and hypoglycemia in a diabetic patient given ciprofloxacin and in several patients receiving gatifloxacin and hypoglycemic agents.[110] These effects were unexpected and not fully explained. Glyburide may be metabolized by CYP2C9, which is not known to be affected by quinolones, and when directly studied, there was no detectable effect of gatifloxacin on glyburide metabolism or glucose tolerance in diabetic volunteers. Furthermore, hypoglycemia has been reported in patients receiving gatifloxacin and clinafloxacin without concomitant hypoglycemic agents, and hyperglycemia has also been reported in patients receiving gatifloxacin. Thus effects in addition to potential drug interactions likely contribute to the occurrences (see "Adverse Events").

Nonsteroidal antiinflammatory drugs (NSAIDs) may affect the central nervous system stimulant effects of some quinolones. Seizures were reported in a group of Japanese patients receiving enoxacin and the NSAID fenbufen. Potentiation of seizures by combinations of quinolones and NSAIDs has also been reported in animals. Assays of the displacement of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) or a related molecule from GABA receptors in rat brain tissue have demonstrated displacement of GABA by quinolones and enhancement of this displacement by fenbufen and by theophylline.[127,129] Clinical experience does not suggest, however, that concurrent use of quinolones other than enoxacin with other NSAIDs will result in central nervous system toxicities, but patients receiving both classes of drugs should be cautioned about and monitored for these potential adverse effects.

Although probenecid reduces the renal clearance of norfloxacin and fleroxacin, no quinolone accumulation occurred. The effect of probenecid might be predicted to be greater with quinolones such as ofloxacin and lomefloxacin, for which renal clearance includes tubular secretion and is the predominant mode of clearance, but data are lacking.

CLINICAL USES

Earlier quinolones such as nalidixic acid, oxolinic acid, and cinoxacin were used almost exclusively for treatment of urinary tract infections, although nalidixic acid was also used for treatment of shigellosis. With the development of the more potent fluoroquinolones, an increasingly broad array of infections is treated with members of the fluoroquinolone class.

Urinary Tract Infections

Although the low pH and magnesium concentrations present in urine may reduce quinolone activity, the concentrations of many quinolones in urine are usually sufficient to provide substantial therapeutic ratios of urinary drug concentration to the MIC of most urinary pathogens.

For uncomplicated urinary tract infections, usually in symptomatic young women with cystitis caused by highly susceptible organisms such as *E. coli,* most quinolones are likely to be highly effective when given for brief courses of 3 to 10 days,[129a–129c] and norfloxacin, ciprofloxacin, and ofloxacin

have been found to be comparable to trimethoprim-sulfamethoxazole (TMP-SMX)[129d] and nitrofurantoin[129b,129e,129f] and better than amoxicillin-clavulanate.[129g] Usually, the lowest dose in the dosage range (see Table 35.6) is sufficient for treatment of these infections. Three-day regimens of norfloxacin, ciprofloxacin, and ofloxacin result in cure rates of 81% to 96%.[129b] An extended-release formulation of ciprofloxacin (500 mg oral [PO] once daily) was comparable to conventional ciprofloxacin (250 mg PO twice daily) when both were given for 3 days.[129h,129i] Limited data on single-dose therapies with ciprofloxacin, ofloxacin, and norfloxacin indicate eradication in 75% to 96% of patients.[129j,129k] Single-dose norfloxacin (800 mg) was equivalent to a 3-day regimen for *E. coli* infections,[129l] but for *Staphylococcus saprophyticus* infections, a 7-day regimen is preferred because of failures with shorter courses.[129m,129n] Women with uncomplicated acute pyelonephritis given norfloxacin, ciprofloxacin (either orally or intravenously),[129o] or ofloxacin for 7 to 10 days have bacteriologic cure rates comparable to or better than those with TMP-SMX, and levofloxacin and ciprofloxacin were comparable to each other with 95% eradication rates.[129p,129q,130]

Increased fluoroquinolone resistance among community uropathogens and associated microbiologic and clinical failure, similar to that observed with TMP-SMX, is a major consideration when deciding to use fluoroquinolone for urinary tract infections.[131,132] A disturbing finding has been the widespread distribution of urinary tract infections caused by multidrug-resistant *E. coli* clonal groups in the United States and worldwide.[133,134] Of interest, strains belonging to this clone were found to harbor the gene *aac(6′)-Ib-cr*.[135] In addition, the potential for collateral damage (i.e., the selection of drug-resistant organisms and colonization or infection with multidrug-resistant organisms) complicates the use of this antimicrobial class. The Infectious Diseases Society of America (IDSA) incorporated these considerations into their guidelines.[130] The recommended antibiotics for acute uncomplicated cystitis are nitrofurantoin, TMP-SMX, fosfomycin, or pivmecillinam, whereas a 3-day fluoroquinolone is a second-line treatment option. For acute pyelonephritis, fluoroquinolones (5–7 days) are an appropriate choice for therapy in settings where the prevalence of resistance of community uropathogens to fluoroquinolones is not known to exceed 10%.[130,136–138] Rates of resistance, however, have been higher (approaching 30%) in recently reported surveys of extraintestinal *E. coli* isolates in the United States, due in part to the emergence of the *H*30 subclone of sequence type ST131.[139] For prophylaxis of recurrent infections in women, norfloxacin (200 mg at bedtime) was highly effective and superior to nitrofurantoin,[140] and low doses of ofloxacin (100 mg), norfloxacin (200 mg), and ciprofloxacin (125 mg) given after coitus have also been effective as prophylaxis.[141] Other agents, such as nitrofurantoin or TMP-SMX, are preferred for this indication, however, because of expense, collateral damage, and the risks of potential pregnancy.

Cystitis occurring in elderly women is more often complicated by comorbid conditions and is more likely to be caused by pathogens, in addition to *E. coli,* that are less susceptible to antimicrobial agents. For such infections, ofloxacin and ciprofloxacin given for 7 days have high eradication rates but have no clear advantage over a short course.[142]

Complicated urinary tract infections occurring in men and in patients with catheters or structural or functional abnormalities of the urinary tract are often caused by more resistant pathogens and have a higher frequency of relapse and reinfection. Bacteriologic cure rates for ciprofloxacin were superior to those for TMP-SMX and aminoglycosides shortly after completion of a 7- to 10-day course, but the low fractions of patients who continued to have sterile urine were similar by 4 to 6 weeks after therapy.[138,143–145] In patients with spinal cord injury, treatment with ciprofloxacin (250 mg twice daily) for 3 and 14 days had similar clinical cure. The percentage of patients with microbiologic cure was, however, significantly higher for the 14-day treatment arm at long-term follow-up and was attributable to the significantly lower rates of both clinical and microbiologic relapse.[146] High cure rates were seen with both ofloxacin and levofloxacin.[147,148] In collected noncomparative trials, infections caused by *P. aeruginosa* were eradicated in 70% of patients given ciprofloxacin and 83% of those given norfloxacin. Development of bacterial resistance has been associated with therapeutic failure in about 2% of patients overall, but resistance rates (10%–20%) are often higher for *P. aeruginosa* infections, and resistance can be selected with fluoroquinolone therapy.[149] Ciprofloxacin is the preferred fluoroquinolone for treatment of *P. aeruginosa* infections, and when chosen, doses of at least 500 mg orally twice daily should be used. In patients with bladder dysfunction resulting from spinal cord injury who used intermittent suprapubic taps or self-catheterization for bladder emptying, ciprofloxacin (100 mg at bedtime) reduced episodes of infection about 10-fold relative to placebo.[150] Whereas infections in the placebo group represented a mixture of enteric and nonenteric bacteria and enterococci, breakthrough infections in the ciprofloxacin group were largely nonenteric bacteria, particularly *P. aeruginosa,* which were often resistant.

Fluoroquinolones are now commonly used in prophylaxis in urologic surgery. When postoperative bacteriuria, but not other secondary outcomes, was the parameter to define efficacy of antimicrobial prophylaxis, single-dose ciprofloxacin (500 mg) or levofloxacin (500 mg) has been shown to be effective after transurethral prostate resection and transrectal prostate biopsies,[151–157] but infections with quinolone-resistant *E. coli* have been seen increasingly with this use (see "Prostatitis"). There is no evidence that prophylaxis should extend beyond 24 hours after a procedure.[158,159] Prophylaxis of urinary tract infection in renal transplant recipients is routinely done because of high risk from infection. Although ciprofloxacin is effective,[160] TMP-SMX is often used because it may be additionally useful as prophylaxis against other opportunistic pathogens in this patient group.

Prostatitis

Fluoroquinolones concentrate in prostatic tissue, with lower levels in prostatic fluid. In one small comparative study of men with predominantly *E. coli* infections, norfloxacin given for 4 to 6 weeks was superior (92% eradication) to TMP-SMX (67% eradication) at 1-month follow-up.[161] In open studies, similar courses of norfloxacin, ciprofloxacin, ofloxacin, and levofloxacin have produced eradication rates of 60% to 86% at follow-up, ranging from 1 to 13 months.[162,163] Similar results were obtained with levofloxacin and prulifloxacin (a fluoroquinolone marketed outside the United States).[164] With 2-week courses of therapy and infections caused by less susceptible organisms, such as *P. aeruginosa* and enterococci, failures appear to be more frequent.[113,165] Recent studies indicate that approximately 11% to 22% of men undergoing transrectal prostate biopsy harbor fluoroquinolone-resistant organisms within the rectum before the administration of antimicrobials, and that fecal carriage of fluoroquinolone-resistant *E. coli* strains was a risk factor for infectious complications after prostate biopsy.[166,167] Fluoroquinolone-resistant *E. coli* sequence type 131 has been the predominant clone causing bloodstream infection after transrectal prostate biopsy.[166] Two strategies for the prevention of infection have recently emerged: "augmented" standard fluoroquinolone or aminoglycoside prophylaxis with a second agent, and targeted prophylaxis guided by prebiopsy screening for rectal colonization with ciprofloxacin-resistant organisms.[168–170] Although prebiopsy rectal screening is favored with respect to antibiotic stewardship goals, its implementation is more difficult to achieve.

Sexually Transmitted Diseases

Neisseria gonorrhoeae is an important pathogen in urogenital diseases, anogenital diseases, and pelvic inflammatory disease (PID). Because patients are managed on the basis of presenting symptoms and signs and associated risk factors (i.e., syndromic approach), stringent clinical efficacy criteria are used to ensure that therapy is effective against this pathogen.[171,172] After the emergence of quinolone resistance among *N. gonorrhoeae* strains, the Centers for Disease Control and Prevention (CDC), in April 2007, ceased recommending fluoroquinolones in its treatment guidelines.[173] Earlier data indicate, however, that susceptible strains can be treated with quinolones. Quinolones have retained activity in vitro against the sexually transmitted pathogens *C. trachomatis* and *Haemophilus ducreyi,* but they appear to lack activity against *T. pallidum*.[174]

Uncomplicated quinolone-susceptible gonococcal urethritis and cervicitis are effectively eradicated by single doses of quinolones (norfloxacin, 800 mg; ciprofloxacin, 250 mg; ofloxacin, 400 mg).[175,176] Rectal infections appear to have virtually complete cure rates with all of these quinolones, and pharyngeal infections are similarly highly cured by ciprofloxacin and ofloxacin.[176,177]

Single doses of quinolones are ineffective for genital chlamydial infections.[143] Seven-day courses of therapy with norfloxacin and ciprofloxacin have failed, but 7-day courses of ofloxacin and levofloxacin were comparable to a similar course of doxycycline for treatment of chlamydial infections.[178–181] In a more recent study, sitafloxacin (which is not currently available in the United States) 100 mg twice daily for 7 days was effective against symptomatic nongonococcal urethritis in heterosexual men.[182]

Small numbers of patients with gonococcal and nongonococcal PID have been cured with 10- to 14-day courses of sequential intravenous and oral ciprofloxacin or oral ofloxacin.[183,184] Results from three more recent randomized clinical trials of treatment for PID, a syndrome with mixed microbiology, including gonococci, chlamydiae, enteric bacteria, and anaerobes, have been published. Ofloxacin (400 mg twice daily for 10 days) and cefoxitin (2 g intramuscularly [IM] once) plus doxycycline (100 mg twice daily for 10 days) were similar, and ofloxacin was highly effective in eradicating gonococci and chlamydiae.[185] Ciprofloxacin (250 mg twice daily) plus clindamycin (300 mg three times daily) given for 14 days was also comparable to ceftriaxone plus doxycycline, with high clinical cure rates, and was effective for *C. trachomatis* eradication (100% in both groups).[186] In randomized trials, moxifloxacin (400 mg once daily) monotherapy was clinically and bacteriologically as efficacious as metronidazole (500 mg twice daily) plus either ofloxacin (400 mg twice daily) or levofloxacin, with all drugs administered for 14 days.[187] In the latter trial, a single intramuscular dose of ceftriaxone was also given if *N. gonorrhoeae* was detected.

In patients with chancroid, *H. ducreyi* was eradicated from genital ulcers by ciprofloxacin (500 mg twice daily for 3 days) in all patients, a result comparable to that with TMP-SMX.[188] A once-daily dose of 500 mg or 250 mg for 5 days was effective in 88% of patients.[188] In another randomized, double-blind, placebo-controlled clinical trial, single-dose ciprofloxacin (500 mg) was comparable to a 7-day course of erythromycin (500 mg three times daily), with an overall cure rate of 92%.[189] The 3-day ciprofloxacin regimen is an option for treatment of chancroid in the CDC guidelines.[190]

Two cases of septic arthritis with susceptible gonococci have responded to ciprofloxacin. For bacterial vaginosis, ofloxacin was less effective than metronidazole.[191] There are no data on treatment of syphilis with quinolones in humans, but in experimentally infected rabbits, ofloxacin lacked efficacy.

Gastrointestinal and Abdominal Infections

Quinolones were active against all bacterial pathogens known to cause gastroenteritis, but resistance has emerged in some settings. Although fecal material may decrease the activity of quinolones, drug concentrations in feces are exceedingly high. The penetration of quinolones into macrophages (see Table 35.5) may also be important for their effectiveness in systemic *Salmonella* infections.

Bacterial gastroenteritis is often a self-limited disease, but in a number of circumstances quinolones have been shown to shorten the duration of diarrhea and to eradicate pathogens from stools.[192] In traveler's diarrhea, which is often caused by enterotoxigenic *E. coli* and *Shigella* spp., norfloxacin (400 mg twice daily for 3 days) and ciprofloxacin (500 mg twice daily for 5 days), begun shortly after the onset of diarrhea, have shortened the duration of loose stools by 1 to 3 days relative to placebo and have been comparable to TMP-SMX.[193,194] Because of resistance to TMP-SMX among *E. coli* in many parts of the developing world, quinolones are often the preferred therapeutic agents in travelers.[195] Single-dose therapies, including ciprofloxacin (500 mg or 1 g)[196,197] and ofloxacin (400 mg),[198] are also effective with or without loperamide for this indication. After the emergence of quinolone resistance, the combination of levofloxacin and loperamide was evaluated in adult US military personnel or their families located in Turkey and who presented with acute noninflammatory diarrhea, and the effectiveness was comparable to that of azithromycin plus loperamide.[199] When given as prophylaxis to travelers, quinolones have produced protection rates ranging from 68% to 92% compared with those in placebo control subjects, but routine use of quinolones or other antimicrobials is not recommended for prevention of diarrhea in travelers.[192,200,201] Contingency treatment at the onset of diarrhea is preferred for travelers, and with this approach, norfloxacin (400 mg twice daily for 3 days)[202] and ciprofloxacin (500 mg single dose)[197] were shown to shorten diarrhea by 1 to 3 days relative to placebo.

In patients with shigellosis, for whom antimicrobials are generally indicated, 5-day courses of norfloxacin, ciprofloxacin, and ofloxacin have been highly effective and generally at least as effective as comparator agents, including ampicillin, TMP-SMX, azithromycin, and ceftriaxone.[192,203] Addition of loperamide to ciprofloxacin may further shorten the duration of diarrhea, but there is risk of clinical worsening and intestinal perforation in this condition.[204] Even a single 750-mg dose of ciprofloxacin may be effective in shigellosis, except those cases caused by *Shigella dysenteriae* type 1, the most virulent of *Shigella* types.[205] Failure rates for ciprofloxacin have increased, however, associated with the emergence of strains resistant to nalidixic acid and ciprofloxacin, particularly in Asia and Africa.[206]

In patients with nontyphoidal *Salmonella* gastroenteritis, symptoms were shortened with ciprofloxacin or norfloxacin in some[207,208] but not all studies.[209] Eradication of stool carriage of *Salmonella* is generally transient,[208,210] but carriage is not necessarily prolonged relative to no treatment.[211] Treatment of *Salmonella* gastroenteritis is generally not indicated, except in immunocompromised and elderly patients because of the risk of invasive disease. In immunocompromised hosts, systemic, nontyphoidal *Salmonella* infections have been successfully treated with ciprofloxacin, but there have been no comparative trials and some relapses have occurred in acquired immunodeficiency syndrome (AIDS) patients.[212]

For *C. jejuni* gastroenteritis, studies of treatment with quinolones have had variable results. Ciprofloxacin (500 mg twice daily for 5 days)[213] and norfloxacin (400 mg twice daily for 5 days)[208] were superior to placebo. Clinical and microbiologic failures have been associated with development of resistant *C. jejuni* in some,[196,214] but not in all, studies using ciprofloxacin.[211] Quinolone resistance in *C. jejuni* has increased. In the 1990s, a CDC survey in selected US counties did not detect ciprofloxacin resistance among a sample of *C. jejuni* isolates from sick people; the resistance rate subsequently rose from 17% in 1997–1999[214a] to 25% in 2012–2014. In a trial done in the years 2000 and 2001 on US military personnel based in Thailand who presented with acute diarrhea, 50% of the *Campylobacter* isolates were resistant to fluoroquinolones, and levofloxacin (500 mg daily for 3 days) was clinically and microbiologically inferior to 1 or 3 days of azithromycin.[215]

For treatment of patients with cholera, norfloxacin (400 mg twice daily for 3 days) was shown to be superior to TMP-SMX, a single dose of doxycycline, and placebo in shortening diarrhea,[216,217] and 3 days of ciprofloxacin (250 mg once daily) and a standard tetracycline (500 mg four times daily) regimen were comparable.[218] A single dose of ciprofloxacin (1 g) or two 500-mg doses of ciprofloxacin has been effective and superior to doxycycline in shortening diarrhea and eradicating *Vibrio cholerae* from stool.[218,219] A single 20-mg/kg dose of ciprofloxacin achieved clinical outcomes similar to, or better than, those achieved with erythromycin (12.5 mg/kg every 6 hours for 3 days).[220] In some areas, such as Bangladesh, however, progressively decreasing susceptibility to ciprofloxacin has been more recently observed along with low efficacy (27%) of single doses of ciprofloxacin.[221,222] In patients with diarrhea caused by *Yersinia enterocolitica, Plesiomonas shigelloides,* and *Aeromonas* spp., quinolones have eradicated the organisms from stool but have not yet been clearly shown to shorten clinical illness.[192]

In studies performed in the late 1980s and early 1990s in patients with enteric fever caused predominantly by *Salmonella enterica* serovar Typhi or serovar Paratyphi, 7-day courses of ciprofloxacin and ofloxacin cured over 90% of patients, shown to be superior to ceftriaxone, with resolution of fever within 5 days.[223] Since the mid-1990s, outbreaks of serovar Typhi strains that were resistant to nalidixic acid (the prototype quinolone that was used for in vitro screening tests) and had reduced susceptibility to fluoroquinolones (ofloxacin MIC of 0.25–1.0 μg/mL) have been reported in a number of countries and in travelers to them.[223,224] Clinical failures and delayed defervescence after short courses (2–3 days) of ofloxacin were more frequent in patients with nalidixic acid–resistant strains versus those with nalidixic acid–susceptible strains.[225–228] Gatifloxacin, which targets both DNA gyrase and topoisomerase IV,

also failed to meet the expectation to be a better alternative for *Salmonella* infections with increased MICs to fluoroquinolones. Two studies were conducted in Nepal in adults and children with uncomplicated enteric fever. In one, 7 days of gatifloxacin or ofloxacin among 170 patients infected by nalidixic acid–resistant isolates were equally effective.[229] In another study, performed in a background of increasing fluoroquinolone resistance, 7 days of gatifloxacin (10 mg/kg/day) was inferior to ceftriaxone among microbiologically confirmed cases.[230] Chronic fecal carriage of fluoroquinolone-susceptible *S. enterica* serovar Typhi has been eradicated in 83% to 93% of small numbers of patients, including a few with gallstones, given norfloxacin, ciprofloxacin, or ofloxacin for 4 weeks.[223,231,232]

Quinolones are active against *H. pylori* in vitro. Several randomized comparative trials have demonstrated that levofloxacin- and moxifloxacin-based multidrug regimens administered for 7 to 10 days were effective in eradication of *H. pylori*. The eradication rate was approximately 80%, similar to other standard non–fluoroquinolone-containing multidrug regimens.[233–239] The presence of quinolone resistance, however, significantly decreased the eradication rate.[240] Current guidelines recognize that antibiotic resistance should be addressed when choosing treatment regimens for *H. pylori* and do not recommend inclusion of fluoroquinolones in first-line regimens or their repeated use.[241,242]

Data are quite limited on the use of quinolones for treatment of biliary tract infections. In a single small study, 83% of patients with cholecystitis and cholangitis, most associated with *E. coli* bacteremia, responded to intravenous and then oral ciprofloxacin.[243] In patients with biliary stents, late blockage has been thought to be due in part to bacterial adherence to the stent with biofilm formation. In small studies, ciprofloxacin (250 mg PO twice daily) and ofloxacin (200 mg twice daily) were not shown to delay stent blockage.[244,245]

In patients with complicated intraabdominal infections caused by disruption of the integrity of the gastrointestinal tract, a mixture of anaerobes and facultative gram-negative aerobes, with or without enterococci, are usually involved. In a randomized, double-blind trial, ciprofloxacin (400 mg IV every 12 hours/500 mg PO every 12 hours) plus metronidazole (500 mg IV/PO every 6 hours) was compared with imipenem (500 mg every 6 hours) for patients with complicated infections largely caused by disease of the colon, small bowel, and appendix. Ciprofloxacin and metronidazole were given either entirely intravenously or with a switch to oral administration after initial response. Clinical outcomes were similar in all three arms of the study. Regardless of treatment, however, there was a significantly higher rate of treatment failure if enterococci were isolated from the site of infection (28%) than if they were not (14%),[246] suggesting that additional agents active against enterococci should be considered if culture results dictate. Ciprofloxacin plus metronidazole also appeared superior to piperacillin-tazobactam in another double-blind trial, although analysis of treatment failures was incomplete,[247,248] and another study found no differences when ciprofloxacin plus metronidazole was compared with ceftriaxone plus metronidazole.[249]

Moxifloxacin, which has activity against enteric gram-negative bacilli similar to that of ciprofloxacin and additional activity against anaerobic bacteria, was studied in double-blind randomized trials as a single agent (400 mg IV/PO once daily) in comparison to piperacillin-tazobactam (3.75 g IV every 6 hours) followed by amoxicillin-clavulanate (914 mg PO every 12 hours), both regimens given for up to 14 days,[250] and in comparison to ceftriaxone (2 g IV once daily) plus metronidazole (500 mg IV twice daily) given for 3 to 14 days.[251] In the first study, clinical cure rates were 80% and 78% in the two arms, respectively. In the second study, although clinical response rates for moxifloxacin met the noninferiority criteria, they were significantly lower than in the ceftriaxone-metronidazole arm (90.2% vs. 96.5%).[251] The high rates of response in both studies would be expected because of the low overall Acute Physiology and Chronic Health Evaluation (APACHE) II scores of the enrolled patients. High rates of susceptibility to moxifloxacin among baseline anaerobic isolates (87%) were found overall in these and related moxifloxacin intraabdominal infection trials.[252] Current IDSA guidelines for intraabdominal infections include moxifloxacin alone and combinations of either ciprofloxacin or levofloxacin with metronidazole among recommended regimens for initial therapy.[253]

Use of quinolones in peritonitis has been evaluated most in patients undergoing chronic ambulatory peritoneal dialysis (CAPD) and in patients with cirrhosis. Oral ofloxacin, 300 mg once daily, and ciprofloxacin, 500 mg once daily, have cured episodes of peritonitis in CAPD patients, but failures in infections caused by the common coagulase-negative staphylococci have occurred. Higher concentrations of ciprofloxacin have been achieved by adding drug to the dialysate (20–50 μg/mL), with outcomes similar to those with standard regimens of intraperitoneal vancomycin plus gentamicin.[254] Oral regimens may have been less effective due to binding of fluoroquinolones to oral phosphate binders, which reduce their bioavailability. A combination of intraperitoneal ciprofloxacin (50 mg/L) and rifampin (50 mg/L) produced a 65% cure rate, which was superior to that with intraperitoneal cephradine.[255] When intraperitoneal vancomycin was used, oral levofloxacin was inferior to an intraperitoneal aminoglycoside in patients with gram-negative CAPD infections, a limitation that appeared to be related to the prevalence of levofloxacin resistance in these organisms.[256] Current International Society for Peritoneal Dialysis guidelines recommend oral treatment for peritonitis due to susceptible bacteria with the following quinolones: ciprofloxacin (250 mg twice daily), levofloxacin (250 mg once daily), moxifloxacin (400 mg once daily), and intraperitoneal administration of ciprofloxacin or ofloxacin.[257]

In treatment of spontaneous bacterial peritonitis in cirrhotic patients, ciprofloxacin has been compared with cefotaxime, ceftriaxone, or ceftazidime; ofloxacin has been compared with cefotaxime; and moxifloxacin has been compared with amoxicillin-clavulanate.[258] With 35 to 123 patients in these studies, no differences in clinical outcomes were found. In patients with cirrhosis at high risk for recurrent spontaneous bacterial peritonitis, norfloxacin, 400 mg once daily given as prophylaxis, reduced recurrences by threefold.[259] Norfloxacin and ciprofloxacin were also studied for up to 1 year for the primary prophylaxis of spontaneous bacterial peritonitis among high-risk patients with low ascitic protein concentration.[260–264] A trend toward a reduced number of episodes of peritonitis was observed in all studies, and in a few, a notable increased probability of survival was also found. Prolonged use of norfloxacin was, however, later associated with increasing occurrence of quinolone-resistant bacteria[263,265]; in this respect, it is interesting that the survival advantage was substantial at 3 months (94% vs. 62%; $P = .003$) but only 60% versus 48% by 1 year ($P = .05$).[261] Patients who have been on this prophylaxis and develop peritonitis should be treated with agents other than a fluoroquinolone because the risk of quinolone resistance is high in this group.[266] Similarly, primary prophylaxis had also been recommended for patients with cirrhosis and gastrointestinal bleeding because in several studies, performed in the 1990s, that tested mainly quinolones, prophylaxis was efficacious in reducing the number of deaths and bacterial infections.[267] In a more recent study,[261] however, oral norfloxacin and intravenous ceftriaxone were compared for prophylaxis in 111 cirrhotic patients with gastrointestinal bleeding. Spontaneous bacteremia or bacterial peritonitis was significantly higher in patients receiving norfloxacin.[268] Thus the risks of selecting resistant enteric bacteria must also be considered before embarking on prolonged use of quinolones in prophylaxis.

Respiratory Tract Infections

A range of respiratory tract pathogens is susceptible to a number of quinolones in vitro. *Haemophilus influenzae, M. catarrhalis,* and many enteric gram-negative bacilli, as well as the agents of atypical pneumonias—*M. pneumoniae, C. pneumoniae,* and *L. pneumophila*—are generally susceptible to systemic quinolones, such as ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin, gemifloxacin, and delafloxacin. High intracellular concentrations of quinolones are likely advantageous for intracellular pathogens, such as *L. pneumophila* and *M. tuberculosis.* Levofloxacin, moxifloxacin, gemifloxacin, and delafloxacin have improved activity against *S. pneumoniae* and have been referred to as respiratory quinolones.

Patients with acute bacterial exacerbations of chronic bronchitis have been treated with many different quinolones, which have generally been effective in eradicating *H. influenzae* from sputum.[269] In studies with ciprofloxacin, eradication rates for *S. pneumoniae* and *P. aeruginosa* were lower, and some failures have been associated with the development

of bacterial resistance. In comparative trials with respiratory quinolones, however, clinical responses have usually been similar to or better than those with ampicillin, amoxicillin, cefaclor, and macrolides.[270] Clinical and microbiologic outcomes were similar for levofloxacin (500 mg once daily) versus cefuroxime axetil[271–273]; moxifloxacin (400 mg once daily) versus clarithromycin,[274,275] azithromycin,[276,277] levofloxacin,[278] or a new quinolone, zabofloxacin[279]; and gemifloxacin (320 mg once daily) versus clarithromycin[280] or levofloxacin.[281] Five- or 7-day courses seemed to be sufficient, with clinical response rates of 85% to 95%, and 5- and 10-day courses of moxifloxacin were comparable to each other (94% responses).[275]

Moxifloxacin and gemifloxacin were superior to clarithromycin in eradicating *H. influenzae* from sputum, probably because of the lesser activity of clarithromycin against this organism.[274,278] In a double-blind, randomized study of patients with acute exacerbations of chronic bronchitis, characterized by dypsnea and increased volume of purulent sputum, moxifloxacin (400 mg once daily for 5 days) was superior to standard therapies (7 days of amoxicillin, 500 mg three times daily; clarithromycin, 500 mg twice daily; or cefuroxime axetil, 250 mg twice daily) in clinical cure and bacteriologic eradication.[282] In a study of more severely ill, hospitalized patients with acute exacerbations of chronic bronchitis, gemifloxacin (320 mg PO once daily for 5 days) had better clinical responses in the intent-to-treat analysis and a shorter time to hospital discharge relative to ceftriaxone (1 g IV once daily for ≤3 days), followed by cefuroxime axetil (500 mg PO twice daily for ≤7 days).[283] Time to next recurrent exacerbation was lengthened after treatment with ciprofloxacin versus clarithromycin[284] but was comparable to that after treatment with cefuroxime axetil,[285] and average infection-free intervals were similar in both comparisons. Moxifloxacin treatment was associated with a significant 13% to 27% increase in the time to next exacerbation relative to conventional therapies with amoxicillin, clarithromycin, or cefuroxime,[282] and the proportion of patients without a recurrence after treatment of an exacerbation with gemifloxacin was significantly lower than that after treatment with clarithromycin (71% vs. 58%, respectively, at 26 weeks).[280] Repeated courses of moxifloxacin (5 days every month) in patients with stable chronic obstructive pulmonary disease were, however, similar to placebo in reduction in bacterial load, and had more treatment-related adverse events.[286]

In patients with community-acquired pneumonias, ciprofloxacin and ofloxacin have consistently eradicated *H. influenzae* and *M. catarrhalis*.[79] Although cures of pneumococcal pneumonias, including a few cases with bacteremia, have been reported with intravenous and then oral ciprofloxacin[287] and ofloxacin[288,289] and with oral ofloxacin alone,[290] failures with both drugs have occurred, and pneumococcal bacteremia has developed during ciprofloxacin therapy of pneumonia.[143] For this reason, ciprofloxacin is generally not used to treat lower respiratory infections, with the exception that it is recommended as part of a combination regimen for treatment of inhalational anthrax (see "Skin and Soft Tissue Infections"). For respiratory quinolones with greater pneumococcal activity, similar or better clinical and microbiologic efficacies have been found for levofloxacin (500 mg IV/PO once daily) versus ceftriaxone (with or without erythromycin), followed by cefuroxime axetil,[291] ceftriaxone alone,[292] amoxicillin-clavulanate alone or together with clarithromycin, and solithromycin or tigecycline.[293–296] Five days of a higher-dose regimen of levofloxacin (750 mg once daily) was comparable in clinical success to 10 days of the lower dose (500 mg once daily),[297] with a higher proportion of patients with resolution of fever and purulent sputum by day 3.[298] Comparable outcomes with generally high cure rates have also been seen in comparisons of moxifloxacin (400 mg PO once daily) versus clarithromycin,[299] ceftriaxone plus levofloxacin,[300] solithromycin (PO and IV-to-PO),[301,302] or amoxicillin[303] and comparisons of gemifloxacin (320 mg PO once daily) versus ceftriaxone, followed by cefuroxime axetil,[304] or versus amoxicillin-clavulanate.[305] A 5-day course of gemifloxacin also appeared to be comparable to a 7-day course.[306]

In addition to the initial study with levofloxacin,[291] a few studies, including one with moxifloxacin[307] and another with gemifloxacin,[308] have also demonstrated superiority relative to the comparator agents in treatment of patients with community-acquired pneumonia. In hospitalized patients requiring initial parenteral therapy, moxifloxacin (400 mg IV/PO once daily) was superior to amoxicillin-clavulanate (IV/PO) with or without clarithromycin, with more rapid defervescence, higher rates of clinical success (93% vs. 85%), and higher rates of bacterial eradication from sputum (94% vs. 82%).[307] For hospitalized patients older than 65 years, moxifloxacin (400 mg IV/PO once daily) produced a trend toward slightly better overall clinical outcomes than levofloxacin (500 mg IV/PO once daily), with a higher proportion of patients with clinical recovery by days 3 to 5.[309] No studies have compared moxifloxacin with the higher dose of levofloxacin. Gemifloxacin (320 mg PO once daily) produced modestly better clinical success in comparison to trovafloxacin (200 mg PO once daily) in an intent-to-treat analysis (88% vs. 81%), with similar responses in the per-protocol analysis (96% vs. 94%).[308]

Clinical responses in the subgroups of patients (usually numbering 10–20 patients) with pneumococci isolated from sputum from various trials were usually similar to the overall response rates (levofloxacin, 81%–100%[292]; moxifloxacin, 100%[299,307]; and gemifloxacin 90%–100%).[304,308] Most studies have included patients with mild-to-moderate pneumonia, but those with levofloxacin and gemifloxacin[304] included patients with severe pneumonia by using clinical criteria similar to those used for stratification of risk of death,[310] with 91% and 87% clinical cures, respectively. Outcomes in patients with pneumococcal bacteremia and pneumonia, a more severe test of drug efficacy, were good overall with levofloxacin (91%, 107 of 117 cured),[291,311] moxifloxacin (88%, 21 of 24 cured; and 70%, 7 of 10 cured),[301,302,307,312] and gemifloxacin (100%, 12 of 12 cured).[304] Clinical failures associated with prior or acquired quinolone resistance in pneumococci have been reported with levofloxacin therapy but have not yet been reported in association with the less extensive use of moxifloxacin or gemifloxacin.[313] In light of the high selection pressure for resistance imposed by broad-spectrum combination therapy for community-acquired pneumonia, a recent study from the Netherlands evaluated the paradigm of combining β-lactams with fluoroquinolones or macrolides.[314] The mortality difference between the β-lactam strategy and the fluoroquinolone strategy was in favor of the fluoroquinolone strategy, but was not statistically significant, and no differences were found in other secondary outcomes.

In aspiration pneumonia and lung abscess, coverage for oral anaerobic bacteria is generally important. Moxifloxacin, relative to other fluoroquinolones, exhibits enhanced activity in vitro against anaerobic bacteria and, at 400 mg intravenously/orally once daily, produced similar clinical responses compared with ampicillin-sulbactam (2 g/1 g IV three times daily), followed by sultamicillin (750 mg PO twice daily), in treatment of patients with aspiration pneumonia (32 of 48 [67%] in both groups) or lung abscess (15 of 19 [79%] vs. 11 of 15 [73%]).[315]

Among atypical pneumonias, smaller numbers of patients with pneumonias caused by *Legionella,*[316,317] *Mycoplasma,*[79] and *C. pneumoniae*[318] have also responded to ciprofloxacin and ofloxacin, but some apparent failures were seen in patients with *M. pneumoniae* and *C. pneumoniae* infection treated with ofloxacin.[289] Clinical responses to levofloxacin,[291] moxifloxacin,[307,319] and gemifloxacin[304,306,310] in usually small numbers (except for the larger number in the trial of gemifloxacin[310]) of patients with *C. pneumoniae, M. pneumoniae,* and *L. pneumophila* infection, usually diagnosed serologically, have generally been high. In observational studies, patients with *Legionella* pneumonia had similar or better clinical responses to levofloxacin relative to macrolides.[320,321] With pneumonia caused by other atypical pathogens—particularly with *M. pneumoniae* infections, which may improve without treatment—assessment of responses is difficult because study designs often do not compare the rapidity of symptomatic improvement between active and inactive agents. Respiratory quinolones appear to be at least as good as if not superior to macrolides for treatment of *Legionella* pneumonia.[322]

Hospital-acquired pneumonias, which are commonly caused by gram-negative bacilli, have responded to intravenous ciprofloxacin[143,323] and ofloxacin,[288] and responses to ciprofloxacin correlated with the level of susceptibility of the infecting organism, with better bacteriologic responses in infections caused by *Haemophilus* spp. and members of the Enterobacteriaceae than in infections caused by the less susceptible *P. aeruginosa.* Comparative trials of imipenem versus ciprofloxacin[324]

or levofloxacin[325] for treatment of patients with nosocomial pneumonia have been published. For hospitalized patients with severe pneumonia, ciprofloxacin in high dose (400 mg IV every 8 hours) was compared with imipenem (1000 mg IV every 8 hours) in a multicenter, randomized, double-blind trial.[324] Most of the patients had nosocomial pneumonia (78%) and were mechanically ventilated (79%). Clinical and microbiologic response rates were somewhat higher in the ciprofloxacin (69% and 69%, respectively) than the imipenem (56% and 59%, respectively) group, and bacterial eradication rates were highest for members of the Enterobacteriaceae. Substantially poorer responses in both treatment groups were seen in patients with *P. aeruginosa* infection, and for both *P. aeruginosa* and *S. aureus,* persistence in sputum was substantial (60% to 67% and 35% of patients, respectively) and was associated with development of resistance. For these reasons, combination therapy and use of maximum approved doses of ciprofloxacin (750 mg PO or 400 mg IV every 8–12 hours) may be preferred when ciprofloxacin is used in patients with *P. aeruginosa* infection. Addition of ciprofloxacin to meropenem did not provide additional clinical benefit over meropenem alone overall in patients with ventilator-associated pneumonia, but in the subset of patients with *P. aeruginosa, Acinetobacter* spp., or multidrug-resistant gram-negative bacilli, bacteriologic eradication was higher in the combination therapy group.[326] In a multicenter, randomized, open-label trial, high-dose levofloxacin (750 mg IV/PO) produced clinical responses similar to those with imipenem (500 mg to 1 g every 6–12 hours), followed by ciprofloxacin (750 mg PO every 12 hours), when both regimens were given for 7 to 15 days (135 of 204 patients [66%] vs. 143 of 206 patients [69%] in intent-to-treat analysis), including a severely ill subset of patients with APACHE II scores less than 20 (mean APACHE II scores were 15.0 vs. 14.8).[325] A later analysis of the subgroup of patients with ventilator-associated pneumonia also found similar efficacy in the two treatment groups.[327] Patients also received additional therapy if they had documented infection with methicillin-resistant *S. aureus* (vancomycin for both study arms) or *P. aeruginosa* (ceftazidime for levofloxacin arm and amikacin for imipenem arm). Microbiologic eradication rates were also similar overall. In patients with *P. aeruginosa* infection, however, there was a trend toward better clinical and microbiologic responses in the levofloxacin arm, suggesting that levofloxacin plus ceftazidime might be somewhat better than imipenem plus amikacin for these infections. No data were included about emergence of bacterial resistance in this study, but prior exposure to levofloxacin has been associated with the emergence of resistance in *P. aeruginosa*.[328] Recent IDSA guidelines for the treatment of hospital-acquired and ventilator-associated pneumonia have limited the role of combination therapy to the setting of definite therapy of infection with *P. aeruginosa* in patients who remain in septic shock or at a high risk for death.[329]

There has been a single reported study of moxifloxacin (400 mg IV/PO once daily) in treatment of hospital-acquired pneumonia in patients who were screened to exclude clinical risk factors for resistant pathogens. The study was terminated early because of slow enrollment and had identified no differences in outcomes relative to ceftriaxone, followed by cefuroxime.[330]

Mild-to-moderate respiratory exacerbations in patients with cystic fibrosis and *P. aeruginosa* in sputum responded clinically to oral ciprofloxacin (750 mg twice daily) and ofloxacin (400 mg twice daily), and similarly to conventional parenteral therapies that use an antipseudomonal β-lactam and tobramycin.[143] In patients with more severe exacerbations, however, conventional parenteral combination therapy may be superior. Rarely is *P. aeruginosa* eliminated from sputum by any regimen in patients with cystic fibrosis. Rotating the use of different regimens may reduce the selective pressure for persistence of resistant bacteria that may emerge with either type of regimen. Levofloxacin inhalation solution is an aerosolized fluoroquinolone licensed for treatment and maintenance therapy in patients with cystic fibrosis and chronic *P. aeruginosa* lung infections. In a randomized noninferiority study, levofloxacin inhalation solution (three 28-day on/off cycles) was noninferior to the same regimen with inhalational tobramycin. Levofloxacin inhalation solution was well tolerated, with dysgeusia (distorted taste) as the most frequent adverse event; a greater than fourfold increase in MIC of levofloxacin was observed in 21% of the *P. aeruginosa* isolates, similar to the comparator group.[331]

Acute purulent sinusitis acquired in the community is often caused by a similar group of pathogens to those that cause acute bacterial exacerbations of chronic bronchitis and may be seen as a complication of viral upper respiratory infections. Anaerobic bacteria are usually present in only a small percentage of patients and are more likely if sinusitis is chronic or associated with dental infections. Establishment of drainage of the infected sinus cavity by use of nasal decongestants or other means is an important adjunctive therapy in addition to antimicrobial agents. There are a number of trials comparing quinolones with other therapies in patients with acute purulent sinusitis, including ciprofloxacin (500 mg PO twice daily) versus cefuroxime axetil,[331a] levofloxacin (500 mg PO once daily) versus amoxicillin-clavulanate[331b] or clarithromycin,[331c] and moxifloxacin (400 mg PO once daily) versus cefuroxime axetil[331d,332] or amoxicillin-clavulanate.[331e] In all of these studies, there were comparable clinical responses between the quinolone and its comparator, with generally high response rates (87% to 96%). A 5-day course of levofloxacin at the higher dose (750 mg once daily) was also comparable to a 10-day course at the lower dose (500 mg once daily).[331f] In these or other studies in which microbiologic samples were obtained by sinus puncture or nasal endoscopy before therapy, *H. influenzae* and *S. pneumoniae* were the predominant pathogens, and clinical responses in the subset of patients from whom *S. pneumoniae* was isolated were high: 100% for levofloxacin[331f] and 97% for moxifloxacin.[332] Thus these quinolones may be an alternative to, but offer no advantage over, nonquinolone therapies for treatment of acute community-acquired purulent sinusitis. A prospective, multicenter, randomized, double-blind trial compared the efficacy and safety of moxifloxacin (400 mg daily for 5 days) versus placebo in the treatment of culture-positive acute bacterial rhinosinusitis.[333] Interestingly, moxifloxacin was not significantly superior to placebo for the primary end point.

Although acute otitis media is caused by pathogens similar to those that cause acute bacterial sinusitis, fluoroquinolones have not been widely used because acute otitis media is usually a disease of children, in whom joint toxicity of fluoroquinolones had been a concern. Invasive otitis externa in diabetics is usually caused by *P. aeruginosa* and may respond to oral ciprofloxacin (750 mg twice daily) given for 6 weeks.[334,335] No studies comparing quinolones with conventional parenteral therapies in adults have been reported.

Bone and Joint Infections

The prolonged antimicrobial therapy usually given for bone and joint infections is facilitated by effective oral agents, and quinolones may fill this role in some cases.[336] For treatment of chronic osteomyelitis, there have been noncomparative trials using ciprofloxacin, ofloxacin, pefloxacin, or levofloxacin, in which treatment was usually for 6 or more weeks and follow-up was for at least 6 months after completion of therapy.[79,143,337,338] Clinical cures after oral ciprofloxacin (750 mg twice daily) were 75% overall in infections in which gram-negative bacilli predominated, and similar rates of cure were reported in the smaller subgroups of patients with *P. aeruginosa* and methicillin-susceptible *S. aureus* (MSSA) infections. Failures were associated with incomplete débridement, the presence of foreign bodies, and the development of resistance in *P. aeruginosa, S. aureus,* and *Serratia marcescens.* Levofloxacin (500 mg once daily) was effective in 9 of 15 patients (60%), with largely polymicrobial (*S. aureus* in 7 and *P. aeruginosa* in 3; all but 3 had other organisms as well) infections; in 4 cases, failures were thought to be due to inadequate débridement.[337] Three months of treatment with ciprofloxacin produced a cure rate of 60% in one study of osteomyelitis in the feet of diabetics[339]; ofloxacin produced a similar response rate.[340] Calcaneal osteochondritis caused by nail puncture wounds of the foot is often due to *P. aeruginosa.* Local débridement in combination with ciprofloxacin (400 mg IV every 12 hours, then 750 mg PO twice daily) given for 14 days appears to be highly effective for this condition, with cures in all 18 patients with *P. aeruginosa* infection and in 2 with *S. aureus* infection.[341]

In four small comparative trials, ciprofloxacin (750 mg twice daily) and ofloxacin (400 mg twice daily) have generally produced apparent rates of cure similar to those of conventional parenteral therapies using β-lactams with or without an aminoglycoside,[143,342] but the power of

these studies to detect differences between the regimens was small. For ofloxacin, cures of MSSA infections were 80% (10 of 12), but cures of *P. aeruginosa* infections were only 25% (1 of 4).[342] For patients with spinal brucellosis, ciprofloxacin (500 mg PO twice daily) plus rifampin (600 mg PO once daily) produced cure rates similar to doxycycline plus streptomycin given for 12 to 24 weeks when compared in consecutive, nonrandomized groups.[343] Randomized, controlled studies do not, however, provide support for the use of quinolone-based combinations as a first-line therapy, and quinolone-based regimens were associated with a higher relapse rate than comparators.[344]

For septic arthritis of prosthetic joints, standard therapy involves staged prosthesis removal and débridement, prolonged antibiotic treatment, and prosthesis reimplantation. Infections of prosthetic joints caused by *S. aureus* and coagulase-negative staphylococci have been treated with ofloxacin (200 mg PO three times daily) plus rifampin (900 mg once daily) for 6 to 9 months using the standard approach as well as débridement with retention of the prosthesis in patients for whom removal was not possible.[345] With prolonged follow-up after completion of therapy, response rates were high in patients after the standard approach (81%–93%) and unexpectedly high in the patients with retained prostheses (54%–70%). Similar results have been reported for ciprofloxacin plus rifampin that also document the importance of rifampin in the antimicrobial regimen, because failure rates were high when ciprofloxacin was used alone.[346] Ceftazidime (1.5 g every 12 hours) given for 6 weeks plus ciprofloxacin (500 mg PO every 8 hours) given for 6 months also appeared to cure *P. aeruginosa*–infected orthopedic implants without prosthesis removal.[347] Patients with infections of prosthetic joints caused primarily by staphylococci have also been successfully treated with a combination of levofloxacin (500 mg PO once daily) and rifampin (600 mg once daily) given for extended periods after débridement with prosthesis retention.[348,349] A randomized comparison of short (8 weeks) and long (3–6 months) durations of treatment with levofloxacin at a higher dose (750 mg PO once daily) and rifampin (600 mg daily) found that at a median of 540 days of follow-up, the short course was no worse than the long course in 63 patients with staphylococcal prosthetic joint infection after débridement and prosthesis retention (73% vs. 58% cure in intent-to-treat analysis and 92% vs. 95% in per-protocol analysis for short and long durations, respectively).[350] For patients with rigid internal fixation devices (plates and screws) infected by staphylococci that were débrided and retained, a levofloxacin-rifampin regimen resulted in an overall cure rate of 72%.[351] For patients with either prostheses or internal fixation devices, failures were associated with a longer duration of symptoms before débridement.[348,351] Thus, for salvage of some patients in whom orthopedic devices cannot be removed, early débridement and extended treatment with a combination of a fluoroquinolone and rifampin (or ceftazidime) could be considered if the infecting organism is susceptible to both agents in the combination.

There are few other data on treatment of septic arthritis with quinolones, most often with ciprofloxacin or ofloxacin.[79] Infections caused by *N. gonorrhoeae* and *E. coli* have responded to oral therapy. Failures have been seen in infections of prosthetic joints and infections caused by *S. aureus* and *P. aeruginosa* infections.

Skin and Soft Tissue Infections

Although the most common causes of cellulitis and pyodermas are streptococci and *S. aureus,* in patients with diabetes and peripheral vascular disease, decubitus ulcers, and some surgical wound infections, soft tissues may become infected with a mixture of bacteria that includes, in addition to streptococci and staphylococci, aerobic gram-negative bacteria and anaerobes. Quinolones have been evaluated as treatment for skin and soft tissue infections in some of these subgroups of patients.[352,353]

For uncomplicated skin infections, comparable clinical response rates (usually 90% or higher in both arms) have been reported for ofloxacin (400 mg PO twice daily) versus cephalexin (500 mg four times daily),[354] levofloxacin (500 mg PO once daily) versus ciprofloxacin (500 mg PO twice daily),[355] and moxifloxacin (400 mg PO once daily) versus cephalexin (500 mg three times daily).[356] *Staphylococcus aureus* and *Streptococcus pyogenes* were the dominant pathogens in these studies, but methicillin-resistant *S. aureus* (MRSA) strains, which are commonly also resistant to quinolones, were infrequently reported. Levofloxacin was significantly better than ciprofloxacin in eradicating *S. aureus* in one study.[355] In these and noncomparative studies,[79] failures have been seen with infections caused by *P. aeruginosa* (11%), streptococci (6%), and *S. aureus* (5%) and have been associated with the development of resistant organisms, which for *P. aeruginosa* may be more frequent in diabetic patients.[354]

For complicated skin infections, comparisons of oral ciprofloxacin (750 mg twice daily)[357,358] and ofloxacin (400 mg twice daily)[359] with intravenous cefotaxime or ceftazidime, given for 9 to 12 days to patients with mixed infections in which gram-negative bacilli predominated, showed similar rates of clinical and bacteriologic efficacy, in the range of 79% to 98% clinical cures. *Staphylococcus aureus* and *P. aeruginosa* were the most common pathogens in these studies, with few MRSA strains identified. Levofloxacin (750 mg IV/PO once daily) compared with ticarcillin-clavulanate (3.1 g IV every 4 to 6 hours), followed by amoxicillin-clavulanate (875 mg PO every 12 hours), produced similar overall clinical success (116 of 138 patients [84%] vs. 106 of 132 patients [80%]).[360] Levofloxacin was superior in eradicating MSSA, the dominant pathogen, and similar in eradicating a mix of gram-negative pathogens. The subgroup with diabetic foot infections was too small to assess adequately. In diabetic foot infections without osteomyelitis, the overall rate for complete healing using ciprofloxacin alone was 50%.[339] The polymicrobial nature of these infections, which may include anaerobes, may be a limiting factor for efficacy of some fluoroquinolones. Use of additional antimicrobial agents with activity against anaerobes should be considered in these patients. In a double-blind, randomized trial, moxifloxacin (400 mg IV/PO once daily) produced comparable clinical responses and bacterial eradication rates relative to piperacillin-tazobactam (3.375 g IV every 6 hours), followed by amoxicillin-clavulanate, in patients with dominant cellulitis or skin abscess.[361] In the subgroups of patients in whom *S. aureus* (only a minority were MRSA), *S. pyogenes,* and enteric gram-negative bacteria were isolated and in the subgroup of diabetic foot infections, clinical response rates were also similar. In a randomized, double-blind trial, delafloxacin (300 mg IV) was compared to linezolid (600 mg IV) and vancomycin (15 mg/kg IV) each given twice daily in 256 patients with acute bacterial skin and skin structure infections.[362] *Staphylococcus aureus* was the dominant pathogen identified, with MRSA exceeding MSSA, and cellulitis (~45%) and major abscess (~28%) were the dominant infection types. Delafloxacin was significantly better than vancomycin in clinical cure (57 of 81 patients [70%] vs. 53 of 98 patients [54%]) and in reduction of local erythema, with no significant differences between delafloxacin and linezolid. In a phase 3, double-blind, randomized trial, delafloxacin was also shown to be noninferior to vancomycin plus aztreonam, with similar objective reductions in erythema (78% vs. 81%), rates of clinical cure (70% vs. 67%), and eradication of MRSA (100% vs. 98%).[363]

The role of quinolones in the treatment of cutaneous and other forms of anthrax received considerable attention as a result of bioterrorism cases of anthrax in the United States in 2001. Activity in vitro is excellent for a number of fluoroquinolones, and ciprofloxacin (500 mg PO twice daily) is the recommended regimen for cutaneous disease and prophylaxis after exposure to anthrax spores.[364] Inhalational anthrax is treated with intravenous ciprofloxacin in combination with other agents.

For patients with uncomplicated cellulitis or pyodermas, in whom streptococci and staphylococci are the most likely pathogens, respectively, conventional therapies with penicillin, semisynthetic penicillins, and cephalosporins, either oral or parenteral, remain the therapy of choice. For infections with MRSA, for which the quinolones were initially hoped to be valuable oral therapies, rapid emergence of quinolone resistance has become a particular problem, and resistance is now highly prevalent in many medical centers.[365,366] In addition, with the increasing frequency of MRSA as a cause of skin infections in the community and the emergence of fluoroquinolone resistance in community strains (along with established resistance in hospital strains),[367] fluoroquinolones cannot be relied on overall for empirical therapy of serious skin infections possibly caused by staphylococci. The exception appears to be delafloxacin, which has in vitro activity against MRSA, and for which efficacy has been shown in MRSA skin infections in initial clinical trials. It remains important to confirm the proper choice of antimicrobial by

isolation and susceptibility testing of the relevant pathogen when possible.

Other Uses

Because of resistance to conventional antituberculosis agents, quinolones are being used for treatment of mycobacterial infections.[368] In patients with multidrug-resistant pulmonary tuberculosis, ofloxacin (300 or 800 mg once daily) has been used in combination with other second-line agents, with sputum conversions and apparent clinical cures,[369] but in cases in which ofloxacin was the only active drug, there was failure of sputum conversion and development of ofloxacin resistance.[369,370] Ciprofloxacin (750 mg PO once daily) is less effective in combination regimens than are other first-line agents.[371,372] Levofloxacin and moxifloxacin, however, are being used in second-line regimens for treatment of multidrug-resistant tuberculosis. For early bactericidal activity against *M. tuberculosis* in sputum, levofloxacin (1 g once daily) and moxifloxacin (400 mg once daily) were similar to isoniazid (300 mg once daily).[373] Moxifloxacin (400 mg) was comparable to ethambutol as the fourth component of a regimen of isoniazid, rifampin, and pyrazinamide for sputum conversion within the first 2 months of therapy, with no difference between dosing 5 days per week versus 3 days per week, after the first 2 weeks of daily therapy.[374] A later study, however, in which almost all patients had lung cavities found that moxifloxacin versus ethambutol, in a four-drug regimen, was associated with accelerated reduction in viable bacilli in sputum when adjusted for patient factors that affect rate of sputum conversion.[375] Three recent randomized controlled trials compared standard 6-month regimens with quinolone-containing 4-month regimens in the treatment of rifampin-sensitive pulmonary tuberculosis, but all three studies failed to show noninferiority of the quinolone-containing regimen.[376–378] Somewhat surprisingly, in extensively drug-resistant tuberculosis, which by definition includes resistance to quinolones, the presence of moxifloxacin in the treatment regimen was associated with 40% higher favorable outcomes in a meta-analysis.[379]

Because of widespread quinolone use for respiratory and other indications, there have been concerns about selection of resistance and masking of symptoms in undiagnosed tuberculosis patients, particularly in areas of high endemicity. Prior quinolone exposure for longer than 10 days has been associated with a sevenfold increased risk of quinolone resistance in *M. tuberculosis* isolates and a 17-fold increased risk if the exposure was greater than 60 days before the diagnosis of tuberculosis.[380] In a randomized trial of patients in Hong Kong with community-acquired pneumonia or exacerbations of bronchiectasis, 4.8% of those treated with amoxicillin-clavulanate were diagnosed with active tuberculosis within 12 months, in contrast to 2.4% of those treated with moxifloxacin for 5 days and 0% of those treated for 10 days, suggesting potential masking by moxifloxacin.[381] In an open-label, randomized controlled trial of patients with tuberculous meningitis, a levofloxacin-containing (10 mg/kg up to 500 mg) regimen produced better survival that a rifampin-containing (10 mg/kg up to 450 mg) regimen, both in combination with isoniazid, pyrazinamide, ethambutol, and prednisolone, but the levofloxacin-containing regimen was associated with significantly more seizures and myoclonus.[382] In a subsequent study, however, addition of levofloxacin to a standard four-drug regimen containing rifampin did not result in improved outcomes and was also associated with an increased occurrence of seizures.[383]

For nontuberculous mycobacteria, ciprofloxacin (750 mg twice daily or 500 mg three times daily) has been used in three-drug (clarithromycin plus amikacin)[384] and four-drug (rifampin, ethambutol, and clofazimine)[385] regimens in patients with AIDS and *M. avium-intracellulare* complex bacteremia. Improvement in symptoms occurred with both regimens, but clearance of bacteremia appeared better with the clarithromycin-containing three-drug regimen.[386] In cutaneous *M. fortuitum* infections, ciprofloxacin used alone was followed by relapse and the development of drug resistance,[143] but ofloxacin has been used successfully in some sternotomy infections caused by this pathogen.[387] With agents used against *M. leprae,* there have been reports of clinical improvement in patients with lepromatous leprosy given ofloxacin (400 mg once daily) and pefloxacin (800 mg once daily),[388] and in a randomized, double-blind study of patients with paucibacillary leprosy, at 10 years follow-up, ofloxacin (400 mg once daily) plus rifampin (600 mg once daily) given for 28 days produced clinical improvements comparable to dapsone (100 mg once daily) plus rifampin (600 mg every month) given for 6 months.[389]

For patients with bacteremias, intravenous ciprofloxacin and ofloxacin have been effective in cases caused by enteric gram-negative bacilli, although responses have been poor for *P. aeruginosa* bacteremias when these drugs were used in relatively low doses of 200 mg twice daily.[390,391] In neutropenic patients with fever, ciprofloxacin in combination with aminoglycosides produced defervescence and cure of documented infections comparable to standard β-lactam–aminoglycoside combinations,[392] but ciprofloxacin monotherapy was less effective than such combinations[393] and should not be used. Although lower doses were used in earlier studies, more recent studies have used regimens of ciprofloxacin (400 mg IV every 8 hours; in one case, followed by 750 mg PO twice daily), either alone[394] or in combination with piperacillin.[395] Ciprofloxacin alone was comparable to ceftazidime plus amikacin, and ciprofloxacin plus piperacillin was comparable to tobramycin plus piperacillin in resolution of fever, but changes in therapy were necessary in more than half of patients in both arms of both studies. Use of quinolones in this setting should be cautious and should be considered principally as an alternative regimen when there are reasons for not choosing standard regimens with combinations of β-lactams and aminoglycosides.

There has been an increase in interest in defining low-risk groups of neutropenic patients who might be safe candidates for oral antimicrobial therapy for fever because of the potential for increased convenience and reduced costs. Quinolones have been components of such oral regimens.[396,397] These trials evaluated a combination of oral ciprofloxacin and amoxicillin-clavulanate compared with intravenous ceftazidime in one trial[397] and with intravenous ceftriaxone plus amikacin in the other.[396] The trials involved patients with fever and neutropenia from cancer chemotherapy and who were able to take oral medications and were considered to have low risk of serious complications, based on the absence of other diseases and documented infection and a projected duration of neutropenia of less than 10 days. Success of treatment in patients given the oral and parenteral regimens was similar in both studies, although the oral regimen was associated unexpectedly with a higher incidence of adverse effects (16% vs. 1%, respectively), largely nausea and vomiting.[397] Renal failure reported in an earlier trial of ciprofloxacin plus clindamycin for treatment of a similar group of patients was not seen in these trials.[398] In a double-blind, randomized trial, moxifloxacin (400 mg PO daily) alone was similar to ciprofloxacin (750 mg twice daily) plus amoxicillin-clavulanate in clinical response (80% vs. 82%).[399] Guidelines of the IDSA recommend that febrile neutropenic patients with low risk, defined as anticipated neutropenia for less than or equal to 7 days and no or few comorbidities, are candidates for oral therapy with a regimen of ciprofloxacin or levofloxacin plus amoxicillin-clavulanate.[400]

Oral ciprofloxacin (500 mg twice daily), ofloxacin (300 mg twice daily), and norfloxacin (400 mg twice daily), given as prophylaxis in neutropenic patients, have consistently reduced the occurrence of gram-negative bacteremia and, in some cases, prolonged the time to first fever, but breakthrough gram-positive bacteremias have occurred, particularly streptococcal bacteremias in bone marrow transplant recipients.[401,402] Ciprofloxacin (20 mg/kg/day) compared to placebo reduced febrile episodes in neutropenic pediatric patients during the acute induction phase but not the consolidation phase of chemotherapy for acute lymphoblastic leukemia and was associated with increased stool colonization with ciprofloxacin-resistant *E. coli* and *K. pneumoniae.*[403] Ciprofloxacin and ofloxacin appear superior to norfloxacin,[404,405] with lower rates of gram-negative and, in the case of ofloxacin, streptococcal bacteremias. Addition of penicillin to norfloxacin reduced breakthrough streptococcal bacteremias,[406] and addition of rifampin to ofloxacin reduced staphylococcal bacteremias.[405] Colonization and breakthrough bacteremias with quinolone-resistant viridans streptococci have also been reported when levofloxacin was used alone as prophylaxis in recipients of autologous stem cell transplants and hematologic malignancies.[407,408] Use of additional agents with activity against gram-positive pathogens in combination with fluoroquinolones in prophylaxis can reduce gram-positive bacteremias but is less well tolerated.[409,410] In

addition, breakthrough bacteremias with quinolone-resistant *E. coli* have occurred as well with fluoroquinolone prophylaxis.[411–413] IDSA guidelines recommend prophylaxis with ciprofloxacin or levofloxacin (the latter preferred in patients with increased risk of mucositis-related streptococcal bacteremia) in high-risk patients, defined as having an expected absolute neutrophil count less than or equal to 100 cells/mm^3 for greater than or equal to 7 days.[400] Relative to historical controls, levofloxacin (10 mg/kg twice daily) reduced febrile episodes in pediatric patients with neutropenia and autologous hematopoietic stem cell transplantation.[414] Reductions in numbers of patients with blood cultures yielding gram-negative bacteria was offset by increases in numbers with blood cultures yielding gram-positive bacteria. Patients who have received quinolone prophylaxis should not be treated with quinolones for fever and neutropenia because of the risk of quinolone resistance.

There is limited experience in using quinolones for treatment of endocarditis.[415] One study reported good responses in intravenous drug abusers with right-sided *S. aureus* (methicillin-susceptible) endocarditis and who complied with the full course of ciprofloxacin (300 mg IV twice daily for 1 week, then 750 mg PO twice daily for 3 weeks), plus rifampin (300 mg PO twice daily for 4 weeks).[416] An additional study compared 28 days of inpatient therapy with ciprofloxacin (750 mg PO twice daily) plus rifampin (300 mg PO twice daily) versus intravenous oxacillin or vancomycin plus gentamicin (given for the first 5 days) for similar patients, with similar response rates for the two regimens when patients were evaluated at 6 to 7 days after completion of therapy.[417] Drug resistance, however, has occurred in this setting, and there have been failures in patients with left-sided *S. aureus* endocarditis. Because of limited case report data, it is not clear if quinolones, such as levofloxacin and moxifloxacin, which would be preferred based on greater activity against susceptible strains of *S. aureus,* would have better outcomes of treatment. There have been a number of single-case reports of patients with gram-negative bacillary endocarditis whose infections have been suppressed with oral quinolones, but there have been failures. Q fever endocarditis, which responds poorly to conventional antimicrobial therapy, has been successfully cured with prolonged courses of ciprofloxacin (12 weeks)[418] or ofloxacin plus doxycycline (4 years).[419] Doxycycline combined with hydroxychloroquine was, however, superior to ofloxacin plus doxycycline.[419] Use of quinolones for endocarditis should currently be limited to circumstances in which established therapies are not possible.

Quinolones vary in their penetration across the blood-brain barrier into CSF.[420] In the presence of meningeal inflammation, concentrations in CSF have reached as high as 39%, 40%, and 60% of peak serum concentrations for ciprofloxacin, levofloxacin, and pefloxacin, respectively.[421,422] In two small studies of patients with predominantly gram-negative bacillary meningitis, pefloxacin (800 mg IV every 12 hours) cured 12 of 16 neurosurgical patients, many of whom had failed to respond to β-lactam therapies,[423] and ciprofloxacin (200 mg IV every 12 hours) cured 18 of 20 similar patients.[424] Ten of 12 neonates with gram-negative bacillary meningitis (and in some cases mixed infections) were also reported to have been cured with intravenous ciprofloxacin (10 to 60 mg/kg/day).[425] Treatment of *P. aeruginosa* meningitis with ciprofloxacin may require very high doses in some patients (800 mg every 8 hours).[426] Use of these quinolones for treatment of meningitis should only be considered in those circumstances in which standard therapies are not possible or have failed. Ciprofloxacin has been used successfully in the treatment of brain abscess caused by *S. enterica* serovar Enteritidis in a small number of case reports.[420] For eradication of nasopharyngeal carriage of *Neisseria meningitidis,* which is indicated in the setting of close contact with patients with meningococcal meningitis, ciprofloxacin (750 mg) or ofloxacin (400 mg) given as a single dose has been highly effective,[427,428] and ciprofloxacin had efficacy similar to rifampin (600 mg twice daily for 2 days) and ceftriaxone (2 g IM).[429] The recent emergence of quinolone-resistant strains of *N. meningitidis,* however, could compromise the efficacy of quinolones for this indication.[430]

Quinolones have been used for treatment of a variety of other infections in small numbers of patients. A few patients with tularemia have responded to ciprofloxacin or levofloxacin,[431,432] and several patients with cat-scratch disease improved more rapidly after ciprofloxacin treatment than might be expected without treatment.[433] Patients with Mediterranean spotted fever caused by *Rickettsia conorii* and Q fever caused by *Coxiella burnetii* may respond to ciprofloxacin or ofloxacin, but doxycycline remains the preferred therapy.[419,434,435] Attempts to treat patients with brucellosis with quinolones have been complicated by a high frequency of relapses,[344,436] but a combination of ofloxacin (400 mg once daily) and rifampin (600 mg once daily) for 6 weeks resulted in a low relapse rate and was comparable to doxycycline plus rifampin.[437] Patients with falciparum malaria have had inconsistent responses to quinolones.[438,439] Ciprofloxacin decreased the BK polyomavirus load in patients who underwent allogeneic hematopoietic stem cell transplantation.[439,440] A double-blind, placebo-controlled randomized study of levofloxacin 500 mg daily as prophylaxis for BK polyomavirus following renal transplant in 154 patients did not find a statistically significant difference in the percentage of patients with viremia at a median of 46 weeks; however, no patient in either group developed BK polyomavirus nephropathy.[441] Ciprofloxacin and metronidazole alone or together have been used in the treatment of inflammatory bowel disease, particularly Crohn's disease, but studies using ciprofloxacin have not shown consistent benefit.[442]

PROBLEMS WITH QUINOLONE RESISTANCE DURING CLINICAL USE

Resistance to fluoroquinolones has been found in many locales throughout the world, but the extent varies in different areas and varies with the pathogen and site of infection.[443] In addition to data from clinical trials, clinical decisions on choice of fluoroquinolones in initial empirical therapy would be best informed initially by local susceptibility data and later by specific susceptibility data on the patient's strain. Development of bacterial resistance among pathogens during clinical use of quinolones is multifactorial. Because spontaneous chromosomal resistance mutations causing resistance increments of fourfold to eightfold for fluoroquinolones may occur at frequencies of 10^{-8} to 10^{-10}, resistance is predicted to occur more often in settings in which there are large numbers of bacteria at the site of infection and the concentration of the drug is below the MIC of the least drug-susceptible mutant subpopulation (a value called the mutant prevention concentration).[31,444,445] Therefore, resistance is more likely in infections caused by less susceptible pathogens, such as *P. aeruginosa* and *S. aureus,* at sites of infection where the bacterial burden is high or at which drug delivery or host eradication mechanisms may be compromised, and in patients who receive inadequate drug doses. Once selected, the rate of amplification of the drug-resistant mutant population will be governed by the degree of resistance conferred by the mutation to the drug being used, the fitness cost that bearing the mutation exacts, the dose of the drug being used, and the duration of treatment.[446]

Epidemiologic factors also affect the extent to which resistant pathogens can spread and thereby amplify the prevalence of resistance.[447] General surveys of resistance patterns occurring over time have found resistance to increase after the introduction of fluoroquinolones and to occur most often with *Pseudomonas* spp. and staphylococci and in soft tissue infections and infections associated with foreign bodies.[448–450] In many medical centers, ciprofloxacin resistance has increased markedly (to <90%) among MRSA but not MSSA strains.[366,451,452] Resistance appears to have been selected in patients colonized with MRSA and given ciprofloxacin for other infections.[366] Clonal dissemination may also contribute to spread of resistance during outbreaks. The community-acquired MRSA strains that have spread into health care systems are more likely ciprofloxacin susceptible, but resistance has occurred in some isolates.[453,454] Susceptibility rates have also decreased among coagulase-negative staphylococci.[455] A similar difference between methicillin-resistant and -susceptible strains has been seen for ciprofloxacin resistance in coagulase-negative staphylococci, and in this setting, cross-selection by exposure to other antibiotics, in addition to direct quinolone selection, appears to augment ciprofloxacin resistance in methicillin-resistant strains, which are usually also multidrug resistant.[456,457]

More surprising has been the emergence of substantial quinolone resistance in initially highly susceptible species of bacteria, particularly *N. gonorrhoeae, C. jejuni,* and *E. coli.* Fluoroquinolone resistance in *N.*

gonorrhoeae was first identified in the 1990s in some countries in the Far East.[458] The first fluoroquinolone-resistant *N. gonorrhoeae* isolate was found in the United States in 1991. Data from a national sentinel surveillance system showed that fluoroquinolone resistance increased from 0.4% in 1999 to 4.1% in 2003 and to 13.8% in 2006 (www.cdc.gov/std/gisp2006/GISPSurvSupp2006Short.pdf).[459] Reduced susceptibility of *N. gonorrhoeae* to ciprofloxacin was associated with increased likelihood of failure with ciprofloxacin treatment.[460–462] Subsequently, in 2007, the CDC changed its guidance and recommended that fluoroquinolones no longer be used as empirical treatment of gonococcal infections.[173] Quinolone resistance has now also been reported in *N. meningitidis.*[430]

Resistance emerged in *C. jejuni* in human and poultry populations in parallel after quinolone use in both groups in Europe.[463] In the United States, travel to Spain or Latin America was a risk factor for acquiring resistant *C. jejuni,* and the occurrence of domestically acquired cases in patients without prior treatment with a fluoroquinolone also increased. Strain typing has shown an overlap in resistant *C. jejuni* strains from humans and poultry, and contamination of food products with resistant *C. jejuni* has been demonstrated,[464] suggesting contaminated poultry products as the source of some resistant infections in humans. Resistance acquired by an initially susceptible isolate of *C. jejuni* during treatment of *Campylobacter* gastroenteritis with a quinolone has also been reported.[214]

Quinolone-resistant strains of Enterobacteriaceae have emerged among inpatients and outpatients in the United States[465] and globally, in association with fluoroquinolone use.[466,467] Hospital-wide fluoroquinolone use correlated with resistance among *E. coli, K. pneumoniae,* and *Proteus mirabilis.*[465,468] Risk factors in Spanish patients with resistant *E. coli* urinary isolates have included use of quinolones, complicated infections, and use of urinary catheters.[469] Clinically important resistance in *E. coli* has also developed in some hematology-oncology units in Europe in which quinolones were used as prophylaxis during periods of neutropenia[470–472] as well as in nonneutropenic patients in Spain.[473] In these units, breakthrough bacteremias with quinolone-resistant *E. coli* have become problems. These bacteremias as well as colonization of the fecal flora with quinolone-resistant *E. coli*[474] have been associated with quinolone use as prophylaxis and were caused by distinct strains, rather than representing clonal spread within the units. In addition, some patients not receiving quinolones were found to be colonized with quinolone-resistant *E. coli,*[474] and a survey suggested that 25% of the population in Spain may have fecal colonization with such strains.[475] These findings, in conjunction with earlier findings of high rates of colonization of poultry with resistant *E. coli* in Spain,[476] raise the possibility that contamination of the food supply with resistant *E. coli* could be a contributing factor in these areas.[477] Surprisingly, in the United States, fluoroquinolone resistance in extraintestinal *E. coli* isolates has been linked to a clonal lineage with distinctive mutant alleles of *gyrA* and *parC.*[478] Quinolone resistance in *E. coli* bloodstream isolates has been linked with a higher likelihood of initial inappropriate therapy[479] and, in some studies, higher mortality.[480]

With increasing use of quinolones for treatment of patients with respiratory tract infections, there have been concerns about the emergence of quinolone resistance in *S. pneumoniae.* In Canada, ciprofloxacin resistance in isolates of *S. pneumoniae* was 0%, 0.6%, 1.7%, and 4.2% in 1993, 1997–1998, 2000–2001, and 2005, respectively,[481] with the increase between 1993 and 1997–1998 preceded by increasing use of quinolones, largely ciprofloxacin. Resistance to levofloxacin and moxifloxacin also increased significantly, from 0.2% and 0% between 1997 and 1998 to 1.1% and 1.0% in 2005, respectively.[482] Rates of resistance to ciprofloxacin of 15.2% in Northern Ireland and 5.3% in Spain[457] have also been reported. In 2000 in Hong Kong, rates of resistance to ciprofloxacin, levofloxacin, and moxifloxacin were as high as 17.8%, 13.3%, and 8.9%, respectively.[483] Resistant isolates were generally reported in adults but not children, who would be less likely to have received a quinolone, and in noninvasive diseases.[482–484] Although resistant isolates in Hong Kong were clonal, indicating spread from person to person, in Canada, increased genetic homogeneity of resistant clones, as well as de novo mutations, were associated with quinolone resistance,[481–485] and in Spain, the strains were polyclonal. Worrisome was the observation that among the Spanish isolates, 30% of the strains belonged to one of two international, epidemic, multidrug-resistant clones (France9V-3 and Spain23F-1), raising concerns of possible future spread. In the United States, between 1999–2000 and 2001–2002, in one survey, there was a twofold increase in the rate of ciprofloxacin (1.2%–2.7%) and levofloxacin (0.6%–1.3%) resistance, with a significant minority related to widespread quinolone-resistant pneumococcal clones.[486] In different surveys, which also included invasive and noninvasive strains from across the United States, resistance to respiratory quinolones has remained consistently low (0.5%–1.1%) since 1996 and through 2004.[484,487–490]

Several factors potentially account for the variable trends in quinolone susceptibilities. Preferential use of quinolones that have better pharmacokinetic and pharmacodynamic profiles against pneumococci relative to ciprofloxacin could slow selection of resistant mutants.[491,492] In addition, introduction of the pneumococcal conjugate vaccine, which targets drug-resistant pneumococcal serotypes, may have limited the spread of resistant clones. Strains that contain single *parC* resistance mutations are circulating,[493] and these strains, which may be the progenitors of fully resistant strains with dual mutations in *parC* and *gyrA,* would be classified as susceptible and thus are difficult to detect with routine testing.[494] In this background, there is no reason to assume that resistance to newer quinolones will not ultimately emerge among *S. pneumoniae.*[484] Because children are a major reservoir of pneumococci, concerns have been raised that possible future increased use of quinolones in children could increase the rate of development of resistance to quinolones in *S. pneumoniae.*[495]

Resistance to quinolones should be monitored, and strategies for minimizing its occurrence, including focused quinolone use, should be used to avoid compromising the future utility of the class. Prevention of emergence of resistance needs to be addressed in several ways, including monitoring of preexisting chromosomal and transferable mechanisms of resistance; better defining the optimal selected quinolone, dosage, and duration of treatment for particular infections; and infection control to prevent spread of resistant organisms.[445,446,496,497]

ADVERSE EFFECTS

The tolerability of the fluoroquinolones is best assessed in double-blind, randomized trials in which the effects of patient populations, methods of ascertainment, and possible bias can be controlled. In some cases, however, such trials may not have the power to detect adverse effects occurring at low frequency or in more diverse patient populations. In an analysis of 56 such trials, in which fluoroquinolones were compared with placebo or other antimicrobial agents, most studies found similar adverse effect profiles.[498] In a minority of studies, there were either significantly fewer or more adverse effects relative to a variety of comparator agents. In a number of instances, increasing doses and durations of therapy were associated with higher rates of adverse effects. The overall adverse effect profile has been recently reviewed.[499]

The most frequent category of adverse effect involves the gastrointestinal tract, occurring in 3% to 17% of patients in clinical trials. In most patients, anorexia, nausea, vomiting, and abdominal discomfort are mild when they occur. Diarrhea is less frequent, and antibiotic-associated colitis has been rare, possibly because most current quinolones have limited effect on the anaerobic bowel flora.[498,500] Fluoroquinolone use has, however, been a strong epidemiologic risk factor for *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea in some studies and was particularly noted in outbreaks of the NAP1 strain, which is resistant to fluoroquinolones.[501]

The next most frequent category of adverse effects involves the nervous system, occurring in 0.9% to 11% of patients.[502] Symptoms of mild headache and dizziness have predominated, followed by insomnia and alterations in mood. Hallucinations, delirium, psychosis, and seizures are rare.[503] Seizures may have resulted in some cases from theophylline accumulation or from the ability of theophylline and NSAIDs to augment the ability of quinolones to displace GABA from its receptors.[504] Postmarketing surveillance has also identified uncommon cases of exacerbations of myasthenia gravis occurring after 0.5 to 10 days (median, 1 day) of exposure to various quinolones. The effect is usually reversible within 24 hours of stopping therapy and has recurred with rechallenge.[505] An increased risk (relative risk, 1.83) of peripheral neuropathy was seen in a case-control study of men ages 45 to 80 years. The onset can be

rapid and resolution variable. The US Food and Drug Administration reissued in 2016 a class warning regarding fluoroquinolone peripheral neuropathy because of additional reports since the 2004 warning that in some cases neuropathy appeared to be permanent. No patient-specific risk factors have been identified, and the mechanism is not known.[506] Although the incidence was very low (1 in 20,000), another case-control study found an increased risk of pseudotumor cerebri in patients receiving fluoroquinolones within 15 to 30 days (adjusted rate ratio, 5.7).[507]

Allergic and skin reactions have occurred in 0.4% to 2.8% of patients in clinical trials overall. Unspecified rashes have been most frequent. With gemifloxacin, rashes developed in 2.8% of patients in clinical trials, but in young women receiving gemifloxacin for 7 or more days, a self-limited, maculopapular rash without biopsy evidence of vasculitis occurred in 14%.[508] In patients with durations of therapy of 5 or less days, rash occurred with low frequency, similar to that of other fluoroquinolones. The occurrence of a rash with gemifloxacin resulted in a somewhat higher rate of rash in response to subsequently given ciprofloxacin (5.9%) relative to placebo (2.0%).[509] Hypersensitivity cross-reactivity among different fluoroquinolones can vary and is difficult to predict,[510] with, for example, reports of patients with prior reactions to moxifloxacin tolerating ciprofloxacin or levofloxacin but not vice versa. Phototoxicity reactions are uncommon with currently used quinolones but can occur in some patients after exposure to ultraviolet A (320–400 nm) light. Earlier quinolones with a halide at position 8, which are no longer available, had higher rates of phototoxicity.[511,512] Delafloxacin appears to have little or no phototoxicity. Drug fever, urticaria, angioedema, vasculitis, serum sickness–like syndromes, and anaphylactoid reactions have been uncommon. Acute interstitial nephritis, probably allergic in origin, also occurs infrequently and has been associated with eosinophiluria but generally not crystalluria. Infiltrates of lymphocytes and eosinophils have been found in the renal interstitium on renal biopsies.[498]

Arthropathy with cartilage erosions and noninflammatory effusions occurs in the weight-bearing joints of juvenile animals given quinolones.[513] Experience with use of quinolones in children has increased, particularly in children with cystic fibrosis given ciprofloxacin.[514] These children and others receiving nalidixic acid, norfloxacin, and ciprofloxacin have only uncommonly had joint symptoms, which have been reversible.[514,515] Studies to identify subclinical cartilage damage by nuclear magnetic resonance imaging of joints of treated children have also been negative.[516] Because of concerns about cartilage toxicity in children, quinolones have not been recommended for routine pediatric use, but there is an evolving view, based on absence of human arthropathy seen over the past decades of fluoroquinolone use, that in some children, particularly those with cystic fibrosis, the benefit of quinolones outweighs what appears to be a small short-term risk of joint toxicity, and expanded use of quinolones in pediatrics is under consideration.[514,517]

Tendinitis with acute onset of pain, swelling, and inflammatory skin changes has been reported in adults given various quinolones[518,519] and has resulted in a highlighted warning in drug safety labeling in the United States. The Achilles tendon is most often involved, and rupture can occur. Symptom onset can be after completion of quinolone therapy. The risks are highest in patients older than 60 years, patients on corticosteroids, and organ transplant recipients.[519–521] In a large patient database, fluoroquinolone use was associated with Achilles tendinopathy (odds ratio, 4.3) and rupture (odds ratio, 2.0).[522] In addition to age and steroid use, female sex and being nonobese were also risk factors. The mechanism of this toxicity is uncertain, but exposure of cultured tendon cells to ciprofloxacin has been associated with increases in expression of matrix metalloproteinases and cellular apoptosis.[523]

Associations with other potential connective tissue toxicities have been reported but cause-and-effect relationships have not been established. Recent large cohort studies show conflicting results with respect to an association of retinal detachment and fluoroquinolone use. In a study of patients who had visited an ophthalmologist in British Columbia, Canada, current use of fluoroquinolones was associated with a 4.5-fold higher risk of developing a retinal detachment, whereas no risk was observed among recent users and past users, and the absolute risk was low.[524] In a study from Taiwan, patients who received oral fluoroquinolones within the preceding 90 days had a twofold higher risk for rhegmatogenous retinal detachment versus amoxicillin users.[525] A larger nationwide, registry-based cohort study in Denmark, however, failed to find such an association; neither current nor recent or past fluoroquinolone use was associated with a significantly increased risk of retinal detachment.[526] In a propensity-adjusted, case-control study from Taiwan, fluoroquinolone use was associated with aortic aneurysm or dissection with an odds ratio of 2.43 for current use and 1.48 for use within 60 days prior.[527] Risk increased with age over 70 years and increased duration of exposure. A cohort study of Canadian patients over the age of 65 years found an adjusted hazards ratio of 2.24 for aortic aneurysm in patients receiving concurrent fluoroquinolones, with a substantially lower risk for amoxicillin.[528] Potential confounding and possible use triggered by symptoms of the condition itself add uncertainty as to any causal relationship with fluoroquinolone use.

Quinolones, to varying extents, can block the potassium channels and thereby delay repolarization in cardiac tissue, an effect that underlies their ability to prolong the QT interval on the electrocardiogram. Prolongation of the QT interval can predispose to ventricular arrhythmias such as torsades de pointes.[529] Older quinolones, sparfloxacin and grepafloxacin, which had QT interval prolongation greater than that of currently available quinolones, were removed from the market in part because of reports of unexpected cardiac events.[529] QT interval prolongation of a lesser magnitude was also found with moxifloxacin, and some increased risk of arrhythmias have been noted in some[530] but not all[531] population-based studies. Ciprofloxacin and levofloxacin have lesser effects on prolongation of the QT interval than moxifloxacin.[532–534] Delafloxacin appears to produce no QT interval prolongation. Additive effects on QT interval prolongation may occur when quinolones are given together with other agents that prolong the QT interval. Thus quinolones should be avoided or used with caution in patients also receiving class III (block potassium channel, e.g., amiodarone, sotalol) or class IA (block potassium and sodium channels, e.g., quinidine, procainamide) antiarrhythmics, or other agents (e.g., erythromycin) that prolong the QT interval. Risk is also potentially increased in the presence of cardiomyopathy, bradycardia, hypokalemia, and hypomagnesemia.[534,535]

Leukopenia and eosinophilia generally occur in less than 1% of patients, and mild elevations in serum transaminases occur in less than 1% to 3% of patients receiving quinolones; these abnormalities are rarely of sufficient severity to require cessation of therapy. An exception occurred with trovafloxacin, which was associated with elevated transaminases in approximately 10% of patients receiving a 4-week course for prostatitis. After release of trovafloxacin for clinical use, rare cases of idiosyncratic, symptomatic hepatitis (1:17,000 reporting incidence) were identified in postmarketing surveillance, some of which were associated with eosinophilic infiltrates and sufficiently severe to cause hepatic failure requiring liver transplantation.[498,536] These events led to the restrictions of trovafloxacin use and later to its withdrawal. Temafloxacin was found through postmarketing surveillance to be associated with rare cases of hemolytic anemia, thrombocytopenia, and renal failure at a reporting incidence of 1 in 5000; such occurrences led to its removal from the market.[537] These severe toxic effects of trovafloxacin and temafloxacin are of uncertain mechanism and have not been seen with currently available quinolones. In a population-based, case-control study of outpatients over age 65 years without a history of prior liver disease, moxifloxacin (adjusted odds ratio, 2.0) and levofloxacin (adjusted odds ratio, 1.85), but not ciprofloxacin, were associated with an increased risk of hospital admission for acute liver injury within 30 days of receiving a prescription relative to clarithromycin.[538]

Although there have been occasional reports of hypoglycemia associated with the use of ciprofloxacin, levofloxacin, and moxifloxacin, cases of severe hypoglycemia associated with use of gatifloxacin in diabetics on oral hypoglycemic agents and other patients have occurred.[539] In addition, hyperglycemia has been reported in elderly nondiabetics receiving gatifloxacin. Various quinolones have been shown to stimulate release of insulin from rat pancreatic islet cells. Although the frequency of dysglycemia appears to be low overall, the relative risk was greater with gatifloxacin than levofloxacin and ciprofloxacin.[540,541] Although systemic gatifloxacin has been withdrawn from the market, topical ophthalmic formulations are still available and have not been associated with dysglycemia.[542]

Safety in pregnancy[498] has not been established for any of the quinolones, but studies of babies born to women exposed to norfloxacin or ciprofloxacin during the first trimester identified no increase in major malformations, stillbirths, or premature births.[543–545] In one prospective case-control study comparing 200 women exposed to fluoroquinolones and 200 women exposed to known nonembryotoxic antibiotics, there were no differences in birth defects, spontaneous abortions, prematurity, or fetal distress, but there was a higher rate of therapeutic abortions, suggesting that concerns about teratogenic risks may exceed the actual risks.[544] Because quinolones can be excreted in breast milk, they should be avoided for nursing mothers.

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The complete reference list is available online at Expert Consult.

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36 Unique Antibacterial Agents

Gerard R. Barber

SHORT VIEW SUMMARY

- The US Food and Drug Administration (FDA) has granted each of several novel antibacterials a Qualified Infectious Disease Product (QIDP) designation. Whether their activity is chiefly directed against gram-negative or gram-positive pathogens, or both, each of these agents has been developed to combat multidrug-resistant organisms/pathogens (MDROs).

Plazomicin (Zemdri)

- A new aminoglycoside (AG)
- Binds to bacterial 30S ribosomal subunit, inhibiting protein synthesis
- Indicated for the treatment of complicated urinary tract infections (cUTI), including pyelonephritis
- Seemed to produce good outcomes and clearance of carbapenem-resistant Enterobacteriaceae (CRE) from bloodstream infections; larger trial numbers are needed
- Predominantly a gram-negative–active agent to MDROs, including CRE. As with other AGs, seems to exhibit synergy when paired with various other β-lactam agents
- Requires therapeutic drug monitoring, serum trough levels <3 μg/mL to avoid nephrotoxicity in treating cUTI
- More involved pharmacokinetics/pharmacodynamics (PK/PD) may be needed to determine optimal dosing for deeper infection
- Boxed warnings for nephrotoxicity, ototoxicity, neuromuscular blockade, and fetal harm

Cefiderocol

- Most advanced among "Trojan horse" antibacterials
- Siderophore (iron-binding) antipseudomonal catechol cephalosporin
- Solely gram-negative activity
- Broad spectrum over gram-negative MDROs across Ambler classes A to D of β-lactamases, demonstrates enhanced stability compared with cefepime, ceftazidime, and meropenem
- Question remains regarding which infectious indications will be pursued
- PK/PD modeling suggests doses of 2 g and 3 g via extended 2.5-hour infusions at every-8-hour intervals for optimization in subjects with normal renal function
- Dose reduction needed for diminished renal function

Meropenem-Vaborbactam (Vabomere)

- Combination antipseudomonal carbapenem/cyclic boronic acid β-lactamase inhibitor
- Broad gram-negative (including MDROs), gram-positive, and solid anaerobe coverage
- Labeled indication: (community-acquired) cUTI, including pyelonephritis
- Off-label use likely to include complicated intraabdominal infections (cIAIs), hospital-acquired bacterial pneumonia (HABP)/ventilator-associated bacterial pneumonia (VABP)
- Niche will be extended-spectrum β-lactamase (ESBL)/CRE infections, especially in penicillin-allergic patients
- Dose: 4 g intravenous piggyback (IVPB) every 8 hours via 3-hour infusion, requires dose and/or interval reduction with reduced renal function

Fosfomycin (Contepo)

- First epoxide antibacterial
- Oral form already approved in United States for single-dose lower tract UTI, IV form long approved outside United States
- Expected New Drug Application (NDA) initially for cUTI, potential for numerous other indications, given characteristics mentioned later
- Potent MDRO activity against gram-negative and gram-positive organisms and has demonstrated activity to ceftazidime-avibactam–resistant *Klebsiella pneumoniae* carbapenemases (KPCs)
- A late April 2019 FDA Complete Response Letter cited concerns for facility inspections, and manufacturing deficiencies could possibly delay potential drug approval date.
- Varied anaerobe activity
- Cross-sensitivity to other antibacterials not reported
- Good distribution into lung, bone, and cerebrospinal fluid
- Evidence of additive or synergistic properties when combined with other antimicrobial classes, such as β-lactams, carbapenems, lipoglycopeptides, AGs, polypeptides, and glycylcyclines
- For severe resistant infections, recommended to be given with other antibacterials and not as monotherapy
- 330 mg sodium per 1 g fosfomycin, so attention needed for electrolyte abnormalities, especially in fragile populations; can perhaps be useful in conjunction with amphotericin B in patients having fungal coinfection

Omadacycline (Nuzyra)

- An aminomethylcycline derived from tetracycline
- Active against pathogens possessing tetracycline resistance mechanisms
- IV and oral formulations advantageous for step-down therapy; one study showed extravasation rates 1.5-fold higher than comparator
- Epithelial lining fluid (ELF) and alveolar macrophage concentrations surpass serum concentrations
- Demonstrated activity against gram-negative, gram-positive, and atypical respiratory pathogens. Activity against other MDROs and selected sexually transmitted disease pathogens. No activity against *Pseudomonas* spp.
- One study demonstrating acceptable outcomes with conventional dosing in high body mass index subjects and diabetic subjects
- Does not require dose adjustment for renal dysfunction, including hemodialysis
- Should have a niche for stewardship and placement among penicillin-allergic or patients with intolerance to other antibacterial classes

Eravacycline (Xerava)

- Fully synthetic fluorocycline with resemblance to tetracycline reference structure
- Active against pathogens possessing tetracycline resistance mechanisms
- IV formulation only
- Indication: cIAI. Anticipate other off-label use on hospital floors, intensive care units, and outpatient infusion centers
- Broad gram-positive and gram-negative coverage, including MDROs. Very good activity against *Bacteroides fragilis* but variable non-*fragilis Bacteroides* activity
- Good atypical respiratory pathogen coverage and achievement of pulmonary concentrations but pneumonia indications not pursued
- Not active against *Pseudomonas* spp.
- Opportunities to spare carbapenems and quinolones for stewardship and intolerant patients, a potent agent with activity against ESBL-, KPC-, New Delhi metallo-β-lactamase (NDM)-, and oxacillinase (OXA)-producing *Klebsiella* spp.
- 1 mg/kg IVPB every-12-hour dosing, renal adjustment not necessary

Lefamulin

- First systemic pleuromutilin
- IV and oral formulations
- NDA for community-acquired bacterial pneumonia anticipated in fourth quarter of 2018

SHORT VIEW SUMMARY—cont'd

- Broad activity—gram-positive and gram-negative community respiratory pathogens, including *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*, as well as atypical bacteria: *Legionella pneumophila*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*
- Small study of healthy subjects suggests 5.7-fold higher ELF concentrations compared with serum, as well as good adipose and skeletal tissue penetration
- Acute bacterial skin and skin structure infection (ABSSSI) studies entering phase III
- Possesses potent in vitro activity against common gram-positive skin organisms, including *Staphylococcus aureus* (methicillin-sensitive *S. aureus* [MSSA] and methicillin-resistant *S. aureus* [MRSA]), coagulase-negative *Staphylococcus* spp., *Streptococcus agalactiae*, and *Streptococcus pyogenes*
- Good MRSA activity and skeletal penetration suggest a potential off-label role for prolonged step-down therapy (IV dosing is every 12 hours)
- Seemingly well tolerated
- Niche for stewardship and reducing selective pressures of quinolones, use in intolerant patients with opportunity for oral step-down therapies

Iclaprim

- Inhibits bacterial dihydrofolate reductase
- Bears some structural similarity to trimethoprim
- A gram-positive active agent demonstrating good activity against β-hemolytic streptococci, *Staphylococcus aureus* (MSSA and MRSA)
- QIDP status ABSSSI and HABP
- NDA for ABSSSI submitted; in February 2019 received an FDA Complete Response Letter citing concerns for hepatotoxicity and the need for additional patient data
- Iclaprim does have FDA orphan drug status for the treatment of *S. aureus* pulmonary infections in patients with cystic fibrosis
- IV formulation only
- Would not require renal dose adjustment
- Older studies using weight-based dosing seemed to show an association of cardiac toxicity associated with higher serum concentrations; for ABSSSI, acceptable serum concentrations obtained used a fixed dosing regimen of 80 mg/kg IV every 12 hours

Antimicrobial resistance long preceded the advent of the penicillin era. This "modern antibiotic age" of antibacterials brought enormous and immediate improvements to global public health. Still, the declaration that infectious diseases were forever cured was short lived, as briefly after the introduction of penicillin was the arrival of penicillin-resistant *Staphylococcus aureus* noted in the hospital environment. Today's rapid and welcome technologic advances have created faster and economical modes of travel, capable computers, and electronic data transfer. Regarding medicine, these advances serve to enable increasingly aggressive medical therapies and diagnostic tools. However, an Infectious Diseases Society of America (IDSA) report in 2009 showed that within the pharmaceutical industry, only three new antibacterial compounds were in advanced stages of clinical development.[1] Until about 2010 drug manufacture for the previous 3 decades shifted away from the creation of "unique antibacterial" agents having novel mechanisms to favor medication development for chronic diseases, oncology, and niche diagnoses. Thus the current challenges of the emergence and distribution of multidrug-resistant organisms (MDROs) pose a significant burden not only to health care institutions but the community as well.

The Generating Antibiotic Incentives Now (GAIN) provisions and US Food and Drug Administration (FDA) stance (Table 36.1) augmented the activism of organizations such as IDSA against the drought in the antibiotic pipeline. In 2010 the IDSA's 10 × '20 Initiative[2] was a call to develop 10 new safe and effective antibacterials by the year 2020. A good deal of the most pressing, current MDROs include methicillin-resistant *S. aureus* (MRSA); penicillin-resistant *Streptococcus pneumoniae*; vancomycin-resistant enterococci; fluoroquinolone-, extended spectrum cephalosporin-, and recently, carbapenem-resistant *Klebsiella pneumoniae*; *Escherichia coli*; and *Pseudomonas* spp. All of these and other clinically relevant gram-negative bacteria[3] are listed among the GAIN provisions' "qualifying pathogens" posing significant threat to public health (see Table 36.1).

Thus there is an urgent need to develop antibacterials that preferably are bactericidal in action and have a high therapeutic index, a targeted spectrum of antimicrobial activity that limits perturbation of the normal host microbiota, and a low potential for promoting drug resistance.[4] In this chapter are summaries of "novel antibacterials," most in later stages of development, with potential for future clinical utility.

A summary in the order in which this chapter's novel antibacterials are listed can be seen in Table 36.2.

TABLE 36.1 The GAIN Provisions

In July 2012 Generating Antibiotic Incentives Now (GAIN) was signed into US law. GAIN provisions are part of the US Food and Drug Administration (FDA) Safety and Innovation Act. The intent of GAIN was to make antibacterial and antifungal antibiotics a more valuable commodity of drug class to pharmaceutical manufacturers.

Brief points about GAIN provisions:

- Allows, by law, a designation of Qualified Infectious Disease Product (QIDP) status to antibacterial or antifungal drugs developed for humans with the intention of treating serious or life-threatening infections;
- QIDP status granted to a new antimicrobial status effects a 5-year market protection or exclusivity to the manufacturer that distances competition from generic manufacturers. This QIDP 5-year protection would be in addition to any other market exclusivities already granted by law;
- QIDP-designated antimicrobials receive FDA fast-track and priority review status and are also granted an expedited FDA-approval process;
- The FDA will compile and at minimum, every 5 years update a list of "qualifying" pathogens that pose significant risk to public health (see link later);
- The FDA will review and update antibiotic guidance from a regulatory and scientific perspective and issue newly developed guidance on pathogen-focused antibiotics;
- The FDA will draft preliminary guidance for manufacturers seeking to develop antibiotics addressing unmet needs, including agents that are narrowly targeted or pathogen focused.

Dalbavancin (Dalvance) was the first antibacterial agent to receive QIDP status and ceftolozane-tazobactam (Zerbaxa) the first antibacterial with gram-negative activity to receive the designation. Every agent in this current chapter and each antibacterial discussed in the full text of Chapter 35, 8th edition, *Principles and Practice of Infectious Disease*, has been granted QIDP status (with the exception of LFF571, which has been deleted from Novartis development).

Federal Register. Establishing a List of Qualifying Pathogens Under the Food and Drug Administration Safety and Innovation Act. https://www.federalregister.gov/documents/2014/06/05/2014-13023/establishing-a-list-of-qualifying-pathogens-under-the-food-and-drug-administration-safety-and. Accessed February 27, 2018.

MULTIDRUG-RESISTANT BACTERIA

Plazomicin (ACHN-490)

Plazomicin (Zemdri; Achaogen, San Francisco) is a next-generation AG derived from the AG sisomicin, which was the parent compound from which 400 derivatives were evaluated.[5] A New Drug Application (NDA) had been filed for plazomicin for the treatment of complicated urinary tract infections (cUTIs), including pyelonephritis and bloodstream infections (BSIs) due to certain Enterobacteriaceae in patients who have limited or no alternative treatment options. However, this "neoglycoside" with Qualified Infectious Disease Product (QIDP) status instead received FDA approval in late June 2018 only for the treatment of cUTIs, including pyelonephritis caused by certain Enterobacteriaceae in patients who have limited or no alternative treatment options.

The increasing frequency of gram-negative MDROs has renewed interest in the utility of the AG class, long ignored with the arrival of agents such as tigecycline, linezolid (LZD), niche β-lactams, and carbapenems, and also because AG-induced nephrotoxicity and ototoxicity are potentially serious concerns. Development of drug resistance to tobramycin and amikacin among clinical gram-negative isolates has been less often observed when compared with advanced-generation cephalosporins, monobactams, and carbapenems. This combined with improved knowledge of pharmacokinetics/pharmacodynamics (PK/PD) of AGs based on monitoring of serum levels has led to markedly improved ototoxicity profiles and their use in combination with β-lactam agents.[6,7]

TABLE 36.2 Summary of Activities of Novel Antibacterial Agents

DRUG NAME	DRUG CLASS	TARGET	ACTIVITY AGAINST RESISTANT GRAM-NEGATIVE PATHOGENS	ACTIVITY AGAINST RESISTANT GRAM-POSITIVE PATHOGENS	INDICATIONS (1 = FDA-LABELED INDICATION) (2 = ANTICIPATED NDA FILING) (3 = ANTICIPATED OFF-LABEL USE)
Plazomicin (Zemdri)	Aminoglycoside	30S ribosomal subunit	Yes	No	**1.** cUTI (adults), including pyelonephritis, caused by certain Enterobacteriaceae in patients who have limited or no alternative treatment options **2, 3.** Deeper-seated infections, such as bloodstream infections, HABPs, VABPs, and cIAIs caused by certain Enterobacteriaceae in patients who have limited or no alternative treatment options
Cefiderocol	Siderophore Cephalosporin	PBP	Yes	No	**2, 3.** Health care–associated and hospital-acquired bacterial pneumonias, VABPs, and carbapenem-resistant organisms at various sites
Meropenem-vaborbactam (Vabomere)	β-Lactam (carbapenem) + β-lactamase inhibitor	PBP; β-lactamase	Yes	No	**1.** cUTIs, including pyelonephritis in adults **2, 3.** cIAIs, HABPs and VABPs with suspicion of MDROs
Fosfomycin (Contepo)	Phosphonic acid derivative	Phosphoenolpyruvate synthetase	Yes	Yes	**2, 3.** cUTI, including pyelonephritis; cIAIs, HABPs, and VABPs; added to existing therapies for demonstrated MDRO infections
Omadacycline	Aminomethylcycline	30S ribosomal subunit	Yes	Yes	**2.** CABP, ABSSSIs, possibly uncomplicated UTIs and cUTIs if trials are pursued
Eravacycline (Xerava)	Fluorocycline	30S ribosomal subunit	Yes	Yes	**1.** cIAIs
Lefamulin	Pleuromutilin	50S ribosomal subunit	No	Yes	**1.** ABSSSIs **2.** CABP
Iclaprim	Diaminopyrimidine	Dihydrofolate reductase	No	Yes	**2.** ABSSSIs **2, 3.** Various bacterial pneumonias (CABP, HABP, VABP)

ABSSSIs, Acute bacterial skin and skin structure infections; *CABP,* community-acquired bacterial pneumonia; *cIAIs,* complicated intraabdominal infections; *cUTI,* complicated urinary tract infection; *HABP,* hospital-acquired bacterial pneumonia; *MDRO,* multidrug-resistant organism; *NDA,* New Drug Application; *PBP,* penicillin-binding protein; *VABP,* ventilator-associated bacterial pneumonia.
Modified from The Pew Charitable Trusts at http://www.pewtrusts.org/-/media/assets/2018/03/antibiotics_clinical_dev_table_february2018.pdf.

A second phase I study, among eight subjects, age 18 to 65 years (the earlier phase I study was among 39 adults), examined the highest dose from the first study of this concentration-dependent agent at 15 mg/kg given once daily for a shorter duration of 5 days, attempting to mimic the same cumulative exposures from the earlier study. In these healthy subjects it appears that the area under the concentration-time curve (AUC) and maximum serum concentration (C_{max}) of plazomicin increase linearly and proportionately in accordance with increasing doses, without accumulation when given in a once-daily regimen; conversely, elimination of the drug is independent of plazomicin dose. Similar to gentamicin, tobramycin, and particularly amikacin, the plasma protein binding of ACHN-490 is relatively low at 16% (±5), with plasma AUC values high relative to potential pathogens. The investigators extrapolate that in *E. coli* having minimal inhibitory concentrations (MICs) of ≤1 μg/mL and *K. pneumoniae* with MICs of ≤0.5 μg/mL, AUC/MIC target attainment of 80 and 160, respectively, should be achievable at a once-daily dose of 7 mg/kg.[8] Renal safety was assessed with estimated creatinine clearance using the Cockcroft-Gault formula. Ototoxicity was measured by pure tone audiometry with bone conduction and otoacoustic emission at baseline, the end of treatment, and at 3 and 6 months posttreatment. Electronystagmography tested vestibular function at the same time intervals. ACHN-490 was well tolerated. Headache, tinnitus, dizziness, and somnolence of mild-to-moderate severity were the most commonly encountered adverse events (AEs), all being transient and rapidly resolving. Tinnitus had occurred in two patients at higher dose ranges, with both subjects retaining normal cochlear and vestibular function.

A phase II multinational study compared plazomicin (10 mg/kg or 15 mg/kg) to IV levofloxacin, 750 mg each once daily for 5 days, in the treatment of cUTI and pyelonephritis. Randomized at a 2:1 ratio, plazomicin to levofloxacin, a total of 145 subjects were enrolled (22, 76, and 47 subjects to plazomicin 10 mg/kg, plazomicin 15 mg/kg, and levofloxacin 750 mg, respectively); median age was 39 and 43 years, respectively, with *E. coli* ($n = 69$) the most common pathogen among Enterobacteriaceae ($n = 84$). Serious adverse events occurred in 1 patient receiving plazomicin deemed unrelated, and 2 levofloxacin study patients (1 related, 1 unrelated). Unilateral, permanent tinnitus was reported for 1 plazomicin patient dosed at 15 mg/kg. Mild increases in serum creatinine (SCr) deemed transient, but clinically relevant, occurred in 5 plazomicin subjects and 1 receiving levofloxacin. However, 1 patient receiving plazomicin did not have resolution of their rising SCr at late follow-up (LFU). In the modified intent-to-treat population, microbiologic eradication rates (MBE) at test-of-cure (TOC) for plazomicin 15 mg/kg and levofloxacin were each approximately 59%, whereas the rate of MBE for plazomicin 10 mg/kg was 50%. Microbiologic eradication rates in the microbiologically evaluable population at TOC were 85.7%, 88.6%, and 81% for plazomicin 10 mg/kg, plazomicin 15 mg/kg, and levofloxacin 750 mg, respectively. Investigators concluded that rates of MBE at TOC were similar across all arms, although rates of microbiologic recurrence were higher among subjects receiving levofloxacin.[9]

Plazomicin demonstrated greater activity against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates having aminoglycoside-modifying enzymes (AGME) than isolates with altered permeability/efflux. From 16 New York City–area hospitals, 407 *A. baumannii* and 679 *P. aeruginosa* isolates from 2009 were analyzed, comparing ACHN-490 to gentamicin, tobramycin, and amikacin. Against *A. baumannii* isolates, the MIC_{90} of ACHN-490 was 16 μg/mL compared with >64 μg/mL for gentamicin, tobramycin, and amikacin, respectively. Against *P. aeruginosa* isolates, the ACHN-490 MIC was 32 μg/mL, compared with >64, 64, and 16 μg/mL of gentamicin, tobramycin, and amikacin, respectively.[10] Breakpoints for Zemdri are not yet established.

A synergy study reviewed the activity of ACHN-490 alone and when combined with either cefepime, doripenem, imipenem-cilastatin, or piperacillin-tazobactam against 25 clinical isolates from the United States and Poland of *P. aeruginosa* from blood, urine, wounds, and the respiratory tract. Four strains exhibited resistant AGME phenotypes, with 3 having *ant*(2")-Ia and another *acc*(6')-Ib.[11] Investigators found identical MICs to plazomicin for susceptible or resistant phenotypes. Synergy was defined as a ≥2-$\log_{10}$ decrease in colony-forming units (CFUs)/mL 24 hours after exposure to the drug combination versus active drug alone. At 24 hours individual MIC ranges were plazomicin, 0.5 to 256 μg/mL; cefepime, 1 to 256 μg/mL; doripenem, 0.12 to 256 μg/mL; imipenem-cilastatin, 0.25 to 512 μg/mL; and piperacillin-tazobactam, 2 to 4096 μg/mL. At 24 hours plazomicin with each of the four agents demonstrated synergy against most strains, imipenem-cilastatin/plazomicin showing the least synergy at 68% of isolates, whereas the piperacillin-tazobactam/plazomicin combination was greatest at 92%. Antagonism was not seen among any of the four combinations. It seems clinical validation assessing the efficacy of plazomicin plus piperacillin-tazobactam is warranted for *P. aeruginosa* and other MDROs.

Among 493 clinical blood and nasal isolates of MRSA collected from 23 US hospitals in 2009 and 2010, plazomicin demonstrated greater activity than either tobramycin or amikacin but similar activity to gentamicin.[12] Unlike gram-negative bacteria, only three AGME are produced by *S. aureus* isolates that may influence variable activity of the AGs.

In addition, the Biomedical Advanced Research and Development Authority has granted funds to the manufacturer of ACHN-490 to investigate plazomicin as potential treatment for *Yersinia pestis* and *Francisella tularensis* biowarfare.[13]

The phase III CARE (Combating Antibiotic Resistant Enterobacteriaceae) trial was an open-label, multicenter, pathogen-specific study comparing plazomicin with colistin in patients with serious carbapenem-resistant Enterobacteriaceae (CRE) infection. A total of 69 patients were divided into two cohorts. In cohort 1 ($n = 39$), 29 of 30 patients with a BSI were confirmed to have CRE infection. Subjects were randomized to plazomicin, 15 mg/kg intravenous piggyback (IVPB) daily, or colistin, 300 mg IVPB load, followed by 5 mg/kg/day, each arm also receiving either high-dose, extended-infusion meropenem or tigecycline for BSI or hospital-acquired bacterial pneumonia (HABP)/ventilator-associated bacterial pneumonia (VABP) infections. Plazomicin, colistin, and meropenem were dose adjusted according to renal function. Cohort 2 enrolled 30 subjects having BSI, HABP/VABP, or cUTI with or without acute pyelonephritis (AP). In this observational arm there were 15 BSI, with 14 confirmed due to CRE. These subjects were generally less acute than those in cohort 1, but several did have concomitant infection with *P. aeruginosa* or *Acinetobacter* spp., which qualified as exclusion criteria in the randomized cohort. Both cohorts received 7 to 14 days IVPB study drug for BSI and HABP/VABP, whereas for cUTI/AP patients in cohort 2, the protocol-specified treatment was 4 to 7 days of IVPB plazomicin, followed by optional oral therapy for 7 to 14 days of total therapy. Follow-up included a 7-day test of cure (TOC; from the last dose of IVPB study drug), 14-day end of study, and 60-day LFU.

Clearance of CRE bacteremia by study day 5 was observed in 12 of 14 (85.7%) plazomicin-based subjects compared with 7 of 15 (46.7%) subjects receiving colistin-based therapy. Persistence of CRE bacteremia more than 5 days after initiation of the study drug was found to be a significant disease-related complication associated with higher rates of 28-day all-cause mortality and also death at 60 days, both of which were higher in the colistin arm. Safety profiles also favored plazomicin with regard to serious adverse events related to the study drug among the randomized cohort, 1 of 14 (7.1%) to 4 of 16 (25%), plazomicin to colistin, respectively; no serious adverse events related to plazomicin were observed in observational cohort 2. Specifically, regarding renal function, postbaseline rise of ≥0.5 mg/dL SCr and overall magnitude of increase of SCr were greater among the colistin-treated subjects. Among plazomicin-treated subjects there was a higher incidence ($n = 6$) of SCr elevations >0.5 mg/dL; 4 subjects were <1 mg/dL and 2 were <1.5 mg/dL. Instances of ototoxicity or death related to the study drug were not noted in either cohort.[14]

In a multinational, double-blinded study termed "EPIC" (European Prospective Investigation into Cancer and Nutrition), among 609 randomized subjects, plazomicin (15 mg/kg IVPB daily) was compared with meropenem (1 g IVPB every 8 hours) for the treatment of cUTI or AP for 7 to 10 days duration. After 4 days of IVPB therapy, transition to oral levofloxacin in either arm was permitted. All drugs were dose adjusted per renal function. A primary efficacy end point was a composite of microbiologic eradication (baseline uropathogen $\geq 10^6$ CFUs/mL reduced to $<10^4$ CFUs/mL) in microbiologically modified intent-to-treat subjects and clinical cure at study day 5 and TOC (approximately day 17 after study initiation). Nearly 64% of the study population ($n = 388$) had a uropathogen susceptible to plazomicin and meropenem. Day 5 composite cure rates were similar: 88% to 91.4%, plazomicin to meropenem, respectively, whereas the composite cure at TOC demonstrated statistical superiority for plazomicin, 81.7% (156/191) to 70.1% (138/197) meropenem. A LFU visit at approximately day 28 also favored plazomicin over meropenem, 77% to 60.4%, respectively. Ototoxicity was observed in 1 subject in each arm, both with complete resolution. Rates of SCr increases ±0.5 mg/dL during IV therapy were similar (3.7% and 3%) among plazomicin and meropenem subjects, respectively, but among plazomicin subjects it was reported that in 2 of 11 subjects SCr had not returned to baseline by the LFU visit.

Also from the EPIC study, regarding MDRO and DROs, in a subgroup of microbiologically modified intent-to-treat patients with an extended spectrum β-lactamase (ESBL)-positive phenotype (MIC >2 μg/mL for aztreonam, ceftazidime, or ceftriaxone), microbiologic eradication was achieved in 42 of 51 (82.4%) and 45 of 60 (75.0%) for plazomicin and meropenem, respectively. Another subgroup of microbiologically modified intent-to-treat patients with AG-nonsusceptible pathogens (nonsusceptibility to amikacin, gentamicin, or tobramycin per 2016 Clinical and Laboratory Standards Institute [CLSI] criteria), microbiologic eradication was achieved in 41 of 52 (78.8%) and 35 of 51 (68.6%) for plazomicin and meropenem, respectively.[15,16]

At the LFU visit (day 28 ± 4 days), 147 of 191 (77.0%) of the plazomicin-treated patients and 119 of 197 (60.4%) meropenem-treated patients achieved composite cure. Clinical cure was similar between the plazomicin and meropenem treatment groups, 169 of 191 (88.5%) and 168 of 197 (85.3%), respectively. Microbiologic eradication was achieved in 161 of 191 (84.3%) and 128 of 197 (65.0%) of plazomicin and meropenem patients, respectively.

In conclusion, Zemdri is a recently approved AG with potent activity against MDR gram-negative pathogens, including CRE. As with other AGs, it is expected that plazomicin will demonstrate synergistic activity when combined with certain other antibacterials. A good understanding of resistance mechanisms most prevalent among an institution's pathogens should be helpful in directing empirical therapy of plazomicin for cUTI and other critical off-label uses. Despite good outcomes and some important lessons learned regarding time to clearance of CRE from blood in the small CARE trial, the FDA issued a complete response letter and denied the drug's approval for deeper-seated MDRO infections. Still, a niche for plazomicin utilization to combat CRE will be as a colistin-sparing agent. For cUTIs Zemdri will require dose adjustment in patients with diminished renal function, and the labeled dosing schema differs somewhat from the other concentration-dependent killing AGs and includes recommendations for therapeutic drug monitoring of serum plazomicin trough levels <3 μg/mL in patients having an estimated creatinine clearance of 15 to 89 mL/min. Zemdri labeling also includes black box warnings for nephrotoxicity, ototoxicity, neuromuscular blockade, and fetal harm.

Cefiderocol (S-649266)

Cefiderocol (Shionogi & Co, Osaka, Japan) is in the most advanced stages of trials among the siderophore, or iron-binding, antibacterials. It is an antipseudomonal catechol-cephalosporin with a novel, "Trojan horse," mechanism. Cefiderocol binds to ferric iron and is actively transported into bacterial cells through the outer cell membrane via bacterial iron transporters whose chief function is to deliver necessary iron nutrients to the bacterial cells (Fig. 36.1).

A small study of S-649266's stability to clinical isolates with β-lactamases, including metallo-β-lactamases (MBLs) and class A and D carbapenemases, showed impressive results, including withstanding hydrolysis by *Klebsiella pneumoniae* carbapenemases (KPCs), MP-1,

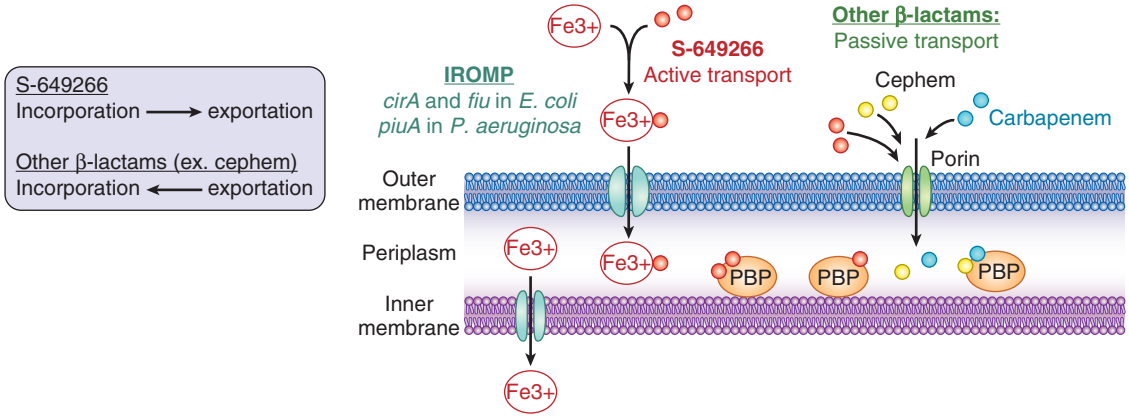

FIG. 36.1 Cefiderocol (S-649266); among Trojan horse antibacterials: a novel way to circumvent gram-negative bacterial resistance. *IROMP,* Iron-regulated outer membrane protein; *PBP,* penicillin-binding protein. *(From Tillotson G. Trojan horse antibiotics: a novel way to circumvent gram-negative bacterial resistance?* Infect Dis (Auckl). *2016;9:45–52.)*

Verona integron-coded metallo-β-lactamase 2 (VIM)-2, L1, and New Delhi metallo-β-lactamase 1 (NDM-1) when compared with cefepime, ceftazidime, and meropenem.[17]

In vitro studies of S-649266 activity against clinical isolates of nonfermenting gram-negative bacteria and Enterobacteriaceae seem to demonstrate the drug's potent activity. Compared with cefepime, meropenem, piperacillin-tazobactam, and levofloxacin against global isolates of *A. baumannii* ($n = 104$), *P. aeruginosa* ($n = 104$), and *Stenotrophomonas maltophila* ($n = 108$), S-649266 exhibited a demonstrably lower range of MICs and MIC_{90} for all isolates than its comparators. For *P. aeruginosa*, β-lactamase–producing strains ($n = 33$) were predominantly VIM and IMP. Composite $MIC_{50/90}$ for S-649266 was 0.5 and 4 μg/mL, respectively, compared with $MIC_{50/90}$ of >64 μg/mL (cefepime), >32 μg/mL (meropenem), and 128 μg/mL and >256 μg/mL (piperacillin-tazobactam).[18] Using a cation-adjusted Mueller-Hinton broth for the siderophore versus CLSI breakpoints for comparators, S-649266 activity was assessed among 850 global isolates of *E. coli, K. pneumoniae, Serratia marcescens, Citrobacter freundii, Enterobacter aerogenes,* and *Enterobacter cloacae*. In a first set of 617 isolates, containing 233 β-lactamase producers, MICs were ≤1 μg/mL, except for 8 isolates having an MIC of ≥8 μg/mL. A second set, including 116 KPC and class B metallo-carbapenemase producers, showed MICs of ≤4 μg/mL for all except 7 isolates, which showed MICs of ≥16 μg/mL. These high-MIC isolates left investigators uncertain but believing reduced porin influx due to deficient iron transport may be responsible.[19] Among 1221 global isolates, including 679 varied oxacillinase (OXA)-producers of meropenem, nonsusceptible Enterobacteriaceae, *P. aeruginosa,* and *A. baumannii* demonstrated cefiderocol having generally lower MICs compared with ceftazidime, ceftolozane-tazobactam, and colistin.[20]

The completed (late 2016) APEKs-cUTI phase III trial was an international multicenter double-blinded comparison of subjects randomized 2:1 cefiderocol, 2 g IVPB every 8 hours, versus imipenem-cilastatin, 1 g IVPB every 8 hours. Both antibacterials were infused over a 1-hour period in a trial of 448 subjects with cUTIs with or without pyelonephritis due to gram-negative bacteria. A median duration of therapy was 9 hospital days among each arm; switch to oral therapy was not permitted. A TOC visit approximately 7 days after the completion of therapy showed cefiderocol treatment noninferior to imipenem-cilastatin meeting composite end points of clinical cure and microbiologic eradication. Among the 371 modified intent-to-treat subjects, these rates were 72.6% (183/252) for cefiderocol to 54.6% (65/119) for imipenem-cilastatin. The FDA composite end points and per patient microbiologic response at TOC both demonstrated statistical superiority as well. Investigators reported a lower number of cefiderocol-related adverse reactions than for its comparator.[21]

One-gram to 2-g doses of cefiderocol exhibit linear PK characteristics. As 60% to 70% of the drug is eliminated by the kidneys, renal dose adjustments will be needed for patients having diminished renal function, as well as supplemental dosing for hemodialysis patients. With a terminal half-life of approximately 2.5 hours, every-8-hours dosing appears appropriate for normal function. Monte Carlo modeling suggests that a 3-hour infusion will optimize bactericidal effect and that perhaps every-6-hours dosing should be considered in subjects having augmented renal function.[22]

As of early 2018, other phase III trials of cefiderocol were ongoing. One, named APEK-NP, is a multicenter double-blind comparison to meropenem, each with adjunctive LZD, for health care–associated bacterial pneumonia, HABP, and VABP, anticipating enrollment of 300 subjects with completion in early 2019 (ACT 03032380).[23] Another, CREDIBLE-CR, is an open-label multicenter investigation of SH-689266 to "best available therapy" (BAT) for the treatment of carbapenem-resistant organisms causing infection at various sites, including pneumonias, cUTI, sepsis, and BSIs, expecting about 150 subjects (ACT 02714595).[24]

In all, the novel mechanism enhancing the penetrability of this siderophore-cephalosporin, as well as demonstrable stability to β-lactamases, presents a gram-negative antibacterial with potent and very broad activity. Should it receive FDA or European Medicines Agency (EMA) approval based on efficacy and safety results, it would be a welcome addition as treatment against drug-resistant gram-negative pathogens, including *Stenotrophomonas* spp.

In conclusion, cefiderocol is an iron-binding, antipseudomonal, catechol-cephalosporin with broad activity against gram-negative aerobic bacteria. It is largely devoid of activity against gram-positive and anaerobic organisms that do not display an affinity for ferric iron uptake. Similar to ceftazidime, cefiderocol displays a decided affinity for penicillin-binding protein 3 among nonfermenting gram-negatives, such as *P. aeruginosa* and *A. baumannii* as well as *Enterobacteriaceae*. However, cefiderocol's potent gram-negative activity thus far demonstrates the ability to withstand resistance mechanisms among CRE, KPC, and MBL-producing organisms. The drug seems to be well tolerated; whether or not the variability of human subject physiology of ferric iron stores and uptake mechanisms will influence outcomes of clinical cure in infection caused by questionably susceptible pathogens remains a subject of debate. Further question remains regarding the submission of which infectious indications are to be pursued by the manufacturer.

Meropenem-Vaborbactam (Vabomere)

Vaborbactam (Melinta Therapeutics, New Haven, CT), formerly known as RPX7009 and developed by Rempex Pharmaceuticals, is classed as a cyclic boronic acid β-lactamase inhibitor (BLI), which is a subclass of serine BLIs. Boronic acids have been studied as inhibitors of serine proteases, such as the serine carbapenemases that can be produced by strains of KPC. Vaborbactam is the second non–β-lactam BLI receiving FDA approval after NXL104 (avibactam) in combination with ceftazidime. Earlier, as RPX7009, it had been tested in combination with biapenem, a carbapenem approved in Japan and studied in complicated intra-abdominal infections (cIAIs).[25–27]

In the United States vaborbactam was mated to the antipseudomonal carbapenem, meropenem, and studied in two phase III clinical trials coined TANGO 1 and TANGO 2.[28] TANGO was the acronym for *T*argeting *A*ntibiotic *N*onsusceptible *G*ram-Negative *O*rganisms. According to the GAIN provisions, meropenem-vaborbactam is an example of a pathogen-focused antibacterial.

TANGO 1 was a multicenter, randomized, double-blind, double-dummy study of adult subjects with cUTI, including AP. There were 550 randomly assigned (1:1) subjects receiving either meropenem-vaborbactam (4 g [as meropenem 2 g plus vaborbactam 2 g] IVPB as 3-hour infusion) or piperacillin-tazobactam (4.5 g IVPB as 30-minutes infusion) each 8 hours for up to 10 days. Subjects meeting defined protocol criteria for improvement could be switched to oral levofloxacin after 5 days of parenteral therapy. Patients were well matched regarding demographics, cUTI strata (AP, cUTI with or without removable source of infection), and among microbiologically modified intent-to-treat subjects ($n = 374$), gram-negative pathogen. The mean duration of IV therapy among both arms was 8 days. With FDA-defined end points, meropenem-vaborbactam was deemed noninferior to piperacillin-tazobactam, actually achieving superiority for clinical cure or improvement and microbiologic eradication ($<10^4$ CFUs/mL) at end of IV therapy visit, 98.4% (183/186 subjects) to 94.3% (165/175 subjects), respectively. Among 12 microbiologically modified intent-to-treat subjects treated with meropenem-vaborbactam having concurrent bacteremia at baseline, clinical and microbiologic response was successful in 10 subjects (83%). For meropenem-vaborbactam, higher rates of transaminitis, headache, and phlebitis were observed, although rates of discontinuation due to study drug AEs were lower than that of piperacillin-tazobactam, 2.9% versus 5.1%, respectively.

TANGO 2 was an open-label, multicenter study across 27 sites in nine countries of meropenem-vaborbactam compared with BAT, randomized 2:1. Chosen clinical sites were known to have a high incidence of KPC-producing CRE, and eligible adult subjects had a confirmed diagnosis of serious infection, such as bacteremia and/or HABP/VABP, cIAI, or cUTI, and expected to receive IV antimicrobial therapy ≥7 to 14 days. Duration of therapy could be extended beyond 14 days, and mean duration was approximately 29 days. Enrollment also allowed patients with impaired renal function, including subjects on hemodialysis.[29]

Meropenem-vaborbactam 4 g (as meropenem 2 g plus vaborbactam 2 g) was given IVPB over 3 hours every 8 hours; BAT was defined as IV antibacterials either alone or in combination: carbapenems (imipenem, meropenem, ertapenem), AGs (gentamicin, tobramycin, amikacin), polypeptides (colistin, polymyxin B), tigecycline, or monotherapy with ceftazidime-avibactam.

Primary efficacy end points differed by infection but included the proportion of patients with a clinical outcome of cure at end of therapy (EOT) and TOC based on assessment of signs and symptoms across all infection types. Also included was all-cause mortality at day 28 across all infection types. The modified carbapenem-resistant Enterobacteriaceae (mCRE) modified intent-to-treat subpopulation ($n = 43$) were subjects meeting the microbiologically modified intent-to-treat criteria and having confirmed meropenem-resistant Enterobacteriaceae at baseline. *K. pneumoniae* accounted for 86% of baseline pathogens. These mCRE–modified intent-to-treat subjects, with 28 in the meropenem-vaborbactam arm and 15 in BAT, were considered as a primary efficacy population.

By all indications, clinical cure at EOT (64.3%–33.3%) and TOC (57.1%–26.7%) favored meropenem-vaborbactam over BAT, respectively, both with statistical significance. Microbiologic cure, evaluated across all infection types of confirmed CRE infection at EOT and TOC visits, also favored meropenem-vaborbactam (63.3% and 50%, respectively) compared with BAT (40% and 33.3%, respectively). Also, across all infection types, all-cause 28-day mortality was higher among the BAT arm compared with meropenem-vaborbactam, as were sepsis-related deaths, numbering 4 to 1, respectively. Renal-related AEs and proportions of subjects with increases in SCr of ≥0.5 mg/dL also favored meropenem-vaborbactam.

In another subset of patients from TANGO 2, 19 subjects deemed immunocompromised (4 leukemia/lymphoma, 5 medication, 10 transplant), 18 of whom were confirmed CRE, clinical cure rates at EOT and TOC, as well as 28-day all-cause mortality, favored meropenem-vaborbactam therapy over BAT.[30]

In conclusion, in August 2017 the FDA granted approval of meropenem-vaborbactam for cUTIs, including pyelonephritis. FDA-recognized antimicrobial susceptibility test interpretive criteria (STIC) for Enterobacteriaceae are (1) Susceptible ≤4/8 μg/mL; (2) Intermediate 8/8 μg/mL; and (3) Resistant ≥16/8 μg/mL. As with meropenem alone, meropenem-vaborbactam will require renal dose adjustment. With favorable disposition for patients having severe penicillin allergy and its broad spectrum of activity, including gram-negative MDROs and solid antianaerobic coverage, meropenem-vaborbactam as monotherapy or combined with other antibacterials will likely be used for numerous off-label indications in intensive care unit (ICU) settings and among other seriously ill patients.

Fosfomycin (ZTI-01, Contepo): Intravenous

Fosfomycin (Nabriva Therapeutics, Dublin, Ireland [formerly Zolyd, Zavante Therapeutics, San Diego, CA]) is the first of the epoxide class of antimicrobials. Originally named phosphonomycin, it is a broad-spectrum bactericidal agent active against resistant gram-negative and gram-positive microorganisms. Through inhibition of phosphoenol-pyruvate synthetase, fosfomycin blocks formation of *N*-acetylmuramic acid, ultimately interfering with early cell wall synthesis.[31,32] Discovered in Spain in the late 1960s, IV and oral formulations have been in clinical use for decades outside the United States, with the oral form approved in the United States since 1996 for uncomplicated UTIs. With phase III trials (ZEUS) completed for cUTI,[33] including AP, ZTI-01 has also received FDA QIDP status for numerous, current late-phase I trials, including cIAI, HABP, VABP, and acute bacterial skin and skin-structure infections (ABSSSI).

The ZEUS phase III trials were multinational, randomized, double-blinded studies among 465 hospitalized subjects with cUTI or AP. Comparing 6 g of ZTI-01 to 4.5 g of piperacillin-tazobactam, each administered every 8 hours as 1-hour infusions, the antibiotics were given for a 7-day duration, although up to 14 days of therapy was permissible in patients with a concurrent bacteremia. Transition to oral therapy was not allowed. ZTI-01 met its noninferiority objective compared with piperacillin-tazobactam, having similar rates of overall success (64.7% vs. 54.5%, respectively), clinical cure at TOC (day 19, 90.8% vs. 91.6%, respectively), and treatment-emergent adverse events (TEAEs). Most TEAEs to ZTI-01 were reported to be mild and with transient gastrointestinal (GI) effects not necessitating discontinuation of study drug. No deaths occurred in the study, and serious AEs were reportedly uncommon (five, ZTI-01 vs. six, piperacillin-tazobactam). Within ZEUS, among the microbiologically modified intent-to-treat cases, 34% of subjects exhibited isolates from urine or blood by MIC indicative of phenotypic resistance for ESBL (31%, ≥2 μg/mL aztreonam, ceftazidime, or ceftriaxone), CRE (6.1%, ≥4 μg/mL imipenem or meropenem), Amino-R (17%, gentamicin or amikacin), or MDR (19%, nonsusceptibility ≥3 classes of antimicrobials). The numbers of resistant isolates to each arm were balanced and clinical cure rates high, but eradication rates were notably higher in the ZTI-01 arm.[34]

The SENTRY surveillance program in 2015 collected a total of 2200 gram-negative and gram-positive isolates from US medical centers. Using established FDA breakpoints for oral fosfomycin tromethamine, the program examined the drug's activity against a total 96% of those isolates, finding high activity against Enterobacteriaceae; 100% of randomly selected *E. coli* were sensitive to fosfomycin—$MIC_{50/90,}$ 0.5/1 μg/mL, whereas *K. pneumoniae* exhibited an $MIC_{50/90}$ of 4/16 μg/mL, 97% of isolates of these isolates having a ≤64 μg/mL. Fosfomycin exhibited less activity against *P. aeruginosa*—$MIC_{50/90}$ of 64/128 μg/mL and *A. baumannii-calcoaceticus* complex, $MIC_{50/90}$ of 128/256 μg/mL. The drug did well against *Enterococcus faecalis* isolates, exhibiting 99% sensitivity, 1% as intermediate, whereas MICs were higher among *Enterococcus faecium* isolates. Among a very small number (<30) of gram-negative anaerobes, fosfomycin MICs to *Bacteroides fragilis* group were variable, whereas activity was described as limited against *Prevotella* spp. As in several in vitro studies using established FDA or CLSI breakpoints of the fosfomycin oral formulation with its limited bioavailability, a further

conclusion was that the IV formulation will necessitate a reevaluation of breakpoints.[35]

Owing to its unique structure and class, to date fosfomycin has not been shown to incur cross-resistance to other antimicrobial classes. This and favorable characteristics for achieving meaningful serum and tissue distribution, including lung, cerebrospinal fluid, and bone, have led to IV fosfomycin being used in combination with a host of other antimicrobial agents against various deep-seated infections due to resistant gram-positive and gram-negative pathogens.[36,37] Del Rio and "FOSIMI" investigators conducted an open-label prospective trial of IV fosfomycin plus imipenem for complicated bacteremia and endocarditis caused by MRSA. Subjects were 16 adults across three teaching hospitals in Barcelona and Madrid from 2001–10, 14 with persistence of MRSA in blood; early subjects had fosfomycin 2 g every 6 hours plus imipenem 1g every 6 hours added to their initial regimen of vancomycin, whereas after 2006 the fosfomycin-imipenem regimen replaced initial therapy of either vancomycin or daptomycin, 6 to 10 mg/kg body weight every 24 hours. Investigators found all patients to have negative blood cultures at 72 hours after initial dosing of fosfomycin-imipenem (a primary end point) without recurrence, with only one death attributed to MRSA.[38] In contrast, studies using other therapies, including combination therapy, have shown a mean clearance of complicated MRSA bacteremia to range from 6 to more than 8 days.[39,40] The clinical success rate at TOC (45 days after EOT) was deemed to be 69% and therapy considered safe, although several patients manifested hypernatremia, and one death was attributed to sodium overload.

Regarding highly resistant gram-negative infections, an in vitro study by Falagas and colleagues[41] demonstrated activity of fosfomycin against ESBL- and MBL-producing isolates of *K. pneumoniae*. A more recent in vitro study of ZTI-01 activity against 50 clinical CRE isolates also used existing oral fosfomycin breakpoints, with an MIC >64 µg/ mL defining resistance. A majority of isolates (42) were *K. pneumoniae* largely consisting of a variety of KPC, but also a variety of strains having OXA, VIM-1, NDM-1 and NDM-6. Also noted were that 36 (72%) and 20 (40%) isolates possessed *ompK*35 and *ompK*36 porin mutations, respectively. Forty-seven (94%) of isolates demonstrated fosfomycin susceptibility, including susceptibility to 14 isolates resistant to ceftazidime-avibactam (Avycaz). Three isolates demonstrated resistance to fosfomycin via modifying enzymes—1 fosA2, 1 fosA3, and another undetermined.[42]

In conclusion, attractive attributes are IV fosfomycin's broad activity against resistant gram-negative and gram-positive pathogens, as well as additive or synergistic properties when combined with a host of other agents from antimicrobial classes such as β-lactams, carbapenems, lipoglycopeptides, AG, polypeptides, and glycylcyclines. It would also find use in patients with intolerance or allergies to some of these more often-used antimicrobials.

Also, despite a route of elimination that is primarily renal excretion, the drug is not associated with overt nephrotoxic effects. In contrast, fosfomycin for severe resistant infections should be given with other antibacterials and not as monotherapy.[43] Monitoring this novel antibacterial in parenteral form should be subject to further close observation as early studies had small sample sizes. Dosing should be reduced in the presence of diminished renal function, and higher doses have been associated with bradycardia.[44] Each 1 g of fosfomycin contains 330 mg of sodium, so even reduced doses can be burdensome, particularly among fragile populations with cardiac, renal, or hepatic failure. Other noted AEs include hypocalcemia, hypokalemia, and phlebitis.

Omadacycline (PTK 0796, Nuzyra)

Omadacycline (Paratek Pharmaceuticals, Boston, MA) is among the aminomethylcyclines derived from the core structure of tetracycline that are protein synthesis inhibitors and represent a novel class of antibiotics with potent in vitro activity against tetracycline-resistant organisms. This first-in-class semisynthetic agent is available in IV and oral formulations; in February 2018 an NDA was submitted to the FDA for omadacycline for the treatment of community-acquired bacterial pneumonia (CABP) and ABSSSI. These indications received a favorable FDA Advisory Committee recommendation for approval in late August 2018, and a followed with FDA approval in October 2018. The MIC range against MRSA isolates, which included several vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus* strains, was 0.06 to 2 µg/mL.[45] Against *Legionella* spp., the MIC was 0.25 µg/mL.[46] In vitro activity also includes vancomycin-resistant enterococci and some resistant gram-negative pathogens, including *A. baumannii* (MIC, 8 µg/mL).[47,48] An in vitro study of PTK 0796 and comparator agents assessed activity against a total of 1024 respiratory isolates, including *S. pneumoniae, H. influenzae*, and *M. catarrhalis,* from European Union centers in 2011. The investigators concluded that omadacycline's activity was relatively unaffected by penicillin or tetracycline susceptibility patterns with demonstrably potent activity against these key respiratory pathogens and that pneumonia studies are warranted.[49]

Additional in vitro susceptibility testing of omadacycline was performed against reference strains and clinical isolates from the United States and China of *Mycoplasma pneumoniae* and *Mycoplasma hominis,* as well as *Ureaplasma parvum* and *Ureaplasma urealyticum*. Comparator agents were tetracycline, doxycycline, azithromycin; moxifloxacin with clindamycin was substituted for azithromycin for *M. hominis* isolates. An MIC_{90} value of 0.063 µg/mL for omadacycline was the lowest among all drugs tested against *M. hominis* with all omadacycline MICs <0.125 µg/mL, including tetracycline-resistant strains. Azithromycin had the highest potency against *M. pneumoniae*, but the omadacycline MIC_{90} was 0.25 µg/mL and 0.125 µg/mL against 10 strains having high-level macrolide resistance with azithromycin MICs >16 µg/mL. Omadacycline was less potent against *Ureaplasma* spp. although its activity was again not lessened by the presence of quinolone or high-level tetracycline resistance. With consistency to an earlier study, the authors concluded omadacycline demonstrated in vitro bacteriostatic activity against the organisms tested.[50,51]

Omadacycline given 100 mg IV every 12 hours for two doses, followed by three doses every 24 hours demonstrated epithelial lining fluid (ELF) and alveolar macrophage (AM) concentrations 1.5 and 25 times greater, respectively, than plasma among 41 healthy subjects.[52]

A phase III randomized, double-blind, multicenter trial across 86 sites termed OPTIC (Omadacycline for Pneumonia Treatment in the Community), compared omadacycline to moxifloxacin in adults with CABP. Omadacycline was loaded IV, 100 mg every 12 hours (two doses), followed by 100 mg every 24 hours, whereas moxifloxacin 400 mg was administered every 24 hours. After 3 days, a possible step-down to oral drug was offered, omadacycline 300 mg or moxifloxacin 400 mg, both every 24 hours for a total treatment duration of 7 to 14 days. A total of 770 subjects were randomized 1:1, and stratification by PORT (Pneumonia Outcomes Research Team) risk scores II to IV was even, both arms with a ≤15% limitation of PORT II score and a limit of ≤25% randomized subjects receiving a prior antibiotic. Subjects were predominantly white, having a mean body mass index (BMI) of 27, with 42% age older than 65 years, another 20% older than 75 years, all evenly matched. Among a total of 685 clinically evaluable subjects clinical success at early clinical response (ECR, days 3–5) 81.1% to 82.7%, EOT (days 7–14) 87.6% to 85.1%, and posttreatment evaluation (PTE, 5–10 days after last dose of drug) 92.9% to 90.4%, omadacycline to moxifloxacin, respectively. Clinical success was also similar against a spectrum of gram-positive, gram-negative, and atypical respiratory baseline pathogens isolated from microbiologically modified intent-to-treat subjects. Safety profiles were also similar, but for diarrhea, which was markedly higher for moxifloxacin and *Clostridioides difficile* (formerly *Clostridium difficile*) infection, all eight cases among the moxifloxacin arm.[53]

The OASIS (Organization to Assess Strategies in Ischemic Syndromes) 1 and OASIS 2 trials were large, randomized, double-blinded multicenter phase III studies comparing omadacycline with omadacycline versus LZD, whereas OASIS 2 compared only oral formulations. Noninferiority for efficacy for omadacycline to LZD and comparable safety profiles were demonstrated in each trial.[54] From OASIS I, an analysis of a subgroup of subjects having a high BMI (≥ 25 kg/m^2) or diabetes mellitus was undertaken to assess the efficacy and safety of the omadacycline dosing schema of 100 mg IV and 300 mg oral dosing. Seventy-nine percent of subjects in either the omadacycline or LZD cohorts had ABSSSI deemed due to IV drug use, 66% of subjects having *S. aureus*, the most common baseline pathogen. As a composite of normal BMI and high BMI subjects, the rate of infusion site extravasation was noted to be

1.5-fold higher among omadacycline subjects. Investigators concluded that IV to oral step-down therapy of omadacycline was efficacious and well tolerated in treating ABSSSI regardless of BMI or diabetic status, with similar outcomes compared with LZD at ECR and PTE. Dose adjustment of omadacycline was not needed.[55]

Omadacycline showed good in vitro activity against common Enterobacteriaceae responsible for UTIs.[56] Not yet determined is whether it would be a good antibacterial to treat UTIs, with 27% of an IV-administered omadacycline dose being eliminated in the urine of healthy subjects. What is known is that overall clearance and volume of distribution were similar among healthy subjects and subjects having end-stage renal disease; thus omadacycline dose adjustment is not required for subjects with renal impairment or those on hemodialysis.[57] As a tetracycline it would be anticipated to have prominent warnings regarding its use among pregnant or lactating women. Similar to tigecycline and eravacycline (discussed later) and the tetracycline class, omadacycline possesses little to no activity against *P. aeruginosa*. Data on activity against *Vibrio* spp., *Aeromonas* spp., and other halophilic gram-negative rods and the *Rickettsiaceae* are also lacking.

In conclusion, the IV and oral dosage forms present an advantage to omadacycline for uniform, step-down therapy of CABP and ABSSSI when needed. Omadacycline displays very good activity against key gram-positive, gram-negative, and atypical respiratory pathogens, including those displaying tetracycline resistance; susceptible organisms also include MRSA. It presents an excellent option for CABP or ABSSSI patients having a penicillin allergy or intolerance to "mainstay" community or hospital antibacterial regimens, including the fluoroquinolones, as well as lessening selective pressures of those regimens. Postmarketing surveillance should include close observation of omadacycline regimens that solely use the oral formulation. In these instances it appears a loading dose of 450 mg for the first 2 days of therapy will initiate the oral regimen, curtailing to remaining daily doses of 300 mg. Pharmacovigilance regarding GI tolerance, patient adherence, and outcomes seems warranted.

Eravacycline (TP-434, Xerava)

Eravacycline (Tetraphase Pharmaceuticals, Watertown, MA) is a fluorinated tetracycline derivative considered to be a novel fluorocycline. Owing to modifications at the C-7 and C-9 positions of its tetracycline core, eravacycline possesses broad-spectrum antimicrobial activity and demonstrates remarkable potency to pathogens, even those with tetracycline-specific efflux and ribosomal protection and inactivation.[58] The C-9 modification also appears to confer oral activity to this compound.[59]

Eravacycline has demonstrated in vitro activity against uropathogens responsible for cUTI. Within 8 hours of the first IV dose of eravacycline, 1.5 mg/kg body weight, urinary concentrations of the drug were shown to be 4- to 14-fold higher than eravacycline's MIC_{90} (0.5 μg/mL–2 μg/mL) to many common cUTI pathogens,[60] although it is not active against *P. aeruginosa*. However, in two phase III clinical trials IGNITE-2 (Investigating Gram-Negative Infections Treated With Eravacycline 2) and IGNITE-3, eravacycline as once-daily IVPB therapy with step-down to oral eravacycline (IGNITE-2), and later in IGNITE-3, to oral levofloxacin, respectively, failed to achieve statistical noninferiority for efficacy end points compared with IV levofloxacin to oral levofloxacin (IGNITE-2) or ertapenem to oral levofloxacin (IGNITE-3) for the treatment of patients with cUTI, including AP. Although well tolerated at the 200-mg every-12-hours oral dose used, Tetraphase has discontinued the oral formulation of eravacycline.

IGNITE-1 was the first of two randomized, controlled, double-blinded, prospective studies in the treatment of cIAI designed to assess the efficacy and safety of the drug. Eravacycline was dosed IV 1 mg/kg body weight every 12 hours and compared with ertapenem, dosed IV 1 g every 24 hours. Eravacycline achieved high cure rates in patients having gram-positive aerobic baseline pathogens, as well as those with infections caused by ESBL-producing Enterobacteriaceae, including a lone carbapenemase-resistant isolate; it achieved 100% clinical cure against a variety of drug-resistant *Acinetobacter* spp. Clinical cure was also high among patients, accounting for 70 isolates of *B. fragilis* and *Bacteroides thetaiotaomicron* (88.6% and 88.5%, respectively) but only 68.4% for *Bacteroides ovatus*.[61]

TABLE 36.3 In Vitro Susceptibility of TP-434 and Comparators to *Escherichia coli* DH10B–Expressing Major Tetracycline-Resistance Genes (MIC in μg/mL)

ANTIBIOTIC	*lacZ*	*tet(M)*	*tet(K)*	*tet(A)*	*tet(B)*	*tet(X)*
Eravacycline	0.063	0.063	0.031	0.25	0.063	4
Tigecycline	0.063	0.13	0.063	1	0.063	2
Doxycycline	2	64	4	32	32	16
Minocycline	0.5	64	1	8	16	4
Tetracycline	2	128	128	>128	>128	128
Ceftriaxone	0.063	0.13	0.063	0.13	0.13	0.13

MIC, Minimal inhibitory concentration.
Modified from Grossman TH, Starosta AL, Fyfe C, et al. Target- and resistance-based mechanistic studies with TP-434, a novel fluorocycline antibiotic. Antimicrob Agents Chemother. *2012;56:2559–2664.*

Eravacycline demonstrated an MIC_{90} of ≤0.5 μg/mL to 445 isolates of *E. coli,* of which 127 (29%) showed intermediate resistance or resistance to third-generation cephalosporins. Among these were a broad range of ESBL- and carbapenemase-producing isolates. The drug exhibited similar potency against good numbers of fluoroquinolone-, AG-, and MDR-resistant (three or more antibacterial classes) and tetracycline-resistant isolates. It performed equally well, besting all comparators except colistin, against 52 isolates of *A. baumannii,* with exquisite activity as low as 0.016 μg/mL (lowest) to carbapenem-intermediate and carbapenem-resistant isolates, as well as fluoroquinolone- and AG-resistant strains.[62] In another in vitro study examining eravacycline activity against major tetracycline resistance mechanisms, it exhibited a fourfold greater potency than tigecycline against a clinical strain of *E. coli* DH10B expressing a widespread tetracycline efflux pump (TetA),[58] as seen in Table 36.3**.** The TetA mechanism predominates in Enterobacteriaceae and certain other pathogens, such as *Vibrio* spp., *P. aeruginosa,* and *Aeromonas* spp. A later in vitro study of activity against Enterobacteriaceae demonstrated eravacycline's potency against ESBL-, KPC-, NDM- and OXA-producing *Klebsiella* spp.; eravacycline's $MIC_{50/90}$ was 0.5/2 μg/mL, including AG- and fluoroquinolone-nonsusceptible isolates. The $MIC_{50/90}$ was 2/8 μg/mL against tigecycline-resistant *Klebsiella*.[62,63] Eravacycline is known to not have meaningful activity against *Pseudomonas* spp.

ELF and AM achieve higher eravacycline concentrations compared with plasma by 6- and 50-fold, respectively, in healthy subjects.[64] The penetration into AM speaks to a potential advantage in treating intracellular organisms such as *Legionella* spp., whereas eravacycline's ELF penetration could indicate optimal activity against extracellular organisms, such as *S. pneumoniae*, *S. aureus,* and *Acinetobacter* spp.

The drug demonstrates potent activity against *S. aureus*, regardless of methicillin susceptibility and *Enterococcus* spp. independent of vancomycin or LZD susceptibility or daptomycin nonsusceptibility. The $MIC_{50/90}$ of eravacycline versus comparators demonstrated excellent in vitro activity to a number of anaerobic pathogens, including *B. fragilis, C. difficile*, and *Finegoldia magna,* although activity against species of non-*fragilis Bacteroides* showed a wider range of MIC values. In vitro activity against several clinically important oropharyngeal anaerobes, including *Peptostreptococcus* spp. and *Fusobacteria* spp., appears excellent.[62]

Like tigecycline, it appears that GI side effects, such as nausea and vomiting, reported at 3.3% and 2.2% of study subjects, respectively, are among the most frequent.

From eravacycline's two phase III cIAI trials (IGNITE 1 and IGNITE 4, compared with ertapenem and meropenem, respectively) the drug's FDA-labeled dosing is as twice-daily IV therapy for the treatment of cIAI, including resistant and MDR pathogens susceptible to eravacycline. As a tetracycline its labeling includes prominent warnings regarding its use among children up to age 8 years and pregnant or lactating women. Eravacycline's PK/PD profile coupled with its broad spectrum of activity, including *Legionella* spp., suggest it might be used in the treatment or studies of hospital-acquired and ventilator-associated

pneumonias. The company also has TP-271, another tetracycline derivative, in oral and IV formulations finishing phase I trials aimed at CABP.

In conclusion, in late August 2018 twice-daily IV Xerava received FDA approval for the treatment of cIAI, including resistant and MDROs susceptible to eravacycline, ESBL-producing strains, and CRE. Somewhat unique to its labeling, the FDA has allowed the statement that eravacycline demonstrated in vitro bactericidal activity against certain strains of *E. coli* and *K. pneumoniae.* Also, as a limitation of use, Xerava's labeling specifies the drug is not indicated for the treatment of cUTI. The oral dose formulation program for oral eravacycline has been discontinued, so its use will be chiefly relegated to hospital floor and ICU settings. Xerava displays broad activity against gram-negative and gram-positive MDRO pathogens, including broad anaerobe activity. It appears that the manufacturer (Tetraphase) will forego formal studies of use of eravacycline for pulmonary infection, instead seeking to advance other synthetic tetracycline moieties for potential pulmonary indications. More detailed PK/PD data on the disposition of eravacycline in blood and penetration into various tissue sites is needed to aid clinician certainty in prescribing this antibacterial for patients having suspected or documented MDRO infections. Regardless of patient allergy status, eravacycline's prescribing could lessen selective pressures by sparing prescribing of antibacterial classes such as β-lactams/carbapenems and quinolones. It would not require dose adjustment for patients having diminished renal function.

Last, as a tetracycline derivative, if supported by PK/PD data for a once-daily dosing schema achieving sufficient bone concentrations, eravacycline could have potential utility for off-label empirical or targeted therapy in osteomyelitis.

Lefamulin (BC-3781)

Lefamulin (Nabriva Therapeutics, Dublin, Ireland) is a novel systemic pleuromutilin antimicrobial inhibiting bacterial protein synthesis by interaction with 23S ribosomal RNA of the 50S bacterial ribosome subunit. Until BC-3781, pleuromutilins, known since the 1950s, have been relegated to veterinary medicine and topical use in humans (retapamulin, Altargo, Altabax; Glaxo-Smith Kline, Philadelphia, PA) due to toxicity. This systemic pleuromutilin antibacterial possesses excellent bioavailability, and both IV and oral formulations are in clinical trials for CABP (phase III completed) and ABSSSI (late phase II).

Lefamulin possesses potent in vitro activity against common gram-positive skin organisms, including *S. aureus* (methicillin-sensitive *S. aureus* [MSSA] and MRSA), coagulase-negative *Staphylococcus* spp., *Streptococcus agalactiae*, and *Streptococcus pyogenes*. It also exhibits in vitro activity against a broad array of gram-positive and gram-negative community respiratory pathogens, including *S. pneumoniae*, *Moraxella catarrhalis,* and *Haemophilus influenzae,* as well as atypical bacteria—*Legionella pneumophila, C. pneumonia*e, and *M. pneumoniae*. An in vitro study of lefamulin activity against 822 isolates of *S. pneumoniae* collected from 58 hospitals across nine US census regions showed an overall $MIC_{50/90}$ of 0.125 μg/mL and 0.25 μg/mL to (1) common serotypes, (2) isolates displaying ceftriaxone or erythromycin nonsusceptibility, or (3) MDR serotypes defined as resistant to three or more antimicrobial classes.[65] Compared with moxifloxacin, azithromycin, erythromycin, tetracycline, and doxycycline against small subsets of macrolide-sensitive and -resistant strains of *M. pneumoniae* from Europe, China, and the United States, lefamulin demonstrated potent bactericidal activity to all and with an MIC_{90} of 0.002 μg/mL to resistant strains.[66]

A small study of 12 healthy male subjects examined lefamulin concentrations in plasma, skeletal muscle tissue, subcutaneous adipose tissue, and pulmonary ELF after a single IV 150-mg dose administered in 400 mL normal saline over 1 hour. Findings were notable plasma levels with rapid and good penetration into skeletal muscle and adipose tissues and also an ELF concentration 5.7-fold higher than free plasma levels of lefamulin. Investigators postulated that, like macrolides, lefamulin as a P-glycoprotein substrate may be actively transported into ELF.[67]

At 20 US centers a phase II study compared 100 mg or 150 mg lefamulin or 1 g vancomycin, each given IVPB every 12 hours for 5 to 14 days, for ABSSSI. Patients (n = 210) were 18 years or older and randomized 1:1:1. ABSSSI characteristics for primary infection type and lesion size were similar, as were other baseline characteristics for age, underlying diabetes mellitus, body weight, and concomitant bacteremia. Rates of clinical success for MSSA and MRSA were similar among all three arms. Clinical success rates among clinically evaluable subjects was similar: 90%, lefamulin 100 mg; 88.9%, lefamulin 150 mg; and 92.2%, vancomycin 1 g. The reduction of lesion size at 72 hours, the first 5 days of therapy, and at EOT and TOC were similar among all three arms. The clinical success rate was also considered equivalent by various patient subgroups and baseline pathogens, including Panton-Valentine leukocidin–positive MRSA and USA300 MRSA. The mean duration of therapy was approximately 7 days for all three groups, with a higher rate of drug-related AE in the vancomycin arm. Among lefamulin subjects the most frequent AEs were headache, nausea, and diarrhea, although local infusion site reactions and phlebitis were more frequent compared with vancomycin. There were no occurrences of *C. difficile* infection. Small increases in QT fixed correction factor (QTcF) interval were noted, although no subject had a QTcF >480 ms, nor increase in QTcF >60 ms from baseline. This was the first study of a systemic pleuromutilin used for ABSSSI and should advance to phase III studies based on FDA stance of the LEAP (Lefamulin Evaluation Against Pneumonia) trials for CABP.[68]

The phase III LEAP I trial was a global multicenter, double-blind, randomized, controlled trial evaluating lefamulin versus moxifloxacin with or without adjunctive LZD in adult subjects with moderate-to-severe CABP. Both antibacterials were administered IVPB, with transition to oral lefamulin or moxifloxacin ± LZD. There were 551 subjects—275 in the lefamulin arms and 276 in the moxifloxacin arm—and were evenly matched regarding PORT scores, with the majority of subjects falling into PORT classes III (lefamulin, n = 196; moxifloxacin, n = 201) and IV (lefamulin, n = 76; moxifloxacin, n = 70). Among modified intent-to-treat subjects, rates of ECR were reported similar among *S. pneumoniae* and *S. aureus* infections.[69]

Regarding safety and tolerability, rates of TEAEs leading to study drug discontinuation were 2.9% for lefamulin versus 4.4% for moxifloxacin with or without LZD. Notable AEs in >2% of patients receiving the study drug (lefamulin vs. moxifloxacin with or without LZD, respectively) were hypokalemia (2.9% vs. 2.2%), nausea (2.9% vs. 2.2%), insomnia (2.9% vs. 1.8%), infusion site pain (2.9% vs. 0%), and infusion site phlebitis (2.2% vs. 1.1%). One subject in each arm displayed an increase in absolute QT interval to >500 ms.

In conclusion, to date BC-3781 has no cross-resistance with other major classes of antibacterials and shows good activity against numerous pathogens, including drug-resistant strains responsible for CABP and ABSSSI. The phase III trial of lefamulin for CABP, LEAP 2 completed enrollment in late 2017, and mid-2018 analysis of results regarding safety and efficacy were also positive. Lefamulin will be initially submitted for FDA and EMA market approval, offering a novel IV/oral antibacterial for mild-to-severe CABP. Relating to previous patient exposure, allergies or intolerance, and antimicrobial stewardship, lefamulin's potential approval provides antibacterial monotherapy with oral step-down, if needed, with little consideration of cross-resistance and avoidance of later-generation cephalosporins and quinolones for CABP therapy.

Iclaprim

Iclaprim (Motif Biosciences, New York, NY) is primarily active against gram-positive organisms. Like trimethoprim, to which it bears some structural similarity, iclaprim inhibits bacterial dihydrofolate reductase. Earlier the drug was branded Mersarex by Arpida Pharmaceuticals (Reinach, Switzerland) and applications to the FDA (ASSIST 1 and 2) and EMA for complicated skin and skin-structure infections were rejected and withdrawn in 2009. Chief concerns were safety owing to QTc prolongation and concomitant antibiotic use before study entry, which could have obscured efficacy.[70] However, in 2015 Motif Biosciences embarked on reexamination of iclaprim, and in late 2017 the FDA granted the drug orphan status for the treatment of *S. aureus* pulmonary infections in patients with cystic fibrosis.[71] With that and PK data pulled from 470 ASSIST I patients, Monte Carlo modeling sought to determine optimal efficacy dosing for MRSA while avoiding serum concentrations exceeding 800 ng/mL, this iclaprim concentration associated with AEs. It was determined in contrast to weight-based dosing used in the ASSIST trials, a fixed dose of iclaprim, 80 mg IVPB over 2 hours every 12 hours could be used for ABSSSI trials.[72]

With clinical breakpoints not yet established, an MIC ≤ 1 μg/mL for MSSA and MRSA was proposed for an in vitro study activity of iclaprim against MRSA nonsusceptible to daptomycin, LZD, and vancomycin. These latter three antibacterials have indications for ABSSSIs and LZD-vancomycin for nosocomial pneumonia. In a 2017 study with a small number of MRSA isolates, iclaprim demonstrated bactericidal activity against a majority of nonsusceptible daptomycin, LZD, and vancomycin organisms: 71%, 100%, and 67%, respectively.[73] This perhaps lends validity to sustained activity of the drug demonstrated in a 2009 in vitro study of 5937 European and US gram-positive clinical isolates from 2004–06. Iclaprim then exhibited bactericidal activity against all MRSA strains and potent activity against β-hemolytic *Streptococcus* spp. and *E. faecalis*.[74]

With an NDA expected to be filed in early 2018 (indication: ABSSSI), iclaprim recently completed a phase III randomized, double-blind, multicenter trial (REVIVE I) of ABSSSI in 598 subjects due to gram-positive organisms. Per recent FDA guidelines, a primary end point was ECR, defined as a ≥20% reduction in lesion size within an early time point of 48 to 72 hours, comparing fixed-dose 80 mg iclaprim versus vancomycin 15 mg/kg, both given IVPB every 12 hours for 5 to 14 days, duration based upon investigator discretion. Median lesion size was approximately 330 cm^2 in each arm, with primary end point of ECRs similar: 80.9% (241/298) iclaprim to 81% (243/300) vancomycin. Clinical cure rates, determined 7 to 14 days after EOT, were also similar: 83% (248/298) to 87% (262/300) for iclaprim and vancomycin, respectively. Organisms recovered were predominantly MSSA and MRSA, followed by β-hemolytic *Streptococcus,* evenly matched in each arm as was an approximate 5% rate of concomitant bacteremia. Wound definition (e.g., major cutaneous abscess, cellulitis/erysipelas, wound infection) and determination of severity were also well matched. Compared with vancomycin, a higher incidence of headache, nausea, and fatigue was noted among subjects receiving iclaprim, with similar rates of study drug–related TEAEs. TEAEs leading to study drug discontinuation was 2.7% for iclaprim compared with 4.4% for vancomycin. One patient in the iclaprim group experienced QTc intervals >500 ms and increased by 60 ms compared with baseline. Iclaprim discontinuation resulted in a return to baseline values.[75]

Iclaprim has received QIDP status for ABSSSI and for HABP. A recent (2017) manuscript of a shortened phase II trial enrolling 70 subjects from 2007 through early 2009 compared two weight-based dosages of iclaprim to vancomycin, randomized 1:1:1, for suspected or confirmed nosocomial pneumonia due to gram-positive pathogens.[76] With comparable rates of clinical cure and safety profile to vancomycin, there are plans to bring iclaprim to phase III trials, coined INSPIRE (*I*claprim for *N*o*S*ocomial *P*neumon*I*a g*R*am-positive pathog*E*ns), of HABP including VABP. An earlier 2007 study of single-dose iclaprim pulmonary distribution did demonstrate ELF and AM concentrations 20- and 40-fold greater, respectively, than plasma.[77]

It appears iclaprim spares patients substantial renal toxicity and spares clinicians the need for renal dose adjustment, but a 50% dose reduction was recommended for patients having moderate hepatic impairment (Child-Pugh class B) in earlier studies using weight-based dosing (EMA letter). Whether or not 80-mg fixed-dosing would require adjustment in this population is uncertain, as well as whether or not it would achieve suitable target attainment activities against pathogens causing infection in sites other than ABSSSI. As a singular entity not combined with a sulfur-containing agent, as is trimethoprim-sulfamethoxazole, iclaprim would avoid issues of patients having a sulfa allergy; however, unlike trimethoprim it is not available in oral formulation.

In conclusion, iclaprim is a gram-positive, active, oral antibacterial demonstrating very good activity against β-hemolytic streptococci and *S. aureus,* including MRSA. It has variable activity against the enterococci, as expected exhibiting greater activity against *E. faecalis* isolates than *E. faecium*, and also of US origin versus Europe, in one in vitro analysis.[74] Dosing adjustments do not appear necessary for diminished renal function but would possibly be recommended in the presence of moderate hepatic dysfunction. As mentioned, iclaprim lacks an oral formulation. It has potential as monotherapy or step-down from other broader parenteral therapies for ABSSSI and for this indication was granted FDA priority review and a PDUFA date of February 13, 2019. However, in February 2019, Motif Bio received an FDA Complete Response Letter holding up potential approval requesting additional data for evaluation of hepatotoxicity in study patients. Iclaprim also demonstrates excellent penetration into the ELF and AMs, so it has potential utility in various pneumonias, given its gram-positive spectrum. However, for indications other than ABSSSI, further PK/PD study is needed regarding dosing demonstrating efficacy and an acceptable profile of adverse drug events.

OVERALL CONCLUSIONS

The antibacterial agents outlined in this chapter appear most promising, with several agents receiving approval for use in the United States only very recently. The present challenge in finding effective treatment for locally invasive or systemic disease caused by MDROs has reached near-alarming concerns. *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa,* and *Enterobacter* spp. were abbreviated as "ESKAPE" to identify problem pathogens that elude treatment with commonly used antibiotics. Economic incentives for industry-sponsored new drug development (Table 36.4) are promoted by a relative ease in gaining market entry, as regulatory agencies recognize limited available

TABLE 36.4 Other Novel Antibacterial Drugs in Advanced Stages of Development

Debio-1452 (formerly AFN-1252, Affinium); Debiopharm Group	Late phase-II (ABSSSIs)	Novel agent, inhibits enoyl reductase (FabI-inhibitor). Inhibits bacterial fatty-acid biosynthesis, targeting the FabI enzyme in staphylococci (SaFabI). Recent findings show Debio-1452 binds to the BpmFabI enzyme, inhibiting growth of *Burkholderia pseudomallei*. Other late-generation FabIs in very early development show inhibitory activity against *Acinetobacter baumannii* and demonstrable synergy with colistin	Multicenter trials in United States and Canada for targeted oral therapy of ABSSSIs due to staphylococci (MSSA and MRSA). Inactive against streptococci, enterococci, and Enterobacteriaceae. In United States, recent (completed 2016) 25-site, double-blind, controlled trial, randomized 1:1:1 (*N* = 330), two separate doses of IV/PO Debio-1452 to IV vancomycin/oral linezolid (V/LZD) for ABSSSI due to staphylococci, including MRSA. Noninferiority to V/LZD was demonstrated in both arms across patient status (cellulitis and diabetes mellitus). Rates of early clinical response (48–72 h) were 94.6% and 90.1% for Debio-1452 to 91.1% V/LZD. Debio-1452 also found safe and well tolerated
Solithromycin (formerly CEM 101); Melinta Therapeutics	III (CABP)	A novel fluoroketolide, a fourth-generation macrolide agent demonstrating activity to pathogens resistant to earlier-generation macrolide agents, such as erythromycin, azithromycin—first- and second-generation agents, respectively. Binds at three sites of 50S subunit of the bacterial ribosome, compared with current macrolide agents binding at one site and has IV and PO formulations; blocks protein synthesis, preventing bacterial growth and reproduction	Met efficacy in phase III trials for CABP in adults. However, an FDA complete response letter relative to both PO and IV formulations for safety regarding hepatotoxicity requested a large-subject supplemental safety study and a comprehensive pharmacovigilance program. In 2017 did not meet noninferiority end points in a phase III trial (SOLITAIRE-U) comparing an oral 1-g dose of solithromycin against parenteral ceftriaxone plus oral azithromycin in uncomplicated gonorrhea with or without *Chlamydia* infection. Status of return to studies remains uncertain

TABLE 36.4 Other Novel Antibacterial Drugs in Advanced Stages of Development—cont'd

Sulopenem (IV and PF-03709270, sulopenem etzadroxil); Iterum Therapeutics	II complete (CABP)	Carbapenem antibacterial. Inhibits cell wall synthesis by binding penicillin-binding proteins. IV formulation and a potential first-in-class oral agent as prodrug	QIDP status granted for uUTI, cUTI, and cIAI. Active against MDROs. Anticipate starting phase III trials of cUTI mid-2018
Cadazolid; Actelion/Janssen	III (*Clostridioides difficile [formerly Clostridiium difficile]*–associated diarrhea)	Novel chimeric quinolinyl-oxazolidinone, having structural elements of both quinolones and oxazolidinones. In vitro testing of resistance development demonstrated cadazolid activity against moxifloxacin-resistant strains	Two phase III double-blinded, randomized, controlled trials (IMPACT 1 and 2, *n* = 1263 adult subjects) comparing cadazolid 250 mg PO bid vs. vancomycin 125 mg PO qid in patients with *C. difficile* infection. Both trials demonstrated safety and tolerability of cadazolid. However, although IMPACT 1 met its primary end point of clinical cure at end of therapy, IMPACT 2 failed to meet the primary end point. A study of cadazolid in pediatric subjects (NCT03105479) is ongoing. Resumption of additional adult phase III trials (???)
POL7080/murepavadin; Polyphor	II (VABP), or nosocomial pneumonias due to *Pseudomonas aeruginosa*). Planned phase III trials (NCT02096315) for early 2018	OMPTA. Pathogen-specific peptidomimetic binding of lipopolysaccharide transport protein D inhibits its export and ultimately causes cell death. A pathogen-specific antibacterial (*Pseudomonas* spp.) with no activity against Enterobacteriaceae or gram-positive pathogens	In an open-label multicenter trial of VABP likely due to *P. aeruginosa* in 25 subjects, POL7080, 2. 5 mg/kg IVPB q8h, was added to standard-of-care therapy for 10–14 days. Preliminary results in 12 subjects having confirmed *P. aeruginosa* at baseline (5 with MDR *P. aeruginosa*) showed 91% rate of clinical cure with 9% all-cause mortality at day 28

ABSSSI, Acute bacterial skin, skin-structure infection; *bid,* twice daily; *BpmFabI, Burkholderia pseudomallei* enoyl-acyl carrier protein reductase; *CABP,* community-acquired bacterial pneumonia; *cIAI,* complicated intraabdominal infections; *cUTI,* complicated urinary tract infection; *FabI,* an enoyl-acyl carrier protein reductase; *FDA,* US Food and Drug Administration; *IV,* intravenously; *IVPB,* intravenous piggyback; *MDR,* multidrug resistant; *MDRO,* multidrug-resistant organism; *MRSA,* methicillin-resistant *Staphylococcus aureus; MSSA,* methicillin-sensitive *S. aureus; OMPTA,* outer membrane protein targeting antibiotic; *PO,* orally; *qid,* once per day; *QIDP,* Qualified Infectious Disease Product; *SaFabI, S. aureus* enoyl-acyl carrier protein reductase; *uUTI,* uncomplicated urinary tract infection; *VABP,* ventilator-associated bacterial pneumonia; *VILZD,* vancomycin/linezolid (combination).

options for cure of conditions such as cancer.[78] It is estimated currently that 500 million to 1 billion dollars are spent in the launching of a new antimicrobial drug for clinical use; this includes costs for biochemical research, preclinical development, and mandatory clinical trials.[79,80] New entries to the market should be shepherded with the best intentions for antimicrobial stewardship and cautious pharmacovigilance, especially to monitor for AEs and toxicities that become apparent during the postmarketing period. As with any prescription medication, time will allow for the distinction of the most useful of these antibacterials. To be considered are all the PK/PD characteristics of the new agents across broad clinical scenarios. These would include the likelihood of multiple off-label indications, application for and receipt of newly labeled indications, patent strategies, and ease of provider and patient use in the face of observations regarding outcomes, safety, and drug interactions.

ACKNOWLEDGMENTS

The author wishes to thank Amar Safdar, MD, MBBS, FIDSA, for his contributions to this chapter in an earlier edition, and Mattie Huffman for assistance with data retrieval.

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37 Urinary Tract Agents: Nitrofurantoin, Fosfomycin, and Methenamine

James M. Horton

SHORT VIEW SUMMARY

Nitrofurantoin

- Nitrofurantoin is a first-line treatment for uncomplicated cystitis, with an efficacy of 88% to 92%. It is not indicated for pyelonephritis.
- The most common side effect is nausea, but the most serious is pulmonary hypersensitivity, which occurs in about 1 in 100,000 cases.
- Nitrofurantoin is a preferred treatment for cystitis during the last two trimesters of pregnancy but should be avoided at term or when delivery is imminent because of the risk of hemolytic anemia in the mother and baby.
- The American College of Obstetrics and Gynecology recommends that nitrofurantoin only be used in the first trimester if there are contraindications to safer antibiotics.

Fosfomycin

- Fosfomycin is administered orally as 3 g of powder mixed with at least one-half cup of water in a one-time dose before a meal.
- Fosfomycin is a first-line treatment for uncomplicated cystitis. A cure rate of 91% has been reported, but other data suggest fosfomycin is slightly less effective than other agents.
- Observational trials have shown that fosfomycin is effective against multidrug-resistant bacteria.

Methenamine

- Methenamine is hydrolyzed to formaldehyde in the acidic urine of the bladder. It is not effective in patients with indwelling Foley catheters or urostomies because of the rapid elimination of the drug from the bladder.
- Methenamine is indicated only for prevention of cystitis.

Nitrofurantoin and fosfomycin are considered first-line treatments for acute cystitis because of their pharmacologic properties.[1] At tolerated doses after oral administration, nitrofurantoin achieves adequate concentrations only in the urine.[2] Although parenteral fosfomycin has been used for systemic infections, the oral formulation is used only for urinary tract infections (UTIs). Methenamine becomes active only after chemical degradation in acidic bladder urine, to generate its active breakdown product, formaldehyde, and is used only for the prophylaxis of UTIs.[3]

NITROFURANTOIN

Nitrofurantoin is a member of a group of synthetic nitrofuran compounds and is a weak acid (pK_a 7.2) (Fig. 37.1).[4,5] A microcrystalline form was introduced in 1952, and macrocrystalline forms were developed in 1967. Mixtures of the microcrystalline and macrocrystalline forms are now available (Macrobid: 25 mg macrocrystals plus 75 mg monohydrate form), as are the macrocrystals alone (Macrodantin).[5]

Mechanisms of Drug Action and Bacterial Resistance

The mechanism of bactericidal activity appears to involve multiple sites, including inhibition of ribosomal translation, bacterial DNA damage, and interference with the Krebs cycle.[6–8] The role of each of these mechanisms is unclear.[7] It is metabolized by bacterial nitroreductases, which convert nitrofurantoin to a highly reactive electrophilic intermediate that attacks bacterial ribosomal proteins, causing complete inhibition of protein synthesis.[9]

Resistance to nitrofurantoin is uncommon, probably because of the multiple sites of action of the antibiotic.[1,2,10–12] A sixfold to sevenfold increase in resistance of *Escherichia coli* has been reported when the bacteria lack nitrofuran reductase enzyme activity.[10]

Spectrum of Activity

Nitrofurantoin is active against more than 90% of *E. coli* strains causing UTIs, but *Proteus* spp., *Serratia* spp., and *Pseudomonas* spp. have natural resistance.[6,8,12] In a study of catheter-associated UTIs, fewer than half of the *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp. are susceptible.[13] The drug has increasingly been used to treat enterococcal infections, including those due to vancomycin-resistant enterococci.[1,12] *Staphylococcus aureus* and *Staphylococcus saprophyticus* are usually susceptible.[1]

Most strains are considered susceptible to nitrofurantoin if the minimal inhibitory concentration (MIC) is 32 µg/mL or less.[14] Testing is indicated only for Enterobacteriaceae, *Staphylococcus* spp., and *Enterococcus* spp. *Pseudomonas aeruginosa* is almost universally resistant.[14]

Pharmacology

Absorption

Orally administered nitrofurantoin is 40% to 50% absorbed; absorption is improved when the drug is taken with food.[4,15] Absorption occurs principally in the small intestine. The microcrystalline form is more rapidly and completely absorbed than the macrocrystalline form (43% vs. 36%) but is associated with more gastrointestinal (GI) side effects.[4,15]

Distribution

Serum concentrations of nitrofurantoin are low or undetectable with standard oral doses.[12] Animal studies with intravenous nitrofurantoin suggest distribution in extracellular and intracellular tissues.[15] Drug concentration in the urine (50–250 µg/mL) easily exceeds the MIC of 32 µg/mL for susceptible organisms.[4] Concentrations in prostatic secretions are too low for effective use in prostate infections.[16] Concentrations in human breast milk are extremely low (0–0.5 µg/mL).[17,18] Biliary concentrations are about the same as those in the serum.[15]

Excretion

Nitrofurantoin is eliminated predominantly in the urine. Renal elimination involves glomerular filtration, tubular secretion, and tubular reabsorption.[15] Alkalinization of the urine can prevent the reabsorption of the nitrofurantoin in the renal tubules, but nitrofurantoin has reduced antimicrobial activity in alkaline urine.[2]

In patients with renal failure, nitrofurantoin excretion is proportionally decreased and should not be used in patients with substantial renal insufficiency.[5]

One study found that the treatment failure rate in elderly women with decreased renal function was the same for nitrofurantoin, ciprofloxacin, and trimethoprim-sulfamethoxazole (TMP-SMX).[19] The package insert recommends against using nitrofurantoin in patients with a creatinine

clearance less than 60 mL/min. The American Geriatrics Society recommends avoiding it if the clearance is less than 30 mL/min or for long-term suppression.[20] In patients with normal renal function, a small proportion of nitrofurantoin is eliminated by metabolism and biliary excretion, but these are minor pathways. No dose adjustment is needed in patients with liver failure.

Dosing

For therapy for UTIs, nitrofurantoin (Furadantin, Macrodantin) in the macrocrystalline formulation is given orally at 50 to 100 mg four times daily. For prophylaxis for recurrent UTIs, it is dosed at 50 to 100 mg once daily. The dose of the mixture of microcrystalline and macrocrystalline formulations (Macrobid) is 100 mg twice a day.

Indications

Nitrofurantoin is indicated only for the treatment and prophylaxis of lower UTIs.

Acute Uncomplicated Cystitis

Nitrofurantoin is now considered a first-line therapeutic agent for acute uncomplicated cystitis[1] because of the efficacy of a 5-day course of nitrofurantoin and the risks for collateral damage to the normal human microbes by fluoroquinolones (Table 37.1).[1]

Studies indicate a clinical cure rate with nitrofurantoin of 88% to 92% and a microbiologic cure rate of 81% to 92%.[1] Four randomized trials have demonstrated that nitrofurantoin has an efficacy equivalent to a 3-day course of TMP-SMX or ciprofloxacin. It is equivalent to one dose of fosfomycin. Past studies with 3 days of treatment with nitrofurantoin have demonstrated persistence of the pathogen in the periurethral, vaginal, and rectal areas,[21] so a 7-day course was recommended.[16] More recent studies have demonstrated the efficacy of a 5-day course of nitrofurantoin.[1] Overall, infections caused by *E. coli* respond well to nitrofurantoin,[2] but infections due to *Proteus* spp. and *Pseudomonas* spp. do not respond. Nitrofurantoin may have a role in treatment of nosocomial UTIs, but studies have been limited.[7]

Nitrofurantoin has been used safely in pregnant women[22] and in children.[4]

Acute Pyelonephritis and Complicated Urinary Tract Infections

Nitrofurantoin should not be used for treatment of pyelonephritis. It not only has failed to successfully treat pyelonephritis, but also two cases of bacteremia have been reported while patients were receiving nitrofurantoin therapy.[2]

Complicated UTIs resulting from anatomic abnormalities, indwelling Foley catheters, or nosocomial infections are more likely to be caused by organisms such as *Pseudomonas* that are resistant to nitrofurantoin. In men with recurrent bacteriuria, nitrofurantoin has reduced the recurrences by 40%, but other agents that achieve higher concentrations in the prostate are more effective.[23]

Many strains of vancomycin-resistant enterococci remain susceptible to nitrofurantoin, so it can be used for cystitis caused by these organisms.[24]

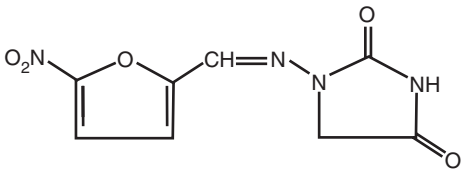

FIG. 37.1 **Chemical structure of nitrofurantoin.**

Prophylaxis for Recurrent Urinary Tract Infections

In young women with two or more episodes of symptomatic UTIs within 12 months, nitrofurantoin (100 mg) was effective and comparable to TMP-SMX in preventing further UTIs.[25,26] In a more heterogeneous population, nitrofurantoin was equivalent to cefaclor (250 mg at bedtime) or norfloxacin (200 mg at bedtime).[27,28] Nitrofurantoin was slightly less well tolerated owing to nausea.

For women in whom recurrence of infection is associated with sexual intercourse, a single dose of nitrofurantoin (100 mg) taken shortly after intercourse has been effective in preventing symptomatic infection.[28,29]

In postmenopausal women with recurrent UTIs, nitrofurantoin (100 mg every day) was more effective than an estriol-containing vaginal pessary in preventing symptomatic and asymptomatic bacteriuria.[30] In one pediatric study of children with intermittent catheterization, nitrofurantoin prevented *E. coli* UTIs, but infections due to resistant uropathogens increased.[25]

Antimicrobial prophylaxis is not of value in patients with long-term indwelling catheters.[22]

Adverse Effects

Pulmonary Reactions

Pulmonary reactions have been classified into acute and chronic forms.[4,31] Most studies cite a frequency of one or fewer cases per 100,000 courses of treatment,[17] although others have reported a rate of 1 in 5000 courses.[32–34] In 13,421 adults older than 65 years who were given short-course nitrofurantoin, there was no measurable increased risk of pulmonary complications; however, chronic administration was associated with pulmonary complications (risk ratio, 1.53).[35] Determining a precise incidence is difficult because the clinical presentation of nitrofurantoin lung disease overlaps with that of many other illnesses, such as pneumonia, exacerbation of bronchitis, heart failure, or chronic pulmonary fibrosis.[33,34]

Acute reactions occur within hours to weeks of drug exposure and are characterized by a reversible hypersensitivity phenomenon.[33,36] The reaction comprises the rapid onset of fever, cough, dyspnea, myalgia, and occasionally a rash. Peripheral blood eosinophilia (83% of cases), lower lobe infiltrates (94% of cases), and pleural effusions (20% of cases) often accompany these signs and symptoms. Sputum production, rash, pruritus, and chest discomfort may also occur. Lung biopsy can show vasculitis, alveolar exudates, and interstitial inflammation. Most

TABLE 37.1 Efficacy of Agents Commonly Used for Uncomplicated Urinary Tract Infections

ANTIBIOTIC	DOSE	CLINICAL EFFICACY (%, RANGE)	MICROBIOLOGIC EFFICACY (%, RANGE)	SIDE EFFECTS
Nitrofurantoin monohydrate/ macrocrystals	100 mg bid, 5–7 days	93 (84–95)	88 (86–92)	Nausea, headache, hepatic and pulmonary toxicity
Trimethoprim-sulfamethoxazole	160/800 mg bid, 3 days	93 (90–100)	94 (91–100)	Rash, hematologic toxicity, nausea
Fosfomycin	3-g single-dose packet	91	80 (78–83)	Diarrhea, headache
Fluoroquinolones	Dose varies, 3 days	90 (85–98)	91 (81–98)	Nausea, diarrhea, insomnia, prolonged QT interval
β-Lactam antibiotics	Dose varies, 3–5 days	89 (79–98)	82 (74–98)	Diarrhea, nausea, rash

Modified from Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update for the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. *2011;52:e103–e120.*

reports have occurred in women, which is consistent with the much greater use of nitrofurantoin in women than in men, and most cases occur in patients older than 40 years.[33,36] The reason for the frequency in older adults is unclear, but the American Geriatrics Society warns against the use of nitrofurantoin in patients older than 65 years with creatinine clearance of less than 30 mL/min or for prolonged courses.[20,37] Symptoms usually improve within 15 days with discontinuation of the drug, although case reports of fatalities have occurred.[31,36] One study cites the mortality from acute pulmonary reaction as 2 in 398 cases (0.5%).[36]

Chronic pulmonary reactions are 10 to 20 times less common, perhaps because the drug is infrequently used for prolonged therapy. These reactions occur after 1 to 6 months of therapy[31] and are characterized by nonproductive cough, dyspnea, interstitial infiltrates, and usually fever. Eosinophilia is less common than in patients with acute reactions. Abnormal results of liver enzyme studies (40%) and positive assays for antinuclear antibodies have been reported. Improvement can occur with discontinuation of the drug, but about half of the affected persons have persistent mild signs of pulmonary fibrosis.[31] An interstitial pattern on chest tomography has been reported but does not correlate with progression of disease.[38] The mortality rate from the chronic pulmonary reaction is reported as 4 in 49 cases (8%).[36]

The most important treatment includes early recognition of the complication and prompt discontinuation of the drug. The chronic pulmonary complications can be insidious, leading to erroneous diagnoses, such as congestive heart failure.[33] Corticosteroids have been used in some patients who recovered, but no large trials exist.[39] Bronchiolitis obliterans and organizing pneumonia have been reported.[40] Desquamative interstitial pneumonitis has been reported in a child.[41] Exacerbation of hereditary angioedema with lip and throat swelling has been described.[42]

Gastrointestinal Reactions

The tolerability of nitrofurantoin is limited by the GI adverse effects, which occur particularly with the microcrystalline formulation.[18] The macrocrystalline formulations are associated with nausea and vomiting in 17% of patients, compared with 39% in patients using the microcrystalline forms at doses of 100 mg four times daily. Slower dissolution of the macrocrystalline formulation is believed to be responsible for its lower frequency of GI side effects. In a double-blind study, ciprofloxacin had fewer episodes of nausea than did nitrofurantoin (3% vs. 11%).[43]

Cutaneous Reactions

Rashes occur in about 1% of patients being treated with nitrofurantoin. TMP-SMX is significantly more likely to cause a rash than nitrofurantoin (4% vs. 0.4%).[43] Most cutaneous reactions with nitrofurantoin are relatively mild, particularly when compared with those that may occur after therapy with TMP-SMX.[18] Cutaneous reactions have included Sweet syndrome, antineutrophilic cytoplasmic antibody-associated vasculitis, and lupus.[44]

Hepatic Reactions

Hepatic reactions occur at about the same frequency as the pulmonary reactions and can occur at the same time.[36] Acute hepatitis associated with short-term use of nitrofurantoin was self-limited and reversible in 8 patients in one study.[45] Prolonged use of nitrofurantoin has been associated with chronic active hepatitis, cirrhosis, and death.[46] In one study of 42 patients with nitrofurantoin-induced hepatitis, two patients (5%) died and 18% developed chronic hepatitis.[47] In these cases antinuclear antibodies are often present, but eosinophilia occurs less frequently.[46]

Hematologic Reactions

Hemolytic anemia in patients receiving nitrofurantoin is most commonly associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency.[48] Hemolysis from deficiencies in enolase and glutathione peroxidases has been described, as have folic acid–responsive anemias.[49] Eosinophilia has been described in patients with pulmonary and hepatic reactions. Other leukocyte dyscrasias are uncommonly reported.[36]

Peripheral Neuropathy

A peripheral sensorimotor neuropathy has been reported uncommonly but is especially noted in patients with renal failure who are receiving the drug.[18,36] The neuropathy is characterized by distal dysesthesias and paresthesias. Distal muscle weakness also can occur.[49] Histopathology shows demyelination and axonal degeneration. Resolution of symptoms is slow and variable after cessation of the drug.

Systemic Inflammatory Response Syndrome

One case of systemic inflammatory response syndrome has been described in a patient on long-term prophylactic therapy with nitrofurantoin. The syndrome resolved with discontinuation of the drug.[50]

Drug Interactions

There has been one case report of fluconazole precipitating nitrofurantoin pulmonary toxicity.[51] Theoretically, nitrofurantoin can precipitate methemoglobinemia; other drugs causing methemoglobinemia, most commonly dapsone or benzocaine, in high-risk patients should be avoided.[52] Nitrofurantoin can inhibit the oral live typhoid or cholera vaccines.[5] Magnesium-containing compounds can inhibit its absorption.[5] It also interacts with probenecid. Nitrofurantoin has been associated with hyperkalemia; thus drugs such as spironolactone and triamterene should be avoided.[53]

Use in Children and During Pregnancy

Adverse events in children appear to be similar to those in adults. Nitrofurantoin is not recommended for neonates.[54] Studies on safety of nitrofurantoin during pregnancy have mixed results. It is the most commonly prescribed medication during pregnancy and is considered a pregnancy category B drug.[55,56] Although the drug crosses the placenta, only low concentrations reach the amniotic fluid.[57] When adverse events in 165 pregnant patients receiving nitrofurantoin were reviewed, no increased incidence was found of fetal loss or fetal abnormality when compared with the general population.[58] Other studies have confirmed these findings.[49,59,60] Although nitrofurantoin is mutagenic in bacterial studies, no teratogenicity or carcinogenicity has been found in animal studies.[18] A meta-analysis of 91,115 exposed cases and 1,578,745 unexposed controls in the first trimester showed no increased risk on nitrofurantoin during pregnancy in cohort studies, but meta-analysis of the three case-control studies did show some increased risk.[61] In a retrospective study, the risk of cleft lip or palate in babies born to mothers who received nitrofurantoin periconceptionally was increased compared with penicillin (odds ratio, 1.97), but the absolute risks remained low compared with the risk of UTIs.[62] The American College of Obstetricians and Gynecologists recommends avoiding nitrofurantoin in the first trimester unless there are no safer alternatives.[63] Case reports of hemolytic anemia in pregnant women with G6PD deficiency exist; the complication can be confused with HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets).[64] Nitrofurantoin should be avoided at term, if labor is imminent, or in neonates because of concerns for hemolytic anemia and jaundice in the baby.[18]

It is excreted in small amounts in the breast milk; theoretically neonates exposed to nitrofurantoin from breast milk could develop hemolytic anemia, although no cases have been reported.[65]

FOSFOMYCIN

Fosfomycin was discovered in 1969 as a member of a novel class of phosphonic antibiotics (Fig. 37.2). It has been used as a parenteral antibiotic for systemic infections but more recently has been available

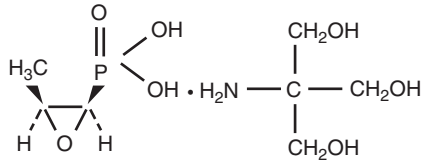

FIG. 37.2 Chemical structure of fosfomycin.

as an oral formulation that is used solely for treatment of uncomplicated cystitis.[66]

Mechanism of Action and Antimicrobial Activity

Fosfomycin blocks cell wall synthesis by inhibiting the synthesis of peptidoglycans. The drug requires transport into the cell wall by two main transport systems: the L-α-glycerophosphate and the hexose phosphate uptake systems.[66] G6PD must be added to the medium to determine the in vitro susceptibilities. Fosfomycin has been available in Europe for parenteral use, but in the United States it is available only in the oral form for treatment of UTIs.[66]

Fosfomycin has broad-spectrum bactericidal activity against staphylococci, enterococci, *Haemophilus* spp., and most enteric gram-negative bacteria. It also has excellent activity against most *E. coli,* including 95.5% of extended-spectrum β-lactamase (ESBL)-producing *E. coli.*[66] ESBL 025b/B2 *E. coli* strains are resistant to fosfomycin.[66] *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp. have higher MICs; fosfomycin has activity against only 57.6% of ESBL-producing *Klebsiella* spp.[67] *P. aeruginosa* is variably susceptible to fosfomycin, with MICs ranging from 4 to more than 512 μg/mL.[66] *Acinetobacter baumannii* is usually resistant. Fosfomycin retains excellent in vitro activity against both *Enterococcus faecalis* (97.7%) and *Enterococcus faecium* (100%).[68] Susceptibilities to fosfomycin should be determined by disk diffusion and not broth dilution.[14]

Most resistance is chromosomally mediated and interferes with the transport of the antibiotic into the bacteria.[69] Three resistance genes carried by plasmids—*fosA* in *P. aeruginosa, fosB* in *S. aureus,* and *fosX* in *Listeria monocytogenes*—confer resistance by breaking the oxirane ring of the fosfomycin molecule.[70]

Pharmacology

Fosfomycin is best absorbed if given before food intake, with up to 58% absorbed and excreted in the urine.[66] The fosfomycin molecule (138 Da) is smaller than other antibiotics, and it diffuses across membranes easily. It is water soluble and hydrolyzed to an active form but not metabolized. After a 3-g oral dose, the peak serum levels are 22 to 32 μg/mL at 4 hours. Fosfomycin achieves high concentrations in the urine of 2000 μg/mL and maintains high levels for more than 24 hours.[69] Its long half-life allows for one dose to treat uncomplicated cystitis.

Dosing

Fosfomycin (Monurol) is administered as a powder containing 3 g of the drug mixed into a slurry with at least one-half cup (4 oz) of water before meals. It should never be taken as the dry powder formulation.[71] It is generally administered as one dose for acute uncomplicated cystitis, but the dose has been repeated every 3 days to successfully treat more complicated UTIs.[71]

Indications

Acute Uncomplicated Cystitis

The clinical efficacy of one (3 g) dose of fosfomycin (91% cure) is comparable to nitrofurantoin (93%), TMP-SMX (93%), and fluoroquinolones (90%) in acute uncomplicated cystitis.[1] The microbiologic cure rate of fosfomycin (80%) is lower than comparable antibiotics at 88% to 94%.[1] One review reports unpublished data that show fosfomycin is slightly less effective than these comparable agents.[1] The Infectious Diseases Society of America lists fosfomycin as first-line therapy for cystitis because of the ease of administration but cautions that it might be slightly less effective than other agents.[1] One meta-analysis demonstrated equivalent clinical success between fosfomycin and other antibiotics for UTIs.[72]

One niche use for fosfomycin is in the treatment of multidrug-resistant organisms. There has been increasing use against ESBL- and *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae.[73] Observational trials indicate that fosfomycin can be effective in treatment of UTIs due to multidrug-resistant organisms such as KPC-producing Enterobacteriaceae with 3-g doses repeated every 48 to 72 hours.[73,74] One study documented a parallel increase in resistance to fosfomycin among ESBL-producing *E. coli* with more frequent use of fosfomycin in the community.[75]

Fosfomycin is not indicated for pyelonephritis.[1] Shrestha and Tomford[69] report one case of the successful treatment of prostatitis with 3 g fosfomycin given orally every 3 days for 3 weeks. It has been used for prophylaxis of transrectal ultrasound-guided prostate biopsy.[76]

Adverse Effects

Fosfomycin has been associated with diarrhea, vaginitis, nausea, and headache at rates comparable to those of other antibiotics used for UTIs.[66,71] Cases of optic neuritis and hearing loss have been reported.[66,71] At least one case of anaphylaxis due to fosfomycin has been reported,[77] as well as a single case of *Clostridioides difficile* (formerly *Clostridium difficile*) diarrhea.[66]

Use During Pregnancy

Fosfomycin is approved for use during pregnancy and is considered a US Food and Drug Administration (FDA) category B drug; it is one of the choices for treatment of cystitis during pregnancy.[71,78] Safety with breast-feeding and in children younger than 12 years has not been established.

METHENAMINE

The chemical structure of methenamine (hexamethylenetetramine) is shown in Fig. 37.3. It is available as a salt of mandelic acid (Mandelamine) or hippuric acid (benzoyl amino acetic acid) (Hiprex) or without these acids (Urised, Prosed/DS, Urimax).[79]

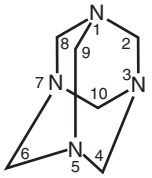

FIG. 37.3 Chemical structure of methenamine.

Mechanism of Action and Antimicrobial Activity

Methenamine itself has little antibacterial activity, but at acid pH each molecule of methenamine is hydrolyzed to produce four molecules of ammonia and six molecules of formaldehyde.[80,81]

The active product in formaldehyde is a nonspecific denaturant of proteins and nucleic acids with broad-spectrum antimicrobial activity. Microbial resistance has not been described, but *Proteus* spp. may produce urease, causing an alkaline urine and preventing the conversion of methenamine to formaldehyde.

Pharmacology

Absorption and Distribution

Methenamine is rapidly absorbed after oral administration, and 82% to 88% is recovered in the urine of normal volunteers in the 24 hours after a 1-g dose.[82] Methenamine may be partially degraded in the presence of gastric acid before absorption.[83] An enteric-coated formulation prevents this degradation but slows absorption. Methenamine is widely distributed to tissues and crosses the placenta, and concentrations in breast milk are similar to those in the plasma.[84] The drug is 95% excreted through the kidneys by glomerular filtration and tubular secretion.[82] The elimination half-life from the serum is 3 to 4 hours. The amount of accumulation of methenamine in patients with renal failure is not known.[85]

Factors Affecting Formaldehyde Concentrations in Urine

Antimicrobial activity correlates with urinary formaldehyde concentrations; those concentrations are determined by (1) the methenamine concentrations in the urine, (2) the rate of hydrolysis of methenamine to formaldehyde, and (3) the rate of urine loss from the bladder by voiding or drainage. Methenamine concentrations usually reach 150 μg/L but are lower with higher volumes of urine. The conversion to formaldehyde

increases with more acidic urine; at a urine pH of 6.8 or greater no hydrolysis occurs. The time required for effective concentrations of formaldehyde is 6 hours at a pH of 6.5 and 2 hours at a pH of 5.6.[86,87] A formaldehyde concentration of 25 µg/mL requires 2 hours of bacterial exposure to be effective. Methenamine is ineffective in the presence of indwelling bladder catheters or frequent catheterization because the rapid elimination of urine does not allow time for the conversion into formaldehyde. It is also ineffective for treatment of upper UTIs.

Acidification of Urine During Methenamine Treatment

A urine pH less than 6 is required for antibacterial activity of methenamine. Ascorbic acid has been given to aid urine acidification.[80]

Dosing

For adults and children older than 12 years, methenamine hippurate is usually given at a dose of 1 g orally twice daily. For children between 6 and 12 years, the dose is 500 mg to 1 g twice daily.[79]

Indications

Methenamine should not be used for treatment of established UTIs and is not effective for pyelonephritis.

Methenamine is effective in preventing recurrent lower UTIs. In young, otherwise healthy women, 1 g of methenamine twice daily reduced the frequency of recurrent cystitis by 73%.[88] Methenamine mandelate (500 mg four times daily) with ascorbic acid reduced cystitis by 56%.[89] This drug is not as effective in suppressing UTIs when compared with TMP-SMX or nitrofurantoin.[90]

Methenamine is not effective in preventing UTIs in patients with indwelling bladder catheters.[91] Trials of methenamine in patients undergoing intermittent catheterization have had variable results.[91,92] In patients undergoing bladder retraining with catheterization, voiding and controlled drinking resulted in a decrease in urinary tract infection over 21 days.[92] More prolonged use may only postpone the bacteriuria.

Adverse Effects

Side effects from methenamine are infrequent and usually mild.[80,89] Nausea, vomiting, rashes, or pruritus have been described.[80] At higher doses, GI intolerance and hemorrhagic cystitis may occur, possibly related to the high bladder concentration of formaldehyde.[93] Methenamine may predispose to the development of urate crystals in the urine and may cause precipitation of sulfonamides. Because of the ammonia produced by the hydrolysis of methenamine, use of this drug should be avoided in patients with liver failure. The safety of methenamine in renal failure has not been established.[94] The data about use in pregnancy are limited; the FDA categorizes methenamine as pregnancy risk C.[95]

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The complete reference list is available online at Expert Consult.

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38 Topical Antibacterials

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SHORT VIEW SUMMARY

- Topical antimicrobial agents include topical antibacterials, such as mupirocin, clindamycin, and metronidazole, and topical antiseptics, such as chlorhexidine gluconate (CHG), alcohol, and povidone-iodine.
- CHG is available in impregnated cloths for bathing, in impregnated sponges and gels for vascular catheter dressing sites, and as a liquid solution for skin site preparation or washing. Preparations that combine CHG with 70% alcohol are recommended for skin site antisepsis in surgical procedures, for vascular access placement, and for blood culture phlebotomy.
- Daily bathing of critically ill patients with CHG-impregnated cloths has been shown to decrease the subsequent development of multidrug-resistant organism colonization and bloodstream infections.
- Topical mupirocin is available for intranasal use and in cream formulations for use on skin.
- Topical mupirocin can be used to treat impetigo.
- Intranasal mupirocin for decolonization may be beneficial as a preventive strategy for surgical site infections in patients undergoing cardiac surgery, joint replacement surgery, and neurosurgery.
- Topical mupirocin applied to the exit site of hemodialysis catheters is recommended for prevention of bloodstream infection.
- Topical povidone-iodine applied to the hemodialysis catheter exit site may also be beneficial in prevention of bloodstream infections.
- Topical mupirocin applied to peritoneal dialysis exit sites may be beneficial in preventing peritoneal catheter site infection and peritonitis.
- Intranasal mupirocin and CHG bathing have been used as a decolonization regimen in outpatients with recurrent methicillin-resistant *Staphylococcus aureus* (MRSA) skin and soft tissue infections and in critically ill inpatients for prevention of hospital-acquired infection.
- The optimal decolonization regimen for MRSA is still not known, and no definitive recommendations on when to attempt decolonization are currently available.
- The cornerstone of management of acne is topical combination therapies that include a benzoyl peroxide and an antimicrobial, such as topical clindamycin or erythromycin.

Topical antibacterial therapy has an important but often undervalued role in the prevention and management of specific infections. Topical antibacterial agents can be subdivided into two types: topical antimicrobials and topical antiseptics. The topical *antimicrobial* agents usually have a primary target site and mechanism of action. They include bacitracin, clindamycin, erythromycin, metronidazole, mupirocin, neomycin, and retapamulin, and they may be administered concomitantly with other systemic antimicrobial agents. Topical antimicrobials have been used to prevent wound infections, treat superficial skin and soft tissue infections (SSTIs), and eradicate carriage of undesirable bacteria, such as *Staphylococcus aureus*. Moreover, these agents may be used to prevent postoperative infections and catheter-related infections in certain patient populations. The topical *antiseptics* (such as chlorhexidine gluconate [CHG], povidone-iodine, and alcohol) have multiple target sites of action against bacteria and are sometimes referred to as biocides.[1] For the purposes of this chapter, the term *topical antibacterials* will refer to both types of agents.

Topical antibacterial therapy has several potential advantages over oral or parenteral antibacterial administration in specific clinical settings (Table 38.1).[2] After the application of small amounts directly to an infection or wound, very high local drug concentrations are achieved, levels that may be toxic if delivered systemically. When administered topically, these agents first enter the skin (the first target organ), and then a variable quantity is distributed throughout the body and finally eliminated. Concentrations of a topical antibacterial decline from the skin surface to the subcutis (after systemic administration, the opposite occurs) (Fig. 38.1).[3] Therefore, topical administration is favored if the pathologic process is in the epidermis or papillary dermis because the highest doses are delivered directly to the site of infection. For infection in the lower dermis or subcutis, it is necessary to determine whether a topically administered antibacterial provides the drug concentrations necessary to effectively eradicate the infection. Topical preparations formulated to contain combinations of topical antibacterial agents may offer the benefits of synergism and delay the selection of resistant microorganisms. In this chapter, we review the general uses of topical antibacterial agents in the therapy and prevention of infections. Topical agents are also effective in treating eye (see Chapters 111 through 116) and ear (see Chapter 61) infections.

GENERAL USES OF TOPICAL ANTIBACTERIALS

Skin Disinfection

Skin disinfection is a critical component of safe and effective patient care and includes disinfection of patients' skin to provide a sterile environment for procedures, and disinfection of health care workers' skin to prevent transmission of pathogenic bacteria through contact with patients. Some topical antibacterial agents, in particular the topical antiseptics, are very effective at decreasing the number of bacteria on the skin. The ideal antiseptic agent should have the following properties: a broad antimicrobial spectrum; rapid bactericidal activity; persistent activity on the skin; an absence of irritating, allergic, or toxic reactions; an absence of systemic absorption; activity in the presence of body fluids (e.g., blood); and cosmetic acceptance.[2] Unfortunately, no single compound meets all these criteria. However, depending on the specific clinical situation, only certain properties may be required. For example, for repeated hand washing (e.g., by medical personnel), lack of irritation and persistence of activity are essential properties. In contrast, for the preparation of operative sites, rapid bactericidal activity is required.

Several topical antiseptic agents are used as skin disinfectants. Hexachlorophene is no longer used as a skin disinfectant for many reasons. The iodophors, in particular povidone-iodine, are widely used as skin antiseptics. Povidone-iodine is an organic complex of polyvinylpyrrolidone and triiodine ions (the antimicrobial component) that slowly liberates iodine on reduction. Iodophors have a broad antimicrobial spectrum; however, antibacterial activity does not persist for prolonged periods on the skin, in wounds, or on mucous membranes, and iodophors

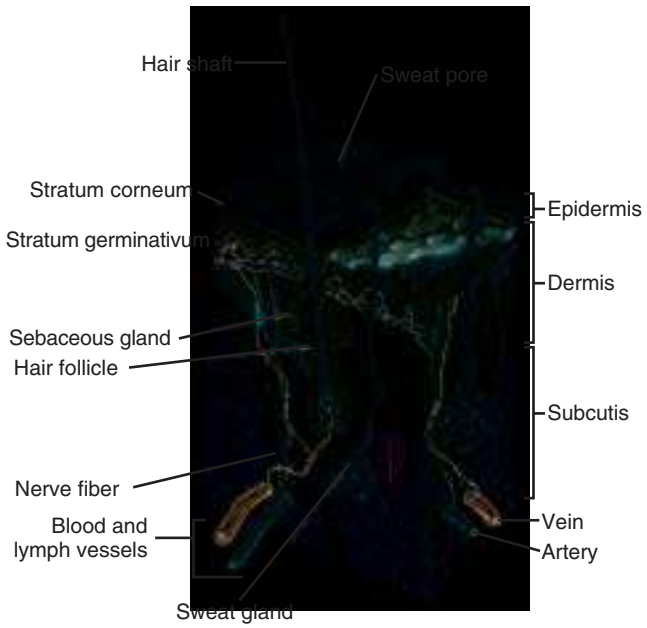

FIG. 38.1 Skin structures.

TABLE 38.1 Advantages of Topical Antibacterial Therapy

Ease of administration
Lower potential for adverse reactions
Lower risk of noncompliance
Delivery of high drug concentrations to site of infection
Decreased risk of bacterial resistance or antimicrobial cross-resistance
Cost savings (depending on agent used)

may be inactivated by blood and body fluids. Their microbicidal effects are the result of cell wall penetration, oxidation, and substitution of microbial contents with free iodine. Povidone-iodine's antibacterial effect is directly related to its desiccation, or drying, after application. Failure to allow the product to dry completely will result in incomplete antisepsis. For proper effect, povidone-iodine must be applied properly, according to the manufacturer's instructions, and allowed to dry thoroughly. Povidone-iodine has been widely used for many years in preoperative skin preparation, preparation of skin for blood culture phlebotomy, certain catheter placements, hand scrubbing, and the treatment and prevention of skin infections.[2]

Povidone-iodine in a 5% cream preparation has been studied for its in vitro activity against various strains of *S. aureus,* including methicillin-resistant strains (MRSA) and mupirocin-resistant strains. It has also been evaluated for its bioavailability in human nasal secretions and has demonstrated rapid bactericidal activity and good bioavailability within the human nares.[4] The cream formulation may play a role in the prevention of infection and eradication of nasal colonization by *S. aureus.*

Alcohols are rapidly bactericidal, but older preparations were not widely adopted because of their transient antiseptic action, local irritation, and excessive drying, especially when associated with repeated use. Over the last 15 years, newer waterless, alcohol-based hand hygiene products have been brought to market in the United States. These products contain 70% alcohol as the active antiseptic ingredient, and also contain emollients to minimize or eliminate the drying or irritating side effects. Alcohol-based hand sanitizers also differ from older preparations in that their antiseptic action is far more prolonged. Because these products are waterless, and there is no need for a sink or paper towels, alcohol-based hand sanitizers can be made widely available in and around patient care areas, which encourages more widespread use. Current guidelines on hand hygiene recommend these products over soap and water as the preferred method for cleaning unsoiled hands before and after patient encounters.[5]

Chlorhexidine, a cationic bisbiguanide that achieves its antiseptic activity by causing disruption of microbial cell membranes and precipitation of cell contents, is an ideal agent for skin cleaning and surgical scrubs. Its noteworthy properties include persistent activity on the skin when used regularly, rapid bactericidal activity, a broad antibacterial spectrum, little evidence of irritation or allergy, activity in the presence of body fluids, and minimal absorption. Chlorhexidine-containing products have become more widely available in the United States since the US Food and Drug Administration (FDA) approved a 2% tincture of a chlorhexidine gluconate (CHG) preparation for use as a skin antiseptic in 2000. CHG formulations containing 0.5% to 0.75% chlorhexidine are more effective than plain soap and water but less effective than preparations containing 4% chlorhexidine.[6] Preparations containing 2% chlorhexidine are slightly less effective than those containing 4%. When 0.5% to 1% CHG is combined with 70% alcohol-based preparations, the greatest antibacterial activity can be observed. In a study comparing 2% chlorhexidine, povidone-iodine, and 70% alcohol for sterile skin preparation of central venous and arterial catheter sites, the 2% chlorhexidine lowered rates of subsequent bloodstream infections (BSIs) significantly more than the other two preparations.[6] Chlorhexidine-containing preparations are not currently recommended for use in children 2 months of age or younger or in surgery involving the inner or middle ear because of the ability of chlorhexidine to cause ototoxicity. Current guidelines for the prevention of vascular catheter–related infections recommend CHG-containing products for preparation of the skin before catheter insertion.[7] Several additional uses of CHG-containing products are discussed later and include use as skin preparation before blood culture phlebotomy, use as a skin antiseptic agent in the preoperative setting, and use as a skin wash to prevent hospital-acquired infections (HAIs) in critically ill hospitalized patients.

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) is a synthetic bisphenol active against a broad range of gram-positive and gram-negative bacteria. It is a biocide that had been used extensively for many years in dental hygiene products and soaps and has been incorporated into plastic kitchenware products, toys, and tea towels.[8,9] In September 2016, the FDA removed triclosan and triclocarban from over-the-counter hand and body washes owing to lack of efficacy and concerns about systemic absorption and development of resistance; however, they are still present in many products.[10] A summary of the antimicrobial spectrum and characteristics of antiseptic agents is provided in Table 38.2.

Prophylaxis of Infection in Clean Wounds

When a wound leads to disruption of epidermal integrity (e.g., secondary to abrasions, cuts, or bites), the application of a topical antibacterial agent to prevent infection from developing can be considered. However, no antibacterial formulation has ever been proven to be efficacious in the prophylaxis of clean wounds because so few clean wounds become infected. Studies of topical preparations (e.g., neomycin alone or in combination with bacitracin, polymyxin, or both) have shown efficacy in the prevention of infection in some circumstances, although these studies have been criticized because of the absence of control groups.[11] Controlled studies are unlikely to ever be performed, and the effects of these agents on the microbiome are not known.

To avoid the difficulties in performing a large randomized controlled trial, a human skin infection model was developed to test the efficacy of topical antibiotic formulations in the prophylaxis of minor skin infections.[11] After the induction of abrasion-type wounds in human volunteers, the wounds were inoculated with either 10^5 organisms of *S. aureus* or 10^7 organisms of *Streptococcus pyogenes* and covered with an impermeable dressing for 6 hours. Both neomycin and bacitracin were more effective in preventing infection with both *S. aureus* and *S. pyogenes* than the placebo ointment vehicle. The treated wounds did not develop pus and reepithelialized within 3 to 5 days. Other investigators have also found that the use of either topical neomycin-bacitracin-polymyxin ointment or bacitracin alone enhanced the epidermal healing of wounds[12] and significantly reduced streptococcal skin colonization and the subsequent infection of small skin trauma in children.[13] Despite these studies, the efficacy of topical antibacterials in the prevention of infections in clean wounds and wound healing remains uncertain. The

TABLE 38.2 Antimicrobial Spectrum and Characteristics of Antiseptic Agents

GROUP	GRAM-POSITIVE BACTERIA	GRAM-NEGATIVE BACTERIA	MYCOBACTERIA	FUNGI	VIRUSES	SPEED OF ACTION	COMMENTS
Alcohols	+++	+++	+++	+++	+++	Fast	Optimum concentration 60%–95%; no persistent activity
Chlorhexidine (2% and 4% aqueous)	+++	++	+	+	+++	Intermediate	Persistent activity; rare allergic reactions
Iodine compounds	+++	+++	+++	++	+++	Intermediate	Causes skin burns; usually too irritating for hand hygiene
Iodophors	+++	+++	+	++	++	Intermediate	Less irritating than iodine; acceptance varies
Phenol derivatives	+++	+	+	+	+	Intermediate	Activity neutralized by nonionic surfactants
Quaternary ammonium compounds	+	++	–	–	+	Slow	Used only in combination with alcohols; ecologic concerns

[a]Hexachlorophene is not included because it is no longer an accepted ingredient of hand disinfectants.
+++, Excellent; ++, good but does not include the entire bacterial spectrum; +, fair; –, no activity or not sufficient.

use of topical antibacterials in superficial wounds for a few days, until the integrity of the epidermis is reestablished, has been recommended because the longer the epidermal barrier remains defective, the more likely it is that infection will occur; however, the strength of the evidence around this recommendation is weak.

The use of topical antibacterials in chronic nonhealing, noninfected wounds, such as pressure ulcers, has been recommended by the US Agency for Health Care Policy and Research.[14] The goal of such therapy is to decrease the bacterial burden in these wounds and possibly to promote healing. However, there is no substantial evidence in the published medical literature that this goal can be achieved.[15] A Cochrane review from 2014 included 45 randomized controlled trials with 4486 participants and found no evidence for the use of topical antiseptics to promote healing of chronic venous leg ulcers.[16] A clinical practice guideline from the American College of Physicians on treatment of pressure ulcers does not recommend use of topical antiseptic or antibiotic agents.[17] Moreover, the chronic use of topical antibacterials may be expected to promote bacterial resistance and should be avoided. None of the currently marketed topical antibacterials has been labeled for use specifically in the setting of chronic nonhealing wounds.[15] Therefore the use of topical antibacterials for chronic nonhealing wounds should be discouraged.

Prophylaxis of Recurrent Skin and Soft Tissue Infections

Some patients with recurrent furuncles, carbuncles, and other SSTIs caused by *S. aureus* may have persistent nasal carriage of this organism. It has been suggested that eradication of *S. aureus* from the nares could reduce the recurrence of these infections. One randomized trial treated such patients with a 5-day course of intranasal mupirocin and then randomized half the patients to receive successive monthly therapy for 1 year, and half to receive a placebo.[18] Intranasal mupirocin reduced the recurrence of furunculosis by approximately 50% in study subjects who were colonized with *S. aureus* in their nares. The small size of the study precluded the authors from making definitive recommendations for the management of recurrent furunculosis and folliculitis in staphylococcal carriers.

A 2011 open-label trial sought to determine whether there is benefit to topical mupirocin alone or in combination with other strategies, in the prevention of recurrent SSTIs caused by *S. aureus*. Fritz and colleagues[19] enrolled 300 patients with community-associated SSTIs and *S. aureus* colonization of the nares, axilla, or inguinal folds and randomly assigned them to one of four groups: no treatment, education only; twice-daily intranasal mupirocin ointment for 5 days; twice-daily intranasal mupirocin plus daily chlorhexidine body washes for 5 days; or twice-daily intranasal mupirocin plus daily 15-minute dilute bleach water soaks (one-fourth cup of bleach per tub of water). All groups received education about personal and household hygiene. Decolonization with mupirocin alone or in combination with chlorhexidine or bleach baths was more effective than education alone at eradicating colonization at 1 month (56%, 55%, and 63% vs. 38%, respectively). However, at 4 months the only regimen more effective than education alone in eradicating colonization was mupirocin plus bleach baths (71% vs. 48%). Recurrent SSTI was common, occurring in 20% of participants at 1 month and 36% of participants at 4 months, suggesting that factors other than endogenous colonization play a role in MRSA infection.[19] Many practitioners, including us, are now recommending dilute bleach baths twice a week for patients with recurrent community-associated MRSA infections. The bath is prepared by adding one-quarter cup of bleach to a bathtub of water. The patient must soak in the bath for 15 minutes twice weekly.[19] Nasal mupirocin is being used as part of a prophylactic regimen that may also include topical CHG washes, bleach bathing, oral antimicrobials, or any combination of the three, as a skin decolonization regimen. This is discussed in further detail later.

Prophylaxis of Infection in Operative Wounds

With more than 30 million surgical procedures performed annually in the United States, surgical site infections (SSIs) have become the most common and costly HAI. Current estimates suggest that 2% to 5% of the 30 million patients will develop an SSI. Most SSIs are caused by endogenous microbiota, including *S. aureus*. *S. aureus* is the etiologic agent of 20% to 30% of all SSIs. SSI prevention strategies have focused on (1) MRSA decolonization; (2) optimal methods to decrease bioburden on the host's skin before the procedure; and (3) determining the best antiseptic agent for skin preparation of the surgical site.

MRSA Decolonization

Several studies have been performed in a variety of surgical patients to evaluate the effectiveness of preoperative decolonization with intranasal mupirocin, alone or in combination with other products, on the subsequent rate of postoperative SSIs.

Universal Decolonization

The first large randomized controlled, multicenter trial examining use of intranasal mupirocin versus placebo enrolled 4030 patients undergoing general, cardiothoracic, neurosurgical, and gynecologic surgical procedures.[20] Of the 3864 patients included in the intention-to-treat analysis, 2.3% of mupirocin-treated patients developed an SSI, compared with 2.4% of the placebo recipients. In a secondary analysis of 891 patients who were noted to have *S. aureus* nasal carriage before surgery, those patients treated with mupirocin had a subsequent SSI rate of 4%, compared with their placebo-treated counterparts, who had a 7.7% rate of subsequent infection, a difference that was not statistically significant. The lack of a statistically significant outcome in this study resulted in part from the fact that the sample size calculation had 85% power to detect a relative reduction of 50% in the rate of *S. aureus* SSIs.[21]

Targeted Decolonization for Patients Who Screen Positive for *Staphylococcus aureus*

A study examined the impact of preoperative *S. aureus* screening and targeted decolonization on the incidence of postoperative MRSA colonization, intensive care unit (ICU) MRSA transmission, and SSIs in cardiac surgery patients. During the intervention period, subjects underwent nasal screening for methicillin-susceptible *S. aureus* (MSSA) and MRSA with polymerase chain reaction (PCR) assay, and colonized patients received intranasal mupirocin twice daily and chlorhexidine baths daily for 5 days. There were 2826 patients in the preintervention period and 4038 patients in the intervention period. Patients found to be colonized with MRSA received vancomycin plus cefazolin for surgical prophylaxis. The authors found that the intervention patients had risk-adjusted reductions in MRSA colonization. Increased duration of preoperative decolonization therapy was associated with decreased postoperative MRSA colonization (odds ratio, 0.73; 95% confidence interval [CI], 0.53 to 1.00; $P = .05$). This study provided evidence that preoperative *S. aureus* screening with targeted decolonization was associated with reduced MRSA colonization, transmission, and SSIs.[22]

A multicenter US study evaluated whether the implementation of MRSA decolonization as part of an evidence-based bundle was associated with a lower risk of *S. aureus* SSIs in patients undergoing cardiac operations or hip or knee arthroplasties. Patients whose preoperative nares screens were positive for MRSA or MSSA were asked to apply mupirocin intranasally twice daily for up to 5 days and to bathe daily with chlorhexidine-gluconate for up to 5 days before their operations. MRSA carriers received vancomycin and cefazolin or cefuroxime for perioperative prophylaxis; all others received cefazolin or cefuroxime. Patients who were MRSA negative and MSSA negative bathed with CHG the night before and the morning of their operations. Rates of SSIs were collected during the preintervention and postintervention periods. The primary outcome was complex *S. aureus* SSIs. These occurred in 36 of 10,000 operations in the preintervention period and 21 of 10,000 operations in the intervention period (relative risk [RR], 0.58; 95% CI, 0.37 to 0.92). The rates of complex *S. aureus* SSIs decreased significantly for hip or knee arthroplasties (difference per 10,000 operations, −17 [95% CI, −39 to 0]). There was not a demonstrated statistically significant decrease in SSIs among the patients undergoing cardiac surgery in this study (difference per 10,000 operations, −6 [95% CI, −48 to 8]; RR, 0.86 [95% CI, 0.47 to 1.57]).[23]

A meta-analysis of 17 studies assessed nasal decolonization (5 were randomized controlled trials and 12 were quasiexperimental studies); 10 studies included cardiac operations, 3 assessed joint arthroplasties, and 4 assessed other orthopedic procedures. In 16 of the 17 studies, mupirocin was used for decolonization, whereas in 1 study nasal CHG was used. A total of 11 studies examined decolonization regardless of whether the participants carried *S. aureus* in their nares. Nasal decolonization was associated with a significant decrease in *S. aureus* SSIs (pooled RR, 0.4; 95% CI, 0.29 to 0.55). In contrast, 6 other studies were designed to decolonize only patients who carried *S. aureus* in their nares. The pooled effect estimate in these 6 studies indicated that this approach was also associated with a significant decrease in *S. aureus* SSIs (RR, 0.36; 95% CI, 0.22 to 0.57). Nasal decolonization plus skin decontamination with CHG or triclosan was assessed in 6 studies. The pooled effect estimate showed protection against *S. aureus* SSIs (RR, 0.29; 95% CI, 0.19 to 0.44). None of the studies compared nasal decolonization alone with nasal decolonization plus skin decontamination.[24]

In summary, the data show that nasal decolonization with mupirocin plus skin decontamination with CHG decreases the risk of *S. aureus* SSIs in both patients with and those without *S. aureus* colonization of nasal passages. Most studies have been performed in cardiac and orthopedic populations. We believe that this is an appropriate strategy and has been considered both cost-effective and clinically effective.

Concerns with mupirocin such as cost and patient compliance (mupirocin must be applied for several days to be effective) have led researchers to seek alternate strategies for nasal decolonization. Povidone-iodine–based ointment for nasal decolonization can be applied 2 hours before surgical incision, and therefore there is less concern regarding patient compliance. A single-center study examined a prospective cohort of patients undergoing elective orthopedic surgery with hardware implantation. Patients were given CHG washcloths and oral rinse and nasal povidone-iodine solution to be used the night before and the morning of scheduled surgery. The SSI rate in the intervention group (1.1%) was significantly lower than in the control group (3.8%).[25]

One randomized trial has performed a head-to-head comparison of mupirocin ointment for 5 days before surgery versus 30-second applications of povidone-iodine 5% solution into each nostril within 2 hours of surgical incision. Both groups also received 2% CHG wipes. In the intention-to-treat analysis, a deep SSI developed after 14 of 855 operations in the mupirocin group and 6 of 842 operations in the povidone-iodine group; *S. aureus* deep SSI developed after 5 operations in the mupirocin group and 1 operation in the povidone-iodine group. This study shows that povidone-iodine may be considered an alternative to mupirocin as part of a multifaceted approach to reduce SSI.[26]

Topical Skin Antisepsis

The routine application of topical antiseptics to the skin before a surgical procedure is a standard practice. The choice of topical antiseptic in this setting has varied in the past. However, several publications and a meta-analysis all have concluded that preoperative skin site preparation with CHG-alcohol preparations are superior to povidone-iodine. In a randomized controlled trial of 849 patients undergoing clean contaminated surgical procedures, the SSI rate was significantly lower in the CHG-alcohol group (9.5%) compared with the povidone-iodine group (16%).[27-29] Chlorhexidine-alcohol may be superior because CHG is not inactivated by blood or serum and has longer residual activity than povidone-iodine. Some experts have suggested that when CHG-alcohol is compared with povidone-iodine plus alcohol, there may be no significant benefit to the CHG-containing product. In a study that compared a povidone-iodine–alcohol preparation with a CHG-alcohol preparation and a standard povidone-iodine paint with an isopropyl alcohol wash in between, researchers determined that SSI rates were the lowest in the period during which the povidone-iodine–alcohol product was in use.[30] A blinded randomized noninferiority trial compared iodine povacrylex–alcohol and chlorhexidine-alcohol for elective clean contaminated colorectal surgery. In the intention-to-treat analysis the use of iodine povacrylex failed to meet criteria for noninferiority for overall SSI prevention compared with chlorhexidine-alcohol.[31] To date, studies have suggested that CHG is superior to povidone-iodine. Whether addition of alcohol to povidone-iodine would make this a product equal to CHG remains debatable. However, studies have shown conflicting results, and therefore more data are needed in order to determine whether povidone-iodine with alcohol is noninferior to CG with alcohol.

Methods to Decrease Bioburden on Skin

The use of chlorhexidine bathing preoperatively, as part of a bundle of interventions, has become a trend in SSI prevention. Patients may be told to bathe the evening before, on the morning of, or on several occasions before the planned surgical procedure. Different CHG-containing products may be used, either a wash or a wipe. Bathing with any antiseptic preparation before surgery has no proven benefit, as concluded in a 2007 Cochrane Database systematic review.[32] A more recent meta-analysis of 16 trials included a total of 17,932 patients; 7952 patients used a chlorhexidine bath, and 9980 patients were allocated to various comparator groups. Overall, 6.8% of patients developed SSI in the chlorhexidine group compared with 7.2% of patients in the comparator groups. Chlorhexidine bathing did not significantly reduce overall incidence of SSI when compared with soap, placebo, or no shower or bath (RR, 0.90; 95% CI, 0.77 to 1.05; $P = .19$).[33] Given that CHG bathing is often part of a several-pronged approach to SSI reduction, it may not be possible to determine its efficacy as a single intervention.

Prophylaxis of Vascular Catheter–Related Infections

The use of topical antibacterial agents to prevent vascular catheter–related infections includes choice of appropriate skin site antiseptic preparation and the use of a topical agent applied to the catheter site as part of line site maintenance. Studies assessing skin site preparation before central venous catheter (CVC) insertion have demonstrated that 2%

CHG-alcohol preparations are superior to povidone-iodine preparations or 70% isopropyl alcohol–only preparations.[6] Current guidelines on the prevention of vascular catheter–related infections recommend CHG-alcohol preparations as the preferred skin site antiseptic.[7] After insertion, guidelines recommend disinfecting catheter hubs, connectors without needles, and injection ports before accessing the catheter with an alcohol-chlorhexidine preparation or 70% alcohol to reduce contamination.

A chlorhexidine-impregnated sponge applied as a standard CVC exit site dressing cover has been shown to reduce BSIs in several studies. A meta-analysis looking at chlorhexidine-impregnated sponge dressings demonstrated that its use was associated with a trend toward reduction of central line–associated bloodstream infections (CLA-BSIs).[33] A large randomized controlled trial comparing the chlorhexidine-impregnated sponge with standard dressings revealed that the impregnated sponge significantly reduced the incidence of CLA-BSIs from 1.3 to 0.4 per 1000 catheter-days.[34] Society guidelines recommend considering the addition of this dressing in several circumstances, including (1) in hospital units or patient populations with CLA-BSI rates higher than the institutional goal, despite compliance with an evidence-based prevention bundle; (2) in patients with limited venous access and a history of recurrent CLA-BSI; or (3) in patients at increased risk for severe sequelae from CLA-BSI, such as patients with a recently implanted intravascular device, prosthetic heart valve, or aortic graft.[7]

More recently, a chlorhexidine-impregnated gel dressing has been developed that allows continuous inspection of the insertion site. No studies have directly compared CHG foam and CHG gel–based dressing. However, a randomized control trial compared CHG-impregnated gel dressings and standard nonchlorhexidine dressings and found that in 1879 patients the rate of vascular catheter–related infection was 67% lower ($P = .0006$). The contact dermatitis rate was 1.1%.[35]

Applying a topical antibacterial agent to the vascular access site to decrease bacterial burden and prevent bacterial colonization of intravascular catheter sites is of unclear benefit. In one prospective evaluation of 827 random catheter insertions in which three regimens of catheter care (neomycin-bacitracin-polymyxin at insertion and every 48 hours vs. iodophor ointment at insertion and every 48 hours vs. no ointment) were used, no differences in catheter-acquired sepsis (2 patients in each group) or local inflammation (38.9% vs. 41.9% vs. 41.7%, respectively) were noted.[36] The only differences were in semiquantitative cultures of catheter tips, with 6 positive cultures in the neomycin-bacitracin-polymyxin group, 10 in the iodophor group, and 18 in the no-treatment group. In contrast, a randomized controlled trial of povidone-iodine in the prevention of infection in subclavian vein hemodialysis (HD) catheters found that povidone-iodine was associated with a significant decrease in the incidence of septicemia (5% vs. 18%; $P < .02$).[37]

Prophylaxis of Peritoneal Dialysis Catheter Infections

Catheter-associated infections in patients undergoing either HD or peritoneal dialysis are one of the most common reasons for hospitalization and catheter removal in this patient population. In patients who undergo peritoneal dialysis, a few strategies have demonstrated efficacy in prophylaxis against exit site and tunnel infections. In the absence of prophylaxis, the average rates of *S. aureus* exit site infections range from 0.34 to 0.41 episodes per patient-year.[38] Patients with *S. aureus* nasal colonization have exit site infection rates that are significantly higher (>0.5 infections per year).[39] Moreover, the rates of peritonitis in patients with one or more positive naris cultures for *S. aureus* have been shown to be significantly higher than they are in patients never colonized (0.24 vs. 0.08 per year).[40] With the use of some prophylaxis regimen, the rates of infection can be reduced to less than half these numbers.[41]

The application of povidone-iodine at the exit site of the peritoneal dialysis catheter was shown to be effective in reducing subsequent infection rates in some published studies.[41] However, this strategy has also failed to demonstrate effectiveness in other published data.[40] The overall effectiveness of povidone-iodine in this setting remains unclear. As discussed earlier, the use of intranasal mupirocin twice daily for 5 days for the treatment of *S. aureus* nasal carriage has been shown to be an effective strategy in decreasing *S. aureus* carriage and catheter infections in patients who are proven nasal carriers.[42-44] The long-term effectiveness of intranasal mupirocin is approximately 60%, and therapy may need to be repeated either monthly or, if routine cultures are performed, whenever cultures are found to be positive for *S. aureus*. In a prospective open trial of intranasal mupirocin, peritoneal dialysis patients were treated with mupirocin when naris cultures were positive for *S. aureus*.[43] Compared with historical control subjects, exit site infections were significantly decreased from 0.22 per patient-year among control subjects to 0.09 per patient-year in the mupirocin-treated patients. Topical intranasal mupirocin also resulted in a significant decrease in the subsequent rates of *S. aureus* peritonitis in this study, although the rates of peritonitis caused by gram-negative organisms increased over the study period. The only randomized, double-blind, placebo-controlled trial of monthly intranasal mupirocin was performed by the Mupirocin Study Group in nine centers throughout Europe.[45] Peritoneal dialysis patients with positive naris cultures for *S. aureus* were randomized to receive twice-daily intranasal mupirocin or placebo for 5 days every 4 weeks and followed for 18 months. Although the rate of *S. aureus* exit site infections was significantly lower in the mupirocin group in this study, the total rate of exit site infections, tunnel infections, and peritonitis was not significantly different between treated and placebo recipients. Of note, in a cost-effectiveness analysis, it was not cost-effective to use prophylactic topical intranasal mupirocin in chronic peritoneal dialysis patients.[46]

Another prophylactic strategy is the application of topical mupirocin to the exit site as part of the routine daily care of the peritoneal dialysis catheter. In a prospective, randomized study comparing oral rifampin with topical mupirocin applied daily to the exit site, the rates of infection were compared with those of historical control groups.[47] The rates of *S. aureus* exit site infections, tunnel infections, peritonitis, and catheter loss caused by *S. aureus* infections were not statistically different between the two treatment groups, but they were significantly lower than those in the historical control group. In another prospective, controlled, historical study, in which a group of peritoneal dialysis patients were treated with topical mupirocin at the exit site three times weekly,[48] there was a significant reduction in *S. aureus* exit site infections (21 vs. 3 episodes) and *S. aureus* peritonitis (35 vs. 11 episodes); however, the patients were not screened for *S. aureus* carrier status. In several smaller studies (none of which were randomized, placebo controlled, or blinded), topical mupirocin was applied to the exit site either daily or three times weekly, and the results were similar with respect to decreased rates of peritoneal dialysis catheter–related infections.[40,49,50] A more recently published experience also advocated exit site mupirocin in peritoneal dialysis patients.[49] However, no cost-effectiveness analyses have been performed for the use of topical mupirocin in this patient population.

A systematic review looked at seven studies that compared topical gentamicin and topical mupirocin for prevention of peritoneal dialysis–associated exit site infections. There were 458 patients in the mupirocin group and 448 in the gentamicin group. The risk of gram-positive exit site infection was similar between the groups. The gram-negative exit site infection rate was higher in the mupirocin group (RR, 2.125; $P = .037$). Of the 7 studies, 6 also assessed the risk of bacterial peritonitis. There was no difference in the gram-positive or gram-negative peritonitis rate.[51]

We agree with the International Society of Peritoneal Dialysis 2016 guidelines, which recommend daily use of either topical gentamicin or mupirocin cream or ointment at the peritoneal dialysis exit site.[52]

Prophylaxis of Hemodialysis Catheter Infections

Catheter-associated BSI rates in the HD patient population remain high, even as BSI rates in hospitalized patients continue to decline.[53] Several potential prophylactic strategies have been evaluated in the HD catheter population, some of which include the application of topical antibacterial agents, including topical mupirocin, topical povidone-iodine, or topical Polysporin, to the exit site, and intranasal mupirocin.[40] Several studies have looked at the use of topical mupirocin at the catheter exit site. Studies of topical mupirocin used at the HD catheter exit site have shown decreased incidence rates of exit site infections and bacteremia and longer times to onset of bacteremia.[54] In one such randomized, controlled, open-label trial of topical exit site mupirocin application in

patients with tunneled, cuffed HD catheters, a total of 50 HD patients were randomized to topical mupirocin applied to the catheter exit site three times weekly versus no treatment.[54] The rates of *S. aureus* nasal carriage in the two groups were similar. Compared with control subjects, the mupirocin-treated patients had significantly fewer incidents of catheter-related bacteremia (7% vs. 35%) and had a longer time interval to the first incident of bacteremia. Median catheter survival was also significantly longer in the treated group.[54] A Cochrane Database systematic review in 2010 concluded that topical mupirocin reduced HD catheter–associated BSIs.[55] The study determined that there was insufficient evidence to support the use of povidone-iodine ointment, Polysporin ointment, honey, or a variety of topical catheter site dressings in prevention of BSI. Topical mupirocin is an effective strategy in prevention of bacteremias in HD catheter patients.

Although not determined to be definitively effective in a Cochrane Database review, povidone-iodine ointment is recommended in the 2011 Centers for Disease Control and Prevention guidelines for prevention of catheter-related BSIs in HD patients.[7] In a randomized open-label study of 129 patients, exit site infection and bacteremia were significantly higher in the control group compared with patients provided povidone-iodine ointment.[37] Another single-site, prospective, randomized, open-label study evaluated the use of povidone-iodine at the exit site of the HD catheter, along with a sterile dressing, versus sterile dressing alone.[56] At the onset of this study, 22% of the patients in the treatment group and 32% of those in the placebo group were positive for *S. aureus* nasal carriage. The incidence of bacteremia was significantly higher in the control group compared with those treated with povidone-iodine—17% versus 2% incidence. Similar results were found with respect to exit site infections in this study. The value of povidone-iodine ointment is likely real, and additional studies in which povidone-iodine ointment is used as part of catheter site care are needed. We believe that topical mupirocin is an effective strategy in prevention of bacteremias in HD catheter patients; however, a contrary view is taken by the authors of Chapter 300, who do not recommend topical mupirocin around tunneled HD catheter exit sites. These differences in approach reflect the paucity of truly comparative data.

The efficacy of intranasal mupirocin as a preventive strategy for HD catheter bacteremias or exit site infections has been studied in several published trials. However, topical mupirocin at the exit site is currently recommended and preferred over intranasal mupirocin. In one study, intranasal mupirocin was used three times daily for 2 weeks after catheter insertion, followed by three times weekly as a maintenance regimen, versus placebo.[57] All patients were positive for *S. aureus* nasal carriage before enrollment and were followed for 9 months. The authors observed a significant decrease in nasal colonization in the mupirocin-treated group and a significant decrease in the rate of *S. aureus* infections. However, the rates of *S. aureus* bacteremia did not differ between the treated group and the placebo recipients. A subsequent prospective open-label study performed by the same group used intranasal mupirocin in HD patients three times weekly for 6 months and then once weekly for 6 months.[58] The study patients were compared with historical control subjects and were found to have had a statistically significant decrease in *S. aureus* bacteremia over the study period. Another prospective open trial assessing intranasal mupirocin applied once weekly in HD patients showed significantly decreased rates of *S. aureus* bacteremia in the mupirocin-treated study patients compared with historical control subjects.[59] A single cost-effectiveness analysis of intranasal mupirocin in HD patients suggested that such a strategy was cost-effective.[60]

Prophylaxis of Health Care–Associated Infections

Despite ongoing prevention efforts over the last decade across the United States and globally, HAIs continue to result in morbidity, mortality, and increased costs. Twenty percent of HAIs are acquired in the critical care setting, and risk of HAI increases with length of critical care stay.[61] The three most common critical care HAIs are catheter-associated urinary tract infection (CA-UTI), CLA-BSI, and ventilator-associated lower respiratory tract infections. Over the last decade, national device-associated HAI rates have declined somewhat, in particular because of implementation of bundled prevention strategies. The use of daily CHG bathing has been proved to be an effective method of both reducing the development of HAIs and preventing colonization of critical care patients with multidrug-resistant organisms (MDROs).[62-65]

In the largest published prospective study to date, daily bathing with 2% CHG–impregnated washcloths was implemented in a multicenter, cluster-randomized, nonblinded crossover trial.[62] Primary outcomes included incidence rates of MDRO acquisition and rates of CLA-BSIs. Nine critical care settings or bone marrow transplant units were used. Patients were randomized to either daily bathing with CHG for 6 months or use of a nonantimicrobial bathing cloth, and in the second 6 months the daily bathing product was alternated. More than 7700 patients were enrolled, and a 23% reduction in MDRO acquisition rate was found. The MDRO acquisition rate was 5.1 cases per 1000 patient-days in the CHG bathing group, compared with 6.6 cases per 1000 patient-days in the standard bathing group; this was statistically significant. CHG-bathed patients had a CLA-BSI rate of 4.78 per 1000 patient-days, compared with 6.6 CLA-BSIs per 100 patient-days in the control group. This translated to a 28% lower rate of CLA-BSIs in those patients bathed with CHG, and this result was also statistically significant.

A 2012 meta-analysis to assess the efficacy of daily CHG bathing in reducing HAI occurrences in ICU patients concluded that there was benefit to the practice.[63] One randomized and 11 nonrandomized trials were included in the analysis. CHG bathing led to a significant reduction in BSI rates and CLA-BSI rates, with a pooled odds ratio of 0.44. The authors noted that there was a wide variation across studies with respect to the type of product and concentration of CHG, along with differences in use of other infection-control practices, such as active surveillance cultures, nasal mupirocin use, and enhanced hand hygiene. Of note, adverse events were extraordinarily rare.

A more recent cluster-randomized crossover study of 9340 patients admitted to five ICUs in a tertiary medical center in the United States used a 10-week period of bathing with 2% CHG washcloths, a 2-week washout period, and then 10 weeks of bathing with nonbacterial washcloths.[66] The primary outcome was a composite of CLA-BSI, CA-UTI, ventilator-associated pneumonia (VAP), and *Clostridioides difficile* (formerly *Clostridium difficile*) rate. The study found no reduction in the primary outcome. However, of note, this study had low rates of HAIs and used a composite end point instead of looking at only CLA-BSI.

In summary, most studies have shown that bathing patients in the ICU with CHG decreases rates of CLA-BSI, acquisition of MRSA and vancomycin-resistant enterococcus (VRE), and blood culture contamination rates. It is unclear what the impact of CHG bathing is on CA-UTI, VAP, and *C. difficile* rates.

Researchers have also addressed the question of whether targeted or universal decolonization to prevent ICU infection or colonization is effective.[64] Forty-three hospitals with 74 ICUs participated in a cluster-randomized trial assessing three strategies: MRSA screening and isolation (standard practice), targeted decolonization (for patients who were MRSA-screen positive), or universal decolonization. Patients in the universal decolonization study group were not assessed for MRSA carrier state. Universal decolonization included daily CHG bathing for the entire ICU stay plus 5 days of nasal mupirocin on admission for all patients. Compared with baseline preintervention rates, universal decolonization reduced MRSA acquisition rates and rates of BSIs caused by any pathogen more often than either standard practice or targeted decolonization. The cost of universal decolonization was approximately $40 per patient. Researchers determined that 181 patients would need to be decolonized to prevent one MRSA infection, whereas 54 patients would require decolonization to prevent one ICU BSI. Because CHG bathing and nasal mupirocin were used together in this study, it is not possible to determine whether the infection reduction was from CHG or mupirocin. This study adds to the growing literature identifying benefits of CHG bathing and nasal decolonization.

Daily CHG bathing in pediatric critical care unit patients has also been studied and resulted in a nonsignificant reduction in subsequent bacteremia.[67] CHG-impregnated cloths were used in all patients older than 2 months during the study period, and no adverse events were observed in this trial.

CHG is generally well tolerated, with a very low incidence of immediate adverse effects. Rarely, CHG may cause an allergic contact dermatitis

or local irritation. With the potential widespread adoption of CHG bathing in ICU settings, the question of CHG resistance is a concern.[68] Multidrug efflux pumps, encoded in plasmid-borne *qacA/B* genes, may lead to CHG resistance.[69] The issues of CHG resistance and the effects on the microbiome will need to be the focus of future studies as CHG bathing becomes a more common practice in health care settings.

Prophylaxis of Infection in Burn Wounds

The prevention of infection in the burned patient is extremely difficult because burn wound sites are favorable for bacterial overgrowth, the epidermal barrier is often defective for extended periods, and the patients are in the hospital, where multiple antibiotic-resistant organisms are found.[2] Frequent débridement and the establishment of an epidermis, or a surrogate such as a skin graft or skin substitute, are essential for the prevention of infection. As a result of the pathogenesis and pathophysiology of the burn wound, the delivery of systemic antimicrobial therapy to the deepest, most severely ischemic areas of the wound cannot be relied on because gradient diffusion from the wound periphery is the sole means of access.[70]

The use of topical antibacterial agents for burned patients is well established. Before the development of effective topical burn wound chemotherapy, burn wound sepsis was diagnosed as the principal cause of death in 60% of lethal burn injuries[71]; the use of mafenide acetate has reduced to 28% the incidence of burn wound sepsis as a cause of death. After administration, high antimicrobial concentrations are found on the wound surface, where the risk of bacterial contamination is the greatest. In patients with deep, extensive wounds, dense bacterial colonization, particularly by gram-positive cocci, often occurs within 24 hours; aerobic gram-negative bacilli typically appear within 3 to 7 days. If this initial bacterial colonization is not treated, deeper spread and ultimately systemic invasion of pathogenic bacteria can occur. Therefore, topical antibacterial therapy should be initiated as soon as possible to delay or prevent these processes.

There is evidence that effective topical antibacterial therapy delays colonization of the burn wound for a variable period (measured in days, not weeks), maintains the bacterial density of the wound at lower levels than those that could otherwise be achieved and for appreciable intervals (measured in weeks), and tends to result in a relatively homogeneous and less diverse wound flora than what would otherwise be expected.[70] The specific antimicrobial agent chosen for topical therapy should have a broad in vitro spectrum of activity against gram-positive cocci (staphylococci, streptococci, and enterococci) and the aerobic gram-negative microbiota (including *Pseudomonas aeruginosa*). Ideally, the agent should penetrate the eschar, but because it may be absorbed, it must have low toxicity[72]; the agent must also remain active in the presence of serum and necrotic debris. Furthermore, with the increasing use of cultured skin grafts in the therapeutic approach to the burned patient, topical antibacterials may be required to prevent microbial colonization and the destruction of grafts containing cultured skin cells. The successful use of topical agents prevents the bacterial conversion of superficial burns to deeper injury, results in the spontaneous healing of wounds that initially appeared clinically to be of full thickness, and decreases the frequency of episodes of systemic sepsis.[73] Specific topical agents for use in burned patients are discussed in Chapter 314.

Treatment of Pyoderma

There is no role for topical antibacterials in the treatment of erysipelas, cellulitis, or furuncles.

However, there is a role for topical agents in the management of one specific type of pyoderma—impetigo. Impetigo is a superficial infection of the skin caused by group A streptococci (see Chapter 197), *S. aureus,* or both. Bullous impetigo is usually caused by *S. aureus.* One of the goals of antimicrobial therapy in impetigo is to prevent the spread of infection to uninvolved skin.[2] Early uncontrolled and controlled trials of topical antibacterial therapy in patients with impetigo suggested the efficacy of topical antibacterials, although other studies found that systemic antimicrobial therapy was more efficacious.[70] Systemic antimicrobial therapy is somewhat superior to topical therapy in the management of streptococcal pyoderma, with swifter healing and fewer failures. However, topical therapy may be used early in infection, when the number of lesions is small and there is a reasonable chance that these agents will be scrupulously and skillfully applied.[74] Exclusions to the use of topical antibacterials in pyoderma include the following: bullous impetigo, because the pathogenesis of this exfoliative infection may lead to continuing infection, rapid spread, recurrence, or all of these, unless *S. aureus* is promptly eradicated; and extensive pyoderma, regardless of the clinical form or bacterial cause.[75] Topical mupirocin has been shown to be as efficacious as systemic antibiotics in the therapy for limited impetigo (see later).

Retapamulin, a newer topical antibacterial, has been approved for use in adults and children 9 months of age and older. Retapamulin administered twice daily for 5 days was compared with placebo or fusidic acid in two separate double-blind, randomized trials in patients with impetigo. The response rates for retapamulin were 85.6% versus 52.1% for placebo and 94.8% for retapamulin versus 90.1% for fusidic acid. Retapamulin was found to be superior to placebo and not inferior to fusidic acid. The two most commonly identified pathogens in these studies were *S. aureus* and *S. pyogenes*, with similar response rates against both organisms.

Topical antibacterial agents may have some efficacy in the therapy for secondary types of pyoderma, although the available studies generally did not include control groups.[74] Despite organism eradication, the underlying process persisted. Therefore a cure (in the sense of complete healing) was not achieved. Because topical antibacterials can lower the bacterial colony counts in acute dermatitis, the use of these agents in combination with topical glucocorticoids is a logical treatment regimen.[2]

Treatment of Erythrasma and Rosacea

Erythrasma is a cutaneous eruption caused by the bacterium *Corynebacterium minutissimum.* The rash of erythrasma is commonly found in intertriginous areas that include axillae, inframammary areas, the interspaces of toes, and intergluteal and crural folds. Once the diagnosis has been confirmed, systemic antibacterial therapy with erythromycin is usually prescribed as first-line therapy.[76] However, there is an important role for topical antibacterial therapy in the management of this dermatologic infection. Most experts recommend the addition of a topical agent to systemic therapy in patients with intertriginous area involvement and the exclusive use of topical therapy in those patients intolerant of the recommended active systemic therapies.[76] Topical 2% clindamycin has been shown to be effective in the treatment of erythrasma and, when it is in an alcohol-based formulation, has the additional advantage of a drying effect.[77] Topical fusidic acid has also been shown to be effective as a topical treatment for erythrasma, but is not available in the United States. Topically administered erythromycin, tetracycline, and chloramphenicol have all been evaluated and found not to be effective in the treatment of erythrasma.[76]

Rosacea is a dermatologic condition with several disease phases that include flushing, followed by erythrosis, papulopustular rosacea, and finally phimosis. The etiology of rosacea remains somewhat controversial and is considered to be multifactorial, depending on the phase of disease. *Helicobacter pylori* has been believed by some to be a potential causative factor, and the *Demodex* mite has been implicated as a significant contributing factor of papulopustular rosacea.[78] There are no clear data that rosacea has a bacterial etiology, although the disease clearly responds to both systemic and topical treatment with antibacterial agents. Metronidazole, in either a 1% cream or a 0.75% gel, is the topical therapy that has been most widely evaluated in the treatment of papulopustular rosacea and the preceding erythrosis. Numerous randomized controlled trials have confirmed the tolerability and superior efficacy of topical metronidazole.[79,80] Because topical metronidazole has no antiinflammatory effects, its mechanism of action with respect to a cure for rosacea remains unknown but is presumably antimicrobial. Topical clindamycin, retinoids, and azelaic acid in a 20% cream formulation have all also been identified as acceptable topical alternatives in the management of rosacea.[78]

Ivermectin is an antiparasitic agent that also targets overgrowth of *Demodex* mites and has antiinflammatory properties. Two randomized, double-blind, placebo-controlled trials have assessed the efficacy and safety of 1% topical ivermectin for papulopustular rosacea. At 12 weeks' follow-up both trials showed "clear" or "almost clear" on the Investigator

Global Assessment of Rosacea Severity Score (IGA-RSS) scale. No adverse effects were reported.[81]

Treatment of Acne Vulgaris

Topical antibacterials are helpful for inflammatory acne.[82,83] The proliferation of *Cutibacterium* (formerly *Propionibacterium*) *acnes* is considered critical for the development of inflammatory lesions. The blocked follicles become an ideal anaerobic culture medium filled with nutrients in the form of lipid substrates; *C. acnes* metabolizes the lipid, producing free fatty acids, and this may be a triggering mechanism that leads to retention hyperkeratosis and microcomedone formation. Benzoyl peroxide exerts its effects by bacteriostatic activity on the proliferation of *C. acnes.* Oxygen is liberated when the drug is decomposed by cysteine in the skin, and bacterial proteins are thus oxidized. After 2 weeks of daily application, a 10% benzoyl peroxide preparation reduces concentrations of free fatty acids by about 50% and *C. acnes* by about 98%, comparable to the levels obtained after 4 weeks of treatment with antibiotics.

Topical antibiotics are used almost universally by dermatologists for the treatment of acne vulgaris.[82,83] These agents also exert their beneficial effects by decreasing the population of *C. acnes* in the follicle, although not as effectively or rapidly as benzoyl peroxide, and they also inhibit the production of proinflammatory mediators by organisms that are not killed.[82] Preparations containing clindamycin and erythromycin are the most commonly used[82,83]; topical tetracyclines have also been used but are less effective than either clindamycin or erythromycin. Topical azelaic acid, a dicarboxylic acid derivative that is bacteriostatic for *C. acnes,* has been shown to reduce colony counts of *C. acnes* by about the same degree as clindamycin. However, the most effective topical antibacterial regimens against *C. acnes* are the combination formulations that include benzoyl peroxide and either erythromycin or clindamycin. A randomized 10-week trial comparing the efficacy of erythromycin (3%)–benzoyl peroxide (5%) gel with that of erythromycin (4%)–zinc (1.2%) solution in 72 acne vulgaris patients revealed that both inflammatory lesions and comedones showed a significantly greater percentage reduction from baseline in those patients receiving the erythromycin–benzoyl peroxide combination[84]; both physician and patient efficacy evaluations were also more favorable for this regimen. Additional studies have also demonstrated the efficacy of the combination formulation of benzoyl peroxide with erythromycin, and this regimen is considered one of the most effective in the treatment of acne. The combination formulation of 1% clindamycin and 5% benzoyl peroxide gel has been marketed and evaluated for its effectiveness in the treatment of acne vulgaris.[85] Several clinical trials have demonstrated that the twice-daily application of this product for 10 to 16 weeks was more effective in reducing the number of inflammatory lesions than 5% benzoyl peroxide, 1% clindamycin, or placebo gel in patients with mild to moderately severe acne.[86,87] In another multicenter blinded trial, clindamycin–benzoyl peroxide gel was directly compared with benzoyl peroxide alone and the erythromycin–benzoyl peroxide combination formulation.[88] The clindamycin–benzoyl peroxide was significantly more effective in reducing the mean number of inflammatory lesions than benzoyl peroxide alone, and it was similar in efficacy to erythromycin–benzoyl peroxide. Both patient and physician assessments at the end of the study indicated that global improvement was significantly greater with clindamycin–benzoyl peroxide than with benzoyl peroxide alone and similar to that identified with erythromycin–benzoyl peroxide. Therefore, the clindamycin–benzoyl peroxide and erythromycin–benzoyl peroxide formulations are equally efficacious and can both be considered first-line topical therapies in the treatment of acne vulgaris. Use of antibiotic-based preparations are typically for 3 months, but studies of long-term effects have not been performed. Dapsone 5% gel has also been studied and has been found to be particularly useful in inflammatory acne and in females.[89,90]

Elimination of *Staphylococcus aureus* Nasal Carriage

Decolonization to eliminate MRSA nasal carriage is a complex topic beyond the scope of this chapter. The general role of decolonization in the prevention of MRSA infections is unclear. Decolonization has been attempted in colonized hospital patients, in outpatients with recurrent MRSA infections, and as part of a universal decolonization protocol without assessment of MRSA carrier state. It has also been used in the prevention of dialysis catheter infections and in the prevention of SSIs, as discussed earlier. The optimal approach to MRSA decolonization for outpatients and inpatients is not known. Whether inpatient decolonization should be attempted only in the setting of outbreaks or more universally is also undecided. Currently, there is no definitive evidence to support routine MRSA decolonization of inpatients. However, decolonization may be reasonable in the setting of hospital outbreaks, for outpatients with recurrent MRSA infections, and within households where ongoing MRSA transmission is documented despite the implementation of other hygiene measures.[91,92,93]

With respect to decolonization in hospitalized patients, it is well known that 20% to 40% of healthy persons carry *S. aureus* in their anterior nares. In hospitalized patients, serious infection caused by *S. aureus* may occur from autoinoculation to susceptible sites or the transfer of organisms from another patient or staff member who is a carrier. Attempts to control hospital outbreaks have included methods to eradicate nasal carriage of staphylococci by means of systemic antimicrobial agents with or without topical treatment; however, recolonization is frequent, and the development of resistance has been reported.[94] Several topical antibacterial agents have been used in the nasal eradication of *S. aureus,* with varied degrees of success.

A systematic review of this subject was published by Ammerlaan and colleagues in 2009.[95] This review included all known studies investigating the eradication of *S. aureus* carriage. Of the 2388 studies identified, 23 met the authors' inclusion criteria, all of which were published between March 1977 and October 2008. Of these, 13 studies evaluated mupirocin, with either placebo or active comparator arms. In summary, short-term nasal application of mupirocin was shown to be effective at eradicating *S. aureus,* with an estimated 90% probability of success 1 week after treatment and approximately 60% after longer-term follow-up (ranging from 14 to 365 days). This compares with an estimated success rate from oral antibiotic administration of 60% 1 week after treatment and 50% after longer follow-up. The effectiveness of mupirocin was comparable among MSSA and MRSA carriers. Long-term duration of effect is not to be expected, and many patients will eventually recolonize. Decolonization using mupirocin has had variable efficacy in other groups, including a study of soldiers, in which no difference was found in MRSA infection rates when mupirocin was compared with placebo.[96]

Many experts recommend combination topical therapy with nasal mupirocin and CHG bathing, with or without oral antibiotics, when MRSA decolonization is desired.[92] A 5- to 10-day total course with the topical therapies is suggested. Longer courses should be avoided because of the potential for adverse effects and development of resistance. Once decolonization is complete, routine screening cultures are not recommended.[85] The value of repeated decolonization attempts is unknown.

A noteworthy concern associated with mupirocin decolonization is the development of acquired drug resistance. Based on the studies evaluated by Ammerlaan and colleagues,[95] the use of mupirocin is associated with a 1% risk of acquiring a drug-resistant strain during therapy. Although this is a low rate, recent surveillance studies have demonstrated mupirocin-resistant MRSA in up to 13% of the patients in institutions that do not practice routine mupirocin use and up to 65% in patients in areas where there is widespread use of mupirocin.[41]

SPECIFIC TOPICAL ANTIBACTERIALS

Numerous topical antibacterial agents are available for clinical use in various concentrations, vehicles, and mixtures (Table 38.3). Although they are used as topical agents, antimicrobials such as clindamycin, erythromycin, tetracycline, and gentamicin are covered in other chapters of this book. This section provides additional information on the four most common topical antimicrobials.

Bacitracin

Mechanism of Effects

Bacitracin is a polypeptide antibiotic produced by *Bacillus subtilis.* There are three bacitracin subgroups: A, B, and C. Subgroup A is the major constituent of commercial preparations.[97] Bacitracin contains a thiazoline ring and peptide side chains. After administration, it forms a complex

TABLE 38.3 Selected Topical Antibacterial Agents in Clinical Use

TOPICAL ANTIBACTERIAL (TRADE NAME)	CONCENTRATION	FREQUENCY	INDICATION
Azelaic acid (Azelex)	20% cream	Twice daily	Acne vulgaris
Benzoyl peroxide	2.5%–10%	Once to twice daily	Acne vulgaris
Clindamycin (Cleocin,[a] Clindagel)	1%, 2%	Twice daily	Acne vulgaris; bacterial vaginosis
Clindamycin–benzoyl peroxide (BenzaClin)	1%–5%	Twice daily	Acne vulgaris
Erythromycin (Emgel)	2%	Twice daily	Acne vulgaris
Erythromycin–benzoyl peroxide (Benzamycin)	3%–5%	Twice daily	Acne vulgaris
Fusidic acid (Fucidin)[b]	2%	Three times daily	Skin infections; eradication of nasopharyngeal carriage of *Staphylococcus aureus*
Mafenide (Sulfamylon)	—	Twice daily	Burns
Metronidazole (MetroGel, MetroCream, MetroLotion, Noritate)[c]	0.075%	Once to twice daily	Inflammatory pustules, papules, and rosacea; bacterial vaginosis
Mupirocin (Bactroban)	2% ointment	Three times daily	Skin infections; elimination of nasopharyngeal carriage of *S. aureus*
Polymyxin B–bacitracin–neomycin (Neosporin)[d]	5000 units/g–400 units/g–3.5 mg/g	One to three times daily	Prevention of infection in minor cuts, scrapes, and burns
Polymyxin B–neomycin–hydrocortisone (Cortisporin)	10,000 units/g–3.5 mg/g–0.5%	Two to four times daily	Corticosteroid-responsive dermatoses with secondary infection
Retapamulin (Altabax)	1% ointment	Two times daily for 5 days	Impetigo caused by *S. aureus* or *Streptococcus pyogenes*
Silver sulfadiazine (SSD, Silvadene)	1%	Once to twice daily	Burns

[a]The 2% formulation is recommended for bacterial vaginosis.
[b]Not licensed in the United States.
[c]Supplied in a 1% cream and given once daily for inflammatory lesions and erythema of rosacea.
[d]Maximum strength formulation contains 10,000 units/g polymyxin B, 500 units/g bacitracin, and 3.5 mg/g neomycin.

with C55-isoprenyl pyrophosphate, a component of the bacterial cell wall. This molecule acts as a carrier involved in the transfer of polysaccharides, peptidoglycans, and lipopolysaccharides to the growing cell wall. Therefore, formation of the bacterial cell wall is impaired.

In Vitro Spectrum of Activity

The activity of bacitracin is primarily against gram-positive organisms: staphylococci, streptococci, corynebacteria, and clostridia.[97] The development of resistance to bacitracin is rare, although it has been reported in *S. aureus*.

Clinical Uses

Topical bacitracin has been used for many years, although its efficacy in controlled clinical trials has never been shown. In impetigo, bacitracin ointment was shown to be 80% effective in clearing pathogenic organisms,[75] although slow or delayed healing was noted in one-third of those patients cured. Bacitracin was the least effective in bullous impetigo, in which four of six patients continued to develop new lesions, requiring systemic erythromycin therapy. Furthermore, in a trial comparing topical bacitracin with topical mupirocin or oral cephalexin for the treatment of impetigo, the treatment failed in most patients (six of nine) treated with bacitracin.[98] Bacitracin has also been evaluated in the eradication of nasal carriage of *S. aureus,* although its efficacy has never been shown.[99] For topical use, bacitracin is often formulated with neomycin, polymyxin B, or both (see Table 38.2).

Adverse Effects

Toxicity with bacitracin is minimal. Minor skin irritation may occur. Cases of anaphylaxis have been reported after the topical administration of bacitracin to open lesions[97]; these patients had previous multiple exposures to the drug. In addition, there have been rare reports of anaphylaxis after the use of bacitracin as an irrigating solution in the intraoperative setting and when used topically in conjunction with nasal packing after rhinoplasty.[100,101] Ready access to the systemic circulation appears to be a prerequisite for the development of anaphylaxis from this externally applied agent. On rare occasions, allergic contact dermatitis has been reported. Because bacitracin and polymyxin B are both derived from the *Bacillus* species, cross-reactivity between the two agents may occur.

Neomycin

Mechanism of Action

Neomycin is an aminoglycoside antibiotic isolated from cultures of *Streptomyces fradiae*.[97] The mechanism of action involves inhibition of protein synthesis by binding to the 30S subunit of the bacterial ribosome, leading to misreading of the genetic code; neomycin may also inhibit the bacterial DNA polymerase.

In Vitro Spectrum of Activity

Neomycin has in vitro activity against many gram-positive and gram-negative bacteria, including *Escherichia coli, Haemophilus influenzae, Proteus* species, *S. aureus,* and *Serratia* species. *P. aeruginosa* is generally resistant.[102] There is minimal in vitro activity against streptococci, although at the high concentrations achieved on the skin, *S. pyogenes* organisms are probably killed by topical neomycin preparations.[2] Resistance to neomycin has been reported in both gram-positive and gram-negative bacteria[74,97] and can be plasmid mediated; resistance to other aminoglycosides, such as kanamycin and gentamicin, may be present on the same plasmid.

Clinical Uses

Neomycin is widely used in combination with other antibiotics, antifungals, and corticosteroids because of its availability, relatively low cost, and perceived efficacy.[10,74] There are few well-controlled clinical trials documenting the efficacy and safety of topical neomycin.[75] Neomycin has been shown to enhance reepithelialization in wound healing.[11] However, in view of its well-documented contact sensitivity, possible systemic toxicity, and cross-reactivity with other antibiotics and because of the emergence of resistance, it is difficult to recommend the use of topical neomycin in the treatment of superficial skin infections.[97]

Adverse Effects

Neomycin is not absorbed through intact skin, although application to denuded or damaged epithelium can lead to sensitization and systemic

toxicity.[102] After systemic absorption, neomycin is excreted by the kidney. Patients with decreased renal function may develop ototoxicity, which is irreversible and may progress after discontinuation of the drug. Allergic contact sensitivity is widely reported, with a prevalence of 1% to 6% and an incidence of 3% to 6%.[102,103] The incidence of hypersensitivity, as assessed with patch testing in 390 patients with suspected contact dermatitis from topical medications, was approximately 36%.[104] Sensitivity is most likely to occur with chronic use on inflamed skin. Sensitivity to neomycin was reported in 49% of patients with a history of allergy to any topical agent.[97] Neomycin may also cause mast cell degranulation and histamine release.

Polymyxin B

Mechanism of Action

Polymyxin B is isolated from the aerobic gram-positive rod *Bacillus polymyxa,* a soil organism (see Chapter 32). The polymyxins are cationic, branched, cyclic decapeptides that destroy bacterial membranes with a surface detergent–like mechanism by interacting with membrane phospholipids and increasing cellular permeability.

In Vitro Spectrum of Activity

The spectrum of activity of polymyxin B is almost exclusively limited to gram-negative organisms. The agent is bactericidal against many aerobic gram-negative organisms, including *P. aeruginosa,* but it is not active against *Proteus* species and is poorly active against *Providencia, Burkholderia,* and *Serratia* species. There is no in vitro activity against gram-positive organisms. Although *Pseudomonas* is usually sensitive, the in vitro activity of polymyxin B against *Pseudomonas* is promptly neutralized by divalent cations at concentrations in body fluids. Organisms resistant to polymyxin B have cell walls that prevent access of the drug to the bacterial cell membrane. There is no cross-resistance with other antimicrobial agents, and resistance rarely develops during therapy.

Clinical Uses

Polymyxin B is used primarily in the prevention and treatment of minor skin infections. It is most often added to neomycin and bacitracin (see earlier) to broaden coverage against gram-negative organisms.

Adverse Effects

Because polymyxin B binds to cell membranes with very high affinity, there is little systemic absorption, and there are few reactions even when applied to open wounds. Contact sensitization has been reported.

Mupirocin

Structure and Mechanism of Action

Mupirocin has a chemical structure unlike that of any other antimicrobial agent,[105,106] containing a short fatty acid side chain (9-hydroxy-nonanoic acid) linked to monic acid by an ester linkage. Mupirocin is formulated in a bland water-miscible ointment base consisting of polyethylene glycol 400 and polyethylene glycol 3350. Mupirocin used to be called pseudomonic acid because its major metabolite is derived from submerged fermentation by *Pseudomonas fluorescens*. Pseudomonic acid A represents 90% to 95% of the pseudomonic acid family and is responsible for most of the antibacterial activity; three other minor metabolites of similar chemical structure and antimicrobial spectrum have been denoted as pseudomonic acids B, C, and D.[98] Mupirocin inhibits bacterial RNA and protein synthesis by binding to bacterial isoleucyl–transfer RNA (tRNA) synthetase, which catalyzes the formation of isoleucyl-tRNA from isoleucine and tRNA.[105,106] This prevents the incorporation of isoleucine into protein chains of the bacterial cell wall, leading to the arrest of protein synthesis. Because of its unique structure and unique mechanism of action, mupirocin does not cross react with other antimicrobial agents.

In Vitro Spectrum of Activity

Mupirocin is bacteriostatic at low concentrations near the minimal inhibitory concentration (MIC) for *S. aureus,* but it is bactericidal at concentrations achieved by topical administration (20,000 µg/mL with the 2% formulation) after 24 to 36 hours of exposure.[105] It is highly active in vitro against MRSA, staphylococcal strains resistant to other antibacterials (e.g., penicillin, streptomycin, neomycin, erythromycin, fusidic acid, lincomycin, chloramphenicol, and tetracycline), and streptococci that are associated with primary and secondary skin infections.[105] The exception to the antistreptococcal activity of mupirocin is the enterococci. Mupirocin is inactive in vitro against *P. aeruginosa,* anaerobes, fungi, and the Enterobacteriaceae. An important feature of the antibacterial spectrum of mupirocin is its weaker in vitro activity against the normal skin flora (e.g., *Micrococcus, Corynebacterium,* and *Propionibacterium*), which is part of the skin's natural defense against infection. The in vitro antibacterial activity of mupirocin is the greatest at an acidic pH, which is advantageous because of the low pH of the skin; in one study, mupirocin was fourfold to eightfold more active in vitro at pH 6 than at pH 7.

Long-term therapy with mupirocin can lead to the development of resistant staphylococci,[105,107] an effect that is irreversible. Staphylococcal isolates with low- or intermediate-level resistance have MICs in the range of 8 to 256 µg/mL, whereas isolates with MICs at or greater than 512 µg/mL demonstrate high-level resistance. This resistance can be induced in *S. aureus* by subculturing the organisms onto media containing increasing concentrations of the drug. Naturally occurring clones of staphylococci with low-level resistance to mupirocin have been described, although their clinical significance is unclear because the concentration of mupirocin in ointment exceeds 20,000 µg/mL. High-level mupirocin resistance, associated with clinical treatment failure, has emerged in both MRSA and *Staphylococcus epidermidis.*[108] Most mupirocin-resistant staphylococcal isolates have been found in patients with chronic skin infections, many of whom had been treated with prolonged courses of mupirocin.[106] Researchers from France reported that a 5-day course of intranasal mupirocin for decolonization of a patient with glycopeptide-intermediate *S. aureus* (GISA) failed to eradicate this isolate, even though the strain was susceptible in vitro to mupirocin.[109] The patient went on to develop nosocomial pneumonia with the GISA strain, and the strain had developed resistance to mupirocin at repeat testing. Several mechanisms have been advanced to explain mupirocin resistance in staphylococci. Low-level resistance is most likely mediated by altered access to binding sites on isoleucyl-tRNA synthetase, whereas high-level resistance appears to be mediated by a transferable plasmid carrying the *mupA* gene, which encodes a modified isoleucyl-tRNA synthetase.[106] It has been suggested that high-level resistance may have evolved by the conjugate transfer of plasmids from enterococci,[110] which are inherently resistant to mupirocin; the conjugate transfer of high-level mupirocin resistance has also been observed among coagulase-negative staphylococci.[111] A study has demonstrated that two different isoleucyl-tRNA synthetase enzymes are present in highly mupirocin-resistant *S. aureus* isolates (MIC ≥512 µg/mL), whereas only a chromosomally encoded isoleucyl-tRNA synthetase enzyme was detected in strains expressing intermediate levels of resistance (MIC 8 to 256 µg/mL). Commercially available Etests (bioMérieux, Durham, NC) to detect mupirocin resistance in *S. aureus* are available and are quite accurate in differentiating low-level from high-level resistance patterns.[112] Etests compare favorably with the more traditional disk diffusion method and MIC determination in the detection of resistance.[113] Adding to the concerns about mupirocin resistance is the identification of the gene encoding high-level mupirocin resistance in USA300 MRSA clones, one of the most common clones found in community-acquired MRSA infections.

Pharmacokinetics

After systemic administration, mupirocin is immediately metabolized to monic acid, which is bacteriologically inactive and rapidly eliminated (plasma half-life, <30 minutes). Mupirocin is not appreciably absorbed after topical administration to intact skin. In one study, a mean of 0.24% of applied radiolabeled ointment was absorbed through intact skin after 24 hours of occlusion.[107] Greater penetration of mupirocin is expected in damaged or diseased skin. However, any drug that is absorbed is converted to monic acid, formed by deesterification of mupirocin at the ester linkage between the side chain and the nucleus, and rapidly eliminated in the urine.[90] Skin can also metabolize mupirocin to its inactive metabolite but at a rate less than 3%. Therefore, because only small amounts of mupirocin penetrate the skin and equally small amounts are degraded, most of the drug is available to act at the skin level.

Because mupirocin is highly protein bound (approximately 95%), its activity decreases in the presence of serum.[98]

Clinical Uses

Mupirocin is used primarily in skin infections, such as impetigo and folliculitis, which are usually caused by *S. aureus* and *S. pyogenes,* to decolonize the nares in outbreak settings and as prophylaxis against a variety of catheter-related infections and SSIs. In trials of mupirocin alone in the therapy for impetigo (772 evaluated patients),[114–116] clinical cure rates ranged from 81% to 100% and bacterial elimination rates from 67% to 100%. Several trials have shown mupirocin to be more effective in the treatment of impetigo than its polyethylene glycol vehicle, which also has antibacterial activity. Clinical cure and bacterial elimination rates have ranged from 85% to 100% and from 80% to 95%, respectively, for mupirocin versus from 12% to 84% and from 12% to 63%, respectively, for the vehicle.[114–117] Of eight comparative trials of mupirocin versus several other topical antibacterials (neomycin, fusidic acid, chlortetracycline, polymyxin B–bacitracin–neomycin),[114,118,119] six found mupirocin to be the most effective agent. Multiple studies have also compared topical mupirocin with systemic antibiotics (erythromycin, cloxacillin, dicloxacillin, flucloxacillin, ampicillin, and cephalexin) in the therapy for impetigo, and topical mupirocin was found to be as or more effective than the oral agent.[98,114] However, in many of these studies, the entry criteria excluded patients who, in the judgment of the investigator, had too many lesions to allow reliable compliance with topical therapy. No studies have shown that topical therapy is as effective as systemic antimicrobial therapy for the treatment of widespread, extensive lesions.

Mupirocin is effective for the treatment of secondarily infected eczema, burns, lacerations, and leg ulcers. In a study in which 33 centers contributed a total of 1030 cases,[120] mupirocin ointment produced significantly better bacteriologic and clinical responses than its vehicle in the treatment of secondary skin infections. Of 851 evaluated patients with 1131 pathogens, mupirocin eliminated 87% (505/583) of pathogens versus only 53% (288/548) for the vehicle. In a double-blind, vehicle-controlled study, mupirocin successfully eradicated 85% of *S. aureus* in 33 patients, compared with a 6% eradication rate in the vehicle-treated group[121] for all pathogens; the success rate of mupirocin versus the vehicle was 69% versus 14%, respectively. Comparative trials have also demonstrated the efficacy of mupirocin compared with other topical antibacterials and systemic antimicrobial agents in the treatment of secondary skin infections.

The use of mupirocin to eliminate nasal carriage of *S. aureus* in a variety of settings that include outbreaks, preoperative patients, and chronic dialysis patients is discussed earlier in this chapter.

Adverse Effects

Mupirocin is not associated with substantial toxicity in humans because of its very low affinity for mammalian isoleucyl-tRNA synthetase. The propylene glycol base may irritate mucous membranes and eroded skin. There is minimal potential for inducing allergic contact dermatitis; only two cases have been reported.[122] The drug is not phototoxic. Local effects, such as itching, stinging, or rash, have been reported when mupirocin is used on broken skin or mucous membranes. Chronic exposure to topical mupirocin rarely has been associated with the rupture of polyurethane peritoneal dialysis catheters, which may be caused by the vehicle portion of the preparation interacting with the polyurethane in the catheter.[123] No photosensitivity reactions have occurred. Mupirocin is listed as a pregnancy category B drug. In experimental animal models, doses were used that were between 14 and 43 times greater than usual human doses, and no teratogenic effects, embryotoxicity, or fertility or reproductive issues were identified.[120] Prolonged use may lead to the overgrowth of nonsusceptible organisms such as fungi.

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The complete reference list is available online at Expert Consult.

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39 Antimycobacterial Agents

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SHORT VIEW SUMMARY

Therapy for Tuberculosis *(Mycobacterium tuberculosis)* (see Chapter 249)

- Isoniazid, rifampin, pyrazinamide, and ethambutol are first-line agents for drug-susceptible tuberculosis. Doses of antiretroviral agents may need adjustment (see Table 39.3).
- Initiation of treatment for human immunodeficiency virus (HIV) infection usually does not need to be delayed in patients starting treatment for tuberculosis (see Table 39.2 and http://aidsinfo.nih.gov/guidelines).
- Rifabutin is used instead of rifampin in patients receiving protease inhibitors and nonnucleoside reverse transcriptase inhibitors for HIV infection (see Table 39.5).
- Rifapentine is recommended for directly observed therapy along with isoniazid, both given once weekly for 12 weeks for latent tuberculosis.
- Fluoroquinolones, amikacin, kanamycin, streptomycin, capreomycin, linezolid, para-aminosalicylic acid, cycloserine, and ethionamide are second-line agents used for drug-resistant *M. tuberculosis* infection. Fluoroquinolones are the most important of these agents.
- Bedaquiline and delamanid are available for drug-resistant tuberculosis.

Therapy for Hansen Disease *(Mycobacterium leprae)* (see Chapter 250)

- Dapsone, rifampin, and clofazimine are first-line agents. Clofazimine use is restricted (see text).

Therapy for Other Mycobacterial Infections

- Macrolides are the most important drugs for treating macrolide-susceptible nontuberculous mycobacteria isolates including *Mycobacterium avium-intracellulare* complex and susceptible *Mycobacterium abscessus* subsp.
- Tetracyclines, macrolides, and quinolones are discussed more extensively in Chapters 26, 29, and 35.

Drugs for mycobacterial infections are discussed in three groups: drugs primarily for the treatment of infections caused by *Mycobacterium tuberculosis,* drugs for infections caused by nontuberculous mycobacteria (NTM), and agents principally for the treatment of Hansen disease (leprosy). Approaches to antituberculous chemotherapy have been affected by the increasing prevalence of multidrug-resistant *M. tuberculosis* (MDR-TB), defined as resistance to at least isoniazid (INH) and rifampin,[1–3,4,5] and extensively drug-resistant *M. tuberculosis* (XDR-TB), defined as resistance to at least INH and rifampin, resistance to any fluoroquinolone, and resistance to at least one second-line injectable drug,[5,6,7,8,9,10] and by the special impact on *M. tuberculosis* of human immunodeficiency virus (HIV) infection.[5,11,12,13]

MDR-TB and XDR-TB strains require the use of drugs that are considered second-line agents as well as drugs that must be administered by injection.[14] The list of agents active against MDR-TB and XDR-TB strains is limited; however, more recent development and introduction of new antituberculous drugs has significantly improved the outlook for successful treatment of *M. tuberculosis* strains resistant to traditional first-line and second-line agents. The current pace of new tuberculosis drug development is unprecedented and cause for optimism even in the presence of increasing MDR-TB and XDR-TB disease prevalence. However, it still must be emphasized that universal supervised therapy is essential to prevent the emergence of acquired drug resistance and to optimally treat both drug-susceptible and drug-resistant *M. tuberculosis* infection.[15,16]

MDR-TB and XDR-TB infections are especially concerning in individuals infected with HIV with or without acquired immunodeficiency syndrome (AIDS) because the host contribution to controlling the infection is severely diminished. HIV-infected patients have a number of other special problems. They are especially prone to adverse drug reactions.[13,17] The susceptibility of protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) to hepatic metabolism induced by rifamycins, especially rifampin and rifapentine, necessitates regimens that exclude these agents.[13,17] In addition, malabsorption of antituberculous drugs can occur in patients with AIDS, resulting in suboptimal antituberculous serum drug levels, which predisposes to acquired drug resistance.[18]

The early years of HIV disease saw a dramatic increase in the prevalence and severity of infections caused by *Mycobacterium avium* and other NTM, especially in their disseminated forms. However, the combination of the macrolide group (clarithromycin and azithromycin) with ethambutol was active against *M. avium* and other NTM even in patients with markedly impaired cell-mediated immune defenses. In recent years, with great advancement in antiretroviral therapy (ART) and the use of disseminated *M. avium* prophylaxis, the incidence of disseminated disease in advanced HIV infection has markedly declined. The effects of HIV infection and AIDS on Hansen disease and its chemotherapy do not appear to be as great as expected.

Traditionally, antimicrobial agents for tuberculosis have been classified as first-line drugs, having superior efficacy with acceptable toxicity, and second-line drugs, having less efficacy and greater toxicity. Several excellent reviews of antimycobacterial agents and therapy are available,[14,19,20,21,22,23,24] including guidelines for therapy for MDR-TB infection.[14,20,21] Effective treatment of both MDR-TB and XDR-TB strains requires detailed information about extended drug susceptibilities to guide individualized treatment regimens.[8,14,20,25]

Antituberculous drugs differ in their mechanism of bactericidal action and in their delivery to tuberculous lesions. Three of the first-line agents—INH, rifampin, and ethambutol—are active against the large populations of tubercle bacilli in cavities. Streptomycin (now considered a second-line agent), other aminoglycosides, and capreomycin penetrate cells poorly and are inactive at acidic pH. Pyrazinamide (PZA), the fourth first-line agent, is inactive at the neutral or slightly alkaline pH that may occur extracellularly and is active only in acidic environments such as within macrophages. Slowly replicating organisms in necrotic foci are killed by rifampin and less readily by INH.

First-line antituberculous agents except ethambutol are bactericidal. The bactericidal activities of both INH and rifampin against tubercle bacilli in cavitary, intracellular, or necrotic foci provide the basis for the efficacy of short-course INH-rifampin regimens. A combination of three bactericidal agents that are active against intracellular organisms—INH, rifampin, and PZA—is essential for the 2-month initiation phase of the standard 6-month regimen currently recommended for drug-susceptible disease in the United States. A residual population consisting

of virtually nonreplicating tubercle bacilli within necrotic foci is especially difficult to eradicate, perhaps explaining the minimum of 4 months of continuation phase therapy needed even in individuals with competent immune defenses.

Much of the following discussion of individual antimycobacterial agents is taken from existing guidelines. In collaboration with other organizations, the US Centers for Disease Control and Prevention (CDC) recently updated treatment guidelines for tuberculosis.[21] Additionally, new technologies such as the widely available tuberculosis GeneXpert and gene sequencing, available through the CDC, offer the clinician the ability to rapidly (within days) detect drug-resistant *M. tuberculosis* strains to limit empirical use of ineffective and toxic second-line tuberculosis medication regimens. The reader is encouraged to contact the CDC website for details about the indications and procedures for using rapid molecular detection of drug resistance identification technologies (http://cdc.gov/tb/topic/laboratory/mddrusersguide).

Chemical structures of the major drugs discussed in this chapter are shown in Fig. 39.1.

FIRST-LINE ANTITUBERCULOUS DRUGS

Isoniazid

Derivation and Structure

Isoniazid, isonicotinic acid hydrazide (INH), a synthetic agent, was introduced in 1952.

Mechanism of Action

INH is bactericidal against actively growing *M. tuberculosis* and bacteriostatic against nonreplicating organisms. It acts by inhibition of synthetic pathways of mycolic acid, an important constituent of mycobacterial cell walls. It also likely inhibits the catalase-peroxidase enzyme coded for by the gene *katG*.

Antimicrobial Activity and Resistance

Against *M. tuberculosis,* 0.025 to 0.05 μg/mL of INH is inhibitory, and higher concentrations are bactericidal against replicating organisms. When INH is administered alone as monotherapy in the presence of active tubercular disease, resistance to INH will emerge. Initially susceptible isolates become resistant in more than 70% of cases treated with INH monotherapy for 3 months. Resistance results from selection under antimicrobial pressure of resistant mutants of *M. tuberculosis* that number 1 in 10^6 among untreated bacillary populations. Large populations such as the 10^9 to 10^{10} bacilli in pulmonary cavities are especially likely to contain significant numbers of inherently resistant tubercle bacilli. Low-level INH resistance, defined as an INH minimal inhibitory concentration (MIC) for *M. tuberculosis* of greater than 0.1 μg/mL but less than 1.0 μg/mL, is most commonly associated with point mutations or short deletions within the catalase-peroxidase gene *(katG)*, which still produces some enzymatic activity, whereas high-level resistance, defined as an INH MIC for *M. tuberculosis* of greater than 1.0 μg/mL, is associated with major deletions within the gene with loss of all enzymatic activity.[26,27] Most experts recommend treating patients with low-level INH resistance in the same manner as if they had higher level in vitro INH resistance with the exception that INH retains sufficient activity in this situation to justify inclusion in the treatment regimen. Resistance in the regulatory region of a second gene involved in mycolic acid synthesis *(inhA)* also confers INH resistance.[26,27] The incidence of INH resistance among new cases of tuberculosis in the United States in 2016 was 8.7%, occurring primarily in people born outside the United States including immigrants from Africa, Southeast Asia,

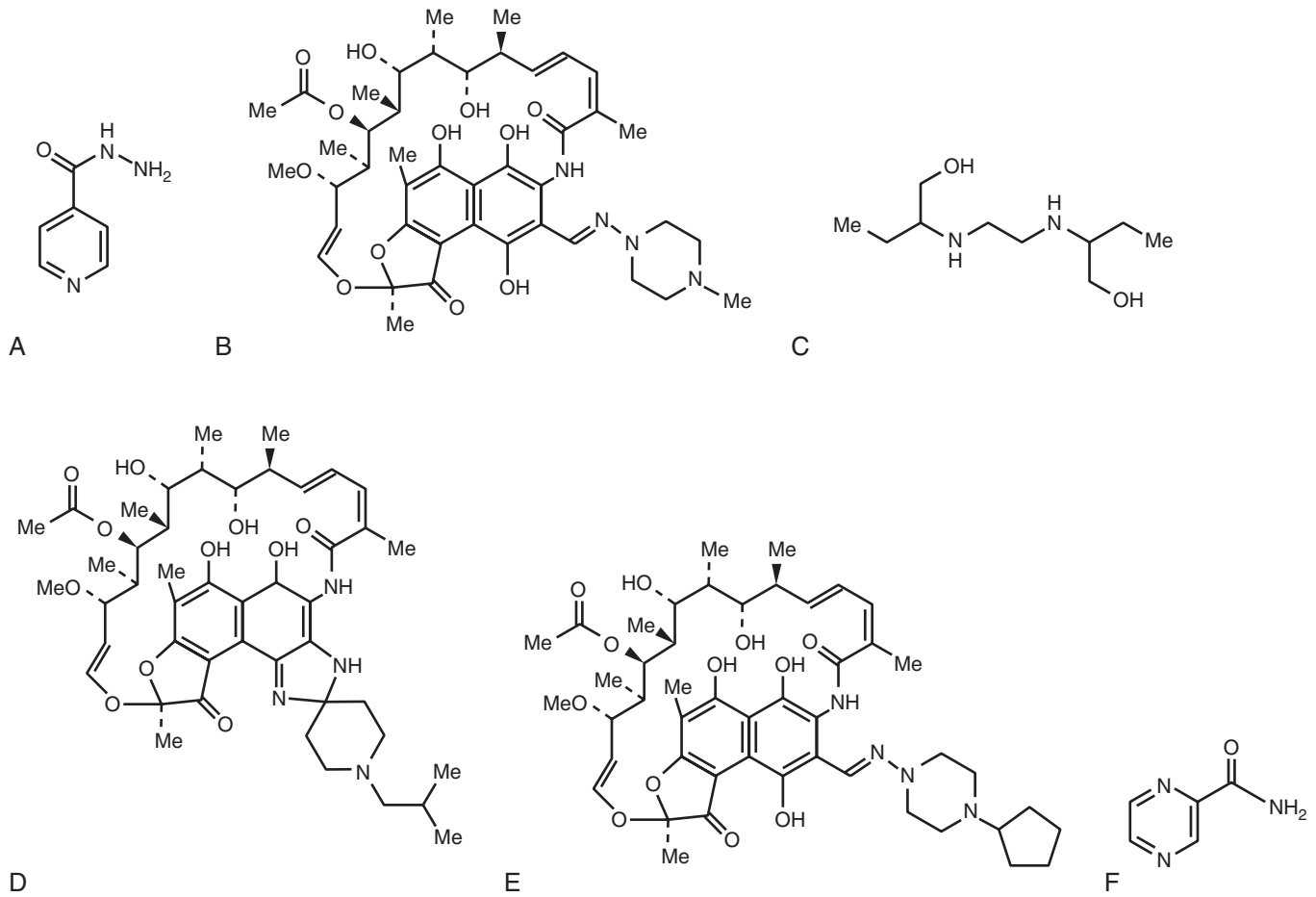

FIG. 39.1 Structures of major antimycobacterial agents. (A) Isoniazid. (B) Rifampin. (C) Ethambutol. (D) Rifabutin. (E) Rifapentine. (F) Pyrazinamide.

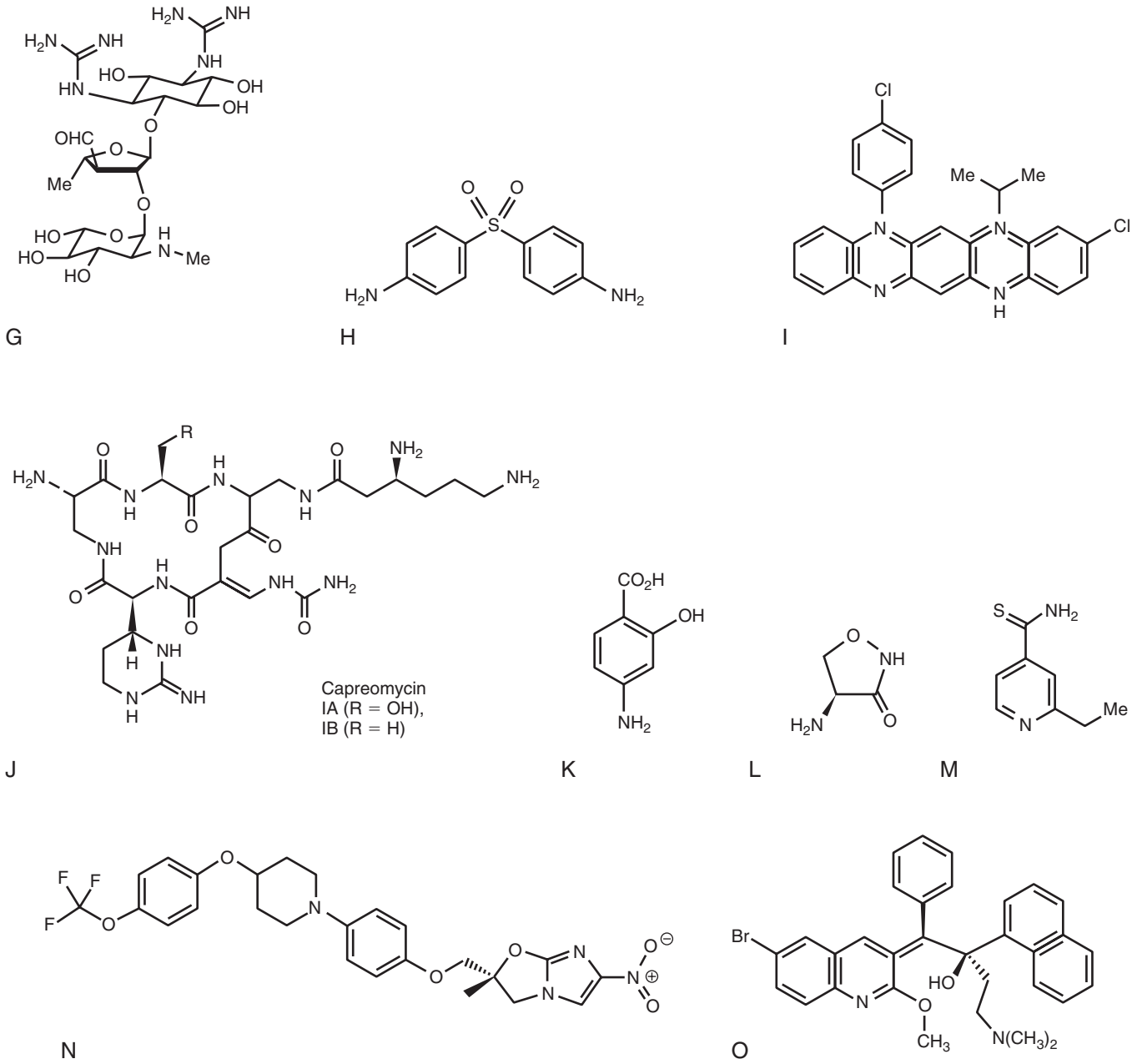

FIG. 39.1, cont'd (G) Streptomycin. (H) Dapsone. (I) Clofazimine. (J) Capreomycin. (K) Para-aminosalicylic acid. (L) Cycloserine. (M) Ethionamide. (N) Delamanid. (O) Bedaquiline.

Eastern Europe, and Central America, where INH resistance is more common.[11,12,28]

Pharmacology

INH is well absorbed orally or intramuscularly and is distributed throughout the body. Cerebrospinal fluid (CSF) levels are generally about 20% of plasma concentrations but may approach plasma levels in the presence of meningeal inflammation. Coadministration with vitamin C appears to inactivate INH suspensions markedly.[29]

Metabolism of INH occurs initially by hepatic *N*-acetyltransferase. Diminished acetylation capacity is inherited as an autosomal recessive trait that varies from a 5% prevalence rate in indigenous people in Canada to 83% in Egyptians. Ten percent to 15% of Asians are "slow" acetylators, as are 58% of whites in the United States. Six hours after a 4-mg/kg oral dose, slow acetylators exhibit plasma INH levels of more than 0.8 µg/mL, and rapid acetylators have levels of less than 0.2 µg/mL.[20] The striking bimodal distribution of plasma half-lives of INH depending on acetylator status generally does not affect the outcome with daily therapy because plasma levels are maintained well above inhibitory concentrations. Metabolically altered INH is principally excreted in urine along with lesser amounts of unaltered drug. Dosage modification in renal insufficiency is not necessary. The benefit of dosage adjustment with significant hepatic disease is not established. Table 39.1 summarizes dosage modifications for INH and other antituberculous drugs in hepatic or renal failure.

Adverse Reactions

Hepatitis. INH has infrequent major toxicities, most notably hepatitis. Of INH recipients, 10% to 20% have asymptomatic minor elevations in serum aspartate aminotransferase levels that usually resolve even with continued therapy.[30] A meta-analysis of six studies estimated the rate of clinical (symptomatic) hepatitis in patients given INH alone to be approximately 0.6%.[31] More recent data indicate that the incidence of clinical hepatitis is even lower. Hepatitis occurred in

TABLE 39.1 Need for Dosage Modification for Antituberculous Drugs in Hepatic or Renal Failure

ANTIMICROBIAL DRUG	MODIFY IN HEPATIC FAILURE	MODIFY IN RENAL FAILURE
Isoniazid	No	No
Pyrazinamide	Yes	Yes
Ethambutol	No	Yes
Rifampin	?Yes	No
Rifabutin	?Yes	No
Amikacin	No	Yes
Capreomycin	No	Yes
Kanamycin	No	Yes
Streptomycin	No	Yes
Quinolones	No	Yes (levofloxacin, not moxifloxacin)
Para-aminosalicylic acid	No	Yes
Ethionamide	Yes	No
Cycloserine	No	Yes

only 0.1% to 0.15% of 11,141 patients receiving INH alone as treatment for latent tuberculosis infection (LTBI) in an urban tuberculosis control program.[32]

Early estimates of the incidence of severe or major INH hepatotoxicity were provided from the results of a large multicenter US National Institutes of Health (NIH)–sponsored trial. Fatal hepatitis occurred in 8 of nearly 14,000 patients receiving INH.[33] All but one of the deaths occurred in one study center, and patients did not receive routine monitoring for toxicity during the trial.[33] More recent studies, however, suggest that the rate of fatal INH-related hepatitis is substantially lower.[32,34,35] The likely explanation is the adoption in the early 1980s of uniform clinical toxicity monitoring for patients receiving INH for treatment of LTBI.[34,35] Hepatotoxicity can occur at any time but generally occurs after weeks to months of therapy rather than days to weeks after treatment has begun. INH hepatotoxicity is correlated with age, presumably owing to a diminished capacity for repair of INH-induced hepatocellular damage in elderly patients. Undernutrition may also play a role in the expression of INH hepatotoxicity.[36] Hepatotoxicity is increased in the following groups: patients who abuse alcohol, patients with preexisting liver damage,[33] pregnant women and women up to 3 months postpartum,[37] patients also taking INH in combination with acetaminophen,[38] patients receiving other potentially hepatotoxic agents such as rifampin,[35] patients with active hepatitis B, and HIV-seropositive patients receiving highly active antiretroviral therapy (HAART).[39] Histologically, hepatocellular damage can progress to submassive necrosis. Although active hepatitis B is considered a contributing factor to INH hepatotoxicity,[40,41] INH has been safely administered to patients with acute hepatitis[42] and for LTBI to patients with chronic hepatitis B and C infection.[39,43,44] Educating patients about the recognition of symptoms of INH-induced liver disease is key in preventing its progression. As noted, routine clinical monitoring of patients receiving INH is mandatory.[35]

Routine monitoring of serum hepatic enzyme concentrations is not indicated for all patients at the start of treatment of LTBI. Baseline testing is recommended for patients whose initial evaluation suggests a liver disorder, patients infected with HIV who are receiving HAART, pregnant women and women in the immediate postpartum period (i.e., within 3 months of delivery), patients with a history of chronic liver disease (e.g., hepatitis B or C, alcoholic hepatitis, or cirrhosis), individuals who use alcohol regularly, and other individuals who are at risk for chronic liver disease.[35,39] Baseline testing is no longer routinely indicated in people older than 35 years of age.[35,39] Laboratory monitoring during treatment of LTBI is indicated for patients whose baseline liver function test results are abnormal and for other individuals at risk for hepatic disease.[35,39,45] Although biochemical monitoring may contribute to patient confidence and adherence with the treatment regimen, the contribution of routine biochemical monitoring to the safety of INH administration is not proven. The importance of routine clinical monitoring is clear, however, and clinical monitoring must be applied rigorously with or without biochemical monitoring.

The most feared INH-related toxicity is fulminant hepatic failure necessitating liver transplantation or resulting in death. The CDC conducted a detailed analysis of 17 patients with severe INH-related hepatotoxicity over a 4-year period.[46] The estimated incidence of death and liver transplantation was 1 in 150,000 to 1 in 220,000 patients receiving INH therapy for LTBI. Although continuation of INH after the onset of hepatitis-related symptoms was associated with severe hepatotoxicity, some patients still developed severe hepatotoxicity after stopping INH within days to a week of symptom onset. Although it is not universally protective, patients should be strongly advised to discontinue INH therapy at the onset of symptoms consistent with incipient hepatitis such as nausea, loss of appetite, and abdominal pain. Perhaps most disconcerting, there were two children younger than 15 years of age in this series of patients. The overall conclusions of this analysis were that severe hepatotoxicity was idiosyncratic, occurred at any time during INH treatment, occurred even with careful clinical and biochemical monitoring and with appropriate (recommended) doses of INH, and could occur in children.[46]

Most hepatotoxicity subsides after discontinuation of INH. Cautious readministration of INH after resolution of hepatitis for selected patients has been reported to be well tolerated and safe,[47] although most experts recommend alternative therapies such as rifampin for LTBI.[35,39] Recognition of the frequency and severity[48] of INH hepatotoxicity has not curtailed therapeutic usage but has led to a revision of indications for treatment of LTBI, with special caution indicated for groups identified at high risk for INH hepatotoxicity (see earlier discussion).[35,39,48]

Neurotoxicity. Peripheral neuropathy has been described in 17% of recipients of 6 mg/kg/day of INH but is less frequent when adults receive the standard dose of 300 mg/day. Poor nutrition or underlying alcoholism, diabetes mellitus, or uremia predisposes to neuropathy, which is more frequent in slow acetylators who have higher plasma levels of unaltered drug. Increased pyridoxine excretion is promoted by INH. Pyridoxine 10 to 50 mg daily can ameliorate neuropathy without interfering with the antimycobacterial effect. Current guidelines suggest that use of pyridoxine is necessary only for patients with underlying predispositions for neuropathy, as outlined earlier, but pyridoxine use is essentially universal for patients receiving INH. Notably, neuropathy is a manifestation of excessive pyridoxine dosing.

INH-induced central nervous system (CNS) toxicity can produce aberrations ranging from memory loss to psychosis or seizures. Optic neuropathy has been reported and can be confused with ethambutol-related optic neuritis in patients receiving both drugs. Toxic CNS reactions are not necessarily related to pyridoxine deficiency but have responded to pyridoxine administration.[20]

Hypersensitivity reactions. Fever, which may be sustained or "spiking"; skin eruptions; and hematologic abnormalities can occur. INH recipients can develop positive antinuclear antibody reactions and rarely manifest a lupus-like syndrome that is reversible on discontinuation of INH.

Miscellaneous adverse reactions. INH-associated arthritic disorders have included Dupuytren contracture and shoulder-hand syndrome.[20,24] Pellagra can occur in malnourished INH recipients.[20,49] Pyridoxine deficiency–related anemia can occur in children or adults.[50]

Overdose

Accidental ingestion of INH by children or ingestion during a suicide attempt may result in metabolic acidosis, hyperglycemia, seizures, and coma. High-dose pyridoxine usually reverses these toxicities.

Significant Drug Interactions

Particular caution is indicated when administering INH to patients with a seizure disorder. INH may alter metabolism of antiseizure medications, so it is important to monitor blood levels of seizure medications for patients taking INH. Phenytoin (Dilantin) toxicity is potentiated by INH. Mental changes, nystagmus, and ataxia can result,

especially in slow acetylators whose high INH levels inhibit phenytoin metabolism. Concomitant use of INH with valproic acid may result in increased serum concentrations of valproic acid and increase the risk for serious adverse reactions such as hepatotoxicity. Theophylline toxicity has been reported with coadministration of INH. INH decreases the efficacy of clopidogrel by decreasing the metabolism of the parent compound to the more biologically active metabolite. Combined INH and rifampin therapy predisposes to elevation of plasma hepatic enzymes. Plasma INH concentrations are increased by para-aminosalicylic acid (PAS) through interference with acetylation. Although significant drug interactions are less frequent with INH than with rifampin, it is important to check for all potential drug-drug interactions for any patient taking INH.

Usage

INH is indicated for all clinical forms of tuberculosis. It is used alone for therapy for LTBI in individuals with selected purified protein derivative skin test or interferon-γ release assay reactions at high risk for developing active tuberculosis disease.[35] The most recent American Thoracic Society (ATS)/CDC/Infectious Diseases Society of America (IDSA) guidelines state that INH is considered safe in pregnancy, but the risk for hepatitis may be increased both before and after delivery. Supplementation with pyridoxine is recommended if INH is administered during pregnancy.[19,35] INH is approved by the US Food and Drug Administration (FDA) for treating active tuberculosis in pregnant patients, but it should be given with caution and then only for selected high-risk patients with LTBI (e.g., HIV-seropositive patients or recent contacts to individuals with active tuberculosis).[35]

Availability and Dosage

INH is available generically (tablets, syrup, injectable solutions) and under brand names (INH tablets or Nydrazid injection solution). Dosage forms include 100-mg and 300-mg tablets, syrup containing 10 mg/mL, 100 mg/mL solution for parenteral injection, and combination capsules combining 150 mg of INH with 300 mg of rifampin (Rifamate) or tablets of 50 mg with 120 mg of rifampin and 300 mg of PZA (Rifater). The usual adult dosage is 5 mg/kg/day (preferably 300 mg once daily). A higher dosage (10–15 mg/kg; maximum 300 mg/day) has been recommended for infants and children.

With directly observed therapy for all patients, the initial induction phase of drug-susceptible tuberculosis includes INH, rifampin, and PZA, preferably given daily for the entire 2-month induction phase of therapy. Intermittent, three-times-weekly, high-dose INH, 15 mg/kg orally (maximum 900 mg), combined with rifampin, 10 mg/kg (maximum 600 mg orally), can be given for the maintenance phase of drug-susceptible tuberculosis therapy. Multiple therapeutic options are available with the caveat that the more drug that is administered, the better the outcome. For most patients in the United States with tuberculosis, these drugs are combined with ethambutol during the induction phase of therapy until susceptibilities are available. A reliable urine test is available to confirm INH ingestion.[51] Although the preferred parenteral route is intramuscular injection, INH for injection can be administered safely intravenously.[52]

Rifampin (see also Chapter 27)

Derivation and Structure

Rifampin (termed *rifampicin* in the United Kingdom) is a semisynthetic derivative of a complex macrocyclic antibiotic, rifamycin B, produced by *Streptomyces mediterranei*. It was introduced for clinical trials in tuberculosis in 1967.

Mechanism of Action

Rifampin inhibits DNA-dependent RNA polymerase. Human RNA polymerase is insensitive.

Antimicrobial Activity and Resistance

Rifampin is bactericidal against actively replicating *M. tuberculosis* to a degree comparable to INH with MICs of 0.005 to 0.2 μg/mL. It is also active against intracellular, slowly replicating bacilli and somewhat against nearly dormant organisms in necrotic foci. Efficacy of rifampin is indicated in susceptible pulmonary tuberculosis by sputum conversion 2 weeks earlier with rifampin-containing regimens than with regimens without the drug. Resistance emerges rapidly if the drug is given as monotherapy. Approximately 95% of resistance to rifampin results from a point mutation or deletion within an 81–base pair region of the gene encoding the β-subunit of RNA polymerase *(rpoB)*.[27,53] Mutations in the *rpoB* gene can be rapidly detected with the tuberculosis GeneXpert technology, which is widely available in the United States. The prevalence of rifampin resistance among new cases of tuberculosis in the United States is currently less than 1%.[11,12] In the past, isolated rifampin resistance in the United States was strongly associated with HIV infection.[54] However, currently in the United States, the discovery of rifampin resistance by GeneXpert analysis, available before phenotypic drug susceptibility results, should be considered an indicator for MDR-TB. Isolates with *rpoB* mutations should be immediately referred to the CDC for molecular detection of drug resistance analysis to detect mutations associated with resistance to other antituberculosis drugs, especially INH. Resistance to rifampin is associated in all instances with cross-resistance to rifapentine and in most instances with rifabutin, especially in the presence of high-level resistance. Rifampin resistance coupled with resistance to INH and other antituberculous agents defines either MDR-TB or XDR-TB isolates, depending on the number and type of antituberculous agents to which the *M. tuberculosis* isolate is resistant.[5,14]

Pharmacology

Rifampin is well absorbed orally, yielding peak plasma concentrations of 7 to 8 μg/mL after a dose of 600 mg. It is widely distributed throughout the body. CSF concentrations range from undetectable to 0.5 μg/mL in healthy individuals and reach 50% of plasma concentrations with meningeal inflammation. The high lipid solubility of rifampin enhances phagosomal penetration. Rifampin is deacetylated to an active form that undergoes biliary excretion and enterohepatic recirculation. Because of autoinduction of rifampin metabolism (cytochrome P-450–coupled),[55] biliary excretion increases with continued therapy. Induction of rifampin's metabolism with consequent reduction in its half-life and plasma concentrations becomes maximal after approximately six doses.[56] Excretion is primarily into the gastrointestinal tract, with lesser amounts excreted in the urine. The plasma concentration and urinary excretion increase in hepatic failure. Probenecid blocks hepatic uptake, causing decreased biliary excretion. There are no recommendations for rifampin dosage modification (i.e., dose reduction) with either hepatic or renal insufficiency. Rifampin is not removed by hemodialysis or peritoneal dialysis.[57]

Adverse Reactions

Minor adverse reactions are frequent with rifampin, but cessation of therapy because of adverse effects was necessary in only 6 of 372 patients taking the drug for 20 weeks.[58]

Hepatitis. The major adverse effect of rifampin is hepatitis. Minimal abnormalities in liver function tests are common in patients taking rifampin and usually resolve, possibly because of autoinduction of its metabolism even with continuation of the drug. Characteristically, elevations of bilirubin and alkaline phosphatase levels result, whereas elevation of hepatocellular enzyme concentrations can be caused by rifampin, INH, or both. Patients who abuse alcohol with preexisting liver damage appear to be especially prone to rifampin-induced liver reactions. There is no clear evidence that rifampin monotherapy for LTBI is associated with fulminant hepatic failure as reported with INH therapy for LTBI.

Effects on immune parameters. Rifampin has widespread effects on humoral and cell-mediated immunity, but they appear to be of no clinical significance.

Hypersensitivity reactions. Flushing, fever, pruritus without rash, urticaria, cutaneous vasculitis, eosinophilia, thrombocytopenia, hemolysis, or renal failure due to interstitial nephritis can occur with rifampin use. A systemic flulike syndrome, at times associated with thrombocytopenia, has been described almost exclusively with intermittent, high-dose therapy.

Miscellaneous adverse reactions. Widespread distribution of rifampin is reflected in an orange color appearing in urine, feces, saliva,

sputum, pleural effusions, tears, soft contact lenses, sweat, semen, and CSF. With overdosage, red man syndrome of skin discoloration has been described. Gastrointestinal upset is frequent but is usually ameliorated by a temporary reduction in dosage.

Significant Drug Interactions

By induction of microsomal cytochrome P-450–mediated enzymatic activities, rifampin causes increased hepatic metabolism of many substances. Rifampin interaction with more than 100 drugs has been described.[13,17,59–61] Despite interactions with many antiretroviral agents, rifampin or rifabutin is an essential component of treating tuberculosis in HIV-infected patients (Table 39.2). A compilation of this expanding list of compounds is given in Table 39.3. The induction period with rifampin may last for weeks after the drug is discontinued. The recent introduction of PIs and NNRTIs for the treatment of HIV infection has complicated the treatment of tuberculosis in this setting (see Table 39.3). Because rifampin induces metabolism of PIs and NNRTIs, rifampin should not be coadministered with these agents.[13,17,62] The most recent guidelines for coadministration of rifamycins with antiretroviral agents are summarized in Table 39.3 and can be found on the NIH website (http://aidsinfo.nih.gov/guidelines),[13] which is a living document with ongoing updates. Assistance can also be found at the CDC website (www.cdc.gov/tb/publications/guidelines/TB_HIV_Drugs/default.htm).[17] As new antiretroviral agents and more pharmacokinetic data become available, treatment recommendations are likely to be modified, so the reader is encouraged to check these websites periodically for updates.

In general, the coadministration of antituberculosis drugs and antiretroviral drugs should be initiated and guided by clinicians experienced in the treatment of these patients and familiar with the potential drug-drug interactions.

Competition for excretion with contrast agents used for biliary tract imaging may cause inability to visualize the gallbladder. Probenecid interferes with renal excretion, whereas PAS may interfere with gastrointestinal absorption.

Usage

Rifampin is indicated for treatment of all forms of pulmonary and extrapulmonary tuberculosis. It is recommended for treatment of LTBI as an alternative choice to INH.[35] The available data suggest that the efficacy of rifampin for treating LTBI appears comparable to that of INH, and, as noted, it appears to be less frequently associated with severe hepatotoxicity. Rifampin in combination with PZA was shown to be effective in HIV-positive patients for LTBI therapy when given for only 2 months,[63] but for reasons that have yet to be elucidated, the combination of rifampin and PZA for treatment of LTBI was associated with an unanticipated high rate (almost 6%) of severe or fatal hepatotoxicity, especially in HIV-seronegative patients.[64,65] Therefore the combination of rifampin and PZA is not currently recommended for treatment of LTBI, although the use of this regimen might rarely be indicated for selected patients under unusual circumstances with close clinical and biochemical monitoring and supervision by experienced clinicians. Rifampin is approved by the FDA for use in pregnant patients with active tuberculosis. As with INH, it should be used in pregnancy only for high-risk patients with LTBI and then only if INH is not appropriate.

Availability and Dosage

Rifampin is supplied in the United States as Rifadin, available in 150-mg or 300-mg capsules and in combination 300-mg capsules with 150 mg of INH (Rifamate) or 120-mg tablets with 50 mg of INH and 300 mg of PZA (Rifater). Rifampin from opened capsules can be suspended (usually 10 mg/mL) in simple or flavored sugar syrups that should not include ascorbic acid, which can inactivate rifampin.[29] Suspensions can be refrigerated up to 2 weeks. Rifampin for intravenous infusion (600 mg/vial; Rifadin) should not be administered intramuscularly. The usual oral dosage is 10 mg/kg/day (maximum 600 mg) for adults and 10 to 20 mg/kg/day for children (not to exceed 600 mg/day). A 600-mg twice-weekly schedule generally has been well tolerated. The current rifampin dosing recommendations have raised concerns that some patients may be receiving suboptimal rifampin doses.[66] For instance, with current recommendations for 10 mg/kg/dose, maximal 600 mg, dosing, a 60-kg individual would receive the same rifampin dose as a 100-kg person. One study has shown that doses of 35 mg/kg/day are safe and well tolerated, with ongoing studies investigating the efficacy of weight-adjusted rifampin doses higher than the 600-mg dose. The most recent ATS/CDC/IDSA tuberculosis treatment guidelines still recommend a maximal rifampin dose of 10 mg/kg/dose with latitude for the prescribing physician to increase the dose on an individualized basis.[66a]

Pyrazinamide

Derivation and Structure

PZA is a synthetic pyrazine analogue of nicotinamide.

TABLE 39.2 *Mycobacterium tuberculosis* Disease in Human Immunodeficiency Virus (HIV) Coinfection

- The principles for treatment of active tuberculosis (TB) disease in HIV-infected patients are the same as those for HIV-uninfected patients.
- All HIV-infected patients with diagnosed active TB should be started on TB treatment as soon as possible.
- All HIV-infected patients with diagnosed active TB should be treated with antiretroviral therapy (ART).
- In patients with CD4 counts <50 cells/mm^3, ART should be initiated within 2 weeks of starting TB treatment.
- In patients with CD4 counts ≥50 cells/mm^3 who present with clinical disease of major severity as indicated by clinical evaluation (including low Karnofsky score, low body mass index, low hemoglobin, low albumin, organ system dysfunction, or extent of disease), ART should be initiated within 2–4 weeks of starting TB treatment. The strength of this recommendation varies on the basis of CD4 cell count:
  - CD4 count 50–200 cells/mm^3 (BI)
  - CD4 count >200 cells/mm^3 (BIII)
- In patients with CD4 counts ≥50 cells/mm^3 who do not have severe clinical disease, ART can be delayed beyond 2–4 weeks of starting TB therapy but should be started within 8–12 weeks of TB therapy initiation. The strength of this recommendation also varies on the basis of CD4 cell count:
  - CD4 count 50–500 cells/mm^3 (AI)
  - CD4 count >500 cells/mm^3 (BIII)
- In all HIV-infected pregnant women with active TB, ART should be started as early as feasible, both for maternal health and for prevention of mother-to-child transmission of HIV.
- In HIV-infected patients with documented multidrug-resistant and extensively drug-resistant TB, ART should be initiated within 2–4 weeks of confirmation of TB drug resistance and initiation of second-line TB therapy.
- Despite pharmacokinetic drug interactions, a rifamycin (rifampin or rifabutin) should be included in TB regimens for patients receiving ART, with dosage adjustment if necessary.
- Rifabutin is the preferred rifamycin to use in HIV-infected patients with active TB disease on a protease inhibitor (PI)–based regimen because the risk for substantial drug interactions with PIs is lower with rifabutin than with rifampin (AII).
- Coadministration of rifampin and PIs (with or without ritonavir boosting) is not recommended (AII).
- Rifapentine is *not* recommended in HIV-infected patients receiving ART for treatment of latent TB infection or active TB, unless in the context of a clinical trial (AIII).
- Immune reconstitution inflammatory syndrome (IRIS) may occur after initiation of ART. Both ART and TB treatment should be continued while managing IRIS (AIII).
- Treatment support, which can include directly observed therapy of TB treatment, is strongly recommended for HIV-infected patients with active TB disease (AII).

Rating of recommendations: A = strong; B = moderate; C = optional.
Rating of evidence: I = data from randomized controlled trials; II = data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes; III = expert opinion.
Modified from Department of Health and Human Services: Guidelines for the Use of Antiretroviral Agents in HIV-1 Adolescents and Adults. Last updated July 14, 2016. For the most up-to-date guidelines, see http://aidsinfo.nih.gov/guidelines.

TABLE 39.3 Recommendations for Coadministering Antiretroviral Drugs With Rifampin

	RECOMMENDED CHANGE IN DOSE OF ANTIRETROVIRAL DRUG	RECOMMENDED CHANGE IN DOSE OF RIFAMPIN	COMMENTS
Nonnucleoside Reverse Transcriptase Inhibitors			
Efavirenz	None (some experts recommend 800 mg for patients >60 kg)	No change (600 mg/day)	Efavirenz AUC ↓ by 22%; no change in rifampin concentration. Efavirenz should not be used during first trimester of pregnancy
Nevirapine	Nevirapine and rifampin should not be used together	Nevirapine AUC ↓ 37%–58% and C_{min} ↓ 68% with 200 mg twice daily	
Rilpivirine	Rifampin and rilpivirine should not be used together	Rilpivirine AUC ↓ by 80%	
Etravirine	Etravirine and rifampin should not be used together	Marked decrease in etravirine predicted, based on data on interaction with rifabutin	
Single PIs			
Ritonavir	Rifampin and ritonavir should not be used together		Significantly ↓ PI exposure (>75%) despite ritonavir boosting
Fosamprenavir	Rifampin and fosamprenavir should not be used together		
Atazanavir	Rifampin and atazanavir should not be used together		Atazanavir AUC ↓ by >95%
Indinavir	Rifampin and indinavir should not be used together		Indinavir AUC ↓ by 89%
Nelfinavir	Rifampin and nelfinavir should not be used together		Nelfinavir AUC ↓ by 82%
Saquinavir	Rifampin and saquinavir should not be used together		Saquinavir AUC ↓ by 84%
Dual PI Combinations			
Saquinavir/ritonavir	Saquinavir 400 mg + ritonavir 400 mg twice daily	No change (600 mg/day)	Use with caution; combination of saquinavir (1000 mg twice daily), ritonavir (100 mg twice daily), and rifampin caused unacceptable rates of hepatitis among healthy volunteers
Lopinavir/ritonavir (Kaletra)	Increase dose of lopinavir/ritonavir (Kaletra) to 4 tablets (200 mg of lopinavir with 50 mg of ritonavir) twice daily	No change (600 mg/day)	Use with caution; this combination resulted in hepatitis in all adult healthy volunteers in an initial study
"Super-boosted" lopinavir/ritonavir (Kaletra)	Lopinavir/ritonavir (Kaletra)—2 tablets (200 mg of lopinavir with 50 mg of ritonavir) + 300 mg of ritonavir twice daily	No change (600 mg/day)	Use with caution; this combination resulted in hepatitis among adult healthy volunteers. There are favorable pharmacokinetic and clinical data among young children
Atazanavir/ritonavir	Standard dose of ritonavir-boosted atazanavir (300 mg once daily with 100 mg of ritonavir) should not be used with rifampin	Atazanavir trough concentration ↓ by >90%	
Tipranavir/ritonavir	Rifampin and tipranavir/ritonavir should not be used together		
Darunavir/ritonavir	Rifampin and darunavir/ritonavir should not be used together		
CCR-5 Receptor Antagonists			
Maraviroc	Increase maraviroc to 600 mg twice daily	No change (600 mg/day)	Maraviroc C_{min} ↓ by 78%. No reported clinical experience with ↑ dose of maraviroc with rifampin
Integrase Inhibitors			
Raltegravir	No change	No change (600 mg/day)	Increase raltegravir dose to 800 mg orally twice daily; monitor for antiretroviral efficacy or switch to rifabutin
Dolutegravir	Double dose from 50 mg once daily to 50 mg twice daily	No change	Limited experience
Elvitegravir/cobicistat/ tenofovir/emtricitabine (Stribild)	Coadministration should be avoided		Cobicistat and elvitegravir concentrations may be significantly ↓. Consider alternative antimycobacterial or alternative antiviral regimen

AUC, Area under the curve; C_{min}, trough concentration; *PI,* protease inhibitor.
Modified from UpToDate, 2013. For the most up-to-date information, see http://www.aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.

Mechanism of Action

The mechanism of action of PZA is unknown.

Antimicrobial Activity and Resistance

PZA is bactericidal for tubercle bacilli at 12.5 µg/mL. Its optimal activity appears to be against semidormant organisms in an acid pH environment, such as that existing intracellularly in phagolysosomes. Despite good activity at acid pH in vitro and inhibitory concentrations within monocytes,[67] PZA exhibits low activity alone in pretreated macrophages.[68] Resistance rapidly evolves if PZA is used alone. Primary resistance is seen in less than 1% of isolates, but nearly 50% of MDR-TB isolates resistant to INH and rifampin are resistant to PZA.[2] Most isolates resistant to PZA have mutations in the gene encoding

TABLE 39.4 Mechanism of Action and Recognized Mutational Resistance in Commonly Used Antituberculous Agents

DRUG	MECHANISM OF ACTION	SITE OF MUTATIONAL RESISTANCE (GENE)
Isoniazid	Inhibits mycolic acid synthesis	*inhA* (regulatory region) (mycolic acid gene)
	Catalase/peroxidase enzyme	*katG* (catalase/peroxidase gene)
Rifampin	Inhibits RNA polymerization	β-subunit *rpoB* (RNA polymerase gene)
Pyrazinamide	Unknown	*pncA* (pyrazinamidase gene)
Ethambutol	Inhibits cell wall synthesis (blocks arabinosyl transferase)	*embB* (gene for arabinosyl transferase enzyme)
Streptomycin	Inhibits protein synthesis	*rpsL* (gene for ribosomal S12 protein); 16S ribosomal RNA gene
Amikacin	Inhibits protein synthesis	16S ribosomal RNA gene (*Mycobacterium tuberculosis* and *Mycobacterium avium* complex)
Capreomycin	Inhibits cell wall synthesis	Unknown
Quinolones	Inhibit DNA structure	*gyrA* (gyrase A gene)
Macrolides	Inhibit protein synthesis	23S rRNA gene (*M. avium* complex, *Mycobacterium abscessus* subsp.)

pyrazinamidase *(pncA)* (Table 39.4).[69,70] This results in the loss of pyrazinamidase activity, an enzyme that converts PZA to the active form of pyrazinoic acid.

Pharmacology

PZA is well absorbed orally and widely distributed throughout the body, attaining concentrations above that needed to inhibit tubercle bacilli. Peak plasma concentrations are approximately 50 µg/mL, with a half-life of 12 hours, making once-daily or less frequent dosing practical. PZA crosses inflamed meninges and has been recommended in combination regimens for tuberculous meningitis.[71] It is metabolized by the liver, and metabolic products including principally pyrazinoic acid are excreted mainly by the kidneys, requiring dosage modification in renal failure. PZA is dialyzable, so dosing is recommended after dialysis sessions consistent with dosing recommendations for INH, rifampin, and ethambutol.[57]

Adverse Reactions

The most common side effects are nausea and vomiting. Hepatotoxicity occurred in nearly 15% of PZA recipients in early trials that employed dosages of 40 to 50 mg/kg/day for prolonged periods. Current regimens of 20 to 35 mg/kg/day are safer,[72] although more recent data suggest that PZA is the most common cause of hepatotoxicity in multidrug regimens also containing INH and rifampin.[73,74] Patients with preexisting liver disease should have symptoms and hepatic function tests monitored closely. PZA is also a frequent cause of hypersensitivity reactions and nongouty polyarthralgia in these multidrug regimens.[73] Other adverse reactions (1% of patients or less) include interstitial nephritis,[75] rhabdomyolysis with myoglobinuric renal failure,[76] and photosensitivity. Asymptomatic urate retention occurs in 50% of PZA recipients, with symptomatic gout usually occurring in patients with preexisting gout.[72]

Significant Drug Interactions

As just noted, the combination of rifampin and PZA for treatment of LTBI is associated with a high rate of severe or fatal hepatotoxicity.[63,64,65] There are no other significant drug interactions with PZA.

Usage

PZA is included as an essential component of multidrug 6-month short-course chemotherapy.[19,73] Without PZA for the first 2-month initiation phase of therapy, relapse rates after 6 months of therapy are unacceptable.[19] Efficacy with intermittent administration makes PZA suitable for directly observed therapy regimens. PZA is a class C drug and should be used with caution in pregnancy. Although PZA is recommended for routine use in pregnant women by the World Health Organization (WHO), the drug has not been recommended for general use in pregnant women in the United States by the FDA and the CDC because of insufficient data to determine safety.[19] The practical consequence of this policy is that pregnant women in the United States with active tuberculosis require 9 months of therapy because PZA is not included in the first 2 months of therapy. Most experts do not hesitate to include PZA in treatment regimens for pregnant patients with drug-resistant tuberculosis.

Availability and Dosage

PZA is available in 500-mg tablets or as 300-mg tablets in combination with INH (50 mg) and rifampin (120 mg) (Rifater). Dosage is 20 to 25 mg/kg/day (maximum 2.0 g) orally once or in two divided doses. PZA has been well tolerated in a twice-weekly dosage of 50 mg/kg (not to exceed 4 g/day) for short-course regimens.

Ethambutol

Derivation and Structure

Ethambutol (ethylenediiminobutanol) was discovered in 1961 among synthetic compounds screened for antituberculous activity.

Mechanism of Action

Ethambutol inhibits arabinosyl transferase enzymes that are involved in arabinogalactan and lipoarabinomannan biosynthesis within the cell wall.[77]

Antimicrobial Activity and Resistance

Ethambutol is bacteriostatic in vitro or within macrophages[67] at concentrations of 1 µg/mL against susceptible strains of *M. tuberculosis*. Primary ethambutol resistance in the United States is extremely unusual.[11,12] The principal role of ethambutol has been as a "companion" drug to curtail resistance. Specifically, ethambutol is included in the usual initial tuberculosis treatment regimen with INH, rifampin, and PZA for the possibility of unrecognized INH resistance. PZA alone is not an effective drug for preventing the emergence of rifampin resistance in the presence of INH resistance. Ethambutol is discontinued when INH and rifampin susceptibility are confirmed. Ethambutol resistance rates of 80% have been demonstrated with isolates with resistance to INH and rifampin, indicating a likely limited utility against MDR-TB.[2] Ethambutol resistance relates to point mutations in the arabinosyl transferase enzyme EmbB, which is coded for by the *embB* gene.[78]

Pharmacology

Ethambutol administered orally is 75% to 80% absorbed, yielding peak plasma concentrations of 5 µg/mL after a dose of 25 mg/kg. It is distributed throughout the body including the CSF. Although little ethambutol crosses normal meninges, levels 10% to 50% of those in plasma occur in CSF with meningeal inflammation. After conversion of approximately 25% of absorbed ethambutol to inactive metabolites, 80% of the parent drug, together with metabolites, is excreted in urine. Consequently, it becomes necessary to modify the dosage in significant renal failure.

Adverse Reactions

The major toxicity of ethambutol is neuropathy, including peripheral neuropathy and retrobulbar optic neuritis. Characteristically, patients complain of bilateral blurry vision and are found to have impairment of visual acuity and red-green color vision. Common in association with high-dose (50 mg/kg/day) therapy with prolonged administration and more common with 25 mg/kg/day than with 15 mg/kg/day dosing, retrobulbar neuritis is usually slowly reversible. Visual loss has rarely occurred in elderly patients receiving 15 mg/kg/day.[79] The administration of ethambutol at 25 mg/kg on a three-times-weekly basis appears to be associated with a reduced risk for visual toxicity in this patient population

compared with daily dosing at 15 mg/kg.[80] This observation is important because baseline visual acuity is often impaired in older patients and is associated with other ocular problems, such as cataracts, also associated with blurred vision. Recipients of ethambutol should be instructed to report symptoms of blurry vision or color vision abnormalities promptly and to discontinue the drug until confirmatory visual testing can be done. Visual acuity and red-green color perception testing is recommended at baseline, whenever a change in visual symptoms occurs, and monthly for patients taking ethambutol for more than 2 months or at higher than usual doses.[19] Monthly testing in patients receiving 15 mg/kg can be useful in establishing the range of visual abnormalities in those already visually impaired.

Gastrointestinal intolerance is infrequent. Hyperuricemia occurs because of decreased renal uric acid excretion. Hypersensitivity reactions are rare and include dermatitis, arthralgias, and fever.

Significant Drug Interactions

There are no significant drug interactions with ethambutol.

Usage

Ethambutol is routinely included as the fourth drug along with INH, rifampin, and PZA in initial therapy for tuberculosis before the availability of drug susceptibility information. As described in "Antimicrobial Activity and Resistance," ethambutol is included to protect against the emergence of rifampin resistance in patients with occult INH resistance who, if receiving only INH, rifampin, and PZA, would be functionally on only INH and PZA. It is also routinely used in treatment regimens for patients with isolates resistant to INH or rifampin or both. Ethambutol has no detectable effects on the fetus and is approved for treatment of tuberculosis in the United States.

Availability and Dosage

Ethambutol is available as ethambutol hydrochloride (Myambutol) supplied in 100-mg or 400-mg tablets. The usual dosage is 15 mg/kg/day as a single daily dose or 25 mg/kg three times weekly.

Streptomycin

Derivation, Structure, and Pharmacology

Streptomycin, an aminoglycoside antibiotic introduced in the 1940s, was the first drug to reduce tuberculosis mortality. Its structure, mechanism of action, and pharmacology are discussed in other chapters. Briefly, intramuscular injection of 1 g yields peak plasma concentrations of 25 to 45 µg/mL. It is virtually excluded from the CNS.

Antimicrobial Activity and Resistance

Streptomycin is bactericidal against *M. tuberculosis* in vitro but is inactive against intracellular tubercle bacilli. Concentrations of 4 to 10 µg/mL of plasma are inhibitory. The rapid emergence of resistance to streptomycin was quickly recognized as a consequence of single-drug therapy. Approximately 1 in 10^6 tubercle bacilli is spontaneously resistant to streptomycin. Primary resistance to streptomycin is seen most often in patient populations having a high incidence of INH resistance. In MDR-TB disease outbreaks, approximately 80% of isolates resistant to INH and rifampin are also resistant to streptomycin.[2] Streptomycin resistance relates to mutational changes involving ribosomal binding protein or the ribosomal binding site.[27,81,82] Isolates resistant to streptomycin are not cross-resistant to amikacin, kanamycin, or capreomycin.

Adverse Reactions

Streptomycin toxicity is similar to that of other aminoglycoside antibiotics but with less renal and auditory toxicity and greater vestibular toxicity than more commonly used aminoglycosides. Patients receiving streptomycin should be instructed to be aware of tinnitus, decreased hearing, and problems with balance, and they should be instructed to notify their caregiver immediately if such reactions occur.

In contrast to other aminoglycosides, allergic or hypersensitivity reactions can be seen with streptomycin. These include fever, chills, eosinophilia, and rash.

Significant Drug Interactions

There are no significant drug interactions with streptomycin.

Usage

Streptomycin is most commonly indicated as part of multidrug therapy for drug-resistant tuberculosis. A high prevalence of streptomycin resistance in patients who have received it as part of antituberculosis therapy outside the United States has limited its utility for treating drug-resistant tuberculosis in the United States. Additionally, the lack of availability of streptomycin levels for guiding streptomycin dosing versus the widespread availability of amikacin levels has further diminished its use in the United States. Dosages greater than 1 g/day should be avoided. Reduction in dosage or frequency of administration or both is indicated in patients older than 50 years, patients with low body weight, and patients in whom renal function is impaired. Streptomycin blood levels may be useful for guiding streptomycin dosing in these circumstances. Special care must be taken when streptomycin is used in combination with other nephrotoxic or ototoxic drugs, such as capreomycin or amikacin. It is a category D drug in pregnancy because of fetal ototoxicity.

Primary resistance to streptomycin is significant in isolates from individuals from some countries. Cross-resistance is not seen between streptomycin and amikacin, so unless patients have had prior treatment with either kanamycin or amikacin, streptomycin-resistant isolates should remain susceptible to amikacin.[66a]

Availability and Dosage

Streptomycin sulfate for intramuscular injection is provided in 1-g single-injection vials. The recommended dosage in younger adults (<50 years old) with normal renal function is 15 mg/kg/day or 0.5 to 1 g daily to 1 g twice weekly. Children receive 20 to 40 mg/kg/day (maximum 1 g) in divided doses every 12 hours. Although not approved for such use, the drug can be safely given intravenously when needed.

ALTERNATIVES TO RIFAMPIN

Rifabutin

Derivation and Pharmacology

Several spiropiperidyl rifamycins have activity against mycobacteria, including *M. tuberculosis, Mycobacterium avium-intracellulare* complex (MAC), and *Mycobacterium kansasii*.[83,84,85,86,87] Rifabutin (Mycobutin), a derivative of rifamycin-S, is more active in vitro and more effective on a weight basis in experimental murine tuberculosis than rifampin.[86] The mechanism of action is inhibition of RNA polymerase, as it is with rifampin. It has a long plasma half-life (45 hours) in humans and marked tissue tropism, producing tissue concentrations 5-fold to 10-fold greater than in serum. Peak serum concentrations of rifampin (5–10 µg/mL) are 5-fold to 10-fold higher than those of rifabutin (0.5 µg/mL).[88]

Adverse Reactions

Polymyalgia syndrome, yellowish tan discoloration of the skin (pseudojaundice), and anterior uveitis have occurred in patients taking rifabutin, usually at doses exceeding 300 mg daily.[89,90] Almost all patients with these side effects have also been receiving clarithromycin, fluconazole, or ritonavir. Symptoms of uveitis include ocular pain and blurred vision.[90] Neutropenia and thrombocytopenia are dose dependent and occur in up to one-third of patients receiving therapy for pulmonary MAC disease.[91] The incidence of rash, hepatitis, and gastrointestinal distress appears comparable to that with rifampin. Rifabutin also can produce an orange-red discoloration of urine, saliva, tears, and contact lenses similar to rifampin.

Significant Drug Interactions

Rifabutin induces the hepatic cytochrome P-450 system, but only about 50% of that seen with rifampin. This induction produces lowered serum levels of numerous drugs normally metabolized in the liver, including PIs (Table 39.5).[13,17] Concurrent administration of delavirdine or saquinavir with rifabutin is not recommended for this reason.[92] Rifabutin is also metabolized by this same system, so enzyme inhibitors such as the PIs fluconazole and clarithromycin increase plasma rifabutin concentrations (see Table 39.3).[13,17,89,92]

TABLE 39.5 Recommendations for Coadministering Antiretroviral Drugs With Rifabutin

	ANTIRETROVIRAL DOSE CHANGE	RIFABUTIN DOSE CHANGE	COMMENTS
Non-Nucleoside Reverse-Transcriptase Inhibitors			
Efavirenz	No change	Change to 450–600 mg (daily or intermittent)	Rifabutin AUC ↓ by 38%. Effect of efavirenz + PI on rifabutin concentration has not been studied. Efavirenz should not be used during first trimester of pregnancy
Nevirapine	No change	No change (300 mg daily or 3 times/wk)	Rifabutin and nevirapine AUC not significantly changed
Rilpivirine	Rifabutin and rilpivirine should not be used together		Rilpivirine AUC ↓ by 46%
Etravirine	No change	No change (300 mg daily or 3 times/wk)	No clinical experience; etravirine C_{min} ↓ by 45%, but this was not thought to warrant a change in dose
Single PIs			
Fosamprenavir	No change	↓ to 150 mg/day or 300 mg 3 times/wk	No published clinical experience
Atazanavir	No change	↓ to 150 mg every other day or 3 times/wk	No published clinical experience. Rifabutin AUC ↑ by 250%
Indinavir	1000 mg q8h	↓ to 150 mg/day or 300 mg 3 times/wk	Rifabutin AUC ↑ by 170%; indinavir concentrations ↓ by 34%
Nelfinavir	No change	↓ to 150 mg/day or 300 mg 3 times/wk	Rifabutin AUC ↑ by 207%; insignificant change in nelfinavir concentration
Dual PI Combinations			
Lopinavir/ritonavir (Kaletra)	No change	↓ to 150 mg every other day or 3 times/wk	Rifabutin AUC ↑ by 303%; 25-*O*-desacetyl rifabutin AUC ↑ by 47.5-fold
Ritonavir (any dose) with saquinavir, indinavir, amprenavir, fosamprenavir, atazanavir, tipranavir, or darunavir	No change	↓ to 150 mg every other day or 3 times/wk	Rifabutin AUC and 25-*O*-desacetyl rifabutin AUC ↑, by varying degrees
CCR-5 Receptor Antagonists			
Maraviroc	No change	No change	No clinical experience; significant interaction is unlikely
Integrase Inhibitors			
Raltegravir	No change	No change	No clinical experience; significant interaction is unlikely
Dolutegravir	No change	No change	No clinical experience; significant interaction is unlikely
Elvitegravir/cobicistat/tenofovir/emtricitabine (Stribild)	Coadministration should be avoided		Consider alternative antimycobacterial or alternative antiviral regimen. If used, consider rifabutin 150 mg once daily or every other day. Monitor rifabutin concentrations, and adjust dose accordingly. Elvitegravir AUC ↓ 21%, C_{min} ↓ 67%; rifabutin active metabolite (25-*O*-desacetyl rifabutin) AUC ↑ 625%

[a]For the most up-to-date information, see https://aidsinfo.nih.gov/guidelines/html/4/adult-and-adolescent-opportunistic-infection/325/tb.
AUC, Area under the curve; *C_{min},* trough concentration; *PI,* protease inhibitor.

Usage

Rifabutin appears as effective as rifampin in the treatment of drug-susceptible tuberculosis.[93,94] In patients with HIV infection, rifampin is replaced by rifabutin if PIs are used (amprenavir, atazanavir, darunavir, fosamprenavir, nelfinavir, lopinavir-ritonavir [Kaletra], tipranavir-ritonavir, darunavir-ritonavir, and ritonavir).[13,17] Current guidelines suggest that rifabutin should be given as 300 mg three times a week or every other day with the option for monitoring rifabutin levels.[13] Rifabutin's potential for treatment of infection with MDR-TB is of interest because approximately 25% of rifampin-resistant tuberculosis strains are inhibited by low concentrations of rifabutin. Molecular methods for rifampin susceptibility testing have identified strains of *M. tuberculosis* with selected mutations that are rifampin resistant but with MIC values only slightly above the usual MIC. These strains may test as susceptible to rifampin on liquid media but usually are reported as resistant if tested on solid media. These isolates usually are reported as susceptible to rifabutin at 2.0 μg/mL. Some data suggest that rifabutin adds to the efficacy of treatment regimens in this circumstance, although other studies have reported poor outcomes including treatment failure, relapse, and further acquisition of drug resistance associated with the presence of these mutations.[95,96] It remains controversial whether patients with rifampin-resistant/rifabutin-susceptible *M. tuberculosis* can be treated for shorter durations than other patients with rifampin-resistant *M. tuberculosis*, especially patients with concomitant INH resistance. Until there is more supportive evidence, it seems prudent to include rifabutin in the regimens of these patients but not to assume that rifabutin has activity in this situation comparable to its activity for *M. tuberculosis* isolates without *rpoB* mutations.[97]

Rifapentine

Derivation and Pharmacology

With the microbiologic success of rifabutin but problems with low serum levels and complex adverse reactions, investigators searched for other rifamycin compounds. The most promising agent is rifapentine.[98–100] In the study leading to licensure in 1998, rifapentine, 600 mg twice weekly, was compared with rifampin, 450 to 600 mg, when both were given with daily INH, pyrazinamide, and ethambutol for 2 months followed by rifapentine, 600 mg, plus INH once weekly or rifampin, 450 to 600 mg, plus INH twice weekly. Results after 6 months were comparable, although the rifapentine relapse rate was slightly higher (10% vs. 5%).[99] When given with a fatty meal, peak blood levels after the administration of 600 mg of rifapentine are 15 μg/mL of native drug and 6 μg/mL of 25-desacetyl rifapentine, the active metabolite. The half-life of rifapentine is 13 hours.

Adverse Drug Reactions
The adverse effects of rifapentine are similar to those associated with rifampin.

Significant Drug Interactions
Rifapentine appears to be a more potent inducer of the cytochrome P-450 system than rifabutin but less than rifampin. Therefore it may increase metabolism of coadministered drugs that are metabolized by these enzymes.

Usage
Rifapentine has been approved for once-weekly use with INH in the continuation phase of therapy for HIV-negative patients without cavitation on chest radiograph and negative acid-fast bacilli smears at 2 months.[19] With recent interest in possible benefits of increased rifamycin exposure, tuberculosis therapy studies are ongoing with daily and higher dose rifapentine-containing regimens. In one study, daily rifapentine was found to be well tolerated and safe. The high rifapentine exposure was associated with enhanced sputum sterilization levels at completion of the intensive phase.[101] Rifapentine is contraindicated in HIV-seropositive patients on ART because of interaction with PIs and NNRTIs and the development of rifamycin monoresistance in some patients.[13,102] Rifapentine in combination with INH given once weekly for 12 weeks (12 total doses) has been shown to be as effective and safe as INH with higher treatment completion rates than INH for treating LTBI in HIV-seronegative and HIV-seropositive patients not on ART.[103,104] This combination in once-weekly therapy has more recently been shown effective in children 2 to 17 years of age.[105] Rifapentine use in LTBI therapy should be administered by directly observed therapy.

Availability and Dosage
Rifapentine is available in 150-mg tablets. The recommended dosage for adults in the continuation phase of therapy for tuberculosis has been 10 mg/kg or a maximum 600 mg per week, although studies are ongoing to determine the optimal dose for rifapentine with active tuberculosis disease. The recommended dose for LTBI therapy is 900 mg per week, but weekly doses of 1200 mg appear to be tolerated well. The drug is not approved for use in children or pregnant women. The pharmacokinetics of rifapentine have not been evaluated in patients with renal impairment. Only about 17% of an administered dose is excreted via the kidneys. The clinical significance of impaired renal function in the disposition of rifapentine is unknown. Similarly, the clinical significance of impaired hepatic function in the disposition of rifapentine and its 25-desacetyl metabolite is not known.

SECOND-LINE ANTITUBERCULOUS DRUGS

Quinolones (see Chapter 35)

Mechanism of Action
Emerging outbreaks of MDR-TB disease[2,3,4,5,6] stimulated the investigation of fluorinated quinolones for their activity against mycobacteria.[106–109] These drugs are bactericidal against both extracellular rapidly multiplying bacteria and intracellular nonmultiplying bacteria with achievable serum levels.[108] They inhibit bacterial DNA gyrase, an enzyme that is essential for the maintenance of DNA supercoils needed for chromosomal replication.[108] The fluoroquinolones penetrate well into alveolar macrophages, respiratory secretions, and body fluids with concentrations equal to or higher than those in serum. CNS penetration is good, allowing these drugs to be used for tuberculous meningitis.[10,14,110] The prolonged half-life (5–8 h for levofloxacin and 9–15 h for moxifloxacin) and significant postantibiotic effect allow once-daily dosing.[108]

Antimicrobial Activity and Resistance
Older quinolones ciprofloxacin and ofloxacin have both been associated with good outcomes in the treatment of drug-susceptible and drug-resistant tuberculosis.[10,14,111,112] Ciprofloxacin and ofloxacin inhibit more than 90% of strains of drug-susceptible tubercle bacilli at concentrations of 0.5 μg/mL and 1.0 μg/mL, respectively.[108,109] Clinical trials of ofloxacin in combination with INH and rifampin indicate activity comparable to ethambutol.[112] Usage of fluoroquinolones as a single agent in animal models or in human trials with inactive drugs has led to the rapid emergence of resistance. Resistance appears to result from mutations in the genes responsible for DNA configuration (DNA gyrase).[27,113]

Ofloxacin and ciprofloxacin have relatively weak activity compared with the newer quinolones.[10,14] Of the quinolones, gatifloxacin (no longer marketed in the United States), moxifloxacin, and levofloxacin, in that order, have the most activity against *M. tuberculosis* with significant early bactericidal activity against *M. tuberculosis* relative to first-line antituberculous drugs.[108,114] Moxifloxacin and levofloxacin have lower MIC_{90} values (MIC required to inhibit the growth of 90% of organisms; 0.25 mg/L and 0.5–1 mg/L) than ciprofloxacin (4 mg/L) and ofloxacin (2 mg/L).[115,116] Furthermore, moxifloxacin has demonstrated early bactericidal activity in sputum that is equivalent to rifampin at 2 days and to INH at 5 days.[117] Despite the absence of prospective clinical trials using fluoroquinolones for MDR-TB, there has been considerable experience with levofloxacin and moxifloxacin, and they are regarded as critical to good treatment outcomes.[10,14,118]

The optimal quinolone and dose are still unclear, as higher doses of levofloxacin (750–1200 mg) are undergoing evaluation. The usual dose of moxifloxacin is 400 mg, which has good bactericidal activity.[115,116] Higher doses of 600 to 800 mg have been used to enhance CNS penetration during the treatment of MDR-TB meningitis and when the MIC of moxifloxacin is >0.5 μg/mL but <2.0 μg/mL.

Two phase II studies sponsored by the CDC have been conducted to evaluate the effect of moxifloxacin substitution for either ethambutol or INH during the first 2 months of treatment of newly diagnosed tuberculosis.[119,120] In the first trial, moxifloxacin substitution for ethambutol resulted in no apparent effect on 2-month sputum conversion rates to negativity but did show a faster median time to sputum conversion.[119] In a second trial, moxifloxacin substituted for INH in the first 2 months of treatment of tuberculosis had no effect on 2-month sputum conversion rates.[120] These studies suggest that substitution of moxifloxacin for ethambutol or INH during the first 2 months of therapy is no less efficacious than standard intensive-phase therapy and may result in more rapid clearance of *M. tuberculosis* from the lungs. A study comparing the replacement of INH with moxifloxacin (and rifampin with rifapentine) with both moxifloxacin and rifapentine given once weekly for the last 4 months of a 6-month course of therapy found this regimen to be noninferior to the current standard 6-month therapy.[121] However, two studies attempting to shorten the 6-month course of therapy for pulmonary tuberculosis to 4 months by replacing INH or ethambutol with moxifloxacin or gatifloxacin did not show noninferiority in any of the regimens compared with the 6-month standard therapy.[122,123]

Ofloxacin is the class drug used for determining quinolone susceptibility. Cross-resistance has been demonstrated among ciprofloxacin, ofloxacin, and levofloxacin. Resistance to quinolones develops rapidly when they are used as monotherapy.[124] Some quinolone-resistant isolates show susceptible MICs for moxifloxacin. The overall significance of this observation is not yet clear, but it suggests that moxifloxacin may still be an important element of therapy for some patients with XDR-TB.

Usage
Quinolones are now routinely incorporated into treatment regimens for MDR-TB disease along with other agents and are the cornerstones of therapy for quinolone-susceptible MDR-TB isolates. The usual dosage is levofloxacin, 750 to 1000 mg/day, or moxifloxacin, 400 mg/day. The use of quinolones in children and adolescents has not been approved, but most experts agree that the drugs should be considered for children with MDR-TB disease.[21] This class of drugs should be avoided in pregnancy. Fluoroquinolones are cleared primarily by the kidney, and dosage adjustment is recommended if creatinine clearance is less than 50 mL/min. They are not cleared by hemodialysis, so supplemental doses after dialysis are not necessary. Drug levels are not affected by hepatic disease. There is also ongoing concern about the use of quinolones as first-line therapy for community-acquired pneumonia, which is the most common misdiagnosis applied to missed cases of tuberculosis. It appears that either multiple or prolonged courses of quinolones are required to promote quinolone-resistant tuberculosis, which is not a circumstance without precedent.

Adverse Reactions

The adverse effects for quinolone include nausea and bloating, dizziness, insomnia, tremulousness, headache, rash, pruritus, and photosensitivity. Toxicity with the quinolone class of drugs most commonly is reported as gastrointestinal upset such as nausea and bloating. Myalgia is relatively common; rarely, tendon rupture has been reported.[10,14] An important consideration for long-term safety of the newer quinolones is their cardiac effect on the QT interval, resulting in a ventricular tachycardia known as torsades de pointes. The optimal approach for monitoring possible cardiac toxicity in patients on long-term quinolone therapy is not established. Whether to continue a quinolone in the presence of QT-interval prolongation or whether to add another agent that also may prolong the QT interval is clearly a risk-benefit decision, especially for patients with MDR-TB disease for which the quinolone may be the most important drug in the treatment regimen. Tendinitis and tendon rupture are also concerns with fluoroquinolones, which has prompted a special warning by the FDA on package inserts for fluoroquinolone use. As with potential cardiac toxicity, the decision to continue a quinolone in a patient with tendinitis requires an individualized risk-benefit analysis. Prolongation of the QT interval has been noted in patients taking moxifloxacin, but to our knowledge or in our experience it has not been reported in patients taking this drug for MDR-TB.

Significant Drug Reactions

Because antacids and other medications containing divalent cations markedly decrease absorption of fluoroquinolones, it is important that a fluoroquinolone not be administered within 2 hours of such medication.

Linezolid (see Chapter 33)

Linezolid is an oxazolidinone with activity against drug-resistant gram-positive bacteria. It has been shown to inhibit all strains of *M. tuberculosis* at concentrations of less than 1 μg/mL in vitro.[125] Although it has potentially severe toxicity with long-term use, it has been shown to have significant activity in multidrug regimens for MDR-TB and XDR-TB. Several studies have shown improved outcomes from linezolid in patients with tuberculosis with extensive resistance, even when added to a chronically failing regimen as salvage therapy.[126,127,128–130] The dosage of linezolid for bacterial infections is 600 mg twice daily, but most studies have used 600 mg once daily for treatment of tuberculosis in an effort to limit toxicity and cost. Serum drug levels are sufficiently above the MIC with this dosage.[126] In a series of 41 patients with XDR-TB unresponsive to therapy, linezolid at initial dosages of 600 mg/day followed by either 600 mg/day or 300 mg/day was associated with a sputum conversion rate of 87%.[127] Acquired linezolid resistance occurred in less than 10% of patients with both 300-mg and 600-mg linezolid dosing. Remarkably, 82% of patients had clinically significant adverse events but only 3 patients permanently discontinued linezolid because of drug toxicity.[127]

Most studies show that frequent significant adverse events occur in patients treated with 600 mg once daily of linezolid. These reactions, which are caused by inhibition of mitochondrial protein synthesis, include myelosuppression, peripheral and optic neuropathy, and lactic acidosis.[131,132,133] Most linezolid toxicity was reversible after stopping the drug but peripheral neuropathy has been especially concerning, as it may persist after stopping treatment.[131,132,133] Favorable outcomes and decreased toxicity compared with the 600 mg/day dosage have been reported for patients treated with a 300 mg/day dosage.[127]

Linezolid is associated with the serotonin syndrome in 25% of patients given selective serotonin reuptake inhibitors or other medications that increase serotonin concentrations in the CNS.[134] Patients should also be counseled to avoid foods, dietary supplements, and beverages high in tyramine.

Linezolid is marketed as Zyvox and is available as 600-mg tablets and infusions. The usual daily dosage for bacterial infections has been 600 mg twice daily. However, the usual dosage for tuberculosis is 300 to 600 mg once daily. The oral form is almost 100% bioavailable. The emergence of mutational resistance is not surprising because the drug inhibits the ribosome, and *M. tuberculosis* has only one copy of this gene.

Even in the context of its potential toxicity, linezolid is an important addition to the list of available medications for treating drug-resistant tuberculosis. It is superior to the relatively weak and toxic older second-line drugs such as cycloserine, ethionamide, and PAS and should be used instead of those medications in the absence of a specific contraindication.

Capreomycin, Amikacin, and Kanamycin

Capreomycin, amikacin, and kanamycin are considered as a group because all are administered by intramuscular or intravenous injection, have similar pharmacokinetics and toxicities, and are excreted by the renal route. These drugs have been used principally as alternative agents for MDR-TB disease. All have additive ototoxicity and nephrotoxicity and in that regard should be given cautiously similar to streptomycin or other aminoglycosides.

Capreomycin

Antimicrobial Activity and Resistance

Capreomycin, a polypeptide antibiotic obtained from *Streptomyces capreolus,* is active against *M. tuberculosis* including most MDR-TB strains[2] at concentrations of 1 to 50 μg/mL (usually 10 μg/mL). Average peak plasma concentrations of 30 μg/mL are achievable. There is no cross-resistance between streptomycin and capreomycin,[135] but some isolates resistant to kanamycin or amikacin are cross-resistant to capreomycin. The site of mutational change resulting in capreomycin resistance is unknown.

Adverse Reactions

Capreomycin can cause hearing loss, tinnitus, and decreased renal function but is considered less toxic than amikacin and especially kanamycin.

Significant Drug Interactions

There are no significant drug interactions with capreomycin.

Usage

Capreomycin has emerged as the first-line injectable agent in regimens for the treatment of drug-resistant tuberculosis, especially in the presence of streptomycin resistance.

Availability and Dosage

Capreomycin sulfate is supplied as Capastat. Dosing is the same as with streptomycin, with a range of 500 mg to 1 g deep intramuscularly five times weekly for 2 to 4 months in patients younger than 50 years and having normal renal function. Thereafter the dosage is reduced to 1 g two to three times weekly. It is a category C pregnancy drug.

Amikacin

Antimicrobial Activity and Resistance

In vitro and in animals, amikacin is among the most active aminoglycosides against *M. tuberculosis.* Because of its expense and greater toxicity, in the past it has been considered after streptomycin and capreomycin for the treatment of MDR-TB. However, several factors have made it the preferred parenteral agent including (1) the high rate of streptomycin resistance in MDR-TB isolates and (2) the ready availability of amikacin levels facilitating amikacin dosing that does not cause early severe toxicity and loss of the parenteral agent for long-term use. It has generally replaced kanamycin in the United States. Cross-resistance to amikacin and kanamycin results from an A-G change at base pair 1408 of the 16S ribosomal RNA gene.[136]

Recognition of this cross-resistance is important because, although kanamycin is rarely used in the United States, it is used more frequently outside the United States so that individuals from outside the country who received parenteral tuberculosis medications cannot be assumed to be susceptible to amikacin. However, kanamycin-resistant but amikacin-susceptible strains have been reported, emphasizing the importance of in vitro susceptibility testing regardless of prior antibiotic exposure.[137]

Adverse Reactions

Common side effects include tinnitus, hearing loss, and nonoliguric renal failure. Hypersensitivity events are rare.

Usage
Amikacin is an alternative injectable agent for the treatment of resistant *M. tuberculosis* infections. The customary dosage is 7 to 10 mg/kg (not to exceed 1 g) five times weekly. Because most pathology laboratories can determine blood levels of amikacin but not kanamycin, streptomycin, or capreomycin, amikacin is especially suited when parenteral therapy is required in patients with renal failure or in elderly patients with preexisting hearing loss. It is a category D drug in pregnancy.

Kanamycin
Kanamycin is an aminoglycoside that has activity against most strains of streptomycin-resistant tubercle bacilli. Except for its lower cost, kanamycin offers no advantage over amikacin in combination therapy and has substantial ototoxicity. In addition, serum levels are not readily available.

Availability and Dosage
Kanamycin sulfate is available as Kantrex, 0.5 g/2 mL, 1 g/3 mL, or 75 mg/2 mL (pediatric formulation) for intramuscular injection. The usual dose is 15 mg/kg (maximum 1.0 g), generally limited to 500 mg/day in adults because of ototoxicity.

Para-Aminosalicylic Acid
Derivation, Structure, and Pharmacology
As a calcium or sodium salt, this synthetic compound inhibits the growth of tubercle bacilli by the impairment of folate synthesis. PAS is incompletely absorbed orally. A 4-g dose yields plasma concentrations of 70 to 80 µg/mL. Of absorbed PAS, 85% is excreted in urine as various metabolic products.

Adverse Reactions
Chief among side effects of PAS is gastrointestinal intolerance, which is often severe and results in poor compliance. PAS can cause reversible drug-induced lupus-like reactivity and, when given as a sodium salt, sodium overload. It can produce lymphoid hyperplasia, and recipients can develop mononucleosis-like syndromes with fever, rash, hepatosplenomegaly, occasionally toxic hepatitis, and adenopathy. Hypersensitivity to PAS is frequent.

Usage
PAS has retained a limited role in multidrug therapy in developing countries because of its low cost. However, it is becoming less favored because of poor compliance and primary resistance. Its use in the United States is limited to the treatment of MDR-TB and XDR-TB disease.

Availability and Dosage
In the United States, PAS is available from the CDC (www.cdc.gov). Dosage forms include 500-mg tablets or 4-g resin packets. The usual dosage in adults is 10 to 12 g/day in three or four divided doses (6–8 g/day of the sodium potassium–free ascorbate) and in children 200 to 300 mg/kg/day in divided doses.

Cycloserine
Derivation and Mechanism of Action
By virtue of inhibiting cell wall synthesis, cycloserine possesses antimicrobial activity against a broad range of prokaryotic organisms including mycobacteria. In vitro, 5 to 20 g/mL inhibits susceptible *M. tuberculosis*.

Pharmacology
Cycloserine is readily absorbed orally. Serum concentration measurements aiming for a peak concentration of 20 to 35 mg/L are often useful in determining the optimal dose for a given patient. Widely distributed among tissues, no blood-brain barrier exists to cycloserine.[10,14,19] Little of the drug is metabolized, and approximately two-thirds is excreted unchanged by the kidneys.

Adverse Reactions
Cycloserine levels are essential for optimal use of this toxic drug. Peak serum concentrations of cycloserine should be kept between 20 and 35 µg/mL.[10,14,19] Cycloserine can cause peripheral neuropathy or CNS dysfunction including confusion, irritability, somnolence, headache, nervousness, vertigo, dysarthria, and seizures. Behavioral alterations include severe depression with suicidal ideation. Cycloserine is contraindicated in patients with a history of seizures or patients with severe underlying depression. Because the CNS toxicity appears to be dose related and only a few specialized laboratories do serum drug levels, the drug is generally avoided even by clinicians experienced in treating tuberculosis, unless no other options are available, such as with some patients infected with MDR-TB and XDR-TB strains.

Usage
Cycloserine is one of several alternatives for re-treatment regimens or for treatment of primary drug-resistant *M. tuberculosis*. It appears to have limited activity against MDR-TB strains.[2,10,14] It is classified as a category C drug in pregnancy.

Availability and Dosage
Cycloserine is provided in the United States as Seromycin in 250-mg pulvules. The usual dosage is 500 to 750 mg/day in two divided doses, with 500 mg/day commonly used. Cycloserine levels can be obtained to guide drug dosage and minimize toxicity.

Ethionamide
Derivation, Mechanism of Action, and Resistance
Ethionamide, a derivative of isonicotinic acid, was first synthesized in 1956. It is tuberculostatic at 0.6 to 2.5 µg/mL against susceptible strains, presumably by inhibition of oxygen-dependent mycolic acid synthesis.[138] The mechanism of ethionamide resistance is unknown, but some isolates are resistant to both INH and ethionamide and harbor mutations in the region of the *inhA* gene, which is involved in mycolic acid biosynthesis.[26]

Pharmacology
Ethionamide is absorbed well orally, yielding peak plasma concentrations of 20 µg/mL. It is widely distributed and penetrates both normal and inflamed meninges to yield CSF concentrations equivalent to those in plasma. It is metabolized by the liver, with metabolites renally excreted. Ethionamide interferes with INH acetylation.

Adverse Reactions
Gastrointestinal distress with nausea and vomiting frequently leads to poor compliance and drug discontinuance. Various neurologic disorders have been caused by ethionamide including peripheral neuropathy and psychiatric disturbances. Neurologic side effects have been reported to be alleviated by pyridoxine or nicotinamide. Reversible hepatotoxicity occurs in approximately 5% of ethionamide recipients. A hypersensitivity-type rash and poor diabetic control are infrequent complications. Its usage also is limited by a high frequency of severe gastrointestinal intolerance. Hypothyroidism is a recognized complication of ethionamide use and is more common when ethionamide is combined with PAS. Patients receiving long-term treatment with ethionamide should have periodic screening for hypothyroidism.

Usage
Ethionamide is among the agents that can be chosen for the treatment of drug-resistant tuberculosis. It appears to be active against most MDR-TB isolates. There is similarity in mode of action between ethionamide and INH, although their modes of action are sufficiently different that cross-resistance with clinical M. tuberculosis isolates usually does not occur. Regardless, confirmation of in vitro susceptibility to ethionamide for INH-resistant isolates must be obtained.

Availability and Dosage
Ethionamide is available in the United States as Trecator-SC in 250-mg coated tablets. The initial dosage is 250 mg twice daily (or as a single

dose at bedtime), which is increased by 250 mg daily until 1 g/day in divided doses is reached. Usually, 500 to 750 mg is the maximal tolerated dose.

β-Lactams

All mycobacteria produce β-lactamase. *M. tuberculosis* is protected from β-lactam antibiotics through its potent β-lactamase–encoded *gbya* gene called BlaC. Several β-lactamase–resistant β-lactam antibiotics are active in vitro against *M. tuberculosis*.[138–141] Clavulanate in particular has demonstrated in vitro the capacity to inhibit the activity of BlaC-encoded products. Meropenem, a carbapenem offering a limited substrate to hydrolysis, has demonstrated high bactericidal in vitro activity when combined with clavulanate against susceptible MDR-TB and XDR-TB strains.[142,143] Reports of successful treatment of tuberculosis with β-lactams have been sporadic. In a study of 31 patients, meropenem-clavulanate was added to a linezolid-containing regimen for treating MDR-TB/XDR-TB. The patients receiving meropenem-clavulanate had a higher percentage of smear and culture conversion than patients who did not.[143] However, activity of β-lactam agents against intracellular mycobacteria is generally poor.[144,145] In concentrations of 50 μg/mL in a macrophage model, ceforanide, active in vitro, was unable to inhibit tubercle bacilli.

Amithiozone

Amithiozone (thiacetazone), a thiosemicarbazole, is active against many strains of *M. tuberculosis*.[20] Because of its low cost, amithiozone has been employed as a first-line drug, particularly in East Africa.[20] However, because of severe toxicity in HIV-infected recipients there, clinical usage no longer seems appropriate.[13]

New Drugs for Tuberculosis

This is an era of unprecedented interest and activity in the development of new antituberculous drugs. At the present time there are more than 15 new compounds with potential antituberculosis activity in preclinical or clinical phases of development. Approximately twice that number of other interesting molecules have been identified in screening, lead identification, or lead optimization.

The diarylquinoline bedaquiline (also referred to as TMC207 or Sirturo) (see Fig. 39.1) is a novel compound that acts by inhibiting the *M. tuberculosis* adenosine triphosphate synthase, becoming bactericidal over several days.[146,147] Bedaquiline oral absorption is doubled in the fed versus the fasting state.[148] The drug is metabolized by the liver, largely by CYP3A4, with negligible renal excretion. The tertiary amino group is progressively demethylated, with the *N*-monodesmethyl compound M2 having reduced antibacterial effect (about fivefold less). The ratio of plasma metabolite, M2, to bedaquiline is 0.25:0.30. The mean terminal half-life of bedaquiline with one dosing regimen was 164 days (range, 62–408 days), and the mean terminal half-life of M2 was 159 days (range, 69–407 days).[149] Protein binding of both compounds is greater than 99%, with little CSF penetration anticipated. Clinical studies with 100-mg tablets have begun with a 2-week loading period of 400 mg once a day, followed by three times a week of 200 mg, with food, up to a total duration of 24 weeks. After loading, doses of 200 mg should be at least 2 days apart. Rifampin reduces bedaquiline levels by 52% and M2 levels by 25%. Lopinavir-ritonavir approximately doubles bedaquiline exposure. Both bedaquiline and M2 cause QT-interval prolongation, which could be a potential lethal toxicity in patients with hypokalemia, in the presence of other drugs that prolong the QT interval such as fluoroquinolones or clofazimine, or when drug interactions increase bedaquiline exposure. Nausea is another side effect. No effect on bedaquiline pharmacokinetics was observed with coadministration of INH, ethambutol, cycloserine, ofloxacin, kanamycin, pyrazinamide, or efavirenz.[148,150]

Bedaquiline demonstrates significant in vitro potency against *M. tuberculosis*—both drug-susceptible and multidrug-resistant strains—as well as multiple other mycobacteria.[146] Animal studies suggested that bedaquiline has significant potential to shorten treatment duration for both drug-susceptible and drug-resistant strains.[151] In initial and follow-up reports of a randomized trial of bedaquiline versus placebo in multidrug regimens for patients with newly diagnosed MDR-TB disease, the patients receiving bedaquiline had significantly reduced time to sputum conversion with an increased proportion of patients with sputum conversion. Bedaquiline also prevented resistance against companion drugs in the course of treatment.[152,153] One caveat that emerged from data filed with the FDA is that in a larger cohort of patients the bedaquiline treatment arm was associated with five times the number of deaths compared with the placebo arm.[154] Investigation of these deaths showed that half of them were due to progressive tuberculosis and that to date there is no direct pathophysiologic link between bedaquiline and the other deaths. The FDA approved bedaquiline for treatment of drug-resistant tuberculosis with a black box warning relating to possible cardiac toxicity and sudden death. Subsequent analyses have failed to show a significant risk of cardiac toxicity with long-term bedaquiline use.[150] As with the quinolones, a risk-benefit analysis is necessary for any patient to receive bedaquiline, but it would seem difficult to withhold bedaquiline to patients with few other antibiotic choices even in the presence of a prolonged QT interval on an electrocardiogram.

OPC-67683, or delamanid (see Fig. 39.1), is a new agent derived from the nitro-dihydro-imidazooxazole class of compounds that inhibits mycolic acid synthesis and has shown potent in vitro and in vivo activity against both drug-susceptible and drug-resistant *M. tuberculosis* strains.[155,156] Delamanid is a prodrug that is converted to the active compound by the mycobacterial cell. The drug is insoluble in water and compounded in 5% gum arabic to facilitate absorption from the gastrointestinal tract. Delamanid is highly protein bound and principally metabolized by a chemical reaction with serum albumin, with little hepatic or renal excretion of native drug.[157] Drug interactions with cytochrome enzymes are thought to be minimal. The most common side effects are nausea, vomiting, and dizziness. In one assessment of early bactericidal activity of delamanid in patients with tuberculosis, doses of 200 mg and 300 mg daily resulted in decreased sputum *M. tuberculosis* burden of a magnitude similar to that of rifampin.[158] In a placebo-controlled trial of delamanid, dosages of 100 mg/day or 200 mg/day administered over 2 months as part of a multidrug regimen for MDR-TB were associated with a significant increase in sputum-culture conversion at 2 months.[159] Most adverse events were mild to moderate in severity and were evenly distributed across groups. QT-interval prolongation was reported significantly more frequently in the groups that received delamanid. Delamanid has also been given safely in combination with bedaquiline. The European Medicines Agency conditionally approved delamanid (Deltyba) for MDR-TB at an adult dosage of two 50-mg tablets, orally twice a day with food.

PA-824, or pretomanid, is a nitroimidazole that has undergone extensive testing in animal models and is currently being studied primarily in patients with MDR-TB.[159,160,161,162] The mechanism of action of delamanid and of PA-824 is not yet known, but both appear to generate radicals with toxic effects on mycolic acids and protein biosynthesis.[163] PA-824 is potent in vitro against drug-susceptible and drug-resistant tuberculosis strains and appears to have activity against nonreplicating or slowly replicating strains. Initial dose-ranging monotherapy studies have shown dose-related early bactericidal activity.[159] An initial clinical study in drug-susceptible tuberculosis demonstrated that the combination of PA-824, moxifloxacin, and PZA had antituberculosis activity in sputum lasting 14 days, comparable with that of the current standard regimen for drug-susceptible tuberculosis.[162] A phase II trial of moxifloxacin, pretomanid, and pyrazinamide as the first 8 weeks of treatment of drug-susceptible and drug-resistant tuberculosis found this regimen well tolerated and superior in bactericidal activity compared with a standard tuberculosis regimen (in patients with drug-susceptible disease).[164] An important implication is that this regimen is effective and does not contain rifampin, and thus there is a low interaction potential with antiretroviral regimens. PA-824 would also be suitable for MDR-TB isolates. Trials involving longer duration of PA-824 administration are ongoing.

WORLD HEALTH ORGANIZATION ANTITUBERCULOSIS DRUG CLASSIFICATION FOR DRUG-RESISTANT TUBERCULOSIS

Core antituberculosis medications for MDR-TB and XDR-TB are grouped by the WHO in categories A through D with subgroups D-1, D-2,

and D-3.[10] Group A drugs include later-generation fluoroquinolones (levofloxacin, moxifloxacin, and gatifloxacin). Group B drugs include second-line injectable agents (amikacin, capreomycin, kanamycin, and streptomycin). Group C includes other core second-line agents listed in order of decreasing usual preference (ethionamide-prothionamide, cycloserine-terizidone, linezolid, and clofazimine). Other drugs that may be used are included in group D, add-on agents that are noted to be "not part of the core MDR-TB regimen" (subgroup D-1 includes PZA and ethambutol; subgroup D-2 includes bedaquiline and delamanid; and subgroup D-3 includes PAS, imipenem-cilastatin, and meropenem). Both carbapenems must be given with amoxicillin-clavulanate, as clavulanic acid is needed for efficacy of the carbapenem against *M. tuberculosis*.

Given the toxicity and relative weakness of the traditional second-line drugs, ethionamide, cycloserine, and PAS, we believe contemporary regimens for treating MDR-TB and XDR-TB should include newer agents in their place. A recent MDR Survival Guide notes the importance of linezolid and clofazimine in the treatment of MDR-TB, with mounting evidence for the efficacy and tolerability of bedaquiline and delamanid.[14]

THERAPEUTIC DRUG MONITORING

The role of therapeutic drug monitoring (TDM) remains controversial, especially in the routine management of most patients with tuberculosis. As pointed out in the most recent ATS/CDC/IDSA guidelines on tuberculosis therapy, "TDM cannot predict who will be cured, fail or relapse; however, it does allow for timely, informed decisions regarding the need for dose adjustment when necessary."[19] For some antituberculosis drugs such as amikacin and cycloserine, TDM is essential for minimizing risk of drug toxicity. It is hoped that prospective studies will be undertaken to better define the role of TDM in tuberculosis management.

COADMINISTRATION OF ANTITUBERCULOSIS THERAPY AND HUMAN IMMUNODEFICIENCY VIRUS MEDICATIONS

The most recent ATS/CDC/IDSA tuberculosis treatment guidelines note that available data show significant reductions in mortality and AIDS-defining illnesses when patients with HIV infection and tuberculosis receive ART in conjunction with daily antituberculosis medications.[19] A meta-analysis reported in this document identified no increase in the risk of other adverse events or poor outcome of tuberculosis therapy. Therefore it was recommended that patients with tuberculosis and HIV infection receive ART during antituberculosis treatment. ART should ideally be started within 2 weeks for patients with $CD4^+$ count <50 cells/mm^3 and by 8 to 12 weeks for patients with $CD4^+$ count ≥50 cells/mm^3.

MAJOR DRUGS FOR TREATMENT OF NONTUBERCULOUS MYCOBACTERIAL INFECTIONS (see Chapters 251 and 252)

NTM vary greatly in susceptibility to antimicrobial agents.[165–167,168] Some such as *M. kansasii* are susceptible to agents used principally for the treatment of tuberculosis; others such as *M. fortuitum* and *M. chelonae* respond to antibiotics used more commonly for pyogenic bacterial infections; still others including MAC, *M. abscessus* subsp., *M. simiae,* and many others are broadly resistant. The role of NTM susceptibility testing has been progressively better defined by the publication of national guidelines for testing by the Clinical Laboratory Standards Institute in 2003, 2011, and 2017.[169,170,209] Hence the indications, feasibility, and limitations of chemotherapy for NTM infections based on susceptibility results are available for many species. An important example of that process is MAC, where susceptibility testing has limited clinically predictive value except for the newer macrolides and amikacin.[168]

Macrolides (see Chapter 29)

Antimicrobial Activity and Resistance

Pretreatment strains of *M. kansasii, M. marinum, M. haemophilum, M. malmoense,* MAC, *M. chelonae,* and *M. abscessus* subsp. *massiliense* are susceptible to achievable therapeutic concentrations of the newer macrolides clarithromycin and azithromycin. This has resulted in a dramatic change in therapy for NTM infections, with a macrolide now part of the treatment regimen for many species.[171–175] These agents are especially efficacious against both pulmonary and disseminated MAC infections.[168,176,177] Both macrolides have proved efficacious for prevention of disseminated MAC infection in patients with AIDS.[178,179] Although some NTM such as *M. fortuitum*, *M. abscessus* subsp. *abscessus,* and *M. abscessus* subsp. *bolletii* may appear to be susceptible to macrolides on routine evaluation, they contain an inducible macrolide resistance gene that becomes apparent and confers macrolide resistance after macrolide exposure either in vitro or in vivo.[180] This intrinsic resistance mechanism is mediated by a series of chromosomal *erm* (erythromycin methylase) genes found in *M. tuberculosis, M. fortuitum, M. abscessus* subsp. *abscessus,* and *M. abscessus* subsp. *bolletii*.[180] This resistance mechanism is not found in the slowly growing NTM species.

Macrolides should not be used as monotherapy for any macrolide-susceptible NTM because of the rapid emergence of acquired mutational resistance, which results from a point mutation at adenine 2058 or 2059 on the 23S ribosomal RNA gene, the presumed macrolide-binding site, and produces cross-resistance to all macrolides.[181,182] The emergence of this type of resistance is inevitably associated with treatment failure and increased mortality.[183,184] Risk factors other than macrolide monotherapy include a lack of adequate companion medications to protect against the emergence of macrolide resistance, especially the combination of macrolide and quinolone without other medications.

Pharmacology

Clarithromycin is metabolized in the liver, and significant concentrations of the 14-OH metabolite are detectable in the serum.[185] Clarithromycin is also excreted in part by the kidneys, and a reduction in dosage is required in elderly patients, patients with low body weight, and patients with reduced renal function.

Adverse Reactions

The most common side effects are nausea, vomiting, and diarrhea, which are generally dose related.[186] Toxic hepatitis occurs with daily doses greater than 1.0 g and is associated with elevated levels of alkaline phosphatase and γ-glutamyltransferase.[186] Temporary hearing loss may also occur with high doses of azithromycin.[187]

Significant Drug Interactions

Clarithromycin is metabolized by the cytochrome P-450 enzyme system, and serum levels are dramatically reduced by enzyme inducers such as rifampin.[185] Clarithromycin is an inhibitor of the P-450 enzyme system, and its use results in increased serum levels and potential increased toxicities of multiple drugs metabolized by these enzymes including rifabutin, carbamazepine, cisapride, astemizole, terfenadine, and theophylline.[86,87] Clarithromycin inhibits the metabolism of PIs as well. In contrast, azithromycin is not metabolized by the cytochrome P-450 system and has no significant drug interactions.[187]

Dosage

The usual daily therapeutic doses are 500 mg twice daily for clarithromycin and 250 mg once daily for azithromycin. The usual therapeutic doses for intermittent, three-times-weekly dosing are 1000 mg (500 mg twice a day) for clarithromycin and 500 mg for azithromycin. For noncavitary MAC lung disease, the usual clarithromycin dosage is three times weekly with 500 mg in the morning and evening; for azithromycin, the dosage is 500 mg given three times weekly. For disseminated MAC prophylaxis, the dosage of azithromycin is 1200 mg once a week.[179]

Rifampin

Antimicrobial Activity

Rifampin is employed for the treatment of many slowly growing NTM infections. In vitro, all untreated strains of *M. kansasii, M. marinum, M. haemophilum,* and *M. xenopi* are inhibited by 0.25 to 1.0 μg/mL.[168,188,189] Other species are much less susceptible. Only one-half of

MAC strains are inhibited in vitro by 4 to 16 μg/mL of rifampin. Synergy between rifampin and other agents has been demonstrable for a number of species in vitro. Its role as a single agent is discouraged because resistance will occur.[189]

Pharmacology, Adverse Events, and Significant Drug Interactions

See the earlier discussion in "Rifampin" under "First-Line Antituberculous Drugs."

Usage

The excellent response of *M. kansasii* infections to rifampin-containing regimens has made rifampin the basis of most treatment regimens.[168] Rifampin doses are the same as those for treatment of tuberculosis. For patients with HIV infection receiving PIs or NNRTIs, rifabutin (300 mg three times per week) is recommended over rifampin[168] because of its lesser effect on the cytochrome P-450 system. The role of rifampin in the treatment of MAC disease is less clear. The apparent benefit of rifampin in multidrug MAC treatment regimens is protection against the emergence of acquired mutational macrolide resistance not due to direct antimycobacterial activity.

Rifabutin

Antimicrobial Activity

Rifabutin is inhibitory against 90% of strains of MAC at a concentration of 2 μg/mL.[83,84] It is concentrated several-fold in tissue and, similar to rifampin, has gastrointestinal toxicity as its most common adverse effect. Rifabutin at a dosage of 300 mg/day has been shown to reduce by 50% the incidence and rate of dissemination of MAC infections in patients with AIDS with $CD4^+$ counts <200 cells/mm^3,[190] although it is probably not as effective as the newer macrolides.

Pharmacology, Adverse Events, and Significant Drug Interactions

The reader is referred to the earlier discussion in "Rifabutin" under "First-Line Antituberculous Drugs."

Usage

Rifabutin is used in place of rifampin in HIV-infected patients receiving PIs and NNRTIs, although it is not recommended with saquinavir or delavirdine.[13] Rifabutin is also an effective prophylaxis agent against disseminated MAC. However, for therapy of disseminated disease in patients with AIDS, a placebo-controlled study showed that through 16 weeks of follow-up, a dose of 300 mg daily did not increase the culture conversion rate over clarithromycin and ethambutol alone, but it did protect against the development of macrolide resistance.[191] Rifabutin was frequently used in multidrug treatment of MAC lung disease in the past but because of poor tolerability was replaced by rifampin in most circumstances. There are no published studies comparing the efficacy of rifabutin versus rifampin in treatment regimens for MAC, although there are theoretical reasons to believe that rifabutin would be more efficacious than rifampin. Rifabutin is more clearly an important element in MAC treatment regimens in patients who have failed a regimen containing rifampin or who have developed macrolide resistance.[168]

Aminoglycosides

Aminoglycoside antibiotics have been used extensively for the treatment of rapidly growing as well as some slow-growing NTM infections. Among *M. kansasii* strains, 86% demonstrated streptomycin susceptibility. Of strains of MAC, 44% have been found to be susceptible to streptomycin. Rapidly growing mycobacteria, including *M. fortuitum* and *M. abscessus,* are resistant to streptomycin.

Amikacin is the most active aminoglycoside against NTM.[168,192–194] However, marked variability exists between mycobacterial species in susceptibility. All strains of *M. marinum, M. kansasii,* and *M. fortuitum* are susceptible to 4 μg/mL or less,[188,193,194] whereas isolates of *M. chelonae, M. abscessus,* and MAC are more resistant but are usually inhibited by 8 to 64 μg/mL of amikacin.[193,194] Doses for streptomycin and amikacin are generally the same as those for treatment of tuberculosis. Tobramycin is the most active aminoglycoside for treatment of *M. chelonae* and is the recommended aminoglycoside for that organism.[193,194] Mutational resistance to amikacin due to a 16S ribosomal RNA gene mutation can occur with MAC therapy and is associated with amikacin MICs greater than 64 μg/mL.[195] Aside from macrolides, amikacin is the only antimicrobial with a demonstrated association between the MIC for MAC and treatment response. Also similar to the macrolides, this possibility of acquired mutational resistance emphasizes the importance of combining amikacin with adequate companion medications to prevent this occurrence. Resistance with amikacin therapy has also been described with *M. abscessus*.[196]

Amikacin (generic) has also been administered by inhalation for treatment of NTM lung disease, especially MAC lung disease. However, despite widespread use, there are very few data supporting inhalational use. An amikacin sulfate preparation encapsulated in liposomes for inhalational delivery has been tested for MAC lung disease. A placebo-controlled phase 2 trial in patients with treatment-refractory MAC lung disease showed that the inhaled liposomal amikacin compound combined with guideline-based therapy achieved early and sustained negative sputum cultures in treatment-refractory MAC lung disease significantly better than guidelines-based therapy alone.[197] Initial results of a phase 3 randomized, open-label study of the liposomal amikacin preparation in adults with treatment-refractory MAC lung disease have shown similarly favorable results.[198]

Ethambutol

Ethambutol has good in vivo activity against *M. kansasii* and to a lesser extent MAC and is included as part of treatment regimens for these organisms.[168] Although MICs for ethambutol with MAC isolates are high compared with *M. tuberculosis,* ethambutol is unquestionably the critical element in preventing the emergence of acquired mutational macrolide resistance and must be included in MAC treatment regimens for the purpose of preventing the emergence of macrolide resistance regardless of the ethambutol MIC. It also has activity against most other slowly growing NTM including *M. kansasii, M. marinum,* and *M. xenopi.* Species of rapidly growing mycobacteria are all highly resistant with the exception of the *M. smegmatis.*

LESS FREQUENTLY USED MISCELLANEOUS ANTIMICROBIAL AGENTS FOR TREATMENT OF NONTUBERCULOUS MYCOBACTERIA

Isoniazid

Drugs such as INH used principally for treatment of *M. tuberculosis* were evaluated relatively early for their activity against NTM. INH inhibits nearly 90% of strains of *M. kansasii* at concentrations of 1 to 5 μg/mL, as contrasted to only 10% to 30% of MAC strains. At the present time INH is included routinely in the therapy for *M. kansasii* in the United States, although it has largely been replaced by macrolide for this organism.[168] INH is also used for *M. xenopi* and *M. szulgai,* but also is frequently replaced by macrolide. It is not currently recommended for treatment of MAC.

Tetracyclines

Approximately 50% of isolates of the rapidly growing species *M. fortuitum* and 20% of *M. chelonae* are susceptible to tetracycline.[195] Minocycline and doxycycline are twofold to fourfold more active than tetracycline[194] and have been effective in therapy when the isolates were susceptible in vitro.[145] Minocycline and doxycycline are also active against *M. marinum*[182,199,200] and have been used successfully in *M. marinum* infections.[168,199] A parenteral tetracycline, tigecycline, with activity against all rapidly growing mycobacteria has been marketed. It is frequently used as part of combination therapy for *M. abscessus* lung disease; however, few data evaluating its efficacy in this role are available.[201] Tigecycline is also associated with frequent and severe side effects, most notably nausea and vomiting.

Sulfonamides

Sulfamethoxazole is active against *M. fortuitum* but not against *M. chelonae* or *M. abscessus*.[193,194] Localized infections have been cured with sulfamethoxazole alone or in combination with trimethoprim.[145]

M. marinum infections have responded to therapy with trimethoprim-sulfamethoxazole,[168] but strains are susceptible in vitro only against a low inoculum.[175]

Both *M. marinum* and *M. kansasii* exhibit very similar drug susceptibilities. Sulfamethoxazole is also active against isolates of *M. kansasii.* It has been curative in combination regimens used for treatment of rifampin-resistant *M. kansasii* infections.[189]

Limited experience indicates some in vitro activity and clinical efficacy of sulfonamides against *M. terrae* complex, *M. haemophilum, M. simiae,* and MAC.[168]

Quinolones

The newer fluorinated quinolones (ciprofloxacin, moxifloxacin, levofloxacin) have in vitro activity against a number of NTM at achievable serum levels.[194,202] *M. fortuitum* strains are the most susceptible, with ciprofloxacin MICs of 0.25 µg/mL or less and good responses clinically.[202] More recent studies have shown levofloxacin to have comparable activity to ciprofloxacin, whereas the newer quinolone moxifloxacin has twofold to fourfold lower MICs than these older agents.[203] A number of species are inhibited by intermediate concentrations of ciprofloxacin (1–4 µg/mL). These include *M. chelonae* (25%), *M. malmoense, M. marinum, M. xenopi, M. kansasii, M. haemophilum,* and some strains of MAC. The newer quinolones gatifloxacin and moxifloxacin are more active than ciprofloxacin.[203] The clinical efficacy of the quinolones for these species has yet to be established due to the lack of in vivo evidence correlating favorable MICs, especially for MAC, with favorable treatment outcomes. There is also a clear risk for developing acquired mutational macrolide resistance for MAC with the combination of macrolide and fluoroquinolone without other companion medications such as ethambutol.[204,205] For other organisms with a clearer correlation between in vitro susceptibility and treatment outcome, resistance to ciprofloxacin after monotherapy has been described and presumably involves the same DNA gyrase mutations observed with quinolone resistance with *M. tuberculosis.*[113]

Linezolid

More recent studies have shown that many NTM are inhibited by 8 µg/mL (susceptible MICs) of linezolid including *M. fortuitum, M. kansasii, M. marinum,* and *M. haemophilum.* Isolates of *M. chelonae* all are susceptible or intermediate to linezolid, and in vitro it is the most active oral drug available for this species after the macrolides. Some isolates of *M. abscessus* subsp. show susceptible MIC levels, which is especially important for these species, for which there are limited choices for oral drugs to include in treatment regimens. Isolates of MAC and *M. simiae* are usually resistant to linezolid.[207]

β-Lactams

All mycobacteria produce β-lactamase, although it can be difficult to detect in MAC. However, β-lactams or combinations of β-lactam and β-lactamase inhibitors have been shown to be active or useful clinically only for the *M. fortuitum* complex. Cefoxitin, cefmetazole, and imipenem-cilastatin are active in vitro against approximately 80% of *M. fortuitum* strains and most isolates of *M. abscessus* subsp. at clinically achievable plasma concentrations.[135,208]

Clofazimine

Discussed more fully in "Drugs for Treatment of Hansen Disease (Leprosy)," clofazimine (Lamprene) possesses in vitro activity against *M. chelonae, M. abscessus,* and MAC. Most strains are inhibited by 1.6 to 2.0 µg/mL.[168] Clinical experience with clofazimine in therapy against MAC in AIDS was disappointing.[168] Clinical experience with MAC lung disease has been infrequently reported, although it is frequently used for this indication. More recent reports suggest that it is generally well tolerated, but its role in MAC treatment regimens is still unclear. At the present time it appears to be similar to rifampin in that it helps protect against the emergence of acquired mutational macrolide resistance.

Susceptibility Tests

Standardized methods for susceptibility testing of NTM were approved for the first time in 2003 by the Clinical Laboratory Standards Institute and subsequently updated.[145] In vitro susceptibility of NTM drugs is no guarantee of therapeutic efficacy. Previously cited failures of fluoroquinolones and ethambutol in MAC infections indicate limitations in extrapolating in vitro data to clinical experience. As a rule, favorable therapeutic results are likely when drugs to which NTM are susceptible in vitro are used, and poor outcomes can be anticipated when there is in vitro resistance. Least predictable are outcomes of MAC infections, for which routine susceptibility testing is *not* recommended except for clarithromycin (or azithromycin).[145,146,168]

DRUGS FOR TREATMENT OF HANSEN DISEASE (LEPROSY)

(see Chapter 250)

Background

The special parasite-host relationship of *M. leprae* (Hansen bacillus), characterized by persistence of the organism in tissue for years, has mandated prolonged chemotherapy to prevent relapse. For years, chemotherapy for leprosy mainly consisted of dapsone alone, which produced gratifying clinical results and was affordable. However, because of self-supervised monotherapy, resistance of leprosy bacilli, both secondary and now primary, became a problem worldwide.[210] At the present time multidrug therapy is the rule for both multibacillary and paucibacillary disease, and this approach has markedly increased cure rates and decreased the incidence of drug resistance. The principal agents used in therapeutic multidrug regimens are dapsone, rifampin, and clofazimine.

Dapsone

Derivation and Structure

Dapsone (4,4′-diaminophenyl sulfone), a synthetic compound, was demonstrated to be effective in rat leprosy in 1941 and soon thereafter was used successfully in human trials.

Mechanism of Action

Sulfones inhibit bacterial dihydropteroate synthase, as do sulfonamides, and presumably inhibit *M. leprae* by the same mechanism.

Antimicrobial Activity

By mouse foot pad inoculation, 0.003 µg/mL of dapsone is estimated to inhibit multiplication of *M. leprae.* Dapsone has been described as "weakly bactericidal" for susceptible leprosy bacilli. In humans, it has been estimated that 99.9% of bacillary populations are killed after 3 to 4 months of dapsone therapy.[211] In lepromatous (multibacillary) patients on monotherapy, secondary dapsone resistance often emerges 5 to 24 years after beginning therapy.[212] Before the usage of current standard multidrug regimens, secondary resistance occurred in approximately 20% of cases.

Pharmacology

Dapsone is well absorbed orally. Distributed throughout body fluids, tissue concentrations are approximately 2 µg/mL. The plasma half-life of dapsone is 21 to 44 hours, with some drug retention for up to 3 weeks. Dapsone becomes acetylated with 70% to 80% excreted as metabolites in urine. The dosage should be reduced accordingly in renal failure.

Adverse Reactions

An oxidant drug, dapsone produces dose-dependent hemolysis, which is not of clinical consequence in patients without a hematologic disorder taking dapsone 50 to 100 mg daily. Hemolysis is greatly enhanced in patients with glucose-6-phosphate dehydrogenase deficiency, especially in its severe forms. Gastrointestinal intolerance occurs with resulting anorexia, nausea, or vomiting. Hematuria, fever, pruritus, rashes, and granulocytopenia can occur.

Dapsone is now being used in patients with AIDS as prophylaxis and treatment of *Pneumocystis jirovecii* pneumonia. These patients usually have preexisting anemia, making dapsone-induced hemolysis less well tolerated. Conversion of up to 20% of erythrocyte hemoglobin to methemoglobin can occur with dapsone doses of 100 mg daily. Although methemoglobinemia is usually asymptomatic, it may

become of clinical concern if the patient develops hypoxemia from lung disease. Rash is common in this patient population. In one study using dapsone, 100 mg daily, for prophylaxis, 33 of 47 patients discontinued the drug.[213]

In patients with leprosy, reactions with dapsone may be difficult to extricate from the reactions associated with the disease itself.[214] A sulfone syndrome occurring 5 to 6 weeks after the initiation of therapy can be characterized by fever, jaundice, dermatitis, and lymphadenopathy—a presentation similar to infectious mononucleosis.[215] During initial dapsone therapy, erythema nodosum leprosum reactions commonly become manifest in patients with multibacillary disease.

Usage

Dapsone and rifampin are the principal therapeutic agents for the treatment of both multibacillary and paucibacillary *M. leprae* infections. Usage in prophylaxis or treatment of *P. jirovecii* pneumonia is discussed in Chapter 269. Dapsone is also useful in dermatitis herpetiformis, but that is beyond the scope of this chapter.

Availability and Dosage

Dapsone is available generically in tablets of 25 or 100 mg. The daily dose for adults is 100 mg and for children is 1.0 to 1.5 mg/kg/day. The WHO recommends administration daily for 6 months for paucibacillary disease and treatment for 1 year for multibacillary disease. The National Hansen's Disease Program (NHDP) recommends 1 year of treatment for paucibacillary disease and a minimum 2 years of treatment for multibacillary disease.

Rifampin

Mechanism of Action and Resistance

The mechanism of action of rifampin is presumed to be inhibition of *M. leprae* DNA-dependent RNA polymerase that produces a relatively rapid bactericidal effect. Its inhibitory concentration of human strains of *M. leprae* tested in mice is 0.3 μg/mL. Acquired rifampin resistance is caused by mutational changes in RNA polymerase.[216]

Usage

Clinical usage of rifampin has confirmed that it is more bactericidal by several orders of magnitude than all other antileprosy drugs either alone or in combination. It is considered the only rapidly bactericidal drug against *M. leprae*. Using a skin biopsy assay, a single 1500-mg dose of rifampin was determined to reduce the viability of leprosy bacilli to undetectable levels by 3 to 5 days.[217] Despite such a dramatic impact on numbers of tissue *M. leprae* organisms, rifampin must be employed with one or more companion drugs to prevent the development of resistance.[211] The high cost of rifampin has discouraged daily usage in economically disadvantaged regions. However, once-monthly therapy with 600 to 1200 mg of rifampin in combination drug regimens has produced satisfactory clinical responses with a minimum of adverse reactions.[218] Current recommendations are that rifampin should be administered in a single monthly supervised dose of 600 mg. This dosage is continued for 6 months in paucibacillary disease and for a minimum of 2 years in multibacillary disease. In the United States, rifampin is given as a 600-mg daily dose in both of the settings described. Reversal and erythema nodosum leprosum reactions with rifampin have been comparable or less severe than with sulfones alone. Daily rifampin treatment may reduce both the serum levels and subsequently the beneficial effects of corticosteroids for reactions by inducing hepatic microsomal enzymes. In patients with reactions requiring steroids, switching rifampin dose from daily to monthly helps to overcome interaction between rifampin and corticosteroids.

Clofazimine (Lamprene)

Derivation and Structure

Clofazimine is a phenazine dye.

Mechanism of Action and Antimicrobial Activity

The precise mechanism of action of clofazimine is unknown. Highly lipophilic and bound to mycobacterial DNA, clofazimine is weakly bactericidal against *M. leprae*. Its action may relate to iron chelation with resulting production of nascent oxygen radicals intracellularly.[219] The inhibitory concentration of clofazimine in mouse tissue is between 0.10 mg/kg and 1.0 mg/kg. A delay of about 50 days ensues before tissue antimicrobial activity can be demonstrated in humans.

Clofazimine is not commercially available in the United States; it is distributed through the NHDP. NHDP holds the Investigational New Drug (IND) application for treatment of leprosy in the United States. To obtain clofazimine, physicians are required to register as an investigator under the NHDP IND. This requires submitting a curriculum vitae and a signed FDA form 1572 to the NHDP. For further information or to request investigator status, physicians should contact the NHDP at 1-800-642-2477 or nhdped@hrsa.gov or go to http://www.hrsa.gov/hansensdisease/index.html.

Pharmacology

Clofazimine pharmacokinetics are complex. Absorption is quite variable, with 9% to 74% of an administered dose appearing in feces. Oral administration results in plasma concentrations of 0.4 to 3 μg/mL with a half-life of approximately 70 days. Clofazimine is widely distributed throughout reticuloendothelial tissues, especially liver, spleen, lung, adrenals, adipose tissue, and skin lesions. Red-orange phagocytized crystals of clofazimine are observed microscopically in macrophages. It is largely unmetabolized and subsequently slowly excreted with less than 1% in urine. Biliary excretion appears to be the major route of excretion. Excretion also occurs in breast milk. Dosage of 100 mg/day has been calculated to result eventually in a total accumulation of at least 10 g in human tissue.

Adverse Reactions

Gastrointestinal intolerance (anorexia, diarrhea, abdominal pain) is the most common therapy-limiting side effect and is generally dose related. Dry mouth and dry skin may occur. Skin pigmentation is quite common, resulting from drug accumulation and producing red-brown to nearly black discoloration, especially in dark-skinned people. Skin discoloration is reversible but may take several months to years after discontinuation of medication. Avoidance of direct sun exposure and use of sun block before sun exposure are recommended. Suicidal ideation (rare) has been reported with skin discoloration. Prolongation of QT interval associated with torsades de pointes has been reported in patients taking >100 mg daily and other QT interval–prolonging drugs.

Usage

The current role of clofazimine is principally in combination with rifampin and dapsone for multibacillary disease. It is also used in combination for sulfone-resistant infections and for individuals who are intolerant to sulfones, usually because of severe sulfone-associated erythema nodosum leprosum or reversal reactions. Such reactions occur much less often with clofazimine than with dapsone[220] possibly because of antiinflammatory properties of clofazimine.

Availability and Dosage

Clofazimine is supplied in 50-mg capsules. For multibacillary disease, it is administered in a dosage of 50 mg/day for a minimum of 2 years in combination with rifampin and dapsone. When used as a dapsone alternative, dosage has usually been 100 to 300 mg/day.

Additional or Second-Line Drugs

Thiacetazone (Amithiozone)

Efficacy of thiacetazone is greater in tuberculoid (paucibacillary) than in lepromatous (multibacillary) disease. It can be administered when sulfones are not tolerated. Considerable cross-resistance occurs with sulfones. Thiacetazone is unavailable in the United States.

Ethionamide and Protionamide

Ethionamide (Trecator-SC) is described in "Second-Line Antituberculous Drugs." Pharmacokinetics and dosing are similar for ethionamide and its congener protionamide (which is unavailable in the United States), and these agents provide alternatives to clofazimine in multidrug regimens for multibacillary disease in patients who are unable to tolerate

clofazimine or refuse it because of skin pigmentation. Ethionamide and protionamide are apparently weakly bactericidal against *M. leprae*. Ethionamide is provided in 250-mg tablets. The usual dosage is 250 mg daily. Both agents are expensive and cause considerable gastrointestinal intolerance and occasional hepatitis.

Other Substituted Rifamycins

Rifabutin (Mycobutin) and rifapentine (Priftin) are substituted rifamycins that are active against *M. leprae*. Both are approved by the FDA for treatment of tuberculosis. In mice, these compounds are even more active than rifampin,[221] which raises interest in their use, especially in intermittent regimens.

Other Sulfones

Acedapsone

Acedapsone (4,4′-diacetyldiaminodiphenyl sulfone) is a long-acting intramuscular repository derivative of dapsone. The parent compound possesses little activity against *M. leprae* but is metabolized into active dapsone. Its half-life is 46 days, and the half-life of the derived dapsone is 43 days.[4,222] A 300-mg intramuscular dose maintains dapsone levels in volunteers above the inhibitory concentration for *M. leprae* for approximately 100 days. Microbiologic and clinical responses are slower than those for daily dapsone. Long-term studies with acedapsone by injection five times yearly have yielded encouraging results. Acedapsone shows promise especially in regions where, or in patients in whom, long-term oral therapy is not practical.

Sulfoxone

Less well absorbed and more expensive than dapsone, sulfoxone, a disubstituted sulfone, may be better tolerated gastrointestinally. It is formulated in 165-mg enteric-coated tablets, with a usual daily dosage of 300 mg.[222]

Newer Agents

Several other agents have shown promising activity against *M. leprae* in mouse foot pad models and early clinical trials. These agents include minocycline (Minocin)[223]; clarithromycin (Biaxin)[223]; and fluorinated quinolones pefloxacin, ofloxacin (Floxin),[224] and especially sparfloxacin.[225] Their roles in replacing drugs in existing multidrug regimens remain to be determined, although none of these agents appears to be as bactericidal against *M. leprae* as rifampin, and neither pefloxacin nor sparfloxacin is on the market. Current efforts with these newer agents are also focusing on short-course therapy,[226] rather than the current standard 2-year regimens.

Chemotherapy-Associated Reactions in Leprosy

Febrile reactions in leprosy can be ameliorated with a conventional dosage of acetylsalicylic acid (aspirin). Immunologic reactions are common during chemotherapy. Reversal reactions associated with swelling and edema in preexisting skin lesions or peripheral neuropathy in more severe reactions usually occur in the first year of therapy. Corticosteroids such as prednisone, 40 to 80 mg/day initially with subsequent tapering of dosage, have been reasonably efficacious for reversal reactions. Thalidomide is not used for reversal reactions.

For patients with erythema nodosum leprosum reactions, thalidomide in an initial dosage of 100 mg four times a day is the treatment of choice. Its beneficial effect for these reactions appears to be mediated by the inhibition of tumor necrosis factor-α.[227] Treatment is initiated at 400 mg/day to attain control of the disease but should then be tapered over the first week with a maintenance dose of 50 to 100 mg/day. Thalidomide is commercially available for the treatment of leprosy, but its usage is tightly regulated (see Chapter 250). Because of its marked teratogenicity, thalidomide should never be administered to women of childbearing potential. In patients with erythema nodosum leprosum for whom thalidomide is unacceptable, high-dose prednisone is an alternative treatment. Patients who manifest puzzling or severe reactions are best managed by HD specialists such as those at the National Hansen's Disease (Leprosy) Clinical Center, Baton Rouge, Louisiana. The NHDP located in Baton Rouge is the only facility in the United States devoted to diagnosis, treatment, and research concerning HD.

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40A Antifungal Agents: Amphotericin B

David A. Stevens

GENERAL FEATURES

Mechanism of Action

Amphotericin B is available in a formulation with deoxycholate and in lipid-associated formulations. For all preparations, the active component is amphotericin B produced by *Streptomyces nodosus*. Amphotericin B is a lipophilic molecule (Fig. 40A.1) that exerts its antifungal effect by insertion into the fungal cytoplasmic membrane, probably orienting as head-to-tail oligomers perpendicular to the plane of the membrane.[1] The drug is closely bound to sterols such as ergosterol. Amphotericin B causes membrane permeability to increase. At lower drug concentrations, potassium ion (K^+)–channel activity is increased.[2] At higher concentrations, pores are formed in the membrane. Loss of intracellular potassium and other molecules impairs fungal viability. The onset of action is rapid and unrelated to the growth rate, consistent with the concept that the drug acts at preformed sites and no metabolic processing is required before a target is exposed.[3] Alternative explanations of amphotericin B action have been postulated. In one, binding to cell membrane lipids is postulated to have primacy over actions at ion channels.[4] Amphotericin B also has effects via oxidative pathways that may enhance antifungal activity. In addition, amphotericin B localizes to endothelial cells and may thereby produce endothelial cell activation.[5] The possible effects of amphotericin B and its lipid formulations on the immune system have been reviewed.[6]

Spectrum of Activity and Mechanisms of Resistance

Amphotericin B is active against most fungi, and its spectrum of activity is not influenced by the choice of formulation. Where resistance occurs, it is generally attributed to reductions in ergosterol biosynthesis and synthesis of alternative sterols that lessen the ability of amphotericin B to interact with the fungal membrane.[7] Resistance appears modulated by sphingolipids,[8] and may also follow from increased production of reactive-oxidant scavengers.[9] Primary resistance is common for *Aspergillus terreus, Scedosporium* spp., and *Trichosporon* spp.[7] Among the *Candida* spp., primary resistance is noted at meaningful frequencies most often for *Candida lusitaniae* and the newly recognized pathogen, *Candida auris*. Development of resistance in isolates of normally susceptible species is rare but has been described for essentially all common pathogens. Although such isolates may exhibit altered growth and reduced pathogenicity,[10] invasive and lethal infections are well described. Cell wall changes in resistant strains may also have immunologic consequences.[11] In studies of amphotericin B deoxycholate as therapy for candidiasis, the principal pharmacodynamic driver of in vivo response has been ratio of the peak achieved serum concentration to the minimal inhibitory concentration.[12]

AVAILABLE FORMULATIONS

There are four principal available amphotericin B formulations. Amphotericin B deoxycholate (ABD; Fungizone) was licensed in 1959 in the United States.[13] Subsequently, three lipid-associated formulations have been marketed: amphotericin B colloidal dispersion (ABCD; Amphotec or Amphocil), amphotericin B lipid complex (ABLC; Abelcet), and liposomal amphotericin B (LAMB; AmBisome).[14] Other lipid amphotericin preparations, including liposomal preparations, have emerged in markets outside the United States; that they are equivalent in efficacy and toxicity to ones marketed in the United States has not been shown. In attempts to produce lower-cost lipid-associated formulations, some reports have advocated mixing ABD with a parenteral fat emulsion at an ABD concentration of 1 to 2 mg/mL. Although less nephrotoxicity in adults has been suggested with this preparation, at a dose of 1 mg/kg daily, than with infusions of ABD in 5% dextrose,[15] no advantage was found in children,[16] and serum amphotericin B concentrations were also lower with the fat emulsion, thus raising the possibility that amphotericin B was simply aggregating in the fat emulsion, but that the resulting cloudiness could not be perceived in the milky-looking lipid. Use of such preparations should be reserved for investigational settings.

Amphotericin B Deoxycholate Formulation

ABD is insoluble in water at physiologic pH. The drug is marketed for intravenous (IV) use as a powder containing amphotericin B, 50 mg; sodium deoxycholate, 41 mg; and sodium phosphate buffer, 25.2 mg. Although a clear yellow solution forms when the powder is hydrated, ABD is colloidal. If a filter with a 0.22-micropore diameter is placed in the infusion line, considerable drug is removed by the filter. ABD must be prepared by dissolving in water or dextrose in water: dissolution in electrolyte-containing diluents (e.g., sodium chloride or sodium bicarbonate) will aggregate the colloids, producing visible clouding, and must be avoided. ABD is currently manufactured by a number of different generic manufacturers, and significant differences in amphotericin A contamination and the ability of the product to induce interleukin-1β production have been reported and may be part of the cause of the intersubject variation in toxicities observed with such compounds.[17]

Pharmacology

Concentrations of amphotericin B in biologic fluids have usually been measured by bioassay,[18] but use of high-pressure liquid chromatography,[19,20] immunoassay,[21] and radiometric respirometry[22] have been described. Despite the proliferation of methods, routine determination of amphotericin B serum, urine, or cerebrospinal fluid (CSF) concentrations has no definite clinical value. Nonetheless, amphotericin B assays have revealed some remarkable pharmacologic properties of ABD. When colloidal amphotericin B is admixed in serum, deoxycholate separates from amphotericin B, and more than 95% of the latter binds to serum proteins, principally to β-lipoprotein. Presumably the drug is bound to the cholesterol carried on this protein. Most of the drug leaves the circulation promptly, perhaps bound to cholesterol-containing cytoplasmic membranes. Amphotericin B is stored in the liver and other organs; the drug appears to reenter the circulation slowly. Most of the drug is degraded in situ, with only a small percentage being excreted in urine or bile. Blood levels are uninfluenced by hepatic or renal failure. Hemodialysis does not alter blood levels, except in an occasional patient with lipemic plasma, who may be losing drug by adherence to the dialysis membrane. Concentrations of amphotericin B in fluid from inflamed areas, such as pleura, peritoneum, joints, vitreous humor, and aqueous humor, are roughly two-thirds of the trough serum level. Cord blood from one infant contained an amphotericin B concentration of 0.37 μg/mL, half the simultaneous maternal trough blood level. Amphotericin B penetrates poorly into CSF (whether meninges are normal or inflamed), saliva, bronchial secretions, brain, pancreas, muscle, bone, vitreous humor, or normal amniotic fluid. The presence of infection and inflammation in some tissues may increase the local concentrations. Urine concentrations are similar to serum concentrations. Peak serum concentrations with conventional IV doses are 0.5 to 2.0 μg/mL and

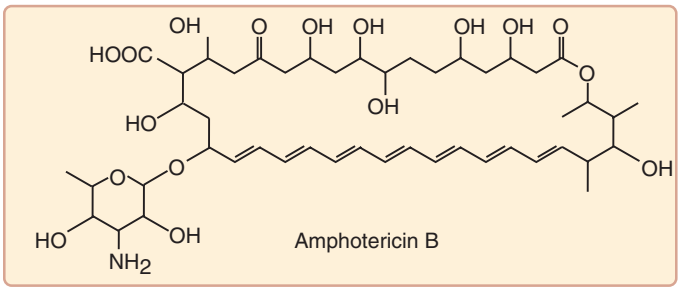

FIG. 40A.1 Structure of amphotericin B.

rapidly fall initially to slowly approach a plateau of 0.2 to 0.5 μg/mL.[18] The initial half-life is about 24 hours; the β-phase half-life is roughly 15 days. Serum concentrations can be detected for at least 7 weeks after the end of therapy, presumably reflecting release from cell membranes and/or lipids. The drug also has complex immunomodulatory properties, potentially of clinical significance but presently undefined.

Toxicity

Nephrotoxicity

ABD causes a dose-dependent decrease in the glomerular filtration rate. The direct vasoconstrictive effect of amphotericin B on afferent renal arterioles results in reduced glomerular and renal tubular blood flow.[23] Other primary or secondary effects on the kidney include potassium, magnesium, and bicarbonate wasting and decreased erythropoietin production. Permanent loss of renal function is roughly related to the total dose, not the level of temporary azotemia, and is due to destruction of renal tubular cells, disruption of tubular basement membrane, and loss of functioning nephron units. Methodologies to detect early signs of kidney damage are under study.[24] Saline loading, such as infusion of 1 L of saline before ABD, has been associated with reduced nephrotoxicity in some studies but not others.[25] Benefits from oral salt loading have also been reported.[26] Serum potassium should be monitored, and losses often require supplemental oral or IV potassium. Renal tubular acidosis from bicarbonate wasting rarely requires base replacement, but other drugs and diseases that promote acidosis may act synergistically.

Amphotericin B is generally devoid of drug-drug interactions, but azotemia caused by amphotericin B is often worse in patients taking other nephrotoxic drugs, such as cyclosporine or aminoglycosides. Hypotension, intravascular volume depletion, renal transplantation, and other preexisting renal disease all magnify the management problems associated with amphotericin B–induced azotemia. These toxicities are lessened by use of the lipid-associated formulations of amphotericin B (LFABs; see "Lipid-Associated Formulations of Amphotericin B" later).

Early in a course of therapy with ABD, azotemia may increase rapidly, often falls a little, and then stabilizes after several days. Adults with no other renal disease may develop an average serum creatinine level of 2 to 3 mg/dL at therapeutic doses, and therapy should not be withheld unless azotemia exceeds this level. Attempting to give ABD to an adult without causing azotemia will usually lead to inadequate therapy. The purpose of tapering therapy is not to avoid drug accumulation, but to reduce insults to already damaged nephrons.

Other Chronic Toxicity

Nausea, anorexia, and vomiting are common. Phlebitis occurs if peripheral vein catheters are used. Normocytic normochromic anemia occurs gradually and is associated with lower plasma erythropoietin levels than anticipated from the level of anemia.[27] The hematocrit rarely falls below 20% to 25% unless other causes of anemia are present. Rarely, thrombocytopenia, modest leukopenia, arrhythmias, coagulopathy, hemorrhagic enteritis, tinnitus, vertigo, encephalopathy, seizures, hemolysis, elevated transaminases, or dysesthesia of the soles of the feet may be observed.

Acute Reactions

About 30 to 45 minutes after beginning the first few ABD infusions, chills, fever, and tachypnea may occur, peak in 15 to 30 minutes, and slowly abate over a period of 2 to 4 hours. A patient with underlying cardiac or pulmonary disease may have hypoxemia. These reactions are less common in young children or patients receiving adrenal corticosteroids. Subsequent infusions of the same dose cause progressively milder reactions. Premedication with acetaminophen or the addition of hydrocortisone, 25 to 50 mg, to the infusion solution can diminish the reactions. Meperidine given early in a chill shortens the rigors but may induce nausea or emesis. Concern about this kind of reaction in an unstable patient had led some physicians to use a test dose of 1 mg given over a 15-minute period to assess the subsequent reaction over 1 hour before deciding whether the next dose should be a full therapeutic dose of at least 0.5 mg/kg or an intermediate dose. Whether or not a test dose is given, patients with rapidly progressive mycoses should receive a full therapeutic dose within 24 hours, without any delay entailed by test or intermediate doses. Equally important, this reaction should not be mistaken for anaphylaxis or otherwise considered a contraindication to further ABD. True allergic reactions are extremely rare. Reversible dilated cardiomyopathy has been reported.[28]

Administration

ABD is infused in 5% dextrose over a 2- to 4-hour infusion interval. Infusion 1 hour in duration appears to generally be safe for persons who have tolerated slower infusions and may be advantageous for outpatient therapy.[29,30] Early in the course of therapy, fever is more pronounced with infusion intervals of only 45 minutes than with infusion lasting 4 hours.[31] Rapid infusion in patients with severely compromised renal function may lead to acute, marked hyperkalemia and ventricular fibrillation. Particularly with infusions of prolonged length, shielding the infusion bottle from light would reduce drug decay from light sensitivity.

Once therapy is well underway, patients receiving a stable daily dose may be changed to an increased double dose on alternate days to reduce the frequency of infusion-associated toxicity, particularly anorexia, and as a convenience for outpatient therapy. Doses greater than 1.5 mg/kg are not generally given on this schedule because the toxicity of such infusions is not well described. As another approach to reduction of toxicity, continuous infusion of amphotericin B with doses up to 2.0 mg/kg per 24 hours has been described based on limited data,[32] but this approach is not consistent with the observation that the principal pharmacodynamic driver of efficacy for amphotericin B is peak drug concentration.[33,34]

Dosage

Daily ABD doses of 0.3 mg/kg often suffice for esophageal candidiasis. A dose of 0.5 mg/kg is appropriate for blastomycosis, disseminated histoplasmosis, and extracutaneous sporotrichosis. Patients with cryptococcal meningitis are generally given doses of 0.6 to 1 mg/kg; those with coccidioidomycosis may require doses of 1 mg/kg. Patients with mucormycosis or invasive aspergillosis are given daily doses of 1 to 1.5 mg/kg until improvement is clearly present. Doses of 0.5 to 1.0 mg/kg are often used in neutropenic patients receiving empirical amphotericin B (see Chapter 306).[35] Local instillation of amphotericin B into CSF, joints, or pleura is rarely indicated. One exception is coccidioidal meningitis, which can be treated with intrathecal amphotericin B because it may produce superior results, particularly in the long term, although with far greater toxicity than seen with systemic azole therapy. Intraocular administration for fungal endophthalmitis is occasionally used; doses of 5 to 10 μg appear to avoid retinal toxicity. Corneal baths with 1 mg/mL in sterile water are useful for fungal keratitis but are irritating. Bladder irrigation with 50 μg/mL in sterile water is useful for patients with *Candida* cystitis and a Foley catheter, particularly as preparation for genitourinary surgery. Equivalent results may be obtained with oral fluconazole.

Lipid-Associated Formulations of Amphotericin B

One of the LFABs (LAMB; trade name AmBisome) is a liposomal formulation. The other two—ABLC (trade name Abelcet) and ABCD (two trade names: Amphotec and Amphocil)—are aggregates of lipid and amphotericin B rather than liposomes. The phrase "liposomal

amphotericin B" is often used mistakenly as a label for the entire class of compounds. Thus this chapter uses the phrase *lipid-associated formulations of amphotericin B* (LFABs) as a general label for the class. Because tolerance of one product does not always translate to tolerance of another, it is important that the physician use an unambiguous name when prescribing these compounds.[36]

ABLC is licensed in the United States for treatment of invasive fungal infections in patients who are refractory to or intolerant of conventional amphotericin B therapy (5 mg/kg/day).[37] LAMB is licensed for empirical therapy for presumed fungal infection in febrile, neutropenic patients (3 mg/kg/day); for treatment of cryptococcal meningitis in human immunodeficiency virus (HIV)–infected patients (6 mg/kg/day); for treatment of patients with *Aspergillus* spp., *Candida* spp., and/or *Cryptococcus* spp. infections refractory to ABD; or in patients in whom renal impairment or unacceptable toxicity precludes the use of ABD. LAMB is also indicated for treatment of visceral leishmaniasis (3 mg/kg/day for immunocompetent patients, 4 mg/kg/day for immunocompromised patients; see Chapter 275 for further details).[38] ABCD is not presently commercially available in the United States.

Pharmacology and Toxicity

The three LFABs have quite different pharmacokinetic patterns.[12,13] When compared on the basis of equal milligram-per-kilogram dosages, the LFABs produce tissue amphotericin B concentrations that range from 10% to 500% of those seen with ABD,[12] with a consistent relative reduction (80%–90%) seen in the kidney concentration. Because the LFABs are typically given at milligram-per-kilogram doses that are 3- to 12-fold higher than those used for ABD, the relevance of these comparisons is uncertain, although it is generally clear that all three LFABs require higher doses in experimental animals to achieve the same therapeutic effect as ABD.

These higher but equipotent doses of the LFABs are notably better tolerated than ABD, with reductions in both the frequency and severity of acute infusion-related reactions and chronic nephrotoxicity.[39] An exception to this rule is ABCD, which generally shows acute infusion-related reactions similar to or worse than those with ABD.

Intolerance to one LFAB but not to another has been rarely reported, and allergy to some component of the offending preparation has been speculated.

Amphotericin B Lipid Complex

ABLC is a complex of almost equimolar concentrations of amphotericin B and lipid, the latter being a 7:3 mixture of dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol. The drug is shipped as a cloudy suspension with particles 1.6 to 11 μm in diameter. Particle shape is not globular but ribbon-like. The manufacturer provides a device for the pharmacy to filter out aggregates larger than 5 μm before dispensing in 5% dextrose solution. The major efficacy data are from the manufacturer's open-label, noncomparative studies.[40,41]

Liposomal Amphotericin B

LAMB comprises a unilamellar liposome about 55 to 75 nm in diameter that contains roughly one molecule of amphotericin B per nine molecules of lipid. The latter is a mixture of hydrogenated soy lecithin–cholesterol-distearoylphosphatidylglycerol in a 10:5:4 ratio. Unlike the other lipid-associated amphotericins, serum concentrations are not lower than those obtained with the same dose of ABD, the circulating drug is liposome-associated after IV infusion, and the amount of unbound amphotericin is less than with ABD.[42] In a randomized three-way trial comparing LAMB at 3 or 6 mg/kg/day and ABD at 0.7 mg/kg/day in HIV-infected subjects with cryptococcal meningitis, equivalent global response rates were reported. A randomized comparison of LAMB versus ABD for histoplasmosis in HIV-infected subjects reported superior efficacy for LAMB.[43] Prospective randomized studies have compared LAMB and ABD in neutropenic patients with fever and found equivalent efficacy.[44,45] Randomized trials in patients with aspergillosis indicated doses of 1 to 3 mg/kg LAMB to be not inferior in efficacy to doses of 4 to 10 mg/kg, and with fewer side effects.[46,47]

These studies generally suggest that LAMB causes less nephrotoxicity and less severe hypokalemia than ABD, a result supported by a direct comparison of the two formulations, and less nephrotoxicity than ABLC.[48,49] Infusion at 1 to 2 mg/mL over a 2-hour period is recommended. Infusion intervals can be shortened to 60 minutes for patients in whom the treatment is well tolerated. A triad of infusion-related reactions—symptoms from the categories of (1) chest pain, dyspnea, and hypoxia; (2) severe pain in the abdomen, back, flank, or leg; and (3) flushing and urticaria—have been reported to occur, most often within the first 5 minutes of infusion and apparently unrelated to infusion speed.[50] These reactions are effectively managed by administration of diphenhydramine and brief interruption of the LAMB infusion. LAMB is the only LFAB that does not contraindicate the use of an in-line filter, although pore size should be at least 1.0 μm. Pseudohyperphosphatemia has been reported in children.[51]

Comparison of Amphotericin B Deoxycholate and the Lipid-Associated Formulations of Amphotericin B

Randomized clinical trials comparing ABD as therapy for a defined mycosis are limited to the demonstrations for LAMB of similar efficacy for cryptococcal meningitis and greater efficacy for histoplasmosis.[43] Randomized comparisons with ABD as therapy in the persistently neutropenic and febrile cancer patient provide consistent demonstrations of a generally better tolerability profile, but there are few data on differential antifungal effect. Consistent with these results, the aggregate open-label data efficacy rates for the LFABs are similar to those for ABD.[13] Although the LFABs are notably more costly (10- to 60-fold) than ABD, the purchase cost of the compound must be balanced against the morbidity and financial costs of monitoring, treating, and managing ABD-related nephrotoxicity. Of importance, such toxicity may be well tolerated in an outpatient with few other comorbidities or in children, whereas ABD-related nephrotoxicity (50% increase in baseline creatinine to a minimum of 2 mg/dL) was associated with a 6.6-fold increased odds of death and an absolute increase in mortality from 16% to 54%.[52] In the majority of patients, LAMB or ABLC is preferred over ABD.

The lipid-associated amphotericins also remain valuable agents when compared with the azoles and echinocandins. Although associated with more adverse events than agents from these two other classes, ABLC and LAMB remain appropriate for acute management of severe disseminated histoplasmosis, initial management of cryptococcosis, and treatment of suspected mucormycosis. They also provide important options for management of the persistently febrile neutropenic patient (particularly when this syndrome develops despite prophylaxis with an azole that has activity against molds) and for treatment of selected cases of candidiasis.[53] The advantage of prolonged blood levels has caused some centers to explore infusions given one to three times per week for prophylaxis in leukopenic or immunosuppressed patients.

Other Routes for Amphotericin B

Inhalation of amphotericin B has been used both therapeutically and prophylactically, but the supporting data for this practice are relatively sparse.[54] Nebulized forms of the lipid-associated amphotericins have been better tolerated than ABD, in part because of the bitter taste of the bile salt. Formal studies on prophylaxis in high-risk populations have shown encouraging results.[55] An argument has been made that the aerosol might decrease *Aspergillus* infections in lung transplant recipients, including infections at the bronchotracheal anastomotic site.[56–58]

Many researchers have tried to develop formulations of amphotericin B that could be given by the oral route, and studies of nanoparticles or microemulsions are promising in avoiding gastric degradation and promoting intestinal uptake.[59,60]

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The complete reference list is available online at Expert Consult.

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40B Antifungal Drugs: Azoles

George R. Thompson III

SHORT VIEW SUMMARY

- Azole antifungals differ from nitroimidazole drugs such as metronidazole in that azole antifungals have no clinical activity against bacteria. Azoles include triazoles, with three nitrogens, and imidazoles, with two nitrogens.
- Azoles inhibit synthesis of ergosterol by blocking lanosterol 14α-demethylase. Azole resistance arises from mutations in the gene (*ERG11* or *CYP51*) coding for the demethylase or by increased drug efflux from the fungal cell.
- A wide variety of imidazoles are available for topical treatment of dermatophyte infections and vulvovaginal candidiasis.
- Triazoles such as fluconazole have replaced imidazoles such as ketoconazole for the treatment of systemic mycoses due to improved oral absorption and more favorable side-effect profiles.
- The systemically active triazoles have overlapping antifungal spectra, with some exceptions.
  - Fluconazole is not active against molds but is used in the treatment of invasive candidiasis, cryptococcosis, and coccidioidomycosis.
  - Voriconazole has proven efficacy in invasive aspergillosis and candidiasis, plus some reports of activity in endemic mycoses and hyaline molds, but is notably not useful in mucormycosis.
  - Posaconazole is effective as prophylaxis in certain immunosuppressed populations and in the treatment of some cases of mucormycosis.
  - Isavuconazole is noninferior to voriconazole in the treatment of invasive aspergillosis and is active against some agents of mucormycosis.
  - Itraconazole remains the azole of choice for blastomycosis, coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis.
- All triazoles have drug-drug interactions (Table 40B.2) primarily through cytochrome P-450 3A4, with itraconazole having the most and fluconazole (and possibly isavuconazole) having the least.
- Of the currently available triazoles, only fluconazole has significant concentrations in urine.
- Fluconazole has the most predictable serum concentrations, and itraconazole and voriconazole have the least predictable serum concentrations, with posaconazole and isavuconazole in between.

The azole antifungal agents contain either two or three nitrogens in the azole ring and are classified as imidazoles (ketoconazole, miconazole, econazole, and clotrimazole) or triazoles (fluconazole, itraconazole, posaconazole, voriconazole, and isavuconazole), respectively (Fig. 40B.1). The imidazoles were previously used for systemic therapy but have been relegated to topical agents in the treatment of dermatophytosis and cutaneous or mucosal *Candida* infections.

The triazoles exert their effects within ergosterol synthesis by complexing the heme group of the cytochrome P-450 (CYP)–dependent lanosterol 14α-demethylase, preventing the conversion of lanosterol to ergosterol.[1] This inhibition leads to an increase in methyl sterols, causing a disruption of the packed acyl chains of fungal phospholipid cell membranes, and reduces the concentration of ergosterol—a sterol essential for normal fungal cytoplasmic membrane function. Following this destabilization of membrane-associated enzymes and the normal membrane structure and function, fungal growth is inhibited.

Techniques for in vitro susceptibility are available as standardized methodologies.[2,3] Reference documents have been composed providing detailed instructions for in vitro susceptibility testing of the most frequently encountered yeasts and molds, and standardization of these processes has facilitated attempts to establish clinically predictive breakpoints for susceptibility testing. This remains a work in progress and a source of ongoing controversy.

The principal pharmacokinetic predictor of triazole efficacy has been the ratio of total drug exposure (area under the curve) to the mean inhibitory concentration. This ratio has been found predictive of in vivo efficacy in studies of *Candida, Cryptococcus,* and *Aspergillus* spp.[4–6] Doses are outlined in Table 40B.1.

The triazoles exhibit significant differences in their affinity for the lanosterol 14α-demethylase enzyme, and these differences are largely responsible for their varying antifungal potency and spectrum of activity. Cross-inhibition of several human CYP-dependent enzymes (CYP3A4, CYP2C9, and CYP2C19) is responsible for most of the clinical side effects and drug-drug interactions within this class.[7] These interactions differ substantially between agents, and key interactions are summarized in Table 40B.2.

MECHANISMS OF TRIAZOLE RESISTANCE

The availability of the triazoles has been a welcome advance in the treatment of invasive fungal infections; however, resistance to these agents represents a significant and ongoing challenge. Resistance to triazoles occurs primarily through increased lanosterol 14α-demethylase expression, alterations in the binding site, or drug efflux.[8] Resistant species, whether inherently resistant or with acquired resistance, complicate management, and invasive fungal infections may break through or develop during antifungal therapy.

Candida albicans remains the most common *Candida* species, and although resistance to fluconazole and other triazoles has been described, it remains relatively uncommon in cases of candidemia (0%–5%).[9] Patients with oropharyngeal candidiasis, particularly recurrent disease, have been found to harbor triazole-resistant *C. albicans,* and overexpression of genes encoding lanosterol 14α-demethylase (*ERG11*) and efflux transporters (*MDR1*, *CDR1*, *CDR2*) have been found to be responsible in most cases, with amino acid substitutions less frequently observed.[10] In some centers, *Candida glabrata* is the second most common cause of invasive candidiasis, and this species exhibits frequent resistance to azoles mediated by changes in drug efflux (Cdr1 and Cdr2).[11] This can often be overcome by higher triazole dosing; however, for invasive infections an alternative antifungal class is recommended.[12] The ability of *C. glabrata* to develop resistance to multiple drug classes including triazoles and echinocandins has been associated with a mutator phenotype caused by a mismatch repair defect prevalent in *C. glabrata* clinical isolates.[13] Fluconazole resistance in *C. glabrata* is largely due to drug efflux. *Candida krusei* is inherently resistant to fluconazole due to changes within *ERG11,* although it typically remains susceptible to alternative triazole agents.[14] Resistance to triazoles among other common *Candida* spp. (e.g., *C. parapsilosis, C. guillermondii, C. tropicalis*) is infrequent.

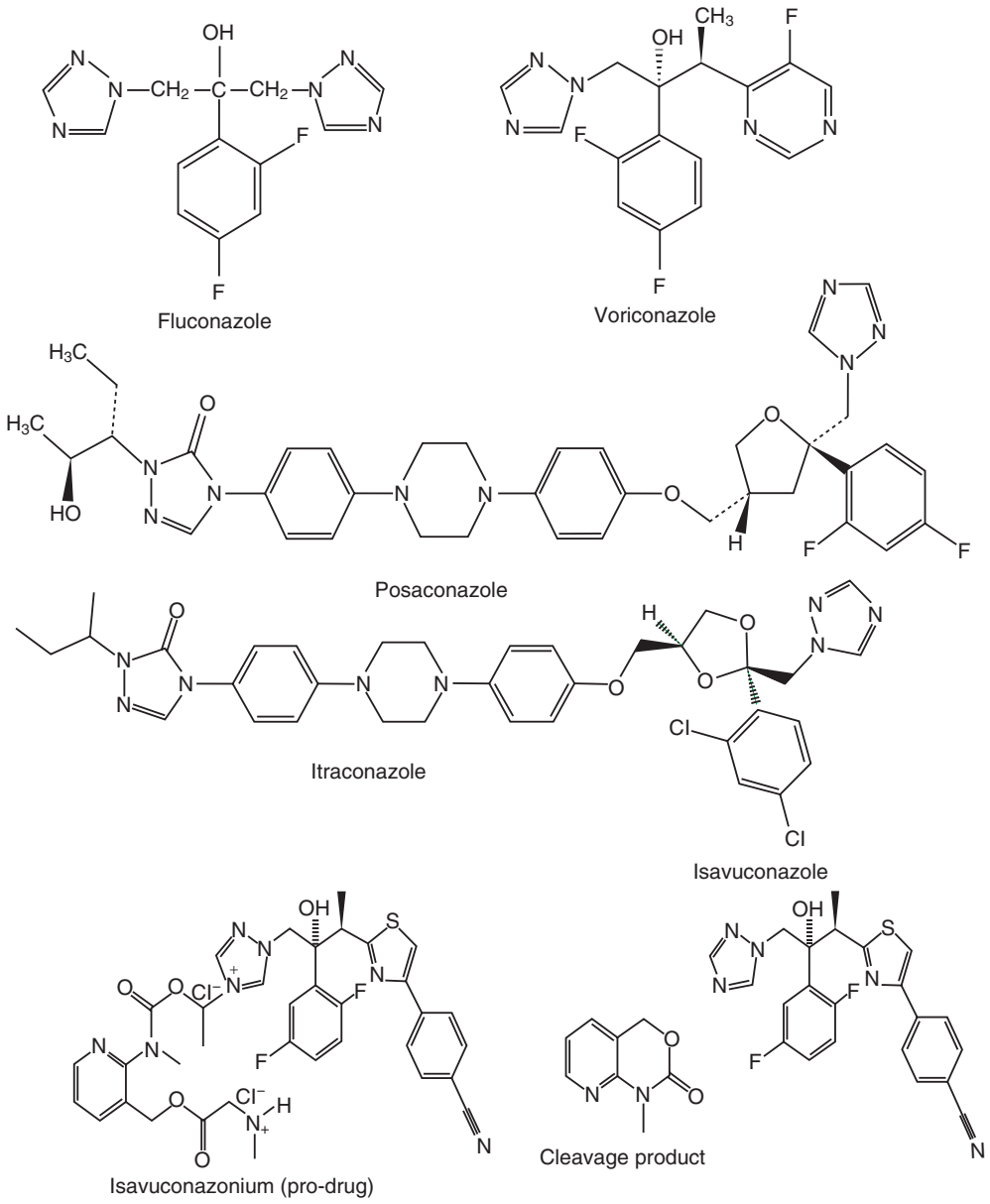

FIG. 40B.1 **Structures of clinically available triazoles used for systemic antifungal therapy.**

TABLE 40B.1 Dosing of Triazoles in Treatment of Systemic Fungal Infection

AZOLE	ADULT DOSE	FREQUENCY	ROUTE	LOADING	NOTE
Fluconazole	400 mg	Daily	PO, IV	None	
Itraconazole	200 mg	Daily or twice daily	PO	None	Suspension or capsule
Conventional formulation: suspension or capsules	200 mg	Daily or twice daily	PO	None	
Enhanced bioavailability SUBA-itraconazole formulation	65 mg	Daily or twice daily	PO	130 mg 3 times daily for first 3 days in life-threatening situations	
Posaconazole					
Suspension	200 mg	Three or four times daily	PO	None	
Tablets	300 mg	Once daily	PO, IV	300 mg q12h on day 1	
Voriconazole	4 mg/kg	Twice daily	PO, IV	6 mg/kg q12h on day 1	
Isavuconazole	200 mg	Once daily	PO, IV	200 mg q8h for 6 doses	

[a]Alternative dosing strategies may be needed in select clinical circumstances.

The emergence of *Candida auris* and subsequent nosocomial outbreaks has been of particular concern, as the large majority of isolates are resistant to fluconazole (due to *ERG11* mutations) with variable susceptibility to other azoles, and amphotericin B and echinocandin resistance rates are approximately 30% to 40% and up to 5% to 10%, respectively.[15] *Candida* isolates developing cross-resistance between triazoles and amphotericin B have been found to harbor mutations within other steps of the ergosterol biosynthetic pathway (*ERG2* and *ERG3*)[16,17] or the acquisition of simultaneous mutations in several genes, resulting in the development of multidrug resistance.[18,19]

TABLE 40B.2 Synopsis of Azole Drug-Drug Interactions

DRUG	FLUCONAZOLE	VORICONAZOLE	ITRACONAZOLE[a]	POSACONAZOLE	ISAVUCONAZOLE
Azole Causes Increased Blood Levels of Other Drug					
Alfentanil		Yes	Yes		
Alprazolam		Yes	Yes		
Astemizole	Yes	Yes[b]	Yes	Yes	
Bortezomib			Yes		
Budesonide	Yes	Yes	Yes	Yes	Yes
Calcium channel blockers		Yes	Yes	Yes	
Carbamazepine	Yes		Yes		
Cisapride	Yes[b]	Yes[b]	Yes	Yes	
Coumarin-type anticoagulants including warfarin	Yes	Yes	Yes		
Cobicistat			Yes		
Cyclosporine	Yes	Yes	Yes	Yes	Yes
Digoxin		No	Yes	Yes	Yes
Disopyramide			Yes		
Dofetilide			Yes		
Ergot alkaloids		Yes[b]	Yes	Yes	
HMG-CoA reductase inhibitors ("statins")		Yes	Yes	Yes	Yes
Ibrutinib	Yes	Yes	Yes	Yes	Yes
Levacetylmethadol (levomethadyl)			Yes		
Methadone	Yes	Yes	Yes		
Midazolam (and other short-acting benzodiazepines)	Yes	Yes[b]	Yes	Yes	Yes
Mycophenolate					Yes
Nevirapine			Yes		
Nisoldipine			Yes		
Nortriptyline			Yes		
Omeprazole		Yes			
Oral hypoglycemics (e.g., glyburide, glipizide)	Yes	Yes	Yes	No for glipizide	
Phenytoin	Yes	Yes	Yes	Yes	
Pimozide	Yes	Yes[b]	Yes	Yes	
Prednisolone		Yes			
Quinidine	Yes	Yes[b]	Yes	Yes	
Rifabutin	Yes	Yes[b]	Yes	Yes	
Rivaroxaban	Yes				
Ruxolitinib	Yes	Yes	Yes	Yes	Yes
Saquinavir, ritonavir, and other protease inhibitors	Yes	Yes	Yes	Yes	
Sirolimus	Yes	Yes[b]	Yes	Yes	Yes
Tacrolimus	Yes	Yes	Yes	Yes	Yes
Terfenadine	Yes[b]	Yes[b]	Yes	Yes	
Theophylline	Yes[c]				
Tofacitinib	Yes	Yes	Yes	Yes	Yes
Triazolam	Yes	Yes	Yes	Yes	
Vinca alkaloids (vincristine, vinblastine)		Yes	Yes	Yes	
Azole Level Is Reduced by Other Drug					
Antacids of any type (H2 blocker or PPI)	No	No	Yes	Yes for suspension[d], no for tablets	
Carbamazepine	Yes	Yes[b]	Yes	Yes	Yes[b]
Efavirenz		Yes[c]	Yes	Yes	

Continued

TABLE 40B.2 Synopsis of Azole Drug-Drug Interactions—cont'd

DRUG	FLUCONAZOLE	VORICONAZOLE	ITRACONAZOLE[a]	POSACONAZOLE	ISAVUCONAZOLE
Fosamprenavir				Yes	
Isoniazid			Yes		
Long-acting barbiturates		Yes[b]			Yes[b]
Metoclopramide				Yes for suspension, no for tablets	
Nevirapine		Yes	Yes		
Phenobarbital		Yes[b]	Yes		
Phenytoin		Yes	Yes	Yes	
Rifabutin		Yes[b]	Yes	Yes	
Rifampin	Yes	Yes[b]	Yes	Yes	Yes[b]
Ritonavir		Yes[b]			
St. John's wort		Yes[b]	Yes		Yes[b]

[a]In addition, itraconazole levels are increased by clarithromycin, erythromycin, indinavir, and ritonavir.
[b]Absolute contraindication.
[c]Concurrent usage requires unusually careful dose adjustments and monitoring.
[d]When given concomitantly with the suspension formulation, cimetidine and esomeprazole have been shown to reduce plasma posaconazole levels.
HMG-CoA, 3-Hydroxy-3-methylglutaryl coenzyme A; *PPI,* proton pump inhibitor.
Note: Azoles are involved in drug-drug interactions either by interfering with metabolism and thus increasing the concentration of other drugs or by having their level reduced via either induction of hepatic metabolism or reduced absorption. The principal interactions are via CYP3A4 (all azoles) plus CYP2C9 (fluconazole, voriconazole) and CYP2C19 (fluconazole, voriconazole). Because of the differential distribution of the cytochrome P-450 enzymes in the liver and gut, the magnitude of drug-drug interactions may be influenced by the route of drug administration. For example, the effect of voriconazole on tacrolimus is even more pronounced (higher tacrolimus levels) when voriconazole is given orally rather than intravenously. Key interactions found in the US prescribing literature are listed above; for more exhaustive reviews, drug-interaction software (e.g., Lexicomp) are also available. Blanks in this table signify absence of data and not absence of interaction.

Triazole resistance has similarly been described in *Cryptococcus* spp., with recurrence of disease during fluconazole maintenance therapy increasingly reported.[20] In a recent review, up to 12% of all cryptococcal isolates were found to have decreased fluconazole susceptibility.[21] In contrast to *Candida* spp., most azole-resistant cryptococcal strains exhibit unstable resistance, described as heteroresistance. Resistance in these isolates represents a transient adaptation to drug pressure, and detailed investigation has found resistance is not due to mutations within *ERG11* or efflux genes, but rather is due to duplications of chromosome 1 (harbors *ERG11* and the efflux pump *AFR1*).[22] This phenomenon is readily observed in vitro for *Cryptococcus* spp.; however, the importance of in vivo resistance by this mechanism is less clear.

The emergence of less common but clinically important non-*Candida*, non-*Cryptococcus* yeasts including *Trichosporon, Saprochaete, Magnusiomyces, Saccharomyces, Wickerhamomyces (Pichia),* and *Rhodotorula* has complicated the treatment and prophylaxis of the expanding immunocompromised population.[23] Although most of these species exhibit in vitro susceptibility to fluconazole, *Rhodotorula* spp. are uniformly resistant,[24] and breakthrough infections despite fluconazole therapy have been described in *Saprochaete* and *Wickerhamomyces (Pichia)* infections.[23]

Triazole resistance in *Aspergillus* has been increasingly described and may occur de novo or after treatment. Epidemiologic surveys have been undertaken to understand the burden of resistant *Aspergillus,* with resistance rates differing substantially between regions.[25] Acquired resistance in *Aspergillus fumigatus* is frequently caused by point mutations in CYP51A, the lanosterol 14α-demethylase enzyme in *Aspergillus* spp. Different mutations cause differential triazole susceptibility, with some causing resistance to voriconazole and isavuconazole, and others causing resistance to posaconazole and itraconazole, whereas others are responsible for pan-azole resistance.[26] De novo resistance has been attributed to the use of agricultural triazole fungicides[27] due to the presence of a 34- or 46-base pair tandem repeat in the promoter coupled with a point mutation in the *cyp51A* target gene. This leads to an amino-acid substitution at codon 98 (TR34/L98H) causing multi-azole resistance, or TR46/Y121F/T289A causing reduced voriconazole susceptibility and attenuation of itraconazole and posaconazole activity.[26,28] Other mutations and non-*cyp51A*–mediated mechanisms have also been described.[29,30] Other species such as *Aspergillus tubingensis* and *Aspergillus calidoustus* may be resistant to triazoles, so susceptibility testing of those cryptic species is typically recommended.[31]

The agents of mucormycosis are inherently voriconazole resistant, and more recent work has identified a characteristic *CYP51* sequence in *Mucorales* spp. responsible for this phenotype.[32] *Scedosporium* and *Fusarium* spp. are also frequently resistant to triazoles, whereas *Lomentospora* (formerly *Scedosporium*) *prolificans* is almost uniformly resistant to all clinically available agents.

FLUCONAZOLE (DIFLUCAN)

Formulations and Pharmacology

Fluconazole is available in 50-, 100-, 150-, and 200-mg tablets; a powder for oral suspension; and an intravenous (IV) formulation of either 200 or 400 mg, both as 2 mg/mL. Fluconazole is an attractive agent because of its low cost, relatively dose-dependent adverse-effect profile, tolerability, and excellent oral bioavailability. Following oral ingestion, most drug can be found in the circulation (>90%), and 80% of drug appears unchanged in the urine. Oral absorption is unaltered by food or gastric pH.[33]

Fluconazole exhibits cerebrospinal fluid (CSF) concentrations approximately 50% to 70% of CSF concentrations found in the serum and penetrates the brain.[34] Fluconazole is widely distributed in body fluids, and excellent concentrations have been found in the saliva, sputum, urine, and other body fluids and tissues and is not significantly protein bound (approximately 10%).[35,36]

The relatively long half-life (approximately 30 hours) allows for once-daily dosing of fluconazole (see Table 40B.1). In patients with a creatinine clearance <50 mL/min a dose reduction of 50% is required, and patients with a creatinine clearance <20 mL/min require a reduction to 25% of the normal dose. A loading dose of twice the daily dose is recommended. Patients receiving dialysis should receive 100% of the normal daily dose after each dialysis session and on nondialysis days a reduced dose according to their creatinine clearance. Preterm infants (body weight <1000 g) prescribed fluconazole prophylaxis are prescribed 3 to 6 mg/kg twice weekly for 6 weeks.

Drug Interactions

Fluconazole is a strong inhibitor of CYP2C19 and CYP2C9 and a moderate inhibitor of CYP3A4, and significant drug-drug interactions can thus occur. Concurrent therapy with medications metabolized

through CYP3A4 with the potential to prolong the QT interval (e.g., cisapride, astemizole, pimozide, and quinidine) is contraindicated.

Side Effects

Adverse effects from fluconazole are generally benign; however, both hepatotoxicity and cardiac toxicity due to prolongation of the QTc interval can occur with any of the triazoles, and neither hepatotoxicity nor cardiac toxicity is clearly dose or time dependent. Headache, alopecia, xerosis, and cheilitis are the most frequent side effects of fluconazole and are reversible after discontinuation of therapy.[37] Neurotoxicity has been reported with very high doses (2000 mg daily) before the availability of alternative triazoles.[38] Anaphylaxis and Stevens-Johnson syndrome have been rarely observed. Fluconazole is a known teratogen and is listed by the US Food and Drug Administration (FDA) as a category D agent. An analysis found no association of fluconazole with birth defects noted in prior reports, although exposure may confer an increased risk of tetralogy of Fallot.[39]

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) of fluconazole therapy is not generally recommended due to its high bioavailability and a lack of defined target serum concentrations.[6] However, at high doses such as those frequently used in coccidioidomycosis[40] or in patients with renal dysfunction or morbid obesity, fluconazole exposure may be profoundly altered and difficult to predict. Monitoring fluconazole serum drug levels may theoretically be useful in these settings.

Indications

Candidiasis

Fluconazole (100–200 mg daily) is one of most effective agents for oropharyngeal candidiasis. Daily therapy (200–400 mg) for 14 to 21 days is also effective in the management of esophageal candidiasis. A single 150-mg tablet is highly efficacious in the treatment of vulvovaginal candidiasis.[12] Symptomatic *Candida* cystitis responds to fluconazole 200 mg daily for 2 weeks. High-risk groups (neutropenic patients, low-birth-weight infants, and patients undergoing urologic procedures) with asymptomatic candiduria similarly respond to fluconazole therapy.[12,41] Although echinocandins are recommend first-line treatment agents in cases of candidemia, fluconazole is an acceptable alternative in selected patients who are not critically ill and are unlikely to have a fluconazole-resistant *Candida* spp. Transition from an echinocandin to fluconazole (step-down therapy) is recommended for patients following clinical improvement and clearance of fungemia.[12] After initial therapy and clearance of blood cultures in candidal endocarditis, fluconazole is often used to prevent relapse.[12]

Cryptococcosis

Preferred induction therapy for acquired immunodeficiency syndrome (AIDS)–associated cryptococcal meningitis includes a lipid formulation of amphotericin B plus flucytosine. Following an appropriate response, consolidation therapy with fluconazole 400 to 800 mg daily should be administered for a minimum of 8 weeks. At the completion of consolidation, maintenance therapy with fluconazole 200 to 400 mg daily for 1 year after diagnosis is frequently recommended and continued until the $CD4^+$ count increases above 100 cells/mm^3 with an undetectable human immunodeficiency virus (HIV) viral load for >3 months.[42] Secondary prophylaxis should be reinitiated if the $CD4^+$ count later decreases again below 100 cells/mm^3. In settings where flucytosine is not available as a component of induction therapy for HIV-positive patients, amphotericin B with fluconazole (800 mg daily) is recommended for 2 weeks followed by fluconazole alone. If no amphotericin B formulations are available, fluconazole 800 to 1200 mg daily plus flucytosine × 2 weeks and fluconazole 800 to 1200 mg daily × 6 weeks are alternative regimens.[43] Treatment of patients with mild-to-moderate pulmonary cryptococcosis for 6 to 12 months with fluconazole 400 mg daily is also recommended.[43]

Other Mycoses

Fluconazole is useful for different manifestations of coccidioidomycosis, including meningitis.[40] Other endemic mycoses have been treated with fluconazole with variable success. In the treatment of histoplasmosis, fluconazole led to improvement in only 54% of cases.[45] Fluconazole treatment of blastomycosis and sporotrichosis has been similarly disappointing.[46,47] Cutaneous dermatophyte infections frequently respond to fluconazole, although other agents are preferred.[48] Fluconazole has no activity against invasive mold infections and should not be used in aspergillosis, mucormycosis, scedosporiosis, and others.

Prophylaxis in Neutropenic Patients

Past reports have evaluated fluconazole in the prevention of fungal infections in bone marrow transplant recipients. These reports illustrate the protective effects of fluconazole against deep-seated candidiasis, although not against aspergillosis.[49,50] Since this time, mold active triazoles have been developed, and multiple trials have been conducted, including two studies demonstrating the superiority of posaconazole compared with fluconazole prophylaxis in high-risk patient populations.[51,52] Other trials have directly compared fluconazole with voriconazole for fungal prophylaxis in allogeneic hematopoietic stem cell transplant recipients, and in this study, fungal-free survival and overall survival did not differ between groups.[53] However, a more recent meta-analysis has suggested that mold-active prophylaxis compared with fluconazole significantly reduces proven and probable invasive fungal infections and fungal-related mortality.[54]

Prophylaxis in Acquired Immunodeficiency Syndrome

Fluconazole, 200 or 400 mg once per week, has reduced the incidence of oral and vulvovaginal candidiasis in patients with advanced HIV infection, but the regimen has not been demonstrated to prevent histoplasmosis, cryptococcosis, or esophageal candidiasis in this population.[55,56] Prophylaxis with 200 mg daily reduces the incidence of oropharyngeal and esophageal candidiasis, as well as cryptococcosis in patients with a $CD4^+$ count of less than 200 cells/mm^3. Cost, lack of effect on survival, and the possibility of azole resistance have led the US Public Health Service/Infectious Diseases Society of America advisory committee to recommend against fluconazole prophylaxis in patients with AIDS. Fluconazole is an alternative to itraconazole for maintenance therapy in patients with AIDS with prior disseminated histoplasmosis.[57]

Prophylaxis in Preterm Neonates

Fluconazole at 3 to 6 mg/kg (every third day for first 2 weeks of life, then every day) until day 30 of life (neonates weighing 1000–1500 g at birth) or day 45 (neonates weighing <1000 g at birth) reduced the rate of invasive *Candida* infection from 13% (placebo) to 2.7% (6-mg group) and 3.8% (3-mg group) in a randomized trial.[58] When evaluated in conjunction with other studies,[59] these data support the use of fluconazole prophylaxis and standard infection control measures for very-low-birth-weight neonates within neonatal care units.

ITRACONAZOLE (SPORANOX) AND ENHANCED BIOAVAILABILITY SUBA-ITRACONAZOLE (TOLSURA/LOZANOC)

Formulations and Pharmacology

Conventional itraconazole is available as a 100-mg capsule, as an oral solution (10 mg/mL) in cyclodextrin as a solubilizing agent, or as an enhanced bioavailability (SUBA-itraconazole) formulation (65-mg capsule). The solution allows for dosing in critically ill patients via the orogastric route or in neonates and children. Oral absorption and daily dosing differs between each formulation. The conventional itraconazole capsule has a bioavailability of approximately 55%, and absorption is improved when given with food and an acidic gastric pH for solubilization. The suspension has 30% to 37% greater bioavailability than that of the capsule, and absorption is not altered by gastric pH, although administration on an empty stomach is optimal for absorption.[60] SUBA-itraconazole should be taken with food, and absorption is enhanced with omeprazole and other acid neutralizing medications. Peak levels are achieved 2 to 6 hours after dosing. Steady state is reached in approximately 14 days, after which time the half-life is 30 to 40 hours. Itraconazole is highly protein bound (99.8%) and undergoes extensive

hepatic metabolism with more than 30 metabolites. One of these, hydroxyitraconazole, has in vitro antifungal activity as well. Itraconazole exhibits poor CSF penetration; however, in both animal and clinical studies, it has proven efficacious in the treatment of fungal meningitis.[61] Renal excretion of itraconazole and hydroxyitraconazole account for less than 1% of the total dose. No dose adjustments have been suggested for use in renal or hepatic impairment, although it should be used with caution in these circumstances.

Drug Interactions

Itraconazole, a strong inhibitor of CYP3A4 as well as P-glycoprotein, is also a substrate for CYP3A4. Significant drug-drug interactions may occur with coadministration of other agents that are metabolized by CYP3A4 or that use P-glycoprotein transporters.[62] In addition, drugs that induce CYP3A4 isozymes may lead to profound increases in itraconazole clearance and subtherapeutic plasma concentrations.[63]

Side Effects

Adverse effects from itraconazole may occur, including hepatotoxicity and QTc prolongation. Gastrointestinal distress is common with the oral suspension. More severe side effects include development of hypertension, hypokalemia, and peripheral edema.[64] This effect was recently determined to be caused by inhibition of human 11β-hydroxysteroid dehydrogenase, causing the syndrome of apparent mineralocorticoid excess.[65] Heart failure has also been described secondary to the negative inotropic effects of itraconazole,[66] and it should be used with caution in patients with a history of ventricular dysfunction of congestive heart failure.[67]

Therapeutic Drug Monitoring

Monitoring of itraconazole serum drug levels is generally recommended due to the erratic absorption and limited bioavailability of current formulations.[6] Target serum levels for itraconazole are generally >0.5 mg/L; however, a concentration effect has been shown in the treatment of most mycoses.[6] Toxicity has similarly been correlated with serum drug levels.[68] It is important to consider the technique used for itraconazole TDM, as bioassay results are typically 2- to 10-fold higher than results obtained by high-performance liquid chromatography (HPLC). HPLC procedures used for TDM quantify both itraconazole and hydroxyitraconazole separately.[69] To make the HPLC assay more reflective of bioassay results, it is common practice to add the concentrations of itraconazole and hydroxyitraconazole together and report a total drug concentration. However, hydroxyitraconazole has more potent activity against the bioassay test organism than itraconazole, a finding that is not necessarily true in patient samples.[70]

Clinical Use

Itraconazole has activity against most cutaneous and invasive mycoses. Allergic bronchopulmonary aspergillosis[71] and invasive aspergillosis[72] both have demonstrated a favorable response to itraconazole therapy. Itraconazole is also useful in the treatment of meningeal[61] and non-meningeal coccidioidomycosis,[73] histoplasmosis,[74] sporotrichosis,[75] blastomycosis,[74] paracoccidioidomycosis,[76] talaromycosis,[77] and the phaeohyphomycoses.[78] Itraconazole is second-line therapy in the treatment of onychomycosis[79] and numerous dermatophyte infections.[80] Chromoblastomycosis has also exhibited a favorable response rate to itraconazole.[81] Itraconazole has been traditionally used for prophylaxis in high-risk neutropenic patients; however, other agents are preferred in this setting following a randomized trial comparing itraconazole or fluconazole prophylaxis with posaconazole solution.[51] Efficacy has also been demonstrated in oral or esophageal candidiasis; however, other agents are preferred, and itraconazole should be reserved for patients intolerant or refractory to other antifungals.[12]

VORICONAZOLE (VFEND)

Formulations and Pharmacology

Voriconazole is available in 50- and 200-mg tablets, a powder for oral suspension, and an IV formulation. Compounded formulations are also available for the treatment of fungal keratitis. Voriconazole has moderate lipophilicity and excellent oral bioavailability (>90%), although absorption is decreased with food by approximately 30%, and administration on an empty stomach is preferred.[7] Voriconazole is widely distributed throughout the body and is able to penetrate the CSF and has demonstrated efficacy in fungal meningitis including the 2012 *Exserohilum* outbreak.[82–84] Voriconazole exhibits nonlinear pharmacokinetics, and the half-life is variable and patient dependent and not useful in predicting the accumulation or elimination of drug. Voriconazole is cleared by hepatic metabolism, and less than 2% of the dose is excreted unchanged in the urine. Dose reduction is required in patients with mild-to-moderate hepatic impairment (Child-Pugh class A or B) with a reduction in the maintenance dosage by 50%. No specific dosage adjustments are indicated with renal impairment. However, the IV form contains a sulfobutyl-β-cyclodextrin for solubility, which is known to accumulate in patients with renal dysfunction, although the clinical implications of this are unclear,[85] and the IV preparation has been safely used in renal dysfunction.[86]

Drug Interactions

Voriconazole undergoes extensive hepatic metabolism and is an inhibitor and substrate of CYP2C19, CYP2C9, and CYP3A4. Significant drug interactions, including bidirectional drug interactions, may occur, and caution with concurrent medications metabolized through these isoenzymes is encouraged.

Side Effects

Adverse effects from voriconazole include hepatotoxicity and QTc prolongation. In addition, there are several unique side effects of voriconazole compared with other triazoles in clinical use. Visual disturbances, including photopsia (the perception of flashing lights), photophobia, and color changes, have been observed and are thought to be secondary to selective and reversible dysfunction of retinal ON-bipolar cells.[87] These effects are usually associated with peak serum concentrations and occur 30 to 60 minutes after oral or IV administration.[88] These effects are reversible and typically resolve over 30 to 60 minutes. No irreversible ocular toxicity has been described. Visual and auditory hallucinations have also been reported and are distinct from photopsia. This effect is usually seen after the first few IV doses of 6 mg/kg and may be more common with elevated serum voriconazole concentrations >5.5 μg/mL.[89] Neurologic toxicity, including confusion, agitation, and myoclonus, are rare and are possibly associated with serum levels exceeding 5.5 μg/mL.

Cutaneous adverse events including rashes and photosensitivity reactions have been seen in approximately 7% of patients.[88] These effects may lead to skin carcinoma following long-term therapy.[90] Although this was initially presumed to be due to a disruption in normal retinol metabolism by voriconazole, this has not been demonstrated.[91] Alopecia, xerosis, and nail changes can also be seen with long-term voriconazole use.[92]

Voriconazole is the only trifluorinated antifungal in clinical use, and long-term administration has been associated with the development of fluoride excess and periostitis/exostoses.[93,94] Bone pain, elevated serum alkaline phosphatase levels, and periosteal elevation is observed on radiographic imaging, and bone scans may be useful to follow the course of disease.[95] Resolution of this manifestation occurs after cessation of voriconazole therapy.[96]

Therapeutic Drug Monitoring

TDM of voriconazole is recommended, given the poor dose-response relationship[89] and variability of patient serum drug levels in patients receiving standard dosing regimens.[97] The usual indications for TDM are poor clinical response or adverse events. Serum drug concentrations should be maintained between 1.0 and 5.5 μg/mL. Children are usually rapid metabolizers, requiring 6 to 8 mg/kg every 12 hours. Variability in children is sufficiently large that monitoring drug levels is essential. Japanese and some other non-Indian Asian populations have a high frequency of slow metabolizers and higher voriconazole levels due to frequency of the CYP2C19*2 genotype. CYP3A4 and CYP2C9 polymorphisms also affect voriconazole serum drug concentrations, but to a lesser degree.[98,99] It has been shown that voriconazole TDM does not impact the incidence of adverse events.[100] In contrast, a meta-analysis reported that patients with therapeutic concentrations were twice as likely to respond to treatment, and patients with supratherapeutic concentrations were four times as likely to experience toxicity.[101]

Clinical Use
Aspergillosis
Voriconazole is licensed in the treatment of invasive aspergillosis following a randomized multicenter trial comparing voriconazole with amphotericin B deoxycholate in the treatment of invasive aspergillosis. At week 12, significant differences were observed, with a successful outcome in 52% of patients in the voriconazole group compared with only 31% in the amphotericin B deoxycholate group.[102] The survival rates also were significantly different between groups (71% in the voriconazole group vs. 58% in the amphotericin B deoxycholate group). Efficacy in central nervous system aspergillosis[103] and other disseminated forms[104] has also been observed, showing the utility of voriconazole even in severe infections. Voriconazole has also been used successfully in noninvasive disease such as allergic bronchopulmonary aspergillosis.[105] Although offering potential advantages over itraconazole, including improved gastrointestinal tolerance and bioavailability, no comparative data are available.

Febrile Neutropenia
In an open-label randomized trial comparing voriconazole with liposomal amphotericin B for persistent fever in neutropenic patients, mortality was similar in both groups. In this study, the overall response was higher in patients treated with liposomal amphotericin B, although voriconazole was associated with fewer documented breakthrough fungal infections, fewer infusion reactions, and less nephrotoxicity.[106] The results of this study are controversial, and the results may have been affected by the open-label design. Empirical treatment with voriconazole during febrile neutropenia may be useful in select patients.

Other Mycoses
Voriconazole has additionally been used successfully in the treatment of other hyaline mold infections, although de novo resistance in these often difficult-to-treat mycoses can be seen. The treatment of these less common and often refractory fungal infections was evaluated in a prospective study, and 46% of fusariosis and 30% of scedosporiosis infections demonstrated a satisfactory response to voriconazole.[83] Voriconazole is also effective in the treatment of mucosal and invasive candidiasis,[83,107] although echinocandins remain first-line agents, and there is little benefit over fluconazole for most *Candida* infections.[12] Voriconazole has been shown to be effective as a prophylactic agent for allogeneic hematopoietic stem cell transplant recipients[53] and in the treatment of *Cryptococcus* and the endemic mycoses—although use in these settings typically follows failure or intolerance to other agents.[83] The phaeohyphomycoses frequently respond to voriconazole.[78] Although voriconazole exhibits a broad spectrum of activity, it is not useful in the treatment of mucormycosis and has poor activity against *Sporothrix* spp. in vitro.

POSACONAZOLE (NOXAFIL)
Formulations and Pharmacology
Posaconazole is available as a 100-mg delayed-release tablet, an oral suspension (40 mg/mL), and an IV formulation. The oral suspension requires a fed state or an acidic carbonated beverage to maximize bioavailability,[108] and saturable absorption with this formulation obviates once-daily dosing.[6] Gastric acid suppressants (H2 receptor antagonists and proton pump inhibitors) significantly decrease serum drug concentrations when administered with the oral suspension.[108] Bioavailability of the tablet formulation is not significantly affected by food or gastric acid. Posaconazole penetrates most sites well, although similar to itraconazole, CSF levels are generally not observed. Clinical experience with posaconazole in the treatment of central nervous system fungal infections is limited, although successful therapy has been observed.[109] The half-life of posaconazole is approximately 27 hours and allows for once-daily dosing with the IV or tablet formulation. The suspension formulation requires more frequent dosing due to decreased bioavailability, and these dosing differences between formulations have led to significant medication errors and prompted warning letters from both US and European regulatory agencies. Most of the drug is eliminated via the fecal route unchanged (77%), and urinary concentrations are negligible. Dose adjustment is not indicated in renal impairment or in Child-Pugh class A, B, or C liver disease.

Drug Interactions
Posaconazole undergoes hepatic metabolism via glucuronidation and has the capacity for drug-drug interactions through inhibition of CYP3A4 isoenzymes.[110] Posaconazole may also inhibit P-glycoprotein transporters, although a clinically relevant effect has not thus far been demonstrated. Concurrent administration with other medications known to prolong the QTc interval metabolized through CYP3A4 is relatively contraindicated. Coadministration with posaconazole may increase the levels of both cyclosporine and tacrolimus.

Side Effects
Adverse effects from posaconazole are primarily gastrointestinal, with nausea, vomiting, and diarrhea more frequent with the oral suspension.[111] Hepatotoxicity and cardiac toxicity due to prolongation of the QTc interval have also been described.[112] The IV formulation of posaconazole contains a cyclodextrin vehicle, and in the setting of renal dysfunction this solubilizing agent may accumulate, although this is of uncertain clinical significance. Hypokalemia, hypertension, and peripheral edema have also been described, with inhibition of 11β-hydroxylase and/or 11β-hydroxysteroid dehydrogenase by posaconazole suggested as the pathophysiologic mechanism.[111]

Therapeutic Drug Monitoring
TDM of posaconazole concentrations is recommended in most current Infectious Diseases Society of America guidelines.[12,113] The tablet formulation has significantly improved drug exposure compared with the solution, and drug levels have correlated with both prophylaxis and treatment efficacy[114,115]; however, toxicity may also be associated with high drug concentrations.[116] Target serum levels are not well defined; however, levels >0.7 μg/mL for prophylaxis and >1.0 μg/mL for treatment are generally recommended. Genetic polymorphisms have not been found to affect posaconazole pharmacokinetics.[117]

Clinical Use
Prophylaxis in High-Risk Patients
Posaconazole decreases the incidence of fungal infections in high-risk patients during graft-versus-host disease following allogeneic bone marrow transplantation.[52] In this study posaconazole was compared with fluconazole for antifungal prophylaxis in severe graft-versus-host disease and was found to be superior to fluconazole in the prevention of invasive aspergillosis (2% vs. 7%) and in prevention of death due to fungal infections (1% vs. 4%). Treatment-related adverse events were comparable between groups (36% vs. 38%). Similarly, posaconazole prophylaxis was compared with fluconazole or itraconazole in patients with neutropenia following chemotherapy (acute myelogenous leukemia or myelodysplastic syndrome).[51] Posaconazole was more effective in prevention of invasive fungal infections than either fluconazole or itraconazole (2% vs. 8%), in prevention of aspergillosis (1% vs. 7%), and in overall mortality (16% vs. 22%). More adverse events occurred in the posaconazole group.

Other Mycoses
Posaconazole has been evaluated in a comparative study with fluconazole in the treatment of oropharyngeal candidiasis in patients with HIV/AIDS and found to be equally effective. Clinical relapse was less common in the group treated with posaconazole.[118] Posaconazole has also been effectively used in a number of other invasive mycoses, including refractory aspergillosis,[114] fusariosis,[119] eumycetoma, chromoblastomycosis,[120] and the phaeohyphomycoses.[78] Additionally, posaconazole is a useful agent in the treatment of coccidioidomycosis[121] and histoplasmosis,[122] although data in the treatment of other endemic mycoses are limited. Following improvement with a polyene, posaconazole is often used as oral step-down therapy during treatment of the mucormycoses.[123]

ISAVUCONAZOLE (CRESEMBA)
Formulations and Pharmacology
Isavuconazonium sulfate (referred to in this chapter as isavuconazole) is available in both an oral capsule (100 mg of isavuconazole) and an IV formulation; the IV formulation does not contain cyclodextrin. Loading doses are required over the initial 48 hours of therapy.

Isavuconazole has a prolonged half-life (approximately 130 hours), which allows once-daily dosing. Oral capsules are well absorbed with a bioavailability of approximately 98% and can be taken with or without food. The isavuconazonium sulfate prodrug is rapidly hydrolyzed in the blood to isavuconazole and an inactive cleavage product by serum esterases, predominately butylcholinesterase. Isavuconazole is widely distributed through body tissues with a steady-state volume of distribution of approximately 450 L and a prolonged half-life of 130 hours. Central nervous system infections have limited available data, but isavuconazole has been useful in the treatment of a few reported patients with cryptococcal and coccidioidal meningitis.[124,124a] Less than 1% of isavuconazole is present in the urine. Dose adjustment is not indicated in renal impairment or in Child-Pugh class A or B liver disease.

Drug Interactions

Significant interactions with drugs metabolized by CYP occur, especially with substrates and inducers of the CYP3A4 enzyme, although preclinical studies and limited clinical data suggest these drug interactions may be less severe than with other triazole agents. Coadministration of methotrexate with isavuconazole increases exposure to 7-hydroxymethotrexate, a potentially toxic metabolite. Tacrolimus and sirolimus levels are likely to be increased by coadministration of isavuconazole, whereas interactions with cyclosporine and glucocorticoids appear to be modest.

Side Effects

The most commonly observed adverse effects are nausea, vomiting, diarrhea, headache, elevated transaminases, and hypokalemia. Overall drug-related side effects in patients receiving isavuconazole are less frequent than in patients receiving voriconazole. In contrast to the other triazoles, isavuconazole is associated with QTc shortening, and although the clinical significance of this remains unclear, it may be useful in patients receiving multiple other QTc prolonging medications.

Therapeutic Drug Monitoring

No definitive recommendations have been made for or against isavuconazole TDM, and clinical experience continues to accumulate. Further studies are needed to clarify whether elevated isavuconazole levels are associated with toxicity and whether TDM is indicated with either the oral or the IV formulation.

Aspergillosis and Mucormycosis

Isavuconazole has been evaluated in a prospective comparative trial with voriconazole in the primary treatment of invasive mold disease caused by *Aspergillus* and other filamentous fungi.[125] In this study, isavuconazole was found to be noninferior to voriconazole in clinical efficacy measured by survival and composite clinical responses, but with a lower incidence of drug-related adverse events (42% vs. 60%). In a single-arm open-label trial, isavuconazole was evaluated in the treatment of mucormycosis and other rare fungi, including the endemic mycoses.[126] Overall, isavuconazole showed activity against mucormycosis and was considered to have similar efficacy compared with historical patients treated with amphotericin B. Of the 18 patients with proven mucormycosis initially treated with isavuconazole, 3 had a complete response, 3 had a partial response, and 15 failed or had the drug discontinued. Additional data are needed.

Other Use

Isavuconazole (IV followed by oral therapy) was also compared with caspofungin followed by oral voriconazole in the treatment of candidemia/invasive candidiasis.[127] In this study a successful response to therapy was observed in only 60% of patients in the isavuconazole arm and 71% in the caspofungin arm—findings confirming the superiority of echinocandins as first-line therapy for invasive candidiasis, as seen in other studies.[128] A limited number of patients with cryptococcosis, paracoccidioidomycosis, coccidioidomycosis, histoplasmosis, and blastomycosis have been evaluated with isavuconazole; although the results are generally favorable, additional data are warranted.[124]

INVESTIGATIONAL AGENTS

Albaconazole, ravuconazole, and non-azole inhibitors of lanosterol 14α-demethylase are currently in various stages of development and may offer advantages over existing antifungals. A new itraconazole formulation (SUBA-itraconazole) has been recently approved and has enhanced oral absorption compared with the liquid or capsule forms.[129] Multiple clinical trials evaluating this new formulation are currently ongoing.

ACKNOWLEDGMENTS

The author acknowledges the contributions of Drs. David Stevens and John Rex to the previous edition of this chapter.

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The complete reference list is available online at Expert Consult.

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40C Antifungal Drugs: Echinocandins

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SHORT VIEW SUMMARY

- Echinocandins are semisynthetic cyclic lipopeptides that exert fungicidal activity against most *Candida* spp. and fungistatic activity against *Aspergillus* spp. by inhibiting the synthesis of cell-wall (1→3)-β-D-glucan.
- Three echinocandins are currently on the market: caspofungin, anidulafungin, and micafungin. Another echinocandin, rezafungin, and a nonechinocandin, the (1→3)-β-D-glucan synthase inhibitor ibrexafungerp, currently are in clinical trials.
- Echinocandins are agents of choice for treatment of candidemia and many other types of deeply invasive candidiasis, and as salvage or combination agents for treatment of invasive aspergillosis. They also have roles in empirical treatment of febrile neutropenia and as antifungal prophylaxis against invasive candidiasis in hematopoietic stem cell transplant recipients and invasive fungal infections after liver transplantation and surgery.
- Half-lives range from 9 to 11 hours for caspofungin, 11 to 17 hours for micafungin, and 24 to 26 hours for anidulafungin. Each of the drugs is dosed intravenously once daily, without need for renal dose adjustment.
- Drug-drug interactions are minimal, and safety profiles are excellent.
- Echinocandin resistance rates are low in general but may be encountered in up to 10% of *Candida glabrata* at high-risk centers. Resistance rates among *Candida auris* isolates are ≈3% to 5%.
- Caspofungin minimum inhibitory concentrations and interpretive breakpoints may overcall *Candida* resistance; anidulafungin or micafungin resistance is an accurate proxy for the class.

The echinocandins are semisynthetic lipopeptides that have emerged as agents of choice for the treatment of many infections by *Candida* spp., salvage and combination agents in the treatment of invasive aspergillosis, empirical treatment options during febrile neutropenia, and empirical or prophylactic agents in patients suspected of, or at, increased risk for invasive fungal infections.[1–5] Echinocandins noncompetitively inhibit (1→3)-β-D-glucan synthase, an enzyme complex that is essential for the synthesis of (1→3)-β-D-glucan, the backbone of the cell wall in ascomycetous fungi. Mammalian cells lack a cell wall, and the target specificity of the echinocandins may help account for reduced toxicity, fewer side effects, and diminished drug-drug interactions compared with earlier classes of systemic antifungals, such as polyene (amphotericin B) or azole agents.[1] Three echinocandins are currently available for clinical use. Caspofungin (Cancidas; Merck, Kenilworth, NJ), micafungin (Mycamine; Fujisawa Healthcare, Osaka, Japan), and anidulafungin (Eraxis; Pfizer, New York, NY) were approved by the US Food and Drug Administration (FDA) in 2001, 2005, and 2006, respectively.[4] Although there are physiochemical and pharmacokinetic differences among these agents, they exhibit almost identical spectra of activity, and practice guidelines generally consider them to be interchangeable.[6,7] In a direct comparison of caspofungin versus micafungin for the treatment of invasive candidiasis, treatment responses were indistinguishable.[8]

This chapter will review the spectra of activity, pharmacology, susceptibility and resistance patterns, and clinical roles of the currently available echinocandins in detail. In a short concluding section, consideration will be given to the newer echinocandin rezafungin (formerly known as CD101; Cidara, San Diego, CA) and the nonechinocandin (1→3)-β-D-glucan synthase inhibitor ibrexafungerp (formerly known as SCY-078; Scynexis, Jersey City, NJ), which are under investigation in clinical trials.[3]

STRUCTURE, MECHANISM OF ACTION, AND SPECTRUM OF ACTIVITY

Caspofungin, micafungin, and anidulafungin have cyclic lipopeptide core structures derived from pneumocandin A_o or pneumocandin B_o that confer antifungal activity (Fig. 40C.1). Side-chain modifications in each of the agents have little effect on antifungal activity compared with parent compounds but improve aqueous solubility.[9–11] The echinocandins bind to the Fks catalytic subunit of (1→3)-β-D-glucan synthase. The resulting inhibition of (1→3)-β-D-glucan synthesis causes an increase in cell-wall permeability and disturbances of intracellular osmotic pressure.[12] The extent of activity against various fungi is determined by cell-wall glucan content. The potency of echinocandins in vivo may be augmented by immunomodulatory activity stemming from unmasking or release of cell-wall glucans, leading to dectin-1 receptor activation of phagocytes and enhanced fungal killing.[13–15]

Echinocandins exert fungicidal activity against *Candida* and *Saccharomyces* spp. by causing cell lysis,[16–18] and they are also the most active class of antifungal agents against *Candida* biofilms in vitro and in animal models.[19–21] Each of the currently approved agents exhibits prolonged postantifungal effects against *Candida,* which are of unclear significance in vivo.[4,22] There is a hierarchy of activity against *Candida* spp., as the lowest minimum inhibitory concentrations (MICs) and most robust effects in animal models are evident against *C. albicans, C. glabrata, C. tropicalis, C. krusei,* and *C. dubliniensis*.[1] *C. parapsilosis* carries an Fks polymorphism that results in decreased echinocandin affinity and higher MICs than observed for the species listed earlier.[1] Echinocandins appear to be fungistatic rather than fungicidal against *C. guilliermondii* and *C. lusitaniae*.[2,23] Breakthrough *C. parapsilosis* infections among patients receiving an echinocandin are well reported,[24,25] although clinical trials of each of the agents show equivalent outcomes for *C. parapsilosis* and *C. albicans* infections. Furthermore, conclusive evidence of poorer outcomes for treatment of *C. guilliermondii* or *C. lusitaniae* infections in clinical trials is lacking. Mouse model data support clinical findings that echinocandins can be used to treat *C. parapsilosis* infections successfully, despite higher MICs than against other species.[26] In vitro susceptibility and mouse model data indicate that echinocandins are the most consistently active class of antifungals against the emerging pathogen *C. auris*.[27]

In general, echinocandins are fungistatic against *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus,* and *Aspergillus niger*, the most common causes of invasive aspergillosis in humans.[1,28] Antifungal activity

correlates with morphologic changes and inhibition of growth at hyphal tips, but viable fungi may remain in tissue.[29–31] Echinocandins have excellent activity against the cyst form of *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*), suggesting a potential role in prophylaxis against *Pneumocystis* pneumonia.[28] Micafungin and anidulafungin were active against *P. jirovecii* during mouse and rat infections,[32,33] but clinical data in humans are limited. Echinocandins are inactive against *Cryptococcus neoformans* and *Trichosporon* spp., in which (1→6)-β-D-glucan, rather than (1→3)-β-D-glucan, predominates in the cell wall.[1,2] Agents are also not reliably active against the yeast forms of endemic mycoses, or Mucorales, *Fusarium* spp., *Scedosporium* spp., and other non-*Aspergillus* molds that have diminished (1→3)-β-D-glucan synthase activity.[2]

PHARMACOLOGY

Pharmacokinetic properties of the commercially available echinocandins are summarized in Table 40C.1. In general, echinocandin pharmacokinetics are similar in bone marrow and peripheral blood stem cell transplant recipients, patients with hematologic malignancies, critical care unit residents, and patients undergoing continuous venovenous hemodialysis.[34–39] Drug clearance may be higher in obese patients and those with candidemia or other forms of invasive candidiasis, but these covariates account for a minority of interpatient variability in pharmacokinetics.[34,40]

Caspofungin, micafungin, and anidulafungin have poor oral bioavailability and are only available in intravenous (IV) formulations.[1] They exhibit linear concentration-dose relationships, although caspofungin may accumulate as doses increase.[41] Each of the agents is highly protein bound in plasma. After initial distribution, caspofungin and micafungin are taken up by the liver and red blood cells (the latter for micafungin only), where they are slowly degraded to inactive metabolites that are largely excreted via bile.[1] Anidulafungin is degraded almost entirely in plasma rather than the liver. The agents distribute well into tissues such as liver, spleen, lungs, and kidneys, but their large molecular weights and high protein binding limit penetration into urine, cerebrospinal

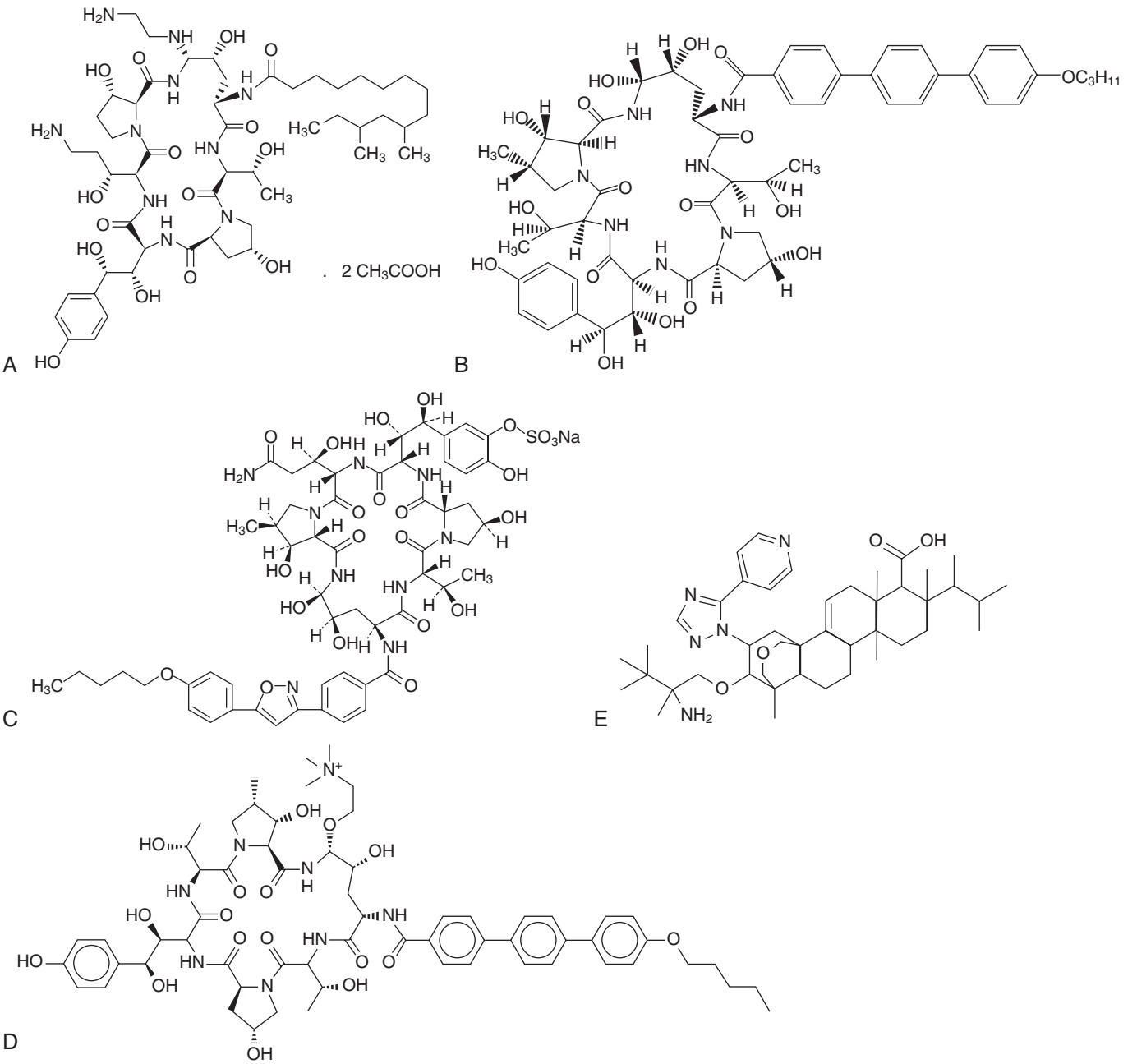

FIG. 40C.1 Structures of echinocandins and ibrexafungerp. Shown are caspofungin (A), anidulafungin (B), micafungin (C), rezafungin (D), and ibrexafungerp (E).

TABLE 40C.1 Echinocandin Pharmacokinetic Parameters

PARAMETER	CASPOFUNGIN	ANIDULAFUNGIN	MICAFUNGIN
Oral bioavailability	<10%	2–7%	<10%
$T_{1/2}$ (hours)	9–11	24–26	11–17
Protein binding (%)	96–97	84–99	≥99
Metabolism	Slow peptide hydrolysis and *N*-acetylation; spontaneous degradation to inactive product	Not metabolized; slow degradation to inactive metabolites	Catechol-*O*-methyltransferase pathway
Elimination	35% in feces, 41% in urine	Primarily in feces, 1% in urine	40% in feces, <15% in urine
Plasma clearance (mL/min)	10–12.5	16.67	10.5
CSF penetration	Low	<0.1%	Low
Renal dose adjustment	None	None	None
Hepatic dose adjustment	None for mild dysfunction (Child-Pugh 5–6); reduce maintenance dose to 35 mg/d for moderate dysfunction (Child-Pugh 7–9); no data for severe dysfunction	None	None for moderate dysfunction (Child-Pugh 7–9); no data for severe dysfunction
C_{max} (mg/L) (50-mg single dose)	7.64	2.07–3.5	4.95
Steady-state AUC_{0-24} (μg/h per mL (70-mg single dose)	87.9–114.8	42.3–53	111.3

AUC_{0-24}, 24-hour area under the (plasma) concentration-time curve; *C_{max}*, maximum concentration; *CSF*, cerebrospinal fluid; *$T_{1/2}$*, elimination half-life.

TABLE 40C.2 Echinocandin Drug Interactions

INTERACTING DRUG	CASPOFUNGIN	ANIDULAFUNGIN	MICAFUNGIN
Tacrolimus	Tacrolimus AUC, peak and 12-h concentrations decreased ≈20%	No significant effect	No significant effect
Sirolimus	No data	No data	Sirolimus AUC increased 21%
Cyclosporine	Caspofungin AUC increased ≈35%	Anidulafungin AUC increased 22%	Cyclosporine clearance decreased 16%
Nifedipine	No data	No data	Nifedipine AUC increased 18%
Rifampin	Caspofungin steady-state concentrations decreased	No significant effect	No significant effect
Voriconazole	No data	No significant effect	No significant effect

AUC, Area under the (plasma) concentration-time curve.

fluid, brain, prostate, and (with the exception of micafungin) ocular fluid. Despite attaining low concentrations in urine, echinocandins have been reported to sterilize azole-resistant candiduria in several case reports,[42–44] and they have been used successfully in experimental models of renal candidiasis.[45] Pharmacokinetic modeling in mice demonstrated that echinocandins accumulated within the kidneys and other organs, which served as reservoirs from which biologically active drug slowly returned to the bloodstream.[46,47] Tissue persistence correlated with ongoing antifungal activity, even after serum concentrations decreased below the MIC.

Elimination half-lives range from 9 to 11 hours for caspofungin, 11 to 17 hours for micafungin, and 24 to 26 hours for anidulafungin; each of the drugs is dosed once daily.[1] Standard doses are as follows: anidulafungin (200-mg loading dose, followed by 100 mg daily; 100-mg loading dose, followed by 50 mg daily for treatment of esophageal candidiasis), caspofungin (70-mg loading dose, followed by 50 mg daily; 50 mg daily for treatment of esophageal candidiasis), and micafungin (100 mg daily; 150 mg for treatment of esophageal candidiasis; 50 mg daily as prophylaxis). IV infusions are typically administered slowly over at least 1 hour to avoid histamine-mediated or histamine-like reactions.[4] No renal dose adjustments are necessary, and the drugs are not dialyzed. Hepatic dose adjustments are not needed for anidulafungin. Dose reductions of caspofungin, but not micafungin, are recommended for moderate hepatic dysfunction; data are lacking for both agents in severe hepatic dysfunction. In general, the echinocandins are well tolerated and similar in types of adverse events. Infusion-related reactions, thrombophlebitis, and mild gastrointestinal (GI) symptoms may occur in <5% of patients.[41] None of the agents are major substrates, inducers, or inhibitors of CYP450 enzymes or the P-glycopeptide transport system, and drug interactions are minimal. The most notable drug interactions are listed in Table 40C.2.

The echinocandins exhibit concentration-dependent fungicidal activity against *Candida* spp. in vivo.[26,48–50] The pharmacokinetic-pharmacodynamic parameters that best correlate with treatment efficacy in mouse models and human studies of candidiasis are the maximum concentration–to-MIC (C_{max}/MIC) ratio or area under the plasma concentration-time curve–to-MIC (AUC/MIC) ratio.[26,45,48–51] Exposure-response relationships for each echinocandin in a study of mice with hematogenously disseminated candidiasis partitioned into fungistatic and fungicidal components at lower and higher doses, respectively.[52] Dosing regimens that mimicked pharmacokinetics in humans receiving currently recommended treatment regimens resulted in fungistatic rather than fungicidal activity.

The pharmacokinetic-pharmacodynamic parameter that best correlates with the fungistatic activity of echinocandins against *Aspergillus* spp. appears to be C_{max}/MIC,[2] although data are less extensive than that against *Candida* spp.

SUSCEPTIBILITY TESTING IN VITRO

Clinicians should be aware of several issues surrounding echinocandin susceptibility testing. Reference broth microdilution methods for testing echinocandins against *Candida* and *Aspergillus* spp. have been developed by the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST).[53,54] There is good interlaboratory agreement in anidulafungin and micafungin MICs determined by these methods against *Candida* and *Aspergillus* isolates.[53–56] In an international study, however, modal caspofungin MICs generated by individual laboratories using either of the reference methods differed by more than four twofold dilutions against *C. albicans, C. glabrata*, *C. tropicalis,* and *C. krusei*.[56] Interlaboratory variability in MICs is most striking for caspofungin against *C. glabrata*.[54,55]

Most hospitals do not use reference broth microdilution methods but rather commercialized assays, such as Sensititre YeastOne (SYO, Trek Diagnostics, Cleveland, OH) and Etest (bioMérieux, Marcy-l'Étoile, France), or automated systems such as Vitek 2 (bioMérieux).[55,57] Anidulafungin and micafungin MICs generated by these methods demonstrate good essential agreement (MICs within a twofold dilution) with those from reference methods against both *Candida* and *Aspergillus*. The commercialized assays also exhibit less interlaboratory disagreement in caspofungin MICs against *Candida*.[58,59] Nevertheless, interpretive breakpoints applied to MICs from these tests may assign disproportionately high rates of caspofungin nonsusceptibility among *C. glabrata* and *C. krusei* isolates, compared with other agents.[60,61] For this reason it has been proposed that anidulafungin or micafungin MICs and susceptibility interpretations against *Candida* be used as proxies for the echinocandin class, rather than relying upon caspofungin MICs.[62] Clinicians should know how echinocandin susceptibility and resistance are tested and defined for *Candida* and *Aspergillus* isolates from their centers. To place MIC results in some context, institutional MIC and resistance patterns for all agents should be compared with those published in the literature.[54] It is also important to recognize that clinical breakpoints proposed by CLSI and EUCAST for certain *Candida* and *Aspergillus* spp. may differ. Clinicians are advised to consult periodically with their clinical microbiology laboratories and review the latest documents from the respective agencies. Suggestions for incorporating echinocandin susceptibility testing into routine clinical practice are offered in the following section on echinocandin resistance.

Echinocandins are well known to demonstrate paradoxical effects in vitro against certain *Candida* and *Aspergillus* isolates, in which growth is inhibited at the MIC but increased at concentrations greater than the MIC. Paradoxical effects have been demonstrated against *C. albicans, C. parapsilosis, C. krusei, C. tropicalis, C. dubliniensis*, and *C. auris* isolates,[22,27,63–70] as well as *A. fumigatus*, *A. flavus*, and *A. terreus*.[71] Each of the commercially available agents has been implicated, but reports are most common for caspofungin. The precise mechanisms accounting for paradoxical effects are unknown but likely involve activation of cell-wall integrity regulatory pathways and upregulated chitin synthesis to compensate for reduced (1→3)-β-D-glucan.[70,72] The majority of studies using mouse models have failed to validate paradoxical growth in vivo.[64,67] In a recent study *C. auris* isolates exhibiting paradoxical growth in the presence of elevated caspofungin concentrations in vitro responded as if fully susceptible in a mouse model of hematogenously disseminated candidiasis.[27] Moreover, paradoxical growth in vitro is eliminated in human serum, and poorer outcomes were not reported among patients receiving higher echinocandin doses in clinical trials. Therefore the phenomenon is likely to be of minimal clinical significance.[8,73]

ECHINOCANDIN RESISTANCE

With increased use of the echinocandins, reports have documented the emergence of resistance.[54,61,74–76] Resistance is most common among *C. glabrata* isolates and remains significantly less common for most other *Candida* and *Aspergillus* spp.[54,77] Diminished susceptibility is mediated by mutations in hot spots of *FKS* genes, with specific mutations conferring higher or lower levels of resistance.[54,77–79] Echinocandin inhibition of (1→3)-β-D-glucan synthase is reduced by 30- to several thousand-fold in isolates harboring various *FKS* mutations.[80–82] Many of these mutations correlate with poor treatment responses in mouse models of disseminated candidiasis.[50,83–85] Selection of resistance mutations in vitro occurs at highest frequency for caspofungin, followed by anidulafungin and micafungin.[86] For the most part mutations confer resistance to all three agents. Agent-specific mutations have been reported,[87] but the clinical relevance of these findings or mutational frequency rates in vitro has not been established.[54]

At some large centers ≈10% of *C. glabrata* strains recovered from sterile sites are *FKS* mutants.[54,60,61,74,75,88] Rates among *C. glabrata* in strain biorepositories are much lower.[54] Molecular resistance typically is seen in the setting of extensive prior echinocandin exposure.[54,60,61,76,88] Particularly worrisome is the emergence of *C. glabrata* isolates that are resistant to both fluconazole and echinocandins, as reported for 12% to 14% of fluconazole-resistant isolates at several centers.[54] Overall echinocandin resistance rates among *C. auris* isolates are ≈3% to 5%, although these may be higher for isolates in some geographic regions.[27,89–91] Echinocandin resistance in *C. auris* invariably is associated with multidrug resistance, as fluconazole and amphotericin B resistance rates are ≥80% and ≈30%, respectively.[89–93]

FKS mutations most often arise in specific settings, in which echinocandin resistance rates are significantly higher than in the broader population with invasive candidiasis. The greatest risk is among patients who develop breakthrough infections during echinocandin treatment, in whom ≥50% of *C. glabrata* or *C. albicans* isolates harbor mutations.[54,94,95] In contrast, *FKS* mutant isolates account for <10% of *C. glabrata* or *C. albicans* infections among patients with remote echinocandin exposure[61]; risk is greatest for prolonged prior exposure and exposure within the preceding month.[54,75] Other risk factors for *FKS* mutant *C. glabrata* isolates include underlying GI disorders, solid-organ transplantation, and recurrent invasive candidiasis.[54,74,75,95] Intraabdominal candidiasis can represent a hidden reservoir for echinocandin resistance at centers that only test bloodstream *Candida* isolates.[95] The detection of *FKS* mutations may be a more reliable predictor of echinocandin treatment failures than elevated MICs in patients with invasive candidiasis, but most clinical microbiology laboratories do not have the capacity to conduct rapid molecular testing.[88,96] An elevated echinocandin MIC in the presence of prior drug exposure is a useful surrogate for the presence of an *FKS* mutation.[54,76,88] In the end, most echinocandin treatment failures are not due to drug resistance but, rather, a combination of factors, such as underlying disease, host immune function, severity of illness, source control, time to initiation of treatment, pharmacokinetic-pharmacodynamic parameters at the site of infection, and isolate fitness.[54,97]

In consideration of the issues raised in the previous paragraph, echinocandin susceptibility testing and/or screening for *FKS* mutations is not likely to be necessary in managing most patients with invasive fungal infections.[54] Such testing may be most useful for the subset of patients with infections that occur during echinocandin exposure or after prolonged remote drug exposure, among patients failing to respond to treatment, and in those infected by *C. glabrata* and *C. parapsilosis*.[7,54] Routine susceptibility testing is important to establish the epidemiology of resistance locally and as a sentinel for detecting increasing rates of resistance.

CLINICAL INDICATIONS

Candidiasis

Echinocandins exert greater anti-*Candida* activity than fluconazole in vitro and in animal models of candidiasis,[98] and they are less toxic and better tolerated than amphotericin B formulations.[99,100] In humans echinocandins are most useful in the treatment of candidemia and invasive candidiasis, as established in a series of randomized, blind, controlled trials. Regardless of the comparator agent in the trials, success rates for the echinocandins were similar to each other.[8,100–103] Individual studies, powered for noninferiority, have shown echinocandins to be comparable to fluconazole, amphotericin B deoxycholate, and liposomal amphotericin B.[8,100–103] In one prospective, randomized, blind, controlled trial, anidulafungin was superior to fluconazole for patients with candidemia and invasive candidiasis, as measured by clinical and microbiologic success at completion of treatment (76% vs. 60%; 95% confidence interval [CI], 3.9 to 27; $P = .01$).[103] A reinterpretation of data excluding the highest enrolling center (which reported exaggerated differences in response between the groups) revealed global response rates of 73% and 61%, respectively (CI, −1.1 to 25.3). Statistical superiority for anidulafungin in the overall cohort was also lost by 6-week follow-up. A patient-level review of data pooled from seven randomized antifungal trials against invasive candidiasis found that echinocandin treatment was associated with significantly improved survival and greater clinical success rates than treatment with an amphotericin B formulation or azole (mortality rates: 27%, 35%, and 36%, respectively; $P < .0001$ for echinocandin vs. others).[100] In subgroup analysis, improved outcomes were evident for patients infected with *C. albicans* and non–*C. albicans* spp.

The latest clinical practice guidelines from the Infectious Diseases Society of America (IDSA) recommend an echinocandin as the preferred initial agent for treatment of candidemia in both neutropenic and

nonneutropenic patients, and for empirical treatment of suspected invasive candidiasis in intensive care unit patients.[7] If patients with documented candidiasis demonstrate a clinical response to an echinocandin, guidelines indicate that step-down therapy with fluconazole can be used to complete a treatment course, usually beginning after 5 to 7 days if patients are infected with an isolate that is known or likely to be fluconazole susceptible. Published experience with echinocandins against deep-seated candidiasis is less extensive than for candidemia, and echinocandins may be limited by pharmacokinetic considerations in the treatment of diseases such as endophthalmitis, meningitis, and urosepsis.[104] Treatment guidelines for intraabdominal candidiasis recommend that antifungal choices follow the considerations laid out for candidemia, with echinocandins as appropriate first-line agents. Antifungal therapy is an adjunct to prompt source control in treatment of intraabdominal candidiasis.[105,106] An echinocandin is endorsed by IDSA as an alternative to amphotericin B formulations for frontline treatment of *Candida* endocarditis and chronic disseminated (hepatosplenic) candidiasis. The most recent IDSA guidelines no longer favor fluconazole over an echinocandin for treatment of *C. parapsilosis* infections, as was recommended previously.[6]

All three echinocandins achieved response rates similar to fluconazole in trials against esophageal candidiasis, but relapse rates were greater, with the exception of high-dose micafungin, which was comparable to fluconazole.[107–109] These findings and the availability of fluconazole as an oral formulation establish the echinocandins as second-line agents against mucosal candidiasis.

Aspergillosis

Treatment guidelines do not endorse echinocandins as primary treatment options against invasive aspergillosis.[110] Most reports of echinocandin treatment of invasive aspergillosis have been salvage studies of patients unable to tolerate or not responding to other antifungal agents, or studies of combination antifungal regimens. In salvage settings most data are for caspofungin, followed by micafungin. In general, the agents were safe, and response rates have ranged from about 40% to 50%.[111–115] Dose escalation studies have shown echinocandins to be well tolerated at higher-than-conventional doses,[111] and it has been proposed that such regimens could prove more effective in treating invasive aspergillosis.[116] There are no data to support this practice, but administration of large doses would be consistent with optimizing C_{max}/MIC, the pharmacokinetic-pharmacodynamic predictor of responses in experimental systems.[2]

Data from in vitro and mouse model experiments have demonstrated synergy between echinocandins (as cell wall–active agents) and mold-active azoles or amphotericin B (as cell membrane–active agents) against *Aspergillus*.[116,117] In keeping with these findings, improved outcomes have been reported in retrospective, single-center studies of patients with aspergillosis who were treated with an echinocandin and an azole or amphotericin B.[118–124] In a randomized, double-blind, placebo-controlled multicenter trial of voriconazole plus anidulafungin (the latter given for the first 2 weeks of treatment) versus voriconazole monotherapy in hematologic malignancy or hematopoietic stem cell transplant patients, there was a nonsignificant trend toward reduced mortality in the combination arm (16% vs. 27%; $P = .09$).[121] Toxicity was not increased with the combination regimen. Although there is no conclusive benefit for combination therapy, IDSA guidelines recommend considering an echinocandin plus voriconazole for the initial stage of primary therapy in the setting of severe disease, especially in patients with hematologic malignancies and those with profound and persistent neutropenia.[110]

Empirical Treatment of Febrile Neutropenia and Antifungal Prophylaxis

Empirical antifungal therapy reduces invasive fungal infection-related mortality among patients with febrile neutropenia who have not responded to antibacterial agents.[125] Several trials have demonstrated that echinocandins are effective and well tolerated in this setting.[126–130] In a recent meta-analysis echinocandins were the most effective class of antifungal agents for empirical treatment of febrile neutropenia, as assessed by mortality and treatment responses.[125] Echinocandins also have been shown to be effective as antifungal prophylaxis against invasive candidiasis after hematopoietic stem cell transplantation[131] and against invasive fungal infections after liver transplantation and surgery.[132–136]

NEW AGENTS: REZAFUNGIN AND IBREXAFUNGERP

Rezafungin is a novel long-acting echinocandin that is stable in plasma and aqueous solution. It is under evaluation as a once-weekly IV formulation.[3] Phase I dose escalation data did not show serious adverse events or safety concerns and confirmed low clearance (<0.28 L/h), long plasma half-life (>80 hours), and minimal excretion.[137] Rezafungin has activity comparable to other echinocandins against *C. albicans, C. glabrata, C. tropicalis*, *C. krusei*, *C. parapsilosis,* and *C. auris*, and it remains effective against some, but not all, *FKS* mutant isolates.[138] Rezafungin is active against *A. fumigatus, A. terreus, A. niger,* and *A. flavus*,[53] and mouse studies have demonstrated protection against *Pneumocystis* pneumonia.[3] Preliminary in vitro data using spontaneous resistance and serial passage–selection methodologies showed that *Candida* spp. develop low-level resistance to rezafungin similar to other echinocandins; passage did not select unique resistance-gene mutations.[139] In a mouse model of intraabdominal infection due to *C. albicans,* rezafungin penetrated the necrotic cores of abscesses significantly better than micafungin.[140] As of this writing the agent was under investigation in a phase III clinical trial for treatment of candidemia and invasive candidiasis, with plans for a phase III clinical trial as prophylaxis in allogeneic bone marrow transplant recipients. In the setting of prophylaxis the activity of rezafungin and other echinocandins against *P. jirovecii* cysts may complement anti-*Candida* and anti-*Aspergillus* activity. Plans for developing a topical application against acute vulvovaginal candidiasis were discontinued after release of unfavorable phase II clinical trial data.

Ibrexafungerp is not an echinocandin but, rather, a semisynthetic derivative of the natural product enfumafungin (a terpenoid and potent inhibitor of [1→3]-β-D glucan synthase). The agent has broad activity against *Candida* spp.,[141,142] including echinocandin-resistant isolates, *C. auris*, and *Aspergillus* spp.[141] As of this writing it is under investigation or scheduled for investigation in phase II and III clinical trials of refractory invasive fungal infections, invasive candidiasis, combination therapy of invasive aspergillosis, and acute and chronic vulvovaginal candidiasis. The agent is being developed in both IV and oral formulations. The latter may facilitate step-down therapy after an initial IV course of ibrexafungerp or an echinocandin. A cyclodextrin-based IV formulation of ibrexafungerp was placed on clinical hold by the FDA in 2017 after thrombotic events in a phase I trial. Clinical trials were scheduled to be reinitiated with a liposomal IV formulation in the latter half of 2018.

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The complete reference list is available online at Expert Consult.

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40D Antifungal Drugs: Flucytosine

Benjamin Colton

SHORT VIEW SUMMARY

- Flucytosine is a fluorinated pyrimidine analogue. The antifungal activity of flucytosine is due to its role as a subversive substrate within the pyrimidine salvage pathway, resulting in inhibition of both protein and DNA synthesis.
- Activity is primarily restricted to candidiasis and cryptococcosis, used in combination with amphotericin B.
- Dosage is 25 to 37.5 mg orally every 6 hours in patients with normal renal function. Blood levels should be monitored in azotemic patients and maintained below 100 μg/mL.
- Flucytosine is well absorbed orally, is eliminated by renal excretion, and penetrates well into cerebrospinal fluid, urine, and other body fluids.
- Toxicities include leukopenia, thrombocytopenia, and colitis, largely related to elevated drug levels. Hepatotoxicity also occurs but is less clearly dose related.

The search for new therapies in the pursuit of a cure for cancer has resulted in many failed chemotherapeutics. Despite falling short of their primary intent, many of these therapies have proven far more capable in fields outside oncology, including infectious diseases. One notable example of this is flucytosine. While attempting to leverage the profound biologic effects of substituting fluorine for hydrogen for the purpose of formulating new chemotherapies, Duschinsky and colleagues synthesized 12 fluoropyrimidines in the research laboratory of Hoffman-La Roche in 1957.[1] As with many pharmaceutical pursuits, only two of these compounds, 5-fluorouracil and 5-fluorocytosine (i.e., 5-flucytosine or flucytosine), would prove of clinical utility. Initial investigations into the activity of both agents by Drs. Duschinsky and Heidelberger reported appreciable antitumor and antibacterial effects for 5-fluorouracil, while noting 5-fluorocytosine's antitumor and antibacterial activity was markedly less.[2] While these early findings proved flucytosine to be a poor antineoplastic agent, its antifungal activity prompted further investigation.[3,4] Following evidence of flucytosine's in vitro and in vivo activity, several investigators set forth to elucidate the mechanism by which it exerted its selective antifungal activity.

PHARMACOLOGY

Flucytosine is a fluoropyrimidine, similar to cytosine and fluorouracil (Fig. 40D.1) The fluoropyrimidines exert their microbiologic activity by serving as subversive substrates within the pyrimidine salvage pathway. In order to accomplish this, flucytosine must first be taken up by fungal cells by the energy-dependent transporter, cytosine permease (Fig. 40D.2).[5] Cytosine permease also serves as the transporter for adenine, hypoxanthine, and cytosine, all of which competitively antagonize the uptake of flucytosine.[6] Once in the fungal cytoplasm, flucytosine is readily deaminated to 5-fluorouracil by cytosine deaminase.[7] The absence of cytosine deaminase in mammalian cells is fundamental to the antifungal selectivity and relative safety observed in humans.[8] Following conversion to fluorouracil, its antifungal effects are mediated through inhibition of both DNA and protein synthesis.[9] As seen in tumor cells and bacteria, fluorouracil is reduced to 5-fluorodeoxyuridine monophosphate by uracil phosphoribosyltransferase (UPRT). 5-Fluorodeoxyuridine monophosphate antagonizes thymidylate synthetase, directly inhibiting the formation of thymidylate.[10] This thymidylate deficiency results in imbalances in the nucleotide pool, impairing DNA replication and repair.

The second metabolic pathway occurs after fluorouracil has been converted to fluorouridine monophosphate by UPRT. Phosphorylation to fluorouridine triphosphate subsequently displaces up to 50% of the uridylic acid in fungal RNA.[7] Incorporation of fluorouridine triphosphate alters the aminoacylation of transfer RNA, disrupting the amino acid pool and inhibiting protein synthesis.[9,11] There exists some debate regarding the significance of either of these two different pathways to the total antifungal activity of flucytosine. Both pathways have been correlated with the degree of flucytosine susceptibility in susceptible *Candida albicans*.[9]

ANTIMICROBIAL ACTIVITY

The antifungal activity of 5-flucytosine was first described by Malbica and colleagues in 1962, with subsequent demonstration of activity in mouse models of invasive candidiasis and *Cryptococcus neoformans* fungemia.[3,4] While those initial studies noted a lack of activity against several dimorphic fungi, later studies would confirm flucytosine's narrow spectrum of antifungal activity as being primarily restricted to pathogenic yeast.

When attempting to understand the epidemiologic trends of resistance to flucytosine, it is important to note the changing definitions of antifungal susceptibility testing for flucytosine. Originally, the British Society for Medical Mycology had proposed breakpoints for interpreting fungal susceptibility to flucytosine, settling upon a minimal inhibitory concentration (MIC) of ≤1 mg/L as sensitive.[12] Similarly, the Clinical and Laboratory Standards Institute (CLSI) had previously defined clinical breakpoints for flucytosine against *Candida* species (sensitive, <4.0 mg/L; intermediate, 8.0–16.0 mg/L; resistant, ≥32 mg/L) based upon historical data and animal studies. In 2017 the CLSI removed the breakpoints, noting that insufficient data exist to set breakpoints or epidemiologic cutoff values.[13] The European Committee for Antimicrobial Susceptibility Testing (EUCAST) has not set interpretive breakpoints for flucytosine for *Candida* species. Despite the lack of clinical breakpoints or epidemiologic cutoff values, investigators have proposed pharmacokinetic/pharmacodynamic breakpoints for disseminated candidiasis. Utilizing a murine pharmacodynamic model of disseminated candidiasis paired with human population pharmacokinetics and Monte Carlo simulation, Hope and colleagues determined that a dosing regimen of 100 mg/kg/day in four divided doses resulted in a resistance breakpoint of 32 mg/L. When compared to the MIC distributions of over 1900 clinical isolates of *C. albicans,* the findings suggested that lower doses may be sufficient to treat disseminated candidiasis due to isolates with lower MICs.[14] While helpful, one cannot forget that pharmacokinetic/pharmacodynamic breakpoints are specific to both the pathogen and the disease state being studied. It is difficult to extrapolate the results obtained from the murine model of disseminated candidiasis to another clinically relevant disease state such as cryptococcal meningitis.

Among the pathogenic yeast, most *Candida* spp. are considered susceptible, with low MICs well within clinically achievable concentrations.[15] The exceptions are *Candida krusei,* in which the MICs are severalfold higher, or *Candida tropicalis,* for which high rates of primary resistance have been published.[16,17] *Cryptococcus* species are considered susceptible, although, like *C. krusei,* the distribution of MICs tends to be higher than in most *Candida* species. An analysis of several thousand multinational isolates of *C. neoformans* and *Cryptococcus gattii* species revealed a minimal inhibitory concentration for 50% of isolates (MIC_{50}) ranging from 1 to 4 μg/mL.[18]

Flucytosine is of little clinical utility against most pathogenic molds. Initial studies of flucytosine against species of *Aspergillus* noted little to no antifungal activity, but subsequent studies have found MIC values within clinically achievable concentrations of flucytosine.[19–22] Specifically, the most recent report of antifungal activity against *Aspergillus* species found a MIC_{50} for *Aspergillus fumigatus* ranging from 8 to 16 μg/mL, while the reported MIC_{50} for both *Aspergillus flavus* and *Aspergillus terreus* was ≤2 μg/mL.[22] Interestingly, the susceptibility of *A. fumigatus* to flucytosine is pH dependent. A lower pH corresponds to lower MIC values, which may indicate enhanced antifungal activity in the acidic environment of abscesses.[23] Current clinical practice guidelines do not recommend the use of flucytosine for the treatment of aspergillosis.[24] Flucytosine's activity against other filamentous fungi is mixed. Against *Mucorales, Fusarium* species, and multiple dermatophytes, flucytosine is of no clinical utility.[25,26] Conversely, flucytosine remains active against phaeohyphomycosis and chromoblastomycosis.[27]

Flucytosine is largely inactive against the dimorphic fungi. Microbiologic studies revealed little to no activity against *Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides* species, *Paracoccidioides brasiliensis,* and *Sporothrix schenckii.*[4,28–30] The only dimorphic fungi for which flucytosine maintains activity is *Talaromyces marneffei.*[31]

RESISTANCE

Development of resistance has been a significant limiting factor in the utility of flucytosine as monotherapy for fungal infections. Of 23 cryptococcal meningitis patients treated with flucytosine alone, 6 of the 12 patients who failed therapy had developed resistance to flucytosine.[32] Polak and Scholer were able to establish that the resistance frequency in sensitive *C. albicans* isolates was sufficiently high to be of concern in infections with a high inoculum.[7] Specifically, the frequencies of resistant isolates of *C. albicans* when grown in 64 μg/mL of 5-fluorouracil for 7 and 14 days were 5.84×10^{-8} and 2.24×10^{-7}, respectively. This work and numerous reports of the development of resistance during therapy remain of concern.

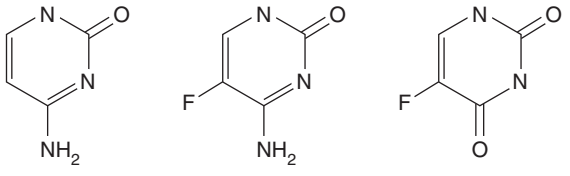

FIG. 40D.1 Comparative chemical structures of pyrimidine analogues.

As early as 1969, Grenson[33] and Jund and Lacroute[34] had described four likely mechanisms of resistance through chemical and ultraviolet mutagenesis studies of *Saccharomyces cerevisiae.* Their studies identified that resistance in *Saccharomyces* could arise from mutations affecting cytosine permease, cytosine deaminase, and UPRT activity, and from mutations giving rise to increased de novo synthesis of pyrimidines. While revealing, later studies have identified that primary resistance to flucytosine among *Candida* species is primarily mediated via decreased activity of either cytosine deaminase or UPRT.[7,35,36] Several researchers have clearly identified a single point mutation in the *FUR1* gene encoding for UPRT as accounting for most of the resistance in isolates of *C. albicans.*[37,38] Specifically, Hope and colleagues were able to demonstrate that the substitution of thymine for cytosine at position 301 of the *FUR1* gene results in a change of arginine to cysteine at amino acid 101 of the UPRTase protein. This amino acid change compromises the enzyme activity as a consequence of alterations of the quaternary structure of the enzyme.[38] Hope and colleagues were also able to describe a homozygous polymorphism in the *FCA1* gene that resulted in a glycine-to-aspartate substitution at position 28 in cytosine deaminase for a single clinical isolate of *C. albicans* resistant to flucytosine.[38] Such an understanding of the molecular mechanisms of resistance has not yet been described for *Cryptococcus* species. Recent studies have suggested that cryptococcal resistance to flucytosine is likely due to changes in an unknown protein or pathway related to regulation of flucytosine metabolism.[39–41]

PHARMACOKINETICS

Flucytosine has excellent bioavailability following oral administration. In healthy volunteers, peak serum concentrations between 30 and 40 μg/mL are seen within 2 hours of a dose of 2 g.[42,43] Similar concentrations are seen in patients with cryptococcal meningitis.[44] Absorption is delayed when following a meal or with concomitant aluminum hydroxide.[45] Overall, 78% to 89% of an oral dose is absorbed by healthy volunteers, but this is reduced in patients with renal failure and late-stage human immunodeficiency virus infection, with bioavailability estimated to be 40% to 76% and 45%, respectively.[45,46]

As a small, hydrophilic drug with minimal protein binding, flucytosine readily distributes throughout the body with a volume of distribution reflective of total body water. Regardless of kidney function, volume of distribution is estimated to be roughly 0.6 L/kg.[42,43,45,47] Protein binding of flucytosine is minimal. Regardless of concentration, studies showed that less than 4% of flucytosine was bound to protein by modified ultrafiltration, and clearance via hemodialysis was nearly indistinguishable from creatinine, corroborating the in vitro findings.[47,48] Flucytosine penetrates well into tissues, including cerebrospinal fluid, peritoneal fluid, bronchial secretions, and articular fluid.[49,50] Single-point estimates of penetration into the cerebrospinal fluid have found concentrations ranging from 60% to 90% that of corresponding serum concentrations.[44,51–53] Peritoneal fluid concentrations have ranged from as low as 5% to nearly 50% of serum concentrations and appear to be increased in the presence of inflammation.[54,55]

In mammals, flucytosine does not undergo traditional routes of metabolism. In rats, fluorouracil and its metabolites were found following oral but not intraperitoneal administration, suggesting that some oral flucytosine is deaminated by intestinal bacterial flora to fluorouracil.[56] In humans, Diasio and colleagues clearly demonstrated that while only 4% of an oral dose of flucytosine was deaminated to fluorouracil, patients

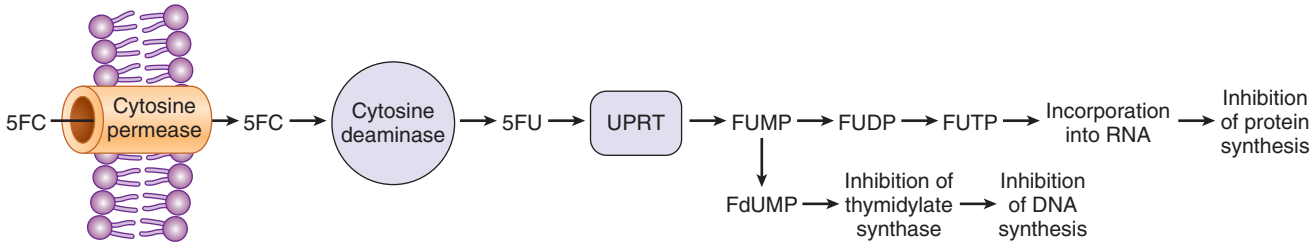

FIG. 40D.2 Mechanism of action of flucytosine. *5FC,* 5-Fluorocytosine; *FdUMP,* 5-fluorodeoxyuridine monophosphate; *5FU,* 5-fluorouracil; *FUDP,* fluorouridine diphosphate; *FUMP,* fluorouridine monophosphate; *FUTP,* fluorouridine triphosphate; *UPRT,* uracil phosphoribosyltransferase.

were exposed to concentrations previously seen with intravenous administration of fluorouracil. Several of the patients in their study developed gastrointestinal and hematologic toxicities associated with these exposures.[57] Finally, Malet-Martino and colleagues correlated the level of intestinal gram-negative bacilli with the appearance or urinary fluorouracil metabolites using fluorine-19 magnetic resonance spectroscopy.[58]

Flucytosine is predominantly eliminated unchanged by the kidneys. Evidence suggests that flucytosine is eliminated via filtration at the glomerulus with little to no tubular reabsorption or secretion.[45,47] In patients with normal kidney function, up to 95% of the dose of flucytosine is recovered unchanged in the urine within the first 24 hours.[43,45,47,56]

PHARMACODYNAMICS

Despite several decades of clinical use, the pharmacodynamics of flucytosine were not elucidated until the turn of the century. In vitro time-kill studies and in vivo mouse models confirmed the fungistatic activity of flucytosine.[59,60] Those same studies demonstrated a significant post-antifungal effect ranging from 2 to 4 hours in the in vitro studies and extended from 3.3 to 15.1 hours in the in vivo murine mouse models. In the in vitro studies, the post-antifungal effect appeared to increase with concentrations up to four times above the MIC for species of *Candida* and *Cryptococcus neoformans*.[60] Given these attributes, Andes and van Ogtrop demonstrated in a neutropenic murine mouse model of disseminated candidiasis that the pharmacokinetic/pharmacodynamic parameter most predictive of microbiologic effect was the time above the minimum inhibitory concentration (T>MIC). Specifically, serum concentrations need only exceeded the MIC for 20% to 25% of the dosing interval for maximal microbiologic effect.[59]

Due to evidence of the rapid induction of resistance, flucytosine has been used almost entirely in combination with a second antifungal. Initial studies suggested combinations of amphotericin B and flucytosine exhibited synergistic fungicidal activity against *C. albicans, C. tropicalis,* and *C. neoformans*.[61] Subsequent animal model studies confirmed the synergistic nature of this combination against *Candida* spp.[62,63] Hope and colleagues further elucidated the additive effects of this interaction in a well-validated pharmacodynamic animal model of invasive candidiasis.[64] In the treatment of cryptococcal meningitis, the combination of fluconazole and flucytosine reduced the dose of flucytosine required for an antifungal effect and abrogated the need for higher doses of fluconazole with increased severity of meningitis.[65,66]

DOSING, ADMINISTRATION, AND MONITORING

In the United States, flucytosine is only available as oral capsules of 250 and 500 mg.[67] Elsewhere, an intravenous formulation is also available.[68] The usual dose in patients with normal renal function for both intravenous and oral administration is 25 to 37.5 mg/kg every 6 hours (100–150 mg/day).[67] The recommendation is similar for pediatric patients, although this dosing regimen tends to result in slightly higher concentrations in neonates. Pharmacokinetic studies have clearly demonstrated that the clearance of flucytosine is almost completely reflective of the glomerular filtration rate, allowing simplified dosing nomograms to be proposed by multiple investigators.[43,47,69,70] Considering the degree of variability in these dosing recommendations, determining the appropriateness of a regimen to ensure clinical efficacy and minimize toxicity is relevant. Ette and colleagues evaluated two different dosing strategies in hospitalized adults utilizing a population pharmacokinetic approach and determined that a dosing regimen starting at 150 mg/kg/day resulted in more concentrations outside the desired therapeutic range and had less convenient dosing intervals.[71] Furthermore, Hope and colleagues evaluated the most appropriate dosing strategy for flucytosine in combination with amphotericin B for the treatment of disseminated candidiasis utilizing a pharmacokinetic/pharmacodynamic approach and found that significantly lower doses of flucytosine (25 mg/kg/day) were able to achieve the target time above the MIC for all isolates with a MIC of less than or equal to 1 μg/mL.[72] These findings suggest the value of pairing individual microbiologic data with pharmacokinetic/pharmacodynamic modeling to allow dosing regimens that minimized toxicity while maintaining optimal microbiologic efficacy.

Therapeutic Drug Monitoring

Early in the study of flucytosine, it became evident that associations between the development of resistance, toxicities of flucytosine, and serum concentrations existed.[35,70] Additionally, decades of experience monitoring serum concentrations of flucytosine have demonstrated that despite a well-delineated pharmacokinetic profile, there remains significant variability in predicted exposures.[73,74] The combination of the association of clinically relevant serum concentrations with microbiologic outcomes and toxicity and the variability in predicted pharmacokinetics make flucytosine an ideal candidate for therapeutic drug monitoring.

The development of resistance through flucytosine therapy was noted early in treatment of pathogenic yeast employing monotherapy.[32,52,75] By exposing *Candida* spp. to progressively increasing concentrations of flucytosine in an effort to select for resistant strains, Normark and Schönebeck noted that the number of resistant mutants was considerably higher in concentrations less than 25 μg/mL.[35] Based upon this observation, they postulated that in vivo concentrations should not be below this concentration. Nearly 30 years would pass before a new understanding of pharmacokinetic/pharmacodynamic relationships would elucidate more well-defined targets for optimizing the efficacy of flucytosine.[59,60] Specifically, targeting concentrations to exceed the MIC of the isolate for at least 20% to 25% of the dosing interval is optimal for disseminated candidiasis.[59] Similar targets have not been defined for cryptococcal disease.

Similarly, an association between elevated concentrations and significant toxicities appeared rather apparent in early clinical experience.[76,77] Multiple investigators have established the association between concentrations greater than 100 μg/mL for periods of 2 or more weeks and the development of myelosuppression and, perhaps, hepatotoxicity.[70,78] Following observations of the detection of 5-fluorouracil and its catabolites in urine and serum of patients receiving oral flucytosine, early studies had suggested that conversion of flucytosine to 5-fluorouracil by gastrointestinal bacteria was responsible for these observed toxicities.[57,58,79,80] While this apparent relationship may describe much of the toxicodynamics of flucytosine, no studies have described an association between 5-fluorouracil concentrations and the toxicities associated with flucytosine.[70,81] Thus from a toxicity perspective, routine monitoring of flucytosine concentrations is of the most utility clinically.

These inherent aspects of flucytosine therapy have resulted in routine monitoring of flucytosine concentrations throughout its clinical history. The British Society for Antimicrobial Chemotherapy Working Party first published guidelines for the therapeutic drug monitoring of antifungals, noting that serum concentrations of flucytosine were useful in all patients, and essential with renal impairment or when used in combination with amphotericin B.[82] Dosing was recommended to maintain peak concentrations of 70 to 80 mg/L and trough concentrations of 30 to 40 mg/L. More recent preclinical infection models have established that serum concentrations need not exceed the MIC for more than 50% of the dosing interval and that little to no benefit is gained from increasing concentrations markedly above the MIC.[14,59] While these findings are promising regarding their implications for dosing strategies that may optimize efficacy and minimize toxicity, there are as of yet no clinical trials evaluating these targets in the disease states of interest. Thus even recent guidelines for the therapeutic drug monitoring of antifungals has continued to recommend trough concentrations of >20 to 40 mg/L and peak concentrations of 50 to 100 mg/L for optimal antifungal activity and to minimize toxicity, respectively.[83]

TOXICITY

As one of the oldest antifungals available for clinical use, flucytosine has a toxicity profile that is rather well understood. Oral flucytosine monotherapy is relatively well tolerated, with most adverse reactions occurring in fewer than 10% of cases.[84] Despite this, over 30% of patients receiving amphotericin B and flucytosine develop one or more toxicities to flucytosine in most trials; this usually occurs in patients with amphotericin B–induced azotemia. The most well-established toxicities include gastrointestinal toxicities, hepatotoxicity, and myelosuppression. While much of the toxicity attributed to flucytosine appears to be largely concentration dependent, concurrent nephrotoxicity is associated with

concomitant amphotericin B. Changes in gastrointestinal flora might also play a contributing role.[85]

Gastrointestinal

One of the most common toxicities of oral flucytosine, gastrointestinal complaints occur in up to 18% of patients and manifest primarily as nausea and diarrhea.[70] Most cases occur during the first week or two of therapy and persist for only a few days before resolving spontaneously or with dose reductions. More severe cases may also develop anorexia, vomiting, profuse diarrhea, and mild diffuse abdominal pain.[84] Rarely, colonic perforation or colitis has led to a fatal outcome.[32,84]

Myelosuppression

Some degree of myelosuppression occurs in roughly 20% to 30% of patients.[53,70,86,87] It primarily manifests as neutropenia or thrombocytopenia. In the setting of neutropenia, bone marrow biopsy demonstrates hypocellularity with decreased granulocyte lineage.[88] Onset is typically within the first 2 to 3 weeks of therapy and has been associated with prolonged exposure to concentrations above 100 μg/mL.[70,78] Notably, Stamm and colleagues found that neutropenia or thrombocytopenia occurred in 12 (60%) of 20 patients with flucytosine concentrations greater than 100 mg/L, and in only 8 (12%) of 65 patients with flucytosine concentrations less than 100 mg/L.[70] Deaths have occurred from leukopenia and from thrombocytopenia,[84] but near-complete resolution occurs within 1 to 2 weeks with discontinuation or dosage reductions.

Hepatotoxicity

Hepatotoxicity is another common adverse reaction associated with flucytosine therapy. Estimates of the incidence of hepatotoxicity vary from as low as 7% to as high as 41%.[70,84,86,87] The significant variation in the estimated incidence of hepatotoxicity may only be reflective of the criteria used to define hepatotoxicity, with more strict criteria reflecting the highest estimates. Flucytosine hepatotoxicity primarily manifests as a transient, mild-to-moderate transaminitis with accompanying elevated alkaline phosphatase.[89] Elevations in bilirubin are uncommon and instances of acute liver injury and hepatic failure appear to be extremely rare. While the exact mechanism has not been fully elucidated, evidence does suggest that it is not dose dependent, with resolution occurring upon discontinuation.[70]

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41 Antimalarial Drugs

James S. McCarthy and Richard N. Price

SHORT VIEW SUMMARY

- The pharmacology of the major drugs is discussed in this chapter. For details of diagnosis and drug doses, the reader is referred to Chapter 274, with treatment summarized in Table 274.3 and prophylaxis in Table 274.4 of that chapter.
- Drugs in the artemisinin class are the first-line treatment of choice for all malaria species because of their rapid action, safety, and activity against all species. They can be given by oral, intravenous (IV), intramuscular (IM), or intrarectal route. They are generally coadministered with partner drugs, such as lumefantrine (Coartem in the United States), amodiaquine, piperaquine, pyronaridine, or mefloquine, so-called artemisinin combination therapy (ACT). The pharmacology of these partner drugs is discussed in this chapter.
- Reduced artemisinin susceptibility has been documented in Cambodia and other countries in the Greater Mekong Region.
- Uncomplicated malaria can also be treated with one of the following: atovaquone-proguanil (Malarone), mefloquine, or quinine plus either doxycycline, tetracycline, or clindamycin.
- Complicated malaria should be treated with IV artesunate (available through the Centers for Disease Control and Prevention), quinidine, or quinine (IV quinine is not available in the United States).
- Options for prophylaxis against chloroquine-resistant falciparum malaria include once-weekly mefloquine, once-daily doxycycline, or once-daily atovaquone-proguanil. Mefloquine use is limited by central nervous system side effects. Doxycycline photosensitivity and cost of atovaquone-proguanil are considerations.
- Primaquine can be used for prophylaxis during travel or used after return from malarious areas to lessen incidence of recurrent vivax malaria. Travelers should be tested for glucose-6-phosphate dehydrogenase deficiency before use. Tafenoquine is a new long-acting drug that is now approved by the US Food and Drug Administration and can be used as a substitute for primaquine.
- The pharmacology of sulfadoxine and pyrimethamine is included in this chapter.

Antimalarial drugs can be classified in the following ways: chemical class, mechanism of action, or life-cycle stage of the parasite against which they are active. In clinical medicine the priority of antimalarial chemotherapy is to clear the blood-stage infection that causes illness and prevent disease progression to severe malaria and death. Although all antimalarials are active against the asexual blood stages, a few also have activity against the preerythrocytic life-cycle stage in the blood (sporozoites) and liver. Such activity makes these drugs useful for chemoprophylaxis and for the elimination of latent infection with hypnozoites of *Plasmodium vivax* and *Plasmodium ovale*. Drugs with activity against gametocytes, the sexual stages of the parasite, have potential to reduce the ongoing transmission of the parasite to the mosquito; this has important public health benefits in areas where *Anopheles* vectors are present. The life-cycle stages targeted by available antimalarial drugs are shown in Fig. 41.1.

Pharmacokinetic properties of major antimalarials are presented in Table 41.1 and discussed with each drug.

ARTEMISININ DERIVATIVES

Artemisinin is the active principle extract of *Artemisia annua* (Qinghao), a plant used for centuries in traditional Chinese herbal remedies for the treatment of febrile illness. In the 1960s Chinese scientists began an extensive search for new antimicrobial compounds from their traditional pharmacopoeia, a search that brought to light the novel antimalarial properties of artemisinin.[1] These compounds are unusual in that they consist of a peroxide bridge within a 1,2,4-trioxane configuration, which contains a sesquiterpene lactone ring. The endoperoxide bridge is essential for their antimalarial activity.[2] Artemisinin and its derivatives are highly potent antimalarials, with a broad-stage specificity of action, resulting in a faster clinical and parasitologic response than any other antimalarial agents in clinical use. The importance of this new antimalarial class of drugs was acknowledged in the 2015 Nobel Prize for Medicine awarded to Tu Youyou for her contribution to the discovery program.

The use of the parent compound artemisinin has been superseded by the development of four main derivatives: water-soluble hemisuccinate artesunate and its active metabolite dihydroartemisinin (DHA), and the lipophilic methyl ethers artemether and artemotil (formerly known as arteether). Artesunate, the most widely used of the derivatives, is available in oral, intravenous (IV), intramuscular (IM), and rectal formulations. It is the only member of this class that can be administered IV. Artemether, a methyl ether derivative, can be administered by IM injection suspended in oil or as capsules for oral administration. Novel synthetic and semisynthetic derivatives with potent antimalarial activity in vitro have also been developed.[3] Two synthetic trioxolanes, arterolane (OZ277) and artefenomel (OZ439), have been developed. Arterolane is marketed in a fixed-dose coformulation with piperaquine called Synriam.[4] Artefenomel is currently under development in combination with ferroquine.[5,6]

Structures of Artemisinin Compounds

The artemisinin derivatives demonstrate excellent efficacy against all human malaria parasites, including multidrug-resistant strains of *Plasmodium falciparum*[7] and *Plasmodium vivax*[8] (Fig. 41.2). These drugs have also been shown to have in vitro activity against other parasites, including *Schistosoma, Fasciola, Opisthorchis, Clonorchis,* and *Leishmania,* although susceptibility is more modest, and they have not received the same degree of preclinical and clinical development for these indications.[9–11] Although the activity of the artemisinins is known to be dependent on the endoperoxide dioxygen bridge, their exact mechanism of action remains unclear. The active endoperoxides accumulate in various parasite compartments, including the cytosol, digestive vacuole, and membranes. The interaction between the drug and intraparasitic heme-derived iron appears to be a crucial step both in vitro and in vivo,[12,13] in which the endoperoxide bridge is cleaved to hydroperoxide, the resultant hydroperoxide-metal complex acting as a powerful oxidizing agent, releasing carbon-centered free radicals and other reactive

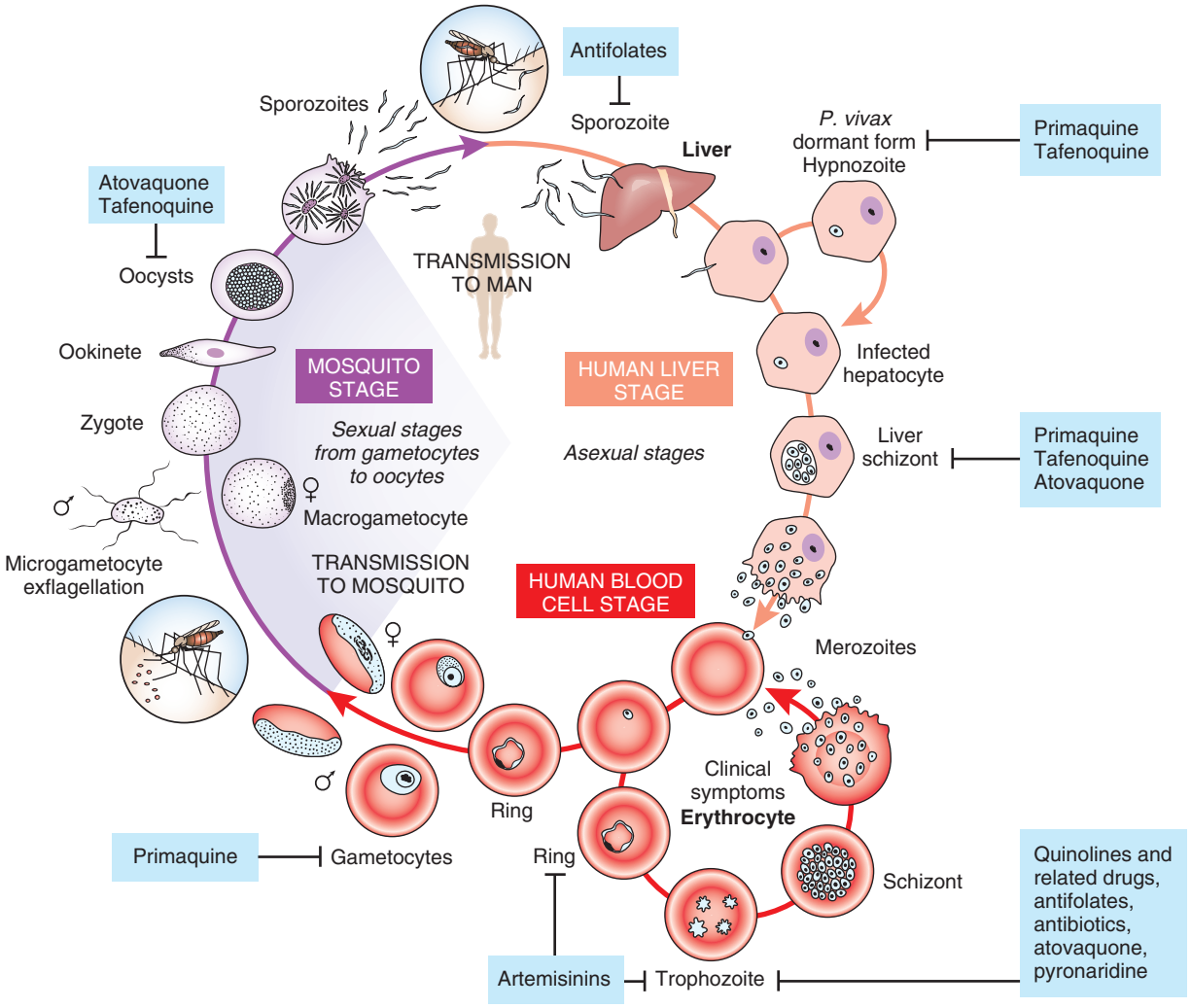

FIG. 41.1 Summary of the activity of the most widely used antimalarials throughout the life cycle of *Plasmodium*. The three main phases (i.e., liver stage, blood stage, and vector stage) of the life cycle of *Plasmodium* are shown. Parasite forms specific to each stage are highlighted, and drugs identified as inhibitors of development of these forms are listed in boxes. *(Modified from Delves M, Plouffe D, Scheurer C, et al. The activities of current antimalarial drugs on the life cycle stages of Plasmodium: a comparative study with human and rodent parasites.* PLoS Med. *2012;9: e1001169.)*

metabolites.[14,15] These reactive metabolites bind with hemin, parasite proteins, and red cell membranes, which are thought to cause critical damage to parasite organelles.[16] Other studies have implicated interference with calcium homeostasis, a translationally controlled tumor protein (TCTP) homologue, the mitochondrial electron transport chain, and inhibition of angiogenesis.

Pharmacokinetics and Dynamics

Bioavailability of the artemisinin compounds varies with the route of administration and duration of therapy. Artesunate is metabolized almost immediately to DHA and therefore can be considered a prodrug; pharmacokinetic studies therefore tend to present the profile of DHA rather than the parent compound. Peak plasma concentrations (C_{max}) of DHA occur at 10 minutes after IV injection of artesunate, 30 to 40 minutes after IM injection, and 1 to 2 hours after oral and rectal administration.[17,18] The bioavailability of oral artesunate is approximately 60%[17,19] but falls to 16% when administered rectally,[18] whereas the bioavailability of DHA after artesunate is 86% to 88% when it is given parenterally.[20] The bioavailability of artemisinin after oral administration is significantly less (32%) and is far more variable than that for oral artesunate,[21] ranging from 43% to 200%.[22,23] Bioavailability of artemether when administered by IM injection is variable; C_{max} vary between 2 and 10 hours after injection.[24]

Host factors can affect the bioavailability of some artemisinin derivatives. The bioavailability of DHA is increased twofold in patients with malaria, compared with healthy volunteers,[25] and is twofold higher during the acute phase of infection compared with convalescence.[19] However, in pregnant women with malaria the bioavailability of artesunate is reduced fourfold compared with nonpregnant malaria patients.[26] The bioavailability of oral artemisinin, but not artesunate, falls by more than 80% after 5 days of treatment, a process thought to relate to autoinduction of metabolism.[27]

Once artemether and artesunate are absorbed, there is extensive first-pass metabolism and rapid biotransformation to DHA. The elimination half-life of DHA is less than 1 hour.[18,20] The elimination half-life of artemether and DHA after artemether, given either orally or intramuscularly, is longer, varying from 2 to 12 hours. Metabolic pathways for the biotransformation of artesunate, artemether, and DHA differ from that observed for the parent compound artemisinin. Artemether is metabolized predominantly by the hepatic cytochrome CYP3A4 to DHA. DHA is metabolized by hepatic cytochrome P-450, involving biotransformation to biologically inert glucuronides that are eliminated in bile.[28]

DHA blood concentrations are reduced after IM and IV administration of artesunate in children weighing between 6 and 10 kg compared with older children and adults, and thus a higher dose is recommended

TABLE 41.1 Pharmacokinetic Properties of the Major Antimalarials

DRUG (METABOLITE)	C_{MAX}	T_{MAX} (h)	AUC	K_A (per h)	V/F (L/kg)	CL/F (L/h per kg)	$T_{½}$
Intravenous artesunate (dihydroartemisinin)	11,346–29,681 ng/mL (1279–3011 ng/mL)	— (0.01–0.2)	558–1146 ng/mL × h (739–2559 ng/mL × h)	— (—)	0.08–0.24 (0.47–1.01)	1.63–4.26 (0.73–2.16)	0.03–0.19 h (0.3–1.1 h)
Rectal artesunate (dihydroartemisinin)	269–507 ng/mL (682–896 ng/mL)	0.8–0.9 (1.5–2.3)	692 ng/mL × h (2402–2786 ng/mL × h)	0.3 (0.7)	2.1 (2.1–4.4)	6.0 (2.2–2.64)	0.9 h (0.7–1.3 h)
Oral artesunate (dihydroartemisinin)	51–244 ng/mL (251–488 ng/mL)	0.5 (2.0)	113–232 ng/mL × h (740–1671 ng/mL × h)	3.3 (1.19–4.27)	6.8 (2.3–4.6)	19.2 (0.6–4.0)	0.36 h (0.64–2.5 h)
Oral artemether (dihydroartemisinin)	34–186 ng/mL (101–165 ng/mL)	1.0–2.0 (1.0–3.0)	65.6–211 ng/mL × h (357–604 ng/mL × h)	— (—)	49.5 (7.8)	25.9 (4.5)	1.5–3.9 h (1.3–1.9 h)
Lumefantrine	5.72–25.7 µg/mL	4.0–544.0	210–636 µg/mL × h	0.06–0.17	3.7	0.12	32.7–79.2 h
Piperaquine	568 ng/mL	5.7	46.9–56.4 µg/mL × h	0.08–0.72	164–614	0.85–1.85	12–28 days
Pyronaridine	130 ng/mL	2.4–3.2	29400 ng/mL × h	0.7	72	0.9	14–18 days
Mefloquine	1.62–2.70 µg/mL	15.0–45.0	307.2–1497 µg/mL × h	0.292	8.14–31.8	0.017–0.174	8.5–19.3 days
Amodiaquine (desethylamodiaquine)	14.1–15.5 ng/mL (235–1185 ng/mL)	– (46.9–47.9)	(28.9–44.3 µg/mL × h) (–)	— (0.13–0.87)	322.7 (75.2–123)	14 (0.61–0.86)	3.3 h (104–216 h)
Chloroquine (desethylchloroquine)	283–1430 ng/mL (89–220 ng/mL)	6.5–6.9 (–)	37–140 µg/mL × h (44–64 µg/mL × h)	0.14 (—)	31.8–154 (—)	0.23–0.80 (—)	4.5–9.7 days (7.3–8.5 days)
Quinine	4–15.3 µg/mL	2.0–16.1	52.6–332 µg/mL × h	0.93–3.50	0.53–1.84	0.05–0.138	7.0–19.7 h
Atovaquone	2.07–13.3 µg/mL	5.1	63–663 µg/mL × h ($AUC_{48h-\infty}$)	0.26–0.46	4.7–10.2	0.07–0.32	31.3–72.9 h
Proguanil (cycloguanil)	363–750 ng/mL (26–67 ng/mL)	4.5–5.2 (6.4–6.9)	5.7–13.5 µg/mL × h ($AUC_{48h-\infty}$) (0.71–1.8 µg/mL × h) ($AUC_{48h-\infty}$)	0.41–0.51 (—)	15.8–29.7 (—)	0.76–1.23 (—)	8.0–17.6 h (6.4–22.6 h)
Primaquine 15 mg daily for 14 days (carboxyprimaquine)	50.7–57.7 ng/mL (291–432 ng/mL)	2.2–2.3 (2.6–7.3)	480–547 ng/mL × h —	— (—)	4.8–5.1 (—)	0.2–0.5 (—)	5.6–6.4 h (21.8 h)
Primaquine 45 mg, single dose (carboxyprimaquine)	167 ng/mL (890 ng/mL)	2.0 (6.1)	— (12,737 ng/mL × h)	— (—)	— (—)	0.5 (—)	6.1 h (—)
Tafenoquine 300 mg single dose	320 ng/mL	15 days	98 µg/mL × h (0–60 days)	0.23	>20	0.06	15 days
Pyrimethamine	280 ng/mL	12.0–19.8	38.0–89.1 µg/mL × h	—	4.25–4.46	0.0174–0.0417	2.8–3.4 days
Sulfadoxine	63.9–130 µg/mL	5.7–13.5	—	0.3	0.242–3.13	0.00068–0.00190	4.1–8.9 days

AUC, Area under the concentration-time curve; *CL/F,* apparent total clearance after oral administration; C_{max}, maximum plasma concentration; K_a, first-order absorption rate constant; $t_{½}$, terminal elimination half-life t_{max}, time after administration to maximum plasma concentration; *V/F,* apparent volume of distribution.
Only metabolites that have been reported are included in this table; some antimalarial agents have active metabolites (e.g., desbutyl-lumefantrine for lumefantrine) that have not been reported in any of the studies summarized here.
Modified in part from http://www.who.int/malaria/publications/atoz/9789241502061/en/; Table 3.1, p. 62.

in young children[29,30] The kinetics of DHA are also modified significantly in late pregnancy, with oral bioavailability reduced by 23%[31]; plasma concentrations are significantly lower than those observed in nonpregnant women.[26] Further dose-optimization studies in pregnant women may be necessary.

Clinical Use

Comparative clinical studies have shown the artemisinin compounds to act faster than any other licensed antimalarial, with typical fever clearance times being approximately 20 hours and parasite clearance achieved within 48 hours.[32] Although this class of drugs is extremely potent, treatment success if administered without a partner drug is dependent upon the duration of therapy, rather than on the dose or dose regimen. Because these compounds are rapidly eliminated, a prolonged course (minimum, 7 days) of monotherapy is required to effect cure,[33] particularly in patients presenting with high initial parasitemia.[34] For this reason World Health Organization (WHO) recommends that artemisinin derivatives always be administered with a longer half-life partner drug to improve cure rates,[35] so-called artemisinin combination therapy (ACT).

Combination regimens with an artemisinin derivative offer several inherent benefits. The rapid clinical response can improve the tolerability and absorption of the combination partner drug, which can often be compromised in an acutely febrile patient.[36,37] The rapid reduction of the pathogen biomass achieved with an artemisinin derivative also reduces the number of asexual parasites exposed to the second drug, thereby reducing the chances of a resistant mutant emerging during treatment.[38] The sustained clinical efficacy of several ACTs suggests that this strategy may have has indeed slowed the rise of antimalarial resistance in areas where use has been widespread.[39] A combination treatment regimen also serves to protect the artemisinin compounds because neither drug will be exposed to the parasites alone.[40–43] Artemisinin derivatives have the additional benefit of reducing the production of gametocytes, the sexual stages of the parasite.[44] Because recrudescent infections are associated with an increase in gametocyte carriage, the reduction in their carriage after ACT may help to slow the emergence of resistance and reduce malaria transmission in settings of low endemicity.[45]

More than 80 malaria-endemic countries have now endorsed the use of ACT as the first treatment of uncomplicated malaria, with huge clinical experience supporting their safety and efficacy.[35] WHO recommends six ACTs for the treatment of uncomplicated falciparum malaria: artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, DHA + piperaquine, artesunate + sulfadoxine-pyrimethamine, and artesunate + pyronaridine.[35]

Artemether-lumefantrine is a fixed-dose combination registered by regulatory authorities in Europe, the United States, and Australia

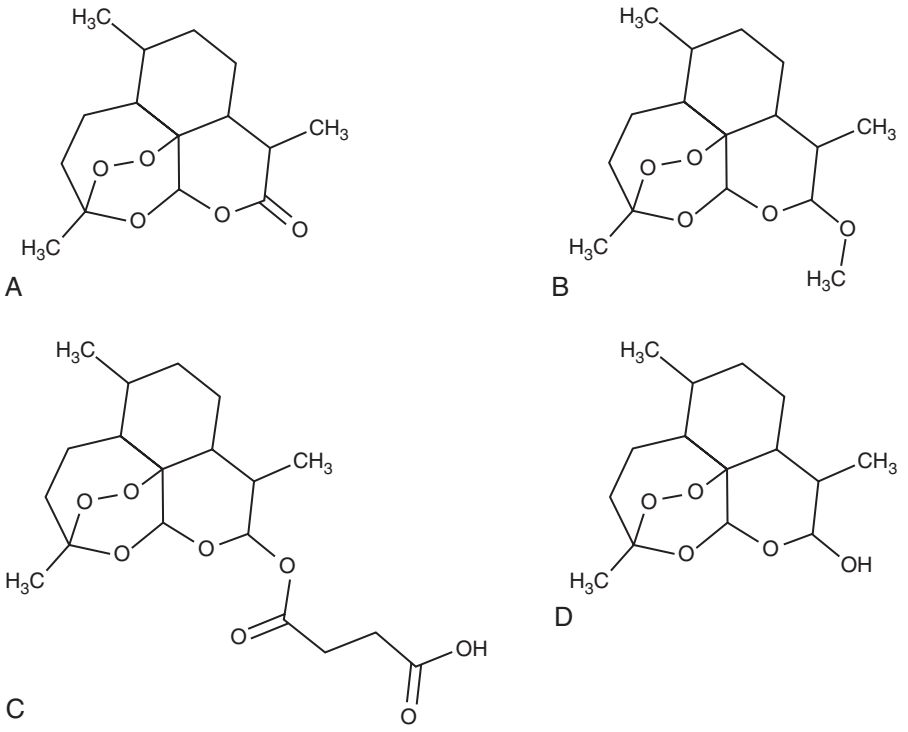

FIG. 41.2 Structures of artemisinin derivatives. (A) Artemisinin. (B) Artemether. (C) Artesunate. (D) Dihydroartemisinin.

(under several trade names, including Coartem and Riamet), where it is approved for the oral treatment of malaria. In addition, four other fixed-dose ACTs have been licensed through the European Drug Agency: DHA-piperaquine (Eurartesim), artesunate-amodiaquine (Winthrop), artesunate-mefloquine (Mefliam plus), and artesunate-pyronaridine (Pyramax), although to date their use in nonendemic countries is limited. The combination artesunate plus sulfadoxine-pyrimethamine is available in a blister pack formulation. The synthetic trioxolane OZ277 has been combined with piperaquine (Synriam) and is now available for use in several African countries, although there is sparse data available from phase III clinical trials.[4]

Severe Malaria

The rapid action and broad-stage specificity of the artemisinin derivatives are critically important for their role in the treatment of severe malaria and for prevention of the development of severe disease. Patients with high parasitemia (>4%) are at significantly greater risk of mortality compared with those with lower parasitemia. Oral artesunate results in faster parasite clearance compared with IV quinine, with potential to arrest the progression to severe disease.[46] The clinical benefits of accelerated parasite clearance have been highlighted in patients with severe malaria in two multicenter, randomized clinical trials comparing IV quinine to artesunate. The first, conducted at four sites in Southeast Asia in 1461 patients, highlighted a 35% reduction in case fatality with IV artesunate (from 22%–15%).[47] The second, conducted in 5425 patients enrolled at 11 African sites, involved a greater proportion of children and showed a 22% reduction in fatality (from 10.9%–8.5%).[48] A meta-analysis of these results, together with other smaller clinical trials, highlights overwhelming evidence for the clinical efficacy of IV artesunate in reducing the mortality from severe malaria in an endemic setting (odds ratio [OR] 0.69; 95% confidence interval [CI], 0.57 to 0.87]).[48]

Artesunate can be given by slow IV or IM injection, at an initial dose of 2.4 mg/kg (≈140 mg in adults), followed by 2.4 mg/kg at 12, 24, and 48 hours, with further daily doses if required. In view of the reduced concentration of DHA after parenteral administration of artesunate to young children, the recommended dose in patients weighing less than 20 kg is 3 mg/kg of artesunate.[35] Once oral treatment can be tolerated, treatment can be changed to appropriate ACT to ensure complete eradication of any remaining parasites. In addition to parenteral formulations, the rapid absorption of rectal artesunate resulting in high plasma concentrations gives it significant advantages over the other artemisinin derivatives in prehospital treatment of malaria.[49] In settings without ready access to parenteral therapy, in children less than 6 years an artesunate suppository at a dose of 10 mg/kg may be administered before reaching a health care facility where more intensive treatment can be given.[50]

Resistance

The artemisinin derivatives have been in clinical use in China and Cambodia for almost 40 years, whereas deployment in the rest of the world has increased substantially over the past 15 years. The first concerns of artemisinin resistance arose from clinical studies in western Cambodia,[51] where delayed parasite clearance rates were recognized as the earliest indication of declining efficacy[52,53]). Large multicenter clinical studies have highlighted the variation in clearance half-lives across the Greater Mekong, ranging from more than 6 hours in western Cambodia and in Srisaket in Thailand, to 3 hours in Vietnam and Myanmar, 2.5 hours in Bangladesh, and more than 2 hours in a variety of African sites.[54] The variation in parasite clearance rates is proposed to be due to reduced activity against immature ring stages of the parasite.[55] Molecular studies have identified a correlation between artemisinin resistance and mutations of the kelch protein gene on chromosome 13 (*kelch 13* [K13]).[54,56] In Cambodia, the epicenter of drug resistance, 17 K13 mutations have been identified. More recent studies in Myanmar have identified further relevant mutations with different geographic distribution.[57] Other molecular surveys have demonstrated that spontaneous K13 mutations have arisen in many endemic locations[58] although not all of these polymorphisms are associated with reduced susceptibility.

The public health consequences of declining treatment efficacy, particularly in areas of high transmission, are alarming, especially when they are combined with resistance to the partner drug. This threat is heightened by the fact that no other antimalarial medicines are currently available that offer the same level of efficacy and tolerability as ACTs.

Toxicity

The artemisinin drugs have an excellent toxicity profile, the most commonly reported adverse effects being nausea, vomiting, and diarrhea, all of which are frequently reported during an acute malaria.[59,60] No

differences in the incidence of possible clinical adverse reactions have been noted between derivatives. There have been a few cases of acute urticaria and anaphylaxis after oral artesunate and artemether alone[61] or in combination[59]; readministration to these individuals should be avoided.

High doses of the lipophilic members of this family, such as artemether, have been associated with neurologic toxicity in animal studies involving rodents, dogs, and monkeys.[62–65] The pattern of neuronal damage is unusual, preferentially affecting the brainstem, in particular the reticular formation, vestibular system nuclei, and trapezoid nucleus. In animals manifestation includes gait disturbance; loss of spinal, brainstem, and pain responses; and cardiorespiratory depression. However, prolonged exposure at high doses (>15 mg/kg/day for more than 15 days) through parenteral administration is required to elicit this toxicity, and the risk is far greater after parenteral rather than oral administration.

There is no conclusive evidence of neurotoxicity in humans exposed to artemisinin derivatives at doses used in clinical practice (generally less than a total of 12 mg/kg). Several studies have reported neurologic abnormalities after artesunate treatment of patients with malaria, some of which have been attributed to artemisinin neurotoxicity[66–68]; however, the doses of drug administered suggest that drug toxicity would be unlikely. Cerebellar dysfunction, although rare, is a well-recognized complication of malaria[69]; hence these reports may reflect the presentation of patients with postmalarial neurologic syndrome (PMNS) rather than drug toxicity.[70]

Artemisinin derivatives have been used in several million patients over the past 20 years, with more than 10,000 enrolled in clinical trials and more than 300 patients undergoing formal neurophysiologic testing. Despite this, there have been no convincing clinical reports of neurotoxicity reliably associated with these drugs.[59,71] These studies have been supported further by the analysis of patients undergoing audiometry and auditory-evoked responses, which have also failed to reveal significant neurologic pathology.[72,73] Large prospective studies of patients with recurrent exposure to coartemether are in progress.

Cardiotoxicity does not appear to be a significant issue with the artemisinins; electrocardiographic changes are confined to minor QT prolongation, sinus bradycardia, and a few cases of transient first-degree heart block.[74] Hematologic abnormalities have been noted, particularly mild transient neutropenia (1.3%), when doses of artesunate in excess of 18 mg/kg total dose have been administered. Delayed onset of anemia has been reported in patients treated with IV artesunate for severe malaria.[75,76] The nadir occurs 8 to 28 days after treatment but generally improves within 4 to 6 weeks. The underlying mechanism is unclear but has been postulated to be due to artesunate killing malaria parasites without destroying the red blood cell, resulting in more deformable cells surviving the acute infection but with a shorter life span.[77] *It would be prudent to check the hemoglobin in such cases about 2 weeks after commencing IV artesunate therapy.*

Embryotoxicity and fetal resorption, but not teratogenicity, have been reported in animal studies at relatively low doses of artemether (7.5–15 mg/kg/day).[78] However, clinical studies from the Thai-Myanmar border and The Gambia have indicated that artesunate and artemether are well tolerated in pregnancy, with no evidence of an increased risk of abortion, stillbirth, congenital abnormality, or premature delivery.[79–82] In one study, where infants were monitored in the first year of life, normal developmental milestones were attained.[83]

Available data are reassuring for the administration of the artemisinin derivatives in later pregnancy,[84] and more recent clinical studies have reported that this safety extends to their use in the first trimester.[85] WHO antimalarial treatment guidelines committee recently revised the guidelines to recommend ACT for uncomplicated *P. falciparum* malaria to be extended to women in all stages of pregnancy, with artemether-lumefantrine being the preferred drug in the first trimester in view of it having the largest amount of safety data available.

ARTEMISININ PARTNER DRUGS

Lumefantrine

Lumefantrine (Fig. 41.3) is an aryl-amino alcohol, currently only available as a fixed-dose oral formulation, combined with artemether

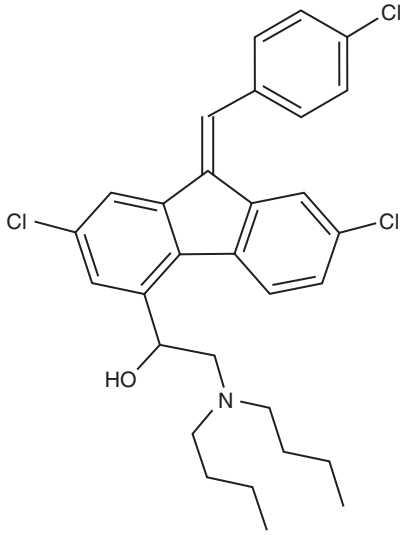

FIG. 41.3 **Structure of lumefantrine.**

(coartemether: 20 mg of artemether and 120 mg of lumefantrine). Artemether and lumefantrine have complementary activity—the potent but short-lived antimalarial activity of artemether resulting in a rapid reduction of parasite biomass over the first 3 days of treatment and the longer-acting lumefantrine providing sustained antimalarial activity to kill the residual blood-stage parasites. The combination has become one of the most highly used ACTs, with more than 3 billion treatments dispensed in malaria-endemic countries over the past decade.

The activity of lumefantrine is well documented against the erythrocytic stages of both *P. falciparum* and *P. vivax,* but does not extend to activity against gametocytes, hypnozoites, or the preerythrocytic stages. Although the mechanism of action is incompletely understood, it is thought that lumefantrine and other members of this class exert their antimalarial effect in the parasite's acid food vacuole by interacting with heme. Desbutyl-benflumetol, the putative metabolite of lumefantrine, has significantly higher blood schizonticidal activity compared with lumefantrine.

Pharmacokinetics

After ingestion, lumefantrine is slowly and erratically absorbed with peak concentrations occurring within 4 to 10 hours. Bioavailability is highly variable and reduced significantly during the acute phase of the infection[86] but increases when administered with food.[87] Only a small amount of fat is necessary to increase bioavailability, with as little as 36 mL of milk (corresponding to 1.2 g of fat) being sufficient for a clinically relevant increment (up to 16-fold) in blood concentrations.[88] Absorption is dose limited and results in the need for twice-daily dosing over 3 days rather than once-daily dosing. Lumefantrine is extensively and rapidly distributed to body tissues and highly protein bound (90%). It is metabolized predominantly by the liver and eliminated through the bile,[87] with a terminal elimination half-life of 3 to 6 days in patients with malaria. Pregnancy is associated with a significantly faster terminal elimination half-life compared with nonpregnant patients (49 vs. 77 hours, respectively).[89] The principal pharmacokinetic determinant of lumefantrine efficacy in patients with malaria is the area under the concentration-time curve (AUC), which is correlated closely with the plasma lumefantrine concentrations on day 7.[90,91]

Clinical Use

Artemether-lumefantrine is registered in many countries for the treatment of uncomplicated malaria, especially of multidrug-resistant *P. falciparum* infection. In view of its excellent tolerability and efficacy against all species of plasmodia and most multidrug-resistant isolates, it has been proposed as standby medication for travelers to countries endemic for malaria.

The dose for adults weighing more than 35 kg is four tablets of the fixed-dose combination per dose (total per dose: artemether 80 mg, lumefantrine 480 mg) given twice daily over 3 days (0, 8, 24, 36, 48, and 60 hours). Patients should be instructed to take their medication with milk or a fat-containing food, such as maize porridge with vegetable oil, particularly on the second and third day of treatment.

The efficacy and safety of artemether-lumefantrine in children weighing 5 to 10 kg is similar to that in older children.[92,93] A dispersible, pediatric tablet, containing 20 mg of artemether and 120 mg of lumefantrine, has been developed for use in infants and young children[35] and shows a similar efficacy and safety profile to that after administration of the crushed standard tablet.[94] The dosing schedule is the same as that for adults, with the body weight–adjusted doses stratified into three groups: 25 to 35 kg, 3 tablets per dose; 15 to 25 kg, 2 tablets per dose; and 5 to 15 kg, 1 tablet per dose. The child should take the medication with a small quantity of fat. A pooled analysis revealed that young children are underdosed with the current dosing schedule, although dosing recommendations have yet to be revised to address this.[95]

Coadministration of antiarrhythmics, β-blockers, macrolides, quinolones, antidepressants, antifungals, and grapefruit juice should be avoided. However, there is no evidence of harmful interactions after coadministration with these drugs. Mefloquine reduces plasma concentrations of lumefantrine by 30% to 40%,[96] whereas ketoconazole increases the AUC by 70%[97]; neither interaction is considered sufficient to warrant altering dosing.

Lumefantrine has good activity against chloroquine-resistant (CQ-R) strains of *P. falciparum* and *P. vivax*.[8] Both polymorphisms and copy number of the *pfmdr1* gene modulate susceptibility to lumefantrine[91,98] and are under selective pressure, with the *86N* allele (wild-type) significantly more prevalent in patients failing treatment.[99,100] A pooled analysis of data from more than 7000 patients demonstrated that *pfmdr1* amplification is an independent risk factor for recrudescence in patients treated with artemether-lumefantrine.[101]

Toxicity

Artemether-lumefantrine has an excellent toxicity profile. The most commonly reported adverse effects are nausea, vomiting, and diarrhea.[102] Pruritus and rash occur in less than 2% of patients. In contrast to the significant cardiotoxicity of halofantrine, despite its structural similarity with lumefantrine, no significant prolongation of the QTc intervals has been observed after administration of coartemether.[96,102] Although data are generally lacking on potential adverse interactions between lumefantrine and other drugs, there is no evidence of an interaction between lumefantrine and either mefloquine[96] or quinine.[103]

Although lumefantrine has not been formally evaluated sufficiently to recommend its widespread use in pregnancy, animal reproductive toxicity studies have failed to demonstrate mutagenic or embryotoxic properties in doses up to 1000 mg/kg, and the coformulation with artemether has been recommended as suitable for treatment of *P. falciparum* infection in pregnancy. Increasing amounts of accumulating data indicate that artemether-lumefantrine is better tolerated than conventional quinine, which is associated with hypoglycemia and poor adherence to a 7-day regimen.[84,104,105] Rates of recurrence of malaria after coartemether are high in pregnant women, a likely reflection of the increased rate of elimination of lumefantrine.[106] A recent multicenter, randomized trial demonstrated inferior cure rates compared with DHA-piperaquine.[107] This is likely associated with lower serum concentrations of lumefantrine.[106,108,109] Although studies are underway, there are as yet no formal recommendations for safe dose adjustment to overcome this. Current WHO guidelines do not preclude lactating mothers from taking artemether-lumefantrine because the amount of either active antimalarial drug entering breast milk is small, with no associated evidence of toxicity to the baby.[35]

Piperaquine

Piperaquine is a bisquinoline 4-aminoquinoline antimalarial structurally related to CQ. It was synthesized independently in France and China in the 1960s[110] and widely used for malaria control activities in China in the 1970s and 1980s.[111] In the 1990s piperaquine was repurposed as a partner drug in artemisinin-based combination therapy, and resulted in the development of a combination formulation of DHA plus piperaquine, with each tablet containing 40 mg DHA and 320 mg piperaquine phosphate (DHP). The mechanism of action of piperaquine has not been well studied but is likely to be similar to those of drugs of the same class.[111]

Pharmacokinetics

The pharmacokinetic properties of piperaquine are similar to those of CQ. It has a large volume of distribution, ranging from 103 to 716 L/kg, values that are significantly larger even than comparable drugs such as CQ.[112–114] It has a long terminal elimination half-life of 22 days in adults and 20 days in children.[115] The prolonged half-life results in a beneficial posttreatment prophylactic period, estimated to be about 28 days, and protects against both *P. vivax* and *P. falciparum*. Although early recurrent infections are reduced, infections treated with DHP are more likely to produce gametocytes than artemether-lumefantrine, an observation hypothesized to reflect the lower dosing of artemisinin derivative in DHP (total ≈7.5 mg/kg of DHA compared with ≈11.5 mg/kg of artemether in artemether-lumefantrine). Body weight influences clearance and volume parameters significantly, resulting in lower piperaquine exposures in small children (<25 kg) compared with larger children and adults,[116] and this is associated with a higher risk of recrudescence and earlier reinfection.[117]

Piperaquine is highly lipophilic, and its oral bioavailability is approximately doubled by administration with a high-fat meal.[118,119] However, data on the food effect of bioavailability of piperaquine are conflicting.[113,120,121] In a study carried out in Papua New Guinea,[122] a surprisingly low efficacy of DHP was reported (88% at day 42), significantly lower than that for artemether-lumefantrine. However, the difference had wide CIs and was apparent at day 28 but not day 42. This reduced efficacy is in contrast to other studies carried out in Africa[123–125] and Asia,[126,127] where DHP had similar or higher efficacy to other ACTs.

Clinical Use

Several fixed-dose formulations of DHP have been marketed, although only Eurartesim has been approved by the European Drug Agency. Eurartesim is available in tablets of two strengths, either 20/160 or 40/320 mg of DHA-piperaquine, respectively. It is used for the treatment of uncomplicated malaria, administered over 3 consecutive days for a total of three doses taken at the same time each day. The recommended dose is 4/18 mg/kg, once daily for 3 days (equivalent to 3–4 40/320 tablets for adults). Some manufacturers recommend four doses (at 0, 6–8, 24, and 32 hours), although there is no evidence that this regimen has any better efficacy than the simpler once-daily dosing. A large pooled analysis of more than 20 clinical trials highlighted the excellent efficacy of DHP, although a slightly higher risk of treatment failure has been identified in children age 1 to 5 years.[117] In 2015 WHO treatment guidelines revised the dose in children to be less than 25 kg, to receive a minimum of 2.5 mg/kg per day of dihydroartemisinin and 20 mg/kg per day of piperaquine, daily for 3 days.[35] Development of a pediatric formulation is underway.

Resistance

Piperaquine has potent activity against highly CQ-R *P. falciparum*[115] and *P. vivax*.[8] Widespread use of piperaquine has mostly been confined to the use in combination with dihydroartemisinin (DHP) introduced into national policy in Cambodia and Indonesia. Although initially showing excellent efficacy against all species of malaria,[126] reports of declining efficacy against *P. falciparum* began to emerge in 2010, although these were almost exclusively in the Greater Mekong region, on a background of the K13 mutations associated with artemisinin resistance.[128,129] Subsequent genomic studies have implicated amplification of the plasmepsin 2 gene and a single nucleotide polymorphism of the exo-E415G on chromosome 13, both of which appear to correlate with clinical efficacy.[130,131]

Toxicity

DHP appears to be better tolerated than artesunate-mefloquine administered with similar tolerability to artemether-lumefantrine

and amodiaquine-artesunate.[115,132] The main side effects reported are gastrointestinal (GI) disturbance such as diarrhea,[126] although this varies considerably according to geographic region.

Electrocardiographic effects of piperaquine have been specifically evaluated in two studies.[133,134] Both demonstrated minor lengthening of the corrected QT interval during treatment (between 11 and 14 ms). Very few patients experienced a prolongation that could be regarded as clinically significant (>60 ms). Therefore, although statistically significant, the QT prolongation observed after piperaquine therapy is unlikely to be clinically significant. European regulatory authorities have, however, required that DHP not be administered with food (to reduce peak concentrations) and caution that prior electrocardiographic monitoring be undertaken in those at risk of QT prolongation.

Pyronaridine

Pyronaridine is a Mannich base antimalarial synthesized in China in the 1970s.[135,136] It demonstrates potent in vitro activity against erythrocytic stages of *P. falciparum*[137–139] and *P. vivax.*,[140] including against parasites with high-grade CQ resistance.[141–143] A fixed-dose formulation of pyronaridine and artesunate combined in a 3:1 ratio has been developed for treatment of multidrug-resistant uncomplicated malaria (Pyramax). This novel ACT was licensed by the European Medicines Agency in 2012; it has been reviewed comprehensively.[144]

Clinical trials have shown potent efficacy of monotherapy against multidrug-resistant falciparum malaria,[145–147] as well as *Plasmodium ovale* and *Plasmodium malariae*.[146] The early therapeutic response was faster when pyronaridine was combined with artesunate.[148] Subsequent multicenter clinical trials have demonstrated excellent efficacy against multidrug-resistant strains of *P. falciparum*[149,150] and *P. vivax*.[151]

Pharmacokinetics

The pharmacokinetic profile of pyronaridine suggests that peak concentrations after single administration are reached within 1 hour, with a large volume of distribution (907 L) and a terminal elimination half-life of between 10 and 13 days.[152] The pharmacokinetic profile in children is similar, with a slightly faster elimination (half-life 6–9 days).

Clinical Use

Pyronaridine is available in combination with artesunate (Pyramax) as an alternate ACT for the treatment of acute, uncomplicated malaria infection caused by *P. falciparum* or by *P. vivax* in adults and children weighing 20 kg or more. Pyramax comes in tablets for adults with pyronaridine 180 mg and artesunate 60 mg. The dose for adults 65 to 90 kg is four tablets once daily for 3 days. A pediatric formulation containing 60 mg pyronaridine and 20 mg artesunate has also been developed with large clinical trials completed.[145,153]

Toxicity

Pyronaridine is generally well tolerated, the most common side effects being headache, vomiting, abdominal pain, bradycardia, and hypoglycemia. Early animal studies and early clinical trials suggested that pyronaridine may cause transient hepatotoxicity.[149–151] However, in a recent study the incidence of adverse effects, particularly hepatotoxicity after repeated courses of pyronaridine-artesunate, was not increased.[154]

Amodiaquine

Amodiaquine (AQ) (Fig. 41.4) is a 4-aminoquinoline that was widely used for prevention of malaria until the mid-1980s, when fatal adverse drug reactions described in travelers using AQ as prophylaxis[155] resulted in a dramatic decrease in its use for these indications. However, reevaluation of the drug for treatment rather than prophylaxis[156] resulted in increased recognition that the drug is not significantly more toxic than CQ when used for treatment. Its activity against CQ-R parasites gives it an important role in the antimalarial pharmacopoeia. However, this activity against CQ-R parasites shows significant geographic variation, activity being greater against isolates from Africa,[157–159] whereas those from Southeast Asia, South America, and Papua New Guinea display high levels of AQ resistance.[160] The apparent geographic specificity of AQ resistance may be due to differences among the haplotypes of the parasite *pfcrt* gene in these locations.[161] The mechanism of action of AQ is believed to be similar to that of CQ (see later).

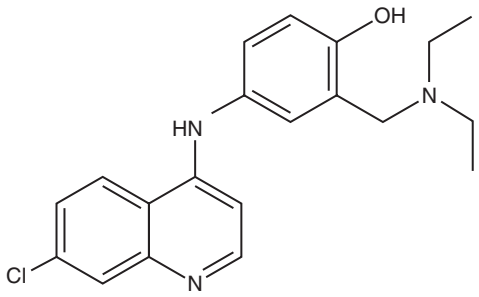

FIG. 41.4 Structure of amodiaquine.

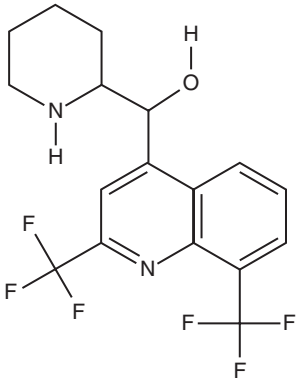

FIG. 41.5 Structure of mefloquine.

Pharmacokinetics

In contrast to CQ, AQ has a relatively short half-life (5 hours), being rapidly cleared by the liver by the action of CYP2C8 to an active metabolite, desethylamodiaquine AQ. The half-life of this metabolite has been variously estimated to be 1 to 3 weeks in adults and 3 to 12 days in children. Thus desethylamodiaquine provides approximately 100-fold-higher total drug exposure than AQ and therefore contributes most of the antimalarial effect of AQ.[162] Polymorphism of this enzyme may be a factor in drug interactions.

Clinical Use

Amodiaquine is used principally in a fixed-dose coformulation with artesunate (so-called AS/AQ). In two trials undertaken in sub-Saharan Africa, AS/AQ had superior efficacy to artemether-lumefantrine, with fewer episodes or recurrent parasitemia.[163,164] Although amodiaquine retains efficacy against CQ-R *P. vivax*,[8] recurrence rates after AQ monotherapy and AQ + artesunate are high.[165,166]

Toxicity

The most important adverse effects of AQ are hepatotoxicity (1/15,000 exposures) and bone marrow toxicity (1/2200 exposures; fatality rate, 1/31,000). Of note, none of these toxic effects have been reported when AQ has been used for malaria treatment but only in more prolonged use as a prophylactic agent. However, the actual incidence of such serious side effects in the setting of treatment is unknown. An important recently reported side effect is the significant risk of severe neutropenia in human immunodeficiency virus–infected children treated for malaria with artesunate-AQ.[167]

Mefloquine

Mefloquine (Fig. 41.5) is a chiral quinoline methanol. It is active against the asexual erythrocytic stages of malarial parasites but not against sporozoites, liver-stage parasites, or gametocytes. Mefloquine forms a complex with heme that may be toxic to the parasite,[168] although its mechanism of action is believed to be distinct from that of CQ. CQ-R parasites are more sensitive to mefloquine and vice versa.[98]

Pharmacokinetics

Mefloquine is only available as an oral tablet formulation. Absorption is adversely affected by vomiting and diarrhea but significantly enhanced when administered with or after food.[169] The mean absorption half-life of mefloquine after a single oral dose is 2.1 hours in healthy volunteers and 5.4 hours in patients with uncomplicated falciparum malaria.[170] About 98% of the drug binds to protein.[171] The terminal elimination half-life is 14 to 28 days. The apparent volume of distribution is compatible with plasma protein binding of greater than 98%.[171] Pharmacokinetic parameters are similar in single- and multiple-dose studies of healthy volunteers, suggesting that there are no significant changes in clearance when mefloquine is taken for prophylaxis. Mefloquine at a dose of 250 mg of the salt taken weekly reaches steady-state concentrations in 10 weeks without any indication of accumulation.[172] Mefloquine pharmacokinetics are highly stereoselective, with the (−) enantiomer demonstrating a significantly greater C_{max}, AUC, and longer elimination half-life than the (+) enantiomer.[173]

No dose adjustment is necessary in renal impairment or hemodialysis. Excretion of the drug and its metabolites is largely in the bile and feces. No studies have examined mefloquine disposition in patients with significant hepatic dysfunction, ascites, or cardiac failure. Because mefloquine is largely metabolized by the liver, the use of usual prophylactic doses over time may cause significant accumulation and consequent increased toxicity in patients with liver disease.

Clinical Use

Mefloquine is indicated for the treatment and prophylaxis of malaria, including CQ-R strains. Treatment regimens initially recommended a single dose of 15 mg/kg, although a higher dose (25 mg/kg) has been recommended to overcome declining efficacy. A combination with artesunate (4 mg/kg/day) as a 3-day regimen has been used in parts of Southeast Asia for almost 20 years although in areas of emerging artemisinin resistance the efficacy of this combination appears to be declining.[39,173a] A fixed-dose combination of artesunate-mefloquine has a target dose of mefloquine of 8 mg/kg per day for 3 days, which improves its tolerability and reduces the risk of early vomiting.[174,175] Mefloquine is under study as a component of so-called triple ACT to combat artemisinin resistance in the Greater Mekong subregion. Evidence indicates that its use in combination with piperaquine may result in better activity against DHP-resistant parasites.[130]

Resistance

High-grade mefloquine-resistant strains of *P. falciparum* have been documented in Southeast Asia, particularly the western border of Thailand, since the early 1990s. Cross-resistance of mefloquine with lumefantrine, halofantrine, and quinine has been documented.[176] *P. falciparum* isolates resistant to mefloquine have both increased copy number of the *pfmdr1* gene and increased expression of the gene product.[177]

Toxicity

The adverse effects of mefloquine have been the subject of much study. More than 20 million doses have been prescribed since 1984. Consistent with the frequency of side effects during other chemoprophylactic regimens, weekly mefloquine is associated with symptoms such as dizziness, nausea, and diarrhea in 20% of travelers.[178] These symptoms are usually mild and self-limiting. Dose-related drug-induced vomiting can be problematic, particularly in young children, in whom rates can reach 30% in infants younger than 2 years. When given as part of an ACT, vomiting can be reduced by delaying the administration of the mefloquine component of the ACT until day 1 or 2 or splitting the dose of mefloquine over 3 days.[175,179] Less serious side effects include rash, pruritus, and oral ulcers.[180] More serious dermatologic reactions have been reported but are exceptionally rare.

The most serious adverse effect of mefloquine is neuropsychiatric toxicity. This can develop at any time during prophylaxis or treatment, and symptoms range from mild to life threatening. The incidence of neuropsychiatric effects appears higher in Caucasians and Africans than in Asians and higher in women than men.[178] Although difficult to assess in the context of an infection that can itself cause profound neurologic disturbances, psychosis, depression, encephalopathy, convulsions, and other severe neuropsychiatric effects occur after the use of mefloquine for treatment of falciparum malaria, with a frequency of 1 : 200 to 1800 courses.[175,181] Symptoms typically resolve within 3 to 4 weeks.[178] Serious reactions similar to those seen after treatment courses can occur during prophylaxis, with rates of 1 : 10,000 to 20,000 courses reported in large trials.[180,181] Sleep abnormalities (e.g., insomnia, abnormal dreams) have occasionally been reported. Mefloquine should not be prescribed to patients with neuropsychiatric conditions, including depression, anxiety, psychosis, schizophrenia, and seizure disorder. If acute anxiety, depression, restlessness, or confusion develops during prophylaxis, the drug should be discontinued. Mefloquine-induced neurotoxicity can be difficult to distinguish from PMNS, which typically lasts 1 to 10 days and consists of acute confusion, psychosis, and tremor, with or without convulsions. PMNS has been reported to occur at a rate of 1 : 1000 patients[182] but appears to be more common among patients with severe malaria, with frequencies of PMNS being reported in 1 in 20. This late complication is almost 10 times more likely to occur after treatment with mefloquine than quinine.[182] In an analysis of neuropsychiatric outcomes among 367,840 US Armed Services members prescribed mefloquine, doxycycline, or atovaquone-proguanil as chemoprophylaxis between 2008 and 2013, the risk of incident anxiety, tinnitus, and posttraumatic stress disorder (PTSD) was higher among recipients of mefloquine (incidence rate ratios of 1.12, 1.81, and 1.83, respectively). A prior neuropsychiatric history resulted in an increased, but not statistically significant, risk for adjustment disorder, anxiety, insomnia, and PTSD among mefloquine recipients.[183] In contrast, in a study of the side effect profile of mefloquine for the treatment of uncomplicated malaria on the Thai-Myanmar/Cambodia borders among 19,850 patients receiving seven different regimens of mefloquine, serious neuropsychiatric side effects associated with mefloquine use were rare, especially among patients administered a split dose over 3 days (7.8/10,000 treatments).[175] Of note, re-treatment with mefloquine within 1 month of a course of therapy markedly increases the risk of adverse effects sevenfold, rising from 1 per 1217 to 1 per 173.[184] Of importance, the incidence of neurotoxicity is significantly higher after severe malaria (1/20), and thus the drug should not be used in this setting.

Mefloquine should not be used concurrently with quinine, quinidine, or drugs producing β-adrenergic blockade owing to the risk of electrocardiographic abnormalities or cardiac arrest. Administration of mefloquine with quinine or CQ may increase the risk of convulsions. Mefloquine may lower plasma levels of anticonvulsants.[185] Minor or inconsistent interactions have been observed between mefloquine and sulfadoxine-pyrimethamine, primaquine, metoclopramide, and oral contraceptives. Mefloquine has been shown to exert variable effects on ritonavir pharmacokinetics.

Although mefloquine prophylaxis is not recommended during pregnancy, the drug is probably safe and effective and discovery of pregnancy during mefloquine prophylaxis is not an indication for pregnancy termination.[186]

Chloroquine

CQ, a 4-aminochloroquine (7-chloro-4-[4-diethylamino-1-methylbutylamino] quinoline) (Fig. 41.6), was first synthesized in Germany in 1934 but not developed until after World War II.[187] Hydroxychloroquine

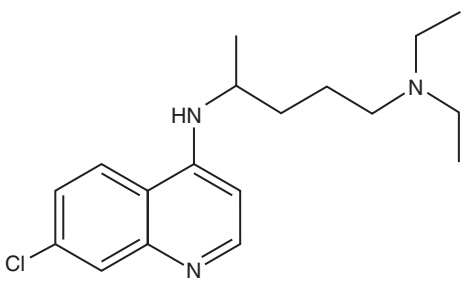

FIG. 41.6 Structure of chloroquine.

(Plaquenil), which differs from CQ only by hydroxylation at the end of the side chain, has identical antimalarial properties to CQ and is increasingly used in parts of the world where the availability of CQ is becoming limited. Hydroxychloroquine has antiinflammatory and antimalarial properties and is less prone to cause ocular toxicity than CQ among those receiving prolonged treatment,[188] and can be used interchangeably with CQ. CQ and other 4-quinoline antimalarials are only active against the blood stages of the parasite life cycle, during which hemoglobin is degraded.

CQ, a weak base, concentrates in the food vacuoles of intraerythrocytic parasites due to a pH gradient between the parasite cytoplasm and the acidic food vacuole of the parasite. Once it enters the food vacuole it is rapidly converted to a membrane-impermeable protonated form, where it is trapped. It binds to heme released after the parasite's digestion of host hemoglobin, preventing its conversion to the inert crystal hemozoin. It is the resulting accumulation of toxic heme monomers or the heme-CQ complex, or both, that is believed to kill the parasite.[189]

Pharmacokinetics

CQ is well absorbed whether given by oral, subcutaneous, or IM routes. Rectal bioavailability exceeds 90% but falls to 22% to 24% of the oral dose[190] unless a noncoated tablet is used, in which case plasma concentrations are only slightly lower than those found with oral administration.[191] CQ is 46% to 74% protein bound; it has an extremely large apparent volume of distribution and very long terminal half-life. As a consequence, a loading dose is required to yield effective plasma concentrations, and its pharmacokinetic profile is largely determined by its distribution rather than the elimination phase. Therapeutic drug levels in plasma are reached 2 to 3 hours after oral administration, with a mean initial half-life of 4 days. As plasma levels decline, the rate of excretion decreases, thus enabling once-weekly administration for prophylaxis. The estimated terminal elimination half-life is 45 to 55 days for CQ and 59 to 67 days for its active metabolite desethylchloroquine. Chloroquine is detectable in the urine for up to a year after drug administration.[192] Urinary excretion is the main route of elimination for both CQ and desethylchloroquine. The elimination half-life of CQ is significantly prolonged in chronic renal disease, with an associated increase in the AUC; hence a dose reduction is warranted in patients with renal insufficiency taking CQ for prophylaxis, although not required for acute malaria.[193]

Clinical Use

CQ remains the drug of choice for the treatment and prophylaxis of *P. falciparum* malaria in the few regions of the world where this species remains sensitive to this drug. (See Table 274.3 in Chapter 274 for chloroquine dosing.) Against sensitive parasites, CQ induces rapid parasite clearance only slightly slower than artemisinin drugs. CQ remains the treatment of choice for both prophylaxis and treatment of the nonfalciparum malarias, although this is under threat for *P. vivax* due to an increasing prevalence of CQ resistance in this species.[194] It lacks activity against sporozoites and mature gametocytes. Although CQ is not active against liver-stage infection, it may potentiate the activity of primaquine as a causal prophylactic agent.[195]

Resistance

CQ accumulates at concentrations 4 to 10 times lower in drug-resistant parasites compared with chloroquine-sensitive (CQ-S) parasites; it is this marked decrease in CQ accumulation that underlies the phenomenon of CQ resistance. CQ-R parasites can be partially resensitized to CQ in vitro by a range of weak bases, including the antihistamine chlorpheniramine and the calcium channel blocker verapamil.[196] This "resistance reversal" effect is characterized by both an increase in CQ accumulation and an increase in the CQ sensitivity of CQ-R parasites. However, the concentration of these reversal agents required to reverse CQ resistance is generally higher than that tolerated in vivo. The key molecular determinants of CQ resistance involve a number of mutations in the so-called CQ resistance transporter gene, or *pfcrt*. Of these, the predominant resistance-conferring mutation (K76T)[197] results in the loss of a positive charge from the putative substrate-binding site on the vacuolar side of the protein.[198] CQ-R *P. falciparum* is now found throughout the malaria-endemic world, with the exception of Central America and the Panama Canal. Of note, evidence indicates that the *pfcrt* mutation exerts a fitness cost on the malaria parasite. Twelve years after the drug was withdrawn from use in Malawi in 1993, a clinical trial undertaken in children in 2005 showed disappearance of the mutant *pfcrt* allele and return of CQ sensitivity.[199] The return of the *pfcrt* K76 CQ-sensitive allele has also been reported in other countries after withdrawal of CQ treatment, including Ethiopia, Tanzania, and Kenya.[200–202]

CQ-R *P. vivax* has developed more slowly than for *P. falciparum*. However, resistance has now been reported in nearly all countries where *P. vivax* is endemic. The geographic distribution of CQ-R *P. vivax* has been recently reviewed,[203] and a complete list of the clinical trials identified in this review is available online.[204] High rates of resistance are particularly notable in the island of New Guinea,[113,126] Indonesia,[205–207] Malaysian Borneo,[208] Cambodia,[129] Vietnam,[209] and Myanmar.[210] Resistance has been reported in India[211] and across countries in the Amazon basin, including Brazil,[212,213] Bolivia,[213] Peru,[214] Colombia,[215] and Guyana.[216] CQ-R *P. vivax* has also been reported in Turkey,[217] Ethiopia,[218,219] and Madagascar.[220]

The molecular mechanisms underlying CQ resistance in *P. vivax* remain uncertain. Although a possible role of orthologous genes in *P. vivax* are associated with resistance in *P. falciparum*, including *pvmdr1* and *pvcrt-o*, no specific genetic marker has been unequivocally demonstrated.[221]

If CQ is used for the treatment of *P. vivax*, close follow-up is essential to monitor for possible treatment failures, as previously unrecognized foci of CQ-resistant *P. vivax* are emerging. CQ should not be used to treat severe vivax malaria due to slower parasite clearance times compared with artemisinin derivatives.[222]

Toxicity

CQ is generally well tolerated.[60] The most commonly reported adverse effect is pruritus, which is more common in dark-skinned populations.[223] It predominantly affects the palms, soles, and scalp and starts within 24 hours of exposure and can last for several days. As systemic equilibration of CQ is relatively slow, this combined with the cardiac and central nervous system (CNS) toxicity of high plasma levels renders parenteral administration or overdose hazardous, with seizures and death from cardiovascular collapse resulting from high plasma levels.[224] Although CQ crosses the placenta,[225] doses used for chemoprophylaxis do not cause a significant increase in fetal abnormalities. CQ is safe for nursing mothers. When used for prolonged periods (>5 years prophylaxis), the cumulative accumulation can cause retinal damage,[226] and regular ophthalmologic examination is appropriate if long-term therapy is undertaken.

Quinine and Quinidine

Derived from the bark of the cinchona tree, quinine comprises a racemic mixture of D and L enantiomers. As the availability of quinine is reducing in the developed world, its D enantiomer, quinidine, which is also used as a cardiac antiarrhythmic, is increasingly being used as a replacement[227] (Fig. 41.7). IV quinine is the second-line alternative to IV artesunate for treatment of severe malaria.[227,228] Parenteral artesunate is more efficacious with a mortality benefit in severe malaria.[47,48] As is the case for CQ, the only life-cycle stage of *P. falciparum* that quinine is active

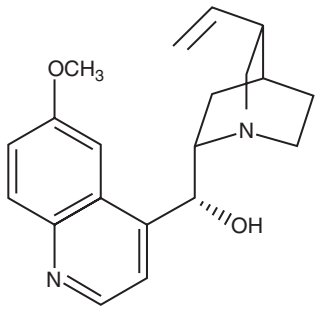

FIG. 41.7 Structure of quinine.

against is the asexual erythrocytic form. It has little effect on sporozoites or liver-stage parasites. It is gametocidal against *P. vivax* and *P. malariae* but not against *P. falciparum*. Quinine is active against the zoonotic primate malaria species *Plasmodium knowlesi.*[229] Although the mechanism of action of quinine against *Plasmodium* spp. is not known, it appears to inhibit the digestion of hemoglobin. It has also been shown to inhibit the adenosine triphosphatase of *P. falciparum* food vacuoles[230]; this mechanism appears to be independent of its alkalinization of the food vacuole.[231]

Pharmacokinetics

The pharmacokinetic properties of quinine have been extensively reviewed.[232] Two clinically important pharmacokinetic issues are the risk of underdosing in severe malaria, necessitating a loading dose (see later),[233] and the variation in pharmacokinetic parameters in patients with severe malaria.[232] Although quinine levels are higher in severe malaria, toxicity is uncommon compared with equivalent doses in healthy subjects. This is due to increased plasma binding to a1-acid glycoprotein, an acute-phase protein whose levels are elevated in acute malaria,[234] which results in a reduction of levels of free quinine by 25% to 30% in uncomplicated malaria and by 40% in severe malaria.[235–237] With increasing severity of malaria, there is also a reduction in the volume of distribution and clearance, particularly hepatic clearance, because of the changes in free quinine levels.[232] Both quinine and quinidine are metabolized by the hepatic microsomal enzyme cytochrome P-450 3A4; quinidine is also metabolized by CYP2D6.[232]

It is important to avoid confusion arising from calculation of the dose of quinine depending on its various salts. The following doses are equivalent: quinine base 100 mg; quinine sulphate 121 mg; quinine bisulphate 169 mg; and quinine dihydrochloride 122 mg. The dose of quinine sulfate for treatment of malaria is 10 mg salt/kg, given three times a day for 7 days.

Pharmacokinetic parameters of quinine in patients with acute renal failure appear to be similar to patients with severe malaria without renal failure.[238] A dose reduction of 30% to 50% is suggested only after 2 days of standard quinine therapy, including standard loading doses, for patients with acute renal failure or who remain severely unwell.[232]

Clinical Use

Quinine is less well tolerated than other antimalarials (see later) and requires prolonged course of treatment (at least 7 days) to effect a cure. Hence completion of a therapeutic course is problematic. It remains an option as second-line oral treatment of all species of malaria, particularly for pregnant women, infants, and patients with suspected drug-resistant infections recurring after a compromised treatment regimen. To reduce the risk of recrudescence, quinine (10 mg salt/kg q8h for 7 days) is often combined with clindamycin (10 mg/kg q12h for 7 days).[239] In patients older than 8 years, clindamycin can be substituted for either doxycycline (100 mg q12h in adults or 2.2 mg/kg q12h in children) or tetracycline (250 mg q6h in adults or 6 mg/kg q6h in children).[240] Further, because of the poor tolerability of quinine, especially after 3 days, many recommended oral regimens for mild malaria entail 3 days of quinine, with a full 7 days of the partner drug. When used for treatment of severe malaria, it is imperative that therapeutic drug concentrations are reached as quickly as possible. Hence an initial loading dose should be administered,[233] followed by a maintenance dose of 10 mg salt/kg q8h, starting 8 hours after the loading dose. Two loading-dose regimens can be used:

- The standard regimen is quinine dihydrochloride 20 mg of salt/kg by IV infusion over 4 hours. (Note: IV quinine is not available in the United States.)
- An alternative regimen has been suggested with quinine dihydrochloride 7 mg of salt/kg by IV infusion over 30 minutes, followed by 10 mg/kg infused over 4 hours.[241]

A common clinical dilemma is whether to administer a loading dose if there is a history of self-medication with antimalarials. The risk of inadequate treatment due to underdosing must be weighed against the risk of serious cardiac toxicity due to overdose. Authorities suggest omitting the loading dose if more than 2 g of quinine has been administered within the past 48 hours.[242,243]

If an IV infusion cannot be safely administered, quinine can also be administered intramuscularly. Although pharmacokinetic studies of IM quinine are lacking in adult patients with severe malaria, a loading dose appears to be well absorbed in patients with uncomplicated malaria[244] and efficacious in severe malaria.[245] Quinine should be diluted with water to reach a concentration for administration of less than 150 mg/mL to reduce local pain and improve absorption, and be administered in divided doses into each thigh.[232,246]

Quinine Resistance

Quinine resistance in *P. falciparum* was first reported in Brazil in 1908.[247] Although quinine resistance of *P. falciparum* varies globally, published data on its prevalence since the increased use of artemisinin antimalarials are sparse. Resistance is more common and severe in Southeast Asia and has paralleled the rise of resistance to mefloquine,[248] suggesting cross-resistance between these two drugs. As is the case for mefloquine, amplification of the drug transporter *pfmdr1* is associated with resistance. However, there is a paucity of data associating *pfmdr1* amplification and clinical outcome.[249] Genetic markers of quinine resistance have been reviewed.[250]

Quinine for Treatment of Babesiosis

Quinine in combination with clindamycin has been the treatment of choice for severe babesiosis at doses similar to those used for treatment of malaria: quinine 8 mg salt/kg (up to 650 mg in adults) every 8 hours, with clindamycin 7 to 10 mg/kg (up to 600 mg in adults) every 6 to 8 hours.[251] Azithromycin with atovaquone is a better-tolerated regimen and equally effective in patients with mild-moderate babesiosis.[252]

Toxicity

The syndrome of cinchonism (tinnitus, high-tone deafness, visual disturbances, headache, dysphoria, vomiting, and postural hypotension) commonly occurs at therapeutic plasma levels, with plasma levels correlating with severity.[253] Reversible deafness, which is predominantly high-tone, also correlates with plasma concentrations.[254–257] The most common cardiovascular adverse events associated with quinine and quinidine are postural hypotension and syncope.[74] Although cardiotoxicity due to quinine is unusual at plasma concentrations in the therapeutic range,[254,258] quinidine causes QT interval prolongation in approximately 3% of patients.[259] Fatal ventricular arrhythmias have only been described in a patient with a preexisting prolonged QT interval (490 ms).[260] Acute toxicity caused by inadvertent rapid infusion may cause life-threatening cardiac arrhythmias, including ventricular tachycardia and ventricular fibrillation.[261] Patients being treated with quinidine should have regular clinical assessment for hypotension, a baseline electrocardiogram for assessment of corrected QT interval, and cardiac monitoring. Patients being treated with quinine do not generally require cardiac monitoring unless there is preexisting heart disease or the potential of interactions with other medications that are known to affect cardiac electrophysiology, or in children younger than 2 years receiving IV treatment. Quinine has been associated with transient retinal toxicity, which typically occurs in drug overdose; the majority of patients report complete blindness, but alterations in color vision, blurring of vision, or visual field restriction are described.[262]

An important adverse effect of quinine treatment in severe malaria is hypoglycemia.[263,264] Pregnant women are particularly susceptible to this complication.[265] All patients receiving quinine or quinidine should be monitored for hypoglycemia, and any deterioration in clinical status (including seizures or altered mental status) should prompt assessment of plasma glucose. Patients receiving IV therapy should also receive an IV infusion of dextrose.

Blackwater fever is the syndrome of massive hemolysis (with visible hemoglobinuria) in the setting of severe malaria, glucose-6-phosphate dehydrogenase deficiency (G6PDd), and quinine treatment. Because malaria is also associated with hemolysis, the relative roles of quinine and G6PD are unclear.[245,266,267]

Allergic reactions, such as drug-induced thrombocytopenia,[268] hepatitis, urticaria, and other more severe dermatologic phenomena (erythema multiforme, fixed-drug eruptions, and toxic epidermal

necrolysis),[261,269,270] are all much less common after malaria chemotherapy than when the drug has been used long term.

The risks of using quinine in pregnancy must be weighed against the known deleterious effects of malaria on pregnancy. Some, but not all, animal studies have demonstrated abnormalities of the inner ear, auditory nerve, and CNS.[186,271–273] At standard doses, quinine does not increase the rate of spontaneous abortion, and its use in the first trimester does not appear to be associated with birth defects.[274] Controlled trials have not found evidence of an increased risk of birth defects.[275] As noted earlier, in pregnant women with severe malaria, hypoglycemia appears to be a common complication of quinine treatment.[276]

8-AMINOQUINOLINES

Primaquine

Primaquine (Fig. 41.8) is the most widely used 8-aminoquinoline antimalarial compound. Drugs in this class are unique in having activity against asexual, sexual, and liver stages of the parasite.[277] Although discovered almost 80 years ago and recommended by most national and international malaria treatment guidelines, primaquine is often underused owing to fear regarding its toxicity and uncertainty regarding the efficacy. This is especially the case in settings where G6PD testing (see later) is not readily available. However, there has been a resurgent interest in this drug and its role in interrupting malaria transmission, achieving the radical cure of *P. vivax,* and reducing the spread of drug resistance.[278]

The mechanism of action of the 8-aminoquinolines is unknown but hypothesized to be due to oxidative metabolites that affect parasite pyrimidine synthesis and disrupt the mitochondrial electron transport chain.

Pharmacokinetics

Primaquine is essentially a prodrug that is extensively and rapidly metabolized, with only a small fraction of the parent drug excreted unchanged. Little is known of the pharmacokinetics of the metabolites that are responsible for both its antimalarial activity and toxicity. Its principal metabolite, carboxyprimaquine, is formed as a result of oxidative deamination, which is thought to involve both the cytochrome-P450 enzyme complex and monoaminooxidases.[279] Primaquine is rapidly and almost completely absorbed after oral administration, with C_{max} of about 3 hours. It is cleared by hepatic biotransformation, with an elimination half-life of 8 hours. Repeated dosing does not alter its pharmacokinetic profile.[280] Although there are no pharmacokinetic studies of the enantiomers in humans, animal studies point to stereoselective metabolism of positive (+) and negative (−) primaquine,[281] with the (+) enantiomer exhibiting a significantly longer elimination half-life.[282] These studies also suggest a difference in toxicity between the two enantiomers that may be relevant to humans. Recent data indicate that the activity of primaquine is dependent on its metabolism by the cytochrome P-450 CYP2D6 metabolic pathway, and that slow metabolism may explain drug failure in some situations.[283]

Clinical Use

Primaquine has three main clinical applications: the radical cure of *P. vivax* and *P. ovale* infection, causal prophylaxis, and the reduction of *P. falciparum* transmission. The dose of primaquine is expressed as the amount of base. There is only one salt, the diphosphate, available for use. A dose of 15 mg primaquine base is equivalent to approximately 26.3 mg primaquine diphosphate salt.

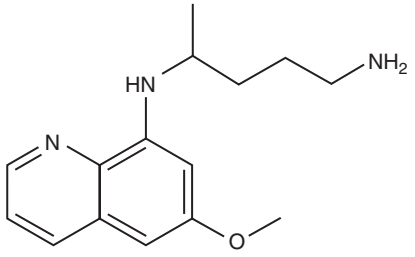

FIG. 41.8 Structure of primaquine.

Radical Cure

Primaquine and now tafenoquine are the two licensed agents for the radical cure of *P. vivax* and *P. ovale.* Despite more than 60 years of continuous use, there is still neither evidence nor consensus on the optimal dose or dosing regimen.[284] The main determinant of primaquine antirelapse efficacy is the total dose of primaquine administered, rather than the dosing schedule.[277] WHO guidelines for the radical cure of vivax malaria currently recommend the use of a daily dose of 0.25 mg/kg per day of primaquine base for 14 days (3.5 mg/kg total dose) taken with food once daily, coadministered with CQ or ACT depending on CQ sensitivity in the region.[35,285] However, this standard dose regimen of primaquine fails to prevent relapses in many different endemic locations.[284] High rates of recurrence can be overcome by administering a higher total dose (7 mg/kg, equivalent to an adult dose of 30 mg/day for 14 days), a regimen that is now recommended for Southeast Asia and Oceania. However, in practice most national guidelines still recommend lower doses. To reduce the risk of hemolysis and improve tolerability from GI disturbance, the target cumulative dose of primaquine is recommended to be administered over 14 days, either as a once- or twice-daily regimen. Advocating such prolonged courses of treatment can result in significant problems with adherence.[286] Short-course, high-dose regimens have the potential to increase patient adherence and thus effectiveness,[287] but appropriately powered prospective multicentered, randomized, controlled trials are necessary.

Primaquine can induce hemolysis in patients with G6PDd. In patients with mild G6PDd, primaquine 45 to 60 mg once weekly for 8 weeks may be better tolerated.[288] In some patients, particularly those who have previously suffered severe adverse effects of primaquine, it may be prudent to withhold primaquine treatment and instead promptly treat relapses. Primaquine use is contraindicated in pregnancy because the G6PD status of the fetus is unknown, and even in normal individuals, fetal red cells are relatively deficient in G6PD. Instead, after treatment of *P. vivax* or *P. ovale* infection, a pregnant woman should take weekly CQ prophylaxis until after delivery, when hypnozoite eradication can be considered. Treatment is contraindicated in breastfeeding women, due to concerns of excretion of primaquine in breast milk. Data on the administration of primaquine to children are limited, although the current WHO guidelines recommend administration to children older than 6 months. The dose is the same as for adults adjusted for body weight.

Reduction of Transmission

Interrupting transmission has become a priority of many malaria control programs now set on achieving malaria elimination. The use of primaquine as a gametocytocidal drug in areas of low malaria transmission has potential to reduce the incidence of infection and retard the spread of drug-resistant parasites. The addition of primaquine to current ACTs reduces gametocyte carriage after ACT administration.[289] Current WHO guidelines recommend a single dose of 0.25 mg/kg at the start of schizonticidal treatment.[35]

Chemoprophylaxis

The activity of primaquine activity against all species of plasmodia makes it a useful agent for prophylaxis. For this indication, a daily dose of 0.5 mg/kg (equivalent to an adult dose of 30 mg base) is well tolerated and effective. It is particularly useful in areas where the risk of *P. vivax* infection is high.[290] When used for primary prophylaxis, it should be taken daily for 1 to 2 days before travel to malarious areas, daily while in the areas, and daily for 7 days after leaving the areas. Primary prophylaxis with primaquine obviates the need for presumptive antirelapse therapy where travel has been to a region of high risk for *P. vivax* infection. If primaquine is used for this purpose, G6PDd must be excluded in advance.

Toxicity

Although primaquine is generally well tolerated, its propensity to cause GI adverse effects and hemolysis warrants caution. Nausea, headache, vomiting, and abdominal pain are relatively common, particularly when doses exceed 30 mg (0.5 mg/kg). These adverse effects can be reduced substantially by taking the drug with food. Diarrhea, weakness, visual disturbance, and pruritus occasionally occur.

Primaquine can induce significant hemolysis, particularly in those with G6PDd.[291] G6PDd is the most common heritable enzymopathy in the world, with a prevalence ranging from 2% to 40%.[292] The gene encoding G6PD is found on the X chromosome, and inheritance is thus sex linked. Individuals with less than 10% of normal enzyme activity are at risk of life-threatening hemolysis,[293] whereas those with milder variants may suffer negligible effects.[277] Primaquine-induced hemolysis is dose dependent, the dose-effect relationship depending on the degree of G6PDd, which is graded according to the level of enzyme activity. This is particularly important in poorly resourced settings where routine G6PD testing is not available. In these circumstances health care providers simply omit radical cure or prescribe lower and prolonged courses and stop treatment in the event of frank hematuria or overt hemolysis.

Primaquine metabolites induce the oxidation of hemoglobin into methemoglobin, a process that is dose dependent and occurs in normal and G6PDd erythrocytes. In most treatment regimens methemoglobin concentrations induced by primaquine are asymptomatic, transient, and rarely apparent. However, prolonged administration of 45 mg (0.75 mg/kg) weekly or 15 mg (0.25 mg/kg) daily may increase methemoglobin levels to more than 30% in some individuals.[294] Individuals with a genetic polymorphism resulting in reduced nicotinamide adenine dinucleotide cytochrome b5 reductase activity are particularly vulnerable to this effect. Quinine appears to offer some protection against methemoglobinemia, whereas combination with CQ can result in methemoglobin levels fourfold higher in healthy subjects.[295]

Primaquine exerts other hematologic side effects, such as mild leukocytosis and leukopenia (specifically granulocytopenia) within several days of starting treatment. It has also been associated with arrhythmias, although these are only induced at supratherapeutic doses.

Tafenoquine

Tafenoquine is an 8-aminoquinoline antimalarial drug developed by the Walter Reed Army Institute of Research (WRAIR) and GlaxoSmithKline Pharmaceuticals (GSK). Like primaquine, it is active against all forms of malaria. Tafenoquine has similar antimalarial properties as primaquine. Because of tafenoquine's long half-life, the radical cure of *P. vivax* can be achieved with a single dose. Tafenoquine can also be administered intermittently for chemoprophylaxis and clearance of circulating gametocytes.

Tafenoquine is approximately 10 times more potent than primaquine against liver-stage hypnozoites in rhesus monkeys. Unlike primaquine, tafenoquine inhibits heme polymerization similar to the 4-aminoquinolines, an effect that may explain its activity against the asexual intraerythrocytic stages of both *P. falciparum* and *P. vivax*.[296] Tafenoquine has additional antiparasitic activity in mosquitoes, preventing sporozoite development in the mosquito.[297,298]

Pharmacokinetics

Tafenoquine is rapidly absorbed; Absorption is increased when taken with food, which also reduces the severity of GI adverse effects.[299] It has a large volume of distribution and a prolonged elimination half-life (≈16 days),[300] thus offering the potential of a longer dosing interval for prophylaxis compared with primaquine. Unlike primaquine, tafenoquine accumulates in erythrocytes, which may contribute to its greater potency compared with primaquine.[301] Like primaquine, the activity of tafenoquine may require CYP2D6 metabolism.[302]

Clinical Use

Tafenoquine is formulated as the succinate salt, with 250 mg of salt equal to 200 mg base. It is marketed as 100- and 150-mg tablets of tafenoquine base. The prophylactic activity of tafenoquine against *P. falciparum* in a variety of weekly regimens varied from 86% to 89%, rising to 95% against *P. vivax*.[303,304] Against *P. vivax* hypnozoites, a single-dose regimen achieves reliable radical cure.[304,305a,305b] A multicentered phase III clinical trial has been completed and showed that a 300-mg dose of tafenoquine was not inferior to a 14-day primaquine regimen (total dose 3.5 mg/kg). The US Food and Drug Administration approved tafenoquine for single-dose radical cure of *P. vivax* malaria in adults in July 2018. Prophylaxis was later approved for adults at a dose of 200 mg once per day for 3 days within 1 week of travel, 200 mg once per week during travel, and 200 mg once within a week after completing travel. The ability of tafenoquine to kill parasites in the mosquito suggests that it may be useful during malaria epidemics by blocking transmission.[298]

Toxicity

The most frequently noted adverse events are mild GI upset, headaches, and myalgia. As with primaquine, the main concern is that of hemolysis in persons with G6PDd, particularly if tafenoquine's slow elimination results in sustained toxicity.[306] Therefore G6PD status should be checked before administering the drug. In view of the lack of data of safety in heterozygote females with intermediate deficiency, the manufacturer recommends that patients should have a G6PD enzyme activity greater than 70%; this currently requires confirmation by spectrophotometry. Tafenoquine should not be used in pregnant women or in lactating mothers with infants of deficient or unknown G6PD status. Tafenoquine at prophylaxis doses is not recommended in those with psychotic disorders. Methemoglobinemia may be observed, as with primaquine.

Atovaquone

Originally developed as an antimalarial agent on the basis of potent in vitro activity against drug-resistant strains of *P. falciparum*,[307] the hydroxynaphthoquinone atovaquone (Fig. 41.9) has subsequently found to be active against a number of other microorganisms, including *Pneumocystis jirovecii*,[308] *Toxoplasma gondii*,[309] *Babesia microti, Cryptosporidium parvum, Encephalitozoon intestinalis, Leishmania donovani, Entamoeba histolytica,* and *Trichomonas vaginalis*. Toxoplasmosis and babesiosis can be effectively treated with atovaquone, when used in combination with pyrimethamine and azithromycin, respectively.

Studies on the potentiation of atovaquone by other antimalarial drugs revealed evidence of synergistic activity with proguanil.[310] After clinical trials, this culminated in the development of a fixed-dose combination (Malarone) for the treatment and prevention of malaria.[311,312] Although atovaquone is active against both the erythrocytic and preerythrocytic (liver) stages of *Plasmodium* spp., it does not eradicate hypnozoites from the liver, so patients with *P. vivax* or *P. ovale* infections require radical cure with an 8-aminoquinoline.

Atovaquone inhibits the electron transport system at the cytochrome bc1 complex.[313] There is obligatory coupling of pyrimidine biosynthesis and electron transport via ubiquinone/ubiquinol. Selectivity is achieved through the difference between mammalian and plasmodial electron transport systems in their sensitivity to the hydroxynaphthoquinones. In addition, plasmodia depend completely on pyrimidine synthesis, whereas mammalian cells use a pyrimidine salvage pathway. The mechanism of potentiation of atovaquone by proguanil appears to be related to proguanil itself; metabolism to cycloguanide is not required.[314]

Pharmacokinetics

Atovaquone is highly lipophilic and shows significant variation in oral bioavailability. After a single oral dose, absorption is slow, increasing twofold to threefold with a fatty meal, and is dose limited above 750 mg.[307] It is highly protein bound (>99.9%).[315] The liver is the main route of elimination, with enterohepatic circulation contributing to its prolonged

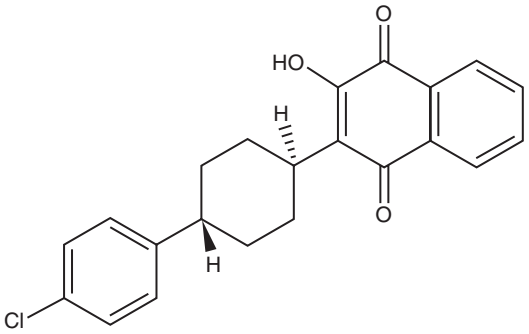

FIG. 41.9 Structure of atovaquone.

half-life. Negligible biotransformation occurs in vivo with atovaquone being eventually eliminated in the feces, with less than 1% excreted in the urine. Its elimination half-life is increased in patients with moderate hepatic impairment, probably due to impaired enterohepatic circulation. Caution is advised in patients with hepatic dysfunction. Because urinary excretion is negligible, no dosage adjustment is necessary in patients with renal impairment. However, because proguanil and its metabolite cycloguanil are primarily excreted in the urine, use of the atovaquone-proguanil combination (Malarone) is contraindicated in patients with creatinine clearance of less than 30 mL/min. Atovaquone is not teratogenic and does not cause reproductive toxicity in rats at plasma concentrations of up to two to three times human levels.

Clinical Use

Atovaquone-proguanil (Malarone), administered as a single tablet daily (250/100 mg), is a highly effective chemoprophylactic regimen. Its major drawback is the cost of the medication due to the high cost of synthesis of atovaquone. It is also an effective treatment for all species of human malaria (in a dose of four tablets daily for 3 days in adults; in children use a weight-based dose of the pediatric formulation: 62.5 mg atovaquone/25 mg proguanil), including drug-resistant strains of *P. falciparum*. However, for the latter it is probably safer to combine it with an artemisinin drug. Of interest, clinical trials have shown that a full treatment course (4 tablets daily for 3 days) of atovaquone-proguanil results in protection from reinfection with *P. falciparum* for 28 to 32 days,[316–319] and a recently undertaken clinical trial showed that a single dose of atovaquone-proguanil results in prophylaxis of sufficient duration to suggest that a weekly dosing schedule may be sufficient. The study also supported previous data indicating activity against incubating liver-stage parasites.[320]

Resistance

Reports of clinical strains of *P. falciparum* resistant to atovaquone-proguanil typically involve changes at codon 268[308] of the cytochrome b gene.[321] Although there have been reports of prophylaxis failure in patients taking atovaquone-proguanil, these have been associated with subtherapeutic drug levels that have permitted the in vivo evolution of drug-resistant parasites.[322] Of note, it appears that even if atovaquone resistance emerges in vivo, it is unlikely to be transmissible through the mosquito due to the requirement for wild-type cytochrome C enzyme in the mosquito vector.[323] Mutations in the cytochrome b gene occur more commonly in patients with *P. jirovecii* infection in the acquired immunodeficiency syndrome (AIDS) after treatment with atovaquone, but its clinical significance is unknown.[324] Phenotypic resistance to atovaquone has not been demonstrated for *P. jirovecii*. Atovaquone-resistant isolates of *T. gondii* also demonstrate mutations in the cytochrome b gene.[325] Clinical failures of azithromycin-atovaquone therapy for babesiosis in immunocompromised patients have been reported.[326]

Toxicity

Atovaquone is generally well tolerated, but reported adverse events include nausea, vomiting, diarrhea, headache, fever, and transient elevations in liver function tests. Caution should be used when administering atovaquone concurrently with other highly plasma protein–bound drugs with narrow therapeutic indices because competition for binding may occur. Coadministration of rifampin results in significant reductions in atovaquone plasma concentrations. Tetracycline and metoclopramide reduce atovaquone plasma concentrations by 40% and 50%, respectively. Atovaquone increases plasma concentrations of zidovudine by approximately 33% due to inhibition of hepatic glucuronidation. Clinically insignificant reductions in both trimethoprim and sulfamethoxazole occur with atovaquone.[327]

Folate Antagonists

This class of drugs has been used for both prophylaxis and treatment of malaria. In view of their ability to act synergistically, they are often used in combination. However, the efficacy and therefore use of the antifolates have fallen due to the development of high-grade resistance. Activity of the antifolates derives from selective inhibition of parasite rather than host enzymes responsible for one-carbon metabolism.

DIHYDROFOLATE REDUCTASE INHIBITORS

Unlike mammalian cells, *P. jirovecii* and protozoan parasites cannot use preformed pyrimidines obtained through salvage pathways but rely completely on de novo pyrimidine synthesis, for which folate derivatives are essential cofactors. This process, thymidine synthesis, is dependent on the enzyme dihydrofolate reductase (DHFR). DHFR inhibitors compete with the substrate, dihydrofolate, by binding reversibly to the active site of DHFR. Although DHFR is found in almost all organisms, the amino-acid sequence of DHFR varies between species, thus explaining the selective action of different DHFR inhibitors.[328]

Proguanil (Chloroguanide)

Although proguanil (Fig. 41.10) is an effective schizonticide, its onset of action is too slow to recommend its use alone for malaria treatment. Proguanil is now almost exclusively used with atovaquone for malaria chemoprophylaxis, and for the oral treatment of uncomplicated malaria. It exerts its antimalarial activity primarily through its metabolites cycloguanil and 4-chlorophenyl biguanide, which inhibit dihydrofolate reductase in the parasite and disrupt deoxythymidylate synthesis, thus interfering with the biosynthesis of pyrimidines required for nucleic acid replication. Proguanil also exhibits intrinsic activity, possibly involving mitochondrial toxicity, that is synergistic in combination with atovaquone.[314] This is further suggested by the finding that cycloguanil-resistant parasites retain their sensitivity to high concentrations of proguanil.[329] In addition, a *P. falciparum* strain transfected with a variant form of human dihydrofolate reductase selectable by methotrexate had increased resistance to cycloguanil but remained susceptible to proguanil.[330]

Pharmacokinetics

Proguanil is extensively absorbed in the fasting or fed state, with peak plasma levels occurring 1 to 6 hours after a single dose. The drug is 75% protein bound; this appears to be unaffected by the concentration of atovaquone. Metabolism of proguanil to cycloguanil is mediated in the liver by cytochromes 3A4 and 2C19.[331] Genetic polymorphism of CYP2C19 leads to a bimodal distribution of proguanil and cycloguanil concentrations in humans. Therefore markedly lower cycloguanil and moderately higher proguanil plasma concentrations occur in poor metabolizers (18%–25% in Asians and Africans; 3% in Caucasians[332]). However, this does not appear to affect therapeutic response.[312] The main routes of elimination are hepatic biotransformation and renal excretion, with 40% of proguanil and 20% of its metabolites excreted in the urine. Patients with renal failure may develop hematologic toxicity due to accumulation of the drug. Drug levels are increased and elimination is impaired in patients with hepatic impairment. There are no clinical data indicating that folate supplementation diminishes drug efficacy; women of childbearing age for whom atovaquone-proguanil

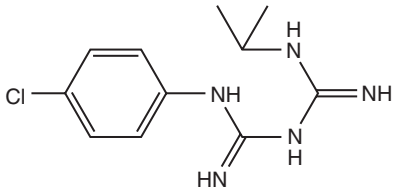

FIG. 41.10 Structure of proguanil.

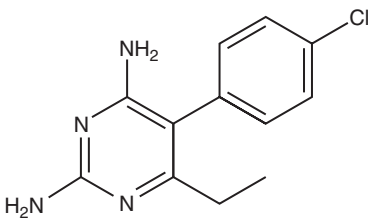

FIG. 41.11 Structure of pyrimethamine.

is prescribed should continue taking folate supplements to prevent neural-tube birth defects. Symptomatic adverse effects are dose related and include mouth ulcers, dyspepsia, and hair loss. Idiosyncratic reactions are rare. No significant clinical drug interactions have been reported.

Pyrimethamine

Pyrimethamine (Fig. 41.11) in combination with short-acting sulfonamides is effective in malaria, toxoplasmosis, and isosporiasis. Drug resistance to pyrimethamine due to mutations in DHFR is an important problem in treatment of *P. falciparum* and *P. vivax*. Pyrimethamine is always used in combination with either a sulfonamide or dapsone.

Pharmacokinetics

Pyrimethamine is well absorbed after oral administration to healthy volunteers, patients with malaria, and in AIDS patients with cerebral toxoplasmosis. Pyrimethamine is about 90% bound to plasma proteins, principally albumin, but this varies with drug concentration; the unbound fraction increases concomitantly with drug concentrations. In healthy volunteers, drug concentration remains at therapeutic levels for up to 2 weeks; drug levels are lower in patients with malaria. In infants with congenital toxoplasmosis, cerebrospinal fluid drug concentrations reach 10% to 25% of serum concentrations.[333] The high lipid solubility of pyrimethamine suggests that it probably crosses the placenta readily, but no data are available. Pyrimethamine is excreted into breast milk, but its concentration appears too low to cause dose-related adverse effects.[334] Pyrimethamine clearance is not reduced by renal disease, but caution is advised in both kidney and liver disease.

Toxicity

Adverse effects are common but rarely serious. In children toxicity is frequently seen with doses greater than 25 mg. Concentration-dependent suppression of marrow function occurs at the higher doses used for toxoplasmosis; at these doses the drug should be administered with folinic acid. Prolonged use and folate deficiency put patients at significant risk for marrow toxicity, glossitis, stomatitis, and exfoliative dermatitis. Rarely, a photosensitive rash can develop; it regresses after treatment stops.[335] When combined with dapsone, agranulocytosis can develop but usually only when the maximum dose is exceeded.[336] When combined with sulfadoxine, severe skin reactions can occur rarely but are probably attributable to the sulfa component. Severe idiosyncratic reactions to pyrimethamine are rare. The synergy of pyrimethamine and sulfadiazine against *T. gondii* may be reversed by zidovudine.[337]

Trimethoprim

For a discussion of trimethoprim, see Chapter 34.

DIHYDROPTEROATE SYNTHETASE INHIBITORS

Unlike multicellular eukaryotes, many unicellular organisms synthesize folate rather than relying on dietary sources of this cofactor. The sulfonamides and sulfones inhibit the enzymatic activity of a key enzyme in the folate synthesis pathway, dihydropteroate synthetase (DHPS). DHPS catalyzes the conversion of para-aminobenzoate to dihydropteroate.

Sulfonamides

Sulfonamides are synthetic drugs with low potency against a wide range of protozoa. No drug of the group is potent enough to have therapeutic activity when used alone for these infections; all are given in synergistic combination with a DHFR-inhibitor such as pyrimethamine or trimethoprim.

Sulfadiazine and Sulfamethoxazole

For a discussion of sulfadiazine and sulfamethoxazole, see Chapter 34.

Sulfadoxine

Sulfadoxine is available as an oral formulation with pyrimethamine.

Pharmacokinetics

When a single oral dose is given to healthy patients, it is rapidly and extensively absorbed from the gut, reaching C_{max} within 4 hours. The C_{max} in healthy patients is about 55% that of nonimmune adults with falciparum malaria. Sulfadoxine is about 94% bound to plasma proteins. It appears to concentrate within malaria-infected erythrocytes and crosses the blood-brain barrier, achieving 30% to 60% of the plasma concentration. The majority of sulfadoxine undergoes glomerular filtration unchanged; however, of the filtered drug, about 70% undergoes tubular resorption. Sulfadoxine is excreted in the bile, but the majority is reabsorbed from the gut. Weekly dosing results in steady-state concentration after seven doses.

Toxicity

The adverse effects of sulfonamides are best considered together; distinctions between drugs typically result from differences in elimination. The most common adverse effects include fever; arthralgias; marrow suppression; rash, including Stevens-Johnson syndrome; methemoglobinemia; and hemolysis in patients with G6PDd. At high doses and in patients with volume depletion, sulfonamides may crystallize in acidic urine, leading to tubular damage and severe renal insufficiency.

Sulfonamides typically cause adverse drug interactions by displacement of a drug from plasma protein binding sites, inhibition of biotransformation, and increasing the pharmacodynamic response. Sulfonamides potentiate the effects of warfarin, sulfonylureas, phenytoin, and other drugs with a narrow therapeutic range through the first two mechanisms.

Sulfones

Dapsone is the only member of this group in regular clinical use. It is active against *P. falciparum, T. gondii,* and *P. jirovecii,* but its potency is limited, and treatment of these pathogens requires combination with a DHFR inhibitor. For a detailed discussion of sulfones, see Chapter 39.

Dapsone is slowly absorbed, with an absorption half-life of 1 hour in healthy subjects; it is more slowly absorbed in children with uncomplicated malaria.[338] Dapsone is extensively metabolized by acetylation, hydroxylation, and conjugation with glucuronic acid to a variety of inactive metabolites. The parent drug and these metabolites are mainly excreted in the bile, and there is significant enterohepatic circulation. The half-life of dapsone is about 30 hours in healthy subjects and young children with malaria.

Agranulocytosis has been commonly reported among individuals taking dapsone with pyrimethamine for malaria prophylaxis. The estimated prevalence of this reaction is 1:2000 to 1:5000 prescriptions. For this reason, this combination was removed from recommendations for malaria chemoprophylaxis. The mechanism of this was unclear but presumed to be due to the dapsone component.

ANTIBIOTICS WITH ANTIMALARIAL ACTIVITY

Doxycycline and Tetracycline

Both doxycycline and tetracycline are important antimalarial agents. For details of their pharmacokinetic properties and adverse effects, see Chapter 26. Neither agent acts rapidly enough to be used alone in patients with acute falciparum malaria. The slow action of these and other antibiotics discussed later is due to the fact that the drugs target the translational machinery of the plastid organelle of the parasite,[339] and therefore a lag of one parasite life cycle is required before antiparasitic activity is apparent. However, both agents are useful as follow-on agents after initial treatment with quinine or artesunate to prevent recrudescence of parasitemia. Daily doxycycline is a preferred prophylactic regimen for malaria.

MACROLIDES

Azithromycin

Azithromycin, a macrolide, has activity against several protozoa. For a detailed discussion of its pharmacokinetics and adverse effects, see Chapter 29. It is an effective treatment of babesiosis when combined with atovaquone.[252] This combination is better tolerated than quinine

and clindamycin as a treatment for this infection. Azithromycin has been used with pyrimethamine to treat patients infected with *T. gondii*, but relapse rates were unacceptably high with maintenance doses used for suppression.[340,341] It may be useful as alternative therapy for ocular toxoplasmosis.[342] Although azithromycin has activity against *Plasmodium* spp., it is not sufficiently potent to be used for prophylaxis or treatment of *P. falciparum*.[343,344] However, it may be efficacious in preventing *P. vivax* infection.[345] Although it has been studied as a partner drug for artemisinins, results of clinical trials do not support a role for this drug in ACT. The drug is under active investigation as an option for intermittent preventive therapy of malaria in pregnancy.

Clindamycin

Clindamycin shows useful activity against several protozoa. Its pharmacokinetics are discussed in Chapter 29. It has been used with quinine for the treatment of CQ-R falciparum malaria; this regimen is the preferred one for treatment of malaria in the first trimester of pregnancy and as a second-line treatment in the second and third trimesters. It is also an option for the oral phase of treatment for severe malaria and is the treatment of choice for severe babesiosis.[346] The combination of clindamycin with artesunate rather than with quinine leads to faster parasite clearance and equivalent cure rates.[347] The major adverse effect of clindamycin is the development of pseudomembranous colitis.

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42 Drugs for Protozoal Infections Other Than Malaria

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SHORT VIEW SUMMARY

Definition
- Drugs are included here for leishmaniasis, trypanosomiasis, cryptosporidiosis, trichomoniasis, amebiasis, giardiasis, and toxoplasmosis. Many of these drugs are available only from the Centers for Disease Control and Prevention (CDC, 800-639-3670). See Table 42.1 for recommended dosages.

Leishmaniasis
- Cutaneous and mucosal leishmaniasis can be treated with the antimonials stibogluconate in the United States and meglumine in Latin America, or liposomal amphotericin B. Miltefosine is an alternative. Visceral leishmaniasis (kala-azar) can be treated with liposomal amphotericin B, miltefosine, or an antimonial.

Trypanosomiasis
- Chagas disease can be treated with either nifurtimox or benznidazole, both of which can cause severe side effects. Treatment of human African trypanosomiasis (sleeping sickness) should only be undertaken with input from physicians with relevant experience.

Amebiasis, Giardiasis, and Trichomoniasis
- These diseases are treated with tinidazole or metronidazole.

Cystoisosporiasis
- Trimethoprim plus sulfamethoxazole (TMP-SMX) or pyrimethamine plus sulfadoxine are the drugs of choice. Ciprofloxacin is a less effective alternative.

Cyclosporiasis
- This disease is treated with TMP-SMX.

Cryptosporidiosis
- In immunocompetent patients cryptosporidiosis can be treated with nitazoxanide.

Toxoplasmosis
- Toxoplasmosis acquired during pregnancy should be treated with spiramycin. Early in the infection, spiramycin may decrease the chance of fetal infection.

This chapter includes the antiprotozoal drugs other than those used to treat malaria, namely drugs to treat infections caused by *Leishmania* parasites—African and American trypanosomes, the causes of human African trypanosomiasis (HAT) and Chagas disease, respectively—and drugs used to treat intestinal protozoal infections, particularly giardiasis and amebiasis. The drugs of choice for these infections are summarized in Table 42.1. Doses and alternative drugs are presented in this chapter and in the chapters describing the parasites and the diseases they cause. Drugs for malaria are covered in Chapter 41, sulfonamide drugs are covered in Chapter 34, and metronidazole is covered in Chapter 28.

DRUGS FOR LEISHMANIASIS AND TRYPANOSOMIASIS

The selection of drugs for leishmaniasis is influenced by the geographic location where the infection was acquired and by the clinical features of the infection, particularly its anatomic location. In patients with African trypanosomiasis the region in which the infection was acquired and the presence or absence of spread to the central nervous system (CNS) are critical elements in drug selection. None of the latter factors are relevant in the choice of treatment for Chagas disease. Many of the agents used for treating these diseases can be difficult to obtain, and in the United States several are only available from the Centers for Disease Control and Prevention (CDC). In addition, many of these agents have significant toxicity profiles and complex treatment regimens, so seeking expert advice is advised.

Amphotericin B

The polyene amphotericin B preferentially binds to the membrane sterol ergosterol, which is the primary sterol of *Leishmania* membranes, and less avidly to the principal sterol of mammalian host membranes, which is cholesterol.[1] The mechanism of action of amphotericin B is thought to result from polymerization of molecules and pore formation at membranes where binding occurs, resulting in membrane leakage of various ions.[2] Treatment failure, although uncommon, may be related to changes in sterol metabolism and an ability to resist oxidative stress.[3]

Conventional amphotericin B has demonstrated efficacy for the treatment of cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL).[4] However, toxicity (primarily renal) limits its use, and liposomal amphotericin B has emerged as the preferred therapy because of its lower toxicity. The latter drug was approved by the US Food and Drug Administration (FDA) in 1997 for the treatment of VL. Accumulating case reports and small case series suggest that this agent may also have a role in the treatment of MCL and CL.[5–7] The FDA-approved regimen for liposomal amphotericin B in immunocompetent patients with VL is 21 mg/kg in seven infusions over a 21-day period (3 mg/kg/day on days 1–5, 14, and 21), although success with a single dose of 10 mg/kg has been reported in India.[8,9] The dosing regimens suggested for CL and MCL vary, with some studies reporting success with doses of 3 mg/kg/day given for 6 to 10 days.[5–7] See Chapter 40A for a full discussion of the use and side effects of amphotericin B.

Antimonials

Antimony has been used for medicinal purposes for several centuries, with efficacy for leishmaniasis reported in the early 1900s.[10] Pentavalent antimony Sb(V) is used for treatment of leishmaniasis and is available as stibogluconate (sodium antimony gluconate, Pentostam; Fig. 42.1) and as meglumine antimoniate (Glucantime, France). Generic preparations of the two formulations of Sb(V) are manufactured, one of which has been approved by the World Health Organization (sodium stibogluconate [SSG], Albert David, India). In the United States neither meglumine nor stibogluconate is commercially available, but stibogluconate can be obtained from the CDC Drug Service for civilian physicians and from the US Army Medical Material Development Activity for US military physicians.

The detailed chemical structure of Sb(V) is not fully known, although analysis by electrospray ionization mass spectrometry (ESI-MS) and

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

TABLE 42.1 Drugs for Protozoa Other Than Malaria

INFECTING ORGANISM	TREATMENT OF CHOICE	ALTERNATIVE TREATMENTS
Leishmaniasis: *Leishmania donovani, L. major, L. infantum, L. chagasi, L. mexicana, L. tropica, L. (Viannia)* spp.		
Cutaneous leishmaniasis	Sodium stibogluconate or meglumine antimoniate, 20 mg/kg/day IV or IM × 20 days, or liposomal amphotericin B, 3 mg/kg/day IV × 7–10 doses[a]	Miltefosine, 50 mg PO 2 (30–44 kg) to 3 (≥45 kg) times daily × 28 days
Mucosal leishmaniasis	Liposomal amphotericin B at 20–35 mg/kg (total dose) given as 3 mg/kg IV once daily	Sodium stibogluconate or meglumine antimoniate 20 mg/kg/day IV or IM × 28 days; miltefosine 50 mg PO 2 (30–44 kg) to 3 (≥45 kg) times daily × 28 days; amphotericin B deoxycholate, 1 mg/kg IV qod or daily to a total dose of 20–40 mg/kg
Visceral leishmaniasis	Liposomal amphotericin B, 3 mg/kg/day IV × days 1–5, then at day 14 and 21	Sodium stibogluconate or meglumine antimoniate, 20 mg/kg/day IV or IM × 28 days; miltefosine 50 mg PO 2 (30–44 kg) to 3 (≥45 kg) times daily × 28 days; amphotericin B deoxycholate, 0.5–1 mg/kg/day or qod to a total dose of 15–20 mg/kg[a]
Amebiasis *(Entamoeba histolytica)*	Tinidazole, 2 g PO once daily × 3–5 days	Metronidazole, 500–750 mg PO or IV tid × 7–10 days
Giardiasis (*Giardia lamblia,* also called *G. duodenalis* or *G. intestinalis*)	Tinidazole, 2 g PO once	Metronidazole, 250 mg PO tid × 5–7 days
Trichomoniasis *(Trichomonas vaginalis)*	Tinidazole, 2 g PO once	Metronidazole, 2 g PO once
Cryptosporidiosis (*Cryptosporidium* spp.)	Nitazoxanide, 500 PO bid × 3 days[b]	
American trypanosomiasis (Chagas disease; *Trypanosoma cruzi*)	Benznidazole, 5–7 mg/kg/day PO in two doses × 60 days	Nifurtimox, 8–10 mg/kg/day PO in three to four doses × 90 days
African trypanosomiasis (*Trypanosoma brucei gambiense* [W. African]; *T. b. rhodesiense* [E. African])	Pentamidine/suramin/eflornithine/nifurtimox/melarsoprol	Drug treatment depends on stage of disease (hemolymphatic stage or CNS disease) and on geographic origin (W. or E. African); consult expert guidelines

[a]Not specifically approved by the US Food and Drug Administration for use in this infection.
[b]Relatively ineffective in immunosuppressed patients.
bid, Twice daily; *CNS,* central nervous system; *IM,* intramuscularly; *IV,* intravenously; *PO,* orally; *qod,* every other day; *tid,* three times daily.

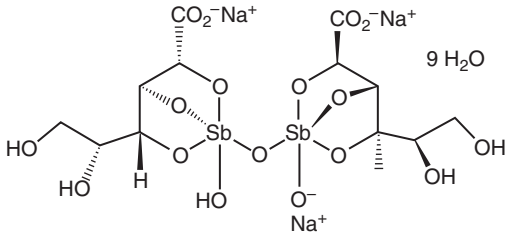

FIG. 42.1 Sodium stibogluconate.

osmolarity measurements suggest that both meglumine antimoniate and stibogluconate contain 1:1, 1:2, 2:2, and 2:3 Sb(V)-ligand complexes. ESI-MS analysis of meglumine antimoniate showed negatively charged 1:1 (mass-to-charge ratio [m/z] 364) and 2:2 (m/z 765) Sb(V)–meglumine antimoniate complexes, supporting the predominance of zwitterionic species in solution. ESI-MS measurements of stibogluconate also demonstrate a mixture of oligomeric structures.[11]

Despite almost a century of use, the mechanism of action of pentavalent antimonials is not well understood. One model suggests that Sb(V) acts as a prodrug and is reduced to the more active/toxic trivalent form of antimony, Sb(III). Sb(III) affects glucose metabolism, fatty-acid beta-oxidation, and adenosine triphosphate (ATP) formation.[12,13] Other specific targets, such as topoisomerase or trypanothione reductase, have been identified.[14,15] Sb(III) also competes with Zn(II) for its binding to the motif CCHC, which is a constituent of zinc-finger domains, suggesting that zinc-finger proteins may be targets of antimony.[16] A second model holds that Sb(V) has intrinsic antileishmanial activity. Sb(V) has been reported to complex with adenine nucleosides, which might act as an inhibitor of *Leishmania* purine transporters or interfere with the purine salvage pathway.[17] A third model suggests that activation of the host immune system is the major mechanism of action for pentavalent antimonials. Sodium antimony gluconate has been shown to induce nitric oxide (NO) synthesis and reactive oxygen species (ROS), with both ROS and NO involved in parasite killing in the early stage of infection and NO in the late stage.[18,19] Sodium antimony gluconate has also been shown to upregulate interferon-γ (IFN-γ) receptors in both *Leishmania donovani*–infected and –uninfected Th1 cells, and in monocytes derived from kala-azar patients treated with sodium antimony gluconate, thus potentially influencing host response by altering IFN-γ responsiveness.[20]

Clinical nonresponse or relapse after treatment with Sb(V) has become problematic in certain geographic areas, most notably the Bihar State, India, and the Terai regions of Nepal.[21] The mechanism of resistance is not defined, and no biomarker for drug resistance has been identified.[22,23] An intriguing association between arsenic contamination of drinking water and antimonial resistance has been proposed in a mouse model and an underpowered and retrospective analysis.[24,25]

SSG and meglumine antimoniate have similar efficacy and side-effect profiles, and because English-speaking countries primarily use SSG, further discussion will focus on that drug. SSG is provided as a 100-mg antimony/mL solution that contains a preservative, *m*-chlorocresol. The solution should be drawn up through a filter before administration due to the presence of particulates formed by an interaction between the product preservative and the antioxidant in the rubber stopper. SSG is administered parenterally, with the intravenous (IV) route being preferable for patient comfort due to the volume of injection. The recommended dose of SSG is 20 mg/kg per day with no maximum daily dose for a duration of 10 to 30 days, depending on patient and parasite factors.[4] Although painful, SSG can also be directly injected into CL lesions, typically at a dose of 1 to 5 mL of SSG solution injected per session every 3 to 7 days for one to five sessions.[26]

SSG is well absorbed after intramuscular (IM) administration, with a first-order absorption rate constant, rapid distribution, and a slower elimination half-life of about 10 hours.[27,28] The drug is renally excreted, with substantial variability among patients. Administration to patients with renal insufficiency is not recommended, and there are no specific guidelines for dose adjustment with renal or hepatic impairment. SSG should be avoided during pregnancy, with one study reporting abortions during the 16th to 22nd weeks of pregnancy in 11 of 16 treated patients.[29]

Side effects of systemic administration of stibogluconate are common and include asymptomatic elevations in amylase and lipase in most patients. Approximately 50% of patients develop elevations of liver aminotransferase levels (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) and/or electrocardiographic changes (most commonly prolongation of the QT interval and/or T wave changes), both of which resolve with cessation of therapy. Arthralgias and myalgias develop in approximately 50% of patients and can persist for weeks after completion of treatment. Rarer, more serious adverse effects

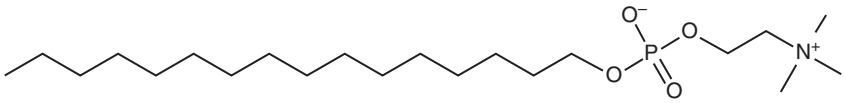

FIG. 42.2 Miltefosine.

include pancreatitis, herpes zoster, and cardiotoxicity.[30–33] To monitor for toxicity, baseline and at least weekly complete blood counts, serum biochemistry, amylase, and electrocardiograms (ECG) are suggested. ECG changes and elevations of amylase, lipase, and hepatic aminotransferase levels are typically transient and may not require cessation of therapy. However, consultation with a provider experienced with the use of SSG is suggested.

Drug interactions with stibogluconate have not been reported, but it is advised that the product be given with caution in patients with cardiovascular disease, a history of ventricular arrhythmias, or other risk factors known to predispose toward QT prolongation, including use of class III antiarrhythmics, such as sotalol and amiodarone. A risk of fatal cardiac arrhythmias has been observed when amphotericin B is administered after stibogluconate during re-treatment of VL.[34]

Triazoles

A variety of triazoles typically used for treatment of fungal infections have been reported to be useful for treating leishmaniasis, most commonly for CL. Fluconazole, itraconazole, and ketoconazole are the drugs most studied in this regard, with varying rates of efficacy.[35] See Chapter 40B for a full discussion of the uses and side effects associated with triazoles.

Miltefosine

Miltefosine (Fig. 42.2) is an oral agent originally developed for treatment of breast cancer and other solid tumors, but its development as an antineoplastic agent was stopped because of dose-limiting gastrointestinal (GI) toxicity. In immunocompetent patients a 28-day regimen has a reported a cure rate of 60% to 80% in MCL and greater than 80% in VL.[36–39] Cure rates for CL have ranged widely by geographic area, perhaps reflecting a species-specific variation in response to therapy.[40] In 2014 the drug was approved by the FDA for the treatment of VL due to *Leishmania donovani*; CL due to *L. braziliensis, L. panamensis*, and *L. guyanensis;* and for MCL due to *L. braziliensis*.

Miltefosine is a member of the alkylphosphocholine class of drugs, which are phosphocholine esters of aliphatic long-chain alcohols. The mechanism of action of miltefosine is incompletely understood but may be related to a disruption of lipid metabolism and other metabolic pathways, with insertion directly into the *Leishmania* cell membrane and/or induction of apoptosis.[41,42] Miltefosine inserts directly within the *Leishmania* plasma membrane, which may be especially relevant when entry into *Leishmania* is inhibited.[43] It inhibits the activity of the AKT protein (also known as protein kinase B [PKB]), which is an important protein within the phosphatidylinositol-3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) intracellular signaling pathway, a pathway that is essential for cell survival.[44,45] It also inhibits phophatidylethanol-amine-PC-*N*-methyltransferase, which decreases the production of phosphatidylcholine, the primary phospholipid component of *Leishmania*.[46]

Resistance of *Leishmania* parasites to miltefosine appears to be related to decreased drug accumulation. Resistant lines achieve low drug levels by two independent mechanisms: either by increasing drug efflux, mediated by the overexpression of the ATP-binding cassette (ABC) transporter P-glycoprotein, or by decreasing drug uptake, which is achieved by the inactivation of any one of the two proteins known to be responsible for the miltefosine uptake—the miltefosine transporter LdMT and its beta subunit LdRos3.[47] Resistance may also be related to alterations in fatty-acid and sterol metabolism, which reduce the ability of miltefosine to insert into the plasma membrane.[48]

Miltefosine is administered for 28 days at a dose of 50 mg orally twice daily for persons weighing 30 to 44 kg or 50 mg orally three times daily for those weighing 45 kg or more. Its oral bioavailability has not been determined, but the absolute bioavailability in rats and dogs is 82% and 95%, respectively.[49] The drug has an extremely long triphasic elimination half-life of 30.9 days, with drug detected at least 5 months posttreatment. No data on drug-drug interactions have been reported.[50] Nausea, vomiting, and diarrhea have been consistently reported, and it is recommended that the drug be taken with meals to decrease GI side effects. Elevated creatinine levels are frequently reported, although severe nephrotoxicity is rare. Mild elevations of aminotransferase levels (both ALT and AST) often occur during the first week of treatment.[37,51] In a cohort of soldiers treated with miltefosine, 70% were unable to complete daily military exercises, and 62% reported temporary diminished ejaculate volume.[52] Miltefosine is teratogenic, and thus administration is contraindicated during pregnancy. Because of the drug's long half-life, it is recommended that females of childbearing age use contraception during and for at least 4 months after the standard 28-day treatment regimen.[53] Miltefosine is not recommended for use in women who are breastfeeding.

Paromomycin

Paromomycin (Aminosidine), an aminoglycoside antibiotic, is the only aminoglycoside with activity against *Leishmania*; it also has activity against some other protozoa and cestodes.[54] Although its utility for the treatment of VL has been reported, there are reports of geographic variation in response rates.[55,56] In some studies the combination of paromomycin and stibogluconate has been shown to be effective.[57] Injectable paromomycin is ineffective for the treatment of CL, and mixed results have been reported with topical paromomycin, formulated as an ointment with various additional components for the treatment of CL.[58–60] A randomized study conducted in Tunisia reported that a topical cream containing 15% paromomycin cured 81% of index lesions caused by *Leishmania major*, whereas a study in Panama with CL lesions due to *Leishmania panamensis* reported a 60% cure rate with 15% paromomycin, which was improved to 87% with the addition of 0.5% gentamicin.[61,62] IV paromomycin is not available for use in the United States, but the oral capsule is FDA approved for treatment of intestinal amebiasis. The topical formulation is not approved or commercially available, although compounding recommendations are available.[4]

The mechanism of leishmanicidal activity of paromomycin is poorly understood, with activity proposed to be mediated through inhibition of parasite metabolism and mitochondrial respiration.[63,64] Resistance to paromomycin is readily induced in vitro with contributions from altered membrane fluidity, decreased drug accumulation, increased expression of ABC transporters, and greater tolerance of parasites to host defense mechanisms.[65]

Paromomycin is given at a dose of 11 to 20 mg/kg IM daily for 10 to 21 days for the treatment of VL. As expected, the important aminoglycoside class adverse drug reactions are renal, cochlear, and vestibular toxicity. The rates of adverse reactions reported in clinical trials have been low, although audiometric studies were not performed in most trials. Insufficient data are available regarding the use of paromomycin in pregnant women, although the drug is thought to be safe during lactation, provided the mother and infant have normal renal function.

Oral paromomycin has useful activity for clearance of cyst carriage of *Entamoeba histolytica*, with a small clinical trial indicating that it is more effective than diloxanide for this indication.[66] The dose for this indication and for treatment of *Dientamoeba fragilis* infections is 25 to 35 mg/kg per day in three divided doses for 7 days. The drug shows some activity in human cryptosporidiosis,[54] with most studies reporting on activity in the setting of human immunodeficiency virus (HIV) infection.[67,68] However, available data indicate that the drug is not curative.

Pentamidine

Pentamidine is an aromatic diamidine that is used to treat the hemolymphatic form (stage 1 disease) of both East and West African

trypanosomiasis. It also is a second-line drug for prophylaxis and treatment of *Pneumocystis jirovecii* pneumonia[69,70] and is also used for treatment of leishmaniasis.[71,72] In addition, pentamidine has activity against *Acanthamoeba* spp. and *Balamuthia mandrillaris*.[73,74]

Although the precise mechanism of action of pentamidine has not been determined, it is known that pentamidine affects a wide range of microbial processes, including interaction with trypanosomal kinetoplast DNA; interference with polyamine synthesis by decreasing the activity of ornithine decarboxylase; and inhibition of RNA polymerase, ribosomal function, and the synthesis of nucleic acids and proteins.

IM pentamidine is well absorbed, highly tissue bound, and is excreted slowly over several weeks. Renal clearance is minimal. Its elimination half-life is 12 days,[75] but it can be detected in plasma up to 8 months after a single dose.[76] A steady-state plasma concentration is not attained in persons given daily injections, with extensive accumulation of the drug in visceral tissues, primarily the liver, kidney, adrenal glands, and spleen. Pentamidine does not penetrate the blood-brain barrier well, reaching only 0.5% to 0.8% of plasma concentrations after a course of treatment.[77] Thus it should never be used to treat patients with the CNS form of trypanosomiasis (stage 2 disease).

Resistance to pentamidine has been found in human African trypanosomes that are also resistant to melarsoprol. Recent evidence suggests that the unconventional aquaglyceroporin AQP2 renders cells sensitive to both melarsoprol and pentamidine, and that loss of AQP2 function could explain cases of innate and acquired pentamidine-melarsoprol cross-resistance.[78]

Dosing of pentamidine is not generally modified in patients with renal or liver dysfunction. There are no data regarding the pharmacokinetics of pentamidine in obese patients. In animal models pentamidine is embryocidal but neither teratogenic nor mutagenic.[79] Treatment with pentamidine should be delayed until after the first trimester of pregnancy. The relapse rate in children with trypanosomiasis treated with pentamidine is higher than in adults,[80] and a delayed response in children with leishmaniasis treated with pentamidine has been noted.[81] These observations suggest that the pharmacokinetics of pentamidine in children differ substantially from those in adults, but there are no experimental data to support this concept.

Sterile abscesses can result from IM injections of pentamidine. Hypotension is seen in roughly 15% of patients given the drug, especially if given IV over less than 1 hour. In up to a third of patients pentamidine causes some degree of renal impairment; this is usually mild to moderate and reversible. Electrolyte abnormalities, including hyponatremia, hyperkalemia, hypomagnesemia, and hypocalcemia, are common. Hypoglycemia, resulting from a cytotoxic effect on beta-islet cells and insulin release, is an unpredictable and occasionally lethal complication seen in 15% to 25% of patients, particularly with prolonged therapy, azotemia, or high pentamidine levels.[82,83] Pentamidine can cause severe pancreatitis, resulting in diabetes over the long term. Neutropenia has been noted; anemia and thrombocytopenia are less frequent. Nausea or vomiting is seen in up to one-half of patients, and abnormalities of liver function tests are also common. A variety of electrocardiographic abnormalities, including *torsades de pointes*, have been reported. Data regarding interactions between pentamidine and other drugs are lacking.

Pentamidine is a second-line therapy for leishmaniasis, predominantly used for CL. Efficacy varies widely, with cure rates ranging from 35% with *L. (Viannia) braziliensis* in Peru, to 90% with *L. (Viannia) guyanensis* in Suriname. Use as secondary prophylaxis for VL in a small number of immunocompromised patients has been reported.[84] When used for treatment of CL, the dose is usually 3 to 4 mg/kg every other day for 3 or 4 doses or 2 mg/kg every other day for 7 days. The dose recommended for treatment of mucosal leishmaniasis is 2 to 4 mg/kg every other day or three times per week for 15 or more doses, and for VL it is 4 mg/kg every other day or three times per week for 15 to 30 doses. See Chapter 269 for a further discussion of the use and toxicities of pentamidine for treatment of *P. jirovecii* infection.

Benznidazole

Benznidazole (Fig. 42.3) is a nitroimidazole derivative that is the drug of choice for treating infections with *Trypanosoma cruzi*. The mechanism of action of benznidazole is not known. Benznidazole is readily absorbed, highly lipophilic, and extensively metabolized, with only 5% of the dose excreted unchanged in the urine.[85–87]

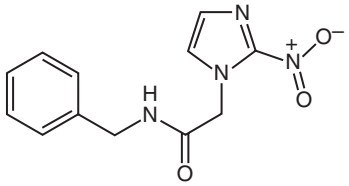

FIG. 42.3 Benznidazole.

Benznidazole is only available in oral form. The dose and duration of treatment are the same regardless of the stage of the infection. No assays are available commercially to determine blood levels, and no data are available to guide dose adjustments in patients with renal or hepatic insufficiency, pregnancy, or in lactating women. It is recommended that benznidazole not be given to such patients. Resistance to benznidazole has been reported[88]; efforts to understand the molecular mechanisms underlying resistance are underway.[89,90] The clinical significance of resistance is unknown, in large measure because compliance is often an issue, and also because assessment of parasitologic cure after treatment is difficult. No assays for testing for resistance to benznidazole are available, and no published data regarding drug-drug interactions are available.

Adverse effects occur in a substantial proportion of patients treated with benznidazole. Peripheral neuropathy and rash are the most commonly reported adverse effects. Granulocytopenia can also occur. On occasion the latter can be severe, and because of this, blood counts should be monitored weekly during the first few weeks of treatment. Adverse effects usually disappear with dose reduction or stopping the drug, although granulocytopenia may take several weeks to resolve. Limited data suggest that there is no relationship between benznidazole levels and the risk of adverse drug reactions.[91] In one sizable study only female gender, increasing age, and duration of treatment were found to be significant risk factors for side effects.[78] It is noteworthy that patients with Chagas disease who undergo cardiac transplantation and who are given benznidazole for management of reactivation of *T. cruzi* have an increased incidence of malignant tumors.[92] However, the incidence of tumors in the general population of patients treated with benznidazole has not been studied.

Benznidazole is widely available in the endemic countries, which include Mexico and all the countries of Central and South America. In the United States benznidazole is available from the CDC Drug Service. Of importance, however, in 2017 the FDA granted accelerated approval to Laboratorio ELEA, a company in Argentina, to market benznidazole for use in children ages 2 to 12 years old with Chagas disease. When benznidazole receives regulatory approval in the United States, obtaining it will become easier.

Fexinidazole

Fexinidazole, a nitroimidazole was discovered in the late 1970s and shown to have useful activity in a murine model of HAT.[93] After a hiatus of 30 years, its development for this indication was resumed by the Drugs for Neglected Diseases initiative (DNDi).[94] Its in vitro inhibitory concentration of 50% against laboratory strains and clinical isolates of *Trypanosoma brucei* ranged between 0.16 and 0.93 μg/mL.[94] Its bioavailability when administered orally ranged from 41% in mice to 10% in dogs. It is well tolerated in single doses from 100 to 3600 mg. It is quickly absorbed and rapidly metabolized in vivo by a wide range of cytochrome P450 (CYP) enzymes, as well as via the human flavin monooxygenase-3 enzyme to at least two biologically active metabolites (a sulfoxide and a sulfone derivative; time of maximum concentration of 2–5 hours and 18–24 hours, respectively). These active metabolites likely account for a significant portion of its antiparasitic effect. Taking the drug with food significantly increases the absorption and plasma concentrations of fexinidazole and its two metabolites by approximately 200%.[95] Its half-life is approximately 11 hours,[95] with most of the drug being excreted by the biliary route. Although fexinidazole shares a class

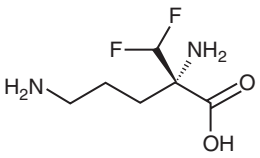

FIG. 42.4 **Eflornithine.**

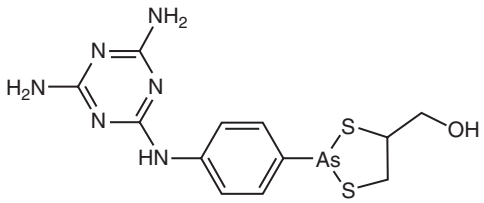

FIG. 42.5 **Melarsoprol.**

effect of nitroimidazoles in being mutagenic in the Ames test, a mutagenic effect has been excluded in mammalian cells and in mice.[94] Distinguishing any side effects of the drug from clinical effects of HAT treatment is difficult. In a phase I study, where the proposed therapeutic dose was administered, the drug was well tolerated, with transient headache and vomiting being reported side effects.[95] In a recently reported phase II/III study,[96] the safety and efficacy of an oral regimen of fexinidazole versus nifurtimox-eflornithine combination therapy (NECT) was evaluated in patients with late-stage HAT caused by *Trypanosoma brucei (T. b.) gambiense*. Patients were randomized 2:1 to once-daily oral fexinidazole in a regimen of 1800 mg/day (days 1–4), then 1200 mg (days 5–10), or to a standard NECT regimen. At 18 months, 239 of 264 (91%) patients given fexinidazole were deemed cured, compared with 124 of 130 (98%) patients given NECT, a difference of −6.4% (97.06% confidence interval [CI], −11.2 to −1.6; $P = .0029$). Treatment-related adverse events, discontinuations, disruptions, or deaths occurred at equivalent rates in the two groups. Based on this study, in November 2018 the European Medicines Agency adopted a positive scientific opinion of this drug as the first all oral agent for treatment of all stages of *T. b. gambiense* HAT.

Eflornithine

Eflornithine (DFMO, Ornidyl; Fig. 42.4) is a fluorinated analogue of ornithine used for treatment of HAT. The drug is effective against all stages of West African HAT, caused by *T. b. gambiense*. However, it lacks activity against East African HAT caused by *T. b. rhodesiense*.

Eflornithine acts as an irreversible suicide inhibitor of ornithine decarboxylase, the first enzyme in the biosynthesis of the polyamines putrescine and spermidine. Interference with polyamine synthesis impairs the ability of the parasite to maintain its redox state and to block reactive oxygen intermediates.[89,97] Polyamines also are essential for parasite cell division. Although the drug has a similar effect on humans, there is a selective effect on trypanosomes because they have a relatively low turnover of ornithine decarboxylase and, as a consequence, a more rapid decrease of polyamines with eflornithine treatment.

Eflornithine can be given IV or orally, but its bioavailability after oral administration is only 54%. Eflornithine readily crosses the blood-brain barrier, and cerebrospinal fluid (CSF) levels are highest in persons with the most severe CNS involvement of the infection.[98] The elimination half-life of eflornithine is 3.3 hours, with greater than 80% excreted in the urine unchanged.[99] Given its predominant renal excretion, dose reduction should occur in patients with renal sufficiency. However, specific data to guide dose adjustments are not available. Dosing need not be adjusted in patients with hepatic dysfunction.

Treatment failures with eflornithine have been reported, with the loss of the gene that encodes the amino-acid transporter TbAAT6 being proposed as the molecular basis of this resistance, at least in *T. b. gambiense*.[100,101] The relative lack of activity of eflornithine against *T. b. rhodesiense* may be attributable to alternate mechanisms.[98,102] At the end of a 14-day course of IV eflornithine, patients who failed therapy had eflornithine CSF trough concentrations of greater than 50 nmol/mL, suggesting parasite resistance to the drug rather than inadequate CSF drug levels as the cause of the failures. The pharmacokinetics of eflornithine in children differ substantially from adults, with mean serum and CSF levels about 60% of levels found in adults; this may explain the higher rate of treatment failure in children.[103]

The most common toxicity of eflornithine is hematologic, with anemia (40%), leukopenia (20% to 30%), and thrombocytopenia (50%) being common. However, these effects are usually mild and without clinical significance. Seizures, associated with higher CSF concentrations, occur more commonly in cases of relapse (12%) than in new cases (4%). An osmotic diarrhea is seen more frequently when eflornithine is given orally. Hearing loss and alopecia have been reported in a few patients. Some patients die during treatment, but this appears to be related to the underlying disease rather than as a result of drug toxicity. In animal models eflornithine is embryotoxic and induces abortions but is not teratogenic[104]; anecdotal data suggest that treatment of pregnant women with eflornithine is associated with abortion.[103]

No data are available concerning interactions between eflornithine and other drugs. However, animal models suggest that eflornithine may be synergistic with other trypanocidal drugs, particularly melarsoprol, because eflornithine reduces the production of trypanothione, a spermidine-glutathione conjugate that is one of the targets of melarsoprol.[105,106]

The use of eflornithine in combination with nifurtimox (NECT) for stage 2 West African HAT is discussed in the subsequent nifurtimox section.

Melarsoprol

Melarsoprol (Fig. 42.5) is an aromatic arsenical that has been used for more than 60 years for treatment of HAT with CNS involvement (stage 2 disease) and for the treatment of hemolymphatic (stage 1 disease) HAT that cannot be effectively treated with suramin or pentamidine. The mechanisms of action of melarsoprol and of drug resistance are complex and have been the subjects of considerable study.[88,89,102] The metabolism of trypanothione appears to be a central effect of the drug on the parasite. Melarsoprol interacts with thiol groups of several key proteins, depriving the parasite of its main sulfhydryl antioxidant and inhibiting trypanothione reductase, also depriving the parasite of the essential enzyme system that is responsible for keeping trypanothione reduced.[97] Melarsoprol enters the parasite via an adenosine transporter, and resistant strains lack this transport system.[107]

Melarsoprol can only be given IV. Penetration into the brain and CSF is low but sufficient for activity because of the drug's high trypanocidal activity. Melarsoprol is rapidly transformed into active metabolites in plasma, with the parent drug having a half-life of less than 30 minutes.[108] Melarsen oxide is the predominant active metabolite, and it is excreted rapidly, with about 80% of the arsenic found in feces.[109]

There are no pharmacokinetic data on melarsoprol distribution and metabolism in patients with renal or liver disease, obesity, or ascites regarding dose adjustments in these settings. Melarsoprol has no known effect on pregnancy.

Melarsoprol is an extremely toxic drug. The most serious adverse reaction of melarsoprol is reactive encephalopathy, which typically develops within 4 days of the start of therapy and affects about 6% of treated patients.[110,111] The mortality of melarsoprol-induced reactive encephalopathy may be as high as 50%. Glucocorticoids administered with melarsoprol reduce the incidence of the encephalopathy. This toxic reaction is more common in East African (5%–18%) than in West African HAT (4%–8%) patients with CSF trypanosomes than in those without and in patients with high CSF white cell counts.[112] Polyneuropathy, probably caused by a direct toxic effect of the arsenic, is seen in up to 10% of patients. It typically presents as a glove-and-stocking paresthesia that progresses proximally. Motor deficits appear and, if neglected, can progress to quadriplegia. Amelioration with thiamine suggests an interaction between toxic and nutritional factors. Other adverse effects include skin rash, tremor, abdominal pain, and fever. Melarsoprol is formulated in propylene glycol. Because this excipient is intensely irritating, care must be taken to avoid extravasation. No information is available about interactions between melarsoprol and other drugs. In the United States melarsoprol is available from the CDC Drug Service.

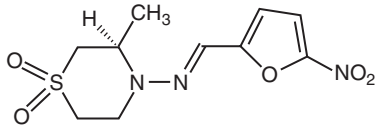

FIG. 42.6 Nifurtimox.

Nifurtimox

Nifurtimox (Lampit; Fig. 42.6) is a nitrofuran derivative that has been used for decades to treat infections with *T. cruzi.* More recently it has been found to be useful for treatment of HAT when used in combination with eflornithine, so-called NECT. The mechanism of action of nifurtimox is only partially understood and may be related to the generation of oxidative stress in trypanosomes.[113] An additional mechanism may involve reductive activation by a type I nitroreductase.[114] Nifurtimox is well absorbed orally, is metabolized in the liver, and less than 0.5% is excreted unchanged in the urine.[115]

Nifurtimox is only available in oral form. The dose is the same for all clinical stages of *T. cruzi* infection, but the recommended dosage varies as a function of age. No assays are available to determine blood levels, and no data are available to guide dose adjustments in patients with renal or hepatic insufficiency, pregnancy, or in lactating women. It is recommended that nifurtimox not be given to such patients. Resistance to nifurtimox in *T. cruzi* occurs,[116] and efforts to understand the molecular mechanisms underlying the resistance are underway.[117] The clinical significance of resistance is not known, in large measure because compliance is often uncertain, and also because confirmation of parasitologic cure after treatment is difficult. No assays are available commercially for testing *T. cruzi* for resistance to nifurtimox, and no data regarding drug-drug interactions have been published.

A sizable percentage of persons given nifurtimox develop adverse reactions.[118–120] Common GI complaints include nausea, vomiting, abdominal pain, anorexia, and weight loss. Neurologic adverse effects include restlessness, insomnia, twitching, paresthesia, seizures, and disorientation. Rashes may also occur. Factors that predispose patients to side effects have not been defined, and pretreatment protocols to reduce their occurrence have not been developed. Side effects usually resolve when the dosage is reduced or treatment is stopped. No information is available regarding the interaction of nifurtimox with other drugs. The development of malignant tumors in rabbits and mice treated with nifurtimox and related drugs is of some concern,[121] but the incidence of tumor in treated patients has not been studied.

As noted, nifurtimox is now used in combination with eflornithine for treating patients with stage 2 West African HAT. The safety and efficacy of the combined regimen, NECT, have been demonstrated.[122–124] In addition, NECT is markedly less complicated to administer and less costly compared with eflornithine monotherapy. In the United States nifurtimox is available from the CDC Drug Service.

Suramin

Suramin is a complex molecule containing eight benzene rings, with a molecular mass of 1297. It has been used to treat HAT for more than 90 years. Suramin is only used to treat the hemolymphatic form (stage 1 disease) of both East and West African trypanosomiasis. It does not penetrate the CNS to any useful degree and should never be used to treat patients with the CNS form of trypanosomiasis (stage 2 disease).

Suramin can only be given IV. It binds extensively to plasma proteins (>99%), accumulates during treatment, and is not metabolized to any great extent. It is slowly excreted in the urine, resulting in its persistence in plasma for several weeks after treatment ends.[125]

Trypanosomes take up only small amounts of suramin and do not actively concentrate the drug.[126] Resistance to suramin is uncommon, and the mechanism of resistance has not been defined.[116,127]

Treatment with suramin should be initiated at low doses that then are gradually increased. No pharmacokinetic data are available regarding the use of suramin in patients with renal or insufficiency, and no assays for monitoring blood levels are available. Given that it is excreted in the urine and is nephrotoxic, it seems prudent to reduce the dose in patients with renal insufficiency. There are no recommendations for dose reduction in liver failure. Suramin has been shown to induce abortions and to be teratogenic in animal models,[128] but no adverse effects in pregnant women have been reported, despite the long history of its use.[129]

Serious treatment-related hypotension is uncommon, occurring in only one of several thousand patients. Mild proteinuria is the most frequent adverse effect, although renal failure is rare. Thus monitoring patients for proteinuria throughout treatment is recommended. Fever occurs in approximately 10% of patients, often a few hours after infusion and may be due to reaction to breakdown products of dead trypanosomes rather than as a direct effect of the drug. Pruritus, urticaria, and stomatitis have been reported. Nausea and vomiting are uncommon if the drug is administered slowly. Optic atrophy and sensorimotor polyneuropathy have also been reported. Although suramin has activity against filarial parasites, aggravation of ocular lesions and allergic reactions has been reported in patients with onchocerciasis,[128] and the availability of ivermectin and tetracycline drugs for treatment of the *Wolbachia* endosymbiont mean that it should not be used for treatment of filariasis.

Suramin has been used in the past as therapy for cancer, albeit with doses substantially higher than those used today to treat patients with HAT. Additional adverse effects reported in such patients included corneal deposits, adrenal insufficiency, coagulopathy, neutropenia, thrombocytopenia, and fatal toxic epidermal necrolysis, but none of these effects have been observed in patients given suramin for HAT, presumably because the dosage is so much lower.

There are few data regarding interactions of suramin with other drugs. Suramin is known, however, to displace other drugs from plasma proteins, such as chlorpromazine, sulfonamides, and anticoagulants.[128] In the United States suramin is available from the CDC Drug Service.

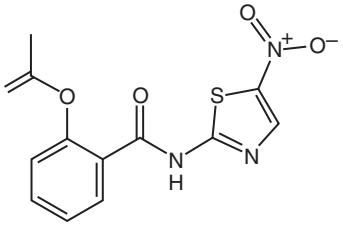

FIG. 42.7 Nitazoxanide.

Nitazoxanide

Nitazoxanide (Fig. 42.7) is a nitrothiazolyl-salicylamide derivative. The benzamide moiety resembles that of the anthelmintic drug niclosamide, whereas the nitrothiazolyl moiety resembles that of the nitroimidazole drugs tinidazole and metronidazole. It was first developed as a veterinary anthelmintic, with its activity against human tapeworms first reported in 1984.[130] Since then the drug has been shown to have activity against a wide range of pathogens, including hepatitis B and C,[131] *Clostridioides difficile* (formerly *Clostridium difficile*)[132] *Helicobacter pylori,*[133] and *Mycobacterium tuberculosis.*[134] Although clinical trials have indicated that it has activity against a broad range of intestinal protozoa and helminths,[135–138] it is approved by FDA only for treatment of giardiasis and cryptosporidiosis.

Trials in patients with cryptosporidiosis indicate that it has useful activity in HIV-negative individuals[139–141] when administered twice daily for 3 days in the following doses: 500 mg (those older than 12 years), 200 mg (children age 4–11 years), and 100 mg (children age 1–3 years). Symptom resolution is expected in 4 to 5 days, with a sustained response to treatment reported after 14 days of treatment. For cryptosporidiosis in the setting of HIV infection, the drug shows significantly inferior efficacy,[142] with randomized[143–145] and uncontrolled[141] studies indicating that prolonged therapy results in a modest-at-best response.

For giardiasis, a 3-day course of therapy, administered in the same dose as for cryptosporidiosis, reduces the duration of diarrhea and shedding of parasites. In a single-case report it cured refractory giardiasis in a patient with HIV-induced immunosuppression who had metronidazole- and albendazole-resistant giardiasis.[146] However, in an open-label, randomized, controlled trial in children, single-dose tinidazole, 50 mg/

kg, was shown to be more effective than a 3-day course of nitazoxanide administered in a dose of 7.5 mg/kg twice daily for 3 days.[147]

Nitazoxanide is active against a variety of protozoan and helminth parasites, including *E. histolytica*,[148] *Cystoisospora* (*Isospora) belli*,[149] *Blastocystis hominis*,[150] microsporidiosis,[151] and intestinal tapeworm[152] and nematode infections.[135–137] In addition, it has been shown to have activity in fascioliasis.[153] However, for all these parasites it has not shown significant superiority to other agents.

Published data indicate that nitazoxanide acts by inhibiting the activity of pyruvate-ferredoxin oxidoreductase, an enzyme essential for electron transfer and energy metabolism in anaerobic bacteria and parasites.[154] Although in vitro induction of drug resistance in *Giardia intestinalis*[155] has been reported, the mechanism of drug resistance has not been clearly defined.[156]

Nitazoxanide is available in both liquid and tablet forms; administration with food is recommended to improve bioavailability. Once absorbed the drug is rapidly deacetylated to its main active metabolite, tizoxanide, which is glucuronidated and excreted in the urine and feces.[157] When administered in the recommended doses, the peak serum concentration (C_{max}) of tizoxanide is between 10 and 17.5 µg/mL, with a half-life of 1.3 hours.[158] Although no significant drug interactions have been reported, the high level of protein binding of tizoxanide (>99%)[157] indicates a potential interaction with other drugs that also are highly protein bound. Nitazoxanide is well tolerated when administered in the recommended dose, with the rate of reported side effects no higher than placebo.[158] There are no data on dose adjustment in renal or hepatic insufficiency. Likewise, there are no data on safety in pregnancy, although reproductive toxicity studies in laboratory animals done by the manufacturer did not reveal any adverse findings.[159]

Diloxanide

Diloxanide furoate is a luminal amebicide whose primary indication is to clear *E. histolytica* cysts. Published data indicate that paromomycin is the preferred drug for this indication.[66,160] Moreover, diloxanide is not effective in amebic colitis or extraintestinal infection because it has little or no activity outside the intestinal lumen.[161]

The mechanism of action of diloxanide is unknown. It acts against trophozoites of *E. histolytica* that eventually form cysts. It has some structural similarities to chloramphenicol, suggesting that it may block protein synthesis.[162] In the gut diloxanide is hydrolyzed into diloxanide and furoic acid by bacterial and gut esterases.[163,164] It is then rapidly absorbed, glucuronidated, and excreted in the urine.[165] The unabsorbed component remains in the gut lumen as the active antiamebic agent that ultimately is excreted in the feces. Flatulence is a common adverse effect, occurring at a frequency of up to almost 90% in some studies.[166] Less common side effects include nausea, abdominal cramping, and diarrhea. The safety of diloxanide in pregnancy and lactation has not been established.

For treatment to clear passage of cysts of *E. histolytica*, after a course of metronidazole or tinidazole for treatment of invasive infection, 500 mg orally three times per day is recommended.[166,167] If required, a second course of treatment may be prescribed. A 5-day course of diloxanide with metronidazole given three times per day was shown to be efficacious in the treatment of symptomatic amebiasis, with or without dysentery. The drug can be given to children weighing more than 25 kg in a dose of 20 mg/kg in three divided doses for 10 days.[166] Doses of 25 mg/kg daily in three divided doses for 10 days have also been used.[161,168]

As noted above, for this indication paromomycin is more effective, requires a shorter duration of therapy (7 vs. 10 days), and is more widely available. Iodoquinol (diiodohydroxyquin) has also been widely used as a luminal amebicide but requires 20 days of therapy and is contraindicated in patients with hepatic impairment or allergy to iodine.[169]

Combined preparations of diloxanide furoate and metronidazole have also been shown to be effective in the treatment of giardiasis.[170] However, there is insufficient evidence to determine whether this regime is more effective than metronidazole alone.

Nitroimidazoles

The nitroimidazoles metronidazole and tinidazole (Fig. 42.8) are the drugs of choice for treatment of giardiasis, amebiasis, and trichomoniasis. The clinical pharmacology of metronidazole is discussed in detail in Chapter 28. Tinidazole is approved by the FDA for treatment of trichomoniasis, giardiasis, as well as intestinal and extraintestinal *E. histolytica* infection. Although its precise mechanism of action is unknown, available evidence indicates that it acts in a similar way as metronidazole—through nitroradicals generated by metabolism of the drug within target organism. Apart from its activity against protozoa, tinidazole, like metronidazole, has in vitro activity against anaerobic bacteria, including organisms associated with bacterial vaginosis (*Gardnerella vaginalis*, *Bacteroides* spp., and *Prevotella* spp.) and *Helicobacter pylori*.

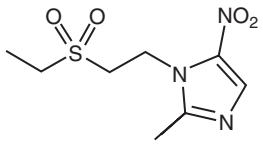

FIG. 42.8 Tinidazole.

Tinidazole is only available for oral administration, as 250- and 500-mg tablets, and as a syrup. Bioavailability is excellent, with almost complete absorption. It is metabolized in the liver by CYP3A4; it has the advantage over metronidazole of having a longer plasma half-life (12–14 hours vs. 8 hours[171]).

When administered as a single 50-mg/kg dose (maximum, 2 g), it is an effective treatment for giardiasis. In a meta-analysis of trials where single-dose (50 mg/kg) tinidazole was compared with metronidazole administered three times daily for 5 to 10 days,[172] the single-dose tinidazole regimen appeared to be more effective. However, the authors commented that the studies were small, enrolling only a total of 179 subjects, and were of low quality.

For intestinal amebiasis, a meta-analysis[173] of eight studies enrolling a total of 477 subjects, indicated that tinidazole administered in a dose of 2 g once daily for 3 days was significantly more effective than metronidazole and caused fewer adverse events. Likewise, tinidazole appeared to be at least as effective as metronidazole for treatment of amebic liver abscesses and better tolerated.[174]

For treatment of trichomoniasis, tinidazole is at least as effective as single-dose metronidazole.[175] In a meta-analysis of eight studies in which metronidazole was compared with tinidazole, although no parasitologic failures were reported, significantly higher rates of treatment and clinical failure and side effects were observed among those treated with metronidazole. Tinidazole may have a role among patients failing metronidazole therapy. In studies comparing tinidazole with metronidazole for treatment of bacterial vaginosis, similar cure rates have been reported.[176]

Reported adverse effects with tinidazole have been generally similar to those reported for metronidazole, although generally less frequent or severe. These include a metallic taste, GI disturbance (anorexia, nausea, vomiting, and epigastric discomfort), and weakness. Seizures and peripheral neuropathy have been reported with tinidazole. Tinidazole is contraindicated during the first trimester of pregnancy and in patients who are allergic to metronidazole.

Although there is a paucity of reports of drug interactions with tinidazole, the similar structure and metabolism of this drug compared with metronidazole suggests that the same precautions regarding interactions with metronidazole should be applied to tinidazole. Because tinidazole is a substrate for CYP3A4, levels of this drug may be affected by drugs that inhibit or induce this enzyme. Tinidazole shares the same propensity to interact with alcohol to cause an Antabuse-like reaction.

In summary, although there are no controlled studies comparing the efficacy of metronidazole and tinidazole for treatment of parasitic infections, tinidazole appears to be at least as effective and is better tolerated.[174] For trichomoniasis tinidazole appears to be more effective.[177] The doses of these drugs for treatment of these infections are listed in Table 42.1.

Spiramycin

Spiramycin is a macrolide antibiotic, first isolated from *Streptomyces ambofaciens*. Although its antimicrobial spectrum is similar to that of other macrolides, such as erythromycin, its principal use is for treatment

of *Toxoplasma gondii* during pregnancy or when first-line anti-*Toxoplasma* agents are inappropriate. Although it has been used for treatment of a range of bacterial infections, its use for treating the latter is limited because there are so many alternative agents. It is formulated as 250- and 500-mg capsules or tablets. Rectal and parenteral formulations are available in Europe. Spiramycin is not commercially available in the United States. It can be obtained at no cost from the Palo Alto Medical Foundation–Toxoplasma Serology Laboratory (650-853-4828), the US National Collaborative Treatment Trial Study (773-834-4152), or the FDA at 301-796-1400.

Although its mechanism of action is not fully defined, it is believed to act as an inhibitor of protein synthesis by binding to the 50S subunit of bacterial ribosomes. Like other macrolides, it is bacteriostatic.[178] Spiramycin has good bioavailability, ranging from 30% to 40%.[178] Administration away from meals is recommended because food reduces bioavailability by 50% and delays the time-to-peak serum concentration.[179] The mean half-life after oral administration is 5.5 to 8.0 hours, and the C_{max} is 0.4 to 1.4 mg/L after a single 1-g dose.[178] Although the drug is selectively concentrated in the placenta, reaching five times the maternal serum concentration, transfer across the placenta is incomplete, resulting in levels in the fetus approximately 50% of the corresponding maternal level. Spiramycin reaches higher concentrations in prostate, muscle, lymph nodes, and lung relative to plasma. The drug is secreted in milk (up to 200 µg/mL). Because the drug does not cross the blood-brain barrier, spiramycin should not be used to treat *Toxoplasma* encephalitis.[180]

Reported side effects of spiramycin include pseudomembranous colitis, cholestatic hepatitis,[181] QT prolongation with dysrhythmia,[182] thrombocytopenia,[183] and hemolysis in glucose-6-phosphate dehydrogenase–deficient patients.[184] Spiramycin has not been shown to be teratogenic and has been safely administered to pregnant women. Excretion is predominantly through the biliary route, with some enterohepatic recirculation. The drug is free of significant drug interactions. Unlike erythromycin, it does not induce cytochrome P450 enzymes. It has been reported to prolong the elimination half-life of L-dopa and carbidopa in healthy volunteers, possibly because of effects on intestinal motility that are well-known effects of macrolides.[185]

As for other macrolides, GI side effects, including nausea, vomiting, and abdominal pain, are common, particularly in high doses. On occasion, skin rashes and pruritus, including urticaria, may occur. Hypersensitivity to spiramycin has been described.[186] Of interest, although cross-sensitivity by skin prick test to other macrolides (erythromycin and clarithromycin) has been observed, oral therapy with these drugs was well tolerated. Nevertheless, it should be used with caution in patients with a history of macrolide hypersensitivity.

The utility of spiramycin in pregnancy rests on the fact that there is a delay between maternal *Toxoplasma* infection, placentitis, and then fetal infection. Thus the goal of spiramycin therapy is to prevent rather than treat fetal infection.[187] Once fetal infection is established, pyrimethamine-sulfadiazine should be used. The recommended dose of spiramycin for prevention of fetal infection (placental prophylaxis) is 1 g three times daily in the first trimester. Uncontrolled studies have shown that spiramycin therapy in newly infected pregnant women reduces transmission by up to 68% in the first trimester, 65% in the second trimester, and 32% in the third trimester.[188–190] Details of the management of infection during pregnancy are available in Chapter 278.

Because the rate of fetal infection rises with each trimester and the apparent effect of spiramycin falls, pyrimethamine and sulfadiazine are generally added later in pregnancy. Ethical considerations have made it difficult to do controlled studies of the efficacy of spiramycin in preventing transmission of *Toxoplasma* from mother to fetus. A meta-analysis published in 2007 was not able to confirm a significant reduction in transmission resulting from spiramycin therapy,[191] possibly because of the small size of the studies and the lack of an untreated control group. A nonrandomized comparative study of 255 live-born prenatally infected infants demonstrated a significant reduction in fetal transmission with treatment initiated within 4 weeks of maternal infection, whether spiramycin or sulfadiazine plus pyrimethamine was used, compared with no treatment.[192] Two recent studies also support the use of monthly screening during pregnancy and treatment of acute toxoplasmosis. In one study it was shown that monthly screening of pregnant women for *Toxoplasma* infection, followed by standard treatment of those found to be infected, was significantly more effective in reducing the rate of vertical transmission than screening every 3 months (29.5%–23.9%).[193] In a nonrandomized study comparing infected neonates who had been diagnosed and treated as a result of prenatal screening with those who have been diagnosed as a result of postnatal screening (untreated), the use of prenatal treatment, including the use of spiramycin for acutely infected women and sulfadiazine plus pyrimethamine when fetal infection was suspected or diagnosed, was associated with a significant decrease in severe neurologic sequelae or death in the neonate.[194]

In an additional nonrandomized study in which 44 patients with *Toxoplasma* chorioretinitis were treated with spiramycin alone or with sulfadiazine plus pyrimethamine, no difference in efficacy between the two groups was observed, but subjects in the spiramycin arm had fewer side effects. Hence spiramycin may be an alternative if standard therapy cannot be tolerated.[195]

Atovaquone

Originally developed as an antimalarial agent on the basis of potent in vitro activity against drug-resistant strains of *Plasmodium falciparum*, this hydroxynaphthoquinone was subsequently found to be active against a number of other microorganisms, including *P. jirovecii*, *T. gondii*, *Babesia microti*, *Cryptosporidium parvum*, *Encephalitozoon intestinalis*, *L. donovani*, *E. histolytica*, and *Trichomonas vaginalis*. Toxoplasmosis and babesiosis can be effectively treated with atovaquone when used in combination with pyrimethamine and azithromycin, respectively. See Chapter 41 for discussion of the pharmacology of this drug.

OTHER DRUGS RARELY USED TO TREAT INTESTINAL PROTOZOAL INFECTIONS

A number of other luminal antiprotozoal agents active against *E. histolytica* and *G. intestinalis* are now rarely used in most settings because of the availability of more active, more easily administered, and less toxic alternatives. These include iodoquinol, also known as diiodohydroxyquin; a halogenated 8-hydroxyquinolone, quinacrine, also known as mecaprine; emetine; and dehydroemetine. None of these drugs is readily available, but they can be obtained in the United States from the CDC Drug Service.

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The complete reference list is available online at Expert Consult.

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43 Drugs for Helminths

James S. McCarthy and Thomas A. Moore

SHORT VIEW SUMMARY

- This chapter includes anthelmintic drugs—drugs to treat infections with roundworms (nematodes) and flatworms, with the latter subdivided into flukes (trematodes) and tapeworms (cestodes). The drugs of choice for these are listed in this summary. Alternative drugs are given in this chapter and in the chapter describing the relevant parasite. Doses are given in Table 43.1 and in the text.

INTESTINAL ROUNDWORMS

- For ascariasis, capillariasis, hookworm (*Necator* and *Ancylostoma* spp.), pinworm *(Enterobius),* and whipworm *(Trichuris),* albendazole is generally the drug of choice, with mebendazole being an alternative.
- For strongyloidiasis, ivermectin is the drug of choice.

TISSUE ROUNDWORMS

- Filariasis
  - For lymphatic filariasis caused by *Wuchereria bancrofti* and *Brugia malayi,* diethylcarbamazine or ivermectin is used.
  - For loaiasis, caused by *Loa loa,* diethylcarbamazine is the drug of choice, with the caveat that posttreatment reactions need to be avoided in high-grade microfilaremia.
  - For onchocerciasis, ivermectin is the drug of choice, potentially followed by doxycycline for its anti-*Wolbachia* effect. Caution is also advised in onchocerciasis if high-grade *L. loa* microfilaremia coexists.
  - Treatment for *Trichinella* is supportive care with or without prednisone plus albendazole.
- For flukes (*Schistosoma, Paragonimus, Clonorchis, Fasciolopsis,* and *Opisthorchis*) and intestinal cestodes (*Diphyllobothrium, Hymenolepis nana, Taenia saginata,* and *Taenia solium*), praziquantel is the drug of choice.
- The exception is *Fasciola hepatica,* for which triclabendazole is the drug of choice.
- For tissue cestodes (*Echinococcus* [hydatid disease] and cysticercosis), albendazole is the drug of choice, with dexamethasone administered in neurocysticercosis to mitigate any posttreatment reaction. Combination therapy with albendazole and praziquantel for both neurocysticercosis and hydatid disease is now commonly used.

Although a large number of drugs have been used for anthelmintic chemotherapy, treatment is dominated by three drugs—albendazole, ivermectin, and praziquantel. This is because of the generally high-level efficacy, relatively low cost, and good safety profiles of these drugs. Their spectrum of activity can be classified according to the class of helminths against which the drugs have specific efficacy: nematodes or roundworms, for which albendazole or ivermectin are generally used; flukes or trematodes, for which praziquantel is generally used; and cestodes, for which intestinal infection is generally treated with praziquantel and tissue infection with albendazole. Drug doses are given in Table 43.1.

BENZIMIDAZOLES

Since the identification in 1961 of the potent antiparasitic activity of thiabendazole, this class of drugs has played a critical role in the treatment of parasitic infections worldwide. The structure of all members of this class is based on a bicyclic ring structure where benzene and imidazole rings are fused. The separate discoveries that thiabendazole is deactivated by hydroxylation of the benzene ring and that activity is enhanced by adding a 2-methylcarbamate moiety to the imidazole ring led to the development of mebendazole and albendazole.

The principal antiparasitic effect of the benzimidazoles (with the exception of triclabendazole) appears to be through binding to β-tubulin. This prevents assembly of microtubules, resulting in disruption of cell division and energy pathways.[1,2] Interference with vital processes therefore results in parasite death. This effect on tubulin also prevents hatching in helminth eggs.[3] Its selectivity for nematodes is attributable to its 25- to 400-fold greater inhibition of nematode tubulin compared with mammalian tubulin.

With the use of albendazole in mass treatment programs, there are concerns about the continued efficacy of the benzimidazoles to treat human infections, particularly given the well-documented reports of resistance from the veterinary literature. Resistance to albendazole in veterinary nematodes is caused by a small number of single nuclear polymorphisms, resulting in amino-acid substitutions in the parasite's β-tubulin protein, the most important of which is a phenylalanine-to-tyrosine substitution at position 200, with less important changes at residues at 167 and 198.[4] Until more recently, these concerns were unsubstantiated by evidence of resistance in human isolates. However, reports suggest that benzimidazole resistance may be developing in *Wuchereria bancrofti,*[5] *Trichuris trichiura,*[4,6] and hookworms,[6,7] particularly in areas where benzimidazoles have been extensively used in lymphatic filariasis control programs. These findings portend potential threats to the future utility of benzimidazoles for treatment of helminth infections.

Albendazole

Albendazole (Fig. 43.1) has a mode of action similar to other benzimidazoles; it is effective against a wide range of helminths and some protozoa. It is directly active against intestinal parasites; thus the relatively poor absorption of the parent drug from the intestine is ideal for an intraluminal effect. For tissue-dwelling helminths, however, albendazole acts as a prodrug, with the metabolite albendazole sulfoxide being responsible for anthelmintic activity outside the intestinal lumen. The efficacy of albendazole against tissue-dwelling helminth infections such as echinococcosis is difficult to reliably predict,[8] in part because of variable levels of the active metabolite in blood and tissues.[9]

Albendazole is administered orally, either as tablets (200 mg or 400 mg) or as a suspension (2% or 4%), with the dose regimen dependent on the target parasite. Tablets may be swallowed whole, chewed, or crushed and mixed with food. The drug is poorly soluble in water, with alcohol increasing solubility. Although no studies have been undertaken in humans to investigate bioavailability and efficacy when the drug is administered with alcohol, studies in rats suggest absorption is inhibited in all but the lowest amounts.[10]

Bioavailability studies of the parent drug have not been possible because of the lack of availability of a parenteral form. An estimated

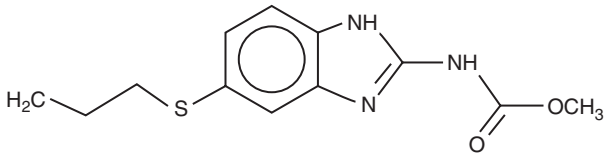

FIG. 43.1 Albendazole.

TABLE 43.1 Drugs Commonly Used for Helminth Infections

DRUG	DOSE	COMMENT
Albendazole	400 mg[a]	Taken with a fatty meal
Mebendazole	100 mg twice daily for 3 days	Less active than albendazole against extraintestinal infections
Triclabendazole	10 mg/kg once or twice	*Fasciola hepatica* infections
Pyrantel pamoate	11 mg/kg base × 1 dose; maximum dose, 1 g	
Ivermectin	150–200 µg/kg once daily × 1–2 days	
Moxidectin	8 mg PO once	Onchocerciasis
Diethylcarbamazine	6 mg/kg/day × 12 days in 3 divided doses	
Praziquantel	40/60 mg/kg/day in 1 or 2 doses × 1 day	40 mg/kg for *Schistosoma haematobium;* 60 mg/kg for *Schistosoma mansoni* and *Schistosoma japonicum*

[a]Dose varies for indication: once for ascariasis and pinworm; once daily for 3 days for hookworm and whipworm; twice daily for 7 days for strongyloidiasis, for 8–30 days for neurocysticercosis, and for 1–6 months for echinococcosis.
PO, Per os (orally).

5% to 10% of the drug is absorbed after oral administration. Once absorbed, the drug undergoes nearly complete first-pass metabolism either in the gut mucosa or in the liver to the active metabolite albendazole sulfoxide, a mixture of R(+) and S(−) enantiomers. Albendazole sulfoxide is then subject to biotransformation by cytochrome P-450 enzymes (primarily CYP3A4)[11] into the inactive metabolite albendazole sulfone.[12] The proportion of enantiomer production is species dependent, but in humans the R(+) enantiomer predominates.[13] In patients with neurocysticercosis, albendazole R(+) sulfoxide accumulates in the cerebrospinal fluid at a higher level than the S(−) enantiomer.[14] Data indicate that the R(+) enantiomer is more active than the S(−) enantiomer against *Taenia solium.*[15]

The pharmacokinetic profile of albendazole differs between men and women. For the main metabolites, albendazole sulfoxide and albendazole sulfone, there is no significant difference in half-life, time to reach peak concentration, and mean residence time. However, the apparent oral clearance and apparent distribution volume are lower in women, and the serum peak concentration (C_{max}), serum concentration-time curve (area under curve [AUC]), and area under the first-moment curve are lower in men.[16]

Food enhances the oral bioavailability of albendazole, presumably by stimulating gastric acid secretion, because albendazole absorption is pH dependent. This is true in both healthy patients[17,18] and subjects infected with tissue cestodes.[19,20] Plasma concentrations of albendazole sulfoxide are up to 5-fold higher when albendazole is administered with a fatty meal (fat content ≈ 40 g) compared with the fasting state. The administration of a single 10-mg/kg oral dose of albendazole with a high-fat meal (57 g fat, 1399 kcal) has been shown to increase the mean C_{max} and AUC by 6.5-fold and 9.4-fold, respectively.[21] The time to reach C_{max} increased from 2.5 to 5.3 hours compared with the fasting state and administration with water. The elimination half-life was not affected.

Tissue and blood concentrations of albendazole are also determined by mucosal cytochrome P-450 enzymes, which metabolize the drug, and by P-glycoprotein (Pgp), which acts as an efflux pump into the intestinal lumen.[22] In studies of patients with *Echinococcus granulosus* infection, albendazole administered orally in a dose of 10 to 14 mg/kg/day resulted in stable plasma concentrations of albendazole sulfoxide after 2 to 4 days of treatment. Significant quantities of this metabolite are measurable in lung and liver tissues and in hydatid cyst fluid obtained at surgery.[23,24] This active metabolite is excreted in bile. Albendazole sulfoxide crosses the blood-brain barrier to achieve levels in the cerebrospinal fluid approximately 43% of plasma levels.[25] However, there is significant interindividual variation because of differences in drug handling that are influenced by age, sex, or inflammation in the subarachnoid space. The high efficacy of albendazole for treatment of neurocysticercosis is most likely due to penetration of the central nervous system (CNS) by albendazole sulfoxide, which reaches higher brain levels than that achieved in plasma.[14]

Albendazole is generally taken as a single dose of 400 mg for mass drug administration for intestinal nematode infections in adults and children older than 2 years. However, apart from ascariasis, single-dose therapy, although it reduces infection intensity, is not curative. The drug has not been fully evaluated in infants, but in one study of children 9 to 23 months of age, no adverse laboratory abnormalities were noted.[26] Albendazole is mostly metabolized by the liver; thus no renal dose adjustments are needed. Conversely, absorption of albendazole and clearance of albendazole sulfoxide are delayed in patients with echinococcosis and significant biliary obstruction. However, a paucity of data limit dosing recommendations in this group.[27] The maximum recommended dose in humans, regardless of total body weight, is 800 mg/day.

In humans, single-dose albendazole therapy is very well tolerated, with an overall frequency of side effects attributable to the drug to be less than 1%.[28] In a large placebo-controlled study comprising 700 patients, the incidence of side effects in both groups was equivalent.[29] It is important to note that it is difficult to reliably differentiate symptoms attributable to the drug itself from the immune response to antigen released from dead parasites. In an analysis of clinical trials in which patients with lymphatic filariasis were administered albendazole alone or in combination with other agents, side effects were almost exclusively limited to patients with microfilaremia.[28]

Liver function abnormalities and bone marrow toxicity have been observed during prolonged courses of therapy given for treatment of echinococcosis.[28] These observations led to the initial recommendation that the drug be administered in treatment cycles of 28 days on/14 days off when prolonged use is anticipated. With increased experience, there is now less concern about continuous treatment, but monitoring is recommended.[30] The most commonly encountered side effects have been transient liver function abnormalities (≤20%) and alopecia (5%).[31] Bone marrow toxicity is rarely observed but can be irreversible. Abnormalities in liver function tests are typically less than five times the upper limit of normal and generally return to normal without stopping treatment.

Animal studies have demonstrated that both albendazole and albendazole sulfoxide are teratogenic in rats and rabbits at doses greater than 6 mg/kg/day and 30 mg/kg/day, respectively.[32] These and other observations have led to the recommendation that albendazole not be administered during pregnancy. However, clinical trials in which single-dose albendazole therapy was administered to pregnant women with hookworm infection (treated after the first trimester) demonstrated no effect on perinatal mortality, congenital malformations, or birth weight.[33] Although a small reduction in human immunodeficiency virus (HIV) viral load was noted,[34] no effect on vertical transmission of HIV was observed.[35] Of note, the trials demonstrated an increase in childhood eczema among treated children.[33,36]

Cimetidine inhibits the absorption of albendazole through reduction of gastric acidity, yet inhibits metabolism of albendazole sulfoxide by interfering with CYP3A4 enzymes, thus prolonging the elimination half-life from 7.4 (±3.3) hours to 19.0 (±11.7) hours.[37] Grapefruit juice inhibits metabolism of albendazole at the intestinal mucosa, but drug concentrations are higher than among patients administered cimetidine.[21] Short-term administration of ritonavir, a potent CYP3A4 inhibitor, does not significantly alter the pharmacokinetic parameters of albendazole. However, long-term administration results in significant decreases in AUC and C_{max}.[38] The blood concentration of albendazole sulfoxide is increased by 50% when administered concurrently with dexamethasone.[39]

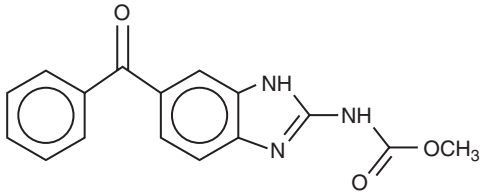

FIG. 43.2 Mebendazole.

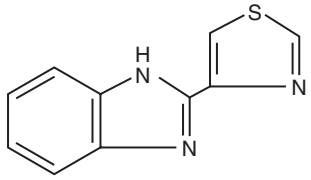

FIG. 43.3 Thiabendazole.

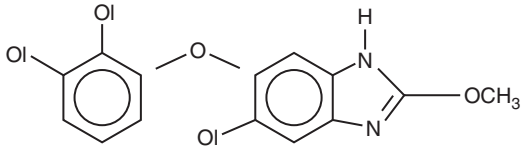

FIG. 43.4 Triclabendazole.

This has clinical relevance in the setting of treatment of neurocysticercosis, where albendazole is usually administered in conjunction with corticosteroid cover to prevent reactive cerebral edema caused by parasite death.

Mebendazole

Mebendazole (Fig. 43.2) was first introduced in 1977 as a veterinary anthelmintic agent for treatment of *Echinococcus multilocularis*. Its mode of action is similar to other benzimidazoles. Although it has been approved for the treatment of both intestinal and tissue helminths, it is less effective than albendazole for treatment of extraintestinal helminths, and therefore it is used almost exclusively for the treatment of common intestinal nematode infections.

Similar to albendazole, mebendazole is poorly soluble, is poorly absorbed, and undergoes extensive first-pass metabolism in the liver. However, it is even less well absorbed than albendazole, with a bioavailability of only 1% to 2% after administration of a single oral dose. The low bioavailability is attributable both to the low solubility of the oral formulation and to the high level of first-pass metabolism in the liver. Ingestion with fatty food increases absorption. A formulation with higher bioavailability is under development. The drug is highly protein bound (≈95%). The absorbed portion of mebendazole is predominantly metabolized by the liver. There are two major metabolites: 2-amino-5-benzoylbenzimidazole, created by amide hydrolysis, and methyl-5[α-hydroxybenzyl]-2-benzimidazole carbamate, a product of ketone reduction. In contrast to albendazole, these and other metabolites are not believed to have significant anthelmintic activity.

Mebendazole reaches its highest tissue concentrations in the liver. About half of the absorbed dose is excreted in the urine as metabolites[40]; however, a significant portion is also excreted in bile as metabolites. Mebendazole crosses the blood-brain barrier but reaches levels significantly lower than serum.

Cimetidine appears to improve the bioavailability of mebendazole.[41] Among patients with cystic echinococcosis, there is significant variability in absorption. Furthermore, concomitant administration of phenytoin and carbamazepine results in lower plasma levels, presumably because of induction of the cytochrome P-450 enzyme CYP3A4. Although no other significant drug-drug interactions have been reported with mebendazole, similar to albendazole, caution is warranted among individuals prescribed prolonged treatment courses and who are also taking medications with effects on the P-450 system.[38]

For the treatment of soil-transmitted helminths, mebendazole is typically administered as 100 mg given twice daily for 3 days. However, its pharmacokinetic profile enables a single 500-mg dose to be administered for mass treatment campaigns to control soil-transmitted helminths. Although mebendazole has not been fully evaluated in children 2 years or younger, it is well tolerated in community geohelminth control programs.[42,43] The maximum recommended dose in humans, regardless of total body weight, is 500 mg/day.

Although metabolites are excreted in the urine, there are no clinical data on the use of mebendazole in patients with kidney disease, and dose adjustment does not appear to be necessary in this setting. Nevertheless, caution seems warranted in these patients. Similarly, there are no clinical data on the use of mebendazole in patients with liver disease, but increased drug levels have been observed in a patient with cholelithiasis.[44]

When given either as a single 500-mg dose or 100 mg twice daily for 3 days, mebendazole is very well tolerated. In a trial involving more than 600 children given treatment for a geohelminth eradication program in Zanzibar, the most common adverse events were abdominal cramps (11%), fatigue (6%), headache (6%), vertigo (4.4%), and nausea (3.7%).[45] This is similar to reports from other large clinical trials. When taken at higher doses for longer periods (50 mg/kg for 3–12 months) for the treatment of echinococcosis, side effects occurred in approximately 20% of patients and were minimal and reversible in all patients without discontinuing treatment (transient elevation of transaminases, abdominal pain, headache, vertigo, urticaria, and dyspepsia).[46,47] There are also case reports of reversible bone marrow suppression.[48]

Although teratogenic in rats, the safety of mebendazole in pregnancy has been examined in three large human studies. In a survey of 170 women who took mebendazole in the first trimester of pregnancy, fetal loss or neonatal death was not significantly higher than that observed in the general population.[49] In a second series, one congenital hand malformation was observed in 112 first-trimester exposures.[49] In a larger retrospective study sponsored by the World Health Organization (WHO) involving more than 7000 Sri Lankan women who had accidentally taken the drug anytime during their pregnancy, there was a significantly lower rate of fetal loss and perinatal death, presumably resulting from reduced levels of maternal anemia.[50] Despite this ostensible beneficial effect, there was a trend toward a higher rate of congenital malformations with exposure during the first trimester (2.5% vs. 1.5%). In a randomized, placebo-controlled trial in Peru where women in the second and third trimesters were enrolled, no differences in the incidence of adverse effects were observed between the treatment arm and placebo arm.[51] The beneficial effect of decreased incidence of very low birth weight was again noted.

Thiabendazole

Although thiabendazole (Fig. 43.3) remains one of the most potent of the benzimidazoles developed, its use has dramatically declined, and it is no longer readily available. This is because thiabendazole is much less well tolerated than other equally effective agents from this class and because of the preference for ivermectin as an agent for treatment of strongyloidiasis.

Triclabendazole

Triclabendazole (Fig. 43.4) is a benzimidazole compound used routinely since 1983 in veterinary practice for the treatment of fascioliasis. It was first used for the treatment of human infection in 1986; however, its use in humans expanded in 1989 during an outbreak of fascioliasis near the Caspian Sea, when Iranian authorities approved the use of the veterinary formulation to treat human infections. In 1997 after the remarkable success of clinical trials using triclabendazole for the treatment of fascioliasis and paragonimiasis, WHO recommended that the drug be placed on the Essential Drugs List. Although the US Food and Drug Administration (FDA) has not approved triclabendazole for use in humans, it is registered in some countries where fascioliasis is endemic.

Triclabendazole is a narrow-spectrum agent and is unique among benzimidazoles in its highly specific activity against *Fasciola* spp. and

Paragonimus spp., with minimal activity against nematodes, cestodes, or other trematodes. Similar to other benzimidazoles, the mechanism of action of triclabendazole results from inhibition of microtubule formation.[52] The β-tubulin protein of *Fasciola hepatica* exhibits species-specific protein changes at position 82 (glutamic acid) and position 91 (threonine), which are unique among nematodes and cestodes. These substitutions appear to cause the fluke β-tubulin to adopt a three-dimensional structure that is relatively unaccommodating to other benzimidazoles, which are flat or L-shaped.[53] Triclabendazole exhibits a nonplanar U-shaped configuration that appears to be uniquely suited to binding to fluke β-tubulin.[52] In addition, triclabendazole-sulfoxide, the active metabolite of triclabendazole, has been shown to disrupt the tegument of both mature and immature stages of *F. hepatica*.[54] Furthermore, it is also a potent inhibitor of protein synthesis.[55]

Resistance to triclabendazole in veterinary use has become widespread since its original description in Australia.[56] A report of apparent triclabendazole-resistant *Fasciola* human infection in The Netherlands is important because of its epidemiologic relationship to resistance in livestock.[57] Susceptibility to the drug appears to be enhanced by ketoconazole[58] and methimazole[59] among strains previously known to be resistant.

After oral ingestion, absorption of triclabendazole occurs rapidly.[60] Similar to albendazole and mebendazole, triclabendazole undergoes extensive first-pass metabolism in the liver. It is converted into the active metabolite triclabendazole sulfoxide, and then the inactive metabolite triclabendazole sulfone.[61] Both metabolites are highly protein bound (>99%).[60]

After oral administration, the parent drug cannot be detected in plasma. Food enhances absorption of triclabendazole but also shortens the elimination half-life of its metabolites.[61]

Although there are no clinical data regarding treatment of patients with renal disease, dose adjustment seems unnecessary in renal disease, given the short course of therapy and the extensive hepatic metabolism of the drug. Although dose adjustment may be necessary in patients with hepatic disease, no data exist to provide insight.

Triclabendazole appears to be remarkably safe. Adverse events, when they have occurred, have been mild; short lived; and limited to abdominal pain, headache, nausea, and fatigue.[62] Abdominal pain was reported to occur in 21.5% of patients who received triclabendazole in a dose of 5 mg/kg daily for 3 days, 6.7% receiving 10 mg/kg twice daily for 1 day, and 31.3% receiving a single dose of 10 mg/kg.[63] A trial in the Bolivian Altiplano reported similar findings.[62] The abdominal pain is transient lasting less than 5 days, is typically located in the right upper quadrant, is relieved by oral spasmolytics, and has been attributed to the expulsion of dead or dying worms from the hepatobiliary system into the intestinal tract.[61,64,65] The contention that nearly all the adverse reactions in human clinical studies can be attributed to the death of adult worms is supported by ultrasound studies that have demonstrated dilated intrahepatic bile ducts caused by transient biliary obstruction associated with expulsion of dying worms. This is further supported by evidence from clinical trials in paragonimiasis, where the only gastrointestinal (GI) side effects reported were rare episodes of diarrhea. Fever has been reported in 6.3% of patients within 4 days of treatment for paragonimiasis. There have been no reports of derangement of liver function tests, renal function, or hematologic indices attributable to triclabendazole in human clinical trials. However, laboratory studies using high doses of the drug in rats and dogs have demonstrated bone marrow depression and increased serum alkaline phosphatase. No evidence of dose-related toxicity or carcinogenicity has been observed in animals. In contrast to the other benzimidazoles, triclabendazole has not been shown to cause birth defects in animal studies. Nevertheless, there are no data regarding its safety in pregnancy. Teratogenicity has been assessed in a zebrafish model, which highlights the potential importance of the sulfoxide metabolite, present at concentrations 30 times greater than the native drug.[66] Where possible, it seems prudent to avoid use in the first trimester. Triclabendazole is known to pass into breast milk, but there are no reports of adverse events in nursing infants. No data exist regarding drug-drug interactions.

Macrocyclic Lactones

Macrocyclic lactone compounds include avermectins and milbemycins. These compounds are widely used due to their broad spectrum of activity, efficacy at low doses, and excellent safety profile (Figs. 43.5 and 43.6).

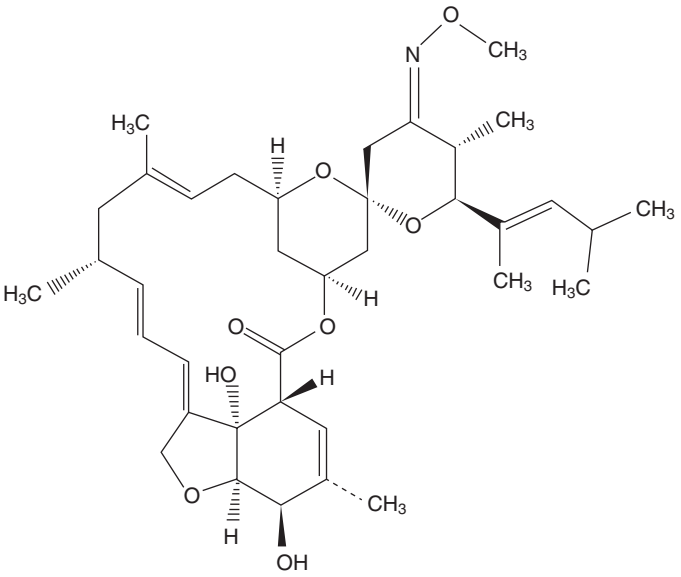

FIG. 43.6 **Moxidectin.**

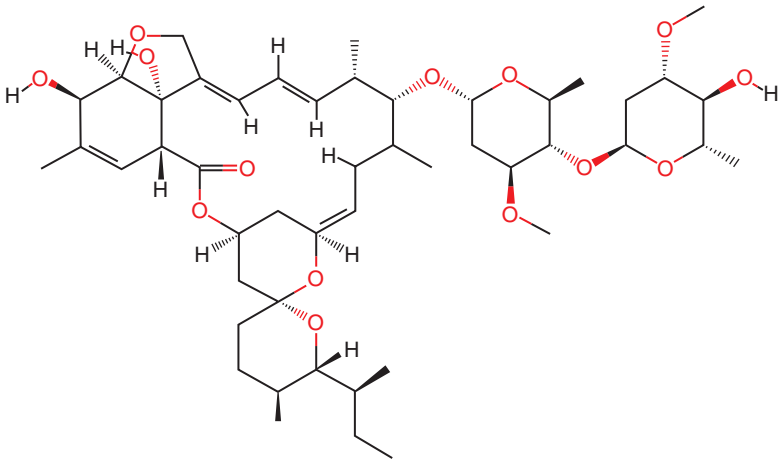

FIG. 43.5 **Ivermectin.**

Ivermectin

Ivermectin is a semisynthetic antibiotic derived from *Streptomyces avermitilis*. The organism was isolated from a soil sample taken from a golf course in Japan. Ivermectin is marketed under the brand names Stromectol (Merck, Whitehouse Station, NJ) in the United States, Mectizan (Merck) in Canada, and Ivexterm (Valeant Pharmaceuticals, Montreal, Canada) in Mexico. It is lipophilic, with poor water solubility. After the discovery of its activity against nematodes of veterinary importance, it was later found to be extremely effective against the cattle parasite *Onchocerca cervicalis;* this led to its development for treatment of onchocerciasis.[67] It is active at low doses against a wide range of helminths and ectoparasites and is the drug of choice for treatment of onchocerciasis and strongyloidiasis. It is an option for treatment of cutaneous larva migrans, head lice, and scabies. Although active against the intestinal helminths *Ascaris lumbricoides* and *Enterobius vermicularis,* it is only moderately effective in trichuriasis when given alone and has limited activity against hookworms.

Ivermectin activates neuromuscular membrane-associated chloride channels, particularly glutamate-gated channels, by binding to α-type channel subunits. The subsequent influx of chloride results in membrane hyperpolarization and muscle spasm particularly affecting the nematode pharynx, thus halting nutrient ingestion. Although ivermectin binding sites are present in the CNS of mammals, its affinity for nematode ligands is about 100 times greater than for mammals, explaining its selectivity.

Ivermectin is available for human use only as an oral formulation (either a 3-mg tablet or 6-mg scored tablet) and is generally administered as a single dose of 150 to 200 μg/kg. The bioavailability of ivermectin is increased twofold with food.[68,69] Plasma concentrations of ivermectin have been shown to decrease with ingestion of orange juice[70] and increase with ingestion of beer.[71] Its absorption half-life is approximately 1 hour.[72] The C_{max} is proportional to dose, with a value of approximately 38 to 46 μg/L reached after a therapeutic 150- to 200-μg/kg dose.[73–75] No significant differences in absorption have been found between healthy volunteers and patients with onchocerciasis. A second rise in plasma levels occurs 6 to 12 hours after ingestion, suggesting significant enterohepatic recycling.[72] Because it is a highly lipophilic drug, ivermectin is distributed widely throughout the body. Animal studies indicate that it accumulates at the highest concentration in adipose tissue and liver. Plasma protein binding is 93%.[76] The terminal half-life of the parent drug is 20 hours.[77] The elimination half-life of the metabolites of ivermectin is longer than that of the parent drug, at about 3 days.[77] It is not known whether the metabolites have antiparasitic activity. In healthy subjects, its volume of distribution is approximately 3.5 L/kg after oral administration.[78] In patients with onchocerciasis, the volume of distribution of the area was 9.9 L/kg after administration of a single 6-mg tablet.[74] The tissue distribution was similar in both groups of patients. Because of its high lipophilicity, a two-compartment model best describes its pharmacokinetic profile, with a high volume of distribution into a peripheral compartment.[79] Ivermectin is extensively metabolized in the liver by the CYP3A4 cytochrome.[80]

Ivermectin is both a substrate for the transporter Pgp[81,82] and a moderately potent Pgp inhibitor at concentrations consistent with doses used in mass treatment campaigns.[83,84] It is highly protein bound; scant pharmacokinetic and pharmacodynamic data exist regarding levels in individuals with conditions that would affect these parameters. The lack of significant macrofilaricidal activity in onchocerciasis cannot be explained by lack of penetration into onchocercal nodules, as the drug penetrates well into nodules when given as a single dose.[73] In an animal model of onchocerciasis, high concentrations were detected in the capsule wall and inside the nodule after subcutaneous administration with 500 μg/kg.[85] Because there is no significant renal handling of the drug, administration in renal failure should not be of concern. There are no data regarding safety in hepatic failure, but it is probably safe for use in this setting, given the lack of significant side effects at high doses.[68] Most of the drug is excreted in the feces as metabolites, mainly as monosaccharide derivatives; these also undergo enterohepatic recycling.[86]

In strongyloidiasis, the difficulty in achieving adequate drug levels of ivermectin in patients with disseminated strongyloidiasis and intestinal ileus can be a serious clinical problem. There is a growing experience with the use of the drug administered by subcutaneous injection of a veterinary formulation of ivermectin in the setting of ileus and disseminated strongyloidiasis.[87–89] In one report[89] in which a dose of 200 μg/kg was given every 2 days, the ivermectin level was 7.9 ng/mL 1 week after the last dose, with evidence of additional metabolite accumulation and a sustained antiparasitic effect. In another report[88] a level of 5.8 ng/mL was measured 16 hours after the first subcutaneous dose, with serum levels of 11.4 to 17.2 ng/mL being measured over the next 15 days, without evidence of significant accumulation.

Pgp appears to be important in preserving the blood-brain barrier and preventing accumulation of ivermectin in mammalian brain tissue.[90] Pgp is an essential component of the intact blood-brain barrier in vivo. In knockout mice deficient for Pgp, the 50% lethal dose of ivermectin is 100-fold lower than in wild-type mice.[81] Collies and some other breeds of dogs are unusually susceptible to neurotoxic effects of ivermectin, resulting from a mutation in the canine multidrug-resistance gene, *MDR1-1,*[91] which results in increased CNS penetration of the drug. Differences in levels of Pgp expression, protein levels, and drug passage across the gut wall and blood-brain barrier have been reported in different age groups both in laboratory animals and in human studies.[92] However, how these relate to potential CNS toxicity is uncertain, given the interspecies variations and limited data available.[93,94] It is unclear whether ivermectin is safe to use in infancy, when expression of Pgp at the blood-brain barrier is likely at its lowest levels; hence it is recommended not to administer the drug to children weighing less than 15 kg. Although not specifically studied, ivermectin has been used without dosage alteration across all ages in mass treatment campaigns. Although ivermectin use in an elderly nursing home population was reported to be associated with an increase in mortality within 6 months after treatment,[95] this report has been criticized for not controlling for comorbidities.

The successful mass distribution programs with ivermectin to control onchocerciasis have prompted the development of programs entailing coadministration with albendazole and azithromycin. The pharmacokinetic interactions of these agents have not been well studied. In a study where azithromycin, ivermectin, and albendazole were coadministered, ivermectin AUC and C_{max} were increased by 31% and 27%, respectively.[69]

Ivermectin is the drug of choice for the treatment of infection with the filarial nematode *Onchocerca volvulus*. Onchocerciasis is endemic in Central and Western Africa and until recently in certain areas of Central and South America. Administered as a single oral dose of 150 μg/kg, it has a rapid microfilaricidal effect, with most microfilariae being cleared by the end of the first week of therapy; within 1 month after treatment with ivermectin, skin microfilarial loads decrease by 95% to 99%.[96] The drug also blocks the production of new microfilariae by the adult female worms, which resume release of microfilariae 3 to 6 months after treatment.[97] This action of ivermectin to block embryonal development explains the prolonged effect in suppressing microfilariae for up to 1 year after treatment. Ivermectin also has a mild macrofilaricidal effect in reducing the longevity of the adult worms when the drug is administered every 1 to 3 months.[98,99] However, as adult *O. volvulus* worms may live up to 10 years, the drug should be administered every 6 to 12 months for the life of the adult worm.

Data indicate that a combination of ivermectin, diethylcarbamazine (DEC), and albendazole administered as a single dose for treatment of bancroftian filariasis has equivalent activity as annual therapy with ivermectin and DEC.[100]

A single dose of ivermectin is similarly microfilaricidal against *W. bancrofti*. With a single oral dose of 10 to 20 μg/kg, microfilaremia disappears for up to 3 months.[101] With higher doses (150–400 μg/kg), microfilaremia takes longer to return, and the level of parasitemia is lower than baseline.[102–104] Ivermectin appeared to be slightly inferior to DEC in producing sustained reduction of microfilaremia, even when given in combination with albendazole.[105] In lymphatic filariasis, as in onchocerciasis, ivermectin has no discernible effect on the adult worm, even when given at a dose of 4800 μg over 6 months.[106] Given these data, it is not surprising that when given as part of a mass treatment campaign, ivermectin resulted in a significantly smaller impact on hydrocele prevalence than DEC.[107,108] Nevertheless, a small trial of a triple-drug regimen with ivermectin added to standard therapy with DEC and albendazole demonstrated greater reductions in microfilaremia

with three drugs. Persistent microfilaremia was present in 11 of 12 people treated with two drugs at 1 year and none of 12 treated with three drugs, although the adverse event rates were 50% and 83%, respectively.[109]

Although the activity of ivermectin against *B. malayi* and *Brugia timori*—the less common lymphatic filarial parasites—is less well studied, it appears to be less effective against these species compared with *W. bancrofti*. Single doses of ivermectin, even as high as 400 µg/kg, result in slower clearance of microfilaremia, and this effect is shorter lived.[110] For treatment of brugian filariasis, the addition of albendazole to both DEC and ivermectin treatments did not result in improved efficacy.[111]

The effect of ivermectin against *Loa loa* appears similar to that seen in brugian filariasis. High doses of the drug (400 µg/kg) are required to clear microfilaremia,[112] and the microfilaremia clears more slowly than in bancroftian filariasis.[113] Serious adverse events, most notably fatal encephalopathy, have been reported when ivermectin was administered as part of a mass drug administration program for control of onchocerciasis in areas where loiasis was highly endemic. Although this required a temporary suspension of ivermectin distribution in these areas, mass drug administration was safely reinstituted after risk mapping tools were implemented. Serious adverse effects have been confined to individuals with high levels of microfilaremia (>30,000 microfilariae/mL); therefore ivermectin should be used with extreme caution in these patients.

A single oral dose of ivermectin resulted in sustained suppression of microfilaremia in patients infected with *Mansonella streptocerca*[114] and in a significant reduction of intensity and prevalence of infection in *Mansonella ozzardi*.[115,116] Ivermectin does not appear to be effective against *Mansonella perstans*.

Ivermectin is the drug of choice for the treatment of *Strongyloides stercoralis* infection, where administration of a single 200-µg/kg dose to children with uncomplicated infections led to a cure rate of 83%.[117] This finding is consistent with other clinical data.[118,119,120] To increase the likelihood of cure, most experts recommend an additional dose given 7 to 10 days later. Repeated courses may be needed in individuals with impaired cellular immunity, particularly patients infected with human T-cell lymphotropic virus type 1.[121] Although uncomplicated infection can be readily treated using orally administered drug, as discussed earlier, in patients with disseminated strongyloidiasis with intestinal dysfunction the drug is poorly absorbed,[87,89] and subcutaneous injection of a veterinary parenteral preparation may be required.[87,89,122,123] Ivermectin has also been given by rectal enema, but a clinical report of two cases found that clinically effective serum levels were not achieved when the drug is administered by this route.[124–126]

Ivermectin is highly effective for treatment of cutaneous larva migrans, a zoonosis usually caused by hookworms of dogs *(Ancylostoma caninum)* or cats *(Ancylostoma braziliense)*. For this indication, it is given at a dose of 150 to 200 µg/kg once daily for one or two doses.[127]

When given at a dose of 200 µg/kg/day for 2 days, ivermectin is effective for the treatment of gnathostomiasis, with a reported cure rate of 100% in one study.[128] When administered as a single dose of 200 µg/kg, cure rates range from 76% to 92%.[129] Among cases not cured, the signs of resurgent infection (as demonstrated by the recurrence of subcutaneous swelling) were not statistically significant compared with placebo.[130] The drug is reasonably well tolerated in this disease, with self-limited adverse effects that are not dose-related.[130]

Ivermectin is safe and highly effective against *A. lumbricoides*. A single dose of 100 to 200 µg/kg results in cure.[131] It is also active against pinworm caused by *E. vermicularis,* with a cure rate of up to 85% after a single oral dose ranging from 50 to 200 µg/kg.[131] However, it has limited activity in hookworm infection, with treatment resulting in reduction of worm burden but not usually cure.[131–133]

Similar to its activity against hookworm, ivermectin is relatively ineffective for the treatment of infections caused by *T. trichiura*. The cure rate after a single dose of 50 to 200 µg/kg ranges from 11% to 67%.[131,132] Higher doses[133] or extending daily treatment to 3 days[117] appears to be more effective. In this infection, combination with albendazole appears to result in a greater efficacy than when either agent is used alone.[134–136]

Although recommended as a second-line alternative to topical permethrin,[137] ivermectin is effective for the treatment of infection caused by *Sarcoptes scabiei,* in which it should be administered orally in a single dose of 200 µg/kg, with a second dose given 2 weeks later.[137,138] Ivermectin is particularly useful for treatment of crusted (Norwegian) scabies,[139] although repeated treatments are recommended. In this setting, it should be combined with a topical ascaricide, such as permethrin, alternating with keratolytic creams, such as salicylic acid or lactic acid/urea, to facilitate breakdown of the skin crusting.[137] A topical formulation of ivermectin 0.4% was approved by the FDA for control of head lice following the report of a randomized, controlled trial in which efficacy of 73.8% was reported.[140] Of note, however, short exposure intervals of body lice to sublethal amounts of ivermectin was shown to induce upregulation of detoxification genes including cytochrome P-450 monooxygenase and adenosine triphosphate–binding cassette transporter genes leading to tolerance.[141] This suggested the vulnerability of this class of drugs to the development of resistance.

Ivermectin given in the absence of helminth infection has few, if any, side effects, a fact that has permitted its use in mass drug administration programs for more than 25 years. Although high doses administered to some animals including beagle dogs and in human overdose can lead to CNS toxicity manifested by emesis, mydriasis, and ataxia, its poor penetration of the blood-brain barrier mitigates any toxic effect.[142] No significant toxicity has been reported in dose finding studies where doses as high as 2000 µg/kg, 10 times the recommended therapeutic dose, were tested.[68,143] However, as noted earlier, patients with a high parasite burden, for example, with high levels of microfilariae in the skin (onchocerciasis) or blood (lymphatic filariasis or loiasis), may have significant posttreatment reactions including postural hypotension and thus should be observed for up to 36 hours after treatment. In onchocerciasis, skin edema, pruritus, and mild eye irritation may also occur. Usually recovery follows rapidly when the patient remains recumbent, and no specific treatment is necessary. Occasionally patients require symptomatic treatment with antipyretics or antihistamines.

As noted earlier, severe complications of ivermectin therapy can occur among patients treated for onchocerciasis but with coincident heavy *L. loa* infection (>30,000 microfilariae/mL blood). A single case of hepatitis associated with ivermectin use has been reported,[144] but there are no other reports of significant immune reactions with this agent.

The use of ivermectin in mass treatment campaigns for more than 2 decades has occasionally resulted in the inadvertent administration of ivermectin to pregnant women.[145] Although no adverse effects have been recorded in retrospective studies,[146,147,148] administration of the drug in pregnancy is not advised. However, the established teratogenicity of the alternative treatment for strongyloidiasis (albendazole) suggests that ivermectin would be the drug of choice if life-threatening hyperinfection occurred in a pregnant woman. Although ivermectin reaches levels in breast milk that are about 35% of those seen in the serum, operational constraints and the absence of reports of toxicity in breastfeeding infants have resulted in a recommendation not to exclude lactating women from mass drug administration programs.[149]

Because of overuse of ivermectin as a single agent for control of parasites in cattle, resistance to ivermectin has developed in *Haemonchus contortus,* the organism for which ivermectin was originally developed as a treatment.[150] The mechanism of resistance in veterinary nematodes has not been precisely defined and may involve both target alteration and drug pumps. More than 1.3 billion tablets have been distributed in Africa, with some individuals having now received up to 20 annual treatments.[151,152] However, in some areas, this program has not resulted in interruption of transmission.[153] Reports of poor parasitologic responses to the drug[154–156] have been followed by parasitologic and epidemiologic evidence of ivermectin resistance.[157] Furthermore, in a study conducted in Cameroon, parasites obtained from individual patients demonstrated changes in the β-tubulin gene before and after the patients were treated with ivermectin.[158] Although these genetic changes developed over the 3 years the study was conducted, these parasites develop slowly, requiring about 1 year to go from birth to sexual maturity. Not surprisingly, the development of resistance to ivermectin results in resistance to related compounds such as moxidectin.[159] Exposure to increasing doses of

ivermectin is also associated with the development of cross-resistance to levamisole and pyrantel.[160]

Moxidectin

Moxidectin is a semisynthetic milbemycin macrocyclic lactone derived from the fermentation of *Streptomyces cyanogriseus* subsp. *noncyanogenus*. Nemadectin was isolated from *S. cyanogriseus* subsp. *noncyanogenus* in a soil sample in Victoria, Australia, and chemically modified by the addition of a methoxime moiety to form moxidectin.[159] Moxidectin was approved by the FDA in 2018 for use in the treatment of onchocerciasis.

Similar to ivermectin, the primary mode of action of moxidectin is believed to be via activation of glutamate gated chloride channels leading to flaccid paralysis as well as activity on related ligand gated ion channels. However, there are likely some differences in binding sites and/or mode of action, as suggested by the finding that an ivermectin-resistant strain of *Caenorhabditis elegans* demonstrates susceptibility to moxidectin.[161,162] In addition, reduced neurotoxicity in mammals of moxidectin compared with ivermectin has been observed.[163,164]

Although there have been reports of resistance to moxidectin in livestock, the resistance is not as widespread as with ivermectin, and moxidectin can often be used effectively against ivermectin-resistant parasites despite the concerns about cross-resistance. This appears in part to be due to the reduced efflux of moxidectin in Pgps[165]; however, resistance may be conferred by other genetic mutations.

Moxidectin is supplied as a pale yellow uncoated oval-shaped tablet containing 2 mg of drug. The recommended dose of moxidectin is a single dose of 8 mg (four 2-mg tablets). Moxidectin is highly lipophilic, is rapidly absorbed (2–6 hours), has a large volume of distribution, and has a very long half-life (>30 days). Administration with a high-fat meal significantly delays absorption.[166] Similar to ivermectin, moxidectin is generally well tolerated; however, like ivermectin it may cause cutaneous, ophthalmological, or systemic reactions of varying severity (Mazzotti reaction) in the treatment of onchocerciasis. These adverse reactions are due to allergic and inflammatory host responses to the death of microfilariae. There is a trend toward an increased incidence of these adverse reactions in patients with higher microfilarial burden.

Clinical trials comparing moxidectin with ivermectin in the treatment of patients with onchocerciasis revealed that some side effects occurred more frequently compared with ivermectin, presumably due to the differences in absorption and lipophilicity. Most notably, the development of symptomatic orthostatic hypotension with inability to stand without support after lying down for 5 minutes (an orthostatic hypotension provocation test) was seen in 5% of patients treated with moxidectin compared with 2% who received ivermectin. Decreases in blood pressure, which most commonly occurred on days 1 and 2 after treatment, were short-lived and alleviated when the patient lay down.[167] Patients who feel dizzy or light-headed after taking moxidectin tablets should lie down until the symptoms resolve. Patients with hyperreactive onchodermatitis (sowda) may be more likely than others to experience severe edema and worsening of onchodermatitis following the use of moxidectin.[168]

As with ivermectin, use of moxidectin in areas endemic for loiasis should be preceded by screening for microfilariae of *L. loa* to avoid the risk of acute encephalopathy. Elevation of bilirubin above the upper limit of normal occurred more often in patients treated with moxidectin than with ivermectin (2.8% vs. 0.6%). Most of the patients had single measurements of hyperbilirubinemia without concurrent elevation in transaminases. Elevation of transaminases greater than five times the upper limit of normal occurred slightly less often in patients treated with ivermectin (0.4% for alanine aminotransferase and 0.6% for aspartate aminotransferase) compared with moxidectin (1% for both alanine aminotransferase and aspartate aminotransferase).[167]

In clinical trials, no clinically significant differences in the pharmacokinetics of moxidectin were observed based on age (18–60 years old), sex, weight (42.7–107.2 kg), or mild-to-moderate renal impairment. Although the pharmacokinetic profile of moxidectin in patients with moderate-to-severe renal impairment is unknown, the fact that renal elimination of intact drug is negligible means that no reduction in the size of single-dose treatment is necessary. The effect of hepatic impairment on the pharmacokinetics of moxidectin is unknown.[169] Moxidectin is concentrated and excreted in breast milk in small quantities (0.7% of maternal dose).[170] In vitro, moxidectin is neither a substrate nor an inhibitor of CYP enzymes; in a clinical trial with midazolam, moxidectin did not alter CYP3A4 activity.[171]

Diethylcarbamazine

DEC remains the treatment of choice for lymphatic filariasis and loiasis. It can also be used for treatment of visceral larva migrans. The piperazine ring of DEC is essential for activity of the drug. Despite the description of activity of DEC against these parasites in 1940s, its mechanism of action remains poorly understood. When filarial parasites are exposed to DEC in vitro, no effect is observed. Proposed mechanisms of action include platelet-mediated triggering of the release of excretory antigen from microfilariae,[172] drug-induced alterations of prostaglandin metabolism in microfilariae and host endothelial cells resulting in immobilization due to inhibition of parasite cholinergic muscle receptors,[173,174] disruption of microtubule formation,[175] and alteration of helminth surface membranes resulting in enhanced killing by the host's immune system. Although efficacy of DEC in filariasis is variable, this has not been established to be due to resistance.

DEC is available only for oral administration. It is well absorbed, with peak plasma concentrations occurring within 1 to 2 hours. There is no significant binding to plasma proteins.[176] The drug is largely eliminated unchanged by renal excretion, with a terminal elimination half-life of 10 to 12 hours; less than 5% is excreted in feces. Alkalinization of the urine increases the half-life of the drug.[176] If more than one dose is to be administered to patients with renal impairment, the dose should be reduced commensurate with the reduction in glomerular filtration rate.[177] The pharmacokinetic profile of the drug is similar in both healthy and infected persons.[178] No data exist regarding the safety of DEC in pregnancy, but the finding of enhanced uterine contractility in rats suggests that caution is warranted.[179]

Worms can be refractory to repeated courses of therapy.[180–182] Monthly administration is known to be an effective chemoprophylactic agent for bancroftian filariasis and loiasis.[183]

Among uninfected individuals, GI upset, characterized by anorexia and nausea, is the most common side effect. Among infected patients, adverse reactions to DEC are common and proportional to the dose administered and intensity of infection. In patients with onchocerciasis, DEC can precipitate a typical side effect termed the *Mazzotti reaction,* characterized by pruritus, fever, and arthralgia.[184] The inflammatory response occurring in both the anterior (cornea) and the posterior (retina) segments of the eye can result in permanent visual damage.[185] In patients with lymphatic filariasis, similar but generally less severe systemic adverse reactions may occur, characterized by fever, headache, malaise, myalgia, and microscopic hematuria.[186] Localized effects in patients with lymphatic filariasis include pain, adenitis, lymphangitis, epididymitis, and lymphedema. As for ivermectin, life-threatening encephalitis can develop in patients with loiasis and high-burden parasitemia.[187]

DEC is active against *Ascaris* spp. Patients with ascariasis may expel live, paralyzed worms after treatment. No significant drug interactions have been reported with DEC.

Piperazine

Piperazine is a little-used anthelmintic that can be used for the treatment of ascariasis and enterobiasis. Piperazine causes an influx of chloride into nematode musculature by acting as an agonist at extrasynaptic γ-aminobutyric acid receptors. The paralyzed worm is expelled in the feces.

Doxycycline

In the past decade, it has been discovered that filarial nematodes depend on endosymbiotic bacteria of the *Wolbachia* spp. for normal development and fertility.[188] More recently, this endosymbiont has been shown to be an important determinant of the clinical manifestations of onchocerciasis[189] and has become an important drug target in patients with lymphatic filariasis.[190,191] Based on its activity against *Wolbachia,* doxycycline has

been shown to be active in onchocerciasis and lymphatic filariasis but not in loiasis because *L. loa* lacks the endosymbiont. The mechanism of action of doxycycline against *Wolbachia,* although not proven, is presumed to be similar to its antibacterial properties. For details of its pharmacokinetic properties and adverse effects, see Chapter 26.

Pyrantel and Oxantel Pamoate

Pyrantel pamoate is used to treat intestinal nematode infections, particularly hookworm and *Ascaris* infections, but is ineffective in trichuriasis. Conversely, its m-oxyphenol analogue, oxantel pamoate, exhibits trichuricidal activity[192] but lacks activity against hookworms and *Ascaris*.[6] For this reason, the drugs are often combined to provide broad coverage against intestinal helminths as part of mass treatment campaigns. A fixed combination dose, however, is no longer produced.

Both pyrantel and oxantel demonstrate a mechanism of action similar to levamisole—that is, by targeting the nicotinic acetylcholine receptor on the surface of nematode somatic muscle, thereby depolarizing the neuromuscular junction of the nematode resulting in irreversible paralysis and natural expulsion of the worm.[193] Although not clearly identified in humans, resistance is seen in parasites of domestic animals and is associated with modification of the nicotinic receptor.[194,195] Both pyrantel and oxantel pamoate are poorly soluble in water and thus are poorly absorbed from the intestine; more than 85% of the dose is passed unaltered in feces.[196] The absorbed portion of pyrantel is metabolized by the liver and excreted in urine; no human data exist for oxantel. Both agents are usually effective in a single dose. Safety in pregnancy and children younger than 2 years has not been established. It has minimal toxicity at doses used to treat intestinal helminth infection. Reported side effects are usually limited to anorexia, nausea, vomiting, abdominal cramps, and diarrhea.[197] Pyrantel has been shown to increase theophylline levels[198] and inhibit the anthelmintic activity of piperazine[199] and thus should not be given concomitantly. Oxantel enhances the activity of albendazole against *T. trichuria,* providing superior efficacy compared with albendazole alone.[6]

Levamisole

Levamisole is a rarely used anthelmintic agent. It is effective against *A. lumbricoides*[200] and *Ancylostoma duodenale*.[201] Similar to pyrantel, levamisole appears to act on nematode muscle, interfering with the function of a nicotinic acetylcholine receptor.[202] This depolarizes the muscle membrane and paralyzes the worm.[203] Resistance appears to be due to ion channel desensitization in the nicotinic acetylcholine receptor.[204] It is well absorbed orally and extensively metabolized in the liver.[205] Levamisole is excreted in the urine mainly as metabolites; only a small amount (<6%) is excreted in the feces.[206] The pharmacokinetics of levamisole have not been evaluated in patients with renal or hepatic disease, in children, or in elderly patients.[207] Animal studies with levamisole have not shown any evidence of teratogenicity. Nevertheless, treatment of pregnant women with levamisole should be deferred until after delivery. When used for the treatment of helminth infections, levamisole is well tolerated except for mild GI distress.[200]

Tribendimidine

Tribendimidine, a diamidine derivative of the aminophenylamidine amidantel, was developed at the National Institute of Parasitic Diseases in Shanghai, China, in the mid-1980s and represents the first new anthelmintic compound in 3 decades. It has a broad spectrum of activity against a wide variety of helminths including *Taenia* spp., intestinal nematodes (*A. lumbricoides*, hookworms, *T. trichiura*), and trematodes (*Opisthorchis viverrini* and *Clonorchis sinensis*).

Molecular studies on *Caenorhabditis elegans* suggest that tribendimidine is a cholinergic agonist that is selective for the nicotinic acetylcholine receptors of nematode muscle.[208] This action is similar to that of levamisole; however, tribendimidine has a much broader spectrum of activity.

Tribendimidine is converted to the active metabolite p-(1-dimethylamino ethylimino)aniline, known more simply as deacetylated amidantel (dADT). In healthy Chinese volunteers, dADT was detectable in plasma; while the parent drug was undetectable.[209] Evaluation of pharmacokinetic properties of tribendimidine in patients infected with *O. viverrini* demonstrated a variable t_{max} between 2–24 hours. Furthermore, AUC measurements of dADT over 24 hours demonstrated substantial differences in acetylation rates. Not surprisingly, patients cured of their infections showed significantly higher dADT serum concentrations and AUC values.[210,211] Food does not appear to affect the pharmacokinetics of tribendimidine when administered in the form of an enteric-coated tablet.[212] Clinical trials demonstrate efficacy of a single dose of tribendimidine alone[213] or in combination with other anthelmintics[214] against hookworm and concomitant soil-transmitted helminth infections. The clinical potential of this drug is yet to be determined, but it offers much promise.

Praziquantel

Praziquantel is highly active against a broad spectrum of trematodes and cestodes, with the exception of *F. hepatica*. It is the major drug used for treatment of schistosomiasis, including for community control programs for this disease. The drug is rapidly and reversibly taken up by flukes and tapeworms but not metabolized. It is a component of dual therapy with albendazole for neurocysticercosis and hydatid disease, as further discussed in Chapter 289.

The mechanism of action of praziquantel is incompletely understood, but evidence from animal models[215] and study of the drug's relative efficacy in schistosomiasis-naïve and endemic human populations[216] indicates that a preformed antiparasitic antibody response is required for optimal activity. Praziquantel disrupts the parasite tegument, causing tetanic contractures with loss of adherence to host tissues and, ultimately, disintegration or expulsion. It also interferes with parasite metabolism, exposing concealed antigens in the parasite.[217]

Reports from Senegal[218] and Egypt[219] of *Schistosoma mansoni* infection resistant to praziquantel and animal models have documented decreased efficacy.[220] The mechanisms of resistance remain unclear, but resistant strains were less susceptible to praziquantel-induced tegumental damage in vivo.[221] An investigation of the role of efflux pumps in this resistance suggested that modulation of Pgp with verapamil reversed resistance in vitro.[222] There have been no plausible reports of resistance in *Schistosoma haematobium* or *Schistosoma japonicum;* oxamniquine would be a treatment option for treatment of praziquantel-resistant *S. mansoni* infection. Praziquantel has been shown to have good activity against *Schistosoma mekongi* and *O. viverrini*.[223]

Praziquantel is rapidly absorbed with an estimated bioavailability of 80% to 100% but undergoes extensive first-pass hepatic metabolism to inactive metabolites so that most of the active drug does not reach the systemic circulation.[224] Praziquantel is completely metabolized, with 80% of the dose recovered as metabolites in urine within 4 days. It is not known to what extent praziquantel crosses the placenta, but only a small amount (<0.001%) is excreted in breast milk.[225] The bioavailability of praziquantel is influenced by other drugs. Phenytoin and carbamazepine both substantially reduce praziquantel levels by induction of first-pass hepatic metabolism and displacement of praziquantel from plasma protein binding.[226] Dexamethasone administered 24 hours earlier reduces the bioavailability of praziquantel by approximately 50%.[227] Praziquantel plasma levels are reduced by chloroquine and glucose. Levels of the drug are increased when it is taken with cimetidine and with food, particularly high-fat, high-carbohydrate meals.

Praziquantel is considered safe in children older than 2 years. Tablets can be divided but should not be chewed. It should be given with food and plenty of water because of its bitter taste. No dose adjustment is required in renal failure.[228] Praziquantel concentrations and side effects increase in proportion to the degree of hepatic impairment[229]; thus dose reduction may be indicated in these patients. In a rodent study, fetal death ensued when the drug was administered in high doses to pregnant rats between the 6th and 10th day of gestation.[230] Although there have been no reports of adverse maternal and fetal outcomes in large-scale treatment programs where inadvertent administration to women in early pregnancy was likely, it is recommended that praziquantel be withheld during the first trimester. It is also recommended that mothers should not breastfeed for 3 days after drug ingestion. A randomized, double-blind, placebo-controlled trial in 370 pregnant women in the

Philippines reported no significant differences with respect to the key indices of abortion, fetal death in utero, and congenital anomalies.[231] The integration of these new data into existing recommendations is awaited.

Praziquantel is well tolerated. The most commonly reported side effects are relatively mild and include transient nausea, vomiting, abdominal pain, dizziness, headache, and lassitude. Symptoms begin 30 minutes after ingestion, may require spasmolytics for relief, and usually disappear spontaneously after a few hours. In general, the adverse effects are related to the intensity and location of the infection, suggesting that they are due to the host immune response to release of parasite antigen.[232] In one study in an African population heavily infected with *S. mansoni,* the frequency of GI symptoms of abdominal pain and bloody diarrhea exceeded 50%.[233] However, in a large retrospective study of patients infected with *S. japonicum,* less than 0.5% had significant side effects attributable to the drug.[234]

Oxamniquine

Oxamniquine is a hydroxylated metabolite of a 2-aminotetra-hydroquinoline with activity against S. *mansoni*. After the development of praziquantel, it has assumed a minor role because of its narrower spectrum of action and its more pronounced toxicity profile. In addition, drug efficacy shows regional variation.[235]

The primary mode of action of oxamniquine seems to be mediated by adenosine triphosphate–dependent generation of an intermediate that alkylates essential macromolecules including DNA. After 4 to 8 days of treatment with oxamniquine, adult schistosomes show tegmental alterations similar to that induced by praziquantel. These changes result in disorganization of the suckers with which the worms attach to blood vessels. The worms lose attachment and then shift from the mesenteric veins to the lungs and liver, where they are destroyed. Female worms may return to the mesenteric veins but are unable to produce eggs.

Oxamniquine is well absorbed after oral administration.[236] Peak plasma concentrations are achieved 1 to 4 hours after oral administration of a 10-mg/kg dose.[237,238] The elimination half-life of oxamniquine is 2 hours. Oxamniquine is administered orally as a single dose and is well absorbed. Food slows absorption and reduces bioavailability.[239] About 70% of an administered dose is excreted in urine as a mixture of pharmacologically inactive metabolites. No dose adjustment is necessary in renal or hepatic disease.[237] Oxamniquine is mildly embryocidal in mice and rabbits at doses an order of magnitude greater than those used in humans. It is suggested that breastfeeding be delayed for at least 4 hours after administration of oxamniquine.

The drug can turn urine bright orange-red. Side effects are uncommon and usually mild at the recommended doses. However, hallucinations and seizures have been rarely reported.[240–242] No data are available on interactions with other drugs, but the ability of oxamniquine to inhibit CYP2D6 may be of clinical importance.[243]

Metrifonate

First introduced as an insecticide, this organophosphate compound has selective activity only against *S. haematobium*. It was introduced as a drug for humans in the 1960s.[244–246] The standard dose regimen is 7.5 to 10 mg/kg given three times at 14-day intervals; the drug is 19% to 48% effective against this parasite,[247] with egg reduction rates of up to 90% 4 weeks after the final dose.[248] Use of metrifonate has dramatically declined with the recognition that praziquantel has superior clinical properties, particularly because it can be given as a single dose and is now relatively inexpensive.[249] Metrifonate has been withdrawn from the WHO Model List of Essential Medicines.[250]

Metrifonate is converted nonenzymatically in the body to the active form, dichlorvos (2,2-dichlorovinyl dimethyl phosphate), which irreversibly inhibits acetylcholinesterase. Dichlorvos is more active against schistosomal cholinesterase than against the human enzyme.

Metrifonate is well absorbed after oral administration; peak plasma concentrations for both metrifonate and dichlorvos are reached within 1 to 2 hours of a single oral dose. Whole-blood concentrations of the active metabolite dichlorvos are about 1% of the parent drug. Elimination of metrifonate depends on its conversion to dichlorvos, which occurs nonenzymatically. Although there are no data in humans, animal studies show that both metrifonate and dichlorvos are degraded largely by hydrolysis. Metrifonate and dichlorvos are predominantly eliminated in the urine as glucuronides.[251]

After a single oral dose, metrifonate produces a 95% decrease in plasma cholinesterase activity within 6 hours, with a fairly rapid return to normal. Erythrocyte cholinesterase levels return to normal more gradually, usually requiring about 2.5 months.[248] Patients should not be exposed to neuromuscular blocking agents or organophosphate insecticides for at least 48 hours after treatment.

No serious side effects have been seen with recommended doses,[251,252] but side effects resulting from cholinergic stimulation (fatigue, muscular weakness, tremor, sweating, salivation, fainting, abdominal colic, diarrhea, nausea, vomiting, and bronchospasm) have been reported. These are relatively rare and subside within a few hours. There is a strong relationship between the occurrence of side effects and plasma concentrations.[253] Metrifonate potentiates the effect of succinylcholine. Food may delay the rate of its absorption.[254]

Niclosamide

Niclosamide is highly effective against a wide variety of intestinal tapeworms but not against tissue cestodes, such as echinococcosis or cysticercosis. Praziquantel is the preferred agent for treatment of tapeworm infection in most settings. Niclosamide has the unique indication for treatment of intestinal *T. solium* infection when it is desired to treat only the intestinal form. The scolex and proximal segments of the tapeworms are killed on contact with niclosamide and may be digested in the gut. There is theoretical concern that autoinfection may result from release of viable ova as the tapeworm disintegrates within the intestinal lumen. As a precaution, a brisk purgative is recommended to be given 2 hours after the first dose given for *T. solium* infections. The drug acts by uncoupling oxidative phosphorylation.[255] In vitro, niclosamide rapidly paralyzes the cestode. Little is known about the pharmacokinetics of niclosamide. Only a small amount of the drug is absorbed from the GI tract.[255]

Use of the drug is limited by side effects, necessary duration of therapy, recommended use of purgatives, and restricted availability. Tablets are given on an empty stomach in the morning after a liquid meal: for *T. solium*, 2 g as a single dose, 500 mg in a child younger than 2 years, and 1 g in a child 2 to 6 years of age. All doses are to be taken as a single dose after a light breakfast. For *Taenia saginata* infection in an adult, 1 g is taken after breakfast, and 1 g is taken an hour later; in a child younger than 2 years, 250 mg is given after breakfast, and 250 mg is given an hour later; and in a child 2 to 6 years of age, 500 mg is given after breakfast, and 500 mg is given an hour later. For treatment of hymenolepiasis in an adult, the initial dose is 2 g on the first day, followed by 1 g daily for 6 days.

Adverse effects of niclosamide are mild and infrequent and may include GI disturbances, lightheadedness, malaise, and pruritus.[255] Alcohol may enhance the absorption of niclosamide, thereby increasing the risk of side effects, and therefore should be avoided when taking this drug. Niclosamide is safe for use in pregnancy.

Nitazoxanide

Discussed in detail in Chapter 42, nitazoxanide is approved by the FDA for the treatment of cryptosporidiosis and giardiasis. Its benzamide moiety resembles that of niclosamide, and it was initially developed as a veterinary anthelmintic agent. It demonstrated acceptable effectiveness (94% after a first course) as an alternative treatment to triclabendazole for fascioliasis in Mexico.[256]

Bithionol

Bithionol is a little-used chlorinated bisphenol with activity against trematodes including *F. hepatica*[257] and *Paragonimus* spp.[258] Praziquantel or triclabendazole is generally preferred. It competitively inhibits electron transfer resulting in impaired anaerobic energy metabolism and trematode death. Its parasite specificity is due to its unique target in the parasite respiratory chain.

Bithionol is readily absorbed from the GI tract and is widely distributed throughout the body. Peak blood concentrations are achieved

within 4 to 8 hours after oral administration. A high blood concentration seems to be maintained when bithionol is administered at intervals of 2 to 3 days.[259] Excretion has been reported to be mainly through the kidneys.

Limited supplies of bithionol are available from the Centers for Disease Control and Prevention Drug Service. Bithionol is given as an oral dose of 30 to 50 mg/kg on alternate days for 10 to 15 doses. Data on dose adjustments in special groups are limited, but one study indicated safety in patients with altered renal or liver function.[260]

Adverse effects of bithionol are usually mild and transient and include anorexia, nausea, abdominal cramps, diarrhea, and urticaria.[257,258,261] Data on drug interactions are limited.

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The complete reference list is available online at Expert Consult.

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44 Antiviral Agents: General Principles

Raphael Dolin and Janet Morgan

Although viruses are among the most common causes of human illnesses, the development of antiviral drugs to treat or prevent viral infections has historically lagged behind the development of drugs against other microbial agents. In part, this has been related to difficulties in detection and characterization of many viruses, and in limitations in techniques for evaluation of laboratory and clinical effects of antiviral agents. However, in recent years the development of antiviral chemotherapy and chemoprophylaxis has markedly expanded, driven in large part by advances in understanding of molecular virology and viral pathogenesis and by the establishment of highly specific, sensitive, and quantitative methods for viral detection. More than 70 antiviral agents are now available to treat various viral infections, including 28 to treat human immunodeficiency virus (HIV) infections (see Chapter 128), and many additional agents are at various stages of preclinical and clinical development. The availability of laboratory data to guide administration of antiviral drugs and to assess their clinical efficacy has also been limited compared with data used in treatment of bacterial infections. However, exemplified by the success of antiviral therapy for HIV and hepatitis B and C virus infections, methods have now become widely available to assess sensitivity of viruses to antiviral drugs, to quantitate viruses in host compartments (virus load), and to determine pharmacokinetics of antiviral drugs. These methods now provide data to guide the appropriate use of antiviral agents.

General principles for the use of antiviral agents are discussed in this chapter. Individual antiviral agents are reviewed in Chapters 45 through 48, and antiretroviral therapy is reviewed in Chapter 128.

MECHANISMS OF ACTION OF ANTIVIRAL AGENTS

The mechanisms of action of antiviral agents generally involve inhibition of a virus-specific step in viral replication. Because viral replication depends primarily on host cell metabolic functions, useful antiviral agents should inhibit virus-specific functions but leave host cell functions intact, or at least preferentially inhibit virus-directed as opposed to host cell–directed macromolecular synthesis. In consequence, antiviral agents typically have a restricted spectrum of activity. Although many compounds can be found that exhibit antiviral activity in vitro, most also affect host cell functions and are associated with low therapeutic ratios or with unacceptable toxicity in humans. Most current antiviral agents inhibit ongoing viral replication and then enable host defenses to eliminate the virus. If "viable" virus persists after the drug is removed, virus replication may resume. Viruses that have established a latent, that is, a nonreplicating, state are generally not affected by currently available antiviral agents.

The search for effective and specific antiviral agents has identified specific cellular sites and macromolecular mechanisms whose interdiction can be used to inhibit virus replication. The individual mechanisms of action through which individual drugs exert their antiviral effects are discussed as follows.

Inhibition of Viral Nucleic Acid Synthesis

Many antiviral compounds are nucleoside or nucleotide analogues whose mechanism of action is inhibition of viral nucleic acid synthesis. Nucleoside analogues such as acyclovir and penciclovir require phosphorylation to the monophosphate, which requires a thymidine kinase coded by herpes simplex virus and not present in uninfected cells.[1] Cellular kinases phosphorylate acyclovir to the triphosphate moiety, which inhibits the viral DNA polymerase and is incorporated into viral DNA as a chain terminator, thus inhibiting viral DNA synthesis. The requirement for a virus-encoded enzyme provides a virus-specific mechanism of action. Herpes simplex viruses that lack a thymidine kinase are resistant to acyclovir and penciclovir.[2]

Cidofovir is a monophosphorylated nucleotide analogue that does not require a virus-coded thymidine kinase for its activity. It uses cellular kinases to convert it to a diphosphate moiety that inhibits viral DNA polymerase and also causes premature chain termination. It is active against viruses that lack thymidine kinase and are therefore resistant to acyclovir and penciclovir.[3]

Foscarnet is not a nucleoside or nucleotide analogue but, rather, a pyrophosphate that blocks the pyrophosphate-binding site on the viral DNA polymerase. Thus it does not require intracellular metabolism for its antiherpesvirus activity.[4]

A novel mechanism of action, inhibition of the helicase-primase complex, is manifested by amenamevir[5] and pritelivir,[6] which are active against herpes simplex viruses types 1 and 2. The helicase-primase complex is a heterotrimer consisting of helicase, primase, and cofactor subunits that are essential for DNA replication. Amenamevir and pritelivir are not nucleoside analogues, do not require phosphorylation by thymidine kinase, and are therefore active against viruses that are resistant to acyclovir and penciclovir because of thymidine kinase deficiencies. Amenamevir and pritelivir are undergoing clinical studies in suppression and treatment of genital herpes (see Chapter 46).

Letermovir is an antiviral agent with activity against cytomegalovirus (CMV) through inhibition of the viral terminase enzyme complex, which results in interference with processing and blockage of viral DNA.[7] It does not inhibit the viral DNA polymerase and is therefore active against CMV strains resistant to ganciclovir, cidofovir, and foscarnet.

In HIV infection nucleoside and nucleotide analogues inhibit viral complementary DNA (cDNA) synthesis through inhibition of reverse transcriptase. These drugs must be phosphorylated to the triphosphate moieties for anti-HIV activity. They include zidovudine, tenofovir, emtricitabine, and abacavir. Nonnucleoside reverse-transcriptase inhibitors (NNRTIs) also bind to reverse transcriptase but do so in a pocket far from the active site, in contrast to the nucleoside and nucleotide analogues. NNRTIs include nevirapine,[8] efavirenz, and rilpivirine.[9]

Integrase Strand Inhibitors

In HIV infection HIV RNA is reverse transcribed in the cytoplasm, and the cDNA is processed by HIV integrase and transported to the nucleus, where integration into the cellular genome occurs through strand transfer, which is blocked by integrase strand inhibitors (INSTIs).[10] Raltegravir, elvitegravir, and dolutegravir are examples of INSTIs. The high efficacy and safety of INSTIs is reflected in their inclusion in currently recommended first-line treatment regimens (see Chapter 128).

CAP-Dependent Endonuclease Inhibitor

Viral RNA transcription in influenza viruses requires a cap dependent endonuclease (CEN), which provides "cap-snatching" activity and primes viral transcription. CEN resides on the N-terminal domain of the polymerase acid (PA) subunit of the RNA-dependent RNA polymerase

of influenza A viruses and is highly conserved among influenza A subtypes. Baloxavir marboxil is a small-molecule inhibitor of influenza A and B CENs. It has recently been shown to be effective in both clinical and virologic parameters in a phase III study of influenza A and B infections.[11–13]

RNA Antisense Nucleotides

Antisense nucleotides inhibit transcription of messenger (m)RNA and can be used to inhibit replication of viruses. Fomivirsen is an antisense oligonucleotide that is complementary to a sequence in mRNA transcripts of the major immediate-early region 2 of CMV.[14] It is administered intravitreally to treat cytomegaloviral retinitis in HIV-infected patients who have failed or are intolerant to other treatments. Because of its unique antiviral mechanism of action, fomivirsen is active against cytomegaloviral strains that are resistant to ganciclovir, foscarnet, or cidofovir. Currently, fomivirsen production has been discontinued and is no longer available in the United States.

Viral Entry Inhibition

Inhibition of attachment of virus to its cellular receptor or subsequent intracellular entry of virus is an important mechanism for antiviral activity. Maraviroc is an allosteric inhibitor that interferes with attachment of HIV-1 glycoprotein (gp)120 to the CCR5 cellular chemokine receptor.[15,16] Enfuvirtide is a synthetic peptide that inhibits gp41-mediated fusion of HIV-1 with the cell membrane and thus blocks virus entry[17] (see Chapter 128).

Protease Inhibition

Many viruses require proteolytic cleavage of polypeptide precursors to activate essential viral proteins. HIV-1 and HIV-2 contain aspartyl proteases that cleave Gag and Gag-Pol polyproteins into essential structural and enzymatic components. Viral particles may still be found in the presence of protease inhibitors, but such particles are rendered noninfectious. For HIV-1, there are 10 clinically used inhibitors of such protease activity, which are important components of combination antiretroviral regimens. These protease inhibitors include ritonavir, darunavir, and atazanavir. Ritonavir is a protease inhibitor that is used at lower doses, primarily to increase the bioavailability and half-life of other protease inhibitors, except for nelfinavir.

Hepatitis C has a polyprotein in which regions contain proteins NS3, NS4A, NS4B, NS5A, and NS5B, which are required for RNA replication and assemble into a membrane-associated replicase complex within the cytoplasm of infected cells. Multiple direct-acting antivirals (DAAs) are available that inhibit the just-mentioned proteins, including NS3/NS4A proteases and NS5A and NS5B proteins, and they have pangenotype activity (sofosbuvir/velpatasvir and glecaprevir/pibrentasvir).[18]

Inhibition of Virus Uncoating

The adamantane compounds amantadine[19] and rimantadine[20] interfere with the function of the M2 protein of influenza A virus and inhibit uncoating of influenza virus shortly after entry of virus into cells.[19,20] Single amino-acid changes can render viruses resistant to the adamantanes, as is the case with currently circulating (2013–17) influenza A viruses.[21]

Inhibition of Virus Release

The neuraminidase inhibitors oseltamivir, zanamivir, and peramivir interfere with release of influenza A and B from infected cells.[22–24] Neuraminidase prevents the clumping of released virus, and thus inhibition of this enzyme retards the cell-to-cell movement of virus.

MODIFIERS OF HOST DEFENSES AGAINST VIRUSES

Appropriate host defenses, including both innate and adaptive immune responses, are essential for prevention and recovery from viral infection. Immunosuppression resulting from organ transplantation, cancer chemotherapy, or HIV infection may be associated with more severe viral infections and with higher rates of recrudescent or chronic viral infections. Antiviral prophylaxis and preemptive therapy have become standard practice in immunocompromised patients, such as hematopoietic stem cell and solid-organ transplant recipients. Responses to antiviral treatment may be delayed in such patients, and the risk for selecting drug-resistant viruses is higher. In addition, some antiviral agents may blunt host immune responses through reductions in viral antigens that would otherwise stimulate such responses. Reduction of immunosuppression should be part of antiviral treatment in immunocompromised patients whenever possible.

Interferons

Interferons are cytokines that do not directly have antiviral activity themselves but induce an antiviral state in host cells through activation of tyrosine kinases (Tyk2, JAK1, JAK2) and phosphorylation of cytoplasmic signal transducer and activator of transcription (STAT) proteins (see Chapter 48). This results in inhibition of multiple steps in viral replication, depending on the particular virus and cell type.[25,26] Interferons have been most widely used in treatment of hepatitis B and C virus infections as part of combination regimens with ribavirin and with protease inhibitors for hepatitis C. Interferon therapy is no longer recommended for treatment of hepatitis C (see Chapter 47).

Innate Immunity Stimulators

Imiquimod is an imidazoquinoline that is a Toll-like receptor (TLR) 7 agonist that activates various cells in the innate immune system.[27] It is topically administered and is approved for treatment of anogenital warts (see Chapter 48). Resiquimod is a structurally related investigational compound that is a TLR7 and TLR8 agonist.[28]

Host Sialidases

Influenza and parainfluenza viruses infect host cells through binding with sialic acid cell receptors. Host sialidases inactivate such receptors and reduce viral infection through inhibition of virus binding. DAS181 is a sialidase attached to a respiratory epithelium–binding domain that has activity against influenza A and B and parainfluenza virus infections in vitro and in vivo.[29] It is being evaluated in clinical studies in influenza and parainfluenza infections in immunosuppressed patients (see Chapter 45).

DETERMINATION OF SENSITIVITY OF VIRUSES TO ANTIVIRAL AGENTS

Determination of the sensitivity of viruses to antiviral agents is essential for appropriate use of antiviral chemotherapy or chemoprophylaxis, just as it has been for use of antibacterials. Sensitive and specific laboratory assays to determine activity of antivirals are now increasingly available, both as licensed, commercial assays as well as in research settings. For certain viral infections for which antivirals are available, epidemiologic-based sensitivity data are widely available, generally through public health agencies, which can guide selection of antivirals. Examples of these are infections with influenza virus, herpes simplex virus, varicella-zoster virus, and hepatitis C virus. For others, such as HIV infection, determination of viral sensitivities may be important in selection of an initial regimen or in its modification.

Originally, laboratory assays of sensitivity to antiviral agents were based entirely on phenotypic assays in cell culture developed for individual viruses.[30] Results in these assays varied with the in vitro system that was used and were often difficult to standardize. These assays are now being largely supplanted by highly sensitive, rapid, and specific molecular genotypic assays (see Chapter 16), in which specific amino-acid changes have been associated with phenotypic resistance.[31] Genotypic assays can detect thymidine kinase mutations, which are associated with resistance to acyclovir and penciclovir in herpes simplex virus and varicella-zoster virus infections. Mutations in phosphotransferase and DNA polymerase genes of CMV are associated with resistance to ganciclovir, cidofovir, and foscarnet. Despite their advantages, genotypic assays rely only on mutations that have been previously recognized to be associated with phenotypic resistance. Thus the probes that are used must be frequently updated to detect newly recognized mutations that are associated with antiviral resistance. In situations in which de novo resistance is uncommon, such as herpes simplex virus or varicella-zoster virus infections in normal hosts, genotypic assays

for resistance are performed when failure of antiviral therapy is suspected (see later). In other clinical settings in which antiviral resistance may be relatively common, such as in HIV-1 infection, genotypic assays of virus isolates may be used to select or monitor an antiviral regimen.

QUANTITATION OF VIRUS (VIRUS LOAD)

Quantitation of virus in body fluids, most commonly in plasma but also in cerebrospinal fluid, is a measure of the extent and possible severity of a viral infection. Highly sensitive, specific, and precise assays for virus load are now commercially available and approved by the US Food and Drug Administration (FDA) and by Conformité Européenne (CE) marking in the European Union. The majority of these assays use nucleic acid amplification tests that report results in copies of nucleic acid per milliliter (see Chapter 16). Virus load measurements are used most frequently to monitor the effect of antiviral therapy, but they also may be used to establish or confirm the diagnosis and to provide assessment of the extent and possible severity of the viral infection. Their most extensive use is in patients with HIV-1 infection, in which plasma virus loads are used to monitor the effectiveness of antiretroviral therapy (see Chapter 128). The goal of antiretroviral therapy is to reduce viral load in plasma to below the level of detection of the assay, generally less than 20 to 80 copies of HIV RNA/mL in commercially available assays. Virus load assays are then periodically performed to ensure that the effect of antiretroviral therapy is maintained, and rises in virus load are investigated for cause, including assays for development of resistance. Virus loads are also used to assess the effect of antiviral therapy for hepatitis B and C infections and are the basis for definition of a sustained viral response to a particular antiviral regimen. Virus loads are also used to follow immunosuppressed patients with potential or established viral infections, including CMV, BK virus, and adenovirus. These may be used to assess whether the particular viral infection needs antiviral therapy or whether preemptive therapy might be desirable, as well as to monitor the effects of antiviral therapy, once instituted.

RESISTANCE TO ANTIVIRAL AGENTS

As is the case with antibacterial antibiotics, resistance to antiviral agents is also increasingly recognized as an important problem in antiviral therapy. The possibility of drug resistance is usually recognized because of a lack of clinical or virologic response to treatment. However, clinical failures of antiviral therapy may also involve drug-sensitive viruses in immunocompromised patients who are unable to mount effective host responses. Factors favoring the emergence of resistant variants include high viral replicative load, as in infections with prolonged and rapid viral turnover; high intrinsic viral mutation rates, which are generally greater in RNA than in DNA viruses; degree of selective drug pressure, which is higher with prolonged or repeated courses of drug therapy, particularly with suboptimal doses; and an antiviral target that can mutate without adversely affecting viral fitness. As a consequence, most drug-resistant viruses (including herpes simplex virus, varicella-zoster virus, CMV, and HIV-1) are recovered from immunocompromised patients, although resistant influenza A virus and hepatitis B and C viruses can be seen in immunocompetent individuals.

The consequences of the emergence of resistance may vary according to the specific virus and to the antiviral drug that is involved. If a resistant virus has similar replicative ability ("viral fitness") to the parent sensitive virus, then a failure of antiviral therapy may ensue and may be associated with prolonged or severe disease in immunocompromised hosts. If resistant variants are at some biologic disadvantage ("less fit") with respect to transmissibility, ability to establish chronic or latent infection, or ability to persist in the absence of selective drug pressure, emergence of resistance may have less overall impact or may be associated with an indolent clinical course.

Although the prevalence of resistant virus is generally related to increasing clinical use of drugs, the emergence and global spread of oseltamivir-resistant influenza A/H1N1 viruses in 2008, before the influenza A/H1N1 pandemic of 2009, did not appear to be related to concurrent oseltamivir use, nor did the subsequent emergence of resistance to the adamantanes. This highlights the limited understanding of factors associated with emergence of resistant strains. The laboratory selection of a drug-resistant strain of virus implies that the drug has a specific antiviral mechanism. The development of resistance results from mutations in the viral genome, and the presence of selective drug pressure leads to the emergence of a resistant virus population. Resistant subpopulations often exist naturally in clinical isolates, but resistant mutations can also arise during drug exposure. Single-nucleotide mutations leading to critical amino-acid substitutions in a target protein are often sufficient to cause antiviral resistance, as has been noted in HIV, hepatitis B and C, and influenza A virus infections.

COMBINATIONS OF ANTIVIRAL AGENTS

Combinations of antiviral agents with different mechanisms of action are used as a means to prevent or reduce the likelihood of development of drug resistance. Viral isolates from treated and even untreated patients may be genetically heterogeneous with respect to mixtures of sensitive and resistant viruses. They may also include viruses with different resistance mutations that affect similar mechanisms of action. In such cases, combinations of antiviral agents may provide broader activity than single agents alone. Combination antiviral therapy is now the standard of care in HIV and hepatitis C virus infections and is likely to become increasingly used in other viral infections.[18]

Combinations of antiviral agents have the potential advantage to increase antiviral activity by additive effects at the same site or at different tissue or cellular sites. This rationale has supported the use of combination therapy for particularly severe cases of viral infection, but evidence of clinical benefit is not available, other than for the infections noted earlier.

The use of combinations of antivirals also offers the opportunity to reduce individual drug dosage and perhaps reduce toxicity. However, combinations of antiviral agents may also result in increased toxicity, as is seen with combinations of interferon and ribavirin.

PHARMACODYNAMICS

Human pharmacokinetic studies that define absorption, stability in body fluids, tissue distribution, and metabolic fate of antiviral drugs are essential for selection of proper dosages and regimens of administration of antiviral agents. Data from such studies that describe relationships between antiviral drug concentrations in blood or other body fluids and clinical effects or toxicities, are not nearly as extensive as those compiled for antibacterial agents. However, such data for various antiviral agents are now becoming increasingly available.

The most extensive studies of pharmacodynamics of antiviral agents have been conducted with antiretroviral drugs. These have associated peak plasma concentration (C_{max}), trough plasma concentration (C_{min}), as well as area under the concentration-time curve (AUC) with virologic responses to antiretroviral agents (see Chapter 19). The AUC has been correlated with virologic effects for protease inhibitors,[32,33] including atazanavir, darunavir, nelfinavir, and lopinavir, as well as for the inhibitor of CCR5 binding by maraviroc.[34] Relationships of C_{min} with virologic responses have been noted for the NNRTIs nevirapine[35] and efavirenz.[36] Defining concentration-effect relationships between nucleoside analogue reverse-transcriptase inhibitors has been difficult because those drugs require intracellular phosphorylation, which may be impacted by different intracellular phosphorylation activities in different cell types and under different metabolic conditions.

Pharmacodynamic relationships have also been established for antiviral treatment of certain herpesvirus infections. Foscarnet, a pyrophosphate analogue that inhibits viral DNA polymerases of CMV and other herpesviruses, has a correlation of AUC with treatment effects and with development of toxicity.[37] In hepatitis C virus infection, virologic suppression by boceprevir and telaprevir is closely related to C_{min} concentrations.[38,39]

Topical application of an antiviral agent to the cornea, skin, mucous membranes, or respiratory tract is intended to provide high concentrations at the site of infection and to avoid the possible toxicity of systemic administration. However, topically applied drugs must be able to penetrate such barriers as stratified epithelium or local secretions to reach the site of active viral replication.

Progress in the development of antiviral drugs has been extraordinary during recent years. It has been accompanied by advances in understanding of the appropriate clinical use of such drugs for antiviral therapy and prophylaxis. Despite this success, antivirals are not yet available for the vast majority of viral infections. Thus this promises to be an area of continued major research activity, building on what has been learned from the considerable accomplishments thus far.

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45 Antiviral Drugs for Influenza and Other Respiratory Virus Infections

Fred Y. Aoki

SHORT VIEW SUMMARY

INFLUENZA A AND B

Neuraminidase Inhibitor Drugs

- Oseltamivir
  - Most influenza A and B viruses are inhibited in vitro at concentrations achievable with standard doses in patients.
  - Drug of choice for prevention and treatment of patients of all ages with influenza A or B.
  - Administered orally and well absorbed even in critically ill patients.
  - Effective in prevention and treatment of uncomplicated influenza in otherwise healthy adults.
  - Therapeutic efficacy is optimal when therapy is initiated as soon as possible after symptom onset.
  - Efficacy for treatment of those with avian influenza uncertain.
  - Observational studies during the 2009 swine flu pandemic indicate it is beneficial up to 5 days after symptom onset in patients with serious illness, including pregnant women.
  - Side effects are modest and primarily gastrointestinal.
  - Substantial data exist on its safety for the pregnant woman and her fetus based on use during the 2009 swine flu pandemic.
  - Failure due to selection of resistant mutants is not uncommon during prolonged treatment of immunocompromised patients.
- Peramivir
  - Intravenously administered neuraminidase inhibitor.
  - Shares cross-resistance of influenza A viruses resistant to oseltamivir.
  - Efficacious as a single-dose intravenous (IV) treatment for uncomplicated influenza in adult patients with symptomatic disease of no more than 2 days' duration.
  - Well tolerated.
- Zanamivir
  - Orally inhaled powder similar to oseltamivir in effectiveness. Active against oseltamivir-resistant strains.
  - May lead to bronchospasm in individuals with asthma or chronic obstructive pulmonary disease.
  - Efficacy of an experimental IV formulation of zanamavir based on case reports and a controlled trial comparing it with oral oseltamivir in hospitalized patients remains uncertain.

Endonuclease Inhibitor Drug

- Baloxavir
  - Inhibits cap-dependent endonuclease of influenza RNA-dependent RNA polymerase.
  - Active against multiple influenza A subtypes and influenza B, including oseltamivir-resistant strains.
  - A single dose of baloxavir was as efficacious as 5 days of oseltamivir, with greater effect in virus shedding.
  - Posttreatment viruses with reduced baloxavir susceptibility developed in 10% of baloxavir recipients and in no placebo recipients.
  - Place in influenza therapeutics is evolving.
  - Licensed in Japan in February 2017.

INFLUENZA A

Investigational Agents

- Laninamivir
  - Orally inhaled neuraminidase inhibitor with prolonged presence and effect in the respiratory tract.
  - Therapeutic efficacy remains uncertain.
  - A single dose inhaled once daily for 1 to 3 days prevents infection in household contacts of index cases when initiated within 48 hours of onset in the index case.
- Favipiravir
  - Inhibits a wide spectrum of RNA viruses in vitro and in vivo in animals.
  - Polymerase inhibitor.
  - Orally administered.
  - Clinical trials are underway, including in patients with influenza and Ebola virus infection.

Adamantanes

- Amantadine and rimantadine
  - Widespread resistance is present in currently circulating influenza A viruses, so the adamantanes should not be used unless sensitivity of isolates is demonstrated.
  - Orally administered and effective against uncomplicated influenza A.
  - Effectiveness in serious illness is not established.
  - Toxicity with amantadine is primarily central nervous system symptomatology, and with rimantadine is gastrointestinal intolerance.

RESPIRATORY SYNCYTIAL VIRUS AGENTS

- Ribavirin
  - A guanosine analogue with activity against a broad variety of viruses, including respiratory syncytial virus (RSV) and influenza.
  - Approved for aerosol administration to children hospitalized with RSV pneumonia or bronchiolitis.
  - Has been administered orally and intravenously to treat respiratory RSV and other virus infections in immunosuppressed patients.
  - Teratogenic, so aerosolized ribavirin should not be used near potentially pregnant staff.
- RSV604
  - An investigational agent that inhibits RSV through interaction with the nucleocapsid protein.
  - Well absorbed orally, and phase II studies are underway.

PARAINFLUENZA VIRUSES AGENT

- DAS181 (Fludase)
  - An investigational compound with activity against parainfluenza and influenza viruses.
  - An orally inhaled sialidase, it reduces virus binding to epithelial cells.
  - Has been used to treat parainfluenza 3 infections in immunosuppressed patients.
  - Development of adverse respiratory side effects may limit treatment to fewer than 7 to 10 days.
  - Development of antibodies may preclude repeated treatment courses.

TABLE 45.1 Antiviral Agents of Established Therapeutic Effectiveness for Respiratory Virus Infection

VIRAL INFECTION	DRUG	ROUTE	USUAL ADULT DOSAGE
Influenza Viruses			
Influenza A and B viruses	Oseltamivir	PO	75 mg bid for 5 d[a]
	Peramivir	IV	300 or 600 mg once
	Zanamivir	Inhalation	10 mg bid by inhaler for 5 d[b]
	Laninamivir octanoate	Inhalation	40 mg once[c]
Influenza A virus	Amantadine	PO	100 mg bid for 5 d for treatment[d]
	Rimantadine	PO	100 mg bid for 5 d for treatment[e]
Respiratory Syncytial Virus			
	Ribavirin	Aerosol	Aerosol treatment 18 h/d for 3–7 d[f]

Note: Please consult text and manufacturer's product prescribing information for dosage adjustments in renal or hepatic insufficiency and in other circumstances.
[a]Pediatric dosages: For infants 2 wk to <1 yr of age, dose is 3 mg/kg twice daily. For children ≥1 yr of age, doses are weight adjusted: 30 mg bid for <15 kg, 45 mg bid for 16–23 kg, 60 mg bid for 24–40 kg, and 75 mg bid for >40 kg. Prophylactic dosage is given once daily (one-half of total daily treatment dosage). Not US Food and Drug Administration (FDA) approved currently for prophylaxis in children <1 yr old or treatment in children <2 wk old.
[b]FDA approved at same dosage for treatment of children ≥7 yr. Prophylactic dose is 10 mg inhaled once daily for adults and children ≥5 yr old.
[c]Adult dose and for children ≥10 yr old. Pediatric dose: 20 mg once for children <10 yr of age.
[d]Maximum recommended dosage for older adults (≥65 yr old) is 100 mg/d. Recommended pediatric dosage is 5 mg/kg/d up to a maximum of 150 mg/d in divided doses. For prophylaxis, the same daily dosage should be given for period of risk.
[e]Pediatric dosage is 5 mg/kg up to a maximum of 150 mg/d in divided doses. Not approved by FDA for treatment in children <13 yr old. For prophylaxis, same daily dosage should be given for period of risk.
[f]Reservoir concentration of 20 mg/mL. Special aerosol-generating device (available from manufacturer) and expert respiratory therapy monitoring for administration are required. Higher reservoir concentration (60 mg/mL) given for 2 h tid is an alternative.

This chapter reviews antiviral agents against influenza viruses and certain other respiratory viruses, such as parainfluenza virus and respiratory syncytial virus (RSV) (Table 45.1). The antivirals are presented in alphabetical order in the text of the chapter and include licensed (approved) as well as investigational agents. Agents that have been investigated in rhinovirus infections but have been utilized primarily in non–respiratory tract infections, such as interferons and pleconaril, are discussed in Chapter 48.

AMANTADINE AND RIMANTADINE

Spectrum

Amantadine (1-adamantanamine hydrochloride; Symmetrel) and rimantadine (α-methyl-1-adamantane methylamine hydrochloride; Flumadine) are symmetrical tricyclic amines (Fig. 45.1) that specifically inhibit the replication of influenza A viruses at low concentrations (<1 μg/mL). Influenza B and C viruses are resistant.[1] In the past, epidemic human and avian strains of influenza viruses have generally been susceptible to amantadine.[2] However, since 2008–2009, influenza A(H1N1) and A(H3N2) isolates, the highly pathogenic avian H5N1 isolate, and the influenza A(H1N1)pdm09 isolate have become resistant to amantadine and rimantadine (discussed further under "Resistance").[3] By plaque assay, inhibitory concentrations of the drugs range from 0.1 to 0.4 μg/mL or less for sensitive human influenza A viruses. Rimantadine is 4 to 10 times more active than amantadine in some assay systems. Both drugs inhibit virus containing the M protein from the 1918 pandemic strain.[4]

Higher concentrations (10–50 μg/mL) inhibit other enveloped viruses in vitro, including parainfluenza, influenza B, rubella, dengue, several arenaviruses (Junin, Lassa, Pinchindé), rabies, and African swine fever virus, but these concentrations are not achievable clinically and can be cytotoxic in vitro.[5] Rimantadine has pH-dependent trypanocidal activity at concentrations of approximately 1 μg/mL[6]; amantadine at the same concentration in combination with doxycycline inhibits *Coxiella burnetii*.[7] Amantadine may transiently inhibit hepatitis C virus (HCV) replication in humans.[8]

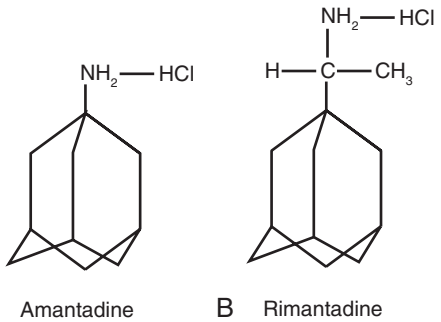

FIG. 45.1 Chemical structures of amantadine hydrochloride (A) and rimantadine hydrochloride (B).

These agents have prophylactic and therapeutic activity in experimental influenza A virus infection of animals after oral or parenteral dosing. Combinations of M2 protein inhibitors and neuraminidase inhibitors (NAIs) and ribavirin show enhanced antiviral and therapeutic effects in vitro or in animal models of influenza.[9–12]

Mechanism of Action

Amantadine and rimantadine share two concentration-dependent mechanisms of antiinfluenza action. Low concentrations inhibit the ion channel function of the M2 protein of influenza A viruses, which affects two different stages in virus replication.[13–15] The primary effect involves inhibition of viral uncoating or disassembly of the virion during endocytosis. For subtype H5 and H7 viruses, a late effect on hemagglutinin maturation and viral assembly is presumably mediated through altered pH regulation of the *trans*-Golgi network. Amantadine and rimantadine block proton permeation and prevent M2-mediated changes in pH. This action probably accounts for inhibition of the acid-mediated dissociation of the matrix protein from the ribonucleoprotein complex within endosomes early in replication and potentiation of acidic pH–induced alterations in the hemagglutinin during its transport late in infection.

Amantadine and rimantadine are also concentrated in the lysosomal fraction of mammalian cells. Drug-mediated increases in lysosomal pH may inhibit virus-induced membrane fusion events and account for the broader antiviral spectrum at higher concentrations. In contrast, the selective anti–influenza A virus effects are quickly lost after removal of the drug from the surrounding medium, which suggests that drug must be present in extracellular fluid early in the replicative cycle.

Amantadine inhibits the ion channel activity of expressed HCV p7 protein at low concentrations,[16] an effect that might account for its reported anti-HCV effects in vivo. Neither amantadine nor rimantadine inhibit HCV enzyme functions or internal ribosome entry in biochemical assays.[17]

Resistance

Amantadine-resistant virus is readily selected by virus passage in the presence of drug. Resistance with more than 100-fold increases in inhibitory concentrations has been associated with single amino acid substitutions at critical sites (positions 26, 27, 30, 31, and 34) in the transmembrane region of the M2 protein.[13] Analysis of 32,251 global M2 protein sequences from 1902 to 2013 for mutations known to mediate adamantane resistance revealed that 45% of influenza A virus H1 through H17 subtypes circulating globally are resistant. The vast majority of resistant viruses possess the S31N M2 protein mutation and about 1% possess the V27A mutation. H1, H3, H5, H7, H9, and H17 subtype influenza A viruses exhibit high-level adamantane resistance. Resistance mutants in H2, H4, H6, H10, and H11 subtypes were rare.

TABLE 45.2 Clinical Pharmacokinetic Characteristics of Amantadine and Rimantadine in Healthy Adults

	AMANTADINE		RIMANTADINE	
CHARACTERISTIC	**Young**	**Elderly**	**Young**	**Elderly**
Relative oral bioavailability (%)	62–93	53–100	75–93	NA
V_d (L/kg) at 200 mg/d	6.1 ± 2.1	3.6 ± 1.1	18.4 ± 9.6	11.5 ± 2.9
Plasma protein binding (%)	67	NA	40	NA
Clearance (mL/min/kg)				
Plasma or total	5 ± 2.1	2 ± 0.9	6.1 ± 1.9	4.7 ± 2
Renal	6.4 ± 3.7	2 ± 1.1	1.2 ± 0.4	NA
Nonrenal	0	0	6.4 ± 1.4	NA
Urinary excretion of unchanged drug (%)	62–93	53–100	8.3–43	NA
Plasma half-life (h)	14.8 ± 6.2	26.1 ± 9.7	29.1 ± 9.7	36.5 ± 14.5
Therapeutic range (ng/mL)				
C_{max}				
200 mg/d	475 ± 110	—	416 ± 108	447 ± 108
100 mg/d	—	362 ± 158	—	—
C_{trough}				
200 mg/d	302 ± 80	—	300 ± 75	310 ± 87
100 mg/d	—	301 ± 75	—	—

NA, Not available; *V_d,* volume of distribution.
Modified from Hayden FG, Aoki FY. Amantadine, rimantadine, and related agents. In: Yu VL, Edwards D, McKinnon S, et al., eds. Antimicrobial Therapy and Vaccines. *2nd ed. Pittsburgh, PA: E Sun Technologies; 2002:714.*

No adamantane-resistant mutants were identified in H8 or H12 through H16 subtypes.[18]

Amantadine and rimantadine share cross-resistance. In avian models, resistant viruses are virulent, genetically stable, and able to compete with wild-type virus so that transmission of drug-resistant virus may occur after cessation of drug use.

Before 2003, a small percentage of untreated patients (<1%) had infection with resistant influenza A virus.[19] However, approximately 30% of drug-treated ambulatory children and adults and 80% of hospitalized children or immunocompromised patients shed resistant virus.[20–22] Immunocompetent individuals shedding resistant virus resolve their illness promptly,[23] whereas immunocompromised hosts may experience prolonged illness associated with persistent virus shedding.[21] Transmission of M2 inhibitor–resistant virus, associated with failure of drug prophylaxis, occurs in household contacts of treated index cases[24] and in nursing home residents.[25] Resistant variants can cause typical influenza illness. It is prudent to avoid contact between treated patients and susceptible high-risk contacts, and to avoid use of treatment (specifically of young children) and postexposure prophylaxis in the same household.

Globally, up to 2003, epidemic influenza A H1N1 and H3N2 strains were M2 inhibitor sensitive. Since 2003, the prevalence of amantadine resistance has increased progressively, although rates vary by virus type and geography.[26,27,28] Among H3N2 isolates, amantadine resistance increased from 12% worldwide in 2003[26] to 91% by 2005 and greater than 95% in 2008–2009.[27] In the United States prior to March 2009, nearly all of the A(H1N1) isolates tested were sensitive to the adamantanes, but subsequently virtually all A(H1N1) isolates have been resistant, including the A(H1N1)pdm09 virus.[29] Among nonpandemic A(H1N1) isolates, the prevalence of amantadine resistance was 4% in 2004–2005 worldwide and 16% in isolates from 2005–2006, with rates ranging from 2% in South Korea to 72% in China.[27,30,31] The reason for the emergence and global spread of amantadine-resistant strains is unclear. Widespread inappropriate use of amantadine[32] and acquisition of undefined advantageous mutations combined with lack of fitness impairment may have been contributing factors. Ribavirin and the NAIs zanamivir and oseltamivir carboxylate inhibit M2 inhibitor–resistant strains.

Adamantane derivatives have been developed that inhibit amantadine-resistant and amantadine-sensitive influenza virus in vitro[33,34] and in vivo in animals with induced influenza.[33]

The triple combination of amantadine, oseltamivir, and ribavirin impedes the selection of drug-resistant influenza A virus in vitro at clinically achievable concentrations[35] compared to double combinations and the agents used singly in vitro.[36] The same combination of drugs was also synergistic in vitro in inhibiting the growth of both amantadine- and oseltamivir-resistant influenza A virus strains at concentrations that had no activity as single agents.[36] The clinical efficacy of this triple combination has been studied (see "Clinical Studies" later).[37]

Pharmacokinetics

The clinical pharmacokinetic characteristics of amantadine and rimantadine are shown in Table 45.2.

Amantadine

Amantadine is well absorbed after oral administration of capsule, tablet, or syrup forms.[5] Steady-state peak plasma concentrations average 0.5 to 0.8 μg/mL with a 100-mg twice-daily regimen in healthy young adults. Older adults require only one-half of the weight-adjusted dosage needed for young adults to achieve equivalent trough plasma levels of 0.3 μg/mL. Plasma protein binding of amantadine is about 67%, and amantadine's volume of distribution is large (4–5 L/kg). Nasal secretion and salivary levels of amantadine approximate those found in the serum. Cerebrospinal fluid (CSF) levels are 52% to 96% of those in plasma, and amantadine is excreted in breast milk.

Amantadine is eliminated largely unchanged in the urine by glomerular filtration and probably by tubular secretion by a bicarbonate-dependent organic cation transporter.[38] The plasma elimination half-life ($t_{½elim}$) is about 12 to 18 hours, ranges widely, and correlates with creatinine clearance (CrCl). Because of age-related declines in renal function, $t_{½elim}$ increases twofold in older adults and even more in patients with impaired renal function. Dosage reductions are required in renal insufficiency (see "Toxicity" later). Amantadine is inefficiently cleared in patients receiving hemodialysis or continuous ambulatory peritoneal dialysis, and additional doses are not required. Monitoring of plasma concentrations in such patients is desirable, but impractical.

Amantadine pharmacokinetics remained unaffected by concurrent administration of oseltamivir and ribavirin in healthy adult volunteers or stable immunocompromised patients.[39]

Rimantadine

Rimantadine is well but slowly absorbed, with the time to peak plasma concentration averaging 2 to 6 hours. Absorption does not seem to be decreased by food. With multiple doses of 100 mg twice daily, the steady-state peak and trough plasma concentrations in healthy adults are approximately 0.4 to 0.5 μg/mL and 0.2 to 0.4 μg/mL, respectively. In infants receiving dosages of 3 mg/kg each day, peak serum levels

range from 0.1 to 0.6 µg/mL. No important age-related changes in pharmacokinetics have been found in healthy older adults or in children. However, steady-state plasma concentrations in older nursing home residents receiving 100 mg twice daily average more than twofold higher (mean 1.2 µg/mL) than concentrations observed in healthy adults, which indicates the need for lower dosages in these patients. Plasma protein binding is about 40%. Rimantadine has a very large volume of distribution (about 12 L/kg), and concentrations in nasal mucus average 50% higher than those in plasma.

In contrast to amantadine, rimantadine undergoes extensive metabolism by hydroxylation, conjugation, and glucuronidation before renal excretion.[5] The plasma $t_{½elim}$ of rimantadine averages 24 to 36 hours. No clinically important changes in pharmacokinetics are found in patients with chronic liver disease without significant hepatocellular dysfunction. In hemodialysis patients with severe renal failure, the clearance of rimantadine is decreased by 40%, and the $t_{½elim}$ is about 55% longer. Reducing dosages by one-half (e.g., to 100 mg/day) is recommended for marked hepatic or renal insufficiency (CrCl <10 mL/min). Hemodialysis removes only a small amount of rimantadine, so supplemental doses are not required.

Interactions

The risks of central nervous system (CNS) adverse effects with amantadine and possibly with rimantadine are increased by concomitant ingestion of antihistamines, antidepressants, anticholinergic drugs, and other drugs affecting CNS function. Concurrent use of trimethoprim-sulfamethoxazole or triamterene-hydrochlorothiazide has been associated with CNS toxicity resulting from decreased renal clearance of amantadine. Cimetidine is associated with 15% to 20% increases, and aspirin or acetaminophen is associated with 10% decreases, in plasma rimantadine concentrations, but such changes are unlikely to be significant. Neither adverse clinical nor adverse pharmacokinetic effects are observed when amantadine and oseltamivir are coadministered.[40]

Concurrent administration of recommended doses of amantadine, oseltamivir, and ribavirin for 10 days was well tolerated.[39]

TABLE 45.3 Amantadine Dosage Regimens for Treatment in Patients With Normal and Reduced Renal Function

CONDITION	SUGGESTED DOSAGE
No Renal Insufficiency	
Children 1–9 yr	5 mg/kg/d in 2 divided doses, ≤150 mg/d
Ages 10–64 yr	100 mg q2d
Ages ≥65 yr	100 mg qd[a]
Creatinine Clearance (mL/min/1.73 m²)[b]	
≥75	100 mg (1.4 mg/kg) twice daily
74–35	100 mg qd
34–25	100 mg q2d
24–15	100 mg q3d
<15	100 mg q7d
Older Adults and Creatinine Clearance (mL/min/1.73 m²)[c]	
≥80	100 mg qd
60–79	100 mg and 50 mg on alternate days
40–59	100 mg q2d
30–39	100 mg twice/wk
20–29	50 mg three times/wk
10–19	100 mg and 50 mg on alternate wk

[a]Use weight-adjusted dosing for smaller patients (<50 kg). Dosages of 1.4 mg/kg/d have been suggested.[5]
[b]Based on adult dosage of 200 mg/d. Proportionate reductions should be made for older adults receiving lower dosages and for children.
[c]This dosing schedule for older adults with renal insufficiency is taken from the Canadian guidelines and has been found to be reasonably well tolerated.[49]
Modified from Wu MJ, Ing TS, Soung LS, et al. Amantadine hydrochloride pharmacokinetics in patients with impaired renal function. Clin Nephrol. *1982;17:19–23.*

Toxicity

Amantadine or rimantadine given in treatment courses of 5 days is generally well tolerated in young healthy adults.[41] Longer periods of administration, such as 6 weeks for seasonal prophylaxis in young adults,[41] or administration to fragile, elderly nursing home residents such as octogenarians for 10 days for outbreak control, are associated with a significant frequency of adverse reactions and drug withdrawals.[42]

A case-control study demonstrated that in children less than 12 months of age, amantadine and rimantadine were well tolerated, as was oseltamivir.[43] No evidence of adverse maternal or neonatal outcomes was observed after influenza antepartum treatment with adamantanes.[44]

The most common side effects related to amantadine ingestion are minor, dose-related gastrointestinal and CNS complaints, including nervousness, lightheadedness, difficulty concentrating, confusion, insomnia, and loss of appetite or nausea.[45] Complaints typically develop within the first week of administration, often resolve despite continued ingestion, and are reversible on drug discontinuation. Central nervous system side effects occur in approximately 5% to 33% of amantadine recipients at dosages of 200 mg/day, but are significantly less frequent with rimantadine. When amantadine is used for influenza prophylaxis in ambulatory adults, dosages of 200 mg/day are associated with excess withdrawals in 6% to 11% of recipients because of drug side effects. Dosages of 100 mg/day are better tolerated and may be protective against influenza illness. Amantadine dosage reductions are required in older adults (100 mg/day), but 20% to 40% of nursing home residents experience significant adverse effects on this lower dosage despite some adjustment for renal insufficiency.[46–48] Consequently, further dosage reductions based on CrCl are warranted in this population[49] (Table 45.3).

In the setting of renal insufficiency or high dosages, serious neurotoxic reactions, including delirium, hostility, hallucinations, tremor, myoclonus, seizures, or coma; cardiac arrhythmias; and death can occur in association with elevated amantadine plasma concentrations (1–5 µg/mL).[50] Neurotoxic reactions may be transiently reversed by physostigmine administration, and lidocaine has been used to treat ventricular arrhythmias. Long-term amantadine ingestion has been associated with livedo reticularis, livedo racemose,[51] peripheral edema, orthostatic hypotension, and, rarely, congestive heart failure, vision loss, corneal edema,[52] or urinary retention. Peripheral edema and livedo reticularis may improve if treatment is switched from amantadine to rimantadine.[53] Patients with preexisting seizure disorders have an increased frequency of major motor seizures during amantadine use, and dosage reductions are advised. Psychiatric side effects in patients with Parkinson disease and psychotic exacerbations in patients with schizophrenia may occur with addition of amantadine. Rash and leukopenia have been described rarely.

Rimantadine administration is associated with dose-related side effects similar to those observed with amantadine, although the risk for CNS side effects is lower with rimantadine at dosages of 200 or 300 mg/day in ambulatory adults.[5] During prophylaxis, excess withdrawal rates are usually less than 5%. In older nursing home residents, dosages of 200 mg/day are associated with higher side effect rates, whereas dosages of 100 mg/day seem to be better tolerated.[46,54] Rimantadine may uncommonly cause exacerbations of seizures in patients not receiving anticonvulsants, and was associated with an unexplained excess mortality in one nursing home study.[54]

The clinical observations of dry mouth, pupillary dilation, toxic psychosis, and urinary retention in acute amantadine overdose suggest that anticholinergic activity is present in humans. Amantadine shows activity on the adrenergic nervous system by affecting accumulation, release, and reuptake of catecholamines in the CNS and in the peripheral nervous system. Malignant ventricular arrhythmia after amantadine overdose has been described in humans.

Amantadine and rimantadine lack mutagenicity in vitro; carcinogenicity studies have not been reported for either. Amantadine is teratogenic and embryotoxic in rats, and rimantadine may cause teratogenic effects in rabbits and maternal toxicity and embryotoxicity at high dosages in

rodents. Both drugs are classified as pregnancy category C. Birth defects have been reported after amantadine exposure during pregnancy.[55] The safety of neither amantadine nor rimantadine has been established in pregnancy. Because of excretion in breast milk, use is not recommended in nursing mothers.

Clinical Studies

Influenza A

Amantadine and rimantadine have been efficacious for the prevention and treatment of influenza A virus infections in young healthy adults.[5,45,56] A systematic review of published studies in children and the elderly concluded that available data only demonstrate amantadine prophylactic efficacy and a modest therapeutic effect in children.[57] In the elderly, no data were available to support a conclusion of prophylactic or therapeutic efficacy of either adamantane. The emergence of widespread and nearly complete amantadine resistance among influenza A(H3N2) isolates,[27] as well as the amantadine resistance of the pandemic A(H1N1)pdm09 strain, precludes the empirical use of adamantanes for management of untyped influenza A outbreaks. Amantadine and rimantadine, both at a dosage of 200 mg/day in adults, are about 70% to 90% protective against clinical illness caused by various susceptible influenza A subtypes, including susceptible pandemic strains.[58] Prophylaxis is effective in preventing nosocomial influenza and possibly in curtailing nosocomial outbreaks caused by such strains. Protection seems to be additive to that provided by vaccine.[59]

Rimantadine was less effective than zanamivir in reducing cases of influenza A illness in adults in a long-term care facility.[60] The difference in protective efficacy was largely due to the emergence of rimantadine-resistant viruses that caused rimantadine prophylactic failure; no zanamivir-resistant viruses were isolated. Rimantadine administration to school-age children (5 mg/kg/day) decreased the risk for influenza A illness in recipients and possibly in their family contacts. Postexposure prophylaxis with these drugs provided inconsistent protection to family contacts, however, in part depending on whether ill index children were treated.[20] A dosage of 100 mg/day seems to protect against influenza A illness and is well tolerated in adults.[61]

Amantadine and rimantadine are also effective therapies for uncomplicated adamantane-susceptible influenza A illness in healthy adults,[5,23] but it is uncertain whether treatment reduces the risk for complications in high-risk patients or is useful in patients with established pulmonary complications. Early treatment in ambulatory adults (200 mg/day for 5 days) reduces the duration of fever and systemic complaints by 1 to 2 days, decreases virus shedding, and shortens time to resumption of usual activities.[23] In illness caused by H3N2-subtype influenza viruses, certain abnormalities of peripheral airways function, but not of airway hyperreactivity, resolve more quickly in amantadine-treated patients. Amantadine or rimantadine treatment in adults with leukemia or stem cell transplantation may reduce the risk for pneumonia,[62] but more recent data suggest that in stem cell transplant recipients, early NAI therapy may be preferred to adamantanes because it may prevent progression to pneumonia and decrease viral shedding, thereby possibly preventing both influenza-related death in index patients and nosocomial transmission to others.[63] In children, rimantadine treatment is associated with lower symptom burden, fever, and viral titers during the first 2 days of treatment compared with acetaminophen administration, but rimantadine-treated children have more prolonged shedding of virus. Treatment generally does not seem to affect immune responses to infection but may blunt secretory antibody levels.[64]

In a randomized controlled trial of 633 individuals,[37] 316 received the combination of amantadine (100 mg), oseltamivir (75 mg), and ribavirin (600 mg), and 317 received oseltamivir (75 mg) each twice daily for 5 days. Of evaluated patients, 80 of 200 (40%) in the combination group and 97 of 194 (50%) in the monotherapy group had influenza detectable by polymerase chain reaction (PCR) ($P = .046$) at day 3, but there were no differences in secondary clinical end points such as median duration of symptoms (4.5 days in the combination group and 4.0 days in the monotherapy group; $P = .21$).

Intermittent aerosol administration of amantadine or rimantadine seems to be therapeutically useful in uncomplicated influenza. No injectable formulation of either drug is available in the United States.



FIG. 45.2 Molecular model of DAS181. The catalytic domain of the *Actinomyces viscosus* sialidase is colored in green and the protruding amphiregulin anchoring domain on the C terminus in blue. *(From Malakhov MP, Aschenbrenner LM, Smee DF, et al. Sialidase fusion protein as a novel broad-spectrum inhibitor of influenza virus infection.* Antimicrob Agents Chemother. *2006;50:1470.)*

Other Viruses

Amantadine has been used in multiple trials for treatment of chronic hepatitis C with inconsistent evidence for increases in sustained viral response (SVR). In treatment-naïve patients, the addition of amantadine (200 mg daily in single or divided doses) to interferon (IFN)[65,66] or to IFN plus ribavirin[67] may modestly increase biochemical responses and the likelihood of SVR. In re-treatment of IFN nonresponders, the combination of IFN plus amantadine is ineffective,[68] but the addition of amantadine to the combination of IFN plus ribavirin may be associated with SVR in 10% to 25%.[69] Amantadine plus combined pegylated IFN (PEG IFN) and ribavirin may increase SVR modestly in treatment-experienced patients compared with PEG IFN plus ribavirin.[70] Reports of possible activity in *Bornavirus* infections and associated neuropsychiatric symptoms require confirmation.

DAS181 (FLUDASE)

DAS181 is an orally inhaled investigational antiviral agent with activity against influenza A and B viruses and parainfluenza viruses types 1, 2, and 3.[71–75] It has a novel mechanism of action in that it is a sialidase from *Actinomyces viscosus* (Fig. 45.2), linked to a respiratory epithelium-anchoring domain.[76] It cleaves the terminal sialic acid residues on the surface of human respiratory cells, thus reducing the binding of respiratory viruses, which use those as receptors. Desialylation is rapid and results in an antiviral effect, which lasts for at least 2 days.[77] The 50% effective concentration against influenza A and B viruses ranged from 0.04 to 0.9 nM.[71] DAS181 is active against influenza viruses that are resistant to NAIs.[78] Low-level resistance to DAS181 can be induced, but resistant variants appear to be reduced in fitness.[79]

In volunteers, adverse respiratory events developed after 7 days of therapy. Rapidly declining serum levels of drug appeared thereafter, consistent with the induction of DAS181 antibodies, which were detected in 15 of 18 subjects in day 30 serum samples. These effects could preclude use of this medication for longer than 7 days or for repeated courses.[80] In a phase II placebo-controlled trial in 177 subjects with influenza A and B virus infections, DAS181 was administered either as a single 10-mg dose or as a daily 10-mg dose for 3 days.[81] Compared to placebo recipients, DAS181 recipients had a statistically significant decrease in virus load determined by PCR between days 1 and 3 and days 1 and 5. However, there were no differences in resolution of clinical illness among the groups. Treatment for up to 3 days was well tolerated, although transient elevations in alkaline phosphatase were seen.[81]

DAS181 has also been utilized to treat parainfluenza virus type 3 infections in lung transplantation and stem cell transplantation

patients.[82,83] These case reports described clinical improvement, increased pulmonary function, and decreased virus loads. Additional clinical studies of DAS181 are being planned.

FAVIPIRAVIR (T-705)

Favipiravir (5-fluoro-2-hydroxypyrazine-3-carboxamide; T-705) is a potent broad spectrum inhibitor of influenza and other RNA viruses[84] (Fig. 45.3). In 2014 in Japan, a tablet formulation was approved as Avigan, an antiinfluenza drug with a mechanism of action different from those of marketed M2 inhibitors (adamantanes) and NAI drugs. It was approved for release at the request of the Minister of Health, Labor and Welfare of Japan for patients infected with novel or reemerging influenza viruses.

Favipiravir is a prodrug that is converted to the antiviral molecule T-705-4-ribofuranosyl-5-triphosphate (T-705) intracellularly.[85] T-705 inhibits RNA virus replication by selectively inhibiting RNA-dependent RNA polymerase and causing RNA chain termination after incorporation into the nascent RNA strand. T-705 inhibits influenza A/PR/8/34 (H1N1),[86] A(H1N1)pdm09,[87] A(H3N2),[87] A(H5N1),[88] A(H7N9),[87] and B viruses[87] both sensitive and resistant to oseltamivir. T-705 plus oseltamivir synergistically inhibit H1N1, H3N2 and H5N1 in vivo,[89,90] and prolong the treatment window for H5N1 infection in mice and in vitro.[91] Combinations of favipiravir and peramivir are more effective for treating pandemic influenza A/California/04/2009 (H1N1) virus infections in mice than suboptimal doses of each compound alone.[92] In 57 pairs of different influenza viruses isolated from patients pre– and post–T-705 administration, no instances of reduced susceptibility to T-705 or neuraminidase inhibition were observed.[87]

Few data on T-705 pharmacokinetics have been published. In hamsters, induced arenavirus hemorrhagic fever infection reduced T-705 plasma concentrations,[93] presaging what was observed in 66 T-705–treated patients with Ebola virus infection.[94] T-705 inhibits acetaminophen metabolism, but the effect is unlikely to be of clinical importance.[95] It does not prolong the QT interval after a single dose of 2400 mg.[96]

The use of T-705 for treatment of Ebola virus infection is discussed in Chapter 164.

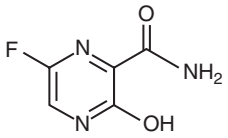

FIG. 45.3 **Chemical structure of favipiravir (T-705, Avigan).**

LANINAMIVIR OCTANOATE

Laninamivir octanoate (Inavir) is an investigational drug except for its approval in Japan. It is the prodrug of laninamivir, an inhibitor of influenza A and B neuraminidases.[97] Laninamivir is derived from zanamivir by replacement of its 7-OH group by a methoxy group.[98] Laninamivir is (2R,3R,4S)-3-acetamido-2-[(1R,2R-2,3-dihydroxy-1-metoxypropyl]-4-guanidino-3,4-dihydro-2H-pyran-6-carboxylic acid (Fig. 45.4D). Laninamivir octanoate consists of an octanoic acid ester side chain attached at the C_3 position of laninamivir. Laninamivir octanoate and polymeric zanamivir conjugates share the pharmacokinetic characteristic of persisting for a prolonged period in the respiratory tract after administration intranasally or intratracheally in animals or by oral inhalation in humans. These observations have presaged therapeutic effects of a single dose in animals with experimentally induced influenza and in patients with naturally acquired infection.

Spectrum

Laninamivir octanoate exhibits 1/30th to 1/50th of the influenza virus neuraminidase inhibitory activity of laninamivir in vitro.[99] However, its hydrolysis product is a potent inhibitor of neuraminidases of N1 to N9 influenza A viruses plus influenza B virus and their replication in cell culture at nanomolar concentrations.[100] These include seasonal and pandemic influenza A(H1N1), highly pathogenic avian influenza (HPAI) H5N1 viruses, and clinical isolates of oseltamivir-resistant A(H1N1), A(H3N2), A(H5N1), and A(H1N1)pdm09. Median inhibitory concentrations in cell culture vary over a wide range and in general appear to be intermediate between those of oseltamivir carboxylate (lower) and zanamivir (higher), but the clinical importance of these differences is not yet known.

In preclinical studies, laninamivir octanoate reduced fever in ferrets, mortality in mice, and virus concentrations in lung in ferrets and mice and in brain in mice after induced influenza with a variety of viruses: A/PR/8/34, HPAI H5N1, A(H1N1)pdm09, and B/Malaysia/2506/2004 as well as oseltamivir-resistant A(H1N1) and HPAI H5N1 clinical isolates possessing the H274Y mutation, as reviewed by Yamashita.[100] In these studies, laninamivir octanoate was administered as a single intranasal dose following intranasal inoculation of virus and was as, or more, efficacious than multiple doses of oral oseltamivir or intranasal zanamivir. The results of these studies in animals with experimental influenza have been replicated in part in therapeutic trials of a single laninamivir octanoate dose in the clinic (see "Clinical Studies" later).

Single doses of laninamivir octanoate are also efficacious prophylactically in mice. One dose prevents mortality and reduces virus concentration in lungs and brain when administered as much as 7 days before virus challenge.[101]

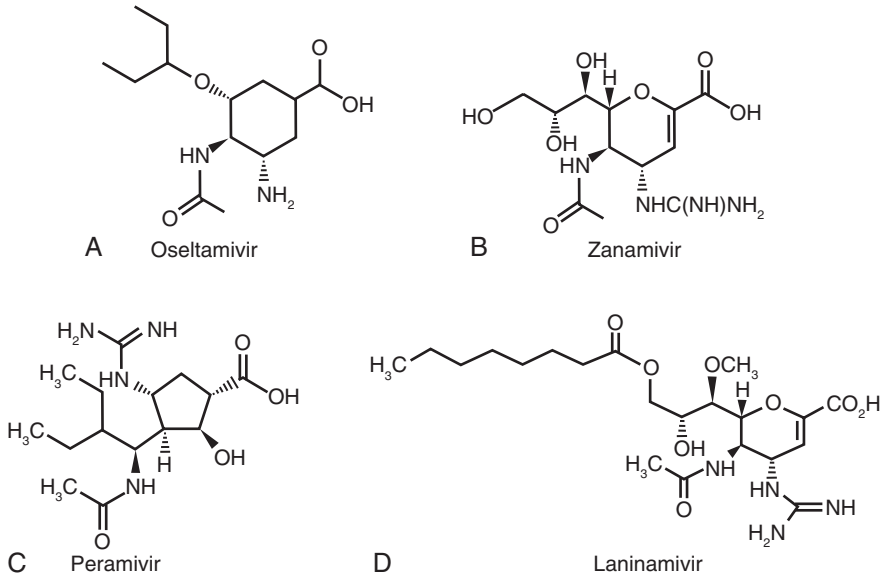

FIG. 45.4 **Chemical structures of oseltamivir carboxylate (A), zanamivir (B), peramivir (C), and laninamivir (D).**

Mechanism of Action

See subsequent discussion of mechanism of action under "Oseltamivir."

The basis for the prolonged persistence of laninamivir in the respiratory tract after intranasal or intratracheal administration of laninamivir octanoate in animals or oral inhalation in humans is not completely understood. In human volunteers, bronchoalveolar lavage samples obtained serially over 24 hours following oral inhalation of a single 40-mg dose of laninamivir octanoate revealed concentrations that exceed influenza virus neuraminidase inhibitory concentrations at all test times.[102] In mice, intranasal administration of ^{14}C-labeled laninamivir octanoate demonstrates prolonged retention of laninamivir in lung tissues. Microautoradiography indicates that laninamivir octanoate is taken into airway epithelial cells, seemingly hydrolyzed to the antiviral molecule laninamivir by intracellular esterases, and then released slowly extracellularly, perhaps as a result of its hydrophobic poor membrane permeability.[103] The cellular and molecular processes underlying these observations are not yet determined.

Resistance

Laninamivir resistance has been demonstrated infrequently. In the four winter seasons from 2010 to 2014 in Japan, where laninamivir has been marketed since 2010, no laninamivir resistance was observed in influenza A(H1N1)pdm09, influenza A(H3N2), and influenza B viruses.[104]

Pharmacokinetics

Epithelial lining fluid (ELF) concentrations of laninamivir octanoate and laninamivir calculated from analysis of bronchoalveolar lavage washings after a single oral inhalation of 40 mg laninamivir octanoate were 102.4 and 8.6 μg/mL, respectively, at 4 hours in healthy adult volunteers.[97] The disappearance half-times in bronchoalveolar lavage were 41 and 141 to 241 hours, respectively. The plasma $t_{½elim}$ values were 2.6 and 45.7 hours, respectively. Laninamivir concentrations in ELF exceeded the median inhibitory concentrations for influenza neuraminidases at all time points for 240 hours after dose inhalation. In other healthy adult volunteers, the pharmacokinetics of laninamivir octanoate and laninamivir were evaluated after oral inhalation of single doses from 5 to 120 mg.[105] Laninamivir octanoate appeared rapidly in plasma with a peak serum concentration (C_{max}) at 0.5 to 1.0 hour compared to 4.0 hours for laninamivir. Plasma $t_{½elim}$ values were 1.8 and 71.6 to 80.8 hours, respectively. The plasma area under the concentration-time curve (AUC) of laninamivir octanoate was linearly related to dose while that of laninamivir increased disproportionately. The mean cumulative excretion in urine over 144 hours was 2.3% to 3.6% and 10.7% to 14.6%, respectively.

After intravenous administration of ^{14}C-laninamivir in rats, almost 90% of the radioactivity was recovered in urine.[106] In human volunteers, the clearance of both laninamivir octanoate and laninamivir is linearly related toCrCl.[107] In subjects with none, mild, moderate, or severe renal impairment given a single orally inhaled dose of 20 mg laninamivir octanoate, the renal clearances of laninamivir octanoate and laninamivir are directly related to CrCl, whereas $t_{½elim}$ values are not. Geometric mean laninamivir octanoate clearance values declined from 26.0 mL/min in normal control subjects to 6.5 mL/min in patients with severe renal impairment. However, $t_{½elim}$ values were 2.3 to 3.5 hours and not different among the four groups. Laninamivir renal clearance declined from 65.0 to 12.7 mL/min across the four groups, while $t_{½elim}$ was not different among the groups, ranging from 53.2 to 57.0 hours. The likely explanation is that the elimination of both laninamivir octanoate and laninamivir reflects slow release of these compounds from tissues into plasma, rather than renal elimination, a pharmacokinetic concept called "flip-flop."[108] These pharmacokinetic data indicate that reduction of laninamivir octanoate doses may be appropriate for patients with renal impairment for pharmacokinetic reasons, but the lack of clear dose-related toxicity (see under "Toxicity") and the minimal absorption of orally inhaled drugs suggest that no dose adjustment will be needed.

Toxicity

Like orally inhaled zanamivir, orally inhaled laninamivir octanoate powder is well tolerated. In a double-blind study in healthy adult volunteers, single doses from 5 to 120 mg or multiple doses of 20 or 40 mg twice daily for 5 days were as well tolerated as placebo.[107]

In clinical trials, patients with influenza were randomized to single laninamivir octanoate doses of 20 or 40 mg in adults or children 10 years old or older, 20 mg in children less than 10 years old, or inhaled zanamivir as the control NAI treatment. Inhaled laninamivir octanoate once was as well tolerated as inhaled zanamivir 20 mg twice daily for 5 days.[109] In a double-blind trial in children 9 years of age or under with influenza, a single dose of inhaled laninamivir octanoate of 20 or 40 mg was as well tolerated as oral oseltamivir at 2 mg/kg body weight twice daily for 5 days.[110] In a phase III double-blind trial in adults 20 years of age or older with influenza, a single dose of inhaled laninamivir octanoate of 20 or 40 mg was as well tolerated as oral oseltamivir at 75 mg twice daily for 5 days.[111] Notwithstanding the lack of data from large, randomized, double-blind, placebo-controlled trials to establish the tolerability of laninamivir octanoate across the range of persons in healthy and high-risk groups, these published data on laninamivir octanoate tolerance plus those from studies of orally inhaled zanamivir collectively suggest that orally inhaled laninamivir octanoate will likely prove to be well tolerated and safe in the clinic.

Postmarketing studies of laninamivir octanoate in Japan concluded that the safety profile of laninamivir octanoate for abnormal behavior/delirium and syncope is similar to that of other NAIs.[112] In Japan, it is recommended in the product labeling that teenage patients inhaling laninamivir octanoate should remain under constant parental supervision for at least 2 days to monitor for behavioral changes to prevent associated self-injury. To avoid syncope, patients should inhale laninamivir octanoate in a relaxed sitting position. In another postmarketing survey for laninamivir octanoate tolerance, 50 of 3542 patients (1.4%) reported an adverse event.[113] Commonly reported adverse events included psychiatric disorders (abnormal behavior), gastrointestinal symptoms, and nervous system disorders such as dizziness, with frequencies of 0.48%, 0.45%, and 0.17%, respectively. These usually appeared on the day of laninamivir octanoate treatment and resolved in 3 days. These adverse reactions and their frequencies were considered comparable to those previously observed during clinical trials, and thus were thought to confirm no noticeable problem with safety.

A single orally inhaled dose of laninamivir octanoate appeared to be safe for pregnant women and did not increase the risk of miscarriage, preterm birth, or congenital malformation.[114]

Clinical Studies

No data from placebo-controlled trials have been published on the efficacy of orally inhaled laninamivir octanoate for influenza treatment, although other studies have demonstrated its efficacy for postexposure prophylaxis. Three randomized, controlled trials on the therapeutic efficacy and tolerance of laninamivir octanoate and one observational study comparing it to other NAIs have been reported. In these trials, laninamivir octanoate has been administered as an orally inhaled powder with a proprietary device that has two containers of 10 mg dry laninamivir octanoate powder. The manufacturer's instructions recommend two inhalations from each 10-mg chamber. For children, 4 inhalations are necessary, while 8 inhalations from two devices are required for adults. Occasionally, young children do not inhale the medication completely due to technical difficulty with the device.[109]

Of 112 pediatric patients with influenza of less than 48 hours' duration, evaluations were completed on 44 who were randomized to treatment with a single inhaled dose of laninamivir octanoate, 20 or 40 mg according to age, and 41 who were randomized to inhaled zanamivir 10 mg twice daily for 5 days.[109] Median times to fever resolution were 36 hours in the laninamivir octanoate groups and 37 hours in the zanamivir-treated group. This relatively small study suggested that a single dose of inhaled laninamivir octanoate was as efficacious as the recommended 5-day treatment with zanamivir. In another study, 184 children 9 years or younger with influenza of less than 36 hours' duration were randomized to a single oral inhalation of 40 ($n = 61$) or 20 mg ($n = 61$) laninamivir octanoate, or oseltamivir 2 mg/kg ($n = 62$) ingested twice daily for 5 days.[110] Of the 184 children, 112 (62%) were infected with influenza A(H1N1) virus, of which all but 4 cases possessed the H274Y mutation, mediating oseltamivir resistance. Thus oseltamivir therapy was likely

not to have been different from placebo. The median times to alleviation of influenza illness in children were significantly less (49.6 and 44.3 hours) in the laninamivir 40- and 20-mg groups, respectively, than in the oseltamivir-treated group (110.5 hours). Treatment effects on virus concentration and persistence in upper airway secretions were inconsistent, although on day 3, 10%, 0%, and 25% of subjects in the three groups, respectively, were still excreting virus. There were no clinical therapeutic or virologic differences among children infected with influenza A(H3N2) or B viruses, but the numbers of cases with these strains were small.

In a randomized, double-blind noninferiority trial, 1003 young healthy adults with febrile influenza for no more than 36 hours were randomized to receive either 40 mg or 20 mg laninamivir octanoate by oral inhalation once or oseltamivir 75 mg twice daily orally for 5 days.[111] The primary end point was time to influenza illness alleviation. Unfortunately, as in the pediatric study of Sugaya and Ohashi,[110] 66% of the subjects were infected with oseltamivir-resistant influenza A(H1N1) virus. The median times to resolution of illness in patients infected with this virus were 74.0, 85.8, and 77.8 hours, respectively, which were not different. Virus was detected by culture significantly less often at day 3 in the groups treated with laninamivir octanoate 40 mg (28%) and 20 mg (32%) than in the oseltamivir-treated group, which might be considered analogous to a placebo-treated cohort. Among cases infected with oseltamivir-susceptible influenza A(H3N2) virus, median times to illness alleviation were not different between the groups treated with laninamivir octanoate 40 mg (73.5 hours) and oseltamivir (67.5 hours) but were significantly longer in the group treated with laninamivir octanoate 20 mg (91.2 hours). There were no differences among the groups in H3N2 virus concentration in upper airway secretions or persistence. The 95% confidence intervals of the pooled analysis of all data were less than the prescribed noninferiority margin. It was concluded that a single inhalation of laninamivir octanoate is effective for treatment of seasonal influenza, including that caused by oseltamivir-resistant virus in adults.

In an observational study, 211 children with febrile influenza of less than 48 hours due to influenza A(H3N2) infection and 45 with A(H1N1) pdm09 infection were treated according to the recommendations of clinicians and the preference of patients or their guardians.[115] Of the 256 children, 119 were treated with oseltamivir in weight-appropriate doses, 124 with zanamivir, 4 with one dose of intravenous peramivir, and 9 with a single dose of orally inhaled laninamivir octanoate of 40 mg for those 10 years or older or 20 mg for those less than 10 years. The primary end point was duration of fever from the first dose of NAI. There were no differences in the duration of fever among the oseltamivir, zanamivir, or laninamivir octanoate groups. The median time to resolution of fever in the peramivir group (17.0 hours) was significantly less than in the other three groups.

The prophylactic efficacy of laninamivir for postexposure prophylaxis in household contacts of index cases who developed laboratory-confirmed influenza has been studied in three randomized, placebo-controlled trials. In the first trial, laninamivir 20 or 40 mg or placebo inhaled once on days 1 and 8 did not reduce the incidence of laboratory-proved influenza in contacts (3.6% [7 of 197 cases] and 3.7% [7 of 188 cases] in the two laninamivir treatment groups, respectively, compared to 6.6% [17 of 183 cases] in the placebo group).[116] Laninamivir 20 mg once daily for 2 days or 3 days reduced the incidence of influenza significantly to 3.9% (19 of 487 cases) and 3.7% (18 of 486 cases) compared to 16.9% (81 of 478 cases) in the placebo-treated group.[116] In another study, laninamivir 40 mg inhaled orally once and 20 mg inhaled once daily for 2 days both reduced the incidence of influenza significantly compared to placebo (4.5% [12 of 267 cases], 4.5% [13 of 269 cases], and 12.1% [32 of 265 cases] in the three groups, respectively).[117] In a third study, laninamivir 20 mg inhaled orally once by household contacts less than 10 years of age reduced the incidence of influenza significantly compared to placebo (11% [18 of 171 cases] compared to 19% [33 of 170 cases] in the two groups, respectively).[118]

In summary, a single inhaled dose of laninamivir octanoate is as efficacious as oral oseltamivir or inhaled zanamivir, both for 5 days, for treatment in children with influenza of less than 48 hours' duration, but this conclusion is not strongly supported by data from controlled trials. Efficacy in other populations, especially those with high-risk conditions, remains to be evaluated, as does the impact on complications of influenza. Laninamivir octanoate 20 or 40 mg inhaled orally is efficacious for postexposure prophylaxis in the household setting. The optimal dose is unclear: 20 mg/day for 2 or 3 consecutive days are also efficacious.

OSELTAMIVIR

Spectrum

Oseltamivir phosphate (Tamiflu) is the ethyl ester prodrug of oseltamivir carboxylate, a sialic acid analogue (Fig. 45.4A) that is a potent, specific inhibitor of the neuraminidases of influenza A and B viruses.[119,120] The metabolite, oseltamivir carboxylate, is approximately 50-fold more potent than the phosphate prodrug.[121] Oseltamivir carboxylate competitively and reversibly interacts with the active enzyme site to inhibit neuraminidase activity at low nanomolar concentrations.[122] Inhibitory concentrations for NAIs in cell culture have a broad range (≥1000-fold), depending on the assay method, and may not correlate with in vivo activity.[123,124] Oseltamivir carboxylate is active against viruses containing all nine influenza A neuraminidase subtypes recognized in nature, including more recent pathogenic avian viruses (H5N1, H7N7, H9N2), reassortant virus-containing neuraminidase from the 1918 pandemic strain, M2 inhibitor–resistant strains,[4,125] and the recently circulating (2009) pandemic A(H1N1) viruses (swine-origin influenza virus).[29] Resistance to oseltamivir has been recently reported in an H7N9 isolate.[126,127]

Influenza B viruses are 10-fold to 20-fold less susceptible to oseltamivir carboxylate than influenza A viruses, and influenza B virus illness responds less well clinically and virologically to oseltamivir than does influenza A illness.[128,129,130] The carboxylate is not cytotoxic and inhibits neuraminidases from mammalian sources or other pathogens only at 10^6-fold higher concentrations. Oral oseltamivir is active in murine and ferret models of influenza.[120,123] A prophylactic regimen given orally twice daily for 10 days completely protected ferrets against morbidity and mortality caused by H5N1 infection and did not interfere with development of protective immunity against subsequent H5N1 infection.[131] Neuraminidase inhibitors combined with M2 inhibitors or ribavirin show enhanced antiviral activity in vitro and in animal models of influenza A virus infection,[132] including H5N1 virus.[133,134] Amantadine combined with oseltamivir prevented the emergence of amantadine resistance in cell culture.[135]

Mechanism of Action

The NAI drugs oseltamivir, zanamivir, peramivir, and laninamivir share a common mechanism of action. Influenza neuraminidase cleaves terminal sialic acid residues on glycoconjugates and destroys the receptors recognized by viral hemagglutinin on cells, on newly released virions, and on respiratory tract mucins. This action is essential for release of virus from infected cells and for spread within the respiratory tract.[136] Inhibition of neuraminidase action causes newly formed virions to adhere to the cell surface and to form viral aggregates. Inhibitors thus limit spread of virus within the respiratory tract, and may prevent virus penetration of respiratory secretions to initiate replication.

Resistance

Resistant variants selected by in vitro passage with oseltamivir carboxylate or zanamivir have point mutations in the viral hemagglutinin or neuraminidase genes.[124,137] Hemagglutinin variants generally have mutations in or near the receptor binding site that make them less dependent on neuraminidase action for release from cells in vitro, and that confer cross-resistance among NAIs. Most of these variants retain full susceptibility in vivo.[124] Neuraminidase variants contain single amino acid substitutions in the framework or catalytic residues of the active enzyme site that alter drug binding and cause approximately 30-fold to more than 1000-fold reduced susceptibility in enzyme inhibition assays.[122] Influenza A variants selected by oseltamivir carboxylate are subtype specific, most commonly Arg292Lys in N2 and H275Y in N1, without cross-resistance to zanamivir. The altered neuraminidases have reduced activity or stability in vitro, and early studies of these variants usually demonstrated decreased infectivity and transmissibility in animals.[138] However, neuraminidase mutations in an A(H7N9)

strain led to enhanced replicative fitness and virulence.[139] Permissive additional neuraminidase mutations improved replication of oseltamivir-resistant A(H1N1) viruses with an H275Y neuraminidase change in vitro.[140]

Globally, prior to the first approval of an NAI drug in 1999, no oseltamivir-resistant strains were detected among clinical isolates.[141] Oseltamivir therapy has been associated with recovery of viruses with reduced susceptibility in about 1% of immunocompetent adult and 18% of pediatric recipients.[142,143] Generally, emergence of resistant variants has not been associated with clinical worsening, although prolonged recovery of resistant variants, sometimes in combination with M2 inhibitor resistance, has been observed in highly immunocompromised hosts.[144] Transmission of oseltamivir-resistant virus has been documented.[145,146] However, in 2007–2008, oseltamivir-resistant seasonal H1N1 virus appeared widely in immunocompetent individuals in Norway in the absence of antiviral pressure.[147] This mutant virus became the transmissible, pathogenic prevalent global H1N1 virus strain. Similarly, during and since the 2009 A(H1N1)pdm09 pandemic, there has been no linkage between prevalent use of oseltamivir in immunocompetent patients and the appearance of oseltamivir-resistant A(H1N1)pdm09 strains, which remain uncommon. Worldwide, from April 2009 to May 2014, the prevalence of influenza A(H1N1)pdm09 isolates with reduced susceptibility to oseltamivir has remained low at less than 3.4%, as reviewed.[148] Among influenza A(H3N2) global isolates to 2014, a very low pooled prevalence of oseltamivir resistance of 0.2% was observed.[148] Among influenza B global isolates from 2008 to 2014, 4 of 17,714 isolates reported in six studies were resistant to oseltamivir.[148] In the 2015–2016 influenza season in Japan, there was no trend toward decreased susceptibility to oseltamivir compared to results from 2010–2011 through to 2015–2016.[148]

On the other hand, oseltamivir-resistant isolates are not uncommonly recovered from immunocompromised patients being treated with the drug.[149] In one study, 17% (4 of 24) of immunocompromised hospitalized patients with influenza A(H1N1)pdm09 infection treated with oseltamivir shed resistant virus with the H275Y neuraminidase mutation.[150] Reports indicated that some of the A(H1N1)pdm09 oseltamivir-resistant strains retained replicative fitness, transmissibility, and pathogenicity comparable to wild-type oseltamivir strains in humans,[146,151] and in murine and ferret models of influenza infection.[152] Clinical illness caused by oseltamivir-resistant H1N1 strains in immunocompetent children responded less well to oseltamivir[153] as evidenced by higher fever at day 4 or 5 of treatment, although some found no evidence of prolonged illness in children infected with drug-resistant virus.[154] Others reported a significantly longer time to achieve nondetectable virus load in patients with oseltamivir-resistant H1N1 compared to oseltamivir-sensitive strains.[155]

Pharmacokinetics

Oral oseltamivir is rapidly absorbed and metabolized by esterases in the gastrointestinal tract, liver, and blood to the active carboxylate. The estimated bioavailability of the carboxylate is approximately 80%,[156] and its time to maximum plasma concentrations averages 2 to 4 hours. Dose proportionality of oseltamivir has been reported over the dose range from 75 to 675 mg. Only low blood levels of the prodrug are detectable. Rarely, possession of a constitutive variant of carboxylesterase 1, the enzyme that normally catalyzes the conversion of oseltamivir phosphate to carboxylate, can markedly impair the hydrolysis of the parent compound, resulting in the potential for a compromised antiviral effect after oseltamivir administration.[157] Ingestion with food delays absorption slightly but does not decrease overall bioavailability. Oseltamivir administered via a nasogastric tube to patients with respiratory failure requiring mechanical ventilation is well absorbed and converted to oseltamivir carboxylate.[158,159] In healthy adults, peak and trough plasma concentrations average 0.35 μg/mL and 0.14 μg/mL, respectively, after 75-mg doses.[160] In infants up to 1 year of age, systemic exposure (AUC_{0-12}) to the carboxylate exhibits decreasing variability while clearance increases.[161] Recommended doses of oseltamivir are 3.0 mg/kg twice daily for infants 0 to 8 months of age and 3.5 mg/kg twice daily for those 9 to 11 months of age. In children older than 1 year, carboxylate exposure increases gradually with increasing age[160] so that weight-based dosing is recommended.[162] In healthy elderly adults, overall drug exposure is about 25% greater than in younger adults, most likely due to differences in renal elimination. Morbid obesity (body mass index ≥40 kg/m^2) does not alter oseltamivir pharmacokinetics to a clinically important degree.[163–165] The effects of pregnancy on the pharmacokinetics of oseltamivir are unclear. One study reported no differences among women in the third trimester of pregnancy and historical controls,[166] whereas two reported 25% to 30% reductions in systemic (AUC_{0-12}) oseltamivir-carboxylate exposure in pregnant women compared to concurrent nonpregnant controls,[167,168] perhaps suggesting a need for 75 mg three times daily of oseltamivir for treatment.[167]

Plasma protein binding of the prodrug (42%) and the carboxylate (<3%) is low.[156] The volume of distribution is moderate (23–26 L). In animals, lower respiratory tract levels are similar to or exceed the levels in blood,[169] and in humans, the carboxylate is detectable in middle ear and maxillary sinus fluid at concentrations similar to those in plasma.[170]

Oseltamivir appears in breast milk.[171] In the ex vivo human placenta model, oseltamivir was extensively metabolized to the carboxylate moiety, but transplacental passage of oseltamivir carboxylate occurred at a low rate, inferring that fetal exposure during maternal treatment with oseltamivir may be minimal.[172] No carboxylate was detected in CSF in one child,[173] whereas C_{max} values in CSF were 2.1% and 3.5% of corresponding plasma concentrations for oseltamivir and oseltamivir carboxylate, respectively, in eight healthy adults after ingestion of 150 mg of oseltamivir.[174] After oral oseltamivir, the plasma $t_{½elim}$ of the carboxylate averages 6 to 10 hours in healthy adults. The prodrug and carboxylate are excreted primarily unchanged through the kidney; the carboxylate is eliminated by glomerular filtration and tubular secretion via a probenecid-sensitive anionic transporter. Clearance varies linearly with CrCl, such that $t_{½elim}$ increases to 22 hours in patients with CrCl less than 30 mL/min, and dosage reductions are needed.[156] Oseltamivir carboxylate is removed with different degrees of efficiency by different renal replacement therapies (peritoneal dialysis, hemodialysis, and continuous renal replacement therapies). Doses of oseltamivir for patients with renal impairment receiving renal replacement therapy have been published.[175] Extracorporeal membrane oxygenation per se does not appear to affect oseltamivir clinical pharmacokinetics,[176] although in another report, extracorporeal membrane oxygenation resulted in diminished clearance and increased volume of distribution.[177]

Uncomplicated influenza illness does not seem to alter the pharmacokinetics of oseltamivir.[156] Cystic fibrosis patients appear to clear oseltamivir carboxylate more rapidly than patients who do not have the disease.[178]

Interactions

Probenecid reduces renal clearance of oseltamivir by about 50%.[179] Few other clinically important drug interactions have been recognized to date. Sotalol appeared to induce a torsades de pointes cardiac arrhythmia during oseltamivir therapy of influenza.[180] Specific studies have found no clinically important interactions with antacids, acetaminophen, aspirin, known inhibitors of selected renal tubular secretion pathways, amoxicillin, cimetidine, cyclosporine, mycophenolate, tacrolimus,[156,181] warfarin,[182] rimantadine,[183] or dexamethasone.[184]

Toxicity

Preclinical studies have found no evidence of mutagenic, teratogenic, or oncogenic effects. High-dose oseltamivir causes renal tubular mineralization in mice and maternal toxicity in rabbits. It is classified as pregnancy category C.

Oseltamivir is generally well tolerated in patients of all ages, including pregnant women and fetuses,[185–187] and no serious end-organ toxicity has been recognized.[123,188–190] Oral administration is associated with nausea, epigastric distress, or emesis in about 10% to 15% of adults receiving 75 to 150 mg twice daily. These gastrointestinal complaints are usually mild to moderate in intensity, resolve despite continued dosing, and are ameliorated by administration with food. Nausea and vomiting (and possibly dizziness) are dose related in adults.[191] Discontinuation rates of 1% to 2% were observed in controlled treatment studies. The mechanism of nausea and/or vomiting is uncertain, but the risk

seems to be lower in older adults. Long-term prophylaxis has not been associated with an increased risk for adverse events,[123,192] although headache may occur in older recipients. Self-injury, delirium, and psychiatric illness have been reported in patients with influenza, primarily pediatric or adolescent, treated with oseltamivir, mostly in Japan.[193] Analyses of neuropsychiatric reactions among patients with influenza treated with oseltamivir in three large US administrative databases did not demonstrate such an association.[194–196] The decline in cases in Japan following a regulatory recommendation to restrict oseltamivir use in children 10 to 19 years of age has been associated with a decline in oseltamivir-related cases but a corresponding rise in cases associated with zanamivir, the inhaled, minimally systemically bioavailable NAI. The latter fact raises further doubts about a causal association between oseltamivir therapy and neuropsychiatric and behavioral adverse reactions in patients with influenza.[197]

Erythematous skin rashes and rare instances of severe eruptions of Stevens-Johnson syndrome, hepatic inflammation, hemorrhagic colitis, anaphylaxis, and thrombocytopenia have been reported, but their relationship to oseltamivir is uncertain. Oseltamivir administered to a patient receiving other serotonergic medication may have contributed to serotonin syndrome–like neurotoxicity.[198] Another case report suggested oseltamivir caused hypothermia and respiratory suppression.[199]

Clinical Studies

Oseltamivir is efficacious for the prevention and treatment of influenza A and B virus infection. In the United States, it is approved for the prevention of influenza in patients 1 year of age and older and the treatment of acute uncomplicated influenza in patients 2 weeks of age and older who have been symptomatic for no more than 2 days.[162]

In early clinical experiments in volunteers with induced influenza, it was demonstrated that oral oseltamivir is highly protective against experimental human influenza, and early treatment is associated with reductions in viral titers, symptoms, nasal cytokines, and middle ear pressure abnormalities.[123] Subsequent controlled trials in patients, largely healthy adults and children with naturally acquired mild to moderately severe seasonal influenza A infection, demonstrated that early oseltamivir treatment of acute influenza reduces the time to illness alleviation by up to 3.5 days, fever duration, and viral titers in the upper respiratory tract.[142,200,201,202–204] Earlier treatment maximizes the speed of resolution of illness.[200,205] Treatment of children reduces the risk for otitis media and decreases overall antibiotic use.[142,200] In healthy and high-risk adults with seasonal influenza, early treatment has been reported to decrease the risk for lower respiratory tract complications leading to antibiotic administration and to hospitalization,[206] but this has been questioned.[207] A meta-analysis of observational studies of high-risk patients with seasonal influenza concluded that oseltamivir treatment may reduce hospitalization, while treatment of hospitalized patients reduces respiratory failure, intensive care unit admission, and mortality.[208,209] In hospitalized adults with more severe illness due to A(H1N1)pdm09, oseltamivir provides similar benefits[210,211] even if treatment is started more than 48 hours after clinical illness has begun.[210,212,213] Experts convened by the European Centre for Disease Prevention and Control in February 2015 came to broadly similar conclusions.[214] In children, meta-analysis of five trials describing results of treatment with oseltamivir concluded that it reduced the duration of influenza illness and reduced the risk of developing otitis media.[201] A review of influenza virus infection in pregnancy concluded that early treatment with oseltamivir is associated with a reduced risk of severe disease.[215] Oseltamivir treatment initiated within 24 hours of illness onset did not reduce transmission to and illness in household contacts in one study,[216] although another study reported a slight reduction.[217]

Treatment with higher doses of oseltamivir (150 to 225 mg twice daily) did not appear to improve clinical[218–220] or virologic[218] outcome compared to the standard dose of 75 mg twice daily.

Combined treatment of influenza-infected patients with oral oseltamivir plus inhaled zanamivir was less effective than oseltamivir monotherapy[221] or zanamivir monotherapy.[222] Two controlled trials comparing oseltamivir plus intravenous peramivir,[223] or inhaled zanamivir, compared to oseltamivir alone[224] were underpowered to rigorously compare combination therapy and monotherapy. Combined therapy with oseltamivir, amantadine, and ribavirin reduced virus shedding at day 3 compared to oseltamivir monotherapy, but this difference was not associated with clinical benefit.[37]

In hospitalized patients with severe influenza, intravenous zanamivir 300 or 600 mg or oseltamivir 75 mg, all twice daily, were not different in efficacy or safety.[225] In an open-label, randomized study, clarithromycin 500 mg, naproxen 200 mg, and oseltamivir 75 mg, all twice daily for 2 days, followed by oseltamivir alone 75 mg twice daily for 3 days, reduced 30- and 90-day mortality more than oseltamivir alone in patients hospitalized with influenza.[226] Azithromycin combined with oseltamivir in hospitalized patients with influenza reduced inflammatory cytokines and C-reactive protein faster than oseltamivir alone, although symptoms did not resolve more rapidly nor were viral negativity rates attained more quickly.[227]

Oseltamivir is less efficacious for the treatment of influenza B than for influenza A virus infection in children[129,228] and adults.[129] An analysis of 284 cumulated cases of influenza A(H5N1) infections in a global registry demonstrated that crude mortality was significantly less in those treated with oseltamivir (40%) than in those not treated (76%) when started up to 6 to 8 days after symptoms onset.[230]

Oseltamivir treatment of hematopoietic stem cell transplant recipients with influenza may prevent the development of pneumonia and virus shedding, thereby preventing both influenza-related death in index patients and nosocomial transmission to others.[231] Of 21 patients with leukemia who developed influenza and were treated with oseltamivir, none died compared to 3 of 8 who were not treated.[232]

Prophylactic administration of once-daily oral oseltamivir (75 mg) is highly effective in reducing the risk for developing febrile illness during influenza season in unimmunized adults (efficacy 84%),[233] immunized nursing home residents (efficacy 92%),[234] and transplant recipients (efficacy 80%).[192] Prevention of influenza may reduce secondary complications in institutionalized older adults.[234] Once-daily oseltamivir for 7 to 10 days is also effective for postexposure prophylaxis in household contacts, including children, when ill index cases do[217,235] or do not[236] receive concurrent treatment. Oseltamivir chemoprophylaxis has been used to control institutional outbreaks of influenza A continuing despite M2 inhibitor use, and an influenza B outbreak.[237]

PERAMIVIR

Spectrum

Peramivir ([1S,2S,3R,4R]-3-[(1S)-1-(acetylamino)-2-ethylbutyl]-4-[(aminoiminomethyl)amino]-2-hydroxycyclopentanecarboxylic acid; Rapiacta) (see Fig. 45.4C) was first approved in 2010 in Japan, China, and South Korea for intravenous treatment of influenza. In 2013 in the United States, it was approved as a single-dose intravenous treatment for uncomplicated influenza in patients 18 years of age or older who have been symptomatic for more than 2 days.

Peramivir is a potent, selective inhibitor of influenza A and B virus neuraminidases, including those of all nine avian neuraminidase subtypes[238] and influenza A(H1N1)pdm09.[239] It is a sialic acid analogue designed to be structurally distinct from oseltamivir and zanamivir such that cross-resistance to it among oseltamivir-resistant and zanamivir-resistant strains is not consistently observed.[240,241] Like oseltamivir and zanamivir, peramivir inhibits influenza neuraminidase in enzyme assays at nanomolar concentrations[242] and requires micromolar concentrations to inhibit influenza replication in cell culture.[243] It is a more potent inhibitor of influenza A and B viruses in vitro than is oseltamivir or zanamivir.[243] The clinical relevance of this difference has not yet been evaluated. In murine and ferret models of influenza infection, peramivir is effective when administered intranasally,[244] orally,[245] and intramuscularly.[246]

Combination treatment of influenza A virus infection with peramivir and ribavirin in cell culture and in mice yields additive or synergistic interactions with no increase in toxicity.[247] The antiviral effect of combinations of peramivir plus rimantadine in vitro is variable, ranging from additive to synergistic.[248] In mice with experimental influenza infections, the combination of peramivir and rimantadine is synergistic.[249] The combination of two NAIs, oseltamivir plus peramivir, is synergistic.[250] At this time, the clinical importance of peramivir combined with another small-molecule inhibitor of influenza remains uncertain.

In a controlled clinical trial, intravenous peramivir at 600 mg daily plus oral oseltamivir did not accelerate symptom resolution more than oral oseltamivir alone.[223] More studies are required to determine whether the benefits of peramivir combined with other influenza virus inhibitors exceed those of monotherapy.

Mechanism of Action

See previous discussion of mechanism of action under "Oseltamivir."

Resistance

Peramivir-resistant influenza virus has been selected in vitro,[240,241,251,252] but not from peramivir-treated mice with experimental influenza infection,[253] healthy volunteers given peramivir for prevention or treatment of experimentally induced influenza A or B infection,[254] or healthy treated patients. A peramivir-resistant virus possessing the H275Y mutation emerged during intravenous therapy of the pandemic 2009 influenza A(H1N1) isolate in an immunocompromised patient.[255] Peramivir-resistant mutants generated in vitro may possess unaltered or diminished virulence and replicative capacity in mice and ferrets.[256] Peramivir resistance associated solely with an alteration in the hemagglutinin gene conferred cross-resistance to oseltamivir and zanamivir, and could cause lethal disease in mice. Infection with the resistant virus in mice was still amenable to peramivir therapy, however.[252]

The prevalence of resistance to peramivir in large collections of influenza virus isolates is low as of reporting to 2015.[257] In a survey of 2015–2016 influenza season outbreaks in Japan, peramivir resistance was low and unchanged from the 2010–2011 season.[258] Notwithstanding, there have been at least two outbreaks caused by peramivir-resistant H275Y neuraminidase mutant influenza A(H1N1)pdm09 isolates.[146,259] Nonetheless, resistance to peramivir arising in patients solely treated with it has not yet been reported.

Naturally occurring oseltamivir-resistant influenza viruses possessing the H275Y mutation have a 100-fold[239] to 661-fold[256] reduced susceptibility to peramivir, less than that of oseltamivir (982-fold), but such mutants are clinically uniformly cross-resistant to both drugs. Thus, although studies in mice[260,261] and high-risk patients[262] suggest that infection due to viruses possessing the H275Y mutation may be successfully treated with higher-dose regimens of injected peramivir, data from a case report[255] and an observational study[262] indicate that intravenous peramivir was not more effective than oseltamivir. In 2009, the World Health Organization recommended that for treatment of infection due to A(H1N1)pdm09 strains possessing the H275Y mutation, intravenous peramivir is likely to be suboptimal and intravenous zanamivir is preferred.[263]

Pharmacokinetics

The absolute oral bioavailability of peramivir is 2%.[264] As a result, clinical development has focused on its efficacy and safety after intramuscular and intravenous injection. Fortunately, its long elimination half-life supports single-dose intravenous treatment regimens. At doses up to 2 mg/kg in adults, plasma $t_{1/2}$ and $AUC_{0-\alpha}$ increase in proportion to dose. At higher doses of greater than 2 mg/kg being used in clinical trials in adults, initial estimate of plasma $t_{1/2}$ in healthy adults was approximately 20 hours,[265] which supports single-dose treatment. More recent studies in healthy adults given up to 800 mg intravenously once a day or 400 mg twice daily for 6 days[266–268] confirmed the dose proportionality of maximum plasma concentration and AUC_{plasma}. Apparent volume of distribution in adults was 0.4 L/kg and systemic clearance 89 mL/h/kg.[268] Approximately 90% of peramivir was excreted unchanged into urine within 12 hours after a dose. Mean plasma elimination $t_{1/2}$ after an intravenous dose of 600 mg ranged from 3.3 hours[268] to 8.8 hours.[269] The corresponding values for children with mean age of 9 years are plasma $t_{1/2}$ 7.7 hours, apparent volume of distribution 0.3 L/kg, and systemic clearance 173 mL/h/kg.[270] The physiologic counterpart of this large apparent volume of distribution is unknown, because no locus of drug sequestration has been identified.

Plasma protein binding is less than 30%. Peramivir concentrations in plasma are 10-fold to 50-fold higher than concurrent levels in nasal wash or pharyngeal gargle solutions.[264] Peramivir is detectable at these sites 24 hours after dosing, at concentrations greater than levels that inhibit neuraminidases of most strains of influenza virus. The clinical relevance of these data is unknown. Influenza infection increased peramivir clearance 18% and reduced volume of distribution 6%.

A 300-mg dose injected intravenously once in young healthy adults with influenza illness of less than 48 hours' duration is efficacious and well tolerated.[271] Infusion of this dose over a median of 38 minutes produced median plasma concentrations of 18,100 ng/mL at the end of the infusion and 14.8 ng/mL 18 to 24 hours later. A 600-mg dose yielded corresponding values of 36,300 and 32.8 ng/mL. The median inhibitory concentration for 50% of isolates (IC_{50}) for the neuraminidase of the patient viruses ranged from 1.15 nmol/L for A(H1N1), 1.36 nmol/L for A(H3N2), and 2.81 nmol/L for influenza B isolates.[262] In pediatric patients 1 month to 15 years of age with A(H1N1)pdm09 influenza infection, an intravenous infusion of 10 mg/kg once daily produced comparable plasma concentrations to those seen in young healthy adults (see earlier): median peramivir plasma concentrations were 33,150 ng/mL at the end of the infusion and 20.7 ng/mL 18 to 24 hours later.[270] The relationship of these plasma concentration data to efficacy is unclear. In mice, plasma AUC of peramivir is the pharmacokinetic characteristic related to efficacy.[272]

Data on peramivir distribution into breast milk in humans are unavailable.[264] In rats, less than 5% of ^{14}C-labeled peramivir administered is recovered in breast milk. Peramivir is eliminated unchanged into urine by glomerular filtration, and probenecid does not affect its excretion. In patients with renal insufficiency, mean $t_{1/2}$ ranges from 24 to 30 hours in subjects with mean CrCl of 21 to 68 mL/min. In individuals with dialysis-dependent renal failure, $t_{1/2}$ averages 79 hours.

In adults with normal renal function, the recommended intravenous dose is 600 mg/day and in children 6 to 17 years of age, 10 mg/kg intravenously once daily. For other age groups and patients with renal impairment, including end-stage renal disease requiring different renal replacement therapies, alternative doses have been suggested.[264,273]

Interactions

Adverse drug-drug interactions have not been reported in subjects given peramivir, but the number of individuals exposed is still modest. No pharmacokinetic interaction of intravenous peramivir and oral oseltamivir or rimantadine was observed in healthy volunteers.[269] Drug-drug interactions in individuals receiving peramivir are unlikely because it neither induces nor inhibits important drug-metabolizing cytochrome P-450 enzymes.

Toxicity

Peramivir is generally nontoxic and well tolerated. Preclinical studies revealed no genotoxicity, reproductive toxicity, or developmental toxicity.[264] In multiple species of animals, the only apparent adverse effect is reversible nephrotoxicity, which is species (rabbit only) and gender (female) specific. The nephrotoxic dose is greater than 200 mg/kg/day intravenously for 9 days.

The largest doses administered to humans, 800 mg orally[254] and 600 mg intravenously,[271] have not been associated with consistent adverse symptoms or laboratory abnormalities compared to placebo.[271] In placebo-controlled clinical trials of peramivir orally up to 800 mg/day for 4 to 5 days,[254] 300 mg/day intramuscularly once,[274] and 600 mg intravenously once,[271] adverse symptoms were not reported more frequently in peramivir recipients than in placebo recipients.

In controlled, blinded trials as well as uncontrolled studies of intravenous peramivir, it has been generally well tolerated and safe. In a randomized, double-blind study comparing a single dose of peramivir of 300 or 600 mg and a matching placebo given intravenously to 300 young healthy adults in an outpatient setting,[271] nausea may have been reported more frequently in drug recipients (3.0%, 6.1%, and 1.0%, respectively, in the three groups). Extensive blood and urine laboratory tests revealed no differences among groups. In a randomized, double-blind, double-dummy trial in young healthy adults with influenza treated with 300 mg and 600 mg peramivir intravenously once or oseltamivir 75 mg orally twice daily for 5 days, the overall incidence of adverse effects was lowest in the 300-mg group: 14.0% compared to 18.1% and 20.0% in the other groups, respectively. Diarrhea (3.8%, 5.5%, and 5.2%), nausea

(0.5%, 1.9%, and 4.4%), and a decreased neutrophil count (2.5%, 3.8%, and 3.6%) all tended to be lowest in the 300-mg peramivir group.[275] In the randomized, unblinded study in 137 hospitalized patients treated for influenza with intravenous peramivir 200 or 400 mg once daily, or oseltamivir 75 mg orally twice daily, all for 5 days, the "incidence of adverse events was low and generally similar among treatment groups."[269]

Assessment of side effects of intravenous peramivir in uncontrolled studies in hospitalized adults with high-risk comorbid conditions[262] also suggested that the drug was generally well tolerated. A single case of dilated cardiomyopathy or myocarditis in a volunteer infected with an influenza B challenge virus and treated with peramivir has been reported.[254] The relationship of the cardiac disorder to the drug is unknown.

Two postmarketing studies confirmed the safety and effectiveness of intravenous peramivir in pediatric patients ($n = 1254$)[276] and patients at high risk of influenza complications ($n = 772$).[277]

Clinical Studies

In a study in serosusceptible volunteers, peramivir prophylaxis with 50 to 800 mg orally daily or placebo, initiated 24 hours before influenza A or B virus challenge and continued for 5 days, tended to prevent illness at doses of 200 mg or greater and to reduce viral shedding and titer in nasal washings in subjects inoculated with influenza A virus.[254] No effect on preventing illness caused by influenza B virus was observed, although the duration of virus shedding tended to be less in individuals receiving 400 mg and 800 mg of peramivir.[254]

In early studies in patients with influenza, oral peramivir therapy with doses of 400 to 800 mg daily for 5 days[254] and single intramuscular doses of 150 mg or 300 mg[264] reduced median times to relief of symptoms, but the differences were not statistically significant from controls. Subsequently, controlled trials with an intravenous formulation demonstrated peramivir therapeutic efficacy and tolerance in patients with influenza due to susceptible virus strains. Peramivir treatment of naturally acquired influenza in young adults with illness of 48 hours' duration or less with 300 mg or 600 mg injected once intravenously versus placebo reduced median time to relief of symptoms significantly from 82 hours in the placebo group to 59 hours and 60 hours in the peramivir 300-mg and 600-mg groups, respectively, in the outpatient setting.[271] Peramivir treatments also significantly reduced the proportion of subjects still excreting virus in nasal and throat secretions at day 3 from 51% in placebo recipients to 26% to 37% in those treated with peramivir 600 or 300 mg, respectively.[271] Intravenous peramivir (300 or 600 mg once) was not different from oral oseltamivir (75 mg orally twice for 5 days) in two randomized, controlled trials in influenza-infected adults.[275,278] In 137 hospitalized patients randomized to 5 days' treatment with intravenous peramivir 200 or 400 g/day compared to historical reports with oral oseltamivir at 75 mg twice daily, the reduction in virus concentration in nasopharyngeal secretion was similar across the three treatments.[269] An additional study utilizing a higher dose of peramivir (300 mg twice daily or 600 mg once daily) in 234 hospitalized patients also showed no differences in virologic or clinical end points among the peramivir-treated regimens or compared to reported patients treated with oseltamivir.[279] A randomized controlled study of intravenous peramivir plus standard of care versus standard of care alone in patients hospitalized with influenza was recently terminated because of futility to show a difference between the peramivir and control groups.[223]

As noted earlier, intravenous peramivir is probably not effective for treatment of patients with oseltamivir resistance due to possession of the H275Y mutation; intravenous zanamivir has been recommended.

RIBAVIRIN

Spectrum

Ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide; Virazole, Rebetol, Copegus) is a guanosine analogue (Fig. 45.5) in which the base and the D-ribose sugar are necessary for antiviral activity. Ribavirin inhibits the in vitro replication of a wide range of RNA and DNA viruses, including myxoviruses, paramyxoviruses, arenaviruses, flaviviruses, bunyaviruses, coronaviruses, togaviruses, reoviruses, herpesviruses, adenoviruses, poxviruses, and retroviruses. By plaque assay, inhibitory concentrations range from 3 to 10 μg/mL for influenza virus, parainfluenza virus, and RSV. High concentrations inhibit group C adenoviruses[280] and pathogenic flaviviruses,[281] including West Nile virus in neural cells. Ribavirin does not inhibit severe acute respiratory syndrome coronavirus in vitro.[282]

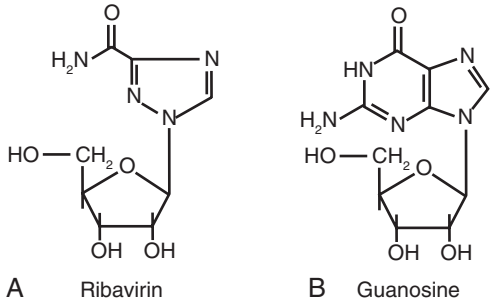

FIG. 45.5 Chemical structure of ribavirin (A) and the nucleoside guanosine (B).

Low concentrations of ribavirin (1–10 μg/mL) reversibly inhibit macromolecular synthesis and the proliferation of rapidly dividing cells.[283] Ribavirin decreases nucleic acid and protein synthesis, inhibits IFN-γ release, and increases apoptosis in human peripheral blood mononuclear cells in vitro,[282,284] but it does not adversely affect polymorphonuclear leukocyte functions.[285] Ribavirin has been postulated to enhance cell-mediated immune responses by increasing type 1 and suppressing type 2 cytokine responses in T cells,[285] and to decrease proinflammatory cytokine elaboration and inflammatory cell numbers. Inhibition of mast cell secretory responses occurs in vitro.

Aerosol administration is more effective than parenteral dosing in animal models of influenza and RSV infection. Parenteral ribavirin has antiviral and therapeutic activity in animal models of infection with Lassa virus, other arenaviruses, and bunyavirus (see Chapters 166 and 167). Combinations of ribavirin with immunoglobulin in RSV infection and with M2 inhibitors or NAIs in influenza A infection or with NAIs in influenza B infection show enhanced antiviral activity.[12] The use of ribavirin in treatment of hepatitis B and C is discussed in Chapter 47.

Mechanism of Action

The antiviral mechanisms of action of ribavirin are complex and most likely vary for different viruses. Ribavirin causes alterations of cellular nucleotide pools, inhibits viral RNA synthesis, and may cause lethal mutagenesis of certain RNA virus genomes.[285–287] Intracellular phosphorylation to the monophosphate, diphosphate, and triphosphate derivatives is mediated by host cell enzymes. In uninfected and RSV-infected cells, the predominant derivative (>80%) is the triphosphate, which is rapidly lost, with an intracellular $t_{½elim}$ of less than 2 hours.

Ribavirin monophosphate competitively inhibits inosine monophosphate dehydrogenase and interferes with the synthesis of guanosine triphosphate (GTP) and with nucleic acid synthesis. Decreased concentrations of competing GTP likely potentiate ribavirin's other antiviral effects. Ribavirin triphosphate inhibits influenza virus RNA polymerase activity and the GTP-dependent 5′-capping of viral messenger RNA. The monophosphate is incorporated inefficiently into viral RNA genomes, and this may lead to lethal mutagenesis and contribute to antiviral activity.[286] HCV RNA polymerase incorporates ribavirin monophosphate into viral RNA, which causes mutations and inhibits viral RNA synthesis.[288] Ribavirin diphosphates and triphosphates also inhibit human immunodeficiency virus (HIV) reverse transcriptase activity.[289]

Ribavirin has immunosuppressive effects in experimental animals, and shows therapeutic activity against transplantable virus-induced tumors and certain autoimmune diseases. Ribavirin increases type 1 cytokine–mediated immune responses in vivo, an effect that may contribute to its therapeutic activities,[285] and seems to augment type 1 cytokine responses ex vivo in peripheral blood mononuclear cells from patients with chronic hepatitis C.[287]

Resistance

Antiviral resistance to ribavirin has been documented only in Sindbis virus and HCV to date. One HCV RNA polymerase variant (F415Y)

selected in genotype 1a–infected, ribavirin-treated patients has been associated with ribavirin resistance in vitro.[290] No ribavirin-resistant RSVs have been detected during aerosol therapy of children.

Pharmacokinetics

Oral ribavirin is well absorbed, but bioavailability averages 45% to 65% in adults because of first-pass metabolism.[291–294] Administration with food increases absorption and peak plasma concentrations by 70%.[291] After single oral doses of 600 mg, 1200 mg, or 2400 mg, peak plasma concentrations occur at 1 to 2 hours and average 1.3 μg/mL, 2.5 μg/mL, and 3.2 μg/mL, respectively. Plasma concentrations average approximately 24 μg/mL and 17 μg/mL after intravenous doses of 1000 mg and 500 mg in patients with Lassa fever. During long-term administration, overall exposure and $t_{½elim}$ increase substantially.[291] Steady-state plasma levels of about 1 to 4 μg/mL occur by about 4 weeks with weight-adjusted dosing in chronic hepatitis C, and higher concentrations at 4 weeks correlate with decline in hemoglobin and likelihood of sustained viral responses.[295] Plasma protein binding is negligible, and ribavirin has a large volume of distribution (>2000 L). At steady state, CSF levels are about 70% of those in plasma.[293]

The disposition of ribavirin is complex, involving renal elimination and metabolism. After rapid initial distribution, there is a prolonged terminal $t_{½elim}$ of 37 to 79 hours.[291–293] Ribavirin triphosphate concentrates in erythrocytes with an erythrocyte-to-plasma ratio of 40 : 1 or greater, and erythrocyte levels gradually decline with an apparent $t_{½}$ of 40 days. Renal excretion accounts for approximately 30% to 60% of ribavirin's overall clearance, but the contribution of hepatic metabolism is significant. About 5% to 10% is recovered unchanged in the urine, and a much greater fraction is excreted as triazole carboxamide and carboxylic acid metabolites.[291] Plasma clearance is reduced threefold in patients with advanced renal impairment (CrCl ≤30 mL/min). Dosage adjustments are needed for renal insufficiency, and ribavirin should be used with caution in patients with CrCl less than 50 mL/min. Hemodialysis and hemofiltration remove small amounts of drug. Higher initial blood levels occur in severe hepatic dysfunction.[294]

With aerosol administration, systemic absorption is low (<1% of deposited dose). Peak plasma levels range from 0.5 to 2.2 μg/mL after 8 hours of exposure, and from 0.8 to 3.3 μg/mL after 20 hours in pediatric patients. Respiratory secretion levels often exceed 1000 μg/mL and persist with a $t_{½}$ of 1.4 to 2.5 hours. A special small particle aerosol generator (SPAG-2; Bausch Health) is needed to produce particles of proper aerodynamic size to reach the lower respiratory tract. The delivered dose is twice as high in infants (1.8 mg/kg/h) as in adults.

Toxicity

Ribavirin causes dose-related anemia because of extravascular hemolysis and, at higher dosages, suppression of bone marrow release of erythroid elements.[296] Reversible increases of serum bilirubin (in one-quarter of recipients), serum iron, and uric acid concentrations occur during short-term oral administration. Long-term use of oral ribavirin at dosages greater than 800 mg daily causes hemoglobin reductions of 2 to 4 g/dL in most recipients, usually within 4 weeks. When used in combination with IFN, hemoglobin levels less than 11 g/dL develop in 25% to 30% of patients.[297] Renal impairment increases the risk for hemolysis. Severe anemia requires dosage reduction or cessation, although erythropoietin has been used effectively.[297] Other reported side effects include pruritus, myalgia, rash, nausea, depression, nervousness, and cough or respiratory symptoms.[298] High-dose intravenous ribavirin is associated with headache, hypomagnesemia, and hypocalcemia.[299] Bolus intravenous dosing may cause rigors, so infusion over 10 to 15 minutes is advised.

Aerosolized ribavirin may cause conjunctival irritation, rash, bronchospasm, reversible deterioration in pulmonary function, and, rarely, acute water intoxication. No adverse hematologic effects have been associated with aerosolized ribavirin. The drug may precipitate on contact lenses, so they should not be worn during aerosol exposure. Ribavirin exposure may occur in health care workers working in the environment of aerosol-treated infants.[299,300] Health care worker exposure is higher during delivery by oxygen hood than by ventilator or vacuum-exhausted hood systems.[299] Use of aerosol containment and scavenging systems, turning off the aerosol generator before providing routine care, and use of personal protective equipment have been recommended.[300]

When ribavirin is used in conjunction with mechanical ventilation, in-line filters, modified circuitry, and frequent monitoring are required to prevent plugging of ventilator valves and tubing with precipitates of ribavirin. However, the effects of such modifications on drug delivery to the lower respiratory tract are undefined.

In preclinical studies, ribavirin is mutagenic, gonadotoxic, and teratogenic.[296] Low oral dosages have been teratogenic or embryotoxic in multiple species. Use of ribavirin is relatively contraindicated during pregnancy, and pregnant women should not directly care for patients receiving ribavirin aerosol. Ribavirin is categorized as pregnancy category X, and contraception for men and women is recommended for at least 6 months after discontinuation of treatment or exposure.

Interactions

Antacids slightly decrease the oral bioavailability of ribavirin. During coadministration clinically, ribavirin, amantadine, and oseltamivir do not interact pharmacokinetically.[39] Ribavirin antagonizes the anti–HIV-1 effects of zidovudine, but enhances the activity of purine dideoxynucleosides. Ribavirin use in patients who are coinfected with HIV and HCV and are taking antiretroviral drugs seems to increase the risk for mitochondrial toxicity and lactic acidosis, particularly when ribavirin is combined with didanosine. Ribavirin may inhibit the effect of warfarin.

Clinical Studies

Ribavirin aerosol is approved in the United States for treatment of RSV bronchiolitis and pneumonia in hospitalized children. Oral ribavirin in combination with various IFNs is approved for treatment of chronic hepatitis C. The following describes only clinical studies on the prevention and treatment of respiratory virus infection with ribavirin. Hepatitis C virus treatment is discussed separately (see Chapter 47).

Respiratory Syncytial Virus

Aerosolized ribavirin (18-hour exposure daily for 3 to 6 days) variably shortens the duration of virus shedding, and may improve certain clinical measures in infants hospitalized with RSV illness.[302] However, no consistent reductions in need for ventilatory support or duration of hospitalization have been documented. In infants receiving mechanical ventilation for RSV-related respiratory failure, no significant reductions in duration of ventilatory support, hospitalization, or mortality have been found.[302,303] Intermittent, high-dose therapy (2-hour exposures three times daily for 5 days) is well tolerated, and may be as effective as prolonged exposure.[304]

Use of aerosolized ribavirin is limited by concerns regarding its efficacy, ease of administration, risk of occupational exposure, and cost. The American Academy of Pediatrics states that aerosol treatment for RSV infection "is not recommended for routine use but may be considered for use in selected patients with documented, potentially life-threatening RSV infection."[305] Decreased RSV-specific serum neutralizing antibody titers, and diminished nasopharyngeal secretion RSV-specific immunoglobulin E and immunoglobulin A responses may occur in ribavirin-treated children. No long-term adverse or beneficial effects of ribavirin therapy have been documented in children.[306]

Combinations of aerosolized ribavirin and intravenous immunoglobulin or palivizumab may be beneficial in treating RSV pneumonia in hematopoietic stem cell transplant recipients,[307–310] whereas intravenous ribavirin alone is ineffective.[311] Aerosolized[312,313,314] and oral[315,316] ribavirin therapy appear to prevent progression from upper to lower respiratory tract illness in such patients. A similar benefit of preemptive treatment of RSV upper respiratory tract infection in lung transplant recipients with oral and inhaled ribavirin has been reported.[317,318]

Other Respiratory Viruses

Intravenous and aerosolized forms of ribavirin have been used to treat severe influenza virus infections.[319,320] Aerosolized ribavirin inconsistently reduces viral titers and illness measures in adults with uncomplicated influenza A or B, and has modest efficacy in children hospitalized with influenza.[321] However, oral ribavirin 300 mg three times per day combined

with amantadine 100 mg twice daily and oseltamivir 150 mg twice daily may possibly be effective for treatment of influenza A(H1N1)pdm09 disease, and more so than oseltamivir alone.[322] Oral, intravenous, and aerosolized ribavirin have been used in immunosuppressed patients with severe parainfluenza virus and adenovirus infections with inconsistent clinical benefits.[319,323,324] Intravenous ribavirin has been used to treat adenovirus-associated hemorrhagic cystitis, pneumonia, and invasive infections in immunocompromised patients, and it may be effective even in severe disease.[325,326] Use of intravenous[327,328] and oral[329] ribavirin for treatment of human metapneumovirus pneumonia in immunocompromised patients has been associated with resolution. Aerosolized ribavirin has been used in treating parainfluenza virus infections in solid-organ transplant recipients, but seems ineffective in parainfluenza virus pneumonia in hematopoietic stem cell transplant recipients.[323] Oral ribavirin was effective in accelerating functional graft recovery and reducing late bronchiolitis obliterans in 38 lung transplant recipients[330] and in a bone marrow transplant recipient[331] with parainfluenza respiratory infection. Intravenous ribavirin therapy was associated with successful treatment of paramyxovirus type 3 respiratory infection in cardiac transplant recipients.[332,333] Ribavirin has been used extensively in treating severe acute respiratory syndrome coronavirus infections without proven antiviral effects in vitro[282] or in patients,[334] and has been associated with frequent adverse effects.[299] Intravenous ribavirin seems to be ineffective in treatment of hantavirus cardiopulmonary syndrome,[335] However, it inhibits Andes virus, an important cause of this syndrome in vitro, and is effective in a hamster model of hantavirus cardiopulmonary syndrome caused by this virus (see Chapter 166).[336]

RSV604

RSV604 is an oral benzodiazepine compound ($C_{22}H_{17}FN_4O_2$) under development for treatment of RSV infections (Fig. 45.6).[337,338] It inhibits both RSV types A and B at submicromolar concentrations. Its antiviral activity is expressed through interaction with the RSV nucleocapsid (N) protein, which is highly conserved.[339] The drug is well absorbed orally, and a single dose per day is sufficient to achieve antiviral 90% effective concentration levels. In vitro resistance can be elicited, at an apparent low rate, and resistant virus appears similarly fit to wild-type RSV in terms of replication.[339] Phase II studies of RSV604 are underway in transplant patients with RSV infections.

ZANAMIVIR

Spectrum

Zanamivir (4-guanidino-2,4-dideoxy-2,3-didehydro-*N*-acetylneuraminic acid; Relenza) is a sialic acid analogue (see Fig. 45.4B) that is a potent and specific inhibitor of the neuraminidases of influenza A and B viruses.[340] It inhibits influenza neuraminidase activity at nanomolar concentration, but requires a higher and broader range of inhibitory concentrations in cell culture.[341,342] Compared with oseltamivir carboxylate, zanamivir is more active against influenza B, and data from a comparative trial in children indicate that this difference is clinically important.[343] Zanamivir is less active against neuraminidases of influenza A N2 clinical isolates,[344] but the clinical importance of this difference is uncertain. Zanamivir inhibits certain influenza A neuraminidase variants that are resistant to oseltamivir carboxylate.[122] Combinations of zanamivir plus rimantadine inhibit strains of influenza A(H1N1) and A(H3N2) viruses synergistically, but some concentrations seem antagonistic when assessed by reductions in cell-associated virus yield.[345] Zanamivir is not cytotoxic and is highly selective for influenza neuraminidase, inhibiting neuraminidases from human[346] and other mammalian sources or other pathogens only at 10^6-fold higher concentrations. Millimolar concentrations inhibit parainfluenza virus type 3 in cell culture, most likely by blocking attachment.[347] Topical zanamivir in the respiratory tract is active in murine and ferret models of influenza.[341] Zanamivir has recently been reviewed in detail.[348]

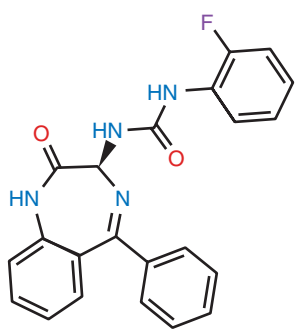

FIG. 45.6 **Chemical structure of RSV604.**

Resistance

Resistance to NAI drugs has developed less frequently than to the adamantane compounds and less frequently to zanamivir than to oseltamivir.

A recent systematic review of the prevalence of NAI resistance among influenza viruses cultured from immunocompetent ambulatory adults enrolled in prophylactic and therapeutic trials of zanamivir found no reports of zanamivir resistance.[349] In surveys of other collections of influenza isolates, a similar absence or dearth of zanamivir resistance was reported: influenza A(H1N1) viruses circulating in the 2008–2009 influenza season in the United States prior to emergence of the 2009 pandemic were resistant to oseltamivir but susceptible to zanamivir.[350] Among 391 nonpandemic A(H1N1) isolates from Australia and Southeast Asia patients from 2006 to 2008, 2.3% were resistant to zanamivir[351] but susceptible to oseltamivir. Zanamivir resistance was not demonstrated among 3359 influenza A(H1N1)pdm09 global isolates[352] nor among 304 oseltamivir-resistant isolates reported by the World Health Organization to August 2010.[353] From 2009 to 2014, the prevalence of zanamivir resistance among global isolates of influenza A(H1N1)pdm09, influenza A(H3N2), and influenza B isolates has been less than 1%.[348] In Japan in the 2015–2016 influenza season, there was no trend toward diminished susceptibility to zanamivir compared to results from 2010–2011 to 2015–2016.[348] Avian influenza A(H5N1) isolates from 2003 to 2005 were susceptible to zanamivir.[354] Of 680 influenza B viruses isolated in China from 2010 and 2011, one with D197N neuraminidase amino acid substitution was resistant to zanamivir.[355]

Several neuraminidase mutations mediate diminished susceptibility to zanamivir: Q136K in an A(H1N1) seasonal virus (300-fold reduction in zanamivir susceptibility),[351] S274N[356] in nonpandemic A(H1N1) virus, and I223R in an A(H1N1)pdm09 isolate (5-fold reduction).[357] The relationship of these virus resistance mutations and prior zanamivir therapy and host immune competence was not consistently apparent. An influenza B virus with an A152L mutation resistant to both zanamivir and oseltamivir was recovered from an immunocompromised child with prolonged virus excretion despite receipt of nebulized zanamivir.[358] The effect of these neuraminidase mutations on infectivity and transmissibility compared to the wild-type parental strains is variable, but only some mutants have been characterized in this regard.[358,359,360]

An observational study in pediatric patients with influenza treated with oseltamivir or zanamivir suggested that the lower prevalence of zanamivir than oseltamivir resistance is more related to the intrinsic properties of the drugs than to differences in the prevalence of use of the drugs.[361]

Pharmacokinetics

The oral bioavailability of zanamivir is low (<5%). The approved formulation is a dry powder containing a lactose carrier delivered by oral inhalation with a proprietary Diskhaler device. The proprietary inhaler device for delivering zanamivir is breath activated and requires a cooperative, trained patient. The use of the Diskhaler device is unreliable in young children, very infirm or elderly patients, or cognitively impaired patients. Although the inhaler has been used effectively in older adults,[362] more than half of hospitalized older adults could not correctly use the device after instruction.[363]

After inhalation of the dry powder using the Diskhaler, approximately 15% is deposited in the lower respiratory tract while the remainder is deposited in the oropharynx.[341,364] Zanamivir concentrations in epithelial lining fluid (ELF) obtained by bronchoalveolar lavage may approximate concentrations in alveoli. Median ELF concentrations of zanamivir 12 hours after oral inhalation of the recommended 10-mg dose by Diskhaler

in healthy volunteers ranged from 0.3 to 0.9 μg/mL.[365] In other uninfected individuals, median zanamivir levels in induced sputum were 1.34 μg/mL, 0.30 μg/mL, and 0.05 μg/mL at 6 hours, 12 hours, and 24 hours after dosing, respectively, with the pulmonary $t_{½elim}$ estimated to be 2.8 hours.[366] Approximately 4% to 17% of an inhaled dose is absorbed systemically, and peak plasma levels are low, averaging 0.04 to 0.05 μg/mL.[341] Because of the low bioavailability of zanamivir inhaled orally, dosage adjustments are not indicated in renal insufficiency.

After intravenous dosing, the plasma $t_{½elim}$ of zanamivir ranges from 1.6 to 2.9 hours,[341,365] with about 90% eliminated unchanged in the urine.[341] Following intravenous administration of 600 mg zanamivir to healthy adults, the median serum C_{max} is 39.4 μg/mL, $AUC_{0–12}$ is 86.6 μg·h/mL, and C_{trough} is 0.6 μg/mL. The pharmacokinetic apparent volume of distribution is independent of dose and averages 14.8 liters.[364] The median ELF concentration 12 hours after dosing is 0.4 μg/mL, very similar to the value after inhalation of 10 mg (see earlier). This is 552 to 1653 times the in vitro IC_{50} for influenza A and B neuraminidases, respectively.[344]

The pharmacokinetics of zanamivir administered intravenously to adults with and without renal impairment were similar to those in previously described studies.[367] Zanamivir renal clearance declines linearly with increasing renal impairment.[368] The suggested dose for adults with normal renal function is 600 mg intravenously given twice daily. Doses for children and for patients with renal impairment who are or are not receiving replacement therapy have been published.[369]

Interactions

No clinically significant drug interactions have been recognized for inhaled zanamivir. Zanamivir at concentrations up to 150 mg/L did not inhibit the metabolism of eight cytochrome P-450 isoenzyme probe substrates by human hepatocyte microsomes in vitro.[370] No clinically relevant pharmacokinetic interaction was demonstrated between oseltamivir 150 mg taken orally twice daily and zanamivir 600 mg administered intravenously every 12 hours in healthy volunteers.[371] Zanamivir does not affect the immune response to injected inactivated influenza vaccine, but similar to all antiviral medications, it has the potential to impair the immunogenicity of live-attenuated influenza virus vaccine administered concurrently. Zanamivir should not be administered from 48 hours before to 2 weeks after intranasal administration of an attenuated influenza vaccine.[372]

Toxicity

Preclinical studies of zanamivir found no evidence of mutagenic, teratogenic, or oncogenic effects. In cell culture, the inhibitory effect of zanamivir on influenza virus replication was not impaired by analgesics, antihistamines, decongestants, or antibacterial drugs.[373] Zanamivir is classified as a pregnancy category C agent.

Orally inhaled zanamivir is generally well tolerated, and the frequencies of complaints are not significantly different from those in placebo recipients among adults and children 5 years old or older.[341,373,374] This includes once-daily oral inhalation for prophylaxis by adults for 16 weeks.[375] Most reported symptoms in treatment studies are likely the result of the underlying illness. Similarly, in high-risk patients receiving zanamivir or placebo, no differences in adverse reactions have been seen in controlled trials.[376] In patients with mild to moderate asthma or chronic obstructive pulmonary disease, orally inhaled zanamivir is associated with fewer bronchitis episodes, similar measurements of forced expiratory volume in 1 second, and more rapid improvement in peak expiratory flow rate than with inhaled placebo.[378] However, postmarketing reports indicate a potential risk for acute bronchospasm, respiratory arrest, or worsening of chronic obstructive pulmonary disease accompanied by pulmonary edema after zanamivir inhalation, particularly in patients with underlying airway disease.[379] Apparent declines in respiratory function have also been rarely reported in patients without recognized airway disease. Consequently, use in patients with underlying airway disease is not generally recommended in the United States, although zanamivir is administered as treatment in at-risk patients in other countries.[380] If used in patients with obstructive airway disease, zanamivir should be administered cautiously under close observation and with availability of fast-acting bronchodilators.

Zanamivir inhaled as an experimental nebulized solution containing 16 mg/mL for 10 minutes four times a day for 5 days for treatment of serious influenza with lower respiratory tract signs in hospitalized patients 10 years or older was well tolerated.[381] However, when the oral formulation containing lactose has been reformulated as a solution and administered into the airway during mechanical ventilation, lactose precipitation in the airway filters has caused obstruction,[382] precluding the reformulation of the powder in the orally inhaled formulation into a solution for nebulization and inhalation.

Zanamivir injected intravenously to healthy volunteers in doses from 50 to 600 mg twice daily for 5 days was also well tolerated.[383] In 130 hospitalized adults with influenza treated with 600 mg zanamivir intravenously twice daily for 5 days or reduced doses in those with renal impairment, no safety signals or clinically significant trends in laboratory values, vital signs, or electrocardiograms were identified that were considered attributable to the drug.[384]

Clinical Studies

Zanamivir has been administered to patients intranasally as a spray, by oral inhalation as a dry powder, by nasal inhalation as an aerosol from a nebulized solution, and by intravenous injection.

Intranasal and intravenous zanamivir are highly protective against experimental human influenza, and early treatment is associated with reductions in viral titers, symptoms, and middle ear pressure abnormalities.[203,341,385] Orally inhaled zanamivir powder is approved in the United States for prevention of influenza in individuals 5 years old and older, and for treatment of influenza in individuals 7 years old and older. Zanamivir (10 mg twice daily for 5 days) inhaled early in the course of illness for treatment of uncomplicated influenza in previously healthy adults and children 5 to 12 years old shortens the times to illness resolution and return to usual activities by 1 to 3 days.[374,386,387] Treatment benefits seem to be greater in patients with severe symptoms at entry, in patients older than 50 years, and in higher-risk patients.[388] Inhaled zanamivir treatment in adults is associated with a 40% reduction in lower respiratory tract events leading to antibiotic use and a 28% overall reduction in antibiotic prescriptions.[389]

Zanamivir inhaled orally is equally efficacious for treatment of influenza A and B infection.[387,390] In individuals with influenza B illness, zanamivir reduces the median duration of fever by 32%, from 53 hours to 36 hours, compared with oseltamivir.[343] In high-risk patients with primarily mild to moderate asthma or other chronic cardiopulmonary conditions, orally inhaled zanamivir treatment reduces illness duration and the incidence of complications leading to antibiotic use.[378,391] It has been used to treat immunocompromised hosts with influenza A and B infections,[392] including a child to whom an aqueous zanamivir solution (16 mg/mL) was administered by aerosol and nebulizer via an endotracheal tube.[393] More recently, in an observational study, orally inhaled zanamivir was more efficacious for treatment of oseltamivir-resistant influenza A(H1N1) than oseltamivir.[394]

Prophylactic administration of once-daily inhaled zanamivir (10 mg) prevents febrile influenza illness during influenza season (84% efficacy),[395] or when used for postexposure prophylaxis in households with or without treatment of the ill index case (82% efficacy).[396,397] In an observational study with limited numbers of patients, orally inhaled zanamivir and oral oseltamivir were not different for prevention of secondary cases during nosocomial outbreaks on pediatric wards.[398] In nursing home residents, 2 weeks of inhaled zanamivir was superior to oral rimantadine in preventing influenza A infection, in part because of a high frequency of rimantadine resistance,[399] and inhaled zanamivir has been used to curtail transmission of amantadine-resistant influenza A in nursing homes.[362]

Orally inhaled zanamivir has been administered in combination with oral oseltamivir. For postexposure prophylaxis in families, such combined zanamivir-oseltamivir administration was not more efficacious than either agent alone.[222] However, a subgroup analysis suggests greater efficacy of the combination treatment among contacts whose prophylaxis was commenced within 24 hours of exposure to the index case compared to oseltamivir or zanamivir alone. For treatment of adults with mainly A(H3N2) influenza, zanamivir-oseltamivir combination treatment was not more efficacious than zanamivir alone and was less efficacious than oseltamivir monotherapy.[221]

Zanamivir has been administered intravenously to treat patients seriously ill with influenza who could not receive, or who had failed, oral oseltamivir therapy. Immunocompetent[400] and immunocompromised[401,402] patients who were infected with oseltamivir-resistant[403] and oseltamivir-susceptible[402,404] A(H1N1) nonpandemic viruses or with oseltamivir-resistant pandemic virus[405] or oseltamivir-sensitive A(H1N1) pdm09 virus[401,406] have been successfully treated with intravenous zanamivir. There is a sense that intravenous zanamivir may be lifesaving.[407] However, an apparent lack of a relationship between intravenous zanamivir treatment-associated reductions in pandemic virus load in upper and lower respiratory tract secretions and mortality have prompted questions about its effectiveness in seriously ill patients.[408] A phase III study comparing intravenous zanamivir at 300 or 600 mg per dose compared to oral oseltamivir 75 mg, all twice daily for 5 days, demonstrated no differences in efficacy or safety in hospitalized patients.[225]

POLYMERIC ZANAMIVIR CONJUGATES

Polymeric zanamivir conjugates are experimental, high-molecular-weight antiinfluenza compounds comprising multiple zanamivir monomers connected at the 7-OH position to backbone or linker molecules of various types and lengths.[409–415] These compounds are second-generation potential inhaled NAIs for influenza chemoprophylaxis and therapy with enhanced potency and prolonged lung retention time compared with zanamivir. In mice, one of these compounds has been associated with prophylactic efficacy for 7 days after a single intranasal administration. Polymeric zanamivir conjugates have recently been reviewed.[348]

Spectrum

Polymeric zanamivir conjugates exhibit broad-spectrum antiinfluenza activity, inhibiting human influenza A N1 and N2 and influenza B viruses and an avian influenza A(H5N1) virus.[409] Inhibitory potency varies according to the length[411] and type of linker molecule,[410] and the number of zanamivir derivatives, whether dimeric,[409] trimeric, or tetrameric.[412] The most potent polymeric zanamivir conjugate is a dimer with a 14-carbon linker, which was 10-fold less potent than zanamivir alone in a neuraminidase enzyme inhibition test (IC_{50} = 7.86 nM vs. 0.76 nM for zanamivir), but is 500,000-fold more potent in inhibiting influenza A/WSN/33 (H1N1) in a cytopathic reduction assay (IC_{50} = 0.0001 nM vs. 56 nM for zanamivir).[409] In mice, this dimeric conjugate is 100 times more potent than zanamivir in preventing influenza virus replication in the lung for 7 days after a single intranasal dose of drug, and 1 day after intranasal virus challenge (drug doses to reduce lung virus titer by 90% were 0.03 mg/kg and 2.92 mg/kg for the dimeric conjugate and zanamivir, respectively). The prophylactic effect is associated with prolonged persistence of dimer conjugate in lung tissue after intranasal administration (see under "Pharmacokinetics"). The specificity of polymeric zanamivir conjugates for influenza A and B neuraminidase is presumed but not yet reported.

A zanamivir polymer can overcome zanamivir resistance. A zanamivir polymer bound to the neuraminidase of zanamivir-resistant avian influenza A viruses possessing a resistance mutation at position 119 bound as much as 2000 times more strongly than did monomeric zanamivir.[416]

Mechanism of Action

The synthesis of polymeric conjugates of zanamivir that retain NAI activity is possible because of the unique position of the molecule when it is docked in the enzymatic pocket, with the 7-OH group pointing out and away from the target site, making it accessible to linkage to different backbone molecules. Electron micrographs show influenza virus clumping in the presence of dimeric zanamivir conjugates. The marked potency of some conjugates is postulated to reflect clumping caused by three types of bivalent binding: binding between two neuraminidase molecules in the tetrameric transmembrane spike protein (intratetramer), binding between sites on different tetramers on the same virion (intravirionic), and head-to-head binding between different neuraminidase sites on separate virions (intervirionic).[409] An additional mechanism for the marked enhancement of potency observed by synthesis of polymer-attached zanamivir is postulated to be the result of interference with intracellular trafficking of endocytosed virus and subsequent virus-endosome fusion.[417]

Resistance

Studies describing attempts to induce resistance in vitro by repeated passage in the presence of drug have not been reported.

Pharmacokinetics

Prolonged retention of polymeric zanamivir compared with monomeric zanamivir in lung tissue accounts for the enhanced antiviral effect of polymeric conjugates. After intratracheal instillation of the same single dose of a polymeric zanamivir conjugate or monomeric zanamivir solution in rats, lung homogenate drug concentrations of the polymeric compound after 48 hours and 168 hours are 35 times and 60 to 113 times greater than zanamivir concentrations, respectively. Generally, lung retention time is directly related to molecular weight because small polar molecules leave the lung by passing through tight junctions between cells. Thus prolonged retention of high-molecular-weight polymeric conjugates compared with monomeric zanamivir is expected. However, the prolonged lung retention time of some smaller conjugates indicates that aqueous insolubility and aggregate formation plus partitioning into cell membrane phospholipids may also play a role in the prolonged retention of zanamivir polymeric conjugates in the lung after inhalation.[409]

Interactions and Toxicity

Toxicity studies have been limited to assessments of in vitro cytotoxicity. For a series of dimeric conjugates, concentrations of 100 to 1000 ng/mL caused no cytotoxicity.[411]

Clinical Studies

No clinical studies have been reported.

BALOXAVIR MARBOXIL

Baloxavir (S-033188/S-033447; Xofluza) is a small-molecule inhibitor of influenza A and B cap-dependent endonuclease, which resides on the N-terminal domain of the PA subunit of the RNA-dependent RNA polymerase of influenza A (Fig. 45.7). Baloxavir interferes with the "cap-snatching" activity of cap-dependent endonuclease that primes viral transcription.[418,419] The N-terminal domain of the PA subunit is highly conserved among subtypes of influenza A, and baloxavir is active in vitro against H1N1, H3N2, H5N1, H7N7, H7N9, and H9N2 strains.[419] It has shown efficacy in the mouse model against influenza A(H5N1) and influenza B infection.[420] The developers of baloxavir have reported activity against oseltamivir-resistant strains of influenza.[421] The drug was stated to have a prolonged half-life, and single-dose oral administration was utilized in clinical trials.

A phase II proof-of-concept, randomized, placebo-controlled trial in influenza found that a single dose of baloxavir at 10 mg, 20 mg, or 40 mg was effective in reduction of time to alleviation of symptoms and resulted in a rapid reduction in viral titer.[420]

A phase III study (CAPSTONE-1) was conducted in 1436 patients 12 to 64 years of age, with onset of influenza-related symptoms within 48 hours, and in whom influenza virus was detected by PCR. Subjects

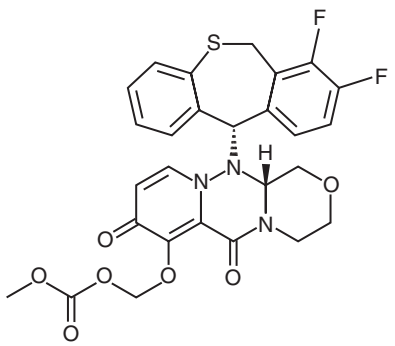

FIG. 45.7 **Chemical structure of baloxavir marboxil.**

received a single dose of 40 or 80 mg of baloxavir according to body weight, 75 mg of oseltamivir twice daily for 5 days, or placebo.[422] Time to alleviation of symptoms was shorter in the baloxavir group (53.7 hours) than in the placebo group (80.2 hours) (P <.0001) but similar to that in the oseltamivir group (53.8 hours). Mean time to cessation of viral shedding was 24 hours in patients who received baloxavir, compared to 72 hours in the oseltamivir group (P <.0001) and 96 hours in the placebo group (P <.0001). Baloxavir administration was generally well tolerated. Polymerase acidic PA 138 T/M protein variants conferring reduced susceptibility to baloxavir developed in 10% of baloxavir recipients and in no placebo recipients.

Baloxavir was licensed in Japan in February 2017 and in the United States in October 2018. Additional studies in immunosuppressed patients and children are planned in the United States.

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46 Antivirals Against Herpesviruses

Fred Y. Aoki

The availability of efficacious and well-tolerated antiviral drugs that collectively inhibit most of the eight human herpesviruses has significantly reduced the morbidity and mortality caused by these viruses in healthy individuals. The availability of these drugs has also contributed to the control of herpesvirus infections resulting from immunosuppression due to diseases such as human immunodeficiency virus (HIV) infection or reticuloendothelial system malignancies such as Hodgkin disease. Moreover and just as important, the availability of these drugs has permitted the increasing and successful use of potent immunosuppressive agents for the management of a wide variety of problems such as transplant rejection, as a substantial proportion of herpesvirus infections are due to reactivation of asymptomatic latent herpesvirus infection. Antiviral drugs that are of established therapeutic effectiveness as evidenced by registration by drug regulatory bodies in various countries are listed in Table 46.1.

ACYCLOVIR AND VALACYCLOVIR

Spectrum

Acyclovir (9-[(2-hydroxyethoxy)methyl]-9H-guanine; acycloguanosine; Zovirax) is a deoxyguanosine analogue that has an acyclic side chain lacking the 3′-hydroxyl group, instead of the cyclic ribose base of natural nucleosides (Fig. 46.1A). Valacyclovir (Valtrex) is the L-valyl ester prodrug of acyclovir. The clinically useful antiviral spectrum of acyclovir is limited to certain herpesviruses. Acyclovir is approximately 10 times more potent against herpes simplex virus type 1 (HSV-1) and HSV-2 than against varicella-zoster virus (VZV), and it is even less active against cytomegalovirus (CMV) (Table 46.2).[1,2] Acyclovir inhibits the replication of Epstein-Barr virus (EBV) in productively infected cells but does not affect latent or persistent infection. Acyclovir has shown antiviral activity in experimental HSV infection when administered topically, parenterally, or orally and in simian varicella when given systemically.[1] Enhanced antiherpesvirus activity occurs when acyclovir is given in combination with interferons (IFNs) and other antiviral agents in vitro and in animal models.[1]

Growth of uninfected mammalian cells is generally unaffected by high acyclovir concentrations. Acyclovir (20 μg/mL) does not reproducibly alter cell-mediated immune responses of human peripheral blood leukocytes or affect human granulocyte progenitor cell growth in vitro.[3]

Mechanism of Action

Acyclovir is the prototype of a group of antiviral agents that are activated by viral thymidine kinase (TK) to become inhibitors of viral DNA polymerase that block viral DNA synthesis.[1,2,4] Acyclovir uptake and intracellular phosphorylation to the monophosphate derivative are catalyzed by HSV TK. Cellular enzymes convert the monophosphate to acyclovir triphosphate, which is present in 40-fold to 100-fold higher concentrations in HSV-infected cells than in uninfected cells. Acyclovir triphosphate competitively inhibits viral DNA polymerase and to a much smaller extent cellular DNA polymerases with respect to deoxyguanosine triphosphate. Acyclovir triphosphate is also incorporated into viral DNA, where it acts as a chain terminator because of the lack of the 3′-hydroxyl group. Formation of a complex between the terminated DNA template containing acyclovir and the enzyme may lead to irreversible inactivation of the DNA polymerase. The DNA polymerases of various herpesviruses differ in their degree of inhibition by acyclovir triphosphate; the polymerases of EBV and CMV seem to be especially insensitive.

Resistance

Acyclovir-resistant HSV, often defined by an in vitro inhibitory concentration greater than 2 to 3 μg/mL, can be readily selected by passage in the presence of acyclovir and is present in native virus populations, with an approximate frequency of 1 in 10^3 to 10^4 infectious virions.[4–6] Three basic resistance mechanisms have been identified: absent or low production of viral TK, altered TK substrate specificity (e.g., phosphorylation of thymidine but not of acyclovir), and altered viral DNA polymerase. Changes in these viral enzymes relate to point mutations or base insertions or deletions in the corresponding genes.[7,8] The most common mechanism found in clinical HSV isolates is absent or deficient TK activity.[4,9] Less commonly, resistant isolates have altered TK activity, and DNA polymerase mutants are rare in clinical strains. Heterogeneous mixtures are commonly found. TK-negative variants are cross-resistant to other agents activated by viral TK (e.g., penciclovir, ganciclovir), but TK-altered and DNA polymerase mutants variably retain susceptibility.[4]

The prevalence of acyclovir-resistant HSV isolates in immunocompetent hosts is about 0.1% to 0.7% but increases to approximately 4% to 14% in immunocompromised patients.[4,7,10] During several decades of use, no increase in the prevalence of acyclovir-resistant variants has occurred in immunocompetent individuals.[4,11] Resistant HSV-2 has been found in 0.2% of human immunodeficiency virus (HIV)–negative and 5.3% of HIV-positive individuals with genital herpes[12] and has been recovered from 11% to 17% of individuals with acquired immunodeficiency syndrome (AIDS) or recipients of allograft transplants receiving acyclovir treatment for 2 weeks or longer.[13] Progressive HSV disease associated with recovery of acyclovir-resistant virus and poor response to acyclovir therapy is well recognized in immunocompromised patients. Painful ulcerating perirectal lesions, often indolent and necrotizing, caused by HSV-2 represent the most common pattern in patients with AIDS. Orofacial disease caused by HSV-1 is common in transplant recipients. Risk factors for resistance emergence include degree of immunosuppression, size of lesions, repeated or prolonged use of acyclovir for treatment rather than prophylaxis, and, possibly, the use of topical acyclovir in genital herpes.[11]

TK-negative HSV strains are less neurovirulent than wild-type strains and are unable to reactivate from latency in animal models, although they may cause extensive mucocutaneous disease in immunocompromised hosts.[4,6] TK-deficient, TK-altered, or DNA polymerase mutants have variable decreases in pathogenicity. Acyclovir-resistant HSV recurrent genital or ocular infections have rarely been found in immunocompetent hosts.[4] One case of possible person-to-person spread of resistant HSV has been reported. Recurrences after cessation of acyclovir are usually caused by sensitive virus.[4] In patients with AIDS, persistent shedding of resistant HSV at the site of initial infection and recurrences with acyclovir-resistant variants have been found in the absence of selective drug pressure.[14] Visceral disease is uncommon, but pneumonitis, meningoencephalitis, esophagitis, hepatitis, retinal necrosis, and disseminated infection have occurred with resistant variants, including instances in neonates.[4,12,15]

Depending on the degree of immunosuppression, resistant HSV infections may undergo spontaneous healing during or after cessation of acyclovir therapy. In patients with progressive disease, intravenous foscarnet therapy is effective, but therapy with vidarabine is not.[14] Intravenous cidofovir also seems to be effective. High-dose continuous infusion of acyclovir,[16] topical trifluridine, topical IFN-α2 alone or in

TABLE 46.1 Antiviral Agents of Established Therapeutic Effectiveness for Herpesvirus Infections

VIRAL INFECTION	DRUG	ROUTE	USUAL ADULT DOSAGE
Cytomegalovirus	Ganciclovir	IV	5 mg/kg/12 h in 1-h infusion for 14–21 days[a]
Retinitis	Valganciclovir	PO	900 mg bid for 21 days[a]
	Cidofovir	IV	5 mg/kg once weekly × 2, then every other week
	Fomivirsen	Intravitreal	330 µg every 2 weeks × 2, then every 4 weeks
	Foscarnet	IV	60 mg/kg/8 h in 1- to 2-h infusion for 14–21 days[b]
HSV			
Genital herpes			
First episode	Acyclovir	PO[c]	400 mg tid or 200 mg 5 times/day for 7–10 days
	Famciclovir	PO	250 mg tid for 7–10 days
	Valacyclovir	PO	1 g bid for 7–10 days
Recurrent	Acyclovir	PO	800 mg tid for 2 days or 400 mg tid or 200 mg 5 times/day or 800 mg bid for 5 days
	Famciclovir	PO	125 mg bid for 5 days or 1000 mg repeated once at 12 h for 1 day
	Valacyclovir	PO	500 mg bid for 3 days or 1 g/day for 5 days
Suppression[d]	Acyclovir	PO	400 mg bid or 200 mg tid
	Famciclovir	PO	250 mg bid
	Valacyclovir	PO	500 mg/day or 1 g/day (≥10 episodes/y) or 250 mg bid
Encephalitis	Acyclovir[e]	IV	10–15 mg/kg/8 h in 1-h infusions for 14–21 days
Mucocutaneous disease in immunocompromised hosts	Acyclovir[f]	IV	5 mg/kg/8 h for 7–14 days[g]
		PO	400 mg 5 times/day for 7–14 days
	Valacyclovir[h]	PO	500 mg or 1 g bid for 7–10 days
	Penciclovir[h]	IV	5 mg/kg/8–12 h for 7 days
	Famciclovir	PO	500 mg bid for 7–10 days
Orolabial herpes			
First episode	Acyclovir	PO	Children: 15 mg/kg 5 times/day for 7 days (maximum 200 mg/dose) Adults: drugs and doses recommended for first-episode genital herpes have been used
Recurrent	Penciclovir 1%	Topical	Apply cream every 2 h while awake for 4 days
	Acyclovir 5%	Topical	Apply cream 5 times/day for 4 days
	Acyclovir 5% with hydrocortisone 1% cream	Topical	Apply cream 5 times/day for 5 days
	Docosanol 10%	Topical	Apply cream 5 times/day until healed
	Valacyclovir	PO	2 g repeated once at 12 h
	Famciclovir	PO	1500 mg once or 750 mg repeated once at 12 h
	Acyclovir	PO	400 mg tid/day for 5 days
Neonatal HSV	Acyclovir[i]	IV	10–20 mg/kg/8 h for 14–21 days
Keratitis	Acyclovir	Topical	1 cm of 3% ophthalmic ointment 4 times/day
	Trifluridine[j]	Topical	1 drop of 1% solution topically q2h, ≤9 drops/day
	Vidarabine	Topical	½-inch ribbon of 3% ointment 5 times daily
Varicella-Zoster Virus			
Varicella in normal children	Acyclovir	PO	20 mg/kg (≤800 mg) qid for 5 days
Varicella in immunocompromised hosts	Acyclovir	IV	10 mg/kg/8 h or 500 mg/m^2/8 h for 7–10 days[k]
Herpes zoster in immunocompromised hosts	Acyclovir	IV	10 mg/kg/8 h in 1-h infusion for 7–10 days[k]
Herpes zoster in normal hosts	Acyclovir	PO	800 mg 5 times daily for 7–10 days
	Valacyclovir	PO	1 g tid for 7 days
	Famciclovir	PO	500 mg tid for 7 days
	Brivudin[h]	PO	120 mg daily for 7 days

Consult text and manufacturer's product prescribing information for dosage adjustments in renal or hepatic insufficiency and in other circumstances.
[a]In patients with AIDS or who are otherwise highly immunocompromised, long-term suppression with valganciclovir 900 mg/day is recommended after acute treatment. IV ganciclovir 5 mg/kg given 7 days/wk or 6 mg/kg given 5 days/wk or oral ganciclovir 1 g tid are alternatives for suppression. These dosages are also approved for prevention of cytomegalovirus disease in transplant recipients.
[b]Long-term suppression with daily infusion of 90–120 mg/kg over 2 h is recommended after initial treatment in patients with AIDS.
[c]In patients with severe initial genital herpes and in patients unable to tolerate oral medicines, IV acyclovir 5 mg/kg/8 h for 5–7 days is recommended before a switch to an oral agent.
[d]Famciclovir 500 mg bid and valacyclovir 500 mg bid are effective in reducing recurrences in human immunodeficiency virus–infected patients.
[e]Higher dosages and 21 days of IV therapy are recommended by some authorities. Additional oral valacyclovir treatment afterward provides no additional benefit.[92a]
[f]In acyclovir-resistant HSV or varicella-zoster virus infections, IV foscarnet 40 mg/kg/8 h seems beneficial. Duration of therapy depends on the clinical response. For limited cutaneous infections in immunocompromised patients, 5% acyclovir ointment can be applied to lesions every 3 h up to 6 times daily for 7 days (about ½-inch ribbon per 4 square inches), using a finger cot or glove.
[g]Higher dosages (30 mg/kg/day) are recommended in progressive or visceral infections. Suggested pediatric dosage in children <12 years old is 10 mg/kg/8 h for 7 days per manufacturer.
[h]Not approved by US Food and Drug Administration for this indication. IV penciclovir is unavailable in the United States.
[i]High dosage (20 mg/kg/8 h) seems superior in disseminated and central nervous system disease in neonates.[92]
[j]An ophthalmic ointment of 3% acyclovir is available in some countries. Idoxuridine 0.1% solution, q1h while awake and q2h at night, or 0.5% ointment 5 times/day is a less effective alternative. Treatment of HSV ocular infections should be supervised by an ophthalmologist.
[k]Pediatric dosage of 500 mg/m^2/8 h for 7–10 days for children ≥1 year old, although some experts recommend 10 mg/kg/8 h for these children.[113]
AIDS, Acquired immunodeficiency syndrome; *HSV,* herpes simplex virus; *IV,* intravenously; *PO,* orally.

TABLE 46.2 Representative in vitro Inhibitory Concentrations of Acyclic Nucleosides and Nucleotides for Clinical Isolates of Herpesviruses in Human Cells

	INHIBITORY CONCENTRATION (μg/mL)			
VIRUS	**Acyclovir**	**Penciclovir**	**Ganciclovir**	**Cidofovir**
Herpes simplex virus 1	0.02–1.9	0.2–1.8	0.05–0.6	0.4–3
Herpes simplex virus 2	0.3–2.9	0.3–2.4	0.05–0.6	0.4–3
Varicella-zoster virus	0.8–5.2	0.9–5.1	0.2–2.8	0.25
Cytomegalovirus	2–57	52	0.2–2.8	0.2–0.9
Epstein-Barr virus	1.6	—	1.5	<0.03
Human herpesvirus 6	7–23	—	0.3–7	—
Human herpesvirus 8	15–17	—	0.9–1.4	0.1–0.23

Data from references 6, 8, 14, 159, 162, 250, 252, 373, 418, 420.

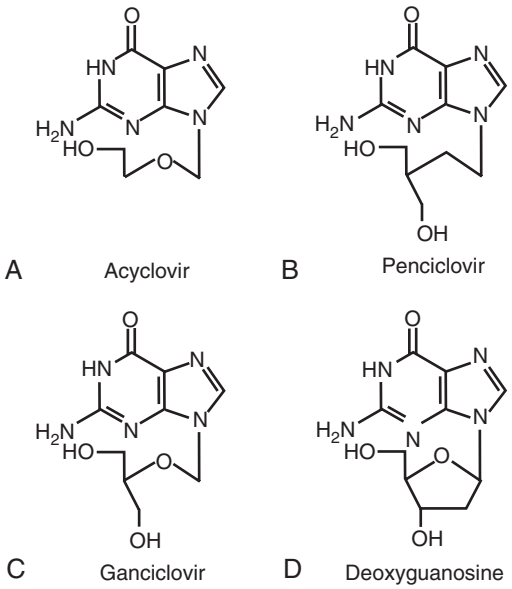

FIG. 46.1 Chemical structures of acyclovir (A), penciclovir (B), and ganciclovir (C) and their natural nucleoside analogue deoxyguanosine (D).

combination with topical trifluridine,[17] topical foscarnet,[18] topical cidofovir gel, and topical imiquimod[19] have been used with variable success.[20]

Acyclovir resistance in VZV isolates, associated with 20-fold to 40-fold increases in inhibitory concentrations, is usually related to mutations in VZV TK with inability to phosphorylate acyclovir or, less often, to mutations in viral DNA polymerase. Although rare, resistant isolates, including the Oka VZV vaccine strain,[21] have been recovered from highly immunocompromised children and adults with chronic disseminated, hyperkeratotic, or verrucous papular lesions that failed to heal with intravenous acyclovir.[22,23] Invasive disease with resistant variants occurs. Long-term suppressive therapy with subtherapeutic dosages of acyclovir seems to be a risk factor. Intravenous foscarnet or cidofovir may be effective for acyclovir-resistant VZV infections.[24,25]

Pharmacokinetics

The bioavailability of oral acyclovir is low (15%–21%) and decreases with increasing dosages.[1] Peak plasma concentrations average 0.4 to 0.8 μg/mL after 200-mg oral doses and increase to about 1.6 μg/mL with 800-mg doses. Bioavailability is lower in transplant recipients in whom doses of 400 mg provide peak levels of 0.7 to 0.9 μg/mL. A liquid suspension has lower oral bioavailability; peak plasma concentrations average 1 μg/mL in children receiving doses of 600 mg/m^2. In neonates and infants younger than 2 years, oral bioavailability averages 12%. Acyclovir kinetics are affected by prematurity and age younger than 1 month, and weight-adjusted dosing is essential.[26] Peak and trough plasma concentrations average 9.8 μg/mL and 0.7 μg/mL, respectively, after intravenous administration of 5 mg/kg every 8 hours and 20.7 μg/mL and 2.3 μg/mL, respectively, after 10 mg/kg every 8 hours. Peak concentrations average 10.3 μg/mL and 20.7 μg/mL after intravenous doses of 250 mg/m^2 and 500 mg/m^2, respectively, in children.

After oral administration, valacyclovir is readily absorbed, most likely via human peptide transporter 1 (hPEPT1), and rapidly converted to acyclovir during first-pass enzymatic hydrolysis in the liver and intestine by an enzyme designated valacyclovir hydrolase.[27,28] The relative bioavailability of acyclovir is three to five times greater after ingestion of valacyclovir, whose absolute bioavailability averages 54% to 70%.[29] Estimated bioavailability is 48% in hospitalized immunocompromised children 5 years old or older.[30] Peak plasma levels of valacyclovir are 0.4 μg/mL or less after 1000-mg doses. Peak plasma acyclovir levels average 5 μg/mL and 8.5 μg/mL after doses of 1000 mg and 2000 mg, respectively, in adults and are estimated to be 7 to 8 μg/mL after doses of 30 mg/kg valacyclovir in children.[29,31,31a] Total acyclovir exposure with administration of valacyclovir is similar to that seen with intravenous acyclovir, although peak plasma concentrations are twofold to fourfold lower.[30] In older adults, peak plasma concentrations increase 15% to 20%, and overall acyclovir exposure increases 30% to 50%,[27] probably because of reduced renal clearance in this population.

Acyclovir is distributed widely in body fluids. Plasma protein binding is less than 20%. Concentrations over time in noninflammatory cerebrospinal fluid (CSF) average 20% of concentrations in serum.[32] Salivary concentrations average 13% of plasma levels, but concentrations in vaginal secretions range from 15% to 170% of those in plasma. Zoster vesicular fluid levels are similar to those in plasma. Aqueous humor levels average 37% of concurrent plasma values. Acyclovir is concentrated in breast milk at approximately threefold higher levels than in maternal serum. Plasma levels in newborns are similar to maternal levels, and amniotic fluid and placental concentrations are several-fold higher.[33] Percutaneous absorption of acyclovir after topical administration seems to be low.

The mean plasma elimination half-life ($T_{½elim}$) of acyclovir is about 2.5 to 3 hours (range, 1.5–6.3 hours) in adults with normal renal function; it is slightly longer (3.8 hours) in neonates and increases to 19.5 hours in anuric patients.[33,34] Renal excretion of unmetabolized acyclovir by glomerular filtration and tubular secretion accounts for 60% to 91% of an administered dose, whereas less than 15% is excreted as 9-carboxymethoxymethylguanine or minor metabolites.[35] Acyclovir and valacyclovir dosage reductions are indicated in patients with a creatinine clearance (CrCl) less than 50 mL/min (Tables 46.3 and 46.4). Hemodialysis removes 33% to 60% of acyclovir during a 6-hour session, whereas peritoneal dialysis removes very little.[36] Dosing is recommended after hemodialysis, but supplementation is not needed during continuous ambulatory peritoneal dialysis. Bioavailability is about 61% after intraperitoneal dosing.[37] Table 46.4 lists dosage adjustments for valacyclovir in patients with impaired renal function.

TABLE 46.3 Acyclovir Dosage Adjustments Suggested for Patients With Impaired Renal Function

CREATININE CLEARANCE (mL/min/1.73 m²)	INTRAVENOUS		ORAL[a]	
	Standard Dosage (%)	Dosing Interval (h)	Dosage (mg)	Dosing Interval (h)
>50	100	8	800	4
>25–50	100	12	800	4
>10–25	100	24	800	8
0–10[b]	50	24	800	12[c]

[a]Oral acyclovir dosage adjustments are needed for severe renal insufficiency. Recommendations are based on the high-dose oral regimen (4000 mg/day). For the low-dose (1000 mg/day) oral regimen, the suggested dosage is 200 mg q12h when creatinine clearance is <10 mL/min/1.73 m².
[b]An alternative in patients with end-stage renal disease is administration of 14% of standard dosage q8h after loading with 37% of the standard dosage. In hemodialysis, use 60%–100% of standard dosage after the hemodialysis run only.
[c]In dialysis-dependent patients, a further dosage reduction to 200 mg/12 h and 400 mg after dialysis is recommended to avoid toxic levels. A dosage of 800 mg orally q24h has been suggested for patients on continuous ambulatory peritoneal dialysis.[36] For adults during continuous renal replacement therapy, give q48h.
Modified from Blum MR, Liao SH, de Miranda P. Overview of acyclovir pharmacokinetic disposition in adults and children. Am J Med. *1982;73:186–192; and Laskin OL, Longstreth JA, Whelton A, et al. Effect of renal failure on the pharmacokinetics of acyclovir.* Am J Med. *1982;73:197–201.*

TABLE 46.4 Dosage Adjustments of Valacyclovir in Patients With Impaired Renal Function

CREATININE CLEARANCE (mL/min/1.73 m²)	HERPES ZOSTER		RECURRENT GENITAL HERPES		RECURRENT OROLABIAL HERPES	
	Dosage	Interval	Dosage	Interval	Dosage	Interval
≥50	1 g	8 h	500 mg	12 h	2 g × 2	12 h
30–49	1 g	12 h	500 mg	12 h	1 g × 2	12 h
10–29	1 g	24 h	500 mg	24 h	500 mg × 2	12 h
<10	500 mg	24 h	500 mg	24 h	500 mg	Once

Based on manufacturer's recommendations. Reductions are also indicated for other dosage regimens (e.g., for genital herpes suppression and initial treatment).

Interactions

Severe somnolence and lethargy may occur with combinations of zidovudine and acyclovir.[38] Concomitant use of cyclosporine and probably of other nephrotoxic agents enhances the risk for nephrotoxicity. Probenecid and cimetidine slow valacyclovir metabolism, decrease renal acyclovir clearance, and increase overall acyclovir exposure by 48% and 27%.[39] By competing for the organic acid secretory pathway, acyclovir may decrease the renal clearance of other drugs eliminated by active renal secretion, such as methotrexate. Thiazide diuretics or the hPEPT1 substrate cephalexin do not substantially alter valacyclovir pharmacokinetics.[27] No pharmacokinetic interaction was observed between tipranavir/ritonavir and single-dose valacyclovir at steady state in healthy volunteers.[28]

Toxicity

Topical acyclovir may cause transient burning when it is applied to genital lesions. The polyethylene glycol base of topical acyclovir may cause mucosal irritation and is not approved for intravaginal use. Acyclovir cream uncommonly causes allergic contact dermatitis.[1]

Intravenous acyclovir is generally well tolerated,[1] although inflammation, phlebitis, and, rarely, vesicular eruption can occur at the injection site after extravasation of the alkaline solution (pH 9–11). Uncommon side effects include rash, diaphoresis, hematuria, hypotension, headache, and nausea. Approximately 1% to 4% of patients receiving intravenous acyclovir have manifested neurotoxicity, characterized by lethargy, confusion, obtundation, tremor, myoclonus, hallucinations, delirium, seizures, extrapyramidal signs, autonomic instability, or coma.[40] Diffuse electroencephalographic abnormalities and increased CSF concentrations of myelin basic protein may occur. Symptoms of neurotoxicity usually develop within 1 to 3 days after starting treatment. Most of these patients have acute renal dysfunction or preexisting renal disease, and neurotoxicity occurs in association with high serum acyclovir concentrations (>25 μg/mL) and detectable CSF levels of 9-carboxymethoxymethylguanine, the main metabolite of acyclovir, which may be the cause of acyclovir neurotoxicity. It has been detected at higher concentrations in the CSF of subjects with neurotoxicity[41] than in asymptomatic normal subjects or subjects with chronic kidney disease.[31] Neurotoxicity occurs more often after valacyclovir. Neurologic side effects usually resolve within several days after drug concentrations decrease. Hemodialysis may be useful in severe cases.

Reversible renal dysfunction has been observed in approximately 5% of patients, including a higher proportion of children, treated with intravenous acyclovir.[1] Acyclovir can cause a crystalline nephropathy and, rarely, interstitial nephritis.[42] Acyclovir solubility decreases to 2.5 mg/mL at 37°C, and crystalluria has been described in adult and pediatric patients. Obstructive nephropathy may manifest as nausea, emesis, flank pain, and increasing azotemia. Co-administration with other nephrotoxic drugs, bolus infusion, dehydration, preexisting renal insufficiency, high doses, and high acyclovir plasma levels are risk factors. Nephrotoxicity usually resolves with drug cessation and volume expansion.

Oral acyclovir has been associated infrequently with nausea, diarrhea, rash, and headache and uncommonly with renal insufficiency or neurotoxicity. Immediate hypersensitivity reactions to acyclovir are rare but may be managed with oral desensitization.[43] Long-term acyclovir suppression for frequently recurring genital or mucocutaneous infections seems well tolerated after long-term use,[44,45] and no adverse effects on sperm production or peripheral blood lymphocyte cytogenetics have been detected.[46] However, oral acyclovir can cause neutropenia in infants.[47] High-dose valacyclovir (8 g/day) is associated with gastrointestinal intolerance, azotemia, and possibly thrombotic microangiopathy in patients with AIDS[48] and with confusion and hallucinations in transplantation patients.[49] Tolerance at lower doses is comparable to that of acyclovir. Localized bullous skin lesions[50] and an acute generalized pustulosis confirmed by patch testing have been reported.[51]

Acyclovir has shown mutagenic activity in some in vitro assays at high concentrations, but no significant immunosuppressive activity, carcinogenicity, or teratogenicity has been noted in animal studies. High doses decrease spermatogenesis and cause testicular atrophy in animals. Acyclovir is classified as pregnancy category B and is present in breast milk. No excess frequency of congenital abnormalities has been recognized in infants born to women exposed to acyclovir during pregnancy, although whether exposure may increase the risk for spontaneous abortion is unresolved.[52,53,54]

Clinical Studies

Acyclovir is the agent of choice for management of many types of HSV and VZV infections because of its efficacy, safety, and ease of administration (see Table 46.1).[1,2] Valacyclovir is comparably effective in most of the conditions in which oral acyclovir is used and offers more convenient dosing regimens.

Herpes Simplex Virus

Acyclovir by various routes is effective in primary genital HSV infections.[55] Topical acyclovir is less effective than oral or intravenous administration, and its use is discouraged. Intravenous acyclovir markedly reduces viral shedding, time to healing, and duration of symptoms in patients with severe primary genital HSV infections. In outpatients, oral acyclovir (200 mg five times daily for 10 days) is associated with significant reductions in virus shedding, symptoms, and time to healing. Higher dosages of oral acyclovir do not increase efficacy,[56] and valacyclovir (1 g twice daily for 10 days) is comparable to acyclovir in efficacy and tolerability for treating first-episode genital herpes.[57] None of these regimens has been associated with consistent reductions in the risk for recurrent genital lesions. Acyclovir therapy decreases the humoral and cellular immune response to HSV after a first episode of genital herpes. Higher oral dosages (400 mg five times daily for 10 days) provide similar benefit in first episodes of HSV proctitis.

In recurrent genital HSV infections, patient-initiated oral acyclovir (200 mg five times daily for 5 days) during the prodrome or at the first sign of lesions is associated with reductions of 1.5 to 2 days in the duration of shedding and time to healing. A 2-day regimen of high-dose acyclovir (800 mg three times daily) is also associated with 2-day reductions in duration of lesions and symptoms.[58] Valacyclovir (500 mg twice daily for 5 days) is comparable to acyclovir and superior to placebo in the treatment of recurrent genital herpes.[59,60] Famciclovir 1000 mg taken twice early in the course of a recurrent episode of genital herpes,[61] a 3-day course of valacyclovir (500 mg twice daily), and a 5-day course of valacyclovir 500 mg twice daily are equally efficacious and well tolerated.[62] Topical acyclovir alone offers minimal clinical benefit in this condition.

In patients with frequently recurring genital herpes, long-term oral acyclovir (400–1000 mg/day in divided doses) reduces the frequency of clinical recurrences by about 90%, protects 65% to 85% of patients from recurrence, and reduces the frequency of subclinical viral shedding and viral DNA detectability.[45,63] Dosages of 400 mg twice daily are effective for longer than 5 years. Once-daily or weekend-only use of acyclovir is inadequate, whereas once-daily valacyclovir (500 mg, or 1000 mg if frequent recurrences) seems to be effective and well tolerated.[64] Valacyclovir suppression (500 mg daily) reduces the risk of transmitting HSV-2 infection to a serosusceptible partner by approximately 50%.[65] Asymptomatic shedding may occur during suppression, and transmission to sexual partners has occurred. After cessation of acyclovir, patients generally return to their previous pattern of recurrent infection. Oral acyclovir and valacyclovir suppression during late pregnancy reduce virus shedding, recurrences, and cesarean delivery rates for HSV after a first episode occurring during pregnancy and in women with recurrent genital herpes.[66,67] Acyclovir 400 mg twice a day from 28 to 36 weeks of gestation tended to reduce premature rupture of membranes from 10% to 4% in placebo recipients and significantly reduced premature delivery from 24% in placebo recipients to 11%.[68]

Long-term suppression may be useful in other patients with disabling recurrences of herpes whitlow or HSV-related erythema multiforme. In patients with recurrent herpes labialis or ocular HSV disease, prolonged oral acyclovir (400 mg twice daily) or valacyclovir (500 mg once daily) reduces the number of recurrences by about half.[69–71] In patients with a history of sun-induced recurrences, short-term prophylaxis (400–800 mg twice daily) inconsistently reduces the risk for recurrence.[72] Short-term prophylaxis during outbreaks in day care centers may be effective in preventing primary infections in children,[73] but the efficacy of postexposure prophylaxis remains to be established.

In recurrent orolabial HSV infections in immunocompetent individuals, topical acyclovir ointment is not beneficial. Topical 5% acyclovir cream is available in many countries outside of the United States, and patient-initiated treatment reduces the duration of an episode by about 0.5 day.[74] A cream consisting of 5% acyclovir and 1% hydrocortisone prevented progression of cold sores to ulcerative lesions and significantly reduced the cumulative lesion area compared with acyclovir cream alone or placebo.[75] High-dose, patient-initiated oral valacyclovir (2 g twice in 1 day) reduces the duration of orolabial herpes episodes and healing time by about 1 day.[76] Oral acyclovir (200–400 mg five times daily for 5 days) provides modest clinical benefit in orolabial HSV but seems to be efficacious in recurrent whitlow.[77] Acyclovir (15 mg/kg five times daily to a maximum of 200 mg/dose for 7 days) is beneficial for treating primary HSV gingivostomatitis in children,[78] and long-term suppression reduces cutaneous recurrences after neonatal infection.

In Bell's palsy, prednisolone improves the chances of complete recovery. Controlled studies have shown that there is no benefit of acyclovir or valacyclovir alone or in addition to prednisolone for treatment of Bell's palsy, but some consultants still use combined oral steroids and valacyclovir in severe disease.[79,80]

Acyclovir in various regimens and valacyclovir have been used successfully for prevention and treatment of mucocutaneous HSV infections in immunosuppressed patients.[2,81] Intravenous acyclovir (250 mg/m^2 every 8–12 hours or 125 mg/m^2 every 6 hours), begun before transplantation and continuing for several weeks, is highly effective in reducing the incidence of HSV disease in seropositive bone marrow transplant recipients.[82] For patients who can tolerate oral medications, oral acyclovir (400 mg five times daily or 600 mg every 6 hours) or valacyclovir (500 mg twice daily) is effective in marrow transplant recipients, and long-term oral acyclovir (400 mg three times daily for 6 months) reduces the risk for HSV, CMV, and VZV infection.[83,84] Low doses of oral acyclovir (200 mg every 6–8 hours) seem to be effective in renal transplant recipients. Valacyclovir (500 mg twice daily) is effective for suppression of recurrent genital herpes in HIV-infected individuals.[85] Serial HSV DNA plasma load was monitored during successful prevention of recurrent HSV disease with intravenous acyclovir and valacyclovir following hepatic transplantation for treatment of fulminant HSV hepatitis in an immunocompetent host.[86]

In immunocompromised patients with established mucocutaneous HSV infection, intravenous acyclovir (250 mg/m^2 every 8 hours for 7 days) shortens healing time, duration of pain, and virus shedding,[87] although recurrences are common after cessation of therapy. Oral acyclovir (800 mg five times daily) is also effective.[88] Intravenous acyclovir may be beneficial in cases of disseminated HSV in pregnant women and in transplant recipients.[89,90]

In HSV encephalitis, acyclovir reduces mortality to 19% to 28% compared with 50% to 54% with vidarabine.[2] In neonates, in immunosuppressed patients, and, uncommonly, in apparently healthy individuals, early relapse of encephalitis may follow initial acyclovir therapy (see Table 46.1). Neurologic deterioration, interpreted as relapsing HSV encephalitis, seems to be unrelated to direct viral cytolysis.[91] High-dose intravenous acyclovir (60 mg/kg/day for 21 days) seems to be more effective than the lower dosage (10–15 mg/kg/day) approved by the US Food and Drug Administration for neonatal central nervous system (CNS) and disseminated HSV disease.[92] In immunocompetent patients older than 12 years with HSV encephalitis treated with IV acyclovir, subsequent therapy with 3 months valacyclovir 6 g/day did not provide additional benefit.[92a]

Continuous infusion of acyclovir was reported to be effective in a neonate with HSV-2 encephalitis who did not respond to the intermittent recommended acyclovir dose regimen.[93] The efficacy of acyclovir for treating primary or recurring HSV-2 meningitis has not been demonstrated. However, valacyclovir 500 mg twice daily was not effective compared with placebo in preventing recurrent episodes of verified and probable HSV-2 meningitis.[94] Acyclovir seems to be effective for treatment of presumed HSV acute retinal necrosis syndrome.[95] Prolonged oral acyclovir (≥14 weeks) was significantly more effective than a shorter duration (<14 weeks) in preventing involvement of the other eye.[96] Fulminant HSV hepatitis in two pregnant women was successfully treated with intravenous acyclovir.[97]

Epidemiologic studies demonstrate a strong synergistic relationship between the dual contemporary epidemics of genital HSV-2 and HIV-1 infection. HSV-2 increases the risk of HIV-1 acquisition twofold or threefold[98] as well as the risk of transmission. However, prophylactic daily acyclovir does not decrease HIV-1 acquisition rates,[99,100] and

administration of acyclovir does not prevent HIV-1 transmission,[101] even though HIV-1 plasma load[102] as well as genital, rectal, seminal,[103] and, perhaps, cervicovaginal[104] concentrations are reduced. Prevalent HSV acyclovir resistance does not likely explain the incomplete antiviral effect,[105] whereas incomplete adherence may contribute to acyclovir inefficacy.[104]

HSV can cause exophytic, ulcerating, hypertrophic genital lesions in HIV-infected individuals.[106,107] These respond best to intravenous acyclovir.[106] Topical imiquimod may augment the effect of oral acyclovir.[107] Acyclovir 4 g daily was superior to oral erythromycin and no less effective than higher dose of acyclovir for treatment of pityriasis rosacea, which may be due to human herpesviruses, mainly human herpesvirus (HHV)-6 and HHV-7.[108,109]

Varicella-Zoster Virus Infections

High-dose oral acyclovir is effective treatment for herpes zoster in older adults and, if begun within 24 hours after rash onset, for varicella in children,[110] adolescents,[111] and adults.[112] In children, effects of acyclovir include reductions of about 1 day in fever and of 15% to 30% in the severity of other illness measures, so routine use for uncomplicated varicella in otherwise healthy children younger than 13 years is not recommended.[113] In adults with varicella, oral acyclovir (800 mg five times daily for 7 days) initiated within 24 hours after the onset of rash reduces fever and the time to total crusting of lesion by approximately 2 days; it does not affect the course of illness if begun later.[112] Postcontact prophylaxis with oral acyclovir (40 mg/kg daily in divided doses) beginning 9 to 11 days after exposure may reduce the risk for varicella in household contacts.[114]

In immunocompetent older adults with herpes zoster, intravenous acyclovir reduces virus shedding, time to healing of skin lesions, and duration of acute pain. Oral acyclovir (800 mg five times daily for 7–10 days) also reduces acute pain and healing time in older adults if treatment is initiated within 72 hours after rash onset and particularly within 1 or 2 days. In a placebo-controlled study, concomitant administration of steroids and antivirals resulted in improved quality of life.[115] A reduction in ocular complications, particularly keratitis and anterior uveitis, occurs with oral acyclovir (800 mg five times daily for 7 days) or valacyclovir (1 g three times daily for 7 days) treatment of zoster ophthalmicus.[116] No consistent effect of acyclovir on the incidence or severity of postherpetic neuralgia has been found[117]; evidence was insufficient to determine whether valacyclovir prevents postherpetic neuralgia. Compared with acyclovir, oral valacyclovir (1 g three times daily for 7 days) speeds resolution of zoster-associated pain and decreases the frequency of persistent pain.[118] Intravenous acyclovir and steroid treatment was associated with recovery from cervical myelitis occurring 1 month after herpes zoster in an immunocompetent adult.[119]

Intravenous acyclovir is effective for varicella and herpes zoster in immunocompromised patients. It also seems to be effective in varicella pneumonia or encephalitis in previously healthy adults.[120] In immunocompromised patients with herpes zoster, intravenous acyclovir (500 mg/m^2 every 8 hours for 7 days) reduces the risk for cutaneous dissemination and visceral complications. In immunosuppressed children with varicella, intravenous acyclovir reduces the risk for visceral complications and time to full crusting. Early relapse of infection may occur after cessation of therapy, and treatment may be ineffective in visceral disease. Early treatment with oral acyclovir (800 mg five times daily for 7 days) may be effective in immunocompromised children.[121] Valacyclovir 1000 mg three times daily is not different from 2000 mg three times daily, both for 7 days, in efficacy and safety for the treatment of uncomplicated herpes zoster in immunocompromised adults.[121a] Herpes zoster recurrence in patients with multiple myeloma treated with bortezomib-based therapy can be successfully prevented with acyclovir.[122] Valacyclovir 1000 mg twice daily for 4 through 24 months after transplantation is more efficacious than placebo in preventing herpes zoster in bone marrow transplant recipients.[123]

Cytomegalovirus

Acyclovir is therapeutically ineffective in established CMV infections. However, for prophylaxis, high-dose intravenous acyclovir (500 mg/m^2 every 8 hours) beginning 5 days before allogeneic bone marrow transplantation and continuing for 30 days afterward is associated with a delayed risk for CMV infection; when high-dose intravenous acyclovir is followed by oral acyclovir (800 mg four times daily for 6 months), overall survival is improved.[124,125] In seropositive allogeneic bone marrow or stem cell transplant recipients, valacyclovir (2 g four times a day after initial intravenous acyclovir) was more effective than high-dose oral acyclovir (800 mg four times a day)[126] and comparable to intravenous ganciclovir[127] in preventing CMV reactivation.

High-dose oral acyclovir also reduces the risk for CMV disease in renal and liver transplant recipients but seems to be less effective than ganciclovir,[128,129] although in one report valacyclovir and valganciclovir were similarly effective.[129] Acyclovir has not proved effective in liver transplant recipients receiving OKT3 therapy or in lung transplant recipients. High-dose valacyclovir prophylaxis (2 g four times a day for 90 days) reduces the risk for CMV disease and of graft rejection in renal transplant recipients including CMV-seronegative recipients of seropositive grafts[130] and seems to be effective in preventing CMV reactivation after heart transplantation.[131] In an uncontrolled study, in HIV-infected pregnant women, valacyclovir 500 mg twice daily begun at 34 weeks of gestation and continued for 1 year did not reduce acquisition of CMV by infants compared with placebo.[132] In another uncontrolled study, in non–HIV-infected pregnant women with a fetus with ultrasound features of intrauterine CMV infection, valacyclovir 8 g/day increased the proportion of asymptomatic neonates to 82% compared with 43% without treatment.[133] However, the American Society for Fetal-Maternal Medicine advises against any such antenatal therapy at this time.[134]

Other Viruses

Valacyclovir is recommended in managing herpesvirus B (*Cercopithecine herpesvirus* 1) exposures, and high-dose intravenous acyclovir (12.5–15 mg/kg/8 h) or ganciclovir is recommended for treating non-CNS herpesvirus B infections; intravenous ganciclovir (5 mg/kg/12 h) is advised for CNS infection (see Chapter 141).[135] High-dose acyclovir has been reported to provide a modest survival benefit in advanced HIV infection, although this remains controversial.[136] Valacyclovir reduced oropharyngeal shedding of HHV-8 in HIV-1–infected individuals, but not as markedly as did antiretroviral drugs.[137]

In infectious mononucleosis, acyclovir is associated with transient suppression of salivary EBV excretion but no important effects on illness parameters.[138] High-dose acyclovir is ineffective in patients with chronic fatigue syndrome.[139] Some cases of post-transplantation EBV-related polyclonal lymphoproliferation may respond to acyclovir.[140] However, a systematic review and meta-analysis concluded that antiviral prophylaxis (acyclovir, valacyclovir, ganciclovir, and valganciclovir) in solid-organ transplant recipients did not prevent EBV-associated post-transplantation lymphoproliferative disease.[141] Long-term acyclovir or related antivirals possibly reduce the risk for AIDS-related lymphoma.[142] EBV-related oral hairy leukoplakia usually regresses with oral acyclovir or valacyclovir treatment but then recurs.[143] Topical 5% acyclovir with 25% podophyllin resin is effective in resolving lesions of oral hairy leukoplakia with a reduced recurrence rate compared with podophyllin resin alone in HIV-1–seropositive patients.[144] Neither intravenous nor oral acyclovir enhances the response to IFN in patients with chronic hepatitis B. Long-term acyclovir does not reduce neurologic deterioration in multiple sclerosis.[145]

BRIVUDIN

Brivudin ([*E*]-5-[2-bromovinyl]-2′-deoxyuridine) is a halogenated thymidine nucleoside analogue that is licensed for treatment of herpes zoster in several European Union countries, but not the United States (Fig. 46.2).[146] Brivudin potently and selectively inhibits replication of VZV and to a lesser extent HSV-1 in vitro. In vitro inhibitory concentrations of brivudin average 0.0033 μM for clinical VZV isolates and are more than 100-fold lower than those for acyclovir and penciclovir.[147] Brivudin-resistant HSV-1 clones can be readily selected in vitro during replication in the presence of drug.[148,149] Cross-resistance to acyclovir is common.[148,149]

When used for early treatment of nonophthalmic herpes zoster in immunocompetent adults, oral brivudin (125 mg once daily) is not

different from famciclovir given at 250 mg three times a day, both given for 7 days.[150] The same brivudin regimen is generally comparable to acyclovir (800 mg five times daily) in effects on acute pain and healing of lesions, although it may accelerate cessation of new lesion formation to a slightly greater extent.[146] In adults 50 years old and older, brivudin treatment seems to reduce modestly the frequency, although not the duration, of postherpetic neuralgia compared with acyclovir.[151] Treatment-related adverse effects are similar to those of acyclovir, with low frequencies of gastrointestinal upset, headache, and dizziness[151]; delirium and hepatitis have been reported during therapy.[152,153] The main metabolite of brivudin is bromovinyluracil, which interferes with the catabolism of fluorinated pyrimidines such as 5-fluorouracil, with the result that potentially serious drug interactions can occur.[154]

CIDOFOVIR

Spectrum

Cidofovir (S)-1-[3-hydroxy-2-(phosphomethoxy) propyl] cytosine dihydrate; HPMPC; Vistide) is an acyclic phosphonate nucleotide analogue of deoxycytidine monophosphate (Fig. 46.3A) with inhibitory activity against human herpesviruses including HSV, CMV, EBV, HHV-6, HHV-7, and HHV-8 and certain other DNA viruses including papillomaviruses, polyomaviruses, poxviruses, and adenoviruses.[155–158] In vitro inhibitory concentrations range from less than 0.2 to 0.7 μg/mL for CMV,[159] 0.4 to 33 μg/mL for HSV,[160] and 0.02 to 17 μg/mL for adenoviruses.[161] Because phosphorylation does not depend on virus-specified enzymes, cidofovir is inhibitory for acyclovir-resistant, TK-deficient, or TK-altered HSV strains and ganciclovir-resistant CMV strains with *UL97* mutations, although not for strains with *UL54* mutations. TK mutants of HSV show 20-fold enhanced susceptibility to cidofovir compared with wild-type HSV because mutant viruses induce smaller elevations in competing deoxycytidine triphosphate pools in infected cells.[162] Cidofovir inhibits parvovirus B19 in vitro.[163]

In vitro, cidofovir shows synergistic inhibition of CMV in combination with ganciclovir or foscarnet.[157] The prolonged intracellular $T_{½}$ of the diphosphate is associated with persistent antiviral activity and enables infrequent dosing regimens. Cidofovir is active in animal models of herpesvirus, papillomavirus, and, given systemically or by aerosol, poxvirus infections.[164–166] Topical cidofovir is active against ocular adenovirus infection in rabbits but is associated with local irritation.[167]

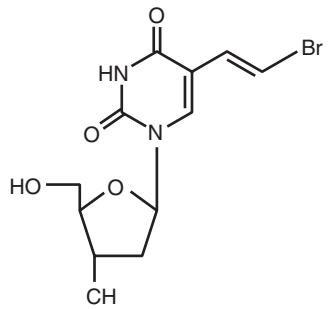

Brivudin

FIG. 46.2 Chemical structure of brivudin.

Mechanism of Action

Cidofovir inhibits viral DNA synthesis. Cidofovir is metabolized intracellularly to its active diphosphate form by cellular enzymes, and the levels of phosphorylated metabolites are similar in infected and uninfected cells. The diphosphate acts as a competitive inhibitor with respect to deoxycytidine triphosphate and as an alternative substrate for viral DNA polymerase. Incorporation of cidofovir slows chain elongation and abrogates it if two consecutive cidofovir molecules are introduced. The diphosphate has a prolonged intracellular $T_{½}$, averaging 17 to 65 hours depending on the cell type, and it competitively inhibits CMV and HSV DNA polymerase at concentrations 8-fold to 600-fold lower than concentrations inhibitory for human DNA polymerases.[156,168] An adduct with prolonged intracellular $T_{½}$ (>2 days) may serve as a reservoir of drug.

Resistance

Resistance to cidofovir selected by in vitro passage of CMV, poxviruses, and adenoviruses relates to point mutations in viral DNA polymerase. Highly ganciclovir-resistant clinical isolates of CMV that possess *UL54* mutations show cross-resistance to cidofovir in vitro with 8-fold to 16-fold reductions in inhibitory concentrations.[169] Foscarnet-resistant CMV and HSV isolates may retain susceptibility to cidofovir, but multidrug-resistant CMV variants with DNA polymerase mutations occur.[170] The development of resistance to cidofovir as a result of cidofovir therapy seems to be uncommon and low level (less than eightfold change in susceptibility).[159,171] In patients with CMV retinitis, reduced cidofovir susceptibility has been detected in about 5% of CMV isolates before treatment and in 29% by 3 months of therapy.[172] Poxviruses selected for resistance to cidofovir in vitro (8-fold to 27-fold reduced susceptibility) seem less virulent than wild-type viruses but are resistant to cidofovir in vivo.[173]

Pharmacokinetics

Oral bioavailability of cidofovir is low (<5%). After intravenous infusion, plasma levels are proportional to dose and decline in a biphasic pattern, with a terminal $T_{½elim}$ that averages about 2.6 hours.[174] The maximum plasma concentration averages 19.6 μg/mL after a dose of 5 mg/kg in conjunction with probenecid.[175] Plasma protein binding is low (<7%), and the volume of distribution approximates total body water (0.5 L/kg). CSF penetration is low.[174] Cidofovir is cleared by the kidney via glomerular filtration and active tubular secretion. More than 90% of the dose is recovered unchanged in the urine, and no significant metabolism has been recognized in humans. High-dose probenecid (2 g 2 hours before and 1 g 2 hours and 8 hours after each infusion) reduces renal clearance by blocking tubular secretion of cidofovir and increases blood levels. Clearance correlates with CrCl, and $T_{½elim}$ increases to 32.5 hours in patients receiving continuous ambulatory peritoneal dialysis. About 50% of a dose is removed by hemodialysis, but cidofovir is not significantly cleared by continuous ambulatory peritoneal dialysis.[175a] After application of cidofovir gel, systemic absorption is low (peak plasma concentrations <0.5 μg/mL) and is related to lesion size.[160]

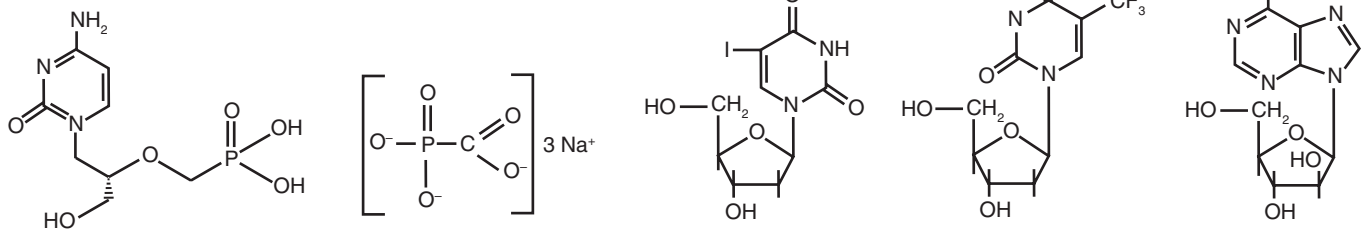

A Cidofovir B Foscarnet C Idoxuridine D Trifluorothymidine E Vidarabine

FIG. 46.3 Chemical structures of cidofovir (A), foscarnet (B), idoxuridine (C), trifluorothymidine (D), and vidarabine (E).

Interactions

Concomitant therapy with other nephrotoxic agents is contraindicated during cidofovir therapy, and an interval of at least 7 days is recommended after aminoglycoside, intravenous pentamidine, amphotericin B, foscarnet, nonsteroidal anti-inflammatory drug, or contrast dye exposure.

Toxicity

Dose-related nephrotoxicity is the principal side effect of intravenous cidofovir.[157] It is characterized by proximal tubular dysfunction including proteinuria, azotemia, glycosuria, metabolic acidosis, and, uncommonly, Fanconi syndrome. Nephrotoxicity occurs as a result of a cidofovir-avid renal organic anion transport protein that causes drug accumulation in the renal cortex. Nephrotoxicity occurs because of apoptosis induced by cidofovir in renal tubular epithelial cells.[176] Concomitant oral probenecid and vigorous saline prehydration reduce the risk for renal toxicity, whereas prior foscarnet therapy and concurrent use of other nephrotoxic agents increase the risk. On maintenance dosing (5 mg/kg every other week), approximately 12% to 39% of patients develop proteinuria, and 15% to 24% have elevated serum creatinine. Severe nephrotoxicity requiring dialysis sometimes occurs. Initiation of cidofovir is relatively contraindicated in patients with CrCl less than 55 mL/min or with significant proteinuria (2+). Dosage reductions (3 mg/kg) are indicated for minor increases in serum creatinine (0.3–0.4 mg/dL), and cessation of administration is indicated for greater creatinine increases or development of proteinuria of 3+ or higher.

Neutropenia develops in approximately 24% of patients, and regular monitoring of neutrophil counts is necessary. Fever, nausea, emesis, diarrhea, headache, rash, asthenia, iritis, uveitis, and ocular hypotony may occur during combined therapy with cidofovir and probenecid.[157,177] Maintenance is withdrawn in approximately 25% to 35% of patients with AIDS because of intolerance.

Mucosal application is associated with dose-related application site reactions (burning, pain, pruritus) in one-third of patients and occasionally with ulceration.[160] Although no evidence of systemic toxicity was reported, topical cidofovir treatment of two severely immunocompromised patients with refractory multidrug-resistant HSV infection resulted in irreversible acute kidney injury.[178] Intravitreal cidofovir may cause iritis, vitreitis, reduced intraocular pressure, and visual loss.[179] Anterior uveitis has been reported after intravenous cidofovir administration.[180] Conjunctival application causes local irritation; persistent epiphora related to lacrimal canalicular blockade has developed in some patients.

Intravesical cidofovir (2.5–5 mg/kg in 50–100 mL normal saline) was administered via a transurethral catheter to treat BK or adenovirus hemorrhagic cystitis.[181,182] In one study, pain was reduced by cidofovir treatment in 8 of 9 patients, but in another study, due to the development of significant lower abdominal pain, only two of six patients could tolerate the 2-hour dwell time.[181] Compared with the reported area under the curve for the first 24 hours after time zero (AUC0–24) for an equivalent intravenous dose, intravesicular cidofovir resulted in 1% to 74% of the corresponding systemic exposure. One patient developed a >50% increase in serum creatinine, suggesting the need for more studies on the renal safety of intravesicular cidofovir.

Preclinical studies indicate that cidofovir has mutagenic, gonadotoxic, embryotoxic, and teratogenic effects. Because cidofovir causes carcinomas in rats, this agent is considered a potential human carcinogen. Intralesional cidofovir in patients with recurrent respiratory papillomatosis does not seem to increase the frequency of laryngeal dysplasia.[183,184] In a case report, progressive laryngeal dysplasia developed during intralesional cidofovir therapy of recurrent laryngeal papillomatosis over 27 months.[185] Safety during pregnancy is uncertain, and cidofovir is classified as pregnancy category C.

Clinical Studies

Intravenous cidofovir is approved for the treatment of CMV retinitis in patients with AIDS. Intravenous cidofovir (5 mg/kg once a week for 2 weeks, followed by every-other-week dosing) significantly increases the time to progression of CMV retinitis in previously untreated patients and in patients failing or intolerant of ganciclovir and foscarnet therapy.[157,186,187] Maintenance dosages of 5 mg/kg every other week are more effective but less well tolerated than 3 mg/kg dosages.[188] Clearance of viruria, but not viremia, has been shown.[189] Intravenous cidofovir seems comparable to a combined regimen of oral ganciclovir plus implant in preventing retinitis progression and mortality.[190] When used as preemptive therapy for post-transplant CMV infection or treatment of established disease including CMV pneumonia, cidofovir has been associated with responses in 50% or more of allogeneic stem cell recipients.[191,192]

Intravenous cidofovir has been used as preemptive therapy for post-transplant CMV infections as an alternative to prophylaxis with ganciclovir or valganciclovir for prevention of CMV disease.[193] Intravenous cidofovir has been used to treat acyclovir-resistant or foscarnet-resistant mucocutaneous HSV infection[155,194,195] and ganciclovir-resistant CMV disease including encephalitis in a stem cell transplant recipient.[196,197] Early treatment has been advocated as being possibly efficacious in controlling severe adenovirus pneumonia in nonimmunocompromised patients[198] and invasive adenoviral infections of transplant recipients,[199–201,202–204] although altered dosage schedules (e.g., 1 mg/kg thrice weekly) may be needed to reduce nephrotoxicity.[205] Low intravenous dosages (0.25–1 mg/kg every 1–3 weeks, with or without probenecid) have been used in treating refractory BK virus–associated nephropathy in renal transplantation recipients[206,207] and BK virus–associated[208] and adenovirus-associated[209] hemorrhagic cystitis in stem cell transplant recipients. Cidofovir has been instilled in the bladder for treatment of BK-hemorrhagic cystitis.[182] The addition of intravenous cidofovir to antiretroviral therapy (ART) has inconsistent effects on neurologic outcomes and survival in HIV-associated progressive multifocal leukoencephalopathy,[210,211] but a review of 33 cases suggests that cidofovir does not increase survival independent of the beneficial effect of ART.[212] Primary HHV-8 disease and Kaposi sarcoma in a liver transplant recipient was successfully treated with combination therapy including glucocorticoids, doxorubicin, and intravenous cidofovir.[213] However, cidofovir did not reduce HHV-8 viral load in peripheral blood mononuclear cells or prevent progression in Kaposi sarcoma in another report.[214] Intracavitary cidofovir was associated with resolution of pleural and peritoneal HHV-8–associated lymphoma in HIV-negative patients.[215–217]

Topical cidofovir gel formulated in polyethylene glycol reduces pain, virus shedding, and lesion healing time in HIV-infected patients and stem cell transplant recipients with acyclovir-resistant mucocutaneous HSV infections.[160,218] Cidofovir 3% oral rinses controlled acyclovir-resistant and foscarnet-resistant HSV-1 orolingual infection in an immunocompromised host.[219] Intralesional cidofovir and aerosolized cidofovir induce remissions in respiratory papillomatosis.[220–222] However, in a randomized, double-blind, placebo-controlled trial, intralesional cidofovir was not more efficacious than placebo.[223] Intralesional cidofovir suppresses recurrent EBV-associated nasopharyngeal carcinoma.[224] Topical cidofovir has been used for the treatment of recurrent anogenital herpes including exophytic pseudotumorous lesions, anogenital warts, refractory condyloma, orf poxvirus lesions,[155,225–228] recurrent HPV-related nasal squamous papillomatosis,[229] high-grade vulvar[230] and anal[231] intraepithelial neoplasia, and trichodysplasia spinulosa due to polyomavirus.[232] Intravenous cidofovir has resulted in resolution of anogenital[233] and generalized[234] warts. Topical and intravenous preparations have been used in recalcitrant molluscum contagiosum in immunosuppressed patients.[155,235] Intravitreal cidofovir injection may be effective for treatment of CMV retinitis[179] but is relatively contraindicated because of toxicity. A 1% ophthalmic solution reduced the risk for corneal opacities in adenoviral keratoconjunctivitis but was associated with severe, dose-dependent local toxicity.[236]

CMX-001 (Brincidofovir)

Alkoxyalkyl ester prodrugs of cidofovir exhibit enhanced cellular uptake and are more potent poxvirus inhibitors than the parent molecule, are well absorbed orally, and are not renally concentrated.[237] In cells, the esters are cleaved to release cidofovir. Two ether lipid esters, hexadecyloxypropyl-cidofovir and octadecyloxyethyl-cidofovir, administered orally, are highly active in experimental poxvirus, CMV, and adenovirus infections in animals.[238–240] Hexadecyloxypropyl-cidofovir (CMX-001 or brincidofovir) has undergone phase I and phase II testing. The renal toxicity of cidofovir is related to its accumulation in renal tubular epithelial cells by organic anion transporter 1 (OAT1). As CMX-001 is not a substrate for OAT1, it has not been associated with nephrotoxicity.[241]

A phase II study of CMX-001 for the prevention of CMV infection in stem cell transplant recipients showed a reduction in new or progressive CMV infections in patients who received 100 mg twice a week. Diarrhea was dose limiting at a dose of 200 mg twice a week.[216] Neither myelosuppression nor nephrotoxicity was observed. In an uncontrolled trial, CMX-001 prevented HSV and VZV infections in human stem cell transplant (HSCT) recipients.[242]

Retrospective case studies suggested that CMX-001 therapy was effective for treating adenovirus viremia in HSCT recipients.[243–245] Brincidofovir was associated with successful treatment of one patient with cancer and acyclovir-resistant HSV and two patients with ganciclovir-resistant (*UL97* gene mutation) CMV infection. One patient with ganciclovir-resistant CMV disease due to a *UL54* mutation did not respond.[246]

DOCOSANOL

Docosanol (*n*-docosanol or behenyl alcohol; Abreva) is a saturated 22-carbon aliphatic alcohol that inhibits a broad range of lipid-enveloped viruses including HSV-1 and HSV-2 at millimolar concentrations in vitro.[247] Docosanol is not directly virucidal, and its principal anti-HSV mechanism of action in vitro apparently relates to interference with viral fusion to host cell membranes early in replication, although other inhibitory effects may be possible.[247] A nonantiviral mechanism of action resulting from anti-inflammatory effects has been described. In a guinea pig model of cutaneous HSV, topical docosanol did not show antiviral or therapeutic benefits and was less active than topical penciclovir and acyclovir.[248] No emergence of resistance has been described to date.

Docosanol 10% cream is available over the counter for treatment of recurrent herpes labialis in immunocompetent individuals. Early treatment (five applications daily until healing up to maximum of 10 days) shortens the time to complete healing by about 0.7 day and time to resolution of symptoms by 0.5 day.[249] Application site reactions occur in about 2%, and its use is generally well tolerated. Whether combined treatment with docosanol and topical nucleosides might provide greater therapeutic effects remains to be determined.

FAMCICLOVIR AND PENCICLOVIR

Spectrum

Penciclovir (9-[4-hydroxy-3-hydroxymethylbut-1-yl] guanine; Denavir) is an acyclic guanosine analogue (see Fig. 46.1B) similar to acyclovir in its spectrum of activity and potency against herpesviruses (see Table 46.2).[250] Famciclovir (Famvir) is a prodrug, the diacetyl ester of 6-deoxy penciclovir, and lacks intrinsic antiviral activity. Because of its dependence on viral TK for initial phosphorylation, penciclovir is inactive against TK-deficient strains of HSV or VZV, but it may be active against some TK-altered or polymerase mutants that are resistant to acyclovir and against some foscarnet-resistant HSV isolates.[251,252] Penciclovir is also inhibitory for hepatitis B virus (HBV) and shows enhanced inhibition in combination with lamivudine or adefovir in vitro.[253,254] Topical, parenteral, and oral penciclovir and oral famciclovir are active in experimental HSV infections.[255] In a guinea pig model of primary cutaneous HSV-1 infection, penciclovir 1% cream was more efficacious than acyclovir 5% cream.[256]

Mechanism of Action

Penciclovir is an inhibitor of viral DNA synthesis. In infected cells, penciclovir is preferentially phosphorylated to its active form, penciclovir triphosphate, which serves as a competitive inhibitor of viral DNA polymerase.[257] In contrast to acyclovir, it is not an obligate chain terminator. Although penciclovir triphosphate is approximately 100-fold less potent in inhibiting viral DNA polymerase than acyclovir triphosphate, it is present in much higher concentrations and for more prolonged periods in infected cells. The prolonged intracellular $T_{½}$ of penciclovir triphosphate, which ranges from 7 to 20 hours, is associated with a sustained antiviral effect in cell culture and in animal models.[258] This effect may allow for infrequent dosing during clinical use. Although not preferentially phosphorylated in HBV-infected cells, penciclovir triphosphate is also a potent inhibitor of HBV DNA polymerase reverse transcriptase.[259]

Resistance

Penciclovir-resistant variants of HSV selected by in vitro passage have mutations in viral TK or DNA polymerase. Acyclovir-resistant, TK-negative mutants are resistant to penciclovir, but some variants with altered TK substrate specificity or with DNA polymerase mutations are susceptible.[4,260] Resistant HSV is detected in about 0.3% of patients with orolabial herpes, and emergence during clinical use has been very low in immunocompetent hosts.[5,261] As seen with acyclovir, higher rates have been observed in immunocompromised patients.[9] Resistance of HBV to penciclovir is associated with point mutations in viral DNA polymerase, particularly L528M.[262] Lamivudine resistance is associated with this mutation and predicts poor virologic response to famciclovir.[263]

Pharmacokinetics[264]

Famciclovir is a prodrug that is well absorbed orally and is rapidly converted to penciclovir by deacetylation and oxidation of the purine. This occurs during and after absorption through the intestinal wall and in the liver.[265] Although penciclovir itself is poorly absorbed, its bioavailability averages 77% after oral administration of famciclovir. Little or no famciclovir is detectable in blood or urine. Penciclovir is less than 20% bound to plasma proteins. The volume of distribution is approximately double that of body water. After single 250-mg and 500-mg doses of oral famciclovir, the peak plasma concentration of penciclovir averages 1.6 to 1.9 μg/mL and 2.7 to 4.0 μg/mL, respectively. Food reduces peak plasma concentrations but does not significantly alter overall bioavailability. After intravenous infusion of penciclovir (10 mg/kg), peak plasma levels average 12 μg/mL.

The plasma $T_{½elim}$ of penciclovir averages 2 to 3 hours, and approximately 70% is recovered unchanged in the urine. About 5% is excreted as the 6-deoxy precursor. Rapid renal clearance of penciclovir suggests elimination by filtration and active tubular secretion. Nonrenal clearance accounts for about 30% of the dose, primarily by fecal excretion of penciclovir and its 6-deoxy precursor. The plasma elimination rate is reduced approximately 4-fold, and penciclovir exposure is increased 10-fold in patients with severe renal failure (CrCl <30 mL/min).[266] Dosage reductions are indicated in moderate or advanced renal failure (Table 46.5). In patients with compensated liver disease, peak plasma levels are reduced by approximately 40%, but overall penciclovir exposure is unchanged, and dosage adjustments are unnecessary.[267] The pharmacokinetics of a single 1500-mg dose of famciclovir in adolescents with herpes labialis are similar to those in adults.[268] The pharmacokinetics of famciclovir in infants[269] and children 1 to 12 years old[270] have been used to develop dosage regimens that yield pharmacokinetic parameters comparable to those observed with 500-mg doses in adults. Older adults have approximately 40% higher penciclovir exposure because of lower renal clearance.

Interactions

No clinically important drug interactions have been identified to date. Famciclovir does not interact pharmacokinetically with cimetidine, theophylline, allopurinol, digoxin, or zidovudine to a clinically significant extent.[271]

TABLE 46.5 Dosage Adjustment of Famciclovir for Renal Insufficiency

STANDARD DOSAGE[a]	CREATININE CLEARANCE (mL/min)[b]	ADJUSTED DOSAGE
500 mg q8h or q12h	40–59	500 mg q12h
	20–39	500 mg q24h
	<20	250 mg q24h
250 mg q12h	≥40	250 mg q12h
	20–39	125 mg q12h
	<20	125 mg q24h
125 mg q12h	≥40	125 mg q12h
	20–39	125 mg q12h
	<20	125 mg q24h

[a]Dosage is based on manufacturer's recommendations.
[b]For hemodialysis patients, give the adjusted dosage for creatinine clearance <20 mL/min after dialysis.

Toxicity

Oral famciclovir is well tolerated but may be associated with headache, nausea, fatigue, and diarrhea.[272] The frequencies of such complaints are generally comparable to frequencies seen with placebo or acyclovir. However, at high doses (1000 mg twice in 1 day) used to treat recurrent genital herpes, headache occurs more commonly (14%) in famciclovir recipients than placebo (5%) recipients.[273] Case reports suggest famciclovir may cause cutaneous vasculitis.[274,275] Urticaria, rash, and, predominantly in older adults, hallucinations or confusional states have been reported. Neutropenia and elevated transaminase values occur in less than 5% of patients. The safety and efficacy of famciclovir are not established in children younger than 18 years. Topical penciclovir, which is formulated in 40% propylene glycol and a cetomacrogol base, is associated with application site reactions at low rates (approximately 1%), comparable to the vehicle.

Famciclovir reduces spermatogenesis and fertility in rodents and dogs, but long-term administration (1 year) does not affect spermatogenesis in men.[276] No teratogenic effects have been observed in animals. Safety of administration in pregnant patients has not been established (pregnancy category B).[53] Very high penciclovir concentrations are mutagenic, and long-term administration of high-dose famciclovir is associated with mammary tumors in female rats but not in mice. The clinical significance of these observations is uncertain. Penciclovir is excreted in the breast milk of animals.

Clinical Studies

Topical penciclovir and oral famciclovir are approved for clinical use in the United States, and intravenous penciclovir has been approved in some countries. In immunocompetent individuals with recurrent orolabial HSV, patient-initiated topical 1% penciclovir cream (applied every 2 hours while awake for 4 days) shortens healing time and symptoms by nearly 1 day.[277]

Oral famciclovir initiated within 1 hour of prodrome in a single dose of 1500 mg or as two doses of 750 mg 12 hours apart shortens cold sore outbreaks by 2 days compared with placebo.[278] A systematic review and meta-analysis concluded that penciclovir and famciclovir do not increase the percentage of aborted herpes labialis episodes as do acyclovir and valacyclovir.[279] Oral famciclovir (250 mg three times daily for 5–10 days) is as effective as acyclovir in treating first episodes of genital herpes.[280] In non–HIV-infected patients with recurrent genital HSV, patient-initiated famciclovir treatment of 125 mg twice daily for 5 days reduces healing time and symptoms and is comparable to acyclovir treatment.[281,282] Famciclovir, 125 mg twice daily for 5 days, is comparable to famciclovir, 500 mg once followed by 250 mg twice daily for 2 days.[283] A single-day treatment with famciclovir, 1000 mg given twice 12 hours apart, reduces median healing times by 1.8 days and increases the percentage of patients whose lesions do not progress beyond the papule stage by 45%—from 13% in placebo recipients to 23% in famciclovir-treated subjects.[273] This famciclovir single-day regimen is not inferior to valacyclovir, 500 mg twice daily for 3 days.[284] Larger doses of 250 mg or 500 mg of famciclovir twice daily do not increase the beneficial effects.[282,285] Suppressive therapy (250 mg twice daily) for 1 year is effective in non–HIV-infected individuals with frequent recurrences, but single daily doses are not as effective.[261] Famciclovir reduces recurrences of genital herpes and asymptomatic genital HSV shedding in individuals with recurring lesions[261,286–289] but does not reduce shedding in asymptomatic seropositive individuals.[287] Famciclovir, 250 mg twice daily, seems to be less effective than valacyclovir, 500 mg daily.[290] Famciclovir suppressive therapy is not different from episodic treatment in terms of quality of life or patient satisfaction with treatment.[291]

In HIV-infected patients, famciclovir suppression (500 mg twice daily) reduces lesional HSV recurrences and asymptomatic viral shedding.[292] Famciclovir (500 mg twice daily for 7 days) is comparable to acyclovir (400 mg five times daily) in the treatment of mucocutaneous HSV infections in HIV-infected patients.[293] Intravenous penciclovir (5 mg/kg every 8 or 12 hours for 7 days) is comparable to intravenous acyclovir in efficacy and tolerance for treatment of HSV infections in non–HIV-infected, immunocompromised hosts.[294]

In immunocompetent adults with herpes zoster of 3 days' duration or less, famciclovir (500 mg three times daily for 7 days) is at least as effective as acyclovir (800 mg five times daily) and is superior to placebo in reducing acute manifestations, particularly in patients older than 50 years.[295–298] The efficacy of famciclovir in preventing postherpetic neuralgia is unclear.[117] Famciclovir and valacyclovir provide comparable therapeutic effects in treating zoster in immunocompetent adults 50 years old or older.[299] Famciclovir is comparable to high-dose oral acyclovir in the treatment of ophthalmic herpes zoster in immunocompetent adults[300] and, given for 10 days, in the treatment of nonophthalmic, localized zoster in immunocompromised patients.[301]

Famciclovir is associated with dose-related reductions in HBV DNA and transaminase levels in patients with chronic hepatitis B[302] but is less effective than lamivudine, with less than 10% of patients experiencing a greater than 100-fold decrease in HBV DNA levels after 12 weeks.[303] Combination therapy with famciclovir and lamivudine may be more efficacious in suppressing HBV replication than either agent alone.[304] Famciclovir induces HBV resistance and is usually ineffective in treating lamivudine-resistant infections.[305] It is ineffective in chronic hepatitis D.[306] It has been used to treat recurrent HBV infection after liver transplantation, with reductions in HBV DNA levels for longer than 18 months in some patients.[307]

Studies of famciclovir combined with prednisone or prednisolone for treatment of idiopathic Bell palsy have shown that it is more effective than the glucocorticoid alone.[308,309] (Controlled studies have not shown a benefit for acyclovir or valacyclovir alone or in combination with steroids compared with steroids alone in treatment of Bell palsy—see earlier.[79,80]) Facial nerve palsy associated with ipsilateral acoustic neuroma resection is not prevented by perioperative famciclovir,[310] and Meniere disease is not ameliorated by famciclovir.[311] Pityriasis rosea may be linked to reactivation of HHV-6 and HHV-7, but famciclovir treatment, 250 mg thrice daily for 7 days, was no more effective in hastening resolution than treatment of historical control subjects with topical corticosteroid.[312] Famciclovir produced resolution of oral hairy leukoplakia for at least 1 year in one HIV-infected patient not receiving ART.[313] Topical penciclovir 1% in 25% podophyllin resin was less effective than acyclovir 5% in 25% podophyllin resin or podophyllin resin alone for treatment of oral hairy leukoplakia in HIV-1–seropositive individuals.[314] Famciclovir reduced oropharyngeal shedding of HHV-8 in HIV-infected individuals but not as markedly as antiretroviral drugs did.[315]

FOMIVIRSEN

Fomivirsen (ISIS 2922; Vitravene) is a 21-nucleotide phosphorothioate oligonucleotide that inhibits human CMV replication through an antisense mechanism.[316] Fomivirsen is complementary to a sequence in the messenger RNA transcripts of the major immediate-early region 2 of CMV, which encodes proteins responsible for regulation of viral gene expression. Other mechanisms of antiviral action may include nonantisense, sequence-dependent inhibition of virus replication and sequence-independent inhibition of virus absorption to the cell.[317] With persistent in vitro passage, it has been possible to isolate CMV clones with 10-fold less susceptibility to inhibition of replication.[318] Fomivirsen, because of its novel mechanism of antiviral action, retains activity against CMV strains that are resistant to ganciclovir, foscarnet, or cidofovir.

Fomivirsen is administered by intravitreal injection. In human eyes, concentrations at 1 hour after injection of 165-μg and 330-μg doses average 5.5 μmol/L and 11.6 μmol/L, respectively.[319] Clearance is first order with a $T_{½elim}$ of approximately 55 hours. The major route of elimination from the eye is metabolism by exonucleases; systemic exposure is not detectable. Similar to other phosphorothioate oligonucleotides, fomivirsen binds readily to proteins.

Fomivirsen is indicated for the intravitreal treatment of CMV retinitis in HIV-infected patients who are intolerant of, have not responded to, or have contraindications to other treatments.[316] Intravitreous injection is associated with delays in time to progression in patients with peripheral retinitis (dosages of 165 μg weekly for 3 weeks, followed by every 2 weeks).[320] In patients with retinitis that reactivated or was persistent despite alternative agents, a comparison of two regimens (330 μg weekly for 3 weeks followed by every 2 weeks vs. 330 μg on days 1 and 15 followed by every 4 weeks) found that the less intense regimen was better tolerated, more convenient, and apparently effective.[321] Dose-dependent inflammation including iritis, uveitis, and vitritis is the most common

adverse ocular effect but usually responds to topical corticosteroids. Increased intraocular pressure is common and needs close monitoring. Retinal pigment epitheliopathy and detachments have been described. Fomivirsen is not recommended for use in patients who have received cidofovir within 2 to 4 weeks because of the increased risk for ocular inflammation.

FOSCARNET

Spectrum

Foscarnet (trisodium phosphonoformate; Foscavir), an inorganic pyrophosphate analogue (see Fig. 46.3B), inhibits herpesviruses and HIV.[322] In vitro inhibitory concentrations vary widely among clinical isolates but are generally 100 to 300 µmol/L for CMV and 80 to 200 µmol/L for HSV, VZV, EBV, and HHV-8.[323] Foscarnet inhibits most ganciclovir-resistant CMV and acyclovir-resistant HSV and VZV strains. Combinations of foscarnet with ganciclovir, acyclovir, or artesunate synergistically inhibit CMV infection in vitro.[324,325] Foscarnet acts synergistically with zidovudine in inhibiting HIV replication.

Concentrations of 500 to 1000 µmol/L reversibly inhibit the proliferation or cellular DNA synthesis of uninfected cells. Foscarnet is active in animal models of herpesvirus and hepadnavirus infection.[322]

Mechanism of Action

In contrast to nucleosides, foscarnet does not undergo significant intracellular metabolism. It directly inhibits herpesvirus DNA polymerase or HIV reverse transcriptase. Foscarnet reversibly blocks the pyrophosphate binding site of the viral polymerase in a noncompetitive manner with respect to deoxynucleotide triphosphates and inhibits cleavage of pyrophosphate from deoxynucleotide triphosphates.[322,326] Concentrations that inhibit cell-free viral polymerases are many times lower than the concentrations required for inhibition of viral replication in cell culture,[322] and cellular uptake is slow. Selectivity of foscarnet relates to its 100-fold greater inhibitory effects against herpesvirus DNA polymerases or HIV reverse transcriptase compared with cellular DNA polymerase-α.[326]

Resistance

Resistance to foscarnet is caused by point mutations in DNA polymerase of HSV and CMV (the *UL54* gene product) or in reverse transcriptase of HIV, which confer 3-fold to more than 10-fold increases in inhibitory concentrations in clinical isolates.[7,327,328] Foscarnet-selected CMV mutations generally are not cross-resistant to ganciclovir or cidofovir, but simultaneous resistance to all three drugs has occurred.[327] Foscarnet-resistant CMV variants, defined by 50% inhibitory concentrations greater than 400 µmol/L in plaque reduction or greater than 600 µmol/L in DNA hybridization assays, develop in 37% of foscarnet recipients by 12 months of therapy and are associated with progressive retinitis.[329] Foscarnet-resistant HSV mucocutaneous and CNS infections have developed during therapy, including dually acyclovir-resistant and foscarnet-resistant variants.[330,331] Some foscarnet-resistant CMV infections respond to ganciclovir or cidofovir, whereas resistant HSV strains usually remain susceptible to cidofovir. The experimental antiviral agent maribavir was effective for treatment of CMV infection resistant or refractory to prior therapy with ganciclovir or foscarnet in hematopoietic stem cell or solid-organ transplant recipients,[332,333] whereas brincidofovir, the oral prodrug of cidofovir, was not.[334] Foscarnet resistance in HIV can phenotypically reverse zidovudine resistance.[335,336]

Pharmacokinetics

Oral bioavailability is low, averaging 7% to 9%. After an infusion of 60 mg/kg every 8 hours, peak and trough plasma concentrations have a broad range but average approximately 450 to 575 µmol/L and 80 to 150 µmol/L, respectively. Peak concentrations range from 490 to 2600 µmol/L after dosages of 90 mg/kg/day. Plasma protein binding is about 15%, and the volume of distribution is 0.4 to 0.7 L/kg. CSF concentrations vary widely but average 66% of plasma values at steady state.[337] Vitreous concentrations average 1.4 times higher than concurrent plasma concentrations.[338]

Foscarnet is eliminated renally with more than 80% of the dose excreted unchanged by glomerular filtration and tubular secretion. Plasma clearance is highly correlated with CrCl, so that dosage adjustments are indicated for small decreases in renal function (Table 46.6). Initial plasma $T_{½elim}$ averages 2 to 3 hours in individuals with normal renal function but increases to a mean of 25 hours in individuals with CrCl less than 25 mL/min.[339] Plasma elimination is complex, with a prolonged terminal $T_{½elim}$ averaging 88 hours, which is attributed to bone deposition that is estimated to account for 15% to 20% of a dose. A hemodialysis run removes about 38% of a dose[339]; dosing after dialysis is recommended. Peritoneal dialysis clears foscarnet to a limited extent.[340]

Interactions

Administration of foscarnet with amphotericin B or other nephrotoxic agents (e.g., aminoglycosides, intravenous pentamidine, intravenous acyclovir, cyclosporine) may cause enhanced renal toxicity,[322] and administration with calcineurin inhibitors may cause neurotoxicity. Probenecid does not affect renal excretion. The risk for symptomatic hypocalcemia is increased by concomitant intravenous pentamidine. Ganciclovir does not alter foscarnet pharmacokinetics. Foscarnet and zidovudine do not affect the clearance of each other, but the risk for anemia is higher with the combination.

Toxicity

Foscarnet has a narrow therapeutic index. Nephrotoxicity with azotemia, proteinuria, and sometimes acute tubular necrosis is the major dose-limiting side effect.[322,341] Approximately one-third of patients develop significant renal impairment (serum creatinine ≥2 mg/dL). Increases in serum creatinine usually occur during the second week of therapy and are reversible within 2 to 4 weeks after cessation in most patients. High dosages, rapid or continuous infusion, dehydration, and concurrent use of nephrotoxic drugs are risk factors. Extra saline hydration before and during infusion seems to reduce the risk for nephrotoxicity.[342,343] Crystalluria, crystalline glomerulopathy, renal tubular acidosis, nephrogenic diabetes insipidus, and interstitial nephritis have also been described.

TABLE 46.6 Foscarnet Dosage Reduction in Renal Insufficiency

CREATININE CLEARANCE (mL/min/kg)	INDUCTION DOSAGES		MAINTENANCE DOSAGES	
	60 mg/kg/8 h	90 mg/kg/12 h	90 mg/kg/day	120 mg/kg/day
>1.4	60 q8h	90 q12h	90 q24h	120 q24h
>1–1.4	45 q8h	70 q12h	70 q24h	90 q24h
>0.8–1	50 q12h	50 q12h	50 q24h	65 q24h
>0.6–0.8	40 q12h	80 q24h	80 q48h	105 q48h
>0.5–0.6	60 q24h	60 q24h	60 q48h	80 q48h
≥0.4–0.5	50 q24h	50 q24h	50 q48h	65 q48h
<0.4	Not recommended	Not recommended	Not recommended	Not recommended

Dosages expressed in mg/kg. Recommendations taken from Aweeka FT, Jacobson MA, Martin-Munley S, et al. Effect of renal disease and hemodialysis on foscarnet pharmacokinetics and dosing recommendations. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1999;20:350–357.

Foscarnet is a potent chelator of divalent cations, and metabolic abnormalities are common, including hypocalcemia (15%–35%), hypomagnesemia (15%–44%), hypokalemia (10%–16%), hypercalcemia, hypophosphatemia, and hyperphosphatemia.[343] Decreased serum ionized calcium may cause paresthesias, arrhythmias, tetany, seizures, and other CNS disturbances.[344] Intravenous magnesium sulfate does not prevent ionized hypocalcemia. Intravenous foscarnet should be administered at a fixed rate (maximum 1 mg/kg/min) by infusion pump to minimize the possibility of acute metabolic abnormalities.[344] Close monitoring with electrolyte supplementation and foscarnet dosage adjustments are often required during induction therapy.

CNS side effects include acute dystonia[345] and headache in about one-fourth of patients, seizures in 10%, tremor, irritability, and hallucinosis. Other reported side effects are fever, generalized rash, diarrhea in 30%, nausea or emesis in up to one-half, abnormal liver function tests, anxiety, fatigue, and painful genital ulcerations.[343] In 59 allogeneic hematopoietic stem cell transplant recipients treated with intravenous foscarnet for 14 days for CMV disease, the incidences of hemorrhagic cystitis and genital ulcers were 37% and 29%, respectively, despite routine pretreatment hyperhydration and postvoiding genital cleaning.[346] In 30% of patients, treatment had to be stopped due to adverse effects. Genital ulcerations are probably caused by high urinary foscarnet concentrations, appear on average after 8 days of treatment, and usually resolve 15 days after therapy is stopped.[346] Although anemia may develop in 20% to 50% of patients with AIDS, granulocytopenia is uncommon. Heart block and electrocardiogram changes occur in 5% or fewer of patients. Oral foscarnet causes dose-related gastrointestinal disturbances.

Preclinical studies indicate that high concentrations are mutagenic and that foscarnet causes fetal skeletal anomalies in rodents and rabbits. It may cause tooth and skeletal developmental abnormalities in growing animals. Safety in pregnancy (pregnancy category C) or in children is uncertain. Foscarnet is excreted in the breast milk of animals.

Clinical Studies

Intravenous foscarnet is approved for treatment of CMV retinitis in patients with AIDS and acyclovir-resistant mucocutaneous HSV infections. With the usual foscarnet regimen (60 mg/kg every 8 hours for 14–21 days, followed by long-term maintenance at 90–120 mg/kg/day), about 90% of patients with retinitis experience clinical stabilization, and a smaller portion of patients cease CMV excretion.[322,347,348] An induction regimen of 100 mg/kg twice daily also is effective but is associated with a higher risk for penile ulceration. Maintenance dosages of 120 mg/kg/day seem to be more effective than 90 mg/kg/day in prolonging survival and controlling retinitis.[349] Comparing foscarnet with ganciclovir, one study found that foscarnet provided comparable control of CMV retinitis in patients with AIDS but improved survival, although patients had to be switched from foscarnet more than three times as often because of side effects.[350] In patients with persistently active or relapsed retinitis, combined foscarnet (90 mg/kg/day) and ganciclovir (5 mg/kg/day) delay progression significantly longer than higher dosages of either single agent.[351] Intravitreal foscarnet has been used.[351a]

Foscarnet was useful in treating CMV encephalitis in a stem cell transplant recipient[352] and patients with ganciclovir-resistant CMV retinitis and other CMV syndromes[347] including gastrointestinal and pulmonary infections in patients with AIDS,[353] but was not useful in treating CMV pneumonia in bone marrow transplant recipients. In allogeneic stem cell transplant recipients with CMV infections, preemptive foscarnet therapy is as effective as intravenous ganciclovir[342,354] and valganciclovir[354] and is associated with less neutropenia.[342] Foscarnet was effective and safe in eradicating viremia in 27 patients among 209 renal allograft recipients who developed ganciclovir-resistant CMV disease following preemptive ganciclovir therapy.[355] Combinations of foscarnet and ganciclovir have been used to treat allogeneic transplant recipients with high CMV loads[356] and solid-organ transplant recipients with ganciclovir resistance.[357] In allogeneic bone marrow, liver, or renal transplant recipients with CMV DNA positivity in blood according to polymerase chain reaction, half-standard doses of foscarnet (90 mg/kg) and ganciclovir (5 mg/kg) given intravenously once daily for 14 days are not more efficacious in eliminating CMV DNA positivity in blood than a standard dose of ganciclovir (5 mg/kg intravenously twice daily for 14 days).[358]

HSV exophytic genital pseudotumors in HIV-1–infected patients respond to foscarnet.[228] In acyclovir-resistant mucocutaneous HSV infections, lower dosages (40 mg/kg every 8 hours) are associated with complete healing in about 75% of patients.[14] Other dosage regimens (e.g., 90 mg/kg every 12 hours) seem to be effective. Acute HSV and VZV retinal necrosis have been successfully treated with intravenous foscarnet.[359] The outcome appears better in patients with HSV compared with VZV infection and with supplemental intravitreal foscarnet injection.[360] Foscarnet is also effective in patients with acyclovir-resistant VZV acute retinal necrosis[361] as well as other VZV infections in patients with AIDS[24] and transplant recipients.[25] An immunocompetent adult with severe encephalomyelitis with integrated HHV-6 recovered after combined foscarnet and ganciclovir therapy.[362] HHV-6 encephalitis[363] and erythrocyte aplasia[364] in transplant recipients responded to foscarnet therapy, but prophylactic foscarnet for 10 days after allogeneic stem cell transplantation did not prevent HHV-6 encephalitis.[365] Foscarnet may reduce the risk for Kaposi sarcoma in HIV-infected patients with CMV disease.[366,367] Severe glandular fever caused by EBV has been treated with intravenous foscarnet and immunoglobulin combined with prednisolone.[368] Foscarnet controlled EBV infection in a lung transplant recipient in whom reduced immunosuppression controlled post-transplantation lymphoproliferative disease.[369] Intravenous foscarnet reduces serum transaminase and viral markers in previously untreated patients with active chronic hepatitis B infection and individuals with lamivudine-resistant HBV infection and severe chronic HBV liver disease.[370] In patients with AIDS, foscarnet administration significantly reduces p24 antigen and HIV RNA levels without clear increases in $CD4^+$ counts.[371] Foscarnet was effective for treatment of HIV-2 disease that did not respond to other antiretroviral drugs.[372] In recurrent orolabial or genital herpes infections of immunocompetent hosts, topically applied foscarnet is not associated with reproducible clinical benefits.

GANCICLOVIR AND VALGANCICLOVIR

Spectrum

Ganciclovir (9-[1,3-dihydroxy-2-propoxymethyl] guanine; Cytovene) is a deoxyguanosine analogue that differs from acyclovir in that it has an additional hydroxymethyl group on the acyclic side chain (see Fig. 46.1C). Valganciclovir (Valcyte) is the L-valyl ester of ganciclovir and is rapidly converted to ganciclovir after oral administration. Ganciclovir has inhibitory activity against herpesviruses (see Table 46.2), but its distinguishing characteristic is potent inhibition of CMV replication.[373] Inhibitory concentrations are 10-fold to more than 50-fold lower than acyclovir for human CMV strains. Combinations of ganciclovir and foscarnet synergistically inhibit CMV replication in vitro.[324] Ganciclovir is about twofold more active than acyclovir for herpes B virus (*Cercopithecine herpesvirus* 1) with an inhibitory concentration of 9 μg/mL.[135] Ganciclovir inhibits some adenoviruses in vitro, and inhibition of HBV occurs in vivo.[374] Systemic ganciclovir is effective at relatively low dosages in animal models of CMV and HSV infections.

Although high concentrations are needed to inhibit the growth of uninfected cells, inhibitory concentrations for human bone marrow progenitor cells are similar to concentrations that inhibit CMV replication. Inhibition of human lymphocyte proliferative responses to mitogen and antigen occurs at concentrations of 1 to 10 μg/mL, so immune responses requiring active DNA synthesis may be depressed at therapeutic ganciclovir concentrations.[3] Cells transfected by viral TK are killed on exposure to ganciclovir in vitro,[375] in animal models in vivo,[376] and in patients with malignancies such as prostate carcinoma[377] and glioblastoma multiforme.[378]

Mechanism of Action

Ganciclovir inhibits viral DNA synthesis.[373,379] Intracellular ganciclovir is phosphorylated to the monophosphate derivative by virus-induced TK during HSV infection and by a viral protein kinase homologue encoded by the *UL97* gene during CMV infection.[380,381] Ganciclovir diphosphate and ganciclovir triphosphate are formed through the

action of cellular enzymes. At least 10-fold higher concentrations of ganciclovir triphosphate are present in CMV-infected than in uninfected cells. Intracellular ganciclovir triphosphate concentrations are also more than 10-fold higher than the concentrations of acyclovir triphosphate in CMV-infected cells, and the intracellular $T_{½}$ of ganciclovir triphosphate is prolonged (16.5 to >24 hours). These differences may account in part for the greater anti-CMV activity of ganciclovir and explain how single daily doses may be effective in suppressing human CMV infections.

Ganciclovir triphosphate is a competitive inhibitor of deoxyguanosine triphosphate incorporation into DNA and preferentially inhibits viral more than host cellular DNA polymerases. Incorporation of ganciclovir triphosphate into viral DNA causes a slowing and subsequent cessation of viral DNA chain elongation. In contrast to acyclovir, ganciclovir is not an obligate chain terminator, and continued viral DNA synthesis results in intranuclear accumulation of short, noninfectious viral DNA fragments.[382] Ganciclovir is incorporated into host cell and viral DNA.

Resistance

Resistance in CMV isolates, often defined by inhibitory concentrations greater than 1.5 to 3 µg/mL in vitro, has been related to two mechanisms: (1) reduced intracellular ganciclovir phosphorylation caused by point mutations or deletions in the phosphotransferase encoded by the *UL97* gene and (2) point mutations in viral DNA polymerase (*UL54* gene).[383,384] Most resistant clinical isolates with 4-fold to 20-fold increases in inhibitory concentrations have single or sometimes multiple *UL97* mutations and usually remain susceptible to foscarnet and cidofovir.[169] Highly resistant CMV strains (inhibitory concentrations >10 µg/mL) typically harbor *UL97* and *UL54* mutations and are cross-resistant to cidofovir but are usually susceptible to foscarnet.[169,170,385]

Ganciclovir resistance is rare in ganciclovir-naïve patients but has been recognized clinically by progressive disease[386] and persistent CMV viremia in patients receiving therapy.[347] Risk factors include prolonged ganciclovir exposure, primary infection, combined immunodeficiency, perhaps subtherapeutic doses,[387,388] and higher immunosuppression including use of antilymphocyte globulin. In patients with AIDS receiving ganciclovir for retinitis, resistant CMV is detectable in about 7% by 3 months and in 28% by 9 months.[389] During the ART era, a 15% frequency of *UL97* mutations has been found by 18 months in valganciclovir recipients.[390] Emergence of resistance, sometimes within several weeks, also occurs with ganciclovir use in children with primary combined immunodeficiency.[391] Ganciclovir-resistant CMV disease developed in 7% of mismatched solid-organ transplant recipients receiving ganciclovir prophylaxis.[392] Valganciclovir prophylaxis may be associated with less risk of ganciclovir-resistant CMV mutants, but the frequency of resistant virus has ranged from 0%[393] to 14%.[394] Mismatched lung transplant recipients are at particular risk for resistance emergence.[395] The transmissibility of ganciclovir-resistant CMV strains is undefined, but patients with such strains may have invasive disease including retinitis, enteritis, polyradiculopathy, or pneumonia.[7,396] Foscarnet or cidofovir therapy may benefit patients with ganciclovir-resistant CMV infections.[347] Successful treatment has been described with leflunomide, which has been postulated to have anti-CMV activity.[397,398] Ganciclovir is more than 40 times less active against acyclovir-resistant, TK-deficient HSV strains than against wild-type strains.

Pharmacokinetics

The oral bioavailability of ganciclovir is about 5% under fasting conditions.[373] Food increases bioavailability to 6% to 9%, so dosing with meals is recommended.[399–401] Peak and trough plasma levels average about 0.9 to 1.2 µg/mL and 0.2 to 0.5 µg/mL, respectively, on an oral regimen of 1000 mg every 8 hours. Valganciclovir is a monovalyl ester prodrug that is well absorbed, most likely by intestinal peptide transporter 1, and rapidly hydrolyzed to the parent molecule by intestinal and hepatic esterases. After administration of oral valganciclovir tablets (900 mg) with food, ganciclovir bioavailability is approximately 60%, prodrug blood levels are low (1%–2% of ganciclovir), ganciclovir peak plasma concentrations average 5.9 to 6.7 µg/mL, and overall ganciclovir exposure is comparable to intravenous dosing of ganciclovir at 5 mg/kg.[402,403] Similarly, in a patient with a small bowel transplant and other patients with stable graft-versus-host disease of the gastrointestinal tract, absolute bioavailability of ganciclovir from valganciclovir is 65%[404] to 75%,[405] and ganciclovir systemic exposure with a 900-mg dose of valganciclovir is not inferior to that of 5 mg/kg of ganciclovir given intravenously.[406] A valganciclovir solution is bioequivalent to the tablet formulation.[407] Valganciclovir has essentially supplanted oral ganciclovir and is commonly used in place of intravenous ganciclovir.

After intravenous administration of doses of 5 mg/kg, peak and trough plasma concentrations average 8 to 11 µg/mL and 0.6 to 1.2 µg/mL, respectively.[373,408] Subcutaneous and intramuscular administration are too irritating for clinical use. Plasma protein binding is only 1% to 2%. After intravenous dosing, aqueous, vitreous, and subretinal fluid levels are similar to those in serum.[338,409] CSF levels are 24% to 70%, and brain tissue levels are 38% of those in plasma.[403] The plasma $T_{½elim}$ averages 2 to 4 hours in patients with normal renal function but increases almost linearly as CrCl declines, increasing to 28 to 40 hours in patients with severe renal insufficiency. Most ganciclovir is eliminated unmetabolized by renal excretion (>90% of dose) by glomerular filtration and tubular secretion. Dosage reductions of ganciclovir (Table 46.7) and valganciclovir (Table 46.8) are necessary in patients with CrCl less than 60 to 70 mL/min. Ganciclovir dosing regimens for patients on continuous venovenous hemodiafiltration are suggested.[410]

A single hemodialysis session reduces the plasma levels of ganciclovir by approximately 50% to 60%, and dosing after dialysis is recommended.[411] In adults on continuous renal replacement therapy, 450 mg every 48 hours produces plasma concentrations comparable to concentrations observed in patients with normal renal function receiving 900 mg daily.[412] In neonates, valganciclovir, 15 mg/kg twice daily, yields plasma concentrations similar to intravenous ganciclovir, 5 mg/kg twice daily.[413] The intravitreal ganciclovir implant is designed to release the drug at a rate of approximately 1 µg/h over 5 to 8 months.[414]

Interactions

Concurrent oral ganciclovir doubles the overall exposure to didanosine, increases zidovudine exposure by a much smaller extent, and may

TABLE 46.7 Ganciclovir Dosage Adjustments in Renal Insufficiency

CREATININE CLEARANCE (mL/min)	IV GANCICLOVIR INDUCTION DOSE (mg/kg)	DOSING INTERVAL (h)	IV GANCICLOVIR MAINTENANCE DOSE (mg/kg)	DOSING INTERVAL (h)
≥70	5	12	5	24
50–69	2.5	12	2.5	24
25–49	2.5	24	1.25	24
10–24	1.25	24	0.625	24
<10	1.25	3 times/wk after hemodialysis	0.625	3 times/wk after hemodialysis

[a]Dosing suggestions are based on manufacturer's recommendations.
IV, Intravenous.

TABLE 46.8 Dosage Adjustments for Valganciclovir in Renal Insufficiency

CREATININE CLEARANCE (mL/min)	INDUCTION DOSAGE	MAINTENANCE DOSAGE
≥60	900 mg bid	900 mg qd
40–59	450 mg bid	450 mg qd
25–39	450 mg qd	450 mg q2d
10–24	450 mg q2d	450 mg 2 times/wk
<10[b]	Not recommended	Not recommended
Hemodialysis[b]	Not recommended	Not recommended

[a]Dosage suggestions are based on manufacturer's recommendations.
[b]Dose <450-mg tablet is needed. Use intravenous ganciclovir.

increase the risk for didanosine concentration–related toxicities.[415] Ganciclovir exposure is reduced about 20% when it is ingested 2 hours after, but not simultaneously with, didanosine. Ganciclovir antagonizes the anti-HIV activity of didanosine and zidovudine in vitro,[416] and zidovudine antagonizes the anti-CMV effects of ganciclovir.[417,418] The clinical significance of these observations is unknown, but didanosine-valganciclovir coadministration resulted in complete suppression of HIV in plasma, a paradoxical $CD4^+$ decline, and didanosine toxicity.[419]

Zidovudine and probably other cytotoxic agents increase the risk for ganciclovir-induced myelosuppression, as do nephrotoxic or other agents (probenecid, trimethoprim-sulfamethoxazole) that impair ganciclovir excretion. In animals, zidovudine (but not amphotericin B, ketoconazole, dapsone, or trimethoprim-sulfamethoxazole) antagonizes the anti-CMV effects of ganciclovir.[417,420] Renal dysfunction may occur in patients given concurrent ganciclovir and either amphotericin B or cyclosporine, and ganciclovir may increase cyclosporine levels.

Toxicity

Myelosuppression is the principal dose-limiting toxicity of ganciclovir and its prodrug. The most common adverse events are neutropenia in 24% to 40% and thrombocytopenia in 15% to 20% of patients with AIDS receiving intravenous ganciclovir or oral valganciclovir.[373] The risk for these toxicities is lower in transplant recipients. Neutropenia occurs in approximately one-fourth of patients receiving oral ganciclovir. Neutropenia is most commonly observed during the second week of treatment and is reversible in most patients within 1 week after drug cessation. Recombinant granulocyte-macrophage colony-stimulating factor may be useful in treating ganciclovir-induced neutropenia.[421]

CNS side effects, ranging in severity from headache, to behavioral changes with confusion or psychosis, to convulsions and coma, have been described in 5% to 15% of patients. Acute neurotoxicity with confusion and hallucinations was associated with serum and CSF concentrations of 3.9 μg/mL and 2.6 μg/mL, respectively, 48 hours after the last valganciclovir dose.[422] One-third of patients receiving intravenous ganciclovir interrupt or prematurely stop therapy because of bone marrow or CNS toxicity, and catheter-related complications are common. Approximately 25% of valganciclovir recipients discontinue maintenance therapy within 10 months for toxicity or other reasons.[423] Increased rates of azotemia occur in transplant recipients receiving intravenous ganciclovir prophylaxis.[424] Oral valganciclovir and ganciclovir are associated with diarrhea and possibly with mild nephrotoxicity.[425]

Anemia, rash, fever, liver function test abnormalities, nausea or vomiting, and eosinophilia have also been reported, as have two cases of lactic acidosis.[426] Phlebitis at the infusion site may be caused by the alkaline pH of the solution. In the event of massive overdosage, hemodialysis and hydration may be effective in reducing plasma ganciclovir levels. Placement of the intravitreal insert and intravitreal injections may be associated with visual changes, hemorrhage, infection, and retinal detachment.[427,428]

Ganciclovir is mutagenic, carcinogenic, and immunosuppressive, and it causes irreversible reproductive toxicity in animals and possibly humans.[373] Teratogenicity, embryotoxicity, testicular atrophy, and bone marrow hypocellularity have been observed in animals at ganciclovir exposures comparable to those in humans. Ganciclovir may be teratogenic in humans (classified pregnancy category C), and mothers should avoid breastfeeding while receiving ganciclovir or valganciclovir. Topical 0.15% ganciclovir ophthalmic gel is well tolerated.[429]

Clinical Studies

Ganciclovir and valganciclovir are currently approved for treatment and long-term suppression of CMV retinitis in immunocompromised patients and prevention of CMV disease in transplant recipients. Because of toxicities, administration is usually limited to patients at risk for or having life-threatening or sight-threatening CMV infections. With initial or induction intravenous dosages of 2.5 mg/kg every 8 hours or 5 mg/kg every 12 hours for 10 to 21 days, about 85% of patients with CMV retinitis experience improvement or stabilization of disease, and funduscopic improvement is usually evident by 10 to 14 days.[373,430] Ganciclovir is comparable to foscarnet in initial control of retinitis.[350] Oral valganciclovir (900 mg twice daily for 3 weeks) is as effective as intravenous ganciclovir for initial treatment of non–sight-threatening disease.[402] Almost all patients with AIDS who respond to initial treatment relapse within weeks without suppressive therapy.[430] High dosages of intravenous ganciclovir (30–35 mg/kg/wk) and oral valganciclovir (900 mg daily)[402] are effective for long-term suppression. Oral ganciclovir suppression (1 g three times daily) seems to be comparably effective to intravenous dosing.[431]

Retinal detachments are common during long-term follow-up. Combined ganciclovir and foscarnet are superior to monotherapy and may be effective when single-agent therapy fails.[351] Intraocular sustained-release ganciclovir implants[414,432] and repeated intravitreal injections are effective in controlling retinitis but do not prevent CMV disease in the other eye or in other sites.

Clinical improvement and virologic responses are also seen in CMV pneumonia and gastrointestinal infections in patients with AIDS and in solid-organ transplant recipients.[379,430] Valganciclovir is as effective as intravenous ganciclovir for viremic CMV disease in solid-organ transplant recipients.[433] Half doses (once daily) each of intravenous ganciclovir combined with foscarnet were not as effective as twice-daily ganciclovir alone for the same indication.[434] CNS syndromes respond less predictably and may progress despite therapy.[435] In biopsy-proven CMV colitis in patients with AIDS, ganciclovir (5 mg/kg every 12 hours for 14 days) is associated with significant antiviral effects, stabilization of weight loss, and lower incidence of extracolonic CMV disease, but no differences in symptoms compared with placebo.[436] In bone marrow transplant recipients, virologic responses (but no reduction in mortality) occur in patients with CMV pneumonia treated with ganciclovir alone or in combination with corticosteroids. In contrast, ganciclovir combined with intravenous immunoglobulin or CMV immunoglobulin reduces the mortality rate among bone marrow transplant recipients with CMV pneumonia (from 80%–90% to 30%–50%). Prolonged ganciclovir and valganciclovir treatment may reduce the risk for hearing loss in symptomatic infants with congenital CMV disease.[437,438]

Intravenous ganciclovir prophylaxis seems to be effective and reasonably well tolerated in preventing CMV disease in bone marrow[439,440] and solid-organ transplant recipients.[424] Preemptive ganciclovir treatment (5 mg/kg every 12 hours for 7–14 days, followed by 5 mg/kg/day for 5–7 days/wk until days 100–120 after transplantation) when CMV is isolated from bronchoalveolar lavage fluid[439] or from other body sites[440] is highly effective in preventing CMV pneumonia and seems to reduce mortality in bone marrow transplant recipients. In seropositive bone marrow transplant recipients, initiation of ganciclovir at the time of engraftment also markedly reduces CMV shedding and disease rates but does not improve survival, in part because of neutropenia-related infections.[441,442]

Short-term ganciclovir administration after transplantation reduces the risk for CMV disease in seropositive allograft recipients undergoing heart, lung, or liver transplantation.[424,443] More prolonged administration provides more sustained protection, but breakthrough CMV infection, toxicity, and resistance can limit its utility.[444] In lung transplant recipients, ganciclovir prophylaxis was associated with less bronchiolitis obliterans, improved survival, and prevention of HSV infection.[444–446] Indefinite

prophylaxis is now recommended in lung transplant recipients.[447] In renal transplant recipients, 24 weeks of prophylaxis was more effective than 12 weeks of prophylaxis in reducing symptomatic CMV disease.[448] Preemptive intravenous ganciclovir during antilymphocyte antibody treatment for 2 weeks reduced the risk for CMV disease in renal transplant recipients.[449] Ganciclovir results in clearance of EBV from oropharyngeal secretions, although a rapid rebound in excretion occurs after cessation of therapy.[450] Valganciclovir was not more effective in preventing blood levels of CMV DNA-emia by PCR compared with valacyclovir in renal transplant recipients.[451]

Oral ganciclovir (1 g three times daily to day 98) prophylaxis markedly reduces the risk for invasive CMV disease in liver transplant recipients including the high-risk group comprising seronegative recipients of organs from seropositive donors.[452] Ganciclovir prophylaxis (1 g three times daily orally for 12 weeks) is also effective in renal transplant recipients.[453] Compared with oral ganciclovir (1 g three times daily), valganciclovir (900 mg once daily) has been associated with a lower rate of CMV viremia on prophylaxis but comparable rates of CMV viremia and disease at 12 months in high-risk mismatched solid-organ transplant recipients in one study,[454] although other investigators have not observed a difference.[455] The optimal duration of prophylaxis remains to be established, and studies of lower doses are underway. Preemptive therapy with oral ganciclovir (1 g three times daily for 8 weeks) when CMV DNA is detectable protects against CMV disease in liver transplant recipients.[456]

Oral ganciclovir (1 g every 8 hours) prophylaxis seems to decrease the incidence of CMV disease in HIV-infected patients with CD4 T-cell counts less than 100 cells/mm,[457] although not when administered with didanosine.[458–460] However, oral ganciclovir is no longer marketed in the United States. In a randomized, placebo-controlled trial, valganciclovir did not reduce CMV end-organ disease in patients with AIDS at high risk (CD4 count <100 cells/mm^3 and CMV viremia),[459] so valganciclovir primary prophylaxis is not recommended for HIV-positive patients who are or are not receiving ART.[460]

In newborn infants with congenital CMV infection, ganciclovir therapy increases "the improvement rate and the rate of CMV infection indexes becoming negative" and decreases the incidence of hearing disturbance with few side effects.[461] In infants with congenital CMV involving the CNS, 6 weeks of intravenous ganciclovir results in fewer developmental delays.[462] In neonates with asymptomatic congenital CMV infection, intravenous ganciclovir for 21 days in the newborn period reduces subsequent hearing loss.[463]

EBV infections have been prevented and treated with ganciclovir and valganciclovir. Primary infection is prevented in high-risk pediatric renal transplant patients receiving a kidney from an EBV-seropositive donor.[464] Preemptive therapy may prevent post-transplantation lymphoproliferative disease in pediatric liver transplant patients.[465] Post-transplantation lymphoproliferative disease of the CNS has been successfully treated with combination therapy including ganciclovir.[466] Severe hepatitis complicating primary EBV infection responded to valganciclovir plus glucocorticoids,[467] as did EBV multicentric Castleman disease.[468]

Intravenous ganciclovir (5 mg/kg every 12 hours) is recommended for initial treatment of herpes B virus infections, particularly for patients with CNS involvement.[135] Intravenous ganciclovir reduces biochemical abnormalities and hepatitis B virus DNA levels by 90% in post-transplantation HBV infection.[374] Additional viral infections treated with intravenous ganciclovir and valganciclovir include varicella acute retinal necrosis,[469] HHV-6 meningoencephalitis or meningoradiculitis,[470–472] HHV-8 Castleman disease,[473,474] and EBV CNS infection and lymphoma.[475,476] Topical 0.15% ganciclovir ophthalmic gel is comparable to acyclovir ointment in treating HSV keratitis[477] but is not different from artificial tears for treatment of adenoviral conjunctivitis.[478]

IDOXURIDINE

Idoxuridine (5-iodo-2′-deoxyuridine, IDU; Herplex) is an iodinated thymidine analogue (see Fig. 46.3C) that inhibits replication of various DNA viruses in vitro, particularly herpesviruses and poxviruses.[479] Plaque production by most clinical isolates of HSV type 1 is inhibited by concentrations of 2 to 10 μg/mL. The median inhibitory concentration for two laboratory vaccinia strains was 0.2 to 0.3 μg/mL.[480] Idoxuridine and cidofovir were reported to synergistically inhibit the growth of a vaccinia virus at concentrations that were not cytotoxic. The antiviral mechanism of action of idoxuridine is not completely defined, but the phosphorylated derivatives interfere with various enzyme systems. The triphosphate inhibits viral DNA synthesis and is incorporated into viral and cellular DNA. Resistance to idoxuridine readily develops under laboratory conditions[481] and occurs in viral isolates recovered from idoxuridine-treated patients with HSV keratitis.

In human patients, extremely low plasma concentrations of idoxuridine (0.1–0.4 ppm) are detected in about half of patients treated topically with 40% idoxuridine in the penetration-enhancing agent dimethyl sulfoxide (DMSO). Idoxuridine is teratogenic, mutagenic, tumor-promoting, and immunosuppressive in preclinical testing. DMSO is teratogenic and can cause adverse ocular effects in laboratory animals.

In the United States, idoxuridine is approved only for topical treatment of HSV keratitis, whereas idoxuridine in DMSO is available in Europe for treatment of herpes labialis, herpes genitalis, and herpes zoster. In ocular HSV infections, topical idoxuridine is more effective in epithelial infections, especially initial episodes, than in stromal infections.[482] In a systematic review of antiviral drugs and other treatments for HSV epithelial keratitis, it was concluded that "trifluridine and acyclovir are more effective than idoxuridine or vidarabine and are similar in effectiveness."[483] Adverse reactions include pain, pruritus, inflammation, or edema involving the eye or lids and, rarely, allergic reactions.

Topical idoxuridine alone in solution is ineffective in mucocutaneous herpesvirus infections. In a small pilot study, optimizing the dispersion of idoxuridine 1% in a 5% liposome in gel formulation increased idoxuridine penetration and retention in vitro compared with a non-optimized formulation and increased healing of HSV skin lesions in patients by 1.6-fold to 2-fold. Itching, burning, and other side effects were much less common with the optimized liposomal gel.[484] Frequent topical application of 5% to 40% idoxuridine dissolved in DMSO seems to hasten healing and shorten pain duration in localized herpes zoster. Topical 30% idoxuridine in DMSO may shorten the duration of viral shedding in recurrent or primary genital HSV infections, but it does not reduce the duration of symptoms or healing time. Topical 15% idoxuridine in DMSO reduces the duration of pain and healing time in recurrent herpes labialis.[485] Mild local burning and aftertaste are common after topical application of DMSO; headache, dizziness, sedation, nausea, and localized and generalized dermatitis have also been reported.

LETERMOVIR (AIC246)

Letermovir inhibits only CMV among human herpesviruses (Fig. 46.4). It was licensed in the United States in 2017 for prevention of CMV infection in adult CMV-seropositive recipients of an allogeneic stem cell transplant. It is a 3,4-dihydro-quinozoline, which inhibits CMV replication by binding to and inhibiting the viral terminase enzyme complex (UL51, UL56, or both).[486] It does not require phosphorylation as ganciclovir does or act by inhibiting viral DNA polymerase similar to ganciclovir, cidofovir, or foscarnet. It is therefore active against most CMV isolates that are resistant to the currently available anti-CMV

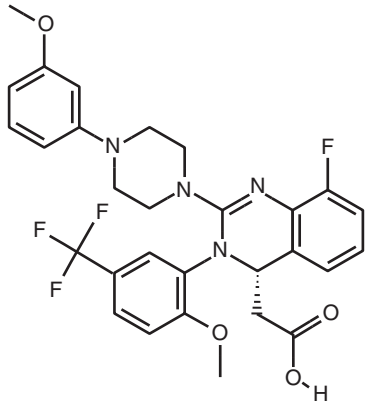

FIG. 46.4 Chemical structure of letermovir.

agents. Letermovir has highly potent anti-CMV activity, with an ID_{50} of 5 μM in tissue culture and a selective lack of cellular toxicity.[487] Drug-resistant virus can be selected by a one-step selection process due to a specific point mutation in the terminase complex.[486]

Letermovir is administered orally or by intravenous injection. It was well tolerated in phase I trials. A phase II study of letermovir was undertaken in stem cell transplant patients as prophylaxis against CMV disease. Once-daily doses of 120 mg or 240 mg or placebo were administered for 84 days. Failure of prophylaxis was significantly less in recipients of either dose of letermovir than in the placebo group. A modest excess of vomiting and edema in the letermovir group was observed.[488] In a phase III study, 495 adults were given 480 mg/day letermovir or placebo a median of 9 days after HSCT and continued for approximately 100 days after transplantation.[489] CMV infection was reduced from 61% (103 of 170) in placebo recipients to 38% (122 of 325) in letermovir recipients. All-cause mortality was also less in letermovir recipients (21% vs. 26%). Peripheral edema and vomiting tended to be less common in letermovir recipients. One patient receiving letermovir developed breakthrough CMV infection; his isolate had a *UL56* gene mutation mediating letermovir resistance. Such CMV mutants remain susceptible to ganciclovir.[490] The use of letermovir to treat CMV infection has been reported only in a small trial with a low dose, 80 mg/day, for preemptive treatment of CMV infection in renal transplant recipients.[491] Letermovir and ganciclovir as a control were similarly effective in controlling CMV DNA in blood. In one case report, letermovir treatment cured a lung transplant recipient with multidrug-resistant CMV disease unresponsive to other treatments.[492]

Letermovir pharmacokinetics are complex.[493] The major route of letermovir clearance is via biliary excretion (93% based on studies in rats) of primarily unchanged (71%) drug by organic anion transporting polypeptide 1B1 (OATP1B1) and OATP1B3 transporters. No letermovir is metabolized by human cytochrome P-450 (CYP) isoenzymes.[494] In healthy adults, AUC and C_{max} increase in proportion to dose over the range from 30 to 120 mg/day for 8 days.[494,495] Plasma $T_{½elim}$ (median 13.8 hours) is independent of dose over this range, as is the apparent volume of distribution at steady state (median 241 ± 221 L for the 3 doses). Systemic exposure was equivalent at doses of 480 mg/day administered orally or intravenously but appeared to increase disproportionately (nonlinearly) to 4.6-fold greater with a dose of 720 mg orally twice daily compared with 480 mg once daily.[496]

Renal impairment increases exposure to letermovir.[495] Moderate hepatic impairment increases exposure to letermovir less than twofold, whereas severe hepatic impairment increases letermovir exposure approximately fourfold compared with results in healthy subjects.[494] Concomitant letermovir increases tacrolimus and cyclosporine exposure. Cyclosporine alters letermovir exposure, whereas tacrolimus does not.[497]

TRIFLURIDINE

Trifluridine (trifluorothymidine, 5-trifluoromethyl-2′-deoxyuridine; Viroptic) is a fluorinated pyrimidine nucleoside (see Fig. 46.3D) that has in vitro inhibitory activity against HSV-1, HSV-2, CMV, vaccinia, and, to lesser extent, certain adenoviruses. Concentrations of 0.2 to 10 μg/mL inhibit replication of herpesviruses, including acyclovir-resistant HSV strains.[17] The antiviral mechanism of action involves inhibition of viral DNA synthesis. Trifluridine monophosphate irreversibly inhibits thymidylate synthetase, and the triphosphate competitively inhibits DNA polymerases with respect to thymidine triphosphate. Trifluridine is incorporated into viral and, to a lesser extent, cellular DNA, and it inhibits cellular DNA synthesis at low concentrations. It also exhibits mutagenic, teratogenic, and antineoplastic activities in experimental systems. Trifluridine-resistant HSV with altered TK substrate specificity can be selected on laboratory passage.[481] The clinical significance of this observation is uncertain.

Clinical use of trifluridine is limited to topical therapy for HSV infections, and it is approved in the United States for treatment of primary keratoconjunctivitis and recurrent epithelial keratitis caused by HSV-1 and HSV-2 (see Table 46.1).[482] A recent systematic review concluded that topical trifluridine and acyclovir are more effective than idoxuridine or vidarabine and are similar in effectiveness.[483] Topical trifluridine is effective in some patients who have not responded clinically to idoxuridine or vidarabine. Adverse reactions include discomfort on instillation; palpebral edema; and, uncommonly, hypersensitivity reactions, irritation, and superficial punctate or epithelial keratopathy.

Topical trifluridine also seems to benefit some patients with acyclovir-resistant HSV cutaneous infections.[483a] Combinations of trifluridine and IFN-α synergistically inhibit HSV replication in vitro and have been used to treat ocular and drug-resistant mucocutaneous HSV infections.[17]

VIDARABINE

Vidarabine (9-β-D-ribofuranosyladenine; ara-A, adenine arabinoside; Vira-A) is an analogue of adenosine (see Fig. 46.3E) that has in vitro antiviral activity against herpesviruses, poxviruses, rhabdoviruses, and some RNA tumor viruses. Plaque formation by most HSV and VZV strains is completely inhibited by 3 μg/mL or less of vidarabine, and it is inhibitory for idoxuridine-resistant and acyclovir-resistant strains.

Vidarabine is phosphorylated by cellular enzymes to the triphosphate, which competitively inhibits the activity of viral and, to a lesser extent, cellular DNA polymerases. Vidarabine triphosphate is incorporated into cellular and viral DNA. Vidarabine triphosphate inhibits other host cell enzyme systems including ribonucleoside reductase, RNA polyadenylation, and *S*-adenosylhomocysteine hydrolase. Resistant variants resulting from mutations in viral DNA polymerase can be selected under laboratory conditions, but drug resistance is not a recognized clinical problem.

Little systemic absorption occurs after ophthalmic application. Absorbed vidarabine is rapidly converted to its hypoxanthine metabolite (araHx), which has 30-fold to 50-fold less antiviral activity. The kidneys excrete vidarabine and araHx.

Vidarabine has been shown to be mutagenic, teratogenic, and oncogenic in preclinical testing. Hypersensitivity reactions including pruritus, erythema, ocular pain, and foreign body sensation may occur after ocular application. Other reported effects include photophobia, keratitis after exposure to ultraviolet light, and increased lacrimation.

Vidarabine has activity in certain life-threatening herpesvirus infections, including herpes simplex encephalitis and neonatal HSV[498] and adenovirus infection[499] in immunocompromised individuals and chronic EBV,[500] but not smallpox. Its use was discontinued in the United States in 1992. An ophthalmic ointment of vidarabine is less effective than trifluridine or acyclovir in HSV keratoconjunctivitis (see Table 46.1).[483] It is effective in patients who cannot receive idoxuridine because of allergy, toxicity, or drug resistance.

MARIBAVIR

Maribavir {L-ribofuranosyl benzimidazole (5,6-dichloro-2-isopropylamino-1-[β-L-ribofuranosyl]-1H-benzimadazole)} inhibits CMV and EBV but not other human herpesviruses in vitro (Fig. 46.5).[501,502] It inhibits replication of clinical isolates of CMV at concentrations of less than 1 μM to approximately 15 μM in cell culture, including most variants resistant to ganciclovir, cidofovir, or foscarnet.[501,503]

Maribavir has a novel mechanism of anti-CMV action that does not require intracellular phosphorylation. It is a highly specific inhibitor of the protein kinase product of the *UL97* gene.[504] Inhibition of *UL97* function reduces the phosphorylation of several viral proteins, including UL44, which is essential for CMV DNA replication.[505] Maribavir thereby inhibits viral DNA synthesis and blocks nuclear egress of virions. As predicted from its inhibitory effect on *UL97* kinase, which phosphorylates ganciclovir to its active metabolite,[506] maribavir antagonizes the anti-CMV

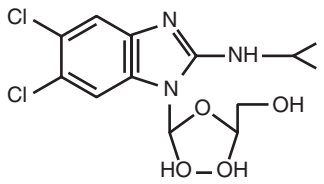

Maribavir

FIG. 46.5 Chemical structure of maribavir.

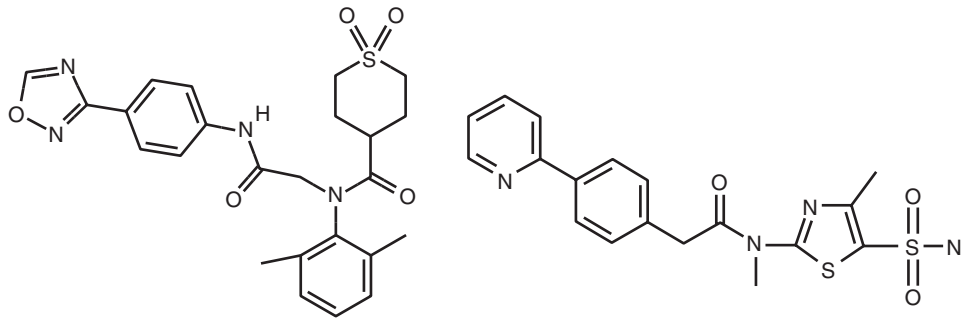

FIG. 46.6 **Chemical structures of amenamevir (A) and pritelivir (B).**

effect of ganciclovir,[506] although others report an additive or indeterminate effect.[507,508] As expected, maribavir does not affect the anti-CMV effect of foscarnet or cidofovir, which are drugs that do not require *UL97*-mediated phosphorylation.[506] Maribavir-resistant CMV strains selected during propagation with drug in vitro possess *UL97* kinase mutations that confer moderate to high-level resistance and *UL27* mutations that confer low-level resistance.[509,510] Maribavir-resistant strains are sensitive to ganciclovir,[505] whereas CMV strains resistant to ganciclovir, foscarnet, and cidofovir are sensitive to maribavir, consistent with their different mechanisms of action.[511]

Oral maribavir is rapidly absorbed and shows dose-proportional kinetics.[503] A high-fat meal decreases absorption by 30%.[512] Maribavir is highly bound to plasma proteins (>97%). The plasma $T_{½elim}$ is about 3 to 6 hours, and 30% to 40% of the dose is cleared in the urine as an *N*-alkylated metabolite and less than 2% as the parent molecule.[512] Maribavir pharmacokinetics are not significantly affected by mild (CrCl 50–80 mL/min) to severe (CrCl <30 mL/min) renal impairment.[513] Maribavir does not accumulate during twice-daily dosing for 10 days.[514] A dose of 400 mg twice daily for 10 days does not alter CYP1A2, CYP2C9, CYP3A, *N*-acetyl-transferase-2, and xanthine oxidase drug-metabolizing activity, although this regimen may inhibit CYP2C19 and CYP2D6 activities.[514] However, clinically important pharmacokinetic interactions have been demonstrated during co-administration of maribavir and tacrolimus (increasing tacrolimus exposure), presumably due to inhibition of CYP3A4 or P-glycoprotein, or both,[515] and rifampin (reducing maribavir), presumably due to induction of CYP3A4 or P-glycoprotein, or both.[516] Voriconazole did not interact pharmacokinetically with maribavir.[517]

In a randomized, placebo-controlled trial, maribavir 100–400 mg twice daily reduced the incidence of CMV DNA-emia in plasma compared with placebo,[518a] but did not prevent CMV disease, although 100 mg twice daily was not different from placebo in another study.[518] Similarly, maribavir was safe but not efficacious compared with ganciclovir in preventing CMV disease in adult liver transplant recipients.[519]

At higher doses than those used in the phase III studies, maribavir may be effective for treatment of refractory or resistant CMV infection in transplant recipients,[520] but resistance to it may appear rapidly and preclude a therapeutic effect.[521] In HIV-infected adults, maribavir (330–2400 mg daily in divided doses for 28 days) is associated with approximately 3 $\log_{10}$ or greater reductions in semen CMV titers.[522] Dose-related taste disturbance and diarrhea occur, and other possible adverse effects include rash, pruritus, headache, nausea, and fever.[522]

HELICASE-PRIMASE INHIBITORS (AMENAMEVIR, PRITELIVIR)

Helicase-primase inhibitors are antiviral agents with a novel mechanism of action against HSV-1 and HSV-2. They inhibit the viral heterotrimeric complex consisting of helicase, primase, and cofactor subunits that is essential for viral DNA replication. Both UL5 and UL52 together with the accessory protein UL8 form the enzyme complex that is the molecular target of these inhibitors.[523] They are not nucleoside analogues and do not require phosphorylation by a TK to inhibit HSV replication. Therefore they are active against TK-deficient HSV, which is the major mechanism of resistance to acyclovir and penciclovir.

Amenamevir (ASP2151)

Amenamevir (ASP2151) is an oxadiazolephenyl derivative (Fig. 46.6A) with potent activity against HSV-1 (median effective concentration [EC_{50}] 0.036 μM), HSV-2 (EC_{50} 0.028 μM), and VZV (EC_{50} 0.047 μM).[524] Amenamevir also inhibits nucleoside-resistant HSV strains. Amenamevir was investigated in a dose-finding placebo-controlled study in 437 patients with recurrent genital herpes.[525] Amenamevir was administered orally at one of four doses: 100 mg, 200 mg, or 400 mg daily for three doses or 1200 mg as a single dose. Another group of subjects received valacyclovir at 500 mg twice daily for 3 days. The duration of the recurrent episodes were 1 or 2 days shorter in both amenamevir-treated groups compared with placebo. The single 1200-mg dose amenamevir-treated group had a comparable efficacy rate to 3 days of valacyclovir. HSV isolates resistant to amenamevir were not detected before or after dosing.[526] In a controlled randomized trial in 751 patients with herpes zoster of fewer than 3 days' duration, amenamivir 200 mg or 400 mg orally once daily was not inferior to valacyclovir 1000 mg three times daily, all in 7 days.[527] Amenamevir 200 mg and 400 mg for 7 days was as well tolerated as valacyclovir in immunocompetent adults.

Clinical pharmacokinetics studies of amenamevir demonstrate dose-proportional AUC, C_{max}, and C_{min} over the dose range from 5 to 2400 mg.[528] Severe renal impairment increases AUC 78%, but the clinical importance of reducing doses in such patients is unclear.[529] Moderate hepatic impairment does not appear to produce sufficient pharmacokinetic changes to necessitate dose reductions.

Pritelivir (AIC316)

Pritelivir (AIC316) is a thiazolylamide (Fig. 46.6B) inhibitor of helicase-primase functions of HSV. It has potent antiviral activity with EC_{50} values of 0.026 μM against HSV-1 and 0.029 μM against HSV-2.[530] It is active against TK and DNA polymerase HSV mutants, which are resistant to nucleoside analogues.

The drug is administered orally and has a long half-life (up to 80 hours). Therefore it can be administered once weekly and appears to be well tolerated. Its effect on suppression of genital HSV-2 DNA shedding was studied in 156 individuals.[531] Otherwise healthy adults with genital HSV-2 infection were randomly assigned to one of four doses of pritelivir, 5 mg, 25 mg, or 75 mg daily or 400 mg weekly, or placebo for 28 days. Pritelivir reduced the rates of genital HSV DNA shedding and days with lesions in a dose-dependent manner.[531] No evidence of pritelivir resistance among HSV-2 isolates was observed.[532] In a subsequent study, pritelivir was compared with valacyclovir, 500 mg twice daily. Pritelivir, 100 mg/day for 28 days, reduced the relative risk of shedding of HSV-2 DNA in genital swabs by 58% and days with genital lesions by 60%.[533] However, this study was terminated before completion based on findings in monkeys in a 39-week toxicity study. The findings included dry and crusted skin lesions, alopecia, and anemia the mechanisms of which were not understood. Of note, no trial participants experienced such effects. One trial on the efficacy and safety of pritelivir for treatment

of acyclovir-resistant mucocutaneous HSV in immunocompromised adults is currently recruiting subjects.[534]

Tenofovir

Tenofovir is a nucleotide analogue that inhibits HSV DNA polymerase,[535,536] HBV DNA polymerase, and HIV reverse transcriptase. It is phosphorylated by cellular enzymes to form tenofovir diphosphate, which competitively inhibits HSV DNA polymerase and acts as a chain terminator. In vitro, tenofovir inhibits HSV-2 replication at a concentration of 125 to 176 μg/mL compared with 0.05 to 0.12 μg/mL for acyclovir. Cytotoxic concentrations are >200 μg/mL for tenofovir and >50 μg/mL for acyclovir.[536]

Tenofovir was approved for treatment of HIV infection in 2001 (see Chapter 128) and for treatment of HBV infection in 2006 (see Chapter 47). It is available as the orally administered prodrug tenofovir disoproxil fumarate, the acyclic nucleotide analogue of adenosine monophosphate.

Tenofovir formulated as a 1% topical gel was studied as an antiherpes drug in a double-blind, randomized, placebo-controlled trial in 422 HSV-2–negative women as part of the CAPRISA 004 study.[537] It was applied intravaginally pericoitally 12 hours before sex with a second dose applied within 12 hours after sex. Efficacy was addressed solely by prevention of HSV-2 seroconversion. Subjects receiving tenofovir demonstrated a 51% reduction in cases of HSV-2 infection ($P = .003$). No significant protective effect was observed in HIV-1–infected women, but the sample was small and compliance with use of tenofovir gel was low. In another study of tenofovir for prophylaxis of HSV-2 infection, daily oral tenofovir–based preexposure prophylaxis alone or in combination with emtricitabine reduced the risk of HSV-2 seroconversion significantly by 27% compared with placebo in the entire study population and by 30% in subjects whose partner was HSV-2–positive.[537] The same regimen was evaluated in a cohort comprising HSV-2–seronegative transgender women and men who have sex with men.[538] In contrast to the aforementioned result in heterosexual HIV-uninfected men and women, daily tenofovir-emtricitabine combination did not reduce the seroincidence of HSV-2 infections. Among the plausible explanations posited for the differences in efficacy in the three studies are differences in tenovir concentrations in the vagina achieved with topical and oral tenofovir and in the rectal-anal tissues with the oral formulation.[538]

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The complete reference list is available online at Expert Consult.

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47 Antiviral Drugs Against Hepatitis Viruses

Jules L. Dienstag

SHORT VIEW SUMMARY

ANTIVIRALS FOR HEPATITIS B (SEE TABLE 47.1)

Pegylated ([Polyethylene Glycol]; PEG) Interferon (IFN)- α2a (Has Largely Supplanted IFN-α2b)

- PEG IFN-α2a is administered subcutaneously, with hepatitis B e antigen (HBeAg) seroconversion in 32% of patients.
- Toxicities include flulike symptoms, cytopenia, emotional lability, and autoimmune events.
- Included in treatment guidelines as first-line therapy, but its use pales in comparison to that of the first-line oral agents.

Lamivudine (Epivir)

- Orally administered and well tolerated but has a low barrier to resistance
- Supplanted by entecavir

Adefovir (Hepsera)

- Orally administered at low doses (10 mg/day) to avoid nephrotoxicity
- Less potent and slower activity than other anti–hepatitis B virus (HBV) drugs
- Supplanted by tenofovir

Entecavir (Baraclude)

- Orally administered and well tolerated
- High barrier to resi:stance
- Currently, first-line therapy for hepatitis B

Telbivudine (Tyzeka)

- Orally administered and generally well tolerated
- Elevated creatine phosphokinase, myopathy, and peripheral neuropathy can occur
- Frequent development of resistance and toxicities limit its use; now discontinued

Tenofovir Disoproxil Fumarate (TDF) (Truvada)

- Orally administered, 300 mg
- Can cause nephrotoxicity and reduced bone density, but rarely encountered in hepatitis B studies
- Active against human immunodeficiency virus and HBV
- High potency and high barrier to resistance against HBV
- Currently, first-line therapy for hepatitis B

Tenofovir Alafenamide (TAF) (Vemlidy)

- Orally administered, 25 mg, prodrug of tenofovir, activated in target organ (liver), >90% reduction in systemic exposure
- Reduced nephrotoxicity and bone density decline
- Active against human immunodeficiency virus and HBV
- High potency and high barrier to resistance against HBV
- Currently, first-line therapy for hepatitis B in patients with evidence for proximal tubular injury, with reduced bone density, with risk of renal disease, and with age older than 60 years (per the European Association for the Study of the Liver [EASL])

ANTIVIRALS FOR HEPATITIS C (SEE TABLE 47.1)

- The availability of highly potent, well-tolerated, orally administered, direct-acting antivirals (DAAs) has supplanted the use of PEG IFN and first-generation protease inhibitors for treatment of hepatitis C. The September 2017 treatment recommendations by the Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America (IDSA) are presented in Chapter 117.

PEG IFN-α2a or PEG IFN-α2b Plus Ribavirin

- Initial treatments historically; now supplanted by other drugs
- Was still recommended with first-generation protease inhibitors and with sofosbuvir (SOF) and ribavirin (RBV) until supplanted by pangenotypic DAA combinations

Boceprevir (Victrelis)

- First-generation inhibitor of hepatitis C virus (HCV) nonstructural 3 (NS3) protease (supplanted by other drugs, discontinued December 2015)
- Most active against HCV genotype 1; limited activity against and not approved for genotypes 2 and 3
- Orally administered with food and metabolized by cytochrome P3A4 (CYP3A4) system, resulting in multiple drug-drug interactions
- Main toxicity from bone marrow suppression additive to PEG IFN/RBV

Telaprevir (Incivek)

- First-generation inhibitor of NS3/NS4A protease (supplanted by other drugs, discontinued October 2014)
- Active against HCV genotype 1; limited activity against and not approved for genotypes 2 and 3
- Orally administered with fatty meal; metabolized by CYP3A4 system, resulting in multiple drug-drug interactions
- Toxicities primarily are anemia, rash, and rectal burning.

Simeprevir (Olysio)

- Inhibitor of NS3/NS4A protease
- Active against HCV genotype 1
- Orally administered once daily with food; metabolized by CYP3A4 system
- Toxicities primarily rash, hyperbilirubinemia
- Supplanted by improved, pangenotypic DAA combinations but still considered an alternative combination regimen along with SOF for genotype 1; should not be used as monotherapy because of emergence of resistance
- In patients with HCV genotype 1a, requires testing for Q80K polymorphism, which is predictive of nonresponse

Sofosbuvir (Sovaldi)

- Nucleoside inhibitor of NS5B polymerase
- Active against HCV genotypes 1 to 6
- Orally administered once daily with or without food; metabolized in the liver to form the active uridine triphosphate
- Negligible toxicity
- Sofosbuvir is a component for recommended DAA regimens as part of combination therapy with simeprevir or daclatasvir and as first-line treatment in combination with ledipasvir, velpatasvir (VEL), and VEL/voxilaprevir (VOX) (see later).

Ledipasvir/Sofosbuvir (Harvoni)

- Fixed-dose combination of ledipasvir (NS5A inhibitor) and SOF (NS5B inhibitor)
- Recommended as first-line regimen for treatment of genotypes 1 and 4 to 6
- Orally administered once daily with or without food
- Well tolerated with very mild side effects of fatigue, headache, nausea, diarrhea, and vomiting
- High rates of sustained virologic response (SVR) in treatment of naïve and experienced patients, both with and without cirrhosis
- Approved in patients with decompensated cirrhosis (genotypes 1 and 4), not for patients with advanced renal insufficiency

Paritaprevir/Ritonavir/Ombitasvir Plus Dasabuvir (Viekira Pak) and Paritaprevir/Ritonavir/Ombitasvir Without Dasabuvir (Technivie)

- Viekira Pak is a fixed-dose combination of paritaprevir (NS3/4A protease inhibitor), ritonavir (pharmacokinetics booster), ombitasvir (NS5A inhibitor), and dasabuvir

SHORT VIEW SUMMARY—cont'd

(nonnucleoside inhibitor of NS5B). Available without dasabuvir (Technivie).
- Was recommended for treatment of genotypes 1a and 1b but is no longer a recommended first-line treatment.
- Technivie (does not include dasabuvir) plus RBV recommended for treatment of genotype 4 but is no longer a recommended first-line treatment.
- Paritaprevir/ritonavir/ombitasvir is given orally once daily, and dasabuvir is given orally twice daily; RBV is given orally twice daily, with or without food.
- Well tolerated but commonly associated with mild nausea, pruritus, insomnia, and asthenia. As with all DAAs that include a protease inhibitor, hepatic decompensation and failure have been reported with both Viekira Pak and Technivie; they should not be used in patients with decompensated cirrhosis.
- Paritaprevir and ritonavir are metabolized by the CYP3A system, resulting in multiple drug-drug interactions. High rates of SVR in treatment of naïve and experienced patients both with or without cirrhosis.

Elbasvir/Grazoprevir (Zepatier)
- Fixed-dose combination of elbasvir, an NS5A inhibitor, and grazoprevir, an NS3/4A protease inhibitor
- Approved and recommended as first-line treatment of genotypes 1 and 4 infection
- Administered orally once daily with or without food
- No dosage adjustments required for renal insufficiency or mild hepatic impairment
- Elbasvir and grazoprevir are substrates of CYP3A, and drug-drug interactions should be considered
- Generally well tolerated, and most common adverse events were fatigue, headache, and nausea
- Highly effective in treatment of genotypes 1 and 4 in treatment-naïve and treatment-experienced patients, with and without cirrhosis
- A preferred regimen in patients with advanced renal insufficiency but cannot be used in decompensated cirrhosis

Daclatasvir (Daklinza)
- Daclatasvir is an inhibitor of HCV NS5A used in combination with SOF.
- Approved for treatment of genotypes 1 and 3 infection and also recommended by AASLD/IDSA for genotype 4 (see Table 47.1). In treatment-naïve and treatment-experienced patients, daclatasvir is no longer approved as a first-line treatment, except in patients with decompensated cirrhosis (genotypes 1–4) and after liver transplantation (genotypes 2 and 3).
- Standard dose is oral administration of one tablet with or without food plus SOF. RBV may be added for decompensated cirrhosis with genotypes 1 to 4 and after liver (genotypes 1–6) or kidney transplantation (genotypes 2, 3, 5, and 6).
- Dosage does not need to be adjusted for renal or hepatic impairment (because daclatasvir is taken along with SOF, the combination cannot be used in patients with severe renal impairment [creatinine clearance < 30 mL/min]).
- Daclatasvir is a substrate for CYP3A and is a moderate inhibitor of organic anion-transporting polypeptide 1B1 (OATP1B1) and OATP1B3. Drug-drug interactions need to be considered.
- Daclatasvir is generally well tolerated. When administered with SOF, most common adverse events were fatigue, headache, nausea, and diarrhea.
- Highly effective in treatment of genotypes 1 to 3 infection. Also recommended for genotype 4. Somewhat less effective in patients with advanced liver disease; recommended as first-line treatment (genotypes 1–4) in combination with SOF and RBV for 12 weeks in patients with decompensated cirrhosis or for 24 weeks without RBV.

Velpatasvir/Sofosbuvir (Epclusa)
- Fixed-dose combination of ledipasvir (NS5A inhibitor) and SOF (NS5B inhibitor)
- Pangenotypic DAA recommended as first-line treatment of genotypes 1 to 6
- Orally administered once daily with or without food
- Well tolerated with very mild side effects of headache, fatigue, nausea, asthenia, and insomnia
- High rates of SVR in treatment of naïve and experienced patients, both with and without cirrhosis
- Recommended in patients with decompensated cirrhosis (genotypes 1–4 and 6), not for patients with advanced renal insufficiency

Glecaprevir/Pibrentasvir (Mavyret)
- Fixed-dose combination of glecaprevir, a high-potency, pangenotypic protease inhibitor and the HCV NS5A inhibitor pibrentasvir.
- Pangenotypic DAA recommended for treatment of genotypes 1 to 6
- Well tolerated with very mild side effects of headache, fatigue, diarrhea, and nausea
- High rates of SVR in treatment of naïve and experienced patients, both with and without cirrhosis
- Like DAA combinations containing a protease inhibitor, this combination is not recommended in patients with decompensated cirrhosis
- A preferred regimen in patients with advanced renal insufficiency

Sofosbuvir/Velpatasvir/Voxilaprevir (Vosevi)
- Fixed-dose combination of ledipasvir (NS5A inhibitor), SOF (NS5B inhibitor), and VOX (protease inhibitor)
- Recommended for retreatment in treatment-experienced patients with genotypes 1 and 3 to 6 and for treatment-naïve cirrhotic patients with genotype 3
- Orally administered once daily with food
- Well tolerated with very mild side effects of fatigue, headache, nausea, and diarrhea
- High rates of SVR in treatment of naïve and experienced patients, both with and without cirrhosis
- Not recommended in patients with decompensated cirrhosis, nor for patients with advanced renal insufficiency

Antiviral drugs for viral hepatitis are reviewed in this chapter and in Chapter 117.

HEPATITIS B

Overview

The first antiviral agent applied to the treatment of chronic hepatitis B was interferon-α (IFN-α), supplanted eventually by pegylated (polyethylene glycol) interferon (PEG IFN; see Chapters 117 and 145). Although IFNs have immunomodulatory properties, data to support a direct immunologic effect of IFN in its antiviral efficacy against hepatitis B virus (HBV) are lacking. Instead, the effect of IFN in HBV infection may be mediated by direct virus inhibition, for instance, by such factors as protean interference at multiple points in the viral life cycle; by stimulation of the IFN signal-transduction pathway, culminating in antiviral activity; and by overcoming the effect of viral infection on suppression of IFN-stimulated genes. IFN therapy, although free of antiviral drug resistance, requires frequent subcutaneous injections and intensive clinical and laboratory monitoring, and tolerability is limited by adverse side effects, including sometimes disabling flulike symptoms, marrow suppression, emotional/psychiatric responses, autoimmune disorders, and a variety of miscellaneous reactions. Currently, potent (micromolar to nanomolar effective concentration for 50% of isolates [EC_{50}], with a high therapeutic index), well tolerated, direct-acting oral nucleoside/nucleotide analogues that inhibit HBV DNA polymerase are the dominant agents used to treat chronic hepatitis B; the most successful have been agents with high genetic barriers to resistance (Table 47.1). These nucleoside analogues require metabolic activation within the cell by phosphorylation (to a nucleoside analogue triphosphate or nucleotide analogue diphosphate). To date, efforts to develop antivirals that target other events in the HBV life cycle remain embryonic.

In chronic hepatitis B, oral antiviral agents can suppress viral replication by up to approximately 4 to 7 $\log_{10}$, which, in turn, translates to immediate biochemical (return to normal of aminotransferase activity), histologic (improvement in grade of necroinflammatory activity and

TABLE 47.1 Antiviral Agents of Established Therapeutic Effectiveness in Hepatitis B and C

DRUG	ROUTE	USUAL ADULT DOSAGE
Chronic Hepatitis B		
IFN-α2b	SC/IM	5 MU/day or 10 MU 3 times weekly for 16 wk
PEG IFN-α2a	SC	180 μg weekly for 48 wk
Lamivudine[a]	PO	100 mg/day
Adefovir	PO	10 mg/day
Entecavir	PO	0.5 mg/day (1.0 mg if lamivudine resistant)
Telbivudine	PO	600 mg/day
Tenofovir disoproxil fumarate (TDF)	PO	300 mg/day
Tenofovir alafenamide (TAF)	PO	25 mg/day
Chronic Hepatitis C[b]		
PEG IFN-α2a *or*	SC	180 μg weekly for 48 wk
PEG IFN-α2b *plus* RBV	SC PO	1.5 μg/kg weekly for 48 wk 800–1400 mg/day, depending on weight and genotype
Boceprevir	PO	800 mg tid (with meals) with PEG IFN plus RBV for up to 44 wk after 4 wk of lead-in therapy with PEG IFN plus RBV (no longer recommended for therapy; see text)
Telaprevir	PO	750 mg tid (with fatty meal) with PEG IFN plus RBV for 12 wk, followed by another 12–36 wk of PEG IFN plus RBV (no longer recommended for therapy; see text)
Simeprevir	PO	150 mg once daily with sofosbuvir ([SOF], 400 mg). Supplanted by improved, pangenotypic DAA combinations but still considered an alternative combination regimen along with SOF for genotype 1; should not be used as monotherapy because of emergence of resistance
Sofosbuvir	PO	400 mg plus simeprevir (150 mg) once daily. SOF is a component for recommended DAA regimens as part of combination therapy with simeprevir or daclatasvir and as first-line treatment in combination with ledipasvir, velpatasvir (VEL), and VEL/voxilaprevir (VOX) (see later).
Ledipasvir/sofosbuvir	PO	Ledipasvir (90 mg)/SOF (400 mg) daily for 12 wk for genotypes 1, 4, 5, and 6
Paritaprevir/ritonavir/ombitasvir and dasabuvir[b]	PO	Paritaprevir (150 mg), ritonavir (100 mg), ombitasvir (25 mg) once daily and dasabuvir (250 mg) twice daily or 12 wk for genotypes 1a with RBV, 1b without RBV (compensated cirrhotic patients with genotype 1a require 24 wk plus RBV). Genotype 4 is treated without dasabuvir (Technivie) without cirrhosis or with compensated cirrhosis Supplanted as first-line agents and are considered alternative regimens
Elbasvir/grazoprevir	PO	Elbasvir (50 mg) combined with grazoprevir (100 mg) for 12 wk for genotypes 1 and 4 without cirrhosis or with compensated cirrhosis
Daclatasvir	PO	60 mg plus SOF (400 mg) for 12 wk Approved for treatment of genotypes 1 and 3 and also recommended for genotype 4 No longer approved as a first-line treatment, except in patients with decompensated cirrhosis (genotypes 1–4) and after liver transplantation (genotypes 2 and 3)
Velpatasvir/sofosbuvir	PO	100 mg combined with SOF (400 mg) for 12 wk in genotypes 1 to 6, with or without compensated cirrhosis. Not recommended in advanced renal insufficiency; recommended in decompensated cirrhosis (genotypes 1–4, 6)
Glecaprevir/pibrentasvir	PO	Glecaprevir 300 mg combined with pibrentasvir 120 mg. A *first-line* treatment for hepatitis C (genotypes 1–6) in treatment-naïve, treatment-experienced, noncirrhotic, and cirrhotic patients, including patients with severe renal impairment (chronic kidney disease, stages 4 and 5, creatinine clearance (CrCl) <30 mL/min) but not patients with decompensated cirrhosis
Sofosbuvir/velpatasvir/voxilaprevir	PO	SOF 400 mg combined with velpatasvir 100 mg and voxilaprevir 100 mg × 12 wk Recommended in noncirrhotic and compensated cirrhotic patients with genotypes 1–6 but not in patients with decompensated cirrhosis or in patients with severe renal impairment (chronic kidney disease, stages 4 and 5, CrCl <30 mL/min). In noncirrhotic and compensated cirrhotic patients with hepatitis C, SOF/VEL/VOX is a *first-line* treatment for (1) treatment-experienced patients with genotypes 1 to 6 and (2) DAA treatment-naïve (not PEG IFN/RBV–naïve) patients with genotype 3. Among treatment-experienced patients, SOF/VEL/VOX is recommended for patients with failed prior protease inhibitor/PEG IFN/RBV treatment who have genotypes 3 to 6; for patients who failed prior SOF but not an NS5A and who have genotypes 1 and 3 to 6; and for patients who failed prior NS5A treatment and who have genotypes 1 to 6.

[a]Dosage in children ≥2 years of age is 3 mg/kg/day to a maximum of 100 mg/day.
[b]US Food and Drug Administration warning letter issued in October 2015 that hepatic decompensation and failure have been reported with paritaprevir/ritonavir/ombitasvir with or without dasabuvir.
DAA, Direct-acting antiviral; *IFN,* interferon; *IM,* intramuscular; *MU,* million units; *PEG,* polyethylene glycol (pegylated); *PO,* oral; *RBV,* ribavirin; *SC,* subcutaneous; *tid,* three times daily.
Note: Please consult text and manufacturer's product prescribing information for dosage adjustments in renal or hepatic insufficiency and in other circumstances.
See recommendations in September 2017 American Association for the Study of Liver Diseases and Infectious Diseases Society of America guidelines for testing, managing, and treating hepatitis C (see Chapter 117: Table 117.9).

grade of fibrosis), and serologic (seroconversion from hepatitis B e antigen [HBeAg] to antibody to HBeAg [anti-HBe, clinical status of an inactive carrier] or, more rarely, from hepatitis B surface antigen [HBsAg] to antibody to HBsAg [anti-HBs, serologic pattern of recovery from infection]) effects, and to long-term improvement in the natural history of disease (progression to cirrhosis, hepatic decompensation, hepatocellular carcinoma [HCC], and death). The more profound the suppression of viral replication and the lower the level of residual viremia, the more frequent the immediate outcomes of therapy and, conversely, the lower the likelihood of antiviral resistance. Combination antiviral therapy is the standard of care in human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection but not in HBV infection. Whereas

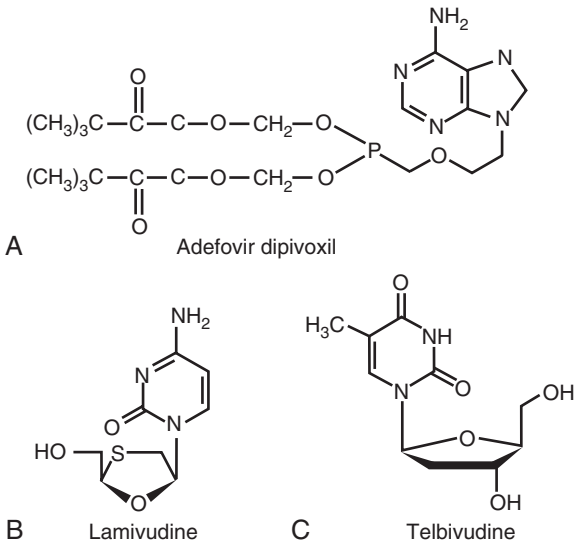

FIG. 47.1 Chemical structures of adefovir (A), lamivudine (B), and telbivudine (C).

combination drug therapy is required for the older, lower-resistance-barrier oral agents after the emergence of resistance, monotherapy suffices in almost all instances with the more potent, high-resistance-barrier agents (entecavir, tenofovir). In addition, although replication must be suppressed to undetectable in patients with HIV and HCV infections, in patients with HBV infection, suppression to a threshold of less than 10^3 IU/mL is usually sufficient to achieve a meaningful clinical change, for instance, from chronic hepatitis to inactive carriage. In practice, HBV DNA can be suppressed to undetectable levels (as determined by sensitive amplification assays) in 70% to 90% of patients treated with current-generation potent oral antivirals.

Individual drugs for hepatitis B are discussed in alphabetic order as follows.

Adefovir

Also see Chapter 128.

Spectrum and Mechanism of Action

Adefovir dipivoxil (9-[2-phosphonomethoxyethyl]adenine; bis-POM, PMEA; Hepsera) is a diester prodrug of adefovir, an acyclic phosphonate nucleotide analogue of adenosine monophosphate (Fig. 47.1A). It is active in vitro against a range of DNA and RNA viruses, including hepatitis B, HIV, poxviruses, and herpesviruses.[1,2] In cell culture, inhibitory concentrations for HBV range from 0.2 to 1.2 µM, whereas concentrations inhibiting growth of uninfected cells are generally greater than 100 µM. Adefovir retains activity against lamivudine-resistant HBV strains and shows dose-dependent inhibition of hepadnavirus replication in animal models.[1] Combinations of adefovir with lamivudine or penciclovir show enhanced antihepadnavirus activity in vitro.[3]

After intracellular transport, adefovir is converted by cellular enzymes to the diphosphate, which acts as a competitive inhibitor of viral DNA polymerases and reverse transcriptases with respect to deoxyadenosine triphosphate and serves as a chain terminator of DNA synthesis.[3,4] Its selectivity relates to higher affinity for HBV DNA polymerase than for host cell polymerases. The prodrug is taken up intracellularly much more readily than the parent. The intracellular half-life ($T_{½}$) of the diphosphate is prolonged, ranging from 5 to 18 hours in different cells, which makes once-daily dosing feasible.[4]

Resistance

Adefovir resistance in HBV is difficult to develop in the laboratory, and no resistant variants have been detected during 48 to 60 weeks of use in chronically infected patients.[5–10] After 144 weeks of dosing, 3.9% of patients show a novel HBV DNA polymerase mutation (N236T or A181V) that confers reduced susceptibility.[6–9] The nucleoside analogues lamivudine, telbivudine, and entecavir are inhibitory for such variants (see Chapter 117).

TABLE 47.2 Dosage Adjustments of Adefovir Dipivoxil in Renal Impairment

CREATININE CLEARANCE (mL/min)	ORAL DOSE (mg)	INTERVAL
≥50	10	q24h
30–49	10	q48h
10–29	10	q72h
Hemodialysis	10	q7 days after dialysis

Note: Dosage is based on manufacturer's recommendations.

Pharmacology/Pharmacokinetics

Adefovir has low oral bioavailability (<12%).[4,11] In contrast, oral adefovir dipivoxil is rapidly absorbed and hydrolyzed to the parent compound by esterases in the intestine or blood or both, with liberation of pivalic acid. The bioavailability of adefovir ranges from approximately 30% to 60%, and after 10-mg doses of adefovir dipivoxil, peak serum concentrations average 0.02 µg/mL. No intact prodrug is detectable in the blood, and ingestion with food does not affect bioavailability. Adefovir has low protein binding (<5%), and has a volume of distribution approximating body water (≈0.4 L/kg).

Adefovir is eliminated unchanged by renal excretion through a combination of glomerular filtration and tubular secretion. After intravenous (IV) dosing, greater than 98% is recovered in urine within 24 hours.[11] After oral administration of adefovir dipivoxil, about 30% to 45% of the dose is recovered within 24 hours, and the serum elimination half-life ($T_{½elim}$) is approximately 5 to 7.5 hours. HIV-infected children have higher clearance than adults. Peak plasma levels increase and clearance decreases with decreasing renal function; therefore dosage reductions are indicated (Table 47.2). Adefovir is removed by hemodialysis (≈35% of the dose during a 4-hour session), and a once-a-week dose after dialysis is recommended, but the effects of peritoneal dialysis are unknown. The pharmacokinetics of adefovir in patients with moderate-to-severe hepatic insufficiency (unrelated to hepatitis B) are not altered.

Interactions

No clinically important drug interactions have been recognized, although drugs that reduce renal function or compete for active tubular secretion could decrease adefovir clearance. Ibuprofen increases adefovir exposure, but no interactions with lamivudine, acetaminophen, or trimethoprim-sulfamethoxazole have been found. An increased risk for lactic acidosis and steatosis may exist when used in conjunction with nucleoside analogues or other antiretrovirals.

Toxicity

Nephrotoxicity at higher doses was the primary adverse event leading to discontinuation of HIV clinical studies in 1999. Adefovir is efficiently transported into tubular epithelium by a probenecid-sensitive human organic anion transporter, and inhibitory effects of the diphosphate on renal adenyl cyclase may contribute to nephrotoxicity. In HIV trials with dosages of 60 mg daily, a Fanconi-like disorder with elevations of serum creatinine, decreases in serum phosphorus and bicarbonate, glycosuria, and proteinuria developed gradually after 20 weeks of dosing. Such nephrotoxicity was generally mild to moderate in severity and usually reversible after a median duration of 4 months. Older age and preexisting renal insufficiency appeared to be risk factors.

In studies of chronic hepatitis B, a lower dose (10 mg daily) has been associated with few adverse events (headache, abdominal discomfort, diarrhea, asthenia) and minimal renal toxicity compared with a higher dose (30 mg).[5,10] Adverse events lead to premature discontinuation in about 2% of patients. At 96 weeks of dosing the estimated risks of an increase in serum creatinine of 0.3 or greater and of an increase of

0.5 mg/dL or greater are 10% and 2%, respectively, but the risk is substantially higher in patients with preexisting renal insufficiency. During therapy, marked increases in aminotransferase levels (>10 times the upper limit of normal) occur less often in adefovir recipients (10%) than in patients taking placebo. Acute, sometimes severe, exacerbations of hepatitis can occur in patients who stop adefovir or other anti-HBV therapies. Close monitoring is necessary after cessation of therapy, and the threshold for resumption of therapy should be low for posttreatment viremia and/or biochemical evidence for reactivation, especially in patients with histologically or clinically advanced liver disease (cirrhosis, advanced fibrosis, borderline hepatic decompensation).

Pivalic acid, a product of adefovir dipivoxil metabolism, can esterify free carnitine and cause reduced free carnitine levels. Although L-carnitine was administered in some HIV studies, supplementation is generally not recommended at the dosages used in chronic hepatitis B.

In preclinical studies, adefovir was genotoxic, and high doses caused renal tubular nephropathy, hepatotoxicity, and toxicity to lymphoid tissues in animals. Carcinogenicity studies in rodents were negative. Adefovir dipivoxil is not associated with reproductive toxicity, although high IV doses of adefovir cause maternal toxicity and embryotoxicity with fetal malformations in rats. Adefovir is classified as pregnancy category C.

Clinical Studies

A detailed discussion of the clinical uses of adefovir dipivoxil in hepatitis B is presented in Chapter 117. Adefovir dipivoxil is approved for treatment of chronic hepatitis B and causes dose-dependent inhibition of HBV replication within 1 week of starting administration. In patients with chronic hepatitis B and who are positive for HBeAg, adefovir (10 mg daily for 48 weeks) results in improved hepatic histology in 53%, reduced serum HBV DNA levels (>3.5 $\log_{10}$ copies/mL), normal aminotransferase levels in 48%, and a 12% rate of HBeAg seroconversion.[10] Continued therapy is associated with sustained viral suppression and increasing frequencies of normalization of aminotransferase and HBeAg loss and seroconversion.[6,7] Similarly, in patients with HBeAg-negative chronic hepatitis B (precore or core promoter mutants), adefovir dipivoxil (10 mg daily for 48 weeks) is associated with significant reductions in HBV DNA (nearly 4 $\log_{10}$), normalization of aminotransferase levels in 72%, and histologic improvement in 64%.[5] Regression of cirrhosis may occur in some patients.[6,7] In addition, adefovir reduces HBV DNA levels comparably across all genotypes,[12] and potential differences in clinical and serologic responses between different genotypes have not been found.

In patients with lamivudine-resistant HBV infections, adefovir dipivoxil monotherapy results in sustained reductions in serum HBV DNA levels comparable to reductions seen with the combination of adefovir and lamivudine, whereas lamivudine monotherapy is ineffective.[13–15] Still, in the face of lamivudine resistance, rather than switching from lamivudine to adefovir, adefovir should be added to lamivudine to reduce the likelihood of biochemical exacerbation and adefovir resistance. In patients with dual HIV and lamivudine-resistant HBV infections, adefovir dipivoxil (10 mg daily) causes sustained decreases in HBV DNA levels.[16] Adefovir dipivoxil has also been used successfully in patients with lamivudine-resistant HBV infections before and after liver transplantation,[17] in whom approximately 4 $\log_{10}$ reductions in HBV DNA levels, improved biochemical markers, and stable or improved histology have been observed.

Although adefovir formerly had an important role in therapy of lamivudine-resistant HBV infection, it has several important limitations. Adefovir is the least potent and slowest acting of the anti-HBV drugs and is ineffective in many patients.[18] Adefovir has been supplanted by tenofovir (see "Tenofovir") and is no longer recommended as a first-line agent.[19,20]

Entecavir

Spectrum and Mechanism of Action

Entecavir (Baraclude) is a cyclopentyl 2′-deoxyguanosine nucleoside analogue that potently and selectively inhibits hepadnaviruses (Fig. 47.2C) and is approved as a first-line drug for the treatment of chronic hepatitis B infection. Entecavir is inhibitory for HBV replication at

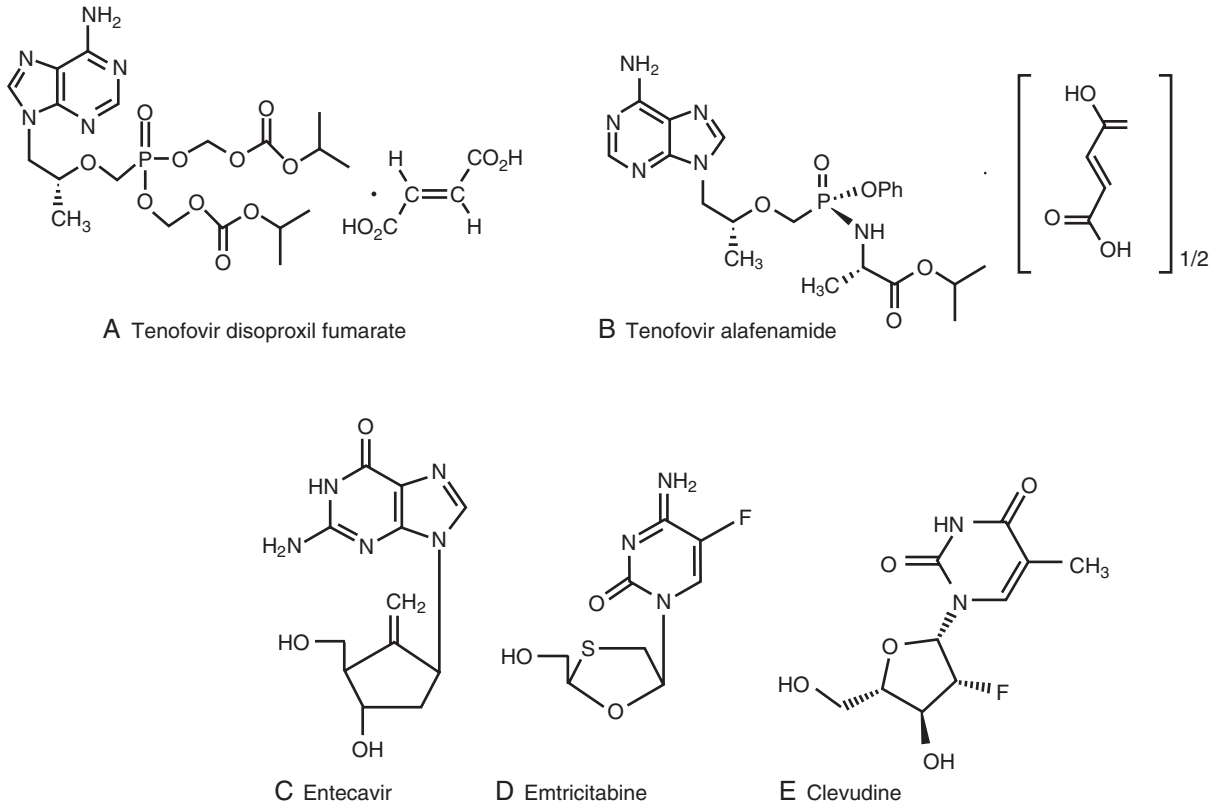

FIG. 47.2 Chemical structures of tenofovir disoproxil fumarate (A) and tenofovir alafenamide (B), entecavir (C), emtricitabine (D), and clevudine (E).

nanomolar concentrations and is 30-fold to greater than 1000-fold more active than lamivudine in cell culture.[21–23] Entecavir is also inhibitory for lamivudine-resistant HBV variants possessing M550I or M550V/L526M mutations, although at 20-fold to 30-fold or higher concentrations.[21,22] After intracellular phosphorylation by cellular enzymes, the triphosphate inhibits several viral DNA polymerase functions, including priming, reverse transcription, and synthesis of positive-strand DNA. The triphosphate accumulates intracellularly at approximately 10-fold to 30-fold higher concentrations relative to extracellular entecavir levels and persists with a $T_{1/2}$ of approximately 15 hours. Oral entecavir is active in several animal models of hepadnavirus infection; prolonged administration for 3 years in hepatitis virus–infected woodchucks resulted in sustained viral suppression without emergence of resistance, in reductions in hepatocyte covalently closed circular (ccc) DNA levels, and in partial protection against HCC.[24] Entecavir inhibits HIV-1 isolates at EC_{50} values ranging from 0.026 to greater than 10 μM. Because its anti–HIV-1 activity can result in development of the M184V mutation, the drug should not be used as monotherapy in HIV-positive patients.[25]

Resistance

Entecavir has a high barrier to resistance; resistance requires the emergence of a YMDD (tyrosine-methionine-aspartate-aspartate) mutation and a second mutation in polymerase domain B, C, or D.[26] In treatment-naïve patients receiving entecavir at 0.5 to 1 mg/day, resistance has rarely been encountered in the first years of therapy and has not exceeded 1.2% after 6 years.[27–30] In patients with previous lamivudine resistance, entecavir is effective, but a higher daily dose (1 mg/day) is required, and resistance to entecavir occurs in 7% at year 1 to 43% at year 4. Therefore, although approved for lamivudine-resistant HBV infection, entecavir has not been adopted for this indication by the treating community; tenofovir is used instead (see "Resistance" under "Tenofovir").

Pharmacology/Pharmacokinetics

Oral administration of entecavir is essentially 100% bioavailable and results in peak plasma concentrations in 0.5 to 1.5 hours. After multiple doses of 1 mg/day, maximum drug concentration (C_{max}) was 8.2 ng/mL, and C_{trough} was 0.5 ng/mL.[31] Administration with food results in a 0.25- to 0.75-hour delay in absorption; therefore, ideally, entecavir should be administered on an empty stomach. The drug has an estimated $T_{1/2}$ of 24 hours and is predominantly (62%–73%) eliminated by the kidneys, probably by glomerular filtration and tubular secretion. Dosage reductions should be undertaken with a creatinine clearance (CrCl) of <50 mL/min (Table 47.3).[32] For patients with liver impairment, the higher 1-mg dose is recommended, but pharmacokinetics are not altered, and dosage reductions are not necessary.

Interactions

Entecavir is not a substrate, inhibitor, or inducer of cytochrome P (CYP) isoenzymes and is not affected by drugs that are metabolized or otherwise affected by that system. Drugs that affect renal function may also affect serum levels of entecavir. Because of its low-level anti-HIV activity, entecavir monotherapy is contraindicated in patients with HBV-HIV coinfection who are candidates for anti-HBV therapy.[33]

TABLE 47.3 Dosage Adjustments of Entecavir in Renal Impairment

CREATININE CLEARANCE (mL/min)	ORAL DOSE (mg)	INTERVAL
≥50	0.5	q24h
30–50	0.5 q48h or 0.25 q24h	q24h or q48h, respectively
10–30	0.5 q72h or 0.15 q24h	q24h or q72h, respectively
<10 or hemodialysis	0.5 once/wk or 0.05 q24h	q7days after dialysis

[a]Doses shown are for treatment-naïve patients; for lamivudine-refractory patients or patients with decompensated liver disease, all doses should be doubled.
Note: Dosage is based on manufacturer's recommendations.

Toxicity

Entecavir is generally well tolerated at dosages of 0.5 to 1 mg/day[34,35] and has a safety profile similar to that of lamivudine. Preclinical toxicity identified hepatic, pulmonary, and brain tumors in rodents but not in other animal species; such toxicity has not been encountered in clinical trials or in postapproval surveillance. In clinical trials the following adverse events were reported: hematuria (9% of patients); glycosuria (4%); and elevations of amylase (8%), lipase (3%), and bilirubin (3%).[32] As is the case for other nucleoside/nucleotide analogues for hepatitis B, a black box warning for entecavir has been issued by the US Food and Drug Administration (FDA) for lactic acidosis and steatosis in patients treated with nucleoside analogues with or without antiretrovirals and for acute exacerbations of hepatitis B when therapy is stopped. Entecavir is classified as pregnancy category C.

Clinical Studies

Short-term administration (0.05–1 mg daily for 4 weeks) is associated with approximately a 2 to 3 $\log_{10}$ suppression of serum HBV DNA levels and at higher doses (0.5 mg and 1 mg) a delay in return of levels to baseline.[36] Entecavir (0.1 mg or 0.5 mg once daily for 24 weeks) reduces HBV DNA levels by more than 4 $\log_{10}$ copies/mL or about 10-fold more than lamivudine.[37] When evaluated in phase III trials against lamivudine as a comparator, entecavir, 0.5 mg/day for 48 weeks, was superior to lamivudine in both HBeAg-positive chronic hepatitis B (HBeAg seroconversion in 21%, 6.9 $\log_{10}$ reduction in HBV DNA, undetectable HBV DNA in 69%, normal alanine aminotransferase [ALT] activity in 68%, improved histology in 72%) and HBeAg-negative chronic hepatitis B (5.0 $\log_{10}$ reduction in HBV DNA, undetectable HBV DNA in 90%, normal ALT in 78%, improved histology in 70%).[35,37,38] Sustained inhibition of lamivudine-resistant HBV infections occurs at dosages of 0.5 mg or 1 mg given daily for 48 weeks[23,39]; however, in this population of lamivudine-experienced/refractory patients, the rate of emerging entecavir resistance becomes prohibitive (reaching 43% at 4 years), rendering entecavir a poor choice for lamivudine-resistant HBV infection.[26,39] Continuation of entecavir for 2 to 5 years or more is well tolerated and is followed by increased HBeAg seroconversion (40% at year 5) and HBsAg loss (5%–6% at years 2–5, respectively).[27,40] Entecavir is effective as well in patients with HBV reinfection after liver transplantation.[23]

The clinical use of entecavir is discussed in more detail in Chapter 117. Because of its efficacy and excellent resistance profile, entecavir is recommended as first-line therapy for chronic hepatitis B.[19,20]

Interferons

The classification, mechanism of action, and pharmacokinetic properties of IFNs are reviewed in Chapter 48. Clinical studies of IFNs in hepatitis B are discussed as follows and in Chapters 117 and 145.

For patients with chronic hepatitis B, parenteral administration of various recombinant IFNs is associated with loss of HBV DNA, loss of HBeAg and development of antibody to HBeAg (anti-HBe), and biochemical and histologic improvement in about 25% to 40% of patients.[41,42] Lasting responses require moderately high IFN dosages and prolonged administration (5 MU/day or 10 MU three times a week for at least 16 weeks).

Responses with seroconversion to anti-HBe are usually associated with aminotransferase elevations and often a hepatitis-like illness during the second or third month of therapy, presumably related to immune clearance of infected hepatocytes. An increased risk for clinical deterioration exists in patients with poor or decreasing hepatic synthetic function, and lower dosages have been suggested for such patients. Factors associated with reduced response rates include high plasma HBV DNA, long-standing infection, male sex, low aminotransferase levels, inactive histology, immunosuppressive therapy, and HIV infection.

Remissions in chronic hepatitis B induced by IFN are sustained in more than 80% of patients, and are frequently followed by later loss of HBsAg and improved long-term clinical outcomes.[43–46] Patients with

precore mutants of HBV may have higher relapse rates.[47] IFN-γ is less effective than IFN-αs and IFN-βs, and combinations do not seem to enhance antiviral effects.[48,49] Combinations of IFNs and lamivudine were not shown to provide consistently greater antiviral effects or clinical benefits than monotherapy.[50,51]

IFN has been covalently linked to a PEG moiety (pegylation) that renders the drug more stable, with a longer half-life, and less immunogenic. PEG IFN-α2a at doses of 180 μg/wk and 270 μg/wk was more efficient than standard IFN-α2a at a dose of 4.5 MU three times per week, which was half the standard dose used in registration trials.[52] The higher dose (270 μg) was associated with more side effects but with no greater efficacy. Large-scale trials of 48 weeks of PEG IFN-α2a (180 μg/wk), lamivudine (100 mg orally daily), or combinations of lamivudine and PEG IFN-α2a showed potent levels of HBV DNA suppression and HBeAg seroconversion in all three groups.[53] Because of the convenience of administration and the results of comparisons in clinical trials, PEG IFN supplanted standard IFN for this indication (see Chapter 117). IFN may improve HBV-associated nephrotic syndrome and glomerulonephritis in some patients.[54]

Standard doses of IFN-α seem to be ineffective against chronic hepatitis D. Longer duration of therapy (12 months) at doses of 9 MU three times a week resulted in return to normal aminotransferase activity and improvement in histology in two-thirds of patients, although reactivation of disease occurred in many patients after therapy was discontinued.[55] PEG IFN has also been shown to have efficacy in chronic hepatitis D infection (see Chapter 146).[56,57]

Lamivudine

Spectrum and Mechanism of Action

Lamivudine (3TC; Epivir) is an L-configuration deoxycytidine analogue with a β-oxathiolane ring that is inhibitory for HIV and HBV (see Fig. 47.1B). It is approved for treatment of HIV (at 150 mg twice daily) and chronic hepatitis B (at 100 mg daily) infection. Its use as an HIV reverse-transcriptase inhibitor is discussed in more detail in Chapter 128, and its use as an anti–hepatitis B drug is discussed in Chapter 117. The triphosphate moiety is a potent inhibitor of the DNA polymerase–reverse transcriptase of HBV, which results in chain termination, and oral lamivudine is active in animal models of hepadnavirus infection. Lamivudine shows enhanced antiviral activity in combination with adefovir or penciclovir against hepadnaviruses.[3]

Resistance

Point mutations in the YMDD motif of HBV DNA polymerase (M550I/V and others) result in 40-fold to 10^4-fold reduction in in vitro susceptibility.[21,58,59] Lamivudine resistance confers cross-resistance to related agents, such as emtricitabine and clevudine (see later), and is often associated with an additional non-YMDD mutation at codon L526M that confers cross-resistance to famciclovir.[60,61] Lamivudine-resistant HBV retains susceptibility to adefovir, tenofovir, and, to a lesser extent, entecavir.[8,22,61,62]

The frequency of lamivudine-resistant variants increases progressively with continued drug administration; cumulative frequencies of 14% to 32%, 38%, 53%, and 67% were observed after 1, 2, 3, and 4 years, respectively, of treatment in chronic hepatitis B.[8,62] Most lamivudine-resistant variants possess L526M/M550V mutations, followed by M550I and L526M/M550I.[22] The risk is higher in posttransplantation infections, and patients coinfected with HIV and HBV have 50% and 90% frequencies of resistance at 2 and 4 years, respectively, of lamivudine therapy.[63,64]

Viruses bearing YMDD mutations are less replication competent in vitro than wild-type HBV and may be associated with lower HBV DNA levels in patients on lamivudine therapy[62]; however, eventually, the antiviral benefit of continued lamivudine therapy degrades. Lamivudine resistance is associated with an increase in HBV DNA levels, decreased likelihood of HBeAg loss or seroconversion, biochemical and histologic deterioration, and graft failure in liver allograft recipients.[58,65,66] Moreover, in patients with histologically advanced chronic hepatitis B, lamivudine resistance reduces the beneficial impact of lamivudine resistance on preventing hepatic decompensation.[67] Emergence of resistant variants may be associated with hepatitis exacerbations in 67% of patients[68] and with rapid clinical deterioration and progressive hepatic fibrosis.

Toxicity

At the dosages used for chronic hepatitis B, lamivudine is well tolerated, including by children; adverse event and laboratory abnormalities are similar to those of placebo.[69] Increases in aminotransferase activity after therapy occur more often in lamivudine recipients, and substantial posttreatment aminotransferase elevations (>500 IU/mL) occur in about 15% of patients after drug cessation.[62] Among the oral antiviral agents for hepatitis B, lamivudine has been used most widely during pregnancy and has an excellent safety record, but the FDA classifies lamivudine as pregnancy category C.

Clinical Studies

(See also Chapter 117.)

In adults with chronic hepatitis B, lamivudine is associated with dose-related, reversible decreases in serum HBV DNA levels.[70] Dosages of 100 mg/day for 1 year suppress HBV DNA levels by a median of 5.5 $\log_{10}$, normalize aminotransferase levels in 40% to 75% or more of patients, and reduce hepatic inflammation in 52% to 56%.[35,62,66] Seroconversion from HBeAg to anti-HBe occurs in only 15% to 17% of adults after 1 year but increases with each year of resistance-free therapy, reaching approximately 50% at 5 years.[71] In children 2 to 17 years of age, lamivudine (3 mg/kg daily for 1 year) is associated with HBeAg loss in 26%, HBeAg seroconversion in 22%, and normalization of aminotransferase levels in 55%.[69] In cases without emergence of YMDD variants, prolonged therapy is associated with sustained suppression of HBV DNA, normalization of aminotransferase activity, continued histologic improvement, and an increase in the proportion of patients experiencing a virologic response (loss of HBeAg and undetectable HBV DNA).[65] In patients coinfected with HIV, higher lamivudine dosages are associated with reductions of HBV replication, reversal of hepatic decompensation, and, in a few patients, HBeAg seroconversion.[63] Administration before and after liver transplantation is useful in preventing or suppressing recurrent HBV infection.[72] Indefinite lamivudine treatment is required until serologic responses are achieved (HBeAg loss/seroconversion in HBeAg-positive patients, HBsAg loss/seroconversion in HBeAg-negative patients), followed by a consolidation period of at least 6 to 18 months of additional therapy. Lamivudine was the first oral antiviral drug introduced against hepatitis B, and, in the absence of other antivirals, lamivudine therapy was continued after YMDD mutations emerged. Currently, newer, more potent, and less resistance-prone anti–hepatitis B drugs are available; therefore lamivudine has been largely supplanted (see Chapter 117 for further details) by entecavir (see earlier) and tenofovir (see later) and is no longer recommended as first-line therapy.[19,20]

Telbivudine

Spectrum and Mechanism of Action

Telbivudine is a synthetic thymidine nucleoside analogue (β-L-2′-deoxythymidine, Tyzeka) (see Fig. 47.1C) that was approved in 2006 in the United States for the treatment of chronic hepatitis B infection.[73] A highly selective inhibitor of HBV, telbivudine is not active against HIV-1. This antiviral agent is phosphorylated by cellular kinases to the active 5′ triphosphate, which competitively inhibits HBV DNA polymerase and causes DNA chain termination.[73,74] Telbivudine inhibits first-strand and second-strand DNA synthesis but may inhibit second-strand synthesis preferentially. The mean EC_{50} against HBV in HCC cell lines is 0.2 μm.

Resistance

Telbivudine is generally cross-resistant with lamivudine and other L-nucleosides to which HBV may be resistant. Although telbivudine resistance after 1 year of treatment was less common than lamivudine resistance,[75,76] by year 2, telbivudine resistance increased to 22% and 9% for HBeAg-positive and HBeAg-negative patients, respectively.[77] Resistant virus usually has the M2041 resistance pattern with or without the rtL80I/V mutant.[75]

Pharmacology/Pharmacokinetics

Telbivudine is rapidly absorbed orally, and C_{max} is reached 1.5 to 4 hours after administration.[73,74] In a steady state after multiple 600-mg doses of telbivudine, C_{max} was 3.7 μg/mL. Plasma concentrations declined in a biexponential manner with a terminal $T_{½elim}$ of 40 to 49 hours.[73]

Approximately 42% of the dose is recovered in the urine, and 50% is recovered in the feces.[73,78] Dosage adjustments for renal impairment are recommended for a CrCl less than 50 mL/min. Telbivudine absorption is not affected by food.[73]

Interactions

Telbivudine does not seem to be metabolized by the CYP450 isoenzymes, and the likelihood for interactions with drugs that use that pathway is low. Drugs that inhibit renal function may cause inhibition of excretion of telbivudine.

Toxicity

In general, telbivudine has a similar tolerability to lamivudine. Asymptomatic elevations of creatinine kinase (7× upper limits of normal) occurred in 13% of telbivudine recipients in phase III studies, compared with 4% of lamivudine recipients.[73] Fatigue and myalgias were also noted in those studies. FDA black box warnings for lactic acidosis and severe hepatomegaly with steatosis are included in the prescribing information as they are for other nucleoside analogues. Exacerbations of hepatitis B upon discontinuation of the drug are noted as they are for other anti-HBV drugs. Myopathy and peripheral neuropathy have been reported. Peripheral neuropathy with telbivudine may be exacerbated with coadministration of PEG IFN-α2a or other IFNs. Telbivudine is classified as pregnancy category B.

Clinical Studies

In large-scale trials telbivudine, at a dose of 600 mg/day for 52 weeks, reduced HBV DNA by a median of 6.4 $\log_{10}$ in HBeAg-positive and 5.2 $\log_{10}$ in HBeAg-negative patients.[76] HBV DNA was reduced to undetectable (<100 copies/mL) in 60% and 88% of HBeAg-positive and HBeAg-negative patients, respectively. ALT returned to normal in 74% to 77%, and histopathology improved in 65% to 67% of patients, respectively.[77] Despite its excellent activity, telbivudine has not been widely used as therapy for hepatitis B because of the frequency of resistance, as noted earlier; its similarity to lamivudine and inferiority to other available hepatitis B antiviral drugs; and reported creatine kinase elevations, myopathy, and peripheral neuropathy. Telbivudine is not recommended as a first-line antiviral agent for the treatment of patients with HBV infection[19,20]; its production and distribution were discontinued in December 2016.

Tenofovir Disoproxil Fumarate

(See also Chapters 117 and 128.)

Spectrum and Mechanism of Action

Tenofovir disoproxil fumarate (TDF, Viread) is a prodrug of tenofovir (see Fig. 47.2A) that has been approved for treatment of HIV-1 infection since 2001 and was approved for treatment of chronic HBV infection in 2008. The prodrug is hydrolyzed to tenofovir, which is an acyclic nucleotide analogue of adenosine 5′-monophosphate. It is phosphorylated by cellular enzymes to form tenofovir diphosphate, which competitively inhibits HIV-1 and HBV polymerases, and, after incorporation into DNA, acts as a chain terminator. The EC_{50} values for tenofovir against HBV in a hepatoma cell line ranged from 0.14 to 1.5 μM.[79] Activity of tenofovir against HIV-1 is discussed in Chapter 128 and activity against HBV in Chapter 117.

Resistance

Tenofovir is active against lamivudine-resistant HBV and generally active against viruses with entecavir-resistant mutations. Development of resistance to tenofovir has not been observed in HBV-infected patients who were HIV negative and were treated for up to 5 years.[80–82] HBV resistance to tenofovir has been noted in patients coinfected with HIV and HBV and who had an rtA194T mutation, but this was not a consistent finding.[83,84] Tenofovir would be the drug of choice for treatment of entecavir-resistant HBV infection, if encountered. When adefovir was used to treat lamivudine resistance, the recommendation was to *add* adefovir to lamivudine to prevent ALT elevations and preempt the emergence of adefovir resistance; however, switching to tenofovir monotherapy, which has not been associated with the emergence of tenofovir resistance, is now recommended for patients with L-nucleoside resistance.[85–87]

Pharmacology/Pharmacokinetics

Oral administration of TDF provides a 25% bioavailability of tenofovir. C_{max} is achieved in 1 ± 0.4 hours at a level of 0.30 ± 0.09 μg/mL. The terminal $T_{½}$ of TDF is 17 hours, and 70% to 80% of the drug is recovered in the urine. Tenofovir's pharmacokinetics and drug interactions are discussed in more detail in Chapter 128.

Toxicity

The general safety profile of TDF is similar to that of adefovir, but renal dysfunction has not been a major problem with TDF at the doses given in registration studies or in clinical practice; nephrotoxicity has been observed in no more than 1% of patients treated for hepatitis B.[82] Renal dysfunction, a proximal tubular injury, is a recognized side effect of TDF,[88] however, and in an observational study, TDF was associated with a greater decline in renal function than that seen with other nucleoside reverse-transcriptase inhibitors.[89] Additional studies with longer-term follow-up continue to support the safety of TDF and the rarity of nephrotoxicity.[81,82] TDF has also been associated, rarely, with reduced bone density (likely related to proximal tubular dysfunction and phosphate wasting), but, in patients treated for hepatitis B, this side effect is uncommon; in a 5-year study of 585 patients, only 3 had evidence of reduced bone density.[82] Still, because kidney and bone toxicity occur occasionally with TDF, patients now have the option of switching to tenofovir alafenamide (TAF) (see later). Tenofovir is classified as pregnancy category B.

Clinical Studies in Hepatitis B Virus Infection

(See also Chapter 117.)

In studies in immunocompetent and immunocompromised patients, treatment with TDF in naïve or lamivudine-resistant patients reduced HBV viral level by greater than 6 $\log_{10}$; achieved DNA suppression rapidly and consistently, with little development of resistance; and was clearly superior to adefovir.[90–94] Given its superiority to adefovir and its high barrier to resistance, TDF supplanted adefovir for the treatment of chronic hepatitis B, and TDF is now recommended as a first-line antiviral agent for hepatitis B.[19,20]

In a large phase III trial[94] 300 mg daily of TDF was compared with, and shown to be superior to, 10 mg daily adefovir for 48 weeks in both HBeAg-positive and HBeAg-negative patients. In HBeAg-positive chronic hepatitis B, tenofovir achieved the following: HBeAg seroconversion in 21%, mean HBV DNA reduction of 6.2 $\log_{10}$, undetectable HBV DNA in 76%, normalization of ALT in 68%, and histologic improvement in 74%. In HBeAg-negative chronic hepatitis B, tenofovir achieved the following: mean HBV DNA reduction of 4.6 $\log_{10}$, undetectable HBV DNA in 93%, normalization of ALT in 76%, and histologic improvement in 72%. In HBeAg-positive patients, HBeAg seroconversion increased from 21% at the end of 1 year to 40% at the end of 5 years, and HBsAg loss occurred in 3% at 1 year, 6% at 2 years, and 8% at 3 and 5 years[81,82,94] in the absence of resistance.[81,82] The long-term benefit of TDF has been demonstrated even in challenging patients with very high levels of HBV DNA—greater than or equal to 10^9 IU/mL.[95] Long-term (5-year) therapy in 348 patients has also been associated with progressive histologic improvement in 87%, regression in fibrosis score in 51%, and, among 96 patients with cirrhosis at baseline, reversal of cirrhosis in 71%.[82] TDF has also been used effectively as salvage therapy in patients with prior adefovir treatment failure (i.e., inadequate suppression of HBV DNA but not adefovir resistance) or nucleoside analogue (lamivudine, entecavir) resistance.[96,97]

Tenofovir Alafenamide

(See also Chapters 117 and 128.)

Spectrum and Mechanism of Action

TAF (Vemlidy) (see Fig. 47.2B) is a prodrug of tenofovir (an inhibitor of HBV and HIV reverse transcriptase) that must be activated to tenofovir in cells (e.g., hepatocytes, peripheral blood mononuclear cells), allowing for higher targeted delivery of the drug to the liver and a 90% reduction

in systemic exposure,[98] reducing significantly the risk of proximal renal injury and loss of bone density (phosphate wasting as a result of proximal tubular injury). TAF is converted by hydrolization within cells to an alaninyl-tenofovir intermediate, then to tenofovir, an acyclic nucleotide analogue of adenosine 5′-monophosphate. Finally, tenofovir is phosphorylated by host nucleotide kinases to the pharmacologically active metabolite tenofovir diphosphate, which is incorporated by HBV reverse transcriptase into HBV DNA. This process results in HBV DNA chain termination.[99–101] At an oral dose of 25 mg daily, TAF is as effective and well tolerated as 300 mg daily of TDF with a comparably high barrier to resistance.

TAF is now one of the first-line antiviral drugs for chronic hepatitis B recommended for patients with impaired renal function (CrCls < 50 mL/min), reduced bone density, anyone at risk for renal injury, and for patients older than 60 years, who are more prone to the potential nephrotoxicity of TDF.[20] Dosage adjustments of TAF are not required for patients with renal impairment; however, TAF is not recommended in patients with CrCls less than 15 mL/min. No dose adjustments are required for patients with mild hepatic impairment; however, TAF is not recommended for patients with severe hepatic impairment (Child-Pugh class B and C).[102]

Resistance

The resistance pattern of TAF is similar to that of TDF (see earlier). Tenofovir has a high barrier to resistance; as noted earlier, no resistance to TDF has been encountered clinically, even after 5 or more years of use, and no resistance to TAF was encountered in phase III clinical trials of TAF (see later).[103,104]

Pharmacology/Pharmacokinetics

After oral administration of TAF, >80% of the dose is metabolized; <1% of the drug is recovered in urine, and 31.7% is recovered in feces. C_{max} for TAF is 0.27 ± 63.3 μg/mL. The terminal $T_{½}$ of TAF is 0.51 hours.[102] Pharmacokinetics and drug interactions are discussed in more detail in Chapter 128.

Drug Interactions

TAF is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters; therefore drugs that influence the activity of P-gp and BCRP may affect TAF absorption. CYP3A metabolism is minimal.

Toxicity

In clinical trials of TAF, low frequencies of mild side effects (headache, abdominal pain, fatigue, cough, nausea, back pain) occurred in 5% to 9% of patients, indistinguishable from frequencies in patients taking TDF. Similarly, nonrenal, nonbone laboratory abnormalities tended to be similar to those recorded for TDF recipients. Patients treated with TAF have a reduced renal and bone toxicity compared with patients treated with TDF (see clinical trial data later).[103,104] Although proximal tubular injury has been reported in patients taking TDF, no such reports have appeared in patients taking TAF. Changes in serum lipids among TAF recipients are similar to those recorded in TDF recipients. Reductions in high-density lipoprotein cholesterol have been noted in TAF recipients. Like all nucleoside analogs for hepatitis B, TAF has a black box warning about the risk of severe acute exacerbations of hepatitis B if the drug is stopped, the importance of close monitoring after discontinuation, and the option to resume therapy should reactivation occur.[102] Like TDF, TAF is classified as pregnancy category B.

Clinical Studies

(See also Chapter 117)

Randomized, double-blind, phase III, 48-week noninferiority trials of TAF versus TDF were conducted in HBeAg-reactive and HBeAg-negative patients.[103,104] In HBeAg-reactive patients for TAF versus TDF, HBV DNA was undetectable by polymerase chain reaction (<29 IU/mL) in 64% versus 67%, ALT was normal (in central laboratory) in 72% versus 67%, HBeAg loss/seroconversion was 14%/10% versus 12%/8%, and HBsAg loss was 4 of 576 (1%) versus 1 of 288 (0.3%). Mean reductions in bone density were lower for TAF (e.g., hip, 0.10%) versus TDF (hip, 1.72%), mean increases in serum creatinine were smaller for TAF (0.01 mg/dL) versus TDF (0.03 mg/dL), and median reductions in CrCl (estimated glomerular filtration rates) were lower for TAF (–0.6 mL/min) versus TDF (–5.4 mL/min).[103] In HBeAg-negative patients for TAF versus TDF, HBV DNA was undetectable in 94% versus 93%, ALT was normal in 83% versus 75%, and no one in either group lost HBsAg. Mean reductions in bone density were lower for TAF (e.g., hip, 0.29%) versus TDF (hip, 2.16%); mean increases in serum creatinine were comparably small for TAF (0.01 mg/dL) and TDF (0.02 mg/dL), but median reductions in CrCl were lower for TAF (–1.8 mL/min) versus TDF (–4.8 mL/min).[104] These findings were similar at week 96, and the more substantial ALT reduction with TAF was sustained over 96 weeks. This sustained, enhanced biochemical advantage of TAF over TDF remains unexplained. Between weeks 96 and 120, when initial TDF recipients were switched to TAF, earlier differences between TDF and TAF in ALT normalization, renal function, and bone density all resolved. No resistance to either TAF or TDF was encountered through 120 weeks of observation.

Other Agents of Potential Interest

Emtricitabine

Emtricitabine ([–]FTC) is a 5′-fluorinated derivative of lamivudine approved for treatment of HIV infection (see Fig. 47.2D; see also Chapter 128), but it is not currently approved for treatment of HBV infection. The potency and selectivity of emtricitabine for HBV are comparable to those of lamivudine in cell culture. It is not inhibitory for lamivudine-resistant HBV variants, and its resistance patterns are similar to those of lamivudine.[22] Emtricitabine is phosphorylated by cellular enzymes to the triphosphate that inhibits HBV DNA polymerase.

Emtricitabine is rapidly absorbed and has a plasma $T_{½elim}$ of about 6 to 9 hours.[105] Once-daily dosing (25–300 mg for 8 weeks) is associated with nearly dose-proportional steady-state plasma concentrations (peak 1.7 μg/mL with 100-mg doses) and rapid antiviral effects with approximate 3 $\log_{10}$ reductions in HBV DNA levels.[105] Resistance mutations develop in 19% of patients within 2 years of treatment.[63] Emtricitabine is generally well tolerated, and its safety profile is similar to that of lamivudine. Clinical studies have shown good activity against HBV in monotherapy[106] and in combination therapy.[107] Although not formally approved as therapy for hepatitis B, emtricitabine (200 mg) is available in combination with TDF (300 mg) or TAF (25 mg) for the treatment of HIV infection.

Clevudine

Clevudine (L-FMAU) is a fluorinated L-arabinofuranosyl nucleoside analogue (see Fig. 47.2E) that is inhibitory for hepadnaviruses and Epstein-Barr virus. Clevudine is about 10-fold more potent than lamivudine against HBV in cell culture, although it is not inhibitory for most lamivudine-resistant variants.[22] Once-daily oral administration reduces woodchuck hepatitis virus replication profoundly, including hepatocyte ccc DNA levels.[108]

In patients with chronic HBV infection, clevudine (10–200 mg once daily for 4 weeks) reduces HBV DNA levels by 2.5 to 3 $\log_{10}$ copies/mL and is associated with sustained biochemical and antiviral effects at 6 months after dosing, that is, delayed HBV DNA rebound after therapy.[109] Administration for 24 weeks resulted in a 4.25 $\log_{10}$ decrease in HBV DNA.[110] Despite these promising early findings, clinical trials demonstrated no advantage in potency or resistance profile over approved drugs, and, in 2009 the discovery that prolonged clevudine administration was associated with severe myopathy resulted in the discontinuation of its development.

The Future

To date, no other nucleoside inhibitors are being developed that are competitive with the leading hepatitis B drugs entecavir or tenofovir (TDF and TAF), and these drugs are so potent and resistance-free that they can be used effectively as monotherapy; tenofovir monotherapy is effective even in patients with lamivudine resistance, without the need to continue lamivudine.[85–87] In general, the current antivirals convert chronic hepatitis into an inactive carrier state with suppressed viral replication as well as biochemical, histologic, and clinical quiescence

and marked reductions in the late, dire consequences of chronic HBV infection (e.g., hepatic decompensation, all-cause mortality, HCC).[111] Still, because almost all patients treated with the current oral agents require long-term, indefinite therapy, a substantial research effort is being devoted both to immunologic interventions and to interference with other components of the HBV replicative life-cycle besides the viral polymerase targeted by nucleoside inhibitors. Immunomodulatory approaches being pursued include reconstitution of innate and adaptive immune responsiveness, Toll-like receptor agonists, T-cell vaccines, checkpoint inhibitors, IFN gene agonists, and retinoic acid–inducible gene-I activation. Viral targeting strategies being studied include inhibitors of viral entry, HBV core assembly, cccDNA, and HBV secretion; RNA interference and gene editing with CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) are novel genetic technologies being pursued. Whether immunomodulatory therapy or novel approaches to direct viral inhibition can overcome host tolerance to HBV infection and lead to recovery from chronic infection, which is now the exception to the rule during antiviral therapy, remains to be seen. So far, none of these imaginative approaches has been successful in "curing" HBV infection and eliminating the need for continued antiviral therapy.[112]

HEPATITIS C

Overview

Extensive advances have been made recently in recommended treatments for hepatitis C, with the approval of the DAAs ledipasvir/sofosbuvir (SOF) in October 2014, ombitasvir/paritaprevir/ritonavir plus dasabuvir in December 2014, daclatasvir in July 2015, elbasvir/grazoprevir in January 2016, SOF/velpatasvir (VEL) in June 2016, SOF/VEL/voxilaprevir (VOX) in July 2017, and glecaprevir (GLE)/pibrentasvir (PIB) in August 2017. These newly approved drugs are discussed subsequently, as well as in Chapters 117 and 154.

Historically, as was true for hepatitis B, IFN-α was the first agent applied to and approved for the treatment of hepatitis C (see Chapters 117 and 145). Approved originally as a 24-week course of therapy, IFN suppressed HCV RNA to undetectable levels during therapy and for greater than or equal to 24 weeks after therapy (sustained virologic response [SVR]) in fewer than 10% of patients. The frequency of SVR doubled when the duration of IFN therapy was extended to 48 weeks and increased again when ribavirin (RBV) was added to IFN (SVR ≈ 40%) and when longer-acting PEG IFNs were introduced (SVR ≈ 55%; lower in genotype 1, ≈45%; higher in genotypes 2 and 3, ≈80%). The addition of RBV added a new dimension of efficacy but also of intolerability, especially hemolytic anemia, to the profile of IFN adverse effects (see earlier under "Hepatitis B"), aggravating the challenge of navigating therapy for both patients and their clinicians. The combination of PEG IFN and RBV remained the standard of care for a decade. In 2011 the first oral protease inhibitors were approved for the treatment of chronic hepatitis C in patients with HCV genotype 1 but were highly prone to resistance when used as monotherapy. When these agents were evaluated in clinical trials and approved, they had to be taken together with the former standard of care, PEG IFN and RBV. For patients with chronic hepatitis C and genotypes 2 and 3, the old standard remained the only option, although the duration of therapy was shorter and its intensity lower (reduced RBV doses) (see Table 47.1).

The first-generation protease inhibitors ushered in a new era of antiviral therapy and catapulted the response rate for genotype 1 from approximately 45% with the old standard of care to greater than or equal to 75%. Moreover, the duration of therapy could be shortened in half to two-thirds of patients. Unfortunately, first-generation protease inhibitor regimens added new and challenging side effects, a high pill burden and thrice-daily dosing along with meals, cumbersome drug-drug interactions, and complicated response-guided therapy (RGT) milestones and stopping rules. Now, however, that novel oral agents, such as later-generation protease inhibitors and drugs that interfere with other targets in the HCV life cycle (polymerase inhibitors, NS5A inhibitors), have become available, as noted earlier, many of the limitations of first-generation protease inhibitors have been overcome. These new agents include pangenotypic drugs with improved pharmacokinetics (once-daily dosing), excellent resistance profiles, very high potency, excellent tolerability, and fewer drug-drug interactions. In addition, the most current of the first-line all-oral drug-combination regimens, which do not require IFN injections and need to be taken for only 8 to 12 weeks, have become the new standard of care as of September 2016 (www.hcvguidelines.org).[113]

As is true for hepatitis B, effective antiviral therapy for chronic hepatitis C has been shown to result in not only virologic improvement but also in biochemical, histologic, and clinical benefit, including a reduction in progression to cirrhosis, hepatic decompensation/liver death, and HCC.

Interferons

The classification, mechanism of action, and pharmacokinetic properties of IFNs are reviewed in Chapter 48. Clinical studies of IFNs in hepatitis C are discussed later and in Chapters 117 and 154.

In acute hepatitis C infection, high-dose IFN monotherapy (IFN-α2b, 5 MU daily for 4 weeks, then three times per week for 20 weeks) markedly reduced the risk for chronicity[114]; lower-dose regimens were less effective.[115] Before the DAA era, PEG IFN plus RBV at conventional doses (see later) for 24 weeks were used to treat patients with acute hepatitis C.[116–118] Efficacy was improved by treating patients as early as possible. In chronic HCV infection, the combination of a PEG IFN and oral RBV is superior to IFN/RBV.[119] Although IFN-α monotherapy (3 MU three times a week for 6–12 months) was associated with serum aminotransferase normalization[120,121] and loss of detectable serum HCV RNA in about 40% to 60% of patients,[122] greater than 50% of responding patients relapsed 1 to 2 months after treatment was stopped, and less than 20% had an SVR predictive of long-term biochemical and histologic improvement.[123–126]

In chronic hepatitis C, combining IFN with oral RBV (1000–1200 mg daily in divided doses) increased the likelihood of SVR to 35% to 45% and was superior to IFN monotherapy in initial treatment and retreatment after IFN failure or relapse, especially in patients with high HCV RNA levels or genotype 1 infections.[127,128] Combined IFN and RBV treatment helped HCV-related systemic vasculitis, cryoglobulinemia, and glomerulonephritis.[129,130] RBV, as discussed later in the chapter, is an oral guanosine that has minimal activity against HCV in vitro and is ineffective against HCV when used alone.[131] Although the mechanism of its effect in treatment of chronic HCV infection is not understood, RBV has been postulated to exert a subtle antiviral effect or an immunomodulatory effect, facilitating a Th2 response.[132] Combined IFN and RBV, but not IFN alone, reduced the risk for recurrent HCV infection after liver transplantation, but treatment resulted in an SVR in only 21% and was not well tolerated.[133] Such antiviral therapy was shown to slow disease progression after liver transplantation.[134]

Two PEG IFNs were approved for treatment of hepatitis C: PEG IFN-α2b, which is 12 kilodaltons (kDa) in size, linear, and partially renally excreted, and PEG IFN-α2a, which is 40 kDa in size, branched, and nonrenally excreted. The 40-kDa molecule has a longer $T_{1/2}$ and a more restricted volume of distribution (8 L), which permits a common dose to be used over a wide range of patient weights. The 12-kDa molecule has a much larger volume of distribution (20 L) and is administered based on weight. PEG IFN monotherapy doubled the frequency of SVR (30%–39%) relative to monotherapy of its nonpegylated counterpart,[123,124] and addition of RBV to PEG IFN increased the frequency of SVR to 55%.[135–137] PEG IFN monotherapy was a treatment option in patients unable to take RBV. Combined therapy with PEG IFN-α2a (180 μg once weekly for 48 weeks) and RBV (1000 or 1200 mg daily in divided doses) yielded an overall SVR of 56%, compared with 44% for IFN and RBV, and was more effective in genotype 1 infections (46% vs. 36%) in previously untreated patients.[138] Similarly, combined PEG IFN-α2b (1.5 μg/kg once weekly for 48 weeks) and RBV (800 mg daily) yielded an overall SVR of 54%, compared with 47% for IFN/RBV.[139]

Higher, weight-adjusted doses of RBV were more effective, particularly in genotype 1 infections. A shorter duration of therapy (24 weeks) and lower RBV dose (800 mg daily) were effective in genotype 2 and 3 infections (84% SVR), but prolonged therapy was needed for genotypes 1 and 4.[140] Failure to achieve an early viral response (undetectable HCV RNA or reduction of ≥2 $\log_{10}$ at 12 weeks) was highly predictive of lack of SVR after further therapy and was relied upon as a stopping point

for futility.[141] Treatment was associated with reductions in steatosis and fibrosis progression, and histologic improvement was shown to occur even in patients who did not achieve SVRs. In patients with compensated cirrhosis, treatment appeared to reverse cirrhotic changes and to reduce the risk for HCC.[142–144] Retreatment for 48 weeks was shown to clear infection in patients relapsing after an initial course.[145] Only 15% to 20% of nonresponders to combined IFN/RBV achieved SVR with PEG IFN/RBV therapy, however.[119]

Age (<40 years), genotype other than 1, host *IL28B* genotype C/C, body weight (<75 kg), disease duration (<5 years), absence of cirrhosis, serum levels of HCV RNA (<2×10^6 copies/mL), and RBV dosage (>10.6 mg/kg) were predictors of response to IFNs.[138,139] Differences in genotype and, for genotype 1, mutations within an IFN sensitivity-determining region of the *NS5A* gene affected virologic responses.[146,147] Individuals coinfected with HIV also seemed to respond better to combined PEG IFN/RBV therapy and could achieve SVRs.[119,145]

As noted earlier, the use of IFNs to treat hepatitis C was supplanted by the recent availability, since 2014, of all-oral DAAs, as discussed later.

Ribavirin

The mechanism of action and pharmacokinetic properties of RBV are presented in Chapter 45. Clinical studies of RBV in hepatitis C are discussed later and in Chapters 117 and 154. RBV played an important role during the IFN era, was still required during the time of the first-generation protease inhibitors, and maintained a niche even during the early IFN-free DAA era before the availability of pangenotypic combinations in 2017. With the advent of these contemporary, high-potency, pangenotypic *first-line* DAAs, the need for RBV in combination with DAAs was reduced substantially, as recommended in the September 2017 guidelines from the American Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America (IDSA) (www.hcvguidelines.org; see Chapter 117) and is now reserved for (1) DAA-naïve compensated cirrhotic patients with genotype 3 being treated with ledipasvir/SOF in the setting of baseline ledipasvir resistance-associated substitution (RAS) Y93H and (2) DAA-experienced cirrhotic patients with genotype 3 who had failed a prior NS5A inhibitor and are being treated with SOF/VEL/VOX. RBV also has a role in several of the alternative DAA regimens (e.g., elbasvir/grazoprevir for genotype 1a in patients with baseline NS5A elbasvir mutations) and in first-line regimens in patients with decompensated liver disease and after liver or kidney transplantation (www.hcvguidelines.org).

RBV is an oral guanosine whose mechanism of action in treatment of hepatitis C is not well understood. Its direct antiviral activity, if present, appears to be relatively weak inhibition of the *NS5B* gene–encoded RNA-dependant RNA polymerase[148]; RBV may promote mutational errors in RNA viruses,[149] and it may inhibit cellular inosine monophosphate dehydrogenase and influence intracellular nucleoside pools. RBV may also stimulate IFN-response genes and modulate Th1/Th2 balance, which may contribute to its clinical effect.[148–151] When administered alone, RBV reduced aminotransferase levels in 21% of patients but had little impact on HCV RNA levels.[152–154]

In adults with chronic hepatitis C, long-term oral RBV therapy reversibly reduced not only serum aminotransferase elevations but also hepatic inflammation and fatigue without, as noted earlier, affecting serum HCV RNA concentrations significantly.[154–156] In earlier studies, for patients with HCV genotype 1, RBV was administered in split (twice-daily) doses of 1000 to 1200 mg for PEG IFN-α2a and doses of 600 to 1400 mg for PEG IFN-α2b. In earlier studies, for patients with genotypes 2 and 3, a daily RBV dose of 800 mg sufficed. The combination of oral RBV and conventional IFNs or PEG IFNs significantly increased the frequency of sustained biochemical and virologic responses compared with IFN monotherapy.[127,157] Increases in sustained responses also occurred in patients nonresponsive to, or relapsing after, previous IFN monotherapy.[128,145,157]

Orally administered RBV causes a dose-related extravascular hemolytic anemia. Dosages greater than 800 mg/day often resulted in decreases in hemoglobin of 2 to 4 g/dL in most patients. When used in combination with IFN, hemoglobin levels less than 11 g/dL developed in 25% to 35% of patients.[158] Renal failure increases the risk for hemolysis. Severe anemia requires dosage reduction or cessation, but erythropoietin treatment can be used.[158] Other reported RBV side effects include pruritus, rash, myalgia, nervousness, depression, and cough. Weight-adjusted dosages of RBV improved tolerability and overall results.

Boceprevir

Boceprevir is no longer recommended for therapy of hepatitis C in guidelines issued by the AASLD and IDSA and by the European Association for the Study of the Liver (EASL) because of the availability of new, better therapies.[113,159]

Spectrum and Mechanism of Action

Boceprevir (Victrelis) is an orally administered ketoamide peptidomimetic inhibitor of the NS3 protease of HCV (Fig. 47.3A).[160] Monotherapy at a dose of 400 mg three times daily resulted in a reduction of HCV RNA of 1.61 ± 0.21 $\log_{10}$ after 1 week.[161] Boceprevir activity was demonstrated

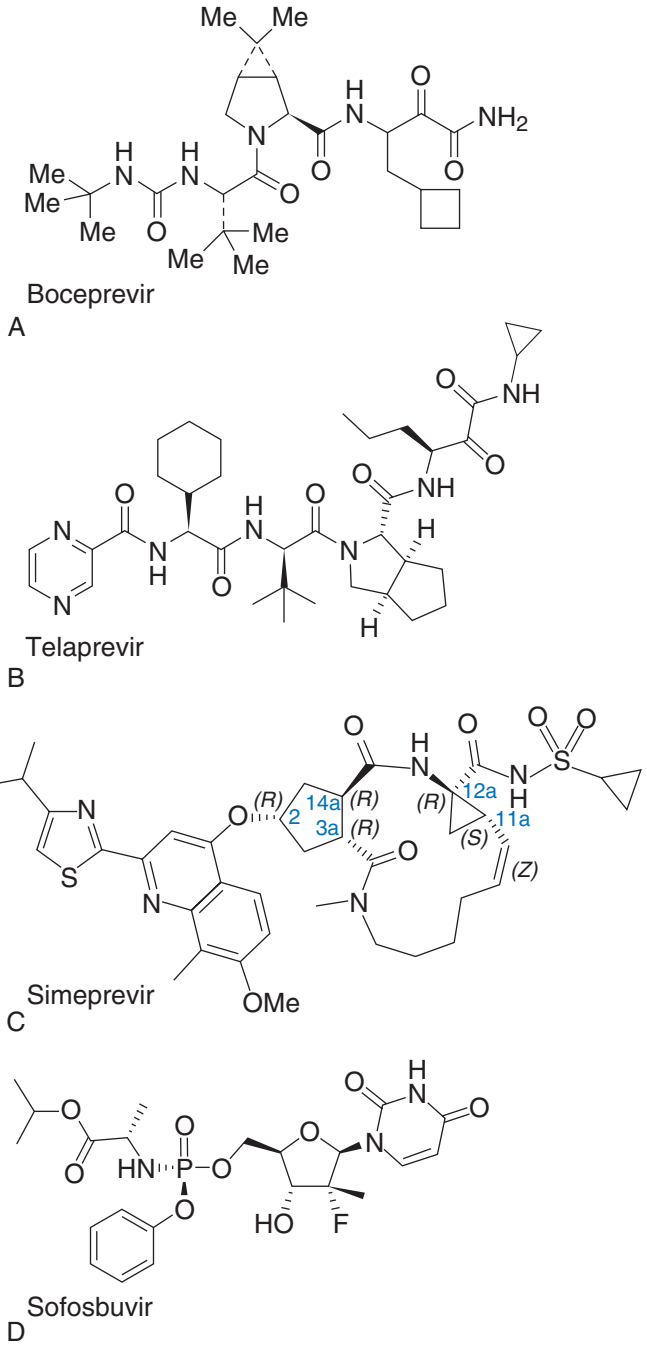

FIG. 47.3 Chemical structures of boceprevir (A), telaprevir (B), simeprevir (C), and sofosbuvir (D).

against HCV genotype 1 (genotype 1b > 1a) and was approved for its treatment; its activity against genotypes 2 and 3 was limited, and boceprevir was not approved for these genotypes. In preliminary trials among patients with genotype 1 chronic HCV infection, when boceprevir was administered at a dose of 800 mg three times daily in combination therapy with weekly PEG IFN and twice-daily weight-based RBV, at week 12 HCV RNA was undetectable in 80% of patients, and after 48 weeks of triple-drug therapy, SVR was as high as 66%.

Mechanism of Action

Boceprevir inhibits the HCV NS3/4A protease by forming a reversible covalent bond with the NS3 serine active site, preventing proteolytic cleavage by NS3 of the HCV polyprotein into NS4A, NS4B, NS5A, and NS5B proteins.[160,162] In an HCV genotype 1b replicon, boceprevir has an EC_{50} of 200 nM; its activity against genotype 1a, relative to genotype 1b, is reduced by approximately twofold; its activity against genotypes 2 and 3 is reduced even more substantially; and its activity is additive—not synergistic—with IFN.[160]

Resistance

In most patients who failed to achieve an SVR after boceprevir treatment, HCV RASs emerged, the most common of which were V36M, T54S, and R155K for genotype 1a and T54A/S, V55A, A156S, and I/V170A for genotype 1b. For the R155K RAS, compared with genotype 1a, which requires only a single amino-acid substitution, genotype 1b has a higher barrier to resistance, requiring two amino-acid substitutions. These RASs were not archived, and reversion to wild-type HCV occurred within 18 to 24 months in almost all cases. Very low-level RASs were present before therapy in almost all patients,[163] but their presence did not predict the emergence of resistance during boceprevir therapy; therefore pretreatment resistance testing was not indicated. Because RASs emerged efficiently and rapidly during boceprevir monotherapy, boceprevir had to be used in combination with other antivirals, and boceprevir antiviral therapy was developed as a triple-drug combination with PEG IFN and RBV.

Pharmacology/Pharmacokinetics

Boceprevir is metabolized primarily by aldoketoreductase to ketone-reduced metabolites but also by oxidative metabolism via CYP3A4, and its pharmacokinetic disposition is the same in normal subjects and in patients with HCV infection. At an oral dose of 800 mg (four 200-mg tablets) three times daily, boceprevir has a C_{max} of 1723 ng/mL, C_{min} of 88 ng/mL, area under the time-concentration curve $(AUC)_{(T)}$ of 5408 ng × h/mL, median absorption T_{max} of 2 hours, plasma $T_{½}$ of approximately 3.4 hours, and mean volume of distribution of approximately 772 L. Boceprevir is metabolized by the liver predominantly; approximately 79% is eliminated in stool and approximately 9% in urine.[160] Because food (independent of fat content) increases boceprevir exposure by as much as 65%, boceprevir is taken with food (fatty meal is not necessary). No dose adjustments were necessary in patients with renal or hepatic impairment.

Interactions

Medications that are CYP3A4 inducers lower boceprevir plasma levels and efficacy, and boceprevir competition for CYP3A4 metabolism can raise plasma levels of multiple medications, leading to toxicity. Thus boceprevir has multiple interactions with commonly prescribed drugs, including statins, benzodiazepines, calcineurin inhibitors, antiretroviral agents, antifungals, and opioid antagonists, to name a few. Before prescribing boceprevir, clinicians were encouraged to consult the boceprevir product insert and/or the website www.hep-druginteractions.org.[164]

Toxicity

The major adverse effects encountered in patients receiving boceprevir-based triple-drug therapy, including PEG IFN and RBV, were anemia (Hgb <10 g/dL) in approximately half of patients and dysgeusia (altered sense of taste) in approximately a third. In the triple-drug combination, all three drugs had an effect on red blood cells: RBV through hemolysis, PEG IFN through mild marrow suppression, and boceprevir through more severe marrow suppression. Patients treated with the triple-drug, boceprevir-based combination also experienced the following side effects that appeared to be more frequent than in patients treated with PEG IFN and RBV (without boceprevir): neutropenia (25%) and vomiting (20%). Boceprevir was not associated with rash and did not have cardiac toxicity (no effect on QT interval).[160] Because of the need to use boceprevir with PEG IFN and RBV, triple-drug boceprevir-based therapy was contraindicated in pregnancy.

Clinical Studies

A detailed review of boceprevir clinical trials appears in Chapter 117. As noted earlier, boceprevir has been supplanted by newly available therapies for hepatitis C.[113,159]

In clinical trials of boceprevir-based triple-drug therapy (in combination with PEG IFN-α2b, 1.5 μg/kg subcutaneously and weight-based RBV, 600–1400 mg/day orally) in treatment-naïve or treatment-experienced patients with genotype 1 chronic hepatitis C, a 4-week PEG IFN/RBV lead-in period preceded the introduction of boceprevir, 800 mg three times daily orally.

In *treatment-naïve* subjects, patients were randomized to 48 weeks of PEG IFN/RBV versus PEG IFN/RBV/boceprevir triple therapy ("SPRINT 2").[164] One group of triple-drug recipients received a full, fixed 48-week course (triple-drug therapy for 44 weeks after the 4-week lead-in period), and another group received RGT after the 4-week lead-in phase, that is, triple-drug therapy for 24 weeks if HCV RNA was undetectable at weeks 8 and 24 or a full 44 weeks of triple therapy if HCV RNA remained detectable at week 8 (as long as it was undetectable at week 24). SVRs occurred in 63% of RGT recipients (44% meeting criteria for shortened, 28-week therapy) and in an indistinguishable 66% of full 48-week boceprevir-based therapy but in only 38% of PEG IFN/RBV recipients, and SVR was achieved in 67% to 69% of non-Black subjects but only 42% to 53% of Black subjects. Among subjects in the RGT arm who did not meet criteria for shortened therapy, SVRs occurred in only 36%, no better than the PEG IFN/RBV control arm.[165]

In *treatment-experienced* patients, prior relapsers and partial responders (≥2 log_{10} reduction but not complete suppression in HCV RNA during previous IFN-based therapy) were randomized to RGT (4 weeks of PEG IFN/RBV lead-in plus 32 [instead of 24 in treatment-naïve subjects] weeks of triple therapy, that is, potential for shortened, 36-week treatment), full 48-week boceprevir-based therapy, or 48 weeks of PEG IFN/RBV ("RESPOND-2").[166] In the RGT group, if HCV RNA was undetectable at weeks 8 through 36, therapy was discontinued at week 36; if this criterion was not met (as long as HCV RNA was undetectable at week 24 and thereafter), subjects received an additional 12 weeks (to week 48) of PEG IFN/RBV. In this trial, 59% in the RGT arm, a statistically indistinguishable 66% in the full 48-week arm, and 21% in the control PEG IFN/RBV arm achieved an SVR. In the RGT arm, among the 46% who met criteria for short-duration therapy, 86% had an SVR, whereas among the 95.4% who did not meet criteria for shortened therapy, 35% had an SVR. Efficacy was substantially higher in prior relapsers (up to 75%) than in prior partial responders (up to 52%)[166]; null responders (<2 log_{10} HCV RNA reduction during prior IFN-based therapy) were not included in this trial, but in a small ($n = 46$) subsequent, open-label trial of a full 48-week regimen (4 weeks of PEG IFN/RBV lead-in, followed by 44 weeks of boceprevir-based triple therapy), an SVR was observed in 38% of prior null responders.

Based on these trials and on additional analyses conducted by the FDA,[167] RGT (28 weeks total if truncation criteria are met vs. an additional 8 weeks of triple therapy and then 12 weeks of PEG IFN/RBV) was approved for noncirrhotic treatment-naïve patients. For noncirrhotic prior relapsers and partial responders, RGT (36 weeks total if truncation criteria are met vs. an additional 12 weeks of PEG IFN/RBV) was approved. For cirrhotic patients who failed to achieve a 1-log_{10} HCV RNA reduction during the 4-week lead-in period and for prior null responders, a full 48 weeks (4 weeks of PEG IFN lead-in, followed by 44 weeks of triple-drug therapy) was the recommended option.[160,168,169] Therapy was stopped if HCV RNA equaled or exceeded 100 IU/mL at week 12 or remained detectable at week 24; in such instances, additional therapy was shown to be futile. Thus, although boceprevir-based triple therapy improved efficacy compared with PEG IFN/RBV therapy, the complexity of the treatment regimen and drug-drug interactions, as

well as the need to follow complicated response-guided and futility milestones, limited the drug's appeal and adoption. Because of the superiority, improved tolerability, and simplicity of use of currently available treatments (see later), boceprevir is no longer recommended in the treatment of chronic hepatitis C.[113,159]

Telaprevir

Telaprevir is no longer recommended for therapy of hepatitis C in guidelines issued by the AASLD, IDSA, and EASL because of the availability of new, better therapies.[113,159]

Spectrum and Mechanism of Action

Telaprevir (Incivek; see Fig. 47.3B)[170] is an orally administered peptidomimetic inhibitor of the HCV NS3/NS4A protease; the ketoamide moiety of telaprevir binds to the active enzymatic site. As monotherapy, it induced a 4 $\log_{10}$ decrease in HCV RNA within 2 weeks, but it was followed by the frequent emergence of resistance.[171,172] Telaprevir is active against HCV genotype 1 (genotype 1b > 1a), much less so for genotype 2, and even less so for genotype 3; it was approved for use in genotype 1. In studies of a combination of PEG IFN and RBV along with telaprevir administered three times daily at a dose of 750 mg (total daily dose 2250 mg), HCV RNA was reduced rapidly (within 4 weeks) in 80% of subjects. Telaprevir could also be administered, with comparable efficacy, at a dose of 1125 mg twice daily (three 375-mg tablets) for the same total daily dose of 2250 mg. In early-phase trials, 12 weeks of triple-drug therapy, followed by 12 weeks of PEG IFN plus RBV, resulted in SVRs in 61% to 68% of patients.[172,173] In these early studies patients who had either relapsed or been nonresponders to PEG IFN or standard IFN had SVRs of 41% to 72%. Responsiveness to combination telaprevir with PEG IFN alone was less than that to the combination with PEG IFN plus RBV; therefore, RBV was an important ingredient of this combination therapy. A severe maculopapular rash was observed in 3% to 6% of patients, and gastrointestinal discomfort and anemia were also seen. See additional details as follows.

Mechanism of Action

Telaprevir inhibits the HCV NS3/4A protease by forming a reversible covalent bond with the NS3 serine active site, preventing proteolytic cleavage by NS3 of the HCV polyprotein into NS4A, NS4B, NS5A, and NS5B proteins.[170] The 50% inhibitory concentration (IC_{50}) in a biochemical assay of HCV NS3 proteolytic activity is 10 nM. In an HCV genotype 1b replicon telaprevir has an EC_{50} of 354 nM; in a genotype 1a replicon it has an EC_{50} of 280 nM. The median IC_{50} of telaprevir in biochemical enzymatic assays is 20 nM for genotypes 1a and 1b, 16 nM against genotype 2, 40 nM against genotype 3a, and 130 nM against genotype 4a. The activity of telaprevir does not interfere with the activity of IFN, and the activity of the two together is additive—not synergistic.[170]

Resistance

Please see the discussion of resistance to boceprevir, which applies as well to telaprevir. Resistance-associated substitutions appeared to account for clinical nonresponse to telaprevir-based therapy. The telaprevir HCV RASs that emerged most commonly during clinical trials were V36M/A/L, T54A/S, R155K, and A156S/T (V36M, R155K, or the combination of V36M and R155K for genotype 1a and V36A, T54A/S, and A156S/T for genotype 1b).[170] Telaprevir RASs are not archived, and reversion to wild-type HCV occurred within 24 to 36 months in almost all cases. Very-low-level RASs were present before therapy in almost all patients,[170] but their presence did not predict the emergence of resistance during telaprevir therapy; therefore pretreatment resistance testing was not indicated. Because RASs emerged efficiently and rapidly during telaprevir monotherapy, telaprevir had to be used in combination with other antivirals, namely PEG IFN and RBV.

Pharmacology/Pharmacokinetics

Telaprevir is metabolized predominantly by ketoamide reduction but also by oxidative metabolism via CYP3A4 and by hydrolysis. At an oral dose of 750 mg (two 375-mg tablets) three times daily, telaprevir has a mean ± SD C_{max} of 3510 ± 1280 ng/mL, C_{min} of 2030 ± 930 ng/mL, AUC_{8h} of 22,300 ± 8650 ng × h/mL, median absorption T_{max} of 4 to 5 hours, plasma $T_{1/2}$ of 4.0 to 4.7 hours after a single dose and at steady state approximately 9 to 11 hours, and mean volume of distribution of 252 L. Telaprevir is metabolized by the liver. Metabolites appear in stool (median, 82%), urine (1%), and expired air (9%).[170] Food, and especially a fatty meal, increases telaprevir exposure by as much as 237% (low-fat meal by 117%, high-fat meal by 330%)[170]; therefore telaprevir was taken with a fatty (≈20 g fat) meal. No dose adjustments were necessary in patients with renal impairment. Because moderate hepatic impairment (Child class B) reduces telaprevir steady-state exposure by 46%, telaprevir was not recommended in moderate-to-severe hepatic impairment; however, mild hepatic impairment, which has a minimal effect on telaprevir exposure, did not necessitate any telaprevir dose adjustment, and no dose adjustment was necessary in patients with compensated cirrhosis.[170]

Interactions

As is the case for boceprevir (see earlier), telaprevir is metabolized via the P450 (CYP3A4) system, accounting for multiple drug-drug interactions. Careful review of coadministered medications was important before initiation of treatment. For example, statins had to be stopped for the duration of telaprevir therapy. Medications that are CYP3A4 inducers lower telaprevir plasma levels and efficacy, and telaprevir competition for CYP3A4 metabolism can raise plasma levels of multiple medications, leading to toxicity. Thus telaprevir had multiple interactions with commonly prescribed drugs, including statins, calcium channel blockers, benzodiazepines, calcineurin inhibitors, antiretroviral agents, antifungals, and opioid antagonists, to name a few.[170] Before prescribing telaprevir, clinicians were encouraged to consult the product insert and/or www.hep-druginteractions.org.[164]

Toxicity

Telaprevir can amplify the anemia associated with PEG IFN/RBV therapy (Hgb ≤ 10 in a third of patients), mandating close laboratory monitoring and occasionally requiring red blood cell transfusions, especially in prior null responders with cirrhosis. Rectal burning and other anorectal discomfort occurred in approximately a third and dysgeusia (altered sense of taste) in approximately 10% of patients. Rash occurred in approximately half, but a severe, confluent, maculopapular, pruritic rash of the trunk and extremities occurred in 3% to 6% of patients at a median of 8 weeks. In clinical trials such severe rash led to withdrawal from treatment and, in some cases, necessitated systemic corticosteroid therapy. If patients were monitored closely, such rashes could be identified when they were sufficiently early and mild, to stop therapy, thus preventing them from becoming severe. Telaprevir had a "boxed warning" regarding serious skin reactions and rashes, including toxic epidermal necrolysis and erythema multiforme, which were reported in postmarketing surveillance.[170] Also more common than in patients treated with PEG IFN/RBV alone were pruritus (≈50%), nausea (≈49%), and diarrhea (≈25%). Telaprevir was not associated with renal, hepatic, or cardiac toxicity (no effect on QT interval). Because of the need to use telaprevir with PEG IFN/RBV, telaprevir triple-drug therapy was contraindicated in pregnancy.

Clinical Studies

As noted earlier, telaprevir has been supplanted by newly available therapies for hepatitis C.[113,159] A detailed review of telaprevir clinical trials appears in Chapter 117.

In registration clinical trials of telaprevir-based triple-drug therapy (in combination with PEG IFN-α2a, 180 μg subcutaneously, and weight-based RBV, 1000– 1200 mg/day orally) in treatment-naïve or treatment-experienced patients with genotype 1 chronic hepatitis C, telaprevir was administered at a dose of 750 mg three times daily orally. Because phase II trials showed no advantage of continuing telaprevir beyond 12 weeks, in phase III trials triple-drug therapy lasted only 12 weeks, and subsequent extension of therapy involved PEG IFN/RBV. Unlike boceprevir trials, telaprevir trials in treatment-naïve subjects did not include a 4-week PEG IFN/RBV lead-in period,[174,175] although a lead-in arm was included in the registration trial among prior nonresponders, and outcomes were indistinguishable between the arms with and without a lead-in period.[176]

In *treatment-naïve* subjects patients were randomized to 48 weeks of PEG IFN/RBV versus response-guided PEG IFN/RBV/telaprevir triple therapy for 8 or 12 weeks, followed by PEG IFN/RBV for up to another 40 or 36 weeks, respectively. Trial subjects with undetectable HCV RNA at week 4 and 12 extended PEG IFN/RBV therapy for only an additional 16 (for the 8-week triple-drug arm) to 12 weeks (for the 12-week triple-drug arm), for a total duration of 24 weeks; trial patients who did not meet the early stopping milestone but who cleared HCV RNA by week 24 continued PEG IFN/RBV therapy for 24 more weeks ("ADVANCE").[174] In the RGT groups an SVR occurred in 75% of the 12-week triple-therapy recipients and 69% of the 8-week triple-therapy recipients but in only 46% of PEG IFN/RBV recipients. Criteria for early stopping at week 24 were met by 58%, among whom the likelihood of an SVR was 83% (8-week telaprevir arm) to 89% (12-week telaprevir arm); those failing to meet early stopping criteria, but in whom HCV RNA was undetectable by week 24, achieved SVR in 50% to 54%, respectively, more frequently than in the PEG IFN/RBV arm, 39%. In a subsequent treatment-naïve follow-up study of RGT ("ILLUMINATE"), of the 65% who met criteria for early discontinuation of therapy (undetectable HCV RNA at weeks 4 and 12), 92% achieved an SVR.[175]

In *treatment-experienced* patients, the registration trial of telaprevir ("REALIZE")[176] contained 12-week, but not 8-week, triple-drug treatment arms in which triple therapy was followed by PEG IFN/RBV for the rest of 48 weeks and involved prior relapsers, partial responders, and null responders; the trial did not have an RGT arm (all trial patients were treated for 48 weeks) but did include a lead-in arm. In the triple-drug, telaprevir-based treatment arms, SVRs occurred in 64% with lead-in therapy and in 66% without a lead-in, compared with 17% in the 48-week PEG IFN/RBV control group. Relapsers had the best response: SVR in 86%, then partial responders (57%), and null responders with the poorest response (31%).[176] In another trial patients who failed PEG IFN/RBV therapy during phase II telaprevir trials were re-treated with telaprevir-based triple-drug therapy: (1) RGT—12 weeks of triple therapy, followed by 12 to 36 weeks of PEG IFN/RBV therapy (based on meeting early stopping criteria) for relapsers and partial responders or (2) a fixed 48-week regimen—12 weeks of three drugs, followed by 36 weeks of PEG IFN/RBV for null responders. SVRs occurred in 97% of relapsers, 55% of partial responders, and 37% of null responders.[177]

Based on these trials and on additional analyses conducted by the FDA,[178] RGT—12 weeks of triple therapy plus 12 weeks of PEG IFN/RBV (total of 24 weeks) if truncation criteria are met versus an additional 24 weeks of PEG IFN/RBV if truncation criteria are not met (total of 48 weeks)—was formerly recommended for noncirrhotic treatment-naïve patients and relapsers. For partial and null responders, a full 48-week course of therapy was recommended (12 weeks of triple therapy, followed by 36 weeks of PEG IFN/RBV) without RGT. For cirrhotic treatment-naïve patients, even if they met RGT criteria for shortened therapy, a full 48-week course was advised.[168–170,178] Therapy was stopped for futility if HCV RNA exceeded 1000 IU/mL at week 4 or 12 or if HCV RNA remained detectable at week 24. After its initial approval at a three-times-daily dose of 750 mg, telaprevir was shown to be equally effective at a twice-daily dose of 1125 mg. Although telaprevir-based triple therapy improved efficacy compared with PEG IFN/RBV therapy, the complexity of the treatment regimen, the high frequency of drug-drug interactions, the need to follow complicated response-guided and futility milestones, and the greater-than-anticipated drug toxicities and morbidity encountered once the drug was approved (especially in cirrhotic prior nonresponders) limited the drug's appeal and adoption. Because of the superiority, improved tolerability, and simplicity of use of newly available therapies (see later), telaprevir is no longer recommended in the treatment of chronic hepatitis C.[113,159]

Simeprevir

Spectrum and Mechanism of Action

Simeprevir (Olysio; see Fig. 47.3C), a second-generation NS3/4A protease inhibitor (approved November 2013) with antiviral activity against HCV genotype 1 (1b > 1a), has improved pharmacokinetic properties compared with those of first-generation protease inhibitors and therefore can be taken once a day; simeprevir must be taken with food. Initial approval of simeprevir was based on trials in combination with PEG IFN and RBV in genotype 1 HCV infections. Based on current recommendations, however, simeprevir has a very limited role and is not part of *first-line* DAA combinations. As an alternative regimen, simeprevir can be used in combination with SOF in genotype 1a and 1b infections in treatment-naïve and PEG IFN/RBV treatment-experienced patients if no cirrhosis is present (www.hcvguidelines.org).[113] Approximately a third of patients with HCV genotype 1a harbor an NS3 polymorphism, Q80K, which renders them refractory to the antiviral activity of simeprevir; if simeprevir therapy is being considered, Q80K testing is advisable, and, if positive, should discourage the use of simeprevir. In early phase III clinical trials, 150 mg of simeprevir along with PEG IFN and RBV was associated with an SVR in 80% of treatment-naïve subjects as well as treatment-experienced subjects who had relapsed after a prior course of PEG IFN and RBV. For treatment-naïve patients and prior relapsers, 150 mg by mouth of simeprevir daily was administered for an initial 12 weeks of triple therapy, followed by another 12 weeks of PEG IFN and RBV dual therapy (total course, 24 weeks); for prior nonresponders, based on data extrapolated from phase II trials, the initial 12 weeks of triple therapy were followed by 36 weeks of dual therapy (total course 48 weeks). These treatment approaches in clinical trials applied to patients with any stage of fibrosis, including those with cirrhosis. If an HCV RNA suppression milestone of ≤25 IU/mL was not met by week 4, further treatment was futile, and therapy was be stopped; if HCV RNA was not suppressed to ≤25 IU/mL at week 12 or 24 (by which time simeprevir had been completed), PEG IFN and RBV were stopped for futility. In Black patients simeprevir therapy was approximately 10% less effective in achieving an SVR than in white patients.[179] (See "Clinical Studies" later for more recent studies.)

Simeprevir inhibits the HCV NS3/A protease directly with an EC_{50} in a genotype 1b replicon of 9.4 nM, which is increased by up to 11-fold in isolates with a Q80K polymorphism. The activity of the drug in vitro is not reduced by IFN, RBV, or polymerase inhibitors (nucleoside or nonnucleoside). The simeprevir plasma concentration is reduced by two-thirds when administered with darunavir/ritonavir. Like first-generation protease inhibitors that needed to be taken with PEG IFN and RBV, simeprevir-based triple-drug therapy was most effective in patients with the IFN-γ–associated *IL28B* C/C haplotype and reduced in patients with non-C/C haplotypes C/T and T/T.[179]

Resistance

The most common treatment-emergent RASs observed during clinical trials of simeprevir among study subjects who failed to achieve an SVR were NS3 substitutions Q80R, S122A/G/I/T/R, R155K, and/or D168E/V/X. For genotype 1a the most common RAS was R155K (alone or along with Q80 S122, and/or D168 substitutions); for genotype 1b, D168V was the most common RAS. For study subjects with genotype 1a and a baseline Q80K substitution (prevalent in 30%–35% of persons with genotype 1a), the most common emerging RAS was R155K. Cross-resistance was demonstrated between simeprevir and the first-generation protease inhibitors boceprevir and telaprevir (e.g., boceprevir- or telaprevir-induced R155K or A156T/V—less so V36A/G and I170A/T—would be expected to reduce simeprevir responsiveness).[179]

Pharmacology/Pharmacokinetics

Simeprevir, formulated as a 150-mg capsule, is metabolized by hepatic cytochrome P450 3A (CYP3A) and excreted by biliary excretion. The $T_{½elim}$ for simeprevir is 10 to 13 hours in healthy subjects and 41 hours in persons with HCV infection. The AUC for plasma reaches steady state after 7 days. In patients with hepatitis C the mean steady-state predose AUC was 1936 ng/mL, and the AUC_{24} was 57,469 ng·h/mL; C_{max} occurs 4 to 6 hours after oral administration, and oral bioavailability is increased by taking the drug with food. Almost all of the drug is plasma protein bound. Because the drug is not renally excreted, no dose modifications are necessary in patients with renal impairment, but, because of its hepatic metabolism, simeprevir should not be used in patients with decompensated liver disease.[179]

Interactions

Simeprevir is metabolized in the liver by CYP3A; therefore concomitant administration of simeprevir along with CYP3A inducers or inhibitors

can amplify or reduce simeprevir exposure; although simeprevir does not induce hepatic CYP3A activity, coadministration of simeprevir with drugs metabolized by CYP3A can increase plasma concentrations of these drugs. Similarly, concomitant administration of simeprevir with drugs that are substrates for hepatic organic anion-transporting polypeptide 1B1 or 1B3 (OATP1B1/3) or P-gp transporters can elevate plasma concentrations of these drugs. Because of these substantial drug-drug interactions, prescribing information and/or the website www.hep-druginteractions.org should be consulted before simeprevir is prescribed.[179]

Toxicity

Potential toxicity encountered during clinical trials included photosensitivity, rash (in 28%; mild-to-moderate in almost all, severe [grade 3] in 1%), and reversible mild elevation of bilirubin (both unconjugated and conjugated; mild-to-moderate in 45% of treated subjects; grade 3 in 4%). Although most other adverse events during simeprevir therapy are attributable to PEG IFN and RBV, the following were slightly more common in the simeprevir group than in the placebo group: pruritus, nausea, myalgias, and dyspnea. Simeprevir has no effect on the QT interval on electrocardiography. Simeprevir could not be used in pregnant women when it was combined with PEG IFN and RBV, which are contraindicated during pregnancy. Simeprevir is unlikely to be used during pregnancy and is classified as pregnancy category C.[179]

Clinical Studies

In phase III clinical trials among treatment-naïve subjects with chronic hepatitis C, simeprevir 150 mg daily plus PEG IFN and RBV for 12 weeks, followed by another 12 weeks of PEG IFN and RBV, an SVR was achieved in 80% (compared with 50% of control subjects treated with PEG IFN and RBV). In subjects with genotype 1a and a Q80K variant, simeprevir triple-drug therapy was no more effective than PEG IFN/RBV, and the efficacy of simeprevir-based triple-drug therapy was reduced to 58% in subjects with cirrhosis. Phase III trials included RGT: If HCV RNA < 25 IU/mL at week 4 and undetectable at week 12, the treatment course could end at week 24; if these milestones were not met, but stopping rules not violated, treatment was extended to 48 weeks; however, in the 8% of study subjects who failed to meet RGT milestones for shortened therapy, only 25% experienced an SVR. Therefore RGT is not recommended for simeprevir. The efficacy of simeprevir-based triple-drug therapy is similar in prior relapsers to IFN-based therapy, achieving an SVR in 79%, compared with 37% in a PEG IFN/RBV-treated control group. Phase III trials did not include treatment-experienced nonresponders, but, based on phase II trials, simeprevir-based triple-drug therapy was approved for partial responders and null responders, for all of whom a full 48 weeks of therapy (12 weeks of simeprevir with PEG IFN and RBV, followed by 36 weeks of PEG IFN and RBV) is indicated; in these phase II trials, an SVR was achieved in 70% of partial responders and 45% of null responders. In more recently conducted studies, the combination of simeprevir and SOF for 12 weeks resulted in SVR_{12}s of 97% in treatment-naïve and treatment-experienced patients without cirrhosis.[180] In patients with compensated cirrhosis, the overall rate of SVR_{12} was 83%. Based on this trial, addition of RBV and expansion of therapy for 24 weeks was recommended in treatment-naïve and treatment-experienced cirrhotic patients,[181] before subsequent revision of treatment guidelines (see later).[113]

The combination of SOF and simeprevir was also found to be very effective in patients with genotype 1 (well tolerated; SVR_{12} after 12 to 24 weeks of therapy, with or without RBV, in 92%–94% of 167 treatment-naïve ["METAVIR F3–F4"] and IFN-experienced prior nonresponders ["METAVIR F0–F2"]),[182] including those with decompensated cirrhosis (well tolerated; 74% SVR_{12}, with or without RBV, in 42 patients with Child-Pugh class B and C)[183] and was a recommended first-line combination regimen until superseded by later-generation DAAs (see later).[113] Currently, all protease inhibitor regimens, including those containing simeprevir, are not recommended in patients with decompensated cirrhosis because of reports of liver deterioration, including liver failure and death.[113] Overall, as noted earlier, based on current recommendations, simeprevir has a very limited role and is not part of *first-line* DAA combinations. As an alternative regimen, simeprevir can be used in combination with SOF in genotype 1a and 1b infections in treatment-naïve and PEG IFN/RBV treatment-experienced patients if no cirrhosis is present (www.hcvguidelines.org).[113]

Sofosbuvir

Spectrum and Mechanism of Action

The first nonprotease inhibitor direct antiviral to be approved (in December 2013) was SOF (Sovaldi; see Fig. 47.3D), a uridine nucleoside polymerase inhibitor with one of the best profiles among the all-oral DAA agents available (and part of three of the five first-line recommended regimens). SOF is very potent, has a high barrier to resistance and pangenotypic activity, is very well tolerated with few adverse events, requires only once-daily oral administration with or without food, and appears to be relatively free from major drug-drug interactions.[184–186] In clinical trials SOF at a daily dose of 400 mg has been studied in all genotypes (1–6); in treatment-naïve subjects and prior null responders to PEG IFN–based therapy; in prior telaprevir and boceprevir nonresponders, with PEG IFN/RBV or in IFN-free regimens; in combination with RBV or with NS5A inhibitors (SOF/ledipasvir, SOF/VEL, see later) and in combination with both an NS5A inhibitor and a protease inhibitor (SOF/VEL/VOX, see later); and for treatment periods as brief as 8 to 12 weeks to as long as 24 weeks.[187,188] Currently, SOF-containing DAA combinations are recommended as *first-line* therapy of both treatment-naïve and treatment-experienced patients, both noncirrhotic and cirrhotic patients (compensated and decompensated) with genotypes 1 to 6 (see Tables 47.1 and 117.9); as noted earlier, SOF plus simeprevir is recommended as an alternative DAA regimen in noncirrhotic patients with genotypes 1a and 1b (www.hcvguidelines.org).[113]

SOF is a prodrug nucleoside inhibitor of HCV RNA NS5B polymerase that is metabolized to the active uridine nucleoside triphosphate, resulting in chain termination. SOF is active against all HCV genotypes and has an EC_{50} of 0.7 to 2.6 μM against recombinant NS5B and of 0.014 to 0.11 μM in NS5B-containing replicons.[186]

Resistance

Susceptibility to SOF is reduced by an S282T substitution in NS5B, but clinically apparent, treatment-emergent resistance with this RAS or others (M289L, L159F, V321A, C316N, S282R) is very rare in patients receiving SOF.[186]

Pharmacology/Pharmacokinetics

In the liver, SOF is metabolized to the active triphosphate nucleoside analog by sequential hydrolysis, phosphoramidate cleavage, and phosphorylation. After oral administration of SOF, peak plasma concentrations of SOF and its active metabolite occur at 0.5 to 2 hours and 2 to 4 hours, respectively; the AUC_{0-24} in normal subjects was 828 ng·h/mL and 6790 ng·h/mL, respectively; and the AUC_{50} in persons with HCV infection was 39% higher and 39% lower, respectively. Food has no effect on SOF metabolism. Approximately 61% to 65% of SOF is bound to plasma proteins, but plasma protein binding of its active metabolite is negligible. Most of SOF and its active metabolite are eliminated by renal clearance; the $T_{1/2}$ for SOF is 0.4 hours and of its metabolite is 27 hours.[186]

Interactions

Clinically relevant drug-drug interactions are rare for SOF. P-gp inducers, which can reduce plasma SOF concentrations, should not be used with SOF; however, P-gp inhibitors do not affect the concentration of the active metabolite of SOF and can be given with SOF, and SOF does not increase exposure of drugs that are a substrate for P-gp. Drugs that can reduce concentrations of SOF and its active metabolite—and that should not be administered with SOF—include carbamazepine, phenytoin, phenobarbital, oxcarbazepine, rifampin, rifabutin, rifapentine, St. John's wort, and tipranavir/ritonavir. Proton pump inhibitors (PPIs) such as omeprazole raise gastric pH, which reduces SOF solubility; if a PPI is to be used, both the PPI (equivalent to ≤20 mg of omeprazole) and SOF should be taken together under fasted conditions.[186]

Toxicity

In initial clinical trials, the most commonly reported side effects (in ≥20% of participants) associated with SOF appeared to be attributable to concomitant PEG IFN and RBV (headache, fatigue, nausea, insomnia)

or concomitant RBV (headache and fatigue). SOF appears to have no effect on hematologic markers that are not attributable to PEG IFN and RBV. SOF does not prolong the QT interval on electrocardiography. SOF is contraindicated in pregnancy when used with RBV (category X); however, when used without RBV, SOF, which has no effect in animals on fetal development, is classified as pregnancy category B.[186] SOF has been associated with severe bradycardia when taken with amiodarone, especially in patients also taking β-blockers; therefore SOF, either alone or in combination with other DAAs, should not be used in patients taking amiodarone. Although no dose reductions are necessary in either decompensated liver disease or mild-moderate chronic kidney disease (stages 1–3), insufficient data are available for, and the DAA combinations containing SOF are not recommended in, patients with severe chronic kidney disease who have a CrCl <30 mL/min (stages 4 and 5).[186]

Clinical Studies

In a phase III IFN-free trial of SOF plus RBV—in patients with genotypes 2 and 3 who were IFN intolerant, ineligible, or unwilling—78% achieved SVR (93% genotype 2, 61% genotype 3).[189] Similarly, among prior PEG IFN/RBV nonresponders with genotypes 2 and 3 treated with open-label SOF/RBV (IFN-free) for 12 or 16 weeks, 50% (86% genotype 2, 30% genotype 3) and 73% (94% genotype 2, 62% genotype 3), respectively, experienced SVRs.[189] In phase III trials, among patients with genotypes 1 and 4 to 6, open-label SOF plus PEG IFN/RBV for 12 weeks yielded SVRs of 90% (compared with 60% in historical control subjects), 89% in genotype 1, and 97% in genotypes 4 to 6.[188] In treatment-naïve subjects with genotypes 2 and 3, SVRs occurred in 67% of patients treated with either SOF and RBV for 12 weeks or PEG IFN/RBV for 24 weeks; again, SVR was more frequent in SOF/RBV recipients with genotype 2 (97%) than in those with genotype 3 (56%).[188] Exploratory trials of IFN-free combinations of SOF plus NS5A inhibitors yielded near-100% SVRs in both treatment-naïve subjects and prior null responders with genotypes 1, 2, and 3 after treatment durations as brief as 12 weeks (ledipasvir) and 24 weeks (daclatasvir). A fixed-dose combination tablet containing SOF and the NS5A inhibitor ledipasvir was approved in October of 2014 (see later).[190] Fixed-dose combinations of SOF were approved as well with another NS5A inhibitor, VEL (approved June 2016) and with both NS5A inhibitor VEL and the protease inhibitor VOX (approved July 2017). The two-pill combination of SOF with the protease inhibitor simeprevir occupies a small niche in DAA guideline (see earlier), as does the two-pill combination of SOF with the NS5A inhibitor daclatasvir approved in July 2015 (see later) (www.hcvguidelines.org).[113] These DAAs and the clinical trials demonstrating their efficacy are described later.

Ledipasvir/Sofosbuvir (Harvoni)

Spectrum and Mechanism of Action

Ledipasvir/SOF is a fixed-dose combination tablet containing 90 mg of ledipasvir and 400 mg of SOF, approved by the FDA in October 2014. Ledipasvir is an NS5A inhibitor, and SOF is an NS5B polymerase inhibitor, reviewed earlier. The EC_{50} of ledipasvir against full-length replicons from genotypes 1a and 1b is 0.031 nM and 0.004 nM, respectively. It has somewhat less activity against genotypes 4, 5, and 6 and substantially lower activity against genotypes 2 and 3.[191]

Resistance

Reduced susceptibility to ledipasvir was seen with NS5A amino-acid substitution at Y93H/N, Q30E, and L31M in genotype 1a, and at Y93H in genotype 1b. Approximately 20% of genotype 1 viruses have polymorphisms associated with decreased susceptibility to ledipasvir; however, these variants do not appear to reduce the efficacy in treatment-naïve patients but may do so in treatment-experienced patients.[191] Ledipasvir is active against SOF-resistant viruses with NS5B substitutions, but resistance to ledipasvir may confer resistance to other NS5A inhibitors. In patients who had failed prior DAA treatment, including those with ledipasvir RASs, clinical trials demonstrated that increasing the duration of ledipasvir/SOF (e.g., from 8–12 weeks to 24 weeks) and/or adding RBV was highly effective in achieving an SVR_{12}. Such addition of RBV and the extension of treatment to 24 weeks are rarely needed now that an even more potent NS5A inhibitor, VEL, has been combined with SOF (see later).

Pharmacology/Pharmacokinetics

The combination of ledipasvir and SOF is administered once daily with or without food. The median terminal half-life of ledipasvir is 47 hours. Ledipasvir is greater than 99.8% bound to human plasma proteins, and biliary excretion is the major route of elimination. No adjustment of dose is needed for mild or moderate renal impairment, but SOF and its metabolite can accumulate in severe renal impairment, and SOF-containing DAA combinations are not recommended for patients with CrCl of <30 mL/min. No dose reduction is needed for moderate (Child-Pugh class B) or severe (Child-Pugh class C) liver dysfunction.[191]

Drug Interaction

Compared with protease inhibitors, ledipasvir and SOF have few clinically important drug-drug interactions. Ledipasvir and SOF are both substrates for P-gp; therefore P-gp inducers can reduce ledipasvir and SOF plasma concentrations. These drugs include rifampin, St. John's wort, phenytoin, and phenobarbital. Neither ledipasvir nor SOF is a substrate for hepatic or renal transporters. Ledipasvir is also an inhibitor of P-gp, may increase levels of tenofovir, and should not be used with the combination of elvitegravir, cobicistat, and emtricitabine.

Toxicity

Ledipasvir/SOF is generally well tolerated, with discontinuation of medication of 0%, less than 1%, and 1% for subjects receiving the combination for 8, 12, and 24 weeks, respectively. The most frequent adverse effects were fatigue, headache, nausea, diarrhea, and insomnia and were mostly of grade 1 severity.[191] As with all SOF-containing DAAs, ledipasvir/SOF should not be administered to patients taking amiodarone, in whom SOF has been associated with severe bradycardia. PPIs such as omeprazole raise gastric pH, which reduces SOF solubility; if a PPI is to be used, both the PPI (equivalent to ≤20 mg of omeprazole) and SOF should be taken together under fasted conditions.[186]

Clinical Studies

Clinical trials of ledipasvir/SOF have resulted in high SVR_{12} rates (91%–100%) in both treatment-naïve ("ION-2" and "ION-3") and treatment-experienced ("ION-2") patients with HCV genotype 1.[190,192–194] The duration of therapy is 12 weeks for treatment-naïve patients and for treatment-experienced patients without cirrhosis; 8 weeks of therapy may be sufficient for treatment-naïve non-Black patients without cirrhosis (and without HIV coinfection) who have HCV RNA levels of $<6 \times 10^6$ IU/mL. For treatment-experienced patients with cirrhosis who were treated with ledipasvir/SOF, clinical trials showed that 24 weeks of therapy was required; however, ledipasvir/SOF has been supplanted by other SOF-containing DAA combinations (SOF/VEL and SOF/VEL/VOX), as well as combinations of SOF-free DAAs (see later) that require only 12 weeks in these patients (treatment-experienced cirrhotic patients). Studies with and without RBV indicate that addition of RBV did not improve efficacy of ledipasvir/SOF. Moreover, in patients with genotypes 1 and 4 this combination DAA has been shown to be effective and is now indicated for patients with decompensated cirrhosis. In one such study in the United States ("SOLAR-1"), 337 patients received 12 to 14 weeks (which turned out to be indistinguishable) of ledipasvir/SOF plus RBV. In the cohort of patients who had not undergone liver transplantation, SVR_{12} was achieved in 86% to 89% in decompensated cirrhotic patients. In the cohort of patients who had undergone liver transplantation, SVR_{12} was achieved in 96% to 98% without cirrhosis or with compensated cirrhosis, in 85% to 88% with Child-Pugh class B, in 60% to 75% with Child-Pugh class C, and in six out of six with fibrosing cholestatic hepatitis.[195] Additional discussion of clinical studies can be found in Chapter 117.[113,159]

Paritaprevir/Ritonavir/Ombitasvir Plus Dasabuvir (Viekira Pak)

Spectrum and Mechanism of Action

Paritaprevir is an inhibitor of the HCV NS3/4A protease, with EC_{50} activities against genotypes 1a and 1b of 1.0 nM and 0.21 nM, respectively. Good EC_{50} activities were also noted against genotypes 4a and 6a (0.09 nM and 0.68 nM, respectively).[196] Paritaprevir was less active against genotype 2A (EC_{50} 5.3 nM) and 3a (EC_{50} 19 nM). Ritonavir is an HIV-1 protease

inhibitor and a CYP3A inhibitor that boosts levels of paritaprevir pharmacokinetically but has no direct activity against HCV. (See Chapter 128 for further discussion of ritonavir.)

Ombitasvir is an inhibitor of HCV NS5A and has EC_{50} activities against replicons of genotypes 1a and 1b of 0.68 pM and 0.94 pM, respectively. EC_{50}s were 1.7 to 19 pM against genotypes 2a, 2b, 3a, 4a, and 5a. The EC_{50} against genotype 6a was 366 pM.[196]

Dasabuvir, a nonnucleoside inhibitor of NS5B RNA-dependent RNA polymerase, targets the palm domain of NS5B and acts as an allosteric inhibitor. The EC_{50} values of dasabuvir against replicons of genotypes 1a and 1b are 0.6 nM and 0.3 nM, respectively. Dasabuvir has reduced activity in biochemical assays against NS5B polymerase from genotypes 2a, 2b, 3a, and 4a (IC_{50} values range from 900 nM– >20 μM).[196]

Because later-generation DAAs consist of fewer individual drugs, have broader genotype specificity, and require once-daily RBV-free dosing, this combination DAA is no longer recommended as a *first-line* treatment for hepatitis C; however, ritonavir/paritaprevir-ombitasvir-dasabuvir remains included among the recommended *alternative* regimens (www.hcvguidelines.org).[113] Still, by and large, the current use of this DAA combination is very limited.

Resistance

Resistance can be generated by exposure of genotype1a and 1b replicons to paritaprevir, ombitasvir, or dasabuvir. In clinical trials of HCV 1a resistance emerged with amino-acid substitutions in NS3 (D168V/any), NS5A (M28A/T/V and Q30E/K/R), and NS5B (S556G/R). Few virologic failures were noted in genotype 1b infections. Resistance-associated substitutions were slightly more frequent among patients who did not achieve SVR, but the presence of such polymorphisms was not anticipated to have a clinically significant effect on treatment responses.[196]

Pharmacology/Pharmacokinetics

Paritaprevir/ritonavir/ombitasvir are formulated as a fixed-combination tablet consisting of 75/50/12.5 mg of each component, respectively. Two tablets are given once daily in the morning (daily dose of 150, 100, and 25 mg, respectively). Dasabuvir tablets (250 mg) are given twice daily (morning and evening) for a total daily dose of 500 mg. In July 2016 an extended-release version (Viekira XR) was approved, consisting of three individual tablets (ritonavir [33.33 mg]-boosted paritaprevir [50 mg], ombitasvir [8.33 mg], and long-acting dasabuvir [200 mg])—three tablets of each taken once daily (daily doses ritonavir 100 mg, paritaprevir 150 mg, ombitasvir 25 mg, dasabuvir 600 mg), with or without twice-daily RBV. Except for noncirrhotic patients with genotype 1b, all others must take RBV in divided doses (twice daily) along with paritaprevir/ritonavir/ombitasvir plus dasabuvir. The regimen is given with meals.

The components of the regimen are highly plasma protein bound. Paritaprevir is metabolized predominantly by CYP3A4 and to a lesser extent by CYP3A5; ritonavir is metabolized predominantly by CYP3A and to a lesser extent by CYP2D6; ombitasvir is metabolized predominantly by amide hydrolysis, followed by oxidative metabolism; and dasabuvir is metabolized predominantly by CYP2C8 and to a lesser extent by CYP3A. The mean plasma half-lives of paritaprevir, ritonavir, and dasabuvir are 5.5, 4, and 5.5 to 6 hours, respectively. The ombitasvir mean elimination half-life was 21 to 25 hours. Nonrenal mechanisms account for the elimination of greater than 85% of the administered drugs. Therefore renal insufficiency does not affect levels of this regimen significantly. If RBV is needed, this DAA cannot be used in renal insufficiency; if RBV is not needed (e.g., treatment-naïve noncirrhotic patients with genotype 1b), this DAA combination can be administered in patients with mild, moderate, or severe renal insufficiency. Dose adjustment is not required for mild hepatic impairment (Child-Pugh class A), but use of this combination therapy is not recommended in moderate hepatic impairment (Child-Pugh class B) and contraindicated in severe (Child-Pugh class C) hepatic impairment.[196]

Interactions

As noted earlier, the components of this treatment regimen are substrates and inhibitors of important metabolic enzymes; therefore the potential for drug-drug interactions is considerable. Individual drugs that may increase levels of the treatment regimen or, alternatively, drugs whose levels may be affected by the treatment regimen are tabulated in the most recent product brochures, to which the reader is referred. Such drugs include rifampin, St. John's wort, anticonvulsants, estradiol-containing products, and salmeterol. Potential elevation of immunosuppressants (cyclosporine, tacrolimus), antiarrhythmics, and statins may occur.[197]

Toxicity

The above regimen has been generally well tolerated. Mild adverse events were common but led infrequently to discontinuation of therapy (generally <1%). Nausea, pruritus, insomnia, and asthenia were most common. In trials in which the treatment regimen included RBV, some of the side effects may have been related to RBV. Hyperbilirubinemia (primarily unconjugated) and elevations of aminotransferases were seen in approximately 1% of subjects. These usually occurred early and resolved shortly thereafter while patients remained on therapy. Mean decreases in hemoglobin of 2.4g/dL, which were noted in the phase III studies when RBV was added to the regimens, generally returned to pretreatment levels after treatment had ended.[196]

In October 2015, the FDA issued a warning to be added to prescribing information for Viekira Pak—that hepatic decompensation and failure have been reported with this medication.[198] Patients who received the medication should be monitored closely for hepatic decompensation, and, if noted, administration of Viekira Pak should be stopped. Like other DAA combinations including a protease inhibitor, Viekira Pak should not be used in patients with decompensated cirrhosis (Child-Pugh class B or C).

Clinical Studies

Clinical trials demonstrated that the regimen is highly effective in treatment-naïve patients, both with and without cirrhosis as well as in those who have failed therapy with PEG IFN and RBV.[197,199–202] High rates of SVRs have been noted in clinical studies, ranging from 93% to 100%, with the highest efficacy seen in noncirrhotic subjects with genotype 1b infections. When approved, the regimen was recommended for treatment of genotypes 1a, 1b, and 4. For genotype 1a, this drug combination was given with weight-based RBV for 12 weeks (no cirrhosis) or 24 weeks (cirrhosis). For genotype 1b, this treatment was given for 12 weeks, without RBV (no cirrhosis) or with RBV (cirrhosis). For genotype 4, the regimen was given for 12 weeks with RBV but without dasabuvir. Clinical studies are discussed further in Chapter 117.

Paritaprevir/Ritonavir/Ombitasvir (Technivie)

Technivie is an oral fixed-dose medication that consists of three of the four components of Viekira Pak. It does not include dasabuvir, which does not have activity against genotype 4. The regimen is two tablets taken daily with or without food, for total daily doses of paritaprevir 150 mg/ritonavir 100 mg and ombitasvir 25 mg. In July 2015 Technivie plus RBV received FDA approval for treatment of HCV genotype 4 in patients without cirrhosis but was also recommended at the time in AASLD/IDSA guidelines for patients with compensated cirrhosis (Child-Pugh class A) (see Table 47.1). In October 2015, the FDA issued the same warning letter that had been issued for Viekira Pak,[198] namely, that hepatic decompensation and failure have been reported with Technivie. Patients receiving the medication should be followed closely for hepatic decompensation, and, if noted, the drug should be stopped. Technivie should not be used in patients with decompensated cirrhosis (Child-Pugh class B or C).

As with Viekira Pak, administration of Technivie has been generally well tolerated, and the most common side effects consist of fatigue, weakness, insomnia, and pruritus, most often in the RBV-containing regimen.[203] Discussion of resistance, pharmacology, and drug interactions is presented in the section on Viekira Pak, earlier.

Technivie administered for 12 weeks with or without RBV was shown to be highly effective against HCV genotype 4. In a clinical trial involving 135 patients without cirrhosis,[204,205] SVR_{12} was achieved in 91% (40/44) of treatment-naïve patients without RBV and in 100% (42/42) with RBV. In treatment-experienced patients, SVR_{12} occurred in 100% (49/49) treated with the RBV-containing regimen. As noted

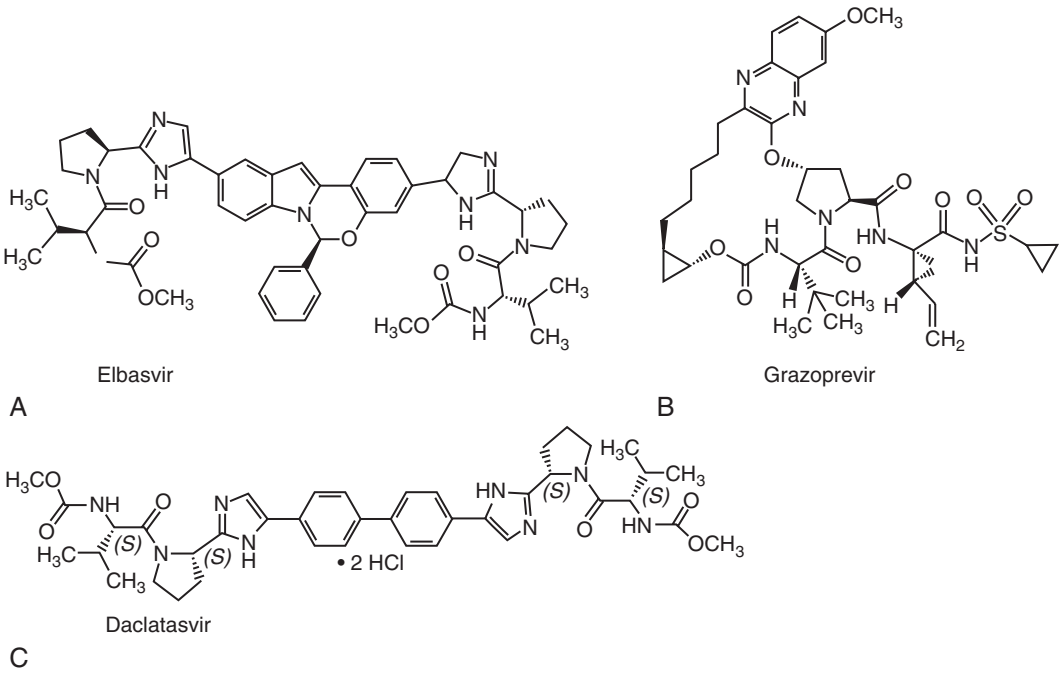

FIG. 47.4 **Chemical structures of elbasvir (A), grazoprevir (B), and daclatasvir (C).**

earlier for Viekira Pak, although retained as an alternative regimen, Technivie is no longer included in AASLD/IDSA guidelines as *first-line* therapy (www.hcvguidelines.org).[113]

Elbasvir/Grazoprevir (Zepatier)

Spectrum and Mechanism of Action

Elbasvir (50 mg)/grazoprevir (100 mg) is an oral fixed-dose combination that was approved in January 2016 for treatment of HCV infections in patients with genotypes 1a, 1b, and 4 (see Table 47.1). Compared with earlier-generation DAAs, it is highly potent and active against some of the major resistance-associated substitutions of earlier DAAs. The standard dosage regimen is one tablet once daily with or without food for 12 weeks. Because of the availability of later-generation DAAs, in patients with genotype 1a infection who have baseline NS5A RASs for elbasvir, elbasvir/grazoprevir is not recommended.

Elbasvir is a DAA inhibitor of the HCV NS5A replication complex (Fig. 47.4A). In HCV full-length replicon assays, it has activity against genotypes 1a, 1b, and 4, with EC_{50} values of 4 pM, 3 pM, and 0.3 pM, respectively.[206]

Grazoprevir is a DAA inhibitor of HCV protease NS3/4A (see Fig. 47.4B). In HCV full-length replicon assays, grazoprevir has EC_{50} values against genotypes 1a, 1b, and 4 of 0.4 mM, 0.5 mM, and 0.3 mM, respectively.[206]

Thus elbasvir and grazoprevir target different mechanisms of action of viral replication. Combined effects of elbasvir and grazoprevir did not show antagonism either by themselves or with the addition of RBV.

Resistance

Resistant polymorphisms against NS5A are present at positions M28, Q30, L31, and Y93 and result in reduced efficacies of 12-week courses of treatment.[207] Before therapy with this DAA, patients with HCV genotype 1a infection should undergo RNA NS5A resistance testing to determine if such polymorphisms are present. If so, an alternative DAA regimen should be selected.

Resistant NS3 polymorphisms in genotypes 1, 1a, and 4, are most commonly at positions A156T and D168A.

Pharmacology/Pharmacokinetics

After oral administration, peak concentrations occur at 3 hours for elbasvir and at 2 hours for grazoprevir.[206] Both elbasvir and grazoprevir are extensively bound to plasma proteins (≥99%). The half-lives are 24 hours for elbasvir and 31 hours for grazoprevir. Excretion is primarily through feces (>90%), and less than 1% is recovered in urine. No dosage adjustment is required for patients with renal insufficiency or for patients with mild hepatic impairment (Child-Pugh class A). Like other DAAs that include a protease inhibitor, elbasvir/grazoprevir should not be used in patients with moderate or severe hepatic impairment (Child-Pugh class B or C) because of potential ALT elevations and increased grazoprevir plasma concentrations; however, this DAA is a preferred regimen for patients with advanced renal insufficiency (CrCl <30 mL/min); no dosage adjustment is necessary for patients with any level of chronic kidney disease, including those undergoing hemodialysis.[206]

Drug Interactions

Elbasvir and grazoprevir are substrates of CYP3A and P-gp, although the role of intestinal P-gp in absorption of elbasvir and grazoprevir appears to be minimal.[206] Coadministration of moderate and strong CYP3A inducers, which may reduce the plasma concentration of elbasvir and grazoprevir, is not recommended. Coadministration of CYP3A inhibitors, which may increase the plasma concentrations of elbasvir and grazoprevir, is also not recommended.

Grazoprevir is a substrate of OATP1B1 and B3, and coadministration of drugs that inhibit these transporters may increase grazoprevir plasma concentrations. Consult the full prescribing information for elbasvir/grazoprevir for further discussion of drug-drug interactions.[206]

Toxicity

Elbasvir/grazoprevir is generally well tolerated. In pooled data from phase II and III clinical trials, the most common adverse events were fatigue (11%), headache (10%), and nausea (5%), similar to rates in placebo recipients.[206–208] Increased ALT levels occurred in 1% of subjects, usually at or after 8 weeks of therapy, and mostly resolved at or after completion of therapy. Anemia has been observed when RBV was included in therapy.

Clinical Studies

(Also see Chapter 117.)

Elbasvir/grazoprevir has been shown to be highly effective in treatment of genotypes 1a, 1b, 4, and 6 infection in both treatment-naïve and treatment-experienced patients with or without cirrhosis. A regimen of one tablet taken daily resulted in SVR_{12} in 92% to 100% of treatment-naïve patients treated for 12 weeks ("C-EDGE-TN" [treatment-naïve])[209] and in 92% to 98% of treatment-experienced patients who received elbasvir/grazoprevir for 12 to 16 weeks with or without RBV ("C-EDGE-TE"

[treatment-experienced]).[210] Patients coinfected with HIV and HCV also had high SVR_{12} responses, 96% ("C-EDGE COINFECTION"),[211] as did patients with renal impairment ("C-SURFER").[212]

Daclatasvir (Daklinza)

Spectrum and Mechanism of Action

Daclatasvir (see Fig. 47.4C) is an orally administered inhibitor of HCV NS5A that is used at a daily dose of 60 mg in combination with 400 mg of SOF. It was approved in July 2015 for treatment of genotype 3 infection, and its approval was expanded in February 2016 to include treatment of genotype 1 infection. The AASLD/IDSA September 2017 guidelines no longer include daclatasvir/SOF as a *first-line* DAA, with rare exceptions (12-week course, except as noted): for (1) patients with decompensated cirrhosis, genotypes 1 to 4, with or without RBV (for 24 or 12 weeks, respectively), and (2) after liver transplantation along with RBV in treatment-naïve or treatment-experienced patients with genotypes 2 and 3, with or without cirrhosis, including decompensated cirrhosis. The September 2017 guidelines do retain daclatasvir/SOF as an *alternative* regimen in (1) treatment-naïve noncirrhotic patients with genotypes 1a and 1b, genotype 2 without or with compensated cirrhosis, and genotype 3 without or with (+RBV) cirrhosis; (2) PEG IFN/RBV-experienced noncirrhotic patients with genotypes 1a, 1b, and 3, as well as in both noncirrhotic and cirrhotic (extended to 16–24 weeks) patients with genotype 2; (3) after liver transplantation in treatment-naïve or treatment-experienced patients with genotypes 1, 4, 5, and 6 (along with RBV) with or without cirrhosis; and (4) after renal transplantation in treatment-naïve or treatment-experienced patients with genotypes 2, 3, 5, and 6 (along with RBV). In general, however, other highly effective DAA regimens are available for all these indications, relegating daclatasvir/SOF to a marginal role in the treatment of hepatitis C (www.hcvguidelines.org).

Daclatasvir inhibits the HCV phosphoprotein NS5A by binding to the N-terminus of domain 1 of the protein, therefore inhibiting both viral RNA replication and virion assembly.[213,214] In hybrid replicons containing NS5A sequences from genotypes 1a, 1b, and 3, median EC_{50} values of 0.008 mM, 0.002 mM, and 0.02 mM, respectively, were found.

Resistance

In genotype 1a–infected subjects with cirrhosis, the presence of an NS5A amino-acid polymorphism at positions M28, Q30, L31, or Y93 was associated with reduced efficacy.[213] Polymorphisms in NS5A were also noted in genotype 1b infections, but SVR_{12} was achieved in all treated with daclatasvir and SOF, with or without RBV. In genotype 3 infections, NS5A polymorphisms appeared to be associated with reduced efficacy of treatment.

Pharmacology/Pharmacokinetics

After one administration of daclatasvir, peak plasma concentration occurs within 2 hours. Administration of food does not have a major effect on absorption.[213] Protein binding of daclatasvir is high (99%) and independent of dose. Elimination of daclatasvir is mostly fecal (88%), and 6.6% is detected in urine. Dosage does not need to be adjusted for renal impairment, and, because of the high plasma protein binding, daclatasvir is unlikely to be removed by hemodialysis. Daclatasvir is recommended only when combined with SOF, however. Thus, because SOF has not been shown to be effective in patients with CrCl <30 mL/min (chronic kidney disease stages 4 and 5), the combination of daclatasvir and SOF is not indicated for patients with advanced renal insufficiency. No dose adjustment is recommended for patients with hepatic impairment; SOF with daclatasvir is one of the recommended first-line DAA regimens for patients with decompensated cirrhosis and genotypes 1 to 4 (www.hcvguidelines.org).

Drug Interactions

Daclatasvir is a substrate of CYP3A, and levels will be reduced when administered with moderate inhibitors of CYP3A. In that situation the dosage of daclatasvir should be increased to 90 mg/day. The use of daclatasvir with strong inducers of CYP3A should be avoided. If a strong inhibitor of CYP3A is given, the dose of daclatasvir should be reduced to 30 mg/day. Daclatasvir is a moderate inhibitor of OATP1B1, OATPB3, and BCRP and thus has the possibility to increase levels of drugs that are substrates for those transporters.

Toxicity

Daclatasvir has been generally well tolerated in reported studies. When administered along with SOF, the most commonly reported adverse events were fatigue (14%), headache (14%), nausea (8%), and diarrhea (5%). Dacalatasvir plus SOF, like any drug combination that includes SOF, may cause serious bradycardia when administered with amiodarone, particularly if a β-blocker is being administered.[215] Therefore concomitant administration of those drugs should be avoided. Because SOF has not been shown to be effective in patients with CrCl <30 mL/min (chronic kidney disease stages 4and 5), the combination of daclatasvir and SOF is not indicated for patients with advanced renal insufficiency. PPIs such as omeprazole raise gastric pH, which reduces SOF solubility; if a PPI is to be used, both the PPI (equivalent to ≤20 mg of omeprazole) and SOF should be taken together under fasted conditions.[186]

Clinical Studies

In a phase II study, among 211 treatment-naïve or treatment-experienced (with first-generation protease inhibitors plus PEG IFN/RBV) noncirrhotic patients treated with SOF plus daclatasvir with or without RBV for 12 to 24 weeks, the SVR_{12} was 98% for genotype 1; 92% for genotype 2; and 89% for genotype 3.[216] Three open-label phase III clinical trials of daclatasvir plus SOF ("ALLY-1," "ALLY-2," and "ALLY-3") showed high rates of SVR_{12} in patients with genotypes 1a, 1b, 2, 3, and 4 HCV infections treated for 12 weeks. In 101 treatment-naïve and 51 treatment-experienced patients with genotype 3 treated with the combination for 12 weeks, SVR_{12} occurred in 90% and 86%, respectively. In these patients with genotype 3, noncirrhotic patients responded substantially more frequently to this regimen than did cirrhotic patients (SVR_{12}, 96% vs. 63%) (ALLY-3).[217]

In patients with cirrhosis with Child-Pugh class A (compensated), B (moderately decompensated), or C (severely decompensated), treatment with daclatasvir plus SOF and RBV for 12 weeks resulted in an SVR_{12} ranging from a high of 93% in Child-Pugh class A and B, down to 56% in Child-Pugh class C (ALLY-1).[218] In this study, rates of SVR_{12} in cirrhotic patients were 82% in genotype 1, 80% in genotype 2, 83% in genotype 3, and 100% in genotype 4. Patients coinfected with HCV and HIV also responded well to daclatasvir and SOF for 12 weeks; across genotypes 1 to 4 the SVR_{12} was 97% in treatment-naïve and 98% in previously treated patients (ALLY-2).[219]

Velpatasvir/Sofosbuvir (Epclusa)

Spectrum and Mechanism of Action

The combination of the pangenotypic, high-barrier-to-resistance, picomolar-EC_{50} NS5A inhibitor VEL (Fig. 47.5A) with the NS5B polymerase inhibitor SOF was shown in clinical trials to be highly effective in patients with genotypes 1 to 6, including in difficult-to-treat patients with genotype 3 (e.g., prior null responders with cirrhosis) and patients who have genotypes 1 to 4 and 6 with decompensated (Childs-Pugh class B) cirrhosis.[220–222] VEL/SOF, a fixed-dose combination tablet containing 100 mg of VEL and 400 mg of SOF taken daily for 12 weeks, was approved in June 2016 for patients infected with HCV genotypes 1 to 6 and in August 2017 for patients with HIV-HCV coinfection. SOF is reviewed earlier.

The mean EC_{50} of VEL against full-length replicons from genotypes 1a and 1b is 0.014 nM and 0.016 nM, respectively. For most other genotypes, the EC_{50} of VEL is lower, accounting for its highly effective, broad pangenotypic activity: genotype 2, 0.002 to 0.016 nM; genotype 3, 0.004 nM; genotype 4, 0.004 to 0.009 nM; genotype 5, 0.021 to 0.054 nM; and genotype 6a, 0.006 to 0.009 nM.[223] The IC_{50} of VEL in biochemical assays ranges from 0.36 to 3.3 μM.[223]

Resistance

Reduced susceptibility to VEL in clinical trials was rare but was seen with NS5A amino-acid substitution at Y93H/N, K24M/T, Q30E, and L31I/V in genotype 1 and Y93H, L314F/I/P in genotype 3 (none in genotype 2).[223] In clinical trials baseline RASs for genotypes 1 to 6 ranged from a low of 9% in genotype 5 to a high of 83% in genotype

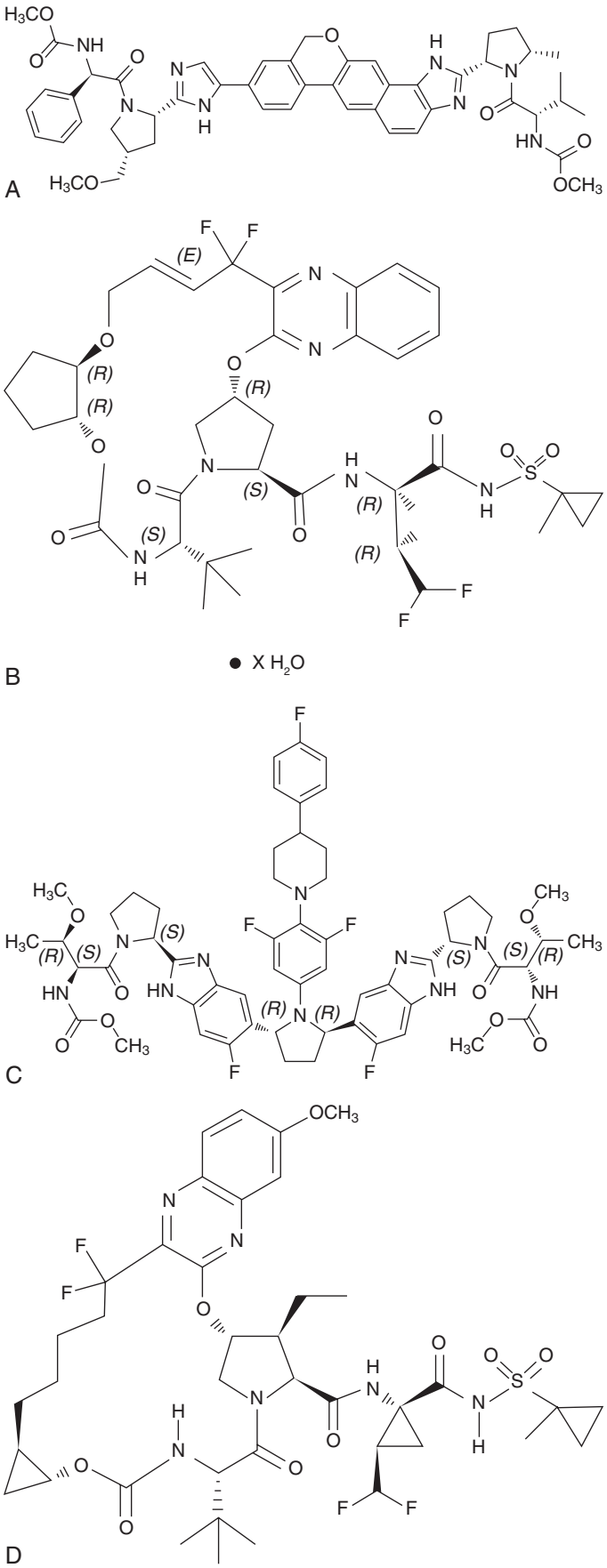

FIG. 47.5 **Chemical structures of velpatasvir (A), glecaprevir (B), pibrentasvir (C), and voxilaprevir (D).**

6 (18%–32% genotype 1, 64% genotype 2, 20% genotype 3). In trials of subjects with or without cirrhosis, relapses occurred in 1 of 75 with genotype 1 and baseline RASs, 4 of 56 with genotype 3 and baseline RASs (20% of 15 with baseline Y93H), but none with genotypes 2 and 4 to 6 with baseline RASs.[223]

Pharmacology/Pharmacokinetics

The combination of VEL and SOF is administered once daily with or without food. The median $T_{1/2}$ of VEL is 15 hours. Mean C_{max} is 259 ng/mL. VEL is greater than 99.5% bound to human plasma proteins, and biliary excretion is the major route of elimination. Because VEL is excreted via the biliary route, severe renal impairment has no impact on VEL pharmacokinetics; however, VEL is administered with SOF, which is renally excreted. No adjustment of the VEL/SOF dose is needed for mild or moderate renal impairment, but SOF and its metabolite can accumulate in severe renal impairment, and SOF-containing DAA combinations are not recommended for patients with CrCl of <30 mL/min. No dose reduction is needed for moderate (Child-Pugh class B) or severe (Child-Pugh class C) liver dysfunction. VEL does not prolong the QTc interval.[223]

Drug Interactions

Compared with protease inhibitors, VEL and SOF have few clinically important drug-drug interactions. VEL is an inhibitor of drug transporter P-gp, BCRP, OATP1B1, OATP1B3, and OATP2B1; if VEL is taken with drugs that are substrates for these transporters, drug levels of these other drugs can be increased.[223] The effect of VEL may also be reduced by moderate to potent CYP inducers.[223] VEL drug-drug interactions occur with rifampin, St. John's wort, phenytoin, and phenobarbital, among others. Neither VEL nor SOF is a substrate for hepatic or renal transporters. Because VEL is an inhibitor of P-gp, coadministration of the two drugs together may increase levels of tenofovir; VEL should not be used with efavirenz. For all DAA treatment, before initiating therapy, checking for drug-drug interactions is advisable.[223]

Toxicity

In clinical trials VEL/SOF was well tolerated; the most common adverse events were headache, fatigue, nausea, asthenia, and insomnia—all but asthenia occurring in a comparable proportion of placebo recipients, and only headache and fatigue occurred in >10% ("ASTRAL-1").[220] The frequency of these adverse events was higher in patients with decompensated cirrhosis ("ASTRAL-4").[222] As with all SOF-containing DAAs, VEL/SOF should not be administered to patients taking amiodarone, in whom SOF has been associated with severe bradycardia, especially in patients also taking β-blockers (see earlier). PPIs such as omeprazole raise gastric pH, which reduces SOF solubility; if a PPI is to be used, both the PPI (equivalent to ≤20 mg of omeprazole) and SOF should be taken together under fasted conditions.[186] Like other SOF-containing DAA combinations, SOF/VEL is not recommended in patients with CrCls <30 mL/min.[186,223] Data on safety of VEL during pregnancy are not available.

Clinical Studies

(See also Chapter 117.)

Efficacy of this pangenotypic combination was demonstrated in a series of trials: ASTRAL 1 to ASTRAL 4[220–222] and ASTRAL-5.[224] In 626 patients (including treatment-naïve, treatment-experienced, noncirrhotic patients and compensated cirrhotic patients) with genotypes 1, 2, 4, 5, and 6, treatment with SOF/VEL achieved SVR_{12} in 97% to 100% (ASTRAL-1). In ASTRAL-1, among patients receiving SOF/VEL, treatment was well tolerated; adverse events occurred in indistinguishable percentages of SOF/VEL recipients (78%) and 116 placebo recipients (77%).[220] In 134 patients with genotypes 2 (ASTRAL-2) and 277 patients with genotype 3 (ASTRAL-3), 26% to 29% of whom had failed prior antiviral treatment, 29% to 30% of whom had compensated cirrhosis, SOF/VEL for 12 weeks achieved SVR_{12} rates of 99% and 95%, respectively, superior to outcomes in control groups receiving the previous standard-of-care, SOF with weight-based RBV for 12 weeks in genotype 2 (n = 132, SVR_{12} 94%) and 24 weeks in genotype 3 (n = 275, SVR_{12} 80%).[221] Moreover, SOF/VEL was highly effective and superior to SOF/RBV in

the most refractory patient subsets, those with genotype 3 who had cirrhosis and/or had failed prior IFN-based therapy. Among treatment-naïve subjects, the rate of SVR_{12} for SOF/VEL versus SOF/RBV was 98% versus 90% in noncirrhotic patients and 93% versus 74% in compensated cirrhotic patients. The differences were even more pronounced in treatment-experienced subjects: 91% versus 71% in noncirrhotic patients and 89% versus 58% in compensated cirrhotic patients.[221]

In 267 patients with Child-Pugh class B decompensated cirrhosis (78% genotype 1, 4% genotype 2, 15% genotype 3, 3% genotype 4, <1% genotype 6; 55% treatment-experienced [44% with PEG IFN/RBV, 11% with a protease inhibitor regimen], 75%–83% with ascites), SOF/VEL achieved SVR_{12} in 83% treated for 12 weeks and in 86% treated for 24 weeks; a third group treated with SOF/VEL/RBV for 12 weeks had a slightly higher SVR_{12} of 94%. Efficacy was comparable across genotypes, except for patients with genotype 3, in whom the SVR_{12} rate for the 12-week and 24-week SOF/VEL regimens were both only 50% but 85% in the 12-week SOF/VEL/RBV regimen.[222] Baseline NS5A RASs did not affect responsiveness, except in cirrhotic patients with baseline Y93H, who had a slightly reduced SVR_{12} of 84% and in whom the addition of RBV has been recommended. In a study of SOF/VEL in 106 patients with HIV-HCV coinfection and genotypes 1 to 4, an SVR_{12} was reported in 95%—i.e., 95% in genotype 1, 100% (11/11) in genotype 2, 92% in genotype 3, and 100% (4/4) with genotype 4.[224]

Based on these data, SOF/VEL for 12 weeks is recommended as initial treatment for all genotypes, for noncirrhotic patients and compensated cirrhotic patients, and for re-treatment in subgroups of treatment-experienced patients with genotype 1 and 2; for re-treatment of patients with genotypes 3 to 6, the more effective triple combination of SOF/VEL/VOX (see later) is recommended (see Table 117.9) (www.hcvguidelines.org).

Glecaprevir/Pibrentasvir (Mavyret)

Spectrum and Mechanism of Action

The single-pill combination of two pangenotypic, high-potency, high-barrier-to-resistance DAAs, the protease inhibitor glecaprevir (GLE, 300 mg, see Fig. 47.5B) and the NS5A inhibitor pibrentasvir (PIB, 120 mg, see Fig. 47.5C), is highly effective across all genotypes. Each pill contains a third of the daily dose of both drugs; therefore daily treatment consists of three pills, which should be taken with food. In treatment-naïve noncirrhotic patients an 8-week course suffices, whereas for treatment-naïve cirrhotic patients and all treatment-experienced patients (noncirrhotic or cirrhotic) a 12-week course is required (www.hcvguidelines.org). This combination DAA was approved for adults 18 years or older in August 2017 and for children ages 12 to 17 in April 2019.[225] This DAA is a *first-line* treatment for hepatitis C in treatment-naïve, treatment-experienced, noncirrhotic, and cirrhotic patients, including patients with severe renal impairment (chronic kidney disease, stages 4 and 5, CrCl <30 mL/min) (www.hcvguidelines.org).[225]

The median EC_{50} of GLE against replicons from genotypes 1 to 6 is 0.08 to 4.6 nM; the median EC_{50} of PIB against replicons from genotypes 1 to 0.5 to 4.3 nM.[225] The IC_{50} of GLE in biochemical assays ranges from 3.5 to 11.3 μM (the IC_{50} of PIB is not provided by the manufacturer).[225]

Resistance

Reduced susceptibility to GLE/PIB and treatment failure in clinical trials were rare across all HCV genotypes. In clinical trials patients with virologic failure who were treatment-naïve or PEG IFN/RBV-experienced and/or SOF-experienced, treatment-emergent NS3 RASs included A156V, and treatment-emergent NS5A RASs included Q30R, L31M, and H58D in two patients with genotype 1a; no RASs in two patients with genotype 2; and NS3 RASs Y56H/N, Q80K/R, A156G, or Q168L/R and NS5A RASs M28G, A30G/K, L31F, P58T, or Y93H in 18 patients with genotype 3.[225] In prior nonresponders to protease (NS3/4A) inhibitors and/or NS5A inhibitors, treatment-emergent RASs in 11 GLE-/PIB-treated clinical trial subjects who failed this DAA therapy included NS3 RASs V36A/M, Y56H, R155K/T, A156G/T/V, or D168A/T and NS5A RASs M28A/G (or L28M for genotype 1b), P29Q/R, Q30K/R, H58D or Y93H/N.[225]

In clinical trials, baseline NS3 RASs were found in 1% of subjects with genotypes 1, 2, and 4; 2% with genotype 3; and 42% with genotype 3. Baseline NS5A RASs were found, in ascending order, in 13% of subjects with genotype 5, 22% with genotype 3, 27% with genotype 1, 50% with genotype 4, 54% with genotype 6, and 80% with genotype 2.[225] For patients with genotypes other than 3, baseline RASs had no influence on treatment efficacy; however, for treatment-naïve, noncirrhotic patients with genotype 3, the NS5A RAS A30K, present in 10%, was associated with a reduced SVR_{12} of 78%. In contrast, SVR_{12} occurred in all patients with genotype 3 harboring baseline Y93H RASs.[225]

Pharmacology/Pharmacokinetics

The combination of GLE and PIB is administered once daily with food. The median $T_{½}$ of GLE is 6 hours and of PIB is 13 hours. The geometric mean C_{max} is 597 ng/mL for GLE and 110 ng/mL for PIB. Percent bound to human plasma protein is 97.5% for GLE and >99.9% for PIB; biliary-fecal excretion is the major route of elimination for both DAA components. Because GLE/PIB is excreted via the biliary route, severe renal impairment has no impact on pharmacokinetics of this DAA. No adjustment of the GLE/PIB dose is needed for mild, moderate, or severe renal impairment, including patients with a CrCl of <30 mL/min. This protease inhibitor–containing DAA is not recommended in patients with moderately decompensated cirrhosis (Child-Pugh class B) and is contraindicated in patients with severely decompensated cirrhosis (Child-Pugh class C). GLE/PIB does not prolong the QTc interval.[225]

Drug Interactions

Compared with first-generation protease inhibitors, GLE/PIB has relatively few clinically important drug-drug interactions. Both GLE and PIB are inhibitors of drug transporter P-gp, BCRP, OATP1B1, and OATP1B3; if GLE/PIB is taken with drugs that are substrates for these transporters, plasma concentrations of these other drugs can be increased, as can plasma concentrations of GLE/PIB. Plasma concentrations of GLE/PIB are reduced when the DAA is taken together with P-gp of CYP3A inducers.[225] GLE and PIB are weak CYP3A, CYP1A2, and UGT1A1 inhibitors.[225] GLE and PIB drug-drug interactions occur with rifampin, carbamazepine, efavirenz, atazanavir, and St. John's wort, among others. Neither drug in the DAA combination is a substrate for hepatic or renal transporters. For all DAA treatment, before initiating therapy, checking for drug-drug interactions is advisable.[225]

Toxicity

In clinical trials GLE/PIB was well tolerated; the most common adverse effects (mild in >80% of instances), encountered in >50% of treated subjects, were headache, fatigue, diarrhea, and nausea. Like other DAAs for hepatitis C, GLE/PIB has a black box warning about reactivation of HBV infection (some cases resulting in fulminant hepatitis, hepatic failure, and death) in patients with HBV and HCV coinfection in the absence of antiviral therapy for hepatitis B.[225] Like other DAAs that contain a protease inhibitor, which can cause elevations of ALT activity and that have been complicated by liver decompensation in patients with advanced disease, GLE/PIB should not be used in patients with decompensated cirrhosis.[225] Data on safety of GLE/PIB during pregnancy are not available.[225]

Clinical Studies

(See also Chapter 117.)

In phase II and phase III trials of >2300 treatment-naïve and treatment-experienced noncirrhotic patients, this DAA achieved SVR_{12} in 99.0% to 99.7% of patients with genotypes 1, 2, and 4 to 6 and 95% with genotype 3; across all genotypes in noncirrhotic patients, 8 weeks of therapy was equivalent in efficacy to 12 weeks (phase III trials "ENDURANCE"-1–4 and phase II trial "SURVEYOR-1" and "SURVEYOR-2").[226–229] In patients with compensated cirrhosis, both treatment-naïve and treatment-experienced (IFN/RBV-based or SOF/RBV ± PEG IFN), 12 weeks of GLE/PIB achieved SVR_{12} in 99% across genotypes 1, 2, and 4 to 6 (phase III "EXPEDITION-1") and in up to 98% of patients with treatment-naïve genotype 3 (phase II SURVEYOR-2). In DAA-treatment–experienced patients (approximately a third each with protease inhibitor experience only, NS5A inhibitor only, and both protease inhibitor and NS5A experience) with genotypes 1 and 4, with or without compensated cirrhosis, GLE/PIB achieved SVR_{12}

just as frequently after 12 weeks of treatment (89%) as after 16 weeks of treatment (91%) (phase II "MAGELLAN-1").[230] In this trial both the number of previous DAA treatments and the number of baseline RASs were associated with degradation of GLE/PIB treatment. For example, in the cohort treated for 12 weeks, the GLE/PIB-associated SVR_{12} fell from 100% for prior protease inhibitor only, to 88% for prior NS5A inhibitor only, to 79% for prior protease inhibitor and NS5A inhibitor; SVR_{12} rates were 100% for patients with no baseline RASs and baseline RASs limited to NS3 (protease inhibitor) only but 83% for patients with NS5A baseline RASs.[230]

In a phase III trial among 104 patients with stage 4 or 5 renal disease (CrCl of <30 mL/min), with or without cirrhosis, whether treatment-naïve or treatment-experienced, the SVR_{12} rate across all genotypes was 98%.[226]

Sofosbuvir/Velpatasvir/Voxilaprevir (Vosevi)

Spectrum and Mechanism of Action

First-line DAA regimens for chronic hepatitis C are so effective that "cure" rates approach 100%; however, a small subset of patients fail to achieve SVR_{12}—for instance, up to 6% for genotype 1 and up to 12% for genotype 3. This efficacy gap is addressed by one of the latest generation combination DAAs, approved in July 2017, a combination of three pangenotypic, high-potency, high-barrier-to-resistance DAAs—SOF/VEL/VOX, that is, with the NS5B polymerase inhibitor SOF (400 mg), the NS5A inhibitor VEL (100 mg), and the NS3/4A protease inhibitor VOX (100 mg; see Fig. 47.5D). The single-pill DAA combination is highly effective across all genotypes.[231,232] Treatment consists of one daily triple-drug–containing pill, which should be taken with food. A 12-week course of SOF/VEL/VOX is recommended in noncirrhotic and compensated cirrhotic patients with all HCV genotypes but not in patients with decompensated cirrhosis (Child-Pugh class B and C) or in patients with severe renal impairment (chronic kidney disease, stages 4 and 5, CrCl <30 mL/min) (www.hcvguidelines.org). In noncirrhotic and compensated cirrhotic patients with hepatitis C, SOF/VEL/VOX is a *first-line* treatment for (1) treatment-experienced patients with genotypes 1 to 6 and (2) DAA-treatment-naïve (not PEG IFN/RBV-naïve) patients with genotype 3 (www.hcvguidelines.org).[177] Among treatment-experienced patients, SOF/VEL/VOX is recommended for patients with failed prior protease inhibitor/PEG IFN/RBV treatment who have genotypes 3 to 6; for patients who failed prior SOF but not an NS5A and who have genotypes 1 and 3 to 6; and for patients who failed prior NS5A treatment and who have genotypes 1 to 6. In the latter treatment-experienced category (prior NS5A treatment), patients with *cirrhosis* should have RBV added to SOF/VEL/VOX (see Table 117.9) (www.hcvguidelines.org).[233]

The EC_{50} values for SOF and VEL are listed earlier; the median EC_{50} of VOX against full-length replicons from genotypes 1 to 6 is 0.2 to 6.6 nM.[233] The IC_{50} of VOX in biochemical assays ranges from 0.36 to 3.3 μM.[233]

Resistance

In the "POLARIS"-1 clinical trial,[231] SOF/VEL/VOX treatment failure occurred in 7 of 263 (3%) patients (all with cirrhosis) who had failed prior NS5A-containing/SOF-containing treatment. All 7 had treatment-emergent NS5A RASs when they failed SOF/VEL/VOX—L31M, Y93H, K24R; the 1 patient with NS5A RAS K24R also had NS3 RAS V36A RAS at the time of failure.[233] In the POLARIS-4 clinical trial,[231] among patients who had failed a non–NS5A-containing DAA regimen, only 1 patient relapsed and had a treatment-emergent NS5A RAS M28T but no NS3/4A or NS5B RASs. In these clinical trials (described later), neither the presence of baseline NS5A RASs nor the presence of baseline NS3 RASs influenced efficacy (SVR_{12} in 98% to 99% without and in 97% to 100% with baseline RASs).[231] Similarly, in these trials the number of baseline RASs (of any kind, NS3, and/or NS5A) had no influence on SVR_{12}, 97% with one RAS, 98% with two, and 100% with three or greater than four.[231]

Pharmacology/Pharmacokinetics

The combination of SOF, VEL, and VOX is administered once daily with food. Pharmacokinetic profiles of SOF and VEL appear above. The median $T_{1/2}$ of VOX is 33 hours. Mean C_{max} is 192 ng/mL. VOX is >99% bound to human plasma proteins, and biliary excretion is the major route of elimination. Because VOX is excreted via the biliary route, severe renal impairment has no impact on VOX pharmacokinetics; however, VOX is administered with SOF, which is renally excreted. No adjustment of the SOF/VEL/VOX dose is needed for mild or moderate renal impairment, but SOF and its metabolite can accumulate in severe renal impairment, and SOF-containing DAA combinations like SOF/VEL/VOX are not recommended for patients with CrCl of <30 mL/min. Like other DAA combinations that include a protease inhibitor, SOF/VEL/VOX is not recommended for patients with moderate hepatic impairment (Child-Pugh class B) or severe hepatic impairment (Child-Pugh class C).[233] VOX does not prolong the QTc interval.[233] Data on safety of VEL during pregnancy are not available.[233]

Drug Interactions

Compared with first-generation protease inhibitors, SOF, VEL, and VOX have few clinically important drug-drug interactions. VOX is a substrate of drug transporters P-gp, BCRP, OATP1B1, and OATP1B3; if VOX is taken with drugs that are substrates for these transporters, drug levels of these other drugs can be increased.[233] VOX is metabolized primarily by CYP3A4 and to a lesser extent CYP1A2 and CYP2C8. The effect of VOX may be reduced by moderate-to-potent CYP inducers.[233] SOF/VEL/VOX administration is not recommended along with P-gp inducers and/or moderate-to-potent CYP inducers.[233] VOX drug-drug interactions occur with rifampin, St. John's wort, and carbamazepine, among others. SOF/VEL/VOX should not be administered with BCRP substrates (including methotrexate, mitoxantrone, imatinib, irinotecan, lapatinib, rosuvastatin, sulfasalazine, and topotecan).[233] Neither SOF, VEL, nor VOX is a substrate for hepatic or renal transporters. VOX has no effect on the metabolism of TDF or TAF or their combinations with emtricitabine and rilpivirine (TAF) or with emtricitabine and raltegravir (TDF). Administration of VOX together with efavirenz is not recommended.[233] For all DAA treatment, drug-drug interactions should be considered before initiating therapy (www.hcvguidelines.org).[233]

Toxicity

In clinical trials ("POLARIS"),[231] side effects in the SOF/VOL/VOX group were mild and uncommon; the four most common (in 12%–27%) were headache, fatigue, nausea, and diarrhea; the SOF/VEL/VOX side-effect profile was similar to that in placebo recipients and SOF/VEL recipients, for instance, for these four adverse events, 8% to 20% and 5% to 28%, respectively.

Like all DAAs that include a PI, SOF/VEL/VOX should not be used in decompensated cirrhotic patients. Similarly, like all DAAs that include SOF, this combination is effective and recommended for patients with mild-to-moderate renal impairment but not with severe renal impairment (CrCl <30 mL/min). Like other DAAs for hepatitis C, SOF/VEL/VOX has a black box warning about reactivation of HBV infection (some cases resulting in fulminant hepatitis, hepatic failure, and death) in patients with HBV and HCV coinfection in the absence of antiviral therapy for hepatitis B.[233] As with all SOF-containing DAAs, SOF/VEL/VOX should not be administered to patients taking amiodarone, in whom SOF has been associated with severe bradycardia, especially in patients also taking β-blockers (see earlier). PPIs such as omeprazole raise gastric pH, which reduces SOF solubility.[186] SOF/VEL/VOX can be taken with 20 mg of omeprazole, but the safety of other PPIs has not been evaluated.[233]

Clinical Studies

(See also Chapter 117.)

In a randomized, double-blind, placebo-controlled, phase III trial involving 263 patients with genotype 1 who had failed a course of an NS5A-containing DAA treatment (114 patients with other genotypes were included as well but were all assigned to the triple-combination group), 96% of SOF/VEL/VOX-treated subjects (12-week course of daily treatment) versus 0% of placebo recipients achieved SVR_{12} (POLARIS-1).[231] The SVR_{12} ranged from 91% for genotype 4, 95% for genotype 3, 97% for genotype 1 (1a and 1b indistinguishable), and 100% for the small numbers with genotypes 5 and 6. Noncirrhotic patients had higher SVR_{12} than compensated cirrhotic patients (99% vs. 93%), but the

presence of baseline NS5A RASs did not influence efficacy (SVR_{12} in 98% without and in 97% with baseline RASs). In a complementary randomized, double-blind, positive-controlled trial among prior nonresponders to DAA regimens that did not contain an NS5A inhibitor (POLARIS-4), patients with genotypes 1 to 3 were randomized to receive 12 weeks of SOF/VEL/VOX ($n = 163$) versus SOF/VEL ($n = 151$); 19 additional patients with genotype 4 were treated with SOF/VEL/VOX without randomization, for a total SOF/VEL/VOX cohort of 182. SVR_{12} occurred in 98% of the triple combination group versus in 90% of the double-combination group.[231] SVR_{12} rates were higher for SOF/VEL/VOX than for SOF/VEL for all genotypes except for 1b (and, in genotype 4, none received SOF/VEL): genotype 1a, 98% versus 89%; genotype 1b, 96% versus 95%; genotype 2, 100% versus 97%; genotype 3, 94% versus 85%; genotype 4 100%. SOF/VEL/VOX responsiveness was indistinguishable between noncirrhotic patients (98%) and compensated cirrhotic patients (96%), whereas, as described earlier for SOF/VEL, responsiveness to SOF/VEL in this trial was reduced in compensated cirrhotic patients (86%) compared with noncirrhotic patients (94%). The presence of baseline NS5A or NS3 RASs did not influence efficacy (SVR_{12} in 99% without and in 100% with baseline RASs) in SOF/VEL/VOX recipients; similarly, in the SOF/VEL arm of POLARIS-4,[231] SVR_{12} rates were the same in those with and without baseline NS5A or NS3 RASs (90% vs. 89%), consistent with the roughly 10% inferiority of SOF/VEL to SOF/VEL/VOX. Between POLARIS-1 and POLARIS-4, the number of baseline RASs (of any kind, NS3, and/or NS5A) had no influence on SVR_{12}—97% with one RAS, 98% with two, and 100% with three or greater than four. In these trials side effects in the SOF/VOL/VOX group were mild and uncommon (see earlier).

The potential for an 8-week course of SOF/VEL/VOX was pursued in treatment-naïve patients. Two SOF/VEL/VOX phase III trials, in which an 8-week versus a 12-week course was compared, showed that 8 weeks was inferior, yielding SVR_{12} rates of 95% versus 98% for all genotypes and for both compensated cirrhotic patients and cirrhotic patients (with the exclusion of cirrhotic patients with genotype 3), primarily because of the lower 8-week course response in genotype 1a (92%) (POLARIS-2). Eight versus 12 weeks of SOF/VEL/VOX was evaluated separately in patients with genotype 3 and cirrhosis; 96% in both duration groups achieved SVR_{12} (POLARIS-3).[232]

These trials established the basis for treatment recommendations. As noted earlier, SOF/VEL/VOX is recommended as a *first-line* DAA combination for patients with genotype 1 who have failed a prior non-NS5A, SOF-containing regimen or an NS5A-containing regimen and for patients with genotypes 3 to 6 who have failed any DAA regimen. For cirrhotic patients with genotype 3 who had failed a prior NS5A regimen, SOF/VEL/VOX efficacy was slightly lower, leading to the recommendation that RBV be added. This triple-drug DAA combination is not recommended for treatment-naïve patients, except for cirrhotic patients with genotype 3 (www.hcvguidelines.org; see Table 117.9).

State of the Art

First-generation protease inhibitors improved antiviral efficacy substantially over that of the previous standard of care (PEG IFN/RBV) but were plagued by the complexity of their regimens, suboptimal resistance profiles, limited genotype specificity, intolerability, and frequent drug-drug interactions. As noted earlier, improved HCV DAAs are available, and gone are the complexities of RGT and futility rules—not to mention the need for PEG IFN injections and the side effects of PEG IFN/RBV that beleaguered first-generation protease inhibitor–based DAA regimens. IFN-based therapy and first-generation protease inhibitors have been supplanted by combinations of simple-regimen, once-daily, orally administered, well-tolerated, pangenotypic, high-barrier-to-resistance DAAs with treatment courses as brief as 8 to 12 weeks and near-100% efficacy (almost always without RBV), the best of which are now recommended as *first-line* treatment (www.hcvguidelines.org). Because the current first-line combinations have been so successful, the impetus for and momentum behind development of new DAAs have been blunted substantially, leading to the abandonment of several DAA candidates that were not competitive with current first-line agents. Thus, the pace of new DAA development for hepatitis C has slowed dramatically, and, because current DAAs are so effective, pursuit of alternative treatment approaches (e.g., RNA interference, gene editing, immunologic manipulation) is no longer competitive. The initial costs of several of the new DAA combinations approved beginning in late 2013 were exorbitant, resulting in restrictions on approval of these therapies by insurers (including state Medicaid) and pharmacy benefits management organizations; however, as competing DAAs were approved, prices came down, and restrictions were relaxed. Still, the goal of universal therapy for patients with hepatitis C remains elusive, because, despite screening recommendations, most persons with HCV infection go undetected, and, even among those with known hepatitis C, many are lost to a porous "cascade of care" and do not find their way to initiating or completing therapy.[234,235]

Finally, worth reiterating are important considerations in selecting a DAA regimen for individual patients: (1) SOF-containing DAA combinations are not recommended for patients with severe renal impairment (CrCls <30 mL/min); the first-line regimens approved for severe renal impairment are grazoprevir/elbasvir and GLE/PIB. (2) Protease inhibitor–containing DAA combinations are not recommended for patients with moderately to severely decompensated cirrhosis (Child-Pugh B and C); for such patients, the recommended first-line DAA combinations are SOF/ledipasvir, SOF/VEL, and SOF plus daclatasvir. (3) SOF-containing DAA combinations should not be taken along with amiodarone, especially in patients taking β-blockers. (4) All DAAs for HCV infection can lead to reactivation of HBV infection (some cases resulting in fulminant hepatitis, hepatic failure, and death) in patients with HBV and HCV coinfection in the absence of antiviral therapy for hepatitis B.

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48 Miscellaneous Antiviral Agents (Interferons, Tecovirimat, Imiquimod, Pocapavir, Pleconaril)

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SHORT VIEW SUMMARY

INTERFERONS

- Interferons are potent cytokines that stimulate antiviral, immunomodulating, and antiproliferative effects.
- They are classified as type I (α, β), II (γ), or III (λ) (see Table 48.1) and administered subcutaneously or intramuscularly, but pharmacokinetic properties are not well linked to physiologic effects.
- Attachment to polyethylene glycol (pegylation) prolongs half-life and possibly decreases immunogenicity and results in more convenient dosing, but toxicities persist including anemia and other cytopenias, depression, thyroid dysfunction, and fatigue.
- Major clinical use is in treatment of hepatitis B, but their use in treatment of hepatitis C has been largely supplanted by direct-acting antivirals (see Chapter 47).
- They are also approved for treatment of anogenital warts.

TECOVIRIMAT

- Tecovirimat is a low-molecular-weight compound active against smallpox virus and other orthopoxviruses.
- It inhibits formation and egress of enveloped virus through inhibition of p37 protein.
- It is effective against monkeypox virus infection in nonhuman primates and rabbitpox virus infection in rabbits.
- A phase 1 clinical safety study showed tecovirimat to be well tolerated in healthy volunteers.
- It was approved by the US Food and Drug Administration (FDA) in July 2018 under the Animal Rule, which allows use if clinical efficacy studies cannot be conducted.

IMIQUIMOD AND RESIQUIMOD

- These agents are topically applied Toll-like receptor (TLR) 7 (imiquimod) and TLR7/TLR8 (resiquimod) agonists.
- Imiquimod is approved for treatment of anogenital warts and results in clearance of these lesions in 37% to 52% of cases.
- Local skin toxicity is common, consisting of erythema, burning, and tenderness.
- Resiquimod is an investigational compound with somewhat greater potency than imiquimod.
- Resiquimod has been studied in genital herpes simplex virus infection, with early suggestion of decreases in rates of recurrences, but a phase III study failed to support an effect on recurrences.

PLECONARIL

- Pleconaril is an antipicornavirus drug that inhibits viral replication by binding to a hydrophobic pocket on the viral capsid.
- In vitro activity is against almost all commonly isolated enteroviruses and 90% of rhinovirus clinical isolates.
- Orally administered and generally well tolerated, pleconaril induces CYP3A isoenzymes and therefore has multiple drug interactions.
- Its use has been studied in enteroviral meningitis, but effects on headache and illness duration were inconsistent.
- Pleconaril has reduced the duration of rhinovirus infection by 1 day but is not approved by the FDA for this indication.
- Pleconaril is no longer under clinical development.

POCAPAVIR

- Pocapavir is an orally available antiviral active against polioviruses 1, 2, and 3 and many enteroviruses.
- Antiviral activity is through binding on specific sites on viral capsid proteins.
- In a clinical trial in healthy volunteers who were challenged with oral poliovirus 1 vaccine, pocapavir reduced duration of shedding of virus in stool, but it was associated with frequent emergence of resistant virus.
- Pocapavir is being considered for future studies in immunodeficient persons at risk for shedding of virulent polioviruses.

Antiviral agents discussed in this chapter have activity against a variety of viral infections in addition to the infections addressed in Chapters 44 to 47. The major current clinical use of interferons is in treatment of hepatitis B, which is discussed in Chapter 47.

INTERFERONS

Classification

Since their discovery in 1957 as mediators of the phenomenon of viral interference (i.e., inhibition of growth of one virus by another), interferons (IFNs) have become recognized as potent cytokines that are associated with complex antiviral, immunomodulating, and antiproliferative actions.[1–4] IFNs are proteins that are synthesized by eukaryotic cells in response to various inducers and that cause biochemical changes leading to a nonselective antiviral state in exposed cells of the same species. Three subfamilies of IFNs are recognized. Type I IFNs are the largest subfamily and include the IFN-αs (13 subtypes in humans) and the IFN-βs. The type II subfamily has only one member, IFN-γ. Type III is a more recently identified subfamily and includes IFN-λ,[5] of which there are three subtypes (λ1, λ2, λ3), also known as interleukin (IL)-28, IL-29, and IL-28R.[6,7] A fourth interferon λ subtype (λ4) has been identified as well.[8] Type I IFNs are clustered on the short arm of chromosome 9 in humans,[9] type II IFN is on chromosome 12, and type III IFNs are encoded on chromosome 19.[10] Formerly designated on the basis of the cell types from which they were derived, the IFN-αs, IFN-β, and IFN-γ are the IFNs currently in clinical use (Table 48.1). Each type is immunologically distinct and has different producer cells, inducers, and biologic effects and unique physicochemical characteristics.[2,5,11]

The IFN-αs and IFN-βs are produced by almost all cells in response to viral infection and various other stimuli including double-stranded RNA (dsRNA); bacteria; protozoa; mycoplasmas; polyanions; several low-molecular-weight organic compounds; and certain cytokines and growth factors such as IL-1, IL-2, and tumor necrosis factor. IFN-γ production is restricted to T lymphocytes ($CD4^+$ T helper cells and $CD8^+$ cytotoxic cells)[12] and natural killer cells responding to antigenic stimuli, mitogens, and certain cytokines such as IL-2. The IFN-λs also appear to be produced by multiple cell types.[13] The principal antiviral IFNs, IFN-αs and IFN-βs, are approximately 30% homologous at the amino-acid level. The human IFN-αs share a high degree of amino-acid sequence homology (>70%) but have differing in vitro antiviral and biologic effects on human cells.[14] Compared with the IFN-αs and IFN-βs, IFN-γ has less antiviral activity but more potent immunoregulatory effects, particularly with respect to macrophage activation, expression

TABLE 48.1 Nomenclature and Classification of Human Interferons

SUBFAMILY	TYPE I	TYPE I	TYPE II	TYPE III
Class[a]	α	β	γ	λ
No. subtypes	13	1	1	4
Receptor	IFNAR1/2	IFNAR1/2	IFNGR1/2	IL10R2, IFNLR1
Human chromosome	9	9	12	19
Commercial formulations	rIFN-α2b (Intron A) IFN-α2a (Roferon-A) Le-IFN-αn3 (Alferon N) Ly-IFN-αn1 (Wellferon) rIFNalfacon-1 (Infergen) Peg-IFN-α2a (Pegasys) Peg-IFN-α2b (PEG-Intron)	rIFN-β1b (Betaseron) rIFN-β1a (Avonex, Rebif)	rIFN-γ1b (Actimmune) rIFN-γ (Immuneron)	Peg-IFNλ1

[a]Type I classes in humans also include IFNε and IFNώ.

of class II major histocompatibility complex (MHC) antigens, and mediation of local inflammatory responses. Most IFNs in clinical use are produced by recombinant DNA techniques (see Table 48.1).

Recombinant interferons are available as interferon alfa-2a and interferon alfa-2b, which differ by a single amino acid. Interferon alfa-n3 is a mixture of subtypes of IFN-α purified from human leukocytes. Interferon alfacon-1 is a bioengineered form of IFN-α based on a consensus amino-acid sequence derived from naturally occurring forms of IFN-α. IFN-β is available as recombinant interferon beta-1a and interferon beta-1b.

Mechanisms of Action

A wide range of animal viruses are sensitive to the antiviral actions of IFNs, although many DNA viruses are relatively insensitive, and considerable differences in potency exist among viruses and assay systems. IFN activity is usually measured in terms of antiviral effects in cell culture. Typically one unit of IFN activity is the amount present in a sample dilution that causes a 50% reduction in virus replication or expression in certain cell lines; this is generally expressed as international units (IU) relative to National Institutes of Health or World Health Organization reference standards.

IFNs are not directly antiviral but cause elaboration of effector proteins in exposed cells, which contribute to a state of viral resistance.[1,15,16] The initial step involves IFN binding to specific cell surface receptors. For the type I IFNs (IFN-αs and IFN-β), the cognate receptor is IFNAR1/2. Type II IFNs (IFN-γ) use the homodimeric receptor, IFNGR1/2. Type III IFNs (IFN-λ) signal through a receptor complex consisting of IL10R2 and IFNLR1.[7,17–19] IFN receptors are linked to the Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling pathways, which, through a multistep process, activate transcription factors that bind selectively to and upregulate approximately 100 IFN-regulated genes.[20–22] The distinct pattern of STAT proteins activated by different IFNs is one mechanism for eliciting different cellular responses. For IFN-α and IFN-β, a three-protein complex known as IFN-stimulated gene factor 3 localizes to the nucleus and binds to a *cis*-acting DNA element (designated IRSE) that activates transcription of the target genes.[1,4]

A family of IFN regulatory factors exists, and other pathways may contribute to regulation of the IFN response. Microarray analysis shows that many genes are upregulated by IFN-β, but not by IFN-α or IFN-γ, in vitro.[20] The onset of IFN-induced antiviral action is rapid, and IFN exposure can induce production of more than 300 cellular proteins.[20] For many viruses, the primary antiviral effect of IFN in vitro is mediated by inhibition of viral protein synthesis. Depending on the virus and cell type, the antiviral actions of IFNs may also include inhibition of viral penetration or uncoating, synthesis or methylation of messenger RNA (mRNA), or viral assembly and release.

Among the better characterized IFN-induced proteins are unique 2′-5′-oligoadenylate (2-5[A]) synthetases and protein kinase R (PKR), either of which can inhibit protein synthesis in the presence of dsRNA.[1] The 2-5(A) synthetase produces adenylate oligomers that activate a latent cellular endoribonuclease (RNase L) to cleave cellular and viral single-stranded RNAs, leading to inhibition of protein synthesis. Activated PKR selectively phosphorylates and inactivates eukaryotic initiation factor-2 to impede translation. Activated PKR also phosphorylates the transcription factor inhibitor IκB and mediates dsRNA-induced activation of nuclear factor kappa B, which is required for IFN-β synthesis. IFNs may also block mRNA capping by inhibiting transmethylation reactions. IFNs also induce human guanylate binding protein-1, which mediates antiviral activity for several RNA viruses; the soluble form of the low-density lipoprotein receptor inhibitory for rhabdovirus assembly[23]; the MxA protein (a guanosine triphosphatase with activity against orthomyxoviruses and certain RNA viruses)[24]; and the RNA-specific adenosine deaminase ADR1, which modifies RNA transcripts after transcription.[1,4]

IFNs also inhibit hepatitis C virus (HCV) internal ribosome entry site–dependent RNA translation in vitro.[25] Induction of nitric oxide synthase seems to mediate a substantial antiviral effect of IFN-γ.[26] Increased levels of 2-5(A) synthetase activity and MxA protein or mRNA in peripheral leukocytes are also used as a marker for IFN exposure or endogenous release.[24]

Except possibly for the Mx proteins and influenza viruses and for 2-5(A) synthetase/RNase-L and picornaviruses, no consistent correlations exist between induction of a particular protein and resistance to a specific virus across a range of cell types.[16] A particular virus may be inhibited at several steps, and the principal inhibitory effect differs among virus families. Many viruses are able to counter IFN effects by blocking signaling and production or activity of selected IFN-inducible proteins.[1,16] The NS5A protein of HCV represses the function of the IFN-induced PKR,[27] and another HCV protein, E2, competitively inhibits PKR kinase activity. The *NS1* gene of influenza is an IFN antagonist that binds dsRNA to inhibit IFN production and dsRNA-activated pathways. IFN exposure may also reduce the expression of certain cellular genes including selected oncogenes and genes involved in collagen synthesis.

The viral and immune IFN systems are functionally nonredundant,[1,4,28] and complex interactions exist between IFNs and between IFNs and other parts of the immune system.[11,29] IFNs upregulate MHC class I expression and promote cytotoxic T-cell responses, regulate the expression of cytokines (IL-12, IL-15, IFN-γ) and chemokines that affect T-cell responses, alter expression of Toll-like receptors (TLRs), enhance natural killer cell cytotoxicity, and promote the differentiation of dendritic cells and Th1 lymphocytes.[15,29] IFN-α is produced by macrophages and can modify macrophage functions, increasing phagocytosis and cytolytic activity. Consequently, IFNs may ameliorate viral infections by exerting direct antiviral effects and by modifying the immune response to infection. IFN-induced expression of MHC antigens may contribute to the antiviral actions of IFN by enhancing antigen presentation and the lytic effects of cytotoxic T lymphocytes. The IFN-αs, the IFN-βs, and IFN-γ lead to increased expression of class I MHC molecules, but only IFN-γ efficiently induces class II MHC molecules.[1,4] Several viruses including cytomegalovirus (CMV) and varicella-zoster virus antagonize IFN-γ–induced MHC expression. In addition, proapoptotic and antiapoptotic genes are induced by IFNs,[20] and IFN-α and IFN-β are important mediators of apoptosis including induction of *TP53*.[1,30]

IFN titers generally appear at the sites of viral replication just after peak titers of virus and before humoral antibody responses. IFNs may

mediate some of the systemic symptoms associated with viral infections and contribute to immunologically mediated tissue damage in certain viral diseases. High IFN titers are usually followed by a reduction of virus titers, although persistently elevated IFN titers have been recognized in certain chronic and acute viral infections (e.g., hemorrhagic fevers).

Pharmacokinetics

The prolonged biologic effects of IFNs are not easily related to serum concentrations or other conventional pharmacokinetic parameters. After intramuscular (IM) or subcutaneous injection of IFN-α, absorption is greater than 80%.[31,32] Plasma levels are dose related, peaking at 4 to 10 hours and returning to baseline by 18 to 36 hours. Levels of 2-5(A) synthetase in peripheral blood mononuclear cells, which have been used as an index of biologic responsiveness to IFN, show increases beginning at 6 hours and lasting through 4 days after a single dose. An antiviral state in these cells is detectable at 1 hour, peaks at 24 hours, and slowly decreases to baseline by 6 days after injection. IM or subcutaneous injections of IFN-β result in negligible plasma levels, although increases in 2-5(A) synthetase may occur. Oral administration does not result in detectable serum IFN levels or increases in 2-5(A) synthetase activity in peripheral blood mononuclear cells.[33]

After systemic administration, low levels of IFNs are detected in respiratory secretions, cerebrospinal fluid, eye, and brain. After intravenous dosing, cerebrospinal fluid levels average less than 1% of serum concentrations.[34] The IFN-αs are stable in most body fluids, whereas the IFN-βs and IFN-γ seem to lose activity readily. It is unknown, however, whether measurable IFN levels at a particular site accurately reflect its antiviral or other biologic activities. The IFN-αs and the IFN-βs are cleared rapidly in a complex fashion. Leukocyte and recombinant IFN-α species have a plasma elimination half-life ($t_{½elim}$) of 3 to 8 hours. The clearance of IFN includes inactivation by various body fluids, cellular uptake, and metabolism by body organs, primarily the kidney, although negligible biologically active IFN is excreted in the urine. Clearance of IFN-α2 is reduced by 64% to 79% in patients on hemodialysis.[35]

The attachment of polyethylene glycol to IFN slows absorption, decreases clearance, increases $t_{½elim}$, and results in higher and more sustained serum concentrations, so that once-weekly dosing is effective. Two types of pegylated (peg) IFN-α are currently approved: peg-IFN-α2a has a 40-kDa branched polyethylene glycol moiety attached by a stable amide bond to lysine residues within the IFN protein, and peg-IFN-α2b has a 12-kDa linear moiety attached to histidine residues. peg-IFN-α2a is more stable and dispensed in solution, whereas peg-IFN-α2b requires reconstitution before use. peg-IFN-α2a is cleared primarily by the liver, whereas about 30% of peg-IFN-α2b is cleared renally.[35] For peg-IFN-α2a (multiple 180-μg doses), a peak serum concentration of 26 ng/mL occurs at about 45 hours after dosing, and $t_{½elim}$ is 80 to 90 hours. Steady-state serum levels are attained 5 to 8 weeks after initiation of weekly dosing. Moderate renal impairment and presence of cirrhosis do not affect pharmacokinetics, although clearance is reduced by 25% to 45% in patients with renal failure on hemodialysis. For peg-IFN-α2b, dose-related maximal plasma concentrations (1.4 ng/mL with multiple doses of 1.5 μg/kg) occur at 15 to 44 hours after dosing and decline with a $t_{½elim}$ of 30 to 40 hours, or about 10-fold longer than for IFN-α2b.[33] Some accumulation occurs with repetitive dosing. Dosage reductions in both peg-IFNs are indicated in end-stage renal disease.

Interactions

IFN and its inducers reduce the metabolism of various drugs by the hepatic cytochrome P-450 (CYP)–dependent mixed-function oxidase system and specifically decrease CYP1A2-mediated clearance of theophylline. IFNs may increase the neurotoxic, hematotoxic, or cardiotoxic effects of other drugs including increased risk for anemia with ribavirin.

Toxicity

Purified natural and recombinant IFNs are associated with dose-related immediate-onset and late-onset toxicities.[36] Adverse effects are generally mild and reversible at dosages of less than 5 million IU/day.[32] IM and subcutaneous injections of IFN doses of 1 to 2 million IU or more are usually associated with an acute influenza-like syndrome including fever, chills, headache, malaise, myalgia, arthralgia, nausea, vomiting, and diarrhea, especially during the first week of therapy. Symptoms begin several hours after administration and are most prominent 8 to 24 hours after dosing. Despite more prolonged blood levels, the duration of influenza-like symptoms after peg-IFN is similar to that after conventional IFNs.[35] Tolerance develops in most patients within several weeks. Febrile responses can be moderated by pretreatment with various antipyretics. Half of patients receiving intralesional therapy for genital warts experience the influenza-like illness. Intralesional IFN also causes discomfort at the injection site and leukopenia. Local reactions consisting of tenderness and erythema also occur after subcutaneous injection, and intranasal IFN causes local irritation.

Major toxicities that limit dosage and duration of IFN therapy are bone marrow suppression with granulocytopenia and thrombocytopenia; neuropsychiatric disturbance manifested by depression, anxiety, somnolence, confusion, behavioral disturbance, electroencephalographic changes, and, rarely, seizures; reversible neurasthenia with profound fatigue, anorexia, weight loss, and myalgia; thyroid dysfunction and autoimmune thyroiditis; and cardiotoxicity with hypotension, arrhythmias, and reversible cardiomyopathy. Psychiatric disturbance and depression are more common in patients with preexisting disorders but can also occur in otherwise healthy individuals. Elevations in hepatic enzymes and triglycerides and retinopathy are common.[37] IFN may lead to the development or exacerbation of various immunologically mediated disorders including sarcoidosis, systemic lupus erythematosus, psoriasis, vitiligo, lichen planus, and eczematoid skin lesions. Rare pulmonary manifestations include interstitial pneumonia, bronchiolitis obliterans, organizing pneumonia, asthma, and pleural effusion.[38] Alopecia, proteinuria, renal insufficiency, interstitial nephritis, autoantibody formation, bacterial infections, and hepatotoxicity occur.[39] Acute allergic reactions are rare. Patients with autoimmune chronic hepatitis, who may have false-positive enzyme immunoassay tests for anti-HCV antibodies, can experience worsening of their disease if treated with IFN.[40]

The adverse effects of peg-IFNs are similar to adverse effects with conventional IFNs, although dose-related neutropenia and thrombocytopenia and injection site reactions are more common with peg-IFNs. About 50% of patients with chronic hepatitis C treated with peg-IFN develop fatigue and systemic symptoms after injections; 20% to 30% experience depression or other psychiatric reactions; and approximately 10% to 16% discontinue treatment because of adverse events, most commonly psychiatric disorders.[41,42] peg-IFN-α2a may be associated with a lesser frequency of depression.[42]

The development of serum neutralizing antibodies to exogenous IFNs varies with the IFN type, dosage, and route of administration but may be more common with IFN-α2a.[43] Neutralizing antibodies may be associated infrequently with loss of clinical responsiveness.[32] Pegylation may reduce the immunogenicity of IFNs, and anti-PEG antibody seems to be rare.

IFNs may impair fertility and alter hormone levels in women. IFN is an abortifacient in monkeys at high dosages and has been used in small numbers of pregnant women, so safety during pregnancy is not established.[44] It is classified as pregnancy category C.

Clinical Studies

IFNs have undergone clinical studies in a broad variety of infections, malignancies, and other diseases. Depending on the IFN type, recombinant and natural IFN-α (see Table 48.1) are approved in the United States for treatment of condyloma acuminatum, chronic hepatitis C, chronic hepatitis B, Kaposi sarcoma in human immunodeficiency virus (HIV)–infected patients, and other malignancies. IFN-β is approved for management of multiple sclerosis. Recombinant IFN-γ is approved for treatment of chronic granulomatous disease. The major clinical uses for IFNs have been in treatments for hepatitis B and C, although they have been supplanted by the use of direct-acting antivirals for hepatitis C. The clinical studies of IFNs for those infections are discussed in detail in Chapters 47 and 117.

Herpesviruses

Although IFN is associated with antiviral effects against herpes simplex viruses (HSVs), no consistent reductions in symptoms or lesion duration have been observed with topical or systemic IFN treatment of genital

herpes.[45] Topical IFN seems to have some activity in combination with trifluridine in drug-resistant mucocutaneous HSV infections. In superficial HSV keratitis, combined administration of topical IFN-α with trifluridine or acyclovir seems to be more effective than single-agent therapy.

In localized herpes zoster in patients with cancer, early treatment with high-dose IFN-α (about 36 million IU/day for 5–7 days) reduces the risk for cutaneous or visceral dissemination, but systemic reactions are frequent, and more effective antiviral agents are available. IFN is ineffective in preventing CMV infection in bone marrow recipients or in treating CMV pneumonia.

Human Immunodeficiency Virus

HIV-infected patients frequently have detectable IFN levels, and plasma inhibitors of IFN activity are often present in patients with acquired immunodeficiency syndrome. High doses of IFN-α induce 10% to 40% response rates in patients with Kaposi sarcoma without benefiting concurrent herpesvirus infections or immune functions.[46] IFN treatment is associated not only with dose-related antiretroviral effects, particularly in early-stage infection, but also with adverse effects.[47] IFN seems to benefit HIV-related thrombocytopenia[48] and eosinophilic folliculitis.

Papillomavirus

Intralesional and systemic forms of administration of IFN produce some regression of anogenital warts,[49] although more cost-effective and better tolerated modalities are available (see Chapter 143).[50] Intralesional injection of various natural and recombinant IFNs is associated with complete clearance of injected warts in 42% to 62% of patients within 12 to 20 weeks.[51] Responders have low relapse rates (20%–30%). Responsiveness is poor in HIV-infected patients and patients with chronic lesions. Intralesional IFN does not reliably increase the response to other local therapies.[51] Mild-to-moderate systemic side effects (8%–10% dropout rate), pain and irritation at injection site, and leukopenia (≤30%) are common with intralesional IFN. Topical IFN gel provides inconsistent effects and does not seem to reduce the recurrence rate substantially after ablative therapies.[50]

Systemic IFN may provide adjunctive benefit in recurrent juvenile laryngeal papillomatosis. Most children have some initial decrease in lesions, but recurrence rates are high after cessation of therapy, and the long-term response to parenteral IFN-α is variable.[52] Laryngeal disease in older patients seems to be more responsive.

Respiratory Viruses

With the exception of adenoviruses, IFNs have broad-spectrum antiviral activity against respiratory viruses in vitro including severe acute respiratory syndrome (SARS) coronavirus.[53] In experimentally induced infections in humans, intranasal administration of leukocyte or recombinant IFN-α is protective against rhinovirus, coronavirus 229E, respiratory syncytial virus, and, to a lesser extent, influenza virus infections.[54] Under natural conditions, prophylactic intranasal IFN-α is protective only against rhinovirus colds, however, and long-term use is limited by the occurrence of nasal side effects. Intranasal IFN-α is ineffective in treating rhinovirus colds. IFN-αs, IFN-βs, and IFN-γ inhibit SARS coronavirus replication in vitro,[53] and the systemic IFN-αs have been used to treat SARS coronavirus illness, but the beneficial effect, if any, is unclear.[55,56]

Enteroviruses

Recombinant IFN-α (IFN-alfa-1b) was evaluated in a randomized, placebo-controlled study of an outbreak of hand-foot-and-mouth disease caused by enterovirus 71 (EV71) in China. The study included 312 children between 0.5 and 5 years of age. IFN-alfa-1b was administered daily for 5 days by ultrasonically nebulized inhalation or by IM injection. Both IM and aerosol IFN-alfa-1b were associated with modest, although statistically significant, reductions in duration of fever, time to healing of skin lesions, and virus load in stool.[57] Another study examined the effect of aerosol administration of IFN-alfa-2b in an outbreak of hand-foot-and-mouth disease in China caused by EV71 or coxsackievirus A16 in 372 children younger than 7 years of age. In the placebo-controlled trial, subjects received two aerosol administrations on day 1 and one

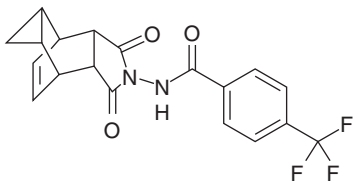

FIG. 48.1 Chemical structure of tecovirimat (ST-246, TPOXX).

administration on days 2 to 7. IFN-alfa-2b recipients had modest, although statistically significant, reductions in duration of fever, rashes, vesicles, or ulcers as well as in virus detection, along with an improvement in appetite.[58]

TECOVIRIMAT

Tecovirimat (ST-246, TPOXX) is an antiviral that is active in vitro against smallpox virus (variola) and was approved by the FDA on July 13, 2018, for treatment of smallpox under Fast Track and Priority Review designations.[59] (See later discussion and Chapter 132.) Smallpox was declared eradicated in 1980, but the possibility remains that clandestine sources of variola exist or that virus may be recreated from published genomic sequences. Thus, the availability of effective antiviral chemotherapy is of considerable interest.

Tecovirimat is an orally bioavailable, low-molecular-weight compound (Fig. 48.1) that has activity against all known orthopoxviruses in vitro including variola,[60,61] but it does not have activity against other known viruses. It was identified after screening a chemically diverse library of more than 350,000 unique compounds.

The EC_{50} (50% effective concentration) of tecovirimat against variola is 0.010 to 0.068 μmol/L.[61] The mechanism of action of tecovirimat is through inhibition of p37, a highly conserved protein in all orthopoxviruses that mediates the formation and egress of enveloped virions.[62–66]

The efficacy of tecovirimat against orthopoxviruses was evaluated in pivotal studies in monkeypox infection in nonhuman primates and rabbitpox infection in rabbits.[60,67–75] Survival was the primary endpoint of the studies, and lesion formation (nonhuman primate model) and virus load were secondary endpoints. In nonhuman primates, treatment with tecovirimat increased survival rates to 95% compared with 5% in placebo recipients. The minimum effective dose was 3–10 mg/kg. The dose of 10 mg/kg had a greater effect on reduction of lesion count and virus load than lower doses tested. Tecovirimat was similarly efficacious in the rabbit model, with a minimum effective dose of 20–40 mg/kg.[60] A treatment-delay study in nonhuman primates showed that survival rate was improved if treatment was begun earlier after challenge (day 4 or 5) compared with later (day 6) after challenge.[75]

A phase 1 safety clinical study was conducted in 451 healthy volunteers who received either 600 mg of tecovirimat or placebo twice daily for 14 consecutive days. Tecovirimat was generally well tolerated, and overall adherence to treatment regimens was similar in tecovirimat and placebo recipients. No serious safety concerns were identified.[60] Pharmacokinetic studies revealed that half-lives of tecovirimat were approximately 23 hours, that steady-state levels were achieved by 6 days, and that elimination of drug was similar in fasting and fed groups.[60] Renal clearance is the major route of excretion. At a dose of 600 mg twice a day, maximum concentration and minimum concentration were 1591 mg/mL and 560 mg/mL at day 1 and 2209 mg/mL and 690 mg/mL at day 14, respectively.[60] This represents a ratio more than four times the minimum concentration associated with efficacy in the nonhuman primate model.[76]

The FDA approved tecovirimat based on the Animal Rule, which accepts evidence of "multiple effective exposures in animal models" when efficacy studies in humans are not possible,[77] along with evidence of safety in humans.

IMIQUIMOD AND RESIQUIMOD

Imiquimod (Aldara) is an imidazoquinoline compound (Fig. 48.2A) that is a TLR7 agonist; it acts as a topical immune response modifier lacking direct antiviral effects. Imiquimod exposure causes activation of immune cells (monocytes, macrophages, natural killer cells) to produce antiviral cytokines, particularly IFN-α and tumor necrosis factor-α and

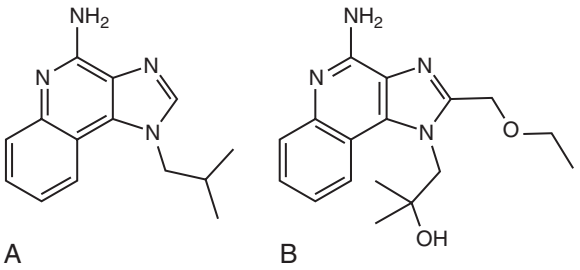

FIG. 48.2 Chemical structure of (A) imiquimod and (B) resiquimod.

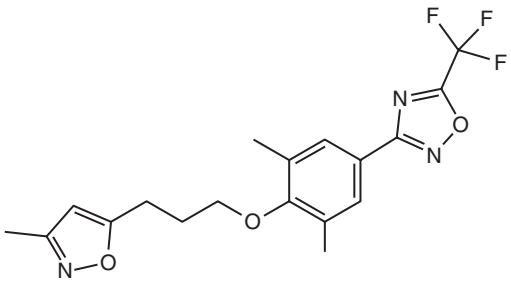

FIG. 48.3 Chemical structure of pleconaril.

IL-12, IL-10, IL-1, IL-6, and IL-8.[78,79] Imiquimod indirectly enhances acquired immune responses through activation of antigen-presenting dendritic cells including Langerhans cells and Th1 lymphocytes. IFN-γ production from T cells stimulates cytotoxic T lymphocytes, which is important in clearance of virally infected cells. Clinical responses in anogenital warts are associated with decreases in human papillomavirus (HPV) DNA copies and RNA transcripts in treated skin.[80] Resiquimod is a structurally related investigational compound (Fig. 48.2B) that is a TLR7/TLR8 agonist and is associated with greater stimulation of cytokines and with activation of dendritic cells.[81]

Topical imiquimod 5% cream is approved for patient-applied treatment of anogenital warts and has been used in other mucocutaneous infections and dermatologic conditions.[78] In immunocompetent patients, imiquimod (three overnight applications [for approximately 8 hours] weekly for up to 16 weeks) leads to complete wart clearance in 37% to 52%.[50,78,82] Clearance rates (39%–52%) are not different with 4, 8, 12, or 16 weeks of treatment.[83] Daily application increases local adverse effects without increasing clearance rates.[84] Imiquimod and podophyllin are similar in efficacy with 50% and 55% clearance rates, respectively.[85] Clearance rates are higher in women than in men and substantially lower (<15%) in HIV-infected individuals.[86–88] In one study in women, clinical outcome was related to HPV type: complete response rates were 76% for HPV-6, 67% for HPV-11, 35% for HPV-6 and HPV-11 coinfection, and 6% for other HPV types.[89] The time to clearance averages 8 to 10 weeks. Recurrences are less common (14%–19%) than after ablative therapies, and retreatment is frequently successful.[78] Imiquimod may be useful as an adjunct to laser or surgical ablative therapies.[78]

Imiquimod has been used for warts at nongenital sites[79] and in other types of HPV-related conditions. Both grade 2 and grade 3 intraepithelial neoplasia of the vulva respond to imiquimod therapy.[90] HPV anal infection in HIV-infected men may[91] or may not[92] respond. Imiquimod seems beneficial in refractory cutaneous leishmaniasis in combination with other drugs,[93,94] although other authors did not observe any benefit from combining standard intravenous antimony therapy with imiquimod.[95] Depending on the level of immunocompetence, imiquimod has been recommended as possibly beneficial for treatment of molluscum contagiosum.[78,79,96] A Cochrane Review concluded that no intervention had sufficient efficacy to be recommended for therapy in molluscum contagiosum in most patients.[97,98] A review of randomized clinical trial data in the product insert also concluded that imiquimod was not effective in treatment of molluscum contagiosum in children.[99] Imiquimod has been used successfully to treat chronic genital herpes resistant to acyclovir[100] and to foscarnet[101] in immunocompromised individuals with HIV and drug-induced immunosuppression.[102] No beneficial effects on lesions or recurrences are seen in immunocompetent adults with genital herpes.[103] Imiquimod 5% cream made recurrent herpes labialis lesions worse compared with the vehicle cream as control but increased the time to the next recurrence 80% from a median of 50 days to 91 days in the control and imiquimod-treated groups.[104] Other viral infections reported to respond to imiquimod include oral hairy leukoplakia,[105] orf,[106] and Kaposi sarcoma.[107] Tinea pedis resolved during imiquimod treatment of a contiguous basal cell carcinoma.[108]

Because of encouraging preliminary findings, topical resiquimod was investigated for treatment of recurrent genital herpes in an effort to reduce recurrences.[109] However, a controlled study did not show an effect of imiquimod on the rate of recurrences.[103] Resiquimod 0.01% gel applied to recurrent genital herpes lesions two times a week for 3 weeks reduced neither healing time nor shedding of HSV DNA by polymerase chain reaction assay compared with a control gel.[110] The same treatment regimen was associated in the subsequent 60 days with a reduction in lesional recurrence rate from 16% to 10% and with a trend toward reduced shedding from 17% to 10%. Seven months later, recurrence rates were reduced from 26% to 10% in control and resiquimod groups.[111] However, a phase III study of resiquimod did not show reduced rates of recurrence, and further development of the drug to treat anogenital herpes has been halted.[110,112]

Topical application of imiquimod 5% cream to affected skin can result in systemic absorption with serum levels of 0.1 to 3.5 ng/mL. Patients treated with imiquimod need to wash the affected area on awakening to remove residual drug. About two-thirds of treated patients experience local erythema. Application site reactions with erythema, irritation, pruritus, burning, tenderness, and scabbing (and less often with erosion or ulceration at the wart site and other exposed areas) are generally mild to moderate in intensity. These usually resolve within 2 weeks of cessation of the drug. The frequency of local reactions relates to frequency of application, and use in genital herpes may delay healing.[103] Severe local reactions including pain, erythema, or scarring are rare.[86] Unusual local reactions include erosive cheilitis[113] and aphthous ulcers,[114] angioedema and urticaria,[115] and worsening of psoriasis.[116] Systemic reactions to imiquimod such as fatigue and influenza-like symptoms have been reported infrequently.[117] Generalized psoriasis,[116] eczema,[118] exacerbations of myasthenia gravis,[119] and worsening of HLA-B27 spondyloarthropathy[120] have been reported during topical therapy with imiquimod.

Systemic reactions may be caused not by the drug itself, but rather by diffusion of cytokines from the skin into the systemic circulation. During repeated applications, small concentrations (<10 ng/mL of imiquimod) can be detected in the blood.[121] Safety during pregnancy has not been established (pregnancy category B), but there are case reports of its safe use in pregnant women.[122,123] Preclinical studies indicate that imiquimod is not genotoxic or teratogenic.

PLECONARIL

Pleconaril (3-[3,5-dimethyl-4([3-(3-methyl-5-isoxazoyl)propyl]oxy) phenyl]-5-[trifluoromethyl]-1,2,4-oxadiazole) is an orally active antipicornaviral agent (Fig. 48.3). Pleconaril inhibits picornavirus replication by binding to a specific hydrophobic pocket within the viral capsid and preventing viral attachment or uncoating of the genome. In cell culture, pleconaril inhibits replication of almost all commonly isolated enterovirus serotypes[124] and approximately 90% of rhinovirus clinical isolates.[125] Pleconaril is active in murine and human models of coxsackievirus infection.[124,126] Pleconaril has been reported to be active in vitro against enterovirus D68 (EV-D68).[127]

In adults, oral bioavailability is about 70% in the fed state, and peak plasma concentrations average 2.2 μg/mL after doses of 400 mg.[128] Pleconaril undergoes hepatic metabolism, and less than 1% is excreted unchanged in the urine. The initial plasma $t_{½elim}$ averages 2 to 3 hours, but there is a prolonged terminal $t_{½elim}$ of approximately 180 hours.[128] Single oral doses of 5 mg/kg in children provide maximal plasma concentrations of 1.3 μg/mL and approximately 40% lower overall drug exposure because of a larger volume of distribution and more rapid

clearance. Neonates seem to require higher dosages in part because of lower bioavailability.[129]

Pleconaril has been generally well tolerated, and the most common adverse events have been headache, nausea, diarrhea, and abdominal discomfort. Pleconaril induces CYP3A isoenzymes, however, and consequently has the potential for multiple drug interactions including with oral contraceptives.[130] In children or adults with enteroviral meningitis, pleconaril has inconsistent effects on headache and illness duration.[128] Pleconaril (400 mg three times a day for 5 days) reduces the duration of uncomplicated rhinovirus colds by about 1 day[130,131] but was not approved by the FDA for this indication. Pleconaril antiviral effects and clinical outcomes are related to in vitro susceptibility.[131] Pleconaril treatment (15 mg/kg/day [children] and 600–1200 mg/day [adults] in divided doses for 7–10 days) seems to be beneficial in some patients with severe or life-threatening enteroviral syndromes including chronic enteroviral meningoencephalitis in agammaglobulinemic patients and possibly neonatal enteroviral sepsis.[128,132–134] The drug was formerly available for compassionate use, but this is no longer the case.

Vapendavir, another capsid binder, has in vitro activity against EV71[135] and against EV-D68, although less so than pleconaril.[136]

POCAPAVIR

Pocapavir (V-073, SCH 48973) is an orally bioavailable antiviral that is an inhibitor of picornaviruses (Fig. 48.4). Its mechanism of action is through binding at a specific site on the viral capsid that inhibits uncoating of the virus and prevents subsequent release of viral RNA.[137,138] Pocapavir was originally developed to be a broad enterovirus inhibitor, but its major emphasis has been in potential management of poliovirus infections, particularly in the emergence of virulent poliovirus after live vaccine administration, particularly in immunodeficient persons (see Chapter 171).

Pocapavir is effective in vitro against all three poliovirus serotypes with EC_{50} of 0.003 to 0.126 μM[138] and showed effectiveness in a mouse model of poliovirus infection.[137] Pocapavir is also active against a broad variety of many, but not all, nonpolio enteroviruses with EC_{50} of 0.009 to 7.08 μM.[137]

The antiviral efficacy of pocapavir against orally administered live monovalent type 1 oral poliovirus vaccine (mOPV1) was examined in a trial in 144 healthy volunteers 18–50 years of age.[139,140] All subjects received one vaccine dose (10^7 $CCID_{50}$ [cell culture infectious dose 50%]) of mOPV1 and then pocapavir or placebo 1 or 3 days later, for a total of 14 days under a randomized, blinded allocation. The daily pocapavir dose was 1600 mg (8 capsules)/day, administered as either one or two doses per day, with or without a high-fat meal. The primary efficacy measure was duration of virus shedding in stool, and a secondary end point was quantity of virus shed. The median duration of virus shedding was 10 days for pocapavir recipients and 13 days for placebo recipients ($P = .0019$). Reduction of quantity of virus shed was significantly lower only in the cohort of pocapavir recipients who received the single daily dose beginning 72 hours after receiving oral polio vaccine ($P = .0138$).[139] The development of resistance to poliovirus was relatively common, occurring in 44% (41 of 93) of pocapavir recipients, but was also found in placebo recipients, indicating that cross-infection was common under the conditions of the study. Resistance of poliovirus to pocapavir in vitro has been previously described, with single amino-acid substitutions in capsid proteins VP1 or VP3.[141] In the current study, 70% of resistant viruses had an amino-acid substitution at VP1; 22%, at VP2; and 4%, at both sites.[139]

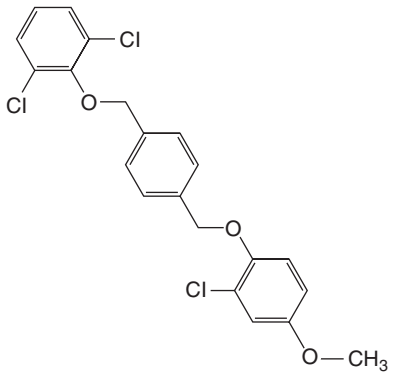

FIG. 48.4 Chemical structure of pocapavir (V-073, SCH 48973).

Administration of pocapavir appeared to be well tolerated, and the rates of adverse events were similar across treated and placebo groups. No significant safety laboratory or electrocardiogram findings were noted.

Interest in pocapavir remains as a part of therapeutic approaches to address the continuing problem of excretion of virulent poliovirus, particularly in immunodeficient persons, either singly or in combination with other antivirals. Discussions for potential studies in such settings are underway.[141]

Pocapavir is classified as an emergency investigational drug, and it has been used to treat serious cases of enteroviral sepsis[142] and enteroviral myocarditis.[143,144] The overall effect of such treatment is difficult to interpret.

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49 Immunomodulators

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SHORT VIEW SUMMARY

IMMUNOMODULATION FOR PROPHYLAXIS/ TREATMENT OF INFECTION

Colony-Stimulating Factors and Granulocyte Transfusions

- Colony-stimulating factors (CSFs) are glycoproteins that regulate the development and/or function of hematopoietic cells, in this case, of the myeloid series.
- Granulocyte colony-stimulating factor (G-CSF) may be used for primary prophylaxis in primary chronic neutropenic conditions, primary and secondary prophylaxis of chemotherapy-induced febrile neutropenia in selected patient populations, and treatment of febrile neutropenia only in highest risk populations.
- Granulocyte-macrophage colony-stimulating factor (GM-CSF) is approved for use in the United States as an alternative to G-CSF in selected patient populations.
- Granulocyte transfusions are recommended only for treatment of antimicrobial-refractory fungal infections in neutropenic patients, albeit with scant supporting evidence.

Interferons

- Type I interferons (IFN-α and IFN-β) have intrinsic antiviral properties. IFN-α has been used in the treatment of hepatitis B virus (HBV), hepatitis C virus (HCV), and human papillomavirus (HPV) infections.
- Type II interferons (IFN-γ) activate macrophages and contribute to granuloma formation and the response to intracellular pathogens. IFN-γ may be used for prophylaxis of infection in patients with chronic granulomatous disease (CGD).

Interleukins

- Interleukin (IL)-2, IL-7, and IL-12 have been studied in viral diseases, including human immunodeficiency virus (HIV). IL-11 has been studied in sepsis and for the prevention of bacterial infection.
- No cytokine therapy is currently recommended for the treatment or prevention of infection.

Intravenous Immune Globulin

- Pooled, nonspecific intravenous immune globulin (IVIG) preparations have been used to replace naturally occurring immunoglobulins in primary immunodeficiencies.
- The use of IVIG is not currently recommended for the treatment of established sepsis in adults.
- Hyperimmune globulin preparations are available for the treatment and/or prophylaxis of cytomegalovirus (CMV), hepatitis B, varicella, rabies, botulism, anthrax, and tetanus.

Monoclonal Antibodies

- Palivizumab, a humanized mouse monoclonal antibody against respiratory syncytial virus (RSV), prevents viral fusion with respiratory epithelial cells and is administered monthly for up to five doses during RSV season to reduce the severity of infection and the need for hospitalization in selected high-risk infants.
- Bezlotoxumab was recently approved for the prevention of recurrent *Clostridioides difficile* (formerly *Clostridium difficile*) infection in high-risk adults already receiving anti–*C. difficile* therapy.
- Raxibacumab and obiltoxaximab are approved, based on animal studies, for use in the treatment of inhalational anthrax in combination with antibacterial therapy, and for prophylaxis of inhalational anthrax in the absence of other appropriate therapy.
- Ibalizumab-uiyk is approved for use in combination with an antiretroviral regimen in heavily treatment-experienced adults with multidrug resistant HIV-1 infection in whom current therapy is failing.

Glucocorticosteroids

- Glucocorticosteroids are used to suppress host immune responses that are contributing to the pathophysiology of an infection and have been shown to be of benefit in HIV-infected patients with moderate-to-severe hypoxia in the setting of *Pneumocystis jirovecii* pneumonia (PJP); paradoxical tuberculosis–immune reconstitution inflammatory syndrome (TB-IRIS) in patients with HIV infection but without Kaposi sarcoma; tuberculous meningitis and pericarditis when combined with antituberculous therapy; and bacterial meningitis, particularly pneumococcal meningitis, when administered with or just before the first dose of antimicrobial therapy in non–resource-constrained settings.
- Glucocorticosteroids have not been shown to be of benefit in HIV-associated cryptococcal meningitis, nor in community-acquired pneumonia.
- Their use in sepsis is currently recommended only for patients with persistent hemodynamic instability despite adequate fluid resuscitation and vasopressor support.

Synthetic Compounds With Immunomodulatory Activity

- The use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors ("statins") has been studied, but they were not found to be of benefit in patients with sepsis, acute respiratory distress syndrome (ARDS), and community-acquired pneumonia.
- Imiquimod, a Toll-like receptor 7 (TLR7) agonist, is approved for the topical treatment of external genital and perianal condylomata acuminata in patients ≥12 years old.
- Thalidomide inhibits tumor necrosis factor (TNF) production by monocytes and macrophages and may be used for the treatment of erythema nodosum leprosum except in women of childbearing age.

Cell-Based Immunomodulatory Therapy

- Mesenchymal stromal or stem cells (MSCs) are immune-privileged pluripotent cells that influence immune cell development and function via cytokine and chemokine production.
- Although not currently used outside of clinical trials, MSCs have been studied in sepsis, ARDS, and HIV.

IMMUNOMODULATION AND RISK OF INFECTION

- Anti-TNF therapy affects the immune system's ability to eradicate intracellular pathogens and those that are sequestered in granulomas, and is therefore associated with risk for TB, bacterial sepsis, invasive fungal infections such as histoplasmosis, and other systemic infections.
- Screening for latent TB and hepatitis B should be undertaken before initiation of therapy.
- Rituximab is an anti-CD20 monoclonal antibody associated with risk of active HBV and progressive multifocal leukoencephalopathy (PML), both potentially fatal, and other bacterial, fungal, and viral infections.
- Immunomodulators targeting solid tumors include checkpoint inhibitors (such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies) which release immune cells from normal inhibition and may result in significant immune-mediated adverse reactions that are treated with conventional immunosuppressive medications.
- Immunomodulators targeting hematologic malignancies include chimeric antigen receptor

SHORT VIEW SUMMARY—cont'd

(CAR) T-cell therapies and bispecific T-cell engager (BiTE) monoclonal antibodies, both of which may result in cytokine release syndromes, and anti-CD30 antibody-drug conjugate therapy, which has been linked to PML and febrile neutropenia. Inhibition of certain PI3K isoforms has been associated with CMV disease and PJP, inhibition of Janus kinases (JAKs) with reactivation of TB and HBV, and inhibition of Bruton tyrosine kinase with invasive aspergillosis, particularly of the central nervous system.

- Immunomodulators are increasingly used to treat autoimmune and connective tissue diseases, inflammatory bowel diseases, multiple sclerosis, respiratory diseases, and other conditions. Specific infectious risks depend on the agent and its immune target (see Table 49.3). Those that target T cells or certain cytokines (IL-1, IL-6, IL-17, IL-23), their receptors (IL-2R, IL-6R), or their intracellular signaling pathways (JAKs) are associated with greater risk of TB reactivation, whereas those that target B cells or integrins are associated with increased risk of development of PML. Anti-T- and anti-B-cell agents and JAK inhibitors have been associated with HBV reactivation, and all agents have been associated with a variety of other serious infections. Monoclonal antibodies targeting IgE, IL-5, or IL-5R may be associated with risk of new or worsening helminthic infection. The anti-C5 antibody eculizumab has been associated with severe and potentially fatal meningococcal infections.

Antimicrobial agents and vaccines are the traditional strategies used for treatment and prevention of infectious diseases. Although both approaches have been remarkably successful, many infectious diseases continue to pose difficult clinical problems. Treatment may be hampered by defects of the immune system resulting from an underlying disease or immunosuppressive medications, and enhancement or reconstitution of the host immune response may be a prerequisite to long-term cure. In contrast, it is the aggressive host immune response that mediates inflammation and tissue damage in syndromes such as sepsis, and in this case downregulation of the host immune response, at least in the initial stages of the disease, may be beneficial.

An immunomodulator is a biologic or nonbiologic agent that alters the host immunoregulatory response. This response results from the actions and interactions of a complex network of cells and soluble mediators from both the innate and acquired arms of the immune system. As a result, immunomodulators can affect or influence a wide variety of targets. Immunomodulators currently in use for the treatment of infectious diseases can be divided into six main groups: (1) naturally occurring proteins or glycoproteins, many of which are reproduced by recombinant DNA technology, including the colony-stimulating factors (CSFs), interferons (IFNs), interleukins (ILs), and thymic hormones; (2) immunoglobulins, used either as replacement therapy in immunoglobulin-deficient individuals or as true immunomodulators to upregulate or downregulate the immune response; (3) monoclonal antibodies (MAbs) that block the action of proinflammatory cytokines, components of the complement cascade, or the pathogen itself; (4) glucocorticosteroids that exert pleiotropic effects on components of both the innate and acquired (adaptive) immune systems; (5) synthetic compounds with immunomodulatory activity, such as the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) pentoxifylline, imiquimod, and thalidomide; and (6) immunomodulatory cell-based therapies, including mesenchymal stromal or stem cells (MSCs).

This chapter focuses on (1) agents that are used in an effort to manipulate the immune system for the treatment or prevention of infection in humans, and (2) agents used to modulate the immune system for the treatment or control of noninfectious diseases that result in increased risk of infections. A number of potentially useful immunomodulators have been investigated in vitro or in preclinical experiments involving animal models of infection. However, because of the complexity of the host immune response, in vitro data may not correlate with in vivo results, and animal models have inherent limitations that compromise their applicability to human disease. Therefore this chapter is limited to immunomodulatory agents that have been investigated in clinical trials in humans. As knowledge of the molecular pathogenesis of inflammation, immunity, and infection evolves, novel immunomodulatory therapeutics and refinement of current immunomodulatory approaches are expected to emerge and change prevention, management, and outcomes of challenging infectious diseases.

COLONY-STIMULATING FACTORS

The CSFs are a group of naturally occurring glycoproteins that regulate the production, differentiation, survival, and activation of hematopoietic cells. Erythropoietin stimulates red blood cell production and is widely used clinically for the treatment of anemia. Thrombopoietin plays a key regulatory role in the growth and differentiation of megakaryocytes. Granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and macrophage colony-stimulating factor (M-CSF) act as growth factors for specific cell types in the myeloid series and have attracted considerable interest as immunomodulators.

Granulocyte Colony-Stimulating Factor

G-CSF is a glycoprotein that acts almost exclusively on neutrophils (polymorphonuclear leukocytes) and neutrophilic precursors to promote cell growth, differentiation, and function.[1] It is produced predominantly by monocytes-macrophages, fibroblasts, and endothelial cells and serves as a potent stimulus to both increase and accelerate neutrophil production.[1] The essential role of G-CSF in normal regulation of neutrophil development has been clearly demonstrated in studies of G-CSF knockout mice, which develop chronic neutropenia. These mice have a 50% reduction in the number of granulocyte precursor cells in the bone marrow, fail to develop sepsis-related neutrophilia, and demonstrate a diminished capacity to control experimental *Listeria monocytogenes* infection.[2] Endogenous G-CSF also influences the functional activity of developing and mature neutrophils, enhancing the inducible oxidative (respiratory) burst, phagocytosis, and chemotaxis, and delaying spontaneous apoptosis.[3–5]

Recombinant G-CSF is available for clinical use in three forms: filgrastim, a nonglycosylated polypeptide produced in *Escherichia coli*; pegfilgrastim and lipegfilgrastim, pegylated forms of filgrastim with long half-lives and single-dose administration; and lenograstim, a glycoprotein produced in Chinese hamster ovary cells, which bears the greatest resemblance to the naturally occurring molecule. Biosimilar forms of both filgrastim and pegfilgrastim have been reported to be comparable in efficacy to the original (reference or originator) forms of G-CSF when used for the prevention of febrile neutropenia in patients with breast cancer receiving myelotoxic chemotherapy.[6,7] Biosimilar forms of filgrastim are approved for use in both the United States and Europe, and a biosimilar of pegfilgrastim was recently approved for use in the United States.[8] Although concerns had been raised over long-term safety and efficacy of biosimilars, particularly when used in healthy stem cell donors, guidelines from the American Society of Clinical Oncology (ASCO) include biosimilar G-CSFs for use in the prophylaxis of chemotherapy-induced febrile neutropenia, which is usually brief.[9] For the purposes of this chapter, however, only the reference forms of G-CSF are discussed.

When administered in vivo, recombinant human G-CSF initially causes a transient decrease in the peripheral blood neutrophil count, followed by a sustained dose-dependent increase that persists while serum G-CSF concentrations remain elevated.[10] After a single subcutaneous dose of G-CSF, the highest neutrophil count is reached in approximately 12 hours (for filgrastim) or 48 hours (for pegfilgrastim), after which there is a slow decline over 2 to 3 days in patients receiving filgrastim or over 1 week or longer in those receiving pegfilgrastim.[11] The increase in neutrophil count results from an enhanced rate of

granulopoiesis combined with a shortened maturation time, culminating in increased release of immature neutrophil precursors from the bone marrow. In addition, recombinant G-CSF may also produce slight increases in monocyte and lymphocyte counts and relatively modest declines in platelet counts.[12]

Because of its potent ability to increase neutrophil counts, G-CSF has emerged as an important therapeutic option for a variety of primary chronic neutropenic conditions, including severe congenital neutropenia, cyclic neutropenia, and severe forms of idiopathic neutropenia, in which the absolute neutrophil count (ANC) may be cyclically or persistently less than $0.5 \times 10^3/mm^3$.[13] In this patient population, prophylactic administration of G-CSF significantly increases the ANC, accompanied by a concomitant reduction in infection, antibiotic use, and hospitalization.[14] It should be noted, however, that although G-CSF induces granulopoiesis in patients with congenital neutropenia via activation of the nicotinamide phosphoribosyl transferase (NAMPT)/sirtuin 1 signaling pathway, GM-CSF is unable to activate this pathway and therefore is not used in the treatment of this patient population.[15]

The most common use of G-CSF is for the prevention or treatment of neutropenia after cancer chemotherapy, in which the risk of infection is related to both the degree and duration of neutropenia. Opportunistic bacterial and fungal infections continue to be a major cause of morbidity and mortality, and each episode of febrile neutropenia has been shown to carry an in-hospital mortality rate of approximately 10%.[16] Therefore G-CSF has been used to both prevent and shorten febrile neutropenic episodes.[9]

G-CSF administration for primary prophylaxis of febrile neutropenia has been studied in multiple patient populations. However, because individual randomized controlled trials (RCTs) enrolling patients with specific malignancies may be underpowered to detect any potential effect on clinically relevant outcomes such as mortality, most of the recent data are derived from meta-analyses and systematic reviews. For instance, in a systematic review of patients who received chemotherapy for solid tumors, non-Hodgkin lymphoma, or acute lymphoblastic leukemia (ALL), administration of filgrastim was found to reduce the incidence of severe neutropenia and febrile neutropenia, and in a systematic review of patients undergoing chemotherapy for breast cancer, CSF support reduced febrile neutropenia and possibly all-cause, but not infection-related, mortality.[17,18] Although primary prophylactic G-CSF has been shown repeatedly to reduce the incidence of chemotherapy-induced neutropenia and febrile neutropenia, its detectable effects on mortality have been inconsistent. A systematic review and meta-analysis of 59 RCTs with a minimum follow-up duration of 2 years found that all-cause mortality was reduced in adult patients receiving primary prophylactic G-CSF during treatment for solid tumors or lymphomas.[19] Reductions in mortality were found in subgroups of older patients and individuals in whom chemotherapy was noncurative in intent. However, mortality reductions were most evident in individuals receiving dose-dense chemotherapy or chemotherapy for lymphoma, and were not observed when the analysis included only trials in which identical chemotherapeutic regimens were used in the G-CSF and non–G-CSF arms. A second meta-analysis including only patients with lymphoma found no reduction in mortality during the chemotherapy period, although the reduction in episodes of febrile neutropenia and documented infection remained significant.[20] A large meta-analysis of 148 studies of a mixed population of adults and children undergoing chemotherapy for solid tumors, for hematologic malignancies, or as conditioning regimens before hematopoietic stem cell transplant (HSCT) and randomized to receive either primary prophylactic G-CSF/GM-CSF or placebo/no treatment reported an overall reduction in febrile neutropenia and documented infections, but no difference in infection-related or all-cause mortality.[21] Important to note, only trials in which both arms used the same chemotherapeutic or conditioning regimen were included. This study also reported a reduction in febrile neutropenia and documented infection in the subgroup of patients with leukemia, albeit in a heterogeneous population of adult and pediatric patients receiving either G-CSF or GM-CSF. In adults with acute myelogenous leukemia (AML), CSF use has been shown to reduce the duration of neutropenia and hospitalization but not the incidence of documented infections or mortality.[17,22] Similarly, children with AML who were randomized to receive G-CSF after inductions 1 and 2 experienced a shorter duration of neutropenia but no reduction in febrile neutropenia, documented infection, infection-related mortality, or 5-year event-free survival, whereas children with ALL who received CSF support experienced fewer infections but not febrile neutropenic episodes.[23,24] Finally, in a meta-analysis of primary prophylactic G-CSF/GM-CSF before neutrophil engraftment during autologous or allogeneic HSCT, CSF use was associated with a reduction in documented infection but not infection-related mortality.[25] No increase in acute or chronic graft-versus-host disease (GVHD) was observed in recipients of allogeneic HSCT.

In aggregate, the available evidence suggests that the use of CSFs for primary prevention in patients undergoing chemotherapy for solid tumors, for hematologic malignancies excluding the acute leukemias, or as conditioning therapy before HSCT, can reduce the incidence of febrile neutropenia and proven infections and may, in certain patient subgroups, reduce infection-related and all-cause mortality. Updated clinical practice guidelines from ASCO recommend that the use of a CSF as primary prophylaxis be reserved for adult patients with a 20% or greater risk of febrile neutropenia and for some patients receiving dose-dense chemotherapy.[9] Calculation of risk for febrile neutropenia should include factors specific to the patient, his or her disease, and the planned treatment, with preference given to treatments that do not require CSF support, when such therapies are available and equivalent. No recommendation could be made for or against CSF support in myelodysplastic syndrome (MDS) or AML. As with the adult population, CSF therapy in pediatric patients should likewise be limited to those with a high risk for febrile neutropenia, and to enable dose-intensive chemotherapy that has been proven to provide a survival benefit. ASCO recommends against the use of CSFs in pediatric patients with non-relapsed ALL or AML.[9] ASCO also recommends that CSFs be used after both autologous and allogeneic HSCT to reduce the duration of severe neutropenia, albeit with the caveat that the evidence, and therefore strength of the recommendation, is much greater for CSF use in autologous HSCT. Although antibiotics have also been studied for the primary prevention of febrile neutropenia, this strategy is not addressed in the ASCO guidelines. A review found only two small studies comparing CSF support versus antibiotics for the primary prophylaxis of febrile neutropenia, and based on such limited evidence, no conclusions could be drawn.[26]

Secondary prophylaxis refers to the use of a CSF to prevent neutropenia in patients who developed a neutropenic complication, typically an episode of febrile neutropenia, during a previous cycle of chemotherapy. Secondary CSF prophylaxis has been shown to reduce episodes of febrile neutropenia and hospitalization in a mixed population of patients with solid and liquid tumors, and to reduce mortality in older patients with non-Hodgkin lymphoma.[18,27] More recent studies have focused on the use of secondary CSF prophylaxis to help achieve a target dose intensity for the chosen chemotherapeutic regimen, although the clinical relevance of this outcome may vary according to underlying disease and treatment regimen.[28] Accordingly, ASCO recommends that secondary prophylaxis be initiated in patients who have experienced a neutropenic complication in a prior chemotherapy cycle in which primary prophylaxis was not used, with the caveat that dose reduction or delay may be an alternative strategy provided it does not adversely affect outcome or survival.[9]

Unlike primary, or even secondary, prophylaxis, G-CSF is not routinely recommended to treat an established episode of febrile neutropenia. A meta-analysis of 14 RCTs that included 1553 participants who received G-CSF or GM-CSF in addition to antibiotics to treat an established episode of febrile neutropenia demonstrated shorter periods of neutropenia, antibiotic use, and hospitalization among those receiving CSFs, but no difference in overall or infection-related mortality.[29] Given the lack of demonstrable improvement in outcome, guidelines from ASCO suggest that G-CSF be considered as part of a treatment regimen for febrile neutropenia only in patients at the highest risk of infectious complications or poor clinical outcomes. Examples of the latter include prolonged or profound neutropenia, older age, uncontrolled malignancy, inpatient status at diagnosis, and infection attributable to pneumonia or invasive fungus or resulting in sepsis.[9] In keeping with these criteria, G-CSF is strongly recommended as adjunctive therapy in neutropenic

patients with hematologic malignancies and mucormycosis (zygomycosis) in clinical practice guidelines developed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and European Confederation of Medical Mycology (ECMM).[30]

Finally, some investigators have reported differences among the various reference formulations of G-CSF when used in the prevention of chemotherapy-induced neutropenia. Neutrophils stimulated with lenograstim in vivo best maintain their phenotype and function when studied ex vivo, and consistent with this finding, use of lenograstim as compared with filgrastim resulted in fewer febrile episodes after initial neutrophil reconstitution (ANC $>0.5 \times 10^3/mm^3$) following high-dose cyclophosphamide-induced neutropenia before peripheral blood stem cell mobilization in patients with multiple myeloma.[31,32] Likewise, meta-analysis of five RCTs comparing once-daily filgrastim versus single-dose pegfilgrastim for the primary prevention of chemotherapy-induced neutropenia in a mixed population of patients with solid organ and hematologic malignancies demonstrated fewer episodes of febrile neutropenia with the use of pegfilgrastim.[33] Nonetheless, there is no strong evidence for clear superiority of any one agent, and they are considered equivalent (when dosed appropriately) for the purposes of the prevention of febrile neutropenia.[9]

In noncongenital, non–chemotherapy-induced neutropenia, the therapeutic benefits of G-CSF are less established. For example, in human immunodeficiency virus (HIV)–infected patients, mild-to-moderate neutropenia commonly occurs as a result of medications, opportunistic infections, and the direct impact of HIV on neutrophil production and function.[34] Neutropenia has been associated with an increased risk of bacterial infections and an increase in mortality in the setting of culture-confirmed bloodstream infections.[35] However, neutropenia has not been shown to increase overall mortality in HIV-infected patients, and G-CSF has not been rigorously studied for the prevention of neutropenia-associated infection in patients receiving the current standard of antiretroviral therapy (ART).[36] Likewise, in a small study of preterm infants with neutropenia, the addition of G-CSF to antimicrobial therapy hastened neutrophil recovery but did not improve in-hospital mortality rates.[37]

Similarly, mixed results have been seen when G-CSF has been studied as a potential therapeutic immunomodulator to enhance microbicidal activity of existing phagocytes in nonneutropenic disease states. For instance, treatment with G-CSF in addition to standard antimicrobial therapy in nonneutropenic patients with severe community-acquired pneumonia reduced neutrophil apoptosis and increased ANCs, expression of cell surface markers of neutrophil activation, and release of antiinflammatory cytokines.[38] However, a systematic review of six RCTs enrolling a total of 2018 patients with pneumonia found no reduction in 28-day mortality or length of stay in the intensive care unit.[39]

In critically ill postoperative patients, G-CSF produced enhanced oxidative burst activity and phagocytosis when neutrophils were studied ex vivo.[40] However, no improved neutrophil function was detected after administration of G-CSF to patients with systemic inflammatory response syndrome (SIRS), and no mortality benefit could be detected in studies of sepsis secondary to pneumonia, severe sepsis secondary to melioidosis, or undifferentiated septic shock.[41–43] In a meta-analysis of 12 RCTs evaluating nonneutropenic patients with sepsis who were randomized to receive either G-CSF/GM-CSF or placebo, administration of a CSF increased the likelihood of resolution of infection but not in-hospital or 28-day survival.[44] In patients with severe sepsis due to suspected melioidosis in Thailand, an RCT demonstrated longer survival but no overall mortality benefit, in contrast to data from prospectively-assembled databases from Northern Australia, in which the addition of G-CSF to standard therapy, contemporaneously with other changes to standard supportive care, reduced mortality in critically ill patients with melioidosis from 92% to 26%.[45,46]

In patients with diabetic foot infections, a meta-analysis of five small RCTs, enrolling 167 patients in total, reported that administration of G-CSF resulted in fewer surgical interventions, including amputations, but did not improve wound healing or resolution of infection.[47] However, disease severity among the enrolled patients varied greatly, from mild cellulitis to limb-threatening osteomyelitis, and guidelines from the Infectious Diseases Society of America (IDSA) concluded that there was insufficient evidence to recommend the routine use of G-CSF in these patients.[48]

TABLE 49.1 North American and European Approved Uses of Cytokines to Prevent or Treat Infection

CYTOKINE	INDICATIONS
G-CSF (filgrastim)	After myelosuppressive chemotherapy for nonmyeloid malignancies After induction or consolidation chemotherapy for AML Myeloid reconstitution after HSCT Severe chronic neutropenia in patients with congenital neutropenia, cyclic neutropenia, or idiopathic neutropenia Neutropenia in advanced HIV disease[a]
Pegylated G-CSF	After myelosuppressive chemotherapy for nonmyeloid malignancies
GM-CSF (sargramostim)[b]	After induction chemotherapy for AML in patients older than 55 yr Mobilization and after transplantation of autologous PBPCs Myeloid reconstitution after autologous HSCT for Hodgkin lymphoma, non-Hodgkin lymphoma, or ALL Following allogeneic bone marrow transplantation from an HLA-matched related donor HSCT failure or engraftment delay
IFN-α	Treatment of chronic HBV infection Treatment of chronic HCV infection Treatment of condylomata acuminata[b] Treatment of HIV-related Kaposi's sarcoma[b]
Pegylated IFN-α	Treatment of chronic HCV infection Treatment of chronic HBV infection
IFN-γ	Chronic granulomatous disease

[a]Canada and Europe only.
[b]United States only.
ALL, acute lymphoblastic leukemia; *AML,* acute myelogenous leukemia; *G-CSF,* granulocyte colony-stimulating factor; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen; *HSCT,* hematopoietic stem cell transplant; *IFN,* interferon; *PBPCs,* peripheral blood progenitor cells.

Table 49.1 summarizes the approved indications for G-CSF and other recombinant cytokines in North America and Europe.

Adverse Effects of Granulocyte Colony-Stimulating Factor

G-CSF is usually well tolerated. The most common adverse effect is mild-to-moderate bone and/or musculoskeletal pain, which is estimated to occur in at least 25% of recipients but can be controlled with acetaminophen or nonsteroidal antiinflammatory drugs (NSAIDs).[1,9,12,17,49] Other commonly reported adverse effects include mild local erythema at injection sites, headache, anemia, splenomegaly, thrombocytopenia, and asymptomatic elevations of lactate dehydrogenase and alkaline phosphatase. As a result of G-CSF–induced upregulation of osteoclast activity, osteopenia or osteoporosis has been reported in approximately 50% of patients receiving long-term G-CSF therapy.[50] More rarely, G-CSF has been associated with cutaneous neutrophilic vasculitis (Sweet syndrome), progression of autoimmune disorders, cancer chemotherapy–induced lung toxicity, severe pulmonary edema, and splenic rupture.[12]

The relationship between years of G-CSF use and subsequent leukemia remains a subject of debate. In severe congenital neutropenia, the overall cumulative incidence of MDS/AML after 15 years of G-CSF therapy was 22%, and increased to 34% in patients who required higher-than-usual doses of G-CSF.[51] However, a causal relationship has not been demonstrated, and it is possible that the specific underlying genetic cause of severe congenital neutropenia may be responsible for the elevated risk of leukemia.[13] Patients with cyclic and idiopathic neutropenia are at little risk of evolution to MDS/AML.[13]

Concern has also been raised about secondary MDS/AML in patients receiving G-CSF support during treatment for a primary malignancy, and caution has been advised for the use of G-CSF in children with

ALL for this reason. In a systematic review of 25 RCTs enrolling patients with solid tumors or lymphoma, both the relative and absolute risks of MDS/AML with G-CSF use were statistically significant at 1.92% and 0.41%, respectively.[52] Mortality, however, was significantly reduced in patients who had received G-CSF support, with relative and absolute risk reductions of 0.9% and 3.4%, respectively. There is currently no evidence that G-CSF is associated with hematologic malignancies in otherwise healthy individuals.[53,54]

Granulocyte Transfusions

Once G-CSF was established as a method of mobilizing large numbers of $CD34^+$ hematopoietic stem cells from bone marrow to peripheral blood (peripheral blood progenitor cells [PBPCs]) in healthy donors for use in HSCT, interest was rekindled in granulocyte (neutrophil) transfusions for the prevention or treatment of serious opportunistic bacterial and fungal infections in patients with neutropenia or inherited disorders of neutrophil function, including chronic granulomatous disease (CGD) and leukocyte adhesion deficiency, in which G-CSF therapy alone is inadequate. A systematic review of granulocyte transfusions in 44 neutropenic neonates with sepsis found no difference in all-cause mortality.[55] A systematic review of granulocyte transfusions for the prevention of infection in individuals with chemotherapy- or HSCT-induced neutropenia included 11 randomized or quasirandomized trials involving 653 participants and conducted between 1978 and 2006.[56] Although there were many caveats regarding risk of bias and imprecise outcome measures in the included studies, no difference was detected in 30-day all-cause mortality or 30-day infection-related mortality between those who received granulocyte transfusions and those who did not. However, bacteremia and fungemia were reduced in granulocyte recipients, as were local or systemic bacterial or fungal infections in those receiving an intermediate dose of granulocytes (1×10^{10} to 4×10^{10} per day). A separate systematic review of granulocyte transfusions for the treatment of established infection in patients with chemotherapy- or HSCT-related neutropenia identified 10 trials for inclusion, with a total enrollment of 587 individuals, conducted between 1975 and 2015.[57] In this instance there was insufficient evidence to determine the overall impact of granulocyte transfusion on 30-day all-cause mortality, but there was no detectable difference in clinical resolution of infection between those who received granulocyte transfusions and those who did not. This systematic review included the most recent randomized, controlled, open-label trial in this field, in which 114 patients with neutropenia due to chemotherapy, HSCT, or underlying bone marrow disease, and presumed, probable, or proven bacterial or fungal infection were randomized to antimicrobial therapy or antimicrobial therapy in combination with daily granulocyte transfusions until recovery of neutrophils or from infection, or to a maximum of 42 days.[58] The median dose transfused was 54.9×10^9 granulocytes per transfusion, and patients received a median of five transfusions. The primary outcome was survival and clinical response at day 42, which was met by similar numbers of patients in both arms, although patients who received higher doses of granulocytes (mean dose $\geq 0.6 \times 10^9$ per kilogram per transfusion) appeared to meet the primary outcome more often than those who received lower doses. Important to note, the study was stopped early owing to low accrual, with approximately half the target number of individuals enrolled.[58] At this time, granulocyte transfusions are not part of routine care for either the prevention or treatment of infection in neutropenic patients.

Nonetheless, granulocyte transfusions are recommended in several guidelines from the IDSA for treatment of refractory infections in patients with neutropenia, including persistent candidemia, although the authors acknowledge the low quality of evidence in support of the recommendation.[59] Likewise, clinical practice guidelines from ESCMID suggest that granulocyte transfusions may be used as adjunctive or salvage therapy in hematologic, cancer, and neutropenic patients with fusariosis (with a caveat in those who may undergo allogeneic HSCT); in HSCT recipients, neutropenic patients, and patients with CGD with scedosporiosis when used in combination with antifungal agents; and in neutropenic patients with disseminated candidiasis or candidemia.[60,61] However, in all cases it is acknowledged that the evidence for the recommendations is relatively weak and often based on small case reports or case series.[62]

Granulocyte-Macrophage Colony-Stimulating Factor

GM-CSF is a 127–amino-acid glycoprotein that is available in various recombinant forms, including sargramostim (produced in yeast), molgramostim (produced in *E. coli*), and regramostim (produced in Chinese hamster ovary cells), which differ slightly in amino-acid sequence and degree of glycosylation. The principal sources of endogenous GM-CSF are T lymphocytes, monocytes-macrophages, fibroblasts, and endothelial cells.[1] Administration of GM-CSF increases peripheral blood counts and stimulates the function of neutrophils, monocytes, and eosinophils.[1] Unlike G-CSF, GM-CSF does not play an essential role in neutrophil development: GM-CSF knockout mice have normal numbers of peripheral blood cells and bone marrow progenitors. However, GM-CSF knockout mice have impaired leukocyte function, increased susceptibility to bacterial and fungal pneumonia, and alveolar proteinosis as a result of impaired macrophage clearance of surfactant.[63,64] Similarly, neutralizing anti-GM-CSF antibodies have been associated with cryptococcal disease (particularly *Cryptococcus gattii*), nontuberculous mycobacterial infection, and idiopathic pulmonary alveolar proteinosis in humans.[65–68]

In the United States, GM-CSF is approved for human use only as sargramostim and has been used primarily as an alternative to G-CSF to prevent neutropenia and its infectious risks in oncology patients after myelosuppressive chemotherapy.[1] Sargramostim is not approved in Canada or Europe, and there are no widely available biosimilars. In healthy volunteers, GM-CSF stimulates the release of mature neutrophils from the bone marrow, resulting in an increase in ANC to 3.5 times baseline levels.[69] GM-CSF also enhances the induced neutrophil oxidative burst in vitro and induces eosinophilia when administered in vivo.

Several studies have reported differences in the clinical effects of G-CSF and GM-CSF when used for the prevention of neutropenia in patients with malignancies. After myelosuppressive chemotherapy, patients who received GM-CSF experienced a longer duration of neutropenia (14 vs. 12 days) than did those who received G-CSF.[70] In a mixed population of patients undergoing chemotherapy for solid tumors or hematologic malignancies, GM-CSF reduced the incidence of febrile neutropenia and documented infections, but less so than G-CSF.[21] However, neither CSF reduced all-cause or infection-related mortality. Compared with G-CSF, hospitalization rates for patients receiving GM-CSF for the prevention of neutropenia have been variously reported to be lower (infection related), equivalent (febrile neutropenia related), or higher (neutropenic complications including both fever and infection) depending on the outcome studied.[28,71,72] In a meta-analysis of patients with lymphoma, G-CSF, but not GM-CSF, reduced the incidence of neutropenia and documented infection; however, few included studies used GM-CSF exclusively.[20]

In post-HSCT patients, the population in whom GM-CSF has been most widely studied, GM-CSF, but not G-CSF, reduced the incidence of documented infection. Once again, however, neither reduced all-cause or infection-related mortality.[25] In contrast, cumulative mortality and transplant-related mortality were lower at 100 days in patients who received GM-CSF while neutropenic after allogeneic HSCT than in those who received G-CSF.[73] Furthermore, although there was no difference in the incidence of invasive fungal infection between the groups at 100 days, those who had received GM-CSF had lower infection-related and fungal infection-related mortality after a median follow-up of 600 days. One-year overall and disease-free survival rates did not differ between the groups. Prolonged use of GM-CSF after allogeneic HSCT may confer some antitumor activity (an effect related to GM-CSF–mediated increases in myeloid dendritic (antigen-presenting) cells and IL-12–stimulated cytotoxic T cells) but does not exacerbate GVHD.[73,74]

When used for treatment of an established episode of febrile neutropenia, GM-CSF, like G-CSF, does not reduce mortality.[75] Neither the 2006 nor the 2015 ASCO guidelines found enough evidence to make recommendations regarding the equivalency of G-CSF and GM-CSF.[9,76]

Nonetheless, like G-CSF, GM-CSF has been studied for use in non-neutropenic patients. In patients with SIRS but without hemodynamic

instability, a 72-hour course of treatment with GM-CSF in addition to standard therapy resulted in multilineage leukocyte activation and a greater number of patients with clinical or microbiologic resolution of infection.[77] However, GM-CSF had no impact on organ failure or 28-day mortality. Similarly, an RCT of a 5-day course of GM-CSF in 18 patients with sepsis and pulmonary dysfunction found no difference in the incidence of acute respiratory distress syndrome (ARDS), extrapulmonary organ failure, or 30-day mortality, despite increased superoxide production by peripheral blood neutrophils.[78] An RCT of 38 patients with severe sepsis or septic shock and sepsis-induced immunosuppression (as defined by monocytes with decreased human leukocyte antigen [HLA] class II expression) found a shorter duration of mechanical ventilation and improved APACHE II scores with GM-CSF treatment.[79] Once again, 28-day mortality was unchanged. Finally, when 132 critically ill patients with acute lung injury (ALI) or ARDS, most commonly due to sepsis and pneumonia, were randomized to receive either GM-CSF for 14 days or placebo, no difference in ventilator-free days or 28-day mortality was found between the groups.[80] Given the small numbers of enrolled patients, all of these studies should be regarded as preliminary and hypothesis generating, and conclusions regarding the use of GM-CSF in adults with sepsis should be withheld pending larger RCTs adequately powered to detect an impact on survival.

Similarly promising but inconclusive findings have emerged from studies of GM-CSF in pediatric sepsis. A meta-analysis of treatment studies using G-CSF or GM-CSF in critically ill neonates found no survival benefit (14-day mortality) with the addition of CSF support to standard antimicrobial therapy, except in the small subgroup with severe neutropenia.[81] In a relatively large RCT of 280 low-birth-weight, preterm infants, a 5-day course of prophylactic GM-CSF started within 72 hours of birth did not reduce the incidence of sepsis or mortality (within 14 days or up to hospital discharge), even among the 21% of participants who were neutropenic.[82] Results of long-term follow-up from this study (at 2 and 5 years, respectively) demonstrated no differences in neurodevelopmental, health. or educational outcomes between the infants who received GM-CSF and those who did not.[83,84] Finally, in a small study of 14 critically ill children with failure of at least three major organs and immunoparalysis (as defined by blunted tumor necrosis factor [TNF] production in response to lipopolysaccharide [LPS] ex vivo), pediatric patients who received GM-CSF had significantly fewer nosocomial infections than those who received the standard of care without CSF support.[85]

Similarly, no definitive benefit to GM-CSF treatment has been found in the other infectious diseases for which its use has been proposed. In postoperative patients in the intensive care unit, 1 to 3 days of therapy with GM-CSF did not reduce infections in the short term (up to postoperative day 9).[86] In contrast, a small RCT of 58 patients with nontraumatic generalized peritonitis who received GM-CSF for 4 days in addition to protocol-specified broad-spectrum antimicrobial therapy for at least 5 days found fewer infectious complications in those receiving GM-CSF therapy.[87] Based on ex vivo studies that demonstrated enhanced antifungal activity in human neutrophils, GM-CSF has been used, both alone and in combination with IFN-γ, as adjunctive therapy for refractory invasive fungal infections in nonneutropenic patients, and ongoing GM-CSF therapy has been used to suppress relapsing *Candida* meningoencephalitis in a patient with CARD9 (caspase recruitment domain-containing protein 9) deficiency.[88,89]

Adverse Effects of Granulocyte-Macrophage Colony-Stimulating Factor

Compared with G-CSF, GM-CSF–associated toxicity in adults is more frequent and more severe, possibly because of stimulation of proinflammatory responses in monocytes-macrophages. Fever is the most common adverse effect, often accompanied by myalgias and malaise, and occurs in more than 20% of recipients.[1] First-dose reactions, consisting of dyspnea, hypoxemia, hypotension, tachycardia, flushing, musculoskeletal pain, nausea, and vomiting, have been reported in 5% of patients receiving GM-CSF.[90] Adverse effects are more common when GM-CSF is administered intravenously and with doses greater than 3 μg/kg. High doses (20 μg/kg/day) of GM-CSF have been reported to cause a generalized capillary leak syndrome.

Macrophage Colony-Stimulating Factor

M-CSF or CSF-1 is a glycoprotein that was first cloned and produced in recombinant form in 1985. It is produced endogenously by monocytes-macrophages, fibroblasts, and endothelial cells. M-CSF acts specifically on cells of the monocyte-macrophage lineage to stimulate their production and enhance their functional activity.[91] Clinical experience with M-CSF for the treatment of infectious diseases has been limited, and it is not currently approved for human use in either the United States or Europe.

INTERFERONS

IFNs are a class of cytokines distinguished by their intrinsic antiviral properties but produced in response to a variety of stimuli, including intracellular pathogens and bacterial toxins. Although three families of IFNs have been identified in humans, this chapter will focus on the two best studied in infectious diseases in humans: type I IFNs (IFN-α and IFN-β), produced primarily by leukocytes and fibroblasts; and type II IFN (IFN-γ), produced primarily by NK cells and T lymphocytes. Whereas IFN-α and IFN-β are primarily antiviral IFNs, IFN-γ activates macrophages and contributes to host defense against intracellular pathogens.

Interferon-α

The antiviral activity of IFN-α is discussed in Chapter 4. The term *IFN-α* actually refers to a class of molecules, of which there are 12 subtypes. Currently, IFN-α is available for clinical use as recombinant IFN-alfa-2a and IFN-alfa-2b, which differ by a single amino acid; IFN-alfa-n3, which is a mixture of subtypes of IFN-α purified from human leukocytes; and IFN-alfacon-1, a bioengineered form of IFN-α based on a "consensus" amino-acid sequence of the most common forms of naturally occurring IFN-α. Pegylated forms of IFN-alfa-2a and IFN-alfa-2b have extended biologic half-lives.

IFN-α has been most widely used as treatment for chronic hepatitis B and C virus infections (see Chapters 48, 143, 145, and 154); however, it is also an effective therapy for various manifestations of human papillomavirus infection, including condylomata acuminata (genital warts) and recurrent respiratory papillomatosis.[92,93]

As an immunomodulatory agent, IFN-α has been studied as an adjunct to conventional antimicrobial agents. A combination of topical trifluorothymidine and IFN-α was reported to be effective in three HIV-infected individuals with refractory cutaneous herpes simplex virus infection, and combined use of IFN-α with foscarnet was reportedly successful in treating a single case of severe acyclovir-resistant perianal herpes simplex virus infection in an individual with advanced HIV disease.[94,95]

In virologically suppressed patients with HIV on ART, fewer patients who received pegylated IFN-α-2a had virologic failure when ART was subsequently stopped for 12 weeks than did historical control subjects.[96] In a phase II, multicenter RCT of 259 patients with HIV in whom current ART therapy was failing (based on a detectable and increasing viral load), pegylated IFN-α-2b administered subcutaneously once weekly for 4 weeks resulted in a significant decrease in plasma HIV RNA levels (mean 0.53 $\log_{10}$ copies/mL) despite an unchanged ART regimen.[97] $CD4^+$ T-cell counts were also unchanged, but 15% of patients discontinued IFN-α therapy owing to adverse events. At this time, there is no clear role for IFN-α as adjunctive treatment in HIV disease.

Given the recent outbreaks of hand, foot, and mouth disease due to enterovirus 71, there has been interest in the use of IFN-α to treat affected individuals. In an RCT of 305 non–critically ill children hospitalized with hand, foot, and mouth disease, IFN-α administered via either aerosolization or intramuscular injection reduced the time to defervescence, time to healing of skin and mucosal lesions, and stool viral load when compared with standard supportive care.[98] Clinical outcomes were not affected by route of IFN-α administration. A second clinical trial randomized 400 non–critically ill children with hand, foot, and mouth disease, primarily due to enterovirus 71 or coxsackievirus A16, to receive either IFN-α2b oral spray for 7 days or standard care and placebo spray.[99] Patients who received the IFN-α spray experienced a shorter duration of fever, oral ulcers, and rash than did those who received

placebo, although the absolute differences between groups were not large. At this time, it is not clear that the small reductions in duration of clinical symptoms (<1 day) with the use of IFNs to treat hand, foot, and mouth disease are either clinically relevant or cost-effective.

IFN-α has also been used to treat severe HSV infections in patients with heritable immunodeficiencies, such as dedicator of cytokinesis 8 (DOCK8) deficiency.[100] Finally, preliminary studies have suggested that IFN-α may have clinical benefit in human T-cell lymphotropic virus type 1 (HTLV-1)–associated myelopathy and pulmonary tuberculosis (TB), the latter in combination with standard antimycobacterial therapy.[101,102]

The major adverse effects of systemic IFN-α therapy are dose-dependent and influenza-like, with fever, headache, myalgias, arthralgias, and nausea common.[103] These symptoms are usually alleviated by pretreatment with acetaminophen or NSAIDs. At higher doses, bone marrow suppression, neuropsychiatric effects (depression, change in mental status or cognition, or seizures), and elevated serum hepatic aminotransferase levels may be observed. Rash and erythema at the injection site are also reported. Intralesional IFN-α can cause local discomfort, inflammation, and mild systemic effects.

Interferon-β

IFN-β is available as IFN-beta-1a and is approved for treatment of relapsing multiple sclerosis. It has been studied in the treatment of infectious diseases less frequently than its type I counterpart IFN-α, but has been explored for its potential antiviral properties in some small studies. For instance, an inhaled form of IFN-β was investigated for the prevention of asthma exacerbations when used at the onset of a viral upper respiratory tract infection.[104] Although not found to be significantly different from placebo in the study population as a whole, those with more severe asthma may have experienced a reduction in symptoms. IFN-β shares many of the potential adverse effects of IFN-α, including depression and suicidal ideation, seizures, liver dysfunction, cytopenias, and thrombotic microangiopathy, along with influenza-like symptoms and injection site reactions.

Interferon-γ

Recombinant human IFN-γ is available as IFN-gamma-1b. In vivo, IFN-γ is produced by $CD4^+$, $CD8^+$, and NK cells and targets monocytes, macrophages, and neutrophils, in addition to nonprofessional host defense cells such as fibroblasts, hepatocytes, astrocytes, microglia, and endothelial cells.[1] IFN-γ plays a critical regulatory role in macrophage-mediated killing and granuloma formation in response to important intracellular pathogens, including *Mycobacterium, Leishmania, Rickettsia, Legionella,* and *Chlamydia* species.[1] Individuals with autoantibodies against IFN-γ develop an adult-onset immunodeficiency syndrome characterized by multiple opportunistic infections by organisms including *Mycobacterium* species (tuberculous and nontuberculous, slow and rapid growing), *Salmonella* species, *Cryptococcus neoformans, Talaromyces marneffei (*previously *Penicillium marneffei),* and varicella-zoster virus.[105,106]

Recombinant IFN-γ is approved by the US Food and Drug Administration (FDA) for the prevention of infection in individuals with CGD, characterized by dysfunction of the NAPDH oxidase needed for the phagocytic respiratory burst (see Table 49.1). In an early clinical trial, IFN-γ–treated patients developed significantly fewer infections and required fewer days of hospitalization.[107] An observational study of 76 patients with CGD documented persistent efficacy for up to 9 years when IFN-γ was used in combination with prophylactic trimethoprim-sulfamethoxazole and itraconazole.[108] However, a more recent study reported decreasing long-term use of IFN-γ, largely due to discontinuation as a result of adverse events.[109] Although this was a nonrandomized study of a small group of patients, a prospective 5-year comparison of those receiving IFN-γ in combination with trimethoprim-sulfamethoxazole and itraconazole versus those receiving standard antimicrobial prophylaxis alone did not demonstrate differences in the rates of infections or severe infections. A subsequent meta-analysis that combined the results of this study with the original 1991 trial for a total of 163 patients found a significant reduction in severe infection with IFN-γ therapy, albeit with the caveat that standard of care with regard to antimicrobial prophylaxis differed significantly between the two trials.[110] To date, no RCTs comparing current standard antimicrobial prophylaxis alone with that with IFN-γ have been published.

Hyperimmunoglobulin E syndrome (hyper-IgE syndrome; previously known as Job syndrome) is a primary (congenital) immunodeficiency syndrome most commonly caused by mutations in the gene encoding STAT3 and characterized by recurrent skin and pulmonary abscesses, pneumonia, eczema, eosinophilia, and elevated serum IgE levels.[111] Lymphocytes of patients with hyper-IgE syndrome have a depressed response to IL-12, resulting in decreased IFN-γ production after challenge with bacterial antigens.[112] On the basis of these observations, a small, uncontrolled trial was performed, and it suggested possible improvement in the clinical course of hyper-IgE patients when treated with systemic IFN-γ therapy.[113] No RCTs have been performed in this patient population.

IFN-γ has also been administered as adjunctive therapy for infections caused by intracellular pathogens in patients without immunodeficiency. Administration of IFN-γ with pentavalent antimony in visceral leishmaniasis was reported to significantly improve the response rate compared with historical control groups treated with pentavalent antimony alone.[114] However, a prospective RCT of a 30-day course of pentavalent antimony, with or without adjunctive IFN-γ, failed to demonstrate any treatment benefit for the combination therapy.[115] Adjunctive IFN-γ has also been reported to be effective for treatment of cutaneous leishmaniasis when combined with pentavalent antimony.[116] When administered in lepromatous leprosy, IFN-γ has been shown to increase the macrophage response to *Mycobacterium leprae* and to enhance clearance of mycobacteria from skin.[117] However, a small RCT involving 21 patients with multibacillary leprosy failed to show a significant beneficial effect on clinical or microbiologic outcomes when IFN-γ was added to standard multidrug therapy.[118]

When administered as adjunctive therapy to HIV-infected patients with disseminated *Mycobacterium avium* complex infection, systemic IFN-γ therapy decreased mycobacterial load.[119] A study of HIV-negative patients with nontuberculous mycobacterial infection of the lungs found significant clinical improvement, persisting for 12 months after completion of therapy, when a 5-month course of IFN-γ was combined with standard antimycobacterial therapy.[120] An aerosolized form of IFN-γ has been used successfully in patients with antimicrobial-refractory nontuberculous mycobacterial infection of the lungs and in patients with pulmonary TB.[121,122] In a meta-analysis of studies of adjunctive IFN-γ for the treatment of TB, the aerosolized form was associated with significantly higher rates of sputum clearance both at 1 month and at the end of treatment as compared with standard therapy alone.[123] Chest radiograph improvement was also more likely in the IFN-γ–treated group. However, none of the included studies were considered to be of high methodologic quality by the authors.

Although many studies have focused on the potential usefulness of IFN-γ for the treatment of invasive fungal infections, few RCTs have been conducted. One notable exception is an RCT of two doses of adjunctive IFN-γ for the treatment of HIV-associated cryptococcal meningitis, which demonstrated significantly faster fungal clearance of the CSF with IFN-γ therapy versus amphotericin and 5FC alone.[124] There was no difference in mortality or adverse events between the groups. A small case series of eight patients with refractory invasive candidiasis or aspergillosis who were treated with IFN-γ three times weekly for 2 weeks in addition to standard antifungal therapy at the discretion of their attending physician revealed an ex vivo improvement in proinflammatory cytokine response and HLA-DR expression on monocytes, the latter a marker of immune paralysis.[125] Published case reports document the successful use of IFN-γ, in combination with G-CSF, GM-CSF, or granulocyte transfusions, to treat or stabilize refractory invasive fungal infections in patients with leukemia or persistent and prolonged neutropenia.[126,127] IFN-γ was also reported to be safe and well tolerated when used in combination with standard antifungal therapy to successfully treat disseminated fungal infections in seven renal transplant patients.[128]

Although IFN-γ has been studied as adjunctive therapy for a variety of infections and infectious syndromes, there have been no large-scale RCTs demonstrating improvement in clinically relevant end points with

the addition of IFN-γ to standard treatment regimens, and therefore its use is not typically recommended in patients other than those with CGD.

Adverse effects of systemic IFN-γ administration include fever, myalgias, and headache, all of which are common. Although IFN-γ–associated influenza-like symptoms are typically mild, decrease over time, and can usually be managed with prophylactic antipyretics, these adverse effects are largely avoided if IFN-γ is administered via inhalation.[1] Reversible neutropenia can occur; however, clinically significant hematologic abnormalities are infrequent, even in patients who have received IFN-γ therapy for years.[1]

INTERLEUKINS

A variety of IL cytokines have been studied for the treatment of infectious diseases—some for their ability to stimulate the immune system in conditions of immunodeficiency, and others for their ability to suppress excessive immune activation in situations of fulminant infection. Clinical trials involving these cytokines as immunomodulatory therapy for the treatment of infectious diseases are outlined in the following sections; however, as of the time of publication, no ILs are in routine clinical use for the treatment of infection.

Interleukin-1

The IL-1 family of cytokine agonists and receptor antagonists plays a pivotal role in multiple aspects of infection and immunity. Three molecules are currently available for clinical use in IL-1 blockade: anakinra, a recombinant human IL-1 receptor antagonist; canakinumab, an IL-1β inhibitor; and rilonacept, a bispecific anti-IL-1β antibody. Although these molecules have been used successfully for the treatment of autoimmune and autoinflammatory diseases (see Table 49.3, later), they have yet to demonstrate similar usefulness in the treatment of infection. RCTs published in 1994 and 1997 explored the use of anakinra as a treatment for severe sepsis but found no impact on overall 28-day survival.[129,130] However, preliminary data may indicate a role of IL-1 blockade in patients with sepsis and certain features of the macrophage activation syndrome. A post hoc analysis from the 1997 trial was published in 2016 and reported improved 28-day survival in the small subgroup of patients with hepatobiliary dysfunction and disseminated intravascular coagulation (DIC), mimicking findings of an eight-patient retrospective observational study of critically ill children with sepsis and features of the macrophage activation syndrome.[131,132] Trials of immunomodulators in patients with sepsis have previously been hampered by the heterogeneity of the patient population, so future studies that focus on specific subgroups may help to define those in whom blockade of a particular cytokine, such as IL-1, is of benefit.

Interleukin-2

IL-2 plays an important role in the proliferation, differentiation, and activation of lymphocytes and, in particular, promotes the development of $CD4^+CD25^+FoxP3^+$ T-regulatory cells (Tregs), and inhibits differentiation of Th17 and follicular helper T cells.[133] By doing so, IL-2 can effectively limit or downregulate the immune response. However, IL-2 can also exert proinflammatory effects, by inducing the production of other proinflammatory cytokines, stimulating the proliferation of activated B cells, and enhancing the activity of NK cells and cytotoxic T lymphocytes. Recombinant human IL-2 was first approved by the FDA in 1992 for the treatment of metastatic renal cell carcinoma and metastatic melanoma but has since been proposed as an antiinfective immunomodulatory therapy. In that role, IL-2 has been best studied as adjunctive treatment for HIV disease. Two multicenter, randomized, open-label trials were conducted in patients with HIV and varying degrees of immunodeficiency: SILCAAT (Subcutaneous IL-2 in HIV Infected Subjects with Low CD4 Counts Under Active Antiretroviral Therapy), which enrolled 1695 patients with $CD4^+$ cell counts between 50 and 299 cells/mm^3, and ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial), which enrolled 4111 patients with $CD4^+$ cells counts greater than or equal to 300 cells/mm^3.[134] In both, participants were randomized to receive ART alone or ART plus IL-2. IL-2 was administered on 5 consecutive days every 8 weeks with trial-specific doses and durations: 4.5 million international units (IU) of IL-2 twice daily for six cycles in SILCAAT and 7.5 million IU twice daily for three cycles in ESPRIT. Despite significant and sustained increases in $CD4^+$ T-cell counts in both studies, addition of IL-2 to standard ART did not change the risk of opportunistic disease, grade 4 clinical events, or death. A third randomized trial (Study of Aldesleukin With and Without Antiretroviral Therapy [STALWART]) enrolled patients with HIV and $CD4^+$ cell counts greater than or equal to 300 cells/mm^3 who had not received ART in at least a year.[135] Participants were randomized to receive no therapy, IL-2 alone, or IL-2 and a maximum of 10 days of pericycle ART beginning 2 days before IL-2 administration. The STALWART study was halted early based on results from SILCAAT and ESPRIT; however, analysis of the preliminary data demonstrated that participants receiving IL-2 therapy experienced a significantly greater increase in $CD4^+$ cell counts compared with those receiving no therapy, but also experienced a greater number of opportunistic diseases and deaths. To assess the longer-term impact of IL-2 therapy, 222 of the 267 patients initially enrolled in the STALWART study were followed for an additional 24 months.[136] No increase in adverse events was detected after discontinuation of IL-2 therapy, nor was there a detectable impact on subsequent $CD4^+$ cell count or viral load responses to ART. IL-2 has also been studied in patients with TB, hepatitis C, and hepatitis C virus (HCV)–HIV coinfection, with similarly negative results.[137–139]

Interleukin-7

IL-7 plays a key role in leukocyte development, most critically for T cells.[140] Secreted constitutively and at low levels by stromal cells, IL-7 provides continuous survival signals to T cells under normal conditions and is markedly increased during periods of lymphopenia, most notably in inverse proportion to levels of $CD4^+$ T cells. In humans, administration of recombinant human IL-7 produced a dose-dependent increase in $CD4^+$ and $CD8^+$ T cells, particularly naïve and central memory T cells, and enhanced the T-cell receptor repertoire but did not expand the $CD4^+CD25^+FoxP3^+$ Treg population.[141] IL-7 has therefore been proposed as an immune stimulant without the confounding enhancement of immunosuppressive Tregs, and in this role has been best studied in patients with HIV.

In patients who were classified as poor or nonresponders to at least 1 year of ART on the basis of $CD4^+$ T-cell counts of 101 to 400 cells/mm^3 for more than 6 months despite virologic control (plasma HIV RNA <50 copies/mL), three weekly doses of IL-7 yielded a sustained, dose-dependent increase in $CD4^+$ T cells that persisted for up to a year.[142] As expected, patients randomized to receive placebo demonstrated no such increase. However, IL-7 administration also resulted in low-level viremia in some patients. Two subsequent phase II trials, enrolling HIV-infected individuals with similar immunologic and virologic parameters (INSPIRE 2 and INSPIRE 3), were conducted with repeated cycles of three weekly doses of IL-7 (every 3 months to a maximum of three cycles in the first year) whenever $CD4^+$ T-cell counts reached <550 cells/mm^3.[143] Although the studies were terminated prematurely because of closure of the sponsoring company, leaving some cycles incomplete, IL-7 administration resulted in increased $CD4^+$ T-cell counts but also increased viral loads. Large-scale RCTs would be needed to determine the clinical impact of these results; at the time of publication, no such studies are registered on ClinicalTrials.gov.

Given its immunobiology and promising initial results in patients with HIV, IL-7 has also been studied in idiopathic $CD4^+$ lymphocytopenia; researchers conducting a phase I/IIa open-label clinical trial of 21 patients reported significant increases in circulating $CD4^+$ and $CD8^+$ T cells above baseline.[144] Similarly, a phase I study in recipients of T-cell–depleted allogeneic HSCT demonstrated increased $CD4^+$ T-cell counts (primarily effector memory T cells) and improved T-cell receptor diversity after IL-7 administration.[145] Finally, two phase II studies were initiated to explore the use of IL-7 for the restoration of lymphocyte counts in patients with septic shock and lymphopenia (ClinicalTrials.gov identifiers NCT02640807 and NCT02797431), although no results are yet available and the latter study was terminated owing to lack of IL-7 availability.

Interleukin-10

Endogenous IL-10 is produced primarily by T cells and acts to suppress the functional activity of macrophages. A clinical trial examining

recombinant human IL-10 in HIV failed to demonstrate a beneficial effect on plasma viral load or $CD4^+$ T-cell counts.[146]

Interleukin-11

IL-11 has been classified as a primarily antiinflammatory cytokine and therefore has been studied as a mediator of the excessive immune response in sepsis. In a case-control study of patients with severe sepsis and thrombocytopenia, those who received recombinant human IL-11 demonstrated lower APACHE II scores from experimental day 3 to day 14, and a significantly lower 28-day mortality rate.[147] IL-11 has also been studied in a small randomized, placebo-controlled trial of 40 patients with hematologic malignancies in which IL-11 reduced the incidence of bacteremia and polymicrobial bacteremia when administered for at least 21 days and until resolution of neutropenia.[148] It should be noted, however, that serious adverse events have been reported. In a small RCT of 13 patients undergoing allogeneic HSCT in whom IL-11 was administered for 21 days from the conditioning regimen, 5 of the 10 recipients of IL-11 died with multiorgan failure and severe, diuretic-resistant fluid retention.[149]

Interleukin-12

IL-12 is produced primarily by macrophages, dendritic cells, and B lymphocytes and regulates activation, IFN-γ production, and cytotoxicity of T cells and NK cells.[150] IL-12 appears to play a critical role in defense against intracellular pathogens, particularly *Mycobacterium* species, and has been studied for the treatment of nontuberculous mycobacterial infection, although the findings have yet to be published. In chronic hepatitis B virus (HBV) and HCV infections, IL-12 has proved clinically ineffective, and in HCV-infected patients may be associated with serious toxicity, including fulminant liver failure.[151,152] Although phase I trials of IL-12 as therapy for HIV infection did not demonstrate clinical benefit, some, but not all, studies that have used IL-12 as an adjuvant for HIV therapeutic vaccines have reported improved $CD4^+$ T-cell cytokine production in response to subsequent HIV-specific antigen stimulation ex vivo.[153–157]

THYMIC HORMONES

Although not currently approved for clinical use in North America or Europe, thymosin-α_1, produced by the thymus and capable of inducing expression of IL-1, IL-2, IFN-α, and IFN-γ, has been proposed as immunomodulatory therapy for sepsis. In a multicenter, randomized, single-blind, placebo-controlled trial of 361 critically ill patients with severe sepsis, those who received a 5-day course of thymosin-α_1 had a significantly lower rate of in-hospital, but not 28-day, mortality.[158] A phase III trial of thymosin-α_1 is currently recruiting participants and is expected to complete data collection in 2019 (ClinicalTrials.gov identifier: NCT02867267).

Based on the cytokine profile stimulated by thymosin-α_1, it has also been proposed as monotherapy or combination therapy for the treatment of chronic hepatitis B. In an early meta-analysis, patients who received 6 to 12 months of thymosin-α_1 therapy were more likely to become HBV DNA and hepatitis B e-antigen (HBeAg) negative (if positive at baseline) than were those who received placebo, but this effect was not seen until 12 months after the completion of therapy.[159] No difference was seen in normalization of liver enzymes between the groups. A more recent meta-analysis included eight studies that enrolled a total of 583 patients and compared thymosin-α_1 plus lamivudine versus lamivudine alone in patients who were HBeAg positive.[160] Patients who received thymosin-α_1 for 6 to 12 months in combination with lamivudine for 1 year were significantly more likely to normalize their liver enzymes and become HBV DNA and HBeAg negative than were those who received lamivudine alone. Consistent with the earlier meta-analysis, the virologic response was sustained for up to a year after treatment. It should be noted, however, that there are as yet no published studies of thymosin-α_1 in combination with newer antiviral agents.

IMMUNOGLOBULINS

Immunoglobulin (pooled immune globulin) therapy has existed in many forms for decades. There are preparations available for intravenous (intravenous immune globulin [IVIG]), subcutaneous, and/or intramuscular use. Pooled products are nonspecific in their composition, whereas hyperimmune products have an augmented concentration of antigen-specific antibodies. Pooled IVIG products are prepared from plasma obtained from thousands of donors per lot, and although the various preparations are thought to be therapeutically equivalent for clinical indications, the individual protocols used to isolate immunoglobulin G (IgG) and the excipients used for stabilization may render certain preparations preferable over others for an individual patient.[161] For instance, sucrose-containing products should be avoided in patients with renal dysfunction, and hyperosmolar products may be avoided in those with cardiac disease. IgA content may play a role in product selection for those with IgA deficiency (see later). With multiple variations in product processing, the composition of the particular product chosen should be matched with the particular risk factor profile for each individual patient.

One of the oldest uses of pooled, nonspecific immunoglobulin therapy is in the treatment of a variety of primary immunodeficiency disorders, including X-linked agammaglobulinemia, severe combined immunodeficiency, hyper-IgM syndrome, Wiskott-Aldrich syndrome, and common variable immunodeficiencies (CVIDs), in which pneumonia and other invasive bacterial infections are significantly reduced by immunoglobulin replacement therapy.[162] Pooled IVIG has also been studied for use in a variety of conditions in which individuals have normal immunoglobulin levels. For instance, infants and young children with HIV infection often have depressed antibody responses and an associated increased incidence of bacterial and viral infections. Infants and children with advanced HIV disease were shown to benefit from IVIG alone (in the pre-antiretroviral era) or in combination with zidovudine.[163] Since that time, however, a double-blind, crossover study of children with HIV disease well controlled with combination ART found that discontinuation of IVIG prophylaxis was safe in the short term.[164] In addition, a retrospective cohort study reported no benefit for IVIG use in the treatment of acute bacterial infections in the same patient population.[165] An HIV hyperimmune globulin product has been studied as adjunctive therapy administered with, and compared with, single-dose nevirapine in HIV-infected pregnant women and their newborns in Uganda. However, the addition of HIV hyperimmune globulin to standard nevirapine therapy was associated with an increased rate of HIV infection in neonates that was statistically significant at 2 weeks but not at 6 months. This study confirmed the negative results of a much earlier study of HIV hyperimmune globulin in a non–breast-feeding American cohort.[166,167]

In preterm or low-birth-weight infants, prophylaxis with IVIG yielded only a small reduction in the occurrence of sepsis and other serious infections, but did not affect mortality.[168] Polyclonal antistaphylococcal immunoglobulin, when administered to very-low-birth-weight infants, failed to reduce the incidence of staphylococcal or other infections.[169] When used for treatment, rather than prophylaxis, of infections in neonates, IVIG has also not been shown to improve outcome. In a landmark multicenter, placebo-controlled, randomized trial (International Neonatal Immunotherapy Study [INIS]) enrolling 3493 neonates with suspected or proven infection, the majority of whom had low birth weight, two weight-based infusions of IVIG failed to affect the incidence of sepsis (secondary outcome) or death/major disability at 2 years (primary outcome).[170] A subsequent meta-analysis of nine studies, albeit dominated by the INIS trial, also concluded that IVIG administration did not reduce in-hospital mortality or death/major disability at 2 years in infants with either proven or suspected infection, and in addition found that IgM-enriched IVIG was also ineffective in reducing in-hospital mortality in neonates with suspected infection.[171]

In adults with established sepsis, both polyclonal IVIG and IgM-enriched IVIG have been proposed as immunomodulatory adjuncts to standard care. A meta-analysis of 10 trials, enrolling 1430 adults with sepsis, found a significant reduction in mortality in those who received polyclonal IVIG when compared with those who received placebo.[172] A reduction in mortality was also seen in a pooled analysis of 7 trials that compared IgM-enriched IVIG and placebo in this patient population. However, when only those studies judged to be at low risk of bias (3 using polyclonal IVIG and 2 using IgM-enriched IVIG) were included in the analysis, the mortality benefit disappeared. Since publication of

the meta-analysis, several other studies have explored immunoglobulins for use in sepsis. A large retrospective study identified mechanically ventilated adult patients with pneumonia and septic shock from a nationwide database in Japan and found no significant difference in 28-day mortality between those who received polyclonal IVIG and those who did not, when analyzed via either the large unmatched cohorts or via 1045 propensity-matched pairs.[173] Using the same methodology, no mortality benefit was associated with IVIG use in propensity-matched pairs of mechanically ventilated patients with septic shock after emergency laparotomy for colonic perforation.[174] Given the lack of proven benefit, current guidelines recommend against the use of immunoglobulins in patients with sepsis and septic shock.[175]

In toxic shock syndromes, exotoxins such as streptococcal pyrogenic exotoxin A and toxic shock syndrome toxin 1 play key pathophysiologic roles as superantigens. In vitro studies demonstrating the ability of pooled immunoglobulin to rapidly neutralize these exotoxins suggest a potential role for IVIG therapy in clinical management, and several observational studies have reported reduced mortality with IVIG (often in combination with clindamycin) in patients with streptococcal toxic shock and/or necrotizing fasciitis (see Chapters 194 and 197).[176,177,178] However, a randomized, double-blind, placebo-controlled trial investigating IVIG therapy in adults with streptococcal toxic shock syndrome was prematurely terminated because of poor patient enrollment and failed to demonstrate a statistically significant benefit of IVIG therapy.[179] A retrospective cohort study of children with streptococcal toxic shock syndrome found no survival benefit in those patients who received IVIG, but both overall numbers and mortality were low.[180] As a result of conflicting evidence, use of IVIG in the setting of streptococcal toxic shock varies widely and is influenced by individual institutional practices.

IVIG has also been studied for host antibody replacement and immunomodulation after immunosuppression, although it is not currently used for these indications. A large systematic review and meta-analysis found no impact of IVIG on infection or mortality after HSCT.[181] Similarly, an open-label phase II study, published after the meta-analysis, of IVIG for the prevention of cytomegalovirus (CMV) infection at day 100 after allogeneic HSCT found no benefit of regular IVIG prophylaxis.[182] In a small randomized, blinded, placebo-controlled crossover trial of lung transplant recipients with hypogammaglobulinemia, monthly infusions of IVIG had no impact on bacterial or other infections during the 3-month trial period.[183]

Finally, polyvalent IVIG has been proposed as therapy for flavivirus infection. There are case reports of therapeutic IVIG use in West Nile virus (WNV) infection in immunosuppressed patients, and a double-blind, placebo-controlled randomized trial of 22 children in Nepal with suspected Japanese encephalitis found an increase in antibodies specific to the Japanese encephalitis virus, but no significant difference in mortality or neurologic recovery among the small number of participants.[184,185] It should be noted that the IVIG selected for use in the trial was obtained from blood donors in a region endemic for Japanese encephalitis virus and therefore known to have high seropositivity.

Adverse effects of IVIG are common and include headache, myalgias, arthralgias, fatigue, and malaise, all or some of which may be alleviated by slowing the infusion rate.[186] Transient acute renal insufficiency and aseptic meningitis can occur, and arterial and venous thrombotic events are being recognized with increasing frequency.[187] In individuals with complete IgA deficiency, anti-IgA antibodies have been implicated in anaphylaxis on receipt of IVIG. However, a review highlighted the lack of a definitive causal role (anaphylaxis can occur in the absence of these antibodies), and the successful receipt of IVIG by some individuals with IgA deficiency and anti-IgA antibodies.[188] In the past, IVIG has been a source of transmitted HCV infection, however, the risk is lower with viral inactivation procedures now required in the production of all IVIG products, and input plasma screening by minipool nucleic acid testing for HBV, HCV, and HIV.[189,190]

In contrast to the pooled, nonspecific IVIG, hyperimmune globulin therapy is designed to provide or replace pathogen-specific antibodies that are lacking in the recipient. Hyperimmune (high specific titer) IgG preparations are routinely available for passive prophylaxis, treatment, or prevention of vertical transmission of a number of viral and toxin-mediated infectious diseases, including CMV, hepatitis B, varicella, rabies, botulism, anthrax, and tetanus (see Chapters 316, 145, 136, 163, 245, 207, and 244). Pathogen-specific hyperimmune globulin was also found to lower both viral load and mortality in a small double-blind RCT in a subgroup of critically ill patients with 2009 pandemic influenza A (H1N1) infection who received the hyperimmune globulin within 5 days of symptom onset.[191] At the time of publication, at least two further studies were recruiting participants: a phase III RCT of antiinfluenza hyperimmune globulin for the treatment of patients hospitalized with influenza A or B (ClinicalTrials.gov identifier: NCT02287467), and a phase II RCT of antiinfluenza immune globulin for the treatment of patients hospitalized with serious influenza A (H3N2) or (H1N1) infection (ClinicalTrials.gov identifier: NCT03315104).

MONOCLONAL ANTIBODIES

Pathogen-specific MAbs are an attractive alternative to nonspecific IVIG and even hyperimmune globulin preparations, although their clinical usefulness has been somewhat hampered by cost and time required for development. Nonetheless, MAbs have been approved for use for both primary and secondary prophylaxis, and treatment, of several viral and toxin-mediated infectious diseases.

Best established among the antiinfective MAbs is palivizumab, a humanized mouse monoclonal IgG1 that binds to a conserved protein in the respiratory syncytial virus (RSV) to prevent both viral fusion with respiratory epithelial cells and cell-to-cell fusion of infected epithelial cells.[192] Palivizumab is administered as a maximum of five monthly intramuscular doses (15 mg/kg) during RSV season (typically November through March in the Northern Hemisphere) to selected high-risk infants with the goal of reducing both the severity of infection and the resulting need for hospitalization (primary data summarized in both a Cochrane Library systematic review and the technical report accompanying the American Academy of Pediatrics [AAP] guidelines).[193,194] There are some data to suggest that palivizumab prophylaxis reduces recurrent wheezing (as per parental report or recall) during the first 1 to 2 years of life depending on the population studied, but none to suggest that it affects mortality or long-term pulmonary outcome.[193,195,196] As a result, the AAP has revised its guidelines on palivizumab use several times in an attempt to better define the pediatric populations at highest risk of RSV-related morbidity. According to those guidelines, palivizumab may be considered for RSV prophylaxis during RSV season in the following high-risk pediatric populations: (1) infants born before 29 weeks, 0 days gestational age and who will be younger than 12 months at the start of RSV season; (2) preterm infants in the first year of life with chronic lung disease of prematurity, and those in the second year of life for whom medical treatment for chronic lung disease of prematurity is required in the 6 months preceding RSV season; (3) infants in the first year of life who have hemodynamically significant congenital heart disease, particularly those with acyanotic heart disease being treated for congestive heart failure (CHF) and awaiting surgery, and those with moderate-to-severe pulmonary hypertension; (4) heart transplant recipients younger than 2 years whose transplantation was performed during RSV season; (5) infants in the first year of life who have pulmonary or neuromuscular abnormalities that impair clearance of secretions; (6) children younger than 2 years who are significantly immunocompromised during RSV season; and (7) infants with cystic fibrosis and chronic lung disease or nutritional compromise in the first year of life, and those with severe lung disease or weight for length less than the 10th percentile in the second year of life. Most significantly, the use of palivizumab in otherwise healthy preterm infants born at or after 29 weeks' gestation is no longer recommended. A large retrospective nonrandomized study published after the AAP guidelines found that, among infants born between 29 and 32 weeks of gestational age, receipt of palivizumab was associated with a small but statistically significant decrease in hospitalization for RSV but a reciprocal increase (of similar magnitude) in hospitalization for bronchiolitis without RSV.[197] Palivizumab had no impact on hospitalization for preterm infants born after 32 weeks. There are insufficient data to support the use of palivizumab for the treatment of active RSV disease in either adults or children, for the prophylaxis of RSV infection in adults, or

for the prevention and control of nosocomial RSV outbreaks. Used in pediatric populations, palivizumab is well tolerated, with idiosyncratic hypersensitivity reactions being the most common serious adverse event.[198]

Bezlotoxumab is an MAb directed against TcdB, the pathogenic exotoxin B of *Clostridioides difficile* (formerly *Clostridium difficile*), and prevents binding of the toxin to host cells.[199] Endogenous anti–toxin B antibodies have been shown to be protective against recurrent *C. difficile* infection, and a single infusion of bezlotoxumab was found to reduce recurrence at 12 weeks in two large double-blind, placebo-controlled, randomized trials (MODIFY I and II) of adults with primary or recurrent *C. difficile* receiving concurrent treatment with metronidazole, vancomycin, or fidaxomicin (4% of patients).[200,201] Patients received bezlotoxumab within a median of 3 days of starting therapy for *C. difficile* infection. Of note, there was a numeric but not statistically significant reduction in recurrent infection in the prespecified subgroups of patients who received bezlotoxumab and who were infected with *C. difficile* strains associated with worse outcome, including those infected with 027 and a composite subgroup of those infected with 027, 078, or 244. However, there was a statistically significant reduction in recurrent infection in these subgroups when bezlotoxumab was administered with actoxumab, an MAb directed against *C. difficile* toxin A. Important to note, participants infected with these strains represented only a small proportion of the total study population, and actoxumab was not found to affect recurrence rates, whether used alone or in addition to bezlotoxumab, in the trial population as a whole. Bezlotoxumab was approved by the FDA in 2016 as adjunctive therapy for the prevention of recurrent *C. difficile* infection in adult patients who are already receiving antibacterial therapy against *C. difficile* and are at high risk of recurrence.[202] A caution regarding the use of bezlotoxumab in patients with CHF was included in the labeling, based on the observation that administration of bezlotoxumab was associated with increased risk of heart failure exacerbation in patients with a prior history of CHF. Because the approval for bezlotoxumab came after the completion of the most recent guidelines for the treatment of *C. difficile* infection by the IDSA and Society for Healthcare Epidemiology of America (SHEA), no recommendations regarding its role in the treatment algorithm were made.[203]

Eculizumab is a humanized MAb that binds C5 of the complement system and prevents its cleavage by C5 convertases into C5a, a potent anaphylatoxin that can mediate degranulation and phagocytosis by neutrophils, and C5b, a terminal complement component and part of the membrane attack complex responsible for cytolysis.[204] Eculizumab is currently approved in the United States and Europe for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), atypical (without *Shiga* toxin–producing *E. coli* [STEC] infection) hemolytic-uremic syndrome (aHUS), and refractory generalized myasthenia gravis in those patients who are anti–acetylcholine receptor antibody positive (see Table 49.3). During the 2011 outbreak of STEC O104:H4 infection in Europe, eculizumab therapy was used for the first time in the treatment of typical, diarrhea-positive, STEC-associated hemolytic-uremic syndrome (HUS).[205] Although administration of eculizumab was not randomized, several matched and unmatched observational studies found no reduction in dialysis, seizures, or death among those who received eculizumab versus those who received best supportive care.[206] Longer-term follow-up of children from the same outbreak (median follow-up time of 3 years) demonstrated no difference in renal outcome parameters between those who received eculizumab and those who did not.[207] Therefore eculizumab is not indicated for the treatment of STEC-induced HUS.

Raxibacumab is a human MAb directed against the protective antigen of *Bacillus anthracis* and approved by the FDA for use in adults and children for treatment of inhalational anthrax, in combination with antibacterial therapy, and for prophylaxis of inhalational anthrax in the absence of other appropriate therapy. Given the near impossibility of studies of inhalational anthrax in humans, raxibacumab was approved on the basis of preclinical animal studies and evaluation of its pharmacokinetics and safety in healthy volunteers.[208] Rabbits treated with prophylactic raxibacumab had a dose-dependent improvement in 14-day survival as compared with placebo, and cynomolgus monkeys given prophylactic raxibacumab demonstrated improvement in 28-day survival and in outcome after rechallenge 1 year later in those animals that survived the initial insult.[209] When administered for treatment, raxibacumab improved survival in both rabbits and cynomolgus macaques as compared with placebo. In a relatively large study of 76 rabbits that survived to 84 hours after receiving 200 times the median lethal dose of *B. anthracis* spores, treatment with raxibacumab in addition to levofloxacin resulted in a numerically but not statistically significant improvement in survival rate when compared with treatment with levofloxacin alone.[210] As a result, raxibacumab has been included as part of anthrax preparedness guidelines from both the Centers for Disease Control and Prevention and the AAP as adjunctive treatment to antimicrobial therapy for systemic anthrax.[211,212] A second anthrax antitoxin MAb, obiltoxaximab, has been approved by the FDA for the same indication. In a rabbit model of inhalational anthrax, use of obiltoxaximab in combination with doxycycline resulted in a numerically but not statistically significant increase in survival at 30 days.[213] Important to note, neither raxibacumab nor obiltoxaximab is expected to cross the blood-brain barrier, and therefore these agents are not expected to be effective for the treatment of meningitis in this setting.

ZMapp is a combination of three humanized chimeric MAbs that reduced mortality in nonhuman primate models of Ebola virus disease (EVD).[214] In a randomized, controlled trial of 72 patients with EVD, three weight-based intravenous infusions of ZMapp every 3 days in addition to standard of care did not meet the a priori established target for superior reduction in 28-day mortality when compared with the standard of care alone.[215] It should be noted that the trial was closed early, before the planned enrollment of 200 individuals, when the EVD outbreak in West Africa was declared over. At the time of publication, ZMapp was not approved for use in either the United States or Europe. Convalescent blood products have also been studied in EVD. A nonrandomized observational study of two infusions of ABO-compatible convalescent plasma in patients with EVD in Guinea found no significant difference in 14-day mortality as compared with usual standard of care.[216] However, levels of neutralizing antibodies in the convalescent plasma were not known before administration.

Finally, several anti-HIV MAbs have been identified and target either epitopes on the HIV envelope or on the T cell itself. Included in the first group are broadly neutralizing antibodies such as the anti-CD4 binding site antibodies VRC01 and 3BNC117, and the anti-V3 glycan supersite antibody 10-1074. In phase I and/or IIa studies, these antibodies appear to be well tolerated and capable of reducing viremia in antiretroviral naïve patients and delaying reemergence of viremia after analytic treatment interruption of ART in previously treated patients.[217–219] However, they were also associated with varying degrees of rebound viremia with antibody-resistant virus when used as monotherapy. As a result, current studies are focused on using the broadly neutralizing antibodies (1) in combination with each other—for example, several phase I studies combining 3BNC117 and 10-1074 in patients with HIV infection (ClinicalTrials.gov identifiers: NCT03526848, NCT02825797, and NCT02824536); (2) as adjuncts to antiviral therapy—for example, a phase I/II study of VRC01 in combination with ART in HIV-infected infants at 12 weeks of age or younger (ClinicalTrials.gov identifier: NCT03208231) and a phase I study of VRC01 with or without ART in adults with acute HIV infection (ClinicalTrials.gov identifier: NCT02591420); and (3) as a means of HIV prevention in at-risk individuals—for example, two phase IIb studies using VRC01 for immunoprophylaxis of HIV infection (ClinicalTrials.gov identifiers: NCT02568215 and NCT02716675).

Monoclonal antibodies that target the T cell include PRO 140, which binds to the CCR5 coreceptor, and ibalizumab-uiyk, which binds to the CD4 receptor. Both prevent entry of HIV-1 into $CD4^+$ T cells. Weekly administration of PRO 140 monotherapy was shown in a phase IIb study to prevent viral rebound at 12 weeks after analytic treatment interruption in 56.1% of patients with CCR5-tropic virus whose viral load was suppressed on their current oral antiretroviral regimen.[220] Phase III trials of PRO 140 are ongoing in virologically suppressed patients and in treatment-experienced patients with detectable viral loads despite oral ART (ClinicalTrials.gov identifiers: NCT02859961 and NCT02483078). Ibalizumab-uiyk received FDA approval in May 2018 for use, in combination with other antiretroviral medications, in the treatment of heavily treatment-experienced adults with

multidrug-resistant HIV-1 infection in whom current therapy is failing.[221] Approval was granted on the basis of a phase III open-label clinical trial (TMB-301; ClinicalTrials.gov identifier: NCT02475629) that enrolled 40 patients who received a loading dose of ibalizumab-uiyk in combination with their failing antiretroviral regimen and were then transitioned to maintenance doses of ibalizumab-uiyk every 2 weeks in combination with an optimized background antiretroviral regimen that included at least one medication to which the virus was susceptible. Virologic suppression was achieved in 83% of patients 1 week after the loading dose was administered, and remained suppressed at <50 copies/mL in 43% of patients 24 weeks later. Immune reconstitution inflammatory syndrome (IRIS) was noted in 1 patient who received ibalizumab-uiyk in combination with other ART.

GLUCOCORTICOSTEROIDS

Glucocorticosteroids have prominent antiinflammatory effects and are used to restrain an overactive host immune response contributing to the pathology of an infection. Glucocorticosteroids have proved beneficial to overall clinical outcome when combined with effective antimicrobial therapy in *Pneumocystis jirovecii* (formerly *P. carinii*) pneumonia (PJP) in HIV-infected individuals, some forms of extrapulmonary TB, and bacterial meningitis. They are recommended for selected patients with sepsis and have been studied with varying effect in other infectious disease syndromes.

In HIV-infected individuals with PJP and moderate-to-severe hypoxia (arterial Po_2 <70 mm Hg), clinical studies have conclusively demonstrated that initiation of glucocorticosteroid therapy within 72 hours after initiation of antibiotics significantly increases arterial oxygenation and decreases the incidence of respiratory failure and mortality (see Chapters 129 and 269).[222]

Adjunctive glucocorticosteroid therapy has also been used to dampen the damaging host inflammatory response in extrapulmonary TB and in paradoxical reactions to appropriate antimycobacterial therapy (see Chapters 129 and 249). A landmark RCT of adjunctive steroids for the management of tuberculous meningitis in individuals older than 14 years demonstrated that patients who received steroids in combination with antituberculous therapy had a reduced risk of death versus those who received antituberculous therapy alone.[223] A subsequent systematic review of nine RCTs enrolling a total of 1337 participants confirmed a significant survival benefit at 3 to 18 months of follow-up in those who received adjunctive steroid therapy, although no impact on disabling neurologic deficit was detected.[224] Five-year follow-up of patients enrolled in one of the included RCTs demonstrated no long-term benefit on either mortality or severe disability in those who had received steroid therapy.[225] Although only one trial in the systematic review included patients with HIV disease, results did not appear to differ between patients with and without HIV infection, and current guidelines suggest that adjunctive steroid therapy be considered in HIV-infected individuals with TB involving either the central nervous system (CNS) or the pericardium.[226] In HIV-negative individuals, steroids likely reduce the risk of death from pericarditis, whereas in HIV-infected individuals, the majority of whom were not receiving ART, steroids may reduce the risk of constrictive pericardial disease.[227] In the largest trial to explore corticosteroids for the treatment of tuberculous pericarditis, in which two-thirds of the patients had HIV disease, the reduction in constrictive pericardial disease in prednisolone-treated individuals was associated with a reduction in hospitalization.[228] However, prednisolone therapy was also associated with an increased risk of HIV-associated malignancy. Unlike in CNS or pericardial TB, adjunctive corticosteroid use in pulmonary TB has not been shown to reduce mortality.[229]

Corticosteroid therapy has been shown to benefit patients living with HIV who have experienced a paradoxical TB-associated IRIS in the setting of ART initiation. In a randomized, double-blind, placebo-controlled trial of prednisone for the treatment of non–life-threatening TB-IRIS, steroid therapy reduced the combined end point of days of hospitalization and outpatient procedures.[230] Steroid therapy, with a gradual taper, is recommended in patients with HIV and TB-associated paradoxical IRIS with significant symptoms or involving the CNS, except when concurrent Kaposi sarcoma is present or when the diagnosis of IRIS is not clear.[223] Steroid therapy is also recommended in patients with HIV and life-threatening unmasking TB-IRIS, owing to preexisting TB unrecognized at the time of initiation of ART, with the caveat that there are no objective data in this patient population. A similar caveat exists regarding the use of corticosteroids in the treatment of IRIS associated with cryptococcal meningitis in patients living with HIV. Although brief courses of steroids might be recommended by some, there are no data to support this practice, and steroids are not recommended for the treatment of cryptococcal meningitis not complicated by IRIS.[223] In a double-blind, placebo-controlled RCT of 451 patients with HIV-associated cryptococcal meningitis in Asia and Africa, those who received dexamethasone in addition to antifungal therapy (amphotericin and fluconazole) experienced a higher rate of mortality at 10 weeks and 6 months and disability at 10 weeks than did those who received antifungal therapy alone.[231] Adverse events were also more common, and fungal clearance of the CSF slower, in those who received dexamethasone, and the trial was concluded early on the basis of safety. Furthermore, no benefit to dexamethasone was detected even in a subgroup analysis of the 86 patients who had initiated ART within the 3 months before enrollment and were presumed to have unmasking IRIS.

In bacterial meningitis, the beneficial effects of adjunctive steroid therapy were first identified in 1988, in a study in which dexamethasone significantly decreased sensorineural hearing loss in infants and children with *Haemophilus influenzae* meningitis.[232] In 2002, a prospective, randomized, double-blind, multicenter trial showed that early treatment with dexamethasone (10 mg administered 15–20 minutes before or with the first dose of antibiotics, followed by 10 mg every 6 hours for 4 days) reduced mortality and improved overall clinical outcome in adults with acute bacterial meningitis, particularly pneumococcal meningitis.[233] Long-term follow-up of patients in this study demonstrated a mortality benefit in those who had received dexamethasone that persisted for a median duration of at least 13 years.[234] A subsequent systematic review, including 25 studies enrolling a total of 4121 adults and children, found that corticosteroid use reduced hearing loss and short-term neurologic sequelae, but not death, among the total mixed population of patients, but not among the subgroup of patients treated in resource-limited settings.[235] Mortality was reduced only in those with meningitis due to *Streptococcus pneumoniae*. Based on the overall evidence, adjunctive dexamethasone administered with the first dose of empirical antimicrobial therapy has become the standard of care in adults with suspected bacterial meningitis in non–resource-constrained settings.

Glucocorticosteroids were also the first immunomodulatory agents studied in patients with sepsis. A regimen of low-dose hydrocortisone and fludrocortisone reduced 28-day mortality when used in patients with adrenal insufficiency by cosyntropin test.[236] However, a second RCT in 499 patients with septic shock, 47% of whom did not have a response to the cosyntropin test, found no impact on 28-day mortality overall or in the predefined subgroup with adrenal insufficiency among patients who received hydrocortisone (50 mg intravenously every 6 hours for 5 days followed by a 6-day tapering dose).[237] A subsequent review of 27 trials enrolling a total of 3176 critically ill patients with sepsis found that steroid therapy reduced 28-day mortality, albeit with a caveat regarding the low quality of the evidence, partly based on the variety of dosing regimens trialed.[238] Subgroup analysis found a survival benefit with long-course (≥3 days), low-dose (≤400 mg/day of hydrocortisone or its equivalent) steroid therapy, but not with short-course, high-dose therapy. Important to note, reversal of shock at both day 7 and day 28 was significantly more likely in those who received steroid therapy. A second, more inclusive meta-analysis assessed trials that enrolled patients with less severe illness, anywhere along the sepsis spectrum from SIRS to septic shock, and found no impact of high- or low-dose corticosteroids on mortality.[239] Similarly, a randomized, double-blind trial of critically ill patients with sepsis found no benefit to low-dose steroid therapy for the prevention of shock within 14 days.[240] Current international consensus guidelines, therefore, recommend adjunctive low-dose hydrocortisone (200 mg/day) only in patients in whom adequate fluid resuscitation and vasopressor therapy have failed to resolve hemodynamic instability.[175] An exception to the rule against high-dose steroid therapy may be typhoid fever, in which mortality was

reduced approximately 50% by chloramphenicol plus high-dose dexamethasone (3 mg/kg followed by 1 mg/kg every 6 hours for eight doses) in a randomized, double-blind, placebo-controlled trial.[241] No trials have been conducted in the era of modern antimicrobial therapy. Glucocorticosteroid use in the treatment of patients hospitalized with community-acquired pneumonia has been an area of recent research interest. In a small RCT, patients who received dexamethasone (5 mg intravenously for 4 days) had no difference in outcome compared with those who received placebo.[242] Dexamethasone did appear to shorten the median duration of hospitalization by 1 day, but also significantly increased the incidence of hyperglycemia. Similarly, a double-blind, placebo-controlled RCT of 785 adults hospitalized for less than 24 hours with community-acquired pneumonia found that prednisone (50 mg/day for 7 days) shortened time to clinical stability and duration of hospitalization by approximately 1 day but resulted in more hyperglycemic episodes requiring insulin than did placebo.[243] A meta-analysis confirmed the reduced length of stay and increased incidence of hyperglycemia that were found in the individual trials, but also reported no reduction in all-cause mortality and an increase in readmission to hospital within 30 days due to community-acquired pneumonia.[244] Without more definitive benefit to clinical outcomes, adjunctive corticosteroid therapy is not considered current standard of care in patients hospitalized with community-acquired pneumonia.

Finally, glucocorticosteroid therapy has been used to reduce the inflammatory sequelae of a variety of other infections. A systematic review and meta-analysis of RCTs in patients aged 5 years and older who presented to either the emergency department or a primary care setting with acute tonsillitis, pharyngitis, or sore throat found that those who were treated with a single low dose of corticosteroid experienced faster pain relief and resolution than did those who received placebo.[245] Adverse events in this patient population were infrequent and similar in both the treatment and placebo groups. Glucocorticosteroids are also often administered in severe type 1 (reversal) and type 2 (erythema nodosum leprosum) reactions to prevent nerve damage in patients with leprosy, although evidence for their use in this context is limited.[246] In addition, glucocorticosteroids are recommended for the treatment of severe inflammatory syndromes, including pericarditis, rheumatologic disease, and mediastinal lymphadenitis associated with histoplasmosis.[247]

SYNTHETIC COMPOUNDS WITH IMMUNOMODULATORY ACTIVITY

The lipid-lowering HMG-CoA reductase inhibitors (statins) have been shown to modulate the immune response through a variety of mechanisms, including alteration of intracellular signaling pathways, decreased cytokine production, and changes in leukocyte trafficking and function.[248] Multiple observational studies have been published that document an association between the use of statin and risk for infection. In a large meta-analysis of published RCTs up to 2011, however, statin therapy had no demonstrable impact on the incidence of infection or infection-related mortality.[249] Nonetheless, multiple studies have been undertaken in the interim in an attempt to better define a specific population in whom statin therapy might improve outcome. Best studied are hospitalized patients with sepsis, in whom several meta-analyses have likewise documented no impact of statin therapy on mortality.[250,251] Critically ill patients with ARDS, most or all of whom have had ARDS on the basis of sepsis, have also shown no improvement in clinical outcomes including 28-day or 60-day in-hospital mortality.[252,253] The latter study followed the patient cohort for a year after enrollment, and likewise found no long-term survival benefit among those who received statins versus those who did not.[254] A retrospective cohort study of 2139 bacteremic patients reported no impact of prehospital statin use on survival after a propensity-matched analysis was performed to account for differences in clinical characteristics between the groups at baseline.[255] At the current time, therefore, there is no evidence to support the initiation of statin therapy to either prevent or treat sepsis or sepsis-associated ARDS.

Observational studies that have found an inverse relationship between statin use and pneumonia have provided the rationale for subsequent RCTs in this patient population. In a small trial of 34 patients who were hospitalized for community-acquired pneumonia, those randomized to receive statins experienced the same time to clinical stability as did patients randomized to receive placebo.[256] In a crossover randomized trial of statins in patients with severe bronchiectasis and chronic *Pseudomonas aeruginosa* colonization, patients randomized to receive statins had no change in the primary end point of cough.[257] Statins have been studied for their preventive impact on infections after stroke or transient ischemic attack (TIA), with similarly negative results.[258]

Imiquimod is a Toll-like receptor 7 agonist that upregulates IFN-α, IFN-γ, TNF, and IL-12. It is available as a cream in multiple strengths and is approved for topical treatment of external genital and perianal condylomata acuminata in patients age 12 and older (see Chapter 48). Imiquimod has not been shown to be effective for the treatment of molluscum contagiosum in children ages 2 to 12, and therefore should not be used for this indication. For the treatment of anogenital warts, topical imiquimod has been shown to be more effective than placebo, and not inferior to other patient- or provider-administered treatments. However, the authors of one systematic review considered the evidence to be of low or very low quality, with a high risk of bias in the included trials.[259,260] Topical imiquimod has also been shown to induce a clinical response in patients with high-grade human papillomavirus–induced vaginal intraepithelial neoplasia, and has also been used successfully in acyclovir-resistant anogenital herpes simplex virus infections in immunocompromised individuals.[261–263] Although not approved for this indication, topical imiquimod has also been demonstrated to enhance the serologic response to the intradermal seasonal influenza vaccine, and in a trial of older adults with a median age of 73 years was also associated with fewer hospitalizations for either influenza or pneumonia.[264]

Pentoxifylline is a methylxanthine derivative that has been shown to inhibit the production and effects of TNF. Pentoxifylline has been studied as an adjunct to antimicrobial therapy in neonates with sepsis, in whom it was found to reduce all-cause in-hospital mortality and length of hospitalization in a systematic review of several small trials.[265] Nonetheless, at least one double-blind RCT published after the systematic review found no survival benefit when pentoxifylline was added to standard therapy in preterm neonates with late-onset sepsis.[266] Pentoxifylline also continues to be studied as an adjunct to pentavalent antimony in adults with cutaneous leishmaniasis, with variable success.[267,268] At this time, pentoxifylline remains an investigational agent for the treatment of infectious diseases.

Thalidomide functions as an immunomodulator via selective inhibition of TNF production by monocytes-macrophages and is well established as an effective treatment for erythema nodosum leprosum.[269] Peripheral neuropathy is a relatively common adverse effect associated with long-term use, whereas arterial thromboembolic events and second primary malignancies are less common but more severe. The teratogenicity of thalidomide is well known, and in the United States it is available only to prescribers registered with the Risk Evaluation and Mitigation Strategies (REMS) program.

CELL-BASED IMMUNOMODULATORY THERAPY

MSC therapy is a promising candidate approach for immunomodulation in sepsis. MSCs are immune-privileged progenitor cells that can differentiate into multiple stromal or supporting cell types, produce a wide variety of cytokines and chemokines that influence immune cell maturation and function, and respond in vivo to proinflammatory stimuli.[270,271] In animal models of sepsis, intravenous administration of MSCs reduces mortality and increases bacterial clearance through enhanced phagocytic activity.[272] A systematic review and meta-analysis of all preclinical animal studies of MSCs in sepsis reported a significant reduction in mortality despite different models of sepsis and outcome measures, albeit with a substantial suspected overestimate of effect due to publication bias.[273] MSCs have already been studied for the treatment of a variety of cardiovascular diseases, and for GVHD, and have been found to be safe for intravascular administration to humans.[274] MSC therapy has been studied in phase I clinical trials for the treatment of sepsis and ARDS, much of which is sepsis-related. In a phase I

safety and dose escalation trial of nine patients with refractory septic shock and median APACHE II scores of 25, enrolled within 30 hours of admission to the intensive care unit, single intravenous infusions of varying doses of allogeneic single-donor bone marrow–derived MSCs were well tolerated, without infusion-related or serious and unexpected adverse events even in patients followed for a year after MSC infusion.[275] Sepsis-related serious and expected adverse events did occur and included bacterial and fungal infections, clinically important bleeding, arrhythmias, and pulmonary embolism. Similarly, in a phase I clinical trial of nine patients with moderate-to-severe ARDS, enrolled within 96 hours of meeting the Berlin definition for ARDS, receipt of a single intravenous infusion of allogeneic bone marrow–derived MSCs was well tolerated without demonstrable infusion-related events or serious treatment-related adverse events, although serious adverse events thought to be unrelated to treatment did occur.[276] Future studies will need to define not only the optimal dose of MSCs to deliver, but also the optimal timing of administration needed to produce a clinically significant benefit.

MSCs have also been studied in other infectious syndromes as well. In a small prospective study, that included 13 patients with HIV and $CD4^+$ T-cell counts <250 cells/mm^3 despite virologic suppression on ART, half of the patients were assigned to receive three monthly transfusions of MSCs and the other half to receive saline. MSCs were well tolerated, and were associated with increased $CD4^+$ T-cell counts.[277] Other cell products have also been explored in HIV disease. In a phase IIB, multicenter, double-blind trial of 54 patients with HIV, $CD4^+$ T-cell counts >450 cells/mm^3, and viral loads <50 copies/mL participants were randomized to receive engineered autologous dendritic cell injections or placebo every 4 weeks for 16 weeks. Monocytes were collected via leukapheresis, transformed into dendritic cells ex vivo, and engineered to express HIV viral proteins based on HIV RNA isolated from each patient's pre-ART plasma. Patients who received the engineered dendritic cells had enhanced $CD8^+$ effector/memory T-cell responses, but no change in viral load, when compared with patients who received placebo.[278] Although still in preclinical stages, an HIV-specific chimeric antigen receptor (CAR) $CD4^+$ T-cell product was able to control viral replication better than infusions of nonengineered T cells in a humanized mouse model of HIV.[279]

IMMUNOMODULATORY THERAPY AND INFECTIOUS RISKS

Immunomodulatory therapy is being used increasingly frequently by physicians in a variety of specialties for the treatment of immune-mediated diseases in rheumatology, gastroenterology, dermatology, neurology, and pulmonary medicine, and to enhance the immune-mediated antitumor response in solid tumors or target a malignant lineage of cells in hematopoietic malignancies. As a result, the population of immunosuppressed patients, and the specific iatrogenic immune deficits those patients may now possess, has grown considerably. This section of the chapter focuses on the infectious risks associated with immunomodulatory therapies that are currently approved for use in humans to treat noninfectious diseases. Conventional chemotherapeutic agents, novel agents whose primary risk relates to the infectious complications of neutropenia, and agents most commonly used in solid organ transplant or HSCT are not addressed here (see Chapters 306, 307, and 308). Instead, this chapter focuses on novel therapeutic agents that manipulate one or more aspects of the immune system to limit the impact of a noninfectious disease, and in doing so allow latent, opportunistic, or even common infections to manifest.

TNF inhibitors were one of the first groups of immunomodulatory agents to be recognized for their significant infectious risk. Anti-TNF therapy can be used for the treatment of a diverse array of noninfectious inflammatory diseases including rheumatoid arthritis, psoriatic arthritis, plaque psoriasis, polyarticular juvenile idiopathic arthritis, ankylosing spondylitis, hidradenitis suppurativa, noninfectious uveitis, ulcerative colitis, and Crohn disease, depending on the particular TNF inhibitor chosen (e.g., adalimumab, certolizumab pegol, etanercept, golimumab, or infliximab). In all cases, however, US labeling carries an FDA boxed warning about the risk of serious infections including TB, bacterial sepsis, invasive fungal infections such as histoplasmosis, and other opportunistic infections. TNF is an important proinflammatory cytokine and is crucial for the formation and activation of macrophages, phagosomes, and granulomas. Its inhibition, therefore, predisposes to infection by intracellular pathogens and pathogens that are controlled by the immune system via sequestration into granulomas.

Data for the association of TNF inhibitors and TB come largely from registries and long-term extensions of RCTs, and indicate lesser risk with screening and prophylaxis for latent tuberculosis infection (LTBI).[280,281,282] Therefore, several specialty societies have issued guidelines to minimize infectious risk with the use of TNF inhibitors. The American College of Rheumatology (ACR) currently recommends screening all patients for active or latent TB before therapy, and that immunosuppressed patients with risk factors for TB with an initial negative test result undergo repeated testing after 1 to 3 weeks.[283–285] Active TB should be treated appropriately before initiation of an anti-TNF agent, and at least 1 month of treatment for latent TB should be completed before initiation of anti-TNF therapy.[283–285] A high index of suspicion for reactivated or de novo TB should be maintained for the entire duration of anti-TNF therapy, regardless of initial screening test results. The ACR currently recommends that screening be repeated intermittently (if the initial test result is negative) if risk factors for TB infection persist.[283–285]

In addition to TB, the ACR identifies other risks with TNF inhibitor use and recommends that TNF inhibitors not be initiated or resumed in the setting of serious bacterial infection requiring antibiotics, febrile and presumed viral upper respiratory tract infection, infected skin ulcer, active and life-threatening invasive fungal infection, active herpes zoster infection, acute hepatitis B or C infection, and chronic hepatitis B or C infection with significant liver injury.[283–285] The 2015 ACR recommendations reference guidelines from the American Association for the Study of Liver Diseases (AASLD) with regard to screening for hepatitis B.[286] The AASLD recommends screening all individual who are preparing to undergo immunosuppression, whether associated with cancer chemotherapy, transplantation, or biologic or corticosteroid therapy (especially at high doses of >20 mg of prednisone or its equivalent per day) for autoimmune or inflammatory diseases, with measurement of hepatitis B surface antigen and hepatitis B core antibody. Risk stratification to guide prophylactic or preemptive therapy can then be undertaken on the basis of each individual patient's serologic profile and planned immunosuppressive regimen.

Highlighted in the AASLD recommendations is the high risk of HBV reactivation, particularly in those who are HBV surface antigen positive, with the anti-CD20 MAb rituximab (see also Table 49.3). Because of the substantial risk of reactivation, prophylactic antiviral therapy is recommended for patients who are surface antigen positive, and for those who are surface antigen negative but core antibody positive.[286] Randomized trials have demonstrated the effectiveness of prophylaxis in both groups of patients.[287,288] Important to note, the risk of HBV reactivation may extend well beyond rituximab use; HBV reactivation has been reported more than 24 months after rituximab was discontinued.[289] Rituximab carries an FDA boxed warning regarding the risk of fulminant hepatitis, hepatic failure, and death in the setting of HBV reactivation associated with therapy, and of progressive multifocal leukoencephalopathy (PML), also potentially fatal.

Other immunomodulators are outlined in Tables 49.2 and 49.3. The infectious risk associated with each immunomodulator depends on its unique target within the immune system.

For instance, the group of immunomodulators collectively referred to as checkpoint inhibitors includes anti-PD-1 antibodies and anti-CTLA-4 antibodies. These molecules differ significantly from traditional chemotherapy, which, either purposefully or as an unintended consequence, reduces the number and function of immune cells. Checkpoint inhibitors, on the other hand, exert their antitumor effects by blocking inhibitory ligands from binding with receptors, thereby releasing target cells from inhibition, and stimulating rather than inhibiting the immune response with the goal of inducing an antitumor immune response. As a result, their direct infectious risks are relatively minimal. However, by releasing immune cells from inhibitory stimuli, these agents often precipitate immune-mediated adverse reactions that in

Text continued on p. 654

TABLE 49.2 Infectious Risks Associated With Selected Immunomodulatory Therapies Used in the Treatment of Malignancy

AGENT	MODE OF ACTION	IMMUNE DEFICIT	INFECTIOUS RISKS, MIMICKERS, AND PREVENTION	FDA-APPROVED CLINICAL INDICATIONS	REFERENCES
Immunomodulators Targeting Solid Tumors					
Atezolizumab (Tecentriq) Avelumab (Bavencio) Durvalumab (Imfinzi)	Monoclonal Abs directed against PD-L1, which may be found on tumor cells or tumor-infiltrating immune cells	Block the interaction between PD-L1 and PD-1 and B7.1 receptors on T cells and APCs, thereby increasing T-cell proliferation, cytokine production, and cytotoxic T-cell function in the tumor microenvironment	UTI is the most common infection in patients with urothelial cell carcinoma with all three agents Neutropenia and febrile neutropenia are rare Immune-mediated pneumonitis, hepatitis, colitis, nephritis, endocrinopathies and other immune-mediated phenomena may occur with all three agents *Atezolizumab:* 38.4% of patients developed infection Pneumonia most common in those with lung cancer Sepsis, herpes encephalitis, and mycobacterial infection have all been reported *Durvalumab:* Sepsis, necrotizing fasciitis, and osteomyelitis have all been reported In a study of durvalumab for use in NSCLC, for which it is not currently approved, pneumonia was the most frequent grade 3 or 4 adverse event, but was not increased with durvalumab over placebo	Locally advanced or metastatic urothelial carcinoma in patients who are not eligible for cisplatin chemotherapy (atezolizumab only) or who have had disease progression during or after platinum-containing chemotherapy or within 12 mo of adjuvant/ neoadjuvant chemotherapy[a] Metastatic NSCLC in patients with disease progression during or after platinum-containing chemotherapy and EGFR or ALK-specific therapies if applicable (atezolizumab only) Metastatic Merkel cell carcinoma in patients older than 12 yr (avelumab only)[a]	290–294
Ipilimumab (Yervoy)	Monoclonal Ab against CTLA-4	Binds and blocks CTLA-4, an inhibitor of T-cell function (checkpoint blockade), leading to T-cell activation and proliferation, and reduced Treg function	Fever in >5% Immune-mediated enterocolitis (with or without bowel perforation), hepatitis, dermatitis, neuropathy, and endocrinopathy can all occur, and in many cases, infectious causes will need to be ruled out[b] High-dose corticosteroids or TNF inhibitors (in the case of colitis) may be required for treatment of immune-mediated adverse reactions, may need to be continued for months, and may predispose to other opportunistic infections such as invasive fungal infections or PJP	Unresectable metastatic melanoma in patients aged >12 yr Cutaneous melanoma with >1 mm involvement of regional lymph nodes, as an adjuvant to complete resection and total lymphadenectomy	295–298
Pembrolizumab (Keytruda) Nivolumab (Opdivo)	Monoclonal Abs against PD-1	Bind and block the PD-1 receptor, which inhibits T-cell proliferation and cytokine production (checkpoint blockade), thereby allowing for enhanced T-cell function and surveillance	Immune-mediated adverse reactions may include pneumonitis, colitis, hepatitis, endocrinopathies, dermatologic disorders (including SJS or TEN), encephalitis, or nephritis High-dose corticosteroids may be required for immune-related adverse reactions Fever reported in ≥20% with anti-PD-1 therapy Active tuberculosis has been reported during both pembrolizumab and nivolumab therapy Allogeneic HSCT after anti-PD-1 therapy may be complicated by severe acute or hyperacute graft-versus-host disease, hepatic veno-occlusive disease, or a steroid-requiring febrile syndrome Postmarketing studies have reported an association of pembrolizumab with rejection of solid organ transplants	*Pembrolizumab:* Unresectable or metastatic melanoma Metastatic NSCLC: Single-agent first-line therapy for tumors with high PD-L1 expression and no EGFR or ALK mutations, or for tumors that express PD-L1 and have progressed on or after platinum-containing chemotherapy or therapy directed against EGFR or ALK mutations In combination with pemetrexed and carboplatin for nonsquamous NSCLC[a] Recurrent or metastatic head and neck squamous cell cancer with disease progression on or after platinum-containing chemotherapy[a] Refractory Hodgkin lymphoma or relapse after three or more lines of therapy[a] Locally advanced or metastatic urothelial carcinoma if not eligible for cisplatin-containing chemotherapy[a] or in patients with disease progression during or after platinum-containing chemotherapy (and within 12 mo if adjuvant or neoadjuvant) Unresectable or metastatic microsatellite instability–high cancer (but not in pediatric patients with CNS tumors), mismatch repair deficient solid tumors that have progressed and have no alternative therapies, and mismatch repair deficient colorectal cancer that has progressed after receipt of a regimen of fluoropyrimidine, oxaliplatin, and irinotecan[a] Recurrent, locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma expressing PD-L1, with progression on two or more lines of therapy including fluoropyrimidine- and platinum-containing chemotherapy and HER2/neu-targeted therapy (where applicable)[a]	299–307

Continued

TABLE 49.2 Infectious Risks Associated With Selected Immunomodulatory Therapies Used in the Treatment of Malignancy—cont'd

AGENT	MODE OF ACTION	IMMUNE DEFICIT	INFECTIOUS RISKS, MIMICKERS, AND PREVENTION	FDA-APPROVED CLINICAL INDICATIONS	REFERENCES
				Nivolumab: Unresectable or metastatic melanoma: single agent for BRAF V600 wild-type (or BRAF V600 mutation-positive[a]) disease, or in combination with ipilimumab[a] Metastatic NSCLC with progression on or after platinum-containing chemotherapy or EGFR- or ALK-specific therapy, if applicable Advanced renal cell carcinoma, after antiangiogenic therapy Hodgkin lymphoma with relapse or progression after autologous HSCT and posttransplantation brentuximab[a]	
Talimogene laherparepvec (Imlygic)	Oncolytic virus for local, intralesional injection	HSV-1 induces lysis of tumor cells, release of antigens, and overexpression of human GM-CSF, thereby increasing $CD8^+$ T cells and reducing inhibitory Tregs	Fever and influenza-like illness reported in ≥25% Herpetic infections (mucocutaneous and keratitis) have been documented. Acyclovir or other antiviral agents may interfere with antitumor activity Necrosis of the local injection site may occur, with secondary cellulitis or systemic bacterial infection reported Live-attenuated HSV may result in disseminated infection in immunocompromised patients and should not be administered to those with primary, acquired, or iatrogenic immunodeficiency or to those who are pregnant Effective contraception is recommended for recipients of childbearing potential owing to possible risk to fetus or neonate during therapy Immunocompromised or pregnant HCWs should not prepare or administer the virus, nor contact dressings, injection sites, or body fluids of recipients Any HCW, close contact, pregnant contact, or newborn should avoid direct contact with injection sites, dressings, and body fluids of the recipient Instructions for infection control and accident exposure precautions are included in the product monograph	Melanoma with unresectable cutaneous, subcutaneous, and nodal lesions that recur after surgery, with the caveat that talimogene laherparepvec has not been shown to affect survival or visceral metastases	308, 309
Immunomodulators Targeting Hematologic Malignancies					
Axicabtagene ciloleucel (Yescarta) Tisagenlecleucel (Kymriah)	CAR T-cell therapy	Autologous $CD8^+$ T cells directed against CD19 (B-lymphocyte antigen) after genetic modification with a transgene-carrying viral vector ex vivo Results in B-cell depletion or aplasia Preceded by lymphodepletion chemotherapy Corticosteroids or tocilizumab may be used to treat CRS	CRS may occur and may be fatal[b] Infections in ≥20% with either agent Bacterial, viral, and fungal pathogens all implicated Neutropenia, hypogammaglobulinemia, or agammaglobulinemia occur and may be prolonged Febrile neutropenia is common HBV reactivation is possible given B-cell target, so screening for HBV, HCV, HIV recommended before cell collection No live virus vaccines from 2 wk (tisagenlecleucel) to 6 wk (axicabtagene) before lymphodepleting therapy and until immune recovery after CAR T-cell infusion For tisagenlecleucel, myeloid growth factors, especially GM-CSF, are not recommended within 3 wk of infusion and until CRS has resolved Sample prophylactic regimen used in a phase I/II single institution study of tisagenlecleucel: Levofloxacin and fluconazole during neutropenia, acyclovir or valacyclovir from lymphodepletion until at least 3 mo after infusion, trimethoprim-sulfamethoxazole from neutrophil recovery until at least 3 mo after infusion, and IVIG for hypogammaglobulinemia when serum IgG levels measured monthly	*Axicabtagene:* Treatment of adults with relapsed or refractory large B-cell lymphoma (but not primary CNS lymphoma) after receipt of at least two lines of systemic therapy. *Tisagenlecleucel:* Treatment of B-cell ALL, refractory or in second or later relapse, in patients up to 25 yr old	310–312

Blinatumomab (Blincyto)	Bispecific T-cell engager (BiTE) monoclonal Ab	Ab binds to CD19 on B cells, both malignant and nonmalignant, and CD3 on T cells, thereby connecting the two and upregulating cell surface adhesion molecules, proinflammatory cytokines, and cytolytic proteins, resulting in lysis of $CD19^+$ B cells	CRS may occur and may be life-threatening or fatal[b] CRS is often accompanied by fever and elevated liver enzymes, and may be accompanied by DIC, CLS, and hemophagocytic histiocytosis/macrophage activation syndrome Serious, life-threatening, or fatal infections including sepsis, pneumonia, bacteremia, and opportunistic and catheter site infections were reported in 25% Neutropenia and febrile neutropenia have been reported Live vaccine should not be administered within 2 wk of therapy or until after immune recovery following final dose	Relapsed or refractory B-cell precursor ALL in adults and children	313–315
Brentuximab vedotin (Adcetris)	CD30-directed Ab-drug conjugate, consisting of a chimeric IgG1 anti-CD30 Ab, a microtubule disrupting agent (MMAE), and a linkage molecule	Binding of brentuximab to CD30 on Reed-Sternberg cells in Hodgkin lymphoma leads to internalization of the Ab-drug conjugate, intracellular release of MMAE, disruption of the microtubule network, and ultimately, cell-cycle arrest and apoptosis CD30 is also expressed by activated T and B cells	PML has been reported, may occur as early as 3 mo after initiation of therapy, and may be fatal; further doses should be held while PML is being investigated and discontinued once diagnosed[b] Neutropenia is common, may be prolonged or severe, and may require dose delays or reductions, discontinuation, or subsequent secondary G-CSF prophylaxis Bacterial, fungal, and viral infections can occur; clinical syndromes including pneumonia, bacteremia, sepsis, and septic shock have been reported CMV reactivation and viremia have been reported In a trial of brentuximab for Hodgkin lymphoma, primary prophylaxis with trimethoprim-sulfamethoxazole, ciprofloxacin, and G-CSF was used In the trial of brentuximab for post-HSCT consolidation, prophylaxis against HSV and VZV was administered for a median duration of 11.1 mo, and prophylaxis against PJP was administered for a median of 6.5 mo MMAE is a substrate of CYP3A4/5, and therefore administration with CYP3A4 inhibitors such as ketoconazole increases MMAE exposure, and administration with CYP3A4 inducers such as rifampin reduces MMAE exposure MMAE is also a substrate for the P-glycoprotein efflux transporter	Hodgkin lymphoma in adults after failure of either autologous HSCT or two or more multidrug chemotherapeutic regimens if HSCT cannot be used Hodgkin lymphoma in adults after autologous HSCT for consolidation therapy in patients at high risk of relapse or progression Primary cutaneous anaplastic large cell lymphoma or CD30-expressing mycosis fungoides, in adults, after receipt of prior systemic therapy Systemic anaplastic large cell lymphoma in adults after failure of one or more multidrug chemotherapeutic regimens[a]	316–319
Copanlisib (Aliqopa) Idelalisib (Zydelig)	Small molecular inhibitors of phosphatidylinositol 3-kinase (PI3K) isoforms, which regulate signals from B-cell, integrin, cytokine, and chemokine receptors	Blockade of PI3Kα and PI3Kδ (copanlisib) or PI3Kδ (idelalisib) leads to disrupted intracellular signaling and B-cell apoptosis	Severe or fatal infections (including CMV and PJP),[b] hepatoxicity,[b] diarrhea/colitis,[b] pneumonitis,[b] and intestinal perforation[b] have all been documented in patients receiving idelalisib; serial monitoring of liver enzymes and blood counts is recommended Idelalisib-related noninfectious pneumonitis and/or colitis may necessitate treatment with corticosteroids Noninfectious pneumonitis may also occur with copanlisib Severe cutaneous reactions, neutropenia, and pneumonia may occur with either agent SJS and TEN have been seen with idelalisib CMV infection and viremia have been reported with idelalisib In those with a history of CMV infection or with positive serology, serial clinical and laboratory monitoring is required If infection or viremia occurs, idelalisib should be held until resolution, and if reintroduced, should be accompanied by monthly CMV PCR monitoring PJP has been reported with both agents, and documented PJP is a contraindication to resumption of idelalisib therapy, even after complete treatment of infection	*Copanlisib:* Relapsed follicular lymphoma despite at least two prior therapies[a] *Idelalisib:* With rituximab for relapsed chronic lymphocytic leukemia in patients for whom rituximab alone would also be indicated Relapsed follicular lymphoma or relapsed small lymphocytic lymphoma, despite receipt of at least two prior therapies[a] Not for use as first-line therapy in any patient or in combination with bendamustine or rituximab in follicular lymphoma	320–325

Continued

TABLE 49.2 Infectious Risks Associated With Selected Immunomodulatory Therapies Used in the Treatment of Malignancy—cont'd

AGENT	MODE OF ACTION	IMMUNE DEFICIT	INFECTIOUS RISKS, MIMICKERS, AND PREVENTION	FDA-APPROVED CLINICAL INDICATIONS	REFERENCES
			Primary prophylaxis for PJP should be given with idelalisib therapy and may be considered with copanlisib Copanlisib should be held during PJP treatment and resumed only after PJP treatment and if secondary PJP prophylaxis is provided Avoid use of either agent with strong CYP3A4 inducers such as rifampin, and monitor or dose adjust (see product monographs) if concomitant administration with strong CYP3A4 inhibitors cannot be avoided		
Elotuzumab (Empliciti)	Monoclonal Ab directed against SLAMF7	Binding of the monoclonal Ab to SLAMF7 leads to NK cell activation and destruction of SLAMF7-expressing cells	Fever, pneumonia, upper respiratory tract infection, and nasopharyngitis reported in ≥20% Opportunistic infections including fungal infections and herpes zoster have also been reported Lymphopenia is common	Multiple myeloma, in combination with lenalidomide and dexamethasone, after receipt of 1–3 prior treatment regimens	326–328
Ibrutinib (Imbruvica)	Covalent inhibitor of Bruton tyrosine kinase	Inhibition of Bruton tyrosine kinase interferes with signaling from B-cell and chemokine receptors, impairing B-cell migration and adhesion	Bacterial, viral and fungal infections may occur HBV reactivation, PML, PJP, and disseminated cryptococcosis have been documented Invasive aspergillosis, particularly of early onset and affecting the central nervous system, has also been identified in patients receiving ibrutinib Neutropenia is common and blood counts should be monitored at least monthly Coadministration with strong CYP3A inducers and inhibitors should be avoided Dose modifications according to indication are provided in the product monograph for concomitant use with azoles	Mantle cell lymphoma after one or more prior therapies[a] CLL or small lymphocytic lymphoma, including those with 17p deletion Waldenström macroglobulinemia Marginal zone lymphoma in patients who are candidates for systemic therapy and have received one or more prior anti-CD20 therapies[a] Chronic graft-versus-host disease after failure of at least one line of systemic therapy	329–332
Inotuzumab ozogamicin (Besponsa)	Anti-CD22 Ab-drug conjugate	Binding of the Ab component of inotuzumab to CD22 leads to internalization and then release of drug which subsequently induces breaks in the DNA, cell-cycle arrest, and apoptosis	Higher day 100 post-HSCT mortality, not related to relapse, hepatotoxicity, and fatal or life-threatening hepatic veno-occlusive disease have been reported[b] Neutropenia, fever, and febrile neutropenia reported in ≥20%	Relapsed or refractory B-cell precursor ALL	333, 334
Obinutuzumab (Gazyva) Ofatumumab (Arzerra) Rituximab (Rituxan) is discussed in greater detail in the body of the text	Monoclonal Ab targeting CD20 on pre-B and mature B cells Each of the three molecules binds to a unique epitope on CD20	B-cell lysis and depletion	Hepatitis B reactivation can occur in both HBsAg- and HBcAb/HBsAb-positive patients, and may be fatal[b] All patients should be tested for HBsAg and HBcAb before initiation of therapy, and those who are positive should be considered for antiviral therapy and monitored during and for several months after therapy Ofatumumab has been associated with HBV reactivation 12 mos after therapy Anti-CD20 therapy should be discontinued in the event of HBV reactivation, and treatment for HBV should be initiated There are no data to indicate whether anti-CD20 therapy can be reinstated after HBV reactivation Fatal new infection with HBV has been reported with ofatumumab PML can occur and has been fatal[b] Anti-CD20 therapy should be discontinued if PML occurs Neutropenia is common, and antimicrobial prophylaxis is recommended for severe neutropenia lasting more than 1 wk Neutropenia may be prolonged (lasting >28 days) or delayed (occurring >28 days after therapy)	*Obinutuzumab:* Previously untreated CLL in adults, in combination with chlorambucil Follicular lymphoma (relapsed or refractory to rituximab-containing therapy), in combination with bendamustine and then as monotherapy Previously untreated follicular lymphoma, stages II bulky, III, or IV, in combination with chemotherapy and followed by obinutuzumab monotherapy in those achieving at least partial remission *Ofatumumab:* Previously untreated CLL, in combination with chlorambucil, if fludarabine cannot be used Relapsed CLL, in combination with fludarabine and cyclophosphamide Extended treatment of recurrent or progressive CLL in complete or partial remission after two or more lines of therapy CLL refractory to fludarabine and alemtuzumab	335, 336, 337–342

			Bacterial, fungal, and viral infections (new and reactivated) can occur and may be serious or fatal, and anti-CD20 therapy should not be administered to those with active infection Serious adverse events, and those graded 3–5 in severity, were more common with obinutuzumab than rituximab when used for follicular lymphoma, although progression-free survival was better with obinutuzumab Trials in RA have screened for latent TB infections as per local guidelines, and excluded those with LTBI, and trials in follicular lymphoma excluded HBsAg-positive patients and included HBcAb-positive patients provided they were willing to undergo monthly HBV testing. HBsAb-positive patients were included. Live vaccines are not recommended during treatment and until B-cell recovery; at least one trial of obinutuzumab excluded patients who had received a live vaccine in the preceding 28 days Immunogenicity of any vaccine after ofatumumab is unknown		
Ruxolitinib (Jakafi) See also: Tofacitinib (Xeljanz)	Inhibitor of Janus kinase (JAK) 1 and 2	Inhibition of JAKs 1 and 2 disrupts cytokine and growth factor signaling, thereby altering immune function, in particular that of dendritic cells Proinflammatory cytokines may also be reduced	Serious bacterial, viral (PML, herpes zoster), mycobacterial, and fungal infections have been reported Therapy should not be initiated until serious infections have resolved Localized and disseminated tuberculosis has been reported with ruxolitinib Prior to therapy, patients should be assessed for risk of active or latent tuberculosis, and testing for LTBI should be performed in those at higher risk HBV DNA titers can increase during therapy with or without concomitant increases in liver enzymes; clinical guidelines for monitoring and treatment of patients with chronic HBV should be observed Neutropenia, including severe neutropenia, may occur Dose modifications are required if coadministered with strong CYP3A4 inhibitors or fluconazole ≤200 mg/day (see product monograph); use with fluconazole >200 mg/day should be avoided	Myelofibrosis, intermediate or high risk, and including primary, post–polycythemia vera, and post–essential thrombocythemia myelofibrosis Polycythemia vera with inadequate response or intolerance to hydroxyurea	343–346

[a]Accelerated approval; confirmatory clinical trials may be required to maintain this indication.
[b]FDA boxed warning.
Ab, Antibody; *ALL,* acute lymphoblastic leukemia; *APCs,* antigen-presenting cells; *ALK,* anaplastic lymphoma kinase; *CAR,* chimeric antigen receptor; *CLL,* chronic lymphocytic leukemia; *CLS,* capillary leak syndrome; *CMV,* cytomegalovirus; *CNS,* central nervous system; *CRS,* cytokine release syndrome; *CTLA-4,* cytotoxic T-lymphocyte antigen 4; *DIC,* disseminated intravascular coagulation; *EGFR,* epidermal growth factor receptor; *FDA,* US Food and Drug Administration; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *G-CSF,* granulocyte colony-stimulating factor; *HBcAb,* hepatitis B virus core antibody; *HBsAb,* hepatitis B virus surface antibody; *HBsAg,* hepatitis B virus surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HCW,* health care worker; *HSCT,* hematopoietic stem cell transplant; *HSV,* herpes simplex virus; *IgG,* immunoglobulin G; *IVIG,* intravenous immune globulin; *LTBI,* latent tuberculosis infection; *MMAE,* monomethyl auristatin E; *NSCLC,* non–small cell lung cancer; *PCR,* polymerase chain reaction; *PD-1,* programmed death receptor 1; *PD-L1,* programmed death ligand 1; *PJP, Pneumocystis jirovecii* pneumonia; *PML,* progressive multifocal leukoencephalopathy; *SJS,* Stevens-Johnson syndrome; *SLAMF7,* signaling lymphocytic activation molecule family member 7; *TB,* tuberculosis; *TEN,* toxic epidermal necrolysis; *TNF,* tumor necrosis factor; *Treg,* regulatory T cell; *UTI,* urinary tract infection; *VZV,* varicella zoster virus.

TABLE 49.3 Infectious Risks Associated With Selected Immunomodulatory Therapies in the Treatment of Nonmalignant Diseases

AGENT	MODE OF ACTION	IMMUNE DEFICIT	INFECTIOUS RISKS, MIMICKERS, AND PREVENTION	FDA APPROVED CLINICAL INDICATIONS	REFERENCES
Immunomodulators Targeting Autoimmune or Connective Tissue Diseases					
Abatacept (Orencia)	Fusion protein composed of the hinge, CH2, and CH3 domains of IgG1 and the extracellular domain of CTLA-4	By binding to costimulatory molecules (CD80 and CD86) on antigen-presenting dendritic cells, abatacept prevents T-cell activation and proinflammatory cytokine release	In clinical trials, the most commonly reported infections have been upper respiratory tract infections, nasopharyngitis, sinusitis, UTIs, cellulitis, and bronchitis, among others Pneumonia and sepsis, including fatal cases, have been observed in patients receiving abatacept Abatacept should not be initiated in the setting of active infection, and should be discontinued if a serious infection develops on therapy Patients should be screened for LTBI, and if positive, anti-TB therapy should be initiated, prior to beginning therapy with abatacept; at least one open-label clinical trial used this protocol and found no cases of active TB at 6 mo Although reactivation of TB with abatacept therapy has been reported, it appears to be rare Screening for HBV should be done before abatacept therapy, because reactivation, even in patients who are isolated HBcAb positive, has been reported; important to note, those who screened positive for HBV were excluded from initial clinical trials Abatacept should not be administered concurrently with TNF inhibitors owing to the increased risk of infection Live vaccines should not be administered during, or within 3 mo after, abatacept therapy Inactivated vaccines may be less effective during abatacept therapy; therefore vaccinations should be up-to-date prior to beginning therapy, in accordance with local guidelines	Moderate-severe active RA in adults Moderately to severely active polyarticular juvenile idiopathic arthritis in children ≥2 yr old Active psoriatic arthritis in adults	280, 347, 348, 349, 350
Anakinra (Kineret)	IL-1 receptor antagonist	By binding to the IL-1 receptor, anakinra competitively inhibits both IL-1α and IL-1β and prevents the resulting proinflammatory cascade	If serious infection occurs during therapy, anakinra should be discontinued for patients with RA; patients with NOMID may be at risk of a flare of their underlying disease and therefore a risk-benefit analysis applies Anakinra should not be initiated in patients with active infections Cellulitis, pneumonia, and bone and joint infections were reported most frequently in clinical trials of anakinra in RA; fungal (including histoplasmosis and *Candida* esophagitis), mycobacterial, and bacterial opportunistic infections have been reported in postmarketing experience Upper respiratory tract infection, sinusitis, otitis media, and nasopharyngitis were the infections most often observed in clinical trials in patients with NOMID Reactivation of TB has been reported, including after negative screening for LTBI prior to anakinra initiation; however, TB reactivation in this setting (with appropriate screening and treatment for LTBI before therapy) appears to be rare HBV reactivation with anakinra use has not been studied Neutropenia can occur and therefore blood counts should be assessed at baseline, monthly for 3 mo, and then every 3 mo for 1 yr Combination use with TNF inhibitors is not recommended owing to increased risk of infection Live vaccines should not be administered during anakinra therapy; no difference in serologic response to Td vaccine was detected between patients receiving anakinra and those receiving placebo	Moderate-to-severe active RA in adults without response to one or more prior DMARDs CAPS, specifically NOMID	280, 351–353
Belimumab (Benlysta)	Monoclonal Ab against B-lymphocyte stimulator (BLyS), previously known as BAFF	By binding to BLyS, required for B-cell survival and maturation, belimumab inhibits autoreactive B cells and B-cell differentiation into plasma cells	Serious infections reported, including at least one episode of fatal CMV pneumonia PML has been reported in patients receiving belimumab, and in some cases has been fatal; belimumab should be discontinued if PML is confirmed Live vaccines should not be administered for 30 days before, or during, belimumab therapy Upper respiratory tract infections are most commonly reported; pneumonia, cellulitis, and UTIs are among the most common serious infections reported Severe hypogammaglobulinemia occurs infrequently	Adult patients with active SLE and positive autoantibodies (ANA or dsDNA) despite standard therapy Belimumab should not be used in CNS lupus, active lupus nephritis, or in individuals receiving other biologics or intravenous cyclophosphamide, as it has not been studied in these patient populations	336, 354, 355

Brodalumab (Siliq) Ixekizumab (Taltz) Secukinumab (Cosentyx)	Monoclonal Abs directed against IL-17 or IL-17 receptor A	By binding either IL-17 or its receptor, these therapies prevent cytokine signaling and subsequent release of other proinflammatory cytokines	In clinical trials, patients receiving anti-IL-17 therapy had a higher rate of infections overall than did those receiving placebo The most commonly reported infections include upper respiratory tract infections, pharyngitis, UTIs, and oral candidiasis One instance of cryptococcal meningitis has been reported with brodalumab Perform a risk-benefit evaluation before initiating anti-IL-17 therapy in patients with chronic or recurrent infections, and suspend anti-IL-17 therapy until infection resolves if serious infection develops on therapy Patients should be screened for LTBI before initiation of therapy with anti-IL-17 agents, and if positive, treatment for LTBI should be started before anti-IL-17 therapy is administered Active TB is a contraindication to anti-IL-17 therapy, and patients should be monitored for active TB during and after therapy With ixekizumab or secukinumab, empirical anti-TB therapy may be considered for those with a past history of TB or LTBI but without clear documentation of adequate treatment Neutropenia can occur with anti-IL-17 therapy Live vaccines should not be administered to patients receiving anti-IL-17 therapy; response rates to inactivated vaccines administered during anti-IL-17 therapy may be reduced, so all age-appropriate recommended, inactivated vaccinations should be completed before therapy as per current guidelines	Moderate-severe plaque psoriasis in adults who are candidates for systemic therapy or phototherapy (ixekizumab and secukinumab) and have not responded adequately to other systemic therapy (brodalumab) Active psoriatic arthritis in adults (secukinumab only) Active ankylosing spondylitis in adults (secukinumab only)	356–361
Canakinumab (Ilaris)	See later				
Guselkumab (Tremfya)	Monoclonal Ab directed against IL-23	Guselkumab prevents binding of IL-23 to its receptor and limits the proinflammatory immune response that would otherwise result	Upper respiratory tract infections, gastroenteritis, tinea infections, and HSV infections have been reported in ≥1% and more commonly than placebo Guselkumab should be discontinued in the event of serious infection Patients should be evaluated for TB before beginning therapy; active TB is a contraindication to guselkumab, and treatment for LTBI should be initiated before beginning therapy Anti-TB therapy may be considered in those with a history of LTBI or active TB in whom an adequate treatment course cannot be confirmed Use of live vaccines should be avoided during therapy; there are no data on the immunogenicity of inactivated vaccines during treatment with guselkumab	Moderate-severe plaque psoriasis in adults who are candidates for systemic therapy or phototherapy	362
Sarilumab (Kevzara)	Monoclonal Ab directed against the IL-6 receptor	Sarilumab binds to soluble and membrane-bound IL-6 receptors, thereby inhibiting the effect of proinflammatory IL-6 on immune effector cells	Serious, life-threatening, or fatal bacterial, viral, fungal and other opportunistic infections have been reported, often during coadministration with corticosteroids, methotrexate, or other immunosuppressive medications; sarilumab should be interrupted if these occur[a] Sarilumab should not be used with other biologic DMARDs owing to risk of additive immunosuppression Invasive candidiasis and PJP have been reported; fungal infections may be disseminated[a] Sarilumab should not be started in patients with an active infection Testing (and initiation of treatment) for LTBI should occur before therapy; pulmonary or extrapulmonary TB has been reported during therapy[a] Treatment for LTBI should be considered if there is a past history of latent or active TB for which adequate treatment cannot be confirmed, or in those with risk factors for TB despite a negative result of testing for LTBI Retest for LTBI during therapy[a] (frequency not specified) Cellulitis and pneumonia are among the most common serious infections Herpes zoster has been reported during therapy with sarilumab Patients at risk for HBV reactivation were excluded from clinical trials, so risk remains unknown Neutropenia may occur; neutrophils should be monitored prior to therapy, 4–8 wk after initiation, and every 3 mo thereafter GI perforation has been reported Live vaccines should not be administered to patients receiving sarilumab Interaction with CYP enzymes is possible, requiring monitoring of coadministered CYP substrates	Moderate-severe active rheumatoid arthritis in which one or more DMARDs have been ineffective or intolerable	363–365

Continued

TABLE 49.3 Infectious Risks Associated With Selected Immunomodulatory Therapies in the Treatment of Nonmalignant Diseases—cont'd

AGENT	MODE OF ACTION	IMMUNE DEFICIT	INFECTIOUS RISKS, MIMICKERS, AND PREVENTION	FDA APPROVED CLINICAL INDICATIONS	REFERENCES
Tocilizumab (Actemra)	Monoclonal Ab directed against IL-6	Tocilizumab binds to both soluble and membrane-bound IL-6, thereby inhibiting its various proinflammatory functions	Life-threatening or fatal infections with mycobacteria (pulmonary or extrapulmonary TB), bacteria, fungi, and viruses have occurred, as have other opportunistic infections, in patients receiving tocilizumab[a] Fungal infections may be disseminated rather than localized, and have included candidiasis, aspergillosis, cryptococcosis, and PJP Tocilizumab should be suspended if an infection develops during therapy, at least until the infection is controlled[a] Tocilizumab should not be started during active infection, even if the infection is localized Patients should be screened for LTBI before therapy, and if result is positive, anti-TB treatment should be initiated before tocilizumab is administered[a] Consider empirical anti-TB treatment in patients with a past history of latent or active TB in whom adequate therapy cannot be confirmed All patients should be monitored for active TB throughout therapy, even in the setting of prior negative test for LTBI[a]; active pulmonary TB after negative screening at the onset of therapy has been reported with tocilizumab Per the product monograph, consider the risk-benefit profile when proposing tocilizumab for use in patients who have chronic or recurring infection, prior severe or opportunistic infection, prior exposure to TB or risk factors for TB or endemic mycoses, or coexisting conditions that increase risk of infection The most common infections observed during tocilizumab therapy are upper respiratory tract infection or nasopharyngitis; the most common serious infections include pneumonia, UTIs, cellulitis, and herpes zoster GI perforation has been reported after diverticulitis Neutropenia may occur, although it has not commonly been associated with infection in patients receiving tocilizumab Patients with HBV and HCV were excluded from clinical trials, so impact on these infections is unknown; tocilizumab is not recommended in those with active hepatic disease or impairment Live vaccines are contraindicated with tocilizumab; patients who received live vaccines within 4 wk of planned study entry were excluded from clinical trials Tocilizumab is presumed, but has not been shown, to reduce the efficacy of other, inactivated vaccines	Moderate-severe active RA in adults without sufficient response to one or more DMARDs Giant cell arteritis in adults Active polyarticular or active systemic juvenile idiopathic arthritis in children >2 yr old	366–370, 371, 372
Tofacitinib (Xeljanz) See also: Ruxolitinib (Jakafi)	Inhibitor of Janus kinases (JAKs), specifically JAK1 and JAK3, and to a lesser extent JAK2	By inhibiting JAKs, which are associated with intracellular signaling of receptors for IL-2, IL-6, IL-7, and IL-12, tofacitinib prevents cytokine signaling and limits lymphocyte activation, differentiation, and function	Tuberculosis (pulmonary, extrapulmonary, or disseminated), bacterial, fungal, viral, and other opportunistic infections, some life-threatening or fatal, have occurred during therapy with tofacitinib; therapy should be interrupted until the infection is controlled[a] Therapy should be interrupted for any active, serious infection, even if localized All patients should be screened for LTBI before starting tofacitinib; if result is positive, treatment for LTBI should be initiated before tofacitinib is started[a] Patient who have had LTBI or TB with unclear treatment or who have risk factors for TB despite a negative test for LTBI may also be considered for empirical anti-TB therapy All patients should be monitored for TB throughout therapy, regardless of initial LTBI screening results[a] Median time to diagnosis of active TB from start of tofacitinib therapy was 10 mo Per the product monograph, consider the risk-benefit profile when proposing tofacitinib for use in patients who have chronic or recurring infection, prior severe or opportunistic infection, prior exposure to TB or risk factors for TB or endemic mycoses, or coexisting conditions that increase risk of infection, including chronic or interstitial lung disease or lymphopenia Fungal infections have included PJP and cryptococcosis, and disseminated disease is possible Other reported opportunistic infections have included esophageal candidiasis, CMV, BK virus infection, and listeriosis Patients with HBV or HCV were excluded from clinical trials; however, HBV reactivation has been reported	Moderate-severe active RA in adult patients for whom methotrexate cannot be tolerated or has not been sufficiently effective	373–378

			Screening for HBV and HCV should occur before tofacitinib treatment The most commonly observed serious infections in patients receiving tofacitinib included pneumonia, cellulitis, herpes zoster, and UTIs; one large retrospective study found a higher incidence of herpes zoster with tofacitinib than with other biologic DMARDs in patients with RA Lymphopenia may occur and may be associated with increasing risk of infection in a dose-dependent manner; tofacitinib should not be started or continued in severe lymphopenia Neutropenia may also occur Blood counts should be monitored at baseline, at weeks 4–8, and every 3 mo thereafter EBV-associated PTLD has been reported more frequently in renal transplant recipients who receive tofacitinib in combination with other immunosuppressive medications[a] Tofacitinib should not be used in combination with biologic DMARDs or other potent immunosuppressive medications, such as azathioprine or cyclosporine Live vaccines should not be administered to patients receiving tofacitinib; dissemination of vaccine strain varicella occurred in a varicella-naïve patient 16 days after vaccination with the live-attenuated zoster vaccine and 2 days after first receipt of tofacitinib Tofacitinib has several drug-drug interactions, including with antiinfective agents such as rifampin, fluconazole, or ketoconazole, for which dose modification may be required in the setting of concomitant therapy		
Ustekinumab (Stelara)	Monoclonal Ab directed against a shared subunit of both IL-12 and IL-23	By inhibiting IL-12 and IL-23, ustekinumab interferes with NK cell activation and $CD4^+$ T-cell differentiation, thereby limiting the immune response	Serious bacterial, fungal and viral infections have been reported with ustekinumab; listeria meningitis and ophthalmic HSV occurred in one patient each in the trials of Crohn disease Ustekinumab should not be started in the presence of significant active infection; if infection develops during treatment, consideration should be given to holding ustekinumab until resolution Genetic deficiencies in IL-12/IL-23 have been associated with infections due to mycobacteria, BCG (vaccinations), and salmonella; the risk of these infections in association with ustekinumab use is unknown Patients should be evaluated for LTBI, and if positive, should begin treatment for LTBI before starting therapy with ustekinumab; anti-TB therapy may be considered empirically in patients with a history of LTBI or active TB in whom prior treatment is unconfirmed In clinical trials, one trial required LTBI therapy to start 3 wk before ustekinumab; the remainder allowed LTBI therapy to begin at or before the first dose of ustekinumab LBTI therapy with isoniazid was well tolerated among patients receiving ustekinumab in the context of clinical trials for psoriasis Patients receiving ustekinumab should be monitored closely for active TB regardless of treatment; reactivation of LTBI in the setting of ustekinumab use despite negative TB skin testing and interferon-γ release assay prior to start of ustekinumab has been reported Patients with HBV, HCV, HIV, or prior nontuberculous mycobacterial or other opportunistic infection were excluded from clinical trials, so impact of ustekinumab in these situations is unknown; a small study documented HBV reactivation in two of seven HBsAg positive patients not receiving antiviral therapy Live vaccines are contraindicated with ustekinumab use, and clinical trials excluded patients with receipt of live vaccine within 12 wk of the planned initiation of ustekinumab therapy, and in at least one trial it was stipulated that live vaccines could not be used in the year following last administration of ustekinumab Also in keeping with clinical trial protocols, BCG vaccines are contraindicated for 1 yr before and 1 yr after therapy with ustekinumab Concern exists about the potential risk of shedding of live vaccines from close household contacts All recommended vaccines should be up-to-date before beginning therapy with ustekinumab as serologic response to vaccination during therapy may not be protective	Moderate-severe plaque psoriasis in patients ≥12 yr old who are candidates for phototherapy or systemic therapy Active psoriatic arthritis in adults ± methotrexate Moderate-severe active Crohn disease in adults who fail to respond to or tolerate one or more immunomodulators, corticosteroids, or TNF inhibitors	379–384

Continued

TABLE 49.3 Infectious Risks Associated With Selected Immunomodulatory Therapies in the Treatment of Nonmalignant Diseases—cont'd

AGENT	MODE OF ACTION	IMMUNE DEFICIT	INFECTIOUS RISKS, MIMICKERS, AND PREVENTION	FDA APPROVED CLINICAL INDICATIONS	REFERENCES
Immunomodulators Targeting Inflammatory Bowel Diseases					
Natalizumab (Tysabri)	See later				
Ustekinumab (Stelara)	See earlier				
Vedolizumab (Entyvio)	Monoclonal Ab directed against the α4β7 integrin	Vedolizumab binds to the α4β7 integrin to prevent its interaction with MAdCAM-1, thereby blocking the migration of a subset of memory T cells into the GI tract	Vedolizumab should not be started in patients with active, severe infections until those infections are controlled, caution should be used before initiating vedolizumab in patients with severe and recurring infections, and vedolizumab should be held if severe infections develop during therapy PML, including fatal episodes, have occurred during treatment with natalizumab, another integrin antagonist; although PML has not been observed in patients treated with vedolizumab and followed for up to 5 yr, PML remains a theoretical risk Upper respiratory tract infections and nasopharyngitis are the most commonly reported infections with vedolizumab; however, serious infections have included anal abscess, sepsis (at times fatal), salmonellosis, *Listeria* meningitis, tuberculosis (all considered primary infections), giardiasis, and CMV colitis Vedolizumab product monograph advises to consider screening for tuberculosis; all patients in clinical trials were screened Live vaccines should be administered concurrently with vedolizumab only if the benefit outweighs the risk; vaccination before therapy is preferred Owing to additive infectious risk, vedolizumab should not be administered with natalizumab or anti-TNF therapy	Moderate-severe active ulcerative colitis or Crohn disease in adults who have not responded adequately or cannot tolerate a TNF inhibitor, other immunomodulatories, or corticosteroids, or who are dependent on corticosteroid therapy	385, 386
Immunomodulators Targeting Multiple Sclerosis					
Daclizumab (Zinbryta)	Monoclonal Ab directed against the IL-2 receptor	Daclizumab binds to CD25, a subunit of the Il-2 receptor, and is presumed to mediate IL-2-induced activation of lymphocytes	Severe liver injury, including autoimmune hepatitis and hepatic failure can occur[a]; daclizumab is therefore contraindicated in those with preexisting hepatic disease or impairment (AST or ALT >2× ULN) Use other hepatotoxic medications with caution Immune-mediated dermatologic disorders, colitis, hemolytic anemia, and lymphadenopathy may occur and may require corticosteroid or other immunosuppressive therapy[a] Infections and serious infections are increased over placebo Upper respiratory tract infections and pharyngitis among most commonly reported adverse events CMV hepatitis, CMV pneumonia, and TB have occurred in patients receiving daclizumab Screening for TB, HBV, and HCV recommended before therapy, and daclizumab should not be administered in those with active, severe infection Live vaccines should not be administered during and up to 4 mo after therapy	Relapsing MS in adults, usually after lack of response to two or more other medications	387–389
Fingolimod (Gilenya)	Sphingosine 1-phosphate receptor modulator	Binding of fingolimod-phosphate (after metabolism by sphingosine kinase) to sphingosine 1-phosphate receptors blocks lymphocytes from exiting lymph nodes and reduces peripheral blood lymphocyte counts	Infection can occur up to 2 mo after discontinuation of fingolimod, and fingolimod should not be initiated in the setting of active infection PML may occur, usually in patients receiving fingolimod for ≥2 yr, although the relationship between treatment duration and risk of PML is not known; fingolimod should be discontinued during evaluation, diagnosis, and management of PML Influenza was reported in ≥10% of patients receiving fingolimod, and at higher rates than in those receiving placebo; bronchitis, sinusitis, pneumonia and herpes zoster were also observed more commonly with fingolimod than with placebo Response to influenza vaccine is reduced in those receiving fingolimod as opposed to those receiving placebo JCV, disseminated HSV and VZV with multiorgan failure, cryptococcosis, atypical mycobacteria, and HHV-8–associated KS have all been reported VZV serology should be done before initiation of fingolimod in anyone without a clear prior episode of VZV (confirmed by a health care professional) or documented complete vaccination series; Ab-negative individuals should be vaccinated and fingolimod postponed until at least 1 mo after last vaccination In clinical trials, fatal disseminated primary HSV and fatal HSV encephalitis were each diagnosed in one patient, both of whom were receiving higher-than-recommended doses of fingolimod	Relapsing MS to reduce exacerbations and delay physical disability	390, 391, 392, 393, 394

			Cryptococcal infections may include cryptococcal meningitis and disseminated cryptococcosis Because fingolimod can result in a dose-dependent decrease in lymphocyte count, a CBC within 6 mo should be available before initiation of therapy Live vaccines should not be administered during or for 2 mo after discontinuation of fingolimod; all vaccines may be less effective during this period Coadministration of fingolimod with ketoconazole raises blood levels of fingolimod and increases risk of adverse events; multiple other drug-drug interactions have been reported		
Natalizumab (Tysabri)	Monoclonal Ab directed against the α4-integrin subunit	Natalizumab binds to the α4 subunit of α4β1 and α4β7 integrins, located on the cell surface of leukocytes (except neutrophils), and blocks integrin binding (and therefore leukocyte adhesion) to cognate receptors such as VCAM-1 and MAdCAM-1; ultimately this prevents leukocyte migration into tissue	Increased risk of PML, with high risk of subsequent morbidity and mortality; duration of therapy (especially >2 yr), prior immunosuppression, and anti-JCV antibodies are all associated with the development of PML[a] Risk of PML in the United States varies from <1/1000 in patients who are anti-JCV Ab negative, or anti-JCV Ab positive with <2 yr natalizumab use and no prior immunosuppression, to 13/1000 for those who are anti-JCV Ab positive, with 4–6 yr of natalizumab use and prior immunosuppression In risk assessment, a single positive anti-JCV Ab is considered a positive result regardless of previous or subsequent testing Testing should be done 2 wk after plasma exchange and ≥6 mo after IVIG Retesting for anti-JCV Ab should be done periodically for the purposes of risk stratification, not for diagnosis of PML or other JCV-related syndrome In one study of patients with MS receiving natalizumab, the anti-JCV Ab seroconversion rate was 7.1% per year Natalizumab product monograph suggests consideration of periodic monitoring for MRI evidence of PML even in asymptomatic patients, and a baseline MRI in patients with MS prior to natalizumab Monitoring for PML should be continued for ≥6 mo after discontinuation of natalizumab, because PML has occurred after discontinuation of therapy Prior PML is a contraindication to natalizumab JCV granule cell neuronopathy can cause cerebellar dysfunction with or without concurrent PML, but is diagnosed and managed similarly IRIS can complicate PML in patients who discontinue natalizumab and often undergo plasma exchange to further remove natalizumab HSV and VZV meningitis, encephalitis, and acute retinal necrosis have been observed with natalizumab, and in some patients have resulted in blindness, life-threatening illness, or death Hepatic failure requiring transplant has occurred Urinary tract and lower respiratory tract infections are reported in ≥10% of patients receiving natalizumab for MS Opportunistic infections such as cryptosporidiosis, PJP, aspergillosis, and others do occur with natalizumab, although infrequently Natalizumab should not be combined with other immunosuppressive medications, including anti-TNF medications, for the treatment of Crohn disease; if corticosteroid therapy cannot be withdrawn within 6 mo of beginning natalizumab, natalizumab should be discontinued	Relapsing MS Moderate-severe active Crohn disease, with evidence of inflammation, in adults who have not responded to or tolerated usual therapy and TNF inhibitors	395, 396, 397, 398, 399
Ocrelizumab (Ocrevus)	Monoclonal antibody directed against CD20	Ocrelizumab binds to CD20 and facilitates lysis of pre-B, mature B, and memory B cells	Screening for HBV is required prior to initiation of therapy; HBsAg positivity is a contraindication to therapy Reactivation in the setting of isolated HBcAb positivity with negative HBV DNA has been reported with ocrelizumab use for rheumatoid arthritis Ocrelizumab should not be initiated in patients with an active infection Live virus vaccines are contraindicated during and after ocrelizumab therapy, until B-cell repletion has occurred; all vaccines should be initiated ≥6 wk before start of ocrelizumab therapy Upper and lower respiratory tract infections and skin infections are among the most common infections reported, depending on the population studied Herpesvirus infections have also been reported more frequently	Relapsing or primary progressive MS	400–403

Continued

TABLE 49.3 Infectious Risks Associated With Selected Immunomodulatory Therapies in the Treatment of Nonmalignant Diseases—cont'd

AGENT	MODE OF ACTION	IMMUNE DEFICIT	INFECTIOUS RISKS, MIMICKERS, AND PREVENTION	FDA APPROVED CLINICAL INDICATIONS	REFERENCES
			Immunoglobulin levels decrease with therapy, but in the clinical trial population studied, no association was found with risk of infection Neutropenia was also observed, but was largely nonsevere in magnitude PML has not been reported with ocrelizumab but has been identified in other patients receiving anti-CD20 therapy Although not mentioned in the product monograph, some trials in rheumatoid arthritis (for which ocrelizumab is not currently approved) required screening for TB before entry, and others in MS excluded patients with known TB or a history thereof		
Immunomodulators Targeting Asthma					
Benralizumab (Fasenra)	Monoclonal Ab directed against IL-5 receptor subunit α	Benralizumab binds to the IL-5 receptor on eosinophils and basophils, and to FcγRIII receptors on NK cells, leading to apoptosis of eosinophils and basophils via antibody-dependent cell-mediated cytotoxicity	Known parasitic infection was an exclusion criteria for clinical trials, and therefore the impact of benralizumab on parasitic infections is not known Helminthic infections should be treated before receipt of benralizumab, and new infections may require discontinuation of benralizumab until infection resolves	Severe asthma in patients >12 yr old with an eosinophilic phenotype, when used for add-on maintenance therapy	404, 405
Mepolizumab (Nucala) Reslizumab (Cinqair)	Monoclonal Abs directed against IL-5	Mepolizumab and reslizumab bind IL-5 and prevent binding to the IL-5 receptor on eosinophils, reducing eosinophil differentiation, activation, and survival	Herpes zoster infections have been reported with mepolizumab; vaccination against VZV may be considered (if appropriate) prior to therapy Known parasitic infection was an exclusion criteria for clinical trials, and therefore the impact of anti-IL-5 therapy on parasitic infections is not known Helminthic infections should be treated before anti-IL-5 therapy, and new infections may require discontinuation of anti-IL-5 therapy until infection resolves Anaphylaxis can occur with reslizumab, and therefore it should be administered only in a health care setting with the means to address anaphylaxis, if required[a]	Severe asthma in patients >12 yr old (mepolizumab) or ≥18 yr old (reslizumab) with an eosinophilic phenotype, when used for add-on maintenance therapy	406–409
Omalizumab (Xolair)	Monoclonal Ab directed against IgE	Omalizumab binds to IgE, lowers free IgE levels, and prevents IgE binding to its cell surface receptor on mast cells and basophils, thereby reducing release of soluble mediators	Viral infections, upper respiratory tract infections, sinusitis, and pharyngitis were among the most common adverse events reported, but were similar between patients with asthma ≥12 yr old receiving omalizumab and placebo Anaphylaxis can occur after the first dose, or longer than 1 yr into a course of therapy[a] Fever, arthralgias, and rash can occur In an RCT of patients at risk for geohelminth infection who received prestudy antihelminthic treatment, more patients receiving omalizumab developed a new intestinal geohelminth infection than did those receiving placebo	Moderate-to-severe persistent asthma despite inhaled corticosteroids in patients ≥6 yr old with a positive skin test result or in vitro reactivity to a perennial allergen Chronic idiopathic urticaria despite H_1 antihistamine therapy in patients ≥12 yr old	410, 411
Immunomodulators Targeting Miscellaneous Conditions					
Canakinumab (Ilaris)	Monoclonal Ab directed against IL-1β	By binding IL-1β and blocking its downstream effector functions, canakinumab reduces the proinflammatory immune response	Increased risk of serious infections with canakinumab therapy; caution should be used when considering canakinumab for those with recurrent infections or conditions that predispose to infections Canakinumab should not be administered during active infections and should be discontinued if a serious infection develops during therapy Opportunistic infections including aspergillosis, mycobacterial infections, herpes zoster, and CMV infections have been reported during therapy with canakinumab Nasopharyngitis, upper respiratory tract infections, and influenza are among the most commonly reported infections Canakinumab has not been studied in patients with LTBI, including those receiving anti-TB therapy; screening for active and latent TB before therapy, and treatment if positive, is recommended, although safety in this population is unknown In a recent trial, patients with HBV and HCV were excluded, as were those with evidence of, risk factors for, or currently treated, active TB Coadministration with TNF inhibitors is not recommended owing to the increased risk of infection Live vaccinations are contraindicated during therapy with canakinumab	Periodic fever syndromes such as CAPS (including FCAS and Muckle-Wells syndrome), in patients ≥4 yr old TNF receptor–associated periodic syndrome (TRAPS) in adults and children Hyperimmunoglobulin D syndrome/mevalonate kinase deficiency in adults and children Familial Mediterranean fever in adults and children Active systemic juvenile idiopathic arthritis in patients ≥2 yr old	412–415

Eculizumab (Soliris)	Binds to C5, preventing conversion into C5a and C5b	Eculizumab reduces terminal complement components and inhibits formation of the membrane attack complex (MAC; C5b-9) required for cytolysis	Life-threatening and fatal meningococcal infections can occur[a] Follow current recommendations with respect to meningococcal vaccination (and revaccination depending on the duration of therapy) for patients with terminal complement deficiencies[a] Meningococcal vaccination should be given at least 2 wk before beginning eculizumab, unless the risks of a meningococcal infection are outweighed by the risks of delayed eculizumab[a] Meningococcal infection has been documented in previously vaccinated patients with both aHUS and PNH, so a high index of suspicion is required throughout therapy; 14 of 16 eculizumab recipients with meningococcal disease in the United States from 2008–2016 had received at least one dose of vaccine Antimicrobial prophylaxis, either temporarily (2 wk after vaccination in those who were not vaccinated 2 wk prior to eculizumab) or lifelong (with penicillin or a macrolide if penicillin allergic), has been proposed for risk reduction, although the risks and benefits of either practice have not been fully studied Eculizumab therapy may predispose children to infection with *Streptococcus pneumoniae* or *Haemophilus influenzae* type b, and therefore vaccination against these organisms should occur as per current guidelines Upper respiratory tract and urinary tract infections were among the commonly reported infections in studies of patients with aHUS	PNH to reduce hemolysis Atypical (but not Shiga toxin–induced) hemolytic-uremic syndrome (aHUS) to inhibit complement-mediated thrombotic microangiopathy Generalized myasthenia gravis in adults with positive anti–acetylcholine receptor antibodies	416–419
Rilonacept (Arcalyst)	Monoclonal Ab directed against IL-1	Rilonacept binds Il-1 and prevents interaction with the receptor	Upper respiratory tract infection was the most commonly reported infection Serious infections can occur Combination therapy with an anti-TNF agent is not recommended owing to additive immunosuppression Treatment should not be initiated in the setting of active or chronic infection, and should be discontinued if a serious infection develops on therapy Reactivation of LTBI may occur, so screening and treatment as per current guidelines should occur before therapy Vaccinations should be given before therapy is initiated, and live vaccines are contraindicated during therapy	CAPS including FCAS and Muckle-Wells syndrome, in patients ≥12 yr old	420
Dupilumab (Dupixent)	Monoclonal Ab directed against the IL-4 receptor subunit α	Dupilumab binds to the shared IL-4 receptor subunit α and inhibits IL-4 and IL-13 signaling, ultimately reducing proinflammatory cytokines and IgE	Conjunctivitis (including allergic, bacterial, viral, and other), keratitis (including due to HSV), and blepharitis were observed in clinical trials HSV infections have been reported with therapy Known helminthic infection was a trial exclusion, and therefore the impact of dupilumab on helminth infections is unknown Patients with HBV, HCV, HIV, or other immunosuppression were also excluded from trials Use of live vaccines is not recommended for patients receiving dupilumab; immune responses 4 wk postvaccination to tetanus toxoid and serogroup C meningococcal polysaccharide were not different in patients treated with dupilumab for 12 wk and then vaccinated with Tdap and a meningococcal polysaccharide vaccine	Moderate-to-severe atopic dermatitis in adults, when topical therapies are ineffective or cannot be used	421, 422

[a]FDA boxed warning.

aHUS, Atypical hemolytic-uremic syndrome; *ALT,* alanine aminotransferase; *ANA,* antinuclear antibody; *AST,* aspartate aminotransferase; *BCG,* bacillus Calmette-Guérin; *CAPS,* cryopyrin-associated periodic syndrome; *CBC,* complete blood count; *CMV,* cytomegalovirus; *CNS,* central nervous system; *CTLA-4,* cytotoxic T-lymphocyte antigen 4; *CYP,* cytochrome P450; *DMARD,* disease-modifying antirheumatic drug; *dsDNA,* double-stranded DNA; *EBV,* Epstein-Barr virus; *FCAS,* familial cold autoinflammatory syndrome; *GI,* gastrointestinal; *HBcAb,* hepatitis B virus core antibody; *HBsAg,* hepatitis B virus surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HHV-8,* human herpesvirus 8; *HIV,* human immunodeficiency virus; *HSV,* herpes simplex virus; *Ig,* immunoglobulin; *IL,* interleukin; *IRIS,* immune reconstitution inflammatory syndrome; *IVIG,* intravenous immune globulin; *JCV,* John Cunningham (JC) virus; *KS,* Kaposi sarcoma; *LTBI,* latent tuberculosis infection; *MAdCAM-1,* mucosal addressin cell adhesion molecule 1; *MRI,* magnetic resonance imaging; *MS,* multiple sclerosis; *NOMID,* neonatal-onset multisystem inflammatory disease; *PJP, Pneumocystic jirovecii* pneumonia; *PML,* progressive multifocal leukoencephalopathy; *PNH,* paroxysmal nocturnal hemoglobinuria; *PTLD,* posttransplant lymphoproliferative disorder; *RA,* rheumatoid arthritis; *RCT,* randomized controlled trial; *SLE,* systemic lupus erythematosus; *TB,* tuberculosis; *Td,* tetanus and diphtheria (vaccine); *Tdap,* tetanus, diphtheria, and acellular pertussis (vaccine); *TNF,* tumor necrosis factor; *ULN,* upper limit of normal; *UTI,* urinary tract infection; *VCAM-1,* vascular cell adhesion molecule-1; *VZV,* varicella zoster virus.

turn require immunosuppressive medications, leading to secondary risk of infection.

Better characterization of the infectious risks associated with immunomodulatory therapy will be an important area of research in future. Identifying high-risk patients, the best method and algorithm for screening, the ideal means and duration of prophylaxis or monitoring, and the impact of immunomodulators on geographically restricted infections all represent key areas for future investigation.

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50 Hyperbaric Oxygen

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SHORT VIEW SUMMARY

Physiologic Effects
- Modulation of inflammation and immune function (cytokine downregulation).
- Angiogenesis and improved wound healing (growth factor upregulation).
- Direct antibacterial properties and increased activity of antimicrobial agents.

Administration
- Monoplace chambers are used more commonly owing to their portability.
- Chamber pressures are usually 2 to 3 atmospheres absolute. Most sessions last 90 to 120 minutes.
- Acute therapy may require one to two sessions, whereas a typical wound-healing protocol may involve more than 40.

Indications
- Diabetic foot ulcers (Wagner grade III or higher without signs of healing after 30 days) are the most common indication for hyperbaric oxygen treatment (HBOT). An accumulating body of clinical evidence (with variable methodologic design) suggests improved healing rates. However, HBOT has not been proven to contribute to resolution of infection. Questions remain regarding patient selection and cost-effectiveness. Routine use is not recommended.
- Necrotizing soft tissue infections and clostridial myonecrosis (gas gangrene) have also been treated with HBOT. Recent studies have reported variable impact on mortality and amputation rates. The role of HBOT in these infections remains uncertain.
- HBOT has been used for refractory osteomyelitis, bacterial intracranial abscess, and mucormycosis, but there is insufficient clinical evidence to support routine use for these indications.

Side Effects and Complications
- HBOT has a good safety record. Prolonged treatment may result in higher rates of adverse events.
- Complications can result from barotrauma due to expansion of gases in enclosed anatomic compartments (including middle ear, paranasal sinuses, lung) or oxygen toxicity (to central nervous system, eye, lung).
- Untreated pneumothorax is the only absolute contraindication for HBOT.
- Seizure incidence has been markedly reduced with routine use of "air breaks."
- Myopia is characteristically mild. Cataract formation may complicate prolonged treatment.
- Careful patient selection and baseline evaluation for underlying conditions (e.g., cataracts, upper respiratory or cardiopulmonary disease, seizures) may reduce the risk for complications.

Conclusions
- The role of HBOT in infectious diseases remains controversial, primarily because advocates have not generated adequate controlled data to define conclusively its appropriate uses.
- Selection of treatment candidates is difficult and should be individualized for each patient and each clinical situation. It is critical to weigh the risks of transport, lack of access to the patient, potential complications, and cost against any potential benefit that the treatment may provide.

Hyperbaric oxygen treatment (HBOT) has been proposed for a wide range of indications. In fact, it has been described as a treatment in search of indications. The indications for its use vary between professional organizations and countries. Most accepted uses are based on experimental models and observational clinical experience. The role of HBOT in infectious diseases remains a contentious issue. In this chapter we review aspects of HBO relevant to the infectious disease field, including physiologic effects, methods of administration, clinical evidence supporting approved indications, side effects and complications, patient selection and evaluation, cost, and certification in hyperbaric medicine. Strengths, weaknesses, and controversial aspects of this treatment are also discussed.

PHYSIOLOGIC EFFECTS IN EXPERIMENTAL MODELS

Individuals breathing ambient air (21% oxygen) at sea level pressure (1 atmosphere absolute [1 ATA]) normally achieve an arterial oxygen pressure (Pao_2) of 90 mm Hg and a tissue oxygen pressure (Pto_2) of 55 mm Hg. HBOT, in which patients breathe 100% oxygen inside a chamber at a pressure higher than sea level (>1 ATA), results in a Pao_2 of up to 2000 mm Hg and a Pto_2 of up to 500 mm Hg.[1] These high oxygen concentrations have been associated with several physiologic effects in experimental models. Those pertaining to infectious diseases are discussed in Table 50.1.

Modulation of Inflammation and Immune Function

Accumulating experimental evidence is shedding light on the effects of HBO at the cellular and molecular levels. Increases in nitric oxide generation triggered by HBO have been associated with decreased neutrophil adhesion and sequestration.[2] These changes reduce reactive oxygen species formation,[3] which in turn decreases production of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α.[4] HBO showed a protective effect on mortality in a murine sepsis model that appeared to be linked to enhanced IL-10 expression[5] and demonstrated a protective effect in an ischemia-reperfusion injury model (in which restoration of blood flow produces a "second hit" phenomenon that may be more damaging than the initial event) by ameliorating neutrophil infiltration to the affected tissues.[2] In addition, HBO has been shown to reduce thrombosis, tissue edema, and compartmental pressures.[6]

Angiogenesis and Improved Wound Healing

Animal models have demonstrated that HBO creates the necessary oxygen gradients between the blood and injured tissues to promote angiogenesis via vascular endothelial growth factor upregulation.[7] A proposed mechanism for improved wound healing is the maintenance of a critical level of hyperoxia required for increased production of

TABLE 50.1 Physiologic Effects of Hyperbaric Oxygen Shown in Experimental Models

Modulation of Inflammation and Immune Function
Decreased neutrophil adhesion and sequestration[2]
Decreased production of reactive oxygen species in neutrophils[3]
Decreased production of proinflammatory cytokines (interleukin [IL]-1, IL-6, tumor necrosis factor-α)[4]
Enhanced IL-10 expression[5]
Protective effect in ischemia-reperfusion injury model[2]
Reduced thrombosis, edema, and compartmental pressures[6]
Improved Wound Healing
Promote angiogenesis via vascular endothelial growth factor upregulation[7]
Increased fibroblast proliferation and collagen synthesis[8]
Increased extracellular matrix synthesis in cartilage[10]
Enhanced osteogenesis and bone mineralization[12]
Antibacterial Properties
Formation of bactericidal toxic oxygen radicals[13]
Decreased α-toxin production by *Clostridium*[14]
Restoration of neutrophil-mediated killing of bacteria by oxidative mechanisms[15]
Activity of Antimicrobial Agents
Potentiated effect of tobramycin, linezolid, vancomycin, tigecycline, cefazolin, penicillin, imipenem, and ciprofloxacin[16–22]
No effect when added to daptomycin plus rifampin in a model of *Staphylococcus aureus* implant-associated osteomyelitis[23]

TABLE 50.2 Indications Approved by the Undersea and Hyperbaric Medicine Society for Hyperbaric Oxygen Treatment

INFECTIOUS DISEASES	NONINFECTIOUS DISEASES
Clostridial myonecrosis (gas gangrene)	Air or gas embolism
Intracranial abscess	Carbon monoxide poisoning
Necrotizing soft tissue infections	Crush injury, compartment syndrome, and other acute traumatic ischemia
Refractory osteomyelitis	Decompression sickness
	Arterial insufficiencies
	Central retinal artery occlusion
	Enhancement of healing in selected problem wounds
	Severe anemia
	Delayed radiation injury (soft tissue and bony necrosis)
	Compromised grafts and flaps
	Thermal burns
	Idiopathic sudden sensorineural hearing loss

growth factors necessary for fibroblast proliferation.[8] It has been shown that when the environment of the fibroblast has a low oxygen tension, the cell can divide but can no longer synthesize collagen, nor can it migrate to where it is needed for healing. When the oxygen tension is increased, the fibroblast can regain these wound-healing functions. Increased expression, deposition, and maturation of collagen have been demonstrated.[9] In murine and rabbit models, positive effects in cartilage were observed, including hydroxyapatite formation, decreased chondrocyte apoptosis, and increased extracellular matrix synthesis.[10,11] There is evidence that HBO may enhance osteogenesis. Increased serum osteoprotegerin levels and improved bone mineralization were observed in a group of patients with femoral head necrosis exposed to HBO.[12]

Antibacterial Properties

The antimicrobial action of oxygen occurs when reduced nicotinamide adenine dinucleotide phosphate–linked oxygenase acts as a catalyst for the production of toxic oxygen radicals, particularly a superoxide ion that kills bacteria. Anaerobic organisms are extremely sensitive to these radicals because most lack the superoxide-degrading enzyme superoxide dismutase.[13] HBO also can affect the production of bacterial toxins. At a Pto_2 of 250 mm Hg, α-toxin production by *Clostridium perfringens* completely ceases.[14] This toxin is a virulence factor that degrades cell membranes and produces liquefactive necrosis. Moreover, neutrophil-mediated killing of *Staphylococcus aureus* is restored when a low Pto_2 in osteomyelitic bone is increased to physiologic or supraphysiologic levels.[15]

Activity of Antimicrobial Agents

Increased tobramycin efficacy was observed in an experimental murine model of *S. aureus* endocarditis.[16] This was likely the result of enhanced transport of aminoglycosides across bacterial cell walls, an oxygen-dependent mechanism. In another murine model of *S. aureus* endocarditis, linezolid plus HBO was found to be more effective in reducing the number of bacterial colonies in aortic valve vegetations than either treatment alone.[17] In a murine model of *S. aureus* mediastinitis, bacterial counts in tissue were significantly lower when vancomycin or tigecycline were combined with HBO ($P < .05$).[18] A positive effect has also been shown for certain cephalosporins. The combination of cefazolin and HBO produced a 100-fold greater reduction in bacterial counts than cefazolin or HBO alone.[19] The combined treatment of penicillin and HBO exerted additive effects in decreasing bacterial counts and increasing survival in a murine model of streptococcal myositis.[20] With in vitro models of *Pseudomonas aeruginosa* infection, HBO enhanced the effect of imipenem[21] and ciprofloxacin in biofilms.[22] These effects are believed to be mediated by stimulation of aerobic respiration and enhanced bactericidal activity by oxidative mechanisms. Conversely, HBO was ineffective when combined with daptomycin plus rifampin in a murine model of *S. aureus* implant-associated osteomyelitis suggesting a lack of effect in device-associated infections.[23]

ADMINISTRATION

HBOT can be carried out in either monoplace (single person) or multiplace (typically 2–14 patients) chambers. In monoplace chambers the entire atmosphere is pressurized (typically 2–3 ATA) with 100% oxygen, and the patient breathes the ambient chamber oxygen directly. Modern monoplace chambers have transparent acrylic walls offering a clear view of the patient and a less secluded feeling. Monoplace chambers are used more commonly because of their portability. In multiplace chambers the pressurized ambient oxygen contains normal room air, and patients breathe 100% oxygen from hoods or masks. Modern multiplace chambers are integrated into hospital architecture to appear and function as ordinary treatment rooms. They incorporate patient monitoring equipment, control panels to regulate air flow, intercom systems, automatic fire suppression, entertainment systems, and other technologic advancements to enhance clinical support, safety, and patient comfort.

Most HBOT sessions are administered over 90 to 120 minutes. Acute therapy may require only one or two treatments, whereas chronic medical conditions, such as those addressed by wound-healing protocols, may warrant more than 40 sessions. In the United States HBOT facilities are relatively widely available and can be found in every state. They vary widely from storefront clinics in strip malls to advanced wound care units in major academic medical centers. Multiplace chambers are typically operated by hospitals, navies, professional diving organizations, and dedicated recompression facilities. The slang term, at some facilities, for a cycle of pressurization inside the HBOT chamber is "a dive."

Directories of clinics and centers listing contact information, type of facility, and chamber types are available.[24] Similarly, directories of HBOT practitioners can be found covering the full range of training and education, from nonphysician wellness entrepreneurs to board-certified specialists. HBOT should not be self-administered in the home or administered by noncertified providers. Critical care monitoring and treatment, including mechanical ventilation, should be readily available during treatments.

INDICATIONS

The Undersea and Hyperbaric Medicine Society (UHMS) publishes a list of approved indications (Table 50.2).[25] Among the infectious diseases listed, treatments for necrotizing soft tissue infections (NSTIs), clostridial myonecrosis, and refractory osteomyelitis are reimbursable by the Centers for Medicare and Medicaid Services and third-party payers. High-grade diabetic ulcers of the lower extremities without signs of healing after

30 days of standard treatment are also reimbursable indications. The American College of Hyperbaric Medicine (ACHM) supports treatment of patients with nonapproved indications in a research setting.[26]

Diabetic Foot Ulcers

Even though HBOT has long been used for diabetic foot ulcers (DFUs), its effectiveness is debated. A number of randomized controlled trials (RCTs) and systematic reviews have been published in the last decade. Most, but not all, suggest a benefit. Significant variability in methodologic design is evident. Results of these reviews are summarized as follows.

A Cochrane Database systematic review of 10 RCTs ($n = 531$ patients) compared wound healing in patients treated with adjuvant HBO versus standard treatment.[27] Even though an increase in the rate of ulcer healing (risk ratio [RR], 2.35) with HBOT at 6 weeks was observed, this benefit was not evident at 1 year. There was no statistically significant difference in major amputation rates. Another pooled analysis that included observational trials and RCTs found that HBOT was associated with reduced risk for amputation (odds ratio [OR], 0.2; 7 studies) and improved healing rates (OR, 9.9; 6 studies).[28] A review of 13 studies ($n = 624$) that included 7 RCTs showed that adjuvant HBOT resulted in a higher proportion of healed ulcers (RR, 2.33) and a significant risk reduction for major amputations (RR, 0.29), compared with standard treatment; however, the rate of minor amputations was not affected ($P = .37$).[29] A recent review of 7 RCTs compared standard care plus HBOT with standard care alone and found a significant difference in favor of standard care plus HBOT in relation to ulcers healed, mixed results for major amputation rates, and no difference in adverse events.[30] Improved mortality (35% vs. 47%) and lower cost of care ($33,100 vs. $66,300) were observed in a cohort of 106 patients with high-grade ulcers treated with a limb salvage protocol that included HBOT, compared with a historical cohort of 53 patients treated primarily with amputation without HBOT.[31]

Other investigators have reported less encouraging results. HBOT was associated with reduced rates of wound healing (hazard ratio [HR], 0.68) and an increased risk of requiring amputation (HR, 2.37) in an observational cohort of 6259 patients (HBOT group = 793 vs. non-HBOT group = 5466) from a US national wound care company.[32] Similarly, in a recent prospective RCT of 103 patients (HBOT = 49 vs. non-HBOT = 54), HBOT did not offer an additional advantage to comprehensive wound care in facilitating wound healing or reducing the indication for amputation.[33]

Professional organizations have issued position statements regarding the role of HBOT in DFUs. The American Diabetes Association Professional Practice Committee reviewed the available data on the use of HBOT in 2016 and did not identify sufficient supporting data on the efficacy of this treatment to recommend its use.[34] The 2015 UHMS practice guidelines stated that HBOT was recommended for patients with Wagner grade III or higher ulcers that have just undergone surgical débridement or ulcers that have shown no significant improvement after 30 days of treatment.[35] The 2012 Infectious Diseases Society of America diabetic foot infection practice guideline stated that a limited number of RCTs are available to support the use of HBOT for wound healing and concluded that no adjunctive therapy, including HBO, has been proven to improve resolution of infection, but for selected wounds that are slow to heal, clinicians might consider using HBOT (strong recommendation, moderate-quality evidence).[36]

Despite the increased number of published studies and systematic reviews, the available evidence remains conflicting and makes it difficult to draw any definitive conclusions regarding the role of HBOT in DFUs. Questions remain regarding patient selection, timing of treatment, optimal protocols, and cost. The impact on morbidity and mortality is unclear. Therefore routine use is not recommended.

Necrotizing Soft Tissue Infections

In a retrospective analysis of 45,913 patients diagnosed with NSTIs in the US Nationwide Inpatient Sample, 405 patients received HBOT (0.9%).[37] These patients had a lower mortality rate (4.5 vs. 9.4%, $P = .001$). This advantage persisted after controlling for other predictors of mortality and possible confounders (OR, 0.49). Higher hospitalization costs ($52,205 vs. $45,464) and longer length of stay (14.3 days vs. 10.7 days) were also observed in the HBOT group. Another systematic review conducted by German investigators found 250 related articles (993 patients treated with HBO).[38] There were only 10 comparative studies. Fifty percent of articles were case series or reports. In this review the authors identified nine chambers enabling intensive care in the country and reported an average cost of €8000 to €25,000/patient. They concluded that the published studies lacked the quality to confirm a therapeutic benefit of HBOT in NSTIs, and the limited resources available and high costs involved did not allow comprehensive clinical care with HBOT in Germany. In a case-controlled study from Australia of necrotizing fasciitis conducted over a 13-year period, mortality was 12% in the HBOT group ($n = 275$) vs. 24.3% in the non-HBOT group ($n = 66$).[39]

Other retrospective studies have not demonstrated a statistically significant impact on mortality, need for amputations, or length of hospital stay in patients with NSTIs treated with HBO.[40–42] In a recent retrospective cohort of 43 patients with necrotizing fasciitis of the head and neck, more complications (63% vs. 25%) and sequelae (77% vs. 40%) were observed in the HBOT group ($n = 30$) compared with the non-HBOT group ($n = 13$).[43] Adjunctive HBO decreased mortality and limited the extent of débridement in an observational study of 70 patients with Fournier gangrene.[44] However, another study of 42 patients with this infection showed increased mortality, morbidity, and cost of care among patients treated with HBO.[45]

HBOT has been recommended as adjuvant to surgical débridement and antimicrobial treatment for NSTIs in many body locations, particularly perineal gangrene. The role of this treatment remains uncertain primarily because published clinical studies are observational and continue to report conflicting results (e.g., variable impact on mortality and amputation rates). Higher costs of care and longer hospital stays have also been observed.

Clostridial Myonecrosis (Gas Gangrene)

Although there has never been an RCT evaluating the role of HBOT in clostridial myonecrosis, experimental animal data and clinical experience suggest a beneficial effect. Evidence has shown that HBO may help define necrotic tissue, facilitating more precise surgical débridement. In a review of 20 clinical series, including more than 1200 patients with clostridial myonecrosis treated with HBO, a cumulative mortality of 23% was reported.[46] Historical controls had a mortality of 45% without HBOT. A Medline review analyzed 650 patients with clostridial myonecrosis treated with HBO from four comparative studies and 13 case series.[47] Most investigators stated that adjunctive HBO was beneficial. However, because of the noncomparative nature of the case series, it is impossible to reliably assess the therapeutic effects of HBO. The reported mortality rates in these studies ranged from 11% to 52%.

Some HBO practitioners recommend aggressive treatment protocols for clostridial myonecrosis (and other anaerobic or mixed bacterial infections), such as two to three daily 90- to 120-minute sessions at 2.8 to 3 ATA started early in the course of treating the infection. These pressures maintain tissue oxygen tension above thresholds needed to inhibit clostridial spore and exotoxin production. As is the case for other NSTIs, the role of HBOT in clostridial myonecrosis is controversial due to the observational nature of the available evidence because no RCTs have been performed.

Refractory Osteomyelitis

Few controlled studies have been performed to confirm the efficacy of HBOT in refractory osteomyelitis. More rapid resolution of infection and prevention of recurrences, when compared with standard treatment, have been reported in patients with sternal, skull, and spinal infections.[48–50] This clinical experience should be viewed with caution. Patient selection and optimal assessment of treatment response remain unsolved questions. HBOT is not routinely recommended as an adjuvant to standard treatment in patients with refractory osteomyelitis.

Bacterial Intracranial Abscess

In a cohort study of 40 patients (HBOT = 20 vs. non-HBOT = 20) with bacterial intracranial abscess, the HBOT group had fewer recurrences (14% vs. 58%, $P < .01$), decreased need for reoperation (10% vs. 45%, $P = .03$), and improved long-term outcomes (higher Glasgow scores in

80% vs. 45%, $P = .04$) compared with the non-HBOT group.[51] In another series of 13 patients, decreased need for reoperations and decreased duration of antimicrobial therapy were reported.[52] Infection was controlled in all patients, and there were no recurrences. A cumulative review of 48 patients treated with HBO reported a 2% mortality.[53]

Adjunctive HBOT has been suggested in selected cases (i.e., multiple or deep abscesses, underlying immunosuppression, suboptimal response to standard treatment, and poor surgical candidates). This recommendation is based primarily on expert opinion.[54] The available clinical evidence is insufficient to support routine use of adjuvant HBOT in bacterial intracranial abscess.

Mucormycosis

A review of 28 published cases indicated that adjunctive HBOT may be beneficial in patients with mucormycosis and underlying diabetes (94% survival), whereas its benefit in patients with underlying hematologic malignancies or bone marrow transplantation is doubtful (33% survival).[55] This experience suggests that it was the underlying immunosuppression, and not HBOT, that ultimately impacted outcomes. A retrospective study examined 14 patients with invasive fungal infections caused by *Mucor* or *Aspergillus* over a 12-year period.[56] Most had rhinocerebral or pulmonary involvement. These patients had a 50% survival with a treatment regimen consisting of surgery, antimicrobial therapy, and HBOT.

There are no controlled studies evaluating the role of HBOT in mucormycosis. Mortality rates for this infection remain high despite treatment. The role of HBOT in mucormycosis remains uncertain.

SIDE EFFECTS AND COMPLICATIONS

Concerns about the safety aspects of HBO have been raised due to the unique atmospheric conditions to which patients are exposed. In a recent database evaluation that contained information on 1.5 million HBO treatments, the overall rate of adverse events per session was 0.68%. Severe events were extremely uncommon, with fewer than 0.05 instances per 1000 treatments.[57] In a retrospective analysis of 2334 patients from Israel, 406 (17.4%) experienced adverse events, but the event-per-session rate was 0.72%.[58] These data support the notion that single-session or short-term HBOT is generally safe. In the elective setting serious complications are rare when patients are properly prepared and monitored and treatment adheres to standard protocols (duration <120 minutes, pressure <3 ATA, and <40 sessions). Complications can result from barotrauma due to expansion of gases in enclosed anatomic compartments (middle ear, paranasal sinuses, lung) or oxygen toxicity (central nervous system, eye, lung) (Table 50.3). Oxygen has been shown to be harmful to tissues via the formation of oxygen free radicals. The higher partial pressures of oxygen used during HBOT are believed to saturate protective enzymes and unfavorably shift oxidative stress reactions in the direction of cellular damage.

The incidence of middle ear barotrauma varies between treatment centers. Most episodes are self-limited, but continued high pressure may cause middle ear hemorrhage and tympanic membrane bulging, resulting in severe pain. In a report of 1115 patients otalgia was described in 157 patients (14%).[59] Ear fullness, hearing loss, tinnitus, and vertigo were uncommon (≤1%). Age older than 60 years and female sex were independent risk factors for otologic symptoms. Half of the patients experienced symptoms at the first treatment session in this study. Among 58 symptomatic patients evaluated, abnormal otoscopic findings were recognized in 58 (50%)/116 ears. Twenty-seven ears required myringotomy or tube insertion, and HBOT had to be stopped in 4 patients. To prevent injury to the tympanic membrane, alert patients need to be appropriately coached to equalize by swallowing to facilitate eustachian tube clearance during decompression, as one does during descent in an aircraft. In the obtunded or mechanically ventilated patient it is recommended to perform a myringotomy before compression. Prophylactic myringotomy is performed at variable rates in the United States.

Paranasal sinus barotrauma has been reported mainly in patients with upper respiratory tract infections or allergic rhinitis. These patients experience difficulty equalizing their sinuses, which can result in sinus congestion or "sinus squeeze" and epistaxis.[58] This problem may be alleviated by pretreatment with nasal decongestants, steroids, or antihistamines. Impaired nasal mucociliary clearance, as measured by a saccharin clearance test, was reported in a cohort of 47 patients receiving long-term exposure to HBOT.[60]

A more serious complication is pulmonary barotrauma causing pneumothorax during decompression.[57] Gas trapped in air spaces not in communication with the diminishing pressure in the chamber (lung cysts or bullae) will expand rapidly. Bronchospasm, which may cause localized air trapping, should be corrected before institution of HBOT. Patients with a history of spontaneous pneumothorax must be evaluated for occult pneumothorax. Untreated pneumothorax is the only absolute contraindication for HBOT. A history of chest surgery is usually not considered a contraindication. However, there is concern that air may be trapped in lesions that were created by surgical scarring. Pulmonary barotrauma is more problematic in monoplace chambers, where the clinician cannot reach the patient until the chamber is decompressed, which may take several minutes even in an emergency.

Pulmonary oxygen toxicity, manifested by chest tightness, cough, dyspnea, or a reversible decline of pulmonary function, occurs most commonly in patients receiving multiple treatments.[58] Cardiovascular responses to HBOT include reduction in cardiac output and systemic vasoconstriction with increased peripheral vascular resistance. Although these effects are well tolerated by normal individuals, cardiogenic pulmonary edema was reported in three patients with underlying cardiac disease, including one fatality.[61]

Breathing oxygen at sufficiently elevated pressures can trigger seizures. Seizures due to oxygen toxicity, however, do not typically result in permanent structural brain damage. In a survey from Israel of 62,614 HBOT therapies administered to 2334 patients, the incidence of seizures was 0.01% per therapy occurring in 0.3% of patients.[62] Seizure incidence has been markedly reduced with the routine use of "air breaks." Should a seizure occur in the chamber, it is important to wait until the seizure has ceased before beginning emergent decompression; rapid decompression with a closed glottis may result in tension pneumothorax. Underlying seizures unrelated to oxygen therapy are not a contraindication to HBOT.

Myopic shift on examination, reported in up to 81% of patients receiving HBOT,[63] may be due to direct oxygen toxicity or deformity of the lens as a result of repeated compressions and decompressions. It is characteristically mild and reversible within few days to 4 weeks after cessation of treatment. Other ocular effects include eyelid twitching, blurry vision, and visual field defects. Cataract formation rarely occurs during commonly used treatment protocols, but it has been described in patients who received intense treatment for extended periods. Cataracts are not reversible after cessation of treatment. Cataract formation may occur due to an excess of reactive oxygen species or a deficiency in antioxidant activity in ocular tissues.[64] Similar mechanisms may contribute to other ocular pathologies, including keratoconus, macular edema, and macular degeneration.[65]

Claustrophobia may occur even in multiplace chambers.[58] Occasionally, anxiolytics are required to facilitate treatment. Decompression sickness with formation of gas bubbles in soft tissues may occur in patients breathing compressed air that contains nitrogen. This complication has also been described among clinical staff in multiplace chambers. The

TABLE 50.3 Side Effects and Complications of Hyperbaric Oxygen Treatment

Otalgia, 14%[59]
Middle ear hemorrhage, tympanic membrane bulging[59]
Ear fullness, hearing loss, tinnitus, vertigo ≤ 1%[59]
Paranasal sinus congestion or "sinus squeeze," epistaxis[58]
Impaired nasal mucociliary clearance[60]
Pneumothorax[57]
Chest tightness, cough, dyspnea, reversible decline of pulmonary function[58]
Cardiogenic pulmonary edema, death (case reports)[61]
Seizures, 0.3%[62]
Myopic shift on examination in up to 81%[63]
Eye twitching, blurred vision, visual field defects
Cataract formation with prolonged treatment, may be irreversible[64]
Dizziness and/or weakness, 1.5%[58]
Claustrophobia[58]
Decompression sickness

likelihood of decompression sickness is reduced by administration of 100% oxygen toward the end of the treatment and by recompression or gradual decompression.

PATIENT SELECTION AND EVALUATION

HBOT providers need to be aware of the potential complications associated with HBOT. Selection of patients mandates a careful risk-benefit analysis. Risk for complications may outweigh benefits in situations where treatment effectiveness is considered uncertain. Patients should be provided with complete information about potential risks as part of their informed consent. A baseline ophthalmology examination is suggested in patients at increased risk for cataract development (age older than 50 years, diabetes, radiation therapy of the head, and systemic corticosteroids). Similarly, evaluation for underlying upper respiratory or cardiopulmonary disease, and seizures may be warranted depending upon the patient's history. Any history of brain, ear, or thoracic surgery should be elicited. HBOT in small children poses particular challenges. Pregnancy is not considered a contraindication for HBOT. Mechanical ventilation is generally considered safe during HBOT, but sedation needs to be optimized to avoid patient-ventilator asynchrony.[66] Hemodynamically unstable patients should not be treated with HBO. Multiplace chambers allow closer monitoring of critically ill patients, whereas monoplace chambers are most appropriate for the treatment of chronic medical conditions in stable patients. The risks associated with transportation of patients for HBOT are acceptable with meticulous monitoring performed by well-trained personnel.[67] It is always important to consider the potential impact of reduced access to the patient during HBOT, which should be balanced against any potential differential benefit over standard care that the treatment may provide.

COST

Cost of an HBOT session in the United States can range from $150 in outpatient clinics to $1000 or more in hospitals. Physician consultation fees, technical supervision, type of insurance carrier, and geographic location all impact costs. A full course of treatment for a conventional diabetic wound healing protocol typically costs $50,000 (Medicare) to $200,000 (private payer).[68] Despite these figures, some economic analyses suggest that HBOT may be cost-effective.[31,69] These analyses, however, rely primarily on outcome data reported from uncontrolled studies. An in-depth analysis of broad implementation of HBOT for DFUs in the United States concluded that the resources consumed by this treatment modality could be better spent on other aspects of management and prevention.[70] HBOT is less expensive in other countries. A 40-session course was reported to cost €6916 in the Netherlands.[71] In the United Kingdom most chambers are financed by the National Health Service, and others are nonprofit. China and Russia treat more than 80 conditions with HBO because their costs are low compared with those in the United States.

CONTROVERSY

Although HBOT has been proposed for many decades, a sense of controversy continues to pervade the field (Table 50.4). Advocates typically quote its beneficial physiologic effects, effectiveness shown by a growing body of clinical experience, indications recognized by professional organizations and third-party payers, and its safety record. Opponents point at the lack of well-designed RCTs for the proposed indications as the main weakness of HBOT. For DFUs in particular, it is important to recognize that numerous parameters affect wound healing. Wounds that exhibit normal tissue oxygenation usually fail to heal only when not appropriately off-loaded or when not properly débrided. Failure to recognize correctable arterial insufficiency will result in a poor response to HBOT. In addition, it is difficult to assess the value of HBOT in patients already receiving multidisciplinary treatment (local wound care, off-loading, antimicrobials, surgical débridement, and revascularization). In addition, there are challenges associated with HBOT administration. Using HBOT in critically ill or unstable patients is difficult. Other standard treatments should never be delayed, access to the patient should never be compromised, and only a patient whose condition is stable should be considered for transfer to a facility with HBOT capability. Last, HBOT consumes substantial resources. How it should be used in practice alongside other treatment modalities in a real world with finite resources is unclear.

TABLE 50.4 Strengths and Weaknesses of Hyperbaric Oxygen Treatment

STRENGTHS	WEAKNESSES
Positive physiologic effects in experimental models	Lack of randomized controlled trials
Effectiveness shown by accumulating clinical experience (mostly uncontrolled)	Cumulative toxicities
Indications approved and recognized by professional organizations and third-party payers	Difficult selection of treatment candidates
Good safety record	Risks of transport and lack of access to the patient
	Delay of standard treatments
	Availability
	Cost

CERTIFICATION IN HYPERBARIC MEDICINE

Physicians with a current certification from one of the member boards of the American Board of Medical Specialties (ABMS) can obtain board certification through the undersea and hyperbaric medicine unit of the American Board of Preventive Medicine (ABPM).[25] Board eligibility requires successful completion of a minimum of 12 months in an accredited hyperbaric medicine fellowship program, and certification requires successful completion of the UHMS examination.

An alternative pathway that certifies added qualification in hyperbaric medicine includes a 40-hour course approved by the ACHM, the US Department of Defense, or the UHMS. Applicants must also submit documentation of supervision of at least 300 hyperbaric treatments for approved indications as noted by either the UHMS or the Centers for Medicare and Medicaid Services, and pass a final examination. This pathway does not replace fellowship training or board certification, which is considered the gold standard for training in undersea and hyperbaric medicine.

The UHMS also provides a program of facility accreditation based on adherence to recognized treatment protocols, facility safety, and operational methodology. Table 50.5 lists professional organizations and other Internet resources on hyperbaric medicine.

CONCLUSIONS

HBOT has been both lauded and questioned, primarily because advocates have not generated adequate controlled data to define conclusively its appropriate uses. It is difficult to assess the efficacy of HBOT in infectious diseases, even those for which use is endorsed by the UHMS and reimbursed by third-party payers. In clinical practice nonhealing wounds,

TABLE 50.5 Internet Resources on Hyperbaric Medicine

RESOURCE	WEBSITE	COMMENT
American College of Hyperbaric Medicine (ACHM)	www.achm.org	Specialty society, education, training
HBO Evidence	http://hboevidence.unsw.wikispaces.net/	Database of clinical trials (non-infectious disease indications, nonhealing wounds)
HyperbaricLink	www.hyperbariclink.com	Independent guide, general information, directory of treatment centers, links
Undersea and Hyperbaric Medical Society (UHMS)	www.uhms.org	Primary professional body, education, training, credentialing, certification, list of approved indications, publications, employment opportunities

HBO, Hyperbaric oxygen.

including complicated DFUs, have become the most common indications for HBOT. Use of HBOT in clinical practice has increased considerably in the last decade and will likely continue to rise if current modes and extent of reimbursement continue. It is not entirely clear which patients with infections, if any, truly stand to benefit from HBOT, and how they should be selected. Prospective RCTs are needed to provide definitive answers regarding efficacy of HBOT in infectious diseases. Until convincing controlled data appear, each patient and each clinical situation must be evaluated individually. Most important, it is critical to weigh the risks of transport, lack of access to the patient, potential complications, and cost against any potential differential benefit over standard care that the treatment may provide.

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The complete reference list is available online at Expert Consult.

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51 Antimicrobial Stewardship

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SHORT VIEW SUMMARY

ANTIMICROBIAL STEWARDSHIP

- Systematic approaches to improve antimicrobial use across the health care spectrum are termed *antimicrobial stewardship.*
- The desired outcomes of antimicrobial stewardship are to ensure that patients receive optimal antimicrobial therapy when it is indicated and to limit adverse events associated with antimicrobial use including *Clostridioides difficile* (formerly *Clostridium difficile*) infection and emergence of resistance at the patient and population levels.

PHILOSOPHY AND SOCIOLOGY OF ANTIMICROBIAL USE

- Antimicrobial use is a key driver of antimicrobial resistance. Most clinicians consider antimicrobial resistance to be an important public health threat but do not view their own prescribing as being a significant driver of resistance.
- Perceived patient expectations, fear of providing inadequate therapy, lack of knowledge, inadequate diagnostics, and local prescribing etiquette contribute to suboptimal antimicrobial prescribing.

ANTIMICROBIAL STEWARDSHIP STRATEGIES

- Educational strategies aim to bring clinicians to a common understanding of principles of antimicrobial use, institutional best practices and processes, and recommended disease management approaches.
- Development of institutional guidelines for antibiotic use to ensure standard prescribing practice is a cornerstone of antimicrobial stewardship program building.
- Antimicrobial restriction strategies limit the prescribing scope of clinicians, either through exclusive use of certain agents at the institution or through requirements for prior authorization by phone call to a stewardship team member or completion of an order set or form before the pharmacy dispenses the agent.
- Postprescription review and feedback strategies involve targeted review of courses of antimicrobials after their initiation. In cases in which antimicrobial use could be optimized, prescribers are contacted with patient-specific suggestions for modifying the current antimicrobial regimen.
- Computer-assisted strategies involve use of information technology tools to make other stewardship processes, such as restriction or postprescription review, both more effective and efficient.

MEASURING OUTCOMES FROM ANTIMICROBIAL STEWARDSHIP PROGRAMS

- Analysis of the impact of antimicrobial stewardship programs on clinical and microbiologic outcomes is complex, but most studies suggest a beneficial or at least a neutral impact. Economic analyses strongly favor antimicrobial stewardship programs, especially when accounting for the entire spectrum of cost savings associated with improving suboptimal antimicrobial use.
- Antimicrobial stewardship programs should determine approaches to track their success, including monitoring antibiotic use and resistance and results of specific interventions designed to improve antibiotic use.

ANTIMICROBIAL STEWARDSHIP PROGRAM STRUCTURE AND IMPLEMENTATION

- Early involvement of administrators and key opinion leaders, dedicated funding, involvement of a range of specialties, extensive use of longitudinal and benchmarking data, development of institution-tailored guidelines for therapy, and constant quality assessment are key strategies for implementing and maintaining a successful stewardship program.

THE FUTURE OF ANTIMICROBIAL STEWARDSHIP

- Governmental and accrediting agencies are instituting requirements for antimicrobial stewardship in hospitals and long-term care facilities. Antimicrobial stewardship in outpatient settings is an emerging area, with the need for different strategies to be tailored to this care environment.

Issues associated with use of antibiotics were recognized shortly after their introduction into clinical medicine in the early 1940s, including a warning from Alexander Fleming about risks of emergence of resistance to penicillin during his Nobel Prize acceptance speech. In his review of the subject in 1956, Jawetz[1] was one of the first to recognize the problems caused by the attractiveness of new antibiotics to physicians, the exaggerated claims by the pharmaceutical industry, and the enormous impact that promotion by the drug companies had on medical practice. The problem has only worsened. In 2011, 262 million courses of antibiotics were prescribed to outpatients in the United States, a rate of 842 per 1000 population.[2] More than half of all hospitalized patients in the United States receive at least one dose of an antibacterial drug during hospitalization.[3] Studies estimate that 30% to 60% of antibacterial use is unnecessary or otherwise inappropriate.[4-6] Among the unwanted consequences of antimicrobial therapy are adverse reactions, *Clostridioides difficile* (formerly *Clostridium difficile*) infection, increased length of stay, increased cost of hospitalization, predisposition to secondary infections, and the emergence of drug-resistant microorganisms.[7] Antimicrobial drug use is a contributor to the rising incidence of serious infections caused by methicillin- and glycopeptide-resistant *Staphylococcus aureus,* vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase–producing Enterobacteriaceae, multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp., and *Klebsiella* spp. expressing *Klebsiella pneumoniae* carbapenemases, and the proliferation of more virulent strains of *C. difficile.*[8,9] Despite recent initiatives to incentivize antimicrobial development, introduction of novel antimicrobial agents active against these resistant organisms has not kept pace with their proliferation; therefore, more effective use of current agents is essential to preserve the value that antimicrobials provide to modern medicine.[10,11]

ANTIMICROBIAL STEWARDSHIP

Programs designed to increase the appropriateness of antimicrobial use in hospitals appeared initially in the 1970s; most had cost reduction as a primary goal because antibiotic expenditures can account for 30% to 50% of a hospital's drug budget.[12] Aside from cost, antimicrobials differ from other drug classes in that their use in one person can lead to selection for resistant organisms that can then be spread to others. In addition, when resistance to a particular antibiotic reaches a certain threshold at the population level, that agent is no longer reliable for use as empirical therapy, driving use toward broader-spectrum agents. Thus, the focus of antimicrobial stewardship programs (ASPs) has shifted

away from cost savings to improvement in the quality of patient care, with the primary goals of improving clinical outcomes, reducing adverse events associated with antibiotic use, and stabilizing or reducing rates of resistance.[13,14]

The last decade has seen widespread support for the implementation of ASPs across all health care settings with publication of updated stewardship guidelines from the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA), recommendations for Core Elements for Stewardship Programs from the Centers for Disease Control and Prevention (CDC), incorporation into the National Action Plan to Combat Antibiotic-Resistant Bacteria, a Standard from The Joint Commission requiring hospitals to have ASPs for accreditation, and requirements for ASPs in long-term care setting from the Centers for Medicare and Medicaid Services (CMS).[15–21] This chapter reviews antimicrobial stewardship strategies that have been reported in the literature, summarizes their goals and the clinical and institutional outcomes of these efforts, and ends with recommendations for the development, implementation, and funding of a successful ASP.

PHILOSOPHY AND SOCIOLOGY OF ANTIMICROBIAL USE

To address the problem of inappropriate antimicrobial usage, we must understand the constraints under which physicians work and the pressures exerted on them to prescribe drugs. The prescription of antibiotics has become as much a psychological or philosophical endeavor as a scientific exercise.[22,23] Physicians must balance the risks of not treating or inadequately treating a patient with antimicrobials against the risks of antimicrobial use in terms of adverse effects, drug costs, and contribution to antimicrobial resistance. Because emergence of antimicrobial resistance and even other adverse consequences such as development of *C. difficile* infection typically are removed in time and place from the original prescribing decision, they often receive little weight. Indeed, a study by Metlay and colleagues found that "risk of contributing to antimicrobial resistance" ranked last among seven factors that physicians were asked to weigh when deciding which antimicrobial to prescribe for a hypothetical patient.[24] In the same study, perceived patient expectations around antimicrobial prescribing was identified as a key factor in overprescribing of antimicrobials. Although studies have demonstrated that patients can be dissuaded from demanding unnecessary antimicrobials without adverse effects on satisfaction with their physician, these interventions may be more time consuming and require more training relative to simply prescribing an antimicrobial.[25] Diagnostic tests for infections may be perceived as overly expensive, invasive, or time consuming relative to simply prescribing an antimicrobial for a suspected infection. When diagnostic tests for infections are ordered, they are often slow to turn around and limited in their sensitivity. Prescribers may be unwilling to take the risks of not prescribing an antimicrobial or prescribing a narrower-spectrum antimicrobial because of malpractice and litigation concerns if a patient truly has an infection. Clinicians may lack knowledge regarding infectious disease diagnostics and management, especially in the face of a growing population of immunocompromised patients (human immunodeficiency virus, stem cell and solid-organ transplantation).[26] Local "prescribing etiquette" may dictate that junior physicians follow the prescribing practices of senior physicians and may dissuade any clinician from modifying antimicrobial (or other) therapy started by another, regardless of its appropriateness.[27] All these factors may lead physicians to fall back on the relative "comfort" of broad-spectrum antimicrobial use.[25,28]

In this environment, some physicians believe that ASPs impose unnecessary or even deleterious constraints on the practice of medicine.[29] Prescribers may fail to appreciate that antimicrobial use has significant ecologic consequences that extend beyond the individual patient under their care and can affect their entire practice population. Their skepticism may arise from the perception that there is a lack of documented efficacy of ASPs across varied health care settings, a paucity of direct evidence demonstrating an improvement in clinical outcomes, limited physician time or incentive to pursue such efforts, and a weak causal link between the emergence of resistance and antibiotic use patterns. Although there are few large multicenter, randomized controlled trials to address all of these questions, there is a growing evidence base that on balance supports the implementation of antimicrobial stewardship efforts.[30]

TABLE 51.1 Antimicrobial Stewardship Strategies for Health Care Institutions Rated by the Infectious Diseases Society of America

STRATEGY	STRENGTH OF RECOMMENDATION AND QUALITY OF EVIDENCE
Recommended Strategies to Implement	
Prior authorization, prospective audit and feedback, or both	Strong recommendation, moderate-quality evidence
Targeting antibiotics associated with a high risk of *Clostridioides difficile* (formerly *Clostridium difficile*) disease	Strong recommendation, moderate-quality evidence
Programs to increase appropriate use of oral antibiotics and intravenous-to-oral switch	Strong recommendation, moderate-quality evidence
Promotion of shortest effective durations of antibiotic therapies	Strong recommendation, moderate-quality evidence
Pharmacokinetic monitoring and adjustment programs for aminoglycosides	Strong recommendation, moderate-quality evidence
Suggested Strategies to Implement	
Facility-specific clinical practice guidelines	Weak recommendation, low-quality evidence
Syndrome-specific interventions	Weak recommendation, low-quality evidence
Regular prescriber self-review of antibiotic appropriateness (e.g., antibiotic time-outs)	Weak recommendation, low-quality evidence
Computerized clinical decision support at point of prescribing	Weak recommendation, moderate-quality evidence
Pharmacokinetic monitoring and adjustment programs for vancomycin	Weak recommendation, low-quality evidence
Pharmacokinetic-pharmacodynamic-based dosage strategies for β-lactams	Weak recommendation, low-quality evidence
Allergy assessment including penicillin skin testing as appropriate	Weak recommendation, low-quality evidence
Stratified antibiograms to supplement traditional antibiograms	Weak recommendation, low-quality evidence
Selective reporting of antibiotic susceptibilities	Weak recommendation, low-quality evidence
Rapid viral testing for respiratory pathogens	Weak recommendation, low-quality evidence
Rapid diagnostic testing for bacterial pathogens in blood cultures	Weak recommendation, moderate-quality evidence
Serial procalcitonin measurements in critically ill patients with suspected infection	Weak recommendation, moderate-quality evidence
Suggested Strategies to Avoid	
Antibiotic cycling	Weak recommendation, low-quality evidence
Reliance on didactic education as sole strategy	Weak recommendation, low-quality evidence

From Barlam TF, Cosgrove SE, Abbo LM, et al. Implementing an antibiotic stewardship program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. Clin Infect Dis. *2016;62:e51–e77.*

ANTIMICROBIAL STEWARDSHIP STRATEGIES

Table 51.1 lists the stewardship strategies evaluated by the IDSA and SHEA guidelines on implementing an ASP in the acute-care setting.[16] A 2015 survey of 4569 US hospitals found that 63% of teaching hospitals and 42% of nonteaching hospitals had all seven of the CDC's Core Elements in place.[31] The most common strategies used among the institutions represented were facility-specific treatment guidelines (77%), prospective audit and feedback (74%), antimicrobial preauthorization

programs (63%), requirements to document an antibiotic indication (26%), and antibiotic time-outs (23%). In addition, 62% reported providing education about resistance to clinicians and other staff.

In the following sections, we provide examples of data supporting major strategies. The reader is referred to many excellent reviews of stewardship programs for a more comprehensive review.[32,33,34-36]

Educational Strategies

Education is a common approach in medicine to impart information with the goal of changing practice. Current undergraduate and postgraduate training in antimicrobial management may be inadequate, because there are significant ongoing deficiencies in clinicians' knowledge base concerning antimicrobial pharmacology, spectrum, and correct use.[29,37-39] A variety of approaches have been used for education regarding appropriate antimicrobial use, including staff conferences, grand rounds, newsletters, and continuing education programs.

Educational programs are difficult to assess because of the complex nature of educational variables, the diversity of efforts, the lack of standardized feedback, and the intricacy of the infectious diseases decision-making process. Nonetheless, several generalizations can be culled from the existing literature. Individual instruction by an antibiotic-utilization expert appears to be the most successful educational strategy, whereas more passive methods such as printed materials and large group instruction are less useful.[40,41] Contemporaneous interventions have a greater impact than those removed from the original antibiotic order by space and time. The development of guidelines and clinical pathways can also be considered a form of education; these are a critical component of all ASPs because they require the relevant institutional stakeholders to gather together and agree on recommendations and approaches. For guidelines and clinical pathways to modify antimicrobial prescribing, however, they must be accompanied with an implementation strategy to ensure their continued adoption by frontline providers.[42,43] Therefore, although education and locally developed guidelines should be cornerstones of any ASP, these approaches should not be used as the sole strategy.[15,16]

Antimicrobial Formulary Restriction Strategies

Limiting the availability of agents on formulary is the most direct method to influence antimicrobial use. The purpose of a limited formulary may be to discourage use of newer, more expensive antimicrobials in favor of older, equally effective drugs or to take advantage of similarities between drugs within a class to obtain preferential manufacturer pricing by exclusively using one agent.[44,45] Beyond the issue of cost, exercising control over which antimicrobials are available for physicians to prescribe also may strongly influence the development of antimicrobial resistance in a health care institution. There is debate as to whether certain antibiotic classes or agents have a lower intrinsic risk of selecting for antimicrobial-resistant pathogens. A national antimicrobial stewardship intervention in Scotland restricted the use of fluoroquinolones, amoxicillin-clavulanate, clindamycin, and cephalosporins.[46] Although the total use of antimicrobials did not change, significant reductions in the prevalence of *C. difficile* infection were found in hospitals and the community. Other studies, however, have found that restriction of certain antimicrobial classes may lead to emergence of resistance to agents that become more commonly used, a phenomenon termed "squeezing the balloon."[47] Design of an antimicrobial formulary should also pay careful attention to an institution's recommended doses, because resistance may be associated with inadequate drug dosing.[48]

Preauthorization Strategies

At a level below institution-wide decisions about which antimicrobials are available are strategies that require physicians to justify their use of antimicrobials in individual patients in some way. These stretch across a spectrum from less restrictive (e.g., requiring use of antimicrobial order forms, mandating that an indication accompany every antimicrobial order) to more restrictive (e.g., requiring physicians to obtain approval from an infectious diseases clinician before restricted antimicrobial release—also known as "preprescription approval" [PPA], mandating formal infectious diseases consultation for use of specific agents). Published studies suggest that these policies are effective at reducing the use of the targeted agents, although sometimes with an increase in use of nontargeted agents. Because agents are frequently targeted based on cost, net cost avoidance for the institution may be observed.[49] The impact on antimicrobial resistance patterns is variable, sometimes resulting in favorable reductions in the prevalence of pathogens such as *C. difficile* or multidrug-resistant gram-negative rods.[50,51,52] For example, in a study at a county hospital in which PPA from an infectious diseases attending physician was required before empirical prescription of broad-spectrum antimicrobials, a 32% reduction in antimicrobial expenditures was associated with increase in *P. aeruginosa* susceptibilities to imipenem from 65% to 83% in the intensive care unit (ICU) and 83% to 95% on the floor. No adverse events such as an increase in mortality or longer hospitalization were observed.[53] Enforcement of more restrictive types of prior-approval programs can be difficult, and the process may be viewed as a punitive exercise. In a survey of residents in a teaching hospital, a majority agreed that a restrictive antimicrobial program was a "good idea" and that such a program "improved individual patient care," but a majority also agreed that the requirement for call approval led to delays in patient receipt of antimicrobial agents and that calling for approval was frustrating.[54] Some studies have found delays in receipt of antimicrobial therapy for agents requiring PPA, although online or electronic medical record (EMR)–integrated approval approaches may reduce some of these issues.[55,56] In private practices, infectious diseases physicians are not enthusiastic about restricting antibiotics because of concerns that the policies will place them in a policing role and thereby damage their traditional referral patterns.[57] Preauthorization strategies offer direct control over antimicrobial use but may be best used in well-staffed programs targeting agents with high cost, particularly niches of use requiring expertise in identification, risk of contributing to resistance, or toxicity. In addition, temporary use of preauthorization strategies may be valuable in the setting of antimicrobial shortages or in response to outbreaks of resistant bacteria.[58,59]

Postprescription Review With Feedback

Postprescription review with feedback (PPRF; also known as prospective audit and feedback) consists of review of prescriptions of targeted antimicrobials (usually at least 2 or 3 days per week) coupled with feedback to physicians to improve antimicrobial use specific to that patient, and is an important stewardship strategy. Feedback should be educational and evidence based, with the goal of appropriate individualized therapy. Interventions occurring as part of a PPRF strategy include switching patients from intravenous to oral therapy, from broad-spectrum and combination therapy to narrower-spectrum therapy (also called *streamlining*), stopping antibiotic therapy if an alternative, noninfectious diagnosis has been identified, determining the optimal duration of therapy, and modifying doses. Prescribers tend to view PPRF strategies as less onerous than prior-approval programs. PPRF has been identified as a core stewardship strategy, and most effective stewardship programs will have at least some form of this strategy, which has been supported by a variety of studies including randomized trials.[60,61]

As an illustrative example, Elligsen and colleagues used an interrupted time-series design to study the impact of a PPRF program implemented at 72 hours on streamlining broad-spectrum antimicrobial use in three ICUs.[62] Review of antimicrobials was performed by a pharmacist with backup from an infectious diseases physician. During the intervention period, suggestions for drug optimization were given for 34% of broad-spectrum antimicrobial orders, and 82% were accepted. Compared with the prior 12 months before the intervention, the mean monthly use of targeted antimicrobials decreased by 22% during the intervention period. Use of nontargeted antimicrobials did not increase during the intervention, leading to an overall reduction in antimicrobial use. The percentage of drug-resistant isolates was similar between the two periods, except for a reduction in meropenem resistance among gram-negative organisms. The monthly rate of *C. difficile* infections was decreased by 31% during the intervention period. Hospital and ICU length of stay and mortality were similar between the two study periods. The authors subsequently expanded their intervention outside of the ICU in the context of a stepped-wedge randomized trial, with a reduction in targeted antimicrobial use of approximately 20% among patients qualifying for audit and feedback.[63] However, targeted antimicrobial use did not significantly

decrease among all patients, a finding that the authors attributed to the shorter length of stay of floor patients. Another approach to PPRF might be termed "self-stewardship." Using approaches adapted from surgical quality improvement, these interventions include use of prescribing checklists or an "antibiotic time-out" to enforce monitoring at a team or prescriber level instead of by ASP personnel. Although intuitively attractive and resource efficient, data on the effectiveness of these approaches are currently limited.[64-67]

Some limitations of PPRF strategies include that in most programs the treating clinician has the option to accept or reject the recommendations made by the ASP. Therefore it is important that ASPs develop collegial relationships with providers and use evidence-based approaches supported by local or national guidelines that are likely to be acted on by clinicians when making recommendations. ASPs must also develop a mechanism to identify patients who have been started on targeted antimicrobials, which can be difficult in the absence of support for information technology. Finally, PPRF strategies do not ensure appropriateness of initial selection of empirical therapy in an individual patient, which can put patients at risk for overtreatment or undertreatment (although an effective PPRF intervention may improve the future empirical prescribing of the receiving clinician).

Some studies have attempted to compare the two major strategies for ASPs. Mehta and colleagues performed a before-and-after quasiexperimental study assessing the impact on use of antibiotics before and after prior approval restrictions (PPA) were lifted and PPRF was instituted for cefepime, piperacillin-tazobactam, and vancomycin.[68] They found that use of cefepime and piperacillin-tazobactam increased significantly after the change ($P = .03$), as did hospital length of stay after antibiotic initiation ($P = .004$), suggesting that a restrictive approach may have been associated with less antibiotic use. Tamma and colleagues conducted a crossover quasiexperimental study among four medicine units in which control units used PPA only and intervention units had PPA removed and PPRF implemented. Overall antibiotic use decreased by approximately 1 day in the PPRF group; however, empirical therapy was more appropriate in the PPA group.[69] Of note, a Cochrane review published in 2013 reported that restrictive strategies such as PPA were more effective in making short-term change than persuasive approaches such as PPRF, but the 2017 update of this review found that nonrestrictive approaches were also effective in reducing antibiotic use.[70,71]

ASPs must determine what are the most effective strategies for improving antibiotic use locally and may elect to use a combination of PPA and PPRF based on program and institutional resources and needs.

Computer-Assisted Antimicrobial Stewardship

Information systems have the potential to be effective adjuncts for antibiotic stewardship and education strategies. Computer order entry affords a unique opportunity for instantaneous feedback, education, and alteration in prescription patterns.[72] A report of an Internet-based ASP developed and studied at the Johns Hopkins Children's Medical and Surgical Center demonstrated improved communication, more timely antimicrobial administration, increased user satisfaction, and significant cost savings over prior stewardship methods.[56] Effective information technology support can also be very effective on the "back end" by helping to target audit and feedback to the highest-yield interventions. These programs have been associated with an increased number of stewardship interventions and reductions in targeted antimicrobial use, "drug-bug" mismatches, and resistant infections.[73-75] Other examples of integration of information technology include sharing of institutional guidelines on intranet or Internet sites or through mobile applications.[76,77]

A few computer-assisted programs offer sophisticated point-of-care decision support incorporating institution- and patient-specific data from the EMR and other systems and applying algorithms to suggest appropriate therapy and provide alerts. Studies of these systems have demonstrated improvements in appropriateness of therapy and patient outcomes; however, they are more complex and require significant investment to integrate into hospital systems.[78,79,80] Nevertheless, with advances in computer systems and artificial intelligence, there is clear potential for growth in this area over time.

MEASURING OUTCOMES FROM ANTIMICROBIAL STEWARDSHIP PROGRAMS

Implementation of programmatic antimicrobial stewardship strategies should be accompanied by well-considered plans to measure the outcomes of these strategies. Indeed, regulatory bodies are beginning to mandate that institutions track stewardship metrics internally, and required external reporting (including the possibility of public reporting) is on the horizon.[81] We briefly examine some of the key considerations for assessing the outcomes of ASPs.

Clinical Outcomes

Most studies of the impact of ASPs have had as their primary outcome the amount of aggregate antimicrobial use, antimicrobial costs, or rates of antimicrobial resistance.[32,33,34-36,71] When clinical outcomes such as clinical cure rate, length of hospital stay, or mortality have been examined, there has frequently been no difference between the stewardship group and the control group, although many of these studies are underpowered to detect these differences. A Cochrane Collaboration meta-analysis found no difference in mortality across 28 randomized controlled trials and an average reduction of 1 day in mean length of stay between stewardship and control arms across 15 randomized controlled trials in hospitalized patients.[71] Given the small average effect sizes, detecting significant improvements in clinical outcomes may not be realistic for most ASPs. Exceptions might be programs specifically targeting transitions of care (e.g., intravenous to oral administration conversion programs) or patients at highest risk of death (e.g., sepsis protocols or ICU-targeted interventions).

Economic Outcomes

Institutional cost avoidance with regard to antimicrobial expenditures is among the most frequently reported outcomes of ASPs.[82,83] However, if savings are calculated as a function of pharmacy expenditures, they tend to plateau over time, probably because of improved antimicrobial-utilization practices and sustained benefits of the program.[84] Thus, comparison of drug expenditure savings relative to projected cost growth in the absence of stewardship is a more relevant comparison than ongoing year-over-year savings.[84,85] Expenditures beyond drug acquisition costs should also be considered in evaluating the impact of ASPs. Gross and colleagues developed a probability pathway model to calculate both the direct and the indirect cost savings of a stewardship program.[86] In this model, total cost was defined as the sum of drug expenditures, microbiology costs, bed costs, and the costs of infectious diseases consultations; additional costs accrued if the initial antibiotic regimen failed. The median drug costs per recommendation were $50 lower for the ASP compared with usual practice, and total costs were $379 lower per recommendation. Based on the probability pathway model, the annualized savings were $363,000 in antibiotic expenditures and $2.7 million in total costs. Thus, institutions implementing ASPs should consider the broad range of expenditures that antimicrobial stewardship activities can affect, and should develop business proposals that provide sustainable support for antimicrobial stewardship program costs.[82] Failing to account for the entire spectrum of antimicrobial stewardship–related costs and savings risks providing an inaccurate economic foundation for a stewardship program's operation.

Microbiologic Outcomes

Studies using robust methodologies, including sophisticated mathematical modeling, have demonstrated convincing relationships between reductions in antimicrobial use and resistance on an aggregate level, including for *C. difficile,* multidrug-resistant gram-negative organisms, and methicillin-resistant *S. aureus* (MRSA).[46,87,88] However, the relationship between antibiotic use in the hospital and rates of antimicrobial resistance is complex. Further work is needed to evaluate the relative impact of infection control interventions to limit transmission and importation of resistant organisms from the community, long-term care facilities, and other health care settings, and the nature of antimicrobial changes within the acute-care institution.[89] Many investigators have reported the impact of antimicrobial stewardship interventions on antimicrobial resistance in terms of the metrics measured by hospital antibiograms—percentage

of clinical testing susceptible over a given time period. However, such measures may not be reflective of the absolute burden of resistance, which is better measured by the rate of resistant isolates in the population.[90,91] In addition, many studies use a "before-and-after" design without following trends in antibiotic use or resistance for sufficiently long periods before and after the intervention in order to establish a causal relationship or adequately controlling for confounding variables.[91] Finally, the relationship between antimicrobial use and resistance may be multifactorial (i.e., use of one antimicrobial may select for resistance to an unrelated antimicrobial if they are carried on the same genetic element) and nonsymmetrical (i.e., the relationship between increasing antimicrobial use and resistance may be different from that observed with decreasing use).[89,92,93] Guidelines for investigators that recommend standards for the conduct and analysis of quasiexperimental interventions to reduce nosocomial infections have been published and may be useful in designing and reporting the impact of antimicrobial stewardship interventions on resistance in an institution.[94]

ANTIMICROBIAL STEWARDSHIP PROGRAM STRUCTURE AND IMPLEMENTATION

Acute-Care Hospitals and Long-Term Care Facilities

As discussed earlier, the CDC has published its Core Elements for acute-care hospital ASPs, which largely agree with the published evidence-based guidelines released by IDSA and SHEA and are reflected in the standards implemented by the CMS and The Joint Commission.[15-17] The CDC has also published Core Elements for long-term care facilities such as nursing homes, which adapt the acute-care hospital Core Elements for the long-term care environment.[18] These Core Elements are summarized in Table 51.2. Among the acute-care hospitals surveyed by the National Healthcare Safety Network (NHSN) in 2014, 48% reported implementing all seven Core Elements.[31] Notably, the single lowest category for which implementation was reported was "hospital leadership commitment," with only 60% reporting having secured this; this is a crucial point. Following are recommendations for the design and implementation of a successful ASP in acute-care and long-term care facilities. Although resources for stewardship implementation (both financial and from the standpoint of having access to infectious diseases physician and pharmacist expertise) in the long-term care settings at this time are generally not as robust as in acute-care settings, the steps listed should be considered and modified based on local resources.[95]

1. Define the philosophy of the program. This may seem to be a trivial step, but the initial approach defined at this early time is likely to form the foundation for the success or failure of the program. Although surveys suggest that prescribers agree that antibiotic resistance is increasing in scope, costly, and related to antibiotic resistance, they tend to view their own antibiotic prescribing as appropriate.[23] Therefore, prescribers are more likely to respond to a program designed to "improve" antibiotic use or patient care rather than as efforts to "restrict" or "control" antimicrobials. Most prescribers will alter their behavior to improve the quality of patient care. Tying program objectives to specific patient care outcomes, such as reducing the incidence of *C. difficile* infection or carbapenem-resistant organisms, may engender more support than programs to generically "reduce antibiotic resistance." Identify key "influencers" of antimicrobial prescribing (e.g., senior or influential physicians) and open a dialogue with them early in the process of program implementation.
2. Involve hospital and long-term care administrators early in the design and implementation process. Provide evidence of the broad effects of suboptimal antimicrobial use beyond antimicrobial costs, and the spectrum of potential benefits to be realized. The support of key officials is critical to the success of the program. Prescribers are more likely to comply with a health system effort rather than with an infectious diseases or pharmacy program that may be perceived to have predominantly financial motives. Maintain an open dialogue with chief administrative personnel, and update them frequently concerning the progress of the program.
3. Develop a budget for all official positions and operating costs, including reasonable compensation for stewardship activities. Almost 500 infectious diseases physicians who participated in the Emerging Infections Network of the IDSA responded to a questionnaire that assessed their roles in ASPs.[57] Overall, half indicated that the hospitals in which they worked required approval of an infectious diseases physician before restricted antimicrobials could be dispensed, but payment for such services was rare (18%). Agree on a formula to calculate cost savings, ideally including savings beyond just antimicrobial costs, from stewardship activities. It may be prudent to involve managed-care organizations in these discussions if they are at risk for pharmacy costs.
4. Define the structure for accountability, reporting, and participation for the ASP. A program lead ultimately accountable for success needs to be identified, along with a core group of personnel, each of whose responsibilities should be defined. A reporting structure to facility administration should be created and a schedule for regular reporting implemented.

TABLE 51.2 Centers for Disease Control and Prevention Core Elements for Acute-Care Hospital and Long-Term Care Facility Antimicrobial Stewardship Programs

Leadership Commitment
Formal statements of support from administration
Dedication of staff time for stewardship activities
Supporting stewardship education and training
Ensuring and empowering participation from groups that can support stewardship activities (e.g., microbiology, infection control, nursing, information systems) *(Nursing commitment may be especially important in long-term care facilities.)*
Accountability
Identification of a single accountable leader for the program (e.g., infectious diseases physician, nursing home medical director)
Drug Expertise
Identification of a pharmacy leader who will colead the program *(For long-term care facilities, engage the facility consultant pharmacist in stewardship or recruit a trained infectious diseases pharmacist to consult with the program.)*
Active Stewardship Strategies
Broad interventions (prior authorization, prospective audit and feedback, antibiotic time-outs)
Pharmacy-driven interventions (e.g., intravenous-to-oral transition, dose optimization, automatic stop orders)
Infection- and syndrome-specific interventions (e.g., pneumonia pathway, asymptomatic bacteriuria policy)
Tracking
Process (e.g., adherence to guidelines) and outcome measures (e.g., *Clostridioides difficile* (formerly *Clostridium difficile*) rate)
Aggregate antibiotic use and facility-wide antimicrobial resistance
Reporting
Regular feedback of tracking metrics to facility personnel and reporting to agencies as required
Education
Education of clinicians about appropriate antimicrobial use, resistance, and infection management

Considerations specific to long-term care facilities are in *italics*.
From Pollack LA, Srinivasan A. Core elements of hospital antibiotic stewardship programs from the Centers for Disease Control and Prevention. Clin Infect Dis. *2014;59(suppl 3):S97–S100.*

5. Gather baseline data concerning antimicrobial expenditures, antimicrobial-utilization patterns, and susceptibilities of nosocomial and community pathogens. Benchmark the antimicrobial budget and antimicrobial use to similar institutions, if possible. Participation in the NHSN's Antimicrobial Use and Resistance Module allows measurement of aggregate antimicrobial use in days of therapy per 1000 patient-days present and enables benchmarking against other participating institutions. Access to antibiotic use data can be challenging in the long-term care setting, but sites should work with consulting pharmacists to identify potential sources of data.
6. Evaluate the antimicrobial formulary for redundancy and seek competitive bidding between therapeutically equivalent antimicrobials as a cost-saving measure when feasible.
7. Develop and publish guidelines for antibiotic use and empirical antimicrobial therapy. Recommendations should be based on local susceptibility profiles. In addition, establish appropriate dosing and dosage intervals based on disease-state and pharmacokinetic principles. This step is critical to the success of the program. Involve key personnel from relevant departments when designing the guidelines in order to build consensus for the recommendations. Be willing to compromise on certain issues, but demand that all recommendations be evidence based. It is also worthwhile to consider multimedia formats. Creation of an institutional website for sharing ASP information and guidelines is recommended; a number of examples are available.[76] The website can include institutional antimicrobial susceptibility, dosing recommendations, and price lists along with the clinical guidelines.
8. Define the strategies that the program will use. Interventions must be tailored to the character of the institution. For example, telephone-approval mechanisms are less likely to be successful at a community hospital staffed by busy private practitioners. Begin with a focus on targeted agents based on frequency of use, cost, toxicity, and contribution to resistance. Consider whether inappropriate laboratory and microbiology testing (e.g., testing for *C. difficile* or urinary tract infection in the absence of signs and symptoms of infection) is leading to overuse of antibiotics, and address these issues with appropriate interventions. In the long-term care setting, consideration should be given to obtaining infectious disease expertise for implementation of stewardship activities through consulting agreements with infectious diseases physicians, pharmacists at local acute-care hospitals, or both or via telemedicine, because involvement of infectious disease expertise has been associated with improved uptake of stewardship activities in this setting.[96,97]
9. Develop mechanisms to arbitrate disagreements. A discussion between the prescriber and the director of the program is usually sufficient if the director has administrative time to handle these issues. A mandatory infectious diseases consultation is another option.
10. Develop innovative educational methods. The Internet can be a useful tool in this regard. Information systems personnel may be helpful in incorporating guidelines, recommendations, or reminders into the hospital computer network. Ensuring that guidance on testing and treatment is available at the point of care is likely to increase their adoption. Especially focus on education of health profession trainees who are eager to improve their knowledge of antimicrobial use.
11. Develop and maintain a database to monitor clinical and institutional outcomes, publish and present results, and share best practices.
12. Continually reevaluate the program, paying particular attention to changes in susceptibility profiles and patterns of use. Drug-utilization evaluations should be a part of this process. Reformulate the program as indicated to address problems as they arise. This should be a dynamic process that is responsive to the needs of prescribers and their patients.

Outpatient and Ambulatory Care

The vast majority of studies of antimicrobial stewardship focus on acute-care hospital settings (and especially academic medical centers), despite the fact that most antimicrobial consumption occurs outside of the hospital.[98] A number of recent studies have demonstrated the feasibility and effectiveness of ASPs at improving antimicrobial use in these settings.[99,100,101] CDC guidelines provide Core Elements for antimicrobial stewardship in ambulatory and outpatient care (Table 51.3).[19] These guidelines recognize the need to adapt traditional antimicrobial stewardship approaches to this different care environment. Examples of comprehensive outpatient stewardship programs are currently uncommon, but studies from the literature suggest certain strategies to consider when implementing a stewardship program in the outpatient setting.

1. Form partnerships with patients and parents. Prescriber perceptions of real or assumed patient or caregiver demands for antimicrobial treatment are a key driver of antimicrobial prescribing in the outpatient setting.[102] Training for providers in communication and shared decision making can reduce inappropriate antimicrobial use while maintaining patient satisfaction.[103,104] Provide a balanced view of the risks and benefits of antibiotic use on patients' health and the health of those around them.
2. Provide aggregated feedback. Traditional patient-level audit-and-feedback approaches are not feasible in the outpatient

TABLE 51.3 Centers for Disease Control and Prevention Core Elements for Outpatient Antimicrobial Stewardship

ELEMENT	CLINICIAN EFFORTS	ORGANIZATIONAL EFFORTS
Demonstrate commitment to appropriate antimicrobial use	Publicly attest to commitment to appropriate antimicrobial use (e.g., signed poster in clinic)	Appoint and hold accountable a single leader for stewardship at the facility Incorporate stewardship-related duties in job functions and descriptions Ensure all clinic staff communicate and demonstrate antimicrobial stewardship messages to patients
Act to improve antimicrobial prescribing	Adhere to evidence-based diagnostic and prescribing standards Incorporate delayed prescribing or watchful waiting into care plans when appropriate	Provide communications skills training for clinicians Require explicit justification for nonrecommended antimicrobial prescribing Provide decision support to facilitate best-practice prescribing Reduce unnecessary clinic visits for low-acuity conditions by using call centers, hotlines, or pharmacist consultation
Tracking and reporting	Self-evaluate antimicrobial prescribing practices Participate in quality improvement activities to track and improve antimicrobial prescribing	Implement an antimicrobial tracking and reporting system Assess and share performance on quality measures
Education and expertise	Use effective strategies to communicate with patients around the need for antimicrobials Educate patients about potential harms of antimicrobial use Provide patient education materials, refer patients to reputable sources of information on appropriate antimicrobial use, or both	Provide face-to-face academic detailing for clinicians Provide continuing education programs for clinicians Ensure timely access to persons with expertise

From Sanchez GV, Fleming-Dutra KE, Roberts RM, et al. Core elements of outpatient antibiotic stewardship. MMWR Recomm Rep. *2016;65:1–12.*

environment, given the greater volume of patient visits, the lower degree of objective data (e.g., microbiologic cultures), and the "one-off" nature of most outpatient encounters. However, providing periodic feedback to prescribers on their aggregate antimicrobial prescribing for targeted conditions, especially if performed with peer benchmarking data or in the context of an in-person academic detailing visit, can be effective in improving prescribing.[105,106,107]

3. Set expectations for prescribing at the practice level and develop approaches for prescribers to follow them. Posting commitment pledges to prescribe antibiotics only when indicated, which are personalized to the practice and provider, have been shown to be effective in improving antibiotic use. Providing clear and easily accessible guidelines for prescribers around common outpatient disease states may help to improve appropriate prescribing, although the impact may wane without reinforcement.[40,109] Designing electronic order entry systems to prioritize or default to guideline-concordant therapies and to require written justification for nonconcordant therapies at the time of prescribing can further encourage appropriate antimicrobial use.[108,110,111]

THE FUTURE

The rising prevalence of antimicrobial resistance and its clinical and economic impacts are being recognized as critical by clinicians, administrators, and health care regulatory bodies.[15-21] Comprehensive ASPs decrease costs while improving both patient and institutional outcomes.[70,71,83] However, further work is needed to clearly identify the relationship between antimicrobial use and the emergence of resistance in a way that colleagues will appreciate and accept. Relevant studies will require sufficient statistical power to characterize baseline resistance, to deal with random variation, and to control for a multitude of confounding variables. The explosion of electronically collected data provides the opportunity to programs to continually monitor their performance and to use benchmarks from other institutions. However, further work needs to be done to identify key metrics, provide risk adjustments, and create estimates of appropriateness of antimicrobial use from aggregate data.

The pace of new antimicrobial development has increased recently from its long nadir, in response to investment from the pharmaceutical industry and changes to government regulations and incentives.[112] However, it is far from clear whether this increased development will keep pace with the growth in antimicrobial resistance. Moreover, indiscriminate use of new agents will compromise their efficacy, too. The focus on antimicrobial stewardship strategies must continue to shift from cost containment to efforts to limit resistance and improve patient safety. It is likely that the increasingly sophisticated information systems being deployed in all hospitals will play a more prominent role in antimicrobial stewardship. Several companies currently market products that can either assist in monitoring antimicrobial therapy and evaluating interventions or actually offer computer decision support for antimicrobial selection and dosing. Advances in the development of rapid diagnostics and biomarkers will likely improve the precision of determining the need for and optimal selection and duration of antimicrobial therapy, and ASPs will be critical in ensuring that providers integrate these new technologies into practice. The development of improved infection diagnostics is at least as essential to the control of antimicrobial resistance as new antimicrobials and should be similarly incentivized. The current crisis of antimicrobial resistance and the paucity of viable therapeutic options mandates that antimicrobial stewardship will become an increasingly important responsibility of all clinicians.

ACKNOWLEDGMENT

The authors and editors wish to thank Drs. Ronald E. Polk and Neil O. Fishman for their contributions to prior editions of this chapter.

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52 Designing and Interpreting Clinical Studies in Infectious Diseases

John H. Powers III

LEXICON OF TERMS IN CLINICAL STUDY DESIGN AND ANALYSIS

Types of Studies

Exploratory studies: Studies that provide preliminary evidence used as the basis for designing further studies to confirm the effects of a test intervention compared with a control intervention; sometimes called "pilot studies," to evaluate operational characteristics of a study (e.g., ability to enroll participants or enrollment processes); often provide erroneous results, given the small study size or outcomes based on biologic activity that does not reflect patient outcomes. Results are sometimes erroneously presented as confirmatory evidence after the conclusion of study. May use descriptive or inferential statistical methods.

Confirmatory studies: Studies intended to provide firm evidence in support of causal relationships between an exposure or intervention and outcomes between a test and a control group; include statement of hypothesis, adherence to protocols, and standard operating procedures. Use inferential statistical methods to analyze results.

Controls in Clinical Studies

Control group: A group in which an intervention is administered for which the effects of the intervention are already known from prior evidence; outcomes in the group that receives the test intervention are compared with the outcomes of this control group. The purpose of a control group is to evaluate whether the effects of the intervention are due to the intervention or to the conditions of the experiment or other factors through *counterfactual evidence*—what would happen to a group that did not receive the test intervention. Control groups are unnecessary only in rare situations. Examples would be diseases in which an unbiased outcome is well known, such as all-cause mortality, or even rarer situations in which patient factors or other factors have limited or no effects on outcomes.

No-treatment concurrent control: No specific treatment prescribed to control group (does not exclude supportive care); cannot be blinded; uses superiority hypotheses.

Placebo concurrent control: Control group receives placebo that appears similar to test intervention; can be blinded; uses superiority hypotheses.

Dose-comparison concurrent control: A higher dose or exposure to the test intervention is compared with a lower dose or exposure of the test intervention; uses superiority hypotheses.

Active concurrent control: Control group receives biologically active intervention known to be effective from prior studies; may or may not be blinded; can be superiority or noninferiority hypotheses; placebo can be used to blind study in add-on trials testing older standard of care plus placebo compared with older standard of care plus new intervention.

External concurrent control or historical control: Study that compares a group receiving the test intervention with a control group external to the study, rather than to an internal control group from the same population assigned to a different treatment. The external control can be a group evaluated at an earlier time (historical control) or a group evaluated during the same time period but in another setting (external control); design is more susceptible to bias in terms of selection of participants (control groups in historical controls, on average, have worse outcomes than concurrent controls) and changes in medical care over time.

Trial Hypotheses

Hypothesis: The investigator's proposed explanation for the relationship between the exposure or intervention (e.g., a drug) and the outcomes of the study (e.g., mortality, symptoms) that is tested in the study; based on prior accrued evidence. The results of a study are compared to the prestated hypothesis of the study to allow valid interpretation of the results. Studies without hypothesis provide exploratory and not confirmatory evidence.

Null hypothesis: The starting point of all trials in which the assumption is that the investigators' hypothesis is *not* true and the study cannot exclude the possibility that the intervention or drug has no effect on outcome; based on the "theory of falsification," in which hypotheses must be rigorously tested to show that they are not false.

Alternative hypothesis: If evidence from the study shows the null hypothesis is unlikely to be true, then investigators can accept (not prove) the alternative hypothesis that the relationship between the exposure or intervention and the outcome is likely to be true.

Noninferiority hypothesis: A hypothesis in an active controlled trial that evaluates whether the test intervention is no worse than the control intervention by a predefined difference. The predetermined difference is selected because it is considered clinically insignificant to patients. The difference must be greater than the known effect of the control intervention compared with placebo or no specific therapy. This hypothesis is misnamed in that the test intervention can be inferior to the control intervention, just by an amount smaller than a predefined amount. The predefined amount of difference in end points between the test and control groups, called the noninferiority margin, or "delta," is the difference for which the test intervention may be permitted to be worse than the control, using 95% confidence limits. If the difference is less than the delta, one may accept the alternative hypothesis that the treatment being compared is not worse than the other treatment by the prespecified amount.

Equivalence hypothesis: A hypothesis in an active controlled trial that evaluates whether the test intervention is no worse *and* no better than the control intervention by a predefined amount. Unlike in noninferiority studies, there is an upper limit of the difference in addition to a lower limit on the difference between the end points in the test and control groups. A classic example is bioequivalence studies, in which a generic drug must have concentrations that are no lower and no higher than some prespecified amount.

Superiority hypothesis: A hypothesis in a no-treatment, placebo, dose-response, or active controlled study that evaluates whether the test group has better outcomes than the control group by some prespecified amount; often designed to rule out no effect (zero absolute difference) but can be designed to rule out some clinically meaningful effect other than zero (e.g., confidence interval (CI) around difference between test and control groups should rule out some nonzero amount for intervention to be clinically useful);

asks that one treatment is at least a certain percentage better than the other treatment.

Types of Error in Clinical Trials

Random error: Error due to chance alone or random sampling error; statistics are used in the study planning to decrease random error through techniques such as selecting a sufficient number of participants for a study (sample size) and to evaluate the results of a study for potential effects of random chance; can be decreased by increasing sample size.

Alpha or type 1 error: The prespecified false-positive error rate or probability (i.e., concluding that the evidence is sufficient to accept that the intervention is effective when in truth it is not) for a study conventionally set at 0.05 or 5%, meaning that for every 100 studies performed, 5 would show results as great as those observed by chance alone in the absence of other biases; relates to the *P* value and hypothesis testing after the study is over because the *P* value should be smaller than the type 1 error chosen.

Beta or type 2 error: The prespecified false-negative error rate (i.e., concluding that the evidence is insufficient to accept that the intervention is effective when in truth it is effective); conventionally set at 0.10 or 0.20 (10% or 20%).

Power: The ability of a study to detect a difference if in truth a difference exists; determined *before* initiation of a study; defined as 1 minus the type 2 error; type 2 error of 10% or 20% corresponds to power of 90% or 80%, respectively. It is inappropriate to perform power calculations on observed results after a study is complete.

Delta, or effect size: The predetermined amount of difference between the test and control groups expected in the study; in superiority hypotheses, the delta equals the expected amount of superiority of the test group compared with the control group; in noninferiority hypotheses, the delta equals the amount of inferiority of the test group compared with the control group that would be the limit one would accept as not being clinically important; in equivalence hypotheses, the delta is the largest difference one would accept as showing equivalence to the comparator.

Sample size: The number of participants or groups planned to be enrolled in a study; based on calculation using the type 1 error, type 2 error, effect size, and standard deviation (spread of values in the data).

Systematic error: Errors that do not occur by chance in the course of designing, conducting, or analyzing studies and can cause the observed results of a study to deviate from true results; addressed by design of study and not by statistical methodologies after study is complete; increasing sample size increases effects of systematic bias, so a larger study is more incorrect.

Selection bias: Systematic differences between participants in test and control groups with regard to factors that independently affect outcomes; can result from lack of randomization or exclusion of participants from randomized studies.

Observer or assessment bias: Bias that is present when persons assessing outcome are influenced by knowledge of treatment assignment that influences assessment of outcomes.

Misclassification bias: Bias that is present when a given measurement is defined in error (e.g., *cure* is called *failure*); occurs when insufficient instructions or training is given to make assessments (e.g., clinician judgment when clinicians are not clearly instructed regarding what to assess in making judgments).

Instrument bias: When instrument (scoring system or laboratory test or clinician judgment) does not make accurate measurements compared with true value.

Confounding: Factors that can affect study independent of test and control intervention such that outcomes are not causally related to interventions tested (e.g., prescribing of concomitant effective medications in the setting of a noninferiority trials obviates assessments of both test and control drug); addressed by randomization in superiority trials; can still occur in randomized noninferiority trials because of factors such as effective therapy prescribed before randomization. This therapy biases the study toward showing no difference. Confounding occurs more often in nonrandomized studies.

Methods to Control Bias in Clinical Trials

Randomization: Nonsystematic method (e.g., flipping a coin or random table of numbers) used to assign participants to test and control groups to decrease selection bias and balance groups by baseline factors that affect outcomes; randomization gives best chance of avoiding confounding differences between the groups and of incorrectly assigning causality of the outcomes to the interventions tested; effective randomization minimizes baseline differences between test and control groups; does not control for sources of bias that occur after randomization, such as misclassification of outcomes; excluding participants from the analysis decreases protection of randomization against selection bias.

Individual randomization: Unit of randomization is the individual participant; used where effects of the intervention have direct effects on individuals but no indirect effects on the group.

Cluster or group randomization: Unit of randomization is a group such as a hospital, institution, neighborhood, or geographic area; used where indirect effects of the intervention may carry over to others in the group (e.g., herd immunity in vaccination studies).

Allocation concealment: Lack of knowledge of the randomization code so that investigators cannot affect who is enrolled in the study groups and who is not (selection bias).

Blinding: Lack of knowledge of the assigned intervention by any or all of these three groups: participants in the study, investigators implementing the study, and those who assess the outcomes of the study; "double-blind" studies usually are those in which there is lack of knowledge of the treatment intervention by both participants and investigators, and in which investigators both implement the study and assess outcomes.

Types of Nonrandomized Studies

Prospective study: Study in which hypotheses precede data collection.

Retrospective study: Study in which hypotheses are formed after data have already been collected.

Case-control study: Retrospective study in which investigator selects outcomes of interest and then looks backward in time to compare exposures between the test and control groups (e.g., examine survivors of disease, then look back in time to see proportion of survivors who did or did not receive a drug).

Cohort study: Prospective or retrospective longitudinal study in which investigator selects exposure of interest and then compares outcomes between test and control groups (e.g., comparing sexual activity of teenagers who did or did not receive the human papillomavirus vaccine).

Methods to Attempt to Control Selection Bias in Nonrandomized Studies

Matched control: The test and control groups are matched for the baseline variables thought to be most likely to influence the outcome being evaluated by using methods such as logistic regression. Unrecognized confounding differences between the two groups that influence outcome are the major limitation of this method of analysis.

Propensity score control group: Assigns a score based on several baseline factors to individual study participants that represents the probability that the person had a given exposure (e.g., received a particular drug), then compares outcomes between test and control group participants with similar propensity scores. Several methods exist for comparing propensity scores (e.g., matching patients with identical scores, matching patients with similar scores, matching patients with scores similar within some range); cannot compensate for large differences in choice of treatment or clustering in choice of treatment or unmeasured confounders. For example, if one treatment was largely confined to intensive care patients, or inpatients, and the other treatment was not, propensity scoring cannot compensate for the differences in outcome.

Outcomes of Clinical Studies

Outcome assessment: The actual measurements performed in clinical trials that are used to define end point(s). Outcome assessments can include survival, biomarkers, patient-reported outcomes (PROs), clinician-reported outcomes (ClinROs), observer-reported outcomes (ObsROs), and performance outcomes (PerfOs).

Clinical outcomes assessments: Direct or indirect measures of how patients feel (symptoms) or function in their daily lives that are based on judgments or efforts of the observers or patients. Include PROs, ClinROs, and PerfOs but not biomarkers or survival.

Patient-reported outcomes (PROs): Direct measures of how patients feel or function obtained directly from patients without interpretation by anyone else.

Clinician-reported outcomes (ClinROs): Indirect measures of patient benefit involving evaluation of physical signs or other observations captured by clinicians based on clinician judgments.

Surrogate outcome assessments: Indirect measures of patient's health status, which are physical signs or biologic parameters, including laboratory measurements used as substitutes for how patients feel (symptoms), function, or survive.

End points: End points of a study include the following: (1) outcome and how it is defined (e.g., survival, cure) or (2) timing when the outcome is to be assessed (single or multiple fixed times, time to event). End points can be composed of a single measurement (e.g., all-cause survival), multiple independent measurements (coprimary), or multiple measurements combined into a single evaluation (composite end points; e.g., all-cause survival plus symptoms plus patient function).

Primary end point: The single specified outcome compared between the test and control groups. The sample size of the study is usually chosen based on this outcome.

Secondary end points: Multiple outcomes that may help explain the primary end point but are not appropriate for statistical analysis unless the study is designed with secondary hypotheses and appropriate sample size, and increase in false-positive rate resulting from multiple testing is controlled for. For example, a new drug may be better than an old drug in terms of the primary end point, and a secondary end point may indicate that this is particularly true for one subset of patients. Secondary outcomes are particularly problematic when used to claim outcomes different from the primary outcome—for instance, finding noninferiority in one group but claiming superiority on secondary end points that were not designed appropriately at study inception.

Analysis Populations

Analysis population: The types of participants in the study who are included in the analysis.

Intention-to-treat (intent-to-treat) analysis (ITT): Includes all randomized participants regardless of intervention actually received in the analysis; maintains protection of randomization from selection bias.

Modified intention-to-treat analysis (mITT): Includes patients in analysis based on information obtained before randomization (such as a particular diagnosis); maintains protection of randomization from selection bias as long as numbers of exclusions compared with ITT analysis are few.

Per-protocol/as-treated/clinically evaluable analysis (PP): Includes patients in the analysis who follow the protocol as specified; based on excluding patients from analysis based on events that occur after randomization. Exclusions can bias study results and decrease randomization's protection from selection bias.

Analysis of Study Results

Number needed to treat: The number of patients needed to treat in order to benefit one patient is defined as the inverse of the absolute difference in outcomes between the test and control groups; can evaluate number needed to treat to benefit one person and number needed to treat to harm one person.

95% confidence interval (CI): A measure of *estimation* used to estimate the difference in a population from the observed results in a sample; based on the theory that, on average, if 100 identical studies were performed, 95 of the studies would include the true difference and 5 studies would not contain the true difference between the test and control groups; any value within the 95% CI is equally likely to be the true mean (it is not a probability), but often the 95% CI is misinterpreted as the upper and lower boundaries around a difference. Results are in the units of the study outcome and so can be readily interpreted in terms of clinical meaning. The range of values useful in interpreting both "positive" and "negative" studies—for instance, a "negative" study with wide 95% CI that does not exclude clinically meaningful differences is inconclusive, but a study with narrow CIs that does exclude clinically meaningful differences can provide stronger evidence.

***P* value:** A measure of *hypothesis testing*; interpreted as the probability that results as extreme as those seen in the study could occur by chance; statistical significance is defined conventionally as *P* values less than .05 (<5% or 1 in 20 probability of results occurring by chance alone); not a measure of bias, so biased studies can show "statistically significant" results; not a measure of clinical meaningfulness because not measured in terms of study outcomes. Studies with larger sample size can show "statistically significant" results that are not clinically meaningful. It is not a measure of the likelihood that intervention is or is not effective—for instance, a *P* value of .01 does not mean that there is only a 1% chance the intervention is ineffective, but it is often misinterpreted as such. False-positive error in *P* values increases with greater number of comparisons between test and control groups (called *multiplicity*).

Inferential statistics: Statistical methods using the results from the sample of patients studied to draw conclusions about the results in the population from which the study patients were drawn. Includes hypothesis testing (*P* values) and CIs around the difference between the test and control groups**.** Used to evaluate effects of medical interventions in the larger population based on the sample of patients studied in a clinical trial.

Descriptive statistics: Statistical methods used to report the results in the sample studied without comparison to another group or inference to a larger population—for example, describing the number of patients in an institution who develop pneumonia in 1 year without comparison to another year or another institution. Not appropriate for evaluating the effect of medical interventions in clinical trials or for making inferences about effects in larger populations based on a single sample.

OVERVIEW

The volume of information available to caregivers and patients increases exponentially with each passing year. The availability of information in textbooks, in journals, and through the Internet requires an ability to sift through data efficiently to determine what is valuable in clinical decision making versus what is hypothetical, hype, or harmful. A comparison of the time necessary to review medical journals compared with the time available for general practitioners showed that clinicians would need to read 19 journal articles per day for 365 days a year to stay current.[1] However, in this same study, clinicians indicated by self-reports that they had well less than an hour a week to review new medical information. The half-life of scientific information is quite short, and therefore there is a continual need to reevaluate as new information emerges. Inappropriately designed studies may be accurate measures of prevailing biases rather than informative for clinical practice.[2,3] In addition, authors have pointed to an increasing inability to confirm the results of clinical research, which may in part be due to the design and analysis of those studies.[4] These issues make the knowledge to critically evaluate evidence a necessity rather than a luxury.

Clinical practice and clinical research continuously interact. Observations in clinical practice form the basis for research questions that are then evaluated to determine the probability of their truthfulness, their generalizability, and their reliability. The results of research are then

applied in practice, leading to a new set of questions. However, clinical practice and clinical research differ in important aspects. Clinical practice refers to interventions designed solely to enhance the well-being of an individual patient and that have reasonable expectations of success. The purpose of clinical practice is to provide diagnosis, preventive treatment, or therapy to particular individuals. By contrast, clinical research designates an activity designed to test hypotheses in groups of research participants, to permit conclusions to be drawn, and thereby to develop or contribute to generalizable knowledge expressed in theories, principles, and statements of relationships.[5] Research is described in a formal protocol that sets forth an objective and a set of procedures designed to reach that objective. Because interventions are used in clinical practice does not necessarily make them more beneficial than harmful. In addition, although in vitro data and pharmacokinetic and animal studies form the building blocks for proofs of principles and generation of important hypotheses concerning effectiveness and harms of medical interventions, these hypotheses require adequate testing in humans to ascertain the benefits and harms of medical interventions, including drugs, biologics (e.g., vaccines, antibodies), diagnostics, and devices. A study showed that 28.1% of investigational new drug (IND) applications for antimicrobials submitted to the US Food and Drug Administration (FDA) were subsequently approved, a higher percentage than in any other therapeutic area.[6] However, all of the 71.9% of drugs that were not approved had promising in vitro data and animal studies, showing the importance of appropriately designed trials in humans to test hypotheses. More than half the recommendations in treatment guidelines are based on opinions or case series, demonstrating the need for appropriate testing of much of what is recommended in the absence of substantial evidence.[7] Clinical research performs the systematic evaluations that allow clinicians, caregivers, patients, payers, and policy makers to understand that interventions truly are helping more than hurting.

HISTORY

Before the 1930s, fields in science based conclusions on personal experience and observations that were then extrapolated widely. This *observationalist-inductivist* view led to incorrect and harmful theories, such as bloodletting for various infections, a practice that lasted for thousands of years.[8] Since the 1930s, however, science has been based on *falsifiability*—that is, testing hypotheses to ascertain whether they might be false.[9] For example, one might conclude by observing a lake full of white swans that all swans are white. Further testing of this hypothesis and finding a single black swan "falsifies" this conclusion. To test the percentage of swans that are white, one would have to make enough observations to draw valid conclusions. This relates to the statistical principle of the "null" hypothesis—starting with the hypothesis that the intervention is not effective, then gathering evidence to show with sufficient probability that the null hypothesis is unlikely to be correct. In this regard, hypotheses are not "proven" but are shown likely to be correct based on the evidence. Inherent in this view is that hypotheses must be appropriately tested in a fair and unbiased manner.[10]

The field of infectious diseases has a distinguished history in advancing the methodology to elucidate descriptions of disease and testing hypotheses by making fair comparisons among medical interventions. The first studies to use control groups, placebos, double-blinding, and randomization were all performed to study various infections.[11] The investigators of these studies were well aware of how the play of chance and bias could affect the validity and clinical usefulness of their results. Although drugs such as penicillin, used in the treatment of various infections, had large treatment effects on decreasing mortality, even penicillin was studied in thousands of patients in order to understand its effects in different infections.[12] The promotion of ineffective and harmful antibiotics in the 1950s prompted infectious disease physician-scientists to lead the way in establishing "adequate and well-controlled trials" as the regulatory standard in law for determining the effectiveness of new drugs, including antibiotics.[13]

DEFINING TERMS

A common language is necessary to communicate about both the design and the results of clinical research studies. Research and science, in general, entail making measurements and applying those measurements to study natural phenomena. Some terms are commonly used in the description of measures. One of the most common is the term *validity*. Validity refers to the extent to which measures actually reflect what they purport to measure. Although all measures have some associated error, validity implies that the amount of error does not appreciably affect the truthfulness and the usefulness of the results.[14] Therefore, even though measures are not "perfect," the goal is to minimize error as much as possible. Validity is not an all-or-none quality but rather is contextual, affected by factors such as the use to which the measure is put (diagnosis vs. prognosis vs. outcome) or the population from which the measures are obtained. A measurement that is valid in one situation may lack validity in another setting. *Internal validity* refers to the validity of the results of an individual study in that the study is capable of providing adequate evidence for the primary research question. *External validity* refers to the generalizability of the results to patients or clinical settings outside the study. Internal validity is a prerequisite for external validity. If the results are not valid for the study itself, it is not possible to extrapolate those results to other settings. *Reliability* is the ability to obtain similar data that are reproducible on repeated measurements. Validity must precede consideration of reliability. If a measure lacks validity, it may be reliable but just reliably wrong. *Responsiveness* is the ability of measurements to detect change over time.

Association, correlation, and causality are terms used to describe the relationships between measures.[15] *Association* implies a general relationship, whereas correlation is a means of quantifying that relationship. *Correlation* is a subset of association and implies that as the value of a measure changes, the value of a related measure also changes, either in the same or the opposite direction. For instance, as the human immunodeficiency virus (HIV) load increases, mortality from HIV/acquired immunodeficiency syndrome (AIDS) increases (a positive correlation), whereas, as CD4 count decreases, mortality increases (an inverse correlation). Neither association nor correlation implies causality. *Causality* means that one measure precedes the other and that the first measure is necessary and sufficient to cause the second measure. Correlation is often sufficient when evaluating questions related to risk factors and prognosis of disease, but correlation alone is insufficient when evaluating effects of interventions on outcomes, wherein assessment of causality is needed.

Treatment effects are measures of the causal impacts of interventions on outcomes, measured by differences in outcomes between baseline comparable test and control groups. Treatment effects are not measured by correlation alone. For instance, there is a correlation between patients with disease caused by organisms with higher minimal inhibitory concentrations (MICs) to a specific drug and increased mortality.[16,17] However, this relationship may not be a measure of treatment effects if patients infected with higher MIC organisms differ from patients with organisms with lower MICs on important factors that influence mortality.[18,19] Therefore, higher MICs are not a measure of treatment effects of the chosen drug because other factors such as age, severity of disease, and comorbidities also affect outcomes. Factors that affect outcomes independent of the chosen factors because of imbalances between study groups are called confounders. *Confounding* can occur when there are baseline imbalances between study groups, most often in nonrandomized, observational studies. For instance, in an observational study comparing interventions, if there are more patients with higher Acute Physiology and Chronic Health Evaluation (APACHE) scores in the test group than in the control group, then an imbalance in severity of disease may be causally related to outcomes rather than the interventions tested. Confounding differs from effect modification. *Effect modification* refers to treatment effects that differ in specific groups of patients that are baseline comparable with regard to other factors. For instance, in a randomized trial, if the treatment effect on mortality (the difference between the test and control groups) of a new drug is greater in an appropriately designed subgroup analysis of patients with lower baseline APACHE scores, but smaller or nonexistent in an appropriately designed subgroup of patients with higher APACHE scores, then the severity of illness as measured with APACHE is a causal effect modifier, not a confounder, because randomization balances other factors between the groups besides the interventions received.

CHOOSING A RESEARCH QUESTION AND DEFINING GOALS OF A STUDY

Before discussing other aspects of a clinical research study, one must first decide on the research question that will be evaluated. Many types of questions form the basis for clinical research, including questions related to biologic, behavioral, cognitive, environmental, and sociologic and cultural aspects of disease. This chapter focuses mainly on biologic questions. Biologic questions include those related to natural history, diagnosis, risk factors for acquisition of disease or outcomes, and prognosis, in addition to testing of interventions to influence the outcomes of disease.[20]

A common error in clinical research is lack of clarity on the end points, the specific question or questions asked by the study, or asking too many questions, resulting in a study that cannot answer any question validly or reliably. Because the type of research question directly affects the choice of the study design, greater specificity about the research question allows better choice of study design.

There are two main categories of research studies. *Descriptive research* makes observations and describes the results of those findings, providing an accounting and delineating components of a problem (such as the number of persons in a hospital with a particular disease) without making inferences regarding causality. There are usually no specific hypotheses in this type of study, and the sample size is defined based on the setting (e.g., all patients in a hospital) or on obtaining a certain degree of precision around the estimate of the described data. Examples of descriptive research include single case reports and case series. An "open-label, noncomparative, single-arm trial" is in fact not a trial but a case series. *Qualitative research,* such as patient interviews, is a form of descriptive research. These types of studies are useful in providing observations that form hypotheses for future testing.

Analytical research studies attempt to make comparisons between groups. Analytical research entails specific hypotheses stated by investigators that are evaluated during the course of the study. Hypotheses are investigators' statements of belief, but they are not decided on haphazardly because they are based on prior studies that have informed both the research question and design of the planned study. However, multiple prior hypothesis-generating studies do not obviate the need to test specific hypotheses.[21]

TYPES OF ANALYTICAL STUDY DESIGNS

Analytical research is divided into *experimental* and *nonexperimental (observational)* research designs.[16] The primary difference is that in nonexperimental designs the investigator does not assign research participants to the study groups. Rather, participants are assigned according to usual practice, such as the drug they would ordinarily be given, which may result in differences in both measured and unmeasured factors between study groups, affecting the comparability of those groups. Nonexperimental research designs include cohort, case-control, and cross-sectional study designs. *Case-control studies* choose participants based on outcomes and then look back in time to evaluate differences in exposures between the groups. Case-control studies are inherently retrospective, meaning data collection occurs before hypotheses are formed. Case-control studies are useful when the outcomes evaluated are uncommon.[22] *Cohort studies* begin with exposures and then evaluate differences in outcomes between the groups. Cohort studies can be prospective, meaning that hypotheses precede collection of the data, or retrospective. Cohort studies can also contain nested case-control studies. Cohort studies are useful when exposures are uncommon. *Cross-sectional studies* evaluate exposures and outcomes at a particular point in time.

In experimental studies, also called *clinical trials,* investigators choose the groups to which participants are assigned, as discussed in more detail later. There are two basic approaches to clinical trials that differ in design characteristics. The first type is called an *explanatory trial,* in which an intervention is evaluated under "optimal" conditions wherein the intervention is expected to have an effect, such as in a narrow population in which the intervention is administered by specialists in the field. These trials test for a causal effect on outcomes but often do not ask the more generalizable question of outcomes with the intervention as used in practice. The other approach is a *pragmatic trial,* which evaluates strategies or approaches under usual care conditions, with the goal of helping to select options for care as interventions as used in practice.[23] In reality, trials often extend along a continuum between explanatory and pragmatic, but investigators should be clear about the particular approach and the reason for choosing elements of trial design for that approach. Explanatory and pragmatic trials differ in their selection criteria for participants, flexibility in application of the interventions, intensity of follow-up and measurement of adherence, and other design factors.

There has been increasing interest in designing studies to acquire "real-world evidence" regarding therapeutics. An exact definition of real-world evidence is not clear, and may mean different things to different stakeholders, However the basic principle is evaluating the effects of interventions under the conditions in which they are used in practice.[24] Real-world evidence may be acquired both from observational studies and from randomized trials designed to be more pragmatic and reflective of real-world practice.[25] Randomization is not inconsistent with real-world evidence, and more pragmatic trials often have broader inclusion and exclusion criteria and patient-centered end points, measuring patient function and symptoms rather than biomarkers.[26]

When testing interventions, investigators should clearly explain if they plan to evaluate whether those interventions will be used for *diagnosis,* to *prevent* disease, or to *treat* patients with existing disease. The design characteristics differ for each of these types of studies.

COMPARISON WITH A CONTROL GROUP

The purpose of control groups is to distinguish the effect of some chosen factor in the test group on outcomes from effects caused by other factors, such as the natural history of disease, placebo effects, or observer or patient expectations.[27,28] A control group demonstrates what would happen to patients who are not in the test group. A control group is necessary except in rare circumstances in which the course of disease is uniform for any given patient or group of patients and outcomes are predictable based on patient characteristics. In most situations, variability exists in patient outcome such that a control group is necessary. Even when diseases are claimed to be "universally fatal," collection of such information for a historical control group, to validate that assumption, usually is necessary. The selection of control groups depends on the goals of the study. For instance, in evaluating a diagnostic test. the test and control groups consist of those with and without a disease diagnosed by some reference standard.

The choice of comparison groups is based on selecting participants who are like those in the test group except for the factor that is analyzed. Some types of control groups, such as patients who refuse a particular therapy or those who receive "inappropriate" therapy, are inherently different from the test group of patients who agree to therapy or receive "appropriate" therapy.[21] Also, for a group to function as a control, the outcomes in that group should be known or expected based on prior evidence. Experimental studies make comparisons between test outcomes that are unknown and control groups in which the outcomes are known or expected. Experimental studies assign participants by using the process of randomization (explained later in this chapter) or nonrandom methods to assign participants to study groups. Control groups can be *concurrent* or *historical.* In concurrent studies, participants in the test and control groups are evaluated over a similar time frame. In historical studies, a current test group is compared with a control group from which the data were collected sometime in the past and therefore are always nonrandomized. Patient characteristics between the historical control and current patients often differ because of changes in medical practice over time or differences in selection criteria for current versus past patients.[28] Historical studies, on average, show lower success rates for their control groups than do concurrent control groups, biasing the studies toward showing a difference that may not exist or toward a larger treatment effect than truly exists.[29]

In concurrent studies, investigators can choose several types of control groups. *No specific treatment* concurrent controls and *placebo* concurrent controls both compare the test group with groups that receive no specific

active interventions. The placebo intervention does not contain the active test intervention but appears similar in all aspects to it. The main difference is that assignment to study groups can be blinded in placebo-controlled studies but not in trials in which treatment of the comparison group is not specified and/or based on current standards of care. The fact that a trial is placebo controlled does not mean that patients receive no treatment at all. In *add-on studies,* patients receive current standard of care plus placebo in the control group, whereas the test group receives current standard of care plus the new intervention. Dose-response trials compare higher and lower exposure of the test intervention. Active controlled trials compare the test intervention and another active intervention.

Types of Hypotheses

Because an investigator often cannot directly test interventions in the entire population of interest, the investigator starts off with the hypothesis that the intervention does *not* have an effect and then gathers evidence to show that this hypothesis of lack of effect is unlikely to be correct. This is consistent with the principles of falsification expounded in the 1930s.[9] An analogous situation is assuming a defendant in a court case is innocent until shown guilty by examining evidence showing beyond a reasonable doubt that the defendant is not innocent. The hypothesis that the intervention does not have the proposed effect is called the *null hypothesis,* termed *null* because the assumption is that there is no proposed effect of the intervention being studied. If there is sufficient evidence from the study, then the null hypothesis is said be "rejected," and the investigators can "accept" the alternative hypothesis that the intervention does have the observed effect. Note that the alternative hypothesis is not "proven" but rather is accepted based on available evidence. The result is the somewhat confusing double-negative terminology of not showing that the intervention is not effective.[30]

No-therapy, placebo-controlled, and dose-response trials are usually designed as *superiority* trials. The null hypothesis in a superiority trial is that the intervention is not more effective than the control regimen, whereas the alternative hypothesis states that there exists a difference between the test and control groups. This difference must be at least greater than zero in absolute terms or different from one in relative terms.[31]

Active controlled trials can be designed to test *superiority, equivalence,* or *noninferiority* hypotheses.[31] Equivalence and noninferiority trials attempt to rule out absolute differences, other than zero, or relative differences between two groups. The null hypothesis is that the intervention is less effective than the control regimen by some amount. In other words, these trials allow that the outcomes in the test group might be slightly inferior to those in the control group, as long as that amount of inferiority is judged not to be clinically meaningful—making them really "not too much inferior" trials. Neither type of hypothesis can show that two interventions are equal or "just as good as" each other because this would require an infinite sample size to rule out any difference at all.[32] Rather, these trials attempt to rule out some amount of inferiority and/or superiority of a chosen magnitude between the groups. Equivalence trials attempt to rule out that the test intervention is both not worse than a chosen amount and no better than a chosen amount when compared with the control group. The classic examples are bioequivalence trials, where a new formulation of a drug is compared with an older formulation with the goal of showing that concentrations with the new formulation are not much lower or higher with the test formulation compared with the control formulation. Equivalence trials entail a choice of an upper boundary and a lower boundary for superiority and inferiority.[33] In most cases, however, investigators would be happy and it would be clinically relevant to show that a test intervention was more effective than the active intervention in the control group. In these situations, investigators wish only to rule out that the test group is not too much inferior to the control group. These types of trials carry the unfortunate name of "noninferiority" trials.[34] This name is misleading because the test group can show frank inferiority or superiority to the control group, yet still be termed "noninferior," as long as the amount of inferiority of the test drug demonstrated in the trial compared with the control drug does not exceed a prechosen magnitude of inferiority.[35,36]

The reason to perform a noninferiority trial is not just the existence of an older intervention that has been shown to be effective or that placebo cannot ethically be used, but that the test intervention is hypothesized to have benefits other than improved effectiveness, such as fewer harms or greater convenience, while preserving most of the effectiveness seen with the control intervention.[37] It is ethically questionable to expose research participants to more toxic and somewhat less effective interventions in serious and life-threatening diseases for which effective therapy exists and when patients may experience irreversible harm, remembering that noninferiority trials cannot demonstrate that a new intervention is "as good as" the control.[38] A noninferiority hypothesis is not appropriate if the hypothesized benefits of the new intervention are superior effectiveness in a population other than the one studied, which should be tested by superiority hypotheses. If the intervention is hypothesized to have advantages in a specific population (those intolerant to other interventions or in whom other interventions fail), it is more logical and ethical to test the intervention in the target group of patients, to avoid exposing the nontarget population to less effective or more toxic interventions.[37] For instance, in the setting in which there is no effective therapy, as in patients infected with pan-resistant organisms, there is no effective control regimen; therefore, interventions should be tested in superiority hypotheses against current standards of care. Showing noninferiority in patients with susceptible organisms does not provide evidence for effectiveness in patients with no options, because the study does not test the hypothesis of superior treatment effects in patients with resistant organisms. Given differences in characteristics between the patient groups, benefit cannot be extrapolated from one type of patient to different patients. It also exposes patients who have other effective options to potentially less effective drugs.[39] For instance, tigecycline was shown to be "noninferior" to control drugs on an outcome of "clinical response," yet it had higher mortality in all patient groups, and excess mortality, compared with the control drugs, was highest in those with resistant pathogens.[40]

Noninferiority trials are justifiable when placebo-controlled trials cannot be used because the demonstrated effect of the control intervention has important benefits for patients on morbidity and mortality and the new intervention is hypothesized to have non-efficacy benefits in a setting where patients are not exposed to irreversible harm. Placebo-controlled trials are still ethical if the benefits of the control intervention are improvements in non–life-threatening symptoms. Placebo-controlled superiority trials are also ethical in the setting of add-on trials, in which all participants receive current standard of care, such as recent clinical trials in multidrug-resistant tuberculosis.[41]

Conclusions from superiority and noninferiority trials have different implications in that demonstration of superiority is direct evidence that an intervention has some effect (whether that effect is clinically meaningful is a different question). Demonstration of noninferiority is indirect evidence of an effect and could mean that the two interventions are either similarly effective within the range of the noninferiority margin or similarly ineffective if a noninferiority trial is not designed properly.[28] This is because noninferiority trials have no "negative control" group, and the evidence that the control intervention is superior to a putative placebo usually is external to the study itself, unless the study has a third placebo group in addition to the two active intervention groups. In this sense, noninferiority trials are like historically controlled trials in that the evidence of the effect in the control group could have changed over time. Patients could receive cointerventions currently that were not prescribed in the past, which could attenuate the effects of the control intervention—for instance, patients may have received prior effective antimicrobial before enrollment in a noninferiority trial.[42]

The risk-benefit assessment for patients differs in noninferiority compared with superiority trials. In superiority trials, patients may be willing to accept increased toxicity if there is superior effectiveness on outcomes such as all-cause mortality. Noninferiority trials are based on the hypothesis of a trade-off of some amount of lesser effectiveness in exchange for some nonefficacy benefit such as fewer adverse effects. Patients may be willing to accept some small amount of lesser effectiveness in non–life-threatening illness in exchange for fewer adverse effects.[43]

Last, bias in trials affects superiority and noninferiority trials differently. Many types of bias that tend to skew results toward no difference between groups tend to move superiority trials toward a negative result, whereas those same biases result in false-positive conclusions for a noninferiority trial.[44] For instance, enrolling participants who have the

common cold in a pneumonia trial would bias a noninferiority trial toward concluding that the two interventions are similar in the treatment of pneumonia, whereas a superiority trial would correctly conclude that the test intervention lacks effect, compared with the control group.

Design of Noninferiority Trials

After meeting the criteria for selection of noninferiority as the ethical and appropriate hypothesis, appropriate design of noninferiority trials entails four considerations.[28,45] First, there must be reliable and reproducible comprehensive quantitative evidence of the effect of the control intervention compared with placebo or no specific therapy in comparable patients, taking into account the variability of that estimate. Prior use in clinical practice or the investigator's belief in effects alone does not justify a noninferiority design. For instance, in nonfatal diseases such as acute bacterial sinusitis, antimicrobials routinely fail to show superiority to placebo; therefore there is a lack of justification for noninferiority trials in this setting.[46] It is irrelevant to show that a new regimen is "not too much worse" than a standard regimen if the standard regimen is ineffective or does not have reliably reproducible effectiveness under the conditions of the planned study.[47] For instance, clinical trials in acute bacterial sinusitis, acute otitis media, and acute exacerbations of chronic obstructive pulmonary disease routinely show "noninferiority" of one antimicrobial to another, yet the effects of the control drug compared with placebo on patient-centered outcomes are unclear in these diseases.[46,48,49] It is insufficient to merely know that a control intervention "works." It is also necessary to know the magnitude of the effect of the control intervention compared with placebo or no specific therapy on specific measureable patient-centered outcomes. Such an analysis should include all information from adequate and well-controlled studies, not only studies that showed favorable effects of the control intervention.

Second, similar to maintaining the conditions of a laboratory test as constant as possible when repeating the test, the design of the planned noninferiority study should be similar in all important aspects (enrollment criteria, dose of the control intervention, comedications and other cointerventions, definition and timing of outcome measures) to the designs of studies that showed the effect of the control intervention. This is done to increase the likelihood that the control intervention will have similar effects in the current study as it did in past studies. For instance, if the timing of an outcome in an acute self-resolving disease that showed the effect of a control drug was seen at 3 days in placebo-controlled trials, moving the timing of the end point to 3 months in a subsequent noninferiority trial may make ineffective drugs appear "noninferior." Similarly in life-threatening diseases, measuring an outcome "too soon" may make interventions appear similar when they differ with longer follow-up.[50] The lack of difference between the groups would be due to natural history of the disease rather than the drug's effects because the patients' disease would resolve spontaneously over this period or because of delayed treatment effects that accrue over time in life-threatening disease.

Third, the investigators must choose a value for how much inferior the test intervention might be while still being considered clinically useful. This value, called the *noninferiority margin,* or *delta,* cannot exceed the magnitude of the effect of the control intervention over placebo in previous studies (a value termed *M1*) and should be somewhat smaller than that value to preserve the important effects of the control intervention (a value called *M2*). The magnitude of the effect of the control drug over placebo or no specific therapy may change based on the types of patients studied owing to heterogeneity of treatment effects.[51] For example, the treatment effect of older effective antimicrobials on mortality in treatment of pneumococcal pneumonia, compared with no specific therapy, in nonbacteremic patients older than 50 years is a 33% absolute reduction in death, with 95% confidence intervals (CIs) from 26% to 40% (M1). In nonbacteremic patients aged 30 to 49 years, the treatment effect on mortality is 10%, with 95% CIs from 5% to 14% (M1).[45] The amount of inferiority for a planned noninferiority trial comparing a new antimicrobial with an older antimicrobial in patients older than 50 years should be less than 26%, but in patients aged 30 to 49 years it should be smaller than 10%—for instance, 5% (M2)—to ensure that a new intervention has any effect at all compared with placebo. Because the goal of noninferiority trials is to preserve the important effects of the control intervention, the chosen margin should be smaller than the total effect of the control. The amount of preservation of the effect of the control intervention should be based on what is clinically meaningful rather than sample size considerations alone or some fixed value, such as half the effect of the control. For instance, in nonbacteremic patients older than 50 years, the margin (M2) would be substantially smaller than 26%, to ensure that patients receiving the new intervention do not have substantially increased probability of death compared with patients receiving the control treatment. The overall margin (M2) in such a case might be 10% or smaller because a 20% increase in death would show the drug has some effect, but it would be clinically unacceptable to allow an increase in death in 1 of every 5 patients treated with the new drug. Smaller noninferiority margins result in larger sample sizes; however, patients in noninferiority trials already have available effective options and therefore cannot ethically be subjected to greatly increased risk of poor outcomes. For instance, for control interventions that decrease mortality, larger noninferiority margins would expose patients to excess mortality in the setting of the trial and in clinical practice. The noninferiority margin should be developed based on patient preferences regarding the inherent trade-offs in noninferiority trials—for example, based on patient interviews of persons with the disease, how much loss of effectiveness is acceptable to them in a trade-off with how much gain in nonefficacy benefits?[43,47]

Fourth, the conduct of noninferiority trials must minimize loss to follow-up and nonadherence to decrease bias toward showing no difference.[52]

SELECTION OF STUDY PARTICIPANTS

Selection of study participants is based on the goals of the study.[53] In explanatory trials, investigators often ensure that patients have a high probability of a diagnosis for the disease under study. In pragmatic trials, investigators may use less stringent criteria that more closely match those that are used in general practice. In diagnostic studies, investigators choose participants who do and do not have the disease, based on some reference standard. In case-control studies, investigators select participants based on the presence or absence of a chosen outcome. In cohort studies, investigators select participants on the basis of presence or absence of the chosen exposure, which can be a drug, a behavior such as smoking, or an environmental factor such as being in a hospital.

When studying interventions in clinical trials to evaluate their treatment effects in treating disease, one must first define the disease and then select a subset of the population for study from the population of patients with that disease. Clear definitions of the disease under study allow (1) consistency among investigators about the types of patients they enroll in a trial, (2) generalization of the results to patients outside the trial, (3) accurate description by regulatory agencies of the intended use of the drug in prescription drug labeling, and (4) appropriate application of the information presented in the trial in clinical practice.

The availability of rapid diagnostics with adequate positive and negative predictive values for the disease under study greatly aids selection of participants. In some situations, the lack of rapid diagnostics has hindered development of new therapies—for instance, in disease caused by resistant pathogens. In this setting, criteria based on signs and symptoms alone lack sufficient sensitivity and specificity, and culture data often are too slow in providing results that would influence study enrollment.

Diagnostic tests with both high sensitivity and high specificity are always preferable in both clinical trials and clinical practice. However, when diagnostic testing is less than optimal and one must choose between high sensitivity or high specificity, the desirable characteristics of a diagnostic test differ significantly between clinical practice and clinical trials. In clinical practice, clinicians may choose to use a diagnostic test with high sensitivity so that clinicians do not fail to diagnose patients with the disease, especially for infections with high morbidity and mortality. In the setting of an explanatory clinical trial, however, tests

with high specificity are needed to ensure that patients enrolled in the trial actually have the disease under study, especially in the setting of noninferiority trials, where inappropriate diagnosis can bias toward a false conclusion of noninferiority. For instance, practice guidelines recommend treating patients with symptoms of uncomplicated urinary tract infections and colony counts as low as 10^2 colony-forming units (CFUs)/mL of urine.[54,55] However, to increase specificity, clinical trials usually enroll only patients with at least 10^5 CFUs/mL of urine, to more accurately differentiate patients with true uncomplicated urinary tract infection from those with symptoms of dysuria caused by other conditions with potentially contaminated urine specimens. In noninferiority trials, investigators must use the same definition of disease as that used in the historical studies that demonstrated the effect of the control intervention compared with no specific therapy.[56]

In some diseases, microbiologic information is not available or helpful in making diagnoses—for instance, in settings wherein culture data have low sensitivity and specificity (e.g., cellulitis) or in culture data from nonsterile body sites where differentiation of colonization from disease is challenging.

Selecting the appropriate patient population often entails striking a balance between including enough different types of patients so that one may extrapolate the results of the trials to clinical practice but not be so inclusive as to blur important distinctions in drug efficacy or safety across patient subgroups. Inclusion of patients with various types of infections caused by the same organism but with differing pathophysiologic features, patient characteristics, and outcome measures could result in uninterpretable results for any type of infection. For example, both sinusitis and pneumonia are respiratory tract infections but differ substantially in pathophysiology and outcomes. Conversely, skin infection studies often include patients with both cellulitis and wound infections, given the similar pathophysiologic features of the diseases. However, investigators wish to be inclusive enough that results of the trial are useful in making treatment decisions for a broad range of patients. For instance, clinical trials often include patients who are elderly or who have mild renal or hepatic insufficiency in an attempt to determine outcomes in these patient populations.

MINIMIZING ERROR IN STUDIES

Two types of error can affect the validity of study results. *Random error* refers to variability that occurs because of chance alone. Common statistical tests are used to evaluate the impact of random error on study results, and studies are designed to minimize random error as much as possible. Increasing the sample size of studies, studying a more homogeneous population, or using more precise measurement tools decreases random error.

Two types of random error exist in trials. *Type 1 error,* or alpha, is the probability of concluding false-positive results. This means rejecting the null hypothesis when, in fact, the null hypothesis of lack of effect of the test intervention is true.[57] This value is conventionally set at 5% for most studies, meaning an error rate of 1 in 20. If one considers the thousands of studies conducted each year, an error rate of 1 in 20 studies is actually a low standard of evidence.

Therefore, independent confirmation of evidence from similar studies is important because a confirmatory study with a type 1 error rate of 5% lowers the overall type 1 error rate of the two studies to 0.25% (5% times 5%). An error rate of 1 in 20 is still substantial, and the inability to confirm results of many studies using *P* values of .05 has led some authors to suggest lowering the threshold for "statistical significance" to .005.[58]

Type 2 error, or beta, is the probability of a false-negative result—that is, not rejecting the null hypothesis when, in fact, the alternative hypothesis is true. This value is usually set at 10% to 20% for most studies.[59]

Power is the ability of the study to detect a difference, should such a difference exist, and is defined as 100% minus the type 2 error. For a study with a type 2 error rate of 10%, the study has 90% power to be able to detect a difference. It is not appropriate to calculate "power" after a study is complete, because power is only the prestudy probability of detecting a difference. Once the study is complete, a difference was detected or it was not.[60]

The type 1 and type 2 error rates are values selected before the study in order to choose a sample size (the number of participants needed for enrollment) for the study.[61,62] In addition, two other variables affect sample size. The first is the hypothesized difference in effect size between the test and control groups, called delta, based on available evidence from similar studies and what is a clinically meaningful difference. This value often represents the investigators' best guess, and the actual measured difference between study groups cannot be known until after the study is over. A common error is selecting a larger difference between study groups than is justifiable based on available evidence or what is clinically meaningful in order to decrease the sample size of the study. However, if the true difference between groups is smaller, then the study will have an insufficient sample size to detect differences. Conversely, investigators may choose a large sample size to be able to detect small differences even if those differences are not clinically meaningful.

The appropriate number of participants to enroll (sample size) for a study is the number that allows one to answer the chosen research question while minimizing random error and achieving adequate precision of the results. Larger sample sizes, however, expose more participants to potential risk and cost more in terms of time, manpower, and money. Therefore the sample size of a study should be carefully chosen before the trial, to maximize valid results while achieving the greatest efficiency. Interim blinded preplanned analyses during the course of a study may show that initial assumptions affecting sample size were incorrect and require adjustment for valid reasons during the study. However, it is ethically and scientifically questionable to start with a sample size in mind and then choose hypotheses that are not clinically meaningful but for which data can be acquired with the chosen sample size. For instance, one could choose to use a laboratory test as an outcome measure because of the ability to show differences between study groups with smaller sample sizes. However, this approach is questionable if the laboratory test does not validly reflect differences on the true outcome of interest, such as mortality, patient function, or patient symptoms. Smaller trials require greater planning and foresight than larger trials.[63]

The second type of error in studies is *systematic error.*[64] Systematic error is any type of error at any point of inference in a study that causes the results to not provide valid inferences related to the research question. Systematic error can be divided into bias and confounding. *Bias* is any type of systematic error at any point in the study that gives results or conclusions that differ systematically from the true values.[65] In other words, the measured values are incorrect. For example, a nonrandomized, nonblinded, placebo-controlled clinical trial of the drug patulin, prescribed for the common cold in the 1940s, showed a 48% (95% CI, 35%–60%; $P = .002$) absolute reduction in symptoms with the drug.[66] However, a subsequent randomized, blinded trial with better definitions of outcomes showed a treatment effect of zero (95% CI, −4% to +4%; $P = .96$).[67] The difference between these two results is due to bias inherent in the design of the first study. *Confounding* is the presence of factors associated with both exposure and outcome that influence causal conclusions related to the interventions resulting from imbalances in the confounding factors between the study groups.[68] With confounding, the measured values are correct, but confounding means the observed effects may be due to factors other than those measured. For instance, in nonrandomized studies of interventions, if there are baseline differences between groups with factors known to influence outcomes, such as age and severity of illness (selection bias), then outcomes may be confounded by those factors and not be due to the interventions under study. The process of randomization addresses the problem of confounding at the time of randomization, but does not address confounding that may occur before randomization (e.g., prior effective therapy) or after randomization (concomitant effective therapy).[69]

Many types of bias can affect the results of clinical studies, and authors have divided bias into various types.[65] *Selection bias* means that subjects in one study group systematically differ from subjects in another group by factors that influence outcomes.[70] One of the major issues with nonrandomized trials is that even when attempting to control for known factors influencing outcomes, one cannot account for unknown factors or the various combinations of known and unknown factors that influence outcomes. Randomization addresses this issue as discussed

later. *Observer bias* is the process by which persons who assess outcomes can influence the measurement of those outcomes. Blinding participants, investigators, and outcome assessors to study group assignment can address this issue. Studies that are not blinded to treatment assignment are called "open label." *Misclassification bias* is related to observer bias and occurs when a value is defined in error—for instance, when participants who experience failure of a study medication are coded as successes and vice versa. Blinding addresses this issue in part, but clearly defined study measures and procedures also help minimize this bias. *Instrument bias* is when the instrument, scoring system, laboratory test, or other measure is systematically incorrect. For example, one study asked clinicians to measure marbles of the same and different sizes used as a substitute for tumors in a blinded manner. The results of the study showed that clinicians systematically erred in measuring marbles of the same size such that there would be a large "treatment effect" on tumor shrinkage, when, in fact, they were measuring marbles of identical size.[71] In infectious diseases, lack of reliability of measurements, such as the Clinical Pulmonary Infection Score (CPIS), could result in biased measurements.[72,73]

Bias is most problematic because there is no way to specifically test for it. Bias cannot be addressed or measured by statistical testing alone, and increasing sample size actually magnifies the effects of bias on outcomes. Therefore, a larger trial will minimize random error but give a more precisely incorrect answer to the research question in the presence of biases. Bias is addressed most effectively at the design and conduct stages of a study. Appropriate design of a study helps to minimize the occurrence of bias before initiation of the study. Appropriate conduct, such as minimizing loss to follow-up, helps control bias during the study. Appropriate analysis, such as including all randomized patients in the primary analysis, also is an important way to minimized bias. Controlling bias is also an important ethical issue in studies. Studies cannot contribute to generalizable knowledge if their results are not valid and free from bias.

BASELINE COMPARABILITY BETWEEN STUDY GROUPS

With some research questions, the goal is to evaluate the differences in baseline factors that influence outcomes between groups. For instance, when evaluating risk factors for disease acquisition or prognostic factors for outcomes, the goal of the research is to find the baseline differences between the groups that may or may not be causal but still bear some relationship to each other. For instance, hospitalization is a risk factor for acquisition of resistant pathogens, but hospitalization alone does not cause colonization with resistant pathogens because other factors may be equally or more important, or hospitalization may be a marker for the presence of other factors.

However, when attempting to evaluate causal relationships between exposures and outcomes, one of the most important aspects of study design and analysis is ascertaining that study groups are as comparable as possible so that investigators can draw valid conclusions about relationships between study groups.[74]

In nonrandomized trials, investigators can attempt to evaluate baseline comparability of known factors that influence outcomes, but it is challenging, if not impossible, to ascertain the influence of selection bias due to unmeasured factors on outcomes. For this reason, nonrandomized studies are often used as a basis for hypothesis generation for future randomized trials, and randomized trials have become the gold standard for evaluating medical interventions.[75] A study that evaluated the importance of randomization and blinding on potential bias in study results showed that at least one prognostic factor was not distributed equally between arms of the trial in 14% of trials that were blinded and randomized, in 26.7% of open-label randomized trials, and in 58.1% of the open-label observational trials. The authors found statistically significant differences in case-fatality rates between the treatment and control groups in 8.8% of the blinded and randomized trials, 24.4% of the open-label randomized studies, and 58.1% of open-label observational trials.[76]

The process of randomization is a systematic way of assigning participants to study groups.[77] These include processes as simple as flipping a coin or using computer-generated number tables. Nonsystematic ways of assignment to study groups, such as alternately assigning participants to study groups or enrollment in groups based on admission date or social security numbers are not random processes and are not defined as randomization. Randomization in studies of sufficient sample size results in an equal probability of similar distribution of both measured and unmeasured factors and thereby minimizes confounding. This addresses the issue of confounding resulting from baseline imbalances between groups and obviates discussion of confounding in randomized superiority trials. As noted previously, confounding is often confused with effect modification in the setting of randomized trials, wherein the effect of interventions may differ in different types of patients or clinical settings. The minimization of selection bias and confounding by randomization allows causal inferences between the interventions studied and outcomes. Differences between study groups in the randomized population causally relate the interventions to observed outcomes, as long as other sources of bias that can occur after randomization are minimized.

It is still possible for baseline imbalances to remain in randomized trials, especially in trials with small sample sizes. Randomization provides a *probability* of equally distributing important variables between arms of a trial prior to enrollment. When important variables are not equally distributed across arms of the trial once the study is complete, randomization has not "failed" because the process of random distribution did occur as planned but without the desired result. However, unequal distribution of factors between the arms of a trial may have an impact on the interpretation of the results. The importance of unequal distribution of various factors depends not on the statistical significance of the difference in the distribution of the factor between the study arms but on the strength of the association of the outcome with that factor.[78,79] In other words, one should consider small differences in the distribution of factors between study arms as clinically significant if the factor has a known large impact on outcomes, even though the baseline differences in patient characteristics are not statistically significant.[80]

The randomization code also should remain blinded so that investigators cannot consciously or unconsciously influence assignment to study groups. This process of blinding of the randomization code is called *allocation concealment*. On average, studies without allocation concealment show larger treatment effects than do those with allocation concealment, indicating the potential for bias influencing results.[81]

Stratification may be used along with randomization to ensure baseline comparability between groups, especially when sample sizes are small.[82] Stratification entails dividing the study group before randomization by the important baseline variables that affect outcomes and then randomizing separately in each stratum. For instance, patients could be divided by age category before randomization and then randomized to study groups within each age category. Stratification ensures equal numbers of participants in each stratum; but unless there are separate hypotheses, sample size calculations, and appropriate adjustment for increasing the false-positive error rate (type 1 error), stratification alone does not allow confirmatory conclusions in each stratum. Keeping the number of strata to a minimum of important categories known to affect outcomes helps simplify trial operations.

The benefit of randomization on decreasing selection bias relies on participants remaining in their assigned study groups and analysis of results based on the randomized groups. Eliminating participants from the analysis or performing analyses that evaluate only subgroups of participants may still involve selection bias. Missing data are an important consideration in clinical studies and an important cause of bias.[83] Minimizing missing data in terms of loss to follow-up or elimination of participants from analyses requires careful study planning. Although the best way to address the situation of missing data is to avoid it, imputation methods are available to perform analyses based on various assumptions related to the reason for missing data. The challenge is that these assumptions are often unverifiable based on the available evidence in the study.[84]

OUTCOME ASSESSMENTS

The specific definitions of outcomes, who does the measuring, when assessments are performed, and how to analyze the outcomes depend on the clinical context and the research questions of the study initiation. In case-control studies, investigators choose the outcomes first and then

look backward in time to examine differences in exposures. In cohort studies and clinical trials, investigators choose exposures and then follow patients forward in time to examine outcomes. In clinical trials, outcomes are used to define *end points.* An end point is the outcome measure used to examine differences between study groups at specific points in time using specific predefined analysis methods, such as differences in proportion of mortality between groups treated with different antimicrobials at 30 days after randomization.

The principles of terminology, development, and application of outcome assessments as end points in clinical trials have been explored.[85] The variables that make up an end point should be clearly defined and reliably measured. Terms such as "clinical response" or "success" and "failure" are meaningful only if the defining of specific variables allows one to determine what is actually measured. In addition, if end points have components related to judgment in measuring or interpreting the variables, there may be substantial variability in the measurements, with resulting misclassification bias.[72,73]

The definitions of end points for trials should be clinically relevant to patients: measuring how patients feel in terms of their symptoms, function in their daily lives, or survive.[86] Direct measures of how patients feel, function, or survive have previously been defined as "clinical" end points; however, in trials the term "clinical" is often misunderstood to mean any measure obtained as part of patient care.[87] Recent guidance has suggested the term "clinical outcome assessments" to encompass both direct and indirect measures of patient benefit.[85] Patient-reported outcomes (PROs) are direct measures of patient benefit, whereas clinician observations including physical signs are indirect measures of patient benefit. Direct measures of patient benefit include resolution or improvement of impaired functioning or symptoms that cause patients to seek medical care, in addition to survival.[88]

Investigators sometimes are concerned about "specificity" of symptom-based outcome measures; however, appropriate diagnosis ensures that patients' symptoms are due to the disease. For instance, many patients with cough do not have pneumonia, but after radiographic confirmation of the diagnosis, cough is no longer a "nonspecific" outcome measure but due to pneumonia. In addition, randomization addresses baseline comparability and balances groups for outcomes unaffected by the intervention such that any remaining differences between groups are causally related to the interventions tested. Examining "all-cause" outcomes is important because interventions may worsen symptoms, function, or survival as a result of off-target effects. For instance, all-cause mortality evaluates the net benefits and harms of an intervention, whereas cause-specific mortality does not because all-cause mortality takes into account adverse effects of interventions that may cause death even if the test intervention "works" according to its specified mechanism of action. In addition, the tools and judgments used to evaluate cause-specific mortality lack sensitivity and specificity. For instance, current diagnostic tools used to diagnose fungal infections are not sufficiently sensitive or specific.[86] Autopsy studies show substantial misclassification of clinicians' judgments regarding causes of death.[89]

Often, more than one manifestation of a disease is clinically relevant, and/or different outcomes may occur at different rates. In these cases it may be appropriate to evaluate relevant symptoms in addition to mortality in a composite end point, either by combining outcomes into a single measure or by ranking outcomes on an ordinal scale.[90,91] For types of diseases or stages of diseases in which mortality is low, a measure of symptoms plus survival may be most useful. The use of a composite end point can provide a comprehensive evaluation of the efficacy of interventions.[89] For example, in clinical trials of patients at low risk of death with community-acquired pneumonia, an appropriate end point may evaluate resolution or improvement of cough, shortness of breath, and chest pain, in addition to mortality.[92] Survival is always measured because it is not possible to evaluate symptoms in patients who do not survive. However, composite end points are driven by the most common outcomes in the composite, so similarity of symptoms may mask differences in outcomes between groups regarding mortality if mortality in the study population is low. Composite end points should be derived from outcomes that are similarly meaningful for patients. For example, combining death and complications of disease is more clinically meaningful than combining death and a surrogate end point of culture results. In noninferiority trials, investigators are limited to using the same definition and timing of end points used in previous studies that demonstrated the effect of the control intervention.[16]

End points in clinical trials also can measure indirect outcomes that are hypothesized to reflect how patients feel, function, and survive. These indirect end points can be events determined by clinician judgment (termed *clinician-reported outcomes* [ClinROs]), such as decisions to switch medications, prescribe additional medications, or decisions on hospital admission or discharge.[93] Such end points assume that the reasons for the event are related to patient symptoms. Biomarkers, defined as laboratory measurements (e.g., radiography, cultures, polymerase chain reaction [PCR] assays, and serologic testing) or physical signs, used as a substitute for direct measures of how patients feel, function, or survive, can also be used as indirect outcomes or "surrogate" end points.[88] Microbiologic end points that measure the suppression of pathogenic organisms below some level of detection are surrogate end points because they do not measure patient outcomes directly. The most useful indirect measures in clinical trials are those that are reproducible (analytically valid), are on the causal pathway of the disease, and have relationships to direct outcomes of interest that are well known.[87,94] Indirect measures that are not on the causal pathway of disease are less useful because they can be affected by interventions other than those that affect the disease process. For instance, in pneumonia, fever is not on the causal pathway of disease because pneumonia causes fever rather than fever causing pneumonia. Therefore, interventions such as antipyretics can lower body temperature without affecting the course of pneumonia. Measurements of pathologic organisms that are on the causal pathway of the disease may be candidates for potential surrogate end points. Surrogate end points are most useful in chronic diseases when direct outcomes cannot be measured for a prolonged period of time.

However, the major issue with surrogate end points is that they may not reflect the outcomes of how patients feel, function, or survive because of off-target benefits or harms or pathways of disease not captured by the indirect measures. Correlation between an indirect outcome and a direct outcome, such as mortality, is necessary but not sufficient to evaluate an indirect measure as a surrogate end point.[95] Correlations often compare patients with and without the indirect measure and then evaluate the relationship to the direct outcome measure independent of the interventions received. However, such *patient-level* evaluations may be measuring differences in patient characteristics rather than differences in the treatment effects of the interventions the patients receive.[96] Of note, such patient-level evaluations are independent and may not be mediated by treatment—that is, they may reflect the natural history in different types of patients rather than the effects of interventions. In addition, correlations often evaluate only one side of the relationship between the direct and indirect measures. For instance, a microbiologic outcome of negative cultures may "correlate" with resolution of symptoms. However, one must also evaluate whether positive cultures also correlate with lack of resolution of symptoms. Therefore, microbiologic success should predict clinical success, and microbiologic failure should predict clinical failure. Although it seems intuitive that suppression of pathogenic organisms below some level of detection should reflect direct outcomes, this is not always the case. Antimicrobials may exert direct effects on the immune system that may affect direct outcomes.[97–99] Antimicrobials may also have effects on organisms, such as rapid lysis with subsequent stimulation of the immune system, which also may influence direct outcomes. The recommendation to administer steroids before antimicrobials in acute bacterial meningitis is based on this phenomenon.[76,77] Immune reconstitution inflammatory syndrome in HIV is another such example. Because these effects are the result of administration of the antimicrobial, they are not solely effects related to the host. In addition, host inflammatory reactions may continue to cause disease manifestations even after decreasing organism burden, thereby contributing to discordance between indirect microbiologic outcomes and direct outcome measures. Measuring direct outcomes is the only way to capture such effects.

A *trial-level* surrogate is when an intervention-mediated treatment effect (the difference between the test and control group) on the surrogate end point also reflects a treatment effect on the direct patient-centered

outcome of interest (symptoms, function, or survival).[96,100,101] This entails comparison of treatment differences between the test and control groups regarding the indirect measure and the direct measure. Biomarkers or clinical outcome assessments that are patient-level correlates are not necessarily trial-level surrogates. This also requires clearly defining the direct measure, in terms of symptoms, function, or survival, for which the indirect measure is a substitute.

Biomarkers have been successful in some settings, such as treatment effects on viral load measurements in trials of HIV/AIDS, reflecting treatment effects on mortality and AIDS-defining opportunistic infections. On the other hand, surrogate end points often fail to reflect direct outcomes. For instance, improved microbiologic outcomes with clarithromycin in *Mycobacterium avium* complex bacteremia showed increased mortality in trials in patients with HIV/AIDS.[102] This reinforces the idea that the utility of surrogate end points is contextual, requiring reevaluation when used in different patient populations or with different interventions.[103]

Biomarkers as surrogate end points are useful in hypothesis-generating studies to demonstrate proof of principle that an intervention has biologic activity. Such trials are appropriate for selecting candidate interventions and to generate hypotheses for further trials that demonstrate that the effect of the biomarker translates into treatment effects on direct measures of symptoms, function, or survival.

The timing of end-point measurements also is important.[104] Investigators should time the measurements based on the natural history of the disease and the timing of clinically relevant treatment effects. For example, placebo-controlled trials show that most cases of traveler's diarrhea will resolve spontaneously within a few days.[105] Therefore, measuring resolution of diarrhea several weeks after the start of symptoms would not allow one to differentiate the effects of antimicrobial therapy from placebo in a superiority trial or would make two agents appear similar in a noninferiority trial. Often in spontaneously resolving diseases, the time to resolution of symptoms may be a more clinically meaningful end point than measurement of clinical outcomes at some fixed time point. However, in life-threatening acute diseases in which death occurs in days to weeks, a fixed time point may be more informative because whether the patient dies earlier or later in the course of a short illness may not be clinically relevant. Also, it is important to follow patients for long enough to evaluate the durability of treatment effects. Again, in noninferiority trials, the timing of the end point should reflect the timing used in prior superiority trials that demonstrated the effect of the control regimen.

When measuring outcomes in diseases that tend to recur, such as diarrhea caused by *Clostridioides difficile* (formerly *Clostridium difficile*) studies should evaluate outcomes in the entire randomized population and not recurrence only in the subgroups of patients who experience resolution of diarrhea. Outcome measures should include both patients in whom interventions initially fail combined with those in whom disease recurs. Evaluating only subjects who are initially cured and then following that subgroup alone for recurrence gives biased estimates when initial cure rates differ between the two interventions. The "recurrence rate" in an intervention that cures no one is zero, but this means the intervention is "preventing" recurrence through being ineffective.[106]

ANALYSIS OF STUDY RESULTS

Investigators and consumers of trial results should evaluate the results in light of the primary research questions posed by the study. Secondary hypotheses or subgroup analyses may pose interesting questions, but the primary analysis is of primary importance, as reflected by the design of the trial.

In addition to presentation of estimates of difference between test and control groups, investigators use statistical tests to examine the likelihood that the results of the trial could have occurred by chance alone (random error), to examine the direction and magnitude of treatment effects, and to determine the precision of the results. If the likelihood of accepting a chance result is low and bias is minimized, as determined by the error rates set up before initiation of the trial and design and conduct of the study, then investigators conclude that the evidence is strong enough to reject the null hypothesis. They then can assume that the alternative hypothesis is likely true.[107]

Analysis of Populations

Before performing statistical calculations, investigators must first decide on the population of patients in the trial that they wish to analyze. In the *intention-to-treat* (ITT) population, patients are assigned according to the group to which they were initially randomized, regardless of whether the patient received the drug, discontinued therapy, or switched to a different therapy.[108] In other words, investigators evaluate patients by the treatment they intended to give. This population preserves the protection from selection bias that randomization offers. It also provides the most clinically meaningful results because in practice patients and clinicians often do not use interventions as prescribed, and the reasons for not adhering to the intervention may be related to the intervention itself, such as adverse effects and tolerability. The ITT analysis also allows appropriate statistical testing based on the principle of randomization. Investigators can use a *modified intention-to-treat* (mITT) population of patients who received at least one dose of the study medication and who have the disease under study. This analysis is valid only if the difference in the number of patients between the mITT and ITT populations is small—for instance, less than 5%. The *per-protocol* (PP) population (sometimes called the "clinically evaluable" population) is a subgroup analysis composed of patients who follow the protocol as specified—for instance, those who received some minimal amount of the study or control drug and returned for an assessment of their outcome. Such analyses may lack the protection of randomization from selection bias if patients are excluded from analyses based on events that occur after randomization and/or if those who drop out of the study systematically differ from those who complete the study. Exclusion of participants from the PP population assumes that the missing data are uninformative, a conclusion that is usually not verifiable or justifiable. Exclusion of subjects based on lack of receiving a "sufficient" duration of the study intervention is not scientifically justifiable and is based on the notion that antimicrobials take some fixed amount of time to exert an effect. Also, excluding participants after randomization may mitigate the protection of randomization from selection bias because investigators are selectively choosing which patients to analyze. Exclusions based on receipt of concomitant medications during the study are also problematic, especially if the subjects received additional antimicrobials because of spread of their disease or new disease elsewhere in the body.

In superiority trials, the ITT population is usually the most appropriate patient population used for analysis of the primary end point because it provides the least likelihood of coming to a false-positive conclusion.[109] However, in a noninferiority trial, if there are large numbers of participants excluded or missing data, then the analysis of the ITT population may make the test and control interventions appear more similar, although more recent studies have demonstrated that this concern may be more theoretical than practical.[110,111] The theoretical concern is that ITT analyses may lead to a false-positive conclusion that the drugs are similar in efficacy when there may be important differences between the drugs. Because the benefits of randomization may be lost in the PP population, this population also may not reflect the true efficacy of drugs in a noninferiority trial. Given the limitations of both the ITT and the PP population in noninferiority trials, it is often most informative to examine analyses of the primary end point in both populations. Differences in the comparative efficacy of the study and control drugs between the ITT and PP populations in a noninferiority trial would require an examination of the reasons for such differences. Trial results are most convincing in noninferiority studies when ITT, mITT, and PP analyses reach similar conclusions.

Examining Baseline Comparability

Although randomization gives a probability of equal distribution of measured and unmeasured baseline factors before trial initiation, it is still necessary to evaluate whether randomization produced such balance once a trial is complete. One should examine baseline variables that can influence outcomes to assess their balance between the test and control groups. Using *P* values to evaluate balance on baseline variables is not appropriate.[79] Factors with "insignificant" *P* values can still affect outcomes, and factors with "significant" *P* values may have no effect on outcomes. *P* values are measures of hypothesis testing, and no

hypothesis is being tested regarding baseline variables in a trial, and any differences are by definition due to chance.

In observational trials, there may be imbalances on both measured and unmeasured baseline factors that affect outcomes independent of the intervention or exposure. There are multiple methods that attempt to balance for such factors after data have been collected. Of increasing interest is the use of *propensity scores* in observational studies, which attempt to evaluate the effect of interventions or exposures on outcomes.[112] The scores are used to fit a statistical model to estimate the individual likelihood that patients would be prescribed a specific intervention or have a specific exposure based on their observed baseline characteristics. The score is derived by combining baseline preintervention characteristics rather than using individual variables as in multivariable logistic regression. The variables used to derive the scores are estimated by logistic regression with treatment or exposure as the dependent variable and the observed potential confounding variables as the independent variables. The propensity "scores" range from 0 to 1.0. Investigators can then "match" patients by their propensity scores using various methods of matching (identical vs. "close enough").[113] It is unclear if propensity score matching is a better method of controlling bias than other methods of adjustment for baseline variables in observational studies. Studies comparing propensity scoring in observational trials versus randomized trials in the same topic area have shown either larger or erroneous treatment effects with use of propensity scores.[114] No method of adjustment can account for unmeasured confounding.[115]

Calculating Differences Between Groups

Once investigators decide which population to analyze, they calculate the *point estimate* for successful outcomes for the primary end point in the study drug and control groups.[116] The point estimate represents the average value in the sample studied, but not necessarily the average value in the population from which the sample was taken. The point estimate is the number of patients with successful outcomes divided by the total number of patients in the analyzed population. For instance, consider an example of a trial in which the primary analysis population contains 300 patients in each arm of the trial, where 225 and 240 patients have successful outcomes in the study drug and control drug arms, respectively. In this case, the point estimates of successful outcomes are 75% (225/300) in the study drug arm and 80% (240/300) in the control arm. Investigators should present numerator and denominator data rather than summaries alone so that readers can perform these calculations on their own.

However, in experimental studies, the *differences* between the test and control groups are of primary importance rather than solely "success rates" in the individual study groups. Clinical trials are comparative, and "success rates" can vary from study to study by random variation alone or because of differences in patient groups between studies. This makes cross-study comparisons challenging.

Investigators can present comparisons of the differences between the test and control groups as absolute differences, relative differences, or odds. *Absolute differences* are the difference between proportions between the groups.[116] In our example, the absolute difference in outcomes between the study and control drugs is 75% minus 80% or a difference of −5%. Absolute differences are easy to calculate and interpret and are symmetrical (number of successes and number of failures sum to 100%). However, when percentages are small (below 1%) these differences are challenging for patients and clinicians to understand.

The inverse of the absolute difference (1 divided by the absolute difference) is the *number needed to treat* (NNT).[117] This value shows the number of patients who are exposed in order to benefit or harm one person. In our example, 1 divided by the 5% absolute difference gives an NNT of 20. This means that 20 patients must be treated with the drug for one person to benefit. This provides a basis for numerically comparing benefits and harms in a single study but does not provide a context for the numbers because the nature of the benefits and harms may differ. For instance, a smaller number needed to treat to harm (NNTH) regarding nausea may be justified if the benefit is a larger NNT regarding mortality, given that nausea would be a tolerable adverse effect in order to achieve benefits in mortality. However, NNT is useful when the nature of benefits is similar to the nature of harms, such as the benefit of decreasing symptoms compared with the harms of headache, skin rash, or diarrhea. There is no single "good" NNT number, and comparing across therapeutic areas and between treatment and prevention studies is challenging, given the different contexts and different balances between benefits and harms. Several studies have shown that patients and clinicians more readily understand absolute differences and the NNT.

Relative differences express the event rates in the test group as a proportion of those measured in the control group, with a range of values between 0 and +1.0 or −1.0. In our example, the rate of unsuccessful outcomes is 25% for the study drug and 20% for the control. Therefore the relative reduction in unsuccessful outcomes is a 20% decrease in treatment failures for the control drug relative to the study drug (1 minus 20% divided by 25%). Relative differences do not reflect the baseline rate of events in the control group. Hence, decreases in the absolute event rate, from 2% to 1% and from 20% to 10%, are both 50% relative decreases. The clinical meaning may differ substantially, however, and may overestimate or underestimate the actual impact of an intervention when event rates are very high or very low. Presentation of relative differences can lead to overinterpretation of treatment benefits.[117]

Odds are expressed as the number of persons experiencing an event divided by the number of persons not experiencing an event. For instance, if 8 of 10 people develop a disease in a test group, the odds are 8 divided by 2, which equals 4. The odds ratio is the odds in the test group divided by the odds in the control group. For instance, if 6 people develop a disease in the control group, the odds in the control group is 6 divided by 4, which is 1.5, and the odds ratio is 4 divided by 1.5, which is 2.7. Odds are intrinsically more challenging to understand. They are not the same as relative risk, but they are often misinterpreted in this way. Odds can range from zero to infinity, unlike relative risks. Odds and relative risks are similar when event rates are less than 20%, but they give different results when events rates exceed this value. Odds are useful in case-control studies in which the denominator of events is unclear. They are also the output of logistic regression analyses and provide a greater "dynamic range" of values than relative risks because they can extend beyond a value of 1.

Examining the Evidence: *P* Values and Confidence Intervals

Investigators use inferential statistical testing to examine the precision and variability of the difference between the test and control groups and the probability that the difference could have occurred by chance alone. The actual statistical tests used depend on the types of data examined. A discussion of various statistical tests and their appropriate use is beyond the scope of this chapter. Investigators commonly use tests such as the chi-square to examine dichotomous variables (cure vs. success) and the Student *t* test to examine continuous variables (e.g., weight and age).[118,119] However, at a minimum, readers of clinical trials and peer reviewers for journals should ensure that investigators identify the appropriate statistical tests used in examining the results of a trial.

One of the most commonly used results in statistical testing is the *P* value.[120,121] Unfortunately, it is often misused and misinterpreted.[122] In one study, only about one-fifth of respondents to a multiple-choice questionnaire understood the meaning of the *P* value.[123] The *P* value is a measure of hypothesis testing. If the *P* value is less than the specified type 1 error, usually set at .05, the result is called "statistically significant." A *P* value of less than .05 means that there is a less than 5% chance that results as extreme as those observed in the trial are due to chance or random error.[124] Therefore the *P* value is not a measure of clinical significance. *P* values do not measure bias that may be inherent in the design of a study or occur during the study. Increasing sample size decreases the *P* value for a given difference while increasing the effects of bias on the results. Authors have pointed out that the view of dichotomizing *P* values above and below .05 as "positive" and "negative" studies often clouds issues related to clinical meaningfulness of the results of studies. A *P* value of .01 does not mean that there is only a 1% chance that the intervention tested is ineffective or that there is a 99% chance that the intervention is effective. As discussed previously, given the thousands of trials performed each year, a *P* value of .05 is

actually a low standard of evidence. The *P* value does reflect the "strength" of the evidence in that it provides evidence toward whether the study (in the absence of other forms of bias) is likely to be confirmed in follow-up studies. A study with a *P* value of .05 has a probability of confirmation in 57% of follow-up studies, meaning that one in every two to three subsequent trials will fail to confirm the original findings.[125] *P* values also are affected by the size of the between-group difference and the sample size. The larger the difference between the study and control drugs, then the smaller the sample size needed to demonstrate statistical significance. Therefore, less effective interventions require larger sample sizes to demonstrate effects. Because *P* values are a unitless measure, they do not directly evaluate the magnitude or the clinical meaning of the treatment effect. Therefore, larger sample sizes may result in statistically significant *P* values but do not necessarily translate into meaningful clinical effects.

In our example, the *P* value for the difference between a study drug with a success rate of 75% and a control drug with a success rate of 80%, with 300 patients per arm, is .14. If one studies 1000 patients per arm and the success rates remain at 75% and 80% for the study and control drugs, respectively, the *P* value decreases to .007, even though the difference in point estimates remains at −5%. However, if the study drug success rate remains at 75% and the success rate in the control increases to 85%, with 300 patients per arm in the trial, the *P* value is .002. The assumption that point estimates will remain constant with increased sample size is often erroneous because the principle of regression to the mean indicates that point estimates will change toward the true value with increasing sample size. Therefore, it is often erroneous to propose that increasing sample size would yield a "significant" result in trials that "just miss" demonstrating statistical significance, or that show a "trend" toward statistical significance (e.g., *P* values of .6 to .9). This is an empirical question that needs evaluation in future studies.

P values as traditionally calculated are of little utility in noninferiority trials because they are based on difference around zero rather than difference around the noninferiority margin. Interventions that meet a noninferiority margin and those that do not will both have *P* values of greater than .05, as traditionally calculated, whenever the interventions are not frankly superior or inferior.

A *P* value of .05 means that 1 in 20 studies will demonstrate a false-positive result. Also it reflects that 1 in 20 comparisons made in a clinical trial may represent a false-positive result. If an investigator makes more than one comparison in a clinical trial, the chance of drawing a false conclusion increases. For 5 independent comparisons the type 1 error increases from 5% to 20%, and for 10 comparisons to 40%. For 20 comparisons, the type 1 error increases to 64%.[124] In other words, by making multiple comparisons with *P* values at a .05 level of statistical significance, one is more likely than not to find a statistically significant result that is false. This is called the problem of *multiplicity*. Examining many *P* values without adjustment for multiplicity is appropriate in exploratory hypothesis-generating studies. However, in confirmatory studies testing medical interventions, it often is appropriate to use a lower *P* value to define statistical significance when making multiple comparisons.

Investigators or readers may use methods such as the Bonferroni procedure[126] or other corrections for *P* values. The Bonferroni method is easy for casual readers to use because one divides the *P* value by the number of comparisons to determine the corrected definition for statistical significance. Therefore, if one makes five comparisons, the *P* value used to define statistical significance decreases from .05 to .01. An important caveat is that one should divide by the total number of comparisons made by the investigators, not just the number of comparisons presented. If the comparisons are not independent, some authors feel that the Bonferroni correction is too conservative.[127] Alternatively, investigators can perform "sequential testing" by spelling out a hierarchy of assessments before study initiation, testing each sequential difference at the same .05 significance level, proceeding from one to the next as long as each test reaches statistical significance. For instance if a study has three hierarchic end points of survival, spread of disease to another organ system, and improved symptoms, each can be tested at the .05 significance level. If the *P* value for survival is less than .05, then the investigator can proceed to test the hypothesis related to spread of disease at the .05 significance level. If this hypothesis does not reach statistical significance, then the investigator cannot test the third hypothesis related to symptoms at the .05 level. The important point for readers of clinical trials is that authors should describe what procedure was used when accounting for multiple comparisons. The most common use of multiple comparisons is in subgroup analyses, but it also occurs with multiple secondary end points or a primary end point measured at multiple times.[128]

CIs are another way of analyzing trial results and provide adjunctive information to *P* values.[129] Unfortunately, studies demonstrate that researchers lack understanding of the interpretations of CIs, just as for *P* values.[130] However, understanding CIs is integral to understanding trial results, especially for noninferiority trials. CIs are measured in the same units as the point estimate of the primary end point—for example, percentage of patients with a successful outcome. Therefore, CIs can provide an estimate of the size of the treatment effect. The range of the CI also provides some estimate of the precision of the result in clinically meaningful terms. With larger sample sizes, the range of the CI decreases. A 95% CI is a measure of estimation rather than hypothesis testing, meaning that the investigator attempts to estimate the true difference in a population from the results in a sample from that population. A 95% CI means that if the same trial hypothetically was repeated 100 times, one could expect the observed treatment effects of 95 of those trials to contain the true treatment difference, and 5 studies would not contain the true difference within their confidence bounds. When evaluating a single study, it is not possible to know if that study is one of the 95 that contain the true difference or one of the 5 erroneous studies. This reinforces why confirmation of evidence from independent studies is important. The CI is not a measure of probability (like the *P* value). Any value within the limits of the CI of a single study is equally likely to represent the true value in the population from which the sample is chosen. On average, 5 studies out of 100 will not contain the true mean at all. Data simulations show that the point estimate for one study does not lie within the confidence bounds for a follow-up study in one in six repetitions.[131] Therefore, posing that the point estimate from the studied sample of patients is the most likely "true" value in the population and that the lower and upper bounds of a given single CI are "unlikely" does not represent the true meaning of CIs.[129] In our example, the difference in point estimates is −5%. For a trial with 300 patients in each arm, the lower bound of the 95% CI is −11.7%, and the upper bound is +1.7%. This means that based on the evidence from this single study, the study drug could be somewhere from 11.7% worse to 1.7% better than the control drug for the measured outcome. Increasing the sample size to 1000 patients in each arm would give greater precision to the results by narrowing the CI to −10.0% to +0.002% if the success rates remained the same. However, the success rates usually will not remain fixed but will move toward the true mean, a phenomenon called *regression to the mean*.

CIs aid in the interpretation of "negative" studies that do not reach statistical significance and in the interpretation of noninferiority trials. CIs whose range includes clinically meaningful differences can mean that further larger studies are needed to rule out an important effect. Narrow CIs that rule out clinically meaningful differences may indicate that future studies are not needed. In this way, CIs provide more information than dichotomous evaluations of statistical significance.

An examination of the upper and lower bounds of the CI allows one to determine, as with *P* values, whether a study drug is statistically superior, inferior, or noninferior to a control drug (Fig. 52.1). The *P* value and the CI are related in that if the 95% CI excludes zero, then the *P* value is less than .05. A statistically superior drug has a lower bound of the 95% CI that is greater than zero for absolute differences. A statistically inferior drug has an upper bound of the 95% CI that is less than zero. To determine whether a study drug is "noninferior" to a control drug, one must compare the bound of the 95% CI to some prespecified margin of noninferiority. In our example, the lower bound of the 95% CI is −11.7%. If the noninferiority margin selected before initiation of the trial was −15%, then one can conclude that the study drug is statistically noninferior to the control. If the prespecified noninferiority margin was −10%, then the study drug fails to meet the statistical definition of noninferiority. Hence, the incentive for some

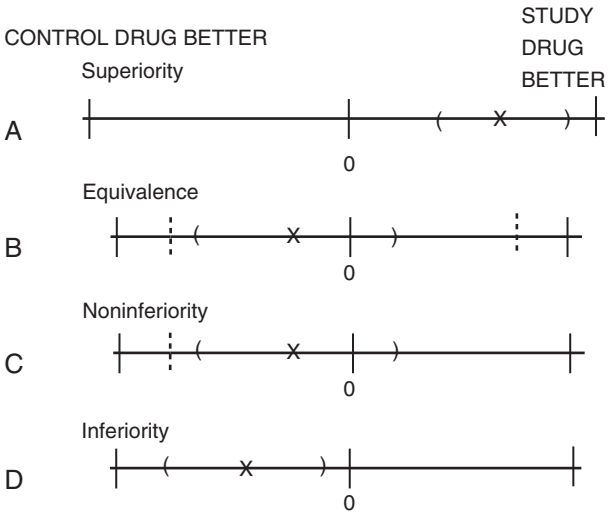

FIG. 52.1 Interpretation of results using confidence intervals (CIs) in clinical trials. *X* represents the difference in the point estimates of two therapies. The central zero mark indicates no difference between drugs. The parentheses represent the upper and lower bounds of the 95% CIs. The dashed lines are the noninferiority and equivalence margins. (A) A study drug that demonstrated superiority over the control has a point estimate and the upper and lower bounds of the 95% CIs greater than zero. (B) A study drug that demonstrates equivalence to a control drug has upper and lower bounds of the 95% CIs within a prespecified margin. The point estimate can be greater or less than zero. (C) A study drug that demonstrates noninferiority to a control drug has a lower bound of the 95% CI greater than some prespecified margin. If the lower bound is more negative than the prespecified margin, the study drug is said not to meet the definition of noninferiority. (D) A drug that is inferior to the control drug has the upper bound of the 95% CI less than zero. (See text for further explanation.)

investigators is to push for larger noninferiority margins to both decrease the sample size of trials and increase the likelihood of "positive" results. However, as discussed previously, investigators should choose noninferiority margins based on ruling out clinically meaningful differences in loss of effect with the test intervention compared with the control intervention rather than sample size considerations alone. For instance, allowing a new drug to potentially increase mortality by 15% to 20%, or 1 in every 5 to 6 patients treated with the new drug, compared with having received the older drug, seems hard to justify.

Measurement of Harms

Many clinical trials are designed to evaluate both the harms and the benefits of an intervention. However, studies often have no specified hypotheses related to harms and do not have a large enough sample size to rule out many adverse events, especially those that are less common. Reporting of harms is usually descriptive only, because often no specific hypotheses are tested, and often inadequate, with more space devoted to authors' affiliations[132] than description of harms. Absence of evidence of a difference is not the same as evidence of absence of a difference between interventions. When a clinical trial shows no cases of a particular adverse event, one can only rule out a rate of 1 in the number of patients examined divided by 3.[133] For example, if one sees no cases of a particular adverse event in 300 patients, that rules out a risk of 1 in 100 or, in other words, a rate greater than 1%. If one does not see an adverse event in 3000 patients, that observation rules out a risk of 1 in 1000, or greater than 0.1%. When there are no specified hypotheses or chosen sample size related to demonstration of harms, use of *P* values is less helpful, and nonsignificance of *P* values does not mean that no difference exists between groups. The goal of analysis of harms is to evaluate signals for toxicity along with data from preclinical, early clinical, and other studies. Spontaneous reporting of harms from postmarketing databases often detects only a small proportion of the total number of harms, estimated at 1% to 10% of the true total. Spontaneous reporting may be helpful in assessing causality to the intervention when the adverse rarely occurs spontaneously—for example, liver failure in otherwise healthy people. Spontaneous reporting is less helpful in assessing causality related to the effects of interventions on common events such as myocardial infarctions or death in seriously ill populations. Therefore, comparisons of rates of adverse events are challenging when the denominators are not known.

Subgroup Analyses

Although the overall results based on the primary research question are the most valid, investigators often express an interest in examining whether differences observed in the overall population are also present in specific subgroups of patients in the study. However, there are several pitfalls that may decrease the validity of subgroups.[134]

Valid subgroups analyses in confirmatory trials are those that (1) are spelled out before the study begins ("prespecified"), (2) have separate hypotheses (delineating what differences are expected in the subgroup compared with the entire study population) and sample size calculations, (3) have appropriate adjustment for multiple comparisons in the study, and (4) perform tests for interactions. Prespecification alone is not sufficient for valid analysis of subgroup analyses. Testing for interaction means comparing the observed differences between the test and the control groups in the subgroup of interest with the observed differences in the complementary subgroup, rather than presenting only the statistical significance of the difference between the test and control groups for the individual subgroup—in other words, evaluating a difference of differences. For instance, if a drug shows a larger effect in older patients than in younger patients, a test for interaction would compare the treatment effects in the test versus control group of older participants with the treatment effects in the test versus control group of younger participants, rather than just evaluating the statistical significance of the test versus control group of older participants.

The issue with subgroup analyses is that the protection of randomization from selection bias applies to the total randomized population but may not apply to specific subgroup analyses. Subgroups chosen based on factors that occur *after randomization,* such as adherence, drug concentrations used in pharmacodynamic analyses, exposure to medication, or treatment success or failure, are particularly problematic.[135] In these types of subgroup analyses, the patients comprising the groups may differ substantially for other factors that influence outcomes. For instance, drug exposure is influenced by severity of illness and concomitant medications. Therefore, comparing outcomes in subgroups of patients with higher drug exposures versus those with lower drug exposures may not be measuring treatment effects of the drugs, and observed differences may reflect baseline differences between patients. The same issues apply to comparing patients who adhere to a medication regimen with less adherent patients because baseline differences exist between such patients. Prior studies show decreased mortality when comparing adherent with nonadherent patients within the placebo group, demonstrating that such differences in patient characteristics influence the observed outcomes rather than effects of the interventions.[136,137] Finally, there is the issue of multiple comparisons discussed previously. The adjustment for multiple comparisons should be based on the number of comparisons made rather than the number of comparisons presented. There is an incentive to present only the "positive" subgroups in publication rather than all the subgroups evaluated. It is common, when subgroup analyses are performed without following the three criteria outlined earlier, that follow-up randomized trials in the subgroup population fail to confirm the results in the subgroup analyses. The results of subgroup analyses are most useful when they confirm the primary findings of the study.

EXAMINING THE CONCLUSIONS

In the discussion section of published studies, investigators summarize the results, discuss the strengths and limitations of the data, and relate the results to other studies of the disease in the field.[138] However, previous studies have shown that authors commonly make claims not justified by the results. A review of trials in obstetrics and pediatrics journals concluded that in only 10% of the trials were the conclusions justified by the results.[139] In another study, the authors found doubtful or invalid

statements in 76% of 196 trials.[140] Authors may tend to highlight statistically significant results over less impressive findings, whether or not those results represent the primary hypothesis of the study or post hoc subgroup analyses.[141]

Some common pitfalls readers should look for in examining the discussion section of clinical trials (Table 52.1) include the following:

1. Conclusions from studies with an unclear objective: Because the results of a study are evaluated based on the research questions chosen before study initiation, it is impossible to evaluate study results without clearly stated objectives or hypotheses. For clinical trials, this should include explanation of the types of error and the sample size. Understanding the chosen objective of the study also ensures that investigators chose the hypotheses before examining the results, rather than examining the results and deciding what the hypotheses "should have been." For instance, clinical trials of monoclonal antibodies in sepsis failed to show decreases in mortality compared with placebo, but post hoc analyses claimed benefits in patients with bacteremia, as if the hypothesis "should have been" related to only participants with bacteremia. Subsequent studies did not support this hypothesis.[142] Readers should also evaluate single-trial results in the context of other similarly designed trials. A single "positive" trial in the context of other trials that do not confirm that result is more likely to represent a false-positive finding.
2. Drawing conclusions about treatment effects of interventions from studies wherein control groups lack baseline comparability: Many types of nonexperimental studies do not evaluate comparability between the test and control groups or make comparisons between groups that are known to differ in important baseline characteristics. For instance, comparing unadjusted outcomes in patients with disease caused by resistant pathogens with outcomes in patients with susceptible pathogens compares people with inherently different baseline characteristics that independently affect outcomes.
3. Concluding noninferiority from a trial designed to demonstrate superiority: Conclusions of superiority or noninferiority of the study drug should relate to the initial hypothesis. A noninferiority trial may show a superior result for one of the drugs when the results show a lower bound of the CI around the difference in the point estimates that is greater than zero. However, it is difficult to claim noninferiority from a superiority trial. In most cases, investigators do not select a noninferiority margin before initiation of a superiority trial or evaluate whether the design of the current study conforms to prior studies that demonstrated the effect of the control intervention. This results in difficulties in interpretation of the results because noninferiority is based on comparison of the lower bound of the CI to the prespecified noninferiority margin. In addition, the sample size of a noninferiority trial is often larger than in a superiority trial; therefore the trial may not have an adequate sample size to conclude noninferiority when designed for superiority. Studies have demonstrated that the reporting of noninferiority trials is often poor, and investigators often have reported failed superiority trials as showing noninferior results.[143,144]
4. Extrapolation of results to types of patients not studied in the trial and who differ substantially from patients in the trial: The results of the trial are most applicable to patients who fit the definition and stage of the disease and the population in the study. It is more difficult to extrapolate results to other populations, especially those that may differ in host immune function, age, and pathophysiology or severity of underlying disease. For instance, demonstration of noninferiority of interventions in populations with disease caused by susceptible pathogens does not justify conclusions of superiority of the test intervention in patients with disease caused by resistant pathogens, given differences in patient characteristics between those with disease caused by susceptible or resistant pathogens.
5. Extrapolating results of explanatory trials broadly to clinical practice when interventions are used under different conditions in practice: Data from explanatory trials may not describe the effectiveness of interventions in the real-world setting. Conversely, pragmatic trials do not rule out effects of interventions in other populations or when used under other conditions. Using evidence from explanatory trials to justify noninferiority trials using pragmatic designs is similarly problematic because it extrapolates effects from one setting to another without evidence.
6. Presenting the results in a way to make them appear more favorable: The presentation of results may influence clinicians' interpretations of trials and their application in clinical practice.[94] A meta-analysis of studies showed that clinicians were more impressed and indicated a higher likelihood of prescribing an intervention to patients when investigators presented the results of trials as a relative difference in outcomes rather than as an absolute difference.[94] One should examine both the relative and absolute differences in outcomes when interpreting the results of trials. Small absolute differences may translate into large relative differences that may be clinically insignificant.
7. Conclusions based on results that do not achieve statistical significance: Authors may describe results that do not achieve statistical significance as a "trend" toward successful outcomes. Readers should interpret such claims with care. A trend assumes that further study of a larger sample size with similar patients would yield similar results. However, the likelihood of confirmatory results in a second trial when the *P* value of the first trial is 0.10 is 37%.[125] Similarly, readers should be wary of conclusions that rely on statements related to the trial being "underpowered" after the trial is complete. Power analyses are a measure of pretrial probability, and are not appropriate when a trial is over. Rather, an examination of CIs related to trial results is more appropriate.
8. Conclusions based on subgroup analyses when results were not significant for the primary end point: The results of the trial are most relevant for the specified primary end point. The most problematic types of subgroups are those based on factors chosen after randomization, such as adherence, drug exposure, or success or failure, because these subgroups select patients with different baseline characteristics.[135] As described earlier, results from subgroup analyses may be biased because of several issues. Subgroup analyses are most useful in confirming the overall results rather than drawing difference conclusions from the primary analysis and for generating hypotheses for study in future clinical trials.
9. Conclusions regarding direct patient-centered outcomes on symptoms, patient function, or survival based on nonvalidated surrogate end points: The relationship between treatment effects

TABLE 52.1 Points to Examine in Interpreting Clinical Trials

- Define goals of study
  - Type of research study—descriptive or analytical
  - Explanatory or pragmatic study
  - Define random error and sample size
  - Type of hypothesis—superiority or noninferiority
- Select control groups
- Select participants by appropriate inclusion and exclusion criteria
- Baseline comparability of participants
  - Randomization
  - Blinding
  - Stratification
- Minimizing bias
  - Selection bias—randomization
  - Observer bias—blinding
  - Confounding—randomization
  - Minimize missing data
- Selection of appropriate end points with direct benefit to patients or valid surrogate end points
- Appropriate analysis of results
- Appropriate conclusions based on data presented in trial

on direct outcomes and indirect outcomes, including biomarkers, should be clearly demonstrated. A correlation between direct and indirect outcomes is not sufficient to evaluate this relationship. For instance, studies that use decrease in organism colonization as the primary end point in a prophylaxis trial or negative cultures in a treatment trial can claim a decrease in direct patient-centered outcomes only if results of the current trial or previous trials show that a treatment effect on colonization reflects a treatment effect on decreasing disease in similar patient populations tested under similar conditions. A correlation between patients who are decolonized or who have negative cultures and direct outcomes does not provide such information. Validation of surrogate end points applies only to the population, disease definition, end–time point timing and definitions, and types of interventions used in the validation studies.

10. Ignoring the impact of bias on study outcomes: Various types of bias can influence the validity of study results and thereby impact clinical practice and patient outcomes through the promotion of ineffective or unsafe interventions. The problem is that the effect of bias is not directly measurable. Demonstration of statistical significance does not measure bias, and studies with larger sample sizes are more influenced by bias. Different scoring systems are available to evaluate the various biases that may occur in randomized trials and may help readers to systematically evaluate the presence and impact of bias.[145] The impact of bias is different in the setting of superiority and noninferiority trials, with many types of bias resulting in false-positive conclusions of noninferiority. The evaluation of study design and results is not solely an academic exercise but one that directly affects patient care. Scientific validity is the basis for ethical testing of interventions involving humans, so minimizing bias is an ethical in addition to a scientific concern.[5]

There can be significant variation in trial results based on differing definitions of disease, outcomes, and patient populations. When making treatment decisions in clinical practice, clinicians should examine a range of trials on a given disease. Reproducible or related confirmatory results in several trials of adequate design are the strongest evidence of a true effect.[12] Also, clinicians should examine all the evidence, including studies that show positive, negative, and neutral results, the potential sources of bias, and the variability and precision in those results. However, investigators are more likely to publish trials with positive results.[146] This publication bias can, in turn, affect meta-analyses and reviews. One study showed that review articles tend to omit trials with negative or neutral results.[3]

Ultimately, clinicians must decide the relevance of a study to particular patients. The distinction between clinical significance and statistical significance is an important one. Statistical significance indicates that the results of the trial are unlikely to occur by chance and that the results likely reflect the outcomes in a population similar to those studied in the trial, as long as the results are not influenced by bias. However, statistics do not reflect the clinical importance of the outcomes of the trial. Clinical significance reflects the value of the outcomes for patients and reflects a balance of both benefits and harms.[48] A trial with a very large sample size may show that small differences in outcomes are statistically significant, but such small differences may be clinically meaningless.

Clinical studies examine average effect on a population of patients. When making individual treatment decisions, however, clinicians should examine the risks and benefits of administering the drug for a particular patient. This entails an evaluation of such factors as the patient's comorbid illnesses, drug allergies and intolerance, concomitant medications, and the severity of the disease under treatment. Whereas a careful consideration of the design, analysis, and results of clinical trials helps guide clinical decision making, there is no substitute for sound clinical judgment based on applying the evidence from appropriately designed studies. Conversely, individual experience is not a substitute for sound evidence.

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The complete reference list is available online at Expert Consult.

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53 Outpatient Parenteral Antimicrobial Therapy

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Outpatient parenteral antibiotic therapy (OPAT) refers to the practice of administering intravenous (IV) antibiotic therapy in a non-inpatient hospital setting. The non-inpatient setting may include a variety of sites, such as home-based therapy, a physician's office or hospital-based ambulatory practice, infusion center, hemodialysis center, emergency department, skilled nursing facility (SNF), or long-term acute care facility. The goal of OPAT programs is to provide the required infrastructure for optimal patient selection, monitoring, and treatment, and to enable patients to complete IV therapy safely and effectively in one of these outpatient settings. Ongoing quality assurance efforts are essential to an effective OPAT enterprise. When successful, these programs shorten hospital stays, thereby lowering health care expenses, reducing the risk for hospital-acquired complication, and increasing patient satisfaction.[1–3]

THE HISTORY AND GROWTH OF OPAT

The practice of providing OPAT in the home was first described in the early 1970s for the treatment of pulmonary infections in children with cystic fibrosis.[4] Over the following years, multiple additional studies demonstrated the use of OPAT for a broad range of conditions in adults, including soft tissue infection, infective endocarditis, osteomyelitis, septic arthritis, pyelonephritis, and pulmonary infection.[5–9] Major advances in vascular access devices and the development of relatively nontoxic antibiotics that may be administered once daily during this time significantly contributed to the ability to deliver antibiotic infusions in the non-inpatient setting.[10–14]

In addition to clinical advancements, health care reform played a major role in the spread of OPAT programs by establishing financial incentives for innovations that reduce inpatient length of stay. The first of such major health care reforms occurred in 1983 with the adoption of diagnostic-related group (DRG) models for payment of clinical services by the United States Healthcare Financing Administration (now Centers for Medicare and Medicaid Services [CMS]). This payment structure transitioned from fee-for-service billing to lump-sum payments based on a weighted estimate of cost for specific diagnoses multiplied by a fixed average inpatient length of stay.[15] In this new payment structure, facilitating earlier discharge for patients requiring prolonged IV therapy offered significant potential for cost savings[8,16] and further promoted spread of the OPAT model. The passage of the Affordable Care Act in 2010 further promoted the trend for payers to transition from fee-for-service to bundled payment models based on the development of a readmission reduction program and value-based purchasing models. These programs have contributed to the development of accountable care organizations, with a focus on creating novel care delivery models that provide health care in a more efficient and cost-effective way.

One major limitation to the continued expansion of OPAT to all patients remains the lack of coverage for home infusion provided by Medicare services. Despite the coverage of OPAT services by most commercial payers, Medicare covers OPAT services as a Part D benefit, meaning that only coverage for the medication is provided and not the required equipment or supplies. Despite these limitations, OPAT represents a significant opportunity for an innovative approach to improve population health and to reduce the per-capita costs of health care.[17,18]

It has previously been estimated that more than 250,000 Americans are treated by OPAT annually,[21] although it is likely this figure now underestimates current practices. The extensive use of OPAT has been characterized by recent surveys of infectious diseases (ID) consultants performed through the Emerging Infections Network. In 2006[19] 94% of 454 respondents (54% response rate) indicated that they "frequently" discharged patients on OPAT, and use was reported throughout North America. An additional survey published in 2014[20] found that the use of OPAT was common among ID physicians, with greater than 80% of respondents discharging one or more patients on OPAT in an average month. However, with the use of OPAT becoming the standard of care for patients requiring prolonged IV antibiotic therapy, continued attention is required to ensure proper patient selection is performed and the adequate programmatic structure is available to provide optimal care.

Despite the continued growth of OPAT in the United States and internationally, there remain limited prospective study and registry data to guide best practices. Available practice guidelines focus on the key elements of a program, appropriate patient selection, and recommended clinical and laboratory monitoring.[1–3]

PATIENT SELECTION FOR OPAT

It is important that all programs develop a standard approach for the assessment of patients before discharge and OPAT program enrollment. Patient selection criteria will vary depending on the model for OPAT care chosen; often this means the focus is on proper selection of the best discharge location (e.g., SNF, infusion center, or home infusion) rather than maintaining an inpatient status for the full duration of IV therapy. The key components of patient selection include an assessment of pertinent medical and patient factors.[1,3,21]

Medical factors include ensuring adequate source control of infection, necessity of IV antibiotic therapy, and readiness for discharge.[1,3,21] Success with OPAT has been demonstrated for a multitude of different infections, including soft tissue infection, bone and joint infection, endocarditis, and pneumonia. OPAT may be optimally used when a clear diagnosis has been made, culture data with antibiotic microbial susceptibility information is available, and a response to treatment can be monitored. If a patient requires source control via drain placement or surgery (e.g., percutaneous drainage of a liver abscess or surgical débridement of devitalized tissue in a diabetic foot infection), this should occur before discharge. Patients with bloodstream infection should have demonstrated clearance of blood cultures and a full assessment for metastatic foci of infection. Those with initial sepsis physiology must have demonstrated clear hemodynamic stability before discharge.

Patient factors to consider before OPAT enrollment include an assessment of the likelihood of adherence to therapy and follow-up, social support, and insurance coverage. The lattermost often determines options for discharge location.[1,3] If a patient is planned to be discharged home for OPAT, he or she or their caregivers must be able to adhere to the required care of vascular access and administration of antibiotic infusions. This additionally includes being able to recognize clinical changes, including new fever, rash, redness or swelling at the vascular access site, and to communicate effectively to ensure timely notification of changes to care providers. Patients should be willing and able to return to an outpatient clinic during regular intervals or if new symptoms develop, and to comply with recommended safety laboratory monitoring and vascular access care. In addition, patients must be fully informed of the economic aspects of OPAT, including their individual insurance coverage before initiation of treatment. It is important that patients be informed of any potential out-of-pocket costs for medication treatment,

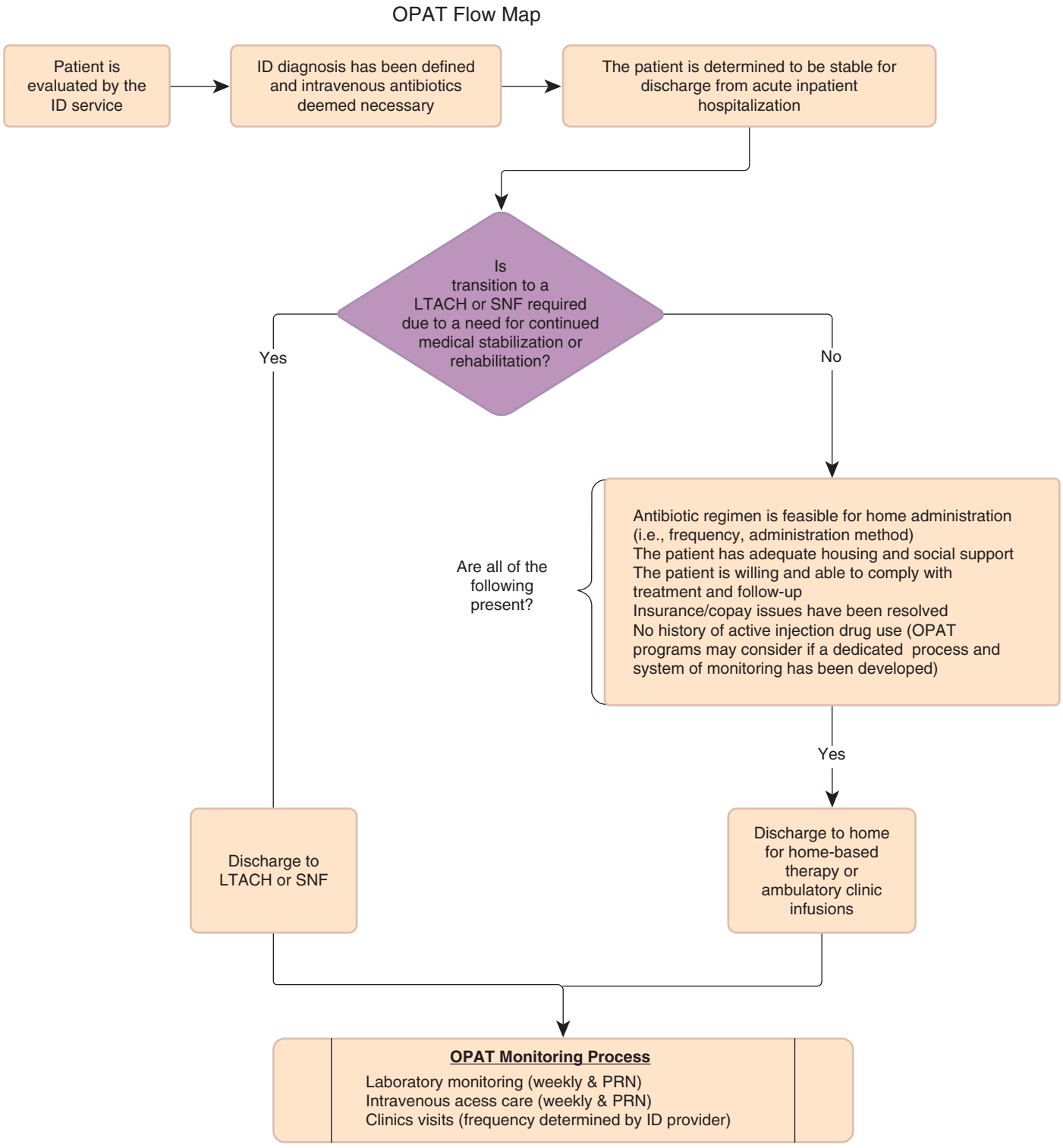

FIG. 53.1 Outpatient parenteral antibiotic therapy enrollment process. *ID,* Infectious diseases; *LTACH,* long-term acute-care hospital; *OPAT,* outpatient parenteral antibiotic therapy; *PRN,* as needed; *SNF,* skilled nursing facility. *(Modified from Tice AD, Rehm SJ, Dalovisio JR, et al. Practice guidelines for outpatient parenteral antimicrobial therapy. IDSA guidelines.* Clin Infect Dis. *2004;38:1651–1672; Muldoon EG, Snydman DR, Penland EC, Allison GM. Are we ready for an outpatient parenteral antimicrobial therapy bundle? A critical appraisal of the evidence.* Clin Infect Dis. *2013;57:419–424.)*

vascular access care, office visits, and laboratory monitoring. If a patient incurs unexpected out-of-pocket costs, it may lead to unexpected treatment interruption or early discontinuation of IV therapy.

It is the combination of the assessment of medical factors and patient factors that inform the decision regarding the optimal modality of OPAT at the time of hospital discharge (Fig. 53.1). If a patient has not yet demonstrated adequate clinical improvement for discharge home but no longer meets criteria for inpatient hospitalization, or is unlikely to comply with home- or office-based therapy due to patient or social factors, a transition of care to a long-term acute-care facility or SNF for continued treatment with ongoing daily monitoring is the most reasonable initial option. If a patient is medically ready for discharge

home, daily treatment in a physician's office or infusion center may be optimal for patients who have a preference for this approach. This may be because of discomfort with home infusion or a lack of caregiver support to assist with medication administration, and the patient may live close to the ambulatory practice or lacks of insurance coverage for treatment at home. If the patient does not live near an available ambulatory practice or infusion center, at times use of a local emergency department is required. For those patients meeting criteria for home infusion, a clear communication plan with an infusion company and/or visiting nurse service is required to ensure continuity of care at the time of discharge. Due to the frequent occurrence and spotty nature of antimicrobial drug shortages, which particularly effect generic injectable antibiotics,[22,23] the OPAT team should confirm that the infusion company has access to sufficient supplies of the recommended agent (or a suitable alternative) to complete the planned course of treatment.

OPAT IN PEOPLE WHO INJECT DRUGS

The current epidemic of opioid abuse has been marked by a rapid increase in the number of people who inject drugs (PWIDs). This, in turn, has been associated with an alarming increase in infectious diseases associated with injection drug use (IDU) from hepatitis C virus and human immunodeficiency virus, along with morbid and life-threatening bacterial infections, such as endocarditis; spinal epidural abscess; and bone, joint, and soft tissue infections.[24] As a result, most ID clinicians are faced with the potentially increasingly consequential consideration of OPAT in PWIDs.[24] The current, somewhat dated, Infectious Diseases Society of America (IDSA) Practice Guidelines for Outpatient Parenteral Antimicrobial Therapy give little practical guidance in this area, suggesting consideration of alternatives to home-based OPAT, such as SNF placement, intramuscular injection, or daily infusion with IV catheter removal for patients likely to manipulate an IV catheter.[1] A more recent OPAT guideline from the United Kingdom does not appear to directly address the issue.[3] High-quality evidence is still sparse; however, some preliminary observations can be made. First, this is a common scenario faced by many ID clinicians.[24] Second, compared with other patients receiving OPAT, PWIDs tend to have more severe infections, although they tend to be younger and have fewer medical comorbidities, as observed by Buerhle and colleagues,[25] who compared their PWIDs OPAT population with an unselected Department of Veterans Affairs (VA) health care facility OPAT population.[26] Third, more recent IDU is associated with an increased risk for OPAT failure.[25] Finally, several reports suggest that careful selection of lower-risk patients and/or use of well-structured and comprehensive OPAT programs designed for this population can produce outcomes that are similar to non-IDU populations.[25,27–29] Elements of successful programs for the provision of OPAT to PWIDs include careful patient selection, contracts of care requiring adherence to follow-up appointments and laboratory testing, prohibition of manipulation of the IV devices, and attention to the substance abuse diagnosis with available medically assisted treatment and harm reduction strategies for ongoing IDU.[27–30] Successful examples include the experience of Ho and colleagues,[27] who reported approximately 97% of the 29 PWIDs (defined as IDU in the past 12 months) they studied were compliant with OPAT, and about 21% required readmission during the study period. Their OPAT program included a contract of care, prohibition of illicit drug use enforced with as-needed urine testing, daily clinic visits for infusions, and the application of decals to prevent manipulation of peripherally inserted central catheter (PICC) lines. Beieler and colleagues[28] reported another successful OPAT program embedded in a residential "respite care center" associated with an urban hospital that also included a care contract and antimanipulation decals for catheters. Harm reduction strategies included allowance of safer injection practices and opiate replacement therapy, but precluded illicit use of the IV catheter. Camsari and colleagues[29] took a potentially more generalizable approach to this problem by using an addiction specialist and a psychiatrist to carefully risk stratify PWIDs, then used that risk assessment to make recommendations regarding the conditions for OPAT. They were able to achieve uniformly successful OPAT in low-risk populations, a 90% success in a moderate-risk population (including prior IDU no less than 12 months ago), and reasonable success (80%) even in the highest-risk population, although most of those went to a SNF. A more cautionary note was provided by a study of OPAT in population of IDUs, lacking some of the structural elements outlined earlier, wherein 61% of patients failed to complete their treatment successfully. Failure was associated with more recent IDU (3 vs. 8 weeks), although patients who had a more remote history of IDU (>5 years) had a relatively low failure rate (21%).[25] The alternative to successful execution of an OPAT strategy for PWIDs is prolonged hospital stays for inpatient treatment. It is clear that we must move past an overly simplistic approach to this population, acknowledging that the data are not robust, and the risk of failure is probably somewhat higher than a general OPAT population; however, that risk can be mitigated using proactive strategies of risk stratification and harm reduction in well-structured OPAT programs.

ALTERNATE OPTIONS FOR OPAT

OPAT is not the preferred approach for all patients, particularly those with a lack of ability to self-administer IV therapy or for those without adequate social support or stable housing to complete the required treatment. Factors such as substance abuse, most prominently active opioid use disorder, may render a patient unsuitable for vascular access device placement in the outpatient setting. In addition, not all patients who fail to meet criteria for home-based therapy may be able to be placed in a SNF due to insurance coverage or patient preference against facility placement.

USE OF ORAL ANTIBIOTIC THERAPY

There are times when use of parenteral antibiotic therapy would be preferable. However, it may not be determined to be a safe approach by the ID service due to concern for challenges with adherence to therapy, active IDU, or needed follow-up. In these instances, offering an alternative of oral therapy may be an acceptable, although a suboptimal, approach. When used, agents with excellent oral bioavailability, such as the fluoroquinolones, metronidazole, trimethoprim-sulfamethoxazole, clindamycin, and linezolid, should be chosen when possible.

Although the role of oral antibiotic therapy for serious infections, such as endocarditis and osteomyelitis, has not been well established, there are limited data that demonstrate the efficacy of this approach.[31–36] For example, there are few studies of oral antibiotic treatment of endocarditis. Limited data suggest that oral ciprofloxacin in combination with rifampin was active against right-sided *Staphylococcus aureus* endocarditis in a small randomized trial.[37] There are a handful of small retrospective and observational studies suggesting that oral penicillins, sometimes combined with probenecid, may be effective for primarily streptococcal endocarditis.[32] Even more limited data suggest that linezolid may be effective treatment for endocarditis.[38] In addition, if initial parenteral therapy has been used to treat endocarditis, a recent large randomized multicenter trial suggested that providers can consider an early transition to oral therapy in some patients without loss of efficacy.[34] When considering use of an oral regimen for osteomyelitis, this is ideally done after any needed surgical débridement has been performed, and the causative pathogens have been identified and are susceptible to a highly bioavailable oral agent. If this approach is taken, typically more prolonged courses of therapy are used.[35,36]

USE OF LONG-ACTING PARENTERAL AGENTS

Although currently only approved for use in skin and soft tissue infection, the long-acting parenteral lipoglycopeptides dalbavancin and oritavancin present an additional option for outpatient antibiotic therapy.[39,40] Their long half-lives obviate the need for placement of long-term IV access, which may be a particularly attractive option in the setting of PWIDs. Available data suggests that these agents may be active in the treatment of bloodstream infections,[41] and excellent penetration in bone and articular tissues has been reported.[42] However, additional clinical studies are required before recommending the use of these agents as a standard practice. The risk of emergence of resistance to these drugs in prolonged courses also requires further study.[43,44] Acquisition costs for these agents are high, and cost-effective implementation of their use requires careful economic analysis.

PROGRAMMATIC REQUIREMENTS

OPAT should be provided through a team approach, led by an ID clinician with experience in managing all of the common modalities of outpatient IV antibiotic therapy. The OPAT multidisciplinary team should optimally also include a pharmacist with ID training, a nurse with expertise in parenteral medication administration and venous access devices, and administrative support to facilitate timely office visits and communication with patients, infusion companies, and care facilities.

INFECTIOUS DISEASES PROVIDER

The physician responsible for oversight of OPAT should have ample knowledge and experience in the diagnosis of infectious diseases and the unique challenges of the management of antimicrobials in the outpatient setting.[1,3] Specific experience is required to allow timely identification of poor clinical responses or complications of therapy, such as adverse drug reaction (ADR) and complications of venous access devices. Experience in performing an assessment of patient factors to help guide optimal selection of OPAT modality or deferral of this approach is needed. In addition, provider oversight of an OPAT program should include a focus on optimization of team management and continuous quality improvement work, including an assessment of complications of therapy and outcomes.

ID consultation has been shown to provide significant benefit in performance of salient medical and patient factors assessment. A major beneficial impact may be deferred IV therapy, if not required. Shrestha and colleagues[45] assessed 263 ID consultations for the purpose of OPAT and found that 84% of patients had an optimization of their antibiotic regimen, 52% had a significant change in assessment, and additional medical care contribution was provided in 71%. In addition, OPAT was found to be unnecessary in 27% of patients. Conant and colleagues[46] retrospectively reviewed 577 requests for OPAT by the ID consult service and found a 10.4% denial rate. Of the 56 patients available for evaluation, the cure rate was 87.5%, with 6 of 7 failures attributed to nonadherence with oral antibiotic therapy or deemed to have an incurable infection.

Once the decision has been made that OPAT is the preferred approach, the OPAT clinician should be responsible for approving all changes in medication orders and final interpretation of safety laboratory values to ensure that change in medication or treatment is not needed.

OPAT PHARMACIST

The role of a pharmacist on the OPAT team serves multiple purposes, including working with the ID provider to determine the most effective, safe, and narrowest spectrum of therapy with the least likelihood of adverse reaction. This includes screening for unrecognized drug interactions and ensuring optimal antibiotic dosing based on changing patient factors, such as fluctuating renal function. In addition, pharmacists have been used to implement IV switch programs to oral therapy, with the goal of shortening the required OPAT duration.[1,3,47,48]

After patient discharge the role of the pharmacist and interactions with the OPAT team often varies because of the site of care chosen and the OPAT program structure. For example, if a patient is discharged to a facility, the OPAT team will often interact with the pharmacists responsible for medication administration at that site. However, if a patient is receiving IV therapy at home via an infusion company, the interaction will be with a pharmacist responsible for acquisition, storage, compounding, and dispensing of antibiotics to the home.

INFUSION NURSE SUPPORT

The role of nursing services varies with the OPAT model chosen and site of care. At the time enrollment in OPAT occurs, nursing services are required to provide patient education regarding IV access devices and medication administration. When home therapy is performed, nursing provides regular home assessments to ensure oversight of medication administration and IV access care by the patient or caregiver, as well as providing notification to the responsible clinician regarding any clinical changes. Regardless of the modality of OPAT chosen, nursing plays a significant role in the coordination of care and ongoing patient education and monitoring.[1]

ANTIMICROBIAL STEWARDSHIP

A policy statement from the Society for Healthcare Epidemiology of America, IDSA, and Pediatric Infectious Diseases Society proclaimed "antimicrobial stewardship must be a fiduciary responsibility for all healthcare institutions across the continuum of care."[49] There is a growing focus on the importance of antimicrobial stewardship programs expanding to the non-inpatient setting, of which OPAT represents a significant opportunity. There is a substantial need for ID consultants and OPAT pharmacists to determine the necessity for OPAT and to balance providing narrow-spectrum therapy with facilitating the convenience of once-daily home infusion when feasible.[46,47]

SYSTEM FOR DATA MANAGEMENT AND COMMUNICATION

For OPAT programs to meet their goals to provide IV therapy safely and effectively in the outpatient setting, a reliable system of communication among the patient, clinician, pharmacy, and nursing staff is required. Programs should have established policies and procedures to detail the responsibility of each team member, as well as to define standards for patient selection criteria, laboratory monitoring, responses to critical or urgent values, and complications of vascular access. All patients should be informed and demonstrate knowledge of the program's emergency access numbers and know which conditions should prompt a telephone call or urgent visit. Programs need to have a system for rapid communication between the patient and care providers, documentation of this communication, and ability to accommodate urgent clinic visits to assess concerns.[1,3,21]

ANTIMICROBIAL CONSIDERATIONS

In addition to the usual considerations regarding antimicrobial activity, pharmacokinetics-pharmacodynamics, drug toxicity, drug interactions, selection for drug resistance, and overgrowth syndromes such as *Clostridioides difficile* (formerly *Clostridium difficile*) infection (CDI), the choice of antimicrobial drugs for OPAT requires consideration of certain logistical issues that are unique to this care setting. Therapeutic drug monitoring (TDM) can be challenging in the OPAT setting as the timing of phlebotomy versus the drug infusion may be logistically difficult. Although the need for TDM does not preclude use of drugs monitored in OPAT, it does make OPAT more complex for those drugs that need to have timely phlebotomy performed (sometimes repeatedly), along with the ability to acquire, record, interpret, and act upon results with new drug orders as necessary. The number of infusions per day and the duration of those infusions must be taken into account when considering a drug regimen for OPAT use. For some drugs, such as vancomycin, infusion time is prolonged in an effort to prevent infusion-relative toxicity, whereas for β-lactams and other drugs with activity related to time above minimum inhibitory concentration (MIC), a prolonged infusion may be used to improve predicted activity.[50] For this reason, prolonged infusion is recommended in the package insert for some newer β-lactam drugs, such as ceftazidime-avibactam (2 hours) and meropenem-vaborbactam (3 hours).[51,52] Drugs administered by a visiting nurse should generally be limited to once- or, at most, twice-daily infusions of a limited duration. If the patient and/or a family member or friend can be trained to administer the IV drug and care for the line before and after, there can be a bit more flexibility, but numerous and/or prolonged infusions may be expected to have a negative impact on the patient's quality of life. Venous access device compatibility is yet another consideration. Certain drugs are thought to be sufficiently caustic to veins (phlebitogenic) that they should be administered through central lines, although there is some debate about which drugs require central access.[53,54] For example, the safety of provision of vancomycin via midline has been debated, and most recent data suggest that this may be a safe practice.[55] Drug stability at room temperature is another factor that must be considered for OPAT. More stable drugs can potentially be administered by a programmable IV pump at more frequent intervals (e.g., penicillin every 4 hours) or as an extended infusion over 24 hours (potentially requiring periodic bag changes). Drug shortages, particularly common among generic injectable antibiotics, are yet another consideration.[23] Because hospitals and infusion companies may have different supply chains, providers should be certain that infusion companies

have adequate supplies of drugs in shortage before discharge on OPAT. Last, but certainly not least, the cost of the drug may be a determinative factor, depending on the patient's insurance coverage, and this should be considered in the context of stewardship of health care financial resources.

DRUG CHARACTERISTICS AND REGIMENS

The ideal OPAT drug is highly active against the target organism(s), has a high barrier to selection for resistance, is sufficiently narrow in spectrum to confer limited selective pressure for CDI, and is inexpensive, stable, and can be infused rapidly and infrequently. Few drugs meet all of these criteria, so compromises are required. Properties of commonly used OPAT antibiotics are detailed in Table 53.1. Common parenteral regimens for methicillin-susceptible *S. aureus* (MSSA) include cefazolin and ceftriaxone, although some providers shy away from once-daily ceftriaxone for the most serious MSSA infections.[56] Antistaphylococcal penicillins (nafcillin and oxacillin) have historically been perceived to be the most active agents for the most serious MSSA infections, but these drugs require frequent infusion, and a number of recent retrospective studies suggest similar clinical outcomes can be achieved with cefazolin with reduced drug toxicity (see "Adverse Drug Reaction") and lower mortality in some studies.[56–58] However, in the absence of randomized controlled clinical data, some experts continue to favor antistaphylococcal penicillins for the most difficult-to-treat MSSA infections.[59] Cefazolin may be used in patients exhibiting non–immunoglobulin E–mediated reactions to antistaphylococcal penicillins. Vancomycin should not be used for MSSA infection in the absence of severe β-lactam allergy, including for patients on dialysis, as clinical outcomes are inferior to β-lactam treatment.[60,61] For methicillin-resistant *S. aureus* (MRSA), vancomycin remains a work-horse agent, although vancomycin treatment is laden with the use of TDM and increased toxicity versus some comparators, as well as concerns about efficacy, resulting in the increased use of daptomycin. Daptomycin has been shown to be a safe and effective OPAT drug for MRSA compared with vancomycin,[62] although some studies suggest both drugs have higher rates of ADRs in OPAT versus comparators.[63] For a variety of reasons, including multiple allergies and drug resistance, there is some OPAT uptake for IV and oral (PO) linezolid and ceftaroline, although both drugs have risk of ADRs, including hematologic toxicity, particularly linezolid.[64–66] Vancomycin and daptomycin are also options for MSSA infection in the setting of a serious β-lactam allergy (see Chapter 194). Once-daily ceftriaxone is an ideal option for most streptococcal infections, and in the setting of serious β-lactam allergies, vancomycin or daptomycin may be considered. For serious enterococcal infections, such as endocarditis due to ampicillin-susceptible enterococci, ampicillin is still a favored agent, despite the need for frequent infusions, whereas vancomycin and daptomycin are also used for allergies or resistance to β-lactams[67] and potentially for logistical reasons in less serious infections. The use of gentamicin for synergistic treatment of ampicillin-susceptible enterococcal endocarditis is covered elsewhere (see also Chapter 80), but it would be worth noting in this section that use of thrice-daily aminoglycosides would be challenging in most OPAT settings, and high-dose ceftriaxone combined with ampicillin or alternatives to ampicillin should be considered for endocarditis treatment. A variety of agents may be chosen for gram-negative rod (GNR) infections, including extended-spectrum cephalosporins—for instance, ceftriaxone, ceftazidime, and cefepime. For patients with some types of antibiotic resistance and some mixed infections, the β-lactam plus β-lactamase inhibitor combination of piperacillin-tazobactam is heavily used for inpatients and sees some use in OPAT, but this requires 3 to 4 daily infusions in patients with normal renal function and makes it a less-than-optimal drug in that setting. The carbapenem ertapenem,

TABLE 53.1 Properties of Commonly Used OPAT Antibiotics

DRUG	HALF-LIFE (H)	STABILITY AT 5°C/25°C	PHLEBITIS RISK	ISSUES OF CONCERN DURING OPAT
Cefazolin	1–2	10 days/1 day	1	
Ceftazidime	1.4–2	21 days/2 days	1	
Ceftriaxone	5.4–10.9	10 days/3 days	1	LFTs should be checked weekly.
Daptomycin	8.1	12 h/48 h	1	Occasional myopathy; CPK should be checked weekly. Eosinophilic pneumonitis is an unusual adverse effect. Can falsely prolong INR without changing anticoagulation; INR should be checked at least 24 h after daptomycin dosing
Ertapenem	4	24 h/6 h	2	LFTs should be checked weekly.
Gentamicin	2–3	30 days/30 days	1	Ototoxicity and nephrotoxicity common, so hearing and vestibular function must be clinically assessed regularly. Drug levels (typically trough concentrations) should be tested weekly, and kidney function must be checked twice weekly.
Meropenem	1.5	24 h/4 h	1	LFTs should be checked weekly.
Nafcillin	0.5–1.5	3 days/1 day	3	Occasional hepatotoxicity; LFTs should be checked weekly. Also risk of allergic nephritis. Frequent dosing generally requires programmable IV infusion device.
Oxacillin	0.3–0.8	7 days/1 day	2	Higher risk of thrombophlebitis and DVTs. Also risk of allergic nephritis. Frequent dosing generally requires programmable IV infusion device.
Penicillin G	0.4–0.9	14 days/2 days	2	Frequent dosing generally requires programmable IV infusion device.
Tobramycin	2–3	4 days/2 days	1	Ototoxicity and nephrotoxicity common, so hearing and vestibular function must be clinically assessed regularly. Drug levels (typically trough concentrations) should be tested weekly, and kidney function must be checked twice weekly.
Vancomycin	4–6	63 days/7 days	2	Occasional nephrotoxicity; kidney function should be checked weekly. Therapeutic drug monitoring (weekly trough concentration) recommended. Avoid rapid infusion to reduce the risk of "red man" syndrome (histamine release reaction).

CPK, Creatine phosphokinase; *DVTs,* deep vein thromboses; *INR,* international normalized ratio; *IV,* intravenous; *LFTs,* liver function tests; *OPAT,* outpatient parenteral antibiotic therapy.

Modified from Tice AD, Rehm SJ, Dalovisio JR, et al. Practice guidelines for outpatient parenteral antimicrobial therapy. IDSA guidelines. Clin Infect Dis. *2004;38:1651–1672.*

which can be given once daily, is a favored agent for OPAT for mixed infections[68,69] and mild–to–moderately severe infections caused by extended-spectrum β-lactamase–producing GNRs. However, unlike the more frequently dosed carbapenems (meropenem, imipenem, doripenem), it lacks activity against *Pseudomonas* spp. For coverage of *Pseudomonas* spp., favored drugs include cefepime, ceftazidime, piperacillin-tazobactam, carbapenems exclusive of ertapenem, and IV/PO levofloxacin and ciprofloxacin. For coverage of anaerobic infections, metronidazole IV/PO can be used, often added to a regimen with activity against gram-negative and gram-positive aerobes for mixed infections. The drug has caused neuropathy in prolonged use, but recent data would suggest that this is uncommon, and ADRs occur typically after more than 4 weeks of treatment.[70,71]

METHODS OF INFUSION AND DRUG DELIVERY

The logistics of home infusion of antibiotics will vary depending on a number of factors, including the duration of treatment, the nature of the drug, patient and caregiver capacity to perform infusions, type of insurance, and the home infusion company.[14] First doses of an OPAT regimen are often given in the hospital before discharge but may be given in an infusion center or, increasingly, at home by home infusion companies staffed by health care personnel "trained and equipped to manage anaphylaxis."[3] Vascular access devices used for OPAT range from peripherally inserted devices, such as peripheral venous catheters for very short-term use (days), to midlines, which typically extend from the antecubital fossa to the axilla, to what is probably the most common type of access for OPAT, the PICC. Most often, when patients requiring prolonged IV treatment are unable to receive a PICC line, a surgically implanted direct central access is used, typically via a cuffed implanted central venous catheter (CVC; brand names include Hickman, Groshong, and Broviac) or totally implanted CVCs with a subcutaneous reservoir accessed by percutaneous needle (e.g., Port-a-Cath).[72] Noncuffed central lines are not favored for home infusion because of a risk of dislodgement and infection. Formats that may be used to prepare drugs for IV infusion will depend on the drug, its stability, dilution requirements, and so forth.[14] Drugs may be prepared in syringes for relatively rapid infusion by a nurse or may be given by a syringe pump, in bags of IV fluid that may be gravity dripped (when the rate of infusion is not critical) or infused by rate-controlled devices, for instance, an elastomeric pump (nonelectronic pump) or an electronic pump. Programmable electronic pumps allow frequent or continuous infusions, although drug stability at room temperature must be taken into account. Some elastomeric devices and electronic pumps are sufficiently portable to allow full patient mobility.

LABORATORY MONITORING OF ANTIBIOTIC THERAPY

Laboratory monitoring of OPAT serves several important purposes: to assess for evidence of progressive resolution of the infection, to assess for changes in the patient's capacity to clear drugs, and to test for possible drug toxicity.[1] In addition to white blood cell count and differential, it may be helpful to regularly (e.g., weekly) track inflammatory markers, such as erythrocyte sedimentation rate and C-reactive protein, over time to provide evidence for progressive resolution of some infections, for instance, endovascular and osteoarticular infections.[73,74] Changes in kidney function, and liver function for some drugs, independent of drug toxicity may alter the patient's ability to clear the drug and warrant dose adjustments. For instance, resolution of kidney injury after hospital discharge may warrant a dose increase, or a decline in creatinine clearance due to treatment of heart failure may warrant a reduction. The routine monitoring of a set of laboratory parameters to assess for drug toxicity is required for the safe administration of OPAT. Blood is typically obtained at least weekly for patients receiving OPAT, and the menu of tests are determined by the more common toxicities and route of clearance of the OPAT regimen components (Table 53.2). For most regimens (with drugs subject to renal clearance), weekly renal function (creatinine and blood urea nitrogen) and a complete blood count with differential to test for hematologic toxicity is appropriate. The differential is important to detect eosinophilia, suggesting hypersensitivity, as well as neutropenia related to direct bone marrow suppression or immune-mediated phenomena. Twice-weekly testing of renal function is appropriate for nephrotoxic agents, such as aminoglycosides. Drugs with significant hepatic toxicity and/or hepatic metabolism (e.g., nafcillin, oxacillin, ceftriaxone, meropenem, and ertapenem) should have a weekly liver panel (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin). Other chemistries may be relevant for particular agents, for instance, electrolytes for drugs with high electrolyte content (typically penicillins such as nafcillin) and creatinine phosphokinase (CPK) for daptomycin due to the muscle toxicity of that drug. Weekly TDM should be performed for vancomycin (trough level), and twice-weekly testing should be performed for aminoglycosides, with troughs for all indications and the addition of peak concentrations for enterococcal and staphylococcal synergy dosing. It is a considerable logistical challenge for OPAT programs to obtain timely blood draws and acquire, document, and communicate laboratory results on numerous OPAT patients, who are often cared for by a variety of home infusion vendors, visiting nurse organizations, and SNFs. The challenge is even greater for TDM, as blood draws have to be timed properly for doses of drugs. Optimally, documentation of test results should be in a cumulative format to reveal trends before more overt toxicity occurs.

TABLE 53.2 Suggested Laboratory Monitoring for Commonly Prescribed OPAT Antibiotics

DRUG	CBC/ DIFFERENTIAL	RENAL FUNCTION (BUN, CREATININE)	LIVER ENZYMES/LIVER FUNCTION TESTS	THERAPEUTIC DRUG MONITORING	OTHER
Vancomycin	Weekly	Weekly		Weekly trough	
Daptomycin	Weekly	Weekly			Weekly CPK
Linezolid	Weekly				
Antistaphylococcal penicillins	Weekly	Weekly	Weekly		
Ceftriaxone	Weekly	Weekly	Weekly		
Carbapenems	Weekly	Weekly	Weekly		
Other β-lactams	Weekly	Weekly			
Fluoroquinolones			Weekly		
Aminoglycosides	Weekly	Twice weekly		Weekly trough	Clinical monitoring for hearing and vestibular function at each visit

BUN, Blood urea nitrogen; *CBC,* complete blood count; *CPK,* creatine phosphokinase; *CRP,* C-reactive protein; *ESR,* erythrocyte sedimentation rate; *OPAT,* outpatient parenteral antibiotic therapy.
Consider weekly ESR/CRP for patients with bone/joint infections and endocarditis or endovascular infections.
Modified from Tice AD, Rehm SJ, Dalovisio JR, et al. Practice guidelines for outpatient parenteral antimicrobial therapy. IDSA guidelines. Clin Infect Dis. *2004;38:1651–1672.*

OPAT PROGRAM OUTCOME MEASUREMENT

The measurement of outcomes is an integral part of an OPAT program's responsibility to assess the safety and efficacy of care and to inform continuous process improvement efforts. At a minimum an assessment should include clinical outcome, response to treatment, and adverse events related to the OPAT course. This should include the course of antibiotic therapy, vascular access device, and need for readmission. The IDSA guidelines have recommended metrics used to assess the safety and efficacy of OPAT, including an assessment of clinical status, infection status, program outcome, antibiotic use, and vascular access complications.[1] The ability of OPAT programs to share data and allow comparison is limited by a lack of standard timing of outcome measurement and lack of a national database for benchmarking. The most robust studies of outcomes of OPAT include an assessment of cost savings and of financial analyses, which can be used to advocate for the programmatic support required for OPAT services. Additional studies assessing patient reported outcomes and experience are needed.

READMISSIONS

One of the major benefits of OPAT programs are a reduction in inpatient length of stay through facilitating ongoing IV antibiotic treatment in the non-inpatient setting. A principal balancing measure of increasing OPAT program use is unplanned hospitalization related to preventable complications of therapy. Unplanned readmissions are common; however, rates are widely variable across studies and range between 3% and 26%.[75–79] Common causes of readmission include worsening infection, ADR, and CVC-related complications. However, in published studies readmissions were frequently non-OPAT related (27%–57%), and thus a significant portion may be less likely to be preventable through enhanced OPAT services. This emphasizes the complex medical illnesses that many OPAT patients suffer.[75,76,80] Several studies have attempted to elucidate the predictors of unplanned hospitalization after OPAT initiation at the time of hospital discharge, to better tailor patient selection and needed patient and programmatic support. Common identified predictors include age and prior hospitalization in the previous 12 months. In addition, drug-resistant organisms, aminoglycoside use, increased planned OPAT duration, and lack of available laboratory monitoring have also been observed as risk factors.[75–78] The incidence of ADR varies widely based on the definition used[62,81] but accounts for up to 25% of readmission in the OPAT population.[76,82] OPAT-related ADRs often occur within the first 2 weeks of discharge from the hospital and frequently lead to need for dosing change, alteration in treatment, or early discontinuation of therapy.[63] The risk of ADRs stresses the importance of frequent safety laboratory monitoring, and guidelines based on expert opinion recommend weekly monitoring.[1] Nonavailability of recommended tests has been associated with an increased risk of readmission during OPAT.[78] Timely antibiotic changes based on responses to the development of ADRs may reduce the need for readmission.[83] Additional multicenter studies are required to elucidate the risk factors associated with readmission—related to patient factors as well as to the site of delivery of therapy—to inform optimal patient selection and future interventions for programmatic improvement.

COMPLICATIONS OF VASCULAR ACCESS DEVICES

The presence of a vascular device for delivery of outpatient IV antibiotics confers risk to the patient of complications that include occlusion, dislodgement, thrombosis, bleeding, and infection. The most common type of catheter used for OPAT is a PICC. However, some patients already have long-term venous access for existing comorbidities, such as malignancy, which require treatment with chemotherapy or for gastrointestinal tract malabsorption requiring total parenteral nutrition, for which a tunneled cuffed catheter (e.g., Hickman) or totally implanted catheter (e.g., Port-a-Cath) may be used. The incidence of complications related to vascular access devices range from 0.4 to 5 complications per 1000 OPAT days.[77,84,85] A recent retrospective cohort study from a large academic medical center found a rate of 4.29 complications per 1000 OPAT days, and catheter occlusion accounted for 53% of complications. In this cohort catheter-related thrombosis and infection were seen in less than 1% of OPAT courses. Complications were more common in PICCs compared with Hickman catheters or Port-a-Caths. Patients with a history of IDU had three times as many complications than those without this history. Of note, vascular access device-related complications were not associated with an increased risk of readmission.[84] A recent study found that midline catheters, commonly used for shorter courses (<4 weeks) of antibiotic therapy, were an independent risk factor for catheter-related complications. However, additional studies are needed to further investigate the impact of catheter type on complications.[54]

Given the risk of vascular access device-related complication, education regarding the signs and symptoms of this complication, such as redness, pain, swelling, drainage, or catheter migration, as well as regular assessment in the outpatient setting, is required. In addition, a structured approach to assessment and management of catheter occlusion is needed as an integral component of an OPAT program, to avoid emergency department visits or readmission.

FINANCIAL ANALYSIS OF OPAT PROGRAMS

The assessment of the full economic impact of OPAT requires estimates of both direct and indirect medical costs. Direct medical costs assessed for OPAT typically include expenses for inpatient care, physician visits, ancillary services, and medical supplies. Indirect costs include absenteeism from work, caregiver labor provided by family members or friends, and limited activity. However, indirect costs are challenging to ascertain for OPAT therapy because of a lack of validated quality-of-life instruments specific for infectious diseases and antibiotic therapy.[16]

The early OPAT literature focused largely on direct costs of health care, demonstrating significant reductions in cost, mainly driven by a reduction in inpatient bed-days.[5,7,16,86,87] However, this approach is limited as it does not take into account differences in incentive for reimbursement across the health care system. Additional factors to consider are the cost savings of the risk reduction of hospital-acquired infection by reducing length of stay,[88] as well as the costs of OPAT-related adverse events, such as readmission or ADRs.

PATIENT SATISFACTION

Available studies assessing patient satisfaction with OPAT therapy demonstrated that nearly all patients reported being satisfied overall with the program and had a willingness to participate in OPAT again, should the need arise.[89–94] Much of the benefit of enhanced patient satisfaction from OPAT stems from the patient's ability to self-administer antibiotic treatment, allowing increased responsibility for treatment and for autonomy with return home. It was common for patients to express fear about outpatient treatment, particularly the need for a vascular access device.[93] Thus adequate patient education and training, as well as visiting nursing support, are necessary components to enhance patient comfort with the process. Additional studies to assess patient satisfaction in OPAT and to benchmark programs would significantly benefit from use of a standard and validated questionnaire.

FUTURE OF OPAT

Current trends in health care that focus on cost-effective high-quality care, are associated with high rates of patient satisfaction, and focus on the aging population are likely to contribute to further growth in OPAT. The burgeoning opioid epidemic will continue to challenge OPAT to provide effective approaches to the care of to these complex patients.

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54 Tables of Antiinfective Agent Pharmacology

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This chapter serves as a centralized source of pharmacologic information on antiinfective agents. The first table provides an index of all the tables that are included in this chapter (Table 54.1). Tables detailing generic and trade names for antiinfective agents are provided in an alphabetized sequence (Tables 54.2 and 54.3). Tables describing existing dosage formulations also are categorized by chemical or pharmacologic class (Tables 54.4 through 54.16). Available information regarding the usual doses in adults and children, pharmacokinetic (PK) information, and dose adjustment in renal impairment is provided according to chemical or pharmacologic class (Tables 54.17 through 54.30). Several antiinfective agents are substrates, inducers, and/or inhibitors of drug metabolism enzymes and transporters. Tables categorizing these potential mechanisms for drug-drug interactions as either the victim or the perpetrator drug are included (Tables 54.31 through 54.36).

DOSAGE GUIDELINES

The selection of an appropriate dose of an antiinfective agent is based on the site of infection, identity and known or presumed antibiotic susceptibility of the infecting organism, dose-related drug toxicity, and patient's ability to eliminate the drug (see Chapter 19). In general, dose selections from the higher end of the dosage range are recommended for severe, life-threatening infections (e.g., sepsis, meningitis). Known organisms with intermediate susceptibility (i.e., high minimal inhibitory concentration [MIC]) also should prompt the use of higher doses. More recent data with various types of antiinfectives suggest that higher doses or use of extended infusions of certain agents may increase the probability of response against some resistant organisms. This may be true with agents that concentrate at the infection site and that concentrate in the phagocytes that clear the organisms, such as quinolones and macrolides or azalides.

The lowest doses are typically used for urinary tract infections or when the isolated pathogen is extremely susceptible to the antiinfective. A sizable range in dosage intervals exists for some antiinfective agents, with longer durations between doses appropriate for less severe infections in which a critical threshold level in serum or other site of infection (e.g., central nervous system) is not mandatory, or the drug concentrates significantly at the site of infection (e.g., urine, bile). Lower doses may be appropriate when the patient has impairment in the function of eliminating organs (but not in all instances).

In the past, knowledge regarding the optimal dosage of an antiinfective agent was limited at the time that the drug was approved by regulatory bodies. However, with use of modern PK and pharmacodynamic (PD) principles, this has changed in many instances. Limited optimal dosing information may still be true in "special populations" including pediatric, geriatric, or pregnant patients; those with liver and kidney disease; and those who are obese. Patients treated with antiinfectives may also concurrently be receiving other medications, minerals, herbal supplements, and foods that may be associated with drug-drug or drug-food interactions. These interactions and resultant untoward effects may not be apparent during drug development. In addition, the pharmacokinetics of some drugs that are metabolized by drug metabolizing enzymes (DMEs) or acted on by transporters, or both, may change as the burden of the infectious agent decreases, with resultant decreases in cytokine production and the downregulation of DMEs and transporters. Hence, dosage recommendations for all agents will change as research into them evolves. To prevent dosage errors, the recommendations discussed in this chapter should be compared with the most recent scientific literature, package labeling, and other current reference sources.

DOSAGE ADJUSTMENT FOR RENAL IMPAIRMENT

Drug half-lives in adults with impaired renal function and changes related to dialysis procedures (hemodialysis, peritoneal dialysis) are summarized for the user. However, renal replacement therapy now encompasses multiple potential modalities, such as (1) high-flux hemodialysis; (2) nocturnal hemodialysis; (3) slow–low-efficiency hemodialysis; and (4) continuous renal replacement therapy, among others. Specific dosage guidance may not be available for these various dialysis modalities, and determination of dosage will require an educated decision based on the pharmacology of the antiinfective. To date, even learned nephrology groups cannot provide useful guidelines for drug dosages in patients receiving renal replacement therapy. Our recommendation is to consider the toxicity of an agent compared with the ability to treat the infection. In many situations the risk-benefit ratio is low, and therefore more aggressive administration often should be used. An alternative to the elongation of the interval between doses is a reduction of the daily dose given at the usual dosing interval. Antiinfective serum concentrations should be determined and patient-specific dosage adjustments made on the basis of these determinations, when appropriate and available. In the absence of therapeutic drug monitoring, a surrogate biomarker of this impaired drug clearance mechanism is used. Serum creatinine is the most commonly used clinical biomarker for estimation of renal function and drug dosage adjustment in patients with chronic kidney disease. The production of creatinine is regulated by numerous factors, such as diet composition and amount, patient muscle mass, liver function, age, and sex, which can bias the value of this biomarker. The elimination of creatinine is regulated by renal function and involves both glomerular filtration and tubular secretion. Creatinine clearance (CrCl) equates to an estimate of both elimination processes when serum creatinine is used. The Cockcroft-Gault equation is the most common equation that has been used to define CrCl and to inform the dose adjustments in the drug labels approved by regulatory bodies. Newer equations to estimate the glomerular filtration rate (GFR) have been developed to estimate kidney function in patients with chronic kidney disease (<60 mL/min/1.73 m^2). Specific estimates of GFR may be automatically reported with the measurement of serum creatinine in the clinical setting by using the Modification of Diet in Renal Disease (MDRD) equation.

The estimated CrCl and GFR calculations derived from these equations may yield comparable results, but it is important to remember that these values may not be interchangeable. Accordingly, this is a source of major controversy when applied to drug dosage calculation. A fundamental point of consideration is that patients with serious infections are likely to be in a dynamic physiologic state that can influence both the production and the elimination of serum creatinine. Calculation of GFR and CrCl with the aforementioned equations serves only as a point estimate for a patient under conditions of physiologic stability. These equations are not reliable in patients with changing renal function (increase or decrease), those with malnutrition (overestimation), elderly patients (overestimation), those with low serum creatinine (overestimation), and those with moderate-to-severe liver disease (overestimation).

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TABLE 54.2 Generic and Trade Names

GENERIC NAME	TRADE NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Abacavir	Epzicom,[a] Triumeq,[a] Trizivir,[a] Ziagen	Antiretroviral	NRTI
Acyclovir	Zovirax	Antiviral	Nucleoside analogue
Adefovir dipivoxil	Hepsera	Antiviral	Nucleotide reverse-transcriptase inhibitor
Albendazole	Albenza	Anthelmintic	Benzimidazole
Amantadine hydrochloride	Symmetrel	Antiviral	Adamantane
Amikacin sulfate	Amikin	Antibacterial	Aminoglycoside
Aminosalicylic acid	Paser	Antimycobacterial	Aminosalicylic acid
Amoxicillin	Amoxil, Prevpac[a]	Antibacterial	Penicillin
Amoxicillin-clavulanate potassium	Augmentin[a]	Antibacterial	Penicillin–β-lactamase inhibitor
Amphotericin B deoxycholate	Fungizone	Antifungal	Polyene
Amphotericin B lipid complex	Abelcet	Antifungal	Polyene
Amphotericin B liposomal	AmBisome	Antifungal	Polyene
Ampicillin sodium	Polycillin-N	Antibacterial	Penicillin
Ampicillin trihydrate	Polycillin	Antibacterial	Penicillin
Ampicillin-sulbactam	Unasyn[a]	Antibacterial	Penicillin–β-lactamase inhibitor
Anidulafungin	Eraxis	Antifungal	Echinocandin
Artemether-lumefantrine	Coartem[a]	Antimalarial	Artemisinin-aryl aminoalcohol
Artesunate	Plasmotrim (CDC[b])	Antimalarial	Artemisinin
Atazanavir	Evotaz,[a] Reyataz	Antiretroviral	Protease inhibitor
Atovaquone	Mepron, Malarone[a]	Antiprotozoal	Naphthoquinone
Atovaquone-proguanil	Malarone[a]	Antimalarial	Naphthoquinone-antifolate
Azithromycin	Zithromax	Antibacterial	Azalide
Aztreonam	Azactam	Antibacterial	Monobactam
Bacitracin	Baci-IM	Antibacterial	Polypeptide
Bedaquiline	Sirturo	Antimycobacterial	Diarylquinoline
Benznidazole	—	Antiprotozoal	Nitroimidazole
Besifloxacin	Besivance	Antibacterial	Fluoroquinolone
Butoconazole nitrate	Femstat 3	Antifungal	Azole
Capreomycin sulfate	Capastat Sulfate	Antimycobacterial	Aminoglycoside
Caspofungin	Cancidas	Antifungal	Echinocandin
Cefaclor	Ceclor	Antibacterial	Cephalosporin
Cefadroxil	Duricef	Antibacterial	Cephalosporin
Cefazolin sodium	Ancef	Antibacterial	Cephalosporin
Cefdinir	Omnicef	Antibacterial	Cephalosporin
Cefditoren pivoxil	Spectracef	Antibacterial	Cephalosporin
Cefepime	Maxipime	Antibacterial	Cephalosporin
Cefixime	Suprax	Antibacterial	Cephalosporin
Cefotaxime sodium	Claforan	Antibacterial	Cephalosporin
Cefotetan disodium	Cefotan	Antibacterial	Cephalosporin
Cefoxitin sodium	Mefoxitin	Antibacterial	Cephalosporin
Cefpodoxime proxetil	Vantin	Antibacterial	Cephalosporin
Cefprozil	Cefzil	Antibacterial	Cephalosporin
Ceftaroline	Teflaro	Antibacterial	Cephalosporin
Ceftazidime	Fortaz	Antibacterial	Cephalosporin
Ceftazidime-avibactam	Avycaz[a]	Antibacterial	Cephalosporin–β-lactamase inhibitor
Ceftibuten	Cedax	Antibacterial	Cephalosporin
Ceftolozane-tazobactam	Zerbaxa[a]	Antibacterial	Cephalosporin–β-lactamase inhibitor
Ceftriaxone sodium	Rocephin	Antibacterial	Cephalosporin

Continued

TABLE 54.2 Generic and Trade Names—cont'd

GENERIC NAME	TRADE NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Cefuroxime axetil	Ceftin	Antibacterial	Cephalosporin
Cefuroxime sodium	Zinacef	Antibacterial	Cephalosporin
Cephalexin	Keflex	Antibacterial	Cephalosporin
Chloramphenicol sodium succinate	Chloromycetin	Antibacterial	Chloramphenicol
Chloroquine phosphate	Aralen	Antimalarial	Quinoline
Ciclopirox	Loprox	Antifungal	Miscellaneous antifungals
Cidofovir	Vistide	Antiviral	Nucleotide analogue
Ciprofloxacin hydrochloride	Cipro	Antibacterial	Fluoroquinolone
Ciprofloxacin lactate	Cipro	Antibacterial	Fluoroquinolone
Clarithromycin	Biaxin, Prevpac[a]	Antibacterial	Macrolide
Clindamycin hydrochloride	Cleocin HCl	Antibacterial	Lincosamide
Clindamycin palmitate hydrochloride	Cleocin	Antibacterial	Lincosamide
Clindamycin phosphate	Cleocin Phosphate	Antibacterial	Lincosamide
Clioquinol-hydrocortisone	Ala-Quin	Antifungal	Miscellaneous antifungal-corticosteroid
Clofazimine	Lamprene	Antimycobacterial	Miscellaneous antimycobacterial
Clotrimazole	Lotrimin	Antifungal	Azole
Cobicistat	Evotaz,[a] Genvoya,[a] Prezcobix,[a] Stribild,[a] Tybost	Antiretroviral	CYP3A inhibitor
Colistimethate sodium	Coly-Mycin M	Antibacterial	Polymyxin
Cycloserine	Seromycin	Antimycobacterial	Miscellaneous antimycobacterial
Daclatasvir	Daklinza	Antiviral (HCV)	NS5A inhibitor
Dalbavancin	Dalvance	Antibacterial	Lipoglycopeptide
Dapsone	—	Antibacterial	Sulfone
Daptomycin	Cubicin	Antibacterial	Lipopeptide
Darunavir	Prezcobix,[a] Prezista	Antiretroviral	Protease inhibitor
Delafloxacin	Baxdela	Antibacterial	Fluoroquinolone
Delavirdine	Rescriptor	Antiretroviral	NNRTI
Demeclocycline hydrochloride	Declomycin	Antibacterial	Tetracycline
Dicloxacillin sodium	Pathocil	Antibacterial	Penicillin
Didanosine	Videx	Antiretroviral	NRTI
Diethylcarbamazine	Hetrazan, DEC (CDC[b])	Anthelmintic	Miscellaneous anthelmintic
Dolutegravir	Tivicay, Triumeq[a]	Antiretroviral	INSTI
Doripenem	Doribax	Antibacterial	Carbapenem
Doxycycline calcium	Vibramycin Calcium	Antibacterial	Tetracycline
Doxycycline hyclate	Vibramycin Hyclate	Antibacterial	Tetracycline
Doxycycline monohydrate	Monodox, Vibramycin Monohydrate	Antibacterial	Tetracycline
Econazole nitrate	Ecoza, Spectazole	Antifungal	Azole
Efavirenz	Atripla,[a] Sustiva	Antiretroviral	NNRTI
Efinaconazole	Jublia	Antifungal	Azole
Eflornithine	Ornidyl, DFMO (CDC[b])	Antiprotozoal	Miscellaneous antiprotozoal
Elbasvir-grazoprevir	Zepatier[a]	Antiviral (HCV)	NS5A inhibitor-NS3/4A inhibitor
Elvitegravir	Genvoya,[a] Stribild[a]	Antiretroviral	INSTI
Emtricitabine	Atripla,[a] Complera,[a] Descovy,[a] Emtriva, Genvoya,[a] Odefsey,[a] Stribild,[a] Truvada[a]	Antiretroviral	NRTI
Enfuvirtide	Fuzeon	Antiretroviral	Fusion inhibitor
Entecavir	Baraclude	Antiviral	NRTI
Ertapenem	Invanz	Antibacterial	Carbapenem
Erythromycin	ERYC, PCE	Antibacterial	Macrolide
Erythromycin ethylsuccinate	E.E.S., EryPed	Antibacterial	Macrolide
Erythromycin lactobionate	Erythrocin	Antibacterial	Macrolide

TABLE 54.2 Generic and Trade Names—cont'd

GENERIC NAME	TRADE NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Erythromycin stearate	Erythrocin Stearate	Antibacterial	Macrolide
Ethambutol hydrochloride	Myambutol	Antimycobacterial	Miscellaneous antimycobacterial
Ethionamide	Trecator	Antimycobacterial	Miscellaneous antimycobacterial
Etravirine	Intelence	Antiretroviral	NNRTI
Famciclovir	Famvir	Antiviral	Nucleoside analogue
Fidaxomicin	Dificid	Antibacterial	Macrolide
Finafloxacin	Xtoro	Antibacterial	Fluoroquinolone
Fluconazole	Diflucan	Antifungal	Azole
Flucytosine	Ancobon	Antifungal	Miscellaneous antifungal
Fosamprenavir	Lexiva	Antiretroviral	Protease inhibitor
Foscarnet sodium	Foscavir	Antiviral	Miscellaneous antiviral
Fosfomycin tromethamine	Monurol	Antibacterial	Phosphonic acid derivative
Ganciclovir sodium	Cytovene	Antiviral	Nucleoside analogue
Gatifloxacin	Zymar	Antibacterial	Fluoroquinolone
Gemifloxacin	Factive	Antibacterial	Fluoroquinolone
Gentamicin sulfate	Garamycin	Antibacterial	Aminoglycoside
Glecaprevir-pibrentasvir	Mavyret[a]	Antiviral (HCV)	NS3/4A inhibitor–NS5A inhibitor
Gentian violet	—	Antifungal	Miscellaneous antifungal
Griseofulvin	Grisactin, Gris-PEG	Antifungal	Miscellaneous antifungal
Hydroxychloroquine sulfate	Plaquenil	Antimalarial	Quinoline
Imipenem-cilastatin	Primaxin[a]	Antibacterial	Carbapenem–dihydropeptidase inhibitor
Indinavir	Crixivan	Antiretroviral	Protease inhibitor
Iodoquinol	Aloquin	Antiprotozoal	Hydroxyquinoline
Isavuconazonium sulfate	Cresemba	Antifungal	Azole
Isoniazid	Rimifon, Rifamate,[a] Rifater[a]	Antimycobacterial	Miscellaneous antimycobacterial
Itraconazole	Sporanox	Antifungal	Azole
Ivermectin	Sklice, Soolantra, Stromectol	Anthelmintic	Avermectin
Kanamycin sulfate	Kantrex	Antibacterial	Aminoglycoside
Ketoconazole	Nizoral	Antifungal	Azole
Lamivudine	Combivir,[a] Epivir, Epivir-HBV, Epzicom,[a] Triumeq,[a] Trizivir[a]	Antiretroviral	NRTI
Levofloxacin	Levaquin	Antibacterial	Fluoroquinolone
Lincomycin hydrochloride	Lincocin	Antibacterial	Lincosamide
Linezolid	Zyvox	Antibacterial	Oxazolidinone
Lopinavir-ritonavir	Kaletra[a]	Antiretroviral	Protease inhibitor, CYP3A inhibitor
Luliconazole	Luzu	Antifungal	Azole
Mafenide	Sulfamylon	Antibacterial	Sulfonamide
Maraviroc	Selzentry	Antiretroviral	CCR5 receptor antagonist
Mebendazole	Vermox	Anthelmintic	Benzimidazole
Mefloquine	Lariam	Antimalarial	Miscellaneous antimalarial
Melarsoprol	Arsobal (CDC[b])	Antiprotozoal	Arsenical
Meropenem	Merrem	Antibacterial	Carbapenem
Meropenem-vaborbactam	Vabomere	Antibacterial	Carbapenem–β-lactamase inhibitor
Methenamine hippurate	Hiprex, Urex	Antibacterial	Urinary antiinfective
Methenamine mandelate	—	Antibacterial	Urinary antiinfective
Metronidazole	Flagyl, Pylera[a]	Antibacterial	Nitroimidazole
Micafungin	Mycamine	Antifungal	Echinocandin

Continued

TABLE 54.2 Generic and Trade Names—cont'd

GENERIC NAME	TRADE NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Miconazole	Monistat	Antifungal	Azole
Miltefosine	Impavido	Antiprotozoal	Alkylphosphocholine
Minocycline hydrochloride	Minocin	Antibacterial	Tetracycline
Moxifloxacin	Avelox	Antibacterial	Fluoroquinolone
Mupirocin	Bactroban	Antibacterial	Pseudomonic acid
Nafcillin sodium	Unipen	Antibacterial	Penicillin
Naftifine	Naftin	Antifungal	Allyamine
Natamycin	Natacyn	Antifungal	Polyene
Nelfinavir	Viracept	Antiretroviral	Protease inhibitor
Neomycin sulfate	Mycifradin, Neosporin[a]	Antibacterial	Aminoglycoside
Nevirapine	Viramune	Antiretroviral	NNRTI
Nifurtimox	Lampit (CDC[b])	Antiprotozoal	Nitrofuran
Nitazoxanide	Alinia	Antiprotozoal	Thiazolide
Nitrofurantoin	Furadantin, Macrobid, Macrodantin	Antibacterial	Nitrofuran
Nystatin	Mycostatin, Nilstat	Antifungal	Polyene
Ofloxacin	Floxin	Antibacterial	Fluoroquinolone
Ombitasvir-paritaprevir-ritonavir	Technivie[a]	Antiviral (HCV)	NS5A inhibitor–NS3/4A inhibitor–CYP3A inhibitor
Ombitasvir-paritaprevir-ritonavir-dasabuvir sodium	Viekira Pak,[a] Viekira XR[a]	Antiviral (HCV)	NS5A inhibitor–NS3/4A inhibitor–CYP3A inhibitor–NS5B inihibitor
Oritavancin diphosphate	Orbactiv	Antibacterial	Lipoglycopeptide
Oseltamivir phosphate	Tamiflu	Antiviral	Neuraminidase inhibitor
Oxacillin sodium	Bactocill	Antibacterial	Penicillin
Oxiconazole nitrate	Oxistat	Antifungal	Azole
Oxytetracycline hydrochloride	Terramycin	Antibacterial	Tetracycline
Paromomycin sulfate	Humatin	Antiprotozoal	Aminoglycoside
Penciclovir	Denavir	Antiviral	Nucleoside analogue
Penicillin G benzathine	Bicillin, Bicillin C-R,[a] Bicillin L-A	Antibacterial	Penicillin
Penicillin G potassium	Pfizerpen	Antibacterial	Penicillin
Penicillin G procaine	Bicillin C-R,[a] Pfizerpen-AS	Antibacterial	Penicillin
Penicillin G sodium	—	Antibacterial	Penicillin
Penicillin V potassium	Pfizerpen VK	Antibacterial	Penicillin
Pentamidine isethionate	NebuPent, Pentam	Antiprotozoal	Miscellaneous antiprotozoal
Peramivir	Rapivab	Antiviral	Neuraminidase inhibitor
Piperacillin-tazobactam	Zosyn[a]	Antibacterial	Penicillin–β-lactamase inhibitor
Pivmecillinam[c]	Selexid	Antibacterial	Penicillin
Polymyxin B sulfate	Aerosporin, Neosporin[a]	Antibacterial	Polymyxin
Posaconazole	Noxafil	Antifungal	Azole
Praziquantel	Biltricide	Anthelmintic	Miscellaneous antihelmintic
Primaquine phosphate	—	Antimalarial	Quinoline
Pyrantel pamoate	Pin-X, Reese's Pinworm Medicine	Anthelmintic	Miscellaneous anthelmintic
Pyrazinamide	Rifater[a]	Antimycobacterial	Miscellaneous antimycobacterial
Pyrimethamine	Daraprim	Antiprotozoal	Miscellaneous antiprotozoal
Quinine sulfate	Qualaquin	Antimalarial	Quinoline
Quinupristin-dalfopristin	Synercid[a]	Antibacterial	Streptogramins
Raltegravir	Isentress	Antiretroviral	INSTI
Retapamulin	Altabax	Antibacterial	Pleuromutilin
Ribavirin	Copegus, Rebetol, Virazole	Antiviral	Nucleoside analogue

TABLE 54.2 Generic and Trade Names—cont'd

GENERIC NAME	TRADE NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Rifabutin	Mycobutin	Antimycobacterial	Rifamycin
Rifampin	Rifadin, Rifamate,[a] Rifater[a]	Antimycobacterial	Rifamycin
Rifapentine	Priftin	Antimycobacterial	Rifamycin
Rifaximin	Xifaxan	Antibacterial	Rifamycin
Rilpivirine	Complera,[a] Edurant, Odefsey[a]	Antiretroviral	NNRTI
Rimantadine	Flumadine	Antiviral	Adamantane
Ritonavir	Kaletra,[a] Norvir, Technivie,[a] Viekira Pak,[a] Viekira XR[a]	Antiretroviral	Protease inhibitor–CYP3A inhibitor
Saquinavir	Invirase	Antiretroviral	Protease inhibitor
Secnidazole	Solosec	Antibacterial	Nitroimidazole
Sertaconazole	Ertaczo	Antifungal	Azole
Silver sulfadiazine	Silvadene	Antibacterial	Sulfonamide
Simeprevir	Olysio	Antiviral (HCV)	NS3/4A inhibitor
Sodium stibogluconate	Pentostam (CDC[b])	Antiprotozoal	Antimonial
Sodium thiosulfate	Versiclear	Antifungal	Miscellaneous antifungal
Sofosbuvir	Sovaldi	Antiviral (HCV)	NS5B inhibitor
Sofosbuvir-ledipasvir	Harvoni	Antiviral (HCV)	NS5B inhibitor–NS5A inhibitor
Sofosbuvir-velpatasvir	Epclusa[a]	Antiviral (HCV)	NS5B inhibitor–NS5A inhibitor
Sofosbuvir-velpatasvir-voxilaprevir	Vosevi	Antiviral (HCV)	NS5B inhibitor–NS5A inhibitor–NS3/4A inhibitor
Stavudine	Zerit	Antiretroviral	NRTI
Streptomycin sulfate	—	Antibacterial	Aminoglycoside
Sulconazole	Exelderm	Antifungal	Azole
Sulfacetamide	—	Antibacterial	Sulfonamide
Sulfadiazine	—	Antibacterial	Sulfonamide
Sulfanilamide	AVC	Antibacterial	Sulfonamide
Suramin	Germanin (CDC[b])	Antiprotozoal	Miscellaneous antiprotozoal
Tavaborole	Kerydin	Antifungal	Oxaborole
Tedizolid	Sivextro	Antibacterial	Oxazolidinone
Telavancin	Vibativ	Antibacterial	Lipoglycopeptide
Telbivudine	Tyzeka	Antiviral	Nucleoside analogue
Tenofovir alafenamide fumarate	Descovy,[a] Genvoya,[a] Odefsey,[a] Vemlidy	Antiretroviral	NRTI
Tenofovir disoproxil fumarate	Atripla,[a] Complera,[a] Stribild,[a] Truvada,[a] Viread	Antiretroviral	NRTI
Terbinafine	Lamisil	Antifungal	Allylamine
Terconazole	Terazol	Antifungal	Azole
Tetracycline hydrochloride	Achromycin, Pylera[a]	Antibacterial	Tetracycline
Tigecycline	Tygacil	Antibacterial	Glycylcycline
Tinidazole	Tindamax	Antiprotozoal	Nitroimidazole
Tioconazole	Vagistat-1	Antifungal	Azole
Tipranavir	Aptivus	Antiretroviral	Protease inhibitor
Tobramycin sulfate	Nebcin	Antibacterial	Aminoglycoside
Tobramycin inhalation solution	Tobi	Antibacterial	Aminoglycoside
Tolnaftate	Tinactin	Antifungal	Miscellaneous antifungal
Trifluridine	Viroptic	Antiviral	Nucleoside analogue
Trimethoprim	Bactrim,[a] Primsol, Septra[a]	Antibacterial	Antifolate
Trimethoprim-sulfamethoxazole	Bactrim,[a] Septra[a]	Antibacterial	Antifolate-sulfonamide
Undecylenic acid	Fungi-Nail	Antifungal	Miscellaneous antifungal
Valacyclovir	Valtrex	Antiviral	Nucleoside analogue

Continued

TABLE 54.2 Generic and Trade Names—cont'd

GENERIC NAME	TRADE NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Valganciclovir	Valcyte	Antiviral	Nucleoside analogue
Vancomycin hydrochloride	Vancocin	Antibacterial	Glycopeptide
Voriconazole	Vfend	Antifungal	Azole
Zanamivir	Relenza	Antiviral	Neuraminidase inhibitor
Zidovudine	Combivir,[a] Retrovir, Trizivir[a]	Antiretroviral	NRTI

[a]A combination product.
[b]US Centers for Disease Control and Prevention (CDC) Drug Service for distribution to US clinicians.
[c]Not available in the United States.
HBV, Hepatitis B virus; *HCV,* hepatitis C virus; *INSTI,* integrase strand transfer inhibitor; *NNRTI,* nonnucleoside reverse-transcriptase inhibitor; *NRTI,* nucleoside reverse-transcriptase inhibitor.

TABLE 54.3 Trade and Generic Names

TRADE NAME	GENERIC NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Abelcet	Amphotericin B lipid complex	Antifungal	Antifungal
Aerosporin	Polymyxin B sulfate	Antibacterial	Polymyxin
Ala-Quin[a]	Clioquinol-hydrocortisone	Antifungal	Miscellaneous antifungal-corticosteroid
Albenza	Albendazole	Anthelmintic	Benzimidazole
Alinia	Nitazoxanide	Antiprotozoal	Thiazolide
Aloquin	Iodoquinol	Antiprotozoal	Hydroxyquinoline
Altabax	Retapamulin	Antibacterial	Pleuromutilin
AmBisome	Amphotericin B liposomal	Antifungal	Polyene
Amikin	Amikacin sulfate	Antibacterial	Aminoglycoside
Amoxil	Amoxicillin	Antibacterial	Penicillin
Ancef	Cefazolin sodium	Antibacterial	Cephalosporin
Ancobon	Flucytosine	Antifungal	Miscellaneous antifungal
Aptivus	Tipranavir	Antiretroviral	Protease inhibitor
Aralen	Chloroquine phosphate	Antimalarial	Quinoline
Arsobal (CDC[b])	Melarsoprol	Antiprotozoal	Arsenical
Atripla[a]	Efavirenz–tenofovir disoproxil fumarate–emtricitabine	Antiretroviral	NNRT-NRTI-NRTI
Augmentin[a]	Amoxicillin-clavulanate potassium	Antibacterial	Penicillin–β-lactamase inhibitor
AVC	Sulfanilamide	Antibacterial	Sulfonamide
Avelox	Moxifloxacin	Antibacterial	Fluoroquinolone
Avycaz[a]	Ceftazidime-avibactam	Antibacterial	Cephalosporin–β-lactamase inhibitor
Azactam	Aztreonam	Antibacterial	Monobactam
Baci-IM	Bacitracin	Antibacterial	Polypeptide
Bactocill	Oxacillin sodium	Antibacterial	Penicillin
Bactrim[a]	Trimethoprim-sulfamethoxazole	Antibacterial	Antifolate-sulfonamide
Bactroban	Mupirocin	Antibacterial	Pseudomonic acid
Baraclude	Entecavir	Antiviral	NRTI
Baxdela	Delafloxacin	Antibacterial	Fluoroquinolone
Biaxin	Clarithromycin	Antibacterial	Macrolide
Bicillin C-R[a]	Penicillin G procaine–penicillin G benzathine	Antibacterial	Penicillin
Bicillin L-A	Penicillin G benzathine	Antibacterial	Penicillin
Biltricide	Praziquantel	Anthelmintic	Miscellaneous anthelmintic
Cancidas	Caspofungin	Antifungal	Echinocandin
Capastat Sulfate	Capreomycin sulfate	Antimycobacterial	Aminoglycoside
Ceclor	Cefaclor	Antibacterial	Cephalosporin

TABLE 54.3 Trade and Generic Names—cont'd

TRADE NAME	GENERIC NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Cedax	Ceftibuten	Antibacterial	Cephalosporin
Cefotan	Cefotetan disodium	Antibacterial	Cephalosporin
Ceftin	Cefuroxime axetil	Antibacterial	Cephalosporin
Cefzil	Cefprozil	Antibacterial	Cephalosporin
Chloromycetin	Chloramphenicol sodium succinate	Antibacterial	Chloramphenicol
Cipro	Ciprofloxacin hydrochloride (oral formulation), Ciprofloxacin lactate (IV formulation)	Antibacterial	Fluoroquinolone
Claforan	Cefotaxime sodium	Antibacterial	Cephalosporin
Cleocin	Clindamycin palmitate hydrochloride (pediatric formulation)	Antibacterial	Lincosamide
Cleocin HCl	Clindamycin hydrochloride	Antibacterial	Lincosamide
Cleocin Phosphate	Clindamycin phosphate	Antibacterial	Lincosamide
Coartem[a]	Artemether-lumefantrine	Antimalarial	Artemisinin-aryl aminoalcohol
Coly-Mycin M	Colistimethate sodium	Antibacterial	Polymyxin
Combivir[a]	Lamivudine-zidovudine	Antiretroviral	NRTI-NRTI
Complera[a]	Rilpivirine–tenofovir disoproxil fumarate–emtricitabine	Antiretroviral	NNRTI-NRTI-NRTI
Copegus	Ribavirin	Antiviral	Nucleoside analogue
Cresemba	Isavuconazonium sulfate	Antifungal	Azole
Crixivan	Indinavir	Antiretroviral	Protease inhibitor
Cubicin	Daptomycin	Antibacterial	Lipopeptide
Cytovene	Ganciclovir sodium	Antiviral	Nucleoside analogue
Daklinza	Daclatasvir	Antiviral (HCV)	NS5A inhibitor
Dalvance	Dalbavancin	Antibacterial	Lipoglycopeptide
Daraprim	Pyrimethamine	Antiprotozoal	Miscellaneous antiprotozoal
DEC (CDC[b])	Diethylcarbamazine	Anthelmintic	Miscellaneous anthelmintic
Declomycin	Demeclocycline hydrochloride	Antibacterial	Tetracycline
Denavir	Penciclovir	Antiviral	Nucleoside analogue
Denvar	Cefixime	Antibacterial	Cephalosporin
Descovy	Tenofovir alafenamide fumarate	Antiretroviral	NRTI
DFMO (CDC[b])	Eflornithine	Antiprotozoal	Miscellaneous antiprotozoal
Dificid	Fidaxomycin	Antibacterial	Macrolide
Diflucan	Fluconazole	Antifungal	Azole
Doribax	Doripenem	Antibacterial	Carbapenem
Duricef	Cefadroxil	Antibacterial	Cephalosporin
Ecoza	Econazole nitrate	Antifungal	Azole
Edurant	Rilpivirine	Antiretroviral	NNRTI
E.E.S.	Erythromycin ethylsuccinate	Antibacterial	Macrolide
Emtriva	Emtricitabine	Antiretroviral	NRTI
Epclusa[a]	Sofosbuvir-velpatasvir	Antiviral (HCV)	NS5B inhibitor–NS5A inhibitor
Epivir, Epivir-HBV	Lamivudine	Antiretroviral	NRTI
Epzicom[a]	Abacavir-lamivudine	Antiretroviral	NRTI-NRTI
Eraxis	Anidulafungin	Antifungal	Echinocandin
Ertaczo	Sertaconazole	Antifungal	Azole
ERYC	Erythromycin	Antibacterial	Macrolide
EryPed	Erythromycin ethylsuccinate	Antibacterial	Macrolide
Erythrocin	Erythromycin lactobionate	Antibacterial	Macrolide
Erythrocin Stearate	Erythromycin stearate	Antibacterial	Macrolide
Evotaz[a]	Atazanavir-cobicistat	Antiretroviral	Protease inhibitor–CYP3A inhibitor

Continued

TABLE 54.3 Trade and Generic Names—cont'd

TRADE NAME	GENERIC NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Exelderm	Sulconazole	Antifungal	Azole
Factive	Gemifloxacin	Antibacterial	Fluoroquinolone
Famvir	Famciclovir	Antiviral	Nucleoside analogue
Femstat 3	Butoconazole nitrate	Antifungal	Azole
Flagyl	Metronidazole	Antibacterial	Nitroimidazole
Floxin	Ofloxacin	Antibacterial	Fluoroquinolone
Flumadine	Rimantadine	Antiviral	Adamantane
Fortaz	Ceftazidime	Antibacterial	Cephalosporin
Foscavir	Foscarnet sodium	Antiviral	Miscellaneous antiviral
Fungi-Nail	Undecylenic acid	Antifungal	Miscellaneous antifungal
Fungizone	Amphotericin B	Antifungal	Polyene
Furadantin	Nitrofurantoin	Antibacterial	Nitrofuran
Fuzeon	Enfuvirtide	Antiretroviral	Fusion inhibitor
Garamycin	Gentamicin	Antibacterial	Aminoglycoside
Genvoya[a]	Elvitegravir–cobicistat–tenofovir alafenamide fumarate–emtricitabine	Antiretroviral	INSTI–CYP3A inhibitor–NRTI–NRTI
Germanin (CDC[b])	Suramin	Antiprotozoal	Miscellaneous antiprotozoal
Grisactin	Griseofulvin	Antifungal	Miscellaneous antifungal
Gris-PEG	Griseofulvin	Antifungal	Miscellaneous antifungal
Harvoni[a]	Sofosbuvir-ledipasvir	Antiviral (HCV)	HS5A inhibitor–NS5A inhibitor
Hepsera	Adefovir dipivoxil	Antiviral	Nucleotide reverse-transcriptase inhibitor
Hetrazan (CDC[b])	Diethylcarbamazine	Anthelmintic	Miscellaneous anthelmintic
Hiprex	Methenamine hippurate	Antibacterial	Urinary antiinfective
Humatin	Paromomycin sulfate	Antiprotozoal	Aminoglycoside
Impavido	Miltefosine	Antiprotozoal	Alkylphosphocholine
Intelence	Etravirine	Antiretroviral	NNRTI
Invanz	Ertapenem	Antibacterial	Carbapenem
Invirase	Saquinavir	Antiretroviral	Protease inhibitor
Isentress	Raltegravir	Antiretroviral	INSTI
Jublia	Efinaconazole	Antifungal	Azole
Kaletra[a]	Lopinavir-ritonavir	Antiretroviral	Protease inhibitor–CYP3A inhibitor
Kantrex	Kanamycin sulfate	Antibacterial	Aminoglycoside
Keflex	Cephalexin	Antibacterial	Cephalosporin
Kerydin	Tavaborole	Antifungal	Oxaborole
Lamisil	Terbinafine	Antifungal	Allylamine
Lampit (CDC[b])	Nifurtimox	Antiprotozoal	Nitrofuran
Lamprene	Clofazimine	Antimycobacterial	Miscellaneous antimycobacterial
Lariam	Mefloquine	Antimalarial	Miscellaneous antimalarial
Levaquin	Levofloxacin	Antibacterial	Fluoroquinolone
Lexiva	Fosamprenavir	Antiretroviral	Protease inhibitor
Lincocin	Lincomycin hydrochloride	Antibacterial	Lincosamide
Loprox	Ciclopirox	Antifungal	Miscellaneous antifungal
Lotrimin	Clotrimazole	Antifungal	Azole
Luzu	Luliconazole	Antifungal	Azole
Macrobid	Nitrofurantoin	Antibacterial	Nitrofuran
Macrodantin	Nitrofurantoin	Antibacterial	Nitrofuran
Malarone[a]	Atovaquone-proguanil	Antimalarial	Naphthoquinone-antifolate
Mavyret[a]	Glecaprevir-pibrentasvir	Antiviral (HCV)	NS3/4A inhibitor–NS5A inhibitor
Maxipime	Cefepime	Antibacterial	Cephalosporin

TABLE 54.3 Trade and Generic Names—cont'd

TRADE NAME	GENERIC NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Mefoxitin	Cefoxitin	Antibacterial	Cephalosporin
Mepron	Atovaquone	Antiprotozoal	Naphthoquinone
Merrem	Meropenem	Antibacterial	Carbapenem
Minocin	Minocycline hydrochloride	Antibacterial	Tetracycline
Monistat	Miconazole	Antifungal	Azole
Monodox	Doxycycline monohydrate	Antibacterial	Tetracycline
Monurol	Fosfomycin tromethamine	Antibacterial	Phosphonic acid derivative
Myambutol	Ethambutol hydrochloride	Antimycobacterial	Miscellaneous antimycobacterial
Mycamine	Micafungin	Antifungal	Echinocandin
Mycifradin	Neomycin sulfate	Antibacterial	Aminoglycoside
Mycobutin	Rifabutin	Antimycobacterial	Rifamycin
Mycostatin	Nystatin	Antifungal	Polyene
Naftin	Naftifine	Antifungal	Allyamine
Natacyn	Natamycin	Antifungal	Polyene
Nebcin	Tobramycin sulfate	Antibacterial	Aminoglycoside
NebuPent	Pentamidine isethionate	Antiprotozoal	Miscellaneous antiprotozoal
Neosporin[a]	Neomycin–polymyxin B		Aminoglycoside-polymyxin
Nilstat	Nystatin	Antifungal	Polyene
Nizoral	Ketoconazole	Antifungal	Azole
Norvir	Ritonavir	Antiretroviral	Protease inhibitor–CYP3A inhibitor
Noxafil	Posaconazole	Antifungal	Azole
Odefsey[a]	Rilpivirine–tenofovir alafenamide fumarate–emtricitabine	Antiretroviral	NNRTI-NRTI-NRTI
Olysio	Simeprevir	Antiviral (HCV)	NS3/4A inhibitor
Omnicef	Cefdinir	Antibacterial	Cephalosporin
Orbactiv	Oritavancin diphosphate	Antibacterial	Lipoglycopeptide
Ornidyl (CDC[b])	Eflornithine	Antiprotozoal	Miscellaneous antiprotozoal
Oxistat	Oxiconazole nitrate	Antifungal	Azole
Paser	Aminosalicylic acid	Antimycobacterial	Aminosalicylic acid
Pathocil	Dicloxacillin sodium	Antibacterial	Penicillin
PCE	Erythromycin	Antibacterial	Macrolide
Pentam	Pentamidine isethionate	Antiprotozoal	Miscellaneous antiprotozoal
Pentostam (CDC[b])	Sodium stibogluconate	Antiprotozoal	Antimonial
Pfizerpen	Penicillin G potassium	Antibacterial	Penicillin
Pfizerpen VK	Penicillin V potassium	Antibacterial	Penicillin
Pfizerpen-AS	Penicillin G procaine	Antibacterial	Penicillin
Pin-X	Pyrantel pamoate	Anthelmintic	Miscellaneous anthelmintic
Plaquenil	Hydroxychloroquine sulfate	Antimalarial	Quinoline
Plasmotrim (CDC[b])	Artesunate	Antimalarial	Artemisinin
Polycillin	Ampicillin trihydrate	Antibacterial	Penicillin
Polycillin-N	Ampicillin sodium	Antibacterial	Penicillin
Prevpac[a]	Amoxicillin-clarithromycin-lansoprazole	Antibacterial (*Helicobacter pylori*)	Penicillin–macrolide–proton pump inhibitor
Prezcobix[a]	Darunavir-cobicistat	Antiretroviral	Protease inhibitor–CYP3A inhibitor
Prezista	Darunavir	Antiretroviral	Protease inhibitor
Priftin	Rifapentine	Antimycobacterial	Rifamycin
Primaxin[a]	Imipenem-cilastatin	Antibacterial	Carbapenem–dihydropeptidase inhibitor
Primsol	Trimethoprim	Antibacterial	Antifolate
Pylera[a]	Bismuth subcitrate–metronidazole–tetracycline	Antibacterial (*H. pylori*)	Antidiarrheal-nitroimidazole-tetracycline
Qualaquin	Quinine sulfate	Antimalarial	Quinoline

Continued

TABLE 54.3 Trade and Generic Names—cont'd

TRADE NAME	GENERIC NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Rapivab	Peramivir	Antiviral	Neuraminidase inhibitor
Rebetol	Ribavirin	Antiviral	Nucleoside analogue
Reese's Pinworm Medicine	Pyrantel pamoate	Anthelmintic	Miscellaneous anthelmintic
Relenza	Zanamivir	Antiviral	Neuraminidase inhibitor
Rescriptor	Delavirdine	Antiretroviral	NNRTI
Retrovir	Zidovudine	Antiretroviral	NRTI
Reyataz	Atazanavir	Antiretroviral	Protease inhibitor
Rifadin	Rifampin	Antimycobacterial	Rifamycin
Rifamate[a]	Rifampin-isoniazid	Antimycobacterial	Rifamycin–miscellaneous antimycobacterial
Rifater[a]	Rifampin-isoniazid-pyrazinamide	Antimycobacterial	Rifamycin–miscellaneous antimycobacterial
Rimifon	Isoniazid	Antimycobacterial	Miscellaneous antimycobacterial
Rocephin	Ceftriaxone sodium	Antibacterial	Cephalosporin
Selexid[c]	Pivmecillinam	Antibacterial	Penicillin
Selzentry	Maraviroc	Antiretroviral	CCR5 receptor antagonist
Septra[a]	Trimethoprim-sulfamethoxazole	Antibacterial	Antifolate-sulfonamide
Seromycin	Cycloserine	Antimycobacterial	Miscellaneous antimycobacterial
Silvadene	Silver sulfadiazine	Antibacterial	Sulfonamide
Sirturo	Bedaquiline	Antimycobacterial	Diarylquinoline
Sivextro	Tedizolid	Antibacterial	Oxazolidinone
Sklice	Ivermectin	Anthelmintic	Avermectin
Solosec	Secnidazole	Antibacterial	Nitroimidazole
Soolantra	Ivermectin	Anthelmintic	Avermectin
Sovaldi	Sofosbuvir	Antiviral (HCV)	NS5B inhibitor
Spectazole	Econazole nitrate	Antifungal	Azole
Spectracef	Cefditoren pivoxil	Antibacterial	Cephalosporin
Sporanox	Itraconazole	Antifungal	Azole
Stribild[a]	Elvitegravir–cobicistat–emtricitabine–tenofovir disoproxil fumarate	Antiretroviral	INSTI–CYP3A inhibitor–NRTI-NRTI
Stromectol	Ivermectin	Anthelmintic	Avermectin
Sulfamylon	Mafenide	Antibacterial	Sulfonamide
Suprax	Cefixime	Antibacterial	Cephalosporin
Sustiva	Efavirenz	Antiretroviral	NNRTI
Symmetrel	Amantadine hydrochloride	Antiviral	Adamantane
Synercid[a]	Quinupristin-dalfopristin	Antibacterial	Streptogramins
Tamiflu	Oseltamivir	Antiviral	Neuraminidase inhibitor
Technivie[a]	Ombitasvir-paritaprevir-ritonavir	Antiviral (HCV)	NS5A inhibitor–NS3/4A inhibitor–CYP3A inhibitor
Teflaro	Ceftaroline	Antibacterial	Cephalosporin
Terazol	Terconazole	Antifungal	Azole
Tindamax	Tinidazole	Antiprotozoal	Nitroimidazole
Tivicay	Dolutegravir	Antiretroviral	INSTI
Tobi	Tobramycin inhalation solution	Antibacterial	Aminoglycoside
Trecator	Ethionamide	Antimycobacterial	Miscellaneous antimycobacterial
Triumeq[a]	Dolutegravir-abacavir-lamivudine	Antiretroviral	INST-NRTI-NRTI
Trizivir[a]	Abacavir-lamivudine-zidovudine	Antiretroviral	NRTI-NRTI-NRTI
Truvada[a]	Emtricitabine–tenofovir disoproxil fumarate	Antiretroviral	NRTI
Tybost	Cobicistat	Antiretroviral	CYP3A inhibitor
Tygacil	Tigecycline	Antibacterial	Glycylcycline
Tyzeka	Telbivudine	Antiviral	Nucleoside analogue
Unasyn[a]	Ampicillin-sulbactam	Antibacterial	Penicillin–β-lactamase inhibitor

TABLE 54.3 Trade and Generic Names—cont'd

TRADE NAME	GENERIC NAME	PATHOGEN CLASS	PHARMACOLOGIC CLASS
Unipen	Nafcillin sodium	Antibacterial	Penicillin
Urex	Methenamine hippurate	Antibacterial	Urinary antiinfective
Vabomere[a]	Meropenem-vaborbactam	Antibacterial	Carbapenem–β-lactamase inhibitor
Valcyte	Valganciclovir	Antiviral	Nucleoside analogue
Valtrex	Valacyclovir	Antiviral	Nucleoside analogue
Vancocin	Vancomycin hydrochloride	Antibacterial	Glycopeptide
Vantin	Cefpodoxime proxetil	Antibacterial	Cephalosporin
Vemlidy	Tenofovir alafenamide fumarate	Antiretroviral	NRTI
Vermox	Mebendazole	Anthelmintic	Benzimidazole
Versiclear	Sodium thiosulfate	Antifungal	Miscellaneous antifungal
Vfend	Voriconazole	Antifungal	Azole
Vibativ	Televancin	Antibacterial	Lipoglycopeptide
Vibramycin Calcium	Doxycycline calcium	Antibacterial	Tetracycline
Vibramycin Hyclate	Doxycycline hyclate	Antibacterial	Tetracycline
Vibramycin Monohydrate	Doxycycline monohydrate	Antibacterial	Tetracycline
Videx	Didanosine	Antiretroviral	NRTI
Viekira Pak,[a] Viekira XR[a]	Ombitasvir–paritaprevir-ritonavir–dasabuvir sodium	Antiviral (HCV)	NS5A inhibitor–NS3/4A inhibitor–NS5B inhibitor–CYP3A inhibitor
Viracept	Nelfinavir	Antiretroviral	Protease inhibitor
Viramune	Nevirapine	Antiretroviral	NNRTI
Virazole	Ribavirin (inhalation)	Antiviral	Nucleoside analogue
Viread	Tenofovir disoproxil fumarate	Antiretroviral	NRTI
Viroptic	Trifluridine	Antiviral	Nucleoside analogue
Vistide	Cidofovir	Antiviral	Nuceoside analogue
Vosevi[a]	Sofosbuvir-velpatasvir-voxilaprevir	Antiviral (HCV)	NS5B inhibitor–NS5A inhibitor–NS3/4A inhibitor
Xifaxan	Rifaximin	Antibacterial	Rifamycin
Xtoro	Finafloxacin	Antibacterial	Fluoroquinolone
Zepatier[a]	Elbasvir-grazoprevir	Antiviral (HCV)	NS5A inhibitor–NS3/4A inhibitor
Zerbaxa[a]	Ceftolozane-tazobactam	Antibacterial	Cephalosporin–β-lactamase inhibitor
Zerit	Stavudine	Antiretroviral	NRTI
Ziagen	Abacavir	Antiretroviral	NRTI
Zinacef	Cefuroxime sodium	Antibacterial	Cephalosporin
Zithromax	Azithromycin	Antibacterial	Azalide
Zosyn[a]	Piperacillin-tazobactam	Antibacterial	Penicillin–β-lactamase inhibitor
Zovirax	Acyclovir	Antiviral	Nucleoside analogue
Zymar	Gatifloxacin	Antibacterial	Fluoroquinolone
Zyvox	Linezolid	Antibacterial	Oxazolidinone

[a]A combination product.
[b]US Centers for Disease Control and Prevention (CDC) Drug Service for distribution to US clinicians.
[c]Not available in the United States.
HBV, Hepatitis B virus; *HCV,* hepatitis C virus; *INSTI,* integrase strand transfer inhibitor; *NNRTI,* nonnucleoside reverse-transcriptase inhibitor; *NRTI,* nucleoside reverse-transcriptase inhibitor.

TABLE 54.4 Penicillin Dosage Forms

	FORMULATIONS		
	ORAL		
GENERIC NAME	Tablets/Capsules (mg)	Liquid (mg/mL)	PARENTERAL (g)
Amoxicillin	125,[a] 250,[a] 250, 500, 775,[b] 875	125/5, 200/5, 250/5, 400/5	0.25[c], 0.5[c], 1[c]
Amoxicillin-clavulanate	200-28.5,[a] 400-57,[a] 250-125, 500-125, 875-125, 1000-62.5[b]	125-31.25/5, 200-28.5/5, 250-62.5/5, 400-57/5, 600-42.9/5	0.5–0.1[c], 1–0.2[c]
Ampicillin	250, 500	125/5, 250/5	0.125, 0.25, 0.5, 1, 2, 10
Ampicillin-sulbactam[d]			1.5, 3, 15
Dicloxacillin	250, 500		
Nafcillin			1, 2, 10
Oxacillin			1, 2, 10
Penicillin G sodium[e]			1 MU, 2 MU, 3 MU, 5 MU, 20 MU
Penicillin G potassium[e]			1 MU, 2 MU, 3 MU, 5 MU, 20 MU
Penicillin G benzathine			0.6 MU, 1.2 MU, 2.4 MU
Penicillin G procaine			0.6 MU
Penicillin G benzathine–penicillin G procaine			0.6 MU–0.6 MU, 0.9 MU–0.3 MU
Penicillin V potassium	250, 500	125/5, 250/5	
Piperacillin-tazobactam[d]			2.25, 3.375, 4.5, 13.5, 40.5
Pivmecillinam[c]	200		

[a]Chewable.
[b]Extended release.
[c]Not available in the United States.
[d]Dose expressed as the sum of the penicillin and β-lactamase inhibitor
[e]400,000 IU = 0.4 MU = 250 mg.

TABLE 54.5 Cephalosporin Dosage Forms

	FORMULATIONS		
	ORAL		
GENERIC NAME	Tablets/Capsules (mg)	Liquid (mg/mL)	PARENTERAL (g)
First Generation			
Cefadroxil	500, 1000	250/5, 500/5	
Cefazolin			0.5, 1, 2, 10, 20, 100, 300
Cephalexin	250, 333, 500, 750	125/5, 250/5	
Second Generation			
Cefaclor	250, 500, 500[a]	125/5, 250/5, 375/5	
Cefotetan			1, 2, 10
Cefoxitin			1, 2, 10
Cefprozil	250, 500	125/5, 250/5	
Cefuroxime	250, 500	125/5, 250/5	0.75, 1.5, 7.5
Third Generation			
Cefdinir	300	125/5, 250/5	
Cefditoren pivoxil	200, 400		
Cefixime	100,[b] 200,[b] 400	100/5, 200/5, 500/5	
Cefotaxime			0.5, 1, 2, 10
Cefpodoxime proxetil	100, 200	50/5, 100/5	
Ceftazidime			0.5, 1, 2, 6, 100
Ceftibuten	400	180/5	
Ceftriaxone			0.25, 0.5, 1, 2, 10, 100
Fourth Generation			
Cefepime			1, 2
Fifth Generation			
Ceftaroline fosamil			0.4, 0.6
Cephalosporin, β-Lactamase Inhibitor Combinations			
Ceftazidime-avibactam			2-0.5
Ceftolozane-tazobactam			1.5[c]

[a]Extended release.
[b]Chewable.
[c]Dose expressed as the sum of the cephalosporin and β-lactamase inhibitor.

TABLE 54.6 Carbapenem, Monobactam, and Polymyxin Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	FORMULATIONS: ORAL Liquid (mg/mL)	FORMULATIONS: PARENTERAL (g)
Monobactams			
Aztreonam		75/1[a]	1, 2
Carbapenems			
Doripenem			0.25, 0.5
Ertapenem			1
Imipenem-cilastatin			0.25-0.25, 0.5-0.5
Meropenem			0.5, 1
Meropenem-vaborbactam			2[b]
Polymyxins			
Colistimethate			0.15[c]
Polymyxin B			500,000 IU[d]

[a]For inhalation.
[b]Dose expressed as the sum of the carbapenem and β-lactamase inhibitor.
[c]Dose expressed in units of colistin base activity.
[d]500,000 IU = 0.5 MIU = 50 mg.

TABLE 54.7 Aminoglycoside Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	FORMULATIONS: ORAL Liquid (mg/mL)	FORMULATIONS: PARENTERAL (g)
Amikacin			0.5, 1
Gentamicin			0.02, 0.06, 0.08, 0.1, 0.12, 0.8
Kanamycin			0.5, 1
Neomycin	500		
Streptomycin			1
Tobramycin	28[a]	300[a]	0.02, 0.08, 1.2, 2

[a]For inhalation.

TABLE 54.8 Tetracycline, Glycylcycline, and Folate Antagonist Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	FORMULATIONS: ORAL Liquid (mg/mL)	FORMULATIONS: PARENTERAL (g)
Tetracyclines and Glycylcyclines			
Demeclocycline	150, 300		
Doxycycline	20, 40,[a] 50, 50,[a] 75, 75,[a] 100, 100,[a] 120,[a] 150, 150,[a] 200[a]	25/5, 50/5	0.1
Minocycline	45,[b] 50, 55,[b] 65,[b] 75, 80,[b] 90,[b] 100, 105,[b] 115,[b] 135[b]		0.1
Tetracycline	250, 500		
Glycylcyclines			
Tigecycline			0.05
Folate Antagonists			
Trimethoprim-sulfamethoxazole	80-400, 160-800	40-200/5	0.080-0.400, 0.160-0.800, 0.480-2.400
Trimethoprim	100	50/5	
Sulfadiazine	500		
Dapsone	25, 100		

[a]Delayed release.
[b]Extended release.

TABLE 54.9 Macrolide, Azalide, Lincosamide, and Miscellaneous Antibacterial Agent Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	ORAL Liquid (mg/mL)	PARENTERAL (g)
Macrolides and Azalides			
Azithromycin	250, 500, 600, 1000	100/5, 200/5, 2000/60[a]	0.5
Clarithromycin	250, 500, 500[a]	125/5, 250/5	
Erythromycin base	250, 250,[b] 500, 500[b]		
Erythromycin stearate	250		
Erythromycin ethylsuccinate	400	200/5, 400/5	
Erythromycin lactobionate			0.5
Fidaxomicin	200		
Lincosamides			
Clindamycin	75, 100,[c] 150, 300	75/5	0.3, 0.6, 0.9, 9
Lincomycin			0.6, 3
Miscellaneous Antibacterial Agents			
Chloramphenicol			1
Metronidazole	250, 375, 500, 750[a]	250/5, 500/5	0.5
Rifaximin	200, 550		
Secnidazole	2000[d]		

[a]Extended release.
[b]Delayed release.
[c]Vaginal suppository.
[d]Oral granules.

TABLE 54.10 Glycopeptide, Lipopeptide, Lipoglycopeptide, Polypeptide, Oxazolidinone, and Streptogramin Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	ORAL Liquid (mg/mL)	PARENTERAL (g)
Bacitracin			50,000 IU[a]
Dalbavancin			0.5
Daptomycin			0.5
Linezolid	600	100/5	0.2, 0.6
Oritavancin			0.4
Quinupristin-dalfopristin			0.15-0.35
Tedizolid	200		0.2
Telavancin			0.75
Vancomycin	125, 250	125/5, 250/5	0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 5, 10

[a]For intramuscular injection.

TABLE 54.11 Fluoroquinolone and Urinary Antiinfective Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	ORAL Liquid (mg/mL)	PARENTERAL (g)
Fluoroquinolones			
Ciprofloxacin	100, 250, 500, 500,[a] 750, 1000[a]	250/5, 500/5	0.2, 0.4
Delafloxacin	450		0.3
Gemifloxacin	320		
Levofloxacin	250, 500, 750	250/5	0.25, 0.5, 0.75, 2.5, 5
Moxifloxacin	400		0.4
Ofloxacin	300, 400		
Urinary Antiinfectives			
Fosfomycin	3000		2,[b] 4,[b] 8[b]
Methenamine[c]	500, 1000		
Nitrofurantoin	25, 50, 100	25/5	

[a]Extended release.
[b]Not available in the United States.
[c]Available as both mandelate and hippurate salt forms.

TABLE 54.12 Antimycobacterial Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	ORAL Liquid (mg/mL)	PARENTERAL (g)
Aminosalicylic acid	4000		
Bedaquiline	100		
Capreomycin			1
Clofazimine	50, 100		
Cycloserine	250		
Ethambutol	100, 400		
Ethionamide	250		
Isoniazid	100, 300	50/5	0.1
Pyrazinamide	500		
Rifabutin	150		
Rifampin	150, 300	125/5	0.6
Rifapentine	150		
Streptomycin			1

TABLE 54.13 Antifungal Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	FORMULATIONS: ORAL Liquid (mg/mL)	FORMULATIONS: PARENTERAL (g)
Polyenes			
Amphotericin B deoxycholate			0.05
Amphotericin B lipid complex			0.1
Amphotericin B liposomal			0.05
Nystatin	100,000 IU,[a] 500,000 IU, 1,000,000 IU	500,000 IU/5	
Echinocandins			
Anidulafungin			0.05, 0.1
Caspofungin			0.05, 0.07
Micafungin			0.05, 0.1
Azoles			
Clotrimazole	10[b]		
Fluconazole	50, 100, 150, 200	50/5, 200/5	0.1, 0.2, 0.4
Isavuconazonium	186		372
Itraconazole	100, 200	50/5	
Ketoconazole	200		
Miconazole	50,[b] 100,[a] 200[a]		0.01
Posaconazole	100	200/5	0.3
Terconazole	80[a]		
Voriconazole	50, 200	200/5	0.2
Allylamines			
Terbinafine	250		
Miscellaneous Antifungals			
Flucytosine	200, 250, 500		2.5
Griseofulvin	125, 250, 500	125/5	

[a]Vaginal tablet, ovule, suppository form.
[b]Oral lozenge, troche or buccal tablet.

TABLE 54.14 Antiparasitic Dosage Forms

GENERIC NAME	FORMULATIONS: ORAL Tablets/Capsules (mg)	FORMULATIONS: ORAL Liquid (mg/mL)	FORMULATIONS: PARENTERAL (g)
Anthelmintics			
Albendazole	200		
Diethylcarbamazine[b]	100		
Ivermectin	3		
Mebendazole	100[a]		
Praziquantel	600		
Pyrantel pamoate	62.5	250/5	
Antimalarials			
Artemether-lumefantrine	20-120		
Artesunate[b]			0.11
Atovaquone-proguanil	62.5-25, 250-100		
Chloroquine phosphate[c]	150, 300		

Continued

TABLE 54.14 Antiparasitic Dosage Forms—cont'd

GENERIC NAME	FORMULATIONS		
	ORAL		
	Tablets/Capsules (mg)	Liquid (mg/mL)	PARENTERAL (g)
Hydroxychloroquine sulfate	200		
Mefloquine	250		
Primaquine phosphate	26.3		
Proguanil	100		
Quinine sulfate	324		
Antiprotozoals			
Atovaquone		750/5	
Benznidazole	12.5,[d] 100		
Eflornithine[b]			20
Iodoquinol	210, 650		
Melarsoprol[b]			0.180
Miltefosine	50		
Nifurtimox[b]	120		
Nitazoxanide	500	100/5	
Paromomycin sulfate	250		
Pentamidine isethionate		300/1[e]	0.3
Pyrimethamine	25		
Sodium stibogluconate[b]			10[f]
Suramin[b]			1
Tinidazole	250, 500		

[a]Chewable.
[b]Must be obtained from the Centers for Disease Control and Prevention in the United States.
[c]Dose expressed as chloroquine base.
[d]Dispersible tablet for pediatric use.
[e]For inhalation.
[f]Dose expressed as grams of pentavalent antimony per bottle.

TABLE 54.15 Antiviral Dosage Forms

GENERIC NAME	FORMULATIONS		
	ORAL		
	Tablets/Capsules (mg)	Liquid (mg/mL)	PARENTERAL (g)
Acyclovir	200, 400, 800	200/5	0.5, 1
Adefovir	10		
Amantadine	100	50/5	
Cidofovir			0.375
Entecavir	0.5, 1	0.25/5	
Famciclovir	125, 250, 500		
Foscarnet			6
Ganciclovir			0.5
Oseltamivir	30, 45, 75	30/5, 60/5	
Peramivir			0.2
Ribavirin	200, 400, 600	200/5, 6000/1[b]	
Rimantadine	100		
Telbivudine	600		
Valacyclovir	500, 1000		
Valganciclovir	450	250/5	
Zanamivir	5[b]		

[a]Not including antivirals active against hepatitis C virus or antiretrovirals. For information on available dosage forms for these antivirals, refer to Tables 54.16 and 54.17.
[b]For inhalation.

TABLE 54.16 Hepatitis C Direct-Acting Antiviral Dosage Forms

GENERIC NAME	FORMULATIONS		
	ORAL		
	Tablets/Capsules (mg)	Liquid (mg/mL)	PARENTERAL (g)
Daclatasvir	30, 60, 90		
Elbasvir-grazoprevir	50-100		
Glecaprevir-pibrentasvir	100-40		
Ombitasvir-paritaprevir-ritonavir	12.5-75-50		
Ombitasvir-paritaprevir-ritonavir-dasabuvir	8.33-50-33.33-200,[a] 12.5-75-50-250[b]		
Simeprevir	150		
Sofosbuvir	400		
Sofosbuvir-ledipasvir	400-90		
Sofosbuvir-velpatasvir	400-100		
Sofosbuvir-velpatasvir-voxilaprevir	400-100-100		

[a]Extended release.
[b]Tablet therapy pack.

TABLE 54.17 Antiretroviral Dosage Forms

GENERIC NAME	FORMULATIONS		
	ORAL		
	Tablets/Capsules (mg)	Liquid (mg/mL)	PARENTERAL (g)
Abacavir	300	20/1	
Abacavir-lamivudine	600-300		
Abacavir-lamivudine-zidovudine	300-150-300		
Atazanavir	50,[a] 150, 200, 300		
Atazanavir-cobicistat	300-150		
Cobicistat	150		
Darunavir	75, 150, 600, 800	100/1	
Darunavir-cobicistat	800-150		
Delavirdine	100, 200		
Didanosine	125,[b] 200,[b] 250,[b] 400[b]	2000, 4000	
Dolutegravir	50, 25, 50		
Dolutegravir-abacavir-lamivudine	50-600-300		
Efavirenz	50, 200, 600		
Efavirenz–emtricitabine–tenofovir disoproxil fumarate	600-200-300		
Elvitegravir–cobicistat–emtricitabine–tenofovir alafenamide fumarate	150-150-200-10		
Elvitegravir–cobicistat–emtricitabine–tenofovir disoproxil fumarate	150-150-200-300		
Emtricitabine	200	10/1	
Emtricitabine–tenofovir alafenamide fumarate	200-25		
Emtricitabine-tenofovir disoproxil fumarate	100-150, 133-200, 167-250, 200-300		
Enfuvirtide			0.09
Etravirine	25, 100, 200		
Fosamprenavir	700	50/1	
Indinavir	200, 400		
Lamivudine	100, 150, 300	5/1, 10/1	
Lamivudine-zidovudine	150-300		
Lopinavir-ritonavir	100-25, 200-50	80-20/1	
Maraviroc	25, 75, 150, 300	20/1	
Nelfinavir	250, 625		
Nevirapine	100,[c] 200, 400[c]	50/5	
Raltegravir	25,[d] 100,[d] 100,[a] 400, 600		
Rilpivirine	25		
Rilpivirine-emtricitabine-tenofovir alafenamide fumarate	25-200-25		
Rilpivirine-emtricitabine-tenofovir disoproxil fumarate	25-200-300		
Ritonavir	100	80/1	
Saquinavir	200, 500		
Stavudine	15, 20, 30, 40	1/1	
Tenofovir alafenamide fumarate	25		
Tenofovir disoproxil fumarate	40,[a] 150, 200, 250, 300		
Tipranavir	250	100/1	
Zidovudine	100, 300	50/5	0.2

[a]Oral powder.
[b]Delayed release.
[c]Extended release.
[d]Chewable.

TABLE 54.18 Antiinfective Agent Pharmacology: Penicillins

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral[p]	Parenteral[p]	Up to 1 wk	1–4 wk	>80	<10
Amoxicillin[b] (74–92)	0.25 PO[c]	3.5–5		0.25–2 q8–12h		4	20–90 mg/kg/day in 3 doses				0.7–1.4	7.4–21
	0.5 PO[c]	5.5–11										
Amoxicillin-clavulanate[f]	0.25 PO	3.7–4.8[g]	381[g]	0.25–2 q8–12h		4	20–90 mg/kg/day in 3 doses[h]				1.1–1.3	7.5
	0.5 PO 0.875 PO	6–9.7 11.6										
Ampicillin[b] (30–55)	0.25 PO[c]	1.8–2.9		0.25–1 q6h	0.5–2 q4–6h	4 PO, 12 IV	50–100 mg/kg/day in 3–4 doses	100–300 mg/kg/day in 3–4 doses[j]	100–300 mg/kg/day in 2–3 doses	100–300 mg/kg/day in 3–4 doses	0.7–1.4	7.4–21
	0.5 PO[c] 2 IV	3–6 47.6										
Ampicillin-sulbactam	1.5 IV	40–71[g]			1.5–3 q6h	12		100–200 mg/kg/day in 3–4 doses[f,p]			1	9
	3 IV	109–150[g]										
Dicloxacillin[b] (35–76)	0.5 PO	10–18		0.125–0.5 q6h		2	12.5–50 mg/kg/day in 4 doses				0.6–0.8	1–2.2
Nafcillin[b,l] (36)	1 IM	7.6			0.5–2 q4–6h	12 IV		100–200 mg/kg/day in 4 doses	50–75 mg/kg/day in 2–3 doses[m]	75–150 mg/kg/day in 3–4 doses[n]	0.5–1.5	1.8–2.8
	0.5 IV[e]	30										
Oxacillin[b,l] (30–35)	0.25 PO	1.65			0.5–2 q4–6h	12 IV		100–200 mg/kg/day in 4 doses	50–75 mg/kg/day in 2–3 doses[m]	75–150 mg/kg/day in 3–4 doses[n]	0.3–0.8	0.5–2
	0.5 PO 0.5 IV q6h[i]	2.6–3.9 52–63										
Penicillin G[b,l] (15–30)	1 MU	25			1–4 MU q4–6h	24 MU IV		50,000-500,000 U/kg/day in 4–6h doses[p]	50,000–450,000 U/kg/day q8–12h[m]	75,000–500,000 U/kg/day q6–8h[n]	0.4–0.9	6–20
	5 MU	400										
Penicillin G benzathine	1.2 MU IM	0.15 U/mL			0.6–2.4 MU IM × 1	2.4 MU		0.6 MU IM × 1		50,000 U/kg IM × 1	Days	
Penicillin G procaine	0.6 MU IM	1.6			0.6–1.2 MU IM	4.8 MU		50,000 MU/kg/day IM in 1 dose		50,000 U/kg/day IM in 1 dose	24	
	1.2 MU IM	1.95										
Penicillin V potassium[b] (60–73)	0.25 PO	2.3–2.7		0.25-0.5 q6h		2	25–75 mg/kg/day in 3–4 doses				0.5	7–10
	0.5 PO	4.9–6.3										
Piperacillin-tazobactam[l]	3.375 IV[q]	209[g]			3.375–4.5 q6–8h	18		200 mg/kg/day in 3–4 doses[f,p]	100 mg/kg q12h[f]	100 mg/kg q8h[f]	0.7–1.1	1.9–3.5
	4.5 IV[q]	224[g]										

[a]Inflamed meninges.
[b]Decreased rate and/or extent of absorption when given with food.
[c]Fasting.
[e]Specified dose should be administered in addition to scheduled dosing after a dialysis session to supplement drug clearance during dialysis.
[f]Dose based on penicillin component only.
[g]Penicillin component only.
[h]Children <40 kg should not receive the 250-mg film-coated tablet.
[i]Children <40–50 kg.
[j]16.7–33.3 mg/kg q4h for meningitis.
[l]Administration by extended (3–4 h) or continuous infusion (23–24 h) should be strongly considered to optimize efficacy. This is especially important in patients who are critically ill, immunosuppressed, or with deep-seated infections due to resistant organisms.
[m]q8h if >2 kg; q12h if <2 kg.
[n]q6h if >2 kg; q8h if <2 kg.
[q]IV push (over 2–10 min).
[p]Dosage should not exceed adult dosage.
[q]Infusion over 15–30 min.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; *PD,* peritoneal dialysis.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT													
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD	PD	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
		0.25–2	8–12	8–12	0.25–0.5 q12h	0.25–0.5 q24h	0.25–0.5 q24h, with 0.25–0.5[e] after HD	0.25 q12h	5–10	25–33	5	100–3000	Negligible
		0.25–2	8–12	8–12	0.25–0.5 q12h	0.25–0.5 q24h	0.25–0.5 q24h, with 0.25–0.5[e] after HD	0.25 q12h	6		Low	1100	
		0.5–2 IV	4–6	4–6	8–12	12–24	0.5–2 q12–24h, with 0.5–2[e] after HD	0.5–2 q12h		100	11	100–3000	2–8
		1.5–3.0	6–8	6–8	8–12	12–24	1.5–3 q12–24h	1.5–3 q12h					
1–2.2	1–2.2	0.125–0.5	6	6	6	6	0.125–0.5 q6h	0.125–0.5 q6h	Minimal	0–10		5–8	
1.8–2.8	1.8–2.8	0.5–2	4–6	4–6	4–6	4–6	0.5–2 q4–6h	0.5–2 q4–6h	9–20	10–15		≥100	Negligible
0.5–2	0.5–2	0.5–2	4–6	4–6	4–6	4–6	0.5–2 q4–6h	0.5–2 q4–6h		10–15	≤3.5	20–30	0
	6–20	1–4 MU	4–6	4–6	4–6	0.5–2 MU q4–6h	0.5-2 MU q4–6h, with 0.5 MU[e] after HD		0–10	100	6	200–800	
		0.6–1.2 MU	× 1	× 1	75% dose × 1	20%–50% dose × 1							
		0.6–1.2 MU	12–24	12–24	75% dose q12–24h	20%–50% dose q12–24h			Minimal				
		0.25–0.5	6	6	6	8	0.25–0.5 q8h, with 0.25[e] after HD						
		3.375–4.5	6–8	6–8	6–8	2.25 q8h	2.25 q8h					>100	

TABLE 54.19 Antiinfective Agent Pharmacology: Cephalosporins

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval[a]		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
First Generation												
Cefadroxil (100)	0.5 PO 1 PO	10–18 24–35	1800	0.5–1 q12–24h		2	30 mg/kg/day in 2 doses				1.1–2	20–25
Cefazolin	1 IV 1 IM	188 64–76	4000		0.5–2 q8h	6		25–150 mg/kg/day in 3–4 doses	25 mg/kg q12h[d]	25 mg/kg q8–12h[d]	1.2–2.2	18–36
Cephalexin (100)	0.25 PO 0.5 PO	9 15–18	2000	0.25–1 q6h		4	25–100 mg/kg/day in 3–4 doses				0.5–1.2	5–30
Second Generation												
Cefaclor[e] (>52)	0.25 PO 0.5 PO	5–7 13–15	600 900	0.25–0.5 q8h		1.5	20–40 mg/kg/day in 2–3 doses[f]				0.5–1	2.8
Cefotetan	1 IV[g] 2 IV[g]	142–179.6 237	1400–2000 3500–4000		1–2 q12h	4		30–50 mg/kg/day in 2 doses[h]			2.8–4.6	12–30
Cefoxitin	1 IM 1 IV[g] 2 IV[g]	22–24 110–125 221	3000		1–2 q6–8h	8		80–160 mg/kg/day in 3–4 doses[i]	35 mg/kg q8–12h[d]	35 mg/kg q6–8h[d]	0.7–1.1	13–22
Cefprozil (95)	0.25 PO 0.5 PO 1 PO	5.6–6.8 8.2–10.4 15.5–19.9	250 1000 2900	0.25–0.5 q12–24h		1	15 mg/kg q12h				0.9–1.5	5.9
Cefuroxime (37–52)[j]	0.5 PO 0.75 IV[f]	7 51.1	1150	0.25–0.5 q12h	0.75–1.5 q8h	1 PO 4.5 IV	50 mg/kg q12h	50 mg/kg q8–12h[d,k]			1–2	20
Third Generation												
Cefdinir[e] (36)	0.2 PO 0.6 PO	0.7–1.7 2.4		0.6/day in 1–2 doses		0.6	14 mg/kg/day in 1–2 doses				1.1–4.4	
Cefditoren pivoxil (14–16)	0.2 0.4	2.5–3 4.4–4.6		0.2–0.4 q12h		0.8	3–6 mg/kg q8h[h]				1.3–2	4.7
Cefixime[e] (30–50)	0.4 PO tabs 0.4 PO susp	3.7 4.6	15.7–305 15.7–305	0.4/day in 1–2 doses		0.4	8 mg/kg/day in 1–2 doses				2.4–4	11.5
Cefotaxime	0.5 IM 1 IV[g] 2 IV[g]	11.7–11.9 102.4 214.1	90–3261		1–2 q6–12h	12		50–200 mg/kg/day in 4–6 doses	50 mg/kg q12h	50 mg/kg q8–12h[d]	0.9–1.7	
Cefpodoxime proxetil[m] (50)	0.1 PO 0.2 PO 0.4 PO	1.4 2.3 3.9	60	0.1–0.4 q12h		0.8	5 mg/kg q12h[n]				1.9–3.2	9.8
Ceftazidime[o,p]	0.5 IV[f] 1 IV[f] 2 IV[f]	42 69 159–185.5			1–2 q8–12h	6		90–150 mg/kg/day in 3 doses	50 mg/kg q12h	50 mg/kg q8–12h[d]	1.4–2	11.9–35
Ceftibuten (80)	0.4 PO	15		0.4 q24h		0.4	9 mg/kg/day in 1 dose				1.5–2.9	18–29

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT													
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[b]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/Serum (%)	Aqueous Humor/ Serum (%)
		0.5–1	12–24	12–24	0.5 q12–24h	0.5 q36h	1 q72h, 1[c] after HD			50	0.9–1.9	22	
		0.5–2	8	8	0.5–1 q8–12h	0.5–1 q18–24h	0.5–1 q24h	0.5 q12h	1–4	35–69	3	29–300	<1.7
		0.25–1	6	6	8–12	24–48	0.25–0.5 q12–24h	0.25–0.5 q12–24h	Minimal	60	2	216	11
		0.25–0.5	8	8	8	8	0.25–0.5 q8–12h, 0.25[c] after HD	0.25 q8–12h			2	≥60	1–3
		1–2	12	12	24	48	25% dose nondialysis days, 50% dose dialysis days				2.3	2–21	
		1–2	6–8	8–12	12–24	0.5–1 q12–48h	0.5–2 q24h, 1–2[c] after HD		2.8	100	≤3	280	4–7
		0.25–0.5	12–24	12–24	50% dose q12–24h	50% dose q12–24h	50% dose q12–24h, 0.25[c] after HD	50% dose q12–24h					
		0.25–0.5 PO 0.75–1.5 IV	12 8	12 8	12–24 8–12	48 24	0.25–0.5 q24–48h, 0.25–0.5[c] after HD 0.75–1.5 q24h, 0.75–1.5[c] after HD	 0.75–1.5 q24h	17–88	20–33	≤3	35–80	10–14
		0.3 0.6	12 24	12 24	0.3 q12–24h	0.3 q24h	0.3 q48h, 0.3[c] after HD						
		0.2–0.4	12	12	0.2 q12–24h								
7												>100	
		1–2	6–8	6–8	8–12	24	1–2 g q24h	1g q24h	1.8–3.1	20–50	≤ 1.5	800–1200	1–6
		0.1–0.4	12	12	12–24	24	0.1–0.4 thrice weekly after HD						
		1–2	8–12	8–12	12–24	24	0.5–1 q24h		27		Up to 3–8	15–7510	0.5–4
	16	0.4	24	24	0.2–0.4 q24h	0.1 q24h	0.4 thrice weekly after HD						

Continued

TABLE 54.19 Antiinfective Agent Pharmacology: Cephalosporins—cont'd

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS						SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval[a]		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Ceftriaxone	1 IV[f]	123.2–150.7	504–995		1–2 q12–24h	4		50–100 mg/kg/day in 1–2 doses	50 mg/kg q24h	50 mg/kg q24h	5.4–10.9	12.2–18.2
	2 IV[f]	223–276										
	2 IV[q]	216–281										
Fourth Generation												
Cefepime[p]	1 IV	81.7			0.5–2 q8–12h	6		100–150 mg/kg/day in 2–3 doses	30–50 mg/kg q12h	30–50 mg/kg q12h	2	13.5
	2 IV	163.9										
Fifth Generation												
Ceftaroline fosamil	0.6	21.3			0.6 q8–12h	1.8		8 mg/kg q8h			1.6–2.7	6
Cephalosporin/β-Lactamase Inhibitor Combinations												
Ceftazidime-avibactam[r]	2-0.5	88.1-15.2			2.5 q8h	7.5					2.76[s]	7.7[s]
Ceftolozane-tazobactam[t]	1-0.5 q8h	74.4-18			1.5 q8h	4.5					3.12/1.10	11.1/2.15

[a]Dosage should not exceed adult dosage.
[b]Inflamed meninges.
[c]Specified dose should be administered in addition to scheduled dosing after a dialysis session to supplement drug clearance during dialysis.
[d]Lower end of dosing range if weight <2 kg and higher end if >2 kg.
[e]Decreased rate and/or extent of absorption when given with food.
[f]Should not exceed 1 g.
[g]IV push (over 2–10 min).
[h]Not FDA approved for use in this population.
[i]Should not exceed 12 g.
[j]52% after food.
[k]Doses up to 200–240 mg/kg/day q6–8h are used for meningitis.
[l]Infusion over 15–30 min.
[m]Should be given with food to increase absorption.
[n]No more than 400 mg/day for otitis or 100 mg/day for pharyngitis, tonsillitis.
[o]Arginine component not approved for children <12 yr.
[p]Administration by extended (2–4 h) or continuous infusion (23–24 h) should be strongly considered to optimize efficacy. This is especially important in patients who are critically ill, are immunosuppressed, or have deep-seated infections due to resistant organisms.
[q]2g q24h at steady state.
[r]Labeled infusion time is 2 h.
[s]Cephalosporin component only.
[t]Labeled infusion time is 1 h.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; *PD,* peritoneal dialysis.

TABLE 54.20 Antiinfective Agent Pharmacology: Monobactams, Carbapenems, and Polymyxins

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS						SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval[n]		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Monobactams												
Aztreonam[b]	1 IV[c]	90–164	3000–3500		1–2 q6–8h	8		90–120 mg/kg/day in 3–4 doses	30–50 mg/kg q8–12h[d]	30–50 mg/kg q6–12h[d]	1.3–2.2	6–9
	2 IV[c]	204–255	5600–6600									
Carbapenems												
Doripenem[b]	0.5 IV	23	601		0.5 q8h	1.5		60 mg/kg/day in 3 doses			1	
Ertapenem (90 IM)	1 IV[c]	155			1 q24h	1		30 mg/kg/day in 2 doses			4	14

		STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT										
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations			
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/Serum (%)[b]	Newborn Serum/Maternal Serum (%)	Breast Milk/Maternal Serum (%)	Bile/Serum (%)	Aqueous Humor/Serum (%)
12.2–18.2	12.2–18.2	1–2	12–24	12–24	12–24	12–24	1–2 q12–24h	1–2 q12–24h	16–32	18–25	3–4	200–500	
13.5	19	0.5–2	8–12	8–12	12–24	0.25–1 q24h	0.5–1 q24h	1–2 q48h			0.5 mg/L		
		0.6	12	12	0.3–0.4 q12h	0.2 q12h	0.2 q12h						
		2.5	8	8	0.94 q12 to 1.25 q8h	0.94 q24–48h	0.94 q24–48h						
		1.5	8	8	0.375–0.75 q8h		0.15 q8h						

		STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT										
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations			
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/Serum (%)[a]	Newborn Serum/Maternal Serum (%)	Breast Milk/Maternal Serum (%)	Bile/Serum (%)	Aqueous Humor/Serum (%)
2.7		1–2	6–8	6–8	8–12	0.5–1 q12h	0.5–1 q12h	0.5–1 q12h	3–52		0.1–0.6	115–405	5–14
		0.5	8	8	0.25 q8–12h							117	
		1	24	24	0.5–1 q24h	0.5 q24h	0.5 q24h, 0.15[e] after HD[f]				Minimal		

Continued

TABLE 54.20 Antiinfective Agent Pharmacology: Monobactams, Carbapenems, and Polymyxins—cont'd

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval[n]		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Imipenem–cilastatin[b]	0.25 IV[c] 0.5 IV[c] 1 IV[c]	14–24 21–58 41–83	50 100 > 100		0.5–1 q6h	4		15–25 mg/kg q6h	20–25 mg/kg q12h[d]	25 mg/kg q8–12h[d]	0.8–1	3.5
Meropenem[b]	0.5 IV[c] 1 IV[c]	26 55–62			0.5–2 q8–12h	6		20–40 mg/kg q8h			0.8–1	6–20
Meropenem/vaborbactam[h,i]	2/2 IV[i]	46.0/50.7			4 q8h	12					1.22/1.33	5.71/11.7
Polymyxins												
Colistimethate[k]	0.15 IM	5–7.5	200–270		2.5–5 mg/kg/day in 2–4 doses	5 mg/kg		2.5–5 mg/kg/day in 2–4 doses	Not recommended	Not recommended	1.5–8	48–72
Polymyxin B[l]	0.5 mg/kg q48–1.25 mg/kg q12h	2.38–13.9			1.25 mg/kg q12h	2.5 mg/kg/days		1.5–2.5 mg/kg/day in 2 doses	Not recommended	Not recommended	4.3–6	48–72

[a]Inflamed meninges.
[b]Administration by extended (2–4 h) or continuous infusion (23–24 h) should be strongly considered to optimize efficacy. This is especially important in patients who are critically ill, are immunosuppressed, or have deep-seated infections due to resistant organisms.
[c]IV infusion over 15–30 min.
[d]Lower end of dosing range if weight <2 kg and higher end if >2 kg.
[e]Specified dose should be administered in addition to scheduled dosing after a dialysis session to supplement drug clearance during dialysis.
[f]A supplemental dose after dialysis is recommended if the maintenance ertapenem dose is administered within 6 h before a dialysis session.
[g]Imipenem-cilastatin is not recommended with CrCl <15 mL/min unless hemodialysis will be initiated within 48 h.
[h]Dose expressed as the sum of both components.
[i]Manufacturer labeling recommends infusion time of 3 h.
[j]IV infusion over 3 h.
[k]Colistimethate is the sulfamethyl derivative of colistin, and the doses are based in milligrams of colistin base activity. Although 300 mg/day is considered to be the maximum daily dose, doses up to 480 mg/day may be necessary but are likely to also increase the risk for toxicity.
[l]Manufacturer labeling expresses doses in units; however, milligrams are more commonly used in clinical practice. For conversion, 10,000 units = 1 mg of polymyxin B.
[m]Manufacturer labeling recommends reducing the dose to <1.5 mg/kg/day in patients with renal impairment; however, recent data suggest that polymyxin B doses should not be adjusted for renal dysfunction.
[n]Dosage should not exceed adult dosage.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; *PD,* peritoneal dialysis.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT													
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
		0.5–1	6	0.5 q6–8h	0.5 q8–12h	0.25–0.5 q12h[g]	0.25–0.5 q12h		1–10			Minimal	3
		0.5–2	8–12	8–12	0.5–1 q12h	0.5–1 q24h	0.5–1 q24h		12			40	
		4	8	8	2 q8–12h	1 q12h	1 q12h						
		5 mg/kg/day	5 mg/kg/day in 2–4 doses	2.5–3.8 mg/kg/ day in 2 doses	1.5–2.5 mg/kg/ day in 1–2 doses	1.5 mg/kg q36h	1.5 mg/kg q24–48h		50	18		25–30	
		1.25 mg/kg q12h	12	12[m]	12[m]	12[m]	12[m]	Minimal					

TABLE 54.21 Antiinfective Agent Pharmacology: Aminoglycosides

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Amikacin[b]	7.5 mg/kg IM	21	832		15–20 mg/kg/day in 1–3 doses[c]	15–20 mg/kg		15–22.5 mg/kg/day in 2–3 doses[c]	15 mg/kg q24–48h	15–17.5 mg/kg q24h	2–3	30–86
	7.5 mg/kg IV[f]	38										
Gentamicin[g]	1 mg/kg IM	4–7.6	113–423		3–7 mg/kg/day in 1–3 doses[c]	3–7 mg/kg		6–7.5 mg/kg/day in 3 doses[c]	4–5 mg/kg q24–48h[c,h]	4–5 mg/kg/q24–36h[c,h]	2–3	24–60
	1 mg/kg IV[i]	4–7.6										
Kanamycin[b] (1)	7.5 mg/kg IM	22			15 mg/kg/day in 1–3 doses[c]	1.5		15 mg/kg/day in 2–3 doses[c]			2–4	27–80
	7.5 mg/kg IV[f]	22										
Neomycin[j] (3)	4 g PO	2.5–6.1		1 PO q6h		4 PO	100 mg/kg/day in 4 doses				2–3	12–24
Streptomycin[k]	0.5 IM	5–12	400 ≥1000		1–2 g/day in 2–4 doses	2					2–3	Up to 110
	1 IM	25–50										
Tobramycin[g]	1 mg/kg IM	4–6	75–100		3–7 mg/kg/day in 1–3 doses[c]	3–7 mg/kg		6–7.5 mg/kg/day in 2–3 doses[c]	4–5 mg/kg q24–48h[c,h]	4–5 mg/kg/day q24–36h[c,h]	2–3	5–70
	1 mg/kg IV[f]	4–6										

[a]Inflamed meninges.
[b]Desired concentrations of amikacin and kanamycin are peak 15–30 μg/mL and trough <5–10 μg/mL. Peak targets generally occur 2 h after a 30–minute infusion using a high-dose, extended-interval approach (total daily dose administered once daily with therapeutic drug monitoring). Divided daily dosing of aminoglycosides is generally reserved for synergy indications (i.e., endocarditis) and special populations including pediatrics and those with renal impairment.
[c]The dosing strategy for aminoglycosides involves the use of ideal (lean) body weight (IBW) for dosage calculation. In obese patients, this approach would result in serum aminoglycoside concentrations less than expected. Alternative dosing recommendations have been proposed that account for the change in drug distribution volume with obesity: (1) IBW + 40% of excess weight, defined as total body weight (TBW) minus IBW (*J Infect Dis.* 1978;138:499–505); (2) IBW + 58% of excess weight (TBW – IBW) (*Clin Pharmacol Ther.* 1979; 26:508); (3) IBW + 38% of excess weight (TBW – IBW) (*Am J Hosp Pharm.* 1980;37:519–522). Extended-interval dosing, where the total daily dose is administered in a single dose, has become increasingly common in clinical practice as a method to optimize pharmacodynamics and improve safety.
[d]Dosing at CrCl ≤10 mL/min should be assisted with serum concentrations.
[e]Specified dose should be administered after a dialysis session to supplement drug clearance during dialysis.
[f]Infused over 30–60 min.
[g]Desired concentrations of gentamicin and tobramycin are peak 4–10 μg/mL and trough <1–2 μg/mL. Peak targets generally occur 2 h after a 30–minute infusion using a high-dose, extended-interval approach (total daily dose administered once daily with therapeutic drug monitoring). Divided daily dosing of aminoglycosides is generally reserved for synergy indications (i.e., endocarditis) and special populations including pediatrics and those with renal impairment.
[h]Lower end of dosing range if weight <2 kg and higher end if >2 kg.
[i]Infused over 2 h.
[j]Parenteral administration of neomycin is no longer recommended.
[k]Desired concentrations: peak 5–25 μg/mL; trough <5 μg/mL.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; *PD,* peritoneal dialysis.

		STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT										
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/Serum (%)	Aqueous Humor/ Serum (%)
		5–7.5 mg/kg	8	8–12	12–48	48[d]	2.5–3.75 mg/kg[e] after HD	2 mg/kg/day	15–24	20		30	Minimal
		1–1.7 mg/kg	8	8–12	12–48	48[d]	1.0–1.7 mg/kg[e] after HD	0.6 mg/kg/day	10–30	30–40		30–60	Minimal
		7.5 mg/kg	8–12	8–12	12–48	≥48[d]	4–5 mg/kg[e] after HD	3.75 mg/kg/day	43	50	35		Minimal
		1 PO	6	6									Minimal
		0.5–1	12	12	24–72	72–96[d]	0.5[e] after HD		20	10–40	<25	40–300	Minimal
		1–1.7 mg/kg	8	8–12	12–48	48[d]	1 mg/kg[e] after HD	0.6 mg/kg/day	14–23	50		10–20	18

TABLE 54.22 Antiinfective Agent Pharmacology: Tetracyclines, Glycylcyclines, and Folate Antagonists

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Tetracyclines and Glycylcyclines												
Demeclocycline[b] (60–80)	0.15 PO	0.9–1.2		0.6/day in 2–4 doses		0.6	7–13 mg/kg/day in 2–4 doses[c]		Not recommended[c]	Not recommended[c]	10–17	42–68
	0.3 PO	1.5–1.7										
Doxycycline[b] (90–100)	0.1 PO	1.5–2.1		0.1 q12h	0.1 q12h	0.2	2–4 mg/kg/day in 1–2 doses[c]	2–4 mg/kg/day in 1–2 doses[c]	Not recommended[c]	Not recommended[c]	14–24	18–30
	0.1 IV[d]	2.5										
Minocycline[b] (90–100)	0.2 PO	2–3.5		0.1 q12h	0.1 q12h	0.2	4 mg/kg/day in 2 doses[c]	4 mg/kg/day in 2 doses[c]	Not recommended[c]	Not recommended[c]	11–26	12–30
	0.2 IV	2.52–6.63										
Tetracycline[b] (75–80)	0.25 PO	1.5–2.2		0.25–0.5 q6h		2	25–50 mg/kg/day in 2–4 doses[c]		Not recommended[c]	Not recommended[c]	6–12	57–120
	0.5 PO	3–4.3										
	0.5 PO[e]	2–5										
Tigecycline	0.1 IV	1.45			0.1 × 1, then 0.05 q12h	0.1–0.2		1.2 mg/kg q12h[c,f]	Not recommended[c]	Not recommended[c]	27–42	27–42
Folate Antagonists												
Trimethoprim-sulfamethoxazole[g] (85–90)	0.16/0.8 PO[h]	1.72/68		0.16/0.8 q12h	10–20 mg/kg/day in 2–3 doses[i]	1.2[i]	6–12 mg/kg/day in 2 doses[i]	6–12 mg/kg/day in 2 doses[i]	Not recommended		8–15/7–12	24/22–50
	0.16–0.8 IV[h]	8.8/105.6										
Trimethoprim (80)	0.1 PO	1	30–60	0.1 q12h		0.2	4–10 mg/kg/day in 2 doses[m]				8–15	24
	0.2 PO	2										
Sulfadiazine[g] (70–90)	3 PO	50		2–4 g/day in 3–6 doses		4	120–150 mg/kg/day in 4–6 doses[n]	Not recommended	Not recommended		17	34
Dapsone	0.2 PO	0.1–7[h]		0.05–0.1 q24h		0.1	1–2 mg/kg/day in 1–2 doses				20–30	

[a]Inflamed meninges.
[b]All tetracyclines should be given 1 h before or 2 h after meals.
[c]The tetracyclines cause a brown discoloration of the teeth and may retard the growth of bone in the human fetus and children. The American Academy of Pediatrics recommends that tetracyclines be used only in children who are 8 years of age or older.
[d]Infused over 60 min.
[e]At steady state.
[f]Not FDA approved for use in pediatric patients. Dose is recommended based on limited pharmacokinetic data for patients 8–12 years of age when no alternative is available.
[g]Decreased rate and/or extent of absorption when given with food.
[h]At steady state.
[i]Based on the trimethoprim component.
[j]Uninflamed meninges.
[k]Amniotic fluid concentrations (μg/mL).
[l]Specified dose should be administered after a dialysis session to supplement drug clearance during dialysis.
[m]Not approved for children <6 months old.
[n]In children 2 mo or older.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; *PD,* peritoneal dialysis.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT													
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
		0.15–0.3	6–12	Not recommended	Not recommended	Not recommended	Not recommended	Not recommended	Minimal		70	200–3200	10–30
18–30		0.1	12	12	12	12	0.1 g q12h	0.1 g q12h	26		30–40	200–3200	10–13
		0.1	12	12	12	12	0.1 g q12h	0.1 g q12h		77	8–26	200–3200	17
		0.25–0.5	6	6	8–12	24			7	60–70	25–150	200–3200	9–11
27–42		0.1 × 1, then 0.05	12	12	12	12	12	12				> 100	
		0.16/0.8	12	12	12–24	Avoid	0.16/0.8 q48h	0.16/0.8 q48h	50/40[j]	80/50[k]	125/10	100–200/ 40–70	10–45/ 20–30
		5 mg/kg IV[i]	6–8	8	8–12	12	5–10 mg/kg after HD[i,l]						
		0.1	12	12	18–24	Avoid			30–50	70–100	100	100	10
		0.5–1	4–6						50–80	≥50			
		0.05–0.1	24								69		

TABLE 54.23 Antiinfective Agent Pharmacology: Macrolides, Azalides, Lincosamides, and Miscellaneous Antibacterial Agents

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)			
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)		With Dialysis	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10	HD	PD
Macrolides and Azalides														
Azithromycin[b] (35–40)	0.5 PO 0.5 IV 0.3 PO	0.09–0.44 3.63 0.5	8.64–26.8	0.25–0.5 q24h	0.25–0.5 q24h	0.5	5–12 mg/kg/day	10–12 mg/kg/day[c]	10 mg/kg/day	10 mg/kg/day	68	68	68	68
Clarithromycin (50–55)	0.25 PO[d] 0.5 PO[d] 0.5 PO[d]	1 2–3 1[e]		0.25–0.5 q12h		1	7.5 mg/kg q12h				5–7			
Erythromycin base[f,g]	0.25 PO	0.1–2		0.25–0.5 q6h		2	30–50 mg/kg/day in 3–4 doses				1.5–2	6		
Erythromycin stearate[g]	0.25 PO	0.1–2		0.25–0.5 q6h		2	30–50 mg/kg/day in 3–4 doses				1.5–2	6		
Erythromycin ethyl succinate[g]	0.4 PO	0.1–2		0.4 q6h		4	30–50 mg/kg/day in 3–4 doses				1.5–2	6		
Erythromycin lactobionate[h,i]	0.2 IV	3–4			0.5–1 q6h	4		15–20 mg/kg/day in 4 doses			1.5–2	6		
Fidaxomicin[j]	0.2 PO	0.0052		0.2 q12h		0.4					11	11	11	11
Lincosamides														
Clindamycin[f] (90)	0.15 PO 0.6 IV[m]	1.9–3.9 10		0.15–0.45 q6–8h	0.3–0.9 q6–8h	12.7 PO/IV	10–40 mg/kg/day in 3–4 doses	20–40 mg/kg/day in 3–4 doses	5 mg/kg q8–12h[k,l]	5 mg/kg/day q6–8h[k,l]	2–3	2–3.5		
Lincomycin[f] (20–30)	0.5 PO 0.6 IM 0.6 IV[n]	1.8–5.3 9.3–18.5 15.9–20.9			0.6–1 q8–12h	8		10–20 mg/kg/day in 2–3 doses	Not indicated	Not indicated	4–6.4	10		
Miscellaneous Antibacterial Agents														
Chloramphenicol[o] (75–90)	1 PO 1 PO[d] 1 IV	11 18 4.9–12			0.25–1 q6h	4			25 mg/kg/day in 4 doses[p]	50 mg/kg/day in 4 doses[q]	1.5–4.1	3–7		3–7
Metronidazole[f] (80)	0.25 PO 7.5 mg/kg[d]	4.6–6.5 26		0.25–0.75 q8h	0.5 q6–8h	4	30–50 mg/kg/day in 3 doses	22.5–40 mg/kg/day in 3 doses	7.5 mg/kg/day q8–12h[l]	7.5 mg/kg/day q6–12h[l]	6–14	8–15		8–15
Rifaximin[j]	0.2 PO q8h	0.00081		0.2–0.55 q8–12h		1.65					5.6–6			
Secnidazole	2 PO	45.4		2 × 1		2								

[a]Inflamed meninges.
[b]Decreased extent of absorption of capsule formulation only when given with food.
[c]No studies to support; extrapolated from adult conversion.
[d]At steady state.
[e]Of 14-hydroxyclarithromycin (active metabolite).
[f]Denotes decreased rate and/or extent of absorption when given with food.
[g]Erythromycin and its derivatives have varying degrees of bioavailability (18%–45%).
[h]Oral erythromycin therapy should replace IV therapy as soon as possible.
[i]Because of the local irritative effects, the drug must not be administered rapidly by direct IV injection (IV push).
[j]Negligible oral bioavailability.
[k]When IV clindamycin is given to neonates and infants, organ system functions should be monitored.
[l]Lower end of dosing range if weight <2 kg and higher end if >2 kg.
[m]Over 20 min.
[n]When given over 2 h.
[o]Chloramphenicol dosage should be administered to maintain plasma concentrations of 10–25 mg/L for peak and 5–10 mg/L for trough.
[p]Age <2 wk.
[q]Age >2 wk.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; *PD,* peritoneal dialysis.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT											
	For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/Serum (%)[a]	Newborn Serum/Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/Serum (%)	Aqueous Humor/ Serum (%)
0.25–0.5	24	24	24	24	24	24			>100		
0.25–0.5	12	12	12–24	24			Minimal		30	7000	
0.25–0.5	6	6	6	6	0.25–0.5 q6h	0.25–0.5 q6h	2–13	5–20	50		
0.25–0.5	6	6	6	6	0.25–0.5 q6h	0.25–0.5 q6h	2–13	5–20	50		
0.4	6	6	6	6	0.4 q6h	0.4 q6h	2–13	5–20	50		
0.5–1	6	6	6	6	0.5–1 q6h	0.5–1 q6h	2–13	5–20	50		
0.2	12	12	12	12	12	12					
0.15–0.45 PO 0.6–0.9 IV	6 8	6 8	6 8	6 8	0.15–0.3 PO q6h 0.6–0.9 IV q8h	0.15–0.3 PO q6h 0.6–0.9 IV q8	Minimal	46	38–50	250–300	
0.6–1 IV	8–12	8–12	8–12	24–36			18	25	13	250–400	8.75
0.25–1 IV	6	6	6	6	0.25–1 IV q6h	0.25–1 IV q6h	45–89	30–80	100	100	33–50
0.25–0.75 PO 0.5 IV	8 6–8	8 6–8	8 6–8	8 6–8	0.25–0.75 PO q8h 0.5 IV q6–8h	0.25–0.75 PO q8h 0.5 IV q6–8h	≥100	97	100		
0.2–0.55	8–12										

TABLE 54.24 Antiinfective Agent Pharmacology: Glycopeptides, Lipopeptides, Lipoglycopeptides, Polypeptides, Oxazolidinones, and Streptogramins

	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
Drug (Oral Absorption, %)	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Dalbavancin[b]	1 IV 1.5 IV	287 423			1.5 × 1	1.5					204	
Daptomycin	4 mg/kg IV 6 mg/kg IV	57.8 93.9			4–6 mg/kg q24h	8–12 mg/kg[c]		5–10 mg/kg q24h[d]			9.4	27.8
Linezolid (100)	0.4 PO 0.6 PO 0.6 IV	8.1 12.7 15.1		0.4–0.6 q12h	0.4–0.6 q12h	1.2	10 mg/kg q8h	10 mg/kg q8h	10 mg/kg q8–12h[e]	10 mg/kg q8h	5.5	7.1
Oritavancin	1.2 IV	138			1.2 × 1	1.2					245	
Quinupristin/dalfopristin	7 mg/kg IV	3/4			7.5 mg/kg q8–12h	22.5 mg/kg/day		7.5 mg/kg q12h[g]			8.6	
Tedizolid (100)	0.2 PO 0.2 IV	2 2.3									12	12
Telavancin	7.5 mg/kg IV 15 mg/kg IV	87.5 186			10 mg/kg q24h	10 mg/kg					6–8	
Vancomycin[i,j]	1 IV	25		0.125–0.5 q6h	15 mg/kg q8–12h	1 PO, 2 IV	40 mg/kg/day in 4 doses[k]	40–60 mg/kg/day in 3–4 doses	7.5–30 mg/kg/day in 1–2 doses[l]	7.5–30 mg/kg/day in 1–2 doses[l]	4–6	44.1–406.4

[a]Inflamed meninges.
[b]Doses are approved either as a single dose or as a two-dose regimen divided on days 1 and 8. The divided regimens are as follows: 1500 mg = 1000 mg on day 1, 500 mg day 8; 1125 mg = 750 mg on day 1, 375 mg on day 8.
[c]Some experts recommend the use of daptomycin 8–12 mg/kg in patients with infective endocarditis, persistent MRSA bacteremia, vancomycin-resistant enterococcal infections, or vancomycin treatment failures in adults. Alternatively, an empirical fixed daily dose of 750 mg may be optimal in critically ill patients, whereas doses >1000 mg/day are likely to increase the probability of skeletal muscle toxicity without significantly increasing the probability of effect.
[d]Doses are recommended for ages 1–17 years for the treatment of cSSSI. Higher doses per unit body weight are recommend for younger patients.
[e]Lower end of dosing range if weight <2 kg and higher end if >2 kg.
[f]Oritavancin has not been studied in severe renal impairment (CrCl < 30 mL/min).
[g]Children >12 years of age.
[h]Use is not recommended in patients with CrCl <50 mL/min unless benefit outweighs risk owing to increased mortality in this subpopulation in a trial of telavancin for nosocomial pneumonia.
[i]Negligible oral bioavailability.
[j]Vancomycin dosing should ideally occur with therapeutic drug monitoring to achieve a target AUC/MIC of 400. Although monitoring of trough levels (target 10–20 μg/mL) as a surrogate for AUC is currently the most common strategy in the clinical setting, this practice likely leads to overexposure in an important proportion of patients. The most rigorous monitoring strategy to optimize safety and efficacy involves obtaining two levels (peak and trough) and calculating the AUC to target values of 400–600.
[k]Not to exceed 2 g/day.
[l]Dosing recommended by the American Academy of Pediatrics is based on neonatal serum creatinine as follows: <0.7 mg/dL = 15 mg/kg q12h; 0.7–0.9 mg/dL = 20 mg/kg q24h; 1–1.2 mg/dL = 15 mg/kg q24h; 1.3–1.6 mg/dL. = 10 mg/kg q24h; >1.6 mg/dL = 15 mg/kg q48h.
[m]Refer to the Matzke nomogram (*Antimicrobial Agents Chemother*. 1984;25:433–437) for more precise recommendations for empirical renal dosing of vancomycin. Therapeutic drug monitoring should occur for safety and efficacy in most patients, but is essential in patients with renal impairment.
[n]Initial doses of 20–25 mg/kg are recommended, with further dosing based on therapeutic drug monitoring. Vancomycin doses in hemodialysis are frequently administered thrice weekly with the last hour or after the dialysis run.
[o]Specified dose should be administered after a dialysis session to supplement drug clearance during dialysis.
AUC, Area under the concentration-time curve; *CrCl,* creatinine clearance; *CSF,* cerebrospinal fluid; *MIC,* minimal inhibitory concentration; *cSSSI,* complicated skin and skin structure infection; *HD,* hemodialysis; MRSA, methicillin-resistant *Staphylococcus aureus*; *PD,* peritoneal dialysis.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT												
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/Serum (%)	Aqueous Humor/ Serum (%)
		1.5 × 1	1.5 × 1	1.5 × 1	1.125–1.5 × 1	1.125 × 1	1.125 × 1						
29.8		4–6 mg/kg	24	24	24–48	48	4–6 mg/kg q48h	4–6 mg/kg q48h					
		0.4–0.6	12	12	12	12	0.4–0.6 q12h	0.4–0.6 q12h	20				
		1.2 × 1	1.2 × 1	1.2 × 1	1.2 × 1[f]								
		7.5 mg/kg	8–12	8–12	8–12	8–12	7.5 mg/kg q8–12h	7.5 mg/kg q8–12h					
		0.2 PO/IV	24	24	24	24	0.4 q24h						
		10 mg/kg	24	24	7.5 mg/kg q24[h]								
		15 mg/kg[m]	8–12	12–18	18–48	Dose by levels[n]	0.25–1[o] after HD[n]	0.5 q48–96h[n]	7.21			50	Minimal

TABLE 54.25 Antiinfective Agent Pharmacology: Fluoroquinolones and Urinary Antiinfectives

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval		Serious Infection Daily Dose (g)						
				Oral	Parenteral		Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Fluoroquinolones												
Ciprofloxacin[b] (50–85)	0.5 PO 0.75 PO 0.4 IV[f]	1.6–2.9 2.5–4.3 4.6	350	0.5–0.75 q12h	0.4 q8–12h	1.5 PO 1.2 IV	10–20 mg/kg q12h[c]	6–10 mg/kg q8–12h[c]	Not recommended	Not recommended	3–5	5–10
Delafloxacin (58.8)	0.45 PO 0.3 IV	7.17 8.94		0.45 q12h	0.3 q12h	0.9 PO 0.6 IV					4.2–8.5 3.7[g]	14–15
Gemifloxacin (71)	0.32 PO	1.48		0.32 q24h		0.32					6.65	
Levofloxacin (99)	0.5 PO 0.75 PO 0.5 IV[f]	5.7 7.1 6.2		0.5–0.75 q24h	0.5–0.75 q24h	1	8 mg/kg q12h[i]	8 mg/kg 12h[i]	Not recommended	Not recommended	6–8	35
Moxifloxacin (90)	0.4 PO 0.4 IV	3.1 3.9		0.4 q24h	0.4 q24h	0.4					9–16	
Ofloxacin[b] (85–100)	0.4 PO 0.2 PO 0.4 IV[f]	2.9–5.6 1.5–2.7 4	200	0.2–0.4 q12h		0.8					4–8	16.9–28.4
Urinary Antiinfectives												
Fosfomycin (37)	3 PO	26.1	706	3 × 1 dose		3					5.7	40–50
Methenamine hippurate[k]	1 PO		40 (formaldehyde)	1 q12h[l]		2	0.5–1 q12h[l]	Not recommended	Not recommended		3–6	
Methenamine mandelate[k]	1 PO	70–100 μmol/L	~50 (formaldehyde)	1 q6h[l]		4	0.5–1 q6h[l]	Not recommended	Not recommended		3–6	
Nitrofurantoin (40–94)[n]	0.1 PO	<2	50–150	0.05–0.1 q6–12h[o]		0.4	5–7 mg/kg/day q6h	Not recommended	Not recommended		0.3	1

[a]Inflamed meninges.
[b]Decreased rate and/or extent of absorption when given with food.
[c]Dose should not exceed the adult dose. Routine use in children is not recommended.
[d]3.2 h during dialysis/5.8 h in between sessions.
[e]Case report.
[f]Infused over 60 min.
[g]Multiple dose PO, single dose IV.
[h]Delafloxacin has not been studied with CrCl <15 mL/min. Recommended dose adjustments for the IV formulation occur for CrCl 15–30 mL/min.
[i]FDA-approved pediatric use is plague in children >6 mo of age. Weight-based dose should not exceed 250 mg/dose in children <50 kg.
[j]8–12 h during dialysis/13–48 h between sessions.
[k]Usually coadministered with an acidifying agent to convert the methenamine salts in urine to ammonia and bactericidal formaldehyde (pH <5.5). Mandelic acid and hippuric acid are mildly antiseptic and contribute to urine acidification.
[l]Use primarily for the prevention of urinary tract infections.
[m]Ineffective urinary concentrations expected with compromised renal function.
[n]Bioavailability is variable but generally sufficient for urinary activity.
[o]Nitrofurantoin is available in two formulations: macrocrystals and microcrystals. The macrocrystalline formulation is administered twice daily, whereas the microcrystalline formulation must be administered four times daily.
[p]Nitrofurantoin accumulates in the serum of patients with renal impairment, which may lead to systemic toxicity. The labeling states that this medication should not be used at CrCl <60 mL/min; however, recent data suggest that it may used until CrCl <30 mL/min.
[q]Although only small amounts of nitrofurantoin have been detected in breast milk, the drug could cause hemolytic anemia in a glucose-6-phosphate dehydrogenase–deficient infant exposed in this manner.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *FDA,* US Food and Drug Administration; *HD,* hemodialysis; *PD,* peritoneal dialysis.

With Dialysis		STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT											
			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
3.2/5.8[d]		0.5–0.75 PO	12	12	0.25–0.5 q12h	0.25–0.5 q18–24h	0.25–0.5 q24h	0.25–0.5 q24h	11–46		400	2800–4500	3–22[e]
		0.4 IV	8–12	8–12	12–24	18–24	0.2–0.4 q24h	0.2–0.4 q24h					
		0.45 PO	12	12	12[h]								
		0.3 IV	12	12	0.2 q12h[h]								
		0.32	24	24	0.16 q24h	0.16 q24h	0.16 q24h	0.16 q24h					
		0.5–0.75 PO/IV	24	24	24–48	0.25–0.5 q48h	0.25–0.5 q48h	0.25–0.5 q48h	15		100		
		0.4	24	24	24	24	24	24					
8–12/ 13–48[j]		0.2–0.4 PO	12	12	24	0.1–0.2 q24h	0.2 × 1, then 0.1 q24h	0.2 × 1, then 0.1 q24h	28–87		96–112	210–1886	
40–50		3	× 1	× 1	× 1								
		1	12	12	Avoid[m]								
		1	6	6	Avoid[m]					50	70–100		
		0.05–0.1[o]	6–12	6–12	6–12[m,p]					100	<25[q]	200–400	

TABLE 54.26 Antiinfective Agent Pharmacology: Antimycobacterial Agents

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)			
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)		With Dialysis	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval		Serious Infection Daily Dose (g)								
				Oral	Parenteral		Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10	HD	PD
Aminosalicylic acid (60–65)	4 PO	76–104		8–12 g/day in 2–3 doses		12	200–300 mg/kg/day in 2–4 doses				1	23		
Bedaquiline[b]	0.4 PO	3		0.4 q24h for 2 wk, then 0.2 thrice weekly		0.4					5.5 mo	5.5 mo		
Capreomycin[c]	1 IM	20–47			0.75–1 IM/IV q24h[d]	1		15–30 mg/kg/day q24h[e]			4–6	29.4–55.5		
Clofazimine[b] (45–70)	0.1 PO[f]	0.76		0.1–0.2 q24h		0.2					8 days, 70 days[g]			
Cycloserine[h] (70–90)	0.25 PO	10		0.25–0.5 q12h[i]		1	10–15 mg/kg/day in 1–2 doses[e]				10–25			
Ethambutol (75–80)	25 mg/kg PO	2–5		15 mg/kg q24h		15 mg/kg	15–20 mg/kg q24h[e]				3.3	≥7		
Ethionamide (80)	1 PO	20		0.5–0.75 q24[i]		1	15–20 mg/kg/day[j] q24h[e]				3	9		
Isoniazid[k,l]	7 mg/kg PO	4.5, 1[m]		0.3 q24h	0.3 IM q24h	0.3	10–2 mg/kg/day in 1–2 doses	15 mg/kg/q24h			0.5–4	2–10		
Pyrazinamide	0.5 PO	9–12		15–30 mg/kg q24h		2	15–30 mg/kg/day in 1–2 doses[e]				10–16			
Rifabutin (≥20)	0.3 PO	0.375		0.3 q24h		0.3	4–18.5 mg/kg q24h[n]				16–69			
Rifampin (100)	0.6 PO	7		0.6 q24h	0.6 q24h	0.6	10–20 mg/kg/day in 1–2 doses				2–5	2–5	Minimal change	
	0.6 IV[o]	17.5												
Rifapentine (70)	0.6 PO	15.05		0.6 twice weekly		0.6					13.19			
Streptomycin	1 IM	25–50	≥1000		0.75–1 IM/IV q24h[d]	1		20–40 mg/kg/day q24h			2–3	100		

[a]Inflamed meninges.
[b]Should be taken with food.
[c]Pharmacokinetics similar to streptomycin.
[d]Dose is 15 mg/kg/day (max 1 g). Dose is reduced to 10 mg/kg/day (max 750 mg) in patients older than 59 years. Dosing is generally 5–7 days out of the week until 2–4 mo after culture conversion, then 2–3 times per week.
[e]Not FDA approved for use in pediatric patients.
[f]In leprosy patients.
[g]8-day serum half-life, 70-day tissue half-life.
[h]Patients are frequently unable to tolerate full dosing, and serum concentrations can be useful to assess exposure (target 20–35 μg/mL).
[i]Recommended dose is 15 mg/kg/day, generally administered as 500–700 mg/day divided in two doses.
[j]Limited evidence suggests that 20 mg/kg daily given as a single dose in children is more likely to produce cerebrospinal fluid concentrations exceeding the minimal inhibitory concentration of 2.5 μg/mL for *Mycobacterium tuberculosis*.
[k]Decreased rate and/or extent of absorption when given with food.
[l]To minimize risk of polyneuritis from isoniazid-induced pyridoxine deficiency, pyridoxine (15–50 mg) is often given concurrently.
[m]4.5 μg/mL in slow acetylators, 1.0 μg/mL in rapid acetylators.
[n]Dose varies significantly by age group; see manufacturer's recommendations before prescribing.
[o]Infused over 30 min.
[p]Desirable serum concentrations: peak, 5–25 μg/mL; trough, <5 μg/mL.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *FDA,* US Food and Drug Administration; *HD,* hemodialysis; *PD,* peritoneal dialysis.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT											
	For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
4	8–12	8–12	12	12	4 q12h		10–50				
0.4, then 0.2	24, then thrice weekly	24, then thrice weekly	24, then thrice weekly	24, then thrice weekly	0.4 q24h for 2 wk, then 0.2 thrice weekly	0.4 q24h for 2 wk, then 0.2 thrice weekly					
0.75–1[d]	24	24	12–15 mg/kg 2–3 times weekly	12–15 mg/kg 2–3 times weekly	12–15 mg/kg 2–3 times weekly					Minimal	
0.1	24	24	24	24	24	24					
0.25–0.5[h]	12	12	0.25–0.5 q24h	0.25 q24h	0.25 q24h	0.25 q24h	80–100	100	72		
15 mg/kg	24	24	15–25 mg/kg thrice weekly	15–25 mg/kg thrice weekly	15–25 mg/kg thrice weekly after HD	15–25 mg/kg thrice weekly	25–50	~100			
0.5–0.75[i]	24	24	0.25–0.5 q24h	0.25–0.5 q24h	0.25–0.5 q24h	0.25–0.5 q24h	100				
0.3 PO/IM	24	24	24	24	0.3 q24h	0.3 q24h	100	High	100		
15–30 mg/kg	24	24	25–35 mg/kg thrice weekly	25–35 mg/kg thrice weekly	25–35 mg/kg thrice weekly	25–35 mg/kg thrice weekly	100				
0.3	24	24	0.15 q24h	0.15 q24	0.15 q24h	0.15 q24h					
0.6 PO/IV	24	24	24	24	24	24	10–20	33	20–60	10,000	
0.6	Twice weekly	Twice weekly									
1[p]	24	24	12–15 mg/kg 2–3 times weekly	12–15 mg/kg 2–3 times weekly	12–15 mg/kg 2–3 times weekly		20	10–40	<25	40–300	

TABLE 54.27 Antiinfective Agent Pharmacology: Antifungal Agents

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval		Serious Infection Daily Dose (g)						
				Oral	Parenteral		Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Polyenes												
Amphotericin B deoxycholate	0.03 IV[b] 0.05 IV[b]	1 2			0.25–1 mg/kg q24h[c,d]	1 mg/kg[e]		1–1.5 mg/kg q24–48h[c]	1 mg/kg q24h[c]	1 mg/kg q24h[c]	≥24, 15 days[f]	≥24, 15 days[f]
Amphotericin B lipid complex	5 mg/kg	1.7			5 mg/kg q24h	5 mg/kg		5 mg/kg q24h	Not recommended	Not recommedned		
Amphotericin B liposomal	5 mg/kg IV	57.6			3–5 mg/kg q24h	3–5 mg/kg		3–5 mg/kg q24h	Not recommended	Not recommended	100–153	
Nystatin (0)	All doses PO	Not detectable		0.4–1 MU q8h		3 MU	0.4–0.6 MU q6h		0.1–0.2 MU q6h	0.1–0.2 MU q6h		
Echinocandins												
Anidulafungin	0.1 IV	7.2			0.1–0.2 × 1, then 0.05–0.1 q24h	0.1		1.5–3 mg/kg ×1, then 0.75–1.5 mg/kg q24h[g]			26–50	26–50
Caspofungin	0.05 IV	8.7			0.07 × 1, then 0.05 q24h	0.05		70 mg/m^2 × 1, then 50 mg/m^2 q24h			9–11	9–11
Micafungin	0.1 IV	8.17			0.1–0.15 q24h	0.15		2–10 mg/kg q24h[h]			15	15
Azoles												
Fluconazole (≥90)	0.4 PO 0.1 IV[i]	6.72 3.86– 4.96		0.2–0.4 q24h	0.2–0.4 q24h	0.8	3–12 mg/kg q24h	3–12 mg/kg q24h			20–50	48
Isavuconazonium (98)	0.372 PO	7.499		0.372 q8h × 2 days, then 0.372 q24h	0.372 q8h × 2 days, then 0.372 q24h	0.372					130	
Itraconazole (99.8)[j,k]	0.2 PO[l]	0.23, 0.35[m1]		0.2 q12h		0.4					21–60[m2]	
Ketoconazole[k]	0.2 PO	4.2		0.2–0.4 q24h		0.4	3.3–6.6 mg/kg q24h				8	8
Posaconazole (54)[n]	0.2 PO[o] 0.3 PO[p]	0.512 1.580		0.2 q8h[o] 0.3 q24h[p]		0.8 0.3					35	35
Voriconazole[q] (96)	0.2 PO[r] 3 mg/kg IV[r]	2.08 3.06		0.4 q12h × 1 day, then 0.2 q12h	6 mg/kg q12h × 1 day, then 4 mg/kg q12h	8 mg/kg	9 mg/kg q12h[s]	9 mg/kg q12h × 1 day, then 8 mg/kg q12h[s]			6	
Allylamine												
Terbinafine (80)	0.25 PO	1		0.25–0.5 q24h		0.5	0.0675–0.25 q24h[u]				22–30	
Miscellaneous Antifungals												
Flucytosine[q,v] (75–90)	2 PO	30-–45		25 mg/kg q6h		150 mg/kg	50–150 mg/kg/day in 4 doses				3-–6	30-–250

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT													
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
		0.25–1 mg/kg	24	24	24	24	0.25–1 mg/kg q24h	0.25–1 mg/kg q24h	3	50			25
		5 mg/kg	24	24	24	24	5 mg/kg q24h	5 mg/kg q24h					
		3–5 mg/kg	24	24	24	24	3–5 mg/kg q24h	3–5 mg/kg q24h					
		0.4–1 MU	8	8	8	8	0.4–1 MU q8h	0.4–1 MU q8h					
26–50	26–50	0.05–0.1	24	24	24	24	0.1–0.2 × 1, then 0.05–0.1 q24h	0.1–0.2 × 1, then 0.05–0.1 q24h					
9–11		0.05	24	24	24	24	0.07 × 1, then 0.05 q24h	0.07 × 1, then 0.05 q24h					
		0.1–0.15	24	24	24	24	0.1–0.15 q24h	0.1–0.15 q24h	Undetectable				Low
	71	0.2–0.4 IV/PO	24	24	0.1–0.2 q24h	0.1–0.2 q24h	0.1–0.2 q24h	0.1–0.2 q24h	50–94		85		
		0.372 IV/PO	24	24	24	24	0.372 q8h × 2 days, then 0.372 q24h	0.372 q8h × 2 days, then 0.372 q24h					
		0.2	12	12	12	12	0.2 q12h	0.2 q12h	<10				
		0.2–0.4	24	24	24	24	0.2–0.4 q24h	0.2–0.4 q24h	Minimal			Minimal	~10
35	35	0.2[o]	8	8	8	8	0.2 q8h[o]	0.2 q8h[o]					
		0.3[p]	24	24	24	24	0.3 q24h[p]	0.3 q24h[p]					
		0.2	12	12	12[t]	12[t]	12[t]	12[t]	42–67				
		4 mg/kg	12	12	12	12	12	12					
		0.25–0.5	24	24							Unsafe		
		25 mg/kg	6	6	12–-24	24–-48	25–-50 mg/kg q48–-72h	25–-50 mg/kg q48–-72h	60–-100				

Continued

TABLE 54.27 Antiinfective Agent Pharmacology: Antifungal Agents—cont'd

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval		Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
				Oral	Parenteral							
Griseofulvin (50[w],> 50[x])	0.5 PO[w]	0.4–2		0.5–1 q24h[v]		1	10–20 mg/kg/day in 2 doses[v]				24	24
	0.25 PO[x]	0.4–2		0.375–0.75 q24h[w]		0.75	5–15 mg/kg q24h[w]					

[a]Inflamed meninges.
[b]Infused over several hours.
[c]A test dose of 1 mg infused over 15 min is often given to assess febrile reactions before proceeding to higher doses.
[d]Should be administered by slow infusion; rapid IV infusion should be avoided because potentially serious adverse effects (e.g., hypotension, hypokalemia, arrhythmias, shock) may occur.
[e]Or 1.5 mg/kg every other day.
[f]Plasma half-life, terminal half-life.
[g]Experience in children is limited. Not FDA approved for pediatric patients.
[h]Higher doses need for patients younger than 8 years.
[i]Infused over 30 min; ascertained on days 6–7.
[j]When given with meals.
[k]Gastric acid–suppressing agents decrease bioavailability to <5%.
[l]Taken twice daily for 15 days.
[m1]Parent drug, active metabolite (hydroxyitraconazole).
[m2]Half-life extends as dosing continues.
[n]Bioavailability of the suspension is highly dependent on food; administration with a full meal is recommended.
[o]Oral suspension.
[p]Delayed-release tablet.
[q]Decreased rate and/or extent of absorption when given with food.
[r]Administered q12h for 10 days after 1 day of loading doses.
[s]Pediatric patients aged 2–12 years. Adult dosing should be used for pediatric patients older than 12 years.
[t]The intravenous formulation of voriconazole contains cyclodextrins, which may accumulate in renal impairment. Oral dosage formulations should be used whenever possible in these patients.
[u]Recommended doses are based on weight: <20 kg = 67.5 mg; 20–40 kg = 125 mg; >40 kg = 250 mg.
[v]Peak concentrations should be 25 μg/mL to avoid development of resistance but should not exceed 100–120 μg/mL to avoid side effects. Levels should be monitored routinely in renal dysfunction.
[w]Microsize.
[x]Ultramicrosize.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *FDA,* US Food and Drug Administration; *HD,* hemodialysis; *PD,* peritoneal dialysis.

With Dialysis		STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT					Dosage With Dialysis		Body Fluid Concentrations				
			For CrCl Ranges (mL/min)										
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
		0.5–1[v]	24	24	24	24				80			
		0.375–0.75[iv]	24	24	24	24							

TABLE 54.28 Antiinfective Agent Pharmacology: Antiparasitic Agents

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS						
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval		Serious Infection Daily Dose				
				Oral	Parenteral		Oral	Parenteral	Up to 1 wk	1–4 wk
Anthelmintics										
Albendazole (minimal)[b,c]		0.4 PO	1.31[c]	0.4 q12h		0.8	15 mg/kg/day 2 doses			
Praziquantel (80)		40 mg/kg PO	0.8–3.57[d]	75 mg/kg/day in 3 doses		75 mg/kg	75 mg/kg/day in 3 doses[e]			
Diethylcarbamazine		0.5 PO	0.15–0.25	6–10 mg/kg/day in 3 doses		10 mg/kg	6 mg/kg/day in 3 doses			
Ivermectin		50–200 μg/kg PO	0.003–0.008	150–200 μg/kg × 1		200 μg/kg	150–200 μg/kg			
Mebendazole (5–10%)		0.1 PO	0.006–0.051	0.1 q12h		0.2				
Pyrantel pamoate				11 mg/kg[f] × 1		1	11 mg/kg[f] × 1			
Antimalarials										
Artemether-lumefantrine[g]		0.08/0.48 PO	0.06/ 7.38	4 tablets × 6 doses[g]		4 tablets × 6 doses[g]	1–4 tablets × 6 doses[g]			
Artesunate		2.4 mg/kg IV	4–5		2.4 mg/kg × 5 doses[h]	2.4 mg/kg		2.4 mg/kg × 5[h]		
Atovaquone-proguanil		0.5/0.2 q12h PO	0.908/0.17	1/0.4 q24h × 3 days		1–0.4	0.125/0.5 to 1/0.4 q24h × 3 doses[i]			
Chloroquine (89)		1 PO	1.0	0.5 × 5 doses[j]		1	5–10 mg/kg × 5 doses[j]		5 mg/kg × 5 doses[j]	5 mg/kg × 5 doses[j]
Hydroxychloroquine (75)		0.4 PO	1.22 nmol/mL	0.4 × 5 doses[j]		0.8	5–10 mg/kg × 5 doses[j]			
Mefloquine phosphate		12.2–18.3 mg/kg PO	1.8	0.75, then 0.5[k]		1.25	15 mg/kg, then 10 mg/kg[k]			
Primaquine phosphate (96)[l]		0.015 PO	0.053	0.015–0.03 q24h		0.03	0.5 mg/kg q24h[m]			
Quinine sulfate (89)[l]		0.648 PO	3.2	1.944/day in 3 doses		1.95	30 mg/kg/day in 3 doses			
Antiprotozoals										
Atovaquone (23–47)		0.75–1.5 PO	12	1.5/day in 1–2 doses		1.5	30–45 mg/kg/day in 2 doses[n]			
Benznidazole		0.1 PO	2.4	5–7 mg/kg/day in 2 doses[o]		7 mg/kg	5–8 mg/kg/day in 2 doses			
Eflornithine					400 mg/kg/day in 4 doses	400 mg/kg/day		400 mg/kg/day in 4 doses		
Iodoquinol (poor)				0.65 q8h		1.95	30–40 mg/kg/day in 3 doses			
Melarsoprol		3.6 mg/kg IV	4.7–6.7		2–3.6 mg/kg q24h	0.2		2–3.6 mg/kg q24h		
Miltefosine		0.050 q12h[p] PO	66.2	0.05 q8h		0.15	0.05 q8–12h[q]			
Nifurtimox				8–10 mg/kg/day in 3–4 doses		10 mg/kg	8–20 mg/kg/day in 3–4 doses[r]			
Nitazoxanide		0.5 PO	3.0	0.5 q12h		1	0.1–0.5 q12h			
Paromomycin (minimal)				25–35 mg/kg/day in 3 doses		35 mg/kg	25–35 mg/kg/day in 3 doses			
Pentamidine		4 mg/kg IV[s]	0.612		4 mg/kg q24h IM/IV	4 mg/kg		4 mg/kg q24h IM/IV		

SERUM HALF-LIFE (h)				STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT											
With Normal and Anuric CrCl Values (mL/min)		With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
>80	<10	HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
8–12[c]				0.4	12	12	12	12	0.4 q12h	0.4 q12h	25–50				
0.8–3				25 mg/kg	8	8	8	8	25 mg/kg q8h	25 mg/kg q8h	14				
10–12				6–10 mg/kg/ day											
18				150–200 µg/kg × 1											
3–6				0.1	12	12	12	12	0.1 q12h	0.1 q12h					
1.6/ 101				4 tablets × 6 doses[g]	4 tablets × 6 doses[g]	4 tablets × 6 doses[g]									
0.05–0.5				2.4 mg/kg × 5 doses[h]	2.4 mg/kg × 5 doses[h]	2.4 mg/kg × 5 doses[h]									
32–84/ 12–21				1–0.4	24	24									
6–60 days				0.5 × 5 doses[j]	0.5 × 5 doses	0.5 × 5 doses	0.5 × 5 doses	0.25–0.5							
40 days				0.4 × 5 doses[j]	0.4 × 5 doses[j]	0.4 × 5 doses[j]									
10–12 days				0.75, then 0.5[k]	0.75, then 0.5[k]	0.75, then 0.5[k]	0.75, then 0.5[k]	0.75, then 0.5[k]	0.75, then 0.5[k]	0.75, then 0.5[k]	High				
4–7				0.015–0.03	24	24									
9.7–20	26			0.648	8	8	0.324 q12h	0.324 q12h	0.324 q12h						
50–84				0.75	12	12	12	12	0.75 q12h	0.75 q12h	<1				
13													30–279%		
				100 mg/kg	6										
				0.65	8										
35				2–3.6 mg/kg	24										
30 days				0.05	8										
				8–10 mg/kg/ day											
1–1.6 h[c]				0.5	12										
				25–35 mg/kg/ day											
6.4				4 mg/kg	24										

Continued

TABLE 54.28 Antiinfective Agent Pharmacology: Antiparasitic Agents—cont'd

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS					
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval		Serious Infection Daily Dose			
				Oral	Parenteral		Oral	Parenteral	Up to 1 wk / 1–4 wk
Pyrimethamine[t]		0.025–0.075 PO	0.26–4.7	0.05–0.075 q24h		0.75	1 mg/kg q24h		
Sodium Stibogluconate		20 mg/kg IV	9–12		20 mg/kg q24h IM/IV[u]	20 mg/kg/day[u]		20 mg/kg q24h IM/IV[u]	
Suramin[w]					1[x]			20 mg/kg q24h[x]	
Tinidazole		2 PO	42–60	2 q24h		2		50 mg/kg q24h	

[a]Inflamed meninges.
[b]Bioavailability is highly dependent on food. Administer with food.
[c]Rapidly converted to its active metabolite by first-pass hepatic metabolism. Plasma concentrations and pharmacokinetics reflect the active metabolite.
[d]Peak serum concentration increased significantly with impaired hepatic function.
[e]Not approved for children younger than 4 years.
[f]Dose expressed as mg/kg pyrantel base.
[g]A 3-day regimen of 6 doses of the coformulated artemether-lumefantrine (20 mg/120 mg per tablet) is recommended based on weight category (adult and pediatric) as an initial oral dose followed by an oral dose 8 h later, then the oral dose twice a day for the following 2 days. The dose is stratified by weight: 5 to <15 kg (1 tablet per dose); 15 to <25 kg (2 tablets per dose); 25 to <35 kg (3 tablets per dose); ≥35 kg (4 tablets per dose).
[h]5-dose regimen given at 0, 12, 24, 48, and 72 h.
[i]Dose depends on weight.
[j]4-dose regimen given at 0, 6, 24, 48 h. Initial loading dose is double subsequent doses.
[k]Initial dose followed by second dose given 6–12 h later.
[l]Used as part of a combination regimen in the treatment of malaria.
[m]Dose expressed as primaquine base.
[n]Dose depends on age and indication.
[o]FDA approved for children aged 2–12 years. Adult use is off label.
[p]After 4 wk of dosing.
[q]Dose based on weight: 30–44 kg (50 mg q12h); >45 kg (50 mg q8h).
[r]Dose based on age: 1–10 years (15–20 mg/kg/day); 11–16 years (12.5–15 mg/kg/day); >17 years (8–10 mg/kg/day).
[s]2-h infusion.
[t]Pyrimethamine is frequently administered with a loading dose on day 1 of therapy (200 mg adults, 2 mg/kg pediatrics). Leucovorin 10–25 mg/day is generally coadministered to prevent toxicity.
[u]Dose expressed in mg of pentavalent antimony.
[v]Beta elimination half-life, gamma elimination half-life.
[w]Suramin treatment courses require single-dose administrations on days 1, 3, 5, 14, and 21.
[x]Specified dose should be administered after a dialysis session to supplement drug clearance during dialysis.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *FDA,* US Food and Drug Administration; *HD,* hemodialysis; *PD,* peritoneal dialysis.

SERUM HALF-LIFE (h)				STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT											
With Normal and Anuric CrCl Values (mL/min)		With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis	Body Fluid Concentrations					
>80	<10	HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
80–96				0.05–0.075	24										
2, 76[v]				20 mg/kg	24										
50 days				1							1				
13.2				2	24	24	24	24	2 q24h, 1 after HD[y] if usual dose given prior						

TABLE 54.29 Antiinfective Agent Pharmacology: Antiviral Agents

	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF-LIFE (h)			
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)		With Dialysis	
Drug (Oral Absorption, %)	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10	HD	PD
Acyclovir (15–30)[c]	0.2 PO[d]	0.83		0.2–0.8 2–5 times daily	5–10 mg/kg q8h	4 PO	1–4/day in 3–5 doses	30–45 mg/kg/day in 3 doses	60 mg/kg/day in 3 doses	60 mg/kg/day in 3 doses	2.1–3.5	19.5		Minimal change
	0.8 PO[d]	1.61				30 mg/kg IV								
	5 mg/kg IV[g]	7.7												
Adefovir (59)	0.01 PO	0.018		0.01 q24h		0.01	0.01 q24h				7.5			
Amantadine (85–90)	0.1 PO[d]	0.302		0.1 q12h		0.2	5 mg/kg/day in 2 doses				16–17		8.3 days	
Cidofovir	10 mg/kg IV	24			5 mg/kg weekly[h]	5 mg/kg					2.5			
Entecavir[i]	0.5 mg PO	0.004		0.5–1 mg q24h		1 mg					128–149			
	1 mg PO	0.008												
Famciclovir (75–77)	0.5 PO	4		0.25–0.5 q8–12h		1.5					2–2.3			
	0.75 PO	5.1–5.3												
Foscarnet	0.09 IV[d]	218 μmol/L			90 mg/kg q12h[k]	180 mg/kg					0.1–17			
Ganciclovir (5)	5 mg/kg IV[g]	9			5 mg/kg q12h[k]	10 mg/kg/day		5 mg/kg q12h	12 mg/kg/day in 2 doses	12 mg/kg/day in 2 doses	2.5–5	10		
	1.0 PO	0.98												
Oseltamivir (75)	0.075 PO	0.065		0.075 q12h		0.15	0.03–0.075 q12–24 h[m]		3 mg/kg q12h	3 mg/kg q12h	6–10			
Peramivir	0.6 IV	46.8			0.6 × 1	0.6		12 mg/kg × 1			20			
Ribavirin (64)	0.6 q12h PO	2.748		0.4–0.6 q12h		1.2 PO	15 mg/kg/day in 2 doses	6/day inhalation[n]			9.5, 298[o]			
	0.82 mg/kg/h[q]	0.275												
	0.82 mg/kg/h[r]	1.1												
Rimantadine (100)	0.2 PO	0.05–0.086		0.1 q12h		0.2	5 mg/kg/day in 1–2 doses				19.8–36.5			
Telbivudine	0.6 PO[d]	3.69		0.6 q24h		0.6					40–49			
Valacyclovir (55)	2 PO[d]	8.49		0.5–2 q8–12h		4	20 mg/kg q8h				2.5–3.6[s]	20		
Valganciclovir[t] (60)	0.45 PO	3.1		0.9 q12[k]		1.8 PO					3.7–4.6			
Zanamivir (2)	0.01 PO	0.017–0.142			0.01 q12h[u]			0.01 q12h[u]			1.6–5.1			

[a]Not including hepatitis C direct-acting antivirals and antiretrovirals, which are listed in Tables 54.30 and 54.31, respectively.
[b]Inflamed meninges.
[c]Bioavailability decreases as dosage is increased.
[d]At steady state.
[e]Specified dose should be administered after a dialysis session to supplement drug clearance during dialysis.
[f]Supplemental dose after dialysis is recommended based on simulation data when dose is 800 mg 5 times daily in normal renal function.
[g]Infused over 1 h.
[h]Weekly for 2 wk, then every other week.
[i]Cidofovir is contraindicated in preexisting renal impairment (SCr >1.5 mg/dL; CrCl <55 mL/min; urine protein >100 mg/dL).
[j]Decreased rate and/or extent of absorption when given with food.
[k]For 14–21 days as induction therapy. Dose should be reduced for maintenance therapy.
[l]Renal dose adjustments are based on modified Cockroft and Gault equation: ([140 – age/Scr[+] 72]). ([+], 0.85 if female). Refer to package insert for dosing table.
[m]Dosing is based on weight: <15 kg (30 mg q12h); 15.1–23 kg (45 mg q12h); 23.1–40 kg (60 mg q12h); >40 kg (75 mg q12h).
[n]Mist of 190 μg/L via SPAG-2 aerosol generator; rate of 12.5 L mist/min × 12–18 h/day for 3–7 days.
[o]Inhaled, oral.
[p]After administration for 4–7 wk in acquired immunodeficiency syndrome (AIDS) or AIDS-related complex patients.
[q]Inhaled over 5 h each day for 3 days.
[r]Inhaled over 8 h each day for 3 days.
[s]Half-life of valacyclovir is <30 min, but its metabolite, acyclovir, has a half-life of 2.5–3.6 h.
[t]Should be taken with food.
[u]Dose administered by oral inhalation.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; *PD,* peritoneal dialysis; *SCr,* serum creatinine.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT

	For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
Usual Adult Dose (g)	**>80**	**80–50**	**50–10**	**<10 (Anuric)**	**HD (g)**	**PD (g)**	**CSF/ Serum (%)[b]**	**Newborn Serum/ Maternal Serum (%)**	**Breast Milk/ Maternal Serum (%)**	**Bile/ Serum (%)**	**Aqueous Humor/ Serum (%)**
0.2–0.8 PO	2–5 times daily	2–5 times daily	2–5 times daily	0.2 q12h	0.2 q12h, 0.4[e] after HD[f]	0.2 q12h	50		≥100		37
5 mg/kg IV	8	8	12–24	2.5 mg/kg q24h	2.5 mg/kg q24h	2.5 mg/kg/day					
0.01	24	24	48–72	0.01 weekly	0.01 weekly	0.01 weekly					
0.1	12	12–24	48 – weekly	0.2 weekly	0.2 weekly	0.2 weekly	50				
5 mg/kg	weekly	weekly	Do not use[i]	Do not use[i]	Do not use[i]	Do not use[i]					
0.5–1.0 mg	24	24	48–72	weekly	0.5–1 weekly	0.5–1 weekly					
0.25–0.5	8–12	8–12	0.125–0.5 q12–24h	0.125–0.25 q24h	0.125–0.25 after HD[e]						
90 mg/kg[k,l]					45–60 mg/kg after HD[e]		13–103				
5 mg/kg[k]	12	2.5 mg/kg q12h	1.25–2.5 mg/kg q24h	1.25 mg/kg thrice weekly	1.25 mg/kg thrice weekly after HD[e]	1.25 mg/kg thrice weekly	24–68		40		
0.075	12	12	0.03 q12–24h	0.03 q48h	0.03 after HD[e]	0.03 q48h					
0.6 × 1	× 1	× 1	0.1–0.2 × 1	0.1 × 1	0.1 × 1 after HD						
0.4–0.6	12	12	0.2–0.4 q24h	0.2 q24h	0.2 q24h		70[p]				
0.1	12	12	12–24	24	0.1 q24h	0.1 q24h					
0.6	24	24	48–72	72	0.6 q96h	0.6 q96h					
0.5–1	8–12	8–12	12–24	0.5 q24h	0.5 q24h	0.5 q24h		170			
0.9[k]	12	12	0.45 q12–48h	Do not use	Do not use	Do not use					
0.01	12	12	12	12	0.05 q12h	0.05 q12h					

TABLE 54.30 Antiinfective Agent Pharmacology: Hepatitis C Direct-Acting Antivirals

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS						SERUM HALF-LIFE (h)	
				Adults			Children: Dose/Interval	Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
				Dose (g)/Interval		Serious Infection Daily Dose (g)					
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Oral	Parenteral		Oral Parenteral	Up to 1 wk	1–4 wk	>80	<10
Daclatasvir (67)				0.06 q24		0.06				12–15	
Elbasvir-grazoprevir[b]				1 tablet q24h		1 tablet				24/31	
Glecaprevir-pibrentasvir[c]	0.3/0.12 q24h PO[d]	0.725/0.018		3 tablets q24h		3 tablets				6–10/13–27	
Ombitasvir-paritaprevir-ritonavir[e] (48.1/52.6)[f]	0.0125/0.075/0.05 q24h PO[d]	0.082/0.194/0.543		2 tablets q24h		2 tablets				21–25/5.5/4	
Ombitasvir-paritaprevir-ritonavir- dasabuvir (48/53/70)[g]	0.0125/0.075/0.05 + 0.25 q24h PO[d,h]	0.068/0.262/0.682/0.667		1 kit q24h[h] 3 tablets q24h[i]		1 kit[h] 3 tablets[i]				21–25/5.5/4/5.5–6	
Simeprevir (62)	0.2 q24 PO[d]	10.9		0.15 q24h		0.15				10–13[j]	
Sofosbuvir	0.4 PO	1.26		0.4 q24h		0.4				0.4, 27[k]	
Sofosbuvir- ledipasvir[l]	0.4/0.09 q24h PO[d]	0.618/0.323		1 tablet q24h		1 tablet				0.5/47	
Sofosbuvir-velpatasvir[m]	0.4/0.1 PO q24h[d]	0.567/0.259		1 tablet q24h		1 tablet				0.5/15	
Sofosbuvir-velpatasvir-voxilaprevir[n]	0.4/0.1/0.1 q24h PO[d]	0.678/0.311/0.192		1 tablet q24h		1 tablet				0.5/17/33	

[a]Inflamed meninges.
[b]One tablet contains 50 mg of elbasvir and 100 mg of grazoprevir.
[c]One tablet contains 100 mg of glecaprevir and 40 mg of pibrentasvir.
[d]In subjects with HCV infection.
[e]One tablet contains 12.5 mg of ombitasvir, 75 mg of paritaprevir, and 50 mg of ritonavir.
[f]Bioavailability of ombitasvir-paritaprevir.
[g]Bioavailability of ombitasvir-paritaprevir-ritonavir reported for bilayer tablet.
[h]Kit composed of 2 tablets containing 50 mg of dasabuvir and 2 tablets containing 12.5 mg of ombitasvir, 75 mg of paritaprevir, and 50 mg of ritonavir. Dosing consists of 2 combination tablets in the morning and 1 tablet of dasabuvir twice daily in the morning and evening.
[i]Bilayer tablet composed of an extended-release layer containing 200 mg of dasabuvir and an immediate-release layer containing 8.33 mg of ombitasvir, 50 mg of paritaprevir, and 33.33 mg of ritonavir.
[j]May be prolonged up to 41 h in patients with hepatitis C.
[k]Parent drug, primary metabolite.
[l]One tablet contains 400 mg of sofosbuvir and 90 mg of ledipasvir.
[m]One tablet contains 400 mg of sofosbuvir, 100 mg of velpatasvir.
[n]One tablet contains 400 mg of sofosbuvir, 100 mg of velpatasvir, and 100 mg of voxilaprevir.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HCV,* hepatitis C virus; *HD,* hemodialysis; *PD,* peritoneal dialysis.

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT													
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD(g)	PD (g)	CSF/Serum (%)[a]	Newborn Serum/Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/Serum (%)	Aqueous Humor/ Serum (%)
		0.06	24	24	24	24	0.06 q24h	0.06 q24h					
		1 tablet	24	24	24	24	1 tablet q24h	1 tablet q24h					
		3 tablets	24	24	24	24	3 tablets q24h	3 tablets q24h					
		2 tablets	24	24	24								
		1 kit[h]	24	24	24								
		3 tablets[i]	24	24	24	24	3 tablets[i] q24h	3 tablets[i] q24h					
		0.15	24	24	24	24	0.15 q24h	0.15 q24h					
		0.4	24	24	24								
		1 tablet	24	24	24								
		1 tablet	24	24	24								
		1 tablet	24	24	24								

TABLE 54.31 Antiinfective Agent Pharmacology: Antiretroviral Agents

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS							SERUM HALF–LIFE (h)	
				Adults			Children: Dose/Interval		Newborn (Parenteral): Dose/Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval		Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
				Oral	Parenteral							
Abacavir (83)	0.3 PO	3.3		0.6/day in 1–2 doses		0.6	16 mg/kg/day in 1–2 doses				1.2	1.2
Atazanavir (60–70%)[b]	0.4 PO	5.226		0.4 q24h 0.3 q24h[c]		0.4	0.15–0.3 mg/kg q24h[c,d]				5–8	
Cobicistat	0.15 PO	0.99		0.15 q24h		0.15					3–4	
Darunavir (82)[b,e]	0.6 PO			0.8 q24[c] 0.6 q12h[c]		1.2	0.2–0.6 q12h[c,d]				15	
Delavirdine (60–100)	0.4 PO[f]	356		0.4 q8h		1.2					2–11	
Didanosine[g] (21–43)[h,i]	33 mg/kg	29.8 μmol/L		0.4/day in 1–2 doses[j]		0.4	120 mg/m^2 q12h			50 mg/m^2 q12h PO	1.3–1.6	4.5
Dolutegravir	0.05 PO	3.67		0.05 q12–24h[k]		0.1	0.035–0.05[d,k]				14	
Efavirenz	0.6 PO[f]	4.5		0.6 q24h		0.6	0.1–0.6 q24h[d]				40–55	
Elvitegravir	0.15 PO	1.7		0.15 q24h[l]		0.15 q24h[l]					12.9	
Emtricitabine (93)	0.2 PO[n] 6 mg/kg[o]	2.1 1.9		0.2 q24h[n] 0.24 q24h[o]		0.2[n] 0.24[o]	0.2 q24h[d,n] 6 mg/kg q24h[o]		3 mg/kg q24h[o] PO	3 mg/kg q24h[o] PO	2.5–7	
Enfuvirtide	0.09 SQ	4.59			0.09 q12h SQ	0.18		2 mg/kg q12h SQ				
Etravirine[b]	0.2 q12h PO	0.297		0.2 q12h		0.4	0.1–0.2 q12h[d]				41	
Fosamprenavir	1.4 PO[e]	7.9		1.4 q12h[p]		2.8	18–45 mg/kg q12h[d,e]				7.7	7.7
Indinavir[g] (30)	0.8 PO[f,j]	12,617 nmol/L		0.8 q8h		2.4					1.5–2	1.5–2
Lamivudine (82–87)	0.15 q12h PO	1.4		0.3/day in 1–2 doses		0.3	4 mg/kg q12h		2 mg/kg q12h PO	2 mg/kg q12h PO	3–7	
Lopinavir-ritonavir[b]	0.4/0.05 PO	6[bb]		0.4/0.1 q12h		0.8/0.2	0.3/0.075 mg/m^2 q12h			0.3/0.075 mg/m^2 q12h PO	5–6	
Maraviroc (23–33)	0.3 PO[f]	0.888		0.15–0.6 q12h[q]		1.2	0.05–0.3 q12h[d,q]				14–18	
Nelfinavir[b]	0.75 PO[f]	2.9		1.25 q12h		2.5	45–55 mg/kg q12h				3.5–5	3.5–5
Nevirapine (> 90)	0.4 PO	2.9–3.4		0.2 q12h[s]		0.4	120–200 mg/m^2 q12h[t]		4–6 mg/kg q12h PO	6 mg/kg q12h PO	25–30	25–30
Raltegravir	0.4 q12h PO[v]	4.5		0.4 q12h		0.8–1.2[u]	0.02–0.1 q12h[d]				9	9
Rilpivirine	0.025 PO	0.079		0.025 q24h		0.025					50	
Ritonavir (80)[v,w]	0.6 PO 1.2 PO	5 11.2		0.1–0.4/day in 1–2 doses		0.4					3–3.5	3–3.5
Saquinavir[b]	0.6 PO	0.066		1 q12h[e]		2					13	13
Stavudine (80)	4 mg/kg PO	4.2		0.04 q12h[x]		0.08	2 mg/kg/day[y]		0.5 mg/kg q12h PO	1 mg/kg q12h PO	0.9–1.6	5.7
Tenofovir alafenamide fumarate	0.0225 q24h PO	0.03		0.025 q24h		0.025					0.51	

STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT													
With Dialysis			For CrCl Ranges (mL/min)				Dosage With Dialysis		Body Fluid Concentrations				
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
		0.3–0.6	12–24	12–24	12–24	12–24	0.6/d in 1–2 doses	0.6/d in 1–2 doses	18–33				
		0.4[c]	24	24	24	24							
		0.15	24	24	24	24	0.15 q24h	0.15 q24h					
		0.8[c] 0.6[c]	24 12	24 12	24 12	24 12	0.8 q24h[c] 0.6 q24h[c]	0.8 q24h[c] 0.6 q24h[c]					
		0.4	8	8	8	8	0.4 q8h	0.4 q8h	0.4				
		0.4/day[j]	1–2 doses	1–2 doses	0.1–0.2 q24h	0.075–0.125 q24h	0.075–0.1 q24h	0.075–0.1 q24h	21–46				
		0.05	12–24	12–24	12–24	12–24	0.05 q12–24h	0.05 q12–24h	<2				
10		0.6	24	24	24	24	0.6 q24h	0.6 q24h	1.19				
		0.15[l]	24	24[m]	Do not use	Do not use	Do not use	Do not use					
		0.2[n] 0.24[o]	24 24	24 24	48–72 0.08–0.12 q24h	96 0.06 q24h	0.2 q96h[n] 0.06 q24h[o]	0.2 q96h[n] 0.06 q24h[o]					
		0.09	12	12	12	12	0.09 q12h SQ	0.09 q12h SQ					
		0.2	12	12	12	12	0.2 q12h	0.2 q12h					
		1.4[p]	12	12	12	12	1.4 q12h[p]	1.4 q12h[p]					
		0.8	8	8	8	8	0.8 q8h	0.8 q8h	5–9	Unsafe			
		0.15–0.3	12–24	12–24	0.05–0.15 q24h	0.025 q24h	0.025 q24h		6–11	~100	~100		
		0.4/0.1	12	12	12	12	0.4/0.1 q12h	0.4/0.1 q12h					
		0.15–0.6	12	12	0.3 q12h[r]	0.3 q12h[r]	0.3 q12h[r]						
3.5–5	3.5–5	1.25	12	12	12	12	1.25 q12h	1.25 q12h	0				
		0.2[s]	12	12	12	12	0.2 q12h	0.2 q12h		60			
		0.4	12	12	12	12	0.4 q12h	0.4 q12h					
		0.025	24	24	24	24	0.025 q24h	0.025 q24h					
		0.1–0.4	12–24	12–24	12–24	12–24	0.1–0.4/day in 1–2 doses	0.1–0.4/day in 1–2 doses	0.2				
		1	12	12	12	12	1 q12h	1 q12h	0.2				
5.3		0.04[x]	12	12	0.02 q12–24h	0.02 q24h[x]	0.02 q24h[x]		24–94				
		0.025	24	24	24								

Continued

TABLE 54.31 Antiinfective Agent Pharmacology: Antiretroviral Agents—cont'd

Drug (Oral Absorption, %)	Serum and Urine Concentration: Selected Doses			DOSAGE RECOMMENDATIONS						SERUM HALF–LIFE (h)	
				Adults			Children: Dose/ Interval		Newborn (Parenteral): Dose/ Interval		With Normal and Anuric CrCl Values (mL/min)	
	Dose (g)	Peak Serum (μg/mL)	Peak or Range, Urine (μg/mL)	Dose (g)/Interval Oral	Dose (g)/Interval Parenteral	Serious Infection Daily Dose (g)	Oral	Parenteral	Up to 1 wk	1–4 wk	>80	<10
Tenofovir disoproxil fumarate[b] (40)	0.3 PO	0.296		0.3 q24h		0.3	8 mg/kg q24h				4–8	
Tipranavir[b]	0.5 PO[e]	94.8 μmol/L		0.5 q12h[e]		1	14 mg/kg q12h[e]				4.8–6	
Zidovudine (50–76)[z]	2 mg/kg PO 1 mg/kg IV	0.41–0.54 0.4–0.68		0.3 q12h	1 mg/kg q4h[aa]	0.6	9–12 mg/kg[d]		2–4 mg/kg PO	2–4 mg/kg PO	0.5–3	1.4

[a]Inflamed meninges.
[b]Give with food.
[c]Administered with ritonavir or cobicistat as a boosting agent.
[d]Dose in pediatric patients depends on weight. Refer to guidelines or prescribing information for specific dosing nomograms.
[e]When administered with ritonavir.
[f]At steady state.
[g]Decreased rate and/or extent of absorption when given with food.
[h]Bioavailability decreases as dosage is increased.
[i]In human immunodeficiency virus (HIV)–positive patients.
[j]Delayed-release tablets administered once daily. Oral solution can be administered once daily or divided twice daily; however, divided dosing is preferred. Dose reduction is necessary for weight <60 kg and with concomitant tenofovir therapy.
[k]Administered once a day in HIV-1 integrase strand transfer inhibitor (INSTI)–naïve patients but should be used twice daily in INSTI experience with certain or clinically suspected resistance substitutions or used in combination with UGT1A/CYP3A inhibitors.
[l]Single-agent elvitegravir is no longer available. Dosing reflects the elvitegravir component of combination tablets, which is boosted with cobicistat.
[m]Renal adjustments for combination tablets depend on the tenofovir component: Tablets with tenofovir disoproxil fumarate should not be initiated with CrCl <70 mL/min and should be stopped if CrCl declines to <50 mL/min. Tablets containing tenofovir alafenamide fumarate should not be used with CrCl <30 mL/min.
[n]Capsule.
[o]Oral solution.
[p]Alternative dosage regimens are available with the addition of ritonavir. The preferred regimen for protease inhibitor–experienced patients is fosamprenavir 700 mg with ritonavir 100 mg twice daily.
[q]Dose depends on concomitant medications and resulting drug-drug interactions. Refer to guidelines or prescribing information to determine dose.
[r]Reduce dose to 150 mg q12h if postural hypotension occurs. Maraviroc is not recommended with CrCl <30 mL/min if patient is taking potent CYP3A inducers or inhibitors.
[s]Once-daily administration is possible with the extended-release formulation.
[t]Dose in pediatric patients depends on age.
[u]Isentress HD formulation can be administered as 1200 mg once daily. Other formulations may not be substituted for Isentress HD for this dosing.
[v]In animals.
[w]Ritonavir has antiretroviral activity but is no longer used as an antiretroviral. Doses listed reflect those used in combination with other antiretrovirals for pharmacologic boosting effect.
[x]Dosage should be reduced for patients weighing <60 kg.
[y]Pediatric patients weighing >30 kg should receive the adult dose for <60 kg.
[z]Reaches systemic circulation as unchanged drug.
[aa]An IV dose of 1 mg/kg q4h is equivalent to an oral dose of 100 mg q4h.
[bb]Lopinavir.
CrCl, Creatinine clearance; *CSF,* cerebrospinal fluid; *HD,* hemodialysis; PD, peritoneal dialysis.

With Dialysis		STANDARD DOSE WITH DOSING INTERVALS IN RENAL IMPAIRMENT					Dosage With Dialysis		Body Fluid Concentrations				
			For CrCl Ranges (mL/min)										
HD	PD	Usual Adult Dose (g)	>80	80–50	50–10	<10 (Anuric)	HD (g)	PD (g)	CSF/ Serum (%)[a]	Newborn Serum/ Maternal Serum (%)	Breast Milk/ Maternal Serum (%)	Bile/ Serum (%)	Aqueous Humor/ Serum (%)
		0.3	24	24	48–96		0.3 weekly						
		0.5	12	12	12	12	0.5 q12h	0.5 q12h					
		0.3	12	12	12	24	0.3 q24h	0.3 q24h	50–70	100	100		

TABLE 54.32 Key Drug Substrates, Organized by Cytochrome P450 (CYP) Drug Metabolizing Isoenzymes

CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A	
amitriptyline	bupropion	amodiaquine	amitriptyline	carisoprodol	alprenolol	acetaminophen	alfentanil	lidocaine
caffeine	cyclophosphamide	**dasabuvir**	celecoxib	**chloramphenicol**	amitriptyline	chlorzoxazone	alprazolam	lovastatin
clomipramine	**efavirenz**	cerivastatin	diclofenac	citalopram	amphetamine	enflurane	amlodipine	**maraviroc**
clozapine	ifosfamide	paclitaxel	fluoxetine	clomipramine	aripiprazole	ethanol	aprepitant	midazolam
cyclobenzaprine	methadone	torsemide	fluvastatin	cyclophosphamide	atomoxetine	halothane	aripiprazole	nateglinide
estradiol		repaglinide	glipizide	esomeprazole	carvedilol	isoflurane	**artemisinins**	**nelfinavir**
fluvoxamine			glyburide	hexobarbital	chlorpheniramine	methoxyflurane	astemizole	nifedipine
haloperidol			ibuprofen	indomethacin	chlorpromazine	sevoflurane	**atazanavir**	nisoldipine
mexiletine			irbesartan	lansoprazole	clomipramine		atorvastatin	nitrendipine
naproxen			lornoxicam	moclobemide	codeine		**bedaquiline**	ondansetron
olanzapine			losartan	**nelfinavir**	debrisoquine		buspirone	**paritaprevir**
ondansetron			meloxicam	nilutamide	desipramine		Cafergot	pimozide
phenacetine			nateglinide	omeprazole	dexfenfluramine		chlorpheniramine	**praziquantel**
propranolol			omeprazole	pantoprazole	dextromethorphan		cilostazol	progesterone
riluzole			phenytoin	phenobarbital	duloxetine		**clarithromycin**	quetiapine
ropivacaine			piroxicam	phenytoin	encainide		cocaine	quinidine
tacrine			rosiglitazone	primidone	flecainide		cyclosporine	**quinine**
theophylline			rosuvastatin	progesterone	fluoxetine		**daclatasvir**	risperidone
tizanidine			S-warfarin	**proguanil**	fluvoxamine		**dapsone**	**ritonavir**
verapamil			tamoxifen	propranolol	haloperidol		**darunavir**	salmeterol
R-warfarin			tolbutamide	rabeprazole	imipramine		dexamethasone	**saquinavir**
zileuton			torsemide	teniposide	lidocaine		dextromethorphan	sildenafil
zolmitriptan				**voriconazole**	methoxyamphetamine		diltiazem	**simeprevir**
					metoclopramide		docetaxel	simvastatin
					paroxetine		domperidone	sirolimus
					perphenazine		**elbasvir**	tacrolimus
					propafenone		eplerenone	tadalafil
					S-metoprolol		**erythromycin**	taxol
					thioridazine		estradiol	terfenadine
					timolol		**etravirine**	terfenadine
							felodipine	testosterone
							fentanyl	**tipranavir**
							finasteride	trazodone
							fosamprenavir	triazolam
							grazoprevir	vardenafil
							haloperidol	verapamil
							hydrocortisone	vincristine
							imatinib	zaleplon
							indinavir	ziprasidone
							irinotecan	zolpidem

[a]Antiinfectives are **bold.**

TABLE 54.33 Drug Inhibitors by Cytochrome P450 (CYP) Drug Metabolizing Isoenzymes

CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A
amiodarone cimetidine **ciprofloxacin** fluvoxamine **interferon** methoxsalen	ticlopidine thiotepa	gemfibrozil montelukast pioglitazone quercetin rosiglitazone **trimethoprim**	amiodarone fenofibrate **fluconazole** fluvastatin fluvoxamine **isoniazid** probenecid sertraline **sulfamethoxazole** teniposide **voriconazole** zafirlukast	cimetidine esomeprazole felbamate fluoxetine fluvoxamine indomethacin **ketoconazole** lansoprazole modafinil omeprazole oxcarbazepine pantoprazole probenecid rabeprazole ticlopidine topiramate	amiodarone bupropion celecoxib chlorpheniramine chlorpromazine cimetidine citalopram clemastine clomipramine cocaine diphenhydramine doxepin doxorubicin duloxetine escitalopram fluoxetine hydroxyzine metoclopramide mibefradil midodrine moclobemide paroxetine perphenazine quinidine ranitidine **ritonavir** sertraline terbinafine ticlopidine	disulfiram	amiodarone aprepitant cimetidine **clarithromycin** **cobicistat** **delavirdine** diltiazem **elvitegravir** **erythromycin** **fluconazole** fluvoxamine gestodene imatinib **indinavir** **isavuconazonium** **itraconazole** **ketoconazole** mibefradil mifepristone nefazodone **nelfinavir** norfluoxetine **posaconazole** **ritonavir** **saquinavir** verapamil **voriconazole**

[a]Antiinfectives are **bold.**

TABLE 54.34 Drug and Chemical Inducers by Cytochrome P450 (CYP) Drug Metabolizing Isoenzymes

CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A
cigarette smoking marijuana smoking modafinil **nafcillin**	phenobarbital **rifampin**	**rifampin**	**rifampin** secobarbital	carbamazepine norethindrone prednisone **rifampin**	dexamethasone **rifampin**	ethanol **isoniazid**	barbiturates carbamazepine **efavirenz** glucocorticoids modafinil **nevirapine** oxcarbazepine phenobarbital phenytoin pioglitazone **rifabutin** **rifampin** St. John's wort

[a]Antiinfectives are **bold.**

TABLE 54.35 Select Drug Transporter Localization With Representative Substrates, Inducers, and Inhibitors

FAMILY	ATP-BINDING CASSETTE (ABC) TRANSPORTERS		SOLUTE-LIKE CARRIER (SLC) TRANSPORTERS				
SUBGROUP			ORGANIC ANION TRANSPORTERS (OAT)		ORGANIC ANION TRANSPORTER POLYPEPTIDE (OAT)		ORGANIC CATION TRANSPORTERS (OCT)
TRANSPORTER	BREAST CANCER RESISTANCE PROTEIN (BCRP)	P-GLYCOPROTEIN (P-gp)	OAT1	OAT3	OATP1B1	OATP1B3	OCT2
Organ or tissue localization	breast intestine liver placenta	adrenal brain intestine kidney liver placenta testes	brain kidney	brain kidney	liver	liver	brain kidney
Substrates	**dasabuvir**, **delafloxacin, glecaprevir**, imatinib, irinotecan, lapatinib, **ledipasvir**, methotrexate, mitoxantrone, **ombitasvir, paritaprevir, pibrentasvir**, rosuvastatin, **sofosbuvir**, sulfasalazine, topotecan, **velpatasvir**, **voxilaprevir**	colchicine, **dasabuvir**, **delafloxacin**, digoxin, fexofenadine, **glecaprevir**, **indinavir**, **ledipasvir**, **ombitasvir, paritaprevir,** paclitaxel**, pibrentasvir**, **sofosbuvir**, topotecan, vincristine, **velpatasvir**, **voxilaprevir**	**adefovir**, captopril, furosemide, **lamivudine**, methotrexate, **oseltamivir**, **tenofovir**, zalcitabine, **zidovudine**	**acyclovir**, bumetanide, **ciprofloxacin**, famotidine, furosemide, methotrexate, **oseltamivir carboxylate**, **penicillin G**, pravastatin, rosuvastatin, sitagliptin, **zidovudine**	atrasentan, atorvastatin, bosentan, ezetimibe, fluvastatin, glyburide, olmesartan, **paritaprevir**, pitavastatin, pravastatin, repaglinide, **rifampin**, rosuvastatin, simvastatin, valsartan, **voxilaprevir**	atorvastatin, **glecaprevir**, **grazoprevir,** olmesartan, **paritaprevir**, pitavastatin, rosuvastatin, telmisartan, valsartan, **voxilaprevir**	**amantadine**, amiloride, cimetidine, dopamine, famotidine, memantine, metformin, oxaliplatin, pindolol, procainamide, ranitidine, varenicline
Inhibitors	cyclosporine, **daclatasvir**, **dasabuvir**, eltrombopag, **glecaprevir**, gefitinib, **ledipasvir**, **paritaprevir**, **pibrentasvir**, **tedizolid, velpatasvir**, **voxilaprevir**	amiodarone, **azithromycin**, captopril, carvedilol, **clarithromycin**, conivaptan, cyclosporine, diltiazem, dronedarone, **erythromycin**, felodipine, **glecaprevir**, **itraconazole**, **ketoconazole**, **pibrentasvir**, quercetin, quinidine, ranolazine, **ritonavir**, **velpatasvir**, verapamil, **voxilaprevir**	**cefadroxil**, **cefamandole**, **cefazolin,** probenecid	cimetidine, diclofenac, probenecid	**atazanavir**, cyclosporine, **daclatasvir**, eltrombopag, gemfibrozil, **paritaprevir**, **rifampin**, **ritonavir**, **saquinavir**, **tipranavir, velpatasvir**, **voxilaprevir**	**atazanavir**, cyclosporine, **daclatasvir, glecaprevir, paritaprevir, pibrentasvir, rifampin**, **ritonavir**, **saquinavir**, **velpatasvir**, **voxilaprevir**	cimetidine, quinidine
Inducers	Not yet identified	avasimibe, carbamazepine, phenytoin, **rifampin**, St. John's wort, **tipranavir-ritonavir**	Not yet identified	Not yet identified	Not yet identified	Not yet identified	Not yet identified

[a]Antiinfectives are **bold.**

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Abacavir With		
Amprenavir	Increased amprenavir effect, toxicity	Increased bioavailability
Methadone	Decreased methadone effect	Mechanism not established
Ribavirin	Increased risk of lactic acidosis	Mechanism not established
Acyclovir, Valacyclovir With		
Aminoglycosides	Increased nephrotoxicity and/or neurotoxicity	Mechanism not established
Cimetidine	Increased acyclovir toxicity	Mechanism not established
Cyclosporine	Increased risk of nephrotoxicity	Mechanism not established
Meperidine	Increased meperidine effect	Decreased renal excretion
Phenytoin	Decreased phenytoin effect	Altered GI transit, pH
Probenecid	Possible increased acyclovir toxicity	Decreased renal excretion
Valproic acid	Decreased valproic acid effect	Altered GI transit, pH
Zidovudine	Increased neurotoxicity (profound drowsiness and lethargy)	Additive toxicity
Albendazole With		
Ginseng	Reduced intestinal albendazole concentration	Increased metabolism
Amantadine With		
Anticholinergics	Hallucinations, confusion, nightmares	Mechanism not established
Antihistamines	Increased CNS adverse reactions	Additive anticholinergic effects
Bupropion	Increased adverse events	Mechanism not established
CNS stimulants	Additive CNS stimulant effects	Mechanism not established
Triamterene	CNS toxicity	Decreased renal clearance
Trimethoprim	CNS toxicity	Decreased renal clearance
Aminoglycoside Antibiotics With		
Acyclovir	Increased nephrotoxicity and/or neurotoxicity	Additive toxicity
Amphotericin B	Increased nephrotoxicity	Synergistic toxicity
Anticoagulants, oral	Potentiation of anticoagulation effects	Decreased GI absorption or synthesis of vitamin K
Bacitracin	Increased nephrotoxicity	Additive toxicity
Capreomycin	Increased nephrotoxicity and/or neurotoxicity	Additive toxicity
Carboplatin	Increased ototoxicity	Additive toxicity
Cidofovir	Increased nephrotoxicity	Additive toxicity
Cisplatin	Increased nephrotoxicity	Mechanism not established
Cyclosporine	Increased nephrotoxicity	Possibly additive or synergistic toxicity
Digoxin	Probable decreased digoxin effect with oral gentamicin or neomycin	Decreased absorption
Loop diuretics	Increased ototoxicity and nephrotoxicity	Additive toxicity
Magnesium sulfate	Increased neuromuscular blockade	Additive toxicity
Methotrexate	Possible increased methotrexate toxicity with kanamycin Possible decreased methotrexate effect with oral aminoglycosides	Mechanism not established Decreased absorption
Methoxyflurane	Increased nephrotoxicity	Additive toxicity
Neuromuscular blocking agents	Neuromuscular blockade	Additive toxicity
NSAIDs	Possible aminoglycoside toxicity in preterm infants with indomethacin given for patent ductus closure	Decreased renal clearance
Polymyxins	Falsely low aminoglycoside levels Increased nephrotoxicity; neuromuscular blockade	In vitro inactivation Additive toxicity
Tacrolimus	Increased nephrotoxicity	Additive toxicity
Vancomycin	Possible increased nephrotoxicity and ototoxicity	Additive toxicity
Aminosalicylic Acid With		
Anticoagulants, oral	Enhanced hypoprothrombinemic effects	Mechanism not established
Ammonium chloride	Increased probability of crystalluria	Acidification of urine
BCG vaccine	Negates BCG vaccine effect	Negates immune response

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Digoxin	Decreased digoxin effect	Decreased absorption
Diphenhydramine	Decreased effect of aminosalicyclic acid	Decreases absorption
Ethionamide	GI distress, hepatotoxicity	Additive toxicity
Isoniazid	Increased isoniazid serum concentrations	Decreased metabolism
Probenecid	Increased aminosalicyclic acid toxicity	Decreased renal excretion
Rifampin	Rifampin effectiveness may be decreased; separate doses by 8–12 h	Decreased absorption due to excipient bentonite
Amphotericin B With		
Aminoglycoside antibiotics	Nephrotoxicity	Synergistic toxicity
Antineoplastics	Possible increased renal toxicity, bronchospasm, and hypotension	Mechanism not established
Capreomycin	Increased nephrotoxicity	Additive toxicity
Cidofovir	Increased nephrotoxicity	Additive toxicity
Cisplatin	Increased nephrotoxicity	Additive toxicity
Corticosteroids	Increased hypokalemia	Additive toxicity
Cyclosporine	Increased renal toxicity	Possible synergism
Digoxin	Increased digoxin toxicity	Hypokalemia
Imidazole antifungals	Possible antagonism in animal models	Mechanism not established
Methoxyflurane	Increased nephrotoxicity	Additive toxicity
Neuromuscular blocking agents	Increased neuromuscular blocking effects	Hypokalemia
Pentamidine	Increased nephrotoxicity	Additive toxicity
Polymyxins	Increased nephrotoxicity	Additive toxicity
Tacrolimus	Increased nephrotoxicity	Additive toxicity
Vancomycin	Increased nephrotoxicity	Additive toxicity
Zidovudine	Potential for increased myelotoxicity and nephrotoxicity	Mechanism not established
Anidulafungin With		
Cyclosporine	Increased anidulafungin exposure	Mechanism not established
Artemisinins With		
CYP3A inducers	Decreased artemether-lumefantrine exposure	Increased metabolism
CYP3A inhibitors	Increased artemether-lumefantrine exposure	Decreased metabolism
Atovaquone With		
Efavirenz	Decreased atovaquone exposure	Mechanism not established
Rifamycins	Decreased atovaquone exposure	Mechanism not established
Azithromycin With		
Aluminum-magnesium antacids	Decreased peak; no effect on overall exposure	Decreased absorption
Cyclosporine	Possible increased cyclosporine effect, toxicity	Mechanism not established
Digoxin	Increased digoxin concentrations	Destruction of intestinal *Eubacterium lentum* in 10% of digoxin patients
Nelfinavir	Increased azithromycin exposure	P-gp competition
Warfarin	Possible increased warfarin effect, toxicity	Decreased vitamin K–producing gut flora
Aztreonam With		
Chloramphenicol	Possible in vitro antagonism; administer a few hours apart	Mechanism not established
Bacitracin With		
Aminoglycosides	Increased nephrotoxicity	Additive toxicity
Anesthetics	Potentiation of neuromuscular blocking effects	Additive toxicity
Neuromuscular blocking drugs	Potentiation of neuromuscular blocking effects	Additive toxicity
Polymyxins	Increased nephrotoxicity	Additive toxicity
Bedaquiline With		
CYP3A inducers	Decreased bedaquiline exposure	Increased metabolism
CYP3A inhibitors	Increased bedaquiline exposure	Decreased metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Capreomycin With		
Aminoglycosides	Increased nephrotoxicity and/or ototoxicity	Additive toxicity
BCG vaccine	Negates BCG vaccine effect	Negates immune response
Polymyxins	Increased nephrotoxicity	Additive toxicity
Vancomycin	Increased nephrotoxicity and/or ototoxicity	Additive toxicity
Carbapenems With		
Cyclosporine	Increased cyclosporine and imipenem-cilastatin exposures, toxicities	Mechanism not established
Ganciclovir	Increased seizure risk with imipenem-cilastatin	Mechanism not established
Probenecid	Increased carbapenem exposure	Decreased tubular secretion
Theophylline	Increased CNS toxicity risk with imipenem-cilastatin	Mechanism not established
Valproic acid	Decreased valproic acid exposure	Mechanism not established
Caspofungin With		
Carbamazepine	Decreased caspofungin effect	Increased metabolism
Cyclosporine	Increased caspofungin effect, toxicity	Mechanism not established
Dexamethasone	Decreased caspofungin effect	Increased metabolism
Efavirenz	Decreased caspofungin effect	Increased metabolism
Nelfinavir	Decreased caspofungin effect	Increased metabolism
Nevirapine	Decreased caspofungin effect	Increased metabolism
Phenytoin	Decreased caspofungin effect	Increased metabolism
Rifamycins	Decreased caspofungin effect	Increased metabolism
Tacrolimus	Decreased tacrolimus effect	Mechanism not established
Cefditoren With		
Antacids	Decreased cefditoren effect	Decreased bioavailability
H_2 receptor antagonists	Decreased cefditoren effect	Decreased bioavailability
Proton pump inhibitors	Decreased cefditoren effect	Decreased bioavailability
Cephalosporins With		
Alcohol	Disulfiram-like effect with cefotetan	Inhibition of intermediary metabolism of alcohol
Ampicillin	In vitro antagonism with ceftazidime versus group B streptococci and *Listeria*	Mechanism not established
Chloramphenicol	In vitro antagonism	Mechanism not established
Diuretics	Increased nephrotoxicity with some cephalosporins	Mechanism not established
Polymyxins	Increased nephrotoxicity	Additive toxicity
Probenecid	Higher and prolonged cephalosporin concentrations	Competitive inhibition of tubular secretion
Salicylates	Decreased cefixime concentration and area under the serum antiinfective AUC	Displacement from protein binding sites
Warfarin	Increased bleeding risk with some cephalosporins	Mechanism not established
Chloramphenicol With		
Acetaminophen	Possible decreased chloramphenicol effect	Increased metabolism
Aminoglycosides	In vitro antagonism; not seen in vivo	Mechanism not established
Aztreonam	Antagonism; administer chloramphenicol separately a few hours later	Mechanism not established
Barbiturates	Increased barbiturate effect; decreased chloramphenicol effect	Decreased, increased metabolism
Cephalosporins	Antagonism	Mechanism not established
Cimetidine	Aplastic anemia	Possibly additive or synergistic toxicity
Cyclophosphamide	Decreased cyclophosphamide effect	Decreased clearance
Etomidate	Prolonged anesthesia	Decreased metabolism
Folic acid	Delayed response to folic acid	Mechanism not established
Iron	Delayed response to iron	Mechanism not established
Lincomycin	Decreased lincomycin effect	Target site antagonism
Penicillins	In vitro antagonism; not seen in vivo	Mechanism not established

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Phenytoin	Increased phenytoin toxicity Possible increased chloramphenicol toxicity	Decreased metabolism Mechanism not established
Rifamycins	Decreased chloramphenicol effect	Increased metabolism
Sulfonylureas	Increased hypoglycemic effect	Mechanism not established
Vitamin B_{12}	Delayed response to vitamin B_{12}	Mechanism not established
Warfarin	Increased warfarin effect	Mechanism not established
Chloroquine With		
Ampicillin	Decreased ampicillin effect	Decreased bioavailability
Cholestyramine	Decreased effect	Decreased bioavailability
Cimetidine	Increased toxicity	Decreased clearance
Cyclosporine	Increased cyclosporine toxicity	Decreased clearance
Magnesium antacids	Decreased efficacy	Decreased bioavailability
Methotrexate	Decreased methotrexate efficacy	Increased clearance
Rabies vaccine	Decreased vaccine effect	Interference with antibody response
Ritonavir	Increased chloroquine toxicity	Decreased metabolism
Succinylcholine	Increased neuromuscular blockade	Decreased clearance
Cidofovir With		
Aminoglycosides	Increased risk of nephrotoxicity	Additive toxicity
Foscarnet	Increased risk of nephrotoxicity	Additive toxicity
Pentamidine	Increased risk of nephrotoxicity	Additive toxicity
Clarithromycin With		
BZDs	Increased CNS toxicity	Decreased metabolism
CBZ	Increased CBZ toxicity	Decreased metabolism
Cimetidine	Decreased clarithromycin concentrations	Prolonged absorption
Cisapride	Increased cisapride effect, toxicity	Decreased metabolism
Clindamycin	In vitro antagonism; not documented clinically	Mechanism not established
Corticosteroids	Increased steroid effect, toxicity	Decreased excretion
Cyclosporine	Increased cyclosporine toxicity	Decreased metabolism
Delavirdine	Increased clarithromycin toxicity	Decreased metabolism
Digoxin	Increased digoxin effect	Decreased gut metabolism, increased absorption
Disopyramide	Increased disopyramide effect, toxicity	Mechanism not established
Ergot alkaloids	Increased ergot effect, toxicity	Mechanism not established
Fluoxetine	Increased fluoxetine effect, toxicity	Decreased metabolism
HMG-CoA reductase inhibitors	Increased risk of rhabdomyolysis	Decreased metabolism
Omeprazole	Increased omeprazole effect, toxicity; increased clarithromycin gastric tissue exposure	Decreased metabolism; mechanism not established
Phenytoin	Possible increased or decreased effect	Altered metabolism
Pimozide	Increased pimozide effect, toxicity	Decreased metabolism
Rifamycins	Decreased clarithromycin concentrations, increased rifamycin toxicity	Increased, decreased metabolism
Ritonavir	Increased clarithromycin effect, toxicity	Decreased metabolism
Saquinavir	Increased saquinavir effect, toxicity	Decreased metabolism
Sildenafil	Increased sildenafil effect, toxicity	Decreased metabolism
Tacrolimus	Increased tacrolimus effect, toxicity	Decreased metabolism
Theophylline	Increased theophylline effect, toxicity	Decreased metabolism
Warfarin	Increased warfarin effect, toxicity	Decreased metabolism
Zidovudine	Decreased zidovudine effect	Mechanism not established
Clindamycin With		
Neuromuscular blocking agents	Increased neuromuscular blockade	Additive toxicity
Saquinavir	Increased clindamycin toxicity	Decreased metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Clofazimine With		
Dapsone	Possible decrease or nullification of clofazimine's antiinflammatory activity	Opposing effects on neutrophil motility and lymphocyte transformation
Isoniazid	Increased clofazimine serum and urine concentrations, decreased skin concentrations	Mechanism not established
Phenytoin	Decreased efficacy	Increased phenytoin clearance
Rifampin	Decreased rate of absorption, time to reach peak, and AUC of rifampin	Mechanism not established
Cobicistat With		
CYP3A substrates	Increased substrate exposure	Decreased metabolism
CYP3A inducers	Decreased cobicistat exposure	Increased metabolism
Cycloserine With		
Alcohol	Increased alcohol effect or convulsions	Mechanism not established
Anticoagulants, oral	Increased effect	Mechanism not established
Ethionamide	Increased neurotoxicity	Additive toxicity
Isoniazid	CNS effects, dizziness, drowsiness; increased neurotoxicity	Mechanism not established, additive toxicity
Phenytoin	Increased phenytoin effect, toxicity	Decreased metabolism
Daclatasvir With		
Amiodarone	Symptomatic bradycardia	Mechanism not established
CYP3A inducers	Decreased daclatasvir exposure	Increased metabolism
CYP3A inhibitors	Increased daclatasvir exposure	Decreased metabolism
Digoxin	Increased digoxin exposure	Mechanism not established
HMG-CoA reductase inhibitors	Increased statin exposure	Decreased metabolism
Dapsone With		
Clofazimine	Possible decrease in or nullification of clofazimine's antiinflammatory activity	Opposing effects on neutrophil motility and lymphocyte transformation in vitro
Delavirdine	Increased dapsone toxicity	Decreased metabolism
Didanosine	Increased incidence of *Pneumocystis jirovecii* pneumonia recurrence	Mechanism not established
Folic acid antagonists	Increased risk of hematologic toxicity	Additive toxicity
Niridazole	Increased risk of hemolysis in G6PD deficiency	Additive toxicity
Nitrofurantoin	Increased risk of hemolysis in G6PD deficiency	Additive toxicity
Primaquine	Increased risk of hemolysis in G6PD deficiency	Additive toxicity
Pyrimethamine	Increased risk of hematologic toxicity	Additive toxicity
Rifamycins	Decreased dapsone serum concentrations	Hepatic enzyme induction
Saquinavir	Increased dapsone toxicity	Decreased metabolism
Trimethoprim	Increased dapsone serum concentrations; increased risk of adverse effects	Mechanism not established
Warfarin	Increased warfarin effect	Decreased metabolism
Zidovudine	Increased risk of hematologic toxicity	Additive toxicity
Daptomycin With		
HMG-CoA reductase inhibitors	Possible increased risk of myopathy	Additive toxicity
Tobramycin	Possible increased exposure to daptomycin, possible decreased exposure to tobramycin	Mechanism not established
Darunavir With		
Antihistamines	Increased antihistamine exposure, toxicity	CYP3A inhibition
Benzodiazepines	Increased benzodiazepine exposure, toxicity	CYP3A inhibition
Cisapride	Increased cisapride exposure, toxicity	CYP3A inhibition
CYP3A substrates	Increased exposure	CYP3A inhibition
Ergot derivatives	Increased ergot exposure, toxicity	CYP3A inhibition
Ombitasvir-paritaprevir-ritonavir	Decreased darunavir exposure	Mechanism not established
Pimozide	Increased pimozide exposure, toxicity	CYP3A inhibition

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Delavirdine With		
Alfentanil	Increased alfentanil effect, toxicity	Decreased metabolism
Amiodarone	Increased amiodarone effect, toxicity	Decreased metabolism
Amprenavir	Decreased amprenavir clearance; increased delavirdine clearance	Cytochrome P-450 (CYP) 3A4 interaction
Antacids	Decreased delavirdine effect	Decreased bioavailability
Anthracyclines	Increased anthracycline effect, toxicity	Decreased metabolism
Azole antifungals	Increased azole exposure, toxicity	Decreased metabolism
Barbiturates	Increased barbiturate effect, toxicity; decreased delavirdine effect	Decreased, increased metabolism
BZDs	Increased BZD effect, toxicity	Decreased metabolism
Calcium channel blockers	Increased calcium blocker effect, toxicity	Decreased metabolism
CBZ	Increased CBZ toxicity; decreased delavirdine effect	Decreased, increased metabolism
Clarithromycin	Increased clarithromycin, delavirdine effect, toxicity	Decreased metabolism
Clindamycin	Increased clindamycin effect, toxicity	Decreased metabolism
Corticosteroids	Increased steroid effect, toxicity	Decreased metabolism
Cyclosporine	Increased cyclosporine effect, toxicity	Decreased metabolism
Cyclophosphamide	Increased cyclophosphamide toxicity; decreased delavirdine effect	Decreased, increased metabolism
Dapsone	Increased dapsone effect, toxicity	Decreased metabolism
Ergot derivatives	Increased ergot effect, toxicity	Decreased metabolism
Erythromycin	Increased erythromycin, delavirdine effect, toxicity	Decreased metabolism
Fluoxetine	Increased delavirdine effect, toxicity	Decreased metabolism
Garlic supplements	Decreased delavirdine effect	Increased metabolism
H_2 receptor antagonists	Decreased delavirdine effect	Decreased bioavailability
HMG-CoA reductase inhibitors	Increased exposure and toxicity	Decreased metabolism
Ifosfamide	Increased ifosfamide effect, toxicity	Decreased metabolism
Lidocaine	Increased lidocaine effect, toxicity	Decreased metabolism
Metronidazole	Increased metronidazole effect, toxicity	Decreased metabolism
Nefazodone	Increased nefazodone effect, toxicity	Decreased metabolism
Paclitaxel	Increased paclitaxel toxicity	Decreased metabolism
Phenytoin	Increased phenytoin effect, toxicity; decreased delavirdine effect	Decreased, increased metabolism
Pimozide	Increased pimozide effect, toxicity	Decreased metabolism
Pioglitazone	Possible increased pioglitazone exposure, effects; possible decreased delavirdine exposure	CYP inhibition; CYP induction
Proton pump inhibitors	Decreased delavirdine effect	Decreased bioavailability
Quinidine	Increased quinidine effect, toxicity	Decreased metabolism
Repaglinide	Increased repaglinide exposure, effects	Decreased metabolism
Rifamycins	Increased rifamycin effect, toxicity; decreased delavirdine effect	Decreased, increased metabolism
Sildenafil	Increased sildenafil effect, toxicity	Decreased metabolism
Sirolimus	Increased sirolimus effect, toxicity	Decreased metabolism
St. John's wort	Decreased delavirdine effect	Increased metabolism
Sulfonylureas	Increased sulfonylurea exposure, effects	Decreased metabolism
Tacrolimus	Increased tacrolimus effect, toxicity	Decreased metabolism
Tolbutamide	Increased tolbutamide exposure, effects	Decreased metabolism
Warfarin	Increased warfarin effect, toxicity	Decreased metabolism
Zolpidem	Increased zolpidem effect, toxicity	Decreased metabolism
Didanosine With		
Allopurinol	Increased didanosine effect, toxicity	Decreased didanosine clearance
Antacids	Increased toxicity caused by ingredients in both	Additive toxicity
BZDs	Increased confusion	Mechanism not established
Bisphosphonates	Decreased effect of bisphosphonates	Decreased bioavailability

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Chloramphenicol	Increased peripheral neuropathy	Additive toxicity
Cisplatin	Increased peripheral neuropathy	Additive toxicity
Dapsone	Decreased dapsone effect, increased peripheral neuropathy	Decreased dapsone bioavailability, additive toxicity
Delavirdine	Decreased delavirdine, didanosine effect	Mechanism not established
Disulfiram	Increased peripheral neuropathy	Additive toxicity
Ethambutol	Increased peripheral neuropathy, ocular toxicity	Additive toxicity
Ethionamide	Increased peripheral neuropathy	Additive toxicity
Fluoroquinolones	Decreased fluoroquinolone, didanosine effects	Decreased bioavailability
Ganciclovir	Increased peripheral neuropathy, pancreatitis	Altered bioavailability of both
Hydralazine	Increased peripheral neuropathy	Additive toxicity
Indinavir	Decreased indinavir effect	Decreased bioavailability
Iodoquinol	Increased peripheral neuropathy	Additive toxicity
Isoniazid	Increased peripheral neuropathy	Additive toxicity
Itraconazole	Decreased itraconazole effect	Decreased bioavailability
Ketoconazole	Decreased ketoconazole effect	Decreased bioavailability
Methadone	Decreased didanosine effect	Decreased bioavailability
Metronidazole	Increased peripheral neuropathy	Additive toxicity
Nitrofurantoin	Increased peripheral neuropathy	Additive toxicity
Pentamidine	Increased risk of pancreatitis	Additive toxicity
Phenytoin	Increased peripheral neuropathy	Additive toxicity
Ribavirin	Increased risk of toxicity	Additive toxicity
Tetracyclines	Decreased tetracycline effect	Decreased bioavailability
Trimethoprim-sulfamethoxazole	Increased pancreatitis	Additive toxicity
Vincristine	Increased peripheral neuropathy	Additive toxicity
Zalcitabine	Increased neurotoxicity	Additive toxicity
Dolutegravir With		
Antacids	Decreased dolutegravir exposure with polyvalent cation–containing medications	Decreased absorption
Carbamazepine	Decreased dolutegravir exposure	Increased metabolism
Efavirenz	Decreased dolutegravir exposure	Increased metabolism
Etravirine	Decreased dolutegravir exposure	Increased metabolism
Fosamprenavir	Decreased dolutegravir exposure	Increased metabolism
Metformin	Increased metformin exposure	Tubular secretion inhibition
Nevirapine	Decreased dolutegravir exposure	Increased metabolism
Oxcarbazepine	Decreased dolutegravir exposure	Increased metabolism
Phenobarbital	Decreased dolutegravir exposure	Increased metabolism
Phenytoin	Decreased dolutegravir exposure	Increased metabolism
St. John's wort	Decreased dolutegravir exposure	Increased metabolism
Tipranavir	Decreased dolutegravir exposure	Increased metabolism
Efavirenz With		
Amiodarone	Decreased amiodarone effect	Increased metabolism
Azole antifungals	Increased efavirenz effect, decreased azole effect, toxicity	Decreased, increased metabolism
Barbiturates	Decreased barbiturate, efavirenz effects	Increased metabolism
Bupropion	Decreased bupropion effect	Increased metabolism
BZDs	Decreased BZD effect	Increased metabolism
CBZ	Decreased CBZ, efavirenz effects	Increased metabolism
Clarithromycin	Increased efavirenz effect, toxicity	Decreased metabolism
Corticosteroids	Decreased efavirenz, steroid effects	Increased metabolism
Cyclosporine	Decreased cyclosporine effect; increased efavirenz effect, toxicity	Increased, decreased metabolism

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Elbasvir-grazoprevir	Decreased elbasvir and grazoprevir exposure	Increased metabolism
Ergot alkaloids	Increased ergot effect, toxicity	Decreased metabolism
Erythromycin	Increased efavirenz effect, toxicity	Decreased metabolism
Fluoxetine	Increased risk of serotonin syndrome	Decreased metabolism
Garlic supplements	Decreased efavirenz effect	Increased metabolism
Indinavir	Decreased indinavir concentrations	Increased metabolism
Methadone	Decreased methadone effect; withdrawal	Increased metabolism
Oral contraceptives	Decreased contraceptive effect	Increased metabolism
Paclitaxel	Decreased paclitaxel effect	Increased metabolism
Phenytoin	Decreased phenytoin, efavirenz effects	Increased metabolism
Repaglinide	Decreased repaglinide effect	Increased metabolism
Rifamycins	Decreased efavirenz effect	Increased metabolism
Ritonavir	Decreased ritonavir concentration	Increased metabolism
St. John's wort	Decreased efavirenz effect	Increased metabolism
Terfenadine	Increased risk of cardiotoxicity	Decreased metabolism
Warfarin	Decreased warfarin effect	Increased metabolism
Zidovudine	Decreased zidovudine concentration	Increased metabolism
Elbasvir-Grazoprevir With		
Cyclosporine	Increased grazoprevir exposure and ALT elevation	OATP1B1/3 inhibition
CYP3A inducers	Decreased elbasvir and grazoprevir exposure	Increased metabolism
Efavirenz	Decreased elbasvir and grazoprevir exposure	Increased metabolism
Eltrombopag	Increased grazoprevir exposure and ALT elevation	OATP1B1/3 inhibition
Etravirine	Decreased elbasvir and grazoprevir exposure	Increased metabolism
HMG-CoA reductase inhibitors	Increased statin exposure	Mechanism not established
Protease inhibitors	Increased grazoprevir exposure and ALT elevation	Mechanism not established
Tacrolimus	Increased tacrolimus exposure	Mechanism not established
Elvitegravir-Cobicistat-Emtricitabine-Tenofovir With		
Alfuzosin	Increased alfuzosin exposure	Decreased metabolism
BZDs	Prolonged sedation or respiratory depression	Decreased metabolism
Ergotamine derivatives	Life-threatening ergot toxicity	Decreased metabolism
HMG-CoA reductase inhibitors	Myopathy including rhabdomyolysis	Decreased metabolism
Pimozide	Cardiac arrhythmias	Decreased metabolism
Rifampin	Decreased elvitegravir exposure	Increased metabolism
Sildenafil	Visual disturbance, syncope, priapism, and hypotension	Decreased metabolism
St. John's wort	Decreased elvitegravir exposure	Increased metabolism
Enfuvirtide With		
Tipranavir	Increased tipranavir exposure	Mechanism not established
Entecavir With		
Probenecid	Increased entecavir exposure	Tubular secretion inhibition
Erythromycin With		
Alfentanil	Increased alfentanil toxicity	Decreased metabolism
Bromocriptine	Increased toxicity	Increased bioavailability
BZDs	Increased BZD toxicity	Decreased metabolism
CBZ	Increased CBZ toxicity	Decreased metabolism
Clindamycin	Antagonism	Target site competition
Clozapine	Increased clozapine toxicity	Decreased metabolism
Corticosteroids	Increased steroid effect, toxicity	Decreased excretion
Cyclosporine	Increased cyclosporine toxicity	Decreased metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Digoxin	Increased digoxin effect	Decreased GI metabolism and increased absorption
Disopyramide	Cardiac arrhythmias	Decreased metabolism
Ergot alkaloids	Increased ergot toxicity	Mechanism not established
Felodipine	Increased toxicity	Decreased metabolism
HMG-CoA reductase inhibitors	Increased risk of rhabdomyolysis	Decreased clearance
Lidocaine	Decreased clearance; increased monoethylglycinexylidide (MEGX) concentration	Decreased metabolism
Phenytoin	Possible increased or decreased effect	Altered metabolism
Quinidine	Increased quinidine effect, toxicity	Decreased metabolism
Ritonavir	Increased erythromycin toxicity	Decreased metabolism
Sildenafil	Increased sildenafil effect, toxicity	Decreased metabolism
Tacrolimus	Increased tacrolimus toxicity	Decreased metabolism
Theophylline	Increased theophylline effect and possible toxicity	Decreased metabolism
Trimetrexate	Increased trimetrexate toxicity	Decreased metabolism
Valproic acid	Increased toxicity	Decreased metabolism
Vinblastine	Increased vinblastine toxicity	Decreased metabolism
Warfarin	Increased warfarin effect, toxicity	Decreased metabolism
Zafirlukast	Decreased zafirlukast effect	Mechanism not established
Ethambutol With		
Aluminum-containing antacids	Decreased ethambutol exposure	Decreased absorption
Ethionamide With		
Aminosalicylic acid	Increased GI distress, hepatotoxicity	Mechanism not established
BCG vaccine	Negates BCG vaccine effect	Negates immune response
Cycloserine	Increased neurotoxicity	Additive toxicity
Ethambutol	Increased ethambutol toxicity	Mechanism not established
Isoniazid	Increased neurotoxicity	Additive toxicity
Pyrazinamide	Increased hepatotoxicity	Additive toxicity
Rifamycins	Increased hepatotoxicity	Additive toxicity
Etravirine With		
Antiarrhythmic agents	Decreased antiarrhythmic exposure	Metabolism induction
Anticonvulsants	Decreased etravirine exposure, effect	Metabolism induction
Atazanavir-ritonavir	Increased etravirine and decreased atazanavir concentrations, effects	Metabolism inhibition and induction
Azole antifungals	Increased etravirine, decreased azole exposure	Decreased, increased metabolism
Clarithromycin	Decreased clarithromycin and increased 14-hydroxyclarithromycin exposures	Metabolism induction
Darunavir-ritonavir	Decreased etravirine exposure	Metabolism induction
Dexamethasone	Decreased etravirine exposure	Mechanism not established
Diazepam	Increased diazepam exposure	Metabolism inhibition
Elbasvir-grazoprevir	Decreased elbasvir and grazoprevir exposure	Increased metabolism
Fosamprenavir-ritonavir	Increased amprenavir exposure, toxicity	Metabolism inhibition
HMG-CoA reductase inhibitors	Decreased lovastatin, simvastatin, increased fluvastatin exposures	Metabolism induction, inhibition
Immunosuppressants	Possible decreased cyclosporine, sirolimus, and tacrolimus exposures	Metabolism induction
Lopinavir-ritonavir	Increased etravirine exposure	Metabolism inhibition
Methadone	Minimal change	—
NNRTIs	Decreased etravirine exposure with efavirenz or nevirapine, increased etravirine exposures with delavirdine	Metabolism induction, inhibition
Phosphodiesterase inhibitors	Possible decreased sildenafil effect	Mechanism not established
Protease inhibitors	Must ensure low-dose ritonavir is given with any etravirine–protease inhibitor combinations	Mechanism not established
Rifamycins	Decreased etravirine exposures	Metabolism induction

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Ritonavir	Significant decrease in etravirine exposure	Metabolism induction
Saquinavir-ritonavir	Decrease etravirine exposure	Metabolism induction
St. John's wort	Decreased etravirine exposure	Metabolism induction
Tipranavir-ritonavir	Decreased etravirine effect	Metabolism induction
Warfarin	Increased warfarin exposure	Metabolism inhibition
Fluconazole With		
Amitriptyline	Increased amitriptyline toxicity	Decreased metabolism
Amphotericin B	Possible antagonism in animal models	Mechanism not established
BZDs	Increased CNS toxicity	Decreased metabolism
Cimetidine	Decreased fluconazole effect	Decreased bioavailability
Cisapride	Increased risk of cardiotoxicity	Decreased metabolism
Cyclosporine	Increased cyclosporine concentration	Mechanism not established
DHP CCB	Increased DHP CCB toxicity	Decreased metabolism
Etravirine	Increased etravirine concentration	Metabolism inhibition
HMG-CoA reductase inhibitors	Increased risk of rhabdomyolysis	Mechanism not established
Phenytoin	Increased phenytoin concentration	Decreased metabolism
Quetiapine	Possible increased quetiapine concentration	Decreased metabolism
Rifamycins	Decreased fluconazole concentration	Mechanism not established
Sulfonylureas	Increased plasma concentration; decreased metabolism of tolbutamide, glyburide, glipizide	Mechanism not established
Tacrolimus	Increased tacrolimus toxicity	Decreased metabolism
Terfenadine	Increased risk of cardiotoxicity	Decreased metabolism
Thiazides	Increased fluconazole concentrations and AUC of fluconazole	Decreased renal clearance
Warfarin	Increased warfarin effect	Metabolism inhibition
Zidovudine	Increased zidovudine concentrations	Decreased metabolism
Flucytosine With		
Amphotericin B	Increased flucytosine toxicity	Increased cellular flucytosine uptake
Zidovudine	Increased hematologic toxicity	Additive toxicity
Fluoroquinolones With		
Antacids	Decreased fluoroquinolone effect with aluminum or magnesium antacids	Decreased absorption
BCG vaccine	Negates BCG vaccine effect	Negates immune response
Chloramphenicol	Inhibition in vitro of norfloxacin bactericidal activity	Mechanism not established
Cyclosporine	Increased risk of nephrotoxicity; increased serum cyclosporine concentration	Mechanism not established
Didanosine	Decreased fluoroquinolone effect	Decreased GI absorption
Iron	Decreased serum fluoroquinolone concentration	Decreased GI absorption
Mineral fortified foods	Decreased fluoroquinolone concentration	Decreased GI absorption
NSAIDs	Possible increased risk of CNS stimulation	Mechanism not established
Oral contraceptives	Possible decreased effect of moxifloxacin	Increased metabolism
Oral hypoglycemics	Increased incidence, severity of hypoglycemia	Mechanism not established
Probenecid	Increased serum concentration and AUC	Decreased tubular secretion
Rifamycins	Inhibition in vitro of norfloxacin bactericidal activity	Mechanism not established
Riluzole	Increased risk of riluzole toxicity	Decreased elimination
Ropivacaine	Increased risk of ropivacaine toxicity	Decreased metabolism
Scopolamine	Decreased rate of fluoroquinolone absorption	Mechanism not established
Sucralfate	Decreased serum fluoroquinolone concentration	Decreased GI absorption
Tetracycline	Inhibition in vitro of norfloxacin bactericidal activity	Mechanism not established
Theophylline	Possible theophylline toxicity	Decreased metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Tizanidine	Increased tizanidine concentrations, toxicity with ciprofloxacin	Metabolism inhibition
Warfarin	Increased warfarin effect	Disruption of vitamin K synthesis
Zinc	Decreased serum fluoroquinolone concentrations	Decreased GI absorption
Fosamprenavir With		
Antacids	Decreased amprenavir exposure	Mechanism not established
Antiarrhythmics	Increased antiarrhythmic exposure, toxicity	Metabolism interaction
Anticonvulsants	Decreased amprenavir exposure, increased anticonvulsant exposure	Metabolism site competition
Azole antifungals	Increased azole and amprenavir exposures	Metabolism interaction
BZDs	Increased benzodiazepine exposure, toxicity	Metabolism interaction
CCBs	Increased CCB exposures, toxicity	Metabolism interaction
Cisapride	Increased cisapride exposure, toxicity	Metabolism interaction
Contraceptives, oral	Decreased amprenavir concentration, change in oral contraceptive exposure	Metabolism interaction
Corticosteroids	Increased corticosteroid exposure	Metabolism interaction
CYP3A inhibitors	Increased amprenavir exposure	Metabolism interaction
Delavirdine	Increased amprenavir and decreased delavirdine exposures	Metabolism interaction
Dexamethasone	Decreased amprenavir exposure	Metabolism interaction
Efavirenz-ritonavir	Increased amprenavir exposure	Metabolism interaction
Ergot derivatives	Increased ergot exposure, toxicity	Metabolism interaction
Esomeprazole	Increased esomeprazole exposure	Metabolism interaction
H_2 receptor antagonists	Decreased amprenavir exposure	Mechanism not established
HMG-CoA reductase inhibitors	Increased HMG-CoA exposure, toxicity; decreased amprenavir exposure	Metabolism interaction
Immunosuppressants	Increased immunosuppressant exposure	Metabolism interaction
Indinavir	Increase in amprenavir and decrease in indinavir exposures	Metabolism interaction
Lopinavir-ritonavir	Decreased amprenavir, increased lopinavir-ritonavir exposures	Metabolism interaction
Methadone	Decreased amprenavir and methadone exposures	Metabolism interaction
Nevirapine	Decreased amprenavir, increased nevirapine exposures	Metabolism interaction
Opiates	Increased opiate exposure, toxicity	Metabolism interaction
Paroxetine	Decreased paroxetine exposure	Metabolism interaction
Phenytoin	Decreased phenytoin exposure	Metabolism interaction
Phosphodiesterase inhibitors	Increased phosphodiesterase inhibitor exposure, toxicity	Metabolism interaction
Pimozide	Increased pimozide exposure, toxicity	Metabolism interaction
Ranolazine	Increased ranolazine exposure, toxicity	Metabolism interaction
Rifabutin	Increased rifabutin and amprenavir exposures	Metabolism interaction
Rifampin	Decreased amprenavir exposure	Metabolism interaction
Saquinavir	Decreased amprenavir exposure	Metabolism interaction
St. John's wort	Decreased amprenavir exposure	Metabolism interaction
Trazodone	Increased trazodone exposure, toxicity	Metabolism interaction
Tricyclic antidepressants	Increased tricyclic exposure, toxicity	Metabolism interaction
Warfarin	Possible change in warfarin exposure	Mechanism not established
Zidovudine	Increased amprenavir and zidovudine exposures	Metabolism interaction
Foscarnet With		
Acyclovir	Increased nephrotoxicity	Additive toxicity
Cidofovir	Increased nephrotoxicity	Additive toxicity
Pentamidine	Increased nephrotoxicity; increased hypocalcemia	Additive toxicity
Probenecid	Increased foscarnet serum concentration; increased possibility of adverse effects	Decreased tubular secretion
Suramin	Increased nephrotoxicity	Additive toxicity
Trimethoprim-sulfamethoxazole	Increased nephrotoxicity	Additive toxicity

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Ganciclovir/Valganciclovir With		
Aminoglycosides	Increased nephrotoxicity	Additive toxicity
Amphotericin B	Increased nephrotoxicity; replication inhibition of rapidly dividing host cells	Additive toxicity; additive
Cyclosporine	Increased nephrotoxicity	Additive toxicity
Cytotoxic antineoplastics	Replication inhibition of rapidly dividing host cells	Additive
Dapsone	Replication inhibition of rapidly dividing host cells	Additive
Didanosine	Increased didanosine effect, toxicity	Increased bioavailability
Flucytosine	Replication inhibition of rapidly dividing host cells	Additive
Imipenem-cilastatin	Generalized seizures	Mechanism not established
Immunosuppressives	Increased suppression of bone marrow and immune system	Additive toxicity
Nucleoside analogues	Replication inhibition of rapidly dividing host cells	Additive
Pentamidine	Replication inhibition of rapidly dividing host cells	Additive
Probenecid	Increased ganciclovir concentration; prolonged AUC	Decrease in tubular secretion
Pyrimethamine	Replication inhibition of rapidly dividing host cells	Additive
Tacrolimus	Increased risk of nephrotoxicity	Additive toxicity
Trimethoprim-sulfamethoxazole	Replication inhibition of rapidly dividing host cells	Additive
Zidovudine	In vitro antiretroviral antagonism; increased risk of hematologic toxicity	Mechanism not established; additive toxicity
Glecaprevir-Pibrentasvir With		
Atazanavir	Increased ALT	Mechanism not established
CBZ	Decreased glecaprevir and pibrentasvir exposure	Increased P-gp–mediated efflux
Cyclosporine	Decreased glecaprevir and pibrentasvir exposure	Mechanism not established
Dabigatran	Increased dabigatran exposure and risk of bleeding	Decreased P-gp–mediated efflux
Estrogens	Increased ALT	Mechanism not established
HMG-CoA reductase inhibitors	Increased statin toxicities	Mechanism not established
Rifampin	Decreased glecaprevir and pibrentasvir exposure	Increased P-gp–mediated efflux
Ritonavir	Increased glecaprevir and pibrentasvir exposure	Decreased P-gp–mediated efflux
St. John's wort	Decreased glecaprevir and pibrentasvir exposure	Increased P-gp–mediated efflux
Griseofulvin With		
Alcohol	Increased alcohol effects, tachycardia and flushing	Mechanism not established
Anticoagulants, oral	Decreased anticoagulant effect	Mechanism not established
Contraceptives, oral	Decreased contraceptive effect	Increased metabolism
Phenobarbital	Decreased griseofulvin concentrations	Decreased absorption or hepatic enzyme induction
Hydroxychloroquine With		
Digoxin	Increased digoxin effect	Mechanism not established
Imipenem-Cilastatin With		
Chloramphenicol	Antagonism; administer a few hours after imipenem-cilastatin	Mechanism not established
Cyclosporine	Increased cyclosporine and imipenem-cilastatin exposures, toxicities	Mechanism not established
Ganciclovir	Generalized seizures	Mechanism not established
Probenecid	Increased imipenem-cilastatin exposure	Decreased tubular secretion
Valproic acid	Decreased valproic acid exposure	Mechanism not established
Isavuconazonium With		
Bupropion	Decreased bupropion exposure	Mechanism not established
CYP3A inducers	Decreased isavuconazonium exposure	Increased metabolism
CYP3A substrates	Increased substrate exposure	Decreased metabolism
Digoxin	Increased digoxin exposure	Mechanism not established
HMG-CoA reductase inhibitors	Increased statin exposure	Decreased metabolism
Immunosuppressants, oral	Increased immunosuppressant exposure	Decreased metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Isoniazid With		
Alcohol	Increased incidence of hepatitis Decreased isoniazid effect in some alcoholic patients	Additive toxicity Increased metabolism
Aluminum antacids	Decreased isoniazid effect	Decreased absorption
Aminosalicylic acid	Increased isoniazid concentration	Reduced acetylation
BCG vaccine	Vaccine may be ineffective	Isoniazid inhibits multiplication of BCG
BZDs	Pharmacologic effects of BZDs may be increased; documented with diazepam and triazolam	Decreased metabolism
CBZ	Increased toxicity of both drugs	Altered metabolism
Cycloserine	CNS effects, dizziness, drowsiness	Mechanism not established
Disulfiram	Psychotic episodes, ataxia	Altered dopamine metabolism
Enflurane	Possible nephrotoxicity	Increased metabolism of enflurane caused increased fluoride concentration
Ethionamide	Increased CNS adverse effects	Additive toxicity
Itraconazole	Decreased itraconazole activity	Increased metabolism
Ketoconazole	Decreased ketoconazole effect	Decreased concentration
Meperidine	Increased risk of serotonin syndrome	Additive toxicity
Phenytoin	Increased phenytoin toxicity	Decreased metabolism
Rifamycins	Possible increased isoniazid hepatotoxicity	Possible increased toxic metabolites
SSRI antidepressants	Increased risk of serotonin syndrome	Additive toxicity
Warfarin	Possible increased anticoagulant effect	Decreased metabolism
Itraconazole With		
Alfentanil	Increased alfentanil exposure	Decreased metabolism
Amphotericin B	In vitro antagonism	Mechanism not established
Antacids	Possible decreased itraconazole bioavailability	Mechanism not established
Aripiprazole	Increased aripiprazole exposure	Decreased metabolism
Barbiturates	Decreased itraconazole effect	Increased metabolism
Buspirone	Increased buspirone exposure	Metabolism interaction
BZDs	Increased CNS effects	Decreased metabolism
CBZ	Decreased itraconazole effect	Increased metabolism
Clarithromycin	Increased itraconazole exposure	Decreased metabolism
Corticosteroids	Increased corticosteroid exposure	Metabolism interaction
Cyclosporine	Possible increased cyclosporine concentrations	Mechanism not established
Didanosine	Decreased itraconazole effect	Decreased bioavailability
Digoxin	Increased digoxin toxicity	Decreased metabolism
DHP CCBs	Increased DHP CCB effect	Decreased metabolism
Docetaxel	Increased docetaxel exposure	Decreased metabolism
Dofetilide	Increased dofetilide effect, toxicity	Decreased metabolism
Eletriptan	Increased eletriptan exposure	Decreased metabolism
Ergot alkaloids	Increased ergot alkaloid exposure, toxicity	Metabolism interaction
Erythromycin	Increased itraconazole exposure	Decreased metabolism
H_2 receptor antagonists	Decreased itraconazole bioavailability	Decreased gastric acidity
HMG-CoA reductase inhibitors	Increased risk of rhabdomyolysis	Decreased metabolism
Indinavir	Increased indinavir toxicity	Decreased metabolism
Nevirapine	Decreased itraconazole exposure	Increased metabolism
Oral hypoglycemics	Increased oral hypoglycemic effects	Decreased metabolism
Phenytoin	Decreased itraconazole effect	Increased metabolism
Phosphodiesterase inhibitors	Increased phosphodiesterase inhibitor exposure	Decreased metabolism

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Pimozide	Increased pimozide effect, toxicity	Decreased metabolism
Protease inhibitors	Increased protease inhibitor exposure, increased itraconazole exposure	Decreased metabolism
Proton pump inhibitors	Decreased itraconazole bioavailability	Decreased gastric acidity
Quinidine	Increased quinidine effect, toxicity	Decreased metabolism
Rifamycins	Decreased systemic bioavailability of itraconazole	Hepatic enzyme induction
Ritonavir	Increased itraconazole concentrations	Decreased metabolism
Saquinavir	Increased saquinavir concentration	Decreased metabolism
Sirolimus	Increased sirolimus exposure	Decreased metabolism
Tacrolimus	Increased tacrolimus toxicity	Decreased metabolism
Terfenadine	Increased risk of cardiotoxicity	Decreased metabolism
Tolterodine	Increased tolterodine exposure	Decreased metabolism
Trimetrexate	Increased trimetrexate exposure	Decreased metabolism
Vinca alkaloids	Increased vinca toxicity	Mechanism not established
Warfarin	Increased warfarin effect	Decreased metabolism
Ivermectin		
Dasabuvir	Increased ivermectin exposure	Decreased BCRP-mediated efflux
Warfarin	Increased warfarin effect	Mechanism not established
Lincomycin With		
Neuromuscular blocking agents	Increased neuromuscular blockade	Mechanism not established
Ketoconazole: Same as Itraconazole *Plus*		
Alcohol	Possible disulfiram-like reaction	Mechanism not established
Delavirdine	Increased delavirdine concentration	Mechanism not established
Donepezil	Increased cholinomimetic effects	Decreased metabolism
Hepatotoxic agents	Increased hepatotoxicity	Additive toxicity
Isoniazid	Decreased ketoconazole effect	Decreased blood concentrations
Quetiapine	Increased risk of quetiapine toxicity	Decreased metabolism
Theophylline	Decreased theophylline concentration	Mechanism not established
Lamivudine With		
Nelfinavir	Increased lamivudine concentration	Decreased clearance
Pentamidine	Increased risk of pancreatitis	Additive toxicity
Sulfonamides	Increased lamivudine concentration	Decreased clearance
Trimethoprim	Increased lamivudine concentration	Decreased clearance
Trimethoprim-sulfamethoxazole	Increased risk of pancreatitis	Additive toxicity
Zidovudine	Increased zidovudine concentration	Mechanism not established
Linezolid With		
Adrenergic agents	Increased adrenergic agent effects	Monoamine oxidase (MAO) inhibition
Bupropion	Increased bupropion exposure, toxicity	Mechanism not established
Entacapone or tolcapone	Decreased catecholamine metabolism	MAO inhibition
MAO inhibitors	Increased MAO inhibitor actions, toxicity	Additive toxicity
Meperidine	Increased risk of serotonin syndrome	MAO inhibition
SNRI antidepressants	Increased risk of serotonin syndrome	MAO inhibition
SSRI antidepressants	Increased risk of serotonin syndrome	MAO inhibition
Tramadol	Increased risk of seizures	Mechanism not established
Tricyclic antidepressants	Increased risk of serotonin syndrome	MAO inhibition
Triptans	Increased triptan exposure, toxicity	MAO inhibition
Maraviroc With		
CYP3A inhibitors	Increased maraviroc exposure	CYP3A inhibition
CYP3A inducers	Decreased maraviroc exposure	CYP3A induction

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Mebendazole With		
CBZ	Decreased mebendazole concentrations	Increased metabolism
Phenytoin	Decreased mebendazole concentrations	Increased metabolism
Mefloquine With		
Anticonvulsants	Increased seizure frequency	Mechanism not established
Lumefantrine	Decreased lumefantrine exposure	Decreased absorption
Rifampin	Decreased mefloquine exposure	Increased metabolism
Methenamine With		
Sulfonamides	Increased risk of crystalluria; precipitate formation between formaldehyde and sulfamethizole	Acidification of the urine
Metronidazole With		
Alcohol	Mild disulfiram-like symptoms	Possible inhibition of intermediary metabolism of alcohol
Azathioprine	Transient neutropenia	Mechanism not established
Barbiturates	Decreased metronidazole effect with phenobarbital	Probably increased metabolism
CBZ	Increased CBZ toxicity	Decreased metabolism
Cimetidine	Possible increased metronidazole toxicity	Decreased metabolism
Disulfiram	Organic brain syndrome	Mechanism not established
Fluorouracil	Transient neutropenia	Mechanism not established
Lithium	Lithium toxicity	Mechanism not established
Warfarin	Increased warfarin effect	Decreased metabolism
Micafungin With		
Cyclosporine	Increased cyclosporine exposure	Mechanism not established
Itraconazole	Increased itraconazole exposure	Mechanism not established
Nifedipine	Increased nifedipine exposure	Mechanism not established
Sirolimus	Increased sirolimus exposure	Mechanism not established
Nafcillin With		
CYP3A substrates	Decreased effect	Increased metabolism
Warfarin	Decreased warfarin effect	Increased metabolism
Nevirapine With		
Amiodarone	Decreased amiodarone effect	Increased metabolism
Azole antifungals	Decreased azole effect; increased nevirapine effect, toxicity	Increased, decreased metabolism
Barbiturates	Decreased barbiturate, nevirapine effects	Increased metabolism
Bupropion	Decreased bupropion effect	Increased metabolism
BZDs	Decreased BZD effect	Increased metabolism
CBZ	Decreased CBZ, nevirapine effects	Increased metabolism
Cimetidine	Increased nevirapine effect, toxicity	Decreased metabolism
Clarithromycin	Increased nevirapine effect, toxicity	Decreased metabolism
Corticosteroids	Decreased nevirapine, steroid effects	Increased metabolism
Cyclosporine	Decreased cyclosporine effect; increased nevirapine effect, toxicity	Increased, decreased metabolism
Erythromycin	Increased nevirapine effect, toxicity	Decreased metabolism
Garlic supplements	Decreased nevirapine effect	Increased metabolism
Indinavir	Decreased indinavir concentration	Increased metabolism
Methadone	Decreased methadone effect; withdrawal	Increased metabolism
Oral contraceptives	Decreased contraceptive effect	Increased metabolism
Paclitaxel	Decreased paclitaxel effect	Increased metabolism
Phenytoin	Decreased phenytoin, nevirapine effects	Increased metabolism
Repaglinide	Decreased repaglinide effect	Increased metabolism
Rifamycins	Decreased nevirapine effect	Increased metabolism

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Ritonavir	Decreased ritonavir concentration	Increased metabolism
St. John's wort	Decreased nevirapine effect	Increased metabolism
Warfarin	Decreased warfarin effect	Increased metabolism
Zidovudine	Decreased zidovudine concentration	Increased metabolism
Nitrofurantoin With		
Antacids	Possible decreased nitrofurantoin effect	Decreased absorption
Fluoroquinolones	In vitro antagonism of fluoroquinolone activity	Mechanism not established
Probenecid	Increased nitrofurantoin serum concentration	Inhibition of renal excretion
Voriconazole	Decreased voriconazole exposure	Mechanism not established
Ombitasvir-Paritaprevir-Ritonavir ± Dasabuvir With		
Angiotensin receptor blockers	Increased angiotensin receptor blocker exposure	Mechanism not established
Antiarrhythmics	Increased antiarrhythmic exposure	Mechanism not established
Atazanavir	Increased paritaprevir exposure	Decreased metabolism
CCBs	Increased CCB effect	Mechanism not established
CYP3A substrates	Increased substrate exposure	Decreased metabolism
Darunavir	Decreased darunavir exposure	Mechanism not established
Digoxin	Increased digoxin exposure	Decreased P-gp–mediated efflux
HMG-CoA reductase inhibitors	Increased statin effect	Decreased metabolism
Immunosuppressants, oral	Increased immunosuppressant exposure	Decreased metabolism
Lopinavir-ritonavir	Increased paritaprevir exposure	Decreased metabolism and P-gp–mediated efflux
Metformin	Increased risk of lactic acidosis	Mechanism not established
Quetiapine	Increased quetiapine exposure	Decreased metabolism
Rilpivirine	Increased rilpivirine exposure	Mechanism not established
Voriconazole	Decreased voriconazole exposure	Mechanism not established
Oritavancin With		
Heparin	Falsely elevated aPTT results	Mechanism not established
Warfarin	Increased warfarin exposure and unreliable PT/INR results	Mechanism not established
Oseltamivir With		
Warfarin	Increased risk of bleeding	Mechanism not established
Oxacillin With		
Same as nafcillin		
Penicillins (Except Nafcillin or Oxacillin) With		
Methotrexate	Increased methotrexate toxicity	Inhibition of renal excretion
Probenecid	Increased penicillin serum concentration	Inhibition of renal excretion
Tetracyclines	Possible decreased penicillin effectiveness	Decreased bactericidal activity
Vancomycin	Increased renal toxicity with piperacillin-tazobactam and vancomycin	Mechanism not established
Warfarin	Increased warfarin effect	Disruption of gut vitamin K synthesis
Pentamidine With		
Aminoglycosides	Increased nephrotoxicity	Additive toxicity
Amphotericin B	Increased nephrotoxicity	Additive toxicity
Capreomycin	Increased nephrotoxicity	Additive toxicity
Cidofovir	Increased nephrotoxicity	Additive toxicity
Cisplatin	Increased nephrotoxicity	Additive toxicity
Foscarnet	Hypocalcemia	Mechanism not established
Methoxyflurane	Increased nephrotoxicity	Additive toxicity
Polymyxins	Increased nephrotoxicity	Additive toxicity
Vancomycin	Increased nephrotoxicity	Additive toxicity

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Polymyxins With		
Aminoglycoside antibiotics	Increased nephrotoxicity; increased neuromuscular blockade	Additive toxicity
Neuromuscular blocking agents	Increased neuromuscular blockade	Additive toxicity
Parenteral quinine/quinidine	Increased neurotoxicity	Additive toxicity
Vancomycin	Increased nephrotoxicity	Additive toxicity
Posaconazole With		
BZDs	Increased benzodiazepine effect, toxicity	Decreased metabolism
CCBs	Increased CCB effect, toxicity	Decreased metabolism
Cimetidine	Decreased posaconazole effect	Unknown mechanism
Ergot alkaloids	Increased ergot effect, toxicity	Decreased metabolism
Halofantrine	Increased halofantrine effect, toxicity	Decreased metabolism
HMG-CoA reductase inhibitors	Increased statin effect, toxicity	Decreased metabolism
Immunosuppressants	Increased immunosuppressant effect, toxicity	Decreased metabolism
Phenytoin	Decreased posaconazole effect, increased phenytoin effect, toxicity	Metabolism interaction
Pimozide	Increased pimozide effect, toxicity	Decreased metabolism
Rifamycins	Decreased posaconazole effect, increased rifamycin effect, toxicity	Metabolism interaction
Quinidine	Increased quinidine effect, toxicity	Decreased metabolism
Vinca alkaloids	Increased vinca effect, toxicity	Decreased metabolism
Praziquantel With		
CBZ	Decreased praziquantel exposure	Increased metabolism
Dexamethasone	Decreased praziquantel exposure	Increased metabolism
Phenytoin	Decreased praziquantel exposure	Increased metabolism
Rifampin	Decreased praziquantel exposure	Increased metabolism
Primaquine With		
Ritonavir	Increased primaquine concentrations	Decreased metabolism
Proguanil With		
Chloroquine	Increased incidence of mouth ulcers	Mechanism not established
Efavirenz	Decreased exposure of active metabolite	Mechanism not established
Warfarin	Increased bleeding risk	Mechanism not established
Protease Inhibitors (Except Ritonavir) With		
Alfentanil	Increased alfentanil effects, toxicity	Decreased metabolism
Antacids	Decreased efficacy of indinavir, tipranavir	Decreased bioavailability
Antiarrhythmic agents	Increased antiarrhythmic effects, toxicities	Decreased metabolism
Azithromycin	Possible decreased nelfinavir efficacy	P-gp interaction
Azole antifungals	Increased azole and protease inhibitor effect, toxicity	Decreased metabolism
Barbiturates	Decreased protease effect, increased barbiturate effect, toxicity	Increased, decreased metabolism
Bupropion	Possible increased bupropion toxicity	Decreased metabolism
BZDs	Increased CNS BZD toxicity	Decreased metabolism
CCBs	Increased CCB effects	Decreased metabolism
CBZ	Decreased protease effect, increased CBZ effect	Increased, decreased metabolism
Clarithromycin	Increased protease toxicity	Decreased metabolism
Clindamycin	Increased clindamycin toxicity	Decreased metabolism
Corticosteroids	Increased risk of hypercorticoidism	Decreased metabolism
Cyclophosphamide	Increased cyclophosphamide toxicity, decreased protease effect	Decreased, increased metabolism
Dapsone	Increased dapsone toxicity with saquinavir	Decreased metabolism
Daunorubicin	Increased risk of cardiotoxicity	Decreased metabolism
Delavirdine	Increased protease toxicity, increased hepatotoxicity, decreased delavirdine effect	Decreased metabolism

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Desipramine	Increased desipramine effect, toxicity	Decreased metabolism
DHP CCBs	Increased DHP CCB toxicity	Decreased clearance
Doxorubicin	Increased risk of cardiotoxicity	Decreased metabolism
Elbasvir-grazoprevir	Increased grazoprevir exposure and ALT elevation	Mechanism not established
Ergot derivatives	Increased ergot toxicity	Decreased metabolism
Erythromycin	Increased erythromycin, protease effect, toxicity	Decreased metabolism
Garlic supplements	Decreased protease effect	Increased metabolism
Grapefruit juice	Decreased indinavir effect, increased saquinavir effect	Increased, decreased metabolism
H_2 receptor antagonists	Decreased indinavir effect	Decreased bioavailability
HMG-CoA reductase inhibitors	Increased statin concentrations, toxicity	Decreased metabolism
Ifosfamide	Increased ifosfamide toxicity	Decreased metabolism
Interferon alfa-2	Increased indinavir toxicity	Decreased metabolism
Isotretinoin	Increased isotretinoin effect, toxicity	Decreased metabolism
Levodopa	Increased levodopa effect, toxicity	Decreased metabolism
Lidocaine	Increased risk of lidocaine toxicity	Decreased metabolism
Loperamide	Decreased protease efficacy	Decreased bioavailability
Methadone	Decreased methadone, protease effect	Increased metabolism
Metronidazole	Increased metronidazole effect, toxicity	Decreased metabolism
Nefazodone	Increased nefazodone effect, toxicity	Decreased metabolism
Oral contraceptives	Altered efficacy of birth control possible	Increased, decreased metabolism
Oral immunosuppressants	Increased immunosuppressant effect, toxicity	Decreased metabolism
Paclitaxel	Increased effect, toxicity of paclitaxel	Decreased metabolism
Phenytoin	Decreased protease, increased phenytoin effects	Increased, decreased metabolism
Phosphodiesterase inhibitors	Increased phosphodiesterase inhibitor effect, toxicity	Decreased metabolism
Pimozide	Increased pimozide toxicity	Decreased metabolism
Primidone	Decreased protease effect	Increased metabolism
Proton pump inhibitors	Decreased effect of indinavir	Decreased bioavailability
Quinidine	Increased quinidine effect, toxicity	Decreased metabolism
Raloxifene	Possible decreased raloxifene effect with nelfinavir	Increased metabolism
Ranolazine	Increased ranolazine effect, toxicity	Decreased metabolism
Repaglinide	Increased repaglinide exposure, effects	Decreased metabolism
Rifamycins	Decreased protease effect, increased rifamycin toxicity	Increased, decreased metabolism
Risperidone	Increased risperidone toxicity	Decreased metabolism
Ritonavir	Increased protease toxicity	Decreased metabolism
SSRI antidepressants	Increased SSRI effect, toxicity	Decreased metabolism
St. John's wort	Decreased protease effect	Increased metabolism
Warfarin	Increased warfarin effect	Decreased metabolism
Zolpidem	Increased zolpidem effect, toxicity	Decreased metabolism
Pyrantel Pamoate With		
Theophylline	Increased theophylline toxicity	Decreased metabolism
Pyrazinamide With		
Cyclosporine	Decreased cyclosporine exposure	Increased metabolism
Ethionamide	Increased hepatotoxicity	Additive toxicity
Probenecid	Increased serum uric acid	Decreased uric acid excretion
Rifampin	Increased hepatotoxicity	Additive toxicity
Zidovudine	Decreased efficacy of pyrazinamide	Mechanism not established
Pyrimethamine With		
Methotrexate	Increased risk of myelosuppression	Mechanism not established

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Trimethoprim-sulfamethoxazole	Increased risk of megaloblastic anemia and myelosuppression	Additive folate inhibition
Zidovudine	Increased risk of myelosuppression	Mechanism not established
Quinine With		
Acetazolamide	Increased quinine serum concentration; increased toxicity	Decreased clearance from increased urinary pH
Aluminum antacids	Decreased quinine serum concentration	Decreased absorption
Cimetidine	Decreased quinine clearance; increased AUC	Hepatic enzyme inhibition
Digoxin	Increased digoxin concentration	Mechanism not established
Flecainide	Increased flecainide toxicity	Decreased metabolism
Heparin	Decreased anticoagulant effect	Mechanism not established
Mefloquine	Increased cardiac events	Additive toxicity
Neuromuscular blocking agents	Potentiation of neuromuscular blocking effects	Additive toxicity
Ritonavir	Increased quinine toxicity	Decreased metabolism
Sodium bicarbonate	Increased quinine serum concentration; increased toxicity	Decreased clearance from increased urinary pH
Warfarin	Increased warfarin effect	Hepatic suppression of synthesis of vitamin K–dependent clotting factors
Quinupristin-Dalfopristin With		
Haloperidol	Increased risk of cardiotoxicity	Decreased metabolism
HMG-CoA reductase inhibitors	Increased risk of myopathy or rhabdomyolysis	Decreased metabolism
Vinca alkaloids	Increased vinca alkaloid exposure, toxicity	Decreased metabolism
Raltegravir With		
Atazanavir	Increased raltegravir exposure	Decreased metabolism
Rifamycins	Decreased raltegravir exposure	Increased metabolism
Tipranavir-ritonavir	Decreased raltegravir exposure	Increased metabolism
Ribavirin With		
Abacavir	Increased risk of lactic acidosis	Mechanism not established
Didanosine	Increased risk of lactic acidosis	Mechanism not established
Lamivudine	Increased risk of lactic acidosis	Mechanism not established
Stavudine	Increased risk of lactic acidosis	Mechanism not established
Warfarin	Decreased warfarin exposure	Mechanism not established
Zidovudine	Increased risk of lactic acidosis; in vitro retroviral antagonism	Mechanism not established; decreased phosphorylation
Rifamycins (Except Rifaximin) With		
Analgesics	Possible decreased analgesic effect	Increased metabolism
Anticoagulants, oral	Possible decreased oral anticoagulant effect	Increased metabolism
Anticonvulsants	Possible decreased anticonvulsant effect	Increased metabolism
Atovaquone	Decreased atovaquone effect	Increased metabolism
Azole antifungals	Decreased azole effect	Increased metabolism
Barbiturates	Possible decreased barbiturate effect	Increased metabolism
BCG vaccine	Negates BCG effect	Negates immune response
β-Blockers	Possible decreased β-blocker effect	Increased metabolism
Chloramphenicol	Possible decreased chloramphenicol effect	Increased metabolism
Clarithromycin	Decreased clarithromycin concentration, increased rifamycin toxicity	Increased, decreased metabolism
Clofibrate	Possible decreased clofibrate effect	Increased metabolism
Contraceptives, oral	Possible decreased oral contraceptive effect	Increased metabolism
Corticosteroids	Possible decreased corticosteroid effect	Increased metabolism
Cyclosporine	Possible decreased cyclosporine effect	Increased metabolism
Dapsone	Possible decreased dapsone effect	Increased metabolism
Delavirdine	Decreased delavirdine concentration, increased rifamycin toxicity	Increased, decreased metabolism
Diazepam	Possible decreased diazepam effect	Increased metabolism

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Digoxin	Decreased digitoxin and digoxin effects	Increased metabolism
DHP CCBs	Decreased DHP CCB effect	Increased metabolism
Disopyramide	Possible decreased disopyramide effect	Increased metabolism
Doxycycline	Decreased doxycycline effect	Increased metabolism
Estrogens	Decreased estrogen concentrations	Hepatic enzyme induction
Ethionamide	Increased hepatotoxicity	Additive
Fluconazole	Decreased fluconazole effect	Increased metabolism
Hepatitis C direct-acting antivirals	Decreased antiviral exposure	Increased metabolism/transport
HMG-CoA reductase inhibitors	Decreased statin effect	Increased metabolism
Hypoglycemics, oral	Possible decreased oral hypoglycemic effect	Increased metabolism
Indinavir	Decreased indinavir effect; increased rifamycin exposure	Increased metabolism; decreased metabolism
Isoniazid	Possible increased hepatotoxicity	Possible increased toxic metabolites
Mexiletine	Possible decreased mexiletine effect	Increased metabolism
Narcotics	Possible decreased narcotic effect	Increased metabolism
Nelfinavir	Decreased nelfinavir effect	Increased metabolism
Nevirapine	Decreased nevirapine effect	Increased metabolism
Praziquantel	Decreased praziquantel effect	Increased metabolism
Progestins	Possible decreased progestin effect	Increased metabolism
Quinidine	Possible decreased quinidine effect	Increased metabolism
Ritonavir	Decreased ritonavir effect, increased rifamycin toxicity	Increased, decreased metabolism
Saquinavir	Decreased saquinavir effect, increased rifamycin toxicity	Increased, decreased metabolism
Tacrolimus	Decreased tacrolimus effect	Increased metabolism
Terbinafine	Decreased terbinafine effect	Increased metabolism
Theophylline	Possible decreased theophylline effect	Increased metabolism
Verapamil	Possible decreased verapamil effect	Increased metabolism
Zidovudine	Decreased zidovudine concentrations, AUCs	Mechanism not established
Rilpivirine With		
Antacids	Decreased rilpivirine exposure	Decreased absorption
Azole antifungals	Increased rilpivirine exposure	Decreased metabolism
Delavirdine	Increased rilpivirine exposure	Decreased metabolism
Efavirenz	Decreased rilpivirine exposure	Increased metabolism
Etravirine	Decreased rilpivirine exposure	Increased metabolism
H_2 receptor antagonists	Decreased rilpivirine exposure	Decreased absorption
Macrolides	Increased rilpivirine exposure	Decreased metabolism
Methadone	Decreased methadone exposure	Increased metabolism
Nevirapine	Decreased rilpivirine exposure	Increased metabolism
Ombitasvir-paritaprevir-ritonavir	Increased rilpivirine exposure	Mechanism not established
Ritonavir	Increased rilpivirine exposure	Decreased metabolism
Ritonavir, Lopinavir-Ritonavir: Same as With All Protease Inhibitors *Plus*		
Albendazole	Altered albendazole efficacy	Increased or decreased metabolism
Atovaquone	Decreased atovaquone effect	Increased phase II metabolism
Chlorpromazine	Increased ritonavir, chlorpromazine effect, toxicity	Decreased metabolism
Clozapine	Decreased clozapine effect	Increased metabolism
Codeine	Decreased analgesic effect	Decreased metabolism
Digoxin	Increased digoxin effect, toxicity	Decreased clearance
Disopyramide	Increased disopyramide effect, toxicity	Decreased metabolism
Disulfiram	Disulfiram-like reactions	Alcohol in protease dosage forms
Ethosuximide	Increased ethosuximide effect, toxicity	Decreased metabolism
Fentanyl	Increased fentanyl effect, toxicity	Decreased metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Flecainide	Increased flecainide effect, toxicity	Decreased metabolism
Gemfibrozil	Increased ritonavir effect, toxicity	Mechanism not established
Haloperidol	Increased haloperidol effect, toxicity	Decreased metabolism
Meperidine	Increased risk of normeperidine toxicity	Increased metabolism of parent
Metoprolol	Increased metoprolol effect, toxicity	Decreased metabolism
Metronidazole	Disulfiram-like reactions	Alcohol in protease dosage forms
Mexiletine	Increased mexiletine effect, toxicity	Decreased metabolism
Morphine	Decreased morphine effect	Increased metabolism
Olanzapine	Decreased olanzapine effect	Increased metabolism
Perphenazine	Increased perphenazine, ritonavir effect, toxicity	Decreased metabolism
Prochlorperazine	Increased prochlorperazine, ritonavir effect, toxicity	Decreased metabolism
Promethazine	Increased promethazine, ritonavir effect, toxicity	Decreased metabolism
Propafenone	Increased propafenone effect, toxicity	Decreased metabolism
Quinine	Increased quinine effect, toxicity	Decreased metabolism
Tamoxifen	Increased tamoxifen effect, toxicity	Decreased metabolism
Terfenadine	Increased risk of cardiotoxicity	Decreased metabolism
Thalidomide	Increased thalidomide effect, toxicity	Decreased metabolism
Theophylline	Decreased theophylline effect	Increased metabolism
Thioridazine	Increased thioridazine, ritonavir effect, toxicity	Decreased metabolism
Timolol	Increased timolol effect, toxicity	Decreased metabolism
Tramadol	Increased tramadol effect, toxicity	Decreased metabolism
Tricyclic antidepressants	Increased tricyclic effect, toxicity	Decreased metabolism
Trifluoperazine	Increased trifluoperazine, ritonavir effect, toxicity	Decreased metabolism
Triflupromazine	Increased triflupromazine, ritonavir effect, toxicity	Decreased metabolism
Simeprevir With		
Amiodarone	Increased risk of symptomatic bradycardia	Mechanism not established
CCBs	Increased exposure of CCBs and simeprevir	Decreased metabolism
Cyclosporine	Increased cyclosporine exposure	Decreased metabolism and efflux transport
CYP3A inducers	Decreased simeprevir exposure	Increased metabolism
CYP3A inhibitors	Increased simeprevir exposure	Decreased metabolism
HMG-CoA reductase inhibitors	Increased statin exposure, toxicity	Decreased metabolism
Ledipasvir	Increased simeprevir and ledipasvir exposure	Mechanism not established
OAT1B1/3 substrates	Increased substrate exposure, toxicity	Decreased OAT1B1/3-mediated efflux
Sofosbuvir With		
Amiodarone	Increased risk of serious bradycardia	Mechanism not established
P-gp inducers	Decreased sofosbuvir exposure	Increased P-gp–mediated efflux
Rifamycins	Decreased sofosbuvir exposure	Increased P-gp–mediated efflux
Sofosbuvir-Ledipasvir: Same as Sofosbuvir *Plus*		
Antacids	Decreased ledipasvir exposure	Decreased absorption
Digoxin	Increased digoxin exposure	Decreased P-gp–mediated efflux
H_2 receptor antagonists	Decreased ledipasvir exposure	Decreased absorption
Proton pump inhibitors	Decreased ledipasvir exposure	Decreased absorption
Simeprevir	Increased ledipasvir and simeprevir exposure	Mechanism not established
Sofosbuvir-Velpatasvir: Same as Sofosbuvir *Plus*		
Antacids	Decreased velpatasvir exposure	Decreased absorption
BCRP substrates	Increased substrate exposure	Decreased BCRP-mediated efflux
Digoxin	Increased digoxin exposure	Mechanism not established
H_2 receptor antagonists	Decreased velpatasvir exposure	Decreased absorption

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Proton pump inhibitors	Decreased velpatasvir exposure	Decreased absorption
Sofosbuvir-Velpatasvir-Voxilaprevir: Same as Sofosbuvir-Velpatasvir *Plus*		
Atazanavir	Increased voxilaprevir exposure	Mechanism not established
Cyclosporine	Increased voxilaprevir exposure	Mechanism not established
Lopinavir	Increased voxilaprevir exposure	Mechanism not established
Stavudine With		
Chloramphenicol	Increased peripheral neuropathy	Additive toxicity
Cisplatin	Increased peripheral neuropathy	Additive toxicity
Clarithromycin	Possible decreased stavudine effect	Decreased bioavailability (?)
Dapsone	Increased peripheral neuropathy	Additive toxicity
Disulfiram	Increased peripheral neuropathy	Additive toxicity
Ethionamide	Increased peripheral neuropathy	Additive toxicity
Fluconazole	Possible decreased stavudine effect	Decreased bioavailability (?)
Glutethimide	Increased peripheral neuropathy	Additive toxicity
Hydralazine	Increased peripheral neuropathy	Additive toxicity
Isoniazid	Increased peripheral neuropathy	Additive toxicity
Iodoquinol	Increased peripheral neuropathy	Additive toxicity
Methadone	Decreased stavudine effect	Decreased bioavailability
Metronidazole	Increased peripheral neuropathy	Additive toxicity
Nitrofurantoin	Increased peripheral neuropathy	Additive toxicity
Pentamidine	Increased pancreatitis	Additive toxicity
Phenytoin	Increased peripheral neuropathy	Additive toxicity
Ribavirin	Increased peripheral neuropathy	Additive toxicity
Rifabutin	Decreased stavudine effect	Decreased bioavailability (?)
Trimethoprim-sulfamethoxazole	Increased pancreatitis	Additive toxicity
Vincristine	Increased peripheral neuropathy	Additive toxicity
Sulfonamides With		
Barbiturates	Increased thiopental effect	Decreased albumin binding
Chloroprocaine	Possible antagonism of sulfonamide action	Competition for PABA site
Cyclosporine	Decreased cyclosporine effect with sulfamethazine	Possible increased metabolism
Hypoglycemics, sulfonylurea	Increased hypoglycemic effect	Mechanism not established
Lamivudine	Increased lamivudine toxicity	Competition for renal clearance
Methenamine	Crystallization of sulfonamides in the urine; precipitate of formaldehyde, sulfamethizole	Acidification of the urine
Methotrexate	Possible increased methotrexate toxicity	Decreased renal clearance and displacement from binding
Paraldehyde	Crystallization of sulfonamides in the urine	Acidification of the urine
Phenytoin	Increased phenytoin effect, except possibly with sulfisoxazole	Decreased metabolism
Procaine	Possible antagonism of sulfonamide action	Competition for PABA site
Tetracaine	Possible antagonism of sulfonamide action	Competition for PABA site
Thiopental	Increased thiopental effect; decreased dose necessary when given with sulfisoxazole	Plasma protein binding competition
Warfarin	Increased anticoagulant effect	Decreased metabolism and displacement from binding sites
Telavancin With		
Heparin	Falsely elevated aPTT results	Mechanism not established
Telbivudine With		
Peginterferon alfa-2a	Increased risk of peripheral neuropathy	Mechanism not established
Tenofovir Alafenamide Fumarate With		
Anticonvulsants	Decreased tenofovir exposure	Increased metabolism
Rifamycins	Decreased tenofovir exposure	Increased metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Tenofovir Disoproxil Fumarate With		
Atazanavir	Decreased atazanavir, increased tenofovir exposure	Mechanism not established
Didanosine	Increased didanosine effect, toxicity	Mechanism not established
Ledipasvir	Increased tenofovir exposure	Mechanism not established
Terbinafine With		
Caffeine	Increased caffeine effect, toxicity	Decreased metabolism
Cimetidine	Increased terbinafine effect, toxicity	Decreased metabolism
Cyclosporine	Decreased cyclosporine effect	Increased metabolism
Rifamycins	Decreased terbinafine effect	Increased metabolism
Tetracyclines With		
Alcohol	Decreased doxycycline effect in alcoholics	Increased metabolism
Aminoglycosides	In vitro antagonism; no in vivo support	Mechanism not established
Antacids, oral	Decreased oral tetracycline effects	Decreased tetracycline absorption
Anticoagulants, oral	Increased anticoagulant effect	Mechanism not established
Antidepressants, tricyclic	Localized hemosiderosis with amitriptyline and minocycline	Possible synergistic toxicity
Barbiturates	Decreased doxycycline effect	Increased metabolism
Bismuth subsalicylate	Decreased tetracycline effect	Decreased absorption
CBZ	Decreased doxycycline effect	Increased metabolism
Contraceptives, oral	Decreased contraceptive effect	Possible decreased enterohepatic circulation of estrogen
Digoxin	Increased digoxin effect	Decreased gut metabolism and increased absorption
Iron, oral	Decreased tetracycline effect, but not with doxycycline; decreased iron effect	Decreased absorption
Kaolin-pectin	Decreased concentrations of tetracyclines	Decreased absorption
Lithium	Increased lithium toxicity	Decreased renal excretion
Methotrexate	Possible increased toxicity	Mechanism not established
Methoxyflurane	Increased nephrotoxicity	Displacement from binding
Penicillins	In vitro antagonism; rare in vivo support for this	Mechanism not established
Phenytoin	Decreased doxycycline effect	Increased metabolism
Rifamycins	Possible decreased doxycycline effect	Increased metabolism
Theophylline	Possible theophylline toxicity	Mechanism not established
Zinc sulfate	Decreased tetracycline effect	Decreased absorption
Tigecycline With		
Warfarin	Increased warfarin exposure without altering the INR	Mechanism not established
Tinidazole With		
Alcohol	Increased alcohol side effects	Decreased metabolism
Cholestyramine	Decreased tinidazole effect, toxicity	Decreased absorption
Cyclosporine	Increased cyclosporine effect, toxicity	Decreased metabolism
CYP3A inducers	Decreased tinidazole effect, toxicity	Increased metabolism
CYP3A inhibitors	Increased tinidazole effect, toxicity	Decreased metabolism
Disulfiram	Increased CNS toxicity	Mechanism unknown
Fluorouracil	Increased fluorouracil effect, toxicity	Decreased clearance
Lithium	Increased lithium effect, toxicity	Mechanism unknown
Phenytoin	Increased IV phenytoin effect, toxicity	Mechanism unknown
Tacrolimus	Increased tacrolimus effect, toxicity	Decreased metabolism
Warfarin	Increased anticoagulant effects	Decreased metabolism
Trimethoprim With		
Angiotensin-converting enzyme inhibitors	Hyperkalemia	Reduced potassium clearance

Continued

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Amiloride	Trimethoprim may potentiate hyponatremia because of concomitant use of amiloride with thiazide diuretics	Additive toxicity
Azathioprine	Leukopenia	Mechanism not established
Cyclosporine	Increased nephrotoxicity	Synergistic toxicity
Dapsone	Increased dapsone toxicity	Altered clearance
Digoxin	Possible increased digoxin effect	Decreased renal excretion and possibly decreased metabolism
Methotrexate	Increased methotrexate toxicity	Decreased clearance
Phenytoin	Increased phenytoin serum concentrations—increased risk of phenytoin toxicity; increased risk of folate deficiency	Decreased metabolism; additive toxicity
Thiazide diuretics	Trimethoprim may potentiate hyponatremia because of concomitant use of amiloride with thiazide diuretics	Additive toxicity
Vancomycin With		
Aminoglycosides	Possible increased nephrotoxicity and ototoxicity	Additive toxicity
Amphotericin B	Increased nephrotoxicity	Additive toxicity
Bacitracin	Increased nephrotoxicity	Additive toxicity
Cisplatin	Increased nephrotoxicity	Additive toxicity
Digoxin	Possible decreased digoxin effect	Possibly decreased absorption
Piperacillin-tazobactam	Increased nephrotoxicity	Mechanism not established
Polymyxins	Increased nephrotoxicity	Additive toxicity
Warfarin	Increased risk of bleeding	Mechanism not established
Voriconazole With		
Amprenavir	Increased amprenavir, voriconazole effect, toxicity	Decreased metabolism
Atorvastatin	Increased atorvastatin effect, toxicity	Decreased metabolism
Barbiturates	Decreased voriconazole effect	Increased metabolism
BZDs	Increased BZD effect, toxicity	Decreased metabolism
CCBs	Increased CCB effect, toxicity	Decreased metabolism
CBZ	Decreased voriconazole effect	Increased metabolism
Ergot alkaloids	Increased ergot effect, toxicity	Decreased metabolism
HMG-CoA reductase inhibitors	Increased statin effect, toxicity	Decreased metabolism
NNRTIs	Increased NNRTI effect, toxicity	Decreased metabolism
Omeprazole	Increased omeprazole effect, toxicity	Decreased metabolism
Phenytoin	Increased phenytoin effect, toxicity; decreased voriconazole effect	Decreased, increased metabolism
Pimozide	Increased pimozide effect, toxicity	Decreased metabolism
Protease inhibitors	Increased protease inhibitor, voriconazole effect, toxicity	Decreased metabolism
Quinidine	Increased quinidine effect, toxicity	Decreased metabolism
Rifamycins	Increased rifamycin effect, toxicity; decreased voriconazole effect	Decreased, increased metabolism
Ritonavir	Increased ritonavir, voriconazole effect, toxicity	Decreased metabolism
Sirolimus	Increased sirolimus effect, toxicity	Decreased metabolism
Sulfonylureas	Increased sulfonylurea effect, toxicity	Decreased metabolism
Tacrolimus	Increased tacrolimus effect, toxicity	Decreased metabolism
Vinca alkaloids	Increased vinca effect, toxicity	Decreased metabolism
Warfarin	Increased warfarin effect, toxicity	Decreased metabolism
Zidovudine With		
Acetaminophen	Granulocytopenia	Mechanism not established
Acyclovir	Neurotoxicity	Mechanism not established
Amphotericin B	Increased risk of nephrotoxicity, hematologic toxicity	Additive toxicity
Antimycobacterials	Possible increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism
Aspirin	Possible increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism
Atovaquone	Possible increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism
Chloramphenicol	Possible increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism

TABLE 54.36 Adverse Drug Interactions Involving Antiinfective Agents[a]—cont'd

INTERACTING DRUGS	ADVERSE EFFECT	PROBABLE MECHANISM
Cimetidine	Possible increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism
Clarithromycin	Decreased zidovudine concentrations and AUCs	Mechanism not established
Cytotoxic or myelosuppressive agents	Increased risk of hematologic toxicity	Additive toxicity
Dapsone	Increased neutropenia	Additive toxicity
Fluconazole	Increased risk of hematologic toxicity	Decreased phase I metabolism
Flucytosine	Increased hematologic toxicity	Additive toxicity
Ganciclovir	Decreased zidovudine effect; hematologic toxicity	Increased clearance; additive toxicity
Hydroxyurea	Increased hematologic toxicity	Additive toxicity
Indomethacin	Possible increased risk of hematologic toxicity	Mechanism not established; additive
Interferon alfa	Increased hematologic toxicity	Additive toxicity
Interferon beta	Increased hematologic toxicity	Additive toxicity
Methadone	Increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism
Nephrotoxic agents	Increased risk of toxicity	Increased serum concentrations; decreased clearance
Nucleoside analogues	Increased risk of hematologic toxicity	Additive toxicity
Oral contraceptives	Increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism
Oxazepam	Possible increased risk of hematologic toxicity	Decreased phase II zidovudine metabolism
Phenytoin	Increased risk of hematologic toxicity	Decreased clearance
Primaquine	Increased risk of peripheral neuropathy	Additive toxicity
Probenecid	Increased risk of hematologic toxicity	Decreased clearance, phase II metabolism
Pyrazinamide	Decreased effect of pyrazinamide	Mechanism not established
Pyrimethamine	Increased hematologic toxicity	Additive toxicity
Ribavirin	In vitro antiretroviral antagonism	Decreased phosphorylation of zidovudine
Rifamycins	Decreased zidovudine effect	Increased phase II zidovudine metabolism
Sulfadiazine	Increased hematologic toxicity	Additive toxicity
Trimethoprim	Increased risk of hematologic toxicity	Decreased zidovudine clearance
Trimethoprim-sulfamethoxazole	Increased hematologic toxicity	Additive toxicity
Valproic acid	Increased zidovudine effect, toxicity	Decreased phase II zidovudine metabolism

[a]This table includes significant adverse drug interactions and is not a comprehensive list of all potential adverse drug interactions.
ALT, Alanine aminotransferase; *aPTT,* activated partial thromboplastin time; *AUC,* area under the concentration-time curve; *BCG,* Bacillus Calmette-Guérin; *BCRP,* breast cancer resistance protein; *BZDs,* benzodiazepines; *CBZ,* carbamazepine; *CCB,* calcium channel blocker; *CNS,* central nervous system; *CYP,* cytochrome P450; *DHP CCB,* dihydropyridine calcium channel blocker; *GI,* gastrointestinal; *G6PD,* glucose-6-phosphate dehydrogenase; *HMG-CoA,* 3-hydroxy-3-methyl-glutaryl coenzyme A; *INR,* international normalized ratio; *MAO,* monoamine oxidase; *NNRTI,* nonnucleoside reverse-transcriptase inhibitor; *NSAIDs,* nonsteroidal antiinflammatory drugs; *OAT,* organic anion transporter; *PABA,* para-aminobenzoic acid; *P-gp,* P-glycoprotein; *PT,* prothrombin time; *SNRI,* serotonin and norepinephrine reuptake inhibitor; *SSRI,* selective serotonin reuptake inhibitor.

This is a very important point to consider, because many clinicians will use these equations even in the face of the aforementioned contraindications, stating that "bad data are better than no data" or "everyone does it this way." We completely disagree with these premises and urge the clinician to use clinical judgement and not rely on data that he or she knows are not accurate, because this could result in serious dosing errors in a patient with resultant morbidity or mortality. In addition, the practice of "rounding serum creatinine to 1.0 mg/dL" to calculate CrCl is inaccurate and should not be done. Improvement in the estimation of kidney function is more likely to occur with the use of exogenously administered substrates (e.g., inulin, iohexol) or endogenous substrates such as cystatin C, which is less confounded by clinical variables. However, use of these novel biomarkers to guide drug dosing will be difficult to validate in the near term.

DOSAGE ADJUSTMENT FOR HEPATIC IMPAIRMENT

In theory, although hepatic impairment can impair drug clearance, accurate biomarkers of hepatic function and correlation with dosage adjustment are generally not available. Several drug labels include dose adjustment recommendations that are based on PK studies performed in patients with cirrhosis and are classified according to the three-category Child-Pugh score (A, B, or C). This scoring system was developed as a tool to predict mortality during surgery and the necessity for liver transplantation. Newer scoring systems such as the Model for End-Stage Liver Disease (MELD) have been developed as prognostic tools. However, these categoric estimates have limited clinical validity in considering dose modification. Many antiinfective agents have a high therapeutic-to-toxic ratio and therefore dosage adjustment in hepatic impairment is not necessary, even in the face of impaired body clearance. In circumstances in which narrow therapeutic-to-toxic ratios are seen, clinical judgment and appropriate treatment of infection should take precedence in drug dosage determinations.

DOSAGE ADJUSTMENT FOR BODY SIZE (OBESE AND UNDERWEIGHT)

The dosage of drugs in adults is often based on a fixed dose (with no regard to body size), weight, or body surface area (height and weight metric). Several antiinfective agents have traditionally been given on the basis of body weight (milligrams per kilogram). Although this approach may be reasonable during maturation (birth to 2 years of age), the reliability of this body size metric has been called into question because drug clearance mechanisms do not increase proportionately with weight (>2 years of age). The underlying mathematical assumptions

and dosing errors that may occur as a consequence of use of weight or an alternate body size descriptor have been recently reviewed (see Pai in Suggested Readings). In general, computing maintenance antiinfective doses on the basis of total body weight has the potential for overdose (in obese individuals) and underdose (in underweight individuals) at the extremes of the weight distribution. Over time, several alternate body size descriptors such as body surface area, ideal body weight, adjusted body weight, and lean body weight have been developed to address this problem. Adjusted body weight and lean body weight are mathematically similar to body surface area as metrics for dosages in adults. In contrast, ideal body weight is computed by sex by using height only, so this body size descriptor is conceptually equivalent to height-based dosing. As an example, if an antiinfective dose is approved at 10 mg/kg/day in an adult, then the dose will be 600 mg (60 kg) and 1200 mg (120 kg) if administered based on total body weight. If the 60-kg and 120-kg adults are male and 69 inches (175 cm) in height, then the ideal body weight is 70.7 kg and both individuals would receive the same dose of 707 mg based on ideal body weight. In contrast, the lean body weight would be 51.1 kg and 73.5 kg in the 60-kg and 120-kg adults, respectively. Therefore the doses would be 511 and 735 mg (1.4-fold difference) across this 2-fold difference in total body weight. If the antiinfective had been approved at 12 mg/kg/day based on lean body weight, the dose would be 613 mg and 882 mg in the 60- and 120-kg individuals, respectively. This last solution meets empirical observations that drug clearance typically increases by approximately 50% across this 2-fold weight distribution. Unfortunately, early-phase clinical trials, which define the dose for evaluation in phase III trials, often exclude obese and underweight participants. The resulting narrow weight distribution limits the ability to define drug-specific dose extrapolation for body size. Regrettably, consensus on a universal approach to dose extrapolation has not been reached and, therefore, specific guidance for antiinfective dosing in obese and in underweight patients should be sought on a drug-by-drug basis.

DRUG-DRUG INTERACTIONS

Drug-drug interactions are categorized into PD and PK interactions. Many interactions deemed PD are really the exposure-response effects of a PK interaction leading to greater pharmacologic effect. These PD interactions are often exploited in antiinfective therapy, and it is through this mechanism that combination therapy can demonstrate synergistic effect. Quantitation of PD interactions is at best difficult. PK interactions are somewhat more "predictable" but still show large variability in the population.

PK drug-drug interactions can occur through a number of different mechanisms. These include:

- Reduction of drug absorption by concurrent drugs: The most common absorption interactions occur by means of chelation of an antiinfective with a cation such as calcium, magnesium, or iron. A useful rule of thumb is that oral antiinfectives should not be administered within 1 hour before or 2 hours after the administration of oral divalent or trivalent cations unless a study has clearly demonstrated a lack of significant interaction with their coadministration.
- Displacement from protein-binding sites: Although this interaction in theory could enhance antiinfective tissue distribution and efficacy, homeostatic mechanisms act to increase the elimination of unbound drug, resulting in no substantial change in overall drug exposure.
- Inhibition, induction, or activation of DMEs or transporters: These drug interactions are by far the most important seen in clinical practice. Most information on drug-drug interactions is generated in a very limited number of individuals, often healthy volunteers, and may not be applicable to the general population. Some basic tenants of these drug interactions are important to remember:
  - Genetic and environmental factors, in addition to the microbiome composition, can affect the baseline activity of DMEs and transporters and thus will affect the potential for drug-drug interactions.
  - Not all patients experience a drug-drug interaction, even if this has been described in the literature. This is determined by both genetic and environmental factors.
  - Patients with low or null DMEs or transporter activity have a low chance of drug-drug interactions; however, if this low baseline activity is due to a reversible pathologic process, the potential for drug-drug interactions will increase as the pathologic process is appropriately treated.
  - Patients with normal or high DME or transporter activity have a high likelihood of drug-drug interactions.
  - Patients with a low genetic potential to make a DME or transporter (poor or intermediate metabolizers or transporters) have a small chance of an induction interaction because they do not have the genetic potential to make more of the enzyme or transporter when stimulated to do so.
  - Patients with normal or high genetic potential to make a DME or transporter are at greater risk of an induction interaction owing to the greater genetic potential to make the enzyme or transporter.
  - Patients can undergo enzyme heterocyclic activation of a DME, which increases the activity of the enzyme approximately 33%. In enzyme heterocyclic activation the peak effect occurs immediately, whereas it can take up to 2 weeks for the peak effect to be seen in induction. Heterocyclic activation is most often seen with CYP3A isozymes and CYP2B6.
  - Over the course of many infectious diseases, patients with infections (acute and chronic) have the potential to substantially change over time in terms of their risk of drug-drug interactions; thus this is not a static process.

We have provided a table of inducers, inhibitors, and substrates for important DMEs and transporters. Data on drug interactions and transporters are much more limited and less understood because specific probes and biomarkers for transporters are not well developed and validated.

Although clinicians request a specific recommendation for dosage adjustment when a drug-drug interaction is identified, it is virtually impossible to provide a specific recommendation because of the previously mentioned factors. An approach that incorporates appropriate treatment of an infection with the goals of optimizing efficacy, reducing the risk of resistance, and attempting to avoid toxicity is recommended. Familiarity with the toxic side effects of antiinfectives and how to monitor them is essential.

Suggested Readings

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Major Clinical Syndromes

II

A Fever

55 Temperature Regulation and the Pathogenesis of Fever

Mohammad M. Sajadi and Andrej A. Romanovsky

SHORT VIEW SUMMARY

Terminology
- Fever is a pyrogen-mediated rise in body temperature above normal.

Clinical Thermometry
- Thermometric measurements are influenced by a host of variables, all too frequently ignored when interpreting the significance of clinical temperature readings.
- There are two basic thermal compartments worthy of special consideration: the shell and the core.
- Oral and tympanic membrane measurements differ from the core temperature as measured rectally but are adequate for the majority of uses.
- Fever may be defined as an early-morning temperature ≥37.2°C (99.0°F) or ≥37.8°C (100°F) at any time.

Thermoregulation
- The thermoregulatory system maintains a relatively constant core body temperature by functioning as a federation of relatively independent effector loops, without a single controller or set point.

The Generation of Fever
- Fever is a thermoregulatory manifestation of the innate immune response, initiated with the binding of pathogen-associated molecular patterns to pattern recognition receptors expressed by immune cells.
- The crucial step is the production of prostaglandin E_2, which crosses the blood-brain barrier and triggers fever.

Acute-Phase Response
- In addition to cytokine-mediated rise in the core temperature, numerous other physiologic reactions, collectively referred to as the acute-phase response, are mediated by members of the same group of pyrogenic cytokines that activate the thermal response of fever.

Biologic Value of Fever
- Questions concerning fever's risk-benefit quotient have generated considerable controversy, and there is as yet no consensus as to the appropriate clinical situations, if any, in which fever or its mediators should be suppressed.

Biologic Value of Fever: The Case of Sepsis
- There is evidence from human studies that suggests that fever in sepsis is associated with improved outcomes.

Biologic Value of Hypothermia
- Like fever, hypothermia is appears to be a regulated response; however, its potential value is still a matter of study and conjecture.

Antipyretic Therapy: General Considerations
- Antipyretics are commonly administered to enhance patient comfort.
- Although antipyretics can decrease postvaccination titers (in the short term) and pathogen clearance, the significance is not known.
- Antipyretics have not been shown to protect against recurrences of febrile seizures.

Antipyretic Therapy: The Case of Sepsis
- Antipyretics are of limited value in the treatment of bacterial sepsis and in critically ill patients with fever.

Antipyretic Therapy: Pharmacologic Agents
- Antipyretics can be prescribed to provide symptomatic relief in febrile patients, reduce the metabolic demands of fever in patients with underlying cardiovascular and pulmonary disorders, and possibly prevent or alleviate fever-induced mental dysfunction in older patients

Antipyretic Therapy: Physical Methods of Antipyresis
- In view of the capacity of external cooling measures to induce a cold pressor response, it is questionable whether this form of antipyretic therapy should ever be administered to febrile patients with infection.

The oldest known written reference to fever exists in Akkadian cuneiform inscriptions from the 6th century BCE, most likely derived from an ancient Sumerian pictogram of a flaming brazier used to symbolize both fever and the local warmth of inflammation.[1] Theoretical constructs of the pathogenesis of fever did not emerge until several centuries later, when Hippocratic physicians proposed that body temperature, and physiologic harmony in general, involved a delicate balance among four corporal humors: blood, phlegm, black bile, and yellow bile.[2] Fever was then believed to result from an excess of yellow bile, a concept in concert with the fact that many infections of that era caused both fever and jaundice. Galen later broadened the classification of fevers and expounded the idea that many types of fever developed from putrification of humors.[3] During the Middle Ages, demonic possession was added to the list of mechanisms thought to be responsible for fever. By the 18th century, Harvey's discovery of the circulation of blood and the birth of clinical chemistry led iatrophysicists and iatrochemists to hypothesize alternatively that body heat and fever resulted from friction associated with the flow of blood through the vascular system and that they resulted from fermentation and putrefaction occurring in the blood and intestines.[4] Ultimately, as a result of the work of Claude Bernard in the 19th century, the metabolic processes occurring within the body came to be recognized as the true source of body heat. Subsequent work established that body temperature is tightly controlled by mechanisms regulating the rate of heat production and the rate of heat dissipation (heat loss).

The origin of the practice of monitoring body temperature as an aid to diagnosis is uncertain. The oldest known references to devices used to measure temperature date to the 1st or 2nd century BCE, when Philo of Byzantium and Hero of Alexandria are believed to have invented several such devices.[5,6] The CE 10th century Persian physician Akhawaynī appears to be the first to propose and use a fever curve.[3] It is reasonably certain that Galileo manufactured a primitive (air) thermometer at about the time that he assumed the chair in mathematics at Padua in 1592.[7] However, thermometry was not fully assimilated into medical practice until 1868, when Carl Reinhold August Wunderlich published a magnum opus entitled *Das Verhalten der Eigenwärme in Krankenheiten (The Course of Temperature in Diseases)*,[8] in which he gave 37°C (98.6°F) special significance with respect to normal body temperature.[9] He described the diurnal variation of body temperature and, in the process, alerted clinicians to the fact that "normal body temperature" is actually a temperature range, rather than a specific value.

TERMINOLOGY

According to the International Union of Physiological Sciences Commission for Thermal Physiology, fever is "a state of elevated core temperature, which is often, but not necessarily, part of the defensive responses of multicellular organisms (host) to the invasion of live (microorganisms) or inanimate matter recognized as pathogenic or alien by the host."[10] The febrile response, of which the temperature rise is a component, is a complex physiologic reaction to disease, involving not only a rise in core temperature, per se, but also the generation of acute-phase reactants and the activation of numerous physiologic, endocrinologic, and immunologic systems. The active rise in body temperature during fever is to be distinguished from that occurring during episodes of passive hyperthermia. During the rising phase of fever, thermoregulation works to increase body temperature by increasing heat production (e.g., by shivering) and decreasing heat loss (e.g., by cutaneous vasoconstriction), and the work of the thermoregulation system is uncompromised. In contrast, during hyperthermia the thermoregulation system works to decrease body temperature by increasing heat loss (e.g., by sweating), and the fact that body temperature nevertheless increases equates to the system's failure. Although basic scientists often distinguish fever from hyperthermia, based on the above-mentioned argumentation, physicians often view fever as an example of hyperthermia (a state with an increased body temperature), similar to how any increase in the heart rate is considered tachycardia.[11]

In the clinical setting, fever is typically defined as a pyrogen-mediated rise in body temperature above the normal range. Although consistent with the popular perception of fever, this definition ignores the fact that a rise in body temperature is but one component of a multifaceted response. This standard clinical definition is further flawed because it implies that body temperature is a single entity, when, in fact, it is a pastiche of many different temperatures, each representative of a particular body part and each varying throughout the day in response to activities of daily living and the influence of endogenous diurnal rhythms.

CLINICAL THERMOMETRY

For more than a century the thermometer has been preeminent among clinical instruments used to distinguish health from disease and to monitor the course of illness. Unfortunately, thermometric measurements are influenced by a host of variables, all too frequently ignored when interpreting the significance of clinical temperature readings.

Anatomic Variability

Although clinicians frequently regard temperature readings from various anatomic sites as equivalent approximations of body temperature,[1] no one temperature provides a comprehensive description of the thermal status of the human body.[12] This is because the body has many different temperatures, each representative of a particular body part. Nevertheless, within the body, there are two basic thermal compartments worthy of special consideration: the shell and the core.[12,13]

The shell, which consists of mostly of skin, subcutaneous fat, and bones, insulates the core from the external environment. The core, of which the brain, thoracic viscera, and arterial and central venous blood are major components, although insulated by the shell, has temperature gradients of its own resulting from differences in the metabolic rates, blood flow patterns, and heat dissipation in the various organs contained therein. Even during baseline conditions, organs with higher metabolic rates have higher temperatures than those with lower metabolic rates and can be up to 1.3°C (2.3°F) higher than the temperature of arterial blood.[14] In addition, tissues close to the skin generally have lower temperatures than those at deeper locations.[13] Although such differences are normally small, muscle temperatures rise markedly during vigorous exercise compared with those of less metabolically active organs. During shock and under extreme environmental conditions, regional anatomic variations in temperature may also be exaggerated. Rectal measurements were once regarded as the most practical and accurate means of obtaining routine estimates of core temperature. However, rectal (and colonic) temperature readings are consistently higher than those obtained at other sites (even pulmonary artery blood), which some authorities have suggested might be caused by heat generated as a result of the metabolic activity of fecal bacteria,[13] even though an early study showed no significant decrease in the rectal temperature after a reduction in the colonic bacterial content. A significant effect on rectal temperature, however, is also exerted by that of the blood returning to the core from the lower extremities, because the internal iliac vein passes near the anorectal canal. Thus, during shock, the core area expands, the shell shrinks,[12] and perfusion of the rectum is markedly impaired, causing the rectal temperature to lag significantly behind a rapidly rising or falling core temperature.[15] For this reason, Houdas and Ring[16] have concluded that the rectal temperature provides a reliable approximation of the core temperature only if the patient is in thermal balance. In neonates and small children, even in the absence of shock, the rectal temperature measured by standard technique has been reported to correlate poorly with the core temperature as measured by a deep rectal (colonic) probe.[17,18]

The right atrium is an ideal site for measuring core temperature because it is the nexus at which venous blood from all anatomic regions joins. However, because it is relatively inaccessible, temperatures of other sites (esophageal, tympanic membrane [TM], and bladder temperature) are more often used as approximations of core temperature.[19,20]

The TM temperature is thought by some to be particularly useful in this regard because at least a portion of this membrane is perfused by a tributary of the artery that supplies the hypothalamus. The ease with which TM measurements can be obtained using modern infrared TM thermometers has made these instruments the devices of choice in many clinics and intensive care units (ICUs). There are two basic types of infrared TM thermometers. One type detects radiant energy emitted from the TM and portions of the ear canal, processes the information, and then displays a value representing tissue temperature in the ear canal (unadjusted mode). The other displays an (adjusted)

estimate of the core temperature (e.g., pulmonary arterial blood temperature) based on comparison data obtained from selected study samples. Readings obtained using the former type of TM thermometer tend to be lower than simultaneously obtained oral readings, whereas those obtained with the latter type are generally higher. Unfortunately, numerous studies of many different TM thermometers have shown that, although convenient, such instruments tend to give highly variable readings that correlate poorly with simultaneously obtained oral or rectal readings. In addition, multiple studies have demonstrated the inaccuracy of TM measurements in patients with hypothermia, hyperthermia, or fever,[21–23] and a careful study showed that there are regional variations on the TM itself.[24] Temporal artery (TA) thermometry (using similar infrared technology as TM measurements) has gained popularity in the last several years. Although some claim that this method measures core temperature, a recent meta-analysis concluded that it tends to underestimate the core and is as inaccurate as TM thermometry.[25]

Several studies have shown that monitoring the skin temperature using temperature-sensitive crystals incorporated into plastic strips placed on the forehead is an insensitive technique for detecting elevations in the core temperature. Studies have consistently demonstrated that axillary temperature measurements do not reflect core temperature,[26–28] even though axillary thermometry continues to be popular in several countries. Finally, the infrared detection of facial temperatures at airports at the present time is too inaccurate for mass screening during epidemics.[29,30]

The detection of fever in children holds its own challenges, as the use of the same sites (TM, TA, axillary) in children seems to be associated with an even greater deviation from the core temperature.[23,31,32]

Because temperatures of the rectum, mouth, and TM are related but not identical, it would be useful to have a reliable formula for converting data from one site to another. In a study of healthy young adults, Rabinowitz and associates[33] determined that, on average, rectal readings exceed concurrent oral readings by 0.4°C (0.8°F) and TM readings by 0.8°C (1.6°F), with high variability. These findings are in agreement with several earlier investigations.[34–37] However, Lorin[38] has shown TM readings to be higher than simultaneously obtained oral measurements. This discrepancy most likely reflects the fact that unadjusted-mode TM thermometers—for example, the IVAC Core—generally give lower readings than adjusted-mode TM thermometers, such as those used in earlier studies.[39]

We recommend consistency in device use, at whichever site is chosen. In addition, practitioners should be aware of the limitations of each modality (hypothermia and hyperthermia for TM readings, mucositis for oral readings), and should measure temperature by another method in those specific instances (e.g., rectal measurement in the case of hypothermia or hyperthermia, or TM measurement in those with mucositis). Currently, oral and TM measurements are the most common modalities used, at least in North America. Although these differ from the core temperature as measured rectally,[33] they are adequate for the majority of uses.

Physiologic Variables

Wunderlich and Seguin believed that "old" people have lower body temperatures than younger persons, and their views in this regard were corroborated by Howell in a report published in *Lancet* in 1948.[39a] There is also a substantial body of data suggesting that thermoregulation is impaired in older persons because of various effects of aging on the autonomic nervous system. Several studies have shown lower oral temperature measurements in the elderly.[40,41] However, core temperatures appear unchanged when comparing healthy older subjects (mean age, 80.3 years; range, 62–99 years) with healthy younger subjects. Comparisons of simultaneous oral, axillary, and rectal temperature readings from these subjects have shown lower average oral and axillary readings in older persons but comparable average rectal temperatures to those in younger subjects.

It has long been known that women exhibit increases in body temperature of about 0.5°C (0.9°F) at the time of ovulation,[16] which is used clinically to time periods of maximal fertility. Wunderlich and Seguin also maintained that women (1) have slightly higher normal temperatures than men overall and (2) often show greater and more sudden changes in temperature. Two other studies have corroborated Wunderlich and Seguin's former (but not latter) observation.[42,43] Further, in a recent study of more than 18,630 white adults age 20 to 98 years (mean, 58.3 years), women were found to have higher oral temperatures (97.5 ± 1.2°F) than men (97.2 ± 1.1°F).[40] Another study suggests that pregnancy affects the core temperature. In this study, rectal temperatures were measured during the first trimester, second trimester, third trimester, and 3 months postpartum. A core temperature of 37.1°C was noted in the first trimester, which progressively decreased and reached a nadir (36.4°C) postpartum.[43a]

Body temperature, like most physiologic functions, exhibits circadian rhythmicity, which is linked to the sleep-wake cycle.[44] Before 8 weeks of age, newborns exhibit hourly oscillations of body temperature.[45] During this time there is a decrease in the core (rectal) temperature that occurs with sleep.[46,47] The typical circadian rhythmicity of temperature seen in adults develops by 10 weeks of age.[48] During normal sleep-wake cycles (i.e., asleep during the night and awake during the day) the core temperature reaches its zenith in the late afternoon or early evening and its nadir in the early morning.[42] Adaptation to night-shift work causes a reversal of this pattern.[49] Thermoregulation has also been reported to be altered in patients with neuropsychiatric disorders, such as chronic depression.[50] Finally, there appears to be a blunting of the circadian amplitude in the elderly,[41,51] which is restored with melatonin therapy.[51] Therefore, when interpreting clinical thermometric measurements, it is important to consider not only the age of the patient, the time of the measurement, the site at which the temperature is taken, but also the sleep-wake cycle of the subject being studied.

In addition to these physiologic variables, exercise, medications (such as corticosteroids), digestion, and underlying disorders such as chronic renal failure, shock, and local inflammation at the site of the thermometric measurement (e.g., proctitis, external otitis, or stomatitis) may alter measured thermoregulatory responses, local temperature, or both. It has, for instance, been shown that the core temperature varies by as much as 3°C (from 36°C–39°C) in states ranging from sleep to moderately high levels of sustained exercise, and this continuum of body temperature is related to a continuum of activity.[52] Mucositis in cancer patients has been associated with a 0.89°C elevated oral temperature measurement compared with TM measurements.[53] Ambient temperature and humidity have been shown experimentally to affect both human sleep stages and body temperature,[54] suggesting that body temperature might also vary according to the time of year and local climate. It is pertinent in this regard that Cheng and Partridge[55] have shown that bundling and warm environments can elevate rectal temperatures of newborns to the febrile range.

"Normal Body Temperature"

A survey of physicians' perceptions of body temperature published in 1995 indicated widespread confusion regarding key features of the human body temperature.[55a] Of 268 physicians and medical students surveyed, 75% gave 37°C (98.6°F) as their definition of normal body temperature. An additional 13% defined the normal temperature as a narrow range of temperatures about a mean of 37°C (98.6°F). Only 10 (4%) subjects in the group as a whole specified a particular body site (e.g., oral or rectal) for temperature measurements in their definition. Also, 98% percent thought that the normal temperature varies during the day, with quantitative estimates of such diurnal variability ranging from 0.2°C (0.4°F) to 2.8°C (5°F). Such misconceptions are not limited to medical students and primary-care physicians. In the latest (2008) joint update from the American College of Critical Care Medicine and the Infectious Diseases Society of America (IDSA) for evaluation of new fever in critically ill adult patients, normal temperature is defined as 37°C.[19]

A 1992 descriptive analysis of 700 baseline oral temperature observations from 148 healthy men and women found a range of 35.6°C (96.0°F) to 38.2°C (100.8°F), with an overall mean of 36.8 ± 0.4°C (98.2 ± 0.7°F); 37°C (98.6°F) accounted for only 56 (8%) of the 700 oral temperature observations recorded (Fig. 55.1). The mean temperature varied diurnally, with a 6 a.m. nadir and a 4 to 6 p.m. peak (Fig. 55.2). The maximum temperature (as reflected by the 99th percentile) varied from a low of 37.2°C (98.9°F) at 6 a.m. to a high of 37.7°C (99.9°F) at 4 p.m. Age did not influence temperature within the range studied (18–40 years). Women

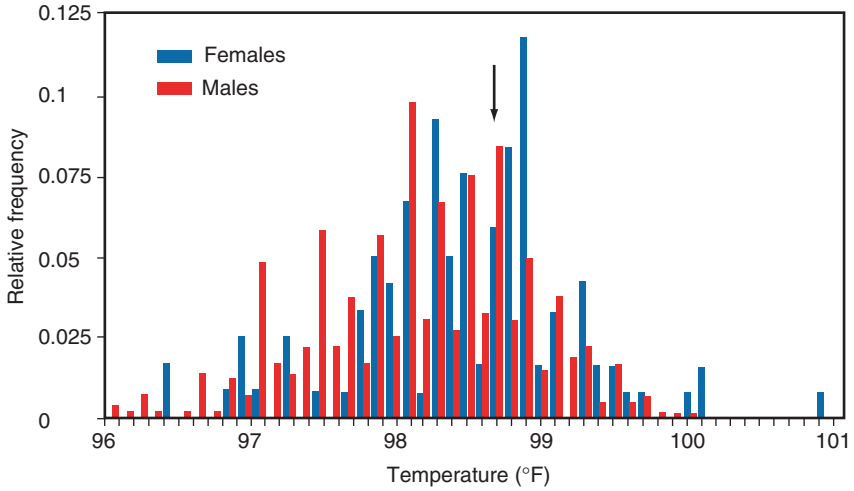

FIG. 55.1 Frequency distribution of 700 baseline oral temperatures obtained during 2 consecutive days of observation in 148 healthy young volunteers. Arrow indicates the location of 98.6°F (37°C). *(From Mackowiak PA, Wasserman SS, Levine MM. A critical appraisal of 98.6°F, the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich.* JAMA. *1992;268:1578–1580.)*

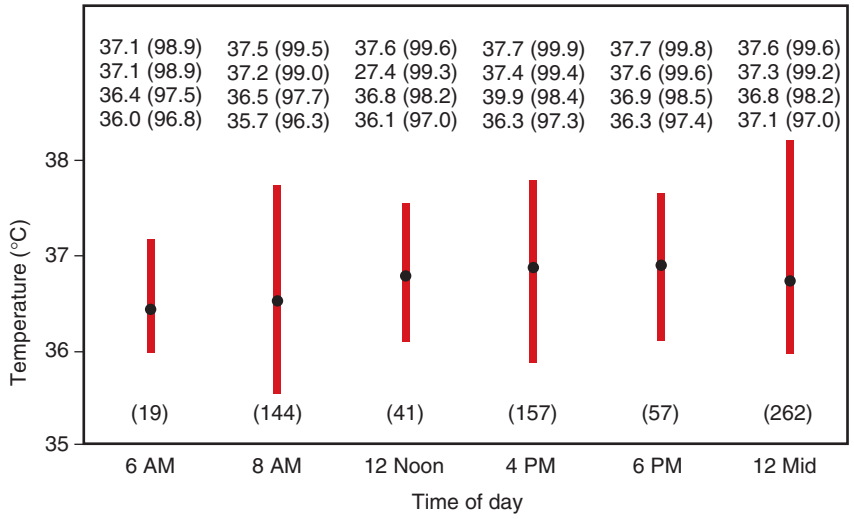

FIG. 55.2 Mean oral temperatures and temperature ranges in 148 healthy young volunteers according to time of day. The four temperatures shown at each sample time are the 99th percentile *(top)*, 95th percentile *(second)*, mean *(third)*, and 5th percentile *(bottom)* for each sample set. (The numbers in parentheses are the temperatures in degrees Fahrenheit.) The numbers in parentheses on the x-axis indicate the number of observations analyzed at each sample time. *(From Mackowiak PA, Wasserman SS, Levine MM. A critical appraisal of 98.6°F, the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich.* JAMA. *1992;268:1578–1580.)*

had a slightly higher average oral temperature than men (36.9°C [98.4°F] vs. 36.7°C [98.1°F]), with similar diurnal temperature oscillations of ~0.5°C (1.0°F). There was a linear relationship between temperature and pulse rate, with an average increase in heart rate of 4.4 beats/min for each 1°C (2.44 beats/min for each 1°F) rise in temperature over a wide range of temperatures examined.

According to Wunderlich and Seguin, "When the organism (man) is in a normal condition, the general temperature of the body maintains itself at the physiologic point: 37°C = 98.6°F."[55b] Although several subsequent investigations have recorded mean temperatures of normal adult populations closer to 36.6°C (98.0°F), Wunderlich's intimation that 37°C (98.6°F) is the most normal of temperatures persists to this day in lay thinking, although to a lessening extent in the thinking of health care workers.

Wunderlich identified 38.0°C (100.4°F) as the upper limit of normal body temperature in his patient population and therefore regarded any temperature higher than 38.0°C (100.4°F) as fever. However, the upper limit of normal body temperature varies among individuals, thereby limiting the applicability of mean values derived from population studies (even those as large as Wunderlich's) to individual subjects. However, the maximum temperature, like the mean temperature, exhibited by a population varies according to the time of day and the site at which the temperature measurement is taken. Because of such variability, no single temperature can be designated as the upper limit of normal. In the study population considered earlier, 37.2°C (98.9°F) was the maximum oral temperature (i.e., the 99th percentile) recorded at 6 a.m., whereas at 4 p.m. the maximum oral temperature observed reached 37.7°C (99.9°F). Thus these data suggest that when modern thermometers are used to monitor oral temperature in young or middle-aged adults, fever is roughly defined as an early-morning temperature of 37.2°C (99.0°F) or higher or as a temperature of 37.8°C (100°F) or higher at any time during the day. It should be noted that this definition is different than the commonly regarded dictum that fever is anything ≥38.3°C (≥101°F), which was endorsed by the IDSA for ICU patients and which is not based on any data we are aware of.

Wunderlich wrote in 1868 that "[temperature] oscillates even in healthy persons according to time of day by 0.5°C = 0.9°F." The next year, Wunderlich and Reeve wrote, "The lowest point is reached in the morning hours between two and eight, and the highest in the afternoon between four and nine."[55c] Modern authorities have generally concurred with these observations. However, Tauber[56] has suggested that the amplitude of diurnal variation might be as high as 1°C (1.8°F).

Nevertheless, the subjects examined in that study exhibited considerable individual variability, with some having daily temperature oscillations as wide as 1.3°C (2.4°F) and others having oscillations as narrow as 0.1°C (0.2°F). A more recent study that used a "temperature holter," a device that measures 24-hour TM and axillary temperature, demonstrated an increase in temperature amplitude in the circadian rhythm of patients with fever, particularly those with tuberculosis.[56a]

THERMOREGULATION

Although the core is only relatively homogeneous, as described earlier, it is reasonable to consider that the thermoregulatory system regulates core (deep) body temperature. This is achieved by use of multiple effector responses, both behavioral and physiologic, that can be activated from multiple thermosensors located within the shell (mostly in the skin) and the core (most importantly in the brain). Thermoregulatory behaviors include heat or cold avoidance and seeking, as well as many others—from simple postural changes, to selecting clothes appropriate for the weather, to complex multiperson operations such as building houses with the desired thermal environment. The principal physiologic cold-defense responses are shivering and sympathetically driven skin vasoconstriction and nonshivering thermogenesis in brown adipose tissue (BAT), the latter two being autonomic responses. Although laboratory rodents, such as rats and mice, exhibit robust shivering responses if core temperature falls, nonshivering thermogenesis is a more important mechanism for heat production in rodents.[57] Conversely, although humans have significant BAT deposits,[58] shivering is thought to be a primary mechanism for heat production in humans.[59] Of interest, BAT also has been shown to be present in adult humans and found to contribute to thermogenesis, especially on a long time scale, such as in cases of cold adaptation, and to affect body mass.[60–64] This highly specialized form of adipose tissue is characterized by its brownish color (due to an abundance of mitochondria) and a profuse vascular supply. In humans multiple small depots of BAT are located in the neck and chest (near the clavicles, along the spinal column, and in other locations). Physiologic (autonomic) heat defenses include cutaneous vasodilation and species-specific responses aimed at evaporating water from the skin or respiratory tract, such as thermoregulatory salivation in rats, panting in dogs, and sweating (and perhaps also hyperpnea[65]) in humans.

How the multisensor, multieffector thermoregulatory system is functionally organized remains a subject of debate. Previously, it was widely believed that the thermoregulation system is unified and fully integrated, as an engineered system for temperature control would be. It was thought that thermoreceptors in different parts of the body detect local temperatures, code them into electric signals, and send these signals to the preoptic anterior hypothalamus (POA). The POA was believed to integrate these temperatures into some functionally meaningful "mean body temperature," to compare this integrated mean temperature with some set point and to recruit the corresponding thermoeffector responses in a coordinated fashion. One of the popular descriptions of such a unified thermoregulatory system with a single set point was given by Hammell.[66] However, no neuronal networks for computing a mean body temperature, computing the set point, comparing the mean temperature with the set point, or coordinating effector responses have been identified, at least not definitively.

A different concept has emerged over the last decade.[18,67,68] This concept is based on the idea that, similar to other physiologic systems,[69] the thermoregulatory system functions as a federation of relatively independent effector loops,[70] without a single controller and without a single set point or its equivalent.[71] Each thermoeffector loop includes a unique efferent neural pathway driving the corresponding effector response,[72,73] and each loop uses negative feedback from the main control variable, core temperature, and feedback (either negative or positive) from the auxiliary (additional) variable, skin temperature.[67] The auxiliary feedback control is similar to the feedforward control in that it allows the body to anticipate thermal disturbances coming from the environment and to maintain the core temperature at a more stable level. By the same token, each thermoeffector is sensitive to a unique combination of shell and core temperatures,[74–78] and each therefore defends a different level of differently distributed body temperatures.[12,79,80] However, the individual activities of each thermoeffector affect the core body temperature, which plays, therefore, a coordinating role for thermoeffector responses.[12] In fact, coordination through the common control variable is probably sufficient to explain all examples of coordinated recruitment of thermoeffectors in a response. This coordination serves as a fundamental unifying force in the thermoregulation system. Of importance, the new concept requires no neural networks for computation of an integrated mean temperature, comparing this temperature with an obvious or hidden set point in a unified system, or for coordinating different thermoeffectors responses with each other. By acting on the thermoreceptive elements of a thermosensitive neuron, a local temperature (whether skin or brain) can change the activity of that neuron and, sequentially, of the entire thermoeffector pathway synaptically linked to that neuron. As explained by Kobayashi,[81,82] a thermosensitive neuron does not code its local temperature into an electric signal to be processed by the central control network. Instead, it generates an effector-driving signal when the local temperature reaches that neuron's threshold; this signal then spreads by a neural pathway and triggers the effector response. (An engineering term for such an element would be "thermostat," not "sensor.") This concept emphasizes the significance of the thermoreceptive elements of thermosensory neurons and gives these elements a principal role in determining whether a thermoeffector response will be triggered. Some of these thermoreceptive elements, especially those located in the skin, are likely to be thermosensitive transient receptor potential channels,[67] whereas the nature of other, especially central, thermoreceptors is largely unknown. The thermoregulatory system also overlaps, or "meshes," with other control systems in multiple ways. Behavioral thermoregulatory responses also involve elements of feedforward control that use nonthermal signals, for instance, selecting weather-appropriate clothes based on a weather forecast.[67] A schematic of thermoregulation by independent effector loops is given in Fig. 55.3. For more information on how the thermoregulation system works, see the recent review by Romanovsky.[82a]

THE GENERATION OF FEVER

Historically, various substances have been shown to raise core temperature and cause fever. They were divided into two general categories: those that originate outside the body (exogenous pyrogens, such as bacterial endotoxin [lipopolysaccharide, LPS]), and those that are derived from host cells (endogenous pyrogens). Endogenous pyrogens are now known as pyrogenic cytokines.[83]

Today, fever is viewed as a thermoregulatory manifestation of the innate immune response.[84] It is initiated with the binding of pathogen-associated molecular patterns, such as LPS, viral RNA, and fungal sugars, to pattern recognition receptors (PRRs) expressed by these immune cell populations.[85] This binding results in the activation of gene expression and synthesis of a broad range of molecules, which, in addition to pyrogenic cytokines, include chemokines, cell adhesion molecules, and immunoreceptors, as well as various enzymes and lipid mediators.[84]

Undoubtedly, LPS is the best-studied example of an exogenous pyrogen. In small laboratory animals (mice, rats, and rabbits), intravenous (IV) injection of LPS produces a monophasic fever (one body temperature peak) at low doses and polyphasic (at least biphasic) fevers, that is, several subsequent body temperature rises, at higher doses.[86–88] Very high doses of LPS (1 mg/kg and higher) often cause hypothermia and shock[89,90]; body temperature is dynamic in these species and dependent on LPS dose and ambient temperature.[11,91] Similarly, low doses of LPS cause monophasic fever in humans, with a single peak in body temperature occurring 3 to 6 hours after injection,[92–94] although there may be a second peak as well.[83,95] High doses of LPS, especially those that cause hypothermia and shock, have not been studied in humans.

The list of pyrogenic cytokines includes interleukin (IL)-1α, IL-1β, IL-6, tumor necrosis factor (TNF)-α, ciliary neurotropic factor, interferon (IFN)-δ, and possibly others.[96–104] Most pyrogenic cytokines have monomeric molecular masses ranging from 17 to 30 kilodaltons. Undetectable under basal conditions in healthy subjects, they are produced by many different tissues in response to appropriate stimuli. Once released, pyrogenic cytokines have short intravascular half-lives. They are pleiotropic, in that they interact with receptors present on many different host cells. They are active in picomolar quantities, induce maximal cellular responses even at low receptor occupancy, and exert

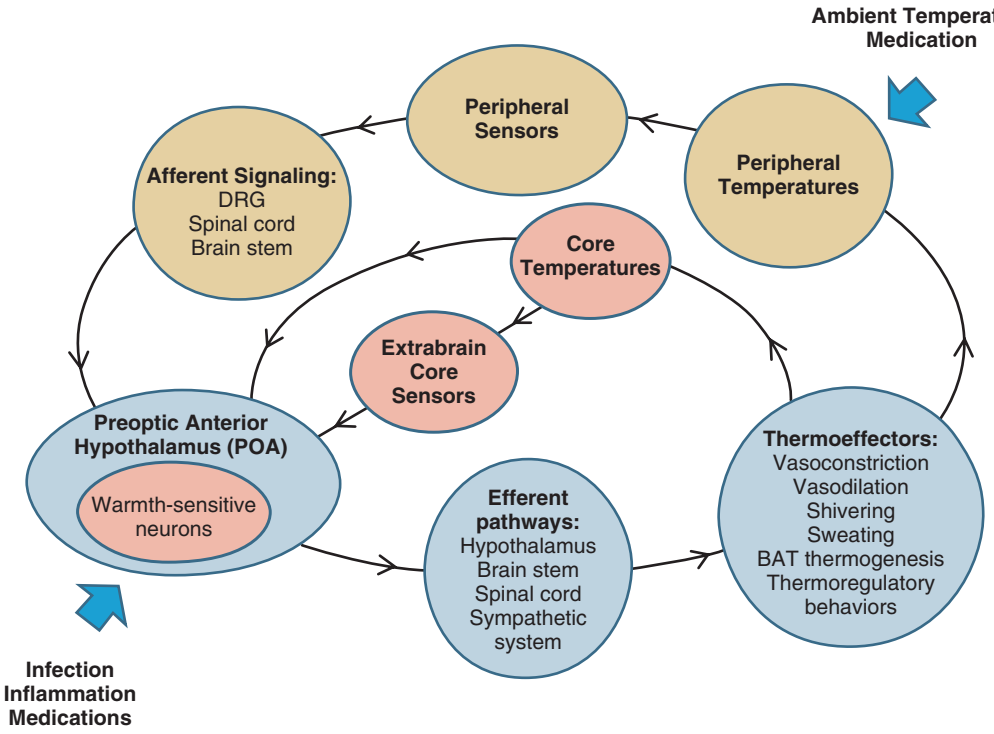

FIG. 55.3 Thermoregulation. Body temperature is regulated by a set of relatively independent thermoeffector control loops. All loops follow the general flow, as outlined in the figure by black arrows. In reality, however, every loop shown in the figure consists of multiple loops, each including only one thermoeffector element and one or more thermoreceptors. Thermoreceptors in the shell and core are activated by changes in peripheral (primarily skin) and core (in the hypothalamus and outside the brain) temperatures, respectively. The resultant signals, through efferent portions of the loops, drive thermoeffector responses. The thermoeffector responses change both core and shell temperatures, thus closing control loops. *BAT,* Brown adipose tissue.

local (autocrine-paracrine) as well as systemic (endocrine) effects.[105] Among the pyrogenic cytokines, complex context-specific relationships exist, with certain members upregulating or downregulating the expression of other members or their receptors.[105] Infusion of pyrogenic cytokines into animals and humans causes fevers.[83]

The crucial step for fever generation by LPS and most other pyrogens is the production of prostaglandin E_2 (PGE_2). For example, in humans, LPS-induced fever can occur even when IL-1 or TNF-α signaling is blocked,[93,95,106] but the vast majority of clinical fevers[107] and all phases of LPS-induced fevers in laboratory animals[108–110] are blocked by pharmacologic or genetic inactivation of cyclooxygenase (COX)-2, an enzyme within the PGE_2-synthesizing cascade; this is how aspirin works.

Binding of LPS to Toll-like receptor 4 (TLR4), one of the PRRs, on pulmonary and hepatic macrophages[111,112] causes massive, rapid induction of COX-2 and microsomal PGE synthase (mPGES)-1 (one of terminal PGE_2 synthases) in the LPS-processing organs, such as the liver and lungs, thus resulting in a rise of PGE_2 concentration in the blood.[112–114] Although PGE_2 is rapidly catabolized in the same organs (liver and lungs), the upsurge in the synthesis is so massive that large amounts of circulating PGE_2 escape the catabolism, and PGE_2 concentration increases even in the aortic blood, that is, immediately downstream of the pulmonary circulation.[154] This peripheral synthesis occurs very early, before body temperature starts rising.[113] The circulating PGE_2, which is bound in the blood to albumin, crosses the blood-brain barrier, probably with the help of a transporter, and triggers fever.[11] Hence the first febrile phase is caused exclusively by the PGE_2 synthesized in the periphery. As the febrile response progresses, PGE_2 synthesis becomes upregulated in the brain as well. For example, during LPS fever in rats the second phase involves the robust upregulation of phospholipaseA_2 (PLA_2; an early enzyme of the PGE_2 cascade), COX-2, and mPGES-1.[113] In the brain PGE_2 is produced by COX-2 in perivascular and endothelial cells.[115–117] Low doses of LPS upregulate COX-2 expression in perivascular cells, whereas higher doses also increase COX-2 in endothelial cells, primarily along small venules.[117,118] However, only endothelial cells have been found to produce mPGES-1 in the adult brain,[119] indicating that they are the source of PGE_2 in the later phases of fever.

This general pattern of febrile pathogenesis, that is, mediation of early phases of fever by peripherally produced PGE_2 and mediation of later phases by both peripheral and central PGE_2, reflects a consensus opinion.[85,120,121] Other authors favor alternative mechanisms, that is, transport of pyrogenic cytokines across the blood-brain barrier, their synthesis in the circumventricular organs (that lack a blood-brain barrier), and neural (via the vagus nerve) signaling by pyrogenic cytokines and PGE_2 from the periphery.[122] The proposed pathways of passage of peripheral pyrogenic signals across the blood-brain barrier are schematically depicted in Fig. 55.4.

When PGE_2, either synthesized in the periphery and transported across the blood-brain barrier to the POA or synthesized locally, gains access to neurons within autonomic thermoeffector loops, it triggers cold-defense responses, thus causing fever. The neurons that are believed to be responsible for fever initiation are located in the median preoptic nucleus (one of the POA nuclei) and express the EP3 receptor to PGE_2.[123–125] They are likely to be γ-aminobutyric acid–ergic warmth-sensitive neurons that tonically inhibit thermogenesis and skin vasoconstriction; PGE_2 removes inhibition from these thermoeffectors, and body temperature rises.[11] Of interest, thermoregulatory behaviors involved in fever do not depend on the POA, and rats with ablated POA develop appropriate fevers when allowed to regulate body temperature by selecting their thermal environment.[126]

ACUTE-PHASE RESPONSE

As noted, a cytokine-mediated rise in the core temperature is but one of many features of the febrile response. Numerous other physiologic reactions, collectively referred to as the acute-phase response, are mediated by members of the same group of pyrogenic cytokines that activate the thermal response of fever. Such reactions include a host of behavioral, physiologic, biochemical, and nutritional alterations (Table 55.1).[127] Stimuli capable of inducing an acute-phase response include bacterial and, to a lesser extent, viral infections, trauma, malignant neoplasms, burns, tissue infarction, immunologically mediated and crystal-induced inflammatory states, strenuous exercise, and childbirth.[128,129] There is also evidence that major depression,[130]

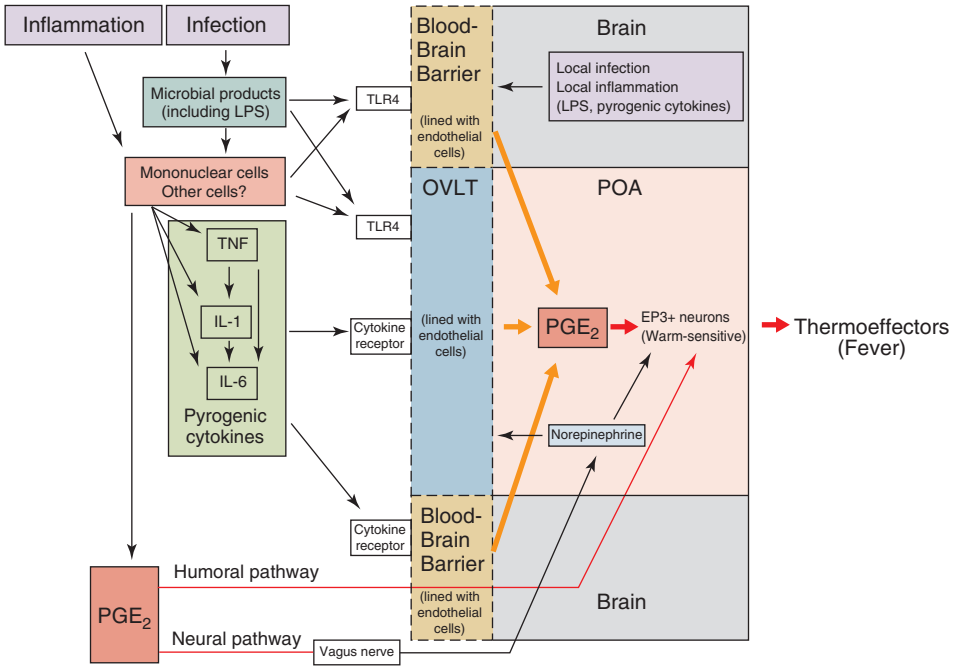

FIG. 55.4 Model for the febrile response. Fever can be initiated by microbial products, which directly or indirectly (through pyrogenic cytokines) activate the production of prostaglandin E_2 *(PGE$_2$)* in the periphery and in the brain. PGE_2 reaches the γ-aminobutyric acid–ergic, warmth-sensitive neurons in the preoptic anterior hypothalamus *(POA),* specifically, in the median preoptic nucleus. By acting on the *EP3* receptor on these neurons, PGE_2 disinhibits cold defenses such as skin vasoconstriction and non-shivering and shivering thermogenesis. The increased heat conservation and heat production results in a body temperature rise (fever). *EP3,* Subtype of prostaglandin E_2; *IL,* interleukin; *LPS,* lipopolysaccharide; *OVLT,* organum vasculosum laminae terminalis; *TLR4,* Toll-like receptor 4; *TNF,* tumor necrosis factor.

TABLE 55.1 Acute-Phase Physiologic Reactions

Neuroendocrine Changes

Fever, somnolence, and anorexia
Increased secretion of corticotropin-releasing hormone, corticotropin, and cortisol
Increased secretion of arginine vasopressin
Decreased production of insulin-like growth factor-1
Increased adrenal secretion of catecholamines

Hematopoietic Changes

Anemia of chronic disease
Leukocytosis
Thrombocytosis

Metabolic Changes

Loss of muscle and negative nitrogen balance
Decreased gluconeogenesis
Osteoporosis
Increased hepatic lipogenesis
Increased lipolysis in adipose tissue
Decreased lipoprotein lipase activity in muscle and adipose tissue
Cachexia

Hepatic Changes

Increased metallothionein, inducible nitric oxide synthase, heme oxygenase, manganese superoxide dismutase, and tissue inhibitor of metalloproteinase-1
Decreased phosphoenolpyruvate carboxykinase activity

Changes in Nonprotein Plasma Constituents

Hypozincemia, hypoferremia, and hypercupremia
Decreased plasma retinol concentrations
Increased plasma glutathione concentrations

From Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. *1999;340:448–454. Copyright © 1999 Massachusetts Medical Society. All rights reserved.*

schizophrenia,[131] and psychological stress[132] are capable of inducing an acute-phase response.

Traditionally, the term *acute-phase response* has been used to denote changes in plasma concentrations of a number of secretory proteins derived from hepatocytes. The acute-phase response is part of the innate immune response triggered through PRRs (such as TLRs). Acute-phase proteins, of which there are many (Table 55.2), exhibit increased synthesis (positive acute-phase proteins) or decreased synthesis (negative acute-phase proteins) during the acute-phase response. IL-6 is the chief stimulator of the production of most acute-phase proteins. Other pyrogenic cytokines, however, also influence the production of various subgroups of these proteins.

Many of the acute-phase proteins are believed to modulate inflammation and tissue repair.[133] A major function of C-reactive protein (CRP), for example, is presumed to involve the binding of phosphocholine on pathogenic microorganisms, as well as phospholipid constituents on damaged or necrotic host cells. Through such binding, CRP might activate the complement system and promote phagocyte adherence, thereby initiating the process whereby pathogenic microbes or necrotic cells are cleared from the host. Such activities are most likely potentiated by CRP-induced production of inflammatory cytokines[134] and tissue factor[135] by monocytes. Nevertheless, the ultimate function of CRP is uncertain, in that several in vivo studies have shown it to have antiinflammatory properties.[136–138]

Another major human acute-phase protein, serum amyloid A, has been reported to potentiate adhesiveness and chemotaxis of phagocytic cells and lymphocytes.[139] There is also evidence that macrophages bear specific receptors for serum amyloid A; serum amyloid A—rich, high-density lipoproteins mediate the transfer of cholesterol to macrophages at sites of inflammation[140]; and serum amyloid A enhances low-density lipoprotein oxidation in arterial walls.[141]

TABLE 55.2 Human Acute-Phase Proteins

Proteins Whose Plasma Concentrations Increase

Complement System

C3
C4
C5
C9
Mycobacterium avium complex
Factor B
C1 inhibitor
C4b-binding protein
Mannose-binding lectin

Coagulation and Fibrinolytic System

Fibrinogen
Plasminogen
Tissue plasminogen activator
Urokinase
Protein S
Vitronectin
Plasminogen-activator inhibitor-1
Kininogen

Antiproteases

α_1-Protease inhibitor
α_1-Antichymotrypsin
Pancreatic secretory trypsin inhibitor
Inter–α-trypsin inhibitors

Transport Proteins

Ceruloplasmin
Haptoglobin
Hemopexin

Participants in Inflammatory Responses

Secreted phospholipase A_2
Lipopolysaccharide-binding protein
Interleukin-1 receptor antagonist
Granulocyte colony-stimulating factor

Others

C-reactive protein
Serum amyloid A
α_1-Acid glycoprotein
Fibronectin
Ferritin
Angiotensinogen

Proteins Whose Plasma Concentrations Decrease

Albumin
Transferrin
Transthyretin
α_2-HS glycoprotein
α-Fetoprotein
Thyroxine-binding globulin
Insulin-like growth factor-1
Factor XII
Retinol-binding protein

HS, Heremans-Schmid.
Modified from Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. *1999;340:448–454. Copyright © 1999 Massachusetts Medical Society. All rights reserved.*

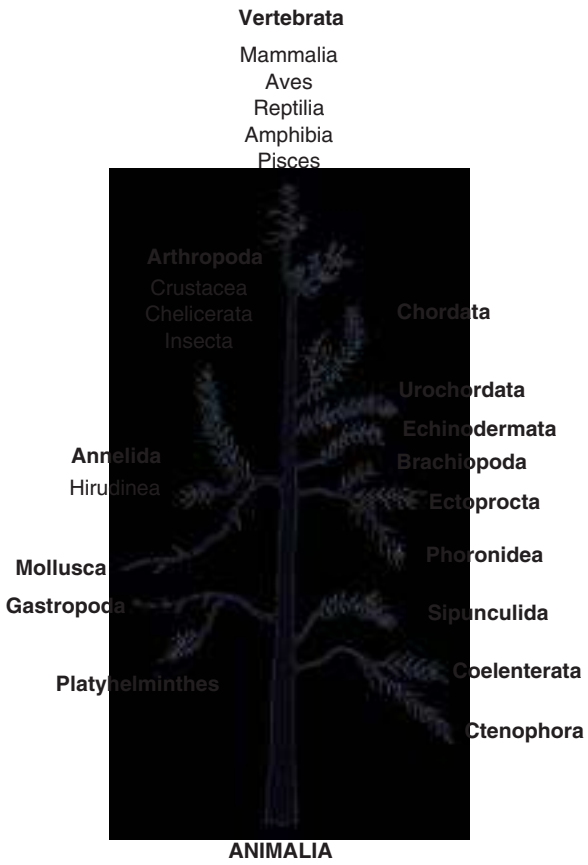

FIG. 55.5 Evolutionary tree of animals. A febrile response has been documented in the Vertebrata, Arthropoda, and Annelida. These observations suggest that the febrile response evolved more than 400,000,000 years ago at about the time that evolutionary lines leading to arthropods and annelids diverged.

Complement components, many of which are acute-phase reactants, induce pyrogenic cytokines and PGE_2; modulate chemotaxis, opsonization, vascular permeability, and vascular dilation; and have cytotoxic effects. Haptoglobin, hemopexin, and ceruloplasmin are all antioxidants and are thought to attenuate infection by binding their respective metals. It is therefore reasonable to assume that, like the antiproteases α_1-antichymotrypsin, and C1-esterase inhibitor, they play important roles in modulating inflammation. However, the functional capacity of such proteins is broad. There is also a growing literature concerned with the acute-phase protein LPS-binding protein, which appears both to enhance and neutralize the biologic activity of LPS through its interaction with the CD14 receptor on macrophages.

Although closely associated with fever, the acute-phase response is not an invariable component of the febrile response. Some febrile patients (e.g., those with certain viral infections) have normal blood levels of CRP. Moreover, patients with elevated blood levels of CRP are not always febrile. Nutritional fatty acids (typically elevated in obesity) can act as TLR4 ligands, generating a chronic acute-phase response.

The acute-phase response, like the febrile response, is a complex response consisting of numerous, separately regulated components. The particular components expressed in response to a given disease process more than likely reflect the specific cytokines induced by the disease.

BIOLOGIC VALUE OF FEVER

Questions concerning fever's risk-benefit quotient have generated considerable controversy. The controversy arises because of data indicating both potentiating and inhibitory effects of the response on resistance to infection. As a result, there is as yet no consensus as to the appropriate clinical situations, if any, in which fever or its mediators should be suppressed. In general the febrile response can be viewed as an adaptive response that has arisen evolutionarily to stymie myriad infectious challenges. Such responses, arising from noninfectious causes (tissue injury from cerebrovascular accidents or myocardial infarction), constitute a separate category that are not dealt with in this chapter; however, mounting evidence points to these being maladaptive responses, harmful to the host, and responding to lowering core and body temperature.[142–144]

Evidence illustrating fever's beneficial effects originates from several sources. Studies of the phylogeny of fever have shown the response to be widespread within the animal kingdom. With few exceptions, mammals, reptiles, amphibians, and fish, as well as several invertebrate species, have been shown to elevate their core temperature after challenge with microorganisms or other known pyrogens (Fig. 55.5). It has been assumed, although not established conclusively, that such elevations in

temperature are the poikilothermic corollary of fever. The prevalence of such febrile responses has been offered as some of the strongest theoretical evidence that fever is an adaptive host defense response, based on the argument that the metabolically expensive increase in body temperature that accompanies the febrile response would not have evolved and been so faithfully preserved in the animal kingdom unless fever had some net benefit to the host.

Further evidence of fever's beneficial effects can be found in numerous investigations demonstrating enhanced resistance of animals to infection with increases in body temperature within the physiologic range. In classic studies involving experimental infection of the reptile *Dipsosaurus dorsalis* with *Aeromonas hydrophila*, Kluger and associates demonstrated a direct correlation between body temperature and survival.[144a] They also showed in their model that suppression of the febrile response with sodium salicylate was associated with a substantial increase in mortality. Covert and Reynolds corroborated these findings in an experimental model involving goldfish.[144b]

In experimental mammalian models, increasing the body temperature by artificial means has been reported to enhance the resistance of mice to herpes simplex virus,[145] poliovirus,[146] coxsackie B virus,[147] rabies virus,[148] *Klebsiella pneumoniae,*[149] and *Cryptococcus neoformans*[150] but to decrease resistance to *Streptococcus pneumoniae.*[151] Increased resistance of rabbits to *S. pneumoniae*[152] and *C. neoformans,*[153] dogs to herpesvirus,[154] piglets to gastroenteritis virus,[155] and ferrets to influenza virus[156] has also been observed after the induction of artificial fever. Unfortunately, because raising the body temperature by artificial means does not duplicate the physiologic alterations that occur during fever in homeotherms, and indeed, entails a number of opposite physiologic responses,[157] evidence obtained using mammalian experimental models must be interpreted with caution when used to understand the febrile response.

Clinical data supporting an adaptive role for fever have accumulated slowly. Like animal data, clinical data include evidence of the beneficial effects of fever and adverse effects of antipyretics on the outcome of infections. In an examination of factors influencing the prognosis of spontaneous bacterial peritonitis, Weinstein and coworkers identified a positive correlation between a temperature reading higher than 38°C (100.4°F) and survival.[157a] In studies of elderly patients with community-acquired pneumonia, those without a fever had higher mortality. In another study, ICU patients with invasive *Candida* infections and temperatures less than 36.5°C had a higher mortality than those with temperatures greater than 38.2°C.[157b]

All data reviewed earlier are subject to interpretation and do not prove a causal relationship between fever and improved prognosis during infection. Nevertheless, they are consistent with such a relationship and, when considered in concert with the phylogeny of the febrile response and the animal data summarized earlier, constitute strong circumstantial evidence that fever is an adaptive response in most situations.

Whereas many of the foregoing investigations examined the relationship between elevation of the core temperature and outcome of infection, others have considered the endogenous mediators of the febrile response. In such studies, all the principal pyrogenic cytokines have been shown to have immune-potentiating capabilities, which might theoretically enhance resistance to infection; many of these capabilities have been shown to be enhanced by elevated body temperature. In vitro and in vivo investigations of these cytokines have provided evidence of a protective effect of IFN, TNF-α, or IL-1, or all of these, against *Plasmodium, Toxoplasma gondii, Leishmania major, Trypanosoma cruzi,* and *Cryptosporidium.*

Several reports have also shown enhancement of resistance to viral and bacterial infections by pyrogenic cytokines. Treatment of normal and granulocytopenic animals with IL-1 has been shown to prevent death in some gram-positive and gram-negative bacterial infections. However, IL-1 is effective only if administered an appreciable time (e.g., 24 hours) before the initiation of infections having rapidly fatal courses. In less acute infections, IL-1 administration can be delayed until shortly after the infectious challenge. Such observations suggest that those physiologic effects of the febrile response that enhance resistance to infection might be limited to localized infections or systemic infections of only mild-to-moderate severity. They may also indicate that the beneficial actions of IL-1 and/or fever are programmed for specific stages in the progression of an infection.

BIOLOGIC VALUE OF FEVER: THE CASE OF SEPSIS

The febrile response's potential for harm was reflected in multiple reports, suggesting that IL-1, TNF-α, IL-6, and IFN mediate the physiologic abnormalities of certain infections. Although proof of an adverse effect of fever on the clinical outcome of these infections has yet to be established, the implication is that if pyrogenic cytokines contribute to the pathophysiologic burden of infections, both the mediators themselves and the febrile response are potentially deleterious. The most persuasive evidence in this regard derives from studies of gram-negative bacterial sepsis.[158] Sepsis is a defined as systemic inflammation due to infection, often resulting in life-threatening organ dysfunction, whereas septic shock is a subset of sepsis, which is accompanied by shock and has higher mortality.[159]

It has long been suspected that bacterial LPS is involved in the pathophysiology of sepsis. Purified LPS induces a spectrum of physiologic abnormalities similar to those occurring in patients with gram-negative bacterial sepsis. In experimental animals, challenge with LPS causes TNF-α and IL-1 to be released into the bloodstream coincident with the appearance of signs of sepsis. Furthermore, patients with sepsis may have detectable levels of circulatory TNF-α, IL-1, and IL-6 independent of culture-documented infection, and these levels correlate inversely with survival.[160] IL-1, alone or in combination with other cytokines, induces many of the same physiologic abnormalities (e.g., fever, hypoglycemia, shock, and death) seen after the administration of purified LPS.[161] In a murine experimental model for septic shock, IFN administered before or as long as 4 hours after LPS challenge increases mortality, whereas pretreatment with anti-IFN antibody significantly reduces mortality.[162] In several investigations, the adverse effects of gram-negative bacterial sepsis, LPS injections, or both, have been attenuated by pretreating experimental animals with IL-1 antagonists[163,164] and monoclonal antibodies directed against TNF-α.[165,166] Furthermore, animals rendered tolerant to TNF-α by repeated injections of the recombinant cytokine are protected against the hypotension, hypothermia, and lethality of gram-negative bacterial sepsis.[167]

The theory derived from these observations—that death from sepsis is the consequence of cytokine-mediated overstimulation of the immune system—unfortunately correlates only loosely with the clinical picture in humans, most likely because the studies cited used large doses of endotoxin or bacteria that induced levels of circulating pyrogenic cytokines exponentially higher than those detected in patients with sepsis. Thus the "cytokine storm" created in such animals most likely has only limited relevance for human sepsis. This perhaps explains why, in clinical trials, inhibition of pyrogenic cytokines in septic patients has had only modest success, improving outcome in patients with a high risk for death but not those with a low risk. Another explanation is that, in the laboratory, experimental sepsis and aseptic systemic inflammation are typically studied in young animals, whereas the majority of human patients with sepsis are elderly, and the clinical course of sepsis and its resistance to therapy is likely to change with age (see reference 168).

There is evidence from human studies suggesting that fever in sepsis is associated with improved outcomes. The first study to show this was a retrospective analysis of 218 patients with gram-negative bacteremia, in which Bryant and associates reported a positive correlation between maximum temperatures on the day bacteremia was diagnosed and survival.[168a] A similar relationship has been observed in patients with polymicrobial sepsis and mild (but not severe) underlying diseases. Another study, by Lee and colleagues,[169] did not show a higher mortality in septic patients with fever, but the results are likewise difficult to interpret due to most of the measurements being taking via the axillary route. A study by Laupland and colleagues[170] showed an association of increased mortality in ICU patients with fever; however, this study is uninterpretable as they did not distinguish those with and without infection in the analysis, even though they had collected the microbiologic data. Two recent studies by Young and colleagues[171,172] did show an association of early peak temperatures (>39°C in one study and >37.5°C

in another study) in septic patients with infection as being associated with improved outcomes. The major problem of all the studies just cited is that they were either retrospective or had limitations related to temperature measurements (e.g., temperature was not recorded or did not reflect core temperature). These problems were addressed in a prospective study measuring core temperatures in patients with severe infection in the ICU.[173] Henriksen and colleagues[173] were able to demonstrate that temperature >38°C was associated with reduced mortality, whereas temperature <36°C was associated with increased mortality.

Hence it appears that fevers in sepsis due to infections appears to be associated with improved outcomes. It should be noted that the development of fever may lead to earlier initiation of antibiotics and hence better outcomes, and this is one additional potential confounding factor that needs to be addressed in future studies. Nevertheless, it is reasonable to assume that the benefits of a febrile response are due to known inhibitory effects of elevated temperature on growth and pathogenesis of bacteria, along with potential benefits from cytokines and other acute-phase reactants involved in the fever response. However, it is also known that cytokines are involved in shock and death, thus separating and stymieing the effects of one (cytokines) and amplifying the effects of the other (elevated temperature) could be a future strategy for improved outcomes. Finally, fever is only one thermoregulatory response to systemic inflammation; the other is hypothermia. While this chapter was in preparation, a comprehensive review on these two thermoregulatory responses was released.[173a]

BIOLOGIC VALUE OF HYPOTHERMIA

Both humans and experimental animals respond to most severe forms of systemic inflammation with hypothermia. Moreover, this response is regulated. For example, it has been established that rats with severe systemic inflammation actively seek a cold environment and that this cold-seeking behavior promotes hypothermia.[79,89,126] Similar to fever, hypothermia in systemic inflammation is an evolutionarily developed response and has an adaptive value when the energy cost of fever exceeds its benefits.[174] Experimental support for this hypothesis has been produced in rats with aseptic systemic inflammation caused by LPS[175,176] or with acute *Escherichia coli* infection.[176] In these studies rats challenged with high doses of LPS or *E. coli* were either allowed to develop hypothermia in a mildly cool environment, which they preferred, or were "forced" to develop fever in a warm environment. Development of hypothermia instead of fever resulted in diminished organ damage and decreased mortality. Similar results have been obtained in mice injected with LPS at a high dose.[177] The mechanisms behind the protection aided by hypothermia in severe forms of systemic inflammation are still poorly understood. However, the metabolic suppression that drives hypothermia occurs concurrently with the earliest decrease in cardiac output in endotoxic shock, so that the balance between oxygen delivery and consumption is maintained and tissue hypoxia does not occur.[178] Tissue hypoxia in this model occurs only when the hypothermic response is prevented.

In contrast with these studies in laboratory animals is the prevailing clinical perception that hypothermia is a dysregulated, detrimental phenomenon in sepsis.[179,180] This perception is rooted in observational studies, which we described earlier in detail, and which identified hypothermia as a poor prognostic factor in septic patients.[181–184] However, the prognostic value of hypothermia does not imply that it is the hypothermia itself that worsens the outcome; those patients who develop hypothermia are usually sicker than those who do not.[173,185–187]

ANTIPYRETIC THERAPY: GENERAL CONSIDERATIONS

Although clinicians have long had at their disposal effective means of lowering the core temperature in febrile patients, the actual benefit of such reductions in temperature is still uncertain. Moreover, it has yet to be shown in humans that increases in the core temperature encountered during fever are actually harmful. Certainly, during the course of heat stroke and other forms of hyperthermia, the core temperature can, and frequently does, rise to levels that are inherently harmful. However, such levels are almost never reached during a febrile rise in temperature, which rarely exceeds 41°C (105.8°F) in humans.[188] Nevertheless, whereas healthy volunteers have been reported to withstand core temperatures of 42°C (107.6°F) for periods as long as 4 hours without apparent ill effects, the possibility remains that in certain patients, even the relatively modest increases in core temperature encountered during fever are deleterious and should therefore be suppressed.

Antipyretic therapy is also commonly administered to enhance patient comfort. General experience with antipyretic drugs, which are usually also analgesic agents, seems to support this notion. However, carefully controlled efficacy studies have not yet established the validity of this contention, and any potential short-term gains must be compared with potential longer-term effects. It has been reported that children with chickenpox who are treated with acetaminophen have a longer time to total crusting of lesions than placebo-treated controls. Stanley and colleagues have reported that adults infected with rhinovirus exhibit more nasal viral shedding when they receive aspirin than when given placebo.[188a] Furthermore, Graham and colleagues have reported a trend toward a longer duration of rhinovirus shedding in association with antipyretic therapy and have shown that the use of aspirin or acetaminophen is associated with suppression of the serum-neutralizing antibody response and with increased nasal symptoms and signs.[188b] A more recent retrospective observational analysis of studies of human volunteers infected with influenza A has found a relationship between antipyretic therapy and prolonged illness, although this was not seen in another study.[188c,188d] In an investigation of children infected with *Plasmodium falciparum*, paracetamol prolonged the parasite clearance time. Of interest, plasma concentrations of TNF and IL-6 in children not receiving paracetamol were no different from those receiving paracetamol, suggesting that elevated temperature itself was responsible for the accelerated parasite clearance. One case-control study showed an increase risk of empyema in children treated with nonsterioidal antiinflammatory drugs (NSAIDs) for acute viral infections.[189] Finally, it should be noted that one recent study and a meta-analysis did not find any adverse effects of adjunctive corticosteroids in the treatment of pneumonia, although both showed a reduction in fever and/or clinical symptoms.[190,191] Corticosteroids are known to have wide-ranging actions, and thus caution and more studies will be needed to determine if these are indeed safe and effective.

In the past several years there have been a number of studies linking certain antipyretics to decreased vaccine responses. In a study by Prymula and colleagues[192] that was designed to study the efficacy of paracetamol on febrile reactions, the authors vaccinated children against *Streptococcus pneumococcus, Haemophilus influenzae, Clostridium tetani, Corynebacterium diphtheriae, Bordetella pertussis,* poliovirus, hepatitis B virus, and rotavrius. Children who had received prophylactic paracetamol (starting at the time of vaccination) had lower opsonophagocytic antibodies against *S. pneumococcus* and lower antibody titers against *S. pneumococcus, H. influenzae, C. tetani, C. diphtheriae, B. pertussis,* and hepatitis B virus. In a follow-up paper from the same group, the investigators showed a trend toward a lower percentage of subjects colonized in the nasopharynx by pneumococcal vaccine serotypes, *H. influenzae,* and nontypeable *H. influenzae* in children who had received paracetamol prophylactically compared with those who did not.[192] A further study with reboosting the same children with *H. influenzae* vaccine showed a trend toward lower antibody titers but no difference in opsonic activity or memory responses, leading the authors to conclude that any changes due to paracetamol therapy are transient.[193] Another study of infants receiving vaccination for *S. pneumococcus, H. influenzae, C. tetani, C. diphtheriae, B. pertussis,* poliovirus, and hepatitis B virus showed that prophylactic paracetamol, started at the time of vaccination, resulted in recipients with lower levels of serotype-specific pneumococcal anticapsular immunoglobulin G than control subjects, whereas ibuprofen recipients had reduced antibody responses to pertussis and tetanus antigens.[194] Like the previous study, these differences did not last, with no difference noted after the dose at 13 months of age. Another recent study in children showed reduced antibody levels to the vaccine antigens within paracetamol- or ibuprofen-treated groups, validating the findings in the previous studies, but of interest, this was not seen if the prophylaxis was started in the booster phase.[195] Finally, a study in adults vaccinated with hepatitis B vaccine demonstrated lower titers of anti-HBs antibodies

if paracetamol was given prophylactically (at the time of vaccination), but not when given therapeutically (6 hours after vaccination).[196] Taken together, until more studies are carried out, antipyretics should be given sparingly during vaccination, especially during the primary series, as decreased levels can have implications for herd immunity.

Another category of patients in whom fever suppression has been studied includes children at risk for febrile seizures, primarily those between the ages of 3 months and 5 years. In such children seizures have been reported to occur during episodes of fever at a frequency as high as 14% in select populations. Although most children with febrile seizures have temperatures of 39°C (102.2°F) or higher at the time of their seizure, many tolerate even higher fevers at later dates without convulsing. Unfortunately, antipyretic therapy has not been shown to protect against recurrences of febrile seizures in the few controlled trials conducted thus far. Camfield and colleagues have conducted a randomized double-blind study comparing a single daily dose of phenobarbital plus antipyretic instruction with placebo plus antipyretic instruction to prevent recurrent seizure after an initial simple febrile seizure.[196a] In children treated with phenobarbital and antipyretics, the febrile seizure recurrence rate was 5%, whereas in those given placebo with antipyretics, the rate was 25%, suggesting that a single daily 5-mg/kg dose of phenobarbital is more effective than counseling parents about antipyretic therapy in preventing recurrent febrile seizures. Acetaminophen has been given to children with fever as prophylaxis against febrile seizure recurrences. Whether given in moderate dosage (10 mg/kg dose four times daily) or in relatively high doses (15–20 mg/kg dose every 4 hours), acetaminophen failed to reduce the rate of febrile seizure recurrence.

Antipyretic therapy is also occasionally given to reverse fever-induced mental dysfunction in frail elderly patients. Beisel and coworkers have shown that aspirin, in combination with propoxyphene, ameliorates fever-induced decrements in mental work performance in young volunteers infected with sand fly fever virus, even in the face of only partial relief of the fever or other symptoms of the illness.[196b] In view of these observations, antipyretic therapy might be expected to have a beneficial effect on fever-induced mental dysfunction in frail elderly patients. However, studies designed to test this hypothesis have not yet been reported.

One of the reasons commonly given to justify suppressing fever is that the metabolic cost of fever exceeds its clinical benefit.[197] Such energy demands are particularly high during the chill phase if shivering is present, as evidenced by increases in the sympathetic tone,[157] oxygen consumption, respiratory minute volume, and respiratory quotient.[198] Because of the potential adverse consequences of these metabolic effects on cardiovascular and pulmonary function, fever has been attacked with particular vigor in patients with underlying cardiovascular and pulmonary diseases.[200] Although antipyretic therapy has theoretical merit in this regard, if it does not induce shivering,[201] the detrimental effects of fever and the salutary effects of antipyretic therapy have yet to be critically evaluated.

External cooling, which is widely used in critically ill patients to suppress fevers unresponsive to antipyretic drugs, has been shown to decrease oxygen consumption by as much as 20% if shivering is prevented by therapeutic paralysis.[201] If shivering is not inhibited, external cooling causes a rise, rather than a fall, in oxygen consumption, which increases energy demand and may exacerbate hypoxemia.[201] Perhaps more important to febrile patients with underlying cardiovascular disease, external cooling has the capacity to cause vasospasm of diseased coronary arteries by inducing a cold pressor response.[202,203]

Finally, we turn to the use of antipyretics for distinguishing serious from nonserious infections, and infections from cancer. Numerous investigators have observed a direct correlation between the height of fevers and the rate of serious bacterial infections in children, with the maximal incidence of such infections at temperatures in excess of 40°C (104°F).[204–207] It has also been suggested that the response of a fever to antipyretic therapy might have diagnostic implications, in that a drop in temperature, improvement in the appearance of a febrile child, or both generally indicates that the fever is not the result of a serious illness. This conclusion, however, is not supported by numerous investigations comparing the temperature response of bacteremic and nonbacteremic infections with antipyretic therapy in children.

Several studies have suggested that an antipyretic response to NSAIDs can distinguish fevers of infectious origin from those caused by cancer by virtue of the fact that the latter fevers are more readily suppressed by such agents. Naproxen was the first such agent to be studied in this regard. Subsequent randomized comparisons have shown naproxen, indomethacin, and diclofenac to be equally effective in inhibiting cancer-induced fever, although the sensitivity and specificity of the naproxen test for differentiating neoplastic from infectious fevers are not yet known. Moreover, there is no physiologic rationale to explain why NSAIDs might be more effective in reducing fever caused by cancer than that caused by infection.

ANTIPYRETIC THERAPY: THE CASE OF SEPSIS

As noted earlier, nowhere are the decisions to be made regarding antipyretic therapy more urgent than in the patient with sepsis. On the one hand, it appears that the presence of fever is associated with better outcomes, although some of its mediators are known to be responsible for the mortality seen in this condition. However, even an association of fever with improved outcomes in sepsis does not necessarily mean that fever should not be treated because the mortality still remains high.

The use of antipyretics in the ICU setting is common, with one recent study showing 64% of patients received at least 1 g of paracetamol.[208] However, data regarding its risks and benefits are just starting to accumulate. Retrospective and observational studies have not been able to answer the question of whether antipyretics in sepsis lower mortality, either showing increased mortality,[169] no benefit,[209] or decreased mortality.[208,210] The first placebo-controlled study to look at this was conducted by Bernard and colleagues,[211] who performed a double-blind placebo-controlled trial to evaluate ibuprofen in patients with sepsis. They did not find any difference in mortality between the two groups, although the placebo group was tainted by the fact that 44% were treated with acetaminophen, making the entire results uninterpretable. Two recent randomized placebo–controlled studies have shown no improvement in mortality with acetaminophen treatment compared with placebo.[212,213] A study by Schortgen and colleagues[214] showed external cooling and sedation improved 14-day, but not longer, outcomes. A recent meta-analysis that analyzed observational and randomized studies has also found that antipyretic therapy does not improve 28-day mortality. Thus antipyretic agents have been shown to be of only limited value for the treatment of bacterial sepsis and critically ill patients with fever.

ANTIPYRETIC THERAPY: PHARMACOLOGIC AGENTS

Although clinicians have long resorted to various forms of antipyretic therapy, there is a dearth of scientific data concerning the actual benefits and relative risks of such treatment. Nevertheless, several tentative conclusions regarding antipyretic therapy seem warranted in light of the limited data available. It is clear, for example, that short courses of approved doses of standard antipyretic drugs carry a low risk for toxicity. Most of these drugs have analgesic as well as antipyretic properties. Therefore, if not otherwise contraindicated (e.g., aspirin in young children because of the risk for Reye syndrome), such drugs can be prescribed to provide symptomatic relief in febrile patients, reduce the metabolic demands of fever in patients with underlying cardiovascular and pulmonary disorders, and possibly prevent or alleviate fever-induced mental dysfunction in older patients. To minimize antipyretic-induced fluctuations in temperature, as well as the risk for recurrent shivering with its associated increased metabolic demands, antipyretic agents should be administered to febrile patients at regular intervals that preclude abrupt recurrences of sweating, chills, and fever, rather than as needed for temperatures above some arbitrary level. Whenever such medications are prescribed, it should also be recognized that each carries its own risk for toxicity and might prolong the course of the illness responsible for the fever while reducing the intensity of its symptoms.

Antipyretic drugs can be grouped into three general categories on the basis of their mechanisms of action. These include corticosteroids, aspirin and the other NSAIDs, and acetaminophen. Each exerts its effects at different points in the febrile response pathway.

Although not generally used for antipyresis, corticosteroids suppress fever through direct and indirect mechanisms. They block the transcription of pyrogenic cytokines and inducible COX via interactions involving the glucocorticoid receptor. They downregulate the synthesis of cytokine receptors and, by inducing lipocortin-1, they secondarily inhibit the activity of PLA_2, a critical enzyme in the PGE_2 synthetic pathway, which leads to fever suppression.

Acetaminophen and aspirin and the other NSAIDs all inhibit COX-mediated synthesis of inflammatory thromboxanes and PGs from arachidonic acid. COX has several distinct isoforms, of which COX-1 and COX-2 are the best studied. The former was long regarded as a constitutively expressed cellular enzyme involved in various housekeeping functions, whereas the latter was touted as an inducible enzyme responsible for hypothalamus-mediated fever and produced as part of the inflammatory process by various cell lines, including macrophages, synoviocytes, and endothelial cells. However, this dichotomous concept of a constitutive COX-1 and an inducible proinflammatory COX-2 has proved to be oversimplified. Not only do some cells express COX-2 constitutively but, under certain conditions, COX-2 has also been shown to promote healing of mucosal lesions and resolution of inflammation. Whereas COX-2 mediates fever, COX-1 mediates hypothermia in experimental systemic inflammation.[215]

The distinctive affinities of the various categories of antipyretic drugs for the different COX variants are thought to determine their relative antipyretic and analgesic potencies. NSAIDS are inhibitors of COX-1 and COX-2, and depending on the individual drug, they are predominantly COX-1 selective (low-dose aspirin and ketorolac), COX-2 selective (rofecoxib, lumiracoxib), or nonselective (ibuprofen, naproxyn sodium, high-dose aspirin). Only aspirin irreversibly inhibits COX via acetylation within the active site of the enzyme. Other NSAIDs and acetaminophen inhibit COX reversibly. Acetaminophen has traditionally been considered a weak inhibitor of COX-1 and COX-2. With the discovery of COX-3,[216] a close relative of COX-1, it was believed that the mechanism for the antipyretic action of acetaminophen was also uncovered. However, it appears that these enzymes vary considerably across mammalian species and that acetaminophen in humans does not reduce fever through COX-3. More recently, one study has shown that acetaminophen is a potent COX-2 inhibitor in humans, thus challenging earlier work.[217] Although the antipyretic activity of NSAIDs through inhibition of COX-2 conforms to current models of fever generation[114] (also see Fig. 55.4), our current understanding of the antipyretic activity of acetaminophen is limited. Studies have shown that aspirin and the NSAIDs also have COX-independent antipyretic activity. Aspirin induces cytochrome P450, which might augment its antipyretic effect by shifting arachidonic acid metabolism toward cytochrome P450–mediated production of antipyretic epoxyeicosanoids. In addition, acetylation of COX-2 by aspirin increases the production of 15R-hydroxyeicosatetraenoic acid, which neutrophils use to form aspirin-triggered lipoxins. These lipoxins have potent antiinflammatory activity independent of aspirin. Heat shock proteins have been shown to reduce the transcription of IL-1β in vitro, and therapeutic doses of aspirin and certain NSAIDs increase heat shock factor 1 concentration in vitro. These same drugs also diminish the activity of transcriptional activator nuclear factor kappa B, which is involved in the transcription of pyrogenic cytokines, adhesion molecules, inducible nitric oxide synthase, and COX-2 in certain cell lines, as well as in fever generation.[154] Production of adenosine, an antiinflammatory mediator produced by leukocytes, is enhanced by aspirin and NSAIDs. The clinical implications of these alternative antipyretic pathways remain to be determined.

Unfortunately, certain antipyretic drugs also appear to cause coronary vasoconstriction in patients with coronary artery disease. Friedman and associates[218] observed significant increases in the mean arterial pressure, coronary vascular resistance, and myocardial arteriovenous oxygen difference after IV indomethacin (0.5 mg/kg) in such patients. Coronary blood flow simultaneously decreased substantially.[218] Thus, in this investigation, myocardial oxygen demand increased in the face of a fall in coronary blood flow after indomethacin administration. The authors believe that indomethacin's vasoconstrictor effect most likely derives from to its capacity to block the synthesis of vasodilatory prostaglandins. Perhaps even more disturbing are reports suggesting that, compared with other NSAIDs, COX-2–selective NSAIDs seem to increase the risk for cardiovascular thrombotic events in patients not taking aspirin. In view of indomethacin's capacity to cause coronary vasoconstriction in patients with coronary artery disease and the possible increased risk for cardiovascular thrombotic events associated with COX-2–selective NSAIDs, it should be used cautiously to suppress fever in such patients.

Although the side-effect profiles of these drugs are well known, there are also recent reports of the association of NSAIDS with inflammatory bowel disease, renal cell carcinoma, and *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.[219–221] Researchers have also noted an association between acetaminophen and development of asthma, although this was not seen in a recent prospective, randomized, double-blind study.[222] Moreover, the relative cost of such symptomatic relief, in terms of drug toxicity and adverse effects of antipyretic agents on the course of the illness responsible for the fever, has rarely been studied.

ANTIPYRETIC THERAPY: PHYSICAL METHODS OF ANTIPYRESIS

In view of the capacity of external cooling measures to induce a cold pressor response, it is questionable whether this form of antipyretic therapy should ever be administered to febrile patients with infection, much less to ICU patients, for whom it is so frequently prescribed, unless future studies can show a clear benefit. If external cooling is used to treat fever, care must be taken to prevent shivering because of its associated increased oxygen consumption. Unfortunately, even if shivering is prevented, there is no guarantee that a cold pressor response will be averted.

Various physical techniques are used to cool febrile patients. These include sponging with various solutions (e.g., tepid water or alcohol), the application of ice packs or cooling blankets, and exposure to circulating fans (most often in conjunction with sponging). With the latter method, Helox (80% helium, 20% oxygen) has been shown to be superior to air in lowering core temperature, at least in experimental animals, because of the greater thermal conductivity of helium compared with that of nitrogen. In contrast to antipyretic drugs, external cooling lowers the temperature of febrile patients by overwhelming effector mechanisms that have been evoked by pyrogens. Therefore, unless concomitant antipyretic agents are used or shivering is inhibited by other pharmacologic means, external cooling is vigorously opposed in the febrile patient by thermoregulatory mechanisms endeavoring to maintain the elevated body temperature. In patients undergoing therapeutic body temperature modulation, shivering can also be prevented by skin warming, similar to how radiative heating from the sun makes people comfortable when exposed to even severe ambient cooling (the so-called Arctic sun).

Physical methods of antipyresis promote heat loss by conduction, convection, and evaporation. Evaporative methods have traditionally been touted as the most effective physical means of promoting heat loss in febrile patients because these methods are deemed to be least likely to induce shivering. However, carefully designed comparative trials have not yet established any one physical method of antipyresis as superior.

Similarly, direct comparisons of pharmacologic and physical methods of antipyresis are all but nonexistent. In the only extant controlled study, Wenzel and Werner reported that salicylates reduced the second phase of endotoxin-induced fever in rabbits, whereas abdominal cooling increased heat production and did not lower the core temperature unless the animals were simultaneously exposed to environmental hyperthermia.[223] Neither antipyretic modality abolished the initial febrile response.

The few available clinical studies of the efficacy of physical methods of antipyresis have differed in their conclusions. Interpretation of the results of these studies has been difficult, because pharmacologic agents have almost invariably been administered concomitantly with external cooling. Steele and coworkers found acetaminophen (in age-adjusted dosages ranging from 80–320 mg) and sponging to be equally effective in lowering fever in children admitted to a pediatric hospital because of fever.[224] However, when combined, the two modalities produced more rapid cooling than either alone. Similar results were obtained by

Aksoylar and colleagues who found that sponging was more effective than medication in the first 30 minutes, after which medications provided more relief.[225] O'Donnell and colleagues have concluded that in adults, although hypothermia blanket therapy adds little to the action of pharmacologic agents in lowering temperature, it induces wider temperature fluctuations and more episodes of rebound hypothermia.[226] For these reasons we believe that cooling blankets and ice water baths should generally be avoided in treating fevers due to infections but not with hyperthermia or fevers due to noninfectious causes, and that wet sponges with tepid water can be used in combination with pharmacologic therapy to make febrile patients comfortable.

ACKNOWLEDGMENT

We dedicate this chapter to Dr. Philip Mackowiak, a superb clinician and researcher, who has worked in this field for many decades, and whose insights are instrumental to our current understanding of the clinical definition of fever. Dr. Mackowiak wrote earlier editions of this chapter, upon which the current chapter is based.

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56 Fever of Unknown Origin

William F. Wright

SHORT VIEW SUMMARY

Definition

- Occurrence of several occasions of fever higher than 38.3°C (100.9°F) with a duration greater than at least 3 weeks despite 1 week of hospital evaluation is still recognized as the classic definition for fever of unknown origin (FUO).
- The advent of improved diagnostic testing modalities coupled with an increasing number of immunocompromised patients led to a revised definition in which cases of FUO are currently codified into four distinct subclasses: classic FUO, health care–associated FUO, immune-deficient FUO, and human immunodeficiency virus–related FUO.

Etiology and Epidemiology

- Infections, neoplasms, connective tissue diseases, miscellaneous causes, and undiagnosed fevers remain the most common causes of classic FUO.
- The frequency with which infections and neoplasms have been identified as the causes of classic FUO has decreased steadily, whereas the proportion of miscellaneous causes and undiagnosed conditions has risen in recent years.

Diagnosis

- A comprehensive medical history with verification of fever and a detailed physical examination are essential in directing formal laboratory testing. Focal inflammatory and infectious processes can readily and more quickly be detected by advanced imaging and biomarker analysis techniques, such as ^{18}F-fluorodeoxyglucose–positron emission tomography (FDG-PET) or diffusion-weighted magnetic resonance imaging.

Therapy

- Empirical therapeutic trials of antimicrobial agents continue to have a limited role in the management of patients with FUO.
- Owing to the relatively high prevalence of serious bacterial infections responsible for fevers in patients with neutropenia, such patients should receive broad-spectrum antipseudomonal therapy immediately after samples for appropriate cultures have been obtained.

Prognosis

- Although the prognosis of a FUO is determined by its etiology and underlying diseases, most patients with prolonged undiagnosed FUO generally have a favorable outcome.

Most episodes of fever in humans are short lived and do not require diagnostic investigation or specific therapy. Some are manifestations of more serious illnesses, most of which can be readily diagnosed and effectively treated. However, small but important subgroups of fevers are both persistent and difficult to diagnose. Such puzzling fevers have fascinated and frustrated clinicians since the earliest days of clinical thermometry,[1] resulting in a welter of clinical publications. The two most important of these, from a historical perspective, are the classic text *Prolonged and Perplexing Fevers,* by Keefer and Leard (published in 1955),[2] and the paper *"Fever of unexplained origin: report on 100 cases,"* by Petersdorf and Beeson (published in 1961).[3]

TERMINOLOGY AND DEFINITIONS

In the United States, the term *fever of unknown origin* (FUO) is generally used.[4] In other countries an alternative term, *pyrexia of unknown origin* (PUO), is often used.[5]

The first formal definition of FUO to gain broad acceptance was proposed by Petersdorf and Beeson nearly six decades ago: "fever higher than 38.3°C (100.9°F) on several occasions, persisting without diagnosis for at least 3 weeks in spite of at least 1 week's investigation in hospital."[3] Later investigators have modified and extended this classic definition to reflect evolutionary changes in clinical practice.[6,7] These changes include the conduct of most diagnostic tests in the outpatient setting rather than in the hospital, the increasing number of immunocompromised patients (especially those with neutropenia), the proliferation of increasingly complex surgical and intensive care treatment protocols, and the advent of human immunodeficiency virus (HIV) infection leading to the acquired immunodeficiency syndrome (AIDS). In response to this evolving environment, Durack and Street,[7] in 1991, proposed a revised definition in which cases of FUO require 3 days of investigation and must be codified into four distinct subclasses of the disorder: classic FUO, nosocomial (health care–associated) FUO, neutropenic (immune-deficient) FUO, and HIV-related FUO. Computed tomography (CT), magnetic resonance imaging (MRI), ultrasound imaging, nucleic acid–based diagnostic testing, and rapid tests for pathogens have enlarged the diagnostic options for FUO. One could argue that the definitions of types of FUO have much overlap and are not as clinically useful as they were in the past. In a prospective study of 80 patients based on these two main definitions, Ergonul and colleagues[8] found that although the 1991 definition included more cases within the infectious diseases group, the overall distribution of diseases among the two definitions was not significantly different.

CLASSIC FEVER OF UNKNOWN ORIGIN

Classic FUO refers to the type of FUO defined by Petersdorf and Beeson in 1961.[3] The only alteration to their definition required to conform to modern medical practice is to incorporate investigation in the outpatient setting, which today has become the preferred venue for evaluation and treatment. Most patients with classic FUO have subacute or chronic symptoms and therefore can be safely investigated as outpatients. In published series of such patients, for example, the median duration of fever before diagnosis was between 40 and 44 days.[1,9]

Of the many publications concerning the etiology of FUO,[3,4,9,10,11,12,13,14,15] most have dealt with classic FUO rather than with the three other more recently defined subclasses listed earlier.[7] Over the years a recurrent theme has become clear: of the myriad disorders causing classic FUO almost all fall within one of five categories: infections, neoplasms, connective tissue diseases, miscellaneous other disorders, and undiagnosed illnesses. The relative frequencies of individual diagnoses within these five categories vary depending on the decade (Fig. 56.1), geographic region, ages of the patients, type of medical practice, and other factors.

In more recent published series, infections have comprised the largest category, accounting for 16% to 55% of cases (Table 56.1).[4] However, in patients older than 65 years, infections become less common, falling into second or third place as a cause of classic FUO.[4,8,10] In the series

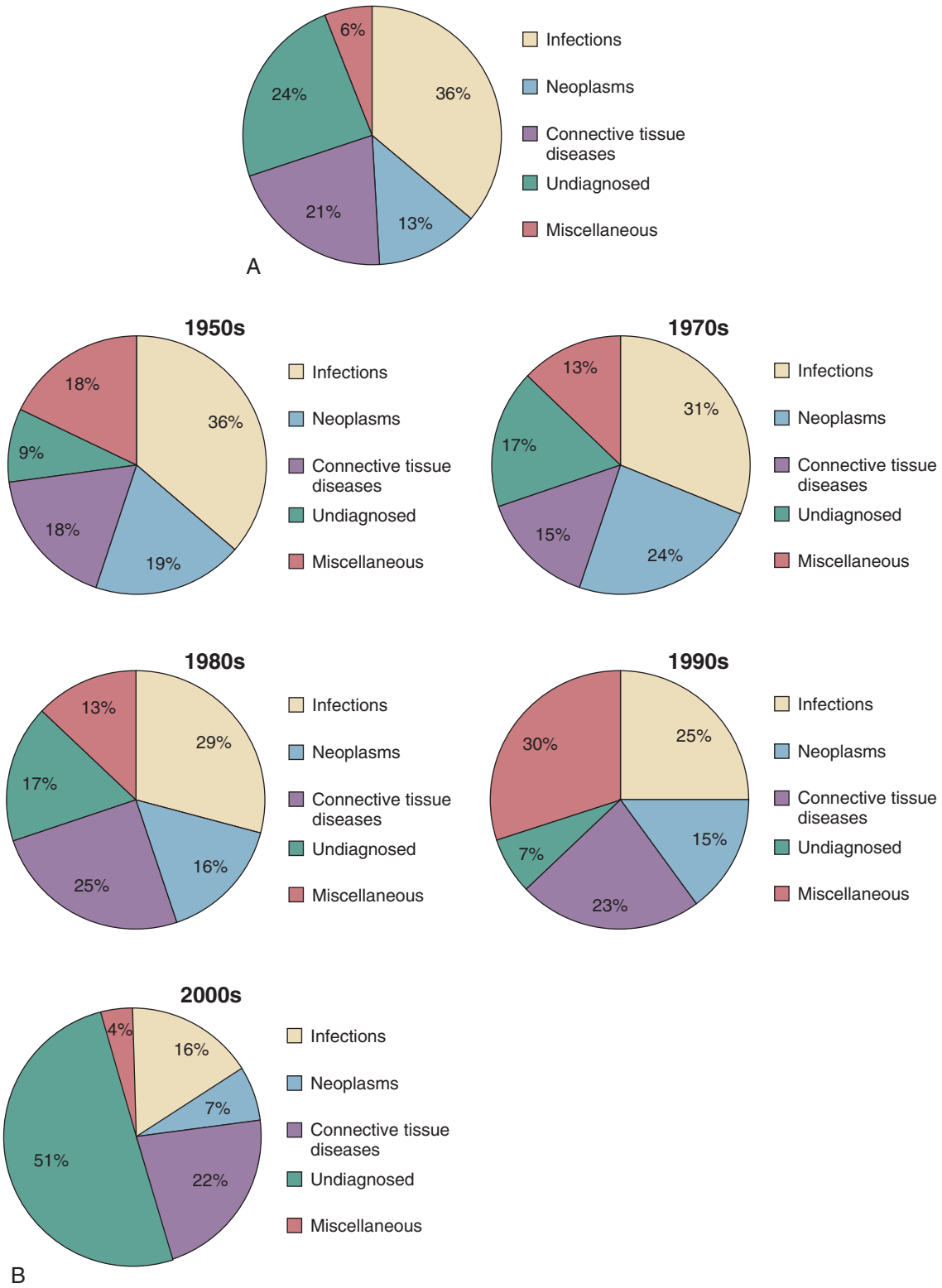

FIG. 56.1 (A) Frequency of the five main etiologic categories of fever of unknown origin. (B) Frequency of the five main etiologic categories of fever of unknown origin by decade. *(A from Hayakawa K, Ramasamy B, Chandrasekar PH. Fever of unknown origin: an evidence-based review.* Am J Med Sci. *2012;344:307–316; B from Mourad O, Palda V, Detsky AS. A comprehensive evidence-based approach to fever of unknown origin.* Arch Intern Med. *2003;163:545–551.)*

of Knockaert and associates,[10] infection was the cause of FUO in only 25% of patients 65 years of age or older; temporal arteritis and various connective tissue diseases accounted for 31% of cases, and tumors accounted for 12%. Only 8% of cases went undiagnosed, which was a percentage similar to that reported by Colpan and colleagues[9] but one substantially lower than that reported in surveys involving younger adults, in which as many as 30% of cases remained undiagnosed.[16] The longer the duration of fever before medical consultation, the less likely that a definitive diagnosis will be made.[17]

Among the infections responsible for classic FUO, abscesses, endocarditis, tuberculosis, and complicated urinary tract infections have consistently been among the most important. These tend to vary

TABLE 56.1 Frequencies of Diagnoses Within the Five Categories of Fever of Unknown Origin (FUO)

PUBLICATION	TOTAL NO. OF PATIENTS	CATEGORY OF FUO (%)				
		Infection	Neoplastic	Rheumatologic	Miscellaneous	Undiagnosed
Petersdorf and Beeson (1961)[3]	100	36.0	19.0	19.0	19.0	7.0
Petersdorf and Larson (1983)[93]	105	30.0	31.0	17.0	10.0	12.0
Larson et al. (1982)[94]	105	30.0	31.0	16.0	11.0	12.0
Knockaert et al. (1992)[72]	199	22.6	7.0	21.5	14.5	25.6
De Kleijn et al. (1997)[16]	167	26.0	12.0	25.0	8.0	30.0
Bleeker-Rovers et al. (2007)[67]	73	16.0	7.0	22.0	4.0	51.0
Colpan et al. (2007)[9]	71	45.1	14.1	26.8	5.6	8.5
Mansueto et al. (2008)[84]	91	31.8	14.2	12.0	9.8	31.8
Bandyopadhyay et al. (2011)[15]	164	55.0	22.0	11.0	3.5	8.5

in incidence according to locale. Visceral leishmaniasis, for example, although absent from most series of classic FUO, accounted for 8% of cases in a study reported in 1997 from Spain.[18] Other examples of causes of classic FUO in distinct populations include familial Mediterranean fever in Ashkenazi Jews; Kikuchi-Fujimoto disease, an unusual form of necrotizing lymphadenitis seen primarily in Japan[19]; and tumor necrosis factor receptor–associated periodic syndrome (TRAPS) fever, formerly called familial Hibernian fever, an inherited periodic fever syndrome described originally in Ireland.[20] The miscellaneous category contains both varied and individually rare causes of classic FUO (Table 56.2).

Of the connective tissue diseases responsible for classic FUO, Still disease (juvenile rheumatoid arthritis), other variants of rheumatoid arthritis, and systemic lupus erythematosus predominate in younger patients, whereas temporal arteritis and polymyalgia rheumatica syndromes are more common in elderly patients.

Malignant neoplasms, another important cause of FUO, can induce fever directly through the production and release of pyrogenic cytokines, as in the case of certain lymphomas. They may also generate fevers indirectly by undergoing induced or spontaneous necrosis and/or by creating conditions conducive to secondary infections.[1] Among the malignant neoplasms responsible for FUO, hematologic cancers, hypernephromas, and gastrointestinal (mainly colorectal) and central nervous system cancers have been documented as common causes.[3,4] In a recent series of 80 patients with FUO, Ergonul and colleagues[8] established a malignant neoplasm etiology in 14 cases (18%).

The relative frequency with which the major diagnostic categories are represented in series of classic FUO varies according to both the era in which the series was published[6,14] and its country of origin.[4,8,15,21,22,23,24] Since the mid-1990s, the frequency with which infections and malignant neoplasms have been identified as causes of classic FUO has fallen steadily, whereas the proportion of miscellaneous causes and undiagnosed conditions has risen.[24] However, in developing countries, the frequency with which infections are diagnosed has changed little.[15] Consequently, in these countries, malignant neoplasms and connective tissue disorders are comparatively less important as causes of classic FUO than in developed countries.[9,15]

Infants and Children

The diseases responsible for classic FUO in infants differ from those in older children and adults. Respiratory infections cause classic FUO in infants more often than in children older than 12 months or in adults.[11] The relative frequency of infections as the cause of FUO in infants is high because connective tissue diseases and cancers are rare in this age group. Kawasaki disease occurs predominantly in children younger than 5 years. Whereas connective tissue diseases are rarely seen in children younger than 12 months, Still disease is a leading cause of FUO in older children and young adults. Joint involvement in children with FUO usually signifies a serious underlying disorder, such as a connective tissue disease, endocarditis, or leukemia.[11]

In a series of 146 pediatric cases of FUO, Jacobs and Schutze[25] established a specific diagnosis in only 84 (57.5%). Of these, 64 (43.8%) had infections, 11 (7.5%) had autoimmune disorders, 4 (2.7%) had malignant neoplasms, and 5 (3.4%) had a variety of other disorders,

TABLE 56.2 Examples of Uncommon or Rare Causes of Prolonged Fever

Addison disease	Infected urachal cyst
Adult-onset Still disease	Inflammatory bowel disease
Alcoholic hepatitis	Kikuchi-Fujimoto disease
Allergic alveolitis	Löfgren syndrome
Aortic dissection	Lymphomatoid granulomatosis
Aortitis	Metal fume fever
Atrial myxoma	Myeloproliferative syndromes
Autoimmune cholangitis	Pancreatitis
Bartonellosis	Parathyroid apoplexy
Behçet syndrome	Paroxysmal hemoglobinurias
Carcinomatous meningitis	Pericarditis
Castleman disease	Periodic fever
Chronic meningitis	Pheochromocytoma
Cirrhotic fever	Polyarteritis nodosa
Cyclic neutropenia	Postpericardiotomy syndrome
Drug fever and other hypersensitivities	Pulmonary emboli
Erythema multiforme	Resorbing hematoma
Fabry disease	Retroperitoneal fibrosis
Factitious fever	Rosai-Dorfman disease
Familial Hibernian fever	Sarcoidosis
Familial Mediterranean fever	Schnitzler syndrome
Giant coronary aneurysm	Serum sickness
Granulomatous hepatitis	Sinusitis
Granulomatous peritonitis	Sjögren syndrome
Hantavirus infection	Subacute necrotizing lymphadenitis
Hemoglobinopathies	Thrombotic thrombocytopenic purpura
Hemolytic anemias	Thyroiditis and thyrotoxicosis
Hemophagocytic syndrome	Venoocclusive disease
Histiocytosis X	Vitamin B_{12} deficiency
Human picornavirus infection	Wegener granulomatosis
Hypereosinophilic syndrome	Whipple disease
Immunoblastic lymphadenopathy	

Modified from Durack DT. Fever of unknown origin. In: Mackowiak PA, ed. Fever: Basic Mechanisms and Management. *2nd ed. Philadelphia: Lippincott-Raven; 1997:237–249.*

such as drug-induced fever, sarcoidosis, and mercury poisoning. The most common infectious diseases diagnosed in their series were Epstein-Barr virus (EBV) infection (15%), osteomyelitis (10%), bartonellosis (5%), and urinary tract infections (4%).

Chow and Robinson[26] analyzed 18 papers concerned with pediatric FUO published between 1968 and 2008. Of 1638 children ages birth to 18 years, 832 (51%) had infections, 93 (6%) had malignant neoplasms, 150 (9%) had noninfectious inflammatory diseases, 179 (11%) had miscellaneous causes such as inflammatory bowel disease and Kawasaki disease, and 384 (23%) had no diagnosis. Although the distribution of diagnostic categories was similar among developed versus developing countries, urinary tract infections, brucellosis, tuberculosis, and typhoid fever were more common in developing countries. The most common infections diagnosed in cases of FUO in developed countries included urinary tract infections, osteomyelitis, tuberculosis, and bartonellosis (e.g., *Bartonella henselae*).

Although a daily rectal temperature greater than 38.3°C (100.9°F) lasting more than 2 weeks despite diagnostic evaluation has been defined as FUO in children, 9 of 18 papers analyzed by Chow and Robinson used the classic definition of FUO.[26]

In a more recent publication, Zhou and colleagues[27] investigated the presence of seven different human herpesvirus (HHV) types in whole blood from pediatric patients with classic FUO. Of 151 children ages 6 months to 15 years, 63 (33.9%) had detectable herpesvirus DNA. Cytomegalovirus (15.1%), HHV-6 (14%), and EBV (18%) were the viruses detected most commonly when compared to a nonfebrile cohort. Whereas fever alone occurred in the majority of these FUO patients, fever of at least 8 days' duration and hepatitis were more strongly associated with EBV and HHV-7.

Elderly Persons

One of the most striking features of classic FUO in patients older than 65 years is the relatively high frequency with which connective tissue diseases are identified as the cause of the illness (Table 56.3).[4,10,28,29,30] In developed countries, connective tissue diseases surpass even infections as the leading cause of classic FUO in the elderly.[4,10,29] This is primarily because the temporal arteritis and polymyalgia rheumatica syndromes are common in this setting.[4,29,30,31] Unfortunately, these diagnoses are frequently missed or delayed because their symptoms are subacute and nonspecific. In elderly patients in whom infections are identified as the cause of FUO, intraabdominal abscesses, complicated urinary tract infections, tuberculosis, and endocarditis have predominated.[10] For unclear reasons, factitious fever is a rare cause of FUO in older adults. Relatively few cases of FUO go undiagnosed in elderly patients (see Table 56.3). The occurrence of classic FUO in an elderly patient has a distinctly poorer prognosis than for the younger patient because of the relatively high incidence of malignancies in the elderly.[32]

Returned Travelers

Fever in returned travelers (see Chapter 319) is most often due to common infections, such as malaria and respiratory or urinary tract infections.[33] However, exotic causes of fever that typically falls short of the duration for FUO, such as amebic liver abscess or dengue, are occasionally diagnosed, especially among international travelers returning from the tropics. Of the many febrile conditions encountered among returning travelers (Table 56.4), malaria, typhoid fever, and acute HIV infection are the ones most likely to manifest as FUO.

TABLE 56.3 Final Diagnosis in Elderly Compared With Younger Patients With Fever of Unknown Origin From Patient Series Pre-1990

DIAGNOSIS	<65 YEARS (*N* = 152)	>65 YEARS (*N* = 201)
Infections	33 (21%)	72 (35%)
Abscess	6	25
Endocarditis	2	14
Tuberculosis	4	20
Viral infections	8	1
Tumors	8 (5%)	37 (19%)
Hematologic	3	19
Solid	5	18
Multisystem diseases[a]	27 (17%)	57 (28%)
Miscellaneous[b]	39 (26%)	17 (8%)
Other	13	12
No diagnosis	45 (29%)	18 (9%)

[a]Rheumatic diseases, connective tissue disorders, vasculitis (including temporal arteritis), polymyalgia rheumatica, and sarcoidosis.
[b]Includes factitious fever (seven cases), habitual hyperthermia (five cases), and drug-induced fever (three cases).
Modified from Iikuni Y, Okada J, Kondo H, et al. Current fever of unknown origin 1982–1992. Intern Med. *1994;33:67–73; and Knockaert DC, Vanneste LJ, Bobbaers HJ. Fever of unknown origin in elderly patients.* J Am Geriatr Soc. *1993;41:1187–1192.*

TABLE 56.4 Causes of Fever in the Returned Traveler

DIAGNOSIS	PERCENT		
	Wilson et al.[95] (*n* = 6957)	O'Brien et al.[96] (*n* = 232)	Antinori et al.[97] (*n* = 147)
Malaria	35.0	27.0	47.6
Hepatitis	1.0	3.0	8.8
Respiratory tract infection[a]	14.0	24.0	2.7
Urinary tract infections	4.0	1.0	1.4
Gastroenteritis	2.0	14.0	4.8
Dengue fever	6.0	8.0	3.4
Enteric (typhoid) fever	2.0	3.0	4.1
Tuberculosis	0.0	0.4	0.7
Rickettsiosis	2.0	2.0	0.7
Acute HIV infection	0.01	0.4	4.5
Amebic liver abscess	0.3	1.0	0.0
Other miscellaneous infections	4.0	4.8	4.3
Miscellaneous noninfectious conditions	10.0	2.4	4.8
Undiagnosed	22.0	9.0	12.2

[a]Includes upper respiratory tract infection, pneumonia, and bronchitis.
HIV, Human immunodeficiency virus.
Modified from Suh KN, Kozarsky PE, Keystone JS. Evaluation of fever in the returned traveler. Travel Med. *1999; 83: 997–1017.*

NOSOCOMIAL (HEALTH CARE–ASSOCIATED) FEVER OF UNKNOWN ORIGIN

Nosocomial (health care–associated) FUO, as the name implies, is a condition in which patients first manifest fever during active medical treatment for some other illness. Such FUO cases, as might be expected, are frequently attributable to risk factors encountered in the health care environment, including surgical procedures, urinary and respiratory tract instrumentation, intravascular devices, drug therapy, and immobilization. Leading examples of causes attributable to health care–associated FUO include drug fever, postoperative complications (i.e., occult abscesses), septic thrombophlebitis, recurrent pulmonary emboli, myocardial infarction, cancers, blood transfusion, and *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.[4,7,34]

Postoperative Patients

Several reports have highlighted the fact that it is often difficult to identify the precise cause of postoperative fevers. In a series of 537 consecutive patients undergoing major gynecologic surgery, 211 (39%)

developed postoperative fever.[35] Of 77 blood cultures performed on these patients, none was positive. Although 11 of 106 (10%) urine cultures were positive and 5 of 54 (9%) chest radiographs were abnormal, a specific pathologic process was detected in only 8% of febrile patients. Of patients with evidence of urinary tract infections and pneumonia, the median length of postoperative fever was 2 days (range: 1–7 days) and 3 days (range: 2–4 days), respectively. In a prospective study by Kendrick and colleagues[36] of postoperative fever among 292 patients admitted to a gynecologic oncology service after abdominal or vaginal operation, 58 (20%) patients developed postoperative fever. Among 37 (16%) low-risk surgical patients developing postoperative fever, only 6 (3%) had an infection diagnosis. The majority of infections occurred within 4 days of the operative procedure and included pneumonia, vaginal cuff cellulitis, and urinary tract infection. The authors proposed that postoperative fever is common and frequently represents the response to surgically induced tissue injury with the release of pyrogenic cytokines and interleukins rather than the result of infection. Although fever is a well-recognized manifestation of some surgical procedures, most episodes are short lived and do not meet the classic definition of FUO. Postoperative fevers generally do not require extensive diagnostic investigation for unusual causes of fever.

In another series concerned with the etiology of persistent postoperative fever in patients undergoing total joint arthroplasty, few definitive diagnoses were established, causing the authors to conclude that postoperative fever (postoperative days 1 through 5) is a normal component of the inflammatory response to this type of major surgery.[37]

Intensive Care Unit Patients

Fever is common in intensive care units (ICUs), most often developing relatively early after admission to the ICU, in which case it tends to be of noninfectious origin and carries a favorable prognosis.[38] Prolonged fever, however, is associated with a worse prognosis. One of the most significant findings is the simultaneous occurrence of multiple infections in the majority of these patients. Health care–associated sinusitis, often a complication of mechanical ventilation arising from supine positioning and the use of endotracheal, gastric, and feeding tubes, is common[39] and should always be considered when evaluating FUO in ICU patients. More often than not, the causes of fever in the ICU are essentially no different from general causes of health care–associated fevers (i.e., abscesses, drug fever, postoperative complications, septic thrombophlebitis, recurrent pulmonary emboli, myocardial infarction, cancers, blood transfusion, and *C. difficile* colitis). Ventilator-associated pneumonia is frequently underdiagnosed among patients with respiratory failure, fever, and nonspecific radiographic pulmonary densities. Patients with acute respiratory distress syndrome (ARDS) tend to develop pneumonia earlier than non-ARDS patients. Pulmonary abscesses are usually readily recognized on radiologic imaging and therefore would be an unlikely cause of FUO.

Stroke Patients

In patients with a recent stroke, fever is usually the result of an infection, most commonly a urinary tract infection related to urinary catheterization or a respiratory tract infection. However, in some cases a focus of infection cannot be identified, and when the fever does not respond to empirical antibiotic treatment, it is presumed to be due to the stroke itself. In a study of 330 patients hospitalized for acute stroke, Georgilis and associates[40] observed that noninfectious fevers were most often associated with intracranial mass effects and tended to occur earlier after the onset of stroke than fevers due to infection.

NEUTROPENIC (IMMUNE-DEFICIENT) FEVER OF UNKNOWN ORIGIN

Obviously, various forms of immunosuppression predispose more or less strongly to a wide variety of infectious complications. Thus it is not surprising that immunosuppressed patients have perhaps the highest incidence of FUO of any group of patients. In a recent series of 116 hematology-oncology patients, for example, Engelhart and associates[41] observed 33 FUOs in 28 patients, for an overall rate of 8.2 episodes per 1000 patient-days. Because of impaired immune responses, signs of inflammation other than fever are notoriously absent or diminished in such patients, leading to atypical clinical features and absence of radiologic abnormalities in what otherwise would be readily diagnosed infections.[42]

In patients with impaired cell-mediated immunity, FUO is often due to conditions other than pyogenic bacterial infections, as illustrated in a prospective evaluation of patients with leukemia and lymphoma by Toussaint and coworkers.[43] In that series, infections were the cause of 319 (67%) of the 477 episodes of fever. The majority of infections included respiratory tract infections (28.8%), secondary bacteremia due to gram-negative bacilli (15.7%), genitourinary tract infections (12.9%), skin and soft tissue infections (11.3%), and primary bacteremia (11.0%). One hundred nine (23%) cases were due to noninfectious conditions: malignant neoplasm, metastatic disease, and drug-induced fever. While noninfectious neoplastic-related fever was more common (41%) among nonneutropenic patients, noninfectious drug-induced fever was more common among neutropenic patients (13%). In 47 (10%) cases, the cause of the fever could not be determined.

Neutropenia and lymphopenia occur frequently among cancer patients undergoing myelosuppressive chemotherapy.[44] Neutropenia is a dangerous condition that can be considered a special subclass of immunodeficiency. The number of patients with episodes of neutropenia resulting from cytotoxic therapy or from various malignancies affecting the bone marrow is rising, even as the median duration of neutropenia in such patients is falling, owing to the increasing application of treatment with colony-stimulating factors. Not only are the duration and nadir of neutropenia correlated with the incidence of fever and infections, but so are outcomes related to morbidity and mortality. The highest percentages of deaths occur among patients with persistent severe neutropenia (<100 cells/mm^3 for more than 7 days) or further declining initial neutropenia (<1000 cells/mm^3). Furthermore, severe infections increase with the increasing proportion of time neutropenia and lymphopenia levels are less than 500 cells/mm^3. However, the incidence of fever and infectious episodes decreases when levels are 1500 cells/mm^3, above which level there is no associated decreased incidence, or the expected duration of neutropenia is less than 7 days.

Episodes of fever are common in patients with neutropenia. Many such episodes are short lived because they either respond quickly to treatment or are manifestations of rapidly fatal infections. Because bacteremia and sepsis are frequent causes, empirical broad-spectrum antibiotics should be administered promptly, without waiting for the results of cultures, when fever develops in neutropenic patients. However, only about 35% of prolonged episodes of febrile neutropenia (usually defined as persistent fevers for >7 days after initiation of empirical antimicrobial therapy in association with a negative workup, and neutropenia expected to last >7 days) respond to broad-spectrum antibiotic therapy. Practitioners often assume that if fever does not respond promptly to antibacterial therapy, fungal infection must be responsible, but other potential causes are at least as likely to be identified.[42,44]

HIV-RELATED FEVER OF UNKNOWN ORIGIN

Episodes of fever are commonplace in patients infected with HIV—a special subgroup of immunodeficient patients now so numerous that a separate category of FUO is justified.[45,46,47,48] The primary phase of HIV infection is characterized by a mononucleosis-like illness in which fever is a prominent feature. All too often, primary HIV infection eludes diagnosis because the illness is nonspecific and precedes seroconversion. For this reason it represents an important cause of HIV-associated FUO. Once symptoms of the primary phase of the HIV infection resolve, HIV-infected patients enter a long period of subclinical infection during which they are usually afebrile.[48] However, in the later phases of untreated HIV infection, episodes of fever become common, often signifying a superimposed illness. Many of these are potentially devastating opportunistic infections, which tend to manifest in atypical fashion owing to the tendency of the disordered immune response or prior therapy, or both,[49] to distort their clinical features.

Once highly active antiretroviral therapy (HAART) has been started and HIV viral load effectively suppressed, the frequency of FUO in

HIV-infected patients falls markedly. In a report of 274 HIV-infected patients, for example, Abellan-Martinez and colleagues[45] observed an incidence range of 2.57 to 3.66 FUO episodes per 100 HIV-infected patients per year prior to the initiation of HAART and an incidence range of 0.84 to 1.24 episodes after the introduction of therapy. Because of the lack of well-established diagnostic criteria during the study period, the immune reconstitution inflammatory syndrome was not reported as a cause of fever. In another study, the frequency of FUO was 3% in untreated patients compared with 0.6% in those receiving therapy.[50] Mycobacterial infections have been the most common cause of FUO in such patients; collagen vascular diseases have been distinctly uncommon.[45,51] In a series reported by Armstrong and colleagues,[52] the etiology of the FUO was identified in 56 of 70 (80%) patients. A single cause was identified in 43 cases; three distinct causes of FUO were identified in 3 cases and two causes in 10 others. The causes of FUO in 70 patients in whom the etiology was determined are listed in Table 56.5. Most cases of FUO in HIV-infected patients are the result of opportunistic infections, the specific frequencies of which are dictated, at least in part, by geographic variation in the prevalence of these infections.[53]

DIAGNOSIS

A point deserving emphasis is that most patients diagnosed with "classic FUO" were not suffering from unusual or rare conditions; rather, they exhibited atypical manifestations of common illnesses in an era when advanced diagnostic laboratory or imaging studies were not available. Changes to the original FUO definition by Durack and Street[7] emphasize that treatment is clearly more time critical for certain classes than others. Despite revised definitions and introduction of improved serologic, laboratory, and imaging technologies, FUOs continue to elude diagnosis, suggesting that fevers may have many origins. The most important lesson learned from the "classic FUO" concept is that, in many instances, available information from the history and physical examination should be utilized more often. The evaluation of a patient with FUO typically includes a comprehensive history, verification that the patient actually has fever, consideration of the fever pattern, repeated physical examinations, and a host of laboratory investigations, key imaging studies, and invasive diagnostic procedures (Table 56.6).

History

It is axiomatic that a comprehensive history is a cornerstone of the evaluation of any complex illness such as FUO. The history can be especially important in determining the choice of the initial laboratory investigations. Particular attention should be given to recent travel, exposure to pets and other animals, the work environment, and recent contact with people exhibiting similar symptoms. The family history should be carefully scrutinized for possible hereditary causes of fever—for example, familial Mediterranean fever. Likewise, the past medical history must be scrutinized for prior episodes of FUO and for any previously diagnosed conditions, such as lymphoma, rheumatic fever, or intraabdominal disorders, complications or reactivation of which might account for the source of fever. In cases of recurrent FUO, rare disorders are more common than in nonrecurrent cases and are also more likely to remain undiagnosed.[54] Finally, a complete list of the patient's medications must be obtained so that each may be evaluated as a potential source of drug-induced fever.

Verification of Fever and Fever Pattern

The next step in the evaluation of the patient with FUO is to verify the presence of fever. The importance of this step should be self-evident, yet it is often overlooked. In fact, in a series of 347 patients admitted to the National Institutes of Health for investigation of prolonged fever, 35% were ultimately determined either not to have significant fever at all or to have fever of factitious origin.[55]

Clinicians have endeavored to diagnose particular diseases by analyzing the associated fever patterns since the earliest days of clinical thermometry.[56] These efforts have given rise to an extensive and frequently arcane terminology, including descriptors such as *remittent, intermittent, hectic, quotidian, picket fence, sustained, quartan,* and *saddleback fevers.* Such terms have been used to codify fever patterns into general categories

TABLE 56.5 Diseases Established as the Etiology of Fever in 70 Cases of HIV-Associated Fever of Unknown Origin

ETIOLOGY	NO. (%) OF TIMES DIAGNOSIS WAS ESTABLISHED
Infection	
DMAC	22 (31)
Pneumocystis jirovecii pneumonia	10 (13)
CMV	8 (11)
Histoplasmosis	5 (7)
Viral (not CMV) [a]	5 (7)
Bacterial	4 (5)
Mycobacterium tuberculosis	4 (5)
Fungal (not histoplasmosis)[b]	2 (3)
Parasitic[c]	2 (3)
Mycobacterium genavense	1 (1)
TOTAL	63 (88)
Neoplasia	
Lymphoma	5 (7)
Kaposi sarcoma	1 (1)
TOTAL	6 (8)
Miscellaneous	
Drug fever	2 (3)
Castleman disease	1 (1)
TOTAL	3 (4)

[a]Includes hepatitis C, hepatitis B, adenovirus pneumonia, herpes simplex esophagitis, and varicella-zoster encephalitis (one case each).
[b]Includes disseminated cryptococcosis and pulmonary aspergillosis (one case each).
[c]Includes cerebral toxoplasmosis and disseminated cryptosporidiosis (one case each).
CMV, Cytomegalovirus; DMAC, disseminated *Mycobacterium avium* complex; HIV, human immunodeficiency virus.
Modified from Armstrong WS, Katz JT, Kazanjian PH. Human immunodeficiency virus–associated fever of unknown origin: a study of 70 patients in the United States and review. Clin Infect Dis. *1999;28:341–345.*

TABLE 56.6 General Diagnostic Evaluation of Patients With Fever of Unknown Origin

Comprehensive history
Repeated physical examinations
Complete blood cell count
Routine blood chemistry determinations, including LDH, LFTs, and ferritin
Urinalysis, including microscopic examination
Chest radiograph
Erythrocyte sedimentation rate
Antinuclear antibodies
Rheumatoid factor
Blood cultures: three or more separate specimens obtained in absence of antimicrobial therapy
Cytomegalovirus IgM antibodies or viral detection in blood
Heterophil antibody test in children and young adults
Tuberculin skin test or whole blood interferon-γ release assay
Computed tomography of abdomen, pelvis, or other sites
Magnetic resonance imaging
Radionuclide scans
Human immunodeficiency virus antibodies or viral detection assay
Further evaluation of any abnormality detected by above tests
Venous duplex imaging of lower limbs

IgM, Immunoglobulin M; *LDH,* lactate dehydrogenase; *LFTs,* liver function tests (for liver enzymes).
Modified from Arnow PM, Flaherty JP. Fever of unknown origin. Lancet. *1997;350:575–580, with permission from Elsevier.*

in an attempt to enhance their diagnostic utility.[57] A few, such as the Pel-Ebstein pattern seen in some cases of Hodgkin disease, the typhus inversus (i.e., reversal of the normal diurnal pattern) in some cases of disseminated tuberculosis, and the pulse-temperature disassociation sometimes seen in typhoid fever, have been posited as having diagnostic value (Fig. 56.2). Unfortunately, with the possible exception of the well-known periodicity of tertian and quartan malaria, these fever patterns are neither sufficiently sensitive nor specific for diagnosis of any disease.

Thus, although fever patterns per se are rarely diagnostic, they occasionally offer useful information[57] and should be considered in the context of other signs, symptoms, and laboratory data. The resolution of fever after the institution of a disease-specific therapy (e.g., empirical therapy for suspected tuberculosis) sometimes provides strong evidence to support a presumptive diagnosis.

In pediatric populations, the height of a fever correlates roughly with the likelihood of bacteremia. McCarthy and coworkers[58,59] reported that in young children with febrile illnesses, the likelihood of bacteremia is 7% in those with temperatures of 40°C (104°F) or less, 13% with temperatures of 40.5°C to 41°C (104.9°F to 105.8°F), and 26% with temperatures of 41.1°C (106°F) or greater. It is commonly believed that there may be a similar relationship between high fever and the likelihood

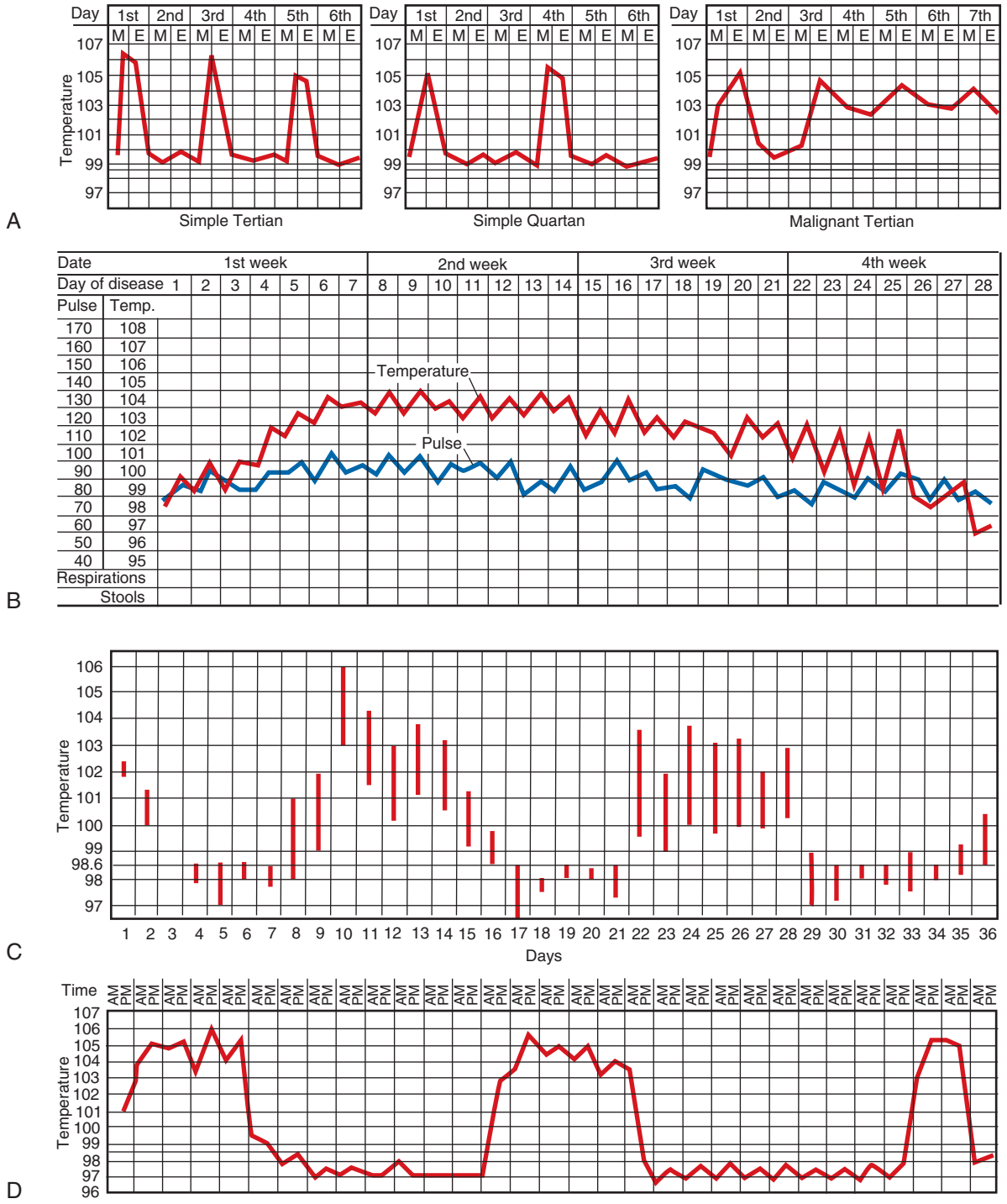

FIG. 56.2 Distinctive fever patterns. (A) Malaria. (B) Typhoid fever (demonstrating relative bradycardia). (C) Hodgkin disease (Pel-Ebstein pattern). (D) Borreliosis (relapsing fever pattern). *(From Woodward TE. The fever pattern as a clinical diagnostic aid. In: Mackowiak PA, ed.* Fever: Basic Mechanisms and Management. *2nd ed. Philadelphia: Lippincott-Raven; 1997:215–236.)*

of bacteremia in adults; this belief has not been substantiated. In any case, the relationship is at best loose, even in children, with numerous examples of bacteremia in which there is little or no fever and nonbacteremic conditions, such as drug-induced fever, thrombophlebitis, and recurrent pulmonary emboli, in which extremely high fevers sometimes are encountered.

Physical Examination

In the investigation of FUO, several aspects of the physical examination should be accorded closer scrutiny than generally given during the evaluation of other illnesses (Table 56.7). Frequently, key physical abnormalities in patients with FUO are so subtle as to require repeated examinations to be appreciated. Examples include the nodular or weakly pulsatile temporal artery of temporal arteritis, the telltale oral ulcers of disseminated histoplasmosis or Behçet syndrome, the choroid granuloma or epididymal nodule of extrapulmonary tuberculosis, the testicular nodule of polyarteritis nodosa, and the vague rectal fluctuance of a perirectal abscess. The diagnostic yield of the physical examination alone in the evaluation of FUO has not been studied directly. Nevertheless, in two pediatric series, abnormal physical findings were reported to have contributed to the diagnosis in 60% of cases of FUO.[60,61] In half of these, the abnormalities were detected only after repeated examinations. A vigorous search for lymphadenopathy is recommended. When enlarged lymph nodes are detected in a patient with FUO, lymph node biopsy is often undertaken. However, with the exception of the lymphomas, the diagnostic yield of lymph node biopsy in FUO is disappointingly low.[60,62]

Laboratory Investigations

The literature is replete with algorithms indicating which laboratory tests should be performed to evaluate FUO.[4,21,29,63,64,65,66,67] Although useful as general guides, blind application of such algorithms may result in performance of an excessive number of tests. They should be selectively applied using clues gleaned from the history and physical examination, also referred to as potentially diagnostic clues, to direct the choice and sequence of tests (Table 56.8). When formulating a diagnostic plan for FUO patients, the clinician should remember the old saying that the cause is more often a common disease presenting in atypical fashion than a rare disease presenting in typical fashion. Inappropriate diagnostic tests in the evaluation of FUO may not only delay identification of the correct diagnosis but also result in false-positive tests leading to misguided treatment plans.[4,21,66] A well-conducted history and physical examination is perhaps more important than any other diagnostic procedure in focusing the FUO evaluation, accelerating initiation of appropriate therapy while minimizing the cost and potential toxicity of unnecessary interventions.

In most series, noninvasive laboratory tests have yielded the diagnosis in approximately a fourth of the cases.[10,24] The most useful of these have been serologic tests for microbial pathogens and for various rheumatologic disorders. Examination of blood smears is occasionally diagnostic, especially in patients with tick-borne or louse-borne relapsing fever, anaplasmosis, and ehrlichiosis. Paradoxically, the advent of enhanced microbial culture systems has had less of an impact on the proportion of successful diagnoses than might have been anticipated. This is because modern culture systems have become so proficient at recovering fastidious bacteria, mycobacteria, and fungi from blood that they provide the diagnosis promptly, before the time required to meet the definitions of FUO has elapsed. Bone marrow examination should be considered for diagnosis of suspected granulomatous diseases (e.g., tuberculosis, histoplasmosis, and sarcoidosis), carcinomatosis, and hemophagocytic syndrome, especially in patients with abnormal complete blood cell counts.[68,69,70] In two series, bone marrow examination contributed to the diagnosis of FUO in approximately a fourth of cases.[68,69] Whereas the majority of HIV-infected patients were diagnosed with an infectious etiology, nonimmunocompromised patients were more likely to have a neoplastic or noninfectious inflammatory condition. In transplant patients, procalcitonin levels may be of use in differentiating infection from acute organ rejection, in that elevated levels are seen in the former but not the latter condition.[71]

TABLE 56.7 Examples of Subtle Physical Findings Having Special Significance in Patients With Fever of Unknown Origin

BODY SITE	PHYSICAL FINDING	DIAGNOSIS
Head	Sinus tenderness	Sinusitis
Temporal artery	Nodules, reduced pulsations	Temporal arteritis
Oropharynx	Ulceration; tender tooth	Disseminated histoplasmosis, periapical abscess
Fundi or conjunctivae	Choroid tubercle, petechiae, Roth spot	Disseminated granulomatosis,[a] endocarditis
Thyroid	Enlargement, tenderness	Thyroiditis
Heart	Murmur	Infective or marantic endocarditis
Abdomen	Enlarged iliac crest lymph nodes, splenomegaly	Lymphoma,[b] endocarditis, disseminated granulomatosis[a]
Rectum	Perirectal fluctuance, tenderness	Abscess
	Prostatic tenderness, fluctuance	Abscess
Genitalia	Testicular nodule	Periarteritis nodosa
	Epididymal nodule	Disseminated granulomatosis
Lower extremities	Deep venous tenderness	Thrombosis or thrombophlebitis
Skin and nails	Petechiae, splinter hemorrhages, subcutaneous nodules, clubbing	Vasculitis, endocarditis

[a]Includes tuberculosis, histoplasmosis, coccidioidomycosis, sarcoidosis, and syphilis.
[b]See text for note on the nonspecificity of lymphadenopathy identified on physical examination.

Imaging Studies

The constant advancement of imaging and biomarker analysis techniques, such as ^{18}F-fluorodeoxyglucose–positron emission tomography (FDG-PET) and diffusion-weighted MRI, provides an opportunity to readily and more quickly identify focal inflammatory or infectious processes prior to patients fulfilling the criteria of FUO. In the past, imaging studies were used primarily to localize abnormalities as a preamble to more definitive invasive tests. Computed tomography of the abdomen and, to a lesser extent, ultrasound imaging of the gallbladder and hepatobiliary system have been used extensively to evaluate cases of FUO. In one series, more than three CT or ultrasound examinations, or both, were performed for each FUO patient evaluated.[72] Nevertheless, the diagnostic yield per test performed was low—about 10%. Of the major diagnostic imaging modalities, another more recent series reported sensitivities of 60% for plain-film chest radiography, 82% for chest CT, 86% for abdominal ultrasound, and 92% for abdominal CT.[67] False-negative CT studies have occasionally been encountered, even in cases of abscesses in solid organs, as a result of distortions of normal anatomy, small abscess size, or failure to use both oral and intravenous contrast agents.[67,73] Computed tomography pulmonary angiograms can be very helpful in diagnosis of pulmonary emboli. Diffusion-weighted MRI is especially useful for evaluation of the central nervous system and, in the abdomen, the spleen and lymph nodes. In a series of 67 patients undergoing evaluation for FUO, Wagner and colleagues[74] reported that application of MRI of the aortic arch and proximal cervical arteries in selected patients improved the diagnosis of large vessel vasculitis by 20%. Although one case did not demonstrate vasculitic changes by cervical imaging, four cases would have remained undiagnosed without the application of MRI. The most common diseases diagnosed by this testing modality were giant cell arteritis (55%), Takayasu arteritis (27%), Wegener granulomatosis (9%), and microscopic polyangiitis (9%). In another series involving prolonged fevers among neutropenic patients,

TABLE 56.8 Examples of Potential Diagnostic Clues to Infections Presenting as Fever of Unknown Origin

ETIOLOGY	HISTORICAL CLUES	PHYSICAL CLUES
Anaplasmosis	Transmitted by the bite of an *Ixodes* tick in association with outdoor activity in north central and eastern United States	Fever, headache, arthralgia, myalgia, pneumonitis, thrombocytopenia, lymphopenia, and elevated liver enzymes
Babesiosis	Transmitted by the bite of an *Ixodes* tick in association with outdoor activity in the northeastern United States	Arthralgias, myalgias, relative bradycardia, hepatosplenomegaly, anemia, thrombocytopenia, and elevated liver enzymes
Bartonellosis	Recent travel to the Andes mountains with fever and bacteremia (Oroya fever; *Bartonella bacilliformis*), association with homelessness in urban settings *(Bartonella quintana)* or contact with either fleas or the scratch of an infected kitten or feral cat *(Bartonella henselae)*	Conjunctivitis, retro-orbital pain, anterior tibial bone pain, macular rash, nodular plaque lesions, and/or regional lymphadenopathy
Blastomycosis	Contact with soil adjacent to the Mississippi and Ohio River valleys, Saint Lawrence River in New York and Canada, and North American Great Lakes, or exposure to infected dogs	Arthritis, atypical pneumonia, pulmonary nodules, and/or fulminant adult respiratory distress syndrome; verrucous, nodular, or ulcerative skin lesions; and prostatitis
Brucellosis	Associated with contact with or consumption of products from infected goats, pigs, camels, yaks, buffalo, or cows and with abattoir work	Arthralgias, hepatosplenomegaly, suppurative musculoskeletal lesions, sacroiliitis, spondylitis, uveitis, hepatitis, and pancytopenia
Coccidioidomycosis	Exposure to soil or dust in the southwestern United States	Arthralgias, pneumonia, pulmonary cavities, pulmonary nodules, erythema multiforme, and erythema nodosum
Ehrlichiosis	Transmitted by the bite of an *Amblyomma, Dermacentor,* or *Ixodes* tick in association with outdoor activity in midwestern and southeastern United States	Pneumonitis, hepatitis, thrombocytopenia, and lymphopenia
Enteric fever (*Salmonella enterica* serovar Typhi)	Recent travel to a Third World country with consumption of potentially contaminated food or water	Headache, arthritis, abdominal pain, relative bradycardia, hepatosplenomegaly, and leukopenia
Histoplasmosis	Exposure to bat or black bird excreta in roosts, chicken houses, or caves in the region surrounding the Ohio and Mississippi River valleys of the United States or regions of Central and South America, Africa, Asia, and Australia	Headache, pneumonia, pulmonary cavities, mucosal ulcers, adenopathy, erythema nodosum, erythema multiforme, hepatitis, anemia, leukopenia, and thrombocytopenia
Leptospirosis	Occupational exposure among workers in sewers, rice and sugar cane fields, and abattoirs; recreational water sports and exposure to contaminated waters or infected dogs	Bitemporal and frontal headache, calf and lumbar muscle tenderness, conjunctival suffusion, hepatic and renal failure, and hemorrhagic pneumonitis
Leishmaniasis (visceral disease)	Associated with recent travel to areas endemic for sand flies	Hepatosplenomegaly, lymphadenopathy, and hyperpigmentation of the face, hand, foot, and/or abdominal skin (kala azar)
Malaria	Recent travel to endemic areas in Asia, Africa, and Central and South America	Fever, headaches, nausea, emesis, diarrhea, hepatomegaly, splenomegaly, and anemia
Psittacosis *(Chlamydia psittaci)*	Associated with contact with birds, especially psittacine birds	Fever, pharyngitis, hepatosplenomegaly, pneumonia, blanching maculopapular eruptions, erythema multiforme, erythema marginatum, and erythema nodosum
Q fever *(Coxiella burnetii)*	Associated with farm, veterinary, or abattoir work; consumption of unpasteurized milk; and contact with infected sheep, goats, or cattle	Atypical pneumonia, hepatitis, hepatomegaly, relative bradycardia, and/or splenomegaly
Rat-bite fever (*Streptobacillus moniliformis*)	Recent bite or scratch by a rat, mouse, or squirrel and/or ingestion of food or water contaminated by rat excrement	Headaches, myalgias, polyarthritis, and maculopapular, morbilliform, petechial, vesicular, or pustular rash over the palms, soles, and extremities
Relapsing fever *(Borrelia recurrentis)*	Associated with poverty, crowding, and poor sanitation (louse-borne); or with camping (tick-borne), particularly in the Grand Canyon	High fever with rigors, headache, delirium, arthralgias, myalgias, and hepatosplenomegaly
Rocky Mountain spotted fever	Associated with outdoor activity in the South Atlantic or southeastern United States and exposure to *Dermacentor* tick bites	Headache, petechial rash involving the extremities, hand palms, and feet soles
Tuberculosis	Recent contact with tuberculosis, recent immigration from an endemic country, and work or residence in homeless shelters, correctional facilities, or health care facilities	Night sweats, weight loss, atypical pneumonia, cavitary pulmonary lesions
Tularemia	Associated with bites by *Amblyomma* or *Dermacentor* ticks, deer flies, and mosquitoes or direct contact with the tissues of infected animals such as rabbits, squirrels, deer, raccoons, cattle, sheep, and swine	Ulcerated skin lesions at a bite site, pneumonia, relative bradycardia, lymphadenopathy, and conjunctivitis
Whipple disease *(Tropheryma whippelii)*	Potential association with exposure to sewage	Chronic diarrhea, arthralgia, weight loss, malabsorption, and malnutrition

diffusion-weighted MRI offered a reliable radiation-free option for diagnosing suspected pulmonary infections.[75]

Scanning with labeled autologous leukocytes has been used for evaluating cases in which infections and malignant neoplasms are the cause of FUO. Although this scan has generally fallen out of favor as contributing very little to diagnosis, there are reports that labeled leukocyte scans can provide a higher diagnostic yield than that obtained by either CT or ultrasound scanning.[76,77] Another technique for evaluating undiagnosed fever, especially FUO, is gallium-67 (^{67}Ga) scanning. Its ability to image inflammation was first described by Lavender and

colleagues in 1971.[78] Shortly thereafter, Hilson and Maisey[79] reported positive results by ^{67}Ga scanning in 50 of 67 patients with FUO, of whom 32 had abscesses. The ^{67}Ga scan was considered at one time to be particularly effective in visualizing chronic infections and lymphomas,[80] but this older test for FUO has been largely displaced by CT and MRI. Disadvantages of this image testing method include a 24- to 72-hour delay period between injection and imaging, relatively high radiation dose to patients, and suboptimal image quality.

Positron emission tomography using the positron-emitting glucose analogue FDG has generally not been helpful in the workup of patients with FUO, but there are reports to the contrary.[81,82,83] In a prospective comparison of the two diagnostic modalities, Meller and colleagues[81] reported a sensitivity of 81% and a specificity of 86% for FDG-PET in detecting the focus of the fever, as compared with 67% and 78%, respectively, for ^{67}Ga scintigraphy. In a meta-analysis reported by Dong and colleagues,[83] FDG-PET/CT exhibited an overall pooled sensitivity of 98.2% and specificity of 85.9% for establishing a diagnosis in patients with FUO. Of the major diagnostic categories, the reported sensitivities were 86.7% for malignant neoplasms, 81.5% for infectious diseases, and 76.3% for noninfectious inflammatory conditions. The authors concluded that FDG-PET/CT should be considered when conventional diagnostic methods have been unsuccessful in the diagnosis of an etiology for FUO.

FDG uptake is related to increased cellular glucose metabolism present in numerous hypermetabolic conditions, including tumors, focal areas of infection, and noninfectious inflammatory conditions. FDG-PET and PET/CT have assumed an increasingly important role in the diagnostic workup of patients with FUO. ^{18}F-fluorodeoxyglucose is especially useful for localizing lesions and areas of interest for further evaluation. In contrast to gallium and labeled leukocyte imaging, recent data indicate that FDG contributes more diagnostically useful information than anatomic imaging such as ultrasound and CT, which leads to earlier institution of appropriate therapy.[82,83] These findings suggest that FDG imaging should be performed earlier, rather than later, in the diagnostic evaluation of the patient with FUO. Diagnostic considerations for which this technology may be most helpful included localized abscesses, osteomyelitis, sinusitis, sarcoidosis, vasculitis, adult-onset Still disease, Crohn disease, and subacute thyroiditis.

Invasive Diagnostic Procedures

Histopathologic examination of tissues obtained by excisional biopsy, needle biopsy, or laparotomy can provide a definitive diagnosis in some cases; however, in most published series of FUO patients, biopsy gave the final answer in fewer than half of cases.[73] The majority of patients with FUO undergo at least one such procedure, even though the diagnostic yield is only fair, with an average of two or three, sometimes even more, separate biopsies required to establish a final diagnosis.[14,84] The diagnostic yield of operative and CT-guided biopsies is higher than that of old-style bedside biopsy procedures.[14] For this reason, bedside biopsies should rarely be performed today unless guided by localizing information gained from imaging studies. An important exception to the injunction against blind biopsies concerns the temporal artery, which may merit biopsy in an elderly FUO patient with an erythrocyte sedimentation rate greater than 50 to 100 mm/h, even in the absence of localizing signs.[10]

Patients in whom FUO remains undiagnosed after extensive noninvasive evaluation generally undergo more invasive procedures in an effort to establish an etiology. In a recent retrospective study of 100 patients continuously observed for classic FUO, Mete and associates[85] successfully identified specific etiologies in 61% of patients based on clinical features and noninvasive tests. While invasive procedures were performed in 79% of patients, a diagnostic benefit was obtained in only 49% of the cases. Biopsy procedures were the most common invasive procedure performed, yielding a diagnosis in 42% of cases. The contribution of laparoscopy and laparotomy to the diagnosis of FUO may be most helpful in patients with solid cancers, lymphomas, and disseminated tuberculosis.[85,86]

Molecular Genetic Testing

Familial Mediterranean fever (FMF) is a hereditary inflammatory disease transmitted in an autosomal-recessive pattern characterized by short, recurrent attacks of fever with abdominal, chest, or joint pain and erysipelas-like erythema.[54,63,64,87] Fever is the main clinical manifestation, lasting up to 96 hours, followed by a relatively long interval between attacks. The disease occurs predominantly among Sephardic Jews, Armenians, Turks, and Arabs. Diagnosis is relatively easy in patients with typical clinical manifestations, family history, and appropriate ethnic origin. In 1997, two consortia independently cloned a gene (*MEFV*) on chromosome 16 that is responsible for FMF.[54,63,64,87] This has led to important diagnostic tools to identify polymorphisms or mutations responsible for the disease, which may help in making a definitive diagnosis of FMF in questionable or atypical cases.[64,88,89]

THERAPY

Therapeutic Trials

In the past, empirical therapy with antiinflammatory agents, such as corticosteroids, aspirin, or antimicrobial agents, was often given with the intent of providing an indirect diagnostic test in patients with unexplained FUO.[1] In rare cases, even antineoplastic drugs were used for this purpose. Today such trials are seldom indicated. However, in carefully selected cases, therapeutic trials employing agents with limited spectrums of activity (e.g., antimycobacterial drugs) continue to be an acceptable means of strengthening a presumptive diagnosis when all other means have failed.

The limitations and risks of empirical therapeutic trials are obvious. Underlying diseases may remit spontaneously during the course of ineffective therapy, giving the false impression of success. Furthermore, empirical treatment is rarely specific. Rifampin, for example, is likely to be included in empirical therapeutic regimens for tuberculosis but is highly active against numerous bacterial species other than *Mycobacterium tuberculosis*. Conversely, fluoroquinolones given for other reasons may have a beneficial effect on tuberculosis or Q fever. Similarly, fevers caused by malignant neoplasms have been reported to respond better to nonsteroidal antiinflammatory agents such as naproxen than fevers of infectious origin,[90,91] but the action of naproxen is nonspecific; the ability of the so-called naproxen test to differentiate malignant from nonmalignant causes of FUO remains unvalidated. For these reasons, therapeutic trials, even when successful in reducing fever, may delay the correct diagnosis and thus the appropriate treatment of FUO. Therefore empirical therapeutic trials should be reserved for those very few patients in whom all other approaches have failed or those so seriously ill that therapy cannot be withheld for a further period of observation, or both. In practice, this occurs most often in the case of suspected tuberculosis.

Management

Traditional classic FUO cases are much less common than the complex and multifactorial FUO cases encountered among ICU patients. The differential diagnosis for prolonged febrile episodes has expanded well beyond infectious causes to what has recently been termed *fever of too many origins*.[92] Because there are few formal guidelines, decisions regarding FUO management have certainly become more complex as infectious disease physicians encounter increasingly vague or inconclusive diagnostic test results.

A fundamental principle in the management of classic FUO is that therapy should be withheld, whenever possible, until the cause of the fever has been determined, so that treatment can be tailored to a specific diagnosis.[1] This approach is based on the oft-repeated observation that nonspecific treatment rarely cures FUO and has the potential to delay reaching a specific diagnosis. This ideal is, however, frequently ignored in clinical practice because the road to diagnosis of FUO is, by definition, often long and frustrating. As a result, clinicians may feel compelled to treat symptoms empirically, even though the agents used may obscure the very signs and symptoms on which the diagnosis depends. An important exception is that empirical treatment with corticosteroids may be appropriate in patients with suspected temporal arteritis to prevent vascular complications such as blindness or stroke. Primary care physicians, who have learned from experience that the most cost-effective approach to many acute febrile illnesses is to try empirical antimicrobial therapy before undertaking expensive diagnostic exercises,

should appreciate that this approach is less likely to succeed in patients with FUO.

In febrile neutropenic patients, the principles of treatment are entirely different. Because of the relatively high prevalence of serious bacterial infections responsible for these fevers, febrile neutropenic patients should generally receive broad-spectrum antipseudomonal antimicrobial therapy immediately after samples for appropriate cultures have been obtained (see Chapter 305).[44]

PROGNOSIS

The prognosis of FUO is determined by the cause of the fever and by the nature of any underlying disease or diseases. The time required to establish the diagnosis is less important. Elderly patients and those with malignant neoplasms have the poorest prognosis.[1] Diagnostic delay affects the prognosis adversely in intraabdominal infections, miliary tuberculosis, disseminated fungal infections, and recurrent pulmonary emboli.[73]

Patients in whom FUO remains undiagnosed after extensive evaluation generally have a favorable outcome, characteristically with resolution of their fever in 4 or more weeks without sequelae.[3,84,87] In a study of 61 patients observed long term for undiagnosed FUO, Knockaert and associates[87] were largely unsuccessful in identifying specific etiologies. Most cases eventually resolved spontaneously, generally obviating the need for corticosteroid therapy. Some patients, however, required nonsteroidal antiinflammatory drugs for symptomatic relief. In this series, the 5-year mortality rate for undiagnosed FUO was only 3.2%. In a series of 91 patients undergoing evaluation for FUO, Mansueto and colleagues[84] reported 29 patients (31.8%) who were discharged without a diagnosis and followed over a period of 48 months. Although a definitive diagnosis was established in eight cases, four of such patients died as a result of noninfectious complications related to neoplastic conditions.

ACKNOWLEDGEMENT

With grateful heart, I remember the person who gave vision and clarity to this chapter for so many editions, and I express my special thanks to Dr. Philip A. Mackowiak, Professor of Medicine (Emeritus) at the University of Maryland. He gave me his friendship, guidance, and mentorship. Dr. Mackowiak cared about each step on the path to knowledge of fever and clinical thermometry, and encouraged me to continue the journey in this academic pursuit.

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57 The Acutely Ill Patient With Fever and Rash

Jonathan J. Juliano, Myron S. Cohen, and David J. Weber

SHORT VIEW SUMMARY

Definition

- Skin lesions are frequently present in acutely ill patients with serious infectious diseases and may provide important clues that aid in early diagnosis and treatment.

Epidemiology

- Acutely ill patients with a potential infectious disease and skin lesions (or rash) should have a history obtained that elicits the following: recent drug ingestion; travel outside the local area; potential occupational exposures; recent immunizations; risk factors for sexually transmitted infections, including human immunodeficiency virus infection; factors affecting host resistance or immunocompromising conditions; prior allergies to antibiotics; recent exposures to febrile or ill persons; exposure to rural habitats, insects, arthropods, and wild animals; and exposure to pets or animals.
- Patients with skin lesions or rashes consistent with a communicable infectious disease (e.g., invasive meningococcal infection) should be immediately placed on appropriate isolation precautions (i.e., contact, droplet, airborne, or special precautions for highly communicable diseases, such as Lassa and Ebola virus infections).
- Infectious disease physicians should be familiar with the skin lesions (or rash) that might accompany a patient with disease that could be the result of the intentional use of a bioweapon (e.g., anthrax, smallpox, plague, viral hemorrhagic fevers).

Microbiology

- Serious bacterial infections with skin lesions include *Staphylococcus aureus* (toxic shock syndrome [TSS], scalded skin syndrome), *Streptococcus pyogenes* (TSS), *Salmonella enterica* serovar Typhi, *Neisseria meningitidis,* and *Rickettsia rickettsii.*
- Potentially serious viral infections with skin lesions include measles, rubella, Epstein-Barr virus infection, cytomegalovirus, human herpesvirus 6, and viral hemorrhagic fevers (e.g., dengue hemorrhagic fever, Ebola, Marburg, Lassa).
- Life-threatening drug reactions may result from antibiotic therapy for disorders such as Stevens-Johnson syndrome/toxic epidermolysis necrosis and from drug reaction with eosinophilia and systemic symptoms (DRESS).

Diagnosis

- Key aspects of skin lesions that aid in a proper diagnosis include (1) primary type(s) of skin lesions, (2) distribution of the lesions, (3) pattern of progression of the rash, and (4) timing of onset of the rash relative to the onset of fever and other systemic signs.
- The appearance of skin lesions may be very useful in the diagnosis of specific infectious diseases. Maculopapular rashes are usually seen in viral illnesses, drug eruptions, and immune complex–mediated diseases. Nodular lesions are suggestive of mycobacterial or fungal infections. Diffuse erythema suggests scarlet fever, TSS, Kawasaki disease, or Stevens-Johnson syndrome/toxic epidermal necrolysis. Bullous lesions suggest streptococcal erysipelas with necrotizing fasciitis, ecthyma gangrenosum, and *Vibrio* infections. Petechial eruptions suggest gram-negative sepsis, invasive *N. meningitidis* infection, rickettsial infections, and viral hemorrhagic fever.
- Consideration should be given to biopsy of skin lesions, if present, in acutely ill immunocompromised patients for appropriate stains (e.g., Gram stain, fungal stain) and cultures and for pathologic study.

Therapy

- Empirical therapy should often be initiated in acutely ill patients with skin lesions based on the clinical diagnosis.
- Most acutely ill patients with skin lesions will require systemic therapy.

Prevention

- Standard vaccines should be provided to children and adults because many vaccine-preventable diseases produce rashes (e.g., measles, rubella, varicella).
- Underlying noninfectious diseases that lead to disruption of skin should be treated because the damaged skin serves as a risk factor for infection.

A recognizable rash can lead to immediate diagnosis and appropriate therapy. Material isolated from involved skin, when properly handled, can confirm a specific diagnosis. Unfortunately, rashes often present a bewildering array of diagnostic possibilities. Dermatologists, who are generally more comfortable with evaluation of the skin, are not always available for immediate consultation. Furthermore, dermatologists and infectious disease specialists frequently differ in their approach to the patient with a rash.

A framework is provided in this chapter for investigation of the cause of rash, with emphasis on the following: (1) a diagnostic approach to patients with fever and rash, (2) categories of skin lesions, and (3) brief descriptions of the most important febrile illnesses characterized by a rash.

APPROACH TO THE PATIENT

In the initial evaluation of a patient with fever and rash, four concerns must be addressed immediately. The first is if the patient is well enough to provide further history or whether cardiorespiratory support is required. The second is whether the nature and presentation of the rash demands immediate institution of isolation precautions. Isolation is required primarily for patients whose illnesses allow droplet or airborne spread of the pathogen, including both viral (e.g., possible viral hemorrhagic fever) and bacterial diseases (e.g., possible invasive meningococcal infection). Isolation precautions should be adhered to scrupulously. Health care personnel should exercise caution in all interactions with patients with undiagnosed infectious diseases, and they should use standard precautions, including the avoidance of direct contact with all excretions and secretions with the exception of sweat.[1–4] Although the vast majority of skin eruptions are noninfectious, gloves should always be worn during the examination of the skin whenever an infectious cause is being considered because some infections (e.g., syphilis, herpes simplex virus [HSV]) may be acquired via direct skin contact. In the event of potential exposure to a pathogen, health care personnel should be evaluated by their occupational health service for postexposure prophylaxis or the need for work restrictions or both.[4–7] The third concern is if skin lesions suggest a life-threatening infection, such as bacterial sepsis, staphylococcal or streptococcal toxic shock, Kawasaki disease, necrotizing fasciitis, toxic epidermal necrolysis, or Rocky Mountain

spotted fever (RMSF).[8–11] Early diagnosis is important because prompt initiation of appropriate therapy may improve survival.[12,13] If skin lesions are consistent with meningococcal disease (see later discussion), the immediate institution of antibacterial therapy is crucial.[14,15–17] Finally, consideration must be given to the possibility that the patient has an exotic disease acquired as a result of travel or the intentional release of an agent of bioterrorism.[18] Bioterrorist agents that may be acquired via person-to-person transmission and characteristically cause a generalized rash include smallpox[1,19] and the viral hemorrhagic fevers (i.e., Ebola, Lassa, Marburg, Crimean-Congo, Bolivian, and Argentinean).[1,20] Patients with plague[1,21] and anthrax[1,22] may present with localized skin lesions.

The history obtained from the patient should elicit the following information:

1. Drug ingestion within the past 60 days
2. Travel outside the local area
3. Occupational exposure
4. Sun exposure
5. Immunizations
6. Sexually transmitted disease exposure, including risk factors for infection with human immunodeficiency virus (HIV)
7. Factors affecting immunologic status, including chemotherapy, corticosteroid use, use of immune modulators, hematologic malignancy, solid-organ or stem cell transplantation, and functional or anatomic asplenia
8. Valvular heart disease, including heart valve replacement
9. Prior illnesses, including a history of drug or antibiotic allergies
10. Exposure to febrile or ill persons within the recent past
11. Exposure to wild or rural habitats, insects, arthropods, and wild animals
12. Exposure to outdoor water sources such as lakes, streams, or oceans
13. Pets, animal exposures, and habits

The clinician should pay particular attention to the season of the year, which dramatically affects the epidemiology of febrile rashes of infectious origin.

Physical examination should focus on the following:

1. Vital signs
2. General appearance
3. Signs of toxicity
4. Presence and location of adenopathy
5. Presence and morphology of genital, mucosal, or conjunctival lesions
6. Detection of hepatosplenomegaly
7. Presence of arthritis
8. Signs of nuchal rigidity, meningismus, or neurologic dysfunction

Key ingredients in arriving at a correct diagnosis, or at least a useful, limited, "working" list of likely diagnoses, include determination of (1) the primary type(s) of skin lesions present, (2) the location and distribution of the eruption, (3) the number and size of the lesions, (4) the pattern of progression of the rash, and (5) the timing of the onset of the rash relative to the onset of fever and other signs of systemic illness.[23–31] It is important for physicians who observe a rash to carefully document the characteristics or take images of the skin lesions in the medical record to aid other providers in the later care of the patient. Although histologic findings from lesional skin biopsies may help to confirm some diagnoses,[29] the patterns observed are frequently not specific for a single organism, the presence of infectious agents may not always be detectable, and laboratory studies often require at least 24 hours to complete. Thus the clinician must attempt to use other diagnostic skills during the early evaluation of a patient with fever and rash. As discussed elsewhere, specific types of primary skin lesions frequently suggest different infectious disorders in patients with fever and rash. For example, palpable purpura, the hallmark feature of leukocytoclastic vasculitis, is the prototypic early finding in meningococcemia and RMSF, whereas rapidly enlarging but asymptomatic red dermal nodules instead suggest candidemia in the appropriate host. Skin nodules noted on very deep palpation are probably located within the subcutaneous fat, suggesting one of several types of panniculitis, including erythema nodosum, a disorder caused by many different types of inflammatory or infectious processes, and erythema induratum, which is a classic tuberculoid reaction.

Examples of differences in the types of primary skin lesions present in the setting of underlying systemic infectious diseases are summarized in Table 57.1, although it should be clear that such a classification, by itself, rarely ever suggests only a single diagnosis. On the other hand, the presence of other more specific lesions, most notably "target" or "iris" lesions (as in erythema multiforme [EM]), may suggest a

TABLE 57.1 Systemic Infections With Prominent Cutaneous Manifestations

ORGANISM/DISEASE	MACULES, PAPULES	VESICLES, BULLAE	PETECHIAE, PURPURA
Viruses			
Human immunodeficiency virus type 1	X		
Echoviruses	X	X	X
Coxsackieviruses	X	X	X
Rubeola (measles)	X		
Atypical measles	X		X
Adenovirus	X		X
Lymphocytic choriomeningitis	X		
Dengue	X		X
Zika virus	X		
West Nile virus	X		
Viral hemorrhagic fevers			X
Rubella (German measles)	X		X
Colorado tick fever	X		
Yellow fever			X
Varicella-zoster (disseminated)		X	
Herpes simplex (disseminated)		X	
Varicella (chickenpox)		X	
Vaccinia		X	

TABLE 57.1 Systemic Infections With Prominent Cutaneous Manifestations—cont'd

ORGANISM/DISEASE	MACULES, PAPULES	VESICLES, BULLAE	PETECHIAE, PURPURA
Variola		X	X
Cytomegalovirus	X		
Congenital cytomegalovirus			X
Epstein-Barr virus	X		X
Hepatitis B virus	X		X (as palpable purpura)
Monkeypox	X		
Parvovirus B19 (erythema infectiosum)	X		
Human herpesvirus 6	X		
Human herpesvirus 7	X		
Bacteria			
Chlamydia psittaci	X	X	
Mycoplasma pneumoniae	X		
Ehrlichia spp.	X		
Rickettsia rickettsii (RMSF)	X		X
Rickettsia akari (rickettsialpox)	X	X	
Rickettsia prowazekii (epidemic/louse-borne typhus)	X		X
Rickettsia typhi (endemic/murine typhus)	X		
Rickettsia tsutsugamushi (scrub typhus)	X		
Bartonella henselae	X		
Bartonella quintana	X		
Salmonella enterica serovar Typhi	X		
Francisella tularensis	X		
Streptobacillus moniliformis (rat-bite fever)	X		X
Treponema pallidum (secondary syphilis)	X		
Mycobacterium haemophilum	X		
Neisseria gonorrhoeae	X		X
Neisseria meningitidis			X
Leptospira spp.	X		
Listeria monocytogenes		X (rare)	
Bartonella bacilliformis	X		
Borrelia spp. (relapsing fever)	X		X
Borrelia burgdorferi (Lyme disease)	X (annular)		
Pseudomonas aeruginosa	X		
Spirillum minus (rat-bite fever)	X		X
Staphylococcus aureus	X		X
Streptococci—group A (scarlet fever)	X		
Capnocytophaga canimorsus			X
Vibrio vulnificus		X	
Fungi (Disseminated Infection)			
Candida spp.	X		
Cryptococcus neoformans	X		
Histoplasma capsulatum	X		
Blastomyces dermatitidis	X		
Coccidioides immitis	X		
Fusarium spp. (agents of mucormycosis)	X		
Protozoa			
Plasmodium falciparum (malaria)			X

single diagnosis, implicating a limited group of underlying infectious diseases as possible causes. Similarly, the presence of some lesions in the setting of fever may immediately exclude an infectious disorder as the cause of rash. For example, high fever accompanying a paucity of tender, red to violaceous, peripherally mammillated plaques suggests Sweet syndrome (acute febrile neutrophilic dermatosis), a rare hypersensitivity reaction frequently associated with selected underlying malignancies,[32] or neutrophilic eccrine hidradenitis, a rare neutrophilic dermatosis most commonly found in patients treated with chemotherapy for malignancies.[33]

Distribution or direction of spread of an eruption may be highly informative. The rash of RMSF and acute meningococcal infection, for example, most often begins on the lower extremities and then spreads centrally (i.e., centripetally), whereas most drug- and viral-infection–associated eruptions (with the exception of those caused by echoviruses and coxsackieviruses) begin on the face or trunk and spread outward (centrifugally). "Streaky" facial involvement, usually without other skin findings, is characteristic of infection due to parvovirus B19 (fifth disease, erythema infectiosum).

The number of lesions can also provide useful insight. For example, "rose spots" (see later discussion), the hallmark cutaneous feature of *Salmonella* infection, are characteristically present in much greater numbers in patients who have paratyphoid fever than in those who have typhoid fever. In contrast, brucellosis may be associated with only one or a few clinically subtle skin lesions, as seen in a fixed-drug eruption.

Finally, timing of the rash may be particularly helpful in allowing the clinician to exclude reactions due to certain drugs as the underlying cause. With the exception of urticarial eruptions, which usually occur within a few minutes to a few hours of the administration of a systemic agent, the more typical generalized maculopapular or morbilliform drug eruption typically occurs within the first 7 to 14 days of the first dose of the offending agent, suggesting the need for a very careful drug history (including start and stop dates for all medications taken within 30 days of the onset of eruption).

It must be emphasized that noninfectious processes often include rash and fever and should be among the diagnostic considerations in the initial evaluation.[34] As noted previously, the presence of some highly specific morphologic types or patterns of skin lesions may quickly suggest a noninfectious cause to the astute clinician, thereby obviating the need to pursue a more extensive clinical and laboratory evaluation.

Between 5% and 15% of all patients to whom a drug is administered experience an adverse reaction.[35–37] Adverse cutaneous reactions to drugs are frequent, affecting 2% to 3% of all hospitalized patients,[38–44] 20% of emergency department visits,[44] and 0% to 8% of all patients placed on medications.[41] Fortunately, only about 2% of adverse cutaneous reactions are severe and very few fatal.[36] The rate of cutaneous reactions to drugs is highest for antibiotics (1%–8% depending on the class of antibiotic), mainly penicillins and cephalosporins.[42,44] Exanthems (75%–95%) and urticaria (5%–6%) account for most drug reactions. Because of their frequency, a drug reaction must be considered in any patient with a generalized maculopapular rash, especially if associated with palmoplantar involvement. Severe cutaneous reactions often induced by drugs include Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN),[11,38,45] hypersensitivity syndromes (urticaria, angioedema, anaphylaxis),[38,46] small vessel vasculitis,[38,39] serum sickness,[11,38] and drug reaction with eosinophilia and systemic symptoms (DRESS).[11,39,47,48] As with other rashes, a morphologic approach to drug eruptions should be used in evaluating the patient.[35,40,43]

Rashes and skin infections associated with occupational exposures,[49–53] athletics,[54–61] animal exposures,[62] international travel,[63,64–69] and working in a clinical or research laboratory[70] have been reviewed in other chapters in this textbook.

PATHOGENESIS OF RASH

Rash with fever can result from a local infectious process due to virtually any class of microbe that has been allowed to penetrate the stratum corneum and multiply locally. A typical example is streptococcal cellulitis. In rare cases such localized inoculations result in more generalized eruptions, and the diagnosis is then relatively straightforward. However, eruptions that begin as generalized exanthems are the "rashes" that constitute the focus of the discussion in this chapter.

An *exanthem* is a cutaneous eruption due to the systemic effects of a microorganism infecting the skin. An *enanthem* is an eruption caused in similar fashion but involving the mucous membranes. Microorganisms may produce eruptions through (1) multiplication in the skin (e.g., HSV); (2) release of toxins that act on skin structures (e.g., in scarlet fever, infections due to *Pseudomonas aeruginosa,* toxic shock syndrome [TSS], staphylococcal scalded skin syndrome [SSSS]); (3) inflammatory response involving phagocytes and lymphocytes, in which the microbicidal/tumoricidal metabolism of host defense cells is directed at the skin; and (4) effects on vasculature, including vasoocclusion and necrosis or vasodilation with edema and hyperemia. Obviously, for many eruptions, several concurrent mechanisms can play a role.

DIFFERENTIAL DIAGNOSIS IN RASH

There are two ways to approach the investigation of infectious rash: either by the type of lesion visualized or by knowledge of individual pathogens and the rashes they produce (Table 57.2). Unfortunately, neither system alone serves both to generate a complete list of diagnostic possibilities to rule out disorders as appropriate. In accordance, both approaches should be incorporated into evaluation of the patient with rash and fever.

Characteristics of the Lesion

Morphologic types of primary skin lesions include macules, papules, nodules, vesicles, bullae, pustules, and plaques. *Macules* are flat, nonpalpable lesions in the plane of the skin. *Papules* are small, solid, palpable lesions elevated above the plane of the skin. Masses that are located deeper within or below the skin are referred to as *nodules. Vesicles* and *bullae* are small and large blisters, respectively, and *pustules* are usually small, palpable lesions filled with pus. *Plaques* are large, flat lesions, usually greater than 1 cm in diameter, that are palpable. In addition to morphology, lesions are characterized by their color and, particularly in the setting of a systemically ill–appearing patient, by the presence or absence of hemorrhage, with hemorrhagic lesions being termed *purpura* or *petechiae.* Lesions may be skin colored, hyperpigmented, or hypopigmented or any of several other colors, of which red is the most common; the presence of such reddening is termed *erythema.* Blanching erythematous lesions are those in which erythema is due to vasodilation, whereas nonblanching erythema may be due to extravasation of blood. For purposes of the following discussion, it is useful to divide eruptions into those that are maculopapular (characterized by both flat and elevated lesions), nodular, vesiculobullous, erythematous, and purpuric. Enanthums and neutrophilic dermatoses are also important in the differential diagnosis of fever and rash.

Maculopapular Rash

Maculopapular rashes (see Table 57.1) (Fig. 57.1) are usually seen in a range of illnesses, including those due to viruses, bacteria, fungi, drug eruptions, and immune complex–mediated syndromes. Descriptions of some of the specific pathogens are included later in the chapter or are included in other chapters. Physicians, in particular, should be familiar with maculopapular rashes associated with EM and SJS/TEN.

EM and its variants may be considered a special category of maculopapular rash. EM is an uncommon acute, immune-mediated, self-limited, usually mild mucocutaneous syndrome that is often relapsing.[71,72,73] The disease is usually related to an acute infection, most commonly a recurrent HSV infection. It is uncommonly related to drug ingestion (i.e., <10%).

The following classification of EM is based on that provided by Bastuji-Garin and colleagues.[73,74] Subtypes of EM include (1) EM major: skin lesions with involvement of mucous membranes, (2) EM minor: skin lesions without involvement of mucous membranes, (3) herpes-associated EM, and (4) mucosal EM (Fuchs syndrome): mucous membrane lesions without cutaneous involvement. Until recently, EM was considered as a variant of the same pathophysiologic process as SJS and TEN. However, currently evidence suggests that EM with mucous membrane involvement and SJS are different diseases with distinct causes.[75] Further, the epidemiology of EM differs from SJS/TEN because

TABLE 57.2 Skin Lesions and Systemic Infections

LESION	COMMON PATHOGENS	HISTOLOGIC FINDINGS	SMEARS POSITIVE FOR PATHOGENS	TIME TO APPEARANCE (AFTER ONSET OF ILLNESS)
Symmetrical peripheral gangrene, purpura fulminans, acrocyanosis	Noninfectious or gram-negative bacteria, *Capnocytophaga canimorsus*	Bleeding in the skin, vascular thrombosis, perivascular infiltration	No	12–36 h
Multiple purpuric lesions in severely ill patients	*Neisseria meningitidis, Capnocytophaga canimorsus, Rickettsia* spp., other gram-negative bacteria	Vascular thrombosis, perivascular hemorrhage	Yes[a]	12–36 h[b]
Ecthyma gangrenosum, other bullous lesions	*Pseudomonas*, gram-negative bacteria, *Vibrio vulnificus*	Veins, mainly involved, intima spared, inflammatory reaction	Yes	Several days
Macronodular lesions	*Candida, Cryptococcus neoformans, Histoplasma capsulatum, Fusarium*	Hyphae, mononuclear perivascular reaction	No	Several days
Delayed-onset rash with nonsymmetrical, scattered maculopapular or vesicular lesions	*Neisseria gonorrhoeae, N. meningitidis* (chronic)	Perivascular mononuclear infiltrate, immune complex	Occasionally (few bacteria only)	3–10 days
Rose spots	*Salmonella* spp.	Perivascular mononuclear infiltrate, or leukocytoclastic vasculitis	No	5–10 days
"Toxic erythema"	*Staphylococcus aureus* (SSSS)	Dilation and perivascular edema	No	At presentation

[a]Except for Rocky Mountain spotted fever, in which therapy, biopsy, and immunofluorescent staining may aid early diagnosis.
[b]In Rocky Mountain spotted fever, 1–7 days.
SSSS, Staphylococcal scalded skin syndrome.
Modified from Kingston ME, Mackey D. Skin clues in the diagnosis of life-threatening infections. Rev Infect Dis. *1986;8:1–11.*



FIG. 57.1 Maculopapular rash. The maculopapular rash of measles showing coalescence of lesions *(From Ferri FF.* Ferri's Clinical Advisor. *1st ed. St. Louis: Elsevier; 2019.)*

patients with EM are younger, are more often male, have frequent recurrences, have less fever, have milder mucosal lesions, and lack association with collagen vascular diseases, HIV infection, or cancer.[76]

Target lesions are the hallmark of EM. The skin eruption generally arises abruptly; most commonly all lesions appear within 3 to 5 days and resolve in approximately 2 weeks. Initially, lesions may begin as round erythematous papules that evolve into classic target lesions. Typical target lesions consist of three components: a dusky central area or blister, a dark red inflammatory zone surrounded by a pale ring of edema, and an erythematous halo on the extreme edge of the lesion. Although there are often a limited number of lesions, in some cases hundreds may form. Most lesions occur in a symmetrical, acral distribution on the extensor surfaces of the extremities (hands and feet, elbows, and knees), face and neck, and, less commonly, thighs, buttocks, and trunk. They often appear acrally and then spread in a centripetal manner. Although the lesions are usually asymptomatic, patients occasionally report burning or itching. Mucous membrane (≈70% oral, ≈25% genital, ≈15% ocular) involvement usually accompanies the cutaneous lesions, although patients may present only with mucous membrane involvement.[77]

Although many factors (e.g., infections, medications, malignancy, autoimmune disease, immunizations, sarcoidosis, and radiation) have been linked to EM, infections account for approximately 90% of cases. HSV has been the infection most commonly linked to EM (≈80% of infectious cases),[78,79] but *Mycoplasma pneumoniae* is an important cause of EM (≈5%–10% of infectious cases), especially in children.[80] The differential diagnosis of target-appearing skin lesions has been reviewed and includes EM, SJS, TEN, ecthyma gangrenosum, syphilis, fixed-drug eruption, contact dermatitis, vasculitis, acute connective tissue diseases, and autoimmune blistering diseases.[81]

SJS and TEN are acute, life-threatening mucocutaneous reactions characterized by extensive necrosis and detachment of the epidermis.[82–87] Although TEN is rare, with an incidence of approximately two cases per million population per year, it is a devastating disease with a mortality rate of 30% to 50%. Owing to the similarity in clinical and histologic findings, risk factors, drug causality, and mechanisms, these two syndromes are considered severity variants of an identical process that differs only in the final extent of body surface involved.[87] TEN involves sloughing of greater than 30% of the body surface, whereas SJS involves less than 10% of the total body surface; total body surface area involvement between 10% and 30% is known as SJS-TEN overlap. SJS and TEN typically have a prodrome of fever and flulike symptoms beginning 1 to 3 days before the development of skin lesions. These may be accompanied by skin tenderness and photophobia. Both SJS and TEN are characterized by rapidly expanding, often irregular macules ("atypical target lesions") and involvement of more than one mucosal site (oral, conjunctival, and anogenital). If untreated, TEN rapidly progresses to widespread full-thickness necrosis of the epidermis, resulting in separation of large sheets of epidermis from the underlying dermis either spontaneously or after the application of minimal lateral traction or pressure to the skin. The Nikolsky sign (i.e., ability to extend the area of superficial sloughing by gentle lateral pressure on the surface of the skin at an apparently uninvolved site) may be positive. Constitutional symptoms and internal organ involvement occur often and may be severe.

A majority of cases in adults are drug induced; medications cause 30% to 50% of cases of SJS and up to 80% of cases of TEN. Although

infections may be an occasional trigger of SJS in adults, they uncommonly cause TEN. Rare precipitating causes include vaccinations, systemic diseases, chemical exposures, and foods. In contrast, in children, although medications are the leading cause of both SJS and TEN, infections such as *M. pneumoniae* and herpesviruses are more common causes of SJS and TEN. Although most of the highest-risk medications are not antiinfective agents, nevirapine is a confirmed high-risk drug.[82] Antiinfective agents most commonly associated with SJS and TEN include amoxicillin, sulfadiazine, and trimethoprim-sulfamethoxazole (TMP-SMX); less common antiinfective agents include cephalosporins, macrolides, tetracyclines, rifampin, thiabendazole, ethambutol, and fluoroquinolones (sulfonamides ≫ penicillins > cephalosporins). Patients infected with HIV have been reported to be at threefold increased risk for SJS/TEN, with a 40-fold increased risk for SJS/TEN due to TMP-SMX. Unfortunately, there is no laboratory test to support the diagnosis of SJS or TEN. The following diseases must always be excluded before making a diagnosis of SJS or TEN: varicella, EM major, SSSS, staphylococcal TSS, skin necrosis from disseminated intravascular coagulation or purpura fulminans, and chemical toxicity.[87] Treatment should always include appropriate fluid and wound care (for extensive disease the patient should be managed in a burn center), strict aseptic care to prevent infections, and consideration for topical antibiotic therapy (avoid silver sulfadiazine).[82,86,88] Adjunctive therapies that are based on extensive use but have not been studied in clinical trials include intravenous (IV) gamma globulin and glucocorticoids.[82,88–90] Plasmapheresis, cyclosporine, and tumor necrosis factor (TNF) antagonists (infliximab, etanercept) have been reported to be of benefit based on small case series.[82,86,88–90] Thalidomide increased mortality in a double-blind randomized placebo-controlled trial.[91]

Last, in several life-threatening infections, the presenting manifestations may include blanching erythematous maculopapular lesions that later evolve into petechiae, making initial diagnosis difficult on the basis of lesion morphology alone. These infections include acute meningococcemia, RMSF, and viral hemorrhagic fevers such as dengue. Although a diagnostic feature of rheumatic fever is an annular or a polycyclic, migrating (or expanding) erythema known as *erythema marginatum,* this disease may also be associated with the presence of a maculopapular eruption and subcutaneous nodules. Patients with enteric fever due to *Salmonella* may develop "rose spots," a transient scattering of rose-colored macules over the abdomen. Typically, the rose spots of typhoid fever are pale pink, oval or circular, completely blanchable, few in number, moderately sized (up to 0.5–1.0 cm in diameter), and usually present on the abdomen or trunk.[92] In contrast, rose spots of paratyphoid fever are typically smaller and more numerous.[93]

Nodular Lesions

A nodule is a palpable, solid, round or ellipsoidal lesion, usually resulting from disease in the dermis and/or subcutis (Fig. 57.2). Nodules may contain various inflammatory cells (as part of a hypersensitivity phenomenon), organisms (most notably fungi, as in septic emboli), or tumor cells (from metastatic cancer, lymphoma, or leukemia cutis). In the appropriate clinical setting, sudden development of dermal nodules may suggest candidal sepsis (see later discussion), but other fungal diseases, including blastomycosis, histoplasmosis, coccidioidomycosis, and sporotrichosis, may produce skin nodules. Bacteria such as *Nocardia* and nontuberculous mycobacteria[94–96] (especially *Mycobacterium marinum*)[97] may also cause nodular lesions that typically later ulcerate. Leishmaniasis can cause nodules. Lesions consistent with ecthyma gangrenosum, typified by the presence of deep, "punched-out" ulcerations with overlying black eschar and peripheral erythema, suggest *Pseudomonas* sepsis. A skin biopsy specimen with appropriate stains and cultures defines the diagnosis.

Subcutaneous nodules pose a real diagnostic challenge because they may reflect the presence of a variety of underlying disorders, including hypersensitivity reactions to systemic infection. The lesions of erythema nodosum are characterized by tender, erythematous nodules that range in diameter from less than a centimeter to several centimeters (Fig. 57.3).[98–100] They are usually multiple and located on the anterior portions of the legs but may be solitary and occur on other parts of the body.



FIG. 57.2 Nodular rash. Multiple erythematous skin nodules of *Mycobacterium abscessus (From Su S, Chen Y-H, Tsai T-Y, et al. Catheter-related* Mycobacterium abscessus *bacteremia manifested with skin nodules, pneumonia, and mediastinal lymphadenopathy.* Kaohsiung J Med Sci. *2013;29:50–54.)*



FIG. 57.3 Erythema nodosum. Erythematous nodules which are ill defined without epidermal changes on the lower leg. *(From Brinster NK, Liu V, Diwan H, McKee PH. Erythema nodosum. Brinster NK, Liu V, Diwan H, McKee PH, eds.* Dermatopathology: High-Yield Pathology. *Philadelphia: Saunders; 2011:170–171.)*

They typically do not suppurate. These lesions often develop in crops and usually heal in days to a few weeks without scarring. Infectious agents are a prominent cause of this lesion (Table 57.3). In contrast, erythema induratum, a known tuberculoid reaction, typically presents as painful, red, subcutaneous nodules over the posterior lower legs and

TABLE 57.3 Known Noninfectious and Infectious Causes of Erythema Nodosum

Noninfectious
Systemic lupus erythematosus
Sarcoidosis
Ulcerative colitis
Crohn disease
Malignancies (Hodgkin and non-Hodgkin lymphoma, leukemia, renal cell carcinoma)
Behçet syndrome
Drugs (especially oral contraceptives, sulfonamides)
Pregnancy
Infectious
Viral
Hepatitis B virus
Hepatitis C virus
Herpes simplex virus
Human immunodeficiency virus
Epstein-Barr virus
Measles
Parvovirus B19
Varicella
Bacterial
Bartonella henselae (cat-scratch disease)
Brucella spp. (brucellosis)
Campylobacter spp. (campylobacteriosis)
Chlamydia trachomatis (lymphogranuloma venereum)
Chlamydia psittaci (psittacosis)
Corynebacterium diphtheriae (diphtheria)
Coxiella burnetii (Q fever)
Francisella tularensis (tularemia)
Haemophilus ducreyi (chancroid)
Mycoplasma pneumoniae
Mycobacterium tuberculosis (tuberculosis)
Mycobacterium leprae (leprosy)
Mycobacterium marinum (atypical mycobacteriosis)
Neisseria gonorrhoeae (gonorrhea)
Neisseria meningitidis (meningitis)
Salmonella spp. (salmonellosis)
Shigella spp. (shigellosis)
Streptococcus pyogenes (respiratory tract infection)
Treponema pallidum (syphilis)
Yersinia spp. (gastroenteritis)
Fungal
Aspergillus spp. (aspergillosis)
Cryptococcus neoformans (cryptococcosis)
Blastomyces dermatitidis (blastomycosis)
Histoplasma capsulatum (histoplasmosis)
Coccidioides immitis (coccidioidomycosis)
Sporothrix schenckii (sporotrichosis)
Protozoal/Helminths
Ascaris lumbricoides (ascariasis)
Giardia lamblia (giardiasis)
Toxoplasma gondii (toxoplasmosis)
Wuchereria bancrofti (filariasis)

ankles. These lesions tend to suppurate, distinguishing them morphologically from erythema nodosum and most other types of panniculitis. Furthermore, erythema induratum can usually be easily differentiated from erythema nodosum on histologic examination of a wedge biopsy specimen. Inflammation can be seen within subcutaneous fat lobules in the former, rather than within septal connective tissue as classically seen in erythema nodosum. Acid-fast bacilli are rarely visible within the lesions of erythema induratum because this condition typically represents reactivation of long-standing infection with, or hypersensitivity to, the tuberculosis bacilli that are present at distant sites.

Diffuse Erythema

Diffuse erythema, especially if desquamation or peeling is present, should lead to consideration of scarlet fever, TSS, mucocutaneous lymph node syndrome (Kawasaki disease), SSSS, SJS, and TEN (Fig. 57.4). Desquamation may occur late in all of these syndromes, and its absence early in the clinical course should not be considered a reason for excluding any disease process. Most of these disorders can be easily diagnosed on the basis of the patient's history and appropriate tests.



FIG. 57.4 Diffuse erythema. Diffuse erythema associated with toxic shock syndrome. *(Courtesy Centers for Disease Control and Prevention Public Health Image Library.)*



FIG. 57.5 Vesicular rash. Vesicles associated with varicella infection. *(From Goering RV, Dockrell HM, Zuckerman M, et al. Infections of the skin, soft tissue, muscle and associated systems. In: Goering RV, Dockrell HM, Zuckerman M, et al, eds.* Mim's Medical Microbiology. *5th ed. St. Louis: Elsevier; 2013.)*

Vesiculobullous Eruptions

A vesicle is a circumscribed, elevated lesion containing free fluid (Fig. 57.5). A vesicular lesion larger than 0.5 cm is termed a *bulla*. Most vesiculobullous eruptions are immunologic in origin; few are associated with infectious systemic infections. Infectious diseases to be considered include varicella, disseminated herpes simplex, eczema herpeticum (HSV superinfection of atopic eczema), and infections due to echoviruses and coxsackieviruses (including coxsackievirus A16, a cause of hand-foot-and-mouth disease). In addition, other poxvirus infections, such as monkeypox, smallpox, and generalized vaccinia, need to be considered (see later). HSV infection, the most common of these infections causing vesiculobullous lesions, is characterized by a grouped clustering of vesicles on an erythematous base that progresses to mucocutaneous ulceration.[101–103] HSV can be detected by a viral culture of a scraping from a blister but more commonly is now accomplished by polymerase chain reaction assay. In addition, the demonstration of multinucleated giant cells in a scraping (Tzanck preparation) of the base of a vesicle indicates infection with HSV or varicella-zoster virus (VZV). Older

vesicles can be easily confused with pustules. A *pustule* is an elevation of the skin enclosing a purulent exudate. Vesicular lesions may at times become pustules, as can occur with HSV or VZV lesions. Diffuse pustular diseases usually represent a noninfectious dermatologic illness (e.g., pustular psoriasis) or a cutaneous infection (e.g., pustular *Pseudomonas* lesions developing after the use of contaminated hot tubs or staphylococcal folliculitis). Pustular skin lesions associated with arthralgias should lead to a consideration of gonococcemia, *Moraxella* bacteremia, chronic meningococcemia, subacute bacterial endocarditis, coxsackievirus infection, and Behçet syndrome.

Bullous skin lesions with sepsis are suggestive of the following infections: group A streptococcal erysipelas with necrotizing fasciitis (gangrenous erysipelas), ecthyma gangrenosum (due to *Pseudomonas aeruginosa* or *Aeromonas* spp.), *Vibrio* infections (especially those due to *Vibrio vulnificus*) (Fig. 57.6), staphylococcal cellulitis or impetigo, and streptococcal cellulitis. Rarely, in immunocompromised patients the initial manifestation of gram-negative sepsis may be the appearance of a solitary hemorrhagic blister. *V. vulnificus* infection should be strongly considered in patients with preexisting liver disease, or other immunocompromising states, who have recently ingested raw seafood, especially oysters.[104,105] *V. vulnificus* sepsis may also occur in persons with open wounds exposed to a marine environment.[106] *Aeromonas hydrophila* skin and tissue infections, which may be acquired from exposure to fresh or brackish water, may also present as cellulitis with bulla formation.[107] Vesicopustular eruptions in the neonate may be due to both noninfectious and infectious causes. Potential infectious causes include congenital and neonatal candidiasis, staphylococcal infections, streptococcal infections, *Listeria monocytogenes* infection, infection with HSV, neonatal varicella, and bacterial sepsis (due to various organisms).[108]

Petechial and Purpuric Eruptions

Petechiae are lesions less than 3 mm in diameter that contain extravasated red blood cells or hemoglobin (Fig. 57.7). Larger lesions are termed *ecchymoses* or *purpura.* Diffuse petechial lesions should always prompt urgent investigation. In critically ill patients these lesions are often associated with symmetrical peripheral gangrene (purpura fulminans), consumptive coagulopathy, and shock. The most common infectious agents include gram-negative organisms, especially *Neisseria meningitidis,* and rickettsiae. Less commonly, *L. monocytogenes* or staphylococci may be associated with a similar clinical picture. Physicians must also consider a hemorrhagic fever if the patient has a history of recent travel to an area experiencing an epidemic or there is a potential household contact with such a traveler. Asplenic patients are at an increased risk for overwhelming sepsis (lifetime risk of ≈5%), which may be accompanied by symmetrical peripheral gangrene.[109–112] The lifetime risk for sepsis in asplenic hosts has been reported to range from 3% to 5%. *Streptococcus pneumoniae* is responsible for 50% to 90% of infections in the asplenic patients and has a mortality rate of approximately 50%. Other important pathogens include *Haemophilus influenzae, N. meningitidis*, and *Capnocytophaga canimorsus.* Additional occasional pathogens include *Staphylococcus aureus,* group B streptococci, *Enterococcus, Escherichia coli* and other Enterobacteriaceae, *Salmonella, Campylobacter, Bacteroides, Bordetella holmesii, Pseudomonas,* and *Babesia* spp. The risks from many of these diseases can be significantly reduced by vaccination of asplenic patients using Centers for Disease Control and Prevention (CDC)/Advisory Committee on Immunization Practices (ACIP) guidelines.[113] Children should also receive *H. influenzae* type b vaccine. Immunizations with pneumococcal vaccines (i.e., PCV13 and PPSV23 as CDC/ACIP recommended) significantly reduces the risk for pneumococcal sepsis.[112]

Viral illnesses associated with petechial rashes include infections due to coxsackievirus A9, echovirus 9, Epstein-Barr virus (EBV), or cytomegalovirus (CMV); atypical measles; and the viral hemorrhagic fevers. Although children with coxsackievirus and echovirus infections are usually nontoxic in appearance, some may appear very ill. In these patients differential diagnosis from acute meningococcemia is difficult. However, in a series of children presenting with fever and petechiae, only 8% had meningococcal infections and 4% had bacterial sepsis secondary to other disorders.[114,115]



FIG. 57.6 *Vibrio vulnificus*. Bullae caused by infection with *Vibrio vulnificus*. (A) Prior to amputation. (B) and (C) Predébridement. (D) Post-débridement. *(From Ralph A, Currie BJ.* Vibrio vulnificus *and* V. parahaemolyticus *necrotising fasciitis in fishermen visiting an estuarine tropical northern Australian location.* J Infect. *2007;54: e111–e114.)*



FIG. 57.7 Petichial rash. Diffuse petechial associated with meningococcemia. *(From Connolly AJ, Finkbeiner WE, Ursell PC, Davis RL. Atlas of gross autopsy pathology. In: Connolly AJ, Finkbeiner WE, Ursell PC, Davis RL, eds. 3rd ed.* Autopsy Pathology: A Manual and Atlas*. St. Louis: Elsevier; 2016:186–319.)*

A diffuse rash is often a prominent characteristic of the tick-borne diseases found in the United States (i.e., infections caused by *Rickettsia, Ehrlichia, Anaplasma, Borrelia,* and *Coxiella*), with the exception of tularemia.[116,117,118] The frequency of a diffuse rash has been reported as follows: RMSF, 99%; *Ehrlichia chaffeensis* (ehrlichiosis), 36%; *Anaplasma phagocytophila* (anaplasmosis), 2% to 11%; *Borrelia* spp. (relapsing fever), 28%; and *Coxiella burnetii* (Q fever), 5% to 21%.[116] Although Lyme arthritis is characterized by EM, a diffuse rash may occur at the time of disseminated infection. Lesions caused by rickettsiae are usually generalized and symmetrical. An eschar *(tache noire)* characteristically develops at the site of inoculation in the following rickettsial infections (infecting species): African tick bite fever *(R. africae),* Mediterranean spotted fever *(R. conorii),* North Asian tick typhus *(R. sibirica),* Queensland tick typhus *(R. australis),* rickettsialpox (due to *R. akari*), and scrub- or chigger-borne typhus *(R. tsutsugamushi).* New rickettsioses continue to be recognized worldwide that are characterized by generalized skin lesions, often with *tache noire* lesions, such as Japanese or Oriental spotted fever *(R. japonica),* Flinders Island spotted fever *(R. honei),* and Astrakhan fever (*R. conorii* Astrakhan).[116]

In patients with an appropriate travel history, infection with *Plasmodium falciparum* must be considered.[119] In addition, clinicians should be aware that malaria may occasionally be acquired in the United States.[120] Rash with petechiae secondary to thrombocytopenia is present in about 5% of affected patients heavily parasitized.

The most important causes of noninfectious petechiae are thrombocytopenia, large and small vessel necrotizing vasculitis (usually presenting as palpable purpura), and the pigmented purpuric eruptions (which usually represent capillaritis).

Enanthems

In attempting to classify the enanthem, it is essential that a thorough search of the mucous membranes (including the mouth, conjunctiva, and, on occasion, also the vagina, rectum, and glans penis) be made for the presence of enanthems. In many allergic reactions the mucous membranes are frequently involved. Koplik spots, diagnostic of rubeola, are tiny, white or blue-gray specks superimposed on an erythematous base, located on the buccal mucosa, most prominently on that adjacent to the molars. A "strawberry tongue" suggests the possibility of Kawasaki disease, TSS, or scarlet fever. Petechiae of the palate are common in scarlet fever and some vasculitides and with thrombocytopenia. In infectious mononucleosis, petechiae of both the hard palate and soft palate are common. Oral ulcers occur in a variety of noninfectious immunologic diseases and also with coxsackievirus A16 infection.

NEUTROPHILIC DERMATOSES, INCLUDING SWEET SYNDROME

The neutrophilic dermatoses are a heterogeneous group of diseases that can occur with localized, generalized, and systemic skin involvement and include Sweet syndrome, pyoderma gangrenosum, Behçet disease, and neutrophilic urticaria. Sweet syndrome, also known as acute febrile neutrophilic dermatosis, is characterized by a constellation of clinical symptoms, physical features, and pathologic findings that include fever, neutrophilia, tender erythematous skin lesions (papules, nodules, and plaques), and a diffuse infiltrate consisting predominantly of mature neutrophils that are typically located in the upper dermis.[121–124] Sweet syndrome occurs in a variety of clinical settings: idiopathic (or classic), malignancy associated, immunodeficiency associated, and drug induced. The classic syndrome is more frequent in women between the ages of 30 and 50 years, is often preceded by symptoms of an upper respiratory tract infection, and may be associated with inflammatory bowel disease and pregnancy. The skin demonstrates one or more tender, red, edematous, urticarial plaques or large papules. Often the border of each plaque is studded with papules (or, infrequently, with vesicles or pustules), giving an irregularly contoured, mammillated appearance reminiscent of that of the areolae of the breast. If solitary and large, such lesions may be confused with those caused by a variety of infectious processes, including primary HSV infection or streptococcal cellulitis. When solitary and present on the dorsum of the hand, a lesion of Sweet syndrome may mimic erysipeloid or a severe reaction to an arthropod bite. On occasion, these plaques become dusky in color and frankly hemorrhagic, suggesting instead EM or leukocytoclastic vasculitis. Some lesions may also become bullous, suggesting bullous EM or fixed drug eruption. Rare bullous lesions may erode or ulcerate, mimicking pyoderma gangrenosum. Mucosal surfaces may rarely be involved. Characteristically, patients with Sweet syndrome have associated fever; other findings may include leukocytosis, malaise, arthralgias, myalgias, conjunctivitis, and episcleritis.

The diagnosis of Sweet syndrome is one of exclusion and includes infectious and inflammatory disorders, neoplastic conditions, reactive erythemas, systemic diseases, and vasculitis. Sweet syndrome responds rapidly to high-dose systemic corticosteroids, but relapse is frequent if tapering is too rapid. Second-line systemic agents include colchicine, dapsone, potassium iodide, TNF-α antagonists, and cyclosporine.[124]

PATHOGENS OR INFECTIOUS CONDITIONS STRONGLY ASSOCIATED WITH RASH

As noted previously, the investigation of infectious rash requires consideration of not only the characteristics of the skin lesions but also the pathogens and infectious processes strongly associated with rash. The following discussion reviews the various skin manifestations of these pathologic processes.

Sepsis

Sepsis is a clinical syndrome that complicates severe infection and is characterized by inflammation, including vasodilation, increased microvascular permeability, and end-organ dysfunction. The inflammatory response has been divided into the systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock,[125] and the pathophysiology has been reviewed.[126] Although SIRS may result from infection or from noninfectious causes (e.g., autoimmune disorder, pancreatitis, vasculitis, thromboembolism, burns, surgery, heat shock), sepsis results from a dysregulated inflammatory response to an infection.

Kingston and Mackey[29] classified the skin lesions associated with sepsis into five pathogenic processes (major categories of infectious causes): (1) disseminated intravascular coagulation (DIC) and coagulopathy (due to *N. meningitidis,* streptococci, enteric gram-negative bacilli), (2) direct vascular invasion and occlusion by bacteria and fungi (*N. meningitidis, P. aeruginosa, Aspergillus* spp., agents of mucormycosis, *Rickettsia* spp.), (3) immune vasculitis and immune complex formation (associated with infection due to *N. meningitidis, Neisseria gonorrhoeae, Salmonella enterica* serovar Typhi), (4) formation of emboli in endocarditis (due

to *S. aureus,* streptococci), and (5) vascular effects of toxins (in SSSS, TSS, scarlet fever). Various systemic bacterial infections may spread to the skin, generally producing discrete lesions from which the organisms can be isolated or recognized on biopsy with special stains. The most characteristic finding in DIC is noninflammatory purpura with extensive microvascular occlusion referred to as purpura fulminans.[127–130] Other manifestations include diffuse bleeding, hemorrhagic necrosis of tissue, and skin necrosis. Although DIC may result from sepsis, it may also be caused by trauma, malignancy, obstetric calamities, severe hepatic failure, and severe toxic or immunologic reactions. Purpura fulminans, a severe skin disorder that is typically associated with DIC, occurs in three clinical settings: (1) as a consequence of severe sepsis, (2) after infection, and (3) in neonates (usually seen in association with homozygous protein C deficiency). *N. meningitidis* is the organism most commonly responsible for symmetrical peripheral gangrene (i.e., ischemic necrosis simultaneously involving the distal portion of two or more extremities without arterial obstruction), but this disorder may also be due to *S. pneumoniae* and other streptococcal species, *H. influenzae, S. aureus, E. coli, Klebsiella* spp., *Proteus* spp., *A. hydrophila,* other gram-negative organisms, and *Aspergillus.* Symmetrical peripheral gangrene is preceded by bleeding into the skin, ecchymosis, purpura, and acrocyanosis (a grayish cyanosis that does not blanch on pressure and occurs on the lips, legs, nose, ear lobes, and genitalia). Subsequently, the ecchymotic lesions become confluent, blister, undergo necrosis and ulceration, and develop overlying eschars. Histologic examination reveals a Schwartzman-like reaction in the skin characterized by diffuse and extensive hemorrhages, perivascular cuffing, and intravascular thrombosis. Bacteria are usually absent from smears of the lesions. Shock rather than DIC appears to be the major factor in the pathogenesis of symmetrical peripheral gangrene. As noted earlier, purpura fulminans may follow a benign infection, especially in children. Common preceding illnesses include scarlet fever, streptococcal pharyngitis, staphylococcal bacteremia, varicella, and measles.

Neisseria meningitidis Infection

With the widespread use of the *H. influenzae* vaccine, *N. meningitidis* is now the second-most common cause of bacterial meningitis after *S. pneumoniae* and has a similar incidence as gram-negative and staphylococcal meningitis.[131] Skin hemorrhages are the hallmark of invasive meningococcal disease.[132–134] *N. meningitidis* has been cultured from 0.5% to 27.5% of children presenting with fever and petechiae, but many of these studies were conducted before the availability of meningococcal vaccines. Hemorrhagic skin lesions have been present in 28% to 77% of patients with invasive meningococcal disease.[135] The lesions characteristically are petechial but may be noted to blanch with pressure early in the course of infection, thus resembling a viral exanthem. The petechiae are irregular and small and are often accompanied by palpable purpuric lesions, some of which may have pale centers. Coalescing lesions, often macular, may have a characteristic gun-metal gray color centrally, consistent with epidermal necrosis. Lesions most commonly occur on the extremities and trunk but may also be found on the head, palms and soles, and mucous membranes. Symmetrical peripheral gangrene may rapidly develop, often in association with DIC. Histologic examination reveals diffuse endothelial damage, fibrin thrombi, necrosis of the vessel walls, and perivascular hemorrhage in the involved skin. Gram staining of aspirates of the involved areas frequently reveals the presence of organisms.[136] In addition to the direct involvement of skin vessels by meningococci, excessive activation of the coagulation system and concomitant downregulation of the fibrinolytic system caused by high concentrations of endotoxin in the blood may lead to cutaneous hemorrhagic lesions.

Chronic meningococcemia is a rare disease. The classic clinical constellation of symptoms includes intermittent or sustained fevers; recurring maculopapular, nodular, pustular, or petechial eruptions; and migratory arthritis or arthralgias with little systemic toxicity.[137] In one large series comprising 148 patients, skin lesions were noted in 93%.[138] A variety of skin lesions may occur in chronic meningococcemia, the most frequently reported being pale to pink macules and papules, seen in greater than 40% of cases. Nodular lesions may occur, mostly on the lower extremities. Petechiae of variable size may be seen, with superimposed vesicles or pustules centrally. Small, irregularly round, subcutaneous hemorrhages with a bluish gray center containing pus cells are a distinctive lesion of this syndrome. Ecchymotic areas or hemorrhagic, tender nodules that are located deep in the dermis may also occur. Lesions associated with chronic meningococcemia tend to appear in showers in association with the onset of fever. In contrast to the lesions associated with fulminant meningococcemia, those of chronic meningococcemia rarely include organisms demonstrable on Gram-stained smear or biopsy specimen. In addition, purpura fulminans is not a typical finding in chronic meningococcemia. A number of diseases with periodic fever, skin lesions, and joint involvement may resemble chronic meningococcemia, including subacute bacterial endocarditis, acute rheumatic fever, Henoch-Schönlein purpura, rat-bite fever, EM, and chronic gonococcemia.

Pseudomonas Infection

Pseudomonas spp. are ubiquitous environmental organisms that are important pathogens in the hospital and in patients with certain underlying host defense abnormalities (e.g., cystic fibrosis). They may also cause infection in normal hosts, especially when the skin has been moistened. *P. aeruginosa* can cause folliculitis ("hot tub" folliculitis), which is characterized by follicular, macular, papular, vesicular, or pustular lesions on any part of the body that has been immersed in contaminated water and has followed bathing in contaminated whirlpools, hot tubs, and swimming pools.[139] Exposure to contaminated water may also result in nodular lesions on the palms and soles ("hot foot" syndrome).[140,141] Of importance, 96% of *P. aeruginosa* isolates from swimming and hot tubs have been reported to be multidrug resistant.[142]

Skin lesions have been reported to accompany *P. aeruginosa* sepsis in 13% to 39% of patients.[143–145] The dermatologic manifestations of *P. aeruginosa* sepsis consist of four types of lesions. First, vesicles and bullae may occur singly or in clusters and frequently are spread in random fashion over the skin. They may become hemorrhagic as they evolve. Second, gangrenous cellulitis may occur as a sharply demarcated, superficial, painless, necrotic lesion. It may also begin abruptly as an acute infection with local pain, swelling, and erythema and involve deep tissue and fascia. Third, macular or papular nodular lesions are located predominantly over the trunk; the lesions are small, oval, and painless. These lesions may resemble the rose spots of typhoid fever. Finally, ecthyma gangrenosum is a lesion characteristic but not pathognomonic of *P. aeruginosa* sepsis. Ecthyma gangrenosum has generally been reported to occur in 1.3% to 2.8% of septic patients,[143,146,147] but one report noted ecthyma gangrenosum in 28% of patients with *Pseudomonas* bacteremia.[145]

Ecthyma gangrenosum lesions begin as painless, round, erythematous macules, with or without adherent vesicles, that soon become indurated and progress to hemorrhagic bluish bullae. Later, the lesion sloughs to form a deep gangrenous ulcer with a gray-black eschar and a surrounding erythematous halo. The process evolves rapidly over 12 to 24 hours. Lesions may be discrete or multiple and are usually found in the groin, axillary, or perianal areas but may occur anywhere on the body. Although most commonly associated with *P. aeruginosa* sepsis, ecthyma gangrenosum–like lesions have also been reported in sepsis associated with other pseudomonal species, *S. pyogenes, S. aureus, A. hydrophila, K. pneumoniae, Serratia marcescens, Stenotrophomonas maltophilia, Morganella morganii, E. coli, Citrobacter freundii, N. gonorrhoeae, Yersinia pestis, Chromobacterium violaceum, Candida* spp., *Aspergillus* spp., *Mucor* spp., and HSV.[148] These lesions may also occur with vasculitis or malignant infiltration.[149] Rarely, ecthyma gangrenosum due to *P. aeruginosa* may be found in the absence of sepsis.[150–152]

Histologically, ecthyma gangrenosum is characterized by three features: bacterial invasion of the media and adventitia of vein walls deep in the dermis, sparing of the intima and lumen, and minimal inflammation.[149] Bacterial invasion results in marked fibrin exudation and frank hemorrhage, followed by bulla formation. Finally, necrosis of the dermis occurs. Bacteria are readily visible in biopsy samples and can be demonstrated in Gram-stained material scraped from the base of the lesion.

Subcutaneous nodules may also result from *P. aeruginosa* bacteremia. Characteristically, the nodules are erythematous and warm, may be

either fluctuant or nonfluctuant, and may be tender. Despite prolonged antibiotic therapy, these lesions may contain viable bacteria weeks after the blood has been cleared of infection. The absence of fluctuance may be due to either the lack of pus in neutropenic patients or the deep location of the abscess, or both. Although successful treatment may require incision and drainage,[153,154] prolonged antibiotic therapy without drainage may result in a cure.[155,156]

Bacterial Endocarditis

Skin lesions have been reported to accompany bacterial endocarditis in 4% to 50% of cases.[157–162] The cutaneous manifestations of bacterial endocarditis are important clues to the diagnosis, although they occur less frequently than in the preantibiotic era.[163] Cutaneous lesions are also less frequent in older patients (i.e., ≥65 years of age).[164] Skin lesions include Osler nodes, Janeway lesions, subungual splinter hemorrhages, cutaneous purpura and petechiae, and conjunctival petechiae (Roth spots). The prevalence of embolic and hypersensitivity lesions in skin and mucous membranes (50%) in heroin-associated infective endocarditis is similar to that described in patients with non–heroin-associated infective endocarditis.[165]

Petechiae are the most common skin and mucous membrane lesions observed in endocarditis, occurring in 20% to 40% of patients. The lesions are small, flat, and reddish brown and do not blanch on pressure. The petechiae may be observed on the skin, especially on the heels, shoulders, and legs. Mucous membrane (oral and conjunctiva) involvement is common. Petechiae frequently occur in small crops. Lesions usually are transient.

In the preantibiotic era, Osler nodes were present in 10% to 90% of patients with bacterial endocarditis.[166] In the 1980s they were seen in 10% to 20% of cases. Recent series suggests that they are now present in less than 10% of patients with endocarditis because endocarditis is now diagnosed more rapidly before the development of Osler nodes. These lesions are tender, indurated, erythematous nodules, with a pale center that is 1.0 to 1.5 mm in diameter.[166–168] Osler nodes most commonly occur on the pads of the fingers or toes but may also occur on the thenar and hypothenar eminences and over the arms. Pain may be elicited by palpating the tips of the digits. Osler nodes tend to occur in crops, are rarely numerous, and tend to be transient. The lesions usually resolve without necrosis or suppuration 1 to 3 days after antibiotic therapy is initiated. Histologically, Osler nodes show microabscesses with microemboli in adjacent arterioles. Osler nodes are most commonly associated with subacute bacterial endocarditis due to infection with streptococci but may occur in endocarditis due to infection with fungi or gram-negative bacilli or in systemic lupus erythematosus, typhoid fever, and gonococcemia. Osler nodes probably represent the sequelae of vascular occlusion by microemboli leading to localized vasculitis.[169]

Janeway lesions consist of small erythematous macules or, less commonly, small nodular hemorrhages in the palms and soles. Although they may be seen in subacute bacterial endocarditis, they are more common in acute endocarditis, especially that due to *S. aureus.* Unlike Osler nodes, Janeway lesions are painless. Histologically, they show microabscesses with neutrophil infiltration of capillaries.

Infections Due to *Staphylococcus aureus* and *Streptococcus pyogenes*

Most commonly, *S. aureus* and *Streptococcus pyogenes* cause local skin infections, including impetigo; folliculitis; furuncles and carbuncles; hidradenitis suppurativa (follicular infection of intertriginous regions); and erysipelas, mastitis, and cellulitis.[170] Both pathogens may produce serious local infection, including abscesses, myositis, and fasciitis. *S. aureus* is responsible for a variety of infectious syndromes that may produce local or diffuse skin lesions.[171–172] Skin lesions arise from (1) production of toxins,[173–175] (2) septic shock, and (3) vascular invasion, often in association with endocarditis. *S. aureus* strains may produce more than 30 different extracellular proteins.[144] Staphylococcal disease syndromes linked to toxins include toxic shock (TSS toxin 1 [TSST-1]), SSSS (mediated by exfoliative toxins, primarily exfoliative toxin A and exfoliative toxin B [ETA and ETB]), staphylococcal food poisoning (due to staphylococcal exotoxins, principally enterotoxin A and LukE-LukD), and community-associated *S. aureus* skin and soft tissue infections (α-toxins, phenol-soluble modulins, leukocidins).[176,177]

Community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains are now the predominant cause of community staphylococcal skin infections.[178–185] In the United States most CA-MRSA strains carry the staphylococcal cassette chromosome (SCC) mec types IV or V, are typed as USA clones 300 or 400, and carry the gene for Panton-Valentine leukocidin. Groups at higher risk for CA-MRSA infections include athletes, HIV-infected persons, homeless persons, household members of infected people, injection drug users, military personnel, prisoners in correctional facilities, tattoo recipients, and urban dwellers who have lower socioeconomic status and live in crowded conditions. CA-MRSA strains are more likely to cause skin and soft tissue infections than methicillin-susceptible strains. Skin lesions may occur as tender red abscesses that are commonly misinterpreted as "spider bites" by the patient. CA-MRSA strains may manifest as furuncles, boils, erythematous patches and nodules, erythematous pustules (folliculitis), crusted plaques (impetigo), or any combination of these. Other soft tissue infections described include chronic external otitis, paronychia, cellulitis, necrotizing fasciitis, and necrotizing myositis. However, the clinical picture is not sufficiently distinct to allow a diagnosis of CA-MRSA based on clinical features.

Staphylococcal Scalded Skin Syndrome

SSSS (Ritter disease) describes a spectrum of superficial blistering skin disorders caused by the exfoliative toxins (also known as epidermolytic toxins, epidermolysins, and exfoliatins) of *S. aureus.*[186] Its severity varies from localized blisters to generalized exfoliation affecting the entire body surface. Although mortality in appropriately treated children is less than 4%, in adults it can reach almost 60%. Approximately 5% of all *S. aureus* strains produce exfoliative toxins; two serotypes have been identified as affecting humans, ETA and ETB.

Bullous impetigo, a disorder usually confined to children that results from toxin-producing strains of *S. aureus,* is characterized by discrete, flaccid bullae containing clear or cloudy yellow fluid. Lesions are frequently localized to the umbilicus, groin, or axillae. The surrounding skin may appear normal or mildly erythematous. The bullae rapidly rupture, leaving raw, denuded erosions that reepithelialize in 5 to 7 days. Affected infants are usually afebrile and lack constitutional signs.

SSSS usually occurs in neonates or young children (<5 years of age) but may affect older children or, rarely, adults. Most cases in adults occur in association with renal impairment, lymphoma, or immunosuppression. A well-characterized animal model exists for SSSS, demonstrating that decreased renal clearance of the causative toxin results in the clinical presentation. Unlike bullous impetigo, in which the staphylococcal infection produces skin lesions at the site of infection, in SSSS the infection produces a toxin (ETA or ETB) that disseminates, causing a diffuse, blanchable erythema in association with marked skin tenderness, fever, and irritability. Light stroking of involved skin causes rupture and separation of the upper portion of the epidermis (Nikolsky sign). Generalized desquamation usually occurs, especially in intertriginous areas. Unless secondary infection intervenes, the skin heals within 10 to 14 days. A skin biopsy specimen (or a frozen section of an induced peel for more rapid diagnosis) may be studied to distinguish between SSSS and TEN. In SSSS the cleavage plane of the early intraepidermal bulla is just beneath the granular cell layer, whereas in TEN the bulla is subepidermal and associated with full-thickness necrosis of the epidermis. In addition, mucous membranes are relatively spared in SSSS in contrast to TEN. Early distinction between these two diseases is important because the therapy for SSSS includes antistaphylococcal antibiotics, whereas in TEN the discontinuation of treatment with the offending drug and initiation of aggressive burn unit intervention may be lifesaving. SSSS due to MRSA has been reported.[186,187]

A mild form of SSSS is characterized by a generalized scarlatiniform eruption with exfoliation ("staphylococcal scarlet fever"). The skin has a sandpaper roughness, and Pastia lines are present, as in streptococcal scarlet fever, but the strawberry tongue and palatal enanthem of streptococcal scarlet fever are absent.

Staphylococcal Toxic Shock Syndrome

TSS is an acute febrile illness characterized by a generalized erythematous eruption that is due to in vivo production of a toxin at the site of localized, often relatively asymptomatic or unnoticed infection by *S. aureus* strains capable of toxin production.[188–192] The multisystem effects observed in TSS patients are induced by TSST-1 and, to a lesser extent, other staphylococcal enterotoxins. The toxins act as superantigens binding directly to the Vβ chain of the T-cell receptor and to the major histocompatibility complex molecule, which triggers clonal expansion of the T cells and an unregulated outpouring of proinflammatory cytokines.[175,193] Although staphylococcal TSS has been most commonly linked to *S. aureus,* TSST-1–producing coagulase-negative staphylococci have also been described.[194]

Although in the early 1980s most cases of TSS occurred in menstruating females, often in association with tampon use, nonmenstrual staphylococcal TSS is now more common.[191] Nonmenstrual TSS has been associated with a variety of infections, including postoperative wounds, cutaneous infections, burn wounds, postpartum complications, and *S. aureus* respiratory infections, often after viral influenza.[195] Recurrent nonmenstrual toxic shock has been described.[196]

TSS may range in severity from a relatively mild disease, often misdiagnosed as a viral syndrome, to a severe life-threatening illness. The most common symptoms include a temperature greater than 40°C (104°F), hypotension, and diffuse erythroderma with desquamation 1 to 2 weeks after the onset of illness. Additional early features include conjunctival, oropharyngeal, and vaginal hyperemia; vomiting and diarrhea; and myalgias. Most patients have abnormalities in three or more organ systems: (1) muscular: rhabdomyolysis, (2) central nervous system: toxic encephalopathy, (3) renal: azotemia, (4) liver: abnormal aminotransferase levels, and (5) hematologic: thrombocytopenia. The rash of TSS is almost always noted within the first 24 hours of illness. Desquamation occurs after 7 to 10 days, most prominently on the hands and feet. Histologically, the epidermis exhibits cleavage in the basilar layers, which differentiates TSS from SSSS and from viral and drug eruptions. *S. aureus* septicemia may be associated with erythematous, petechial, or pustular lesions. In addition, lesions associated with endocarditis, such as Osler nodes, Janeway lesions, and splinter hemorrhages, may occur. Such skin lesions have been reported in 10% to 64% of patients with staphylococcal septicemia. Purpuric lesions may in some cases be so extensive as to suggest meningococcemia or RMSF. Gram-stained smears of the material in these lesions usually demonstrate gram-positive cocci.

Streptococcal Infections

The group A streptococci *(S. pyogenes)* cause a wide variety of local (e.g., impetigo, erysipelas, cellulitis, lymphangitis) and invasive syndromes (e.g., bacteremia, necrotizing soft tissue infections, streptococcal TSS) associated with cutaneous manifestations.[197,198] These manifestations of streptococcal infection occur via three distinct mechanisms[199]: (1) direct infection of the skin, (2) immunologically mediated disease, and (3) toxin-mediated disease. Rheumatic fever affects up to 3% of people with untreated group A hemolytic streptococcal infections of the nasopharynx. Cutaneous manifestations include erythema marginatum (occurring in 10%–20% of cases), subcutaneous nodules (in up to 30%), and erythema papulatum (rare).

Scarlet fever is a diffuse erythematous eruption that results from the production of pyrogenic exotoxin (erythrogenic toxin) produced by *S. pyogenes,* most commonly in the setting of pharyngitis. There appear to be three distinct exotoxins (types A, B, and C) produced by approximately 90% of group A strains. The rash of scarlet fever requires both the presence of pyrogenic exotoxin and the existence of delayed-type skin reactivity to streptococcal products. The latter requires prior exposure to organism. The pharynx is usually beefy red with edema involving the tonsillar area extending anteriorly to the soft palate and uvula. The rash of scarlet fever usually starts on the head and neck and then rapidly expands to cover the trunk and finally the extremities. The palms and soles are usually spared. The rash is a diffuse erythema, blanching on pressure, with numerous small (1–2 mm) papular elevations, giving a "sandpaper" quality to the skin. The rash is most marked in the skin folds of the inguinal, axillary, antecubital, and abdominal areas and about pressure points (e.g., buttocks). The rash often exhibits a linear petechial character in the antecubital fossae and axillary folds (Pastia lines). The rash of scarlet fever may be confused with rashes due to erythema infectiosum (fifth disease), rubella, rubeola, EBV, hepatitis B, HIV, enteroviruses, and *Streptobacillus moniliformis* (rat-bite fever). The cutaneous eruptions of TSS and secondary syphilis may also appear similar to that of scarlet fever, but vasomotor instability in the former and positive serology in the latter should suffice to differentiate them from scarlet fever. Noninfectious syndromes that should be considered include Kawasaki disease, acute systemic lupus erythematosus, and juvenile rheumatoid arthritis.

In the later part of the 1990s an increased incidence of severe group A streptococcal infections was reported.[197] Invasive group A streptococcal infections are life threatening, especially when associated with streptococcal TSS. Similar to staphylococcal TSS, this syndrome is due to the production of highly potent exoproteins that act as superantigens. The term *streptococcal toxic shock syndrome* is now used for those patients with hypotension and organ dysfunction as a result of toxin-mediated streptococcal disease (Table 57.4).[191,198,200] Many, but not all, patients have a rash at the time of presentation. Skin manifestations include generalized erythroderma with desquamation and localized cellulitis with vesiculation or bulla formation. The differential diagnosis of a desquamative rash includes Kawasaki disease, EM, toxic epidermal necrolysis, leptospirosis, and RMSF. Unlike staphylococcal TSS, a focus of pyogenic inflammation is usually present, and a large proportion of the patients have documented bacteremia. Commonly, the local focus of infection results in necrotizing fasciitis manifest by diffuse swelling and tenderness, a peau d'orange appearance, and erythema with subsequent formation of bullae. Later the skin color changes from red to purple or black as the skin becomes necrotic. However, initially the clinical symptom of the local site of infection often is only severe pain, with tenderness and other physical findings appearing later. Rarely, group B[201] and group G[202] streptococci have been reported to cause myositis with TSS.

Rickettsial Infections

Rickettsiae comprise several important pathogens transmitted by ticks in the United States. Cutaneous manifestations often provide important and early clues in the diagnosis of tick-borne diseases.[203–206] Rickettsiae are obligate intracellular parasites whose primary target in humans appears to be the endothelial cell. After parasitization of the endothelial cell, necrosis of the media and intima results in thrombosis, formation of microinfarcts, and extravasation of blood. The end result is increased vascular permeability and vasculitis.

TABLE 57.4 Staphylococcal Versus Streptococcal Toxic Shock Syndrome

FEATURE	STAPHYLOCOCCAL	STREPTOCOCCAL
Age	Primarily 15–35 yr	Primarily 20–50 yr
Gender	Higher frequency in women	Men and women equally affected
Severe pain	Rare	Common
Hypotension	100%	100%
Erythroderma rash	Very common	Less common
Renal failure	Common	Common
Bacteremia	Low frequency	60%
Tissue necrosis	Rare	Common
Predisposing factors	Tampons, surgery	Cuts, burns, varicella
Thrombocytopenia	Common	Common
Mortality rare	<3%	30%–70%

Data from Stevens DL. The toxic shock syndromes. Infect Dis Clin North Am. *1996;10:727–746.*

Rash is a hallmark of RMSF,[207,208] the most common serious rickettsial disease in the United States. Initially, a maculopapular rash develops. Subsequently, the rash becomes more petechial. Characteristically, the rash appears between the second and sixth days of illness (average, 4 days). However, the rash may be absent in 5% to 17% of patients, and in up to 50% it may not appear within the first 3 days of illness.[209–211] Failure to initiate proper therapy within 5 days of onset of symptoms[212] and failure to use a tetracycline[213] have been associated with an increased mortality rate. Independent predictors of failure by the physician to initiate therapy the first time a patient was seen include absence of a rash, presentation between August 1 and April 30, and presentation within 3 days of illness.[213] Most commonly, the rash begins on the extremities, often around the wrists and ankles, and spreads centripetally to the trunk, with relative sparing of the face. However, the rash may begin on the trunk (10%) and spread centrifugally or may have a diffuse distribution at the time of onset (10%). Characteristically, the rash involves the palms or soles or both in the later stages of infection (Fig. 57.8). Over time the rash, which begins as maculopapular lesions, may progress to become petechial or ecchymotic. Rarely, gangrene or skin necrosis that requires amputation occurs.[214] The rash may rarely be urticarial or pruritic. Because the mortality rate for infection may be decreased from 15% to 3% with appropriate treatment, institution of antibiotic therapy should never be delayed in the absence of rash. Signs and symptoms similar to those of RMSF may occur with ehrlichiosis and anaplasmosis (see later discussion). The rash of rickettsialpox typically begins as maculopapular and in its early phase may be confused with that of RMSF, but it quickly becomes papulovesicular, which is not characteristic of the RMSF eruption. The rash of RMSF may also be confused with measles, mononucleosis, viral hepatitis, streptococcal infection, primary HIV infection, secondary syphilis, parvovirus infection (fifth disease), Kawasaki disease, and roseola. If a penicillin or cephalosporin is administered before the appearance of the rash, the subsequent rash may be incorrectly diagnosed as a drug eruption rather than the rash of RMSF.

Ehrlichia and *Anaplasma* Species

Important tick-borne diseases that occur in the United States include RMSF, Lyme disease, tick-borne viral encephalitis, babesiosis, and tularemia (see Chapter 296). Recently, *Ehrlichia* spp. and *Anaplasma* spp. have emerged as important and potentially life-threatening pathogens.[215–220]

In the United States ehrlichiosis caused by *Ehrlichia chaffeensis* (human monocytotropic ehrlichiosis) is most common in the southern and mid-Atlantic, and north- and south-central states and in isolated areas of New England. Common early signs and symptoms of infection include fever, headache, malaise, and myalgia. Rash occurs in approximately 60% of children but in less than 30% of adults. The rash pattern varies from petechial to maculopapular to diffuse erythema and typically occurs late in the course of the disease (median, 5 days after onset). The rash pattern might involve the extremities, trunk, face, or rarely, the palms and soles. A newly recognized human pathogen, *Ehrlichia ewingii*, also may cause rash, but it is uncommon.

Anaplasmosis is caused by *Anaplasma phagocytophilum* (human granulocytotropic anaplasmosis). In the United States it is most common in New England and the north-central and Pacific coastal states. Common signs and symptoms include fever, headache, malaise, myalgia, and vomiting. Rash is reported in less than 10% of cases. The clinical and epidemiologic features of anaplasmosis and ehrlichiosis overlap with those of RMSF. Rash is less common with ehrlichiosis and anaplasmosis than with RMSF, but it is important to remember that at presentation less than 50% of patients with RMSF will have a rash, and 15% will never demonstrate a rash (i.e., so-called Rocky Mountain spotless fever). As with RMSF, the rash of these other tick-borne diseases may resemble that of mononucleosis, thrombotic thrombocytopenic purpura, hepatitis A, and drug eruptions.



FIG. 57.8 Rocky Mountain spotted fever. An example of late stage rash in an RMSF patient. *(From Lin L, Decker CF. Rocky Mountain spotted fever.* Dis Mon. *2012;58:361–369.)*

Borrelia burgdorferi Infection

Lyme disease is a tick-borne borreliosis with broad distribution and myriad manifestations.[221–224] In early localized or disseminated Lyme disease, fever is present in approximately 15% of patients, although it is usually low grade. Skin lesions are prominent clinical manifestations of all stages of Lyme disease.[225–228] Clinically, the disease is divided into three stages of illness: early localized disease, early disseminated disease, and persisting late disease. The most common manifestation of early localized Lyme disease is EM, which usually appears at the site of the tick bite within 7 to 10 days (range, 3–30 days). Seventy percent to 80% of infected patients will demonstrate EM.[203] Most patients (75%–80%) who exhibit EM in the United States have only a single (primary) lesion. EM initially begins as a red macule or papule that is usually homogeneous in its redness and may remain so until it heals. More commonly, the lesion partly or totally clears centrally, leaving an annular erythema that spreads centrifugally. The EM skin lesions are typically 5 cm or more in their largest diameter. EM may develop anywhere but is most frequently located around the knees, in the axillae, and in the groin. Although the lesion may last from a few days to about 1 year, it usually disappears within a few weeks to months. In about half of the patients, itching, dysesthesia/hyperesthesia, or sensations of heat may develop at the site of erythema. Many patients who develop early disseminated disease exhibit EM-like lesions. The disseminated lesions are usually smaller than the primary lesion and often multiple. These lesions may be ring shaped but are often homogeneous and nonmigrating.

Syphilis

Physicians should be familiar with the cutaneous manifestations of syphilis because primary and secondary syphilis and congenital syphilis continue to occur in the United States, especially in the southern states. The primary skin lesion (chancre) typically develops about 21 days after exposure. The differential diagnosis of patients with a genital ulcer in addition to syphilis includes genital herpes and chancroid. Secondary syphilis (Fig. 57.9) is often accompanied by a rash with highly variable morphology. Lesions may be macular, papular, maculopapular, papulosquamous, or pustular. On occasion, all types of lesions may be present in the same patient. A characteristic presentation of secondary syphilis is that of a pityriasis rosea–like eruption appearing as numerous, tan to reddish brown, scaly macules, usually distributed along skin tension lines on the trunk and, to a lesser extent, other body sites. Typically, no herald patch (a hallmark feature of pityriasis rosea) is present when this eruption is caused by syphilis, and usually the patient with secondary syphilis lacks associated pruritus and may have concurrent "copper penny" macules or plaques on the palms or soles. The differential diagnosis for secondary syphilis skin manifestations includes RMSF, pityriasis rosea, psoriasis, lichen planus, and exanthematous drug/viral eruptions. Condylomata lata, which are grayish, raised, broad, flat-appearing papular lesions, may occur in skin folds or apposed skin in



FIG. 57.9 Secondary syphilis. Rash of secondary syphilis on patient's sole. *(From Miller AC, Rashid RM, Khachemoune A. Secondary syphilis.* J Emerg Med. *2008;35:83–85.)*

moist areas, such as the anus, vulva, and scrotum. Condylomata lata need to be distinguished from condylomata acuminata (genital warts), squamous cell carcinoma, molluscum contagiosum, and micropapillomatosis of the vulva.

Candidiasis

The incidence and relative frequency of health care–associated infections due to *Candida* has risen dramatically in the recent past, and this increase has been accompanied by a shift in the infecting pathogen away from *Candida albicans* to non–*C. albicans* spp.[229–231] The most recent data from the CDC (2011–14) reported that non-*glabrata,* non-*albicans Candida* spp. accounted for 4.9%, *C. glabrata* for 3.4%, and *C. albicans* for 6% of central line–associated bloodstream infections.[232] Predisposing factors for disseminated or invasive infection are mucosal colonization with *Candida;* malignancy with cytotoxic therapy; neutropenia; antimicrobial therapy, especially with broad-spectrum agents; parenteral hyperalimentation; severe burn injuries; very low birth weight; use of IV catheters; systemic administration of adrenocortical corticosteroids; acute renal failure; prolonged care in an intensive care unit; and complicated intraabdominal surgery.[229–231]

An older study reported that 13% of patients with disseminated candidiasis have skin lesions,[233] whereas a more recent study reported 35.8%.[234] Typical skin lesions are 5 to 10 mm, erythematous or purpuric, nontender, firm, deep dermal papules that characteristically do not develop a central eschar or necrosis.[234,235] The lesions may have the appearance of papules, nodules, or plaques. In about half of the cases, they show characteristic central pale vesicular or pustular centers. Uncommonly, they may have necrotic centers. Usually the lesions are diffuse, involving the trunk and proximal extremities, but they may be localized to a small area. The face is usually spared. The diagnosis of systemic candidiasis may be confirmed by punch biopsy for touch preparation, histology, and culture in approximately 70% of patients.[234,236] Congenital candidiasis is a rare disease with fewer than 100 cases reported that result from acquisition of infection in utero.[237] It is characterized by a diffuse erythematous skin eruption with or without vesicles and pustules. Systemic infection, including pneumonia, may occur with or without skin lesions.

Many other fungi produce nodular lesions identical to those caused by *Candida* and must be considered as possible pathogens in immunocompromised patients. In patients with acquired immunodeficiency syndrome (AIDS), cryptococci may cause umbilicated nodules that mimic the lesions of molluscum contagiosum.

Measles

Measles remains endemic in multiple regions of the world. However, in recent years we have seen increases in the numbers of cases in countries where there have been decreases in the frequency of measles vaccination.[238] Thus physicians need to become reacquainted with the skin manifestations of the disease. Measles is characterized by a prodrome of fever (as high as 105°F) and malaise; cough, coryza, and conjunctivitis (the three "C"s); and a pathognomonic enanthem (Koplik spots, tiny white spots that appear inside the mouth), followed by a maculopapular rash. The classic morbilliform rash associated with measles typically occurs at the end of the prodromal phase of disease (see Fig. 57.1). The rash typically develops on the face and then proceeds to spread down the body to involve extremities, including the palms and soles. The rash usually lasts 5 days and will become confluent, especially on the face. During the healing phase, the rash may desquamate and typically clears first from the regions initially involved. Typically, measles patients are most ill during the first 1 to 2 days of the rash.

Measles also presents in an atypical form, usually associated with inactivated measles vaccination.[239,240] The atypical measles syndrome is characterized by pneumonia, often with lobular infiltrates, hilar lymphadenopathy, and pleural effusion. The pulmonary syndrome is associated with a maculopapular rash that progresses to vesicular petechial or purpuric lesions. Despite appearing ill, spontaneous recovery is expected with atypical measles syndrome.

NEW AND EMERGING INFECTIOUS DISEASES

The Institute of Medicine defines *new and emerging diseases* as "new, reemerging or drug-resistant infections whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future."[241] Many would also include infections whose geographic range is increasing. The factors leading to the development of new and emerging diseases have been reviewed in the literature.[241–244] Approximately 75% of emerging or reemerging diseases are known to be zoonotic.[245] It is important for infectious disease clinicians to realize that international travel has dramatically increased; in accordance, patients may present with diseases only rarely seen in the United States. Many of these diseases may be associated with either local or generalized skin lesions, including dengue fever, yellow fever, viral hemorrhagic fevers, Zika virus, malaria, and leptospirosis.

Dengue, Chikungunya, and Zika Viruses

The etiologic agents of dengue fever are four serologically related RNA viruses belonging to the family Flaviviridae.[246–248] Dengue is widely distributed with endemic transmission in Africa, Asia, and South and Central America. Most cases in the continental United States have involved travelers, but transmission has been reported in Hawaii and Texas. Classic dengue fever begins after an incubation period of 3 to 15 days (average, 5–8 days) with an abrupt onset of fever that may be accompanied by chills, headache, and general malaise. The fever usually lasts 3 to 7 days and may be biphasic. Dermatologic findings do not occur in all patients with classical dengue. When they occur, erythema may appear shortly before the onset of fever, concurrently with fever onset or 24 to 48 hours later. This rash may be noted as a flushing or erythematous mottling beginning on the trunk and spreading centrifugally to the face, neck, and extremities. Flushing may disappear after 1 or 2 days or may blend into an erythematous macular or maculopapular rash that develops at any time during the course of illness. Pruritus and desquamation, especially on the palms and soles, may follow termination of the eruption. Dengue hemorrhagic fever/dengue shock syndrome, a more severe disease, has more pronounced dermatologic findings, including petechiae, purpura, ecchymoses, epistaxis, and gum bleeding.

Chikungunya virus is an arbovirus (genus *Alphavirus*, family Togaviridae) that is prevalent in Africa and Asia, especially in India and islands in the Indian Ocean and has recently spread to the Caribbean and South America.[249] Disease has been reported in the United States in travelers returning from endemic areas, with local transmission in Puerto Rico and Florida.[250] After an incubation period of 2 to 4 days, patients have an abrupt onset of high fever, headache, back pain, myalgia, and arthralgia that may be intense. Skin involvement is present in 20% to 50% of cases and consists of a pruriginous maculopapular rash mostly located on the face, trunk, and extremities. The rash is transient. Facial edema and pruritus may accompany the rash. In children a bullous rash with pronounced sloughing may occur.

Hemorrhagic fever has been reported in Chikungunya-infected patients from Thailand.

The rash from Zika virus is morphologically very similar to that of dengue virus, with the majority of Zika virus infections presenting with a morbilliform rash with islands of sparing.[251–253] The rash can be pruritic[254–255], descending,[256] and/or blanching.[257–258] The rash typically occurs after the initial 3 to 4 days of symptoms, with reports of the rash occurring as early as the first day of illness or even preceding other symptoms.[259–261] The rash typically lasts for an average of 6 days (range, 2–14 days) and can spread to any part of the body, including the torso, extremities, palms, soles, and face.[259,262] Fever tends to be less prominent than with dengue virus infection, often occurring in less than 50% of individuals.[256]

Viral Hemorrhagic Fever

Hemorrhagic fever may be caused by viruses belonging to several families, including the Arenaviridae (e.g., New World arenaviruses, such as Argentine hemorrhagic fever, Bolivian hemorrhagic fever, Venezuelan hemorrhagic fever; Lassa fever), Bunyaviridae (hantaviruses, Crimean-Congo fever, Rift Valley fever), Filoviridae (Ebola, Marburg), and Flaviviridae (yellow fever, dengue hemorrhagic fever, Omsk hemorrhagic fever).[263–265]

Hantaviruses are RNA viruses that belong to the family Bunyaviridae and include Hantaan, Seoul, Puumala, Dobrava, Sin Nombre, Bayou, Black Creek Canal, and New York viruses.[266–268] Hemorrhagic fever with renal syndrome (HFRS) may be caused by seven hantaviruses (e.g., Hantaan, Seoul, Dobrava, Saaremaa, Amur, Puumala, and Far East).[267] Severe forms of HFRS have characteristic phases that may not be seen with milder disease. After an incubation period of 2 to 3 weeks, patients present with abrupt onset of fever in association with malaise, headache, myalgias, back pain, abdominal pain, nausea, and vomiting. Conjunctival injection or hemorrhage with palatal and upper torso petechiae is commonly seen on physical examination. During this phase, a characteristic erythematous flush that blanches with pressure may be observed, usually affecting the face, neck, and upper torso. After the 3- to 7-day febrile phase, a period of hypotension and severe shock ensues that is characterized by hemorrhagic manifestations. Overall, about 20% of the patients manifest severe disease, with death from shock and renal failure in 5% to 10% of cases. The hantavirus pulmonary syndrome may be caused by multiple hantaviruses, including Sin Nombre virus.[267] Some forms of hantavirus pulmonary syndrome (e.g., Andes) can present as conjunctivitis, facial flushing, and variable numbers of fine petechiae on the trunk, axillary folds, soft palate, or neck.

Filoviruses include the agents that cause Marburg and Ebola hemorrhagic fever.[269–272] Ebola virus is more virulent than Marburg virus and causes more severe clinical disease, with an accelerated course and high morbidity and mortality rates. Patients present with an acute onset of fever, severe frontal headache, anorexia, malaise, and myalgias. These signs and symptoms are followed 2 to 3 days later by clinical deterioration heralded by pharyngitis, conjunctivitis, severe nausea and vomiting, abdominal pain, and watery diarrhea. Five days later, patients develop a maculopapular rash on the trunk and back that is followed by the appearance of petechiae, ecchymoses, subconjunctival hemorrhages, epistaxis, hemoptysis, hematemesis, and melena. Hemorrhagic shock may progress to death. Patients with Marburg virus infection may develop a scarlatiniform rash rather than a maculopapular rash. Considerations in the differential diagnosis of African hemorrhagic fevers also include yellow fever and Lassa fever, but these illnesses are not accompanied by a rash.

Bacterial Diseases Due to *Bartonella* Species

Bartonella are small, fastidious, intracellular gram-negative bacteria.[273–275] The genus now includes more than 20 distinct species, several of which are capable of causing human disease. Most of our current knowledge on *Bartonella* infections is restricted to *B. henselae, B. quintana,* and *B. tribocorum*. *Bartonella* spp. that cause human disease are generally associated with well-defined reservoirs, usually domestic and wild animals.

Infection with *B. henselae* results in disease syndromes of variable severity, ranging from lymphadenopathy to systemic disease. Cat-scratch disease occurs primarily in children and young adults and is generally a benign self-limited disease. Cat-scratch disease typically begins with a cutaneous lesion at the site of inoculation.[276–278] The lesion that develops 3 to 10 days after introduction of the organism evolves through erythematous, vesicular, and papular crusted stages. Less commonly, it can be pustular or nodular. Approximately 2 weeks after inoculation, regional adenopathy develops proximal to the skin lesion. The nodes are usually tender, often have overlying erythema, and may suppurate. Low-grade fever and malaise occur in about 30% of children. Uncommon cutaneous manifestations include a transient maculopapular rash, EM, erythema nodosum, and purpura. The differential diagnosis includes other diseases associated with regional lymphadenopathy and cutaneous inoculation lesions (e.g., nontuberculous mycobacteria, *Nocardia, Y. pestis, Bacillus anthracis, Francisella tularensis, Erysipelothrix rhusiopathiae*), pyogenic infections due to streptococci and staphylococci, viral-associated lymphadenopathy (e.g., CMV, HIV, EBV), and lymphoma.

B. henselae and *B. quintana* are capable of causing a variety of diseases in immunocompromised patients, principally patients with late-stage HIV-1 infection, including bacillary angiomatosis, bacillary peliosis, splenitis, osteomyelitis, and bacteremia. On occasion, bacillary angiomatosis has been reported in immunocompetent individuals.[279] Skin lesions are the most frequent clinical manifestation of bacillary angiomatosis, with a prevalence of 55% to 90%. The typical lesion is solitary or disperses all over the body. The reddish purple papules may be difficult to distinguish clinically from Kaposi sarcoma and pyogenic granuloma. The lesions of bacillary angiomatosis may appear as smooth, warty, and pedunculated papules; subcutaneous nodules; and hyperkeratotic plaques. They rarely ulcerate or bleed.

Orthopoxviruses

The genus *Orthopoxvirus* contains multiple species that infect humans, including variola, monkeypox, vaccinia (includes buffalopox), and cowpox.[280] Variola and monkeypox are often life-threatening diseases, whereas vaccinia and cowpox generally are associated with local lesions. The eradication of smallpox represents one of the greatest public health achievements of the 20th century. After the successful eradication of smallpox, the routine use of vaccinia vaccine was discontinued. However, the threat of bioterrorism raises the prospect for an intentional use of smallpox.[281–283] Given the threat of bioterrorism, all health care personnel should be familiar with the skin manifestations of the category A bioterrorist agents (i.e., anthrax, plague, tularemia, Ebola, Marburg, Lassa, Machupo).[284] Multiple outbreaks of monkeypox in humans have been reported,[285,286] including a 2003 outbreak in the United States traced back to a consignment of rodents (giant Gambian rats) from Ghana. Thus the clinician is now confronted with having to distinguish the skin lesions of several possible poxvirus infections, including smallpox, complications of vaccinia (i.e., generalized or progressive vaccinia, eczema vaccinatum), and monkeypox. These lesions must be distinguished from varicella, disseminated herpes simplex, and other disorders characterized by a similar eruption, including meningococcal septicemia, coagulation disorders, and typhus.

After a 12- to 14-day incubation period (range, 7–17 days), the patient with smallpox typically develops high fever, malaise, and prostration with headache and backache. A maculopapular rash then appears on the mucosa of the mouth and pharynx, face, and forearms and spreads to the trunk and legs. Within 1 to 2 days, the rash becomes vesicular and later pustular. The pustules are characteristically round, firm, and deeply embedded in the skin. Crusts begin to form after 7 to 9 days; the eschars later separate, leaving pits and scars. Bacterial superinfection of skin lesions may complicate smallpox. The rash of variola differs from that of varicella in several ways. First, the lesions of variola appear during a 1- to 2-day period and evolve at the same time, whereas the lesions of varicella demonstrate different stages of maturation and generally appear in crops every few days. Second, the lesions of variola tend to involve the extremities and face, whereas the lesions of varicella have a centripetal predilection with a greater concentration of lesions on the trunk than on the face and extremities.

Also, varicella lesions are almost never found on the palms and soles. Finally, the lesions of variola are much more deeply embedded than the rash of varicella, where the lesions are more superficial. The rash of smallpox may be confused with SJS, measles, and coxsackievirus infections.

Vaccinia vaccine is still being provided to members of the US military. Recognition and management of the complications of vaccinia vaccination have been summarized.[287,288] Vaccinia vaccination leads to the local lesion at the site of immunization. Local complications include satellite lesions, lymphangitis, secondary bacterial infections, lesions from inadvertent remote inoculation, and progressive vaccinia at the site of the vaccination most commonly in immunosuppressed persons. Disseminated lesions include generalized vaccinia and eczema vaccinatum. Generalized vaccinia refers to a relatively benign generalized eruption in which each lesion is identical to its primary smallpox vaccination. The incidence has been reported to range from 23 to 242 per 1 million first-time vaccinees. Severe cases can be treated with variola immune globulin, cidofovir, or both. After vaccinia vaccination, patients also may develop a generalized EM-like rash. This is a benign condition and does not require therapy.

Eczema vaccinatum occurs in persons with a history of eczema or atopic dermatitis regardless of disease activity or severity. It has also been reported in persons without a history of dermatologic conditions. Eczema vaccinatum is characterized by high fever and generalized lymphadenopathy with an extensive vesicular and pustular eruption. The syndrome begins concurrently or shortly after the onset of local vaccinial lesions in the vaccinee. It may also occur in contacts 5 to 19 days after exposure. There is a significant risk for secondary bacterial or fungal infections. This adverse reaction is associated with a poor prognosis and high mortality. Therapy consists of multiple doses of variola immune globulin, hemodynamic support, and treatment of secondary infections. Cidofovir might be useful for therapy, but there are no published clinical data on its efficacy. Historically, the rate of eczema vaccinatum per 1 million vaccinees was reported as 10.4 to 41.5.

Monkeypox is enzootic in squirrels and monkeys in the rain forests of western and central Africa. The disease appears to be endemic in these regions of Africa, but multiple outbreaks have been described. Clinical signs of monkeypox include respiratory distress, lymphadenopathy, and a centrifugally distributed vesiculopustular rash (Fig. 57.10). The case-fatality rate has been reported to be approximately 10% in persons not vaccinated against smallpox.

Skin Lesions in Immunocompromised Patients

The diagnosis of skin lesions in the immunocompromised patient is complex because of the wide range of potential microbial pathogens that may cause disease in patients with abnormal immune responses (Table 57.5).[289–297] In addition, in immunocompromised persons, common infections may have unusual manifestations, and such patients often have an insufficient response to conventional therapy. Johnson[289] recommends the following approach to cutaneous lesions suspected to be infectious. First, the most rapid and sensitive methods for detecting



FIG. 57.10 Monkeypox. Patient with pox during a monkeypox outbreak in the Democratic Republic of the Congo. *(From Jahrling PB. Smallpox and related orthopoxviral infections. In: Guerrant RL, Walker DH, Weller PF, eds.* Tropical Infectious Diseases: Principles, Pathogens and Practice. *3rd ed. Philadelphia: Saunders Elsevier; 2011:369–377.)*

TABLE 57.5 Types of Skin Infections in Immunocompromised Hosts by Pathophysiologic Events

TYPE OF INFECTION	PATHOGEN	SITE OF INFECTION	HEALTHY HOST	COMPROMISED HOST
Primary skin infections (common pathogens)	Group A *Streptococcus*	Epidermis, hair follicles	Impetigo, ecthyma, folliculitis	Soft tissue infection, necrotizing
Cellulitis, abscess	*Staphylococcus aureus*	Dermis	Abscesses, intertrigo	Soft tissue infection
Unusually widespread cutaneous infection	Dermatophytes, *Candida* spp. *Candida* spp. HSV VZV EBV MCV HPV	Epidermis, intertriginous sites, hair follicles Oropharynx, esophagus, genitalia	Dermatophytosis; epidermal (limited), folliculitis Candidiasis; intertrigo, genital Localized herpes, resolves spontaneously Herpes zoster (mild) MCV (localized, nonfacial) Common and mucosal warts	Dermatophytosis; epidermal (extensive), folliculitis Candidiasis; intertrigo, folliculitis, mucosal Chronic herpetic ulcers Herpes zoster (extensive) Hairy leukoplakia Widespread MCV, resistant to therapy Widespread warts; squamous cell carcinoma in situ
Opportunistic primary cutaneous infection	NTM *Nocardia* Molds *Prototheca*	Dermis, hypodermis	Swimming pool granuloma	Soft tissue infection ± necrosis Septicemia
Systemic infection metastatic to cutaneous and subcutaneous sites	Bacteria, fungal pneumonitis with fungemia	Dermis, hypodermis	Soft tissue infection ± necrotic nodules	Soft tissue infection ± necrotic nodules

EBV, Epstein-Barr virus; *HPV,* human papillomavirus; *HSV,* herpes simplex virus; *MCV,* molluscum contagiosum virus; *NTM,* nontuberculous mycobacteria; *VZV,* varicella-zoster virus.

Data from Johnson RA. The immune compromised host in the twenty-first century: management of mucocutaneous infections. Semin Cutaneous Med Surg. *2000;19:19–61.*

microbes both histologically and immunologically should be used. Second, appropriate cultures and stains should be obtained to optimize the chance for identifying the pathogen. A 6- or 8-mm punch biopsy is usually adequate. Half of the tissue is sent for histopathologic evaluation by routine methods and by special stains for fungi, mycobacteria, and bacteria. The other half is sent to the microbiology laboratory for culture of aerobic and anaerobic bacteria, mycobacteria, and fungi (at 25° and 37°C) and for Gram stain, acid-fast, modified acid-fast, and direct fungal stains of touch preparations or ground tissue. Recommendations for processing specimens have been published.[292]

Solid-organ transplant recipients are at high risk for disease due to opportunistic bacterial, viral, and fungal pathogens. The risk for infection and the most likely infecting pathogen depend on the type of transplant; type and dose of immunosuppressive medications; time since transplantation; presence of coexisting diseases, including viral infections; and epidemiologic exposures.

Cutaneous infections in immunocompromised persons may be categorized into four groups based on pathophysiology: (1) infection originating in skin that is typical of those occurring in immunocompetent persons, albeit with the potential for more serious illness; (2) extensive cutaneous involvement with pathogens that normally produce trivial or well-localized disease in immunocompetent patients; (3) infection originating from a cutaneous source that is caused by opportunistic pathogens that rarely cause disease in immunocompetent patients but may cause either localized or widespread disease in immunocompromised patients; and (4) cutaneous or subcutaneous infection that represents metastatic spread from a noncutaneous site. Only life-threatening infections with cutaneous manifestations are discussed further here. In general, most cases of skin infection result from secondary dissemination after initial infection of the lungs or other organ systems. Primary cutaneous infection resulting from direct inoculation is less common. In transplant patients infections with viruses and fungi are probably more common than skin infections caused by bacteria.

Immunocompromised patients are at increased risk for the development of cellulitis due to *S. pyogenes* and *S. aureus*. Neutropenic patients are also susceptible to more unusual pathogens, such as members of the family Enterobacteriaceae and *Pseudomonas* spp. Patients with leukemia or impaired cell-mediated immunity may develop erysipelas-like lesions due to *Candida* spp. or *Cryptococcus neoformans*. Both local and diffuse skin infections with herpesviruses, especially HSV and VZV, are very common. Cutaneous CMV infection has a highly variable appearance that may include nodules, ulcers, indurated plaques, maculopapular eruptions, vesicles, and petechiae.

In immunocompromised patients cutaneous lesions resulting from hematogenous spread of infection are caused by three classes of organisms: (1) *P. aeruginosa* and other bacteria; (2) the endemic systemic mycoses caused by *Histoplasma capsulatum, Coccidioides* spp., and, rarely, *Blastomyces dermatitidis;* and (3) the opportunistic organisms *Aspergillus, C. neoformans, Candida, Rhizopus,* and *Nocardia. P. aeruginosa* may cause either cellulitis or ecthyma gangrenosum, which may develop in the absence of bacteremia. Patients with malignancy may develop sepsis associated with a variety of uncommon bacteria that are also associated with skin lesions (e.g., *A. hydrophila, C. canimorsus, Clostridium septicum,* mycobacteria, and *Salmonella enterica* serovar Typhimurium). Most commonly, *H. capsulatum* causes cellulitis, but it may also cause papules, nodules, pustules, and hemorrhagic lesions. Metastatic spread to the skin from noncutaneous sites of infection most commonly occurs with *Aspergillus* spp., *C. neoformans, Candida* spp., *Rhizopus* spp., and *Nocardia.* With the exception of *Candida,* the initial portal of entry is the respiratory tract. However, the respiratory tract infection may be asymptomatic, with the initial signs of illness seen in the skin. In neutropenic patients cutaneous lesions due to *Aspergillus* spp. are often found in association with the sino-orbital form of disease. Neutropenic patients may also develop disseminated infection with other fungi, such as *Fusarium* spp. and *Trichosporon beigelii.*

HIV infection commonly results in dermatologic disorders in both adults and children.[298–302] Clinically, the skin lesions associated with HIV infection may be classified by morphologic appearance, stage of HIV infection, pathophysiology (infectious, neoplastic, vascular, miscellaneous), and, for infectious diseases, etiologic agent. Skin eruptions are observed in greater than 50% of patients with primary HIV infection, generally developing on day 1 to 5 of the acute illness. The rash characteristically consists of 10 to hundreds of 5- to 10-mm, oval or round, pink to deep-red macules or slightly raised papules. Other skin manifestations have included diffuse urticaria, vesicular and pustular exanthema, desquamation of palms and soles, and alopecia. Like other immunocompromised patients, individuals with AIDS develop infections with opportunistic pathogens that rarely, if ever, cause infection in immunocompetent people. These include unusually severe or persistent herpes viral infections (HSV, VZV, CMV), staphylococcal infections including CA-MRSA, nontuberculous mycobacterial infections, disseminated candidiasis, infections with endemic fungi, ectoparasites, and soil-dwelling fungi.

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B Upper Respiratory Tract Infections

58 The Common Cold

Yehuda Z. Cohen

SHORT VIEW SUMMARY

Definition
- The common cold is a clinical syndrome of mild to moderate severity that includes sore throat, sneezing, rhinorrhea, nasal congestion, and cough.
- A precise definition of the common cold is difficult because numerous viruses serve as etiologic agents, and there can be significant variability in symptoms and severity of illness.

Microbiology
- Rhinoviruses are responsible for approximately half of common colds.
- Coronavirus, adenovirus, respiratory syncytial virus, parainfluenza virus, influenza, and metapneumovirus also cause the common cold.

Epidemiology and Transmission
- Common colds occur four to six times per year in children and one to three times per year in adults.
- There is a seasonality to the viruses that cause the common cold. In temperate climates, rhinovirus infections peak in the spring and fall.
- The nasal and conjunctival mucosa are reliable portals of entry for rhinoviruses, in contrast to the mouth and throat.

Diagnosis
- The diagnosis of the common cold is a clinical diagnosis.

Therapy
- Directed symptomatic therapy is the mainstay of treatment.
- Vitamin C and *Echinacea* have not been shown to be effective for treatment of the common cold. Zinc lozenges may reduce the duration of the common cold.

Prevention
- No currently available interventions, including hand hygiene, have been shown to consistently prevent the common cold.

The common cold is a clinical syndrome of mild-to-moderate severity that includes sore throat, sneezing, rhinorrhea, nasal congestion, and cough. The common cold is universally experienced and easy to self-diagnose, although there may be significant variability in the prominence of symptoms and severity. Infection with any of numerous viruses causes the illness, and the frequency of each virus can vary over time and geographic site. Although there has been great progress in understanding the etiology of the common cold, efforts to prevent and treat the common cold have had little success.

ETIOLOGY

The common cold has been ubiquitous since at least ancient times, and references to common cold syndromes appear in early Chinese and Egyptian medical texts. The use of the term *common cold* first appeared in the 1530s, and derives from the similarity of these symptoms to symptoms that occur on exposure to cold weather.[1] The notion that the common cold was related to a chilling of the body persisted into the 20th century. In the early 20th century, evidence began to accumulate that a nonbacterial "filterable agent" was the likely agent of the common cold. Experiments in which bacteria-free filtrates of nasal secretions transferred cold symptoms from one person to another demonstrated that these filterable agents, later known as viruses, caused the common cold.[2]

Epidemiologic studies conducted in New York and Seattle in the 1960s began to provide some information about which viruses were responsible for the common cold, but the laboratory techniques needed to reliably identify these viruses were still being developed.[3] A large epidemiologic study performed in Tecumseh, Michigan, was the first large prospective study to make extensive use of serology to diagnose respiratory infections. Approximately 1000 people were followed longitudinally over several years in the 1960s and 1970s. The study found that the most frequent cause of respiratory illness was rhinovirus.[4] Coronavirus, influenza, parainfluenza virus, respiratory syncytial virus (RSV), and adenovirus were also associated with upper respiratory infections.

With the emergence of polymerase chain reaction (PCR) and other molecular techniques, additional respiratory pathogens such as human bocavirus and human metapneumovirus have been discovered. Human bocavirus was first reported in children with wheezing and pneumonia.[5,6] The role of human bocavirus as a cause of the common cold remains uncertain. Although it is often detected by PCR, particularly in young children, concurrent respiratory illness is often not present (see Chapter 147).[7,8] Human metapneumovirus infects most children by 5 years of age and accounts for a small but significant proportion of respiratory infections in children and adults (see Chapter 159).[9–11]

Longitudinal, community-based studies using PCR have confirmed rhinovirus as the cause of approximately half of respiratory illnesses. The next leading cause is often influenza, adenovirus, or coronavirus.[8,12–16] There are more than 156 rhinovirus antigen types, with 15 to 30 types typically circulating simultaneously at a given site, although up to 74 rhinovirus types have been found to cocirculate.[17–19] There does not appear to be a correlation between particular rhinovirus antigen types and severity of illness.[18] Influenza is frequently found to be a significant cause of respiratory illness in epidemiologic studies, but it is not generally associated with the common cold. Nonetheless, symptoms of influenza can range from mild to severe and may manifest similarly to symptoms of the common cold.[20]

In early epidemiologic studies that relied solely on cell culture or serology, an infectious agent could not be detected in the vast majority of cases.[3] With the use of PCR, however, multiple respiratory viruses are often found in individuals with respiratory infections.[21–23] When PCR was performed on 225 children in childcare centers during episodes of respiratory illnesses, more than one virus was detected in most children.[13] PCR has also been shown to detect high rates of infection

with respiratory viruses among asymptomatic individuals.[8,24,25] The detection of multiple pathogens appears to be at least partially related to prolonged shedding of some respiratory viruses. The significance of the detection of multiple viruses in the etiology of respiratory illnesses and the common cold remains uncertain.

EPIDEMIOLOGY

The Cleveland Family Study, conducted in the 1940s and 1950s, was the first study to reliably demonstrate that the incidence of respiratory infections is highest in children younger than 4 years of age. The study also found that there was an increased risk of illness when another family member was sick and that children were most often responsible for introducing the infection to the family.[26] In the Tecumseh, Michigan, study, children 4 years of age and younger experienced an average of four to six respiratory illnesses per year, and adults experienced an average of one to three respiratory illnesses per year. Common cold incidence was also found to decline with age, with persons older than 60 years of age having the lowest incidence.[27] Daycare attendance significantly increases the frequency of respiratory illnesses in children, although this difference diminishes the longer the child remains in daycare.[28–30]

There is a seasonality to the viruses that cause the common cold, the reasons for which are not completely understood. In temperate climates, rhinovirus infections peak in the fall but occur year-round. Influenza and RSV infections occur mainly in winter and early spring, and parainfluenza peaks in the spring and fall.[31,32] In tropical climates, influenza and RSV infections peak in the rainy season.[33] Hypotheses for the seasonality of these and other viral infections have included seasonal changes in effective contact rates (e.g., the start of the school year) and seasonal changes in the durability of the pathogen in the environment.[34]

It has been estimated that 500 million noninfluenza viral respiratory infections occur each year in the United States. Although the common cold is typically a mild-to-moderate illness, its economic impact is enormous. The total economic impact of the common cold approaches $40 billion annually, with most of these costs due to days missed from work. Based on these estimates, the economic burden of the common cold is greater than conditions such as hypertension, chronic obstructive pulmonary disease, and heart failure.[35]

Transmission

Early studies demonstrated that very low doses of rhinovirus deposited in the nose reliably produced a cold.[36] The conjunctival mucosa also serves as a good portal of entry, in contrast to the mouth or throat.[37] Following inoculation, virus is typically first recovered in the nasopharynx and subsequently spreads anteriorly. Virus can be recovered from the nasal mucosa for up to 3 weeks.[38] Close contact is needed for efficient transmission of rhinovirus. Transmission is infrequent between childless couples, unless the infected individual sheds large amounts of virus and spends many hours with his or her partner.[17] Additionally, there is poor transmission in office settings.[39] The predominant mode of transmission for rhinovirus infection is not fully established. There is debate in the literature regarding whether the aerosol route or direct contact (self-inoculation after contact with the environment) is the most important mode of transmission, although direct contact appears to be most efficient. Surprisingly, low yields of rhinovirus are recovered from the coughs and sneezes of individuals with rhinovirus colds. In a number of studies in which uninfected individuals were placed in close proximity to infected individuals, few infections were observed, suggesting that direct contact was the primary mode of transmission.[40] However, another experiment found high rates of transmission via the aerosol route and no transmissions after contact with fomites.[41] It is worth noting that there is significant variability among individuals in the amount of aerosolized droplets that are produced during coughing or even quiet breathing.[42,43] This variability may account for at least some of the conflicting findings reported from transmission studies.

The optimal conditions for transmission differ among the numerous viruses that cause the common cold. Influenza and RSV, which are enveloped viruses, are more susceptible to degradation and remain infectious on surfaces for only minutes to hours. Rhinoviruses, which are nonenveloped, can be recovered from contaminated surfaces for days.[44] Rhinoviruses and adenoviruses survive best at high humidity, whereas survival is increased for RSV, parainfluenza, and influenza A at low humidity.[45,46]

Predisposing Factors

Exposure to children is the most significant risk factor for spread of the common cold. Early community-based studies found consistently higher frequencies of respiratory illness in women compared with men, which was likely a consequence of the women having more frequent exposure to children.[3] Cold weather has long been thought to be a risk factor for the common cold, but this idea was mostly dismissed in the 20th century. Although some studies have supported an association between cold weather and the common cold, others have not.[47] The possibility that cold weather directly contributes to the pathogenesis of the common cold has continued to intrigue some investigators, who generally hypothesize that cold weather decreases respiratory immune defenses.[48] It has been shown that antiviral defenses are reduced in the nasal cavity as a result of its reduced temperature (compared with core body temperature) and that rhinovirus preferentially replicates in the nasal cavity for this reason.[49] However, there is currently no evidence that exposure to cold weather further reduces these defenses.

Crowding and poor ventilation are frequently cited as risk factors for respiratory infection. Severe crowding is associated with an increased rate of respiratory infection, and outbreaks of respiratory infection have been reported in crowded shelters following natural disasters.[50] It is less clear that the "crowding" that occurs in schools or other common indoor settings significantly increases the risk of the common cold. Little evidence exists that poor ventilation is a risk factor for the common cold. In one experiment, there was no difference in rates of the common cold between poorly ventilated and well-ventilated offices.[51] Recirculation of aircraft cabin air on 2-hour flights, as opposed to ventilation with fresh air, does not increase the risk of the common cold.[52]

Respiratory infections occur most frequently in children, yet there is significant variability in incidence among children, suggesting genetic risk factors. A number of studies have found that polymorphisms that result in low levels of mannose-binding lectin, a pattern recognition receptor, increase susceptibility to respiratory infections, particularly in young children.[53–55] Pattern recognition receptors recognize conserved molecular structures of particular pathogens and play a key role in the innate immune system. Polymorphisms in Toll-like receptors, another pattern recognition receptor, have also been associated with increased or decreased risk of respiratory infection in children.[55] However, associations between pattern recognition receptor deficiencies and risks of viral and bacterial infections have been inconsistent, and the significance of these findings remains uncertain. Mutations in the viral RNA-sensing protein MDA5 appeared to increase susceptibility to rhinovirus infection in a child with recurrent respiratory infections.[56]

Unique attributes of the common cold have allowed for studies that examine risk factors not typically associated with infectious diseases. Because the common cold is a mild, self-limiting illness, experimental challenge studies can readily be performed. Additionally, the common cold recurs on a frequent basis, which facilitates the conduct of observational studies. For these reasons, a significant body of work has been published on how factors such as stress, social ties, and sleep duration contribute to the risk and severity of the common cold. In a study in which 294 healthy participants were challenged with common cold viruses, psychological stress was associated with increased rates of both viral infection and clinical colds in a dose-response manner.[57] The duration of stress has also been shown to be important, with severe chronic stress, as opposed to acute stress, associated with an increased risk of colds in a challenge study.[58] In other challenge studies, participants with greater social ties to family, friends, work, and community and sociability in general (a combination of traits including extraversion) had a decreased risk of developing a cold.[59,60] In challenge studies that measured sleep duration, individuals who slept on average fewer than 7 hours a night had a significantly greater risk of developing a cold.[61,62] The physiologic mechanisms by which these factors increase or decrease susceptibility to the common cold are yet to be fully elucidated but may be related to inappropriate regulation of inflammation.[63]

Immunity

The common cold recurs on a frequent basis. This is in contrast to many other viral infections in which protective immunity, typically mediated by neutralizing antibodies, is conferred after a single infection. The frequent recurrence of the common cold is primarily a result of the fact that the common cold is caused by different viruses, each with numerous viral antigenic types. (Table 58.1) Rhinovirus alone consists of at least 156 types, and there are at least 57 adenovirus types. Neutralizing antibodies produced against one virus or serotype will not prevent infection with another. Another factor is that the antibody response generated following respiratory virus infection is often not fully protective, even on rechallenge with the same serotype.

Rhinovirus infection induces neutralizing antibodies in both nasal secretions and serum after a period of 1 to 2 weeks.[64] These antibodies are extremely type-specific and can persist for years.[65,66] The fact that the incidence of the common cold decreases with age supports the notion that seroconversion confers some protection against infection. Challenge studies have confirmed that high levels of serotype-specific neutralizing antibody titers can be protective. In a rhinovirus serotype 39 challenge study, 70% of participants with high baseline serum antibody titers (>1:16) against this serotype became infected, compared with greater than 95% of participants with low or intermediate titers. The participants with high baseline serum titers who became infected demonstrated the lowest degree of viral shedding and the fewest symptoms, whereas participants with low titers demonstrated the greatest viral shedding and the most symptoms.[66] In another study, protection on reinfection with rhinovirus serotype 2 was associated with higher levels of rhinovirus serotype 2–specific immunoglobulin A in nasal secretions or serum.[64] Trials of inactivated rhinovirus vaccines conducted in the 1960s demonstrated that such vaccines could provide protection against homologous virus.[67,68] However, no protection was conferred against heterologous serotypes.

It is not clear why respiratory virus infection does not typically induce protective immunity. With regard to rhinovirus, one possibility may be that the antibody response is primarily directed against a nonprotective epitope located inside the viral capsid, which is exposed only on binding of rhinovirus to its cellular receptor intracellular adhesion molecule-1.[69] Evasion of the host immune system by these viruses likely plays a role in the failure of the host to establish protective immunity.[70,71] Human metapneumovirus, which is known to have only two genotypes, also exhibits high reinfection rates.[72,73] Although reinfection with respiratory viruses is common, the subsequent infection is typically of reduced severity.

CLINICAL MANIFESTATIONS

Incubation periods vary for the different viruses that cause the common cold.[74] The incubation period for rhinovirus is typically 2 to 4 days. The common cold can manifest with a wide variety of symptoms based on the causative virus and the age and immune status of the host.

TABLE 58.1 Viruses Associated With the Common Cold

VIRUS GROUP	ANTIGENIC TYPES
Rhinoviruses	>156 types
Coronaviruses	5 types
Parainfluenza virus	5 types
Respiratory syncytial virus	2 types
Influenza virus	3 types[a]
Adenovirus	57 types
Metapneumovirus	2 types
Other viruses: enteroviruses, bocavirus	

[a]Multiple subtypes.

Modified from Turner R. Rhinovirus. In: Bennett J, Dolin R, Blaser M, eds. Principles and Practice of Infectious Diseases. *8th ed. Philadelphia: Elsevier; 2013:2113–2121.*

Common cold symptoms often begin with a sore or scratchy throat. Rhinorrhea and sneezing typically follow and persist for several days, after which nasal congestion and cough become the dominant symptoms.[75] Mucus color often changes from clear to yellow or green during the course of a cold. This does not represent the development of a bacterial infection but is a result of myeloperoxidase and other enzymes produced by neutrophils.[76] The mean duration of symptoms is 7 to 10 days, but nasal congestion and cough can persist for weeks. Other symptoms that may be experienced include headache, hoarseness, chilliness, and malaise. Fever is uncommon in adults but common in children.[77,78]

PATHOGENESIS

The symptoms of a common cold caused by rhinovirus infection are a result of the host innate immune response and not virus-induced tissue damage. Although influenza is known to damage epithelial cells, the nasal epithelium remains intact during rhinovirus infection, and the only distinguishing feature on biopsy is an increased number of neutrophils in the submucosa and epithelium.[79,80] Additionally, very few cells in the nasal epithelium and nasopharynx are infected during rhinovirus colds.[81] The role of neutrophils in the pathogenesis of the common cold and viral infections in general remains unclear.[82,83] Following rhinovirus infection of human epithelial cells in vitro, levels of cytokines increase,[84] and elevated levels of inflammatory mediators and cytokines, including interleukin-1β (IL-1β), IL-6, and IL-8, have been found in nasal secretions during colds.[85,86] In rhinovirus challenge studies, symptom severity has been correlated with IL-8 and bradykinin levels in nasal secretions.[87,88] The roles of particular cytokines in the pathogenesis of the common cold have not been fully elucidated. Production of bradykinin appears to contribute to both sore throat and rhinorrhea, and administration of intranasal bradykinin has been shown to cause sore throat and rhinorrhea in healthy participants.[89,90] Histamine does not play a role in the pathogenesis of the common cold.[89]

The watery nasal discharge that is characteristic of the early phase of rhinorrhea is derived from a mix of nasal glandular secretions and plasma transudate. Nasal glands are activated to produce secretions through cholinergic stimulation.[91,92] The nasal congestion that occurs later in the course of the common cold is a result of the dilation of large capacitance veins (sinuses) in the nasal epithelium that can swell and block the nose.[93] Sympathetic stimulation of these venous sinuses results in vasoconstriction and decreased swelling. Interestingly, the nasal venous sinuses are similar to erectile tissue, with some data suggesting that they may have once played a role in sexual behavior.[94,95] Even in healthy individuals, the nasal venous sinuses undergo phases of congestion and decongestion, known as the nasal cycle. During this cycle, resistance increases in each nasal airway on a cyclical basis. In the setting of the inflammation and increased mucus secretion that occurs with the common cold, the effects of this cycle are amplified, resulting in restricted airflow and nasal obstruction.[96] As a result of the nasal cycle, it is common during a cold for one nasal airway to be patent while the other is obstructed.

The pathogenesis of cough as part of the common cold is not well understood. A dry cough may be the result of virus-induced hypersensitivity of airway sensory nerves. This increased sensitivity may be mediated by bradykinin and tachykinin.[97,98] Also, rhinovirus can invade the lower respiratory tract, causing lower respiratory tract symptoms in some individuals.[99] In the later phase of a cold, cough is often a result of irritation of the larynx with mucus.

DIFFERENTIAL DIAGNOSIS

The common cold is a straightforward diagnosis, and the vast majority of patients will not seek medical care. Sometimes patients may seek medical care for the sore throat that occurs at the start of a cold, which may persist for days in the absence of other common cold symptoms. As opposed to bacterial pharyngitis, patients with pharyngitis as part of a cold generally feel otherwise well and do not have fever or tonsillar exudates. A rapid streptococcal antigen test will be negative. Rhinorrhea can occur in some noninfectious syndromes, particularly allergic and nonallergic rhinitis, but patients will frequently differentiate these syndromes from the common cold on their own. Patients with allergic

rhinitis typically have concomitant allergic conjunctivitis, which is characterized by itching, tearing, and conjunctival edema. Nonallergic rhinitis is a chronic condition in which the most prominent symptoms are nasal blockage and postnasal drip.[100]

Multiplex PCR assays for the diagnosis of upper respiratory tract infections have been adopted in many hospitals. These assays can test for more than 20 respiratory pathogens at once including viruses and bacteria. Multiplex PCR assays are used in emergency departments and hospital wards to guide treatment decisions and do not have a place in the diagnosis of the typical common cold. Even in the hospital setting, the optimal implementation strategy for these assays has been a matter of debate.[101]

COMPLICATIONS

The common cold is a self-limiting illness. However, infection with common cold viruses may increase the risk for bacterial infection. Otitis media in particular is highly associated with upper respiratory viral infections, with one study finding that 37% of upper respiratory tract infections in young children were complicated by acute otitis media.[102] The most important factor in the development of acute otitis media in the setting of viral respiratory infections is congestion, which results in eustachian tube dysfunction. This dysfunction has a number of consequences, including decreased drainage of secretions produced in the middle ear and loss of protection of the middle ear from nasopharynx secretions.[103] Other factors also appear be involved in the development of otitis media, including an alteration of host immune defenses (see Chapter 61).[104] Specific viruses may differ in their propensity to cause otitis media,[102] and the risk for otitis media may be increased by certain combinations of viruses and bacteria.[105]

Sinusitis, which typically manifests as nasal congestion, purulent nasal discharge, and facial pain or pressure, is a common complication of the common cold. The vast majority of these cases are viral and typically resolve after approximately 10 days.[106,107] Bacterial sinusitis is suggested by symptoms that persist for longer than 10 days or a biphasic illness that begins to resolve but then worsens. Bacterial sinusitis comprises only 0.5% to 2% of episodes of sinusitis in adults[108] but may complicate 8% of upper respiratory tract infections in young children.[109]

A clear association exists between viral respiratory infections and asthma and chronic obstructive pulmonary disease exacerbations. In both adults and children with a history of asthma, approximately 85% of colds were associated with asthma symptoms.[110,111] Rhinovirus infections in particular have also been associated with the onset of wheezing illness in children and may play a direct role in the pathogenesis of asthma.[112,113]

Cough may persist for weeks after the resolution of other cold symptoms. This persistent cough has been referred to as a postviral or postinfectious cough. The pathogenesis of postinfectious cough is unclear but may be related to increased cough sensitivity as a result of inflammation. Postinfectious cough does not typically last longer than 8 weeks.[114] There is no specific treatment.

THERAPY

Symptomatic Therapy

The symptoms of the common cold evolve over the course of the illness. Therefore treatment should be directed at specific symptoms (Table 58.2). Studies that have demonstrated the effectiveness of symptomatic treatments have been performed in adults, and the few studies conducted in children have failed to show benefit. For this reason, the US Food and Drug Administration has recommended against the use of antihistamines, decongestants, and cough medicines in children younger than 4 years of age.

Treatment of sore throat includes the use of acetaminophen, nonsteroidal antiinflammatory drugs, or a topical anesthetic such as benzocaine. Anticholinergics are used to treat rhinorrhea. Anticholinergics block the cholinergic stimulation that causes nasal glandular secretion, and intranasal administration of the anticholinergic agent ipratropium is effective at reducing rhinorrhea.[115–117] The most common side effects of intranasal ipratropium are nasal dryness and nasal bleeding.

TABLE 58.2 Symptomatic Treatment for the Common Cold

SYMPTOM	ETIOLOGY	TREATMENT	EXAMPLES
Sore throat	Bradykinin?	Analgesics, topical anesthetics	NSAIDs Acetaminophen Benzocaine lozenges
Rhinorrhea	Nasal glandular secretions	Anticholinergics	Diphenhydramine Doxylamine Ipratropium nasal spray
Nasal obstruction	Engorgement of nasal venous sinuses	Adrenergic agents	Pseudoephedrine Oxymetazoline nasal spray

NSAIDs, Nonsteroidal antiinflammatory drugs.

First-generation antihistamines are frequently used for treatment of the common cold. Their use developed from the mistaken idea that common cold symptoms are mediated by histamine.[118] Nevertheless, the anticholinergic properties of first-generation antihistamines make them effective for the treatment of rhinorrhea.[119–121] The most common side effect of first-generation antihistamines is sedation. This attribute can be useful in the setting of the common cold, and first-generation antihistamines are typically included in nighttime cold medicine formulations. Second-generation antihistamines such as loratidine and cetirizine lack anticholinergic properties and are not effective for treatment of the common cold.[122–124]

Adrenergic agents effectively constrict the nasal venous sinuses and thereby relieve congestion. The nasal veins are five times more sensitive than the heart to the effects of epinephrine,[125] which allows for the treatment of nasal congestion with adrenergic agents without pronounced cardiovascular side effects. Both oral and topical adrenergic agents have been shown to be effective treatments for nasal congestion,[126–129] but topical agents such as oxymetazoline are more potent and reliable.[130] Phenylephrine, which has gained prominence in over-the-counter preparations in recent years, does not appear to be as effective as pseudoephedrine.[131,132] Dextromethorphan is commonly used for the treatment of cough, although it has not been specifically studied in the setting of the common cold. There is insufficient evidence regarding the utility of expectorants such as guaifenesin.

Homeopathic Remedies

Many remedies are marketed for the prevention and treatment of the common cold including vitamin C, *Echinacea*, and zinc as well as a host of "immune-boosting" formulations, which often contain vitamins and herbal supplements. There is no evidence to support the use of vitamin C or other vitamins in otherwise healthy individuals for the treatment or prevention of the common cold.[133,134] *Echinacea* has similarly been shown to be of no benefit.[135,136] The efficacy of zinc, administered as zinc gluconate or zinc acetate lozenges, is controversial. Zinc has been shown to have antiviral properties against a number of respiratory viruses in vitro,[137–140] but the results of clinical trials have been mixed, with some trials demonstrating benefit and others showing no effect.[141–144] Systematic reviews and meta-analyses have generally concluded that zinc lozenges may shorten the duration of the common cold by about 2 days.[145–147]

Antiviral Therapy

Studies conducted in the 1980s found that intranasal administration of interferon could reduce the risk of developing a cold by 78% to 88% when administered prophylactically following rhinovirus challenge or in the setting of an ill family member.[148–150] However, when intranasal interferon was begun after the start of symptoms, it was ineffective.[151] Additionally, after taking interferon for 1 to 2 weeks, participants developed nasal stuffiness and bleeding. For these reasons, further development of interferon for the treatment or prevention of the common cold was abandoned.[152] Other efforts to develop antiviral therapies for the common cold have focused specifically on rhinovirus. Strategies have included blockade of the intracellular adhesion molecule-1 receptor, capsid-binding agents, and 3C protease inhibitors. Pleconaril, a

capsid-binding agent, was found to reduce the duration of common cold symptoms by 1 day in two trials.[153] However, pleconaril was not granted US Food and Drug Administration approval in 2002 due to safety concerns. An intranasal formulation of pleconaril failed to show efficacy for the prevention of rhinovirus infection (ClinicalTrials.gov Identifier: NCT00394914).

Hand Hygiene

Hand hygiene is universally recommended to reduce the transmission of infectious diseases. However, the efficacy of hand hygiene for the prevention of the common cold has not been consistently demonstrated in clinical trials. The implementation of hand hygiene programs was shown to reduce rates of the common cold in individuals in daycare centers and military trainees.[154–156] In a randomized controlled trial conducted in the homes of families with children in daycare, however, a hand hygiene intervention did not reduce the rates of respiratory illness.[157] In another randomized trial that enrolled university students, the use of an alcohol lotion previously shown to be highly effective in removing rhinovirus from the hands was not found to reduce the incidence of rhinovirus infection.[158] As most colds are self-limited, the use of any potential therapy should assess the benefit-risk ratio as well as the cost.

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The complete reference list is available online at Expert Consult.

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59 Pharyngitis

Anthony R. Flores and Mary T. Caserta

SHORT VIEW SUMMARY

Definition
- Pharyngitis is defined as the triad of sore throat, fever, and pharyngeal inflammation.
- Generally a primary disease, pharyngitis may be associated with systemic disorders.

Epidemiology
- Pharyngitis is one of the most common disorders in adults and children, with more than 10 million ambulatory visits per year.
- The highest burden of disease is found in children and young adults, with 50% of cases identified between the ages of 5 to 24 years.
- In temperate climates most cases occur in winter months, corresponding with peaks in respiratory viruses.

Microbiology
- Viruses are the single most common cause of pharyngitis, with adenovirus commonly identified (see Table 59.1).
- Group A *Streptococcus* (GAS) is the bacterial cause for which ample evidence exists for antibiotic therapy to prevent postinfectious sequelae.
- *Fusobacterium necrophorum* is now recognized as a cause of pharyngitis in adolescents and young adults with potential severe complications (i.e., Lemierre syndrome).
- *Arcanobacterium haemolyticum* may cause pharyngitis associated with a rash, seen particularly in adolescents and young adults.

Diagnosis
- Essential to diagnosis is the identification of treatable causes (e.g., GAS) to prevent complications.
- Signs and symptoms of GAS pharyngitis include acute onset of sore throat with tonsillar or pharyngeal exudates, tender anterior cervical lymphadenopathy, and fever (see Table 59.2).
- Signs and symptoms consistent with viral etiologies include conjunctivitis, coryza, oral ulcers, cough, and diarrhea.
- Testing for GAS pharyngitis should not be pursued in those with signs and symptoms indicative of a viral etiology (see Table 59.3).
- Rapid antigen detection tests (RADTs) or throat culture should be used to confirm the diagnosis of GAS in adults; a negative RADT should be backed up by throat culture in children.
- Specific techniques should be used to identify other causes where appropriate.

Therapy
- Treatment of pharyngitis is focused on prevention of postinfectious sequelae (e.g., acute rheumatic fever) from GAS.
- Penicillin and its derivatives remain the primary treatment for GAS pharyngitis (see Table 59.4).
- Antimicrobial therapy should not be used to prevent GAS pharyngitis except in special circumstances.
- Given the potential severity of complications from pharyngitis caused by *F. necrophorum*, signs of bacteremia or neck swelling warrant expansion of antibiotic therapy and further evaluation.

DEFINITION

Acute pharyngitis is typically described as the triad of sore throat, fever, and pharyngeal inflammation characterized by erythema and edema, although exudates, vesicles, or ulcerations may also be present.[1] Although pharyngitis may be a primary disorder, sore throat and pharyngeal erythema may also be prominent in systemic disorders, such as the acute retroviral syndrome, or part of a more generalized upper respiratory tract infection. Most cases of acute pharyngitis are due to common viral infections and are benign, self-limited processes. The appropriate recognition of patients with more complicated infections that require diagnostic evaluations and treatment is one of the challenges of primary care medicine.

Etiology

Viruses are the single most common cause of pharyngitis and account for 25% to 45% of all cases, often occurring with other signs or symptoms of upper respiratory tract infection (URI).[2,3,4] Depending on patient characteristics, including age and the presence of documented pharyngitis versus sore throat, rates up to approximately 60% have been identified, primarily in children and young adults.[5,6] Essentially all viruses known to cause URIs have been described in both adults and children with pharyngitis (Table 59.1). Although the methodology between different studies is highly variable, adenovirus and rhinovirus are frequently identified as the most prevalent viral cause of pharyngitis, reported in 12% to 27% of all cases.[2,5,6,7,8] Other respiratory viruses that cause pharyngitis include enteroviruses, influenza A and B, parainfluenza viruses, respiratory syncytial virus, coronaviruses, human metapneumovirus, and human bocavirus.[2,3,7,9,10,11] Several human herpesviruses, such as Epstein-Barr virus, herpes simplex virus (HSV) 1 and 2, and human cytomegalovirus (CMV), have also been reported to cause pharyngitis, as well as human immunodeficiency virus type 1 (HIV-1).

Streptococcus pyogenes, group A *Streptococcus* (GAS), is the bacterial etiology of greatest concern in cases of acute pharyngitis because of the association between GAS and acute rheumatic fever (ARF). GAS is responsible for approximately 10% to 15% of cases of pharyngitis in adults[12,13] and 15% to 30% of cases in children.[14] *Fusobacterium necrophorum*, a gram-negative, non–spore-forming anaerobe, is a bacterial cause of sore throat in 10% to 20% of cases of pharyngitis in adolescents and young adults[15,16,17] and the etiologic agent in up to 23% of cases of peritonsillar abscess.[18] The organism has also been implicated in recurrent or chronic sore throat syndromes and may be identified in up to 21% of such cases.[19] *Arcanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*), a gram-positive bacillus, has been recognized as a cause of pharyngitis for more than 60 years. *A. haemolyticum* has an incidence ranging from 0.2% to 0.5%, with the highest frequency of infection in adolescents and young adults.[20,21] *Corynebacterium diphtheriae* is also a cause of pharyngitis and is of particular concern for travelers to areas where vaccination programs are not well established or have failed.[22] Nontoxigenic strains of *C. diphtheriae* have been reported with increasing frequency in individuals with sore throat, but their contribution as a causative agent of pharyngitis remains in question.[23] Pharyngitis caused by gonorrhea should be considered in sexually active adolescents and adults. Throat cultures yield *Neisseria gonorrhoeae* in as many as 1% to 6% of individuals in sexually transmitted disease clinics.[24,25] *Mycoplasma pneumoniae,* identified in 3% to 14% of cases of pharyngitis, and *Chlamydia pneumoniae*, less frequently detected at

TABLE 59.1 Microbial Causes of Acute Pharyngitis

PATHOGEN	ASSOCIATED DISORDER(S)
Bacteria	
Streptococcus, group A	Pharyngitis, tonsillitis, scarlet fever
Streptococcus, group C and G	Pharyngitis, tonsillitis
Mixed anaerobes	Vincent angina
Fusobacterium necrophorum	Pharyngitis, tonsillitis, Lemierre syndrome
Neisseria gonorrhoeae	Pharyngitis, tonsillitis
Corynebacterium diphtheriae	Diphtheria
Arcanobacterium haemolyticum	Pharyngitis, scarlatiniform rash
Yersinia pestis	Plague
Francisella tularensis	Tularemia, oropharyngeal form
Treponema pallidum	Secondary syphilis
Viruses	
Rhinovirus	Common cold
Coronavirus	Common cold
Adenovirus	Pharyngoconjunctival fever
Herpes simplex types 1 and 2	Pharyngitis, gingivostomatitis
Parainfluenza	Cold, croup
Enteroviruses	Herpangina, hand-foot-mouth disease
Epstein-Barr virus	Infectious mononucleosis
Cytomegalovirus	CMV mononucleosis
Human immunodeficiency virus	Primary HIV infection
Influenza A and B	Influenza
Respiratory syncytial virus	Cold, bronchiolitis, pneumonia
Human metapneumovirus	Cold, bronchiolitis, pneumonia
Mycoplasma	
Mycoplasma pneumoniae	Pneumonia, bronchitis, pharyngitis
Chlamydia	
Chlamydia psittaci	Acute respiratory disease, pneumonia
Chlamydia pneumoniae	Pneumonia, pharyngitis

CMV, Cytomegalovirus; *HIV,* human immunodeficiency virus.
Modified from Alcaide ML, Bisno AL. Pharyngitis and epiglottitis. Infect Dis Clin North Am. *2007;21:449–469, vii; reproduced with permission.*

3% to 8%, should also be considered as potential etiologic agents of pharyngitis.[3,7,26]

EPIDEMIOLOGY

Pharyngitis is a common disorder in adults and children. In a prospective family study, 16% of adults and 41% of children reported an illness with sore throat over a 1-year time frame.[27] The incidence rate of medically attended tonsillitis in children has been estimated at 15 to 25 cases per 1000 children per year.[28] The National Ambulatory Medical Care Survey and the National Hospital Ambulatory Medical Care Survey have documented 6.2 to 9.7 million visits to primary care physicians, clinics, and emergency departments each year for children with pharyngitis and more than 5 million visits per year for adults.[29–31]

Four factors affect the epidemiology of pharyngitis reported in the literature. These include the age of the population studied, laboratory methods used to identify the causative microorganisms, season of the year, and the clinical severity of the illness. Despite these caveats, the highest burden of disease from pharyngitis is consistently found in children and young adults, with approximately 50% of cases diagnosed in patients from 5 to 24 years of age.[32] School-aged children from 5 to 18 years of age usually account for the greatest overall number of cases of pharyngitis, similar to disease from GAS.[27] The reported prevalence of GAS pharyngitis is influenced both by the age of the patient and the examination setting, with higher rates found in younger people evaluated in urgent care and emergency centers.[33] The most recent studies show GAS prevalence in cases of pharyngitis approaching 40% in children[34,35] and 17% in adults.[36] Population-based data demonstrate that serologically proven GAS pharyngitis occurs at a rate of 0.14 cases per child-year in the developed world and is estimated to be 5 to 10 times greater in developing communities.[37]

In temperate climates, most cases of pharyngitis occur in the winter and early spring, corresponding to peak times of respiratory virus activity. This is also true for GAS pharyngitis, where up to half of the cases in children may be due to this agent during these peak months.[26,38]

Between 49% and 62% of children[39] and 66% to 78% of adults[4,39] evaluated for sore throat or pharyngitis receive an antibiotic prescription, a rate much higher than the prevalence of GAS infection for which treatment is indicated.[29–31] In addition, recent surveys have demonstrated a significant increase in the use of broad-spectrum antibiotics for the treatment of pharyngitis, a practice that is thought to contribute to the growing problem of antibiotic resistance and the "medicalization" of a generally benign illness.[31]

Pathogenesis

The exact mechanisms responsible for the development of the signs and symptoms of pharyngitis have not been fully delineated. Early studies have demonstrated that bradykinin is induced in symptomatic rhinovirus infections and that bradykinin challenge in healthy volunteers produces significant sore throat when delivered either to the oropharynx or the nasal mucosa.[40,41] Other inflammatory mediators, including prostaglandins, have been postulated to play a role with bradykinin via their actions on sensory nerve endings in the pharynx.[42] Several randomized controlled trials have demonstrated a beneficial effect of either nonsteroidal antiinflammatory drugs or corticosteroids on throat pain, also suggesting that inflammatory mediators play a key role in the pathophysiology of sore throat.[43–47]

Among bacterial causes of pharyngitis, the pathogenesis of GAS has been studied most extensively. Multiple virulence factors have been identified that ultimately lead to the manifestation of acute pharyngitis.[48] Despite this growing fund of knowledge, major gaps exist regarding the events leading to tonsillopharyngeal disease. Furthermore, the contribution and mechanism of underlying asymptomatic carriage has been the subject of much speculation.[49,50] The role the immune system and possible molecular genetic changes in GAS play in asymptomatic carriage remains elusive. Proteins involved in immune avoidance (M protein, hyaluronic acid capsule, C5a peptidase), adherence to epithelial cells (pilus, fibronectin binding proteins, lipoteichoic acid), spread through host tissues (hyaluronidase, streptokinase, deoxyribonucleases), and numerous exotoxins (streptolysins, superantigenic toxins) have been described[48] but are beyond the scope of this chapter. Expression of these virulence factors leads to symptomatic pharyngitis and complications, including invasive disease, acute rheumatic fever, and acute glomerulonephritis. The mechanism by which GAS pharyngitis results in acute rheumatic fever is unknown. However, autoimmunity through molecular mimicry is suspected.[51] A growing body of evidence supports the existence of rheumatogenic GAS serotypes. Comparing M-type distribution between two periods separated by 40 years, Shulman and coworkers[52] were able to demonstrate that decreases or complete disappearance of certain M types were associated with the decline in incidence of acute rheumatic fever. Whether other strain-specific GAS virulence factors are involved is unknown.

MICROBIOLOGY

Although it is well documented that the etiology of pharyngitis in individual patients cannot be accurately discerned based on clinical characteristics alone, certain pathogens may cause more readily recognizable syndromes as outlined as follows.

Group A *Streptococcus*

Pharyngitis attributable to GAS is sudden in onset in older children and adults. Sore throat associated with GAS may result in difficulty

swallowing. Fever, headache, and gastrointestinal symptoms (nausea, vomiting, abdominal pain) are also associated with strep throat but are not always present. Physical examination generally reveals pharyngeal erythema, tonsillar enlargement, and a gray-white exudate covering the posterior pharynx and tonsillar pillars. Petechiae are sometimes observed on the soft palate, with erythema and edema of the uvula. Anterior cervical lymphadenopathy, often at the angle of the jaw, is typical of GAS pharyngitis, and nodes may be quite large and tender. Patients may also present with a characteristic scarlatiniform rash that typically begins on the trunk, spreads to the extremities, and spares the palms and soles. The rash is usually described as confluent with a sandpaper-like quality. Scarlet fever is caused by one or more of the pyrogenic exotoxins produced by pharyngeal strains of GAS. Signs and symptoms most indicative of GAS pharyngitis are tonsillar or pharyngeal exudates, tender anterior cervical nodes, fever or history of fever, and absence of cough.[33]

Non–group A *Streptococcus*

Group C and G streptococci are commonly found as normal microbiota in the human pharynx; however, they have also become increasingly recognized as potential causes of pharyngitis. *S. dysgalactiae* subsp. *equisimilis* (group C) is the most commonly isolated non-GAS associated with sore throat,[53] although recently, *S. equi* subsp. *zooepidemicus* has emerged as a potentially important human pathogen.[54] Group C streptococci are known to cause endemic,[55] whereas group G is more frequently associated with epidemic pharyngitis[56] after ingestion of contaminated food, including salads (especially those with eggs) and milk products. Signs and symptoms from pharyngitis caused by group C and G streptococci may be indistinguishable from GAS infection. The need for treatment in these cases is unclear because they have not been associated with the development of acute rheumatic fever.

Fusobacterium necrophorum

Although current guidelines emphasize the identification of GAS in the diagnosis and management of acute pharyngitis, *F. necrophorum* is being more frequently recognized as an agent of endemic pharyngitis in young adults. A recent study in a university health clinic found *F. necrophorum* in 20.5% of patients with pharyngitis and in 9.4% of asymptomatic students.[16] An accompanying editorial pointed out the challenges in interpretation of these findings.[57] A study from the Children's Hospital Los Angeles[17] showed a similarly high proportion of *F. necrophorum* in patients 14 to 20 years (13.5%) compared with younger children (1.9%). The clinical signs and symptoms of pharyngitis caused by *F. necrophorum* may be indistinguishable from those causing GAS pharyngitis. However, the clinician should maintain a high index of suspicion because of the potential for the severe complication of Lemierre syndrome. Patients with Lemierre syndrome may initially present with symptoms of pharyngitis, tonsillitis, or peritonsillar abscess and show initial clinical improvement (see Chapter 64). A recent study from Denmark identified *F. necrophorum* as the most frequently detected bacteria in peritonsillar abscess.[18] Approximately 4 days after clinical improvement of pharyngitis, the signs and symptoms of bacteremia (e.g., rigors) associated with Lemierre syndrome may appear. It has been suggested that *F. necrophorum* be a major consideration in the treatment of pharyngitis in adolescents and young adults based on the severity of complications caused by *F. necrophorum,*[58] in contrast to the markedly decreased incidence of acute rheumatic fever.

Arcanobacterium haemolyticum

Throat findings in patients with *A. haemolyticum* infection include pharyngeal erythema and exudate, fever, and cervical lymphadenopathy, similar to GAS pharyngitis. The distinguishing clinical feature of pharyngitis caused by *A. haemolyticum* is the rash that may occur in up to one-half of infected individuals. The rash is scarlatiniform, macular or maculopapular and is most frequently seen in adolescents and young adults.[21] The rash begins on the distal extremities, typically involving the extensor surfaces but sparing the palms and soles, followed by centripetal spread.[59] Rarely, *A. haemolyticum* may cause more severe infection (e.g., pneumonia and pyomyositis) but in these cases is most often a coinfecting agent.[60]

Corynebacterium diphtheriae

Diphtheria is rare in developed countries because of widespread vaccination. The majority of respiratory infections caused by *C. diphtheriae* are tonsillopharyngeal. Sore throat is one of the most common symptoms of diphtheria and is usually accompanied by low-grade fever and malaise.[61] Formation of a membrane on the tonsil or pharyngeal surface is the hallmark of diphtheria but occurs in only one-third of patients. A relative lack of fever and the formation of a membrane distinguish diphtheria from pharyngitis caused by group A β-hemolytic streptococci and viral etiologies. The membrane that forms in diphtheria is described as white early in the course of the illness, becomes dark gray and leather-like, with attempts to dislodge the membrane potentially causing bleeding.[62] Membrane formation is the result of local toxin production, and spreading of the membrane indicates more systemic toxicity. Extensive spreading of the membrane may lead to tonsillar, anterior cervical, and submandibular lymphadenopathy, as well as swelling of the neck (so-called bull neck). Continued progression may lead to respiratory distress and death.

Neisseria gonorrhoeae

Although pharyngeal infection with *N. gonorrhoeae* is often asymptomatic, sore throat is reported by patients with tonsillar involvement. A review of published cases of oropharyngeal gonorrhea found that more than 10% were classified as tonsillitis.[63] Fever is uncommon, as is cervical lymphadenopathy. Among patients with tonsillitis, a whitish-yellow exudate was observed in 20%.[63] Because the clinical presentation of pharyngitis caused by *N. gonorrhoeae* is nonspecific and symptoms may be mild, a thorough history, including risk factors for sexually transmitted infections, should be obtained in adolescents and young adults with pharyngitis to make this diagnosis.

Atypical Bacteria

Both *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have been identified as a cause of pharyngitis in all age groups, with a higher prevalence generally noted for *M. pneumoniae.*[7,64] Disease occurs year-round, but seasonal peaks and community outbreaks occurring every few years have also been described.[65] Most adult cases appear to present as an undifferentiated acute respiratory infection or an influenza-like illness; however, isolated pharyngitis has also been noted.[66] In an outbreak of respiratory disease caused by *M. pneumoniae* within a military unit, sore throat was reported in 35% to 70% of patients, with fatigue, headache, and cough noted more commonly. The only risk factor for symptomatic disease identified after the outbreak was cigarette smoking.[67] Esposito and colleagues[7] have described several case series of children with pharyngitis caused by *M. pneumoniae* or *C. pneumoniae* and identified dysphagia in 25% to 36%, tonsillar hypertrophy in 76% to 83%, cervical adenopathy in approximately half, and exudate in 25% to 39%. Although these findings were not specific to pharyngitis caused by atypical bacterial infection compared with common viral causes of pharyngitis, children with infection caused by *M. pneumoniae* or *C. pneumoniae* were significantly more likely to have a history of recurrent pharyngitis.[68] In addition, children with pharyngitis caused by atypical bacterial infections treated with azithromycin had lower rates of subsequent respiratory infections, including lower tract disease, compared with children given symptomatic treatment alone.[69] A more recent study of older adolescents and young adults at a University Health Clinic did not identify *M. pneumoniae* as a substantial cause of pharyngitis.[16]

Epstein-Barr Virus

Infectious mononucleosis (IM) is a multisystem disorder caused by primary infection with Epstein-Barr virus (EBV) and defined by the classic symptoms of fever, fatigue, pharyngitis, and adenopathy.[70,71] Among more than 200 young adults with serologically confirmed acute EBV infection, three-quarters reported sore throat with approximately half noting cervical adenopathy at their initial visit.[71,72] Fatigue was common and noted in 65% to 75%. Other symptoms included fever, headache, cough, myalgia, arthralgia, and nausea. Rash was uncommon and is typically described as a diffuse maculopapular eruption in patients given ampicillin or related compounds. On examination, pharyngitis with mildly painful anterior and posterior cervical lymphadenopathy

was detected in 75% of patients, whereas splenomegaly and/or hepatomegaly were detected in approximately 35% of patients during the first two weeks of the illness with minimally elevated transaminase levels in more than half of the group.[71,72] The pharyngitis that accompanies IM is subacute in onset and may be accompanied by mild-to-moderate enlargement of the tonsils as well as exudates and palatal petechiae.[70] Symptoms substantially improve over the first month of illness and after 6 months are almost completely resolved.[72]

Although IM has been traditionally described in adolescents and young adults, children also commonly develop fever, exudative pharyngitis, and cervical adenopathy during primary infection with EBV.[73,74] In addition, rash and splenomegaly are more common in young children with primary EBV infection than in adolescents or adults.[74] Periorbital or eyelid edema, as a symptom of primary EBV infection, appears to be unique to children.[74] A mononucleosis-like illness caused by primary infection with CMV, human herpesvirus 6, HSV-1, and HIV-1 has also been described.

Human Immunodeficiency Virus

Symptoms associated with primary HIV-1 infection develop in 40% to 90% of infected individuals and are referred to as the acute retroviral syndrome.[1,75,76] This illness is a multisystem disorder, typically occurring 5 to 29 days after infection, and is characterized by the acute onset of one or more of the following complaints: fever, rash, pharyngitis, fatigue, weight loss, myalgia, arthralgia, headache, night sweats, cervical adenopathy, nausea, vomiting, or diarrhea.[75] Hecht and coworkers[77] identified 145 patients with either primary HIV-1 infection or recent seroconversion and found that the most sensitive symptoms of primary infection were fever (80%) and malaise (68%), with the majority of patients reporting an illness lasting 1 to 2 weeks. The combination of fever and rash were identified as significant independent predictors of primary HIV-1 infection, with the rash described most commonly as a nonpruritic polymorphous eruption beginning on the face and chest and spreading outward.[1,70] Pharyngitis is recognized in 50% to 70% of patients, whereas cervical adenopathy is noted in 25% to 50%.[76,77] Although extensive descriptions of the pharyngeal findings associated with primary HIV infection are lacking, exudates appear to be present in a minority of patients.[1,77] In addition, the adenopathy tends to be nontender and may be generalized.[70] Painful oral ulcerations are one of the least common symptoms in patients with primary HIV-1 infection, identified in only 10% to 35%, but they are highly specific.[77] Ulcerations can be found almost anywhere in the mouth, including the floor of the mouth, inner lips, buccal mucosa, gingiva, hard and soft palate, as well as the esophagus, anus, and penis.[76,78] Concomitant oral thrush has also been described.

Based on the common presenting symptoms of fever, pharyngitis, rash, and lymphadenopathy, it is easy to understand how primary HIV-1 infection may be confused with infectious mononucleosis, secondary syphilis, acute hepatitis A or B, toxoplasmosis, or other viral syndromes. In fact, Schacker and coworkers[75] noted that only one-quarter of patients with symptoms of primary HIV-1 infection had the diagnosis suspected at the initial medical evaluation. A report estimating the prevalence of primary HIV-1 infection in symptomatic adolescent and adult ambulatory patients found that pharyngitis was due to primary HIV-1 infection in 1.3 patients per 1000 cases.[79] Because up to one-half of all new HIV-1 infections occur in adolescents, physicians who care for adults and children should be familiar with the clinical characteristics of primary HIV-1 infection to maintain a high index of suspicion for this disorder.[78] Early diagnosis via virus-specific tests, such as p24 antigen or the detection of plasma HIV-1 RNA, reliably identify people with primary HIV-1 infection before seroconversion and can aid in both the control of virus transmission and treatment decisions for individual patients (see Chapter 120).

Enteroviruses

Enteroviruses classically cause an undifferentiated febrile illness but are also recognized as a cause of pharyngitis and upper respiratory tract infections, with most disease occurring in the summer and fall. Non-polio enteroviruses have been identified in 8% to 29% of cases of pharyngitis in children by using reverse-transcriptase polymerase chain reaction (RT-PCR).[6,80,81] Fever is common, but the throat examination typically reveals only mild erythema without significant adenopathy. Although exudates are not generally described, enteroviruses were found in 16% of children with exudative pharyngitis in one report.[8]

Two specific pharyngeal syndromes typically associated with enterovirus infections are herpangina and hand-foot-mouth disease (HFM). Among children with fever and clinical signs of pharyngeal or tonsillar infection, 24 were identified with herpangina, of whom 75% had an enterovirus detected in their throat swab.[80] The majority of cases of herpangina are due to group A coxsackieviruses; however, group B coxsackieviruses, echoviruses, enterovirus 71, adenovirus, and HSV have also been detected.[82,83] Both endemic and epidemic herpangina are well described, with young children affected more commonly than newborns, older children, and adults. The clinical manifestations include hyperemia of the pharynx, with discrete 1- to 4-mm erythematous-based vesicles or ulcerations sparsely distributed on the tonsillar pillars, uvula, soft palate, or posterior pharynx.[84] Sore throat and fever are invariably present, but symptoms typically resolve spontaneously in about a week. Similar to herpangina, HFM is characterized by the presence of erythematous-based vesicles and ulcerations in the pharynx in a patient with significant sore throat. In contrast to herpangina, vesicles are also noted on the hands, feet, and buttocks in patients with classic HFM, and the fever tends to be less prominent.[84] Recently, cases of atypical HFM have been described with widespread vesiculobullous lesions involving the extremities, trunk, buttocks, face, hands, and feet.[85,86] Crusting of the lesions followed by acral desquamation and nail changes, including onychomadesis (nail shedding) approximately 4 to 6 weeks after the illness, are also described.[87] Most cases have been identified in young children, but adults with atypical HFM have also been reported and almost all have been attributed to infection with coxsackie A6.[88] Although most cases are self-limited, severe multisystem disease, particularly involving the central nervous system, accompanying HFM and herpangina has been described during outbreaks associated with enterovirus 71 (see Chapter 172).[89,90]

Adenovirus

Respiratory infections with adenovirus are well described in children and young adults, occur year-round, and cause both upper and lower tract disease. Examining sore throat or pharyngitis specifically, adenovirus infections are identified as the etiologic agent in up to 25% of cases in children and 3% of ambulatory adults.[3,7,8,91] Not only are adenoviruses a common cause of pharyngitis, but infections with adenovirus also commonly cause pharyngitis. Retrospective reviews have demonstrated that pharyngitis or tonsillitis is reported in 40% to 88% of children with adenovirus infections.[92,93] Exudates are noted in about half of the cases and are often described as thick and white with marked throat pain. In addition, almost three-quarters of children with adenovirus infections have fever higher than 39°C that persists for a mean of 6 days.[94] Among military recruits followed prospectively, approximately 35% of those with culture-confirmed adenovirus infection had sore throat, and 29% were febrile.[95] Bilateral cervical lymphadenopathy (32%), conjunctivitis (17%), and rash (12%) have also been described in patients with adenovirus respiratory tract infections.[93]

Pharyngoconjunctival fever is a specific syndrome caused by adenovirus infections, often occurring in outbreaks and associated with swimming or bathing.[96] Patients typically present with fever, conjunctivitis, pharyngitis, and cough but may also complain of headache, myalgia, and malaise. Lymphadenopathy is found on examination in about half of the patients, whereas one-quarter also have coryza.[97] This disorder is highly contagious, with an attack rate of approximately 50% and spread via direct inoculation into the conjunctiva. Although the conjunctivitis may be quite intense and last for 1 to 2 weeks, there is invariably complete resolution of all symptoms with no sequelae.[97]

Herpes Simplex Virus

Primary infection with HSV-1 commonly causes gingivostomatitis in young children, whereas pharyngitis is noted among adolescents and young adults due to both HSV-1 and HSV-2. In a series of 35 college students with HSV pharyngitis, infections occurred year-round, with the majority of patients presenting with fever, pharyngeal erythema, exudates, and enlarged tender cervical adenopathy.[98] Approximately

TABLE 59.2 Clinical and Epidemiologic Findings Associated with Group A *Streptococcus* Pharyngitis

Suggestive of Group A *Streptococcus*
Sudden onset
Sore throat
Fever
Headache
Nausea, vomiting, and abdominal pain
Inflammation of pharynx and tonsils
Patchy discrete exudates
Tender, enlarged anterior cervical nodes
Patient aged 5–15 yr
Presentation in winter or early spring
History of exposure
Suggestive of Viral Etiology
Conjunctivitis
Coryza
Cough
Diarrhea
Discrete ulcerative lesions
Suggestive of Complications of Pharyngitis
Dysphagia
Stridor
Drooling
Dysphonia
Marked neck swelling
Respiratory distress
Pharyngeal pseudomembrane
Hemodynamic instability
HIV behavioral risk
Travel to or exposure to individuals from a region endemic for diphtheria
Lack of diphtheria immunization

HIV, Human immunodeficiency virus.
Modified from Shulman ST, Bisno AL, Clegg HW, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin Infect Dis. *2012;55:e86–e102; and Kociolek LK, Shulman ST. In the clinic. Pharyngitis.* Ann Intern Med. *2012;157:ITC3-1–ITC3-16; reproduced with permission.*

TABLE 59.3 Modified Centor Score and Culture Management Approach for Pharyngitis

CRITERIA	POINTS
Temperature >38°C	1
Absence of cough	1
Swollen, tender anterior cervical nodes	1
Tonsillar swelling or exudate	1
Age	
3–14 yr	1
15–44 yr	0
45 yr or older	–1

SCORE	RISK OF STREPTOCOCCAL INFECTION	SUGGESTED MANAGEMENT
≤0	1%–2.5%	No further testing or antibiotic
1	5%–10%	
2	11%–17%	Culture all: antibiotics only for positive culture results
3	28%–35%	
≥4	51%–53%	Treat empirically with antibiotics and/or culture

From McIsaac WJ, Kellner JD, Aufricht P, et al. Empirical validation of guidelines for the management of pharyngitis in children and adults. JAMA. *2004;291:1587–1595; reproduced with permission.*

one-third also had symptoms more characteristic of HSV, including either ulcerations of the mouth, lips, or pharynx; or swollen, tender, erythematous gingiva. One clue to the diagnosis of HSV pharyngitis is that esophagitis may also be present in immunocompetent adolescents and young adults and should be considered in patients complaining of substernal chest pain and dysphagia in addition to sore throat. Acyclovir treatment is often used in severe cases.

DIAGNOSIS

Because pharyngitis is one of the most common complaints a physician may encounter, diagnosis of treatable etiologies is paramount. The prevention of rheumatic fever requires antimicrobial treatment and eradication of GAS from the pharynx.[99] Certain clinical findings help to distinguish GAS from viral causes of pharyngitis (Table 59.2). As noted, tonsillar or pharyngeal exudates, tender anterior cervical lymphadenopathy, and fever are commonly associated with GAS. Alternatively, symptoms such as conjunctivitis, coryza, oral ulcers, cough, and diarrhea suggest a viral cause.

Multiple clinical prediction rules have been developed to aid in the diagnosis of GAS pharyngitis. Scoring systems attempt to use clinical and epidemiologic data to assign a probability that acute pharyngitis is attributable to GAS (Table 59.3).[13,100,101] Prediction rules for the diagnosis of GAS pharyngitis are limited because the signs and symptoms of many viral causes of acute pharyngitis overlap with infection caused by GAS, and the rules are best at identifying patients with a low probability for GAS infection. A large-scale study evaluating the modified clinical prediction rule (see Table 59.3) confirmed that even in subjects with all clinical features, streptococcal pharyngitis could be confirmed in only 57% of cases.[102] Further studies demonstrate that with the presence and increasing number of viral features (e.g., cough, rhinorrhea, oral ulcers/vesicles) GAS was progressively less prevalent.[35] Thus the most recent guidelines from the Infectious Diseases Society of America (IDSA) and the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP) recommend confirmation of GAS infection by rapid antigen detection testing (RADT), throat culture, or both.[103,104] In contrast, the 2001 guidelines issued by the Centers for Disease Control and Prevention (CDC) and the American College of Physicians (ACP)-American Society of Internal Medicine suggested empirical treatment based on a pharyngitis score alone with or without microbiologic confirmation.[105]

The difference in these guidelines had been the subject of intense debate, and empirical therapy based on the use of predication rules has been implicated in the overuse of antibiotics for the treatment of pharyngitis.[101] A study on the effectiveness of these strategies showed that empirical treatment is a reasonable strategy for those least likely to have GAS pharyngitis, but microbiologic confirmation was the most effective and least expensive when all factors were considered.[106] In addition, in 78% of cases, physicians did not adhere to any guidelines, leading to overuse of antibiotics for the treatment of pharyngitis.[107] More recently, improved accuracy of the Centor score[100] was achieved by adding real-time local biosurveillance for GAS pharyngitis. However, real-time biosurveillance is impractical in most settings. In 2016 the CDC and the ACP updated their guidelines to recommend that only patients with confirmed GAS pharyngitis by RADT and/or throat culture be treated with antibiotics.[108]

Culture for Group A Streptococci

Assaying for the presence of GAS by throat culture on sheep blood agar plate (BAP), as first described by Breese and Disney,[109] has been accepted practice for diagnosing streptococcal pharyngitis for more than 50 years. Selective streptococcal media are used by some laboratories and reduce the number of contaminating normal flora and may increase the sensitivity and specificity of culture.[110] However, the use of selective media may also reduce the likelihood of recovery of other bacterial etiologic agents. Ideally, specimens should be obtained from bilateral tonsillar surfaces and the posterior pharynx while avoiding the mouth, tongue, and other surfaces of the pharynx. The major disadvantage of throat culture for the confirmation of GAS pharyngitis is the 24 to 48 hours required for accurate detection.

Rapid Antigen Detection Test

RADT is readily available and, in some instances, has been reported to equal or exceed the sensitivity and specificity of throat culture. Rapid testing may lead to more timely treatment and, in so doing, can reduce the spread of GAS, time missed from school or work, and overtreatment of viral causes of pharyngitis and can minimize suppurative and nonsuppurative sequelae of GAS. The original RADTs were based on the detection of GAS cell wall carbohydrate antigen by enzyme immunoassays. Newer assays use molecular biology methods to detect DNA specific to GAS by using chemiluminescence or real-time PCR. A recent meta-analysis assessing the sensitivity and specificity of RADT among 48 studies showed an overall sensitivity of 86% and specificity of 96%.[111] Highest sensitivity and specificity has been observed in molecular tests, with most recent PCR-based assays taking as little as 15 minutes.[112] Of importance, RADT specimens must be collected in a manner similar to BAP culture. Swabs obtained from the mouth and subjected to RADT have a sensitivity of less than 20% versus 80% for those obtained properly from the posterior pharynx and tonsils.[113] Currently, it is recommended that a negative RADT be confirmed by BAP culture for all children.[103,104] Because the incidence of a first attack of rheumatic fever is low in adults in the United States and the prevalence of GAS pharyngitis is minimal, the need to back up a negative RADT in adults continues to be questioned.[101] In a study assessing the utility of a reflexive culture after a negative RADT in adolescents and young adults with pharyngitis, RADT failed to identify 55% of patients with modified Centor score ≥2, suggesting that in the absence of a backup method, many patients who may benefit from treatment would be missed.[114]

Other Diagnostic Tests

The diagnosis of non-GAS pharyngitis is specific to the etiologic agent involved. A high index of suspicion must be maintained for alternative diagnoses in the appropriate epidemiologic setting because many pathogens are not screened, even in large clinical laboratories. Culture on either standard BAP or selective streptococcal media will identify both group C and G *Streptococcus;* however, they may be identified only as non–group A β-hemolytic *Streptococcus*. *A. haemolyticum* also grows on standard blood agar or selective streptococcal media but may be missed because colonies generally take up to 72 hours to appear and are small and dry.

Specific media and techniques are necessary to identify other causes of pharyngitis. If diphtheria is suspected, the laboratory must be notified so that selective media are used for isolation. Recently, multiplex PCR has been used for the identification of *C. diphtheriae* and to differentiate toxin-producing from nontoxigenic strains[115] but requires further investigation before use in a clinical setting. The diagnosis of *Fusobacterium* pharyngitis can be made by isolating the organism in anaerobic culture media, although most clinical laboratories rely on commercial kits and automated systems for identification. The accuracy of these systems is variable and may lead to initial misidentification. Molecular detection of *Fusobacterium* has been used in some studies,[17,116] and has the advantage of detecting low concentrations of bacteria, but is not commercially available. The diagnosis of pharyngitis caused by *Neisseria gonorrhoeae* is confirmed by isolation of the organism from a throat swab on selective media. Nucleic acid amplification tests are both sensitive and specific for urogenital specimens. Ease of testing pharyngeal samples may be simplified by nucleic acid amplification of salivary samples, but this is still in an investigational stage.[117]

Serologic testing of acute and convalescent serum samples has been the standard procedure for diagnosing pharyngitis caused by *M. pneumoniae* or *C. pneumoniae*; however, PCR is becoming readily available. The diagnosis of primary EBV infection is confirmed by serology, either via a heterophil antibody test (monospot or monoslide) or detection of immunoglobulin M (IgM) antibodies to EBV viral capsid antigen in an acute serum specimen. Although 85% of adolescents and adults develop heterophil antibodies, usually at about 1 week into illness, specific serology for EBV is usually necessary to make the diagnosis in children, especially those younger than 4 years.[70,74] Common respiratory viruses that cause pharyngitis can be identified either by viral culture of a nasopharyngeal swab or molecular detection techniques such as PCR or RT-PCR. Ultimately, as new technologies evolve it is likely that pharyngitis molecular panels will become available, allowing rapid diagnosis of a broad range of bacterial and viral etiologies analogous to those that have been developed for patients with respiratory symptoms, diarrheal illness, and meningitis/encephalitis.[118]

THERAPY

Prescribing antibiotics for patients with sore throat is a common practice and is often done in an effort to prevent potential complications of pharyngitis. A systematic review of the use of antibiotics for sore throat that included almost 13,000 patients found that antibiotics did reduce the incidence of otitis media, acute sinusitis, peritonsillar abscess, and acute rheumatic fever.[119] However, only 7 of the 58 studies included in the review were published since 1996, and the populations included were very heterogeneous, including those with and without GAS. A more recent evaluation used a national database of more than one million cases of sore throat and found that although there was a decrease in the incidence of quinsy (peritonsillar abscess) after the use of antibiotics, the number needed to treat to prevent one case was 4300, suggesting that the small decrease in risk of an uncommon complication did not warrant the widespread use of antibiotics for a self-limited disease.[120]

The goal of therapy for GAS pharyngitis is to decrease the time to resolution of symptoms, reduce risk of transmission, and reduce the incidence of suppurative and nonsuppurative sequelae. This is achieved by the elimination of GAS from the pharynx. Penicillin has been the mainstay of therapy for GAS pharyngitis for more than 60 years. Despite this long-term use, there has yet to be a confirmed instance of penicillin resistance in GAS. A 10-day course of penicillin or amoxicillin is the treatment of choice and is recommended by the IDSA and AAP for the treatment of pharyngitis caused by GAS (Table 59.4).[103,104] Penicillin-allergic patients should be given a macrolide (erythromycin) or first-generation cephalosporin for non–IgE-mediated allergy. Currently, the use of broad-spectrum cephalosporins, such as cefixime and ceftibuten, although approved by the US Food and Drug Administration for the treatment of GAS pharyngitis, is not endorsed.

The use of amoxicillin for the treatment of GAS pharyngitis has increased because of improved taste and less frequent dosing intervals compared with penicillin, leading to better patient compliance. Two relatively small studies have shown that treatment of GAS pharyngitis with once-daily amoxicillin for 10 days achieved similar clinical and bacteriologic outcomes compared with traditional penicillin dosing.[121,122] Furthermore, a larger study confirmed a once-daily amoxicillin regimen as noninferior to twice-daily penicillin.[123] Given the evidence, the most

TABLE 59.4 Antimicrobial Therapy for Group A Streptococcal Pharyngitis

DRUG	DOSE	DURATION
Oral Regimens		
Penicillin V	Children: 250 mg bid or tid Adolescents and adults: 250 mg tid or qid or 500 mg bid	10 days
Amoxicillin	50 mg/kg once daily (maximum 1000 mg) Alternative: 25 mg/kg bid (maximum 500 mg)	10 days
For Penicillin-Allergic Patients		
Erythromycin	Varies with formulation	10 days
First-generation cephalosporins	Varies with agent	10 days
Intramuscular Regimens		
Benzathine penicillin G	600,000 units for patients <27 kg 1.2 million units for patients ≥27 kg	1 dose 1 dose
Mixtures of benzathine and procaine penicillin G	Varies with formulation	1 dose

Modified from Alcaide ML, Bisno AL. Pharyngitis and epiglottitis. Infect Dis Clin North Am. *2007;21:449–469, vii; reproduced with permission.*

recent guidelines endorse the use of amoxicillin if greater compliance is anticipated.[104]

Antimicrobial therapy should not be used for the prevention of GAS pharyngitis except in special circumstances. Culture or RADT for diagnosis, coupled with treatment, is indicated in those with a previous episode of rheumatic fever, during an outbreak of acute rheumatic fever or poststreptococcal glomerulonephritis, or in close contact with persons with invasive infections, such as necrotizing fasciitis or streptococcal toxic shock syndrome.[103,104] There is also no need to routinely obtain throat cultures at the end of treatment in asymptomatic patients, to document clearance of GAS except in those situations noted earlier.

Recommendations for treatment of *Fusobacterium* infections include a penicillin in combination with a β-lactamase inhibitor (e.g., ampicillin/sulbactam) together with metronidazole.[124] *Fusobacterium* species are universally resistant to macrolide antibiotics (e.g., azithromycin)[125] and have been increasingly noted to show resistance to clindamycin. In addition, up to one-quarter of *Fusobacterium* species isolates will produce a β-lactamase.[126] Penicillin and erythromycin are the only two agents recommended for treatment of *C. diphtheriae,* although newer macrolides, such as azithromycin, are commonly used in clinical practice. Treatment of *A. haemolyticum* should include either a macrolide or β-lactam antibiotic. Penicillin resistance has been reported and appears to be more common in cases of pharyngitis.[127] Treatment of pharyngitis caused by *N. gonorrhoeae* is problematic because pharyngeal eradication of the organism is more difficult than eradication from the urogenital tract. As such, it is recommended that repeat cultures be obtained at the end of therapy to confirm eradication. Specific treatment regimens for gonorrhea are discussed in Chapter 212.[128]

Symptomatic treatment is the mainstay for most cases of viral pharyngitis. Significant improvement in pain relief with the use of 25 mg ibuprofen lozenges compared with placebo has recently been reported in a large double-blind randomized control trial of adults with sore throat not requiring antibiotics.[43] The use of a single dose of oral corticosteroids along with antibiotics was associated with a modest reduction in duration of throat pain by a mean time of 14 hours, as reported in a meta-analysis of studies.[45] A more recent meta-analysis reported a mean reduction of pain of 11 hours in patients with pharyngitis treated with a single dose of corticosteroids without antibiotics.[129] The IDSA guidelines[130] and the UK National Institute for Health Care Excellence do not recommend the routine treatment of pharyngitis with corticosteroids.[131]

Complications

The potential suppurative complications of pharyngitis (see Table 59.2) include peritonsillar abscess, parapharyngeal space abscess, lymphadenitis, sinusitis, otitis media, mastoiditis, and invasive infections (e.g., necrotizing fasciitis and toxic shock syndrome with GAS). Peritonsillar abscess typically occurs in adolescents and young adults but has been described in all age groups. Patients present with fever, malaise, sore throat, and dysphagia. There may be trismus or ipsilateral ear pain. Physical examination reveals drooling and a muffled voice ("hot potato voice") with tender cervical adenopathy and swelling of the anterior tonsillar pillar and soft palate on the affected side. The uvula is displaced to the contralateral side by the abscess.[132] In older adults the signs and symptoms of a peritonsillar or parapharyngeal space abscess may be subtle, and disease appears to be more common in those with underlying immunocompromising conditions.[133] In a series of 14 patients older than 50 years, fever, trismus, and voice changes each were present in less than one-third of patients with peritonsillar abscess or parapharyngeal space abscess.[133] Drainage of purulent material coupled with antibiotics are the standard of treatment.[134] Because peritonsillar and parapharyngeal abscesses are often polymicrobial, involving aerobic and anaerobic bacteria, one suggested agent for treatment is ampicillin/sulbactam.[132]

Acute rheumatic fever (ARF) and acute glomerulonephritis are potential nonsuppurative complications of pharyngitis caused by GAS (see Chapter 198). Rheumatic heart disease and its complications affect almost two million individuals each year, primarily in developing countries.[37] Acute rheumatic fever has become rare in the United States except for sporadic outbreaks of rheumatogenic strains of GAS.[135] Acute glomerulonephritis is associated with GAS skin infections and uncommonly associated with pharyngitis caused by GAS. Rarely, acute glomerulonephritis occurs after group C or G streptococcal pharyngitis, but these organisms have never been associated with ARF.

Lemierre syndrome is an uncommon complication of pharyngitis in adolescents and young adults, characterized by septic thrombophlebitis of the internal jugular vein and metastatic lesions (septic emboli) of distant sites after acute sore throat, most commonly caused by *F. necrophorum.*[124] The clinical characteristics of pharyngitis caused by *Fusobacterium* are nonspecific and similar to GAS, and systemic illness may present after pharyngeal symptoms have subsided.

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The complete reference list is available online at Expert Consult.

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60 Acute Laryngitis

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SHORT VIEW SUMMARY

Definition
- Acute laryngitis is a clinical syndrome characterized by a hoarse voice with decreased phonation and voice projection, usually occurring after an upper respiratory tract infection with cough.

Epidemiology
- Approximately 1% of medical care claims are due to dysphonia, with 42% of those classified as acute laryngitis.
- Two percent of individuals with acute respiratory symptoms are diagnosed with acute laryngitis.
- Acute laryngitis is diagnosed more frequently in women (mean age, 36 years) than men (mean age, 41 years).
- More cases are diagnosed in the colder months of the year.

Microbiology
- A viral upper respiratory tract infection is often associated (see Table 60.1).
- Bacterial infections of the upper respiratory tract have also been implicated.
- Unusual causes include tuberculosis, blastomycosis, histoplasmosis, coccidioidomycosis, cryptococcosis, and herpesvirus infections of the larynx.

Diagnosis
- Clinical diagnosis is based on the appropriate history and changes in the voice.
- Visualization of the larynx reveals edema and vascular engorgement of the mucous membranes with hyperemic and erythematous vocal folds.

Therapy
- Treatment is based on the underlying cause of the laryngeal pathologic process.
- Often, symptomatic therapy with voice rest, analgesics, and humidification is sufficient.

DEFINITION

Acute laryngitis is a clinical syndrome commonly encountered by primary care physicians. The symptoms are often described as the recent onset of hoarseness or a husky voice with decreased voice projection often associated with a dry cough.[1] There may be voice breaks or episodes of aphonia that frequently occur in the context of an upper respiratory tract infection with rhinorrhea and sore throat. The duration of symptoms is difficult to discern from the literature; however, in a study of 80 adults with the common cold, hoarseness was reported for a median of 3 days, and 5.5 days represented the 75th percentile.[2] Although most reports describe acute laryngitis as a mild and self-limited syndrome, a survey of intercollegiate athletes found substantial morbidity associated with laryngitis.[3] These students reported laryngitis significantly more often as a cause of missed practice, compared with cough, nasal discharge, or myalgia, and as having an adverse effect on their athletic performance.

EPIDEMIOLOGY

The incidence of acute laryngitis reported in the literature varies and is highly dependent on the research methods used. A study using a large medical claims database found that approximately 1% of people presenting for care did so because of dysphonia, with 42% receiving a diagnosis of acute laryngitis.[4] Further study of the same database found that over 20% of adults aged 65 years or older were diagnosed with acute laryngitis at least once during a 5-year period.[5] When the National Health Interview Survey was used to inquire regarding voice problems in the preceding year, approximately 18 million adults or 7.6% of the US population reported a voice disturbance. Acute laryngitis secondary to a respiratory infection was the most common diagnosis in 17.8%.[6] In reports including almost 5000 children and adults with acute respiratory symptoms, 2% were given a primary diagnosis of laryngitis.[7,8] In other studies, 38% of patients with pneumonia reported hoarseness as a symptom, as did 53% of adults with colds and 67% of children with bacterial tracheitis.[2,9,10] Laryngitis has also been noted in approximately 22% of adolescents or school-aged children with nonstreptococcal sore throat.[11] Despite this demonstration that laryngitis affects patients of all ages, a report of more than 800 patients seen in an ear, nose, and throat clinic showed that most patients with acute laryngitis presenting for care were women with a mean age of 36 years.[12] In addition, the study showed that the frequency of laryngitis during the colder months was almost double that observed in the warmer seasons.

MICROBIOLOGY

All of the major respiratory viruses have been etiologically associated with laryngitis. In the study of patients older than 5 years of age with a primary diagnosis of laryngitis, 21% had infection with parainfluenza virus, 15% had rhinovirus, 3% had influenza virus, and 3% had adenovirus.[7] The risk of developing laryngitis with a particular type of respiratory tract infection is summarized in Table 60.1. McMillan and colleagues[11] reported that laryngitis and cough were noted significantly more often among patients with influenza (29%) than among patients with group A β-hemolytic streptococcal infection (2.3%). In a retrospective review of an epidemic of influenza in the United Kingdom, the rate of laryngitis or tracheitis reported by general practitioners peaked at approximately 100 per 100,000 population, coincident with the peak of influenza illness.[13] Overall 10% of children with human parainfluenza virus (HPIV) infection noted hoarseness in a retrospective review from a large medical center in Taiwan. The symptom was more commonly associated with human parainfluenza virus 2 infection, with hoarseness noted at presentation of 23.9% of children with HPIV2 infection versus only 5.5% of children with HPIV3 disease.[14] Younger patients were significantly more likely to report hoarseness than elderly subjects in a study of human metapneumovirus infection.[15] Hoarseness was reported in 91% of young adults with human metapneumovirus infection compared with 42% of similar-age subjects with respiratory syncytial virus infection. Acute laryngitis was the primary diagnosis in 3.3% of children from 1 month to 14 years of age hospitalized with acute respiratory symptoms and infection with human metapneumovirus.[8] Among older adults admitted to the hospital for respiratory disorders, hoarseness was reported by 25% of subjects with illness resulting from rhinovirus or coronavirus.[16] Hoarseness or laryngitis has not been reported as a symptom in patients

TABLE 60.1 Frequency of Laryngitis Associated With Common Respiratory Pathogens

PATHOGEN	FREQUENCY (%)	REFERENCES
Rhinovirus	25–29	16, 46
Influenza	28–35	11, 16, 46
Parainfluenza	5–25	14, 47
Adenovirus	22–35	48
Coronavirus	25	16
Mycoplasma pneumoniae	3–37	9, 16
Chlamydophila pneumoniae	12–30	21, 49
Group A β-hemolytic streptococcus	2.3–19	11, 46
Human metapneumovirus	3–91	8, 15, 50

with severe acute respiratory syndrome secondary to human pneumonia–associated coronavirus.[17]

Bacterial respiratory infections have also been associated with acute laryngitis. Several authors have noted the presence of hoarseness in patients with acute streptococcal pharyngitis (see Table 60.1). Laryngitis secondary to diphtheria has been virtually eliminated in the United States, although diphtheria continues to be an important cause of laryngeal disease worldwide. The possible etiologic role of *Moraxella catarrhalis* (formerly *Branhamella catarrhalis*) in adults with acute laryngitis was investigated in several reports from Sweden. In a case-control study of 40 adults with hoarseness and symptoms of upper respiratory tract infection, 55% of the patients and 14% of controls had *M. catarrhalis* isolated from a nasopharyngeal culture.[18] *Haemophilus influenzae* was the second most frequently recovered bacterial pathogen from patients with laryngitis (8%–20%), which suggests that the organism may also play a role in this condition. However, treatment of patients with *M. catarrhalis* with oral penicillin or erythromycin for 5 days failed to show any objective clinical benefit over placebo, despite a significant rate of bacteriologic eradication, casting doubt on the significance of the association.[19,20] Laryngitis was diagnosed in 12.2% of children presenting with real-time polymerase chain reaction–confirmed *Chlamydophila pneumoniae* acute respiratory infections in a study from Egypt.[21] Infection with *Bordetella pertussis* or *Mycoplasma pneumoniae* has been suggested as a cause of chronic laryngitis in a single report of adults with symptoms of hoarseness and throat clearing for more than 6 weeks.[22] Methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa,* and *Serratia marcescens* were also identified as possible causes of infectious laryngitis at laryngeal biopsy of patients with over 3 weeks of voice changes and evidence of laryngeal inflammation in a small retrospective review.[23]

Uncommon causes of acute laryngitis include herpesviruses, parvovirus B19, mucosal candidiasis, *Coccidioides immitis, Anncaliia algerae, Cryptococcus neoformans, Sporothrix schenckii*, mucormycosis, leishmaniasis, MRSA, actinomycosis, *Clinostomum complanatum,* and group G β-hemolytic streptococci in normal and immunocompromised patients.[2,4–29,30,31,32,33,34,35] Clinical findings in patients with laryngitis secondary to herpes simplex virus types 1 or 2, varicella-zoster virus, or cytomegalovirus include edema and inflammation of the glottic or supraglottic region with vesicles or ulcerative lesions with or without vocal cord paralysis.[33]

Laryngitis secondary to tuberculosis (TB) and blastomycosis is usually a chronic complication of pulmonary infection.[30,36] Although in the past laryngeal TB was frequently detected in young patients with recognized pulmonary TB, more recent reports have described changes in the epidemiology and clinical features of TB of the larynx. In a study of 31 patients with biopsy-confirmed laryngeal TB, only 55% were referred because of a previous diagnosis of pulmonary TB, whereas 33% had odynophagia or a suspicion of carcinoma.[37] Historically, patients with laryngeal TB had a large burden of organisms in their sputum. In a more recent study from India, patients with laryngeal TB were no more likely to have positive sputum results than patients with pulmonary TB without laryngeal disease.[36] Clinical findings reported in laryngeal TB range from the classic description of cranial nerve palsies with ulcerative lesions of the posterior larynx to anterior tumor-like masses. Given this changing clinical picture, a high degree of diagnostic suspicion is warranted in order to make a diagnosis of laryngeal TB.

Laryngeal histoplasmosis is usually a complication of disseminated infection but may be primary and manifests as hoarseness of indolent onset without cough[38] Blastomycosis and histoplasmosis of the larynx can be mistaken for squamous carcinoma because of the indolent onset, gross appearance at laryngoscopy, and pseudoepitheliomatous hyperplasia at biopsy. Fever is low grade or absent. Diagnosis depends on demonstration of the fungi in the submucosa. Hoarseness may also be noted as a component of other laryngeal infections, such as croup, acute epiglottitis, or supraglottitis. The latter conditions are discussed separately in Chapters 63 and 158. Other noninfectious causes of acute laryngitis include voice abuse, gastroesophageal reflux disease, and laryngeal malignancy.

DIAGNOSIS AND THERAPY

The diagnosis of acute laryngitis caused by an upper respiratory tract infection can often be made from the history alone. Examination of the larynx reveals hyperemic and erythematous true and ventricular vocal folds resulting from edema and vascular engorgement of the mucous membranes.[39] Treatment needs to be directed at the underlying infectious cause of hoarseness but generally is symptomatic, with voice rest, analgesic therapy, and humidification.[39] Occasionally resection or laser ablation may be required for lesions resulting from uncommon pathogens.[40] As noted previously, studies evaluating the use of antibiotics for patients with acute laryngitis have not shown objective benefit, and a Cochrane review in 2013 concluded that antibiotics should not be prescribed for patients with typical laryngitis.[41] An updated Cochrane review in 2015 similarly concluded that antibiotics did not "appear to be effective in treating acute laryngitis when assessing objective outcomes."[42] Despite these findings, antibiotics are still prescribed with some frequency for acute laryngitis.[43]

COMPLICATIONS

Long-term sequelae of laryngitis are uncommon, but prolonged hoarseness has been noted most frequently after infection with uncommon pathogens. Superior laryngeal neuralgia has also been described as a rare complication of acute laryngitis.[44] This disorder is characterized by painful paroxysms of the throat induced by head turning, swallowing, or voice straining and is associated with a trigger point on the lateral aspect of the neck overlying the thyrohyoid membrane. Various treatments, including injections of local anesthetic, have been used to treat this complication. An additional unusual complication of acute laryngitis is idiopathic ulcerative laryngitis.[45] Criteria for diagnosing this condition include a history of a preceding upper respiratory tract infection with cough, the presence of bilateral ulcerations at the midmembranous vocal folds at physical examination, and a lack of response to treatment with corticosteroids, antibiotics, and antireflux medications. Healing usually occurs over a minimum of 6 weeks with complete resolution of symptoms.

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The complete reference list is available online at Expert Consult.

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61 Otitis Externa, Otitis Media, and Mastoiditis

Stephen I. Pelton

SHORT VIEW SUMMARY

DEFINITION

Otitis Externa

- Otitis externa is an infection and inflammation of the external auditory canal.
- Infections of the pinna may occur with piercing ornaments.

Otitis Media

- Acute otitis media (AOM) is an acute illness defined by moderate-to-severe bulging of the tympanic membrane (TM) or new onset of otorrhea not due to acute otitis externa, accompanied by acute signs of illness and signs or symptoms of middle ear inflammation.[1]

Mastoiditis

- Mastoiditis is infection and inflammation of the mastoid air cells and usually evolves from an episode of severe AOM.

EPIDEMIOLOGY

Otitis Externa

- Acute diffuse otitis externa or swimmer's ear occurs in hot humid weather.
- Invasive or malignant otitis externa occurs in diabetic, immunocompromised, and debilitated patients.
- Children are prone to place foreign objects in the external ear canal, which may cause maceration and infection of the skin lining the external canal and herald the development of acute otitis externa.

Otitis Media

- Otitis media (OM) occurs at all ages, but the peak age group is children in the first 3 years of life.
- Children at risk for severe and recurrent OM are more frequently male, have a genetic predisposition to ear infections, and may be in large-group daycare exposed to frequent respiratory viruses and bacterial pathogens.

Mastoiditis

- The epidemiology of mastoiditis parallels that of OM.

MICROBIOLOGY

Otitis Externa

- The microbial microbiota of the external ear canal responsible for otitis externa is predominantly staphylococcal species, and anaerobic bacteria.
- *Pseudomonas aeruginosa* is a frequent cause of swimmer's ear, infections of the pinna, and malignant otitis externa.

Otitis Media

- *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae* are the most frequent bacterial pathogens in all age groups.
- *Moraxella catarrhalis* has become more frequent after universal immunization with pneumococcal conjugate vaccine; group A streptococci and *Staphylococcus aureus* are less frequent causes of AOM.
- Respiratory viruses are frequent causes of AOM, most often associated with bacterial pathogens.

Mastoiditis

- The microbiology of mastoiditis is dominated by *S. pneumoniae* but includes group A streptococci, *S. aureus,* and anaerobic bacteria.
- Patients with persistent perforation of the TM may have invasion of the mastoid by organisms present in the external ear canal, including *S. aureus* and *P. aeruginosa.*

DIAGNOSIS

Otitis Externa

- Acute localized otitis externa may occur as a pustule or furuncle that is visualized in the canal.
- Swimmer's ear is identified by edema, swelling, and erythema of the canal wall, the auricle, or both.
- Infections of the pinna may be associated with piercing ornaments to cartilaginous portions of the ear.
- Malignant otitis externa is associated with severe pain and tenderness of the tissues around the pinna and mastoid; pus may be present in the canal.

Otitis Media

- AOM is an acute illness with fluid in the middle ear space and bulging or decreased mobility and inflammation of the TM.

Mastoiditis

- The signs of mastoiditis include swelling, redness, and tenderness over the mastoid bone.
- The pinna is often displaced downward and outward, and a purulent discharge may emerge through a perforation of the TM.

THERAPY

Otitis Externa

- The primary approach to treatment involves cleaning the external canal, managing pain, and treating infection and inflammation.
- Initial cleaning involves removal of cerumen and debris under direct observation and irrigation with 3% hydrogen peroxide (when the TM is intact).
- Topical therapy, including fluoroquinolone eardrops, is indicated for mild and moderate disease; a topical preparation of acetic acid and hydrocortisone can be used in mild cases.
- Systemic antimicrobial therapy, including activity against *P. aeruginosa,* is necessary to manage invasive external otitis.

Otitis Media

- The American Academy of Pediatrics (AAP) and the American Academy of Family Physicians (AAFP) recommend that infants younger than 6 months receive antibacterial therapy for AOM. Children aged 6 months to 2 years should receive antibacterial therapy if the diagnosis is certain, or if the diagnosis is uncertain and disease is severe. If the diagnosis is uncertain and disease is not severe, observation is an option.
- Children 2 years of age or older should receive antibacterial therapy if disease is severe. If disease is not severe or if the diagnosis is uncertain, observation is an option. See Table 61.1.
- Management of pain should be part of the approach to treatment.
- High-dose amoxicillin is the preferred drug for patients with AOM without recent exposure to antimicrobial treatment or concomitant conjunctivitis.
- If amoxicillin fails, amoxicillin-clavulanate or parenteral ceftriaxone is preferred.
- Most children with AOM improve without use of antimicrobial agents; initial observation may be warranted in children older than 2 years of age with mild or moderate symptoms as noted earlier.
- Placement of tympanostomy tubes may be warranted for children with OM, those who are unresponsive to antimicrobial therapy, or those with severe and recurrent episodes of AOM.

Mastoiditis

- Parenteral antimicrobial therapy is indicated for initial management of mastoiditis, and targets primarily *S. pneumoniae,* group A streptococci, and anaerobic bacterial species.
- Incision and drainage may be necessary when abscess is present in the mastoid air cells.

SHORT VIEW SUMMARY—cont'd

PREVENTION

Otitis Externa

- Patients should be dissuaded from placing foreign objects, including cotton-tipped applicators, in the external canal.
- Use of earplugs, shaking ears dry after swimming, or use of drops with acetic acid, alcohol, or both may be of benefit in individuals with recurrent disease associated with bathing or swimming.

Otitis Media

- Chemoprophylaxis may be of value for prevention of episodes of AOM in children with severe and recurrent disease.
- Pneumococcal conjugate vaccines have been effective in reducing episodes of AOM due to vaccine serotypes.
- Influenza virus vaccines reduce the incidence of AOM during the winter respiratory season.

Mastoiditis

- Prevention is similar to that of AOM.

OTITIS EXTERNA

Infection of the external auditory canal (otitis externa) results most often from fluid (water) being trapped in the external canal and causing irritation and maceration of the superficial tissues, permitting local invasion of skin flora. The pain and itching that result may be severe because of the limited space for expansion of the inflamed tissue. Infections of the external canal may be subdivided into four categories: acute localized otitis externa, acute diffuse otitis externa, chronic otitis externa, and malignant otitis externa. Reviews by Senturia and coworkers,[2] Hirsch,[3] and Rubin and Yu[4] provide more complete information.

Pathogenesis

The external auditory canal is approximately 2.5 cm long from the concha of the auricle to the tympanic membrane (TM). The outer half of the canal is cartilaginous; the medial half tunnels through the temporal bone. A constriction, the isthmus, present at the juncture of the osseous and cartilaginous portions, limits the entry of wax and foreign bodies to the area near the TM. The skin of the canal is thicker in the cartilaginous portion and includes a well-developed dermis and subcutaneous layer. The skin lining the osseous portion is thinner and firmly attached to the periosteum and lacks a subcutaneous layer. Hair follicles are numerous in the outer third and sparse in the inner two-thirds of the canal. Cerumen and debris from epithelial cells accumulate in the canal and are extruded by normal cleansing mechanisms. On occasion, the material may become inspissated and obstruct the canal.

The microbial flora of the external canal is similar to the flora of skin elsewhere. There is a predominance of *Staphylococcus epidermidis, Staphylococcus aureus,* corynebacteria, and, to a lesser extent, anaerobic bacteria such as *Propionibacterium acnes*.[5,6] Pathogens responsible for infection of the middle ear (*Streptococcus pneumoniae, Haemophilus influenzae,* or *Moraxella catarrhalis*) are rarely found in cultures of the external auditory canal when the TM is intact.

The epithelium absorbs moisture from the environment. Desquamation and denuding of the superficial layers of the epithelium may follow. In this warm moist environment, the organisms in the canal may flourish and invade the macerated skin. Inflammation and suppuration follow. Invasive organisms include those of the normal skin flora and gram-negative bacilli, particularly *Pseudomonas aeruginosa.* Invasive otitis externa is a necrotizing infection frequently associated with *P. aeruginosa.* The organism gains access to the deeper tissues of the ear canal and causes a localized vasculitis, thrombosis, and necrosis of tissues. Diabetic microangiopathy of the skin overlying the temporal bone results in poor local perfusion and a milieu for invasion by *P. aeruginosa.*

Clinical Manifestations and Management

Acute localized otitis externa may occur as a pustule or furuncle associated with hair follicles; the external ear canal is erythematous, edematous, and may be filled with pus and flakes of skin debris. *S. aureus* is the most frequent pathogen. Erysipelas caused by group A streptococcus (GAS) may involve the concha and the canal. Pain may be severe. Bluish-red hemorrhagic bullae may be present on the osseous canal walls and also on the TM. Adenopathy in the lymphatic drainage areas is often present. Local heat and systemic antibiotics are usually curative. Incision and drainage may be necessary to relieve severe pain.

Acute diffuse otitis externa (swimmer's ear) occurs mainly in hot humid weather. The ear itches and becomes increasingly painful. The skin of the canal is edematous and red. Gram-negative bacilli, mainly *P. aeruginosa,* may play a significant role. A severe hemorrhagic external otitis caused by *P. aeruginosa* has been associated with mobile redwood hot tub use.[7] Gentle cleansing to remove debris, including irrigation with warm tap water, should reduce symptoms; alternatively, hypertonic saline (3%) and cleansing with mixtures of alcohol (70% to 95%) and acetic acid may be used. Hydrophilic solutions, such as 50% Burow solution, may be used for 1 to 2 days to reduce inflammation. A cotton wick may be of value in enhancing distribution of the ototopical agent when the canal is significantly swollen. A 10-day regimen of a fluoroquinolone otic solution, such as ofloxacin[8] or ciprofloxacin-dexamethasone otic[9] or eardrops of neomycin alone or with polymyxin combined with hydrocortisone, is effective in reducing local inflammation and infection.

Chronic otitis externa is caused by irritation from drainage through a perforated TM. The underlying cause is chronic suppurative otitis media (OM). Itching may be severe. Management is directed to treatment of the middle ear disorder. Rare causes of chronic otitis externa include tuberculosis, syphilis, yaws, leprosy, and sarcoidosis.

Invasive ("malignant") otitis externa is a severe, necrotizing infection that spreads from the squamous epithelium of the ear canal to adjacent areas of soft tissue, blood vessels, cartilage, and bone.[4,10] Severe pain and tenderness of the tissues around the ear and mastoid are accompanied by the drainage of pus from the canal. Older, diabetic, immunocompromised, and debilitated patients are at particular risk. Life-threatening disease may result from spread to the temporal bone and then on to the sigmoid sinus, jugular bulb, base of the skull, meninges, and brain. Permanent facial paralysis is frequent, and cranial nerves IX, X, and XII may also be affected.[11] *P. aeruginosa* is almost always the causative agent (see Chapter 219). The extent of damage to soft tissue and bone may be identified and monitored with the use of computed tomography and magnetic resonance imaging.[4] Diagnostic testing for comorbid disease should be routine. The canal should be cleansed, and devitalized tissue removed. Topical eardrops with antipseudomonal activity combined with a steroid are instilled into the external auditory canal. Systemic therapy with regimens including activity for *Pseudomonas* spp. should be used for 4 to 6 weeks, in combination with topical therapy. The combination of ceftazidime, cefepime, or piperacillin with an aminoglycoside (gentamicin or tobramycin) should be considered.[11] Oral quinolones with activity against *Pseudomonas* spp., such as ciprofloxacin, have been effective therapy early in the course of invasive external otitis.[12]

Aspergillus species, particularly *Aspergillus niger,* may grow in the cerumen and desquamated keratinaceous debris in the external auditory canal, sometimes forming a visible greenish or blackish fluffy colony. Role of the mold in acute otitis externa is usually modest, if it has any role at all, although in the severely immunocompromised patient *Aspergillus* can cause necrotizing otitis externa.[13] *Candida albicans* is also a frequent cause of external otitis in children with chronic mucocutaneous candidiasis.

Infections of the pinna may arise from placement of piercing ornaments in the cartilaginous portions of the ear, unlike the ear lobule where earrings are placed. This may result in chronic infection of the cartilage, often with *P. aeruginosa,* and can lead to severe cosmetic damage to the pinna unless treated early and effectively. Treatment involves removal of the ornament, drainage of fluctuant swelling, and administration of systemic antibiotics. Consultation with an otolaryngologist is advised, and reconstructive surgery may be required.

OTITIS MEDIA

Acute otitis media (AOM) is an acute illness defined by moderate-to-severe bulging of the TM or new onset of otorrhea not due to acute otitis externa, accompanied by acute signs of illness and signs or symptoms of middle ear inflammation.[1] Otitis media with effusion (OME) is defined by the presence of middle ear fluid without acute signs of illness or inflammation of the middle ear mucosa. It usually follows AOM but may also occur as a result of barotrauma or allergy. The peak incidence occurs in the first 3 years of life. The disease is less common in school-aged children, in adolescents, and in adults. Nevertheless, infection of the middle ear may be the cause of fever, significant pain, and impaired hearing in all age groups. In addition, adults experience the sequelae of OM of childhood: hearing loss, cholesteatoma, adhesive OM, and chronic perforation of the TM.

Three recent factors have altered the incidence, microbiology, and management of OM:

1. Introduction of the 7-valent conjugate pneumococcal vaccine (PCV7) in the United States in 2000 and subsequently the 13-valent vaccine (PCV13) in 2010 has reduced the number of episodes of vaccine serotype pneumococcal AOM and has decreased the incidence of severe and recurrent disease, in addition to altering the distribution of pathogens recovered from the middle ear in children with AOM.
2. Developed in 2004 and updated in 2013, management guidelines from the American Academy of Pediatrics (AAP) and the American Academy of Family Physicians (AAFP) for initial management of AOM are available, including observation without antimicrobial agents in selected cases[1,14] (Table 61.1).
3. These guidelines also emphasize the management of pain as part of the initial approach to treatment of AOM. Further details of the AAP/AAFP guidelines for the management of AOM and OME are discussed in the therapy section and in the accompanying references.[1,14,15,16]

Epidemiology

The epidemiology of AOM has been altered by the universal immunization of birth cohorts of children with pneumococcal conjugate vaccine (PCV) in the United States since 2000. An overall decline in AOM and complex OM has been observed in children.[17,18] Recently, the cumulative incidence of OM at 7 years was reported to be 60.6% in Danish children, with 16.2% reporting their first OM episodes at 0 to 6 months of age, 44.3% at 7 to 18 months, and 39.5% at 19 months to 7 years. Four or more OM episodes by age 7 years were reported by 64.0% of those who had their OM debut at 0 to 6 months, supporting the concept of "early and often" as a strong predictor of the child at risk for recurrent or complex disease.[19] OM is infrequent in adults, but the bacteriologic characteristics and therapy are similar to those in children.[20]

Longitudinal studies have provided information about the characteristics of children at greatest risk for recurrent and severe episodes of AOM. The vast majority of children have no obvious anatomic defect or immunologic deficiency responsible for severe and recurrent OM, but a small number have anatomic changes (cleft palate, cleft uvula, submucous cleft), alteration of normal physiologic defenses (patulous eustachian tube), or congenital or acquired immunologic deficiencies. An increased incidence of AOM occurs in children with Down syndrome.[21] Children with acquired immunodeficiency syndrome have a higher age-specific incidence of OM, beginning at 6 months of age.[22] Pichichero and colleagues have reported subtle T-cell and B-cell defects in children with recurrent OM manifesting as reduced responses to vaccine antigens.[23]

AOM occurs more often in males than in females and is observed more frequently when a family history (sibling or parent) is present. Concurrence in development of recurrent OM among identical twins supports a likely genetic susceptibility. Proinflammatory cytokine gene polymorphisms and polymorphisms in immunoresponse genes were also associated with recurrent AOM.[24,25] The age at the time of the first episode of AOM appears to persist as one of the most powerful predictors of recurrent middle ear infections. Breast-feeding for 3 or more months is associated with a decreased risk of AOM in the first year of life. Native Americans, Alaskan and Canadian Eskimos, and Aboriginals have an extraordinary high incidence and severity of OM.

The role of increased exposure to infectious agents and the importance of environmental pollutants have been identified in studies of the incidence of infection in group daycare and the effects of passive smoking on children. The introduction of infants into large daycare groups increases the incidence of respiratory infections, including OM. The daycare risk of infection is associated with the number of children in the facility. Children in daycare not only have more episodes of AOM than children in home care but have more complex disease, as measured by the need for more surgical procedures. A study of Pittsburgh children observed from birth through the second year of life noted that myringotomy and tympanostomy tube placements were performed in 21% of children in group daycare and in only 3% of children in home care.[26] Exposure to tobacco smoke documented by measuring a nicotine metabolite, cotinine, in saliva and urine was correlated with an increased incidence of new episodes of OME and the duration of effusion.[27] Kim and coworkers[28] have identified an association of invasive pneumococcal disease and OM with atmospheric conditions, air pollution (identified by levels of sulfur dioxide), and the isolation of respiratory viruses.

Pathogenesis

The middle ear is part of a continuous system that includes the nares, nasopharynx, and eustachian tube medially and anteriorly and the mastoid air cells posteriorly. These structures are lined with a respiratory epithelium that contains ciliated cells, mucus-secreting goblet cells, and cells capable of secreting local immunoglobulins.

Anatomic or physiologic dysfunction of the eustachian tube appears to play a critical role in the development of OM. The eustachian tube has at least three physiologic functions with respect to the middle ear: protection of the ear from nasopharyngeal secretions, drainage into the nasopharynx of secretions produced within the middle ear, and ventilation of the middle ear to equilibrate air pressure with that in the external ear canal. When one or more of these functions is compromised, the results may be the development of fluid and infection in the middle ear. Most episodes of AOM occur in the following sequence: congestion of the mucosa of the upper respiratory tract, most often caused by a

TABLE 61.1 Criteria for Initial Antibacterial-Agent Treatment or Observation in Children With AOM

AGE	UNILATERAL/BILATERAL AOM[a] WITH SEVERE SYMPTOMS[b]; AOM WITH OTORRHEA[a]	BILATERAL, NONSEVERE AOM[a] WITHOUT OTORRHEA	UNILATERAL, NONSEVERE AOM WITHOUT OTORRHEA[a]
< 6 mo	Antibiotic treatment	Antibiotic treatment	Antibiotic treatment
6 mo to 2 yr	Antibiotic treatment	Antibiotic treatment	Antibiotic treatment or initial observation[c]
2 yr	Antibiotic treatment	Antibiotic treatment or initial observation[c]	Antibiotic treatment or initial observation

AOM, Acute otitis media.
[a]Applies only to children with well-documented AOM with high certainty of diagnosis.
[b]A toxic-appearing child, or persistent otalgia more than 48 hours, or temperature ≥39°C (102.2°F) in the past 48 hours, or if there is uncertain access to follow-up after the visit.
[c]This plan of initial management provides an opportunity for shared decision-making with the child's family for those categories appropriate for additional observation. If observation is offered, a mechanism must be in place to ensure follow-up and begin antibiotics if the child worsens or fails to improve within 48 to 72 hours of AOM onset.
Modified from Lieberthal AS, Carroll AE, Chonmaitree T, et al. The diagnosis and management of acute otitis media. *Pediatrics.* 2013;131:e964.

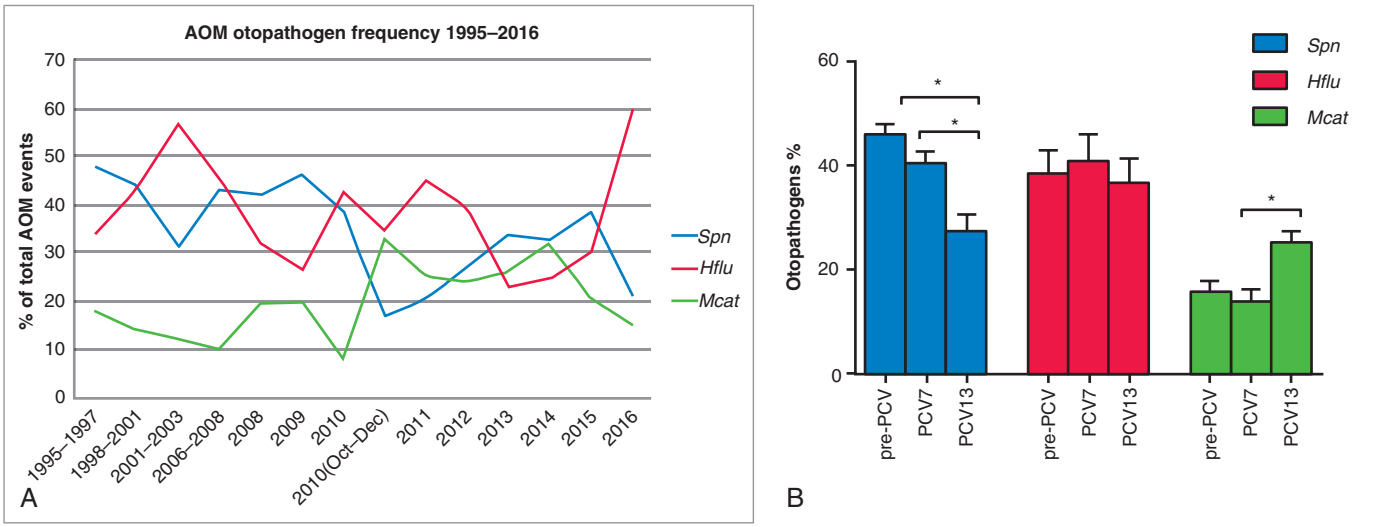

FIG. 61.1 Bacterial pathogens isolated from middle ear fluid in children with acute otitis media (AOM). (A) Frequency of otopathogens from MEF during AOM from 1995 to 2016. (B) Changes in otopathogen prevalence in different vaccine eras. *Hflu, Haemophilus influenzae; Mcat, Moraxella catarrhalis; PCV,* pneumococcal conjugate vaccine; *Spn, Streptococcus pneumoniae. (From Kaur R, Morris M, Pichichero ME. Epidemiology of acute otitis media in the postpneumococcal conjugate vaccine era.* Pediatrics. *2017;140:e2070181.)*

respiratory viral infection and swelling of the mucosa of the eustachian tube, progressing to obstruction of the tube at its narrowest section, the isthmus. Persistence of fluid for weeks to months after episodes of adequately treated AOM is commonplace. Recent evidence supports the presence of bacterial biofilms on the middle ear mucosa as playing a major role in development of OME and complex OM.[29]

Microbiology

Bacteria. The bacteriology of OM has been documented through appropriate cultures of middle ear effusions obtained with needle aspiration. Many studies of the bacteriology of AOM have been performed. The results are remarkably consistent in demonstrating the importance of *S. pneumoniae* and *H. influenzae* in all age groups. Initial studies after the introduction of PCV suggest that *H. influenzae* had replaced *S. pneumoniae* as the most frequently isolated pathogen of AOM in children (Fig. 61.1).[30,31] The most recent studies[32] report a nearly equal distribution of bacterial pathogens—*S. pneumoniae,* nontypable *H. influenzae,* and *M. catarrhalis*. *S. pneumoniae* remains an important cause of bacterial OM in most regions of the world.[33] Wherever PCV has been introduced, a decline in disease due to vaccine serotypes has been observed and serotypes 15A, 15B, 15C, 21, 23A, 23B, and 11A have emerged as most common.[34,35]

OM caused by *H. influenzae* is associated with nontypeable strains in the vast majority of patients. Before the introduction of *H. influenzae* type B conjugate vaccines, 10% of otitis cases were caused by *H. influenzae* type B; the condition was frequently severe and accompanied by bacteremia or meningitis. Type B is now rare because of the efficacy of the conjugate polysaccharide vaccine. Nontypeable strains of *H. influenzae* are a significant cause of OM in patients of all ages.[20] *H. influenzae* is the primary pathogen in the common conjunctivitis-AOM syndrome.[36]

M. catarrhalis was isolated from approximately 10% of children with AOM[37] in the past and was usually associated with a mild form of disease; however, recent studies have suggested that an increased proportion of cases are due to this pathogen. It is unclear, though, whether the incidence has increased. Today, most strains produce β-lactamase and are resistant to penicillin G, ampicillin, and amoxicillin.

S. aureus, including methicillin and multidrug-resistant strains, is an uncommon cause of AOM but is common in cases with persistent otorrhea that follows insertion of tympanostomy tubes.[38]

During the preantibiotic era, AOM caused by GAS was a frequent cause of severe AOM, frequently complicated by mastoiditis and often associated with scarlet fever. For reasons unknown, AOM caused by GAS is now uncommon. A survey in Israel identified GAS as being responsible for 3.1% of 11,311 episodes of AOM.[39] There is an increased proportion of cases due to GAS in children with otorrhea.

Viruses

Virologic and epidemiologic studies have suggested that viral infection is frequently the initial event in the development of AOM.[40,41,42,43,44,45] Respiratory viruses have been isolated from the nasopharynx in up to 50% of children with AOM and have been detected in approximately 25% of middle ear fluids of children with AOM. Respiratory syncytial virus (RSV), influenza virus, enteroviruses, coronaviruses, and rhinoviruses were the most common viruses found in middle ear fluids.[40,41,42,43] Combined viral and bacterial infections are frequent and may be more severe than bacterial infection alone.[43,44,45] Chonmaitree and coworkers[44] noted that in a higher proportion of patients with viruses and bacteria in middle ear fluids, the bacteria had failed to clear 2 to 4 days after initiation of therapy, compared with the group in which bacteria alone were present.

Mycoplasma, Chlamydia, and Unusual Organisms

Mycoplasma pneumoniae was responsible for hemorrhagic bullous myringitis in a study of nonimmune volunteers inoculated with the organism.[46] However, the middle ear fluid of a large number of patients (771) has been studied, and *M. pneumoniae* was isolated in only one case.[47,48] Although mycoplasmas do not appear to play a significant role in AOM, some patients with lower respiratory tract disease caused by *M. pneumoniae* may have concomitant OM.

Chlamydia trachomatis is associated with acute respiratory infections in infants younger than 6 months and is a cause of acute infection of the middle ear in this age group. The organism has been isolated from middle ear fluid of infants with acute infection.[49]

Uncommon forms of otitis include diphtheritic otitis, tuberculous otitis, otogenous tetanus, otitis caused by *Mycobacterium chelonae,*[50] and otitis caused by *Ascaris lumbricoides* or Wegener granulomatosis. Fungi are frequently associated with external otitis but rarely cause AOM; *Candida* and *Aspergillus* spp. have been isolated from middle ear fluids of immunodeficient patients who develop chronic suppurative OM.

Immunology

The importance of host defenses in protecting the middle ear is supported by the observations that AOM is more common in children with immune deficiency and may be the presenting complaint, especially when the defect is related to antibody production against encapsulated

bacterial pathogens.[51] Protection against bacterial OM has been observed after passive immunization with immune globulin and after immunization with polysaccharide conjugate vaccines, further demonstrating the role of host defense in protecting the middle ear from bacterial infection.[52,53–54]

Diagnosis and Clinical Course

AOM is an acute illness defined by moderate-to-severe bulging of the TM or new onset of otorrhea not due to acute otitis externa, accompanied by acute signs of illness and signs of middle ear inflammation. Signs and symptoms may be specific, such as ear pain, ear drainage, or hearing loss, or nonspecific, such as fever, lethargy, or irritability. Vertigo, nystagmus, and tinnitus may occur. Redness of the TM is an early sign of OM, but erythema alone is not diagnostic of middle ear infection because it may be caused by inflammation of the mucosa throughout the upper respiratory tract. However, AOM may manifest with intense erythema of the TM as the only otoscopic finding.[1]

The presence of fluid in the middle ear is determined by the use of pneumatic otoscopy, a technique that permits an assessment of the mobility of the TM. The motion of the TM is proportional to the pressure applied by gently squeezing and then releasing the rubber bulb attached to the head of the otoscope. Normal mobility is apparent when positive pressure is applied and the TM moves rapidly inward; with release of the bulb and the resulting negative pressure, the membrane moves outward. Fluid or high negative pressure in the middle ear dampens the mobility of the TM. Adjunctive techniques are available to confirm the results of otoscopic examinations and assist in the accuracy of diagnosis. Tympanometry uses an electroacoustic impedance bridge to record compliance of the TM and middle ear pressure. This technique presents objective evidence of the status of the middle ear and the presence or absence of fluid.[55] Acoustic reflectometry measures sound reflectivity from the middle ear and is able to distinguish an air- or fluid-filled space. Spatial gradient analysis is correlated with the probability of middle ear effusion in children.[56] In addition to a professional model, a consumer model (Innovia Medical, Omaha, NE) is available that permits home monitoring of the development or persistence of middle ear fluid.

Fluid persists in the middle ear for prolonged periods after the onset of AOM, even though symptoms usually resolve within a few days after the initiation of antimicrobial therapy. About 70% of children with OM have fluid in the middle ear 2 weeks after the onset of disease, 40% still have fluid 1 month after the onset, and 10% still have fluid 3 months after the first signs of middle ear infection.[15]

Patients with middle ear effusion develop hearing loss of variable severity. On average, a patient with fluid in the middle ear has a 25-dB (pure-tone average) loss.[57] Because the development of speech, language, and cognitive skills is dynamic during infancy, when the incidence of AOM is highest, there is concern that any impediment to reception or interpretation of auditory stimuli might have an adverse effect. Children with histories of recurrent episodes of AOM score lower in tests of speech, language, and cognitive abilities than their disease-free peers.[58,59]

The results of microbiologic studies of middle ear effusions in patients with AOM are so consistent that the choice of antimicrobial agents may be based on knowledge of the bacteriologic characteristics of OM acquired from the many investigations, rather than on the results of cultures from other sites, such as the throat or nasopharynx (see Fig. 61.1). If the patient is in a toxic condition or has focal infection elsewhere, cultures of samples of the blood and of the focal infection are warranted. Needle aspiration of the middle ear effusion (tympanocentesis) to define the microbiologic characteristics of the infection should be considered in select patients—the patient who is critically ill at the onset, the patient who has not responded to initial antimicrobial therapy in 48 to 72 hours and is in a toxic condition, and the patient with altered host defenses (e.g., an immunologic defect, including the newborn infant).

Management

Acute Otitis Media

Systemic and Topical Treatment of Otalgia

Pain is a common feature of AOM and may be severe. The AAP recommends treatment to reduce ear pain in children with AOM whether or not they are treated with antibiotics. Acetaminophen or ibuprofen is effective analgesia for mild-to-moderate pain. Topical procaine, or lidocaine preparations (if available) are an alternative for children 2 years of age or older but should not be used in children with TM perforation. Performance of incision and drainage of the middle ear abscess by means of tympanostomy or myringotomy usually requires otolaryngologic support but provides immediate relief.[1]

Antibiotic Therapy Versus Observation

In addition to pain control, there are two strategies for initial management of AOM: (1) immediate treatment with antibiotics and (2) observation with initiation of antibiotic therapy if the symptoms and signs worsen or fail to improve after 48 to 72 hours.

The choice of strategy depends on the age of the child, the severity of illness, and parental preference (Table 61.1). AAP guidelines recommend that children younger than 6 months with AOM be treated with an appropriate antibiotic at the time of diagnosis. In addition, those younger than 60 days or with high fever or severe irritability may require additional diagnostic evaluation for systemic illness.

Randomized trials support initial treatment for children 6 months to 2 years old with unilateral or bilateral AOM.[60,61] In children 2 years of age or older who are otherwise healthy children with mild symptoms and signs and no otorrhea, AAP guidelines support initial observation as an appropriate option; initial antibiotic treatment is recommended for those who appear toxic, have had persistent otalgia for more than 48 hours, have had a temperature of 102.2°F (39°C) or higher in the past 48 hours, or have bilateral AOM or otorrhea.

Selection of Antimicrobial Agents

The preferred antimicrobial agent for the patient with AOM must be active against *S. pneumoniae, H. influenzae*, and *M. catarrhalis*. GAS and *S. aureus* are infrequent causes of AOM and need not be considered in initial therapeutic decisions. Gram-negative enteric bacilli and methicillin-resistant *S. aureus* must be considered when OM occurs in the newborn infant, in the patient with a depressed immune response, and in the patient with a tympanostomy tube in place. The antimicrobial agent should achieve concentrations in middle ear fluid above the expected minimal inhibitory concentration of the likely pathogens. Craig and Andes[62] have examined the relationship between bacteriologic cure in OM and serum and middle ear fluid concentrations for various antimicrobial agents. For β-lactam antibiotics, they found that a bacteriologic cure required the presence of serum concentrations above the minimal inhibitory concentration for at least 40% of the dosing interval.

There are now 19 antimicrobial agents approved by the U.S. Food and Drug Administration (FDA) for AOM. Amoxicillin remains the drug of choice for initial treatment because of its 25-year record of clinical success, acceptability, limited side effects, and relatively low cost. The drug is ineffective against β-lactamase–producing strains of *H. influenzae* and *M. catarrhalis*. In situations such as the otitis conjunctivitis syndrome or in children recently treated with amoxicillin, the likelihood of disease due to β-lactamase–producing nontypeable *H. influenzae* is increased (in the United States), and initial therapy with amoxicillin clavulanate may be warranted.

The recommendation of increasing the dose of amoxicillin to 90 mg/kg/day achieves higher concentrations in middle ear fluid and further reduces the number of children in whom amoxicillin therapy will fail because of resistant pneumococci. Alternatives to amoxicillin include amoxicillin-clavulanate, sulfa-containing regimens or trimethoprim-containing preparations (erythromycin plus sulfisoxazole, trimethoprim, and trimethoprim-sulfamethoxazole), two macrolides (azithromycin and clarithromycin), nine oral cephalosporins (cephalexin, cefaclor, cefixime, ceftibuten, cefprozil, cefpodoxime, cefuroxime axetil, loracarbef, and cefdinir), and one parenteral cephalosporin (ceftriaxone). Two topical fluoroquinolones, ofloxacin and ciprofloxacin-dexamethasone otic, are effective in children who have tympanostomy tubes and develop acute otorrhea. Currently only high-dose amoxicillin and amoxicillin-clavulanate and parenteral administration of ceftriaxone achieve sufficient middle ear concentrations to be effective against penicillin-resistant *S. pneumoniae*.

If amoxicillin therapy fails, preferred regimens include the increased dose of amoxicillin clavulanate (90 mg/kg/day in two doses) or intramuscular ceftriaxone (50 mg/kg once a day for 1 to 3 days). For children with severe and recurrent AOM, the use of tympanocentesis to identify the bacterial pathogen and susceptibility pattern may be necessary in order to choose the most effective drug. For children with known and severe allergy to β-lactam antibiotics, a macrolide (erythromycin plus sulfisoxazole, azithromycin, or clarithromycin) is preferred, but trimethoprim-sulfamethoxazole may be useful in regions where pneumococcal resistance to this combination is not a concern.

Some children with AOM caused by a bacterial pathogen improve without the use of antimicrobial agents.[63,64–65] Howie and Ploussard[65] performed sequential aspirations of middle ear fluid in children with AOM 2 to 7 days apart, with placebo given instead of an antibacterial drug. They found that 19% of middle ear fluids infected initially with pneumococci became sterile, and 48% of middle ear fluids infected initially with *H. influenzae* became sterile. The discrepancy between the proportions of infections becoming sterile after infection with the two bacterial species indicates that a simple mechanical effect (drainage of the infected fluid via a patent eustachian tube or a perforated TM) was unlikely to be responsible for the microbiologic effect. It is more likely that host defenses, probably based on humoral or cellular immunity, act preferentially to rid the infected ear of *H. influenzae* more frequently than *S. pneumoniae*.

The microbiologic results indicating that many children have AOM caused by a viral pathogen, and that some of the episodes of bacterial OM resolve without antibacterial drugs, prompted some European physicians to withhold initial antibiotic therapy from children with ear infections.[66,67] The option of observation of children with AOM rather than initial antimicrobial therapy is practiced extensively in Western Europe.[68] Two randomized controlled studies of initial antimicrobial therapy with amoxicillin-clavulanate compared with initial observation for children younger than 2 years with AOM demonstrated increased rates of treatment failure and TM perforation in those assigned initial observation.[60,61]

With appropriate antimicrobial therapy, most children with AOM are significantly improved within 48 to 72 hours. In children with persistent fever or bulging of the TM after 48 hours, antimicrobial treatment should be initiated in those managed with initial observation, or a change in antimicrobial treatment should be considered for those initially managed with amoxicillin.

Treatment of Otitis Media With Effusion

OME is defined as fluid in the middle ear without signs or symptoms of acute illness. Most episodes resolve spontaneously within months; however, some patients have persistent disease that may last for 3 to 6 months, or longer. Persistent middle ear effusion may be associated with hearing loss, balance problems, sleep disorders, or poor school performance. The American Academy of Otolaryngology–Head and Neck Surgery Foundation, the AAP, and the AAFP have updated the 2004 guidelines for the management of OME.[69] The guidelines support improved diagnostic criteria including use of pneumatic otoscopy and observation in otherwise healthy children with middle ear effusion for a minimum of 3 months, and recommend against the use of antihistamines and decongestants, intranasal and systemic steroids, and antibiotics for treatment of persistent of middle ear effusion. The guidelines recommend that special consideration be given to children with underlying speech and language disorders; tympanostomy tubes be placed only (in the absence of other indications for tonsillectomy or adenoidectomy such as sleep apnea) when surgery is performed in children younger than 4 years; and adenoidectomy with tympanometry tubes be considered when surgery is indicated in children older than 4 years.[69]

Surgical Management

Surgical management of recurrent episodes of AOM and persistent effusion of the middle ear includes use of myringotomy, adenoidectomy, and the placement of tympanostomy tubes. Myringotomy, or incision of the TM, is a method of draining middle ear fluid. Before the introduction of antimicrobial agents, myringotomy was the primary method of managing suppurative OM. Today, the use of myringotomy is limited to the relief of intractable ear pain, hastening resolution of mastoid infection, and drainage of persistent middle ear effusion that is unresponsive to medical therapy.

Tympanostomy tubes resemble small collar buttons. They are placed through an incision in the TM to provide drainage of fluid and ventilation of the middle ear. The placement of these tubes is now one of the most common surgical procedures in children. Hearing improves dramatically after placement of the ventilating tubes. Placement of tympanostomy tubes has also been part of the management of children with AOM due to multidrug-resistant *S. pneumoniae*.[74] The major complication of tympanostomy tube insertion is persistent otorrhea.[81] This may involve staphylococcal species and biofilm formation on the tube.

Prevention of Acute Otitis Media

Prevention of severe and recurrent episodes of AOM includes chemoprophylaxis, use of bacterial and viral vaccines, and surgery.

Chemoprophylaxis

Chemoprophylaxis has been shown to be of value for the prevention of acute illness in children who have had recurrences of middle ear infections. A variety of studies, including various antimicrobial agents and a placebo, have documented the efficacy of an antimicrobial agent in modified dosage in reducing the number of episodes of acute febrile illnesses caused by OM.[70,71] However, a modified dosage form of an antimicrobial agent may select resistant strains in the nasopharynx, and chemoprophylaxis should be considered only for children with severe and recurrent infections. Children should be considered for prophylaxis if they have had two episodes of AOM in the first 6 months of life or, in older children, three episodes in 6 months or four episodes in 1 year. Amoxicillin, 20 to 40 mg/kg, or sulfisoxazole, 50 mg/kg, may be administered once daily. Chemoprophylaxis may suppress symptoms of OM, but asymptomatic middle ear effusion may persist. The physician who chooses to use chemoprophylaxis to prevent acute recurrent disease must examine the patient at approximately 1-month intervals for middle ear effusion.

Because of concern for development of multidrug-resistant bacteria in patients receiving chemoprophylaxis, the 2013 guidelines of the AAP and the AAFP discourage chemoprophylaxis. The guidelines suggest prevention by placement of tympanostomy tubes, although it necessitates a surgical procedure and anesthesia.[1] Physicians must discuss with the parent the risk-benefit ratio of antibiotic prophylaxis versus surgery for prevention of further severe AOM.

Pneumococcal Vaccines

Pneumococcal polysaccharide vaccines were inconsistently immunogenic and demonstrated limited protection against pneumococcal OM.[72,73] However, PCVs have had a significant impact on manifestations of OM associated with pneumococcal serotypes represented in the vaccine (Fig. 61.2).

A 7-valent conjugate pneumococcal polysaccharide vaccine, using a diphtheria toxin mutant (CRM197) as the protein carrier (Prevnar; Wyeth Pharmaceuticals [a subsidiary of Pfizer], Madison, NJ), demonstrated efficacy and effectiveness against vaccine-type invasive disease and resulted in a 60% decline in overall invasive pneumococcal disease. PCV7 was also efficacious in reducing vaccine serotype pneumococcal OM but had a more modest effect on overall AOM.[52] The vaccine reduced the number of episodes of AOM by 7% and reduced the number of procedures for placement of ventilating tubes by 23%, reflecting a decline in recurrent episodes and complex OM. The reduction in number of episodes of pneumococcal AOM was 34%, but the reduction in episodes caused by vaccine serotype disease was 57%. Of concern were increases in the number of episodes of AOM caused by nonvaccine organisms in children who received PCV7, a 33% increase in nonvaccine serotype pneumococcal AOM, and an 11% increase in episodes caused by *H. influenzae*. These data suggested that the vaccine was successful in reducing carriage of vaccine serotypes but that pneumococcal carriage was replaced with nonvaccine serotypes, which subsequently spread from the upper respiratory tract to the middle ear to cause AOM. An example of this serotype replacement was the emergence of

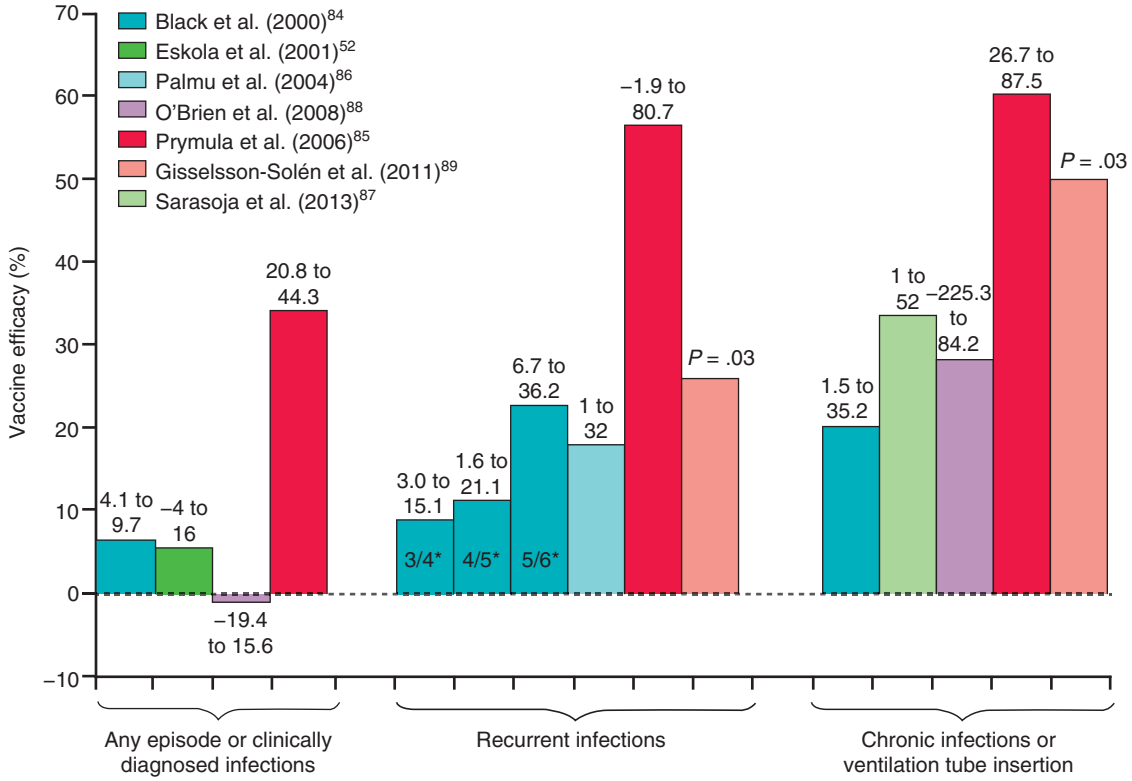

FIG. 61.2 Impact of pneumococcal conjugate vaccine on acute otitis media, recurrent otitis media, and chronic infection or tympanostomy tube insertions. *Data are number of episodes in 6 months and 12 months in the Northern California Kaiser Permanente study.[52] †Parents were not blinded to their child's allocation to the vaccine or control group, although they were asked not to reveal this to the study personnel. *(From Dagan, Pelton S, Bakaletz L, et al. Prevention of early episodes of otitis media by pneumococcal vaccines might reduce progression to complex disease.* Lancet Infect Dis. *2016;16:480–492.)*

multidrug-resistant serotype 19A, a strain not included in PCV7, as an increasing cause of invasive disease and OM.[74]

PCV13 (Prevnar 13, Wyeth Pharmaceuticals) was introduced in 2010 and replaced PCV7 in the United States. In addition to the serotypes in PCV7, the additional serotypes, including types 1, 3, 5, 6A, 7V, and 19A, were added. Dagan and colleagues[35] reported declines in AOM due to the unique vaccine serotypes in PCV13 with no significant increase in disease due to non-PCV13 serotypes. Marom and colleagues observed an overall downward trend in OM-related health care visits after the introduction of PCV7. They also observed a significant reduction in OM visit rates, tympanostomy tube insertions, and mastoiditis in 2010 and 2011 in children younger than 2 years in concert with the introduction of PCV13.[18] The proposed mechanism for the reduction in complex OM and tympanostomy tube insertion is the prevention of early episodes of pneumococcal OM and a reduction in subsequent episodes.[17] Unfortunately, studies of PCV7 administered to children who had already had two or more episodes of OM in the prior year failed to demonstrate any reduction in otitis.[75] A 10-serotype vaccine with most of the pneumococcal polysaccharides conjugated to protein D from nontypeable *H. influenzae* with serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23 was studied and subsequently licensed (Synflorix; GlaxoSmithKline Biologicals). A large clinical trial demonstrated a 16.1% vaccine efficacy against clinically confirmed AOM and 67% against vaccine serotype clinically confirmed AOM. The vaccine has been licensed globally but is not available in the United States.

Respiratory Virus Vaccines

Because of the importance of respiratory viruses in the pathogenesis of AOM, viral vaccines could be of preventive value. Inactivated parenteral influenza virus vaccines were documented to decrease the incidence of AOM in children in daycare in Finland[76] and North Carolina.[77] A reduction of 30% of episodes of febrile OM was also reported in children after the receipt of live-attenuated intranasal influenza vaccine.[78] High-titer RSV immune globulin and RSV monoclonal antibody (palivizumab) have been studied for prevention of RSV respiratory disease.[79,80] The RSV immune globulin, but not the monoclonal antibody, was effective in reducing the number of episodes of AOM.

Additional Complications of Acute Otitis Media

Perforation of the TM is the most common complication of AOM and occurs most frequently in younger children. Certain ethnic groups, such as Alaskan Eskimos and Native Americans, have a higher rate of spontaneous perforation with AOM. Differentiation between AOM with perforation and acute otitis externa can be difficult. In general, the history of increasing pain with relief when otorrhea occurs is found with AOM, whereas increasing pain without relief in the face of otorrhea is seen with otitis externa. The natural history of AOM with perforation is usually complete resolution with healing of the TM. Topical otic suspensions, either ofloxacin or ciprofloxacin administered through a tympanostomy tube, are the preferred therapy for uncomplicated episodes of acute otorrhea.

Facial palsy is less frequent with the routine use of antibiotic therapy. Facial weakness and earache are the predominant symptoms. Management with antimicrobial agents and myringotomy (with or without tube insertion) is usually sufficient to achieve complete resolution.

Labyrinthitis develops when AOM spreads (through the round window) into the cochlear space. The process may be suppurative or serous (due to toxins). The onset of labyrinthitis is often sudden, with vertigo and hearing loss being characteristic. Acute surgical intervention (myringotomy with tube insertion) with antimicrobial therapy is the treatment of choice.

Gradenigo syndrome is a rarely seen complication of AOM in which infection spreads to the apex of the petrous temporal bone. A triad of symptoms consisting of unilateral periorbital pain due to trigeminal nerve involvement, diplopia due to sixth nerve palsy, and persistent otorrhea is present. This classic triad has become very uncommon in the antibiotic era.

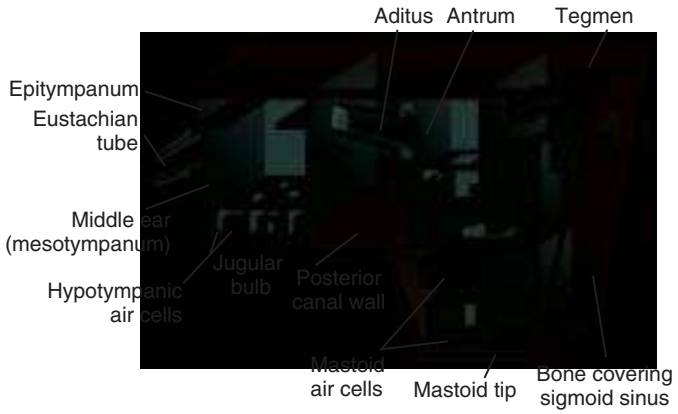

FIG. 61.3 Diagrammatic representation of the anatomy of the middle and mastoid air cell system showing the narrow connection (aditus and antrum) between the two.

MASTOIDITIS

The mastoid is the portion of the petrous temporal bone that lies superior to the middle ear cavity. The mastoid is composed of interconnecting air-filled cells. The mastoid antrum serves as an open passage between the middle ear and mastoid air cells (Fig. 61.3). Thus, most cases of AOM with fluid filling the middle ear space are associated with some degree of inflammation of the mastoid air cells. The incidence of clinically significant mastoiditis, however, declined dramatically once antimicrobial therapy became available for AOM. Nevertheless, acute mastoiditis still occurs and may be responsible for severe illness.

At birth, the mastoid consists of a single cell, the antrum, connected to the middle ear by a small channel. Pneumatization of the mastoid bone takes place soon after birth and is extensive by 2 years of age. The clinical importance of the mastoid is related to contiguous structures, including the posterior cranial fossa, the middle cranial fossa, the sigmoid and lateral sinuses, the canal of the facial nerve, the semicircular canals, and the petrous tip of the temporal bone. The mastoid air cells are lined with modified respiratory mucosa, and all are connected with the antrum. Mastoiditis can occur at any age and may be particularly severe in older adults.[82,83]

Infection in the mastoid follows middle ear infection. Initially, there is hyperemia and edema of the mucosal lining of the air cells followed by serous and then purulent exudate. Necrosis of bone with destruction of the bony septa followed by coalescence of pus results in abscess cavities.

The bacteriology of mastoiditis is the same as that of AOM, including *S. pneumoniae* and *H. influenzae* as the major pathogens. Patients with persistent perforation of the TM may have invasion of organisms from the ear canal, including *Pseudomonas* spp. A pattern similar to the changes in pneumococcal isolates responsible for AOM, the emergence of multidrug-resistant serotype 19A, after the introduction of PCV7 was also observed in children with acute mastoiditis.[83]

Clinical Manifestations

Acute mastoiditis is usually but not universally accompanied by AOM. During early stages, the signs are those of AOM with otalgia, and fever. Subsequently, swelling, redness, and tenderness are present behind the auricle, over the mastoid bone. The pinna may be displaced outward and downward. A purulent discharge may emerge through a perforation in the TM.

Chronic mastoiditis can develop, usually in association with chronic OM. Infection within the mastoid cells may slowly evolve into an indolent, painless chronic process, with chronic drainage from the external auditory canal through a perforated TM. Decreased hearing is usual and may be accompanied by a cholesteatoma or osteomyelitis of the ossicles. Extension through the tympanum or apex of the middle ear may cause an abscess in the contiguous temporal lobe or may extend posteriorly, causing septic thrombosis of the lateral sinus. Organisms include *S. aureus,* mycobacteria, or molds, usually *Aspergillus.* Mastoidectomy and resection of the cholesteatoma are essential for treatment, which may include tympanoplasty and sometimes resection of the ossicles. Antimicrobial therapy may need to continue for 6 weeks or more in order to prevent relapse.

Diagnosis

Radiographs of the mastoid area may show a loss of sharpness, demineralization of bony septa, and cloudiness. Computed tomography is helpful in delineating the extent of disease and determining if abscess is present. If the TM is not perforated, tympanocentesis should be performed to obtain material from the middle ear. If otorrhea is present, culture can be performed once the canal has been cleaned and fresh pus obtained as it exudes from the TM.

Management

The antimicrobial drugs of choice for acute infection are similar to those for AOM: antibiotics with activity against *S. pneumoniae* and *H. influenzae.* Commonly, initial therapy is parenteral ceftriaxone. If the disease in the mastoid has had a prolonged course, coverage for *S. aureus* and gram-negative enteric bacilli may be considered for initial therapy until the results of cultures become available. A mastoidectomy is performed if an abscess has formed in the mastoid bone. The procedure should be performed after initial antimicrobial agents have controlled sepsis.

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62 Sinusitis

Gregory P. DeMuri and Ellen R. Wald

SHORT VIEW SUMMARY

Definition
- Sinusitis is defined as an inflammatory disorder of the paranasal sinuses.

Epidemiology
- Bacterial infection of the sinuses is estimated to occur in 0.5% to 2% of cases of viral upper respiratory infection (URI) in adults and in 6% to 13% of children.

Microbiology
- Classic studies of the bacteriology of sinusitis have obtained a specimen of sinus secretions by puncture of the maxillary antrum to reduce the risk of nasal contamination.
- Historically, *Streptococcus pneumoniae* was the most frequently isolated organism, followed by nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis*.
- The frequency of isolation of *S. pneumoniae* has decreased recently, along with an increase in β-lactamase–producing *H. influenzae*.
- *Staphylococcus aureus* is not likely a significant cause of acute sinusitis but does play a role in the complications of sinusitis.

Diagnosis
- Imaging studies are not indicated for the routine diagnosis of acute sinusitis but may be useful when complications are suspected.
- The following three clinical presentations will identify patients with acute bacterial sinusitis:
  - Onset with *persistent* symptoms or signs, lasting at least 10 days without evidence of substantial clinical improvement
  - Onset with severe symptoms or signs of high fever (≥39°C) and purulent nasal discharge lasting for 3 to 4 consecutive days
  - Onset with worsening symptoms or signs characterized by the new development of fever, headache, or increase in nasal discharge after a typical viral URI that lasted 5 to 6 days with initial improvement

Therapy
- For most adults and children, amoxicillin with or without potassium clavulanate remains an excellent first-line agent for the treatment of sinusitis.
- Second-line agents include fluoroquinolones, cefdinir, cefuroxime, or the combination of cefixime with either clindamycin or linezolid.
- The duration of therapy should be for 7 days after the patient becomes free of signs and symptoms.
- Adjunctive therapies, such as antihistamines, decongestants, nasal steroids, and nasal washes, provide minimal improvement in acute sinusitis.
- Surgical drainage is indicated for the complications of acute bacterial sinusitis.

Sinusitis is defined as an inflammatory disorder of the paranasal sinuses. This condition is one of the most common reasons for a patient to seek care from their primary care physician and is responsible for more than 20 million antibiotic prescriptions per year in the United States.[1] Sinusitis may be classified as acute or chronic. Because most cases of acute bacterial sinusitis are secondary to a viral upper respiratory infection (URI) or allergic inflammation, nasal inflammation is a common finding, and some experts refer to this complex as rhinosinusitis. However, in these instances of bacterial superinfection of the paranasal sinuses, the nose is merely a conduit for the secretions originating in the sinuses. The preferred term is acute community-acquired bacterial sinusitis.[2]

Evidence of maxillary sinusitis has been found in human archeologic specimens discovered in Africa, North America, and Europe.[3] Hippocrates recognized the association between high arched palate, nasal obstruction, headache, and discharging ears—likely rhinosinusitis associated with otitis media. Medieval physicians believed nasal discharge emanated from fluid at the base of the brain. In fact, the pituitary gland is named from the Latin word for slime or mucus as it was believed that the source of yellow discharge from the nose came from the hypophysis. The first accurate description of the paranasal sinuses was by Vesalius in the 16th century, and the first documented cases of suppurative sinusitis were described by Antonio Molinetti in Venice in 1697.[4] The unique and troublesome drainage of the maxillary sinus was recognized by William Cowper in 1707, who eloquently described how "the antra have small openings situated high up in the cavity so that peccant humors could not escape into the nose unless the antrum were full to the top or the head held to one side."[4] Early treatment of sinusitis was mainly surgical, often involving either puncture of the sinus with a trocar or the removal of a molar with drainage through alveolar bone (Fig. 62.1).

ANATOMY AND PHYSIOLOGY OF THE PARANASAL SINUSES

Infections of the paranasal sinuses are a direct consequence of their unique anatomy and physiology. An appreciation of the anatomic relationships of the sinuses is necessary to understand the pathogenesis and complications of sinusitis. The paranasal sinus cavities consist of the maxillary, ethmoid, frontal, and sphenoid sinuses. The maxillary and ethmoid sinuses develop during the third month of gestation and thus are present, albeit small, at birth.[5] The maxillary sinus originates as a slitlike cavity parallel to the middle turbinate and elongates into a pyramidal shape with a final volume of 15 to 30 mL in the adult. The floor of the maxillary sinus lies over the alveolar ridge of the maxilla, which holds the upper dentition. The walls of the maxillary sinus extend from the lateral wall of the nasal cavity to the zygomatic arch and the roof of the sinus is the floor of the orbit. The proximal location of the paranasal sinuses to structures such as the teeth (maxillary sinus) and eye (ethmoid and frontal sinuses) explain, at least in part, some of the clinical manifestations in the affected patient. The outflow tract of the maxillary sinus is located at the highest part of the medial wall of the sinus where it opens into the nasal cavity. This unfortunate positioning inhibits gravitational drainage of secretions and requires an intact mucociliary apparatus to move secretions and debris from the body of the sinus to the nose, thereby predisposing to infection. The ostium of the sinus empties by the way of the hiatus semilunaris into the nasal cavity via a 7- to 11-mm long passage called the infundibulum, which drains into the middle meatus.[6] The anterior ethmoid and frontal sinuses also empty into this same location, known as the osteomeatal complex (Fig. 62.2). The ethmoid sinuses are a complex group of 5 to 15 tiny air cells separated from one another by very thin bony partitions. The lamina papyracea, named for its paper-like thinness, comprises the

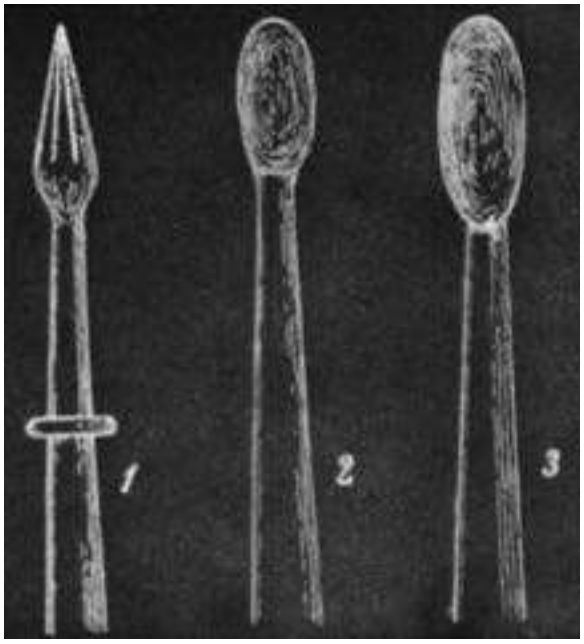

FIG. 62.1 Trocars used in the early surgical treatment of sinusitis. *(From Blegvad NR. History of the treatment of maxillary sinusitis.* J Laryngol Otol. *1957;71:806–823.)*

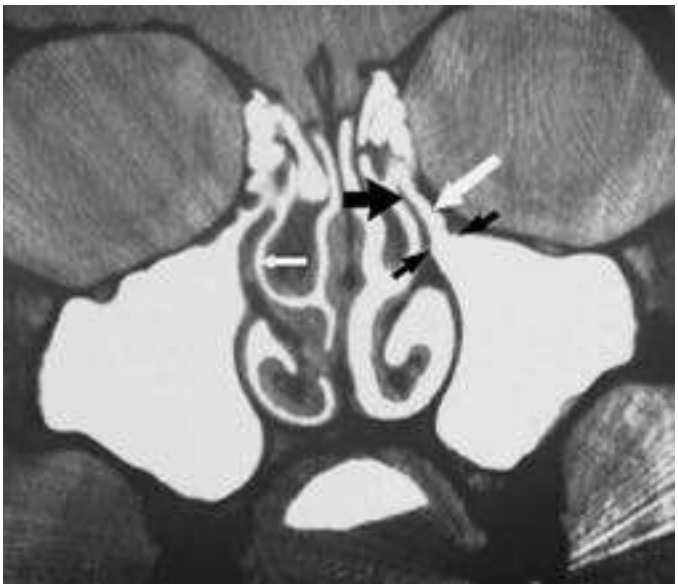

FIG. 62.2 Selected features of the anatomy of the drainage system of the maxillary sinus shown on computed tomography scan. The ostium is located between the two small white arrows. The ostium opens into a tubular structure, the infundibulum, shown by the large black arrow. The large white arrow indicates the upper part of the uncinate process, which forms the inferior portion of the infundibulum. The infundibulum empties into the middle meatus. The small black arrow indicates the contralateral middle meatus, which is narrowed as a result of turbinal distention from the normal nasal cycle.

medial wall of the orbit (lateral wall of the ethmoid) and provides a minimal barrier for spread of infection from the sinuses to the orbit. The ethmoid sinuses are divided into anterior and posterior cells. The larger anterior group empties into the middle meatus and the posterior cells into the superior meatus. The frontal sinuses develop from an anterior ethmoid cell and are present above the orbital ridge by the fifth or sixth birthday. Up to 5% of individuals lack one or both frontal sinuses.[7] The posterior wall of the frontal sinus is the anterior wall of the cranial fossa, and the floor of the frontal sinus forms the roof of the orbit. The frontal sinuses drain into the middle meatus via the frontonasal duct.

TABLE 62.1 Factors That Predispose to Sinus Ostial Obstruction

MUCOSAL SWELLING	MECHANICAL OBSTRUCTION
Systemic Factors	Choanal atresia
Viral upper respiratory infection	Deviated septum
Allergic inflammation	Nasal polyps
Cystic fibrosis	Foreign body
Immune disorders	Tumor
Ciliary dyskinesia	Ethmoid bullae
Tobacco smoke	
Local Insult	
Facial trauma	
Swimming, diving	
Rhinitis medicamentosa	
Nasal intubation	

The location of the frontal sinuses allows for the easy and rapid spread of infection from the sinus cavity to the central nervous system (CNS) and/or the orbits. The sphenoid sinuses are located just anterior to the pituitary fossa and are surrounded by several vital structures, including the optic nerve, internal carotid arteries, and the cavernous sinuses. The sphenoid sinus drains via a narrow duct into the superior nasal meatus. The sphenoid sinuses are infrequently the only site of infection; rather, they usually accompany a pansinusitis. The sphenoid sinus, similarly to the frontal sinus, is a source for the spread of infection from the sinuses to the CNS.

The paranasal sinuses are lined with a pseudostratified columnar (respiratory) epithelium, which also lines much of the nasal cavity. This epithelial lining contains four types of cells: basal cells, which adhere to the basement membrane; columnar cells, which possess cilia; goblet cells, which produce mucus to protect and lubricate the epithelial surface; and inflammatory cells. These inflammatory cells consist of T and B lymphocytes as well as antigen recognition cells.[6,7]

Mucus and other material produced in the maxillary sinus cavity are transported by ciliary action in a spiral direction up to and through the infundibulum into the middle meatus. These cilia beat at a frequency of 1000 times per minute and move material at a rate of 3 to 25 mm per minute.[8] The mucus blanket in the sinus turns over two to three times per hour and mucus normally does not accumulate in the sinus cavity.[9]

The paranasal sinuses are sterile under normal conditions unlike the nasal passages, which are heavily colonized with bacteria.[10–12] Sterility is maintained in the sinus by mechanisms that are not fully understood but are believed to include mucociliary clearance, the immune response, and possibly antibacterial concentrations of nitric acid in the sinus cavity.[13] Nasal secretions contain the immunoglobulins (Igs) IgA, IgG, IgM, and IgE, enzymes such as lysozymes; and proteins such as lactoferrin and complement, all of which exert an antibacterial effect.[14]

Although the exact function of the paranasal sinuses in humans is unknown, multiple roles have been suggested. These include contributing to the resonance of the voice, warming and humidifying inspired air, and acting as a shock absorber for the brain by absorbing energy during trauma.[6]

PATHOGENESIS

The pathogenesis of rhinosinusitis involves three key elements: the patency of the sinus ostia, the function of the ciliary apparatus, and the character of sinus secretions. The narrow caliber of the sinus ostia sets the stage for obstruction to occur. Factors that predispose the ostia to obstruction include those that result in mucosal swelling and those that cause direct mechanical obstruction. Table 62.1 lists the most common factors that predispose to ostial obstruction. Of these multiple causes, viral URI and allergic inflammation are the most frequent and most important. During episodes of acute rhinitis, a completely patent ostia is present only 20% of the time.[15]

When obstruction of the sinus ostium occurs, there is a transient increase in pressure within the sinus cavity. As oxygen is depleted in this closed space, the pressure in the sinus becomes negative relative to atmospheric pressure. This negative pressure may allow the

introduction of nasal or nasopharyngeal bacteria into the sinuses during sniffing or nose blowing.[16] When obstruction of the sinus ostia occurs, secretion of mucus by the mucosa continues, resulting in accumulation of fluid in the sinus. A study of adult volunteers investigated the role of nose blowing in introducing nasal fluid, and thus possibly microbes, into the sinus cavities.[17] Serial computed tomography (CT) scans showed that up to 1 mL of viscous fluid was propelled into the sinus when volunteers blew their noses. In accordance, this serves as one potential mechanism for nasal fluid and flora to contaminate the sinuses, particularly during a common cold. It should be noted, however, that young children who do not blow their noses still develop acute bacterial sinusitis. Thus there must be multiple factors that play a role in the development of acute infection.

Dysfunction of the mucociliary apparatus also contributes to the pathogenesis of sinusitis. During viral colds, both the structure and function of the mucociliary apparatus are impaired. In a study of children with viral URI, subjects had nasal mucosal biopsies performed for examination of the ultrastructure of the cilia. Dysmorphic ciliary forms involving microtubular abnormalities were observed during the acute phase (7 days) of illness. Progressive loss of ciliated cells was noted throughout the illness in a patchy pattern.[18] In a study of documented viral URIs in adults, mucociliary clearance was measured using a solution of blue-colored saccharin. Mucociliary clearance times, measured by taste and color, were significantly slower during the acute phase of illness.[19] Presumably, these same changes in the structure and function of the nasal mucosa during viral URI occur in the sinus mucosa. This contributes to the reduced clearance of fluid and material that increases the likelihood of infection of the sinus cavity.

The quality and character of sinus secretions also plays a role in the pathogenesis of sinusitis. Cilia can only beat in a fluid media. The mucus blanket in the respiratory tract consists of two layers. The sol phase is a thin, low-viscosity layer that envelopes the shaft of the cilia and allows the cilia to beat freely. A more viscous layer, the gel phase rides on the sol phase. Alterations in the mucus layer, which occur in patients with cystic fibrosis or allergy, may impair ciliary function. The presence of inflammatory debris, which is found in an infected sinus, may further impair ciliary movement.

Historically, it has been stated that a reduction in airflow through the nasal passages contributes to the development of rhinosinusitis. An extensive review of this hypothesis, however, found no convincing evidence that diminished airflow is a factor in sinus pathology.[20]

Until recently the histologic findings during acute sinusitis had not been well characterized except in experimental models. In a rabbit model of acute sinusitis, histologic changes include epithelial desquamation, edema, and goblet cell hyperplasia. Of note is the distinct loss of ciliated cells from the epithelium.[21,22] Berger[23] examined biopsies of 11 humans who had acute sinusitis. Surprisingly, the epithelial layer of the sinus remained intact. In contrast, the lamina propria showed edema and massive infiltration of neutrophils and mononuclear cells, including lymphocytes and plasma cells. Occasional aggregates of inflammatory cells with microabscesses were also detected. Thrombosed blood vessels and deep necrotic foci were observed in patients with complications of acute sinusitis. Immunohistologic staining showed T lymphocytes scattered throughout the lamina propria with dense aggregates of B lymphocytes.[23]

The role of viral URI preceding episodes of acute bacterial sinusitis should be underscored. Like otitis media, most cases of sinusitis are directly preceded by a viral URI.[24–26] Cytokine production by respiratory epithelial cells is important in viral URI. Interleukin (IL)-1β, IL-8, IL-6, and tumor necrosis factor-α (TNF-α) are markedly elevated in nasal lavage fluid during acute URI compared with baseline.[27] These and other chemokines are responsible for the inflammatory response that predisposes to acute sinusitis. An analysis of cytokine production in sinusitis has shown that IL-8, a potent chemoattractant for neutrophils, is upregulated in the sinus during acute infection.[28] In addition, viral URI has been demonstrated to increase the density and frequency of colonization with known sinus pathogens.[25]

In patients with acute sinusitis, healing of the mucosa occurs over a period of weeks after infection. In a study in which serial magnetic resonance images (MRIs) were performed in patients with acute bacterial sinusitis, clinical symptoms resolved within 3 days of treatment in most patients. Radiographic changes took much longer to show improvement, with only half of the sinuses showing resolution of opacification by 10 days. It took up to 56 days for 80% of the sinuses to be completely aerated.[29]

MICROBIOLOGY

Knowledge of the microbiology of acute community-acquired sinusitis is essential in choosing appropriate antimicrobial therapy. Studies performed to examine this issue require close attention to the method of specimen collection. The nasal cavity is heavily colonized with respiratory flora, which may easily contaminate material obtained from the paranasal sinuses. Classic studies of the bacteriology of sinusitis have obtained a specimen of sinus secretions by puncture of the maxillary antrum to reduce the risk of nasal contamination. In this method the maxillary sinus is accessed by puncture through a transnasal approach. A trocar is placed beneath the inferior nasal turbinate through the lateral nasal wall. Meticulous efforts to sterilize the mucosal area through which the trocar is placed avoids contaminating the specimen with nasal flora. In a further effort to discriminate true infection from contamination, quantitative methods are used to enumerate the density of microbes. Infection is defined as a colony count of at least 10^4 colony-forming units per milliliter of aspirated material. [30]

Because of the invasive nature of this procedure, there has been considerable interest in obtaining cultures of sinus material through less invasive means. Cultures obtained via an endoscope have been compared with those obtained by sinus aspiration. In this method the sample is obtained from the middle meatus adjacent to the sinus ostia via swab or aspiration through the endoscope. Because the endoscope is passed through the nonsterile anterior nasal cavity, the potential for contamination is great. Many studies correlating sinus puncture with middle meatal culture attempt to improve the results of cultures obtained endoscopically by analyzing data only for *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis,* while dismissing other bacteria. This may ignore potential pathogens such as β-hemolytic streptococci and anaerobes. Most studies of the performance of endoscopic middle meatal culture in adult patients show a sensitivity of approximately 80%, specificity of 90%, positive predictive value of 80% to 90%, and negative predictive value of 80% to 90%, with maxillary sinus aspiration considered the gold standard.[31–34] (Table 62.2) It should be noted however, that the sensitivity and specificity of cultures obtained by endoscope is much lower in children. This is likely due to the smaller nasal cavity and more difficult technical aspects of the procedure.[33] Furthermore, middle meatal cultures obtained from a group of normal children without respiratory symptoms frequently showed the presence of the sinus pathogens *S. pneumoniae* and *H. influenzae*.[35] In accordance, sinus puncture remains the gold standard in investigating the microbiology of sinusitis in children.

TABLE 62.2 Comparison of Endoscopic Middle Meatal Culture Versus Sinus Puncture in Acute Sinusitis

STUDY	*N*	SENSITIVITY %	SPECIFICITY %	POSITIVE PREDICTIVE VALUE %	NEGATIVE PREDICTIVE VALUE %	POPULATION
Talbot[31]	46	85.7	90.6	8.0	93.5	Adults
Joniau[32]	24	80.0	100.0	100.0	78.6	Adults
Benninger[34]	126	80.9	90.5	82.6	89.4	Adults
Hsin[33]	41	75.0	88.9	96.0	50.0	Children

In studies of acute community-acquired bacterial sinusitis in which sinus puncture has been performed with attention to reducing contamination and analyzing results quantitatively, *S. pneumoniae* is the most frequently isolated organism, followed by nontypeable *H. influenzae* and *M. catarrhalis*. Streptococci, *Staphylococcus aureus,* and anaerobes are isolated much less frequently (Table 62.3). In children with acute bacterial sinusitis, *M. catarrhalis* is isolated with a greater frequency than in adults.

The predominance of pneumococci, *Haemophilus,* and *Moraxella* as pathogens in cases of acute sinusitis in children has not changed in more than 50 years.[12,36–42] However, the relative role of *S. pneumoniae* has diminished since the introduction of universal infant immunization with pneumococcal conjugate vaccine, and the serotypes have shifted.[43] This has not been confirmed by direct sinus aspirates, which are rarely performed in children with uncomplicated acute sinusitis. Rather, inferential data may be obtained by performance of tympanocentesis in children with acute otitis media. The similarity in pathogenesis of acute otitis media and acute bacterial sinusitis in children permits this inference to be made. The middle ear is, after all, a paranasal sinus.[44]

Accompanying the decrease in the isolation of *S. pneumoniae* has been an increase in the rate of isolation of nontypeable *H. influenzae* from middle ear isolates. The most recent study from a center performing tympanocentesis in the United States demonstrates a dramatic decrease in the recovery of *S. pneumoniae.* In contrast, *H. influenzae* and *M. catarrhalis* have increased in frequency to account for up to 90% of isolates. The surprising data are that the prevalence of β-lactamase producing organisms is higher than expected, in the range of 70% to 80%.[45] Studies of nasopharyngeal colonization have demonstrated a similar decrease in carriage of pneumococcal serotypes contained in the 13-valent pneumococcal conjugate vaccine and a relative increase in nonvaccine strains.[46] Of importance, the rate of colonization with penicillin- and ceftriaxone-resistant *S. pneumoniae* has decreased recently.[45,47,48] These shifts in microbiology have important implications for the selection of appropriate antimicrobial agents for the treatment of sinusitis.

TABLE 62.3 Bacterial Etiology of Acute Sinusitis

	ADULTS (*n* = 339)		CHILDREN (*n* = 30)	
ORGANISM	NO. OF ISOLATES	% OF ISOLATES	NO. OF ISOLATES	% OF ISOLATES
Streptococcus pneumoniae	92	41	17	41
Haemophilus influenzae	79	35	11	27
Anaerobes	16	7		
Streptococcal species	16	7	3	7
Moraxella catarrhalis	8	4	9	22
Staphylococcus aureus	7	3		
Other	8	4	1	2

Data from references 12 and 30–42.

The role of *S. aureus* in the etiology of sinusitis has been controversial. Some authors have purported that this organism should be considered a major pathogen of the sinuses. This would have important implications for antimicrobial selection as most guidelines do not recommend agents that are directed at this organism.[34,49,50] However, when examined carefully, the role of *S. aureus* as an etiologic agent of acute bacterial sinusitis is doubtful.[51] In sinus aspiration studies of adults done over a 15-year period, *S. aureus* was detected in only 7 of 226 (3%) positive specimens obtained from 339 patients.[40] In the two studies in which maxillary sinus aspirates were performed in 50 children with acute bacterial sinusitis, no isolates of staphylococci were detected.[37,52] Most studies that argue that *S. aureus* is a pathogen in this setting are based on middle meatal cultures. To critically analyze these studies it is imperative to understand the microbiology of the nose and middle meatus in healthy individuals. The nasal vestibule is a location that is frequently heavily colonized with *S. aureus.* In a study of healthy children in a community setting, *S. aureus* was detected in greater than 65% of cultures obtained from the anterior nares.[53] Any study of the microbiology of the middle meatus will involve passage of the endoscope through this highly colonized region. Even with antiseptic measures, contamination of the specimen is possible. Studies of the middle meatus have also shown similarly high levels of detection of *S. aureus*. Gordts and colleagues[54] performed middle meatal cultures on healthy children undergoing surgery for reasons unrelated to the sinuses. *S. aureus* was found in 20% of children, indicating that this region is also frequently colonized.

Thus caution must be exercised in interpreting studies that highlight the role of *S. aureus* as a major pathogen in acute bacterial sinusitis as there is serious concern that these studies have a high rate of contamination of specimens with normal nasal flora. Nonetheless, although *S. aureus* is a very infrequent cause of acute bacterial sinusitis in children, it is a frequent etiologic agent when complications (intracranial and/or orbital) occur. The reason for this discrepancy is unknown.

Despite their prominent role in the pathogenesis of acute community-acquired bacterial sinusitis, viruses have been isolated from patients with acute sinusitis infrequently. This may relate, at least in part, to the timing of sinus aspiration, which is usually performed when the patient has been symptomatic for at least 7 to 10 days, by which time the viral infection may be waning. Respiratory viruses such as adenovirus, parainfluenza, and rhinovirus have been recovered from approximately 10% of sinus aspirates.[12,37] Approximately 30% to 40% of sinus aspirates in patients with acute sinusitis do not yield bacteria. It is presumed that many of these infections are in fact viral. However, a detailed evaluation of sinus aspirates using modern molecular techniques, such as polymerase chain reaction assay, to establish a viral etiology is lacking.

Fungi have long been recognized as important pathogens in selected patients with either acute or chronic sinusitis. Fungal sinusitis may take one of three forms (Table 62.4). Invasive fungal sinusitis is a fulminant disease, often with dissemination. Most cases occur in patients with serious underlying diseases, such as diabetes mellitus, malignancy, and associated neutropenia, or those using high-dose corticosteroids. Local invasion of the sinus cavity and surrounding structures of the skull occurs and is often accompanied by systemic fungal infection. The second form is mycetoma, or fungus ball, which typically appears in the maxillary or sphenoid sinuses. A dense accumulation of hyphae is

TABLE 62.4 Types of Fungal Sinus Disease

		NONINVASIVE	
INVASIVE		MYCETOMA	ALLERGIC FUNGAL SINUSITIS
Underlying Condition	Immunocompromise Diabetes	None	Atopy, nasal polyps
Histopathology	Mucosal hyphal invasion	Mycelial mass	Eosinophilic inflammatory infiltrate
Etiological Agents	*Mucor, Rhizopus, Fusarium, Pseudallescheria boydii, Alternaria, Bipolaris, Cladophialophora, Curvularia*	*Mucor, Rhizopus, Fusarium, Pseudallescheria boydii, Alternaria, Bipolaris, Cladophialophora, Curvularia*	*Aspergillus, Bipolaris, Curvularia, Drechslera*
Therapy	Surgery Systemic antifungal	Surgery	Antiinflammatories Antihistamines Surgery

TABLE 62.5 Microorganisms Associated With Nosocomial Sinusitis Based on Sinus Puncture

MICROORGANISM	% OF ISOLATES
Pseudomonas spp.	10.7
Escherichia coli	5.9
Proteus mirabilis	5.9
Klebsiella spp.	7.2
Enterobacter spp.	7.2
Other gram negatives	8.4
Staphylococcus aureus	9.5
Viridans streptococci	8.3
Streptococcus pneumoniae	2.4
Other gram positives[a]	22.7
Anaerobic bacteria	3.6
Candida spp.	8.4
Total	**100**

[a]Organisms of low pathogenicity, such as coagulase-negative *Staphylococci* and *Corynebacterium* spp.
From George DL, Falk PS, Umberto Meduri G, et al. Nosocomial sinusitis in patients in the medical intensive care unit: a prospective epidemiological study. Clin Infect Dis. *1998;27:463–470.*

present, and local invasion may occur, but dissemination is rare. Allergic fungal sinusitis occurs in patients who are immunocompetent but atopic and is the third form of fungal infection. An exuberant immune response to fungal spores mediated by IgE results in mucinous material collecting in the sinus. Local invasion is not present, but a mucoid inflammatory infiltrate fills the sinus cavity. The causal agents in invasive fungal sinusitis are the filamentous fungi, including *Aspergillus*, *Mucor*, *Rhizopus*, *Fusarium*, *Pseudallescheria boydii,* and the dematiaceous fungi, such as *Alternaria*, *Bipolaris, Cladophialophora,* and *Curvularia* spp.[55–62] *Aspergillus, Bipolaris, Curvularia,* and *Drechslera* have been associated with allergic fungal sinus disease. Sinus zygomycosis refers to infection with members of the phylum Zygomycota, of which *Mucor* and *Rhizopus* spp. are the most common.

Nosocomial sinusitis is a relatively frequent complication of medical care, usually observed in the critical care setting. The cumulative incidence of sinusitis in critically ill patients has been estimated to be 7.7% to 32%.[63,64] Nasal intubation has long been recognized as a risk factor for this infection. In addition, the use of a nasoenteric tubes increase the risk of sinusitis. A prospective study found an incidence of 15.7 cases of sinusitis per 1000 patient days in patients with nasoenteric tubes versus 1.6 cases per 1000 patient days in patients without such tubes.[64] It is hypothesized that the presence of a tube in the nose irritates the nasal mucosa, resulting in inflammation, edema, and subsequent obstruction of the sinus ostia. Other risk factors include nasal colonization with gram-negative enteric bacilli, sedative use, and a Glasgow Coma Score less than 8. *S. aureus*, gram-negative organisms including *Pseudomonas*, and *S. pneumoniae* are frequently isolated in patients with nosocomial sinusitis (Table 62.5). Ventilator-associated sinusitis has been described in critically ill patients and may be a cause of unexplained fever. Nasotracheal intubation increases the risk for this complication.[65]

CHRONIC SINUSITIS

Chronic rhinosinusitis, which is often referred to as chronic sinus disease, is defined as symptoms and signs of sinus inflammation that persist for at least 12 weeks. Despite the prevalence of this disorder in the general population, its pathogenesis is incompletely understood. However, many of the same factors that play a role in acute bacterial sinusitis are found in chronic sinus disease, namely obstruction of the sinus ostia, mucociliary impairment, and thickening of secretions. In addition, the risk factors for chronic disease are similar to those of acute sinusitis[66] (see Table 62.1). The histopathology of the sinus in biopsies of patients with chronic sinusitis demonstrates an infiltration of T and B lymphocytes, macrophages, and eosinophils in the mucosa.[67] The microbiology of chronic sinusitis has been studied extensively, but the exact role of microorganisms is unclear. The same bacterial species (*S. pneumoniae* and *H. influenzae*) that are found in acute sinus disease are occasionally found in chronic sinusitis, especially in patients with acute exacerbations of chronic sinusitis. Other bacteria such as *S. aureus*, gram-negative enteric organisms, and anaerobes have been isolated with a greater frequency in sinus puncture studies.[68–72] Despite the isolation of such bacteria, there has been doubt in the medical literature as to their pathogenic role.[40,73] It is possible that in chronic sinusitis mucociliary clearance and host defenses are impaired to the point that the sinus cavity loses its normal sterility and becomes colonized with nasal flora. Thus chronic sinusitis may not truly be an "infectious process" but an aberration of the normal anatomy responsible for drainage with secondary damage to the mucosa of the sinus cavity.[73] The frequent isolation of bacteria of low pathogenicity, such as *Corynebacterium* spp. and coagulase-negative *Staphylococci,* as well as the unsatisfactory response to antimicrobials, support this hypothesis.

Biofilms, in which bacteria form specialized communities of microorganisms encased in complex extracellular polymeric substances, have been found in many patients with chronic rhinosinusitis and may play a role in both chronic inflammation and in exacerbations.[74] Biofilms offer important survival advantages to bacteria. They are more resistant to the effects of antibiotics than free-floating planktonic bacteria. This is accomplished by several mechanisms: (1) greater cell-cell contact to facilitate plasmid exchange for the evolution of resistance, (2) production of β-lactamases, (3) slow bacterial growth resulting in decreased effectiveness of antibiotics that rely on rapid cell growth and turnover for killing, and (4) the presence of "persister" cells that re-form the biofilm when the antibiotic is discontinued.[75] Although biofilms have been demonstrated on the mucosa of patients with chronic sinusitis, their precise role remains to be determined as they are not present in all cases of chronic rhinosinusitis, and limited biofilms are present in some healthy controls.

In addition, it is also important to mention that although most cases of chronic sinusitis are not thought to be an "infectious process," when patients with chronic sinusitis develop CNS complications these are often caused by *S. aureus* and respiratory anaerobes.

Recently, there has been intense interest in the role of fungi in the pathogenesis of chronic sinus disease. One study reported the isolation of multiple species of fungi in 95% of patients with chronic sinusitis. However, similar rates of isolation of fungi were present in control patients.[76] It has been hypothesized that chronic rhinosinusitis is the result of the allergic response to fungi present in the sinus cavity. Immunotherapy in these patients has been disappointing, as have trials of topical or systemic antifungal agents.[77,78]

EPIDEMIOLOGY

The epidemiology of sinusitis parallels that of the common cold. In the United States the incidence of the common cold is two to three events per person per year in adults and one to eight in children.[26,79] Bacterial infection of the sinuses is estimated to occur in 0.5% to 2% of cases of viral URI in adults and 6% to 13% of children.[80–82] A recent prospective study of URI in children demonstrated that sinusitis complicated URI 8.8% of the time.[26] This makes sinusitis one of the most common infections presenting to the primary care physician. In a national health survey in the United States, 11% of adults reported having been diagnosed with sinusitis by a health care provider in the past year.[83] A study done in 1996, estimating the economic burden of sinusitis, calculated that $5.8 billion in health expenditures was attributable to sinusitis in the United States. It was estimated that sinusitis and its complications are responsible for 23 million visits to health care providers annually and result in more than 20 million prescriptions for antibiotics.[1] Although sinusitis is primarily an outpatient disease, acute sinusitis is associated with more than 8000 inpatient admissions per year in children in the United States.[84] The impact of sinusitis on quality of life is also substantial. In a study of 15 adults with chronic sinusitis, compared with those with other chronic illnesses, scales of bodily pain and social function showed significantly more impairment than in patients with congestive heart failure, angina, chronic obstructive pulmonary disease, and back pain.[85]

Sinusitis is diagnosed more frequently in adult women than men at a ratio of 1.8 : 1. [86]

Risk factors for the development of sinusitis include allergic rhinitis and asthma, swimming, and nasal obstruction due to polyps, foreign body, and tumor. Immunodeficiencies (agammaglobulinemia, human immunodeficiency virus, chronic granulomatous disease), structural defects (polyps, cleft palate), and functional disorders of mucociliary clearance (ciliary dyskinesia, cystic fibrosis) are associated with sinusitis. A weak association between gastroesophageal reflux disease and sinusitis has been documented.[87]

CLINICAL MANIFESTATIONS

Because the pathogenesis of sinusitis and viral URI are similar, the clinical manifestations of these two diseases overlap greatly and must be compared. The uncomplicated URI will have a typical course of 5 to 10 days, and although the symptoms may not have completely resolved by the 10th day of illness, they have usually peaked in severity on days 3 to 6 and are showing improvement.[88] Nasal symptoms such as congestion and discharge are prominent in viral URI. Nasal discharge has a predictable pattern in its progression, from clear and watery to mucoid and thick and finally colored and opaque before resolving. Mild fever, when present, usually occurs in the first 48 hours of illness and is more common in children presenting with URI than in adults.

The clinical presentation of acute community-acquired bacterial sinusitis falls into one of three predictable patterns.[89] The first is that of persistent symptoms characterized by nasal discharge and/or cough that lasts more than 10 days without improvement. It is expected that the symptoms of a viral URI will have improved by the 10-day mark. Therefore it is the lack of improvement that is a sign of an acute bacterial process. Accompanying symptoms may include periorbital edema, malodorous breath, or low-grade fever. The nasal discharge may vary in character from thin and mucoid to thick and purulent. The second presentation is characterized by the onset of severe symptoms. Fever will accompany purulent nasal discharge that is present over a 3- to 4-day period.[90] These patients are often ill appearing. Worsening symptoms, referred to as "double sickening" in the Scandinavian literature, characterize the third presentation. These patients experience an initial improvement of symptoms of cough, nasal discharge, and congestion, but then worsen again within the first 10 days of illness.[89,91] Worsening may be signaled by new onset of fever, increasing nasal discharge, congestion, or daytime cough.

Patients with chronic rhinosinusitis have symptoms for at least 12 weeks. The presentation of such patients is characterized by anterior or posterior mucopurulent drainage and nasal obstruction. Facial pain or pressure, as well as hyposmia, are frequently present in patients with chronic sinus disease, as are alterations in taste.

The physical examination is of limited usefulness in the diagnosis of acute sinusitis, mainly because of the similarity of findings between patients with a viral URI and those with a bacterial process. Mucopurulent discharge may be found on the nasal mucosa. The mucosa itself is erythematous and mildly edematous. Facial tenderness over the maxillary or frontal area may be present but is an unreliable finding. Periorbital edema and mild discoloration of the skin below the eyelids is an occasional sign. Malodorous breath in the absence of dental disease or exudative pharyngitis may accompany acute sinusitis. Patients with chronic sinusitis may experience facial discomfort and mucopurulent nasal discharge. Polyps may be present in the nasal cavity or the middle meatus.

DIAGNOSIS OF SINUSITIS

Clinical Diagnosis

The challenge to the clinician when faced with the patient with upper respiratory symptoms is to identify those patients who have acute bacterial sinusitis and thus would benefit from an antimicrobial agent. The clinician faces a particular challenge in that the symptoms and signs of viral URI (i.e., the common cold) so closely overlap those of a bacterial process. Several studies have attempted to correlate signs and symptoms with the results of radiographic studies or cultures obtained through endoscopy; however, neither of these are reliable in the diagnosis of sinusitis, which brings the validity of these studies into question.

The duration of respiratory symptoms is the single most useful factor in discerning which patients have probable acute bacterial sinusitis. Studies on the microbiology of sinusitis in children showed that if rhinorrhea persisted at least 10 days with no improvement, bacterial burden in the sinuses was high.[37]

Sinusitis is best diagnosed based on the type, duration, and severity of symptoms. Although not entirely specific, the type of symptoms include nasal discharge or congestion reported by the patient or seen on physical examination. In children a persistent cough, especially when present during the daytime, may also be a signal of bacterial infection of the paranasal sinuses.

The following three clinical presentations will identify patients with acute bacterial sinusitis: (1) onset with *persistent* symptoms or signs, lasting at least 10 days without evidence of substantial clinical improvement; (2) onset with severe symptoms of high fever (≥39°C) and purulent nasal discharge lasting for 3 to 4 consecutive days; and (3) onset with worsening symptoms or signs characterized by the new development of fever, headache, or increase in nasal discharge after the initial improvement of a typical viral URI (double sickening).[90,92]

Imaging

Although imaging (plain film radiography, CT, MRI, and ultrasonography) historically has been used as a confirmatory or diagnostic modality in patients suspected to have acute bacterial sinusitis, it is no longer recommended. The membranes that line the nose are continuous with the membranes (mucosa) that line the sinus cavities, the middle ear, the nasopharynx, and the oropharynx. When an individual experiences a viral URI, there is inflammation of the nasal mucosa and often the mucosa of the middle ear and paranasal sinuses as well. The continuity of the mucosa of the upper respiratory tract is responsible for the controversy regarding the usefulness of images of the paranasal sinuses in contributing to a diagnosis of acute bacterial sinusitis.

As early as the 1940s observations were made regarding the frequency of abnormal sinus radiographs in healthy children without signs or symptoms of current respiratory disease.[93] It was hypothesized that sinus radiographs were frequently abnormal in healthy children because viral URIs are so common in children, and probably the so-called "normal" children either had a cold or were recovering from a URI and had persistent mucosal changes.[94] In the 1970s and 1980s several investigators demonstrated that children with uncomplicated viral URI had frequent abnormalities of the paranasal sinuses on plain radiographs.[94–96] These abnormalities were exactly the same as those usually interpreted as consistent with and emblematic of acute bacterial sinusitis (mucosal thickening of at least 4 mm, complete opacification, or an air-fluid level).

As technology advanced and CT scanning of the CNS and skull was performed, several studies reported on incidental abnormalities of the paranasal sinuses.[95,97,98] Manning and colleagues[99] evaluated children undergoing either CT or MRI of the head for indications other than respiratory complaints or suspected sinusitis. Each patient underwent rhinoscopy and otoscopy before imaging, and each patient's parent was asked to fill out a questionnaire regarding recent symptoms of URI. Sixty-two percent of patients overall had physical findings or history consistent with an upper respiratory inflammatory process, and 55% of the total group showed some abnormalities on sinus imaging; 33% showed pronounced mucosal thickening or an air-fluid level. Finally, Kristo and colleagues,[100,101] performed MRIs in children with uncomplicated viral URI and confirmed the high frequency (68%) of major abnormalities seen in the paranasal sinuses.

Gwaltney and colleagues[102] showed very similar findings in young adults. Previously healthy subjects were recruited within 48 to 96 hours of developing symptoms of an uncomplicated cold. These patients were followed prospectively, and CT scans of the sinuses were performed at the initial presentation of the cold, and a follow-up scan was done 2 weeks later. Almost 90% of the subjects had abnormalities of one or both maxillary sinuses, and 65% had abnormal ethmoid sinuses. The sphenoid and frontal sinuses were abnormal less often. The authors concluded that the common cold is associated with frequent and striking abnormalities of the respiratory mucosa lining the paranasal sinuses, including air-fluid levels.

Thus, when the paranasal sinuses are imaged, either with plain radiographs, CT, or MRI in children or adults with uncomplicated URI, the majority of studies will be significantly abnormal with the same kind of findings that have been associated with bacterial infection of the sinuses. Because at the time of presentation with clinical symptoms patients are likely to have had a recent URI, abnormal imaging studies will be common. In accordance, an abnormal image cannot confirm the diagnosis of acute bacterial sinusitis and is not necessary to perform in patients with uncomplicated episodes of clinical sinusitis. In contrast, a normal image of the paranasal sinuses assures that any respiratory symptoms that are present are not the result of an infection of the sinuses.

In summary, the diagnosis of acute sinusitis should be made on clinical grounds in most patients. CT of the sinuses is useful for the evaluation of patients with intraorbital or intracranial complications of sinusitis, and for the evaluation of patients in whom sinus surgery is being considered to define the patient's anatomy. In addition, CT has an important role in the diagnosis and management of chronic rhinosinusitis.[89,103,104] MRI may have a role in the diagnosis of fungal sinusitis and is useful in the diagnosis of the intracranial complications of sinusitis.

THERAPY

Antimicrobial

Antibacterial agents are the primary therapy for acute bacterial sinusitis. In the past 40 years there have been numerous randomized, placebo-controlled trials of antimicrobials in patients with sinusitis. When evaluating the quality of such studies, it is imperative to consider the diagnostic criteria used to enter patients into the trial. If the diagnosis is made on clinical grounds but criteria for subject entry are too liberal, a large number of patients will be included who have viral URI rather than bacterial disease. This will bias the trial toward showing a lack of effectiveness of antibacterial agents.

There has been a recent trend in the literature to publish studies that mimic the "real-world" office setting in which the practitioner is making the diagnosis of sinusitis based on clinical criteria that are not stringent and without any kind of microbiologic confirmation. Table 62.6 summarizes randomized, placebo-controlled trials of antimicrobials in the treatment of sinusitis.[105–122] These trials have shown variable benefit to antibiotics over placebo. In one study, in which clinical criteria such as purulent nasal discharge for at least 2 days and pus visualized on rhinoscopy were required for entry, 40% of patients included in the study had normal radiographs, strongly suggesting that these patients did not have sinusitis.[112]

Further complicating the interpretation of antimicrobial efficacy studies is the fact that patients with sinusitis will have a high rate of spontaneous improvement within 2 weeks of presentation. Studies that included patients who had more severe or prolonged symptoms (i.e., >10 days) or those that used adjunctive diagnostic tests to confirm the diagnosis of sinusitis were more likely to show a benefit to antimicrobials versus placebo.[105,108,118,119,121] When patients enrolled in efficacy studies are followed closely over time, those who received antimicrobials show a more rapid improvement in symptoms compared with patients receiving placebo (Fig. 62.3). A recent trial of amoxicillin-clavulanate versus

TABLE 62.6 Randomized, Controlled Trials of Antibiotic Versus Placebo

STUDY	POPULATION	DIAGNOSTIC CRITERIA	ANTIBIOTIC	NO. (%) IMPROVED-ANTIBIOTIC	NO. (%) IMPROVED-PLACEBO	TIME AT FOLLOW-UP (DAYS)	MEASUREMENT OF SEVERITY	BENEFIT SHOWN TO ANTIBIOTICS?
Garbutt 2012[122]	Adults	Clinical	Amoxicillin	78/85(92)	80/85(94)	10	Yes	No
Wald 2009[121]	Children	Clinical	Amox/clav	18/28 (64)	9/28 (32)	14	Yes	Yes
Williamson 2007[111]	Adults	Clinical	Amoxicillin	71/100 (79)	71/107 (66)	10	No	No
Meltzer 2005[109]	>12 yr	Clinical	Amoxicillin/ steroids	NS	NS	≥15	No	+/–[a]
Merenstein 2005[110]	Adults	Clinical	Amoxicillin	32/167 (48)	25/168 (37)	14	No	+/–[b]
Kristo 2005[113]	children 4–10 yr	Ultrasound/ radiograph	Cefuroxime	22/35 (63)	21/37 (57)	14	No	No
Varonen 2003[114]	Adults	Clinical	Amoxicillin Penicillin Doxycycline	70/88 (80)	39/59 (66)	14	No	Yes
Bucher 2003[112]	Adults	Clinical[b]	Amox/clav	94/124 (76)	93/127 (74)	14	No	No
DeSutter 2002[115]	≥12 yr	Purulent rhinorrhea	Amoxicillin	59/170 (35)	47/164 (29)	7–10	No	+/–
Kaiser 2001[117]	Adults	Clinical + nasal culture	Azithromycin	29/40 (73)	17/37 (47)	7	No	Yes
Garbutt 2001[116]	1–18 yr	Clinical	Amoxicillin *or* amox/clav	83/103 (80)	43/55 (79)	14	No	No
Hansen 2000[118]	Adults	Clinical + ESR/CRP	Penicillin V	50/71 (71)	23/62 (37)	3–7	No	Yes
Haye 1998[119]	Adults	Clinical + radiograph	Azithromycin	50/86 (58)	26/83 (31)	10	No	Yes
Stalman1997[120]	Adults	Clinical	Doxycycline	84/198 (85)	78/94 (83)	10	No	No
vanBuchem 1996[107]	Adults	Radiograph	Amoxicillin	87/105 (83)	78/101 (77)	14	No	No
Lindbaek 1996[105]	Adults	Clinical/CT	Amoxicillin *or* penicillin V	71/83 (86)	25/44 (57)	10	Yes	Yes
Wald 1986[108]	Children	Clinical + radiograph	Amoxicillin Amox/clav	38/58 (65)	15/35 (43)	10	Yes	Yes
Rantanen 1973[106]	Adults	Clinical + radiograph	Doxycycline	24/33 (72)	36/44 (81)	14	No	No

[a]There was a benefit in some individual symptom scores but not in overall score.
[b]Median time to improvement day 8 in amoxicillin treated vs. day 12 placebo, $P = .005$.
Amox/clav, Amoxicillin plus clavulanate; *CRP,* C-reactive protein; *CT,* computed tomography; *ESR,* erythrocyte sedimentation rate; *NS,* not stated.

placebo in children, in which sinusitis was diagnosed based on careful clinical criteria in an office setting, demonstrated a cure or improvement in 64% of patients receiving antibiotic compared with 32% of those receiving placebo.[121]

Several meta-analyses of clinical trials of antimicrobials in sinusitis have been published. These analyses have consistently found a benefit for antimicrobials over placebo despite much heterogeneity in the diagnostic methods, exclusion criteria, and outcome measures found within the studies that were included (Table 62.7). Overall, antimicrobial agents reduce the rate of clinical failure by 25% to 30% within 7 to 14 days of initiating therapy.[123] In most studies evaluating the role of antimicrobials, the adverse event rate is higher in the antibiotic arm of the study. Diarrhea is the most common adverse event noted and is usually self-limited.

A recent Cochrane review deserves mentioning as an example of the difficulties in interpretation of results.[124] In this review 15 trials of antimicrobial versus placebo or no treatment in adults were analyzed.

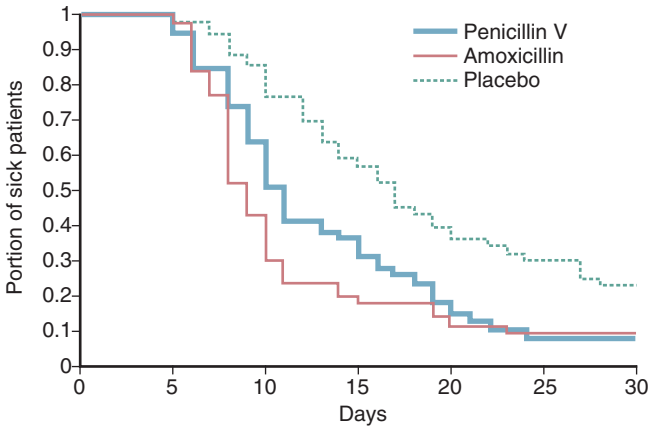

FIG. 62.3 Kaplan-meir curve of antimicrobials versus placebo. *(From Lindbaek M, Hjortdahl P, Johnsen UL. Randomised, double blind, placebo controlled trial of penicillin V and amoxicillin in treatment of acute sinus infections in adults.* BMJ. *1996;313:325–329.)*

TABLE 62.7 Meta-Analyses of Antibiotics Versus Placebo in the Treatment of Acute Rhinosinusitis

STUDY	NO. OF STUDIES	NO. OF PATIENTS	OR	95% CI	NNT
Lemiengre 2018[124]	15	3057	1.38	1.15–1.65	19
Falagas 2008[169]	17	2648	1.64[a]	1.35–2.0	
Young 2008[170]	9	2547	1.35[a]	1.15–1.59	8–15
Ahovuo 2008[171]	5	631	0.66[b]	0.44–0.98	
Rosenfeld 2007[172]	13	NS	NS	NS	7
Ip 2005[123]	39	15,739	0.69[b]	0.53–0.89	

[a]In these studies an OR >1 favored antibiotics.
[b]In these studies an OR <1 favored antibiotics.
CI, Confidence interval; *NNT,* number needed to treat; *NS,* not stated; *OR,* odds ratio.

The authors conclude that the benefit of antimicrobials was too modest, given the spontaneous resolution rate and risk of adverse events. However, the majority of studies in this metanalysis either used overly broad clinical criteria for eligibility or included imaging as criteria for the diagnosis of acute sinusitis. The antimicrobials studied were varied in class and dose. In addition, only one study was performed in the postpneumococcal immunization era.

Guidelines developed by various organizations in the United States have been published on the use of antimicrobials in sinusitis.[90,92,104,125,126] Guidelines published by the Infectious Disease Society of America (IDSA), the American Academy of Pediatrics (AAP) and the American Academy of Otolaryngology–Head and Neck Surgery Foundation (AAO-HNSF) (Table 62.8) recommend making the diagnosis of bacterial sinusitis on the basis of clinical criteria and treating acute sinusitis with antimicrobials.[90,92,126] Two guidelines offer watchful waiting as an option for the clinician. The AAP gives an option to observe for 3 days before starting treatment for nonsevere (onset with persistent symptoms) sinusitis in children, although the AAO-HNSF gives an option to observe up to 7 days and provide a safety-net prescription for all patients with uncomplicated bacterial sinusitis, regardless of severity of symptoms. The IDSA guidelines recommend that treatment be initiated as soon as the diagnosis of acute bacterial rhinosinusitis is made, and that amoxicillin-clavulanate be the first choice of antibiotic to be used. The AAP and AAO-HNSF guidelines recommend amoxicillin with or without potassium clavulanate as first-line therapy.

Patients who present with severe sinus disease or complications of sinusitis should be managed with parenteral antimicrobial therapy. Patients who have mild or moderately severe sinusitis may be treated with an oral antibiotic. Despite the heterogeneity of results in clinical studies, antimicrobial therapy in the treatment of acute sinusitis fits with our understanding of the pathogenesis of this infection. When there is an acute bacterial infection, effective agents exist that should provide levels of antimicrobial that will adequately treat the expected pathogens. Table 62.9 lists oral antibiotics used in the treatment of acute bacterial sinusitis. Antimicrobial therapy is directed at the three major pathogens of sinusitis: *S. pneumoniae, H. influenzae,* and *M. catarrhalis.* Choosing an appropriate antibiotic in patients with sinusitis is a balance between clinical efficacy, toxicity, and minimizing the emergence of resistant organisms. The current lack of up-to-date microbiologic data from studies of sinusitis or otitis media creates a conundrum in selecting the most appropriate antibiotic for the treatment of sinusitis. If the proportion of patients infected with *S. pneumoniae* that are nonsusceptible to penicillin are decreasing, as the most current data suggests, then standard-dose amoxicillin (45 mg/kg/day) rather than high-dose (80–90 mg/kg/day, maximum 4 g/day) may be used. However, if rates of isolation of β-lactamase–producing *H. influenzae* are increasing, then a β-lactamase–stable drug, such as amoxicillin-clavulanate or an advanced-generation cephalosporin, would be indicated.

For most adults and children amoxicillin with or without clavulanate remains an excellent first-line agent.[92,127–131] High-dose amoxicillin or amoxicillin-clavulanate should be used when the patient is at high risk for penicillin-nonsusceptible *S. pneumoniae* (≥10% nonsusceptible pneumococci, attendance at day care, age <2 years or >65 years, recent hospitalization, antibiotics in the past month). A recent controlled trial of high-dose amoxicillin-clavulanate versus standard-dose amoxicillin-clavulanate showed only modest benefit of the high-dose formulation.[132] In the child with a significant penicillin allergy, cefuroxime or cefpodoxime proxetil may be used and is preferred over cefdinir, which is

TABLE 62.8 Comparison of Two Major Guidelines for the Diagnosis and Treatment of Acute Bacterial Sinusitis

	DIAGNOSIS	TREAT	ANTIMICROBIAL OF CHOICE	AMOXICILLIN DOSE
IDSA[92]	Clinical	All patients	Amoxicillin/clavulanate	40–45 mg/kg/day 80–90 mg/kg/day or 2 g/day for high risk[a]
AAP[90]	Clinical	All severe patients Treat or wait 3 days for mild–moderate	Amoxicillin with or without clavulanate	40–45 mg/kg/day 80–90 mg/kg/day or 2 g/day for high risk[a]

[a]High risk: ≥10% nonsusceptible pneumococci, severe infection, attend day care, age <2 years or >65 years, recent hospitalization, antibiotics in the past month.
AAP, American Academy of Pediatrics; *IDSA,* Infectious Diseases Society of America.

TABLE 62.9 Oral Antimicrobial Agents for Acute Bacterial Sinusitis

ANTIMICROBIAL	ADULT DOSAGE	PEDIATRIC DOSAGE
Amoxicillin	500–875 mg q12h	40–80 mg/kg/day divided q12h
Amoxicillin/clavulanate[a]	875 *or* 2000 mg 12h	40–80 mg/kg/day divided q12h
Cefpodoxime proxetil	200 mg 12h	10 mg/kg/day divided 12h
Cefixime[b]	400 mg q12–24h	8 mg/kg/day divided q12–24h
Cefdinir	300 mg q12h *or* 600 mg q24h	14 mg/kg/day divided 12–24h
Azithromycin[c]	500 mg daily for 3 days	10 mg/kg daily for 3 days
Clarithromycin[c]	500 mg q12h for 14 days 1000 mg daily for 14 days	15 mg/kg/day divided q12h
Levofloxacin	500 mg daily	16–20 mg/kg/day divided every 12h[b]
Moxifloxacin	400 mg daily	400 mg daily for adolescents[b]

[a]Dosages specify amoxicillin component.
[b]Not US Food and Drug Administration approved for this indication.
[c]Macrolides not preferred because of poor activity against *Haemophilus influenzae*.

less potent. Alternative agents in adults include a respiratory fluoroquinolone, such as levofloxacin or moxifloxacin, or doxycycline.

The macrolides have become popular in the treatment of sinusitis due to their once-daily dosing and shorter courses. Despite recommendations against these agents, a study of prescribing habits in patients with sinusitis demonstrated that they are the second most commonly prescribed antibiotic after amoxicillin.[133] However, because of increasing resistance and the change in epidemiology of respiratory pathogens, their use cannot be endorsed. Neither azithromycin nor clarithromycin have adequate coverage for *H. influenzae;* furthermore, they are considered second-line therapy for *S. pneumoniae.*[104]

Patients treated with an antimicrobial may be expected to improve within 48 to 72 hours of starting therapy. If the patient fails to improve during this time frame, either the diagnosis of sinusitis should be suspect or a broader-spectrum agent should be used. Regimens in the child or adult failing therapy with high-dose amoxicillin-clavulanate should include a respiratory fluoroquinolone or cefixime in combination with linezolid.

The exact duration required for the treatment of sinusitis is unknown. Recommendations based on clinical observations have varied widely, from 10 to 28 days of treatment. A treatment course of 10 days is typical. Some guidelines suggest that therapy be continued for 7 days after the patient becomes free of signs and symptoms. The advantage of this strategy is that it results in a minimum of 10 days and avoids prolonged antimicrobial therapy in patients who are asymptomatic.[90] It is a particularly advantageous regimen when the patient responds more sluggishly to the antimicrobial regimen.

The efficacy of shorter courses of antimicrobials, particularly quinolones and macrolides, has been reported. For example, azithromycin given for 3 days was reported to be equivalent to 5 days, and 5 days of gatifloxacin was equivalent to 10 days.[123] Five-day courses of cefuroxime have also been compared with a 10-day course. Falagas and colleagues[134] performed a metanalysis of short course (3–7 days) versus long course (6–10 days) of antimicrobial treatment and found no difference between these groups. The diagnosis of sinusitis in these studies was based on radiographic findings, and thus many patients with uncomplicated URI may have been included, biasing the results against showing a difference.

ADJUNCTIVE TREATMENT

Corticosteroids

A number of nonantimicrobial therapies have been used to provide symptom relief to patients with sinusitis. The literature on adjunctive treatment of sinusitis has the same limitations as that of antimicrobial trials; many studies lack stringent definitions of sinusitis. In accordance, many of these studies include patients with uncomplicated viral URI, and the results must be interpreted cautiously. Intranasal corticosteroids have been studied with and without antimicrobials. Placebo-controlled trials of intranasal steroids have shown conflicting results.[109,135–139] Two recent meta-analyses of such studies demonstrated no difference between groups. Zalmanovici[139] found an overall resolution rate of symptoms of 73% in corticosteroid-treated patients versus 66% in placebo controls. Hayward's[140] analysis showed a 7% increase in improvement rate when patients were treated with intranasal corticosteroids compared with placebo. This modest benefit does not warrant endorsement when it adds substantially to the cost and complexity of treatment, although the AAO-HNS guidelines offer this as an option for symptomatic relief in adults.[126] In contrast, intranasal corticosteroids should be offered to those patients with chronic rhinosinusitis, particularly when there is a history of atopic nasal disease.

Antihistamines/Decongestants

Antihistamines have not been shown to be consistently effective in the symptomatic treatment of sinusitis. However, they do have a role in patients with underlying allergic disease. Topical and oral decongestants, which are α-adrenergic agonists, are used frequently as adjunctive therapy but have received little systematic study. Decongestants have a modest effect on decreasing nasal airway resistance. These agents may cause increased blood pressure, CNS stimulation, insomnia, or urinary retention. A recent review of the use of antihistamines and decongestants in children with sinusitis found a lack of well-controlled studies to determine the efficacy of these treatments.[141] Because of concern for adverse events, antihistamines and decongestants should not be used for symptomatic relief in children.[90,142]

Saline

Nasal saline spray, large-volume saline irrigation, and hypertonic saline have been popularized as a measure to reduce symptoms in patients with sinusitis. Such irrigations act by improving mucociliary function, decreasing mucosal edema, and mechanically reducing crusting and debris formation. This may serve to keep the osteomeatal complex clear and facilitate drainage. There is evidence in clinical trials that intranasal saline does provide a modest improvement primarily in adults with chronic symptoms. Large-volume irrigation is more effective than saline sprays. Because adverse events are minimal and mainly include slight nasal irritation, such measures are an option in providing symptomatic relief.[143–146] Data are limited for the effect of nasal saline for children with acute sinusitis but also support a modest benefit.[141,147–150]

Sinus Surgery

Surgical intervention in acute sinusitis may be indicated when there are intracranial or orbital complications or in the patient with severe acute infection refractory to antimicrobial therapy. Surgery is used frequently in patients with chronic sinusitis unresponsive to medical therapy.[90,104,151] Functional endoscopic sinus surgery is the most common procedure currently performed for this condition. In this procedure the emphasis is on restoring the normal anatomic and physiologic drainage of the sinuses through the osteomeatal complex. Diseased tissue, polyps, and bone within the ethmoid or frontal sinus cavities and sinus ostia is removed under endoscopic visualization. Recent attention has been focused on preserving the mucoperiosteum and normal structures as much as possible. Benefit from surgical intervention has been demonstrated primarily in uncontrolled trials in which improvement in both symptoms and quality of life have been noted.[152–155] Randomized, controlled trials have not demonstrated the advantage of functional endoscopic surgery compared with aggressive medical therapy.[156,157]

COMPLICATIONS

The complications of sinusitis result from the close proximity of the sinuses to critical structures of the skull and face, and may be divided into intracranial and extracranial manifestations. Intracranial complications include subdural empyema, epidural abscess, intraparenchymal brain abscess, meningitis, and venous sinus thrombosis. Intracranial

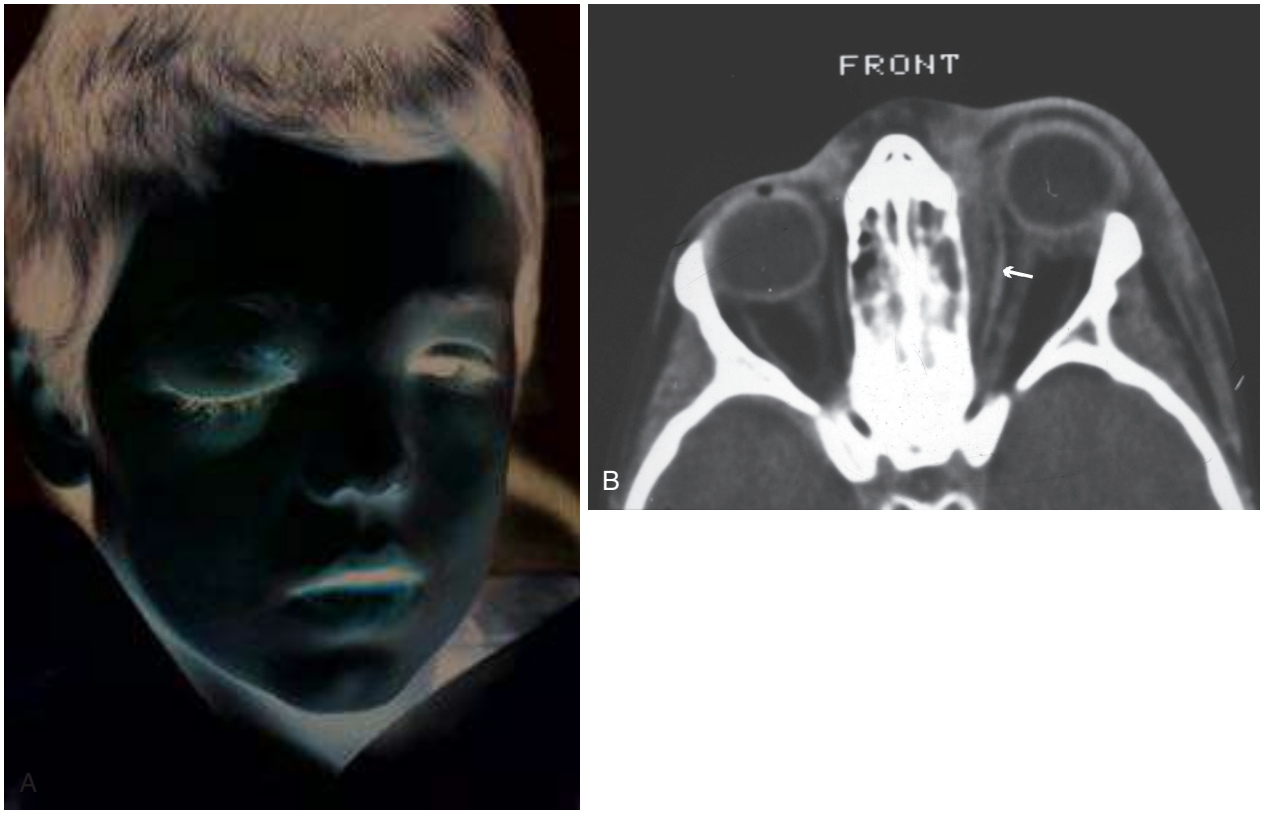

FIG. 62.4 (A) A child with orbital abscess as a complication of ethmoid sinusitis. Note the marked edema and proptosis. (B) Computed tomography scan of the orbit. White arrow demonstrated an orbital abscess. *(A courtesy Gary Williams, MD.)*

complications must be considered in any patient who presents with fever, altered mental status, seizures, or focal neurologic findings. Extracranial complications include orbital cellulitis, orbital abscess, and subperiosteal abscess.[158–161] The Pott puffy tumor is a subperiosteal abscess of the frontal bone that presents as a swelling over the forehead secondary to an underlying osteitis.[162] Orbital and intracranial complications are present together in up to 45% of complicated cases. The majority of complications of sinusitis are a consequence of infection of the frontal and ethmoid sinuses.[163] The pathogenesis of complications involves either direct extension from the sinus cavity or retrograde thrombophlebitis from the valveless venous network.[164]

Acute bacterial sinusitis is occasionally complicated by periorbital edema, particularly when the ethmoid sinuses are involved. These patients will have findings of discoloration and soft nontender edema of the upper and lower eyelids. The overlying skin may have a purple or reddish discoloration. This must be distinguished from signs of true orbital infection, such as orbital abscess or orbital cellulitis. Although these serious complications may also be associated with periorbital edema, accompanying proptosis or impairment of extraocular movements should alert the clinician to a true intraorbital infection (Fig. 62.4). A CT scan of the orbits and sinuses should be performed when an orbital infection is suspected. Immediate surgical intervention is indicated for many orbital and intracranial abscesses. However, small abscesses associated with sinusitis may be managed with a trial of antimicrobial therapy. If clinical improvement does not occur in 24 to 48 hours, then surgical drainage should be undertaken.

Anaerobic bacteria, viridans streptococci, gram-negative organisms, and *S. aureus* are frequently isolated from intracranial collections of purulent material.[164,165] The role of *Streptococcus anginosus* in the complications of sinusitis is being increasingly appreciated.[160] Empirical antibiotic therapy should include vancomycin, ceftriaxone, and metronidazole for intracranial infections. Orbital infections may be treated with ampicillin-sulbactam and vancomycin, or the combination of ceftriaxone plus clindamycin and vancomycin.

Complications of acute bacterial sinusitis are rare, and thus studies that show an impact of treatment of sinusitis on the prevention of complications are difficult to perform and interpret. A recent ecologic study of antimicrobial use in Sweden suggested there was no effect of antimicrobial use on the prevention of orbital or intracranial complications.[166] Often, the initial presentation of the child or adult with complications of acute sinus infection is with signs and symptoms of the complication.

PREVENTION

The prevention of rhinosinusitis is dependent on diagnosing and treating underlying conditions that predispose patients to recurrent or ongoing infection. Such conditions include allergic rhinitis, cystic fibrosis, gastroesophageal reflux disease, ciliary dyskinesia, or anatomic defects. These patients may present with multiple episodes of sinusitis or sinusitis recalcitrant to medical therapy. These conditions should be sought in the patient with recurrent sinus disease and treated aggressively. Attendance of a child in day care is a risk for recurrent URI and subsequent sinusitis. Children attending day care have twice the rate of URIs than those who do not attend day care.[167]

Vaccination has not played a major role in the prevention of acute sinusitis in children. Vaccination against influenza virus will prevent only one URI per year that may predispose the patient to an episode of acute bacterial sinusitis. Studies of the microbiology of the nasopharynx in adults and children have shown a reduction in the isolation of *S. pneumoniae* in the years since the pneumococcal conjugate vaccine has been in use.[43,168] A reduction in the actual incidence of sinusitis, however, has not been demonstrated after introduction of this vaccine.

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The complete reference list is available online at Expert Consult.

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63 Epiglottitis

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SHORT VIEW SUMMARY

Epidemiology and Etiology

- *Pediatric epiglottitis* is a localized, invasive *Haemophilus influenzae* type b infection of the supraglottic area, including the epiglottis, that can be associated with bacteremia (60%–98%); routine conjugate vaccination has largely eliminated this form of epiglottitis.
- *Adult epiglottitis* often involves more of the supraglottic structures (aryepiglottic folds, vallecula, tongue base) and is not associated with bacteremia (<15%); when a bacterial pathogen is identified, it is more likely to be *Streptococcus pneumoniae, Streptococcus pyogenes,* or *Neisseria meningitidis.*
- Before routine infant immunization with *H. influenzae* type b conjugate vaccines, 65% to 75% of all patients with epiglottitis were children 1 to 4 years of age; currently, 90% to 95% are adults.
- Incidence of adult epiglottitis in the United States and Europe is approximately 2 cases per 100,000 population.

Clinical Manifestations

- *Pediatric epiglottitis* is an abrupt illness in a febrile young child with a toxic appearance, dysphagia or sore throat, a muffled or hoarse voice, stridor, drooling, and often a distinctive posture—the "tripod position," comprising apprehension, sitting very still, preferring to lean forward with hyperextension of the neck, and protruding the chin. Cough is distinctly uncommon.
- In *adult epiglottitis,* 80% to 95% of patients have odynophagia and sore throat; only 20% to 40% have fever, drooling, or stridor.

Diagnosis

- Airway management should be promptly evaluated as soon as the diagnosis is considered. Laboratory and radiologic testing in a child with suspected epiglottitis should be performed only in a safe environment (i.e., in the operating room, emergency department, or intensive care unit, with an individual trained in pediatric airway intubation), because of the propensity to develop acute airway obstruction (although airway obstruction is much less common in adults).
- Peripheral leukocytosis is common but not universal. Lateral and anteroposterior neck radiographs show enlargement of the epiglottis (the "thumb sign," as opposed to the "pencil-point narrowing" of the airway in viral croup).
- Direct or indirect laryngoscopy and fiberoptic nasopharyngeal endoscopy are the definitive diagnostic tests.

Differential Diagnosis

- Stridor with toxicity and drooling but lack of cough favors epiglottitis; the presence of stridor, barking cough, and lack of drooling favors viral croup (laryngotracheobronchitis). Other conditions that mimic infectious epiglottitis include bacterial tracheitis, thermal epiglottitis (scald burn from smoke or hot beverages), possibly angioneurotic edema, retropharyngeal or peritonsillar abscesses, uvulitis, and diphtheria.

Therapy

- In *pediatric epiglottitis,* ideally the diagnosis is confirmed with visualization at the time the airway is secured by intubation, at which time laryngeal and blood cultures and complete blood cell counts may be obtained. Emergent tracheotomy or cricothyroidotomy is rarely required.
- In *adult epiglottitis,* in contrast to pediatric epiglottitis, patients generally tolerate direct visualization of the epiglottis for diagnosis. Hospitalization in an intensive care unit during the acute phase of the illness is suggested; however, in most adults (75%–80%) the infection is successfully managed without endotracheal intubation or tracheotomy.
- Empirical therapy for epiglottitis includes intravenous cefotaxime, ceftriaxone, or ampicillin-sulbactam to treat streptococci, pneumococci, *H. influenzae,* and meningococci. In areas with a high proportion of drug-resistant pneumococci, empirical therapy should be broadened.
- Therapy directed against *Staphylococcus aureus* should be considered if bacterial tracheitis cannot be excluded.

Prevention

- Chemoprophylaxis for household contacts of children with *H. influenzae* type b epiglottitis should be given in those households containing underimmunized or nonimmunized children younger than 4 years of age or immunocompromised children.
- Contacts of adults with epiglottitis are unlikely to require any prophylaxis, except in the rare instance of proven *H. influenzae* type b or meningococcal infection.

Acute epiglottitis (supraglottitis) is an invasive cellulitis of the epiglottis and its adjacent supraglottic structures (aryepiglottic folds, vallecula) that has the potential to cause abrupt, complete airway obstruction.

EPIDEMIOLOGY AND ETIOLOGY

Although epiglottitis was reported in both children and adults in the first half of the 20th century, it became known as a classic pediatric infection by the 1970s.[1] It likely is the modern explanation for the illness known in the 18th century as "cynanche trachealis" (literally, "dog strangulation"), now thought to have been the cause of death of George Washington in 1799 (albeit complicated by iatrogenic bloodletting).[2,3]

Epiglottitis has most often been reported to exhibit a slight male predominance (roughly 60% of both children and adult cases occur in males) and a slight excess in cases during the spring and summer.[1,4–7]

Before routine infant immunization with *Haemophilus influenzae* type b conjugate vaccines began in 1990, 65% to 75% of reported cases of epiglottitis occurred in children 1 to 4 years of age (peak, 3 years of age).[1,6–10] Data from geographic areas in which routine *H. influenzae* type b conjugate vaccination is practiced show that 90% to 95% of all reported cases of epiglottitis now occur in adults and that the causative bacterial agent is often not found.[1,4,9,11–13] Thus, the remarkable change in the epidemiology of epiglottitis involves both an upward shift in the average age of patients and a shift in the likely microbiologic cause.

The virtual disappearance of classic pediatric epiglottitis is a direct consequence of the high protective efficacy of *H. influenzae* type b conjugate vaccines, along with the fact that pediatric epiglottitis was, almost uniquely (60%–98% of reported cases in various series), a bacteremic, invasive *H. influenzae* type b infection.[6–14] In the prevaccination era, population-based, age-adjusted estimates of the annual incidence of invasive *H. influenzae* type b infection (the majority of which was meningitis, but also epiglottitis, bacteremia, and bone, joint, and soft tissue infections) in the United States and Scandinavian countries

commonly ranged from 3 to 6 cases per 100,000 children from birth to 18 years of age.[5–13,15–19] The adjusted annual incidence rate for the population of children most at risk was even greater at 21 to 41 cases per 100,000 children from birth to 4 years of age.[15–19] Since the advent of routine conjugate immunization, there has been a 95% to 99% decline in pediatric epiglottitis incidence, such that current rates are 0.02 to 0.6 cases per 100,000 children from birth to 18 years of age.[11–19]

In contrast, in adults, the US and European annual incidence of epiglottitis has remained stable, at 1.8 to 2.0 cases per 100,000 adults, likely because adult epiglottitis tends to be associated with *H. influenzae* type b bacteremia in less than 10% of cases.[4,5,7,11–15] In general, no distinct pathogen is isolated; on occasion, other pathogens unrelated to *H. influenzae,* including *Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus,* or *Neisseria meningitidis,* are recovered from surface cultures of the epiglottis and rarely from blood cultures.[1,7,11–14,20–22] A few studies have reported mild increases in adult disease rates, both before and after conjugate pediatric vaccination, perhaps explained by better recognition of the syndrome.[4,14]

Unusual causes of epiglottitis include *Pseudomonas aeruginosa* and *Candida albicans,* especially in immunocompromised adults or in those with significant preceding antibiotic therapy that might allow opportunistic pathogen overgrowth. In addition, injury from consumption of very hot beverages or illicit substances such as inhaled crack cocaine can result in burns of the epiglottis ("thermal epiglottitis").[23–26]

The pathophysiology of epiglottitis is not completely understood. It is possible that local physical trauma or irritation from preceding viral infection predisposes the epiglottis to bacterial infection. The clinical observation that classic pediatric epiglottitis was far more likely than adult epiglottitis to lead to severe respiratory distress and respiratory arrest is often assumed to be explained by a "ball-valve" effect of the child's epiglottis, obstructing the anatomically smaller (with respect to the adult) airway. However, such an obstruction would not explain the success of bag-mask ventilation before intubation of these children. Instead, it seems more likely to be a consequence of pediatric airway physiology. Laminar airflow in the small bronchioles is governed by the Poiseuille equation, which can be expressed as stating that resistance to airflow is inversely proportional to the fourth power of the luminal radius; however, pharyngeal airflow is turbulent, which is less efficient.[27,28] Resistance to turbulent airflow is inversely proportional to the fifth power of the luminal radius. Thus, 1 mm of circumferential edema in the epiglottic region of a small child's airway might reduce its diameter from 4 mm to 2 mm; the cross-sectional area is decreased by 75%, and the resistance to turbulent flow is increased 32-fold. Yet, in an older child or young adult with an 8-mm airway, the same 1 mm of circumferential edema reduces the cross-sectional area by only approximately 44% and increases the resistance only about fivefold.[27,28]

CLINICAL MANIFESTATIONS

The classic presentation of pediatric epiglottitis was a preschool-aged child who acutely developed fever, irritability, and rapidly progressive respiratory distress with stridor.[1,6,7,13,29] These children frequently appeared toxic, were drooling oral secretions, and assumed a "tripod-like" or "sniffing" position, leaning forward in an upright position with hyperextension of the neck and protrusion of the chin, and perhaps bracing themselves with their hands on knees. Such children also exhibited a muffled, "hot potato" voice or hoarseness.[1,6,7,13,29] After the institution of routine *H. influenzae* type b conjugate vaccination, the typical age of children diagnosed with epiglottitis increased, as noted previously; in addition, a more indolent or subacute presentation of epiglottitis has been increasingly recognized in older children and adults. These older patients typically have a longer duration of symptoms before presentation, predominately complain of sore throat and odynophagia, exhibit less frequent respiratory distress at presentation, and do not have *H. influenzae* type b as the etiologic agent.[4–14,20–22,30,31]

Classic *H. influenzae* type b acute epiglottitis in children was a life-threatening illness, with a high incidence of airway compromise and bacteremia but curiously few metastatic sites of infection (i.e., it was rare to see concurrent meningitis, septic arthritis, or facial cellulitis, which were other well-known forms of pediatric invasive *H. influenzae* type b infections).[6,7] However, pneumonia or epiglottic abscesses have been reported.[6,7,30,31] In contrast, modern epiglottitis in adults is typically a nonbacteremic, somewhat less severe illness. Although most patients are in good health before onset of the disease, underlying conditions that compromise immunity may be a factor in some patients.[20–22] In addition, the morbidity and mortality of adult epiglottitis can be significant—5% to 38% of adults required mechanical ventilation and up to 6% died according to several reports from France, Israel, and Canada. In these series, surface cultures of the epiglottis were positive in approximately 20%, most often recovering *Streptococcus* spp. (including groups A, C, F, and *Streptococcus viridans*), *Haemophilus* spp., and gram-negative bacilli.[20–22] Laboratory tests in individuals with acute epiglottitis often reveal leukocytosis with a left shift.[5,7,13,15,31] Blood cultures yielded *H. influenzae* type b in 60% to 98% of children with classic epiglottitis, yet are positive for any pathogen in fewer than 10% of modern cases of epiglottitis in older children and adults.[5,7,20–22,32]

Lateral neck radiographs in patients with epiglottitis show an enlarged epiglottis ("thumb sign"), with ballooning of the hypopharynx and prevertebral soft tissue swelling.[33–37] Fig. 63.1A shows a schematic view of the normal epiglottis anatomy, with a bold line outlining the position of an edematous epiglottis, which easily could provoke greatly increased airway resistance. An endoscopic view of such an inflamed epiglottis is shown in Fig. 63.1B. Typical lateral neck radiographs of a child without (Fig. 63.1C) and with (Fig. 63.1D) epiglottitis contrast the appearance of the normal and "thumblike" swollen epiglottis. Fig. 63.2A is a lateral radiograph of the neck of a 65-year-old woman with a "thumb sign" of acute epiglottitis, and Fig. 63.2B is a computed tomography (CT) scan showing swelling and edema of the epiglottis.[35] Although the lateral neck radiograph thus can be very helpful in diagnosing epiglottitis, radiographs were not recommended to be performed in children with classic acute epiglottitis because of the risks in delay in securing the airway and the requirement for sending an ill and potentially unstable child to the radiology department (bedside neck radiographs were generally not sufficient for proper interpretation of the anatomic borders).[7]

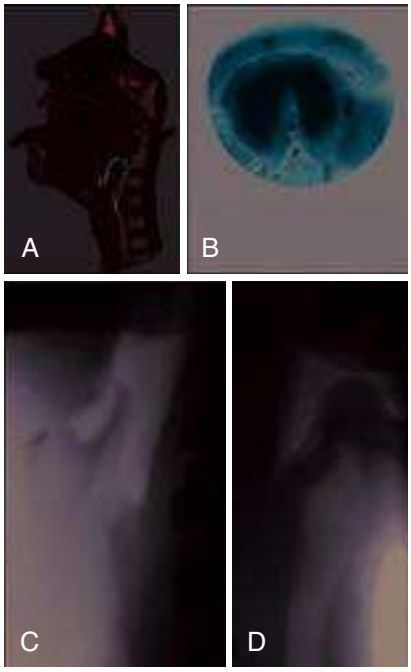

FIG. 63.1 Epiglottitis. Schematic (A) and endoscopic (B) views of swollen epiglottis. The typical lateral neck radiographs of a child without (C) and with (D) epiglottitis, demonstrating the "thumb sign" of the swollen epiglottis, are shown. *(From Hammer J. Acquired upper airway obstruction.* Paediatr Respir Rev. *2004;5:25–33.)*

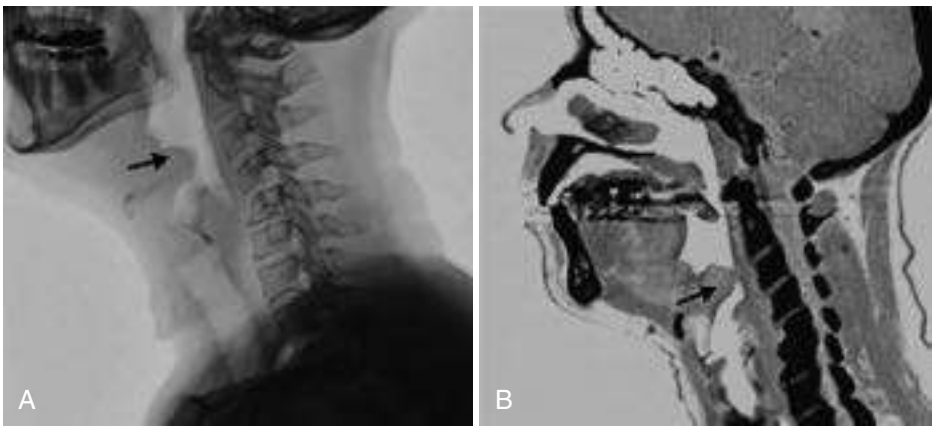

FIG. 63.2 Epiglottitis. Lateral neck radiograph of a 65-year-old woman with acute epiglottitis showing the "thumb sign" (A) and a computed tomography scan showing swelling and edema of the epiglottis (B). *(From Angirekula V, Multani A. Epiglottitis in an adult.* N Engl J Med. *2015;372:e20.)*

DIAGNOSIS

The diagnosis of epiglottitis is definitively established by visualization of an edematous, cherry-red epiglottis by direct or indirect laryngoscopy or by nasal fiberoptic endoscopy.[5–7,12,13,30] In children with classic epiglottitis, or in older children or adults with signs of respiratory distress, this examination should take place in a setting where the airway can be immediately secured—that is, in an intensive care unit or operating room—because airway obstruction may be sudden and precipitated by the examination.

As many as 29% of adults with epiglottitis have sought medical care previously for the same illness without the correct diagnosis being made.[5] Most were assigned a diagnosis of pharyngitis, without undergoing examination of the epiglottis. This emphasizes the need for a complete examination of the epiglottis in adults with severe sore throat or severe pharyngitis with odynophagia.[5] The epiglottis should be visualized directly, even if the radiograph is negative, in patients who are suspected to have epiglottitis. In adults, examination in the sitting-forward position, with a tongue depressor used to gently depress the tongue, may reveal the epiglottis without the need for direct or indirect laryngoscopy.

In subacute epiglottitis of the older child or adult, lateral neck radiographs should be obtained. CT of the neck is generally not required but may be useful for evaluation of epiglottic abscess. Cultures of the blood or epiglottis may be of use in defining a causative organism, although the yield of blood cultures in adult epiglottitis is poor and interpretation of pharyngeal cultures may be complicated by a high carrier rate of certain organisms in the upper respiratory tract.

DIFFERENTIAL DIAGNOSIS

Croup (viral laryngotracheobronchitis) is the most frequent and important diagnosis that mimics classic pediatric epiglottitis; the presentation is sometimes similar to epiglottitis, but the treatment of each syndrome is radically different. Although both illnesses can present as stridor, a toxic appearance is more common in epiglottitis. In a comparative analysis of 203 ill children with either epiglottitis or croup, the presence of drooling oral secretions was 79% sensitive and 94% specific for the diagnosis of epiglottitis; the absence of coughing was similarly predictive (98% sensitivity and 100% specificity).[29] Conversely, the presence of coughing and the absence of drooling heavily favored the diagnosis of croup.[29] Croup is frequently preceded by an upper respiratory tract infection, has a more gradual onset, involves somewhat younger children (median age of 18 months as opposed to median age of 34 months in epiglottitis),[29] and, in general, is a less febrile illness. However, differentiation of epiglottitis from croup is sometimes difficult unless the epiglottis is visualized.

Retropharyngeal abscess, peritonsillar abscess, and uvulitis (invasive bacterial infections generally caused by *S. pyogenes* or oropharyngeal microaerophilic or anaerobic bacteria, rather than *H. influenzae* type b) can mimic epiglottitis but can usually be differentiated by means of physical examination and radiographically. Bacterial tracheitis may be confused with epiglottitis, especially in a patient who has already had antibiotic therapy and may have staphylococcal overgrowth in the trachea; the appearance on laryngoscopy or intubation should clarify the diagnosis. Diphtheria can be differentiated from epiglottitis through the presence of a pseudomembrane in the respiratory tract and the presence of typical organisms on direct smear and culture of the membrane. Noninfectious diagnoses that mimic epiglottitis include angioneurotic edema and foreign-body aspiration; findings from the patient's history and physical examination should help differentiate these entities from epiglottitis.

THERAPY

Initial Management

Maintenance of an adequate airway should be the primary concern as soon as the diagnosis of epiglottitis at any age is even suspected, but especially in a young child. Children who are suspected of having acute epiglottitis should be handled as a medical emergency because of the potential for rapid deterioration to complete respiratory obstruction. Painful or anxiety-provoking procedures (including phlebotomy and lateral neck radiography) should be minimized until the airway is secured or the diagnosis has been excluded. Patients being transported between and even within medical facilities must be accompanied by personnel capable of securing the airway in case obstruction occurs. At the same time, bag-mask ventilation should not be forgotten if intubation or cricothyroidotomy is not possible, because the obstruction is usually not absolute.

Appropriate management of the child with classic pediatric epiglottitis requires the immediate insertion of an endotracheal tube; this procedure is both therapeutic and diagnostic, because visualization of the cherry-red epiglottis is made possible during intubation. Case series and widespread experiential data report that 70% to 100% of children with classic pediatric epiglottitis have undergone intubation for airway management, and generally a small number (<5%) have emergent tracheotomy or cricothyroidotomy.[6,7,15,30,32] However, older children with subacute non–*H. influenzae* type b epiglottitis might be able to be safely managed conservatively without immediate intubation; fewer data exist to guide this decision.

In contrast, it appears reasonably safe for the adult with epiglottitis to be managed without intubation but with careful observation in an intensive care unit with the capability to secure an artificial airway immediately on impending airway compromise (increasing stridor or dyspnea). Most case series of adult epiglottitis report intubation rates of 10% to 25%.[a]

At the same time, adults or older adolescents with epiglottitis accompanied by a rapid onset of symptoms, dyspnea, tachycardia, or tachypnea may benefit from early intubation.[12,13]

[a]References 4, 5, 7, 11–15, 20, 22.

Antibiotic Therapy

After the establishment of an airway, cultures should be obtained from blood and, if possible (i.e., immediately after intubation), the surface of the epiglottis, and empirical intravenous antibiotic therapy should be begun. Antibiotic therapy is directed at the most likely causative bacterial pathogens (*H. influenzae* type b, *S. pneumoniae* [including those with penicillin resistance], β-hemolytic streptococci, and possibly *S. aureus*), although with the recognition that an etiologic agent may not be recovered. This is best accomplished with the use of a third-generation cephalosporin such as ceftriaxone or cefotaxime or a β-lactamase inhibitor combination drug such as ampicillin-sulbactam; for immunocompromised individuals, piperacillin-tazobactam would offer increased coverage for gram-negative organisms while still including the more typical causes. Vancomycin should be added if high-grade penicillin-resistant pneumococci are prevalent in the area or if methicillin-resistant staphylococcal bacterial tracheitis is a diagnostic possibility. For individuals allergic to β-lactams, a combination of a fluoroquinolone (levofloxacin or moxifloxacin) and clindamycin is suggested. Trimethoprim-sulfamethoxazole and clindamycin in combination might also be considered for the β-lactam–allergic patient, depending on the local prevalence of resistance among pneumococci.

The decision to extubate is based on the patient's clinical condition, evidence of resolution on direct visualization of the epiglottis with a fiberoptic laryngoscope, and the presence of an increasing air leak around the endotracheal tube. Pediatric patients with acute epiglottitis usually improve approximately 48 hours after the initiation of appropriate antibiotic therapy. Depending on the patient's progress, the artificial airway can usually be removed within this period. Antibiotics should be continued for 7 to 10 days; in the past they were given intravenously, but it is likely that oral third-generation cephalosporins or respiratory fluoroquinolones, with or without clindamycin, could be used, depending on the individual patient's culture results, response, and status.

The role of nebulized epinephrine, corticosteroids, or other adjuncts to therapy is unknown; evidence of benefits from such interventions is lacking.[7,13,31]

PREVENTION

Household contacts of children with *H. influenzae* type b epiglottitis may require chemoprophylaxis if the household contains unvaccinated or incompletely vaccinated children younger than 4 years; for households without other young children, or if all children are fully immunized, chemoprophylaxis is not indicated.[38] The suggested chemoprophylaxis regimen for young children is rifampin (20 mg/kg/day administered orally once daily for 4 days, maximum daily dose of 600 mg). For pregnant women in whom rifampin use is contraindicated, intramuscular ceftriaxone is an alternative.[38] If a young underimmunized child is present in the household unit, prophylaxis is given to all household contacts, regardless of age or immunization status, to prevent "ping-pong" spread of carriage among individuals.[38] When the index child with invasive *H. influenzae* type b epiglottitis is treated with ceftriaxone or cefotaxime, colonization is successfully eliminated, such that the ill child does not require rifampin therapy. However, children who are treated with ampicillin-sulbactam or other agents will require "terminal prophylaxis" with rifampin at the end of their primary treatment, to prevent reintroduction of the organism into the household.[38] In general, chemoprophylaxis is no longer considered for school contacts of children with *H. influenzae* type b disease.

Contacts of older children and adults with epiglottitis do not require chemoprophylaxis at all, except in the rare case of documented meningococcal or *H. influenzae* type b epiglottitis, in which case rifampin, ceftriaxone, or ciprofloxacin would be used as appropriate.[38]

Primary prevention of *H. influenzae* type b and most pneumococcal serotypes causing pediatric infection is now afforded by routine conjugate vaccination.[38] Routine quadrivalent meningococcal vaccination of adolescents may also lessen the rare cases of meningococcal epiglottitis.[38]

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The complete reference list is available online at Expert Consult.

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64 Infections of the Oral Cavity, Neck, and Head

Anthony W. Chow

SHORT VIEW SUMMARY

Definition
- Infections of the oral cavity, neck, and head are diverse in etiology and clinical presentation.
- Although uncommon in the postantibiotic era, deep fascial space infections and vascular complications are potentially life threatening. These include Lemierre syndrome and Ludwig angina.
- A clear understanding of their interrelationships, anatomic routes of spread, and salient clinical features is critical to diagnosis and management.

Epidemiology
- Infections of the oral cavity are most commonly odontogenic in origin and include dental caries, periodontal disease, and deep fascial space infections.
- Nonodontogenic infections of the oral cavity include those of the oral mucosa, tonsils, epiglottis, supraepiglottic structures, and infections of the major salivary glands.
- Miscellaneous infections of the head and neck most commonly result from human or animal bites, irradiation, or surgical procedures but may also arise from suppurative adenitis, infected embryologic cysts, and suppurative thyroiditis.

Microbiology
- The microbiota associated with odontogenic infections generally reflect the indigenous oral microbiota and are typically polymicrobial, involving both strict anaerobes and facultative bacteria.
- Dental caries originate from "cariogenic" bacteria residing within the supragingival plaque or biofilm, whereas periodontal disease arises from "periodontopathic" bacteria residing within the subgingival plaque or biofilm.
- Important differences within these complex bacterial compositions support the concept of the "specific" plaque hypothesis of dental caries and periodontal disease.

Diagnosis
- Microbiologic investigation requires proper specimen collection, taking care to minimize contamination by resident commensal microbiota.
- Needle aspiration of loculated pus by an extraoral approach is desirable, and specimens should be transported immediately to the laboratory under anaerobic conditions.
- Tissue biopsy specimens should be routinely examined for histopathologic evidence of acute or chronic inflammation and infection.
- Immunofluorescence staining and rapid molecular diagnostic tools, such as DNA probes or polymerase chain reaction, are valuable for the detection of fastidious or noncultivable pathogens.
- Computed tomography (CT) with contrast is the most effective tool for localizing and evaluating the extent of deep space infections of the oral cavity, head, and neck.
- Magnetic resonance imaging with or without angiography is more sensitive than CT for assessing soft tissue and bone involvement, as well as vascular complications.
- Technetium bone scans in combination with gallium- or indium-labeled white blood cells may be useful for the diagnosis of acute or chronic osteomyelitis.

Therapy
- Surgical drainage of loculated infection and removal of necrotic tissue are the keys to management of deep fascial space infections of the oral cavity, head, and neck.
- Antimicrobial therapy is important in halting the local spread of infection and in preventing hematogenous dissemination.
- Choice of antimicrobial regimens is empirical, depending on the primary source (e.g., odontogenic or oropharyngeal vs. rhinogenic or otogenic), anticipated causative microorganisms, and immunity of the host (see Tables 64.2 and 64.3).

Prevention
- Oral hygiene and dental treatment to prevent caries and advanced periodontal disease
- Dietary counseling and use of topical fluorides and chlorhexidine oral rinses for patients at high risk for dental caries
- Behavioral modification of risk factors, such as tobacco smoking

Infections of the oral cavity are most commonly odontogenic in origin. Odontogenic orofacial infections include dental caries, pulpitis, periapical abscess, gingivitis, periodontal disease, and infections in the deep fascial spaces. Complications such as intracranial, retropharyngeal, or pleuropulmonary extension and hematogenous dissemination to heart valves, prosthetic devices, and other metastatic foci, although rare, clearly indicate the potentially serious nature of these infections. Nonodontogenic infections of the oral cavity include ulcerative mucositis, which complicates radiation and chemotherapy; noma (gangrenous stomatitis); and infection of the major salivary glands. Suppurative orofacial infections can also arise from the oronasopharynx, tonsils, middle ear and mastoids, and paranasal sinuses; these are discussed in Chapters 59, 61, and 62, respectively.

Infections of the neck and head in the adult most commonly result from human or animal bites, trauma, irradiation, and surgical procedures. In children cervical adenitis and thyroiditis caused by bacteria or viruses are more common. Rarely do embryologic cysts in the neck region become secondarily infected. These are considered separately from oral infections because they frequently involve different microbiota and necessitate alternative approaches to diagnosis and therapy.

MICROBIOLOGIC CONSIDERATIONS

The microbiota associated with odontogenic infections are complex and generally reflect the indigenous oral microbiota. Such infections are typically polymicrobial, and invasiveness is often influenced by synergistic interactions of multiple microbial species. Moreover, certain species or combinations may be more invasive or more resistant to therapy than others. Despite this complexity, there is strong evidence of a causative role of specific microorganisms in different forms of odontogenic infections.[1] Because the microbiota associated with these infections are typically polymicrobial, the components of these complex microbiota do not necessarily have equal pathogenic potential, and the numerically predominant cultivable microbiota may not be the most pathogenic. Furthermore, it may not be necessary to eradicate the complete microbiota for effective therapy. In addition, results of surveys with molecular tools such as culture-independent nucleic acid technologies indicate a level of diversity in the human subgingival microbiota that cannot be recognized by conventional culture techniques.[2] More than 1000 distinct bacterial species from the oral cavity have been identified.[3] In most instances the cultivable microbiota probably represent

less than 1% of the total extant population, as estimated by microscopy or other means.[4] Nevertheless, an appreciation of the indigenous oral microbiota and the host factors that may modify its composition, as well as knowledge of the most common microorganisms implicated in different odontogenic and nonodontogenic infections, should provide a more rational approach to the management of such infections arising from the oral cavity.

Unique Niches of the Indigenous Oral Microbiota

The oral cavity cannot be regarded as a single, uniform environment. Although representative species of microorganisms can be isolated from most areas of the mouth, certain sites, such as the tongue, tooth surface, gingival crevice, and saliva, are favored for colonization by specific organisms (Table 64.1).[5]

Quantitative studies indicate that obligate anaerobes constitute a large and important part of the residential oral microbiota. In the gingival crevice of healthy adults, for example, the total microscopic counts averaged 2.7×10^{11} microorganisms per gram of wet weight. The total cultivable anaerobic bacteria averaged 1.8×10^{11} microorganisms per gram, whereas facultative bacteria averaged 2.2×10^{10} microorganisms per gram, which is an eightfold difference. Overall, *Streptococcus, Finegoldia, Peptostreptococcus, Veillonella, Lactobacillus, Corynebacterium,* and *Actinomyces* account for greater than 80% of the total cultivable oral microbiota. Facultative gram-negative rods are uncommon in healthy adults but may be more prominent in seriously ill, hospitalized, and elderly patients. Unique ecologic niches have been observed.[5] For example, *Streptococcus sanguis, Streptococcus mutans,* and *Streptococcus mitis,* as well as *Actinomyces viscosus,* preferentially colonize the tooth surface. In contrast, *Streptococcus salivarius* and *Veillonella* spp. have a predilection for the tongue and buccal mucosa.[6] *Fusobacterium, Porphyromonas, Prevotella,* and anaerobic spirochetes appear concentrated in the gingival crevice.[6] The cultivable microbiota in the saliva most closely resemble those on the dorsum of the tongue. Factors that appear to govern these localization patterns include selective adherence characteristics of certain bacteria for various types of cells, local environmental conditions such as oxygen tension, oxidation-reduction potential (Eh) and pH, interbacterial coaggregation, and microbial inhibition.[7] Apart from anatomic considerations, numerous factors, such as age, pregnancy, diet and nutrition, eruption of deciduous dentition, oral hygiene, smoking habits, the presence of dental caries or periodontal disease, antimicrobial therapy, hospitalization, and genetic or racial factors, may influence the composition of the oral microbiota.[8]

Microbial Specificity in Odontogenic Infections

Of importance is that the normal commensal microbiota are closely adapted to their unique ecologic niches in the oral cavity within well-established structures known as *biofilms.* These highly organized microorganisms are encased in an extracellular matrix composed mainly of polysaccharides and exist in a relatively protected environment. Under normal "healthy" conditions, these commensal bacteria maintain an effective and nondestructive inflammatory barrier against potential pathogens.[9] Under pathologic conditions, however, this microbial homeostasis is disrupted, and the commensal microbiota shifts to a dysbiotic pathogenic form, which results in inflammation and tissue destruction.[10] Only certain microorganisms residing within dental plaques or biofilms are cariogenic or periodontopathic (i.e., the "specific" plaque hypothesis of dental caries and periodontal disease).[11] This microbial specificity demonstrated for different odontogenic infections probably reflects the acquisition of unique microbiota during the development of a supragingival dental plaque and its progression to a subgingival dental plaque. Plaques or biofilms that accumulate above the gingival margin are composed mainly of gram-positive facultative and microaerophilic cocci and rods; plaques or biofilms that accumulate below the gingival margin are composed mainly of gram-negative anaerobic rods and motile forms, including spirochetes (Fig. 64.1).[12] Microorganisms residing within the supragingival plaque are characterized by their ability to adhere to the tooth surface and by their saccharolytic activity. Microorganisms in the subgingival plaque are frequently asaccharolytic but proteolytic, and they need not be adherent.

Important differences in bacterial compositions have been noted for dental caries, gingivitis, and different forms of periodontitis in comparison with cultures from healthy tissues.[11] An etiologic association of *S. mutans* in dental caries has been firmly established.[13] *S. mutans* is the only organism consistently isolated from all decayed dental fissures and is the only organism consistently found in greater numbers in carious teeth than in noncarious teeth. The infectious and transmissible nature of this organism in dental caries has been demonstrated in both experimental animals and in longitudinal studies in humans.[14]

Similarly, in gingivitis and periodontitis a unique and specific bacterial composition of the subgingival plaque has been identified.[15] In the

TABLE 64.1 Predominant Cultivable Bacteria From Various Sites of the Oral Cavity

TYPE	PREDOMINANT GENUS OR FAMILY	TOTAL VIABLE COUNT (mean %) Gingival Crevice	Dental Plaque	Tongue	Saliva
Facultative					
Gram-positive cocci	*Streptococcus* spp. *S. mutans* group *S. sanguis* *S. mitis* *S. salivarius* group	28.8 (0–30) (10–20) (10–30) (0–1)	28.2 (0–50) (40–60) (20–40) (0–1)	44.8 (0–1) (10–20) (10–30) (40–60)	46.2 (0–1) (10–30) (30–50) (40–60)
Gram-positive rods	*Lactobacillus*	15.3	23.8	13.0	11.8
Gram-negative cocci	*Moraxella*	0.4	0.4	3.4	1.2
Gram-negative rods	Enterobacteriaceae	1.2	ND	3.2	2.3
Anaerobic					
Gram-positive cocci	*Peptostreptococcus*	7.4	12.6	4.2	13.0
Gram-positive rods	*Actinomyces, Eubacterium, Leptotrichia*	20.2	18.4	8.2	4.8
Gram-negative cocci	*Veillonella*	10.7	6.4	16.0	15.9
Gram-negative rods	*Fusobacterium* *Prevotella* *Porphyromonas*	16.1 1.9 4.7	10.4 4.1 ND	8.2 0.7 0.2	4.8 0.3 ND

ND, Not detected.
Data modified from Hull MW, Chow AW. Indigenous microflora and innate immunity of the head and neck. Infect Dis Clin North Am. *2007;21:265–282.*

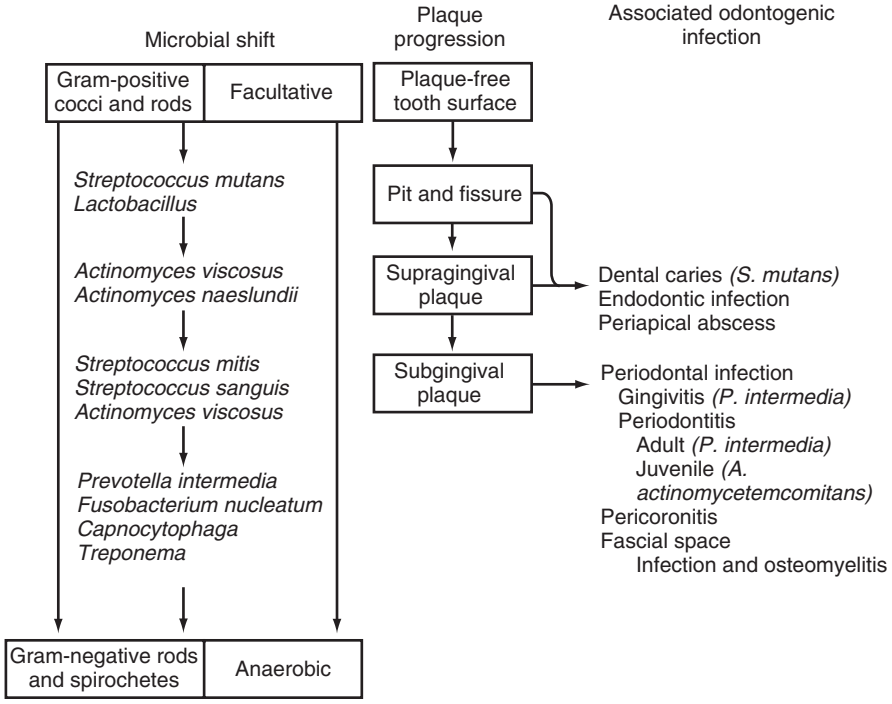

FIG. 64.1 Microbial specificity in odontogenic infections. A unifying hypothesis demonstrating a microbial shift from a plaque-free tooth surface and progression to supragingival and subgingival plaque organisms. *(Modified from Chow AW. Odontogenic infections. In: Schlossberg D, ed.* Infections of the Head and Neck. *New York: Springer; 1987:148.)*

healthy periodontium the microbiota is sparse and consists mainly of gram-positive organisms, such as *Streptococcus oralis, S. sanguis,* and *Actinomyces* spp. In the presence of gingivitis the predominant subgingival microbiota shift to a greater proportion of anaerobic gram-negative rods, and *Prevotella intermedia* (formerly *Bacteroides intermedius*), *Capnocytophaga* spp., *Finegoldia magna,* and *Peptostreptococcus* spp. are most commonly isolated. In adults with "established" periodontitis the microbiota further increases in complexity, with a preponderance of anaerobic gram-negative and motile organisms and spirochetes. *Porphyromonas gingivalis* (formerly *Bacteroides gingivalis), P. intermedia, Aggregatibacter actinomycetemcomitans, Tannerella forsythensis* (formerly *Bacteroides forsythus*), and *Treponema denticola* are most commonly isolated. In juvenile or "early-onset" periodontitis, a clinical variant seen primarily in adolescents, the subgingival plaque consists mainly of saccharolytic organisms, with *A. actinomycetemcomitans* and *Capnocytophaga* spp. as the most common identifiable species. *P. gingivalis* is rarely found in this condition.

In suppurative odontogenic infections, such as periapical abscesses or deep fascial space infections, polymicrobial microbiota are usually present; the predominant isolates are *Fusobacterium nucleatum,* pigmented *Bacteroides* spp., *Peptostreptococcus* spp., *Actinomyces* spp., and *Streptococcus* spp.[16] Except in patients with serious underlying illnesses, facultative gram-negative bacilli and *Staphylococcus aureus* are uncommonly isolated.

PATHOGENETIC MECHANISMS

The mechanisms by which pathogenic microorganisms in the oral cavity can cause disease are varied. To some extent these microbes must be able to adhere to mucosal or tooth surfaces; resist elimination by mechanical means, such as flushing by oral fluids; compete for space and nutrients with other resident microbiota; evade host defenses; and penetrate host tissues. The ability to attach to mucosal and tooth surfaces and form biofilms appears important for both commensal and pathogenic microbes within the dental plaque.[17,18] Commensal biofilms are health promoting and protect against a destructive host inflammatory response elicited by pathogenic biofilms.[9] Homeostasis of the plaque biofilm and its symbiotic relationship with the host is critical for oral health. Disequilibrium or dysbiosis within the plaque biofilms is the initiating event that leads to major oral diseases, such as dental caries and periodontal disease.[19,20]

Microorganisms that cause dental caries, such as *S. mutans* and *Streptococcus sobrinus,* reside within the supragingival plaque and are both acidogenic (able to produce acid) and aciduric (able to grow at low pH). They readily colonize the tooth surface shortly after tooth eruption but do not become cariogenic until they are exposed to dietary sucrose.[17] Fermentation of dietary sucrose by acidogenic plaque bacteria lowers the pH on the tooth surface, promoting demineralization and eventually tooth decay. *S. mutans* can also use sucrose to produce extracellular adhesive polymers, known as *glucans,* which enable *S. mutans* to stick avidly to the tooth surface, facilitating cariogenesis in the underlying structures.[21] In the healthy host at least three mechanisms protect the tooth from carious decay: (1) the cleaning action of the tongue and buccal membranes, which removes any food particles from the proximity of the tooth; (2) the buffering effect of saliva, which has a neutral pH that washes away bacterial acids and provides essential substrates for remineralization and repair of damaged tooth surfaces; and (3) the protective effect of an acellular bacteria-free coating of salivary origin on the tooth surface, known as the *acquired pellicle,* which acts as a surface barrier to most dietary and bacterial acids and other proteolytic substances. In the absence of tooth brushing and flossing, the acquired pellicle becomes rapidly colonized and is replaced by the bacterial plaque or biofilm. It is not surprising, therefore, that carious lesions occur most often in areas inaccessible to the self-cleaning mechanisms of the mouth and on the occlusal surfaces and sites that are protected from the reaches of the toothbrush.

Periodontal disease is caused mainly by selective periodontopathic microorganisms within the subgingival dental plaque, which penetrate the gingival epithelium, elicit an inflammatory host response, and ultimately cause destruction of the periodontium.[20,22] This tissue destruction results in apical migration of gingival tissues (gingival recession), loss of periodontal attachment, and an increase in the depth of the gingival crevice (periodontal pockets). Specific virulence factors, such as lipopolysaccharide and proteolytic enzymes, play a role in this destruction. For example, several oral microorganisms associated with periodontitis, including *A. actinomycetemcomitans,* produce a leukotoxin that destroys polymorphonuclear leukocytes and macrophages and is

believed to be a key virulence factor.[23] Host and environmental factors, such as smoking, malnutrition; underlying disease such as diabetes mellitus; and certain genetic factors may play an even bigger role.[24] In particular, patients with neutrophil defects, such as Chédiak-Higashi syndrome, agranulocytosis, and cyclic neutropenia, have a higher incidence of periodontal disease.[23] Other factors include various hormonal effects that may exacerbate disease activity during puberty, menstruation, and pregnancy. Two major predisposing factors are poor oral hygiene and increasing age.[25] In contrast to its role in dental caries, dietary carbohydrate intake does not appear to have a significant role in the pathogenesis of periodontal disease.

MUCOSAL IMMUNITY OF THE ORAL CAVITY

The oral cavity has three major host defenses against bacterial invasion: the oral mucosa as a physical barrier, nonspecific (innate) immunity, and adaptive (acquired) immunity (Fig. 64.2).[26] The oral mucosa consists of a layer of interconnected epithelial cells containing mainly keratinocytes resting on a basal membrane. The oral epithelium constantly undergoes cellular renewal and turnover. Microorganisms seeking to colonize mucosal surfaces must develop a strategy to counteract the constant turnover of the epithelial cell layer.

Keratinocytes also have an innate system for recognizing pathogenic microbes by the activation of Toll-like receptors (TLRs). TLRs function as pathogen-recognition receptors that interact with conserved domains on microorganisms (so-called pathogen-associated molecular patterns [PAMPs]).[27] Activation of TLRs results in a cascade of signaling pathways, ultimately leading to the upregulation of various proinflammatory (e.g., interleukin [IL]-1α, IL-6, IL-12, tumor necrosis factor-α [TNF-α]) and antiinflammatory (e.g., IL-10, transforming growth factor-β [TGF-β]) cytokines and chemokines (e.g., RANTES [regulated on activation, normal T-cell expressed and secreted], macrophage inflammatory protein-1 [MIP-1], IL-8), which are crucial for nonspecific defense in the oral cavity. Phagocytic cells such as leukocytes and macrophages are abundant in the lamina propria and serve as the first line of defense against pathogenic microbes. Keratinocytes also produce a variety of antimicrobial peptides, including histatins and β-defensins, which have broad antibacterial and antifungal properties. Human β-defensin (hBD)-1 is expressed constitutively in epithelial tissues, whereas hBD-2 and hBD-3 are expressed in response to bacterial stimuli or inflammation.

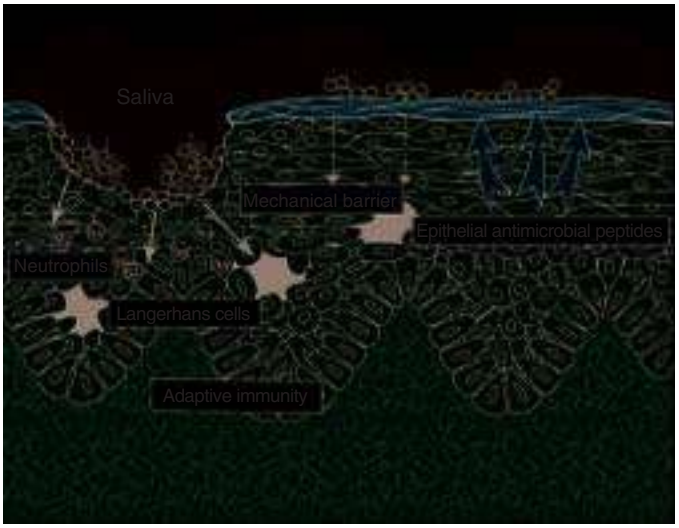

FIG. 64.2 Mucosal defenses against invading bacteria in the oral cavity. The oral mucosa has three types of antimicrobial defenses: physical barrier of the epithelial layer; nonspecific (innate) immunity derived from salivary constituents, neutrophils, and epithelial antimicrobial peptides; and adaptive immunity associated with mucosa-associated lymphatic tissues. *(Modified from Abiko Y, Saitoh M, Nishimura M, et al. Role of beta-defensins in oral epithelial health and disease.* Med Mol Morphol. *2007;40: 392–402.)*

Commensal and pathogenic bacteria have been found to use different signaling pathways in hBD-2 induction; this finding suggests that epithelial cells from different body sites may use common signaling mechanisms to distinguish between commensal and pathogenic bacteria.[28]

In addition, the lamina propria within the mucous membrane contains a full complement of immunocompetent cells that are responsible for adaptive immunity. These cells, which include lymphocytes, macrophages, dendritic cells, natural killer cells, and eosinophils, constitute a common mucosal immune system known as *mucosa-associated lymphoreticular tissue* (MALT). Dendritic cells within MALT (Langerhans cells) process foreign antigens for presentation to and activation of T cells, as well as differentiation of B cells into immunoglobulin-secreting plasma cells. The primary immunoglobulin secreted at these sites within salivary and other exocrine glands is secretory immunoglobulin A (sIgA), whose major function is bacterial agglutination, inhibition of bacterial adherence, toxin neutralization, and antigen exclusion at the mucosal surface.[29] IgA also downregulates the proinflammatory cytokine response by binding with the crystallizable fragment-α receptor (Fc-αR) on phagocytic cells. A number of oral microorganisms implicated in periodontitis, including *P. gingivalis, P. intermedia, Prevotella melaninogenica, Capnocytophaga* spp., *S. sanguis,* and *S. mitis,* are found to secrete IgA proteases.[30] It has been suggested that cleavage of IgA by microbial IgA proteases may impair local mucosal immunity of the host. It remains to be seen whether similar or other defects of host resistance can be identified in different forms of destructive odontogenic infections.

Finally, saliva also acts as an important source of antimicrobial activity against oral pathogens.[31] Mechanically, saliva serves to coat the teeth and contribute to the protective pellicle. In addition, it flushes the oral cavity, clearing away bacteria and their by-products, as well as food debris that may aid bacterial growth and colonization. The buffering capacity of saliva contributes to the maintenance of salivary pH. Numerous chemical constituents that inhibit bacterial growth, such as lysozyme, lactoferrin, defensins, and the peroxidase system, are found within the saliva.[32] Lysozyme is able to lyse bacteria by catalyzing breakdown of the bacterial cell wall in a manner akin to the action of penicillin. It is also active against gram-negative bacteria in the presence of complement and antibody, and it disrupts the lipopolysaccharide coat in the cell wall.[33] Lactoferrin sequesters iron from the environment, thus inhibiting the growth of various facultative and aerobic bacteria that are dependent on iron for metabolism. Salivary lactoperoxidase and myeloperoxidase are generated by polymorphonuclear leukocytes within the gingival crevices and have potent bactericidal properties. Lactoperoxidase generates a hypothiocyanite (HOSCN) molecule that is toxic to bacteria.[34]

ANATOMIC CONSIDERATIONS

Soft tissue infections of odontogenic origin tend to spread along planes of least resistance from the supporting structures of the affected tooth to various potential spaces in the vicinity. Accumulated pus, therefore, must generally perforate bone at the site where it is thinnest and weakest before it extends into the periapical areas or deeper fascial spaces. In the mandible this is usually in the region of the molar teeth on the lingual aspect and, more anteriorly, on the buccal aspect. In the maxilla the bone is weakest on the buccal aspect throughout and relatively thicker on the palatal aspect. If pus perforates through either the maxillary or the mandibular buccal plate, it does so intraorally if inside the attachment of the buccinator muscle to the maxilla or mandible and extraorally if outside this muscle attachment (Fig. 64.3A).[35] When a mandibular infection perforates lingually, it does so in the sublingual space if the apices of the involved teeth lie above the attachment of the mylohyoid muscle (e.g., mandibular incisor, canines, premolars, first molars) and in the submandibular space if the apices lie below the attachment of this muscle (e.g., second and third molars) (see Fig. 64.3B). Thus these local anatomic barriers of bone, muscle, and fascia predetermine the routes of spread, the extent, and the clinical manifestations of many orofacial infections of odontogenic origin. The clinically important "fascial spaces" most often involved are illustrated in Figs. 64.4 and 64.5. These are potential spaces between layers of fascia normally bound together by loose connective tissue. The breakdown of these

attachments by a spreading infective process results in a fascial space infection. These spaces intercommunicate with one another to varied degrees, and the potential pathways of extension from one space to another are illustrated in Fig. 64.6. A thorough understanding of the potential *anatomic routes* of infection not only provides valuable information on the nature and extent of infection but also suggests the optimal surgical approach for effective drainage.

CLINICAL MANIFESTATIONS AND MANAGEMENT

Orofacial Odontogenic Infections

Odontogenic infections originate in either the dental pulp or the periodontium (Fig. 64.7). The most common site is the dental pulp, and the most common infections are dentoalveolar. Deep fascial space infections are rare. Their clinical manifestations and management are briefly reviewed here. Antimicrobial therapy is further discussed later in the section "Therapeutic Considerations" (Tables 64.2 and 64.3).



FIG. 64.3 Routes of spread of odontogenic orofacial infections along planes of least resistance. (A) Coronal section in the region of the first molar teeth: *a,* maxillary antrum; *b,* nasal cavity; *c,* palatal plate; *d,* sublingual space (above the mylohyoid muscle); *e,* submandibular space (below the mylohyoid muscle); *f,* intraoral manifestation with infection spreading through the buccal plates inside the attachment of the buccinator muscle; *g,* extraoral manifestation to buccal space with infection spreading through the buccal plates outside the attachment of the buccinator muscle. (B) Lingual aspect of the mandible: *a,* apices of the involved tooth above the mylohyoid muscle, with spread of infection to the sublingual space; *b,* apices of involved tooth below the mylohyoid muscle, with spread of infection into the submandibular space. *(From Chow AW, Roser SM, Brady FA. Orofacial odontogenic infections.* Ann Intern Med. *1978;88:392–402.)*

Dentoalveolar Infections

Pulpal infection most frequently results from carious exposure, rarely from physical or chemical injury. The carious process most frequently begins in pits and fissures on the occlusal surfaces of molars and premolars, in which food is likely to be retained. Interproximal sites and the gingival margin are the next most common areas where the carious process begins. Demineralization of the enamel results in discoloration, which is the first visible evidence of carious involvement. Destruction of the enamel and dentin and invasion of the pulp produce either localized or generalized pulpitis. If drainage from the pulp is obstructed, pulpal necrosis and rapid proliferation of endodontic microorganisms ensue and lead to invasion of the periapical areas (periapical abscess) and alveolar bone (acute alveolar abscess).

Clinically, the tooth is sensitive to percussion and to both heat and cold during early or reversible pulpitis, although the pain stops abruptly when the stimulus is withdrawn. During late or irreversible pulpitis, the tooth is exquisitely painful in response to a hot stimulus; the application of cold provides prompt relief. If drainage is established through the tooth before extension into the periapical region, chronic irritation from the necrotic pulp may result in periapical granuloma or cyst formation that may be relatively asymptomatic. Dental radiographs are particularly helpful for the detection of silent lesions, particularly those caused by interproximal caries, that are difficult to detect clinically.

The principles of treatment of dentoalveolar infections include prompt elimination of the infected pulp, deep periodontal scaling, or extraction of the affected tooth.[36] Dentoalveolar abscess should be surgically drained at the same time. Other supportive measures include hydration, a diet of soft foods, analgesics, and oral hygiene. Antibiotic therapy is indicated primarily if drainage cannot be adequately established or when infection has perforated the cortex and spread into surrounding soft tissue (see Table 64.2).

Gingivitis and Periodontal Infections

Periodontal disease is a general term that refers to all diseases involving the supporting structures of the teeth (periodontium), including the gingiva, periodontal ligament, alveolar bone, and cementum. In the early phase of periodontal disease, infection is confined to the gingiva (gingivitis). Later, the underlying supporting tissues are affected (periodontitis), ultimately leading to complete destruction of the periodontium and a permanent loss of teeth. Periodontal infections tend to localize in intraoral soft tissues and seldom spread into deeper structures of the face or neck.

Gingivitis

Acute and chronic inflammation of the gingiva is initiated by local irritation and microbial invasion associated with subgingival plaque. In *simple gingivitis,* there is a bluish-red discoloration, with swelling and thickening of the free gingival margin. A tendency for the gums to bleed after eating or toothbrushing may be one of the earliest findings. There is usually no pain, but a mild fetor oris may be noticed. In *acute necrotizing ulcerative gingivitis,* also known as *Vincent angina* or *trench*

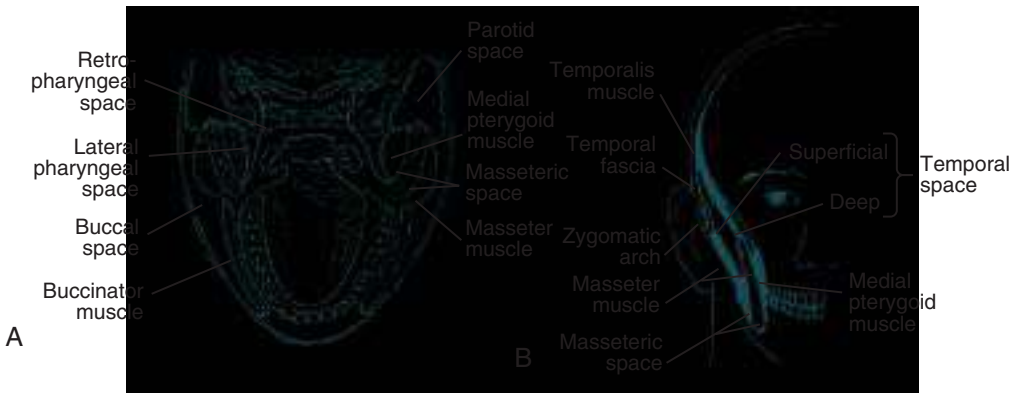

FIG. 64.4 Fascial spaces around the mouth and face. (A) Horizontal section at the level of the occlusal surface of the mandibular teeth. (B) Frontal view of the face. *(From Chow AW, Roser SM, Brady FA. Orofacial odontogenic infections.* Ann Intern Med. *1978;88:392–402.)*

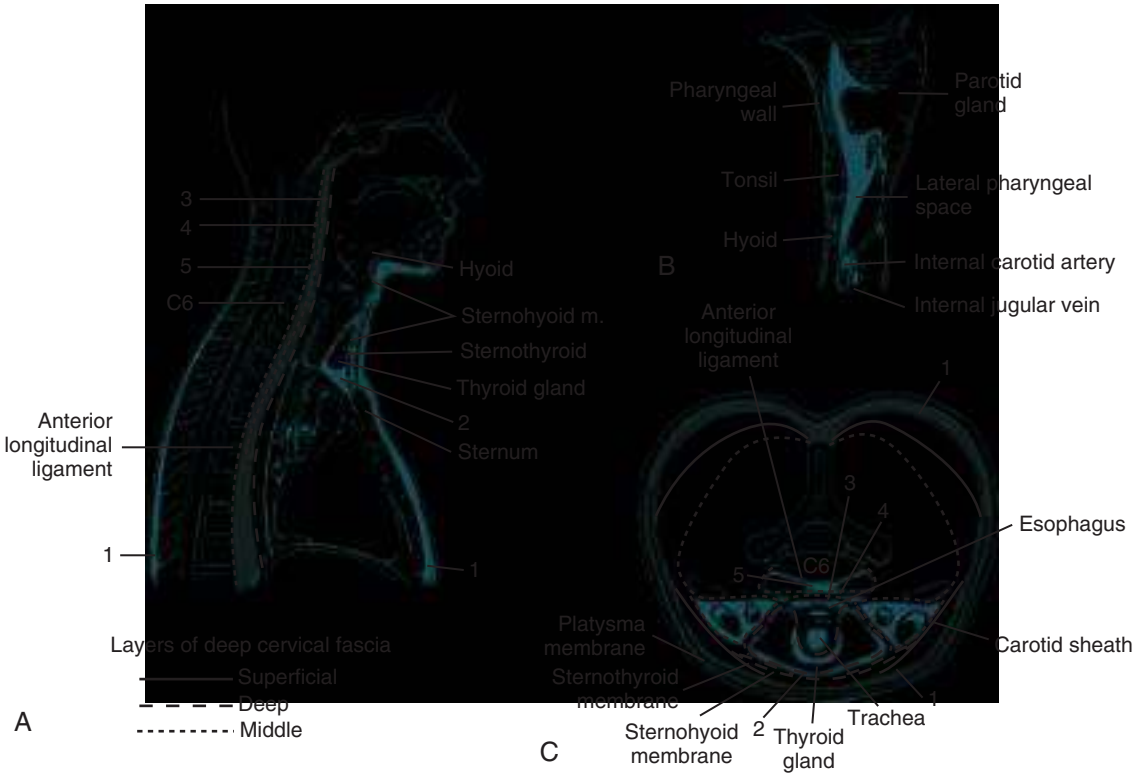

FIG. 64.5 Relation of lateral pharyngeal, retropharyngeal, and prevertebral spaces to the posterior and anterior layers of the deep cervical fascia. (A) Midsagittal section of the head and neck. (B) Coronal section in the suprahyoid region of the neck. (C) Cross section of the neck at the level of the thyroid isthmus. In all illustrations, *1*, superficial space; *2,* pretracheal space; *3,* retropharyngeal space; *4,* danger space; *5,* prevertebral space.

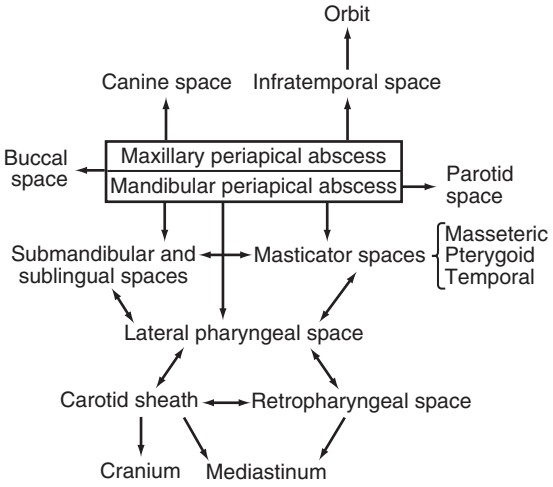

FIG. 64.6 Potential pathways of extension in deep fascial space infections.

mouth, the patient typically experiences a sudden onset of pain in the gingiva that interferes with normal mastication. Necrosis of the gingiva occurs mainly in the interdental papilla and results in a marginated, punched-out, and eroded appearance. A superficial grayish pseudomembrane is formed, and a characteristic halitosis with altered taste sensation is present. Fever, malaise, and regional lymphadenopathy are usually associated. Treatment includes local débridement and lavage with oxidizing agents, which usually alleviates pain within 24 hours. Antibiotic therapy with penicillin or metronidazole is indicated and is highly effective during the acute phase of infection.[37,38]

Periodontitis

Chronic inflammation of the periodontium is the major cause of tooth loss in adults. The destructive process proceeds insidiously, usually beginning in early adulthood. With inflammation, subgingival plaque is always present, and both supragingival and subgingival calculi are usually abundant. Unlike pulpal infection, in which drainage is frequently obstructed, periodontal infections drain freely, and affected patients experience little or no discomfort. Associated sensations include pressure and an itchy feeling in the gums and between the teeth, a bad taste in the mouth, sensitivity to hot and cold, and vague pains in the jaws. The gingiva is inflamed and discolored, bleeds readily, and appears as periodontal pockets around the affected teeth. Frank pus can be readily expressed by digital pressure, or it may exude freely from the pockets. As periodontitis advances, the supporting tissues are destroyed, ultimately leading to loosening and exfoliation of teeth. *Localized juvenile periodontitis* is a particularly destructive form of periodontitis seen in adolescents and is characterized by rapid vertical bone loss affecting the first molar and incisor teeth. Plaque is usually minimal, and calculus is absent. Excellent therapeutic results have been obtained with systemic tetracycline or metronidazole therapy combined with local periodontal treatment involving root débridement and surgical resection of inflamed periodontal tissues.[39] An effective plaque control program with scaling and root planing may be necessary for long-term management of the diseased periodontium.[40]

Periodontal Abscess

Periodontal abscesses may be focal or diffuse and manifest as red, fluctuant swelling of the gingiva, which is extremely tender to palpation. These abscesses are always in communication with a periodontal pocket from which pus can be readily expressed after probing. Treatment is surgical and aimed at drainage of loculated pus. After abscess resolution, endodontic or periodontal infections should continue to be treated by removal of necrotic infected pulpal tissues or by subgingival scaling and root planing. Apical surgery may sometimes be necessary to reach the apical part of the root for débridement.

Pericoronitis

Pericoronitis is an acute localized infection associated with gum flaps overlying a partially erupted or impacted wisdom tooth. Food debris

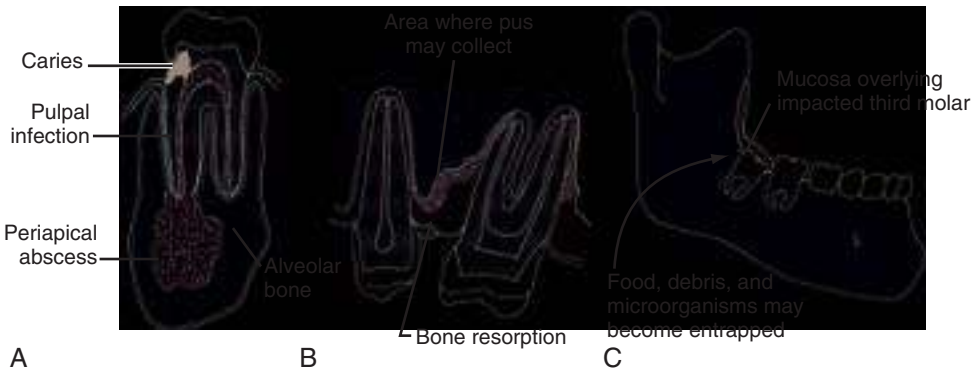

FIG. 64.7 Odontogenic infections. (A) Dental caries, pulpal infection, and periapical abscess. (B) Periodontal infection. (C) Pericoronal infection overlying impacted tooth. *(From Chow AW, Roser SM, Brady FA. Orofacial odontogenic infections.* Ann Intern Med. *1978;88:392–402.)*

TABLE 64.2 Antimicrobial Regimens for Various Odontogenic and Nonodontogenic Orofacial Infections

CLINICAL ENTITY	COMMON CAUSATIVE ORGANISMS	ANTIMICROBIAL REGIMENS
Odontogenic		
Supragingival dental plaque and dental caries prevention	*Streptococcus mutans,* other streptococci, *Actinomyces* spp.	Any one or combinations of the following topical applications: Fluoride-containing toothpaste or oral rinses (e.g., sodium fluoride 1.1% or stannous fluoride 0.4%) two or three times daily Fluoride-containing varnishes (e.g., sodium fluoride 5%) applied three or four times yearly Chlorhexidine 0.12% oral rinses three times daily, or varnishes applied once daily
Acute simple gingivitis	Streptococci, *Actinomyces* spp., oral spirochetes	Amoxicillin-clavulanate, 500 mg PO q8h (or ampicillin-sulbactam, 1.5–3 g IV q6–8h) *plus* metronidazole, 500 mg PO or IV q8h *or* Clindamycin, 450 mg PO q6–8h or 600 mg IV q6–8h
Acute necrotizing ulcerative gingivitis or Vincent angina	*Prevotella intermedia, Fusobacterium* spp., *Tannerella forsythensis, Treponema denticola*, other oral spirochetes	Metronidazole, 500 mg PO or IV q8h *or* Amoxicillin-clavulanate, 500 mg PO q8h *or* Ampicillin-sulbactam, 1.5–3 g IV q6h *or* Clindamycin, 450 mg PO q6h or 600 mg IV q6–8h
Early-onset, "aggressive," or "localized juvenile" periodontitis	*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia*	Amoxicillin-clavulanate, 500 mg PO q8h (or ampicillin-sulbactam, 1.5–3 g IV q6–8h) *plus* metronidazole, 500 mg PO or IV q8h
Adult or "established" periodontitis	*Treponema denticola,* other oral spirochetes, black-pigmented *Bacteroides* spp. (*Porphyromonas gingivalis* and *Prevotella melaninogenica*), *Tannerella forsythensis*	Topical application of minocycline microspheres (Arestin) *or* Topical application of doxycycline hyclate periodontal extended-release liquid (Atridox)
Nonodontogenic		
Gangrenous stomatitis (noma)	*Fusobacterium nucleatum, Borrelia vincentii, Prevotella melaninogenica,* other oral anaerobes	Ampicillin-sulbactam, 1.5–3 g IV q6–8h *or* Amoxicillin-clavulanate, 500 mg PO q8h *or* Clindamycin, 450 mg PO q6–8h or 600 mg IV q6–8h Each *plus* metronidazole, 500 mg PO or IV q8h
Severe oral mucositis in immunocompromised hosts	Viridans and other streptococci, *Bacteroides* spp., *Peptostreptococcus* spp., and other oral anaerobes, facultative gram-negative bacilli	Topical chlorhexidine (0.12%) mouth rinses tid *plus one of the following:* Cefotaxime, 2 g IV q6h *or* Piperacillin-tazobactam, 3.375 g IV q6h *or* Imipenem, 500 mg IV q6h *or* Meropenem, 1 g IV q8h
Sialadenitis and suppurative parotitis	*Staphylococcus aureus*[a] *Streptococcus viridans* and other streptococci, *Bacteroides* spp., *Peptostreptococcus* spp., and other oral anaerobes	Nafcillin, 2 g IV q4h, or vancomycin, 1 g IV q12h *plus either* Metronidazole, 500 mg IV q6h *or* Clindamycin, 600 mg IV q6–8h

[a]For *Staphylococcus aureus* infections in which methicillin-resistant *S. aureus* is suspected, replace nafcillin with vancomycin, 1 g IV q12h; in immunosuppressed hosts, cefotaxime or ceftriaxone or imipenem, as described in the table, can be added.
IV, Intravenously; *MU,* million units; *PO,* orally; *tid,* three times a day.

TABLE 64.3 Initial Empirical Antimicrobial Regimens for Suppurative Infections of the Head and Neck

INFECTION	USUAL CAUSATIVE ORGANISMS	ANTIBIOTIC REGIMENS, NORMAL HOST[a]
Suppurative orofacial odontogenic infections, including Ludwig angina	*Streptococcus viridans* and other streptococci, *Peptostreptococcus* spp., *Bacteroides* spp., and other oral anaerobes	Amoxicillin-clavulanate, 500 mg PO q8h (or ampicillin-sulbactam, 1.5–3 g IV q6–8h) *plus* metronidazole, 500 mg PO or IV q8h *or* Clindamycin, 600 mg IV q6h *or* Cefoxitin, 1–2 g IV q6h
Lateral pharyngeal or retropharyngeal space infections		
Odontogenic	*S. viridans* and other streptococci, *Staphylococcus* spp., *Peptostreptococcus* spp., *Bacteroides* spp., and other oral anaerobes	Amoxicillin-clavulanate, 500 mg PO q8h (or ampicillin-sulbactam, 2 g IV q4h) *plus* metronidazole, 500 mg PO or IV q8h *or* Clindamycin, 600 mg IV q6h
Rhinogenic	*Streptococcus pneumoniae, Haemophilus influenzae, S. viridans* and other streptococci, *Bacteroides* spp., *Peptostreptococcus* spp., and other oral anaerobes	One of the following: (1) Ampicillin-sulbactam, 2 g IV q4h, *or* levofloxacin, 500 mg IV q24h, *or* ciprofloxacin, 750 mg IV q12h *plus* Metronidazole, 500 mg IV q6h, *or* clindamycin, 600 mg IV q6h *or* (2) Moxifloxacin, 400 mg IV q24h
Otogenic	Same as for rhinogenic space infections	Same as for rhinogenic space infections
Suppurative cervical adenitis and infected embryologic cysts	*Streptococcus pyogenes, Peptostreptococcus* spp., *Fusobacterium* spp., oral anaerobes	One of the following: (1) Ampicillin-sulbactam, 2 g IV q4h, *plus* metronidazole, 500 mg IV q6h *or* (2) Clindamycin, 600 mg IV q6h *or* (3) Cefoxitin, 1–2g IV q6h
Suppurative thyroiditis	*Staphylococcus aureus, Streptococcus pyogenes, S. pneumoniae, Haemophilus influenzae, S. viridans* and other streptococci, oral anaerobes	Nafcillin, 2 g IV q4–6h, or vancomycin, 1 g IV q12h *plus either* Metronidazole, 500 mg IV q6h *or* Clindamycin, 600 mg IV q6h
Cervicofacial actinomycosis	*Actinomyces israelii, Arachnia propionica, Aggregatibacter actinomycetemcomitans*	One of the following: (1) Penicillin G, 2–4 MU IV q4–6h *or* (2) Doxycycline, 200 mg PO or IV q12h *or* (3) Clindamycin, 450 mg PO q6h or 600 mg IV q6h
Human or animal bites	*S. pyogenes, S. aureus, Eikenella corrodens, Pasteurella multocida,* oral anaerobes	One of the following: (1) Ampicillin-sulbactam, 2 g IV q4h *or* (2) Amoxicillin-clavulanate, 500 mg PO q8h *or* (3) Moxifloxacin, 400 mg IV or PO q12h
Maxillofacial trauma, postsurgical wound infections	*S. aureus, S. pyogenes, Peptostreptococcus* spp., other oral anaerobes, *Pseudomonas aeruginosa, Enterobacteriaceae* spp.	Nafcillin, 2 g IV q4h, *or* vancomycin, 1 g IV q12h *plus one of the following*: (1) Piperacillin-tazobactam, 3.375 g IV q6h *or* (2) Imipenem-cilastatin, 500 mg IV q6h *or* (3) Meropenem, 1 g IV q8h *or* (4) Moxifloxacin, 400 mg IV or PO q24h *or* (5) Tigecycline, 100 mg IV, then 50 mg IV q12h
Suppurative jugular thrombophlebitis (Lemierre syndrome)	*Fusobacterium necrophorum;* same as for odontogenic space infections	Same as for odontogenic space infections
Suppurative cavernous sinus thrombosis	Same as for odontogenic, rhinogenic, or otogenic space infections	Same as for odontogenic, rhinogenic, or otogenic space infections
Mandibular osteomyelitis	Same as for odontogenic space infections	Clindamycin, 600 mg IV q6h *or* Moxifloxacin, 400 mg PO or IV q24h
Extension of osteomyelitis from prevertebral space infection	*S. aureus*[b] facultative gram-negative bacilli	Either nafcillin, 2 g IV q4h, or vancomycin, 1 g IV q12h *plus* *either* tobramycin, 1.7 mg/kg IV q8h, *or* ciprofloxacin, 400 mg IV q12h

[a]For immunocompromised hosts, consider replacing penicillin G with one of the following: cefotaxime, 2 g IV q4h; ceftriaxone, 1 g IV q12h; or cefepime, 2 g IV q12h. Other regimens to consider are piperacillin/tazobactam, 3.375 g IV q6h; imipenem, 500 mg IV q6h; meropenem, 1 g IV q8h; moxifloxacin, 400 mg IV q24h; or tigecycline, 100 mg IV, then 50 mg IV q12h.

[b]For *Staphylococcus aureus* infections in which methicillin-resistant *S. aureus* is suspected, replace nafcillin with vancomycin, 1 g IV q12h; in immunosuppressed hosts, cefotaxime or ceftriaxone or imipenem, as described in the table, can be added.

IV, Intravenously; *MU,* million units; *PO,* orally.

and microorganisms become entrapped under the affected gingival tissues. If drainage is interrupted by sudden swelling or trauma, infection extends along fascial planes of least resistance into adjacent soft tissues. The underlying alveolar bone is usually not involved. Clinically, the pericoronal tissues are erythematous and swollen. Digital pressure produces a small amount of exudate from under the infected flap. Because the masticator spaces are often involved, marked trismus secondary to irritation of the masseter or medial pterygoid muscle is a prominent presenting feature. Treatment of pericoronitis includes incision of the lesion and irrigation of the pericoronitis pouch with antiseptics. Systemic antibiotics may be necessary if cellulitis of fascial space infection occurs. Excision of the operculum or extraction of the involved tooth may also be considered.

Deep Fascial Space Infections

Infections of either odontogenic or oropharyngeal origin may extend to potential fascial spaces of the lower part of the head and upper portion of the neck. These "space infections" can be conveniently categorized as those around the face (masticator, buccal, canine, and parotid spaces); those in the suprahyoid region (submandibular, sublingual, and lateral pharyngeal spaces); and those involving the infrahyoid region or the total neck (retropharyngeal, danger, and pretracheal spaces) (see Figs. 64.4 and 64.5).[41]

Space Infections Around the Face

Masticator spaces. Masticator spaces consist of the masseteric, pterygoid, and temporal spaces, all of which are well differentiated but intercommunicate with each other, as well as with the buccal, submandibular, and lateral pharyngeal spaces (see Fig. 64.4). Infection of the masticator spaces begins most frequently around molar teeth, particularly the third molars (wisdom teeth). Clinically, the hallmarks of masticator space infection are trismus and pain in the area of the body or ramus of the mandible. Swelling may not be prominent, especially in the masseteric compartment, inasmuch as infection exists deep in large muscle masses, which obscures or prevents clinically apparent swelling. When present, swelling tends to be brawny and indurated, which could indicate cervicofacial actinomycosis or mandibular osteomyelitis. If infection extends internally, it can involve an area close to the lateral pharyngeal wall and result in dysphagia. A true lateral pharyngeal space infection, however, is accompanied by displacement of the lateral pharyngeal wall toward the midline, a finding not present in masticator space infections. Infection of the deep temporal space usually originates from involvement of the posterior maxillary molar teeth. Very little external swelling is observed early in the course of the infection; if present, it usually affects the preauricular region and an area over the zygomatic arch. As infection progresses, the cheek, eyelids, and whole side of the face may be involved.[42] Infection may extend directly into the orbit via the inferior orbital fissure and produce proptosis, optic neuritis, and abducens nerve palsy.

Buccal, canine, and parotid spaces. As noted previously, infections arising from mandibular or maxillary premolar and molar teeth tend to extend in a lateral or buccal direction. The relation of the root apices to the origins of the buccinator muscle determines whether infection exits intraorally into the buccal vestibule or extraorally into the buccal space (see Fig. 64.3). Infection of the buccal space is readily diagnosed because of marked cheek swelling with minimal trismus and systemic symptoms. Antibiotic therapy alone tends to resolve infection. Drainage, if required, is superficial and should be performed extraorally.

Involvement of the maxillary incisors and canines may result in a canine space infection, which manifests as dramatic swelling of the upper lip, canine fossa, and, frequently, the periorbital tissues. Pain is usually moderate, and systemic signs are minimal. On occasion, a purulent maxillary sinusitis may result from direct extension of infection into the adjoining antrum. Treatment consists of antibiotics and drainage, which can be accomplished intraorally.

Parotid space infection from an odontogenic cause generally represents secondary spread from a masseteric space infection in the area of the ramus of the mandible (see Fig. 64.4). Marked swelling of the angle of the jaw occurs without associated trismus. Pain may be intense and accompanied by high fever and chills. Because of its close relationship with the posterior aspect of the lateral pharyngeal space, a parotid space infection carries the potential risk of direct extension into the danger and visceral spaces and hence to the posterior mediastinum (see Fig. 64.5).

Suprahyoid Space Infections

Submandibular and sublingual spaces. These two spaces are separated by the mylohyoid muscle (see Fig. 64.3), and the submandibular space is further divided into the submaxillary and submental spaces. Infection in these spaces usually arises from the second and third mandibular molar teeth because their root apices lie inferior to the mylohyoid muscle. Swelling is typical, although much less trismus is present, in contradistinction to masseteric space infection, because the major muscles of mastication are usually not involved. Submandibular odontogenic infection should be distinguished from submandibular sialadenitis and lymphadenitis that arise from other causes. Therapy includes antibiotics, dental extraction, and extraoral surgical drainage.

Infection of the sublingual space generally arises from mandibular incisors because their root apices lie above the mylohyoid muscle. Clinically, this space infection manifests as a brawny, erythematous, tender swelling of the floor of the mouth that begins close to the mandible and spreads toward the midline or beyond. Some elevation of the tongue may be noted in late stages. Surgical drainage of the sublingual space should be performed intraorally by an incision through the mucosa parallel to the Wharton duct. If the submandibular space is also to be drained, both spaces can be reached through a submandibular approach.

Ludwig angina. The term *Ludwig angina* has been loosely applied to a heterogeneous array of infections involving the sublingual, submaxillary, and submandibular spaces.[43,44] However, for therapeutic and prognostic purposes, it is desirable to restrict this diagnosis to cases that conform to the following classic description: (1) The infection is always bilateral, (2) both the submandibular and sublingual spaces are involved, (3) the infection is a rapidly spreading indurated cellulitis without abscess formation or lymphatic involvement, and (4) the infection begins in the floor of the mouth. A dental source of infection has been found in 50% to 90% of reported cases. The second and third mandibular molars are most commonly involved because they have roots below the mylohyoid muscle. Clinically, affected patients have a brawny, boardlike swelling in the submandibular spaces that does not pit on pressure (Fig. 64.8). They usually hold their mouths open, and the floor is elevated,



FIG. 64.8 Early appearance of a patient with Ludwig angina with a brawny, boardlike swelling in the submandibular spaces. *(From Reynolds SC, Chow AW. Life-threatening infections of the peripharyngeal and deep fascial spaces of the head and neck.* Infect Dis Clin North Am. *2007;21:557–576.)*

which pushes the tongue to the roof of the mouth. Eating and swallowing are difficult, and respiration may be impaired by obstruction from the tongue. A rapid progression of the infection results in edema of the neck and glottis and may precipitate asphyxiation; therefore patients must be monitored and treated aggressively. Fever and systemic toxicity are usually present and may be severe. Treatment entails high doses of parenteral antibiotics, such as ampicillin-sulbactam or penicillin G plus metronidazole; airway monitoring; early intubation or tracheostomy when required; soft tissue decompression; and surgical drainage. Systemic antibiotics combined with aggressive surgical intervention have lowered the rate of mortality from Ludwig angina dramatically from greater than 50% in the preantibiotic era to 0% to 4% currently.[43]

Lateral pharyngeal space. The lateral pharyngeal space (also known as the parapharyngeal or pharyngomaxillary space) in the lateral aspect of the neck is shaped like an inverted cone, with its base at the skull and its apex at the hyoid bone (see Fig. 64.5). Its medial wall is contiguous with the carotid sheath, which contains several vital structures (including the internal carotid artery, the internal jugular vein, and the vagus nerve) and lies deep to the pharyngeal constrictor muscle. Infection of the lateral pharyngeal space may result from pharyngitis, tonsillitis, parotitis, otitis, or mastoiditis, as well as from odontogenic infection, especially if the masticator spaces are primarily involved. The source of infection is best investigated through computed tomography (CT) or magnetic resonance imaging (MRI). If the anterior compartment is infected, the patient exhibits fever, chills, marked pain, trismus, swelling below the angle of the mandible, dysphagia, and medial displacement of the lateral pharyngeal wall. The contiguous parotid gland is usually swollen. Dyspnea can occur, although it is not prominent. Posterior compartment infection is potentially life threatening because of the carotid sheath; it is characterized by septicemia with little pain or trismus. Swelling is usually internal and deep and can often be missed because it is behind the palatopharyngeal arch. Complications include respiratory obstruction from edema of the larynx, thrombosis of the internal jugular vein, and erosion of the internal carotid artery. Suppuration may advance rapidly to other spaces, particularly the retropharyngeal and danger spaces, thus reaching directly to the mediastinum inferiorly or the base of the skull superiorly (see Fig. 64.5). Because respiratory obstruction from laryngeal edema can occur suddenly, the patient must be closely observed, and prophylactic tracheostomy may be required. Treatment includes high doses of antibiotics and surgical drainage. It is usually prudent to wait for the infection to localize before drainage is attempted unless respiratory obstruction or hemorrhage necessitates early surgical intervention.

Infrahyoid Space Infections

Retropharygeal and danger spaces. The retropharyngeal space comprises the posterior part of the visceral compartment, in which the esophagus, trachea, and thyroid glands are enclosed by the middle layer of deep cervical fascia (see Fig. 64.5). It lies behind the hypopharynx and the esophagus and extends inferiorly into the superior mediastinum to about the level of T1 to T2. Posterior to this compartment lies the *danger space,* which descends directly into the posterior mediastinum to the level of the diaphragm. Infection of the retropharyngeal space may result from contiguous infection of the lateral pharyngeal space or from lymphatic spread of infection from more distant sites to involve the retropharyngeal lymph nodes. Dysphagia, dyspnea, nuchal rigidity, and esophageal regurgitation, as well as high fever and chills, may be present. Bulging of the posterior pharyngeal wall may be observed. Lateral soft tissue radiographs of the neck may reveal marked widening of the retropharyngeal space. Infection of the retropharyngeal space is potentially life-threatening, and prompt surgical drainage is required. Complications include hemorrhage and spontaneous rupture of retropharyngeal space contents (abscess) into the airway with asphyxiation, laryngeal spasm, bronchial erosion, and thrombosis of the jugular vein.

Pretracheal space. The pretracheal space comprises the anterior portion of the visceral compartment and completely surrounds the trachea. Infections reach this space most commonly through perforations of the anterior esophageal wall, occasionally through contiguous extension from a retropharyngeal space infection. The clinical presentation is characterized by severe dyspnea, but hoarseness may be the first complaint. Swallowing is difficult, and fluids may be regurgitated through the nose. A pretracheal space infection is always serious because of possible extension into the mediastinum, and prompt surgical drainage is crucial.

Complications of Odontogenic Infections

Complications of odontogenic infections can occur either by hematogenous spread or by direct extension. Transient bacteremia is common during or after various dental procedures, especially the extraction of infected teeth.[45] The temporal relationship between these procedures and subsequent bacterial endocarditis and cardiovascular prosthetic infections is well documented.[46] Reports of infected total hip replacements after dental procedures are of further concern.[45] Prophylactic antibiotic treatment during dental procedures, although frequently used, remains a controversial issue, especially in the absence of preexisting valvular heart disease.[47] Complications of odontogenic infections secondary to direct extension include mediastinal spread,[48] intracranial suppuration (especially cavernous sinus thrombosis),[49] suppurative jugular thrombophlebitis,[50] carotid artery erosion,[49] maxillary sinusitis,[51] and osteomyelitis.[52] Acute mediastinitis and intracranial suppuration secondary to odontogenic infections are relatively uncommon in the postantibiotic era.

Suppurative Jugular Thrombophlebitis (Lemierre Syndrome) and Carotid Artery Erosion

These complications of oropharyngeal or odontogenic infections are uncommon since the introduction of antibiotics. Extension of infection to the carotid sheath, which encloses both the internal jugular vein and the internal carotid artery, usually arises from the lateral pharyngeal space. Because the carotid sheath space in this area is relatively compact with little areolar connective tissue, there is little tendency to spread up and down this vascular sheath, with the exception of possible retrograde thrombophlebitis and intracranial extension. The major concern is protracted septicemia and erosion of the carotid artery or one of its branches.

The onset of suppurative jugular thrombophlebitis (also known as *Lemierre syndrome* or *postanginal sepsis*) is acute, with shaking chills, spiking fevers, and profound prostration.[50] However, symptoms of pharyngitis, which may be the initial presentation of infection, may be resolving before the onset of the septic syndrome. Localizing signs of pain and swelling at the angle of the jaw, tenderness and induration along the sternocleidomastoid muscle, and swelling of the lateral pharyngeal wall with dysphagia and neck rigidity are usually present. However, these findings may be subtle, and their clinical significance may not be fully recognized until postmortem examination. Dysphagia and dysphonia may also occur. Ipsilateral vocal cord paralysis or other neurologic signs representing lower cranial nerve involvement may be present. Systemic evidence of infection includes septic pulmonary emboli and metastatic abscesses to the brain, lungs, kidneys, and joints. Empyema may also occur. Contrast medium–enhanced CT may reveal the normal carotid artery and an enlarged jugular venous wall surrounding a more lucent intraluminal clot (Fig. 64.9). Thrombosis of the jugular vein can also be demonstrated by magnetic resonance angiography (MRA).[53] The organisms most frequently involved are *Fusobacterium necrophorum,* anaerobic streptococci, and *Bacteroides* (now including *Prevotella*) spp.[49] Most cases of suppurative jugular thrombophlebitis can be managed medically without the need for ligation or surgical resection of the infected vein. Prolonged courses of intravenous (IV) antibiotics (3–6 weeks) are required. Anticoagulants have sometimes been used in this setting, but their efficacy is unconfirmed.[49] Surgical ligation of the internal jugular vein, the only available therapeutic option before antibiotics were available, is now required only in the rare patient who fails to respond to antibiotic treatment alone.

Erosion of the carotid artery is a rare complication after odontogenic infection involving the carotid sheath. Infection may arise by spread from the lateral pharyngeal space, Ludwig angina, or suppuration of deep cervical lymph nodes.[54] The initial pathologic process is an arteritis caused by contiguous inflammation that eventually forms a false aneurysm. The patient is extremely ill with a fever of undetermined origin. Trismus is absent, and signs of local suppuration may be subtle

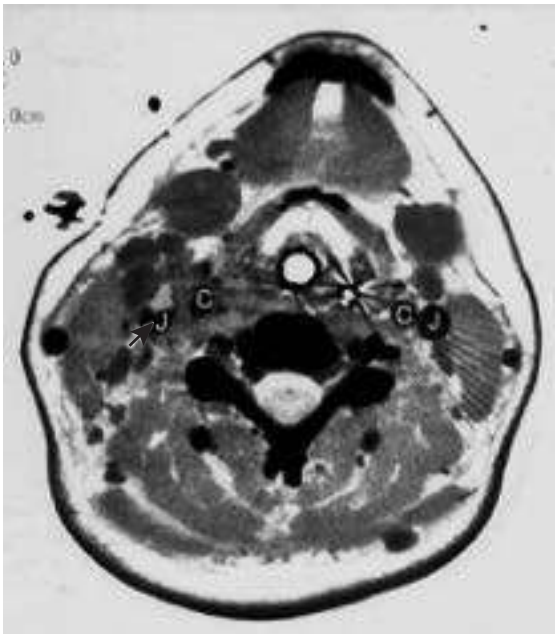

FIG. 64.9 Jugular venous thrombosis associated with a right peritonsillar abscess in a young adult. Contrast medium–enhanced axial computed tomographic scan showing a normal right common carotid *(C)* artery but an enlarged right internal jugular vein *(J) (arrow)* with a dense or enhancing wall that surrounds the more lucent intraluminal clot. *(From Chow AW. Head and neck infections. In: Baddour L, Gorbach SL, eds.* Therapy of Infectious Diseases. *Philadelphia: Saunders; 2003:37.)*



FIG. 64.10 Cavernous sinus thrombosis associated with pansinusitis in a young adult. Contrast medium–enhanced axial computed tomographic scan revealing opacification of the sphenoid and ethmoid sinuses and thrombus in the right cavernous sinus *(arrow). (From Chow AW. Life-threatening infections of the head, neck, and upper respiratory tract. In: Hall JB, Schmidt GA, Wood LH, eds.* Principles of Critical Care. *2nd ed. New York: McGraw-Hill; 1998:899.)*

because of the tight connective tissue around and within the carotid sheath. Rupture of the carotid artery may be heralded by recurrent minor hemorrhages from the nose, mouth, or ear ("herald bleeds"). This is followed by hematoma formation in the surrounding tissues of the neck, a protracted clinical course, and eventually shock caused by exsanguinations. Emergency ligation of the carotid artery may be necessary in cases of major hemorrhage, but the risk of stroke is significant. The mortality rate ranges from 20% to 40%, irrespective of treatment.[42]

Septic Cavernous Sinus Thrombosis

This dreaded complication is, fortunately, rare with the availability of antibiotics. Facial furuncles and purulent paranasal sinusitis were the major predisposing conditions. Infection of the maxillary teeth was the most common dental cause. Eagleton[55] described six criteria for the diagnosis of septic cavernous sinus thrombosis to help distinguish it from other less lethal infections, particularly those of the ethmoid sinus and the orbit: (1) a known site of infection; (2) evidence of bloodstream invasion; (3) early signs of venous obstruction in the retina, conjunctiva, and eyelid; (4) paresis of cranial nerves III, IV, and VI as a result of inflammatory edema; (5) abscess formation in neighboring soft tissue; and (6) evidence of meningeal irritation. Clinically, the onset is abrupt, with diplopia, photophobia, orbital edema, and progressive exophthalmos. Involvement of cranial nerves III, IV, V, and VI produces ophthalmoplegia, a midposition fixed pupil, loss of corneal reflex, and diminished sensation over the upper face. Obstruction of venous return from the retina results in papilledema, retinal hemorrhage, and visual loss. Contrast medium–enhanced CT (Fig. 64.10) and MRI are useful diagnostic tests, but MRA most reliably demonstrates the thrombosed cavernous sinus (see Chapter 93). Successful treatment requires early recognition, high-dose IV antibiotics, and surgical decompression of the underlying predisposing infection. Anticoagulation and steroid therapy are not indicated.[49] The mortality rate remains high, approximately 15% to 30%.[56]

Maxillary Sinusitis

In many people the roots of the maxillary molars lie proximal to the maxillary antrum. At times, congenital bony defects occur, with the root adjacent to the sinus membrane. In these cases sinusitis can result from direct extension of an odontogenic infection or from perforation of the sinus floor during extraction of a maxillary tooth.[51] The clinical manifestation of secondary sinus involvement is similar to that of primary sinus disease.

Osteomyelitis of the Jaws

The mandible is much more susceptible to osteomyelitis than is the maxilla, mainly because the cortical plates of the mandible are thin, and its medullary tissues have relatively poor vascular supply. In view of the large number of odontogenic infections and the intimate relationship of teeth to the medullary cavity, it is surprising that osteomyelitis of the jaws is not more frequent. When osteomyelitis occurs, there is usually a predisposing condition that affects host resistance, such as a compound fracture, previous irradiation, osteopetrosis, Paget disease, diabetes mellitus, or steroid therapy. Osteomyelitis after dental extraction in irradiated bone is termed *osteoradionecrosis.* Prior high-dose radiation from squamous cell carcinoma of the tongue or oral mucosa commonly precedes osteoradionecrosis. Chronic treatment with bisphosphonate in cancer patients, women with osteoporosis, or patients with Paget disease can lead to a condition known as bisphosphonate-related osteonecrosis of the jaw (BRONJ).[57] The precise mechanisms leading to BRONJ remain unclear but may be linked to drug-induced immune dysfunction associated with circulatory γδ (gamma-delta) T-cell depletion.[58,59] At the initiation of mandibular osteomyelitis, the intramedullary pressure markedly increases, which further compromises blood supply and leads to bone necrosis. Pus travels through the haversian and perforating canals, accumulates beneath the periosteum, and elevates it from the cortex. If pus continues to accumulate, the periosteum is eventually penetrated, and mucosal and cutaneous abscesses and fistulas may develop. As the inflammatory process becomes chronic, granulation tissue is formed. Spicules of necrotic and nonviable bone may become either totally isolated (sequestrum) or encased in a sheath of new bone (involucrum).

Severe mandibular pain is a common symptom and may be accompanied by damage to the mandibular nerve, which can result in anesthesia or hypoesthesia on the affected side. In protracted cases mandibular trismus may develop. A clinical variant is chronic sclerosing osteomyelitis associated with a proliferative periostitis.[60] This entity is more common in children and young adults after a periapical infection of the mandibular first molar. It is a nonsuppurative form of osteomyelitis characterized

by a localized, hard, nontender swelling over the mandible. On radiographs the newly formed periosteal bone looks like layers outside the cortex, with a characteristic "onion skin" appearance.[61] Actinomycosis and radiation necrosis are two common causes of this form of osteomyelitis of the jaws. Actinomycosis may be associated with obvious or occult dental disease, usually progresses slowly, and is manifested by rock-hard induration that can drain spontaneously through the skin. Prolonged antibiotic treatment is usually required (see Chapter 254).

Association With Cardiovascular Risk

An association between chronic odontogenic infection and cardiovascular disease has been described in a number of studies.[62] Of importance is that intensive periodontal treatment appears to result in improvement of endothelial function, as well as in oral health.[63] The mechanisms by which chronic periodontitis predisposes to coronary heart disease are not well understood, but an adverse effect of systemic inflammation on endothelial function may play a role. In addition to local infection, other factors may contribute to systemic inflammation, including intermittent bacteremia and release of bacterial endotoxins of oral origin into the bloodstream, an effect that can be induced by gentle chewing.[64]

Orofacial Nonodontogenic Infections

Nonodontogenic infections of the oral cavity are most frequently secondary to chemical, thermal, or traumatic injury. Virtually all infectious microorganisms, particularly sexually transmitted agents and childhood viral enanthems, can produce intraoral manifestations. Patients with cancer who develop mucositis from cytotoxic drugs and human immunodeficiency virus (HIV)-infected individuals are especially susceptible to acute and chronic opportunistic infections of the oral cavity, particularly candidiasis, aspergillosis, mucormycosis, herpetic gingivostomatitis, and mixed gram-negative infections.[65] In this section some of the conditions affecting primarily the oral mucosa and salivary glands, in which an infectious cause is either proved or suspected, are briefly discussed. Antimicrobial therapy is further reviewed in the section "Therapeutic Considerations" (see Tables 64.2 and 64.3).

Infections of the Oral Mucosa

Noma (Gangrenous Stomatitis)

Also known as cancrum oris, noma is an acute, fulminating, and gangrenous infection of the oral and facial tissues. It usually occurs in the presence of severe debilitation and malnutrition, and children are most often affected.[66] The earliest lesion is a small, painful, red spot or vesicle on the attached gingiva in the premolar or molar region of the mandible. A necrotic ulcer rapidly develops and undermines the deeper tissue. Painful cellulitis of the lips and cheeks is observed as the lesion extends outward in a conelike manner. Within a short period, sloughing of necrotic soft tissues occurs and exposes underlying bone, teeth, and deeper tissues.

Noma is thought to be an infectious disease, but its cause remains unknown. Fusospirochetal organisms, such as *Borrelia vincentii* and *F. nucleatum,* are consistently cultured from noma lesions.[67] *P. melaninogenica* may also be present. Biopsy specimens of tissue from the advancing lesion show a mat of predominantly gram-negative threadlike bacteria that cannot be positively identified. Thus this lesion bears a similarity to acute necrotizing ulcerative gingivitis in several respects but appears to be more focal and destructive, involving deeper tissues beyond the gingiva. Treatment of noma requires high doses of IV antibiotics (see Table 64.2). Every effort should be directed to correct the dehydration and underlying malnutrition and debility. Loose teeth and sequestra may be removed, but saucerization should be avoided. Healing is by secondary intention. Serious mutilation and facial deformity may necessitate subsequent cosmetic surgery.[68]

Aphthous Stomatitis

Aphthous ulcers are the most common causes of recurrent oral lesions and must be distinguished from other conditions, such as those caused by herpes simplex virus or coxsackievirus infections, agranulocytosis, and Behçet syndrome. The cause of recurrent aphthous ulcers remains uncertain, although a number of infectious agents, including viruses, have been implicated. Recent data suggest multiple etiologies, including vitamin deficiencies, oral microbiota derangements, autoimmune conditions, stress, and genetic polymorphisms to oxidant-antioxidant imbalances, among others.[69]

Three major clinical variants are recognized: (1) minor aphthous ulcers, (2) major aphthous ulcers, and (3) herpetiform aphthous ulcers. In their most characteristic form, minor aphthous ulcers appear as a number of small ulcers on the buccal and labial mucosa, the floor of the mouth, or the tongue. The palatal soft tissues are rarely involved. Moreover, the ulcers are concentrated in the anterior part of the oral cavity, whereas the pharynx and tonsillar fauces are rarely implicated. A prodromal stage usually occurs. The ulcers appear gray-yellow, often with a raised and erythematous margin, and are exquisitely painful. Lymph node enlargement is seen only with secondary bacterial infection. The course of ulceration varies from a few days to a little more than 2 weeks and is followed by spontaneous healing. Major aphthous ulcers are more protracted and last up to several months. All areas of the oral cavity, including the soft palate and tonsillar areas, may be involved. Long periods of remission may be followed by intervals of intense ulcer activity. Herpetiform aphthous ulcers are small and multiple and characteristically affect the lateral margins and tip of the tongue. The ulcers are gray, without a delineating erythematous border, and are extremely painful, which makes eating and speaking difficult. Despite its name, there is little clinical resemblance to an acute herpetic gingivostomatitis. Although intranuclear inclusions have been demonstrated in herpetiform aphthous ulcers, there is no evidence to suggest that these inclusions bear any relationship to the presence of viruses.

The treatment of aphthous ulcers is primarily symptomatic. Strict oral hygiene should be maintained, and the use of antiseptic mouthwashes may be helpful in temporarily reducing secondary infection. Local anesthetic lozenges or gels may be used as a last resort for brief periods of pain relief. Amlexanox, a topical immunomodulating agent, appears to provide symptomatic relief and shorten the duration of ulcers.[70] Topical or systemic steroids may be beneficial in selected people with extensive disease, but caution must be exercised in their administration.[71] Thalidomide (100–200 mg/day orally for 2–6 weeks) has been reported to be effective for the treatment of large aphthous lesions in patients with acquired immunodeficiency syndrome (AIDS).[72,72a]

Mucositis and Stomatitis in the Severely Immunocompromised Patient

Much of what is known about the management of oromucosal infections has been studied in patients with cancer being treated with radiotherapy, chemotherapy, and bone marrow transplantation.[73] Mucositis involving the nonkeratinized oral epithelium is a frequent complication after irradiation or during chemotherapy for acute leukemia. Other patients who develop oromucosal complications include those undergoing solid-organ transplantation, patients with AIDS, and those with autoimmune diseases associated with xerostomia and systemic immunosuppression. The underlying mechanism appears to be a breakdown of the mucosal epithelium that leads to mucositis and secondary bacterial or fungal infection, or reactivation of latent viral infection. Oral candidiasis, herpes simplex, varicella-zoster, and cytomegalovirus infections may occur concomitantly. Four stages of disease progression are described: (1) inflammatory or vascular phase, (2) epithelial phase, (3) ulcerative/bacteriologic phase, and (4) healing phase.[74] Ulceration and pseudomembrane formation are evident, usually between 4 and 7 days after the initiation of chemotherapy, and they commonly involve the buccal and labial mucosa, soft palate, oropharynx, floor of the mouth, and the ventral and lateral surfaces of the tongue (Fig. 64.11). The clinical manifestations may be quite variable. The lesions are often protracted in duration and may not be associated with an obvious inflammatory reaction. Pain or tenderness may be the only abnormal finding.

A number of strategies are currently recommended for the prevention of oral mucositis in specific patient populations.[75] These include cryotherapy (the placement of ice chips in the mouth during delivery of chemotherapy) in patients receiving bolus 5-fluorouracil (5-FU), and recombinant human keratinocyte growth factor-1 in patients undergoing autologous stem cell transplantation for hematologic malignancy. A systematic review of cryotherapy for the prevention of mucositis found strong evidence that it results in large reductions in the incidence of

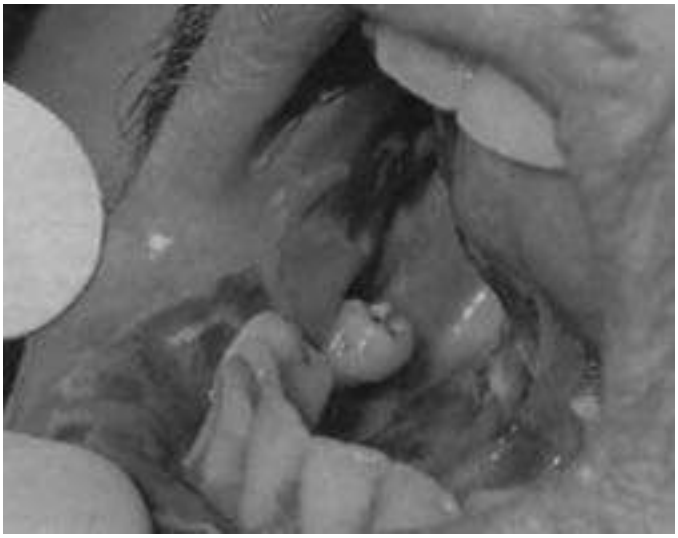

FIG. 64.11 Severe mucositis with marked erythema, loss of epithelial barrier, and extensive ulceration of the labial and buccal mucosa in a patient undergoing radiation therapy. *(From Epstein JB, Chow AW. Oral complications associated with immunosuppression and cancer therapies.* Infect Dis Clin North Am. *1999;13:901–923.)*

mucositis in patients receiving 5-FU.[76] Similarly, recombinant human keratinocyte growth factor-1 is effective in the prevention of oral mucositis in adults who are receiving radiotherapy to the head and neck with cisplatin or 5-FU, or those receiving chemotherapy alone for mixed solid and hematologic malignancies.[77] Topical antiseptic application with chlorhexidine has been shown to have a moderate effect on reducing both the frequency and duration of severe oral mucositis.[78] Because secondary infection is common but the etiologic agents cannot be readily predicted on clinical grounds alone, specific microbiologic diagnosis by culture, histopathologic examination, or molecular or antigen detection techniques may be necessary to guide appropriate antimicrobial treatment.

Frequent saline or bicarbonate rinses may reduce mucosal irritation, remove thickened secretions or debris, and increase moisture in the mouth. Ice chips, topical anesthetics or analgesic gels (e.g., benzydamine, viscous lidocaine), and cytoprotective coating agents (e.g., milk of magnesia or aluminum hydroxide gel) have provided symptomatic relief of painful oral lesions. Meticulous oral and dental hygiene, effective management of xerostomia, selective suppression of oropharyngeal microbial colonization, and early control of reactivation by latent viral infections appear to be key for prevention and reduction of the overall morbidity of oromucosal infections in severely immunocompromised patients.

Infections of the Salivary Gland

Sialadenitis, or infection of salivary tissue, is a relatively common disease. Sialolithiasis in elderly patients (particularly calculi in the Wharton duct) often leads to ductal obstruction and secondary infection. Other predisposing factors for ductal occlusion include dehydration, sialogogic drugs, general debility, and trauma.

Suppurative Parotitis

Acute bacterial parotitis is a specific clinical entity affecting primarily elderly, malnourished, dehydrated, or postoperative patients.[79,80] The onset of firm, erythematous swelling of the preauricular and postauricular areas is sudden and extends to the angle of the mandible. This is accompanied by exquisite local pain and tenderness. Systemic findings of high fevers, chills, and marked toxicity are generally present. Progression of the infection may lead to massive swelling of the neck, respiratory obstruction, septicemia, and osteomyelitis of the adjacent facial bones. Staphylococci have been the predominant isolates, and antibiotic therapy should include an antistaphylococcal agent (see Table 64.2). Enterobacteriaceae, other gram-negative bacilli, and anaerobes have also been reported to cause parotitis. Early surgical drainage and decompression of the gland are generally required because spontaneous drainage is uncommon.

Chronic Bacterial Parotitis

In this condition, parotitis is recurrent with intermittent acute exacerbations. Chronic, low-grade bacterial infection results in functional destruction of the salivary gland. Pus, when obtained directly from the gland, usually reveals the growth of staphylococci or mixed oral aerobes and anaerobes. Sialography during remission may reveal a sialectatic pattern of pooling of contrast medium that suggests multiple cystic cavities in place of the normal acinar pattern. Chronic parotitis may be confused with Sjögren syndrome, a noninfectious illness characterized by the triad of xerostomia, keratoconjunctivitis, and systemic autoimmune disease, such as rheumatoid arthritis, lupus erythematosus, scleroderma, periarteritis nodosa, and polymyositis. The presence of associated temporomandibular arthritis or arthralgia is strongly suggestive of Sjögren syndrome rather than chronic bacterial parotitis.

Therapy for chronic parotitis should initially be conservative and consists of systemic antibiotics and ductal saline or antibiotic irrigations. Parotidectomy may eventually be required for people with long-standing infection.[81]

Viral Parotitis

Mumps parotitis is characterized by the rapid, painful swelling of one or both parotid glands within 2 to 3 weeks after exposure to the mumps virus. A prodromal phase of preauricular pain, fever, chills, and headache may be present (see Chapter 157). Other viral causes of parotitis include influenza and enteroviruses, and virus cultures or serologic examinations may be required for distinguishing these from true mumps. Mumps parotitis usually resolves spontaneously in 5 to 10 days. Symptomatic relief of pain and fever is necessary, and prevention of dehydration and secondary bacterial infection is essential.

Miscellaneous Infections of the Neck and Head

Since the advent of antibiotics, dental causes have outnumbered oropharyngeal and tonsillar sources of deep neck infections. Peritonsillar abscess (quinsy) is discussed separately in Chapter 59. Other miscellaneous infections of the neck and head include suppurative cervical adenitis, infected embryologic cysts of the neck, suppurative thyroiditis, and those secondary to human and animal bites, irradiation, or surgical procedures of the head and neck. Their clinical manifestations and management are briefly reviewed here. Antimicrobial therapy is further discussed in the section "Therapeutic Considerations."

Suppurative Cervical Adenitis

The six groups of cervical lymph nodes (occipital, mastoid, parotid, facial, submandibular, and submental) form a collar at the junction of the head and neck. Within this collar near the base of the tongue lie the sublingual and retropharyngeal nodes. The anterior and lateral cervical nodes form a chain along the front and side of the neck, respectively. The lateral cervical chain serves as a common root for drainage. The final conduit from all lymphatic vessels in the head and neck is the large deep chain situated along the carotid sheath. Cervical adenitis that arises unilaterally is usually caused by pyogenic bacterial infections. Its anatomic location in relationship to major cervical landmarks provides the clinical clues to the primary source of infection. Bilateral acute cervical adenitis is generally suggestive of a nonspecific or viral cause, toxoplasmosis, or group A streptococcal infection. A more chronic or recurrent cervical adenitis is suggestive of typical or atypical mycobacteria, HIV infection, Epstein-Barr virus or cytomegalovirus mononucleosis, cat-scratch fever, actinomycosis, sarcoidosis, or lymphoproliferative and neoplastic disorders.

Infected Embryologic Cysts

Three distinct embryologic abnormalities can manifest with infection in the neck: (1) cystic hygroma or lymphangioma, (2) branchial cleft cysts, and (3) thyroglossal duct cysts.[80] Cystic hygroma is associated with a diffuse tumor mass, usually evident within the first 2 years of

life. It commonly involves the lower aspect of the neck, but it can appear anywhere in the cervical region. It is probably an abnormal development of lymphatic vessels from the jugular lymphatic sacs. Sudden enlargement by infection or hemorrhage into a lymphangioma may cause obstruction of the upper airways. Branchial cleft cysts can develop from the first, second (most common), or third branchial clefts. They usually manifest in childhood as fistulas or masses just posterior to the angle of the mandible along the anterior border of the sternocleidomastoid muscle. The mass can fluctuate in size, and enlargement can be associated with upper respiratory infection. Thyroglossal duct cysts originate from the foramen cecum of the tongue and descend through the body of the hyoid bone into the anterior portion of the neck. Any residual secretory lining may give rise to a thyroglossal duct cyst that is midline. It can cause respiratory obstruction or fistula formation if secondarily infected. Successful treatment of these congenital abnormalities during secondary bacterial infection requires broad-spectrum antibiotics, such as a penicillin or cephalosporin, in addition to clindamycin targeting the oral microbiota. Definitive surgical excision to prevent recurrence should be performed after complete resolution of the acute process.

Suppurative Thyroiditis

Although infections of the thyroid gland are rare, they are potentially life threatening. Such infections may arise by a variety of pathways, including hematogenous dissemination, direct spread from an adjacent deep fascial space infection, an infected thyroglossal fistula, or anterior perforation of the esophagus.[80] Preexisting diseases of the thyroid gland, such as a goiter or adenoma, are frequently present. Acute suppurative thyroiditis is characterized by fever, local pain, tenderness, warmth, erythema, and symptoms of dysphagia, dysphonia, hoarseness, or pharyngitis. The infection may involve a single lobe or both lobes, and fluctuance may not be apparent until late in the course. Subacute thyroiditis may produce similar local findings, but systemic manifestations are not as severe and tend to be more self-limiting. Laboratory investigation of thyroid infections should include ultrasonography, radionuclide scanning, and lateral radiography or computed tomographic scanning of the neck for evidence of peritracheal extension; thyroid function tests; and diagnostic needle aspiration for histopathologic and microbiologic diagnosis. *S. aureus, Streptococcus pyogenes*, and *Streptococcus pneumoniae* are most frequently isolated. Other isolated pathogens include *Haemophilus influenzae, Streptococcus viridans, Eikenella corrodens,* and *Bacteroides, Peptostreptococcus,* and *Actinomyces* spp. Successful treatment requires specific antimicrobial agents and appropriate surgical drainage.

Infections from Bites, Irradiation, and Surgical Wounds

Human and Animal Bites

Human and animal bite wounds to the head and neck are relatively common. Although they may look innocuous initially, serious complications can occur (see Chapter 315). For this reason, empirical antibiotic therapy is recommended when the bite wound involves the face, head, or neck. According to researchers who used adequate anaerobic culture techniques, indigenous oral microbiota rather than the skin microbiota may be the major source of bite wound infections.[82,83] Streptococci, *E. corrodens,* and *S. aureus* are the most prevalent facultative organisms, and *Bacteroides* and *Peptostreptococcus* spp. are the most common anaerobic isolates. Penicillin-resistant gram-negative rods are infrequently found. *E. corrodens* is unique in that it is susceptible to penicillin and ampicillin but resistant to oxacillin, methicillin, nafcillin, and clindamycin.[84] In animal bite wounds, *Pasteurella multocida* has been a common cause of infection.[85] It is susceptible to penicillin, cefoxitin, and fluoroquinolones but resistant to clindamycin and erythromycin. In view of these findings, penicillin, amoxicillin-clavulanate, or moxifloxacin is a reasonable antibiotic choice of initial therapy for either human or animal bite wounds (see Table 64.3).

Irradiation and Postsurgical Wounds

Malignancies of the head and neck are frequently treated with a combination of irradiation, chemotherapeutic agents, and surgical resection. Infectious complications are particularly common after such procedures.[86] Pharyngocutaneous fistulas, osteonecrosis of the mandible, or radionecrosis of the laryngeal cartilage may occur. *S. aureus* and *Pseudomonas aeruginosa* are pathogens commonly involved. Prolonged courses of IV antibiotics should be selected according to culture and sensitivity data, and frequent wound débridement and cleansing are indicated. Although some controversy still exists, immunocompromised patients undergoing oropharyngeal surgery for cancer should receive perioperative antibiotics because they are at particularly high risk for infection. A broad-spectrum antibiotic such as piperacillin-tazobactam or a carbapenem appears appropriate in this setting (see Table 64.3). Coverage of methicillin-resistant *S. aureus* (MRSA) may be prudent for known carriers.

DIAGNOSTIC APPROACHES

Microbiologic Investigation

A major challenge in the microbiologic investigation of oral infections is how to distinguish true pathogens from commensal microbiota. The mere presence of an organism is insufficient to assume that it is a cause of a polymicrobial endogenous infection. However, microorganisms with low virulence potential and usually considered harmless in a healthy host (e.g., coagulase-negative staphylococci, diphtheroids, enterococci) may nonetheless cause invasive disease in an immunocompromised patient. This possibility underscores the importance of proper specimen collection and the need to correlate laboratory data with clinical information. Aerobic and anaerobic cultures should always be obtained. Surface cultures obtained from mucosal sites are regularly contaminated by resident commensal microbiota and are generally not recommended. For closed-space infections, it is imperative that the normal resident oral microbiota be excluded during specimen collection so that the culture results can be interpreted appropriately. Needle aspiration of loculated pus by an extraoral approach is desirable, and specimens should be transported immediately to the laboratory under anaerobic conditions. For intraoral lesions, direct microscopic examination of stained smears often provides more useful information than do culture results from surface swabs. Gram and acid-fast stains for bacteria and potassium hydroxide preparations for fungi should be routinely performed. Tissue biopsy specimens should be routinely examined for histopathologic evidence of acute or chronic inflammation and infection. In chronic osteomyelitis soft tissue swelling and draining fistulas are frequently present. Aspirates from the adjacent soft tissue swellings may be valuable, but cultures from the sinus tracts may be misleading because these sinus tracts are often colonized by organisms that do not reflect what is actually occurring within the infected bone. Bone biopsies for histopathologic examination and culture are often necessary for a definitive diagnosis.

Immunofluorescence staining and DNA probes or polymerase chain reaction (PCR) are increasingly used to analyze pathogens that are fastidious or noncultivable.[87,88] The benzoyl-D,L-arginine naphthylamide (BANA) test is a useful tool for the diagnosis of anaerobic periodontal infections.[89] This test detects a trypsin-like enzyme (BANA) produced by various periodontopathic bacteria, including *P. gingivalis, T. denticola*, and *Tannerella forsythensis*. A positive BANA test result is correlated both qualitatively and quantitatively with the presence of these microorganisms and facilitates the diagnosis in the appropriate clinical setting.

Imaging Techniques for the Localization of Infection

Orthopantomography ("wingbite" radiography) may reveal the true extent of advanced periodontitis or the presence of periapical abscess. High-resolution ultrasonography is useful for assessing the parotid gland or calculi within the Stenson duct. Ultrasonography, radionuclide scanning, CT, and MRI are particularly useful for the localization of deep fascial space infections of the head and neck.[53] CT can both localize a process and define its extent, particularly invasion into the cranial vault, mediastinum, or the bone; therefore it is an invaluable tool for guiding needle aspiration or open drainage. A lateral radiograph of the neck may demonstrate compression or deviation of the tracheal air column or the presence of gas within necrotic soft tissues. The soft tissues of the posterior wall of the hypopharynx are approximately 5 mm deep, less than one-third the diameter of the fourth cervical vertebra

(C4). The retropharyngeal soft tissues should be approximately two-thirds the width of C4, and the retropharyngeal space slightly less.[53] CT can determine whether the soft tissue swelling or an abscess originated from the retropharyngeal space or the prevertebral space. The former suggests an odontogenic source, whereas the latter likely indicates hematogenous involvement of the cervical spine. Bone scanning with technetium, used in combination with gallium- or indium-labeled white blood cells, is particularly useful for the diagnosis of acute or chronic osteomyelitis. In acute osteomyelitis, both the bone scan and the gallium scan are likely to yield positive findings. In chronic osteomyelitis, the gallium or indium scans may yield negative findings, but the result of the technetium scan may be positive. MRI is more sensitive than CT and probably more accurate than bone scan in detecting bone involvement.[90] T2-weighted images may identify and localize areas of pus for drainage or aspiration. Gadolinium enhancement is important for accurately defining the soft tissue component. Finally, MRA is useful for imaging vascular lesions, such as jugular thrombophlebitis or cortical venous thrombosis.[49]

THERAPEUTIC CONSIDERATIONS

Dental Caries and Periodontitis

For both caries prevention and the treatment of periodontitis, the most important strategy is the effective control of the supragingival and subgingival biofilm or plaques through active promotion of and meticulous attention to oral hygiene. The diet should be scrutinized to eliminate or discourage frequent snacking on carbohydrate-rich foods or the intake of sugar-containing beverages. Various antiseptic and antimicrobial regimens are used for the prevention of dental caries and treatment of different clinical forms of periodontal disease (see Table 64.2). Fluoride-containing dentifrices and rinses (e.g., 1.1% sodium fluoride or 0.4% stannous fluoride) and dental flossing should be encouraged after each meal. Fluoride forms a complex with the apatite crystals in dentin by replacing the hydroxyl group, thereby lending strength to the entire structure. Further, fluoride promotes remineralization of the carious lesions and also exerts a bacteriostatic effect. Oral antimicrobial rinses with 0.12% chlorhexidine are also effective for the control of dental plaque bacteria that lead to caries.[91] Chlorhexidine acts as a cationic detergent that kills a wide range of bacteria and is retained on the oral surfaces for prolonged periods to prevent plaque advancement. A synergistic antibacterial effect has been demonstrated with the combination of chlorhexidine and fluoride, greater than that of either agent alone.[92] A disadvantage of chlorhexidine is that it has a bitter taste and stains the enamel and tongue. Prolonged application may also promote the emergence of resistant microorganisms. Among topical antibiotics, although both penicillin and tetracycline have cariostatic effects in animal models, only the topical application of vancomycin has been shown to reduce dental caries with some degree of success in humans.[93] None of these measures is routinely applied in clinical practice, but they are useful for the control of dental plaques in selected patients with rampant caries. With the development of improved restorative dental care and dental restorative materials, such as bonding and fluoride-releasing agents, the need for dental extractions has become much less frequent.

Acute simple gingivitis may be treated with (1) penicillin plus metronidazole, (2) clindamycin, or (3) ampicillin-sulbactam. Acute necrotizing ulcerative gingivitis responds well to metronidazole alone. Clindamycin, ampicillin-sulbactam, or amoxicillin-clavulanate is an alternative choice (see Table 64.2). Certain types of severe periodontitis are amenable to systemic antimicrobial therapy in conjunction with mechanical débridement (scaling and root planing).[39,94] This protocol has often obviated the need for radical surgical resection of periodontal tissues. In double-blind, randomized and prospective clinical studies of moderate-to-advanced periodontitis, systemic metronidazole (500 mg PO three times daily) plus amoxicillin (500 mg PO three times daily) for 7 days in conjunction with mechanical débridement of the root surfaces significantly improved clinical outcome compared with mechanical débridement alone at 1-year follow-up.[95] A topical antibiotic approach is also feasible. The US Food and Drug Administration has approved a powder containing minocycline microspheres (Arestin [Orapharma Inc., Bridgewater, NJ]), which releases controlled amounts of the antibiotics beneath the gum, for use in conjunction with scaling and root planing to reduce pocket depth in adults with "established" periodontitis.[96] Alternatively, topical application of an extended-release doxycycline hyclate liquid (Atridox; DenMat, Lompoc, CA) may also be beneficial.[97] In localized juvenile periodontitis systemic tetracycline therapy directed against *A. actinomycetemcomitans*, combined with local periodontal treatment, has yielded excellent results. Unfortunately, the administration of tetracycline to children younger than 9 years can cause staining of the permanent dentition and is not generally recommended. Furthermore, tetracycline resistance among periodontal pathogens has been increasingly recognized. Oral metronidazole plus amoxicillin is an alternative and the current regimen of choice.[95] The routine use of systemic antimicrobials prophylactically during oral or periodontal surgery in a healthy host is unwarranted because the risk of postoperative infections is low and overuse of antimicrobials leads to added cost and emergence of antimicrobial resistance.[98,99]

The need for definitive restoration or extraction of the infected tooth, the primary source of an odontogenic infection, is readily apparent. Deep periodontal scaling and endodontic treatment with root fillings are required in most instances. The key for the prevention and control of dental caries and advanced periodontitis is the active promotion of oral hygiene, including the following:

1. Rigorous brushing and dental flossing after each meal.
2. Dietary counseling to reduce the ingestion of carbohydrate-rich foods or beverages.
3. Use of topical fluorides and oral antimicrobial rinses, such as chlorhexidine, for patients at high risk for dental caries.
4. Behavioral modification of risk factors, such as tobacco smoking.
5. Overcoming the reluctance for regular visits to dental professionals.

Vaccines based on various immunogens derived from *S. mutans,* the principal bacterial agent associated with dental caries, have been explored, commercialization of an effective and safe vaccine for clinical application is unlikely in the near future.[100]

Suppurative Soft Tissue Infections

The most important therapeutic modality for pyogenic odontogenic or nonodontogenic orofacial infections is surgical drainage and the removal of necrotic tissue. Needle aspiration by the extraoral route can be particularly helpful for both microbiologic sampling and evacuation of pus. Effective surgical management requires a thorough understanding of the anatomic routes by which infection is most likely to spread. The neighboring potential fascial spaces should be carefully and systematically surveyed. For effective drainage, the incision site should be in the most dependent location. It is equally important that the timing for incision and drainage be optimum. Premature incision into an area of poorly localized cellulitis in an ill-conceived search for pus can disrupt the normal physiologic barrier and cause further spread of infection.

Antimicrobial therapy is important in halting the local spread of infection and in preventing hematogenous dissemination. The initial choice of specific antibiotics is based more on knowledge of the indigenous organisms that colonize the teeth, gums, and mucous membranes than on the results of culture and sensitivity testing.[101] Whereas endogenous oral pathogens were almost universally susceptible to penicillin in the past, this is no longer the case. β-Lactamase–producing anaerobic gram-negative bacteria in the oral cavity have been increasingly recognized.[102] They include pigmented *Prevotella* spp., *Porphyromonas* spp., and *Fusobacterium* spp. Failure of penicillin therapy for odontogenic infections caused by these microorganisms has been well documented.[101] Thus a combination of a β-lactam and β-lactamase inhibitor (e.g., ampicillin-sulbactam, amoxicillin-clavulanate) should be considered. Apart from penicillin, there are also increasing reports of in vitro resistance to metronidazole and azithromycin among *Fusobacterium* spp. and other anaerobic gram-negative bacteria isolated from odontogenic infections.[103] Their clinical significance at this time is unclear, however. Nevertheless, the emergence of resistance among oral bacteria is disconcerting and probably results from widespread and inappropriate use of broad-spectrum antibiotics. In accordance, in vitro susceptibility testing of suspected oral pathogens has become more

critical, particularly if the clinical response to initial empirical therapy is suboptimal.

The initial selection of antimicrobial agents should be guided by knowledge of the most likely causative organisms, their predicted antibacterial spectrum, and bioavailability in oral or parenteral formulations (see Tables 64.2 and 64.3). For normal hosts, metronidazole plus penicillin, combinations of β-lactam and β-lactamase inhibitors (e.g., ampicillin-sulbactam, amoxicillin-clavulanate), or clindamycin is generally the agent of choice. Cefoxitin, cefotetan, or moxifloxacin are alternative agents, although for each, the spectrum of anaerobic activity is limited. Erythromycin, other macrolides, and tetracycline are not recommended because of increasing resistance among some strains of streptococci and their lack of optimal anaerobic activity. Severely immunocompromised patients are at increased risk for unhalted and rapidly spreading infection, and coverage for nosocomial pathogens, including facultative gram-negative bacilli and MRSA, may be required. The antibiotic regimen must be broad spectrum, bactericidal, and appropriate in dose and schedule. Ciprofloxacin or a third- or fourth-generation cephalosporin (e.g., cefotaxime, ceftriaxone, ceftizoxime, cefepime), each in combination with metronidazole, is recommended. Monotherapy with an extended-spectrum penicillin β-lactamase inhibitor (i.e., ampicillin-sulbactam or piperacillin-tazobactam); a carbapenem (i.e., imipenem-cilastatin, meropenem, or doripenem); or tigecycline is an alternative choice.

Osteomyelitis

Treatment of osteomyelitis of the jaws is complicated by the presence of teeth and persistent exposure to the oral environment. Antibiotic therapy needs to be prolonged, often for weeks to months. Clindamycin and moxifloxacin have excellent bioavailability in bone tissue, and either is recommended. Adjuvant therapy with hyperbaric oxygen may prove beneficial in hastening the healing process, but its overall efficacy remains controversial. In one study of 33 patients with early chronic osteomyelitis of the jaw, 79% were free of symptoms 10 to 34 months after hyperbaric therapy combined with surgical débridement.[104] Osteoradionecrosis (ORN) refers to osteomyelitis occurring after dental extraction, usually from the mandible that was included in a radiation port. ORN responds poorly to antimicrobial therapy alone. Surgical removal of necrotic bone, closure of the mucosa over the socket, and prolonged antibiotic therapy may be required. In other forms of osteomyelitis surgical management, including sequestrectomy, saucerization, decortication, and closed-wound suction irrigation is also occasionally necessary. In a few advanced cases the entire segment of the infected jaw may have to be resected. For vertebral osteomyelitis with or without extension into the prevertebral space, the most common causative organism is *S. aureus,* followed by facultative gram-negative bacilli in at-risk patient populations such as those with a history of IV drug abuse. Coverage with nafcillin or vancomycin (if MRSA is suspected) plus ciprofloxacin is recommended.

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C Pleuropulmonary and Bronchial Infections

65 Acute Bronchitis

Edward E. Walsh

SHORT VIEW SUMMARY

Definition
- Acute bronchitis is a self-limited syndrome characterized by acute cough with or without sputum but without signs of pneumonia.

Epidemiology
- Acute bronchitis occurs year-round and is caused by a large number of respiratory pathogens according to the typical epidemiology of each pathogen.

Microbiology
- Acute bronchitis is primarily caused by viral infections. Most common are rhinovirus, influenza viruses, respiratory syncytial virus, metapneumovirus, coronaviruses, and adenovirus. Fewer than 10% of cases are caused by *Mycoplasma pneumoniae, Chlamydia pneumoniae,* and *Bordetella pertussis.*

Diagnosis
- Diagnosis is primarily made by the clinical presentation in the absence of signs and symptoms of pneumonia.

Therapy
- Therapy is symptomatic because antibiotics are uncommonly required and unnecessary in the majority of cases.

Prevention
- Prevention is directed at specific pathogens when possible (e.g., influenza and pertussis vaccination).

Acute bronchitis refers to a clinical syndrome distinguished by a relatively brief, self-limited inflammatory process of large and midsized airways not associated with evidence of pneumonia on chest radiography. It is characterized by a dry or productive cough of less than 3 weeks' duration, is most prevalent in winter, and is primarily caused by viruses. This definition does not pertain to acute exacerbations of chronic bronchitis, a clinical syndrome also frequently associated with viral infection but in which bacteria play a more important role (see Chapter 67). It should also be distinguished from acute *bronchiolitis,* a clinical syndrome involving small airways most closely linked to respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) infection in infants (see Chapters 158 and 159).

Acute cough is one of the most common reasons for physician visits in all age groups.[1] Incidence data may be imprecise because symptoms overlap considerably with those of the common cold, pneumonia, and asthma. Estimates from the National Health Interview Survey of US households have found that overall, 5% of persons annually report physician-diagnosed acute bronchitis, with highest rates during the winter months and in those younger than age 5 years.[2] Studies from the United Kingdom have reported similar rates of 54 cases per 1000 persons, ranging from 36 per 1000 in younger men to 225 per 1000 in those older than 85 years of age.[3,4]

MICROBIAL ETIOLOGY

Despite failing to uncover a specific pathogen in most case series, acute bronchitis is believed to be most commonly caused by a wide range of viruses (Table 65.1). Approximately 10% or less of cases are attributed to bacterial pathogens, principally *Mycoplasma pneumoniae, Chlamydia pneumoniae,* and *Bordetella pertussis.*[5] However, the recent increase in pertussis cases among adolescents and adults should be considered when evaluating illnesses. The relative proportion of cases caused by different pathogens varies according to age and the season studied but, importantly, is also influenced by the diagnostic methods used. Molecular tests, rather than standard culture, identify a greater number and wider range of viral pathogens, especially in adults, in whom many illnesses represent reinfection with common pediatric viruses (RSV, hMPV, parainfluenza viruses) often associated with low virus shedding in adults.[6,7] Influenza A and B viruses are most closely associated with winter outbreaks of acute bronchitis in both children and adults because of a high relative incidence of infection and the efficiency of influenza virus to infect and damage bronchiolar epithelial cells.[8,9] Although cough associated with most coronavirus and rhinovirus infections is commonly caused by postnasal congestion, these agents—including recently identified strains of human enterovirus D68, human rhinovirus C, and coronavirus—can infect the lower airway, causing acute bronchitis.[10,11,12,13,14] The higher temperature of lower airways was considered inhospitable to rhinovirus replication; however, recent evidence has convincingly documented virus presence in bronchiolar epithelial cells, although cytopathology is less than that with influenza virus infection.[14] Although found less frequently than other viruses in the general population, adenoviruses, including adenovirus types 4, 7, and 14, are important causes of bronchitis in military recruits.[15] Enteroviruses, primarily echovirus and coxsackievirus but also the newly emerged D68 strain, can cause acute bronchitis.[10] Simultaneous infection with two or more viruses occurs in 10% to 30% of cases, especially in small children, as is frequently the case with bocavirus infection. Worldwide, measles virus remains an important respiratory tract pathogen causing cough during the early prodromal phase; especially vulnerable are malnourished children in developing countries lacking resources for vaccination.[16] Although herpes simplex virus is commonly identified in respiratory secretions of patients with respiratory illness and is generally considered to be clinically insignificant, it has been associated on occasion with acute hemorrhagic tracheobronchitis in normal persons and has been described in critically ill intubated adults.[17] Human papillomavirus types 6 and 11 can produce symptoms of hoarseness, stridor, and chronic cough.[18]

TABLE 65.1 Viral and Bacterial Causes of Acute Bronchitis

PATHOGEN	SEASONALITY	COMMENTS
Influenza viruses	Winter	Local epidemics last 6–8 wk, during which clinical illness of cough and fever has high predictive value; laboratory diagnosis readily available; early neuraminidase inhibitor therapy effective
Rhinoviruses	Fall and spring	Most frequent cause of common cold syndrome; immunity is serotype specific
Coronaviruses	Winter to spring	Cause common cold syndrome; newer strains are difficult to culture and require RT-PCR for diagnosis
Adenoviruses	Year-round, winter epidemics	High attack rates in closed populations such as persons living in military barracks or college dormitories; serotype-specific immunity
Respiratory syncytial virus (RSV)	Late fall to early spring	Attack rates approach 75% in neonates, 3%–5% in adults; associated with wheezing in all age groups; rapid antigen test accurate in children but requires culture or RT-PCR to diagnose in adults
Human metapneumovirus (hMPV)	Winter to early spring	Associated with wheezing in adults and in infants; difficult to isolate in tissue culture and often requires RT-PCR
Parainfluenza viruses	Fall to winter	Similar to RSV and hMPV, parainfluenza viruses are primarily pediatric pathogens but can cause severe acute disease in some adults
Measles virus	Year-round	Can cause respiratory disease in malnourished children; illness causes transient immune suppression
Mycoplasma pneumoniae	Year-round, fall outbreaks	Long incubation period (10–21 days) results in staggered epidemic pattern in families; nonproductive persistent cough typical; diagnosed by IgM serology; treated with macrolide, quinolone, or tetracycline antibiotics
Chlamydia pneumoniae	Year-round	Associated with sinusitis; diagnosis by RT-PCR not readily available
Bordetella pertussis	Year-round	Severe illness in nonimmunized children; illness milder in partially immune adults but can be associated with prolonged cough; adults often reservoir for epidemics; early therapy with antibiotics can reduce spread

IgM, Immunoglobulin M; *RT-PCR,* reverse transcriptase–polymerase chain reaction.

Mycoplasma pneumoniae, C. pneumoniae, and *B. pertussis* are the bacteria most associated with acute bronchitis, implicated in perhaps 10% of cases.[3,5] Cough caused by these agents, especially *B. pertussis,* may persist in some cases for several months. Recently, *Bordetella holmesii* has been recognized as a cause of pertussis syndrome (see Chapter 230). In an acellular pertussis vaccine trial in adults, the incidence of pertussis was 0.7% in the placebo group.[19] However, the number of cases in adults has been increasing in recent years, attributed to incomplete vaccine uptake as well as failure of the vaccine to induce durable immunity.[20] This has resulted in a recommendation to provide a vaccine booster dose to adults and pregnant women (see Chapter 321). *Bordetella bronchiseptica,* a canine pathogen, can occasionally cause disease in humans, especially those who are immunocompromised.[21] Although *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis* can each be isolated from sputum in some patients with acute bronchitis, their pathogenic role in the illness is unclear since antibiotic therapy directed at these pathogens has not been shown to alter the clinical course. The recent presence of normal oropharynx colonizers, such as these pathogens, in the lower airways of normal healthy persons by use of bacterial 16S ribosomal DNA sequencing has altered the long-held concept that the lower airway is sterile.[22] Although many viruses enhance bacterial adherence to respiratory epithelial cells in vitro, the actual incidence of postviral bacterial infection in clinical situations has not been well defined.

PATHOGENESIS

The pathogenesis of acute bronchitis is the result of a combination of direct cytopathology of the pathogen and host immune response. Because cell tropism and pathogenic mechanisms vary for each of the viruses, it is not surprising that the location and extent of cytopathology in the airways also vary. Histopathologic changes in the airways during infection are best characterized for influenza virus.[23,24,25] In a recent autopsy series of 47 children with fatal influenza, submucosal congestion, hemorrhage, mononuclear cell infiltration, and epithelial necrosis were noted in 50% of cases.[24] Similar findings have been reported in adults, along with occlusion of airways by desquamated necrotic debris.[25] Respiratory syncytial virus has also been demonstrated to infect terminal bronchiolar lining cells with extensive debris occluding the lumen in infants, but similar data are lacking in adults.[26] Rhinovirus, which is primarily associated with upper respiratory common cold symptoms, produces patchy inflammation of bronchial epithelial cells up to 15 days after experimental challenge in adults.[14]

There is ample evidence that immune responses contribute significantly to disease pathogenesis in acute bronchitis. The innate immune response, characterized by virus-induced release of proinflammatory cytokines and chemokines by respiratory epithelial cells and immune cells, contributes to systemic and local symptoms. Early after infection with influenza virus or RSV, types I and III interferons, tumor necrosis factor, and interleukin-6 and interleukin-8 can be detected.[27] Interleukin-8 is a chemotactic factor for neutrophils, one of the earliest cells to migrate to the nasal mucosa. In experimental influenza virus infection, symptoms correlate temporally with the kinetics of cytokine secretion.[28] Cytokine induction by rhinovirus may also explain the important role that this virus plays in asthma episodes.[29] The immune response is thought likely to be responsible for the prolonged airway hyperreactivity that can be demonstrated for up to 6 weeks after influenza virus or RSV infection in normal adults.[30] Among the bacterial causes of acute bronchitis, *B. pertussis* is unique in that its expression of toxins may play a role in clinical symptoms, including the prolonged characteristic cough. However, a defined and specific pertussis "cough" toxin has not yet been identified.

CLINICAL MANIFESTATIONS

In most children and adults, acute bronchitis begins with signs and symptoms typical of the common cold syndrome. Nasal congestion, rhinitis, sore throat, malaise, and low-grade fever typical of the common respiratory viral pathogens are noted first, followed shortly by the onset of cough, which becomes the dominant sign in acute bronchitis. In mild cases the illness lasts only 7 to 10 days, whereas in others, cough may persist for up to 3 weeks or longer. Wheezing is not uncommon with acute bronchitis; in one study from the Netherlands, 37% of patients with acute bronchitis ultimately were diagnosed with asthma.[31] Disease severity and dominance of various symptoms may vary according to the specific pathogen. Fever is more common and higher with influenza virus or adenovirus infection, compared with rhinovirus or RSV infection at all ages. In contrast, RSV- or hMPV-infected persons are more likely to wheeze than influenza virus–infected patients.[7,32]

Various host factors such as age, underlying medical conditions, immune status, and environmental factors such as secondhand smoke exposure can influence clinical presentation and illness severity. Older patients and those with underlying cardiopulmonary conditions infected with influenza virus, RSV, or hMPV remain ill for an average of 16 to 17 days, in contrast to younger persons whose illness lasts an average

of 7 to 10 days.[7,32,33] In *M. pneumoniae* infection, cough can be particularly persistent and irritating, often with minimal phlegm, causing severe chest discomfort.

DIAGNOSIS

Acute bronchitis should be suspected in any person with an acute respiratory tract illness in which cough is the dominant complaint. Patient evaluation includes identification of underlying chronic obstructive pulmonary disease or asthma, and taking note of the season and the presence of influenza or other circulating viruses in the community. During community outbreaks of influenza, the presence of an acute illness with fever and cough can have a predictive value of 79% in young healthy persons.[34] Among hospitalized older persons with acute respiratory tract illness, similar clinical parameters increase the odds of influenza approximately threefold.[35] Exposure to environmental irritants should be sought, especially in the absence of typical upper respiratory tract symptoms. Travel history or exposure to ill family members or close contacts may provide useful information about the incubation period and thus the etiology. *Mycoplasma pneumoniae,* with an incubation period of 7 to 21 days, will progress slowly through the family in contrast to influenza, with its 2- to 4-day incubation period and close clustering of illnesses. Recent vaccination does not eliminate influenza from consideration, especially in older persons in whom protective efficacy is poor.[33,36] Prolonged cough longer than 4 weeks, with or without paroxysms or vomiting, should suggest *B. pertussis,* even among vaccinated adolescents and adults, because immunity is not durable.[19,20]

Physical examination should be attentive to signs of pneumonia, because a primary goal of evaluation is to discriminate acute bronchitis from pneumonia. A concern for clinicians is the occurrence of bacterial infection that can follow or occur concurrently with viral infection. This is best exemplified by the severe bacterial pneumonias that followed influenza infection during the 1918 H1N1 and 1957 H2N2 pandemics. Similarly, one report described bacteremic *S. pneumoniae* pneumonia in long-term care patients after antecedent parainfluenza virus infections.[37] Despite concerns about postviral bacterial pneumonia, several large studies have indicated that only approximately 7% of patients diagnosed with acute bronchitis will have radiographic evidence of pneumonia.[38] Combinations of signs and symptoms or overall clinical judgment is imprecise, with a sensitivity of only 23%.[39,40] One study has found that in the absence of any vital sign abnormality, radiographic evaluation could be safely omitted.[39] Standard laboratory tests, such as white blood cell count or inflammatory markers such as serum C-reactive protein, do not reliably discriminate viral from bacterial infection.[40] However, a serum procalcitonin level below 0.1 ng/mL may be a more reliable indicator of patients with a variety of acute respiratory syndromes, including acute bronchitis, that do not require antibiotic therapy.[41,42]

Ultimately, etiologic diagnosis requires laboratory confirmation. Identification of viral pathogens is most commonly performed using multiplex reverse transcriptase–polymerase chain reaction (RT-PCR) assays, especially useful in adults who may shed low levels of many of the most prevalent viruses (RSV, hMPV, coronaviruses, parainfluenza viruses).[6,7,33] Diagnosis of *M. pneumoniae* rests on demonstrating pathogen-specific immunoglobulin M in serum or with RT-PCR assay. *Bordetella pertussis* diagnosis can be made by serology, RT-PCR assay, or culture. *Chlamydia pneumoniae* is difficult to diagnose and requires RT-PCR assay or serology.

THERAPY

Therapy for patients with acute bronchitis is generally symptomatic, directed at relief of troublesome upper respiratory symptoms, cough, and wheezing. As noted, acute bronchitis and asthma can present with similar manifestations, and therapy directed toward bronchospasm may be required. Several approaches to controlling acute cough have included narcotic cough suppressants, expectorants, antihistamines, decongestants, and β_2-agonists.[43,44] Reviews of randomized controlled trials have not clearly concluded that they are beneficial. In a double-blind, placebo-controlled trial in experimental rhinovirus infection, the combination of ibuprofen (400 mg) plus chlorpheniramine (12 mg), administered every 12 hours for 4.5 days, reduced cough significantly, although the effect was optimal when combined with an intranasal antiviral compound (interferon-α).[45]

After their introduction after 1940, antibacterial agents were rapidly embraced for treatment of acute bronchitis; currently, 60% to 80% of patients receive antibiotics for this diagnosis.[46,47] However, empirical antibacterial therapy for acute bronchitis without regard for demonstrated pathogen-specific therapy has repeatedly been shown to provide no benefit for most patients.[48,49] A recently published meta-analysis of 17 randomized controlled trials in patients without chronic respiratory disease, but who had acute bronchitis, concluded that treatment with antibiotics failed to show a significant clinical benefit.[48] However, acute bronchitis remains one of the five most frequently cited rationales for excessive antibiotic use in outpatients. Acute bronchitis and other acute respiratory syndromes (otitis media, sinusitis, pharyngitis, upper respiratory tract infections) account for approximately 75% of ambulatory prescriptions in the United States.[1] Current guidelines, endorsed by a number of national societies—including the American College of Physicians, the Centers for Disease Control and Prevention, and the Infectious Diseases Society of America—do not recommend the routine use of antibiotics for uncomplicated acute bronchitis in otherwise normal persons.[50] Various strategies to reduce antibiotic use, including patient and physician education or delayed prescription filling, can reduce antibiotic use by approximately 50%.[51,52] Recent randomized controlled studies using levels of serum procalcitonin to guide therapy in acute respiratory infections, including acute bronchitis, have also demonstrated that antibiotic use can be partly reduced without adversely affecting outcome.[41,42] If a specific viral diagnosis can be confirmed using a rapid RT-PCR test, perhaps physicians would be more willing to forgo antibiotic treatment. Even patients with documented *M. pneumoniae* or *C. pneumoniae* infection may not benefit from specific therapy with macrolides or quinolones in the absence of pneumonia. In adults and adolescents, early therapy for infection with *B. pertussis* with macrolides or tetracyclines is indicated to prevent transmission, although the clinical findings early in infection are indistinguishable from viral causes of bronchitis.

It may be useful to seek a specific diagnosis of influenza during community outbreaks, because specific treatment with neuraminidase inhibitors active against influenza A and B strains can reduce the duration of illness when administered early in its course. In one study of adults with influenza, subsequent antibacterial use was 11% in zanamivir-treated patients compared with 17% in placebo-treated controls.[53] However, bacteriologic and radiologic confirmation of bacterial pneumonia was not sought in this study.

PREVENTION

Preventive measures to reduce transmission of agents known to cause acute bronchitis should be limited to standard respiratory and contact precautions when a specific etiology is unknown. Immunization against specific pathogens, such as influenza and pertussis, is of benefit and can reduce the incidence of symptomatic infection with these agents (see Chapter 321).

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The complete reference list is available online at Expert Consult.

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66 Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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SHORT VIEW SUMMARY

Definitions
- Chronic obstructive pulmonary disease (COPD) is a chronic lung disease with irreversible airflow limitation with reduced forced expiratory volume in 1 second (FEV_1) and FEV_1/FVC ratio.
- An acute exacerbation is an acute increase of the patient's dyspnea, sputum volume, or sputum purulence. Each symptom contributes to the severity of the COPD exacerbation.

Epidemiology
- There is an increased risk for an acute exacerbation of COPD in winter months.
- Risk factors include cigarette smoking, environmental particulate matter, genetic predisposition, influenza, and other circulating viral infections that affect the respiratory tract.

Pathogenesis
- Intermittent progressive airway inflammation, remodeling, and loss of lung function occur.
- Ciliary dysfunction, excess mucous production, and impaired phagocytosis leading to bacterial colonization also occur.

Microbiology
- Airways of stable COPD patients are frequently colonized with *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*; respiratory syncytial virus is the most frequent colonizing virus.
- Microaspiration in stable COPD introduces oral anaerobes such as *Prevotella* spp. and *Veillonella* spp. into the lower airway.
- During acute exacerbations, bacteria, viruses, or both may be isolated, with more gram-negative rods found with worsening lung function. "Atypical" bacteria are not frequently isolated. Acquisition of a new pathogen is associated with exacerbation.

Diagnosis
- Change in respiratory symptoms are the cornerstone of diagnosis, but procalcitonin may be useful in determining the need for antibiotics in acute exacerbations.

Treatment
- Increased bronchodilators (including long-acting β-agonists and long-acting muscarinic antagonists) are used for mild exacerbations, without antibiotics.
- Early empirical antibiotics and oral or intravenous steroids are administered for moderate-to-severe exacerbations.

Prevention
- Exposure to particular matter should be avoided, and patients should stop smoking.
- Influenza, pneumococcal, and pertussis vaccines are used for prevention.
- Prophylactic daily azithromycin may be considered in patients with advanced disease with a history of exacerbation and no cardiac risk factors.

Chronic obstructive pulmonary disease (COPD) is characterized by airway inflammation and parenchymal destruction. Airflow obstruction produced by structural changes in the lung predisposes to acute declines in patient well-being associated with increasing dyspnea and sputum production; these are the essence of the diagnosis of acute exacerbation of chronic obstructive pulmonary disease (AECOPD). Smoking cessation and irritant avoidance are essential to reduce the risk of exacerbation. Currently, prevention of exacerbation with inhaled steroids and combination long-acting bronchodilators has become the cornerstone of management of patients at high risk for AECOPD. Once AECOPD is established, its management includes a combination of antibiotics and steroids.

EPIDEMIOLOGY

COPD, including emphysema and chronic bronchitis, has been the third leading cause of death since 2008 in the United States.[1] It will become the third leading cause of death in the world by 2020.[2,3] By 2030, 10% of the world's population will be expected to develop COPD. COPD prevalence has increased owing to increased longevity and long-term exposure to respiratory irritants such as tobacco smoke and, to a lesser extent, inhaled particulate matter (PM).[4] Smoking tobacco is the largest risk factor; 50% of smokers will develop COPD.[5–8] Consequently, COPD is an important driver of increased health care utilization, producing significant social and economic costs.[9–11] COPD affects predominately older individuals, with study cohorts having mean ages older than 60 years; however, presentation in young adulthood occurs in susceptible individuals.[12,13,14]

COPD is caused by multiple factors, including environmental exposures, infections, inflammation, and genetic predisposition. Newer conceptualization of COPD characterizes it as a syndrome that includes multiple different processes with distinct pathophysiologic derangements.[15–17] Tobacco smoking is the largest environmental risk factor in the United States. In other countries, occupational dust exposures, outdoor air pollution, and poor indoor air quality from burning biomass fuels also are major COPD risk factors.[18] Among immunocompetent adults, increased airway bacteria produce airway inflammation and accelerate airway obstruction.[19] Immunosuppression is an independent risk factor for COPD. Immunoglobulin A (IgA)-deficient individuals have repeated lower respiratory tract infections during childhood and poor adult lung function.[20] Human immunodeficiency virus (HIV) infection promotes chronic pulmonary inflammation and increased pulmonary matrix metalloprotease expression leading to smoking-related emphysema.[21,22] Even after effective antiretroviral therapy, patients with acquired immunodeficiency syndrome (AIDS) have an increased frequency of pulmonary infection and accelerated lung function decline.[23,24] Genetic susceptibility is another risk factor for development of COPD. Mutation of the α_1-antitrypsin gene leads to low serum antiprotease activity, causing a much higher risk of COPD in smokers and workers exposed to PM.[25] Homozygous α_1-antitrypsin deficiency (PI*ZZ) occurs in 1% to 4.5% of COPD patients, and the heterozygous form (PI*MZ), with less severe deficiency, occurs in 17.8% of COPD patients.[26] Ethnic origin is an important risk factor for COPD, raising the possibility of differential group-level genetic susceptibility

to lung injury.[27] For most individuals, predisposition to abnormal lung function is polygenic; more than 20 risk genes have been identified.[28,29] Poor socioeconomic status, chronic asthma, intrauterine growth retardation, poor nourishment, and history of pulmonary tuberculosis are other risk factors for COPD.[27,30–32]

Acute Exacerbation of Chronic Obstructive Pulmonary Disease

AECOPD produces significant morbidity and mortality. Frequent exacerbators may be defined as COPD patients who have two or more exacerbations per year and likely constitute a distinct subgroup within COPD with poor outcome.[33,34] Risk factors include viral and bacterial infections, change in environmental conditions such as smog, gastroesophageal reflux, lack of compliance with maintenance treatment, and severity of baseline disease.[33] Important to note, the best predictor for exacerbations is a past history of exacerbations.[35] AECOPD occurs approximately two times more frequently in winter than in summer, both in northern and southern latitudes. AECOPD is responsible for the greatest proportion of health care–related costs associated with COPD.[9–11] This chapter focuses on the impact of AECOPD on disease course, infectious etiologies of AECOPD, treatment of AECOPD, and AECOPD prevention, the central goal of current management strategies.

CLINICAL PRESENTATION

AECOPD manifests with progressive shortness of breath, cough, sputum production, reduced energy, and exercise limitation. These symptoms are associated with accelerated decline in lung function, which may continue despite smoking cessation. The dyspnea usually starts during exercise but can occur with minimal exertion or at rest as disease progresses. Cough and sputum production are usually intermittent and more pronounced in the morning. Other chronic pulmonary diseases with similar clinical presentation and acute exacerbations should be differentiated from COPD because treatment differs. Examples of these include asthma, cystic fibrosis, bronchiectasis, diffuse panbronchiolitis, and obliterative bronchiolitis. Worsening symptoms, increased sputum volume, and transition of sputum color from clear to green or yellow suggest AECOPD, which more commonly occurs during winter months.[36] The main differential diagnosis of exacerbations in patients with COPD includes pneumonia and congestive heart failure, both of which are common comorbidities in these patients.[5,8]

DIAGNOSIS

COPD is characterized by progressive airflow obstruction defined by reduction in forced expiratory volume in 1 second (FEV_1) and a postbronchodilator ratio of FEV_1/forced vital capacity (FVC) less than 70% on pulmonary function tests (PFTs). A highly affected subgroup of patients have characteristics of both COPD and asthma (asthma-COPD overlap) with partial bronchodilator reversibility of airflow obstruction.[37] Severity of stable COPD is based on pulmonary function (Table 66.1).[5,8]

TABLE 66.1 Spirometric General Classification of Chronic Obstructive Pulmonary Disease (COPD)

SEVERITY	FEV_1/FVC	FEV_1 % PREDICTED
GOLD 0: At risk Patients who: • smoke or have exposure to pollutants • have cough, sputum, or dyspnea • have family history of respiratory disease	>0.7	≥80
GOLD 1: Mild COPD	≤0.7	≥80
GOLD 2: Moderate COPD	≤0.7	50–79
GOLD 3: Severe COPD	≤0.7	30–49
GOLD 4: Very severe COPD	≤0.7	<30

FEV_1, Forced expiratory volume in 1 second; *FVC*, forced vital capacity; *GOLD*, Global Initiative for Chronic Obstructive Lung Disease criteria.

Acute Exacerbation of Chronic Obstructive Pulmonary Disease

Although there is no universal agreement on how to define or diagnose AECOPD, it is commonly defined as an acute event with worsening respiratory symptoms beyond normal day-to-day variation. The definition often requires increased rescue β-agonist inhaler use or addition of a long-acting muscarinic antagonist (LAMA) to control symptoms.[38,39] The differential diagnosis must include acute coronary syndrome, congestive heart failure, pulmonary embolism, and pneumonia.[8] One widely used scale to diagnose the presence and severity of AECOPD requires patients to have at least one of the following clinical presentations: upper respiratory infection symptoms within the prior 7 days, increased wheezing, fever without another identified cause, or an increase in heart rate or respiratory rate >20% from baseline. This scale then categorizes patients into three groups based on whether they have worsening dyspnea, increase in sputum purulence, increase in sputum volume, or a combination of these. A severe exacerbation meets all three criteria, a moderate exacerbation meets two, and a mild exacerbation meets only one criterion.[40] Although clinical presentation is the most important component in establishing the diagnosis of AECOPD, procalcitonin is a biomarker that may be useful in determining the need for antibiotics.[41] No biomarkers of systemic inflammation reproducibly predict future exacerbations.[42]

Risk factors associated with COPD exacerbations include having two or more COPD exacerbations in the previous year, reduced FEV_1, smoking, and nonadherence with O_2 therapy.[43,44] The risk of severe exacerbation depends on each patient's medical history, including baseline FEV_1, number of prior exacerbations, prior need for mechanical ventilation, and comorbidities. Although the vast majority of AECOPD can be managed in the outpatient setting, the presence of severe exacerbation should prompt consideration of hospital admission.[5,8] The clinical signs of risk for respiratory failure are the most important measure of the current exacerbation's severity. Tachypnea (especially with a respiratory rate above 25), tachycardia, inability to speak full sentences, and fatigue are indications for hospitalization. Oxygen saturation above 90% can be misleading because hypoxemia is frequently a late event in the progression to respiratory failure. In an acutely symptomatic patient, arterial blood gas measurement is more useful than oximetry because only the former can enable diagnosis of hypoventilation with hypercapnia. Use of accessory muscles with paradoxical breathing characterized by inward motion of the abdomen during inspiration indicates diaphragmatic fatigue and impending respiratory failure. Abdominal paradoxical breathing, progressive hypercapnia, or deteriorating mental status usually indicates the need for ventilatory support in an intensive care unit (ICU) with noninvasive or invasive positive pressure ventilation.[5,8]

Additional diagnostic tests include chest radiography to identify pulmonary infiltrates and electrocardiography to assess for cardiac ischemia and arrhythmias, particularly paroxysmal atrial tachycardia. The basic metabolic panel is helpful to assess severity of AECOPD. Elevated bicarbonate is a sign of chronic hypercapnia. Increased anion gap is a sign of anaerobic metabolism of the respiratory muscles, sepsis syndrome, or both. Hyponatremia sometimes occurs as a result of the syndrome of inappropriate antidiuretic hormone secretion. Hyperglycemia is a response to stress or systemic steroids, and renal insufficiency is a manifestation of reduced cardiac output in end-stage pulmonary hypertension. The presence of these derangements may warrant hospitalization.[5,8]

Radiology

Although chest radiography is an insensitive test to diagnose AECOPD, it is the usual first step in the evaluation of patients with progressive dyspnea and cough. It is essential for evaluation of alternative diagnoses such as pneumonia, congestive heart failure, lung cancer, or pulmonary fibrosis. However, computed tomography (CT) is more sensitive for defining alternate diagnoses, and quantitative CT allows estimation of the risk for exacerbation frequency and allows determination of indices of disease impact.[45] Pulmonary emboli can mimic AECOPD, and if they are suspected, a helical CT scan with contrast can reveal this alternate diagnosis.

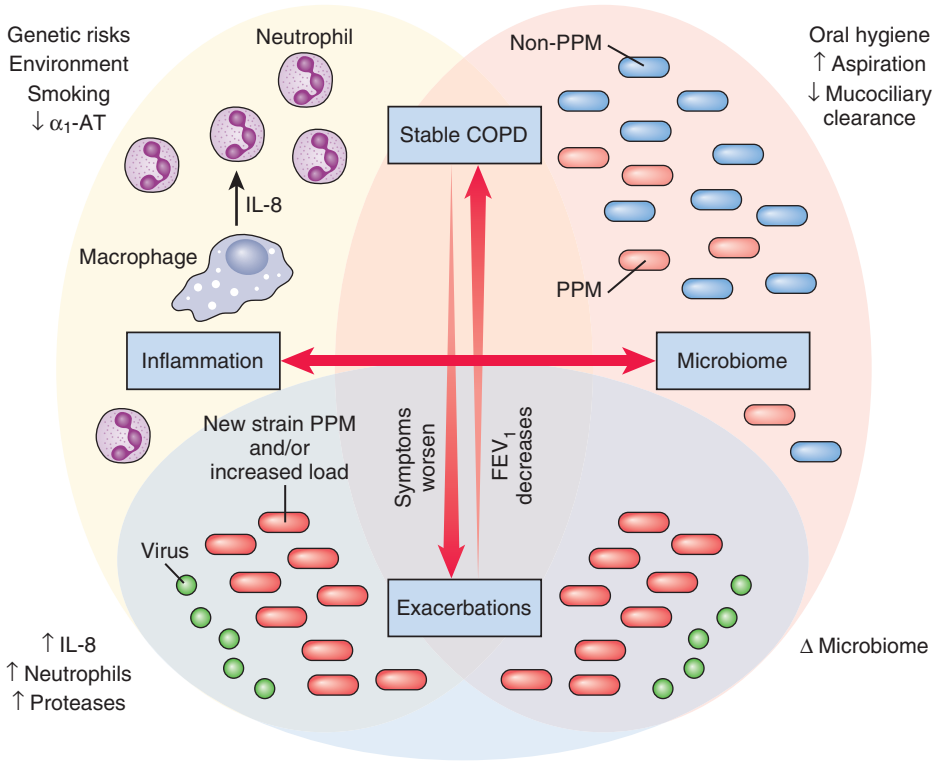

FIG. 66.1 Chronic obstructive pulmonary disease *(COPD)* pathophysiology. α_1-*AT,* α_1-Antitrypsin; *FEV_1,* forced expiratory volume in 1 second; *IL-8,* interleukin-8; *PPM,* potentially pathogenic microorganism.

PATHOPHYSIOLOGY

Impaired lung function in COPD is caused by destruction and remodeling of large and small airways due to chronic inflammation caused by complex interactions among the inhalants (dust or smoke), the lung microbiome, and the immune response in airway mucosa. Both inflammatory changes and lung function deterioration become more prominent with each exacerbation (Fig. 66.1). Early pathologic condition is produced by inflammation in bronchioles less than 2 mm in diameter followed by parenchymal remodeling.[46,47] In early stages, the central airway walls are infiltrated with $CD8^+$ lymphocytes producing bronchial wall thickening evidenced on chest CT.[48] Apoptosis and necrosis of epithelial and endothelial cells are also present, as are activated $CD4^+$ T cells.[49,50] As disease progresses, neutrophils become prominent and release neutrophil elastase, leading to parenchymal destruction.[51,52] Persistent airway injury also produces squamous metaplasia with loss of cilia function in affected bronchial segments. Disease of both the large and small airways contributes to airflow obstruction and ventilation heterogeneity.[53] Progressive disease is marked by increased airway mucin production.[54] Pulmonary hypertension due to loss of the pulmonary capillary bed can also develop in COPD. Chronic hypoxia produces vasoconstriction, which leads to fixed structural changes that worsen pulmonary hypertension.[55] As disease progresses, frequent exacerbations of COPD further contribute to increased lung inflammation and persistent loss of lung function.[56,57,58–60] The biology of exacerbations in COPD is heterogeneous and involves varying degrees of inflammation and microbial pathogenesis.[61]

Mucosal Inflammation

COPD patients have a 20-fold increase in alveolar macrophages (AMs) in alveoli, bronchioles, and small airways.[62,63] Because AMs are a major source of inflammatory cytokines and growth factors, recruitment of inflammatory cells into the lung accelerates the vicious inflammatory cycle produced by microaspiration. In spite of increased numbers, AMs have impaired phagocytosis, reducing their ability to clear bacteria from the lower airway, thereby causing further inflammation and oxidative stress.[64–67] Compared with smokers without COPD, those with COPD have higher levels of neutrophils, eosinophils, macrophages, B lymphocytes, and $CD4^+$ and $CD8^+$ T cells in biopsies of peripheral airways, highlighting the role of innate and adaptive immune responses in disease progression.[68] A proinflammatory subset of $CD4^+$ T cells, known as Th17 cells, are frequently found at the mucosal interface and are implicated in the pathogenesis of chronic inflammatory conditions, including COPD and asthma. Th17 cells secrete interleukin (IL)-17 and IL-22, which synergize on tissue parenchyma to elicit production of inflammatory cytokines and chemokines, often leading to the recruitment of neutrophils and other inflammatory cells. Levels of IL-17A and IL-22 are increased in the sputum of individuals with COPD,[69–71] and Th17 cells have been identified in bronchial biopsy specimens of patients with COPD.[72] IL-17 increases the expression of mucin-encoding genes (*MUC5AC* and *MUC5B*) in human airway epithelial cells.[73] A study has demonstrated that numbers of IL-17A- and IL-22-producing cells in bronchial submucosa are significantly increased in patients with stable COPD.[72] Neutrophils are then recruited and activated by nonresolving pulmonary inflammation, particularly during exacerbations. Neutrophil elastase and metalloproteinases lead to lung parenchyma destruction. Neutrophil elastase is also a potent mucous secretagogue leading to mucous gland hyperplasia.[74] Because neutrophils have a short tissue lifespan, the airway neutrophilia observed in COPD requires continuous neutrophil recruitment.[75] Smoking also impairs neutrophil phagocytic function.[76] Collectins, pentraxins, and complement also are deficient,[77] further impairing mucosal immunity and predisposing to lower respiratory tract infections. More recently, there has been increasing attention to the role of eosinophils in COPD. Eosinophilic airway inflammation occurs in approximately 15% to 40% of individuals with COPD.[78] Increased eosinophils are a risk factor for exacerbation after deescalation of inhaled corticosteroid therapy.[79] During exacerbations, there is an increase in the number of eosinophils in sputum.[80] Elevated eosinophils are associated with patients' responsiveness to corticosteroids during both acute exacerbations and stable disease.[79,81–83] High eosinophils may define a distinct host Th2-predominant endotype with asthma overlap that is more responsive to corticosteroids.[84] Investigations that have excluded patients with a history of asthma have shown that high blood

eosinophils do not predict steroid responsiveness.[85] The use of eosinophil levels in directing therapy requires further investigation.[86]

Pulmonary inflammation continues after smoking cessation, in part because of permanent structural damage, or proinflammatory epigenetic changes.[87,88] Bacterial colonization in the lower airways is also an important determinant of the degree of airway and systemic inflammation in stable COPD.[89] Inflammatory mediators "spill over," producing systemic inflammation with elevated levels of C-reactive protein (CRP) or fibrinogen or elevated leukocyte count, or a combination of these factors. Elevated CRP is most strongly associated with ischemic heart disease in smokers. Systemic comorbidities such as vascular disease are major risk factors for mortality during COPD exacerbations.[90,91] Systemic inflammation caused by COPD is also hypothesized to be associated with anemia, osteoporosis, depression, and metabolic syndrome.[92–94]

Microbes in Stable Chronic Obstructive Pulmonary Disease

Increased bacterial colonization of the lower airways in COPD occurs as a result of chronic microaspiration, impaired clearance of bacteria, and frequent COPD exacerbations.[95,96] Patients with COPD frequently have microaspiration owing to gastroesophageal reflux caused by incoordination between breathing and swallowing.[97–99] Impaired mucociliary clearance in smokers reduces the ability to clear oral microbes from the lower airways, exacerbating inflammation. This leads to chronic cough with progressive incoordination of breathing with swallowing. Inhaled medications may also carry oral bacteria into the lower airway. This vicious cycle could explain the association of poor oral health and increased airway bacterial load, COPD exacerbations, and reduced lung function.[100–104]

Viruses and bacteria in the lower airway perpetuate the inflammation in COPD, causing damage through a number of mechanisms.[19,105,106] They may be ciliotoxic, invade epithelial cells and cause apoptosis, increase mucin production, and/or degrade humoral immunity via secretion of IgA proteases.[107,108] Bacterial molecules such as endotoxins, membrane lipoproteins, peptidoglycan fragments, and lipoteichoic acid activate the innate immune response, exacerbating inflammation.[109] Patients with COPD whose airways are heavily colonized with bacteria have higher concentrations of inflammatory cytokines and neutrophils in respiratory secretions.[106] High bacterial burden in the lower airway is associated with accelerated FEV_1 decline, more comorbidity, more exacerbations, and worse symptoms during exacerbations.[23,110–113]

In stable COPD, the rates of positive routine bacterial cultures of sputum vary between 22% and 83%.[114–116] However, interpreting sputum culture is difficult because of upper airway contamination, which reduces specificity, and failure to grow fastidious bacteria from the complex lower airway microbiome, which reduces sensitivity. In stable COPD, non–potential pathogenic microorganisms (non-PPMs) are isolated much more frequently than potential pathogenic microorganisms (PPMs).[114] Non-PPMs are usually oropharyngeal microbes such as *Corynebacterium* spp., *Neisseria* spp., *Enterococcus* spp., coagulase-negative staphylococci, viridans-group streptococci, and fungi such as *Candida* spp.[114] The most commonly isolated PPMs are *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis.*[58,114,117] Culture-independent technologies that assay microbial nucleic acids and antigens often have found potential pathogens in culture-negative respiratory specimens.[118,119] For example, one-third of patients with stable COPD have *H. influenzae* within airway epithelial cells and AMs.[120] High-throughput sequencing of microbial 16S rRNA genes yields relatively unbiased estimates of the relative abundance of uncultivable and cultivable bacteria.[121,122] The lower airways of healthy individuals harbor low levels of oral bacteria such as *Prevotella* spp. and *Veillonella* spp.[123,124] Culture-independent techniques have challenged the dogma that the lower airway is normally sterile, and provide evidence that there are residential organisms, especially in individuals with already damaged lungs. Oral anaerobes likely modulate the pulmonary immune response in health and disease. Important to note, enrichment of the lower airway microbiota with oral bacteria anaerobes is associated with augmented lung inflammation characterized by a Th17 phenotype.[125] Through use of culture-independent techniques, a distinct lower airway microbiota, characterized by enrichment with Gammaproteobacteria (which includes *Haemophilus* and *Moraxella*) and the presence of upper airway taxa, has been found in advanced-stage COPD (Global Initiative for Chronic Obstructive Lung Disease [GOLD] score of 3 or 4).[121,122,126,127] Multiple mechanisms present in this disease may affect the microbial composition of the lower airways. Patients with COPD frequently have microaspiration due to gastroesophageal reflux,[97–99] in addition to impaired bacterial clearance.[95,96] This vicious cycle could explain the association of poor oral health and increased airway bacterial load, COPD exacerbations, and reduced lung function.[100–104,128] Investigations into possible mechanisms using mouse models have shown that lower airway dysbiosis can lead to increased Th17 inflammatory changes and COPD phenotype.[129,130]

Respiratory viruses are also frequently found in patients with stable COPD. With culture or polymerase chain reaction (PCR) techniques to assess sputum, the most commonly found respiratory virus is respiratory syncytial virus (RSV), found in up to 23.5% of patients with COPD, followed by non-RSV viruses, such as rhinovirus, coronavirus, and parainfluenza virus, in 16.2% of samples.[131] Increased inflammation has also been reported with adenovirus.[132] High-throughput complementary DNA (cDNA) sequencing can identify viral transcripts in an unbiased approach. A fuller understanding of the role of the virome in stable COPD and in AECOPD awaits resolution of the technical challenges of high-coverage RNA sequencing in lower respiratory tract samples.

Microbes in Acute Exacerbation of Chronic Obstructive Pulmonary Disease

Patients with acute exacerbations have accelerated loss of lung function, which emphasizes the importance of identifying the underlying causes of these episodes.[133] Two-thirds of patients with a COPD exacerbation have bacteria, viruses, or both cultured from lower airway secretions. Aerobic bacteria are isolated in one-half of patients, respiratory viruses are isolated in one-third, and bacterial-viral coinfection is present in one-quarter of patients with acute exacerbations.[58,134] The increased incidence of AECOPD during winter months may reflect the significant role that viruses play in the pathogenesis of AECOPD.

H. influenzae, S. pneumoniae, and *M. catarrhalis* are the bacterial pathogens most commonly isolated during COPD exacerbations. The same three also colonize stable COPD patients, and higher bacterial loads have been associated with AECOPD.[58,117,118,135–138] Acquisition of a new bacterial strain frequently precedes AECOPD.[106,119,135,138,139] The increase in total bacterial load during a COPD exacerbation is relatively small compared with the total bacterial load.[138] In this conceptual model, the humoral and cellular immune responses to the new bacterial or viral strain likely drive the increased inflammation that causes the COPD exacerbation. Newer culture-independent investigations evaluating longitudinal changes in sputum microbiota showed that AECOPD is associated with decreased microbial diversity and increased proportion of Proteobacteria.[140] Furthermore, changes in lung microbiota are associated with levels of IL-8 in sputum, a key neutrophil chemoattractive cytokine. Distinct microbial populations can be identified in sputum between subjects with exacerbations marked by positive bacterial culture or elevated eosinophils.

Individuals with greater degrees of functional impairment, recent antibiotic use, or systemic steroid therapy have higher rates of isolation of gram-negative bacteria such as *Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and members of the Enterobacteriaceae family from sputum.[141,142] Patients with an $FEV_1 > 35\%$ of predicted value and no systemic steroid or antibiotic use within the preceding 3 months have a low probability of Enterobacteriaceae or *P. aeruginosa* in sputum culture.[142] *H. influenzae* and *P. aeruginosa* are more common in patients with poorer lung function.[137] Polymicrobial exacerbations occur with advanced pulmonary dysfunction and severe exacerbations.[134,143]

The role of "atypical" bacterial pathogens such as *Mycoplasma pneumoniae, Chlamydia pneumoniae,* and *Legionella pneumophila* in exacerbation is poorly defined, but they are rarely found in AECOPD.[56,131,144] Variations in reported rates of atypical pathogens could be related to the technical or geographic differences among reports.[112,145] When serology or *M. pneumoniae* antigen detection has been used to indicate the presence of *M. pneumoniae* as a pathogen, PCR and culture

frequently do not confirm its presence.[131,146,147] Alternately, two reports from Italy and Greece indicate demonstration of *M. pneumoniae* in 7% to 9% of patients with AECOPD with use of serologic and PCR techniques.[148,149] Controversy also exists regarding the role of *C. pneumoniae* in COPD exacerbations. Several well-conducted studies detected no *C. pneumoniae* or *L. pneumophila*,[131,147] whereas others detected *C. pneumoniae* in 6% to 9% of patients with AECOPD.[112,148] Chronic colonization with *C. pneumoniae* occurred in one-third of patients and was associated with worse pulmonary function in one study.[112]

Rhinovirus is the virus frequently associated with AECOPD and is present approximately 20% to 34% of the time.[131,134,150] Coronavirus, parainfluenza virus, adenovirus, influenza virus, and human metapneumovirus also occur but are less prevalent.[143,150] Viruses can predispose to bacterial infection with higher bacterial burden, more sputum eosinophils, greater lung function impairment, and longer hospitalization.[134,143] It is likely that viruses and bacteria induce independent inflammatory pathways, accounting for more severe presentations and poorer outcome of patients with coinfection.

TREATMENT

Nonantimicrobial Treatment of Steady-State Chronic Obstructive Pulmonary Disease

Treatment of stable COPD should improve the patient's symptoms and functional status, reduce the risk of exacerbations, and slow the decline of lung function.[5,8] Smoking cessation and avoidance of environmental exposure are the most important interventions to prevent disease progression and should be encouraged at every medical visit. Multiple different behavioral and pharmacologic treatments should be explored, including varenicline, nicotine replacement, and bupropion.[151–154] Pneumococcal, influenza, and combined tetanus, diphtheria, and acellular pertussis (Tdap) vaccination is also recommended in every COPD patient.[5,155]

Nonantimicrobial Treatment of Acute Exacerbation of Chronic Obstructive Pulmonary Disease

Systemic corticosteroids (either oral or intravenous) and bronchodilators are the cornerstones of pharmacologic treatment of AECOPD. Short-acting bronchodilators are preferred because they allow titration of the dose required and because there is no clinical trial that has evaluated the use of long–acting bronchodilators during AECOPD. Although for sicker patients nebulized bronchodilators are routinely used in the hospital, data suggest that similar bronchodilator effects occur with metered-dose inhalers.[156] A 10- to 14-day course of systemic corticosteroids reduces treatment failure by 46% during both inpatient and outpatient management of COPD exacerbations.[157] Systemic corticosteroids are preferred during an exacerbation because they reduce recovery time, improve lung function, and increase arterial oxygenation.[158,159,160,161,162] Systemic steroids also reduce the rate of early relapse and length of hospital stay. Although there are no data to support a specific dose or route of administration for steroids, GOLD guidelines recommend 30 to 40 mg of oral prednisolone per day for 10 to 14 days.[5,162–164]

Antibiotic Treatment of Acute Exacerbation of Chronic Obstructive Pulmonary Disease

Rationale for Antibiotics

Early antibiotic treatment of AECOPD in both the outpatient and inpatient settings has become nearly routine in clinical practice even though the role of bacteria in many cases of AECOPD is uncertain. The choice of antibiotic has changed during the past several decades; in part reflecting the changing resistance patterns of infecting bacteria and the availability of newer antibiotics that are taken less frequently, have improved antimicrobial activity, are less toxic, and are heavily marketed to health care providers. Studies evaluating the effect of antibiotic treatment are mostly small and are difficult to compare because of heterogeneous patient populations, diverse outcome measures, and varied definitions of failure. In addition, many different antibiotics and treatment durations have been evaluated, reducing comparability among individual studies. Two meta-analyses and a Cochrane review concluded that antibiotics improve outcome in AECOPD in inpatients, especially those requiring ICU admission.[157,165,166] Among hospitalized patients, antibiotics reduced hospital mortality by 78%.[167–169] Antibiotics also led to improved peak expiratory flow rate by 22%.[170] The largest study to date evaluating antibiotic efficacy is a retrospective cohort of 84,621 inpatients from 413 acute-care centers in the United States.[171] Administration of antibiotics within the first 48 hours reduced the need for mechanical ventilation after 2 hospital days (1.07% vs. 1.80%), lowered rates of inpatient mortality (1.04% vs. 1.59%), reduced readmissions (7.91% vs. 8.79%), and produced fewer treatment failures (9.77% vs. 11.75%). However, there was a significantly higher rate of *Clostridioides difficile* (formerly *Clostridium difficile*) in patients who were treated with antibiotics (0.19% vs. 0.94%). The weight of evidence supports antibiotic use for hospitalized patients; particularly those admitted to the ICU.

As opposed to relatively high-quality evidence supporting the use of antibiotics in the inpatient setting, there has been less convincing evidence to recommend antibiotics for outpatients. In the aforementioned Cochrane review, outcome was improved with antibiotic use when all outpatient studies were evaluated, but was not seen when the analysis was restricted to studies using currently available antibiotics.[40,166,172,173,174] However, a multicenter double-blind, placebo-controlled clinical trial of mild-to-moderate AECOPD documented that amoxicillin-clavulanate improves response to therapy within 9 to 11 days (74.1% vs. 59.9%) and increased the median time to the next exacerbation from 160 days in the placebo group to 233 days in those receiving antibiotics.[173] This study, the findings of which were published after the most recent meta-analysis,[166] suggests that antibiotics may be beneficial for some patients with mild-to-moderate AECOPD. Several other studies have demonstrated a delayed time to relapse or exacerbation with the use of antibiotics to treat AECOPD. In a retrospective cohort outpatient study of 270 patient visits (with relapse defined as a return visit within 14 days), antibiotics reduced the relapse rate to 19% (50/270) compared with 32% (29/92) among patients who did not receive antibiotics. Patients who received amoxicillin, however, had an even higher relapse rate of 54% (20/37) compared with those who did not receive antibiotics.[175] In another study, antibiotics added to oral corticosteroids increased the median time from second to third exacerbation from 189 days to 258 days and reduced mortality.[176] The limited effectiveness of antibiotics for less severe exacerbations may be a result of lower bacterial burden in this healthier group of patients. Doxycycline may not provide similar benefits. A very recent randomized, double-blind, placebo-controlled study comparing 7 days of doxycycline with placebo for outpatients receiving oral prednisolone among patients with GOLD stage 1 to 3 disease demonstrated neither a better initial response (87% in the doxycycline group vs. 83% in the placebo group) nor an increased time to second exacerbation (161 days vs. 138 days).[177] Determination of outpatients with mild-to-moderate AECOPD who would benefit from antibiotic treatment remains an area requiring further investigation.

There are few data comparing cost-effectiveness of using outpatient antibiotics versus withholding antibiotics. Ronaldson and colleagues have reported an observational study using the electronic medical records of AECOPD documented in the National Health Services database of the United Kingdom. The comparator groups were overall well matched, and >75% of patients had mild-to-moderate COPD. Use of antibiotics led to a decrease in costs, mainly driven by fewer hospitalizations, referrals to community respiratory teams, infections, and general practitioner visits.[178] This is an area that merits considerably more study given the potential problem of inducing greater levels of antibiotic resistance.

Whom to Treat

Given the large numbers of patients with COPD who develop AECOPD and the potential economic impact both in terms of antibiotic cost and toxicities of treatment, there has been much effort to identify patients who do not require antibiotics for AECOPD. Clinical investigations have used procalcitonin as a biomarker for bacterial infection in acute exacerbation in an attempt to limit unnecessary antibiotic use and reduce

the emergence of antibiotic resistance and *C. difficile* infection.[179–181] Whereas earlier studies included patients with AECOPD among the patient population with lower respiratory tract infections, several more recent studies have evaluated the effects of using procalcitonin measurement on patients hospitalized with the specific diagnosis of AECOPD. Corti and colleagues randomized patients to point-of-care procalcitonin testing or no testing; they used an algorithm that discouraged antibiotic use when the level was <0.25 ng/mL.[182] These investigators found significantly fewer days of antibiotic use among patients who were randomized to undergo procalcitonin testing. Wang and colleagues randomized patients with AECOPD who had a procalcitonin level <0.1 ng/mL to antibiotics versus no antibiotics and found no difference in outcome.[170] A meta-analysis demonstrated that procalcitonin-based protocols led to less initiation of antibiotics and less total exposure to antibiotics without a difference in multiple outcomes. However, the authors noted that the quality of the available evidence is low to moderate owing to the small study size and to methodologic issues.[183] A more recent population-based study evaluated the use of antibiotics over two time periods among hospitalized patients with AECOPD (505 US hospitals and 2,013,177 patients) in which procalcitonin testing was or was not incorporated. The authors found that there was a weak association toward less antibiotic use among hospitals that incorporated procalcitonin testing (performed 85% of the time on day 1 or 2 of hospitalization).[184] Procalcitonin testing was not associated with the duration of antibiotic treatment.[184] A study evaluating two institutions compared antibiotic use before and after introduction of procalcitonin testing and demonstrated that the use of this test was associated with a significantly decreased duration of antibiotic therapy (5.3 ± 3.2 days before vs. 3 ± 2.9 days after; $P = .01$). The number of patients receiving ≤1 day of therapy decreased from 43.8% to 14.5% in the after period, and there was no increase in readmission.[185] As procalcitonin is increasingly used in practice, its real-life impact on antibiotic usage and outcomes will need to be further studied.

The authors of the GOLD guidelines note that treatment with antibiotics is controversial but recommend antibiotic therapy for patients who have AECOPD who meet one of the following criteria: (1) have all three "cardinal" symptoms—increased dyspnea, increased sputum volume, and increased sputum purulence; (2) have two of the cardinal symptoms if increased purulence of sputum is one of the two symptoms; (3) have severe exacerbation that requires mechanical ventilation (invasive or noninvasive).[5]

In studies, patients who do not meet these criteria frequently have been prescribed antibiotics. Studies involving outpatients and inpatients have demonstrated that although approximately 80% of patients receive antibiotics, purulent sputum is found in only 39% to 64% of patients.[186–188] In a study from the United States, there was no correlation between the use of antibiotics and the presence of an indication for their use.[189] In a more recent study, 20% of patients who did not meet GOLD criteria for antibiotics received antibiotics before hospitalization, whereas approximately 20% who met criteria did not receive antibiotics.[190] A European COPD audit from 13 countries demonstrated that in 2010 and 2011, 38.6% of patients admitted to the hospital for a COPD exacerbation did not meet GOLD criteria for antibiotic use.[191] Although the use of bacterial cultures for treatment of AECOPD is debated, the use of purulence to define the need for antibiotics is based on several studies demonstrating a relationship between sputum color and/or neutrophils and positive bacterial cultures.[192–195] Sputum purulence has been an excellent guide for antibiotic use in several studies. In one, if sputum purulence was not present and antibiotics were withheld, therapy in only 2 of 32 patients failed.[195] In another study in which purulence was used to determine whether antibiotics would be prescribed, the therapeutic failure rate was 9% among those who had nonpurulent sputum and did not receive antibiotics versus 10% among those with purulent sputum who received antibiotics.[196] It is clear that many patients are prescribed antibiotics who do not need them, but the current state of knowledge does not clearly define the group who will be harmed more than they will be helped by a course of antibiotics. The GOLD recommendation for using antibiotics is based on symptoms, in particular sputum purulence, which remains the best guide to decide which patients with AECOPD require antibiotics.

Choice of Antibiotic

Most recent comparative studies have used noninferiority designs to demonstrate equivalency between antibiotics. Among the more commonly studied antibiotics are macrolides (azithromycin and clarithromycin), second-generation cephalosporins (cefuroxime, cefpodoxime, cefdinir), penicillin or penicillinase inhibitors (ampicillin-clavulanate), quinolones (ciprofloxacin, levofloxacin, moxifloxacin, gemifloxacin), trimethoprim-sulfamethoxazole, and tetracyclines (doxycycline). Most of these trials have been performed in patients with acute exacerbation of chronic bronchitis. Several meta-analyses have been performed evaluating randomized controlled antibiotic comparison trials for treatment of AECOPD.[197–199] No agent or class is consistently superior in comparison to others in terms of clinical end points, although in any single study one agent may appear superior in terms of microbiologic eradication of one bacterium or overall microbiologic eradication.[197,200,201] There may be higher relapse rates when acute exacerbations are treated with amoxicillin compared with other antibiotics.[175] Increasing β-lactamase production by *S. pneumoniae, H. influenzae,* and *M. catarrhalis* challenges the use of amoxicillin or ampicillin as first-line empirical treatment of AECOPD.[202,203] An evaluation of 19,608 patients who were treated with either a quinolone antibiotic (13,469 patients) or macrolide antibiotic (6,139 patients) taken from a larger retrospective cohort study of hospitalized patients with AECOPD showed similar rates of treatment success, although less diarrhea occurred in the macrolide cohort.[204] Antimicrobial recommendations for the larger group of COPD patients with acute exacerbations are extrapolated from these data (Fig. 66.2).

Treatment is empirical, is based on risk factors for both antibiotic resistance and infection with Enterobacteriaceae or *P. aeruginosa,* and to some extent is dependent on the need for hospitalization (see Fig. 66.2). The specific choice of antibiotic should be made with an understanding of local bacterial resistance patterns, toxicity, allergies, drug interactions, and comorbidities. A sputum sample is not recommended routinely in either the GOLD guidelines or those of the American College of Physicians–American Society of Internal Medicine and the American College of Chest Physicians.[5,205] Sputum cultures and Gram stains are not reliable in terms of indicating the infecting pathogen. Furthermore, early sputum cultures do not seem to affect outcome with antibiotic treatment among inpatients.[171] An exception to this recommendation should be considered for patients in whom prior therapy has failed and patients at high risk for infection due to Enterobacteriaceae or *P. aeruginosa.*

Patients with AECOPD generally do not have significant systemic illness at presentation. By definition, chest radiographs do not demonstrate a new pulmonary infiltrate. However, because up to one-fifth of patients hospitalized for community-acquired pneumonia (CAP) have normal admission chest films but develop an infiltrate within 48 hours, providers may choose to dismiss a normal chest radiograph and prescribe antibiotics.[206] The combination of overlap between AECOPD and CAP along with antibiotic resistance leads to frequent use of broad-spectrum antibiotics in many hospitalized AECOPD patients.[189] If chest radiographs do not demonstrate an infiltrate at 48 hours, antibiotics can be tailored or stopped but still may be useful for AECOPD that meets other criteria.[207]

Despite increasing resistance to many of the older antibiotics, patients still respond to them clinically. Trimethoprim-sulfamethoxazole and ciprofloxacin were equally effective in a double-blind trial among patients with severe AECOPD requiring mechanical ventilation.[208] Surprisingly, bacterial susceptibility did not predict clinical success.[208] This may be due to the poor sensitivity and specificity of sputum samples in defining the bacteria that caused the AECOPD. Alternately, AECOPD may be driven by changes of a complex lower airway microbiome and not by a single microorganism. A shift in the lung microbiome could produce a flare of inflammation. Given the polymicrobial nature of the microbiome, organisms may not need to be effectively treated to reduce inflammation. In the near future, unbiased culture-independent techniques will more accurately describe the lung microbiome in COPD. A better understanding of microbiome changes preceding AECOPD and alterations produced by antibiotics will improve our understanding of the antibiotic response.

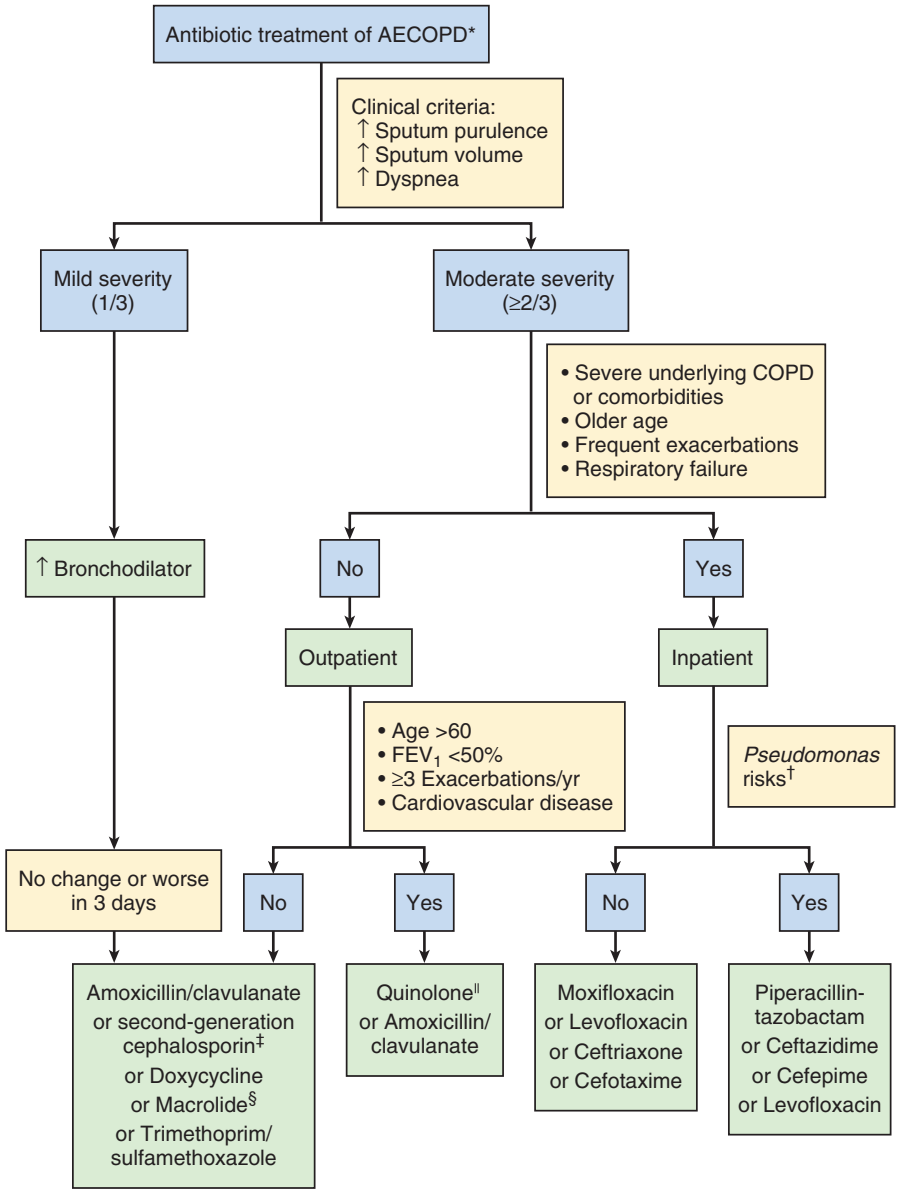

*If antibiotics used within past 3 months, use antibiotics from different class.
†*Pseudomonas* risk factors: >4 episodes antibiotics in past year, hospitalization within past 90 days, prior culture for *Pseudomonas*, FEV_1 <50% predicted.
‡cefpodoxime, cefuroxime, cefdinir.
§azithromycin, clarithromycin.
‖levofloxacin, moxifloxacin, gemifloxacin.

FIG. 66.2 Flowchart for antibiotic treatment decision in chronic obstructive pulmonary disease *(COPD)* exacerbation. *AECOPD,* Acute exacerbations of chronic obstructive pulmonary disease; *FEV_1,* forced expiratory volume in 1 second.

Although nebulized antibiotics have been used for the treatment and prevention of exacerbations in cystic fibrosis and bronchiectasis, there are limited published reports evaluating the efficacy of inhalational antibiotics for the treatment of AECOPD. One study of only 13 patients published in 2008 demonstrated that twice-daily nebulized tobramycin for 2 weeks decreased proinflammatory markers and resulted in a 42% decrease in COPD exacerbations compared with the 6 months before nebulizer use.[209] In a more recent study, 36 patients who were infected with *P. aeruginosa* were followed for 5 years on nebulized colistin. The authors reported a decrease in hospitalizations and duration of hospitalization but no decrease in exacerbation for patients not requiring admission.[210] Such therapy is enticing because if successful, it might limit adverse reactions caused by systemic levels of antibiotics. However, prolonged use of nebulized therapy may lead to microbial resistance. Nebulized amoxicillin-clavulanate for patients with COPD also has been shown to achieve adequate sputum concentrations and to have a good safety profile.[211] Larger, randomized, controlled efficacy studies in COPD patients are warranted.

Duration of Antibiotic Treatment

Outpatient studies have compared duration of therapy in acute exacerbations of chronic bronchitis. A regimen of 5 days of therapy with a quinolone, second-generation cephalosporin, or macrolide is as efficacious as and is associated with fewer adverse reactions than 7 days of antimicrobial therapy.[212–215] The GOLD guidelines currently recommend 5 to 7 days of therapy. Although there are no comparable inpatient trials, durations of therapy have varied between 7 and 14 days; 10 days of treatment have been used in studies of hospitalized AECOPD patients.[168,208] Eight days of therapy, which is currently recommended for ventilator-associated pneumonia, may suffice even in critically ill patients.[216] If tolerated, oral therapy is as effective as intravenous antibiotics.[168]

Treatment of Viral Infection

Patients with COPD would be considered at high risk for severe influenza infection and should be offered neuraminidase inhibitors such as oseltamivir or zanamivir when they develop an influenza-like illness during influenza season, even in the setting of a negative rapid virus detection study result.[217] Nonimmunized people with COPD who have had close family contact with a person with influenza should receive chemoprophylaxis with a neuraminidase inhibitor.[217]

Although numerous viruses other than influenza virus have been associated with AECOPD as noted earlier, there are no treatment options for these viruses currently. Inhaled ribavirin has been used therapeutically in infants and in patients with transplant infections with severe RSV infection, but reports from studies using this agent in patients with COPD are not available. As diagnostic and therapeutic modalities improve, specific antiviral therapy may become part of the armamentarium to decrease the duration of a COPD exacerbation.

PREVENTIONS OF ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

The goal of therapy is to avoid acute exacerbations because these are associated with accelerated declines in lung function.[133] β_2-Agonists (long-acting β_2-agonists [LABAs]) and anticholinergic bronchodilators (LAMAs) frequently combined in a single inhaler are effective in reducing exacerbations.[85] Oral phosphodiesterase inhibitors also reduce the frequency of acute exacerbations (Table 66.2).[5,218] These drugs relax airway smooth muscle, producing bronchodilation with improved lung function. They also have antiinflammatory activity, although the clinical significance of this is not clear.[219] Anticholinergic mediations also reduce sputum production in patients with chronic bronchitis. Corticosteroids in steady-state COPD are restricted to adjunctive therapy complementing long-acting bronchodilators in more severe cases of COPD and may be most effective in patients with characteristics of both COPD and asthma.[220] Inhaled corticosteroids do not reduce mortality but do reduce COPD exacerbations,[221] decrease inflammation, and stabilize lung function in moderate and severe COPD.[222] Unfortunately, inhaled corticosteroids increase the risk for pneumonia, which must be balanced with the benefit of less frequent exacerbations.[221,223] Other drugs such as nonselective and selective phosphodiesterase inhibitors (e.g., theophylline and roflumilast, respectively) may also be considered in cases of severe COPD with frequent exacerbations that are not adequately controlled with long-acting bronchodilators.[224,225]

Antibiotic Prophylaxis in Steady-State Chronic Obstructive Pulmonary Disease

Because bacterial colonization of lower airways plays a significant role in AECOPD, prophylactic use of antibiotics has been investigated. Successful use of prolonged, continuous oxytetracycline in a small number of patients was attempted in Britain as early as the mid-1950s.[226] The effectiveness of prophylaxis with oxytetracycline was not corroborated in later studies.[227,228] More recently, erythromycin, clarithromycin, and

TABLE 66.2 Modified GOLD Treatment Recommendations According to Stable State Patient Group Category

PATIENT GROUP	FIRST CHOICE	SECOND CHOICE	ALTERNATIVE CHOICE
Pharmacologic Treatment Options			
A	SABA prn	LABA or LAMA	Theophylline
Symptoms low	or	or	
Exacerbation: 0 or 1 (not leading to hospital admission)	SAMA prn	SABA and SAMA	
B	LABA	LAMA and LABA	SABA and/or SAMA
Symptoms high	or		Theophylline
Exacerbation: 0 or 1 (not leading to hospital admission)	LAMA		
C	LAMA	LABA and ICS	PDE4 inhibitor
Symptoms low	or LAMA and LABA		SABA and/or SAMA
Exacerbation: ≥2 or ≥1 leading to hospital admission			Theophylline
D	LAMA and LABA	ICS and LAMA	Carbocysteine
Symptoms high	or LAMA and LABA and ICS	or ICS and LABA and LAMA	Roflumilast
Exacerbation: ≥2 or ≥1 leading to hospital admission		or ICS and LABA and PDE-4 inhibitor or LAMA and LABA or LAMA and PDE-4 inhibitor	Theophylline Azithromycin
PATIENT GROUP	**ESSENTIAL**	**RECOMMENDED**	
Nonpharmacologic Treatment Options			
A	Smoking cessation	Physical activity Influenza and pneumococcal vaccination; Tdap	
B–D	Smoking cessation Pulmonary rehabilitation	Physical activity Influenza and pneumococcal vaccination; Tdap	

Symptoms low = mMRC 0–1 or CAT <10. Symptoms high = mMRC ≥2 or CAT ≥10.
CAT, COPD Assessment Test; *GOLD,* Global Initiative for Chronic Obstructive Lung Disease criteria; *mMRC,* modified British Medical Research Council questionnaire; *ICS,* inhaled corticosteroid; *LABA,* long-acting β_2-adrenergic agonist; *LAMA,* long-acting muscarinic antagonist; *PDE-4,* phosphodiesterase type 4; *SABA,* short-acting β_2-adrenergic agonist; *SAMA,* short-acting muscarinic antagonist; *Tdap,* combined tetanus, diphtheria, and acellular pertussis vaccine.
Modified from Vogelmeier CD, Criner GJ, Martinez FJ, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report: GOLD Executive Summary. Eur Respir J. *2017;49:1750214.*

azithromycin have been the most widely studied prophylactic agents. Observational and placebo-controlled trials suggested that long-term macrolide prophylaxis is effective in reducing exacerbations and hospitalizations.[229–232] Macrolide use for 3 months has not resulted in significantly decreased rates of hospitalization or exacerbations of COPD.[233–235] A large-scale randomized, placebo-controlled study of azithromycin 250 mg daily for 1 year reduced AECOPD from 1.82 exacerbations per patient-year in the placebo group to 1.48 exacerbations per patient-year in the azithromycin group. Antibiotic treatment also extended time to first exacerbation from 174 to 266 days and improved quality of life.[232] The azithromycin group, however, had more hearing loss.[232] Among patients who became colonized, macrolide resistance occurred in 81% of the treated group versus 41% of the placebo control. Important to note, this trial excluded patients with cardiovascular risks including a resting heart rate >100 beats/min, prolonged QT (QTc) interval, or medications that increase the QTc interval. Therefore the potential for adverse cardiac reactions reported with macrolides was minimized in this study. There is still controversy about the increased risk for cardiovascular events reported with azithromycin use.[236,237] In addition, intermittent short courses of macrolides produced a fourfold increase in *S. pneumoniae* within 6 months.[238] The mechanisms that reduce exacerbation go beyond their antibacterial effect. These drugs are directly antiinflammatory, decreasing proinflammatory cytokine production, adhesion molecules, and reactive oxygen species.[239,240] In addition, macrolide administration in early emphysema leads to changes in microbiota and decreased levels of inflammatory cytokines in lower airways.[241] Important to note, the net antiinflammatory effect seemed to be mediated more by bacterial stress-induced metabolites than by direct macrolide effects on the host. Long-term macrolides have also been studied in cystic fibrosis and bronchiectasis, where they have led to fewer exacerbations of disease and stabilization of lung function.[242,243] For patients with low cardiovascular risk and frequent exacerbation in spite of adequate routine therapy, macrolides should be considered (see Table 66.2).[244] Intermittent (thrice-weekly) and daily azithromycin appear to provide similar benefits.[245] However, more widespread use of macrolides in less severe COPD cases is not currently recommended.

Another strategy to prevent AECOPD is intermittent antibiotic therapy. Moxifloxacin 400 mg for 5 days every 8 weeks for a total of six courses reduced the odds of exacerbation by 20% in the intention-to-treat analysis of the enrolled COPD patients and by 45% among patients with baseline purulent sputum. The authors reported no increased resistance to moxifloxacin among cultured bacteria but did find more gastrointestinal problems (4.7% with moxifloxacin vs. 0.7% with placebo).[246] Further research is needed to confirm that the benefits of this approach outweigh the risks of resistance and *C. difficile* colitis before this strategy gains widespread use.

Vaccination

The influenza, pneumococcus, and Tdap vaccines are recommended for patients with COPD. Influenza vaccination is recommended annually for persons with COPD by both the Centers for Disease Control and Prevention (CDC) and the Advisory Committee on Immunization Practices (ACIP).[5,8,155,247,248] The pneumococcal polysaccharide vaccine 23 (PPSV23) and the pneumococcal conjugate vaccine 13 (PCV13) are both recommended for patients older than 65 years and the PPSV23 is recommended for those younger than 65 with COPD and other comorbidities.

A systematic review of the literature evaluating influenza vaccine, including observational studies and randomized controlled trials in patients with COPD, concluded that the vaccine is immunogenic in patients with COPD and had no significant adverse reactions. Six of the seven studies evaluating effectiveness of the influenza vaccine revealed that seasonal influenza vaccine decreased the frequency of AECOPD, hospitalizations, outpatient visits, and all-cause and respiratory mortality.[249] Two other studies reported since the systematic literature review confirmed these findings.[250,251] It is of note that the efficacy or effectiveness of the influenza vaccine will be affected by the match between the circulating strains and the vaccine strains.

Pneumococcal vaccine is also recommended for all persons with COPD.[5,8,155] Pneumococcus is a common colonizing organism in addition to being a pathogen in this population. Some observational studies have demonstrated a decrease in pneumococcal bacteremia in the elderly and in hospitalization for pneumonia and mortality among elderly patients with chronic lung disease.[252,253] Others have not been able to corroborate these findings.[254] Based on a meta-analysis including 12 studies, there was a significant reduction in the likelihood of developing CAP but not specifically pneumococcal pneumonia, and a decrease in the likelihood of COPD exacerbation.[255] However, there was neither a decrease in all-cause mortality or cardiorespiratory mortality nor in the likelihood of all-cause or cardiorespiratory hospitalization. Studies evaluating the recent guidelines for sequencing the PPSV23 and the PCV13 vaccines in the COPD population are warranted.[256]

Several studies have reported that there is an additive effect when both the influenza vaccine and pneumococcal vaccine are used among patients with COPD. There was a significant reduction in hospitalization (63%) and in mortality (81%) when both vaccines were administered compared with no vaccine.[257] Two more recent cohort studies from Japan demonstrated better outcomes when both influenza vaccine and pneumococcal vaccine were administered compared with influenza vaccine alone.[258,259]

Given the relatively low rates of vaccination for influenza among patients with COPD in 2012 (48.5%) and pneumococcus among patients older than 65 years in 2016,[260] identifying barriers to vaccination among patients with COPD and evaluating interventions are critical to improve vaccination rates.

Although there have been no studies regarding efficacy of Tdap among patients with COPD, *Bordetella pertussis* has been reported to occur in a significant portion of patients with AECOPD when serologic diagnosis was used in Switzerland.[261] However, all cultures and PCR assay results for *Bordetella* spp. were negative in this study. Therefore it is not certain how to interpret the serologic data. Tdap is recommended by the CDC and ACIP for all adults ages 19 to 64 years, and it is reasonable that all patients with COPD receive this vaccine, particularly in light of the recent surge in cases of pertussis reported among adults.[155]

High rates of herpes zoster infection, due to either immune alterations or frequent steroid use, have been reported in patients with COPD.[260,262] In the population-based study by Yang and colleagues, the adjusted hazard ratio was 1.68 for COPD patients not taking steroids compared with the general population.[262] The hazard ratio increased to 2.09 for COPD patients taking inhaled corticosteroids, and was highest for those on oral steroids (adjusted hazard ratio of 3.00). This study evaluated only patients older than age 50 years, and it is not clear what the risk is for those diagnosed with COPD at a younger age. Zoster vaccine should be administered to patients with COPD per current ACIP guidelines.

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The complete reference list is available online at Expert Consult.

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67 Acute Pneumonia

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SHORT VIEW SUMMARY

Epidemiology and Etiology

- Acute pneumonia is the most common cause of infection-related death.
- Predominant pathogens of community-acquired pneumonia (CAP) in adults in the developed world include *Streptococcus pneumoniae, Mycoplasma pneumoniae,* and community respiratory viruses.
- *Legionella* species, *Staphylococcus aureus,* and enteric gram-negative bacilli are less frequent causes that can produce more severe disease.
- Predominant pathogens of patients recently hospitalized or nursing home residents include *S. aureus,* aerobic gram-negative rods including *Pseudomonas aeruginosa,* and mixed aerobic and anaerobic organisms.

Diagnosis

- Typical clinical manifestations are cough—the *sine qua non* of pneumonia—sputum production, dyspnea, chest pain, fever, fatigue, sweats, headache, nausea, myalgia, and occasionally abdominal pain and diarrhea.
- Analyses of sputum samples by Gram stain and culture remain valuable diagnostic assays.
- Blood cultures should be obtained in all patients who are immunocompromised, have health care–associated pneumonia (HCAP) or hospital-acquired pneumonia, or are hospitalized with severe CAP.
- Analysis of pleural fluid should be performed on all effusions with imaging characteristics atypical of fluid overload.
- Chest radiographs should be obtained in all adult patients suspected to have pneumonia.
- Several biomarkers including procalcitonin and C-reactive protein are under assessment as discriminatory assays to define populations with a higher likelihood of bacterial infection that could benefit from antibiotic therapy, but the clinical usefulness of such assays has not yet been established.

Management

- One of three severity index scores (pneumonia severity index [PSI]; confusion, *u*rea, *r*espiratory rate, low *b*lood pressure plus age >65 [CURB-65]; or CURB-65 score without the urea level [CRB-65]) can be used to assess the need for hospitalization in immunocompetent patients with CAP, and similar indices can be used to define the need for intensive care unit admission.
- Antibiotic therapy for pneumonia should be started as soon as the diagnosis is considered likely.
- Advanced macrolides, respiratory fluoroquinolones, and β-lactam agents are the principal antibiotics used for the treatment of CAP. Coverage for *S. aureus* and mixed anaerobes should be considered in select situations (see Table 67.5 for suggested agents and dosages).
- The duration of intravenous treatment, inpatient hospitalization, and total intravenous and oral antibiotic therapy for CAP should be guided by patient's clinical response.

Prevention

- Immunization with the influenza and pneumococcal vaccines should be performed as appropriate.
- Encourage cessation of tobacco smoking.

In 1901, Sir William Osler noted in the fourth edition of his book *The Principles and Practice of Medicine* that "the most widespread and fatal of all acute diseases, pneumonia, is now Captain of the Men of Death."[1] Despite ongoing advances in medical care, over a century later the prominence of pneumonia as a clinical entity remains. It remained among the top 10 most common causes of death among all age groups worldwide in 2015 and the single most common cause of infection-related mortality.[2] The clinical challenge of community-acquired pneumonia (CAP) involves the wide array and ever increasing number of microbial agents that can cause disease (Table 67.1A–D), the difficulty in making a clinical and etiologic diagnosis, and the fact that no single antimicrobial regimen can cover all the possible causes. Because a specific etiologic diagnosis is often not possible at the time initial treatment is begun, the clinician must decide which empirical therapy is most appropriate. The increasing prevalence of antibiotic resistance among many of the most common pathogens has made this challenge more difficult. An understanding of the pathogenesis of the disease, evaluation of relevant data from a careful history and physical examination, recognition of common clinical patterns of infection, and information from the microbiology laboratory all aid in narrowing down the possible etiologic agents of pneumonia, thereby allowing reasonable therapy to be selected empirically.

HOST DEFENSES AND PATHOGENESIS

The lung is constantly exposed to the mixture of gases, particulate material, and microbes that constitutes inspired air. Although the lower respiratory tract has traditionally been considered sterile, investigations using culture-independent techniques have shown that in healthy individuals there is a similar microbiota in the upper and lower respiratory tract, although with a lower concentration of microorganisms within the lung.[3] A more complex microbiota has been demonstrated in individuals with chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), bronchiectasis, lung transplant, and altered mucociliary transport, and there can be significant variations in the microbiota at different locations within the lungs of individuals.[4–6] The development of acute pulmonary infection appears to arise when there is a defect in host defenses, exposure to a particularly virulent microorganism, or an overwhelming inoculum. Infectious agents gain entry to the lower respiratory tract through aspiration of upper airway resident microbiota, inhalation of aerosolized material, and, less frequently, metastatic seeding of the lung from blood.

Pulmonary Defense Systems

The pulmonary defense system involves both innate and adaptive immunity including anatomic and mechanical barriers, humoral

TABLE 67.1A Causative Agents of Acute Pneumonia: Bacteria

COMMON	UNCOMMON
Streptococcus pneumoniae	*Acinetobacter* var. *anitratus*
Staphylococcus aureus	*Actinomyces* and *Arachnia* spp.
Haemophilus influenzae	*Bacillus* spp.
Mixed anaerobic bacteria (aspiration)	*Moraxella catarrhalis*
Bacteroides spp.	*Campylobacter fetus*
Fusobacterium spp.	*Eikenella corrodens*
Peptostreptococcus spp.	*Francisella tularensis*
Peptococcus spp.	*Neisseria meningitidis*
Prevotella spp.	*Nocardia* spp.
Enterobacteriaceae	*Pasteurella multocida*
Escherichia coli	*Proteus* spp.
Klebsiella pneumoniae	*Burkholderia pseudomallei*
Enterobacter spp.	*Salmonella* spp.
Serratia spp.	*Enterococcus faecalis*
Pseudomonas aeruginosa	*Streptococcus pyogenes*
Legionella spp. (including *L. pneumophila* and *L. micdadei*)	

TABLE 67.1B Causative Agents of Acute Pneumonia: Viruses

CHILDREN	ADULTS
COMMON	**COMMON**
Respiratory syncytial virus	Influenza A virus
Parainfluenza virus types 1, 2, 3	Influenza B virus
Influenza A virus	Respiratory syncytial virus
Influenza B virus	Human metapneumovirus
Rhinovirus	Adenovirus types 4 and 7 (in military recruits)
Bocavirus	Rhinovirus
Human metapneumovirus	
UNCOMMON	**UNCOMMON**
Adenovirus types 1, 2, 3, 5, 14	Coxsackievirus
Coxsackievirus	Echovirus
Echovirus	Coronavirus (SARS, MERS-CoV)
Hantavirus	Hantavirus
Measles virus	Epstein-Barr virus
Coronavirus (SARS, MERS-CoV)	Cytomegalovirus
	Parainfluenza virus
	Herpes simplex virus
	Human herpesvirus 6
	Varicella-zoster virus

MERS-CoV, Middle East respiratory syndrome coronavirus; *SARS,* severe acute respiratory syndrome.

TABLE 67.1C Causative Agents of Acute Pneumonia: Fungi

COMMON	UNCOMMON
Histoplasma capsulatum	Agents of mucormycosis
Coccidioides immitis	*Rhizopus* spp.
Cryptococcus neoformans	*Absidia* spp.
Aspergillus spp.	*Mucor* spp.
Blastomyces dermatitidis	*Cunninghamella* spp.
	Candida spp.

TABLE 67.1D Causative Agents of Acute Pneumonia: Other Agents

Rickettsia
Coxiella burnetii
Rickettsia rickettsiae
Mycoplasma* and *Chlamydia
Mycoplasma pneumoniae
Chlamydia psittaci
Chlamydia trachomatis
Chlamydia pneumoniae (TWAR)
Mycobacteria
Mycobacterium tuberculosis
Nontuberculous Mycobacteria
M. abscessus
M. avium complex
M. kansasii
M. chelonae
M. fortuitum
M. xenopi
M. simiae
M. scrofulaceum
M. malmoense
M. seoulense
Parasites
Ascaris lumbricoides
Pneumocystis jirovecii
Strongyloides stercoralis
Toxoplasma gondii
Paragonimus westermani

immunity, cell-mediated immunity, and phagocyte activity (Table 67.2).[7–9] The upper airways, including the nasopharynx, oropharynx, and larynx, are the sites first exposed to inhaled microorganisms. The nasal mucosa contains ciliated epithelium and mucus-producing cells. Mechanical clearance of entrapped organisms occurs through the nasopharynx via expulsion or swallowing. In the oropharynx, the flow of saliva, sloughing of epithelial cells, local production of complement, and bacterial interference from resident microbiota serve as important factors in local host defense. Secretory immunoglobulin A (IgA) is the major immunoglobulin produced in the upper airways and accounts for 10% of the total protein of nasal secretions. It possesses antibacterial and antiviral activity despite being a relatively poor opsonin. Despite some controversy, low IgA levels are probably not associated with increased bacterial infection. IgG and IgM enter the airways predominantly via transudation from the blood. Their roles in bacterial opsonization, complement activation, agglutination, and neutralization activity are similar to those noted in serum.

Adherence of microorganisms to epithelial surfaces of the upper airways is a critical initial step in colonization and subsequent infection. Changes in fibronectin secretion and in binding characteristics of epithelium for various lectins occur as a response to underlying diseases. This may help to explain why colonization occurs in some clinical settings and not in others. Particles larger than 10 μm are efficiently filtered by the hair in the anterior nares or impact onto mucosal surfaces because of the configuration of the upper airways and the nasal turbinates. The cough and epiglottic reflexes also keep large particulate matter from reaching the central airways. The trachea and conducting airways of the transbronchial tree are usually effective in entrapping particles from 2 to 10 μm in size. The sharp angles at which the central airways branch cause particles to impact on mucosal surfaces, where they are entrapped by endobronchial mucus. Once entrapped, particles are removed by ciliated epithelium to the oropharynx.

Epithelial cells, which line the conducting airways, submucosal glands, and alveoli, produce airway surface liquid—a complex mixture of proteins and peptides mixed with plasma transudate. Airway surface liquid contains lysozyme, lactoferrin, and secretory leukocyte proteinase inhibitor, all of which possess microbicidal activity.[10,11] Respiratory epithelial cells produce other potent antimicrobial peptides including cathelicidins and β-defensins.[12] These peptides possess individual antimicrobial activity and synergistic antimicrobial activity with one another. In addition, the β-defensins may act as chemokines for memory T cells and dendritic cells, thereby serving as a link between the innate and adaptive immune systems.

Most bacteria are 0.5 to 2 μm in size. This size particle may reach the terminal airways and alveoli. No mucociliary apparatus exists at this level, yet a variety of humoral and cell-mediated host defenses function here. The alveolar lining fluid contains surfactant, fibronectin, IgG, and complement, all of which are effective opsonins. Surfactant is composed of several components (SP-A, SP-B, SP-C, SP-D) that serve to increase the microbicidal capacity of macrophages. These compounds may also affect free-radical production and lymphocyte activity.[13] SP-A and SP-D are collectins—a family of collagenous carbohydrate-binding

TABLE 67.2 Pulmonary Host Defenses

LOCATION	HOST DEFENSE MECHANISM[a]
Upper Airways	
Nasopharynx	Nasal hair Turbinates Anatomy of upper airways Mucociliary apparatus Immunoglobulin A (IgA) secretion
Oropharynx	Saliva Sloughing of epithelial cells Cough Bacterial interference Complement production
Conducting Airways	
Trachea, bronchi	Cough, epiglottic reflexes Sharp-angled branching of airways Mucociliary apparatus Airway surface liquid (lysozyme, lactoferrin, secretory leukocyte proteinase inhibitor, antimicrobial peptides) Dendritic cells[b] } Antigen processing and presentation → stimulation of memory and effector T cells and B cells Bronchus-associated lymphoid tissue (BALT) } Immunoglobulin production (IgG, IgM, IgA)
Lower Respiratory Tract	
Terminal airways, alveoli	Alveolar lining fluid (surfactant, fibronectin, immunoglobulin, complement, free fatty acid, iron-binding proteins) Alveolar macrophages Interstitial macrophages Neutrophil recruitment[c] (pattern recognition receptors → transcription factor stimulation → proinflammatory and antiinflammatory cytokine and chemokine production) Dendritic cells[b] } Antigen processing and presentation → stimulation of memory and effector T cells and B cells Bronchus-associated lymphoid tissue (BALT) }

[a]Aspects of native and adaptive immunity play a role throughout the respiratory tract.
[b]Major component of adaptive immunity and important in response to vaccines and prior infections.
[c]Major component of innate immunity.

proteins. These proteins bind a variety of organisms, including viruses, gram-negative and gram-positive bacteria, mycobacteria, and fungi, which may decrease their virulence or enhance phagocytosis by neutrophils and alveolar macrophages.[14] Free fatty acids, lysozyme, iron-binding proteins, and defensins are also present and may be directly microbicidal.

Phagocytic cells including macrophages and neutrophils play a major role in pulmonary host defense. Four distinct populations of macrophages exist in the lung and vary in their location and function.[15,16] The alveolar macrophage is located in the alveolar-lining fluid at the interphase between air and lung tissue. It serves as the resident phagocytic cell in the lower airway and is the first phagocyte encountered by inert particles and potential pathogens entering the lung via inspired air. Alveolar macrophages play several critical roles.[8] As phagocytic cells, they can eliminate certain organisms. If the numbers of organisms increase beyond the macrophages' capability to handle them or if the organisms involved are particularly virulent (e.g., *Pseudomonas aeruginosa*), the macrophage becomes a mediator of an inflammatory response by producing cytokines that recruit neutrophils into the lung.[17] Interstitial macrophages are located in the lung connective tissue and serve both as phagocytic cells and antigen-processing cells. Dendritic cells derive from monocytes and are located within the epithelium of the trachea, conducting airways, terminal airways, alveolar septa, pulmonary vasculature, and visceral pleura.[18] These cells are therefore positioned to interact with antigens in inhaled air. Dendritic cells (and a specialized subpopulation termed Langerhans cells) possess an enhanced capacity to capture, process, and present class II antigens. They can migrate to lymphoid tissue, where they can stimulate T-cell immune responses. Dendritic cells can also produce a variety of cytokines and chemokines including interleukin (IL)-12, which serves to stimulate B-cell immune function.[19] The intravascular macrophage is located in the capillary endothelial cells. These cells are actively phagocytic and remove foreign or damaged material entering the lungs via the bloodstream.

Neutrophil recruitment is crucial for the inflammatory response in the lung. The mechanisms involved in the initial detection of organisms in the lung and the generation and subsequent resolution of a response to them are now being more clearly delineated.[9,20–24] Other lung parenchymal cells may also help regulate the inflammatory response.[25] In addition to epithelial cells, interstitial macrophages, and dendritic cells, endothelial cells, pulmonary smooth muscle cells, and fibroblasts produce both proinflammatory (e.g., colony-stimulating factors, chemokines) and antiinflammatory (IL-10) factors.

Microorganisms express molecular recognition patterns that are unique and different from those of the host. Pattern recognition receptor families such as Toll-like receptors are present on epithelioid cells, alveolar macrophages, dendritic cells, and other cells that are located in strategic areas of the lung and either individually or in groups serve to recognize molecular patterns of invading organisms.[22,25] This recognition leads to the generation of early response cytokines such as tumor necrosis factor-α (TNF-α) and IL-1, which then activate transcription factors such as mitogen-activated protein kinase, phosphoinositide 3-kinase, nuclear factor kappa B (NF-κB), and interferon-regulatory factors. These transcription factors serve as a common pathway for pattern-recognition receptors and orchestrate the development of the inflammatory response by mediating the transcription of chemokines, adhesin molecules, and other cytokines. This signal cascade serves two purposes. The first is to generate and maintain the inflammatory response to recruit neutrophils into areas of microbial invasion. The other goal is to activate antiinflammatory response mediators, which lead to the shedding of receptors, neutralization of cytokines, and inhibition of macrophage recruitment, all of which serve to ensure that the inflammatory response is held in check and that noninvolved areas of lung are not injured. It is this balance of proinflammatory and antiinflammatory cytokines and effector molecules that allows for sterilization of an infected area of lung without gross destruction of the lung itself. In addition, it is now recognized that polymorphisms and defects are not uncommonly found for both pattern recognition receptors and the inflammatory and antiinflammatory mediators, and that these genetic variations can contribute to an individual's susceptibility to pneumonia.[24]

Cell-mediated immunity via lymphocytes and macrophages is central to adaptive immune responses in the lung and is especially important against certain pathogens, including viruses and intracellular organisms that can survive within pulmonary macrophages (e.g., *Mycobacterium, Legionella*).[16] Lymphocytes within the lung are found along the epithelial

surfaces and within the interstitial and intravascular spaces. Lymphocytes at the epithelial surface are predominantly memory T cells and interact both with epithelial cells and with dendritic cells. Interstitial cells are similarly predominantly T cells but with a different CD4/CD8 ratio than seen in either lymphocytes at the epithelial surface or intravascular lymphocytes, and with an abundance of natural killer (NK) cells. In addition, although uncommon in adults, in childhood there are organized lymphoid tissue collections in the lung located in follicles along the bronchial tree termed bronchus-associated lymphoid tissue (BALT) collections. These collections appear to be morphologically similar to Peyer patches in the intestine and are similarly associated with both the vasculature and the mucosal epithelium. Inhaled antigens therefore are able to cross the epithelial surface and immediately encounter cells involved with antigen processing. Once these antigens are processed and presented, B and T lymphocytes localize and are stimulated to become memory cells and effector cells, with antibody production occurring in this tissue.

Antigens inhaled into the alveolus and captured by antigen-presenting cells subsequently activate intraalveolar lymphoid cells. These cells can stimulate the migration of memory lymphocytes into the area, leading to a localized accumulation of antigen-specific T and B lymphocytes, many of which possess effector cell function. As is true in other anatomic areas, binding of T cells to endothelium is a critical first step in the inflammatory process and is mediated by the interaction of leukocyte function–associated antigen 1 (LFA-1) integrins on the lymphocyte cell surface with ligands exposed by endothelium in areas of inflammation (intercellular adhesion molecules 1 and 2 and vascular cell adhesion molecule 1). Expression of these ligands on pulmonary endothelium is upregulated by inflammatory mediators such as IL-1, interferon-γ, and TNF-α, and by bacterial lipopolysaccharides.

Lymphocytes in the lung have several major roles in the lung including the production of antibody, cytotoxic activity (including killing of virally infected cells), production of inflammatory mediators, and mediating immune tolerance. The lung contains a variety of cytotoxic T cells including NK cells (antigen nonrestricted), antibody-dependent cytotoxic cells, and antigen-restricted cytotoxic cells. Pulmonary T cells produce a large number of cytokines. Mouse models suggest that unstimulated T cells produce mainly IL-2. After stimulation and conversion to memory T cells, two distinct groupings of cytokines are produced. The helper T-cell 1 (Th1) and 2 (Th2) pattern of cytokine production noted in murine models occurs in humans, although it appears to be less restrictive. Th1 cells produce interferon-γ, IL-2, IL-6, and IL-10 and contribute to cell-mediated immunity, whereas Th2 cells produce IL-4, IL-5, IL-10, and IL-13 and contribute to humoral immune function. Furthermore, IL-3, TNF-α, granulocyte-macrophage colony-stimulating factor, and chemokines are secreted by both Th1 and Th2 phenotypes. Th1 cells are involved in cell-mediated inflammatory reactions, whereas Th2 cells stimulate antibody production, especially IgE, and stimulate eosinophil activity. However, there appear to be both Th1 and Th2 responses in many immune responses. The interaction of T-regulatory cells with mucosal dendritic cells appears to mediate the phenomenon of immune tolerance in the lung.

Impairment of Pulmonary Defenses

The defenses of the lung, when they are functioning normally, are extremely efficient in maintaining low microbial concentrations in the lower airways. However, several factors are known to interfere with these defenses and predispose the host to infection. Alterations in the level of consciousness from any cause (stroke, seizures, drug intoxication, anesthesia, alcohol abuse, and even normal sleep) can compromise epiglottic closure and lead to aspiration of oropharyngeal microbiota into the lower respiratory tract.[26] Cigarette smoke, perhaps the most common agent involved in compromising natural pulmonary defense mechanisms, disrupts mucociliary transport and alters macrophage and B- and T-lymphocyte functionality.[27,28]

Alcohol not only impairs the cough and epiglottic reflexes but also has been associated with increased colonization of the oropharynx with aerobic gram-negative bacilli, decreased mobilization of neutrophils, abnormal phagocyte oxidative metabolism, and abnormal chemotaxis.[29,30] Alcohol effectively blocks the TNF response to endotoxin, with decreased recruitment of neutrophils to the lung. Furthermore, alcohol enhances monocyte production of 1L-10, a cytokine with antiinflammatory properties.[31]

Infections with *Mycoplasma pneumoniae* or *Haemophilus influenzae* may interfere with normal ciliary function.[32] Viruses may actually destroy respiratory epithelium and may disrupt normal ciliary activity. Neutrophil function, including chemotaxis, phagocytosis, and stimulation of oxidative metabolism and alveolar macrophage function, may also be inhibited by certain viral infections.[33,34] Sepsis associated with extrapulmonary infections may undermine lung defense mechanisms. In animal models, exposure to lipopolysaccharide or endotoxin decreases lung clearance of a bacterial challenge.[35] Infection with human immunodeficiency virus (HIV) compromises many of the components of pulmonary host defense. Quantitative defects involve the naïve CD4 T cells initially, with the memory CD4 T cells depleted more rapidly later in infection. Functional defects caused by the virus include impaired response to remote recall antigens, inhibited response to soluble antigen followed in time by decreased T-cell response to alloantigens and mitogens, impaired IL-2 and interferon-γ production, and decreased immunoglobulin production.[36,37] In BALT, destruction of dendritic cells and degeneration of lymphoid follicles have been noted. Defective antigen presentation by dendritic cells has also been observed. Abnormal chemotaxis, phagocytosis, and oxidative metabolism in neutrophils of patients with acquired immunodeficiency syndrome (AIDS) have been described.

A variety of commonly prescribed drugs including aspirin, erythromycin, and aminophylline have been shown to alter host defenses in vitro or in models, but the clinical significance of this is uncertain.[38,39] Data with macrolides suggest that they have immunomodulatory activity that could have beneficial effects in some settings.[40,41] Other classes of agents including proton pump inhibitors, histamine type 2 (H2) receptor antagonists, and antipsychotic agents have been associated with pneumonia in population-based studies, although the associations have been challenged and the exact pathophysiologic mechanisms have not been determined.[42–44]

Other factors that impair pulmonary host defenses include hypoxemia, acidosis, toxic inhalations, particulate air pollutants, pulmonary edema, uremia, malnutrition, immunosuppressive agents, and mechanical obstruction.[45,46] Recent clinical studies have also shown an increased risk of pneumonia, with therapeutic hypothermia now being used for management of cardiac arrest and head trauma.[47]

Older adults are at increased risk for the development of pneumonia (see Chapter 310). Although numerous factors play an important role in this regard, including an increased number and increased severity of underlying diseases and an increased number of hospitalizations, there are age-related impairments in host defenses.[48] Less effective mucociliary clearance and abnormal elastic recoil may lead to less effective coughing and clearing of the upper airways. Some populations of elderly patients have an increased incidence of microaspiration. Changes in humoral immunity and cell-mediated immune function have been documented in older persons, although their role in the development of infection remains unclear. Immune dysregulation has been shown to occur in the elderly such that low-grade inflammation occurs in the lung in the absence of clinically detectable infection.

Recurrent episodes of bacterial pneumonia suggest the presence of specific predisposing factors.[49–51] In children and young adults, recurrent pneumonia is associated with defects in host defenses, including recurrent aspiration, asthma, congenital cardiac or pulmonary disease, and altered immune function.[52–55] Congenital defects in ciliary activity and CF are other clinical entities associated with recurrent pneumonia in young persons.[56,57] Structural lung abnormalities such as bronchiectasis and pulmonary sequestration are also important predisposing factors for both younger and older patient populations. As more has become known about the molecular basis of the inflammatory response, it has become clear that a variety of genetic polymorphisms exist that are associated with predisposition to the development of pneumonia. It is important to recognize that these defects may be associated with a narrow range of potential pathogens, which may aid in the identification of the defect.[22,24,55]

Although most congenital defects in host defenses appear in childhood, common variable hypogammaglobulinemia may first appear in

adulthood with recurrent pneumonia. Acquired host defense defects are more varied and include malignancies (lymphoma, chronic lymphocytic leukemia, multiple myeloma), infection (AIDS), and iatrogenic causes (immune suppression associated with solid organ or marrow transplantation, cancer chemotherapy, high-dose corticosteroid treatment, and TNF inhibitors). Underlying respiratory tract disorders such as COPD, bronchiectasis, adult-onset CF, bronchopulmonary sequestration, and tracheobronchiomegaly may manifest with pneumonia. Bronchial obstruction due to intrinsic compression (adenocarcinoma) or extrinsic compression (lymphadenopathy due to sarcoidosis or malignancy) has also been associated with recurrent episodes of pneumonia. Underlying diseases that predispose to aspiration lead to an increased incidence of pneumonia. These may be associated with gastrointestinal diseases (tracheoesophageal fistula, esophageal diverticula, esophageal reflux, esophageal stricture), neuromuscular disorders (myasthenia gravis, dementia, amyotrophic lateral sclerosis), and cancer of the head and neck. Most systemic illnesses, including chronic renal failure, diabetes, and sickle cell disease, have been associated with pneumonia.

CLINICAL EVALUATION

History

The history should attempt to define (1) symptoms consistent with the diagnosis of pneumonia or not, (2) the clinical setting in which the pneumonia takes place, (3) defects in host defense that could predispose to the development of pneumonia, and (4) possible exposures to specific pathogens.

Respiratory symptoms are commonly encountered in primary care practices but are usually not associated with pneumonia and may have a number of infectious and noninfectious causes.[58] Clinicians need to differentiate pneumonia from other clinical entities with which it may be confused. The clinician should ask the patient about symptoms that are often associated with pneumonia including cough, sputum production, dyspnea, chest pain, and fever.[59] They should also ask if patient has hemoptysis, hoarseness, vomiting, or trouble swallowing and should inquire about recent travel, weight change, and smoking. In addition, nonrespiratory symptoms are commonly present including fatigue, sweats, headache, nausea, and myalgia, and occasionally abdominal pain and diarrhea.[60] With increasing age, both respiratory and nonrespiratory symptoms of pneumonia become less frequent. In children younger than 6 years, chest radiographs may reveal pneumonia in the absence of lung findings at physical examination.[61] Unfortunately, symptoms at presentation elucidated by a careful history may not always aid in distinguishing pneumonia from other respiratory problems.

Specific etiologic agents of pneumonia have been associated with certain underlying diseases and patient populations. Pneumonia due to *M. pneumoniae* occurs more often in younger people, but in older patients it may be a cause of pneumonia severe enough to necessitate hospitalization.[62] Gram-negative bacterial pneumonia tends to occur in older adults, especially those who are debilitated with comorbid diseases or are ill enough to require intensive care unit (ICU) care. Tuberculosis should be suspected in persons who have lived in countries where tuberculosis is endemic, are homeless, are infected with HIV, have a history of latent tuberculosis, or have been exposed to others with the disease. Staphylococcal pneumonia classically has been noted during epidemics of influenza,[63] yet was shown to cause only 1.6% in a study of over 2000 adults in the United States with CAP, with only 0.7% of the total cohort being due to methicillin-resistant *Staphylococcus aureus* (MRSA).[64] Despite this fact, the study noted that 30% of the adults received antibacterials targeting MRSA.

Pneumonia has been noted to occur with increased frequency in patients with a variety of underlying disorders such as congestive heart failure, diabetes, alcoholism, and COPD. In one series of 292 patients with pneumonia, only 18% were found to have no underlying disease.[65] Certain lifestyle factors have also been associated with an increased risk of pneumonia. These include cigarette smoking, alcohol use (especially in males), contact with children and pets, and living in a household with more than 10 people.[66] Viral upper respiratory tract infections can predispose to pneumonia, and may be associated with more severe disease.[67,68] Recent dental manipulations, sedative overdoses, seizures, alcoholism, or loss of consciousness for any reason should raise the suspicion of anaerobic infection caused by aspiration of oral contents.[26]

Special note needs to be made of the relationship between pneumonia and patients with COPD.[69] Although well-controlled studies are lacking, it does appear that patients with COPD have an increased incidence of pneumonia. However, because the tracheobronchial tree is often colonized with *Streptococcus pneumoniae* and *H. influenzae,* it has been difficult to distinguish clearly between colonization and infection in many studies. Although these organisms play a key role as etiologic agents of pneumonia in this patient population, most of the clinical studies were carried out before it was recognized that other, less common pathogens including *Moraxella catarrhalis, Legionella, Chlamydia,* and aerobic gram-negative rods including *P. aeruginosa* also play a significant role in causing disease.[69–71] Patients with CF more often have pneumonia due to *Pseudomonas* and staphylococci,[57] and *Burkholderia* spp., *Stenotrophomonas* spp., *Achromobacter xylosoxidans,* and atypical mycobacteria. Pulmonary alveolar proteinosis can be associated with *Nocardia* infection.

Patients infected with HIV are at high risk for the development of pulmonary infections.[72–75] Although the incidence of pneumonia has decreased notably in the developed world with the advent of highly active antiretroviral therapy, pneumonia remains a common HIV complication. Principle risk factors for pneumonia in this population include low current CD4 count, nadir CD4 count, injection drug use, smoking, increasing age, and lack of highly active antiretroviral therapy and anti-*Pneumocystis* prophylaxis.[73,76] In considering the etiology of pulmonary infection in patients infected with HIV, providers should consider geographic exposures, demographic characteristics of the patient, and the degree of immune suppression. With the development of highly active antiretroviral therapy and effective prophylactic strategies, the incidence of *Pneumocystis jirovecii* pneumonia in patients with AIDS has decreased to less than 1 per 100 patient-years from 70% to 80%.[77] It is now predominantly seen in individuals who have a CD4 count less than 100 per mm^3 and are either unaware of having HIV infection or are not receiving care.[75] Bacterial pneumonia was a significant complication for HIV-infected individuals in the preantiretroviral era, with an incidence 5- to 10-fold that seen in the general population, and the incidence of invasive pneumococcal disease was more than 50-fold higher in in HIV-infected patients than in non–HIV-infected controls.[76,78] The incidence of these infections has now decreased, although there remains a high risk in patients not on treatment and in those who present unaware of their HIV infection.[75,77] The incidence of pneumonia due to *P. aeruginosa* and *S. aureus* has also been notably higher in HIV-infected patients.[75] Although relatively less common in the developed world, in developing countries, *Mycobacterium tuberculosis* is now viewed as the major pulmonary pathogen in patients with AIDS.[74] The use of highly active antiretroviral therapy has led to a decreased incidence of disease, but in endemic settings its overall importance as a pulmonary pathogen remains.[79] In the severely immunosuppressed HIV population, fungal infections can play a major role, and depending on the patient's exposure history, cryptococcosis, histoplasmosis, blastomycosis, and coccidioidomycosis should be considered. *Pneumocystis* and disseminated tuberculosis are associated with CD4 counts below 200/mm^3, and disseminated nontuberculous mycobacterial and fungal infections occur with CD4 counts less than 50 to 100/mm^3.[80] Pulmonary infections in HIV-infected patients are discussed in more detail in Chapter 123.

Pneumonia developing in hospitalized patients may involve Enterobacteriaceae, *P. aeruginosa,* and *S. aureus,* organisms that are unusual in community-acquired disease.[81] Pneumonia in older adults, especially those who are bedridden or who have chronic diseases, had been felt to be more often associated with gram-negative bacilli than is pneumonia in younger populations, but this association remains unclear.[82,83] In general, elderly patients most frequently have infection due to *S. pneumoniae*, nontypeable strains of *H. influenzae,* or *M. catarrhalis* or aspiration pneumonia. Pneumonia is a manifestation of aging: the annual incidence of pneumonia necessitating hospital admission rises with age, and those aged 80 years or older have the highest rate (164 per 10,000 adults).[84] It has been recognized that patients with outpatient contact with the health care system develop pneumonia with etiologic

TABLE 67.3 Pneumonia: Etiology Suggested by Exposure History

EXPOSURE HISTORY	INFECTIOUS AGENT
Exposure to concurrent illness in school dormitory or household setting	*Neisseria meningitidis,* Mycoplasma pneumoniae
Environmental Exposures	
Exposure to contaminated aerosols (e.g., air coolers, hospital water supply)	Legionnaires' disease
Exposure to goat hair, raw wool, animal hides	Anthrax
Ingestion of unpasteurized milk	Brucellosis
Exposure to bat droppings (caving) or dust from soil enriched with bird droppings	Histoplasmosis
Exposure to water contaminated with animal urine	Leptospirosis
Exposure to rodent droppings, urine, saliva	Hantavirus
Potential bioterrorism exposure	Anthrax, plague, tularemia
Zoonotic Exposures	
Employment as abattoir worker or veterinarian	Brucellosis
Exposure to cattle, goats, pigs	Anthrax, brucellosis
Exposure to ground squirrels, chipmunks, rabbits, prairie dogs, rats in Africa or southwestern United States	Plague
Hunting or exposure to rabbits, foxes, squirrels	Tularemia
Bites from flies or ticks	Tularemia
Exposure to birds (parrots, budgerigars, cockatoos, pigeons, turkeys)	Psittacosis
Exposure to infected dogs and cats	*Pasteurella multocida,* Q fever *(Coxiella burnetii)*
Exposure to infected goats, cattle, sheep, domestic animals, and their secretions (milk, amniotic fluid, placenta, feces)	Q fever *(C. burnetii)*
Travel Exposures	
Residence in or travel to San Joaquin Valley, southern California, southwestern Texas, southern Arizona, New Mexico	Coccidioidomycosis
Residence in or travel in Mississippi or Ohio river valleys, Caribbean, central America or Africa, South Asia	Histoplasmosis, Blastomycosis
Residence in or travel to southern China	SARS, avian influenza
Residence in or travel to Arabian peninsula	MERS-CoV
Residence in or travel in Southeast Asia	Paragonimiasis, Melioidosis
Residence or travel to West Indies, Australia, or Guam	Melioidosis

MERS-CoV, Middle Eastern respiratory syndrome coronavirus; *SARS,* severe acute respiratory syndrome.

agents that may be seen in both CAP and nosocomial pneumonia.[85–87] See further discussion under "Pneumonia Syndromes."

Important aspects of a patient's history that may suggest specific potential infectious agents include occupational, animal, and travel history (Table 67.3). A carefully obtained history may also suggest the presence of noninfectious pulmonary disease, such as tumors, sarcoidosis, granulomatosis with polyangiitis (GPA; previously known as Wegener granulomatosis), or pulmonary emboli, all of which may masquerade as pneumonia. Patients with a history of several episodes of "pneumonia" with abnormal imaging that does not resolve need to be evaluated for a noninfectious cause.

Physical Examination

Most, but not all patients with acute pneumonia appear ill, although the elderly can appear apathetic. Fever is reported to be present in 65% to 90% of patients with pneumonia. It may be sustained, remittent, or at times hectic. Fever patterns per se, however, are not useful for establishing a specific diagnosis. Oral temperature assessment should be avoided to reduce error caused by rapid mouth breathing. Recording of postural changes in blood pressure and pulse rate is useful in assessing hydration and intravascular fluid volume. The pulse usually increases by 10 beats per minute for every degree (centigrade) of temperature elevation. A pulse-temperature deficit (e.g., a relative bradycardia for the amount of fever) should suggest viral infection, mycoplasmal infection, chlamydial infection, tularemia, or infection with *Legionella.* Cyanosis, a rapid respiratory rate, the use of accessory muscles of respiration, sternal retraction, and nasal flaring suggest serious respiratory compromise.

Cutaneous abscesses or "track marks" from injection drug use may signal a source of bacteremia with subsequent pneumonia or septic emboli to the lung via hematogenous spread. Bullous myringitis is an infrequent but significant finding in mycoplasmal pneumonia (was seen in volunteers given intranasal infections). The presence of poor dentition should suggest a mixed infection due to aspiration of anaerobes and aerobes that colonize the oropharynx. Although edentulous patients may develop anaerobic pneumonia as a result of aspiration, it is uncommon.[88]

Examination of the thorax may reveal "splinting," or an inspiratory lag on the side of the lesion, that is suggestive of bacterial pneumonia. Deep breathing can provoke cough. Early in the disease process, definitive signs of pulmonary involvement may be lacking or may manifest only as fine rales. Chest examination may reveal these early signs of pneumonia even though the chest film is normal. Evidence of consolidation (dullness on percussion, bronchial breath sounds, and egophony [E to A changes]) is highly suggestive of bacterial infection but may be absent in two-thirds of patients ill enough to be hospitalized and may be absent more often in patients treated as outpatients.[89] Patients with *Mycoplasma, Pneumocystis,* tuberculosis, or viral infection may exhibit few abnormalities at physical examination despite the presence of impressive infiltrates on chest images.

The overall usefulness of the history and physical examination to detect the presence of pneumonia has been questioned.[90] The probability of detecting pneumonia varies with the patient population, the prevalence of pneumonia in that population, the threshold values for defining a vital sign as abnormal, and the ability of the clinician to detect abnormal physical findings. However, a great deal of interobserver variation has been shown to exist. In one series, three examiners seeing the same patients could not consistently agree on the physical examination findings. The diagnosis of pneumonia could be made with a sensitivity of only 47% to 69% and with a specificity of 50% to 75%.[90]

Rare findings such as egophony and asymmetrical chest movements have a high predictive value for pneumonia, but occur so infrequently that they are of limited usefulness. Several studies have assessed the use of clinical prediction rules for determining the presence or absence of pneumonia based on multiple physical findings.[91] The absence of any vital sign abnormalities (i.e., respiratory rate >20 breaths/min, heart rate >100 beats/min, and temperature >37.8°C) has been associated with a less than 1% chance of a patient's having pneumonia, assuming a pneumonia prevalence of 5% in the population under study. In contrast, a constellation of cough, fever, tachycardia, decreased breath sounds, and crackles raises the possibility that pneumonia is present to 40% to 50%. Therefore, although variable and nondefinitive, a complete history and physical examination may be extremely helpful in guiding the workup of pneumonia (see Table 67.3).

Diagnostic Testing

Clinical features derived from a careful history and physical examination and confirmed with radiographs of the chest that show a pulmonary infiltrate suggest the presence of pneumonia. The role of microbiologic tests to identify the specific cause is an important although controversial element of care. Most empirical antibiotic regimens are successful in the therapy of CAP, especially mild-to-moderate cases. Studies comparing empirical therapy and laboratory-guided pathogen-directed care have shown no differences in efficacy, although increased side effects were noted in the patients receiving empirical therapy.[92] Efforts to determine the specific cause of CAP are justified by the fact that they (1) may enable the clinician to narrow the antibiotic spectrum and to use fewer

agents, thereby decreasing exposure of the patient to potential side effects and potentially reducing the development of resistance; (2) may aid in the specific antibiotic choice for an individual patient depending on the specific epidemiology of infection and the specific resistance patterns of the locale; and (3) may reveal pathogens not usually suspected and therefore not usually covered by empirical therapy. On a broader scale, identifying specific causes may help define new agents, trends in antibiotic resistance in established agents, and epidemiology of infectious outbreaks. The combined use of the standard microbiologic testing in conjunction with nucleic amplification assays can define the cause of CAP in up to 89% of cases as compared with only 39% with culture.[93] However, molecular techniques to identify bacteria are not widely available, add to the cost of care, and include falsely positive findings that represent colonization rather than disease.[94] Guidelines from the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) have suggested diagnostic testing "whenever the result is likely to change individual antibiotic management" or in patients in whom "the diagnostic yield is thought to be greatest."[95]

Sputum Examination and Examination of Other Respiratory Tract Samples

Microscopic examination and culture of expectorated sputum remain the mainstays of the laboratory evaluation of pneumonia despite ongoing controversy concerning their sensitivity and specificity. Of patients admitted to the hospital with CAP, 40% to 60% will not be able to produce sputum. Of those who do, approximately 40% to 60% of samples may be judged to be inadequate for further study because of oropharyngeal contamination.[96,97] Many patients have received antibiotics before the studies are carried out, which drastically reduces the diagnostic yield. A variety of organisms cannot be detected with Gram stain, including *Legionella* spp., *Mycoplasma* spp., and *Chlamydia* spp. However, in patients who produce sputum of adequate quality to be examined (minimal or no oropharyngeal contamination), and who have not received prior antibiotics, diagnostic yields of 80% for sputum Gram stain have been reported in the small fraction of patients with bacteremic *S. pneumoniae* pneumonia.[98] Despite its pitfalls, the sputum Gram stain is noninvasive, can be carried out at no risk to the patient, and under the right circumstances may aid in the diagnosis and choice of empirical therapy in patients with CAP.[99,100] For example, the absence of MRSA in sputum culture or nasal swab may allow the clinician to stop empirical therapy for this pathogen.[101]

Examination of the sputum should include observation of the color, amount, consistency, and odor of the specimen. Mucopurulent sputum is most commonly found with bacterial pneumonia or bronchitis. However, sputum of a similar nature has been described in one-third to one-half of patients with mycoplasmal or adenovirus infections.[102] Scant or watery sputum is more often noted with these and other atypical pneumonias. "Rusty" sputum suggests alveolar involvement and has been most commonly (although not solely) associated with pneumococcal pneumonia.[103] Dark red, mucoid sputum (currant-jelly sputum) suggests Friedlander pneumonia caused by encapsulated *Klebsiella pneumoniae* (Fig. 67.1).[104] Foul-smelling sputum is associated with mixed anaerobic infections most commonly seen with aspiration or lung abscess.[88]

To maximize the diagnostic yield of the sputum examination, most microbiology laboratories use a scoring system to process only samples with minimal oropharyngeal contamination and reject those with likely oral pharyngeal contamination. There are no definitive guidelines, but the number of neutrophils and epithelial cells should be quantitated under low power (×100), with further examination reserved for samples containing 25 or more neutrophils and 10 or fewer epithelial cells.[105] Samples with more epithelial cells and fewer neutrophils are usually nondiagnostic and should be discarded. These criteria are problematic in neutropenic patients, and require notification of the laboratory if the provider believes that the culture should be processed. Morphologic and staining characteristics of any bacteria seen should be recorded and an estimate made of the predominant organisms (Figs. 67.2 through 67.6). When no bacterial predominance exists, this should also be noted.

In the appropriate clinical setting, a predominance of gram-positive, lancet-shaped diplococci should suggest pneumococcal infection (see Fig. 67.2). When strict criteria for Gram stain positivity are used (the finding of a predominant organism or more than 10 gram-positive, lancet-shaped diplococci per oil immersion field [×1000], or both), the specificity of the Gram stain for identifying pneumococci has been shown to be 85%, with a sensitivity of 62%.[106] Because pneumococci may be part of the nasopharyngeal microbiota in 10% to 50% of healthy adults and often colonize the lower airways in patients with chronic bronchitis, identification of the organism does not mean that it is the cause of disease.[107] However, it is our experience that the large number of pneumococci necessary to produce a positive Gram stain is unusual in carriers.

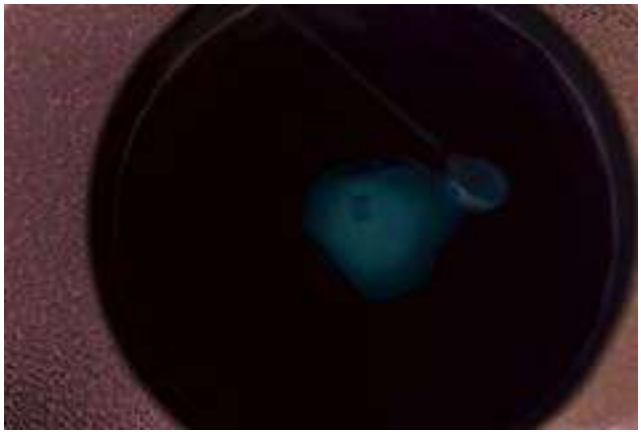

FIG. 67.1 **"Currant-jelly" sputum associated with *Klebsiella pneumoniae* pneumonia.**



FIG. 67.2 **Expectorated sputum with gram-positive, lancet-shaped diplococci from a patient with pneumococcal pneumonia.**



FIG. 67.3 **Expectorated sputum demonstrating a positive quellung reaction in a patient with pneumococcal pneumonia.**

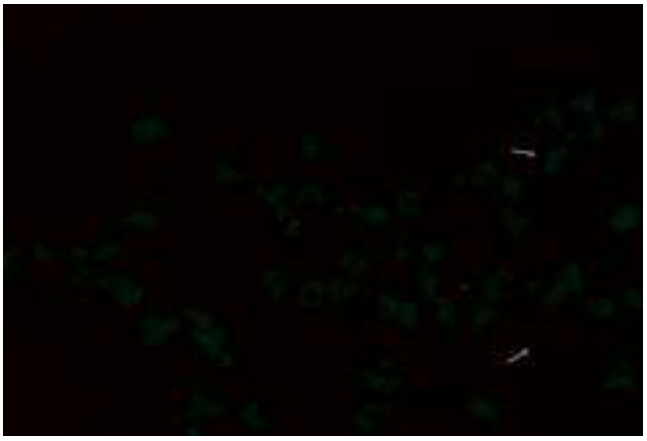

FIG. 67.4 **Expectorated sputum with gram-negative coccobacillary forms *(arrows)* from a patient with *Haemophilus influenzae* pneumonia.**

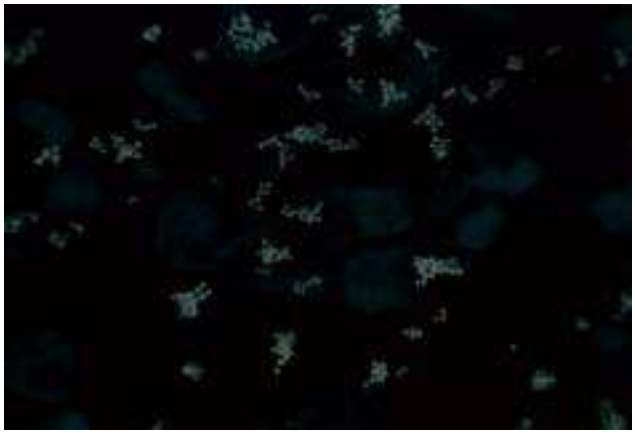

FIG. 67.5 **Expectorated sputum with clusters of gram-positive cocci in a patient with *Staphylococcus aureus* pneumonia.**

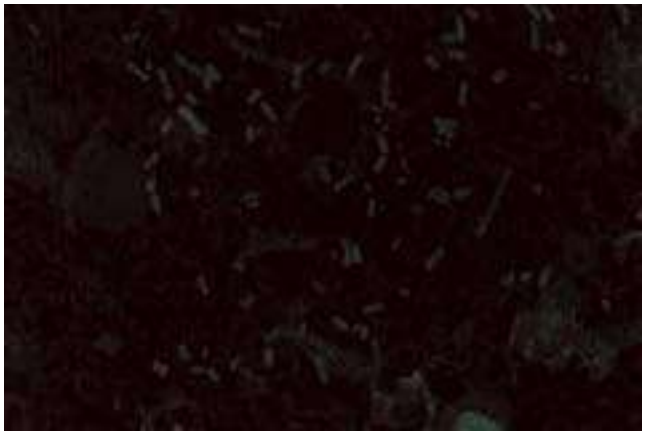

FIG. 67.6 **Expectorated sputum with gram-negative rods in a patient with *Klebsiella pneumoniae* pneumonia.**

Microscopic sputum examination can be helpful to identify organisms other than pneumococci. The finding of small gram-negative coccobacillary organisms on sputum Gram stain is characteristic of *H. influenzae* (see Fig. 67.4). However, the sensitivity of the sputum Gram stain for detecting *H. influenzae* is usually less than that for *S. pneumoniae* and has been reported to be 40% to 80%. Staphylococci appear as gram-positive cocci in tetrads and grapelike clusters (see Fig. 67.5). Organisms of mixed morphology are characteristic of anaerobic infection. Few bacteria are seen with Legionnaires' disease, *Mycoplasma* pneumonia, and viral pneumonia. Examination of induced sputum obtained after patients undergo nebulizer treatment with 3% saline solution and PCR techniques on oropharyngeal wash has been a useful means of diagnosing *Pneumocystis* pneumonia in patients with AIDS[108] and may be useful in other immunocompromised patients who have trouble producing sputum. Special sputum staining techniques are important in identifying other organisms such as mycobacteria (Fig. 67.7) or *Nocardia* species.

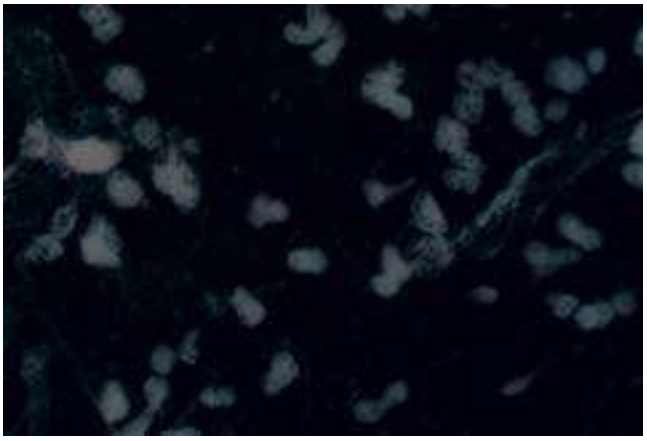

FIG. 67.7 **Expectorated sputum with acid-fast bacilli in a patient with *Mycobacterium tuberculosis* infection.**

Sputum culture as a means of diagnosing pneumonia is as controversial as the sputum Gram stain. Not all patients with pneumonia will produce sputum. Even when they do, studies of patients with bacteremic pneumococcal pneumonia have found sputum culture positivity rates varying between 29% and 94%.[98] Similarly, only 35% to 73% of sputum cultures are positive with proven *H. influenzae* pneumonia.[109,110] Both *S. pneumoniae* and *H. influenzae* are relatively fastidious and the sensitivity of cultures decreases with the prior use of antibiotics or with delays in transport of specimens to the clinical microbiology laboratory. Beyond these concerns with test sensitivity, sputum cultures have frequently been shown to yield more bacterial species than more invasive methods of obtaining respiratory tract secretions.[111]

Several key parameters have been identified in efforts to maximize the diagnostic yield from sputum culture. Procurement of adequate sputum samples is an essential first step. With increasing numbers of epithelial cells and decreasing numbers of neutrophils, an increased amount of oropharyngeal contamination is present, as indicated by the isolation of more bacterial species. The presence of alveolar macrophages does not alter the bacteriologic findings when substantial numbers of epithelial cells are present, indicating that otherwise adequate samples of sputum can be contaminated with oropharyngeal contents and thereby rendered nondiagnostic. This type of initial screening has proved helpful in differentiating adequate sputum samples from saliva, thereby increasing the diagnostic yield of sputum culture.

When culture of sputum is delayed, the isolation of pneumococci is less likely because of overgrowth by oropharyngeal microbiota. Rapid processing of samples is therefore another factor leading to higher diagnostic yield. Some reports suggest that with adequate sputum samples and prompt culture of specimens, the diagnostic yield of the sputum culture may be improved.[98]

Antigen detection in respiratory secretions has been used for over several decades to try to maximize the diagnostic yield of sputum, especially for infections caused by *S. pneumoniae, Pneumocystis, Legionella pneumophila,* and a variety of respiratory viruses. The direct fluorescent antibody assays for *L. pneumophila* and *P. jirovecii* are the most commonly used, with sensitivities of 25% to 75% for *Legionella* and 80% for *Pneumocystis.*[112,113] The sensitivity for *Pneumocystis* may be less for patients with causes of immunosuppression other than HIV disease.[114] Non-*pneumophila* and *pneumophila* non–serogroup 1 strains of *Legionella* may be missed in these assays, and the test needs to be performed by experienced technologists. For other organisms such as *Chlamydia*, problems with colonization versus infection, varying sensitivities, and cross-reactivity with nonpathogens have limited the usefulness of the study.

Detection of microbial nucleic acid in respiratory tract secretions, both nasopharyngeal secretions and sputum, remains an area of ongoing study.[115–118] Nucleic acid amplification assays, especially polymerase chain reaction (PCR) assays, are particularly attractive because they have the capability of detecting minute amounts of material from potential pathogens, do not appear to be greatly influenced by prior antibiotic therapy, and can be performed quickly. Although a variety of PCR techniques have been described, US Food and Drug Administration (FDA)–licensed assays exist for only *M. tuberculosis, Legionella* spp., and respiratory viruses. Assays for more commonly encountered organisms such as *S. pneumoniae, H. influenzae,* and *Mycoplasma* and *Chlamydia* spp. have been developed, and study protocols have advanced our understanding of the etiology of pneumonia, but lack of standardization and also cost have limited usefulness clinically. The FDA has approved multiplex PCR techniques that test for over 20 viral and bacterial pathogens with use of nasopharyngeal swabs and that can be performed at the point of care. Further studies have been proposed in order to investigate the usefulness of such testing in clinical practice.[118] PCR assays can also detect *P. jirovecii,* although the usefulness of PCR remains under debate because it appears sufficiently (overly) sensitive to detect asymptomatic colonization in addition to clinical disease, and a quantitative PCR technique will be necessary for clinical usefulness.[119] PCR techniques have been used to identify DNA from *M. tuberculosis* in both sputum and lavage fluid. Sensitivities of 90% to 100% have been seen with patients who are acid fast bacilli (AFB) smear positive, and 50% to 70% in patients who are AFB smear negative even in low-incidence settings; specificities as high as 99% have been noted.[120,121] However, PCR assay results may remain persistently positive in patients recently treated for tuberculosis and with no apparent active disease. Several individual pathogen and multiplex real-time PCR assay systems have become commercially available for the detection of community respiratory viruses.[116] The test systems differ for viral pathogens that they detect. Available assays can detect influenza A, influenza B, parainfluenza viruses, respiratory syncytial viruses (RSVs), human metapneumovirus, coronaviruses, adenovirus, rhinovirus or enterovirus, and bocavirus in respiratory secretions with high sensitivity and specificity for the presence of viral nucleic acid. However, it is unclear if positive results indicate upper rather than lower respiratory tract infection, colonization, or true infection of the lung; or even the presence of infectious virus particles. These molecular assays have clear utility for research purposes.[67,93] However, they remain expensive, and although they may be of benefit in the management of severely ill hospitalized patients and in select clinical settings, the cost-effectiveness for the general management of acute pneumonia has not yet been defined.

Fiberoptic Bronchoscopy

Although the sputum examination should always be included in the initial evaluation of patients with pneumonia, it may be inadequate for a presumptive diagnosis, particularly in the immunocompromised host or the patient on mechanical ventilation, in whom there is a broader range of potential pathogens. Fiberoptic bronchoscopy allows for the collection of lower respiratory tract cultures through the use of protected brush catheters, and the performance of bronchoalveolar lavage (BAL) or transbronchial biopsy or both.[122] BAL, in which a segment of the lung is washed with sterile fluid, samples approximately 100 million alveoli and consequently enables examination of a larger segment of the lung than either the protected specimen brush or a transbronchial biopsy.

The use of the protected brush catheter and quantitative culturing of material obtained from the procedure have both minimized the problem of oropharyngeal contamination and helped to differentiate colonization from true infection. Approximately 10^6 to 10^8 organisms per milliliter are present in lung tissue involved with pneumonia. Accounting for dilution of samples, a bacterial count of more than 10^3 to 10^4 has been used as a breakpoint for determining the clinical significance of an isolate. When studied prospectively early in the course of CAP, bronchoscopy has yielded a diagnosis in approximately 50% of patients.[123]

Bronchoscopy with a protected specimen brush has been shown to have sensitivities as high as 82% to 100% and as low as 36%, with specificities as high as 60% to 77% and as low as 50% for the diagnosis of bacterial pneumonia.[124–126] Differences in exclusion and inclusion criteria, different definitions of pneumonia, and the acceptance or rejection of patients with recent antibiotic changes may explain the different results.[127] The use of antibiotics markedly diminishes the diagnostic yield of the procedure. Most bacterial species initially found with a protected specimen brush are undetectable after 72 hours of antibiotic therapy, and the majority of organisms found are resistant to the antibiotics given. These may have no role in the infection. However, in a patient with ongoing pneumonia despite antibiotic therapy, bronchoscopy with a protected specimen brush should pick up resistant organisms that may be playing a role in infection.[124] BAL has also been used for the diagnosis of atypical pneumonias, including those caused by *Legionella* species and *M. pneumoniae.* In research settings, BAL with multiplex PCR may be helpful and lead to targeted treatment and improved antibiotic stewardship.[128] Only the specific nasal pharyngeal tests for *Chlamydia* and *Mycoplasma* are FDA approved, but the broadly diagnostic BioFire panel has also been approved.

Bronchoscopy with BAL has been particularly valued for the immunocompromised host including patients with AIDS. In patients with AIDS, diagnostic yields for *Pneumocystis* pneumonia of 89% to 98% have been reported.[129] Excellent yields have also been noted in detecting cytomegalovirus (CMV) in patients with AIDS and in bone marrow and solid organ transplant recipients, although detection of this agent alone does not prove it the cause of pneumonia because it frequently blooms in immunocompromised hosts; most patient with CMV pneumonitis have evidence of CMV by PCR in blood.

BAL has also been shown to be useful for diagnosis of pulmonary *M. tuberculosis* and fungal infections. Culture of BAL material has a sensitivity of approximately 85% for *M. tuberculosis,* even in the setting of negative culture of expectorated sputum and gastric aspirate samples.[130] With use of strict diagnostic definitions, PCR and galactomannan assays on BAL have approximate sensitivities and specificities of 77% and 93% for invasive pulmonary aspergillosis.[131] Bronchoscopy with calcofluor staining and fungal culture can also be helpful in the diagnosis of pulmonary histoplasmosis, cryptococcosis, and coccidioidomycosis.[122,132,133]

Both bronchoscopy and BAL have been used widely in patients with ventilator-associated pneumonia (VAP).[134] The IDSA/ATS guideline on VAP recommends noninvasive sampling or endobronchial aspiration to help determine the specific bacteriologic cause. However, a prospective multicenter trial found that the use of bronchoscopy with BAL and quantitative culture did not improve clinical outcomes as compared with nonquantitative culture of endotracheal secretions in patients with suspected VAP.[135]

Bronchoscopy is not without risk. It can induce respiratory failure and the need for mechanical ventilation in hypoxemic patients. There is a risk of bleeding with both the use of protected brush catheters and transbronchial biopsies, in addition to the lesser risk of pneumothorax. In patients with gram-negative pneumonia, a sepsis-like picture with increased temperature and decreased mean arterial pressure may follow the procedure. It should not usually be considered in patients with CAP unless the infection is severe or unresolving, or if there is a clear failure of antibiotic therapy. This might suggest an occult process such as an obstructing malignancy, a minor obstructing lesion, or a foreign body not seen at diagnostic imaging.[122]

Other Techniques

A variety of less invasive techniques have been used in attempts to determine the cause of pneumonia without resorting to bronchoscopy. Blind endotracheal suctioning with quantitative cultures has compared favorably with bronchoscopic procedures in investigation of VAP in some studies.[136] With a threshold of greater than 10^5 colony-forming units (CFUs) per milliliter, the sensitivity for predicting VAP was comparable to that of lavage or protected brush procedures, although the specificity was somewhat lower.[136] Furthermore, no differences in mortality, length of stay in the ICU, or duration of mechanical ventilation were noted when quantitative endotracheal cultures were used as the sole means of diagnosis compared with BAL and with protected specimen brush. Others have reported false-negative rates of over 30% and many more organisms isolated with endotracheal suctioning than with

brushing.[137] In addition, in the setting of VAP, concern remains about sampling error and the potential for differing pathogens in different lung segments. At present, none of these techniques has been shown to increase the accuracy in diagnosing VAP, and studies of clinical outcome have found that mortality from VAP is unchanged independent of whether bronchoscopic or nonbronchoscopic procedures are used for diagnosis.[138,139]

Lung Biopsy

Direct means of obtaining diagnostic material in patients with pneumonia include percutaneous lung aspiration, transbronchial lung biopsy, video-assisted thoracoscopy, and open lung biopsy. These procedures are usually reserved for cases of severe pneumonia in impaired hosts and in pediatric populations, in whom sputum is not routinely available.

Biopsy procedures are rarely indicated in the previously well patient with acute pneumonia. The indications and usefulness of these invasive procedures remain controversial. Computed tomography (CT)–guided percutaneous lung aspiration has been shown to be effective in diagnosing focal fungal infections in the transplant population.[140] Open lung or video-assisted lung biopsy remains the definitive invasive procedure for making an etiologic diagnosis of pneumonia in immunosuppressed patients, with diagnostic yields of 60% to 100%.[141,142] In immunocompromised patients, the incidence of unexpected diagnoses that can lead to a change in treatment can be over 50%.[143] The incidence of pneumothorax and bleeding is usually less than 10%,[141] although the overall complication rate is 22% to 28% in patients with acute respiratory distress syndrome (ARDS) or after solid organ transplantation.[143]

Examination of Pleural Effusions

The characteristics of pleural effusions and their importance in the differential diagnosis of pulmonary disease are discussed in Chapter 68. Pleural effusion or parapneumonic effusion will occur in 20% to 40% of hospitalized patients with pneumonia, and the incidence of severe pleural involvement has been increasing in recent years.[144–146] The incidence of pleural effusions associated with pneumonia varies with the etiologic agent, from approximately 40% to 57% with pneumococci, to 50% to 70% with gram-negative bacilli, and up to 95% with β-hemolytic streptococci.[103,147] Pleural fluid cultures, when positive, are specific for the organism causing the underlying pneumonia. Furthermore, analysis of pleural fluid may play a major role in determining when drainage is necessary and in differentiating other causes of pulmonary infiltrates that may mimic bacterial pneumonia, including tuberculosis, tumors, pulmonary emboli, and collagen vascular diseases. Thus, pleural fluid analysis should be strongly considered in patients with apparent pneumonia and pleural effusion when the radiologic findings are not consistent with fluid overload. If neutrophils are not the predominant cell type seen in the pleural space, a diagnosis other than bacterial pneumonia should be sought. Pleural biopsy specimens from patients with acute bacterial pneumonia are nonspecific and are therefore of little use in the differential diagnosis.

Parapneumonic effusions can be divided into three stages.[144,147] The first stage, or exudative stage, is culture negative, has a pH of greater than 7.2, glucose greater than 60 mg/dL, and a lactate dehydrogenase (LDH) level that is less than three times the upper limit of normal. This stage is due to pulmonary interstitial fluid entering the pleural space and increased permeability of the capillaries in the pleura. These uncomplicated pleural effusions usually resolve with therapy for the underlying disease. Without appropriate therapy, pleural effusions become infected with the organisms causing the underlying pneumonia and develop into the second stage or fibropurulent stage. This stage is associated with positive microbial cultures, pH less that 7.2, glucose less than 60 mg/dL, and LDH that is greater than three times the upper limit of normal. Such complicated pleural effusions require drainage. The most sensitive finding in determining whether a pleural effusion needs drainage is a pleural fluid pH less than 7.2. This usually occurs before the other chemical parameters associated with complicated pleural effusions develop.[144] If pH is used to determine if an effusion is to be drained, it must be measured with a blood gas machine, not a pH meter or pH indicator strip, which can be inaccurate. If left untreated, fibropurulent pleural effusions will develop into stage three effusions wherein a thick pleural rind is formed, restricting normal lung expansion.

Empyema is defined as pus in the pleural space and represents a late manifestation of complicated pleural effusions. The presence of empyema mandates draining the pleural space. Complicated pleural effusions can have a positive culture result in up to 24% of cases, making thoracentesis and culture of fluid a valuable means of making an etiologic diagnosis of the underlying pneumonia.[148,149] Other diagnostic tools have proven useful in identifying organisms associated with pleural effusions. PCR technology can be useful in detecting *M. tuberculosis* and in defining the cause in culture-negative cases.[149,150] Adenosine deaminase, an enzyme associated with lymphocytes, may also be used to detect *M. tuberculosis,* with reported sensitivity and specificity of over 90%,[151,152] but actual performance in the field appears to be lower.

Blood Culture, Serologic Studies, and Urine Studies, Including Antigen Detection

Blood cultures are positive in 4% to 17% of patients hospitalized with CAP, with the frequency of positive results increasing with the severity of illness.[93,123,153–155] Some studies have suggested that positive blood cultures add little to the management of patients hospitalized with CAP and are not predictive of increased mortality.[156–158] However, the presence of true positive blood cultures is highly specific, may be helpful in narrowing antibiotic use, and may identify the presence of unusual organisms that would not be adequately covered with routine empirical antibiotic coverage.[159,160] Work has shown that several clinical features can be used to predict patients with a higher likelihood of having bacteremia.[154,155] In particular, patients who have two or more of the findings of chronic liver disease, pleuritic pain, tachycardia, tachypnea, or systolic hypotension and the absence of prior antibiotic therapy have at least a 14% incidence of bacteremia, with a bacteremia incidence of up to 63% in those with four or more of these findings.[155] It is clear that blood cultures should be obtained before antibiotic administration in all patients with CAP who are ill enough to be hospitalized who have two or more of these features, and in those patients who are immunocompromised, who have been admitted with health care–associated pneumonia (HCAP), or who acquire pneumonia in the hospital. The IDSA and ATS have also recommended blood cultures for patients who are admitted to an ICU and have a cavitary lesion, leukopenia, active alcohol abuse, asplenia, a positive pneumococcal urinary antigen, or a pleural effusion.[95] Furthermore, because the cause of pneumonia is not always found, assessment of clinical response to initial therapy is important, and blood cultures should be obtained in patients not responding to antibiotic therapy.[160]

A variety of assays have been used to detect pathogens that have been difficult to isolate with routine culture techniques. Serologic assays have been used to diagnose infections caused by *Legionella* species, *M. pneumoniae, Chlamydia* species, and *Coxiella burnetii.* The sensitivity and specificity of the assays vary, and their overall usefulness for making a rapid diagnosis is limited. The Centers for Disease Control and Prevention (CDC) and the Laboratory Centre for Disease Control (LCDC) have established diagnostic standards for *Chlamydia* assays.[161] Microimmunofluorescence (MIF) for serum chlamydial antigens has been recommended, although enzyme immunoassays (EIAs) are also available and may be more sensitive and specific.[162] For the MIF assay, an IgM titer of greater than 1:16 or a fourfold rise in IgG value is used to define positivity. Use of a single IgG value is not viewed as a definitive test. Because the present assays show day-to-day variation, it has been suggested that acute and convalescent titers be assayed at the same time. A fourfold rise in IgG rather than a single clinical titer is accepted as a positive test for *M. pneumoniae.*[163] Although an elevated IgM titer suggests a recent infection, reinfection with *Mycoplasma* occurs frequently, and a rise in IgM may not always be seen.[164] Cold agglutinins may be elevated in infections with *M. pneumoniae.* Titers greater than or equal to 1:4 are suggestive of *M. pneumoniae* infection. For both mycoplasmal and chlamydial infections, nucleic amplification technologies are being examined as alternative diagnostic modalities, and in general have resulted in notably lower rates of positivity than serologic

studies, suggesting that the latter likely have much lower specificity for acute infection than was previously recognized.[163,165,166]

S. pneumoniae produces a variety of antigens and surface markers that are type or species specific.[167] Although both antigen and antibody detection methods in serum have been studied, none have become clinically significant. PCR techniques have been applied to whole blood for the detection of pneumococci, but the assays remain experimental.[168]

Serum assays for cryptococcal capsular antigen have relatively low sensitivity for cryptococcal pneumonia, but are highly specific and of benefit in the management of immunocompromised patients in addition to immunocompetent individuals suspected of having infection with *Cryptococcus gattii.*[169] Serum assays for (1,3)-β-D-glucan, a component of the cell wall of fungi except for *Cryptococcus* spp. and zygomycetes, have high specificity for invasive fungal infections and can be used for detection of invasive pulmonary aspergillosis in immunocompromised hosts, and for the detection of pneumonia due to the endemic fungi *Histoplasma capsulatum, Coccidioides immitis,* and *Blastomyces dermatitidis* in patients who have appropriate geographic exposure.[170–172] In addition, β-D-glucan is also a component of the cell wall of *P. jirovecii,* and serum assays have a sensitivity of over 95% and specificity over 80% for *Pneumocystis* pneumonia in both HIV-positive and HIV-negative immunocompromised patients.[171,173]

A variety of cytokines are released into the circulation as a result of infection.[174–177] Evidence suggests that these biomarkers may be useful adjuncts in diagnosing pneumonia and predicting severity of disease.[174–177] The calcitonin family of gene products, especially procalcitonin, C-reactive protein (CRP), and soluble triggering receptor expressed on myeloid cells (STREP-1) have been the markers most often associated with pneumonia. Procalcitonin appears to be the earliest marker to appear during the course of infection. Clinical trials have now found that the presence of an elevated procalcitonin level (>0.25–0.5 μg/L) can be used to identify patients requiring treatment for pneumonia, and, based on whether levels fall, how long antibiotics should be continued without increasing the risk of an adverse outcome.[178–180] Still, the usefulness of this marker in antibiotic stewardship programs remain unresolved.[181,182] Procalcitonin has also been used as a gauge of pneumonia-related mortality.[183] CRP is an acute-phase reactant produced in the liver as a response to a variety of stimuli, including infection. Normal values of less than 10 mg/L are unusual in patients with pneumonia and can be used to exclude the diagnosis. Levels of >100 mg/L or greater suggest the diagnosis of pneumonia and have been associated with an increased 30-day mortality and a greater likelihood of need for ventilator or vasopressor support (i.e., severe pneumonia).[177] In comparative trials with procalcitonin, CRP appears to have a better ability to define infection and procalcitonin the clinical severity, although definitive studies are lacking.[176] Other cytokines studied include IL-6 and TNF-α, but their correlations with pneumonia appear less consistent. Cortisol levels have also been shown to predict the severity of pneumonia and the chance of survival.[184,185] Although the clinical trials with procalcitonin have shown a reduction in antibiotic costs, there can be significant expense in performing these biomarker assays, and large-scale randomized studies of their cost-effectiveness are lacking. Thus, their role in diagnosis and severity assessment in pneumonia has not been clearly defined.[182,186]

Antigen detection in urine rather than blood or sputum has become a successful means of detecting some important pulmonary pathogens. Soluble *L. pneumophila* antigen can be detected in urine using a commercially available EIA. Although it is useful only for detecting *L. pneumophila* serogroup 1, this assay offers the advantage of being rapid and noninvasive and has a sensitivity of 80% to 95% and a specificity estimated to be 99% for this serogroup.[112] An additional relative limitation of this assay is that antigenuria may persist for weeks to months after therapy.

An immunochromatographic membrane test has been developed to detect the C polysaccharide cell wall antigen found in all *S. pneumoniae* in urine of patients with pneumonia (Binax NOW).[187] This has been reported to be an extremely useful means of diagnosing pneumococcal pneumonia. With use of a variety of standard diagnostic tests as controls, overall sensitivities of 65.5% to 100%, specificities of approximately 94% to 100%, and positive predictive values of 62% have been noted.[188–190] Sensitivities have, in general, been high in bacteremia episodes, with the yields increased slightly by concentrating the urine. The test is not affected by the prior use of antibiotics. Potential problems with the urinary antigen assay include weakly positive results caused by non-pneumococcal organisms, false-positive results in children with nasopharyngeal carriage rather than true infection, and positive results lasting for weeks after the infection has resolved.[161,189] Shortfalls of the test are that no organism is isolated, and thus no antibiotic susceptibilities can be carried out. In addition, retrospective analysis has not found an impact of the routine use of the test on antibiotic prescribing practices for patients with suspected pneumonia, although in hospitalized patients it may allow deescalation of antibiotics.[191]

Radiologic Examination

Chest radiography plays a critical role in the diagnosis of pneumonia, and it represents the gold standard of making a clinical diagnosis. Demonstration of an abnormal chest radiograph with pulmonary infiltrates consistent with pneumonia differentiates a patient population that may benefit from antibiotic therapy from the populations that will not. Because overuse of antibiotics for therapy of upper respiratory infections has been documented and may contribute to the growing problem of antibiotic resistance, identifying patients who really should be receiving antibiotic therapy is important. The chest radiograph is readily available, is reasonably reliable (despite interobserver variability), and should be obtained in many patients suspected of having pneumonia.[95,192,193] The extent and nature of radiographic abnormalities may define patients who are more seriously ill and may need close monitoring.

The infiltrate patterns found on chest radiographs in patients with pneumonia usually are not helpful in making a specific etiologic diagnosis (Fig. 67.8A–B).[193] However, certain features may be of some diagnostic aid (Table 67.4). Lobar consolidation, cavitation, and large pleural effusions support a bacterial cause (Figs. 67.9 and 67.10). Most lobar pneumonias are pneumococcal, although pneumococcal pneumonias are not necessarily lobar. When bilateral diffuse involvement is noted, *Pneumocystis* pneumonia, *Legionella* pneumonia, or a primary viral pneumonia should be suspected. Staphylococcal pneumonia may result from infection metastasizing from a primary focus unrelated to the lung. In these cases, multiple nodular infiltrates throughout the lung may be seen. Staphylococci may cause marked necrosis of lung tissue with ill-defined thin-walled cavities (pneumatoceles), bronchopleural fistulas, and empyema, especially in children (Fig. 67.11). *S. aureus* producing the Panton-Valentine leukocidin, whether methicillin resistant or not, is associated with necrotizing pneumonia with multilobar cavitary lesions and is frequently associated with pleural effusions and empyema.[194,195] Although pneumatoceles are diagnostically significant findings in staphylococcal pneumonia, they may be seen in pneumonias with other causes, including *K. pneumoniae, H. influenzae, S. pneumoniae,* and, more rarely, *Pneumocystis.* Pulmonary infections due to *Pseudomonas* may cavitate. *Pseudomonas* and other gram-negative bacilli most commonly cause lower lobe pneumonia.

Aspiration pneumonia should be considered along with gram-negative and staphylococcal pneumonias as a source of necrotizing pneumonia, cavitation, and empyema. Aspiration pneumonia commonly involves either the superior segment or the basilar segment of either lower lobe, or the posterior segment of the upper lobes, depending on whether aspiration occurred in the dependent or the upright position. Chronic aspiration most commonly results in bilateral lower lobe pneumonia, although it may involve one side more than the other.

Viral infection of the lower airway involves respiratory epithelium and parenchyma adjacent to terminal respiratory bronchioles. Diffuse hemorrhagic congestion of alveolar septa may occur.[196] The radiographic concomitants of these pathologic findings usually involve patchy areas of peribronchial ground-glass opacity, air-space consolidation, and poorly defined small nodules. Diffuse and localized involvement with both interstitial and alveolar patterns has been noted (Fig. 67.12).[196] There is little radiologic distinction among the various viral causes of pneumonia. Influenza pneumonia is associated with poorly defined, patchy air-space consolidation with rapid confluence. Varicella pneumonia usually involves peribronchial involvement with nodular infiltrates. Adenovirus, herpes simplex, and CMV, all of which are more common in immunocompromised hosts, may be associated with diffuse bilateral

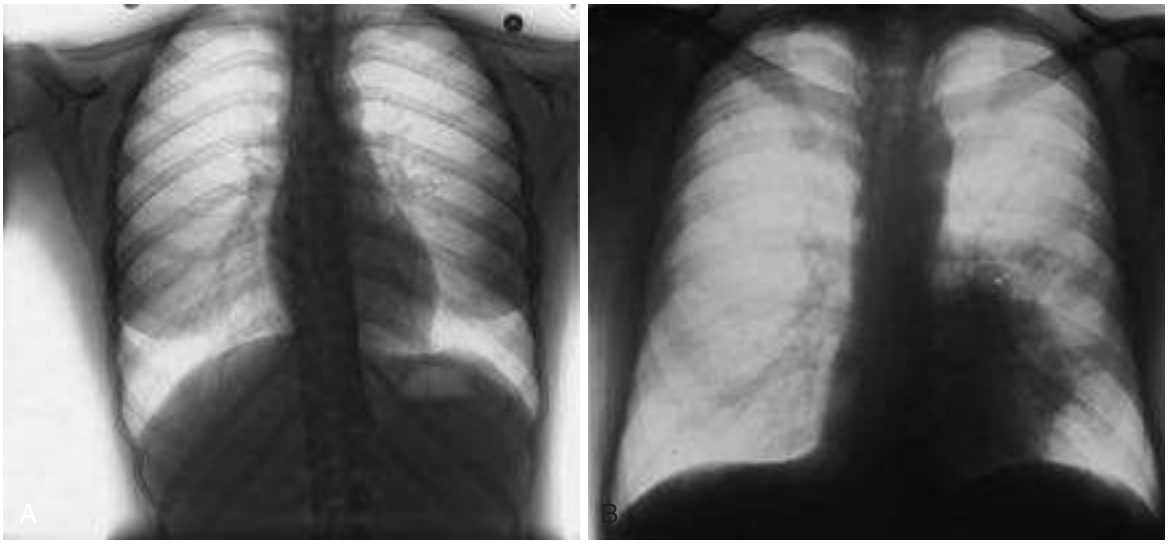

FIG. 67.8 (A) Normal chest radiograph. (B) Patchy infiltrate representing bronchopneumonia in a patient with *Streptococcus pneumoniae* infection.

bronchopneumonia, areas of overinflation, atelectasis, and nodular opacities. Lobar or subsegmental consolidation mimicking bacterial pneumonia may also be seen with adenovirus and herpes simplex. Hantavirus pneumonia usually manifests with interstitial edema, which may progress to consolidation representing a pulmonary capillary leak syndrome. Bilateral involvement and pleural effusion are common and when present are associated with a worse clinical outcome.[197] Both the severe acute respiratory syndrome (SARS) coronavirus and a newer novel coronavirus identified in 2012 can cause pneumonia that begins predominantly with bilateral interstitial basilar infiltrates and progresses to severe symmetrical air-space disease.[198–200] Other recently defined viral pulmonary pathogens are the human metapneumovirus and bocavirus. Most cases of human metapneumovirus involve upper respiratory tract infections in children; pneumonia in adults has been described.[201,202] Multilobar infiltrates have been noted in 50% of cases, and pleural effusions are not uncommon. Bocavirus pneumonia is more frequently reported in children and has been associated with patchy or interstitial infiltrates, similar to the other common respiratory viruses.[203]

Mycoplasmal pneumonia often manifests with an interstitial pattern in a peribronchial and perivascular distribution.[204] Consolidation is noted in approximately 38% of patients, usually in the lower lobe. Once this consolidation stage is reached, radiologic differentiation between bacterial and mycoplasmal pneumonia is difficult. Cavitation is rare, although pleural effusions may be seen in approximately 20% of cases.[204,205] *Chlamydia pneumonia*e predominantly causes unilobar disease with associated air bronchograms.[193]

Legionnaires' disease may initially manifest with a radiographic picture similar to that of mycoplasmal pneumonia. A patchy interstitial or finely nodular pattern is seen in the lower lobe.[206] However, unlike the situation with mycoplasmal pneumonia, pneumonia with more than two-lobe involvement is commonly seen. Rapid progression and pleural effusions are also common. Pneumonia caused by *Legionella micdadei* may manifest with pulmonary nodules, either single or multiple, and with segmental infiltrates. As in pneumonia caused by *L. pneumophila,* rapid radiologic progression of the disease is characteristic.[207]

Chest CT scanning has been shown to improve radiographic characterization of lung infection.[208–210] In the immunocompetent host, chest CT may reveal infiltrates not present on chest radiograph at the time of initial presentation to an emergency room and may rule out CAP in some patients.[211] Chest CT is helpful in evaluating recurrent pneumonia, a chest radiographic abnormality such as a carcinoma that predisposes to pneumonia, or infections unresponsive to therapy. Pneumonia developing behind an obstruction caused by a tumor, other masses, or a foreign body, and lung abscess are better defined on CT scans than on routine chest radiographs.[208] However, exposure to more radiation (the radiation from one CT scan equals that from six to seven chest radiographs) and the increased expense has limited its use as the initial radiographic procedure. Furthermore, it is unclear if all abnormalities found on the chest CT scan truly represent pneumonia.[209] In the immunocompromised host in whom infection is only one of the possible causes of abnormal chest radiographs, chest CT may aid in better defining a "questionable" chest radiograph and may be helpful in localizing involved areas of lung as a guide to biopsy procedures. CT scans are also more sensitive in defining parenchymal disease in the ICU setting and in patients older than 65 years. Certain infections, such as those caused by *Aspergillus, M. tuberculosis,* and *Pneumocystis,* have characteristic appearances on CT that in the correct clinical setting may make invasive procedures unnecessary. Both ultrasound and CT imaging may be more sensitive in defining pleural effusions than plain films.[210] Techniques such as perfusion magnetic resonance imaging and nuclear medicine procedures are generally not used in patients with CAP. Early in the AIDS epidemic, gallium scans were used to help diagnose *Pneumocystis* infection even in patients with normal chest radiographs.

PNEUMONIA SYNDROMES

Acute Community-Acquired Pneumonia

An extensive list of bacterial, fungal, viral, and protozoal agents may cause pneumonia. Because initial evaluation rarely results in a specific etiologic diagnosis, antibiotic therapy is usually begun empirically. Defining pneumonia syndromes on the basis of clinical, epidemiologic, radiographic, and laboratory parameters, with a limited number of organisms commonly associated with each syndrome, has helped the clinician to select rational empirical therapy for the most likely organisms involved. Many of the syndromes have overlapping signs and symptoms, which at times makes clear identification of a specific syndrome in an individual impossible.[212,213] Increases in numbers of patients living longer, more and varied comorbidities, the increasing use of biologic immunomodulators such as TNF inhibitors, expanded contact with various aspects of the health care system, and decreases in the prevalence of *S. pneumoniae* and *H. influenzae* type B due to vaccine usage have led to a wider array of presentations, etiologic agents, and strategies for empirical therapy. Newly described microbial agents are being recognized as potential causes of CAP. Subgrouping syndromes under the general description of CAP may be made based on patient age, severity of illness, comorbidities, need for hospitalization, and epidemiologic setting.

Patients with acute CAP are usually in their mid fifties to late sixties. Peak incidence of bacteremic pneumococcal disease in general occurs in midwinter and early spring, and disease due to *Legionella* is more frequent in the summer.[211,214] Still, there is no "pneumonia season," and disease occurs throughout the year. Most patients (58%–89%) have one or more chronic underlying diseases. Immunosuppression related to HIV infection, malignancy, neutropenia, the chronic use of steroids, myelosuppressive agents, and newer immunosuppressive agents for

TABLE 67.4 Guide to Differential Diagnosis of Pneumonia Based on Radiologic Characteristics

IMAGING CHARACTERISTICS	POSSIBLE PATHOGENS
Chest Radiograph	
Dense segmental or lobar consolidation	More likely bacterial pathogens
Unilateral or bilateral homogenous consolidation	*Streptococcus pneumoniae, Legionella* spp., *Mycoplasma pneumoniae*
Lower lobe	Aspiration-anaerobes, gram-negative rods
Unilobar with air bronchograms	*Chlamydia*
Bulging fissure sign	*Klebsiella* spp.
Bronchopneumonia—result of bronchial inflammation, epithelial ulceration, fibropurulent exudate	*Staphylococcus aureus, Haemophilus influenzae*, fungi
Interstitial infiltrates or interstitial-alveolar infiltrates (diffuse pneumonitis)	More likely viral or *Mycoplasma*
Bilateral with hypoxia out of proportion to imaging abnormalities	*Pneumocystis jirovecii* (PCP)
Patchy, peribronchiolar opacities or ill-defined reticulonodular opacities	Primary viral infection including CMV, HSV, adenovirus *M. pneumoniae*
Diffuse bilateral bronchopneumonia	CMV, HSV, adenovirus,
Unilateral or bilateral interstitial basilar infiltrates progressing to severe symmetrical air-space disease	Coronavirus including SARS, MERS
Cavitary Lung Lesions	
Upper lobe	Tuberculosis or nontubercular mycobacteria
Unilateral or lower lobe	Anaerobic lung abscess
Multiple, may be pleural based	Endemic and opportunistic fungi
Cavities that evolve from lobar consolidation and coalescence of latencies	*S. aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*
Pleural effusion	Large effusions support a bacterial cause
In association with necrosis, pneumatoceles or empyema	*S. aureus*, *Streptococcus pyogenes, S. pneumoniae*
With multilobular infiltrates	Primary viral especially metapneumovirus, bocavirus
Pneumatoceles	*S. aureus*, *Klebsiella* spp., *Haemophilus* spp., *S. pneumoniae*
Multiple nodules	Bacteremic spread of *S. aureus* Endemic or opportunistic fungi
Parabronchial	Varicella zoster virus
Hilar adenopathy	
With upper lobe infiltrate	*Mycobacterium tuberculosis*
With homogenous opacities, may cavitate	Coccidioidomycosis
With signs of obstruction	Malignancy with bacterial infection
Computed Tomography	
Ground-glass opacities—localized increase in lung attenuation	*Pneumocystis, Mycoplasma,* fungi, CMV and other viruses
Tree-in-bud pattern—reflects presence of bronchioles filled with inflammatory material	Bacteria, mycobacteria, fungi, viruses including RSV and parainfluenza virus, and other atypical pathogens

CMV, Cytomegalovirus; *HSV,* herpes simplex virus; *MERS,* Middle East respiratory syndrome; *PCP, P. jirovecii* pneumonia; *RSV,* respiratory syncytial virus; *SARS,* severe acute respiratory syndrome.

Data from Franquet T. Imaging of pneumonia: trends and algorithms. Eur Resp J. *2001;18:196–208, and Franquet T. Imaging of community-acquired pneumonia.* J Thorac Imaging. *2018;33:282–294.*

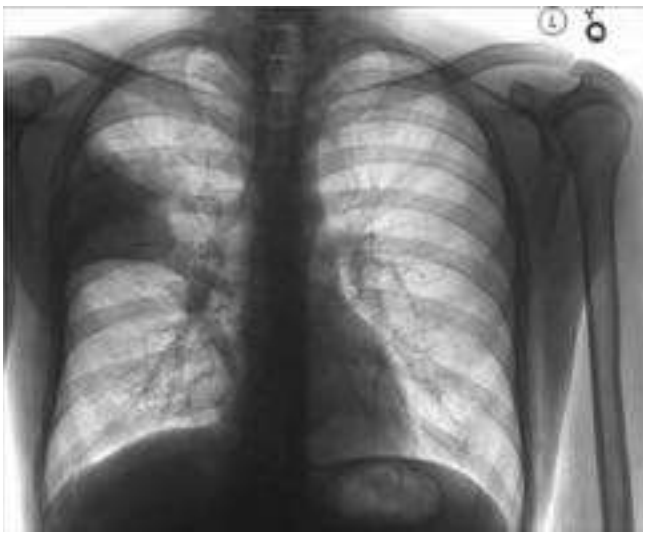

FIG. 67.9 Focal airspace consolidation within the right upper lobe posterior segment, compatible with bacterial pneumonia in a patient with *Streptococcus pneumoniae* infection.

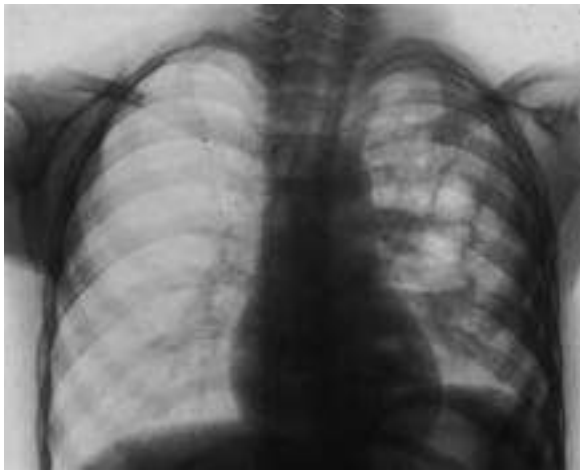

FIG. 67.10 Pneumatocele formation in the left upper lobe of a patient with staphylococcal pneumonia.

treatment of rheumatologic, dermatologic, and gastrointestinal disease is being increasingly observed.

On a historic basis, CAP manifested with a sudden onset of a chill followed by fever, pleuritic chest pain, and cough that produced mucopurulent and rusty sputum. While this classic presentation is still seen occasionally, the signs, symptoms, and physical findings vary according to the age of the patient, therapy with antibiotics before presentation, and the severity of illness. Patients typically present after several days of symptoms.[215] Cough is noted in more than 80% to 90% of patients and is productive in over 60%.[59,215–217] Chest pain is present in approximately 35% to 48% of patients, chills in 40% to 70%, and hemoptysis in approximately 15%.[59,215,216]

A variety of nonrespiratory symptoms are associated with pneumonia, including fatigue (91%), anorexia (71%), sweats (69%), and nausea (41%).[59] Both respiratory and nonrespiratory findings occur less frequently in older age groups.[60]

Physical examination reveals fever in 68% to 78% of patients, but it may be seen less commonly in older populations. Tachypnea (respiratory rate greater than 24–30 breaths/min) is noted in 45% to 69% of patients and may be more frequently seen in older age groups.[60] Tachycardia (pulse rate greater than 100 beats/min) is noted in approximately 45% of patients, rales in approximately 70% of patients, and

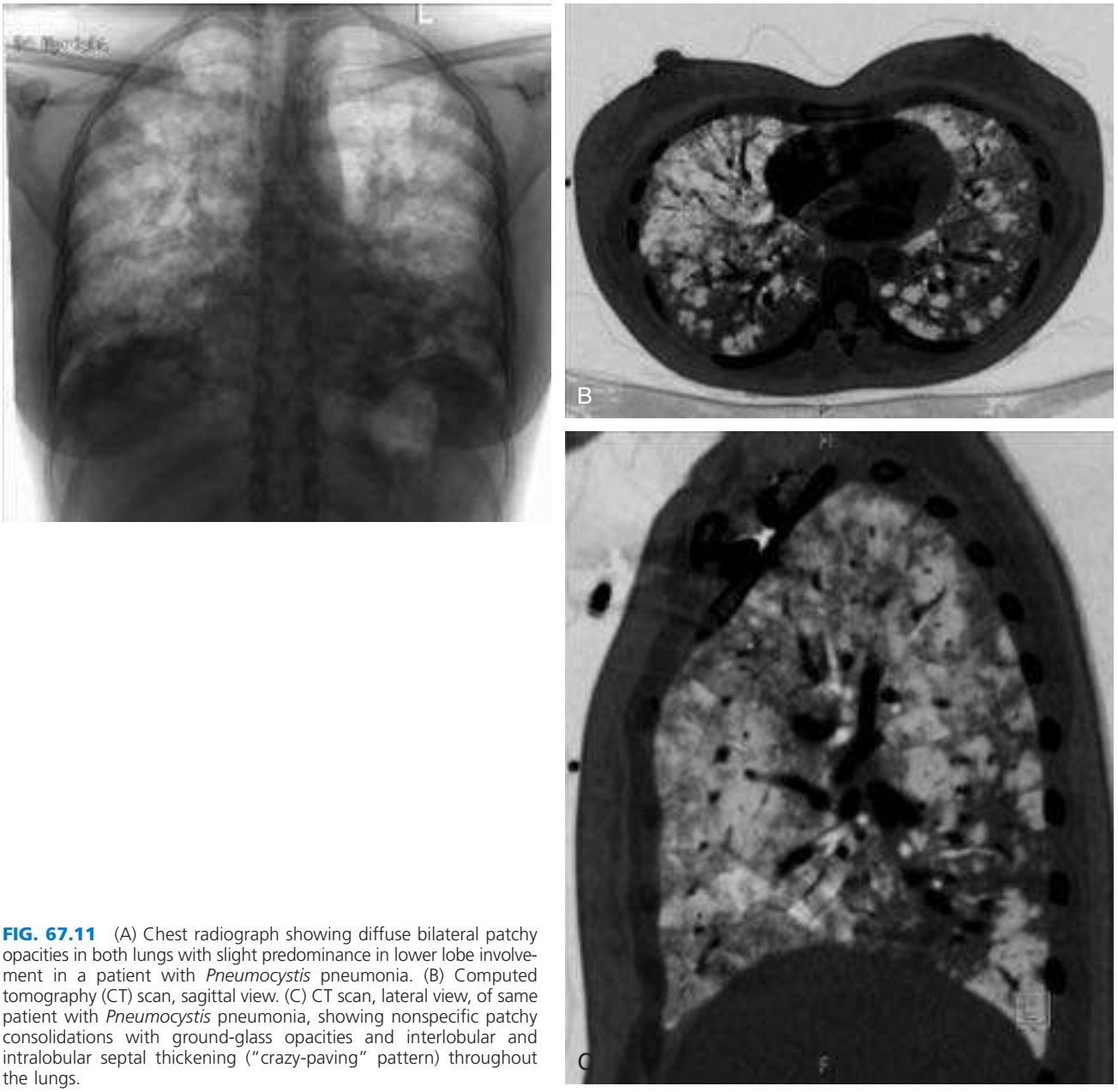

FIG. 67.11 (A) Chest radiograph showing diffuse bilateral patchy opacities in both lungs with slight predominance in lower lobe involvement in a patient with *Pneumocystis* pneumonia. (B) Computed tomography (CT) scan, sagittal view. (C) CT scan, lateral view, of same patient with *Pneumocystis* pneumonia, showing nonspecific patchy consolidations with ground-glass opacities and interlobular and intralobular septal thickening ("crazy-paving" pattern) throughout the lungs.

signs of consolidation in 20%.[215] However, no combination of physical findings has been found to be adequate to confirm a diagnosis of pneumonia.[59]

Most commonly, the white blood cell count is in the range of 15,000 to 35,000/mm^3, and the differential cell count reveals an increased number of juvenile forms. Leukopenia may be noted and is a poor prognostic sign.[95] The hematocrit and the red blood cell indices are usually normal.

With classic CAP, the sputum is thick and purulent and may be rust colored. The sputum Gram stain reveals numerous neutrophils and bacteria, often with a single organism predominating. Chest films show areas of parenchymal involvement, usually with an alveolar-filling process. There is moderate hypoxemia due to ventilation perfusion abnormalities.

In the early 1900s, microbiologic studies defined *S. pneumoniae, H. influenzae, S. aureus,* and enteric gram-negative bacilli as the predominant causes of the typical CAP syndrome. Then in 1938, Hobart Reimann described a small number of patients with a clinical picture that was "atypical" in that episodes began as a mild respiratory tract illness that was followed by pneumonia with dyspnea and cough without sputum.[218] Subsequent investigations have shown that this syndrome can be seen with a number of different pathogens, with *M. pneumoniae, C. pneumoniae, L. pneumophila,* and respiratory viruses being the most significant (characteristics of illness with these pathogens are detailed later). Other agents, such as *Chlamydia psittaci, Francisella tularensis, M. tuberculosis,* and *C. burnetii,* may also cause atypical pneumonia. In patients with AIDS, *Pneumocystis* and nontuberculous mycobacteria should also be included. Historically, the epidemiology and clinical features of the atypical pneumonias were thought to be sufficiently distinct to differentiate them clearly from other causes of CAP. It is now clear that differentiation between atypical agents and typical bacterial causes of CAP is imprecise; and in addition, in approximately one-third of cases more than one pathogen can be identified, most commonly a combination of viral and bacterial agents.[84,219]

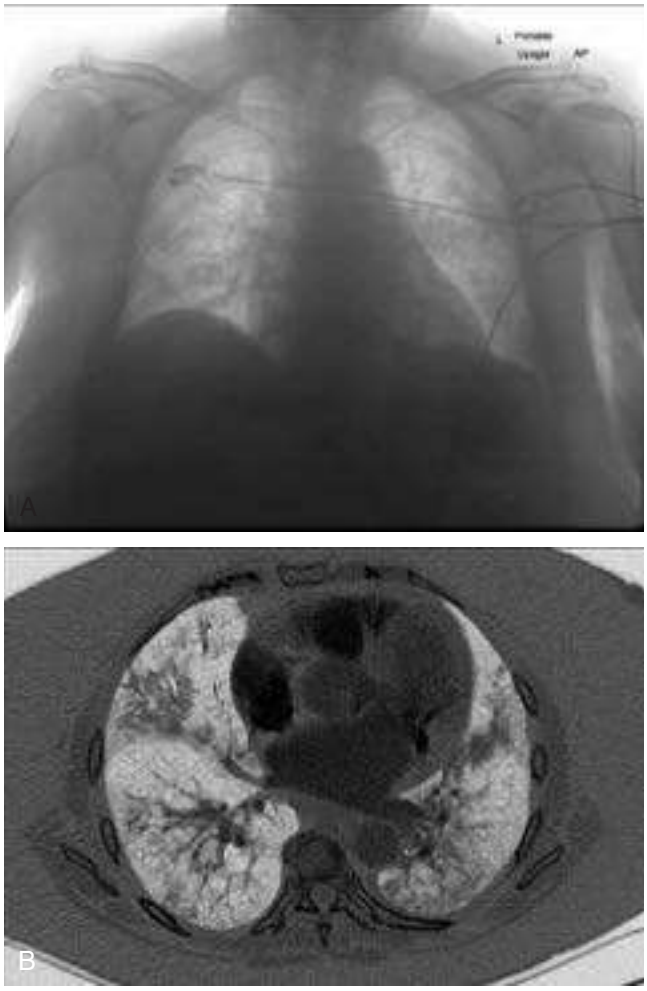

FIG. 67.12 (A) Chest radiograph showing patchy opacities in the right lower and left mid and lower lung zones in a patient with human metapneumovirus pneumonia. (B) Computed tomographic scan, sagittal view, showing extensive patchy ground-glass consolidations in all lobes of the lungs of same patient with human metapneumovirus, obtained 3 days after chest radiograph.

In the past, 40% to 80% of cases of acute CAP appear to have been caused by *S. pneumoniae.*[99,220] More recent studies have found that the relative frequency has diminished in the developed world, although not in developing countries.[84,219,221–223] It has been defined as the cause of pneumonia in as few as 4% to 6% of ambulatory patients and hospitalized patients.[84,224,225] It has been hypothesized that the apparent decreased incidence of pneumococcal pneumonia has been related to the recognition of newer pathogens and diminished use and performance of microbiologic studies.[99] However, through a herd immunity effect, the increasing use of pneumococcal vaccine in children has reduced the incidence of pneumococcal disease in both children and adults in the developed world.[226,227] Severe pneumococcal infections, including pneumonia, have been associated with prior splenectomy due either to trauma or to staging for Hodgkin disease; abnormal immunoglobulin responses (myeloma, lymphoma, HIV infection); and functional asplenia due to systemic lupus erythematosus or marrow transplant.[228,229]

Improved diagnostic testing with more sensitive nucleic acid amplification tests has shown that community respiratory viruses play a significant role in pneumonia both as single agents and in dual infections.[84,219,222,230] Studies have suggested a viral cause in up to one-third of adults and children hospitalized with pneumonia. Influenza A and B, RSV, human metapneumovirus, parainfluenza virus, and coronavirus are the most frequently identified viral pathogens. In addition, up to half of the patients with a viral pathogen identified will have concurrent bacterial infection, and multiple concurrent viral pathogens can also be seen.[84,219,222] Whether rhinoviruses are a direct cause of pneumonia remains unresolved, but they have been found in patients with severe pneumococcal disease, and in vitro have shown increased adherence of *S. pneumoniae* to human tracheal epithelial cells.[231,232] Consequently it is possible that these viruses play a role as facilitators for bacterial infection rather than roles as true pulmonary pathogens.

S. aureus accounts for 1% to 2% of acute CAP cases,[84,224,225] and may take on increased importance as a cause of pneumonia in older adults and in those with influenza.[63,233] Patients who develop postinfluenza pneumonia are usually younger and have less underlying disease than most other patients with CAP. Although it had been felt that bacterial pneumonia in the setting of influenza develops following clinical influenza, studies during the 2009 H1N1 pandemic indicated that bacterial coinfection most likely arises at the peak of viral replication, with patients presenting an average of 6 days after symptom onset.[63] An elevated white blood cell count with a shift to the left, physical signs of pulmonary consolidation, and radiographic evidence of focal parenchymal disease develop, and the sputum Gram stain is consistent with bacterial pneumonia.

S. aureus may also cause lung infection secondary to a bacteremic source, producing multiple bilateral round lesions that will frequently cavitate. Although this presentation has been characteristically associated with right-sided endocarditis in injection drug users, it can also be seen in association with infections of intravascular catheters, and with staphylococcal soft tissue infections.[234,235]

As noted previously, since the late 1990s there has been an increase in the incidence of pneumonia due to community-associated *S. aureus* strains.[195,236] Patients have been young, have had few if any comorbidities, and usually have presented after a flulike illness with high fevers, leukopenia, tachycardia, tachypnea, hemoptysis, and rapid evolution on radiographs to multilobar disease. These cases appear to be associated with *S. aureus* strains carrying the Panton-Valentine leukocidin toxin, regardless of whether or not they are methicillin resistant.[237] The adult respiratory distress syndrome has been a frequent complication in such cases, and mortality rates of over 50% have been seen.[195]

Aerobic gram-negative bacteria, exclusive of *H. influenzae,* may cause anywhere from 2% to 10% of pneumonia cases. *K. pneumoniae, P. aeruginosa,* and *Enterobacter* spp. are the organisms isolated most often.[84,224,225,238] Gram-negative bacilli are particularly important pathogens in older adults, especially those with chronic underlying disease and those who are bedridden and recently hospitalized. *Pseudomonas* infection should be suspected in patients with pulmonary comorbidities and recent hospital stays.

Legionella species are the most important water-related pulmonary pathogens in the United States with regard to mortality and morbidity. The importance of *Legionella* species in causing pneumonia has varied greatly in different geographic areas, with incidences ranging from 0.6% to 23%.[212,213,215,221,239] Since 2000 there has been a fourfold increase in incidence of legionellosis in the United States, although it is not clear how much of this increase has been related to increased diagnostic testing.[240] Although infection may occur at any age, those aged 45 to 64 now appear to be at greatest risk. The presence of a high fever (>40°C), male sex, previous β-lactam therapy, multilobar involvement, rapid progression of radiographic abnormalities, a need for intensive care, gastrointestinal and neurologic abnormalities, elevated liver enzyme levels, and increased creatinine levels have all been associated with *Legionella* pneumonia.[213,239] However, no clinical features reliably distinguish *Legionella* species pneumonia from that caused by other bacteria.

Historically an estimated 5% to 7% of cases of acute CAP were attributed to *H. influenzae.*[221,224,225] The true incidence of this organism was obscured by the difficulty of isolating it from sputum and identifying it in sputum Gram stain, and by the difficulty of distinguishing colonization from infection. However, the use of the *H. influenzae* conjugate vaccine, by decreasing the reservoir in children, has markedly decreased the incidence of invasive disease caused by *H. influenzae* type b, and

although there has been a relative increase in the incidence of invasive infections caused by nontypeable strains, population-based studies have indicated that the pathogen causes only 1% to 5% of pneumonia necessitating hospitalization in adults and children.[84,219,222]

M. catarrhalis has also been identified as a cause of pneumonia.[219,221,224,225,241] The overall incidence of disease caused by this bacterium is low, but it is an important pathogen in older adults with COPD and various forms of immunosuppression.

A number of additional pathogens including *M. pneumoniae, Chlamydia* species, *C. burnetii,* and community respiratory viruses can cause an atypical pneumonia syndrome. In addition, it is not infrequent for a patient to have pneumonia either sequentially or concurrently due to several pathogens, such as influenza virus or *C. pneumoniae* infection being followed by *S. pneumoniae.*[221]

Community-Acquired Pneumonia in the Older Adult

Pneumonia in the elderly has become an increasingly important clinical entity as the world's population has aged.[242] Pneumonia is one of the leading reasons for hospitalization in those 65 and older and represents a major cause of morbidity and mortality. In some series, pneumonia represents the leading cause of death in this population (see Chapter 310). For those over the age of 60, pneumonia is a predictor of increased mortality after the specific episode has resolved and for several years thereafter.[243]

The clinical presentation of pneumonia in older adults (especially those over 80 years of age) may be subtler than in younger populations, with more gradual onset of symptoms and fever and the classic signs of pneumonia.[60,83,213] Fever occurs less commonly in older adults, and temperature elevation is muted. The classic findings of cough, fever, and dyspnea may be absent in over half of older adults.[82,244] Chills and rigors may also be less frequently seen. Tachypnea (respiratory rate of greater than 24–30 per minute) and rales are more frequent findings in older adults and have been observed in up to 65% of patients.[60,83] Nonrespiratory symptoms may be the major presenting feature. The initial presentation of older adults with pneumonia may include decline in functional status, weakness, subtle changes in mental status, and anorexia or abdominal pain. It has been suggested that the nonspecific presentation of pneumonia in older adults may result in great part from the prevalence of dementia in this population.[244] The development of in-hospital complications and death are more frequent in older populations.[83]

Specific etiologic diagnoses are made less frequently in older adults, with approximately 20% to 50% of patients having an etiologic agent defined.[83] The absence of productive cough and common prior use of antibiotics may explain this observation. Etiologies have varied in different series depending on the means of diagnosis, the patient population studied (outpatient vs. institutionalized older adults), and the geographic location.[84] In general, the cause of CAP in the older population follows the general trend of infection in younger populations. Community respiratory viruses and *S. pneumoniae* remain the predominant pathogens, and there is an increased frequency of aspiration pneumonia.[83,242] Major viral pathogens in the elderly include influenza A and B, parainfluenza virus, human metapneumovirus, RSV, and coronaviruses. In addition, rhinovirus is also frequently identified, as both a single pathogen and a copathogen, and appears to potentiate bacterial copathogens if not pathogenic in the lower respiratory tract.[231,232] *H. influenzae,* usually a nontypeable strain, was the second most common agent in the past, accounting for 5% to 10% of episodes,[83,245] but appears to be a less frequent pathogen today.[84] The importance of other aerobic gram-negative bacilli in causing pneumonia in older adults remains a question in part because the criteria for diagnosis of true pneumonia versus colonization vary. In most but not all recent studies, 1% to 3% of cases of pneumonia have been attributed to non-*Haemophilus* gram-negative bacilli. Although increased oropharyngeal colonization with aerobic gram-negative bacilli has been documented in the older population and is thought to be a predisposition to development of pneumonia caused by these organisms, colonization appears to be related to debility of the patient rather than age.[246] Other factors reported to be associated with increasing colonization with gram-negative organisms include prior use of antibiotics, severe bronchopulmonary disease, decreased activity, alcoholism, and incontinence.[238] In this regard, one study of apparent aspiration pneumonia in a nursing home population older than 65 years of age that used protected BAL to define the microbiologic etiology identified gram-negative bacteria as the primary pathogen in 49%, followed by mixed anaerobes in 16%.[247] Older adults are at greater risk for infection with group B streptococci, *M. catarrhalis,* and *Legionella* species, although the overall incidence of these agents in the older population is relatively low. *Legionella* has been described as a cause of severe pneumonia in the elderly. Polymicrobial infections and pneumonia due to aspiration have both been noted to occur more frequently in older adults.[82,83]

It is not completely clear which agents cause atypical pneumonia in the older population. Most series suggest that *M. pneumoniae* pneumonia is unusual, although it has been documented to be a cause of pneumonia leading to hospitalization in older adults.[62,82–84] It is not clear if this significant variation is related to differing epidemiologic characteristics of study populations or to the accuracy of diagnostic methods. *Chlamydia* infections have been reported in the older population, and in one study were associated with 32% of pneumonias on the basis of serologic testing; but again, there is a significant variation in incidence in differing studies and the relative incidence has been lower in more recent studies that have used nucleic acid amplification assays.[62,82–84,219,220,248]

Severe Community-Acquired Pneumonia

Approximately 10% of patients with CAP will develop severe disease as defined by admission to an ICU owing to the presence of shock necessitating vasopressors or respiratory failure necessitating mechanical ventilation.[249] Early identification of patients who are at higher risk for developing severe pneumonia is important because these patients have a higher mortality rate and require more supportive care. Furthermore, patients with severe pneumonia are infected with a different spectrum of etiologic agents and would therefore benefit from different empirical antibiotic strategies than patients with less severe disease. Advanced age, presence of significant comorbidities, nursing home residence, immunosuppression, and altered mental status have all been thought to be associated with the development of severe CAP.[249] Approximately one-third of patients with severe pneumonia would have been previously healthy.

S. pneumoniae was the organism classically associated with severe pneumonia. However, in patients requiring ICU admission, there is an increased incidence of *S. aureus, L. pneumophila,* gram-negative bacilli (especially *Klebsiella* species), and *H. influenzae.*[221,250,251] As with CAP in general, newer molecular tests indicate that the community respiratory viruses are frequent copathogens with severe pneumonia and can be the sole identified pathogen.[252] *Pneumocystis* is increasingly being recognized as a cause of severe pneumonia in non–HIV-infected patients who have impaired cell-mediated immunity owing to organ transplantation, malignancy, severe malnutrition, or receipt of immunosuppressive therapies including corticosteroids, antineoplastic chemotherapeutic agents, and newer agents including TNF-α inhibitors and rituximab.[253–255] As with CAP in general, there can be significant geographic differences in the relative incidence of differing pathogens.

A meta-analysis of 127 studies published through 1995 indicated that despite the overall mortality rate for CAP of 13.7%, it was 36.5% for patients with disease severe enough to require ICU care.[256] Unfortunately, more recent studies have continued to find comparably high mortality in this patient population.[257,258] Prognostic risk factors for death included male sex, pleuritic chest pain, hypothermia, systolic hypotension, tachypnea, diabetes mellitus, neoplastic disease, neurologic disease, bacteremia, leukopenia, and multilobar radiographic pulmonary infiltrates. Although shock and respiratory failure are usually evident and serve as major criteria for defining severe pneumonia, patients without these findings may also benefit from ICU care. During the last 2 decades a number of prediction rules have been developed to assess severity and prognosis of patients with pneumonia, including but not limited to the pneumonia severity index (PSI)[249]; the *c*onfusion, *u*rea, *r*espiratory rate, low *b*lood pressure (CURB) score[259]; the CURB plus age >65 (CURB-65) score[260]; the CURB-65 score without the urea level (CRB-65)[261]; the severe community-acquired pneumonia (SCAP) score[262]; the SMART-COP score[263]; and the Risk of Early Admission to the Intensive Care Unit (REA-ICU) index.[264] These rules vary in complexity

and in their sensitivity and specificity for defining the need for ICU care, but use a combination of factors including age, sex, comorbid conditions, vital sign parameters, and laboratory and radiographic findings to predict either the need for ICU care or the patients' prognosis. Further use of these scoring systems will be discussed under the "Management and Therapy of Pneumonia" section of this chapter. In rare cases, severe CAP may be the presentation for anthrax, plague, tularemia, or zoonotic viral infections: the epidemiologic setting is important in this regard.

Health Care–Associated Pneumonia

In the past, a basic distinction in the epidemiology of pneumonia has been whether the infection developed in the community or in the hospital. The distinction was clinically relevant because the importance of various etiologic agents differed, as did antibiotic susceptibilities. Consequently, the guidelines for empirical antibiotic therapy differed depending on where the infection developed. In the 21st century, there has been a shift of health care delivery from inpatient to outpatient settings, and even complex medical conditions may be handled without hospitalization. Subsequently, a growing number of patients develop pneumonia after extensive outpatient contact with various aspects of the health care system. The recognition of this situation in the mid-2000s led the ATS and IDSA to propose a new clinical classification of pneumonia termed *health care–associated pneumonia* that was felt to represent a distinct syndrome that is a hybrid of CAP and hospital-associated pneumonia.[85,86,265] The exact definition used in studies supporting the concept varied, but in general it has been defined as pneumonia developing in patients who have been hospitalized for 2 or more days within 90 days of developing infection; patients attending hospital or hemodialysis clinics; patients receiving intravenous antibiotic therapy, wound care, or chemotherapy at home within 30 days of developing infection; and residents of long-term care facilities or nursing homes.[85] Important to note, the etiology has shifted from typical CAP, with an increased incidence of infections due to aerobic gram-negative bacilli including *P. aeruginosa*; *S. aureus* including MRSA; *S. pneumoniae*; and mixed aerobic-anaerobic pathogens associated with aspiration most commonly reported.[86,266] In addition, the overall mortality is higher in patients with HCAP (10.3%–19.8%) than in CAP (4.3%–10%), and generally comparable to that of hospital-acquired pneumonia.[86,265,266] Because of this concern, relatively broad-spectrum antibiotic therapy including coverage for both MRSA and *P. aeruginosa* has become common for patients meeting the HCAP definition. However, more recent work has challenged the association of HCAP with a high incidence of MRSA and *P. aeruginosa,* and the association of increased mortality with more antibiotic-resistant pathogens.[267,268] It remains unclear whether mortality in patients with HCAP is due to increased comorbidities in patients, more virulent organisms causing infection, an increased incidence of inappropriate antibiotic use in the first 48 hours of care, or some combination of these factors.

Residents of skilled nursing facilities represent an important subpopulation of older adults at risk for pneumonia. Pneumonia has been reported to be the second most frequent infection in this setting, carries the highest mortality of any infection in this population, and is a common cause for hospitalization.[269,270] Silent aspiration is a major risk factor, as are poor functional status, nasogastric feeding, confusion, the presence of obstructive lung disease, the presence of a tracheostomy, and advancing age.[271] Key modifiable risk factors are inadequate oral care and swallowing difficulties.[272] The subtle presentation noted in other older adult populations also occurs in those in a nursing home setting. *S. pneumoniae* had been considered the predominant cause, but newer studies have identified respiratory viruses as frequent pathogens, in addition to *S. aureus* and gram-negative bacilli in those with severe pneumonia.[248,271] Outbreaks of pneumonia have occurred in nursing homes and have involved *Legionella,* influenza, parainfluenza, RSV, and rhinovirus.[273,274]

"Atypical" Pneumonia Pathogens

Between 10% and 30% of cases of CAP have been attributed in the past to *M. pneumoniae,* with the highest percentage noted in patients well enough to be treated as outpatients, and several studies performed in North America and Europe have suggested that cyclic epidemics occur every 3 to 5 years.[163] *M. pneumoniae* CAP is most likely to occur in children older than 5 years, adolescents, and young adults. The majority of cases occur in those younger than 40 years, although this organism can cause pneumonia necessitating hospitalization in those older than 60.[62,163,275] An increased incidence of disease and true epidemics have been documented in relatively enclosed populations of young adults at military bases, colleges, and boarding schools. Although the disease severity may be mild, owing to the long incubation of approximately 3 weeks, these outbreaks can be quite prolonged. Mycoplasmal infection occurs throughout the year, although a relative increase in incidence is noted in the late summer and fall.

The course of *M. pneumoniae* pneumonia is characterized by up to 10 days of symptoms before presentation, as is true with many of the other agents involved in atypical pneumonia. In its classic form, mycoplasmal infection manifests with constitutional symptoms and a progression from the upper to the lower respiratory tract. Sore throat is often the initial finding. Up to one-third of patients may have ear symptoms. Although bullous myringitis has been historically linked to mycoplasmal infection, this appears to be a rare finding. Fever, malaise, coryza, headache, and protracted nonproductive cough represent the major clinical findings. Pleuritic chest pain, splinting, and respiratory distress are not usually seen. Moist or crepitant rales may be heard. Sputum production is variable, and the sputum is purulent in one-third to one-half of cases. Gram stain and culture of sputum usually reveal mouth microbiota. White blood cell counts greater than 10,000/mm^3 are uncommon, occurring in approximately 20% of patients.[102] An elevated sedimentation rate is noted in about 25% of cases. Pulmonary involvement seen on radiographs is commonly more extensive than the physical examination would indicate. Unilateral or bilateral patchy infiltrates in one or more segments, usually in the lower lobes, are noted in a bronchial or peribronchial distribution. Upper lobe involvement and pleural effusions are less common but may be seen in up to 20% to 30% of cases.[204,205] Progression of the radiographic picture, despite a stable clinical picture, may be seen. The overall clinical course in most cases is benign. Disappearance of constitutional symptoms is usually noted in the first and second weeks, although cough and radiographic changes may persist for several weeks. Occasionally, *M. pneumoniae* infection causes severe CAP, necessitating intensive care.[276] A large number of extrapulmonary manifestations may occur with *M. pneumoniae,* including involvement of skin, central nervous system, blood, and kidneys (see Chapter 183).

C. pneumoniae has been considered an important cause of atypical pneumonia and on the basis of serologic studies was estimated to account for between 6% and 20% of all CAP cases.[62,93,212,215,277–280] Although disease is uncommon in those younger than 5 years, serologic evidence of infection has been noted in over 50% of adults.[62,83,248,281] Disease usually occurs sporadically, but epidemics have been well documented. The majority of infections are either asymptomatic or produce mild symptoms. As with mycoplasmal infection, sore throat and hoarseness herald the onset of pneumonia, although the progression of symptoms appears slower than that noted with mycoplasma or viral pneumonia. Cough may begin after several days to weeks, suggesting a biphasic illness. Hoarseness and sinus tenderness appear more commonly than in patients infected with *Mycoplasma* or viruses. The white blood cell count is rarely elevated. Pneumonia with *C. pneumoniae* is usually mild, although complete recovery may be slow. Cough and malaise may persist for weeks to months. Reinfection occurs and appears to be milder than primary infection and is usually not associated with pneumonia. Chronic and latent infections have also been described. Infection with *C. pneumoniae* has been associated with exacerbations of COPD and asthma. In general, few features distinguish chlamydial pneumonia from infection caused by other atypical agents or other bacteria. *C. pneumoniae* infections have been associated with extrapulmonary manifestations, including otitis, sinusitis, pericarditis, myocarditis, and endocarditis. It has also been associated with coronary artery disease, although the definitive relationship remains unclear (see Chapter 182).

Of the viral agents associated with atypical pneumonia in adults, influenza A and B, adenovirus types 3, 4, and 7 (especially in military recruits), human metapneumovirus, RSV, (especially in older adult and

immunosuppressed patients), and parainfluenza virus have been considered to be the most common.[201,202,230,282,283] The advent of multiplex real-time PCR assays is now rapidly expanding our understanding of the role of viral pathogens in acute pneumonia and has shown that rhinoviruses and coronaviruses can be significant pathogens in adults, and human bocavirus and human metapneumovirus in children younger than 5 years.[84,222,284–287] Moreover, the presence of two or more viral pathogens is not uncommon. Other viral agents that are less common causes of pneumonia include enteroviruses, parechoviruses, all the herpesviruses, hantaviruses, mimiviruses, and measles.[288] Epidemic disease is predominantly linked to influenza, but the SARS coronavirus caused worldwide disease in 2002 and 2003, and a second similar coronavirus, the Middle East respiratory syndrome coronavirus (which can cause severe pneumonia), was identified in 2012 (see Chapter 155).[200] Elderly patients, especially those with comorbidities, are frequently the population at greatest risk for viral pneumonias.

Legionella is now recognized as an important cause of the atypical pneumonia syndrome, although patients infected with *Legionella* may also present with the syndrome of acute bacterial CAP. The incidence of pneumonia varies regionally, but it can account for up to 8% of cases involving hospitalization.[221] *Legionella* species are among the top three our four organisms causing pneumonia that necessitate ICU care.[221,250,251] An international study found that *L. pneumophila* causes over 90% of cases of *Legionella* pneumonia, with approximately 84% of all cases caused by *L. pneumophila* serogroup 1.[289] Inhalation of aerosolized organisms after exposure to environmental reservoirs, such as fresh water and moist soil, has been the usual means of acquiring the organism, although aspiration is now thought to be an alternate route of infection.[290]

Cigarette smoking, chronic lung disease, and immunosuppression are consistently noted risk factors for the development of disease. Although early symptoms of malaise, muscle aches, headaches, and nonproductive cough resemble the onset of a viral syndrome, the rapid progression of pulmonary symptoms and relatively high fever, often exceeding 40°C, is noteworthy.[290]

L. pneumophila pneumonia is associated with a variety of extrapulmonary findings and laboratory abnormalities, including mental status changes, abdominal complaints (loose stools or diarrhea), headache, bradycardia, elevation of hepatic enzyme levels, hypophosphatemia, hyponatremia, elevated serum LDH levels, and elevated serum creatinine levels. These findings mostly reflect the severity of the pneumonia rather than specificity to *Legionella* infections. Extrapulmonary infection is unusual, but when it does occur, it usually involves the heart with myocarditis, pericarditis, and postcardiotomy-like syndrome.[290] Unfortunately, none of these findings distinguishes between pneumonia due to *L. pneumophila*, other atypical agents, or more typical bacterial pathogens. Similarly, radiographic manifestations do not distinguish *Legionella* infections from those of other causes. Patchy interstitial infiltrates, or nodular infiltrates that may progress rapidly even with adequate therapy, are characteristic. Pleural effusions may be noted in up to one-third of patients.

Pneumonia in the Setting of Aspiration

The clinical setting in which aspiration occurs includes any disease state in which consciousness is altered and the normal gag and swallowing reflexes are abnormal; illnesses predisposing to dysphagia either from neurologic disease or upper gastrointestinal tract disease or surgery; or conditions leading to mechanical disruption of glottic closure such as tracheostomy or nasogastric tubes. A prospective population-based study in a Canadian province analyzed 1946 patients hospitalized for pneumonia and identified aspiration as the cause in 10% of cases from the community and in 30% of cases from continuing care facility cases.[291] In the community setting, 43% of the cases were related to an impaired level of consciousness due to alcohol, drugs, or hepatic failure, and 35% of cases were due to dysphagia. In continuing care facilities, the predominant risk factor was dysphagia from neurologic disease in 72% of cases, and impaired level of consciousness was the major risk factor in an additional 22% of patients.

The pathogenesis of lung injury due to acid aspiration has been delineated.[292,293] The presence of acidic contents in the lung induces the release of proinflammatory cytokines including TNF-α and IL-8. These and other cytokines recruit neutrophils into the lung. Activated neutrophils appear to be the key mediators of acute lung injury after acid aspiration, although a role for complement has also been demonstrated.[294]

Although aspiration may be a witnessed event, the majority of episodes are silent and are brought to medical attention because of their sequelae.[293] Three major syndromes are recognized as a consequence of aspiration: chemical pneumonitis, bronchial obstruction secondary to aspiration of particulate matter, and bacterial aspiration pneumonia. Aspiration may be associated with ARDS, atelectasis, bronchial hyperreactivity, and fibrosis. Although chemical pneumonitis and mechanical obstruction usually cause acute symptoms, aspiration pneumonia is more insidious, with symptoms usually occurring gradually several days after the initial episode of aspiration. Pneumonitis, necrotizing pneumonia, abscess, and empyema are common. Symptoms often include fever, weight loss, and productive cough. Foul-smelling or putrid sputum occurs commonly.[295] Anemia and an elevated white blood cell count are frequently associated findings. The bacteriologic findings in aspiration pneumonia reflect the microbiota of the oropharynx, and the importance of periodontal disease in this regard has been noted. Studies performed in the 1970s in patients with indolent disease using the technique of transtracheal aspiration and analysis in anaerobic research laboratories documented anaerobic involvement in the majority of cases, either alone or in combination with oral aerobic or facultative anaerobes.[296] *Bacteroides* species, *Porphyromonas* species, *Prevotella melaninogenica, Fusobacterium* species, and anaerobic gram-positive cocci are the predominant anaerobes isolated. In community-acquired aspiration pneumonia, *Streptococcus* species and *H. influenzae* are the most common aerobic isolates. In contrast, gram-negative bacilli (including *P. aeruginosa*) and *S. aureus* are the most commonly isolated aerobes from nosocomial aspiration pneumonia including VAP, and in nursing home patients.[85,247]

Eosinophilic Pneumonias

Pulmonary infiltrates with eosinophilia (PIE), also termed eosinophilic pneumonia, is a syndrome associated with a variety of clinical entities, only some of which have an infectious cause.[297] Pulmonary eosinophilia with transient, peripheral pulmonary infiltrates and minimal symptoms (Löffler syndrome) has been associated with *Ascaris, Strongyloides,* and hookworm infections. *Ascaris* is probably the leading parasitic cause of the syndrome worldwide. Prolonged pulmonary eosinophilia associated with weight loss, fever, cough, and dyspnea may be due to tuberculosis, brucellosis, psittacosis, coccidioidomycosis, histoplasmosis, and parasitic infections including ascariasis, strongyloidiasis, paragonimiasis, echinococcosis, visceral larval migrans, cutaneous larva migrans, and infections with *Schistosoma, Dirofilaria immitis,* and *Ancylostoma* species. Noninfectious causes include drug allergy, sarcoidosis, eosinophilic leukemia, Hodgkin disease, paraneoplastic syndromes, and hypersensitivity pneumonitis (e.g., pigeon breeder's disease). A PIE syndrome has been associated with *Pneumocystis* pneumonia.[298]

Acute eosinophilic pneumonia is a distinct clinical entity occurring in younger (20- to 45-year-old), otherwise healthy individuals.[299] It is marked by the acute onset of dyspnea, nonproductive cough, fever, severe hypoxia, and chest pain, and patients may require ICU care and mechanical ventilation. Although leukocytosis is common, peripheral eosinophilia is typically minimal. Bilateral, diffuse pulmonary infiltrates are commonly seen. Radiographic abnormalities usually begin as interstitial infiltrates that progress to alveolar infiltrates. Chest CT reveals bilateral opacities. BAL yields marked (27%–81%) eosinophilia, which is the diagnostic feature of the disease. Although most patients have received antibiotics, rapid stabilization occurs with steroid use.

It has been suggested that chronic eosinophilic pneumonia may represent a unique clinical entity that may be on a continuum between asthma and Churg-Strauss syndrome.[300] A subacute onset of cough, dyspnea, fever, and weight loss associated with peripheral eosinophilia are the common features. Unlike the situation in acute eosinophilic pneumonia, respiratory failure is rare. Peripheral and migratory infiltrates are commonly seen on radiographs. Interstitial infiltrates and alveolar exudates with a predominance of eosinophils are characteristic pathologic features. A rapid response to steroids has been reported.

Tropical pulmonary eosinophilia consists of myalgia, fatigue, weight loss, and anorexia associated with cough, frequently with nocturnal exacerbations, wheezing, dyspnea, and marked peripheral eosinophilia in patients who have lived in or visited the tropics. Most cases are thought to represent immunologic hyperresponsiveness to microfilarial infection with *Wuchereria bancrofti* or *Brugia malayi*. Radiographic changes are distinctive and include increased interstitial markings with 2- to 4-mm nodules throughout the lungs with preferential involvement of the bases. Therapy is with diethylcarbamazine (see Chapter 287).

Other causes of PIE syndrome include bronchopulmonary mycosis, which should be suspected when a patient with PIE presents with asthma in conjunction with bronchiectasis, recurrent expectoration of brown mucus plugs, and peripheral eosinophilia.[297,301] Although predominantly associated with chronic bronchial colonization with *Aspergillus* species, it can be seen in conjunction with other fungi such as *Scedosporium apiospermum* and *Cladosporium herbarum*. Patients with the Churg-Strauss syndrome frequently have eosinophilia along with allergic angiitis and granulomatosis and present with asthma, diffuse pulmonary infiltrates, and multiorgan involvement. Hypereosinophilic syndrome, eosinophilic granuloma (also known as primary pulmonary Langerhans cell histiocytosis granulomatosis), bronchiolitis obliterans with organizing pneumonia (BOOP), Sjögren syndrome, and postradiation pneumonitis are unusual causes of PIE.

Hospital-Acquired Pneumonia

Hospital-acquired pneumonia has been the second most common type of nosocomial infection and is associated with significant morbidity and mortality.[181,302,303] It is a leading cause of infection-related deaths in hospitalized patients, with attributable mortality rates of 20% to 33% reported. Higher mortality rates have been observed when patients are bacteremic or have pneumonia caused by *P. aeruginosa* or *Acinetobacter* species. The morbidity associated with nosocomial pneumonia includes longer duration of mechanical ventilation and ICU and hospital stays, in addition to an attributable cost of about $40,000.

Risk factors for the development of nosocomial pneumonia have been categorized as patient related, infection control related, or intervention related. Patient-related risk factors include age greater than 70 years, severe underlying disease, malnutrition, coma, metabolic acidosis, and the presence of any of a number of comorbid illnesses (COPD, alcoholism, azotemia, central nervous system dysfunction). Infection control–related risk factors include a lack of hand hygiene and glove-use practices, and the use of contaminated respiratory equipment. Intervention-related risk factors involve those procedures and therapies that undermine normal host defenses or allow the host to be exposed to large inocula of bacteria. Sedatives and narcotics may lead to aspiration, corticosteroids and cytotoxic agents blunt the normal host response to infection, and the prolonged use of antibiotics engenders resistance. Surgical procedures, especially involving the chest and abdomen, are associated with changes in host defenses that predispose to pneumonia. The use of ventilator support is perhaps the greatest risk factor for the development of nosocomial pneumonia, with VAP occurring in 9% to 40% of intubated patients.[302] Data suggest that there is a 1% to 3% per day risk for developing pneumonia while on a ventilator, with a higher risk during the first 5 days of intubation.[304]

The use of antacids and histamine type 2 blockers that raise the gastric pH has been shown to increase stomach colonization with aerobic gram-negative rods.[305] Whether this leads to an increase in nosocomial pneumonia remains controversial.[42,43] The percentage of patients with VAP caused by organisms initially found in the stomach ranges from 0% to 55%.[305]

Aerobic gram-negative bacilli cause approximately 50% to 60% of cases of nosocomial pneumonia, with members of the family Enterobacteriaceae (*K. pneumoniae, Escherichia coli, Serratia marcescens, Acinetobacter* species, *Enterobacter* species) and *Pseudomonas* species accounting for the majority of these.[81] There is an increasing prevalence of high-level antibiotic resistance among these gram-negative bacilli, and the relative incidence of pneumonia due to multidrug-resistant bacteria varies among institutions, and occasionally among units within an institution.[302] Risk factors for such pathogens include the length of hospitalization, prior antibiotic exposure, and local epidemiologic factors. *S. aureus* causes 13% to 40% of nosocomial pneumonia, and MRSA strains now are major pathogens in this setting.[81,85] In contrast to their prominent role in CAP, *S. pneumoniae* and *H. influenzae* together cause only about 5% to 15% of nosocomial pneumonias in most studies and are predominantly seen in infections developing early in the hospital course. There is only limited information comparing the bacteriology of VAP and non–ventilator-associated hospital-acquired pneumonia, but the available data indicate that the general distributions of aerobic pathogens are relatively comparable, although there is an increase in the relative prevalence of gram-negative pathogens in patients with VAP, particularly nonenteric gram-negative bacilli.[81,306] Although the use of sedatives, feeding tubes, and endotracheal tubes are all risk factors for the development of aspiration pneumonia, the lack of support for anaerobic microbiologic testing has led to a paucity of data on the roles of anaerobic bacteria in hospital-acquired pneumonia.[293] One study performed in the early 1970s at a Veterans Administration hospital with bacteriologic analysis in a research laboratory documented anaerobes in up to 35% of cases of nosocomial pneumonia, and a more recent study identified anaerobes in conjunctions with aerobic microbiota in 23% of patients with VAP.[307,308] These organisms should be considered when aspiration is likely to have occurred. Pneumonia caused by *Legionella* species may occur sporadically or as part of outbreaks. In addition, the respiratory viruses including rhinoviruses, influenza, parainfluenza, adenovirus, and RSV can cause sporadic nosocomial pneumonia and occasional institutional outbreaks.[309] There has been the recognition that the Herpesviridae herpes simplex virus and CMV can reactivate and be identified in patients with severe VAP or ARDS. The significance of this reactivation remains uncertain at this time.[302]

Consensus guidelines have been established concerning the risks, etiologies, diagnostic workup and therapies for nosocomial pneumonia and VAP. A more in-depth review will be found in Chapter 301.

Pneumonia in the Immunosuppressed Host

Pneumonia in the immunocompromised host is perhaps the most complex of all the pneumonia syndromes, because it represents the interaction of host defense defects engendered by the underlying disease and the chemotherapy for that disease, exposure to potential pathogens in the community and within the hospital setting, and reactivation of infectious processes that had previously been dormant. CAP, atypical pneumonia, aspiration pneumonia, and nosocomial pneumonia all occur in the compromised host. A large number of bacterial, fungal, viral, and noninfectious etiologies must be considered. A review of the topic is found in Chapters 305 to 308.

MANAGEMENT AND THERAPY OF PNEUMONIA

The first decision confronting the clinician is whether the patient with respiratory symptoms in fact has pneumonia. The difficulties in establishing a diagnosis on clinical grounds and the potential problem of overprescribing empirical antibiotics for all patients with respiratory findings have been reviewed. A chest radiograph is usually necessary to establish a definitive diagnosis of pneumonia, and should be obtained in patients considered ill enough to be considered for hospitalization.[310]

The next decisions are whether the patient is to be hospitalized, and if hospitalized whether the patient needs admission to an ICU, both of which have consequences as to the level of treatment, the cost of care, and potential complications. Inpatient management can increase the cost of care for CAP up to 25-fold, is less desirable to patients, and for low-risk patients is associated with comparable clinical outcomes.[256,311,312] Numerous severity assessment tools have now been developed to identify patients with more severe disease who require hospitalization or ICU admission.[249,259–264] The earlier assessment tools incorporated a combination of clinical, epidemiologic, laboratory, and radiographic parameters to assess; and the more recently developed tools have focused on clinical parameters alone that can be evaluated at the bedside.

One of the earliest developed and most widely used assessment tool is the PORT score, also known as the pneumonia severity index (PSI).[249] This system uses 20 clinical parameters in categories of age, presence

of comorbidities, vital sign abnormalities, and laboratory and radiologic findings. Based on a point system, five prognostic groups (I–V) were defined. The lowest scores (Group I) are associated with low mortality (0.1%), and the highest scores (Group V) are associated with the highest mortality (27%). As a guideline for hospitalization, patients in Groups I and II are usually treated as outpatients, patients in Group III are in a "borderline" group, and patients in Groups IV and V are admitted to either a routine ward or the ICU. The PORT score or PSI has been validated and widely endorsed.[95,313,314] A randomized controlled trial has confirmed that patients in PSI groups II or III who do not have respiratory failure, complicated pleural effusions, or unstable comorbid conditions have comparable clinical outcomes whether managed as inpatients or outpatients.[312] A limitation of the PSI system is its relative complexity, and several alternative scoring systems have been developed that use more readily obtainable parameters. These include the CURB score, the CURB-65 score, and the CRB-65 score.[259–261] The CURB score was formulated from the British Thoracic Society (BTS) study and uses four clinical parameters, which include new onset of confusion, urea >7 mmol/L, respiratory rate >30 breaths/min, and systolic blood pressure <90 mm Hg or diastolic blood pressure <60 mm Hg. The presence of two or more criteria suggested an increased mortality and defined severe pneumonia. The CURB-65 score, which was developed later, added age >65 years to the system, with the presence of more than three parameters leading to prediction of increased mortality; and CRB-65 modified this index to eliminate inclusion of blood urea determination, making the index laboratory free and with the patient assessment done completely at the bedside.

Several comparative trials have examined the various severity-assessment indices and assessed their usefulness.[315] These trials have indicated that the PSI, CURB-65, and CRB-65 tools appear relatively comparable in predicting high- and low-mortality groupings. Both the IDSA/ATS and BTS guidelines now support the use of these three illness severity scores for the assessment of patients with CAP.[95,310] The CRB-65, which does not require laboratory testing, appears optimal for community or primary care settings.

There is evidence that the use of these severity-assessment indices is increasing the percentage of patients with CAP who are receiving outpatient treatment.[316] However, it is critical to recognize that any severity assessment index serves only as a guideline, not as an absolute. Clinical judgment regarding presence of other comorbid conditions, hypoxia, stability of the home situation, ability to take oral medications, reliability in taking medication, likelihood of returning for follow-up, and likelihood of calling for help when needed all play a role in deciding whether a patient can be treated at home or in a hospital. In addition, the initial validation studies for the PSI, CURB-65, and CRB-65 indices excluded patients who were HIV infected or otherwise immunocompromised, or who had recently been hospitalized. There have been several studies on the usefulness of these indices in patients with HCAP that indicate that they can be used for such patients who are not immunocompromised,[317,318] but the data are still very limited for HCAP and none are applicable for immunocompromised patients.

The PSI, CURB-65, and CRB-65 indices all predict the risk of mortality due to CAP, and not the appropriate level of inpatient admission required for a patient. As noted previously, approximately 10% of patients with CAP are admitted to ICUs. Several additional indices have more recently been devised to define those patients who could benefit from this level of care. These include the SCAP score, the SMART-COP score, and the REA-ICU index, and the use of the admission serum lactate level in conjunction with the CURB-65 score.[261,263,264,319] In addition, the IDSA/ATS guideline has recommended major and minor criteria to define patients who should be admitted directly to an ICU; these have been independently validated.[95,320] The major criteria are either septic shock necessitating vasopressor support or acute respiratory failure for which invasive mechanical ventilation is required. The presence of three of the following minor criteria also is indicative of the need for ICU care: increased respiratory rate ≥30 breaths/min, low Pao_2/fraction of inspired oxygen ratio (≤250), multilobar infiltrates, confusion or disorientation, uremia (blood urea nitrogen [BUN] level ≥20 mg/dL), leukopenia (white blood cell count <4000 cells/mm^3), thrombocytopenia (platelet count <100,000 cells/mm^3), hypothermia (core temperature <36°C), and hypotension for which aggressive fluid resuscitation is required. Limited data suggest that these newer scoring systems have better discriminatory power to assess the need for ICU care.[321] Still, the complexity of these additional scoring systems limits their present use, although they should be of value if they can be incorporated into diagnostic/therapeutic algorithms within electronic medical record systems. Again, they remain guidelines, and their application must be supplemented with clinical judgment.

Antimicrobial Therapy

Although mild cases can be self-limited, the use of antimicrobial agents is the mainstay of treatment for pneumonia. In reducing the microbial burden, antimicrobial therapy can reduce the duration of illness, risk of complications, and mortality rate. If diagnostic studies, as described previously, yield a likely cause, then specific narrow-spectrum agents can be used. However, for most patients a specific diagnosis cannot be established with certainty before the onset of therapy, and an antibiotic regimen must be selected empirically.

In addition to targeting the likely expected pathogens, primary considerations in selecting specific agents for treating pneumonia are the intrapulmonary penetration of differing agents, and pharmacokinetic and pharmacodynamic characteristics. With a few exceptions, most commercially available antimicrobial agents achieve adequate intrapulmonary concentrations to be used for treatment of pneumonia, although there can be significant differences in tissue penetration.[322] One agent, daptomycin, has been shown to bind to pulmonary surfactant, thereby decreasing its efficacy in treating pneumonia.[323]

Pharmacokinetics and pharmacodynamics are important in defining appropriate antibiotic dosing. β-Lactam compounds are time-dependent killers. When a penicillin, cephalosporin, or carbapenem is being used, the active drug levels need to be above the minimal inhibitory concentration (MIC) of the organism being treated for approximately 40% to 50% of the dosing interval for an optimal outcome.[324,325] Parenteral administration of aminoglycosides will lead to low concentrations in bronchial fluids when given using traditional dosing schedules, and high serum peak levels of at least 6 μg/mL for gentamicin or tobramycin and 24 μg/mL from amikacin are needed for successful outcomes in treating gram-negative pneumonia.[326] However, as aminoglycosides show concentration-dependent killing with a significant postantibiotic effect, improved clinical outcomes can be achieved with use of pharmacodynamic modeling to optimize dosing.[324,327] A retrospective pharmacodynamic and pharmacokinetic analysis of the efficacy of vancomycin for treatment of *S. aureus* pneumonia indicated that clinical cure correlates with a 24-hour area under the curve (AUC)/MIC ratio of ≥400, and indicates that optimal dosing should target a vancomycin trough level of 15 to 20 μg/mL.[328,329] Unfortunately, even this high level of vancomycin therapy may not be effective in treating strains of *S. aureus* that have MICs ≥2 μg/mL.[330]

The empirical antimicrobial regimen selected to treat acute pneumonia is dependent on the clinical situation. Several professional societies including the IDSA, the ATS, the BTS, and the Pediatric Infectious Diseases Society have published guidelines for management of CAP, and the IDSA and ATS have published joint guidelines on managing hospital-acquired pneumonia, VAP, and HCAP in adults.[a]

For adults with CAP, the IDSA/ATS guidelines and BTS both recommend stratifying patients for outpatient versus inpatient treatment based on PSI or CURB-65 scoring systems, although the BTS recommends the use of the CRB-65 score for patients seen in the community or primary care setting. In all of the guidelines, recognition of the most likely etiologic agent in any given clinical situation and recognition of the organisms most likely to cause morbidity and mortality are emphasized. Finally, prevalence of common antibiotic resistance patterns and risks of acquisition are recognized. Empirical antibiotic therapy for CAP in children and adults and for HCAP is reviewed in Tables 67.5 and 67.6. Refer to Chapter 301 for recommendations for empirical management of hospital-acquired pneumonia.

For a patient who does not require hospitalization and for whom no clear distinction between typical (i.e., pneumococcal) and atypical

[a]References 85, 95, 181, 310, 331, 332.

TABLE 67.5 Guide to Empirical Choice of Antimicrobial Agent for Treating Adult Patients With Community-Acquired Pneumonia (CAP) or Health Care–Acquired Pneumonia (HCAP)

PATIENT CHARACTERISTICS	PREFERRED TREATMENT OPTIONS
Outpatient	
Previously Healthy	
No recent antibiotic therapy	Macrolide,[a] or doxycycline (100 mg 2 times/day)
Recent antibiotic therapy[b]	A respiratory fluoroquinolone[c] alone, an advanced macrolide[d] plus oral β-lactam[e]
Comorbidities (COPD, Diabetes, Renal Failure or Congestive Heart Failure, or Malignancy)	
No recent antibiotic therapy	An advanced macrolide plus oral β-lactam or a respiratory fluoroquinolone
Recent antibiotic therapy	A respiratory fluoroquinolone alone or an advanced macrolide plus a β-lactam
Suspected aspiration with infection	Amoxicillin-clavulanate or clindamycin (600 mg IV q8h or 300 mg PO q6h)
Influenza with bacterial superinfection	Vancomycin, linezolid, or other coverage for MRSA or CA-MRSA[f]
Inpatient	
Medical Ward	
No recent antibiotic therapy	A respiratory fluoroquinolone alone or an advanced macrolide plus an intravenous β-lactam[g]
Recent antibiotic therapy	An advanced macrolide plus an intravenous β-lactam, or a respiratory fluoroquinolone alone (regimen selected will depend on nature of recent antibiotic therapy)
Intensive Care Unit (ICU)	
Pseudomonas infection is not a concern	A β-lactam[g] plus either an advanced macrolide or a respiratory fluoroquinolone
Pseudomonas infection is not a concern, but patient has a β-lactam allergy	A respiratory fluoroquinolone, with or without clindamycin
Pseudomonas infection is a concern[h] (cystic fibrosis, impaired host defenses)	Either (1) an antipseudomonal β-lactam[i] plus ciprofloxacin (400 mg IV q8h or 750 mg PO q12h), or (2) an antipseudomonal agent plus an aminoglycoside[j] plus a respiratory fluoroquinolone or a macrolide
Pseudomonas infection is a concern but the patient has a β-lactam allergy	Aztreonam (2 g IV q8h) plus aminoglycoside plus a respiratory fluoroquinolone
Health Care–Associated Pneumonia[k]	Either (1) an antipseudomonal β-lactam plus ciprofloxacin or levofloxacin, or (2) an antipseudomonal agent plus an aminoglycoside plus a respiratory fluoroquinolone or a macrolide plus vancomycin or linezolid (for MRSA coverage)

All dosages are usual adult doses and may require adjustment in relation to renal or hepatic function, a patient's body mass index, or drug-drug interactions.
[a]Azithromycin, clarithromycin, or erythromycin.
[b]That is, the patient was given a course of antibiotic(s) for treatment of any infection within the past 3 months, excluding the current episode of infection. Such treatment is a risk factor for drug-resistant *Streptococcus pneumoniae* and possibly for infection with gram-negative bacilli. Depending on the class of antibiotics recently given, one or another of the suggested options may be selected. Recent use of a fluoroquinolone should dictate selection of a nonfluoroquinolone regimen, and vice versa.
[c]Moxifloxacin (400 mg once daily), gemifloxacin (320 mg once daily) or levofloxacin (750 mg once daily).
[d]Azithromycin (500 mg once daily), clarithromycin (250–500 mg 2 times/day), erythromycin (250–500 mg 4 times/day).
[e]High-dose amoxicillin (1 g, 3 times/day), high-dose amoxicillin-clavulanate (2 g, 2 times/day), cefpodoxime (200 mg, 2 times/day) or cefuroxime (500 mg, 2 times/day).
[f]Vancomycin dosing should target a vancomycin trough level of 15 to 20 μg/mL; linezolid, 600 mg 2 times/day.
[g]Cefotaxime (1–2 g IV q4–8h), ceftriaxone (1 g IV daily), ampicillin (1–2 g IV q4–6h), ampicillin-sulbactam (1.5–3 g IV q6h) or ertapenem (1 g IV daily).
[h]Risk factors for *Pseudomonas* infection include severe structural lung disease (e.g., bronchiectasis) and recent antibiotic therapy, health care–associated exposures or stay in hospital (especially in the ICU). For patients with CAP in the ICU, coverage for *S. pneumoniae* and *Legionella* species must always be considered.
[i]Piperacillin-tazobactam (3.375 g IV q6h), imipenem (500–1000 mg IV q6h), meropenem (1–2 g IV q8h), ceftazidime (2 g IV q6–8h), or cefepime (1–2 g IV q8h) are excellent β-lactams and are adequate for most *S. pneumoniae* and *Haemophilus influenzae* infections. They may be preferred when there is concern for relatively unusual CAP pathogens, such as *Pseudomonas aeruginosa, Klebsiella* species, and other gram-negative bacteria.
[j]Data suggest that older adults receiving aminoglycosides have worse outcomes. Traditionally dosed aminoglycosides should achieve peak levels of at least 8 μg/mL for gentamicin or tobramycin, and 25–35 μg/mL for amikacin, and troughs less than 2 μg/mL for gentamicin and tobramycin and less than 10 μg/mL for amikacin. Once-daily dosing for gentamicin or tobramycin is 5–7 mg/kg IV with trough target <2 μg/mL, and 15–20 mg/kg IV for amikacin with trough target <4 μg/mL.
[k]Pneumonia developing in patients who have been hospitalized for 2 or more days within 90 days of developing infection; patients attending hospital or hemodialysis clinics; patients receiving intravenous antibiotic therapy, wound care or chemotherapy at home within 30 days of developing infection; and residents of long-term care facilities or nursing homes.
CA-MRSA, Community-associated methicilllin-resistant *Staphylococcus aureus*; *CAP,* community-acquired pneumonia; *COPD,* chronic obstructive pulmonary disease.
Modified from Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. *2007;44(suppl 2):S27–S72.*

(e.g., mycoplasmal, chlamydial) pneumonia can be made, both types of organisms should be covered. Risks for the presence of drug-resistant *S. pneumoniae* should be assessed. Use of previous antibiotics, especially a β-lactam, macrolide, or fluoroquinolone in the prior 3 to 6 months, and residence in a long-term care facility are predictive of the presence of resistance to β-lactams, macrolides, and fluoroquinolones.[333–335] Where risk of drug-resistant *S. pneumoniae* is low, oral β-lactam agents (high-dose amoxicillin, amoxicillin-clavulanic acid, cefuroxime axetil), azalides and macrolides (azithromycin, clarithromycin, or erythromycin), or respiratory tract quinolones (levofloxacin, gemifloxacin, moxifloxacin) are all adequate choices. Doxycycline and trimethoprim-sulfamethoxazole may be used, but there is a concern of an increasing incidence of resistance to both of these agents in strains of pneumococci.[95,310] Increased resistance to the azalide-macrolide agents due to blockage of the ribosomal binding area encoded by the *erm* (B) gene is also becoming a problem in *S. pneumoniae,* and therapeutic failures have been noted.[336] It has been suggested that azalide-macrolide agents may be used as long as the high-level resistance rate in *S. pneumoniae* in the community is less than 25%; however, analysis has suggested that use of this cutoff could be associated with increased morbidity and mortality in patients with pneumococcal disease.[337]

For patients with an increased risk for poor outcome because of age or underlying disease, or in whom the risk for infection with resistant pneumococci exists because of prior antibiotic use, the respiratory tract quinolones are the agents most likely to be effective. They currently are active against over 99% of strains of *S. pneumoniae* including penicillin-resistant strains, and they have the added benefit of activity against atypical agents. However, extensive use of these agents has led to increased

TABLE 67.6 Guide to Empirical Choice of Antimicrobial Agent for Treating Children With Community-Acquired Pneumonia (CAP)

PATIENT CHARACTERISTICS	PREFERRED TREATMENT OPTIONS
Outpatient	
<5 Years of Age	
Presumed bacterial	Oral amoxicillin (90 mg/kg/day) in 2 doses or oral amoxicillin-clavulanate (90 mg/kg/day amoxicillin component) in 2 doses
Presumed atypical	Oral azithromycin (10 mg/kg on day 1, followed by 5 mg/kg/day on days 2–5) or oral clarithromycin (15 mg/kg/day in 2 doses) or oral erythromycin (40 mg/kg/day in 4 doses)
≥5 Years of Age	
Presumed bacterial	Oral amoxicillin (90 mg/kg/day in 2 doses to a maximum of 4 g/day) or oral amoxicillin-clavulanate (amoxicillin component, 90 mg/kg/day in 2 doses to a maximum dose of 4000 mg/day)
Presumed atypical	Oral azithromycin (10 mg/kg on day 1, followed by 5 mg/kg/day once daily on days 2–5 to a maximum of 500 mg on day 1, followed by 250 mg on days 2–5) or oral clarithromycin (15 mg/kg/day in 2 doses to a maximum of 1 g/day) or erythromycin or doxycycline for children >7 yr old
Inpatient (All Ages)	
***Fully Immunized Against* Streptococcus Pneumoniae *and* Haemophilus Influenzae, *and Low Local Level of Antibiotic Resistance in* S. Pneumoniae**	
Presumed bacterial	Ampicillin or penicillin G or ceftriaxone or cefotaxime; add vancomycin or clindamycin for suspected community-associated MRSA
Presumed atypical	Azithromycin (add β-lactam if diagnosis of atypical pneumonia is in doubt); or clarithromycin or erythromycin; or doxycycline for children >7 yr old; or levofloxacin for children who have reached growth maturity, or who cannot tolerate macrolides
***Not Fully Immunized Against* S. Pneumoniae *and* H. Influenzae, *or High Local Level of Antibiotic Resistance in* S. Pneumoniae**	
Presumed bacterial	Ceftriaxone or cefotaxime; addition of vancomycin or clindamycin for suspected community associated-MRSA; alternative: levofloxacin; addition of vancomycin or clindamycin for suspected community associated-MRSA
Presumed atypical	Azithromycin (add β-lactam if diagnosis in doubt); or clarithromycin or erythromycin; or doxycycline for children >7 yr old; or levofloxacin for children who have reached growth maturity or who cannot tolerate macrolides

Modified from Bradley JS, Byington CL, Shah SS, Alverson B, Carter ER, Harrison C, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. Clin Infect Dis. *2011;53:e25-e76.*

antibiotic resistance.[338] Combination therapy with a β-lactam plus a macrolide is a comparable regimen.

Regardless of the initial choice of antibiotic, once an organism is isolated, coverage should be narrowed down, if possible, on the basis of susceptibility test results.

Patients who are ill enough to require hospitalization should be treated with parenteral agents that cover the likely pathogens. Whether there is a benefit for antibiotic combinations in this setting remains an ongoing question. Combination therapy with β-lactam antibiotics and macrolides, especially azithromycin, had been associated with decreased mortality in adult patients with CAP compared with β-lactam monotherapy.[339,340] One hypothesis is that the immunomodulatory effects of macrolides may contribute to this effect, and the benefit may be more pronounced when macrolides are given before a β-lactam agent.[40,41,341] However, the relative benefit of β-lactam–macrolide combination therapy versus a β-lactam alone has been low or inapparent in randomized controlled trials in adults and children in which guideline-concordant therapy was addressed, using potentially more relevant clinical end points such as clinical stability or hospital length of stay.[342–347] The potential benefit of combination therapy with azithromycin is also counterbalanced by a small increased risk of sudden death due to cardiovascular events in individuals with preexisting cardiovascular risk factors.[348–350] Overall, at this time our choice for most individuals would be azithromycin plus a β-lactam (ceftriaxone or cefotaxime) except in patients at high risk of cardiovascular disease, in whom a respiratory fluoroquinolone seems preferable. If there are factors that suggest a specific cause, or if a Gram stain is revealing, specific antibiotic coverage should be used.

Although these regimens represent the basic course of therapy, specific clinical circumstances may warrant variation. For example, *S. aureus* pneumonia including community-associated MRSA should be considered in patients who have severe necrotizing pneumonia during an influenza outbreak, even though *S. pneumoniae* is still the major etiologic agent.[63] Agents with activity against MRSA should be used if there is reason to suspect its presence as the cause of pneumonia. Linezolid and vancomycin are the best-studied agents for treatment of MRSA pneumonia, and clindamycin has also appeared effective in children.[332,351] A prospective controlled trial comparing linezolid and vancomycin with hospital-acquired pneumonia or HCAP due to MRSA found a better initial clinical outcome for patients treated with linezolid, but no difference in mortality at 60 days.[352] A new cephalosporin, ceftaroline, has good in vitro activity against MRSA isolates and may prove to be another alternative for treatment of MRSA pneumonia, although clinical trials of its use in this setting are not currently available.[353] Should the patient be found to have methicillin-susceptible *S. aureus* pneumonia, treatment with nafcillin or oxacillin is preferred. There are no current clinical efficacy data on the use of trimethoprim-sulfamethoxazole, fluoroquinolones, doxycycline, or tigecycline for treatment of staphylococcal pneumonia.

If anaerobic aspiration pneumonia is a possibility, such as in patients developing pneumonia after loss of consciousness due to drugs, alcohol, or neurologic disease, agents with activity against oral anaerobes are needed, including ampicillin-sulbactam or clindamycin. Otherwise, clinical trials have suggested that targeted anaerobic coverage is not required for the majority of cases of CAP.[95]

Aerobic gram-negative bacilli including *P. aeruginosa* cause 7% to 18% of CAP cases. Risk factors previously noted for gram-negative pneumonia should therefore be sought. Where gram-negative bacilli are suspected, infection with *P. aeruginosa* should be a concern, and therapy with an antipseudomonal β-lactam compound (e.g., cefepime, ceftazidime, piperacillin-tazobactam, imipenem, or meropenem) is a reasonable choice. When *Pseudomonas* involvement can be excluded, agents such as cefotaxime, ceftriaxone, or ertapenem could be considered. Debate exists as to whether combination therapy with both a β-lactam agent and either an aminoglycoside or a quinolone will improve the outcome of gram-negative pneumonia. Data exist to support both sides of the controversy, although there is increasing evidence that initial combination therapy decreases the risk of initially inappropriate therapy.[354–357] We favor initial combination therapy for patients who are severely ill, at least until culture results from sputum and blood are available to confirm that an agent with in vitro activity against the presumed organisms is being given. In patients who are allergic to penicillin, aztreonam with a respiratory tract fluoroquinolone, with or without an aminoglycoside, could be used.

In the patient admitted to an ICU, therapy should be directed against *S. pneumoniae,* penicillin-resistant strains, *Legionella* species, gram-negative rods, and *M. pneumoniae.* If infection with *P. aeruginosa* is unlikely (no recent hospitalization, no recent antibiotic use, no pulmonary comorbidities, no gram-negative rods on Gram stain), a β-lactam plus either an azalide/macrolide or a respiratory tract fluoroquinolone would be therapies of first choice. Ceftriaxone or cefotaxime would be reasonable choices for the β-lactam. If *Pseudomonas* infection cannot be excluded, an antipseudomonal β-lactam (cefepime, imipenem, meropenem, doripenem, or piperacillin-tazobactam) plus a respiratory tract

fluoroquinolone or azalide/macrolide could be used. We favor cefepime or piperacillin-tazobactam plus a respiratory tract fluoroquinolone. An aminoglycoside could be added as a third agent for synergy against *Pseudomonas*. Evidence in the literature favoring one regimen over any other is lacking. New agents, such as ceftazidime-avibactam, that are active against ceftazidime-resistant isolates of *P. aeruginosa* have now been approved, although there are limited data available on their clinical efficacy.[358]

Timing of Antibiotics

In 1997, a retrospective review of over 14,000 Medicare patient hospitalizations suggested that antibiotic therapy given within 8 hours of presentation was associated with decreased mortality.[359] A second retrospective study of similar design in 2004 showed that antibiotics given within 4 hours of presentation resulted in lower mortality.[360] Neither study corrected for pneumonia etiology nor antibiotics used. Despite the lack of a prospective randomized study, advising and regulatory agencies including The Joint Commission and the Centers for Medicare and Medicaid Services began to use the 4-hour rule as a core quality measure. Subsequent studies found that attempting to meet this performance standard led to increased misdiagnoses and potentially inappropriate antibiotic prescribing in emergency department patients, and failure of hospitals to meet this standard was not associated with an increase in inpatient mortality in patients admitted with CAP.[361–363] This hospital performance standard has subsequently been eliminated. Still, there is clear evidence that delays in antibiotic therapy can affect the outcome of patients with both pneumonia and sepsis.[340,364] The IDSA/ATS guidelines currently recommend that antibiotic therapy for pneumonia should be started as soon as the diagnosis is considered likely.[95]

Duration of Treatment and Use of Clinical Practice Guidelines

Until recently, the duration of antibiotic therapy for pneumonia has been based on anecdotal patterns of behavior. There have been few studies addressing the appropriate duration of treatment, but the classic 10- to 14-day duration of care is unsupported by evidence.[365] Data now indicate that clinical stability (defined as normalization of previously abnormal physiologic parameters, including heart rate, respiratory rate, oxygenation, blood pressure, mental state, and ability to care for oneself) occurs relatively quickly for patients hospitalized with CAP (Table 67.7).[366] Most physiologic abnormalities will correct in 2 to 3 days, and normalization of all physiologic abnormalities generally occurs in 5 to 7 days. Patients with more severe illness generally take longer to stabilize. The addition of monitoring for at least a 50% reduction in CRP and determination of procalcitonin levels have been suggested as additional measures to define clinical stability, but therapeutic effectiveness and cost-effectiveness of using these approaches has not been carefully assessed.[180,367,368] Overall, once stability is achieved, clinical relapses serious enough to warrant ICU care occur less than 1% of the time.

Oral antibiotic therapy is safe after clinical stability has been reached, even in patients with severe CAP.[369–371] There is no clear usefulness for observing a patient within the hospital after a switch to oral therapy.[372] However, it is important to recognize that discharging patients before stability has been reached may lead to increased rehospitalization and mortality.[373,374] Using the same definitions of clinical stability, it has been shown that the greater the number of factors remaining abnormal at discharge, the greater is the chance of readmission or death.

There are few studies on the duration of therapy for pneumonia that are prospective, are well controlled, use the same antibiotic and dosing schedule, and vary only the duration of therapy. However, these few studies have found that durations of less than 7 days and as short as 3 days of azithromycin are just as effective as longer durations of therapy for mild-to-moderate CAP.[375–377] With age, presence of underlying comorbidities including immune compromise, and more virulent pathogens, clinical stability may be delayed, and therefore duration of antibiotic therapy may be lengthened. Currently for adult patients with CAP the IDSA/ATS guidelines recommend a minimum of at least 5 days of antibiotic therapy, with the patient being afebrile for between 48 and 72 hours, and lacking no more than one sign of clinical stability.[95] Similarly, the BTS guidelines recommend 7 days of appropriate antibiotic therapy for patients with low- or moderate-severity CAP treated either as outpatients or inpatients.[310] Longer therapy should be considered for patients who have high severity disease, bacteremic *S. aureus* pneumonia, or cavitary disease. The Pediatric Infectious Diseases Society/IDSA guidelines for CAP in children note that 10 days of treatment is best studied in children, although shorter-course treatment is likely effective.[332]

Although early studies found limited benefit to concordance of process of care measures and clinical outcomes in pneumonia, more recent evidence indicates that compliance with clinical practice guidelines for both CAP and HCAP is associated with decreased inpatient mortality and inpatient length of stay.[158,342,344,378–381] The use of inpatient critical pathways (care bundles) based on clinical practice guidelines can reduce inpatient length of stay and 30-day inpatient mortality without increasing adverse effects.[313,382–384]

Once the patient has been discharged, outpatient follow-up should be coordinated, because most patients with CAP will have some related residual symptoms, including fever, cough, shortness of breath, chest pain, sputum production, fatigue, or gastrointestinal symptoms. Comorbidities, particularly cardiopulmonary or neurologic disease, are the most frequent reason for subsequent early readmission among patients who achieve clinical stability.[374,385]

Adjunctive Therapy

A robust inflammatory response to an invading pathogen can lead to a potentially worse outcome in pneumonia, and the use of antiinflammatory agents could have potential benefits as demonstrated with the improved outcome with the addition of corticosteroid therapy for *Pneumocystis* pneumonia. Several randomized controlled trials have now investigated the efficacy of corticosteroid therapy for CAP using differing dosages and agents. To date, steroids appear to shorten the time to clinical stability and overall inpatient length of stay by 1 day; but are also associated with an increase in hyperglycemia and subsequent rehospitalization.[386–389] Statins also possess antiinflammatory properties, and their impact on CAP has been assessed in observational studies. Although there was initial suggestive evidence of benefit, those studies were not been randomized and did not control for other potentially important variables such as underlying health or socioeconomic status.[390] More recent studies have found no impact of recent statin use on clinical outcomes in CAP.[391,392] Although other adjunctive therapies have been described—including the use of activated protein C, noninvasive mechanical ventilation, anticoagulants, immunoglobulin, granulocyte colony-stimulating factor, probiotics, chest physiotherapy, antiplatelet drugs, over-the-counter cough medications, β_2-agonists, inhaled nitric oxide, and angiotensin-converting enzyme inhibitors—in clinical trials, none of these approaches has been shown to have a significant role in therapy.[393]

TABLE 67.7 Evidence of Clinical Stability or Improvement

Temperature ≤37.8°C
Pulse ≤100 beats/min
Respiratory rate ≤24 breaths/min
Systolic blood pressure ≥90 mm Hg
Arterial oxygen saturation ≥90% or Po_2 ≥60 mm Hg on room air
Ability to maintain oral intake
Normal mental status

Modified from Halm EA, Fine MJ, Marrie TJ, Coley CM, Kapoor WN, Obrosky DS, et al. Time to clinical stability in patients hospitalized with community-acquired pneumonia: implications for practice guidelines. JAMA. *1998;279:1452–1457; and Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults.* Clin Infect Dis. *2007;44 Suppl 2:S27–S72.*

PREVENTION OF PNEUMONIA

Vaccinations against influenza and *S. pneumoniae* are important interventions in preventing pneumonia. In older adults, influenza vaccine can decrease the incidence of hospitalization, pneumonia, and mortality, and efficacy has been demonstrated over 10 consecutive influenza seasons.[394,395] Influenza vaccine is suggested for any person 6 months

of age or older, who, because of age or underlying disease, is at risk for influenza-related complications. This includes persons older than 50 years; nursing home residents; people with chronic pulmonary or cardiac disease, or with chronic diseases such as diabetes, renal failure, or hematologic disorders; patients who are immunosuppressed; those taking chronic salicylate therapy; and women in their second or third trimester of pregnancy. Health care workers, workers in nursing homes, and those who provide care to older adults or debilitated persons should also be targeted for influenza vaccination.[396]

A 23-valent pneumococcal polysaccharide and both 7-valent and 13-valent pneumococcal conjugate vaccines are licensed in the United States. Good clinical data show that these vaccines provide protection against bacteremia and invasive pneumococcal disease, and the introduction of the pneumococcal vaccine into the US childhood immunization program has been associated with a notable reduction in pneumonia incidence in all age groups, indicating a ("herd") community effect.[227] Limited data now show a direct effect of adult pneumococcal immunization in decreasing pneumonia incidence.[397,398] Both the 23-valent pneumococcal polysaccharide and 13-valent pneumococcal conjugate vaccines have now been approved for use in adults older than 50 years, and pneumococcal vaccine is recommended for patients older than 65 and those who have recovered from CAP. The efficacy and sequence of administration of these vaccines are discussed further in Chapter 199.

Active smoking is a clear risk factor for bacterial pneumonia, and promotion of smoking cessation should be a component of pneumonia prevention.[66,399,400]

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The complete reference list is available online at Expert Consult.

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68 Pleural Effusion and Empyema

Mark Parta

SHORT VIEW SUMMARY

Physiology

- The small volume of fluid in the pleural space at baseline increases with infection or other inflammatory conditions.
- Multiple cytokines and factors contribute to this change, including vascular endothelial growth factor, interleukin-1α and interleukin-1β, tumor necrosis factor-α, and interleukin-6.
- These factors should be taken into consideration because of the increasing therapeutic use of drugs/agents that antagonize them.
- Coagulation and fibrin clots contribute to the evolution of effusions into loculated collections of material that become progressively more resistant to medical therapies.

Diagnosis

- Community-acquired pneumonia is the most common predisposing condition to pleural infection. Symptoms in addition to those related to the pneumonia may include chest pain and splinting on the affected side, but symptoms and signs are both insensitive and nonspecific. Conventional chest x-rays may be insensitive for effusions, which occur in >40% of cases of community-acquired pneumonia.
- Computed tomography scans and ultrasound may enhance detection of fluid, but only ultrasound reliably detects the organization of an effusion by septation/loculation. Ultrasound improves sampling accuracy.
- Only pleural fluid sampling reliably characterizes effusions. Fluid should be stained and cultured for bacteria, fungi, and mycobacteria, and studied for cell content, pH, and chemistry (lactate dehydrogenase [LDH], glucose, and protein).
- The clinical context should dictate additional testing, such as pneumococcal antigen in the context of lobar pneumonia, molecular assays for viruses or mycobacteria, or pleural biopsy for tuberculosis. Surrogate markers such as adenosine deaminase have a role in certain settings.

Fluid Analysis

- Fluids are divided into transudates or exudates on the basis of chemistries. Transudates are characteristic of noninfectious processes such as congestive heart failure, but occasionally occur early in the course of parapneumonic effusion and may respond to medical therapy alone.
- Exudates, with high protein and LDH content, are more likely to require drainage by tube thoracostomy early. Tuberculosis is the exception, since most tuberculous effusion is exudative but resolves spontaneously even without treatment. Low pH is a strong predictor of the need for aggressive drainage of a parapneumonic effusion and requires submission for testing in a closed syringe like a blood gas.
- Empyema is not characterized by chemistry, but by appearance (purulence, pus) and/or positive microbiologic studies.

Microbiology

- Infecting agents in pleural fluid are often associated with pneumonia. Tuberculous pleurisy is common when tuberculosis is prevalent. *Streptococcus pneumoniae* remains an important cause of community-acquired bacterial pneumonia and pleural disease. Members of the *Streptococcus anginosus* group are important empyema pathogens but infrequent causes of pneumonia.
- In adults, especially older adults, pleural infection is often the result of aspiration or obstruction related to malignancy. In that context, methicillin-resistant *Staphylococcus aureus,* gram-negative organisms, and anaerobic upper aerodigestive tract organisms predominate.
- Fungal empyema is most often caused by *Candida,* and *Candida* recovered from a pleural effusion suggests disruption of the gastrointestinal tract, often the esophagus, as a source.

Management

- Thoracentesis is diagnostic and rarely therapeutic. Effusions that require drainage almost always require placement of a chest tube (tube thoracostomy).
- Antimicrobial therapy should be appropriate to the setting, especially when organisms such as methicillin-resistant *S. aureus,* gram-negative organisms, and anaerobes are potential pathogens.
- Although fibrinolytic therapy, usually recombinant tissue plasminogen activator combined with recombinant DNase, may aid in achieving drainage, the settings in which it contributes to clinical outcomes are poorly defined.
- Medical and video-assisted surgical thoracoscopy are intermediate-stage interventions that allow for directed evacuation of infected space without full surgical intervention by thoracotomy. These are appropriate when fluid is no longer free flowing or the pH is <7.3, but at some poorly defined stage of pleural thickening, operative management by thoracotomy is necessary.
- Chronic empyema requires thoracoscopic treatment or thoracotomy and is a high-morbidity and high-mortality condition. Additional surgical measures are often necessary, especially when bronchopleural fistula is present.

Thoracic empyemas appear in the Hippocratic corpus.[1] In the 16th century, the famous essayist and physician Michel de Montaigne wrote in his essay on the fear of death, "I omit to speake of agues and pleurisies…," recognizing their routine role in mortality.[2] In the early 20th century, one of medicine's most famous practitioners, Sir William Osler, died of complications of empyema.[3]

PHYSIOLOGY AND STAGING

Three factors control the flow of the small volume of fluid that lubricates the pleural space. The pulse of fluid (hydrostatic pressure) is accompanied by osmotic pressure between the vessels in the parietal pleura and the pleural space. The outflow of fluid is in a dependent direction through lymphatic stomata in the lower parietal pleura. The pleura itself consists of collagen and elastic fibers that contain interspersed mesothelial cells. These are metabolically active and mediate some portion of the inflammatory response, as well as provide part of the barrier function of the pleura.[4] The quantity of fluid in the pleural space, measured by in vivo pleural lavage, is 0.26 ± 0.1 mL/kg whole body weight, with 1.7×10^6 white blood cells/mL. Macrophages are the predominant cell (75%), followed by lymphocytes (23%) and mesothelial cells (1%). Smokers have a small increase in neutrophils.[5] In response to infection or injury, the fluid content of the space increases. Uncomplicated effusions usually

respond to measures directed at their cause, for instance, treatment of a pneumonia accompanied by a parapneumonic effusion. Complicated effusions represent a middle point, with the development of fibrinous material over time that will cause septations and loculation of the fluid if the cause is not successfully addressed. Complicated effusions and empyema are likely to require drainage and therapy directed at the pleural complication itself.[6]

Multiple staging systems have been developed to characterize the evolution of pleural disease. Pleural fluid is a transudate when it is primarily influenced by changes in hydrostatic pressure in heart failure and fluid overload states such as renal failure or cirrhosis. Exudates are characteristic of effusions associated with cancer, infection, and many noninfectious causes, as further discussed in "Fluid Analysis" (see later). A three-stage division of the evolution of pleural disease is practical and can be integrated into most of the medical literature, as seen in British Thoracic Society (BTS) and American Association for Thoracic Surgery (AATS) guidelines, which reference an American Thoracic Society statement from 1962.[7–9] The first stage (exudative, stage I [AATS]; simple parapneumonic effusion [BTS]) shows clear fluid with a low cell count and reexpandable lung. The pH is >7.2, lactate dehydrogenase (LDH) is <1000 IU/L, and glucose is >2.2 mmol/L. The second stage is fibrinopurulent (AATS) or complicated parapneumonic effusion (BTS), with high cell count, fibrin deposition, and biochemical parameters of pH <7.2, LDH >1000 IU/L, and glucose <2.2 mmol/L, and there may be a positive culture. The third phase (organized [AATS]; empyema [BTS]) is frankly purulent fluid, which is thick and may present with trapped lung and membranes on the pleural surface. It is important to note that empyema is still defined as fluid that is purulent or has positive microbiologic studies. For parapneumonic effusion, the risk for outcome associated with pleural space anatomy, pleural fluid bacteriology, and pleural fluid chemistry yielded a four-category assessment as documented in a 2000 statement from the American College of Chest Physicians.[10] A classification from Light includes seven stages.[11]

The physiology of infected pleura changes as infective organisms interact with the immune system. Staphylococcal peptidoglycan induces the production of β-defensins in a murine model, and signaling cascade antagonists interfere with this.[12] Vascular endothelial growth factor (VEGF) increases permeability and fluid in the pleural space.[13] The consequence of VEGF antagonism in clinical practice confirms the importance of this mechanism. Levels of VEGF are higher in parapneumonic effusions than in those caused by congestive heart failure (CHF). Experiments with mesothelial monolayers show that *Staphylococcus aureus* causes a response similar to that induced by recombinant VEGF, with a decrease in electrical resistance across the cells and protein leaks across the monolayers.[14]

The evolution of infected effusions may differ with the infecting organism, the difference between bacterial and mycobacterial disease being illustrative. Bacille Calmette-Guérin, in an in vitro model, decreases the tight junction between cells by downregulating β-catenin, an adherens junction protein. This leads to increased permeability due to VEGF production and then the protein influx characteristic of exudative effusions.[15] Inflammatory mediators, such as cytokines, are produced by mesothelial cells and pleural macrophages. In 70 patients with various etiologies of effusion, interleukin (IL)-1β was higher in parapneumonic than malignant effusion, and was produced by pleural macrophages in response to lipopolysaccharide. Interferon-γ in particular is elevated in the pleural fluid of patients with tuberculosis.[16] Variations in these cytokine profiles have led to attempts to use IL-1α, IL-6, and tumor necrosis factor-α (TNF-α) as diagnostic tools that would differentiate transudates from exudates. IL-6 differentiated exudates (high levels) from transudates and was significantly higher in tuberculous than malignant or parapneumonic effusions.[17] Some of the differences between nonspecific effusions and those due to tuberculosis are attributable to the known role of $CD4^+$ lymphocytes in the immunology of tuberculosis, as discussed in Chapter 251.[18]

Cell wall constituents of *Mycobacterium tuberculosis,* such as the protein-peptidoglycan complex and lipoarabinomannan, cause the release of TNF-α from pleural fluid mononuclear cells in a dose-dependent manner, which may account for the classical manifestations of tuberculous pleurisy, such as fever, an exudative effusion, and tissue necrosis.[19] Cytokine changes drive the accumulation of neutrophils. TNF-α induces neutrophil chemotactic activity driven by IL-8, which is higher in empyema than in nonpurulent effusions of any etiology. TNF-α levels and activity diverge, though, with high levels in both empyema and tuberculous fluid, but increased bioactivity only in empyema.[20]

An additional two components of progression in effusion and empyema are coagulation and fibrosis. In animal models, blocking transforming growth factor-β with an antibody decreases purulence and fibrosis.[21] A loculated effusion with fibrous septations is linked to procoagulant activity and decreased fibrinolytic activity in exudates, mediated by mesothelial cells. This important in vitro observation supported clinical trials of fibrinolytics in the treatment of pleural effusion, as delineated later.[22]

NONINFECTIOUS EFFUSION AND EMPYEMA

Pleural effusion can be caused by myriad noninfectious processes. In some of these, there are sufficient descriptions of the likelihood and character of the effusion to increase diagnostic certainty. General states of fluid overload, such as CHF, renal failure, and cirrhosis, are common causes of effusion.[23] Inflammatory noninfectious diseases such as rheumatoid arthritis, systemic lupus erythematosus, and pancreatitis are important differential considerations. Cancers, generally secondary but occasionally primary in the pleura, with mesothelioma prominent among them, are common causes of exudative effusion.[24] Tunneled indwelling pleural catheters (PleurX Drainage System) increasingly are used for chronic drainage in malignant effusions from solid tumors or hematologic disease. These of necessity carry an infection risk, reported as 7.7% in hematologic malignancies and 3.7% in other malignant effusions.[25] The report of longer survival in melanoma patients whose effusions become infected in this context is of dubious significance.[26] Thoracic surgical procedures, from cardiac surgery to lung transplantation, are usually followed by an accumulation of exudative pleural fluid, which decreases more rapidly after cardiothoracic surgery than lung transplantation.[27–29] Elevated neutrophil counts are the most sensitive and specific indicators of infection.[30] Drugs may be associated with effusion, with or without parenchymal involvement. Of note, immune checkpoint inhibitors, while associated with pneumonitis, are rarely if ever the causes of pleural effusion. Thus an effusion in the context of these therapies requires investigation for potential infectious causes.[31] Distinguishing characteristics of some noninfectious causes of pleural effusion are presented in Table 68.1.

The epidemiology of infectious pleural effusion is influenced by the rigor of attempts to detect effusions, the era reported, the age of the population studied, and the prevalence of tuberculosis and other endemic diseases. The outcome of bacterial pleural disease is heavily influenced by the success of treating an associated bacterial pneumonia. In a pediatric hospital in the United States, temporal trends from before and through the initial years of the antibiotic era show a drop in empyema from 1934 to 1958, with decreases in cases caused by *Haemophilus influenzae,* streptococci, and pneumococci but a rise in *S. aureus* in later years, coincident with an influenza epidemic.[32] In adults from 1933 to 1972 (a survey of selective, noncontinuous sample years), a similar decline in pneumococcal disease occurred, and the same increase in *S. aureus* was seen in 1955, with a decrease through 1965. As disease caused by antibiotic-susceptible organisms decreased, mortality associated with empyema increased, as did the age of the population affected.[33] Hospital-acquired disease, described in a separate report on duration of hospitalization, had already shown a trend to an increase in disease caused by gram-negative bacilli, *S. aureus,* and enterococci. Improvements in antibiotic therapy may have been responsible for shorter hospitalizations in the later years compared to those in the preantibiotic era.[34] Later influenza epidemics may have had a different spectrum of bacterial complications. In children, *Streptococcus pneumoniae* and *Streptococcus pyogenes* were the most frequent causes of secondary bacterial pneumonia in the 2009 influenza A(H1N1) epidemic.[35]

In a Spanish report of empyema from 1984 to 1990, adults had underlying disease in 82% of cases, including alcoholism, malignancy, and diabetes mellitus.[36] Anaerobic bacterial disease is increased in this population as a consequence of aspiration and obstruction. In a

TABLE 68.1 Noninfectious Pleural Disease

	PLEURAL DISEASE FREQUENCY	FLUID CHARACTERISTIC	COMMENT	REFERENCE
Inflammatory Diseases				
Sarcoidosis	Rare	Serous exudate by LDH/protein, paucicellular	Consider other causes; diagnosis by pleural biopsy	218
Rheumatoid arthritis	3%–5%	Exudative, low glucose, sometimes chylous	Does not necessarily correlate with joint disease	219, 220
Adult-onset Still disease	17%–53%, literature summary 21%	Sterile exudate, neutrophil predominant	Arthralgia inevitably present	221, 222
Sjögren syndrome	Rare	Exudate but normal pH, glucose, and low ADA	Secondary most common; concern about concomitant lymphoma	223, 224
Granulomatosis with polyangiitis	Rare but summary 12.4%	PMN leukocytes predominant exudate		225, 226
Eosinophilic granulomatosis with polyangiitis	17.6%	Bloody effusion, high eosinophil count	Disease-associated heart failure may contribute	227, 228
Systemic lupus erythematosus	5% at presentation, 30%–50% during the course of disease	High protein exudate, higher glucose/lower LDH than RA, neutrophils early	Likely to be symptomatic, and complement/ANA on effusion not diagnostic	229, 230
Behçet syndrome	Rare (5%) but in 70% of cases with lung disease	Transudate and chylothorax	Presumed relation to thrombosis of central veins	231
TAFRO syndrome	CR, almost all from Japan	NR	IL-2 and IL-6 implicated in pathogenesis	232
Other				
Eosinophilic effusion	Cancer > PPE > tuberculosis	Eosinophils >10%	Inverse relation to likelihood of malignancy	233
Trauma	Rib fracture in 71%, pulmonary contusion in 43%	Hemothorax 39%	Retained hemothorax (higher with medical vs. surgical management) and late effusions risk for empyema	234, 235
Pulmonary embolism	48%	Small	Ipsilateral to embolus, predicted by infarction and CRP	236, 237
Post–cardiac injury syndrome	56%–92%	70% bloody exudate, cells transition from neutrophils to mononuclear cells	Usually with ipsilateral pneumonitis, usually left sided but can be bilateral	27, 28
Radiation therapy	29.1%; symptoms associated with whole lung volume receiving ≥5 Gy	Free flowing early, loculated late	Always associated with pneumonitis	238, 239
Rounded atelectasis	23%	Exudative	Frequently with asbestos-related pleural thickening	240
Superior vena cava syndrome	Rare	Transudate when result	Effusion cause or result of SVC syndrome	241, 242
Hematopoietic stem cell transplant	9.9%	56% exudative	Engraftment syndrome early; GVHD late	243
Pancreatitis	22%–29%	Hemorrhagic; high LDH and protein, amylase Neutrophil predominance	Late or persistent effusion suggests fistula	244
Meigs syndrome	Defining	Exudative	Responsive to removal of tumor	245
Drugs				
Daptomycin	66.7%	NR, bilateral lung infiltrates, bronchial lavage >25% eosinophils	Not clearly related to dose or duration	246
Dasatinib and other tyrosine kinase inhibitors	35%	Usually bilateral, hemorrhagic or chylous, asymptomatic, lymphocyte predominant	Dose and frequency dependent	247

ADA, Adenosine deaminase; *ANA,* antinuclear antibodies; *CR,* case report; *CRP,* C-reactive protein; *GVHD,* graft-versus-host disease; *IL,* interleukin; *LDH,* lactate dehydrogenase; *NR,* not reported; *PMN,* polymorphonuclear neutrophil; *PPE,* parapneumonic effusion; *RA,* rheumatoid arthritis; *SVC,* superior vena cava; *TAFRO, t*hrombocytopenia, *a*nasarca, *f*ever, *r*enal failure or *r*eticulin fibrosis, and *o*rganomegaly.

more restricted referral center for cardiothoracic disease in the United Kingdom (predominantly male, median age 53 years), most empyema was community acquired and the microbiology was diverse, with 16.3% streptococci, 15.5% staphylococci, and gram-negative, anaerobic, pseudomonad, mycobacterial, and polymicrobial infections in lesser proportions. Tuberculosis still caused 9.1% of cases.[37] Other reports with geographic restrictions are informative. Decreases in tuberculosis in Europe and the United States have not occurred to the same extent elsewhere. Tuberculous effusion accounted for 49 of 100 effusions in a series from Malaysia in 1991.[38] In East Asia, despite high rates of *Klebsiella pneumoniae* pleural infection, the majority of empyema is not caused by hyperviscous serotypes.[39]

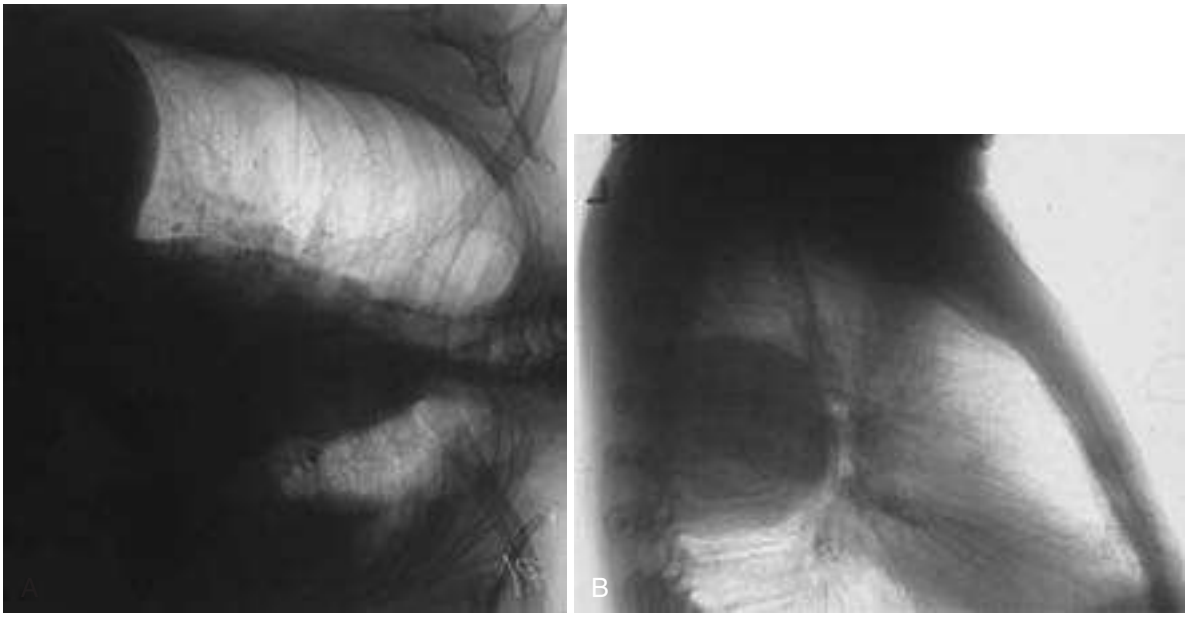

FIG. 68.1 Pleural effusions. (A) Empyema fluid is seen layering out along the dependent chest wall of a patient with left lower lobe pneumonia (left lateral decubitus film). (B) D-shaped mass representing a loculated empyema at the site of a former right upper lobectomy.

OUTCOMES OF EFFUSION AND EMPYEMA

The consequences of an infected pleural effusion depend on the population, on the infection, and, critically, on the vigor with which the physician makes the diagnosis and chooses the right therapy. Most tuberculous effusion (see below) is a low-mortality disease and remits even without therapy, but treatment decreases the likelihood of further tuberculous disease.[40] At the other end of the spectrum, postpneumonectomy empyema affects an older population with a high incidence of malignancy, so that the 5-year survival after surgical therapy was 44.5% in a series of 84 patients treated with the Clagett procedure.[41] In the UK Multicenter Intrapleural Sepsis Trial (MIST) 1, a clinical risk score was developed with levels of low (0–2), medium (3–4), and high risk (5–7). The derived score accurately predicted death at 3 months, but in a validation cohort from MIST 2 the significance of the medium score was lost.[42]

Early diagnosis and therapy are of paramount importance, yet assessment and management often are not adequate.[43,44] A retrospective analysis of prognostic factors over a 9-year period in 158 patients from Denmark (mean age 63 years) found, with an overall mortality of 27%, that nosocomial infection, predisposing conditions, and insufficient initial antimicrobial therapy correlated with poor outcome. Only 36% of patients were appropriately assessed, and the authors commented on the poor adherence to recommended diagnostic approaches in both emergency rooms and internal medicine wards.[45] Three studies of note examined the outcomes and factors affecting morbidity and mortality from empyema or pleural infection. Outcome was related to delays in drainage, with patients with the most severe disease (requiring decortication) suffering the highest mortality when the procedure was delayed.[46] A nonblinded study of 179 patients established the frequency of the need for decortication and the results of less aggressive therapy. Ninety percent of those deemed eligible for thoracocentesis alone were cured, but chest tube therapy alone (closed thoracostomy) was successful in only 62% and had a mortality of 11% in 90 patients, with second procedures needed in 24 cases. Primary or secondary decortication had a cure rate of 88% and mortality of 1.3% in another study with 76 patients.[47] Finally, risk stratification for the need for surgery was studied in 85 patients who received drainage and intrapleural fibrinolytics. There were medical failures (need to proceed to surgery) in 15%, and the absence of purulence predicted medical success but the presence of purulence did not predict failure. The overall survival was 86%.[48]

MICROBIOLOGY

Tuberculosis

The pleural manifestations of tuberculosis have been studied throughout the modern era. In the United States, pleural disease was found to be secondary only to lymphadenitis in extrapulmonary cases from 1993 to 2006. Because of its remitting nature and the difficulty of microbiologic diagnosis when only pleural fluid is sampled, pleural tuberculosis in the early 20th century was not uniformly considered tuberculous disease, but was followed by pulmonary/extrapulmonary tuberculosis in 34.8% of children and 52.6% of adults in a 1912 summary of cases from 1881 to 1908.[49] At the transition from a prechemotherapeutic era to effective drug therapy, Roper and Waring clarified the role of pleural disease as the antecedent to complicated disease (pulmonary or extrapulmonary).[50] Bed rest, once standard therapy, did not decrease this rate of progression, and Falk and Stead established that drug therapy did.[40]

In an urban setting in which the demographics were not yet dominated by human immunodeficiency virus (HIV) infection, pleural disease occurred in older adults and was associated with reactivation in 19%, and comorbidities made the diagnosis difficult.[51] With further evolution of the HIV epidemic, both the breadth and adverse consequences of tuberculosis in HIV-infected persons became apparent, with pleural disease as a common manifestation.[52,53] The $CD4^+$ T-lymphocyte depletion characteristic of HIV disease has proven the perfect storm in all manifestations of tuberculosis, including pleural disease. The role and characteristics of $CD4^+$ lymphocytes in pleural effusions (Fig. 68.1) reactive to purified protein derivative have been established, and interferon-γ, a product of activated T cells, induces the expression of mesothelial cell–derived monocyte attractants (as does bacille Calmette-Guérin).[54–57] The clinical consequences of ineffective $CD4^+$ cell-mediated defense has been shown in Rwanda, where pleural effusion was significantly more frequent in HIV-infected than in HIV-uninfected persons (43% vs. 9%). This may be true with higher $CD4^+$ T-cell counts, and appears in association with weight loss and lower lobe parenchymal disease.[58]

The clinical features of pleural tuberculosis, whether coincident with primary pulmonary disease or the primary site of disease alone (see later), include fever, dyspnea, malaise, diaphoresis, and pain antecedent to the effusion, sometimes by months. Effusions are usually unilateral. The diagnosis of pleural tuberculosis is difficult unless multiple modalities are used, including the analysis of pleural fluid, pleural biopsy, pleural fluid and pleural tissue culture, and sputum culture. Pleural fluid specificity can be maximized on exudative effusions with the parameters

of lymphocytes >80%, protein >5 g/dL, and adenosine deaminase (ADA) >45 U/L (see later), but the sensitivity remains inadequate at 34.9%.[59]

Pleural biopsy clearly established the infectious (rather than allergic) nature of pleural disease, and the use of Cope needle biopsy in 40 patients on the Columbia service at Bellevue Hospital found necrotizing granulomas in 63% and positive cultures of pleural tissue in 55%, with a combined diagnostic yield of 80%.[60] Multiple biopsies may increase the yield (6 biopsies with a sensitivity of 100%).[61] A contemporary study from Brazil found that tissue yielded a histopathologic diagnosis in 78% of cases, pleural tissue culture in 62%, and induced sputum in 52%, emphasizing the frequent occurrence of concomitant pulmonary disease, which should be sought by sputum culture even in the patients with clinically isolated pleural disease.[62] The incidence of parenchymal disease in patients with pleuritis has been underestimated.[63] The likelihood of a positive pleural fluid culture varies with the stage of disease. Tuberculous effusions evolve from polymorphonuclear cell predominance to the more characteristic lymphocyte predominance, and the yield of culture (including sputum) in the former has been higher.[64,65] Interferon-γ production by T lymphocytes has been studied as a surrogate diagnostic, and levels are elevated in tuberculous effusions.[66] Cells responsive to the antigens targeted in commercially available interferon-γ release assays are 15 times higher in tuberculous than nontuberculous effusions.[56] However, commercial assays remain appropriate for the diagnosis of latent tuberculosis but not tuberculous disease in the pleural space. A review and meta-analysis of 14 studies concluded that they were of poor quality, with substantial variation in thresholds for positivity and pooled sensitivity and specificity of 72% and 78%, respectively.[67]

New molecular and other microbiologic assays have been studied. The microscopic observation drug susceptibility assay, recently commercialized, showed greater sensitivity than culture on standard solid media. The study results are confounded by disproportionate loss of sensitivity with decontamination, which process may be of questionable utility on a sterile specimen such as pleural fluid.[68]

Of the surrogate markers studied and of potential clinical utility, by sheer volume of literature ADA would require a chapter by itself. This only serves to emphasize the common problem of a continuous variable used to determine a categorical outcome. The test is inexpensive and widely available. In circumstances in which tuberculosis is prevalent and the differential diagnosis is principally diseases that do not routinely cause a lymphocyte-predominant exudative effusion, an ADA level above 40 U/L and a lymphocyte-to-neutrophil ratio >0.75 support the diagnosis of tuberculous effusion.[69]

The Amplified Mycobacterium Tuberculosis Direct Test (Hologic, Inc., San Diego, CA) had a sensitivity of only 36.4%, but the performance was better in neutrophilic effusions (see earlier) and those of <18 days' duration.[70] Finally, the important GeneXpert product Xpert MTB/RIF (Cepheid, Sunnyvale, CA) has been evaluated in multiple studies. The sensitivities range from 14.2% to 28.7%, but specificities are high and results are rapid, although costs are high.[71,72]

The specificity of pleural radiographic abnormalities is poor in the absence of characteristic pulmonary disease. The pulmonary abnormalities prominent by computed tomography (CT) scan of the chest include micronodules in subpleural and peribronchovascular interstitium. Interlobular septal thickening suggests the lymphatic spread characteristic of the disease.[73]

The treatment of pleural tuberculosis is the same as that of pulmonary disease. Six-month therapy with isoniazid and rifampin led to no relapses in 161 patients, even when associated with smear-negative/culture-positive pulmonary disease.[74] Corticosteroids are not indicated for the treatment of pleural disease because long-term benefit is uncertain due to many confounders.[75] Therapeutic thoracocentesis may relieve dyspnea in large effusions but is otherwise not indicated.[76]

Paradoxical responses, including unmasking or worsening pleural disease at the initiation of antiretroviral therapy, are well described in HIV/tuberculosis-coinfected patients.[77] This may also occur in HIV-negative persons, as described in 16% of isolated pleural disease cases in which effusions worsened at 2 months after the initiation of therapy, but 68% of these patients were asymptomatic.[78]

Tuberculous empyema accounts for a small proportion of cases of pleural tuberculosis but has severe consequences. It may be a chronic condition that degenerates with the development of a bronchopleural fistula or empyema necessitatis (the extension of empyema fluid through the parietal pleura and out the chest wall) (Fig. 68.2). Often the symptoms are chronic, with low-grade fever, night sweats, and weight loss. Fluid is by definition grossly purulent with high neutrophilic cell counts, acidic pH, and low glucose. Therapy is by drainage and chemotherapy.[79] Outcomes of surgical intervention vary by indication, with low morbidity associated with diagnostic procedures, but high morbidity and some mortality following complex surgical procedures such as placement of an Eloesser flap.[80,81]



FIG. 68.2 Spontaneous drainage (empyema necessitatis) from the posterior chest wall of a 55-year-old man with malignant mesothelioma and a loculated empyema. A PleurX catheter had been placed anteriorly. Culture from the draining sinus grew *Pseudomonas aeruginosa, Actinomyces odontolyticus, Granulicatella adiacens,* and *Finegoldia magna.*

Other Bacteria

Lobar pneumonia commonly is caused by *S. pneumoniae*, and so pneumococcal empyema accounted for 68.3% of cases at the Boston City Hospital in 1930. The disease was "meta-pneumonic" in that empyema occurred several days after the onset of pneumonia and progressed over a few days from turbid fluid to purulence, and the turbid and fibrinous character of the fluid impeded drainage. Bronchopleural fistula and abscess may ensue. Even with the bacteriology of the early 20th century, 25% of cases were bacteremic and these showed increased mortality.[82] A historical survey of selected years from 1935 to 1972 noted a declining but still high prevalence of pneumococcal empyema during the early penicillin era (46% decreasing to 15% from 1935 to 1957), and pneumococcal disease still accounted for 12% to 16% in the later years studied.[33] In a prospective study from 1978, vigorous assessment of 35 cases of pneumococcal pneumonia found that 57%

had parapneumonic effusions and 3 were empyemas at admission. The authors suggested that patients with effusions had pneumonia for longer periods before diagnosis and therapy.[83]

The modern literature (roughly the last 50 years) on pneumococcal empyema is dominated by pediatric studies, not addressed here in detail. In adults, 128 cases of pneumococcal empyema occurred in 1808 patients with invasive disease, and the rate increased across the periods studied (1996–2001 and 2005–2009). These years were chosen to coincide with the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7), in parallel with pediatric reports that invasive disease and especially empyema had increased coincident with the introduction of the vaccine.[84,85] Additional population-specific factors contribute to the severity and character of complicated/invasive pneumococcal disease, especially HIV infection. A survey of global epidemiology, specifically addressing serotypes in complicated pneumococcal pneumonia from 1990 to 2012, supported increases in both the disease and geographic variation in serotypes responsible. The summary supported several factors as contributing to this, including better detection methods and diagnostics, and perhaps increases in invasive serotypes not targeted by PCV7.[86] However, other data demonstrate that invasive disease increased prior to the introduction of PCV7 and the increase in serotypes association with invasive potential.[87,88] With the introduction of 13-valent conjugate vaccine (PCV13), which includes many of the dominant invasive serotypes noted previously, observational evidence in patients <18 years of age did not yet support a decreased incidence of complicated parapneumonic effusion. Of vaccine serotypes, in an area of high PCV13 coverage, however, only serotype 3 persisted as a cause of empyema.[89,90]

Specific considerations in the diagnosis of pneumococcal effusion and empyema relate to the deficiencies in standard culture-based methods (sometimes associated with prior antibiotic use), and so antigen detection and molecular methods are used increasingly. In a pediatric literature review, polymerase chain reaction (PCR) and pneumococcal antigen detection in 78 empyema cases found that PCR added 17 diagnoses to 23 made by culture.[91] Pneumolysin-targeted PCR was used for specificity after 16S ribosomal DNA PCR. Latex detection using the BinaxNOW *S. pneumoniae* antigen detection test (Alere, LLC, Orlando, FL) detected 90% of the cases. A subsequent study from Spain noted that the same antigen assay on pleural fluid increased detection by 38% over culture alone and had a sensitivity of 70.6% with specificity of 93.3%.[92]

Finally, the specifics of treatment of pneumococcal pleural disease broadly follow those of the treatment of pneumococcal pneumonia in terms of antimicrobial therapy. In Spain, with a high prevalence of pneumococcus isolated from pleural fluid with elevated minimal inhibitory concentrations to penicillin from 1997 to 2008, oral penicillin nonsusceptibility decreased as PCV7 serotypes decreased, but increases in serotype 19A occurred, one of 3 serotypes (1, 3, 19A) responsible for empyema in 2008, and penicillin nonsusceptibility in 19A was 82.4%.[93]

Staphylococcus aureus remains an important pulmonary and empyema pathogen because of its virulence and drug resistance characteristics. Its prevalence waxes and wanes prominently with influenza epidemics.[94] In a report from Brazil of 332 cases of community-acquired pneumonia from 1992 to 2003, *S. aureus* (95% methicillin-susceptible) accounted for 24 cases, but empyema occurred in half. Predisposing conditions reported were skin infections, septic abortion, and a history of upper respiratory tract viral infection.[95] Empyema resulting from *S. aureus,* especially methicillin-resistant *S. aureus* (MRSA), is a critical component of health care–associated and hospital-associated empyema.[96] *Staphylococcus aureus* may complicate hemothorax secondary to trauma (late at 10 days postdrainage), but gram-negative organisms colonizing the upper respiratory tract cause empyema early (within 4 days of drainage) after pneumo- or serothorax.[97]

Other gram-positive organisms of note in large surveys are often referred to as β-hemolytic streptococci, often not otherwise specified, especially in older literature. *Streptococcus pyogenes* (group A β-hemolytic streptococcus) was prevalent in the preantibiotic era, accounting for 18% to 34% of empyema. The disease is characteristically aggressive and associated with much morbidity and mortality.[33,82]

In current taxonomy the *Streptococcus anginosus* group (formerly the *Streptococcus milleri* group), of which some members are β-hemolytic, are infrequent causes of pneumonia but are increasing as causes of pleural infection. Predisposing conditions such as diabetes mellitus and malignancy are often present in these cases, and mortality has been reported at 14%.[98–100]

Dedicated studies of infections with anaerobic organisms in the 1970s identified multiple species as contributing to pleural infection. Bartlett and Finegold found that anaerobic bacteria are characteristic of pulmonary processes and pleural complications in cases of aspiration and necrotizing pneumonitis, which may evolve to lung abscess. Anaerobic empyema was present in 17 of 43 cases reported, with only one exception associated with parenchymal disease, and 33 required open thoracotomy. Anaerobes only were present in 28 cases, with *Fusobacterium nucleatum, Prevotella melaninogenica* (formerly *Bacteroides melaninogenicus*), and other spp., and microaerophilic streptococci as the dominant species. In mixed infections, *Escherichia coli*, *S. aureus,* and *Pseudomonas aeruginosa* were present.[101] Twenty years later, the same institution reported on 37 cases (19 purely anaerobic) in which 161 isolates from 46 samples cultured anaerobically (3.5 per patient) were found. *Fusobacterium*, *Prevotella, Bacteroides,* and *Peptostreptococcus* (*P. magnus,* now often classified as *Finegoldia magna*) were the dominant species. Susceptibility data noted β-lactamase production in 33% of the isolates.[102] An analysis using a library of cloned fragments without culture studied 42 specimens from 26 patients. Among these, 43.8% had dominant anaerobic phylotypes, and 6 of 7 of these were not detected by culture; 9 of 26 cases showed discordance between molecular and culture results.[103] With the infrequent application of culture methods appropriate to anaerobic organisms and frequent lack of susceptibility testing, these results emphasize the need to consider the presence of organisms that may represent a risk of failure of treatment. For instance, resistance of *Peptostreptococcus* to metronidazole, the frequent production of β-lactamases, the general inadequacy of cephalosporins alone for the treatment of gram-negative anaerobes, and increasing rates of resistance to clindamycin should be taken into consideration.[104,105]

Other gram-positive streptococci and enterococci are less frequent pathogens in monomicrobial empyema, although they may contribute to the microbiology of polymicrobial infections when pleural disease results from aspiration and/or lung abscess. These infections, even when hospital acquired, may cause less morbidity and mortality than those due to pathogens such as MRSA or gram-negative bacteria that define much of the difference between community- and hospital-acquired infections.[106] Other recognized causes of community-acquired pneumonia are less often associated with pleural effusion and empyema, and the literature is dominated by pediatric cases. If a serologic diagnosis is accepted for disease caused by *Chlamydia pneumoniae,* 18 of 34 patients in a radiographic survey from Japan had mixed infections; of 24 of 30 who underwent CT scanning, 25% had parapneumonic effusion, similar to the rate found in the same population with *Mycoplasma pneumoniae* or *S. pneumoniae* pneumonia.[107] Molecular diagnosis of *M. pneumoniae* infection, problematic in the respiratory tract, might be more accurate in pleural fluid. The parapneumonic effusion associated with *M. pneumoniae* is usually lymphocyte predominant, and disease with effusion tends to occur in younger patients and lead to longer hospital stays.[108] *Haemophilus influenzae* in adults most often causes lower respiratory tract infections in patients with chronic obstructive pulmonary disease. The frequency of parapneumonic effusion in a recent report from Greece was 22%. Outcomes were strongly linked to the underlying disease.[109] Effusion occurred in 8 of 109 patients reported from Japan with *Moraxella catarrhalis* pneumonia. Emphysema was present in 67.9% and malignancy in 37.6%, which raises the issue of confounding diagnosis in the etiology of effusions.[110]

Legionella pneumophila and other *Legionella* species, *Legionella micdadei* prominent among them, may be associated with parapneumonic effusion. In a report of 61 nosocomially acquired cases of legionnaires' disease, effusions were present in the majority and were bilateral in 19%.[111] Hemorrhagic mononuclear cell–predominant effusion can occur as reported with *L. micdadei,* the Pittsburgh agent.[112]

The dominance of gram-negative empyema in reports from East Asia is remarkable. Although *K. pneumoniae* is the most frequent pathogen, it should be noted that diabetes mellitus is a major risk factor and the majority of isolates are not hyperviscous capsular types.[39,113]

Table 68.2 presents select infectious agents reported in empyema. Several categories emerge, including zoonotic pathogens (*Franciscella*

TABLE 68.2 Select Microorganisms Associated With Infected Pleural Effusion

PATHOGEN	ASSOCIATION	PLEURAL RATE	FLUID CHARACTERISTIC	RADIOLOGY	REFERENCE(S)
Extrapulmonary Disease					
Fusobacterium necrophorum, Prevotella spp.	Remote disease (septic thrombophlebitis)	15%–54%	31% empyema	Septic pulmonary emboli	248–250
Clostridioides difficile (formerly *Clostridium difficile*)	Remote disease (severe colitis)	35%–40%		In patients with abdominal CT, bilateral	251
Campylobacter jejuni	Dialysis	CR	Exudate, neutrophil predominant	Effusion without pneumonia	252
Campylobacter lari	Probable gastrointestinal source	CR	Empyema	Effusion without pneumonia	253
Salmonella enterica subsp. *enterica*	Contiguous	CR	Culture negative	Effusion without pneumonia	254, 255
Salmonella enterica subsp. *enterica*	Dissemination	CR	Empyema, peritonitis	NR	256
Salmonella enterica serovar *typhi*	Bacteremia	CR	Empyema	Bilateral effusion without pneumonia	257
Geographic/Environmental Association					
Burkholderia (Pseudomonas) pseudomallei	Coastal Asia, northern Australia Farming Diabetes mellitus	21%–36% PPE	Usually lymphocytic Transudate	PPE > effusion without pneumonia	258–260
Leptospira spp.	Water exposure	12.5%	Bloody fluid	Diffuse alveolar hemorrhage	261
Orientia tsutsugamushi	Coastal Asia, farmers	Pneumonia 21.6%, rare effusion	NR	Bronchopneumonia	262
Paragonimus kellicotti	Eating raw crayfish Eosinophils	In blood and pleural fluid in lung		Fever ± nodule in lung	263
Rickettsia rickettsii	Tick exposure	23% pulmonary manifestation 7% effusion	NR	Interstitial and alveolar infiltrates	264
Zoonoses					
Brucella spp.	Endemic zoonosis	10.8%–30.8% with effusion	Exudate by protein, culture positive, lymphocyte and mononuclear cell predominant	Interstitial > lobar pneumonia	265, 266
Coxiella burnetii	None reported	CR	Transudate, eosinophil predominant	Upper/middle lobe pneumonia	267
Chlamydia psittaci	Birds (pigeon breeder)	CR	Lymphocytic exudate, high ADA	PPE, consolidation	268
Francisella tularensis subsp. *tularensis*	Rabbits, cats	30%	Lymphocytic exudate, empyema, high ADA	Interstitial and lobar opacities, cavity rarer	269–271
Bartonella spp.	Zoonosis	CR	Exudate, lymphocyte predominant	Effusion without pneumonia	272
Immunocompromised State					
Rhodococcus equi	HIV infection Animal exposure not required	7.7% effusion	Frequent empyema	Upper lobe pneumonia, cavitation	273
Nocardia spp.	AIDS, renal transplant	10%–33%	Empyema in two-thirds	Cavitary pneumonia, upper to lower lobe 55%/35%	274–276
Campylobacter fetus	Immunoglobulin deficiency	CR	Exudate, lymphocyte predominant	Effusion without pneumonia	277
Cancer and Systemic Debility (Cardiac, Hepatic, and Renal Disease), Including Aspiration Risk					
Eikenella corrodens	CHF	31.5% with pleuropulmonary CA	Polymicrobial empyema	Pneumonia ± cavity	278
Listeria monocytogenes	CA; immunosuppressives	88.9% with CA	Mononuclear-predominant exudate	Effusion without pneumonia	279
Mycoplasma salivarium	CA	CR	Empyema	PPE	280
Actinomyces spp.	CA, hematologic malignancy	CR	Lymphocyte-predominant (ALL) empyema	Nodules, contiguous effusion	281

TABLE 68.2 Select Microorganisms Associated With Infected Pleural Effusion—cont'd

PATHOGEN	ASSOCIATION	PLEURAL RATE	FLUID CHARACTERISTIC	RADIOLOGY	REFERENCE(S)
Proteus mirabilis	CA, CHF	CRs	High pH (7.8–8.1) polymicrobial empyema	Effusions without pneumonia	282
Lactobacillus rhamnosus	COPD	CR	Empyema	Lung abscess	283
Campylobacter curvus	Bronchiectasis	CR	Empyema, polymicrobial	Upper lobe cavitation	284
Pasteurella multocida	Elderly, comorbidities	CRs, 62% with tube, 45% mortality	87% empyema	CPE	285
Campylobacter fetus subsp. *fetus*	Aspiration	CR	Empyema, mononuclear predominant	Contiguous abscess	286
Francisella tularensis subsp. *holarctica*	Immunocompromised	CR	Exudate, lymphocyte predominant	PPE, lobar infiltrate	287
Tropheryma whipplei	Unknown	CR, effusion in all 4 patients	NR	PPE	288
Lactococcus lactis subsp. *cremoris*	Aspiration, dairy products	CR	Empyema	Necrotizing pneumonia	289
Burkholderia (Pseudomonas) cepacia	Cancer, colonization	36%	NR	Cavitary pneumonia, PPE	290
Stenotrophomonas maltophilia	Health care acquired, immunocompromised	65% postsurgery, 30% fistula	Empyema, 77.5% polymicrobial	5% PPE	291
Clostridium perfringens	Cirrhosis	CR	Exudate, neutrophil predominant	Effusion without pneumonia	292
Bacillus cereus/ Clostridium bifermentans	Alcoholism	CR	Empyema	Necrotizing pneumonia	293
Mycoplasma hominis/ Ureaplasma urealyticum	Postsurgical mediastinitis, possible primary genitourinary source	CR	NR	Mediastinitis and pericarditis	294
Bioterrorism					
Bacillus anthracis	Inhalation	81.8%	NR	72.7% with infiltrates but with mediastinal widening	295

ADA, Adenosine deaminase; *AIDS,* acquired immunodeficiency syndrome; *ALL,* acute lymphoblastic leukemia; *CA,* cancer; *CHF,* congestive heart failure; *COPD,* chronic obstructive pulmonary disease; *CPE,* complicated parapneumonic effusion; *CR,* case report; *CT,* computed tomography; *HIV,* human immunodeficiency virus; *NR,* not reported; *PPE,* parapneumonic effusion.

spp.), organisms with restricted or dominant geographic distribution (*Burkholderia pseudomallei*), infections predominantly associated with immunocompromising conditions (*Listeria, Nocardia* spp.), and pathogens that incidentally involve the pleural space during dissemination or involvement of contiguous structures. The data in case reports are often compromised by the interest of the author. For instance, radiographic literature often omits fluid analysis and other clinical information.

Viruses

An important consideration in viral disease and pleural effusion is the relatively recent development of rapid and sensitive molecular assays for diagnosis. In a study from 2013 in children with community-acquired pneumonia and effusion, 50% of cases had a viral diagnosis, and combined viral-bacterial infections were present in an additional 22%. Rhinovirus, enterovirus, influenza virus, respiratory syncytial virus, parainfluenza virus type 1, and several mixed infections were found.[114] Viral respiratory pathogens may be associated with effusion in lower respiratory tract disease. Adenovirus with effusion occurs in longer febrile illnesses, in which clinical management may be complicated and immunosuppressive conditions may be present at baseline.[115] Influenza in the 2009–2010 A(H1N1) epidemic was associated with effusions in all hospitalized cases in a radiology review, and respiratory failure was more likely in cases with radiographic evidence of pneumonia.[116] This was again demonstrated by CT scanning, but predominantly in immunocompromised hosts.[117] New types of influenza virus that have not so far demonstrated epidemic capacity and are associated with exposure to infected poultry, such as influenza A(H7N9), were associated with effusions in three of six cases, of which four died of adult respiratory distress syndrome.[118]

The resurgence of measles internationally necessitates mention of this preventable disease, which can cause respiratory distress associated with pleural effusion.[119] Viruses associated with capillary leak and pulmonary hemorrhagic syndromes have associated effusions, which may be bloody. Dengue virus is prominent among these, and a retrospective analysis of 363 dengue hemorrhagic fever patients found that 57.4% of abnormal chest x-rays had pleural effusions. The effusions sampled were transudative, suggesting a role for the vascular permeability problem common in this disease. Lassa fever, hantavirus pulmonary syndrome, and Crimean-Congo hemorrhagic fever are also reported.[120–123]

Herpesviruses, uncommon pulmonary pathogens, occasionally have manifestations that lead to effusion. Varicella-zoster virus has been detected in the pleural fluid of a patient receiving chemo- and radiotherapy for lymphoma.[124] Epstein-Barr virus, with an increasing spectrum of disease beyond infectious mononucleosis, was identified in the pleural fluid of 20 cases, all lung transplants. Viral burdens in pleural fluid were low but there was polymorphous lymphocytosis in >70% of cases.[125] Finally, Kaposi sarcoma–associated herpesvirus causes Kaposi sarcoma, primary effusion, or body cavity lymphoma. Virus is present in the effusion, though diagnosis of lymphoma is by cytology or flow cytometry.[126]

Newly identified syndromes with some geographic restriction may present with effusion, as reported in the severe acute respiratory syndrome coronavirus epidemic from 2004 in which 15% of patients had effusion at the initial presentation. The Middle East respiratory syndrome coronavirus, reported in 2015, was associated with effusion in 33% of patients in a small series, and there was increased mortality in the group with effusion.[127]

Finally, although the physiology is not obvious, hepatitis viruses may be associated with effusion. Hepatitis A infection may produce a sympathetic effusion from hepatic inflammation.[128] Hepatitis B has been associated with pleural effusion principally in case reports, for instance, when the effusion appeared in the context of exacerbations of chronic infection and without concomitant ascites.[129]

Mycobacteria Other Than Tuberculosis

Nontuberculous mycobacteria are sometimes associated with pleural disease. Nevertheless, the more frequent occurrence of pleural effusion with tuberculosis is a dominant differentiating feature in most reports that include disease caused by *Mycobacterium kansasii* or *Mycobacterium avium* complex (MAC).[130] In a report of nontuberculous mycobacteria pleurisy from Taiwan in the years 2000–2007, MAC most commonly showed more extrapleural involvement and occurred in patients with immune dysfunction. Of 19 cases, 9 were MAC, 5 rapidly growing mycobacteria, and 5 not otherwise characterized.[131] A new species, *Mycobacterium lentiflavum,* has been reported from Zambia, and in a second case report there was acute necrotizing pneumonia and parapneumonic effusion.[132,133] *Mycobacterium abscessus*, *Mycobacterium ulcerans* (after contiguous chest wall disease), and *Mycobacterium bovis* all appear in case reports with pleural effusion.[134–136]

Fungi

Fungal empyema may occur as a parapneumonic process, but increasingly is a complication of medical procedures and immunocompromising conditions. In an analysis from Taiwan from 1990 to 1997, 67 patients with fungal pleural infection were identified. An underlying condition was present in 85% and *Torulopsis glabrata* (now *Candida glabrata*) accounted for 60 of 73 isolates. The most common predisposition was abdominal disease or perforation. Patients who underwent surgery and pleural irrigation (11) survived, but the crude mortality was 73%. Determining attributable mortality is often not possible in such cases.[137] *Candida* spp. continue to be major pathogens in the context of gut disruption, with 55.6% of 63 cases of *Candida* empyema being associated with a gastrointestinal source and perforation.[138] Even with drainage, the mortality was high (62.5%) in a small series, revealing the association of this complication with high-morbidity/mortality predisposing conditions. *Candida* was responsible for 44% of pleural space infections in a series of consecutive lung transplant recipients whose pleural fluid was sampled in the 90 days after transplantation.[30]

Cryptococcus spp. are predominantly central nervous system and pulmonary pathogens in identified or uncharacterized immunodeficiencies but can involve the pleura in dissemination.[139] Pleural involvement is seldom a critical manifestation of disease, even in HIV infection.[140]

In HIV infection, *Pneumocystis jirovecii* is a major pulmonary pathogen but is rarely associated with pleural effusion.[141] *Pneumocystis* pneumonia with associated bilateral pleural effusions has been reported after hematopoietic stem cell transplantation.[142]

A newly identified dimorphic yeast-like fungus which forms hyphae in tissue, *Tilletiopsis minor,* has been found in the parapneumonic effusion of a child whose presentation suggests an undiagnosed immunodeficiency. Voriconazole may have been effective therapy when amphotericin B failed.[143]

Invasive molds involve the pleura by extension from infected lung. Pleural effusion is one of many factors that influences the outcome of invasive aspergillosis.[144] Late invasive aspergillosis occurred in 27 hematopoietic stem cell transplantation patients, with chronic corticosteroid use or graft-versus-host disease as the predisposing factor in 24, and effusions were present in 7.[145] The agents of mucormycosis have a characteristic pathology in pulmonary cases and in those that are associated with pleural effusion. Hemorrhage, gangrene, necrosis, infarction, and arterial thrombosis were present in 32 cases of invasive mucormycosis, of which 6 had unilateral and 3 bilateral pleural effusions.[146] The microbiologic differential of invasive molds in cancer patients may be influenced by an increased prevalence of pleural fluid in mucormycosis compared to aspergillosis (63% vs. 33%).[147]

Endemic or dimorphic mycoses may cause parapneumonic or isolated pleural effusions. *Histoplasma capsulatum* most commonly causes a self-limited illness that does not require therapy. Extensive disease, rupture of a juxtapleural cavity, or fibrosis may occur and be associated with pleural involvement.[148,149]

Coccidioides spp. are also associated with a self-limited acute illness, but pleural manifestations in disseminated disease follow the same risk factors as for dissemination in general. A pediatric series of 33 cases, however, noted effusions in 13, of which 4 were empyema.[150] The surgical pathology of pleural disease suggests that ruptured juxtapleural cavities are a major factor in cases in which spherules may be seen in biopsy or autopsy material from the pleural cavity.[151]

Blastomyces dermatitidis, in a large military survey from 1964, remains an uncommon pulmonary pathogen, and pleural manifestations are rare.[152] However, when pulmonary disease is present, a radiologic report of 63 cases found effusion in 13.[153]

Sporothrix schenckii rarely is a pulmonary or disseminated pathogen. A case report of a parapneumonic effusion found pleural fluid parameters consistent with an empyema (predominantly neutrophilic fluid with positive culture). The authors noted that this finding contradicted their presumption that such an effusion would be lymphocyte predominant.[154]

The literature on *Paracoccidioides brasiliensis* is sparse. A pathology series from Venezuela analyzed 11 autopsy and 20 surgical specimens and noted pleural involvement in 8, all with pulmonary disease.[155]

Talaromyces (previously *Penicillium*) *marneffei* is a cause of disseminated disease, mainly in HIV infected persons, in Southeast Asia. Disease diagnosed by pleural biopsy or pleural fluid analysis is reported.[156]

Parasites

Trichomonas spp. (usually *Trichomonas tenax*), which are flagylated protozoan parasites, are commensals of the oral cavity and thus are involved in pneumonias and associated effusion in the context of aspiration.[157]

Nematodes

Roundworms may infect the pleural space, either incidentally (*Toxocara canis, Gnathostoma* spp., and *Anisakis simplex*) or in relationship to their role as intrathoracic pathogens *(Dirofilaria immitis)*.[158–161] *Strongyloides stercoralis* may involve the pleural space during dissemination.[162]

Filarial pathogens *(Loa loa, Wuchereria bancrofti)* appear in case reports with pleural involvement, the former with and the latter without eosinophilic fluid.[163,164]

Cestodes

Echinococcus spp. tapeworms cause pulmonary hydatid cyst, and the pleura can be involved by rupture of a lung cyst, but the more common complication is transdiaphragmatic involvement from a ruptured liver cyst. Uncomplicated effusion (sometimes eosinophilic) or empyema can occur in 1% to 16% of cases of hepatic hydatid disease.[165,166]

Trematodes

The lung fluke (*Paragonimus* spp.) causes pleural complications as a natural component both of its role as a pulmonary pathogen and the presumed route of pulmonary infection, which may involve migration out of the gastrointestinal tract through the diaphragm. Disease may be mistaken for pulmonary tuberculosis. Subpleural or subfissural parenchymal disease is present in 87% of cases.[167,168] Effusion, sometimes eosinophilic and occasionally massive and persistent, appears in many reports. Worms may be found in the pleural fluid.[169] Pulmonary and pleural disease from *Paragonimus kellicotti,* usually associated with ingestion of inadequately cooked crayfish in North America, had an eosinophilic pleural effusion as a major manifestation in a series of eight patients from a single institution. CT may demonstrate a track connecting the pleura to the parenchyma.[170,171]

Other Parasites

In a report from California, hepatic amebiasis was complicated by pleural effusion in 9 of 30 patients, of whom 60% were symptomatic. Effusions, when sampled, were exudative, with high protein and LDH ratios and neutrophil predominance, but no parasites were detected.[172]

Parasitic infections may disseminate and involve the pleural space in HIV infection. Microsporidia (*Enterocytozoon bieneusi*), *Trypanosoma cruzi* (Chagas disease), and *Leishmania donovani* have been found in

TABLE 68.3 Evolution of Studies Defining Pleural Fluid Characteristics and Diagnoses

AUTHOR, REFERENCE	POPULATION	TEST/FINDING/INTERPRETATION
Paddock[296]	Defined cardiac, neoplastic, tuberculous, and infected effusions	Specific gravity as a test performs poorly in cardiac and nontuberculous infected effusions, neoplastic effusions both transudative and exudative.
Carr and Power[297]	Defined cardiac, neoplastic, and tuberculous effusions	Testing protein, a concentration greater than 3 g/dL was present in 92.8% of neoplastic and all tuberculous effusions but cardiac effusions perform variably.
Chandrasekhar et al.[298]	Defined malignant, pyogenic (tuberculosis and other), and transudative effusions	Testing lactate dehydrogenase (LDH) and protein, all transudative effusions were cardiac and hepatic, LDH cutoff an arbitrary 550 IU/mL; 22/24 cancer and all infectious effusions identified, but protein performed poorly in transudates.
Light et al.[185]	Defined cancer, heart failure, tuberculosis, and others (parapneumonic)	Protein alone poor (19% error with malignancy). Effusion to serum protein <0.5 accurate for transudates. LDH <200 IU/L accurate for transudates, effusion to serum LDH >0.6 IU/L more accurate for exudates. Combine LDH, protein, and LDH ratios for best accuracy.
Light et al.[188]	Infected effusions	To predict which parapneumonic effusions will become empyemas, low pH (cutoff 7.20) separated intervention-requiring disease better than cell count and protein.
Potts et al.[299]	Infected effusions	pH was the only nonoverlapping factor, more predictive than negative Gram stain or negative culture.
Light et al.[183]	Admitted pneumonias screened for parapneumonic effusion and progression to complicated effusion/empyema	Incidence of parapneumonic effusion 44.4%, 10/37 sampled were complicated (5% of the population). All pH <7.0 and glucose <40 mg/dL were complicated, all pH >7.2 or LDH <1000 IU/L were uncomplicated.
Sahn and Light[300]	Editorial on defining the need for intervention, relevant only to parapneumonic effusions	Summary standards: uncomplicated effusion pH >7.30, glucose >60 mg/dL, pleural fluid-to-serum protein ratio >0.5, LDH <1000 IU/L. Complicated effusion (with empyema) pH <7.10, glucose <40 mg/dL, LDH >1000 IU/L. Drainage for positive Gram stain, pH <7.10, glucose <40 mg/dL.
Valdes et al.[301]	Defined effusions in cardiac, cancer, tuberculosis, and miscellaneous/infected (parapneumonic) disease	All transudates had pleural cholesterol <55 mg/dL, but this misclassified 11.9% of cancer cases, 6% of tuberculosis cases, and 8.9% of miscellaneous cases (exudates called transudates). Superior to LDH and LDH ratio but not significantly different than Light (4). Strong positive predictive value, negative predictive value only 79%.
Burgess et al.[302]	Defined population, reconciling acknowledged misclassification of some transudates as exudates after therapy (diuresis) or chronic	In patients in whom a transudative process (heart failure, cirrhosis, or renal failure) is miscategorized by Light criteria as an exudate, the serum-to-effusion albumin gradient (serum albumin minus effusion albumin <1.2 g/L) may reconcile misclassification (especially with diuretics), but overall is less accurate than Light criteria.
Vives et al.[303]	Record review to classify transudates and exudates in defined population	Attempts to improve misclassification by raising the protein ratio, LDH ratio, or absolute LDH value provided little benefit.

pleural fluid in this context.[173–175] Rarely, in HIV-infected or non–HIV-infected persons, *Leishmania chagasi,* an agent of visceral leishmaniasis, may be found in effusions and mimic tuberculosis.[176,177]

Infestation by the causative agent of cutaneous myiasis was associated with recurrent migratory subcutaneous nodules and an eosinophilic pleural effusion.[178]

DIAGNOSIS

Diagnostic tests identify causes but also stratify risk and direct interventions to prevent adverse outcomes. A parapneumonic effusion should be sought in cases of pneumonia, since 40% or more of patients have pleural fluid and the presence of effusion is associated with adverse outcomes, the risk of which is amplified by inadequate evaluation.[179] Physical findings may decrease the likelihood of pleural fluid (normal chest expansion, no dullness to percussion), but positive findings (vocal fremitus, percussion and breath sound changes) are insufficiently specific.[180]

Obtaining bilateral decubitus films or performing more sensitive tests such as CT scans may reveal effusions not visible on posterior-anterior or lateral views. Lower lobe pneumonias may cause standard films to miss 10% of effusions.[181] CT and ultrasound both have roles in identification of effusions, but importantly, neither has the ability to adequately characterize the fluid.[182] Fluid analysis is required. Radiographic indications for fluid sampling might include fluid thickness >1 cm on routine chest x-ray or >2 cm on CT.[183] Ultrasound improves the accuracy of fluid sampling, even by experienced physicians, and can identify septations/loculations that may be missed by CT scan.[184]

As discussed elsewhere, fluid characteristics (transudate, exudate, empyema) direct therapies and interventions. Small transudative effusions may require no further sampling or drainage, especially if the course of treatment for the primary cause (pneumonia, for instance) is satisfactory. Exudative effusions in an early phase, without loculation, may respond to closed thoracostomy (chest tube drainage). Empyemas routinely require drainage and, because they are often loculated, early aggressive therapy may be necessary to avoid adverse outcomes and more aggressive surgical techniques such as open thoracotomy.

Fluid Analysis

The evaluation of pleural fluid should include simultaneous measurements of serum and pleural fluid LDH, albumin, protein, and glucose. Specimens should be stained and cultured for bacteria, fungi, and mycobacteria. Molecular assays and other surrogates are used in the appropriate context. The classic criteria identified by Light and colleagues in 1972 continue to perform with high sensitivity and specificity, but have been modified, particularly for the evaluation of parapneumonic effusions.[183,185] The historical sequence of testing is presented in Table 68.3 to illustrate a very important point. The clinical scenario has always defined the likely character of an effusion. Studies have been performed, as noted in the table, in a "defined" population (known cancer, heart failure, tuberculosis, etc.) with an effusion characteristic of the disease (transudate in CHF, exudate in tuberculosis) to determine the degree to which any test is consistent with clinical judgment. Thus a change in fluid parameters outside of the presumed range for a clinical condition means that causes for the change must be sought. It should be noted that these studies do not conform to a contemporary standard by which a population would be studied, parameters established, and then a second population used to validate the results (see the earlier discussion of the risk score derived from the MIST studies).

Failure of Light's criteria was recognized early and is usually related to misclassification of fluid that should be associated with a noninfectious process (CHF) as an exudate. Diuresis may promote this problem. In the context of low clinical suspicion for infection and the presence of CHF, the addition of serum-to-effusion protein and albumin gradients can be informative.[186] Multiple additional parameters have been suggested, but no addition to Light's criteria, with the possible exception of the serum-to-effusion cholesterol ratio, either is practical or performs well.[187]

The addition of pleural fluid pH is an important parameter in parapneumonic effusions.[188] Very low pH outweighs many other parameters in predicting the need for aggressive management.[183] Determination of pH should be performed on a blood gas analyzer immediately after the pleural fluid is drawn, and residual air and lidocaine or heparin should be minimized because they alter results.[189] Physicians routinely are unaware of the technology used in their institutions for pH measurement, and results are compromised by this lack of knowledge.[190]

Three automated systems utilizing inoculation of pleural fluid into liquid media are available (Biomerieux BacT/Alert, Becton Dickinson BACTEC MGIT [for mycobacteria], and Thermo Scientific VersaTrek). These may hasten results and improve the sensitivity of culture.[191–193] Inoculation of solid media may be necessary to isolate mixed flora, such as obligate anaerobes mixed with more rapidly growing facultative anaerobes. Fluid samples should be obtained directly from the effusion, not drawn from chest tubes or other drainage devices. Additional specific microbiologic diagnostics were discussed earlier in the chapter.

Pleural biopsy was established in the 1950s as a high-yield procedure, especially for the diagnosis of tuberculous pleurisy, because the additional histopathologic evidence and culture of tissue increase the sensitivity of microbiologic diagnosis substantially.[194,195]

TREATMENT

Because of the variety of infectious agents and the changing patterns of drug susceptibilities and available antimicrobials, drug therapy can only be addressed in broad terms.

Two general trends in the treatment of bacterial empyema have emerged. First, gram-negative organisms and MRSA are important, especially in the same settings in which they are responsible for hospital-acquired pneumonia, so those organisms' drug susceptibility should be taken into account early.[96,113,196] Second, in adults with a predisposition to aspiration or obstructive disease from cancer that leads to effusion and empyema, limiting the spectrum of treatment to a noncarbapenem β-lactam without a β-lactamase inhibitor or a cephalosporin without metronidazole is inappropriate.[102,103] Other considerations should be made as discussed in organism-specific chapters of this text.

Data on the specifics of antibiotic activity and pharmacokinetics in the pleural space are limited. Aminoglycosides should not be used to treat empyema.[197] Daptomycin's protein binding, purportedly the cause of its limited role in pulmonary infection in general, might play a negative role in exudative effusions. A wide spectrum of drugs, many no longer commonly used, have been studied.[83] Quinolones have been found to have variable penetrance into pleural effusions, but the clinical consequences of the reported levels remain unclear.[198] Clarithromycin, azithromycin, linezolid, and ertapenem have been studied in experimental settings and levels achieved are consistent with inhibition of growth of organisms normally targeted by these agents.[199–201]

Although repeat thoracentesis has been practiced, it is no longer recommended, and empyema should be treated with more aggressive drainage measures. Small-bore catheters have proven effective in tube thoracostomy, and flushing of catheters (e.g., with 20 mL of saline every 6 hours) may facilitate continued patency of the tube.[202,203] Additional measures have been studied in an attempt to avoid surgery. The physiology of complicated effusion and empyema was demonstrated early, with streptococcal fibrinolysin (streptokinase) and deoxyribonuclease (DNase) improving drainage and outcomes in a study in 1949.[204] DNase predominantly decreases pus viscosity.[205] The role of fibrinolytics in breaking down loculations to improve chest tube drainage has been reported in multiple studies. Notably, MIST 1 found no effect on mortality, rate of surgery, or length of stay, but was criticized on the grounds of enrollment criteria, radiographic evaluation and stratification, and some management decisions.[206] MIST 2 used the same definition of loculation (chest x-ray presence of pleural fluid or thickening not distributed in accordance with gravity), with a different fibrinolytic (recombinant tissue plasminogen activator) and recombinant DNase. The goal was to decrease the area of pleural opacity; 210 subjects were enrolled, and combination therapy was significantly effective compared to placebo. Significant secondary effects of reduced surgical referral and length of stay, but not reduced mortality, were found.[207] Nevertheless, the heterogeneity of patients, perhaps as defined in the criticism of MIST 1, have led both the BTS and the AATS to consider the results inconclusive and recommend against the routine use of fibrinolytics.[7,8]

The transition to more aggressive measures depends on the severity, extent, and chronicity of disease. Video-assisted thoracoscopic surgery (VATS) is a procedure that requires surgical anesthesia, double-lumen intubation, and deflation of the affected lung. In patients unable to tolerate this, medical thoracoscopy, although less studied as an intervention, seems to achieve acceptable rates of success with conscious sedation and without the use of an operating room. When expertise in the procedure is available, VATS, medical thoracoscopy, or open surgery are procedures whose aim is to achieve full evacuation of infected fluid and decompression of the affected lung.[208]

Although much studied in pediatrics, indications for VATS in adults are more poorly defined.[209,210] Medical failure must be defined and the morbidity and cost of VATS as an intermediate-stage intervention before thoracotomy must be considered. A randomized trial enrolling 20 patients in 1997 compared tube thoracostomy with fibrinolytics versus VATS for loculated, complex fibrinopurulent parapneumonic empyema. VATS showed a higher success rate, but VATS was also successful in rescuing failed chest tube/fibrinolytic therapy, clouding the issue of whether VATS was necessarily the first-line therapy.[211]

A prospective trial of 179 adults with empyema established that 42% ultimately needed decortication, with progression to decortication in 24 (decortication was the primary therapy in 52), but this was in a setting in which VATS was not offered. Without decortication, nevertheless, 103 patients (58%) were cured.[47] Delaying VATS reduces its success rate because visceral pleural thickening progresses and impedes lung reexpansion. At this stage, empyema is longer amenable to VATS even when microbiologic treatment has been successful (Fig. 68.3). When VATS is used earlier, there is faster postoperative recovery than with thoracotomy.[212]

An important retrospective review of 104 cases from 2000 to 2006 is illustrative of the general tendency in the literature and its shortcomings. This study stratified patients according to American Thoracic Society criteria, but further stratified stage II disease into types A and B based on the presence of a pleural peel in the latter. The choice of simple drainage (pigtail catheter or chest tube) was the strongest predictor of death or need for further procedure, but was still adequate/definitive treatment in 60 patients with stage I empyema, and the study did not attempt to address better staging. In addition, there was no control for conditions that determined VATS versus thoracotomy, thus the resultant inability to determine that the division in stage II (A/B) made a difference.[213] Additional confounding factors are present in a population-based analysis of 4424 patients hospitalized with pleural infection. In addition to showing increased rates of disease during the period studied and significant imbalances related to younger age and lower comorbidity in patients undergoing surgery, adjustment for those factors still yielded a 58% lower risk of death in those undergoing operative therapy.[214]

Options related to the management of stage III empyema are related to the achievement of adequate evacuation of infected space and reexpansion of affected lung. The organization present in chronic empyema requires VATS or thoracotomy. There is no evidence to support antibiotic instillation into an infected space, although this was the basis of the Clagett procedure, in which a hole in the chest wall was created for periodic antibiotic instillation.[8,215,216]

A problem can arise when the lung cannot expand to fill empyema space in the thoracic cavity because of lung resection or pleural fibrosis. When infection cannot be controlled, closing the space could be beneficial. Resection of the anterior ends of the lower ribs, called *thoracoplasty,* was designed to bring the chest wall toward the remaining lung. A persistent bronchopleural fistula was a relative contraindication for thoracoplasty and needed surgical repair. An even more intractable empyema can occur in a postpneumonectomy thoracic cavity. When empyema treatment fails and long-term chest tube drainage is used, an alternative is formation of an opening through the skin into the base of the thoracic cavity, allowing spontaneous drainage. Although rarely used now, this approach is termed an *Eloesser flap.*[217] Fortunately, improvements in antimicrobial therapy and early, effective treatment of empyema have made these procedures rare.

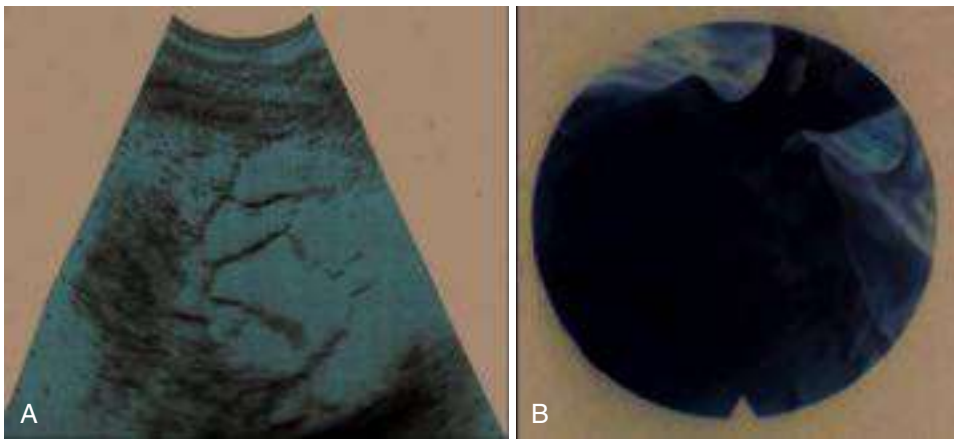

FIG. 68.3 Multiloculated pleural empyema. (A) Ultrasonographic image of a multiloculated pleural empyema and (B) extensive fibrin deposition with septation and pockets of pus as seen during a medical thoracoscopy in a patient with multiloculated pleural empyema (category 7 according to Light[11]). Such septae prevent a successful evacuation of pus by simple chest tube drainage. *(From Brutsche MH, Tassi GF, Gyorik S, et al. Treatment of sonographically stratified multiloculated thoracic empyema by medical thoracoscopy.* Chest. *2005;128:3303–3309.)*

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69 Bacterial Lung Abscess

Joanna K. Nelson and Julie Parsonnet

SHORT VIEW SUMMARY

Definition
- Lung abscess is defined as localized necrosis of infected lung tissue resulting in one or more cavities.

Epidemiology
- Primary lung abscess occurs in the setting of aspiration, usually with altered consciousness and gingival disease.
- Secondary lung abscess occurs as a result of airway obstruction or immunosuppression.

Microbiology
- Lung abscesses are polymicrobial, typically involving oral microbiota including streptococci and anaerobes.

Diagnosis
- Chest imaging shows a thick-walled cavity with an air-fluid level in the appropriate clinical setting.

Therapy
- Treatment entails weeks of antibiotic therapy with a β-lactam/β-lactamase combination, clindamycin, or moxifloxacin.

Prevention
- Aspiration precautions and maintenance of oral hygiene can be helpful in preventing aspiration pneumonia and lung abscess.

DEFINITION

Lung abscess is a localized area of infected, necrotic lung tissue with one or more cavities. Lung abscess is part of a continuum of necrotic lung infections previously known as "lung gangrene."

Lung abscesses can be classified as primary or secondary. Primary lung abscesses, which comprise 80% of all lung abscesses, occur as a result of aspiration of oral or gastric contents into the lungs. In contrast, the inciting factor in secondary abscesses is underlying disease—for example, intrinsic (e.g., tumor or infectious mass) or extrinsic (foreign body) airway lesions, deficient host defense mechanisms, or thoracic surgery. Lung abscesses can also be divided into acute (symptoms for ≤4 weeks) or chronic (symptoms for >4 weeks). The term "putrid" lung abscess is used to describe those with foul-smelling sputum. Lung abscess can also be defined according to the causative organism.

PATHOPHYSIOLOGY

A delicate interplay among the host microbiome, potential pathogens, and local immunity in the lung is at work in a healthy respiratory system. Any disturbance of this homeostasis can result in lung pathology including pneumonia and lung abscess.[1] Within the microbiome of the respiratory tract as a whole, there are discrete anatomic microbiomes, each with its own subset of commensals. Next-generation sequencing of samples taken from the respiratory tract has shown variable combinations of bacteria, fungi, and viruses based on their anatomic location in the respiratory tree, likely resulting from local environmental factors (Fig. 69.1).[2] Despite the overall diversity of microbes found along the respiratory tract, the microbiome of the lung often reflects that of the upper respiratory tract, specifically the oropharynx.[3] Accordingly, colonization of the upper respiratory tract is often a prerequisite for a pathogen to later cause lower respiratory tract infection.[4] However, the lung does not merely represent the oropharynx; local environmental factors in the lung can result in selection of particular organisms, and nonoral microorganisms have established unique niches in the lung itself.[5]

Bacteria can enter the deeper lung airways by means of microaspiration from the upper respiratory tract.[6] Approximately half of healthy individuals demonstrate subclinical aspiration during sleep.[7,8] This aspiration is infrequently of clinical consequence, presumably owing to the low burden of virulent bacteria in aspirated material and to intact local immune defenses, including cough and ciliary transport, and humoral and cellular immune responses. Any condition that increases the bacterial inoculum of aspirated material or impairs host defense mechanisms, however, may lead to aspiration pneumonia and occasionally lung abscess.[9]

Most lung abscesses are polymicrobial infections involving organisms from the oropharynx.[10] In experimental animal models from early in the 20th century, bacteria isolated from lung abscesses at autopsy resembled those in the gingival crevice. Based on this finding, aspiration of oral flora was concluded to be the mechanism of infection.[11,12] Inoculation of animals with four anaerobic bacteria together, but not singly, reproduced the disease, supporting synergistic effects of the bacteria in inducing necrosis and abscess.[11,12]

Once oral contents have been aspirated, ensuing pathology varies from chemical pneumonitis to pneumonia, necrotizing pneumonia, and lung abscess.[11] Aspirated bacteria are carried by gravity to dependent portions of the lung. In a supine individual, the right main stem bronchus is larger and at less of an angle than the left; consequently, lung abscesses occur most frequently in the posterior segment of the right upper lobe. Chemical injury from aspirated gastric acid or obstruction from aspirated particulate matter further predisposes to infection. Resulting inflammation may also provide a favorable environment for certain pathogenic bacteria, particularly gammaproteobacteria, allowing these organisms to grow and outcompete other microbes on the mucosal surface. In a vicious cycle, these bacteria may then contribute to further inflammation and lung destruction.[3] Studies of animal models have shown that tissue necrosis with lung abscess formation takes at least 6 to 7 days.[11,12] In humans, sequential monitoring of chest radiographs after a known aspiration revealed that abscesses typically required at least 7 to 14 days to develop.[13]

Other processes that can lead to anaerobic lung infections include secondary infections of bland pulmonary infarct; postobstructive processes, from either neoplasm or foreign body; or bronchiectasis. The common theme is stasis or necrosis of tissue that presumably serves as a nidus for polymicrobial infection.[14] Pulmonary infarction due to embolism through the pulmonary artery usually does not lead to lung cavitation in the absence of either congestive heart failure or septic pulmonary emboli.

Although less common, monomicrobial abscesses occasionally do occur, usually in the setting of septic embolization from right-sided endocarditis or septic thrombophlebitis. Abscesses arising from a hematogenous source typically manifest as multiple peripheral lesions on chest images (Fig. 69.2).[15] Monomicrobial lung abscesses without



FIG. 69.1 Physiologic and microbial gradients along the respiratory tract. Physiologic and microbial gradients exist along the nasal cavity, nasopharynx, oropharynx, trachea, and lungs. The pH gradually increases along the respiratory tract, whereas most of the increases in relative humidity *(RH)* and temperature occur in the nasal cavity. The partial pressures of oxygen *(PO_2)* and carbon dioxide *(PCO_2)* have opposing gradients that are determined by environmental air conditions and gas exchange at the surface of the lungs. These physiologic parameters determine the niche-specific elective growth conditions that ultimately shape the microbial communities along the respiratory tract. The unit by which bacterial density is measured varies per niche; the density in the environment is depicted as bacteria per cubic centimeter of (indoor) air; density measures in the nasal cavity and nasopharynx are shown as an estimated number of bacteria per nasal swab; and the densities in the oropharynx and the lungs represent the estimated number of bacteria per milliliter of oral wash or bronchoalveolar lavage (BAL) fluid, respectively. *(From Man WH, de Steenhuijsen Piters WA, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health.* Nat Rev Microbiol. *2017;15:259–270.)*

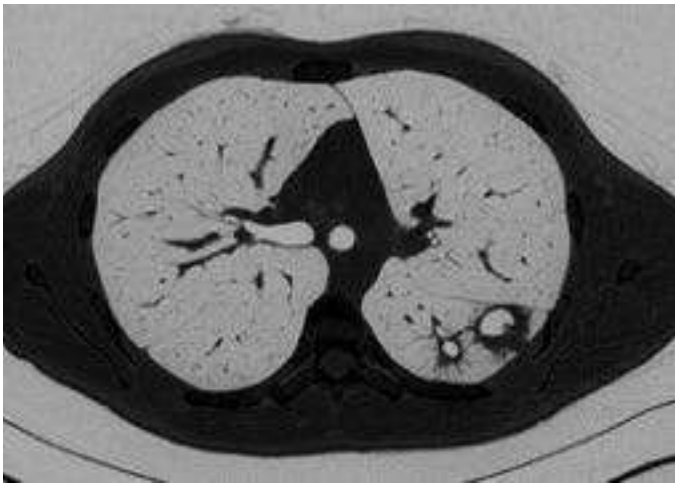

FIG. 69.2 A 28-year-old man with a history of intravenous heroin and cocaine use presented with 5 days of pleuritic chest pain. At computed tomography of the chest, he was found to have 18 cavitary lung nodules. An axial image containing two of the largest lesions is shown. He was ultimately diagnosed with *Staphylococcus aureus* tricuspid valve endocarditis complicated by septic pulmonary emboli. *(Courtesy Daniel Taupin, MD, NYU Langone Health.)*

prior insult to the lung are also more likely to be found in immunocompromised patients, although results from these studies may be biased by difficulties in isolating anaerobic bacteria.[16,17] Lung abscess can also occasionally form as a complication of intrathoracic surgery or flexible bronchoscopy. Nosocomial virulent organisms, as opposed to anaerobes, are typically involved in these iatrogenic lesions.[18,19]

EPIDEMIOLOGY

Because the great majority of lung abscesses result in hospitalization, hospital discharge summaries can be used to understand the incidence of the disease. Based on data from the National Hospital Discharge Survey of the Centers for Disease Control and Prevention for 2000 and 2010, between 10,000 and 15,000 hospital discharges in the United States per year included a diagnosis of lung abscess (International Classification of Diseases, Ninth Revision [ICD-9] code 513.0). The proportion of discharges with this diagnosis changed little over this time: 3.3 per 100,000 discharges in 2000 and 4.0 per 100,000 in 2010. Because the diagnosis of lung abscess in the discharge surveys is relatively nonspecific (cases of staphylococcal bacteremia or *Aspergillus* lung cavitation may be included), these rates overestimate the true occurrence rate of necrotic lung abscess. Consequently, lung abscess is a relatively uncommon diagnosis, with 21st century incidence rates lower than those reported in reviews from the 1970s.[20]

Lung abscesses are more common in males than in females. Surveys from the 1970s show a male to female ratio of 3:1 to 4:1. This sex difference in incidence was speculated to be related to higher rates of alcoholism, and therefore aspiration, among males.[21–23] In the more recent CDC discharge data, however, the male predominance was somewhat less prominent (69% male in 2000 and 52% in 2010). This equalization of the rates between sexes may reflect the increasing proportion of secondary abscesses occurring in immunocompromised hosts. In all studies, lung abscess is a disease of midlife or older. In the CDC discharge data, the mean ages of patients with lung abscess were 57.7 years (2000) and 50.1 years (2010). Approximately three-quarters of lung abscess diagnoses occurred in adults between the ages of 40 and 70 years.

Patients with primary lung abscess typically have risk factors for aspiration (Table 69.1). As mentioned previously, half of healthy individuals aspirate during sleep, and this increases to 70% of patients with altered consciousness.[7] Up to 78% of patients with lung abscess have a preceding episode of loss of consciousness typically due to alcohol use, seizure, stroke, drug use, or general anesthesia.[21,22,24–26] Alcohol use particularly enhances risk because it not only alters consciousness, but also induces vomiting and impairs neutrophil function.

Dysphagia caused by neurologic disease, such as amyotrophic lateral sclerosis or Parkinson disease, or esophageal disease, such as dysmotility or stricture, predisposes to aspiration even in the absence of altered consciousness.[23] Nasogastric tubes and other mechanical obstruction of anatomic barriers to aspiration also increase risk. Enteral tube feeding through the nasogastric tube magnifies the problem, particularly in the critically ill, who often have gastroesophageal reflux and decreased gastric

TABLE 69.1 Predisposing Factors for Development of Lung Abscess

Factors Associated With Aspiration
Alcoholism
Drug abuse
Sedating medications: general anesthesia, antipsychotics
Seizure
Stroke
Esophageal abnormality
Respiratory muscle dysfunction: Parkinson disease, amyotrophic lateral sclerosis
Periodontal disease
Enteral tube feeding
Factors Associated With Immunocompromise
Diabetes
HIV/AIDS
Steroids and immunosuppressive drugs
Local Factors
Bronchial carcinoma
Bronchiectasis
Pulmonary infarction

motility.[27] Furthermore, enteral tube feeds may have an impact on the oral microbiome, resulting in higher proportions of pathogenic flora in the oropharynx.[28] Proton pump inhibitors and histamine type 2 receptor antagonists, by increasing the pH of the stomach, can impair natural host defenses, resulting in higher bacterial burden.[29] If a subsequent aspiration event occurs in these settings, patients are more likely to deliver an inoculum of bacteria large enough to cause clinical infection.[27,29] Increasing the gastric pH can also alter the colonizing microbiota of the stomach and airway, favoring gram-negative organisms and modifying the causative organisms in aspiration pneumonia or lung abscess.[30]

Patients with lung abscess often have poor dentition. Historically, the majority (up to 82%) of patients with lung abscess have periodontal disease.[21,23] Gingivitis coincides with a high density of oral anaerobic organisms in the gingival crevice, leading to a potentially larger inoculum in the setting of aspiration. In contrast, primary lung abscess is rare in edentulous people. Because, in some studies, over 40% of edentulous patients with lung abscess had underlying bronchogenic carcinoma, a diagnosis of a lung abscess in an edentulous patient should prompt evaluation for underlying malignancy.[31]

As mentioned earlier, secondary abscess can occur in patients with underlying immunocompromise, such as those with diabetes mellitus or who are taking immunosuppressive medications including steroids. The presence of a primary lung pathologic condition that would predispose to stasis or necrosis, such as bronchiectasis, is also a risk factor.

CLINICAL PRESENTATION

Patients with lung abscess typically present with cough productive of foul-smelling sputum and fever.[11] Malaise, night sweats, and pleuritic chest pain are also reported. Bad breath can be a complaint, especially in the setting of putrid sputum. Patients typically have an indolent and subacute presentation, with the median duration of symptoms before presentation being 14 days but sometimes as long as 6 weeks.[11,14,22] Hemoptysis can occasionally be a presenting complaint.[22,32] In patients with chronic abscess, weight loss, anemia, and digital clubbing can be observed.[12,21] On physical examination, poor dentition is often noted. Patients with necrotizing pneumonia, particularly from a hematologic source, can occasionally present more acutely, within 1 week of symptom onset, with high fever.[10]

MICROBIOLOGY

The lung has an extant microbiome composed of upper respiratory organisms that transiently colonize the lower airways.[1,3] In adults, most organisms in the lung are those of the oropharynx (e.g., *Streptococci, Prevotella,* and *Veillonella*). Children's lungs also can be colonized with organisms from the nasopharynx such as *Moraxella, Haemophilus,* and *Staphylococcus.* These organisms are thought to enter the lung through microaspiration and to reside there only transiently with constant migration and elimination, never establishing rich, resident microbial communities. Organisms within the alveoli may prime the immune system and facilitate rapid microbial clearance.[1]

According to the findings of culture-based techniques, anaerobic pleuropulmonary infections contain an average of 3.5 different species of anaerobes and 1.7 different aerobes.[33] In the late 20th century, anaerobic bacteria accounted for 85% to 93% of organisms in lung abscess, with the predominant isolates being *Peptostreptococcus* species, *Fusobacterium nucleatum,* and *Prevotella melaninogenica,* formerly known as *Bacteroides melaninogenica.*[10,14,34,35,36,37] Microbiologic analysis from samples obtained by percutaneous drainage of abscesses confirmed the prevalence of anaerobic bacteria.[38] Obligate anaerobes including *Bacteroides* and *Prevotella,* which do not tolerate oxygen, are unlikely to grow in small lesions adjacent to well-oxygenated lung. Facultative anaerobes, which can use but do not require oxygen, constitute most of the organisms in a lung abscess and can grow and create the anaerobic environment for the obligate anaerobes.

Culture-based methods, however, may not tell the entire story. With 16S rRNA sequencing of bronchoalveolar lavage samples from patients with lung abscess, two-thirds of samples were classified as polymicrobial infections, and one-third were monomicrobial. Obligate anaerobes, which comprised the predominant phylotypes in 42% of samples, comprised at least 5% of microbiota in 86.5% of the polymicrobial infections. Among the pathogens identified by 16S sequencing, *Fusobacterium* species were the predominant phylotype, followed by *Streptococcus anginosus.*[38] *S. anginosus* was most likely of the frequent pathogens to be associated with monomicrobial infection.

The microbiology of lung abscess also varies by geography. For example, transthoracic aspiration from 90 patients with community-acquired lung abscess in Taiwan revealed a monomicrobial process in 79% of cases; *Klebsiella pneumoniae* was isolated in 33% of monomicrobial lesions, followed by *Streptococcus milleri* in 16%.[39] In a second study from Taiwan, only 26.2% of abscesses yielded anaerobic bacteria, as either a sole or a participating causative agent. *Streptococcus* spp. were the most commonly isolated organisms (59.8%); *Klebsiella* spp. accounted for 8.2% of community-acquired lung abscesses in this center.[23] Limitations of these studies included prior antibiotic use and the fastidiousness of anaerobes.[36] Despite these limitations, studies do suggest a change over time in the organisms involved in lung abscesses, from predominantly anaerobic to more virulent aerobic bacteria, and that regional variability in abscess microbiology may be important in determining etiology.[27,36,40]

Microbiology may also be quite different in nosocomial settings.[41] In critically ill intubated patients, gram-negative bacteria were found in tongue biofilm samples. These findings correlated with tracheal aspirate cultures in those who developed ventilator-associated pneumonia or aspiration pneumonia.[42] Similarly, patients on acid-suppressing medications have a greater propensity to colonization, and therefore aspiration pneumonia, with gram-negative organisms.[30] If necrotizing pneumonia or lung abscess develops in hospitalized patients, treatment against gram-negative organisms should be strongly considered.

Abscess etiologies in immunocompromised patients—with acquired immunodeficiency syndrome (AIDS), on chemotherapy, or after organ transplantation—are broad, as noted in Table 69.2. A retrospective study comparing immunocompromised patients with nonimmunocompromised patients noted a trend toward a greater proportion of aerobic bacteria isolated and a larger number of patients with multiple bacterial isolates. Conversely, in this group a low number of anaerobes were isolated, possibly in part owing to the methods of specimen collection.[16] Atypical pathogens can also cause abscess in immunocompromised hosts. In patients with impaired cell-mediated immunity, *Legionella, Nocardia,* and *Rhodococcus* are known causes of cavitary lesions. In patients with neutropenia, aerobic bacteria and molds such as *Aspergillus* and Zygomycetes are important pathogens. These pathogens are discussed in other chapters in this book.

DIAGNOSIS

Diagnosis of lung abscess is generally made through chest radiographs that show a lung cavity with an air-fluid level (Fig. 69.3). CT with contrast shows a thick irregular wall with a smooth internal border and

TABLE 69.2 Differential Diagnosis of a Cavitary Lesion on Chest Radiograph

Infectious Causes
Bacteria
Oral anaerobes Less commonly: *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,* enteric gram-negative rods, *Pasteurella multocida, Burkholderia cepacia, Burkholderia pseudomallei, Haemophilus influenzae* types b and c, *Legionella,* group A *Streptococcus, Streptococcus pneumoniae, Streptococcus anginosus, Nocardia, Rhodococcus equi, Actinomyces, Clostridium, Capnocytophaga*
Septic Pulmonary Embolism
Originating in septic phlebitis, including Lemierre syndrome and vegetations from tricuspid endocarditis
Mycobacteria
Tuberculosis and nontuberculous pathogens
Fungi
Endemic mycoses (*Histoplasma, Coccidioides,* blastomycosis), *Cryptococcus, Aspergillus,* Zygomycetes
Parasites
Paragonimus westermani, Entamoeba histolytica, Echinococcus
Noninfectious Causes
Neoplasm
Primary lung cancer, metastatic carcinoma (particularly squamous cell) Pulmonary infarction caused by bland embolus
Vasculitis
Wegener granulomatosis, rheumatoid lung nodule
Airway Disease
Bullae, blebs, or cystic bronchiectasis Developmental cause Pulmonary sequestration
Other
Sarcoidosis, achalasia or transdiaphragmatic bowel herniation giving appearance of cavity with air-fluid level

Modified from Lorber B. Bacterial lung abscess. In: Bennett JE, Dolin R, Blaser M, eds. Principles and Practice of Infectious Diseases. *8th ed. Philadelphia: Elsevier; 2014;855–859.*

peripheral enhancement of the abscess. Consolidation in the adjacent parenchyma is found in half of all cases.[43] The majority of patients—70% to 96% in early case series—present with a single abscess.[21,22] The main localizations of these lesions mirror those of aspiration pneumonia, namely, the posterior segments of the right upper lobe, the middle lobe, and the superior segments of the inferior lobes.[21,22,43] These radiographic findings in a patient with aspiration risk factors and classic subacute presentation of fever, particularly with putrid sputum, are strongly suggestive of lung abscess and sufficient to clinch the diagnosis. In this case, empirical therapy targeting anaerobic organisms can be started in the absence of microbiologic studies.

If obtained, sputum cultures from patients with lung abscess typically yield polymicrobial respiratory flora. Specialized techniques such as transtracheal aspiration, transthoracic aspiration, and bronchoalveolar lavage with quantitative cultures are required for culturing of anaerobic organisms, but these are rarely performed in clinical practice. If empyema has developed, putrid smell of empyema fluid can be diagnostic and culture can be helpful in determining the microbes involved. If patients do not have the classic presentation or if they have secondary lung abscess, then cultures of expectorated sputum or bronchoalveolar lavage should be performed to evaluate for aerobic bacteria, mycobacteria, fungi, and occasionally parasites in the appropriate clinical situation. Furthermore, early bronchoscopy to evaluate for tumor should be considered if the abscess is in an atypical location or if the patient lacks risk factors for aspiration, is edentulous, lacks classic presenting symptoms, or has risk factors for lung cancer.

THERAPY

For primary lung abscess, prolonged treatment with antibiotics that cover oral microbiota, and anaerobes in particular, is necessary. The use of penicillin was a major advance in the treatment of primary lung abscess and for many years was the treatment of choice. In recent decades, production of penicillinase has led to penicillin resistance for many oral anaerobes, and other therapies have proved to be more efficacious. In the 1980s a small randomized clinical trial showed that 3 to 6 weeks of clindamycin was more effective than penicillin with a shorter febrile period, fewer days of putrid sputum, and a higher rate of clinical cure.[44] In a second randomized, controlled trial, 44% of patients who received penicillin failed to respond to therapy compared with only 5% of patients who received clindamycin. Furthermore, cultures obtained through transthoracic needle aspiration and/or bronchoscopic brushing revealed penicillin resistance in *Bacteroides* species that were all sensitive to

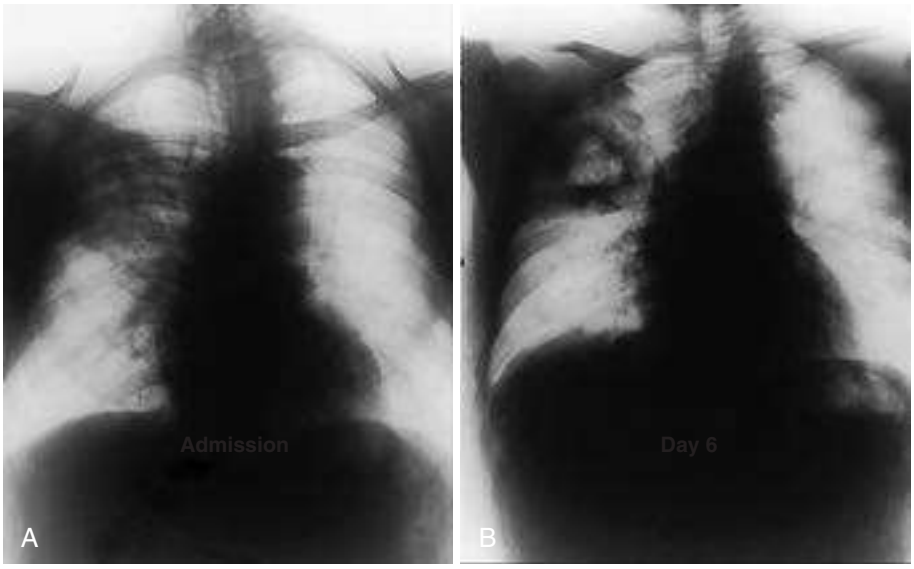

FIG. 69.3 (A) Admission chest radiograph from a 61-year-old man with fever, cough, and putrid sputum of 4 weeks' duration and a 12-pound weight loss. A large infiltrate, without obvious cavitation, is visible in the right lung. (B) A repeat radiograph on day 6 of hospitalization, after the patient began to produce copious sputum, shows a large, thick-walled cavity with an air-fluid level. Because the patient was a heavy smoker and had no aspiration risk and excellent oral hygiene, a bronchoscopy was performed, which showed a partially obstructing carcinoma. *(From Lorber B. Bacterial lung abscess. In: Bennett JE, Dolin R, Blaser M, eds.* Principles and Practice of Infectious Diseases. *8th ed. Philadelphia: Elsevier; 2014:855–859.)*

clindamycin.[38] Similarly, clindamycin compared with metronidazole showed a 90% versus 46% cure rate, respectively.[45] This discrepancy was likely secondary to metronidazole's lack of activity against the microaerophilic streptococci and anaerobic cocci that are often involved in anaerobic lung infections. Consequently, metronidazole is not considered adequate anaerobic therapy for lung abscess. More recently, treatment has shifted to β-lactam–β-lactamase combinations and fluoroquinolones. Intravenous, followed by oral, amoxicillin-clavulanate resulted in cure of 100% of patients.[46] Amoxicillin-clavulanate and clindamycin plus a cephalosporin were shown to have similar efficacy.[47] Moxifloxacin has also been shown to have equal efficacy to amoxicillin-clavulanate, and both treatments were well tolerated.[48,49] Of note, anaerobic resistance to clindamycin and moxifloxacin has been rising in the past few years.[50]

Other agents with excellent anaerobic coverage that should be considered, especially when resistant gram-negative organisms are present (i.e., in the setting of immunocompromise or nosocomial infection), include piperacillin-tazobactam and carbapenems.[51,52] Few data exist for these agents for lung abscess, but for aspiration pneumonia both have been shown to have good efficacy.[53,54]

Although patients have been cured with only oral therapy,[54] the general practice is to treat with intravenous antibiotics initially and then transition to oral therapy once clinical improvement is seen.

Duration of Therapy

Limited data direct duration of therapy for treatment of lung abscess. Levison and colleagues reported one relapse in patients receiving 3 weeks of penicillin compared with no relapse in patients receiving either 3 or 6 weeks of clindamycin or 6 weeks of penicillin.[44] In another study, moxifloxacin and amoxicillin-clavulanate were given through clinical and radiologic resolution of infection with mean duration of 30 and 35 days, respectively.[49] Other investigators advocate that the infection should be treated until there is clearance or small stable residual lesion on chest images.[11] In the absence of a body of clinical trials to guide decision making, a minimum of 3 to 4 weeks of treatment can be assumed, with close clinical monitoring to guide cessation of therapy. A longer duration of therapy should be considered for treatment of particularly large abscesses or in patients with underlying immunocompromise.

Surgical Intervention

Unlike abscesses in other areas of the body where drainage is required for cure, lung abscesses can be treated successfully with medical therapy alone in at least 80% of cases.[55,56] Spontaneous release of pus into the airway, as evidenced by putrid sputum expectoration, may serve as "natural" drainage.[11] In some instances, however, intervention may be required, such as in the setting of antibiotic failure, abscess secondary to neoplasm, or pulmonary hemorrhage.[56–58] Features that suggest risk for failure of medical therapy include associated malignancy, abscess diameter >6 cm, and involvement of resistant organisms.[58,59] Historically, patients who failed medical therapy underwent lobectomy (preferred), segmentectomy, or pneumonectomy. Mortality associated with surgical intervention, however, is as high as 15%.[60,61] More recently, minimally invasive approaches, including percutaneous and endoscopic drainage, have been explored.[62,63] Percutaneous drainage has a reported success rate of 84% with a mortality of 4%, suggesting that it is a reasonably safe alternative to surgical intervention.[63] Endoscopic drainage with pigtail catheter placement with or without antibiotic lavage has also been described with good outcomes.[62,64] This modality may be preferred in patients with coagulopathy, obstructing airway lesions, or centrally located abscesses.[63,64]

Response to Therapy and Prognosis

Typically, patients feel better within a few days of receiving appropriate antibiotics. Defervescence occurs within 7 to 10 days, although chest radiographs can show worsening in approximately one-third of patients during the first week of treatment.[10,22,65,66] Radiographic improvement often lags behind clinical resolution. Time to cavity closure is variable. The majority of cavities resolve on images by 6 weeks, but on rare occasions can take longer than this to close. Surrounding infiltrates may also take longer to resolve, typically at least 8 weeks.[66]

In the preantibiotic era, mortality associated with lung abscess was 33%. Since the advent of antibiotic therapy, mortality has fallen to 4% to 5%.[11,21,22] Prognosis is poorer in patients with obstructing airway lesions or in immunocompromised hosts.[67] Larger abscess size has also been associated with higher mortality rate.[59] In addition, abscesses involving resistant and/or more virulent organisms such as *Pseudomonas aeruginosa, Staphylococcus aureus,* and *K. pneumoniae* tend to have worse outcomes.[59] A recent study in geriatric patients suggested that oral microbiota dominated by *Prevotella* and *Veillonella* species had a higher pneumonia-related mortality rate than those dominated by *Neisseria* and *Fusobacterium*.[68] This suggests that the microbiome of the respiratory tract might play a more important part in the pathophysiology of and recovery from respiratory infection than previously thought.

PREVENTION

Sobriety, maintenance of good dentition, and prevention of aspiration are the best protection against lung abscess. Patients otherwise at risk should follow aspiration precautions. There is no evidence that administering antibiotics to patients who have been observed to aspirate prevents lung infections. Early recognition of lung infections and rapid initiation of antibiotic therapy, however, are crucial in preventing development of lung abscess.

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70 Chronic Pneumonia

Peter G. Pappas

SHORT VIEW SUMMARY

Definition

- There can be persistent or progressive cough, dyspnea, often with chronic sputum production, with or without fever, lasting weeks or months rather than days.
- It is always associated with abnormal chest radiography.
- Constitutional symptoms, including weight loss and anorexia, are typical.
- Definition does not include asymptomatic solitary pulmonary nodules.
- It may be infectious or noninfectious.

Epidemiology

- Age, race/ethnicity, and gender are important considerations
- Underlying health issues and comorbid conditions
- Current and past residence, occupation, travel, and recreation history may be important
- Recent hospitalization or history of imprisonment
- Drug and alcohol history
- Recent prescription drug exposure

Microbiology

- Bacterial: anaerobes, microaerophilic and viridans streptococci; *Staphylococcus aureus;* selected community-acquired and nosocomial gram-negative bacilli; *Burkholderia pseudomallei*
- Higher-order bacteria: *Mycobacterium tuberculosis, Mycobacterium kansasii,* and other nontuberculous mycobacteria; *Nocardia* spp.; *Rhodococcus equi; Actinomyces* spp.
- Fungi: *Histoplasma capsulatum, Blastomyces* spp., *Emergomyces* spp., *Emmonsia crescens, Coccidioides immitis/posadasii, Paracoccidioides spp., Cryptococcus neoformans,* and *Cryptococcus gattii, Aspergillus* spp., *Lomentospora prolificans, Scedosporium* spp., *Mucorales* spp.
- Parasites: *Dirofilaria, Echinococcus granulosus, Paragonimus westermani,* filariasis
- Noninfectious causes: autoimmune diseases, neoplasia, drug induced, sarcoidosis, pulmonary alveolar proteinosis, silicosis, berylliosis, and a variety of other conditions

Diagnosis

- Careful clinical assessment, including detailed history, is essential.
- Clinical findings are generally nonspecific; rash, osteoarticular findings, and mucocutaneous and neurologic findings may provide helpful clues.
- Radiographic imaging, including routine chest radiograph or chest computed tomography, or both, are critical to diagnosis.
- Routine microbiologic studies, including sputum examination by Gram stain, potassium hydroxide, acid-fast stain, and preparations for ova and parasites, are important.
- Culture for routine bacteria, fungi, and acid-fast bacilli when possible. Multiplex polymerase chain reaction panels are available for respiratory specimens but are most useful for acute community-acquired pneumonia.
- Biopsy and culture of nonpulmonary specimens (e.g., urine, skin, bone, liver, prostate, or brain biopsy) in clinically relevant settings. Potentially important serologic studies include the interferon-γ release assays for tuberculosis; urine and serum *Histoplasma* antigen; serum and cerebral spinal fluid cryptococcal antigen; and serum galactomannan (GM).
- Bronchoalveolar lavage for acid-fast, modified acid-fast, and Gram stain; wet mount or calcofluor stain; cultures for bacteria, mycobacteria, and fungi; GM assay; and cytopathology.

Therapy

- Specific therapy is entirely dependent on the most likely etiologies, of which there are many possibilities, usually including antimicrobials.

Prevention

- In this broad category of disease, prevention generally entails the recognition and avoidance of circumstances that increase the likelihood of developing chronic pneumonia. In selected settings, such as high-risk patients receiving chemotherapy, prophylactic antimicrobials may be warranted.

Chronic pneumonia syndrome is a pulmonary parenchymal process that can be infectious or noninfectious, has been present for weeks to months rather than for days, and is manifested by abnormal chest radiographic findings and chronic or progressive pulmonary symptoms. Abnormal chest radiography, which may reveal any of several radiologic patterns, is probably the most important consideration in the diagnosis of chronic pneumonia. Indeed, in many patients, the diagnosis is based more on the pulmonary radiographic findings than on the pulmonary symptoms. However, asymptomatic patients who have abnormal radiographic findings, such as solitary or multiple nodules, should not be considered to have chronic pneumonia.

The emphasis in this chapter is on the chronic pneumonias caused by infectious agents. However, it is important to recognize the importance of noninfectious causes of chronic pneumonia, including the autoimmune diseases such as granulomatosis with polyangiitis; Churg-Strauss syndrome; Goodpasture syndrome and microscopic polyangiitis[1–6]; idiopathic pulmonary fibrosis and rheumatoid arthritis; neoplastic processes such as bronchogenic carcinoma, adenocarcinoma, and lymphoproliferative disorders[7,8,9–14]; prescription drugs, illicit drugs, and other chemicals[15–24]; radiation[17]; amyloidosis[25–27]; sarcoidosis[28,29,30,31]; pulmonary alveolar proteinosis[32] and forms of lipoid pneumonia[32–36]; chronic organizing pneumonia and hypersensitivity pneumonitis due to a wide variety of agents[37–40]; and other idiopathic interstitial pneumonias.[41–43]

CAUSES

The infectious causes of chronic pneumonia can be divided into two main groups: (1) those agents that typically cause acute pneumonia and are unusual causes of chronic pneumonia and (2) infectious agents that typically cause chronic pneumonia. Among those agents that typically cause acute pneumonia, anaerobic bacteria, selected microaerophilic streptococci,[44,45,46] *Staphylococcus aureus,* group F *Streptococcus, Haemophilus influenzae, Klebsiella pneumoniae, Burkholderia pseudomallei,* and *Pseudomonas aeruginosa* are the organisms most likely to produce persistent symptoms and chronic pneumonia. In these instances the pneumonia reflects a chronic necrotizing process that most commonly

occurs in patients with significant underlying disease (e.g., alcoholism, diabetes mellitus, intrathoracic malignancy, chronic obstructive lung disease), hospitalized patients, those requiring long-term ventilatory assistance, patients with chronic swallowing and reflux disorders, and others at risk for recurrent aspiration, such as patients with Parkinson disease and other neurologic disorders.[44,45–55] Acute pneumonia caused by most viral agents and by *Streptococcus pneumoniae, Mycoplasma pneumoniae, Legionella* spp., *Coxiella burnetii,* or *Chlamydia pneumoniae* rarely progresses to a chronic pulmonary infection.[56,57]

Table 70.1 lists the most common infectious and noninfectious causes of chronic pneumonia. In the otherwise healthy host, the most common considerations are tuberculosis[58–61] and nontuberculous mycobacteria (NTM)[59,62–66,67]; cryptococcosis due to either *Cryptococcus neoformans* or *Cryptococcus gattii*[68–70]; and the endemic fungal infections, including histoplasmosis,[71–73] coccidioidomycosis,[74–76] and blastomycosis.[77–80] In the appropriate geographic area, paracoccidioidomycosis, adiaspiromycosis, emergomycosis, and talaromycosis should be considered.[81–85] Lung abscess with mixed aerobic and anaerobic bacterial infections may warrant consideration,[45–47] as well as actinomycosis.[86–89] Aspergillosis (multiple species), scedosporiosis (caused by *Scedosporium apiospermum* and *Lomentopora [Scedosporium] prolificans),*[90,91] sporotrichosis,[92] emergomycosis (due to *Emergomyces africanus, Emergomyces pasteurianus,* and *Emergomyces canadiensis*), and adiaspiromycosis (caused by *Emmonsia crescens*)[93–96] are rare causes of chronic fungal pneumonia in the normal host. In the immunocompromised host, mycobacteria, especially *Mycobacterium tuberculosis,* remain common causes of chronic pneumonia.[97,98] Classic opportunistic infections associated with pneumonia, including nocardiosis,[99–102] *Rhodococcus*,[103] cryptococcosis,[69,104] aspergillosis,[105,106] and infections caused by other molds, such as the agents of scedosporiosis and mucormycosis, are also important in this population.[107,108] In the appropriate geographic areas, coccidioidomycosis, histoplasmosis, and blastomycosis are also important considerations in immunocompromised and noncompromised hosts.[73–78,109,110] In persons with acquired immunodeficiency syndrome (AIDS), coccidioidomycosis and histoplasmosis may be seen and are common AIDS-defining illnesses. Furthermore, in patients with AIDS, chronic pneumonia may be caused by *Rhodococcus equi, Pneumocystis jirovecii, Mycobacterium avium* complex, or noninfectious disorders, such as Kaposi sarcoma, lymphoma, and nonspecific interstitial pneumonitis.[111–117] The protozoa and helminths listed in Table 70.1 are uncommon causes of chronic pneumonia syndrome in persons living in industrialized countries, but they are important considerations for those who live in or have traveled to areas in which these agents are endemic.

EPIDEMIOLOGY

Age, Gender, and Race

A detailed history is an important first step toward establishing a specific diagnosis in patients with a chronic pneumonia syndrome. The significance of age and gender for patients with chronic pneumonia from other causes also usually relates, indirectly, to relevant epidemiologic factors. For example, an older adult is at higher risk of having a cerebrovascular accident, which in turn might predispose this patient to an aspiration episode and subsequent chronic bacterial pneumonia and abscess. Older debilitated patients are at higher risk for development of chronic necrotizing pneumonia caused by aerobic gram-negative bacteria.[49,51,54] Similarly, the gender of a given patient is likely to affect

TABLE 70.1 Causes of Chronic Pneumonia Syndrome

Infectious Agents That Typically Cause Chronic Pneumonia

Bacteria

Mixed aerobic and anaerobic bacteria (e.g., *Streptococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus anginosus* group, *Prevotella* spp., *Bacteroides* spp.)
Actinomyces spp.
Nocardia spp.
Rhodococcus equi
Burkholderia pseudomallei

Mycobacteria

Mycobacterium tuberculosis
Mycobacterium kansasii
Mycobacterium avium complex
Mycobacterium chelonae-abscessus complex
Mycobacterium szulgai
Mycobacterium xenopi
Mycobacterium malmoense
Mycobacterium terrae

Fungi

Aspergillus spp.
Blastomyces dermatitidis, B. gilchristii, B. helicus
Coccidioides immitis/posadosii
Cryptococcus neoformans
Cryptococcus gattii
Emergomyces spp. (*africanus, canadiensis,* and *pasteuriana*)
Emmonsia crescens, E. helica
Histoplasma capsulatum
Lomentospora prolificans
Paracoccidioides brasiliensis
Talaromyces marneffei
Pneumocystis jirovecii
Pigmented (dematiaceous) molds (see Chapter 268)
Scedosporium apiospermum
Sporothrix schenckii complex

Parasites

Dirofilaria spp.
Echinococcus granulosus, E. multilocularis
Filaria (tropical pulmonary eosinophilia)
Paragonimus westermani

Noninfectious Causes of Chronic Pneumonia

Neoplasia
Carcinoma (primary or metastatic)
Hodgkin disease and non-Hodgkin lymphoma
Other lymphoproliferative disorders
Cystic fibrosis
Sarcoidosis
Amyloidosis
Vasculitis (autoimmune diseases)
Systemic lupus erythematosus
Polyarteritis nodosa
Granulomatosis with polyangiitis (Wegener granulomatosis)
Allergic angiitis and granulomatosis (Churg-Strauss syndrome)
Goodpasture syndrome
Microscopic polyangiitis
Lymphomatoid granulomatosis
Progressive systemic sclerosis
Rheumatoid arthritis
Mixed connective tissue syndrome (overlap syndrome)
Chemicals, drugs
Radiation
Recurrent pulmonary emboli
Bronchial obstruction with atelectasis (e.g., tumor, foreign body)
Pulmonary sequestration
Pulmonary infiltration with eosinophilia syndrome
Löffler syndrome: usually transient
Pneumonia plus asthma (e.g., allergic bronchopulmonary aspergillosis)
Bronchocentric granulomatosis
Chronic eosinophilic pneumonia
Pneumoconiosis: asbestosis, berylliosis, silicosis, anthracosilicosis
Chronic form of extrinsic allergic alveolitis (hypersensitivity pneumonitis)

Other Lung Diseases: Cause Unknown

Chronic organizing pneumonia
Chronic interstitial pneumonia (fibrosing alveolitis, idiopathic pulmonary fibrosis)
Usual interstitial pneumonia (UIP)
Desquamative interstitial pneumonia (DIP)
Lymphocytic interstitial pneumonia (LIP)
Giant cell interstitial pneumonia (GIP)
Eosinophilic granuloma (pulmonary Langerhans cell histiocytosis X)
Lymphangioleiomyomatosis
Pulmonary alveolar proteinosis
Pulmonary alveolar microlithiasis
Idiopathic pulmonary hemosiderosis
Angiocentric immunoproliferative lesions

or determine occupation and hobbies and therefore the likelihood of exposure to certain infectious agents or other causative vehicles. Moreover, gender may predispose to certain rare chronic pulmonary disorders. For example, pulmonary lymphangioleiomyomatosis, a cause of chronic pneumonia, is a rare neoplastic disorder that occurs almost exclusively in adolescent and young adult women.[118] Racial and genetic characteristics are increasingly recognized as predisposing factors to severe disease manifestations from a variety of pathogens.[82,119–121] For example, chronic cavitary pulmonary tuberculosis is more common in blacks,[58,59] and disseminated coccidioidomycosis is much more likely in darker-skinned persons, including blacks, Filipinos, and Asians who have lived in or have traveled to an area endemic for *Coccidioides immitis* or *Coccidioides posadasii*.[74,76] Conversely, chronic cavitary histoplasmosis is much more likely in older white men with a history of emphysema.[71] Fungus ball (aspergilloma) of the lung occurs in a previously existing apical cavity in patients with prior sarcoidosis, histoplasmosis, tuberculosis, or other fibrocavitary lung disease.[88] Chronic necrotizing and invasive aspergillosis may occur in men with underlying chronic obstructive pulmonary disease and has been recognized as a postinfluenza complication.[88,122]

Occupation and Hobbies

An example of a condition linked to occupational and recreational behavior includes tuberculosis among health care workers and volunteers who work abroad in high-risk settings. Coccidioidomycosis may occur in desert rock collectors, laboratory technicians, archeologists conducting excavations, construction workers, hikers and campers, and others exposed to desert dust in the endemic area, which includes portions of northern Mexico, Argentina, California, Arizona, New Mexico, Utah, western Texas, and eastern Washington state. Histoplasmosis may occur in persons exposed to pigeon or starling roosts, those who clean out old chicken houses with dirt floors, those who cut and clear hollow trees, and those who demolish old buildings or explore caves inhabited by bats. Blastomycosis more often occurs in forestry workers, those who are earth-moving and heavy equipment operators, hunters, and other outdoorsmen. Unlike endemic fungal infections, cryptococcosis has *not* been strongly linked to occupational and recreational behavior,[123] perhaps reflecting the fact that *Cryptococcus neoformans* is a ubiquitous pathogen that is not geographically restricted. Moreover, good data suggest that a large proportion of the population is exposed to this organism early in life.[124] Furthermore, there has been no strong association between exposure to pigeon droppings and development of disease. Other associations include the following: echinococcosis in sheep herders; berylliosis in workers in the aircraft, electronics, and nuclear industries; the pneumoconioses (e.g., silicosis, asbestosis) in sandblasters and shipyard workers; and both chronic and acute pulmonary disease from repeated occupational or environmental exposure to the aerosolized organic antigens associated with extrinsic allergic alveolitis (hypersensitivity pneumonitis)[2,40,41] or to irritant gases, such as phosgene, ammonia, ozone, and nitrogen dioxide. Hypersensitivity reaction to *Mycobacterium avium* complex contaminating an indoor hot tub and causing interstitial pneumonia ("hot tub lung") occurs primarily in immunocompetent hosts.

Residence and Travel

Because the initial exposure to the microbiologic agents of many chronic or indolent infectious diseases may have occurred months or years before the disease appears, a detailed travel history is essential for any patient with chronic pneumonia. For example, if the patient has lived in the eastern half of the United States, especially the mid-South or upper midwestern regions, chronic pulmonary histoplasmosis and blastomycosis should be considered because the causative agents are endemic to that area. Likewise, coccidioidomycosis should be a strong consideration in a person with chronic cavitary pneumonia who has lived in or visited an area endemic for *Coccidioides immitis/posadasii*. Among patients with chronic pneumonia and a history of living in or travel to the US Pacific Northwest or British Columbia, infection due to *C. gattii* should be considered. Unlike some of the other endemic mycoses, where travel to the endemic area is sufficient to confer risk,[69,70] paracoccidioidomycosis should be realistically considered only for patients who have ever lived in Mexico, Central America, or the regions of South America that are endemic for *Paracoccidioides* spp.

In the setting of a potentially relevant travel history, in addition to identifying specific regions visited, it is often necessary to explore in detail rural versus urban exposure, the type of lodging, sources of drinking water, exposure to local foods, working environment, and other activities such as swimming and hiking. For example, a person who has lived or traveled extensively in Southeast Asia, particularly in low-lying or rice-growing areas, who manifests chronic pneumonia with pulmonary radiographic abnormalities resembling those of tuberculosis or a pulmonary mycosis, may be suffering from melioidosis.[125,126] Pulmonary paragonimiasis should be considered in the visitor to Southeastern Asia or the Philippines who consumes raw or partly cooked shellfish and who has chronic pulmonary symptoms plus dense, nodular lung opacities and ring shadows on the chest radiograph.[127]

Contacts, Habits, and Drugs

In the past few decades, most cases of pulmonary tuberculosis in the United States have been diagnosed in homeless persons, alcoholics, older adults, patients with human immunodeficiency virus (HIV), and immigrant population groups.[128,129] In patients and health care workers in whom tuberculosis is suspected, contacts among companions, relatives, or patients with tuberculosis should be sought. In addition, tuberculosis should be suspected in persons living or working in closed environments, such as jails and prisons, homeless shelters, drug rehabilitation centers, and nursing homes. Inquiry should be made into the patient's smoking and drinking histories and other personal habits. The likelihood of cancer of the lung and one of the invasive mycoses, such as aspergillosis and histoplasmosis, is greater in a smoker than in a nonsmoker. Aspiration pneumonia, chronic gram-negative bacillary pneumonia, tuberculosis, and pulmonary sporotrichosis are more likely to occur in an alcoholic than in a nondrinker. Intravenous drug users who inject heroin or other illicit agents are at risk for not only infection with HIV and subsequent development of AIDS but also septic pulmonary emboli associated with tricuspid or pulmonic valve infective endocarditis, necrotizing pneumonia, single or multiple lung abscesses, or an interstitial granulomatous reaction to the injected material, resulting in pulmonary hypertension (see Chapter 312). Similarly, frequent use of free-base (crack) cocaine has been reported to cause chronic organizing pneumonia, eosinophilic lung disease, interstitial pneumonitis, and pulmonary hemorrhage or infarction.[24,130]

More than 100 different pharmaceutical agents have been reported to cause acute and chronic pulmonary symptoms with radiographic abnormalities.[15–23,131–133] Early in the course of drug-induced pulmonary disease, the chest radiographic findings may be normal; later, an interstitial, nodular, or alveolar pattern (or a combination of these) may be present. Still later, chest radiography may reveal only a fibrotic pulmonary process. The agents that are most likely to cause chronic pulmonary disease include cytotoxic agents, such as bleomycin, busulfan, cyclophosphamide, methotrexate, nitrosoureas, noncytotoxic agents such as amiodarone, gold salts, nitrofurantoin, and penicillamine, as well as disease-modifying agents for rheumatologic disease, such as etanercept, infliximab, and leflunomide.[17–23] Because drug-induced pulmonary disease may develop after drug therapy has been discontinued, the physician should inquire not only about all drugs the patient is presently taking but also about those taken in the last 6 months.

Questions about recent antimicrobial therapy are critically important. Which antimicrobial(s) was used? Did the therapy result in radiographic or clinical improvement? If not, was the antimicrobial drug used in sufficient quantity and duration to cure the suspected process or alter its course? Was the appropriate agent used? What effect did the antimicrobial agent have on the results of cultures? Does the report of "normal flora" from the sputum culture merely reflect the elimination of a specific pathogen by antimicrobial therapy?

Underlying Disease

Pulmonary complications, including acute and chronic or refractory pneumonia, are especially common in persons with AIDS[111–116] and other conditions associated with impaired host immunity, such as high-dose corticosteroid therapy, cytotoxic therapy, hematopoietic stem cell and organ transplantation, Job syndrome, and chronic granulomatous disease.[134,135] Patients with poorly controlled diabetes mellitus or

preexisting chronic obstructive pulmonary disease are at higher risk for development of chronic or persistent bacterial pneumonia. Similarly, chronic obstructive lung disease commonly precedes fibrocavitary histoplasmosis or *M. avium* complex infection. Structural lung disease, such as preexisting bullae, bronchiectasis, and endobronchial lesions, may also predispose to chronic pneumonia. For example, recurrent or persistent pneumonia in the same area of the lung raises the suspicion of a local endobronchial lesion that may not be apparent on routine chest radiographs. Because aspiration may predispose to chronic pneumonia, inquiry should be made into a history of recent dental problems or manipulation, sinusitis with chronic nasal congestion, disorders of swallowing resulting from neurologic or esophageal disease, seizure disorders, recent anesthesia, quantity and frequency of alcohol consumption, and any illness leading to an unconscious state. Finally, it should be determined whether the chronic pneumonia is most likely community acquired or health care associated.

CLINICAL FEATURES

Symptoms

There are many causes of chronic pneumonia, and no single symptom complex is common to all causes. Often, nonspecific and constitutional symptoms, including fever, chills, and malaise, are present initially, followed by progressive anorexia and weight loss, indicating chronic illness. Pulmonary symptoms may be present early but frequently appear later in the course of the illness. Any patient with a prolonged illness and nonspecific constitutional complaints plus pulmonary symptoms, including a new or persistent cough, sputum production, hemoptysis, chest pain (especially pleuritic pain), or dyspnea, deserves medical evaluation, including a routine chest radiograph and, when findings on these studies are nonspecific and suggestive of a chronic parenchymal process, a computed tomography (CT) examination of the chest.

Evidence of extrapulmonary involvement should be explored with each patient. For example, chronic pneumonia with skin lesions should suggest coccidioidomycosis, blastomycosis, or, in the appropriate epidemiologic setting, paracoccidioidomycosis. Similarly, cryptococcosis, nocardiosis, histoplasmosis, talaromycosis, and Kaposi sarcoma should be important considerations for patients with skin lesions and with AIDS or other conditions associated with significant impairment of cell-mediated immune function. Mucous membrane lesions should also raise the possibility of histoplasmosis, paracoccidioidomycosis, talaromycosis, or Kaposi sarcoma. Monoarticular or polyarticular arthritis, polyarthralgia, or localized bone tenderness or pain may indicate systemic vasculitis or sarcoidosis. A history of chronic pneumonia with persistent headache and abnormal cerebrospinal fluid (CSF) should raise the suspicion of tuberculosis, cryptococcosis, or coccidioidomycosis involving the lungs and central nervous system (CNS). The presence of focal neurologic signs and symptoms is strong clinical evidence for a space-occupying lesion in the CNS; such findings in a patient with a cavitary infiltrate seen on a chest radiograph suggest the possibility of a brain abscess associated with chronic suppurative lung disease caused by nocardiosis[101–103] and microaerophilic or anaerobic bacteria, or an endemic fungal infection, such as blastomycosis, adiaspiromycosis, or histoplasmosis.[44,45,46,47] Similarly, the triad of skin nodules, pulmonary nodules, and CNS abnormalities suggests lymphomatoid granulomatosis.[7,8,9]

Signs

Although the findings on physical examination of the chest are usually not helpful in differentiating specific causes of chronic pneumonia, the presence of generalized wheezing or other signs of bronchospasm, in the absence of underlying lung disease, indicates an asthmatic component to the pulmonary illness and raises the possibility of a disorder causing both pneumonia and asthma, such as extrinsic allergic alveolitis, allergic bronchopulmonary aspergillosis, or allergic angiitis and granulomatosis (Churg-Strauss syndrome). Similarly, localized wheezing suggests the presence of an endobronchial obstructing lesion. The findings of tachycardia, cardiomegaly, gallop rhythm, and ankle edema provide evidence of cardiac disease, suggesting that the pulmonary symptoms and signs result at least in part from cardiovascular causes. The presence of skin lesions, clubbing, cyanosis, or phlebitis is not specific for any single pulmonary disorder but may help narrow the differential diagnosis, especially when considered along with other clinical and epidemiologic information. The presence of abnormal liver function, lymphadenopathy, hepatomegaly, and/or splenomegaly with chronic pneumonia suggests a systemic disorder involving the reticuloendothelial system, such as non-Hodgkin lymphoma, sarcoidosis, chronic disseminated histoplasmosis, or tuberculosis.

DIAGNOSTIC PROCEDURES

Initial Laboratory Studies

Routine laboratory studies can provide important clues to diagnosis. Pancytopenia suggests miliary tuberculosis, disseminated histoplasmosis, or a myelophthisic disorder, such as metastatic tumor or lymphoma involving the bone marrow. Isolated anemia is commonly associated with chronic pneumonia but is a nonspecific finding. A normal leukocyte count does not exclude infection. In particular, chronic fungal pneumonia may be associated with a normal or minimally elevated leukocyte count. Leukopenia or lymphopenia should raise the suspicion of an HIV infection. In addition, leukopenia is consistent with a diagnosis of sarcoidosis, systemic lupus erythematosus, tuberculosis, histoplasmosis, or neoplasia. A leukemoid reaction is nonspecific and may be seen in conjunction with almost any infectious cause of chronic pneumonia. Leukocytosis with polymorphonuclear cell predominance is suggestive of, but not specific for, a bacterial cause, including actinomycosis, but is also seen in granulomatosis with polyangiitis.

Routine laboratory tests that measure the function of other organs may provide more helpful information. Liver function studies, including bilirubin, alkaline phosphatase, and serum aspartate aminotransferase determinations and prothrombin time, should be performed for most patients. Urinalysis, with particular attention to the urinary sediment, plus tests of renal function, including measurement of blood urea nitrogen and creatinine, should also be done. Abnormalities of liver function (especially elevated enzyme levels), kidney function, or both should raise the suspicion of disorders that are not limited to the lung but are known to involve multiple other organs, including the liver and kidney. Such disorders include disseminated histoplasmosis and disseminated mycobacteriosis, as well as the vasculitides, sarcoidosis, and certain neoplastic diseases, especially the lymphoproliferative disorders.

In a patient with an abnormally low serum globulin level, a quantitative serum immunoglobulin determination should be obtained to evaluate for common variable immunodeficiency disorder or other disorders associated with hypogammaglobulinemia. Studies that should be performed in patients with suspected vasculitis include serologic tests for antinuclear antibodies, rheumatoid factor, cytoplasmic antineutrophil cytoplasmic autoantibodies (C-ANCAs), C-reactive protein, and erythrocyte sedimentation rate. In addition, measurement of serum angiotensin-converting enzyme may be useful, although it is a nonspecific test for which levels are increased in patients with a number of granulomatous disorders, including 30% to 80% of patients with sarcoidosis.[28,29]

Additional Studies

Basic core studies should be performed on all patients with chronic pneumonia, regardless of the suspected cause, but there should be flexibility in choosing additional tests or procedures to confirm a specific diagnosis. The orderly sequence of diagnostic studies described in the following sections necessarily results in oversimplification and consequently overlooks the unique aspects of a given patient's illness.

Chest Radiographic Studies

The chest radiograph, including posteroanterior and lateral films, is a reasonable screening procedure, but high-resolution CT[136] provides invaluable information. On occasion, magnetic resonance imaging is helpful, particularly in the evaluation of the noninfectious causes of chronic pneumonia. Positron emission tomography (PET) scanning (fluorodeoxyglucose PET), which measures metabolism by glucose uptake, has often proved disappointing in distinguishing malignant from infectious lung lesions. In Table 70.2, disorders are grouped according to the type of radiologic abnormality that is characteristic of the disease. In some disorders there is a spectrum of radiologic manifestations, and these disorders appear more than once in the table. Typical radiographic findings may provide clues to specific diagnoses. For

TABLE 70.2 Radiologic Patterns of Diseases: Common Causes of Chronic Pneumonia.

PATTERN OF DISEASE	FEATURES
Diseases That Cause Patchy Infiltrates and/or Bronchopneumonia or Lobar Consolidation	
Infectious Processes	
Aspiration pneumonia secondary to mixed aerobic and anaerobic infection	Usually dependent portions; superior or basilar segments of lower lobes, or posterior segments of upper lobes; pleural involvement with empyema common
Necrotizing pneumonia secondary to infection by Enterobacteriaceae, *Pseudomonas aeruginosa, Staphylococcus aureus,* or *Nocardia*	Any lobe or segment
Actinomycosis	Commonly involves lower lobes; cavitation frequently present; pleural involvement with empyema common, may extend into chest wall
Tuberculous exudative pneumonia	Not restricted to upper lobes; often bilateral, with perihilar distribution
Blastomycosis	Often a dense area of lobar or segmental consolidation; cavities may form; calcification and pleural disease infrequent
Cryptococcosis	Single or multiple infiltrates, often with sharp border, occasionally cavitary, rarely calcified; pleural effusions
Paracoccidioidomycosis	Asymptomatic bilateral fluffy infiltrates; may be extremely indolent and asymptomatic at presentation
Pneumocystis jirovecii	In addition to causing an acute diffuse pneumonia, *Pneumocystis* pneumonia may appear as dense infiltrates or nodules with granulomas resembling histoplasmosis on histopathology
Noninfectious Processes	
Chronic eosinophilic pneumonia	Rapidly progressive, dense infiltrates; usually peripheral (pattern is the reverse of pulmonary edema)
Bronchiolitis obliterans organizing pneumonia	Patchy nonsegmental areas of consolidation, often subpleural and bilateral; large irregular nodules
Diseases That Cause Pulmonary Cavitation	
Infectious Processes	
Pyogenic lung abscess complicating aspiration pneumonia	Usually single cavity; location same as aspiration pneumonia; air-fluid level common
Complicated necrotizing pneumonia	May involve any lobe; often multiple and bilateral, depending on route of acquisition of pneumonia
Tuberculosis-reactivation or adult type	Usually upper lobes; often bilateral; may be multiple; fibrosis and calcification common
Atypical mycobacterial disease	Radiologically indistinguishable from tuberculosis, except that cavitation may be more frequent
Melioidosis	May be acute or chronic and involve any lobe
Rhodococcal pneumonia	Simulates tuberculosis or nocardiosis; cavitation common
Histoplasmosis, chronic cavitary	Mimics tuberculosis; upper lobes frequently involved but any lobe can be involved; unilateral or bilateral
Coccidioidomycosis	Usually a single, thin-walled cavity with minimal involvement of surrounding lung; occasionally a thick-walled cavity surrounded by extensive parenchymal disease
Sporotrichosis	May mimic tuberculosis but can involve any lobe; cavitation is frequent
Aspergillosis	Single or multiple areas of pneumonia with or without central cavitation; not to be confused with pulmonary mycetoma ('fungus ball')
Paragonimiasis	Cystlike lesions and cavities, usually associated with linear or patchy infiltrates, fibrosis, and/or calcification
Echinococcosis	Single or multiple discrete, sharply defined, round lesions (cysts) with little surrounding inflammatory response; cavitation and/or calcification may occur
Noninfectious Processes	
Granulomatosis with polyangiitis and lymphomatoid granulomatosis	Often, multiple nodules with cavitation; may be unilateral or bilateral granulomatosis
Silicosis	Associated with conglomerate nodular densities, frequently in upper lobes; usually superimposed on background of diffuse nodulation; rarely, eggshell calcification of hilar nodes
Bronchogenic carcinoma	Thick-walled cavitation more common in squamous cell type
Lymphoma, especially Hodgkin's disease	Cavitation may occur in peripheral parenchymal nodules
Kaposi sarcoma	Small or large nodules associated with peribronchial cuffing and "tram track" opacities
Diseases That Cause One or More Dense, Well-Circumscribed Nodules	
Dirofilariasis (usually single)	—
Histoplasmosis (histoplasmoma)	May have calcification in center or in hilar nodes
Coccidioides spp. (coccidioidoma)	May have calcification in center or in hilar nodes
Cryptococcosis (cryptococcoma)	Often multiple, cavitation occasionally, calcification rare
Tuberculosis (tuberculoma)	May have calcification in center or in hilar nodes
Malignancy	—

TABLE 70.2 Radiologic Patterns of Diseases: Common Causes of Chronic Pneumonia.—cont'd

PATTERN OF DISEASE	FEATURES
Infectious and Noninfectious Diseases That Cause Chronic Diffuse Pulmonary Infiltration and Fibrosis	
Alveolar Pattern	
Bronchioloalveolar carcinoma	
Intrapulmonary bleeding (e.g., Goodpasture syndrome, diffuse alveolar hemorrhage)	
Pulmonary alveolar proteinosis	
Ground-Glass Pattern	
Sarcoidosis	
Early asbestosis or berylliosis	
Mycobacterium avium complex "hot tub lung" (hypersensitivity)	
Chronic organizing pneumonia (formerly bronchiolitis obliterans organizing pneumonia)	
Nodular Interstitial Pattern, Including Miliary Spherical Nodules	
Granulomatous infectious diseases (e.g., miliary tuberculosis, disseminated histoplasmosis)	
Sarcoidosis	
Lymphangitic carcinomatosis	
Granulomatosis and polyangiitis	
Lymphomatoid granulomatosis	
Allergic angiitis and granulomatosis	
Rheumatoid lung disease	
Pneumoconiosis (including asbestosis, silicosis, and berylliosis)	
Linear Interstitial Pattern, Including Fine Reticular Markings and Dense Fibrosis	
Chronic form of hypersensitivity pneumonitis	
Idiopathic pulmonary hemosiderosis	
Radiation injury—chronic	
Progressive systemic sclerosis	
Sarcoidosis	
Rheumatoid arthritis	
Idiopathic pulmonary fibrosis	
Honeycombing (Coarse Reticular Pattern With Cystic Air Spaces)	
Advanced form of fibrosing alveolitis	
Bronchiectasis	
Eosinophilic granuloma (pulmonary Langerhans cell histiocytosis X)	
Sarcoidosis	
Idiopathic pulmonary interstitial pneumonia	

example, demonstration of anterior mediastinal involvement argues strongly in favor of neoplasia, including lymphoma, thymoma, and metastatic carcinoma, as the cause of chronic pneumonia syndrome and argues against an infectious cause.

Tuberculosis and nontuberculous mycobacterial diseases, histoplasmosis, coccidioidomycosis, sporotrichosis, paragonimiasis, and the pneumoconioses, especially silicosis, are characteristically associated with fibrocavitary disease—a contracted area of lung with linear fibrosis, nodular or rounded densities, and cavitation.[137] In addition, tuberculosis, chronic fibrocavitary histoplasmosis, pulmonary mycetoma or "fungus ball," and silicosis characteristically involve the upper lobes. Many experts believe that anterior segment upper lobe involvement argues strongly against tuberculosis as a cause. A thin-walled parenchymal cavity is suggestive of coccidioidomycosis, sporotrichosis, or paragonimiasis, whereas a thick-walled cavity surrounded by an area of pneumonitis is more typical of tuberculosis, other mycobacterial infections, histoplasmosis, aspergillosis, melioidosis, nocardiosis, actinomycosis, pyogenic lung abscess, lung disease caused by *R. equi,* and squamous cell carcinoma. The presence of the "halo sign," a ring of enhancement surrounding a cavitary pulmonary lesion on chest CT in a severely immunocompromised patient, suggests invasive mold infection, especially invasive aspergillosis or mucormycosis.[138,139] The "reverse halo sign" may also be useful in this group and suggests pneumonia due to Mucorales.[140,141] Cavitation is seen but is less common in blastomycosis and cryptococcosis. Mediastinal and/or hilar lymph node calcification and occasionally parenchymal calcification are typical of tuberculosis, histoplasmosis, and coccidioidomycosis but are rare in actinomycosis, nocardiosis, blastomycosis, and cryptococcosis. Abscess of the chest wall or osteomyelitis of a rib adjacent to the pneumonia or pleural effusion (empyema necessitans) may be seen in actinomycosis, nocardiosis, blastomycosis, and tuberculosis. Although these radiographic manifestations of selected pulmonary diseases are typical in most patients, experience during the AIDS pandemic has shown that pulmonary diseases in these patients may be highly atypical in radiographic appearance and clinical course. Representative routine chest radiographs and chest CT images are shown in Figs. 70.1 through 70.15.

Patients With Radiographic Evidence of Localized Infiltrates or Cavitation

In all patients with radiographic evidence of localized infiltrates or cavitation in the setting of chronic pneumonia, examination of the sputum is essential. This is in striking contrast to the questionable value of sputum in the setting of acute community-acquired pneumonia.[142–144] The specimen of sputum must be a representative sample (i.e., a deep, coughed specimen). If the expectorated sputum is of adequate volume and is acceptable after cytologic screening, other procedures to obtain sputum may not be necessary. Microscopic examination of sputum may include the following:

1. Gram staining for bacteria and actinomycetes
2. Acid-fast staining for mycobacteria and modified acid-fast staining for *Nocardia*
3. Wet mount for fungi and eggs of *Paragonimus* (calcofluor white or potassium hydroxide preparation with phase contrast may enhance detection of fungi)
4. Cytologic preparations for neoplastic cells, eosinophils, and fungi

Generous volumes of expectorated sputum should also be sent to the microbiology laboratory for culture of bacteria, fungi, and mycobacteria. In addition, contacting the microbiology laboratory personnel directly to alert them to specific etiologic considerations is often helpful in confirming a suspected diagnosis. In this way, specimens can be inoculated on the most appropriate media, and the microbiologists can be made more aware of the likely pathogens.

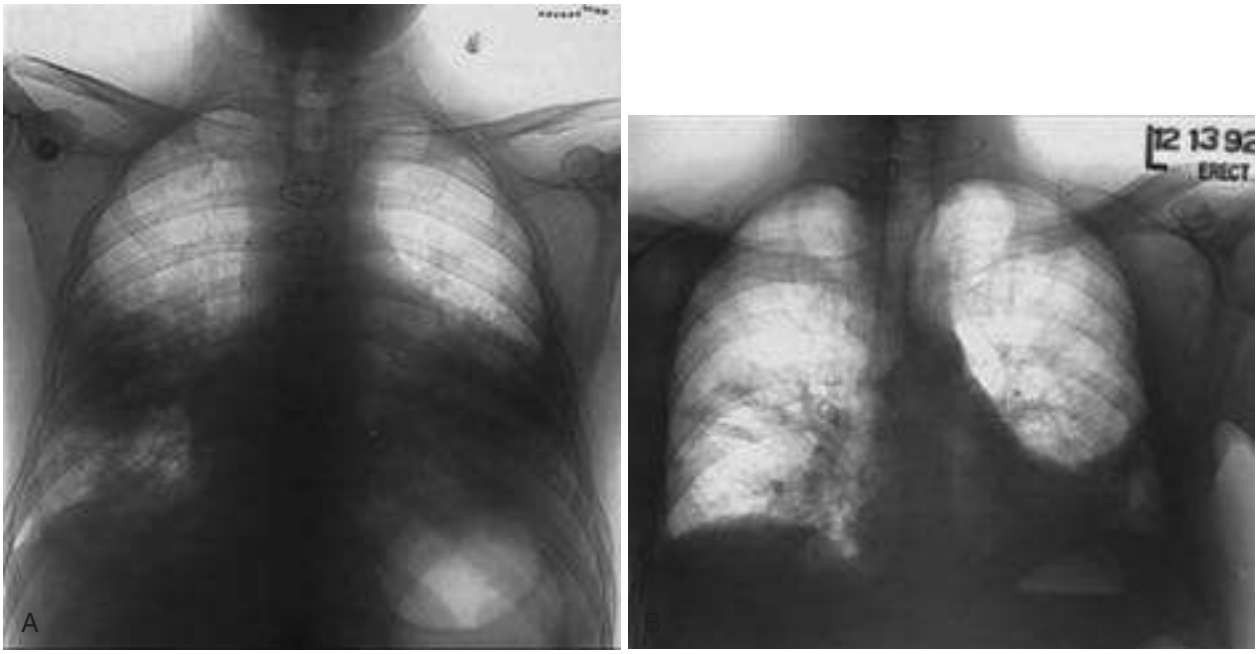

FIG. 70.1 (A) Chronic organizing pneumonia (COP) in a 32-year-old man who developed progressive chronic pneumonia several weeks after an open pericardiectomy to relieve cardiac tamponade associated with renal failure. Note the bilateral airspace consolidation. Bronchoscopy with transbronchial biopsy was nondiagnostic. The diagnosis of COP was established from tissue obtained at open lung biopsy. Corticosteroid therapy was dramatically beneficial. (B) COP in a 60-year-old man with a 1-month illness manifested by fever, malaise, weight loss, nonproductive cough, dyspnea, and bilateral subpleural nodular opacities. Both symptoms and radiographic abnormalities progressed during antibiotic therapy. A bronchoscopy with transthoracic biopsy was nondiagnostic. The diagnosis of COP was made from tissue obtained at open lung biopsy. Corticosteroid therapy was beneficial.

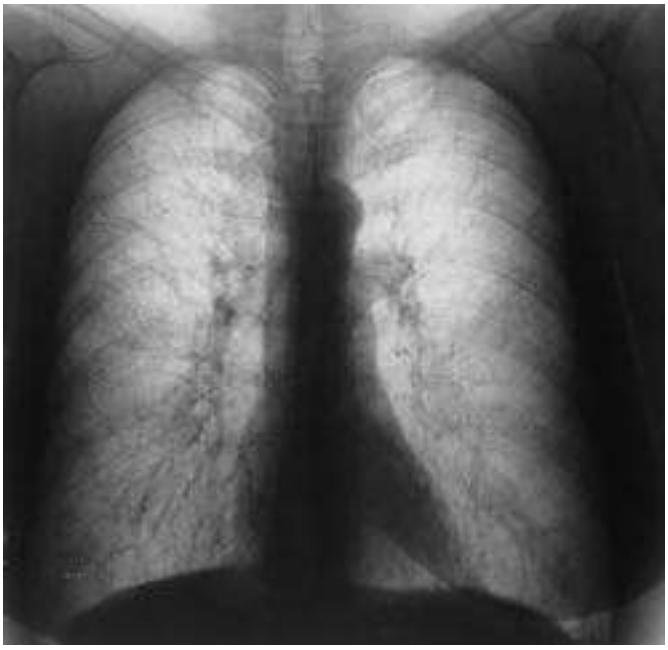

FIG. 70.2 **Lymphangioleiomyomatosis.** This 42-year-old woman had a 6-month history of progressive dyspnea and intermittent blood-streaked sputum. She denied fever or history of pneumothorax. Note the diffuse reticular pattern with areas of cystic dilatation and enlarged lung volumes. A pathologic diagnosis was made from lung tissue obtained by transbronchial biopsy.



FIG. 70.3 **Atypical tuberculosis caused by *Mycobacterium avium* complex.** This 52-year-old man had chronic obstructive pulmonary disease. He had an 18-month history of fever, weight loss, cough, intermittent hemoptysis, and progressive dyspnea associated with persistently positive acid-fast smears and cultures of expectorated sputum and worsening chest films despite appropriate antimycobacterial therapy. Note the bilateral lower lobe fibronodular disease, worse in the right lung, associated with cavitation.

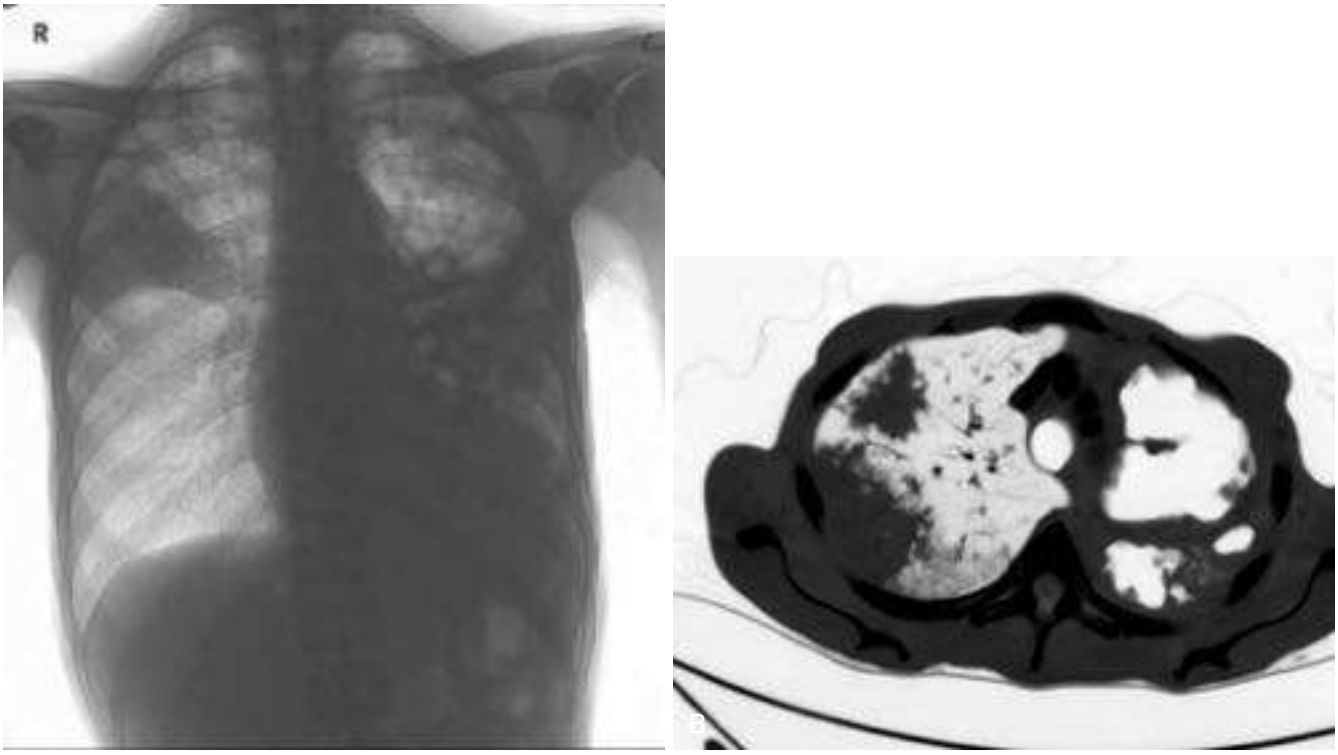

FIG. 70.4 (A) Pulmonary tuberculosis in a 45-year-old man with a 6-month history of cough, hemoptysis, fever, and a 35-lb weight loss. He was HIV negative. Sputum smears were positive for acid-fast bacilli, and cultures were positive for *Mycobacterium tuberculosis*. (B) Note bilateral parenchymal disease and near-total destruction of the left lung.

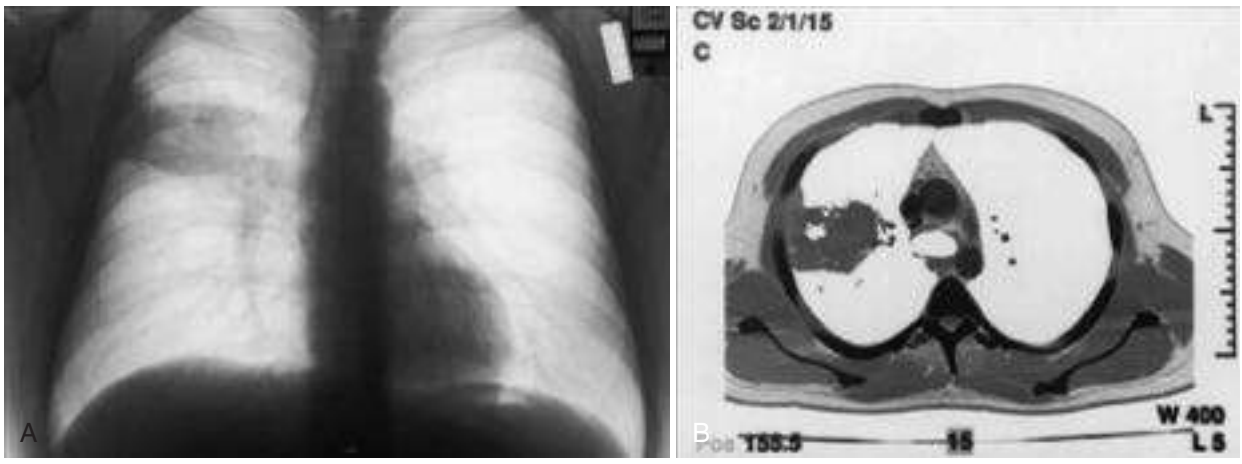

FIG. 70.5 (A) Pulmonary blastomycosis in a 24-year-old man who worked as a heavy equipment operator. He was also an avid hunter. He reported fever, night sweats, and a productive cough for 6 weeks unresponsive to outpatient antibacterial therapy. He had lost 25 lb during this time. He denied skin lesions or bone pain. Note the right upper lobe infiltrate on plain chest film. (B) Chest computed tomography scan shows a dense right upper lobe infiltrate with a central cavity not seen on plain films. Diagnosis was established by video-assisted thoracoscopy biopsy. Histopathology revealed broad-based budding yeasts, and cultures were positive for *Blastomyces dermatitidis*.

With appropriate communication, newer diagnostic techniques (e.g., rapid culture techniques, molecular probes, polymerase chain reaction [PCR], antigen detection assays, enzyme immunoassay [EIA]) can be used in a rational and thoughtful manner to facilitate laboratory diagnosis.[145–151] In particular, serum or urine antigen assays for the diagnoses of cryptococcosis, histoplasmosis, coccidioidomycosis, and blastomycosis are useful.[145–150] Nucleic acid–based tests for detection of *M. tuberculosis* are commercially available and diagnostically helpful (see Chapter 249).

When an infectious cause is being considered, cultures from other appropriate sources should be obtained. These may include the following: blood, urine, and pleural fluid from all patients with pleural effusion (pleural tissue should also be obtained for culture); CSF from all patients with CNS symptoms or signs; synovial fluid from all patients with joint effusion; and samples of skin, subcutaneous aspirate, mucous membrane, or any tissue obtained at biopsy.

If adequate sputum cannot be readily produced via spontaneous expectoration by the patient, consider these methods: (1) sputum induction by hypertonic aerosol and ultrasonic nebulization, hydration, chest physiotherapy, or postural drainage and (2) bronchoscopy for bronchial brushing, transbronchial biopsy, bronchoalveolar lavage (BAL), or protected specimen brush sampling of lower respiratory tract secretions.

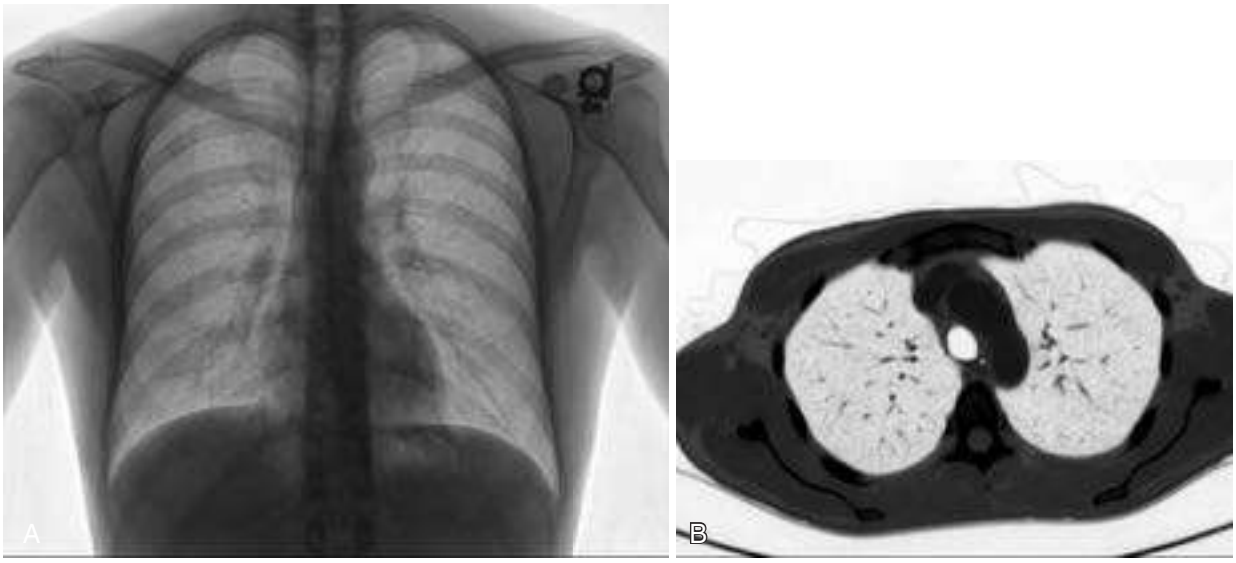

FIG. 70.6 (A) 68-year-old with fever, cough, and 20-lb weight loss over the past 6 months. Routine chest radiograph reveals a classic miliary pattern. Transbronchial biopsy revealed granulomata without organisms; cultures were positive for *Mycobacterium tuberculosis*. (B) Chest computed tomography on this patient reveals diffuse interstitial nodular findings consistent with miliary tuberculosis.

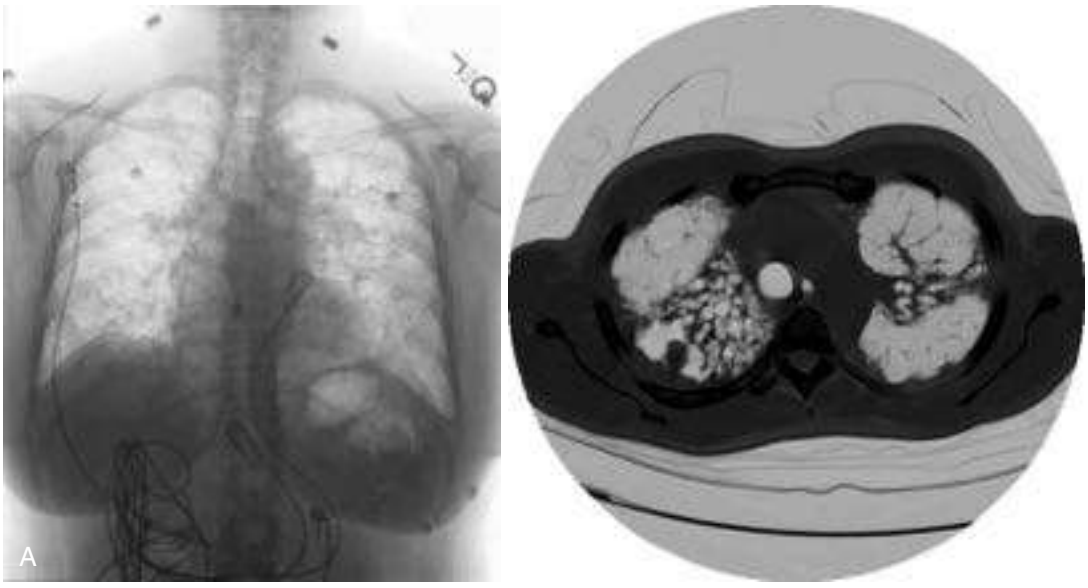

FIG. 70.7 (A) This 45-year-old woman with long-standing pulmonary sarcoidosis has had progressive exertional dyspnea and occasional cough productive of scant hemoptysis. She has received intermittent oral glucocorticosteroids with symptomatic relief but insidious disease progression. (B) A computed tomography scan of this patient reveals extensive fibrocavitary disease with honeycombing and bronchiectasis. There is a pulmonary aspergilloma (mycetoma) in the superior segment of the right lower lobe, noted to be the likely source of hemoptysis.

Quantitative PCR for *P. jirovecii* on BAL specimens is more sensitive than direct fluorescent microscopy, but low-level positivity occurs in patients who are pulmonary carriers (see Chapter 269). PCR on BAL specimens for *Aspergillus* have been used in some centers, largely in Europe, as one of the many factors considered in the diagnosis of invasive pulmonary aspergillosis, along with BAL galactomannan (GM). Standardization of the technique across centers is in progress.[152] Commercially available PCR kits for *Aspergillus* are available in some overseas countries.

Skin tests are appropriate when certain infectious causes are being considered. The tuberculin skin test with purified protein derivative and the interferon-γ release assays on blood are commonly used to detect exposure, although seriously ill patients may be anergic. Skin test antigens for the detection of infection with NTM and sporotrichosis are not commercially available. Skin tests of the tuberculin type are no longer commercially available for patients with suspected histoplasmosis. Coccidioidin is also no longer available, but the spherusol skin test has been US Food and Drug Administration approved for detecting exposure to *Coccidioides immitis*.

Serologic tests for HIV should be performed for all patients with unexplained chronic pneumonia. In addition, serologic tests may be helpful when other infectious causes, especially fungi, are considered. However, there may be problems with some of these tests, including delays in obtaining results and limited sensitivity and specificity. The lateral flow assay (LFA) for *Coccidioides* is a rapid new test that can screen for any form of coccidioidomycosis, but a positive result should

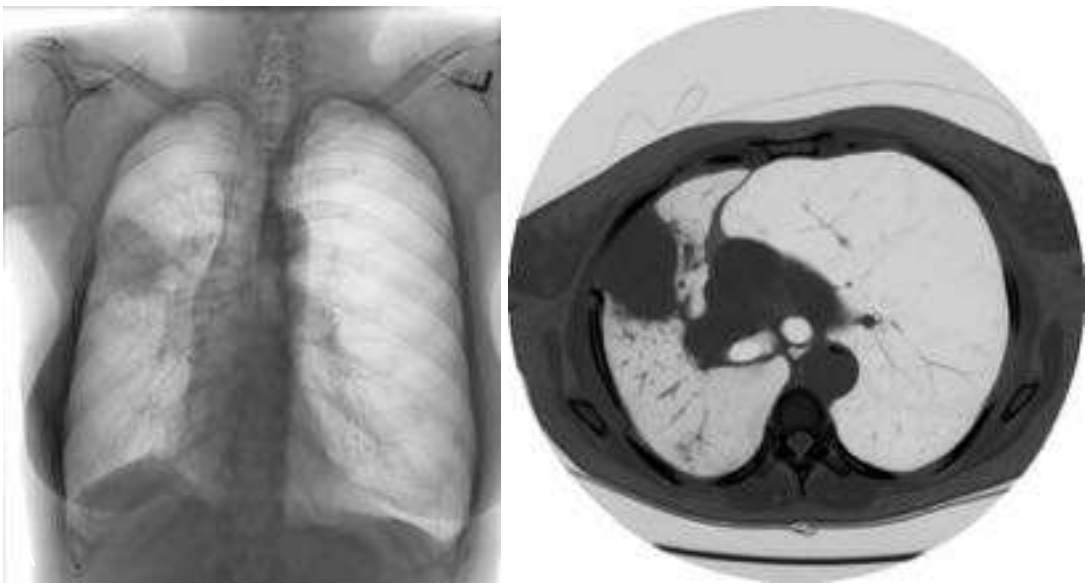

FIG. 70.8 (A) This 64-year-old black female who underwent single-lung transplantation for chronic obstructive pulmonary disease presented with fever and cough for 10 days. The chest radiograph revealed a dense and well-circumscribed nodular infiltrate in the right midlung zone. Blood and bronchoalveolar lavage cultures were positive for *Rhodococcus equi*. (B) A computed tomography scan of the same patient reveals a pleural-based, large, masslike lesion without cavitation in the transplanted lung.

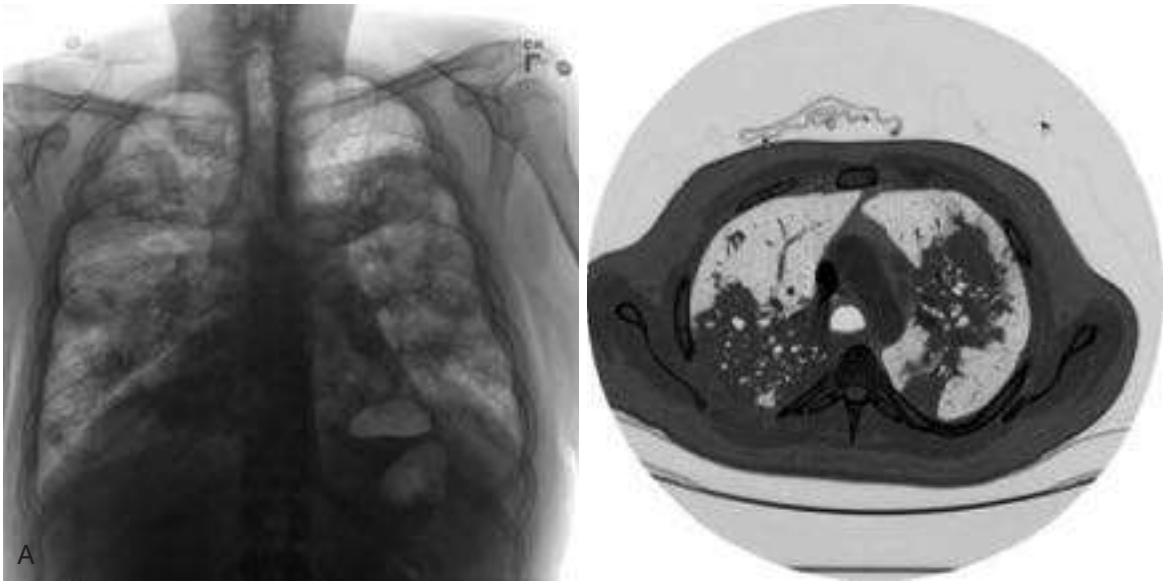

FIG. 70.9 (A) This 38-year-old white male landscaper with poorly controlled diabetes had progressive dyspnea, cough productive of yellowish sputum, fever, and weight loss over 2 months. His chest radiograph on presentation reveals diffuse infiltrates of both lungs with a somewhat nodular character. Sputum and bronchoalveolar lavage (BAL) fungal cultures were positive for *Blastomyces dermatitidis.* (B) A computed tomography scan obtained of the same patient reveals bilateral, dense, multilobar infiltrates with small areas of cavitation. A diagnosis of blastomycosis was initially suspected on the basis of finding broad-based budding yeasts on special stains of BAL fluid.

be confirmed with a more traditional EIA complement fixation or immunodiffusion assay for antibody to *Coccidioides* spp. These assays are especially helpful in patients with disseminated or fibrocavitary coccidioidomycosis but are usually not above background positivity in patients with a solitary pulmonary cavity (see Chapter 265). Serum cryptococcal antigen may be detectable in greater than 50% of nonimmunocompromised patients with pulmonary cryptococcosis; a greater proportion of patients with extrapulmonary disease will have positive serum cryptococcal antigen.[69] The availability of the LFA for cryptococcal antigen should facilitate the performance of this test in resource-poor environments.[153] This new assay is easily performed in 15 minutes, and it is generally more sensitive than the more cumbersome latex agglutination and EIA assays.[153] *Histoplasma* antigen in serum or urine is helpful in disseminated histoplasmosis but less commonly positive in infection confined to the lung, especially in nonimmunocompromised patients. Sensitivity depends on the laboratory performing the test. There is no standardization between laboratories. Serologic tests for paracoccidioidomycosis are available in the endemic area and useful. The serum and BAL EIA GM assay for early diagnosis of acute invasive aspergillosis is useful in hematologic malignancy and stem cell transplantation patients, but its utility in other patient populations is poorly established.[152,154–156] Among patients with suspected tuberculosis but with negative culture

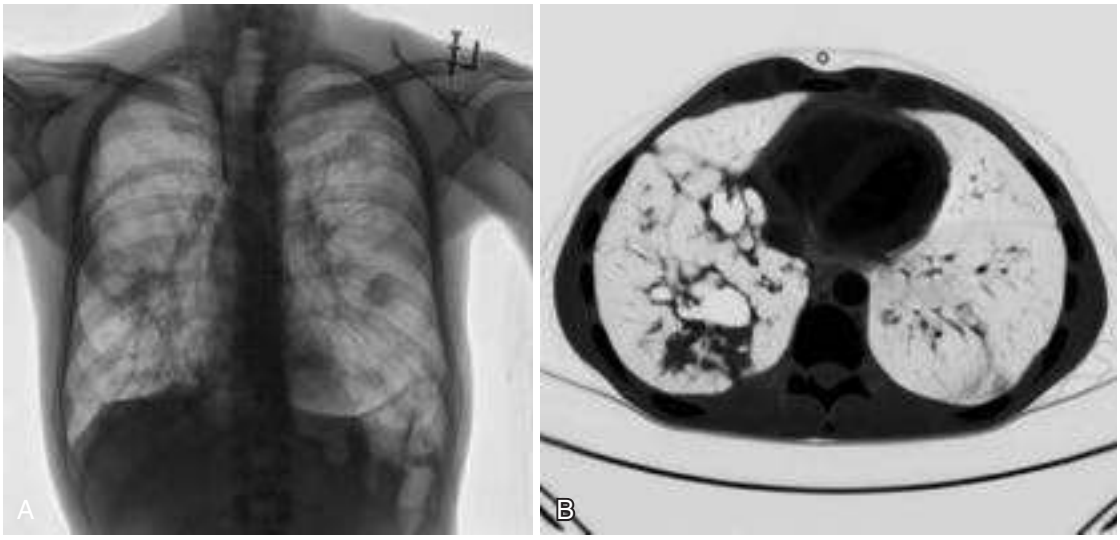

FIG. 70.10 (A) This 68-year-old white male with acute myelogenous leukemia had recently undergone myeloablative chemotherapy as part of his induction course. He experienced profound and prolonged neutropenia, and on the 21st day of fever and neutropenia he complained of cough and scant hemoptysis. A routine chest radiograph revealed multiple nodular lesions in both lungs, some with cavitation. A diagnosis of invasive aspergillosis was suspected, and a serum and bronchoalveolar lavage galactomannan were both positive (1.1 and 2.3, respectively), thus establishing a diagnosis of probable invasive pulmonary aspergillosis. (B) A computed tomography scan from the same patient reveals multiple cavitary lesions from the right lung consistent with invasive pulmonary aspergillosis. Extensive cavitation such as shown is not usually observed until neutropenia has improved or immunosuppression has lessened.

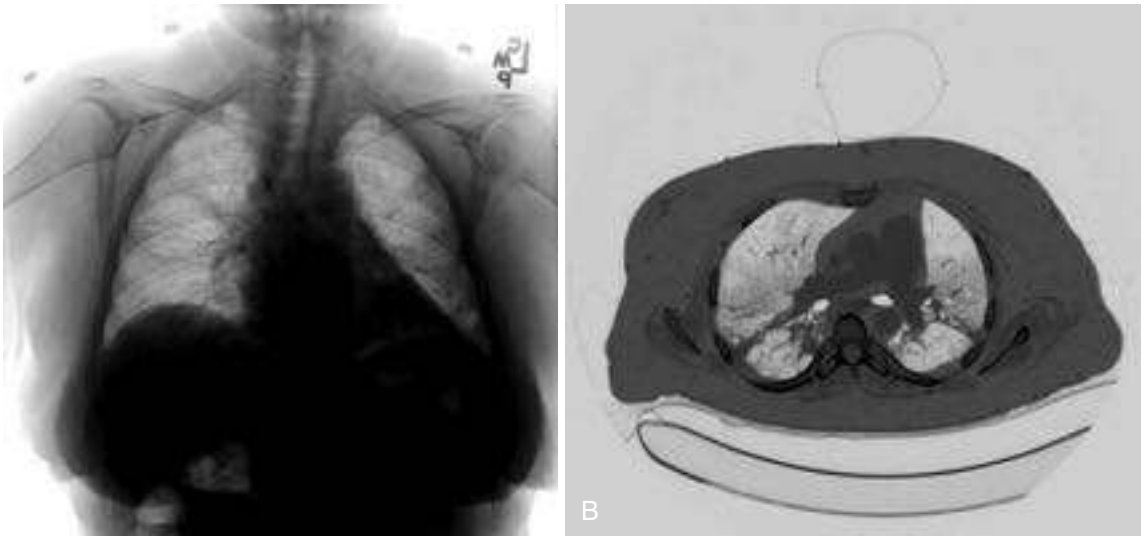

FIG. 70.11 (A) This 32-year-old white female is status postrenal transplant approximately 10 years ago and now presents with progressive, nonproductive cough and low-grade fever over the past 2 months. A routine chest radiograph reveals diffuse interstitial infiltrates without pulmonary nodules or a focal pulmonary infiltrate. (B) A computed tomography scan obtained on the same patient reveals diffuse interstitial infiltrates bilaterally with the "crazy-paving" pattern typical of pulmonary alveolar proteinosis. Diagnosis was confirmed by bronchoscopy with the finding of copious milky-white material on bronchoalveolar lavage. At that time she was also found to have a concomitant *Mycobacterium avium* complex pulmonary infection.

and histopathologic studies, the quantiFERON-Gold (Qiagen; Germantown, MD) in-tube test or the T-Spot TB assay may be a useful alternative to skin testing for detecting exposure to tuberculosis.[157–159] If hypersensitivity pneumonitis is suspected, serum can be examined for precipitating antibodies to various inhalant antigens and, if allergic bronchopulmonary aspergillosis is suspected, total serum immunoglobulin (Ig)E and serum IgG anti-*Aspergillus* antibody levels can be measured. Finally, serologic tests for rare causes of chronic pneumonia, such as *Legionella* spp., *Chlamydia pneumoniae,* and *Coxiella burnetii,* should be considered for appropriate patients. Multiplex PCR panels for respiratory samples are able to detect a variety of microbial agents but are most useful in acute, community-acquired pneumonia. Urine antigen testing for *Legionella* also should be considered in pneumonia of more acute onset.

Invasive Procedures

Certain clinical situations dictate a more aggressive diagnostic approach. In patients who are unable to raise sputum spontaneously and in whom attempts to induce sputum production are unsuccessful, invasive procedures may be necessary. Fiberoptic bronchoscopy is usually the initial procedure. It is diagnostically most helpful when accompanied by BAL and transbronchial biopsy, with appropriate microbiologic and histologic studies. Analysis of BAL fluid may increase the diagnostic yield of bronchoscopy, especially in immunocompromised persons, such as patients with AIDS and suspected opportunistic infections or patients with suspected noninfectious causes of chronic pneumonia. Continued bloody return on BAL confirms the diagnosis of diffuse alveolar hemorrhage in patients after allogeneic hematopoietic stem cell

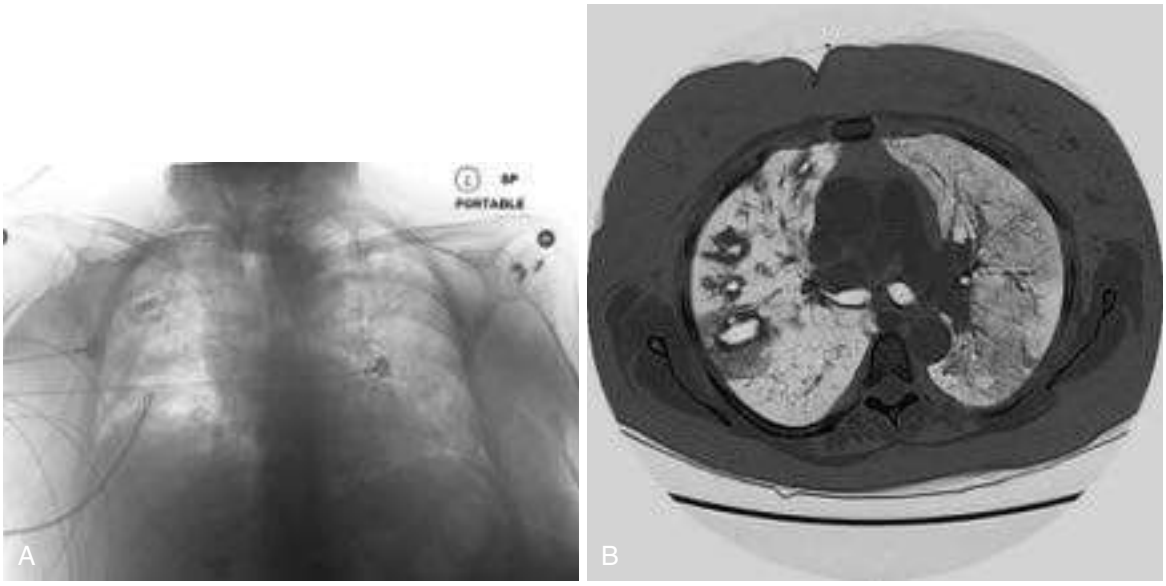

FIG. 70.12 (A) A 68-year-old former nurse presented with fever and cough productive of yellowish, blood-tinged sputum for 2 weeks. She is a smoker with chronic obstructive pulmonary disease and asthma. She received intermittent glucocorticosteroids for asthma exacerbations. Methicillin-susceptible *Staphylococcus aureus* (MSSA) was recovered from sputum, and a bronchoalveolar lavage revealed 4+ gram-positive cocci in clusters among sheets of neutrophils. Cultures were positive for MSSA. (B) A computed tomography scan from the same patient reveals bilateral alveolar infiltrates with well-circumscribed cavitary lesions consistent with the diagnosis of community-acquired MSSA pneumonia.

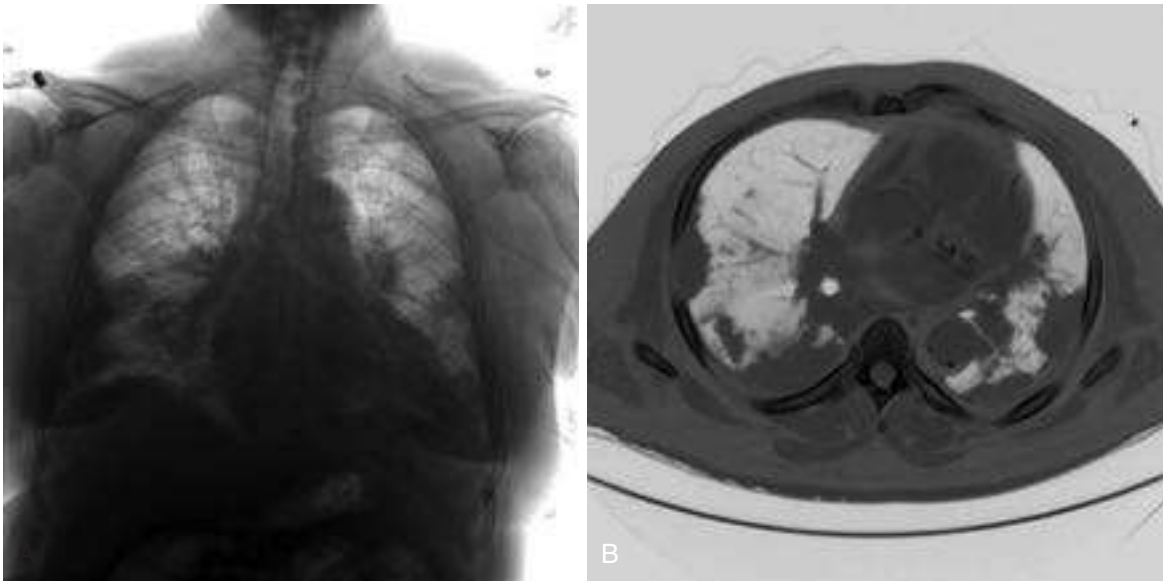

FIG. 70.13 (A) This 71-year-old black male with history of a renal transplant approximately 5 years ago presents with fever, cough, exertional dyspnea, and mild headache over the past 4 weeks. He is stable on his current immunosuppressant regimen. He works as a dairy farmer. Bronchoalveolar lavage and blood cultures at baseline were positive for *Cryptococcus neoformans.* Note the bilateral diffuse nodular lesions. Cerebrospinal fluid cultures and cryptococcal antigen assay were negative. (B) A computed tomography scan from the patient reveals multiple, bilateral parenchymal and pleural-based, noncavitary pulmonary nodules, consistent with a diagnosis of extensive pulmonary cryptococcosis.

transplantation.[160] Transbronchial biopsy can be especially helpful in patients with diffuse pulmonary infiltrates. In a patient with extensive pleural involvement, thoracentesis and pleural biopsy (or rigid thoracoscopy in selected situations) may be more helpful diagnostically than bronchoscopy. In some institutions, open lung biopsy is the procedure of choice for patients with interstitial lung disease and for immunosuppressed patients with unexplained pulmonary disease because of the large sample size, expediency of diagnosis, and safety of the procedure.[161–163] In contrast, in other institutions with experienced operators, CT-guided transthoracic fine-needle aspiration of solid lesions in the lung, particularly those near the pleura, can be diagnostic.[164,165] In patients with adequate ventilatory reserve, video-assisted thoracoscopic surgery (VATS)[166] is preferred to open lung biopsy and is associated with a low risk of complications and high sensitivity. All tissue specimens, regardless of source, should be submitted for the Gram stain, special stains for acid-fast bacilli and fungi, and culture. In patients with extrapulmonary disease, specimens from the extrapulmonary site(s) should be obtained for culture and histologic studies. In such patients the results from a diagnostic arthrocentesis, abdominal paracentesis, lumbar puncture, bone marrow biopsy, liver biopsy, lymph node biopsy, or skin biopsy may preclude the necessity of pursuing more invasive techniques.

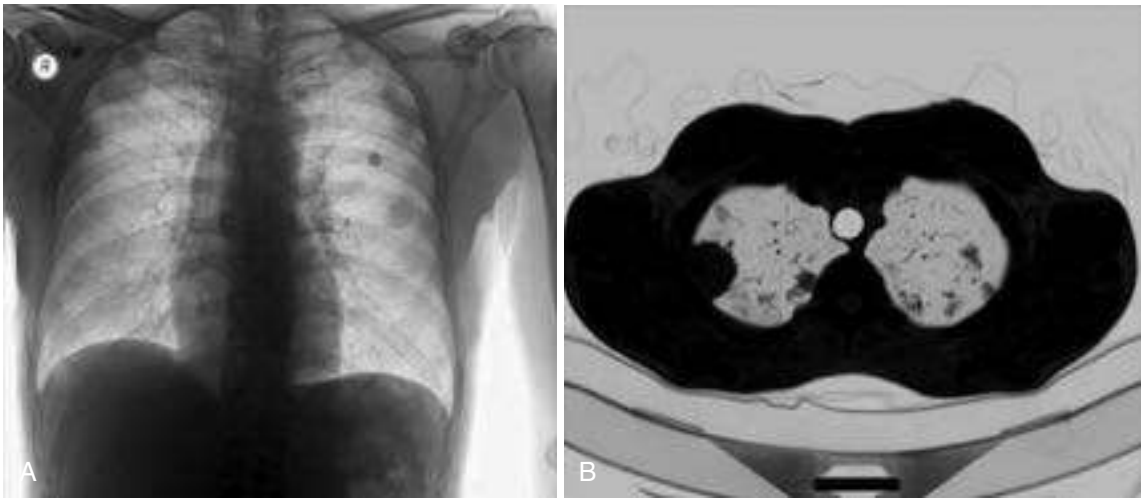

FIG. 70.14 (A) This 48-year-old white male with fever, nonproductive cough, and frontal headache has an extensive travel history, including military service in Southeast Asia, the Philippines, Australia, and California. Chest radiograph reveals multiple bilateral, nodular pulmonary lesions. Both serum and cerebrospinal fluid (CSF) and cryptococcal antigen assay were positive, and CSF eventually revealed *Cryptococcus gattii*. (B) A computed tomography scan of the chest in the same patient reveals multiple parenchymal, poorly defined nodules and one large pleural-based nodule typical for *C. gattii* pulmonary infection.

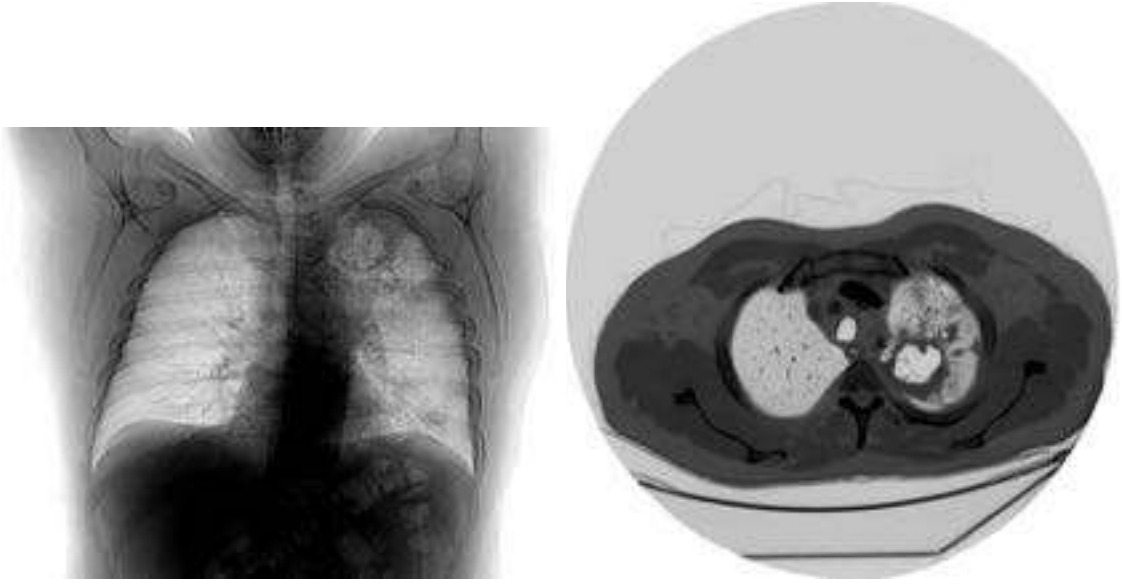

FIG. 70.15 (A) This 59-year-old white male has a recent diagnosis of small cell carcinoma upper lung involving the right lung and mediastinum. He is also noted to have a cavitary lesion and cough productive of yellowish sputum progressive over the past 6 months. He lives in southern Texas near the Mexican border. Serology was positive for *Coccidioides,* and the organism was isolated from multiple expectorated sputum samples. (B) A computed tomography scan from the same patient reveals a large cavitary lesion in the left upper lobe, with some surrounding parenchymal infiltrates consistent with a diagnosis of chronic fibrocavitary pulmonary coccidioidomycosis.

Patients With Radiographic Evidence of Diffuse Pulmonary Infiltration and Fibrosis

In patients whose chest radiographs show a predominantly diffuse infiltrative pattern of either the alveolar or interstitial type (see Table 70.2), pulmonary function studies may be critically important. These studies not only quantify the degree of pulmonary insufficiency but may help delineate the disease processes by virtue of the patterns of pulmonary function impairment. Pulmonary function studies are particularly useful in characterizing those diseases that impair gas transfer and predispose to ventilation perfusion inequalities, such as sarcoidosis or other interstitial lung diseases.[28,29]

Studies that may be especially useful in this group of patients include the following:

1. Arterial blood gas studies and exercise oximetry
2. Tests of pulmonary function, including spirometric measurements, measurements of lung volume, and measurement of pulmonary diffusing capacity
3. Studies on sputum, as previously outlined (cytologic examination is especially important)
4. Lung biopsy—the procedure of choice to make an accurate morphologic diagnosis (fiberoptic bronchoscopy with transbronchial biopsy, VATS, or [uncommonly] conventional thoracotomy for open lung biopsy)

THERAPY

Antimicrobial Agents

Most patients with a chronic, indolent illness and who are clinically stable do not require immediate empirical therapy. A methodical and thorough diagnostic evaluation is the initial priority. In a patient with bilateral upper lobe cavitary disease in whom the initial microscopic examinations are nonrevealing, the leading considerations include tuberculosis, histoplasmosis, and, in the right setting, coccidioidomycosis. If such a patient has a positive tuberculin skin response and/or a positive quantiFERON-Gold assay, then tuberculosis should be presumed

until proven otherwise, and the patient should be kept in respiratory isolation until the diagnosis can be reasonably excluded. Disseminated tuberculosis should be strongly suspected in any patient with unexplained fever and a chest radiograph showing a nodular interstitial pattern; prompt institution of antituberculosis therapy may be lifesaving in this otherwise fatal condition. Similarly, empirical antifungal therapy, usually with an amphotericin B formulation, may be indicated in any immunocompromised patient with severe or rapidly progressing chronic pneumonia due to the high risk of death associated with these infections.

Corticosteroids

The use of glucocorticosteroids for the treatment of a patient with chronic pneumonia is controversial. If the cause of the illness is an infectious agent, particularly a bacterium or fungus, steroids are not routinely indicated. However, some experts advocate a short course of glucocorticosteroid therapy, along with antituberculosis therapy for patients with advanced pulmonary tuberculosis and severe inanition. In general, higher-dose corticosteroids (>20 mg methylprednisolone equivalent daily) are beneficial in chronic pneumonia from noninfectious causes, such as the vasculitides,[3–6] sarcoidosis,[28,29] chronic eosinophilic pneumonia,[2] radiation injury, chronic organizing pneumonia,[36–39] and many of the fibrotic lung diseases, including chronic hypersensitivity pneumonitis, along with avoidance of exposure to the offending antigen.[40] Other immunosuppressive drugs, such as cyclophosphamide and azathioprine, may also be effective for some patients, especially those with pulmonary vasculitis or granulomatosis with polyangiitis.

Bronchoscopy and Surgery

Bronchoscopy is frequently used as a therapeutic adjunct, especially for patients who have thick, tenacious secretions that cannot be raised by noninvasive techniques. In other patients, mucus plugs or foreign bodies may predispose to atelectasis and chronic pneumonia, and therapeutic bronchoscopy may be necessary to expand the atelectatic lung.

Surgery plays a limited role in the management of chronic pneumonia. Lobectomy or pneumonectomy should be considered in a patient with chronic destructive pneumonia, multiple macroabscesses or microabscesses involving an entire lobe or lung, and a ventilation-perfusion scan indicating nonfunction of the involved lung (e.g., pulmonary gangrene).[167] Thoracotomy may also be indicated to decorticate the pleura in patients with significant pleural reaction and resultant restrictive lung disease.

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The complete reference list is available online at Expert Consult.

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71 Cystic Fibrosis

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SHORT VIEW SUMMARY

Definition

- Cystic fibrosis (CF) is a genetically inherited autosomal-recessive disease resulting from mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), located on the long arm of chromosome 7. It is a multisystem disorder in which respiratory and gastrointestinal manifestations predominate.

Epidemiology

- CF is the most common genetically inherited orphan disease involving Caucasians. Current treatment strategies have led to improved clinical outcomes, in which the majority of CF patients now reach adulthood.

Microbiology

- Chronic airway infection and inflammation, most commonly associated with *Pseudomonas aeruginosa,* are strongly associated with increased morbidity and mortality. Many other pathogens, such as methicillin-resistant *Staphylococcus aureus, Burkholderia cepacia* complex, *Stenotrophomonas maltophilia,* and others, are also implicated in chronic progressive lung disease.

Diagnosis

- Newborn screening is now available in all 50 states.
- Measurement of immunoreactive trypsinogen and *CFTR* gene mutation analysis are the mainstays for newborn screening in CF. Positive screening is followed by sweat chloride testing.
- Acute pulmonary exacerbations are usually linked to progression of disease. Respiratory viruses may trigger pulmonary exacerbations.

Therapy

- Maintenance therapy for CF-related lung disease includes mucolytics, airway hydrating agents, chest physiotherapy, antiinflammatory drugs, and a combination of oral and inhaled antibiotics (see Table 71.1).
- Acute pulmonary exacerbation may be treated with oral and nebulized antibiotics as needed; more serious exacerbations will require a combination of intravenous antibiotics, intense airway clearance accompanying mucolytics and airway hydrating agents, and aggressive nutritional management while monitoring for complications.
- In addition to the US Food and Drug Administration–approved CFTR modulators—ivacaftor, lumacaftor-ivacaftor (Orkambi), and tezacaftor-ivacaftor (Symdeko)—for those with specific CFTR genotypes, new triple-combination small-molecule therapies will provide disease-modifying treatment to nearly 90% of CF patients.

Prevention

- Genetic counseling to help avoid offspring with CF and early diagnosis through newborn screening.
- Early eradication strategies may prevent chronic airway infection with PA and other pathogens associated with chronic progressive lung disease.

Chronic airway infection and inflammation remains a major contributor to progressive pulmonary disease in patients with cystic fibrosis (CF) and is strongly associated with increased morbidity and mortality. Better understanding of the underlying pathophysiology, role of early intervention, improved identification and treatment of acute pulmonary exacerbations (APEs), advances in chronic pulmonary therapies, a shift in treatment paradigms, and the use of cystic fibrosis transmembrane conductance regulator (CFTR) modulator drugs, have led to improvements in clinical outcome and life expectancy in CF patients, with greater than 50% of patients now being older than 18 years.[1,2] Despite these advances, there remain significant challenges to the management of acute and chronic lung infection.

CF is associated with mutations in the *CFTR* gene, the most common of which is F508del. Inheriting two mutations on opposite alleles, from the more than 1800 *CFTR* mutations identified, leads to a variable degree of salt and water imbalance in the majority of organs expressing CFTR. These mutations are categorized according to the functional defect: no CFTR translation (class I), defect in processing (class II), impairment in CFTR regulation (class III), abnormal conductance (class IV), decreased production (class V), or increased degradation (class VI).[3] For drug development and practical purposes, mutations outside of F508del and gating mutations are classified as minimal function and residual function, based on their responsiveness to available modulator drugs. Although pancreatic insufficiency is not typically associated with class IV, V, and VI defects, the ability to make other clinical predictions based on mutation profiles is limited.[4] In regard to manifestations of lung disease, there is unpredictability of phenotype based on genotype, with both rapid progressive phenotype patients (those with a steeper decline in forced expiratory volume in 1 second [FEV_1] over time compared to average) and long-term nonprogressive phenotype patients (those with stable FEV_1 over time) in cohorts carrying the same *CFTR* mutation, suggesting additional genetic and environmental factors are involved in health decline.[5,6]

The diagnosis of CF relies on clinical presentation in combination with diagnostic testing. Since it was first introduced in Wisconsin in 1994, all states in the United States have adopted newborn screening for CF, joining CF centers across Europe and Australia along with a growing number of other countries.[7–9] A positive screen includes evidence of elevated immunoreactive trypsinogen or identification of one or more disease-causing *CFTR* mutations.[10] Sweat chloride testing via pilocarpine iontophoresis and *CFTR* mutation analysis follows a positive screening test. A chloride concentration greater than 60 mEq/L is considered positive and concentrations are considered indeterminate down to a cutoff of 30 mEq/L, although rarely a patient diagnosed with CF can have chloride concentrations below the diagnostic range,[11] making the diagnosis of CF more challenging in certain cases. New terms to better define those patients who do not completely meet the diagnostic criteria have been established to identify those at risk for complications of CF and who continue to require close attention. Methodologies to help differentiate those at risk include the β-adrenergic sweat secretion rate and the nasal potential difference (a measure of bioelectric properties that reflect CFTR function using harvested epithelial cells from human bronchi and nasopharynx or intestinal organoids), which are offered at limited CF centers to provide additional insight but currently are available as research tools only.[12,13] *CF-related metabolic syndrome* is a term reserved for asymptomatic individuals who do not meet the diagnostic threshold of sweat chloride testing and *CFTR* mutation analysis. Depending on their clinical presentation or new diagnostic

results, these individuals may be redefined as having CF, having CFTR-related disorder (e.g., a patient with CF-related metabolic syndrome who is symptomatic), or not having CF.[10]

Pulmonologists are often the primary providers for CF patients, with infectious disease physicians involved as consultants. Each discipline approaches the disease according to its background training and experience, but we believe their cooperation is critical to optimally treat those suffering from CF. Increasing evidence-based data are available to help guide the CF caregiver. The Cystic Fibrosis Foundation (CFF), founded in 1955 to serve the CF community, created a Therapeutic Development Network in the late 1990s to support clinical trials, whether in cooperation with industry or physician initiated, harnessing the pooled resources of CF care centers. The armamentarium for the modern CF clinician includes new oral and inhaled antibiotics, airway hydrating agents, mucolytics, antiinflammatory drugs, CFTR modulators, and nutritional supplements (see Table 71.1 under "Treatment" later).

The present chapter describes key CF-related infectious diseases issues that are relevant to infectious diseases clinicians.

CLINICAL DISEASE

The clinical course of CF is frequently described by chronic progressive lung disease punctuated by APEs. These exacerbations may be triggered by infection, environmental exposures, or other events.[14] There is no universally accepted definition of an APE, and responses to a change in symptoms differ among clinicians (chest physiotherapy only, oral antibiotics, intravenous antibiotics, etc.).[15] However, for the purpose of clinical trials, an APE is frequently defined as the presence of four or more of the following criteria[16]:

- an increase in sputum volume or change in color
- new or increased hemoptysis
- increased cough
- increased dyspnea
- malaise
- fatigue or lethargy
- a temperature greater than 38.0°C
- anorexia or weight loss
- sinus pain or tenderness
- a change in sinus discharge
- a change in findings on physical examination of the chest
- a decrease in FEV_1 by 10% or more from baseline
- radiographic changes in support of a pulmonary infection

The focus of CF care is to avoid or reduce APEs, and a standardized definition of APEs would allow caregivers to recognize exacerbations and treat them early. Every APE has an impact on recovery of lung function.[17] In a cohort study involving the CFF Patient Registry, Sanders and colleagues demonstrated a 25% failure in FEV_1 recovery among 8479 exacerbations.[17] In a retrospective study of FEV_1 following an APE included 440 patients and a total of 1667 exacerbations requiring hospitalization and antibiotics, nearly one-third of all patients failed to return to baseline lung function.[18] Both studies correlated poor recovery with a greater drop in FEV_1 from baseline prior to treatment initiation; allergic bronchopulmonary aspergillosis (ABPA); concomitant infection with *Burkholderia cepacia* complex (BCC), *Pseudomonas aeruginosa* (PA), or methicillin-resistant *Staphylococcus aureus* (MRSA); and lower body mass index (BMI).

Prior to the widespread use of newborn screening, the vast majority of CF diagnoses were made based on respiratory symptoms, along with failure to thrive and steatorrhea and other gastrointestinal symptoms. Despite lack of pulmonary symptoms, there is evidence supporting neutrophilic inflammation in the airways of asymptomatic infants. Sly and coworkers observed the presence of increased neutrophil elastase activity in bronchoalveolar lavage (BAL) fluid from infants as young as 3 months of age, and it was associated with subsequent development of bronchiectasis at 36 months.[19]

In more typical CF patients, respiratory manifestations include a chronic suppurative cough, obstructive lung disease by spirometry, and hyperinflation on chest radiographs. Symptoms of cough and wheezing continue in varying degrees and correlate with progressive bronchiectasis. APE presents on top of chronic stable symptoms as noted earlier. Many patients present with digital clubbing, but it is by no means universal. Chronic sinus disease develops in most CF patients as soon as the paranasal sinuses develop.[20] Nasal polyposis is found in 10% to 32% of patients.[21] Chronic sinus disease may be found in patients with minimal lung disease or even those who do not quite meet CF diagnostic criteria and tends to constitute a reservoir for infection.

ABPA can be described as a hypersensitivity response to *Aspergillus fumigatus* and may be a cause of an APE. Although other fungi may produce a similar response, *Aspergillus*-related hypersensitivity is the most commonly recognized. ABPA leads to airway obstruction, bronchiectasis, and pulmonary fibrosis if not managed successfully and further impacts CF-related lung disease.[22] The diagnostic criteria include[23]:

1. An acute or subacute exacerbation (cough, wheeze, decline in pulmonary function, increased sputum) not attributable to another etiology
2. Cutaneous reactivity to *Aspergillus* or elevated *Aspergillus*-specific immunoglobulin (Ig)E antibodies
3. Elevated serum IgE greater than 1000 IU/mL
4. *Aspergillus* serum precipitins or elevated *Aspergillus*-specific IgG antibodies
5. New chest imaging abnormalities that do not resolve with antibacterial treatment or chest physiotherapy

The mainstay of treatment involves corticosteroid use with taper over a course of 2 to 3 months while monitoring response (IgE, respiratory symptoms, spirometry, chest radiography). It is important to manage concomitant infection, particularly in cases of poor response to therapy. Itraconazole, voriconazole, posaconazole, or isavuconazole can be used when there is a poor response or a relapse in ABPA, or when use of corticosteroids is not possible. Triazole-related drug-drug interactions are important to monitor with these agents, particularly in the age of CFTR modulators, but isavuconazonium may be a best option. Response to antifungal treatment is associated with pretreatment isolation of *A. fumigatus* from the respiratory tract.[24] Despite a lack of demonstrable evidence in CF patients, omalizumab is a non–US Food and Drug Administration (FDA)-approved option for some patients resistant to traditional ABPA therapy or suffering untoward side effects from steroids.[25]

Common nonrespiratory manifestations of CF include pancreatic insufficiency, which is progressive and found to some degree in up to 90% of patients by age 1.[26] It is characterized by frequent, copious, foul-smelling, greasy stools, as well as failure to thrive due to malabsorption of fat and protein. Untreated with pancreatic enzyme replacement therapy (PERT), patients may present with electrolyte abnormalities, protein-calorie malnutrition, and fat-soluble vitamin deficiency. Pancreatitis is a complication most commonly associated with those patients who have adequate or borderline pancreatic function. CF-related diabetes afflicts one-quarter of young adults and up to 50% of adults with CF.[27] Meconium ileus or bowel obstruction at birth in a nonpremature infant is highly suggestive of CF.[28,29] When this occurs in adolescents and adults, it is called distal intestinal obstructive syndrome or meconium ileus equivalent. Quick, aggressive medical attention to relieve the obstruction is crucial to avoid the surgical intervention required in severe cases. Rectal prolapse occurs less frequently and is associated with malnutrition, constipation, and poor adherence to PERT and also manifests in stool incontinence. Another symptom similarly impacting quality of life is urinary incontinence. Constipation can be a significant contributor, but often other measures are necessary to manage cough-associated urinary incontinence, such as physical therapy and cognitive behavior therapy.[30,31] Liver disease is common and related to biliary dysfunction due to inspissated bile and is associated with laboratory abnormalities such as elevations in alkaline phosphatase. Liver disease progresses in a small number of patients, rarely beyond early adulthood, leading to cirrhosis, portal hypertension, and development of esophageal varices. Rarely does cholelithiasis require surgical intervention other than prophylactic removal prior to lung transplantation.[32]

There are many risk factors for osteoporosis in men and women with CF. Malabsorption of vitamin D, malnutrition, decreased physical activity, glucocorticoid treatment, and hypogonadism contribute to the development of bone disease.[33] Pain associated with CF-related arthropathy occurs in a small number of patients but causes significant morbidity.

Hypertrophic osteoarthropathy is associated with abnormal proliferation of soft and osseous tissue at distal parts of extremities. It appears to share the same process as digital clubbing but is much less common, and its exact pathogenesis is unclear. Nephrolithiasis and nephrocalcinosis are common in CF patients as well. They are most frequently due to hyperoxaluria due to oxalate absorption in setting of inadequate PERT and decreased citrate excretion.[34]

PATHOGENESIS OF CYSTIC FIBROSIS–RELATED LUNG DISEASE

The CFTR protein, residing on apical membranes of epithelial cells, regulates salt and water balance. The mechanism of its impact on airway surface liquid is the subject of research, but it is recognized that the absence or dysfunction of CFTR in epithelial cells lining various ducts in the body leads to a hyperabsorptive state, which in turn is due to the osmotic gradient created by impaired chloride, sodium, and bicarbonate transport. In the lung, the depletion and dehydration of the airway surface liquid layer lead to impaired mucociliary transport with the development of thick, tenacious secretions. This in turn leads to an inability to clear bacteria and other debris from the lower airways. More recent data link reduced secretion of bicarbonate in the pathogenesis of CF. CFTR impacts pH by altering bicarbonate secretion, leading to reduced pH and a reduced ability for innate mechanisms to combat bacteria.[35] This impaired clearance leads to a chronic infection state, most commonly associated with *S. aureus* and PA. The negative bearing on innate defense results in an inflammatory response that includes polymorphonuclear leukocytes and antibodies.[36–38] While communicating with the lower airway, the sinuses can provide a separate environment for bacteria to inhabit. Segregated intrapulmonary habitats can also exist in focal areas of the lung, making it challenging to target specific organisms that may be responsible for APEs. These bacterial communities are only a small number of organisms representing the complex microbiome of the CF lung.

Similarly aged patient cohorts with the same *CFTR* mutation can have mild, moderate, or severe lung disease, thus genetic modifiers have been implicated, as well as environmental ones (smoke, socioeconomic class), but also variable microbiology.[39] It is difficult to identify and detect genetic modifiers due to significant variations in disease presentation and response to external stressors in the environment.

THE CYSTIC FIBROSIS MICROBIOME

Haemophilus influenzae and *S. aureus* are frequently cultured from the upper airway of children with CF, while worsening clinical outcomes are usually associated with chronic infections with PA, as well as chronic infections with other bacteria, including BCC species, *Achromobacter xylosoxidans,* and *Stenoptrophomonas maltophilia.*[40,41] There is an increased recognition of lung disease caused by nontuberculous mycobacteria (NTM) in CF patients. NTM are recovered with increasing frequency as they age, with *Mycobacterium abscessus* being the most concerning infection associated with worse outcomes.[42] Anaerobic organisms such as *Streptococcus, Prevotella, Actinomyces,* and *Veillonella* are also increasingly recognized as possible pathogens inhabiting the low oxygen concentration of mucoid airway surface liquid.[43,44]

The growing CF literature on airway microbiology reveals an undeniably complex and dynamic interaction between the CF airway and organisms inhabiting this environment.[41,45] Even organisms associated with CF lung infection for decades are recognized to be genetically and phenotypically diverse, even within the same patient and different parts of the lung.[46] Infection diversity likely impacts management, and the presence of two or more species inhabiting the same space appears to change the way individual bacterial species behave, through quorum sensing, and work in concert to drive disease progression. Limoli and associates elegantly demonstrated the change in PA motility via type IV pili and flagella activity when exposed to *S. aureus* in ex vivo sputum samples.[46a] In vitro coculture models involving PA and *S. aureus* also demonstrate enhanced antimicrobial resistance.[47] Their interactions, including sharing genetic data, have an impact on antibiotic susceptibility.[48] Particular bacterial pairs may be associated with better or worse outcome. New tools of molecular diagnostics have helped inform caregivers and researchers, including the role of viruses in APEs.[49,50]

Given the poor relationship between in vitro susceptibility and clinical outcome among CF patients, our current antimicrobial susceptibility testing may be inaccurate, and culture procedures may fail to identify other potential pathogens with diverse susceptibility patterns from different colonies of the same bacterial species. Biofilm susceptibility testing has not proven to be superior to conventional testing but warrants further evaluation.[51,52] DNA- and RNA-based deep sequencing methods can identify a greater diversity of bacterial species and variants, but the genomic knowledge determinant of antibacterial resistance remains limited.[53,54]

Beyond the traditional CF bacterial pathogens, there have been ongoing data highlighting the potential role of other bacteria and their association with CF exacerbations.[55] Anaerobes and oral flora are found in very high densities, including *Prevotella* and *Veillonella. Streptococcus milleri* has been associated with worse outcomes in CF, but is an organism not usually sought out by CF microbiology laboratories. Newly identified organisms such as *Gemella* and *Rothia mucilaginosa* have been associated with worse outcomes with *Rothia* detected more often than PA in many patients.[56,57]

Bacterial pathogens in CF airway are shown in Fig. 71.1. In the early days of CF, *S. aureus* was the only bacterium identified in lungs, but successful treatment soon led to the predominance of chronic infections with PA and BCC, which are associated with poor clinical outcomes. Even before the advent of CFTR modulators, there had been a steady decline in the prevalence of PA despite its being the predominant organism in adults over 25 years of age, while chronic infection with *S. aureus* continues to increase in prevalence. Chronic infection with MRSA and *Achromobacter* has plateaued over the last several years based on data in the CFF Patient Registry data from 2016 (see Fig. 71.1).[57a] We are able to identify bacteria from the respiratory tract utilizing standard culture techniques as well as molecular typing. A major question remains current: how do we identify whether bacteria identified in respiratory cultures are disease producing and impacting clinical outcomes? For example, it is well known that in sputum from CF patients, different bacterial colony morphotypes can be found that arise from the same bacterial strain.[58] This suggests that the bacteria in the lungs are constantly adapting a variety of described mechanisms (auxotrophy, motility, type III secretion system, lipopolysaccharide, antibiotic resistance, biofilm, hypermutability, quorum sensing, small-colony variants [SCVs]) that lead to these different phenotypes, and a dominant single bacterial strain may evolve.[59] We are beginning to understand the evolution of clonal populations. Multiple lineages coexist and multiple clones evolve separately with different lineages. Genomic and proteomic approaches may become helpful to better understand these communities.[60,61] These novel insights remain difficult to translate into clinical outcomes at this time.

Lungs themselves are also very heterogeneous in disease severity. Understanding the differences in regions of the lungs may help explain patient heterogeneity. Regions with mild and severe disease are infected by different subpopulations of PA. As an example, we can target factors that select infections with milder PA subpopulations. Most regions, however, contain a mixture of isolates and subpopulations, confounding this investigation.

The study of CF exacerbation with proteomics has contributed to our understanding of changes in bacteria during APEs.[46] While bacterial density remained stable, a decrease in bacterial diversity was associated with declining FEV_1. Looking at 27 consecutive sputum samples from the same patient, Carmody and colleagues demonstrated that microbial communities change.[56] They showed a decrease in abundance of dominant species, including PA, BCC, and *Achromobacter,* following antibacterial treatment. Zhao and coworkers found that interventions led to a change in bacterial diversity and were associated with age, disease severity, and antibiotic exposure.[62] Density of bacteria remained the same, while microbial diversity decreases.[56] In general, microbial diversity decreases with antibiotic exposure, but bacterial communities generally return to pretreatment configurations by 1 month after completion of antibacterial courses. Others have also demonstrated an association between microbial diversity and FEV_1 (Fig. 71.2), as well as the lack of correlation with total microbial abundance (Fig. 71.3).[63]

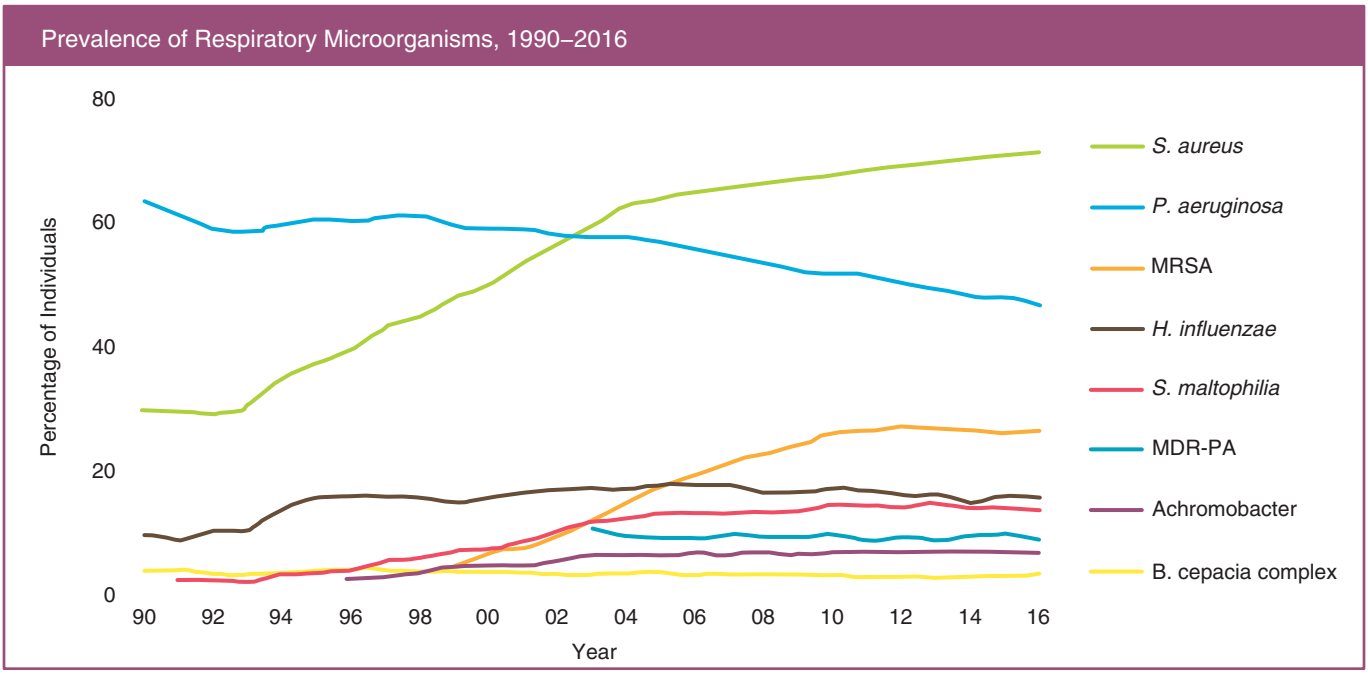

FIG. 71.1 The proportion of individuals in various age groups who cultured positive for the bacterial species indicated during 2016. *MDR-PA,* Multidrug-resistant *Pseudomonas aeruginosa; MRSA,* methicillin-resistant *Staphylococcus aureus. (Data from Cystic Fibrosis Foundation.* Patient Registry 2016 Annual Data Report. *Bethesda, MD: Cystic Fibrosis Foundation; 2016:29.* https://www.cff.org/Research/Researcher-Resources/Patient-Registry/2016-Patient-Registry-Annual-Data-Report.pdf. *Accessed January 3, 2019.)*

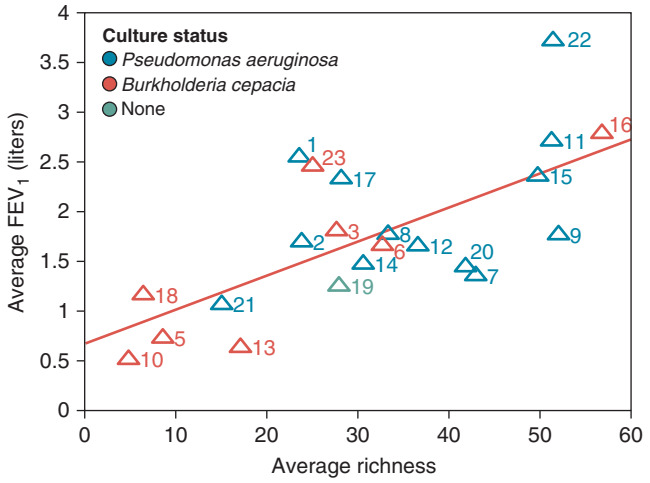

FIG. 71.2 Microbial richness in sputum samples is associated with decreased lung function. Shown is a plot of the average forced expiratory volume in 1 second (FEV_1) compared with average microbial richness in sputum for each patient. FEV_1 and microbial richness values were averaged across all time points for each patient. Numbers next to each symbol indicate patient ID. Symbols are color coded on the basis of patient culture status (*Pseudomonas aeruginosa*, *blue; Burkholderia cepacia* complex species, *red;* culture negative for both, *green*). Results from linear regression analysis (*red line*) indicate a significant correlation (coefficient of determination [r^2] = 0.42, $P = .0009$). *(Data from Fodor AA, Klem ER, Gilpin DF, et al. Adult CF airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations.* PLoS One. *2012;7:e45001.)*

Even with changes in clinical status, neither the Zhao and coworkers nor the Fodor and associates study observed a significant change in airway microbial community structure or bacterial abundance. The 23 patients included in the study by Fodor and associates were chronically infected with either PA or BCC, along with isolation of species belonging to the genera *Streptococcus, Prevotella, Rothia, Veillonella, Actinomyces,* and *Granulicatella.* The progression of disease with age, microbial diversity, and resistance to antibiotics supports this observation. As in the study by Zhao and coworkers, APEs or disease progression was not associated with increase in bacteria numbers, overgrowth of a specific organism, or the isolation of a new bacterial infection. Fodor and associates suggested that an APE represents the spread of infection to previously unaffected airways or adaptation by organisms that may lead to a change in the expression of virulence traits. For example, during chronic CF airway infection, PA can undergo conversion from a smooth to a mucoid colony phenotype, discussed in more detail below.[64] The coexistence of mucoid and nonmucoid PA variants suggests a protective benefit in evasion of host defense.[65,66] Data presented here provide insights into the CF airway microbiota, including initial diversification events in younger patients and establishment of specialized communities of pathogens associated with poor pulmonary function in older patient populations.

How do we decide on therapy based on this information? There is evidence that uncouples susceptibility testing from clinical outcome, and the evolving microbiota data may help explain the disconnect between in vitro testing and clinical outcomes[67,68] However, irrespective of susceptibility, including multidrug-resistant PA, similar clinical responses are observed to usual antimicrobial therapies.[69]

MAJOR CYSTIC FIBROSIS PATHOGENS

Staphylococcus aureus

The most common pathogen among CF patients in contemporary cohorts is *S. aureus,* with recovery from sputum at its height in children and adolescents (see Fig. 71.1).[57a] *S. aureus* was the dominant infection leading to respiratory failure in patients during the early to mid-20th century. The introduction of antistaphylococcal antibiotics led to selection of gram-negative organisms and observation of chronic infection with PA. Like PA, CF patients may culture methicillin-sensitive *S. aureus* (MSSA) from sputum repeatedly and it may represent the same isolate over time.[70] Outside of the United States, chronic management of CF at some centers includes routine treatment against MSSA with similar prevalence rates, but surprisingly, MRSA levels are significantly less in comparison.[71] Although some evidence suggests reduced exacerbation rates,[72] the CFF consensus guideline committee on chronic therapies

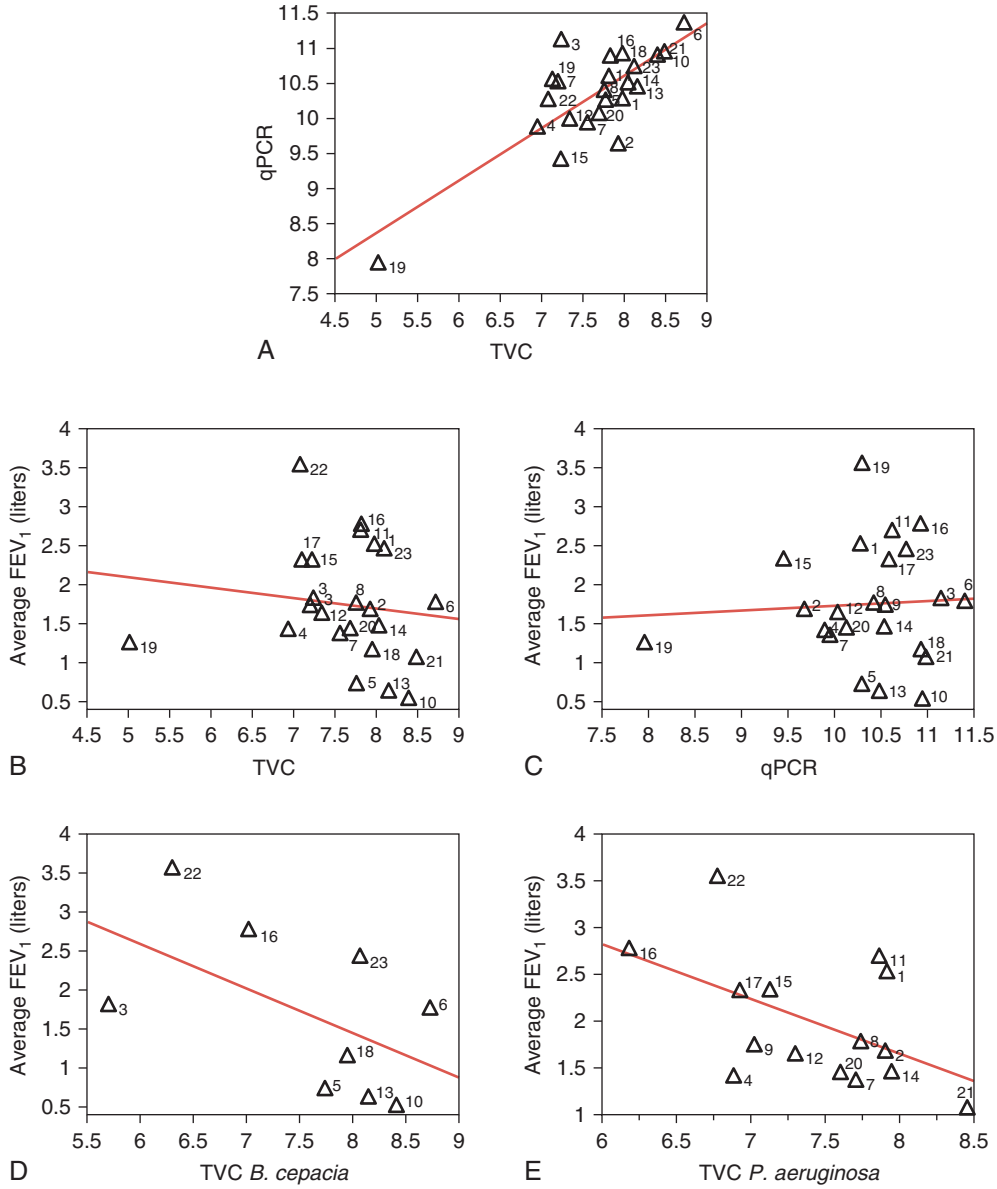

FIG. 71.3 Lung function is not correlated with total bacterial abundance. Measurements of total bacterial abundance in sputum by (A) timed vital capacity (TVC) and quantitative polymerase chain reaction (qPCR) are well correlated ($r^2 = 0.63$, $P < .0001$; $n = 23$). Forced expiratory volume in 1 second (FEV_1) is not correlated with (B) TVC ($r^2 = 0.017$, $P = .55$; $n = 23$) or (C) qPCR ($r^2 = 0.003$, $P = .8$; $n = 23$) and only modestly correlated with TVC from (D) *Burkholderia cepacia* complex species ($r^2 = 0.29$, $P = .13$, $n = 8$) and (E) *Pseudomonas aeruginosa* ($r^2 = 0.26$, $P = .05$; $n = 14$). Measurements for bacterial abundance and FEV_1 were averaged across time points for each of the 23 patients. Labels in each panel indicate patient ID. Lines indicate regression fit by linear least squares. Only TVC values >0 were included for *B. cepacia* and *P. aeruginosa* comparisons. TVC values represent log10 of total bacterial colony-forming units recovered per gram of sputum. qPCR values represent log10 copies of the bacterial 16S ribosomal RNA gene detected per gram of sputum. *(Data from Fodor AA, Klem ER, Gilpin DF, et al. Adult CF airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations.* PLoS One. *2012;7:e45001.)*

to maintain respiratory health recommended against routine antistaphylococcal treatment, given the observation of greater prevalence of PA in groups receiving chronic antistaphylococcal treatment and its consequences.[73] The annual prevalence of *S. aureus* was 71.1% (includes MRSA) in 2016 and increasing, while PA rates have continued to slowly decline.[57a] Despite the increased prevalence of MRSA over the last 20 years, the levels plateaued about 3 years ago. The prevalence mirrors that of community-acquired MRSA in our communities in general, although it is unclear whether this is the source of MRSA infection in CF.[74–76] Prevalence can vary; however, the most recent CFF registry data from 2017 suggest an overall prevalence of 26% and that prevalence is highest among adolescents and young adults.[76a]

With chronic infection and treatment of both MSSA and MRSA, the detection of SCV *S. aureus* is increasingly being recognized in patients with CF. These bacteria do not grow well on standard culture media and are not routinely identified by most clinical laboratories. A recent multicenter trial in the United States was conducted to evaluate the prevalence and clinical significance of SCV *S. aureus* and noted that 28% of patients enrolled were infected with SCV *S. aureus*. Those infected had significantly lower lung function, had increased odds of having an APE, and were more likely to have been treated by a sulfonamide antibiotic prior to detection. It is unclear whether SCV *S. aureus* infection causes or is an indicator of worse lung function. Additional studies are needed to evaluate whether prevention or treatment of SCV *S. aureus* improves clinical outcomes.[77] Our microbiology laboratory uses mannitol–salt agar to help identify this slow-growing variant. There is a positive association with the presence of SCV *S. aureus* and use of trimethoprim-sulfamethoxazole (TMP-SMX) and infection with PA.

Two recent studies demonstrated an association of SCV *S. aureus* with more advanced lung disease in children and adults.[78,78a]

Methicillin-Resistant *Staphylococcus aureus*

A study by Dasenbrook and colleagues reported worse survival in patients with sputum culture positivity for MRSA,[79] and others have reported lower absolute lung function when MRSA is compared to MSSA.[80] In the Dasenbrook and colleagues cohort study of longitudinal data obtained from the CFF Patient Registry that analyzed 19,833 patients, the reported risk of death was 1.27 times greater for individuals with at least one culture positive for MRSA reported compared to those in whom MRSA was never detected, adjusting for PA infection.[79] New and persistent infection with MRSA revealed a more rapid rate of decline in lung function among 8- to 21-year-olds, but this effect was not observed in those older than 21 years of age (Fig. 71.4).[81] The increased prevalence of MRSA in the last decade may explain the flattening of the overall CF median mortality curve.[57a] These findings underscore the importance of persistent MRSA infection in CF.

Routine MSSA-directed antibacterial prophylaxis is not uncommon in the United Kingdom and other parts of Europe. Despite this, the overall MRSA prevalence in the United States is much higher. Distribution of MRSA types in CF and non-CF differ for a given country.[82–84] A cross-sectional study showed that FEV_1 values declined faster in patients who became chronically infected with MRSA compared to those who experienced intermittent MRSA infection.[86] There is fluidity between community- and hospital-associated MRSA. The STAR-CF study looked at chronic MRSA infection based on staphylococcal protein A gene, staphylococcal chromosomal cassette mec (SCCmec), and Panton-Valentine leukocidin (PVL).[87] SCCmec type II is usually hospital associated, SCCmec type IV is usually community associated, and a majority of the pediatric CF population studied was determined to be positive for SCCmec II and PVL negative. There were no differences in gender, FEV_1, BMI, or rates of MSSA. The number of clinic visits was increased among SCCmec II–positive patients, while hospitalizations did not differ. Elizur and coworkers noted an increased rate of decline in FEV_1 and necrotizing pneumonia associated with PVL positivity.[88]

Several recent clinical trials have addressed questions related to acute and chronic infection with MRSA. The STAR-Too trial was a randomized, controlled, open-label study that compared the implementation of an early MRSA eradication protocol (two oral antibiotics, topical antibiotics, and instructions for environmental decontamination techniques) to an observation group receiving current standard of care. The study was stopped early by the data safety monitoring board due to a significant decrease in MRSA-positive screens at 28 days in the treatment group compared to controls.[89] The PMEP study was designed to look at eradication of persistent MRSA infection. Subjects were randomized to receive either inhaled vancomycin (250 mg twice daily) or placebo (taste-matched saline), along with rifampicin plus a second agent (TMP-SMX or doxycycline) and topical agents (mupirocin intranasal cream and chlorhexidine washes).[89a]

There is controversy over management of chronic MRSA infection, but directed treatment is usually employed for all exacerbations using same antibiotic choices as in non-CF patients. Teicoplanin and fusidic acid are used in Europe but are not available in the United States.[71]

Linezolid is active against most strains of MRSA and has been tested for the treatment of MRSA pneumonia in non-CF patients with overall similar outcomes when compared to intravenous vancomycin.[71,90,91] Successful use of linezolid for treatment of MRSA-associated APE has been reported in CF patients,[92] but development of breakthrough linezolid resistance has been frequently reported in CF patients.[93–95] Linezolid use for greater than 2 weeks is frequently associated with myelosuppression and other adverse effects, so longer term use in patients with MRSA infection may be difficult, but we use it in cases of a history of vancomycin hypersensitivity reactions or as step-down treatment in patients for whom intravenous administration is problematic.

Telavancin is a new antibacterial drug with several comparative studies with vancomycin in non-CF patients. Although not currently approved for CF, a recent study by Roch and associates demonstrated excellent bactericidal activity with low resistance profile in vitro against MRSA samples obtained from CF patients.[96] Another recent case series describes the utilization of telavancin for APE in three adolescents.[97] Currently there are ongoing investigations to further delineate the pharmacokinetics of telavancin in adults with CF.[97a]

Ceftaroline is a new antibacterial drug with activity against MRSA. Like telavancin, ceftaroline has not been approved for APE in CF; however, there is growing interest in its use. Autry and colleagues and Barsky and associates recently completed separate pharmacokinetic and pharmacodynamic studies investigating the dosing of ceftaroline in patients with CF. Their studies suggested 600 mg (15 mg/kg in children) every 8 hours administered over 60 minutes should be considered to reach a free drug concentration greater than the minimal inhibitory concentration (MIC) for 60% of the dosing interval.[98,99] It remains unclear, though, how ceftaroline should be adjusted in patients with impaired renal function, the effects of other antibiotics on the ceftaroline pharmacokinetic profile, and if this dosing regimen is associated with clinically significant outcomes.

Ceftobiprole is another new antimicrobial that may have a role in treatment of MRSA-associated APE.[100] Daptomycin is not recommended for pulmonary infections since it binds to surfactant. As noted earlier, there is a high prevalence of MRSA in the United States, and chronic infection may be associated with worse outcome and a risk factor in CF for increased health care utilization. The use of tedizolid or dalbavancin for treatment of MRSA infections in CF patients remain to be determined.

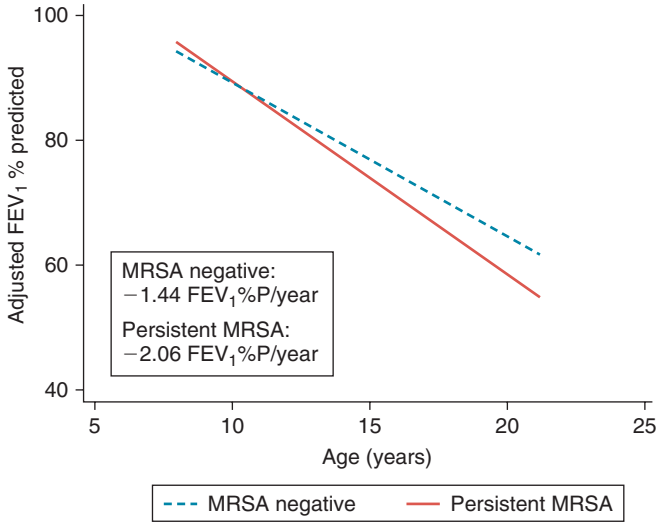

FIG. 71.4 Persistent methicillin-resistant *Staphylococcus aureus* (MRSA) cultures and forced expiratory volume in 1 second (FEV_1) percentage. Linear predictor of percent predicted decline of FEV_1 per year (FEV_1%P/year) for cystic fibrosis patients ages 8 to 21 years with persistent MRSA (≥3 MRSA cultures; *solid line*) compared with those without MRSA *(dotted line).* Average follow-up for each individual in the cohort was 5.3 years. FEV_1 was adjusted for group differences in the confounders. *(Data from Dasenbrook EC, Merlo CA, Diener-West M, et al. Persistent methicillin-resistant* Staphylococcus aureus *and rate of FEV1 decline in cystic fibrosis.* Am J Respir Crit Care Med. *2008;178:814-821.)*

Pseudomonas aeruginosa

In prospectively collected oropharyngeal cultures obtained at least every 6 months from patients diagnosed through neonatal screening,[101] PA has been recognized to be quite adaptable to inhabiting the CF respiratory tract. Since the first description of CF, PA has become the second most prevalent pathogen found in the respiratory tract of CF patients.[57a] The establishment of CF centers and cohorting of patients in hospitals is somewhat responsible for the emergence of this organism.[102] This observation has led to adoption of strict infection control and practice guidelines.[103–106] The neonatal screening program underway since 1985 was a collaboration involving two CF centers in Wisconsin, and its inception had implications for the prevalence

of PA infection.[7] Observation of screened and control groups were similar overall, but one center, which was located in an urban setting, showed significantly younger age for acquisition of PA. This clinic did not segregate patients by age, and perhaps allowed more opportunity for social interaction outside of the clinic. Although the overall study did not indicate a significantly increased risk for PA acquisition, it suggested risk factors associated with an urban center clinic. Patients identified with CF through neonatal screening had a median of 52 PA-free weeks in the urban CF center, while PA-free survival was 289 weeks at the nonurban center. The acquisition of PA impacts survival and is strongly associated with a decrease in FEV_1 and life expectancy.[107–109] Kerem and coworkers showed significant association of decreased lung function at age 7 in patients with PA infection compared to those uninfected with PA.[109] Also, a decrease in PA-positive cultures at a Danish center occurred when PA-infected and noninfected patients were segregated.[109a]

Despite the impaired but robust host inflammatory response and treatments directed toward PA, this organism comprises 60% to 70% of all CF respiratory infections by age 18,[57a] and chronic infection eventually leads to respiratory failure and death. Initial strains are usually unique and begin with strains from an environmental source.[110] This is followed by repeated airway infection and eventually chronic airway infection, as well as parenchymal infection, chronic bronchopneumonia, and necrotizing pneumonia.[49] The aggressive use of antipseudomonal antibiotics can substantially delay chronic infection.[38] The Early Pseudomonal Infection Control (EPIC) study was designed to determine the impact of the acquisition of PA and of four separate antibiotic regimens to eradicate the first positive PA culture: cycled therapy or culture-based therapy with inhaled tobramycin (300 mg twice daily) and ciprofloxacin or placebo.[112–114] No one therapy was superior to the others, but each led to decrease rates of PA recurrence.[115]

Strategies to prevent early PA acquisition from developing into chronic infection remain of interest. Despite efficacious eradication protocols, PA recurrence still develops in one-third of patients within 18 to 27 months. Nearly one-half of the children receiving eradication therapy experience pulmonary exacerbation within 18 months. Macrolide antibiotics have previously been shown to reduce the risk of pulmonary exacerbations and reduce PA biofilm formation. The OPTIMIZE trial investigated whether the addition of azithromycin to inhaled tobramycin during eradication therapy would reduce the risk of pulmonary exacerbation and delay recurrence of PA. Patients were randomized to receive 28 days of inhaled tobramycin with azithromycin or inhaled tobramycin monotherapy with plans to follow for 18 months. The study was stopped early by the data safety monitoring board after the azithromycin arm reached a prespecified monitoring boundary for efficacy. The study was amended and is currently being completed as an open-label study. The study revealed that the addition of azithromycin to eradication therapy with inhaled tobramycin increased the duration of time to pulmonary exacerbation, but did not reduce the frequency of exacerbations or affect the time to PA recurrence.[116]

There is evidence that respiratory syncytial virus (RSV) infection promotes biofilm formation and enhances PA antibiotic resistance (see "Respiratory Viruses" later).

The eventual chronic PA infection can occur through a separate, genomically distinct organism or, in a quarter of the cases, through the same genomic variant.[117–119] The source of recurrent pulmonary PA can be a persistent environmental source or a nonpulmonary reservoir, such as the sinuses. The eventual chronic infection is likely due to adaptive changes to PA and is associated with increased degree of inflammation and recruitment of neutrophils and other destructive proteases.[110]

The term *colonization* is frequently used to describe persistently positive bacterial cultures from the CF airway, but is not an accurate description. Although large bacterial colony counts exist in the airway (1×10^8/mL) without an acute illness and during periods of clinical stability, there is still an exaggerated inflammatory response leading to airway destruction and disease progression. The term *colonized* would suggest that failure to eradicate would not be associated with increased morbidity. Thus we prefer the term *chronic infection*. Extended airway residence leads to changes in gene expression, including formation of anaerobic biofilms such as alginate and progression of disease due to chronic infection.

Antibacterial discovery and use has been associated with improved survival in patients with CF, although the development of antibiotic resistance has not been shown to increase the rate of decline.[120] Even though they may arise from similar strains, PA phenotypic changes have an impact on response to therapy.[120] PA can adapt by altering gene regulation and proteomic expression to the environment in which it lives. The CF airway has significant complexity and promotes a chronic PA infection model. The regulatory pathways are very complex. One such mechanism is alginate overproduction, leading to mucoid-type PA. This is a result of *algT,* a gene that negatively regulates flagella, pili, and quorum sensing (Rhl signal and rhamnoloid). The type three secretion system positively regulates heat shock response, oxidative stress response, osmotic stress response, and alginate production. Neutrophils also cause reactive oxygen species and reactive nitrogen intermediates expression to promote stress on bacteria and PA.[121] CF epithelial cells are unable to efflux glutathione, the most prevalent antioxidant, and they poorly absorb other dietary antioxidants. Reactive oxygen species and reactive nitrogen intermediates can lead to damage in DNA, lipids, and proteins, which in turn can lead to selection of variants that helps PA survive in the microaerobic or increasingly anaerobic environment.[122,123]

Antibiotic tolerance or resistance is directly related to antibiotic exposure. PA was rarely seen early on when severe *S. aureus* infection led to morbidity in the early days of CF lung disease antibacterial treatment. However, effective antistaphylococcal treatment led to the emergence of PA as a dominant CF-associated pathogen. The use of antibiotics has also led to resistance.[124,125] Resistance to all clinically relevant classes of antibiotics is increasingly exhibited by PA isolates from patients with CF.[126] Given the clonal nature of most CF-associated PA infections and the apparently infrequent transmission between patients in the setting of strict infection control practices, it would seem that de novo evolution of antibiotic resistance in individual patients is common.[127–130] Folkesson and coworkers demonstrated the lineage of a single strain of PA evolving or diversifying into mucoid and nonmucoid.[120] These sublineages, found in different regions (sinus, central airway, and specific lobe), reflect the distribution and isolation in different compartments of the lung. The phenotype can be homogeneous despite the complicated evolution genetically. If anaerobic conditions or stress with high salt are added to culture media, PA can change from a nonmucoid to a mucoid phenotype. When the environment becomes favorable again, PA reverts to a nonmucoid phenotype. This is not observed with wild-type PA, but happens to PA obtained from CF airways, which have adapted to their changing environment.

When PA is grown in CF sputum, increased expression of quorum sensing signaling genes is observed, leading to increased expression of virulence factors.[131] PA does not grow alone in the CF airways, and spatial localizations are impacted by other bacteria around them.[132] When PA is in proximity to gram-positive bacteria, it becomes more virulent (e.g., by making piocyanin). Treating MSSA chronically may help keep PA quiescent and may explain the benefit of antistaphylococcal therapy on individual CF patients.

New antimicrobial agents are currently being investigated to target multidrug-resistant PA. Ceftolozane-tazobactam is a novel cephalosporin/β-lactamase inhibitor with activity against multidrug-resistant PA. Currently ceftolozane-tazobactam is only indicated for intraabdominal infections and complicated urinary tract infections in adults, although its utility in nosocomial pneumonia has been described in case reports. However, a study by Grohs and associates examined the in vitro activity of ceftolozane and ceftolozane-tazobactam against 58 nonduplicate clinical isolates of gram-negative bacilli (35 *P. aeruginosa,* 11 *A. xylosoxydans,* and 12 *S. maltophilia*) acquired from CF patients. Ceftolozane was found to be the most active agent against PA (MIC for 50% of isolates [MIC_{50}], 4 μg/mL, and MIC for 90% of isolates [MIC_{90}], 128 μg/mL), with 54% of multidrug-resistant strains being susceptible.[133] In a US surveillance study by Farrell and colleagues of hospital-acquired PA, 96.1% of isolates were inhibited with MIC ≤4 μg/mL and maintained good activity against multidrug-resistant PA, including 77% of ceftazidime-nonsusceptible isolates.[134,135] Two case

reports have described using ceftolozane-tazobactam in CF patients during APE[136,137]; further studies are needed to determine its clinical efficacy in CF APE.

Ceftazidime-avibactam is another novel cephalosporin/β-lactamase inhibitor with good activity against multidrug-resistant PA. In US surveillance studies, it was found to have greater than 90% susceptibility rates, including approximately 80% of ceftazidime-nonsusceptible isolates. In vitro studies of multidrug-resistant PA from CF patients revealed 71.9% susceptibility.[138] Further studies are needed to determine the clinical efficacy of this drug in CF APE.

Currently interest has increased in gallium and its role as an antiinfective and antiinflammatory treatment agent for patients chronically infected with PA. Gallium is structurally similar to iron, and its uptake by PA disrupts PA iron metabolism by blocking the further uptake of iron. Furthermore, gallium also functions by inhibiting bacterial DNA synthesis and catalase activity, which in turn increases its sensitivity to oxidants. In vitro studies have shown it to be effective against PA, including multidrug-resistant strains, stationary-phase bacteria, and biofilms. A recently completed study, the IGNITE trial, randomized adult CF patients to receive either 5-day continuous infusion of gallium or placebo therapy and evaluated its effects on lung function, PA colony density, and drug safety. The study revealed a statistically significant improvement from baseline FEV_1 at days 6 and 14 of therapy and a decrease in PA density at day 28 of therapy. The inflammatory marker data were collected, and the results are currently pending.[139]

Burkholderia cepacia Complex

BCC organisms pose a significant health risk to CF patients. Although BCC was described much earlier, its association with CF was not appreciated until the 1980s.[140–142] In a longitudinal study in Belfast, 19 patients chronically infected with BCC and 19 controls chronically infected with PA were studied over a 4-year period.[143] The FEV_1 and BMI deteriorated much more in the BCC-infected group as compared to the PA-infected group ($P = .001$ and $P = .009$, respectively). Pathogenic differences exist among BCC species. Those infected with *B. cenocepacia* tend to have a much greater decline in FEV_1 compared to patients infected with *B. multivorans*.[143,144] Most of the more than 60 species belonging to the genus *Burkholderia* are not pathogenic to humans, but we pay attention to those that can result in serious sinopulmonary infections in patients with CF. Identified by 16S ribosomal DNA and *recA* gene analysis, many belong to the BCC group, consisting of 17 species at the moment, with new ones being identified. Outside of the BCC group, *B. gladioli, B. pseudomallei,* and *B. fungorum* have been identified as pathogens causing disease in CF patients.[145]

The increasing prevalence and the first of many descriptions of the rapid spread of BCC infection were reported in the CF center in Toronto.[146] They also provided one of the first descriptions of the so-called *cepacia syndrome.* Cepacia syndrome is characterized by rapidly deteriorating lung function, fever, leukocytosis, elevated markers of inflammation, and BCC bacteremia. Although *B. multivorans* behaves more like PA, it too has been associated with cepacia syndrome.

B. cenocepacia and *B. multivorans* are the most frequent isolated species belonging to the genus *Burkholderia.* Our center has a particular interest in *B. dolosa* due to its significant virulence and transmissibility. Caraher and colleagues compared *B. dolosa*'s ability to invade lung epithelial cells and form biofilm in vitro along with other BCC organisms, specifically *B. cenocepacia* and *B. multivorans*.[147] Its behavior is similar to other clinically relevant BCC species that infect CF patients, and it resulted in an epidemic spread across a large CF center. Thirty-one patients chronically infected with *B. dolosa* were compared to unmatched patients with *B. multivorans* and those not infected with *Burkholderia* species. The baseline FEV_1 and rate of decline were similar but the decline in FEV_1 after acquisition of *B. dolosa* was significant (−7.1% per year vs. −2.1% prior to infection) compared to no significant change associated with *B. multivorans* and those infected with non-*Burkholderia* species. A recent retrospective review by Boyer and coworkers revealed that 11 patients infected with *B. dolosa* who underwent lung transplantation had survival rates of 73%, 53%, and 30% at 1, 3, and 5 years, respectively, with a median survival of 44 months.[148] Among the 42 patients eventually identified during the epidemic, only 12 are alive at this time. Of the 11 patients who received transplants, 4 remain alive, and of 8 remaining pretransplantation patients, 5 are either awaiting lung transplantation or facing end of life.

The prevalence of BCC or *B. gladioli* infection can reach 8% in some adult CF cohorts, but usually hovers around 3% to 4% (see Fig. 71.1). The treatment of these drug-resistant strains is difficult and typically requires three to six concurrent antibacterials, including simultaneous use of more than one β-lactam antibacterial. A typical regimen for patients being admitted for APE associated with chronic infection with *B. dolosa* includes prolonged infusion of ceftazidime and meropenem, minocycline, and TMP-SMX, with the addition of chloramphenicol in cases of treatment failure. Fluoroquinolones and other β-lactam antibiotics, such as piperacillin-tazobactam, cefepime, aztreonam, and imipenem, have been used. Most strains of BCC are resistant to colistimethate.

Other Bacteria

With new advances in bacterial taxonomy and genetics-based methodologies, we are identifying new species, including those previously identified as either *Pseudomonas* or BCC. *Herbaspirillium* is one such organism previous identified as belonging to the BCC.[149] Other organisms not explored in depth include *A. xylosoxidans* and *B. gladioli,* which may be associated with progression of lung disease and morbidity.

S. maltophilia is an increasingly recognized pathogen impacting CF patients. Chronic infection is an independent risk factor for recurrent need for antibiotics.[150] Patients who acquire *S. maltophilia* have more advanced disease and may experience an exaggerated decline in lung function.[151] A study by Waters and colleagues looked at the impact of chronic infection with *S. maltophilia* on mortality and the need for lung transplantation.[152] A longitudinal cohort study of 687 patients revealed a nearly threefold increase in mortality for those chronically infected with *S. maltophilia* (≥2 positive sputum cultures in the previous 12 months) and did not bear out in those with intermittent infection (1 positive culture in the previous year or history of culture positivity). Unfortunately, antibiotic resistance is common in *S. maltophilia* infections. This resistance is multifactorial and related to *S. maltophilia* having limited outer membrane permeability as well as the presence of multidrug efflux pumps and antibiotic-modifying enzymes (aminoglycoside-modifying enzymes and carbapenemases).[151] A Cochrane review was performed in 2016 to assess for the effectiveness of antibiotic treatment for *S. maltophilia* in patients with CF, but the review did not reveal any supportive evidence.[153] The decision to treat is driven largely by clinician judgment, previous provider experience, and current and previous sputum or BAL culture results or both. In our center, we routinely utilize ceftazidime, levofloxacin, TMP-SMX, and minocycline.

Nontuberculous Mycobacteria

During the mid-1990s the prevalence of NTM infection among CF patients was 13%, and most were identified as *Mycobacterium avium* complex (72%), followed by *M. abscessus* (16%).[154] Risk factors associated with NTM included older age and geographic location (higher incidence in the Southwest compared to the Northeast) as well as a relatively higher association in those patients infected with *S. aureus* and *A. fumigatus* as compared with PA. The relation of spirometry results to the likelihood of finding NTM-positive cultures is unclear, and studies have found no association, a positive association, or a negative association. CFTR dysfunction may, of itself, predispose to NTM infection, since the rates of heterozygosity for *CFTR* mutations within the non-CF population with pulmonary NTM disease is between 30% and 50%.[155] Recent data from the CFF registry revealed that the prevalence rate of NTM-positive cultures was 13%.[76a] Esther and coworkers reviewed 4862 CF culture data from patients older than 8 years and also found an 11% NTM prevalence rate.[156] However, 55.6% were due to *M. abscessus* and associated with a sharper decline in FEV_1. The risk factors leading to this increased prevalence may coincide with the increased use of azithromycin in CF patients. Interestingly, a study that included over 27,000 patients detected NTM in 20% of cases (64% *M. avium* complex and 36% *M. abscessus*), with chronic azithromycin use less likely to be associated with NTM culture positivity.[157] Despite this, it is still important to screen patients for NTM to help avoid drug resistance to azithromycin.

Recent reports cite the transmissibility of *M. abscessus* among CF patients at care centers in the United States and the United Kingdom.[158,159] Gross and associates at the Tripler Army Medical Center Hospital (Hawaii) described an outbreak among a large portion of their CF cohort.[160]

The diagnosis and treatment of NTM infections remain challenging given the clinical presentation of patients with CF. Active NTM infection should be considered in patients with repeatedly smear-positive respiratory cultures, compatible radiographic findings, and lack of clinical response despite appropriate antibacterial therapy.[161]

Respiratory Viruses

The impact of viral infection on CF lung disease cannot be underestimated.[162,163] Fifty to sixty percent of exacerbations are associated with respiratory virus infections in children and are associated with FEV_1 decline, prolonged antibiotic use, and increased respiratory symptoms. With rhinovirus (69%) and influenza A/H1N1 (16.7%) leading the way, there was a 9.7% prevalence of documented viral infection detected by polymerase chain reaction among a cohort of adult patients presenting with a pulmonary exacerbation.[164] These exacerbations were associated with more severe lung disease and less response to standard therapies, and time to next pulmonary exacerbation was significantly reduced (64 days vs. 107 days). Respiratory virus coinfection may be implicated in planktonic-to-mucoid PA phenotype switching, specifically related to RSV.[49,165,166] Biofilm growth is enhanced during RSV infection compared to absence of RSV infection.

Fungal Disease

The role of fungi in the respiratory tract, particularly *A. fumigatus*, is still being elucidated.[167] When not causing ABPA, there is an increased association with disease severity when measured by FEV_1, hospitalization rates, and radiographic abnormalities.[168] A retrospective cohort study of CF patients with *A. fumigatus* infection, defined as 2 or more sputum or BAL cultures positive within a given year, found a decreased FEV_1 when compared to uninfected subjects; *A. fumigatus* was an independent risk factor for hospitalizations, the primary outcome measure of the study.[167] The role of fungal markers in serum and airway secretions can be helpful in raising suspicion for invasive fungal disease, but false-positive test results due to concomitant antibiotic use, presence of PA, or environmental contaminants should also be considered. Treatment decisions should be based on a full clinical picture, including nodular lung disease and other computed tomography–based radiologic patterns.

TREATMENT (Table 71.1)

Antimicrobial Treatment

A relationship exists between antibacterial use and improvement in lung function and survival in CF patients.[169,169a] Improved survival correlates with the discovery and introduction of additional antibiotics over the last century. It may also be evident that CF care centers with current best pulmonary functions in CF populations are also associated with higher rates of drug resistance.[170] Interestingly, several comparative trials have not shown the impact of antibacterial therapy on lung function. However, the benefits of combination therapy and incongruity with clinical experience are probably due to study design, sample size, poor definition of respiratory exacerbation, and the tremendous variability of the CF study population. Some CF patients have been on chronic antibiotics that may not have been accounted for in these studies as well. Conway and colleagues demonstrated some benefit, but this study included three antibiotics, suggesting that different combinations of antibiotics may be necessary for minimizing FEV_1 decline.[170a] These studies likely placed too much importance on the microbiology data from a single sputum culture; the end points for studies comparing single versus combination therapies are likely also flawed.

TABLE 71.1 Summary of Recommendations for Chronic Pulmonary Therapies for Patients With Cystic Fibrosis, 2013

TREATMENT	RECOMMENDATION	CERTAINTY OF NET BENEFIT	ESTIMATE OF NET BENEFIT	RECOMMENDATION[a]
Inhaled tobramycin: moderate to severe disease[b]	For individuals with CF, 6 yr of age and older, with moderate-to-severe lung disease and *Pseudomonas aeruginosa* persistently present in culture of the airways, the CF Foundation strongly recommends the chronic use of inhaled tobramycin to improve lung function, improve quality of life, and reduce exacerbations.	High	Substantial	A
Dornase alfa: moderate to severe disease[b]	For individuals with CF, 6 yr of age and older, with moderate-to-severe lung disease, the CF Foundation strongly recommends the chronic use of dornase alfa to improve lung function, improve quality of life, and reduce exacerbations.	High	Substantial	A
Ivacaftor[c]	For individuals with CF, 6 yr of age and older, with at least one G551D *CFTR* mutation, the Pulmonary Clinical Practice Guidelines Committee strongly recommends the chronic use of ivacaftor to improve lung function, improve quality of life, and reduce exacerbations.	High	Substantial	A
Inhaled aztreonam: moderate to severe disease[b]	For individuals with CF, 6 yr of age and older, with moderate-to-severe lung disease and *P. aeruginosa* persistently present in cultures of the airways, the CF Foundation strongly recommends the chronic use of inhaled aztreonam to improve lung function and quality of life.	High	Substantial	A
Dornase alfa: mild disease[b]	For individuals with CF, 6 yr of age and older, with asymptomatic or mild lung disease, the CF Foundation recommends the chronic use of dornase alfa to improve lung function and reduce exacerbations.	High	Moderate	B
Azithromycin with *P. aeruginosa*	For individuals with CF, 6 yr of age and older, with *P. aeruginosa* persistently present in cultures of the airways, the CF Foundation recommends the chronic use of azithromycin to improve lung function and reduce exacerbations.	High	Moderate	B
Inhaled tobramycin: mild disease[b]	For individuals with CF, 6 yr of age and older, with mild lung disease and *P. aeruginosa* persistently present in cultures of the airways, the CF Foundation recommends the chronic use of inhaled tobramycin to reduce exacerbations.	Moderate	Moderate	B

Continued

TABLE 71.1 Summary of Recommendations for Chronic Pulmonary Therapies for Patients With Cystic Fibrosis, 2013—cont'd

TREATMENT	RECOMMENDATION	CERTAINTY OF NET BENEFIT	ESTIMATE OF NET BENEFIT	RECOMMENDATION[a]
Inhaled hypertonic saline	For individuals with CF, 6 yr of age and older, the CF Foundation recommends the chronic use of inhaled hypertonic saline to improve lung function, improve quality of life, and reduce exacerbations.	Moderate	Moderate	B
Inhaled aztreonam: mild disease[c]	For individuals with CF, 6 yr of age and older, with mild lung disease and *P. aeruginosa* persistently present in cultures of the airways, the CF Foundation recommends the chronic use of inhaled aztreonam to improve lung function and quality of life.	Moderate	Moderate	B
Chronic use of ibuprofen (age <18 yr)	For individuals with CF, between 6 and 17 yr of age, with an FEV_1 ≥60% predicted, the CF Foundation recommends the chronic use of oral ibuprofen, at a peak plasma concentration of 50–100 μg/mL, to slow the loss of lung function.	Moderate	Moderate	B
Azithromycin without *P. aeruginosa*	For individuals with CF, 6 yr of age and older, without *P. aeruginosa* persistently present in cultures of the airways, the CF Foundation recommends the chronic use of azithromycin should be considered to reduce exacerbations.	Moderate	Small	C
Inhaled corticosteroids	For individuals with CF, 6 yr of age and older, without asthma or allergic bronchopulmonary aspergillosis, the CF Foundation recommends against the routine use of inhaled corticosteroids to improve lung function or quality of life and reduce pulmonary exacerbations.	High	Zero	D
Oral corticosteroids	For individuals with CF, 6 yr of age and older, without asthma or allergic bronchopulmonary aspergillosis, the CF Foundation recommends against the chronic use of oral corticosteroids to improve lung function or quality of life, or reduce exacerbations.	High	Negative	D
Other inhaled antibiotics	For individuals with CF, 6 yr of age and older, with *P. aeruginosa* persistently present in cultures of the airways, the CF Foundation concludes that the evidence is insufficient to recommend for or against the chronic use of other inhaled antibiotics (i.e., carbenicillin, ceftazidime, colistin, gentamicin) to improve lung function and quality of life or reduce exacerbations.	Low	—	I
Oral antipseudomonal antibiotics	For individuals with CF, 6 yr of age and older, with *P. aeruginosa* persistently present in the culture of the airways, the CF Foundation concludes that the evidence is insufficient to recommend for or against the routine use of chronic oral antipseudomonal antibiotics to improve lung function and quality of life or reduce exacerbations.	Low	—	I
Leukotriene modifiers	For individuals with CF, 6 yr of age and older, the CF Foundation concludes that the evidence is insufficient to recommend for or against the routine chronic use of leukotriene modifiers to improve lung function and quality of life or reduce exacerbations.	Low	—	I
Inhaled or oral *N*-acetylcysteine, or inhaled glutathione	For individuals with CF, 6 yr of age and older, the CF Foundation concludes that the evidence is insufficient to recommend for or against the chronic use of inhaled or oral *N*-acetylcysteine or inhaled glutathione to improve lung function and quality of life or reduce exacerbations.	Low	—	I
Inhaled anticholinergics	For individuals with CF, 6 yr of age or older, the CF Foundation concludes that the evidence is insufficient to recommend for or against the chronic use of inhaled anticholinergic bronchodilators to improve lung function and quality of life or reduce exacerbations.	Low	—	I
Chronic use of ibuprofen (age ≥18 yr)	For individuals with CF, 18 years of age and older, the CF Foundation concludes that the evidence is insufficient to recommend for or against the chronic use of oral ibuprofen to slow the loss of lung function or reduce exacerbations.	Low	—	I
Chronic inhaled β_2-adrenergic receptor agonists	For individuals with CF, 6 yr of age and older, the CF Foundation concludes that the evidence is insufficient to recommend for or against chronic use of inhaled β_2-adrenergic receptor agonists to improve lung function and quality of life or reduce exacerbations.	Low	—	I
Oral antistaphylococcal antibiotics, chronic use	For individuals with CF, 6 yr of age and older, with *Staphylococcus aureus* persistently present in cultures of the airways, the CF Foundation concludes that the evidence is insufficient to recommend for or against the chronic use of oral antistaphylococcal antibiotics to improve lung function and quality of life or reduce exacerbations.	Low	—	I

TABLE 71.1 Summary of Recommendations for Chronic Pulmonary Therapies for Patients With Cystic Fibrosis, 2013—cont'd

TREATMENT	RECOMMENDATION	CERTAINTY OF NET BENEFIT	ESTIMATE OF NET BENEFIT	RECOMMENDATION[a]
Ivacaftor	For individuals with CF, 2 yr of age and older, with class III gating mutations other than G511D or R117H (G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P, G1349D)	• Age 2–5: N/A • Age 6–18+ & FEV_1 <40: Very Low–Low • Age 6–11 & FEV_1 40–>90: Low • Age 12–18+ & FEV_1 40–>90: Moderate	—	• Age 2–5: Recommended For • Age 6–18+: Conditional For
Ivacaftor	For individuals with CF, 6 yr of age and older, with the R117H mutation	• Age 6–11 & FEV_1 <40–90: Very Low • Age 6–11 & FEV_1 >90: Low • Age 12–18+ and FEV_1 <40–>90: Very Low • Age 18+ & FEV_1 <40: Very Low • Age 18+ & FEV_1 40–90: Moderate • Age 18+ and FEV_1 >90: Low	—	• Age 6–11 & FEV_1 <40–90: Conditional For • Age 6–11 & FEV_1 >90: Conditional Against • Age 12–17 & FEV_1 <40–90: Conditional For • Age 12–17 & FEV_1 >90: Conditional Against • Age 18+: Conditional For
Ivacaftor-lumacaftor	For individuals with CF, 6 yr of age and older, with two copies of F508del	• Age 6–11: Very Low • Age 12–18+ and FEV_1 <40–90: Moderate • Age 12–18+ and FEV_1 >90: Low	—	• Age 6–11: Conditional For • Age 12–18+ & FEV_1 <40–90: Strong For • Age 12–18+ & FEV_1 >90: Conditional For

[a]Strength of Pulmonary Clinical Practice Guidelines Committee recommendations: *A,* substantial net benefit; *B,* moderate net benefit; *C,* small net benefit; *D,* no net benefit; *I,* the committee concludes that the current evidence is insufficient to assess the balance of benefits and harms of the service. Evidence is lacking, of poor quality, or conflicting, and the balance of benefits and harms cannot be determined.
[b]Severity of lung disease is defined by FEV_1 percent predicted as follows: normal, >90% predicted; mildly impaired, 70%–89% predicted; moderately impaired, 50%–69% predicted; and severely impaired, <40% predicted.[176]
[c]CF Foundation personnel did not participate in any activity related to ivacaftor.
CF, cystic fibrosis; *CF Foundation,* Cystic Fibrosis Foundation; *FEV_1,* forced expiratory volume in 1 second.
Data from Mogayzel et al.,[177] *Rowe et al.,*[213] *and Sawicki et al.*[212]

Azithromycin

Reduction in morbidity and mortality from macrolide antibiotics in diffuse panbronchiolitis, a rare inflammatory lung disease similar to CF in many ways, led to a number of studies in CF patients, including a randomized controlled study involving 185 patients.[171] Participants in the treatment arm experienced fewer respiratory exacerbations at the end of 168 days and their FEV_1 improved. The mechanism of macrolide-associated improvement in lung disease is still uncertain, but azithromycin is thought to modulate inflammation and may in fact have some direct antimicrobial impact.[172,173] Assays to emulate the airway environment and bacterial biofilm, and nonconventional biofilm susceptibility testing, have been used to demonstrate azithromycin inhibition of PA in biofilm. The antiinflammatory impact of azithromycin has been described using distinct models and includes demonstration of a decrease in C-reactive protein and a reduction in BAL fluid neutrophil cell count, neutrophil elastase, interleukin-1β, interleukin-8, and other mediators.[174] Despite a recent study contradicting some of these observations,[175] there is overall wide acceptance of azithromycin's role as an antiinflammatory in CF. In 2007, guidelines on chronic pulmonary therapies were published and included a high recommendation for chronic azithromycin in those CF patients chronically infected with PA.[176] A follow-up publication in 2013 reiterated this recommendation and also reflected on studies of azithromycin for those not infected with PA.[177]

Inhaled Antibiotics

Aerosolized antibiotics have become a mainstay of acute and chronic therapy in patients with CF. Prior to FDA approval of various formulations of inhaled antibiotics, several drugs meant for intravenous use were being nebulized to treat airway infection.[178] Since FDA approval of tobramycin inhalation solution (TIS) in 1997 and the study on intermittent aerosolization of 300 mg of preservative-free tobramycin for patients chronically infected with PA, two other inhaled antibiotic formulations have been FDA approved, and a number of other formulations are in the CFF clinical trials pipeline.[179] The 24-week study of 520 patients receiving either TIS or placebo demonstrated improved FEV_1, decreased density of PA in expectorated sputum, and decreased rates of hospitalization. Despite an increase in drug resistance, there was no significant impact of emergent drug resistance on lung function. The recent EAGER trial studied the safety, efficacy, and convenience of tobramycin inhalation powder (TIP), and results led to FDA approval of TIP in 2013.[180] There was a higher drop-out rate in the TIP group due to cough, but overall, the new formulation has demonstrated better adherence since administration takes 4 to 6 minutes compared to 15 to 20 minutes with TIS.

Aztreonam lysine for inhalation (AZLI) was FDA approved in 2010 based on results from multiple studies, including a randomized, double-blind, placebo-controlled clinical trial.[181–183] AZLI resulted in significant improvement in FEV_1, reduced density of PA, and improvement in respiratory symptoms as measured by the Cystic Fibrosis Questionnaire–Revised (CFQ-R) Respiratory Scale in a study that involved 164 CF patients with moderate-to-severe lung disease.[181]

Colistin is the most well known antipseudomonal antibiotic not FDA approved, but widely used to treat acute exacerbations and chronic infection with PA. A recent death attributed to inhalation of colistimethate, the inactive prodrug of colistin, as noted in an FDA Alert in 2007, has led to more hesitancy to use inhalation of drugs intended for parenteral use. A number of other inhaled antibiotics are in various phases of active clinical trials, including inhaled levofloxacin and vancomycin.[179]

Liposomal inhaled amikacin (LIA) recently received limited approval by the FDA for patients infected with NTM disease. The approval was in part based on a phase II, randomized, double-blind, placebo-controlled clinical trial for treatment-refractory NTM with two treatment arms; one arm received LIA in addition to a multidrug antimicrobial regimen while the second arm did not receive LIA.[184] Despite not achieving the primary end point, more patients in the LIA group had negative sputum cultures at the end of the study period, and it was determined to be safe in a limited patient population with unmet medical needs, as

postulated by the Limited Population Pathway for Antibacterial and Antifungal Drugs under the 21st Century Cures Act.[184a] Further studies are required to determine whether LIA will be efficacious for those patients with acute or chronic infection with PA.

Cystic Fibrosis–Specific Antibiotic Treatment Approaches

There are many questions yet to be answered regarding the treatment of CF respiratory infections. Given susceptibility data in particular patients, how should providers go about selecting antibiotics and what is the optimal length of therapy? The latter question was studied during the STOP trial, a prospective observational clinical trial sponsored by the CFF to evaluate current physician treatment practices and patient outcomes. The multicentered study revealed substantial variation in treatment duration and regimen. The average treatment length was 15.9 days, and individuals with more advanced lung disease received longer treatment courses. Of note, the study revealed that the mean absolute increase in FEV_1 from admission was 9% predicted at the end of antibiotics, and only 39% of participants fully recovered lung function, and only 65% recovered 90% of lung function that was lost.[185] This study laid the ground work for the STOP2 trial, which is an ongoing randomized trial evaluating the effectiveness of different intravenous treatment lengths based upon changes in lung function.[185a] In many cases, susceptibility data from recent and prior bacterial colonies isolated in sputum or BAL are still used to help determine antimicrobial choices. However, the combination of previous patient experience, drug toxicities, and allergies, in addition to the susceptibility data, should also be incorporated into making decisions regarding APE treatment.

Due to the increased volume of distribution and enhanced renal clearance of antibiotics in CF patients, they generally require larger doses of antibiotics compared to the general population.[186]

Patients who develop APE receive treatment directed at current or recent respiratory bacterial isolates. Patients who have chronic PA infection receive a combination of at least two antipseudomonal agents, with the intent of minimizing emergence of resistance given high baseline bacterial density and treating subpopulations of PA that may have not been identified on sputum sampling. Patients chronically infected with *S. aureus* receive treatment directed at MSSA or MRSA. In addition, patients with acute pulmonary infections should be tested for respiratory viruses, especially during influenza season. Patients may develop noninfectious complications related to CF that need to be contemplated in the differential diagnosis of APE, such as hemoptysis, pneumothorax, lobar atelectasis, and pulmonary embolism.

The negative impact of chronic infection with PA on CF patients, and the benefit of delaying chronic acquisition, has led to investigations to identify and treat early infection with PA and improve efficacy of eradication protocols, as discussed earlier. The largest of these trials is the randomized EPIC study investigating four separate treatment arms.[112–115] All patients received an initial course of TIS with or without ciprofloxacin, followed either by cycled antibiotic therapy regardless of respiratory cultures or symptoms or by culture-based therapy only when PA was isolated. No significant differences in clinical or microbiologic outcomes were found, but the EPIC study has led to more universal acceptance of eradication as a strategy. Subsequent studies have confirmed the decrease in PA recurrence after early identification, but this practice has not necessarily led to decreased hospitalization rates when compared to less aggressive protocols predating the EPIC trial.[115] Caregivers continue to aggressively treat patients with new-onset PA, but treatment regimens continue to vary despite the EPIC data.

Once-daily aminoglycoside administration has been widely adopted following the results of the TOPIC study.[187] Once-daily administration of tobramycin (10 mg/kg/day) was shown to be as efficacious as three times daily in treating pulmonary exacerbations and potentially less nephrotoxic. It is not clear if there is increased risk for sensorineural hearing loss, associated with increased prevalence in CF patients exposed to topical or inhaled aminoglycosides, but once-daily dosing is currently under further examination.[188]

A recent study has raised concerns regarding the coadministration of azithromycin and tobramycin. A study looking at PA-associated cutaneous thermal injuries led to the observation that azithromycin reduced the antimicrobial activity of tobramycin.[189] This antagonism was not demonstrated with ciprofloxacin. Another recent study looked at the change in lung function, as well as time to subsequent antibiotic requirement, quality of life (as measured by the CFQ-R Respiratory Scale score), and change in sputum PA density.[190] The data for the study were based on the Gilead Sciences randomized comparator trial involving 268 patients comparing AZLI to TIS.[191] In vitro data suggested a potential drug-drug interaction between tobramycin and azithromycin, based on the effects of combination drugs (azithromycin plus tobramycin and azithromycin plus aztreonam lysine). Subjects randomized to TIS and on chronic azithromycin therapy demonstrated less improvement in FEV_1, decrease in time to next exacerbation, less improvement in disease-related quality of life, and no change in sputum PA density. Though not enough to change treatment practice just yet, these findings are provocative and may impact future management of PA infection in CF.

Prolonged or continuous infusion of β-lactam antibiotics has been utilized with more frequency. The short half-life and lack of significant postantibiotic effect of β-lactam antibiotics makes them an attractive alternative, and duration above MIC helps determine efficacy.[192–194]

Non–Antibacterial-Based Treatments

In addition to antimicrobials, a combination of antiinflammatories, mucolytics, airway hydrating agents, airway clearance techniques, and nutritional supplements, among others, have been studied and have been incorporated into CF clinical practice.

Recombinant human DNase (dornase alfa) has been a mainstay of chronic respiratory therapy in CF since the early 1990s.[195] Dornase alfa helps break down large amounts of DNA (human, bacterial) and improves the rheology of airway secretions, aids in mucolysis, and facilitates airway clearance. The use of dornase alfa is strongly recommended for CF patients, including asymptomatic patients or those with mild disease.[176]

Hypertonic saline improves hydration of the airway surface liquid layer and improves mucociliary clearance in patients with CF.[196] In a multicenter randomized controlled trial, Elkins and coworkers demonstrated the benefits of 7% saline for inhalation.[196] Although the improvement in lung function did not achieve significance, there was a significant reduction in pulmonary exacerbations and need for antibacterial treatments. Given the impact of inhaled hypertonic saline on the airway surface liquid layer and on the basic defect causing CF manifestation in the airway, another multicenter randomized controlled trial involving CF infants ages 4 to 60 months, the Infant Study of Inhaled Hypertonic Saline in CF (ISIS), was pursued. Though the study did not achieve it primary outcome, this was likely due to lack of reliable outcome measures in this age group.[197]

Given the association of an exuberant immune response and advancing lung disease, antiinflammatory agents continue to be commonly used to treat CF lung disease.[198] Inhaled steroids were used indiscriminately given their ease of delivery and presumed benefit without significant downside. Although no studies were able to demonstrate a benefit in patients other than those with airway hyperreactivity or ABPA, an inhaled corticosteroid withdrawal study did not demonstrate a difference between exacerbation rates among subjects.[199] Oral glucocorticoids are not recommended for routine use outside of ABPA or asthma. Despite the benefit in lung function stabilization, the observation of hyperglycemia and diabetes, growth retardation, cataracts, osteoporosis, and higher rates of PA culture positivity indicate that the risks outweighed the potential benefits.[200,201] Konstan and associates performed a randomized, double-blind, placebo-controlled trial that demonstrated a significant reduction in the rate of FEV_1 decline, with the impact greatest in subjects younger than age 13 and with no difference observed in an older cohort.[202]

The concern for renal toxicity exists for patients receiving other agents associated with nephrotoxicity (e.g., ibuprofen) or dehydration, but a recent study did not observe an elevation in biomarkers of kidney injury.[203] Despite one small randomized, placebo-controlled trial that showed an improvement in FEV_1 of 8%, the CFF guidelines committee recommended against routine use of ibuprofen.[177]

Restoring CFTR Function

CF has been an exemplar of precision medicine, which was underscored by the invitation of a medical student with CF to the 2015 State of the

Union, where President Barack Obama announced the National Institutes of Health Precision Medicine Initiative known as the All of Us Research Program.[205] The promise of gene therapy for CF was at the forefront following the discovery of the *CFTR* gene in 1989, but has failed to deliver a much-anticipated cure. While there is still hope for traditional gene therapy, recent advances in gene editing have demonstrated real promise. High-throughput screening of small molecules helped launch a new treatment paradigm. Instead of treating symptoms and the manifestations of the disease, new FDA-approved therapies target basic *CFTR* defects at the protein level, while promising clinical trials targeting DNA/RNA and stem cell biology are in progress. Our knowledge of the mechanism by which a mutation leads to a particular abnormal CFTR protein expression, or lack of it, has led to mutation-specific therapies. G551D, a mutation shared by just over 4% of CF patients, results in a gating defect or class III mutation.[57a] The small molecule ivacaftor (corrector molecule) was shown to correct the mutated CFTR protein on the cell surface.[206] A 48-week randomized, double-blind, placebo-controlled trial demonstrated significant improvements in lung function (FEV_1) and BMI, along with reduction in respiratory symptoms based on the CFQ-R, APE, and sweat chloride test.[207] FDA approval came in 2012, and ivacaftor became the first small molecule in a therapeutic pipeline to expand treatment eligibility from 4% to 90% by 2020.

The most common *CFTR* mutation—F508del, with a near 85% prevalence—was the next target of small-molecule therapy. A total of 1108 patients enrolled in two phase III clinical trials of ivacaftor in combination with lumacaftor, a potentiator molecule to "chaperone" the misfolded protein resulting from *CFTR* mutation F508del, led to a modest but significant improvement in lung function as well as exacerbation-free days and reduced hospitalizations.[208] However, drug-drug interactions, including oral contraceptives and antidepressants, as well as complications of airway hyperreactivity in a small subset of patients led to development of a second corrector molecule. Once-daily tezacaftor, in combination with twice-daily ivacaftor, was introduced as an alternative to lumacaftor and led to a similar but slightly greater improvement in lung function.[209] While lumacaftor-ivacaftor was approved for those patients who are F508del homozygous only, tezacaftor-ivacaftor expanded coverage to compound heterozygotes with F508del and a *CFTR* residual function mutation. While these therapies increased accessibility of modulator therapies to nearly 50% of the CF population, a significant portion are still left out. Next-generation correctors to complement tezacaftor-ivacaftor have demonstrated significantly superior outcomes in comparison to tezacaftor-ivacaftor alone. In addition to the results of the phase II trial, a recent press release from Vertex Pharmaceuticals Inc. reveals the promise of the triple combination therapies. Next-generation correctors VX-659 and VX-445, when combined with tezacaftor-ivacaftor for patients who are heterozygotes for F508del and the minimal function *CFTR* mutation (F508del/MF), demonstrated a 13.3-point and a 13.8-point increase in percent predicted FEV_1 ($ppFEV_1$), respectively.[210,211] For those patients with two copies of F508del (F508del/F508del) and already on tezcaftor-ivacaftor, addition of VX-659 and VX-445 resulted in a 9.7-point and an 11.0-point increase in $ppFEV_1$, respectively. Preliminary results from recently completed phase III clinical trials involving VX-659, tezacaftor, and ivacaftor revealed a 14.0-point increase in $ppFEV_1$ after 4 weeks for F508del/MF patients and a 10.0-point increase in $ppFEV_1$ for F508del/F508del patients. With the successes of small-molecule CFTR modulators, there are four other sponsors of modulators in early phase II clinical trials.[179] These therapies will not reverse damaged, bronchiectatic lungs, while children and those with very mild disease experience the biggest impact on morbidity and mortality. Earlier introduction of these therapies may significantly delay the impact of infectious complications and reduce the rate of disease progression, as observed with ivacaftor in patients with at least one copy of G551D[212] (Fig. 71.5). These effective therapies provide an opportunity to study the change in microenvironment and airway surface liquid of the lung and its impact on bacteria infecting the airways.

Ongoing longitudinal observation studies are in progress for patients on various combinations of modulators, including PROMISE, a new study to follow those patients on the next generation of CFTR modulators (awaiting submission). The G551D Observational Study (GOAL) involving patients with the *CFTR* mutation G551D prescribed ivacaftor is still providing a wealth of information on various outcome measures, including lung function, nutrition, mucociliary clearance, intestinal pH, sputum inflammation and microbiome, and sweat secretion rate, among others.[213] Though there are conflicting data from a cohort of 12 subjects in Ireland, where an eradication of or sustained reduction in PA sputum density was observed, the much larger GOAL study reveals a significant reduction in PA culture positivity: a 35% reduction compared to the year prior to initiation of ivacaftor, particularly associated with patients with a higher FEV_1 prior to starting ivacaftor[214] (Fig. 71.6). The impact on CFTR and alteration in the microenvironment of the airway may be playing a role, but antimicrobial properties of ivacaftor may also be

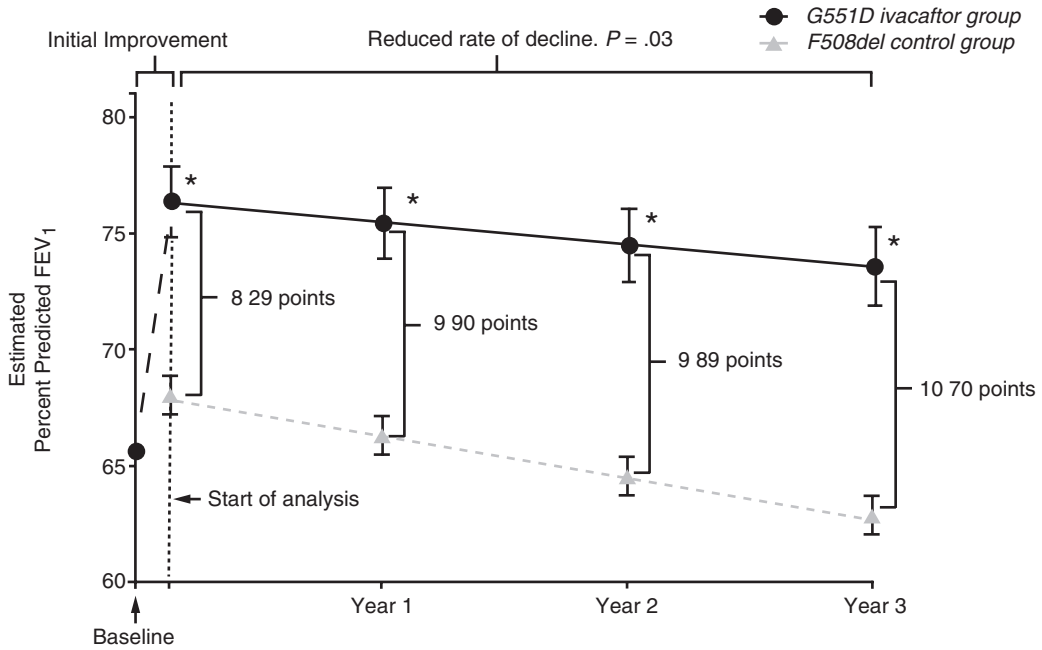

FIG. 71.5 Estimated percentage predicted FEV_1 measures (±SE) at years 1, 2, and 3. *$P < .001$. *FEV_1*, Forced expiratory volume in 1 second; *SE*, standard error. *(From Sawicki GS, McKone EF, Pasta DJ, et al. Sustained Benefit from ivacaftor demonstrated by combining clinical trial and cystic fibrosis patient registry data.* Am J Respir Crit Care Med. *2015;192:836-842.)*

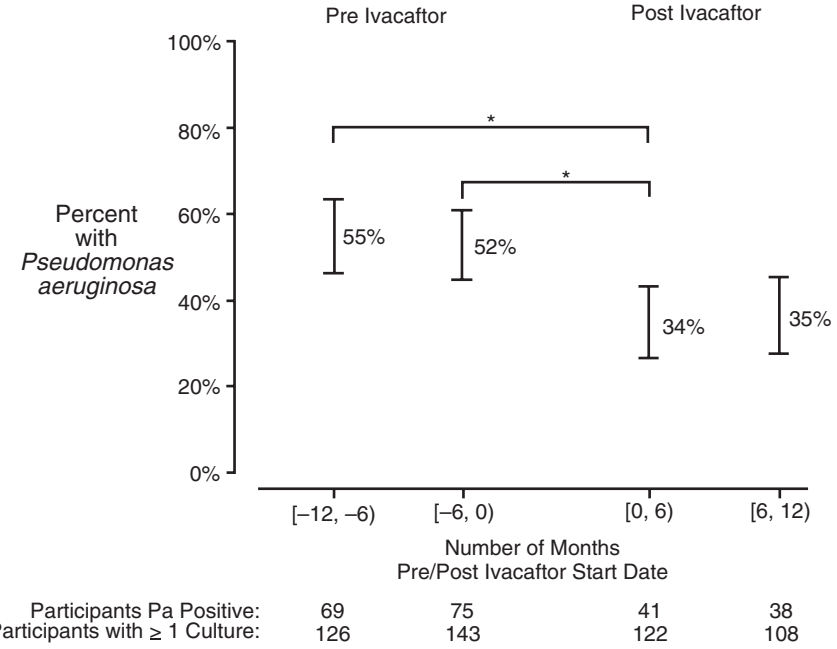

FIG. 71.6 Clinical measures obtained through the Cystic Fibrosis Foundation National Patient Registry in 6-month intervals before ivacaftor and after ivacaftor. *Pseudomonas aeruginosa* (*Pa*) positivity among those with a recorded respiratory culture in 6-month intervals. Means and 95% confidence intervals; *$P < .001$. *(From Rowe SM, Heltshe SL, Gonska T, et al. Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis.* Am J Respir Crit Care Med. *2014;190:175-184.)*

implicated.[208] Ivacaftor has a quinoline ring similar in structure to the quinolone antibiotics, and has demonstrated antimicrobial activity via reduced bioluminescence of bacteria such as *S. aureus* and PA. These properties were enhanced in combination with other antibiotics. The earlier introduction of more effective modulators will very likely have an impact on the epidemiology of infection in CF in years to come.

The therapeutic pipeline also includes a host of preclinical and phase I clinical trial studies, including gene editing techniques such as CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9) and stem cell biology. Despite the nearly 90% of CF patients worldwide who may have access to modulator therapies by the year 2020, there are a large number of CF patients with no clear therapeutic options in the near future. There is a great emphasis on developing *CFTR* mutation agnostic approaches to fixing CFTR to address this increasingly desperate option. Though some nonsense mutations may respond to small-molecule modulators, the great majority will require a different treatment paradigm that may also apply to other genetically inherited orphan diseases.[215]

INFECTION CONTROL

Transmission of pathogens among patients is a significant concern for CF centers and their patients. The CFF Consensus Conference on Infection Control Practices published recommendations in 2003 to help prevent patient-to-patient transmission.[216] Their suggestions apply not only to ambulatory clinics and inpatient setting, but to non–health care environments as well. Acquisition of new CF pathogens may come from the environment, but transmission from direct and indirect contact with CF patients has been demonstrated.[144] Outbreaks have involved PA, BCC, MSSA and MRSA, *Achromobacter, M. abscessus,* and others.[159,217] Updated 2013 CFF guidelines recommend that all CF centers review their quarterly surveillance reports to assess the incidence and prevalence of respiratory tract pathogens at their centers and perform molecular typing utilizing an appropriate genotyping method.[218] Recent studies have shown that aerosolized PA particles travel greater than 3 feet and can survive on surfaces for more than 2 hours.[219–222] Therefore the CFF Infection Prevention and Control 2013 guidelines recommend that all people with CF, regardless of their respiratory tract culture results, should be separated by at least 6 feet from other people with CF in all settings and wear a surgical mask when in health care settings to reduce the risk of droplet transmission. Contact precautions and placing patients with CF in a single-patient room, for both inpatient and ambulatory patients, remain mainstay measures. In the clinic setting, patients with CF should have minimal time in common waiting areas and be placed into an examination room immediately. As with all patients, health care providers should perform hand hygiene with an alcohol-based hand rub, or with antimicrobial soap and water, when hands might be contaminated with pathogens.[218]

LUNG TRANSPLANTATION

Lung transplantation is a treatment option for CF patients who develop end-stage lung disease, and successful transplantation is associated with an increased quality of life and probably increased life expectancy. Here we comment on infectious disease considerations relevant to CF patients who undergo lung transplantation.

Lung transplantation is a contaminated surgery, especially in patients with CF, during which airway and pulmonary secretions are expected to spill into the chest cavity. Bilateral lung transplantation is the norm for these patients. Most early infections in lung transplant recipients are surgical site infections associated with the pleural space, fractured ribs, or soft tissue and, more rarely, anastomotic site infections.

As part of the pretransplantation evaluation, all recent respiratory tract bacterial isolates are taken into consideration in preparing a tailored perioperative antimicrobial regimen. Similar to pretransplantation treatment of APE, two antipseudomonal antibacterials are administered perioperatively. If patients are chronically infected with multidrug-resistant PA strains (susceptible only to colistin), we typically administer a recent regimen that was associated with clinical improvement or stabilization, even if it does not contain colistin. This antibacterial regimen is continued for 2 weeks postoperatively and is adjusted by day-of-transplant bronchial cultures obtained from the recipient and donor. In addition, we give antibacterial prophylaxis for MRSA and continue treatment for 4 weeks if MSSA or MRSA is isolated on day-of-transplant cultures. All patients receive inhaled colistin or tobramycin during their initial hospitalization to minimize the risk of anastomotic site infection.

A majority of lung transplantation centers adopted "universal" prolonged voriconazole prophylaxis when this antifungal was approved in 2002. Although this has probably led to lower incidence of fungal

disease, most chronic toxicities of voriconazole use have been described in this population, especially phototoxicity, and periostitis probably from voriconazole-associated fluorosis. Analysis of fungal disease epidemiology in lung transplantation patients at our center pointed to the fact that most fungal disease was related to perioperative contamination with *Candida* and *Aspergillus,* and was not a result of increased immunosuppression. Given these data, we implemented a "targeted" strategy in which all patients receive micafungin as part of their perioperative antimicrobial regimen, beginning with induction of anesthesia and continued for 7 to 10 days. Patients also received inhaled amphotericin twice daily during their initial hospitalization or if they receive treatment for acute rejection. Once the explant pathology and day-of-transplant cultures are available, patients who had positive cultures for yeast or molds are treated with fluconazole or voriconazole, depending on fungal species isolated and susceptibilities, and are continued on this regimen for 3 to 6 months.[223] Patients whose cultures remain negative go home without any systemic antifungal. With this strategy, around 10% of transplanted patients go home on voriconazole, and the incidence of fungal disease is comparable to centers that use "universal" strategies.[224] *M. abscessus* infection in patients who undergo lung transplantation is infrequent, but highly problematic.[225] We continue TMP-SMX prophylaxis for life as *Pneumocystis jirovecii* and antibacterial prophylaxis.

Given infection control measures in place and younger age, CF patients tend to be cytomegalovirus (CMV) seronegative (R–), and thus are at higher risk of CMV disease when receiving lungs from CMV-seropositive donors (D+). The most common approach is to use valganciclovir prophylaxis for at least 1 year for patients who are CMV D+/R–, but 6 months of prophylaxis is likely sufficient for patients who are CMV R+.[226,227] Similarly, CF patients may be Epstein-Barr virus (EBV) seronegative at the time of lung transplant. This confers a very high risk (≥50%) of EBV-associated posttransplantation lymphoproliferative disease if they receive lungs from an EBV-seropositive donor.

Posttransplantation, lungs no longer contain CFTR-affected cells, but other CFTR-affected mucosal surfaces remain. Management of chronic sinusitis and polyposis associated with CF needs to continue posttransplantation. Sinusal chronic infections remain a reservoir for CF-associated bacteria. This requires judicious interpretation of cultures obtained during surveillance bronchoscopies, since cultures may represent oral or sinus contamination at the time of the procedures. Although some centers treat all BAL isolates, we do not pursue treatment of positive cultures in the absence of lung parenchymal or compatible respiratory symptoms.

FUTURE DIRECTIONS

Despite the numerous advances that have been made in CF care and treatment, there is still more work to be done. The Cystic Fibrosis Foundation is currently engaged in assisting with the development of the next generation of medications to aid in the treatment of CF. This drug development pipeline is robust and includes new modulator therapies, antiinflammatories, antiinfectives, mucociliary clearance medication, and gastrointestinal therapies (https://www.cff.org/Trials/pipeline).

At the 2018 North American Cystic Fibrosis Conference, the Cystic Fibrosis Foundation announced a commitment of 100 million dollars for the development of the Infection Research Initiative.[228] This initiative is envisioned to be a comprehensive approach to improve outcomes with infection through enhanced detection, diagnosis, prevention, and treatment. The Infection Research Initiative will take a multifaceted approach to advancing infection research, including identification of novel approaches to detect microorganisms and diagnose infection, and to enhance understanding of CF microorganisms and their acquisition. This is intended to develop safe and effective antiinfective agents, optimize currently utilized treatment regimens, evaluate the long-term impact of chronic antibiotic utilization, and characterize the effect of modulator therapy on infections.[228]

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The complete reference list is available online at Expert Consult.

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72 Urinary Tract Infections

Jack D. Sobel and Patricia Brown

SHORT VIEW SUMMARY

PATHOLOGIC CHARACTERISTICS

- With acute pyelonephritis there is suppurative necrosis or abscess formation.
- With chronic pyelonephritis there is uneven scarring.
- With papillary necrosis one or more pyramids may slough.

PATHOGENESIS

- Most urinary tract infections (UTIs) are the result of retrograde ascending infection, consequent to numerous behavioral factors.
- Because of efficient host defense mechanisms, bacterial pathogens capable of causing UTIs (uropathogens) are selected by virtue of genetically determined virulence factors.
- Virulence factors facilitate microorganism persistence in the urinary tract.
- Virulence factors include adhesins, bacterial capsules, aerobactin, cytotoxic necrotizing factors, hemolysins, and siderophore receptors.
- In functional and structurally compromised urinary tracts, there is reduced requirement for virulence factors in order to produce infection.

HOST DEFENSES

- Nonimmune mechanisms, especially antibacterial activity of urine, shearing force of micturition, and urine flow, are highly effective in reducing UTI frequency.
- Adaptive immunity—both antibody- and cell-mediated mechanisms—have a limited protective role.
- Innate immunity based on uroepithelial cells and response to adherent microbes reflects a complex recognition and reactive proinflammatory response consisting of multiple cytokines and inflammatory cells.
- Protective cytokine response is genetically determined and influences outcome spectrum from asymptomatic bacteriuria to suppurative renal disease.
- Genetic susceptibility to UTI is still minimally recognized and understood.

EPIDEMIOLOGY AND NATURAL HISTORY

- *Escherichia coli* is the most common infecting organism.
- Resistant *E. coli* and other resistant bacteria are increased in health care–related infection compared with community infection.
- Resistant bacteria are increased in complicated UTI.
- Asymptomatic bacteriuria is common and of little consequence except in a few groups.
- UTIs occur in 1% to 2% of infants, about 5% of girls, and 0.5% or less of boys.
- Renal damage is related to vesicoureteral reflux, which occurs in 30% to 50% of preschool children with infection; structural or functional obstruction also causes damage.
- UTIs are much more common in women than men and are most often asymptomatic.
- Up to 60% of women have symptomatic UTIs during their lifespan, and 10% of women have UTIs each year.
- Two percent to 5% of women have recurrent UTIs with a genetic predisposition.
- UTI in men is uncommon but often associated with structural or functional abnormality.
- Renal infection rarely causes end-stage renal disease without other underlying disease.

DIAGNOSIS

- Urine dipsticks for pyuria and bacteriuria are useful screening tests.
- A negative test for pyuria makes UTI unlikely.
- Negative tests for bacteriuria are common in UTI because of low titers of bacteria.
- Two urine cultures with greater than or equal to 10^5 of the same uropathogen/mL urine are required for diagnosis of asymptomatic bacteriuria.
- One culture with 10^2/mL or more of a gram-negative bacillary uropathogen is diagnostic in symptomatic UTI.
- One-third of young women with cystitis have fewer than 10^5 bacteria/mL urine.

MANAGEMENT AND TREATMENT

(see Table 72.6)

- All symptomatic UTIs are usually treated for relief of symptoms.
- Most asymptomatic infections should be neither sought nor treated because of lack of benefit; exceptions are during pregnancy and those persons who are to have traumatic urologic procedures. Controversial exceptions are some young children and after renal transplantation.
- Nonantimicrobial prevention measures are directed at reinfections and reducing risk factors. They include avoidance of spermicidal contraceptives and catheterization and use of topical estrogens in postmenopausal women.
- Treatment for infants younger than 3 months involves a β-lactam and an aminoglycoside intravenously.
- Treatment for infants older than 3 months is as mentioned previously for those seriously ill and an oral β-lactam or trimethoprim-sulfamethoxazole (TMP-SMX) for others.
- Treatment for acute, uncomplicated pyelonephritis in nonpregnant women is a urine culture and then fluoroquinolone.
- Treatment for uncomplicated cystitis in women is short-course therapy with nitrofurantoin, TMP-SMX, fosfomycin, or pivmecillinam.
- Treatment for complicated UTI is a urine culture and then fluoroquinolone. For cystitis, nitrofurantoin and fosfomycin are options.
- Treatment of relapses is prolonged therapy or chronic suppression. Complicated UTI or prostatitis is possible.
- Reinfections can be self-treated per episode or prevented using single-dose prophylaxis for intercourse or long-term prophylaxis.

PREGNANCY

- Asymptomatic bacteriuria of pregnancy requires therapy and posttreatment surveillance to reduce the risk of maternal pyelonephritis, hypertension, and preterm delivery.
- UTI treatment options are reduced given lack of availability of quinolones, tetracyclines, and sulfonamides (at term) as treatment options.

IMAGING

- The ever-improving quality and availability of renal ultrasonography has facilitated the diagnosis of renal complications and underlying urologic abnormalities. Excretory urography (intravenous pyelography) has fast disappeared, replaced by computed tomography scan and magnetic resonance imaging examinations.
- The use of imaging studies in infants and preschool children with febrile UTIs remains controversial, with reduced reliance on cystourethrography and the preference shifting to a "top-down" approach to selecting children who would most benefit from investigation.

Bacteriuria is a frequently used term and literally means "bacteria in the urine." The probability of the presence of infected urine in the bladder can be ascertained by quantifying the numbers of bacteria in voided urine or in urine obtained via urethral catheterization. *Significant bacteriuria* is a term that has been used to describe the numbers of bacteria in voided urine that usually exceed the numbers caused by contamination from the anterior urethra (i.e., $\geq 10^5$ bacteria/mL). The implication was that in the presence of at least 10^5 bacteria/mL of urine, infection must be seriously considered, and that with less than 10^5/mL, infection was unlikely. The 10^5 criterion was of significance historically. Currently it is of value only for diagnosing asymptomatic bacteriuria, which is important to treat in only limited circumstances.

TERMINOLOGY

The term *cystitis* has been used to describe the syndrome involving dysuria, frequency, urgency, and occasionally suprapubic tenderness. However, these symptoms may be related to lower tract inflammation without bacterial infection and can be caused by urethritis (e.g., gonorrhea, chlamydial urethritis). Furthermore, the presence of symptoms of lower tract infection without upper tract symptoms by no means excludes upper tract infection, which may also be present.

Acute pyelonephritis describes the clinical syndrome characterized by flank pain, tenderness, or both, and fever, often associated with dysuria, urgency, and frequency. However, these symptoms can occur in the absence of infection (e.g., in renal infarction or renal calculus). A more rigorous definition of acute pyelonephritis is the previously described syndrome accompanied by an indication of acute infection in the kidney.

Uncomplicated urinary tract infection (UTI) refers to infection in a structurally and neurologically normal urinary tract. The generally accepted definition of *complicated UTI* includes infection in the presence of factors that predispose to persistent or relapsing infection, such as foreign bodies (e.g., calculi, indwelling catheters or other drainage devices); obstruction; immunosuppression; renal failure; renal transplantation; and urinary retention from neurologic disease.[1,2] In addition, infection in men, pregnant women, children, and patients who are hospitalized or in health care–associated settings may be considered complicated. In the patient with complicated infection, infecting microorganisms are more likely to be resistant to antimicrobial agents. Recurrences of UTI may be relapses or reinfections. *Relapse* of bacteriuria refers to a recurrence of bacteriuria with the same infecting microorganism that was present before therapy was started. This is caused by the persistence of the organism in the urinary tract. *Reinfection* is a recurrence of bacteriuria with a microorganism different from the original infecting bacterium. It is a new infection. Reinfection may occur with the same microorganism, which may have persisted in the vagina or feces. This can be mistaken for a relapse.

The term *urosepsis* has been commonly used to describe the sepsis syndrome caused by UTI. Although used in the literature, this term should not be used in clinical documentation because it is not considered synonymous with sepsis for the purposes of coding. Utilizing the term *sepsis* and indicating the specific clinical syndrome to which the sepsis is secondary (e.g., pyelonephritis or acute prostatitis) is a better way to refer to sepsis secondary to infection in the urinary tract.

Increasingly the term *febrile urinary tract infection* is utilized; these infections can occur with or without concomitant sepsis.[3]

The term *chronic UTI* has little meaning in many patients. True chronic infection should really mean persistence of the same organism for months or years with relapses after treatment. Reinfections do not mean chronicity any more than repeated episodes of pneumonia indicate chronic pneumonia.

The term *chronic pyelonephritis* means different things to different authors. To some, chronic pyelonephritis refers to pathologic changes in the kidney caused by infection only. However, identical pathologic alterations are found in several other entities, such as chronic urinary tract obstruction, analgesic nephropathy, hypokalemic nephropathy, vascular disease, and uric acid nephropathy. Pathologic descriptions do not (and cannot) differentiate between the changes produced by infection versus those produced by these other entities.

Papillary necrosis from infection is an acute complication of pyelonephritis, usually in the presence of diabetes mellitus, urinary tract obstruction, sickle cell disease, or analgesic abuse. Papillary necrosis can occur in the absence of infection in some of these conditions. The necrotic renal papillae may slough and cause unilateral or bilateral ureteral obstruction. Intrarenal abscess may result from bacteremia or be a complication of severe pyelonephritis. Perinephric abscess occurs when microorganisms from the renal parenchyma or blood are deposited in the soft tissues surrounding the kidneys. Acute lobar nephronia, also called acute focal bacterial nephritis, is a radiologic diagnosis (most commonly on computed tomography scanning) consisting of edema and inflammation without liquefaction of one infected kidney lobe in a patient with the clinical syndrome of acute pyelonephritis.

PATHOLOGIC CHARACTERISTICS[4]

Acute Pyelonephritis

In severe acute pyelonephritis, the kidney is somewhat enlarged and discrete, yellowish, raised abscesses are apparent on the surface. The pathognomonic histologic feature is suppurative necrosis or abscess formation within the renal substance.

Chronic Pyelonephritis (Chronic Interstitial Nephritis)

In chronic pyelonephritis, one or both kidneys contain gross scars, but even when involvement is bilateral, the kidneys are not equally damaged. This uneven scarring is useful in differentiating chronic pyelonephritis from diseases that cause symmetrical contracted kidneys (e.g., chronic glomerulonephritis). There are inflammatory changes in the pelvic wall with papillary atrophy and blunting. The parenchyma shows interstitial fibrosis with an inflammatory infiltrate of lymphocytes, plasma cells, and occasionally neutrophils (Fig. 72.1). The tubules are dilated or contracted, with atrophy of the lining epithelium. Many of the dilated tubules contain colloid casts, which suggest the appearance of thyroid tissue ("thyroidization" of the kidney). There is also concentric fibrosis about the parietal layer of the Bowman capsule (termed *periglomerular fibrosis*) and vascular changes similar to those of benign or malignant arteriolar sclerosis.

Many studies have found little correlation between these pathologic findings and evidence for past or present UTI. Clearly, a better term for this pathologic entity would be *chronic interstitial nephritis* to encompass all the clinical states that can cause these changes. To incriminate infection as the sole cause of chronic interstitial nephritis, one needs evidence of past or present UTI and the absence of any other condition that can cause the pathologic picture of chronic interstitial nephritis. These criteria are seldom met and, even if they are, it is frequently impossible to establish whether infection is complicating interstitial nephritis of some unrecognized cause.

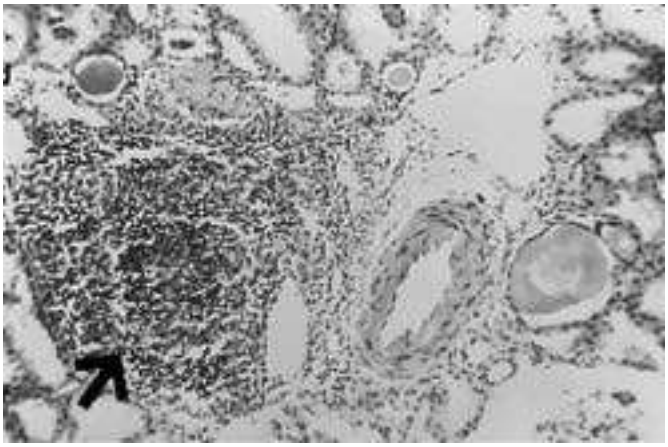

FIG. 72.1 Chronic pyelonephritis. Tubules are filled with eosinophilic casts and surrounded by a dense infiltrate of lymphocytes and plasma cells *(arrow)*.



FIG. 72.2 Necrotizing papillitis (papillary necrosis) complicating acute pyelonephritis in a diabetic patient. The pelvis is hemorrhagic. Note large and irregular defects. *(Courtesy Dr. M. Bergeron.)*

Papillary Necrosis Caused by Infection

Frequently both kidneys are affected, and one or more pyramids may be involved (Fig. 72.2). The pyramids are replaced by wedge-shaped areas of yellow necrotic tissue, with the base located at the corticomedullary junction. As the lesion progresses, a portion of the necrotic papilla may break off, producing a calyceal deformity that results in a recognizable radiologic filling defect. The sloughed portion may be voided and, in some cases, can be recovered from the urine. Microscopically, edema is initially seen in the interstitium. Eventually, the lesion resembles an infarct with coagulation necrosis involving the entire pyramid. The collecting tubules are filled with bacteria and polymorphonuclear leukocytes.

PATHOGENESIS OF URINARY TRACT INFECTION

Urinary tract infections occur as a result of the interaction of bacterial virulence and host biologic and behavioral factors, superseding highly efficient host defense mechanisms. There are three possible routes whereby bacteria can invade and spread within the urinary tract: the ascending and hematogenous pathways.

For a discussion of catheter-associated UTIs, see Chapter 302.

Ascending Route

The ultimate source of uropathogenic *Escherichia coli* (UPEC) and other uropathogens is the intestinal tract acting as a fecal reservoir. Especially apparent in women, the next phase of the ascending route is bacterial colonization of the vaginal introitus and periurethral meatus tissue. Evidence links new and increased colonization with uropathogens originating from rectal microbiota just before UTI. Colonization occurs in parallel with concomitant loss of the normally predominant and protective vaginal *Lactobacillus* species. Both culture and microbe genomic-based studies confirm the prevalence of *Lactobacillus* spp. in vaginal microbiota.[5] Lactobacilli express protective efficacy by achieving low pH (4.0–4.5); by interference with uropathogen adherence to vaginal epithelial cells; and by production of bacteriocins, H_2O_2 and chloride anions, and myeloperoxidase.

The urethra is usually colonized with bacteria. Studies using suprapubic puncture techniques have revealed the occasional presence of small numbers of microorganisms in the urine of uninfected persons. Massage of the urethra in women and sexual intercourse can force bacteria into the female bladder. Condom use may heighten the traumatic effects. Furthermore, just one catheterization of the bladder results in UTI infection in about 1% of ambulatory patients,[6] and infection develops within 3 or 4 days in essentially all patients with indwelling catheters with open drainage systems. Both the contraceptive diaphragm with nonoxynol-9 contraceptive jelly in women and the condom catheter in men have been shown to predispose to infection.[7] Studies have implicated the spermicide rather than the diaphragm. Spermicides increase colonization of the vagina with uropathogens. Although the dominant *Lactobacillus* vaginal flora are more sensitive to nonoxynol-9 than is *E. coli,* it has not been proved that the high coliform presence in nonoxynol-9 users is caused by a loss of lactobacilli. Spermicide use also increases adherence of *E. coli* to vaginal epithelial cells.[5] Estrogen deficiency is now recognized as a predisposing factor to recurrent UTIs in postmenopausal women because of consequent vaginal microbiome changes with loss of protective lactobacilli, which are replaced by coliforms and other uropathogens.[8] As with younger women, recent sexual intercourse is strongly associated with incident UTIs in postmenopausal women.[9] Uropathogenic *E. coli* is not infrequently shared between heterosexual sex partners.

The fact that UTI is much more common in women than in men gives support to the importance of the ascending route of infection. The female urethra is short and is in proximity to the warm, moist, vulvar and perianal areas, making contamination likely. It has been shown that the organisms that cause UTI in women colonize the vaginal introitus and the periurethral area before urinary infection results. Once within the bladder, bacteria may multiply and then pass up the ureters, especially if vesicoureteral reflux is present, to the renal pelvis and parenchyma. Animal studies have also confirmed the importance of ascending infection. If bladder bacteriuria is established after unilateral ureteral ligation, only the unligated kidney develops pyelonephritis. Finally, studies have correlated intestinal carriage of urovirulent *E. coli* and susceptibility to UTIs in children.[10]

In addition to the fecal reservoir, experimental mouse models of UTI have demonstrated that UPEC invade the bladder epithelium and form intracellular bacterial communities. These communities constitute quiescent intracellular reservoirs associated with resistance to clearance by antimicrobial treatment and recrudescence of bacteriuria.[11]

Hematogenous Route

Infection of the renal parenchyma by bloodborne organisms clearly occurs in humans. The kidney is frequently the site of abscesses in patients with *Staphylococcus aureus* bacteremia or endocarditis, or both. Experimental pyelonephritis can be produced by the intravenous (IV) injection of several species of bacteria and *Candida.*[12] However, the production of experimental pyelonephritis by the IV route with gram-negative enteric bacilli, the common pathogens in UTI, is difficult. Additional manipulations, such as the creation of ureteral obstruction, are often necessary.[12] It would appear that in humans, infection of the kidney with gram-negative bacilli rarely occurs by the hematogenous route.

Urovirulence in Bacteria

Although UTIs are caused by many species of microorganisms, most are caused by *E. coli.* However, only a few serogroups of *E. coli*—01, 02, 04, 06, 07, 08, 075, 0150, 018ab—cause a high proportion of infections. This has led to the concept of UPEC clones, or lineages, to differentiate pathogenic populations from commensals, and clonal analysis has facilitated epidemiologic studies of the spread of antibiotic-resistant clones of *E. coli* and identified environmental sources of *E. coli,* including households.[13] Certain O, K, and H serotypes also correlate with clinical severity, especially pyelonephritis. Accordingly, certain strains of *E. coli* are selected from the fecal microbiota by the presence of virulence factors that enhance both colonization and invasion of the urinary tract. Virulence factors allow evasion of host defenses and have the capability to produce disease. Cystitis and pyelonephritic *E. coli* isolates are genetically distinct, exhibiting differences in O, K, and H antigens. Genetic differences among uropathogens may be responsible for different clinical outcomes. Johnson and colleagues[14] have confirmed that certain O, K, and H serotypes are associated with urovirulence and with the presence and expression of multiple chromosomal virulence factor determinants. Virulence is a critical determinant of clinical presentation. Recognized virulence factors include increased adherence to vaginal and uroepithelial cells[15]; resistance to serum bactericidal activity; a higher quantity of K antigens (K1, K5, K12) in capsules; and the presence of aerobactin *(iucC),* cytotoxic necrotizing factor type 1 *(cnf),* hemolysin *(hly)* production, and a siderophore receptor *(iroN).*[15]

A variety of bacterial toxins have been reported that breach epithelial barriers, including an α-hemolysin that inhibits protective cytokine production by bladder epithelial cells, and the importance of extracellular polysaccharide as a virulence factor has been suggested.[16,17] Capsular polysaccharide contributes significantly to bacterial survival by countering lytic effects of complement and phagocytes. Induction of indoleamine 2,3-dioxygenase by UPEC attenuates innate response to uroepithelial cell invasion, facilitating colonization and establishment of infection. Genes for the various urovirulence factors are often duplicated in uropathogens and frequently linked as large, multigene, chromosomal segments called *pathogenicity islands,* and are absent in coliforms found in normal fecal flora.[18,19] Other virulence genes for *E. coli* UTI include *usp,* coding a uropathogenic specific protein, and *iroN,* coding a catechol siderophore receptor homologue.[20] Urine is poor in iron and other minerals. All uropathogens are able to use urine as a growth medium. Urine is, however, an incomplete growth medium; hence, the synthesis of one or more nutritional factors by UPEC is essential. Bacterial synthesis of guanine, arginine, and glutamine is required for optimal growth in urine.[21] *Escherichia coli* with mutations that are insufficient to generate resistance, as measured in the clinical microbiology laboratory, may have a selective advantage in the physiologic conditions provided by urine.[22]

Adhesins

Fimbriae

Adhesive properties of bacteria influence the selection of bacteria capable of colonizing the colon[10,23] and reaching and colonizing the normal urinary tract, and they influence the anatomic level of infection in the urinary tract (Table 72.1). Accordingly, bacteria with enhanced adherence to vaginal and periurethral cells would be selected to colonize the anatomic regions adjacent to the urethral orifice. *Escherichia coli* pyelonephritis isolates adhere better than *E. coli* cystitis isolates, and urinary isolates tend to adhere more strongly to uroepithelial cells than do random fecal *E. coli* isolates. The adhesins of UPEC exist as filamentous surface organelles termed *pili* or *fimbriae* or as nonfilamentous proteins in the outer membrane (see Chapter 218). Numerous uropathogenic strains adhere in the absence of fimbriae.

P Fimbriae

The binding of *E. coli* to epithelial cell receptors containing globoseries glycosphingolipid accounts for the attachment of most strains causing kidney infection and is not inhibited by mannose; this binding is called mannose resistant. Fimbriae attaching to globoseries receptors are termed *P fimbriae* because the receptor is a constituent of the P blood group antigen complex present in human erythrocytes and uroepithelial cells. The glycosphingolipids, synthesized by specific glycosyltransferases, are components of the glycocalyx surrounding epithelial cells and consist of an oligosaccharide moiety on the cell surface covalently linked to a lipid position embedded in the outer leaflet of the plasma membrane. Glycosphingolipases are highly specific to a given host and play an important role in determining tissue tropism for microbial pathogens and an individual host's susceptibility to UTIs. The globoseries glycosphingolipid receptors (Gal-Gal) are distributed throughout the urinary tract, particularly in the kidney.

P fimbriae are frequently present in uropathogens; they augment the virulence of UPEC at different stages of infection, including remaining longer in the intestinal tract and spreading more efficiently to the urinary tract for purposes of colonizing and producing ascending infection.[10,24] Once in the urinary tract, P-fimbriated strains adhere, persist, and, despite enhancing epithelial cytokine response, invade the kidney and induce bacteremia.[24] P fimbriae invariably show the strongest association to acute disease severity with 90% or more of acute pyelonephritis–causing strains, but less than 20% of asymptomatic carriers express this phenotype. The *pap* gene *EFG* sequences encode the adhesin complex. Three molecular variants of PapG adhesin, encoded by *PapG* class I through IV alleles, exhibit subtle receptor-binding preferences influencing the clinical outcome. Allele class II predominates among strains causing pyelonephritis and bacteremia, and class III allele is more commonly found in children and women with cystitis.[25,26] P fimbriae confer enhanced ability of UPEC clones to colonize the colon and spread to the perineum. Although relatively resistant to phagocytosis by neutrophils, *E. coli* with P fimbriae paradoxically enhance the host inflammatory response after engaging Toll-like receptor 4 (TLR4) by inducing the elaboration of proinflammatory cytokines.[27,28] Neutrophils lack receptors for P fimbriae. P-fimbriated *E. coli* dominates as a cause of pyelonephritis and urosepsis, especially among blood isolates; nevertheless, downregulation of P fimbriae expression may occur in the bacteria when they are in the kidney, hence facilitating parenchymal persistence.[29] In the mouse model of pyelonephritis, antibodies directed against P fimbriae that block bacterial adherence to uroepithelial cells in vitro prevent upper tract infection in vivo. With the same model, a vaccine using P fimbriae demonstrated some encouraging results; however, no progress in humans has followed.

Type I Fimbriae

Binding of *E. coli* to mannose-containing host epithelial receptors, glycoproteins uroplakin I and II, is universal in UPEC strains and is essential for establishment of cystitis.[30] In fact, strains from cystitis

TABLE 72.1 Uropathogenic *Escherichia coli* Adhesins and Corresponding Epithelial Receptors

ADHESIN	GENETIC SEQUENCE	RECEPTOR	COMMENTS
Type 1 fimbriae (MS)	*Pil, fimH, fimB, fimE*	Mannosylated proteins on epithelial cells (uroplakin Ia) and PMNs	Bind to THP and SIgA
P fimbriae (MR)	*papG* (class Ia) ($papG_{J96}$) *papGAP* (class II) ($papG_{IA2}$) *papG* (class III) ($prsG_{J96}$)	Gal-α 1-4 (P blood group antigen)	Rare Strongly associated with pyelonephritis and bacteremia Cystitis; predominates among patients with urinary tract abnormalities and males
S/F1C fimbriae (MR)	*Sfa/fac*	Sialyl-(α-2-3) galactoside	Adherence inhibited by THP
Type 1c (MR)	*Foc*	Undetermined	Possibly associated with pyelonephritis
G fimbriae (MR)		Terminal *N*-acetyl-D-glucosamine	
M fimbriae (MR)		Galactose-*N*-acetylgalactosamine	
Type 3 fimbriae	*(mrkABCDF)*	Blood group M (glycophorin A)	Contribute to biofilm formation; *E* gene present in 16% of first-time cystitis isolates
Dr family (fimbriated and nonfimbriated)	*Drb* operon; adhesin (*E* gene); *AfaE1-5; AfaF*	Dr blood group antigen component of DAF (decay accelerating factor) and type IV collagen	

MR, Mannose-resistant; *MS,* mannose-sensitive; *PMN,* polymorphonuclear neutrophil; *SIgA,* secretory immunoglobulin A; *THP,* Tamm-Horsfall protein.

patients are more likely to bind than those from pyelonephritis patients. Fimbriae attaching to mannosylated proteins via *FimH* subunits are the common type 1 fimbriae (pili), and attachment is inhibited in the presence of mannose (mannose sensitive). Type 1 fimbriae bind mannose epitopes on secreted glycoproteins such as secretory immunoglobulin A (IgA) and urinary mucus, Tamm-Horsfall protein (THP), bladder uroplakin protein, integrins, and fibronectin.[31] Type 1 fimbriae are encoded by the *pil* or *fim* gene cluster, including nine genes that encode structural and regulatory proteins.[32] The gene *fimA* encodes the fimbrial subunit protein, which can be expressed independently of the *fimH*-encoded adhesin protein.[33] The *fim* DNA sequences encoding type 1 fimbriae occur in most clinical isolates; consequently, epidemiologic evidence of an association between type 1 fimbriae and the site or severity of infection is more difficult to obtain. Expression of type 1 fimbriae is not especially prevalent among pyelonephritogenic strains. Clinical correlation in childhood, use of human bladder epithelial lines, and experimental animal studies have correlated type 1 fimbriae with persistence of *E. coli* in the urinary tract, and the use of a *fimH* null mutant of a type 1–positive *E. coli* isolate resulted in rapid elimination of the mutant from the urinary tract. Adherent bacteria multiply into biofilm-like inclusions known as *intracellular bacterial communities* or enter a quiescent phase for later reemergence. Such organisms may be relatively protected from host immune defense, and may contribute to clinical relapse. Similarly, clinical correlations in childhood urinary infections suggest that type 1 fimbriae contribute to virulence in the urinary tract when expressed in the background of a fully virulent uropathogen. Paradoxically, *fimH* also promotes adhesion to phagocytic cells that should presumably result in early bacterial clearance because of enhanced intracellular killing. In fact, antibody-opsonized and internalized type 1 fimbriae–bearing *E. coli* are rapidly killed. This is likely to occur within renal parenchyma, and hence type 1–fimbriated *E. coli* are programmed to shed their fimbriae on reaching the renal pelvis. In contrast, *E. coli* internalized only by a type 1 fimbrial mechanism survive intracellularly within the phagocyte, resulting in parasitism. Once inside the macrophage, the bacterium is safe from antibiotic assault, only to emerge later and possibly contribute to the relapse of bacteriuria. Bacterial adherence to urinary catheters is also type 1 fimbriae dependent. Type 1 fimbriae–mediated attachment to mast cells and lymphocytes results in further release of cytokines, causing cell proliferation and secretion of antibodies.

P and type 1 fimbriae are inversely and coordinately regulated in individual strains, explaining how invading bacteria adapt extemporaneously and sequentially to different environmental conditions prevalent in the human urogenital tract (e.g., P fimbrial expression downregulates type 1 fimbrial expression).[34] Type 3 fimbriae in UPEC are thought to contribute to biofilm formation and to be important in urethral catheter infections.[35]

Other Adhesins

In addition to type 1 and P fimbriae, various adhesins, including S (7% of uropathogenic strains), type 1c, G, and Dr fimbriae and M and X adhesins,[36] with differing molecular binding specificities and serologic properties, have been identified on UPEC and are expressed in vivo in urine (see Table 72.1). S fimbriae bind sialic acid epitopes present in renal sialylated lipoproteins. The Dr hemagglutinin family includes fimbrial and nonfimbrial adhesins. Four genes (*draA, B, C,* and *D*) encoding the structural proteins and adhesins of the fimbriae have been identified. The adhesins bind to the Dr blood group antigen component of decay-accelerating factor, which is widely distributed along the urinary tract and mediates cellular invasion.[37] Dr-expressing uropathogens are of relatively low invasive potential and demonstrate low multiplication rates; however, Dr-positive *E. coli* persists in renal infections and may play a role in chronic pyelonephritis and interstitial nephritis.[36] Type 1 pili and the Dr adhesin have been linked to bladder epithelial cell invasion and intracellular persistence by UPEC.[14]

Studies with other species of bacterial uropathogens (e.g., *Proteus mirabilis, Klebsiella* spp.) have similarly demonstrated the significance of adherence in the pathogenesis of urinary infections. *Staphylococcus aureus* uncommonly causes cystitis and ascending pyelonephritis; in contrast, *Staphylococcus saprophyticus* is a frequent cause of lower UTIs. *Staphylococcus saprophyticus* adheres significantly better to uroepithelial cells than does *S. aureus* or *Staphylococcus epidermidis.*

Expression and Selection of Virulence Factors

Evaluation of urinary isolates for virulence characteristics in the presence of underlying structural abnormalities (e.g., severe reflux) frequently fails to demonstrate the typical bacterial virulence factors. Similarly, *E. coli* blood isolates obtained from patients with urosepsis after bladder instrumentation often lack virulence factors. Virulence determinants are more frequently expressed by urinary isolates of *E. coli* obtained from women with cystitis compared with fecal isolates from healthy women. No difference in the prevalence of *E. coli* virulence determinants was found between subjects with first-time cystitis and those with recurrent cystitis, suggesting that host rather than bacterial factors determine the risk for recurrent infection. It has been demonstrated that *E. coli* isolates that cause cystitis in women using diaphragms with spermicides have fewer virulence determinants than do isolates of nonusers, suggesting that the use of diaphragms with spermicides may allow infection with less virulent *E. coli.* In one study, quinolone-resistant UPEC was less virulent with decreased invasive capacity. In contrast, *E. coli* strains isolated from men with febrile UTI exhibited a significantly higher prevalence of virulence-associated phylogenetic groups, serotypes, and virulence genes.[38] Resistant organisms have been shown to have reduced type 1 fimbriae expression and proteolytic toxin Sat elaboration.[39] Trimethoprim-sulfamethoxazole (TMP-SMX), extensively used to prevent urinary infection, reduces the synthesis, expression, and adhesive function of type 1 fimbriae at concentrations well below the minimal inhibitory concentration. The importance of adherence as a virulence factor is not complete without consideration of the role of the host. A difference in adherence receptor density and specificity is linked to a difference in genetic susceptibility. In women and children with recurrent UTI, an increased avidity of bacterial attachment to vaginal, periurethral, and uroepithelial cells has been found.

Other Virulence Factors of Uropathogenic *E. coli*

Certain other characteristics of bacteria may be important in the production of upper tract infection. Motile bacteria, especially those with flagellae, can ascend in the ureter against the flow of urine, and the toxins of uropathogenic gram-negative bacilli have been shown to decrease ureteral peristalsis and possibly contribute to the renal parenchymal inflammatory response by phagocytic cell activation.[40] As a general principle, UPEC promote a more intense inflammatory response through greater activation of the innate immune system, resulting in symptomatic urinary tract inflammation. In *Proteus* spp., the production of urease by infecting microorganisms has been correlated with the ability to cause pyelonephritis. The presence of K capsular antigen protects bacteria from leukocyte phagocytosis. Most uropathogenic strains produce hemolysin, which facilitates tissue invasion and causes renal tubular epithelial and parenchymal cell damage, possibly making iron available to invading *E. coli.* The hemolysin gene is frequently located adjacent to genes encoding for serum resistance and sialic acid–specific (S) fimbriae, but the pathogenic role of hemolysis in pyelonephritis remains controversial. Aerobactin, an iron-scavenging protein or siderophore, is present with increased frequency in uropathogenic strains of *E. coli.*[12] Iron acquisition systems are often found in uropathogenic *E. coli,* presumably because conditions in the bladder are iron poor. Uropathogenic *E. coli* strains contain a greater number of iron acquisition systems than do fecal or commensal strains, which is reflective of the adaptation to the iron-limiting urinary tract environment. Genes have been identified that encode for these virulence factors: *hlya* (encoding hemolysin), *cnf1* (encoding cytotoxic necrotizing factor 1), and *iutA* (encoding aerobactin receptor). Cytotoxic necrotizing factor 1 has been associated with UTIs and prostatitis in clinical epidemiologic studies and causes pronounced bladder inflammation in animal models.[41] The iron-regulated gene homologue adhesin (Iha), an outer membrane protein encoded by *iha,* was shown to be a virulence factor in a mouse model of UTI.[42] Recent studies have indicated a fitness advantage for flagellated *E. coli* in mouse models of UTI facilitatory colonization. Of note, *E. coli* strains causing asymptomatic bacteriuria

appear to have lost their ability to express functional virulence-associated genes.[43]

Some UPEC strains downregulate responding neutrophil activity, hence evading the dominant acute immune response (i.e., suppressing neutrophil transepithelial movement and therefore recruitment), providing an important advantage for establishing infection. Additionally, UPEC resists phagocytic killing and dampens production of antibacterial reactive oxygen species by neutrophils. Downregulation is achieved by reduced expression of polymorphonuclear neutrophil (PMN) genes.

The greater the number of organisms delivered to the kidneys, the higher the chance of producing infection. The kidney itself is not uniformly susceptible to infection because few organisms are necessary to infect the medulla, whereas 10,000 times as many are necessary to infect the cortex. The greater susceptibility of the medulla may be caused by the high concentration of ammonia, which may inactivate complement, and by poor chemotaxis of PMNs into an area of high osmolality, low pH, and low blood flow.

Host Factors in Urinary Tract Infections

Host behavioral factors that contribute to UTI include sexual intercourse, including frequency; use of spermicides for contraception; a new sexual partner within the last year; and recent antimicrobial exposure. The latter factor not only increases risk of UTI but profoundly increases risk of acquisition of resistant bacteria. Finally, lack of estrogen is a major factor contributing to increased risk in postmenopausal women not receiving hormone replacement therapy. Loss of estrogen results in decreased or complete loss of vaginal lactobacilli. Key urogenital mucosal host defense mechanisms are also dependent on estrogen, including bladder contractibility and hence emptying capacity as well as antimicrobial peptides, which are increased following estrogen supplementation.[44] Finally, estrogen enhances epithelial cell barrier function, especially bladder cells.

HOST DEFENSE OF THE URINARY TRACT

With the exception of urethral mucosa, the normal urinary tract is resistant to colonization by bacteria and, for the most part, efficiently and rapidly eliminates pathogenic and nonpathogenic microorganisms that gain access to the bladder[45] (Table 72.2).

Innate Immunity

The antibacterial defense of the urinary tract relies almost entirely on innate immunity. Susceptibility to UTI is largely controlled by specific innate immune signaling and by promotor polymorphisms and transcription factors that modulate the expression of genes controlling these pathways. Innate immune response of the host is critically important, and bacterial clearance normally proceeds without sequelae. On the other hand, dysfunction—usually genetic in origin and often only slight—may actually contribute to enhancing inflammation and cause tissue destruction, especially in the kidney. Defects in innate immunity drastically increase UTI susceptibility both in mice carrying single gene deletion and in patients prone to pyelonephritis.[46,47] Uropathogenic clones elicit an inflammatory response at all levels in the urinary tract by stimulating uroepithelial and other cells to produce cytokines and other proinflammatory factors. The intensity and efficiency of the innate host response are genetically regulated and are critical factors for the host-organism interaction in the urinary tract.[48] The innate immune response is activated in a pathogen-specific manner (e.g., P fimbriae–mediated adhesion). Toll-like receptor 4 is essential for the defense against infection with gram-negative uropathogens leading to cytokine and chemokine responses with recruitment of inflammatory cells.[49] Systemic elaboration of interleukin (IL)-1β transcription factors such as interferon regulatory factor 3 and IL-6 may lead to fever and activation of the acute-phase response. Urine and serum IL-6 concentrations reflect the severity of infection, with highest levels in pyelonephritis and bacteremia.[50] The chemotactic cytokine IL-8 is released at the mucosal site recruiting PMNs, resulting in pyuria and contributing to the eradication of bacteriuria. Interleukin-8 receptor deficiency confers susceptibility to acute pyelonephritis.[51] Toll-like receptor 4 is activated by bacteria in urine either directly by lipopolysaccharide or following bacterial adhesion. This stimulates local urinary production of chemokines such as CXCL8, leading to migration of neutrophils and other responding inflammatory cells. Intensity of this response is one determinant of whether infection resolves or progresses as asymptomatic or symptomatic bacteriuria. Infection also stimulates expression of chemokines CXCR1 and CXCR2 by urothelial cells. CXCR1 is essential for the increased neutrophil migration across infected cell layers in vitro. Secretion of chemokines and cytokines, including CCL2, CXCL6, and CXCL8, follows.[52] Reduced TLR4 expression has been reported in children with asymptomatic bacteriuria.[53] By evading or modulating the activation of the host mucosal urothelial innate response, UPEC may provide a pathogenic advantage in reaching and persisting in the kidney, but paradoxically, if the inflammatory response is absent or attenuated, renal abscesses and symptomatic pyelonephritis may not follow.[54,55]

Direct contact between adherent bacteria and uroepithelial cells may also result in bacterial growth suppression. This antibacterial epithelial defense function is activated by transmembranous signals from bacteria attached to the host cell surface and involves adenylate cyclase activity.

Effectors of the innate immune response also include an array of molecules elaborated by epithelial cells or PMNs, which inhibit a wide range of microorganisms. These include α-defensins (HD5) in the kidney and upper ureter, β-defensins (HBD) in nephron tubules and collecting ducts, cathelicidin, and hepcidin.[56]

Urine and Bladder Defenses

An emerging concept is that the bladder, rather than representing a sterile environment, may in fact host a "urinary microbiome" of commensal organisms that may influence UTI. Although urine is generally considered to be a good culture medium for most bacteria, it does possess antibacterial activity. Anaerobic bacteria and other fastidious organisms that make up most of the urethral flora generally do not multiply in urine. It has been shown that extremes of osmolality, a high urea concentration, and low pH levels are inhibitory for the growth of some of the bacteria that cause UTI. Furthermore, the pH and osmolality of urine from pregnant women tend to be more suitable for bacterial growth than those from nonpregnant women, which in turn are more suitable for bacterial growth than those from urine from men. The presence of glucose makes urine a better culture medium, whereas the addition of prostatic fluid to urine inhibits bacterial growth. Furthermore, urine has been shown to inhibit the migrating, adhering, aggregating, and killing functions of PMNs.

The epithelial surface of the urinary tract is covered by a thin layer of urine and fluid secreted by the epithelial cells. The epithelial secretions possess antimicrobial properties, mainly the product of neutrophils providing a surveillance function. Neutrophil defensins are 3- to 5-kDa disulfide cationic peptides; their presence on epithelial surfaces may play a role in clearance of adherent bacteria. Another weapon against UTI is antimicrobial peptides; an example is ribonuclease 7, but its production is inhibited by certain bacterial strains.[57]

TABLE 72.2 Antibacterial Host Defenses in the Urinary Tract

- Urine (osmolality, pH, organic acids)
- Urine flow and micturition
- Urinary tract mucosa (bactericidal activity, peptides, cytokines)—innate immunity
- Inflammatory response
  - Polymorphonuclear neutrophils
  - Tamm-Horsfall protein
  - Cytokines
  - Low-molecular-weight oligosaccharides
  - Secretory immunoglobulin A
  - Lactoferrin
  - Peptides
- Adaptive immune system
  - Humoral immunity
  - Cell-mediated immunity
- Miscellaneous
  - Prostatic secretions

By means of its mannose-containing side chains, THP, secreted by cells of the ascending loop of Henle, binds strongly to *E. coli* expressing type 1 and S fimbriae. Tamm-Horsfall protein is the most abundant protein of renal origin in urine and may function as a urinary antibacterial defense mechanism by binding to mannose-sensitive strains of *E. coli,* preventing them from attaching to epithelial cell receptors (Fig. 72.3). Tamm-Horsfall protein, a kidney-specific glycoprotein, activates complement and dendritic cells but also has immunoregulatory function in UTI via a TLR4-dependent mechanism (see Fig. 72.3).[58] Significantly reduced levels of THP in older adults occur during episodes of UTI and may contribute to susceptibility to infection.

The flushing mechanism of the bladder exerts a major protective effect. When bacteria are introduced into the bladders of humans, there is a tendency for spontaneous clearance. Because flushing alone would probably not completely clear the bacteria, there must be additional protective factors. Certain host factors, including bladder catheterization, increase the susceptibility of uroepithelial cells to attachment by uropathogens, which in turn increases susceptibility to bacteriuria.

In a study of bladder defense mechanisms in dogs, it was demonstrated that there is an active antiadherence mechanism of bladder mucosa. Pretreatment of the bladder with acid increases bacterial adherence 20- to 50-fold independent of the bacterial species used. Histochemical studies revealed that bacterial adherence is increased by the removal of glycosaminoglycan, a surface mucopolysaccharide that seems to be responsible for the natural resistance to adherence. Thus normally small inocula of bacteria are probably unable to adhere, remain suspended in urine, and are removed by voiding. In the presence of a larger bladder inoculum of bacteria, especially with good adhesive qualities, the primary defense of antiadherence may be overcome, colonization can occur, and subsequent bladder infection may result. Following bladder infection, secondary defense mechanisms such as mobilization of leukocytes, phagocytosis, and bacterial destruction remove bacteria. Bacteria stimulate PMNs to secrete IL-8, IL-1β, and tumor necrosis factor and stimulate lymphocytes to elaborate immunoregulatory cytokines, ultimately resulting in immunoglobulin synthesis and modifying epithelial cell responses to bacteria. Epithelial cells participate in PMN recruitment by secreting neutrophil chemoattractants (e.g., IL-8) and by the expression of adhesion molecules involved in PMN transmigration (intercellular adhesion molecule 1).[51]

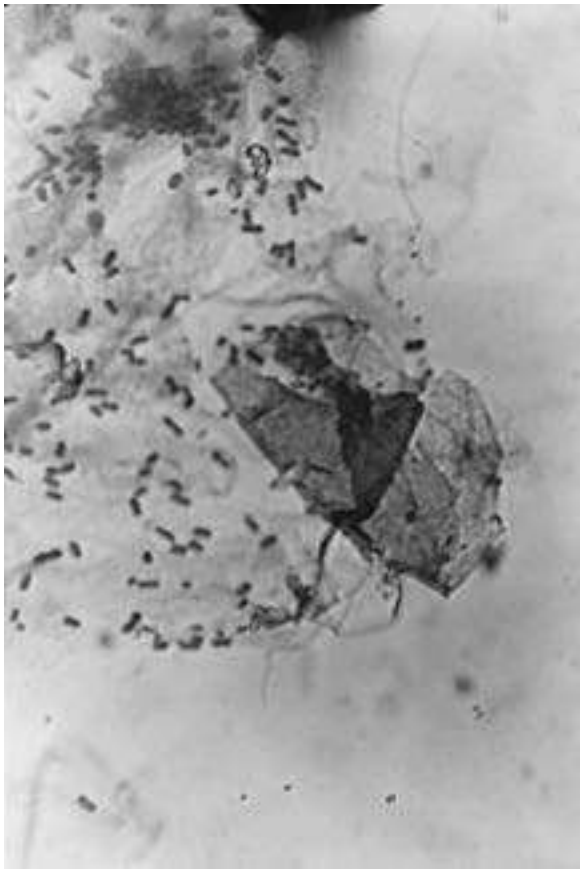

FIG. 72.3 Light microscopic specimen of urine showing uromodulin (Tamm-Horsfall protein) with large numbers of adherent uropathogenic bacteria.

As mentioned previously, upon reaching the bladder, UPEC bind to superficial epithelial cells in a type 1 pili–dependent manner. A subset of adherent bacteria are then internalized into epithelial cells, which in turn undertake active expulsion of internalized UPEC. This process is complex and involves exocytosis of UPEC-containing lysosomes, and activation of TLR4 by internalized UPEC. The efficiency and protective role of expulsion of intracellular bacteria in humans is under investigation[59]; however, uropathogens can occupy the cytoplasm of bladder epithelial cells and using this mechanism can replicate and persist, protected from infiltrating neutrophils. The bladder epithelial surface may serve as yet another bacterial reservoir and a source of recurrent UTI, especially since these cells are protected from urinary antibiotics.

Adherence and Colonization

In women, bacterial adherence and colonization of the vaginal introitus and periurethral region by Enterobacteriaceae are critical in the pathogenesis of UTIs. Periurethral colonization with the same organism almost invariably precedes episodes of significant bacteriuria. Microbiologic studies have demonstrated that the urethra, periurethral region, and vaginal vestibule of women with recurrent UTIs tend to be more commonly colonized with coliform bacteria.[60,61] Stamey has postulated that such colonization is often the prelude to new infection and that women with recurrent UTIs have a biologic predisposition to colonization.[60] In a series of studies, the effects of several factors in vaginal secretions on colonization were examined. A low vaginal pH level was the most important factor reducing colonization with UPEC. *Escherichia coli* was more resistant to low pH levels and less susceptible to the inhibitory effects of vaginal fluid than *P. mirabilis* or *Pseudomonas aeruginosa.* Finally, it was noted by others that *E. coli* adhere more avidly to vaginal epithelial cells of women and young girls with recurrent UTIs. These observations, together with experimental studies using inbred mice, indicate predisposition of some women to UTI because of genetically determined host epithelial cell receptors for UPEC. This is supported by several epidemiologic studies demonstrating that women who are epithelial antigen and receptor nonsecretors have an increased risk of UTI, and their uroepithelial cells bind *E. coli* more avidly than do cells from secretors.[62] The secretor gene, *Se,* encodes a glycosyltransferase that transfers a fucose residue to specific acceptor molecules, resulting in detectable blood group antigens in secretions of people carrying the gene.[63] Lewis gene nonsecretors appear to have an increased susceptibility to urinary infections and express two unique globoseries glycosphingolipids present on vaginal and uroepithelial cells, which preferentially bind UPEC expressing class II *pap*-encoded adhesin.

Other endogenous factors, such as estrogenic hormones, may influence bacterial attachment to uroepithelial cells and affect the risk of UTI.[64] From an acquired or behavioral point of view, both colonization of the vaginal introitus and bacteriuria caused by *E. coli* have been strongly associated with diaphragm and spermicide use, which may contribute to the increased risk of UTI associated with sexual activity. Others have concluded that the decisive predisposing factor is not the colonization of the periurethral area per se, but rather the ability of these organisms to ascend the urethra, including the ability of infecting organisms to adhere to uroepithelial cells and withstand normal host defense mechanisms.

Humoral and Cellular Immunity

The role of humoral immunity in the host's defense against infection of the urinary tract, although extensively studied, is poorly understood. The defense against acute UTI does not rely on specific immunity defined by antigen-specific lymphocyte activation. During acute pyelonephritis,

there is a systemic antibody response to O antigen and occasionally the K antigen of the infecting strain, and antibodies to type 1 and P fimbriae have been described. Immunoglobulin M (IgM) antibodies dominate in the response to the first upper tract infection but not to subsequent episodes. High levels of IgG antibodies to lipid A correlate with the severity of renal infection and the progression of renal parenchymal destruction. An antibody response consisting of IgG and secretory IgA antibodies can be detected in the urine. In contrast to upper UTI, lower UTI is usually associated with a reduced or undetectable serologic response, reflecting the superficial nature of the infection. Macrophages are well distributed in the submucosa throughout the urinary tract, and uroplakin Ia–expressing cells, analogous to Langerhans cells, have been identified in the urinary mucosa, including renal tubules. Macrophage subsets regulate neutrophil-mediated defense in UTIs. Immunoglobulin A–producing lymphocytes are found in the submucosa of infected rat bladders. Similarly, high numbers of IgA-producing plasma cells have been observed in the bladder submucosa of patients with bacterial cystitis in comparison with healthy controls. Finally, in animal models, antibody-secreting cells and B lymphocytes migrate to kidney and urinary tract submucosa during UTIs. The reduced immunologic response to the infecting organism in cystitis may contribute to reinfection with the same strain. However, in a monkey model, systemic and urinary IgG and IgA were observed to accompany experimental cystitis. Antifimbrial antibodies are absent in the urine in lower tract infection. At least one-third of females with a second UTI have an identical strain isolated, indicating incomplete or inadequate immunity with a single episode of infection.[65]

In spite of the impressive systemic and local urinary antibody production that follows acute pyelonephritis, the protective role of these antibodies is unclear. When bacteria persist in the kidney for several months, antigenic drift may occur. Antibodies against several bacterial structures, including O and K antigens and, more recently, fimbrial antigens, have been found to protect against hematogenous or ascending pyelonephritis in experimental animals. Animal recipients of vaccines based on *Pap A* fimbriae were protected against experimental pyelonephritis caused by homologous and heterologous Gal-Gal–binding uropathogenic *E. coli* strains. Mitigating against an important role for urinary and systemic antibodies are experimental studies with mice exhibiting profound B-cell immunodeficiency and a normal capacity to clear experimental ascending UTI.

Antibodies may be of value in limiting the damage incurred in the kidney or preventing colonization preceding recurrence. Svanborg-Eden and Svennerholm[66] have reported that the urine of patients with pyelonephritis inhibits the adherence of *E. coli* to uroepithelial cells and that this activity is removed by absorption with O antigen. Antibodies have not been shown to protect against bladder infection. Cell-mediated immunity has not been shown to play a major role in host defenses against UTI. Urinary tract mucosa contains few T lymphocytes, although both $CD4^+$ and $CD8^+$ T cells can be found in the submucosa and lamina propria; however, they generally lack γδ T cells.[67] Experimental studies in athymic mice have shown similar resistance to intravesical infections when compared with normal controls. Similarly, clinical experience in human immunodeficiency virus–infected women with severe defects in cell-mediated immunity, including low CD4 lymphocyte counts, does not indicate increased susceptibility to or severity of urinary infection.[68] Nevertheless, a useful role for cell-mediated immunity may still exist in that T-cell–derived proinflammatory cytokines (e.g., interferon-γ) stimulate epithelial cells to produce IL-6 in a similar manner to Th2 lymphocyte responses involving IL-4, IL-5, IL-8, IL-10, and IL-13.[28] Interleukin-8 is an inflammatory cytokine that promotes neutrophil migration across the infected uroepithelial cells. Thus urinary tract T cells producing immunoregulatory cytokines may influence the mucosal epithelial cell response to bacterial adherence and invasion. The IL-6 secreted by renal tubular epithelial cells[28] may contribute to mucosal antibacterial activity by increasing IgA secretion by committed B cells. During pyelonephritis, an acute inflammatory exudate consisting predominantly of PMNs is present. Although the inflammatory reaction is directed at limiting bacterial spread and persistence in the kidney, infiltrating phagocytic cells may contribute to tissue damage[48] and renal scarring, as evidenced by reduced parenchymal kidney destruction in experimental neutropenia. It has been suggested that chronic pyelonephritis and persistent renal damage may develop after successful eradication of bacterial pyelonephritis with antimicrobial therapy. According to these concepts, bacterial remnants or antigen or THP persistence induces a chronic humoral immunologic response resulting in cryptogenic renal scarring. Secretory IgA is one of three glycoproteins that bind type 1 *E. coli* and could affect vaginal colonization.[69]

Genetic Factors

It is apparent that multiple genetic risk factors exist for UTIs, influencing host defense mechanisms operative at different levels of the urinary tract.[70,71] Investigators have reported the increased incidence of UTI in the immediate female family members of women with recurrent UTIs, supporting a genetic propensity to uncomplicated cystitis and pyelonephritis.[72,73] Overall, genetic determinants of familial susceptibility are not well characterized. Genetic influences relate to uroepithelial cell receptors (secretor status) and to the inflammatory response and antibacterial peptide elaboration. Studies of experimental UTIs in C3H/HeJ female mice have shown diminished resistance in this mouse strain, which is genetically unresponsive to the biologic effects of *E. coli* lipopolysaccharide, although several genes are likely to be operative. Candidate genes include the *ABH* secretor gene and the IL-8 receptor *CXCR1* gene. Knockout mice lacking CXCR1 were unable to clear bacteria from the kidney and developed bacteremia. Lundstedt and colleagues[70] reported familial associated pyelonephritis, but not cystitis, in girls and women with polymorphisms having significantly lower expressions of CXCR1, an IL-8 receptor. In addition, levels of CXCR2 expression on neutrophils of women with recurrent UTI were significantly lower than those of control patients.[74] Similar findings were observed in family members of acute pyelonephritis–prone children. As noted, the innate immune system involves the TLR family of receptors in microbial recognition. This recognition is through bacteria-specific common antigens (e.g., lipopolysaccharide) rather than a specific ligand for TLR4. Studies have identified single nucleotide polymorphisms of the TLR4 molecule affecting innate immune responses to uropathogenic *E. coli*.[75] Selected polymorphisms that reduce TLR4 functions are associated with asymptomatic bacteriuria, whereas genotype variants of interferon regulatory factor 3, a transcription factor, are associated with increased familial incidence of pyelonephritis. Although women with recurrent urinary infections were shown to have lower levels of urinary secretory IgA, the clinical significance is highly questionable. Bates and associates[76] have shown that knockout mice for THP inoculated with type 1 fimbriated *E. coli* have a longer duration of bacteriuria and more intense bladder colonization compared with $THP^{+/+}$ mice.

Structural Abnormalities

Several abnormalities of the urinary tract interfere with its natural resistance to infection. Obstruction to urine flow is the most important of these. Extrarenal obstruction can result from the following: congenital anomalies of the ureter or urethra, such as valves, stenosis, or bands; calculi; extrinsic ureteral compression from various causes; and benign prostatic hypertrophy. Intrarenal obstruction may be produced by entities such as nephrocalcinosis, uric acid nephropathy, analgesic nephropathy, polycystic kidney disease, hypokalemic nephropathy, and the renal lesions of sickle cell trait or disease. Obstruction inhibits the normal flow of urine, and the resulting stasis is important in increasing susceptibility to infection. Men of any age and pregnant women are the most prone to lesions that result in obstruction to the free flow of urine.

In animals, obstruction of a ureter markedly increases susceptibility to ipsilateral hematogenous infection. Intrarenal obstruction, experimentally produced by scars in various ways, also increases the susceptibility of the kidney to infection. Medullary scars, which produce greater amounts of obstruction than cortical scars, increase the susceptibility of animals to infection more than cortical scars. Calculi may increase susceptibility to UTI by producing obstruction (Fig. 72.4). However, not all stones obstruct, and local irritative phenomena may also be



FIG. 72.4 Staghorn calculus visible in the dilated pelvis of a hydronephrotic kidney. *(Courtesy Dr. M. Bergeron.)*

important. Furthermore, calculi may develop secondary to infection. It has been observed clinically and experimentally that *Proteus* spp. and other urea-splitting organisms are most likely to produce calculi. Furthermore, bacteria survive deep within the calculi and associated biofilm and are extremely difficult to eradicate, even by artificial means such as by incubating in solutions containing antibiotics or iodine and alcohol. This may account for the well-known difficulties encountered clinically in trying to cure UTI in the presence of stones.

Vesicoureteral reflux and UTI are also intricately related. The significance and management of reflux have undergone considerable review (see "Imaging Studies" later). Reflux caused by a congenital abnormality, bladder overdistention, or unknown causes probably contributes to upper tract infection via the ascending route. On the other hand, clinical observations have demonstrated that infection may, in fact, produce reflux, especially in children. Reflux tends to perpetuate infection by maintaining a residual pool of infected urine in the bladder after voiding. It is probable that severe reflux, especially in young children, plays an important role in the production of upper tract infection and subsequent scarring. Patients with incomplete emptying of the bladder for mechanical reasons (bladder neck obstruction, urethral valves, urethral strictures, prostatic hypertrophy) or neurogenic malfunction (diabetic neuropathy, cord injuries) are prone to frequent UTIs. These patients are subject to bladder overdistention, which may interfere with local defense mechanisms, and, most importantly, frequent instrumentation of the urinary tract.

EPIDEMIOLOGY AND NATURAL HISTORY OF URINARY TRACT INFECTION[77]

Infecting Organisms

More than 95% of uncomplicated UTIs are caused by a single bacterial species. There is a difference between the bacterial flora of the urine in patients with an initial episode of UTI compared with the flora from those with frequent recurrences of infection. *Escherichia coli* is the most frequent infecting organism in initial infections.[78,79] In recurrent UTIs, especially with complicated UTI (e.g., obstructive uropathy, congenital anomalies, neurogenic bladder, fistulous communication involving the urinary tract), the relative frequency of infection caused by bacteria such as *Proteus, Pseudomonas, Klebsiella,* and *Enterobacter* spp., by antibiotic-resistant *E. coli,* and by enterococci and staphylococci increases greatly. In the presence of complicated UTI, it is not uncommon to isolate multiple organisms from the urine. Because instrumentation and repeated courses of antimicrobial therapy are common in these patients, antibiotic-resistant isolates might be expected.[1]

The hospital and long-term care facility environments are an important determinant of the nature of the bacterial flora in UTI. *Proteus, Klebsiella, Enterobacter,* and *Pseudomonas* spp., as well as staphylococci and enterococci, are more often isolated from inpatients, compared with a greater preponderance of *E. coli* in an outpatient population.[80] Cross-infections are important in the pathogenesis of hospital-related UTIs, especially with indwelling catheters.[81] *Corynebacterium urealyticum* (formerly known as *Corynebacterium* group D2) has been recognized as an important nosocomial pathogen.[82,83] This gram-positive, urea-splitting, slow-growing bacillus may cause infected mucosal encrustations of the bladder and urinary collecting system, including struvite stones, especially in immunosuppressed patients and in particular in renal transplant recipients. It is highly resistant to antimicrobials, although usually sensitive to vancomycin. It should be considered in the presence of a high urine pH, urologic problems, previous UTI, and recent antibiotic treatment.

In recent years, highly antibiotic-resistant uropathogens such as *Acinetobacter* species and extended-spectrum β-lactamase (ESBL)–producing, AmpC β-lactamase–producing, and carbapenemase-producing Enterobacteriaceae (e.g., New Delhi metallo-β-lactamase 1) have been increasingly reported as causes of health care–related complicated UTIs.[84,85,86,87] There has also been the worldwide spread of antibiotic-resistant uropathogenic clones of *E. coli,* such as CTX-M-15 ESBL *E. coli* O25:H4 sequence type ST131, which has emerged as a significant cause of community-acquired UTI.[88–90]

Anaerobic organisms are rarely pathogens in the urinary tract. Fungi (particularly *Candida* spp.) occur in patients with indwelling catheters who are receiving antimicrobial therapy.[91,92] Coagulase-negative staphylococci are a common cause of UTI in some reports. *Staphylococcus saprophyticus* tends to cause infection in young females who are sexually active, accounting for 5% to 15% of acute cystitis episodes in the United States.[93] Coagulase-positive staphylococci most often invade the kidney from the hematogenous route, resulting in intrarenal or perinephric abscesses. *Actinobaculum schaalii* and other *Actinobaculum* spp. are facultative anaerobic gram-positive bacilli and are often missed in urine cultures because of their slow growth or dismissed as genital flora.[94–96] *Actinobaculum schaalii* is a part of normal skin and vaginal flora. Most cases of urosepsis have occurred in patients older than 65 with renal stones undergoing lithotripsy or other instrumentation. *Actinobaculum schaalii* has been reported as susceptible to ampicillin, cephalosporins, and vancomycin but resistant to fluoroquinolones and TMP-SMX.

Adenoviruses (particularly type 11) have been strongly implicated as causative agents in hemorrhagic cystitis in pediatric patients (especially boys) and in allogeneic hematopoietic stem cell transplant recipients, and in UTI in renal transplant recipients.[97,98] Although various investigators using special media have isolated fastidious organisms from women with lower tract symptoms, the causal role of these organisms is controversial. Similarly, *Gardnerella vaginalis, Ureaplasma urealyticum,* and *Mycoplasma hominis* are possible but unproven causes of UTI.

One group has reported nanobacteria as tiny (0.05- to 0.5-mm) cell wall–possessing bacteria that are associated with renal stones.[99] In vitro, these agents produce carbonate apatite that can fix calcium. These have been found to be self-propagating mineral complexes and not bacteria.[100,101]

Asymptomatic Bacteriuria Versus Symptomatic Infection

The methodical studies of asymptomatic bacteriuria in different populations (described later) were important in understanding the epidemiology

of UTI. However, except in pregnancy, in those patients undergoing invasive genitourinary tract procedures, it is uncommonly followed by symptomatic infection and is of little consequence. With the exception of these groups, there is rarely a reason for determining the presence of or treating asymptomatic bacteriuria.[2,102] With the exception of these groups and other rare exceptions, the only reason for taking urine cultures or treating infection is for symptomatic infection and for the purpose of relieving symptoms. Screening has not been shown to improve clinical outcome, and asymptomatic bacteriuria does not lead to hypertension, chronic renal failure, or decreased survival. Asymptomatic bacteriuria is used as a marker for poor overall health status in diabetics and in elderly inpatients. In contrast, asymptomatic bacteriuria in pregnancy (4%–7% incidence) is associated with progression to symptomatic pyelonephritis,[103] the most common nonobstetric cause of hospitalization during pregnancy.

Urinary Tract Infection in Children

The problem of UTI in pediatric patients spans all age groups, beginning with neonates.[104,105] The frequency of UTI in infants is about 1% to 2%. It is more common in boys during the first 3 months and thereafter occurs more often in girls. The highest incidence of febrile UTIs occurs during the first year of life in both males and females, whereas nonfebrile UTIs (i.e., cystitis) are most common in females older than 3 years of age. The significance of fever is that it implies renal parenchymal infection, which is associated with an increased likelihood of underlying urologic abnormalities and renal scarring.[106] Most studies have found that a lack of circumcision predisposes to UTIs in infants and young boys.[107–109] The risk of UTI is increased in those with underlying urinary tract abnormalities, such as vesicoureteral reflux and voiding dysfunction, and this risk may be increased when bladder and bowel dysfunction are also present.[106,110,111]

During the preschool years and thereafter, UTI is much more common in girls than in boys. In preschool boys, it is frequently associated with congenital urologic abnormalities. The annual incidence of UTI in this age group is 4.5% for girls and about 0.5% for boys. Infections during this period are often symptomatic, and it is believed that much of the renal damage that occurs in association with UTI takes place in infancy and the preschool period (Fig. 72.5).[112] However, adult kidneys can also acquire scars due to pyelonephritis, as is seen in children with transplanted kidneys.

About 7% to 8% of girls and 2% of boys have a febrile UTI during the first 8 years of life.[106] Renal scars may be produced by primary renal damage in utero from obstruction, genetic, and developmental factors and can be indistinguishable from those produced by infection in postnatal life.[106] In fact, antenatal ultrasound studies have demonstrated that intrinsic renal disease and not infection is the major cause of chronic renal disease in children.[106] The risk of recurrent infection is 12% to 30% in the first 6 to 12 months after the first UTI and is increased in the presence of urologic abnormalities.[110]

In school-age girls bacteriuria is common, is often asymptomatic, and frequently recurs.[113,113a] For example, the prevalence of bacteriuria among schoolgirls was about 1.2%, and about 5% of the schoolgirls had significant bacteriuria at some time before leaving high school. About one-third of these patients had some symptom referable to the urinary tract when the bacteriuria was first detected. Bacteriuria was rare in schoolboys (prevalence, 0.03%). When many of these girls who had had bacteriuria previously (usually asymptomatic) were married or became pregnant, bacteriuria recurred at a rate far above that expected for the general population. More than 50% developed bacteriuria within 3 months after marriage. Thus the presence of bacteriuria in childhood defines a population at higher risk for the development of bacteriuria in adulthood.

In general, children with UTIs without structural or functional obstruction or severe vesicoureteral reflux have a very good prognosis.[112,114] In the presence of obstruction (e.g., urethral valves), severe injury of renal parenchyma can occur.[105,115] Vesicoureteral reflux is found in 30% to 50% of young children with symptomatic UTI (see Fig. 72.5).[116] Reflux may be primary, which results from delayed development of the vesicoureteral junction or a short intravesical ureter, or it may occur secondary to increased pressure in the bladder due to abnormal bladder function or obstruction. Renal scarring associated with reflux is called *reflux nephropathy* (small scarred kidneys). Acquired reflux nephropathy, which is a result of pyelonephritis, is more common in female children, whereas congenital reflux nephropathy, which results from renal dysplasia, is more common in male children.[117] Children with reflux are more likely to develop pyelonephritis than those without

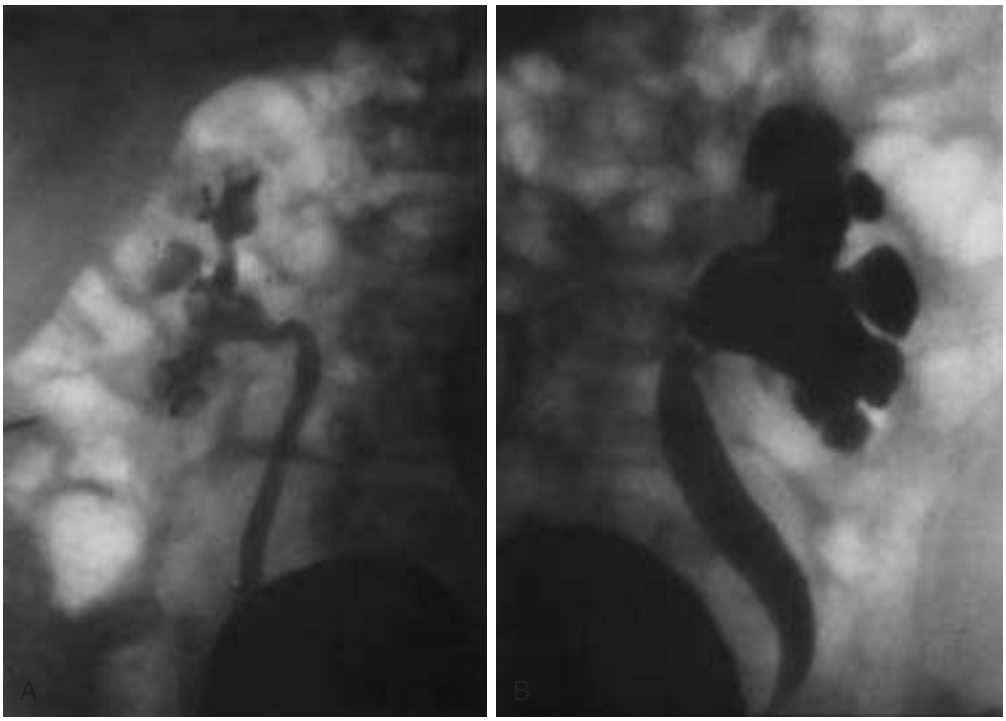

FIG. 72.5 Vesicoureteral reflux in a young girl with recurrent urinary tract infections. (A) Right kidney demonstrates grade II reflux. (B) Left kidney shows dilation of the ureter, grade III reflux, and calyceal clubbing. *(Courtesy Dr. T. Slovis.)*

reflux.[118] Reflux in the presence of infection can be associated with the development of scarring.[115,119] Those with reflux are more likely to develop scarring than those without reflux, and those with higher grades of reflux (grade IV or greater) are more likely to develop scarring than those with lower grades.[118] Renal scarring is 27 times more likely in kidneys with grade IV vesicoureteral reflux as compared to kidneys with no reflux.[120] Unlike the previously published studies, the RIVUR study, which included children ages 2 to 71 months, reported significantly more renal scarring in older children (median age of 26 months) as compared to a median age of 11 months in those with no scarring.[120] Complications of renal scarring include hypertension and proteinuria, and end-stage renal failure may occur in some patients. Scarring has also been associated with delay in antibacterial therapy of UTI.[115]

It should be emphasized that the contribution of reflux alone compared with reflux plus infection in the progression of renal scarring has not been clearly delineated. Severe reflux alone may be associated with renal damage and insufficiency. Studies in uninfected animals have demonstrated that reflux alone, and in particular intrarenal reflux, can produce "pyelonephritic" scars. It has also been shown that the immature kidneys of infants are more prone to intrarenal reflux. However, it is probable that reflux is more likely to lead to severe damage and scarring when infection is also present.[119]

Mild to moderate degrees of reflux (and occasionally even severe reflux) are likely to disappear with time, probably in relation to maturation of the vesicoureteral junction (see "Imaging Studies" later).[119] Severity (grade) of reflux is the strongest predictor of lack of resolution of reflux.[121] Progression of scars already present or the development of new ones is uncommon after the age of 5 years.[119] Treatment options are not well defined. In a multisite study of 607 children with vesicoureteral reflux, antimicrobial prophylaxis did reduce the rate of recurrence, but not scarring. Concerns remain about identifying an appropriate subgroup of patients who would benefit from prophylaxis, in order to decrease unnecessary prescription and selective pressure that favors drug-resistant bacteria.[122] Screening of children for asymptomatic bacteriuria and treatment of asymptomatic bacteriuria are not recommended.

Urinary Tract Infection in Adults

According to the most recently available combined data from the Centers for Disease Control and Prevention's annual National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, in 2007 there were 8.6 million ambulatory visits for UTI in both men and women in the United States.[123] Pyelonephritis is far less common than cystitis, comprising approximately 250,000 cases per year in the United States.[77]

UTIs are much more common in women than in men. Premenopausal women are at especially high risk for acute cystitis; the incidence is 0.5 to 0.7 case per person-year among sexually active women. The strongest risk factors in premenopausal women include sexual intercourse, use of spermicides, and history of previous UTI, as well as diabetes. In this population, risk factors suggesting a genetic component are a history of maternal UTI and age of first UTI. Many of these patients previously had UTIs as children and continue to have infections as adults. Once adulthood is reached, the prevalence of asymptomatic bacteriuria increases in the female but not in the male population. The prevalence of asymptomatic bacteriuria in young nonpregnant women is about 1% to 3%.[124] Each year, about 25% of these bacteriuric women clear their bacteriuria and an equal number become infected, often women who have had urinary infection previously. Up to 60% of the female population (5% in men) will experience at least one symptomatic UTI at some time during their life, and up to 10% of women in the United States have at least one episode of symptomatic infection each year.[78] Worldwide 150 million individuals develop UTIs. The peak incidence of symptomatic infections occurs in young, sexually active women ages 18 to 24 years, with one-third to one-half of women self-reporting at least one UTI by age 32. Recurrent episodes are common.[78,125,126] Frequent sexual intercourse; a new sex partner; diaphragm use, especially with a spermicide; lack of urination after intercourse; and a history of previous infection are risk factors for urinary infection in women.[78,125,127,128] The diaphragm can cause urinary obstruction in some women, but its main effect is probably a change in vaginal flora caused by the spermicide.[125]

About 2% to 5% of healthy women have recurrent UTIs during their lifetime.[129] It seems that there may be a genetic predisposition for recurrent cystitis or pyelonephritis in these women because UTI in female relatives is strongly associated with recurrent UTI.[72,129] Once a woman develops infection, she is more likely to develop subsequent infections than a patient who has had no previous infections. For example, in several studies, after a first episode of cystitis in young women caused by *E. coli,* 24% had a second episode within 6 months. With one or more episodes, the risk of another within 1 year was 70%.[130] Recurrences after the first *E. coli* infection with a different uropathogen (*E. coli* or other) were twice as frequent as recurrences with the same *E. coli* strain.[128] Women with acute pyelonephritis, a much less common infection than cystitis, also tended to have recurrences.[130] It may be a simple matter to cure an individual episode, but recurrence, most often reinfection, is common. The type of infection that recurs (i.e., asymptomatic bacteriuria, cystitis, or pyelonephritis) depends on a complex interaction between genetic factors and virulence characteristics of the uropathogen.[131] It must be stressed that in the nonpregnant adult female with a normal urinary tract, asymptomatic bacteriuria infrequently progresses to symptomatic infection and that cystitis uncommonly progresses to pyelonephritis. *Escherichia coli* causing UTI may be transmitted between sexual partners because the same strains have been found in the urine of men who are sexual partners of women with UTI.[132]

The prevalence of asymptomatic bacteriuria in adult men is low (≤0.1%), as is the occurrence of symptomatic infection, until older age, when it rises.[133] Men with UTI frequently have anatomic abnormalities of the urinary tract and therefore should be considered to have complicated UTI until proven otherwise.[78] In young men, a lack of circumcision increases the risk of UTI caused by uropathogenic strains of *E. coli,* including the development of symptomatic urethritis.[134] Male homosexuality is a risk factor, probably related to rectal insertive intercourse.[135]

It is clear that UTI in adults can lead to progressive renal damage in the presence of obstruction. However, recurrent infection in adults in the absence of obstruction rarely, if ever, leads to renal failure.[124] The United States Renal Data System, which reports causes of renal failure in patients undergoing dialysis, does not even mention infection other than reflux nephropathy, which may or may not have involved infection.[136]

Autopsy studies have shown that it is difficult to implicate infection per se (i.e., in the absence of other renal abnormalities) as an important pathogenic factor in the production of severe renal disease in adults. One exception might be severe papillary necrosis secondary to infection. One group of investigators was unable to find any case of uncomplicated pyelonephritis that progressed to end-stage renal disease among 173 patients admitted to dialysis programs.[137] In prospective studies, hundreds of patients with persistent or recurrent infections in the absence of other underlying renal disease have been followed for years without documenting the progression of renal disease from infection alone.

The role of infection in the progression of clinically or radiographically diagnosed interstitial renal disease has also been examined.[112,124] In general, the studies indicate that infection is rarely, if ever, the major factor leading to further renal decompensation. However, infection may occasionally accelerate the progression of the primary underlying disease process. In summary, except for perhaps rare cases, there is no evidence to indicate that uncomplicated UTI alone produces renal failure in adults.

Urinary Tract Infection in the Elderly

At least 10% of men and 20% of women older than 65 years have asymptomatic bacteriuria. In contrast to young adults, in whom bacteriuria is 30 times more frequent in women than in men, in those older than 65 years the ratio alters dramatically, with a progressive decrease in the female-to-male ratio.[138,139] In both men and women, the prevalence of asymptomatic bacteriuria rises substantially. Possible

reasons for the high frequency of asymptomatic bacteriuria in older patients include obstructive uropathy from the prostate (with resultant instrumentation) and loss of the bactericidal activity of prostatic secretions in men, poor emptying of the bladder because of prolapse in women, soiling of the perineum from fecal incontinence in demented women, and neuromuscular diseases and increased instrumentation and bladder catheter usage in both genders.[139] The majority of older men with UTI have underlying urologic abnormalities.[133] Urinary incontinence also contributes to UTI in postmenopausal women.[140] There is a high rate of spontaneous cure and reinfection with asymptomatic bacteriuria in women and men.[139] The spectrum of microorganisms is unaltered in the older adult population. Symptomatic infection uncommonly follows asymptomatic bacteriuria, and asymptomatic bacteriuria is much more frequent than symptomatic UTI in this age group.

Asymptomatic bacteriuria in older persons does not seem to have any deleterious effects.[141,142] Furthermore, there is no evidence to suggest that treatment of asymptomatic bacteriuria in older patients has any beneficial effects, including decreasing urinary incontinence.[142,143] Therefore routine treatment of asymptomatic bacteriuria in older patients is not indicated.[142–144] Effective management of symptomatic episodes in older males requires determining whether the site of infection is the kidney, bladder, or prostate.

Urinary Tract Infection in Other Conditions

There is a higher prevalence of asymptomatic and symptomatic UTI in hospitalized and nursing home patients than in outpatients. The general ill health of these patients and the higher probability of urinary tract instrumentation are probably the major contributors to these differences. A single catheterization causes UTI in only about 1% of ambulatory persons. However, after a single catheterization of hospitalized patients, infection occurs in at least 10%.

Race apparently does not appreciably affect the prevalence of asymptomatic bacteriuria. However, socioeconomic status is important, with pregnant women from lower socioeconomic groups having a higher prevalence of bacteriuria than those from higher socioeconomic groups.[145]

Various underlying diseases have been associated with an increased frequency of UTI. Diabetic women but not men have been found to have a higher prevalence of asymptomatic bacteriuria than nondiabetic patients[146–148]; they also have an increased frequency of symptomatic infection. Diabetics also have a higher incidence of severe infection, including severe pyelonephritis and uncommon complications such as emphysematous pyelonephritis and perinephric abscess. However, studies that followed diabetic women for up to 3 years found no advantage in screening for and treating asymptomatic UTI; it was concluded that diabetes should not be an indication for screening for or treatment of asymptomatic bacteriuria.[102,149]

African-American women with sickle cell trait had been reported to have a higher prevalence of bacteriuria during pregnancy than African-American women without the sickle trait. A more recent report did not find this association.[150] Other conditions stated to be associated with UTI (but without documentation) include chronic potassium deficiency, gout, hypertension, and other conditions causing interstitial renal disease. There is an increased frequency of symptomatic and asymptomatic UTIs in human immunodeficiency virus infection and particularly with low CD4 counts with a higher incidence of nontypical bacterial pathogens.[151,152]

At least 50% of renal transplantation patients develop UTIs in the early postoperative period; up to 14% develop pyelonephritis, and up to 7% develop bacteremia of UTI origin in the first year.[153–155] Antibacterial prophylaxis is often used in the early transplantation period to prevent UTI. Recent studies have emphasized the emergence of enterococci and drug-resistant Enterobacteriaceae as posttransplantation uropathogens.[156] There is a growing perspective that asymptomatic bacteriuria should not be treated because it is not generally associated with development of symptomatic disease or graft dysfunction.[157]

Table 72.3 lists risk factors for UTIs.

TABLE 72.3 Risk Factors for Urinary Tract Infection (UTI)

Age	Female	Male
All ages	Previous UTI	Lack of circumcision (children and young adults)
	Urologic instrumentation or surgery	Urologic instrumentation or surgery
	Urethral catheterization	Urethral catheterization
	Urinary tract obstruction, including calculi	Urinary tract obstruction, including calculi
	Neurogenic bladder	Neurogenic bladder
	Renal transplantation	Renal transplantation
Adults	UTIs in female relatives	Insertive rectal intercourse
	Sexual intercourse	Vaginal *Escherichia coli* colonization in partner
	New sex partner	
	Lack of urination after intercourse	
	Spermicidal contraceptive jellies	
	Diaphragm use	
	Pregnancy	
	Lower socioeconomic group	
	Diabetes	
	Possibly sickle cell trait in pregnancy	
Older age	Functional or mental impairment	Functional or mental impairment
	Estrogen deficiency (loss of vaginal lactobacilli)	Prostatic enlargement
	Bladder prolapse	Condom catheter drainage

Infection in the Obstructed Kidney After Urologic Treatment of Hydronephrosis

Bacteria or *Candida* may reach the renal pelvis through a percutaneous nephrostomy catheter or retrograde from the bladder through a ureteral stent, particularly if the catheter or stent is in place for several weeks. This colonization of the renal pelvis is generally asymptomatic unless the urine flow is obstructed. This can happen when a stent or nephrostomy tube is removed and significant ureteral stricture remains. Infection of the renal pelvis and calyces can develop rapidly, with fever and pain. Pus can fill the renal pelvis and rapidly destroy the kidney. Sometimes pus will exit the renal pelvis along a prior nephrostomy tract and form a perirenal abscess. Emergency drainage of the renal pelvis and systemic antimicrobial therapy are indicated. As a preventive measure, if there is concern about inadequate ureteral drainage after removing a stent or percutaneous nephrostomy catheter, it is useful to treat the organism in the urine just before the procedure. When *Candida* is in the urine, systemic fluconazole may be useful because of its high urinary concentrations.

Infections in Polycystic Kidneys

Patients with autosomal-dominant polycystic kidney disease are prone to recurrent episodes of infections of their renal cysts, manifesting as fever, leukocytosis, and flank pain. Urine cultures may be negative, but bacteremia is common. *Escherichia coli* is the most common causative organism. Lipid-soluble antibiotics such as fluoroquinolones or TMP-SMX are thought to penetrate best into cysts. Computed tomography (CT) may show intracystic gas or a contrast-enhanced wall. Magnetic resonance imaging (MRI) is useful if renal function permits gadolinium contrast. Distinguishing infection from hemorrhage into a cyst, which is common, can be challenging. Patients not responding to antimicrobial therapy may need ultrasound-guided aspiration of infected cysts.[158]

CLINICAL MANIFESTATIONS

Symptoms

Urinary tract infection in children tends to manifest with different symptoms, depending on the age of the child. Symptoms in neonates and children younger than 2 years are nonspecific.[105,107,108,110] Failure to thrive, vomiting, and fever seem to be the major manifestations. When

children older than 2 years (and, more consistently, older than 5 years) develop infection, they are more likely to display localizing symptoms such as frequency, dysuria, and abdominal or flank pain.

The manifestations of UTI in adults are usually easy to recognize. The lower tract symptoms result from bacteria producing irritation of urethral and vesical mucosa, causing frequent and painful urination of small amounts of turbid urine. Patients sometimes complain of suprapubic heaviness or pain. Occasionally, the urine is grossly bloody or shows a bloody tinge at the end of micturition. Fever is generally absent with cystitis and, if present, should suggest upper tract infection. In a male, presence of fever with only symptoms of cystitis may indicate acute prostatitis.

The classic clinical manifestations of pyelonephritis include fever (sometimes with chills), flank pain, and frequently lower tract symptoms (e.g., frequency, urgency, and dysuria). At times the lower tract symptoms antedate the appearance of fever and upper tract symptoms by 1 or 2 days. It should be recognized that the symptoms described, although classic, may vary greatly. In fact, pyelonephritis may show protean clinical manifestations in adults as well as in children. Flank tenderness or discomfort is frequent in upper tract infection in adults and is more intense when there is obstructive disease. Severe pain with radiation into the groin is rare in acute pyelonephritis per se and suggests the presence of a renal calculus. The pain from the kidney is occasionally felt in or near the epigastrium and may radiate to one of the lower quadrants. These manifestations may offer difficulties in differential diagnosis and suggest gallbladder disease or appendicitis. Functionally independent older adults will present with typical symptoms of UTI.[159] Symptoms may not be diagnostic, however, because noninfected older patients often experience frequency, dysuria, hesitancy, and incontinence. In the very old or frail elderly, worsening of cognitive impairment, delirium, or falls may be the only presenting symptom.[159] Older age is an independent risk factor for severe sepsis and septic shock in individuals with acute complicated pyelonephritis.[160] The effect of asymptomatic bacteriuria on the general sense of well-being, appetite, and urinary continence has been studied, but no association could be demonstrated.[138,161–163]

Silent upper tract infection may be accompanied by lower tract symptoms only or no symptoms at all. Patients with UTIs in the presence of an indwelling urinary catheter usually have no lower tract symptoms, but flank pain or fever may occur. Urinary tract infection is the most common source of bacteremia produced by gram-negative bacilli, which may result in the sepsis syndrome. Bacteremia may occur with no urinary symptoms, especially in the presence of an indwelling catheter.

Symptoms of UTI are frequently difficult to elicit in older adults because of the presence of dementia, indwelling urinary catheters, and the atypical symptoms often seen in this population. Therefore a diagnosis of sepsis due to UTI may be made erroneously in the absence of urinary symptoms because of the presence of unrelated fever and asymptomatic bacteriuria, which is often present in this population.

Alterations in Renal Function

In experimentally produced pyelonephritis, the only consistent abnormality of renal function is the inability to concentrate the urine maximally.[164] The mechanism of the concentrating defect is not clear but seems to be related in experimental animals to inflammation and perhaps to the increased production of prostaglandins. The concentrating defect occurs early in the course of experimental infection and is rapidly reversible with antimicrobial therapy and after the administration of prostaglandin inhibitors. The same phenomenon occurs in humans.

Progressive destruction of the kidney in the presence of obstruction may occur and give rise to clinical manifestations of renal insufficiency. Occasionally, bilateral papillary necrosis can lead to rapidly progressive renal failure. Acute kidney injury in the setting of pyelonephritis may occur secondary to concomitant severe sepsis (acute tubular necrosis).

Pyelonephritis in childhood, even with no evidence of compromised renal function in adolescence, has been shown to be a risk factor for renal disease in adulthood.[165] Among adult women hospitalized with pyelonephritis, renal scars could be demonstrated with sensitive imaging techniques; however, no correlation with hypertension or chronic renal disease was seen.[166]

DIAGNOSIS

Presumptive Diagnosis of Urinary Tract Infection

Using the preferred definition of pyuria, which is at least 10 leukocytes/mm^3 of midstream urine by counting chamber, the vast majority of patients with symptomatic or asymptomatic bacteriuria have pyuria. In fact, with symptomatic infection, most have hundreds of leukocytes per cubic millimeter. A less reliable method uses a urine specimen that is centrifuged for 5 minutes at 2000 rpm and then the sediment is examined under high power. With this method, 5 to 10 leukocytes/high-power field in the sediment is the upper limit of normal. It should be emphasized that the finding of pyuria is nonspecific, and patients with pyuria may or may not have infection.[167] The absence of pyuria should strongly call into question a diagnosis of UTI.

The dipstick leukocyte esterase test is a rapid screening test for detecting pyuria and has largely replaced microscopic methods. Although the sensitivity and specificity are high for detecting more than 10 white blood cells/mm^3 of urine (75%–96% and 94%–98%, respectively), a positive test by no means indicates UTI; in patients with a negative leukocyte esterase test and UTI symptoms, a urine microscopic examination for pyuria or a urine culture should be considered.[168,169]

Microscopic or sometimes gross hematuria is occasionally seen in patients with UTI (i.e., hemorrhagic cystitis). However, red blood cells may be indicative of other disorders, such as calculi, tumor, vasculitis, glomerulonephritis, and renal tuberculosis. White cell casts in the presence of an acute infectious process are strong evidence for pyelonephritis, but the absence of white cell casts does not rule out upper tract infection. White cell casts can also be seen in renal disease in the absence of infection. Proteinuria is a common although not universal finding in UTI. Most patients with UTI excrete less than 2 g of protein/24 h; excretion of 3 g/24 h or more suggests glomerular disease.

Microscopic examination of a urine specimen for bacteria can be useful for the presumptive diagnosis of UTI. The ability to identify bacteria in the urine depends on whether the specimen has been centrifuged and on whether it has been stained with Gram or methylene blue stain (Table 72.4). Smaller numbers of bacteria can be detected microscopically in a stained than in an unstained specimen, and smaller numbers can be detected in centrifuged than in uncentrifuged urine. The presence of at least one bacterium per oil immersion field in a midstream clean-catch, Gram-stained, uncentrifuged urine specimen correlates with 10^5 bacteria/mL of urine or more. Because this titer is regarded to represent significant bacteriuria, Gram staining of an uncentrifuged specimen is an easy, rapid, and relatively reliable way to detect significant numbers of organisms. The absence of bacteria in several fields in a stained sedimented specimen indicates the probability of fewer than 10^4 bacteria/mL.

A number of rapid indirect methods have been devised to detect bacteriuria for presumptive diagnosis. Most common are tests (e.g., dipstick) that detect the presence of urine nitrite, which is formed when bacteria reduce the nitrate that is normally present.[168] False-negative test results are common, especially in the detection of low-count bacteriuria (10^2–10^3/mL) and with certain bacterial species, but false-positive results are unusual. The sensitivity and specificity of screening tests for UTI, such as dipsticks, depends on the likelihood of infection in the group being studied (e.g., acutely symptomatic patients vs. those

TABLE 72.4 Bacterial Count by Direct Examination of Urine

Sample	Unstained (×400)	Stained (×1000)
Uncentrifuged sample	$\geq 0^6$	$\geq 10^5$
Centrifuged sample	$\geq 10^5$	$\geq 10^4$

[a]Colony-forming units (CFU)/mL extrapolated from the finding of one bacterium per microscopic field.

who are asymptomatic) and range widely.[170–172] A negative leukocyte esterase test plus a negative nitrite test are strongly predictive of the absence of UTI.

Women with acute onset of symptoms of cystitis and without factors that would make the infection complicated can be diagnosed and safely managed on the basis of history only, without diagnostic testing.[173]

Diagnosis of Urinary Tract Infection by Culture

General Considerations

Because the urethra and periurethral areas are difficult to sterilize, even the most carefully collected specimens (including those obtained by catheterization) are frequently contaminated by organisms that are normal colonizers in these sites. By quantitating bacteria in midstream clean-voided urine, it is possible statistically to separate contamination from UTI. Most patients with infection usually have at least 10^5 bacteria/mL in urine in the bladder, and therefore voided urine usually contains at least 10^5 bacteria/mL. In patients without infection a properly collected, voided urine sample usually contains less than 10^4 bacteria/mL. However, it is important to remember that about one-third of young women with cystitis have fewer than 10^5 bacteria/mL of urine (see "Urinary Tract Infection with Low Numbers of Organisms" later). It is likely that a significant proportion of other patients with symptomatic and asymptomatic infection have fewer than 10^5 bacteria/mL of urine. The Infectious Diseases Society of America consensus culture definition of cystitis for use in antibiotic treatment studies is 10^3 colony-forming units (CFU)/mL or more of a uropathogen (sensitivity 90% and specificity 90%) and, for pyelonephritis, 10^4 CFU/mL or more (sensitivity 90% and specificity 90%).[174] In a subsequent set of practice guidelines, 10^2 CFU/mL or more of a uropathogen was used.[175] These concentrations of microorganisms can be identified by standard microbiologic techniques in most clinical laboratories. The belief that bladder urine is normally sterile has been increasingly challenged by work that demonstrates the existence of a urinary microbiome.[176,177]

Calibrated loops serve as a simple, inexpensive way to examine the bacteriologic characteristics of urine specimens quantitatively. Platinum loops that deliver 0.01 and 0.001 mL are used to streak urine onto agar plates. After incubation at 37°C for 24 hours, the number of CFU is counted, and the total number of organisms originally present in the specimen is estimated by multiplying the colony count by 10^2 or 10^3, respectively. Other methods, such as the dip inoculum method, in which agar-coated glass slides or paddles are dipped into urine and then incubated, have excellent correlations with calibrated-loop techniques.

Acceptable methods for urine collection include (1) midstream clean-catch, (2) "in and out" catheterization, and (3) suprapubic aspiration. The clean-catch method has traditionally been preferred for the routine collection of urine for culture. It avoids the risk of infection inherent in catheterization. The patient must be instructed in the proper technique of obtaining the urine; this is especially important for women. The woman should wash her hands, straddle the commode (facing the back of the commode), wash her vulva from front to back four times with four different sterile gauze pads soaked in green soap or another appropriate cleansing agent, and then rinse with two more sponges soaked in sterile distilled water. She should then spread her labia and void, discarding the first portion of urine and collecting the second. The urine should be processed immediately or, if refrigerated at 4°C, it can be cultured within 24 hours.

Some have challenged the need for cleansing or for using a midstream specimen from women when collecting urine for culture. Similar contamination rates were noted when midstream urine was collected after cleansing versus voiding into a container without cleansing.[178] A more recent study demonstrated similar rates of contamination for midstream clean-catch, midstream, and random samples.[179]

If there are more than 10^5 bacteria/mL in a clean-catch urine specimen from an asymptomatic woman, there is an 80% probability that this represents true bacteriuria. If two different specimens demonstrate at least 10^5 of the same bacterium/mL, the probability increases to 95%. Thus two clean-catch specimens should be obtained in an asymptomatic woman to confirm the diagnosis.[144] When the number of bacteria per milliliter is between 10^4 and 10^5 in an asymptomatic woman, a confirmatory second specimen will contain 10^5 or more bacteria/mL in only 5% of cases. Thus in asymptomatic women, 95% of the time 10^4 to 10^5 bacteria/mL represents contamination, with occasional infection manifested by fewer than 10^5 bacteria/mL of urine. In asymptomatic men, in whom contamination is less likely, 10^3 or more organisms/mL in one culture is suggestive of infection, and 10^5/mL defines bacteriuria.[144] False-positive cultures are caused by contamination or incubation of urine before processing. False-negative cultures may be caused by the use of antimicrobial agents, soap from the preparation falling into the urine, total obstruction below the infection, infection with a fastidious organism, renal tuberculosis, and diuresis.

These criteria apply only to the Enterobacteriaceae. Gram-positive organisms, fungi, and bacteria with fastidious growth requirements may not reach titers of 10^5/mL in patients with infection and may be in the 10^4 to 10^5/mL range. The organism recovered often helps distinguish contamination from true bacteriuria. Samples with counts of less than 10^4 organisms/mL often contain saprophytic skin organisms, such as diphtheroids, *Neisseria,* and staphylococci. Pure growth of Enterobacteriaceae is uncommonly found in low-titer specimens but is present in over 90% of urine samples containing more than 10^5 bacteria/mL. High colony counts containing more than one species of bacteria from the urine of asymptomatic persons often represent contamination but may be more significant in the presence of symptoms. Mixed infection occurs in about 5% of cases.

In patients with symptoms of UTI, one titer of 10^5 or more bacteria/mL of urine carries a 95% probability of true bacteriuria. With titers below 10^5/mL but in the presence of frequency, urgency, and dysuria, women have a 33% chance of having bacterial infection (see "Urinary Tract Infection with Low Numbers of Organisms" later). The presence of low numbers of Enterobacteriaceae (i.e., 10^2–10^5/mL) in such women correlates highly with infection. These lower cutoffs do not apply to infection with gram-positive organisms. In women with acute uncomplicated cystitis, enterococci and group B streptococci isolated from midstream urine were often not found in catheterized urine obtained at the same time.[180]

Samples obtained by catheterization from noninfected patients are less likely to become contaminated. According to the guidelines, 10^2 CFU/mL or more is consistent with bacteriuria.[144] For diagnosis with an indwelling catheter, see Chapter 302.

Urinary Tract Infection With Low Numbers of Organisms

Most women with an acute onset of frequency, urgency, or dysuria, or all of these, have UTI with 10^5 or more bacteria/mL of urine (Fig. 72.6). However, up to one-half are found to have fewer than 10^5 bacteria/mL of urine,[78,181] and the term *urethral syndrome* has been used to refer to this entity. As shown in Fig. 72.6, Stamm and associates have demonstrated

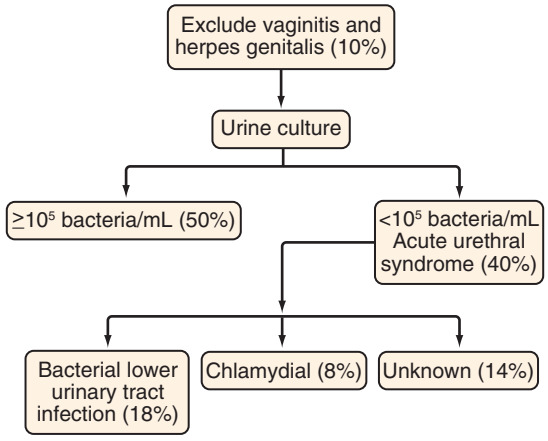

FIG. 72.6 Relative frequencies of causes of acute onset of frequency or dysuria, or both, in young women. *(Modified from Stamm WE, Wagner KF, Amsel R, et al. Causes of acute urethral syndrome in women.* N Engl J Med. *1980;303:409–415.)*

that many of these women have UTI but with low numbers of organisms. When suprapubic bladder aspirates are compared with voided midstream urine in acutely dysuric women, 10^2 or more coliforms/mL in midstream urine have a sensitivity and specificity of 95% and 85%, respectively, for UTI. These women have urinary infections mainly restricted to the lower tract. Furthermore, up to one-third of young women with symptomatic infection localized to the lower urinary tract have fewer than 10^5/mL bacteria in their urine.

A study of women coming to a university health clinic for any reason demonstrated that stepwise increases in bacterial counts from 10^2 to 10^5 CFU/mL are significantly associated with an increased incidence of symptoms and pyuria[182]; it was postulated that low-count bacteriuria represents an early phase of urinary infection. This hypothesis appears to have been supported in a clinical study in which 21 women with low-count bacteriuria (10^2–$<10^5$ CFU/mL) had therapy delayed for 2 days, and reculture showed that 10 of 21 (48%) developed concentrations of more than 10^5 CFU/mL.[182a]

The remaining women with the urethral syndrome, after excluding those with bacteria in the bladder and those with genital herpes infection or vaginitis, can be divided into two groups: (1) those with pyuria from urethritis (e.g., caused by *Chlamydia trachomatis, Neisseria gonorrhoeae,* or *Mycoplasma genitalium* infection); and (2) those without pyuria in whom all bacterial cultures are negative. The pathogenesis of this latter symptom complex is unknown, but various fastidious microorganisms and noninfectious factors (traumatic, psychological, allergic, and chemical) have been suggested as causes. Patients with *C. trachomatis, N. gonorrhoeae,* and *M. genitalium* urethritis respond to antibacterial therapy. Vaginitis is a common cause of dysuria and, accordingly, patients should be questioned regarding vaginal symptoms, particularly if the complaint of burning is external, such as pain felt in the inflamed labia during micturition. If vaginitis is suspected, a pelvic examination should be performed. Dysuria has also been described in 10% of women with initial genital herpes infection.

Although symptoms and the clinical settings cannot reliably distinguish between the causes of dysuria in women, they can be suggestive.[169] Bacterial UTIs tend to have a sudden onset of severe dysuria with frequency and urgency; suprapubic pain and/or hematuria may be present, and there is pyuria. Clinical clues to urethritis (chlamydial, gonococcal, or mycoplasmal) include the following: a patient with a gradual onset of milder dysuria, with or without frequency and urgency, who is sexually active with a recent new sexual partner; hematuria is absent but vaginal discharge or bleeding may be present from chlamydial or gonococcal cervicitis; and/or pyuria is present. With herpes, there are usually lesions in the periurethral area. The diagnosis of urethral chlamydial or gonococcal infection may be confirmed by nucleic acid amplification tests on urine. With vaginitis, the dysuria tends to be mild with gradual onset and is felt externally, frequency and urgency are absent, there is often a vaginal discharge, and pyuria is usually absent in a midstream specimen.

The diagnostic testing for UTI described in the previous sections has changed very little in over 60 years, and new diagnostic techniques are currently under investigation to improve the sensitivity and specificity of diagnosis. Enhanced quantitative urine culture has been reported to detect bacteria in specimens that are reported as sterile by standard culture techniques, including in almost one-half of women with severe symptoms.[183,184] Real-time quantitative polymerase chain reaction and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry have been studied to increase the detection of difficult-to-culture pathogens and decrease the time to positive results; however, neither of these techniques supplies data on antimicrobial susceptibility. More specific markers of infection-associated pyuria—including neutrophil gelatinase-associated lipocalin, a protein from neutrophil granules—are under investigation.[185,186]

Because clinical microbiology laboratories process a high volume of urine cultures and the majority of these cultures are negative, flow cytometry techniques to screen samples for pyuria and bacteriuria and only culture those likely to be positive have been utilized.[187–189] Some of these studies have confirmed the excellent performance of traditional urinalysis, underscoring the value of not submitting urine cultures in patients who do not have pyuria.

Localization of Site of Infection

Invasive diagnostic techniques to localize the site of infection to the kidney versus the bladder have been utilized to understand the epidemiology and response to therapy of UTI.[190] In clinical practice, localization relies mainly on clinical symptoms and signs. Many studies have looked at the use of both serum and urinary biomarkers to distinguish cystitis from pyelonephritis, especially in infants and very young children, in whom the inability to elicit symptoms increases the diagnostic challenge. A Cochrane Database review did not find evidence to support the routine use of procalcitonin, C-reactive protein, or erythrocyte sedimentation rate to differentiate pyelonephritis from cystitis in children.[191] At present, there is no biomarker that has sufficient evidence to support routine use in clinical practice.

MANAGEMENT OF URINARY TRACT INFECTION

General Considerations

The diagnosis of infection in the asymptomatic patient should be made on no fewer than two cultures of clean-voided midstream urine in which the same microorganism is present in titers of 10^5/mL or greater. If the patient is symptomatic, one specimen suffices.

All symptomatic UTI is usually treated because the relief of symptoms is a clear-cut benefit. However, symptoms of cystitis often subside spontaneously or following symptomatic treatment.[79,192–194] On the other hand, there has been no benefit demonstrated or currently postulated in the treatment of asymptomatic bacteriuria in any group outside of pregnant women; those patients who are to undergo traumatic genitourinary procedures associated with mucosal bleeding, such as transurethral prostatectomy; and those who have recently had renal transplantation. In one study, treatment of asymptomatic bacteriuria in young women with recurrent UTI increased the frequency of symptomatic infection.[195] A rational approach to the treatment of UTI depends on an appreciation of the prognosis of the untreated infection and the long-term results to be expected from therapy. The side effects, cost, and inconvenience of different therapeutic regimens must also be considered. Because there is no demonstrated benefit of treatment of asymptomatic UTI in nonpregnant adult women, therapy of asymptomatic bacteriuria in this group is not recommended.[144]

Although asymptomatic bacteriuria in older adults is associated with degenerative and debilitating diseases and in some old reports with mortality, there is no convincing evidence for a cause-and-effect relationship.[139,141,143,196] There certainly is no evidence that treatment of the bacteriuria alters the patient's course. Asymptomatic bacteriuria serves as a marker for debilitating diseases, which in turn may contribute to mortality. In addition, bacteriuria is common in older adults, and many of these patients become reinfected or relapse after antimicrobial therapy. Furthermore, a higher frequency of side effects from chemotherapy would be expected in an older age group because of preexisting renal, auditory, and other diseases. Considering the large number of patients involved, antimicrobial therapy may lead to an unwarranted financial burden and danger of drug toxicity. Treatment is not indicated for asymptomatic bacteriuria in older patients.[138,144] In contrast, bacteriuria in pregnancy may have serious implications. Treatment of pregnant women with asymptomatic bacteriuria is most likely to be beneficial and is strongly recommended; however, it must be acknowledged that the overall quality of evidence to support this recommendation is not high.[197] A recent study found lower rates of pyelonephritis among pregnant women with untreated asymptomatic bacteriuria than did prior studies on which treatment recommendations have been based; however, these women were still more likely than those without asymptomatic bacteriuria to have a subsequent and recurrent UTI requiring treatment with antibiotics during the pregnancy.[198]

Hospitalized patients with asymptomatic bacteriuria have higher mortality rates than those without bacteriuria.[199] This observation may be related to deaths from bacteremia in patients with indwelling urinary catheters.

Some patients have such frequent symptomatic episodes (either relapses or reinfections) that they are almost chronically incapacitated. In these patients, it may be necessary to give prolonged therapy or prophylaxis to prevent recurrent symptoms.

In an adult male with a UTI, the possibility of complicated UTI is much higher than in a female, and the existence of a correctable obstructive lesion must be considered. At a minimum, a postvoiding ultrasound of the urinary tract should be obtained to evaluate bladder emptying. For management of patients with an indwelling catheter, see Chapter 302.

Nonantimicrobial Therapy

Although there is a theoretical basis on which to postulate a benefit from hydration and acidification of the urine in the setting of symptomatic UTI, there is no evidence to support the efficacy of these nonpharmacologic interventions, and they should not be recommended. Two recent randomized controlled trials have studied the potential role of nonsteroidal antiinflammatory agents (NSAIDs) in the management of acute uncomplicated cystitis.[200,201] In both studies, NSAIDs were inferior in terms of rapidity of symptom resolution, and women who received NSAIDs had a higher rate of subsequent pyelonephritis. Although the initial use of analgesics clearly results in less antibiotic use, symptom duration is prolonged and the risk of complications (pyelonephritis) may be higher, so this approach should not be routinely recommended at this time. There may be a role for delayed antibiotic treatment in special circumstances such as multiple antibiotic allergies, or recurrent *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea, since the rate of spontaneous resolution of acute cystitis is substantial. Urinary analgesics such as phenazopyridine hydrochloride (Pyridium) are not recommended because of lack of evidence to support additional benefit in addition to antibiotic therapy. Systemic analgesics are indicated in patients with severe dysuria and those with severe flank pain due to acute pyelonephritis.

Prevention of Urinary Tract Infection

Prevention is aimed primarily at recurrent symptomatic reinfections in women because this is the group that most often has reinfections. Although elimination of some risk factors such as use of spermicidal contraceptive jellies may decrease the numbers of episodes, many women continue to have symptomatic reinfections. Antimicrobial agents have been the mainstay in approaching reinfections, but with increasing resistance of uropathogens to antimicrobial agents, other approaches have been increasingly evaluated.

The use of vaginal estrogens has been shown to be an effective strategy to prevent recurrent UTIs in postmenopausal women and is recommended in a clinical practice guideline.[202,203] The benefit of topical estrogens is believed to be related to decreasing the vaginal pH and increasing colonization of the vagina with lactobacilli, thereby decreasing colonization with Enterobacteriaceae. There continue to be conflicting data on the use of cranberry products for UTI prevention, with some studies reporting effectiveness in reducing infections and others not. A Cochrane Database review concluded that there was little or no benefit of cranberry juice.[204] However, a meta-analysis published the same year found that there was a protective effect while noting substantial heterogeneity across the included clinical trials.[205] A more recently published meta-analysis also suggested a protective effect; however, again the heterogeneity of both the patient populations studied and cranberry products used must be acknowledged.[206] At present, there are not sufficient data to make a clear recommendation, and further large trials utilizing a standardized cranberry product are needed.

At present, there is also insufficient evidence to support a recommendation for either systemic or intravaginal probiotics as a strategy to prevent recurrent UTI.[207] Vaccines to prevent UTI have been under study for several decades, and several promising candidate vaccines are currently in phase III clinical trials.[208,209]

Principles of Antimicrobial Therapy

In most cases, many available agents are satisfactory for the treatment of UTI. Given two or more drugs with good activity against the probable infecting microorganism, the agent with the least toxicity, the least likelihood of affecting the normal flora of the vagina and gastrointestinal tract, the narrowest spectrum, and favorable cost should be chosen.

There is no evidence to support any superiority of bactericidal drugs over bacteriostatic agents in UTI. However, there may be theoretical reasons for using bactericidal drugs in the treatment of relapsing UTI.

Serum, Tissue, and Urine Concentrations of Antimicrobial Agents

A poor correlation exists between the response of bacteriuria and serum levels of antimicrobial agents. In the dosages commonly used for UTI, some oral antimicrobial agents do not achieve serum levels above the minimal inhibitory concentration for most urinary pathogens.

The disappearance of bacteriuria is closely correlated with the sensitivity of the microorganism to the concentration of the antimicrobial agent achieved in the urine.[210] It is probable that the area in which it is most important to reach antibacterial concentrations in pyelonephritis is the intramedullary part of the kidney. Urine concentrations that are inhibitory for sensitive microorganisms are achieved after oral administration of essentially all antimicrobial agents commonly used for UTIs. Although serum levels do not seem to be important in the treatment of UTI, they may be critical in patients with bacteremia and may be important in the cure of patients with renal parenchymal infection who relapse.

In patients with renal insufficiency, dosage modifications are necessary for agents that are excreted primarily by the kidneys and cannot be cleared by any other mechanism. In renal failure, the kidney may not be able to concentrate an antimicrobial agent in the urine, and there may be difficulty in eradicating bacteriuria.[211] This may be an important factor in the failure of therapy for UTI with aminoglycosides.

In addition, high concentrations of magnesium and calcium, as well as a low pH level, can raise the minimal inhibitory concentration of aminoglycosides for gram-negative bacilli to levels above those achievable in the urine of patients with renal failure. In general, the penicillins, cephalosporins, and many fluoroquinolones attain adequate urine concentrations, despite severely impaired renal function. From the point of view of safety, the penicillins and cephalosporins should be considered first-line agents in the treatment of UTIs in patients with renal insufficiency.

Response to Therapy

If therapy is appropriate, clinical response should occur within 24 hours with treatment of cystitis. With pyelonephritis, response should occur by 48 to 96 hours. Lack of response by 72 hours should be an indication for imaging studies. There are four patterns of response of bacteriuria to antimicrobial therapy—cure, persistence, relapse, and reinfection.

Bacteriologic Cure

This term is defined as negative urine cultures on chemotherapy and during the follow-up period, usually 1 to 2 weeks. However, it must be understood that many of these patients will develop reinfection at a later time.

Bacteriologic Persistence

This term has been used in two ways to describe a response to therapy: (1) persistence of significant bacteriuria after 48 hours of treatment, and (2) persistence of the infecting organism in low numbers in urine after 48 hours. Significant bacteriuria usually persists only if the urinary levels of the antimicrobial agent are below the concentration of the drug needed to inhibit the microorganism. This can occur when the infecting strain is resistant to the urinary levels attained (i.e., a resistant organism) or because the urinary levels of the drug are inordinately low (i.e., from not taking the agent, insufficient dosage, poor intestinal absorption, or poor renal excretion, as in renal insufficiency). Persistence of the infecting microorganism in low titers in voided urine may mean persistence in the urinary tract or contamination from the urethra or vagina. Bladder-puncture cultures would be necessary to evaluate the significance of low titers of bacteria obtained when the patient is receiving therapy, but this procedure is rarely indicated. Bacteria may persist in the urinary tract during therapy without excretion of organisms in the urine. Sites of persistence in the urinary tract are the renal parenchyma, calculi, and prostate. Persistence in bladder cells is a theoretical possibility.[212]

Bacteriologic Relapse

This usually occurs within 1 to 2 weeks after the cessation of chemotherapy and is often associated with renal infection, structural abnormalities of the urinary tract, or chronic bacterial prostatitis. Relapse indicates that the infecting microorganism has persisted in the urinary tract during therapy. However, an apparent relapse can be related to reinfection (new infection) with the same microorganism. In spite of eradication from the urinary tract, the original infecting organism may still be present in the periurethral area, vagina, or intestine and then may cause a new infection. Delayed relapses (more than 1–2 weeks after stopping therapy) are much more likely to be the result of this phenomenon or of chronic bacterial prostatitis than of true relapse. Relapses occurring within 1 week are usually true relapses (i.e., from within the urinary tract).

Reinfection

After initial sterilization of the urine, reinfection may occur during the administration of chemotherapy (also called *superinfection*) or at any time thereafter. Reinfection is easy to identify when there is a change in bacterial species. However, there may be reinfection with a different strain of the same species (usually *E. coli*) or even the same strain.

Considerations in Choice of Therapy

With upper tract infection, agents that give antibacterial serum activity, such as fluoroquinolones, TMP-SMX, β-lactam antibiotics, and aminoglycosides, should be used. With lower tract infection, additional agents such as nitrofurantoin (with attention to a requirement for creatinine clearance of at least 60 mL/min) and oral fosfomycin can be used.

Amoxicillin and ampicillin are not recommended as reliable agents for empirical therapy because high levels of resistance have been reported for over 2 decades; many more recent studies of antibiotic resistance among urinary isolates do not even include data for these agents. In female outpatients in the United States, resistance of *E. coli* urinary isolates to TMP-SMX was 30% among those 18 to 64 years of age and 26% percent among those 65 years of age and older; resistance to ciprofloxacin was 12% and 22% in those two age groups, respectively.[213] Nitrofurantoin resistance was less than 5% in both age groups. Studies from other parts of the world show a similar pattern, although the absolute levels of TMP-SMX and fluoroquinolone resistance in some areas of the world are much higher.[214–217] The most concerning resistance problem for the management of UTIs is the increasing incidence of community-acquired infection due to ESBL-producing *E. coli*. While the incidence of community-acquired UTI in the United States is currently low, it is increasing.[218,219]

There is evidence that cell wall–active agents (e.g., penicillins, cephalosporins) are not as effective in eradicating infection in the kidneys, or for that matter anywhere in the urinary tract, as are TMP-SMX, fluoroquinolones, or aminoglycosides.[175,181]

Classification and Antimicrobial Therapy for Different Groups

Most often when therapy for UTI is initiated, the infecting microorganism and its susceptibilities to antimicrobial agents are not known. Furthermore, it may not be known if there is an abnormality present that would make the UTI complicated (e.g., calculi). Therefore the approach to therapy is usually to follow established clinical practice guidelines when available for choice of initial antimicrobial therapy. In the case of nonresponse, therapy is changed according to culture and sensitivity results.

The guidelines must be periodically revised because of the continuously evolving resistance of uropathogens to antimicrobial agents, development of new agents, and results of studies regarding superiority/inferiority of agents, as well as studies redefining appropriate lengths of therapy.

Infection in Children

UTI in children younger than 3 months of age is usually caused by *E. coli* or *Enterococcus faecalis*. After obtaining urine (and if indicated, blood) cultures, recommended empirical therapy is usually a β-lactam antibiotic and an aminoglycoside, such as ampicillin and gentamicin, intravenously. Therapy is modified, if necessary, on the basis of culture results. After response, therapy is changed to oral agents such as a β-lactam or TMP-SMX, on the basis of susceptibility studies, for a total period of 7 to 14 days.[110,220–222]

After 3 months of age, IV therapy is used in seriously ill children as for those younger than 3 months; third-generation cephalosporins are a reasonable choice. Oral therapy with a β-lactam such as a second- or third-generation cephalosporin or TMP-SMX (pending cultures for definitive therapy) is recommended for those not seriously ill, administered for 3 days for afebrile cases and 7 to 14 days for febrile cases.[105,110,220–222]

Prophylaxis with an antimicrobial agent following treatment for febrile UTI in infants and young children is controversial. If prophylaxis is given, it should be reserved for children with associated congenital renal anomaly such as high-grade vesicoureteral reflux, particularly with renal scarring.[106] Antireflux procedures (endoscopic or open surgery) are considered in those older than 12 months of age with high grades of reflux and scarring.[223]

Recommendations for imaging in infants and young children have become increasingly stringent over recent years, especially since prenatal ultrasonography is often available and will select out many with significant urologic abnormalities. Postnatal ultrasounds are mainly restricted to those children at highest risk for kidney damage and underlying abnormalities. These children include those who are seriously ill, those with possible obstruction (e.g., poor urine flow, abdominal or bladder masses, elevated creatinine, lack of response to antibiotics), those infected with non–*E. coli* organisms, and those with recurrent UTIs. In practice, many physicians obtain renal ultrasounds for most children after the first UTI to detect obstructive uropathy. ^{99m}Technetium-labeled dimercaptosuccinic acid (^{99m}Tc-DMSA) renal scans are ordered selectively to detect presence of renal scars. Micturating cystourethrograms may be ordered to detect vesicoureteral reflux (see "Imaging Studies" later).[110] When vesicoureteral reflux is found, management is controversial and includes antimicrobial prophylaxis, surgical intervention, or surveillance only.[106,117]

Acute Uncomplicated Pyelonephritis in Women

All patients with pyelonephritis should have a urine culture with antimicrobial susceptibilities to confirm the appropriateness of therapy chosen. Patients who are severely ill with pyelonephritis should be hospitalized and treated intravenously; because the response to therapy is often rapid, initial admission to an observation unit is often appropriate. The role of blood cultures in patients who require hospitalization continues to be debated and, provided that the urine culture is obtained prior to the receipt of antibiotic therapy, the utility of blood cultures is questionable. However, for those patients ill enough to require hospitalization, we continue to recommend blood cultures. Others can be treated orally if they are reliable, adherent, hemodynamically stable, and able to take oral therapy. If a Gram stain of the urine is available, it will help direct therapy toward gram-negative bacilli, by far the most common causes of pyelonephritis, or gram-positive cocci (usually enterococci), but in the majority of cases the selection of antimicrobial therapy is empirical. If gram-positive cocci in chains are seen on Gram stain, ampicillin or amoxicillin is probably the agent of choice. When staphylococci are implicated on Gram stain, it would be most prudent to use an agent such as vancomycin for inpatients and linezolid or TMP-SMX for outpatients because of the increasing frequency of infection with community-acquired methicillin-resistant staphylococci.

Oral Therapy

There is good evidence that 7 days of a fluoroquinolone and, with levofloxacin, 5 days of therapy is sufficient for the treatment of uncomplicated pyelonephritis.[224,225] For oral therapy, current international guidelines recommend a 7-day course of ciprofloxacin 500 mg twice daily or 1 g once daily, or a 5-day course of levofloxacin 750 mg once daily if local fluoroquinolone resistance is under 10%.[79] When local resistance is greater than 10% fluoroquinolone, therapy should start

with an initial, additional, single dose of a parenteral antibiotic such as a 2-g dose of ceftriaxone or a dose of an aminoglycoside or ertapenem to provide coverage while awaiting results of the urine culture. With use of any agent other than a fluoroquinolone, 14 days of therapy is generally recommended. A recent retrospective study has suggested that shorter (7 day) courses of TMP-SMX may be effective; however, prospective, randomized control trials are needed before this approach can be recommended.[226] When a fluoroquinolone cannot be used, oral TMP-SMX (160 mg/800 mg twice daily) is reasonable, but an initial injection of ceftriaxone, ertapenem, or an aminoglycoside is recommended pending results of cultures. This is because of the frequency of high levels of resistance of *E. coli* to TMP-SMX in the community. If an oral β-lactam agent must be used, an initial dose of ceftriaxone, ertapenem, or aminoglycoside should also be administered. Routine imaging studies are not required for women with acute uncomplicated pyelonephritis. Follow-up urine cultures are not indicated except in pregnancy.

Parenteral Therapy

Patients hospitalized for apparent uncomplicated pyelonephritis should receive initial treatment with parenteral antibiotics. Options are a fluoroquinolone, an extended-spectrum cephalosporin such as ceftriaxone or piperacillin-tazobactam with or without an aminoglycoside initially, or a carbapenem. From a stewardship prospective, ceftriaxone is an appropriate choice in most patients. In those with severe sepsis or septic shock, initial therapy with a carbapenem should be considered. Again, from a stewardship perspective, since the microbial etiology of a UTI in this setting should always be eventually confirmed by urine and/or blood cultures, initial broad-spectrum empirical antibiotic therapy in severely ill patients should not be considered problematic. Patients who develop pyelonephritis in a hospital or long-term care facility setting should be assumed to be infected with the resistant flora of that facility, and therapy should be initiated with antibiotics that are likely to be effective. When the susceptibility pattern of the infecting organism is known, therapy can be altered accordingly. Oral treatment can be used to complete the course of antimicrobial therapy once a response has occurred.[79,175,181] Those with bacteremia who respond appropriately do not require a more prolonged course of either parenteral or total antibiotic therapy.

The finding of continuing positive blood cultures or persistent high fever and toxicity past the first 3 to 4 days suggests the need for investigation to exclude complications such as urinary obstruction or intrarenal or perinephric abscess formation. Investigation should include renal ultrasonography, CT or MRI scan, and, according to the findings, a urologic consultation. The availability of sensitive noninvasive studies has resulted in the early diagnosis of intrarenal or perinephric abscess formation that may respond to antibiotic therapy alone.

Uncomplicated Cystitis in Women

In the woman with classic symptoms of cystitis, urine dipstick or cultures are not necessary for management. However, if obtained, a negative dipstick or microscopic examination for pyuria would raise great suspicion that the diagnosis of cystitis is incorrect. Women with acute uncomplicated cystitis can be treated with short courses of antibiotic therapy. The advantages of short-course therapy include a lower cost, better adherence, fewer side effects, and perhaps less intensive selective pressure for the emergence of resistant organisms in gut, urethral, or vaginal flora.

The international clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women recommend treatment of uncomplicated cystitis with nitrofurantoin (100 mg every 12 hours for 5 days), fosfomycin (a single dose of 3 g with 3–4 oz of water), and if local resistance is under 20% of isolates, TMP-SMX (1 double-strength tablet every 12 hours for 3 days).[79] Clearly one of the challenges in following these recommendations is that local resistance rates are usually not known and data obtained from hospital antibiograms likely overestimate resistance rates. Pivmecillinam (400 mg twice daily for 3–7 days) is also recommended but is not available in the United States. The different durations of administration are based on comparative clinical trials. Fosfomycin, a bacterial cell wall–active antibiotic, is approved by the US Food and Drug Administration for single-dose (3 g oral powder) treatment of uncomplicated lower UTI. Nitrofurantoin, TMP-SMX, or fosfomycin represents an appropriate initial choice of therapy for cystitis because of generally low rates of resistance (variable with TMP-SMX) and low impact on microbiologic flora. It has been reported that the efficacy of single-dose fosfomycin is inferior to 7 days of nitrofurantoin and to other standard short-course regimens, and this agent is the most expensive of the three options.[79] A meta-analysis of 27 trials found no difference in efficacy between fosfomycin and other antibiotics for treatment of cystitis, and it found that fosfomycin was associated with significantly fewer adverse reactions in pregnant women.[227] A potential advantage of fosfomycin is activity against multidrug-resistant pathogens, including ESBL-producing *E. coli,* which may be important in certain epidemiologic settings outside the United States.

The guidelines list fluoroquinolones such as ciprofloxacin, ofloxacin, and levofloxacin given for 3 days as highly efficacious but recommend reserving them for other uses because of "collateral damage"—ecologic adverse effects of antimicrobial therapy.[79] Since these guidelines were published, the US Food and Drug Administration has issued a warning discouraging the use of fluoroquinolones when other agents are available because of safety concerns.

The guidelines also list β-lactam agents other than pivmecillinam, including amoxicillin-clavulanate, cefdinir, cefaclor, and cefpodoxime-proxetil, in 3- to 7-day regimens as appropriate (but not preferred) choices for therapy when other recommended agents cannot be used.[79] Because of inferior efficacy and higher incidence of adverse events, β-lactams should be considered only if nitrofurantoin, fosfomycin, and TMP-SMX cannot be used, with fluoroquinolones reserved as the last resort for therapy.

When acquired in a hospital or long-term care facility, empirical therapy should be based on the usual susceptibility patterns of organisms causing UTI in that facility. Short-course therapy may not be appropriate for women who have a history of previous urinary infection caused by antibiotic-resistant organisms or more than 7 days of symptoms. In these patients, who have an increased likelihood of upper tract infection, 7 to 10 days of therapy are recommended. Some of the risk factors for multidrug-resistant organisms are recent health care facility exposure, recent antimicrobial agent exposure (especially fluoroquinolones and antipseudomonal penicillins), and, in two US-based studies, travel outside the United States in the preceding 3 to 6 months.[79,228]

If symptoms do not respond, or if they recur, a urine culture should be obtained and therapy chosen according to antimicrobial susceptibility testing. A symptomatic response followed by relapse after therapy is discontinued indicates the probability of upper tract infection and the need for a culture and therapy for upper tract infection.

Complicated Urinary Tract Infection, Including Infection in Men

Because of the relative infrequency of UTI in men and the relatively high proportion of complicated UTI in men who get infected, UTI in men should be managed as a complicated infection.[1,2,229,230] In a male, presence of fever with only symptoms of cystitis may indicate acute prostatitis. In addition to complications related to urinary obstruction from diseases of the prostate in men, chronic bacterial prostatitis may be difficult to cure.

The management of complicated UTI is problematic for a number of reasons.[1,2,229,230] Resistant bacteria (such as ESBL-producing *E. coli;* urease-producing bacteria, including *Proteus, Providencia,* and *Morganella*; *Pseudomonas;* and *Acinetobacter*) and polymicrobial infection are found much more commonly in complicated UTI than in uncomplicated disease. The complication may make persistent or relapsing infection much more likely. For example, in the presence of an infected calculus, relapse from within the calculus is usual after therapy is stopped; the presence of a foreign body, such as a drainage device, makes eradication of infection difficult, probably because of formation of biofilms; and renal insufficiency may result in subinhibitory levels of antibiotic in the urine, as well as more antibiotic side effects.

Asymptomatic bacteriuria is often present in complicated UTI. However, excluding pregnancy, no benefit has been demonstrated in

screening for or treating asymptomatic bacteriuria except in two groups of patients: those with renal transplantation, especially in the early posttransplantation period; and those who will have procedures traumatic to the urethra, in whom elimination of bacteriuria can reduce the occurrence of symptomatic infection.[2,144,152,154] Long-term suppressive antimicrobial therapy has been advocated for struvite stones that cannot be removed in an attempt to prevent stone enlargement.

Urine cultures and antimicrobial susceptibility tests should be obtained in all patients with complicated UTI who require treatment. Imaging by ultrasound, CT scan, and/or MRI is often important to rule out obstructive uropathy and other abnormalities. A urologic consultation is often indicated. With complete urinary obstruction or ureteral obstruction, the patient may be septic, and prompt relief of the obstruction is important. Correction of a complication, if feasible (e.g., removal of a calculus or a catheter), may make cure much easier.

Accurate initial empirical therapy is paramount in the severely ill patient in whom the urinary tract may be the primary source of illness. Fluoroquinolone resistance rates in many communities are high, and there is little evidence that concentration of the drugs in the urinary tract makes them effective in vivo against resistant organisms.[231] *Escherichia coli* and *Klebsiella pneumoniae* have a high rate of carriage of ESBLs, which inactivate extended-spectrum cephalosporins. These species may also be dominant sources of carbapenemase production, but resistance mechanisms are too varied to predict. Current recommendations support the use of in vitro susceptibility rather than mechanism in choosing effective treatment.

Some knowledge of local and individual resistance patterns should be used to guide therapy. Consideration should include the increased risk of drug-resistant pathogens in hospital-acquired cases, in men, and in patients ages 65 years or older, and decisions should incorporate the results of urine cultures obtained within the previous 6 months.[218,228,233,234] The strength of association between intestinal colonization and subsequent disease is still difficult to interpret, but the conjunction of colonization with a strain (e.g., *E. coli* ST131-H30Rx) with high pathogenic potential and subsequent disease suggests that colonization should be taken into consideration.[235] The use of a cephamycin such as cefoxitin, a drug less susceptible to inactivation by ESBLs, has been addressed only in small studies.[236] New β-lactam–β-lactamase combinations (ceftazidime-avibactam and ceftolozane-tazobactam) may be of use against some multidrug-resistant pathogens.[237–240] On an empirical basis in the critically ill patient, carbapenems, aminoglycosides, polymyxins (colistin or polymyxin), or IV fosfomycin (not available in the United States) may be necessary.

Our preference for empirical initial therapy for lower tract infection is a fluoroquinolone pending antimicrobial susceptibility studies, but nitrofurantoin and fosfomycin are reasonable choices. Fosfomycin in particular has maintained activity against many multidrug-resistant bacteria.[87] The duration of therapy should be at least 7 days with a fluoroquinolone or nitrofurantoin. When fosfomycin is used to treat complicated cystitis, there are data to support a regimen of three doses, administered every other day.[228]

With creatinine clearances of less than 50 mL/min, nitrofurantoin and sulfamethoxazole urine levels will likely be subtherapeutic, and nitrofurantoin is contraindicated in renal failure related to resultant peripheral neuropathy. Urine concentrations of TMP used alone should be high enough to allow its use in patients with impaired renal function.[241] With severe renal insufficiency, the β-lactams are the safest agents to use. In general, in the presence of severe renal insufficiency, doses of virtually all antimicrobials must be adjusted. In addition, with renal insufficiency, levels of antimicrobial agent in the urine may be insufficient to inhibit the infecting organism.[241]

When upper tract infection is complicated by abscesses, more prolonged therapy and perhaps drainage are indicated (see "Perinephric Abscess" and "Intrarenal Abscess" later).

Renal infection is a special problem in adults with hereditary polycystic disease. Although parenchymal infections respond well to appropriate antibiotics, cyst infections frequently fail to improve and may require antibiotics that diffuse into these closed sites (e.g., TMP-SMX, fluoroquinolone) or aspiration or surgical drainage. Emphysematous pyelonephritis is most often seen in older female diabetic patients with chronic urinary infections and renal vascular disease (Fig. 72.7). Because of a high mortality rate in spite of appropriate antibiotics and supportive therapy, immediate nephrectomy is frequently indicated for this condition.[242,243] However, advances in percutaneous drainage techniques have allowed some individuals to be successfully managed with antibiotics and drainage alone.[244]

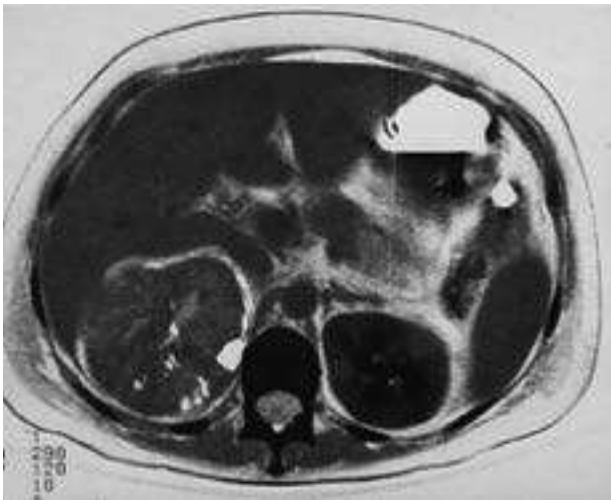

FIG. 72.7 Emphysematous pyelonephritis in a patient with diabetes. This computed tomography scan shows an enlarged, inflamed right kidney with air in the parenchyma and subcapsular space. *(Courtesy Dr. M. Bergeron.)*

Asymptomatic Bacteriuria

Because no benefit has been demonstrated, screening for or treatment of asymptomatic bacteriuria is not recommended for any group of adult patients, including diabetics, except in renal transplantation patients (see "Reinfection of the Urinary Tract and Antimicrobial Prophylaxis" later) and during pregnancy (see "Urinary Tract Infection in Pregnancy" later).[2,138,144,149]

A major exception to not screening for and treating asymptomatic bacteriuria is in those patients who are to undergo traumatic genitourinary procedures associated with mucosal bleeding, such as transurethral prostatectomy and percutaneous lithotomy. There is a high rate of postprocedure bacteremia and sepsis in these patients. To reduce the incidence of bacteremia, a urine culture is obtained several days before the procedure and therapy with a third-generation cephalosporin or another appropriate agent is started 12 hours to just before the procedure. The therapy is usually stopped after the procedure, but some practitioners continue the therapy until any urethral catheter is removed.[2,144,245]

The agents used for management of asymptomatic bacteriuria are those used for uncomplicated bacteriuria, as described earlier in "Uncomplicated Cystitis in Women."

Relapsing Urinary Tract Infection

If a patient relapses after therapy for UTI, the most likely possibilities are that the patient has (1) renal involvement (pyelonephritis); (2) chronic bacterial prostatitis; or (3) a structural abnormality of the urinary tract (e.g., calculi). Relapses, especially in the absence of structural abnormalities, could be related to renal infection that may require a longer duration of therapy or to chronic bacterial prostatitis, which is difficult to cure even with long-term therapy. Patients who have a symptomatic relapse should have a urine culture and sensitivity study and be treated with a course of antimicrobial therapy for upper tract infection. In the past, it was demonstrated that a 6-week course of therapy with a β-lactam results in a higher cure rate than a 2-week course in patients who relapse after 2 weeks of therapy. These were mainly women who presumably had renal infection.

Structural abnormalities of the urinary tract predispose to relapse. Obstructive lesions can be corrected surgically and should be sought in the evaluation of patients with relapsing infection. Calculi may be a cause of relapse of UTI. The ultimate success of chemotherapy is dependent on the removal of stones. The same is true with drainage devices or other foreign bodies.

Some patients continue to relapse despite surgical correction of urologic abnormalities. In others, surgical correction may not be indicated or feasible or no abnormality may be found. Symptomatic relapses must be treated. In men, chronic bacterial prostatitis should be ruled out.

In carefully selected patients, such as those with frequent symptomatic relapses, prolonged periods of therapy such as 4 weeks or longer should be considered. Some agents that can be used for longer-term therapy are amoxicillin-clavulanic acid, cephalexin, TMP-SMX, TMP, and ciprofloxacin in usual doses, and nitrofurantoin in full dosage for 1 week and then half the usual dosage. Rarely patients may require long-term suppressive therapy to keep them asymptomatic. Nitrofurantoin and TMP-SMX have been used for long-term chronic suppression in many older studies. Fosfomycin would seem to be useful for this purpose, but there have been few long-term studies. For example, one study used 3 g of fosfomycin every 10 days.[246]

Reinfection of the Urinary Tract and Antimicrobial Prophylaxis

Patients with symptomatic reinfections can generally be divided into two groups: (1) those who have relatively infrequent reinfections, perhaps only once every 2 or 3 years to several times a year (the more common situation); and (2) those who develop frequent reinfections. The latter are usually young and middle-aged sexually active women who have recurrent cystitis. With infrequent reinfections, each episode is approached as a new episode of infection. Women with reinfections associated with lower tract symptoms can be managed with self-administration of standard short-course therapy at the onset of symptoms.[247,248]

In some women, symptomatic reinfections are associated with sexual activity. Voiding immediately after intercourse may help prevent reinfection. However, single-dose prophylactic chemotherapy taken after sexual intercourse (e.g., a single-strength TMP-SMX tablet; 100 mg of nitrofurantoin) is a more effective method of decreasing these episodes.[248–250]

In other patients with frequent symptomatic reinfections, no precipitating event is apparent; in these patients, when symptoms are severe, long-term chemoprophylaxis may be instituted.[248,250] Although these courses seem to decrease the frequency of reinfections and symptoms in most patients, it is impossible to completely prevent reinfection in many patients.

In older studies, long-term chemoprophylaxis was advocated for asymptomatic patients who reinfect frequently and who were thought to be at risk of developing renal parenchymal damage with each reinfection (e.g., young children with severe vesicoureteral reflux and children and adults with obstructive uropathy). This approach is controversial and not generally recommended.[251] In a large study in young children, long-term antimicrobial prophylaxis was not associated with decreased risk of UTI but was associated with an increased risk of resistant infections.[252] Screening for bacteriuria and chemoprophylaxis has been considered for all renal transplantation patients in the postoperative period to prevent symptomatic infection.[2,153–155] Transplantation populations demonstrate an increased frequency of multidrug-resistant pathogens, complicating decisions for therapy.[253] Definitive studies are lacking, but some evidence supports the contention that routine surveillance beyond the second month post–kidney transplantation may not change a primary outcome of acute pyelonephritis during 24 months of follow-up.[254]

Full antimicrobial dosage is not necessary for successful prophylaxis. One 50-mg capsule of nitrofurantoin or one half-tablet of TMP-SMX (40 mg of TMP, 200 mg of sulfamethoxazole) nightly suffices. Fluoroquinolones and other agents have been used with good results.

Fungal Infections

Most *Candida* UTIs occur in patients with indwelling catheters. Removal of the catheter may result in cure of 30% to 40% of patients with candiduria.[255] Continuous amphotericin B bladder irrigation or oral fluconazole, 200 to 400 mg/day for 7 days, in association with removing (if possible) or replacing the catheter may be effective short term in eliminating candiduria.[91] However, with longer follow-up, oral fluconazole is no more effective than no therapy.[255] There is no demonstrated benefit in the treatment of asymptomatic infection, and therefore therapy is not recommended. Exceptions include only patients who are to undergo procedures involving the urinary tract that result in mucosal bleeding. In these cases, attempts should be made to eliminate or at least suppress the candiduria with oral fluconazole. In the past, asymptomatic candiduria in renal transplant recipients was treated with fluconazole on the basis of a perceived risk of a destructive process in the grafted kidney. However, a large study of posttransplantation patients failed to show excessive morbidity associated with candiduria or any benefit from eradication of candiduria.[256] Infrequently, ascending infection resulting in pyelonephritis occurs, especially in patients with diabetes or obstruction. This obviously requires systemic antifungal therapy and attention to obstruction. Fluconazole-susceptible organisms can be treated with this agent at a dose of 200 to 400 mg daily for 14 days.[257] Pyelonephritis due to azole-resistant *Candida glabrata* and *Candida krusei* should be managed with systemic amphotericin B desoxycholate, with or without oral flucytosine.[257] Symptomatic cystitis is uncommon and is treated with oral fluconazole 200 mg daily for 14 days if the *Candida* species is azole susceptible.[257] *Candida glabrata* and *C. krusei* symptomatic infections are best treated with systemic or amphotericin B irrigation.[257] Azoles other than fluconazole and echinocandins are not recommended for the management of UTIs due to low concentrations of active drug in the urine; for the same reason, lipid formulations of amphotericin B are also not recommended.

Urinary Tract Infection in Pregnancy

Physiologic Alterations in the Urinary Tract

During pregnancy, there is dilation of the ureters and renal pelves, with markedly decreased ureteral peristalsis.[145] These changes begin as early as the seventh week of gestation and progress to term. The bladder also decreases in tone so that, late in gestation, it can contain twice its normal contents without causing discomfort. These changes vary from patient to patient. They are more marked on the right side and are more likely to occur during the first pregnancy or when pregnancies occur in rapid succession. The urinary tract tends to revert to normal by the second month after delivery. The urinary tract alterations may be at least partly related to hyperestrogenism. Other possible explanations for the alterations are obstruction of the ureters by the gravid uterus and hypertrophy of muscle bundles at the lower end of the ureter. In addition to host factors, unique gestational bacterial virulence factors are now recognized for a narrow group of genetically related *E. coli*.[258] Each trimester appears to be associated with specific DNA fingerprints and serotypes of *E. coli,* as well as trimester-specific virulence patterns.

Epidemiology

The incidence of bacteriuria during pregnancy is similar to that seen in nonpregnant women. The prevalence of asymptomatic bacteriuria in pregnancy ranges from 4% to 7%. However, recurrent episodes are more common in pregnant women who had bacteriuria documented at their initial prenatal visit. The prevalence of bacteriuria rises with age, sexual activity, in diabetes mellitus, in women with sickle cell trait, and in women with a past history of UTI; the association of parity and that of socioeconomic status are inconsistent.[259,260] Most women who develop bacteriuria during pregnancy have infection at the first prenatal visit. However, 1% to 1.5% of pregnant women, or about 25% of those with bacteriuria of pregnancy, develop infection in the later trimesters. Acute cystitis and acute pyelonephritis have been shown to complicate 1% to 2% of pregnancies with a microbial spectrum similar to that of asymptomatic bacteriuria in pregnancy and uncomplicated UTI in nonpregnant women. The development of symptomatic pyelonephritis late in pregnancy is usually an expression of asymptomatic bacteriuria that was present earlier in the pregnancy. The marked dilatation of the ureters during the later stages apparently allows bacteria in the bladder to reach the upper tract and to produce symptomatic pyelonephritis.

It has been reported that as many as 40% of the patients with untreated bacteriuria early in pregnancy develop acute symptomatic pyelonephritis later in pregnancy, although, as discussed previously, more recent studies have reported lower rates of pyelonephritis. In contrast, less than 1% of patients whose urine is uninfected early in pregnancy develop acute infection. Screening and treatment for asymptomatic bacteriuria in pregnant patients is recommended by multiple professional organizations, including the US Preventive Services Task Force, the American College of Obstetrics and Gynecology (ACOG), and the Infectious Diseases Society of America. It has also been noted that those whose bacteriuria fails to respond to treatment are at the highest risk of developing symptomatic infection. A lack of cure is probably an indication of upper versus lower tract infection. Untreated asymptomatic bacteriuria has been associated with preterm birth and low birth weight, although the association is inconsistent across studies; the association is most likely due to the increased risk of pyelonephritis.[260]

Postpartum studies of patients with bacteriuria of pregnancy demonstrate a high frequency of bacteriuria, even with treatment during pregnancy. Postpartum intravenous pyelography (IVP) of these patients has shown that 10% to 30% have radiologic changes of chronic pyelonephritis and other abnormalities. These abnormalities are most common in patients in whom renal bacteriuria has been demonstrated or in whom bacteriuria during pregnancy was difficult to eradicate with antimicrobial therapy.[145] However, pyelographic abnormalities should not necessarily be attributed to the infection that occurred during the pregnancy. In fact, these abnormalities probably antedate the pregnancy and, in most cases, are related to childhood infection. Treatment of bacteriuria of pregnancy has little effect on the long-term course of the patient. When patients who had bacteriuria of pregnancy were studied 10 to 14 years later, there were no differences between those who were treated and those who were not.

Management of Bacteriuria of Pregnancy

All women should be screened at 12 to 16 weeks' gestation or at the first prenatal visit, if later. The goal of therapy is to maintain sterile urine throughout pregnancy and thereby avoid the complications associated with UTI. In the treatment of asymptomatic bacteriuria and cystitis, treatment modalities include single-dose fosfomycin trometamol 3 g or cephalexin 500 mg four times a day for 3 to 5 days. A 7-day course of nitrofurantoin or TMP-SMX for 3 days (when susceptibility tests reveal absence of resistance) can be used; however, ACOG has recently released an opinion bulletin discouraging the use of nitrofurantoin and TPM-SMX during the first trimester due to concern regarding the possibility of previously underappreciated risk of birth defects.[261] Acute pyelonephritis in pregnancy should be managed initially with parenteral antibiotics, such as third-generation cephalosporins for 14 days. In selected patients with mild disease, a trial of oral antibiotic therapy with cefixime can be given for 14 days with close follow-up. If cefixime cannot be used, TMP-SMX can be substituted, if the bacterium is susceptible.

Urine cultures should be obtained 1 to 2 weeks after discontinuing therapy and then at regular intervals (monthly) for the remainder of the pregnancy. Some experts recommend prophylactic antibiotic therapy until delivery; however, a Cochrane Review did not find evidence of additional benefit as compared with close follow-up alone.[262] If relapses or multiple reinfections occur during pregnancy, an imaging evaluation should be considered postpartum. Guidelines from ACOG recommend intrapartum penicillin prophylaxis for all women with group B streptococcus bacteriuria at any point during their pregnancy to prevent early-onset disease in the newborn.

Perinephric Abscess and Intrarenal Abscess

Perinephric Abscess

Perinephric abscess is an uncommon complication of UTI.[263] The most common predisposing factors are urinary tract calculi and diabetes mellitus. It usually occurs secondary to obstruction of an infected kidney or calyx or, occasionally, secondary to bacteremia. It may occur insidiously, and up to one-third of cases may not be diagnosed until autopsy. The infecting bacteria are usually gram-negative enteric bacilli and occasionally gram-positive cocci when the infection is of hematogenous origin. Multiple bacterial species are present in about 25% of cases, and occasionally fungi, especially *Candida* spp., can be cultured from the abscess. The abscess is usually confined by the Gerota fascia to the perinephric space but may extend throughout the retroperitoneum to affect adjacent structures.[264]

Patients have a syndrome suggestive of acute pyelonephritis, with fever, abdominal and flank pain (usually unilateral), and often symptoms of lower tract infection. However, presenting symptoms are frequently nonspecific. The patient has often been ill for 2 or more weeks. The diagnosis should be strongly considered in any patient with a febrile illness and unilateral flank pain who does not respond to therapy for acute pyelonephritis. A palpable mass may or may not be present. Pyuria and proteinuria are frequently found, but about 30% of patients have a normal urinalysis and about 40% have sterile urine cultures.[264]

Intrarenal Abscess

Intrarenal abscess may occur as a consequence of bacteremia, often caused by *S. aureus*. Hematogenous lesions are usually unilateral, single, and located in the cortex. However, these focal suppurative lesions are being recognized with increasing frequency as a complication of classic acute pyelonephritis and are located in the cortex, medulla, or both. The clinical setting is usually that of acute pyelonephritis with high fever, severe flank pain, and tenderness, but with no or slow response to appropriate antimicrobial therapy. In the early stages of abscess formation, contrast-enhanced CT may detect intense parenchymatous inflammation and edema in a lobe of the kidney, termed *acute lobar nephronia* or *acute focal bacterial nephritis* (Fig. 72.8). Although antibiotics may arrest progression at this stage, coalescence of microabscesses (multifocal bacterial nephritis) can lead to intrarenal abscess (Fig. 72.9).

Emphysematous pyelonephritis is an uncommon, severe, life-threatening necrotizing form of acute multifocal bacterial nephritis in which retroperitoneal, extraluminal gas is seen in the renal parenchyma, urinary collecting system, or perirenal space on an abdominal radiograph or CT scan (see Fig. 72.7). The presence of gas suggests a gas-forming, gram-negative facultative anaerobic uropathogen and occasionally *Candida* species. *Escherichia coli* is the most common organism associated with this complication, but *Klebsiella* spp., *P. mirabilis,* and *Citrobacter* spp. may be involved. This condition occurs most commonly in diabetic patients with or without urinary obstruction.[265] Mortality rates have improved significantly in the past 2 decades, and "nephron-sparing" management (percutaneous drainage of localized gas collections as opposed to nephrectomy) is increasingly utilized.[266,267] Emphysematous pyelitis is a distinct clinical entity (class 1 emphysematous pyelonephritis) that occurs as a sequela of infection when gas accumulates in the pelvicalyceal system. Diabetes and urinary tract obstruction are common

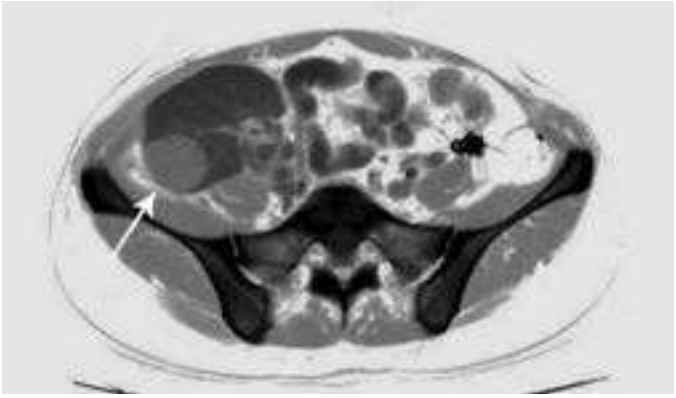

FIG. 72.8 Computed tomography scan of cadaveric kidney transplant showing acute lobar nephronia or acute focal bacterial nephritis. Note the bulging renal capsule, reflecting edema of the lobe *(arrow).*

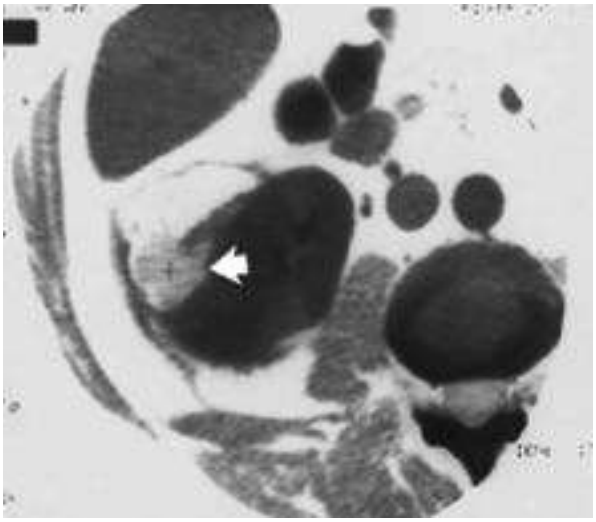

FIG. 72.9 Intrarenal abscess. This computed tomography scan shows an intrarenal abscess, evident as a well-delineated hypodense lesion *(arrow),* extending into the intrarenal space. *(Courtesy Dr. M. Bergeron.)*

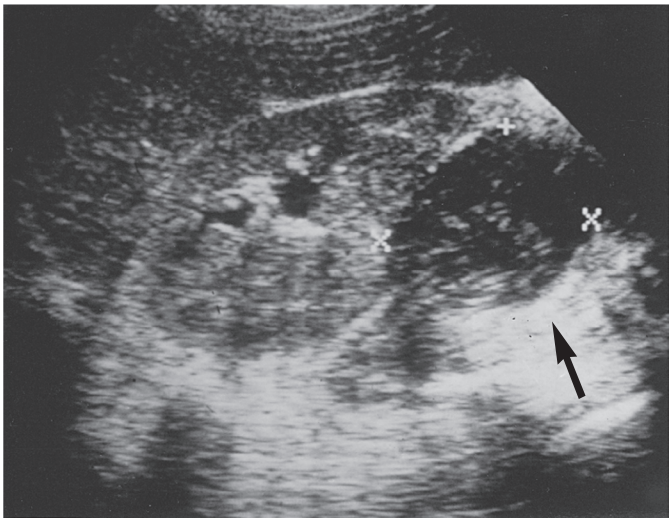

FIG. 72.10 Perinephric abscess. Ultrasound examination reveals a perinephric abscess *(arrow). (Courtesy Dr. M. Bergeron.)*

predisposing factors, and *E. coli* and *K. pneumoniae* are commonly implicated pathogens. These patients are generally far less ill and respond well to antibiotics alone in the majority of cases.[267]

Xanthogranulomatous pyelonephritis (XGP) is an uncommon but severe focal or diffuse chronic infection of the renal parenchyma. Destroyed tissue is replaced by granulomatous tissue containing lipid-laden macrophages (foam cells). Predisposing factors include renal calculi, urinary obstruction, lymphatic obstruction, renal ischemia, secondary metabolic alterations in lipid metabolism, an abnormal host immune response, and diabetes mellitus.[268] XGP has a predilection to extend into the retroperitoneal space, leading to internal and external fistulae. The latter complications and mass effect of XGP often mimic those of a genitourinary malignancy.[269]

Diagnosis and Therapy

Urinalysis is abnormal in 70% of patients with a corticomedullary abscess, whereas it is usually normal in the patient with a hematogenous cortical or perinephric abscess. Confirmation of the diagnosis requires imaging techniques.

The introduction of renal ultrasonography and in particular CT scans added additional sensitivity and specificity, permitting the early diagnosis of intrarenal and perinephric abscesses (Figs. 72.10 and 72.11; see Fig. 72.9).[214,215] The most common CT findings include thickening of the Gerota fascia, renal enlargement, focal parenchymal decreased attenuation, and fluid or gas, or both, in and around the kidney.

In patients with a clinical or radiographic suspicion of perinephric abscess, diagnostic percutaneous needle aspiration can be safely performed by using ultrasonography or CT guidance. When an abscess is confirmed, small catheters can be introduced to provide immediate decompression and continuous and definitive drainage without the need for surgery.[265] Drainage is recommended for abscesses 5 cm or greater in size; drainage or medical management alone are options for abscesses 3 to 5 cm, while abscesses less than 3 cm can often be managed with antibiotics alone.[219,265] Advantages to guided percutaneous drainage compared with open surgical drainage include earlier diagnosis and treatment, the avoidance of general anesthesia and surgery, less expensive therapy, easier nursing care, and greater patient acceptance of closed drainage. Accordingly, it is now recommended that after antimicrobial therapy directed against the most likely pathogens is started, a trial of percutaneous drainage should be the initial mode of therapy for perinephric abscess. Surgical intervention should be undertaken only when percutaneous drainage fails or is contraindicated. Parenteral antimicrobial therapy directed against the infecting organism isolated from blood or urine should be initiated before drainage, but if additional organisms are isolated at the time of drainage, treatment directed against these organisms must be added. Therapy must also be instituted for the underlying disease (e.g., obstructive uropathy).



FIG. 72.11 Left perinephric abscess. This contrast-enhanced computed tomography scan demonstrates a large left perinephric abscess containing gas *(arrow).*

When antimicrobial therapy with appropriate agents fails with infected renal cysts or abscesses, percutaneous drainage should be tried. Agents that diffuse into these closed sites, such as TMP-SMX and fluoroquinolones, may have an advantage.[270,271] Percutaneous drainage often avoids the previously recommended approach of open surgical drainage or nephrectomy.[272–274]

Successful percutaneous treatment has been reported in 90% of patients with renal or perinephric abscess formation.[274] In the past, delay and missed diagnosis resulted in mortality rates of 20% to 50% in patients with perinephric abscess, with approximately one-third of cases diagnosed only postmortem. Today, with early recognition using modern imaging techniques, together with prompt drainage and antibiotic

therapy, the mortality is extremely low. Most patients with intrarenal abscess respond, although slowly, to antimicrobial therapy, but fever and severe flank pain may persist for days. Open surgical drainage is reserved for nonfunctioning kidneys, multilocular abscesses, and patients who fail initial management with percutaneous drainage.

Imaging Studies

Imaging procedures play an important role in the diagnosis and management of patients with UTI,[275] both in the control of complicated episodes of acute pyelonephritis and in the investigation of patients of all ages in whom the clinician suspects the presence of underlying structural abnormalities that may be surgically correctable. In adults, uncomplicated acute renal infections do not require imaging. Studies are useful when the diagnosis is in doubt, in severely ill or immunocompromised patients, in those patients with pyelonephritis who fail to improve after 72 hours of appropriate antibiotic therapy, or when complications are suspected. Imaging may also be indicated when certain bacterial species such as *C. urealyticum* are isolated because infection with these organisms may be a clue to the presence of renal stones.

In the past, excretory urography in the form of IVP was the initial and definitive investigatory study, but this has been replaced by ultrasonography and CT (Figs. 72.12 and 72.13). In general, ultrasonography serves as a rapid, noninvasive, and relatively inexpensive means of evaluating the renal collecting system, parenchyma, and surrounding retroperitoneum.[273]

Johnson and associates[276] have confirmed that renal swelling, as demonstrated by ultrasonography, characteristically occurs in almost all women with acute pyelonephritis. Enlargement may be unilateral or bilateral and correlates with protracted pretreatment symptoms, leukocytosis, high fever, focal suppurative complications, and prolonged hospitalization. They also indicated that the frequencies of underlying abnormalities and focal complications are low.

Guidelines from the American College of Radiology recommend contrast-enhanced CT as the study of choice.[265] Computed tomographic imaging is more panoramic and more sensitive than ultrasonography in detecting calculi and underlying urinary tract anatomic abnormalities.[277] The increased sensitivity of contrast-enhanced CT, especially helical CT, is particularly apparent in identifying renal parenchyma abnormalities, especially in the mildest cases of acute pyelonephritis.[277] Ultrasound techniques such as power Doppler and pulse inversion harmonic imaging are now widely used to overcome this lack of sensitivity by demonstrating renal perfusion. Ultrasonography is normal in most uncomplicated cases with acute pyelonephritis. A common CT finding is decreased opacification of the affected renal parenchyma, usually in a patchy, wedge-shaped, or linear pattern. Different patterns may coexist, and abnormalities vary in size. Non–contrast-enhanced CT is often normal but may show focal areas of decreased attenuation. Areas of markedly decreased attenuation should raise a suspicion of abscess formation, and then contrast material should, if possible, be administered. Other CT findings in pyelonephritis include diffuse or focal kidney enlargement, perinephric stranding, and mild collecting system dilatation. In moderate and severe cases of acute pyelonephritis, CT scan abnormalities usually persist for several weeks, well after clinical symptoms and laboratory findings have returned to normal.[277,278] After adequate therapy, most cases eventually demonstrate complete resolution of imaging abnormalities. In recurrent infection associated with chronic reflux, the affected renal lobes develop changes of reflux nephropathy. Recurrent infection results in deformity and dilatation of calyces and focal cortical loss, with upper and lower poles severely affected.

Both CT and ultrasonography are sensitive in diagnosing intrarenal and perirenal suppuration; however, contrast-enhanced CT is preferred if there is a high suspicion for abscess, unless there are contraindications.[265] On contrast-enhanced CT, the hallmark of an abscess is a focal area that fails to enhance, indicating an avascular state. Abscesses are typically sharply demarcated and round or ovoid and contain a low-density center. The abscess wall enhances after contrast injection, resulting in the rind sign caused by the presence of inflamed dilated vessels.[272] When detected, gas within a low-density mass is pathognomonic for abscess formation. In contradistinction to pyelonephritis, intrarenal abscesses reaching 2 to 3 cm are well evaluated by ultrasonography, showing sharp demarcation and the presence of liquefaction. Ultrasonography demonstrates a well-marginated, hypoechoic mass with good through-transmission, an irregular interior wall margin, and scattered echogenic foci within the mass representing debris. Gas formation is highly echogenic. Both these procedures may be used for the guidance of percutaneous needle aspiration.

Neither CT scans nor ultrasonography reliably distinguish an uninfected obstructed renal collection system (hydronephrosis) from pyonephrosis. Suggestive findings on CT scan include an increased thickness of the renal pelvis wall and the presence of increased density within the renal pelvis indicative of pus or debris. The strongest indication of pyonephrosis on CT is the presence of gas within the collecting system, but this is uncommon. Ultrasonography, especially contrast enhanced, may identify echogenic contents or debris (Fig. 72.14).

Gas formation within the renal parenchyma as a consequence of severe infection by facultative anaerobes and occasionally *Candida* spp. is termed *emphysematous pyelonephritis.* Although gas may be seen on plain radiographs, it is often mistaken for bowel gas. Computed tomography is exquisitely sensitive in the detection of gas, which appears as small bubbles or as linear streaks. Gas often collects in a subcapsular

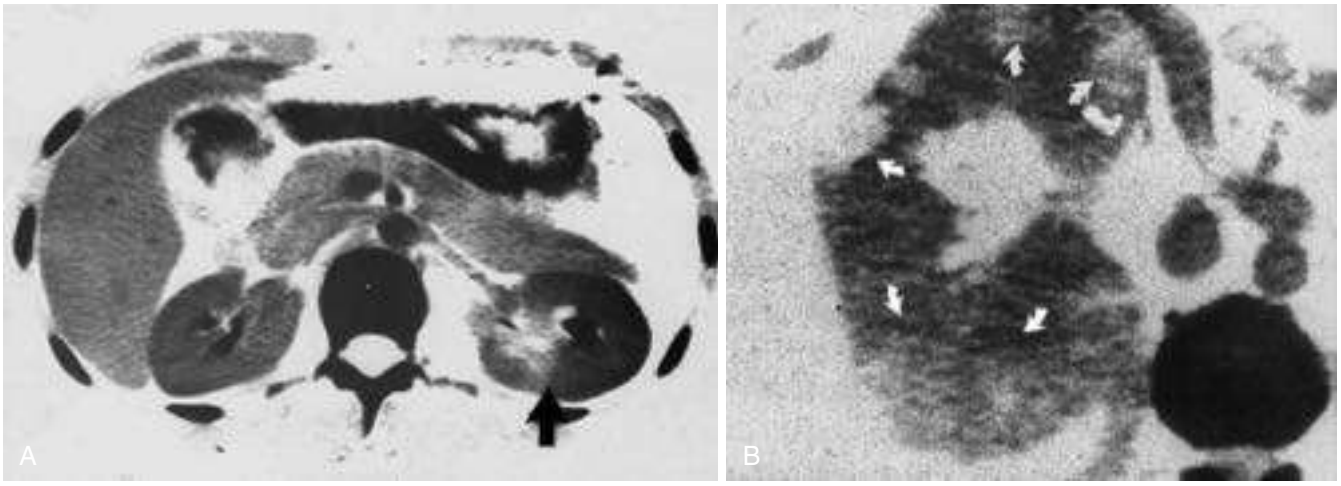

FIG. 72.12 Computed tomography scan in acute pyelonephritis. The masslike lesion of pyelonephritis is often well defined because it is less dense than the cortex. (A) It can be irregular with a nonhomogeneous center *(arrow),* as seen in the left kidney. The contralateral kidney is normal. (B) Acute pyelonephritis can also appear as multifocal with diffuse masslike lesions *(arrows). (Courtesy Dr. Huang.)*

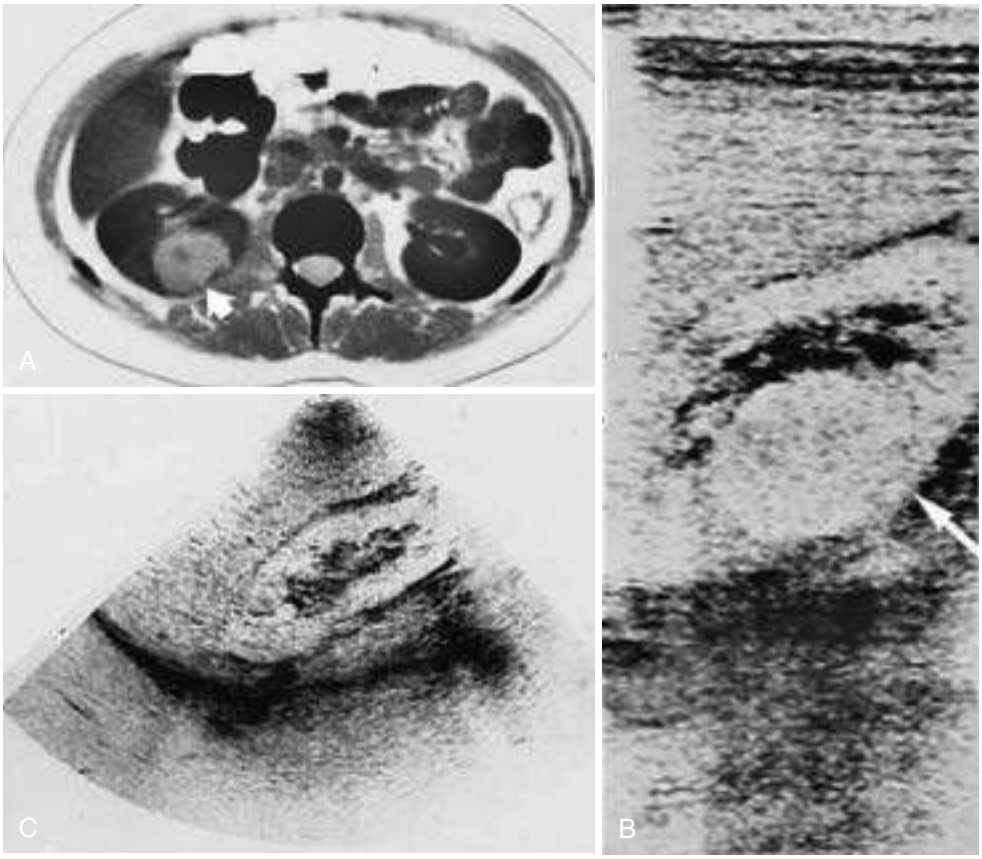

FIG. 72.13 Acute pyelonephritis with a masslike lesion. (A) On a computed tomography scan, acute pyelonephritis with a (B) ultrasound examination of the right kidney shows the same masslike lesion *(arrow)*. (C) The mass has resolved on the follow-up ultrasound study performed 2 months later. *(Courtesy Dr. T. Slovis.)*



FIG. 72.14 Ultrasound study demonstrating pyonephrosis (i.e., hydronephrosis) and echogenic content of the collecting system *(arrow).*

location, forming a sharp line around the margin of the kidney, or may be seen within the renal collecting system.

For detecting lesions of the collecting system, CT provides physiologic information similar to that obtained with IVP, with much better parenchymal delineation but less optimal delineation of the collecting system.[275] For cases in which renal calculi may be present, the CT study should also include noncontrast images through the kidney. Helical CT scanning of the abdomen and pelvis without contrast material is now considered the standard approach to confirm or exclude the presence of obstructing ureteral calculi ("stone protocol").[265,279] The accuracy and sensitivity of a non–contrast-enhanced CT scan for renal, ureteral, and bladder stones approach 100%.[280]

All studies requiring the parenteral administration of contrast material are associated with some risk of allergy or contrast-induced renal insufficiency. Predisposing factors for renal insufficiency include myeloma, diabetes mellitus, preexisting renal failure, severe intravascular volume depletion, and the recent administration of large doses of iodinated contrast material. Magnetic resonance imaging offers no advantage over CT in the diagnosis of intrarenal or perinephric abscess but may be considered as an alternative to contrast-enhanced CT in patients with a contraindication to iodinated contrast administration. Previously, the use of gadolinium was thought to be risk free compared with alternative contrast agents, but there is now a recognized risk of systemic fibrosis and, more recently, concern about toxicity related to deposition in brain and bone.[281,282] Radioisotope studies play only a small role in the investigation of the urinary tract. Indium 111–labeled white blood cell studies and renal scintigraphy using ^{99m}Tc-DMSA scans occasionally prove useful in localizing inflammation or infection to the kidneys in patients with fever of unknown origin, especially patients with spinal cord injuries (Fig. 72.15). Radionuclide scanning may be of value, after ultrasonography or CT scanning has identified a solid renal mass, in suggesting the inflammatory nature of the lesion. However, neither of these radionuclide studies distinguishes pyelonephritis from abscess.

Another important contribution provided by these imaging modalities is the detection of surgically correctable abnormalities of the urinary tract. Investigation should be considered in patients at the greatest risk of having critical surgically correctable abnormalities. Patients included in this higher risk category are those with pyelonephritis, regardless of age, who relapse after therapy. The routine use of noninvasive renal ultrasonography in women with acute uncomplicated pyelonephritis appears excessive because focal complications are rare and underlying

structural abnormalities occur in only about 5% of cases.[276,283] Women with bacteriuria of pregnancy in whom eradication of infection is difficult should be evaluated. Whereas ultrasonography can be safely performed during pregnancy, accurate delineation of the urinary tract should be delayed until at least 2 months after delivery, by which time the physiologic alterations to the urinary tract that occur during pregnancy should be reversed.[145] Ultrasound examination is also useful in diagnosing lower urinary tract obstruction and quantifying residual volume of urine in the bladder. A radionuclide diethylenetriaminepenta-acetic acid (DTPA) scan with furosemide to increase urine flow is useful in determining whether there is structural as opposed to functional ureteropelvic junction obstruction.

In addition to delineating lesions amenable to surgical correction, imaging frequently provides information previously unknown to the patient or physician. For example, unsuspected renal scarring may be seen, suggesting the possibility of undiagnosed (or even diagnosed) UTI in childhood. Occasionally, an unusual or unsuspected type of renal infection such as tuberculosis, papillary necrosis, or XGP may be discovered.[275] Two major radiologic patterns of XGP are seen, a localized mass and diffuse nodularity (Fig. 72.16). When a mass lesion is present, differentiation from pyogenic abscess, tuberculous abscess, or avascular carcinoma may not be possible. Additional findings include nephromegaly, thickening of the Gerota fascia, and infiltration into the perinephric space and surrounding retroperitoneal tissues.

Children with UTI are evaluated with imaging studies to identify those with congenital anomalies who may be at risk of chronic renal damage and those who require corrective surgery to prevent recurrent UTI and preserve renal function (Fig. 72.17).

Imaging protocols in pediatric UTIs are evolving. Renal ultrasound examination is commonly done in children with UTI, particularly those with recurrent UTI. Influenced by the new ability for antenatal diagnosis,



FIG. 72.15 Renal gallium-67 scan in a young girl with bilateral acute pyelonephritis and increased uptake in both kidneys caused by inflammation. *(Courtesy Dr. M. Bergeron.)*

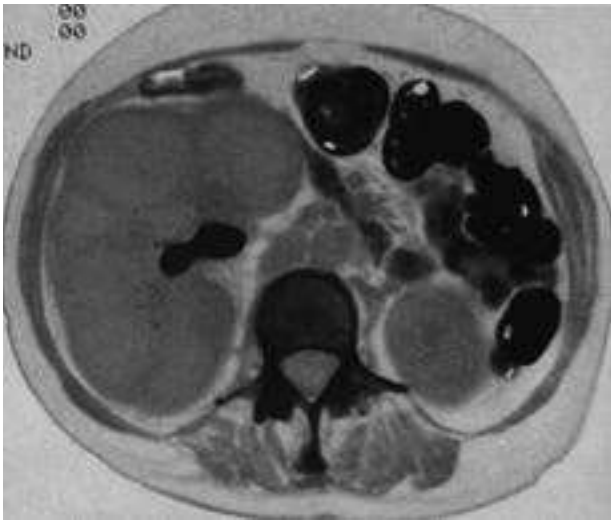

FIG. 72.16 Computed tomography scan of a patient with right-sided xanthogranulomatous pyelonephritis. A huge multilobulated inflammatory mass replaces the right kidney. *Proteus mirabilis* was grown from the urine. *(Courtesy Dr. L. E. Nicolle.)*

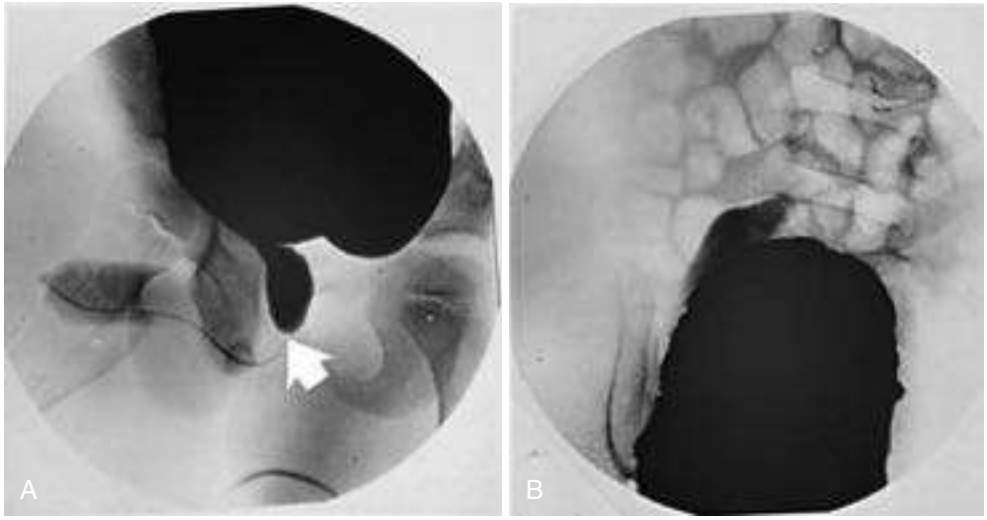

FIG. 72.17 Voiding cystourethrogram. (A) Posterior urethral valve on computed tomography scan. Note the discrepancy in size of the anterior *(arrow)* and posterior urethra. (B) There is irregular trabeculation of the bladder, with right ureteric reflux. *(Courtesy Dr. T. Slovis.)*

strategy has changed from extensive investigations, including cystography for all children younger than 7 years with UTI, to strategies focused on children at risk of developing renal damage as indicated by National Institute for Health and Care Excellence recommendations.[284–286] The main focus in the past was identifying vesicoureteral reflux (VUR). Traditional management consisted of prompt treatment of UTI, long-term antimicrobial prophylaxis until VUR resolved, or surgical intervention for those patients with both persistent high-grade VUR and recurrent UTIs in spite of prophylaxis with antimicrobials. What has changed involves questioning recommendations that were not evidence based and recognition of widespread overinvestigation. The debate regarding prophylaxis in children has moved to "selective prophylaxis".[287] The consistent message that emerges is that investigation of the urinary tract in children with UTIs should be limited to identifying a small select population that has a high risk of developing renal complications, with a lesser role for extensive investigation, surgical treatment, and antibiotic prophylaxis for most patients.[288]

As noted, the natural history for lower grades of VUR (grades I, II, and III) as opposed to higher grades is spontaneous resolution at a rate of 13% per year.[289] Furthermore, more recent data have supported the notion that mild and moderate VUR does not significantly increase the incidence of UTIs, pyelonephritis, or serious renal scarring.[290] In one study, the presence of VUR did not identify a susceptible population with an abnormal kidney on DMSA scan. In the context of a normal ultrasound result, cystography contributes little to the treatment of children younger than 1 year with a UTI.[291]

Although DMSA scans are of value in confirming the clinical diagnosis of acute pyelonephritis, the results uncommonly alter management and hence these scans are not routinely recommended. A DMSA scan performed 36 months after UTI may identify permanent loss of renal parenchyma or renal scars (Fig. 72.18).[292] Children at high risk of structural urinary tract abnormalities and of renal damage in whom renal ultrasonography should be obtained are listed in Table 72.5. Some experts believe that children with their first uncomplicated febrile or nonfebrile symptomatic UTI caused by *E. coli* who respond well to treatment do not require imaging unless they have recurrent infection.[284] Most experts, however, recommend ultrasonography of the urinary tract in all children with febrile UTIs. Accordingly, children with nonfebrile UTI do not need an initial imaging of their urinary tracts unless recurrent, in which case imaging that focuses on bladder function would be indicated—that is, prevoiding and postvoiding ultrasonography followed by voiding cystourethrography or indirect radioisotope cystography (Fig. 72.19).[284,293] Indirect cystography (nuclear) is particularly useful for follow-up evaluation. Children with abnormal findings on renal ultrasound likely will need further imaging such as a voiding cystourethrogram and/or a diuretic DTPA renal scan. Even if the renal ultrasound is normal, some experts recommend a DMSA renal scan to determine if the patient should have a voiding cystourethrogram, also called the "top-down approach."[284] A DTPA scan, particularly with furosemide administration, is used for the diagnosis of ureteropelvic junction obstruction. This study visualizes the passage of tracer through the urinary tract.[292] Some specialists think that cystourethrography should be avoided unless imaging (DMSA scan) shows evidence of renal scars.

Surgical Management

Surgical therapy in the management of UTI consists of the elimination of obstructive lesions or calculi and endoscopic or open surgical correction of severe reflux. An obstruction may be intrinsic (such as renal cysts), or it may be extrinsic anywhere along the urinary conduit from the ureteropelvic junction to the external urethral meatus. Surgical therapy should be directed toward eliminating the obstruction and preserving renal function. After the obstruction is eliminated, the patient should be followed with urine cultures. Urinary tract infection should be treated before surgery to render the urine sterile at the time of surgery; this decreases the possibility of bacteremia occurring in association with the surgery. For the management of perinephric or intrarenal abscess, see "Perinephric Abscess and Intrarenal Abscess" earlier.

Summary of Treatment Approaches

Fig. 72.20 summarizes the approach to the management of UTI. Table 72.6 summarizes the recommendations for initial therapy of UTI in adult outpatients.

TABLE 72.5 Findings in High-Risk Children for Whom Radiologic Imaging Is Indicated

Recurrent infection
Clinical signs such as poor urinary stream or palpable kidney
Unusual organisms (non–*Escherichia coli*)
Bacteremia or septicemia
Prolonged clinical course with failure to respond fully to antibiotic therapy within 48 h
Unusual clinical presentation (e.g., older boy)
Known dilatation or abnormality on antenatal ultrasound screening of urinary tract

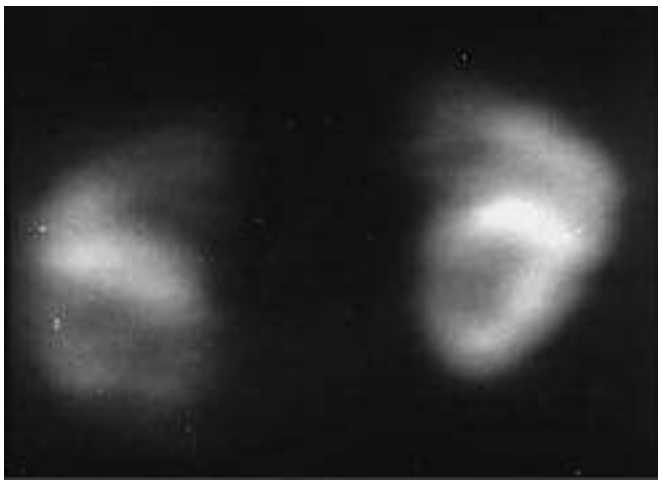

FIG. 72.18 Acute pyelonephritis. This technetium-99m glucoheptonate–labeled, single-photon emission computed tomography scan shows markedly decreased visualization of the upper and lower poles of the right kidney. The midportions are normal. *(Courtesy Dr. T. Slovis.)*



FIG. 72.19 Grades II and III reflux in a voiding cystourethrographic study from a young boy who presented with recurrent urinary tract infection. Note early clubbing of the calyces and dilation of the ureter on the left side. *(Courtesy Dr. L. E. Nicolle.)*

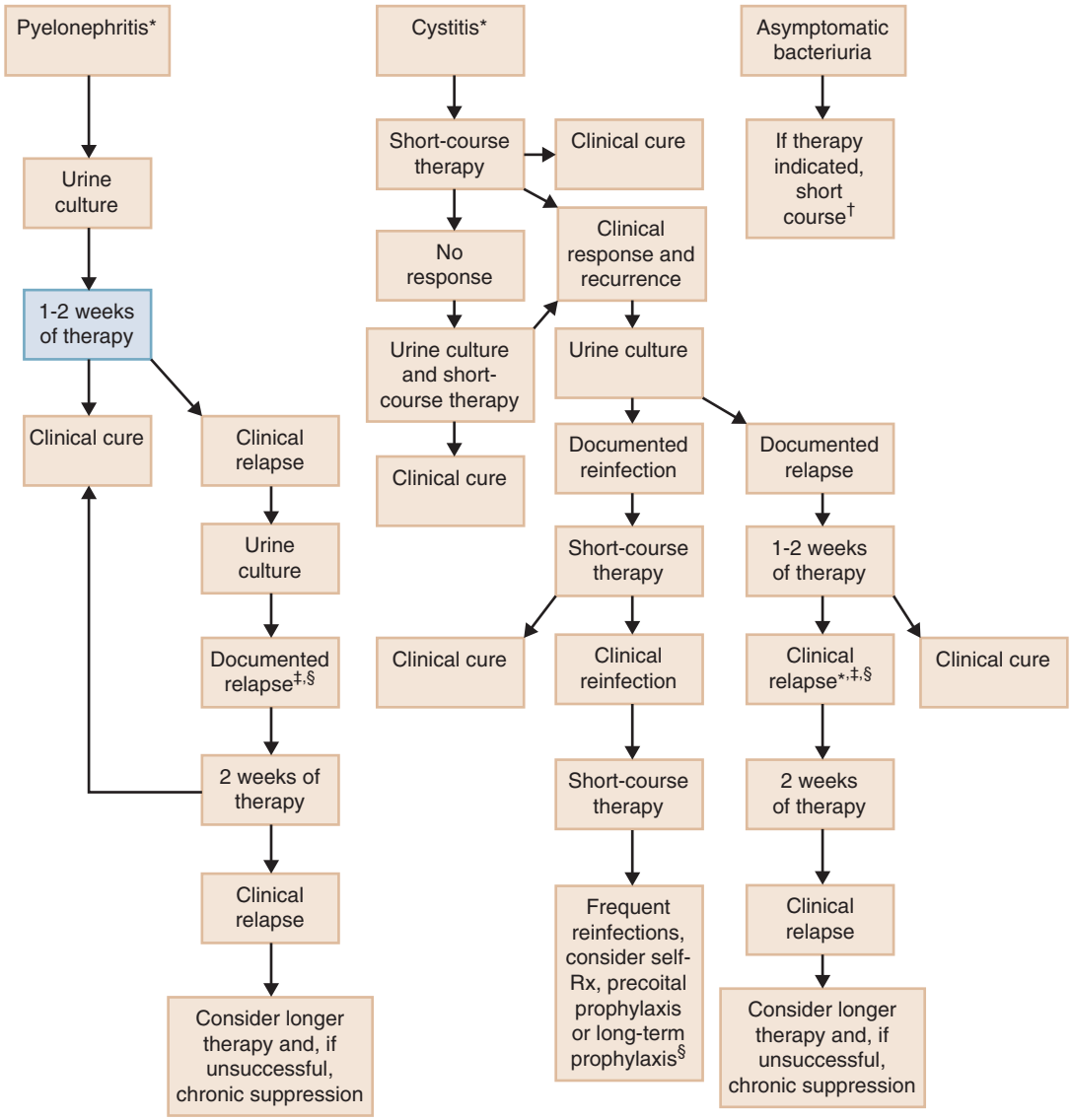

*Consider imaging studies in all men and in women with complicated urinary tract infection.
†No therapy except for renal transplant patients or prior to urologic procedures. Follow-up culture only in transplant patients.
‡Evaluate men for chronic bacterial prostatitis.
§Consider imaging studies in women.

FIG. 72.20 Approach to the management of urinary tract infection in nonpregnant adults.

TABLE 72.6 Recommendations for Initial Therapy of Urinary Tract Infection in Adults

PARAMETER	ORAL[a]	PARENTERAL (SWITCH TO ORAL WHEN RESPONSE OCCURS)
Uncomplicated Pyelonephritis		
GNB or no urine Gram stain available	CP 7 days, LV 5 days; if cannot use FQ, TMP-SMX 14 days + 1 dose CT or AM	FQ or extended-spectrum β-lactam (e.g., CT) ± AM or carbapenem
GPC in chains	Amoxicillin, 14 days	Ampicillin
GPC in clusters	Linezolid or TMP-SMX, 14 days	Vancomycin
Complicated Pyelonephritis		
Nonpregnant women or men	As for uncomplicated pyelonephritis; duration 10–14 days	Extended spectrum β-lactam ± AM, FQ, carbapenem[d]
Pregnant women	Extended spectrum β-lactam; or TMP-SMX[b] only if known sensitive; both for 14 days	Extended spectrum β-lactam ± AM
Uncomplicated Cystitis		
Nonpregnant women	Nitrofurantoin, 5 days; or fosfomycin, 1 dose; or TMP-SMX, 3 days; or pivmecillinam, 3–7 days	

TABLE 72.6 Recommendations for Initial Therapy of Urinary Tract Infection in Adults—cont'd

PARAMETER	ORAL[a]	PARENTERAL (SWITCH TO ORAL WHEN RESPONSE OCCURS)
Complicated Cystitis		
Women or men	FQ or nitrofurantoin, 7 days; or fosfomycin, 1 dose	
Pregnant women	Cephalexin, 3–5 days; or fosfomycin, 1 dose; or nitrofurantoin,[c] 7 days; or TMP-SMX,[b] 3 days if sensitive	

[a]Preferred if the patient is reliable, compliant, hemodynamically stable, and able to take oral therapy.
[b]TMP-SMX should be avoided in the first and third trimesters.
[c]Nitrofurantoin should be avoided in the first trimester.
[d]Ceftazidime-avibactam is a potential option for infections due to carbapenem-resistant Enterobacteriaceae or multidrug-resistant (MDR) *Pseudomonas;* ceftolozane-tazobactam is another potential option for MDR *Pseudomonas.*
GNB, Gram-negative bacilli; *GPC,* gram-positive cocci.
Oral Drugs and Dosages:
CP, ciprofloxacin 500 mg bid or 1000 mg daily.
LV, levofloxacin 750 mg daily.
TMP-SMX, trimethoprim-sulfamethoxazole 160/800 mg bid.
FQ, fluoroquinolone—ciprofloxacin 500 mg bid or 1000 daily or levofloxacin 750 mg daily.
Oral Drugs and Dosages:—cont'd
Amoxicillin 875 mg bid.
Linezolid 600 mg bid.
Nitrofurantoin 100 mg bid.
Fosfomycin 3 g once.
Pivmecillinam 400 mg bid.
Cephalexin 500 mg qid.
Parenteral Drugs and Dosages:
FQ, fluoroquinolone—ciprofloxacin 400 mg q12h or levofloxacin 500 mg daily.
CT, ceftriaxone 2 g/d.
AM, aminoglycoside (e.g., gentamicin 5 mg/kg/day).
Ampicillin 2 g q4h.
Vancomycin 15 mg/kg bid.
Carbapenem (e.g., imipenem 500 mg q6h or ertapenem 1 g daily).

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The complete reference list is available online at Expert Consult.

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73 Sepsis and Septic Shock

Tom van der Poll and Willem Joost Wiersinga

SHORT VIEW SUMMARY

Definition
- Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection.

Clinical Signs and Symptoms
- Pneumonia, peritonitis, urinary tract infections, and soft tissue infections are—in descending order—the most important sources of sepsis.
- Organ failure is the hallmark sign of sepsis and most frequently occurs in the respiratory and cardiovascular systems, followed by renal, central nervous system, hematologic, and hepatic dysfunction.

Epidemiology
- The incidence of sepsis has increased at least in part due to the aging population and aggressive therapies for chronic diseases.
- The case-fatality rate of sepsis has decreased, at least in high-income countries, likely in part through implementation of the Surviving Sepsis Campaign guidelines and improvement of standard intensive care.
- The risk of acquiring sepsis is influenced by age, sex, comorbidity burden, and socioeconomic status.

Microbiology
- A causative microorganism can be identified in up to two-thirds of patients admitted to an intensive care unit for sepsis.
- Gram-negative bacteria are identified in 38% to 62% of cases, gram-positive bacteria are identified in 40% to 52%, and fungi are identified in 5% to 19%. Viral infections are detected in 1% to 7% of cases.

Pathogenesis
- Pattern-recognition receptors are essential for sensing invading bacteria and subsequent initiating of the immune response. These receptors recognize both pathogen-associated molecular patterns (conserved motifs expressed by pathogens) and danger-associated molecular patterns or alarmins (host molecules released during inflammatory stress).
- The host response to sepsis is characterized by both proinflammatory and antiinflammatory reactions and varies among individuals. Proinflammatory responses include cytokine release and activation of the complement system, coagulation system, and vascular endothelium; antiinflammatory responses can result in immunosuppression at least in part due to apoptotic loss of lymphoid cells.
- The host response to infection and noninfectious injury is not fundamentally different.

Diagnosis
- There is no gold standard for diagnosing sepsis. A clinical pattern based on the patient's history and physical examination combined with laboratory, radiology, and microbiology testing is typically required for the diagnosis.
- Host response biomarkers can be of diagnostic value, discriminating infectious from noninfectious conditions or determining the causative pathogen; of prognostic value, assigning risk profiles and predicting outcome; or of theranostic (combining diagnosis and therapy) value, aiding in selection and monitoring of therapy (see Fig. 73.6).

Therapy
- Implementation of the Surviving Sepsis Campaign guidelines for management of sepsis has been associated with improved outcome.
- Intravenous antibiotics should be started as soon as possible after recognition of sepsis and within 1 hour for septic shock.
- The choice of empirical antibiotic treatment depends on the suspected source of infection, global geography, setting (e.g., community or hospital), comorbidity, and local microbial susceptibility patterns.

Prognosis
- Patients who survive sepsis often have long-term complications including cognitive and physical impairments, higher readmission rates, and increased mortality.

Sepsis is a life-threatening condition with organ failure caused by a dysregulated host response to an infection. Septic shock is sepsis accompanied by persistent hypotension. The most common infections causing sepsis and septic shock are pneumonia and peritonitis. Any infection that overrides the protective innate immune response initiated in response to a potential pathogen can result in sepsis. Sepsis is most often caused by bacteria, but fungal and viral infections can also instigate sepsis. A key pathogenic feature of sepsis is that the multifaceted innate immune response fails to return to normal homeostasis in the context of an unsuccessful attempt to eradicate the invading pathogen. Concurrent hyperinflammation and immunosuppression ensue, affecting different cell types and organs. The case-fatality rate of sepsis has improved in recent years, probably as a result of better general clinical practices and development and implementation of sepsis treatment guidelines. Nonetheless, sepsis continues to be the most frequent cause of death in hospitalized patients, and it is expected to remain an important clinical problem in the future, owing to the aging population, aggressive therapies for chronic diseases, and antimicrobial resistance. Moreover, with more patients surviving sepsis, attention has been directed toward long-term sequelae, which include cognitive and physical impairments and cardiovascular disease.

DEFINITION

Sepsis is a broad term used for an incompletely understood process, and there is no gold standard for its diagnosis. The term *sepsis* originates from the ancient Greek word *sêpsis* ("putrefaction" or "decay of organic matter") and was first used in a medical context in Homer's *Iliad,* written more than 2700 years ago. In the early 1990s sepsis was clinically defined by a consensus definition generated by a group of key experts.[1] The Sepsis-1 definition was centered around four systemic inflammatory response syndrome (SIRS) criteria consisting of tachycardia (heart rate >90 beats/min); tachypnea (respiratory rate >20 breaths/min); fever or

hypothermia (temperature >38°C or <36°C); and leukocytosis (white blood cells >1200/mm^3), leukopenia (white blood cells <4000/mm^3), or bandemia (≥10%) (Table 73.1). Sepsis-1 was defined as (documented or suspected) infection leading to the onset of SIRS as reflected by the presence of two or more SIRS criteria. Severe sepsis was defined as sepsis complicated by organ dysfunction, which could progress to septic shock, defined as "sepsis-induced hypotension persisting despite adequate fluid resuscitation."[1] By incorporating the SIRS criteria in the definition, the Sepsis-1 definition sought to capture the then-prevailing view that sepsis resulted from a systemic inflammatory response to infection. In 2001 a new task force was assembled to readdress the definition of sepsis.[2] Although the limitations of Sepsis-1 were recognized, alternatives were not offered owing to absence of supporting evidence. The Sepsis-2 definition expanded the list of diagnostic criteria, encompassing a set of 24 general, inflammatory, hemodynamic, organ dysfunction, or tissue perfusion parameters. In the Sepsis-2 definition the criteria for severe sepsis remained similar, whereas septic shock was defined more explicitly as refractory hypotension (systolic blood pressure <90 mm Hg or mean arterial blood pressure <70 mm Hg) despite adequate fluid resuscitation.[2]

The most recent Sepsis-3 definition, published in 2016,[3] seeks to confine important limitations of Sepsis-1 and Sepsis-2, which include a disproportionate emphasis on inflammation, poor specificity and sensitivity of the SIRS criteria, and the incorrect concept that sepsis follows a continuum through severe sepsis to shock. The Sepsis-3 definition, in contrast to Sepsis-1 and Sepsis-2, partially relies on large data sets, which were used to obtain quantitative information in support of the new criteria. The new definition abandoned the use of SIRS criteria in the diagnosis of sepsis. In addition, in the new definition the presence of organ dysfunction is a requirement for a sepsis diagnosis, and therefore the term *severe sepsis* was eliminated in Sepsis-3. According to the Sepsis-3 definition, sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection.[3] In the clinic, organ dysfunction can be represented by an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more; the baseline SOFA score should be presumed zero unless the patient is known to have preexisting organ dysfunction before the onset of infection (Table 73.2). Septic shock is now defined as a subset of sepsis in which strong circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone; these patients can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia.

The 2016 Task Force also introduced the quick SOFA, or qSOFA, score, composed of three components that are easy to measure at the bedside: respiratory rate of 22 breaths/min or greater, altered mentation, and systolic blood pressure of 100 mm Hg or less.[3] Evidence indicated that in out-of-hospital, emergency department, and general hospital ward settings, adult patients with suspected infection and a higher risk for poor outcomes typical of sepsis can be rapidly identified by the presence of at least two qSOFA criteria. Importantly, qSOFA is *not* part of the new definition of sepsis. Moreover, failure to meet two or more SOFA or qSOFA criteria should not lead to a delay of

TABLE 73.1 Sepsis Definitions

TERM	DEFINITION
1991 Consensus Conference[1]	
SIRS	At least two of the following: • Temperature >38°C or <36°C • Heart rate >90 beats/min • Respiratory rate >20 breaths/min or arterial CO_2 <32 mm Hg • White blood cell count >12 × 10^9/L or <4 × 10^9/L or >10% immature forms
Sepsis	Infection[a] + SIRS
Severe sepsis	Sepsis + acute organ dysfunction
Septic shock	Sepsis + persistent hypotension after fluid resuscitation
2001 International Sepsis Definitions Conference[2]	
No significant changes from 1991 definitions, with the addition that signs and symptoms of sepsis are more varied than captured by 1991 definitions; this resulted in the presentation of a list of these signs and symptoms for the diagnosis of sepsis	
2015 Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[3]	
Sepsis[b]	• Life-threatening organ dysfunction caused by dysregulated host response to infection • Organ dysfunction can be identified as acute change in total SOFA score ≥2 points
Septic shock	• Sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality • Clinically defined as sepsis with persisting hypotension requiring vasopressors to maintain mean arterial pressure ≥65 mm Hg and with serum lactate >2 mmol/L

[a]Suspected or proven.
[b]In the new sepsis definition, the presence of organ dysfunction is central and a requirement; previously, organ dysfunction identified "severe" sepsis, a term that was abandoned in the Sepsis-3 definition.
CO_2, Carbon dioxide; *SIRS,* systemic inflammatory response syndrome; *SOFA,* Sequential [Sepsis-related] Organ Failure Assessment.

TABLE 73.2 SOFA Score

ORGAN SYSTEM	SCORE
Respiration	
Pa_{O_2}/Fi_{O_2}, mm Hg (kPa)	
<400 (53.3)	1
<300 (40)	2
<200 (26.7) with respiratory support	3
<100 (13.3) with respiratory support	4
Central nervous system	
GCS score	
13–14	1
10–12	2
6–9	3
<6	4
Cardiovascular	
MAP or use vasopressors (μg/kg/min)	
MAP <70 mm Hg	1
Dopamine <5 or dobutamine (any dose)[a]	2
Dopamine 5.1–15 or epinephrine ≤0.1 or norepinephrine ≤0.1	3
Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1	4
Liver	
Bilirubin, mg/dL (μmol/L)	
1.2–1.9 (20–32)	1
2.0–5.9 (33–101)	2
6.0–11.9 (102–204)	3
>12.0 (204)	4
Coagulation	
Platelets, × 10^3/μL	
<150	1
<100	2
<50	3
<20	4
Renal	
Creatinine, mg/dL (μmol/L) or urine output, mL/day	
1.2–1.9 (110–170)	1
2.0–3.4 (171–299)	2
3.5–4.9 (300–440) or <500	3
>5.0 (440) or <200	4

Fi_{O_2}, Fraction of inspired oxygen; *GCS,* Glasgow Coma Scale (scores range from 3 to 15; higher score indicates better neurologic function); *MAP,* mean arterial pressure; *Pa_{O_2},* partial pressure of oxygen; *SOFA,* Sequential [Sepsis-related] Organ Failure Assessment.
The SOFA score can be used to measure the severity of organ dysfunction. The aggregate score is calculated by summing the worst scores for each of the organ systems.
[a]Catecholamine doses are given as μg/kg/min for at least 1 hour.
From Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. *1996;22:707–710.*

treatment of infection or any other intervention deemed necessary by physicians.

The Sepsis-3 definition and qSOFA have limitations, and criticism exists regarding their use in clinical practice. Although large cohorts were used to support Sepsis-3 and qSOFA, patient data were almost exclusively derived from high-income countries (especially the United States), which leaves uncertainty with regard to extrapolation to resource-poor settings. In addition, supporting data were generated in adult patients and cannot be readily extrapolated to pediatric sepsis. The measurement of serum lactate is required for the diagnosis of septic shock, which is not feasible in many areas of the world. Finally, the Sepsis-3 definition does not entail a molecular or pathogenic component that may stratify patients into more homogeneous subgroups based on their predominant pathophysiologic abnormality and thereby guide individualized therapies.

CLINICAL SIGNS AND SYMPTOMS

Correctly recognizing a septic patient can be challenging, as clinical signs and symptoms at presentation can be variable and nonspecific.[4,5] The manifestations of sepsis depend on the source of infection, the causative pathogen, the type and extent of organ dysfunction, drug use and comorbidity of the patient, and the delay before consulting a physician or before start of treatment. The most common underlying comorbidities of patients admitted for sepsis include chronic obstructive pulmonary disease, neoplasm, human immunodeficiency virus (HIV) infection, chronic liver disease, chronic renal disease, diabetes, peripheral vascular disease, and autoimmune disease.[6] General variables include fever, tachycardia, tachypnea, altered mental status, significant edema, or positive fluid balance (>20 mL/kg over 24 hours).[2] Hypothermia is observed in 9% to 35% of patients with sepsis and is associated with adverse outcomes.[7,8]

Source of Sepsis

Pneumonia is the most common source of sepsis in adults, followed by abdominal, urinary tract, and skin/soft tissue infections (Fig. 73.1A).[6,9,10,11,12] These preferred sites account for 80% to 90% of all adult sepsis cases, the remainder being caused by bone/joint infections, ear-nose-throat infections, and others. More than one source is found in approximately 6% of episodes.[9] The primary source of infection often depends on the comorbidity of the patient and the presence of indwelling catheters or devices. For instance, the presence of obstructive lung disease is a significant predictor of hospitalizations caused by respiratory tract infections but not of hospitalizations due to infections outside the respiratory system.[13] Likewise, in patients on long-term renal replacement therapy, sepsis more often is caused by central venous catheter infections, peritonitis, or an ischemic bowel.[14] However, in most series, no obvious source of infection can be found in one-sixth of patients.[6,9,10,11,12]

Organ Failure in Sepsis

Organ failure is the hallmark sign of sepsis, and respiratory and cardiovascular failure most frequently occurs, followed by renal, central nervous system (CNS), hematologic, and hepatic failure (Fig. 73.1B).[5,6,10,15,16] Respiratory failure, shock, and kidney failure are the most common reasons for admission to the intensive care unit (ICU).[10,15] Most patients have failure of a single organ, whereas approximately 20% have failure of two organs, and approximately 5% have acute failure of three or more organs.[6,11] There is a direct relationship between the number of organs failing in patients with sepsis and mortality.[10] A study from across Europe showed mortality rates were 26% among patients with dysfunction of two organs on ICU admission, whereas mortality rates were 65% among patients with failure of four or more organs.[10] Severity of organ dysfunction has been assessed with various scoring systems that quantify abnormalities according to clinical findings, laboratory data, or therapeutic interventions.[3] The predominant system in current use is the SOFA score (see Table 73.2).[17] A higher SOFA score is associated with an increased probability of mortality.[18]

Acute Lung Injury

Respiratory failure commonly manifests as acute respiratory distress syndrome (ARDS), defined as hypoxemia and bilateral infiltrates of noncardiac origin (Fig. 73.2).[19] ARDS can be classified as mild (200 mm Hg < partial pressure of arterial oxygen [Pao_2]/fractional inspired oxygen [Fio_2] < 300 mm Hg), moderate (100 mm Hg < Pao_2/Fio_2 ≤ 200 mm Hg), or severe (Pao_2/Fio_2 ≤ 100 mm Hg).[19] The underlying pathology is diffuse alveolar epithelial injury, with increased barrier permeability and exudation of protein-rich fluid into the interstitial and airspace compartments.[20] Neutrophils and monocytes accumulate in the lungs and may form cellular aggregates in pulmonary vessels. Significant right-to-left shunting occurs. Dead space volume increases and compliance decreases, augmenting the work of breathing. Mechanical ventilation is needed in most severe cases. In the past 2 decades, mortality among patients with ARDS has decreased from approximately 60% to 25%, which can be accounted for in part by the application of a lung-protective ventilation strategy (with low airway pressure and low tidal

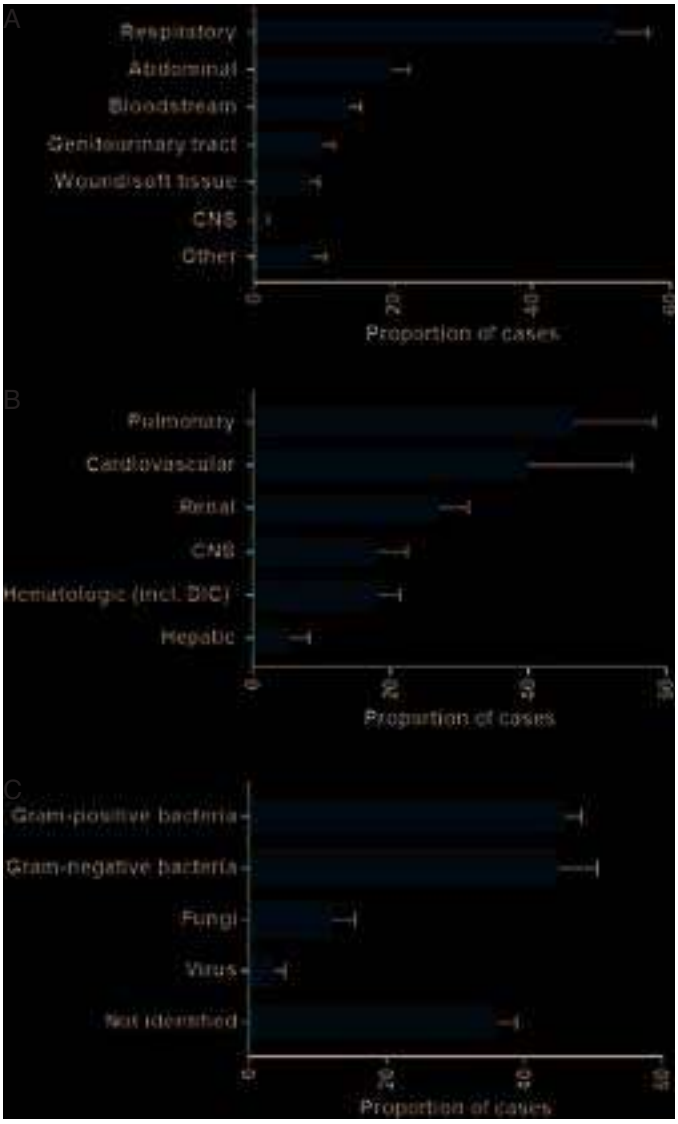

FIG. 73.1 Most common sites of infection, types of organ failure, and causative organisms identified in patients with sepsis. (A) Most common sites of infection. Bars show the means plus standard error of data from six representative studies.[6,9,10,11,12,70] The lung and abdomen are the most common primary sites. (B) Most common types of organ failure in sepsis. Bars show means plus standard error of data from four representative studies.[6,10,15,16] The definitions used to diagnose organ dysfunction varied from study to study. Central nervous system *(CNS)* dysfunction was uncommon in some series and very common in others. (C) Incidence of sepsis by causative organism. Bars show means plus standard error of causative organisms isolated in patients with sepsis from four representative studies.[10,16,57,70] A significant number of patients have two or more causative organisms identified. *DIC,* Disseminated intravascular coagulation.

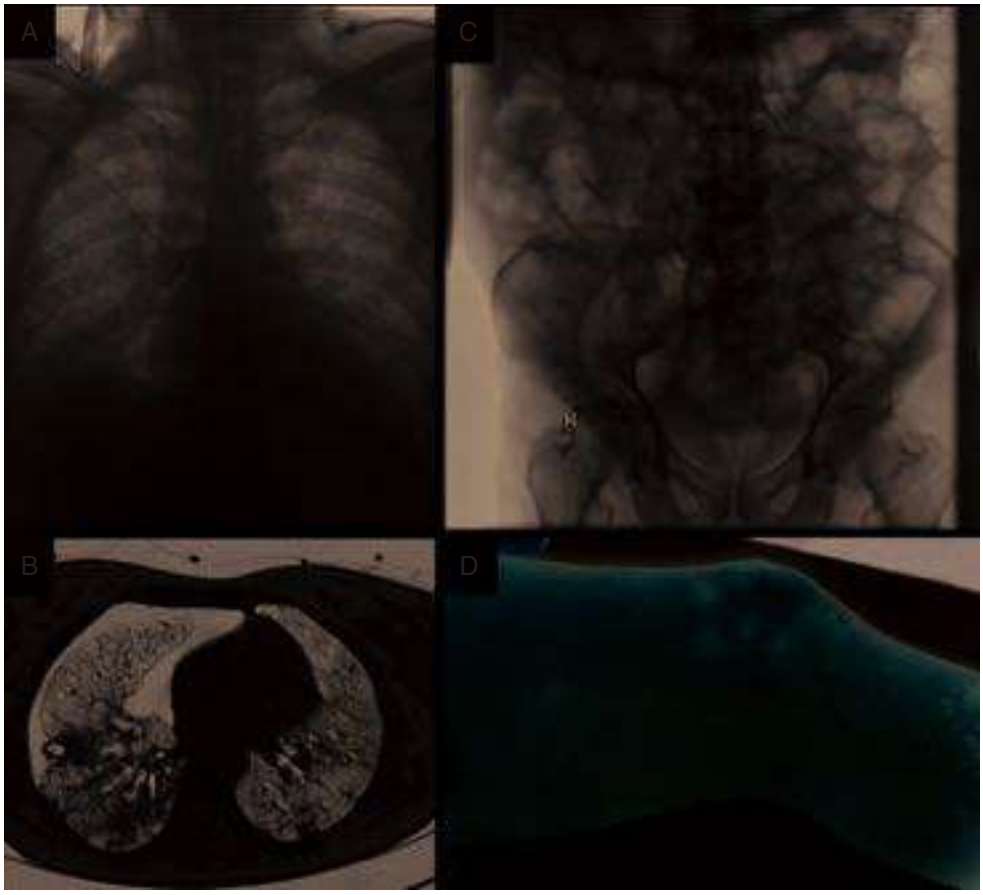

FIG. 73.2 Common complications of sepsis. The most common sepsis complications include brain dysfunction, acute lung injury, acute kidney injury, ileus, cardiovascular dysfunction (including shock), disseminated intravascular coagulation (with thrombocytopenia), and liver injury. (A) Typical chest x-ray and (B) computed tomography scan of acute respiratory distress syndrome showing patchy opacification, loculated pneumothoraces, and bilateral chest drains. (C) Plain abdominal x-ray showing an ileus (note dilated loops of small and large bowel with no cutoff). (D) Purpura as a manifestation of severe disseminated intravascular coagulation. *(A from Khan A, Kantrow S, Taylor DE. Acute respiratory distress syndrome.* Hosp Med Clin. *2015;4:500–512; B from Randhawa R, Bellingan G. Acute lung injury.* Anaesth Intensive Care Med. *2007;8:477–480; C from Glancy DG. Intestinal obstruction.* Surgery. *2014;32:204–211; D from Piette WW. Purpura. In: Callen JP, Jorizzo JL, Zone JJ, Piette W, Rosenbach MA, Vleugels RA, eds.* Dermatological Signs of Systemic Disease. *5th ed. Philadelphia: Elsevier; 2017:109–116, courtesy Dr. Neil A. Fenske, Tampa, FL.)*

volumes) in addition to better supportive care in the ICU.[21] However, up to half of patients who recover from ARDS may have significant functional impairment related to the healing process, which can produce restrictive defects, reduced diffusing capacity, and an inability to return to work.[21,22]

Cardiovascular Dysfunction

The cardiovascular impact of sepsis has two components: myocardial dysfunction and relative hypovolemia resulting from vasodilation.[23,24] Sepsis-associated myocardial dysfunction includes reduced left and right ventricular ejection fractions, increased left and right ventricular end-diastolic volumes, and an elevated heart rate and cardiac output. The cardiac depression associated with septic shock reflects the effects of inflammatory mediators on cardiac myocyte and microcirculatory function, is not caused by ischemia, and usually does not require inotropic therapy. However, if hypovolemic shock cannot be reversed by administering intravenous fluids, the use of vasopressors is indicated to restore tissue perfusion pressure.

Adrenergic agonists are the first-line vasopressors, norepinephrine being the first-choice agent.[25] In cases of refractory septic shock, another vasopressor with a different mechanism of action (nonadrenergic) can be added. Mechanisms implicated in the development of sepsis-induced myocardial depression include alterations in calcium homeostasis, mitochondrial dysfunction, apoptosis, circulating cardiosuppressant mediators, and nitric oxide. A postmortem study found that cardiac myocyte cell death was rare, whereas sepsis-induced focal mitochondrial injury was present in many cells.[26]

Renal Dysfunction

Acute kidney injury (AKI) is characterized by decreased urinary output with increasing serum creatinine levels; many patients with sepsis require renal replacement therapy.[27] Sepsis-associated AKI is associated with a high burden of morbidity (e.g., dependency on dialysis at the time of hospital discharge) and mortality in patients with critical illness. The renal abnormalities range from minimal proteinuria to profound renal failure; postmortem studies have found focal acute tubular injury and minimal glomerular damage.[26] The underlying pathogenic mechanisms include hypovolemia, hypotension, renal vasoconstriction, and toxic drugs (e.g., aminoglycosides) or contrast agents used for medical imaging.[27] In most patients, sepsis-induced renal injury is largely reversible. Nonetheless, even small acute increases in serum creatinine are associated with decreased long-term survival in critically ill patients.[28] Not surprisingly, survival in patients with septic shock is directly linked to the severity of AKI.[29]

Novel promising biomarkers in urine and plasma for the early diagnosis of sepsis-associated AKI include cystatin C, neutrophil gelatinase-associated lipocalin, tissue inhibitor of metalloproteinase 2, and insulinlike growth factor–binding protein 7. At the present time, however, data on these biomarkers are insufficient to support their use in clinical management of patients with sepsis-associated AKI.[30]

Dysfunction of Brain, Peripheral Nerves, and Muscles

Brain dysfunction in sepsis may manifest as coma or delirium. Formally, sepsis-associated encephalopathy (SAE) is defined as diffuse cerebral dysfunction that accompanies sepsis in the absence of direct CNS infection, structural abnormalities, or other types of encephalopathy.[31] Delirium occurs in 30% to 50% of patients with severe sepsis. In general, the severity of SAE parallels the severity of other manifestations of sepsis. Because there are no specific markers for SAE, the diagnosis relies on excluding primary CNS infections and other causes of encephalopathy. Proposed underlying mechanisms include microscopic brain injury, blood-brain barrier and cerebral microcirculation dysfunction, altered CNS metabolism, and impaired cholinergic neurotransmission. Patients who experience severe sepsis may have cognitive and functional defects that last for years.[32] Critical illness polyneuropathy (CIP) and critical illness myopathy (CIM) are other common complications in patients with prolonged ICU stay. Clinical features include difficulty in weaning from a ventilator, generalized wasting of the limbs, and diffuse weakness.[33] The diagnosis of CIP and CIM relies on clinical, electrophysiologic, and muscle biopsy investigations.[33] The septic inflammatory response is thought to play an important role in their pathogenesis.[34] CIP and CIM are associated with prolonged ICU stays and increased mortality.[33,34]

Coagulopathy and Disseminated Intravascular Coagulation

Sepsis is frequently complicated by coagulopathy. Abnormalities range from subclinical coagulation disorders to prolongation of clotting times (most notably prothrombin time and partial thromboplastin time), low platelet counts, and elevated D-dimer levels. In patients with sepsis the reported prevalence of the most severe form of coagulopathy, disseminated intravascular coagulation (DIC), is 35%.[35] Commonly used screening assays for DIC include (1) a reduced or downward trend in the platelet count (usually <100,000/mm^3); (2) the presence of fibrin-related markers including fibrin degradation products, D-dimers, or soluble fibrin in plasma; (3) prolongation of PT or APTT (>1.2 times the upper limit of normal); and (4) low plasma levels of endogenous anticoagulants such as antithrombin and protein C.[36] Clinically, severe DIC can be characterized by widespread thrombosis in small and midsize vessels with simultaneous hemorrhage from various sites (Fig. 73.2). Earlier studies have shown that the development of DIC in patients with sepsis can double the risk of death.[35] At the present time, no specific therapy for DIC exists apart from treatment of the underlying disorder.[25] Studies on the potential benefit of the use of antithrombin (of which the plasma activity is decreased in patients with sepsis and DIC), soluble thrombomodulin, or heparin in patients with sepsis and DIC were negative or are still ongoing.[37–39] Recombinant activated protein C, which was originally recommended in the 2004 and 2008 Surviving Sepsis Campaign guidelines, was not shown to be effective in adult patients with septic shock in the PROWESS-SHOCK trial and was subsequently withdrawn from the market.[25,40]

Gastrointestinal Tract and Hepatic Injury

Gastrointestinal tract injuries include disruption of the intact intestinal epithelium, which may lead to the translocation of inflammatory mediators; the occurrence of erosions of the gastric and duodenal mucosa that predispose to upper gastrointestinal bleeding; and the development of ileus, which may persist for several days after the resolution of septic shock (Fig. 73.2).[41,42] The gut microbiota of patients with sepsis are characterized by lower diversity, lower abundances of key commensal genera, and in some cases overgrowth by one bacterial genus, a state otherwise known as dysbiosis.[42] Preclinical work underscores the role of the microbiota in maintaining gut-barrier function and suggests that impaired communication across the gut-organ axes is associated with brain, lung, and kidney failure.[42]

Hepatic dysfunction includes cholestatic jaundice characterized by elevations in conjugated and unconjugated bilirubin, often seen in association with elevated levels of alkaline phosphatase and aminotransferase levels. Preexisting liver disease can aggravate these values. A "shock liver" is unusual, but if the duration of septic shock is prolonged, a massive rise in serum transaminases may follow hypoxic necrosis of centrilobular liver cells.

At the end, the function of every organ may be affected. Skin manifestations of sepsis include a cutaneous reaction at a local inoculation site (pustule, eschar), lesions that appear at sites of hematogenous seeding of the skin or underlying soft tissue (petechiae, pustules, ecthyma gangrenosum, cellulitis), diffuse eruptions caused by bloodborne toxins (e.g., toxic shock syndrome toxin), and hemorrhagic or necrotic lesions. Other common manifestations of sepsis include altered glycemic control, adrenal dysfunction, and sick euthyroid syndrome.[5]

EPIDEMIOLOGY

In May 2017 the World Health Organization recognized sepsis as a global health priority by adopting the "Improving the Prevention, Diagnosis, and Management of Sepsis" resolution, underscoring the recognition of the substantial burden of sepsis at the individual, health system, and societal level.[43,44] Nonetheless, the true burden of sepsis remains unknown. There is substantial variability in the reported incidence and mortality of sepsis depending on the case definitions and diagnosis codes used to identify patients.[45,46]

Incidence

The current global estimates of 31.5 million episodes of sepsis per year comes from a systematic review that extrapolated data from selected high-income countries (United States, Germany, Australia, Taiwan, Norway, Spain, and Sweden).[47] For these countries, the aggregate population incidence rate of sepsis hospitalization was 270 per 100,000 person-years with a large confidence interval.[47] Estimates for the incidence of sepsis in the United States range from 300 to more than 1000 cases per 100,000 person-years depending on method of database abstraction.[48] There are no population-level sepsis incidence estimates from lower-income countries, which limits the prediction of global cases and deaths.

In high-income countries, sepsis represents approximately 6% of adult hospitalizations and 10% to 37% of ICU admissions.[6,10,49] The overall incidence rate of hospitalization among emergency medical services encounters is greater for sepsis than for acute myocardial infarction or stroke.[50] A retrospective cohort study of 173,690 adult patients with sepsis admitted to hospitals across the United States in 2014 showed that 54.7% required ICU care during hospitalization, and 15.8% had septic shock.[49] Median ICU length of stay was 5 days (range, 2–6 days).[49] Median hospital length of stay was 10 days (range, 8–12 days).[49] The cost of treating sepsis in US hospitals was estimated to be $24 billion in 2013, making it the most expensive condition treated in US hospitals in that year.[44]

Mortality

Sepsis is estimated to account for more than 5.3 million deaths around the world each year.[47] In the United States, sepsis contributes to one in every two to three in-hospital deaths[51] and represents the most frequent cause of death in noncoronary ICUs.[46] The case-fatality rate depends on the setting and severity of disease. US data from 2014 show that of all patients admitted for sepsis, 15.0% died in the hospital, and 6.2% were discharged to a hospice.[49] Data from Australia and New Zealand show hospital fatality rates for sepsis and septic shock of 14% and 22%, respectively.[52] The extent and number of organ failures are the strongest denominators of mortality in sepsis.[46,49] There are significant differences in reported acute mortality rates around the globe. As an example, a 1-day point prevalence study among 227 ICUs in Brazil identified 794 patients with sepsis, in whom mortality was observed in more than 50%.[53] In this setting, low availability of resources and adequacy of treatment were independently associated with mortality.[53]

Trends in Time: Incidence and Mortality

In the past 2 decades, numerous studies have suggested that the incidence of sepsis is increasing over time, while mortality is decreasing.[52,54–57] An observational study from the period 2000–2012 using an ICU registry of more than 100,000 patients with severe sepsis in Australia

and New Zealand provided compelling evidence for gradually declining mortality rates from 35% in 2000 to 18.4% in 2012.[52] Each year absolute mortality decreased 1.3% throughout the study period. In the absence of comorbidities and older age, the case-fatality rate of severe sepsis or septic shock was less than 5% in 2012.[52] This key investigation, which used strict diagnostic criteria during the entire observation period, confirmed the declining mortality rates derived from large retrospective analyses.[46]

In contrast, one more recent study from the United States that used clinical data from electronic health record systems of greater than 170,000 sepsis cases from 2009 to 2014 could not demonstrate any change in the incidence of sepsis or mortality.[49] The shorter observation period in this study together with differences in coding and registration practices can partly explain the variation in the reported incidence and mortality of sepsis compared with previous studies.[5,46,49,52]

The steady decrease of sepsis-related mortality over time most likely reflects the overall improvement in ICU practice in the last 20 to 30 years. This is supported by the finding from the above-mentioned Australian and New Zealand ICU registry study that the observed annual reduction in mortality did not differ between patients with severe sepsis and other diagnoses.[52] Key factors that contribute to the overall rise in the worldwide incidence of sepsis include the aging of the population, the emergence of antimicrobial resistance, the growing use of immunosuppressive drugs and therapies, and the increased number of patients who are at risk for developing sepsis.

Risk Factors

Any infection can trigger sepsis in a susceptible host. People at the highest risk of developing sepsis include infants and elderly adults as well as patients with chronic or serious illnesses such as diabetes and cancer and patients with an impaired immune system.[46] The most important risk factors for the development of sepsis are listed in Table 73.3.

Demographic risk factors for sepsis include age, male sex, and black race. Patients 65 years of age and older account for nearly 65% of sepsis cases.[58] In addition, advancing age is a major risk factor for poor outcome, which is explained in part by the presence of comorbidities and immunosenescence, which refers to the gradual age-related deterioration of the immune system.[58,59] Most studies report a lower risk for women to develop sepsis,[6,52,57,60] although it is uncertain whether this decreased risk is caused by a relative protection against the acquisition of an infection or the development of organ failure. Potential explanations for this sex difference include gender dissimilarities in behavior, social factors, chronic comorbidities, and the effect of sex hormones.

Sepsis occurs more frequently in blacks than in whites.[61] Moreover, reported sepsis-related ICU case-fatality rates are also higher in blacks than in whites.[61] These racial differences in sepsis are explained by both a higher infection rate and a higher risk of acute organ dysfunction in blacks relative to whites.[62] A study in the United States showed that mortality rates adjusted for patient characteristics were higher for patients in all minority groups compared with white patients.[63] In this cohort, racial disparities in sepsis mortality could be explained by differences in hospital characteristics for white, black, and Hispanic patients but not for Asian/Pacific Islanders.[63] The fact that racial differences are not entirely explained by differences in socioeconomic factors or access to care suggest that genetic variation plays a role.

Environmental factors and socioeconomic status also influence the incidence of sepsis. For example, poor socioeconomic status enhances the risk for bloodstream infection.[64] Data from the United States illustrate that communities with higher poverty rates as determined by ZIP codes have a higher incidence of sepsis.[61] In addition, infections causing sepsis show seasonal variation, with pneumonia occurring more frequently in the winter and genitourinary tract infections occurring more often in summer.[46,65] The outcome of sepsis also shows seasonal differences, with higher case-fatality rates in winter despite comparable disease severity.[65]

Chronic comorbidities are present in most patients with sepsis.[66] Certain diseases increase the risk for sepsis including chronic obstructive pulmonary disease, chronic renal or liver disease, diabetes, cancer, and HIV infection. Patients with cancer have a threefold to fourfold increased risk to be hospitalized for sepsis compared with the overall population.[67] This risk can even be 10 times higher in patients with certain hematologic malignancies. The risk of death in patients with cancer compared with patients without cancer who acquire sepsis is approximately 50% higher.[67,68] Similarly, the use of immunosuppressive therapies increases the likelihood of infection and thereby sepsis. Even so, there is evidence that chronic comorbidities have an impact on both infection risk and sepsis outcome. For example, patients with diabetes have an increased risk of developing infections and sepsis.[69] Most common among diabetic patients with sepsis are urinary tract and skin/soft tissue infections compared with nondiabetic patients.[66] The influence of diabetes on sepsis outcome is less clear, however. Some studies have shown an association with increased mortality, others found no effect, and still others found improved survival.[69]

TABLE 73.3 Risk Factors for Sepsis

Demographic Factors
Older age (>65 years old)
Male sex
Black race
Nutrition
Vaccination status
Genetic polymorphisms
Environmental Factors
Poor socioeconomic status
Seasonal variation and contacts
Disease outbreaks
Travel
Comorbidities
Diabetes
Chronic obstructive pulmonary disease
Cancer
Chronic renal disease
Chronic liver disease
Human immunodeficiency virus
Use of immunosuppressive agents
Hospital Factors
Duration of hospitalization
Antibiotic resistance
Catheters (e.g., urine catheters, intravenous lines)
Complications of surgery (wound infection, emergency vs. elective surgery)

MICROBIOLOGY

Virtually any microbe can trigger sepsis. Most cases today occur in patients with previous morbidities and are caused by opportunists from the patient's own microbiome.[4] It is important to determine the exact cause of sepsis, investigate microbial resistance both in a given patient and in the population, and have insights into how these microorganisms express their pathogenicity by means of their virulence.

Main Causative Agents

A causative microorganism can be identified in up to two-thirds of patients admitted to an ICU for sepsis.[5,10,70] Blood cultures are positive in approximately one-third of patients.[5] An epidemiologic study of sepsis showed that during the period 1979–2000, the incidence of sepsis caused by gram-positive microorganisms steadily increased and overtook gram-negative microorganisms as the predominant causative microorganisms.[57] However, a large survey done in 2007 that aimed to investigate the patterns of infection in ICUs and included more than 14,000 patients from 75 countries showed that gram-negative bacteria accounted for 62% of positive isolates in the ICU, gram-positive bacteria accounted for 47%, and fungi accounted for 19% (Table 73.4).[70] An aggregate of recent representative studies on the main groups of causative agents in patients with sepsis is shown in Fig. 73.1C. Viral infections account for 1% to 7% of cases.[10,57,70] In a significant number of patients, two or more microorganisms are identified. The most common isolated gram-positive

bacterial pathogens are *Staphylococcus aureus, Streptococcus pneumoniae,* and *Enterococcus* spp.; the most common gram-negative pathogens are *Escherichia coli, Klebsiella* spp., *Pseudomonas* spp., and *Acinetobacter* spp.[10,57,70] In patients with multiple comorbidities or sepsis acquired in the ICU, an increase in the incidence of antimicrobial-resistant staphylococci, *Acinetobacter* spp., *Pseudomonas* spp., and *Candida* spp. is seen.[10,70]

Significant differences in these patterns are seen around the globe. Compared with Western Europe and North America, the prevalence of gram-negative infections in Eastern Europe, Central and South America, and Asia is significantly higher, mainly caused by higher numbers of *Klebsiella* spp., *Pseudomonas* spp., and *Acinetobacter* spp. (see Table 73.4).[70] Regional differences can show an even more diverse picture. For example, in northeast Thailand, melioidosis is among the most common causes of community-acquired sepsis, as illustrated by the finding that its causative agent, *Burkholderia pseudomallei,* is the cause of 20% of community-acquired bloodstream infections.[71] A meta-analysis that included greater than 15,000 patients admitted to hospitals across Africa for a bloodstream infection showed that *Salmonella enterica* was found in 29% of isolates, making it the most prevalent nonmalaria bloodstream infection.[72] Most of these isolates were nontyphoidal *Salmonella* spp.[72]

The increase of fungal infections over the last 2 decades could not be prevented by the introduction of new antifungals.[57,73] This is worrisome, as fungal sepsis is associated with a high mortality. *Candida* spp. are the most prominent of all the fungi that can cause sepsis. Reported ICU mortality is more than 1.5 times higher in patients with *Candida* bloodstream infections compared with bacterial bloodstream infections.[74] Important risk factors for candidemia include immunosuppressed or neutropenic state, prior intense antibiotic therapy, indwelling vascular catheters, prolonged ICU stay, and colonization in multiple sites.

Virulence Factors of Bacteria Causing Sepsis

Invading microorganisms employ multiple mechanisms to escape antimicrobial defenses and overwhelm the host in the cascade of events that can lead to sepsis. First, they must adhere to and traverse the mucosal barrier, after which they must multiply and overcome antimicrobial host defense systems.[75] To do so, these pathogens have a formidable armory of virulence factors.

Bacteria can coordinate their gene expression according to the density of their local population in a process known as quorum sensing.[76,77] Through these signaling networks, individual bacteria communicate with each other by releasing and sensing small signal molecules. Activation of quorum sensing can help a bacterial population to remain inactive and not express virulence factors to avoid detection by the host or—at the other side of the spectrum—increase the expression of virulence factors and launch a coordinated overpowering attack on the host.[77] Quorum sensing activation can also lead to the production and maintenance of biofilms (slimy layers of bacteria that can protect themselves against the immune system and antibiotics), the production and release of proteins needed for tissue invasion, and the activation of toxins.

Pathogens can also injure the host by the expression of toxins that damage natural barriers such as the mucosa to enable further bacterial spread. Toxic shock syndrome is caused by the production of so-called superantigens during an invasive streptococcal infection or a localized staphylococcal infection. The bacterial production of these exotoxins cause nonspecific activation of T cells, resulting in the massive release of proinflammatory cytokines.[78] *S. aureus* secretes 24 different superantigens, and group A streptococcal strains are capable of producing 11 superantigens.[78] These bacterial toxins can be injected into the cytosol of host cells by specialized nanosyringes called type III secretion systems.

Lastly, microorganisms that cause sepsis express multiple pathogen-associated molecular patterns (PAMPs) that are able to inflict damage to host tissues through the generation of an excessive and damaging host inflammatory response.[73] Examples of bacterial PAMPs are lipopolysaccharide (LPS), peptidoglycan, lipopeptides (constituents of many pathogens), lipoteichoic acid (a cell wall component of gram-positive bacteria), flagellin (factor in the mobility of bacteria), and bacterial DNA. Systemic release of these PAMPs can activate innate immune cell

TABLE 73.4 Distribution in Percentages of Identified Organisms in Culture-Positive Infected Patients Included in the EPIC II Study According to Geographic Region

ORGANISM	WESTERN EUROPE	EASTERN EUROPE	CENTRAL/SOUTH AMERICA	NORTH AMERICA	OCEANIA	AFRICA	ASIA
Gram-Positive							
Staphylococcus aureus	20%	22%	19%	27%	28%	30%	16%
Staphylococcus epidermidis	11%	12%	9%	12%	8%	15%	9%
Streptococcus pneumoniae	5%	5%	3%	4%	3%	6%	2%
Enterococcus spp.	13%	15%	4%	10%	9%	0%	6%
Other	7%	4%	4%	11%	9%	7%	4%
Gram-Negative							
Escherichia coli	17%	15%	14%	14%	13%	11%	17%
Enterobacter	7%	8%	9%	8%	3%	7%	5%
Klebsiella spp.	10%	21%	16%	9%	12%	19%	21%
Pseudomonas spp.	17%	29%	26%	13%	15%	15%	30%
Acinetobacter spp.	6%	17%	14%	4%	4%	15%	19%
Other	18%	15%	17%	11%	21%	20%	15%
Anaerobes	5%	3%	1%	8%	3%	2%	3%
Fungi							
Candida	19%	19%	13%	19%	13%	11%	16%
Aspergillus	2%	1%	1%	3%	2%	0%	1%

Parasites accounted for 1% or fewer of all isolates in all regions. Percentages do not necessarily equal 100 because patients may have had more than one type of infection or microorganism.

Data from Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA. *2009;302:2323–2329.*

signaling, coagulation, and complement activation and eventually can contribute to the induction of septic shock.

Antimicrobial Resistance Trends in the Setting of Sepsis

The widespread emergence of antibiotic resistance genes among bacteria, viruses, fungi, and even protozoa has led to enormous challenges in the treatment of sepsis. Not only will empirical antibiotic treatment be less efficient, the same holds true for the chosen definitive antibiotic treatment. Equally worrisome is the finding that antibiotic-resistant bacteria can increase the total burden of sepsis.[79] For example, a Dutch study that analyzed trends in nosocomial bloodstream infections from 1998 to 2007 found an increase in nosocomial bloodstream infections due to antibiotic-resistant bacteria that occurred in addition to—instead of in replacement of—infections caused by antibiotic-susceptible bacteria.[79] As a result, the total disease burden increases.[79]

This clinical implication of the increasing antimicrobial resistance rates is best illustrated by the results from a recent surveillance study conducted in Malawi between 1998 and 2016 in which 29,183 pathogens derived from 194,539 blood cultures were analyzed.[80] In this 18-year time period, extended-spectrum β-lactamase resistance increased from 1% to 30% in *E. coli* and from 12% to 90% in *Klebsiella* spp. Resistance to ciprofloxacin rose from 3% to 31% in *E. coli* and from 2% to 70% in *Klebsiella* spp.[80] By contrast, more than 92% of common gram-positive pathogens remained susceptible to either penicillin or chloramphenicol. Methicillin-resistant *Staphylococcus aureus* (MRSA) represented 18% of *S. aureus* isolates (an increase from 8%). A similar study from India analyzed 18,695 bacterial pathogens from 35,268 blood cultures derived from a large private laboratory network in the period 2008–2014.[81] Carbapenem resistance increased in both *E. coli* (8%–12%) and *Klebsiella pneumoniae* (42%–57%). Carbapenem resistance was 70% for *Acinetobacter* spp. and 49% for *Pseudomonas aeruginosa*.[81] MRSA rates were relatively stable at 44%. These results highlight the growing challenge of bloodstream infections that are effectively impossible to treat in resource-limited settings.

Regional differences in the prevalence of antibiotic resistance are significant. In the Netherlands, for example, the proportion of extended-spectrum cephalosporin-resistant *E. coli* and *K. pneumoniae* isolates in blood samples collected from 2008 to 2012 increased from 4% to 7% and from 6% to 10%, respectively.[82] MRSA prevalence in blood culture isolates remains low (<1%).[82]

Increasing resistance has been associated with higher mortality rates. In ICU patients, MRSA infection was reported to be independently associated with an almost 50% higher likelihood of hospital death compared with methicillin-resistant *S. aureus* infection.[83] A retrospective cohort study from the United States also reported increased hospital stays and increased mortality when the initial antibiotic therapy was inappropriate in patients with gram-negative sepsis.[84] However, other studies were unable to demonstrate a link between antibiotic resistance and outcome. As an example, a study from Western Europe did not find an association between inadequate treatment and mortality of *S. aureus* bacteremia.[85] Moreover, a retrospective analysis of the EPIC II study demonstrated that being hospitalized in an ICU in a region with high levels of antimicrobial resistance (Greece, Israel, Italy, Malta, Portugal, Spain, and Turkey) was not associated with a worse outcome compared with countries with low resistance levels (Denmark, Finland, the Netherlands, Norway, and Sweden).[86]

PATHOGENESIS

Sepsis is the result of a dysregulated host response that at the very start of the infection was initiated to eliminate invading pathogens.[87] The innate immune system consists of a variety of cell types that are able to "sense" pathogens by virtue of their capacity to recognize PAMPs through a range of pattern-recognition receptors (PRRs).[88] Cells involved in the early response to pathogens include not only leukocytes but also parenchymal cells such as epithelial and endothelial cells and especially populate parts of the body from which they constantly can sample their surroundings. In most cases the first encounter between pathogens and host innate immune cells results in a balanced reaction that entails inflammatory, antiinflammatory, and repair responses, with elimination of microorganisms and return to normal homeostasis. In sepsis the pathogen has succeeded in evading protective immunity, while continuing to stimulate host cells, resulting in an unbalanced and harmful immune response and a failure to return to homeostasis. Knowledge of the course of immune dysregulation during infection is almost exclusively derived from animal studies, which are limited by lack of sufficient relevance for sepsis in humans. Most investigations on the host response in patients during sepsis have been done on admission to the hospital, thereby not affording information about the time course and character of the immune response before clinical recognition of severe disease and the diagnosis of sepsis.

Patients with sepsis show signs of both hyperinflammation and immunosuppression, two seemingly opposite reactions that involve partially different cell types and organ systems (Fig. 73.3). Likely, this disturbed immune response is not only the result of persistent stimulation by pathogens and their virulence factors but also the release of damage-associated molecular patterns (DAMPs), or alarmins, which are molecules derived from host cells released into the extracellular environment on injury. DAMPs can trigger many of the PRRs that also sense PAMPs, giving rise to a vicious cycle with sustained immune activation and dysfunction.[89] Patients who fail to recover after early therapeutic measures and continue to require intensive care often develop a chronic critical illness that has been named "persistent inflammation, immunosuppression, and catabolism syndrome."[90] The notion that the course of sepsis as seen by clinicians in the hospital nowadays is more chronic than acute is important for understanding the pathophysiologic mechanisms at play. In patients with sepsis with a prolonged clinical course, the disturbances in the host response involve different leukocyte subsets and parenchymal cells, encompassing key functions at both intercellular and intracellular levels, such as barrier function of epithelium and endothelium and cellular metabolism and mitochondrial dysfunction. respectively. In the next section, we describe the most important features of the septic host response.

Hyperinflammation

Instigation of Inflammation

PRRs are germline-encoded sensors that are especially expressed by innate immune cells such as macrophages, monocytes, neutrophils, dendritic cells, and epithelial cells. PRRs are grouped according to their localization, ligand specificity, and function. Prominent PRR families include Toll-like receptors (TLRs), C-type lectin receptors, retinoic acid–inducible gene-like receptors, and nucleotide-binding oligomerization domain-like receptors. Nucleotide-binding oligomerization domain-like receptors include inflammasomes, which are cytosolic multimolecular complexes that sense intracellular microbial danger signals and metabolic perturbations. Inflammasome activation results in activation of caspase-1 and the release of proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 as well as cell death. Recognition of pathogens by PRRs is an important defense mechanism against invading pathogens, inducing inflammatory gene transcription and initiation of innate immunity. In sepsis the inflammatory response is disproportional due to concurrent recognition of multiple PAMPs and DAMPs resulting in activation of multiple PRRs and subsequent triggering of downstream signaling cascades. Activation of nuclear factor kappa B is key for induction of the inflammatory response, resulting in the transcription of multiple early activation genes including those encoding cytokines. Cytokines are small proteins (5–20 kDa) that regulate immune responses locally and systemically. Proinflammatory cytokines implicated in sepsis pathogenesis include tumor necrosis factor (TNF), IL-1β, IL-12, and IL-18. These cytokines are released in the circulation in animals challenged with high doses of bacteria or their products, and in these models their inhibition or elimination protects against organ damage and mortality.[91]

Examples of DAMPs involved in proinflammatory responses during sepsis include high mobility group box 1 (HMGB1) and S100A8/9 (calprotectin). HMGB1 is a nuclear protein that is released passively during cell injury or secreted actively on inflammatory stimuli.[92] Depending on specific posttranslational redox modifications, HMGB1 can act as a cytokine via receptors such as Toll-like receptor 4 or as a chemotactic factor. HMGB1 levels are elevated during sepsis, and postponed treatment (24 hours after the challenge) of mice with

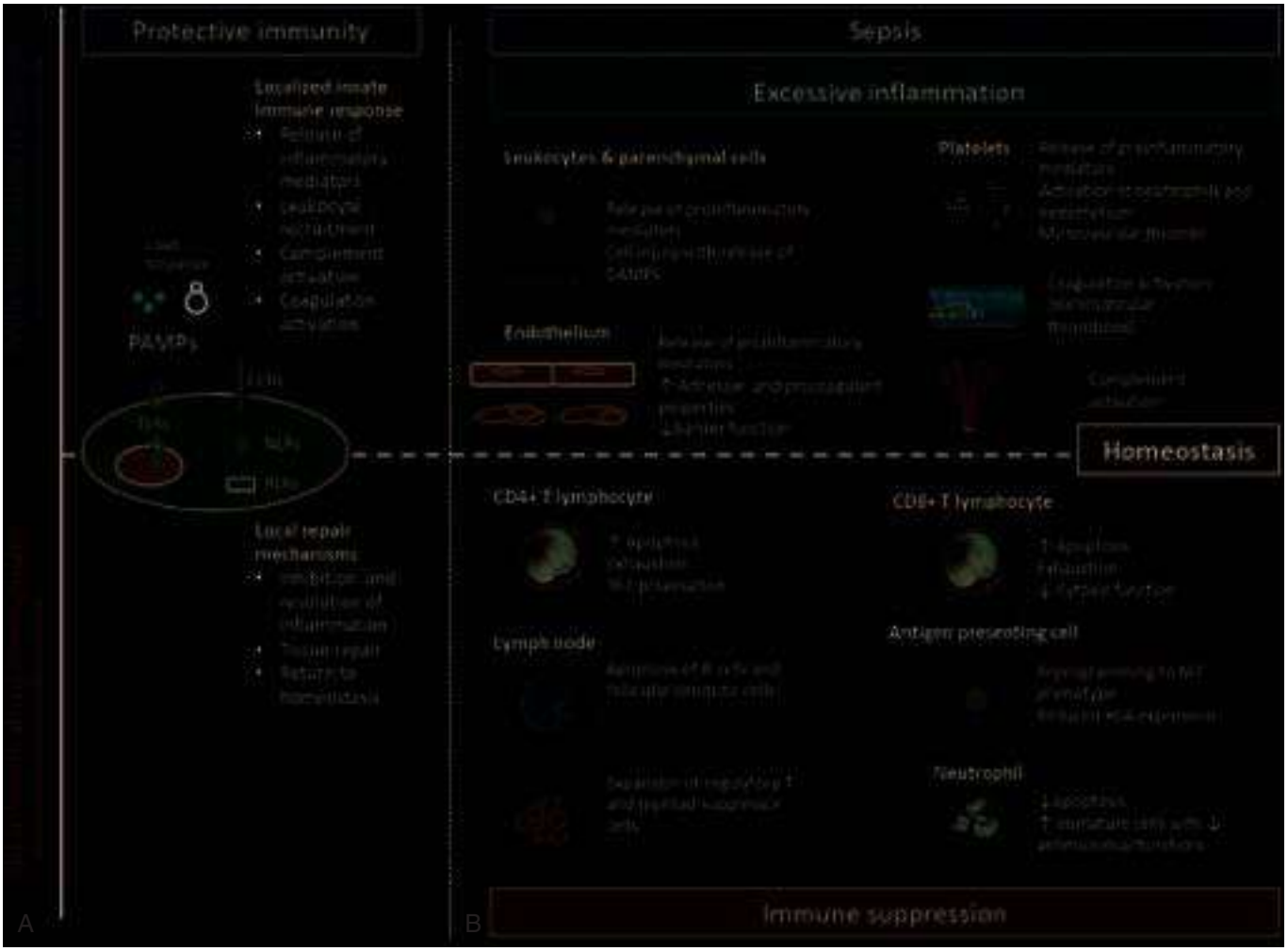

FIG. 73.3 Host response to infection and during sepsis. (A) During a protective immune response, innate immune cells recognize invading pathogens by sensing pathogen-associated molecular patterns *(PAMPs)* through a collection of cell surface and intracellular pattern recognition receptors including Toll-like receptors *(TLRs)*, nucleotide-binding oligomerization domain-like receptors *(NLRs)* (which include inflammasomes), retinoic acid–inducible gene-like receptors *(RLRs)*, and C-type lectin receptors *(CLRs)*. A balanced response entails a variety of proinflammatory reactions such as release of cytokines, influx of phagocytes, and local activation of the complement and coagulation systems, followed by a return to homeostasis by a set of compensatory mechanisms aimed at tempering the initial inflammation and tissue repair. (B) If the pathogen succeeds in multiplying, the immune response becomes unbalanced and harmful to the host. The host response during sepsis is characterized by concurrent hyperinflammation *(top)* and immunosuppression *(bottom)*. See text for description of hyperinflammatory and immunosuppressive responses in sepsis. *DAMPs,* Damage-associated molecular patterns.

antibodies to HMGB1 diminished lethality of experimental abdominal sepsis.[92] S100A8/9 is a heterodimeric protein especially expressed in neutrophils.[91] S100A8/9 can stimulate systemic inflammation through activation of Toll-like receptor 4. Patients with sepsis display elevated circulating levels of S100A8/9, and mice deficient for this protein are protected from endotoxin shock and *E. coli*–induced abdominal sepsis.

This harmful hyperinflammatory response, initiated by proinflammatory cytokines and referred to as the "cytokine storm," has long been held responsible for injury and death associated with sepsis, resulting in a large number of clinical trials with antibodies or inhibitors of TNF and IL-1β or other antiinflammatory strategies.[93,94] At the present time, it is appreciated that systemic challenge models in animals have limited relevance for human sepsis, although early mortality in sepsis, generally due to cardiovascular collapse and multiple organ dysfunction, likely is mainly driven by hyperinflammation. Besides cytokines, several other mediator systems have received attention with regard to their potential involvement in sepsis pathogenesis, most notably the complement system, the coagulation system, and the vascular endothelium.

Activation of the Complement System

The complement system comprises a group of small proteins mostly synthesized by the liver that typically circulate as inactive precursors. The complement system is triggered on exposure to PAMPs and DAMPs, mediated by an interaction with complement components C1q, mannose-binding lectin, and ficolins.[95] Although according to traditional concepts the complement system can be initiated by three distinct pathways (classical, lectin, and alternative), it should be noted that many intracellular proteases (e.g., elastase, neutral proteases) and extracellular proteases (e.g., thrombin, several activated clotting factors) can also generate C3a and C5a, exemplifying the complex nature of inflammation. On complement activation, small activation fragments known as anaphylatoxins are generated, particularly C3a and C5a, which exert strong proinflammatory effects on leukocytes, endothelial cells, and platelets.[95] The main activities of complement activation in protective immunity are opsonization of pathogens by cleavage products of C3 (C3b and iC3b) and C4 (C4b), attraction and activation of leukocytes by C3a and C5a, direct elimination of pathogens through phagocytosis via complement receptors or cell lysis mediated by the so-called membrane attack complex (C5b through C9), and regulation of adaptive immune responses by stimulation of B cells and T cells. Uncontrolled activation of complement can cause collateral damage to surrounding tissues and multiple organ failure at the systemic level. Inhibition of C5a improved the outcome in several animal sepsis models including *E. coli* sepsis in baboons and polymicrobial abdominal sepsis in rats.[96] The C3 convertase inhibitor compstatin

not only suppressed complement activation during *E. coli* sepsis in baboons but also attenuated other inflammatory responses, coagulation activation, and multiple organ failure.[97]

Activation of Coagulation and Vascular Endothelium

Hemostasis, the termination of bleeding, is a continuous process that under physiologic conditions favors an anticoagulant state. In the early stages of host-pathogen interaction, activation of coagulation elicits immune defense mechanisms including the release of antimicrobial peptides, recruitment and activation of phagocytizing cells, and induction of innate immune responses through activation of protease-activated receptors (PARs).[98] Accordingly, inhibitors of elements of the coagulation system were shown to impair microbial clearance and increase mortality during a variety of experimental infections.[98] The term *immunothrombosis* has recently been proposed to reflect the codependency of the coagulation and innate immune systems.[99]

Sepsis is associated with a strong activation of coagulation, which, together with an impairment of endogenous anticoagulant mechanisms, results in a net procoagulant state and a tendency toward microvascular thrombosis (Fig. 73.4).[100] The most severe manifestation of coagulopathy in sepsis is DIC, which clinically can be associated with both microvascular thrombosis and hemorrhage, owing to widespread fibrin depositions and consumption of clotting factors and platelets, respectively. Tissue factor drives coagulation activation after infection via the so-called extrinsic pathway, which involves activation of factor VII and subsequent formation of factor Xa, thrombin, and fibrin. Perivascular cells such as fibroblasts, pericytes, and epithelial cells express tissue factor constitutively, thereby safeguarding hemostasis and vessel integrity. In sepsis, injury of vessels exposes tissue factor to blood coagulation factors, inducing clotting. Cells in close contact with blood such as endothelial cells and macrophages normally do not express large quantities of tissue factor but can be triggered to do so by PAMPs and proinflammatory cytokines. Tissue factor also can be present in microparticles derived from leukocytes, endothelial cells, vascular smooth muscle cells, and platelets. The interaction between coagulation and inflammation is augmented by virtue of the capacity of tissue factor and clotting factors VIIa, Xa, thrombin, and fibrin to induce proinflammatory signaling. The G protein–coupled PAR family plays a major role herein. Inhibition of the tissue factor–factor VIIa pathway in humans and nonhuman primates strongly attenuated coagulation activation after infusion of endotoxin or bacteria, whereas in a model of otherwise lethal sepsis in baboons, tissue factor inhibition in addition prevented multiple organ failure and mortality.[100]

In addition to the tissue factor–mediated extrinsic route, coagulation can be activated via the so-called intrinsic pathway mediated by the contact system.[101] The contact system is initiated by factor XII, which can activate coagulation via factor XI and thrombin and stimulate inflammation via the kallikrein-kinin system. Inhibition of factor XII limits thrombosis in experimental settings without causing hemorrhage. This has raised interest in factor XII as a therapeutic target, although clinical evaluation in sepsis has not been reported.

The tendency toward thrombosis during sepsis is increased by concomitantly compromised activity of three main anticoagulant pathways: antithrombin, tissue factor pathway inhibitor (TFPI), and protein C system.[100] The anticoagulant properties of antithrombin (the main inhibitor of thrombin and factor Xa) and TFPI (the main inhibitor of the tissue factor–factor VIIa complex) are supported

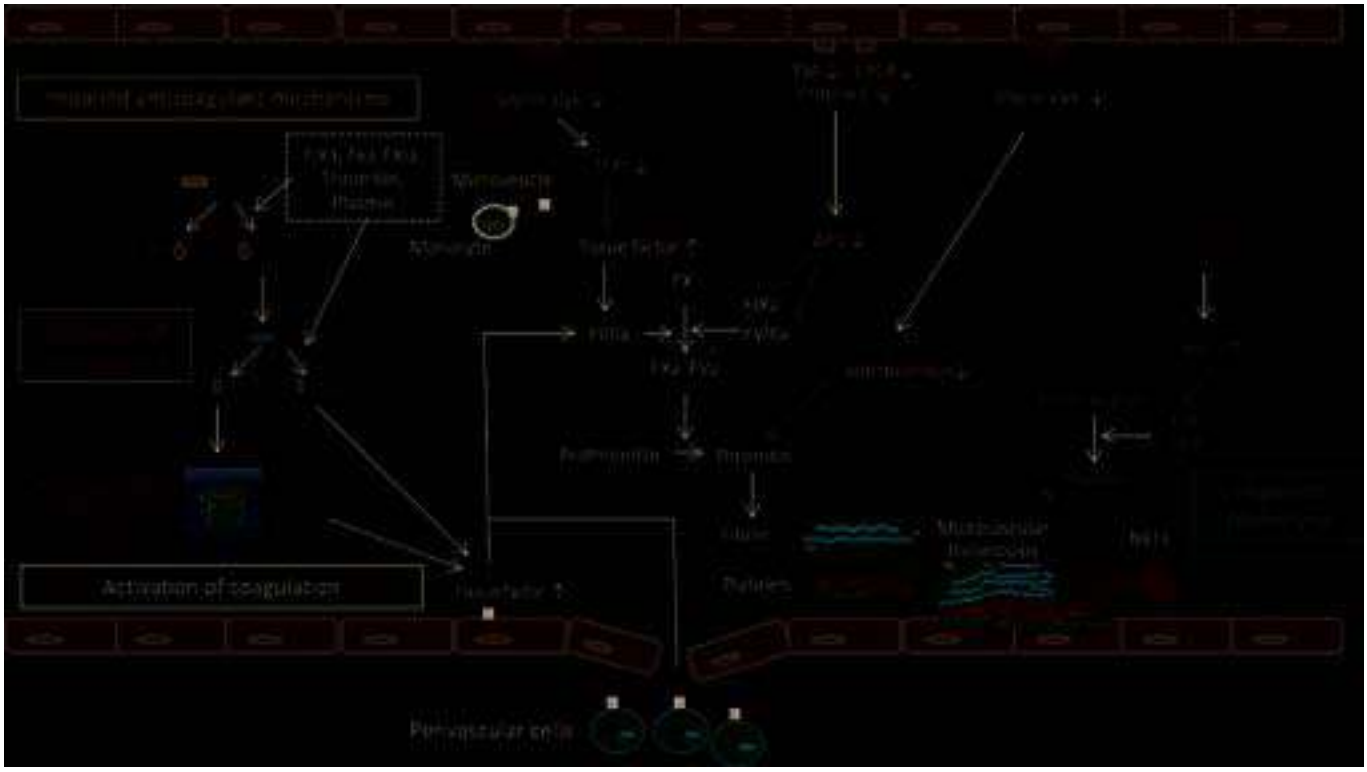

FIG. 73.4 Activation of the coagulation and complement systems during sepsis. Sepsis results in a procoagulant state via at least three mechanisms: tissue factor–mediated thrombin generation *(black font)*, dysfunctional endogenous anticoagulant mechanisms *(green font)*, and impaired fibrin removal due to suppression of the fibrinolytic system by plasminogen activator inhibitor 1 *(PAI-1) (red font)*. Besides in cell-associated form, tissue factor can reside in microparticles. The anticoagulant properties of antithrombin and tissue factor pathway inhibitor *(TFPI)* are supported by the glycocalyx, a glycoprotein-polysaccharide layer covering the endothelium, the continuity of which is disturbed in sepsis. Activated protein C *(APC)* is generated from protein C at the surface of resting endothelial cells, a process mediated by binding of thrombin to thrombomodulin *(TM)* and amplified by the endothelial protein C receptor *(EPCR)*. Multiple interactions exist between the coagulation and complement systems, and coagulation proteases can activate the complement system and vice versa. Vascular inflammation and coagulation are augmented by the release of neutrophil extracellular traps *(NETs)* by neutrophils. *tPA,* Tissue-type plasminogen activator; *uPA,* urokinase-type plasminogen activator.

by the glycocalyx, a glycoprotein-polysaccharide layer covering the endothelium. Sepsis is associated with a disruption of the continuity of the endothelial glycocalyx, which impairs the function of antithrombin and TFPI and increases vascular permeability. Activated protein C (APC) is generated from protein C at the surface of endothelial cells by a process that is accelerated by the endothelial protein C receptor (EPCR) and inhibits coagulation by inactivating the coagulation cofactors Va and VIIIa. Many investigations have supported the anticoagulant potency of the protein C system in vivo, showing that weakening its function converts nonlethal bacteremia into lethal sepsis with DIC.[102] APC also has antiinflammatory, antiapoptotic, and vasculoprotective properties,[102] and the anticoagulant effects of APC are not essential for protection against lethality in experimental sepsis as demonstrated in studies using nonanticoagulant APC mutants with selective cytoprotective properties.[103] Considering the impaired activity of antithrombin, TFPI, and the protein C system, there is a clear rationale to supplement these anticoagulant pathways in sepsis. However, clinical trials in patients with sepsis in whom antithrombin, recombinant TFPI, or recombinant APC was infused did not show a consistent benefit.[37,40,104,105]

Interaction Between Complement and Coagulation Systems

Evidence has accumulated that coagulation proteases can activate the complement system and vice versa (see Fig. 73.4).[106] Components of the coagulation and fibrinolytic systems can activate C3 and C5. Specifically, clotting factors IXa, Xa, and XIa as well as thrombin and the central fibrinolytic protease plasmin can convert C3 and C5 into C3a and C5a, respectively. Proteases of the coagulation cascade can also activate the complement system upstream of C3 and C5; for example, C1 can be activated by factor XIIa, resulting in instigation of the classical complement pathway. Ficolin and mannose-binding lectin can interact with fibrinogen/fibrin, which can modulate the activation of C3 and C4 and complement deposition on the surface of microorganisms. Chondroitin sulfate expressed at the surface of platelets can contribute to binding of C1q and regulators of the complement system C1 inhibitor, C4b-binding protein, and factor H. In a reciprocal way, complement products can activate the coagulation system.[106] C5a and the membrane attack complex can stimulate expression of tissue factor in endothelial cells. Complement products can also cause structural changes in the endothelium that can modify the clotting tendency of blood. For example, C5a can facilitate the release of the endothelial surface proteoglycan heparan sulfate, which can result in a procoagulant shift due to a reduced function of antithrombin.

Endothelial Dysfunction

In health the vascular endothelium affords a semipermeable barrier that regulates the movement of fluids, solutes, gases, macromolecules, and blood cells. Normal barrier function of the endothelium is preserved by the cell cytoskeleton, the glycocalyx, intercellular adhesion molecules, and other supportive proteins.[107] Connections between cells that comprise the vascular lining are upheld by adherens junctions such as vascular endothelial–cadherin and tight junctions (zona occludens), predominantly consisting of occludins and claudins.[108] On infection, leukocytes and platelets adhere to the vascular endothelium and migrate to locations of proliferating microorganisms. In sepsis, exaggerated inflammation enhances these processes, which can result in barrier incompetency. In fact, sepsis is almost invariably associated with a disruption of the integrity of the endothelial barrier, which can expose underlying collagen fibers and tissue factor to circulating blood, eliciting activation of platelets and the coagulation system. Moreover, sepsis is associated with breakdown of the glycocalyx, further damaging barrier function and in addition impeding anticoagulant mechanisms.[107]

The mechanisms underlying vascular leak have been the subject of many investigations. Activation of PARs has been implicated in alterations in vascular barrier function.[109] PARs can be activated by various serine proteases, a process that involves the cleavage of the N-terminal part of the receptor, which exposes a new previously cryptic sequence. The exposed sequence remains tethered to the receptor, acting as a receptor-activating ligand. Four PARs (PAR1 through PAR4) have been identified, which can either disrupt or protect endothelial barrier function, depending on which intracellular signaling pathway is activated. Each PAR can be activated by several proteases. Thrombin is the best-characterized PAR activator, capable of cleaving PAR1, PAR3, and PAR4; these receptors can also be activated by plasmin, trypsin, or cathepsin G. PAR1 signaling on endothelial cells is controlled by the activating protease and heterodimerization with PAR2 or PAR3. Activation of endothelial cell PAR1 by thrombin can contribute to endothelial dysfunction by inducing cytoskeletal derangements, inducing endothelial cell contraction and rounding and thereby destabilizing cell-to-cell contacts and increasing vascular permeability. Notably, thrombin linked to PAR1 at relatively low doses can protect barrier function by transactivating PAR2 into a PAR1-PAR2 heterodimer.[110] PAR1 can also be activated by APC, which results in antiinflammatory, antiapoptotic, and vasculoprotective signals in endothelial cells when APC remains attached to EPCR, and several studies have shown that the APC-EPCR-PAR1 pathway plays an important cytoprotective role in sepsis and endotoxemia.[102] APC potently inhibits thrombin-induced vascular hyperpermeability by a mechanism dependent on transactivation of the sphingosine 1 phosphate (S1P) receptor 1 (S1P1), whereas thrombin induces vascular hyperpermeability dependent on another S1P receptor, S1P3.[111] Activation of endothelial cell S1P1 preserves vascular integrity through cytoskeletal reorganization, adherens junction, and tight junction assembly. Another mechanism implicated in maintaining vascular integrity is angiopoietin 1–induced activation of the endothelial Tie2 receptor.[107] Angiopoietin 2 is a functional antagonist of angiopoietin 1 that can disrupt endothelial barrier function. Matrix metalloproteinase (MMP) 1 and MMP13 can activate PAR1 on cells through noncanonical sites.[109] MMP1 has been detected at high levels in the circulation of patients with sepsis. Cleavage of PAR1 by MMP1 results in barrier disruption, and MMP1-induced PAR1 signaling is associated with poor outcomes in experimental models of septic shock.[112]

Neutrophil Extracellular Traps

Vascular inflammation and coagulation are amplified by so-called neutrophil extracellular traps (NETs) released by neutrophils.[113] NETs are composed of histones, DNA, and neutrophil-derived proteases and can contribute to protective immunity by capturing and killing microorganisms. However, NETs have also been implicated in responses that are detrimental to the host, including collateral tissue damage and thrombosis. NETs can facilitate coagulation and thrombus formation by serving as a scaffold for platelets and proteins such as fibrinogen, fibronectin, and von Willebrand factor and by activating the intrinsic pathway of the coagulation system, thereby stabilizing blood clots. Accordingly, disassembling NETs reduced thrombosis in animal models. Bystander injury and thrombus formation caused by NETs are at least in part mediated by their histone components, which can exert cytotoxic effects, induce thrombin generation, and activate platelets.

Platelets

Platelets are small circulating anucleate cells that are of vital importance in hemostasis. More recently it has become clear that platelets also play an important role in inflammation and immunity (Fig. 73.5).[114,115] Sepsis is associated with activation of platelets through a variety of mechanisms.[114] Thrombin, generated as a consequence of coagulation activation, is an important platelet activator via PAR1, PAR3, and PAR4. In addition, von Willebrand factor can activate platelets by binding platelet glycoprotein Ibα. During normal hemostasis, von Willebrand factor stabilizes the adhesion of platelets at sites of vascular injury, a process regulated by ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif 13), which cleaves large von Willebrand factor multimers. Sepsis is accompanied by a relative deficiency of ADAMTS13, resulting in increased concentrations of ultra-large von Willebrand factor multimers, which facilitate platelet adhesion to injured endothelium. Additional platelet activation during sepsis can be induced by subendothelial collagen (via platelet glycoprotein VI), complement products (via the C1q receptor), bacteria, and DAMPs (e.g., histones).[114] On

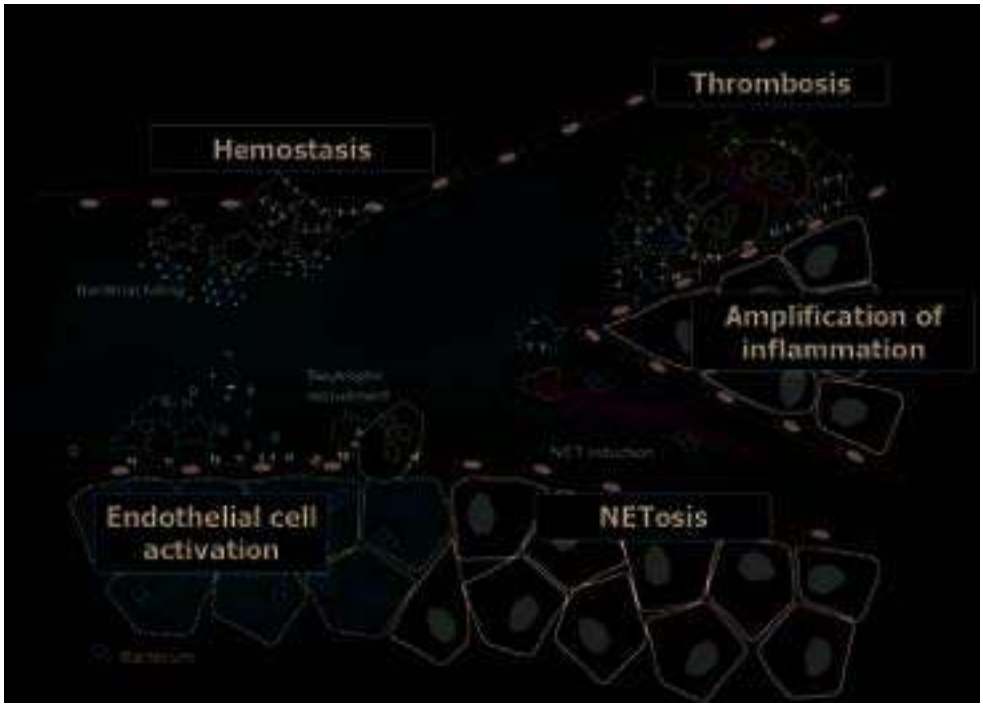

FIG. 73.5 Role of platelets in sepsis. Activated platelets release several regulatory proteins including cytokines, chemokines, coagulation mediators, and antimicrobial peptides. Platelets play an important role in hemostasis and maintenance of the endothelial barrier, and platelets bound to endothelium support adhesion of neutrophils following selectin-mediated tethering and rolling, thereby guiding neutrophils to transmigrate. Platelets can amplify inflammation by interacting with leukocytes and by facilitating the formation of neutrophil extracellular traps. Platelets play an additional role in immunothrombosis, in which platelets and products of the coagulation pathway regulate the effector function of innate immune cells and recruit additional cells. *NET,* Neutrophil extracellular trap. *(Illustration by Sacha de Stoppelaar.)*

activation, platelets release molecules such as thromboxane A_2 and adenosine diphosphate, which further augment platelet activation. Platelet activation triggers platelet-platelet aggregation, platelet-leukocyte complex formation, and the release of granular content, which includes coagulation factors, chemokines, adhesive proteins, mitogenic factors, and regulators of angiogenesis.

Low platelet counts are independently associated with mortality in patients with sepsis,[116] and thrombocytopenic mice demonstrated an impaired host defense during pneumonia-derived sepsis.[117] Platelets likely play a protective role in the initial stages of an infection by virtue of their capacity to release bactericidal products, to activate leukocytes, and to induce the formation of NETs. However, during sepsis, excessive platelet activation contributes to the development of complications such as DIC, acute lung injury, and AKI. Platelets have been implicated in the pathogenesis of organ failure in sepsis by contributing to leukocyte recruitment and hyperinflammation, by driving the development of vasoocclusive thrombi in the microvasculature, and by direct cell toxic effects of platelets and platelet-derived microparticles.[114] Several antiplatelet agents improved survival in sepsis models, and prior use of the platelet inhibitor acetylsalicylic acid was associated with reduced mortality of sepsis in a recent meta-analysis.[118] The potential therapeutic effect of platelet inhibitors in human sepsis has not been investigated in randomized trials.

Antiinflammatory Mechanisms and Immunosuppression

A balanced immune response to an infection entails early induction of antiinflammatory reactions meant to limit excessive and potential harmful activity of inflammation as well as resolution of inflammation and instigation of tissue repair. In sepsis, sustained antiinflammatory reactions can result in immunosuppression involving different cell types and caused by a variety of pathologic mechanisms. Patients admitted to the ICU with sepsis show signs of concurrent hyperinflammation and immunosuppression; a clear separation in time between SIRS and so-called compensatory antiinflammatory response syndrome, a concept for a two-staged host response to sepsis proposed in the late 1990s,[119] does not seem to exist. Rather, as discussed earlier, patients with sepsis who continue to require intensive care experience a condition referred to as persistent inflammation, immunosuppression, and catabolism syndrome.[87,90] Although the inflammatory response during early sepsis likely is mainly driven by PAMPs and DAMPs, the etiology of persistent inflammation is relatively unknown. Sustained release of DAMPs probably plays a role.

Clinically the consequences of sustained immunosuppression may be the occurrence of secondary infections often caused by weakly virulent organisms such as *Stenotrophomonas, Acinetobacter, Enterococcus,* and *Candida* and by reactivation of latent viruses such as cytomegalovirus and herpes simplex virus.[120] We discuss the main features of immunosuppression and their underlying mechanisms in sepsis further on.

Suppression of Innate Immune Cell Functions

Several cell types involved in innate immunity show strong phenotypic and functional changes in patients with sepsis.[87,120] Although neutrophils clearly contribute to sepsis-induced inflammation, at least in part through the release of NETs, they also demonstrate dysfunctional features that impair their antimicrobial capacities. Key findings in sepsis are delayed apoptosis of neutrophils, defects in neutrophil chemotaxis and recruitment to sites of infection, and an impaired capacity of neutrophils to produce essential effector molecules such as reactive oxygen species and cytokines. In addition, antigen-presenting cells such as monocytes, macrophages, and dendritic cells display a reduced expression of HLA-DR and a diminished capacity to release proinflammatory cytokines on stimulation. Although this latter phenomenon has been referred to as "immunoparalysis" or "endotoxin tolerance," these terms are not correct, as these cells seem reprogrammed rather than generally suppressed. Indeed, certain functions remain intact or even are enhanced, such as the release of antiinflammatory mediators (e.g., IL-10). Alterations in the function of antigen-presenting cells during sepsis are at least in part caused by epigenetic changes. Regulation of gene transcription occurs

through organization of gene loci on chromatin into transcriptionally active or silent states.[121] Histones "wrap" DNA in a chromatin structure, and specific histone modifications can wind or unwind chromatin to make it inaccessible (heterochromatin) or accessible (euchromatin) for transcription, respectively. Histone acetylation of lysines typically promotes transcription, whereas methylation can support either active euchromatin or silent heterochromatin formation, depending on the lysine that is methylated.[121] Immune cell function can also be regulated at the posttranscriptional level by noncoding RNAs including microRNAs that can reduce protein expression through targeted degradation of specific messenger RNAs.

The term *trained immunity* has been introduced to reflect the fact that the function of innate immune cells can be influenced by previous encounters with pathogens or their products.[122] Previous exposure to microbial products can render macrophages less responsive to a second stimulation (endotoxin tolerance) but can also prime them for stronger second responses. Both phenotypes are mediated by specific alterations in cellular metabolism and epigenetic reprogramming. Manipulation of trained immunity may reverse some of the effects of immunosuppression in sepsis, as suggested by a more recent study reporting that induction of trained immunity by β-glucan can restore the epigenetic, transcriptional, and functional program of monocytes during endotoxin-induced tolerance.[123] Alterations in cellular metabolism have been linked to reprogramming of antigen-presenting cells in sepsis.[124] A shift from oxidative phosphorylation to glycolysis (the so-called Warburg effect) leads to succinate accumulation, which stabilizes hypoxia-inducible factor 1α to increase IL-1β transcription. Whereas LPS induces a classic Warburg effect, whole microorganisms can trigger an increase in both glycolysis and oxidative phosphorylation in monocytes.[125] Likewise, the defects of monocyte metabolism in patients with sepsis with immunosuppression not only involve glycolysis but also entail a broad inhibition of metabolic processes including glycolysis, fatty acid oxidation, and oxidative phosphorylation.[126] Hence, although a disbalance in cellular metabolism is involved in immunosuppression in sepsis, the mechanisms seem more complex than a simple shift between oxidative phosphorylation and glycolysis.

Inhibition of inflammation can occur through the so-called neuroinflammatory reflex, which involves peripheral sensory input transmitted through the afferent vagus nerve to the brainstem, with subsequent stimulation of the efferent vagus nerve and activation of the splenic nerve in the celiac plexus. This results in norepinephrine release in the spleen and acetylcholine secretion by a subset of $CD4^+$ T cells.[127] Acetylcholine limits proinflammatory cytokine release by macrophages. In experimental endotoxemia, vagotomy increases proinflammatory cytokine release and lethality, whereas stimulation of the efferent vagus nerve attenuates systemic inflammation.[127] Whether the neuroinflammatory reflex contributes to immunosuppression in sepsis remains to be established.

Suppression of Adaptive Immune Cell Functions

Sepsis is associated with a profound reduction in the number of $CD4^+$ and $CD8^+$ T cells, B cells, and dendritic cells caused by apoptosis (programmed cell death).[120,128] Postmortem studies of patients who died of sepsis in addition demonstrated signs of T-cell exhaustion; T cells obtained from the spleen shortly after death produced lower amounts of interferon-γ (IFN-γ) and TNF than splenic T cells harvested from patients who had died of a noninfectious cause.[128] Inhibition of lymphocyte apoptosis improved the outcome of sepsis in several experimental models, suggesting a causal role for the loss of lymphocytes in sepsis lethality. Checkpoint molecules may be an appealing target for therapeutic intervention in patients with immunosuppression. Checkpoint molecules are normally expressed at low levels on various cell types and can be upregulated in a variety of circumstances. Examples important for sepsis include programmed cell death 1 (PD-1) and its ligand (PD-L1), which are found mainly on surfaces of T cells and antigen-presenting cells, respectively. An interaction between PD-1 and PD-L1 results in broad immunosuppressive effects. $CD4^+$ T cells of patients with sepsis displayed enhanced expression of PD-1, whereas macrophages and endothelial cells displayed enhanced expression of PD-L1, which may impair T-cell function at the local tissue level.[128] Inhibition of the PD-1–PD-L1 pathway improved survival in experimental sepsis, pointing at an unfavorable causal role for T-cell exhaustion and identifying the PD-1–PD-L1 axis as a potential therapeutic target in sepsis.[129] Another mechanism that may contribute to reduced T-cell function is an increase in the fraction of regulatory T cells (Tregs) and expansion of myeloid-derived suppressor cells, which are immature myeloid cells that can impede immune responses, particularly T-cell functions. Tregs can also inhibit monocyte and neutrophil functions, and blocking of Tregs improved immune function and increased microbial killing in experimental sepsis.[120]

DIAGNOSIS

There is no gold standard for diagnosing sepsis. A clinical pattern based on the patient's history and physical examination combined with laboratory, radiology, and microbiology testing is typically required for the diagnosis. Once the diagnosis of sepsis is made, it is critical to determine the cause of the underlying infection and the extent of organ dysfunction. We present a set of tools consisting of some basic laboratory, microbiology, and imaging examinations that are important to help establish the diagnosis, detect any level of organ failure, and identify the causative agent. In addition, host response biomarkers can be of diagnostic value (to discriminate infection from noninfectious conditions or to determine the causative pathogen), of prognostic value (assigning risk profiles and predicting outcome), and of potential theranostic value (aid in selection and monitoring of therapy).[130]

Hematologic and Biochemical Evaluation

All patients suspected to have sepsis should have blood drawn for a complete blood count, metabolic panel, renal and liver function, and coagulation parameters. Inflammatory variables classically used as helpful criteria for the diagnosis of sepsis include leukocytosis (white blood cell count, >12,000/mm^3), leukopenia (white blood cell count, <4000/mm^3), normal white blood cell count with >10% immature forms, an elevated plasma C-reactive protein (>2 SD above the upper limit of the normal range), and elevated plasma procalcitonin (>2 SD above the upper limit of the normal range).[5]

High procalcitonin levels are associated with bacterial infection and sepsis.[130] However, the usefulness of procalcitonin in diagnosing sepsis is limited; a meta-analysis reported a sensitivity of 0.77 and specificity of 0.79 of procalcitonin to differentiate between sepsis and noninfectious critical illness,[131] which is not good enough for the decision to start or withhold antibiotics in patients admitted to the ICU with suspected sepsis. Procalcitonin may, however, help to guide the decision on when to stop antibiotic treatment in critically ill patients with infections. Procalcitonin guidance to reduce the duration of antibiotic treatment has been shown to be feasible and safe in patients with an established diagnosis of sepsis.[132,133]

First signs of AKI are an increase in creatinine (>0.5 mg/dL or 44.2 μmol/L) with or without acute oliguria (urine output <0.5 mL/kg/h for at least 2 hours despite adequate fluid resuscitation).[5] Marked hyperbilirubinemia (plasma total bilirubin >4 mg/dL or 70 μmol/L) can indicate liver dysfunction. Renal and liver tests should be monitored during the course of sepsis.

Thrombocytopenia (platelet count <100,000 μL^{-1}) is seen in up to 50% of patients with sepsis.[116] Low platelet counts can suggest DIC, which is also characterized by coagulation abnormalities (international normalized ratio >1.5 or activated partial thromboplastin time >60 seconds), low fibrinogen levels, and elevated markers of fibrinolysis (fibrin degradation products or D-dimer levels).[36] Coagulation disorders in sepsis are discussed in more detail in "Pathogenesis."

Lactate levels can be elevated (>1 mmol/L or >9 mg/dL, or twice these values in septic shock) and signal poor tissue perfusion. High levels are associated with poor outcome.[134] Arterial blood gas analysis often shows a metabolic acidosis with compensatory respiratory alkalosis; acidosis and hypoxia are markers of severe disease. Other laboratory features often seen in sepsis include hyperglycemia (plasma glucose >140 mg/dL or 7.7 mmol/L) in the absence of diabetes and low albumin levels due to capillary leakage, altered hepatic metabolism, or poor nutrition.

Pathogen Detection

Routine microbiologic cultures (blood, urine, sputum, wound, cerebrospinal fluid, joint fluid) should be obtained before starting antimicrobial therapy in patients with suspected sepsis or septic shock.[25] Two sets of blood cultures (aerobic and anaerobic) should always be included. In patients with an intravascular catheter (in place >48 hours), at least one blood culture set should be obtained from the catheter (along with simultaneous peripheral blood cultures) to assist in the diagnosis of a potential catheter-related bloodstream infection.[25]

The culprit pathogen can be identified only in approximately 60% to 65% of patients with sepsis.[5] Often the reason for this will be the start of antibiotic treatment before cultures were obtained. In addition, culture-based pathogen identification is relatively slow, and sensitivity can be low. To overcome these problems, rapid culture-independent diagnostic methods including matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), polymerase chain reaction (PCR), and microarrays have been introduced.[135] These methods can be very helpful for quicker and more accurate identification of microorganisms. MALDI-TOF MS has received much attention, permitting fast and correct identification of bacteria and fungi based on the generation of unique mass spectral protein fingerprint signatures. A recent investigation has suggested that use of MALDI-TOF MS may reduce the time to appropriate antibiotic therapy, the length of ICU stay, and the mortality of patients with bacteremia.[136] Early detection of antimicrobial resistance is essential to reduce the risk of inappropriate therapy. MALDI-TOF MS coupled with a PCR-based rapid diagnosis of MRSA diminished unnecessary coverage of MRSA.[137] A study in six European countries in critically ill patients with bloodstream infections or pneumonia showed the enhanced sensitivity to identify a pathogen when using the combination of PCR and electrospray ionization MS compared with standard culture techniques.[138] Using PCR/electrospray ionization MS also demonstrated the ability to rule out infection with a negative predictive value of 97% at 6 hours from sample acquisition.[138] Several studies have evaluated the use of rapid point-of-care testing for respiratory viruses showing its benefit in better pathogen detection and improved use of antimicrobials.[139,140] As these techniques are emerging, their clinical value and cost-effectiveness have to be assessed as well as their potential to determine antimicrobial resistance for which isolation and direct testing of viable pathogens remain key. Nevertheless, these molecular assays are expected to eventually replace present-day conventional microbiologic techniques for the detection of infections causing sepsis.

Regarding invasive fungal infections, blood cultures are positive in a minority of cases and often late in the course of infection. Non–culture-based laboratory techniques may contribute to early diagnosis. The use of galactomannan and β-D-glucan to assist in the assessment of invasive *Aspergillus* (and a broad range of fungal pathogens) has become well accepted in patients with high-risk hematologic conditions.[141] Regarding invasive candidiasis, the value of antigen-based tests that target the *Candida*-specific cell wall component mannan or the nonspecific fungal element β-D-glucan is still poorly defined in an ICU population.[142] The same applies for the use of *Candida*-specific PCR in blood and serum.

Diagnostic Imaging

Imaging studies should be performed promptly to confirm a potential source of infection.[25] In addition to chest radiography for the evaluation of pneumonia, standard computed tomography can be helpful to assess infection in the sinuses, lungs, liver, and abdomen. Ultrasonography may be useful for evaluating gallbladder and kidney dysfunction.

Host Response Biomarkers

Biomarkers are molecules, genes, or other factors that can assist in the identification of physiologic or pathologic processes.[130] The ideal biomarker provides specific and sensitive information about a process or disease state and can be measured rapidly by a point-of-care test with low production costs. To be clinically useful, a biomarker needs to assist the physician in therapeutic decision making. Biomarkers that have been evaluated in patients with sepsis can be arbitrarily divided into diagnostic and prognostic markers (Fig. 73.6). Diagnostic biomarkers can aid in distinction between sepsis and noninfectious critical illness or between causative organisms; such biomarkers could be helpful in antibiotic stewardship and reduction of inadequate use of antibiotics. Prognostic biomarkers can help to predict adverse outcomes, including specific complications. In recent years, efforts have been made to use biomarker profiles to stratify patients with sepsis in different subgroups or endotypes based on particular pathophysiologic features; this approach likely is key for personalized medicine with implementation of individualized treatment based on the patient's unique characteristics.[87] The term *theranostics* has been introduced to indicate biomarker-guided selection of patients for a specific therapy followed by biomarker-guided assessment of treatment response.

Despite the fact that numerous host response biomarkers have been evaluated in sepsis, none are routinely used in sepsis management,[143] and the Surviving Sepsis Campaign guidelines list procalcitonin only as potentially useful in guiding antibiotic treatment in critically ill patients.[25] Other well-studied protein sepsis biomarkers include C-reactive protein, LPS binding protein, IL-6, soluble triggering receptor expressed on myeloid cells 1, and soluble urokinase-type plasminogen activator receptor, all with a lower sensitivity and specificity relative to procalcitonin.[130] To date, the most robust biomarkers of sepsis-induced immunosuppression have been measured by flow cytometry.[144] Of these, decreased expression of monocyte HLA-DR is considered a good surrogate marker of monocyte anergy. Reduced monocyte HLA-DR expression is an independent predictor of mortality in patients with sepsis and has been used as an inclusion criterion for immunostimulatory trials. Overexpression of PD-1 on lymphocytes and overexpression of its ligand PD-L1 on monocytes have also been used as markers of immunosuppression and risk prediction.[144]

In more recent years the search for sepsis biomarkers has moved from individual proteins to sets of (potential) markers using systems-based approaches.[87,130] The "omics" field includes genomics, epigenetics, transcriptomics, proteomics, and metabolomics. Increasingly, "omics"-based methodologies are used to study host-pathogen interactions and biomarkers in sepsis.[87,145] Especially transcriptomics has been used for the discovery of biomarkers that can discriminate between sepsis and noninfectious causes of critical illness,[146–148] and one such test has been registered in the United States with this indication.[146] Host gene expression signatures may also help in identifying causative pathogens in patients with infections; for example, in adults presenting to the emergency department with suspected community-acquired acute respiratory tract infections, different RNA signatures were revealed in patients with diseases of bacterial, viral, or noninfectious origin.[149] Blood leukocyte gene expression profiles that can assist in the discrimination between bacterial and viral infection have also been reported in other clinical

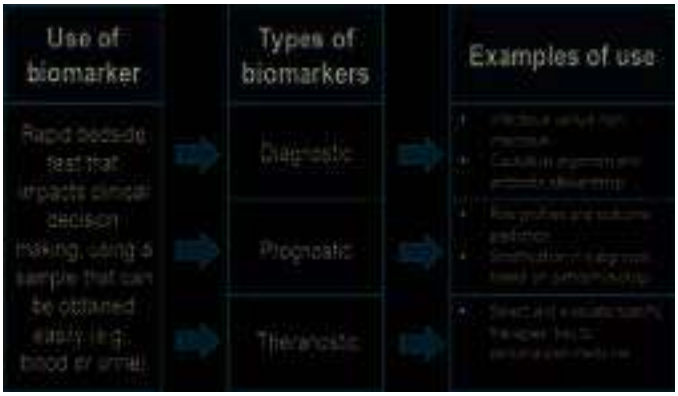

FIG. 73.6 Sepsis biomarkers. Biomarkers can be used to differentiate sepsis from noninfectious critical illness or to determine causative pathogens, thereby contributing to antibiotic stewardship. Furthermore, biomarkers can assist in stratifying patients based on risk profiles, predicting outcome, or identifying pathophysiologic pathways that can be the target for personalized therapy. Theranostics entails biomarker tests that select and monitor specific therapies.

settings including in the ICU.[150] In addition, transcriptomic signatures have been used to stratify patients with sepsis into endotypes with pathophysiologic and prognostic significance.[151,152] These signatures could be the key to targeted therapy and theranostics in the future. RNA biomarkers can be incorporated in PCR-based bedside tests with limited or no hands-on time, which—if proven valuable—make them attractive for implementation in clinical practice. Proteomics, metabolomics, and lipidomics represent other new areas of biomarker research.[153]

THERAPY

Sepsis is a medical emergency. Early clinical suspicion, rigorous diagnostic measures, aggressive initiation of appropriate antimicrobial therapy, comprehensive supportive care, and measures aimed at reversing predisposing causes are the cornerstones of successful management.

Surviving Sepsis Campaign Guidelines

The Surviving Sepsis Campaign consists of a group of international critical care and infectious disease experts that aims to improve the outcome of sepsis and septic shock. The third revision of the Surviving Sepsis Campaign guidelines was published in 2017.[25] Of note, this last revision was conducted before publication of the Sepsis-3 definitions[3] and does not incorporate them. Adherence to the Surviving Sepsis Campaign guidelines has been associated with reduced hospital mortality rates.[154,155,156] Table 73.5 summarizes key recommendations for the initial management of sepsis, which are aimed to provide rapid cardiorespiratory resuscitation and diminish the immediate danger of the underlying infection.

Cardiorespiratory resuscitation involves intravenous fluids, vasopressors, and mechanical ventilation where needed. Compared with the 2012 version of the guidelines,[157] the current version deemphasizes protocolization of care and invasive monitoring, instead suggesting that patients be reevaluated frequently. An earlier landmark study from 2001 showed that early goal-directed therapy (EGDT), a well-defined intervention to guide hemodynamic resuscitation according to targets for central venous pressure, mean arterial blood pressure, urinary output, and central venous oxygen saturation, decreased mortality of patients with severe sepsis and septic shock.[158] However, follow-up multicenter, randomized controlled trials from the United States (ProCESS),[159] Australia (ARISE),[160] and the United Kingdom (ProMISe)[161] could not demonstrate a survival benefit of EGDT compared with usual resuscitation in patients with septic shock. Also, measurements of central venous pressure and central venous oxygen saturation were not beneficial in patients with lactate values greater than 4 mmol/L.[159,160,161] Finally, a patient-level meta-analysis of the ProCESS, ARISE, and ProMISe trials not only confirmed that EGDT did not result in better outcomes than usual care but also showed that this strategy was associated with higher hospitalization costs.[162]

The initial management of infection involves identifying the most likely source of infection, obtaining cultures (whenever possible before start of antibiotic therapy), administration of empirical antimicrobial therapy, and source control (*ubi pus, ibi evacua* ["where there is pus, there evacuate it"]) where appropriate. The importance of source control cannot be overstated.[163] Common occult sources are found in the lungs, urinary tract, or abdomen (e.g., diverticulitis, cholecystitis). Signs of septic arthritis, endocarditis, and osteomyelitis should be looked for. Intravascular access devices that are a possible source of sepsis should be promptly removed after other vascular access has been established. The same holds true for urinary catheters. The Surviving Sepsis Campaign recommendations on antimicrobial therapy and source control are listed in Table 73.6.[25] It should be noted that the formulation of these recommendations has stirred an ongoing debate.[164]

Empirical Antibiotic Therapy

The Surviving Sepsis Campaign guidelines recommend that intravenous antibiotics should be started as soon as possible after recognition and within 1 hour for both sepsis and septic shock.[25] If the patient is infected and is severely ill or has shock, there is little margin for error. However, in patients with less severe disease, this recommendation has been criticized, as uncertainty exists if a 1-hour delay in the administration of antibiotics will negatively impact outcome in all patients with sepsis.[165] A recent study from the Netherlands could not demonstrate an effect on survival in patients with sepsis if prehospital antibiotics were already administered in the ambulance.[166] In addition, one may fear that using a fixed time period may lead to antibiotic overuse.[165] In patients who are not critically ill there is often more time to generate additional diagnostic information and—in some cases—prevent the administration of broad-spectrum antibiotics to patients who do not have infections but present with a sepsis-like syndrome. Taken together, the benefits of treating patients with infections need to be balanced against the harms of treating patients who at first appear as if they may have infections but in fact do not.[164] Other guidelines have proposed that a 3-hour target for the initiation of empirical antibiotic therapy for sepsis is reasonable.[164] One should keep in mind that the recognition of sepsis can be difficult; broad-spectrum antibiotics given to patients with syndromes that look like infections who do not actually have infections should be stopped as soon as possible.

Inadequate empirical antibiotic therapy in patients with sepsis is associated with increased mortality,[167] indicating that antibiotics covering all probable pathogens should be given as early as possible. The choice of empirical antibiotic treatment depends on the suspected source of

TABLE 73.5 Key Recommendations of the Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016

- Sepsis and septic shock are medical emergencies, and treatment and resuscitation should begin immediately.
- Routine microbiologic cultures are obtained before starting antimicrobial therapy in patients with suspected sepsis. These always include at least two sets of blood cultures (aerobic and anaerobic).
- Patients with hypoperfusion should receive at least 30 mL/kg of IV crystalloid within 3 hours and should be reassessed frequently.
- For patients who require vasopressors, initial target mean arterial pressure should be 65 mm Hg.
- Norepinephrine is the first choice for patients who need vasopressors. Vasopressin or epinephrine can be added. For patients who remain unstable, dobutamine is recommended.
- IV hydrocortisone (200 mg/day) is suggested for patients who are hemodynamically unstable despite fluids and vasopressors.
- Blood transfusion should be reserved for patients with hemoglobin concentration <7.0 g/dL except in special circumstances such as hemorrhage and myocardial ischemia. Platelets should be given if the platelet count is <10,000/mm^3 or <20,000/mm^3 with bleeding.
- Mechanical ventilation with a target tidal volume of 6 mL/kg predicted body weight is recommended compared with 12 mL/kg in adult patients with sepsis-induced ARDS. An upper limit goal for plateau pressures of 30 cm H_2O over higher plateau pressures is recommended in patients with sepsis-induced severe ARDS. The use of higher PEEP over lower PEEP is recommended in patients with sepsis-induced moderate-to-severe ARDS.
- A protocolized approach to blood glucose management in ICU patients with sepsis is recommended, initiating insulin dosing when two consecutive blood glucose levels are >180 mg/dL. This approach should target an upper blood glucose level ≤180 mg/dL.
- Either continuous or intermittent renal replacement therapy can be used in patients with sepsis and acute kidney injury.
- Sodium bicarbonate should not be used for most patients with pH ≥7.15.
- Pharmacologic prophylaxis against venous thromboembolism with unfractionated heparin or LMWH should be given in the absence of contraindications for these agents.
- Stress ulcer prophylaxis should be given to patients with sepsis or septic shock who have risk factors for gastrointestinal bleeding.
- Administration of early parenteral nutrition alone or parenteral nutrition in combination with enteral feedings is not recommended in critically ill patients who can be fed enterally. The administration of parenteral nutrition is not recommended over the first 7 days in critically ill patients with sepsis or septic shock for whom early enteral feeding is not feasible.
- Goals of care and prognosis should be set and discussed with patients and families as early as feasible.

See Table 73.6 for specific recommendations on antimicrobial therapy and source control.

ARDS, Acute respiratory distress syndrome; ICU, intensive care unit; IV, intravenous; LMWH, low-molecular-weight heparin; PEEP, positive end-expiratory pressure.

Data from Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Crit Care Med. *2017;45:486–552.*

TABLE 73.6 Surviving Sepsis Campaign Recommendations on Antimicrobial Therapy and Source Control

	RECOMMENDATION	GRADE
	Antimicrobial Therapy	
1	Intravenous antimicrobials should be initiated as soon as possible after recognition and within 1 hour for both sepsis and septic shock.	Strong recommendation, moderate quality of evidence
2	Empirical broad-spectrum therapy is recommended with one or more antimicrobials for patients presenting with sepsis or septic shock to cover all likely pathogens (including bacterial and potentially fungal or viral coverage).	Strong recommendation, moderate quality of evidence
3	Antimicrobial therapy should be narrowed once pathogen identification and sensitivities are established or adequate clinical improvement is noted.	Best practice statement
4	Sustained systemic antimicrobial prophylaxis is not recommended in patients with severe inflammatory states of noninfectious origin (e.g., severe pancreatitis, burn injury).	Best practice statement
5	Dosing strategies of antimicrobials should be optimized based on accepted pharmacokinetic/pharmacodynamic principles and specific drug properties in patients with sepsis or septic shock.	Best practice statement
6	It is suggested to use empirical combination therapy (using at least two antibiotics of different antimicrobial classes) aimed at the most likely bacterial pathogens for initial management of septic shock.	Weak recommendation, low quality of evidence
7	It is suggested that combination therapy is not routinely used for ongoing treatment of most other serious infections including bacteremia and sepsis without shock.[a]	Weak recommendation, low quality of evidence
8	The use of combination therapy for routine treatment of neutropenic sepsis/bacteremia is not recommended.[a]	Strong recommendation, moderate quality of evidence
9	If combination therapy is used for septic shock, it is recommended to deescalate with discontinuation of combination therapy within the first few days in response to clinical improvement or evidence of infection resolution. This applies to both targeted (for culture-positive infections) and empirical (for culture-negative infections) combination therapy.[a]	Best practice statement
10	Antimicrobial treatment duration of 7–10 days is adequate for most serious infections associated with sepsis and septic shock.	Weak recommendation, low quality of evidence
11	Longer courses are appropriate in patients who have a slow clinical response, undrainable foci of infection, bacteremia with *Staphylococcus aureus,* some fungal and viral infections, or immunologic deficiencies including neutropenia.	Weak recommendation, low quality of evidence
12	Shorter courses are appropriate in some patients, particularly patients with rapid clinical resolution following effective source control of intraabdominal or urinary sepsis and patients with anatomically uncomplicated pyelonephritis.	Weak recommendation, low quality of evidence
13	Daily assessment for deescalation of antimicrobial therapy in patients with sepsis and septic shock is recommended.	Best practice statement
14	Measurement of procalcitonin levels can be used to support shortening the duration of antimicrobial therapy in sepsis patients.	Weak recommendation, low quality of evidence
15	Procalcitonin levels can be used to support discontinuation of empirical antibiotics in patients who initially appeared to have sepsis but subsequently have limited clinical evidence of infection.	Weak recommendation, low quality of evidence
	Source Control	
1	A specific anatomic diagnosis of infection requiring emergent source control should be identified or excluded as rapidly as possible in any patient with sepsis. Required source control interventions should be implemented as soon as medically and logistically possible.	Best practice statement
2	Prompt removal of intravascular access devices that are a possible source of sepsis or septic shock is recommended after other vascular access has been established.	Best practice statement

[a]Combination therapy is defined in these guidelines as the use of multiple antibiotics with the specific intent of covering the known or suspected pathogen or pathogens with more than one antibiotic (e.g., piperacillin/tazobactam and an aminoglycoside or fluoroquinolone for gram-negative pathogens) to accelerate pathogen clearance rather than to broaden antimicrobial coverage. Other proposed applications of combination therapy include inhibition of bacterial toxin production (e.g., clindamycin with β-lactams for streptococcal toxic shock) or potential immunomodulatory effects (macrolides with a β-lactam for pneumococcal pneumonia). These statements do not preclude the use of multidrug therapy to broaden antimicrobial activity. Consult local guidelines.
Recommendations according to Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016.[25] Uncertainty about the effect of some of these recommendations on clinical outcome has resulted in widespread variation in clinical practice. See text for a summary of the criticism of some aspects of these guidelines.[164]
Data from Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Crit Care Med. *2017;45:486–552.*

infection, global geography, location where the infection was acquired (e.g., community, nursing home, or hospital), medical history, and local microbial susceptibility patterns. As a result empirical antibiotic guidelines for sepsis are different in different parts of the world. It should be emphasized that local guidelines should be referred to first. For example, multidrug empirical antibiotic therapy is appropriate for patients with risk factors for multidrug-resistant organisms but are often not required for patients with less severe disease who present to a hospital with low levels of antibiotic resistance.[164] Once culture results become available, deescalation of initial broad-spectrum antibiotic therapy is safe and may reduce the appearance of resistant microorganisms, possible drug toxicity, and costs.

Empirical antifungal therapy should be limited to patients at high risk for invasive candidiasis. A randomized trial from France showed that among nonneutropenic critically ill patients with ICU-acquired sepsis, *Candida* spp. colonization at multiple sites, and multiple organ failure, empirical treatment with micafungin did not increase fungal infection–free survival at day 28 compared with placebo.[168] Novel biomarkers or clinical risk algorithms are needed to identify patients who would benefit from empirical antifungal therapies.

Immunomodulation

Based on the assumption that sepsis mortality is driven by excessive inflammation, many trials have been performed with a variety of

antiinflammatory agents. None of these trials showed benefit, strongly suggesting that inhibition of specific components of the inflammatory response does not improve survival in all-comers with sepsis selected based on severity of disease.[93,94] Following the more recent observations of sustained immunosuppression in patients with sepsis, several investigators now suggest use of immunostimulants in the treatment of sepsis, arguing that this would restore normal immune functions and thereby reduce the incidence of secondary infections and late sepsis mortality.[120,169] However, a recent investigation estimated that overall sepsis mortality in the ICU is only 10% attributable to secondary infections.[170] Although this finding in Dutch ICUs needs to be confirmed in different settings, including settings with higher incidences of antimicrobial resistance, it does raise doubt about the potential benefit of immunostimulation in unselected sepsis populations. The key to success of any type of immunomodulation, either antiinflammatory or immunostimulatory, is to identify the patients who may benefit from a particular intervention. Considering the complexity of the host response during sepsis and the diversity of pathophysiologic mechanisms at play, it is unlikely that the present "one-target" and "one-size-fits-all" strategy will ever be successful. In addition, clinical researchers who support inhibiting inflammation and clinical researchers who propose stimulating the immune system in sepsis may well both be right. Subgroups of patients with sepsis may benefit from combined treatments that seek to constrain certain inflammatory responses, while stimulating others. Ideally, each therapeutic should be connected with a specific biomarker that provides insight into the expression of the target, a strategy referred to as theranostics as noted earlier. In the following paragraphs we discuss therapeutics that have been tested in patients with sepsis, roughly divided into antiinflammatory and immunostimulating agents.

Therapeutics That Seek to Inhibit Hyperinflammation

The Surviving Sepsis Campaign guidelines suggest the use of hydrocortisone (200 mg/day intravenously) for patients who are hemodynamically unstable despite fluids and vasopressors.[25] This recommendation is graded as weak with low quality of evidence. Indeed, several systematic reviews that have examined the use of low-dose hydrocortisone in septic shock have shown contradictory results.[171–,173,174] The most recent randomized controlled trial on this subject, a trial from Australia comprising 3800 patients with septic shock undergoing mechanical ventilation, could not demonstrate a 90-day survival benefit of a continuous infusion of hydrocortisone at a dose of 200 mg/day given for 7 days compared with placebo.[175] In addition, low-dose hydrocortisone in patients with septic shock can cause hyperglycemia and hypernatremia as side effects.[175]

Other antiinflammatory agents tested in clinical sepsis trials over the past decades include corticosteroids, TNF antibodies, soluble TNF receptors, IL-1 receptor antagonist, nitric oxide inhibitors, and platelet-activating factor inhibitors; none of these improved mortality.[93,94] Moreover, clinical trials that determined the efficacy of antithrombin, APC, and TFPI (anticoagulants that also exert antiinflammatory effects) showed no consistent benefit in patients with sepsis.[37,40,104,105] Several techniques have been developed seeking to remove bacterial and host inflammatory products from the circulation of patients. Strategies to remove LPS from blood include polymyxin B hemoperfusion, of which the effect on the outcome of patients with sepsis was not consistent.[176–178] CytoSorb is an extracorporeal cytokine adsorber that was evaluated in a small randomized trial in 100 patients with sepsis.[179] Hemadsorption did not lower plasma IL-6 concentrations, and 60-day-mortality was higher in the treatment group, although the difference was not significant after adjustment for baseline imbalances.[179] AB103 is a peptide that impedes the interaction between bacterial superantigens and the T-cell receptor CD28.[180] Besides reducing mortality in mouse models of superantigen-induced shock, AB103 also improved outcome of experimental polymicrobial sepsis,[180,181] suggesting that this peptide may have activity beyond T cells. AB103 is currently being evaluated in patients with necrotizing soft tissue infections.[182] Other antiinflammatory therapeutics studied in clinical sepsis trials are a humanized anti-C5a monoclonal antibody (CaCP29; phase II) and soluble recombinant human thrombomodulin (ART-123; phase III). An attractive therapeutic strategy in sepsis is to restore endothelium barrier function. Several agents have been studied in patients with diseases other than sepsis, including angiopoietin-1/Tie-2 modulators, S1P1 agonists, fibrinopeptide $B\beta_{15–42}$, and a PAR1 pepducin.[107]

Therapeutics That Seek to Stimulate the Immune System

Several interventions that may stimulate the immune system in sepsis have been evaluated in small clinical trials.[120,169] Immunostimulating cytokines have been studied most extensively in this regard. IFN-γ primarily targets antigen-presenting cells and can enhance the capacity of phagocytosis and killing of phagocytes. IFN-γ administration to patients with sepsis partially reversed two hallmark features of immunosuppression—reduced HLA-DR expression on monocytes and the diminished capacity of blood leukocytes to produce proinflammatory cytokines on stimulation.[183,184] IL-7 targets T cells and may reverse immunosuppression in sepsis by stimulation of T-cell proliferation, inhibition of lymphocyte apoptosis, and augmentation of the expression of adhesion molecules resulting in improved cell trafficking.[185] Addition of IL-7 to T cells from patients with sepsis was associated with enhanced proliferation and IFN-γ production, mediated at least in part through alterations in cellular metabolism initiated by mechanistic target of rapamycin activation,[186,187] suggesting that IL-7 may impact T-cell dysfunction in human sepsis. Recombinant IL-7 had a good safety profile in patients with HIV infection and patients with cancer and increased $CD4^+$ and $CD8^+$ T-cell counts[188] and is currently being tested in small trials in patients with sepsis. Granulocyte-macrophage colony-stimulating factor (GM-CSF) enhances the production of neutrophils, monocytes, and macrophages. GM-CSF administration to patients with sepsis restored several features of immunosuppression in the patients, enhancing monocyte HLA-DR expression and the capacity of blood leukocytes to release TNF-α on stimulation,[189,190] which in children with sepsis was associated with a diminished incidence of secondary infections.[190] A larger trial assessing the ability of GM-CSF to avert secondary infections in patients with sepsis with reduced monocyte HLA-DR expression is ongoing. Granulocyte colony-stimulating factor, which enhances the production of granulocytes, did not impact sepsis mortality in several trials.[191] So-called checkpoint inhibitors have been suggested as sepsis treatment as a method to restore T-cell functions.[120] In this respect antibodies against PD1 and PD-L1 have received the most attention. These antibodies have been widely used in patients with cancer,[192] and an anti-PD-L1 antibody is now being studied in patients with sepsis. Thymosin alpha 1 is a thymic peptide that increased HLA-DR expression on blood monocytes and tended to reduce mortality in patients with sepsis.[193]

Mesenchymal Stem Cells

Administration of allogeneic mesenchymal stem (or stromal) cells (MSCs) represents a potential immunomodulatory therapy in sepsis that cannot be readily classified as antiinflammatory or immunostimulatory. MSCs improved survival in animal models of sepsis by mechanisms that comprise antimicrobial, antiapoptotic, immunomodulatory, and barrier-preserving properties.[194] Administration of MSCs to humans appears to be safe, and their effect is currently being studied in patients with sepsis or ARDS or both.

PROGNOSIS

Patients who survive sepsis often have long-term complications after admission, a higher readmission rate for sepsis, and an increased mortality. The magnitude of this problem increases as the number of patients who survive sepsis treatment increases. Impaired quality of life in the physical and mental domains can persist for months to years after a sepsis episode.[195] Sepsis survivors have an increased risk for physical disability, cognitive impairment, renal failure, and cardiovascular events. In the year after sepsis, cardiovascular events were reported to occur in 30% of patients, which was higher compared with matched control populations.[195] As a result, health care use will increase nearly threefold compared with levels before sepsis.

One study from the United States comprising almost 3500 patients with severe sepsis found that 43% of survivors were rehospitalized within 90 days.[196] The most common readmission diagnoses included heart failure, pneumonia, chronic obstructive pulmonary disease exacerbation,

and urinary tract infections. Readmissions for a primary diagnosis of infection (sepsis, pneumonia, urinary tract infection, and skin/soft tissue infection) occurred in 12% of severe sepsis survivors compared with 8% of patients with matched acute medical conditions.[196] Of the patients readmitted with sepsis within 90 days, about two-thirds have an infection at the same site as their initial admission.[197]

Sepsis increases the risk of death for up to 5 years after the initial sepsis episode even after accounting for comorbidities.[195] Not surprisingly, survival after sepsis is strongly modified by age; for instance, reported 1-year survival was 94% for patients <45 years old and 54% for patients ≥85 years old in a retrospective study from California.[198]

Taken together, these poor long-term outcomes in terms of physical and cognitive impairment add to the total burden of sepsis-related death and disability.[195] Recently, more emphasis has been placed on recovery from sepsis. Suggestions made to enhance sepsis recovery include early referral for treatment of new physical, mental, and cognitive problems and thorough evaluation of treatable conditions that commonly result in hospitalization such as infection, heart failure, renal failure, and aspiration. Rehabilitation with physical and occupational therapy may benefit a subset of patients after sepsis.[195] An observational study from Taiwan that included more than 15,000 ICU patients who survived sepsis and received rehabilitation within 3 months after discharge showed that referral to rehabilitation within 90 days of hospital discharge was associated with lower risk of 10-year mortality compared with propensity-matched control subjects.[199]

FUTURE PERSPECTIVES

Despite the recent decline in case-fatality rates, sepsis will remain a major health burden worldwide due to its increasing incidence and the emergence of antibiotic resistance. Better recognition of the sepsis syndrome, faster administration of antibiotics and cardiopulmonary resuscitation, and a general increase in the standard of ICU care most likely all have contributed to the better early survival of patients with sepsis.

Nonetheless, many questions remain. Main research topics include faster detection of causative microorganisms, development of novel treatment strategies that save lung and kidney function, and development of more individualized treatment approaches. The development of new biomarkers will be essential for any personalized medicine approach in sepsis. Fig. 73.7 gives an overview of current and future advances in

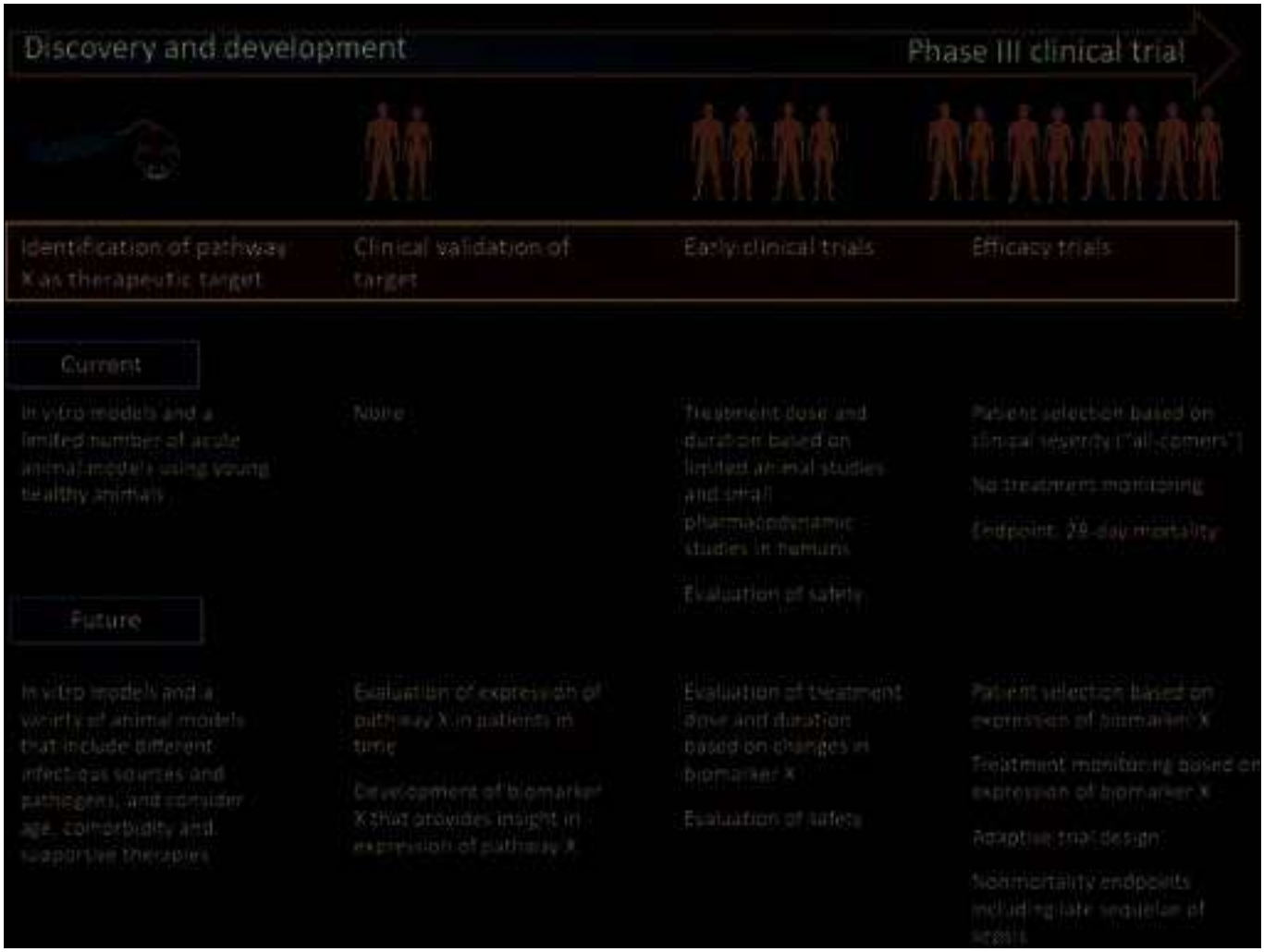

FIG. 73.7 Current and future advances in sepsis therapy development. Therapeutic targets for sepsis have traditionally been identified by studies in animals challenged with high-dose endotoxin (or other bacterial components) or viable bacteria or subjected to infection of the lungs, abdomen, or other body sites. Although such animal sepsis models may be useful in finding potential therapeutic targets, they do not adequately resemble the lengthy course of sepsis in patients and usually do not include common variables in human sepsis such as old age, comorbidities, and supportive therapies. Possible therapeutic targets derived from animal models should be verified in patients by detailed measurements over time, which is also important to develop host response biomarkers that afford insight into the expression of the targeted pathway. Such biomarkers could be implemented in inclusion of patients and treatment monitoring, an approach that has been termed theranostics. Thus far clinical sepsis trials used 28-day all-cause mortality as their primary end point. However, many patients with sepsis are still hospitalized at day 28. Adaptive trial designs and end points that take into account late physical and cognitive sequelae could alter the focus for drug development, moving away from efforts to modify the early host response and instead aiming to support faster and more complete recovery.

sepsis therapy development. Prevention of sepsis is an emerging research area. A better understanding of the long-term health sequelae of sepsis will be instrumental to develop therapies that truly enhance sepsis recovery.

In the future, for any patient presenting with a sepsis-like illness, rapid bedside genetic sequencing of known causative pathogens and simultaneous analysis of the host immune response could be performed with a blood sample. The results—combined with the clinical signs and symptoms and laboratory, imaging, and other phenotype data—could be analyzed with smart computer tools that use continuing learning algorithms to design individualized treatment options, which will help the attending physician, patients, and family members to initiate and monitor the optimal treatment to improve the outcome of sepsis.[200]

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F Intraabdominal Infections

74 Peritonitis and Intraperitoneal Abscesses

Larry M. Bush and Matthew E. Levison

SHORT VIEW SUMMARY

Definition

- Infection in the abdominal cavity encompasses a wide spectrum of entities and may involve any intraabdominal organ or space.
- Peritonitis is an inflammatory response within the peritoneal cavity as the result of contamination with microorganisms, chemicals, or both.
- Infective peritonitis is classified as either diffuse or localized, and primary *(spontaneous),* secondary, or tertiary, and is further characterized as community acquired or health care acquired, and "uncomplicated" or "complicated."
- In primary peritonitis there is no intraabdominal source, whereas in secondary peritonitis there is an intraabdominal source of infection.
- Uncomplicated infections are contained within a single organ without anatomic disruption. Complicated infections involve extension beyond the organ, either localized or generalized peritonitis, with spillage of microorganisms into the sterile peritoneal space. Community-acquired infections may be further subdivided into low- or high-risk infections depending on the probability of involvement of drug-resistant bacteria and severity of infection (mild, moderate, severe), together with any significant comorbidities the patient may have.
- Peritonitis is a common complication occurring in patients undergoing continuous ambulatory peritoneal dialysis (CAPD).
- Intraperitoneal abscesses are focal collections of pus complicating either primary or secondary peritonitis, with locations generally related to the site of primary disease and the direction of dependent peritoneal drainage.
- The majority of intraabdominal infections (80%) are "community acquired" and are graded from "mild to moderate" to "more severe" on the basis of physiologic scoring systems (Acute Physiology and Chronic Health Evaluation II [APACHE II]), the patient's comorbid conditions, underlying immune status, and an inability to achieve adequate source control.
- Health care–associated intraabdominal infections are most commonly acquired as complications of previous elective or emergency abdominal operations.

Epidemiology

- Primary peritonitis accounts for approximately 1% of all peritonitis cases.
- Adult patients usually have cirrhosis and ascites and are at greater risk if there is a coexisting gastrointestinal hemorrhage, previous episode, or low ascitic protein concentration.
- Secondary peritonitis is the most common intraabdominal infection (80%–90%) and is caused by microbial or chemical contamination of the sterile peritoneal cavity from multiple disease processes (see Table 74.3).
- Tertiary peritonitis refers to a persistent or recurrent infection without a surgically treatable focus, may be due to a disturbance in the host's immune response, and often is associated with less virulent and potentially resistant microorganisms.
- CAPD peritonitis is the major complication of peritoneal dialysis and most often is due to touch contamination or catheter-related infection.
- CAPD peritonitis recurs in 20% to 30% of patients and is the primary reason for a switch to hemodialysis.
- Intraperitoneal abscesses occur secondarily as a consequence of diseased organs, penetrating trauma, or a surgical procedure.

Microbiology

- Primary peritonitis is a monomicrobial infection; more than 60% of episodes in cirrhotic patients are caused by gram-negative enteric bacteria.
- In children, hematogenous spread of *Streptococcus pneumoniae* and other streptococcal species is more frequent.
- *Staphylococcus aureus* is rarely isolated in primary peritonitis.
- Variants of primary peritonitis include ascites that grows a single type of organism but has fewer than 250 polymorphonuclear (PMN) white blood cells (WBCs) per cubic millimeter (monomicrobial nonneutrocytic bacterascites) or has more than 250 PMN WBCs/mm^3 with negative cultures (culture-negative neutrophilic ascites).
- Secondary peritonitis is characteristically polymicrobial (see Table 74.4) and depends on the microflora associated with the primary disease process, which may be altered by previous antibiotic therapy, other medications, and specific host factors.
- Enterococci and *Candida* spp. can often be isolated; the significance of this is controversial, but treatment is warranted under certain circumstances.
- CAPD peritonitis is caused by gram-positive organisms (*Staphylococcus epidermidis, S. aureus,* and *Streptococcus* spp.) in 60% to 80% of cases.
- Gram-negative bacteria (e.g., Enterobacteriaceae, *Pseudomonas* spp., and *Acinetobacter* spp.) make up 15% to 30% of cases, often derived from urinary tract, bowel, skin, or contaminated water.
- Fungal and mycobacterial species are occasionally involved.
- Polymicrobial peritonitis in CAPD is assumed to be secondary to an intestinal process (e.g., bowel perforation).
- Intraperitoneal abscesses are mostly polymicrobial and involve the same microorganisms that cause secondary peritonitis.
- Pathogens include obligate anaerobic species (e.g., *Bacteroides fragilis,* anaerobic cocci, and clostridia) along with facultative gram-negative bacilli (e.g., *Escherichia coli* and *Proteus* and *Klebsiella* spp.)

Diagnosis

- Primary peritonitis is diagnosed by excluding a primary source of intraabdominal infection.
- Ascitic fluid studies include WBC count with differential, protein concentration, Gram stain, and culture.
- The difference between serum and ascitic fluid albumin concentration is greater than 1.1 g/dL, which is correlated with portal hypertension.
- Ascitic fluid glucose, amylase, and lactate dehydrogenase measurements may help to distinguish primary from secondary peritonitis.
- Patients suspected of having secondary peritonitis or an intraperitoneal abscess on the basis of clinical findings or peritoneal fluid

SHORT VIEW SUMMARY—cont'd

examination require radiologic evaluation with a computed tomography scan and/or ultrasonography.
- When clinically suspected, CAPD peritonitis is confirmed by a finding of greater than 100 WBCs/mm^3 (predominantly PMN cells) in mostly cloudy dialysate, together with the isolation of a microorganism (90%–95% of cases).

Therapy
- Pending confirmatory studies, empirical antibiotic therapy for suspected primary peritonitis should be initiated (see Table 74.1) on the basis of the most likely pathogens.
- Five days of antibiotic therapy is sufficient in most instances, and oral antibiotic therapy may be an option in selected cases.
- The treatment of secondary peritonitis requires antibiotic therapy (see Table 74.5), along with appropriate medical support, source control, and removal of a diseased organ, necrotic tissue, purulence, blood, feces, and other intraperitoneal foreign material, when present.
- CAPD peritonitis is treated with intraperitoneal antibiotics (see Table 74.6), usually for 10 to 21 days.
- CAPD peritonitis due to fungi is preferably treated with systemic antifungal agents (see Table 74.6).
- Peritoneal dialysis catheter removal is necessary in 10% to 20% of patients, particularly with fungal and nontuberculous mycobacterial infections (see Table 74.7).
- Intraperitoneal abscesses require drainage (with percutaneous catheter or surgical), antibiotic therapy (see Table 74.5), and possibly source control through either radiologic intervention or a surgical procedure.

Prevention
- Primary peritonitis prophylaxis is recommended in cirrhotic patients with ascites who are having a gastrointestinal hemorrhage.
- Long-term antibiotic prophylaxis (e.g., with norfloxacin, ciprofloxacin, or trimethoprim-sulfamethoxazole) is indicated in patients with one or more episodes of primary peritonitis, and in cirrhotic patients with ascitic fluid protein concentrations less than 1.5 g/dL, creatinine ≥1.2 mg/dL, blood urea nitrogen (BUN) ≥25 mg/dL, serum sodium ≤130 mEq/L, or hepatic failure (Child-Turcotte-Pugh score ≥9 and bilirubin ≥3 mg/dL) (see Table 74.2).

Intraabdominal infections can take several forms and include a variety of entities resulting from disease or trauma to both hollow and solid organs located in either the peritoneal cavity or retroperitoneal space. Although the gastrointestinal tract is most commonly involved, the urinary and gynecologic tracts may also serve as the primary source of infection. Intraperitoneal infection may be diffuse or localized into one or more abscesses. Intraperitoneal abscesses may form in dependent recesses, such as the pelvic space or Morison pouch; in the various perihepatic spaces; within the lesser sac; or along the major routes of communication between intraperitoneal recesses, such as the right paracolic gutter. In addition, infection may be contained within the intraabdominal viscera, as in hepatic, pancreatic, splenic, tubo-ovarian, or renal abscesses. Abscesses also frequently form around diseased viscera (pericholecystic, periappendiceal, pericolic, and tubo-ovarian) and between adjacent loops of bowel (i.e., interloop abscesses). In addition, intraabdominal infections are classified according to whether the infection is community acquired (approximately 80%) or health care associated. Moreover, community-acquired infections may be further subdivided into low- or high-risk infections depending on the probability of the presence of drug-resistant bacteria, severity of infection (mild, moderate, severe), and any significant patient comorbidities. These categories help to assist in the management of the patient, selection of antimicrobial therapy, and prediction of outcomes; health care–associated infections are most commonly acquired as complications of previous elective or emergency intraabdominal operations. These are caused by nosocomial isolates particular to the site of the operation and to the specific hospital and unit, all of which possess an increased probability of the presence of antimicrobial-resistant pathogens and bacterial species and fungi not common to the intraabdominal cavity. Intraabdominal infections also can be categorized as uncomplicated or complicated. Although the distinction is not always clear, *complicated* intraabdominal infections historically have been defined as extending beyond the hollow viscus of origin into the peritoneal space, with associated abscess formation or peritonitis, whereas those referred to as *uncomplicated* generally involve intramural inflammation of the gastrointestinal tract or a single organ but have a substantial likelihood of advancing to complicated disease if not properly treated.[1,2] The infection can also be graded from mild to moderate to more severe forms on the basis of accepted physiologic scoring systems and according to the patient's underlying immune status and comorbid conditions.

ANATOMY AND PHYSIOLOGY

The anatomic relationships within the abdomen are important in determining possible sources and routes of spread of infection. The peritoneal cavity extends from the undersurface of the diaphragm to the floor of the pelvis. In men, the peritoneal cavity is a closed space. In women, the peritoneal cavity is perforated by the free ends of the fallopian tubes. The stomach, jejunum, ileum, cecum, appendix, transverse and sigmoid colons, liver, gallbladder, and spleen lie within the peritoneal cavity, some being suspended by a mesentery.

The peritoneal reflections and mesenteric attachments compartmentalize the intraperitoneal space and route, spreading exudate to sites that are often distant from the source (Fig. 74.1). The transverse mesocolon divides the peritoneal cavity horizontally into an upper and a lower space. The greater omentum, extending from the transverse mesocolon and lower border of the stomach, covers the lower peritoneal cavity and further separates the upper from the lower peritoneal cavity (Fig. 74.2). The small bowel mesentery divides the lower peritoneal space.

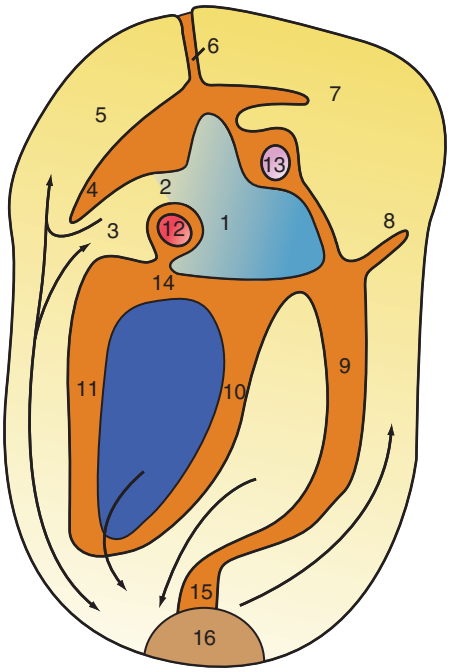

FIG. 74.1 Schema of the posterior peritoneal reflections and recesses of the peritoneal cavity. *1,* Lesser sac; *2,* foramen of Winslow; *3,* Morison pouch; *4,* right triangular ligament; *5,* right subphrenic spaces; *6,* falciform ligament; *7,* left subphrenic space; *8,* phrenocolic ligament; *9,* bare area of the descending colon; *10,* root of the small bowel mesentery; *11,* bare area of the ascending colon; *12,* duodenum; *13,* esophagus; *14,* root of the transverse mesocolon; *15,* bare area of rectum; *16,* bladder.

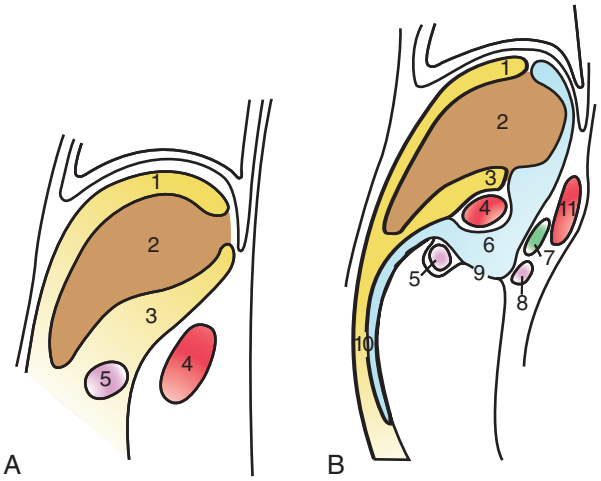

FIG. 74.2 Schema of a sagittal section of the peritoneal cavity. (A) Right upper quadrant. *1,* Subphrenic space; *2,* liver; *3,* subhepatic space; *4,* right kidney; *5,* transverse colon. (B) Left upper quadrant. *1,* Subphrenic space; *2,* liver, left lobe; *3,* subhepatic space; *4,* stomach; *5,* transverse colon; *6,* lesser sac; *7,* pancreas; *8,* duodenum; *9,* transverse mesocolon; *10,* omentum; *11,* left kidney.

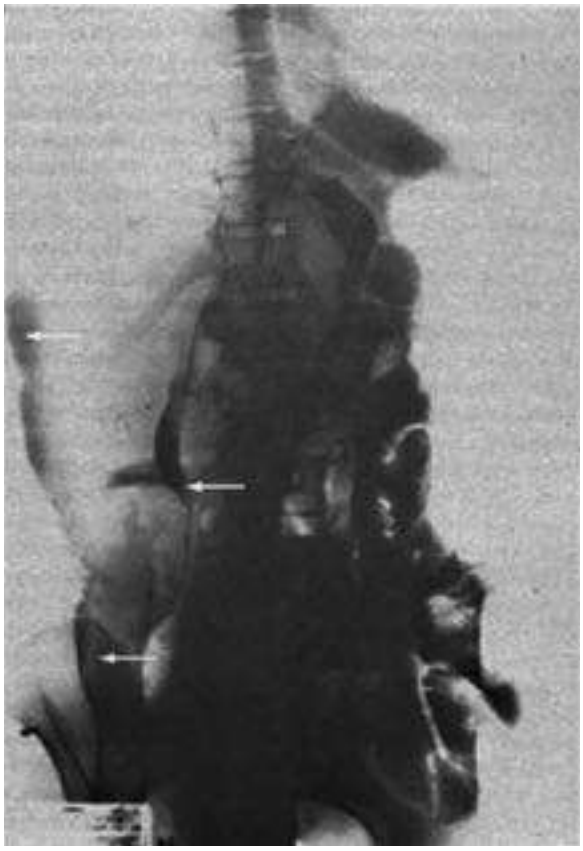

FIG. 74.3 Abdominal radiograph (right decubitus position) after oral administration of radiopaque contrast medium (Gastrografin) to a patient with dehiscence of an esophageal-gastric anastomosis. Radiopaque Gastrografin *(arrows)* can be seen in the subhepatic space, right paracolic gutter, and right subphrenic space and within the lumen of the intestinal tract.

The peritoneal cavity has several recesses into which exudate may become loculated. The most dependent recess of the peritoneal cavity in the supine position is in the pelvis. Between the rectum and bladder in men is a pouch of peritoneal cavity that extends slightly below the level of the seminal vesicles. In women, the uterus and fallopian tubes project into the pelvic recess. Between the rectum and the body of the uterus is the pouch of Douglas, which lies above the posterior fornix of the vagina. On either side of the rectum and bladder are the pararectal and paravesical fossae. The pelvic recess is continuous with the right and the left paracolic gutters.

The phrenicocolic ligament, which fixes the splenic flexure of the colon to the diaphragm, partially bridges the junction between the left paracolic gutter and the left perihepatic space. The right paracolic gutter, in contrast, is continuous with the right subhepatic space and the right subphrenic space. A posterior superior extension of the right subhepatic space, the Morison pouch, is the most dependent portion in the supine position of the right paravertebral groove and lies just above the beginning of the transverse mesocolon. The horizontal posterior reflection of the serosal surface of the liver onto the diaphragm (the right triangular and coronary ligaments) and the vertical reflection (falciform ligament) divide the right perihepatic space into the right subphrenic and right subhepatic spaces (see Figs. 74.1 and 74.2A). The left subphrenic and subhepatic spaces communicate freely around the smaller left lobe of the liver and its more superiorly placed left triangular ligament (see Figs. 74.1 and 74.2B). The right and left subphrenic spaces are separated by the falciform ligament, which probably prevents the spread of pus to the opposite side and explains why only about 5% to 15% of subphrenic abscesses are bilateral. The left subhepatic space is divided by the gastrohepatic omentum into an anterior space and the lesser sac (see Fig. 74.2B). Abscesses within the perihepatic spaces become localized by pyogenic membranes. Abscesses lie anteriorly or posteriorly in the right subphrenic space and superiorly or inferiorly in the subhepatic space. Abscesses of the left perihepatic space are either in the single left subphrenic space or in the lesser sac.

The lesser sac, the largest recess of the peritoneal cavity, is connected to the main peritoneal space by the foramen of Winslow, an opening situated between the free border of the gastrohepatic omentum and the posterior parietal peritoneum. The lesser sac is surrounded posteriorly by the pancreas and kidneys, anteriorly by the stomach, and laterally by the liver and spleen. It may also extend to a variable extent between the folds of the greater omentum. Because of the limited communication from the lesser sac to the major cavity via the foramen of Winslow, suppuration in the lesser sac may exist with little or no involvement of the major cavity. Abscesses in the lesser sac lie between the stomach and the pancreas but may spread to the right and lie anterior to the right kidney and inferior to the liver.

Through intraperitoneal injection of water-soluble contrast material selectively into various intraperitoneal spaces, the right paracolic gutter is demonstrated to be the main communication between the upper and the lower peritoneal cavities. Fluid introduced into the right upper peritoneal space gravitates toward the Morison pouch and then into the right subphrenic space and along the right paracolic gutter into the pelvic recess (Fig. 74.3). Flow of fluid in the left upper peritoneal space is mainly into the left subphrenic space. The phrenicocolic ligament limits flow inferiorly into the left paracolic gutter. Fluid introduced into the lower peritoneal cavity first gravitates to the pelvic recess and then ascends, whether in the supine or erect position, along the right paracolic gutter into the right subhepatic space, especially into the Morison pouch, and into the right subphrenic space. Ascension of fluid from the pelvic space along the left paracolic gutter is minimal and is limited by the phrenicocolic ligament. Although gravity would account for the pooling of fluid in the dependent peritoneal recesses, such as the pelvic recess and Morison pouch, ascension of fluid from the pelvis to the subphrenic space is probably caused by hydrostatic pressure differences between the upper and the lower peritoneal cavities created by diaphragmatic motion. Normal intestinal and abdominal wall motion also accounts for some spread of intraperitoneal fluid.

The retroperitoneal space lies between the posterior peritoneal membrane and the transversalis fascia, extending from the diaphragm to the pelvic brim. In the anterior retroperitoneal space between the peritoneum and anterior renal fascia lie the ascending and descending colons, duodenum, and pancreas. The kidneys and ureters lie within the posterior retroperitoneal (perinephric) space, between the anterior and posterior renal fasciae. The renal fascia encloses the kidneys and

adrenal glands superiorly and laterally, but not inferiorly; this arrangement is favorable for spread of infection in this space inferiorly.

The parietal peritoneum, mainly the anterior portion, is well supplied by somatic afferent nerves and is sensitive to all forms of stimuli. The ability of the anterior parietal peritoneum to sense sharp, well-localized pain in response to local inflammation is crucial for diagnosing abdominal infection and may be associated with involuntary abdominal muscle contraction, tenderness, and rebound tenderness. Irritation of the peripheral diaphragmatic peritoneum is felt as pain near the adjacent body wall, and irritation of the central portion is felt as pain referred to the shoulder. Stimulation of the visceral peritoneum, usually by distention of an organ, causes poorly localized, dull pain.

The peritoneal cavity is lined by a serous membrane. The surface area of this membrane approximates that of the skin. The membrane consists of a monolayer of flat mesothelial cells, beneath which are lymphatic vessels, blood vessels, and nerve endings. Normally, the peritoneal space contains only enough fluid (approximately 100 mL) to maintain moistness of the surface, facilitating movements of the viscera. Noninflamed serous fluid is clear yellow with a low specific gravity (<1.016) and low protein content (usually <3 g/dL). The protein is predominantly albumin. Fibrinogen is not present, and serous fluid does not clot. Solute concentrations are almost identical to concentrations in plasma. A few leukocytes (<250/mm^3), mostly mononuclear cells, and desquamated serosal cells may be present.

The peritoneal membrane is highly permeable. Bidirectional transfer of substances across this membrane is rapid and, because of the large surface area involved, potentially great in quantity. The peritoneal surface, with an approximate area of 1 square meter, has been used extensively as a dialyzing membrane for the treatment of uremia and has been used as a site for the administration of fluid, electrolytes, antibiotics, and blood. The effective serum oncotic pressure and the hydrostatic pressure in the portal veins and lymphatic vessels are major determinants of the rate and direction of fluid movement. The rate of movement of water and solutes between blood and peritoneal fluid also depends on concentration gradients between these compartments and has been studied in detail.[3] Water and solutes diffuse via blood capillaries and, to a lesser extent, through the lymphatic vessels. Lymphatic vessels are involved primarily in removal of nonirritating colloids and particles into the bloodstream. Absorption of particulate matter into lymphatic vessels is thought to occur mostly from the diaphragmatic surface and is aided by the pumping action of diaphragmatic motion. After infusion of radioactive sodium chromate–labeled red blood cells into the peritoneal cavity of dogs, Rochlin and associates[4] found absorption of about 70% of the labeled cells by 48 to 96 hours. This absorption occurred mostly through the lymphatic vessels. In humans, two-thirds of intraperitoneally injected red blood cells in anticoagulated blood have been found in the circulation 8 to 12 days after infusion.[5] The quantity of resorbed cells was lower when no anticoagulant was used with the transfused cells, presumably because of trapping of red blood cells in intraperitoneal clots.[5] Transport of other particulate matter, such as intraperitoneal bacteria, may be similarly impeded because of trapping in fibrinous intraperitoneal exudate.

In addition, communications exist between the peritoneal and pleural cavities that are independent of the bloodstream. In patients with Meigs syndrome, fluid and cells originating in the peritoneal cavity appear in the pleural space, probably as a result of transdiaphragmatic lymphatic transport.

PERITONITIS

Inflammation of the peritoneum may be the result of contamination of the peritoneal cavity with microorganisms, irritating chemicals, or both. Infective peritonitis has been categorized as primary, secondary, or tertiary.[6] Peritonitis complicating peritoneal dialysis can be considered an additional category. In the primary variety, the peritoneal infection is not related directly to other intraabdominal abnormalities. In the secondary variety, peritoneal cavity infections may result from a variety of intraabdominal events, such as a perforation of a hollow viscus (e.g., ruptured appendix, diverticulitis, or a perforated peptic ulcer), ischemic necrosis, or other adverse processes involving the gastrointestinal, gynecologic, or urinary tracts. Tertiary peritonitis has been conceived as a later stage of the disease, when clinical peritonitis and signs of sepsis and multiorgan failure persist or recur after treatment for primary or secondary peritonitis, and no pathogens or only low-grade pathogens (e.g., coagulase-negative staphylococci) or nosocomial, frequently multidrug-resistant, pathogens (e.g., enterococci, *Candida* and *Enterobacter* spp., methicillin-resistant *Staphylococcus aureus*) are isolated from the peritoneal exudate.[7–9] It has been defined as intraabdominal infection that persists or recurs ≥48 hours after successful and adequate surgical source control of secondary peritonitis.[6] Although the transition from one category to another may not always be clear, tertiary peritonitis has a worse prognosis with an estimated mortality rate of 30% to 64%.[10]

Primary Peritonitis

Etiology

Primary peritonitis, sometimes referred to as *spontaneous bacterial peritonitis,* is probably not a specific entity with a common cause but instead represents a group of diseases with different causes that have in common only infection of the peritoneal cavity without an evident source. Primary peritonitis occurs at all ages. The prevalence of primary peritonitis in children apparently has been decreasing.[11] Before the advent of antibiotics, primary peritonitis occurred in about 10% of all pediatric abdominal emergencies; it now accounts for less than 1% to 2%.[12] The decline has been attributed to widespread use of antibiotics for minor upper respiratory tract illness. Although primary peritonitis may occur in children without predisposing disease,[11] it is known to occur particularly in children with postnecrotic cirrhosis[11,13] and in 2% of children with the nephrotic syndrome.[14] In one study, it was also frequently associated with urinary tract infections.[12] In some children with nephrotic disease, repeated episodes of peritonitis occur and peritonitis may precede other manifestations of nephrosis.[11]

Among adults, primary peritonitis has usually been reported in patients with cirrhosis and ascites. The prevalence of primary peritonitis in hospitalized patients with cirrhosis and ascites has been estimated at 10% to 30%.[15] Primary peritonitis occurs in patients with alcoholic cirrhosis,[16–20] postnecrotic cirrhosis,[13] chronic active hepatitis,[16] acute viral hepatitis,[21] congestive heart failure,[22] metastatic malignant disease,[23] systemic lupus erythematosus,[24] or lymphedema and, rarely, in patients with no underlying disease. The presence of ascites resulting from portal hypertension, hypoalbuminemia, peritoneal disease, or other causes (e.g., chylous, myxedema, hemoperitoneum) seems to be the common link among these various conditions. The risk of developing primary peritonitis is greater in patients with high-volume ascites due to advanced cirrhosis (Child-Turcotte-Pugh class C disease, or high Model for End-Stage Liver Disease [MELD] scores) but also increases with a coexisting gastrointestinal hemorrhage, a previous episode of primary peritonitis, a low protein concentration in ascitic fluid (<1 g/dL), elevated serum bilirubin (>2.5 mg/dL), and use of proton pump inhibitors.[15,25–27]

Bacteriologic Characteristics

Primary peritonitis is a monomicrobial infection. Before the 1970s, the organisms reported to cause primary peritonitis in children were *Streptococcus pneumoniae* and group A streptococci.[11,12] By the 1970s, the number of nephrotic children with streptococcal peritonitis had declined and the relative frequency of peritonitis caused by gram-negative enteric bacilli[12,14] and staphylococci[11] had apparently increased.

In cirrhotic patients, microorganisms presumably of enteric origin account for 69% of the pathogens.[28] *Escherichia coli* is the most frequently recovered pathogen, followed by *Klebsiella pneumoniae, S. pneumoniae,* and other streptococcal species including enterococci, and other Enterobacteriaceae.[16,28,29] *Pseudomonas* species are rarely involved.

S. aureus is an unusual isolate in primary peritonitis, accounting for 2% to 4% of cases in most studies, and it has been noted to occur in patients with an erosion of an umbilical hernia. Anaerobes and microaerophilic organisms are reported infrequently. Possible explanations include the intrinsic bacteriostatic activity of ascites against *Bacteroides* spp., the relatively high partial pressure of oxygen in ascitic fluid, and the lack of optimal anaerobic bacteriologic techniques to study patients with primary peritonitis in the past.[30] In a review of 126 cases of primary peritonitis in cirrhotic patients recorded in the literature,

only 8 patients (6%) had disease caused by anaerobic or microaerophilic bacteria, including *Bacteroides* spp., *Bacteroides fragilis, Clostridium perfringens, Peptostreptococcus* spp., *Peptococcus* spp., and *Campylobacter fetus.*[31] Polymicrobial infection was present in four of these eight cirrhotic patients with peritonitis caused by anaerobes, in contrast to the relatively low frequency of polymicrobial infection (only 10 of 118 cases of peritonitis) when aerobes alone were involved.

Three variants of primary or spontaneous peritonitis have been described.[32] One of them, termed *monomicrobial nonneutrocytic bacterascites,* is characterized by ascitic fluid with positive cultures but containing few neutrophils; this condition manifests in patients without clinical findings of peritonitis.[33] It may represent early bacterial colonization before a host response ensues and resolves spontaneously in 62% to 86% of cases, with the remainder progressing to spontaneous bacterial peritonitis, at times within hours. The causative bacteria are similar to those seen in classic primary peritonitis, and patients with a low leukocyte response have the same mortality rate as patients with a greater response.[20] Conversely, several series have identified cases of primary peritonitis with negative ascitic fluid cultures, referred to as *culture-negative neutrocytic ascites.*[34] In one series, sterile cultures occurred in 35% of patients with clinical findings consistent with primary peritonitis, ascitic fluid neutrophil counts greater than 500 cells/mm^3, and no evident source of intraabdominal infection.[34] This variant of peritonitis has been redefined by an elevated ascitic fluid neutrophil count of greater than 250 cells/mm^3, a negative ascitic fluid culture (in the absence of antibiotic therapy or pancreatitis), and no evident intraabdominal, surgically treatable source of infection. Other disorders capable of producing a somewhat similar picture include tuberculous peritonitis, malignancy-related ascites, and any process that leads to death of cells and thereby activates complement or cytokines that can attract leukocytes into the peritoneal cavity. However, in the absence of bacterial infection, the predominance of neutrophils—almost always seen with spontaneous bacterial peritonitis—is not present. Blood cultures have been found to be positive for bacteria in one-third of patients with culture-negative neutrocytic ascites.[34] The frequency of culture-negative ascitic fluid may be decreased by inoculating blood culture bottles with 10 or 20 mL of ascitic fluid at the bedside (see "Laboratory Findings" section).[35]

Last, the third variant of spontaneous bacterial peritonitis, *polymicrobial bacterascites,*[36] is caused by a traumatic paracentesis in which the bowel is entered by the paracentesis needle and bacteria leak, usually transiently, from the gut into the ascitic fluid. This variant occurs in less than 1% of paracentesis procedures, with risk factors that include ileus, the presence of multiple abdominal surgical scars, and intestinal adhesions. In this scenario, various bacterial forms are seen on the Gram stain or grow on culture of the ascitic fluid, which contains fewer than 250 cells/mm^3. If the peritoneal fluid protein concentration is greater than 1 g/dL and the opsonic activity of the fluid is adequate, polymicrobial bacterascites is reported to resolve spontaneously.

Bacteremia is present in 75% of patients with primary peritonitis caused by aerobic bacteria,[28] but it is rarely found in patients with peritonitis caused by anaerobes.[31] Usually the same organisms isolated from the peritoneal fluid are recovered from the blood.[16,31] On occasion, peritonitis results from infection with *Mycobacterium tuberculosis, Neisseria gonorrhoeae, Chlamydia trachomatis,* or *Coccidioides immitis,* but this is usually the result of disseminated infection or sometimes spread from adjacent foci of infection such as the genital tract (see "Pathogenesis" next).

Pathogenesis

The route of infection in primary peritonitis is usually not apparent; it is often presumed to be hematogenous, lymphogenous, or transmural migration through an intact gut wall from the intestinal lumen or, in women, from the vagina via the fallopian tubes. Enteric bacteria have been postulated to migrate from the bowel lumen into mesenteric lymph and to enter the systemic circulation via the thoracic duct; this process is termed *bacterial translocation.*[37] The role of intestinal microbiota bacterial overgrowth is uncertain because different studies have provided conflicting results.[38,39] Enteric bacteria could also enter the systemic circulation from the portal vein by passage through the liver or by portosystemic shunts in patients with portal hypertension.[16] The hepatic reticuloendothelial system is known to be a major site for removal of bacteria from blood. Conn and Fessel[16] postulated that organisms removed from the systemic circulation by the liver contaminate hepatic lymph and pass through the permeable lymphatic walls into the ascitic fluid. Results of animal studies, however, have suggested that destruction of bloodborne bacteria by the reticuloendothelial system is impaired in experimental cirrhosis[40] and in alcoholic liver disease. Infection of ascitic fluid would be facilitated by impaired reticuloendothelial clearance of bacteria from the bloodstream, which would tend to perpetuate bacteremia and increase the opportunity to cause metastatic infection at susceptible sites, such as the ascitic collection. Primary bacteremia, usually caused by coliforms, is a common complication in cirrhosis, and metastatic infection in the pleural space has been reported in cirrhotic patients.[41] An increased frequency of gram-negative endocarditis has also been noted in cirrhotic patients.[42]

In addition, alcohol abuse and cirrhosis have been reported to be associated with impaired intracellular killing by monocytes and neutrophils and with impaired opsonization and low levels of serum complement. The decrease in phagocytic activity seen in alcoholic cirrhosis is proportional to the severity of the liver disease.[43] Impaired local defenses in the peritoneal cavity also facilitate infection of ascites. Opsonic activity, as reflected by low levels of complement and immunoglobulins, is reduced in the ascitic fluid of patients with the nephrotic syndrome and cirrhosis.[44]

Enteric bacteria may also gain access to the peritoneal cavity by directly traversing the intact intestinal wall. In an animal model, *E. coli* passes from the bowel into the peritoneal cavity after the introduction of hypertonic solutions into the peritoneum.[45] A similar mechanism may explain the enteric bacterial peritonitis that frequently complicates peritoneal dialysis. The infrequent occurrence of bacteremia and the multiplicity of species in peritoneal fluid when anaerobic bacteria are involved suggest that transmural migration of bacteria is the probable route of infection of ascitic fluid in most of these patients.[31] In addition, the occurrence of polymicrobial anaerobic peritonitis in two patients after infusion of vasopressin into the superior mesenteric or gastroduodenal arteries suggested that arterial vasoconstriction decreased the intestinal mucosal barrier and permitted transmural migration of enteric organisms.[46] Colonic microorganisms are also known to colonize the upper small bowel in cirrhotic patients.

When pneumococci are present simultaneously in vaginal secretions and peritoneal fluid in prepubertal girls, an ascending infection of genital origin is likely in these patients. The alkaline vaginal secretions of prepubertal girls may be less inhibitory to bacterial growth than the acidic secretions of postpubertal women. Transfallopian spread is also suggested by the development of peritonitis in women with intrauterine devices.[47] In women with gonococcal or chlamydial perihepatitis (Fitz-Hugh–Curtis syndrome), the route of spread is presumably from the fallopian tubes and paracolic gutters to the subphrenic space, but it may also be hematogenous. In the one man documented with this syndrome, *N. gonorrhoeae* was recovered from a liver biopsy specimen and the infection presumably spread by means of bacteremia.[48]

Although tuberculous peritonitis may result from direct entry into the peritoneal cavity of tubercle bacilli (from the lymph nodes, intestine, or genital tract in patients with active disease of these organs), it is more likely to result from hematogenous dissemination from remote foci of tuberculosis, most commonly in the lung. Tuberculous peritonitis can become clinically evident after the initial focus has healed completely.

Bacteria originating from other foci of infections distant from the abdominal or pelvic cavities including the skin and soft tissues, upper and lower respiratory tracts, and odontogenic disease may also hematogenously gain entry into the ascitic fluid.[49]

Infection of ascites stimulates a dramatic increase in proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, interferon-γ (IFN-γ), and soluble adhesion molecules in the serum, and to a much greater extent in the peritoneal exudate.[50] These cytokines are produced by macrophages and other host cells in response to bacteria or bacterial products, such as endotoxin. In an experimental model of peritonitis,[51] antibodies to endotoxin, but not to TNF-α, were found to prevent death and reduce bacterial numbers in the peritoneal exudate. Another potential source is direct translocation of cytokines through

the intestinal barrier. Undoubtedly, many of the systemic and abdominal manifestations of peritonitis are mediated by these molecules. Furthermore, the presence of these cytokines may lead to further reduction of effective arterial blood volume, as indicated by an increase in plasma renin activity and the development of renal insufficiency. Approximately 30% of patients with primary peritonitis develop renal insufficiency, which has been found to be the most sensitive predictor of in-hospital mortality.

Clinical Manifestations

Primary peritonitis is an acute febrile illness often confused with acute appendicitis in children. Fever, abdominal pain, nausea, vomiting, and diarrhea are usually present with diffuse abdominal tenderness and rebound tenderness, and bowel sounds are hypoactive or absent. In cirrhotic patients with primary peritonitis, preexisting ascites is present. In some patients, the clinical manifestations are atypical. The onset may be insidious, and findings of peritoneal irritation may be absent in an abdomen distended with ascites. Fever (temperature >37.8°C [>100°F]) is the most common presenting sign, occurring in 50% to 80% of cases,[16,20] and may be present without abdominal signs or symptoms, or the process may be clinically silent. Primary peritonitis in cirrhotic patients is generally associated with other features of end-stage liver disease (hepatorenal syndrome, progressive encephalopathy, and variceal bleeding). Primary peritonitis should always be considered in the differential diagnosis of decompensation of previously stable chronic liver disease. In approximately 50% of patients with primary peritonitis, mentation is altered, often subtly, because of infection and/or hepatic decompensation. Poor prognostic signs include hypothermia, hypotension, and paralytic ileus.

Gonococcal perihepatitis (Fitz-Hugh–Curtis syndrome) most often occurs in women. It manifests with sudden onset of pain in the right upper quadrant of the abdomen, at times referred to the right shoulder. Low-grade fever, right upper quadrant tenderness, guarding, and a friction rub over the liver may be present. Gonococcal cervicitis or salpingitis may or may not be clinically evident. Chlamydial perihepatitis and gonococcal perihepatitis are clinically indistinguishable.

Primary tuberculous peritonitis is usually gradual in onset, with fever, weight loss, malaise, night sweats, and abdominal distention. The abdomen may not be rigid and is often characterized as being "doughy" on palpation. The findings at operation or laparoscopy consist of multiple nodules scattered over the peritoneal surface and omentum. Adhesions and a variable amount of peritoneal fluid are usually present. Similarly, *C. immitis* can cause a granulomatous peritonitis with a variable clinical manifestation.

Laboratory Findings

Fluid obtained by paracentesis should be examined for cell count, differential count, and total protein and albumin concentrations, and a Gram stain and culture of the fluid should be obtained.[52] It is important that fluid for analysis be obtained before the administration of antibiotics. A polymorphonuclear (PMN) leukocyte count in peritoneal fluid greater than 250 cells/mm^3 is considered diagnostic of primary peritonitis, even when culture of ascitic fluid yields negative results.[15] One potential source of error in the PMN leukocyte count is related to hemorrhage into the ascitic fluid resulting from a traumatic paracentesis, leading to red and white blood cell entry into the fluid. A corrected PMN leukocyte count should be calculated if fluid is bloody, with one PMN leukocyte subtracted from the count for every 250 red blood cells/mm^3 present. The leukocyte count in ascitic fluid may increase, however, during diuresis in patients with chronic liver disease. Studies in which testing for leukocyte esterase has been conducted with use of dipsticks designed for urine have yielded both high and low sensitivities,[53] but this method cannot be recommended at this time.

Nonetheless, the consistently high negative predictive value found with leukocyte esterase reagent strips may support their use as a preliminary screening tool for diagnosis of primary peritonitis.[54] Gram staining of the sediment, when positive, is diagnostic, but it is negative in 60% to 80% of patients with primary peritonitis.[16,19] If multiple bacterial forms are encountered, perforation of the gut needs to be considered. Culture of ascitic fluid may yield negative findings in 40% of patients with clinical manifestations of primary peritonitis and an elevated ascitic fluid PMN leukocyte count.[15,18] In order to optimize the chances of isolating a bacterial pathogen from the fluid, appropriately prepared blood culture bottles should be inoculated with at least 10 mL of ascitic fluid. Culturing ascitic fluid in this manner, as if it were blood, has been shown to increase the culture positivity of patients with an ascitic fluid PMN leukocyte count of greater than 250 cells/mm^3. A positive culture rate increase from about 50% to 77% (with delayed inoculation) to 80% to 100% with immediate inoculation has been demonstrated in individuals who did not receive prior antibiotic treatment and did not have pancreatitis, tuberculous peritonitis, or malignancy-related ascites.[55] Measurement of the ascitic fluid total protein concentration helps distinguish primary peritonitis from secondary bacterial peritonitis. In the latter, the concentration is generally greater than 1 g/dL. Total protein concentration is inversely related to the risk for developing spontaneous peritonitis. Dilute ascitic fluid with a protein concentration less than 1 g/dL poses the highest risk for the development of spontaneous peritonitis because concentrations of opsonins in the fluid are low. The ascitic fluid protein concentration may be low,[16] perhaps because of (1) hypoalbuminemia or (2) dilution of ascitic fluid with transudate from the portal system when cirrhosis is present or the portal vein is obstructed. Calculation of the serum-ascites albumin gradient (SAAG) is useful for indirectly measuring portal pressure. When serum and ascites concentrations are measured simultaneously, a difference of greater than 1.1 g/dL is correlated with portal hypertension with almost 100% accuracy.[56] This difference may be seen with cirrhosis, alcoholic hepatitis, congestive heart failure, the Budd-Chiari syndrome, advanced hepatic metastases, and constrictive pericarditis. Ascitic fluid in tuberculous peritonitis may have an elevated protein concentration (>3 g/dL) and a lymphocytic pleocytosis, but neither may be present, especially in cirrhotic patients.

Various other parameters of ascitic fluid generally considered as optional tests have been suggested to help in diagnosing primary bacterial peritonitis and assist in separating this entity from secondary peritonitis. These include ascitic fluid glucose concentration, which is generally correlated inversely with the number of PMN leukocytes in the fluid. With spontaneous bacterial peritonitis, the glucose concentration usually remains above 50 mg/dL compared with levels that approach zero when peritonitis is secondary to events such as intestinal perforation.[57] Lactate dehydrogenase (LDH) levels (derived from the lysis of PMN leukocytes in ascitic fluid), although increased in spontaneous bacterial peritonitis, are lower than the upper limit of normal for serum. The finding of an elevated amylase concentration in the ascitic fluid is suggestive of gut perforation or pancreatitis. Ascitic fluid found to be dark (orange to brown) in color suggests gallbladder perforation, in which case the ascitic fluid bilirubin concentration that otherwise is low should be measured. Lactoferrin is an iron-binding protein thought to help protect against enteric pathogens by contributing to the antimicrobial effect of neutrophils; although further validation studies may be necessary, levels of lactoferrin have been found to be correlated with the presence of spontaneous bacterial peritonitis.[58] Previously published literature claimed to show the utility of ascitic fluid pH and lactate concentration in helping diagnose primary bacterial peritonitis. However, subsequent larger-scale studies[59] using more accurate methods failed to confirm the usefulness of these tests.

Diagnosis

Primary peritonitis is diagnosed by ruling out a primary intraabdominal source of infection that might result in secondary peritonitis. Distinguishing primary from secondary peritonitis is clinically important, because the latter almost always necessitates either surgical or interventional radiologic source control as a critical component of treatment. In addition, secondary peritonitis usually requires the use of broader spectrum or combination antimicrobial therapy. The signs and symptoms of both forms of peritonitis are frequently not separable. Ascitic fluid analysis and radiologic imaging studies are relied on to distinguish primary from secondary infection. In secondary cases, the ascitic fluid typically contains a total protein >1 g/dL, glucose <50 mg/dL, and LDH level elevated above the upper limit of normal for serum.[57] Polymicrobial infection or the demonstration of multiple morphologic bacterial forms

is suggestive of gastrointestinal perforation. Ascitic fluid carcinoembryonic antigen (CEA) levels >5 ng/mL or alkaline phosphatase quantities >240 units/L are fairly sensitive and specific in the detection of gut perforation.[60] Computed tomography (CT) with oral and intravenous contrast material greatly enhances the detection of intraabdominal sources of secondary peritonitis. Surgery often can be directed toward a potential source of infection identified on the basis of CT findings, rather than by a full exploratory laparotomy, which is known to be associated with high rates of mortality in certain groups of patients, such as cirrhotic patients. A large proportion of cirrhosis in patients with primary peritonitis is due to alcohol ingestion. Alcoholic hepatitis may also manifest with fever and abdominal pain, but the peripheral leukocytosis often observed with the latter does not result in an elevated ascitic fluid PMN count, which suggests concurrent primary peritonitis, if present.[61] Patients with primary peritonitis usually respond within 48 to 72 hours to appropriate antimicrobial therapy.[62] The observation of an exponential rate of decline in the number of ascitic fluid leukocytes after the initiation of antimicrobial therapy for primary peritonitis has also been found to help differentiate primary from secondary bacterial peritonitis.[63]

The finding of pneumococci in peritoneal fluid may not indicate primary peritonitis, as illustrated by a case report of appendicitis and secondary peritonitis caused by pneumococci.[64] For this reason, some surgeons have considered the differential diagnosis between appendicitis and primary peritonitis too difficult to make in children without operative examination, even when gram-positive bacteria are identified in peritoneal fluid. Paracentesis for smear and culture is indicated in all cirrhotic patients with ascites and in children with gross proteinuria and abdominal pain, regardless of whether the diagnosis of nephrotic syndrome has been established. Paracentesis is not without hazard, however, especially in patients with hemorrhagic tendencies and bowel distention. In a retrospective analysis of 242 consecutive diagnostic abdominal paracenteses in patients with liver disease and ascites, major complications were reported in 7, including perforation of the bowel with generalized peritonitis or abdominal wall abscess.[65] Fortunately, hemorrhagic complications, except in the setting of disseminated intravascular coagulation, are rare, as demonstrated in one large study,[66] despite the presence of significantly elevated international normalized ratio (INR) values and diminished platelet counts.

In patients with a subacute or chronic course of primary peritonitis, other pathogens must be considered. The diagnosis of tuberculous peritonitis usually can be made at surgery or laparoscopy and confirmed by the histologic characteristics of the peritoneal biopsy specimen and by bacteriologic examination of the peritoneal biopsy specimen and fluid. The use of polymerase chain reaction (PCR) testing of fluid and tissue samples can aid in the diagnosis of tuberculous peritonitis.[67] The diagnosis of *C. immitis* peritonitis is best established with culture of ascitic fluid. The laboratory should be apprised of this diagnostic possibility because isolation of the fungus is a biohazard.

Prognosis

The treatment of primary peritonitis has been reported to be successful in more than half of cirrhotic patients, but because of the frequency of accompanying end-stage cirrhosis, the overall mortality rate in cirrhotic adults has been 95%.[16] Subsequent studies, however, reported lower mortality rates of 70% and 57%,[19,20] and 28% and 40%, respectively, died from the primary peritonitis. Patients with the poorest prognosis were found to have renal insufficiency, hypothermia, hyperbilirubinemia, and hypoalbuminemia. The lower mortality rates in these later series can perhaps be explained by the less frequent occurrence of hepatic encephalopathy. The lowest hospitalization- and infection-related mortality rates (37.8% and 2.2%, respectively), reported later, were attributed to early diagnosis and treatment.[62] Septic shock due to primary peritonitis had an overall in-hospital mortality rate of 82%, but chance of survival is greatly improved in patients who receive earlier, adequate, and timely antimicrobial therapy.[68] However, patients with severe enough liver disease to develop spontaneous bacterial peritonitis have a poor long-term prognosis; 1- and 2-year mortality rates are 70% and 80%, respectively.[69] Therefore, liver transplantation should be considered for survivors of spontaneous bacterial peritonitis who meet other criteria for transplantation. Treatment of peritonitis caused by gram-positive organisms and of early infections has been more frequently successful than treatment of gram-negative or late infections. In patients with nephrotic disease and gram-positive infections and in patients who do not have a preterminal underlying illness, the survival rate is higher than 90%.[11]

TABLE 74.1 Primary Peritonitis: Indications for Initiation of Therapy

Temperature >37.8°C (100°F)
Abdominal pain and/or tenderness
An unexplained change in mental status
Laboratory abnormalities suggestive of infection (e.g., renal failure, acidosis, or peripheral leukocytosis)
Peritoneal fluid neutrophil count ≥250 cells/mm^3

Therapy

Primary peritonitis is managed medically unless secondary peritonitis is suspected on the basis of clinical, laboratory, or imaging findings, in which case either exploratory laparotomy or laparoscopy, or percutaneous radiologic interventions are performed. In primary peritonitis, indications for initiation of therapy are listed in Table 74.1. Delays in treatment pending culture results may negatively affect clinical outcomes. Other than in emergent situations, it is best that antibiotics not be administered until after ascitic fluid has been obtained for culture in order to optimize the chance of isolating the pathogenic organism.

Because the Gram stain is frequently negative in primary bacterial peritonitis, the initial choice of antimicrobial drug is often empirical, based on the most likely pathogens. Relatively few data exist to guide the optimal selection and dose of antibiotics for treatment of spontaneous bacterial peritonitis.[70] The antimicrobial regimen can be modified when the results of the culture and susceptibility tests are available. Cefotaxime and similar third-generation cephalosporin antibiotics (e.g., ceftriaxone) have been shown to be as efficacious as the combination of ampicillin plus an aminoglycoside for empirical therapy in primary bacterial peritonitis.[71] In addition to high rates of resolution of infection, the third-generation cephalosporins also avoid the risk of nephrotoxicity, which is sufficiently common in this group of patients to warrant the avoidance of aminoglycosides if an equally effective alternative antimicrobial regimen can be used.[20] These extended-spectrum cephalosporins may also be effective in treating patients who are suspected of being infected with a fluoroquinolone-resistant pathogen as a consequence of their having taken oral fluoroquinolone prophylaxis.[72] Other antimicrobial agents, such as β-lactam/β-lactamase combinations (e.g., piperacillin-tazobactam, ticarcillin-clavulanate, ampicillin-sulbactam) and carbapenems (e.g., imipenem, meropenem, doripenem, ertapenem), are potential alternatives.[15] The extended-spectrum fluoroquinolones (e.g., levofloxacin and moxifloxacin) may also be used in patients with contraindications to β-lactam drugs. However, these agents should be avoided if patients were previously receiving a fluoroquinolone for prophylaxis, because there is a high probability that the infecting bacteria are resistant to similar medications. Although intravenous antimicrobial therapy may be preferred, oral administration of antibiotics, such as amoxicillin-clavulanate or fluoroquinolones, may be equally efficacious in patients with uncomplicated disease,[15] assuming the prevalence of resistance to *E. coli* and other Enterobacteriaceae is not high.

Primary bacterial peritonitis caused by either *S. pneumoniae* or group A streptococci is treated best with high-dose penicillin, ceftriaxone, or cefotaxime. If the pneumococcal strain is highly resistant to these drugs, vancomycin is the preferred drug and it may be prudent to include vancomycin until sensitivities of pneumococci are ascertained. Peritonitis suspected of being caused by methicillin-sensitive *S. aureus* should be treated with a penicillinase-resistant penicillin (e.g., nafcillin) or with a first-generation cephalosporin (e.g., cefazolin); if the strain is methicillin resistant or if the patient is allergic to penicillin, vancomycin, daptomycin, telavancin, or linezolid can be used. If *Pseudomonas aeruginosa* is isolated, an antipseudomonal penicillin, ceftazidime, cefepime, ceftolozane-tazobactam (may be active against strains of multidrug-resistant *P.*

aeruginosa), ceftazidime-avibactam, aztreonam, a carbapenem (e.g., imipenem, meropenem, or doripenem), or a fluoroquinolone with good antipseudomonal activity (e.g., ciprofloxacin) could be combined with another antipseudomonal antibiotic, although use of aminoglycosides is discouraged because of nephrotoxicity.[73] More recently, multidrug resistance (mainly via extended-spectrum β-lactamase [ESBL] production) has been encountered among strains of gram-negative bacilli isolated in primary peritonitis. Although this appears to occur with greater frequency in nosocomially acquired and health care–associated cases, resistance to ceftriaxone was found in 7% of community-acquired primary peritonitis cases.[74] In this setting, a carbapenem would be the antimicrobial agent of choice.

In cases in which there is a strong clinical suspicion of primary bacterial peritonitis but all cultures are sterile, antimicrobial therapy should be continued. Clinical improvement together with a significant decline in the ascitic fluid PMN leukocyte count of greater than 25% should occur after 24 to 48 hours of antimicrobial therapy if the diagnosis is correct.[62,63,75] Likewise, a follow-up fluid analysis to document sterility of an initial positive culture and a marked decrease in PMN leukocyte count is not necessary in most patients with spontaneous bacterial peritonitis. However, a repeat paracentesis should be considered in cases in which an expected clinical response is lacking or an unusual organism is present. If the PMN leukocyte count in ascitic fluid continues to be elevated, other diagnoses should be considered. Historically, antimicrobial therapy generally was continued for 10 to 14 days if improvement was noted; however, shorter courses (5 days) of therapy have been shown to be as efficacious.[62] Longer treatment courses may be warranted and should be considered in the setting of resistant bacterial pathogens, and when the clinical response is slower than expected or when results of follow-up of ascitic fluid analysis remain significantly abnormal. The administration of intraperitoneal antimicrobials is not necessary. A meta-analysis of four controlled trials found that intravenous albumin infusion along with antibiotics resulted in a significant decrease in the incidence of acute kidney injury (8% vs. 31%), and a reduction in mortality (16% vs. 35%).[73] Renal insufficiency, as a consequence of further reduction in effective arterial blood volume with a resultant increase in activity of the renin-angiotensin-aldosterone system, occurs in about one-third of patients with primary peritonitis. Patients with ascitic fluid PMN leukocyte cell counts of at least 250 cells/mm^3 and in whom primary peritonitis is clinically suspected should receive 1.5 g albumin/kg body weight within 6 hours of detection and 1.0 g/kg on day 3. Albumin administration is also indicated when the serum creatinine is >1 mg/dL, blood urea nitrogen is >30 mg/dL, or total bilirubin is >4 mg/dL.[76] Nonselective β-blockers have been determined to significantly increase the risk of mortality and rates of hepatorenal syndrome in patients with cirrhosis who developed primary peritonitis and therefore should not be used.[77]

Patients with peritoneal fluid PMN leukocyte cell counts less than 250 cells/mm^3 and signs or symptoms of infection should receive antibiotic therapy as detailed earlier for primary peritonitis, while awaiting results of cultures, because symptomatic patients with monomicrobial nonneutrophilic bacterascites variant are prone to progress to primary peritonitis, even though at the time of the paracentesis it is not known whether the cultures will yield bacteria. Because only 15% of asymptomatic patients with monomicrobial nonneutrophilic bacterascites progress to primary peritonitis, this group of otherwise asymptomatic patients usually does not need antibiotics, and observation is appropriate. Therefore it is recommended that in these asymptomatic patients the paracentesis be repeated as soon as the first culture yields bacteria. Antibiotics are initiated only if signs or symptoms of infection develop or if the second paracentesis demonstrates neutrocytic ascites. The majority of patients with culture-negative neutrocytic peritoneal fluid (PMN count >250 cells/mm^3) have primary peritonitis. In those instances, the same empirical antimicrobial therapy should be given.

Prevention

Because of the common occurrence and high mortality of primary peritonitis in the presence of cirrhosis and ascites, prevention is a desirable strategy. This is particularly true for patients who are awaiting liver transplantation. Patients with a heightened risk of developing primary peritonitis include those with ascitic protein concentrations of less than 1 g/dL, variceal bleeding, or a prior episode of primary peritonitis. In randomized controlled trials, the use of antibiotic prophylaxis has been proven to decrease infection episodes and lower mortality rates in such individuals. Similarly, prophylaxis is recommended in patients with cirrhosis and gastrointestinal bleeding, and in those admitted to the hospital for unrelated reasons but who are known or found to have low ascitic fluid protein concentrations (Table 74.2). Short-term (7 days) inpatient intravenous ceftriaxone should be given to prevent primary peritonitis in hospitalized patients with advanced cirrhosis and gastrointestinal bleeding. This can be changed to oral double-strength trimethoprim-sulfamethoxazole, ciprofloxacin 500 mg every 12 hours, or norfloxacin 400 mg twice daily to complete the course once bleeding has ceased and the patient's condition is stable. Because active bleeding is the risk factor for infection, patients who are undergoing sclerotherapy or variceal banding to prevent bleeding do not require antibiotic prophylaxis.[78]

TABLE 74.2 Prevention and Prophylaxis of Primary Peritonitis (Cirrhotic Patients With Ascites)

INDICATION	ANTIBIOTIC REGIMEN
Variceal or gastrointestinal bleeding	Ceftriaxone 1 g IV for 7 days, can be changed to oral TMP-SMX DS or ciprofloxacin 500 mg or norfloxacin 400 mg twice daily to complete the 7-day course, once bleeding has ceased and the patient is stable
Prior episode of primary peritonitis	Oral TMP-SMX DS, or ciprofloxacin 500 mg, or norfloxacin 400 mg once daily
Ascitic fluid protein <1 g/dL	Oral TMP-SMX DS, or ciprofloxacin 500 mg, or norfloxacin 400 mg once daily
Ascitic fluid protein <1.5 g/dL with impaired renal function[a] or liver failure[b]	Oral TMP-SMX DS, or ciprofloxacin 500 mg, or norfloxacin 400 mg once daily

[a]Creatinine >1.2 mg/dL, blood urea nitrogen >25 mg/dL or serum sodium <130 mEq/L.
[b]Child-Turcotte-Pugh score >9 and bilirubin >3 mg/dL.
TMP-SMX DS, Trimethoprim-sulfamethoxazole double strength.

Patients who have survived one episode of primary peritonitis have an increased 1-year probability of another episode. A combined meta-analysis of 13 trials in which antibiotic prophylaxis was given to hospitalized patients with cirrhosis who had various risk factors for infection (i.e., low-protein ascitic fluid, gastrointestinal bleeding, and history of primary bacterial peritonitis) showed an overall decrease in mortality and a decrease in bacterial infection.[79] Subsequent meta-analyses have reached similar conclusions.[80,81] One concern with prolonged antibiotic prophylaxis is the potential selection of resistant gut bacterial flora, which can subsequently cause spontaneous infection, and a change in the flora of bacterial infections. High-risk patients who are receiving intestinal decontamination regimens are found to have a predominance of gram-positive organisms compared with the usual predominance of gram-negative organisms.[82] In randomized trials, researchers have studied both intermittent and continuous prophylaxis and found that intermittent dosing schedules pose a higher risk of promoting bacterial resistance. Currently, continuous prolonged outpatient double-strength trimethoprim-sulfamethoxazole, ciprofloxacin 500 mg, or norfloxacin 400 mg, each given once daily, are the preferred antibiotic prophylactic regimens for otherwise asymptomatic patients with a prior history of primary peritonitis. The same long-term preventive antibiotics are recommended for patients with cirrhosis and ascites with a protein concentration <1.5 g/dL, along with either impaired renal function (creatinine >1.2 mg/dL, blood urea nitrogen >25 mg/dL, or serum sodium ≤130 mEq/L) or liver failure (Child-Turcotte-Pugh score ≥9 and bilirubin >3 mg/dL) (see Table 74.2).[25,83–86] A similar approach to prevent infection in patients awaiting liver transplantation but lacking documented risk factors is often undertaken. However, randomized trials supporting this practice are lacking.

Proton pump inhibitors have been associated with increased rates of primary peritonitis and should be avoided unless clearly indicated and required.[87] Other prevention strategies include the treatment of unrelated infections to prevent potential hematogenous spread to the ascitic fluid. Diuretic therapy effectively increases the opsonic activity in the normally dilute ascitic fluid of cirrhotic patients, leading to improved antibacterial action.[88]

Secondary and Tertiary Peritonitis

Etiology

Secondary intraabdominal infection is usually caused by spillage of gastrointestinal or genitourinary microorganisms into the peritoneal cavity secondary to loss of the integrity of the mucosal barrier. It is the most common intraabdominal infection and accounts for approximately 80% to 90% of such infections. The primary intraabdominal processes that can give rise to secondary peritonitis are numerous (Table 74.3) and include diseases or injuries of the gastrointestinal or genitourinary tract, such as perforation of a peptic ulcer; traumatic perforation of the uterus, urinary bladder, stomach, or small or large bowel; spontaneous perforation associated with typhoid, tuberculous, amebic, *Strongyloides,* or cytomegalovirus ulcers in immunocompromised persons; appendicitis, diverticulitis, or intestinal neoplasms; gangrene of the bowel from strangulation, bowel obstruction, or mesenteric vascular obstruction; suppurative cholecystitis; bile peritonitis; pancreatitis; operative contamination of the peritoneum or disruption of a surgical anastomosis site; septic abortion, puerperal sepsis, postoperative uterine infection, or endometritis complicating an intrauterine device; gonococcal salpingitis or gonococcal vulvovaginitis in children; suppurative prostatitis; and rupture of an intraperitoneal or visceral abscess, such as renal or perinephric, tubo-ovarian, liver, splenic, or pancreatic abscess. Peritonitis is a major hazard of continuous ambulatory peritoneal dialysis (CAPD) used in the management of renal failure, fluid and electrolytic imbalance, and certain intoxications. Bacterial peritonitis commonly occurs secondary to the use of peritoneovenous and ventriculoperitoneal shunts.[89]

Peritoneal signs suggestive of appendicitis in immunocompromised patients (e.g., patients with acquired immunodeficiency syndrome, organ transplant recipients, patients receiving chemotherapy or corticosteroids for neoplasms, especially myelosuppressive drugs) may be caused by typhlitis, also referred to as *neutropenic enterocolitis,*[90] an inflammation of the cecum. Cecal ulceration in these patients may progress to perforation and secondary peritonitis with colonic flora.

Tertiary peritonitis has been conceptualized as a later stage in the disease, when clinical peritonitis and systemic signs of sepsis persist after treatment for primary or secondary peritonitis. Many times no organisms or low-virulence pathogens, such as enterococci (including vancomycin-resistant strains), coagulase-negative *Staphylococcal* species, Enterobacteriaceae, anaerobes, and fungi (mostly *Candida* species), are isolated from the peritoneal exudate.[9,91] This category of peritoneal infection is more common among critically ill or immunocompromised patients and is characteristically without a surgically treatable focus, following an earlier surgical intervention and source control. In health care–associated intraabdominal infections, which typically encompass tertiary peritonitis, more resistant nosocomial pathogens may also be playing a major role in the infectious process.[9] These organisms may gain access to the peritoneal cavity through contamination during operative interventions, through selection from the initial polymicrobial peritoneal inoculum by antibiotic therapy, or through translocation of bowel flora.

TABLE 74.3 Causes of Secondary Peritonitis

Distal esophagus	Boerhaave syndrome Malignancy Trauma Iatrogenic[a]
Stomach	Peptic ulcer perforation Malignancy Trauma Iatrogenic[a]
Duodenum	Peptic ulcer perforation Trauma Iatrogenic[a]
Biliary tract	Cholecystitis Stone perforation from gallbladder or common duct Malignancy Trauma Iatrogenic[a]
Pancreas	Pancreatitis (e.g., alcohol, drugs, gallstones) Trauma Iatrogenic[a]
Small bowel	Ischemic bowel Incarcerated hernia Crohn disease Malignancy Meckel diverticulum Trauma
Large bowel and appendix	Ischemic bowel Diverticulitis Malignancy Ulcerative colitis and Crohn disease Appendicitis Volvulus Trauma (mostly penetrating) Iatrogenic[a]
Uterus, salpinx, and ovaries	Pelvic inflammatory disease (e.g., salpingo-oophoritis, tubo-ovarian abscess, ovarian cyst) Trauma Malignancy Iatrogenic[a]

[a]Dehiscence of surgical anastomosis or inadvertent injury during a procedure (e.g., endoscopy).

Modified from Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clin Infect Dis. *2010;50:133–164.*

Microbiologic Characteristics

Infrequently, exogenous microorganisms, such as *S. aureus, N. gonorrhoeae,* or *M. tuberculosis,* which cause infection in intraabdominal or adjacent viscera and spread to involve the peritoneum, cause secondary peritonitis. Most cases of secondary peritonitis are endogenous in origin, however, and are caused by the large number and variety of microorganisms that normally colonize mucous membranes lining certain viscera within the abdominal cavity. Characteristically, secondary peritonitis is a polymicrobial infection involving both facultative and obligate anaerobes. Although forming a continuous surface, the mucous membranes of the stomach, upper small bowel, lower small bowel, and large bowel each have characteristic microbiota in terms of type of microbial species, total number of different species, and microbial density. The vagina also has distinct microbiota. Normally, invasive activities of indigenous bacteria are controlled by the intact mucosa of the gastrointestinal tract and vagina. Disturbances in this mucosal barrier can occur as a result of spontaneous disease, trauma, or surgical operations that permit escape of indigenous bacteria and cause an infection of the peritoneum, the abdominal viscera, or the retroperitoneal space. The frequency with which various indigenous organisms are found in intraabdominal infections varies according to the site of the primary process and whether the primary process is associated with an alteration of the indigenous microbiota. Changes in the microflora may result from previous antibiotic therapy, the use of other medications (e.g., those that modify the acidity or alkalinity of gastric and intestinal secretions), and specific host factors, such as any degree of immunodeficiency. In addition, the anticipated microbiota in these infections is determined by whether the infection is community acquired or health care associated. In community-acquired intraabdominal infections, the location of the inciting event generally defines the infecting microbiota, whereas intraabdominal infections categorized as health care associated often involve nosocomially acquired pathogens specific to the diseased organ or postoperative event and at least one multidrug-resistant pathogen.[92,93] The prevalence of Enterobacteriaceae other than *E. coli,* or non–lactose-fermenting gram-negative aerobic bacilli (e.g., *Pseudomonas* species), is increasing. In addition to aerobic streptococci, health care–associated intraabdominal infections in postsurgical patients who

received prior antimicrobial therapy are more likely to involve other gram-positive cocci such as enterococci and staphylococci, some of which may be vancomycin and methicillin resistant. The risk of *Candida* species being present is also increased in these clinical settings. Over time, the bacterial species in community-acquired infections have not significantly changed. However, depending on the patient's geographic location, the antimicrobial susceptibility patterns of these microorganisms, particularly among the Enterobacteriaceae group, have significantly changed.[94] Once rare, extended-spectrum β-lactamase producing *E. coli* and *Klebsiella* species are now increasingly encountered in community-acquired intraabdominal infections.[95]

The bacteria that cause intraabdominal infections are derived from the indigenous microbiota of the gastrointestinal tract. Because gastrointestinal perforation is the most common precipitating event, knowledge of the types and quantities of the normal microflora at these various anatomic sites is key to understanding the spectrum of intraabdominal infections that may ensue.[96] The stomach normally contains 10^3 colony-forming units (CFUs) of microorganisms per milliliter in the fasting state. If bacteria are present, they consist of a few relatively more acid-resistant species or yeast made up mostly of facultative, gram-positive, salivary microorganisms, such as *Candida* spp., lactobacilli, and oral streptococci derived from oropharyngeal flora. The numbers of these organisms in stomach contents increase transiently after a meal. Gastric microbiota is more numerous and may be composed of different organisms when achlorhydria is present (e.g., from type 2 antihistamine or proton pump inhibitor medications), obstruction exists, or blood is in the stomach. Under these circumstances, gastric colonization with oropharyngeal anaerobes such as *Prevotella,* non-*fragilis Bacteroides,* and *Fusobacterium* spp., along with facultative organisms, such as viridans streptococci, microaerophilic streptococci, *Candida* spp., and lactobacilli, may greatly increase. Because of the cleansing activity of gastric acidity and rapid small bowel motility, the duodenum and proximal small bowel contain a sparse microbiota in the fasting state, mostly consisting of salivary microorganisms. In the presence of achlorhydria, intestinal obstruction, or other processes affecting intestinal motility or absorption, however, the microbiota of the small intestine is more profuse and varied. Conditions conducive to small bowel stasis include scleroderma, regional enteritis, small bowel strictures, nontropical sprue, tropical sprue, duodenal and jejunal diverticula, presence of an afferent loop of the Billroth II gastrojejunostomy, and intestinal pseudo-obstruction. Large-bowel flora has been found in the proximal small bowel of cirrhotic patients. The ileum normally contains *E. coli,* enterococci, and an equal number of microorganisms that are obligately anaerobic, such as *B. fragilis.* It is the colon, however, in which profuse microflora exist in concentrations of about $10^{11–12}$ bacteria per gram of feces, a wet sludge of practically pure bacteria. The colonic microbiota is composed predominantly of the obligate anaerobes *B. fragilis* and *Bifidobacterium* spp., which outnumber facultative microorganisms, primarily *E. coli,* by 10^3:1 to 10^4:1. Other colonic bacteria are *Streptococcus viridans* and other streptococci, enterococci, *Eubacterium* spp., *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., and *C. perfringens.* The large-bowel microbiota are relatively stable but may be altered significantly by antibiotic therapy or in patients who have been hospitalized recently or have spent time in other health care facilities.[1,97] Under these circumstances, organisms such as *P. aeruginosa* and *Acinetobacter* spp. may be commonly isolated in hospital-acquired intraabdominal infections.[93] In addition, the prevalence of multidrug-resistant gram-negative bacilli, especially those that produce ESBLs, has significantly increased.[98] The differences in microorganisms observed when the source is in the upper versus lower gastrointestinal tract may partially account for the differences in severity of septic complications after injuries or diseases along the gastrointestinal tract, with sepsis resulting from the more proximal gastrointestinal tract causing less morbidity and mortality than that seen with colonic disease processes.

With loss of the integrity of the mucosal barrier at some point along the gastrointestinal tract, a variable amount of bacteria (in terms of bacterial density and number of different species) is found in the peritoneal cavity, depending on the level of the mucosal defect and comorbid conditions. With perforation of the colon, initially a total of more than 10^{11} CFUs/mL of hundreds of different species spills into the peritoneal cavity. A simplification of the microflora occurs so that when peritoneal infection is established, only about five species are isolated from peritoneal exudate, usually three anaerobic and two aerobic species, even when care is exercised to ensure recovery of the obligate anaerobes. The obligate anaerobes isolated from clinical specimens have been found to be more oxygen tolerant and to have more identifiable virulence factors than the rest of the anaerobic microflora in the gut. The facultative anaerobes isolated also have virulence factors. *B. fragilis* is the obligate anaerobe most frequently isolated after colonic perforation, and *E. coli* is the facultative anaerobe most frequently isolated.

As would be anticipated from the nature of the gastrointestinal microbiota, anaerobes are recovered in 96% of cases of peritonitis secondary to acute appendicitis with perforation. *Prevotella melaninogenica* and anaerobic gram-positive cocci are the most frequent isolates. With the use of modern bacteriologic techniques that provide an anaerobic environment during collection, transport, and incubation, studies of the bacteriologic characteristics of intraabdominal infections[99–101] have confirmed the findings that anaerobes play a major role. Finegold[99] reported that in a series of 73 intraabdominal infections, including 16 cases of peritonitis, there were on average 4.5 isolates per case (range, 1–12 organisms), with 2.5 anaerobes and 2 aerobes or facultative organisms. The most common isolate was *E. coli,* followed by *B. fragilis* (the most common anaerobic isolate), enterococci, other *Bacteroides* spp., *Fusobacterium, C. perfringens,* other clostridia, *Peptococcus* spp., *Peptostreptococcus* spp., and *Eubacterium* spp. Similar findings were reported by Gorbach and coworkers[100] in a series of 43 patients, including 10 with peritonitis, in 93% of whom anaerobes or a mixture of anaerobes and facultative organisms were isolated, and by Swenson and colleagues[102] in a series of 64 patients, including 26 with peritonitis, in 81% of whom anaerobes were isolated. In these series, bacteremia was reported in 20% to 30% of patients. Organisms recovered from blood frequently included *B. fragilis* or *E. coli.* In a series of patients with *Bacteroides* bacteremia, 14% to 62% had a gastrointestinal source.[103] Table 74.4 lists the major bacterial organisms identified and their approximate prevalence from multiple complicated intraabdominal infection antibiotic trials.[1,2,104]

In a study of perforated appendicitis in which careful anaerobic culture techniques were used, an average of 9.4 species of anaerobes were isolated from each patient.[105] *Bilophila wadsworthia,* an anaerobic gram-negative bacillus, was the fourth most common obligate anaerobe isolated and was found in one-third of patients with gangrenous appendicitis and half of patients with perforated appendicitis.[106]

Relatively antibiotic-resistant organisms, such as *Candida* spp., enterococci, *Enterobacter* spp., *Serratia* spp., *Acinetobacter* spp., and *P. aeruginosa,* are isolated more frequently from patients whose intraabdominal infection developed while they were in the hospital, after they received broad-spectrum antimicrobial agents.[107,108] Reported isolation rates for these uncommon intraabdominal pathogens vary; one large study found that nonfermenting gram-negative bacteria (*P. aeruginosa, Stenotrophomonas maltophilia,* and *Acinetobacter baumannii*) accounted for less than 6% of all intraabdominal infections, and a second study found *P. aeruginosa* in about 9% of patients diagnosed with secondary peritonitis.[109,110] Several studies have revealed, however, that *P. aeruginosa* makes up a more significant portion of the aerobic isolates in community-acquired intraabdominal infections[111,112] than had been noted previously. Whether or not the isolation of *P. aeruginosa* represents contamination rather than a true pathogen is subject to question, with one study demonstrating that coverage of *Pseudomonas* did not add to higher rates of clinical success.[110]

Monomicrobial infections with microorganisms that have relatively low pathogenicity, such as *Candida* spp., enterococci, or coagulase-negative staphylococci, have also been observed in what was thought to be new-onset peritonitis in patients with severely impaired defenses.[113] Penetrating injuries to the liver and spleen are followed by infection only rarely because of the usual sterility of these organs.[114]

Quantitative studies[115,116] of sexually active women of childbearing age have revealed that the predominant vaginal microbiota are composed of five to seven different microorganisms and that anaerobes are approximately 10 times more numerous than facultative organisms. There are about 10^8 to 10^9 CFUs of anaerobes and about 10^7 to 10^8 CFUs of facultative organisms per milliliter of vaginal secretions. The most

TABLE 74.4 Organisms That Cause Complicated Intraabdominal Infections

ORGANISM	PATIENTS (%)
Aerobes only[a]	17
Anaerobes only[a]	1
Anaerobes + aerobes[a]	82
Facultative and Aerobic Gram-Negative Bacilli[b]	
Escherichia coli	71
Klebsiella spp.	14
Pseudomonas aeruginosa	14
Proteus mirabilis	5
Enterobacter spp.	5
Anaerobic[b]	
Bacteroides fragilis	35
Other *Bacteroides* spp.	71
Clostridium spp.	29
Prevotella spp.	12
Peptostreptococcus spp.	17
Fusobacterium spp.	9
Eubacterium spp.	17
Gram-Positive Aerobic Cocci[b]	
Streptococcus spp.	38
Enterococcus faecalis	12
Enterococcus faecium	3
Enterococcus spp.	8
Staphylococcus aureus	4

[a]*From Stone HH, Strom PR, Fabian TC, et al. Third-generation cephalosporins for polymicrobial surgical sepsis.* Arch Surg. *1983;118:193–200.*
[b]*Modified from Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America.* Clin Infect Dis. *2010;50:133–164.*

frequent isolates in titers of 10^5 per milliliter or more are obligate or facultative anaerobic lactobacilli, streptococci, anaerobic gram-positive cocci, Bacteroidaceae other than *B. fragilis* (e.g., *P. melaninogenica, Prevotella bivia, Prevotella ruminicola*), and a group of unidentified catalase-negative facultative bacilli. Diphtheroids and *Staphylococcus epidermidis* also have been found to be frequent vaginal isolates. When specifically looked for, *Gardnerella vaginalis* in high counts has also been found to be only slightly less common than lactobacilli in the vaginal secretions of healthy women.[115,116]

Colonic organisms, such as *B. fragilis,* Enterobacteriaceae, and enterococci, are found rarely as predominant components of the normal vaginal microbiota and probably proliferate at this site only under exceptional circumstances. These organisms tend to appear in vaginal secretions in the immediate postoperative period after vaginal operations, and *C. perfringens* is found more frequently in vaginal secretions after difficult labor or abortion. *Bacteroides* spp. and anaerobic gram-positive cocci are infrequent commensals in the vagina of healthy women prenatally, whereas during the puerperium these organisms are found to be more prevalent in women with postpartum endometritis than in noninfected women.[117] Factors favoring colonization by these anaerobes after surgery and in the puerperium are unknown but are possibly related to blood or necrotic tissue, which provides the reduced, enriched environment required by these anaerobes.

Sequential sampling of vaginal secretions during the menstrual cycle reveals constant levels of anaerobes, although recovery of specific organisms varies from specimen to specimen in each individual woman. In contrast, levels of facultative organisms decrease 100-fold in the premenstrual week. This variation in microbiota may reflect cyclic fluctuation in the vaginal environment caused by changes in hormonal activity during the menstrual cycle. Because the vaginal microbiota vary under certain conditions, and members of these flora have differing pathogenicity, the frequency of indigenous intraabdominal infections of gynecologic origin and the types of pathogens involved vary accordingly. The frequency of vaginal colonization with group B streptococci increases during pregnancy, and infections caused by these organisms are relatively common in the postpartum period. In addition, in women with trichomoniasis, *Bacteroides* spp. may be found more often in vaginal secretions.[116] Postpartum infection, presumably caused by anaerobes, has been reported to be more common in women who had trichomoniasis during pregnancy.[118]

The bacteriologic characteristics of intraabdominal infections that complicate infections of the female genital tract are similar to those of secondary peritonitis from a gastrointestinal source except for the occurrence of *N. gonorrhoeae* in cul-de-sac aspirates. According to data compiled by Swenson and associates,[119] Thadepalli and coworkers,[120] and Chow and colleagues,[121] anaerobes were found in 72% of 200 gynecologic infections. Anaerobes were especially frequent (92%) in closed space infections, such as tubo-ovarian and pelvic abscesses. *Bacteroides* spp., particularly *B. fragilis* and *P. melaninogenica,* and anaerobic gram-positive cocci were the most frequently isolated anaerobes. *E. coli* and streptococci were the most prevalent facultative organisms. Bacteriologic studies have shown the presence of anaerobes, usually gram-positive cocci in cul-de-sac aspirates, in most patients, even those with acute salpingitis, despite the recovery of gonococci from the endocervix.[122] The data are interpreted as supporting the concept of superinfection with anaerobes late in the course of this disease, after initial infection with *N. gonorrhoeae.*[123] In children, gonococcal peritonitis has been reported only rarely with gonococcal vulvovaginitis.[124]

Intraperitoneal rupture has been reported in 10% of cases of amebic liver abscess, and it may cause acute generalized peritonitis or, less commonly, a localized intraperitoneal abscess, with a mortality rate of about 18%.[125] Perforation of the colon with bacterial peritonitis resulting from fulminant amebic colitis is also unusual but often fatal. Similarly, *Strongyloides stercoralis* infestation of the small bowel may, in rare cases, cause fatal peritonitis, with or without concurrent bacterial contamination.[126] Intestinal perforation complicating penetrating cytomegalovirus enterocolitis has been described as a cause of an acute abdomen in these patients. *Candida* spp. have been isolated from the abdominal fluid of patients undergoing peritoneal dialysis, as have *S. aureus,* Enterobacteriaceae, and *P. aeruginosa.*[127] *Candida* peritonitis may also be observed as a complication of gastrointestinal surgery or in perforation of a viscus,[128] and its occurrence is related to numerous factors that increase the rate of colonization of *Candida* organisms in the gastrointestinal tract. These factors include immunosuppression, prolonged hospitalization, and antimicrobial or antacid therapy. *Candida* spp. are most commonly isolated from the peritoneum after perforation of a gastric or duodenal peptic ulcer or after spillage of colonic contents into the peritoneum as a result of trauma, mesenteric artery occlusion, or dehiscence of a surgical anastomosis.

Pathogenesis

The virulence of the bacteria that cause peritonitis is enhanced when certain microorganisms are either combined intraperitoneally with substances such as mucus, enzymes, or hemoglobin or are combined with certain other microorganisms. Chemical peritonitis can be produced by escape of bile or of gastric or pancreatic secretions into the peritoneal cavity. When gastric acid escapes into the peritoneal cavity, there is an outpouring of serum protein and electrolytes from the blood into the peritoneal cavity. The acidity is neutralized quickly by these buffers and by diffusion of hydrogen ions into the body fluids. Widespread necrosis may result from enzymatic digestion after intraperitoneal spillage of potent pancreatic enzymes. Escape of bile into the peritoneal cavity is generally considered to be a grave, often fatal situation. The severity of peritonitis after escape of these intestinal secretions results in subsequent bacterial peritonitis. In a dog model with experimentally produced partial biliary diversion into the peritoneal cavity, fatal effects were reduced by oral nonabsorbable or parenteral antibiotics.[129] Bacteria may enter

the peritoneal cavity with contaminated intestinal secretions through perforations in the gastrointestinal wall or by migration through the wall of the intact gastrointestinal tract in response to irritation of the serosal surface by bile and possibly other intestinal tract secretions.

Establishment of an anaerobic infection requires an environment in which the oxygen tension is low, the oxidation-reduction potential is low, and abundant nutrients are available to support anaerobic metabolism. Obligate anaerobes are sensitive to oxygen in the molecular form and to bound oxygen, as in organic peroxides. Survival and growth of anaerobes also depend on the oxidation-reduction potential (i.e., the oxidizing capacity of the environment). Most pathogenic anaerobes require a negative potential of at least −150 mV. Low oxidation-reduction potentials are thought to occur in many abscesses, and oxidation-reduction potentials of −150 mV or less are measured in abscesses from which anaerobes are recovered.[130] Some anaerobic organisms have additional requirements, such as vitamin K, arginine, serum, blood pigments, or bile, before they can grow. These requirements are usually met by tissue devitalized as a consequence of ischemia, trauma, or neoplastic growth. When proper conditions are obtained, anaerobic organisms can achieve doubling rates equivalent to rates seen with aerobic enteric bacilli. In vivo, the rapidly expanding bacterial and inflammatory cell mass, frequently accompanied by gas production, can interrupt the blood supply to the immediately surrounding tissue and cause further tissue necrosis.

Gram-negative anaerobic cocci and bacilli (including *B. fragilis* and *P. melaninogenica*) possess endotoxins, although with much weaker biologic activity in comparison with endotoxins extracted from their aerobic counterparts, and they have low or absent 2-keto-3-deoxyoctanoate content. In addition, certain anaerobes elaborate collagenase, other proteolytic enzymes, and deoxyribonuclease. Certain Bacteroidaceae are capable of degrading heparin, a capability that may be responsible for the suppurative thrombophlebitis frequently seen in infections caused by these microorganisms. These factors tend to provide more areas well adapted to the growth requirements of the anaerobe; as a result, the infection progresses.

In addition, anaerobes may be resistant to host defenses. PMN leukocytes have been shown to have bactericidal activity under aerobic and anaerobic conditions against several anaerobic species, including *B. fragilis,* presumably by mechanisms other than those dependent on the superoxide anion O_2^- or H_2O_2. However, Keusch and Douglas[131] found that granulocytic killing of *C. perfringens* was impaired under anaerobic conditions. Also, the capsule demonstrated on *B. fragilis* and *Porphyromonas asaccharolytica* (formerly *Bacteroides melaninogenicus* subsp. *asaccharolyticus*) might protect the organisms from phagocytosis and favor abscess formation.[132] Some anaerobes, especially *B. fragilis,* may be resistant to the normal bactericidal activity of serum.

Many anaerobic infections seem to be synergistic. Although it is probable that most bacteria isolated in mixed infections are nonpathogenic by themselves, their presence nevertheless may be essential for the pathogenicity of the bacterial mixture. These examples of bacterial synergism in infection were shown in periodontal infection by Socransky and Gibbons[133] and in peritonitis by Altemeier.[134]

Facultative organisms in mixed infections may be essential because they provide a sufficiently reduced environment for the growth of obligate anaerobic organisms. Another mechanism of bacterial synergy is the generation of a substance by one organism that is essential for the growth of another (e.g., the production of vitamin K, a required growth factor for *P. melaninogenica,* by diphtheroids). Anaerobes such as *Bacteroides* spp. also have shown the ability to protect aerobic bacteria from phagocytic killing[135] and from otherwise effective antibiotic therapy (e.g., via β-lactamase production).[136]

In addition, each component of the pathogenic mixture may contribute in different ways to the clinical picture. In a series of experiments, Weinstein and coworkers[137] clarified the sequence of events that occurs after contamination of the peritoneum with fecal flora. In this model, after implantation of fecal contents intraperitoneally into rats, Onderdonk and associates[138] observed that *E. coli* initially predominated in the peritoneal exudate. Bacteremia, caused by *E. coli* during this phase, was uniformly present and frequently fatal. Rats that survived developed indolent intraabdominal abscesses in which *B. fragilis* predominated. Elimination of *E. coli* by early administration of gentamicin reduced early mortality rates but did not prevent late intraabdominal abscess caused by obligate anaerobes; elimination of obligate anaerobes with clindamycin did not prevent early mortality from *E. coli* bacteremia, but it did reduce late abscess formation in survivors. These findings indicate that although *E. coli* is responsible for early mortality, *B. fragilis,* in concert with *E. coli* and perhaps other microorganisms (e.g., enterococci),[139] is responsible for late intraperitoneal abscess formation. This synergy between obligate and facultative anaerobes has long been recognized in mixed infections.[140] Studies in animal models of intraperitoneal infection have also shown that bacterial interactions with host components, specifically activation of IL-17–producing $CD4^+$ T lymphocytes by constituents of abscess-inducing bacteria such as *B. fragilis,* are necessary for abscess formation.[141]

Pathophysiologic Responses

Whatever the initiating cause of peritonitis, a similar series of reactions takes place locally and systemically.

Local Response

The local inflammatory response of the peritoneum is similar to that in other tissues, but the peritoneal lining presents a large exudative and absorptive surface. At sites of irritation, there is an outpouring of fluid into the peritoneal cavity that, in contrast to normal serous fluid, has a high protein content (>3 g/dL) and many cells, primarily PMN leukocytes, that phagocytose and kill organisms. The exudate contains fibrinogen that polymerizes, and plaques of fibrinous exudate form on the inflamed peritoneal surfaces. This exudate glues together adjacent bowel, mesentery, and particularly omentum. Localization of the inflammatory process is aided further by inhibition of motility in involved intestinal loops. Experiments have demonstrated that radiopaque medium injected intraperitoneally at one locus spreads over much of the greater peritoneal sac within a short time. The extent and rate of intraperitoneal spread of contamination depend on the volume and nature of the exudate and on the effectiveness of the localizing processes.

If peritoneal defenses aided by appropriate supportive measures control the inflammatory process, the disease may resolve spontaneously. A second possible outcome is a confined abscess. A third course results when the peritoneal and systemic defense mechanisms are unable to localize the inflammation, which progresses to spreading diffuse peritonitis. Factors favoring spread of the inflammatory process are (1) greater virulence of bacteria, (2) greater extent and duration of contamination, and (3) impaired host defenses.

The cytokine response in peritonitis has been the subject of excellent reviews.[142,143] Many of the local abdominal and systemic manifestations of peritonitis undoubtedly are mediated by cytokines, including TNF-α, IL-1, IL-6, and IFN-γ. Cytokines appear in the peritoneal exudate to a much greater extent than in systemic circulation of patients with peritonitis.[144] These cytokines are produced by macrophages and other host cells in response to bacteria or bacterial products, such as endotoxin, and by tissues traumatized during abdominal operative procedures.

Cytokine responses have been studied in the peritoneal exudate in experimental animal models of peritonitis,[51] in patients with spontaneous bacterial peritonitis,[145] in patients undergoing CAPD,[146] and in patients with severe secondary bacterial peritonitis who were undergoing planned relaparotomy.[147] Anti-TNF antibody failed to prevent death[148] and failed to reduce serum levels of IL-1 and IL-6 in an experimental model of peritonitis.[149] In contrast, in this model, antiendotoxin antibodies were found to prevent death[150] and to reduce bacterial numbers in the peritoneal exudates and serum levels of TNF, IL-1, and IL-6. Anti-IFN-γ antibodies also afforded a protective effect in this model of experimental peritonitis and after intravenous injection of endotoxin.[151]

Systemic Response

Peritonitis leads to changes not only locally in the peritoneal cavity but also throughout the body.

Gastrointestinal. Peritonitis begins with hypermotility and is followed by paralysis of the bowel. Accumulation of fluid and electrolytes in the lumen of the adynamic bowel continues until distention is sufficient to inhibit capillary inflow and secretion ceases.

Cardiovascular. Because of the large surface area of the peritoneum, shifts of fluid into the peritoneal cavity, combined with fluid shifts into the bowel lumen, can produce a profound decrease in circulating blood volume and elevation of the hematocrit. Fluid and electrolyte loss is exaggerated further by coexistent fever, vomiting, diarrhea, and loss of aspirated gastrointestinal fluid. As the process continues, the decreased venous return to the right side of the heart leads to a decrease in cardiac output, with resulting hypotension. Usually there is evidence of increased adrenergic activity, such as sweating, tachycardia, and cutaneous vasoconstriction (i.e., cold, moist skin and mottled, cyanotic extremities).

With adequate replacement of blood volume, cardiac output may be maintained above normal. Cardiac output of two or three times normal may be necessary to satisfy the increased metabolic needs of the body in the presence of infection. Failure to sustain increased cardiac output results in progressive lactic acidosis, oliguria, hypotension, and ultimately death if the infection cannot be controlled.

Respiratory. The intraperitoneal inflammation results in relatively high and fixed diaphragms and considerable pain on respiration. This condition results in basilar atelectasis with intrapulmonary shunting of blood. Satisfactory compensation is possible only if the increase in energy demands does not exceed the respiratory reserve. Heavy cigarette smoking, chronic bronchitis, emphysema, and obesity compound the problem. With decompensation in respiratory function, hypoxemia is accompanied first by hypocapnia (respiratory alkalosis) and later by hypercapnia (respiratory acidosis). In some patients, pulmonary edema develops, not because of left ventricular failure but perhaps because of increased pulmonary capillary leakage as a consequence of hypoalbuminemia or direct effects of bacterial toxins (adult respiratory distress syndrome). In these patients, progressive hypoxemia develops with decreasing pulmonary compliance. This condition necessitates volume-cycled ventilatory assistance with increasingly higher concentrations of inspired oxygen and positive end-expiratory pressure.

Renal. Low renal perfusion may be followed by acute tubular necrosis and progressive azotemia.

Metabolic. The excretion of cortisol is increased during the first few days of peritonitis and subsequently returns to normal. The increased energy demands of infection rapidly deplete body stores of glycogen; this leads to catabolism of protein (muscle) and fat, which accounts for the rapid weight loss of severely infected patients. Prolonged intra-abdominal infection is associated with extreme wasting. Heat production eventually may fail, and then body temperature decreases. Exhaustion and death may ensue.

Clinical Manifestations

Symptoms

The early manifestations of peritonitis that results from disease of abdominal viscera are frequently those of the primary disease process. Moderately severe abdominal pain is almost always the predominant symptom. Any motion, even respiration, aggravates the pain. The progression of abdominal pain is a function of the rate of dissemination of the material producing the pain stimulus. Rupture of a peptic ulcer with massive spillage of gastric contents produces severe epigastric pain that within minutes may spread to involve the entire abdomen. In contrast, the spread of pain from a lesion such as a ruptured appendix is much more gradual. Decreased intensity and extent of pain with time usually suggest localization of the inflammatory process.

Anorexia, nausea, and vomiting commonly accompany abdominal pain. Patients may also complain of feverishness, sometimes with chill, thirst, scanty urination, inability to pass feces or flatus, and abdominal distention.

The formation and progression of an intraperitoneal abscess are often gradual. The patient who seemed to be recovering from peritonitis or an abdominal operation stops improving, fever returns, and localizing symptoms may develop.

Physical Findings

Patients with peritonitis characteristically lie quietly in bed, supine, with the knees flexed and with frequent limited intercostal respirations because any motion intensifies the abdominal pain. The patient is alert, restless, and irritable early in the course but later may become apathetic or delirious.

Body temperatures may reach 42°C. A subnormal temperature of about 35°C is often present in the early stages of chemical peritonitis. This is a grave sign late in the course in patients with continuing intraabdominal sepsis or septic shock.

Increasing tachycardia with weak, thready peripheral pulses reflects decreased effective blood volume. The blood pressure is maintained within normal limits early in the disease process. As peritonitis progresses, the blood pressure decreases to shock levels. Respiration is increasingly rapid and shallow.

Marked abdominal tenderness to palpation is present, usually maximally over the organ in which the process originated. Direct and referred rebound tenderness signifies parietal peritoneal irritation. This finding is sometimes more accurate than direct palpation in locating the point of maximal tenderness and delineating the extent of peritoneal irritation.

Muscular rigidity of the abdominal wall is produced by voluntary guarding and reflex muscular spasm. Hyperresonance caused by gaseous intestinal distention can usually be demonstrated with percussion. Pneumoperitoneum from a ruptured hollow viscus may produce decreased liver dullness to percussion. Bowel sounds, initially hypoactive, later disappear. Rectal and vaginal examination may reveal tenderness and the presence of a pelvic abscess and may indicate a primary focus in the female pelvic organs.

Abdominal pain and muscle spasm may be deceptively absent in some patients. Patients with lax abdominal musculature (e.g., patients in the postpartum period, patients with ascites caused by cirrhosis, patients with marked cachexia) may not have abdominal rigidity. Similarly, patients who are in shock, who are receiving glucocorticosteroid therapy, or in whom loculated intraabdominal abscesses are not in contact with the anterior abdominal wall (e.g., subphrenic, lesser sac, pelvic abscesses) may not exhibit marked abdominal pain and spasm. Absence of bowel sounds may be the only manifestation of peritonitis in these patients, and a high index of suspicion is necessary.

Diagnostic Studies

The differential diagnosis in patients with symptoms and signs of peritonitis includes pneumonia, sickle cell anemia, herpes zoster, diabetic ketoacidosis, tabes dorsalis, porphyria, familial Mediterranean fever, plumbism, systemic lupus erythematosus, and uremia. Routine laboratory studies include a complete blood count, serum chemistry profile, liver profile, coagulation studies, urinalysis, and amylase and lipase determinations. If an ectopic pregnancy is suspected, a urinary β-human chorionic gonadotropin (hCG) determination will be necessary. A peripheral blood leukocyte count of more than 11,000 cells/μL is usual in the majority of patients with acute peritonitis; the differential count exhibits PMN predominance and a moderate to marked shift to the left. Reliance on the significance of the total leukocyte count may be misleading, however. Massive peritoneal inflammation can mobilize leukocytes into the diseased area; there may be fewer than 5000 leukocytes/mL in the circulating blood, but the differential smear in this situation may show an extreme shift to immature PMN forms.

Hemoconcentration and dehydration are reflected by increased hematocrit and blood urea nitrogen values. Patients with severe sepsis who have disseminated intravascular coagulopathy (DIC) may have thrombocytopenia and elevated prothrombin time (PT), partial thromboplastin time (PTT), or D-dimer concentration. Blood chemistries may reveal dehydration, azotemia, and acidosis. Hyperglycemia and glycosuria are usually not present in peritonitis but may be present in diabetic acidosis and acute pancreatitis, which can manifest with signs suggestive of peritonitis. Hematuria and pyuria without bacteriuria may reflect intraabdominal inflammatory disease, such as appendicitis adjacent to the urinary tract. Levels of serum amylase and lipase may be elevated in peritonitis from almost any cause, but levels are very high (greater than three times the upper limit of normal for either enzyme) only in acute pancreatitis. Serum amylase and lipase levels may be elevated in the absence of pancreatitis because they can be caused by transmural absorption in intestinal infarction and transperitoneal absorption with perforated viscus and peritonitis. Hyponatremia may occur in patients

given water to replace isotonic fluid losses, but it is also characteristic of porphyria. Metabolic and respiratory acidosis is present in severe and late peritonitis.

Supine, upright, and lateral decubitus radiographs of the abdomen may reveal distention of the small intestine and the colon, with adynamic loops of bowel or features of mechanical intestinal obstruction, volvulus, intussusception, or vascular occlusion. Inflammatory exudate and edema of the intestinal wall produce a widening of the space between adjacent loops. Peritoneal fat lines and psoas shadows may be obliterated. Free air may be visible if a viscus is ruptured. CT scans are frequently obtained as initial studies in many centers and provide superior detail of intra-abdominal processes. Chest radiographs always should be obtained to investigate whether a pulmonary or thoracic problem is the cause of abdominal distress. The presence of air beneath the diaphragm may be defined best in these images. Trapped gas with a fluid level or mottling caused by gas may be visible in intraperitoneal or visceral abscesses. Calcification in the gallbladder or other organs may also be noted on radiographs.

Ultrasonography is frequently the initial step in evaluating intra-abdominal sepsis, especially for detecting processes in the right upper quadrant, retroperitoneum, and pelvis.[152] Dilated air-filled loops of bowel, obesity, overlying lungs, bandages, wounds, drains, and ostomies can interfere with the quality of ultrasound images. CT of the entire abdomen and pelvis, with oral and intravenous contrast agents, has become invaluable for evaluating patients with a suspected intraabdominal infection.[153] CT has frequently replaced exploratory laparotomy for this purpose. CT can facilitate detection of lesions outside the suspected area on the basis of clinical findings and guidance of percutaneous drainage of peritoneal fluid or abscesses. Although CT is more costly than ultrasonography, it is the preferred initial study except when the lesion is suspected to be in the right upper quadrant, retroperitoneum, or pelvis.[152,154] Ultrasonography is also more operator dependent than is CT, and abdominal tenderness may preclude the use of the external pressure necessary to visualize the intraabdominal contents adequately. Magnetic resonance imaging (MRI) examination may be particularly useful if additional detail of soft tissue characteristics is necessary or for provision of a three-dimensional depiction of a lesion. It is particularly sensitive for investigation of pancreatic, hepatic, or adrenal disease.[155] Laparoscopic evaluation of patients with suspected secondary peritonitis has also been reported to be useful.[156,157]

Needle aspiration of the peritoneal cavity is often helpful. If no fluid can be aspirated, peritoneal lavage with Ringer's lactate solution should be done to obtain fluid for examination. In performing a paracentesis, the operator should avoid the region of abdominal scars, where bowel may be adherent to the underside of the scar. The aspirate is examined grossly for content of blood, pus, bile, or digested fat; chemically for amylase content; and microscopically, with Gram staining, for bacteria. In lower-risk community-acquired infections resulting from processes such as perforated appendicitis, Gram staining and culturing of samples have not been routinely recommended because the encountered flora have generally been susceptible to recommended antibiotic treatment regimens with expected good treatment responses. However, increases in multidrug-resistant aerobic bacilli isolates from community-acquired infections, as seen with *E. coli* strains insensitive to extended-spectrum β-lactam antibiotics and to fluoroquinolones, have hindered the ability to reliably predict the susceptibility of isolates on the basis of knowledge of their presence alone. For other intraabdominal infections, particularly those involving the colon and health care–associated cases, failure rates are substantially higher if empirical therapy is not active against any identified isolate. Therefore, representative specimens collected from the intraabdominal focus of infection should be processed appropriately to best identify the aerobic and anaerobic bacteria present. Furthermore, Gram staining may be of value in these cases in defining the need for specific therapy for possible presence of methicillin-resistant gram-positive organisms.[158] Blood cultures have not proved clinically relevant in community-acquired infections, but they may add diagnostic information in health care–associated and tertiary peritoneal infections. A positive finding of paracentesis is meaningful; a dry or negative finding of paracentesis is of little significance. It is important to exercise caution in interpreting the relevance of bacterial isolates obtained from nonsterile sites including chronic drains or fistulas, especially when collected after prolonged periods of antibiotic exposure. Isolates obtained in this fashion generally represent colonization, demonstrate increased resistance profiles, and in the majority of cases are of no clinical importance. Guidance for the paracentesis may be obtained through ultrasonography or CT.

Prognosis

Survival of a patient with secondary peritonitis depends on many factors, including the age of the patient, nutritional status, albumin levels, comorbid conditions, the presence of malignancy, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, the duration of peritoneal contamination and time to initial intervention, the presence of foreign material (e.g., bile or pancreatic secretions, barium), the primary intraabdominal process and ability to achieve adequate débridement or control of the source of infection, the microorganisms involved, and whether the infection was community or health care acquired.[159–161] Patients with community-acquired peritonitis have been further stratified into lower- and higher-risk categories. Low-risk patients admitted from the community have less than a 10% to 20% incidence of suboptimal outcomes and lower rates of death.[162,163] The more organisms present in peritoneal exudate, the worse the prognosis is, although there appears to be no correlation between severity of infection and the presence of any particular organism. However, involvement with resistant or opportunistic bacterial pathogens that are more likely to occur in health care–acquired or postoperative infections has been determined to be an independent risk factor leading to less favorable outcomes.[164] Individuals who have been hospitalized, resided in a skilled nursing or other long-term care facility, underwent home infusion therapy or wound care, underwent dialysis within the previous 30 days, or received ≥5 days of broad-spectrum antimicrobial therapy during the preceding 90 days are included in the high-risk category as having a greater potential for harboring antibiotic-resistant pathogens.[108,165] Mortality increases with more distal gastrointestinal sources of contamination.[166] In pediatric patients, because of the relatively small omentum, the walling-off process is less effective and diffuse peritonitis occurs more frequently than in adults. In elderly patients, preexisting conditions, such as emphysema, diabetes, or cardiovascular disease, reduce the capacity to meet the demands on the cardiovascular, respiratory, and renal systems during this period of intense metabolic activity. Mortality rates range from 3.5% among patients with early infection caused by penetrating abdominal trauma to more than 60% among patients with established intraabdominal infection and secondary organ failure.[167] Persistent peritoneal contamination; leakage of pancreatic enzymes; septicemia; fluid and electrolyte abnormalities; pneumonia; and cardiovascular, renal, and respiratory failure are the principal causes of death.

With a disease process that has widely varying mortality rates, the ability to predict outcome and stratify severity of disease is important for clinical decision making and for ensuring comparability in evaluating different management strategies with surgical protocols or antimicrobial agents.[168] Outcome has been found to be dependent mainly on host-related phenotypic and physiologic risk factors (e.g., preoperative nutritional status, organ impairment, severity of the patient's systemic response, and the patient's premorbid physiologic reserves, as predicted with the APACHE II scoring system),[169] rather than on type and source of the infection.[170] The Mannheim Peritonitis Index (MPI) is a simple, disease-specific, and easily used scoring system developed to predict the individual prognosis of patients with secondary peritonitis. By assigning weighted points to eight easily determined and simple clinical parameters (age, sex, organ failure, malignancy, preoperative duration of peritonitis >24 hours, origin of sepsis not colonic, diffuse generalized peritonitis, and character of exudate), the MPI score can accurately predict mortality and assist in allocating the intensity of medical and surgical management.[171]

Death from intraabdominal infection, especially when the tertiary phase is reached, is thought to result from an exaggerated, uncontrolled cytokine release that is unresponsive to all therapeutic attempts.[172] This cytokine release has led to the use of endotoxin and cytokine levels in circulation to predict outcome. The magnitude of levels of TNF-α and IL-6 in circulation in patients with peritonitis has not been related invariably to prognosis[173]; however, determination of cytokine levels in

the peritoneal exudate, rather than in blood, has been suggested to reflect better the severity of the compartmentalized peritoneal infection and to predict outcome.[147]

Therapy

Antimicrobial Therapy

Medical management includes use of antimicrobial therapy and supportive measures to maintain vital functions (e.g., improve or maintain circulation, nutrition, and oxygenation to vital organs). The clinical efficacy of immunomodulators, such as anti-TNF-α antibodies, anti-endotoxin antibodies, and IL-1 receptor antagonists, is still unproven. Recombinant human activated protein C (drotrecogin alfa), when used in the treatment of severe sepsis, had been previously demonstrated to reduce the relative risk for mortality.[174] In the subgroup of patients in the original trial who had undergone a surgical procedure and developed severe sepsis of abdominal origin, further analysis revealed an absolute reduction in risk of mortality that was still statistically significant among patients who had received active medication.[175] The greatest benefit was observed in patients with an APACHE II score of 25 points or higher (also see Chapter 75). However, in the fall of 2011, the drug manufacturer, Eli Lilly and Co., voluntarily withdrew this medication from the market in the wake of the release of the PROWESS-SHOCK trial,[176] which found no statistically significant reduction in mortality in patients treated with the sepsis drug compared with placebo.

Secondary peritonitis is typically polymicrobial, and the pathogens in most cases are derived from the gastrointestinal tract, even in patients with a primary gynecologic process. Typically, the facultative microorganisms are *E. coli, Klebsiella* or *Enterobacter* spp., *Proteus* spp., and enterococci, and the obligate anaerobes are *B. fragilis, P. melaninogenica, Peptococcus* spp., *Peptostreptococcus* spp., *Fusobacterium* spp., *Eubacterium lentum,* and *Clostridium* spp. Less commonly isolated pathogens include *S. aureus, P. aeruginosa,* and *Candida* spp.

It is extremely difficult to assess the role of antimicrobial therapy in the outcome of infection caused by anaerobes or by a mixture of anaerobes and facultative microorganisms. Dramatic response to surgical drainage and débridement alone often occurs when infection is localized. Nevertheless, appropriate antimicrobial therapy has been shown to reduce mortality significantly among patients with bacteremic infections caused by Bacteroidaceae or Enterobacteriaceae.[1,103] Antimicrobial drugs are expected to control bacteremia and early metastatic foci of infection, to reduce suppurative complications if the drugs are given early, and to prevent local spread of existing infection. When suppuration has occurred, it may be difficult to cure the infection if antimicrobial drugs are used without drainage; also, antimicrobial drugs used alone may mask some of the clinical manifestations of abscess formation. Some intraabdominal abscesses can, however, be treated successfully with antibiotics alone.[177]

Antimicrobial agents must penetrate to the site of infection in concentrations that are sufficient to overcome the effects of a high bacterial density, the metabolic inactivity and slow growth rate of probably more than 90% of the bacterial inoculum, low pH, low redox potential, necrotic tissue, and bacterial products that may lower the drug's activity. Aminoglycosides and clindamycin are less active at acid pH, aminoglycosides are less active at low redox potentials, and β-lactams are less active against high bacterial densities.

Although the results of many antimicrobial trials for the treatment of intraabdominal infections have been published, caution must be exercised in interpreting these studies because of the possibility of inadequate study design and analysis of data.[178,179] Some of the variables that must be considered are differences in patient populations, types and severity of underlying illnesses, community-acquired versus hospital-acquired infection, and the pathogens isolated. Table 74.5 lists many of the antimicrobial regimens that may be recommended alone or in combination as initial treatment for intraabdominal infections; this list is divided by agents that should be adequate for mild-to-moderate infections and those more appropriate for infections of higher severity. The designation "high risk" describes patients with increased likelihood of treatment failure and a greater potential severity of infection according to clinical assessment criteria.[161] Such patients include those with anatomically unfavorable infections or health care–related infections.[1]

TABLE 74.5 Recommended Agents for Treatment of Complicated Intraabdominal Infections

TYPE OF THERAPY[a]	FOR MILD TO MODERATELY SEVERE INFECTIONS[b]	FOR HIGH-RISK OR HIGHLY SEVERE COMMUNITY-ACQUIRED, HEALTH CARE–ASSOCIATED, AND TERTIARY INFECTIONS[c]
Single Agent		
β-Lactam/β-lactamase inhibitor combinations	Ticarcillin-clavulanic acid Piperacillin-tazobactam	Piperacillin-tazobactam[d]
Fluoroquinolone[e]	Moxifloxacin[f]	Moxifloxacin[f]
Carbapenems	Ertapenem	Imipenem-cilastatin[d] or meropenem or doripenem or meropenem-vaborbactam
Glycylcyclines	Tigecycline[f]	Tigecycline[f]
Fluorocycline	Eravacycline	Eravacycline
Combination Therapy		
Cephalosporin based	Cefazolin, cefuroxime, cefotaxime, ceftriaxone *plus* metronidazole	Third- or fourth-generation cephalosporin (ceftazidime or cefepime) *plus* metronidazole Ceftazidime/avibactam *plus* metronidazole[g] Ceftolozane/tazobactam *plus* metronidazole
Fluoroquinolone based[e]	Ciprofloxacin or levofloxacin, each in combination with metronidazole[g]	Ciprofloxacin in combination with metronidazole[h]
Monobactam based		Aztreonam *plus* vancomycin[f] *or* clindamycin *plus* metronidazole

[a]Empirical antimicrobial regimens should be adjusted according to culture and susceptibility reports to ensure activity against the predominant pathogens isolated in culture.
[b]Applies mainly to low-risk patients with community-acquired intraabdominal infections.
[c]Applies to health care–associated and tertiary peritonitis cases, and some high-risk and severe community-acquired intraabdominal infections. **However, tigecycline should NOT be used as empirical therapy for health care–associated or tertiary infection owing to inactivity of tigecycline against *Pseudomonas aeruginosa.***
[d]In vitro activity may be less, compared to alternative antibiotics; 4.5 g dose recommended.
[e]Fluoroquinolone-resistant *Escherichia coli* has become common in some communities, and fluoroquinolones should not be used unless hospital surveys indicate at least 90% susceptibility of *E. coli* to fluoroquinolones.
[f]Risk of mortality may be greater compared with alternative antimicrobials.
[g]Clinical cure rates were lower than those achieved with meropenem in a phase III trial, with baseline creatinine clearance (CrCL) of 30 to 50 mL/min. However, patients treated with ceftazidime-avibactam received a 33% lower daily dose than is currently recommended for patients with this CrCL.
[h]Because *Bacteroides* strains are resistant to these fluoroquinolones, addition of metronidazole is necessary.

The designation "high risk" is also assigned to community-acquired infections in patients who are elderly, have significant medical comorbidities including immunocompromising conditions, diffuse peritonitis, or delay or inability in achieving adequate débridement or drainage.[2] Travel to global geographic areas with higher rates of resistant or known colonization with such organisms, and a history of extensive or recent antibiotic exposure are also factors that characterize a community-acquired peritoneal infection as high risk.[180,181] No one regimen has been consistently demonstrated to be superior or inferior. Table 74.6 lists antimicrobial agents and regimens that have been adequately studied in clinical trials for use in the treatment of intraabdominal

TABLE 74.6 Antibiotic Dosage for Peritonitis During Peritoneal Dialysis

DRUG	INTERMITTENT (PER EXCHANGE, ONCE DAILY)[a]	CONTINUOUS (ALL EXCHANGES)[b]
Aminoglycosides		
Gentamicin	0.6 mg/kg	LD: 8 mg/L MD: 4 mg/L
Tobramycin	0.6 mg/kg	LD: 8 mg/L
Amikacin	2.0 mg/kg	MD: 4 mg/L
Cephalosporins		
Cefazolin	15 mg/kg	LD: 500 mg/L MD: 125 mg/L
Cefepime	1 g	LD: 500 mg/L MD: 125 mg/L
Ceftazidime	1–1.5 g	LD: 500 mg/L MD: 125 mg/L
Cefotaxime	1 g	LD: 500 mg/L MD: 125 mg/L
Penicillins		
Ampicillin	ND	MD: 125 mg/L
Oxacillin	ND	MD: 125 mg/L
Penicillin G	ND	LD: 50,000 U MD: 25,000 U
Piperacillin	ND	LD: 500 mg/L MD: 250 mg/L
Quinolones		
Ciprofloxacin	ND	LD: 50 mg/L MD: 25 mg/L
Others		
Vancomycin	15–30 mg/kg every 5–7 days[c]	LD: 1000 mg/L MD: 25 mg/L[d]
Aztreonam	ND	LD: 1000 mg/L MD: 250 mg/L
Daptomycin	ND	LD: 100 mg/L MD: 20 mg/L
Linezolid	200–300 mg/day PO	MD: 200–300 mg/day PO
Trimethoprim-sulfamethoxazole	160 mg TMP/800 mg SMX (double strength) 2 × day PO	MD: 160 mg TMP/800 mg SMX (double-strength tablet) 2 × day PO
Combinations		
Ampicillin-sulbactam	2 g every 12 h	LD: 1000 mg/L MD: 100 mg/L
Imipenem-cilastatin	1 g every 12 h	LD: 500 mg/L MD: 200 mg/L
Antifungals[e]		
Amphotericin B	NA	1.5 mg/L
Fluconazole	200 mg every 24–48 h	200 mg/L IP or PO daily

[a]Dwell time for dialysate exchange with antibiotic(s) must be ≥6 hours.
[b]Increase dose by 25% if patient urine output >100 mL/day.
[c]Based on serum drug levels.
[d]Adjust dose based on serum drug levels.
[e]Systemic administration preferred. Fluconazole, 200 mg/day IV or PO; amphotericin B, 0.6 mg/kg per day IV; caspofungin, 70 mg day 1, then 50 mg/day IV; micafungin, 100 mg/day IV; anidulafungin, 200 mg day 1, then 100 mg/day IV; voriconazole, 400 mg twice daily day 1, then 200 mg/day PO.
IP, Intraperitoneal; *LD,* loading dose; *MD,* maintenance dose; *NA,* not applicable; *ND,* no data; *PO,* orally; *SMX,* sulfamethoxazole; *TMP,* trimethoprim.
Modified from Li PK, Szeto CC, Piraino B, et al. ISPD peritonitis recommendations: 2016 update on prevention and treatment. Perit Dial Int. *2016;36(50);481–508; Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2016 update by the Infectious Diseases Society of America.* Clin Infect Dis. *2016;62(4):E1–E50.*

infections. However, most intraabdominal infection antibiotic trials were conducted before the emergence of multidrug-resistant organisms, which are now more frequently encountered as significant pathogens in these infections.[182] Along with this, the determination of antimicrobial susceptibility of bacteria based on interpretive susceptibility breakpoint criteria established by the Clinical Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has been modified in recent years.[93,183] Such alterations may serve to lessen the reliability of previously studied and recommended antibiotics used to treat intraabdominal infections because agents that were once believed to be adequate may now be less dependable, taking into account established pharmacodynamic principles.[184] In general, antibiotics from the carbapenem class have been the most active agents against pathogens involved in intraabdominal infections. Nevertheless, in certain geographic areas the spread of carbapenem-resistant genes, resulting in the emergence of various carbapenem-resistant phenotypes (*K. pneumoniae* carbapenemase [KPC], IMP, Verona integron-encoded [VIM], and New Delhi metallo-β-lactamase [NDM]), has increased and potentially could threaten the available options for treating multidrug-resistant organisms.[185]

Antimicrobial therapy should be started immediately after appropriate specimens (blood and, if possible, peritoneal fluid) are obtained for culture. Antimicrobial therapy is often started before the completion of in vitro antimicrobial sensitivity testing of the specific facultative pathogens. In addition, rapid isolation, identification, and in vitro sensitivity testing of anaerobes, in contrast to testing of facultative bacteria, are not possible in many community hospitals. Several factors account for the delay in obtaining anaerobic bacteriologic results. Infections caused by anaerobes frequently involve mixtures of five or more microorganisms, and cultures require long periods for growth and isolation. In addition, in vitro sensitivity testing by the conventional disk diffusion technique has not been standardized for anaerobes.[130] The results of these tests are influenced to a large extent by the growth rate of the bacteria, inoculum size, pH and type of medium, duration of incubation, and carbon dioxide concentration in the atmosphere. In vitro studies of the stability of the β-lactam antibiotics when exposed to reducing agents such as mercaptoamines (cysteine), which frequently are incorporated in media used for the growth of anaerobes, have revealed that these compounds are able to open the β-lactam ring and to inactivate penicillins. Susceptibility of anaerobic organisms can be determined reliably, however, with the broth or agar dilution technique with the use of appropriate media. Because these tests are generally performed by research laboratories, knowledge about the antimicrobial susceptibility of anaerobes is obtained from periodically published reports on anaerobic isolates by centers that specialize in performing these tests. Initial chemotherapy is usually empirical, based on the most reliable and least toxic antimicrobial agents for the most probable anaerobic and facultative pathogens. Reports on in vitro sensitivity (usually reliable only for the facultative or aerobic organisms) enable physicians to subsequently adjust the initial regimen to more specific therapy.

Because these infections are commonly polymicrobial, a broad spectrum of antimicrobial activity is required. Data suggest that survival in patients with sepsis, including that due to intraabdominal infection, is diminished if initial therapy is inadequate, regardless of the adequacy of subsequent treatment.[158,186] The ideal regimen remains controversial. The animal model of intraabdominal sepsis showed the necessity of treating the facultative enteric gram-negative bacillus *(E. coli)* and the anaerobic gram-negative bacillus *(B. fragilis).* Empirical efficacy of an antimicrobial regimen active against *E. coli* and *B. fragilis* has been well established. The need for intraoperative cultures to document the etiologic microorganisms and their antimicrobial susceptibilities has been controversial because postoperative changes based on results of intraoperative cultures may not improve outcome.[187]

Drugs active against anaerobic bacteria may be inactive against the accompanying aerobic or facultative pathogens in the mixed infections and vice versa. For this reason, combinations of usually two or three drugs are used. These combinations of antimicrobial agents are selected for their activity against most of the more virulent pathogens in the infective mixture (e.g., the Enterobacteriaceae and *B. fragilis*), although monotherapy for polymicrobial intraabdominal infection is now possible

because of the availability of broad-spectrum agents with activity against aerobes and anaerobes.

Antibiotics need not be active against every pathogen isolated. It is apparent that if only some of the organisms can be eliminated, the synergistic effect may be removed and the patient's defenses may be able to eradicate the remaining organisms. Although not recommended, clindamycin alone (which has no activity against Enterobacteriaceae or enterococci) has been reported to be sufficient treatment for some patients with infections resulting from a mixture of Enterobacteriaceae, enterococci, and anaerobes.[188]

Although enterococci are found in about 20% of intraabdominal infections, the exact role they play in polymicrobial intraabdominal infection and the need for an antimicrobial regimen specific for these organisms are controversial.[189–191] In several studies, patients were treated successfully with clindamycin and gentamicin despite absence of activity of this therapeutic regimen against enterococci.[192,193] Selective therapy against *E. coli* and *B. fragilis* that has no or borderline in vitro antimicrobial activity against enterococci has been found to be sufficient to reduce enterococcal counts.[194] Nevertheless, in animal models of experimental polymicrobial intraabdominal infection, enterococci have been found to be a significant component of the inoculum; as such, they enhance abscess formation, weight loss, bacteremia with *B. fragilis* and *E. coli,* and mortality.[194] Similarly, clinical reports have emphasized the importance of enterococci in intraabdominal infection[195] and noted the emergence of enterococcal abscesses and bacteremia supervening after treatment of intraabdominal sepsis with antimicrobial agents that lack significant in vitro antienterococcal activity.[196–199] Enterococci have emerged as major nosocomial pathogens, undoubtedly as a result of their inherent resistance to many commonly used antimicrobial agents and their acquisition of resistance to previous standard therapy (i.e., ampicillin, aminoglycosides, and the glycopeptides vancomycin and teicoplanin).[200–202] Only clinically unproven older antimicrobial agents (e.g., doxycycline, colistin, chloramphenicol) and a few newer antimicrobial agents (e.g., a combination of two streptogramin antibiotics [quinupristin-dalfopristin], linezolid, daptomycin, tigecycline) are available as potential therapy for infections caused by these multidrug-resistant and vancomycin-resistant strains of enterococci.

The efficacy of agents with antienterococcal activity in preventing emergence of enterococcal superinfection is unknown. A multicenter study of intraabdominal infection[203] demonstrated that the presence of enterococci in the initial culture, independently from the APACHE II score, was predictive of the failure of broad-spectrum antimicrobial regimens that lacked specific enterococcal activity. APACHE II score, age, length of preinfection hospital stay, and postoperative infections were predictive of the presence of enterococci. It is unclear whether inclusion of antienterococcal therapy would improve outcome in these high-risk patients, although one study suggested that postoperative addition of antienterococcal antimicrobial therapy after results of intraoperative peritoneal cultures were known did not lower the mortality rate, whereas other studies have suggested that this practice may prove beneficial.[204–206] At present, the available evidence suggests that routine coverage against enterococci is not necessary for patients with community-acquired intraabdominal infections but should be given to patients with prior cephalosporin or fluoroquinolone use (which may select for enterococci), immunocompromised patients, patients with prosthetic heart valves or vascular heart disease, those with hospital-acquired intraabdominal infection including postoperative peritonitis and tertiary peritonitis, and those with health care–associated infections from whom enterococci are recovered.[1,207] In these instances, empirical therapy should be directed against the probable species and strains that may be present with the knowledge that *Enterococcus faecium* has increasingly developed resistance to β-lactam antimicrobial agents and thus requires a glycopeptide drug for adequate activity.[208] Vancomycin-resistant enterococci (especially *E. faecium*) should be considered, particularly if the patient is at very high risk for an infection due to this organism, such as those who have had a previous surgical procedure, prolonged length of hospital stay, or extensive exposure to antibiotics, or individuals known to be colonized with vancomycin-resistant enterococci. Solid-organ transplant patients, especially recipients of liver transplants, with an intraabdominal infection originating in the hepatobiliary tree have a greater risk of vancomycin-resistant infections and colonization.[1,209]

In immunocompetent patients with low-risk community-acquired intraabdominal infections, *S. aureus* is rare, but it has been found to be more prevalent in health care–acquired infections, and many isolated strains exhibit methicillin-resistance. Its presence may be based on preinfection colonization or acquisition in the hospital or in patients with prolonged, complicated disease such as tertiary peritonitis.[7,190] Empirical therapy including agents with anti–methicillin-resistant *S. aureus* activity should be provided to patients with health care–associated intraabdominal infection who are known to be colonized or are at risk of having an infection due to this organism because of prior treatment failure and significant antibiotic exposure.[1,6]

Candida spp. are cultured from about 20% of patients with acute perforations of the gastrointestinal tract.[1] The majority of candida peritonitis occurs in the health-care setting. The highest incidence is observed in patients who have more than one perforation, disruption in the upper gastrointestinal tract, surgically treated pancreatitis, or previous treatment with antibiotics.[210,211] Although *Candida albicans* remains the predominant species, *Candida glabrata* and other species have become more common.[212–214] Nonetheless, management of *Candida* spp. in polymicrobial infections is also controversial.[215,216] Published risk factors for invasive candidiasis in hospitalized patients, including the demonstration of *Candida* colonization at multiple other body sites,[217] have not been specifically proven to contribute to *Candida* peritoneal infections.[218,219] Although the isolation of *Candida* spp. from peritoneal fluid in cases of intraabdominal infection has been associated with increased mortality,[220] other than candidemia and other sites of invasive infection, the issue of attributable mortality remains unresolved.[213,214] Isolation of this microorganism from blood cultures—as the sole organism within residual or recurrent intraabdominal infection or as the predominant organism on Gram staining of peritoneal exudate—represents an indication for specific antifungal therapy plus drainage of abscesses, if present. Most authorities agree that even when *Candida* organisms are isolated in intraabdominal infections, antifungal agents are unnecessary unless the patient has recently received immunosuppressive therapy for malignancy, transplantation, or inflammatory disease or has postoperative or recurrent intraabdominal infection.[1] However, many experts recommend antifungal therapy if *Candida* is grown from intraabdominal specimens in patients with severe community-acquired and hospital-acquired intraabdominal infections.[207] Although no comparative trials in *Candida* peritonitis exist, an echinocandin (caspofungin, micafungin, anidulafungin) is the first antifungal agent of choice. Although fluconazole was previously recommended as initial therapy for invasive candidiasis, including peritonitis, accumulating data have suggested that the use of echinocandins results in improved outcomes.[221,222] However, fluconazole, 800-mg loading dose, then 400 mg daily, still remains an acceptable alternative for nonneutropenic patients who have had no recent azole exposure and are not colonized with azole-resistant *Candida* species.[223,224] Toxicities associated with amphotericin B have limited the role of this polyene antifungal in treating intraabdominal fungal infections.[223] Voriconazole may also be an appropriate alternative (also see Chapter 39).

The rise in bacterial resistance among aerobic gram-negative bacilli involved in health care–associated and, to an increasingly greater extent, community-acquired intraabdominal infections adversely affects outcomes. Antimicrobial resistance directly prolongs length of hospital stay, contributes to escalated overall costs, and increases morbidity and mortality, thus posing new challenges for selecting an adequate antimicrobial regimen for intraabdominal infections, particularly in those characterized as complicated and severe. The burgeoning resistance of aerobic gram-negative bacilli against generally used antimicrobial agents is heavily driven by the increased prevalence of ESBL production in Enterobacteriaceae isolates commonly involved in these infections.[225] The 2011 Study for Monitoring Antimicrobial Resistance Trends (SMART) found that 12.7% and 9.7% of *K. pneumoniae* and *E. coli,* respectively, produced ESBLs.[226] Although the signature phenotypic property of ESBL enzymes is the hydrolysis of extended-spectrum cephalosporins, the overexpression of other β-lactamases such as AmpC (the genetics of which typically are chromosomal but are capable of transfer to other bacteria via plasmids) further limits effective treatment of complicated

intraabdominal infections. Antibiotic resistance mediated by this enzyme is not overcome by the addition of β-lactamase inhibitors (e.g., clavulanate or tazobactam). In addition, resistance mechanisms such as altered drug targets, decreased outer membrane porin expression, and efflux pump regulation frequently coexist in β-lactamase–producing isolates, leading to multidrug-resistant, and at times extremely drug-resistant, pathogens. The use of older polymyxin and aminoglycoside antibiotics as well as newer combination β-lactam agents may need to be relied on when circumstances such as known colonization or involvement with such organisms exists.

The activities of various antimicrobial agents against the usual peritoneal pathogens and the results of clinical trials are discussed in the following sections.

Chloramphenicol. At a concentration of 16 μg/mL, chloramphenicol has activity against more than 99% of the anaerobic pathogens involved in intraabdominal infections, especially *B. fragilis.* The availability of equally effective and potentially less toxic antimicrobial agents to treat anaerobic infections (e.g., metronidazole, β-lactam/β-lactamase inhibitors, carbapenems) has all but eliminated the need for chloramphenicol.

Clindamycin. Clindamycin had been reported previously to inhibit more than 95% of the anaerobes, including *B. fragilis,* at a concentration of 8 μg/mL, and early clinical trials established the efficacy of clindamycin plus aminoglycoside. About 15% of the strains of *Clostridium* spp. other than *C. perfringens, Peptococcus* spp., and rare strains of *Fusobacterium* spp. have been reported to be resistant to clindamycin. Plasmid-mediated, transferable clindamycin resistance in anaerobic bacteria has been shown in vitro,[227] and clindamycin resistance among *B. fragilis* has become increasingly recognized in most medical centers.[228] Clindamycin is active against certain facultative gram-positive cocci, such as *S. aureus* and *Streptococcus pyogenes,* but not against enterococci, and it has virtually no activity against Enterobacteriaceae.

Diarrhea is reported to be the most common side effect of clindamycin therapy,[229] occurring at an incidence of 2% to 20%. The severity of the diarrhea varies, but it may be associated with pseudomembranous colitis in half of patients with diarrhea, as reported in one study.[230] Toxic megacolon, colonic perforation, and death have been reported rarely and were attributed to exotoxins produced by clindamycin-resistant strains of *Clostridioides difficile* (formerly *Clostridium difficile*). For these reasons and because agents with more reliable in vitro antianaerobic activity are available, clindamycin is no longer recommended for use in combination with other antimicrobial agents in the treatment of intraabdominal infections, except in patients in whom other agent options are either contraindicated or intolerable.

Metronidazole. Metronidazole is active against strict anaerobes; inhibits most *B. fragilis* strains, *Fusobacterium* spp., and *Clostridium* spp.; and has a unique bactericidal action against *B. fragilis* and *C. perfringens.* Resistance among *B. fragilis* to metronidazole is rare, although it was detected among 12.3% of *B. fragilis* isolates in one South African survey.[231] The in vitro activity of metronidazole is poor, however, against aerobes, microaerophiles, and anaerobes that may become aerotolerant on subculture (i.e., certain anaerobic gram-positive cocci and sporeless gram-positive rods), although there is some in vivo evidence in animal models and humans that metronidazole has activity against *E. coli* and other aerobes in mixed aerobic-anaerobic infections.[232] The mechanism for this is poorly understood but may be related to the conversion by *B. fragilis* of metronidazole into metabolites with activity against *E. coli* and other aerobes. Nonetheless, because facultative gram-negative bacilli and microaerophilic gram-positive cocci (which are frequent copathogens in polymicrobial anaerobic infection) are resistant to metronidazole, the drug should be used in combination with another agent that is active against these pathogens to compensate for these defects in its spectrum.

In a retrospective study, the relative incidence of *C. difficile*–associated diarrhea and colonization was found to be lower after use of metronidazole than after clindamycin.[233] Metronidazole is one of the drugs of choice for treatment of this disease.

Tetracyclines. The large number of resistant anaerobes, especially *B. fragilis,* precludes the use of tetracycline. These antibiotics currently have limited if any utility in the treatment of intraabdominal infections.

Cephalosporins. *B. fragilis* and other *Bacteroides* spp. are usually resistant to the first-generation cephalosporins (e.g., cefazolin) and to some second-generation cephalosporins (e.g., cefuroxime). Cefoxitin is distinctly more active than any of the other second-generation cephalosporins against *Bacteroides* spp., but cefoxitin resistance has become a problem at many medical centers.[227,234] Cefotetan has activity similar to that of cefoxitin except that it is less active against the *B. fragilis* group (not including *B. fragilis*). These first- and second-generation cephalosporins are also active against most strains of *E. coli, Proteus mirabilis,* and *K. pneumoniae.* However, cefoxitin and cefotetan cannot be recommended for use in treating intraabdominal infections[1] because *B. fragilis* has become increasingly too resistant to these agents. The third-generation cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) and fourth-generation cefepime have shown significantly better activity against the Enterobacteriaceae. Only ceftazidime and cefepime have activity against *P. aeruginosa.* With a few exceptions, the third- and fourth-generation cephalosporins have relatively poor activity against *B. fragilis* and other *Bacteroides* spp., and the addition of metronidazole is required in order to ensure antianaerobic activity.

Regimens in which a third-generation cephalosporin is substituted for the aminoglycoside compare favorably with clindamycin plus aminoglycoside. Under selective pressure of antimicrobial therapy with third-generation cephalosporins, however, resistance emerges readily among certain gram-negative bacilli that produce chromosomal-encoded inducible β-lactamases, such as *Enterobacter, Serratia, Citrobacter, Morganella*, and *Acinetobacter* spp. and *P. aeruginosa.* These organisms have a high spontaneous mutation rate for constitutive production of large amounts of these β-lactamases, which confer resistance to all β-lactams except imipenem, meropenem, ertapenem, doripenem, and possibly cefepime (depending on the interpretive susceptibility breakpoint applied, CLSI vs. EUCAST[93]) and are poorly antagonized by the clinically available β-lactamase inhibitors, sulbactam, clavulanic acid, and tazobactam. Patients who are likely to be infected with these organisms (patients with prolonged hospital stays, prior antibiotic treatment, postoperative peritonitis, or tertiary peritonitis) are treated best with imipenem, meropenem, ertapenem, doripenem, a fluoroquinolone, tigecycline, or an aminoglycoside. Some authorities recommend against using cefepime as first-line therapy for ESBL-producing organisms, particularly because of the effect of greater concentrations of bacterial organisms, which raise minimal inhibitory concentrations (MICs) against such organisms.[235]

Strains of *K. pneumoniae* and to a lesser extent *E. coli* have acquired plasmid-encoded, ESBLs that inactivate all third-generation cephalosporins, especially ceftazidime, extended-spectrum penicillins, and aztreonam. These strains are also frequently resistant to the fluoroquinolones. These β-lactamases may be inactivated to some extent by sulbactam, clavulanic acid, and tazobactam, which confer activity to the β-lactam/β-lactamase inhibitor combinations containing these agents. The cephamycin antibiotic agents cefoxitin and cefotetan demonstrate in vitro activity against several ESBL-producing Enterobacteriaceae but lack clinical trial data assessing their effectiveness in infections with these pathogens. ESBL rates for the two most common aerobic gram-negative bacilli, *E. coli* and *Klebsiella* spp., isolated from intraabdominal infections were found to have increased from 1.7% and 3.2% in 2005 to 7.3% and 13.1% in 2012, respectively, in North America,[98] with even greater increases occurring in various other parts of the world.[93] Imipenem, meropenem, ertapenem, doripenem, and to a lesser degree cefepime (see earlier), along with tigecycline, are most active against these strains. Two new cephalosporin/β-lactamase inhibitor combinations with improved activity against resistant gram-negative bacilli have been approved for the treatment of intraabdominal infections. Ceftolozane is a fifth-generation cephalosporin administrated with tazobactam. This oxyimino-aminothiazolyl cephalosporin's unique chemical structure improves its antimicrobial activity against AmpC β-lactamase–producing Enterobacteriaceae, and the β-lactamase inhibitor tazobactam component affords activity against class A and C β-lactamases. However, this combination antibiotic remains susceptible to degradation by *K. pneumoniae* carbapenemase (KPC) and metallo-β-lactamase (MBL)–producing strains of bacteria. Currently, its greater affinity for attachment to penicillin-binding proteins and ability to evade efflux pumps provides ceftolozane-tazobactam the distinction of being the most potent agent against *P. aeruginosa,* including many strains with documented resistance to ceftazidime and carbapenems. Similarly, ceftazidime in combination

with the novel non–β-lactam/β-lactamase inhibitor avibactam has proven active against most strains of Enterobacteriaceae including ESBL- and AmpC β-lactamase–producing isolates, with the added feature of considerable in vitro activity against KPC-producing Enterobacteriaceae but not MBL producers. Owing to their insufficient activity against anaerobic bacteria, both of these agents require the addition of metronidazole in treating intraabdominal infections and have been proved to be noninferior compared with meropenem in complicated intraabdominal infection phase III clinical treatment trials.[236–238] However, for reasons not entirely clear, both of these combination antibiotics were found to be less effective than meropenem in subgroups of patients with renal insufficiency.[239]

Penicillins. Penicillin G and ampicillin have excellent activity against anaerobes, with the exception of β-lactamase–producing anaerobic gram-negative bacilli, such as *Bacteroides* spp. (especially *B. fragilis*) and *Prevotella* spp. Ampicillin had been active against 70% to 80% of the strains of *E. coli,* although the percentage of resistant strains has been significantly increasing. Almost all strains of *P. mirabilis* are still inhibited by ampicillin. There is some evidence that penicillin G may fail to achieve inhibitory concentrations at sites of *B. fragilis* infection because of a reduction in penetration of penicillin into infected sites and inactivation of the drug by *B. fragilis.* Therapeutic failures despite high doses of penicillin for *B. fragilis* bacteremia have been well documented. These drugs currently have limited use, if any, in the treatment of intraabdominal infections.

About 80% to 90% of strains of *B. fragilis* may be sensitive to high concentrations (MIC ≤125 μg/mL) of the available extended-spectrum penicillins piperacillin and ticarcillin,[240,241] and the clinical experience with these drugs in polymicrobial anaerobic infections has been favorable.[242] The spectrum of these penicillins also includes most aerobic enteric gram-negative bacilli and *P. aeruginosa* but is not likely to include *K. pneumoniae* and many strains of *E. coli* as a consequence of β-lactamase production. In addition, ticarcillin is inherently much less active than piperacillin against enterococci and *P. aeruginosa.* The combination of ticarcillin or piperacillin with the β-lactamase inhibitors clavulanic acid or tazobactam confers activity against a large proportion of β-lactamase–producing strains of anaerobic gram-negative bacilli, *E. coli,* and *K. pneumoniae* (greater for piperacillin-tazobactam)[243–245] but does not confer significant activity against the chromosome-encoded, inducible, β-lactamase–producing strains of *Serratia* spp., *Enterobacter* spp., *P. aeruginosa, Citrobacter* spp., *Acinetobacter* spp., and *Morganella* spp. Newer ESBL-producing strains of these aerobic bacilli will not be inhibited by these extended-spectrum penicillins, and there are few proven clinical efficacy data for these combination agents against such ESBL producers in the treatment of high-inoculum infections that may overcome the action supplied by the β-lactamase inhibitor. With the CLSI's lowering of MIC breakpoints that are currently used in most microbiology laboratories for cephalosporins and monobactams,[246] specific additional tests to confirm the presence of β-lactamase are now not necessarily reported. However, because such breakpoints for β-lactam/β-lactamase inhibitor combination antibiotics, in particular piperacillin-tazobactam, remain high (i.e., susceptible <16/4 mg/L, resistant >128/4 mg/L), this agent is commonly chosen with confidence to treat higher-risk patients or those with more severe intraabdominal infections, discounting the concern that the pharmacodynamic action needed to optimize bacterial killing may not be achievable for these higher-MIC isolates.[247] Combining the β-lactamase inhibitor sulbactam with ampicillin restores activity against many community-acquired β-lactamase–producing bacteria that had developed resistance to ampicillin, including *E. coli, Klebsiella* spp., and *Bacteroides* spp.[243] This combination agent is inactive against nosocomial pathogens, such as *Enterobacter* spp., *Serratia* spp., and *P. aeruginosa.* Monotherapy for polymicrobial anaerobic intraabdominal infection is possible with β-lactam/β-lactamase combinations, such as ticarcillin-clavulanate (no longer available in the United States) and piperacillin-tazobactam, because of their broad spectrum of activity against aerobes and anaerobes. However, because of the significant rise in global resistance rates to Enterobacteriaceae, including 34% and 45% of health care– and community-acquired strains of *E. coli,* respectively, found to be nonsusceptible to ampicillin-sulbactam,[94] this combination agent can no longer be recommended for treatment of intraabdominal infections.[1,6]

Carbapenems and aztreonam. The carbapenem antibiotics imipenem, meropenem, ertapenem, and doripenem have a broad antimicrobial spectrum,[248] with activity against almost all aerobic and anaerobic pathogens, although *E. faecium* are resistant. Meropenem is slightly more active than imipenem against gram-negative bacilli and slightly less active against gram-positive cocci. Doripenem combines the broad-spectrum coverage of imipenem and meropenem with more potent activity against *P. aeruginosa.* The activity of ertapenem is similar to that of the other carbapenems, but ertapenem is not active against enterococci and *P. aeruginosa,* thereby relegating its use to lower-risk patients with community-acquired infections.[249] Its once-daily dosing (facilitating outpatient intravenous therapy) and possible decreased induction of multidrug-resistant gram-negative bacilli[250] may make it appealing. Conversely, experts have expressed concern that broad use of ertapenem may hasten the appearance of carbapenem-resistant Enterobacteriaceae, *Pseudomonas,* and *Acinetobacter* species.[1,94] The carbapenems are resistant to most β-lactamases, including chromosome-encoded, inducible β-lactamases produced by bacteria, such as *Enterobacter* spp., *Serratia* spp., and *P. aeruginosa,* and the plasmid-encoded, ESBLs produced by *K. pneumoniae* and *E. coli.* The carbapenems are susceptible, however, to metallo-β-lactamases produced by rare strains of *B. fragilis* and *S. maltophilia.*[248] Monotherapy for polymicrobial anaerobic intraabdominal infection is possible with the carbapenems because of their broad spectrum of activity against aerobes and anaerobes.[251–253] Subsequently, taking into account their reliable activity against multidrug-resistant organisms, the carbapenems (except for ertapenem because it lacks activity against *P. aeruginosa*) may be the drugs of choice for treatment of complicated intraabdominal infections, especially those that are health care associated. Unfortunately, as a consequence of growing resistance issues, there has been an increased dependence on carbapenems when confronted with high-risk patients having more severe complicated intraabdominal infections, which has created selection pressure for carbapenem resistance. A growing number of Enterobacteriaceae have become resistant to the carbapenems as a result of hydrolyzing enzymes that belong to the KPC serine β-lactamase, and the VIM and NDM metallo-β-lactamases, and this development may lead to extensively drug-resistant bacteria, which will limit the ability to treat patients with complicated intraabdominal infections.[185,254] Although in North America carbapenem resistance among Enterobacteriaceae species is uncommon, and only minor fluctuations in susceptibility to ertapenem and imipenem have been reported,[93,255,256] the surveillance network database for US hospitals from 2000 to 2009 found that in complicated intraabdominal infections, approximately 15% of *P. aeruginosa* strains demonstrated multidrug resistance, and 2% of aerobic gram-negative bacilli were carbapenem-resistant Enterobacteriaceae, mostly KPC-producing strains.[257] The same may not be able to be said for many other parts of the world. On such occasions, tigecycline and older antibiotics such as colistin and fosfomycin may be the only available treatment options.

The carbapenem β-lactamase combination drug meropenem-vaborbactam is highly active against many strains of carbapenemase-resistant Enterobacteriaceae, including KPC-producing isolates, but does not expand coverage to carbapenem-resistant *A. baumannii, P. aeruginosa,* or *S. maltophilia*.[258] Approved for treatment of complicated urinary tract infections and intraabdominal infections, it may be a viable alternative for treating cases of intraabdominal infections suspected or proven to involve multidrug-resistant bacterial pathogens.

Aztreonam, a monobactam antibiotic, has a spectrum of activity limited to aerobic gram-negative bacilli including *P. aeruginosa,* but inadequate activity against ESBL-producing strains of *E. coli* and *K. pneumoniae*.[259] Thus, it would be necessary to add an antibiotic with activity against microaerophilic and aerobic gram-positive cocci, such as vancomycin or clindamycin.[260] Activity against anaerobes would also be needed. Although clindamycin provides some antianaerobic activity, as previously mentioned, the antianaerobic activity of clindamycin is no longer adequately dependable to recommend its routine use. Thus, an antianaerobic drug such as metronidazole would be necessary in addition to aztreonam along with either vancomycin or clindamycin. An aztreonam-based regimen is a useful option in those patients with known serious allergic reactions to other β-lactam antibiotics, because

cross-reactivity in β-lactam–hypersensitive individuals is not known to occur with this monobactam agent.[261]

Aminoglycosides. Aminoglycosides are active against many gram-negative aerobic bacilli and were once considered a major component of intraabdominal infection treatment regimens. Over the decades of aminoglycoside use, aminoglycoside resistance has increased, however, and other antibiotic classes with predictable activity against gram-negative aerobic bacilli have become available. Amikacin, which is less susceptible to some gentamicin-modifying and tobramycin-modifying enzymes, nevertheless may be active against gentamicin-resistant or tobramycin-resistant pathogens. In some medical facilities, many gram-negative bacilli are currently found to be resistant to aminoglycosides, however, and the MICs of sensitive pathogens have increased steadily close to breakpoint concentrations. For optimal pharmacodynamic effects of aminoglycoside antibiotics, serum levels should be at least 10 times the MIC of the pathogen. Persistent inhibition of growth of the pathogen after brief exposure to an aminoglycoside (the postantibiotic effect) allows the serum levels of aminoglycosides to decline to less than the MIC of the pathogen during the dosing interval without loss of efficacy. Because aminoglycoside nephrotoxicity and perhaps ototoxicity are dependent on time, not concentration, high peak serum levels to maximize efficacy can be achieved without excess toxicity if the dosing interval is lengthened. This is the rationale for giving the total daily aminoglycoside dose as a single dose every 24 hours in patients with normal renal function, rather than giving the daily dose in two (amikacin) or three (gentamicin or tobramycin) equally divided doses every 24 hours. Single daily dosing of the aminoglycoside may be necessary to achieve serum levels that are at least 10 times the MIC of sensitive pathogens that have MICs close to breakpoint concentrations. The serum concentrations of gentamicin are unpredictable after a dose based on the body weight, so peak and trough serum levels must be confirmed by any of the various assay methods available.[262] The numbers of severely ill patients with intraabdominal sepsis studied are too limited, however, to permit recommendation of the general use of single daily dosing of these drugs.

Aminoglycosides are inactive against obligate anaerobes, and their activity against sensitive pathogens is antagonized by an anaerobic environment and by reducing substances, such as sulfhydryl compounds. Aminoglycosides are also not active in acidic conditions. Anaerobic and acidic conditions are frequently present in intraabdominal abscesses. In contrast, the β-lactams are relatively nontoxic, can be used in concentrations that are many times higher than the MIC for the pathogen, and are active under anaerobic or acidic conditions. The β-lactams are probably more reliable antibiotics than the aminoglycosides against sensitive pathogens. In two meta-analyses of well-conducted trials, aminoglycoside-based regimens were deemed inferior compared with several other regimens for the treatment of patients with intraabdominal infections.[263,264] For efficacy reasons, and because of toxicity concerns, the use of aminoglycosides to treat intraabdominal infection has been questioned; aminoglycosides are no longer recommended for routine use.[1,265] If indicated on the basis of in vitro sensitivity testing, β-lactams should be used in preference to aminoglycosides. An aminoglycoside may be included with a β-lactam antibiotic in the initial antimicrobial regimen for patients who are critically ill or in whom a resistant pathogen (e.g., *P. aeruginosa*) is suspected, as in empirical treatment of complicated health care–associated infections. They may also be necessary in the severely β-lactam–allergic patient infected with a multidrug-resistant aerobic gram-negative bacilli when no alternative active antibiotic exists.

Fluoroquinolones. The fluoroquinolones (norfloxacin, ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin) are active against almost all aerobic gram-negative bacilli. Ciprofloxacin remains the most potent fluoroquinolone against *P. aeruginosa.* Levofloxacin and moxifloxacin are more active than the older fluoroquinolones against gram-positive cocci, although enterococci and methicillin-resistant *S. aureus* tend to be less susceptible than other gram-positive cocci. In contrast to other fluoroquinolones, moxifloxacin is active against obligate anaerobes.[266,267] Concerns about resistance of anaerobes to moxifloxacin have been raised, although no existing data demonstrate suboptimal clinical outcomes or lower anaerobic susceptibility to this fluoroquinolone.[6,268]

Because of their ability to kill bacteria in the exponential and the stationary phases of growth, the fluoroquinolones are valuable antimicrobial agents for the treatment of intraabdominal infections, including abscesses. However, fluoroquinolones should be used judiciously; especially because *E. coli* resistance has emerged internationally.[94,269] Fluoroquinolones have now been found to have plasmid-mediated resistance. In treating patients with community- or health care–acquired intraabdominal infections of high severity, fluoroquinolone antibiotics should not be used unless local and hospital microbiologic surveys indicate that more than 90% of *E. coli* strains remain susceptible to these agents.[1,6]

Currently, three fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) are available for parenteral administration. These fluoroquinolones are also well absorbed after oral administration and are concentrated in tissues such that tissue levels greatly exceed the MICs of many sensitive pathogens. With current fluoroquinolone dosing regimens, serum levels may be inadequate to treat susceptible pathogens with relatively high MICs (e.g., >0.5 μg/mL of ciprofloxacin), which include some strains of *P. aeruginosa,* enterococci, and *S. aureus,* especially the methicillin-resistant strains. If such organisms can be anticipated (e.g., in nosocomial infections), additional antimicrobial agents may be necessary to broaden the spectrum of an empirical regimen. The addition of an antimicrobial agent active against anaerobic bacteria and aerobic or microaerophilic gram-positive cocci (e.g., clindamycin, ampicillin-sulbactam, amoxicillin–clavulanate acid) would be required if the use of ciprofloxacin were considered for secondary intraabdominal infection. The use of levofloxacin, even combined with a second agent with reliable activity against anaerobic gram-negative bacilli for the treatment of intraabdominal infections, does not have any published support, and perhaps this fluoroquinolone should be reserved for respiratory and urinary tract infections for which it may be better suited.[270] Moxifloxacin is the only fluoroquinolone that has been studied and approved as a single agent for the treatment of complicated intraabdominal infections. It has been determined to be noninferior to β-lactam/β-lactamase inhibitors, cephalosporin-based regimens, and carbapenems in several comparator studies.[271–273] However, its use should be cautioned against in patients who have recently been treated with any fluoroquinolone antibiotic because these individuals may harbor moxifloxacin-resistant strains of *B. fragilis* and other fluoroquinolone-resistant microorganisms.[274] β-Fluoroquinolone–based intraabdominal infection regimens are practical treatment options in lower-risk patients who are severely allergic to β-lactam antibiotics in settings where the degree of *E. coli* fluoroquinolone resistance is not high.

Tigecycline. The first glycylcycline antibiotic approved for the treatment of complicated intraabdominal infections was tigecycline.[275] With an expanded spectrum of activity against aerobic and facultative gram-positive and gram-negative bacteria, in addition to anaerobic bacteria, this parenteral agent was found to be at least as efficacious as imipenem in a trial treating patients with complicated intraabdominal infections.[276] However, a pooled analysis of trials involving tigecycline, including one trial in complicated intraabdominal infection, demonstrated an increased risk of death among patients receiving tigecycline compared with patients receiving comparator antibiotics, which has since led to a black box warning from the US Food and Drug Administration (FDA) and a recommendation against the use of tigecycline in severe infection.[7,274] It nevertheless has a distinct mechanism of antibacterial action that is not affected by resistance encountered with β-lactam and fluoroquinolone agents and therefore may serve a need in treatment of patients with intraabdominal infections with multidrug-resistant gram-negative bacilli, including ESBL- or carbapenemase-producing organisms. In addition, tigecycline has reliable activity against vancomycin-resistant strains of enterococci and methicillin-resistant *S. aureus* and in vitro activity against *A. baumannii,* but not *P. aeruginosa.* One limitation with the use of tigecycline may be the relatively high incidence of nausea and vomiting experienced by patients treated with this agent.

Eravacycline. Eravacycline, an injectable synthetic fluorocycline antibiotic, belongs to the tetracycline group of antibiotics. Like tigecycline, eravacycline has a broad spectrum of antibacterial activity against a wide variety of multidrug resistant gram-negative, gram-positive, and anaerobic bacteria, including methicillin-resistant staphylococci,

vancomycin-resistant enterococci, extended-spectrum β-lactamase and some carbapenem-resistant Enterobacteriaceae, as well as resistant *A. baumanni*.[276a] Eravacycline demonstrates 2- to 8-fold more in vitro potency than tigecycline, but, similar to this agent, it also lacks reliable antipseudomonal activity. Based on the results of two pivotal phase 3 trials (IGNITE 1 and IGNITE 4) demonstrating noninferiority to ertapenem and meropenem, respectively, it has been approved for use as monotherapy for the treatment of complicated intraabdominal polymicrobial infections.[276b,276c] The most common adverse reactions observed in clinical trials were infusion site reactions, nausea, and vomiting. Dosing is weight-based given as an intravenous infusion at 1 mg/kg every 12 hours, with no required adjustment of dose required in patients with renal impairment.

Pharmacodynamic Considerations

A Cochrane Collaboration review of antimicrobial therapy for intraabdominal infections was unable to demonstrate clinical superiority of any one regimen.[277] Using Monte Carlo simulations to model the probability of adequate coverage of pathogens commonly isolated from patients with intraabdominal infections, the researchers revealed substantial variability among regimens.[92] Pharmacodynamic data modeled against MIC information for a variety of bacteria, obtained from a large database for each antimicrobial agent, were expressed as the cumulative fraction of response (CFR), which is defined as the expected population probability of target attainment for a specific drug and dose against a specific population of pathogens.[92] Target attainment, in turn, is based on the percentage of the dosing interval during which free drug concentration remains above the MIC (free T [time] > MIC), for agents whose killing is time dependent (e.g., β-lactams), or the ratio of the area under the concentration-time curve to the MIC for compounds whose killing activity is concentration dependent (e.g., fluoroquinolones). Whereas the carbapenems, piperacillin-tazobactam, ceftazidime, and cefepime all yielded CFRs exceeding 90%, ceftriaxone, ciprofloxacin, and levofloxacin each yielded a CFR of less than 82%. In cases in which exact clinical efficacy data are ambivalent, pharmacodynamic modeling may be a useful adjunct in choosing a treatment regimen.[92]

Duration of antimicrobial therapy. Antibiotic therapy should be given before, during, and after surgery to ensure adequate tissue and blood levels at which the antibiotic can combat local and metastatic spread of the infection. The duration of antimicrobial therapy after adequate surgery is usually 4 to 7 days and depends on severity of infection, clinical response, and normalization of the leukocyte count.[1,6] A recent study involving adults with community-acquired localized peritonitis observed that patients treated for 3 days had similar outcomes to those treated for 5 to 10 days.[278] In another study involving complicated intraabdominal infection, patients who had undergone an adequate source-control procedure had similar outcomes after being treated with a fixed-duration antibiotic course of approximately 4 days compared with those after a longer course of antibiotics of approximately 8 days.[279] Patients with secondary bacteremia from an abdominal source who received shorter courses of antibiotics, defined as 7 or fewer days, have been shown to have similar outcomes to those given traditional longer courses of antiinfective therapy.[280] Only a short course of antimicrobial therapy (24 hours) is required for sterile peritonitis that occurs in the peritoneal space around an infected intraabdominal organ that can be resected, such as the appendix in nonperforated appendicitis, or the gallbladder in acute cholecystitis.[281,282] Rates of infection in patients receiving perioperative antibiotics alone compared with longer courses of treatment in cases of nonperforated or ischemic gastrointestinal diseases have proven the same.[283] Similarly, contamination of the peritoneum with bacteria from a defect in the intestinal wall (e.g., immediately after penetrating abdominal trauma) also may necessitate only (1) operative intervention to remove the diseased organ and to stop continued peritoneal contamination and (2) a brief course of antimicrobial therapy, as would be indicated for surgical prophylaxis, limiting antibiotic administration to no more than 24 hours.[284,285] Persistent sepsis is suggestive of several complications: formation of an intraabdominal abscess, which necessitates drainage; continued contamination of the peritoneum from an inadequately controlled source; superimposed nosocomial infection with a resistant pathogen; or tertiary peritonitis.

The utility and proven benefit of antibiotic therapy to decrease secondary infection in noninfectious intraabdominal inflammatory disease has been an area of controversy. Quite often antibiotics are given to patients diagnosed with acute necrotizing pancreatitis who do not have a secondary infection complication. However, meta-analyses have failed to demonstrate any significant proven benefit, and the risks of bacterial resistance and complications, such as *C. difficile* infection, are increased when antibiotics are administered in this prophylactic fashion.[286–288] Studies looking at the need for antibiotics as part of the management of acute, uncomplicated sigmoid diverticulitis have found no difference in outcomes in those patients who were treated conservatively without antibiotics.[289,290] However, it may be prudent to apply this strategy only in lower-risk patients diagnosed with acute, uncomplicated left-sided diverticulitis. Similarly, a 5-year follow-up of antibiotic therapy alone for uncomplicated appendicitis had similar outcomes as in patients who underwent an appendectomy; the likelihood of late recurrence within 5 years was 39.1%.[291] (See Chapter 78.)

The antibiotic regimen should be adjusted to include the most efficacious, least toxic, and least expensive agents, when cultures have been finalized, with the proviso that therapy must have activity against anaerobes even when they are not isolated, because anaerobic bacteriologic techniques are frequently inadequate.

Patients able to tolerate oral intake may switch to oral therapy after an initial response to intravenous therapy.[1,203] Oral therapy allows earlier discharge for some patients and reduces costs and risks of infusion-related complications. When ileus no longer precludes oral intake, efficacy of oral therapy depends on the adequacy of absorption after oral administration of the antimicrobial agents, the availability of potent oral agents against the significant aerobic and anaerobic gram-negative bacilli and gram-positive cocci pathogens (i.e., *E. coli, B. fragilis,* and streptococcal species), and the effects of oral versus intravenous administration of these agents on bowel microbiota. The potential oral agents most studied for this purpose include amoxicillin-clavulanate, ciprofloxacin with metronidazole, and moxifloxacin, with recognition of the heightened prevalence of *E. coli* resistance to these agents. Levofloxacin is often substituted as the fluoroquinolone component but has never been studied for this purpose. Likewise, the oral cephalosporin drugs and trimethoprim-sulfamethoxazole have little proven evidence as antibiotics for the treatment of intraabdominal infections, but may be potential treatment options in specific cases. Although in many studies the orally administered antibiotics allowed treatment courses longer than the current recommendation of 4 to 7 days, the purpose of the switch should be the intention to shorten the length of parenteral antibiotic administration.[292,293]

The addition of antibiotics or antiseptics to intraperitoneal lavage fluid continues to be debated, and its efficacy intraoperatively or by continuous peritoneal lavage postoperatively remains unclear.[294,295] One study showed that irrigation of the peritoneal cavity with povidone-iodine decreased the frequency of intraabdominal abscess formation, in comparison with saline irrigation,[296] and other studies have suggested that including antibiotics in lavage fluid can reduce the incidence of postoperative infections. Povidone-iodine has been shown to be a potent inactivator of neutrophil functions such as chemotaxis and phagocytosis, however, and may have a detrimental effect.

Hyperbaric Oxygen

The increased oxygen tension attainable with hyperbaric oxygen therapy inhibits and kills *C. perfringens* and reduces the production of *C. perfringens* α-toxin. Hyperbaric oxygenation has been used clinically and experimentally for clostridial myonecrosis with some reported success. *C. perfringens* is a relatively oxygen-tolerant pathogen in comparison with other obligate anaerobes, and it is postulated that hyperbaric oxygen therapy may be at least as efficacious with anaerobic infections caused by these more oxygen-sensitive anaerobes. However, except for a few reports,[297] almost no clinical or experimental data are available. Hill[298] reported suppression of experimental liver abscesses caused by anaerobes in mice after treatment with hyperbaric oxygen therapy alone. In one study, it seemed that the use of hyperbaric oxygen therapy favorably affected the outcome of experimental sepsis in a rat model, perhaps by enhancing host defense mechanisms.[299] Consideration

also should be given to the hazards of hyperbaric oxygen therapy. See Chapter 50 for a more detailed discussion.

Gastrointestinal Drainage

In the presence of peritonitis, the patient should receive nothing by mouth. If no distention is present when treatment is instituted, continuous gastric suction is usually sufficient. For patients with distention when treatment is started and for patients who acquire distention despite gastric drainage, the small intestine should be intubated.

Water and Electrolyte Administration

The type of fluid replacement is determined in large part by the chemical abnormalities found. Early goal-directed volume resuscitation is essential in treating uncomplicated intraabdominal infections but becomes critical in cases of severe sepsis or septic shock. Regardless of whether the end point for resuscitation is mixed venous oxygen (>65%), correction of lactic acidosis, or normalization of base deficit, the goal of volume resuscitation is correction of cellular oxygen. In general, hypovolemia, dehydration, and metabolic acidosis predominate; therefore, plasma or albumin, Ringer's lactate solution, and 5% dextrose in water usually suffice. With regard to the types of fluids, timing, and amounts to be given, the recommendations put forth in the Surviving Sepsis Campaign should be followed.[186]

Blood and Plasma Transfusion

Although many patients recover from an illness satisfactorily with a hemoglobin of 8 or 10 g/dL, some surgeons recommend that the patient undergo transfusion to maintain levels of 12 to 13 g/dL in order to provide a margin of safety in the event of some complication, such as septic shock or upper gastrointestinal hemorrhage. A less liberal blood transfusion policy has been adopted by many medical centers.[300]

Respiratory Support

Fluid sequestered in the abdomen and loops of bowel distended by gas may elevate the diaphragm. Inflammation of the parietal peritoneum, including the diaphragmatic surface, leads to guarding and splinting of the muscular wall, which interferes with deep breathing and coughing. A subphrenic abscess may be responsible for splinting of the diaphragm. Retained bronchial secretions may lead to atelectasis and subsequent pneumonitis. These factors impair the ability to augment respiratory exchange in the presence of the increased expenditure of energy required by the inflammatory process, and this leads to hypoxemia and respiratory alkalosis. When the patient tires, the combination of metabolic and respiratory acidosis may develop and prove fatal.

Arterial blood gas studies are necessary to detect and quantitate respiratory decompensation. Measures aimed primarily at gastrointestinal decompression, elevation of the head of the bed, and control of the inflammation may improve respiration sufficiently. Administration of oxygen may improve arterial oxygen saturation. If these measures are inadequate, endotracheal intubation or tracheostomy should be performed without delay. A volume-cycled respirator should be used and adjusted to produce a partial pressure of oxygen of 80 to 100 mm Hg and a normal pH. If the partial pressure of carbon dioxide is then not normal, metabolic acidosis or alkalosis may be present and must be treated. As the intraabdominal process subsides, the patient may be able to breathe spontaneously again and may be weaned from the ventilator. In certain severe cases, positive end-expiratory pressure may also be necessary.

Operative Approach

Surgical intervention is aimed at controlling the source of peritoneal contamination and at débridement of necrotic tissue and foreign intraperitoneal matter.[301] Immediate surgical intervention within hours of presentation even in the medically unstable septic patient has been found to have a significant survival advantage.[302] In the majority of cases, intervention to control the source, and to restore normal gastrointestinal function and anatomy should be carried out within 24 hours; studies have indicated that postponement of source control beyond this time period has been associated with a greater risk of death.[303] Optimal management includes (1) bowel decompression (e.g., through proximal colostomy for perforation, diverticulitis, or colonic carcinoma); (2) closure of traumatic perforations and resection of a diseased, perforated viscus to stop continued peritoneal contamination with bacteria and adjuvants; and (3) drainage of any purulent collections, which reduces the bacterial inoculum, removes excessive levels of proinflammatory cytokines and adjuvants (e.g., fecal matter, food, blood, bile, barium) that would enhance the virulence of peritoneal infection, and eliminates anaerobic conditions. In the absence of perforation, when the disease process (e.g., acute appendicitis, necrotic bowel) is anticipated to advance, the involved organ is resected. Acute increase in intraabdominal pressure, which itself can lead to multiorgan dysfunction (e.g., acute pulmonary and renal failure, shock), necessitates decompression laparotomy; if necessary, the abdomen is left open and covered with a protective dressing.[92]

In complicated cases or severely ill patients, it may be necessary to perform an initial laparotomy for immediate source control with temporary abdominal closure followed later by a reexploration for definitive treatment.[304,305]

Operative intervention is generally not indicated for patients with primary peritonitis; in patients in whom the disease process subsides and localizes while the patient is being prepared for surgery; in moribund patients; or in patients with pelvic peritonitis caused by pelvic inflammatory disease, which usually responds to medical management. Stable, noncritical patients with localized intraabdominal infections including perforated appendicitis with localized phlegmon or periappendiceal abscesses; acute colonic diverticulitis with inflammation into the adjacent colonic tissues or small abscesses; and localized upper gastrointestinal perforations, may be treated conservatively with antibiotics alone, at times.[306–308] A similar treatment approach followed by a delay before surgery of days or even weeks may be warranted in other scenarios.[309,310]

Intraoperative peritoneal lavage with saline, after drainage of purulent peritoneal exudates, fecal matter, food, and other foreign debris, is standard procedure during laparotomy for peritonitis. Continuous postoperative peritoneal lavage for 48 to 72 hours or until the fluid is clear, with the use of large volumes of fluid to ensure dispersion of the fluid and to prevent loculations, has, however, not been effective.[294,295] Radical peritoneal débridement of all fibrinous deposits on peritoneal surfaces is, likewise, no longer thought to be effective.[311]

Initial reports were favorable for planned reexplorations in which laparotomies were performed at frequent intervals until the abdomen was macroscopically clean, regardless of the patient's condition, with additional surgical procedures (e.g., resections) performed as needed.[312] The abdominal fascia would be left open between laparotomies, with the abdominal wall defect bridged by saline-soaked gauze or by a temporary abdominal closure device such as mesh. On resolution of the septic process and establishment of granulation, the mesh would be removed and a skin graft would be applied to the granulating bed. These demanding and costly procedures have been complicated by multiple fistulas, wound contamination, incisional hernias, and secondary peritonitis with organisms such as enterococci or *Candida* spp.[313] Repeated entry into the inflamed peritoneum may escalate the cascade further. A review concluded that in the absence of randomized, controlled prospective trials with appropriate stratification of patients by severity of illness, evidence is now insufficient to determine whether these procedures improve outcome in severe diffuse peritonitis; nevertheless, they may be lifesaving in some patients.[313,314] In localized infection, local drainage alone is adequate because the risk of disseminating infection outweighs any possible benefit of removing foreign material that may have escaped mechanical removal.

Less invasive source-control procedures, including interventional radiologic percutaneous drainage of intraabdominal and pelvic fluid collections, are increasingly relied on when the infection is localized and a safe window for introduction of the drainage tube exists.[315] This treatment approach may also be the safest alternative in patients who could not tolerate a surgical procedure owing to their medical condition or who refused operation.

Use of multiple drains for drainage of the general peritoneal cavity is physically impossible because exudate and adhesions rapidly isolate and occlude the drains and may increase secondary infections. Drains are often placed, however, in a dependent point to which fluid can be

expected to gravitate or in an area of devitalized tissue that cannot be removed.

Prevention

To prevent postoperative peritonitis, the peritoneum must not be contaminated with gastrointestinal or vaginal secretions. In addition to use of good surgical technique, contamination can be avoided with early treatment of an intraabdominal infection. Leigh and coworkers[316] noted that the rate of wound infection in patients with perforated appendix was greater than 50% if no antimicrobial therapy was used but only 15% with appropriate therapy. Similarly, early use of antibiotics in penetrating wounds of the abdomen, especially wounds involving the colon, has been efficacious.[317] Surgeons also have used various means to reduce the complex gastrointestinal or vaginal flora before performing clean, contaminated surgery. Mechanical cleansing of the bowel with a low-residue diet followed by a liquid diet, cathartics, and enemas can reduce the total fecal mass and coliform count in the colon, although not the predominant anaerobic flora. The use of oral antibiotics preoperatively to reduce bowel flora is well accepted. *E. coli* in the colonic flora is sensitive to oral neomycin or kanamycin, whereas *B. fragilis* is frequently sensitive to erythromycin or metronidazole. Combinations such as neomycin and erythromycin base have been shown to be effective in reducing total bowel flora preoperatively and in decreasing the incidence of postoperative infection.[318]

Parenteral antibiotics have also been used in gastrointestinal and vaginal surgery prophylactically when there is a chance of contamination with normal microflora at the operative site (clean, contaminated surgery). Of these types of operations, 30% may be complicated by infections. The procedures involve cutting through the large bowel without significant spillage, compromising the blood supply of the large bowel, cutting through the stomach or small bowel when there is anticipated intraluminal bacterial overgrowth, appendectomy for appendicitis without rupture, repair of penetrating wounds of the abdomen, gallbladder surgery in an elderly patient, cesarean section after rupture of the membranes and labor, vaginal hysterectomy in a premenopausal woman, and radical pelvic surgery for gynecologic malignancy. Several studies have shown significant reduction in the frequency of postoperative infection, from as high as 30% to as low as 4% after prophylactic antibiotic use in clean, contaminated surgery.[284,319,320] The basic principle of antibiotic prophylaxis is to provide adequate tissue levels at the site of contamination and adequate blood levels during the procedure and for 24 hours after the procedure. See Chapter 313 for a more detailed discussion.

Peritonitis During Peritoneal Dialysis

Long-Term Peritoneal Dialysis

Peritoneal dialysis has been used successfully to treat uremia in patients with end-stage renal disease since the mid-1940s. Peritonitis was a frequently associated side effect that hindered the acceptance of chronic peritoneal dialysis until an improved access catheter was developed by Henry Tenckhoff in 1968. This catheter significantly decreased the incidence of peritonitis, but initial reports of patients undergoing CAPD with this catheter indicated peritonitis rates of more than six episodes per patient-year.[321] This rate has appeared to decline with the introduction of collapsible plastic bags, improved adapters (Y system), and better techniques.[322] However, peritonitis remains the major complication of CAPD today and is the chief reason for peritoneal catheter loss, discontinuation of peritoneal dialysis, and switch to hemodialysis.[323] Historically, it occurred at a rate of about one episode per patient-year (range, <0.5 to ≥3). Of patients, 45% experienced peritonitis at least once during their initial 6 months of CAPD treatment. This rate increased to 60% to 70% during the first year.[324,325] Centers now routinely report infection rates of less than one episode in 24 patient-months and as low as one episode in 60 patient-months.[326] Peritonitis recurs in 20% to 30% of patients. A small proportion of patients seem to have an unusually high frequency of peritonitis.

This disparity has been attributed partly to faulty sterile technique on the part of patients during self-administration of CAPD (touch contamination). The patient undergoing CAPD needs to perform a great number of sterile exchanges per year; a patient on continuous cycling peritoneal dialysis (CCPD), in comparison, performs fewer exchanges. With CCPD, the rate of peritonitis would be presumably lower because manipulation of the connections is required less frequently; this concept is supported by most but not all studies.[327,328]

Source of Infection and Risk Factors

The origin of infection in most cases seems to be contamination of the catheter by common skin organisms.[321] Alterations of skin flora in CAPD recipients[329] may lead to peritoneal contamination with enteric pathogens. A higher incidence of peritonitis seems to occur in patients undergoing dialysis who are carriers of nasal *S. aureus.* This carriage state increases the likelihood of developing exit site infections and tunnel infections in comparison with noncarriers, but the overall peritonitis rate of the two groups was not different in one study, although all cases of *S. aureus* peritonitis occurred in the carriers.[330] Pathogens may also contaminate the peritoneum from exit site infections and subcutaneous tunnel (periluminal) infections, transient bacteremia, and contamination of the dialysate delivery system during bag exchanges. As mentioned previously, enteric bacteria may also gain access to the peritoneal cavity by means of transmural migration through an intact intestinal wall after the introduction of hypertonic solutions into the peritoneum. This mechanism may account for enteric bacterial peritonitis in patients undergoing dialysis. On rare occasions, a vaginal leak may serve as a source of peritonitis. Endoscopic gastrointestinal (colonoscopy, sigmoidoscopy), gynecologic (hysteroscopy), and urologic (cystoscopy) procedures have been associated with a greater risk for the development of peritonitis in CAPD patients when a biopsy, polyp removal, or placement of an intrauterine device was performed.[322,331] The risk of peritonitis is also higher in CAPD patients who are chronically constipated, smoke, or have depression.[332,333] Polymicrobial infection with fecal organisms is suggestive of bowel perforation as a complication of catheter placement or secondary peritonitis from other causes.

Alterations in peritoneal defenses may increase the risk of peritonitis in patients undergoing CAPD. The antimicrobial function of peritoneal macrophages and PMN cells generally requires the presence of opsonins. A reduction in the levels of immunoglobulin G and C3 has been noted in peritoneal dialysis effluents in comparison with serum, and the concentrations of these crucial opsonizing agents are related inversely to the frequency of peritonitis.[334] Other important factors that impair host defense mechanisms are the low pH and high osmolality of peritoneal dialysis fluid, both of which can impair PMN leukocyte function and antibiotic efficacy. Newer peritoneal dialysis fluids containing glucose polymers (e.g., icodextrin) may be less detrimental to macrophage and PMN leukocyte activity. However, the use of more biocompatible dialysis fluids has not conclusively been found to decrease the risk of peritonitis.[335,336] The formation of biofilm on the catheter appears to contribute to relapsing or recurrent infection, and to decreased therapeutic responses and development of antimicrobial resistance.

Microbiology

Gram-positive organisms historically have constituted 60% to 80% of isolates, most commonly *S. epidermidis,* followed by *S. aureus, Streptococcus* spp., and diphtheroids. More recent surveys have found a decrease in single gram-positive organism cases and a concomitant increase in other, less previously involved pathogens. Staphylococcal isolates have been noted to grow on polymer surfaces and frequently produce an extracellular slime substance or biofilm (as just described) that may protect these bacteria from host defenses.[337] Vancomycin-resistant enterococci as nosocomial pathogens are now more frequently recognized in peritonitis cases and when encountered should raise concern for another possible intraabdominal process. Gram-negative organisms are obtained from 15% to 30% of isolates. *E. coli* is the most common, followed by *Klebsiella* and *Enterobacter* spp., *Proteus* spp., and *Pseudomonas* spp. Cases caused by gram-negative organisms have increased, according to a retrospective study of peritoneal dialysis-related peritonitis over a decade-long period.[338] Sources may include contaminated water, skin, urinary tract, bowel, and animal contacts (e.g., *Pasteurella multocida*). Less common pathogens include *Acinetobacter* spp., *C. albicans,* and anaerobic bacteria. Rare isolates include atypical mycobacteria (usually *Mycobacterium chelonae* or *Mycobacterium fortuitum*), *M. tuberculosis, Candida parapsilosis, Aspergillus fumigatus, Nocardia*

asteroides complex, and *Fusarium* spp. Although it is unclear whether peritoneal dialysis–related peritonitis can be caused by viruses, the risk of bacterial infection is believed to increase in patients with a viral infection.[339,340]

Polymicrobial peritonitis in patients undergoing peritoneal dialysis is usually assumed to be secondary to a primary intestinal process (e.g., bowel perforation) and usually necessitates surgical exploration.

Diagnosis

Diagnosis of peritonitis is made when microorganisms and an increased number of leukocytes are present in the dialysate in combination with a constellation of clinical findings that include abdominal pain and tenderness (60%–80% of patients), nausea and vomiting (30%–50%), fever (25%–50%), and diarrhea (10%).[321,341] Not all these criteria need to be met, however, for the diagnosis to be made.

The dialysate is almost always cloudy, and microscopic examination reveals a leukocyte count greater than 100 cells/mm^3 (in approximately 85% of cases, >500/mm^3), with neutrophils predominating. The length of the dwell time has an impact on the number of effluent cells. A low leukocyte count in dialysate may also be indicative of a tunnel infection and not peritonitis. Although a predominance of lymphocytes may be encountered with fungal and mycobacterial infections, the majority of cases still manifest a larger number of neutrophils in the peritoneal dialysis effluent. A preponderance of eosinophils in the peritoneal fluid is seen in a self-limited condition called *eosinophilic peritonitis* that often follows placement of the Tenckhoff catheter and may represent allergy to the tubing. Peritoneal eosinophilia may also be present in fungal and parasitic peritonitis, may be related to chemical and drug (i.e., vancomycin) effects, and may be associated with icodextrin. Gram staining of the fluid reveals organisms in 9% to 50% of cases.[321] Peripheral leukocytosis is a poor indicator for peritonitis in this group of patients. Blood cultures are rarely positive, in contrast to the 30% to 50% positive rate in other types of intraabdominal infections.

Peritonitis with negative cultures occurs in 5% to 10% of cases. Constant flow of dialysis fluid into and out of the peritoneal cavity dilutes the microbial density and may falsely lower the rate of positive results of dialysate culture. Negative cultures also may result from infection with fastidious organisms, from previous antimicrobial treatment, or from inadequate culture techniques (e.g., the collection of too little effluent). Culturing the sediment after centrifuging 50 mL of effluent dialysate or placing 5 to 10 mL of effluent dialysate in each of two blood culture bottles will enhance the recovery rate of organisms.[322] All cultures should be performed aerobically. Fungal, mycobacterial, and anaerobic cultures should be performed if clinically indicated (e.g., negative aerobic cultures). Causes of turbid dialysate, such as hemorrhage, fibrin or other proteins, chylous ascites, lymphoma and other malignancies, and prolonged dwell time, should be considered if the leukocyte count is below 300 to 500 cells/mm^3.[342,343]

Radiologic imaging studies are neither specific nor particularly helpful in the diagnosis of peritoneal dialysis–associated peritonitis. Small amounts of free intraperitoneal air can, at times, be discovered in asymptomatic patients.

Treatment and Prognosis

The prognosis of peritonitis in dialysis recipients is generally favorable. However, in one retrospective study, death occurred in 6% of 565 patients with a total of 693 episodes of peritonitis.[344] Mortality rates have also been found to vary according to the specific etiologic pathogen.[345] The duration of illness and positive peritoneal fluid cultures after institution of antimicrobial therapy is usually 1 to 4 days. Some infections, especially infections caused by *S. aureus, Pseudomonas* spp., or fungus, resolve more slowly, however, and may cause relapse more frequently.

Adequate levels of antimicrobial agents necessary to treat peritonitis successfully can be obtained in the peritoneal fluid through either the systemic or intraperitoneal route. Because CAPD-associated peritonitis is a localized infection, however, the intraperitoneal route is preferred and in fact has been found to be superior to the intravenous route.[346] The increased use of intraperitoneal antibiotic therapy for peritonitis has allowed most patients to be treated on an ambulatory basis. Hospitalization is indicated for patients who are severely ill or who are unable to manage the administration of intraperitoneal antibiotics at home. Although a variety of dosages and drugs can be found in the literature, the initial dosages recommended in Table 74.6 for intraperitoneal administration result in effective peritoneal fluid drug concentrations. Subsequent dosing is used to maintain these levels. The aim of the dosing regimen is to maintain a concentration of the drug in the peritoneal cavity fluid greater than the MIC of the offending pathogen for most, if not all, of the dosing interval. However, intermittent dosing regimens (antimicrobials given once daily) and continuous dosing regimens (given in each exchange) have been found to produce largely equivalent results.[346] With intermittent dosing, the antimicrobial must dwell for at least 6 hours. Physicians must exercise caution when reviewing the MIC and minimal bactericidal concentration data because these concentrations have been markedly increased when peritoneal dialysis effluent is used as the in vitro growth medium.[347]

Because of the lack of comparative, prospective clinical trials, no antimicrobial regimen is superior to another.[348] After cultures are obtained, initial antimicrobial therapy should be based on the results of Gram staining or, if the Gram stain is not helpful, directed against the most likely pathogens, providing coverage against both gram-positive and gram-negative organisms. A reasonable initial empirical regimen would be vancomycin in combination with an aminoglycoside. Vancomycin is preferable to a cephalosporin because of the frequency of β-lactam resistance (i.e., methicillin resistance) in staphylococci, which is also predictive of resistance to cephalosporins. The earlier finding of superior cure rates with glycopeptides compared with early generations of cephalosporins was unable to be confirmed in later trials.[346] Alternatively, ceftazidime, cefepime, or a carbapenem can be used in place of an aminoglycoside for empirical coverage of gram-negative organisms. Fluoroquinolones such as ciprofloxacin have also proven to be very effective agents against gram-negative pathogens, but owing to the increase in fluoroquinolone-resistant enteric bacteria, they should be avoided when known local fluoroquinolone resistance rates are greater than 10%.[349] Initial antibiotic choices should be modified, if necessary, after culture results are obtained. *P. aeruginosa* peritonitis is associated with high rates of treatment failure and relapses. Although the use of a combination of antipseudomonal agents pending susceptibility data is prudent practice, prolonged dual therapy has not been found to improve treatment outcomes. In addition to antibiotics, catheter removal is generally needed when *Pseudomonas* is the infecting pathogen. If vancomycin-resistant enterococci are determined to be the causative microorganism, linezolid or daptomycin should be administered.[350] The minimal length of therapy needed for dialysis-related peritonitis has not been determined, but the usual duration ranges from 10 days to 3 weeks. Longer antibiotic treatment courses (≥21 days) are required for peritonitis due to *S. aureus* and *P. aeruginosa.* Most patients with CAPD-associated peritonitis exhibit clinical improvement within 48 to 96 hours after initiation of antimicrobial therapy. If the signs and symptoms of peritonitis persist after 96 hours of therapy, reevaluation is warranted; the possibilities of resistant pathogens, unusual organisms (e.g., mycobacterial, fungal), and other intraabdominal processes should be considered.

In peritoneal dialysis patients who present with typical findings of peritonitis but have negative cultures, the same treatment regimens should be administered with durations of therapy similar to that for staphylococci.

Fungal peritonitis, usually caused by *C. albicans,* historically has been treated with amphotericin B,[351,352] although echinocandins are currently the preferred initial antifungal agent,[223] particularly in patients with prior exposure to azole antifungal agents. Patients should be transitioned to fluconazole when clinically stable and susceptibility to fluconazole has been confirmed. Initial therapy with fluconazole may be considered in selected patients, including those who are not critically ill.[223] If a mold is isolated, initial therapy with amphotericin B is warranted, although for *Aspergillus* spp., voriconazole given orally is a reasonable alternative. Some molds, including *Fusarium* spp., may be resistant to amphotericin, however. If CAPD is continued, amphotericin B should be given intraperitoneally, but it can cause appreciable abdominal pain when given by this route. There is also concern that amphotericin B–induced inflammation may cause adhesions that reduce the effective

TABLE 74.7 CAPD Peritonitis: Indications for Catheter Removal

Persistent infection at skin exit site or tunnel
Relapsing peritonitis with same organism—within 4 weeks
Refractory peritonitis—failure to respond within 5 days
Fungal, mycobacterial, *Pseudomonas aeruginosa* peritonitis
Intraperitoneal abscess
Catheter malfunction

CAPD, Continuous ambulatory peritoneal dialysis.

dialyzing surface. In most patients, CAPD-associated fungal infections fail to respond unless the catheter is removed; amphotericin B should be given intravenously for 10 days after catheter removal. Oral or intravenous fluconazole penetrates adequately into the peritoneal fluid to treat peritonitis due to susceptible fungi in CAPD recipients after the catheter has been removed. Flucytosine is not recommended in azotemic patients because of potentially lethal toxicity to the colon and bone marrow. Ketoconazole is no longer used.

Additional nonantimicrobial interventions such as routine peritoneal lavage, the use of fibrinolytic agents, and the instillation of intraperitoneal immunoglobulins have not proved beneficial and therefore serve no role in the management of peritoneal dialysis–associated peritonitis.[346] There is no benefit to stopping peritoneal dialysis when peritonitis is diagnosed, and its discontinuation may lead to complications such as injury to the peritoneal surface.[353]

Catheter Removal and Prevention

Removal of the catheter is necessary in 10% to 20% of patients (Table 74.7). The indications for catheter removal include persistent infection at the skin exit site or tunnel; fungal, fecal, or mycobacterial peritonitis; *P. aeruginosa* peritonitis; persistent peritonitis despite 5 days of treatment; recurrent peritonitis with the same organism; and catheter malfunction (e.g., poor flow). The catheter should also be removed in patients with intraperitoneal abscess. In the setting of relapsing peritonitis, a new catheter can be placed at the time of removal if the fluid is clear, but not when removed for recalcitrant or difficult-to-sterilize cases such as fungal infection. Use of oral or intraperitoneal antibiotics has not been shown to be effective in preventing peritonitis during peritoneal dialysis. An antibiotic given just before placement of the peritoneal catheter may decrease the incidence of peritonitis and wound infection. Antibiotic prophylaxis has been suggested for patients before extensive dental procedures (although peritonitis caused by dental flora is unusual) and before colonoscopy with biopsy or polypectomy. Also, it has been suggested with upper endoscopy and certain gynecologic procedures, including cervical and hysteroscopic biopsies and placement of intrauterine devices.[322,331] In addition, topical mupirocin applied to the exit site or intranasal application of mupirocin has been used to eliminate nasal carriage with *S. aureus* and has been shown to reduce exit site infections, but it has not yet been shown to significantly reduce the incidence of CAPD-related peritonitis.[354] Advances in CAPD instrumentation, such as titanium adapters, connector systems with disinfectant, and in-line filters, may decrease the frequency of peritonitis but add to the overall cost of CAPD.

Acute Peritoneal Dialysis

The incidence of peritonitis during acute peritoneal dialysis has remained stable since the 1980s. Innovations in technique, which began during the 1960s, reduced the rate of peritonitis from 50% to lower levels. These innovations included closed drainage systems, small-bore catheters, limitation of dialysis to no longer than 72 hours, incorporation of a Millipore filter into the tubing, and development of closed automatic systems. Use of dry-heat incubators to warm the dialysate also decreases the risk of contamination that may occur when water baths are used for this purpose.

Some authorities have recommended that cultures of dialysate be obtained every 8 to 24 hours during acute peritoneal dialysis and at its termination. Culture of dialysate from the last exchange is more useful than culture of the catheter tip at the end of dialysis because the catheter tip is frequently contaminated at the time of its removal. Results of these routine cultures, in the absence of symptoms or cloudy fluid, are of doubtful value for initiation of therapy. Of more importance, dialysate samples should be cultured and examined microscopically (cell count, Gram stain) if the dialysate becomes cloudy or if the patient develops signs or symptoms of peritonitis (e.g., fever, abdominal pain). Cultures are obtained best by syringe from the port closest to the catheter.

Antibiotic-resistant, hospital-acquired, gram-negative bacilli and staphylococci frequently cause peritonitis during acute peritoneal dialysis. It is recommended that therapy be initiated with intraperitoneal vancomycin and gentamicin (or tobramycin), with or without concurrent or subsequent parenteral infusion of the same antibiotics, depending on the severity of the illness and the response to initial therapy (see Table 74.6 for dosages). The antibiotic regimen should be modified when the culture results become available. The clinical manifestations, prognosis, and response to therapy are similar to those described previously for peritonitis associated with chronic peritoneal dialysis.

INTRAPERITONEAL ABSCESSES

Etiology

Intraperitoneal abscess can complicate either primary or secondary peritonitis. Diseases causing secondary intraperitoneal abscesses include appendicitis, diverticulitis, biliary tract lesions, pancreatitis, perforated peptic ulcers, inflammatory bowel disease, trauma, and abdominal surgery. The relative frequency of abscess formation associated with appendicitis may be declining, and the frequency of trauma and diverticulitis may be increasing.[355] The location of an abscess is generally related to the site of primary disease and the direction of dependent peritoneal drainage. Appendicitis has been reported to be associated most commonly with right lower quadrant and pelvic abscesses; colonic diverticulitis, with left lower quadrant and pelvic abscesses; and pancreatitis, with lesser sac abscesses. In one large series of 194 intraperitoneal abscesses in 1973,[356] about 44% were in the right lower quadrant, 14% in the left lower quadrant, and 14% in the pelvis, whereas 20% were perihepatic. In a 1983 series reported by Saini and associates,[357] the frequencies of various abscess locations had changed, perhaps reflecting the change in the relative frequency of the various etiologic diseases: subphrenic, 26%; pelvic, 20%; paracolic, 13%; periappendicular, 13%; retroperitoneal, 10%; hepatic, 7%; interloop, 4%; and lesser sac, 4%.

Of the various perihepatic (right subphrenic, right subhepatic, left perihepatic, and lesser sac) abscesses, the most common is in the right subphrenic space, but the difference in numbers between the right and left sides has been decreasing. In 1977, in one large series of 267 cases of intraabdominal abscesses, about half were in the subphrenic space, 60% of which were noted in the left perihepatic space.[358] This increased frequency of left perihepatic space abscess has also been noted more recently. That finding is in contrast to the series of Ochsner and DeBakey in 1939,[359] when right subphrenic abscesses were most common owing to the numerous ruptured appendices.

In children, appendicitis is still responsible for more than 50% of the cases of subphrenic abscess. In adults, perihepatic abscesses currently occur mainly as postoperative complications, rather than in neglected primary intraabdominal infections, such as appendicitis or perforated peptic ulcer. This fact may explain the increasing frequency of subphrenic abscess, especially on the left side, in comparison with other intraperitoneal sites.[358] Usually the surgery has been in the gastroduodenal and biliary tracts. One group of investigators[360] noted that abscesses that occurred after gastric operations were in the left subphrenic space if incidental splenectomy had been performed but in the right subhepatic space if splenectomy had not been performed. The subhepatic space is involved less frequently than the subphrenic spaces. Lesser sac abscesses usually follow pancreatitis or perforation of the stomach or duodenum. Multiple perihepatic space abscesses have been reported in 5% to 26% of the patients.[360]

Bacteriologic Findings

These infections are typically polymicrobial. In studies in which bacteriologic techniques permitted isolation of anaerobes, anaerobes were found in 60% to 70% of cases.[356,357,361] In one study, anaerobes were recovered in 20 of 24 subphrenic abscesses and *B. fragilis* was the most common pathogen; anaerobic cocci and clostridia were found in 50%

of the patients. Other bacteria frequently recovered are *E. coli, Klebsiella* and *Enterobacter* organisms, *Proteus* spp., *P. aeruginosa, S. aureus,* and enterococci.[356]

Pathogenesis

Intraperitoneal abscesses develop as a result of localization of diffuse peritonitis, usually in the pelvis, perihepatic spaces, and paracolic gutters. In addition, abscesses may develop around diseased organs (e.g., periappendiceal or pericholecystic abscesses) or after a penetrating wound (stabbing, gunshot, auto accident, or other trauma) or surgical procedure. These abscesses are termed *secondary* and account for most intraperitoneal cases. In contrast, the pathogenesis of primary abscesses is unknown and is presumably similar to that of primary peritonitis.

Clinical Manifestations

An acute course, with a high intermittent fever, shaking chills, abdominal pain, and tenderness over the involved area, is characteristic. The clinical pattern may be that of an acute secondary illness occurring after surgery for primary abdominal disease or a prolonged recuperative course in a patient who has been receiving antibiotics after abdominal surgery. Various authors[359,360] have emphasized the occasional chronicity of subphrenic abscesses and have speculated that the course is often modified by antibiotics. Subphrenic abscesses have been described with 6 months or more of an indolent illness.[362] Some patients may present with fever of unknown origin.

Local symptoms and signs vary widely with the location and source of the abscess. Subphrenic abscesses are usually accompanied by chest findings with costal tenderness and pleural or pulmonary involvement, whereas subhepatic abscesses have more dominant signs of upper abdominal or subcostal involvement and fewer pulmonary changes.

Diagnosis

Noninvasive diagnostic procedures, including ultrasonography and CT, have provided greater sensitivity and specificity than have routine radiography and radionuclide scanning.[363] These latter techniques are occasionally useful, however, and sometimes a combination of diagnostic tests is the optimal approach to confirm the diagnosis of intraabdominal abscess.

Plain radiographs of the abdomen can suggest the location of abscesses in 50% of patients.[364] Radiologic findings associated with a subphrenic abscess may include pleural effusion, elevation of the hemidiaphragm, or loss of diaphragmatic movement at fluoroscopy. Routine radiography also may reveal displacement of viscera by an abscess. These findings can be enhanced with contrast media. The stomach may be outlined with barium or air to indicate displacement caused by a left perihepatic or lesser sac abscess. The presence of gas, either as a single air-fluid level or as mottling within the abscess, may aid in localization on routine abdominal radiography.

Leukocytes tagged with gallium 67 and indium 111 are used in radionuclide scans, which at times may be helpful in detecting intraabdominal abscesses, although they have been largely supplanted by CT and MRI. In contrast to the technetium 99m sulfur colloid liver-spleen scan, which visualizes the entire organ and delineates abnormal areas as "cold" spots caused by decreased uptake of the isotope, gallium 67–tagged and indium 111–tagged leukocytes accumulate in areas of inflammation, such as abscesses, and appear as areas of increased radioactivity or "hot" spots.[365] Gallium is excreted into the intestinal tract and can accumulate in any inflammatory process and in certain neoplasms. For these reasons, false-positive scan readings can occur when radioactivity within the lumen of the bowel, within the wall of an inflamed bowel, or within a noninfected operative site in the process of healing is misinterpreted as an intraabdominal abscess.

Indium 111–tagged leukocyte scans are as sensitive as but more specific than gallium 67–tagged scans. The labeled leukocytes tend to concentrate only in areas of inflammation because, in contrast to gallium 67, indium 111 is not secreted into the bowel.[366] Abscesses in the liver and spleen may be difficult to detect solely on gallium 67–tagged or indium 111–tagged leukocyte images because normal accumulation of activity in these organs may mask an adjacent inflammatory focus. This problem can be overcome by comparing gallium 67 or indium 111 images with technetium 99m scans.

Ultrasonography is a noninvasive technique that is helpful in the determination of the size, shape, consistency, and anatomic relationships of an intraabdominal mass. The appearance of abscesses may vary widely from echo-free lesions to highly echogenic masses, but they typically appear as a fluid collection with an irregular wall and the presence of a few internal echoes. Ultrasound images may be obscured by overlying gas-filled viscera and by postoperative wounds and drains.

CT has proved especially well suited for the diagnosis of intraabdominal abscess.[367,368] Definition is unimpeded by intraluminal gas and postoperative changes except in the presence of surgical metallic clips or residual barium that may disrupt the image. Observed findings consistent with abscess include a low-density tissue mass and a definable capsule (Fig. 74.4). CT can reveal extraluminal gas, a finding highly suggestive of abscess. Contrast material is commonly administered orally and intravenously in attempts to diagnose intraabdominal abscess. The

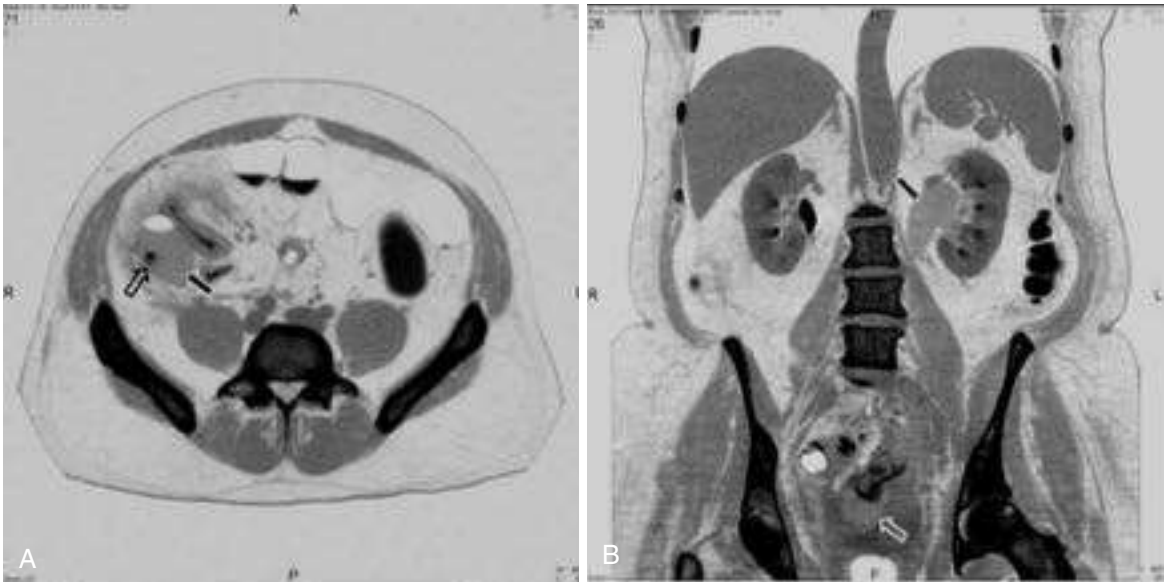

FIG. 74.4 Computed tomographic scans of abdomen and pelvis after administration of oral and intravenous contrast medium. (A) Perforated appendicitis: right lower quadrant collection with an air-fluid level *(solid arrow)* surrounding a dilated appendix *(open arrow).* (B) Perforated diverticulitis associated with abscess *(open arrow),* producing obstructive hydronephrosis *(solid arrow).*

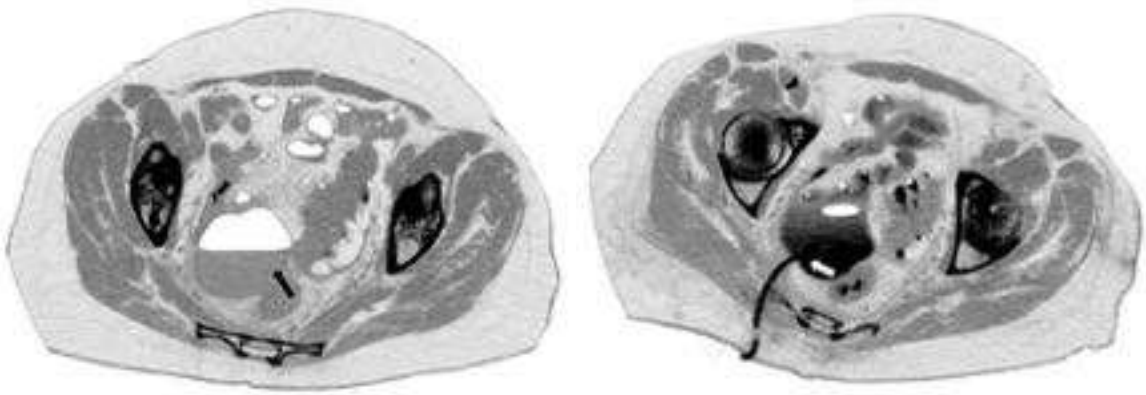

FIG. 74.5 Computed tomographic scans of abdomen-pelvis. (A) Perforated diverticulitis: pelvic collection with an air-fluid level *(white arrow).* (B) Same patient after interventional percutaneous drain placement of the diverticular abscess *(black arrow).*

intraluminal contrast material helps to distinguish loops of bowel from abscess cavities, and the parenteral contrast material may enhance a surrounding capsule, allowing for easier identification.

MRI has the potential to display normal anatomy and to reveal abnormal conditions in many of the body's organ systems and anatomic regions.[369,370] Only a few trials have compared MRI with older radiologic procedures. In one study, MRI more clearly delineated the extent of inflammatory changes than did CT, and it distinguished the abscess better from the surrounding structures.[371] In addition, the use of MRI does not require the administration of contrast medium and eliminates exposure to radiation, but it may be more costly than radiologic techniques.

Arteriographic localization has also been helpful. Overreliance on any one of these techniques is dangerous, and results should be confirmed with other methods and the clinical findings.

Prognosis

The period of morbidity is unusually prolonged in patients with intraperitoneal abscesses. Altemeier and colleagues[356] reported average hospital stays of 21 to 47 days. The presence of residual recurrent infection caused by inadequate surgical drainage, more common in patients with multiple or bilateral abscesses, is associated with significantly greater mortality.

Therapy

The main therapy for any intraperitoneal abscess is drainage. Effective management depends on accurate localization of the abscess, discrimination between single and multiple abscesses, and early and adequate drainage. Conventional therapy for intraperitoneal abscesses usually has included surgical drainage. Since the 1980s, successful therapy has been accomplished with percutaneous catheter drainage as an alternative to surgery (Fig. 74.5).[372–375] This method has become possible with the use of refined imaging techniques, especially ultrasonography and CT.[376]

The general requirements for CT-guided or ultrasonography-guided percutaneous catheter drainage include (1) an abscess that can be approached adequately via a safe percutaneous route; (2) an abscess that is unilocular; (3) an abscess that is not vascular and the absence of coagulopathy; (4) joint radiologic and surgical evaluation, with surgical backup for any complication or failure; and (5) the possibility of dependent drainage through the percutaneously placed catheter.[315] CT also allows detection of an unsuspected additional intraabdominal problem that would otherwise necessitate surgical intervention. Percutaneous catheter drainage can be used as an initial approach in a patient too unstable to withstand immediate surgery. Definitive surgery can be postponed until the patient is in better condition. Percutaneous drainage of peridiverticular or appendiceal abscesses may permit a subsequent one-stage procedure of primary resection and immediate anastomosis, rather than the more costly and complicated multistage procedure.[377–379] After percutaneous placement of the catheter with CT or ultrasound guidance and aspiration of the abscess cavity, the catheter is placed for drainage by gravity or low suction until the daily drainage volume is minimal. Clinical response and collapse of the abscess cavity, evident on repeat scanning, should follow successful drainage. Some patients with percutaneous catheter drainage can be managed at home with their catheters in place. In 80% to 90% of the patients who fit these criteria, percutaneous drainage has been successful.[373] Attempts at drainage of loculated, poorly organized, multiple, or extensive collections are less successful. In most series, the frequency of complications ranges from 5% to 15%,[372] including septicemia, hemorrhage, peritoneal spillage, and fistula formation. In addition, failure may occur because of undrained abscesses or pus too viscid to drain via the catheter. Reports indicate that the morbidity and mortality associated with percutaneous drainage may be lower than with surgical treatment.[380]

Antimicrobial therapy should be started immediately after appropriate specimens (e.g., blood) are obtained for culture; usually they are obtained before drainage. Because the pathogens are usually similar to those involved in secondary peritonitis, initial antibiotic therapy is directed similarly at the anaerobes, especially *B. fragilis* and the Enterobacteriaceae. The antimicrobial regimens discussed in the section on treatment of secondary peritonitis should be appropriate initial therapy (see Table 74.5). This antibiotic regimen should be adjusted to conform to results of in vitro testing of the infecting organisms isolated from blood or purulent material obtained at surgery or from catheter drainage. During the course of a prolonged illness, repeated cultures of blood and purulent collections, when clinically indicated, should provide a basis for change in antimicrobial therapy.

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The complete reference list is available online at Expert Consult.

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75 Infections of the Liver and Biliary System (Liver Abscess, Cholangitis, Cholecystitis)

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SHORT VIEW SUMMARY

Definition
- A pyogenic liver abscess is the end result of a number of pathologic processes resulting in a focal, purulent bacterial collection in the liver.
- An amebic liver abscess is an invasive complication of intestinal amebiasis resulting in a focal collection of nonpurulent fluid in the liver.
- Cholecystitis is inflammation/bacterial infection of the gallbladder often resulting from obstructing gallstones. Acalculous cholecystitis is a similar process in the absence of gallstones.
- Cholangitis is inflammation/infection of the bile ducts.

Epidemiology
- An estimated 1 to 4 cases of pyogenic liver abscess occur per 100,000 persons each year.
- Amebic liver abscesses are rare—about 1 case per million persons annually in the United States—but are more common in endemic regions. They affect men about 10 times more frequently than women.
- Tens of millions in the United States have gallstones; 1% to 3% are complicated by acute cholecystitis.
- Approximately 120,000 cholecystectomies occur each year in the United States.
- Between 2% and 15% of cases occur without gallstones, known as "acalculous cholecystitis."
- In endemic regions, parasites such as *Ascaris* and *Clonorchis* may cause biliary disease.

Microbiology
- Pyogenic liver abscesses may be monomicrobial, especially when caused by bacteremia, or polymicrobial, involving aerobic gram-negative bacilli and anaerobes.
- Hypermucoviscous *Klebsiella pneumoniae* (typically K1 and K2 strains) are now an important cause of pyogenic liver abscess, particularly in parts of Asia but increasingly elsewhere.
- Amebic liver abscesses are caused by invasive strains of *Entamoeba histolytica.*

Diagnosis
- Symptoms are often nonspecific, and a high index of suspicion is required.
- Diagnostic imaging, especially by ultrasonography, radionuclide cholescintigraphy, computed tomography, or magnetic resonance imaging, is essential. For pyogenic liver abscesses, it is often coupled with diagnostic/therapeutic aspiration.

Therapy
- Some pyogenic processes require prompt recognition and urgent image-guided drainage or definitive surgery.
- Antibiotics directed at the suspected pathogens play an important role as adjuncts to surgery, or, in limited cases, they may be the sole form of therapy, (e.g., small pyogenic liver abscesses [see Tables 75.4 and 75.6], amebic liver abscess).

LIVER ABSCESS

Liver abscesses fall broadly into two categories: amebic and pyogenic. Amebic liver abscess represents a distinct clinical entity caused by invasive *Entamoeba histolytica* infection. It has a distinct pathogenesis that is characterized by the specific induction of hepatocyte apoptosis by the organism. Further discussions of the epidemiology, genetics, and biology of *E. histolytica* and of intestinal amebiasis can be found elsewhere in this volume (see Chapters 98 and 272). Pyogenic liver abscess, by contrast, does not represent a specific liver disease but is the end result of a number of pathologic processes that cause a suppurative infection of the liver parenchyma.

Epidemiology/Etiology

Amebic Liver Abscess

In the United States, amebic liver abscess has become a rare disease that is found almost exclusively in travelers and immigrants. In 1994, the last year in which incidence data were collected, there were only 2983 cases of amebiasis; the percentage of cases complicated by abscess is unknown, but it is probably well under 10%, roughly 1 case per million persons per year.[1] Worldwide, contamination of food and drinking water has maintained *E. histolytica* infection second only to malaria as a cause of death from parasitic disease. The epidemiology of this disease has been greatly informed by the appreciation that a closely related nonpathogenic species, *Entamoeba dispar,* colonizes between 5% and 25% of persons.[2,3] *E. dispar* has no apparent propensity for invasive disease, even among patients with acquired immunodeficiency syndrome (AIDS). In industrialized countries, most asymptomatic individuals with *Entamoeba* in their stool are colonized with *E. dispar,* whereas in highly endemic regions, asymptomatic infection with *E. histolytica* may exceed the rate of *E. dispar* colonization.[4–11] In addition, other *Entamoeba* species have been identified; these include *Entamoeba moshkovskii,* which causes noninvasive diarrhea, and *Entamoeba bangladeshi,* which is of unknown virulence. Because these species are indistinguishable by light microscopy, the presence of *Entamoeba* in the stool is not sufficient to establish the cause of a liver abscess. Men and women experience equivalent rates of *E. histolytica* infection, but adult men are at a 10-fold higher risk for invasive disease (colitis or extracolonic disease) than other populations.[12] The reason for this disparity remains uncertain, although gender differences in complement-mediated killing of *E. histolytica* and cytokine responses to amebic liver infection have been proposed.[13,14] In addition, testosterone has been shown to be a risk factor for the development of amebic liver abscesses in experimental animals.[15,16]

Pyogenic Liver Abscess

The incidence of pyogenic liver abscess is about 1 to 4 cases per 100,000 individuals annually in the United States and Europe.[17–20] This incidence has been relatively stable, with a slight increasing trend in more recent case series that may reflect changes in the true incidence or improved detection.[19] In Asia, the incidence of pyogenic liver abscess may be 5- to 10-fold higher and is associated with the emergence of community-acquired *Klebsiella pneumoniae* infection.[21,22] Pyogenic liver abscess is a disease of middle-aged persons, with a peak incidence in the fifth and sixth decades of life; this pattern mirrors the prevalence in the population of biliary disease, which is now the major cause of pyogenic liver abscess. No significant sex, ethnic, or geographic differences seem to exist in disease frequency, in contrast to the epidemiology of amebic liver abscess. About one-half of patients have a solitary abscess. Right-sided

abscesses are most common, followed by left-sided abscesses and then abscesses involving the caudate lobe. This distribution probably reflects the relative mass of each lobe, although more complicated explanations, such as patterns of hepatic blood flow, have been proposed.

Pathogenesis and Pathophysiology

Amebic Liver Abscess

Infection with *E. histolytica* results from ingestion of cysts in fecally contaminated food or water. Excystation occurs in the intestinal lumen, and trophozoites migrate to the colon, where they adhere by means of a lectin that specifically binds galactose *N*-acetyl-D-galactosamine (the Gal/GalNAc lectin) on colonic epithelium and multiply by binary fission.[23,24] At high densities, trophozoite encystation is initiated, and newly formed cysts are released into the stool to complete the life cycle. Most individuals experience asymptomatic infection, but approximately 10% develop symptomatic colitis when invasion of the colonic mucosa occurs. Spread to the liver via the portal system occurs in less than 1% of cases.[2] A number of virulence factors have been implicated in the development of amebic liver abscess, including small proteins (amoebapores) that punch holes in lipid bilayers of target cells, cysteine proteases, and the Gal/GalNAc lectin.[25–31] *E. histolytica* induces apoptosis in hepatocytes and neutrophils, forming large, nonpurulent, "anchovy paste" abscesses that grow inexorably without treatment.[32,33] The mechanism by which *E. histolytica* induces apoptosis is unknown, but its importance is underscored by the resistance of caspase-3–deficient mice to amebic liver abscess formation.[34,35] Recently, an *E. histolytica* surface protein that participates in apoptotic corpse phagocytosis, the phagosome-associated transmembrane kinase (PATMK), was shown to be required for intestinal amebiasis in a murine infection model; however, PATMK was found to not contribute to the development of experimental amebic liver abscess.[36] By contrast, amoebapore expression was found to be required for the formation of liver abscesses but not amebic colitis.[29] These findings suggest that the contribution of particular amebic virulence factors to pathogenesis may be tissue specific, and many of these factors have been recently reviewed.[37]

Predisposition to liver disease is influenced by host genetics. In studies of Mexican mestizo children and adults, individuals with the major histocompatibility complex haplotype HLA-DR3 have an increased frequency of amebic liver abscess compared with healthy populations of the same socioeconomic background.[38,39] Host factors thought to be critical in the containment and clearance of invasive amebae include complement, neutrophils, interferon-γ, nitric oxide, and adaptive immune responses (see Chapter 272).[13,14,40–44]

Pyogenic Liver Abscess

Pyogenic liver abscess does not represent a distinct pathophysiologic process but occurs whenever the initial inflammatory response fails to clear an infectious insult to the liver. Pyogenic liver abscesses are usually classified by presumed route of hepatic invasion: (1) biliary tree, (2) portal vein, (3) hepatic artery, (4) direct extension from contiguous focus of infection, and (5) penetrating trauma. Ideally, this approach defines the microbiology of the abscess and therefore guides empirical antibiotic choice, but it is limited by the high frequency of cryptogenic abscesses. The frequencies of the presumed routes of hepatic invasion are presented in Table 75.1.[18,45–76]

TABLE 75.1 Frequency of Route of Infection in Hepatic Abscess

ROUTE OF INFECTION	FREQUENCY (%)
Biliary tree	40–50
Hepatic artery	5–10
Portal vein	5–15
Direct extension	5–10
Trauma	0–5
Cryptogenic	20–40

Modified from references 17, 18, 42–45, and 46–71.

1. *Biliary tree.* Cholangitis (discussed later) is now the major identifiable cause of pyogenic liver abscess. In such cases, multiple abscesses are usually present and anaerobes are infrequent. The underlying biliary obstruction is usually a result of gallstone disease, but it can also be caused by an obstructing tumor, an occluded stent, overwhelming cryptosporidiosis, or *Ascaris lumbricoides* migration into the biliary tree.
2. *Hepatic artery.* Any systemic bacteremia (e.g., endocarditis, line sepsis) can spread to the liver via this route. Patients who die of overwhelming sepsis frequently have extensive microabscess formation in their livers at autopsy, but macroscopic liver abscess formation in patients who recover from septic shock is uncommon, underscoring the capacity of the liver to clear even large insults.
3. *Portal vein.* The portal venous system drains almost all of the abdominal viscera. Pylephlebitis from diverticulitis, pancreatitis, omphalitis, inflammatory bowel disease, or postoperative infection can result in pyogenic liver abscess. Untreated appendicitis was historically a major cause in this category but was greatly diminished in importance with the introduction of antibiotics.
4. *Direct extension from a contiguous focus of infection.* This may occur with cholecystitis or subphrenic, perinephric, or other intraabdominal abscesses.
5. *Trauma.* Any penetrating trauma to the liver, even as subtle as ingestion of a toothpick, can result in abscess formation. Blunt trauma can also predispose to pyogenic liver abscess formation, presumably because of hepatic hematoma formation and subsequent increased risk of seeding by bacteria. Similarly, hepatic destruction from sickle cell disease, tumor necrosis (either spontaneous or following iatrogenic embolization), or cirrhosis can predispose to abscess formation.

Host factors that predispose to abscess formation from "routine" hepatic bacterial insults may be present in many cryptogenic abscesses. Systemic illness such as diabetes mellitus, cardiopulmonary disease, malignancy, and cirrhosis are common in patients with liver abscesses and may be predisposing factors. Diabetes was shown in one retrospective study to impart a greater than threefold risk of development of pyogenic liver abscess.[77] Conditions of neutrophil dysfunction are associated with a marked predisposition for abscesses of the liver and elsewhere. For example, one-third of patients with chronic granulomatous disease followed at the National Institutes of Health Clinical Center over four decades developed pyogenic liver abscesses; 47% had recurrent abscesses.[78] Finally, hemochromatosis conveys a particular susceptibility to abscesses caused by *Yersinia enterocolitica*.[79]

Microbiology

Amebic Liver Abscess

Although *E. histolytica* is the only species of *Entamoeba* known to cause invasive infections, certain strains may be more proficient at causing liver disease than others. In surveys of clinical *E. histolytica* isolates, genetically distinct strains recovered from amebic liver abscesses were infrequently found to cause asymptomatic intestinal colonization or intestinal disease.[80,81] More recently, Ali and colleagues[82] analyzed paired isolates from patients with concurrent amebic liver abscesses and intestinal infection. In all cases, the liver and intestinal amebae had discordant genotypes, suggesting either that amebae undergo genetic reorganization during invasion or that only a subset of strains is capable of metastasizing to the liver.

Pyogenic Liver Abscess

In light of the diverse pathologic processes that have been discussed, it is understandable that sweeping generalizations about the microbiology of pyogenic liver abscess are difficult. The picture is further complicated because abscess material is rarely obtained before the administration of antibiotics. Even in the preantibiotic era, a high rate of sterile cultures was seen, probably reflecting inadequate culture techniques. Despite these difficulties, abscess cultures are positive in 80% to 90% of cases.

TABLE 75.2 Microbiology of Liver Abscess

TYPE OF ORGANISM	COMMON (>10%)	UNCOMMON (1%–10%)
Gram-negative	*Escherichia coli* *Klebsiella* spp.	*Pseudomonas* *Proteus* *Enterobacter* *Citrobacter* *Serratia*
Gram-positive	*Streptococcus* (anginosus group) *Enterococcus* spp. Other viridans-group streptococci	*Staphylococcus aureus* β-Hemolytic streptococci
Anaerobic	*Bacteroides* spp.	*Fusobacterium* Anaerobic streptococci *Clostridium* spp. Lactobacilli

The demonstration of anaerobic organisms in 45% of pyogenic liver abscesses by Sabbaj and colleagues[83] in 1972 led to an increased awareness of fastidious pathogens, and in recent case series, anaerobes were recovered 15% to 30% of the time.[a] Some of this decrease in the isolation of anaerobic organisms is probably attributable to the emergence of biliary tract disease as the major underlying cause of pyogenic liver abscess, but because of the difficulty in obtaining adequate culture data, these should be viewed as minimum estimates. There has also been an increased appreciation that many liver abscesses are polymicrobial, with estimates ranging from 20% to 50%, depending on the case series. If the source of the abscess is considered, abscesses with a biliary source are most likely to be polymicrobial, and cryptogenic abscesses are most frequently monomicrobial.[61] Some observers have noted that solitary abscesses are more likely to be polymicrobial than are multiple ones.[54,55] Although this has not been universally observed[61] and the small size of these studies prevents conclusive statements, it does suggest two alternative mechanisms for abscess formation. In the first, a synergistic combination of organisms converges by chance to form a single abscess, and in the second, a highly pathogenic organism forms abscesses wherever it was seeded.

In terms of specific pathogens (Table 75.2), *Escherichia coli* and *K. pneumoniae* are by far the most common isolates. The latter has been increasingly recognized as a cause of community-acquired monomicrobial liver abscesses (discussed later). Enterococci and viridans-group streptococci are also common, primarily in polymicrobial abscesses. *Staphylococcus aureus,* by contrast, is more commonly associated with monomicrobial abscesses. Although pyogenic liver abscesses due to fungi, particularly *Candida* species, have been reported, they are comparatively rare and are excluded from most case series.

16S ribosomal RNA sequencing may be a useful technique to identify the etiologic agent in instances in which cultures are negative. In a cohort of 20 Korean patients, only 45% of abscess fluid bacterial cultures were positive, compared with 90% of samples tested using 16S ribosomal RNA sequencing. Of interest, all samples were found to contain sequences of unknown bacteria.[84] Whether these unknown organisms play a role in the pathogenesis of pyogenic liver abscess remains to be determined.

Epidemic *Klebsiella pneumoniae* Pyogenic Liver Abscess

In the mid-1980s, investigators in Taiwan first noted a distinctive syndrome of monomicrobial *K. pneumoniae* pyogenic liver abscess in individuals who were often diabetic but had no biliary tract disorders.[85–87] Subsequently, community-acquired *K. pneumoniae* liver abscess has become a major health problem in parts of Asia, accounting for 80% of all cases of pyogenic liver abscess in Taiwan and South Korea,[88–90] and has been reported sporadically elsewhere in Asia, North America, Europe, Africa, and Australia, initially in persons of Asian ethnicity (raising the possibility of genetic predisposition), but more recently also in individuals of non-Asian descent.[90–97] Although mortality rates for epidemic *K. pneumoniae* liver abscess are generally low, metastatic infections such as meningitis and endophthalmitis occur in 10% to 16% of all cases.[98–100] Not surprisingly, *K. pneumoniae* has also become a frequent cause of pylephlebitis in this region.[101]

Infections are primarily caused by hypermucoviscous strains of *K. pneumoniae* of the capsular K1 (or occasionally K2) serotype.[98,99] Chuang and colleagues[102] have identified a 25-kb chromosomal element containing 20 open reading frames that directs K1 capsular polysaccharide synthesis (CPS). Mutagenesis of one *cps* gene, *magA* (for mucoviscosity-associated gene A), abolishes hypermucoviscosity, increases sensitivity to phagocytosis and serum-mediated lysis, and reduces virulence in mice.[103] *magA* can also be used as a genetic marker for invasive serotype K1 *K. pneumoniae* strains. Epidemic hypermucoviscous strains of *K. pneumoniae* often produce other virulence determinants, including the regulator of mucoid phenotype protein RmpA and the iron-acquisition system aerobactin, encoded on a 170- to 180-kb large virulence plasmid (pLVPK) not typically found in other *K. pneumoniae* strains.[104] Isolates are characteristically highly drug sensitive, but recent outbreaks of often fatal ventilator-associated pneumonia in China due to carbapenemase-producing hypermucoviscous *K. pneumoniae* raise the specter of life-threatening, highly drug-resistant *K. pneumoniae* infections not only in hospitals, but potentially in the community.[105]

TABLE 75.3 Signs and Symptoms of Liver Abscess

FEATURE	AMEBIC LIVER ABSCESS[16,46–75,117]	PYOGENIC LIVER ABSCESS[17,72,106–115]
Epidemiology		
Male-to-female ratio	5–18	1–2.4
Age (yr)	30–40	50–60
Duration (d)	<14 (≈75% of cases)	5–26
Mortality (%)	10–25	0–5
Symptoms and Signs (Approximate % of Cases)		
Fever	80	80
Weight loss	40	30
Abdominal pain	80	55
Diarrhea	15–35	10–20
Cough	10	5–10
Jaundice	10–15	10–25
Right upper quadrant tenderness	75	25–55
Laboratory Tests (Approximate % of Cases)		
Leukocytosis	80	75
Elevated alkaline phosphatase	80	65
Solitary lesion	70	70

Clinical Manifestations

Amebic Liver Abscess

Patients with amebic liver abscess typically present with fever and a dull, aching pain localizing to the right upper quadrant, but jaundice is rare. Only 15% to 35% of patients present with concurrent gastrointestinal symptoms, including nausea, vomiting, abdominal cramping, and diarrhea (Table 75.3).[18,46–75,106–113] Symptoms are acute (<2 weeks' duration) in about two-thirds of cases but can develop months to years after travel to an endemic area.[2,3] The presentation is indistinguishable from pyogenic liver abscess on clinical grounds, and a careful search for epidemiologic risk factors is of paramount importance. Corticosteroid use and male sex are well-established risk factors for invasive amebic disease.[12,106]

Pyogenic Liver Abscess

Only 1 patient in 10 presents with the classic triad of fever, jaundice, and right upper quadrant tenderness. Fever is common, often without localizing signs but only with a general failure to thrive, including malaise,

[a]References 18, 52–54, 57, 65, 69, 70, 72, and 76.

fatigue, anorexia, or weight loss (see Table 75.3). When present, localizing symptoms such as vomiting or abdominal pain are not specific. The duration of symptoms before presentation varied widely in most case series, and there was seldom agreement on an average duration. Butler and McCarthy[48] attempted to address this issue by stratifying according to acute and chronic presentations. They found the former to be typically associated with acute, identifiable abdominal pathology such as cholangitis or appendicitis, whereas abscesses that presented chronically were often cryptogenic. Other series support an association between etiology and chronicity. For example, Seeto and Rockey[61] found that hematogenous liver abscesses presented most acutely (3 days), and those secondary to pylephlebitis had the longest duration of symptoms (42 days).

Diagnosis

The diagnosis of liver abscess should be suspected in all patients with fever, leukocytosis, and a space-occupying liver lesion. Because of the nonspecific symptoms on presentation, the initial clinical impression is frequently wrong and may include cholangitis, pneumonia, hepatic malignancy, intraabdominal catastrophe, or pneumonia.[50] Before the widespread use of noninvasive imaging, liver abscess was among the most frequently identifiable causes of fever of unknown origin. Leukocytosis is present in most patients; recent studies[69,70,73–75,77,114,115] report leukocytosis in 68% to 88% of all patients, with mean white blood cell counts of 15,000 to 17,000/mm.[3] An elevated alkaline phosphatase concentration is the most frequently abnormal liver function test, occurring in about two-thirds of patients with liver abscess, but a normal value does not exclude the diagnosis. Abnormalities of alanine aminotransferase, aspartate aminotransferase, and bilirubin are generally small, although they may be more pronounced in some patients with biliary disease. Albumin concentration and prothrombin time tend to be normal or nearly so. Procalcitonin levels are typically elevated. Chest radiographs are abnormal about half of the time but are of no real value in making the diagnosis. Therefore, although laboratory studies may suggest liver abnormalities, they are of no use in making the diagnosis of pyogenic liver abscess, and a high index of suspicion is required if the diagnosis is to be made in a timely fashion. Laboratory abnormalities may be of prognostic significance, most likely as markers of comorbidities. A multivariate analysis of risk factors found that a hemoglobin concentration of less than 10 g/dL and a blood urea nitrogen concentration greater than 28 mg/dL were independent predictors of mortality in patients found to have pyogenic liver abscess (odds ratios of 13 and 14, respectively).[65]

Once the diagnosis is suspected, radiographic imaging studies are essential to diagnose pyogenic liver abscess.[66] Ultrasonography and computed tomography (CT) scanning have proved particularly useful for demonstration and drainage of abscesses. Ultrasonography is the study of choice in patients with suspected biliary disease and in those who must avoid intravenous contrast or radiation exposure; it has a sensitivity of 70% to 90%. Contrast-enhanced CT scanning has improved sensitivity (≈95%) and is superior for guiding complex drainage procedures. Intravenous contrast is required for optimal imaging in two-thirds of patients.[116] Magnetic resonance imaging (MRI) studies are seldom required, but they may be better at distinguishing abscesses from noninfectious liver lesions such as neoplasia. Fine-needle aspiration is the definitive diagnostic procedure, and MRI is a cumbersome tool for guiding drainage procedures.

In patients with pyogenic liver abscess, blood cultures are positive about half of the time. It is imperative that multiple sets of anaerobic and aerobic cultures be obtained because these are frequently the only cultures obtained before antibiotic administration, and in about 7% of cases they are the only positive culture data obtained.[54,61,73] The diagnosis ultimately rests on obtaining purulent material from an aspiration of the abscess cavity, usually under radiographic guidance. Failure to obtain the expected pus should prompt a reevaluation of the differential diagnosis, considering liver cyst, malignancy, and amebic liver abscess. Purulent material should always be sent for Gram stain, which may provide the only clue to a mixed infection in patients heavily treated with antibiotics. The importance of prompt delivery of anaerobic specimens under proper conditions cannot be stressed enough.

Patients with amebic liver abscess classically present with a single large abscess in the right lobe of the liver, but the abscess can be anywhere and multiple lesions are not infrequent. Moreover, there is near-complete overlap in the symptomatology of the two diseases (see Table 75.3). A Spanish study found that age 45 or younger, presence of diarrhea, and a solitary right lobar abscess favored amebic etiology.[117] Distinguishing amebic from pyogenic liver abscess is not possible with the use of clinical criteria alone; however, a presumptive diagnosis of amebic liver abscess can be made in a patient with positive serology and a space-occupying liver lesion on CT or ultrasonography. Serum amebic serology by enzyme immunoassay has a sensitivity of about 95% and is highly specific for *E. histolytica* infection, especially in persons from nonendemic areas.

While *E. histolytica* serology can be negative in a minority of acute presentations (symptom duration <2 weeks), a repeat serology determination is usually positive.[118–121] It should be remembered that a positive serology result only confirms present or prior *E. histolytica* infection and cannot distinguish colitis from extraintestinal disease. Antigen detection represents a complementary diagnostic method. A commercially available enzyme-linked immunosorbent assay (ELISA) that detects the Gal/GalNAc lectin has been shown to be positive in more than 95% of serum samples and about 40% of stool or abscess specimens in patients with amebic liver abscess.[122,123] Several other US Food and Drug Administration (FDA)–approved ELISA antigen detection assays are also available, but some may cross-react with *E. dispar.*[124,125] Microscopic examination of the stool for cysts is of little value because *E. histolytica* cannot be distinguished from *E. dispar.* Examination of aspirated liver abscess pus is also not recommended, given that trophozoites are identified in only 11% to 25% of cases.[122,126]

There is growing interest in new diagnostic technologies for amebic liver abscesses.[127,128] While nucleic acid amplification of fecal specimens (in the form of FDA-approved molecular multiplex panels for enteric pathogens) is a highly sensitive method for the diagnosis of amebic colitis,[129] patients with amebic liver abscesses generally do not have concurrent intestinal *E. histolytica* infection.[130] Polymerase chain reaction has been shown to be a potential diagnostic tool for aspirated pus or other body fluids from patients with amebic liver abscess, but its use is currently limited to research laboratories.[125,128,130–133] Some investigators have argued that in areas of low endemicity, suspected amebic liver abscess should be aspirated to exclude pyogenic liver abscess. Certainly, if there is no response to initial therapy, the abscess should be aspirated to confirm the diagnosis and to exclude pyogenic liver abscess. Bacterial superinfection of amebic liver abscess has been described in about 1% to 5% of cases, frequently as a complication of drainage procedures.[134]

Echinococcal cysts of the liver, which typically do not cause fever or tenderness, are distinguishable from amebic and pyogenic abscesses by CT but require separate consideration for aspiration (see Chapter 289).

Therapy

Amebic Liver Abscess

Amebic liver abscess can almost always be treated with medical therapy alone. Metronidazole (750 mg three times daily) is typically given for 7 to 10 days.[2,3] An alternative, tinidazole (2 g daily for 3 days), has been used extensively in Europe and the developing world and has been approved for use in the United States for the treatment of amebiasis.[135] Other nitroimidazoles with extended half-lives that are efficacious include secnidazole and ornidazole; neither drug is approved in the United States for treatment of amebic liver abscess, although secnidazole recently received FDA approval for the treatment of bacterial vaginosis in adult women. Decreased fever and abdominal pain are usually observed within 3 to 5 days after initiation of therapy. Patients frequently remain colonized with *E. histolytica* despite nitroimidazole treatment and should be treated with paromomycin, a nonabsorbable aminoglycoside with *E. histolytica* activity, to eliminate this condition.[136,137] It is generally accepted that uncomplicated amebic liver abscess does not require drainage. Percutaneous image-guided aspiration has replaced surgical drainage and should be employed if there is no response to appropriate therapy or the diagnosis is uncertain, to exclude pyogenic liver abscess and bacterial superinfection. Drainage should also be considered for large lesions at risk for rupture, particularly left-sided abscesses that can rupture into the

pericardium.[127] Percutaneous drainage has not been demonstrated to shorten hospital stay or to hasten clinical improvement, with the exception of one randomized controlled trial that found a salutary effect in patients with large abscesses (>300 mL).[107,138–143]

Pyogenic Liver Abscess

Unlike amebic liver abscess, pyogenic liver abscesses usually require drainage in addition to antibiotic therapy. Surgical drainage was traditionally the treatment of choice and, in the preantibiotic era, the only hope for cure. As early as 1953, McFadzean and associates[144] reported the use of percutaneous drainage with antibiotic therapy to treat 14 patients with liver abscess. After ultrasonography and CT became widely available, multiple studies confirmed this approach and made image-guided drainage procedures the preferred primary therapy, although some still advocate surgical drainage (Fig. 75.1). Surgical intervention should be considered if percutaneous drainage fails or management of concurrent intraabdominal disease is required, and also for some patients with multiple large or loculated abscesses.[145,146] Percutaneous catheter drainage

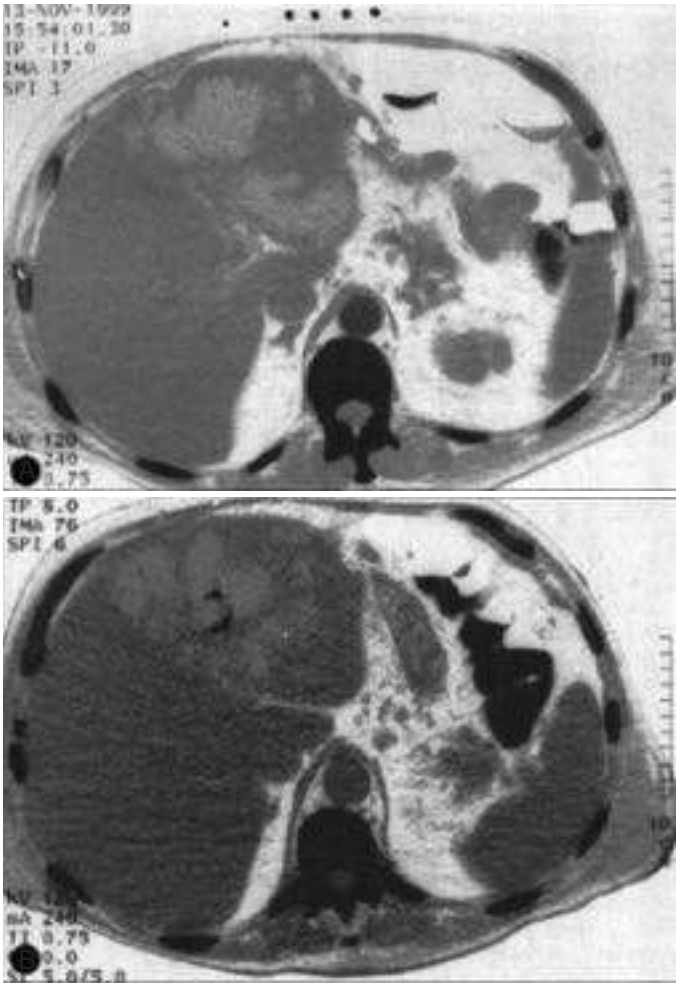

FIG. 75.1 Drainage of liver abscesses by percutaneous aspiration guided by computed tomography (CT). A 67-year-old man with a history of diabetes mellitus and rheumatoid arthritis and a distant history of a Billroth II gastric resection, recently diagnosed with adenocarcinoma of the stomach, presented with fever. A blood culture grew *Escherichia coli*. The liver was imaged by CT (A), revealing multiple hypodense lesions in the right hepatic lobe. The lesion was aspirated under radiologic guidance, and more than 500 mL of frank pus were drained. Cultures produced *E. coli* and several anaerobic bacterial species. Cytologic findings were consistent with metastatic adenocarcinoma. A radiodense percutaneous drainage catheter was left in place (B), and the patient was treated with intravenous antibiotics and continued drainage. Note the position of the catheter and the decrease in size of the abscess. *(Courtesy Lindsey Baden, MD, Brigham and Women's Hospital, Boston. From Johannsen EC, Sifri CD, Madoff LC. Pyogenic liver abscesses.* Infect Dis Clin North Am. *2000;14:547–563, vii.)*

is successful in 69% to 90% of cases; the procedure is generally well tolerated and usually can be performed at the time of radiographic diagnosis.[60–62,64,147] Aspirated material should be sent for Gram stain and cultured for aerobic and anaerobic bacteria. Meticulous handling of the specimen and rapid transportation to a qualified laboratory are essential for efficient recovery of anaerobes. Histopathologic biopsy specimens should also be obtained if possible. Depending on host and epidemiologic factors, microbiologic evaluation for fungi, mycobacteria, and *E. histolytica* should be considered. The catheter is usually left in place until drainage becomes minimal, typically 5 to 14 days. Recent studies suggest that the success rate for percutaneous drainage with antibiotic treatment is approximately 80% to 95%, even for large (>10 cm) pyogenic liver abscesses.[148–150]

Percutaneous aspiration without catheter placement has received recent attention, with several studies reporting success rates between 58% and 88% for solitary abscesses 5 cm or smaller in diameter, similar to those observed with catheter placement.[61,62,64,151–153] Success rates of 94% to 98% were reported in two studies in which percutaneous aspiration without catheter placement was followed by close clinical monitoring and serial ultrasound studies as indicated. Both investigations found that reaspiration was required in about 50% of cases, and a minority of patients required three or more aspiration procedures.[154,155] Therefore catheter placement may not be required in some patients, but prospective, randomized control trials are necessary to clarify the role of aspiration alone versus catheter placement in the management of hepatic abscess.

Attempts to treat pyogenic liver abscess with antibiotics but no drainage have met with some success. Two early studies with a combined total of 25 patients found cure rates of 87% to 90%.[156,157] These studies have been criticized because 68% of the patients underwent diagnostic aspiration and therefore had at least partial drainage. Moreover, this success rate was substantially higher than the conventional experience at the time, when undrained abscesses carried a mortality rate of 60% to 100%. The results may be subject to selection bias. Most patients did not have drainage because they were deemed poor surgical candidates. Even severely debilitated patients can tolerate percutaneous drainage, but the procedure may not always be required for cure: success rates of 44% and 100% with medical therapy alone have been reported in small case series.[61,149,153,158] Recent studies have also shown that chronic granulomatous disease–associated pyogenic liver abscesses may respond to conservative management with antibiotics and high-dose corticosteroids, delaying and perhaps reducing the future need of percutaneous or open surgical drainage.[159,160] However, until criteria for patient selection are better defined, medical management of pyogenic liver abscess should generally be reserved for patients with small abscesses not amenable to drainage and those patients in whom the risk of drainage is unacceptably high.

Treatment with empirical antibiotics should begin as soon as the diagnosis of pyogenic liver abscess is suspected. Multiple blood cultures should be sent before antibiotic initiation, but delaying antibiotic therapy until abscess material is obtained is not necessary and is potentially dangerous. Antibiotic choice should be guided by the suspected source of the abscess (Table 75.4). Abscesses arising from a biliary source frequently involve enterococci and enteric gram-negative bacilli, whereas abscesses from a colonic or pelvic source are more commonly caused by enteric gram-negative bacilli and anaerobes. Metronidazole at appropriately high doses should be included if amebic liver abscess is a consideration. Fluoroquinolones may be substituted for gentamicin, but this may not be advisable in cases complicated by enterococcal bacteremia. If a hematogenous (hepatic artery) source is suspected, coverage should include an antibiotic with activity against *S. aureus*.

Pyogenic liver abscesses are usually treated parenterally for 2 to 3 weeks, and a 4- to 6-week total course is completed with oral agents. Some have reported successful treatment with less than 2 weeks of antibiotic therapy. The patient's clinical response and follow-up imaging should be monitored to judge response to therapy for considerations of antibiotic duration and the need for further aspiration. One-third of patients are readmitted or have emergency room visits following discharge from the hospital, which suggests that these individuals may benefit from close outpatient follow-up and a multidisciplinary approach to care.[161] Abscess cavities usually resolve completely after therapy, but

TABLE 75.4 Antibiotic Therapy for Pyogenic Hepatic Abscess

TYPE OF THERAPY	AGENTS
Monotherapy	
β-Lactam–β-lactamase inhibitor combination[a]	Piperacillin-tazobactam
Carbapenem[a]	Imipenem-cilastin, meropenem, ertapenem, doripenem
Combination Therapy	
Cephalosporin-based	Third- or fourth-generation cephalosporin (cefotaxime, ceftriaxone, ceftizoxime, ceftazidime, cefepime) *plus* metronidazole
Fluoroquinolone-based	Fluoroquinolone (ciprofloxacin, levofloxacin, moxifloxacin) *plus* metronidazole

[a]Metronidazole or tinidazole should be included for presumptive therapy of amebic abscess if suspected.

occasionally they persist despite prolonged courses of antibiotics. In such cases, patients should be observed closely. Recurrent symptoms such as fever or abdominal pain should prompt repeat imaging and possible reaspiration.

INFECTION OF THE BILIARY SYSTEM

Infections of the biliary tract, including the common bile duct and gallbladder, are most often associated with obstruction to the flow of bile. In the United States and many developed countries, gallstones are common and most often asymptomatic. In the United States, for example, it is estimated that 20 to 25 million adults have gallstones.[162] In a small percentage of cases, gallstones may obstruct the cystic or common bile ducts, resulting in inflammation. Approximately 120,000 cholecystectomies are performed every year in the United States for acute cholecystitis, most commonly secondary to impacted gallstones.[163] Other causes of biliary obstruction include tumors of the biliary tree or adjacent structures, strictures secondary to surgery or other injury (including prior infection), and, in many geographic areas, infection by parasites including *Ascaris* and *Clonorchis.* Stasis of bile, inflammation, and loss of mechanical barriers can lead to bacterial infection of the bile, which can result in severe morbidity and death.

Pathogenesis

Cholecystitis

Only about 20% of patients with gallstones experience biliary colic, typified by right upper quadrant pain after a fatty meal when the contracting gallbladder is prevented from emptying by an obstructing stone.[163] Biliary colic must be distinguished from the far more serious acute cholecystitis, which occurs in 1% to 3% of persons with symptomatic gallstones. In this disease, biliary obstruction is accompanied by an intense inflammatory reaction. Obstruction is thought to lead to increased intraluminal pressure; may lead to compromised blood supply and lymphatic drainage; and, in the setting of supersaturated bile, leads to acute inflammation. This process is mediated, at least in part, by prostaglandins, particularly prostaglandins I_2 and E_2 (Fig. 75.2).[164] Infection is not thought to precipitate acute cholecystitis, but it may complicate 20% to 50% of cases. In a microbiologic study of biliary tract processes, 46% of patients presenting with acute cholecystitis had positive bile cultures, compared to 22% of patients with symptomatic gallstones but no evidence of acute cholecystitis and no positive cultures in normal controls.[165] Untreated cholecystitis may resolve spontaneously, but serious complications also occur at a high rate. Once infection is established, complications include gangrenous cholecystitis, emphysematous cholecystitis, gallbladder empyema, pyogenic liver abscess (discussed earlier), and bacteremia.

Acalculous Cholecystitis

In 2% to 15% of cases, cholecystitis occurs in the absence of gallstones, although usually in the presence of other predisposing conditions. These include critical illnesses such as trauma, burns, and sepsis, as well as human immunodeficiency virus (HIV) infection, immunosuppression, diabetes, nonbiliary surgery, and childbirth.[166] Some of these conditions predispose to ischemia in the gallbladder wall or stasis of bile, or both, resulting in concentration of bile salts and leading to inflammation and necrosis of the gallbladder wall.

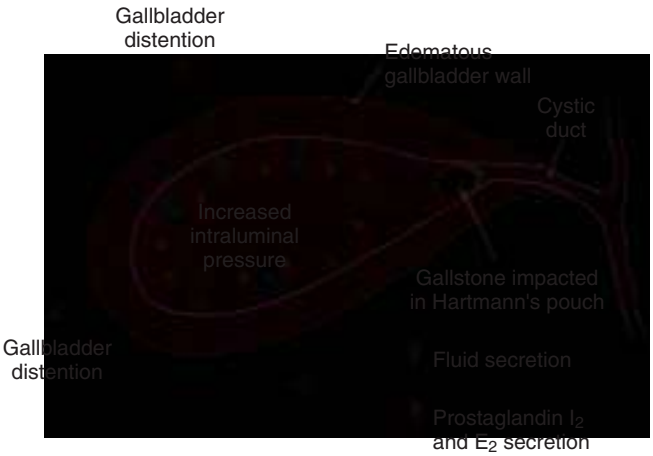

FIG. 75.2 Pathogenesis of acute cholecystitis. Impaction of a gallstone leads to obstruction of the cystic duct and increased intraluminal pressure. An acute inflammatory response is in part mediated by prostaglandins. Infection may then result from biliary stasis. *(Modified from Indar AA, Beckingham IJ. Acute cholecystitis.* BMJ. *2002;325:639–643.)*

Cholangitis

Cholangitis refers to inflammation or infection of the common bile duct. The normally sterile bile may become infected because of the loss of protective factors, including the flow of bile. Obstruction of the common bile duct leads to stasis, which favors the growth of bacteria; increased pressure predisposes to bacteremia. Other factors may involve the loss of antibacterial activity of bile on the proximal small intestine, allowing for greater growth of bacteria. Bacteria may then ascend to the biliary tract (hence the terms *ascending* and *suppurative* cholangitis). Other routes of infection have been proposed, including via the portal system or the lymphatics. Anastomosis of the bile duct to the small intestine, such as in a Whipple procedure, can lead to cholangitis. Primary sclerosing cholangitis is a disease of immunologic origin and is not covered here.[167] AIDS cholangiopathy, which has many features of acute cholangitis, is discussed later in this chapter.

Clinical Manifestations

Patients with biliary tract disease most often present with pain in the right upper quadrant of the abdomen, although occasionally localizing findings are absent. The pain may radiate to the infrascapular region. Cholecystitis and cholangitis are distinguished from simple biliary colic by the continuous nature of the pain. The finding of tenderness in the right upper quadrant on physical examination and the presence of the Murphy sign (inhibition of inspiration by pain when the area of the gallbladder fossa is palpated), with or without a mass, are highly suggestive of biliary tract disease. Fever and tachycardia are frequent findings. Complications of acute cholecystitis, which occur in 10% to 15% of cases, include hepatic or intraabdominal abscess, necrosis or gangrene of the gallbladder, and perforation, which in turn lead to sepsis and peritonitis. Emphysematous cholecystitis, usually diagnosed radiologically, occurs when the wall of the gallbladder is infected with gas-forming organisms, including *Clostridia,* as well as aerobic and facultative gram-negative and gram-positive bacteria. It often occurs in diabetics, and it may suggest a more ominous prognosis.[168,169]

Acalculous cholecystitis, particularly in a critically ill patient who is unresponsive, may produce subtle findings, such as unexplained fever or vague abdominal pain.[170] A high index of suspicion is required because serious complications such as gangrenous gallbladder and perforation frequently occur.

Acute or ascending cholangitis is suggested by the Charcot triad (right upper quadrant or epigastric abdominal pain; fever or chills, or both; and jaundice), reported in 50% to 70% of patients.[171] The additional, less frequent signs of hypotension and altered mental status, in combination with the Charcot triad, constitute the Reynolds pentad, which is reportedly seen in less than 14% of patients with ascending cholangitis.[172] Symptoms and signs of an inflammatory response are usually present and are reflected in the presence of fever, leukocytosis, and other markers.

Diagnosis

Leukocytosis with a left shift is the most frequent laboratory abnormality in acute cholecystitis. Alkaline phosphatase and bilirubin are not usually elevated unless the common bile duct is involved. However, in one study of 217 patients with acute cholecystitis, 25% of patients without obstruction of the common bile duct had elevated bilirubin.[173] Several also had elevations in amylase. The mechanism underlying these abnormalities is unclear but may be the passage of small stones or sludge through the common bile duct. The role of bile and blood cultures in the diagnosis of acute cholecystitis is not well established. International consensus practice guidelines (the "Tokyo Guidelines") recommend obtaining bile and, when available, tissue cultures from patients with cholangitis or moderate-to-severe acute cholecystitis.[174] The utility of routine blood cultures in cases of mild cholecystitis is less clear and are not routinely recommended.[174] Similarly, guidelines of the Surgical Infection Society and the Infectious Diseases Society of America recommend against routinely collecting blood cultures from patients with community-acquired intraabdominal infections, unless the patient is immunocompromised or systemically toxic.[175]

Acute cholangitis results in presentation with the clinical findings mentioned earlier. Abnormalities suggestive of sepsis syndrome, including leukocytosis, are frequently observed. Additional laboratory abnormalities include cholestatic liver function tests with elevations in alkaline phosphatase and bilirubin, particularly conjugated bilirubin. Elevations in γ-glutamyl transpeptidase are often seen as well. Abnormal amylase may suggest an associated pancreatitis, and elevations in transaminases may indicate associated inflammation or infection of the liver parenchyma, or both. The Tokyo guidelines recommend obtaining aerobic and anaerobic cultures of bile aspirates and blood in cases of acute cholangitis.[174] Bile cultures are positive in 59% to 93% of patients, and blood cultures are positive in 30% to 40%.[174,176]

Imaging Studies

Ultrasonography can frequently establish the diagnosis of cholecystitis and is usually the first study obtained.[175] A sonographic Murphy sign (i.e., pain when the ultrasound transducer probes the gallbladder) is a useful diagnostic clue. In addition, the testing may be done at the bedside of critically ill patients, is relatively inexpensive, and can directly visualize stones, particularly in the gallbladder. Abnormalities such as gallbladder wall thickening of greater than 4 mm, pericholecystic fluid, and intramural gas or ductal dilation are suggestive of cholecystitis (Fig. 75.3).[163] The combination of stones and either wall thickening or a sonographic Murphy sign in the appropriate clinical picture were shown to have positive and negative predictive values exceeding 90%.[177]

Radionuclide cholescintigraphy (hepatobiliary iminodiacetic acid [HIDA] scanning) may be used if ultrasound fails to ascertain a diagnosis. A technetium 99m (^{99m}Tc)–labeled derivative of acetanilide iminodiacetic acid is injected intravenously and is secreted into the bile. It is taken up by the gallbladder, which can then be visualized. Failure of the gallbladder to accumulate the marker is highly suggestive of acute cholecystitis due to obstruction of the cystic duct. Normally, visualization of the common bile duct and small bowel occurs within 30 to 60 minutes; failure to visualize these structures indicates obstruction within the common bile duct or at the ampulla. In one study of cholescintigraphy in the diagnosis of acalculous cholecystitis in 62 critically ill patients, ultrasonography had a sensitivity of only 30%, whereas HIDA scanning had a sensitivity of 100% and a specificity of 88%.[178]

CT is not commonly used for the initial evaluation of cholecystitis, and its sensitivity and specificity for detection of this condition are not known.[179,180] CT findings associated with acute cholecystitis include gallstones, particularly within the cystic duct; gallbladder distention and mural thickening; and enhancement of the liver adjacent to the gallbladder, which is the CT equivalent of the scintigraphic "rim sign."

Magnetic resonance (MR) cholangiography is a noninvasive technique that has been used to visualize the bile ducts. In one study of 35 patients comparing ultrasonography with MR cholangiography, the latter modality showed 100% sensitivity for the presence of stones but was less sensitive than ultrasound in the detection of gallbladder wall thickening (69% vs. 96%).[181] In a meta-analysis of 67 studies of MR cholangiography, the technique was highly sensitive (99%) for the detection of biliary obstruction and 92% sensitive for the detection of stones.[182] A more recent meta-analysis of 57 studies found the highest sensitivity (96%) with radionuclide cholescintigraphy, while ultrasonography had a sensitivity of 81%, and MRI had a sensitivity of 85%.[183] The study showed no important differences in specificity among the techniques.

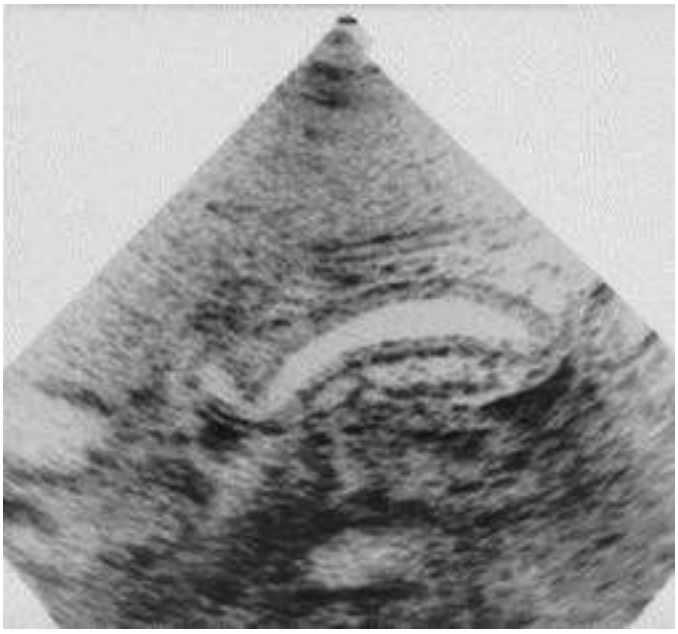

FIG. 75.3 Pathogenesis of acalculous cholecystitis. Ultrasonographic visualization of the gallbladder (sagittal view) in a case of acalculous cholecystitis, demonstrating mural thickening and hypoechoic regions within the gallbladder wall. *(From Gore RM, Yaghmai V, Newmark GM, et al. Imaging benign and malignant disease of the gallbladder.* Radiol Clin North Am. *2002;40:1307–1323, vi.)*

TABLE 75.5 Microbiology of Acute Cholecystitis and Cholangitis

BACTERIA	FREQUENCY (%)
Gram-negative organisms	
Escherichia coli	31–44
Klebsiella spp.	9–20
Pseudomonas aeruginosa	0.5–19
Enterobacter spp.	5–9
Gram-positive organisms	
Enterococcus spp.	3–34
Streptococcus spp.	2–10
Staphylococcus aureus	0–4
Anaerobes	4–20

Modified from Gomi H, Solomkin JS, Schlossberg D, et al. Tokyo Guidelines 2018: antimicrobial therapy for acute cholangitis and cholecystitis. J Hepatobiliary Pancreat Sci. *2018;25:3–16.*

Microbiology

Bacterial cultures from the bile and surgical sites of patients with acute cholecystitis and acute cholangitis typically yield constituents of the normal intestinal microbiota (Table 75.5).[174,184] These include gram-negative bacilli such as *E. coli, Klebsiella* spp., and *Enterobacter* spp.

TABLE 75.6 Recommended Agents and Regimens for Empirical Treatment of Acute Biliary Infections

TYPE OF INFECTION	FOR MILD TO MODERATELY SEVERE INFECTION	FOR HIGHLY SEVERE INFECTION
Community-acquired acute cholecystitis	Cephalosporin-based therapy (cefazolin,[a] cefuroxime,[a] or ceftriaxone)	Imipenem-cilastatin, meropenem, doripenem, piperacillin-tazobactam, or cefepime *plus* metronidazole
Acute cholangitis following biliary-enteric anastomosis	Imipenem-cilastatin, meropenem, doripenem, piperacillin-tazobactam, cefepime *plus* metronidazole, ciprofloxacin[b] *plus* metronidazole, levofloxacin[b] *plus* metronidazole, or moxifloxacin[b]	Imipenem-cilastatin, meropenem, doripenem, piperacillin-tazobactam, or cefepime *plus* metronidazole
Health care–associated biliary infection	Imipenem-cilastatin, meropenem, doripenem, piperacillin-tazobactam, cefepime *plus* metronidazole, ciprofloxacin[b] *plus* metronidazole, levofloxacin[b] *plus* metronidazole, or moxifloxacin[b] Vancomycin[c] added to each regimen	Imipenem-cilastatin, meropenem, doripenem, piperacillin-tazobactam, or cefepime *plus* metronidazole Vancomycin[c] added to each regimen

[a]Local antimicrobial susceptibility profiles and, if available, isolate susceptibility should be reviewed.
[b]Addition of metronidazole is necessary due to increasing resistance of *Bacteroides* to fluoroquinolones other than moxifloxacin. Local antimicrobial susceptibility profiles and, if available, isolate susceptibility should be reviewed due to increasing resistance of *Escherichia coli* to fluoroquinolones.
[c]Linezolid or daptomycin may be considered in cases where vancomycin-resistant *Enterococcus* (VRE) is known to be colonizing the patient, if previous treatment included vancomycin, and/or if VRE is common in the community.
Modified from Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clin Infect Dis. *2010;50:133–164; and Gomi H, Solomkin JS, Schlossberg D, et al. Tokyo Guidelines 2018: antimicrobial therapy for acute cholangitis and cholecystitis.* J Hepatobiliary Pancreat Sci. *2018;25:3–16.*

Anaerobes, most frequently *Bacteroides* spp., *Fusobacterium* spp., and clostridia, are recovered less frequently but are isolated more often from patients with prior biliary tract surgical procedures and those with biliary-intestinal anastomoses. Finally, enterococci are not uncommonly found in infected bile, usually in association with other organisms.[185]

Parasites that infect the biliary tree include *Clonorchis sinensis, Opisthorchis felineus, Opisthorchis viverrini,* and *Fasciola hepatica.*[186] These organisms are prevalent in regions of Asia and can cause acute and relapsing cholangitis with associated stricture and stone formation. *Clonorchis* and *Opisthorchis* infection can lead to the development of carcinoma of the biliary tract. *Ascaris lumbricoides,* a more cosmopolitan parasite, occasionally obstructs the biliary tree, resulting in acute cholangitis.[187] Echinococcal disease can cause biliary obstruction due to mass effect. Parasites that complicate HIV disease may also invade the biliary tree (see discussion following).

AIDS Cholangiopathy (AIDS-Related Sclerosing Cholangitis)

Biliary tract disease is a complication of advanced HIV infection[188] (discussed in Chapter 125). In the era of potent antiretroviral therapy, AIDS cholangiopathy is now a rare clinical entity.[189] Although it is possible that this condition is caused by infection of the biliary tract with HIV per se, more likely it results from opportunistic infection of the biliary system. The pathologic findings of AIDS cholangiopathy include stenosis of the distal common bile duct and irregularities of the intrahepatic and extrahepatic bile ducts.[190] The most common pathogen associated with cholangitis in HIV is *Cryptosporidium.* After an outbreak of cryptosporidiosis in Milwaukee, Wisconsin, in 1995, 29% of patients with HIV infection and intestinal cryptosporidiosis developed cholangiopathy as well.[191] Cytomegalovirus is also a major cause of AIDS-related cholangitis, having been described in up to 42% of patients. Other pathogens associated with AIDS cholangiopathy include *Enterocytozoon bieneusi, Cystoisospora belli,* and *Mycobacterium avium-intracellulare.* The clinical manifestations of biliary tract disease do not differ markedly from those in non–HIV-infected individuals and include right upper quadrant pain and fever. Diagnosis is usually made by characteristic findings on ultrasound, with dilatation of the common bile duct observed in approximately two-thirds of cases. Endoscopic retrograde cholangiopancreatography (ERCP) demonstrates dilatation and irregularities of the ducts and has become the gold standard for the diagnosis.[192] It is also useful in treatment; sphincterotomy provides symptomatic benefit for many patients.

Therapy

Because biliary disease results most commonly from obstruction of the bile ducts, definitive treatment involves removal of the obstruction or the infected material. This can be accomplished surgically, percutaneously, or endoscopically (i.e., by ERCP).[163] Antibiotics and other supportive measures are considered temporizing.

Acute Cholecystitis

The role of antibiotics in acute cholecystitis has not been well established. Many cases of acute cholecystitis remit spontaneously, and retrospective studies of patients with uncomplicated cholecystitis have failed to demonstrate reduced complications such as pericolic abscess or perforation with antibiotic administration.[193,194] In addition, two recent prospective, randomized controlled trials of patients with mild-to-moderate acute cholecystitis managed with early cholecystectomy demonstrated no increase in complications when postoperative antibiotics were withheld, but neither study reached the primary end point of noninferiority.[195,196] Similarly, a recent review of published studies concluded that there was no clear indication that antibiotics are beneficial for patients with acute cholecystitis who were treated conservatively or with delayed cholecystectomy, although the level of evidence was low.[197] In summary, the data suggest that antibiotics can be safely discontinued within 24 hours after cholecystectomy for acute uncomplicated cholecystitis, as long as there is no evidence of infection outside the gallbladder wall.[193]

Antibiotics are clearly indicated for patients with complications of cholecystitis, such as gangrenous cholecystitis or perforation. Guidelines recommend a total of 4 to 7 days of therapy once source control is obtained and anatomic issues have been addressed, although longer courses of treatment may be necessary for complications such as pyogenic liver abscess.[174,175] Antibiotics directed against the enteric flora (as described earlier) should also be given to patients who are debilitated, severely ill, elderly, immunocompromised, or jaundiced (Table 75.6).[174,175] Despite the relatively frequent isolation of enterococci from bile cultures, successful treatment of community-acquired cholecystitis typically does not require antimicrobial therapy against enterococci.[174,175,185] This finding may be a result of the low pathogenic potential of enterococci in this setting; elimination of other pathogens effectively treats the infection even in the setting of persistent enterococcal contamination. However, enterococci may be important pathogens in high-risk patients, such as liver transplant recipients; in these cases, treatment that also targets enterococci may be indicated.[174,175] Institutional drug resistance patterns should be considered when selecting an initial antimicrobial regimen for health care–associated severe cholecystitis. Use of an agent active against gram-negative anaerobes, such as metronidazole, is not required for community-acquired cholecystitis, unless a biliary-enteric anastomosis is present.[174,175]

In the presence of suspected gangrene or perforation, immediate surgery is indicated. Ideally, this includes cholecystectomy with intraoperative cholangiogram. Alternatively, in unstable patients, cholecystotomy (percutaneous drainage of the biliary system with removal of obstructing stones) may be performed as a temporizing measure.[198] This procedure can be performed under local anesthesia and at the bedside in critically ill patients and can be lifesaving. In some circumstances, further surgery may not be required.

In the absence of severe complications, the timing of definitive surgical intervention in the management of acute cholecystitis is the subject of

some debate. Traditionally, in the group of patients who are stable and respond to conservative management, surgery has been delayed for 6 to 12 weeks. However, several studies have shown that patients who undergo early surgical intervention (most often laparoscopic cholecystectomy) have a lower complication rate, a shorter hospital stay, and, if laparoscopic cholecystectomy is performed, a lower requirement for conversion to an open procedure, compared with patients undergoing delayed surgery.[199,200] Several recent meta-analyses of randomized controlled trials found early surgery to be as safe and effective as delayed surgery and associated with lower hospital costs, fewer work days lost, and lower risk of wound infection.[201–203] For patients with high surgical risk and those who are unstable, delayed surgery may still be preferable.

Acute Cholangitis

Acute cholangitis remains a disease with substantial mortality, and therapy should include antibiotics and supportive measures along with decompression/drainage of the biliary system for cases that do not respond promptly to conservative therapy. A variety of antibiotic regimens have been used in the management of acute cholangitis (see Table 75.6).[172,174,175] Empirical coverage should be directed against enteric gram-negative bacilli. A β-lactam–β-lactamase inhibitor such as piperacillin-tazobactam is appropriate initial empirical therapy.[174] Cephalosporins, carbapenems, and fluoroquinolones have also proved efficacious.[172,174,176] Selection of empirical antibiotic therapy in cases of health care–associated disease should be influenced by local nosocomial resistance patterns, as well as the patient's prior antibiotic exposures and known microbial colonization.[174,175] Although anaerobes are only infrequently cultured from bile in acute cholangitis, the addition of metronidazole to empirical third- or fourth-generation cephalosporin-based regimens is advisable (see Table 75.6).[174,175] Like severe acute cholecystitis, acute cholangitis is treated for a total of 4 to 7 days once source control is obtained and anatomic problems have resolved, although longer courses of treatment may be necessary due to complications such as pyogenic liver abscess or gram-positive bacteremia.[174]

Drainage of the biliary tract may be accomplished surgically, endoscopically, or percutaneously. ERCP has, to a large extent, supplanted open surgical procedures in the management of acute cholangitis and is successful in more than 90% of cases.[204,205] (Paradoxically, ERCP is not infrequently the cause of acute cholangitis when it is used in an attempt to decompress an obstructed but not frankly infected bile duct.[206]) ERCP is generally thought to have a lower morbidity rate than open surgery, and it is more likely to offer definitive treatment (e.g., by removal of an impacted gallstone) than percutaneous biliary drainage. Some cases of acute cholangitis will respond to conservative therapy with drainage deferred until 24 to 48 hours to monitor response.[207,208]

Treatment of parasitic infections affecting the biliary tree is covered in Chapters 287, 289, and 290.

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76 Pancreatic Infection

Miriam Baron Barshak

SHORT VIEW SUMMARY

Definition
- Infection of pancreatic tissue.
- Most commonly develops as a complication of acute pancreatitis (AP), in tissue that has been damaged by inflammation (see Table 76.2).
- Pancreatic abscess is a circumscribed collection of pus usually in or near the pancreas, arising as a consequence of AP or pancreatic trauma.

Epidemiology
- Approximately 275,000 admissions for AP per year in the United States, up to 20% of which may have significant pancreatic necrosis.
- Up to 70% of patients with pancreatic necrosis may develop pancreatic infection.

Microbiology
- Gastrointestinal bacterial microbiota most common (aerobes and anaerobes, gram-positive, and gram-negative).
- Use of preemptive antibiotics may select for more resistant bacterial species and fungal pathogens.

Diagnosis
- Must be distinguished from sterile pancreatic necrosis, which can be associated with a sepsis-like syndrome.
- Tissue sampling may be required to identify infection.

Therapy
- Antimicrobial therapy targeting organisms identified in cultures of the infected site, in combination with catheter drainage and/or surgical removal of the infected material (see Table 76.3).
- Delay in surgery for infected pancreatic necrosis until it has evolved to walled-off necrosis may allow a more stable patient with better-demarcated areas of necrotic tissue and is now the favored approach.
- Newer surgical approaches include video-assisted retroperitoneal débridement and endoscopic transgastric necrosectomy.

Prevention
- Enteral nutrition rather than parenteral nutrition, starting as soon as possible (within 72 hours of admission for patients with severe AP); nasogastric or nasojejunal tubes are equivalent.
- A consensus favoring use of early (preemptive) systemic antibiotics emerged in the 1990s and 2000s, using carbapenem alone or quinolone plus metronidazole as first-choice therapy.
- More recent randomized controlled studies have not demonstrated a benefit from this practice, and most guidelines advise *against* the use of preemptive antibiotics for patients with AP.

Inflammation of pancreatic tissue commonly manifests as severe acute upper abdominal pain and elevated serum levels of pancreatic enzymes. Most episodes of acute pancreatitis (AP) are associated with gallstones or alcohol abuse. Other causes of AP are hypercalcemia, hypertriglyceridemia, anatomic abnormalities, familial syndromes, autoimmune disease, ischemia, pancreatic carcinoma, trauma, endoscopic retrograde cholangiopancreatography, and drugs, among which are antimicrobial agents such as tetracycline, pentamidine, sulfonamides, didanosine, erythromycin, nitrofurantoin, and metronidazole.[1,2] Infection of damaged pancreatic tissue may complicate disease initiated by any of these mechanisms, conferring significant morbidity and mortality risks. Less commonly, many different microorganisms may infect the pancreas directly, with or without inciting a syndrome of AP. This chapter reviews the infectious agents associated with primary pancreatic infection before focusing on infectious complications of AP. Issues of nonpancreatic infection related to gallstones are addressed in Chapter 75.

INFECTIOUS CAUSES OF ACUTE PANCREATITIS

Many organisms have been reported to cause pancreatic disease. Case reports in this area have been reviewed and assessed for adequate documentation of both pancreatitis and infection (Table 76.1).[3] The reports of "definite" or "probable" association with pancreatitis described patients with coxsackieviruses, cytomegalovirus (CMV), varicella-zoster virus, herpes simplex virus 2 (HSV-2), mumps virus, hepatitis B virus, and with infections caused by *Mycoplasma, Leptospira, Legionella, Salmonella enterica* serotype Typhi, *Aspergillus, Toxoplasma, Cryptosporidium*, and *Ascaris.*

Among these, ascariasis commonly causes pancreatic-biliary disease in countries with high infection rates; in some tropical areas, ascariasis ranks second to gallstones as a cause of pancreatitis.[4] After hatching in the duodenum, *Ascaris* larvae penetrate the small bowel mucosa, enter the venous circulation, and arrive in the lungs, where they enter the alveolae, ascend the bronchial tree, and are swallowed. In the gastrointestinal (GI) tract they mature into adult worms and then may cause clinical and pathologic AP by migrating across the ampulla of Vater to obstruct the common bile duct or the pancreatic duct.

Other organisms, including Epstein-Barr virus, vaccinia, rubella, adenovirus, and rubeola, have been cited as causes of pancreatic infection in case reports but without adequately rigorous investigation to qualify for definite or probable association with pancreatitis.[3]

Another group of organisms causes pancreatic infection, forming microabscesses or macroabscesses but without inducing the signs, symptoms, or pathology of AP (see Table 76.1).[3]

Thus pathologic or radiographic evidence of pancreatitis associated with well-documented infection has been noted with viruses, bacteria, fungi, and parasites. However, the frequency with which these organisms contribute to idiopathic pancreatitis is unclear.

PANCREATITIS AND HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Although human immunodeficiency virus (HIV) itself has not been identified in pancreatic tissue by molecular methods, HIV infection has been associated with an increased risk of clinical pancreatitis. One study reports a 14% incidence of mild-to-moderately severe AP among patients with HIV between 1993 and 1994, with an inverse correlation between serum pancreatic enzyme level and number of CD4 lymphocytes.[5] Etiologies of pancreatitis frequently seen in HIV-infected patients include drugs (e.g., pentamidine, didanosine, and

TABLE 76.1 Infectious Causes of Pancreatic Disease

Definite[a] or Probable[b] Pancreatitis
Ascaris
Aspergillus
Coxsackievirus B, B3, B4
Cryptosporidium
Cytomegalovirus
Hepatitis B virus
Herpes simplex virus 2
Legionella
Leptospira
Mumps virus
Mycoplasma
Salmonella Typhi
Toxoplasma
Varicella-zoster virus
Probable Pancreatic Infection[c] Without Acute Pancreatitis
Actinomyces
Candida spp.
Clonorchis sinensis
Coccidioides immitis
Cryptococcus neoformans
Echinococcus granulosus
Entamoeba histolytica
Histoplasma capsulatum
Leishmania donovani
Mucormycosis
Mycobacterium avium-intracellulare
Mycobacterium tuberculosis
Nocardia asteroides
Paracoccidioides brasiliensis
Paragonimus westermani
Pneumocystis jirovecii
Schistosoma haematobium
Strongyloides stercoralis

[a]Pancreatitis at surgery or autopsy, or radiographic evidence.
[b]Threefold increase in amylase and/or lipase as well as characteristic symptoms.
[c]Culture of the organism from blood or pancreatic juice, or serologic diagnosis in a characteristic clinical or epidemiologic setting.
From Parenti DM, Steinberg W, Kang P. Infectious causes of acute pancreatitis. Pancreas. *1996;13:356–371.*

trimethoprim-sulfamethoxazole) and opportunistic infections (e.g., CMV, HSV, and others listed earlier). For the most part, however, the infections (microabscesses or macroabscesses) in these reports were noted incidentally at autopsy, without prior associated symptoms or pathologic evidence of AP. In addition, advanced HIV infection is associated with an increased risk of pancreatic malignancies, including Kaposi sarcoma and lymphoma, which may lead to signs, symptoms, or laboratory abnormalities suggesting pancreatitis. Finally, isolated pancreatic enzyme elevations have been noted in HIV-infected patients lacking other signs or symptoms of pancreatitis.[6] In the study discussed earlier[5] the most frequent causes of pancreatitis were gallstones, alcohol or intravenous (IV) drug abuse, pentamidine intake, and infections with *Pneumocystis jirovecii* and *Mycobacterium avium-intracellulare* complex. Of note, no pancreatic tissue sampling was performed to document the presence of these microorganisms.

INFECTION COMPLICATING ACUTE PANCREATITIS

Background

Most pancreatic infections occur as complications of AP initiated by noninfectious causes. Regardless of the inciting event, the pathogenesis of AP involves activation and release of toxic materials, including pancreatic proteolytic enzymes and vasoactive substances (trypsin, cathepsin B, phospholipase, chymotrypsin, elastase, cytokines, and the kallikrein-kinin, coagulation, and fibrinolysis cascades) that injure pancreatic cells and blood vessels. Such damage increases vascular permeability and leads to pancreatic swelling, a condition described clinically as interstitial edematous pancreatitis. Accounting for 80% of AP cases,[7] this disorder usually responds well to supportive care. In more severe disease, liberation of these toxic materials into the surrounding retroperitoneal spaces, lesser sac, and peritoneal cavity causes chemical irritation and contributes to third-space losses of protein-rich fluid, leading to hypovolemia and hypotension. In 5% to 15% of AP cases, hypoxia, free radicals, and ongoing release of pancreatic enzymes cause disruption of the pancreatic microcirculation, which leads to more severe pancreatic tissue injury and ultimately pancreatic necrosis. In addition, recruited inflammatory cells release substances such as phospholipase A_2, polymorphonuclear cell elastase, interleukins, leukotrienes, and complement factors, which contribute to a systemic inflammatory response syndrome (SIRS) that may include fever, acute respiratory distress syndrome, pleural effusions, renal failure, shock, myocardial depression, and metabolic abnormalities.[8] Necrotizing pancreatitis is associated with mortality rates of 30% to 40%.[9] Twenty percent of the deaths occur during the first week of illness in the setting of this inflammatory milieu and associated multiple organ failure. Later deaths from AP often occur in association with local and systemic infectious complications.[10] Predisposition to infection in severe AP may be related in part to altered immune responses; in one report, aberrant monocyte signaling profiles were identified that could alter immune defenses, including monocyte transmigration.[11]

Significance of Infection in Acute Pancreatitis

Some reports suggest that infection increases mortality rates. In one series of 114 patients with pancreatic necrosis, intestinal microorganisms were cultured from the necrotic tissue in 39.4% of cases. Mortality rates in patients with less than 50% gland necrosis rose from 12.9% to 38.9% if the necrotic tissue was infected, whereas mortality rates in patients with greater than 50% necrosis rose from 14.3% to 66.7% in the presence of infection.[12] Of note, other studies have reported that the mortality rates among patients with severe sterile necrosis are equal to those among patients with infected necrosis.[13] Nonetheless, the association of mortality with infection in AP in some studies suggests that preventing, identifying, and treating infections might decrease the risks of adverse outcomes. A 2010 meta-analysis further supports this idea with the finding that among nearly 1500 patients with AP, the absolute influence of organ failure and infected pancreatic necrosis on mortality is comparable, and thus the presence of either indicates severe disease—that is, the relative risk (RR) of mortality doubles when organ failure and infected pancreatic necrosis are both present and indicates extremely severe disease or critical AP.[14] A 2014 prospective study found organ failure to have more effect on mortality than infection among patients with necrotizing pancreatitis but confirmed that each was a significant, independent predictor of death in a multivariate analysis.[15]

Although infection is rare in mild pancreatitis, severe pancreatitis is associated with infection rates as high as 70%.[12,16–19] This difference in infection risk has driven extensive efforts to identify patients with severe pancreatitis early in the course of illness. Multiple criteria have been suggested, with some agreement that severe AP is characterized by abnormalities in physiology (an Acute Physiology and Chronic Health Evaluation [APACHE II] score <8 or equivalent for other scoring systems), in conjunction with computed tomography (CT) evidence of less than 30% pancreatic necrosis, chest radiographic evidence of pleural effusions, and C-reactive peptide (CRP) value less than 150 mg/L.[20] Of importance, the risk of infection increases with the extent of necrosis.[12,21]

Defining Pancreatic Infections

Pancreatic collection nomenclature, derived by consensus at the International Symposium on Acute Pancreatitis in Atlanta in 1992,[22] and revised in 2012,[23] is summarized in Table 76.2. In early AP[13] local infection may arise in necrotic pancreatic and peripancreatic tissue without significant pus collections.[24] The incidence of infection increases with the extent of necrosis and with time.[25] In one study 49% of infections in necrotizing pancreatitis developed within the first 2 weeks of illness, whereas 71% of infections developed within the first 3 weeks of illness.[13] Another group reported the incidence of infection was highest among patients undergoing surgery 15 to 21 days into illness.[12] The contamination rate was 24% within the first week after onset of symptoms and then rose to 36% and 71% within 2 and 3 weeks, respectively.

TABLE 76.2 Definitions Derived From the International Symposium on Acute Pancreatitis, 1992 and Revised in 2012

TERM	DEFINITION
Acute pancreatitis	Acute inflammatory process of the pancreas with variable involvement of other regional tissues or remote organ systems
Necrotizing pancreatitis	Inflammation associated with pancreatic parenchymal necrosis and/or peripancreatic necrosis
ANC (acute necrotic collection)	A collection containing variable amounts of both fluid and necrosis associated with necrotizing pancreatitis; necrosis can involve the pancreatic parenchyma and/or the peripancreatic tissues
APFC (acute peripancreatic fluid collection)	Peripancreatic fluid associated with interstitial edematous pancreatitis with no associated peripancreatic necrosis; term applies only to areas of peripancreatic fluid seen within the first 4 weeks after onset of interstitial edematous pancreatitis and without the features of a pseudocyst
Pancreatic pseudocyst	An encapsulated collection of fluid with a well-defined inflammatory wall usually outside the pancreas with minimal or no necrosis; entity usually occurs >4 weeks after onset of interstitial edematous pancreatitis to mature
WON (walled-off necrosis)	A mature, encapsulated collection of pancreatic and/or peripancreatic necrosis that has developed a well-defined inflammatory wall; WON usually occurs >4 weeks after onset of necrotizing pancreatitis

From Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. *2013;62:102–111.*

Later in the course of AP (weeks 4–7), after the serum markers of pancreatitis resolve, pancreatic necrosis liquefies into fluid collections; sterile necrosis thus develops into pancreatic pseudocysts, whereas infected necrosis matures into walled-off necrosis (WON) in about 3% of patients with necrotizing pancreatitis.[12,17] Pseudocysts may subsequently become superinfected and develop into abscesses as well. Pancreatic abscess typically is seen with fever, abdominal pain, and leukocytosis in patients recovering from recognized pancreatic disease. In general, more systemic toxicity and mortality occur with infected necrosis than with pancreatic abscess. Specifically, mortality rates of 26% to 32.1% have been reported for infected necrosis versus 12% to 22.2% for abscess.[13,24]

Diagnosis of Pancreatic Infection

Identifying infection in AP can be difficult because patients with extensive necrosis of pancreatic and peripancreatic tissue frequently display a sepsis-like syndrome without a septic focus. Such patients may develop physiologic abnormalities indistinguishable from those associated with infection, including systemic organ failure syndrome involving the lungs, kidneys, liver, and cardiovascular systems. In one study[26] 60% of AP patients developed fever; in 22% the fever was related to pancreatitis per se; in 33% it was attributed to extrapancreatic infections; and in 45% it was due to infected pancreatic necrosis. Imaging by CT can aid in diagnosing infected pancreatic necrosis only if gas is seen in and around areas of pancreatic necrosis. Several laboratory parameters have been evaluated as markers of pancreatic infection; the most promising of these is procalcitonin, a 116–amino-acid propeptide of calcitonin, but more data from larger studies must be obtained.[27,28] A 2007 study addressed the value of routine clinical tests (white blood cell [WBC] and CRP) in predicting the development of infected pancreatic necrosis in severe AP. The authors reported that if CRP and WBC values were below cutoff values (81 mg/L for CRP and 13×10^9/L for WBC), the risk for infected necrosis was approximately 1.4%. The authors suggest that invasive testing to diagnose infection may be unnecessary in this subset of patients.[29] In other patients, however, tissue sampling can be helpful to identify infection; Gram stain and culture of pancreatic tissue sampled by CT-guided fine-needle aspiration (FNA) provides high diagnostic sensitivity and specificity with minimal risk of introducing infection or disseminating organisms by intestinal puncture.[13] This procedure has been recommended for patients with necrotizing AP and persistent systemic toxicity or organ failure, or both, in the first 7 to 14 days. Similarly, culture of material retrieved by FNA of collections, seen by CT or ultrasound imaging, allows diagnosis of extrapancreatic infection. Such collections may spread from the pancreas into the retroperitoneum, mesentery, mediastinum, and elsewhere, through tissue damaged by activated proteases, vasoactive substances, and inflammatory mediators.[30] Positron emission tomography/CT with ^{18}F-fluorodeoxyglucose–labeled leukocytes may be helpful in distinguishing infected from uninfected collections less invasively.[31]

More recently a 2014 Dutch study found that infected necrosis could generally be diagnosed based on clinical or imaging signs of infection with positive predictive value of 80% to 94%, but FNA may be useful in patients with unclear clinical signs and no imaging signs of infected necrosis.[32] However, a 2014 study from Boston found from open necrosectomy that the false-negative rate of percutaneous sampling to assess for infection was 20%. The authors conclude that the decision for débridement therefore should not be predicated on proven infection from a percutaneous sample.[33]

Microbiology of Pancreatic Infection

Pancreatic superinfection usually involves GI microbiota, including aerobes and anaerobes. Historically, both gram-negative (most commonly *Escherichia coli* and *Klebsiella* spp., less commonly *Enterobacter, Pseudomonas, Proteus*, and others) and gram-positive (enterococcal, streptococcal, and staphylococcal species) bacteria contribute, and infections may be monomicrobial or polymicrobial. In a collected series of 45 articles, representing more than 1100 cases of secondary pancreatic infections reported as of 1990,[34] the causative organisms were *E. coli* (35%), *Klebsiella pneumoniae* (24%), *Enterococcus* spp. (24%), *Staphylococcus* spp. (14%), and *Pseudomonas* spp. (11%). A more recent (2014) single-center review noted enterococci (45%), Enterobacteriaceae (42%), and fungi (22%).[35]

One group[36] has reported a different microbiologic picture of infected pancreatic necrosis in alcoholic and biliary pancreatitis among 70 patients with similar degrees of necrosis who underwent surgery for pancreatitis. These authors note a higher rate of infection overall and a preponderance of gram-negative organisms in the biliary disease patients, compared with a tendency toward more gram-positive pancreatic infections in the alcoholic group. The authors hypothesize that these differences might be explained by a biliary origin of pancreatic infection among patients with biliary disease, in contrast to a hematogenous origin of infection from catheter contamination in the alcoholic population. Of note, other work has shown that mortality from pancreatitis is related to the severity of the attack and not the underlying cause.[37]

Earlier exposure to broadly active antimicrobial therapy changes the microbiota that cause infections, and increasingly resistant bacterial infections and fungal infections have become more common.[38] A study of 479 patients with acute pancreatitis, of whom 17 had intraabdominal fungal infections, identified administration of prophylactic antibiotics on admission as a statistically significant risk factor.[39] Fungal infection in AP is associated with a higher incidence of systemic complications and perhaps also mortality. In addition to broad-spectrum antibiotic exposure, additional risk factors for fungal infection include prolonged hospitalization, surgical/endoscopic interventions, use of total parenteral nutrition (TPN), and mechanical ventilation.[40]

Overall, the profile of organisms suggests most pancreatic infections originate in the GI tract and seed the pancreas via the bowel, the biliary tree, the lymphatics, or the bloodstream. Initially, bowel microbiota may translocate across a GI mucosal barrier damaged by ischemia during the hypovolemic phase of AP.

Management of Pancreatic Infection

In most cases treating pancreatic infection requires antimicrobial therapy directed at organisms identified in cultures of the infected site, in combination with mechanical removal of the infected material. Although

some groups[41] have reported successful medical management of infected necrosis, most have traditionally believed that removal of infected necrosis and all associated fluid collections is necessary.

Several studies suggest that infected pancreatic necrosis may be managed successfully via catheter drainage, without surgical intervention. In one report primary CT-guided percutaneous catheter drainage was successful for approximately one-half of the patients with acute necrotizing pancreatitis, and the presence of multisystem organ failure was a more important indicator of outcome than the presence of infection.[42] There is some support for the initial drainage efforts to be made endoscopically (i.e., transluminally) rather than percutaneously, with the possible benefit of preventing development of an external pancreatic fistula that could be promoted by percutaneous drainage.[43]

For patients in whom initial drainage is inadequate, a "step-up" to surgical removal of infected material should be pursued using either minimally invasive approaches (endoscopic, laparoscopic, retroperitoneal, percutaneous) or more traditional surgical approaches (i.e., necrosectomy with continuous closed lavage, débridement with open packing, or necrosectomy and drainage with planned reoperation).[44,45] Surgical management often requires multiple staged operations to remove all necrotic pancreatic and peripancreatic material. A 2014 study from Boston found that open débridement was associated with low in-hospital mortality (8.8%).[33]

The timing of surgical removal is a matter of some debate in the older literature. The intended benefit from early intervention is prompt removal of the infected material in hopes of more rapid resolution of the inflammatory processes. However, newer studies demonstrate a benefit from a delay in surgery that may allow for a more stable patient with better-demarcated areas of necrotic tissue.[46,47] For this reason, practice has evolved toward waiting on surgical intervention until the 4-week point if possible, to allow walling off of the necrotic collection and softening/liquefaction of its contents. There is some experience with local administration of antimicrobials via catheter into areas of necrosis that cannot be removed.[48]

In contrast to infected necrosis, postpancreatitis abscesses remote from the pancreas itself and superinfected pancreatic pseudocysts are unlikely to be associated with significant amounts of necrotic tissue; these may be managed successfully by catheter drainage or by surgical drainage in conjunction with appropriate antimicrobial therapy.[30]

Prevention of Pancreatic Infection

Because infection may be associated with increased mortality rates in AP despite aggressive medical and surgical therapy, major efforts have been oriented toward preventing infection. Studies have focused on maintaining the gut barrier function, probiotics, selective decontamination of the digestive tract (SDD) with oral, nonabsorbable antibiotics, and early therapy with systemic antibiotics. A reasonable aim for such studies is to identify interventions that decrease the frequency of pancreatic infection, leading to fewer surgical procedures and lower mortality rates.

Early Enteral Feeding

Several groups have studied enteral feeding in AP for its ability to lower infection risk both by reducing dependence on central venous access for parenteral alimentation and by sustaining the integrity of the intestinal barrier. In animal studies of AP[49] enteral nutrition was associated with less bacterial and/or endotoxin translocation into mesenteric lymph nodes but did not influence pancreatic healing or overall survival. In one study of humans, 38 patients with severe AP were randomized to parenteral nutrition or to enteral nutrition via nasojejunal tube (NJT) within the first 48 hours after admission. The NJT patients suffered fewer complications ($P < .05$) and fewer infectious complications ($P < .01$).[50] Of note, all patients received imipenem from admission until clinical recovery and restoration of normal CRP concentrations. The authors speculate that enteral feeding "improves the gut immune system, restores normal gut structure and microbiota, and aids the mucosa in withstanding challenges… it is, in fact, still unclear whether enteral feeding improves the rate of septic morbidity or whether TPN itself causes an increase in septic complications." Another study of 89 patients with pancreatitis observed that enteral nutrition significantly reduced septic complications but did not affect the rates of multiple organ failure or death.[51] In a randomized trial of 208 patients with severe pancreatitis, early enteric feeding initiated within 24 hours after randomization was not superior to an oral diet initiated 72 hours after presentation in reducing rates of infection or death.[52]

Probiotics have been evaluated in addition to enteral feeding. A randomized, double-blind study showed significantly less pancreatic sepsis and fewer surgical interventions in 22 AP patients given live *Lactobacillus* for 1 week, compared with 23 control patients given heat-killed *Lactobacillus*. Both groups received early enteral feeding with oat fiber supplementation as a substrate for the lactic acid bacteria.[53] In a subsequent double-blind, placebo-controlled study, 298 patients with severe AP received a multispecies probiotic product or placebo, administered via NJT along with tube feeding. Infection rates were similar between groups, but mortality was higher in the probiotic group (16% vs. 6%; RR, 2.53). Nine patients in the probiotic group developed bowel ischemia (8 of whom died), compared with none in the placebo group ($P = .004$). The researchers concluded that this probiotic should not be administered to patients with predicted severe AP.[54] Commentators provide several possible explanations for these unexpected results, including a potentially harmful interaction between the probiotic and the enteral feeding formula used. They also speculate that "patients with multiorgan dysfunction who need vasopressors to maintain systemic circulation might have severe splanchnic hypoperfusion. Probiotics with enteral feeding might result in intestinal oxygen consumption exceeding supply, resulting in overt ischemia."[55] In a follow-up study, bacteremia, infected necrosis, organ failure, and mortality were all associated with intestinal barrier dysfunction early in the course of AP. This was determined by measuring excretion of intestinal fatty acid binding protein (a parameter for enterocyte damage), recovery of polyethylene glycols (a parameter for intestinal permeability), and excretion of nitric oxide (a parameter for bacterial translocation) in urine samples that were collected 24 to 48 hours after randomization to probiotic or placebo treatment and 7 days thereafter from 141 patients with predicted severe AP. The specific combination of probiotic strains used in this study reduced bacterial translocation overall but was associated with increased bacterial translocation and enterocyte damage in patients with organ failure.[56]

Selective Decontamination of the Digestive Tract

SDD aims to eradicate carriage of potentially pathogenic organisms from the GI tract. Animal studies of SDD, reviewed by Ratschko and coworkers,[7] show a mortality benefit in AP. Of some concern, however, studies in rats suggest that eliminating gram-negative bowel microbiota may facilitate overgrowth of *Enterococcus* spp. in the gut and increase the risk of gram-positive infections from translocation of GI microbiota.[57]

Only one controlled study in human patients evaluated the effects of SDD on infection rates in pancreatitis (Table 76.3).[58] In this trial patients with severe AP, but without pancreatic necrosis on CT, were randomized to receive conventional therapy or conventional therapy plus SDD (enteral colistin, norfloxacin, and amphotericin B). The SDD group also received IV cefotaxime until aerobic gram-negative bacteria were eliminated from the oral cavity and rectum. There was no difference in mortality. However, correcting for illness severity yielded a narrow survival benefit for the SDD group ($P = .048$) that was attributable to a decrease in infected necrosis (18% vs. 38%, $P = .03$), with an effect on gram-negative pancreatic infection in particular (8% vs. 33%, $P = .003$). Of note, sterile pancreatic necrosis was documented in 11 of 16 patients who died within 2 weeks of onset (mostly from multiple organ dysfunction syndrome), suggesting that mortality was unrelated to infection. The administration of IV cefotaxime along with SDD in this study obscures interpretation of the effect of SDD alone.

In a follow-up report[59] the authors of this trial noted that among patients in both the SDD and control groups, gram-negative infection of pancreatic necrosis was associated with a mortality rate 15-fold higher than that associated with sterile necrosis, whereas gram-positive pancreatic infection was not associated with an increase in mortality rate. Of interest, there was no significant difference in the incidence of

TABLE 76.3 Pancreatic Infection Incidence and Mortality Rate in Controlled Trials with Antibiotics and Meta-analyses

AUTHOR	ANTIBIOTIC	NO. OF PATIENTS	PANCREATIC INFECTION RATE (%) CONTROL/CASE	MORTALITY RATE (%) CONTROL/CASE	COMMENTS
Luiten et al.[58]	SDD: enteral colistin, norfloxacin, and amphotericin B, until clinical recovery plus IV cefotaxime until gram-negative bacteria are eliminated from the oral cavity	102 severe AP	38/18[a]	35/22	Nonblinded. Necrosis not defined. Mortality difference statistically significant when disease severity differences between the groups are taken into account. Predominant effect on gram-negative pancreatic infection (8% in SDD group vs. 33% in conventional treatment group). Treatment with IV cefuroxime as well as enteral SDD obscures interpretation of the effect of enteral treatments alone
Pederzoli et al.[21]	Imipenem, 500 mg IV tid, 14 days	74 necrotizing pancreatitis, mostly of biliary origin	30/12[a]	12/7	Not placebo-controlled. Nonpancreatic infections also reduced (15% vs. 49%, $P < .01$). No change in multiorgan failure rate, need for surgery, or survival compared with no early antibiotic treatment
Sainio et al.[66]	Cefuroxime, 1.5 g IV tid, until clinical recovery and normalization of CRP level	60 severe acute alcoholic pancreatitis and necrosis of at least one-third of the pancreas by contrast-enhanced CT	40/30	23/3[a]	Not placebo-controlled. Reduction in total infections, infections per patient, and operations also statistically significant. Rate of culture-proven sepsis not statistically significant. Only urinary tract infections were reduced significantly
Delcenserie et al.[67]	Ceftazidime, amikacin, and metronidazole IV, 10 days	23 alcohol-induced severe AP	58/0[a]	25/9	CT with two or more fluid collections, necrosis not defined
Schwarz et al.[68]	Ofloxacin and metronidazole IV, 10 days	26 CT-confirmed pancreatic necrosis	53/61	15/0	Not placebo-controlled. FNA performed on days 1, 3, 5, 7, and 10 in all patients; antibiotics given to control patients with evidence of infection. Better clinical course in intervention group, but no effect on development of pancreatic infections
Bassi et al.[38]	Pefloxacin (400 mg bid) vs. imipenem (500 mg tid), 14 days	60 severe necrotizing pancreatitis	Pefloxacin: 33[a] Imipenem: 10[a]	Pefloxacin: 24 Imipenem: 10	Nonblinded. CT confirmed at least 50% necrosis
Isenmann et al.[72]	Ciprofloxacin (400 mg bid) and metronidazole (500 mg bid) IV, *or* placebo, 14 or 21 days	114 AP plus serum CRP >150 mg/L and/or necrosis on contrast-enhanced CT	12/9	5/7	Randomized, double-blinded, placebo-controlled; 28% of CIP/MET group and 46% of placebo group required open antibiotic treatment; most placebo group switches motivated by extrapancreatic infections; overall mortality and rates of pancreatic infection low, number of patients with pancreatic necrosis low
Golub et al.[69]	Meta-analysis of eight controlled trials, including trials from the 1970s[21,58,61,101–103]				Findings: Early antibiotic administration reduced mortality from AP but only for patients with severe pancreatitis who received broad-spectrum antibiotics reaching therapeutic levels in pancreatic tissue
Sharma et al.[70]	Meta-analysis including only randomized, controlled, nonblinded studies evaluating patients with necrotizing pancreatitis who received either no preemptive antibiotics or preemptive treatment with antibiotics reaching therapeutic levels in necrotic pancreatic tissue[21,66,67]	84 received antibiotic prophylaxis; 76 did not; of note, the total of 160 patients in the pooled data set is inadequate for detection of a 50% reduction in the rate of infections[60]			Findings: Nonsignificant trend toward decreased local infection in patients given early imipenem, cefuroxime, or ofloxacin. Incidence of sepsis and overall mortality significantly lower (absolute risk reductions, 21.1% and 12.3%, respectively, for a relative risk reduction of 72%) for antibiotic treatment. On this basis, the authors recommend that "all patients with acute necrotizing pancreatitis…be given prophylaxis with an antibiotic with proven efficacy in necrotic pancreatic tissue."

TABLE 76.3 Pancreatic Infection Incidence and Mortality Rate in Controlled Trials with Antibiotics and Meta-analyses—cont'd

AUTHOR	ANTIBIOTIC	NO. OF PATIENTS	PANCREATIC INFECTION RATE (%) CONTROL/CASE	MORTALITY RATE (%) CONTROL/CASE	COMMENTS
Villatoro et al.[75]	Cochrane Review including RCTs comparing antibiotics with placebo in AP with CT-proven necrosis	294 (from 5 evaluable studies)			Findings: Significantly less mortality with therapy (6%) vs. controls (15.3%). No differences in rates of infected pancreatic necrosis, operative treatment, nonpancreatic infection, and fungal infection. Subgroup analysis of β-lactam treatment: Significantly less mortality (6.3% vs. 16.7%) than in controls, without significant differences in rates of operative treatment or nonpancreatic infections. No significant differences with quinolone plus imidazole treatment. β-Lactams associated with significantly decreased mortality and infected pancreatic necrosis
Rokke et al.[76]	Prospective RCT, imipenem (500 mg tid for 5–7 days) vs. no antibiotics	73 patients total: 36 received imipenem; 37 received no antibiotics	43/14[a]	11/8	Underpowered study (slow patient accrual). No differences in length of hospital stay, need of intensive care, need of acute interventions, need for surgery, or 30-day mortality rates. Authors conclude that "the study, although underpowered, supports the use of early prophylactic treatment with imipenem in order to reduce the rate of septic complications in patients with severe pancreatitis."
Dellinger et al.[77]	Multicenter, prospective, double-blind, placebo-controlled randomized study set in 32 centers within North America and Europe	100 clinically severe, confirmed necrotizing pancreatitis: 50 received meropenem (1g IV q8h); 50 received placebo within 5 days of symptom onset, for 7–21 days	12/18	18/20	No statistically significant difference between the treatment groups for pancreatic or peripancreatic infection, mortality, or requirement for surgical intervention
Jafri et al.[78]	Meta-analysis assessing the clinical outcome of patients with severe AP treated with prophylactic antibiotics compared with that of patients not treated with antibiotics	502 (from 8 studies)			Findings: No effect of antibiotics on mortality, rates of infected necrosis, or frequency of surgical intervention. Apparent benefit regarding nonpancreatic infections (RR, 0.60; 95% CI, 0.44–0.82), with a RR reduction of 40% (95% CI, 18% to 56%); absolute risk reduction of 15% (95% CI, 6% to 23%), and number needed to treat of 7 (95% CI, 4 to 17)
Villatoro et al.[80]	Cochrane Review including RCTs comparing antibiotics with placebo in AP with CT-proven necrosis	404 (from 7 evaluable studies)			Findings: No benefit of antibiotics in preventing infection of pancreatic necrosis or mortality, except when imipenem was considered on its own, in which case a significant decrease in pancreatic infection was found ($P = .02$; RR, 0.34; 95% CI, 0.13 to 0.84) without effect on mortality. "None of the studies included in this review were adequately powered. Further better-designed studies are needed if the use of antibiotic prophylaxis is to be recommended."
Xue et al.[79]	Imipenem-cilastatin 500 mg IV tid for 7–14 days	56	37/27.6	10.3/14.8	Incidence of operative necrosectomy, extrapancreatic infections, organ complications, and hospital courses were also not significantly different. A significantly increased incidence of fungal infection was observed in the study group (36.1% vs. 14.2%)
Wittau et al.[81]	Meta-analysis of 14 RCTs including 841 patients, comparing antibiotics vs. placebo				Findings: No significant differences in mortality, incidence of infected pancreatic necrosis, nonpancreatic infection, surgical intervention

[a] $P < .05$.

AP, Acute pancreatitis; *CI,* confidence interval; *CIP/MET,* ciprofloxacin/metronidazole; *CRP,* C-reactive protein; *CT,* computed tomography; *FNA,* fine-needle aspiration; *IV,* intravenous; *RCTs,* randomized controlled trials; *RR,* relative risk; *SDD,* selective decontamination of the digestive tract.

Modified from Toouli J, Brooke-Smith M, Bassi C, et al. Guidelines for the management of acute pancreatitis. J Gastroenterol Hepatol. *2002;17(suppl):S15–S39.*

gram-positive infected necrosis between the SDD and the control groups. These issues may be related to the treatment with systemic cefotaxime, which protects from some of the more virulent gram-positive organisms but has relatively poor coverage against gram-negative organisms such as *Pseudomonas aeruginosa*. In fact, the most frequent gram-negative isolates in this study were *P. aeruginosa* and *E. coli* (13 patients each), whereas the most frequent gram-positive isolates were enterococci and *Staphylococcus epidermidis*.

Preemptive Systemic Antibiotic Therapy

Multiple groups have examined the efficacy of early treatment with systemic antibiotics in diminishing the risks of infection in AP. This literature generally describes an approach such as "prophylactic" antimicrobial therapy, but the strategy might be described more accurately as "early" or "preemptive" therapy, and these terms will be used preferentially here. Numerous animal pancreatitis studies have shown a benefit from early antibiotic administration, as reviewed by Ratschko and coworkers[7] and Sand and Nordback.[55] Among other aspects, the ability to administer antibiotics very early in the course of pancreatitis distinguishes these animal models from the treatment of human patients, who did not appear to benefit from antibiotic therapy in the initial prospective randomized studies from the 1970s. However, the human studies enrolled patients with mild disease who were at low risk for infection; among the aggregate of 199 patients in these studies, only 1 patient died. In addition, the treatment groups received monotherapy with ampicillin, which provides suboptimal activity against the relevant organisms and also penetrates poorly into pancreatic tissue and fluid.[7]

Several groups have evaluated antimicrobial penetration into pancreatic tissue. In human studies antibiotic levels in pancreatic tissue and fluid tend to correlate with the degree of inflammation; thus higher levels occur in patients with pancreatitis than in members of control groups.[7] In general, antimicrobials can be assigned to the following categories: (1) poor pancreatic penetration (aminoglycosides, first-generation cephalosporins, cefoxitin, and ampicillin); (2) variable pancreatic penetration, reaching minimal inhibitory concentrations (MICs) for some but not all relevant organisms (mezlocillin [not available in the United States], piperacillin, and cefotaxime); and (3) pancreatic penetration reaching MIC for most relevant organisms (fluoroquinolones, imipenem, ceftazidime, cefepime, metronidazole, clindamycin, chloramphenicol, doxycycline, and fluconazole).[60,61,62–64] One group developed an efficacy factor incorporating each drug's pancreatic penetration and activity against organisms typically associated with pancreatic infection, with a factor of 1.0 considered optimal.[61] The calculated factors were 0.98 for imipenem, 0.86 to 0.87 for fluoroquinolones, and 0.71 to 0.78 for piperacillin and third-generation cephalosporins. However, the relationship between antibiotic levels in pancreatic tissue or fluid and antibiotic efficacy in preventing or treating infections in necrotic pancreatic parenchyma or in the retroperitoneal fat outside the pancreas remains unclear.

Observational reports[13,65] have described single-institution experiences with lower rates of infected necrosis after introduction of early antibiotic therapy. One such report[65] describes a retrospective cohort review evaluating three groups of patients with necrotizing pancreatitis: 50 patients from 1982–89 receiving no early antibiotics, 55 patients from 1990–92 receiving variable antibiotic regimens at the discretion of their physicians, and 75 patients between 1993 and 1996 treated with imipenem until discharge or for up to 4 weeks, according to standard protocol, based on an APACHE score less than 6 and pancreatic necrosis (<15% of the gland), peripancreatic necrosis, or peripancreatic collection. The frequency of pancreatic infection was significantly lower among patients receiving preemptive antibiotics (76% in the earliest group vs. 45% in the middle group [$P = .003$] and 27% in the latest group [$P < .001$]). In addition, the difference in infection rates between the group of patients receiving nonprotocol antibiotics and the group receiving imipenem by protocol was statistically significant ($P = .04$). The mortality rates trended downward from 16% to 7% to 5% ($P = .11$). Mortality rates ranged from 19% to 40% for patients developing infection within the first 4 weeks, compared with 0% to 8% for those infected after 4 weeks. Time to infection, however, was the same across all groups. The authors conclude that early imipenem, or possibly ciprofloxacin, for a 4-week course should be the standard of care for patients with severe AP. Although this study has been invoked in support of routine administration of imipenem, the results are confounded by several factors. The middle group may have received antibiotics with an inadequate spectrum of coverage, later in the course of illness, or for an inadequate duration. Because the causative organisms were not reported, the importance of spectrum of coverage cannot be assessed. In addition, the longitudinal retrospective design of this study impairs understanding of the effects of non–antibiotic-related changes in detection and management of severe AP that may have influenced mortality rates. Of note, lower infection rates have not been associated with improved survival in retrospective studies.

Four initial randomized studies in the 1990s and early 2000s addressed the efficacy of systemic antibiotics in diminishing infection in AP, focusing on patients with severe disease and using broad-spectrum antibiotics with reasonable pancreatic penetration, as summarized in Table 76.3. The results of these studies, however, were not definitive because of problems in study design and sample size.

In one trial of 74 patients with necrotizing pancreatitis, early imipenem treatment reduced morbidity by decreasing the rates of tissue-confirmed pancreatic infection and nonpancreatic sepsis but did not influence time to onset of pancreatic sepsis, rate of multiorgan failure, need for surgery, or survival in comparison to patients receiving no early antibiotic treatment. Preemptive antibiotic therapy was especially effective for patients with mild-to-moderate pancreatic necrosis; no patient in the treated group with less than 50% necrosis developed septic complications versus 29% in the control group.[21]

In another trial[66] 60 patients with severe AP and necrosis were given no preemptive antibiotics or cefuroxime. The treated group suffered significantly fewer total infections, infections per patient, operations, and deaths (3% vs. 23%). However, the rate of culture-proven sepsis was not significantly different between groups, and only urinary tract infections were reduced significantly. The study has been criticized because of the large numbers of urinary tract infections and of *S. epidermidis* "infections" with questionable clinical significance. In addition, 67% of the treatment group patients changed antibiotics after a mean of 9.2 days, 3 because of strong clinical suspicion for (culture-negative) sepsis, and 17 for unstated reasons.[7] Finally, it is unclear whether the deaths were related to infection. The authors acknowledge that the reasons for mortality benefit from early cefuroxime treatment in this study are unclear because cefuroxime would not have been expected to alleviate most of the infections in the control group.[66]

A third group evaluated the effects of a 10-day course of IV ceftazidime, amikacin, and metronidazole in 23 patients with alcohol-induced severe AP. Infections occurred among 0% of patients in the treatment group versus 58% in the placebo group ($P = .03$). Mortality and multiple organ failure rates were not significantly different.[67]

The fourth study randomized 26 patients with pancreatic necrosis to early IV ofloxacin and metronidazole or no antibiotics. All patients underwent serial FNA; antibiotics were administered to the control patients who showed evidence of infection. There was no difference in the incidence or timing of infected necrosis. The mortality rate trended downward from 15% to 0% in the antibiotic-treated group.[68]

Thus, of the four initial randomized studies comparing routine early antibiotic treatment with no treatment in AP, three showed lower sepsis rates, two showed lower rates of pancreatic infection, and none showed any effect on the operation rate. Only one study[66] showed a statistically significant reduction in mortality rate associated with preemptive antibiotics; this particular study found no associated change in pancreatic infection rates. The inconsistencies among trials may arise from the relatively small numbers of patients, providing inadequate power to detect significant differences. Additional discrepancies might be related to the differences in patient populations and in nonantibiotic management among the various populations, such as rates, timing, and techniques of surgery; enteral nutrition; fluid resuscitation; and other factors.

Findings from meta-analyses of the published trials are summarized in Table 76.3.[69,70] The validity of meta-analysis for these studies is unclear because the enrollment criteria, antibiotic regimens, and methods of

diagnosing infections used in the trials are not uniform. Finally, one author points out that the absence of blinding in these studies may have encouraged clinicians to offer surgical débridement preferentially to control patients; such surgery might convert sterile necrosis to infected necrosis, which might increase mortality.[71]

A randomized, double-blinded, and placebo-controlled study was designed to demonstrate with a power of 90% that ciprofloxacin/metronidazole (CIP/MET) reduces the incidence of infected pancreatic necrosis from 40% to 20%.[72] After an interim analysis showed increased frequency of need for open antibiotic treatment in the placebo group, recruitment was stopped and results were reported: no differences in rates of infected pancreatic necrosis or mortality were found. The results of this study have been controversial because of the low rates of infected necrosis in both groups and the high rate of switch to open antibiotic treatment in the placebo group. The authors suggest that both of these issues are related to the close monitoring for signs of SIRS or progressive severe pancreatitis, which led to high switching rates and low rates of pancreatic infection. Brown[73] notes, "It will always be difficult to perform a randomized trial in subjects with severe AP in which the control group does not receive antibiotics" and comments further that the study was underpowered to detect the more meaningful end point of mortality.[73] Additional critiques include the relatively small number of patients with pancreatic necrosis and the substantial number of infections with coagulase-negative staphylococci, raising concern about whether these isolates are true pathogens versus contaminants.[74] Because mortality rates were similar in the two groups, the authors suggest that these data support the approach of antibiotic treatment "on demand" based on development of sepsis, SIRS, failure of two or more organ systems, proven pancreatic or extrapancreatic infection, and an increase in CRP along with evidence of pancreatic or extrapancreatic infection. The authors calculate that use of these criteria, rather than the preemptive antibiotic treatment given in the CIP/MET group, would have reduced antibiotic costs for this group by approximately 34%.[72]

A 2006 Cochrane Review of data from these five evaluable studies found no benefit of antibiotics in preventing infection of pancreatic necrosis or mortality, except for when imipenem was considered on its own, in which case a significant decrease in pancreatic infection but not mortality was found.[75]

A subsequent underpowered prospective randomized controlled trial (RCT)[76] measured outcomes in 73 patients with severe AP, of whom 36 received early imipenem (500 mg tid for 5–7 days) and 37 received no antibiotics. Imipenem treatment was associated with significant reduction in pancreatic infections (43% vs. 14%), but there were no differences in length of hospital stay, need of intensive care, need of acute interventions, need for surgery, or 30-day mortality. The authors conclude that "the study, although underpowered, supports the use of early prophylactic treatment with imipenem to reduce the rate of septic complications in patients with severe pancreatitis."

A larger multicenter, prospective, double-blind, placebo-controlled randomized study set in 32 centers within North America and Europe included 100 patients with clinically severe, confirmed necrotizing pancreatitis, of whom 50 received meropenem (1g IV q8h), and 50 received placebo within 5 days of the onset of symptoms for 7 to 21 days. In this study there were no differences in rates of pancreatic or peripancreatic infections, overall mortality, or need for surgical intervention.[77] Although there was a high percentage of patients in the placebo group (54%) who were treated with IV antibiotics, on average the antibiotic exposure was nearly 3 weeks after randomization. Howard[78] calculates that with the rates of "placebo group infection rate of 20% with a reduction to 10% using prophylactic antibiotic, accurate powering of a definitive study of prophylactic antibiotics patterned after [this] trial would now require the screening of approximately 8383 patients and randomization of 1006. Based on the disease frequency and heterogeneity, combined with the logistical problems found in the current study, this seems like an insurmountable task." He comments further that "the combined weight of these last two clinical trials will undoubtedly skew any future meta-analysis against routine prophylactic antibiotic use."[78]

A later RCT from China, published in 2009, included 276 patients with severe acute pancreatitis, of whom 56 had 30% or more necrosis and were randomized to IV imipenem within 72 hours of onset of symptoms for 7 to 14 days versus no antibiotic prophylaxis. There was no benefit for antibiotic prophylaxis in preventing infected pancreatic necrosis, mortality, or the incidence of operative necrosectomy. The rates of extrapancreatic infection, organ complications, and hospital course were also unchanged. However, the incidence of fungal infection was significantly higher in the study group (36% vs. 14%).[79]

As predicted, several subsequent meta-analyses of trials looking at benefits of antibiotic prophylaxis in severe AP have shown no effect of antibiotics on mortality, rates of infected necrosis, or frequency of surgical intervention. In one meta-analysis there was apparent benefit regarding nonpancreatic infections (RR, 0.60; 95% confidence interval [CI, 0.44 to 0.82), with a RR reduction of 40% (95% CI, 18% to 56%), absolute risk reduction of 15% (95% CI, 6% to 23%), and number needed to treat of 7 (95% CI, 4 to 17).[80] A 2011 meta-analysis of 14 RCTs including 841 patients found no significant differences in mortality, incidence of infected pancreatic necrosis, nonpancreatic infection, or surgical intervention.[81]

A 2015 meta-analysis of published RCTs of "early" administration of prophylactic antibiotics concluded that this was associated with a reduced incidence of infected pancreatic necrosis and with lower mortality.[82] "Early" refers to administration within 72 hours of onset of symptoms or within 48 hours after hospital admission. However, in addition to the difficulties with the design and interpretation of the individual RCTs in this study and the variability of antibiotic regimens/durations among them, an added limitation of this meta-analysis is that the RCTs compared the use and nonuse of antibiotics, not the timing of administration, making this study more strictly a sensitivity analysis of the RCTs rather than a meta-analysis per se. A 2010 Cochrane Review of seven randomized studies that included studies in which administration may have occurred after 72 hours of onset concluded that patients randomized to receive prophylactic antibiotics for acute necrotizing pancreatitis had no statistically significant reduction in infections.[83]

A meta-analysis that included both RCTs and cohort studies found no significant decrease in the incidence of infected pancreatic necrosis with the use of prophylactic antibiotics but did find a reduction in all-cause mortality.[84] However, this reduction in all-cause mortality was not observed when the cohort studies were excluded from the analysis.

Additional Data

One trial has addressed the selection of optimal antibiotic therapy by comparing a course of pefloxacin (not available in the United States) to treatment with imipenem in 56 patients. Infected necrosis and extrapancreatic infection rates were significantly lower in the imipenem group. Rates of death, all resulting from sepsis, did not differ significantly.[38]

Another trial addressed the timing of preemptive meropenem by comparing early treatment (started 1.07 ± 0.6 days after admission, group A) to treatment started after demonstration of pancreatic necrosis by CT (4.56 ± 1.2 days from admission, group B). Pancreatic infection rates were not significantly different between groups, but extrapancreatic infection occurred significantly less frequently in group A than in group B (16.6% vs. 44.8%). Need for surgery and length of hospitalization were also higher in group B. Mortality rates were similar in the two groups, but three of four patients with infected necrosis in group A, and only two of nine in group B, died. The authors conclude that early antibiotic treatment improves the prognosis of necrotizing AP by reducing the rate of septic complications.[85] Concerns about these conclusions include the lack of mortality difference between groups, the fact that organisms resistant to meropenem were responsible for all infections in the early antibiotic group patients, the use of TPN rather than enteral nutrition in this study, and the risks of *Clostridioides difficile* (formerly *Clostridium difficile*) associated with antibiotics.[86]

Review Article Recommendations

Between 1986 and the mid-2000s multiple groups made specific recommendations largely supporting use of carbapenem alone or quinolone plus metronidazole as first-choice preemptive antibiotic therapy, with third-generation cephalosporins or ureidopenicillins as alternative aerobic coverage and clindamycin as alternative anaerobic coverage. Most authors recognized that optimal duration of preemptive antimicrobial therapy

had not been studied, but some recommended 1 to 4 weeks, depending on the course of disease.[7]

In contrast, other investigators favored minimizing antimicrobial exposure. One group[87] recommended efforts to avoid imipenem and to limit the duration of empirical coverage to 14 days because of concerns about facilitating fungal superinfection. Another group[88] concluded that the risks of fungal infection warrant a policy of limiting treatment with broad-spectrum antibiotics to "as short a period as seems prudent (typically 5–7 days)." One review suggested an approach of "on-demand" antibiotics rather than uniform administration of antibiotics for all patients with severe AP with necrosis. Specifically, this group advocated limiting antibiotics to patients with extensive (rather than focal) necrosis and to patients who have undergone surgical procedures for extensive sterile necrosis because of the risks of gram-negative superinfection associated with surgical necrosectomy.[89]

Guidelines

On the basis of more recent studies, in 2013 several major guidelines (American College of Gastroenterology and International Association of Pancreatology) recommended avoiding preemptive antibiotic and antifungal use in AP.[90,91] Multiple other groups have also provided guidelines and recommendations recently, as summarized in Table 76.4.[92–96]

The only guidelines published since 2013 recommending prophylactic antibiotics routinely in severe AP are the Japanese guidelines from 2015,[92] in which authors cite in support of this recommendation the sensitivity analysis termed meta-analysis[82] looking at administration of antibiotics within 72 hours after onset of symptoms or 48 hours after admission.

The Italian Association for the Study of the Pancreas Guideline[93] raises the possibility of antibiotic prophylaxis for patients with pancreatic necrosis involving greater than 50% of the gland "on a case-to-case basis due to the high risk of infection" and favors carbapenem as first-line empirical treatment for patients with suspected infected pancreatic necrosis. No recent guidelines recommend routine antifungal prophylaxis or SDD.

Actual Practice

Despite multiple guidelines now recommending against use of prophylactic antibiotics in AP, substantial use of antibiotics continues in practice, both in mild AP and in more severe AP. In one 2016 study from the United Kingdom, 44% of patients with clinically mild AP received antibiotics.[97] In another 2016 study from Canada, 25.5% of non–intensive care unit (ICU) patients and 48.1% of ICU patients received prophylactic antibiotics.[98]

A 2016 review sums up the current understanding of the role of prophylactic antibiotics and its relationship to clinical practice as follows: "Although the development of infected pancreatic necrosis confers a significant risk of death, well-designed trials and meta-analyses have shown no benefit of prophylactic antibiotics. Prophylaxis with antibiotic therapy is not recommended for any type of acute pancreatitis unless infection is suspected or has been confirmed. Nonetheless, many patients continue to receive prophylactic antibiotics despite guidelines to the contrary."[99]

CONCLUSIONS

In general, mild-to-moderate AP is self-limited and rarely complicated by infection. Severe AP is associated with significant morbidity and mortality rates that may be related to pancreatic superinfection with GI microbiota. The management of such infections requires optimal diagnosis by way of thoughtful clinical assessment, imaging studies, and consideration of percutaneous aspiration of necrotic pancreatic material, as well as aggressive medical and surgical care, with current recommendations favoring efforts to postpone surgical intervention until the stage of WON (i.e., after 4 weeks) if possible. Enteral feeding prevents infection in AP. A consensus favoring early antibacterial treatment aimed at decreasing pancreatic infection risk for patients with severe AP emerged in the 1990s to 2000s with some support from the literature. However, small size and design flaws rendered these studies difficult to interpret. More recently, several larger studies with fewer design flaws have failed to show significant benefits from preemptive

TABLE 76.4 Recommendations From Review Articles and Guidelines Regarding Early Administration of Antibiotics in Patients with Acute Pancreatitis

AUTHORS	YEAR	INCLUSION CRITERIA	TIMING/DURATION
Tenner,[90] American College of Gastroenterology Guideline	2013	All pancreatitis	Routine use of prophylactic antibiotics in patients with AP is not recommended. Use of antibiotics in patients with sterile necrosis is not recommended.
Da Costa, Boerma, van Santvoort, et al.[96]	2013	All pancreatitis	Antibiotics are not indicated as part of initial management.
Yokoe et al.[92]	2015	Severe acute pancreatitis and necrotizing pancreatitis	Start within 72 h of onset or 48 h after admission, continue for no more than 2 weeks if there are no signs of infection.
Italian Association for the Study of the Pancreas[93]	2015	Severe acute pancreatitis	Routine prophylaxis with antibiotics/antifungals is not recommended in severe AP. In the case of pancreatic necrosis involving >50% of the gland, antibiotic prophylaxis might be considered on a case-by-case basis due to the high risk of infection. Among the different antibiotics used, a carbapenem-based prophylaxis has a trend toward efficacy. This class of antibiotics should be considered as a first line empirical treatment for patients with suspected infected pancreatic necrosis.
Greenberg et al.[94]	2016	Mild or severe AP	None unless patient is unstable with suspected sepsis and no source identified. "Treatment with broad-spectrum antibiotics on speculation may be indicated while an appropriate workup (bacterial and fungal cultures, CT) is carried out." Otherwise antibiotics should be prescribed only in patients with infected necrosis confirmed by FNA or if there is gas within a collection visualized on CT scan.
Janisch and Gardner[95]	2016	All pancreatitis	Prophylactic antibiotics are not recommended for use in AP and should not be administered in the first 24 hours of the episode unless there is clinical suspicion for concurrent extrapancreatic infection. Patients may present initially with sepsis, SIRS, and/or multiorgan failure. Treatment with antibiotics is appropriate if evaluation of the patient, via blood cultures and fine needle aspirations of pancreatic necrosis, reveals infection. However, if there is no obvious source of infection, antibiotics should be discontinued.

AP, Acute pancreatitis; *CT,* computed tomography, *FNA,* fine-needle aspiration; *SIRS,* systemic inflammatory response syndrome.

antibiotics, and these have led most experts to recommend against using antibiotics routinely for all patients with severe AP. The lack of benefit from preemptive antibiotics in these more recent studies may result in part from the fact that "modern care is associated with a reduction in the risk of necrosis becoming infected. This reduction would decrease the relative potential clinical impact of prophylactic antibiotics."[78] Residual enthusiasm for preemptive or prophylactic antibiotic treatment must be tempered also by concerns about selecting for increasingly resistant organisms, which have been associated with longer ICU stays and courses of antibiotics.[100]

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77 Splenic Abscess

Lawrence C. Madoff

SHORT VIEW SUMMARY

Definition
- Abscesses comprise one or more focal collections induced by bacteria, mycobacteria, or fungi in the spleen.
- Predisposing conditions include immunosuppression, trauma, diabetes, hemoglobinopathy, Felty syndrome, intravenous drug use, and endocarditis.

Epidemiology
- Splenic abscesses are uncommon and have been reported only in small numbers in the medical literature.
- Splenic abscesses are present in 0.2% to 0.7% in general autopsy series.
- Splenic abscesses are a frequent finding in bacterial endocarditis and are present in up to one-third of patients at autopsy.
- There is a bimodal age distribution, with peaks in the third and sixth decades of life.

Microbiology
- Streptococci, staphylococci, salmonellae, and *Escherichia coli* are important causative agents, as well as fungi and mycobacteria increasingly.
- In specific geographic areas of Asia, *Klebsiella pneumoniae* and *Burkholderia pseudomallei* are important causative agents.
- Anaerobes are rare.

Clinical Findings and Diagnosis
- Fever is present with abdominal pain in the left upper quadrant but a paucity of specific symptoms and findings.
- Ultrasound, computed tomography, and magnetic resonance imaging are used for diagnosis.

Therapy
- Splenectomy has been the gold standard of treatment along with antibiotic treatment of underlying infection elsewhere.
- More recent emphasis has been on the use of percutaneous drainage with antibiotic treatment or, in some cases, antibiotic treatment alone in an attempt to reduce morbidity and preserve the spleen.

EPIDEMIOLOGY

The spleen is a highly vascular hematopoietic lymphoid organ that is part of the reticuloendothelial arm of the immune system. If the spleen is surgically removed, its absence is marked by heightened susceptibility to overwhelming infection by encapsulated bacteria and intraerythrocytic parasites (see Chapter 311). Abscesses of the spleen usually result from bacteremia, particularly in the setting of abnormalities caused by trauma, embolization, or hemoglobinopathy. Immunodeficiency such as that resulting from human immunodeficiency virus (HIV) infection is also a risk factor.[1] Occasionally splenic abscess results from extension of a contiguous focus of infection.

Splenic abscesses are relatively uncommon. For example, in one series of 540 intraabdominal abscesses, none were in the spleen.[2] Autopsy series have placed the incidence of splenic abscesses at 0.2% to 0.7%.[3,4] Only approximately 600 splenic abscesses had been reported in the literature as of 2008.[5] It has been suggested that the incidence may be increasing because of improved detection, increasing use of illicit intravenous drugs, and the increased number of immunocompromised individuals.[6–8] Splenic abscess has a bimodal age distribution, with peaks in the third and sixth decades of life.[8]

PATHOGENESIS

Bacteremic infection from a variety of sites is the most common cause of splenic abscess. Classically, infective endocarditis has been most strongly associated with splenic abscess, and in most series, endocarditis is identified as the leading cause.[9–12] In an autopsy series, 29% of patients with endocarditis between 1986 and 2008 were found to have splenic abscesses.[13] Other common sources of infection are the urinary tract, surgical wounds, and the gastrointestinal tract. Immunodeficiency has become a more important risk factor for the development of splenic abscess. In large reviews, 18% to 34% of patients were immunosuppressed (from disease, cancer chemotherapy, or steroid use) including 9% who were infected with HIV.[11,14,15] Trauma to the spleen, either iatrogenic or accidental, accounts for 7% to 30% of cases, with lower numbers in more recent studies, and contiguous spread of infection (e.g., from an adjacent intraabdominal process) continues to account for a small percentage of cases (2%–7%).[14] Other conditions associated with splenic abscess include splenic abnormalities such as Felty syndrome or amyloidosis, intravenous drug use, hemoglobinopathy, and diabetes mellitus.

Complications of splenic abscess can be life threatening and include perforation into the peritoneum, which occurred in 19 (6.6%) of 287 patients in one series.[14] Rupture into adjacent organs can occur, with resulting fistulas into the gastrointestinal tract, pleural space, or lung parenchyma. Overall mortality rates of 0% to 14% have been reported with appropriate therapy (see "Therapy"), although higher rates occur among immunocompromised patients, and mortality is strongly associated with signs of sepsis using several sepsis scoring systems.[16]

MICROBIOLOGY

Streptococci, staphylococci, salmonellae,[17] and *Escherichia coli* have been the major causative agents of splenic abscess for the past century (Table 77.1). However, with the increased number of immunocompromised patients, more recent series have shown greater numbers of fungal isolates including *Candida* spp., *Aspergillus* spp., and agents of mucormycosis. Mycobacteria have also become more common. Anaerobic bacteria remain a relatively infrequent cause of splenic abscess compared with other intraabdominal abscesses, despite improvements in culture techniques.

In HIV-infected patients, *Salmonella* spp. and *Mycobacterium tuberculosis* are common causes of splenic abscess, as are the opportunistic pathogens *Mycobacterium avium-intracellulare* complex, *Leishmania* spp., *Rhodococcus equi,* and *Pneumocystis jirovecii.* Sickle cell anemia has classically been associated with *Salmonella* infections of the spleen, but more recent series noted a predominance of staphylococcal infection associated with this condition.[8] Many other organisms have been described as causative agents of splenic abscess in case reports including *Bartonella henselae,*[18–20] *Streptobacillus moniliformis, Campylobacter*

TABLE 77.1 Organisms Cultured From Splenic Abscesses: Comparison of Five Time Periods

ORGANISM	% OF POSITIVE CULTURES				
	1900–1977 (*N* = 129)[a]	1977–1986 (*N* = 159)[b]	1987–1995 (*N* = 225)[c]	2000–2011 (*N* = 28)[d]	2012–2016 (*N* = 16)[e]
Aerobic bacteria					
Streptococcus spp.	21.7	6.9	10.2	7	25.0
Staphylococcus spp.	20.2	15.7	17.3	7	6.25
Salmonella spp.	10.9	10.7	16.1	7	6.25
Pseudomonas	0	1.3	6.3	3.5	
Escherichia coli	24.1[f]	10.7	12.5		6.25
Enterococcus spp.		6.3	3.9	7	6.25
Klebsiella		1.9	1.9	7	6.25
Proteus		1.3	3.1		
Other aerobes		8.2	7.1	10.7	
Anaerobic bacteria	5.4	17.6	7.1	7	
Mycobacteria					
Mycobacterium tuberculosis	0.8	0	5.5	3.5	6.25
Mycobacterium avium-intracellulare complex	0	0	1.9	3.5	6.25
Fungi	0.8	25.8	7.1	10.7	6.25
Sterile cultures	28.7	11.9	11.4	32.1	31.25
Bartonella henselae				3.5	

[a]Modified from Chun CH, Raff MJ, Contreras L, et al. Splenic abscess. *Medicine (Baltimore)*. 1980;59:50–65.
[b]Modified from Nelken N, Ignatius J, Skinner M, et al. Changing clinical spectrum of splenic abscess: a multicenter study and review of the literature. *Am J Surg*. 1987;154:27–34.
[c]Modified from Ooi LL, Leong SS. Splenic abscesses from 1987 to 1995. *Am J Surg*. 1997;174:87–93.
[d]Modified from Liu YH, Liu CP, Lee CM. Splenic abscesses at a tertiary medical center in Northern Taiwan. *J Microbiol Immunol Infect*. 2014;47:104–108.
[e]Modified from Lee MC, Lee CM. Splenic abscess: an uncommon entity with potentially life-threatening evolution. *Can J Infect Dis Med Microbiol*. 2018;2018:8610657.
[f]Total percentage for *E. coli, Enterococcus* spp., *Klebsiella, Proteus,* and other aerobes.

jejuni,[21] and *Nocardia* spp.[4] In a series from Thailand, the agent of melioidosis, *Burkholderia pseudomallei,* was the cause of splenic abscess in 24 of 41 cases from which a pathogen was isolated.[22] In a more recent series from Singapore, there was serologic evidence for melioidosis in 15 of 21 patients over a 10-year period, although 7 of these patients had negative blood cultures.[23] Of interest, nearly all the patients with melioidosis involving the spleen had diabetes mellitus (see Fig. 221.14 in Chapter 221). *Klebsiella pneumoniae* has become an increasingly common cause of community-acquired infection including splenic abscess in Taiwan and elsewhere in Asia.[10,20,24,25] In one series, *K. pneumoniae* was the most common cause of splenic abscess in a hospital in southern Taiwan.[24] Overall, blood cultures were positive in 24% to 60% of cases.[9,26] Splenic abscesses caused by enteric bacteria have been reported in patients with *Plasmodium vivax* infection, whose splenomegaly was otherwise attributed to malaria.[27,28] Occasionally aseptic abscesses have been described in the spleen, particularly in patients with inflammatory bowel disease.[29] Patients with these apparently noninfectious inflammatory lesions of unknown etiology present with fever and respond to treatment with corticosteroids but not to antibiotics. These aseptic abscesses are often accompanied by aseptic abscesses in other locations, but the spleen is the most frequent site.

CLINICAL MANIFESTATIONS

Fever may be the only manifestation of splenic abscess, and fever is present in 95% of cases. Another frequent finding is abdominal pain, which is either generalized or localized to the left upper quadrant and may radiate to the left chest or shoulder. Nausea, vomiting, anorexia, and weakness are often present. Abdominal tenderness is present in only half of the cases, most often in the left upper quadrant. Splenomegaly can be detected in a similar number of cases. Chest findings including dullness, rales, or both at the left base have been detected in many patients. Other findings that are less frequently present include splenic friction rubs, hepatomegaly, tenderness at the costovertebral angle, and ascites. The only laboratory abnormality that is frequently present is leukocytosis, which is seen in 60% to 80% of cases.

DIAGNOSIS

Because the symptoms and findings of splenic abscess are most frequently nonspecific, diagnosis depends on appropriate imaging studies. Plain radiographs are surprisingly sensitive, with abnormalities detected in 50% to 80% of chest radiographs and 25% of abdominal radiographs (e.g., basilar infiltrates, pleural effusion, elevated hemidiaphragm, shift of viscera, presence of gas)[26]; however, the findings are most often nonspecific. The use of radionuclide scans as described in earlier literature, primarily technetium 99m sulfur-colloid liver-spleen scans, has been largely supplanted by ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI).

Ultrasonography

The advantages of ultrasonography in the evaluation of splenic abscess include low cost, portability, and relatively high sensitivity, reportedly ranging from 75% to 93%.[8,14,26,30] It is therefore appropriate in the initial assessment of most suspected splenic abscesses. Ultrasonography typically demonstrates an area of decreased or absent echogenicity, sometimes with irregular areas of echodensity (debris) or a gas pattern within the lesion.[31] Splenomegaly can frequently be demonstrated. High-resolution (7.5 MHz) ultrasonography can detect microabscesses in patients infected with HIV that are missed with conventional ultrasonography.[1]

Computed Tomography

CT appears to be the most sensitive modality for the detection of splenic abscess, particularly if enhancement by intravenous contrast medium is used.[30] A sensitivity of greater than 90% has been seen in most series.[14] The abscess is seen as an area of low-density fluid or necrotic tissue within the relatively homogeneous spleen. Enhancement of the rim of the abscess cavity is seen in a few cases, so that splenic infarctions are difficult to distinguish from splenic abscesses on contrast-enhanced CT. Splenic infarction adjacent to intraabdominal abscesses elsewhere in the abdomen has been reported.[32]

Magnetic Resonance Imaging

There is far less experience with MRI for the detection of splenic abscesses, and its role in their management is not yet established. MRI is sensitive for the detection of other abdominal abscesses, and its sensitivity in the discovery of splenic abscesses is probably similar. Nonetheless, although MRI has been successfully coupled with drainage procedures, it appears to be more cumbersome and to offer fewer advantages than ultrasonography or CT.[33]

In a setting in which drainage may not be necessary, for example, in immunocompromised patients in whom pathogens other than pyogenic bacteria are more common, or if a noninfectious diagnosis such as malignancy or cyst is likely, fine-needle aspiration under the guidance of diagnostic imaging may be useful.[34,35] The sensitivity of this method is variable, and its role remains the subject of investigation.

THERAPY

Untreated splenic abscess has a high mortality rate.[8] Splenectomy has been the traditional modality for treatment and remains the gold standard against which other therapies must be assessed. Antibiotics play an important role in the treatment of associated endocarditis and sepsis, in stabilization of the patient for splenectomy or a drainage procedure, and in treatment of selected pathogens (e.g., mycobacteria, fungi), but they are rarely curative alone for splenic abscess caused by pyogenic bacteria. Broad-spectrum empirical antibiotic therapy should be initiated as soon as splenic abscess is suspected, pending surgical or percutaneous drainage. Antibiotics should include agents active against streptococci, staphylococci, and aerobic gram-negative rods. Vancomycin or oxacillin plus an aminoglycoside, a third-generation or fourth-generation cephalosporin, a fluoroquinolone, or a carbapenem would be reasonable empirical therapy. After blood or abscess culture results are obtained, antibiotic coverage can be narrowed accordingly. If splenectomy is a possibility, it is advisable to administer vaccinations for encapsulated bacterial pathogens as early as possible.

Experience with CT-guided and ultrasonography-guided percutaneous aspiration of splenic abscesses has grown in recent decades. These procedures have the advantage of lower initial morbidity and mortality than splenectomy and allow for preservation of the spleen. Success rates have ranged from 50% to 90% in several series.[8,14,36–42] In general, smaller (<3 to 4 cm), solitary, or unilocular abscesses have a higher rate of successful percutaneous drainage. In contrast, microabscesses; complex, phlegmonous, or multilocular processes; and abscesses with thick fluid tend to fare more poorly with this approach. Percutaneous drainage may be useful, at least initially, in patients who are unstable or who present an unacceptably high surgical risk. Failure to achieve effective drainage or failure of the patient's condition to improve is an indication for definitive surgery. Aspirated abscess fluid should be sent for Gram stain; bacterial, fungal, and mycobacterial culture; and other studies as clinically indicated.

The optimal duration of antibiotic therapy for splenic abscess has not been established in any clinical trial. For some patients, such as patients with bacterial endocarditis, the duration is dictated by the underlying condition. If splenectomy is performed and the focus of infection is eradicated, briefer durations may be possible. With percutaneous drainage, duration of therapy needs to be tailored to the clinical course, including resolution of the abscess as assessed by diagnostic imaging.

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The complete reference list is available online at Expert Consult.

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78 Appendicitis

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SHORT VIEW SUMMARY

Definition
- Appendicitis is acute inflammation of the vermiform appendix that is often related to obstruction and may be complicated by polymicrobial infection.
- Complications include perforation, peritonitis, and intraabdominal abscesses.

Epidemiology
- Acute appendicitis is a relatively common disease, often presenting in adolescence and early adulthood.
- Lifetime risk for appendicitis is 8.6% in men and 6.7% in women.
- More than 300,000 appendectomies are performed annually in the United States.

Biology
- The microbial community of the appendix resides in a mucus-rich biofilm that is continuously shed into the intestinal tract and may serve to repopulate the gut after acute diarrhea illness.

Microbiology
- The inflammation is usually polymicrobial, frequently involving aerobic and anaerobic gram-negative bacilli.
- The human appendiceal microbiome is a highly diverse community of organisms, with notable differences in taxonomic composition compared with other locations in the lower intestinal tract.
- Genomic analysis has shown that several taxa, including Fusobacteria, are enriched in the inflamed appendix.
- Infections by *Yersinia* spp., parasites, including *Entamoeba histolytica,* and viruses may mimic appendicitis (e.g., mesenteric adenitis) or cause obstruction leading to acute appendicitis.

Diagnosis
- Clinical diagnosis is enhanced by the use of imaging, especially computed tomography and ultrasonography.
- Diagnosis is more difficult in women of childbearing age in whom gynecologic processes may mimic appendicitis.

Therapy
- Surgical removal of the appendix, often performed laparoscopically, is curative.
- Adjunctive use of broad-spectrum antibiotics, such as piperacillin-tazobactam, ceftriaxone, and metronidazole, may be required.
- An "antibiotic first" strategy for acute uncomplicated appendicitis may be considered for carefully selected patients who have a strong preference for nonoperative treatment or who are poor surgical candidates.

In 1886 Fitz first described the natural course of acute inflammation of the appendix. In his seminal report he coined the term *appendicitis* and advocated early surgical intervention.[1] Today appendectomy is among the most common surgical emergencies of the abdomen; more than 330,000 nonincidental appendectomies are performed annually in the United States, accounting for 1 million inpatient hospital days.[2,3] With improvements in clinical assessment, antibiotic therapy, and surgical management, the overall mortality rate of acute appendicitis is less than 1%, although rates increase to 5% or more in the elderly.[3–5]

EPIDEMIOLOGY

The lifetime risk for appendicitis is 8.6% in men and 6.7% in women.[3] Although appendicitis is rare in infants, its incidence steadily increases through childhood and reaches a peak at 15 to 25 years of age for men and women.[3,5,6] The incidence of appendicitis declines through adulthood, and less than 25% of cases occur in individuals older than 45 years.[3] Men have slightly higher rates of appendicitis than women; the overall male-to-female rate ratio is 1.4 : 1.[3]

PATHOGENESIS

The appendix is a tube-shaped structure, usually 5 to 10 cm long in adults, which arises 2 to 3 cm below the terminal part of the ileum along the medial posterior wall of the cecum.[7] Once thought to be simply a vestigial remnant of evolution, recent studies suggest that the appendix may play an important role in the development and homeostasis of the intestinal microbiome and mucosal immune system.[8] Commensal bacteria reside in the appendix within a mucus-rich biofilm that is intermittently shed into the intestinal tract at a rate of approximately 3 mL per day. Given this, the appendix has been hypothesized to act as a microbial reservoir that can act to repopulate the intestinal tract when needed, for example, during recovery from acute diarrheal illnesses.[9,10] Microbe-host interactions between the resident microbiota and gut-associated lymphoid tissue of the appendix are also hypothesized to aid in the maturation of normal mucosal immune responses.[8,11]

Typically, the appendix lies in an ascending retrocecal position, but atypical positions, such as descending pelvic, transverse retrocecal, and ascending postileal, are common and can alter the typical clinical features of acute appendicitis.[7] Physical obstruction of the appendiceal lumen by fecaliths or other causes (e.g., foreign bodies, tumor, stricture, or parasites) has classically been thought to be the primary pathogenic mechanism of acute appendicitis.[12–16] Mucus accumulates within the obstructed appendiceal lumen, and intraluminal pressure increases, leading to compression of lymphatic and vascular drainage and causing ischemic damage of the mucosa, followed by microbial invasion and inflammation. Continued inflammation and ischemia, if left untreated, may lead to gangrene and eventual perforation. The pathologic hallmark of acute appendicitis is the presence of polymorphonuclear cells within the appendiceal wall, accompanied by other markers of inflammation, such as edema and vascular congestion.

In recent years, however, this classic model of pathogenesis of acute appendicitis has been called into question. In most modern case series fecaliths or other causes of obstruction are found in only a minority of cases.[17,18] The efficacy of medical rather than surgical treatment of acute appendicitis (see later) also suggests that physical obstruction may not be the primary pathogenic mechanism. This has led to the speculation that other inciting etiologies or cofactors, such as primary infectious agents, dietary fiber, genetic susceptibility, or hypersensitivity, lead to lymphoid hyperplasia and inflammation of the appendix.[18–21]

MICROBIOLOGY

Conventional cultures of inflamed or gangrenous appendices typically yield 10 to 14 different organisms, which generally reflect the colonic microbiota. A mixture of colonic anaerobic and facultative bacteria is usually recovered, predominantly *Escherichia coli, Bacteroides fragilis*

group, pigmented *Prevotella* spp., *Bilophila wadsworthia, Peptostreptococcus* spp., Enterobacteriaceae, and viridans streptococci, particularly the *Streptococcus anginosus* group.[22–24] *Pseudomonas aeruginosa* has also been found in a minority of cases (4%–15%) but is less frequently sensitive to routinely used antibiotics.[25,26]

Recently, culture-independent 16S ribosomal RNA gene sequencing-based studies have demonstrated that the microbial community of the appendix is remarkably diverse, consisting of more than a dozen phyla and hundreds of different species of bacteria.[27] Furthermore, these studies have shown that the appendiceal microbiome is distinct, with both quantitative and qualitative differences in composition compared with elsewhere in the digestive tract.[28] Although the diversity of bacteria in the healthy appendix is large, a relatively small number of taxa predominate, including those of the phyla Firmicutes, Proteobacteria, and Bacteroidetes.[27,28] A few taxa, including *Fusobacterium*, *Peptostreptococcus*, and *Parvimonas*—all typically considered to be oral commensal bacteria and occasional periodontal pathogens—are enriched in the inflamed appendix.[29,30] This has led some investigators to hypothesize that rather than being caused by physical obstruction of the appendiceal lumen, acute appendicitis may be the consequence of dysbiosis (microbial imbalance) of the appendix.[27,29]

In some instances intestinal pathogens have been associated with acute appendicitis. For example, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are believed to have a causative role in some cases of acute appendicitis.[31–33] More common, however, nonplague *Yersinia* causes ileocecitis or mesenteric adenitis, which mimics acute appendicitis with fever, leukocytosis, and acute right lower quadrant pain.[34–36] Similarly, *Campylobacter* and nontyphoidal *Salmonella* can also cause ileocecitis and mesenteric adenitis.[35,37] Appendiceal or ileocecal tuberculosis, actinomycosis, and histoplasmosis are more likely to cause subacute or recurrent disease rather than classic acute appendicitis. Viral causes of mesenteric adenitis and, rarely, appendicitis include measles, Epstein-Barr virus, cytomegalovirus, and adenovirus.[38–41] Amebiasis may also cause appendicitis.[42] In many cases of mesenteric adenitis, an infectious cause is not identified but is probably present.[43] The disorder is usually discovered at the time of surgery for suspected appendicitis; mesenteric lymph nodes of the right iliac fossa are enlarged, the adjacent bowel is only mildly inflamed, and the appendix appears normal. Nevertheless, an appendectomy should be performed to avoid the need to differentiate a recurrent attack from true appendicitis in the future.

CLINICAL MANIFESTATIONS

The clinical manifestations of acute appendicitis are distinctive and, in many cases, diagnostic. Appendicitis classically starts as colicky, visceral periumbilical pain that evolves for the next 6 to 24 hours to localized, somatic right lower quadrant abdominal pain after inflammation extends to the parietal peritoneum. If the inflamed appendix lies in the anterior position, tenderness is often maximal at or near the McBurney point, which lies two to three fingerbreadths above the right anterior superior iliac spine on a line with the umbilicus. If the appendix lies in a position relatively hidden from the parietal peritoneum, pain may remain poorly localized, and migration to the right lower quadrant may be delayed or absent.[44] Pelvic appendices can cause pelvic or left lower quadrant pain. Third-trimester pregnancy or intestinal malrotation may shift pain to the right upper quadrant. Pain is often accompanied by mild fever, anorexia, nausea, and vomiting.

Guarding is usually seen on examination of the abdomen. Rebound tenderness in the right lower quadrant with palpation of the left lower quadrant, known as the Rovsing sign, may be elicited. Other maneuvers that support the diagnosis of appendicitis include pain with active extension of the right hip, termed the *psoas sign,* and pain with internal rotation of the right hip, termed the *obturator sign.* High fever or a sudden reduction in pain suggests perforation, whereas abdominal rigidity suggests diffuse peritonitis. A palpable right lower quadrant mass may indicate a phlegmon or walled-off periappendiceal abscess[4,45] or, alternatively, a cecal carcinoma.[46] Most instances of perforation occur as the result of delays in seeking medical care.[47]

Acute appendicitis in the elderly can present as subtle and muted clinical signs.[48,49] Pain may be generalized and of longer duration. This may result in delay of surgery and overall poorer outcome.

DIAGNOSIS

The diagnosis of acute appendicitis primarily is suggested by the history and physical examination findings. Acute onset of abdominal pain migrating over several hours to the right lower quadrant, with guarding and tenderness over the McBurney point, is most predictive of acute appendicitis; a history of previous pain, long duration of symptoms, and lack of migration to the right lower quadrant argue against it.[4,50] The serum concentration of β-human chorionic gonadotropin should be measured in all women of reproductive age to rule out a uterine or ectopic pregnancy. Most patients have a mild leukocytosis and elevated C-reactive protein value. If inflammation extends to the ureter or bladder, sterile pyuria may be noted on urinalysis. Elevation in procalcitonin levels has not been found to add diagnostic value to leukocyte counts or to the C-reactive protein level but may suggest complicated appendicitis.[51] Acute salpingitis with a tubo-ovarian abscess or impaction of a stone in the right ureter can be confused with acute appendicitis.

The appendix is found to be normal in 8% to 25% of patients who undergo emergency appendectomy.[3,5,52–54] This "negative appendectomy rate" is significantly higher in women of childbearing age, young children, and elderly individuals.[53,55,56] Conversely, 20% of all cases of appendicitis are initially missed on presentation.[57–59] In an effort to improve diagnostic accuracy, scoring systems based on clinical findings and laboratory data have been developed to assist in the diagnosis of acute appendicitis.[60] In practice, however, these systems lack sufficient power to discriminate reproducibly acute appendicitis from other causes of pain in the right lower quadrant (Table 78.1).[61]

Observation, laparoscopy, and imaging are additional methods used to improve the diagnostic accuracy of acute appendicitis. If the clinical presentation is ambiguous, observation and reassessment can distinguish cases of evolving appendicitis from other causes of abdominal pain without increasing the risk for perforation.[62] Diagnostic laparoscopy is particularly effective in women of childbearing age, in whom gynecologic causes of abdominal pain are common.[63,64] Diagnostic imaging, by ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI), has been shown to be very effective in diagnosing acute appendicitis. Ultrasonography is rapid and noninvasive, requires no contrast material, uses no ionizing radiation, and is excellent at visualizing gynecologic abnormalities. As such, it has proved to be particularly useful in the evaluation of young women, pregnant women, and children with suspected appendicitis.[65–68] Ultrasonography fails to visualize the appendix in 10% of cases[69] and is not as accurate as CT in diagnosing an abscess or phlegmon.[67]

TABLE 78.1 Causes of Right Lower Quadrant Pain

- Gastrointestinal
  - Appendicitis
  - Crohn ileitis/cecitis
  - Cecal diverticulitis
  - Meckel diverticulitis
  - Mesenteric lymphadenitis
  - Bacterial *(Yersinia, Campylobacter, Salmonella)* ileocolitis
  - Amebic colitis
  - Tuberculous colitis
  - Ileocecal actinomycosis
  - Biliary colic
  - Epiploic appendagitis
  - Typhlitis (neutropenic cecitis)
- Urinary tract
  - Pyelonephritis
  - Renal colic
- Neoplastic
  - Cecal adenocarcinoma
  - Carcinoid
  - Lymphoma
  - Metastasis
- Gynecologic
  - Ovarian cyst
  - Ectopic pregnancy
  - Endometriosis
  - Cervicitis
  - Uterine leiomyoma
  - Tubo-ovarian abscess
  - Pelvic actinomycosis
- Nonspecific abdominal pain

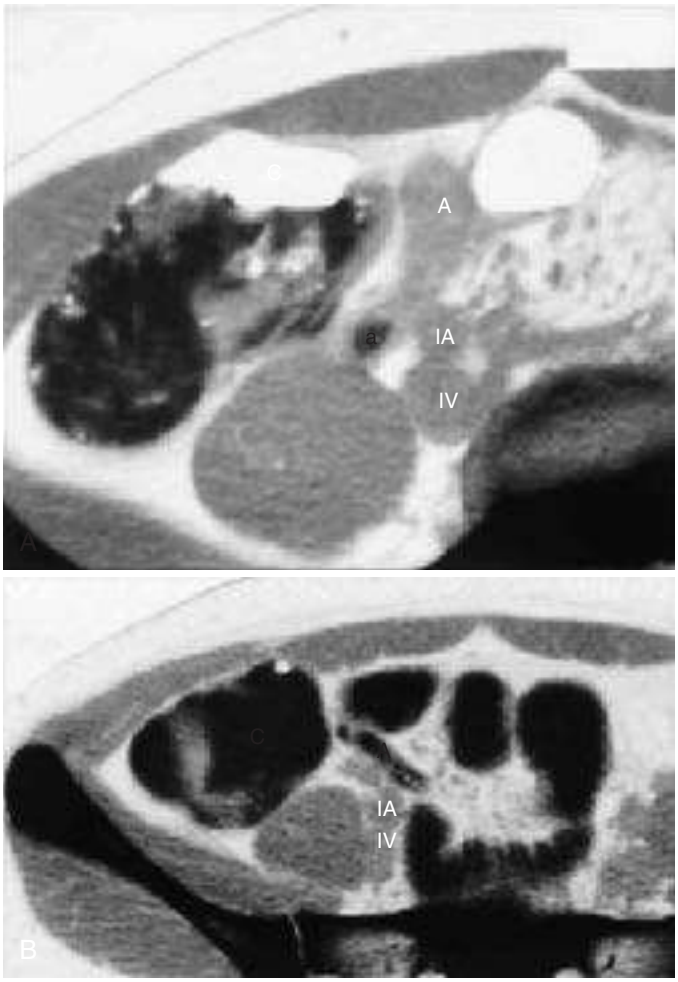

FIG. 78.1 Appendicitis. (A) Computed tomography (CT) scan of a 17-year-old boy with appendicitis. Axial image shows an inflamed, unopacified appendix *(A),* 15 mm in diameter, with proximal appendicolith *(a).* Also shown, and also in part B, are the cecum *(C),* common iliac artery *(IA),* and common iliac vein *(IV).* (B) CT scan of a 21-year-old woman with appendicitis. Axial image shows a tubular, opacified normal appendix *(A). (From Rao PM, Rhea JT, Novelline RA, et al. Effect of computed tomography of the appendix on treatment of patients and use of hospital resources.* N Engl J Med. *1998;338:141–146. Copyright © 1998 Massachusetts Medical Society. All rights reserved.)*

CT, particularly focused helical appendiceal CT, has a reported sensitivity of 94% to 99% and a specificity of 95% to 98% in patients with suspected appendicitis (Fig. 78.1).[70–72] CT can avoid delays before necessary appendectomy, diagnose other pathologic conditions such as right-sided diverticulitis, reduce negative appendectomy rates, and prevent unnecessary hospital admissions.[70,72] The addition of enteral contrast to intravenous contrast does not improve the sensitivity of CT for the diagnosis of acute appendicitis.[73] Low-dose CT, which reduces exposure to ionizing radiation, has been found to be noninferior to standard-dose CT.[74] Use of CT to improve the diagnostic accuracy of suspected appendicitis has been less consistent, however, in clinical practice.[75,76]

MRI can also aid in the diagnosis of appendicitis. Using rapid unenhanced imaging protocols, MRI has a diagnostic accuracy for acute appendicitis that is better than ultrasound and comparable to CT (sensitivity, 96%–97%; specificity 95%–99%).[77–79] Like ultrasound, MRI offers the advantages of avoiding exposure to ionizing radiation and radiographic contrast media and therefore may be particularly attractive in certain populations, such as pregnant women and persons with contrast allergies.[80,81]

Many experts recommended a graded approach to the evaluation of possible acute appendicitis, where factors such as history, examination, patient characteristics (e.g., age, pregnancy status), and clinical risk scores are used to determine diagnostic and management strategies.[45,82,83] An example of one algorithm is presented in Fig. 78.2.[82]

THERAPY

The primary treatment of appendicitis is surgery. Preoperative treatment includes fluid resuscitation and parenteral use of antibiotics. In cases of uncomplicated acute appendicitis, routine perioperative antibiotic prophylaxis for gastrointestinal surgery usually suffices (see Chapter 313).[84–86] In cases of appendicitis with rupture, gangrene, abscesses, or secondary peritonitis, an antibiotic regimen that provides broad coverage for facultative and anaerobic colonic flora should be used: piperacillin-tazobactam, ceftriaxone plus metronidazole, imipenem-cilastatin (see Chapter 74).[85,86] Acute appendicitis, either without rupture or with perforation and secondary peritonitis, requires prompt surgical intervention. There is no clear consensus regarding the optimal operative approach (open vs. laparoscopic) in patients with suspected appendicitis. Laparoscopic appendectomy offers the advantage of allowing for further diagnostic evaluation if a normal appendix is found.[64] In addition, a Cochrane Database systematic review of more than 50 studies suggested that patients have fewer wound infections, less postoperative pain, shorter hospital stays, and quicker returns to normal activity after laparoscopic appendectomy than after open appendectomy.[87] A population-based study from Taiwan of more than 65,000 patients showed a lower readmission rate and shorter hospital stay for laparoscopic versus open appendectomy.[88] Laparoscopic appendectomies are associated, however, with more frequent intraabdominal abscesses, longer operative times, and higher operative and total hospital costs.[87,88] In many cases surgery for acute, uncomplicated appendicitis can be safely deferred for a short period of time (overnight).[87]

There is even less consensus on the optimal postoperative treatment of perforated appendicitis, including the duration of antibiotic therapy, use of parenteral versus oral agents, and duration of hospitalization.[89,90] Patients generally are treated with intravenously administered antibiotics for 4 to 7 days until fever resolves, the white blood cell count normalizes, and bowel function returns.[85,86] However, recent data from an observational study comparing 3 days versus 5 days of antibiotics after appendectomy for complicated appendicitis suggests that 3 days may be sufficient.[91] Institutional clinical practice pathways have been shown to reduce infectious complications and the cost of care.[92,93]

In cases of appendicitis complicated by a contained perforation or phlegmon, antibiotic therapy and percutaneous drainage of the periappendiceal abscess, if present, can be performed. If these maneuvers produce a favorable response, interval appendectomy can be performed 6 to 8 weeks later after inflammation subsides.[86,94,95] Historically, untreated appendicitis was a major cause of portal vein thrombophlebitis (pylephlebitis) and pyogenic liver abscess (see Chapter 75), but these complications are rare in the antibiotic era.[96]

Recently, the need for an appendectomy in all cases of uncomplicated appendicitis has been called into question.[97,98] In fact, "antibiotic first" therapy has recently been advanced as an alternative approach for the management of clinically suspected appendicitis in young men with equivocal imaging, as long as they are observed in the hospital for at least 48 hours and show clinical improvement within 24 hours.[85] It has been known for decades that some individuals with a clinical diagnosis of appendicitis and lacking access to immediate surgical care, such as personnel on submarines, may fully recover spontaneously or with antibiotic treatment alone.[99] More recent studies have confirmed that radiographically proven appendicitis can resolve with conservative therapy.[100,101] If routine, a predominantly nonsurgical approach to the treatment of appendicitis would avoid complications of surgery and lead to substantial health care cost savings. Several more recent studies suggest that medical treatment of appendicitis can be successful, at least in certain patient populations.[101–108] Recurrent appendicitis has been reported to occur in 5% to 38% of all patients, however, of which 4% to 100% progress to perforation or secondary peritonitis.[101–105] Other prospective studies have shown antibiotic therapy alone to be inferior to surgery.[109,110] Systematic reviews and meta-analyses have come to conflicting conclusions, and editorials have weighed in on both sides.[111–117] The most appropriate study design by which to compare antibiotics to

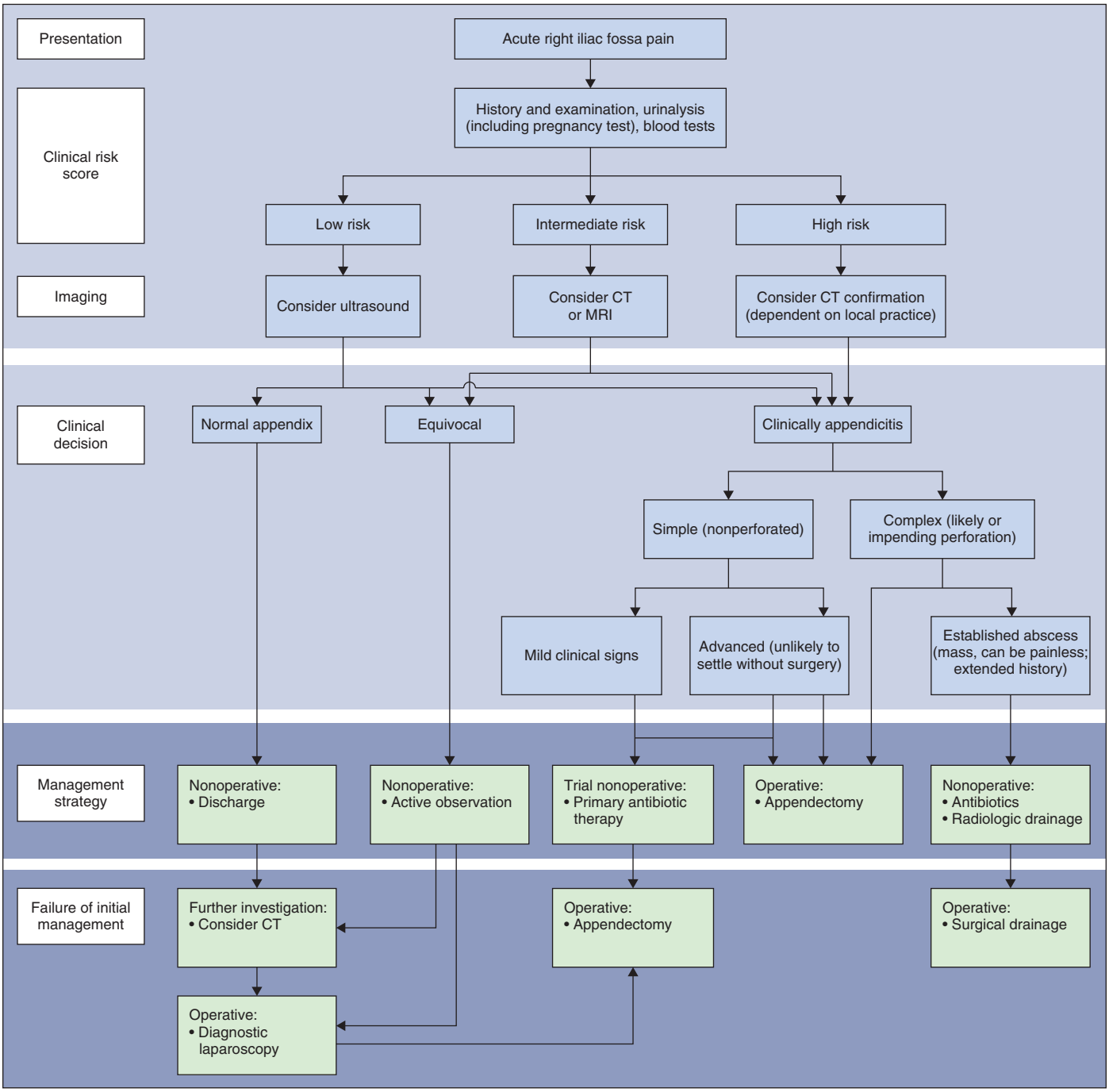

FIG. 78.2 Flowchart of guidance for a stratified approach to preoperative management of suspected appendicitis. *CT,* Computed tomography; *MRI,* magnetic resonance imaging. *(From Bhangu A, Søreide K, Di Saverio S, et al. Acute appendicitis: modern understanding of pathogenesis, diagnosis, and management.* Lancet. *2015;386:1278–1287.)*

surgery is also a matter of debate, and a recent editorial[118] and letter[119] have emphasized that future studies should use well-defined clinical criteria to judge risks and benefits. Conservative therapy may delay the diagnosis of other pathologic processes, such as appendiceal tumors. Unless large prospective randomized trials with appropriate follow-up demonstrate equivalent efficacy of a nonoperative approach to the treatment of acute appendicitis, surgery will likely remain the standard approach except in carefully selected patients.[85]

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The complete reference list is available online at Expert Consult.

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79 Diverticulitis and Neutropenic Enterocolitis

Costi D. Sifri and Lawrence C. Madoff

SHORT VIEW SUMMARY

Definition
- Diverticulitis is inflammation and infection of the bowel wall, leading to microperforation of colonic diverticula.
- Neutropenic enterocolitis (typhlitis) is characterized by abdominal pain and fever during periods of neutropenia resulting from mucosal inflammation, degeneration, and microbial invasion.

Epidemiology
- Diverticulitis occurs in 10% to 25% of all individuals with diverticulosis, which has a prevalence of 30% to 40% in Western society.
- Diverticulitis leads to 150,000 hospital admissions and 24,000 elective operations annually in the United States.
- The incidence of neutropenic enterocolitis is unknown but has been estimated to be 5% in hospitalized neutropenic adults with cancer.
- Bacteremia or fungemia occurs in 14% to 44% of patients with neutropenic enterocolitis.

Microbiology
- Acute diverticulitis is a polymicrobial infection caused by endogenous anaerobic and facultative bacteria, including *Bacteroides* spp., *Peptostreptococcus* spp., Enterobacteriaceae, viridans-group streptococci, and enterococci.
- *Pseudomonas aeruginosa,* Enterobacteriaceae, *Bacteroides fragilis,* viridans-group streptococci, enterococci, and *Candida* spp. are common causes of bloodstream infections in patients with neutropenic enterocolitis.

Diagnosis
- Radiographic imaging (computed tomography and ultrasonography) is essential for the evaluation of suspected diverticulitis and neutropenic enterocolitis.
- More than half of patients with clinically suspected diverticulitis are diagnosed with an alternative condition after diagnostic imaging.
- Radiographic findings of diverticulitis include pericolic fat stranding, diverticula, bowel wall thickening, and phlegmon or abscess formation.
- Neutropenic enterocolitis can be defined as bowel wall thickening, usually of the cecum, greater than 4 mm, with neutropenia, fever, and abdominal pain.

Therapy
- Acute uncomplicated diverticulitis can be safely treated in select outpatients with a short course of oral antibiotics. However, the necessity of antibiotic therapy for the treatment of uncomplicated diverticulitis is controversial.
- Complicated diverticulitis or patients with comorbidities or other predictors of worse outcomes should be hospitalized.
- Percutaneous drainage of large abscesses (>5 cm in diameter) can be temporizing in acutely ill patients.
- Management of neutropenic enterocolitis includes bowel rest, intravenous fluids, nutritional support, and broad-spectrum parenteral antibiotics that cover enteric facultative and anaerobic flora, *P. aeruginosa,* and perhaps yeast.
- Surgery may be required for patients with diverticulitis or neutropenic enterocolitis with intraperitoneal perforation, uncontrolled sepsis, persistent gastrointestinal bleeding, or obstruction, or for those who fail to respond to medical therapy.

Prevention
- High dietary fiber and physical activity may be associated with reduced symptomatic diverticular disease.
- Prolonged nonsteroidal antiinflammatory drug use appears to be a risk factor for diverticular disease.

DIVERTICULITIS

Most colonic diverticula are pseudodiverticula that occur when the mucosa and submucosa herniate through the muscularis propria. These saccular herniations develop in areas of relative structural weakness of the colonic wall, where small nutrient arteries supplying the colonic mucosa, the *vasa recta,* penetrate the circular muscle layer. In the West, the sigmoid and descending colons are most commonly affected. In Asia, colonic diverticula are predominantly right sided, involve all layers of the colonic wall, and likely represent a distinct clinical condition. *Diverticulitis,* defined as inflammation and infection of the bowel wall associated with diverticula, is the most frequent complication of this disorder.

Epidemiology

Only 4% to 25% of patients with diverticulosis manifest clinical disease, making its true prevalence difficult to measure.[1,2] Although the disorder occurs worldwide, diverticulosis is particularly prominent in Western society, where the prevalence has been estimated to be 30% to 40%.[3,4] In the United States alone, there were over 150,000 hospital admissions for acute diverticulitis and an estimated 24,000 elective operations for diverticulitis in 2005, based on hospital discharge records.[5] By contrast, the prevalence of diverticulosis in rural Asia and Africa is estimated to be much less than 1%.[6] Increased prevalence is observed in populations that become westernized, presumably because of dietary changes or other environmental factors.[7–9] The prevalence of diverticulosis increases with age in the West, affecting 50% of those older than 70 years of age and up to two-thirds of individuals by 85 years of age.[1,6,10] The complications of diverticulosis, including diverticulitis, also increase with age,[10] although some studies suggest that diverticulitis may recur more often or be more malignant in patients who are younger than 40 years of age.[2,11–13] Diverticulitis appears to be increasing in the United States, but it is not clear if this reflects demographic shifts, changes in diet, improved recognition resulting from increased use of computed tomography (CT), or other factors.[5]

The most extensively studied risk factor for the development of diverticular disease is low dietary fiber intake. Diets with reduced dietary fiber are associated with more colonic diverticulosis[6] and with symptomatic diverticular disease.[14,15] Other risk factors for the development of diverticulitis are less well understood. Use of corticosteroids, nonsteroidal antiinflammatory drugs, or opiates may be positively associated with diverticulitis, perforation, or both.[16–18] Lack of physical activity and obesity also appear to be independent risk factors for symptomatic disease.[12,19,20] By contrast, vigorous physical activity independent of body mass may reduce symptomatic diverticular disease.[21] Contrary to popular belief, popcorn, corn, and nuts do not appear to increase the incidence of diverticular disease. In fact, nuts and popcorn consumption were

shown to be protective in one study.[22] Tobacco, alcohol, and caffeine consumption also do not appear to increase diverticular disease.[23]

Pathogenesis

The formation of diverticula is thought to be caused by a combination of increased intraluminal pressure and weakening of the bowel wall.[24] High dietary fiber intake increases stool volume and reduces bowel transit time, leading to decreased intracolonic pressures. For over a century, the pathogenesis of diverticulitis has been thought to be very similar to that of acute appendicitis: a fecalith obstructs the neck of the diverticulum,[25] causing mucus accumulation, bacterial overgrowth, perforation, and inflammation of the bowel wall and adjacent tissues. Recent speculation has turned to whether changes in the intestinal microbiota—a consequence of a low-dietary-fiber Western diet—alters mucosal immune homeostasis, leading to low-grade chronic inflammation, particularly in chronic diverticular disease.[26,27,28,29] Microperforation may remain well localized, leading to limited colonic wall inflammation and the formation of a small peridiverticular phlegmon or abscess. Macroperforation results in larger abscesses; if not confined, its complications include distant abscesses, extension to other organs, spreading peritonitis, large inflammatory masses, and fistula formation.[10,24] Less commonly, gross fecal contamination can occur with free rupture of an unobstructed and uninflamed diverticulum into the peritoneal cavity. Rupture of a localized peridiverticular abscess into the peritoneal cavity does not result in gross fecal contamination, presumably because the diverticular neck is obstructed by a fecalith or inflammation.[24]

Microbiology

Like most other cases of secondary peritonitis, acute diverticulitis is a polymicrobial infection caused by a variety of endogenous anaerobic and facultative bacteria. Commonly isolated organisms include *Bacteroides* spp., *Peptostreptococcus* spp., Enterobacteriaceae, viridans streptococci, and enterococci.[30,31]

Clinical Manifestations

The clinical manifestations of uncomplicated diverticulitis resemble those of appendicitis but with findings typically on the left side of the abdomen. Diverticulitis often starts with visceral hypogastric pain that evolves to somatic pain that is localized, in the case of sigmoid disease, to the left lower quadrant. In contrast to acute appendicitis, the pain is often recurrent and is present for several days before presentation.[32] Fever, nausea and vomiting, changes in bowel habits, and urinary symptoms often accompany the pain. Leukocytosis is common but not invariably present (69%–93% of cases).[33] Urinalysis may reveal sterile pyuria if inflammation extends to the urinary system. Trace blood may be present in the stool, but hematochezia is uncommon and should raise suspicion for an alternative diagnosis. Low-grade fever is common in uncomplicated disease, and the physical examination typically reveals abdominal tenderness, guarding, and rebound tenderness in the left lower quadrant, the suprapubic area, or both; bowel sounds may be hypoactive or normal. A palpable abdominal mass may be caused by an inflammatory process but may also be indicative of cancer. High fever and abdominal rigidity suggest generalized peritonitis after perforation. Hyperactive bowel sounds suggest obstruction. Fistulization of the bladder or ureter with the colon may lead to pneumaturia and fecaluria[34]; passage of feces and flatus through the vagina occurs with fistula formation with the vagina or uterus.[35] Recurrent diverticulitis can lead to stricture and obstruction. Pyogenic liver abscess and pylephlebitis are rare complications.[36]

Diagnosis

Although the diagnosis of acute diverticulitis can be made on the basis of the clinical evaluation,[10,33] more than half of patients with clinically suspected diverticulitis are found to have alternative conditions.[37] For this reason, diagnostic studies are performed in most cases of suspected diverticulitis,[18,38] and they are particularly important in the patient with an atypical presentation, a suspected complication, severe illness, or clinical deterioration.[10,33] Imaging modalities include CT and ultrasonography.

CT is now considered to be the diagnostic procedure of choice for patients with suspected acute diverticulitis.[10,24,33,39] Tomographic evidence of diverticulitis includes pericolic fat stranding (98%), diverticula (84%), bowel wall thickening (70%), and phlegmon or abscess formation (35%).[40] CT also offers the opportunity for therapeutic intervention: CT-guided percutaneous drainage can be temporizing in acutely ill patients with large abscesses (>5 cm in diameter), permitting curative surgery on an elective basis.[10,24,41,42] Immediate surgery is indicated, however, if the abscess cavity contains gross fecal material. Abdominal CT for suspected diverticulitis has traditionally used oral, rectal, and, if not contraindicated, intravenous contrast. Helical CT using colonic contrast alone has been shown to be highly effective in the evaluation of patients with left lower quadrant pain. Similar to appendiceal CT, "diverticular CT" has been shown to have high sensitivity (97%), specificity (100%), and overall diagnostic accuracy (99%) in the prospective evaluation of suspected diverticulitis.[37] CT findings include arrowhead-shaped collections of contrast and presence of an inflamed diverticulum.

Magnetic resonance imaging (MRI) has also been utilized in diagnosis of diverticulitis with high sensitivity (86%–94%) and specificity (88%–92%), and with findings similar to those seen with CT.[43,44] MRI has the advantage of absence of radiation exposure, which may be a consideration if repeated examinations are necessary. However, the inability to drain abscesses percutaneously is a disadvantage of using MRI alone. Comparative studies of CT and MRI would be helpful to further guide their use in this setting.

Acute diverticulitis can also be confirmed by ultrasonography, which is capable of delineating inflamed colonic segments, phlegmons, and abscesses.[45] When performed by experienced operators, the diagnostic accuracy of ultrasonography rivals that of CT.[46] In practice, however, ultrasonography is used less frequently than CT for the evaluation of left lower quadrant pain, in contrast to right abdominal pain. The use of a contrast enema to diagnose acute diverticulitis has largely been supplanted by CT and ultrasound; when used, water-soluble contrast should be used in the event of unrecognized macroperforation. Double-contrast enemas should be avoided, because insufflation of air could dislodge an obstructing fecalith and cause perforation.[24] Given the risk of perforation, endoscopy is also avoided in the initial evaluation of patients with suspected acute diverticulitis. On occasion, limited sigmoidoscopy with minimal air insufflation is performed in ambiguous cases to exclude other diagnoses, such as inflammatory bowel disease, carcinoma, or ischemic colitis.[10,24] A complete colonic evaluation should be performed 6 to 8 weeks after resolution of an episode of acute diverticulitis to rule out coexisting lesions, including colon cancer.

Therapy

A trial of outpatient therapy is indicated for acute, uncomplicated diverticulitis or for a well-localized, small (≤5 cm in diameter) peridiverticular abscess, provided the patient can tolerate oral hydration. Outpatient treatment may also include a limited (e.g., 7- to 10-day) course of an oral broad-spectrum antibiotic agent or regimen active against facultative and anaerobic colonic microbiota.[10,24] Antibiotic regimens recommended by the Surgical Infection Society are shown in Table 79.1. Success rates of 94% to 97% have been reported for patients treated in this manner.[47,48,49,50] In one retrospective study, predictors of failure of outpatient therapy included free fluid on tomographic imaging and female sex.[49] A systematic review supported the use of outpatient management in mild or uncomplicated diverticulitis.[51] However, the necessity of treating acute uncomplicated diverticulitis with antibiotics has recently been challenged. Several retrospective case-control series and one open, multicenter, randomized controlled trial of hospitalized patients with CT-proven uncomplicated diverticulitis failed to demonstrate that antibiotics prevented complications, sped recovery, or reduced recurrent diverticular disease.[52–54] A Cochrane review also supported the view that antibiotics may not be necessary in this setting, though it calls for more high-quality trials.[50] Guidelines from the American Gastroenterological Association Institute suggest that "antibiotics should be used selectively, rather than routinely, in patients with acute uncomplicated diverticulitis."[55] Probiotics, mesalamine (5-aminosalicylic acid), and nonabsorbable antibiotics, such as rifaximin, are examples of therapies under investigation that target the potential roles of intestinal dysbiosis and chronic low-grade inflammation in diverticular disease pathogenesis.[27,56–58] If the patient is unable to tolerate oral hydration,

TABLE 79.1 Agents and Regimens That May Be Used for the Initial Treatment of Diverticulitis

	COMMUNITY-ACQUIRED INFECTION IN ADULTS	
REGIMEN	**Mild-to-Moderate Severity[a]**	**High Risk or Severity: Severe Physiologic Disturbance, Advanced Age, or Immunocompromised State[a]**
Single agent	Ertapenem Moxifloxacin	Imipenem-cilastatin Meropenem Doripenem Piperacillin-tazobactam
Combination	Ceftriaxone + metronidazole Cefotaxime + metronidazole Ciprofloxacin + metronidazole Levofloxacin + metronidazole	Cefepime + metronidazole Aztreonam + metronidazole + vancomycin

[a]Because of increasing resistance of *Escherichia coli* to fluoroquinolones, local population susceptibility profiles and, if available, isolate susceptibility should be reviewed.
Modified from Manzuski JE, Tessier JM, Addison MK, et al. The Surgical Infection Society revised guidelines on the management of intra-abdominal infection. Surg Infect. *2017;18:1–76.*

requires narcotic analgesics, or fails to improve despite appropriate outpatient therapy, admission to the hospital for nasogastric tube placement, bowel rest, and parenteral antibiotics is indicated. Elderly patients and those with compromised immune systems or other comorbidities also should be hospitalized. Parenteral antibiotics should be started for the treatment of secondary peritonitis, as shown in Table 79.1 (see Chapter 74).[59] Failure to respond to medical therapy within 48 to 72 hours should prompt repeat investigation for complications, consideration of alternative diagnoses, and surgical evaluation.[10,24]

Conservative medical therapy is successful in 70% to 80% of patients hospitalized with acute diverticulitis.[24,33] Indications for emergency colonic resection include uncontrolled sepsis, generalized peritonitis, acute clinical deterioration, persistent obstruction, and failure to respond to medical therapy. Under these circumstances, a two-stage operation, such as the Hartmann procedure, is usually performed.[33,60] The Hartmann procedure entails resection of the diseased colon, end colostomy, and closure of the distal loop or creation of a mucous fistula; colonic continuity can be restored several months later by elective anastomosis.[60] An alternative two-stage operation is primary resection of the diseased segment of colon with immediate anastomosis after intraoperative colonic lavage and proximal fecal diversion, followed later by elective stoma closure.[61]

Elective colonic resection is usually performed in cases of fistula formation or recurrent attacks of diverticulitis or for complicated diverticulitis brought under control with conservative therapy.[24,33] In these cases, a one-stage procedure with primary resection and anastomosis can be performed, with lower morbidity and mortality and a shorter hospital length of stay than a two-stage procedure. Elective surgery should also be considered for patients at high risk for complications of recurrent diverticulitis, including those with immunosuppressive conditions (e.g., organ transplantation, chronic corticosteroid therapy, renal failure)[62,63,64,65] and perhaps those younger than 40 years,[11] although these considerations are controversial.[66–68] Laparoscopic sigmoidectomy is an alternative to laparotomy in cases of mild-to-moderate disease.[69,70] Retrospective studies have reported that a one-stage procedure, combined with intraoperative colonic lavage but no protective diverting colostomy, may be feasible even in the moderate- to high-risk patient with colonic perforation.[71,72] Peritoneal lavage without sigmoidectomy has also emerged as a treatment for perforated diverticulitis. To date, three randomized trials have compared laparoscopic lavage to the Hartmann procedure, which involves sigmoid resection and end colostomy. In three trials, laparoscopic lavage was not found to be superior to the Hartmann procedure, with one trial demonstrating increased morbidity in the laparoscopic lavage group.[73–75] In one trial, laparoscopic lavage reduced the need for reoperation and had a safety profile similar to that of the Hartmann procedure.[76] The disparate findings in these trials may have been due to differences in primary outcome variables.[77] However, more recent guidelines no longer advocate laparoscopic lavage for perforated diverticulitis.[78]

The classic three-stage procedure, consisting of drainage and proximal diverting colostomy, interval resection of diseased bowel with primary anastomosis, and stoma closure, has largely been abandoned because failure to eliminate the source of peritoneal infection resulted in higher mortality rates compared with the two-stage procedure.[60]

NEUTROPENIC ENTEROCOLITIS (TYPHLITIS)

Neutropenic enterocolitis is also referred to as typhlitis, from the Greek *typhi* or "blind," referring to the blind-ended cecum. It is a life-threatening complication of myelosuppressive chemotherapy administered for the treatment of hematologic and, less commonly, solid-tissue malignancies.[79–83] It is also rarely seen in individuals with human immunodeficiency virus infection[84] and in those with neutropenia caused by aplastic anemia, drug-induced agranulocytosis, cyclic neutropenia, or acute leukemia before the initiation of chemotherapy.[81,85–87] Although estimates vary greatly between studies, the incidence rate of neutropenic enterocolitis has been estimated to be approximately 5% for neutropenic adults hospitalized with hematologic or solid malignancies or with aplastic anemia.[79,88]

Pathogenesis

The pathologic characteristic of neutropenic enterocolitis is marked thickening and edema of the bowel wall, typically involving the cecum and often extending to the ascending colon, terminal ileum, or both.[85] Although the cecum is most commonly involved, other segments of the large or small bowel may also be affected.[89] Examination of the bowel wall reveals discrete or coalesced mucosal ulceration, intramural thrombocytopenic hemorrhage, and degeneration of the muscularis mucosa. Histologic specimens demonstrate mononuclear infiltrates and a variety of invading bacteria and fungi. The pathogenesis is multifactorial and incompletely understood. Cytotoxic chemotherapy and irradiation lead to activation of nuclear factor kappa B, production of proinflammatory cytokines, epithelial cell apoptosis, and increased mucosal permeability.[79,90] Rarely, ulceration and necrosis resulting from direct leukemic infiltration of the intestinal tract can occur.[91] The combination of neutropenia, mucosal injury, and possibly ischemia resulting from cecal distention allows gut flora to opportunistically invade the bowel wall. In the setting of impaired host immunity, these organisms proliferate and cause local destruction by elaboration of exotoxins.[92] Complications include sepsis resulting from translocation of the resident microflora and endotoxins across the injured mucosal barrier, uncontrolled hemorrhage, and perforation.

Clinical Manifestations

Clinical manifestations are variable and depend on the extent of disease. Patients typically present with fever, nausea, vomiting, abdominal pain and tenderness, and diarrhea that is often bloody. Given the prominence of cecal involvement, neutropenic enterocolitis can mimic acute appendicitis, with localization of abdominal pain and tenderness to the right lower quadrant.[93] Right lower abdominal pain is absent in 40% to 55% of cases, however,[94,95] and corticosteroid therapy may mask the symptoms of abdominal pain altogether.[85] Abdominal distention may be present in up to two-thirds of patients, but paralytic ileus is uncommon.[79] Severe stomatitis and pharyngitis may be present and are markers

of diffuse mucosal injury. Rapid progression to the development of an acute abdomen is not uncommon. Symptoms typically develop after 7 to 14 days of neutropenia (absolute neutrophil count, <500/mm^3). In a study of patients with newly diagnosed acute myeloid leukemia treated with cytarabine and idarubicin, 26% developed neutropenic enterocolitis at a median of 17 days after starting chemotherapy.[96] Alternative diagnoses, in addition to acute appendicitis, include chemotherapy-induced mucositis, pseudomembranous colitis, graft-versus-host disease, chronic mucormycosis or aspergillosis, intussusception, ischemic colitis, bowel obstruction, Ogilvie syndrome (colonic pseudo-obstruction), ileus, acute cholecystitis, acute pancreatitis, cytomegalovirus enterocolitis, and herpes zoster.[79,97]

Diagnosis

Ultrasonography and CT have largely supplanted plain radiography of the abdomen in the evaluation of suspected neutropenic enterocolitis.[82,97] Proposed diagnostic criteria for acute neutropenic enterocolitis include bowel wall thickening of greater than 4 mm, fever, and abdominal pain.[88] The degree of bowel wall thickening, as measured by ultrasonography or CT, correlates with severity of disease: patients with bowel wall thickness greater than 10 mm had a 60% mortality rate compared with 4% for those with a thickness of less than 10 mm.[98]

Diagnostic tests for *Clostridioides difficile* (formerly *Clostridium difficile*) should be performed, and blood and stool cultures should be obtained. Bacteremia or fungemia occurs in 14% to 44% of patients, most commonly with *Pseudomonas aeruginosa,* Enterobacteriaceae, *Bacteroides fragilis,* viridans-group streptococci, enterococci, and *Candida* spp.[81,82,95,99,100] Patients with *Clostridium septicum* bacteremia may have a more fulminant, lethal course,[86,101] and this infection may represent a different syndrome.[102]

Therapy

The management of neutropenic enterocolitis has evolved over the last several decades. Although a number of early studies reported mortality rates greater than 50% and advocated early surgical intervention,[103,104] recent studies have reported mortality rates of less than 20% with conservative medical therapy.[81,82,95,99,100] Management includes bowel rest, decompression, intravenous fluids, nutritional support, and broad-spectrum parenteral antibiotics. The antimicrobial regimen should cover enteric facultative and anaerobic flora, *P. aeruginosa,* and perhaps yeast. Examples include cefepime or ceftazidime with metronidazole, piperacillin-tazobactam, or meropenem, all with the possible addition of an antifungal agent with activity against *Candida,* especially if there is a lack of response at 72 hours.[59,83,88,105] The regimen should also effectively treat *C. difficile* if it has not been excluded as a possible cause. The indications for immediate surgical intervention include persistent gastrointestinal bleeding despite resolution of hematologic and clotting abnormalities, free intraperitoneal perforation, and clinical deterioration, suggesting uncontrolled sepsis.[100]

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The complete reference list is available online at Expert Consult.

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G Cardiovascular Infections

80 Endocarditis and Intravascular Infections

Thomas L. Holland, Arnold S. Bayer, and Vance G. Fowler, Jr.

SHORT VIEW SUMMARY

Definition
- *Infective endocarditis* (IE) is an infection of the endocardial surface of the heart.

Epidemiology
- It is traditionally associated with heart valves damaged by rheumatic heart disease.
- In the current era, health care contact and injection drug use are the primary risk factors.

Microbiology
- *Staphylococcus aureus* is now the leading cause of IE in most of the industrialized world.
- Historically, viridans-group streptococci were the most common cause of endocarditis.
- *Bartonella* spp. are the most common cause of culture-negative IE in the United States. Other common causes of culture-negative IE are summarized in Table 80.6.

Diagnosis
- Results of blood cultures remain the cornerstone of diagnosis of endocarditis.
- Clinical evaluation alone is insufficient to exclude the possibility of endocarditis.
- Echocardiography, particularly transesophageal echocardiography, has greatly improved the clinician's ability to identify endocarditis.
- Diagnostic schema, such as the modified Duke criteria, are useful in establishing the presence of endocarditis.

Therapy
- Cardiac surgery is required in up to half of patients with endocarditis and improves patient outcome.
- Cardiac surgery is especially important in patients with endocarditis who have heart failure, paravalvular abscess, recurrent embolic events, or ongoing sepsis or who are infected with highly resistant or fungal pathogens.
- Although the timing of cardiac surgery, particularly after embolic events involving the central nervous system, remains controversial, emerging evidence supports the benefit of early valve replacement surgery for endocarditis.
- Antibiotic therapy involves extended courses of antibiotics. Treatment is highly pathogen specific and is summarized in Table 80.7. Guidelines for treatment of IE were updated in 2015.[1]
- Addition of adjunctive low-dose, short-course gentamicin to standard antibiotic treatment of *S. aureus* native valve IE has been shown to confer high risk for nephrotoxicity without significant improvement in clinical outcomes and is not encouraged.
- Several observational studies support the use of high-dose ceftriaxone in combination with ampicillin for the treatment of ampicillin-susceptible, aminoglycoside-resistant enterococcal endocarditis or for patients with underlying renal disease.

Prevention
- Prevention of endocarditis involves reduction of bloodstream infections, especially in the health care setting.
- The role of antibiotic prophylaxis for the prevention of endocarditis is controversial. Guidelines were published by the American Heart Association in 2015.

INFECTIVE ENDOCARDITIS

The term *infective endocarditis* (IE) denotes infection of the endocardial surface of the heart and implies the physical presence of microorganisms in the lesion. Although the heart valves are affected most commonly, the disease also may occur within septal defects or on the mural endocardium. Infections of arteriovenous shunts and of arterioarterial shunts (patent ductus arteriosus) and infections related to coarctation of the aorta are included in the following discussion because the clinical manifestations are similar. The term *infective endocarditis,* first used by Thayer and later popularized by Lerner and Weinstein,[2] is preferable to the former term *bacterial endocarditis,* because nonbacterial pathogens, including fungi and perhaps even viruses, may be responsible for the syndrome.

In the past, IE was classified as *acute* or *subacute.* This distinction was based on the usual progression of the untreated disease and is mainly of historical interest. The acute form follows a fulminant course, usually with high fever, systemic toxicity, and leukocytosis; death occurs in several days to less than 6 weeks. It classically is associated with infection caused by *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae,* or *Neisseria gonorrhoeae.* The subacute form (death occurring in 6 weeks to 3 months) and the *chronic* form (death occurring later than 3 months) usually are considered together. They commonly occur in the setting of prior valvular disease and are characterized by a slow, indolent course with low-grade fever, night sweats, weight loss, and vague systemic complaints. These two forms of IE classically are caused by the viridans streptococci.

Although useful conceptually, this classification ignores the nonbacterial forms of IE and the frequent overlap in manifestations of infection by specific organisms, such as the enterococci. A classification based on the etiologic agent responsible is preferable because it has implications for the course usually followed, the likelihood of preexisting heart disease, and the appropriate antimicrobial agents to use.

Although IE is relatively uncommon, it has received considerable attention from clinicians and scientists for the past century. The clinical manifestations of IE are so varied that they may be encountered in any of the medical subspecialties. Successful management depends on the close cooperation of medical and surgical disciplines. Endocarditis services and therapeutic protocols have been created at several tertiary care centers in the United States and Europe. IE has attracted considerable investigative interest. Although the factors that influence its development now are identified more clearly, many questions remain about the unique aspects of this infection, in particular:

1. Why do organisms lodge specifically on the cardiac valves rather than elsewhere in the vascular tree?
2. What enables the microorganisms to survive on the valve surface after colonization?

3. What are the primary host defenses against induction and progression of the infection?
4. Why do only a relatively few strains of bacteria produce most cases of IE whereas many others produce only bacteremia?
5. What factors are responsible for the marked variation in the manifestations of IE?
6. Why is the infection so difficult to eradicate with antibiotics even though the infecting organisms often are exquisitely sensitive to the drugs in vitro?

These questions are discussed in detail in the following sections.

Epidemiology

The incidence of IE is difficult to determine, because the criteria for diagnosis and the methods of reporting vary with different series.[3,4] An analysis based on strict case definitions often reveals that only a small proportion (approximately 20%) of clinically diagnosed cases are categorized as definite. In a systematic review of IE epidemiology studies from 1980 to 2008, crude incidence of IE ranged between 1.5 and 11.6 cases per 100,000 people.[5] Of note, high-quality data were available only from 10 countries, most of which are high-income areas; the epidemiology of IE in lower-income regions is poorly characterized. With the use of the Nationwide Inpatient Sample, which approximates about a 20% sample of all US acute-care hospitals, several studies have reported significant increases in hospitalizations for IE, with most of this increase being attributable to *S. aureus*.[6] In the International Collaboration on Endocarditis–Prospective Cohort Study (ICE-PCS), the proportion of acute cases had increased from approximately 20% in the preantibiotic era to more than 75% in most of the industrialized world.[7]

Age

The mean age of patients with IE has increased gradually in the antibiotic era. In 1926, the median age was younger than 30 years[8]; by 1943, it was 39 years, and currently more than half of patients are older than 50 years.[7,9,10] In ICE-PCS, among more than 2700 patients from 58 centers in 25 countries with definite IE according to the modified Duke criteria, the median age was 57.9 years.[7] The disease is uncommon in children, in whom it is associated primarily with (1) underlying structural congenital heart disease, particularly septal defects or complex lesions involving septal defects; (2) surgical repair of these defects; or (3) nosocomial catheter-related bacteremia, especially in infants.[11,12]

The mean age for men is 6 to 7 years older than that for women, and men are affected more commonly (54%–69% of cases); the mean male-to-female ratio is 1.7:1, with a range of 1:1 to 3:1 in 18 large series.[13] Among patients younger than 35 years, more cases occur in women. Many factors may be related to this shift in age distribution. First, there has been a change in the nature of the underlying heart disease owing to a decline in the incidence of acute rheumatic fever and rheumatic heart disease, countered by the increasing importance of degenerative heart disease in elderly patients. Second, the age of the population has been increasing steadily, and people with rheumatic or congenital heart disease are surviving longer. In addition, such patients increasingly are being subjected to prosthetic valve surgery, an important etiologic factor in the pathogenesis of IE.

Health Care–Associated Infective Endocarditis

A relatively new form of the disease, health care–associated IE, has emerged secondary to the introduction of new therapeutic modalities (e.g., intravenous catheters, hyperalimentation lines, pacemakers, dialysis shunts).[3,7,10,14–16,17] In a prospective, multinational cohort study of more than 1600 patients with native valve endocarditis and no injection drug use, more than one-third of patients had health care–associated endocarditis, which in many cases was community acquired.[17] The emerging importance of health care–associated IE in industrialized nations has also influenced the microbiology of IE, with an increasing prevalence of *S. aureus* and decreasing prevalence of viridans streptococci in much of the industrialized world.

Pattern of Valvular Involvement

The heart valve involved by the infection varies considerably with the proportion of acute cases reported in each series. The distribution ranges from 28% to 45% of cases for the mitral valve alone, 5% to 36% for the aortic valve alone, and 0% to 35% for the aortic and mitral valves combined. The tricuspid valve rarely is involved (0%–6% of cases), and the pulmonary valve even less often (<1%).[7,18] Involvement of the aortic valve alone is increasing in frequency and correlates with the increase in acute cases; the incidence was 5% in 1938 and increased to 38% by 2000.[7]

Predisposing Factors

Almost any type of structural heart disease may predispose to IE, especially if the defect results in turbulence of blood flow. Rheumatic heart disease was the underlying lesion in 37% to 76% of infections in the past, and the mitral valve is involved in more than 85% of cases related to rheumatic heart disease.[13] If the mitral valve alone is involved, women outnumber men by 2 to 1. The aortic valve is affected in approximately 50% of these cases; if it alone is involved, men outnumber women by 4 to 1. Right-sided endocarditis is rare (except in injection drug users and patients with indwelling transvenous pacemakers) and accounts for fewer than 10% of all cases occurring in patients with rheumatic heart disease. In developed countries, the proportion of cases related to rheumatic heart disease has continued to decline (to 5% or less in the past 2 decades),[7] whereas in developing countries rheumatic heart disease[19,20] remains the most common predisposing cardiac condition.[21]

Congenital heart disease (especially patent ductus arteriosus, ventricular septal defect, coarctation of the aorta, bicuspid aortic valve, tetralogy of Fallot, and, rarely, pulmonic stenosis) is responsible in 6% to 24% of endocarditis cases.[7] IE is uncommon in the secundum atrial septal defects, probably because this lesion results in a low-pressure shunt with little turbulence. The congenitally bicuspid aortic valve is an important condition in elderly patients (especially men).[22] In one prospective multicenter analysis, it was present in 16% of 310 cases of definite native valve endocarditis. Half of the patients with bicuspid aortic valve had perivalvular abscess, and 72% required valve surgery.[23] Surgical closure of a ventricular septal defect lowers the risk for IE.[24]

The degenerative cardiac lesions (e.g., calcified mitral annulus, calcific nodular lesions secondary to arteriosclerotic cardiovascular disease, post–myocardial infarction thrombus) assume greatest importance in the 30% to 40% of IE patients without underlying valvular disease. The actual contribution made by these lesions is unknown, but they occur with an increased incidence in the elderly. In one series, degenerative lesions were present in 50% of patients older than 60 years with native valve IE.[25] The contribution of these degenerative cardiac lesions to the development of IE was apparent in an analysis of 148 patients receiving treatment in London after 1970.[26,27] The underlying structural cardiac defects were as follows: rheumatic heart disease in 39 patients, congenital defects in 13, and normal or degenerate valves in 65. Although a calcified mitral annulus is fairly common in elderly women, this lesion rarely is complicated by IE (only 3 of 80 patients in one report).[28] When patients with acute IE are considered separately, more than 50% have no recognized underlying cardiac disease.[29]

Many other conditions, such as bicuspid aortic valve,[22] luetic heart disease, arterioarterial fistulas, hemodialysis shunts or fistulas, intracardiac pacemaker wires, and intracardiac prostheses, may predispose to IE. Prosthetic valve endocarditis is increasing in incidence in proportion to other forms of endocarditis. For example, it was present in one-fifth of the 2781 adults with definite IE in ICE-PCS (see Chapter 81).[7] Intravascular infections involving cardiac devices (e.g., permanent cardiac pacemakers, defibrillators) also have increased significantly since the 1990s and are discussed in Chapter 82.[30,31] IE also occurs more frequently among patients with extensive contact with the health care system.[10,17] As noted previously, injection drug users constitute another group with an increased risk for IE (see later discussion). In this population, there is the added problem of a rapidly rising prevalence of IE among persons with human immunodeficiency virus (HIV) infection. In addition, injection drug users are the group at greatest risk for recurrent and polymicrobial IE.[13,32] Although the contribution of invasive procedures (e.g., sigmoidoscopy, colonoscopy) has been debated, native valve IE seems to be more common among patients with active inflammatory bowel disease (6 of 213 patients in one report[33]).

Although it is not classically recognized as a condition leading to bacterial endocarditis, up to 5% of patients with idiopathic hypertrophic subaortic stenosis develop IE.[34] IE is more common in the subset of these patients who have hemodynamically severe forms of the disease, as manifested by a higher peak systolic pressure gradient and a high prevalence of symptoms. New murmurs develop in 36% of patients with idiopathic hypertrophic subaortic stenosis complicated by IE, and this new physical finding correlates with a higher mortality rate.[34] Among seven cases examined histologically, the infection was found on the aortic valve in three cases, on the mitral valve in two cases, on both valves in one case, and on the subaortic endocardium in one case. This distribution probably is related to the associated mitral regurgitation caused by displacement of the anterior leaflet by the abnormal ventricular architecture and by the turbulence of the jet stream affecting the aortic valve distal to the intraventricular obstruction.

An association has also been recognized between IE and mitral valve prolapse. Among 896 Olmsted County residents with mitral prolapse, the 15-year cohort risk of IE was 1.1%, which was more than eightfold higher than in the general population.[35] All IE cases occurred in patients with concurrent mitral regurgitation.

Mitral valve prolapse should be suspected in patients who have midsystolic clicks with or without a late systolic murmur. The condition is common and has been recognized in 0.5% to 20% of otherwise healthy people, especially young women. It has become apparent that mitral valve prolapse is only one component of a developmental syndrome. This lesion often is associated with a distinct habitus in women,[36] with von Willebrand disease, or with ophthalmoplegia. Some of these characteristics may be useful in identifying patients at high risk for IE. All 25 patients in one series who developed IE on a prolapsing mitral valve had a holosystolic murmur, and none had the isolated click without a murmur.[37]

The risk for IE seems to be increased in the subset of patients with mitral valve prolapse who exhibit thickened leaflets with valvular redundancy.[25] In addition, men older than 45 years who have mitral valve prolapse are at increased risk for IE.[38] Nevertheless, the risk for IE is higher in patients with mitral valve prolapse. In a careful retrospective, epidemiologic, matched case-control analysis, the calculated odds ratio (8.2; 95% confidence interval [CI], 2.4–28.4) indicated a substantially higher risk for the development of IE in these patients than in controls.[39] It seems that when IE develops in people with mitral valve prolapse, the symptoms and signs are more subtle and the mortality rate is less than in left-sided IE of other types.[40]

Pathogenesis and Pathophysiology

In vitro observations and studies in experimental animals have shown that development of IE probably requires the simultaneous occurrence of several independent events, each of which may be influenced by a cadre of distinct factors. The valve surface first must be "altered" to produce a suitable site for bacterial attachment and colonization. Valve surface changes may be produced by various local and systemic stresses, including blood turbulence (from underlying structural or inflammatory valvular disease) and the offending organism itself. These alterations result in the deposition of platelets, fibronectin, fibrin, and other matrix ligands in the formation of so-called sterile vegetation—the lesions of nonbacterial thrombotic endocarditis (NBTE). Bacteria then must reach this site and adhere to and invade the involved tissue to produce colonization and persistence. Certain strains seem to have a selective advantage in adhering to platelets, fibronectin, or fibrin and produce the disease with a lower inoculum. After microbial colonization, the surface is covered rapidly with a protective sheath of fibrin and platelets to produce an environment conducive to further bacterial multiplication and vegetative growth. It is important to point out that, at least for left-sided IE, neutrophils play little role in the pathogenesis of or defense against evolution of IE. The interaction of these events is depicted in Fig. 80.1. In the following sections, these factors are considered independently (for in-depth discussions, see references 41–45).

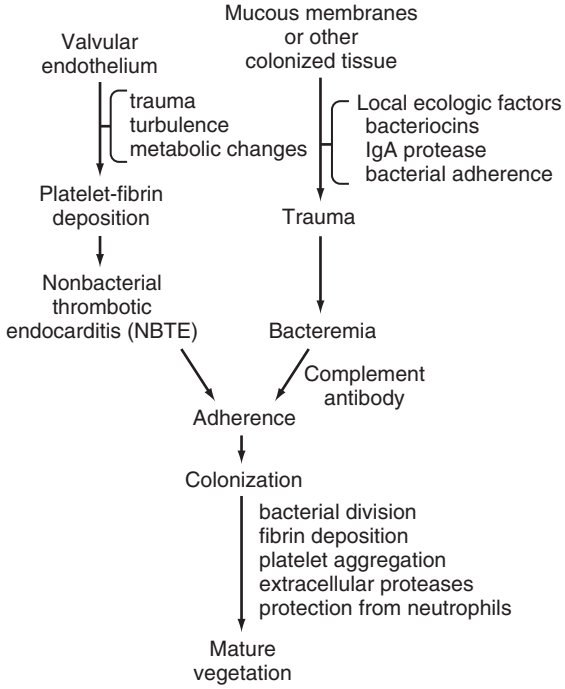

FIG. 80.1 Proposed scheme for the pathogenesis of infective endocarditis. *IgA,* Immunoglobulin A.

Nonbacterial Thrombotic Endocarditis

Luschka, in 1852, first suggested that IE resulted when septic coronary emboli lodged in the vessels of the cardiac valve.[46] This hypothesis was discarded, because cardiac valves are poorly vascularized.[43,47,48] It now is clear that the initial colonization occurs on the damaged endothelial surface of the valve. In experimental animals, it is almost impossible to produce IE with intravenous injections of bacteria unless the valvular surface is first perturbed. If a polyethylene catheter is passed across the aortic valve of a rat or rabbit, IE is produced with intravenously injected bacteria or fungi.[49,50] Microscopic examination of this early lesion shows the organisms intimately adherent to fibrin-platelet deposits overlying interstitial edema and mild cellular distortion that have formed in areas of valvular trauma.[51] Scanning electron micrographs of the damaged valvular surface confirm the adhesion of microorganisms to these areas of fibrin-platelet deposition early in the disease course.[52] The organisms are covered rapidly by fibrin.[53]

Opossums and pigs are the only animals known to develop spontaneous IE readily (i.e., without experimentally induced valvular alteration).[44,54] The stress of captivity is apparently sufficient in these animals to produce subtle valvular changes that lead to spontaneous endocarditis and a markedly increased susceptibility to the disease after intravenous injection of bacteria. In other animals and probably in humans, alteration of the valve surface is a prerequisite for bacterial colonization. Angrist and Oka[47] first recognized the importance of these deposits as the crucial factor in allowing bacterial colonization of valve surfaces and suggested the term NBTE. Many forms of exogenous stress produce these lesions experimentally, including infection, hypersensitivity states, cold exposure, simulated high altitude, high cardiac output states, cardiac lymphatic obstruction, and hormonal manipulations.[44] These procedures all increase the susceptibility of the animals to IE.

NBTE has been found in patients with malignancy (particularly pancreatic, gastric, or lung adenocarcinoma) and other chronic wasting diseases, rheumatic and congenital heart disease,[47] uremia, and connective tissue diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis; after the placement of intracardiac catheters (e.g., Swan-Ganz); and after a self-limited acute illness. NBTE generally reflects one of two pathogenic mechanisms: hypercoagulability (especially related to secondary antiphospholipid syndromes) or endothelial damage. In a careful analysis performed in Japan, NBTE was found in 2.4% of 3404 autopsies, especially in elderly people with chronic wasting disease.[55] Among patients at high risk for NBTE, the rate may be greater. Cardiac valvular vegetations were found in 19% of 200 nonselected ambulatory

patients with solid tumors undergoing prospective echocardiographic screening.[56] Valvular lesions were most common among patients with carcinoma of the pancreas or lung or lymphoma.

NBTE is most common on the low-pressure side of the cardiac valves along the line of closure, precisely the site most often involved in IE. Whether this lesion is always essential for the development of IE in humans is unknown. Secondary hypercoagulable states (e.g., as seen in secondary antiphospholipid syndromes in SLE) may contribute to the development and propagation of the NBTE lesion.[57]

Hemodynamic Factors

When associated with valvular insufficiency, IE characteristically occurs on the atrial surface of the mitral valve and the ventricular surface of the aortic valve. Rodbard[58] showed that this localization is related to a decrease in lateral pressure (presumably with decreased perfusion of the intima) immediately downstream from the regurgitant flow. Lesions with high degrees of turbulence (e.g., small ventricular septal defect with a jet lesion, valvular stenosis resulting from insufficient valves) readily create conditions that lead to bacterial colonization, whereas defects with a large surface area (large ventricular septal defect), low flow (ostium secundum atrial septal defect), or attenuation of turbulence (chronic congestive heart failure [CHF] with atrial fibrillation) rarely are implicated in IE. Cures of IE achieved with ligation of an arteriovenous fistula or patent ductus arteriosus without further treatment also highlight the importance of hemodynamic factors. A hyperdynamic circulation itself, such as that developing after experimentally induced arteriovenous fistulas in dogs or after creation of fistulas and shunts in hemodialysis patients, may lead indirectly to IE by producing NBTE.[43,44] Finally, implantable intracardiac prosthetic material may well contribute to a turbulent blood flow state, as seen in intraventricular pacemakers and implantable cardioverter-defibrillators.

The degree of mechanical stress exerted on the valve also affects the location of the IE.[59] In 1024 autopsy cases of IE reviewed through 1952, the incidence of valvular lesions was as follows: mitral, 86%; aortic, 55%; tricuspid, 19.6%; and pulmonic, 1.1%. This incidence correlates with the pressure resting on the closed valve: 116, 72, 24, and 5 mm Hg, respectively.

Transient Bacteremia

In the setting of preexistent NBTE, transient bacteremia may result in colonization of these lesions and may lead to the development of IE.[60] Transient bacteremia occurs whenever a mucosal surface heavily colonized with bacteria is traumatized, such as occurs with dental extractions and other dental procedures or with gastrointestinal, urologic, or gynecologic procedures (Table 80.1).[60,61] The degree of bacteremia is proportional to the trauma produced by the procedure and to the number of organisms inhabiting the surface; the organisms isolated reflect the resident microbial flora. The bacteremia usually is low grade (≤10 colony-forming units [CFUs]/mL) and transient; the bloodstream usually is sterile in less than 15 to 30 minutes.

In two studies in which samples for blood cultures were drawn from patients with severe gingival disease before the dental procedure, spontaneous bacteremia was identified in 9% to 11% of the patients. Other studies have shown an even higher frequency of spontaneous bacteremia. Of the blood cultured from healthy people, 60% to 80% of specimens were positive when filters and anaerobic techniques were used.[62] The degree of bacteremia was low, however, with only 2 to 10 CFUs/5 mL of blood isolated. "Nonpathogenic" organisms, such as *Propionibacterium* (now *Cutibacterium*) *acnes, Actinomyces viscosus, Staphylococcus epidermidis,* and other *Actinomyces* or streptococcal species, were responsible. Frequent episodes of silent bacteremia also are suggested by the identification of circulating humoral antibodies to the resident oral flora and by the noted increase in sensitized peripheral T cells to the flora of dental plaque. The frequency of such silent bacteremias in the probable pathogenesis of IE has contributed to the current concept that individual dental procedures are uncommonly the cause of such infections.[63] In contrast, it is currently believed[64] that underlying gingivodental disease results in intermittent, low-level bacteremia that can seed damaged valvular endothelial lesions in an unpredictable scenario.

TABLE 80.1 Incidence of Bacteremia After Various Procedures

PROCEDURE OR MANIPULATION	% POSITIVE BLOOD CULTURES
Dental	
Dental extraction	18–85
Periodontal surgery	32–88
Chewing candy or paraffin	17–51
Tooth brushing	0–26
Oral irrigation device	27–50
Upper Airway	
Bronchoscopy (rigid scope)	15
Tonsillectomy	28–38
Nasotracheal suctioning or intubation	16
Gastrointestinal	
Upper gastrointestinal endoscopy	8–12
Sigmoidoscopy or colonoscopy	0–9.5
Barium enema	11
Percutaneous needle biopsy of liver	3–13
Urologic	
Urethral dilation	18–33
Urethral catheterization	8
Cystoscopy	0–17
Transurethral prostatic resection	12–46
Obstetric or Gynecologic	
Normal vaginal delivery	0–11
Punch biopsy of the cervix	0
Removal or insertion of intrauterine (contraceptive) device	0

Data from Everett ED, Hirschmann JV. Transient bacteremia and endocarditis prophylaxis: a review. Medicine (Baltimore). *1977;56:61.*

Another crucial factor during the transient bacteremia stage is susceptibility of the potential pathogen to complement-mediated bactericidal activity. Only "serum-resistant," gram-negative aerobic bacilli (e.g., *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens*) reliably produce experimental IE in rabbits,[65,66] and this property is found in all isolates from human cases of IE. Although experimental IE can be induced in rats with "serum-sensitive" *E. coli,* the organisms are eliminated rapidly on catheter removal.[66]

Microorganism–Nonbacterial Thrombotic Endocarditis Interaction

The ability of certain organisms to adhere to NBTE lesions is a crucial early step in the development of IE. Gould and associates[67] showed that organisms commonly associated with IE (enterococci, viridans streptococci, *S. aureus, S. epidermidis, P. aeruginosa*) adhered more avidly to normal canine aortic leaflets in vitro than did organisms uncommon in IE *(Klebsiella pneumoniae, E. coli).* In addition, *S. aureus* and the viridans streptococci produce IE more readily than does *E. coli* in the rabbit model of IE.[68] This observation correlates with the relative frequency with which these organisms produce the disease in humans. The rarity of IE due to gram-negative aerobic bacilli also may be related to their serum sensitivity, as noted previously.

Differences in the propensity to cause IE are apparent even within a single species. For example, specific clones of *S. aureus,* including clonal complexes 30[69,70] and 22,[71] have been reported to be associated with an increased risk for IE. In addition, only 2 of the 11 capsular serotypes of *S. aureus* described to date, serotypes 5 and 8, account for

approximately 75% of clinical isolates, whereas highly mucoid strains (e.g., serotypes 1 and 2) are rarely recovered. Nevertheless, mutant strains devoid of microencapsulation had significantly lower median infective dose (ID_{50}) values in a rat (catheter-induced NBTE) IE model,[72] compared with wild-type parent strains. Microcapsule expression may attenuate *S. aureus* IE production by blocking bacterial cell surface adhesins, but this hypothesis requires confirmation. In addition, an increasing number of reports suggest that other specific pathogen characteristics in *S. aureus* are associated with the severity of infection caused by these strains in humans.[73–77]

Other studies using an elegant experimental model of IE after dental extraction in rats with sucrose-induced periodontitis, which closely resembles the presumed pathogenetic sequence in humans, also have suggested an important role for bacterial adhesion to NBTE in the early events. Although viridans streptococci were isolated much more commonly than group G streptococci in blood cultures obtained 1 minute after extraction, the latter strains caused 83% of the IE episodes in this rat model.[78,79] This propensity to cause IE was associated with an increased adhesion of group G streptococci to fibrin-platelet matrices in vitro.[79]

The adherence of oral streptococci to NBTE may depend on the production of a complex extracellular polysaccharide, dextran. This polymer plays an essential role in the pathogenesis of dental caries by *Streptococcus mutans*.[80] Dextran allows the organism to adhere tightly to the surface of dental enamel. The enhanced ability to adhere to inert surfaces also may be important in IE. In an analysis of 719 cases of streptococcal infections in the United Kingdom, 317 cases of IE were found.[81] The most common etiologic agents were *Streptococcus sanguinis* (16.4% of the cases), previously identified in *Streptococcus* subacute bacterial endocarditis, and *S. mutans* (14.2%). Ratios of endocarditis to nonendocarditis bacteremia caused by particular organisms have been derived (Table 80.2), allowing prediction of the relative propensity for a particular organism to cause IE. The ratios range from 14.2 : 1 for *S. mutans* to a reversed ratio of 1 : 32 for *S. pyogenes*. Only the first four organisms listed in Table 80.2 (all with ratios >3 : 1) produce extracellular dextran. This finding suggests that dextran production may be another virulence factor in the pathogenesis of IE.

The role of dextran in the adherence of oral streptococci to NBTE also has been studied in vitro with the use of artificial fibrin-platelet matrices (simulating NBTE). The amount of dextran produced by organisms grown in broth correlated with adherence; the amount was increased by incubating the organism in sucrose (which stimulates dextran production) and decreased by the addition of dextranase (which removes the dextran from the cell surface). The addition of exogenous dextran to *S. sanguinis* grown in sucrose-free media increased adherence. Dextran production also correlated directly with the ability of these organisms to produce IE in vivo in the rabbit model.[82] The strain of *S. sanguinis* produced IE less readily when incubated in dextranase than did control strains, and a strain that produced large quantities of dextran produced endocarditis more easily than did a strain that produced relatively small quantities of dextran. Dextran production also increased the adherence of *S. mutans* to traumatized canine aortic valves in vitro,[83] an effect that was dependent on polymers of higher molecular weight.[84] Dextran formation (or, more properly, exopolysaccharide or glycocalyx production) by oral streptococci may be a virulence factor for the production of IE by these organisms.[85] Continued in vivo synthesis of exopolysaccharide during experimental IE correlated with vegetation size and resistance to antimicrobial therapy.[86,87] Measurement of cell-adherent glycocalyx with a quantitative spectrophotometric tryptophan assay among viridans streptococci isolated from blood cultures has potential value as an independent predictor of the likelihood of IE.[88] Non–dextran-producing streptococci may produce IE in humans and adhere to artificial fibrin-platelet surfaces in vitro,[89] suggesting that other microbial surface characteristics are instrumental for this early event. Whatever the role of the extracellular glycocalyx in microbial adhesion, its presence may retard antimicrobial therapy for streptococcal endocarditis (see later discussion).[86,87,90]

FimA, a surface adhesin expressed by viridans streptococci, has been shown to mediate the attachment of such organisms to platelet-fibrin matrices in vitro and to experimental NBTE lesions in the animal model of IE.[91] Homologues of the *fimA* gene are widely distributed among clinical strains of viridans streptococci and enterococci, suggesting its importance in IE.[92] Several lines of experimental evidence have confirmed further the key role of FimA in the pathogenesis of IE. Inactivation of the *fimA* gene has yielded viridans streptococcal mutants exhibiting a significant decrease in virulence in experimental IE compared with the parental strain having intact FimA expression.[91] In addition, animals either passively immunized with anti-FimA antibody or actively immunized with a FimA vaccine were significantly less susceptible to experimental IE than nonimmunized controls.[92]

TABLE 80.2 Ratio of Infective Endocarditis Cases to Nonendocarditis Bacteremia Cases for Various Streptococci and Enterococci

ORGANISM	ENDOCARDITIS:NONENDOCARDITIS RATIO
Streptococcus mutans	14.2 : 1
Streptococcus bovis I	5.9 : 1
Dextran-positive *Streptococcus mitior*	3.3 : 1
Streptococcus sanguinis	3 : 1
S. mitior	1.8 : 1
Unclassified viridans streptococci	1.4 : 1
Enterococcus faecalis	1 : 1.2
Miscellaneous streptococci	1 : 1.3
S. bovis II	1 : 1.7
Streptococcus anginosus	1 : 2.6
Group G streptococci	1 : 2.9
Group B streptococci	1 : 7.4
Group A streptococci	1 : 32

Modified from Parker MT, Ball LC. Streptococci and aerococci associated with systemic infection in man. J Med Microbiol. *1976;9:275.*

Viridans-Group Streptococcal Interactions With Platelets

The viridans-group streptococci interact with human platelets through several mechanisms that may contribute to the pathogenesis of IE. A locus of *Streptococcus gordonii*, important for binding to human platelets,[93] encodes a large, serine-rich repeat (SRR) cell wall–anchored glycoprotein adhesin (GspB), which comprises four proteins mediating the glycosylation of the adhesion,[94–96] and a specialized, multicomponent transporter (the accessory Sec system) that mediates the export of the adhesin to the bacterial surface. GspB binds to platelets via its interaction with sialoglycans on the platelet receptor glycoprotein Ib.[97,98] Subsequent studies have shown that the GspB-accessory Sec locus is highly conserved in other species of oral streptococci implicated as causes of IE, including *S. sanguinis*, *Streptococcus mitis*, and *Streptococcus oralis*.[99] Most homologues of GspB in these organisms also appear to be sialoglycan-binding adhesins, but they can differ in the type and range of sialylated glycan bound.[99,100] Loss of GspB expression was linked with decreased virulence in experimental IE.[101] Similar results have been reported for Hsa, a homologue of GspB expressed by another strain of *S. gordonii*,[102,103] indicating that at least some of the SRR adhesins are conserved virulence factors in IE. Of note, several other SRR adhesins of gram-positive pathogens have been linked to virulence in the setting of IE and other infections.[104–107]

A second mechanism for the binding of viridans-group streptococci to platelets is through the surface expression of bacteriophage-encoded proteins. Studies with *S. mitis/oralis* strain SF100 found that three proteins of a lysogenic phage (SM1) enhanced platelet binding in vitro and contributed to virulence for IE.[108,109] PblA is a "tape measure protein" (important for phage morphogenesis), whereas PblB is a tail fiber protein.

Both proteins are linked to the bacterial surface through their interactions with choline groups within the cell wall. In addition, the phage lysin protein becomes similarly associated with the cell wall, and mediates platelet binding through its interaction with fibrinogen.[110,111] One puzzling issue was how these bifunctional phage proteins exit the bacterium and adhere to its surface. It appears that there is some constitutive, low-level expression of the phage lytic cycle, which results in the expression of phage holin and lysin.[112] The net effect is to render the bacteria more permeable, so that PblA, PblB, and lysin leak from the bacteria and then bind back to the surface, where they can serve as adhesins. This is a remarkable system, because it is perhaps the first example of a phage-encoded bacterial surface structure and adhesin, and because it suggests a coadaptation or coevolution of the bacterium and the phage, wherein the bacterium is essential for phage replication and the phage gives the bacterium a selective advantage by providing it with a critical adhesin to colonize cognate ligands on platelets.

Adhesion to Nonbacterial Thrombotic Endocarditis by Other Organisms

A similar important role of adhesion to NBTE in the pathogenesis of IE has been shown for yeasts. *Candida albicans* adheres to NBTE in vitro and produces IE in rabbits more readily than does *Candida krusei,* a nonadherent yeast rarely implicated in IE in humans.[113] Although microbial adhesion is a crucial early event in the pathogenesis of IE, the precise intracardiac loci are unknown and may differ among organisms. Most organisms probably adhere initially to a constituent of NBTE; some evidence implicates fibronectin as the host receptor within NBTE.[114] More recent studies[115,116] have supported this concept. Low–fibronectin-binding mutants of *S. aureus* and *S. sanguinis* had decreased ability to produce IE in rats, compared with high–fibronectin-binding parent strains. Other normal constituents of damaged endothelium or NBTE (e.g., fibrinogen, laminin, type IV collagen[117]) also may serve to bind circulating bacteria. *Abiotrophia defectiva,* a major species isolated in cases of IE caused by *Abiotrophia* and *Granulicatella* spp.[118] (previously referred to as nutritionally variant streptococci, and discussed later), bound the extracellular matrix of fibroblasts and endothelial cells in a saturable-specific manner, whereas *Granulicatella adiacens* and other *Granulicatella* strains did not bind.[119] A study also documented binding of *S. mutans, S. mitis, S. sanguinis,* and *Enterococcus faecalis* to this extracellular matrix. Laminin-binding proteins (e.g., a 145-kDa protein found in *S. gordonii*) were identified on the cell walls of organisms recovered from patients with IE,[120] and the level of protein expression seemed to be regulated by the presence of extracellular matrix proteins.

Pathogenesis of *Staphylococcus aureus* Infective Endocarditis

Other organisms may bind directly to, or become ingested by, endothelial cells as the initial event.[121–124] This sequence appears to be important in the initiation of IE by *S. aureus* on "normal" cardiac valves or on native endothelial surfaces adjacent to damaged endothelial sites. Many studies in experimental IE using *S. aureus* as the study organism have shed additional light on the importance of microbial binding to specific matrix proteins found within the NBTE lesion on the development of IE. It seems that the key adhesin possessed by the organism for induction of IE is one or more of its several fibrinogen-binding proteins (e.g., clumping factor, coagulase[125,126]). Adhesins for other matrix molecules (e.g., fibronectin, collagen, thrombospondin[127–129]) are not involved pivotally in initial attachment of the organism to damaged endothelium but are crucial in persistence of the microbe at this site. Additional virulence factors produced by this organism (α-toxin[130]) have been identified in the experimental IE model as important for persistence and proliferation of the organism within maturing vegetations in the next stage of infection, after valvular colonization. The fibronectin-binding adhesins of *S. aureus* seem to be crucial in the ability of the organism to invade cardiac endothelium and induce endothelial apoptosis,[131–133] although the specific microbial surface–host receptor ligand relationship remains incompletely defined for all the major IE pathogens. This is an active area of investigation, because inhibition of these events may provide novel prophylactic strategies.

Recently, the expression, structure, and binding activity of the two major *S. aureus* fibronectin-binding proteins (FnBPA, FnBPB) were determined in clinical isolates from patients with persistent bacteremia (frequently IE) or resolving bacteremia.[134] The persistent bacteremia isolates formed significantly stronger bonds with immobilized fibronectin as determined by dynamic binding measurements performed with atomic force microscopy. Several notable differences were also observed when the results were grouped by clonal complex 5 (CC5) strains versus CC45 strains. Fibronectin-binding receptors on CC5 formed stronger bonds with immobilized fibronectin. The *fnbA* gene was expressed at higher levels in CC45, whereas *fnbB* was found in only CC5 isolates. Sequencing of *fnbA* revealed discrete differences within high-affinity, fibronectin-binding repeats (FnBRs) of FnBPA that included (1) 5-amino-acid polymorphisms in FnBR-9, FnBR-10, and FnBR-11 involving charged or polar side chains; (2) an extra, 38-amino-acid repeat inserted between FnBR-9 and FnBR-10 exclusively seen in CC45 isolates; and (3) CC5 isolates that had the SVDFEED epitope in FnBR-11 (a sequence shown to be essential for fibronectin binding), whereas this sequence was replaced in all CC45 isolates with GIDFVED (a motif known to favor host cell invasion at the cost of reduced fibronectin binding). These complementary sequence and binding data suggest that differences in *fnbA* and *fnbB,* particularly polymorphisms and duplications in FnBPA, give *S. aureus* two distinct advantages in human endovascular infections: (1) FnBPs similar to that of CC5 enhance ligand binding and foster initiation of disease, and (2) CC45-like FnBPs promote cell invasion, a key attribute in persistent endovascular infections.

Effect of Antibiotics on Development of Infective Endocarditis

The importance of adherence characteristics in the development of IE also has been examined through the use of preincubation of organisms with antibiotics. Many classes of drugs, after incubation even at subinhibitory concentrations, decrease the adhesion of streptococcal species to fibrin-platelet matrices and damaged canine valves in vitro.[135] Several elegant studies in animal models verified the significance of this in vitro observation: preincubation of the organism in subinhibitory antibiotic concentrations prevented the development of IE in vivo.[136,137] This finding has direct relevance to the chemoprophylactic prevention of IE (see Chapter 83). In one study, subinhibitory concentrations of penicillin were found to result in a loss of streptococcal lipoteichoic acid, with reduced adhesion to NBTE-involved tissue and an impaired ability to produce IE in vivo.[138] Antibiotics may prevent IE by at least two mechanisms: bacterial killing and inhibition of adhesion to NBTE-involved tissue.[139]

Platelets and the Pathogenesis of Infective Endocarditis

Because platelets and fibrin are the major constituents of NBTE, the role of the platelet in the pathogenesis of IE also has been studied. Some strains of bacteria have been found to be potent stimulators of platelet aggregation and the release reaction.[140] In general, IE-producing strains of staphylococci and streptococci more actively aggregate platelets than do other bacteria that less frequently produce IE. Bacteria-platelet aggregates have been found in the peripheral blood of patients with bacteremia. The importance of these aggregates in the formation of the vegetation (or, conversely, the effect of the aggregation on the rate of removal of organisms from the circulation) is unknown. In one study, even small numbers of platelets greatly increased the adherence of oral streptococci to fibrin in vitro.[81] Other studies[141] showed that *S. sanguinis,* an important cause of IE, aggregates platelets and adheres to these blood components by means of protease-sensitive components, not dextrans. A platelet receptor for ligands on certain strains of *S. sanguinis* was suggested. However, this platelet aggregation by viridans streptococci requires direct platelet binding and plasma components.[142] Other experiments implicated immunoglobulin G (IgG) in this specific streptococcal bacteria-platelet interaction and suggested that platelet activation is mediated through the platelet surface Fc receptor, with a molecular weight of 40,000 daltons.[143]

After colonization of the valve occurs and a critical mass of adherent bacteria develops, the vegetation enlarges by further platelet-fibrin

deposition and continued bacterial proliferation. There is a complex interplay among factors responsible for bacteria-platelet adhesion and aggregation. The ability of *S. sanguinis* to induce platelet aggregation in vitro is conferred by two bacterial cell surface antigens: (1) class I antigen, which promotes adhesion of *S. sanguinis* to platelets (adh^+), and (2) coexpression of class II antigen, which promotes platelet adhesion or platelet aggregation (agg^+). At least nine adh/agg phenotypes have been identified among naturally occurring variants, reflecting a range of platelet interactivity. Intravenous inoculation of agg^+ *S. sanguinis* strains into rabbits with catheter-induced aortic valve trauma led to larger vegetations, a more severe clinical course, more gross lesions in major organs, and greater mortality than inoculation with an agg^- strain or with the agg^+ strains pretreated with Fab fragments specific for the platelet interactivity phenotype.[144] Platelet aggregation induced by *S. sanguinis* in vivo seems to be an important virulence determinant of vegetation development and disease progression. Streptococcal exopolysaccharide production inversely correlates with platelet adhesion while inhibiting aggregation,[145] indicating that these surface molecules may enhance endocarditis at some pathogenic steps but not at others.

The manner in which *S. aureus* interacts with platelets in the pathogenesis of IE differs substantially from that of the viridans streptococci. This interaction does not require the presence of specific antistaphylococcal antibody and is not amplified by the platelet Fc receptor.[146] Platelet–*S. aureus* interactions for executing aggregation require fibrinogen as a bridging molecule but are independent of the primary platelet fibrinogen-binding site, the glycoprotein IIb/IIIa integrin receptor. In addition, it seems that *S. aureus* can bind to platelets via platelet-derived von Willebrand factor or directly to von Willebrand factor receptor, at von Willebrand factor–binding domain.[147–149] In addition, platelet surface–expressed gC1qR can serve as a key "docking site" for staphylococci, predominantly through bridging molecules such as protein A and von Willebrand factor.[150] In experimental IE, transposon inactivation of the putative *S. aureus* platelet-binding adhesin gene resulted in mutants with diminished capacity to adhere to platelets in vitro in either suspension or surface-bound monolayers.[151] In experimental IE caused by such low–platelet-binding mutants, the induction rates of IE were equivalent to the induction rates of the parental strain, presumably because of the microbe's ability to attach to damaged endothelium by multiple adhesive mechanisms. However, the capacity of the mutant to persist and proliferate within experimental vegetations and to disseminate hematogenously to the kidneys was markedly impaired in the mutant strain.[151] This transposon defect was found to reside within the staphylococcal fibrinogen adhesin gene, clumping factor A *(clfA)*.[152]

Platelets also may play a role in host defense within the cardiac vegetation during IE.[153] It is underappreciated that platelets can actually phagocytose circulating staphylococci into engulfment vacuoles, in which the organism can persist.[154] Moreover, after specific exposure to thrombin (which is plentiful at the surface of damaged endothelium), release of α-granule–derived platelet microbicidal proteins (PMPs) or thrombocidins with bactericidal activity against most gram-positive cocci that cause IE has been shown.[155] PMPs appear to be homologues of platelet factor 4,[156] whereas thrombocidins evolve from the platelet basic peptide lineage and are truncates of the chemokines NAP-2 and CTAP-3.[157] Although the ability of *S. aureus* to adhere to and aggregate platelets is a related property, the resistance to PMPs is an independent phenotypic characteristic and a potential virulence factor.[158] PMPs are low-molecular-weight (8–10 kDa) cationic proteins that act primarily on the bacterial cell membrane or cell wall, synergistically with antibiotics, to kill bacteria. PMPs also have shown fungicidal activity against some yeasts in vitro.[159]

Microbial resistance to the microbicidal activity of PMPs may contribute to the pathogenesis of IE. This hypothesis was supported by a reduction in vegetation weight and bacterial concentration in rabbits with experimental aortic valve *S. aureus* endocarditis after treatment with aspirin.[160] In addition, three studies in experimental IE confirmed the importance of the relationship of PMP resistance and the pathogenesis of IE. In experimental viridans streptococcal IE and *S. aureus* IE, PMP-resistant strains exhibited an enhanced capacity to persist at sites of valvular damage.[161,162] In addition, *S. aureus* strains exhibiting the PMP resistance phenotype in vitro were able to proliferate within the vegetation and hematogenously seeded extracardiac foci (kidneys, spleen) to a significantly greater extent than their isogenic counterparts, which were PMP susceptible in vitro.[163,164]

Several clinical studies also emphasized the important association between PMP resistance and the pathogenesis of intravascular infections. Bloodstream isolates of viridans streptococci and *S. aureus* from patients with IE tended to be substantially more resistant to PMPs in vitro.[162] *S. aureus* bloodstream isolates arising from an intravascular source (catheter or IE) were significantly more resistant than bloodstream isolates arising from a deep tissue source.[165] Furthermore, PMP-resistant *S. aureus* bloodstream isolates from patients with IE were significantly more likely to have arisen from an intravascular catheter source.[166] In addition, data have shown fusion of PMP-rich α-granules with platelet engulfment vacuoles noted earlier, implicating the intrinsic PMP-resistance phenotypes of engulfed staphylococci in their ultimate intraplatelet survival outcomes.[154] Should the engulfed organism be intrinsically PMP-resistant, the platelet could serve as a "Trojan horse" vehicle to disseminate the organism. In contrast, if the phagocytosed staphylococci were PMP susceptible, this mechanism could eliminate the organism from the circulation.[154] Lastly, when methicillin-resistant *S. aureus* (MRSA) isolates derived from patients with "persistent bacteremia" (>7 days of positive blood cultures despite in vitro susceptibility to the antibiotic being administered [usually vancomycin]) are examined, such strains tend to be more PMP resistant than MRSA from patients with rapidly resolving bacteremia.[167,168] Not surprisingly, such "persistent bacteremia" patient populations are enriched for underlying IE.[169]

Interactions Between Bacteria and the Growing Vegetation

In IE, the bacterial colonies are found beneath the surface of the vegetation (at variable depth, depending on the intracardiac location[170]) and infiltration by phagocytic cells is minimal; the vegetation creates an environment of impaired host resistance. These conditions allow for relatively unbridled bacterial growth, resulting in extremely high colony counts of 10^9 to 10^{11} bacteria per 1 g of tissue. Bacteria deep within the fibrin matrix have been shown by autoradiography to reach a state of reduced metabolic activity.[171] Studies by Yersin[172] and Meddens[173] and their colleagues suggested that impairment of host defenses (e.g., neutropenia, corticosteroids) potentiates progression of the disease when the tricuspid but not the aortic valve is involved but is largely dependent on the intracardiac location of the vegetation.[174]

The role of granulocytes within the vegetation is unknown. When vegetation formation is retarded with anticoagulants in experimental animals with IE, the organisms seem to divide on the surface, total bacterial titers are lower, and the clinical disease is more explosive.[175,176] In addition, it has been suggested that phagocytosis of microorganisms by monocytes on or within the vegetation generates tissue thromboplastin formation; thromboplastin then acts as a stimulant to fibrin deposition and growth of the vegetation.[177] However, the best evidence suggests that coagulation activation initiated by tissue factor,[178] with subsequent local thrombus formation, is responsible for the initiation of vegetation growth and persistence on the cardiac valve. It seems that some organisms (e.g., *S. aureus*) induce tissue factor production by endothelium without the necessity for host cytokines.[179]

Many important studies have elucidated the interactions among the invading microbe, the endothelium, and the monocyte in the pathogenesis of IE. After internalization by endothelial cells in vitro, microbes such as *S. aureus* evoke a potent proinflammatory chemokine response, including, for example, increased expression of interleukin (IL)-6 or IL-8 or of monocyte chemotactic peptide.[180,181] This event also is associated with increased expression on the endothelial cell surface of several key adhesion molecules, especially intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).[124,180,181] Among other cells, monocytes are drawn into this endothelial cell microenvironment; via their appropriate counterreceptors, monocytes can bind avidly to such microbe-activated endothelial cells.[181] Extracellular bacteria circulating in the vascular system then bind directly to the monocyte surface, inducing the release of tissue thromboplastin (tissue factor).[182,183] This latter molecule participates in the catalytic conversion

of prothrombin to thrombin, amplifying the procoagulant cascade at the site of endothelial cell colonization and leading to progressive evolution of the vegetative lesion in IE. Several studies have emphasized that organisms with low protease production (e.g., enterococci) are associated with larger, more friable vegetations with an increased tendency for embolization. This property was underscored in an elegant animal study by inducing IE with an enterococcus with low proteolytic activity and virulence to analyze the role of host proteases in vegetation growth. Matrix metalloprotease 9, elastase, and plasminogen activators were all present at higher concentrations in septic vegetations. These results suggest that the continuous attractant signals coming from bacterial colonies can result in chronic injury of myocardial tissues by host proteases.

Immunopathologic Factors

IE results in stimulation of humoral and cellular immunity, as manifested by hypergammaglobulinemia, splenomegaly, and the presence of macrophages in the peripheral blood. The possibility that preformed antibody can increase the likelihood of the development of IE was suggested by the spontaneous occurrence of IE in horses receiving repeated immunizations with live pneumococci.[184] It was suggested that these antibodies produced bacterial agglutination in vivo, which increased the chances of valvular colonization. Studies in animals have suggested a protective role for circulating antibody. Rabbits preimmunized with heat-killed streptococci plus Freund adjuvant had a significantly higher ID_{50} than that noted for nonimmunized controls after aortic valve trauma.[185] Other studies yielded similar results with *S. sanguinis, S. mutans,* and *S. pneumoniae.*[186,187] In additional experiments, antibody directed against cell surface components (including mannan) reduced the adhesion of *C. albicans* to fibrin and platelets in vitro and reduced IE production in vivo.[188] This effect may depend on the infecting organism, however, because antibody to *S. epidermidis* or *S. aureus* did not prevent the development of IE in immunized animals and did not result in reduced bacterial concentrations in infected vegetations or kidneys,[189] perhaps because of the inability of immune sera to enhance opsonophagocytosis of staphylococci. The role of preformed antibody in the pathogenesis of IE is unclear. Intravascular agglutination of bacteria may decrease the frequency of IE by reducing the actual number of circulating organisms, but cross-protection was not achieved with passive transfer of high-titer immune globulin from *Streptococcus defectivus*–immunized rabbits to control animals.[187] Nitrogen mustard–treated immunized rabbits lost their ability to clear *S. defectivus* efficiently from the circulation, a process partially restored by neutrophil transfusion.[190]

The role of the glycocalyx of *S. aureus,* and of antibodies directed against this exopolysaccharide, in the pathogenesis of IE is controversial. Most experimental studies suggest that microencapsulation of strains by the common capsular types (5 and 8) may mitigate virulence of the organism in IE by obscuring key surface-expressed adhesins involved in colonization or persistence at endovascular damage sites.[191]

Several more recent studies suggested a salutary effect of active or passive immunization strategies against this glycocalyx in diminishing the induction, progression, or metastatic infection phases of experimental IE.[192,193] However, large clinical trials have not been able to document salutary outcomes using active or passive immunization strategies directed against the staphylococcal capsule or candidate surface adhesins (e.g., clumping factor).[194–197]

One relatively unexplored area of pathogenesis of IE is dietary impacts on susceptibility to this infection. It is known that diet, and specifically dietary metals, can modify the risk of infection. However, for example, the mechanisms by which manganese (Mn), a common dietary supplement, alters infection remain undefined. The Skaar laboratory[198] has reported that dietary Mn levels can dictate the outcome of experimental *S. aureus* cardiac infections. Mice fed a high-Mn diet displayed increased Mn levels, with preferential localization in and around cardiac abscesses. Although the canonical mammalian Mn-sequestering protein calprotectin surrounded such lesions, it was not released into the abscess nidus and did not limit Mn within cardiac abscesses. Consequently, excess Mn was bioavailable to the organism in the depths of these lesions. Bioavailable Mn is used by *S. aureus* to detoxify reactive oxygen species and protect the organism from neutrophil killing. Therefore a single dietary modification (i.e., Mn restriction) may be able to help amplify host antistaphylococcal defenses in patients at risk for *S. aureus* IE. Of interest, companies are focusing vaccine efforts to include components designed to raise antibodies against the *S. aureus* Mn receptor.[199]

Rheumatoid factor (anti-IgG IgM antibody) develops in about 50% of patients with IE of longer than 6 weeks' duration.[200] Rheumatoid factors were found at the time of admission in 24% of patients with acute staphylococcal IE (<6 weeks' duration), and the frequency increased to 40% if fever persisted for 2 weeks after the initiation of antibiotic therapy.[201] More than two-thirds of the patients became seronegative after 6 weeks of therapy, and two patients with a second episode of acute IE promptly redeveloped positive rheumatoid factors. The titers correlated with the level of hypergammaglobulinemia and decreased with therapy. Rheumatoid factor may play a role in the disease process by blocking IgG opsonic activity (i.e., by reacting with the Fc fragment), stimulating phagocytosis, or accelerating microvascular damage. Rheumatoid factor (IgM) has not been eluted from the immune complex glomerulonephritis associated with IE.[202] Antinuclear antibodies also occur in IE and may contribute to the musculoskeletal manifestations, low-grade fever, or pleuritic pain.[203]

Similar to malaria, schistosomiasis, syphilis, kala-azar, and leprosy, IE is associated with a constant intravascular antigenic challenge, and the development of several classes of circulating antibody is not unexpected. Opsonic (IgG), agglutinating (IgG, IgM), and complement-fixing (IgG, IgM) antibodies and cryoglobulins (IgG, IgM, IgA, C3, fibrinogen); various antibodies to bacterial heat-shock proteins; and macroglobulins all have been described in IE.[204–206] Antineutrophil cytoplasmic antibodies (ANCAs) were present in 12 of 50 patients (24%) in a recent series and were associated with longer duration of symptoms before IE diagnosis.[207] Circulating immune complexes have been found in high titers in almost all patients with IE.[208] Circulating immune complexes are found with increased frequency in connection with a long duration of illness, extravalvular manifestations, hypocomplementemia, and right-sided IE. Levels decrease and become undetectable with successful therapy. Patients with IE and circulating immune complexes may develop a diffuse glomerulonephritis that is analogous to the nephritis seen with infected ventriculoatrial shunts.[209] Corticosteroids have been used in a few patients with glomerulonephritis associated with IE.[210] Immune complexes plus complement are deposited subepithelially along the glomerular basement membrane to form a "lumpy-bumpy" pattern. Immune globulin eluted from these lesions has been shown to cross react with bacterial antigens.[211] In addition, bacterial antigens have been shown within circulating immune complexes.[212]

Some of the peripheral manifestations of IE, such as Osler nodes, also may result from a deposition of circulating immune complexes. Pathologically, these lesions resemble an acute Arthus reaction. The finding of positive culture aspirates in Osler nodes[213] suggests, however, that they may be caused by septic emboli rather than immune complex deposition. In some diffuse purpuric lesions in IE, immune complex deposits (IgG, IgM, and complement) have been shown in the dermal blood vessels by immunofluorescence.[214] Quantitative determinations of serum immune complex concentrations are useful in gauging the response to therapy. Effective treatment leads to a prompt decrease, with eventual disappearance of circulating immune complexes.[215] Conversely, therapeutic failures or relapses are characterized by rising titers or a reappearance of circulating immune complexes.[216]

Pathologic Changes

Heart

The classic vegetation of IE usually is located along the line of closure of a valve leaflet on the atrial surface of atrioventricular valves or on the ventricular surface of semilunar valves. Vegetations may be single or multiple; they are a few millimeters to several centimeters in size and vary in color, consistency, and gross appearance. Microscopically, the lesion consists primarily of fibrin, platelet aggregates, and bacterial masses; neutrophils and red blood cells are rare. Killed bacteria detectable with Gram stain within these vegetations sometimes persist for months after therapy.[217] Destruction of the underlying valve may coexist. With treatment, healing occurs by fibrosis and occasionally calcification. The

vegetation in acute cases is larger, softer, and more friable and may be associated with suppuration, more necrosis, and less healing than in subacute cases.[46,218]

This infection may lead to perforation of the valve leaflet or rupture of the chordae tendineae, interventricular septum, or papillary muscle. Staphylococcal IE frequently results in valve ring abscesses[219] with fistula formation into areas of the myocardium or pericardial sac. Aneurysm of the valve leaflet or sinus of Valsalva also is common. Valvular stenosis may result from large vegetations. Myocarditis, myocardial infarction, and pericarditis[218,219] are found frequently at autopsy. Myocardial abscesses are found in 20% of the autopsy cases and are associated primarily with acute staphylococcal IE with hectic fever, a rapid onset of CHF, and conduction disturbances. Myocardial infarcts are found in 40% to 60% of the autopsied cases, often without diagnostic changes on the electrocardiogram. Pericarditis is much more common in patients with acute IE.

Echocardiographic abnormalities are commonly observed in patients with acquired immunodeficiency syndrome (AIDS), but pericardial disease (pericarditis, effusions), myocardial disease leading to heart failure or arrhythmias, NBTE, and Kaposi sarcoma all are generally more frequent than IE. AIDS patients with IE usually are injection drug users with right-sided involvement and have an increased prevalence of cases due to *S. aureus* or fungi.[220] The clinical course in AIDS patients often is more fulminant than that of IE in injection drug users without AIDS; pneumonia and sepsis are common. IE also has been described in the transplanted heart.[221]

Embolic phenomena are common in IE. In the preantibiotic era, 70% to 95% of patients had clinically demonstrable embolic events, but this has decreased to 15% to 35% today. Pathologic evidence of embolization still is detected in 45% to 65% of autopsies, most frequently involving the renal, splenic, coronary, or cerebral circulation. Emboli and immune complex deposition contribute to the extracardiac manifestations of IE and may involve almost any organ system. If large emboli occlude major vessels, fungal endocarditis, marantic endocarditis, or an intracardiac myxoma should be suspected.

Kidney

Three pathologic processes may be found in the kidney in patients with IE: abscess, infarction, and glomerulonephritis. Abscesses are uncommon, but infarctions have been seen in 56% of the autopsy cases.[2] The kidney usually is normal in size but may be slightly swollen, and petechiae may be found in the capsule. When renal biopsy specimens are obtained during active IE, the renal architecture is abnormal in *all* cases,[222] even in the absence of clinical or biochemical evidence of renal disease. "Focal" glomerulonephritis is found in 48% to 88% of the cases but is rare in acute IE. It is a focal, local, and segmental process characterized by endothelial and mesangial proliferation, hemorrhage, neutrophilic infiltration, fibrinoid necrosis, crescent formation, and healing by fibrosis. Diffuse glomerulonephritis is found in 17% to 80% of the cases and consists of generalized cellular hyperplasia in all glomerular tufts. A less common condition, termed *membranoproliferative glomerulonephritis,* is associated with IE due to *S. epidermidis* and is characterized by marked mesangial proliferation and by splitting of the glomerular basement membrane. Renal interstitial cellular infiltration is common.[222]

Between 10% and 15% of patients with IE exhibit an immune complex glomerulonephritis similar to that seen in SLE.[209,211,215,216] The evidence for immune complex deposition rather than a recurrent embolic phenomenon as the primary pathogenic mechanism includes the following:

1. Bacteria are rarely, if ever, seen in the lesions.
2. Glomerulonephritis can occur with right-sided IE.
3. Glomerulonephritis is rare in acute IE, even though large, friable vegetations result in widespread metastatic abscess formation.
4. Immunofluorescent staining with antiimmunoglobulin antibody reveals the typical lumpy-bumpy distribution seen in other forms of immune complex nephritis.
5. In diffuse glomerulonephritis, subepithelial electron-dense deposits are seen with electron microscopy and IgG, IgM, IgA, or complement is shown in these deposits with immunofluorescence.
6. Specific antibacterial antibody can be eluted from the lesions.[211]
7. Anti–glomerular basement membrane antibody has been found in a single case of IE with nephritis.
8. The glomerulonephritis often is accompanied by hypocomplementemia, with a positive result on serum assay for rheumatoid factor.
9. All of these abnormalities usually resolve with successful antimicrobial therapy as the concentration of circulating immune complexes declines.

Mycotic Aneurysms

Mycotic aneurysms usually develop during active IE but occasionally are detected months or years after successful treatment. They are more common with viridans streptococcal infections and are found in 10% to 15% of autopsied cases. They may arise by any of several mechanisms: (1) direct bacterial invasion of the arterial wall with subsequent abscess formation or rupture, (2) septic or bland embolic occlusion of the vasa vasorum, or (3) immune complex deposition with resultant injury to the arterial wall. The aneurysms tend to occur at bifurcation points. They are found most commonly in the cerebral vessels (primarily the peripheral branches of the middle cerebral artery), but they also occur in the abdominal aorta; the sinus of Valsalva; a ligated patent ductus arteriosus; and the splenic, coronary, pulmonary, and superior mesenteric arteries. Mycotic aneurysms usually are clinically silent until rupture occurs; consequently, their true incidence in active IE is unknown.[223] Magnetic resonance angiography is a sensitive method for detection of mycotic aneurysms. For example, a prospective single-center study that evaluated 130 consecutive IE patients with cerebral magnetic resonance imaging (MRI) with angiography regardless of symptoms found 10 additional aneurysms that were clinically silent.[224]

Central Nervous System

Cerebral emboli are the most common neurologic manifestation of IE.[225] Although 20% to 30% of patients with IE have clinically apparent cerebral emboli, the actual rate of cerebrovascular complications is significantly higher. In a study from Sweden, patients with left-sided IE were prospectively evaluated with cerebral MRI regardless of neurologic symptoms. The total cerebrovascular complication rate was 65%, including 35% that were symptomatic and 30% that were clinically silent.[226] This finding was externally validated in a French study that prospectively evaluated 130 IE patients with cerebral MRI with angiography within 7 days of admission and prior to any potential surgical intervention. MRI identified cerebral lesions in 82% of patients and led to changes in the diagnostic or therapeutic plan in 28% of the study patients.[224] A multinational, prospective cohort investigation of 1437 cases of definite left-sided IE found that the crude incidence of stroke in patients receiving appropriate antimicrobial therapy was 4.82 cases per 1000 patient-days in the first week and fell to 1.71 per 1000 patient-days in the second week.[227] The middle cerebral artery and its branches are involved most commonly.[46] Three percent of the cerebral emboli from all causes are secondary to IE. Cerebral infarction, arteritis, abscesses, mycotic aneurysms, intracerebral or subarachnoid hemorrhage, encephalomalacia, cerebritis, and meningitis have been reported.[228] Hemorrhagic transformation of an ischemic infarct due to septic emboli is the most common mechanism leading to fatal intracerebral hemorrhage during IE.[229] True acute purulent meningitis is rare except in pneumococcal endocarditis, but multiple microabscesses (cerebritis) due to *S. aureus* are relatively common in acute staphylococcal IE.

Spleen

Splenic infarctions have been reported in 44% of autopsy cases but often are clinically silent.[46] Splenic abscesses are an uncommon complication of IE and typically manifest as fever, left upper quadrant abdominal pain, and leukocytosis. Diagnosis is established with computed tomography (CT) or ultrasonography.[230,231] Although splenectomy is a standard therapy for splenic abscess, percutaneous drainage may be an alternative in selected patients.[232] Splenic enlargement is common, and virtually all cases are associated with hyperplasia of the lymphoid follicles, an increase in secondary follicles, proliferation of reticuloendothelial cells, and scattered focal necrosis.[218] Spontaneous rupture of the spleen has occasionally been observed.

Lung

When right-sided IE is present, pulmonary embolism with or without infarction, acute pneumonia, pleural effusion, or empyema is common. These septic pulmonary emboli commonly manifest on chest radiographs as rounded, "cannonball" lesions. Emboli may be septic or bland.[220]

Skin

Petechiae are found in 20% to 40% of cases (Fig. 80.2) (see later discussion). Osler nodes consist microscopically of arteriolar intimal proliferation with extension to venules and capillaries and may be accompanied by thrombosis and necrosis. A diffuse perivascular infiltrate consisting of neutrophils and monocytes surrounds the dermal vessels. Immune complexes have been shown in the dermal vessels. Janeway lesions consist of bacteria, neutrophilic infiltration, necrosis, and subcutaneous hemorrhage (Fig. 80.3). Janeway lesions (see later description) are caused by septic emboli and reveal subcutaneous abscesses at histologic examination.[233]

Eye

Roth spots consist microscopically of lymphocytes surrounded by edema and hemorrhage in the nerve fiber layer of the retina (Fig. 80.4).[234]

Clinical Manifestations

The interval between an event likely to produce high-grade bacteremia and the onset of symptoms of IE, contrary to older estimates, is quite short. The so-called incubation period in 84% of 76 cases of streptococcal IE was less than 2 weeks.[235] On the other hand, the time from onset of symptoms to diagnosis in the subacute form of IE is quite long, with a median interval of approximately 5 weeks. Symptom duration of cases managed in community hospitals is often shorter than in patients referred to a tertiary care center, reflecting referral bias.[4]

The symptoms and signs of IE (Table 80.3) can be protean, and essentially any organ system may be involved. Four processes contribute to the clinical picture[46]: (1) the infectious process on the valve, including the local intracardiac complications; (2) bland or septic embolization to virtually any organ; (3) constant bacteremia, often with metastatic foci of infection; and (4) circulating immune complexes and other immunopathologic factors.[41–44,46] As a result, the clinical presentation of patients with IE is highly variable and the differential diagnosis often is broad. Because of its many manifestations, the diagnosis of IE may be delayed; occasionally, it is not clinically suspected and is identified only at postmortem examination.[236–238] In a recent postmortem study of IE cases, in 38.2% IE was not diagnosed until autopsy. Important to note, Fernández Guerrero and colleagues found no significant difference in rates of "clinically occult" IE diagnoses before (1970–1985) and after (1986–2008) the availability of echocardiography at their institution (35% vs. 42.8%, respectively).[239] Fever is common but may be absent (5% of cases), especially in the setting of CHF, renal failure, a terminal disease, older age,[240,241] or previous antibiotic therapy. The fever pattern is usually remittent, and the patient's temperature rarely exceeds 40°C (104°F), except in acute IE. Persistent fever during antimicrobial therapy for IE is



FIG. 80.2 Conjunctival petechiae in a patient with bacterial endocarditis.



FIG. 80.3 Janeway lesions in a patient with *Staphylococcus aureus* endocarditis. *(From Sande MA, Strausbaugh LJ. Infective endocarditis. In: Hook EW, Mandell GL, Gwaltney JM Jr, et al, eds.* Current Concepts of Infectious Diseases. *New York: Wiley Press; 1977.)*

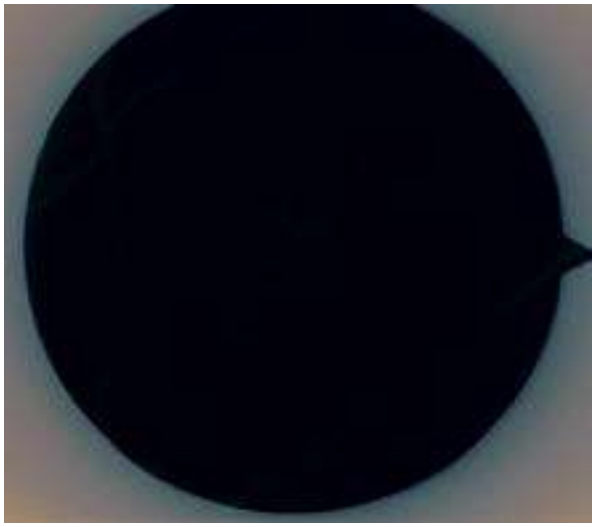

FIG. 80.4 Retina from a patient with viridans streptococcal endocarditis showing Roth spots. *(From Sande MA, Strausbaugh LJ. Infective endocarditis. In: Hook EW, Mandell GL, Gwaltney JM Jr, et al, eds.* Current Concepts of Infectious Diseases. *New York: Wiley Press; 1977.)*

TABLE 80.3 Clinical Findings in More Than 2700 Patients With Definite Endocarditis

SIGNS	PATIENTS (%)
Fever	96
Heart murmur	85
Changing murmur	20
New murmur	48
Vascular embolic event	17
Osler nodes	3
Splinter hemorrhages	8
Janeway lesion	5
Splenomegaly	11
Roth spots	2
Hematuria	26
Conjunctival hemorrhage	5

Modified from Murdoch DR, Corey GR, Hoen B, et al. Clinical presentation, etiology, and outcome of patients with infective endocarditis in the 21st century: the International Collaboration on Endocarditis–Prospective Cohort Study. Arch Intern Med. *2009;169:463.*

relatively uncommon but may be an ominous sign. In a review[242] of 123 cases of IE managed in Cleveland from 1972 to 1984, approximately half of the patients became afebrile within 3 days after the initiation of antibiotics; approximately 75% had defervesced after 1 week of treatment and 90% did so after 2 weeks of treatment. Prolonged fever (of 2 weeks' duration) is associated with specific etiologic agents (i.e., *S. aureus,* gram-negative bacilli, fungi, culture-negative IE) and, perhaps more important, with microvascular phenomena, embolization of major vessels, intracardiac (e.g., myocardial abscess) or peripheral complications, tissue infarction, a need for cardiac surgery, and a higher mortality rate.[242,243] Pulmonary emboli (bland), drug reactions, and nosocomial infection unrelated to IE also are causes of prolonged fever in this patient population.

Nonspecific symptoms, such as anorexia, weight loss, malaise, fatigue, chills, weakness, nausea, vomiting, and night sweats, are common, especially in subacute cases. These nonspecific symptoms often result in an incorrect diagnosis of malignancy, collagen vascular disease, tuberculosis, or other chronic diseases.

Cardiac Murmurs

Traditionally, audible heart murmurs occurred in more than 85% of IE cases. However, recent changes in the epidemiology and microbiology of IE appear to have altered the presentation of IE. For example, in the ICE-PCS experience with more than 2700 prospectively identified IE patients, only 48% had a detectable "new" cardiac murmur, whereas a further 20% exhibited worsening of an existing murmur.[7] Moreover, murmurs may well be absent with right-sided or mural IE. When present, the classic "changing murmur" and "new regurgitant murmur" (usually aortic insufficiency) are diagnostically useful signs, often complicating IE caused by more virulent valvular pathogens such as *S. aureus,* group B streptococci, or pneumococci. New or changing murmurs are less common in elderly patients and often lead to diagnostic confusion.[240,244] More than 90% of patients who show a new regurgitant murmur develop CHF. CHF is the leading complication of IE.[245] Pericarditis is rare but, when present, usually is accompanied by myocardial abscess formation as a complication of staphylococcal infection. Although valvular regurgitation is the most important hemodynamic complication of IE, hemodynamically significant valvular obstruction requiring surgery may occur rarely, even without a prior history of valvular stenosis.[246]

Peripheral Manifestations of Infective Endocarditis

Although the classic peripheral manifestations previously were found in half of cases, their prevalence has decreased in recent years. Clubbing is present in 10% to 20% of patients with subacute IE, especially if the disease is of long duration, and may recede with therapy. The complete syndrome of hypertrophic osteoarthropathy is rare. Splinter hemorrhages are linear, red-to-brown streaks in the fingernails or toenails and are found commonly in IE. They are a nonspecific finding and are seen often in elderly patients and in patients with occupation-related trauma. These lesions are most suggestive of IE when they are located proximally in the nailbed. Petechiae are found in 20% to 40% of cases, particularly after a prolonged course, and usually appear in crops on the conjunctivae (see Fig. 80.2), buccal mucosa, palate, and extremities. These lesions initially are red and nonblanching but become brown and barely visible in 2 to 3 days. Petechiae may result from local vasculitis or from emboli. Osler nodes are small, painful, nodular lesions usually found in the pads of fingers or toes and occasionally in the thenar eminence. They are 2 to 15 mm and frequently are multiple and evanescent, disappearing in hours to days. Osler nodes are rare in acute cases of IE but occur in 10% to 25% of subacute cases. They are not specific for IE, because they may be seen in SLE, marantic endocarditis, hemolytic anemia, and gonococcal infections and in extremities with cannulated radial arteries. Janeway lesions (see Fig. 80.3) are hemorrhagic, painless macules with a predilection for the palms or soles. They persist for several days and are believed to be embolic in origin; they occur with greater frequency in staphylococcal IE. Roth spots (see Fig. 80.4) are oval, pale, retinal lesions surrounded by hemorrhage and usually are located near the optic disk. They occur in fewer than 5% of patients with IE and also may be found in patients with anemia, leukemia, or connective tissue disorders such as SLE.

Splenomegaly

The frequency of splenomegaly in IE patients has declined significantly in the current era. Among 2662 prospectively enrolled patients with definite IE in whom the finding was recorded, 11% had splenomegaly.[7] In part, this changing frequency may reflect the much shorter time to diagnosis and treatment today and the predominance of acute rather than subacute IE. Splenic septic emboli are common during IE, but localized signs and symptoms are absent in approximately 90% of patients with this complication.[247] Abdominal CT is highly sensitive and should be employed if prolonged fever or sepsis is present. Serial CT studies usually can be used to distinguish between bland septic emboli and splenic abscess.[248]

Musculoskeletal Manifestations

Musculoskeletal manifestations are common in IE. In a review of 192 cases,[249] 44% of patients had musculoskeletal symptoms. These symptoms usually occurred early in the disease and were the only initial complaint in 15% of cases. They included proximal oligoarticular or monarticular arthralgias (38%), lower-extremity monarticular or oligoarticular arthritis (31%), low back pain (23%), and diffuse myalgias (19%). The back pain may be severe, limiting movement, and may be the initial complaint in 5% to 10% of cases.[29] These findings may mimic those of rheumatic disease, resulting in a diagnostic delay.

Embolic Events

Major embolic episodes, as a group, are second only to CHF as a complication of IE and occur in almost one-fourth of cases.[7] Splenic artery emboli with infarction may result in left upper quadrant abdominal pain with radiation to the left shoulder, a splenic or pleural rub, or a left pleural effusion. Renal infarctions may be associated with microscopic or gross hematuria, but renal failure, hypertension, and edema are uncommon. Retinal artery emboli are rare (<2% of cases) and may manifest with a sudden complete loss of vision. A panophthalmitis has been reported with pneumococcal IE. Pulmonary emboli secondary to right-sided IE constitute a common feature in narcotic addicts (see later discussion). Coronary artery emboli usually arise from the aortic valve and may cause myocarditis with arrhythmias or myocardial infarction. This finding may be overlooked, especially given the time constraints of interventions such as thrombolytic therapy or angioplasty during acute myocardial infarction, resulting in serious complications in patients with IE presenting with an acute myocardial infarction.[250] Major vessel emboli (affecting the femoral, brachial, popliteal, or radial artery) are more common in fungal endocarditis.

Neurologic Manifestations

Neurologic manifestations occur in 20% to 40% of all IE cases. In IE cases admitted to intensive care units, however, neurologic manifestations are the most common IE complication, occurring in 55% of patients.[251] Patients with *S. aureus* IE, mitral valve IE, or nonneurologic embolic events are at increased risk for neurologic complications. Of patients with neurologic complications, 50% present with these signs and symptoms as the heralding features of their illness.[252,253] In a Finnish survey of 218 episodes of definite or possible IE, neurologic complications were identified in 55 episodes (25%), with an embolic event as the most common manifestation (23 of 55 patients, or 42%). Among critically ill IE patients, however, over half may have a neurologic complication.[251] In most episodes (76%), the neurologic manifestation was evident before antimicrobial treatment was started, being the first sign of IE in 47% of episodes.[254] The development of clinical neurologic deterioration during IE is associated with a twofold to fourfold increase in mortality for the implicated etiologic microbe.[215] Mycotic aneurysms of the cerebral circulation occur in 2% to 10% of cases. They usually are single, small, and peripheral and may lead to devastating subarachnoid hemorrhage. Other features include seizures, severe headache, visual changes (particularly homonymous hemianopsias), choreoathetoid movements, mononeuropathy, and cranial nerve palsies. Toxic encephalopathy with symptoms ranging from a mild change in personality to frank psychosis may occur, especially in elderly patients.

Renal Manifestations

Patients with IE may have symptoms of uremia. In the preantibiotic era, renal failure developed in 25% to 35% of the patients, but presently fewer than 10% are affected. When uremia does develop, diffuse glomerulonephritis with hypocomplementemia usually is found, but focal glomerulonephritis also has been implicated. Renal failure is more common with long-standing disease but usually is reversible with appropriate antimicrobial treatment alone. IE may be confused with thrombotic thrombocytopenic purpura if neurologic signs, fever, renal failure, anemia, and thrombocytopenia are present.[255]

Infective Endocarditis in Drug Addicts

Acute infection accounts for approximately 60% of hospital admissions among injection drug users, and IE is implicated in 5% to 15% of these episodes.[256] It has proved difficult to predict accurately the presence of IE in a febrile drug addict,[257] especially from history and physical examination findings alone,[258] although cocaine use by an injection drug user should heighten the suspicion of IE.[259] Cocaine was associated strongly with the presence of IE in 102 injection drug users in San Francisco when findings were analyzed with logistic regression analysis, but no such correlation was found among febrile addicts who did not use cocaine. The most reliable predictors of IE in febrile parenteral drug users are visualization of vegetations with echocardiography and the presence of embolic phenomena.[258] Although many of the aforementioned clinical manifestations are seen in addicts with IE, several distinctions are noteworthy. In this group of patients, two-thirds have no clinical evidence of underlying heart disease and there is a predilection for the infection to affect the tricuspid valve. Only 35% of addicts who ultimately proved to have IE showed heart murmurs on admission.[256] The frequency of valvular involvement is as follows: tricuspid alone or in combination with others, 52.2%; aortic alone, 18.5%; mitral alone, 10.8%; and aortic plus mitral, 12.5%. Left-sided involvement has been more common in some series, however.[260]

Of patients with tricuspid valve infection, 30% have pleuritic chest pain; pulmonary findings may dominate the clinical picture, and the chest radiograph shows abnormalities (e.g., infiltrates, effusion) in 75% to 85% of the cases.[261] Radiographic evidence of septic pulmonary emboli is eventually present in 87% of cases.[262] Signs of tricuspid insufficiency (gallop rhythm, systolic regurgitant murmur louder with inspiration, large V waves, or a pulsatile liver) are present in only one-third of cases. Most of these patients are 20 to 40 years old (80%), and men predominate in a ratio of 4:1 to 6:1. Almost two-thirds of these patients have extravalvular sites of infection, which are helpful in the diagnosis.[261–263] The course of acute staphylococcal IE in an addict tends to be less severe than in nonaddicts,[261] although this may not be true in AIDS patients. HIV-seropositive patients acquire IE by one of two mechanisms: through injection drug abuse or as a complication of long-term central venous catheterization for administration of medications (e.g., for cytomegalovirus [CMV] retinitis). In either situation, *S. aureus* is the most common etiologic organism. IE is more common among injection drug users with advanced HIV immunosuppression ($CD4^+$ count, <200 cells/mm^3), even after accounting for injection drug use behaviors.[264] Conversely, among HIV-infected persons who do not practice injection drug use, IE is rare despite the increasing number of HIV-infected patients worldwide.[220] In the absence of injection drug abuse, HIV-seropositive patients develop left-sided and right-sided IE with equal frequency.[265] In contrast, in the setting of injection drug abuse, HIV-seropositive patients develop predominantly right-sided IE. The IE-related morbidity and mortality rates in HIV-seropositive patients who do not have an AIDS-defining illness or criteria are similar to the rates in HIV-seronegative counterparts.[265]

Laboratory Findings

Hematologic parameters often are abnormal in IE, but none is diagnostic. Anemia is almost always present (70%–90% of cases), especially in subacute IE, and has the characteristics of the anemia of chronic disease, with normochromic normocytic indices, a low serum iron concentration, and a low iron-binding capacity. The anemia tends to worsen with the duration of the illness. Thrombocytopenia occurs in 5% to 15% of cases but is common in neonatal IE. Leukocytosis is present in 20% to 30% of cases but is rare in the subacute variety, whereas counts of 15,000 to 25,000 cells/mm^3 are common in acute IE. The differential count usually is normal, but there may be a slight shift to the left. Leukopenia is uncommon (5%–15%); when present, it usually is associated with splenomegaly. Large mononuclear cells (histiocytes) can be detected in the peripheral blood in approximately 25% of patients, but the yield is higher in blood obtained by earlobe puncture. This finding is nonspecific, because similar cells have been found in malaria, typhus, typhoid fever, and tuberculosis.

The erythrocyte sedimentation rate is elevated in approximately 60% of contemporary IE cases.[7] In the absence of renal failure, CHF, or disseminated intravascular coagulation, a normal erythrocyte sedimentation rate is evidence against a diagnosis of IE. Hypergammaglobulinemia is detected in 20% to 30% of the cases and may be accompanied by a plasmacytosis in the bone marrow aspirate. A positive result on assay for rheumatoid factor is found in 40% to 50% of cases, especially when the duration of the illness is more than 6 weeks.[200] Hypocomplementemia (reported in 5%–15% of cases) parallels the incidence of abnormal renal function test results (elevated creatinine concentration in 5%–15%). A false-positive result on Venereal Disease Research Laboratory (VDRL) testing is uncommon (0.2%). Lyme serologic test results may be positive in patients with IE who are living in areas endemic for Lyme disease and may be reflective of remote exposure to the pathogen, leading to diagnostic confusion and delay.[266]

The urinalysis frequently is abnormal; proteinuria occurs in 50% to 65% of cases, and microscopic hematuria occurs in 30% to 60% of cases. Red blood cell casts may be seen in 12% of cases. Gross hematuria, pyuria, white blood cell casts, and bacteriuria also may be found.

Circulating immune complexes can be detected in most cases of IE but also are found in 32% of patients who have septicemia but no endocarditis, in 10% of healthy persons, and in 40% of noninfected narcotic addicts.[200] However, levels of aggregated human immune globulin equivalent to or greater than 100 μg/mL were found only in IE (35% of the cases). Detection of high levels of immune complexes may be useful in the diagnosis of right-sided IE in narcotic addicts or in culture-negative cases. In addition, because the levels decline with appropriate treatment, serial measurement of immune complexes may assist in management of the disease.[215,216] Mixed-type cryoglobulins are detectable in 84% to 95% of patients with IE, but this also constitutes a nonspecific finding. Serial determination of the serum C-reactive protein concentration, although nonspecific and virtually always elevated in IE, may be useful to monitor therapy and detect intercurrent complications or infections.[267]

A number of nuclear imaging studies have been evaluated in the diagnosis of IE, including gallium 67 (^{67}Ga) myocardial,[268] technetium 99m (^{99m}Tc)–labeled antibacterial antibody,[269] indium 111 (^{111}In)–labeled platelets[270]; and fluorine 18 (^{18}F)-fluorodeoxyglucose–labeled positron emission tomography.[271] This last technique has been recommended to enhance diagnostic acumen in cases of suspected prosthetic valve IE.[272]

The blood culture is the most important laboratory test performed in a diagnostic workup for IE. The bacteremia is usually continuous and low grade (80% of patients have <100 CFUs/mL of blood).[273] When bacteremia is present, the first two blood cultures yield the etiologic agent more than 90% of the time. In a prospective cohort of more than 2700 prospectively identified contemporary patients with definite IE, 88.8% had positive blood cultures.[7] In streptococcal endocarditis, the first blood culture was positive in 96% of cases, and one of the first two cultures was positive in 98%. When antibiotics had been administered during the previous 2 weeks, the rate of positive cultures declined from 97% to 91% ($P < .02$).[274] The influence of outpatient antibiotic administration on blood culture positivity was more significant in another retrospective analysis[275]; 64% of 88 cultures were positive in 17 patients receiving antibiotics before hospitalization, compared with 100% of cultures in 15 patients without antibiotic exposure. In nonstreptococcal IE, the first blood culture was positive in 86% of the cases; when two cultures were taken, the first culture was positive in 100%. Most blood cultures contained only a few organisms; more than 50% contained 1 to 30 bacteria per milliliter. Only 17% of the cultures yielded more than 100 bacteria per milliliter. The bacteremia also was constant, with little variation in quantitative culture determinations in any individual patient. The sensitivity of blood cultures for the detection of streptococci is particularly susceptible to prior antibiotic therapy and is affected by the media used.[276] Continuous-monitoring blood culture systems such as BACTEC (Becton Dickinson, Mississauga, Ontario) and BacT/ALERT (Organon Teknika, Scarborough, Ontario) are significantly more sensitive than conventional methods.

On the basis of these studies, the following procedures for culturing blood are recommended. At least three blood culture sets (no more than two bottles per venipuncture) should be obtained in the first 24 hours. More specimens may be necessary if the patient has received antibiotics during the preceding 2 weeks. At least 10 mL of blood (if feasible) should be injected into trypticase soy (or brain-heart infusion) and thioglycolate broth.[277,278] Supplementation with 15% sucrose (in an attempt to isolate cell wall–deficient forms) or the use of prereduced anaerobic media is unrewarding.[279] The newer commercial media are effective, but comparative data are few. In general, culture of arterial blood offers no advantage over use of venous blood. Inspection for macroscopic growth should be performed daily, and routine subcultures should be done on days 1 and 3. Specialized methods, and not extended incubation times, are recommended for recovery of fastidious agents of IE.[280] When gram-positive cocci grow on the initial isolation but fail to grow on subculture, *Abiotrophia* or *Granulicatella* spp. should be suspected.[281] In this event, subculture inoculation should be onto media supplemented with either 0.05% to 0.1% L-cysteine or 0.001% pyridoxal phosphate.

Ribitol teichoic acids are major constituents of the cell wall of staphylococci. Gel diffusion and counterimmunoelectrophoresis techniques have been used to detect teichoic acid antibodies in the serum of patients with suspected *S. aureus* IE. Because of problems with false-positive and false-negative results, this test rarely is used now.

Special Diagnostic Tests

Special diagnostic tests are not used routinely (with the exception of echocardiography) in all cases of IE but may be useful in the diagnostic approach to culture-negative IE and in decisions about surgical intervention during active infection. The incidence of so-called blood culture–negative endocarditis has ranged from 2.5% to 31% in published series.[282,283] If the patient has not received previous antibiotic therapy and the blood cultures are obtained as outlined, these cases should represent less than 5% of the total.[3] Some of the aforementioned tests (e.g., assays for rheumatoid factor and teichoic acid antibodies, examination of earlobe blood specimens for histiocytes) may be helpful in identifying such cases, but other procedures often are necessary. If the patient has received antibiotics, blood cultures in hypertonic media may allow detection of cell wall–defective organisms.

Supplementation of media with vitamin B_6 or with cysteine may assist in the recovery of *Abiotrophia* and *Granulicatella* spp. The lysis-centrifugation blood culture technique assists in the detection of staphylococci[284] and fungi, but *Abiotrophia* and *Granulicatella* spp. do not survive this procedure, and yields of pneumococci and anaerobes are decreased.[285] Routine use of this technique is not indicated, but it may be helpful in suspected culture-negative cases of IE. Because of improvements in blood culture media and automated blood culture incubation systems, extended incubation times of blood cultures are no longer necessary to recover HACEK organisms (see later discussion).[280,286] Special efforts to neutralize or inactivate antimicrobial agents present in blood, such as the addition of penicillinase or of antibiotic-removal resins, do not substantially enhance the yield of positive blood cultures in IE, and they increase the incidence of laboratory contamination of the blood culture. These maneuvers are not recommended routinely.[285]

Cultures of bone marrow or urine rarely may be positive when blood cultures are negative. Serologic studies are necessary for the diagnosis of Q fever or murine typhus.[287] Psittacosis endocarditis usually is diagnosed with serologic methods, but one case[288] yielded positive blood and pharyngeal cultures. Special culture techniques (e.g., for *Legionella* spp.[289]) are indicated in patients with suspected prosthetic valve IE if initial cultures are "negative."

Bartonella, recognized as an important cause of apparent blood culture–negative IE (especially in homeless alcoholic patients), also can be isolated by prolonged incubation and subculture of the aerobic broth media (see Chapter 234).[290–294] Serologic strategies also may assist in the diagnosis of *Bartonella* IE.[291] In addition to blood culture and serodiagnostic strategies, as outlined previously, culture of valvular tissue or vegetations that have embolized to peripheral arteries and have been removed surgically may yield the causative organism. Microscopy of these tissues, including direct fluorescence antibody techniques and electron microscopy, may assist in making the etiologic diagnosis, particularly in cases caused by fastidious or intracellular pathogens, such as *Tropheryma whipplei,*[295] *Chlamydia, Coxiella burnetii,*[296] and *Legionella.*[297]

Molecular techniques to recover specific DNA or 16S ribosomal RNA from valve tissue samples have been useful diagnostically in selected cases,[298] and polymerase chain reaction (PCR) studies performed on blood or serum may be highly useful for the diagnosis of IE caused by difficult-to-grow pathogens.[299] In an investigation comparing broad-range PCR results on resected endocardial specimens from 49 patients with suspected IE with results of culture and Gram staining of resected specimens and Duke criteria, bacterial DNA was shown within cardiac tissue in 18% of patients with sterile blood cultures.[300] Other tests to exclude collagen vascular diseases usually are necessary in patients undergoing evaluation for culture-negative native valve IE.[301]

Although still rare, fungal IE is increasing in frequency and usually affects narcotic addicts, patients with prosthetic valves, or hospitalized patients receiving antibiotics or hyperalimentation or both.[302] Low-birth-weight neonates seem particularly prone to *Candida* IE, predominantly on the tricuspid valve or right atrial mural endocardium.[303] The rate of prosthetic valve endocarditis among fungemic patients with prosthetic heart valves was approximately 25% in one retrospective series.[304] Historically, over half of cases of fungal IE exhibited negative blood cultures.[305] The Castaneda principle (a culture of blood in a bottle containing agar and liquid broth) has been shown to increase the yield of fungal cultures.[279]

Various serologic procedures have been used in an attempt to substantiate a diagnosis of fungal IE. Tests for the determination of anti-*Candida* antibody are poorly standardized, variably sensitive, often nonspecific, and difficult to interpret.[306] Tests for mannan antigenemia (a constituent of the cell wall of *Candida*) or enolase by hemagglutination inhibition and by enzyme-linked immunosorbent assay[307,308] have been reported as helpful in the diagnosis of disseminated candidiasis. In addition, a reliable radioimmunoassay for the detection of *Aspergillus* antigenemia is under investigation. If embolism to major vessels occurs, an embolectomy should be performed, and the material should be

examined with special fungal stains and culture. Identification of the fungus by either technique is diagnostic of fungal IE even if blood cultures are sterile.

Echocardiography

The use of echocardiography in the diagnosis of IE first was reported in 1973.[309] The use of two-dimensional (2D), cross-sectional, real-time techniques improved the diagnostic accuracy over M-mode methods.[310] The characteristic finding is a shaggy, dense band of irregular echoes in a nonuniform distribution on one or more leaflets, with full unrestricted motion of the valve. The smallest vegetation detected was approximately 2 mm, but the acoustic impedance of the mass relative to the surrounding structures is a more important factor than size in identifying the vegetation. If the vegetation is calcified (which may occur early and independent of the healing process), the sensitivity of echocardiography may be increased. Echocardiography has localized vegetations correctly in culture-negative cases. Echocardiography may be of special value in detection of the large, friable vegetations characteristic of fungal IE. Use of M-mode or 2D transthoracic techniques with prosthetic heart valves has been disappointing, however, because of the difficulty in resolution around the prosthetic device.

Many reports[311] have evaluated the role of transthoracic echocardiography (TTE) in the diagnosis and management of suspected IE and have been summarized in cogent analyses.[312] It seems from most analyses that TTE should be performed in all patients in whom IE seems to be a reasonable diagnosis. In contrast, TTE is not an appropriate screening test in the evaluation of febrile patients in whom IE is unlikely on clinical grounds or in bacteremic patients with organisms that rarely cause IE (e.g., *E. coli*), particularly if there is another obvious focus to explain the clinical syndrome; TTE may be overused in such low-risk situations.[313] TTE may be technically inadequate in 20% of adult patients owing to obesity, chronic obstructive pulmonary disease, or chest wall deformities. These studies also suggest the following: (1) TTE has variable sensitivity for the detection of vegetations (<50% to >90% positive), indicating that a negative study does not exclude IE; (2) the sensitivity of TTE for detecting vegetations is highest in right-sided IE, because the tricuspid and pulmonic valves lie relatively close to the chest wall; (3) false-positive results are extremely rare; (4) only technically adequate studies are of value, a characteristic that depends on examiner experience; (5) echocardiography is extremely valuable in assessing local complications of IE, especially surrounding the aortic valve, although the sensitivity for detecting these complications is relatively low for TTE (see later discussion); and (6) patients with a "vegetation" identified with echocardiography are at an increased risk for subsequent systemic emboli, CHF, need for emergency surgery, and death, especially with aortic valve involvement. This apparent influence on prognosis has hastened earlier surgery in some cases,[314] but this point is controversial.[315]

Positive findings on the echocardiogram in a patient with IE should serve as adjunctive evidence, together with clinical parameters, in favor of surgical intervention. In one analysis from the Mayo Clinic,[316] emboli were not statistically more common in patients with left-sided native valve IE and echocardiographically documented vegetations within 72 hours after beginning antimicrobial therapy than in patients without vegetations visualized by transthoracic techniques. The occurrence of emboli was correlated positively with the infecting microorganism, being more common in IE due to viridans streptococci than in IE due to *S. aureus*. Most studies have suggested that mitral valve vegetations (particularly vegetations attached to the anterior leaflet), regardless of size, are associated with higher rates of embolization (25%) than are aortic valve vegetations (10%). This association implicates the mechanical effects of abrupt mitral valve leaflet excursions, occurring twice per heartbeat, in enhancing the embolic potential of vegetations.[317] Visualization of vegetations by echocardiography is not sufficient to prompt early surgery.[318] Serial echocardiograms often reveal the persistence of vegetations after successful therapy, but sequential studies may be useful in the timing of surgical intervention. Although the finding is still controversial, larger vegetation size has been associated with an increased risk for cerebral emboli. One large meta-analysis incorporating data from 10 studies involving 738 patients showed that large vegetation size (>1 cm) was associated independently with an increased risk for stroke.[319] Short-term changes in vegetation size during therapy do not correlate well with clinical outcome.[320]

One study suggested that an increase in vegetation size, as seen with echocardiography during treatment of IE, can identify a subset of patients with a higher rate of complications, independent of the presence of persistent bacteremia or overt clinical stigmata of IE.[321,322] Some studies have suggested that highly mobile vegetations constitute an independent increase in risk for complications in IE.[323] In other studies, vegetation mobility at echocardiography has not been an important independent risk factor for embolic events in IE, because it is correlated strongly with vegetation size.[288,314]

One problem in considering the significance of these echocardiographic characteristics is the high degree of interobserver variability in interpreting the echocardiographic images.[324] Inexperienced physicians do not understand this issue. In one investigation involving four readers independently interpreting TTE studies from 41 cases of IE, Heinle and colleagues reported that investigators agreed on vegetation mobility in 57% of the cases, vegetation shape in 36%, and vegetation attachment in 40%.[325] In a more recent study of intraobserver and interobserver agreement for a random sample of 110 echocardiograms from the ICE registry, interobserver agreement was highest for aortic abscess (kappa = 1.0) and vegetation location (kappa = 0.95) and lowest for mobility (kappa = 0.69).[326] This overall improved agreement relative to the Heinle study was attributed to digital image acquisition and inclusion of transesophageal echocardiograms.

Transesophageal Echocardiography

Transesophageal echocardiography (TEE) has altered the diagnostic approach to some patients with suspected IE.[327–332] TEE uses a 5-MHz phased-array transducer with Doppler and color flow encoding capabilities mounted on the tip of a flexible endoscope. Biplane (horizontal and longitudinal) or omniplane imaging is preferred over TTE imaging because of (1) better spatial resolution with a higher-frequency transducer, (2) lack of acoustic interference (e.g., from lungs, chest wall), and (3) proximity to posterior structures (e.g., mitral valve, left atrium, interatrial septum, descending aorta).[329] TEE has proved useful in a wide variety of clinical situations, including detection of possible sources of emboli, diagnosis of thoracic aortic dissection, detection of prosthetic valve dysfunction, and evaluation of IE.[327–329] Intraoperative TEE imaging has become a valuable new tool, often providing real-time feedback to the surgical team during the procedure. Three-dimensional (3D) TEE has emerged as a potential adjunct to characterize valvular lesions. However, in one prospective cohort of 144 consecutive patients, 3D TEE was less sensitive than 2D TEE, with only one additional case of a vegetation detected with 3D TEE but not 2D TEE.[333]

TEE is also more sensitive than conventional TTE in the detection of intracardiac vegetations (approximately 95% vs. 60%–65%, respectively), particularly in the setting of prosthetic valves (see Chapter 81). In one report of 96 patients with IE,[330] the sensitivity of TEE was 100%, compared with 63% for TTE, and the specificity values were identical (98%). The advantage of TEE was particularly evident for vegetations smaller than 10 mm in diameter. In another study,[331] vegetations were detected with TEE in 82% of the cases, compared with 69% for TTE. Although negative results on TEE do not exclude IE,[327–329,332] the procedure should be considered in patients with suspected IE and negative results on TTE. Potential sources of false-negative TEE studies include small vegetations and previous embolization of vegetations. If the clinical suspicion of IE is high and the results of an initial TEE examination are negative, another TEE study is warranted within 7 to 10 days.[334] TEE also has become the procedure of choice for the detection of perivalvular extension of infection in patients with IE.[335,336] Daniel and colleagues[336] reported a diagnostic sensitivity of 87% and a specificity of 95% for TEE in the detection of IE-related abscess, compared with 28% and 99% for TTE. Other investigations have shown TEE to be superior in the diagnosis of valvular perforation,[337] pacemaker IE,[338] eustachian valve IE,[339] IE in the elderly,[340] and other presentations. TEE should be performed (unless contraindicated, as by significant esophageal disease) in all IE patients with a complicated course if perivalvular extension is suspected. MRI also seems promising for the detection of

these complications,[335] but clinical experience with this modality is limited.

TEE is not a screening or noninvasive procedure but is generally safe[341] in experienced hands, and it may alter management in selected patients with proved or suspected IE. Two cost-effectiveness analyses favored the increased use of TEE.[342,343] Using outcomes among a cohort of consecutive patients with intravascular catheter–associated *S. aureus* bacteremia (SAB), Rosen and colleagues[343] found that TEE was a cost-effective method of defining the duration of antibiotic therapy (2 weeks vs. 4 weeks), compared with empirical courses of either 2 weeks or 4 weeks. Similarly, a decision analysis by Heidenreich and coworkers[342] found TEE to be more cost-effective than TTE among patients with a high pretest probability of endocarditis (defined as 4%–60%).

In an effort to identify a subset of patients with suspected native valve IE who may not require TEE, one study compared a standard definition of a negative TTE (no vegetation present) with a strict definition (at least moderate ultrasound quality, normal cardiac anatomy, no valvular stenosis, at most trivial valvular regurgitation, at most mild simple pericardial effusion, absence of implanted hardware or central venous catheter, and no evidence of vegetation). In a retrospective analysis of 790 TTE/TEE pairs, 104 (13.2%) met strict negative criteria. Use of the strict definition improved sensitivity (from 43% to 98%) and negative predictive value (from 87% to 97%), at the expense of specificity (from 90% to 16%) and positive predictive value (from 53% to 22%).[344]

In conjunction with the physical examination, phonocardiography, and electrocardiography, the echocardiogram may play an important role in assessing the severity of acute aortic insufficiency in cases of active IE.[345] In this setting, classic signs, such as a wide pulse pressure and bounding pulses, often are absent; however, there is usually a reduction in intensity of the first heart sound, and Austin Flint murmurs may be audible. Findings on the chest radiograph and electrocardiogram may be normal. The degree of mitral valve preclosure (as determined with echocardiography) correlates with the acute elevation in left ventricular end-diastolic pressure. If this event occurs before the Q wave on the electrocardiogram, urgent surgical intervention is recommended.

Cardiac Catheterization

Cardiac catheterization provides valuable hemodynamic and anatomic information in patients with IE when surgical intervention is being considered.[263] If properly performed, the procedure is safe, as was shown by the lack of postcatheterization emboli or hemodynamic deterioration in 35 consecutive patients in one series.[346]

Diagnostic Criteria

Diagnostic criteria for IE (the Beth Israel criteria) were published in 1982 by von Reyn and colleagues,[3] but these criteria did not use echocardiographic findings in the case definitions, despite major improvements in echocardiographic technology (see previous discussion). In addition, the isolation of a "typical" IE pathogen from blood cultures was not considered in the Beth Israel definitions. Many presumptive cases of IE were classified as not *definite* but *probable*. With improved methodology and recognition of the central role of echocardiography in the evaluation of suspected IE, new case definitions and diagnostic criteria (the Duke criteria), initially were proposed in 1994.[347] Modifications to the Duke criteria were published in 2000[348] and are now used widely (Table 80.4). The Duke criteria (modeled after the Jones criteria for diagnosing rheumatic fever[349]) improve on the Beth Israel criteria by including echocardiographic demonstration of vegetations or paravalvular complications of IE and the isolation of typical IE pathogens from blood cultures as "major criteria" for the clinically definite categorization of IE. In addition, the presence of recent injection drug use is included in the Duke criteria as a "minor criterion" for diagnosis of IE, recognizing the increased risk for IE in this patient population. Direct comparisons of the Duke and Beth Israel criteria have been done in 11 major studies, including almost 1400 patients. Patient populations from diverse geographic areas with presumed IE have been studied, including young, middle-aged, and elderly adults; pediatric patients; patients with native or prosthetic valve involvement; and patients with and without a history of injection drug use. These studies confirmed the increased

TABLE 80.4 Definition of Infective Endocarditis (IE) According to Modified Duke Criteria

Definite Infective Endocarditis

Pathologic Criteria

- Microorganisms demonstrated by culture or histologic examination of a vegetation, a vegetation that has embolized, or an intracardiac abscess specimen; *or*
- Pathologic lesions; vegetation or intracardiac abscess confirmed by histologic examination showing active endocarditis

Clinical Criteria (See Below for Definitions)

- 2 major criteria; *or*
- 1 major criterion and 3 minor criteria; *or*
- 5 minor criteria

Possible Infective Endocarditis

- 1 major criterion and 1 minor criterion; *or*
- 3 minor criteria

Rejected

- Firm alternate diagnosis explaining evidence of IE; *or*
- Resolution of IE syndrome with antibiotic therapy for ≤4 days; *or*
- No pathologic evidence of IE at surgery or autopsy, with antibiotic therapy for ≤4 days; *or*
- Does not meet criteria for possible IE, as above

Major Criteria

Blood Culture Positive for Infective Endocarditis

- Typical microorganisms consistent with IE from two separate blood cultures: viridans streptococci, *Streptococcus bovis,* HACEK group, *Staphylococcus aureus; or*
- Community-acquired enterococci, in the absence of a primary focus; *or*
- Microorganisms consistent with IE from persistently positive blood cultures, defined as follows:
  - At least two positive cultures of blood samples drawn >12 h apart; *or*
  - All of three or a majority of four or more separate cultures of blood (with first and last sample drawn at least 1 h apart)
  - Single positive blood culture for *Coxiella burnetii* or antiphase I IgG antibody titer >1 : 800

Evidence of Endocardial Involvement

- Echocardiogram positive for IE (TEE recommended in patients with prosthetic valves, rated at least "possible IE" by clinical criteria, or complicated IE [paravalvular abscess]; TTE as first test in other patients), defined as follows:
  - Oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; *or*
  - Abscess; *or*
  - New partial dehiscence of prosthetic valve
- New valvular regurgitation (worsening or changing of preexisting murmur not sufficient)

Minor Criteria

- Predisposition, predisposing heart condition or injection drug use
- Fever, temperature >38°C (100.4°F)
- Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, and Janeway lesions
- Immunologic phenomena: glomerulonephritis, Osler nodes, Roth spots, and rheumatoid factor
- Microbiologic evidence: positive blood culture but does not meet a major criterion as noted above[a] or serologic evidence of active infection with organism consistent with IE
- Echocardiographic minor criteria eliminated

[a]Excludes single positive cultures for coagulase-negative staphylococci and organisms that do not cause endocarditis.

HACEK, Haemophilus, Aggregatibacter (formerly *Actinobacillus*), *Cardiobacterium, Eikenella, Kingella*; *IgG,* immunoglobulin G; *TEE,* transesophageal echocardiography; *TTE,* transthoracic echocardiography.

Modified from Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. *2000;30:633.*

sensitivity of the Duke criteria in the clinical diagnosis of IE and the diagnostic usefulness of echocardiography in the identification of clinically definite cases.[334] Modifications of the Duke criteria, to provide a floor for diagnosis of putative cases as possible IE, have added more specificity to the schema.[348]

Etiologic Agents

Streptococci

A plethora of microorganisms have been implicated in IE, but streptococci and staphylococci account for 80% to 90% of the cases in which an identification is made. The most common etiologic agents are listed in Table 80.5. Streptococci were once the most common overall cause of IE and continue to be the predominant etiologic agents in the developing world.[19,20] Staphylococci have assumed increasing importance among isolates in community hospitals in recent years, however (see later discussion).[29,350] Viridans streptococci remain the major cause of IE in children. The disease usually runs a subacute course with multiple nonspecific symptoms (see Table 80.3). More than 80% of these patients have underlying heart disease. Viridans streptococci are the most commonly isolated pathogens in IE cases associated with mitral valve prolapse,[351] and IE in young women with isolated mitral valve involvement is almost universally caused by viridans streptococci. Approximately 20% of cases that come to the physician's attention are seen because of embolic phenomena. With modern medical and surgical management, the cure rate should exceed 90% in nonenterococcal streptococcal IE, although complications may ensue in more than 30% of cases.

The nomenclature of the streptococci is confusing, and the terminology varies among authors. As detailed in Chapter 202, current names for the α-hemolytic streptococci other than *S. pneumoniae* (i.e., viridans streptococci) causing IE are *S. mitis, S. sanguinis, S. mutans, Streptococcus salivarius, Abiotrophia* and *Granulicatella* spp., and some isolates of the *Streptococcus intermedius* group (*S. intermedius* and *Streptococcus anginosus*). *Streptococcus morbillorum* now is classified as *Gemella morbillorum*; *Streptococcus mitior* now is considered to be a "genospecies" of *S. mitis.* The name *S. mitior* is embedded so deeply in the endocarditis literature, however, that it is retained in the discussion that follows. Group D streptococci are sometimes α-hemolytic, depending on the conditions and the isolate, but are not included among the viridans streptococci. Streptococci of the viridans group (not a true species) are α-hemolytic and usually nontypeable by the Lancefield system.

The streptococci most commonly isolated from cases of IE are *S. sanguinis, Streptococcus bovis* (we will continue to use this name here, although the human strains have been reclassified as *Streptococcus gallolyticus*), *S. mutans,* and *S. mitior.*[81] In a series of 317 cases of streptococcal IE, the breakdown was as follows: α-hemolytic, 45%; nonhemolytic, non–group D, 21%; group D, 25%; pyogenic (groups A, B, C, G), 5%; miscellaneous, 3%; and aerococci, 1.3%. The α-hemolytic strains included *S. sanguinis* (16.4% of all cases of IE), non–dextran-producing *S. mitior* (13.2%), dextran-positive *S. mitior* (7.3%), and an unclassified group (7.9%). Some isolates of *S. sanguinis,* formerly called *Streptococcus* SBE, are in Lancefield group H; however, most are nontypeable. *S. mutans* (14.2%), *S. anginosus* (5.4%), and *S. salivarius* (1.3%) are the nonhemolytic, non–group D strains. Group D organisms include the enterococci (8%) and *S. bovis* (17%). In another analysis,[352] viridans streptococci caused 58% of cases of IE at The New York Hospital from 1970 to 1978. The various responsible species were as follows: *S. mitior,* 31%; *S. bovis,* 27%; *S. sanguinis,* 24%; *S. mutans,* 7%; vitamin B_6–dependent *S. mitior,* 5%; *S. anginosus,* 4%; and others, 2%—all of which were slightly different from the species reported from the United Kingdom. By contrast, group D streptococci constituted 25% of all cases of IE within six regions of France representing 16 million inhabitants.[353] Among 1242 consecutive episodes of IE from 1996 to 2013 in three Spanish centers, 294 were streptococcal, of which 47 were *S. bovis* (3.8%), 134 viridans-group streptococci (10.8%), and 113 enterococcal (9.1%).[354]

There seems to be no correlation between clinical outcome and the species involved,[268] with the exception of nutritionally deficient strains (see later discussion). The relative role of each species overall is problematic, however, because species designations of identical strains among laboratories often are disparate, and most blood and cerebrospinal fluid isolates of viridans or nonhemolytic streptococci are not from patients with IE.[355]

S. mutans, a normal member of the oral microbiota, is microaerophilic, pleomorphic, and fastidious. Two-thirds of *S. mutans* strains hydrolyze bile-esculin,[356] a test used to identify group D organisms, and may be confused with enterococci. Other characteristics of *S. mutans* include the absence of group D antigen (some strains are positive for group E), production of acid from mannitol, failure to hydrolyze hippurate, and formation of gelatinous deposits (dextran) in media containing 5% sucrose. This organism may be difficult to isolate and to identify. It often requires more than 3 days for primary isolation, grows best on horse blood agar in 5% to 10% carbon dioxide on subculture, and is pleomorphic, resulting in confusion with diphtheroids. *S. mutans* first was isolated in 1924 by Clark from dental caries lesions of humans and first was reported in 1928 to cause IE. The central importance of this organism in dental caries has been amply documented.

S. bovis is a normal inhabitant of the gastrointestinal tract of humans and many animal species. The genetic diversity among organisms historically classified as *S. bovis* has been clarified to include biotypes I and II, *S. salivarius,* and *Streptococcus macedonicus.*[357] In addition, *S. bovis* has been subdivided further into *S. gallolyticus* subsp. *gallolyticus, S. gallolyticus* subsp. *pasteurianus,* and *Streptococcus infantarius.*[358] It is important to differentiate *S. bovis* from the other members of group D (the enterococci), because the respective therapeutic approaches to infection with these organisms are different (see later discussion). Group D organisms are identified presumptively by bile-esculin hydrolysis.[359,360] Only the enterococci (*E. faecalis* and its varieties, *Enterococcus zymogenes* and *Enterococcus liquefaciens*; *Enterococcus faecium*; and *Enterococcus durans*) grow in 6.5% sodium chloride, whereas *S. bovis* and *Streptococcus equinus* (a rare cause of IE) are salt sensitive. Seventy-five percent of strains of *S. bovis* are heat tolerant, and they may grow and produce acid in *E. faecalis* broth; these methods are unreliable for separation.[361] Arginine hydrolysis by enterococci and starch hydrolysis by *S. bovis* are other means for reliable separation. The association of bacteremia due to *S. bovis* with carcinoma of the colon and other lesions of the gastrointestinal tract suggests that a colonoscopy should be performed if this organism is isolated from blood cultures.[362,363] Interesting to note, this association appears to be higher with *S. gallolyticus* subsp. *gallolyticus.*[358]

TABLE 80.5 Etiologic Agents in 1779 Patients With Definite Infective Endocarditis

AGENT	CASES (%)
Staphylococcus	
Staphylococcus aureus	31.6
Coagulase-negative staphylococci	10.5
Streptococcus	
Viridans-group streptococci	18.0
Streptococcus bovis	6.5
Other streptococci	5.1
Enterococci	10.6
HACEK group	1.7
Non-HACEK gram-negative bacteria	2.1
Fungi	1.8
Polymicrobial	1.3
Other species	3.1
Culture negative	8.1

HACEK, Haemophilus, Aggregatibacter (formerly *Actinobacillus*), *Cardiobacterium, Eikenella, Kingella.*
Data from Fowler VG Jr, Miro JM, Hoen B, et al. Staphylococcus aureus *endocarditis throughout the world: a consequence of medical progress. The International Collaboration on Endocarditis Prospective Cohort Study.* JAMA. *2005;293:3012.*

Enterococci

Enterococci are normal inhabitants of the gastrointestinal tract and occasionally of the anterior urethra. All enterococci are in Lancefield group D; they are catalase negative and nonmotile; and they may exhibit

α-, β-, or γ-hemolysis on blood agar. They grow well in sodium azide (SF *[Streptococcus faecalis]* broth), 40% bile, 6.5% sodium chloride, and 0.1% methylene blue and can survive at 56°C for 30 minutes or at a pH of 9.6.[364] Enterococci are responsible for 5% to 18% of the cases of IE, and the incidence seems to be increasing.[7,365,366,367] The disease usually runs a subacute course and affects older men (mean age, 59 years) after genitourinary manipulation or younger women (mean age, 37 years) after obstetric procedures. The mean duration of nonspecific symptoms, such as malaise, fatigue, anorexia, and weight loss, was 140 days in one review. More than 40% of the patients have no underlying heart disease, although more than 95% develop a heart murmur during the course of the illness. Classic peripheral manifestations are uncommon (<25% of the cases). Bacteriuria with enterococci is a helpful diagnostic clue and was found in 4 of 15 patients in one study.[364] Cure is difficult because of the intrinsic resistance to many antibiotics, and a high mortality rate persists in this disease. With the increasing use of third-generation cephalosporins, which are relatively inactive against enterococci in vitro, and other contributing factors (e.g., use of indwelling urinary catheters), an alarming increase in enterococcal bacteremias has been documented during the past 2 decades.[367–369] Most enterococcal bacteremias are health care associated, are often polymicrobial (42% in one large series[370]), and are associated with serious underlying disorders. Factors that suggest IE in patients with enterococcal bacteremia include (1) community acquisition, (2) preexistent valvular heart disease, (3) cryptogenic source, and (4) absence of polymicrobial bacteremia.[370] Antibiotic usage patterns, the aging of the population, and more invasive procedures in hospitalized adults all portend a continued increase in serious enterococcal infections, including IE, in the future.

Streptococcus pneumoniae

Before 1945, *S. pneumoniae* was responsible for approximately 10% of the cases of IE, but the prevalence has decreased to approximately 1% to 3% currently.[371] The course usually is fulminant and often (in approximately one-third of cases) is associated with perivalvular abscess formation or pericarditis or both. Left-sided involvement is the rule, and there is a predilection for the aortic valve (approximately 70% of cases). Many patients with pneumococcal IE are alcoholics (approximately 40%), and concurrent meningitis is present in about 70% of cases.[371–373] The overall mortality rate is approximately 50% (60%–65% in children[374]), with death usually caused by rapid valvular destruction and hemodynamic compromise,[375] although indolent presentations of pneumococcal IE with a favorable outcome have been described.[376]

Abiotrophia and *Granulicatella* spp. can cause difficulties in isolation and were implicated in 1.5% of all IE cases at one Spanish medical center over a 15-year period.[377] The organisms do not grow on subculture unless L-cysteine or pyridoxal vitamin B_6 is provided. IE due to nutritionally deficient streptococci is almost always indolent in onset and associated with preexisting heart disease.[378] Therapy is difficult, because systemic embolization, relapse, and death are common (occurring in 17%–27% of cases). A closely related species, *S. mitis,* although not nutritionally deficient, also causes serious infections, including IE, in adults[379] and has emerged as an important causative agent of IE among drug addicts in some areas (e.g., New York City[380]).

β-Hemolytic Streptococci

Group B streptococci *(Streptococcus agalactiae)* are normal inhabitants of the mouth, vagina, and anterior urethra in 5% to 12% of the general population. In 149 patients with group B streptococcal infections, the serotypes isolated were Ia in 46%, II in 22%, and III in 11%.[381] Although the organism has long been recognized as a cause of bacteremia and meningitis in neonates, serious *S. agalactiae* infections in adults also have been emphasized.[382,383] Risk factors for group B streptococcal sepsis and IE in adults include diabetes mellitus, carcinoma, alcoholism, hepatic failure, elective abortion, and injection drug use.[383–385] As with *S. bovis,* occasional cases occur in association with villous adenomas of the colon.[386] More than 90 cases of group B streptococcal IE have been reported.[384–388] Underlying heart disease is common, the male-to-female ratio is 1.4:1, the mean age is approximately 54 years, and left-sided involvement predominates. The overall mortality rate is almost 50%. The organism does not produce fibrinolysin, which may be responsible for the large, friable vegetations and frequent major systemic emboli.

A similar clinical picture with a destructive process, left-sided predominance, frequent complications, and high mortality (approximately 40%) has been observed in the 47 cases of group G streptococcal IE reported in the literature.[389,390] Most human infections with *Streptococcus suis* have presented as meningitis (with a striking predilection to cause deafness as a sequela) and sepsis with accompanying arthritis or endophthalmitis, but two cases of IE due to serotype 2 have been described.[391] A history of pig or raw pork contact was a feature in both patients. Several cases of IE due to *Streptococcus canis,* a zoonotic group G streptococcal species associated with dogs, have also been reported.[392]

Group A streptococci remain a rare cause of IE associated with a high complication rate in adults and children.[393] *S. anginosus* is a rare cause of IE (6%–7% of cases), but this species is unusual among these streptococci in that it has a predilection to cause suppurative complications, including brain, liver, perinephric, and other abscesses and cholangitis, peritonitis, and empyema.[394–396] Some of these complications occur during IE due to this organism and may require surgical attention.

Approximately 50% of *S. anginosus* strains carry the group F antigen.[395] IE caused by *S. anginosus* may result in virulent intracardiac complications (e.g., myocardial abscess, purulent pericarditis) more typical of *S. aureus* infections.[397]

Gemella

IE due to *Gemella haemolysans* also has been reported.[398] This organism now is placed in genus V of the family Streptococcaceae. *Gemella* should be suspected if blood cultures reveal a variable morphology (with some organisms resembling diphtheroids) and an indeterminate Gram stain. The antimicrobial susceptibility of *Gemella* spp. is similar to that of the viridans streptococci. Although misidentification in the microbiology laboratory is presumably common, six cases of IE due to *G. haemolysans* have been reported since 1982.[399]

Aerococci

Aerococci are uropathogens frequently misidentified as streptococci, staphylococci, or enterococci. With improved microbiologic techniques, IE due to *Aerococcus urinae* and *Aerococcus sanguinicola*, primarily occurring in older patients with underlying urinary tract diseases, has been described.[400,401]

Staphylococci

S. aureus is now the most common cause of IE in most of the industrialized world.[10] Rates of *S. aureus* IE are rising in the United States.[350] A primary cause of this fundamental shift in the microbiology of IE relates to the growing importance of health care contact as a risk factor for bacteremia and, increasingly, IE.[10,17] This species is the causative agent in most cases of acute IE, but only 10% to 12% of patients with SAB have IE.[402,403] The organism may attack normal heart valves (defined as those having no clinically detectable cardiac disease) in approximately one-third of the patients. The course frequently is fulminant when it involves the mitral or aortic valve, with widespread metastatic infection, and results in death in approximately 40% of patients.[404–407]

As noted, the proportion of cases of IE due to *S. aureus* seems to be increasing at both community and university hospitals.[14,15,29,365,408] Traditionally, most cases of *S. aureus* IE were believed to be community acquired.[409] In the late 1990s, however, a small report of *S. aureus* IE from Duke University suggested a demographic shift among patients with this infection.[410] Over half of cases were associated with health care contact, and many were associated with intravascular catheters. These observations were validated in a multinational cohort of more than 1700 patients with definite IE. *S. aureus* was the most common cause of IE, accounting for 32% of all cases. Among the *S. aureus* cases, 39% were presumed to be health care associated, and in 28% the presumed source was an intravascular device.[10] These statistics emphasize that the finding of health care–associated SAB should prompt a complete evaluation for underlying IE.

Myocardial abscesses (with conduction disturbances), purulent pericarditis, and valve ring abscesses are more common in staphylococcal

endocarditis than in other forms of IE. Peripheral foci of suppuration (e.g., lung, brain, spleen, kidney) are common and affect more than 40% of these patients.[404,407,408] These extravascular sites of involvement may offer clues to an early diagnosis, especially in narcotic addicts.[261,262] This disease often is clinically unsuspected in older patients, and mortality rates frequently exceed 50% in patients older than 50 years, especially when the infection is nosocomially acquired.[408,411] The rare entity of neonatal IE also is often caused by *S. aureus*[412]; survival is unusual.

IE in narcotic addicts usually is due to *S. aureus,* but the disease tends to be less severe, with mortality rates of 2% to 6%.[261,262] The lower mortality is related directly to the preponderance of cases affecting the tricuspid valve, a syndrome that responds to antimicrobial therapy in most cases.[409] The emergence of MRSA in addicts with staphylococcal IE was first documented in the Detroit area in the mid-1980s.[256,413] Among 180 bacteremic addicts admitted to the Detroit Medical Center in 1 year, 24% grew MRSA and 41% of the patients overall had IE. Previous hospitalizations, long-term addiction (particularly in men), and nonprescribed antibiotic use were predictive of MRSA acquisition (odds ratio, 8.6:1).[413] Injection of newer psychoactive substances, including synthetic cannabinoids and synthetic cathinones, was associated with higher incidence of *S. aureus* IE and cavitating pulmonary lesions compared with findings in those who injected only opiates.[414]

Coagulase-Negative Staphylococci

Although *S. epidermidis* was traditionally an important agent in prosthetic valve IE (see Chapter 81) and in infants with IE related to umbilical venous catheters in neonatal intensive care units,[415] studies from the ICE-PCS have confirmed that it also is an emerging cause of native valve IE. Approximately 8% of patients with definite native valve IE and no history of injection drug use were infected with coagulase-negative staphylococci.[416,417] Health care contact was the primary risk factor for infection. Many of these patients also had preexisting valvular disease. Although surgical treatment occurred more frequently in patients infected with coagulase-negative staphylococci than in those with *S. aureus* infection, mortality in these two groups was similar.[416] Data from a *Caenorhabditis elegans* model support the possibility that *S. epidermidis* isolates from patients with native valve IE constitute a more virulent subset within this species.[418]

Separation of IE from uncomplicated bacteremia due to *S. epidermidis* (implicated in approximately 50% of cases of native valve coagulase-negative staphylococcal IE) can be difficult. IgG antibodies to a novel 30-kDa cloned protein, termed *staphylococcal secretory antigen,* were demonstrated in patients with *S. epidermidis* IE but not in patients with uncomplicated *S. epidermidis* bacteremia or IE due to other pathogens.[419] Extensive laboratory evaluation[420] revealed that most *S. epidermidis* IE isolates are distinct and do not represent common-source outbreaks, despite the frequent shift to a small-colony variant by many strains in vivo[421] and the description of polyclonal *S. epidermidis* IE.[422]

Rare cases of IE due to other coagulase-negative staphylococci (e.g., *Staphylococcus saprophyticus, Staphylococcus capitis*) have been reported.[423,424] A growing number of reports of IE caused by the coagulase-negative *Staphylococcus lugdunensis* have been published.[425–428] This organism tends to cause a substantially more virulent form of IE than coagulase-negative staphylococci, with high morbidity rates despite almost uniform in vitro susceptibilities to most antibiotics, including penicillins and cephalosporins.[425–428] These strains frequently are misidentified as *S. aureus* because they often are yellow pigmented and yield complete hemolysis on blood agars. Their in vitro hemolytic capacities relate to the production of synergistic hemolysin. Differentiation of *S. lugdunensis* from *S. aureus* in the microbiology laboratory depends on the tube coagulase test and the ability of the former strains to cause ornithine decarboxylation. Distinguishing *S. lugdunensis* from other coagulase-negative staphylococci can be difficult with commercial identification schemata.[429]

Gram-Negative Bacilli

Gram-negative aerobic bacilli now cause approximately 2% of cases of IE. Among more than 2700 patients with definite endocarditis prospectively enrolled into ICE-PCS from 60 centers in 28 countries, 49 patients (1.8%) had endocarditis due to non-HACEK gram-negative bacilli.[430] Traditionally, injection drug use has been regarded as the primary risk factor for enteric gram-negative bacterial endocarditis[413,431–436] and injection drug users remain a high-risk population in specific locations.[437] However, experience from the large, contemporary, and multinational ICE-PCS demonstrated that health care contact, and not injection drug use, was the most common risk factor for enteric gram-negative IE. In this definitive study, more than half of the patients with IE due to non-HACEK gram-negative bacilli had health care–associated infection, whereas injection drug use was rare (4%). Prosthetic valves and implanted endovascular devices were frequently associated with non-HACEK gram-negative bacillary IE. Patients with cirrhosis[438] also seem to be at an increased risk for the development of gram-negative bacillary IE.

The duration of illness is usually less than 6 weeks, most patients are 40 to 50 years old, and men and women are affected equally.[439] In gram-negative septicemia, the bloodstream usually is cleared by therapy with appropriate antimicrobial agents. In contrast, in IE due to gram-negative bacilli, persistent bacteremia is common, even with high levels of antimicrobial activity. CHF is common, and the prognosis is poor. Older series reported a mortality rate of 60% to 83% for IE due to gram-negative bacilli.[439] However, contemporary experience indicates a substantially better prognosis, with in-hospital mortality rates of 24%.[430] This improved mortality rate is perhaps due in part to high rates of cardiac surgery (51%).

A heart murmur noted during an episode of gram-negative sepsis with unexplained anemia or the persistence of positive blood cultures despite adequate levels of antibiotics may indicate IE. In the early postoperative period after prosthetic valve replacement, sustained gram-negative bacteremia does not imply IE,[440] and other foci of infection (e.g., sternal wound, pneumonia, urinary tract, intravenous catheters) should be sought carefully.

Traditionally, *Salmonella* spp. were important causes of gram-negative bacterial IE among the Enterobacteriaceae. These organisms have an affinity for abnormal cardiac valves, usually on the left side of the heart.[439,441] Although many serotypes have been implicated, most cases are caused by *Salmonella enterica* serotypes Choleraesuis, Typhimurium, and Enteritidis. Valvular perforation or destruction, atrial thrombi, myocarditis, and pericarditis are common, and the outlook is grave. Salmonellae also may produce endarteritis in aneurysms of major vessels (see later discussion).

Although historically regarded as a rare cause of endocarditis because of its poor endothelial cell–binding capacity, *E. coli* now appears to predominate among the Enterobacteriaceae as a cause of IE.[430] This may reflect the growing importance of health care contact as a risk factor for enteric gram-negative IE. Other species of Enterobacteriaceae can also rarely cause IE.[439] In the 1970s, a total of 36 cases of IE due to *S. marcescens* were reported from San Francisco; 32 (88%) were associated with injection drug use.[431,432] The cases were characterized by large vegetations with near-total occlusion of the valve orifice in the absence of significant underlying valvular destruction. The overall mortality rate ranged from 68% to 73%, because a cure of left-sided IE caused by a member of the Enterobacteriaceae is uncommon with medical therapy alone. Valve replacement after 7 to 10 days of antibiotics was recommended for these difficult infections.[432] Approximately 12 cases of IE due to *Campylobacter fetus* have been reported since the first case in 1955.[442]

The first case of *Pseudomonas*-induced IE was recognized in 1899; more than 200 cases have been reported since then.[256,413,433–438] Although historical reports of pseudomonal IE (95%) were primarily associated with injection drug use,[413,433–436] more contemporary reports confirm health care contact as the current primary risk factor.[17,443] The male-to-female ratio is 2.5:1, and the mean age is 30 years. Major embolic phenomena, inability to sterilize valves, neurologic complications (53%), ring and annular abscesses, splenic abscesses, bacteremic relapses, and rapidly progressive CHF are common. Ecthyma gangrenosum, the necrotizing cutaneous lesion characteristic of *Pseudomonas* bacteremia, occasionally has been noted, especially in cases of IE due to *Pseudomonas (Burkholderia) cepacia.*[444] The disease carries the highest mortality if the patient is older than 30 years (73%, compared with 33% in younger

patients), if the duration of illness is less than 5 days (76%, compared with 41% for shorter durations), and if there is left-sided cardiac involvement.[434,436,445] Because of the poor prognosis and frequent complications,[256] early surgery is recommended by many authorities for left-sided pseudomonal IE.[334] In contrast, high-dose regimens of antipseudomonal penicillins combined with aminoglycosides have had a salutary effect in many patients with isolated right-sided pseudomonal IE (see "Antimicrobial Therapy"). Almost all addicts with *P. aeruginosa* IE in more recent reports[65,413,435,436] have abused tripelennamine and pentazocine ("Ts and blues").

HACEK Group Bacteria (*Haemophilus, Aggregatibacter* [Formerly *Actinobacillus* spp.], *Cardiobacterium, Eikenella, Kingella*)

Haemophilus spp. historically accounted for 0.8% to 1.3% of all cases of IE,[446–449] although some have been reclassified into the genus *Aggregatibacter.* These infections usually run a subacute course and occur in the setting of preexisting valvular disease. Emboli to major peripheral arteries were found in six of seven cases of *Haemophilus parainfluenzae* IE.[446] Major central nervous system complications are relatively common.[449] *Aggregatibacter aphrophilus* produces a similar clinical pattern and has been transmitted from dogs to humans. Single cases of IE due to *Aggregatibacter segnis* and *Haemophilus influenzae* biogroup *aegyptius* have been reported from Denmark and Israel.[450,451] A closely related organism, *Aggregatibacter actinomycetemcomitans,* is a rare cause of subacute IE (with approximately 35 cases reported) and carries a mortality rate of 34%.[452,453] IE due to *Cardiobacterium hominis*[454] resembles the disease caused by *Haemophilus* spp.; 26 cases of *C. hominis* IE were reported by 1983. Only one extravascular infection due to *C. hominis* has been documented (meningitis during IE).

At least 28 cases of *Kingella* endocarditis (caused by *Kingella kingae*, 25 cases; *Kingella denitrificans,* 2 cases; and *Kingella indologenes,* 1 case) have been reported.[455] Invasive *Kingella* infections are increasingly recognized in children. Approximately 50% of patients develop complications, including acute stroke in approximately 25%. A dozen cases of IE due to *Eikenella corrodens* have been reported; injection drug use (especially of amphetamines) was implicated in five patients.[456,457] Dental infection or a history of dental procedures may be a feature, and drug users often have "cleaned" the injection site with saliva; *E. corrodens* is a usual habitant of the oropharynx. The disease generally has an indolent course, but presentation may be acute.[457]

All of the aforementioned HACEK bacteria can be reliably isolated with standard blood culture incubation periods using conventional automated blood culture systems.[280,286] The clinical syndrome produced by this group is virtually identical to that observed in subacute IE: large friable vegetations, frequent emboli, development of CHF, and often the eventual need for valve replacement.[458]

Other Gram-Negative Bacteria

IE produced by several other gram-negative species has received attention recently. *N. gonorrhoeae* was responsible for at least 5% to 10% of the cases of IE before the introduction of penicillin but now is implicated rarely. In the older series, half of the patients with gonococcal IE had involvement of the right side of the heart and exhibited the characteristic double-quotidian fever pattern. Of the cases of gonococcal IE reported since 1949,[459–461] most occurred in young men. Skin manifestations consistent with the gonococcal arthritis-dermatitis syndrome or IE have been documented in only 20% of the cases. Most cases of gonococcal IE now follow an indolent course, in contrast to the often fulminant progression in the preantibiotic era. Aortic valve involvement, large vegetations seen with TTE, associated valve ring abscesses, CHF, and nephritis are common.[461] A high frequency of late complement component deficiencies has been observed in patients with gonococcal IE. Sudden hemodynamic deterioration despite appropriate therapy may occur,[459–461] and the mortality rate is approximately 20%. "Nonpathogenic" *Neisseria* spp.—*N. perflava, N. flava, N. pharyngis, N. mucosa, N. sicca, N. flavescens,* and especially *Moraxella (Neisseria) catarrhalis,* and *Neisseria elongata* subsp. *nitroreducens*—now are isolated more frequently in IE than are gonococci, but they usually produce infection on abnormal or prosthetic heart valves.[462–464]

Gram-Positive Bacilli

IE due to various species of corynebacteria (diphtheroids) is uncommon and usually occurs on damaged or prosthetic valves,[465] although native valve infections (e.g., *Arcanobacterium [Corynebacterium] haemolyticum* in a drug addict) are reported rarely. About 19 cases of IE due to *Corynebacterium pseudodiphtheriticum* (previously *Corynebacterium hofmannii*) have been reported; native valves were involved in approximately half of these cases.[466] *Corynebacterium xerosis* is a very rare cause of native valve IE. Nontoxigenic *Corynebacterium diphtheriae* IE has been reported in more than 40 patients. A cluster of 7 cases in 1 year from New South Wales, Australia,[467] emphasized the aggressive nature of the infection, including major vascular complications, the frequent occurrence of septic arthritis (in four of seven patients), and involvement of native valves. Injection drug use also is a predisposing factor.

The isolation of *Listeria monocytogenes* has been reported in 44 cases of IE.[468,469] Most cases of IE due to *Listeria* spp. have occurred in patients without any underlying defect in host defenses, although preexisting heart disease is present in approximately 50%. The mean age in the reported series was 51 years, and the overall mortality rate was 48%.[469]

Propionibacterium (now called *Cutibacterium)* species are typically part of normal skin microbiota, but have been implicated in approximately 70 cases of IE, generally involving prosthetic material.[470]

Lactobacilli also have been reported to cause a subacute form of IE, but these cases are rare (only 41 reported).[471,472] Despite an initial response to therapy, relapse of this infection is not unusual (approximately 60% of cases). Most cases occur on structurally abnormal native valves after dental manipulation.[472] Therapy with single agents is often unsatisfactory because lactobacilli, similar to enterococci, are tolerant to penicillins. Medical cure has been difficult to achieve in the past. These organisms also may require several weeks for isolation on blood culture.

More than 90% of 49 serious infections caused by *Erysipelothrix rhusiopathiae* were characterized as IE.[473] Occupational or vocational animal or fish exposure is a major risk factor, and approximately one-third of patients are alcoholic. Most patients are men. A characteristic erysipeloid skin lesion is present in approximately 40% of cases, and the organism exhibits significant aortic valve tropism (involved in 70% of patients).[473] The overall mortality rate is 38%.

Most cases of *Bacillus* IE involve the tricuspid valve in narcotic addicts, but nonaddicts and prosthetic valve recipients also have been affected.[474] *Rothia dentocariosa* is a rare cause of IE (approximately 20 cases reported) but has led to significant central nervous system complications.[475]

Anaerobic Bacteria

Nonstreptococcal anaerobic bacteria were responsible for 1.3% of all the cases of IE in 1970.[476] *Bacteroides fragilis* was the predominant pathogen in a review of 67 cases from the literature.[477] The following organisms were isolated: *B. fragilis,* 35.8%; *Bacteroides oralis,* 3%; *Prevotella melaninogenica (Bacteroides melaninogenicus),* 3%; *Fusobacterium necrophorum,* 13.4%; *Fusobacterium nucleatum,* 9%; *Clostridium* spp., 13.4%; *P. acnes,* 7.5%; *Dialister granuliformans,* 1.5%; and unidentified, 16.4%. More than one-third of the unidentified cases also were believed by the authors to represent *B. fragilis.* Approximately 25% of these cases were polymicrobial, usually mixed with anaerobic or microaerophilic streptococci. The portal of entry for *B. fragilis* was probably the gastrointestinal tract, whereas *B. oralis, P. melaninogenica,* and fusobacteria originated from the mouth or upper respiratory tract. Two-thirds of the patients were older than 40 years and had preexisting heart disease.

The course usually is subacute except for that with infection due to *F. necrophorum,* which characteristically produces a more fulminant disease. These organisms usually cause extensive valve destruction, CHF, and major systemic emboli (in 60%–70% of the cases). Thromboembolic episodes are especially common in infections caused by *B. fragilis,* a phenomenon that may be related to the heparinase produced by this organism. The mortality rate in cases of anaerobic IE has ranged from 21% to 46%.[477] One series from California noted no deaths among seven patients with anaerobic or microaerophilic IE[478]; these cases constituted 10.6% of the IE cases seen. This is similar to a 7.7% incidence reported

by other investigators[3] and suggests that anaerobic IE may be more prevalent now than it was in 1970.[479] Isolation of these organisms may be improved by the newer anaerobic culture techniques currently in use.

Other Bacteria

Coxiella burnetii

IE due to *C. burnetii* (the cause of Q fever) is well documented in the United Kingdom, the United States, Canada, France, the Middle East, and Australia[480–482] (see Chapter 188). Ten cases of Q fever IE were recognized in four Dublin teaching hospitals during a period of 3 years.[481] Q fever usually is a self-limited respiratory illness caused by the inhalation of infected aerosols.[483] The first IE cases were reported in 1959. Men outnumber women by 6 to 1, and 90% of patients have preexisting heart disease. Most cases of *C. burnetii* IE are chronic, with a history of an influenza-like illness occurring 6 to 12 months previously. Risk factors may include exposure to parturient cats or rabbits, previous valvulopathy, and pregnancy.[482] The aortic valve is involved in more than 80% of the cases. Hematuria is uncommon, although it is observed frequently in acute Q fever. Hepatosplenomegaly and hepatitis, common features in other types of Q fever, are seen in about half of patients with IE caused by this organism. Other important clues are thrombocytopenia (seen in 90% of cases) and hypergammaglobulinemia. Immune complex–mediated glomerulonephritis develops in approximately 25% of cases.[480,481,484] Histologically, *C. burnetii* IE is characterized by significant fibrosis and calcifications, slight inflammation and vascularization, and small or absent vegetations.[296] *Coxiella* were shown histologically in the valve tissue in 62% of the cases, and the organism was isolated in 83%, although this poses a significant laboratory hazard.[298] The diagnosis is best made serologically; a positive titer of antibody to the phase I antigen as measured with complement fixation or enzyme-linked immunosorbent assay indicates chronic infection, whereas a fourfold rise in titer of antibody to the phase II antigen is associated with active current infection. A phase I antibody titer (usually IgG or IgA or both) greater than 1 : 800 is considered virtually diagnostic of *C. burnetii* IE and may be useful to monitor the response to therapy.[480,485] Isolation of *C. burnetii* by inoculation of valve suspensions into a human fetal diploid fibroblast cell line seems to be a promising technique.[486] DNA probes are under development. The prognosis with medical therapy alone is poor, and valve replacement often is necessary for a cure (see later discussion). This agent also may cause endarteritis. A single case of IE due to the causative agent of murine typhus has been reported.[287]

Chlamydia psittaci

Chlamydia psittaci, the agent of psittacosis, has been implicated in at least 10 well-documented cases of IE.[487] This organism also may cause myocarditis or pericarditis. Most cases have been associated with psittacine bird exposure; in one case, chlamydiae were found in the liver of the suspected budgerigar. Transmission from pet cats also has been proposed. The course is subacute, and the diagnosis often is made retrospectively. Most patients had preexisting heart disease, with a striking propensity for aortic valve involvement, and rapid valvular destruction leading to surgical intervention or death. A diagnosis can be established with the demonstration of complement-fixing antibodies. Cure usually requires valve replacement and prolonged antibiotic therapy. The mortality rate in this small group was 40%. Two well-documented cases of IE due to *Chlamydia trachomatis* have been reported.[488] Microimmunofluorescence tests are necessary for a diagnosis. Several cases of probable *Chlamydia pneumoniae* IE have been reported.[489] IE due to *Mycoplasma pneumoniae* was proposed in one case report, but cultural confirmation was lacking.[490]

Tropheryma whipplei

T. whipplei is the causative agent of Whipple disease. Although its precise ecologic niche is unclear, there is increasing evidence to suggest that *T. whipplei* may be a component of the microbiota of the human respiratory tract, especially in patients with HIV. *T. whipplei* is an occasional cause of IE and has been cultivated from human valvular tissue.[491] In a review of 35 reported cases of Whipple IE, CHF, fever, and preceding valvular abnormalities all were less common in patients with Whipple IE than in patients with IE due to other pathogens.[295]

A study from Germany involving heart valves found that *T. whipplei* was the most common cause of culture-negative IE. The study evaluated 1135 heart valves, which were analyzed for bacterial infection by means of conventional culture techniques, PCR amplification of the bacterial 16S rRNA gene, and subsequent sequencing. *T. whipplei*–positive heart valves were confirmed with specific PCR, fluorescence in situ hybridization, immunohistochemistry, histologic examination, and culture for *T. whipplei.* The investigators found that *T. whipplei* was the most common cause of culture-negative IE, accounting for 6.3% of such cases.[492] However, other investigators have emphasized the principal role of *Bartonella* and *C. burnetii* as etiologic agents in culture-negative IE.[493] Thus the etiology of culture-negative IE may vary significantly by geographic region.

Brucella species

Brucella spp. continue as important etiologic agents in Spain and in Saudi Arabia, where these organisms are responsible for approximately 10% of IE cases.[494] Aggressive medical therapy with valve replacement usually is necessary for a cure of *Brucella* IE.[495]

Unusual Bacterial Causes of Infective Endocarditis

Five cases of IE due to *Spirillum minus,* a spirochete, have been reported.[496] This organism is distributed widely in nature, especially in fresh or salt water with organic debris. *S. minus* is the etiologic agent of rat-bite fever (sodoku), but rodent transmission was not documented in the cases of IE. Preexisting heart disease or severe underlying disease (e.g., aplastic anemia) was usually present, although one case occurred in an otherwise healthy person.

Many other bacteria have been described in cases of IE; however, consideration of these organisms separately is beyond the scope of this chapter. These infectious agents include *Acinetobacter* (approximately 20 reported cases; a maculopapular rash on the palms and soles may be present),[497] *Actinomyces,*[498] *Alcaligenes, Bordetella, Flavobacterium, Micrococcus, Moraxella, Paracolon, Stomatococcus mucilaginosus* (4 cases of IE),[499] *Streptobacillus moniliformis* (16 cases; usually damaged native valves are involved),[500] *Vibrio,* and *Yersinia.*

Fungi

Fungal IE is rare. Data from ICE-PCS showed that fewer than 1% of more than 2700 patients with definite IE were infected with *Candida* species.[501] Although injection drug use was traditionally an important risk factor for candidal IE,[305] health care contact has now emerged as the primary risk factor for most patients with this infection.[302,501] For example, 51% of patients from the ICE-PCS data with candidal IE had extensive health care contact as a risk factor, whereas only 12% had a history of injection drug use.[501] *Candida parapsilosis* and other non–*C. albicans* species have been reported to be more common in addicts, whereas *C. albicans* and *Aspergillus* spp. tend to predominate in health care–associated infections.[302,305]

The overall cure rate in cases of fungal IE is poor. The poor prognosis may be due to (1) large, bulky vegetations; (2) tendency for fungal invasion of the myocardium; (3) widespread systemic septic emboli; (4) poor penetration of antifungal agents into the vegetation[502]; (5) low toxic-to-therapeutic ratio of the available antifungal agents; and (6) usual lack of fungicidal activity with these compounds. A cure is almost impossible without surgical intervention (see later discussion).[503] The role of promising antifungal agents, including caspofungin[504] and voriconazole,[505] in the management of fungal IE remains to be defined.

In a review of 25 cases of *Aspergillus*-induced IE in which cultures were made,[506] the organisms isolated were as follows: *A. fumigatus,* 14; *A. flavus,* 4; *A. niger,* 3; and *A. ustus, A. sydowi, A. terreus,* and *A. glaucus,* 1 each. Only 5 of 34 patients in this series had positive blood cultures, and only one patient survived. A few cases, usually fatal, of *Aspergillus* IE after coronary artery bypass surgery have been described; *A. clavatus* was isolated in 1 case.

Other fungi that have caused IE include *Histoplasma, Blastomyces, Coccidioides, Cryptococcus, Hansenula, Fonsecaea (Hormodendrum), Lomentospora prolificans* (formerly *Scedosporium prolificans*), *Mucor,*

Paecilomyces, and *Phialophora. Histoplasma capsulatum* IE has been diagnosed rapidly using direct application of AccuProbe (Hologic Gen-Probe, San Diego, CA) on an excised cardiac valve.[507] Of the eight reported cases of IE due to *Trichosporon beigelii,* six occurred on prosthetic valves; only two patients have survived with a combined medical-surgical approach.[508] *Pseudallescheria boydii* has caused IE in approximately five reported patients; all were immunosuppressed (e.g., liver transplant, AIDS) or had received prosthetic heart valves.[509] A single case of *Trichoderma longibrachiatum* has been reported from a cardiac device–related IE.[510]

IE also has been rarely caused by higher bacteria, such as *Actinomyces, Nocardia,* and *Mycobacterium* spp.

Viruses

The role of viruses in IE is unknown. Experimentally, coxsackievirus B has been shown to produce valvular and mural endocarditis in mice and cynomolgus monkeys.[511] In these studies, the viral antigen was identified in the valvular tissue by immunofluorescence techniques. Although the enteroviruses commonly are implicated in cases of myocarditis or pericarditis in humans, there is no proof that viral infections produce IE in humans. Adenoviruses also are capable of producing IE in mice. Persand[512] described a case of "cytomegalovirus endocarditis," but bacteria also were cultured from a mural lesion. Another potential case of CMV endocarditis was reported in a patient with CMV viremia and viral inclusions in resected valve leaflet tissue.[513] In this case, the patient had also been recently treated for group G streptococcal bacteremia, limiting the ability to confidently establish CMV as the primary pathogen.

Culture-Negative Endocarditis

As discussed earlier, sterile blood cultures have been noted in 2.5% to 31% of cases of IE.[514,515] However, blood cultures are negative in only approximately 5% of patients who have IE confirmed by strict diagnostic criteria.[516,517] Sterile blood cultures may be the result in several conditions: (1) subacute right-sided IE; (2) cultures taken toward the end of a chronic course (>3 months); (3) uremia supervening in a chronic course; (4) mural IE, as in ventricular septal defects, post–myocardial infarction thrombi, or infection related to pacemaker wires; (5) slow growth of fastidious organisms, such as anaerobes, *Haemophilus* spp., *Actinobacillus* spp., *Cardiobacterium* spp., *Abiotrophia* and *Granulicatella* spp., or *Brucella* spp; (6) prior administration of antibiotics[514,517]; (7) fungal IE; (8) IE caused by obligate intracellular parasites, such as *Coxiella,* Chlamydiae, *T. whipplei,*[295] and perhaps viruses; or (9) noninfective endocarditis or an incorrect diagnosis. Table 80.6 summarizes common causes of culture-negative IE. Attention to the proper collection of blood culture specimens, care in the performance of serologic tests, and use of newer diagnostic techniques may reduce the proportion of culture-negative cases.

TABLE 80.6 Causes of Culture-Negative Endocarditis

ORGANISM	EPIDEMIOLOGY AND EXPOSURES	DIAGNOSTIC APPROACHES
Aspergillus and other noncandidal fungi	Prosthetic valve	Lysis-centrifugation technique; also culture and histopathologic examination of any emboli
Bartonella spp.	*B. henselae:* exposure to cats or cat fleas *B. quintana:* louse infestation; homelessness, alcohol abuse	Most common cause of culture-negative IE in United States; serologic testing (may cross-react with *Chlamydia* spp.); PCR assay of valve or emboli is best test; lysis-centrifugation technique may be useful
Brucella spp.	Ingestion of unpasteurized milk or dairy products; livestock contact	Blood cultures ultimately become positive in 80% of cases with extended incubation time of 4-6 wk; lysis-centrifugation technique may expedite growth; serologic tests are available
Chlamydia psittaci	Bird exposure	Serologic tests available but exhibit cross-reactivity with *Bartonella*; monoclonal antibody direct stains on tissue may be useful; PCR assay now available
Coxiella burnetii (Q fever)	Global distribution; zoonosis, wide range of mammals	Serologic tests (high titers of antibody to both phase I and phase II antigens); also PCR assay on blood or valve tissue
HACEK spp.	Periodontal disease or preceding dental work	Although traditionally a cause of culture-negative IE, HACEK species are now routinely isolated from most liquid broth continuous monitoring blood culture systems without prolonged incubation times
Legionella spp.	Contaminated water distribution systems; prosthetic valves	Serology available; periodic subcultures onto buffered charcoal yeast extract medium; lysis-centrifugation technique; PCR assay available
Abiotrophia and *Granulicatella* spp.	Slow and indolent course	Supplemented culture media or growth as satellite colonies around *Staphylococcus aureus* streak; antimicrobial susceptibility testing often requires processing specialized microbiology laboratory
Tropheryma whipplei (Whipple disease)	Typical signs and symptoms include diarrhea, weight loss, arthralgias, abdominal pain, lymphadenopathy, central nervous system involvement; IE may be present without systemic symptoms	Histologic examination of valve with periodic acid–Schiff stain; valve cultures may be done using fibroblast cell lines; PCR assay on vegetation material

HACEK, Haemophilus, Aggregatibacter (formerly *Actinobacillus*), *Cardiobacterium, Eikenella, Kingella*; *IE,* infective endocarditis; *PCR,* polymerase chain reaction.

Polymicrobial Endocarditis

The proportion of IE cases due to more than one pathogen may be increasing. In a literature review spanning the 1980s,[518] 101 cases of polymicrobial IE were found. *S. aureus* and viridans-group streptococci were the most common identified organisms. The mean age was 36.5 years, the male-to-female ratio was almost 2:1, and 71 of the patients were injection drug users. As expected, tricuspid valve involvement with septic pulmonary emboli was common. Left-sided involvement, two organisms (vs. three or more), and older age were associated with a higher mortality rate.

Etiology of Infective Endocarditis in Injection Drug Users

The organisms responsible for IE in injection drug users require separate consideration, because the distribution differs from that in other patients with IE. The frequencies of the etiologic agents isolated before 1977 in seven major series were as follows: *S. aureus,* 38%; *P. aeruginosa,* 14.2%; *Candida* spp., 13.8%; enterococci, 8.2%; viridans streptococci, 6%; *S. epidermidis,* 1.7%; gram-negative aerobic bacilli, 1.7% to 15%; other bacteria, 2.2%; mixed infections, 1.3%; and culture negative, 12.9%. In addition, there was an unexplained geographic variation in the causal agents of IE associated with injection drug use. *S. aureus* predominated in New York City, Chicago, Cincinnati, and Washington, DC; *P. aeruginosa* IE was traditionally encountered in Detroit and has recently resurfaced there,[437] but MRSA still predominates. A 1990 compilation from Detroit indicated the distribution of causative agents in addicts with IE (n = 74) as follows: *S. aureus,* 60.8%; streptococci, 16.2%; *P. aeruginosa,* 13.5%; polymicrobial, 8.1%; and *Corynebacterium* JK, 1.4%.[519]

Polymicrobial IE (as many as eight different pathogens have been recovered from blood cultures of an individual patient) is fairly common among drug addicts. Some authors have speculated that HIV infection

predisposes injection drug users to IE owing to unusual bacteria, including *Corynebacterium* and *Neisseria* spp.[519] Although *S. aureus* IE in this population usually was tricuspid, streptococci infected left-sided valves significantly more often than the other pathogens. Biventricular and multiple-valve infections occurred most commonly in *Pseudomonas* IE, and all of these patients abused "Ts and blues." Left-sided IE due to *P. aeruginosa* is a devastating disease and usually manifests as an acute illness refractory to seemingly optimal antimicrobial regimens. Complications, including ring and annular abscesses, neurologic sequelae, CHF, and splenic abscesses, are common; surgery is often necessary for cure.[520] The overall mortality rate still approaches 60%, however.

S. marcescens was an important historical pathogen in San Francisco,[432] although *S. aureus* now predominates in this patient population (HF Chambers, personal communication). These differences do not correlate with contamination of "street" heroin.[521] The high incidence of staphylococcal endocarditis may be explained partially by an increase in nasal and oral carriage of this organism.[522] Heroin use during the previous week was associated with an *S. aureus* isolation rate of 35% from skin, nose, or throat cultures; this rate declined to 11% (not significantly different from that in controls) if heroin had not been injected in the preceding 2 weeks. This suggests an endogenous source for the infecting organism, because *S. aureus* is infrequently (in <5% of cases) isolated from street heroin or injection paraphernalia.

The exact incidence of IE in injection drug use is unknown. A conservative estimate is 1.5 to 2 cases of IE per 1000 addicts at risk per year.[523] Injection drug use is the most common risk factor for the development of recurrent native valve IE; 43% of 281 patients surveyed from 1975 to 1986 with this syndrome were addicts (see earlier discussion).[524] A nested case-control study from Johns Hopkins University found that IE was more common among injection drug users with advanced immunosuppression, even after accounting for injection drug use behaviors. Analyses have shown that although *S. aureus* remains the most common cause of right-sided IE in injection drug users, cases of left-sided IE in this population are also commonly caused by viridans-group streptococci.

THERAPY FOR INFECTIVE ENDOCARDITIS

The response to antimicrobial therapy for IE is unique among bacterial infections. Although the organisms may exhibit exquisite susceptibility in vitro to the antibiotics used, complete eradication takes weeks to achieve, and relapse is not unusual. There are two possible explanations for these phenomena: (1) The infection exists in an area of impaired host defense and is encased tightly in a fibrin meshwork in which the bacterial colonies divide relatively free from interference from phagocytic cells, and (2) the bacteria in these vegetations reach tremendous population densities (often 10^9 to 10^{10} CFUs/g). At these high populations, the organisms may exist in a state of reduced metabolic activity and cell division, as was suggested by Durack and Beeson[171] in studies of L-alanine incorporation into bacterial cell walls. A similar finding is observed in broth in vitro after 18 hours of incubation. In both situations, the bacteria are less susceptible to the bactericidal action of penicillin or other drugs that require cell wall synthesis and division for maximal activity. The relative importance of antimicrobial penetration into vegetations in the response to therapy is unresolved. Although multiple studies have examined antibiotic concentrations in human cardiac valve tissue obtained during surgery[525] and they have usually been found to be in close agreement with concurrent serum concentrations, the relevance of these data to therapy for IE is unknown, so current recommendations remain unaltered.

Information on antimicrobial concentration in vegetations, either in experimental models or in humans with IE, is sparse. Experiments involving a single dose of a radiolabeled antimicrobial agent with autoradiographic analysis of drug dispersion within vegetations of animals with experimental IE revealed three patterns[526]: (1) concentration at the periphery of the vegetation without diffusion into the core (e.g., teicoplanin); (2) progressive diffusion, but with a high gradient from periphery to core (e.g., ceftriaxone); or (3) homogeneous diffusion throughout the vegetation (e.g., several fluoroquinolones). The predictive value of these observations with regard to therapeutic efficacy is unknown. The suboptimal clinical efficacy of teicoplanin in several clinical trials treating intravascular *S. aureus* infections may relate to the maldistribution of this agent within vegetations.[527] Analysis of pharmacodynamic variables (e.g., concentration-dependent bactericidal activity, postantibiotic effect) also may assist in the rational selection of regimens for the treatment of IE.[526] Studies in animals have confirmed that when vegetation formation is inhibited with anticoagulants, the organisms are eradicated more rapidly with penicillin treatment than in control animals with larger vegetations.[175] Enzymatic modification of the glycocalyx in the vegetations of experimental streptococcal IE by in vivo dextranase administration facilitated the bactericidal activity of penicillin by more rapid sterilization of the lesion.[528] In contrast, tissue-type plasminogen activator produced a concentration-dependent lysis of fibrin clots or vegetations infected with *S. epidermidis* or *S. sanguinis* but did not enhance antimicrobial activity in in vitro models.[529,530] Several studies in experimental IE have confirmed the utility of aspirin in reducing the size of vegetations and the microbial densities within aortic vegetations.[531,532] In addition, one clinical study in patients with established IE suggested that aspirin could mitigate the growth of vegetative lesions (as monitored echocardiographically) and prevent cerebral emboli.[533]

General Principles

Certain general principles have been accepted that provide the framework for the current recommendations for treatment of IE. Parenteral antibiotics are recommended over oral drugs in most circumstances because of the importance of sustained antibacterial activity. Erratic absorption with many classes of agents makes oral drugs less desirable. Short-term therapy has been associated with relapse, and most current recommendations emphasize extended drug administration. Early studies by the British Medical Research Council[534] first emphasized the necessity for prolonged treatment. Bacteriostatic antibiotics are generally ineffective in the treatment of bacterial IE. Their use has been associated with frequent relapses, failure to control the infection, or both. A symptomatic response to agents such as tetracycline, erythromycin, or in some cases clindamycin should not be accepted as indicative of successful treatment, because relapse is common after treatment with these agents is discontinued. Likewise, antibiotic combinations should produce a rapid bactericidal effect. This is seen with synergistic combinations, such as penicillin plus an aminoglycoside effective against most viridans streptococci or enterococci. In experimental animals, the rate of bactericidal action expressed by a drug or combination of drugs in broth is predictive of the relative rate at which the organisms are eradicated from the cardiac vegetations in vivo. Antagonistic combinations, such as penicillin plus chloramphenicol, which are less rapidly bactericidal, are less effective in experimental IE than the single bactericidal drug (penicillin) alone.[535] General guidelines for the evaluation of new antimicrobial agents for the treatment of IE were published in 1992.[536] Guidelines for outpatient parenteral antibiotic therapy for IE have been published. These guidelines outline a conservative approach (inpatient or daily outpatient follow-up) during the critical phase (weeks 0–2 of treatment), when complications are most likely, followed by outpatient parenteral antibiotic therapy for the continuation phase of antibiotic therapy.[537]

Patients with IE may have an associated myocarditis complicated by cardiac arrhythmias and CHF. These patients (with IE plus CHF) require close observation in an intensive care unit with electrocardiographic monitoring. As discussed later, the selection of antibiotics should be based on antimicrobial susceptibility tests and the treatment should be monitored clinically and with determination of antimicrobial blood levels when indicated. Blood cultures should be obtained during the early phase of therapy to ensure eradication of the bacteremia and in patients with persistent or recurrent fever during therapy. The use of anticoagulants during therapy for native valve IE has been associated with fatal subarachnoid hemorrhage and other bleeding complications. Most authorities agree that anticoagulant administration in this setting is contraindicated, but this conclusion is controversial. In cases of IE localized to mechanical prosthetic valves, many clinicians maintain anticoagulation within therapeutic range, provided that the patient has no evidence of major vascular emboli (e.g., central nervous system

signs or symptoms). One study concluded that oral anticoagulation should be discontinued in patients with *S. aureus* prosthetic valve IE until the septic phase of the disease is resolved (approximately 10 days after initiation of antimicrobial therapy).[538]

Patients with left-sided IE should be managed at least initially in facilities with access to cardiothoracic surgery. Although persistent or recurrent fever despite appropriate antimicrobial therapy may be due to pulmonary or systemic emboli or drug hypersensitivity, the most common cause is extensive valve ring or adjacent structure infection or metastatic infection.[539] Approximately one-third of patients with left-sided IE require surgery during the acute stages of infection for either valve replacement or metastatic infection.[347] Close monitoring of and early surgical consultation for patients with IE, particularly those with signs of heart failure or persistent fever, are essential. The critical role of early cardiac surgery in many cases of IE has been clearly reiterated by a large, well-designed propensity study[540] and one randomized trial.[541] However, this latter study has been criticized for study design issues that limited generalizability, including infrequency of *S. aureus* IE and a relatively young and healthy patient population.

Tests Useful for Antimicrobial Treatment Monitoring

Management of IE demands careful consideration of the choice, dose, and duration of antimicrobial therapy. The following laboratory tests can help the physician to monitor treatment and can aid in rational therapeutic decisions. In every case of bacterial IE, the etiologic agent must be isolated in pure culture and the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) must be determined for the usual antibiotics used (see later discussion). Standard disk sensitivity testing is unreliable in the context of treating IE, and results may be misleading without the quantitative information provided from the MIC and MBC.

In some forms of IE, combinations of antibiotics are used routinely.[542] These regimens are based on synergy studies performed in vitro and on results obtained in experimental animal models of IE. In difficult cases with a slow therapeutic response and in cases caused by unusual organisms, a determination of synergistic combinations of antibiotics may be helpful. In these cases, tests for bactericidal synergism may be undertaken by standard techniques, such as broth dilution, microtiter "checkerboards," or time-kill curves in broth. Proper attention to standardized techniques, especially inoculum size, is crucial for a meaningful interpretation of the results.

When aminoglycosides are used in therapy, the concentration of antibiotic in the serum should be determined periodically. These agents have a low toxic-to-therapeutic ratio, especially in elderly patients and in patients with renal disease. Peak and trough concentrations should be measured, and the dose should be altered accordingly. This method is superior to reliance solely on nomograms for dosage changes. If synergy with another agent is demonstrable, serum concentrations of the aminoglycosides lower than those generally considered "therapeutic" may be adequate, lessening the potential for toxicity.

Antimicrobial Therapy

The treatment recommendations contained in this chapter are summarized in Table 80.7 and are based on published guidelines from the American Heart Association Committee on Endocarditis published in October 2015.[1] The role of aminoglycosides and other agents in combination therapy for IE also has been reviewed.[543]

TABLE 80.7 Summary of Treatment Options for Endocarditis

ORGANISM	REGIMEN[a]	COMMENTS
Staphylococcus aureus		
Native Valve		
Methicillin-susceptible	Nafcillin or oxacillin, 2 g IV q4h × 4–6 wk Cefazolin, 2 g IV q8h × 4–6 wk	Use of gentamicin in native valve *S. aureus* IE is associated with significant nephrotoxicity without clear clinical benefit and is discouraged. Acceptable in setting of penicillin allergy other than immediate hypersensitivity. See above cautions about gentamicin use.
Methicillin-resistant	Vancomycin, 15–20 mg/kg IV q8–12h × 6 wk[b] Daptomycin, 6 mg/kg IV qd × 4–6 wk[b]	Also acceptable in setting of immediate hypersensitivity or anaphylaxis to penicillin; goal vancomycin trough level 10–20 μg/mL is recommended with careful monitoring of renal function. Daptomycin is FDA approved for treatment of right-sided *S. aureus* IE; for adults, some experts recommend 8-10 mg/kg IV.
Prosthetic Valve		
Methicillin-susceptible	Nafcillin or oxacillin, 2 g IV q4h × ≥6 wk, *plus* gentamicin, 1 mg/kg IV q8h × 2 wk, *plus* rifampin, 300 mg PO/IV q8h × ≥6 wk	Some authorities recommend delaying the initiation of rifampin therapy for several days in an attempt to prevent treatment-emergent resistance to rifampin.
Methicillin-resistant	Vancomycin, 15–20 mg/kg IV q8–12h × ≥6 wk *plus* gentamicin, 1 mg/kg IV q8h × 2 wk, *plus* rifampin, 300 mg PO/IV q8h × ≥6 wk	Goal vancomycin trough level 10–20 μg/mL is recommended with careful monitoring of renal function. Some authorities recommend delaying the initiation of rifampin therapy for several days in an attempt to prevent treatment-emergent resistance to rifampin.
Injection Drug Use		
Methicillin-susceptible	Nafcillin or oxacillin, 2 g IV q4h × 2 wk; *plus* gentamicin, 1 mg/kg IV q8h × 2 wk	Two-week regimen only for use in injection drug users with infection limited to tricuspid valve, no renal insufficiency, and no extrapulmonary infection. Two weeks of monotherapy with antistaphylococcal penicillin has also been successfully used in these patients.
Methicillin-resistant	Vancomycin, 15–20 mg/kg IV q8–12h × 4 wk Daptomycin, 6 mg/kg IV qd × 4–6 wk	Use of gentamicin in this setting is not recommended. Goal vancomycin trough level 10–20 μg/mL is recommended with careful monitoring of renal function. Daptomycin is FDA approved for treatment of right-sided *S. aureus* IE; for adults, some experts recommend 8-10 mg/kg IV.
Coagulase-Negative Staphylococci		
Native Valve		
Methicillin-susceptible	Nafcillin or oxacillin, 2 g IV q4h × 4–6 wk	
Methicillin-resistant	Vancomycin, 15–20 mg/kg IV q8–12h × 6 wk	Also acceptable in setting of immediate hypersensitivity or anaphylaxis to penicillin. Goal vancomycin trough level 10–20 μg/mL is recommended with careful monitoring of renal function.

TABLE 80.7 Summary of Treatment Options for Endocarditis—cont'd

ORGANISM	REGIMEN[a]	COMMENTS
Prosthetic Valve		
Methicillin-susceptible	Nafcillin or oxacillin, 2 g IV q4h × ≥6 wk, *plus* gentamicin, 1 mg/kg IV q8h × 2 wk, *plus* rifampin, 300 mg PO/IV q8h × ≥6 wk	Some authorities recommend delaying the initiation of rifampin therapy for several days in an attempt to prevent treatment-emergent resistance to rifampin.
Methicillin-resistant	Vancomycin, 15–20 mg/kg IV q8–12h × ≥6 wk, *plus* gentamicin, 1 mg/kg IV q8h × 2 wk, *plus* rifampin, 300 mg PO/IV q8h × ≥6 wk	Goal vancomycin trough level 10–20 μg/mL is recommended with careful monitoring of renal function. Some authorities recommend delaying the initiation of rifampin therapy for several days in an attempt to prevent treatment-emergent resistance to rifampin.
Penicillin-Susceptible Viridans Streptococci (MIC ≤0.1 μg/mL) and *Streptococcus bovis (S. gallolyticus)*		
	Penicillin, 2–3 million units IV q4h × 4 wk, *or* ampicillin, 2 g IV q4h × 4 wk Ceftriaxone, 2 g IV qd × 4 wk	Also effective for other penicillin-susceptible nonviridans streptococci (e.g., group A streptococci) For penicillin allergy; patients with uncomplicated viridans streptococcal IE are candidates for outpatient therapy.
	Penicillin, 2–3 million units IV q4h × 2 wk, *plus* gentamicin, 1 mg/kg IV q8h × 2 wk	Uncomplicated native valve IE only; not acceptable for *Abiotrophia* and *Granulicatella* spp.
Abiotrophia and *Granulicatella* spp.	Penicillin, 2–4 million units IV q4h × 4 wk, *plus* gentamicin, 1 mg/kg IV q8h × 2 wk Vancomycin, 15–20 mg/kg IV q8-12h × 4 wk	For prosthetic valve IE, give 6 wk of penicillin. *Abiotrophia* and *Granulicatella* spp. are often penicillin tolerant. For penicillin allergy. No published guidelines exist for vancomycin trough targeting for treatment of streptococcal or enterococcal infections.
Relatively Penicillin-Resistant Viridans Streptococci (MIC 0.12 to <0.5 μg/mL)		
	Penicillin, 4 million units IV q4h × 4 wk, *plus* gentamicin, 1 mg/kg IV q8h × 2 wk Vancomycin, 15–20 mg/kg IV q8–12h × 4 wk	For penicillin allergy or to avoid gentamicin. No published guidelines exist for vancomycin trough targeting for treatment of streptococcal or enterococcal infections.
Enterococci[c] and Penicillin-Resistant Viridans Streptococci (Penicillin MIC >0.5 μg/mL)		
Penicillin-susceptible, aminoglycoside-susceptible enterococci	Penicillin[d] 3–5 g IV q4h × 4–6 wk, *plus* gentamicin, 1 mg/kg IV q8h × 4–6 wk; *or* Ampicillin, 2 g IV q4h, *plus* gentamicin, 1 mg/kg IV q8h × 4–6 wk	Increase duration of both drugs to 6 wk for prosthetic valve infection or for enterococcal IE with symptoms >3 mo. For older patients and those with underlying renal disease, can consider shortening the duration of gentamicin to 2 wk.
Penicillin-resistant, vancomycin-susceptible, aminoglycoside-susceptible enterococci	Vancomycin, 15–20 mg/kg IV q8–12h × 6 wk, *plus* gentamicin, 1 mg/kg q8h × 6 wk[e]	Also for patients with penicillin allergy. This regimen is associated with enhanced risk of nephrotoxicity. Penicillin desensitization should be considered as an alternative to this regimen when possible. No published guidelines exist for vancomycin trough targeting for treatment of streptococcal or enterococcal infections.
Penicillin-susceptible, aminoglycoside-resistant enterococci	Ampicillin, 2 g IV q4h, *plus* ceftriaxone, 2 g IV q12h	Useful for patients with significant underlying renal disease.
Penicillin-resistant, vancomycin-resistant enterococci	No standard therapy; daptomycin, linezolid, and quinupristin-dalfopristin have been used	Consult infectious diseases specialist.
HACEK Strains		
	Ceftriaxone, 2 g IV qd × 4 wk Ampicillin-sulbactam, 3 g IV q6h × 4 wk (if β-lactamase producing strain)[a]	Increase duration to 6 wk for infections involving prosthetic valves. Increase duration to 6 wk for infections involving prosthetic valves.
Non-HACEK Gram-Negative Bacilli[f]		
Enterobacteriaceae	Extended-spectrum penicillin (e.g., piperacillin-tazobactam) or cephalosporin *plus* aminoglycosides for susceptible strains	Treat for a minimum of 6-8 wk. Some species exhibit inducible resistance to third-generation cephalosporins. Valve surgery is often required for patients with left-sided IE caused by gram-negative bacilli, especially for prosthetic valve IE. Consultation with an infectious diseases specialist is recommended.
Pseudomonas aeruginosa	Antipseudomonal penicillin (e.g., piperacillin) *plus* high-dose tobramycin, 8 mg/kg/day IV or IM in once-daily doses; *or* High-dose ceftazidime, cefepime, or imipenem	Goal tobramycin peak and trough concentrations of 15–20 μg/mL and ≤2 μg/mL, respectively. Treat for a minimum of 6–8 wk. Early valve surgery usually required for left-sided *Pseudomonas* IE; consultation with a specialist in infectious diseases is recommended.
Fungi[f]		
	Treatment with a parenteral antifungal agent (usually an amphotericin B–containing product) is usually recommended as initial therapy	Fungal endocarditis is usually an indication for valve replacement surgery. Long-term/lifelong suppressive therapy with oral antifungal agents is often required. Consultation with a specialist in infectious diseases is recommended.

[a]Dosages assume normal renal function. For patients with renal insufficiency, adjustments must be made for all antibiotics except nafcillin, rifampin, and ceftriaxone. Gentamicin doses should be adjusted to achieve a peak serum concentration of approximately 3 μg/mL 30 minutes after dosing and a trough gentamicin level of <1 μg/mL.
[b]Primarily relevant to left-sided IE.
[c]Enterococci must undergo antimicrobial susceptibility testing. These recommendations are for enterococci susceptible to penicillin, gentamicin, and vancomycin except as indicated.
[d]Ampicillin, 12 g/day, can be used instead of penicillin.
[e]The need to add an aminoglycoside has not been demonstrated for penicillin-resistant streptococci.
[f]Limited data exist.
FDA, US Food and Drug Administration; *HACEK, Haemophilus, Aggregatibacter* (formerly *Actinobacillus*), *Cardiobacterium, Eikenella, Kingella*; *IE,* infective endocarditis; *MIC,* minimal inhibitory concentration.
Modified from Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation. *2015;132:1435.*

Penicillin-Sensitive Streptococcal Endocarditis

Most strains of viridans streptococci, "other" streptococci (including *S. pyogenes*), and nonenterococcal group D streptococci (primarily *S. bovis*) are exquisitely sensitive to penicillins, with an MIC of less than 0.12 μg/mL. Of viridans streptococci, 15% to 20% are "resistant" to this arbitrary concentration of penicillin.[352,377] In addition, approximately 15% of the strains of *S. mutans* show a low MIC for penicillin (<0.1 μg/mL), but the MBC is considerably higher (1.25–50 μg/mL).[356] These organisms probably should be considered "penicillin tolerant" and should be treated accordingly. Although results depend on the in vitro methodology employed,[544] studies beginning in the 1980s suggested that tolerance to penicillin among viridans streptococci was more prevalent than previous reports had indicated.[545] Of viridans streptococci cultured from gingiva and blood after dental procedures, 19% were tolerant,[546] especially among *S. mutans* (27%) and *S. mitior* (20%) isolates. Almost identical figures were reported among blood culture isolates of viridans streptococci,[547] with tolerance defined as a penicillin MBC-to-MIC ratio exceeding 10:1. Almost all strains of nutritionally dependent streptococci were tolerant to penicillin.[548,549] The influence of the tolerance phenomenon on the response to penicillin therapy in experimental IE is not known; two studies yielded conflicting results.[550,551] Data on human infections with tolerant strains and the therapeutic results are unavailable. Except for *Abiotrophia* and *Granulicatella* spp., we do not believe that the demonstration of tolerance by an isolate of viridans streptococci influences therapeutic decisions.

With broth dilution susceptibility tests, the usual MBC determinations for the so-called penicillin-sensitive streptococci are as follows: penicillin, 0.1 to 1 μg/mL; cephalothin, 0.15 to 1.25 μg/mL; vancomycin, 0.15 to 0.4 μg/mL; streptomycin, 6.25 to 50 μg/mL; and gentamicin, 1.56 to 3.12 μg/mL.[552,553] *S. bovis* is 10 to 5000 times more susceptible to penicillin than the other group D species (enterococci). They also are relatively susceptible to oxacillin, methicillin, and lincomycin, whereas the enterococci are resistant.[360] Most streptococci in this group show synergism in vitro between penicillin or vancomycin and streptomycin, gentamicin, or kanamycin (see later discussion).[554,555] The first strains of viridans streptococci with high-level streptomycin resistance (MIC ≥1000 μg/mL) were reported in 1982 from Paris. Although these strains are rare (2%–8% of isolates in some locales),[556] the documentation of aminoglycoside-modifying enzymes and the lack of penicillin-streptomycin synergy in vitro and in experimental animal models[556,557] are alarming. These penicillin-susceptible strains may be killed synergistically by penicillin-gentamicin combinations. Significant antibiotic antagonism was shown with the combination of clindamycin and gentamicin for *S. mutans* IE. The in vitro synergism between penicillin and aminoglycosides was found to correlate with a more rapid rate of eradication of bacteria from cardiac vegetations in vivo in the rabbit endocarditis model[558,559] for the common viridans streptococci. All of these studies have been summarized in reviews.[560–562] Low-level penicillin resistance (defined in this study as MIC 0.2–2 μg/mL) was found in 31% of viridans streptococci in Madrid in 1988 and 1989, and an additional 17% of strains showed high-level resistance.[563] Perhaps most important, streptomycin and cefotaxime resistance also was found to be "common."[22] The authors suggested a vancomycin-gentamicin regimen for IE due to viridans streptococci in Spain.

The combination of penicillin and streptomycin was used in more than 200 historical cases at The New York Hospital–Cornell Medical Center without a documented relapse.[554] This clinical experience has been confirmed elsewhere, but the overall reported relapse rate is 1.4%.[561] This regimen is as follows: aqueous penicillin G, 10 million to 20 million units given intravenously daily, or procaine penicillin G, 1.2 million units given intramuscularly every 6 hours, for 4 weeks, combined with streptomycin, 0.5 g intramuscularly every 12 hours for the first 2 weeks. Studies by Wilson and colleagues at the Mayo Clinic[564,565] showed that a 2-week course of intramuscular procaine penicillin (1.2 million units every 6 hours) and streptomycin (0.5 g every 12 hours) cured at least 99% of patients with penicillin-sensitive streptococcal IE. These results were similar to those obtained with therapy consisting of β-lactams alone for a total of 4 weeks[566,567] but significantly better than results obtained with penicillin alone for 2 weeks. The latter regimen was associated with a 50% relapse rate if low doses of penicillin were used and improved to 17% with higher penicillin dosages. The 2-week penicillin-streptomycin regimen has been the most cost-effective and the preferred therapy among these three regimens in uncomplicated penicillin-sensitive (MIC ≤0.12 μg/mL) streptococcal IE in young patients. However, since the advent of ceftriaxone-based regimens (featuring 2 weeks of ceftriaxone plus an aminoglycoside), many clinicians have opted for this approach (see later discussion).[568,569] Four weeks of penicillin (or ceftriaxone; see later discussion) alone is recommended for patients with impaired renal function and for those who are particularly susceptible to the low risk for streptomycin-induced ototoxicity (i.e., the elderly). The Cornell regimen (4 weeks of penicillin with an initial 2 weeks of streptomycin) has been recommended for patients with a complicated course, a history of disease exceeding 3 months' duration, or prosthetic valve IE caused by these sensitive strains and when susceptibility testing reveals the rare penicillin-resistant streptococci.[570] The preferred regimen for IE due to penicillin-tolerant streptococci is not established.

Most of the published clinical data with β-lactam–aminoglycoside regimens for the treatment of viridans streptococcal IE involve the use of penicillin or ampicillin plus either streptomycin or gentamicin. On the basis of extensive in vitro and animal data and for a variety of other reasons, however, the American Heart Association has deemed gentamicin preferable to streptomycin in this context.[237] First, gentamicin currently is more widely available and is used more often clinically than streptomycin in viridans streptococcal IE. Second, gentamicin (but not streptomycin) serum level determinations are performed routinely in most hospital laboratories. Because in vitro synergy against most viridans streptococci with penicillin or ampicillin in combination with gentamicin occurs at low gentamicin concentrations (1–3 μg/mL), most authorities recommend using gentamicin at a total daily dose of no more than 3 mg/kg/day (IM or IV), split into either a twice-daily (every 12 hours) or a thrice-daily (every 8 hours) dosage regimen. However, one study using ceftriaxone plus gentamicin given as a once-daily dose reported excellent efficacy against penicillin-susceptible streptococcal IE with no increases in aminoglycoside-associated nephrotoxicity.[569] Despite the apparent safety of once-daily aminoglycoside dosing in this study and in clinical trials involving patients with other infectious disorders,[571] the total number of patients receiving such regimens for 2 weeks or longer has been relatively limited. As with any patients receiving aminoglycosides, appropriate clinical and blood level monitoring to mitigate ototoxicity and nephrotoxicity should be used. Nomograms for prudent monitoring and adjustment of aminoglycoside doses when once-daily regimens are used have been published.[570]

We believe that gentamicin, at a total daily dose of 3 mg/kg, should be substituted for streptomycin in the aforementioned regimens when combination therapy is deemed advisable. In addition, a penicillin-gentamicin regimen is indicated for viridans streptococcal IE if high-level streptomycin resistance is present[556,557] and for strains that are relatively resistant to penicillin (MIC >0.12 μg/mL and ≤0.5 μg/mL).[334] Infections caused by strains with a penicillin MIC greater than 0.5 μg/mL should be treated as for enterococcal IE (see later discussion). Because of the enhanced rate of bacterial killing in animal models[572,573] and the high relapse rate of about 17%,[574] we also believe that the Cornell regimen (using gentamicin as the preferred aminoglycoside) should be employed for all patients with IE due to *Abiotrophia* and *Granulicatella* spp. Although temafloxacin plus tobramycin was as effective as penicillin plus tobramycin against experimental IE caused by *G. adiacens* in rabbits,[575] quinolones are best avoided for IE due to streptococci pending further data.

In a penicillin-allergic patient for whom a cephalosporin is deemed safe, several regimens are acceptable for IE caused by penicillin-susceptible viridans streptococci: (1) cefazolin, 2 g IM or IV every 8 hours for 4 weeks, combined with streptomycin, 0.5 g IM or IV every 12 hours; or (2) gentamicin, administered IM or IV in two or three daily doses for a total of no more than 3 mg/kg daily, for the initial 2 weeks; or (3) ceftriaxone alone, 2 g IV or IM given daily for 4 weeks. This last regimen has been proven efficacious against penicillin-sensitive streptococcal IE.[576,577] In one uncontrolled trial in Europe, 55 of 59 patients completed treatment with 4 months to 5 years of follow-up; treatment was completely

uneventful in 71% of patients, whereas valve replacement eventually was required in 10 patients. This regimen may permit outpatient therapy in most stable patients with this disease. In addition, ceftriaxone plus an aminoglycoside has shown in vivo synergy in experimental viridans streptococcal IE.[578] Combination regimens of 2 weeks, including ceftriaxone (2 g once daily) plus an aminoglycoside (netilmicin or gentamicin), which allow for shortened, predominantly outpatient regimens, have been studied in penicillin-susceptible viridans streptococcal IE.[568,569] These trials, performed in Europe and North and South America, documented efficacy in this form of IE comparable to that achieved with other 2-week or 4-week regimens.[568,569]

If treatment with β-lactams is contraindicated, the regimen of choice is vancomycin, 30 mg/kg/24 h in two equally divided doses (not to exceed 2 g/24 h, unless serum concentrations are inappropriately low) for 4 weeks.[334] Because patients, particularly obese patients, who are receiving a fixed dose of vancomycin regularly received inadequate doses of the antibiotic, weight-based dosing strategies are preferred.[579] A 4-week regimen of high-dose teicoplanin has been efficacious in a few patients with streptococcal IE,[580] but the occurrence of drug fever and infection with teicoplanin-tolerant *S. bovis* is cause for concern.

Endocarditis Caused by Streptococci With Penicillin Minimal Inhibitory Concentration Greater Than 0.5 μg/mL or by Enterococci

Enterococci are a common cause of IE and can be highly resistant to therapy. The mortality rate still approximates 20%,[578] and relapses are common. With broth dilution susceptibility tests, the MIC determinations for many enterococci are as follows: penicillin, 0.4 to 12.5 μg/mL; ampicillin, less than 0.4 to 3.1 μg/mL; cephalothin, 12.5 to 25 μg/mL; vancomycin, 0.78 to 3.1 μg/mL; streptomycin, 3.1 to greater than 50 μg/mL; and gentamicin, 6.25 to 25 μg/mL. Ampicillin is approximately twice as active as penicillin by weight. In contrast, the usual MBC determinations are as follows: penicillin, greater than 6.25 μg/mL (in 80% of cases, >100 μg/mL); cephalothin, greater than 100 μg/mL; streptomycin, greater than 25 μg/mL; vancomycin, greater than 100 μg/mL; and gentamicin, 25 μg/mL or less.[581] *E. faecium* strains are more resistant to β-lactams than are *E. faecalis* strains.[582] In general, these agents are bacteriostatic against the enterococci and should not be administered alone in this disease. This bacteriostatic action of agents known to inhibit cell wall synthesis is the result of a defective bacterial autolytic enzyme system.[583,584] As stated earlier, all β-lactams, including imipenem, are bacteriostatic against enterococci in vitro, and combination regimens are used in treating IE whenever possible.[585]

A new mechanism of penicillin resistance among *E. faecalis* strains was described in 1983: plasmid-mediated β-lactamase production. These strains remain relatively rare.[586,587] *E. faecalis* predominates, but β-lactamase production was documented in *E. faecium*.[588] Ampicillin-sulbactam overcomes the β-lactamase production and appears to be equivalent to vancomycin[589,590] (or teicoplanin[591]) in experimental IE involving these organisms and superior to therapy with ticarcillin-clavulanate.[591] Most β-lactamase–producing organisms also display high-level aminoglycoside resistance, further compromising and complicating the choice of available regimens (see later discussion). Still other enterococci, particularly *E. faecium* and *Enterococcus raffinosus,* may display high-level penicillin resistance in the absence of β-lactamase production related to perturbations in various penicillin-binding proteins; experimental IE caused by these organisms responds to therapy with daptomycin or vancomycin.[592] To date, the number of reported cases of enterococcal IE caused by β-lactamase–producing strains or cases with high-level penicillin resistance on a nonenzymatic basis is low. The traditional view of β-lactam tolerance among enterococci has been challenged[593] in that some strains show "paradoxical" tolerance (i.e., there is a higher percentage of survivors at higher antibiotic concentrations). This phenomenon is shown more easily in vitro at high bacterial densities in stationary growth phase, a situation presumably reflecting the in vivo conditions in the vegetation, and may be important in bacterial persistence or relapse during or after therapy for enterococcal IE.

Cell wall–active antibiotics plus an aminoglycoside are synergistic and produce a bactericidal effect in vitro against most enterococcal strains. Although successful treatment of enterococcal IE requires such combinations, aminoglycoside-associated nephrotoxicity and ototoxicity confer significant risk to patients whose antibiotic regimen includes an aminoglycoside. Studies in experimental models[594] suggest that "low-dose" streptomycin (peak serum concentrations of 9.1 μg/mL) in combination with penicillin is sufficient to treat streptomycin-susceptible enterococcal IE. "High-level" streptomycin resistance (MIC >2000 μg/mL) is demonstrable in at least 60% of current enterococcal strains. This resistance correlates with an inability to show in vitro synergism between penicillin and streptomycin.[595] These highly resistant strains show synergism between a penicillin and gentamicin in vitro[596,597] at clinically achievable serum concentrations. Enhanced activity with the penicillin and gentamicin combination was seen in vivo for streptomycin-resistant and streptomycin-sensitive enterococci in the rabbit model of IE. No differences in results were seen when penicillin was combined with low-dose versus high-dose gentamicin (peak serum levels of 3.06 and 8.05 μg/mL, respectively) in the treatment of experimental streptomycin-resistant enterococcal IE.[598] Early reports[599,600] revealed high-level gentamicin resistance among enterococci in 14% of isolates beginning in 1979. This phenomenon has become increasingly prevalent in many areas[601–603] beginning with *E. faecalis,* but now also includes *E. faecium*.[603–605] High-level gentamicin resistance (MIC ≥2000 μg/mL) now accounts for at least 35% of enterococcal blood isolates in many hospitals. The resistance is plasmid mediated through the production of aminoglycoside-modifying enzymes and can be transferred among strains. A clonal dissemination is not the cause of the increased frequency of these resistant strains, because gentamicin resistance appears in a wide variety of different conjugative and nonconjugative plasmids among enterococci.[603] Although these strains seldom cause IE, they present major problems in nosocomial infections,[606] and, because resistance to multiple aminoglycosides is common, they represent a formidable therapeutic challenge.[607] In addition, penicillin-aminoglycoside or vancomycin-aminoglycoside synergy is not apparent against these organisms in vitro.

The optimal therapy for IE due to these highly resistant strains has not been established. None of the currently recommended regimens is bactericidal against these isolates, and valve replacement[608] may be necessary for a cure. When these isolates are encountered, all available aminoglycosides must be tested separately, because the organism may be susceptible to one but resistant to others. Some isolates are sensitive to quinolones or daptomycin, but these agents have received scant attention in human enterococcal infections. Traditionally, long-term therapy (8–12 weeks) with high dosages of penicillin (20–40 million units IV daily in divided doses) or ampicillin (2–3 g IV every 4 hours or by continuous infusion) for IE due to these multiple aminoglycoside-resistant enterococci was recommended. However, in vitro data[609] and two open-label trials (see later discussion) of high-dose ceftriaxone (2 g IV twice daily) plus ampicillin[610,611] now strongly support the efficacy of a double β-lactam combination in the setting of ampicillin-sensitive, aminoglycoside-resistant enterococcal IE data. Although results vary among experimental animal models of IE with these strains,[607,612] continuous infusion of high-dose ampicillin throughout the 24-hour period may be more effective than a divided-dose regimen and merits a trial in recalcitrant cases. Even higher-dose aminoglycosides and trimethoprim-sulfamethoxazole were ineffective in animal models[613,614] and cannot be recommended.

Vancomycin also is bacteriostatic against enterococci and exhibits synergy with the aminoglycosides in vitro. The vancomycin-streptomycin combination synergistically kills 40% to 80% of enterococcal strains, whereas the vancomycin-gentamicin combination shows synergy against 93% to 98%.[615] In one study, vancomycin therapy alone was ineffective in eradicating enterococci from cardiac vegetations in the rabbit model of IE, but the combination of vancomycin plus gentamicin rapidly achieved a bactericidal effect.[598] Vancomycin combined with rifampin had an indifferent effect against enterococci (43 of 48 strains) in vitro; antagonism was observed rarely.[616] Glycopeptide-resistant strains of enterococci first were isolated in France in 1986 and since then have emerged rapidly in many geographic areas worldwide (e.g., New York City[617]) as an important cause of nosocomial infections. The genetics of vancomycin resistance[618] is described elsewhere (see Chapter 30) and

has been reviewed,[619] but multiple phenotypes exist that may confer cross-resistance to related agents (e.g., teicoplanin, daptomycin). Molecular analyses suggest that a highly mobile genetic element (i.e., a transposon) is responsible for the rapid spread of vancomycin-resistant enterococci.[617] The molecular basis for some forms of this resistance (substitutions of D-alanine-D-lactate for D-alanine-D-alanine in the terminal pentapeptide chain) has been defined. IE due to vancomycin-resistant enterococci is unusual[620] but has been reported in a variety of clinical settings,[621,622] including transplanted hearts.[623]

The therapy for vancomycin-resistant enterococcal IE is not established.[334] All suspected strains should be tested quantitatively (e.g., by determination of MIC or MBC) in vitro for susceptibility to glycopeptides, penicillins, and aminoglycosides. Teicoplanin—or, potentially, linezolid or daptomycin[624–626]—may be useful, in combination with gentamicin, against some isolates with low-level vancomycin resistance that do not exhibit cross-resistance. Anecdotal cases have been reported of cures after therapy with linezolid,[621] a combination of quinupristin-dalfopristin plus ampicillin and gentamicin in addition to aortic root replacement,[627] and chloramphenicol plus minocycline.[622] A triple combination of high-dose penicillin plus vancomycin plus gentamicin seems promising in animal models of IE induced by such resistant enterococci[628,629] and should be considered if in vitro susceptibility test results suggest multiply resistant isolates. The cephalosporins are relatively inactive against enterococci, even in combination with an aminoglycoside, and should not be used in this disease except potentially in place of an aminoglycoside and in combination with ampicillin (see later discussion).[610,611] The older-generation quinolones (e.g., ciprofloxacin) do not seem promising for the treatment of enterococcal IE.[630]

Native or prosthetic valve enterococcal IE caused by strains susceptible to penicillin, gentamicin, and vancomycin should be treated with combination antimicrobial therapy for at least 4 to 6 weeks.[560] The recommended regimen is as follows: aqueous penicillin G, 18 to 30 million units IV daily in divided doses (or ampicillin 12 g/24 h in six equally divided doses), plus gentamicin 3 mg/kg/24 h in three equally divided doses, for 4 to 6 weeks. Gentamicin should not be given once daily for enterococcal IE. Experimental studies in enterococcal IE models show a greater reduction in vegetation bacterial densities when the aminoglycoside is administered in multidose rather than once-daily regimens.[631,632]

Wilson and associates[633] analyzed the experience at the Mayo Clinic with 56 patients who received treatment for 4 weeks with aqueous penicillin G (20 million units IV daily) combined with either streptomycin, 0.5 g IM every 12 hours, for enterococcal IE due to streptomycin-sensitive strains, or gentamicin, 1 mg/kg IM every 8 hours, for IE due to streptomycin-resistant strains. Relapse rates were high (12.5%) for both regimens; however, all patients who experienced relapse had had symptoms suggestive of IE for longer than 3 months. Relapses also occurred only in patients with mitral valve involvement. All patients who received more than 3 mg/kg daily of gentamicin developed reversible nephrotoxicity (defined as a twofold increase in serum creatinine concentration), and 19% of patients receiving streptomycin for 4 weeks developed irreversible vestibular toxicity. Although this was not a prospective randomized trial, we believe that in selected cases enterococcal IE may be managed with 4 weeks of combination therapy. Exceptions include mitral valve involvement, duration of symptomatic illness exceeding 3 months, enterococcal prosthetic valve endocarditis, and relapses of enterococcal IE.

A penicillin-allergic patient presents the clinician with a difficult therapeutic dilemma. Vancomycin as a single drug in a dose of 30 mg/kg/24 h in two equally divided doses up to 1 g IV every 12 hours, unless serum vancomycin levels are inappropriately low, is recommended for the treatment of enterococcal IE when patients are unable to tolerate penicillin or ampicillin, or if the infecting pathogen is resistant to these preferred agents.[334] Experience is limited, however, and because of its lack of bactericidal activity in vitro and poor performance in experimental IE, vancomycin should be combined with gentamicin or streptomycin.[334] This combination is potentially more nephrotoxic, and clinical proof of the superiority of such regimens over vancomycin alone is not available. The other therapeutic option in the allergic patient is "penicillin desensitization" followed by the administration of penicillin and an aminoglycoside. The treatment of enterococcal IE caused by highly aminoglycoside-resistant or glycopeptide-resistant strains was discussed earlier.

Alternatives to Full-Course Aminoglycoside Therapy for Enterococcal Infective Endocarditis

The significant risk for nephrotoxicity accompanying the traditional 4- to 6-week course of aminoglycoside therapy recommended for enterococcal IE has prompted a number of investigators to consider potentially safer alternatives. Olaison and colleagues[634] conducted a 5-year nationwide prospective cohort study in Sweden in which 93 episodes of enterococcal IE were identified. Although current American Heart Association guidelines for the treatment of enterococcal IE recommend combined treatment with penicillin or ampicillin plus an aminoglycoside for 4 to 6 weeks, these investigators evaluated the clinical outcomes when the total duration of aminoglycoside therapy was reduced. Patients in this observational study had a median of 15 days of combined penicillin-ampicillin plus aminoglycoside. The overall cure rate of 81% (75 of 93) compared favorably with most historical control studies of enterococcal IE.[635,636] The authors concluded that reducing the aminoglycoside duration to approximately 2 weeks would maintain clinical efficacy while reducing potential toxicities in this high-risk patient population with enterococcal IE.[634] A study from Denmark has essentially confirmed the equivalent efficacies and reduced renal toxicities of the shorter-term aminoglycoside strategy for enterococcal IE.[637]

A second aminoglycoside-sparing strategy for ampicillin-susceptible enterococcal IE involves pairing ampicillin with high-dose ceftriaxone (2 g IV every 12 hours) instead of gentamicin. This approach takes advantage of the fact that the combination of ceftriaxone and ampicillin can saturate at least four of the five major penicillin-binding proteins of enterococci. In a prospective observational cohort study involving 13 centers in Spain, Gavalda and coworkers[610] initially reported a 3-month cure rate of 67.4% in 43 patients with ampicillin-susceptible *E. faecalis* IE who received a 6-week course of intravenous ampicillin, 2 g every 4 hours, plus intravenous ceftriaxone, 2 g every 12 hours. This experience was replicated in a second observational, nonrandomized cohort study involving 246 consecutive adults with *E. faecalis* IE from 18 Spanish and Italian hospitals.[611] Of the 246 patients, 159 patients received ampicillin and ceftriaxone and 87 received ampicillin and an aminoglycoside. Although there were no significant differences between the two patient groups in 3-month mortality or relapses, interruption of antibiotic treatment because of adverse events was much more frequent in patients treated with ampicillin and an aminoglycoside than in those receiving ampicillin and ceftriaxone (25% vs. 1%, $P < .001$), primarily owing to the emergence of new renal failure (23% vs. 0%, $P < .001$). Based on these reports, we believe that the combination of ampicillin and ceftriaxone appears to be effective and safe for treating high-level aminoglycoside-resistant ampicillin-susceptible *E. faecalis* IE and represents a reasonable option for patients with non–high-level aminoglycoside-resistant *E. faecalis* IE who are at increased risk for nephrotoxicity.

Staphylococcal Endocarditis

The mortality rate in acute staphylococcal IE still approximates 25% to 40%, and the preferred antibiotic regimen is controversial. Mortality is highest for men, for patients older than 50 years, and for patients with left-sided involvement or central nervous system manifestations. In addition, injection drug users seem to have a lower mortality than do nonaddicts. Most *S. aureus* isolates, whether community or hospital acquired, are now resistant to penicillin G (MIC >0.2 μg/mL). The current recommended regimen includes a penicillinase-resistant penicillin (nafcillin or oxacillin, 2 g IV every 4 hours) or a cephalosporin (cephalothin, 2 g IV every 4 hours, or cefazolin, 2 g IV every 8 hours) given for 6 weeks.[638] The addition of gentamicin produced a synergistic effect against *S. aureus* in vitro and in experimental staphylococcal IE in rabbits.[639] However, the combination did not improve the survival rate (60%) over that observed with a penicillin derivative alone in a small group of patients.[407] Combination therapy did not improve the results of therapy for staphylococcal IE in injection drug users,[640] but the

mortality rate is low (2%–8%) in this subgroup of patients with this disease.

A number of studies have evaluated whether combination therapy permitted a shorter duration of therapy in injection drug users with *S. aureus* IE. Two weeks of nafcillin (2 g IV every 4 hours) plus tobramycin (1 mg/kg IV every 8 hours) cured 47 (94%) of 50 injection drug users who had right-sided IE[641] without evidence of renal failure, extrapulmonary metastatic infectious complications, aortic or mitral involvement, meningitis, or infection with MRSA. After this original experience with "short-course" parenteral therapy for right-sided *S. aureus* IE in addicts,[641] the findings of three randomized, prospective trials of short-course regimens in this disease were subsequently published, comprising 121 patients (summarized by DiNubile).[642,643] The combination regimens included intravenously administered cloxacillin with either gentamicin or amikacin.[644,645,646] The overall clinical and microbiologic cure rates exceeded 90% in these studies. In patients with HIV seropositivity (most having $CD4^+$ counts greater than 300×10^6 cells) and in patients with large tricuspid vegetations (≥10 mm in diameter), such regimens had excellent efficacy.[646] Ribera and coworkers[646] showed an efficacy of intravenously administered cloxacillin that was equivalent to that of the cloxacillin plus gentamicin regimen. These data suggested that parenteral β-lactam short-course therapy, with or without aminoglycoside, is adequate for the treatment of uncomplicated right-sided IE caused by methicillin-susceptible *S. aureus* (MSSA).[334] By contrast, another investigation confirmed the relative ineffectiveness of a short-course regimen based on a glycopeptide (teicoplanin or vancomycin) plus gentamicin for right-sided *S. aureus* IE.[647]

Daptomycin for Staphylococcal Infective Endocarditis

In 2006, daptomycin, 6 mg/kg IV once daily, was shown to be noninferior to low-dose, short-course gentamicin plus either vancomycin or nafcillin, as appropriate, for the treatment of SAB and right-sided IE.[648] Based on the results of this clinical trial, daptomycin received approval from the U.S. Food and Drug Administration (FDA) for the treatment of SAB, including right-sided native valve IE. Because of concerns about treatment-emergent resistance to daptomycin, some authorities use daptomycin at higher doses (e.g., 8–10 mg/kg IV once daily) than those currently approved when treating complicated *S. aureus* infections. Although a single observational study from the ICE-PCS described the use of high dose (e.g., ≥8 mg/kg daptomycin) for the treatment of left-sided IE,[649] there are currently insufficient data on the use of daptomycin for left-sided IE to provide a recommendation for this indication.

Other Antibiotics for Staphylococcal Infective Endocarditis

Two studies have evaluated the use of predominantly oral 4-week antibiotic regimens (ciprofloxacin plus rifampin) for the treatment of uncomplicated right-sided *S. aureus* IE in injection drug users.[650,651] In each study, including one in which more than 70% of patients were HIV seropositive,[651] cure rates exceeded 90%.

Anecdotal case reports in nonaddicts with staphylococcal IE suggest that the addition of gentamicin may be beneficial if there is no response to nafcillin therapy.[652] In a multicenter, prospective trial comparing nafcillin alone with nafcillin plus gentamicin (for the initial 2 weeks) in the treatment of IE due to *S. aureus,*[653] most of the patients were nonaddicts who had left-sided IE. Although the combination therapy resulted in a more rapid rate of eradication of the bacteremia, the incidence of nephrotoxicity was increased and no improvement in mortality was achieved. The 2015 American Heart Association treatment guidelines concluded that adjunctive gentamicin therapy should not be used for either left- or right-sided *S. aureus* native valve IE,[1] based in part on a report that even brief courses of gentamicin for these syndromes are associated with increased risks of nephrotoxicity.[654] In that report, 236 patients from 44 centers in four countries were randomized to receive either daptomycin monotherapy or initial low-dose gentamicin (e.g., 3 mg/kg/24 h IV in three equally divided doses for 3–5 days). In a post hoc analysis of the study results, Cosgrove and colleagues addressed the issue of gentamicin-induced nephrotoxicity. In their analysis, 22% of patients receiving initial low-dose gentamicin, versus 8% of patients not receiving this adjunct therapy, experienced decreased creatinine clearance ($P = .005$). Receipt of any gentamicin was independently associated with clinically significant decreases in creatinine clearance.[654] Based on these results and on the minimal existing data supporting its benefit, we believe that initial low-dose gentamicin should no longer be used for SAB or native valve endocarditis in most clinical scenarios. The role of initial low-dose gentamicin remains unresolved in a number of other settings not addressed in Cosgrove and colleagues' study, including pediatric populations, patients with left-sided IE or prosthetic valve IE, infections caused by high–vancomycin MIC MRSA isolates (e.g., vancomycin-intermediate-resistant *S. aureus* [VISA]), and infections with persistent bacteremia.[655] If the organism is susceptible to penicillin (MIC <0.1 μg/mL), this agent in a dose of 20 to 24 million units IV daily should be used. The response to treatment may be slow, often with fever and positive blood cultures lasting 1 week.[404]

The therapy for staphylococcal IE in penicillin-allergic patients and in cases in which the isolate is methicillin-resistant (i.e., MRSA) is problematic. A decision analysis concluded that patients with a questionable history of immediate-type hypersensitivity to penicillin and methicillin-sensitive *S. aureus* IE should be skin tested before starting antibiotic therapy.[656] First-generation cephalosporins (as noted previously) generally are recommended in patients with non–life-threatening penicillin allergy, but nafcillin is more active in experimental MSSA endocarditis[657] and is preferred if the results of skin tests for penicillin allergy are negative. With the exception of ceftazidime, the in vivo efficacy of cephalosporins in experimental *S. aureus* IE has a positive correlation with in vitro activity (MIC) and the percentage of time during the dosing interval in which the drug serum level exceeds the MIC.[657] Vancomycin still is recommended for the treatment of *S. aureus* IE in patients with life-threatening penicillin allergy (e.g., anaphylaxis) or anaphylactoid reactions (e.g., giant urticaria) and when MRSA strains are involved; however, more recent experience suggests caution, because suboptimal outcomes have been associated with the use of this agent in MRSA IE. Vancomycin is less rapidly bactericidal than nafcillin in vitro against *S. aureus,* especially at high inocula (approximately 10^7 CFUs), mimicking intravegetation densities. Vancomycin therapy for complicated SAB also has been associated with relapses, as confirmed with pulsed-field gel electrophoresis.[658] Failure rates of approximately 40% have been documented in patients with *S. aureus* IE treated with vancomycin[659] despite right-sided involvement. In another study[660] using vancomycin-based regimens (with or without rifampin), blood cultures remained positive after 7 days of therapy in approximately 50% of patients, despite right-sided involvement and negative results in many echocardiographic studies. Patients with bacteremia or IE due to MRSA with high, but still "fully susceptible," vancomycin MICs (defined as ≥1.5 μg/mL[661] or as 2 μg/mL[662]) had a worse clinical outcome than patients whose bloodstream MRSA isolates had a lower vancomycin MIC value.[663] The full implications of these findings, however, are not yet established,[664] because the same association of worse clinical outcome in infections caused by such "high vancomycin MIC" *S. aureus* strains has also been documented in patients infected with MSSA who were treated with antistaphylococcal penicillins instead of vancomycin.[665,666] Daptomycin is an alternative to vancomycin in the treatment of SAB and IE.[667] When daptomycin is used for the treatment of *S. aureus* IE, many authorities use higher doses than the FDA-approved dose of 6 mg/kg.[667] Clinicians should be aware of the possibility of treatment-emergent resistance during daptomycin therapy, and patients should be carefully monitored for this possibility.[668] Of interest, a recent investigation reported improved clinical outcomes in patients with "high vancomycin MIC" MRSA bacteremia who received early substitution of daptomycin for vancomycin.[669] For MSSA IE in patients with anaphylactoid-type β-lactam allergy and suboptimal responses to vancomycin, the need for β-lactam desensitization should be considered.[670,671]

The use of clindamycin to treat staphylococcal IE is associated with an unacceptable relapse rate and is not recommended.[672] The optimal therapy for IE due to "tolerant" strains of *S. aureus* is controversial.[673,674] One retrospective study[675] suggested that patients with IE due to these tolerant strains had a more complicated course; however, combination

therapy did not seem to be beneficial. Tolerance does seem to influence the response to therapy in some experimental animal models of *S. aureus* IE,[676] and the use of combination regimens seems prudent when these strains are recovered from patients, but this is not universally accepted. Another controversial area is the adjunctive role of rifampin.[677] Because of the emergence of resistant strains, this drug is ineffective alone. Results of in vitro studies on rifampin combinations with either β-lactam agents or vancomycin frequently are contradictory, and clinical outcomes with experimental IE induced by *S. aureus* depend on which drug in the combination exerts the greatest bactericidal activity in vivo.[678] The question of the role of rifampin for SAB was definitively addressed in the ARREST trial, a randomized double-blind trial of 758 adults with SAB (including 33 with IE), who were randomized to receive either rifampin or placebo, in addition to standard therapy. Adjunctive rifampicin did not provide any clinical benefit but was associated with an increased frequency of adverse events.[679] Given the results of the ARREST trial, rifampin should not be recommended in patients with SAB or native valve IE.

Therapy for Highly Resistant Staphylococci

The therapy for IE due to *S. aureus* displaying borderline susceptibility to antistaphylococcal penicillins, first described by McDougal and Thornsberry in 1986,[680] is also a matter of debate. Experimental models of IE induced with these isolates suggest that nafcillin (or oxacillin) or ampicillin-sulbactam should be effective.[681,682] Several agents, including teicoplanin, fosfomycin, and fluoroquinolones, are active against MRSA in vitro and are as rapidly bactericidal as vancomycin in experimental animal models of IE due to MRSA,[683–685] although resistance to the quinolones has emerged during therapy, and frank failures have been recorded.[671,686,687] For patients with MRSA IE not responding to vancomycin, several choices are available, including the addition of rifampin or gentamicin (or both) or other regimens including daptomycin,[648] linezolid, quinupristin-dalfopristin, minocycline, trimethoprim-sulfamethoxazole,[688] fosfomycin plus imipenem,[689] or ceftaroline alone or in combination with trimethoprim-sulfamethoxazole.[690,691] Ceftaroline is active against MRSA with reduced susceptibility to vancomycin and daptomycin, including heteroresistant VISA (hVISA), VISA, vancomycin-resistant *S. aureus* (VRSA), and daptomycin-nonsusceptible *S. aureus* (DNSSA). *S. aureus* also displays a "seesaw" effect in which isolates display increased susceptibility to nafcillin as vancomycin susceptibility decreases—also seen with ceftaroline—which led to improved results with ceftaroline against hVISA and VISA strains in an in vivo model. Experience with these drugs in humans with left-sided or prosthetic *S. aureus* IE is limited.

Treatment of *S. aureus* Bacteremia Without Proven Infective Endocarditis

Bayer and associates[671,692] identified four parameters predictive of the presence of IE in 72 patients with SAB in a prospective study: (1) the absence of a primary site of infection, (2) community acquisition of infection, (3) metastatic sequelae, and (4) valvular vegetations detected with echocardiography. In another study of 724 prospectively identified patients with SAB, Fowler and associates observed key clinical identifiers of complicated bacteremia, including (1) community acquisition, (2) persistent fever at 72 hours, (3) skin examination suggesting the presence of acute systemic infection, and (4) positive blood culture 48 to 96 hours after the initial positive blood culture.[402] Although these clinical identifiers are useful methods to assist in the identification of clinically unsuspected IE, the role of echocardiography increasingly is being recognized.

Two decision analyses have shown the role of TEE in identifying the presence of IE and in helping to define the duration of antibiotic therapy. Heidenreich and colleagues[342] found that in patients whose prior probability of IE was between 4% and 60%, initial use of TEE provided the greatest quality-adjusted survival at a cost within the range for commonly accepted health interventions. Rosen and colleagues[343] found that the use of TEE to determine the duration of therapy (2 weeks vs. 4 weeks) in patients with intravascular catheter–associated SAB was cost-effective and provided better outcomes than empirical 2-week or 4-week treatment strategies. While definitive trials are awaited, short-term therapy should be used only if IE can be excluded reasonably by methods previously discussed. Although older studies suggested that occult IE in patients with nosocomial SAB was uncommon, the increasing importance of health care–associated *S. aureus* IE has now been definitively established.[10,14–16,693,694] In these patients, classic manifestations of IE are uncommon and TEE is useful in diagnosis of IE. IE due to glycopeptide-intermediate *S. aureus* has been reported,[695,696] and soft tissue infections due to vancomycin-resistant *S. aureus*[697,698] have been reported and are cause for concern.

Coagulase-Negative Staphylococci

S. epidermidis is the second most common etiologic agent in cases of prosthetic valve IE.[668] Most of these strains (87%) are methicillin resistant when isolated within 1 year after valve implantation. One study[699] suggested that the optimal antimicrobial regimen for these infections is vancomycin plus rifampin, usually with the addition of an aminoglycoside. The emergence of vancomycin resistance among coagulase-negative staphylococci[700] is cause for concern. These concepts are discussed further in Chapter 81.

Endocarditis Due to Enterobacteriaceae or *Pseudomonas* Species

Approximately 2% of contemporary IE cases are caused by aerobic gram-negative bacilli.[227] Health care contact and cardiac devices seem to have largely replaced injection drug use as the primary risk factors for acquisition of IE due to these bacteria. The prognosis is especially poor with left-sided cardiac involvement. Certain combinations of penicillins or cephalosporins and aminoglycosides have been shown to be synergistic against many of these strains and usually are recommended.[334] For IE caused by most strains of *E. coli* or *Proteus mirabilis,* a combination of a penicillin, either ampicillin (2 g IV every 4 hours) or penicillin (20–24 million units IV daily), and an aminoglycoside, usually gentamicin or a broad-spectrum cephalosporin, is suggested. Third-generation cephalosporins are extremely active against *E. coli* in vitro, and some (e.g., ceftriaxone) have proved effective in experimental animal models of *E. coli* IE,[701] even when long dosing intervals were used. This group of agents merits further evaluation in humans for IE due to susceptible gram-negative bacilli.

A combination of a third-generation cephalosporin and an aminoglycoside (either gentamicin or amikacin) is recommended for *Klebsiella* IE. IE due to carbapenem-resistant *Klebsiella* has been successfully treated with a combination of colistin and gentamicin in one case report. Certain β-lactam/β-lactamase inhibitor combinations (e.g., piperacillin-tazobactam,[702] but not ceftriaxone-sulbactam[703]) are active in vivo in experimental models of *Klebsiella* IE in animals induced by TEM-3–producing isolates and merit further evaluation in combination with an aminoglycoside in humans with this disease. The specific aminoglycoside used is a crucial variable and cannot be predicted totally from MIC data alone, because pharmacodynamic characteristics differ markedly in animal models of IE caused by gram-negative aerobic bacilli.[704,705] Endovascular *Salmonella* infections, including IE, also may respond to third-generation cephalosporins (see later discussion).[706] Left-sided IE due to *S. marcescens* is often refractory to medical therapy alone.[432]

P. aeruginosa is a rare but potentially devastating cause of IE in at-risk populations. Medical therapy may be successful in *P. aeruginosa* IE involving the right side of the heart in 50% to 75% of cases. If the disease is refractory to antibiotics, tricuspid valvulectomy or "vegetectomy"[707] without valve replacement is indicated.[708] Although valve replacement often is necessary for a cure of left-sided IE due to *P. aeruginosa,*[709] medical therapy alone occasionally is curative.[256,430] Studies in animals with experimental *Pseudomonas* IE[710] offer a partial explanation for these disparate results: the penetration into vegetations and the time during which antibiotic concentrations exceeded the MBC were significantly greater with tricuspid vegetations than with aortic vegetations for ceftazidime and tobramycin.

Problems have emerged with all potential regimens in animal models of this disease: (1) therapy with β-lactams (e.g., ceftazidime) has failed, owing to the selection of clones within valve vegetations that exhibit constitutive hyperproduction of type Id β-lactamase[711]; (2) isolates

showing aminoglycoside resistance due to permeability defects emerge during therapy[712]; (3) no postantibiotic effect of β-lactams against *P. aeruginosa* is evident in vivo,[713] necessitating frequent (or continuous) drug administration; and (4) the alginate exopolysaccharide inhibits clearance of mucoid strains from the vegetation via cellular or antimicrobial mechanisms. This inhibition can be reversed partially by the coadministration of alginase in animal models of *Pseudomonas* IE.[714]

Treatment failures in *Pseudomonas* IE in humans also have been related to the selection of isolates with an enhanced production of type Id β-lactamase.[715] Based on clinical experience,[413,433,434] however, the preferred regimen for IE due to *P. aeruginosa* is high-dose tobramycin (8 mg/kg/day IV or IM in divided doses every 8 hours), with maintenance of peak and trough concentrations of 15 to 20 μg/mL and 2 μg/mL or less, respectively, in combination with either an extended-spectrum penicillin (e.g., ticarcillin, piperacillin, azlocillin) or ceftazidime or cefepime in full doses. The toxicity associated with this regimen is low; combination treatment should be given for a minimum of 6 weeks. The use of quinolones (in combination with an aminoglycoside) seems promising for the treatment of *Pseudomonas* IE on the basis of favorable results in animal models[711] and in humans,[716] but the development of stepwise resistance during therapy may limit the efficacy of this class of drugs in the future. Based on limited experimental data,[717] ceftazidime-tobramycin is preferred over aztreonam-tobramycin for this disease. Approximately seven cases of *P. aeruginosa* IE have been treated successfully with imipenem plus an aminoglycoside,[718] but the potential for the development of resistance exists with any of these regimens.

Ceftriaxone or ampicillin-sulbactam should be considered treatments of choice for IE due to HACEK group bacteria.[334] Despite limited clinical data, ciprofloxacin is an accepted alternative therapy.[334] Duration of treatment for HACEK IE should be at least 4 weeks for native and prosthetic valve infections.[334] The previously mentioned recommendations offer only a rough guide for initial treatment. It is imperative that each isolate be subjected to quantitative sensitivity testing in vitro to ensure the optimal selection of antibiotics.

Endocarditis Due to Anaerobic Bacilli

Although IE caused by anaerobic bacilli is uncommon, the mortality rate is high. *B. fragilis* is isolated in many of these cases and is responsible for most fatalities. Most strains of anaerobic bacilli, with the exception of *B. fragilis,* are sensitive to penicillin in vitro, and use of this agent, at a dose of 20 to 24 million units IV daily, is the recommended therapy.[476,477] However, only about one-third of *B. fragilis* strains show an MIC for penicillin that is less than 0.25 μg/mL; and penicillin is only a bacteriostatic agent against these strains (the MBC is invariably >100 μg/mL), and relapse is common. Although clindamycin and chloramphenicol readily inhibit most strains of *B. fragilis,* they lack bactericidal activity and they are poor therapeutic choices, although several patients have been cured with high-dose penicillin, chloramphenicol (1 g IV every 6 hours), or clindamycin (600 mg IV every 6 hours). Owing to excellent bactericidal activity in vitro and the serum concentrations attained, metronidazole, ticarcillin plus clavulanic acid, piperacillin plus tazobactam, imipenem, and meropenem are reasonable choices for therapy for anaerobic IE.[478]

Pneumococcal, Gonococcal, and Meningococcal Endocarditis

IE caused by pneumococci, gonococci, or meningococci is now very rare. Pneumococcal IE must be considered in any patient with pneumococcal bacteremia, especially if meningitis is present. This form of IE is most common in alcoholics; the organism generally attacks the aortic valve and results in valvular insufficiency, often with perivalvular abscess formation or pericarditis. Type 12 pneumococci cause more than 20% of the cases of pneumococcal IE but are a rare (5% of cases) cause of pneumococcal pneumonia. Penicillin, 20 to 24 million units IV daily, cefazolin (2 g IV every 8 hours), or a third-generation cephalosporin for 4 weeks is recommended to treat IE due to highly penicillin-susceptible pneumococcus. Although the impact of penicillin resistance on the outcome of pneumococcal IE is unresolved, one series of 63 patients with pneumococcal IE found that left-sided heart failure, but not penicillin resistance, was associated independently with a higher risk for death.[719] IE without meningitis due to intermediate (MIC >0.1–1.0 μg/mL) or high-level (MIC ≥2.0 μg/mL) penicillin-resistant pneumococci can be treated with high-dose penicillin or a third-generation cephalosporin.[334] If IE and meningitis are simultaneously present, high doses of cefotaxime or ceftriaxone (2 g IV every 12 hours) should be used.[334] IE due to pneumococcal isolates resistant to cefotaxime (MIC ≥2.0 μg/mL) should be treated with vancomycin plus rifampin. Important to note, these recommendations are based on current levels of resistance and could be revised based on future MIC characteristics.[334]

The gonococci that cause systemic infection usually are highly susceptible to penicillin.[720] IE due to these organisms and the meningococci can be treated effectively with the same penicillin regimen recommended for pneumococcal IE. Although IE due to penicillin-resistant gonococci (on the basis of either β-lactamase production or chromosomally mediated mechanisms) has not been reported, ceftriaxone has been used successfully to treat gonococcal IE.[721]

Fungal Endocarditis

Since the 1990s, the incidence of fungal sepsis has undergone a striking increase. Fungal IE now occurs principally in the setting of health care contact (e.g., after surgery or prolonged parenteral therapy, in compromised hosts such as neonates), whereas the importance of injection drug use as a risk factor for this condition has apparently diminished in recent series.[501] Although the survival rate in patients treated before 1974 was less than 20%,[305] survival in the current era has increased to 40% to 70%, coincident with improved diagnostic techniques.[501,722] The preferred mode of therapy has not been determined. The use of antifungal agents alone has been almost universally unsuccessful in achieving a cure of this disease. The addition of surgical measures to antifungal therapy may result in an improvement in prognosis, but to date there is insufficient clinical experience. If fungal IE is diagnosed, a combined medical-surgical approach is usually recommended.[334,503,723]

Historically, the mainstay of antifungal drug therapy is amphotericin B. This agent is toxic and produces multiple side effects, including fever, chills, phlebitis, headache, anorexia, anemia, hypokalemia, renal tubular acidosis, nephrotoxicity, nausea, and vomiting. Drug toxicity is common and often necessitates alterations in the regimen. To reduce toxicity, lipid formulations of amphotericin B are preferred.[724] Dosages and the technique of administration are given in Chapter 40A.

After 1 to 2 weeks of amphotericin B therapy at full dosages, surgery probably should be performed. If isolated tricuspid IE is present, total tricuspid valvulectomy usually can be considered in individual cases. Rarely, removal of the vegetation alone is curative. Valve replacement is necessary for left-sided fungal IE. The duration of antifungal therapy after surgery is empirical, but 6 to 8 weeks usually is recommended.

It is possible that combination antifungal therapy may improve the poor survival rate associated with fungal IE. Some strains of *Candida* spp. and *Cryptococcus neoformans* are inhibited in vitro by concentrations of 5-fluorocytosine achieved with the oral administration of 25 mg/kg/dose 4 times daily. Synergism between 5-fluorocytosine and amphotericin B has been documented for these yeasts in vitro and in the treatment of cryptococcal meningitis. This combination was fungicidal and perhaps instrumental in the cure of one case of *Aspergillus* IE. In the rabbit model of IE due to *C. albicans,* however, the addition of 5-fluorocytosine did not improve the rate of eradication of fungal organisms from the vegetation over the rate observed with amphotericin B alone. Potentiation of amphotericin B activity by rifampin has been noted for virtually all strains of *Candida* spp. tested and for a few isolates of *H. capsulatum.* The therapeutic advantage of the addition of 5-fluorocytosine or rifampin to amphotericin for fungal IE requires further investigation, but initial results in animal models of disseminated candidiasis are not encouraging.[725] On the basis of animal model data,[726] high-dose itraconazole may be of value in the treatment of *Aspergillus* IE but valve replacement probably will remain imperative for a cure.[727] Amphotericin B is more effective than fluconazole for the prophylaxis and treatment of experimental *Candida* IE,[728] and it remains the agent of choice. However, the use of fluconazole apparently has led to long-term cures of *Candida* IE in a limited number of patients[503,729–731] when valve replacement was considered to be contraindicated. This agent should be tried after an initial course of amphotericin B in this

setting or used for long-term suppressive therapy. The roles of lipid and liposomal amphotericin complexes and voriconazole in the treatment of fungal IE are largely unknown. Recent data of echinocandins for the treatment of fungal IE are encouraging, however, and suggest that this class of antifungal agents may be reasonable in selected cases of fungal IE.[732]

Q Fever Endocarditis

More than 1300 well-documented cases of Q fever have been reported[733]; the mortality rate exceeds 65%.[480,481,483,484,485] Prolonged therapy (≥4 years) with doxycycline and either trimethoprim-sulfamethoxazole or rifampin was considered to be the regimen of choice in the past (see Chapter 188).[480,734–736] A fluoroquinolone may be a useful addition to doxycycline.[480,736] The acidic conditions of the phagolysosome, where the organism resides, may inhibit antibiotic activity.[736] Cure of the IE after treatment with a combination of doxycycline and hydroxychloroquine to alkalinize the phagolysosome for 1 year was reported in 20 patients.[480] Valve replacement often is required, and long-term prognosis is guarded. Careful follow-up to detect recrudescence of infection is essential. A combination regimen of doxycycline plus hydroxychloroquine for at least 18 months allowed shortening the duration of therapy and resulted in reduction in the number of relapses.[733] The Raoult group recently published their results with more than 100 patients with Q fever endocarditis followed over 26 years (median follow-up, 100 months). Based on their results, we believe that doxycycline plus hydroxychloroquine are the drugs of choice and that 18 months of therapy is adequate for treatment of native valve Q fever endocarditis versus 24 months for prosthetic valve endocarditis unless certain serologic markers persist (requiring sequential monitoring of these parameters).

Infective Endocarditis Due to Chlamydiae

Based on limited experience, a combination of valve replacement and prolonged (>3 months) tetracycline therapy seems justified in IE due to chlamydiae. Rifampin has cured at least one case of chlamydial IE after therapy with tetracyclines failed, but exposure to this agent rapidly induces the emergence of drug resistance in *C. trachomatis* in tissue culture.[737] The role of combination regimens (e.g., rifampin plus erythromycin or tetracycline) merits further study.

Culture-Negative Endocarditis

The therapy for culture-negative IE in nonaddicts is controversial, but the regimen usually used "covers" enterococci, the HACEK group, and *Abiotrophia* and *Granulicatella* spp. For native valve culture-negative IE, this regimen consists of a combination of ampicillin-sulbactam, 12 g/24 h in four equally divided doses, plus gentamicin 3 mg/kg/24 h in three equally divided doses plus ciprofloxacin for 4 to 6 weeks.[334] Early (≤1 year) culture-negative prosthetic IE should be treated with appropriately dosed vancomycin (for 6 weeks) plus gentamicin (at doses indicated previously for 2 weeks) plus cefepime (6 g IV daily in three equally divided doses for 6 weeks) plus rifampin (900 mg PO or IV daily in three equally divided doses for 6 weeks).[334] Culture-negative IE involving prosthetic valves that have been in place for longer than 1 year should be treated as listed previously for native valve endocarditis.[334] If staphylococcal IE is likely, a penicillinase-resistant penicillin or a cephalosporin in full dosage should be substituted in the aforementioned regimen. If clinical improvement occurs, some authorities recommend discontinuation of treatment with the aminoglycoside after 2 weeks. The other agents should be continued for a full 6 weeks of treatment. Continued surveillance for the causative agent and careful follow-up are mandatory. An analysis of any correlation between the response to empirical antimicrobial therapy and survival was performed in 52 patients with culture-negative IE[518]: 92% of the patients who became afebrile within the first week of therapy survived, compared with only 50% of those whose fever persisted longer than 7 days. Most deaths were caused by major systemic emboli or uncontrollable CHF due to valvular insufficiency.

Surgical Therapy

Valve replacement has become an important adjunct to medical therapy in the management of IE and now is used in almost half of the cases.[7] Decisions regarding surgical intervention in patients with IE should be individualized and should be made with input from the infectious diseases physician, the cardiologist, and the cardiovascular surgeon.[334] Patients with IE and CHF should be immediately evaluated for potential surgical therapy.[334,738] Other generally accepted indications for potential surgical intervention during active IE are as follows: (1) more than one serious systemic embolic episode; (2) uncontrolled infection; (3) ineffective antimicrobial therapy (e.g., in fungal IE); (4) resection of mycotic aneurysms; (5) most cases of prosthetic valve IE caused by more antibiotic-resistant pathogens (e.g., staphylococci, enteric gram-negative bacilli); and (6) local suppurative complications, including perivalvular or myocardial abscesses. The major indications in the past have been persistent infection and CHF[739–741] in adults and children.[742] CHF during active IE was the indication for surgery in 86% of 108 patients undergoing valve replacement at Stanford University Medical Center from 1963 to 1984.[741] The importance of individualizing decisions for valve replacement surgery among patients with *S. aureus* prosthetic valve IE, basing these decisions on the presence of traditional indications (e.g., heart failure), has been recently emphasized.[743]

Despite the widespread use of TTE and TEE in patients with IE, the indications for surgical intervention based on echocardiographic features remain controversial. The American Heart Association Committee on IE, working from data reported in the recent literature, identified the following echocardiographic features in IE as being associated with a potential increased need for surgical intervention: (1) persistent vegetations after a major systemic embolic episode; (2) increase in vegetation size after appropriate antibiotic therapy; (3) acute mitral insufficiency; (4) valve dehiscence, perforation, or rupture; (5) periannular extension of infection (e.g., paravalvular abscess or fistula); and (6) large (>1 cm in diameter) anterior mitral valve vegetations.[334] However, findings of a recent study suggest that early surgery based solely on large vegetation size in patients with left-sided native valve IE was associated with worse clinical outcome.[744] The most common causes of death in IE, in approximate order, are CHF,[738] neurologic events,[227] septic complications,[745] embolic phenomena, rupture of a mycotic aneurysm, complications of cardiac surgery, lack of response to antimicrobial therapy, and prosthetic valve IE.[334]

Owing to the inherent differences between medically managed patients with IE and patients undergoing surgical intervention for IE, the optimal role and timing of valve surgery in IE are controversial. However, a growing body of evidence supports the importance of early surgical intervention. A large, well-designed propensity analysis[540] found that early valve surgery is associated with increased survival in patients with IE. These findings were confirmed in a pivotal clinical trial[746] randomizing 76 patients with left-sided endocarditis, severe valve disease, and large vegetations to early surgery (within 48 hours of randomization) or conventional treatment. The primary end point—a composite of in-hospital death, embolic events within 6 weeks of randomization, or recurrence of IE at 6 months—was significantly less frequent in the early-surgery group than in the conventional-treatment group (3% vs. 28%; $P = .02$). Limitations of this trial, such as the infrequency of cases caused by *S. aureus* and the relatively young and otherwise healthy study subjects, reduced the generalizability of the findings. A retrospective cohort study stratified adults with complicated left-sided native valve IE, using baseline features, into four groups of prognostic severity.[747] When acute aortic regurgitation complicated by CHF supervenes in IE, the mortality rate still exceeds 50%. The classic physical findings associated with chronic aortic regurgitation often are absent in these patients.[345] The current trend is to perform early surgery in this group of patients, because nothing is gained by delay. The merits of early valve replacement surgery were confirmed in 1972.[748] In a series of 28 patients from Birmingham, Alabama, with acute aortic regurgitation, 4 had no CHF and were managed medically, and all survived. In contrast, 7 of 11 patients with mild CHF and 7 of 8 patients with moderate-to-severe CHF died during medical therapy, often suddenly and with pathologic evidence of coronary emboli and myocardial infarction. Four of five patients with moderately severe CHF who underwent surgery survived. These data suggest that early surgical intervention may improve survival in this setting. Valvular regurgitation on Doppler echocardiography is not predictive of death in the absence of CHF.[749]

The hemodynamic status of the patient, not the activity of the infection, is the crucial determining factor in the timing of cardiac valve replacement; that is, development of CHF in the setting of IE generally dictates valvular surgery regardless of the acuteness of the infection or the amount of antibiotics already received by the patient.[750] The hemodynamic severity of the acute aortic regurgitation may be assessed by determining the degree of mitral valve preclosure by echocardiography. If premature closure of the mitral valve occurs before the Q wave of the electrocardiogram, the left ventricular end-diastolic pressure is very high, and surgical intervention is required urgently. Nothing is gained by temporizing, even if only a few hours of antibiotics can be administered. If CHF persists despite digoxin, diuretics, and other therapeutic modalities (e.g., left ventricular afterload reduction), surgery also is indicated. In 80 patients subjected to aortic valve replacement for IE, the surgical cure rate was 72%. There were no instances of subsequent infection of the prosthesis, but 16% of the patients developed paravalvular regurgitation. This latter complication usually was controlled easily with medical therapy. Organisms visible on Gram stain, positive cultures, or annular abscesses at the time of surgery are associated with late complications.[745] Although the topic has not been systematically studied, most authorities suggest that if there is evidence of active IE at the time of valve replacement surgery, antibiotic therapy should be continued postoperatively for at least several weeks. Such evidence might include vegetations that remain culture positive and vegetations with significant polymorphonuclear inflammation. An investigation by Morris and colleagues[217] found that dead bacteria may persist for months in sterile vegetations and concluded that only valve cultures should be considered when determining the duration of antibiotic therapy after valve replacement for IE. By contrast, routine valve cultures in patients undergoing native valve replacement for indications other than clinically suspected IE do not seem warranted.[751]

The optimal timing of valve replacement surgery for left-sided IE is another unresolved clinical question. Because of the observed decrease in embolic risk during the initial 1 to 2 weeks of medical therapy, early surgical intervention could potentially avoid a devastating embolic event while subjecting the patient to the risks of valve replacement surgery. Therefore the strategy for surgical intervention to avoid major embolization remains individualized.[334] The importance of timing of surgery following cerebral emboli in IE patients was evaluated in 198 patients. Fifty-eight patients who underwent surgery within 1 week of stroke were compared with 140 patients who underwent surgery 8 or more days after stroke. After adjustment for other confounding factors, no statistically significant difference in 1-year mortality was present among the early-surgery recipients (95% CI, 0.802–1.650).[752] The high risk of exacerbation is prohibitively high in the first month after hemorrhagic stroke. In a multicenter study of IE with hemorrhagic stroke, mortality was higher when valve surgery was performed within 4 weeks of the hemorrhagic event, compared with later surgery (75% vs. 40%, respectively).[753] Based on these data, the American Heart Association Committee on Endocarditis has made the following recommendations: Valve surgery may be performed in IE with stroke or subclinical cerebral emboli without delay if intracranial hemorrhage has been excluded with imaging studies and neurologic damage is not severe (i.e., coma). In patients with major ischemic stroke or intracranial hemorrhage, it is reasonable to delay valve surgery for at least 4 weeks.[1] It is important to emphasize that these recommendations are intended to serve as general guidelines in the absence of definitive evidence.

In contrast to left-sided IE, in which CHF is the usual indication for surgical intervention, persistent infection is the indication for surgery in more than 70% of patients with right-sided IE. Most patients are injection drug users, with IE caused by organisms that are difficult to eradicate with antimicrobial therapy alone (e.g., fungi, gram-negative aerobic bacilli). Tricuspid valvulectomy or vegetectomy with valvuloplasty is the procedure of choice for refractory right-sided IE.[754,755] Valve replacement at a second operation is advised only if medical management fails to control the hemodynamic manifestations and the patient has ceased using illicit drugs. Combination antimicrobial therapy should be continued for 4 to 6 weeks postoperatively. These patients may develop mild-to-moderate right-sided heart failure, but this is tolerated easily, and the success rate with this approach is greater than 70%. Eventual tricuspid valve replacement usually is required, however, owing to progressive right-sided heart failure. Persistent fever, recurrent pulmonary emboli, or vegetations demonstrable with echocardiography usually do not necessitate tricuspid valvulectomy in this setting.[756] In addition, many surgeons contend that a return to the use of illicit drugs and reinfection of the valve after initial cure are contraindications to reoperation.[757]

Outstanding reviews on the indications for surgery during therapy for IE are available.[740,758–761] The rationale for surgical intervention, including major and minor criteria for valve replacement, is discussed in detail. A point system weighting multiple factors was devised by Alsip and colleagues[758] to assist in decision making concerning surgery in patients with active IE. The value of this system remains to be defined. It has become apparent that most patients with prosthetic valve IE (except patients with late disease caused by penicillin-sensitive viridans streptococci) require valve replacement for consistent cures. Valve replacement also is necessary in a significant proportion of patients with IE on native valves after a medical cure; aortic involvement is a predictor of the need for surgery.[762]

Suppurative Thrombophlebitis

Suppurative thrombophlebitis is an inflammation of the vein wall that is caused by the presence of microorganisms and frequently associated with thrombosis and bacteremia. In the following discussion, suppurative thrombophlebitis is differentiated from catheter-related sepsis. Suppuration of the vein wall usually is absent in intravenous catheter–related sepsis and in bacteremia secondary to contaminated intravenous fluid, although it does occur. Suppurative thrombophlebitis may be classified into four forms: superficial, central (including pelvic), cavernous sinus, and infection of the portal vein (pyelophlebitis). The last two conditions have become rare since the introduction of antibiotics. In contrast, superficial suppurative thrombophlebitis has been increasing steadily in incidence since the introduction of the plastic intravenous cannula. Superficial suppurative thrombophlebitis secondary to intravenous fluid therapy first was described in 1947,[763] when 93 cases were reported, 43 of which were amenable to surgical therapy.

Epidemiology

In 1973, approximately 1 of every 4 hospitalized patients received intravenous therapy, for a total of more than 10 million patients annually in the United States.[764] It is estimated that more than half of the 40 million patients admitted to US hospitals each year undergo intravascular catheterization.[765] Suppurative thrombophlebitis is a particular problem in burned patients, for whom it represents a common cause of death due to infection. In several large series of burned patients,[766–769] suppurative thrombophlebitis developed in 4% to 8% and increased in frequency if cutdowns were performed. Suppurative thrombophlebitis also is found in other hospitalized patients (especially patients with cancer and those receiving corticosteroid therapy).[770,771] Seven cases were recognized during an 18-month period in Charleston, South Carolina, and 35 cases were identified over 7 years in Louisville, Kentucky.[772] Eight cases were encountered during an 8-month period in Johannesburg, and suppurative thrombophlebitis was estimated to represent a minimum incidence of 0.12% of all admissions.[773] In a study using strict diagnostic criteria, 29 episodes of suppurative thrombophlebitis in 27 patients were identified in a large Air Force hospital over a period of 4 years.[774] Using data from the National Nosocomial Infection Study, Rhame and associates[775] estimated the overall incidence of suppurative thrombophlebitis as 88 cases per 100,000 discharges, but this disease is underreported. Suppurative thrombophlebitis also is common among drug addicts, particularly when injections are made in large, central veins (e.g., jugular or subclavian veins).[776] This condition is unusual during childhood[777] but may occur as a complication related to intravenous therapy.

Catheter-related sepsis without suppurative thrombophlebitis is much more common and affects at least 50,000 to 100,000 patients per year in the United States.[778,779] The risk for this complication is approximately 40 times higher with plastic cannulas (8%) than with steel or "scalp vein" cannulas (0.2%). Irritation to the vein wall, with the subsequent development of suppurative thrombophlebitis, is more common with

polyethylene catheters than with catheters constructed of Teflon or Silastic material. Central venous catheterization has been employed for more than 35 years for hemodynamic monitoring, total parenteral nutrition, and infusion of drugs. The exact incidence of suppurative thrombophlebitis of the central veins commonly cannulated (i.e., jugular, subclavian, venae cavae) is unknown. However, recent evidence clearly shows that catheter-associated thrombosis is common in specific populations. For example, in a recent prospective cohort study, venous ultrasonography and targeted physical examination were blindly performed in 48 patients with upper torso central venous catheter–associated SAB to identify the prevalence of venous thrombosis. With ultrasonography, definite or possible thrombosis was present in 34 (71%) of 48 patients. The sensitivity of all physical examination findings, either alone or in combination, was low (≤24%).[780] Autopsy series have revealed central venous thrombosis in 37% of catheterized subjects, but this diagnosis rarely is recognized, because most patients are asymptomatic. When examined with phlebography at the time of catheter withdrawal, 42% of catheters were found to have sleeve thrombi and another 8% revealed veno-occlusive thrombi.[781] In addition, sepsis has been reported in approximately 7% of patients receiving total parenteral nutrition and other medications by the central route.

When thrombosis and bacterial or fungal contamination or sepsis coexist, suppurative thrombophlebitis may intervene.[782] The role of hypercoagulability due to gene polymorphisms in catheter-associated infection and thrombosis has been evaluated[783] but is unresolved. At least 50 cases of suppurative thrombophlebitis of the great thoracic veins have been reported in the literature,[784–786] but this is almost certainly a gross underestimate of the problem. Eight cases in 8 years due to *Candida* spp. alone were observed at the University of Wisconsin.[787] As another example, 53 cancer patients with catheter-related SAB were identified from 1986 to 1989 at the MD Anderson Cancer Center; septic thrombosis was diagnosed in 12 (23%) and suspected in another 3 (6%) of these patients. Five of the 12 patients developed deep-seated complications, including septic pulmonary emboli and endocarditis, compared with 2 of the 38 patients without septic thrombosis ($P < .01$). Persistent fever despite appropriate antistaphylococcal agents was an early clue to the diagnosis.[788] Septic atrial thrombosis, occasionally with a coexistent Budd-Chiari syndrome, has complicated Broviac catheter insertion in infants.[789]

Superficial suppurative thrombophlebitis is a complication of either dermal infection or use of an indwelling intravenous catheter. Pelvic suppurative thrombophlebitis is associated with parturition, abortion, gynecologic surgery, or a pelvic abscess. This is a disease of women of childbearing age, with most cases occurring between the ages of 15 and 40 years (mean, 20 years). In 123 cases in two reports,[790,791] the predisposing conditions were as follows: vaginal delivery, 39 cases; cesarean section, 19 cases; abortion, 33 cases; and major gynecologic surgery, 32 cases. During a 9-year period in Atlanta, 27 cases of postpartum septic pelvic thrombophlebitis were identified in more than 54,000 deliveries.[791] The relative risks for this condition were as follows: parturition, 1 in 2000 (highest in the inner-city population); septic abortion, 1 in 200; and major gynecologic surgery, 1 in 800. The incidence of suppurative thrombophlebitis increases proportionally with the degree of trauma to the pelvic tissues.

Pathogenesis

The pathogenesis of suppurative thrombophlebitis (discussed in detail by Tornos and colleagues[762]) is poorly understood. A thrombus may act as a nidus for local entrapment and colonization of bacteria that gain access to the site from another focus. This is analogous to the proposed role of NBTE in the pathogenesis of IE. When superficial suppurative thrombophlebitis is associated with intravascular cannulas, the route of infection may involve (1) migration from the skin between the catheter wall and perivascular tissue, (2) contamination of intravenous fluid, (3) contamination of the hub, or (4) hematogenous dissemination from an infected focus elsewhere. The relative contributions of these four routes are unknown, although most investigators believe that migration of organisms down the external surface of the catheter is the most important route of invasion.[765] The predominant organism in burn wounds, *P. aeruginosa,* is a rare cause of suppurative thrombophlebitis, and suppurative thrombophlebitis usually develops days to weeks after the cutdown incision is healed,[767,768] arguing against a local cutaneous source in burn patients.

The venous system draining the pelvis includes the intervertebral venous plexus, the lumbar venous plexus, the superficial and deep veins of the abdominal wall, and the hemorrhoidal plexus. Any component of this system may be affected in pelvic suppurative thrombophlebitis, but the veins draining the uterus, including the ovarian veins and the inferior vena cava, are involved most often.[792] Thrombus formation may result from stasis of blood flow due to the gravid uterus or from the hypercoagulable state of parturition. Normal residents of the vaginal or perineal bacterial flora gain access to the thrombus via the bloodstream or regional lymphatics. There often is an associated endometritis or parametritis. Septic pulmonary emboli and metastatic abscess formation are common. Septic thrombosis of the portal vein often is associated with hepatic abscess (occurring in five of seven patients in one series[793]); an obvious extrahepatic source of intraabdominal infection is usually absent.

Pathologic Changes

Regardless of the vein involved, the pathologic changes are similar. The vein is enlarged, tortuous, and thickened. There may be associated perivascular suppuration or hemorrhage or both, and the vein lumen usually contains pus and thrombus. Microscopically, endothelial damage, fibrinoid necrosis, and thickening of the vein wall are evident. Microabscesses may be present in the vein wall or in the surrounding tissue.[772,794] Gross periphlebitic abscesses are not unusual and may be evident on physical examination. Thrombi frequently extend beyond the area of suppuration. In an autopsy series of peripheral suppurative thrombophlebitis in burned patients, extension of the clot into the great central veins was found in 18% of the cases.[767,769] Metastatic abscess formation and septic pulmonary emboli with infarction are found in more than 50% of the fatal cases. These conditions may result from bacterial liquefaction and fragmentation of affected thrombi within the vein, because clot liquefaction is noted commonly in autopsy series.

Clinical Manifestations

Superficial suppurative thrombophlebitis often is difficult to identify, because local findings of inflammation may be absent. The disease occurs more frequently when plastic catheters are inserted in the lower extremities, a common practice in burned patients. In 132 cases of superficial suppurative thrombophlebitis reported from the burn center at Fort Sam Houston, Texas, the distribution of affected vessels was as follows: lower extremity (predominantly saphenous system), 100; upper extremity (predominantly antecubital fossa), 32; jugular vein, 7; and iliac vein, 4. The mean duration of preceding venous cannulation was 4.81 days, and the latent interval from removal of the catheter to the development of symptoms ranged from 2 to 10 days.[766,768] Fever was present in more than 70% of the cases, but rigors were rare. Local findings, such as warmth, erythema, tenderness, swelling, or lymphangitis, were present in only 32% of the patients; however, bacteremia with signs of systemic sepsis was found in 84%. Septic pulmonary emboli with secondary pneumonia—often the first diagnostic clue—occurred in 44%. Pneumonia, sepsis, or metastatic abscess formation was the only manifestation of this disease in two-thirds of the cases. The late onset of pneumonia or sepsis in a burned patient demands the careful inspection of all previously cannulated veins, because untreated suppurative thrombophlebitis is associated with a high mortality rate. In another report, a dramatic increase in the overall insulin daily requirement heralded the onset of suppurative thrombophlebitis.[795] In these series, less than 50% of the cases were diagnosed antemortem.[767]

In contrast to the experience with suppurative thrombophlebitis in burned patients, most medical and postoperative patients develop the disorder in the upper extremities and signs of local inflammation are present more commonly (94% in one series).[776] In a retrospective series of 21 children with superficial suppurative thrombophlebitis, 48% involved an upper extremity.[796] Many of the affected patients are elderly with debilitating diseases, and often they are receiving antibiotics when superficial suppurative thrombophlebitis supervenes. As noted, the duration of intravenous catheterization is an important risk factor; 68%

of implicated cannulas had been left in place for at least 5 days.[775,776] The frequency of catheter manipulations also has been linked to catheter infections.[797]

Subperiosteal abscesses of adjacent long bones may complicate superficial suppurative thrombophlebitis in children.[798] The local findings in this condition, including bone tenderness, erythema, warmth, and limitation of motion with occasional extension into the joint space, may overshadow the suppurative thrombophlebitis itself. Septic deep vein thrombosis of the femoral vessels with swollen, tender, and inflamed inguinal areas has been described in injection drug users of heroin and cocaine. Contiguous pelvic bone osteomyelitis is unusual.

Suppurative thrombophlebitis of the thoracic central veins occurs in critically ill patients with central catheters in place, in patients receiving total parenteral nutrition, and in patients after long-term cannulation with Broviac, Hickman, and other devices. The systemic findings associated with sepsis overshadow any local findings in venous occlusion (e.g., superior vena cava syndrome), which are rare in this setting. This syndrome should be suspected in any septic patient if bacteremia or fungemia fails to resolve on removal of the central catheter and institution of appropriate antimicrobial therapy.

Pelvic suppurative thrombophlebitis usually develops 1 to 2 weeks after delivery or postoperatively and is associated with high fever, chills, anorexia, nausea, vomiting, abdominal pain, and a protracted course.[791] Flank pain may result from ureteral obstruction by enlarged veins. Abdominal tenderness, usually in the right lower quadrant, may be mild to severe. Approximately 80% of cases are unilateral on the right side, 14% are bilateral, and only 6% are unilateral and left sided. This distribution is believed to result from compression of the right ovarian vein at the pelvic brim by the enlarged uterus with retrograde flow on the left and protection from ascending infection. The physical examination findings may be normal, however. A tender vein can be palpated in 30% of the cases at pelvic or abdominal examination.[790,792] The uterus usually is freely movable. Spread of the process to the femoral vein with edema and tenderness of the lower extremity is unusual. Many of these patients are extremely ill, with an acute or chronic course characterized by little or no response to antibiotics and the development of multiple small septic pulmonary emboli. Because many of the manifestations are nonspecific, the differential diagnostic listing is broad and includes acute appendicitis, ureteral obstruction, torsion of an ovarian cyst, pyelonephritis, broad ligament hematoma, parametritis, endometritis, perinephric abscess, pelvic abscess, small bowel volvulus, pelvic inflammatory disease, sickle cell crisis, and ectopic pregnancy.

Laboratory Findings

Bacteremia is a hallmark of superficial suppurative thrombophlebitis, occurring in 80% to 90% of patients with the diagnosis. Gross pus within the vein lumen is found in about half of the cases, and this finding establishes a diagnosis of suppurative phlebitis. If infection of a venous catheter is suspected, the catheter should be removed and cultured. The results may be misleading, however, because even though bacteria are isolated in 60% of the cases, a positive culture does not correlate with inflammation.[799] The following semiquantitative culture technique was developed in an attempt to differentiate catheter-related sepsis from suppurative thrombophlebitis. After preparing the skin with alcohol, the catheter is removed with sterile forceps (avoiding skin contact) and is placed in a sterile tube for transport. The catheter then is cut aseptically into 5.7-cm pieces, and each section is rolled across the surface of a 5% sheep blood agar plate. The growth of more than 15 colonies on the plate correlates well[799] with the presence of venous infection. In the few cases of suppurative thrombophlebitis studied by means of this technique, all catheters yielded confluent growth. Because the standard 5.7-cm catheter retains 0.7 to 1.5 mg of moisture on its surface and the plate growth has exceeded 1000 colonies in every case of suppurative thrombophlebitis, bacterial counts must exceed 10^6 organisms per gram in the catheter wound. These titers are similar to those found with other types of infected wounds. This technique is simple, rapid, and inexpensive and may prove useful in establishing the need for exploratory venotomy. Simple needle aspiration of the suspected vein also may be diagnostic.[126] ^{111}In-labeled leukocyte imaging studies have been used to detect superficial suppurative thrombophlebitis, but experience is limited.

Other laboratory findings in patients with superficial suppurative thrombophlebitis (e.g., leukocytosis) are nonspecific. The chest radiograph may reveal multiple peripheral densities or a pleural effusion consistent with pulmonary emboli, infarction, abscess, or empyema. The diagnosis of an associated subperiosteal abscess is difficult; bone and gallium scans usually reveal hyperperfusion without definite osteomyelitis, routine radiographs almost always show no abnormalities, and CT scans often show only soft tissue swelling with obliteration of tissue planes. The use of high-resolution CT scans may improve these results.[798] The diagnosis of deep central vein suppurative thrombophlebitis in the thorax is established with venography, with the demonstration of thrombi in a patient with positive blood cultures, but CT with contrast enhancement is probably just as sensitive and is noninvasive. CT scans are useful in the diagnosis of suppurative phlebitis of the great central veins[800,801] and the portal vein[778]; gas may be detected in the venular lumen, which is diagnostic of this condition. Experience with MRI[802] and ^{111}In-labeled leukocytes is meager.

In most cases of pelvic suppurative thrombophlebitis, there is a peripheral blood leukocytosis, and the urinalysis is usually normal. The chest radiograph may reveal multiple septic pulmonary emboli. Intravenous pyelography can be useful in disclosing ureteral obstruction. Real-time ultrasonography is helpful in delineating the location and extent of the thrombus, but the ileus that often is associated with this infection may render interpretation difficult. Ultrasonography also may show the presence of a periuterine, adnexal, or tubo-ovarian mass. CT reveals low attenuation with contrast enhancement in suppurative venous thrombosis and is sensitive in the diagnosis of pelvic suppurative thrombophlebitis.[803,804] MRI may be even more sensitive and can differentiate fresh thrombus (≤1 week old) from organizing or subacute thrombus.[805] These sensitive and noninvasive techniques may lead to an increased recognition of pelvic suppurative thrombophlebitis, earlier diagnosis, and improved outcome. The roles of newer diagnostic techniques, such as pelvic venography, transuterine phlebography, ^{111}In-labeled leukocyte scanning, and laparoscopy, still are undefined. Because bacteremia is shown in only 20% to 30%[754,755,770] of cases of pelvic suppurative thrombophlebitis, negative blood cultures do not exclude the diagnosis.

Etiologic Agents

S. aureus was the causative agent in 65% to 78% of the cases of superficial suppurative thrombophlebitis reported before 1968. Many cases now are also caused by a member of Enterobacteriaceae, especially *Klebsiella-Enterobacter* spp.[770,776] These agents are acquired nosocomially and often are resistant to multiple antibiotics. Almost all patients with superficial suppurative thrombophlebitis due to gram-negative aerobic bacilli or fungi are receiving broad-spectrum antibiotics at the time the disease manifests. In a review of 86 cases compiled from the literature reported in the 1970s, the organisms isolated were as follows: *Klebsiella-Enterobacter* spp., 34 cases; *Providencia* spp., 5 cases; *Proteus* spp., 5 cases; *Serratia* spp., 3 cases; *E. coli,* 6 cases; *P. aeruginosa,* 3 cases; *S. aureus,* 15 cases; *C. albicans,* 9 cases; *S. epidermidis,* 4 cases; and enterococci, 2 cases.[775,776]

Suppurative thrombophlebitis due to gram-negative pathogens and *E. faecalis* is more common (than *S. aureus*) in patients with significant intraabdominal pathology.[774] *S. aureus,* other gram-positive cocci, and *Candida* spp. were more common when this risk factor was absent. Multiple organisms are isolated in 14% of cases. Anaerobic isolates are extremely rare but have been described in pediatric patients.[806] A distinct clinical syndrome of suppurative thrombophlebitis due to *C. fetus,* typically in immunocompromised patients, has been described. An increase in the incidence of superficial suppurative thrombophlebitis due to *Candida* spp. has been reported[807,808]; all patients were receiving antibiotics without hyperalimentation. None was neutropenic, and none was receiving corticosteroids. In one series of seven patients observed in a 15-month interval,[808] all had concomitant or preceding bacterial infections and had received multiple antibiotics (mean, five antibiotics) for at least 2 weeks. Preceding candidal colonization at other sites (e.g., sputum, urine) often was present.[808]

Malassezia furfur also is seen as an opportunistic pathogen of deep vein catheters, especially in premature infants[809] and other pediatric patients receiving lipid emulsions, but this risk factor is not present in all patients.

The responsible agents in pelvic suppurative thrombophlebitis are poorly defined because blood cultures often are negative, and most investigators did not use adequate anaerobic techniques. The organisms that have been isolated, in approximate order of frequency, are *Bacteroides* spp., microaerophilic or anaerobic streptococci, *E. coli* and other coliforms, and β-hemolytic streptococci. The predominance of *Bacteroides* may be related to the heparinase produced by this organism. A prolonged latent period (3 weeks) may occur before blood cultures become positive. The more extensive use of anaerobic isolation techniques and routine culturing of surgical specimens may serve to clarify the role of anaerobic bacteria in this entity.

Presumptive Therapy

Superficial suppurative thrombophlebitis is a lethal iatrogenic disease, and surgery often is necessary for cure. The first reported successful cure of suppurative thrombophlebitis followed surgical ligation of the vein by Hunter in 1784.[810] All authorities strongly endorse surgical excision as an integral part of treatment. In a review of 24 patients,[767] 14 were managed medically alone, and all died, either directly from suppurative thrombophlebitis with persistent bacteremia or secondary to metastatic complications. Of 10 patients who underwent surgical exploration, 7 survived, and only one of the three deaths was attributable to suppurative thrombophlebitis. Antibiotics also should be used in the treatment of this disease; initial empirical treatment with a semisynthetic penicillin (e.g., nafcillin, 2 g IV every 4–6 hours) plus either an aminoglycoside (e.g., gentamicin, 1.0–1.7 mg/kg IV or IM every 8 hours) or a third-generation cephalosporin (e.g., cefotaxime) or a quinolone (e.g., ciprofloxacin) is recommended, because members of Enterobacteriaceae or staphylococci are the usual etiologic agents. The optimal duration of therapy is unknown and largely empirical. The role of antifungal therapy for superficial suppurative thrombophlebitis due to *C. albicans* is controversial.[807,808] Most of these infections can be cured by vein excision. However, because of the propensity of this pathogen to disseminate hematogenously to organs (e.g., retina, kidneys), a 10- to 14-day course of amphotericin B, echinocandin, or fluconazole is advised postoperatively, pending further data. Antifungal therapy is mandatory in immunosuppressed patients or if signs of metastatic complications (e.g., endophthalmitis) develop.

If superficial suppurative thrombophlebitis is a likely diagnosis, an exploratory venotomy may be necessary. This procedure should be performed proximal to the suspected site; the vein should be ligated and then "milked" in an attempt to express purulent material for inspection by Gram stain and culture. If no pus is apparent, further surgical exploration is necessary to establish the diagnosis. In older literature, simple ligation was thought to be sufficient, but the rate of relapse with ongoing sepsis was high. The segment of vein and all its involved tributaries should be totally excised. Radical surgery from the ankle to the groin may be required in some burn patients. Nevertheless, local or regional anesthesia alone often is sufficient (approximately 90% of cases) for vein excision. Backbleeding, indicative of a patent lumen, should be evident at the point of vein transection. Vein excision usually is followed by prompt (≤24 hours) defervescence. If systemic symptoms, bacteremia, or marked local manifestations persist after vein excision, reexploration is necessary, with careful attention to total removal of all involved veins and drainage of contiguous (e.g., periphlebitic, subperiosteal) abscesses.

The role of less radical surgery in therapy for superficial suppurative thrombophlebitis has not been addressed adequately. Although the literature supports vein excision, this experience stems largely from burn centers. Despite infection with gram-negative bacilli or *Candida* spp., six of eight children with superficial suppurative thrombophlebitis were cured by means of local incision and drainage of the involved site plus parenteral antimicrobial therapy.[774] Radical surgery with extensive excision perhaps can be reserved for patients in whom these measures fail. Delayed closure is preferred over primary wound closure. If osteomyelitis is documented in the adjacent long bones, antimicrobial therapy should be continued for at least 6 weeks. Resection of the involved vasculature in most patients with suppurative thrombophlebitis of the great central veins is technically impossible. Medical therapy is usually sufficient.[784,787,810] The recommended approach is catheter removal, full-dose anticoagulation with heparin,[810,811] and parenteral antibiotic therapy. Although tissue plasminogen activator therapy has been used successfully in this setting,[812] experience is limited, and its use must be considered experimental. Septic thrombosis of the portal vein usually responds to systemic antimicrobial therapy directed at bowel flora with or without percutaneous drainage of any associated hepatic abscesses.[793]

The duration of therapy for septic phlebitis of deeper veins is unsettled: 2 to 3 weeks after catheter removal is suggested, with at least 4 weeks for *S. aureus* disease.[788] Experience with more potent agents (e.g., third-generation cephalosporins) for suppurative thrombophlebitis due to gram-negative bacilli is scant, but trials are indicated. Because heparin may precipitate vancomycin with a partial loss of antibacterial activity at concentrations present in intravenous lines,[811] these drugs should not be administered simultaneously through the same intravenous access line. In contrast to *Candida* IE, suppurative thrombophlebitis of the great central veins due to *Candida* spp. is curable medically, but antifungal regimens must be continued longer than is usually adequate for superficial suppurative thrombophlebitis. Based on limited data,[787] amphotericin B at a daily dose of 0.7 mg/kg, to a total dose of at least 22 mg/kg, plus 5-fluorocytosine (25 mg/kg/dose 4 times daily), is recommended after catheter removal. A lipid formulation of amphotericin is usually preferred to conventional amphotericin. Fluconazole (400 mg/day) or an echinocandin for 4 to 6 weeks is an alternative in patients who are not able to tolerate amphotericin B. Surgery may be essential in patients with suppurative thrombophlebitis of the thoracic or neck veins if perivascular collections are present.

The optimal therapy for pelvic suppurative thrombophlebitis still is controversial. Because anaerobic streptococci and *Bacteroides* spp. predominate, the initial antibiotics of choice are aqueous penicillin G (20 million units IV daily) plus either clindamycin (450–600 mg IV every 6 hours) or metronidazole (500–750 mg IV every 8 hours). The use of heparin is debated. The addition of heparin after several days of unsuccessful treatment with antibiotics itself may produce an antipyretic effect.[813] In one series of 46 patients with pelvic suppurative thrombophlebitis,[791] including 7 with massive ovarian vein involvement and 15 with septic pulmonary emboli, 42 patients become afebrile within 7 days (mean, 2.5 days) while receiving penicillin, chloramphenicol, and heparin. Four patients required exploratory laparotomy, and pelvic abscesses were found in 3 of them. These results argue strongly that medical therapy alone often is effective, but no controlled studies on the use of heparin have been done. If medical therapy is unsatisfactory, surgery with drainage of abscesses and, usually, ligation of the implicated venous system must be performed. Some authorities[792] believe that ligation of the inferior vena cava or ovarian vein, or both, should be performed in all of these cases, but the evidence for this approach is inconclusive.

Prevention

The incidence of superficial suppurative thrombophlebitis can be reduced by the same preventive procedures that are used for intravenous cannulas in general (see Chapter 300). These include the use of "scalp vein" cannulas whenever possible; avoidance of lower-extremity cannulations; insertion under aseptic conditions; secure anchoring of the cannula; and frequent replacement (at least every 48–72 hours) of intravenous fluid bottles, cannulas, and connecting tubing. Although neomycin–polymyxin B–bacitracin ointment is effective in reducing the incidence of cutdown infections,[814] use of this combination agent has not shown consistent benefit with intravenous cannulas.[815]

A detailed discussion of prevention strategies for vascular catheters has been published by the Centers for Disease Control and Prevention.[816] When clinical signs of bacteremia occur in a patient receiving intravenous fluids, the following steps should be taken: (1) blood culture specimens should be obtained; (2) intravenous administration should be discontinued and all cannulas removed; (3) the intravenous fluid should be cultured; (4) the cannula should be cultured semiquantitatively on blood

agar, as described by Maki and associates[799]; and (5) appropriate antibiotic therapy should be instituted. If clinical signs of sepsis and bacteremia persist despite appropriate antibiotic therapy, an intravascular focus (e.g., suppurative thrombophlebitis at a previously cannulated vein) should be sought, as discussed previously.

INFECTIVE ENDARTERITIS AND MYCOTIC ANEURYSMS

The term *mycotic aneurysm* was coined by Osler in 1885 to describe a mushroom-shaped aneurysm that developed in a patient with subacute bacterial endocarditis. At that time, the term *mycotic* was used to refer to all microorganisms. At present, the use of *mycotic* has been restricted specifically to fungal infections, but *mycotic aneurysm* still is used for all extracardiac (or intracardiac) aneurysms of infectious etiology except for syphilitic aortitis. This term also has been used to describe preexisting aneurysms secondarily infected from contiguous or distant foci and pseudoaneurysms arising from trauma or iatrogenic causes. *Endarteritis* refers to inflammation of the arterial wall, which may occur with or without coexistent aneurysmal dilation. Unless an aneurysm or coarctation of the aorta is present, infective endarteritis is usually a postmortem diagnosis. Because infected aneurysms differ in their pathogenesis, the various classifications (Table 80.8) are examined separately in the following discussion.[817] Infections of arterial prosthetic devices are discussed in detail in Chapter 82 and are not considered here.

Epidemiology

Although incidence figures are unavailable, a localized suppurative process of the arterial wall is rare. Estimates derived from autopsy series of aortic aneurysms are available but ignore infections at other locations in the arterial tree. In a review of more than 22,000 autopsies performed at the Boston City Hospital from 1902 to 1951,[818] aortic aneurysms were found in 1.5%. Mycotic aneurysms constituted only 2.6% of these lesions, however. In another review of 178 aneurysms found among more than 20,000 autopsies at the Mayo Clinic from 1925 to 1954,[819] only 6 were believed to be of infectious origin. Similarly, in a review[820] of 77 pure iliac artery aneurysms in 48 patients from a 21-year period, only 2 aneurysms (4.2%) were mycotic in origin. In the preantibiotic era, infected aneurysms were confined predominantly to patients with IE; in a series of 217 cases reported in 1923,[821] 86% were associated with IE. With the advent of antibiotics, mycotic aneurysms in IE have become less prevalent and hematogenous seeding of a previously damaged arteriosclerotic vessel constitutes the most common mechanism. In a retrospective review of all emergency department cases seen at one city public hospital from 1994 to 1999, the annual prevalence of arterial mycotic aneurysms among injection drug users was 0.03%.[822]

Because most of these lesions arise in areas of severe atherosclerosis, they occur in men more often in a ratio of 3:1, and the average age at presentation has been 65 years. The mean age for mycotic aneurysms that occur with IE is younger (approximately 40 years), and men and women are affected approximately equally. Estimates of the incidence of mycotic aneurysms in patients with IE range up to 15%.[823–826] Two percent to 4% of IE patients develop intracranial mycotic aneurysms,[826,827] although a neurologic presentation is common in patients with IE (noted in 16%–23% of cases), and at least 30% of the patients develop neurologic manifestations.[828,829] As discussed previously, the presence of such manifestations during IE has an adverse effect on the ultimate mortality rate. These lesions remain a significant cause of morbidity and mortality due to intracerebral and subarachnoid hemorrhage, especially in young people in developing countries, where acute rheumatic fever, rheumatic heart disease, and resultant IE still are prevalent.[830] Nine intracranial mycotic aneurysms associated with IE were treated in one neurosurgical unit in South Africa during an 18-month period, and five patients died.[830]

In addition, aortic root complications, including abscess or mycotic aneurysm, are associated with a poor outcome from IE. In one review,[831] aortic root complications were documented in 23 (46%) of 50 cases of aortic valve IE over a 6-year period; prosthetic valve involvement was common, and the surgical mortality rate and incidence of postoperative aortic regurgitation were higher in the group with aortic root complications.

Mycotic aneurysms are extremely rare in childhood[832] and when present are usually associated with IE, cardiovascular malformations, or connective tissue disorders. A specific disease entity first described in 1970 is aneurysm associated with umbilical artery catheterization in neonates.[833] The infecting organism is usually staphylococcal. By 1992, 34 cases had been reported, with the following distribution[833]: descending thoracic aorta, 14 cases; abdominal aorta, 10 cases; iliac arteries, 6 cases; and multiple sites, 4 cases.

TABLE 80.8 Classification of Mycotic Aneurysms

PREEXISTENT ARTERIAL STATUS	SOURCE OF INFECTION
Normal	Intravascular
Atherosclerotic	Septic embolism from the heart
Aneurysm	Bacteremia with seeding
Arterial prosthesis	Extension from adjacent endocardial focus on erosion
	Extravascular
	Contiguous site of infection
	Iatrogenic

Pathogenesis

Four different mechanisms have been postulated to produce infection of the arterial wall: (1) formation of mycotic aneurysms secondary to septic microemboli to the vasa vasorum ("embolomycotic aneurysms"),[834] (2) extension from a contiguous infected focus, (3) hematogenous seeding of the intima during bacteremia originating from a distant infection, and (4) trauma to the arterial wall with direct contamination.[835] Embolomycotic aneurysms usually occur in patients with active IE, and the incidence of this type has declined since antibiotics became available. The source of infection is the cardiac vegetation, with production of arterial emboli that lodge in the vasa vasorum, often at points of bifurcation of the affected artery.

Contiguous foci of infection (e.g., a caseous tuberculous lymph node or pyogenic vertebral osteomyelitis) may extend directly to major vessels, with subsequent aneurysm formation. The normal arterial intima is very resistant to infection. If this lining is altered by congenital malformations (e.g., coarctation of the aorta) or by acquired disease (especially atherosclerotic plaques or ulcers), resistance to infection is lowered and the surface may become colonized by bloodborne organisms. This hypothesis is analogous to the central role of NBTE in the pathogenesis of IE. An intraluminal thrombus associated with an atherosclerotic vessel also may serve as a nidus for colonization. Atherosclerosis accounts for more than 74% of secondarily infected aneurysms. Luetic arteritis and cystic medial necrosis also have been associated with secondary infection.[823]

Trauma to the arterial wall with subsequent infection has been documented in injection drug users (needle trauma)[836] and has been associated with gunshot wounds, vascular surgery, cardiac catheterization, percutaneous transluminal coronary angioplasty,[837,838] intravascular stent placement,[839] radial artery catheterization,[840] implantable ports for intraarterial chemotherapy,[841] and puncture of a femoral artery for analysis of arterial blood gases.[817] These events, if associated with contamination, usually lead to pseudoaneurysm formation in a peripheral artery and a contiguous abscess in extravasated blood.

Pathologic Changes

Infection of the arterial tree has been recognized by pathologists for more than a century. Virchow first showed local dilation of the arterial wall at the site of a septic embolus in 1847. Infection superimposed on an atherosclerotic aorta first was reported by Koch in 1851. Stengel and Wolfroth[821] collected 217 cases of mycotic aneurysms in 1923. These lesions probably are underreported, and pathologic material has been scant in recent years.

Most mycotic aneurysms that develop during the course of IE are situated in the sinus of Valsalva or in the supravalvular proximal thoracic aorta (>70% develop proximal to the aortic arch). Aneurysms are more

common in the right or posterior sinus and may be complicated by acquired shunts (rupture into the right ventricle is the most common), tamponade, coronary artery occlusion, or an atrioventricular conduction block.[842] Less commonly, major visceral, intracranial, and peripheral arteries are involved. Intracranial mycotic aneurysms characteristically develop in the distribution of the middle cerebral artery at peripheral bifurcation points,[829,831] as opposed to a more proximal location for most congenital aneurysms. Multiple intracranial lesions may be present. Mycotic aneurysm of the extracranial carotid arteries is rare (26 case reports[843]), but most develop in association with IE, usually due to *S. aureus*. Fewer than 10% are found in the upper extremities, but these arteries usually are not examined adequately with pathologic or radiologic techniques. Infrafemoral aneurysms during IE or after its treatment[844] also are unusual. Multiple lesions are identified in many IE patients with mycotic aneurysms.[834] Saccular forms seem to be more common than fusiform ones.[818] The aneurysms vary in size from 1 mm to more than 10 cm. As mentioned earlier, many of these aneurysms arise from emboli to the vasa vasorum, and occasionally the embolus can be shown grossly and microscopically. Acute and chronic inflammation is found diffusely through the arterial wall; necrosis, hemorrhage, abscess, and bacterial colonies all may be present in the sections. The elastica and muscularis layers usually are obliterated, but the intima often is intact. Rupture with surrounding hemorrhage and infection may be present.

Secondary infection of a preexisting aneurysm is found most commonly in the abdominal aorta (accounting for 70% of the cases), because this is the area most frequently and severely damaged by atherosclerosis. Ascending and descending aortic aneurysms each account for about 15% of the cases. The primary bacteremia most commonly originates from distal infections in soft tissue, lung, bone, or joint. The arterial infection usually begins in the distal abdominal aorta or iliac arteries as a focus of inflammation on an ulcerated atheromatous plaque. The wall of the aneurysm is thinned, and there is focal acute and chronic inflammation that may lead to arterial rupture. Even so-called bland aortic aneurysms commonly have some mild inflammation (characterized by a predominance of lymphocytes and mononuclear cells) in the wall; however, infected atherosclerotic aneurysms are characterized by acute inflammation with a predominance of polymorphonuclear leukocytes, necrosis, abscess formation, hemorrhage, and visible bacterial colonies. This lesion probably is underreported, because the focal suppuration may be limited in extent and overlooked unless routine culture and histologic sections are examined on every aortic aneurysm specimen. Erosion and rupture may be present without aneurysmal dilation. Lumbar or thoracic osteomyelitis is present in one-third of the cases[823] and may precede the aneurysm or develop secondary to contiguous spread from the vascular infection.

When contamination accompanies arterial injury, an infected pseudoaneurysm may result. These lesions are located in the extremities in more than 80% of the cases and are characterized by more extensive local tissue inflammation than is seen with the two types mentioned previously. Infection as a cause of pseudoaneurysm formation is increasing: 17 of 57 (30%) lesions seen in the 1980s[845] were infected. When endarteritis develops after angioplasty, it usually follows a second procedure or repuncture, and this scenario should suggest the diagnosis; all cases have been due to *S. aureus*.[833,838] Distal emboli, pseudoaneurysm, and coexistent osteomyelitis are present in more than 50% of the cases. Infective aortic root aneurysm also has occurred after coronary artery bypass graft surgery, with disastrous results.[834,846,847] Subclavian artery aneurysms may be present, with systemic findings plus unilateral upper extremity rash or splinter hemorrhages.[848] Nineteen cases of intracavernous carotid artery aneurysms have been reported,[849] usually occurring with meningitis with or without IE.

Of special interest are mycotic aneurysms in patients undergoing renal transplantation. Among 640 renal transplants performed at the University of Minnesota over 8 years, perinephric infections developed in 28 patients, and 8 of these patients developed mycotic aneurysms.[850] These lesions were evident clinically 1.5 to 4 months after transplantation. Six were located in the external iliac artery and one each in the internal iliac artery and aorta. All of these lesions were secondary to contiguous foci of infection in the deep tissues of the transplant wound.[851,852]

Clinical Manifestations

When mycotic aneurysms occur during the course of IE, manifestations of the underlying disease may be evident. Peripheral middle cerebral artery aneurysms constitute 2.5% to 6.2% of all intracranial aneurysms[826–830,852] and usually are secondary to infection. Intracranial mycotic aneurysms are usually clinically silent. Hemorrhage results in severe headache of sudden onset with rapid deterioration in the level of consciousness. The time interval from diagnosis of IE to the onset of hemorrhage is variable (0–35 days), with a mean of 18 days.[827]

Some lesions produce premonitory or herald neurologic signs, including focal deficits and seizures. However, these findings are relatively common in patients who have IE without intracranial aneurysms, and the differential diagnosis and decisions regarding arteriography are difficult.[853] A sudden focal deficit consistent with embolism is seen in approximately 23% of patients and should prompt arteriography.[853] A high proportion of patients with intracranial mycotic aneurysm with severe, unremitting, localized headache, often in association with homonymous hemianopsia (as a herald sign), was reported in one series.[854] Stroke syndromes may be seen and manifest as focal neurologic deficits, headache, confusion, meningismus, seizures, or coma.[828,829] Contrary to popular belief, most intracranial hemorrhages associated with IE were found to be caused not by ruptured mycotic aneurysms but by septic necrotic arteritis.[828] Symptomatic intracranial hemorrhage was associated with a mortality rate of 60% to 90% in that study. Patients also may present with bilateral cortical blindness.

Unusual location or etiology of an intracranial mycotic aneurysm suggests a diagnosis other than IE.[855] Mycotic aneurysms tend to occur more commonly in women of a younger age than does IE in general. They must be differentiated from aneurysms secondary to tumor emboli (especially choriocarcinoma or atrial myxoma), trauma, arteritis, or moyamoya disease and congenital aneurysms. Visceral artery aneurysms are uncommon but when present are almost uniformly caused by infection[835] or by polyarteritis nodosa. The most common location is in the superior mesenteric artery. Although superior mesenteric artery aneurysms account for only 8% of visceral artery aneurysms overall, most are of infectious origin.[856] Symptoms include colicky abdominal pain of acute onset, but the presentation is variable. Hepatic artery aneurysms may produce colicky right upper quadrant pain, fever, jaundice, and gastrointestinal hemorrhage[857] or hemobilia.[858] More than 190 cases of this entity have been reported in the literature; 75% were extrahepatic, and 25% were intrahepatic. Ruptured mycotic aneurysm of the celiac artery may manifest as hemoptysis or hemothorax.[859]

If the external iliac artery is involved, a triad of clinical signs may be present: (1) pain in the lower extremity (especially the anterior aspect of the thigh) with quadriceps muscle wasting and a depressed knee jerk; (2) arterial insufficiency of the extremity with coolness, pallor, and depressed pulses; and (3) bacteremia.[860] Distal aneurysms (e.g., affecting the femoral artery) occasionally have unusual presenting manifestations, including arthritis and purpura in the affected limb. If more peripheral arteries are involved (usually with a pseudoaneurysm), a tender, diffusely indurated mass is present in 92% of cases. The mass is pulsatile, with an associated bruit, in 50% to 60% of patients, and 20% to 30% have decreased peripheral pulses, skin changes, or even frank gangrene.[836] Local suppuration, petechiae, and purpura are often present, and the lesion may be confused with localized cellulitis or an abscess without consideration of vascular involvement. In users of illicit drugs, the brachial, radial, or carotid arteries or arteries of the lower extremity may be involved.[822] Only 50% of these patients are febrile on admission.[822] A superimposed septic arthritis also may be present.[861]

Although most infected aortic atherosclerotic aneurysms occur in elderly men, no pathognomonic findings exist to separate these patients from patients with bland, uninfected aneurysms. Fever is the most helpful differentiating sign (present in >70% of patients), because it is uncommon in patients with bland aneurysms. Back pain or abdominal pain occurs in about one-third of the cases. A draining cutaneous sinus may be present. Differentiation of an infected aneurysm from the entity of inflammatory abdominal aortic aneurysm may be difficult. Inflammatory abdominal aortic aneurysms first were described in 1935 and

account for 5% to 10% of abdominal aortic aneurysms; the lesions are usually infrarenal and often lead to ureteral obstruction, owing to the densely adherent fibrotic mass surrounding the vessel.[862] In a large series of 2816 patients undergoing repair of abdominal aortic aneurysms, 127 (4.5%) had inflammatory abdominal aortic aneurysms.[863] Most patients (123 of 127) were men and heavy smokers. Inflammatory abdominal aortic aneurysms are associated with an elevated erythrocyte sedimentation rate (73% of cases), weight loss, symptoms (back or abdominal pain in 30%–50%), and a high operative mortality rate.

Continuing bacteremia despite "appropriate" antimicrobial therapy in an elderly (especially diabetic) patient who has no signs of IE suggests an infected intravascular site. The aneurysm is palpable in 50% to 60% of the cases.[824,835] In most cases, the onset is insidious, and a low-grade fever may be present for several months before diagnosis. The nonspecificity of the clinical manifestations is reflected by the 75% preoperative rupture rate for this entity. Rupture may occur into the retroperitoneal space or peritoneal cavity (56%), pleural cavity (9%), duodenum (12%), esophagus (6%), mediastinum (3%), or pericardium (3%). The most common site of aortoenteric fistula is between the aorta and the third portion of the duodenum. Short periods of herald bleeding are common warning signs before exsanguinating hemorrhage occurs.[864] Severe pain and the rapid onset of shock usually accompany rupture of the aneurysm.

Laboratory Findings

There are no characteristic laboratory abnormalities in this group of diseases. When mycotic aneurysms occur with IE, alterations suggesting the underlying disease may be present. CT is useful in patients with neurologic manifestations of IE, especially for the demonstration of intracranial hemorrhage. CT is not sufficiently sensitive for the detection of intracranial mycotic aneurysms[828,829]; however, these lesions are not likely when the appearance on CT scan is completely normal. Diagnosis of intracranial mycotic aneurysm can be established by four-vessel cerebral arteriography, although 2D and 3D helical CT angiography,[865] magnetic resonance angiography,[829,866] and the less dangerous and invasive procedure of intravenous digital subtraction angiography[867] are promising. Magnetic resonance angiography may detect aneurysms only 2 to 3 mm in diameter, but false-negative results occur in 8% to 10% of such studies; this modality cannot substitute for selective angiography, although techniques and resolution are evolving.[868]

Patients with infected aortic aneurysms usually have a leukocytosis (65%–83%), but this is nonspecific and may be present even if the aneurysm is bland. Bacteremia is found in 53% to more than 90% of the cases, is continuous, and usually does not clear with antibiotic therapy alone. Evidence for a primary source of bacteremia (e.g., pneumonia, osteomyelitis) may be present but is absent in 46% of the cases.[823] The abdominal aorta is noted to be calcified on abdominal radiographs in 47%,[825] and anterior vertebral body erosion has been shown in 18%. A lack of calcification suggests infection, because 70% to 80% of bland aneurysms show calcification on abdominal radiographs. Certain procedures (e.g., intravenous pyelography, ultrasonography, CT[869]) may reveal the presence of an aneurysm but often are not satisfactory for preoperative detail. The absence of intimal calcification, an associated perianeurysmal fluid collection or osteomyelitis (usually shown by CT), and the sudden appearance of an aneurysm in a septic patient are all features suggesting an infected abdominal aortic aneurysm.[870,871] Gas in the aortic wall is diagnostic but rare.

Although the sensitivity is unknown, ^{67}Ga- and ^{111}In-labeled leukocyte imaging have been used to localize intraarterial infections.[872] Occult infected aneurysms have been identified in patients with fever of unknown origin and negative results on CT or MRI studies with gallium[873] or leukocyte scintigraphy,[874] and these procedures may enable seroma or hematoma to be distinguished from adjacent infection. Leukocyte imaging with ^{99m}Tc-labeled cells also seems promising, but false-positive results have been noted.[875] Positron emission tomography labeled with ^{18}F-fluorodeoxyglucose shows great potential for the detection of endarteritis and infected vascular grafts and will likely become the nuclear medicine study of choice for this group of diseases. Nevertheless, preoperative angiography often is preferred to delineate precisely the extent of aneurysmal involvement.[876] This information may alter the operative approach and may minimize complications.

Two-dimensional echocardiography (TTE or TEE) is a useful noninvasive technique for documenting mycotic aneurysms in the vicinity of the aortic valve (e.g., sinus of Valsalva, supravalvular, subvalvular), and this technique is adjunctive to aortic root angiography preoperatively.[877] Infective endarteritis or mycotic aneurysm in the vicinity of a patent ductus arteriosus also has been visualized successfully with 2D and Doppler echocardiography.[878,879] Intraoperative epicardial echocardiography has been used to facilitate the surgical approach. If a hepatic aneurysm is suspected, liver scanning and ultrasonography performed before angiography may be helpful.[857]

Etiologic Agents

Before the antibiotic era, mycotic aneurysms associated with IE usually were caused by the more "virulent" organisms, such as the β-hemolytic group A streptococci, pneumococci, or *H. influenzae.* With the decline of these organisms as causal agents in IE, most are now due to streptococci or staphylococci (≥60% of cases).

When bacteria seed a preexisting atherosclerotic vessel, the etiologic agents are markedly different from those found in mycotic aneurysms associated with IE. Gram-positive organisms cause approximately 60% of these lesions, but gram-negative bacilli (chiefly salmonellae) are isolated in 35%. Staphylococci are implicated in 40% of the cases overall,[825] and more than two-thirds of these are *S. aureus.* The risk for vascular infection in adult patients with non-Typhi *Salmonella* bacteremia has been reviewed.[880] Salmonellae cause 20% of the cases and involve, in order of frequency, the aorta and femoral and iliac arteries. Only 1 in 24 such cases reported before 1974 was above the renal arteries.[881] Lumbar osteomyelitis due to *Salmonella* was present in one-third of cases. The presumed portal of entry is the gastrointestinal tract.[882] *S. enteritidis* strains are isolated in 40% of cases, which is proportional to their overall rate of isolation in the United States. *S. enterica* serotype Choleraesuis, an uncommon clinical isolate, seems to be particularly pathogenic for this condition, because this species was isolated in 32% of the cases.[883] *S. enterica* serotype Typhi rarely is implicated in this disorder. *Salmonella* infections of aortic aneurysms first were reported in 1948. The predilection for involvement by this organism is not understood, but salmonellae tend to seed abnormal tissues during bacteremia (e.g., hematomas, malignant tumors, cysts, gallstones, bone infarcts, altered endothelium, aortic aneurysms). It has been estimated that 25% of patients older than 50 years with *Salmonella* bacteremia have an intravascular focus of infection.[884,885]

Arizona spp. (especially *Arizona hinshawii*) are closely related to *Salmonella* spp., cause similar clinical syndromes, and infect aortic aneurysms in elderly diabetic men.[886] The following organisms also produce infection in atherosclerotic aneurysms: *E. coli, P. aeruginosa,*[887] *Proteus* spp., *Citrobacter freundii, Klebsiella-Enterobacter* spp., *Brucella* spp.,[888] *S. marcescens, C. fetus,*[889] *L. monocytogenes* (17 reported cases[890,891]), *B. fragilis,* gonococci, group B streptococci,[892] corynebacteria, *C. burnetii,*[893] *Clostridium septicum,*[894] enterococci, and pneumococci.[895] *Mycobacterium tuberculosis* is a rare cause of aortic mycotic aneurysms. Of the 41 cases reported in the literature from 1945 to 1999, 75% seemed to result from erosion of the aortic wall by a contiguous focus and 25% seemed to result from direct seeding of the aortic intima or via the vasa vasorum.[896]

Fungal mycotic aneurysms are rare in the intracranial compartment, and only 13 definite cases had been reported by 1981.[897] The most common etiologic agents are *Aspergillus* spp., agents of mucormycoses, and *Candida* spp. The first two agents may involve intracranial arteries by direct extension from foci of sinusitis. One case of multiple intracranial aneurysms due to *Coccidioides immitis* that occurred during therapy for basilar meningitis has been described. Fungal mycotic aneurysms tend to involve larger, more proximal vessels at the base of the brain (11 [61%] of 18 cases were carotid or basilar), compared with those involved in bacterial cases of IE,[898] and may complicate intracranial surgery. *Aspergillus* mycotic aneurysms have occurred after transsphenoidal resections. Fungi also may cause endarteritis in the aorta or on aortic grafts, including *Aspergillus* or *Bipolaris* spp.[899,900] Mycotic aneurysms with subarachnoid bleeding may complicate the course of neurobrucellosis.[901] Tuberculous aneurysms are now uncommon; when present, they originate from contiguous foci of infection.

Pseudoaneurysms resulting from intraarterial or perivascular injection of illicit street drugs, often in addicts with sclerosed veins due to repeated intravenous inoculation, are associated with contiguous abscesses. The causative agents are *S. aureus* (in 76% of cases), *P. aeruginosa* (in 18%), and many others.[836]

Therapy

No uniformly acceptable approach has been devised for the treatment of mycotic aneurysms in IE. The treatment of intracranial mycotic aneurysms is particularly controversial. Some of these lesions seem to resolve with antimicrobial therapy alone. In a review of 56 aneurysms occurring in 45 patients,[902] 3 of 20 patients died when treatment was limited to antibiotics alone. Mild-to-moderate neurologic deficits were observed in 8 of the 17 survivors. Likewise, 6 of 25 patients for whom treatment included antibiotics and surgery died and 9 of 19 survivors were left with mild-to-moderate neurologic deficits. In other studies, the investigators reported a different experience, with a higher mortality in the nonsurgical group,[827] but patients were selected only after subarachnoid hemorrhage had occurred. In a review of 13 intracranial mycotic aneurysms,[827] 6 of 8 patients who received treatment with antibiotics alone died; no deaths were observed in the surgical treatment group. In a review of 85 cases treated between 1954 and 1978, 20 of 38 patients managed solely with antibiotics died, compared with 8 of 30 patients who underwent surgery.[826] Endovascular stent-grafts combined with antibiotic therapy have been used in a few patients with mycotic aneurysms of the descending thoracic aorta.[903] The distal location of most intracranial mycotic aneurysms associated with IE may permit ligation and excision with fewer complications than are observed with surgery for berry aneurysms in the circle of Willis. The mortality rate was low (4 of 15) in patients with multiple aneurysms who received treatment with antibiotics alone. In one series, the mortality rate was 29% after rupture of an intracranial mycotic aneurysm.

The most important factor in the management of intracranial mycotic aneurysms is whether rupture is present. A definitive review found that mortality rates of medically and surgically managed patients with unruptured intracranial mycotic aneurysms due to IE were similar. If rupture was present, however, surgical or endovascular therapy appeared to be indicated, because the outcome of medically managed patients was poor. Based on these data, most authorities advise a conservative approach if the intracranial mycotic aneurysm is unruptured. Other factors influencing treatment decisions for intracranial mycotic aneurysms include aneurysm location, presence of increased intracranial pressure, and the extent of perfusion supplied by the affected artery. Interesting to note, aneurysm size is not helpful in determining when to operate immediately because small mycotic aneurysms may rupture and some large mycotic aneurysms may regress with medical management.

Serial imaging with angiography, magnetic resonance angiography, or CT angiography may be useful in monitoring these patients, because the aneurysms may change in size or new lesions may develop. Among 21 patients studied with angiography, the mycotic aneurysm increased in size in 5 patients, did not change in 1 patient, became smaller in 6 patients, and completely resolved in 11 patients; new aneurysms developed in 2 patients. More than 50% of these peripheral intracranial aneurysms resolved with antibiotic therapy alone during the treatment of IE.[902] Surgery is indicated for aneurysms that are increasing in size on serial angiographic studies[904–906] but may be deferred for 4 to 6 weeks for aneurysms that are remaining the same size (if the patient is an acceptable medical risk). The definitive treatment for aneurysms that are decreasing in size on serial angiographic studies repeated every 2 weeks is unclear. CT is not helpful in localizing the aneurysm but provides important information if hematomas, infarcts, or abscesses develop. The choice of antibiotics is governed by the etiologic agent of the IE, but therapy for intracranial mycotic aneurysms, especially multiple lesions, must be individualized (see earlier discussion).[828,829]

Peripheral vessels usually are involved when arterial trauma (needle trauma, gunshot wound, iatrogenic injury) results in pseudoaneurysm formation with infection. Therapy with antibiotics, proximal ligation of the vessel, resection of the pseudoaneurysm, and appropriate drainage results in cures in 75% of the cases. Vascular reconstruction through uninfected tissue planes with autogenous grafts is necessary if limb viability depends on the affected vessel. This situation is encountered more frequently in the lower extremity. Severe ischemia developed in 9 of 28 patients after excision of mycotic aneurysms of the common femoral artery in one series of 52 cases.[836] Amputation was required in only 11% in a large series of 54 aneurysms among drug addicts seen at the Henry Ford Hospital; there were no deaths.[907]

The mortality rate in patients with infected atherosclerotic aneurysms often exceeds 90%; approximately 40 long-term survivors were reported from 1962 to 1988.[908–911] A high index of suspicion is necessary to allow surgical intervention before rupture occurs, because this complication is uniformly fatal and occurs in about 80% of the cases. If gram-negative bacilli are the cause of the infection, early rupture (e.g., within 2 weeks after the first positive blood culture) occurs much more frequently (84%) than if gram-positive bacteria are isolated (10%). Survival after surgery also is more common (75%) for patients with aneurysms infected with gram-positive cocci than for patients with gram-negative bacilli (25%). Antibiotics should be used in this disease, however, even if the lesion is sterilized (reported in only three cases). The aneurysm still may continue to enlarge and rupture, and surgery is required.

At surgery, the aneurysm and any intraluminal thrombus must be sectioned and Gram stain performed, and specimens must be submitted for culture. If infection is present, all aneurysmal tissue and surrounding areas of inflammation must be resected before grafting. Basic principles of grafting in this situation include the use of autogenous rather than synthetic grafts and insertion only in clean, noninfected tissue planes. If the graft is placed in the infected area, continued infection, leakage, thrombus formation, abscess formation, or rupture usually results. Although some authorities have achieved a successful result through restoration of vascular continuity in situ after radical débridement,[908,910] this approach is not recommended in most cases. Nevertheless, the type of reconstruction must be individualized, because results of in situ repair seem to be better for suprarenal[912–915] than for more distal aortic aneurysms if reconstruction is combined with prolonged courses of intravenously administered antimicrobial agents. Radical resection of intraabdominal aortic aneurysms without prosthetic material also has been used in a few cases.[916]

In a review of 24 patients with abdominal aortic aneurysms infected with salmonellae, 10 died after rupture without surgery and another 7 survived grafting only to die because of continued leakage from the anastomosis (only 5 patients were long-term survivors). If a graft is inserted in situ and persistent fever with bacteremia or embolism in the lower extremities ensues, reoperation with extraanatomic grafting is mandatory. Because the resected area is contaminated, special bypass techniques—especially for thoracoiliac, transpubic, and axillofemoral bypass—usually are required. If an axillofemoral approach is used, a single graft should be inserted for both lower extremities, because patency is prolonged under these circumstances.[909,911]

Bactericidal antibiotics should be continued for 6 to 8 weeks postoperatively. The choice of agents depends on the isolated organism (or the morphologic characteristics of the organisms in the surgical specimen) and on the results of in vitro susceptibility testing. Implantation of antibiotic-releasing carriers with in situ reconstruction has been used,[917] but only in a few patients without controlled trials; use of such carriers remains of unproved benefit in therapy for mycotic aneurysm.

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The complete reference list is available online at Expert Consult.

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81 Prosthetic Valve Endocarditis

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SHORT VIEW SUMMARY

Definition
- Prosthetic valve endocarditis (PVE) is a potentially life-threatening infection that involves a valve prosthesis or annuloplasty ring.

Epidemiology
- PVE is an uncommon but well-recognized complication of valve replacement or repair.
- Health care–associated PVE is increasing in incidence.
- *Staphylococcus aureus* is the most common cause of PVE.

Microbiology
- Early-onset (illness onset within 1 year of valve surgery) PVE is usually caused by microorganisms acquired perioperatively, such as *S. aureus* and coagulase-negative staphylococci (CoNS). Nosocomial gram-negative pathogens (many of which are multidrug resistant), enterococci, and fungi may cause early-onset PVE.
- Late-onset PVE is usually caused by organisms that are part of endogenous microbiota such as CoNS, *S. aureus,* viridans-group streptococci, and enterococci.
- *S. aureus* and CoNS have become common pathogens of late-onset PVE, in part because of an increase in frequency of health care–associated exposure in the late period.

Diagnosis
- Modified Duke criteria, initially proposed for epidemiologic purposes, have been validated clinically in evaluation of patients for diagnosis of PVE.
- Blood cultures are critical in both diagnosis and management of PVE. The identification of microbial pathogen and drug susceptibility testing are crucial for selection of appropriate pathogen-directed antibiotic therapy.
- Transesophageal echocardiography (TEE) is the preferred imaging modality to support a diagnosis and to identify complications such as severe valve dysfunction and perivalvular extension of infection, which may warrant surgical intervention.

Therapy
- Parenteral antimicrobial therapy specifically targeting the identified microbe for a minimum of 6 weeks is recommended (see Table 81.1).
- During antimicrobial therapy, serial clinical evaluations are important; if complications that would prompt surgical intervention are clinically suspected, TEE may need to be repeated in order to identify them.
- Early surgical intervention should be considered in complicated PVE with perivalvular extension, severe heart failure, severe valve dysfunction or dehiscence, multiple emboli, unresponsive infection, and PVE due to multidrug-resistant organisms or fungi (see Table 81.6).

Prevention
- Perioperative antibiotic prophylaxis, strict infection control measures, good surgical technique, and limiting the use of central venous catheters are important in preventing early-onset PVE.
- Maintenance of good oral hygiene is important to prevent late-onset community-acquired PVE.
- Although there has been no randomized, placebo-controlled trial to define the efficacy and safety of antibiotic prophylaxis, it is recommended for any dental procedure that involves manipulation of the gingival or periapical region of teeth or perforation of oral mucosa, targeting viridans-group streptococci.
- Antibiotic administration may be reasonable for invasive procedures involving the respiratory tract or infected skin or skin structures and/or musculoskeletal tissue; in such cases, antibiotics should be administered before and after the invasive procedure ("after" as a treatment regimen). Coverage for viridans-group streptococci and possibly additional organisms may be needed in the setting of respiratory tract procedures, contingent on known colonization status or presence of infection. For infected skin or skin structures and/or musculoskeletal tissue, coverage for *S. aureus* and β-hemolytic streptococci should be provided.

Prosthetic valve endocarditis (PVE), a microbial infection involving the valve prosthesis or repaired native heart valve with placement of an annuloplasty ring, is an uncommon but potentially lethal complication of prosthetic valve surgery. Despite diagnostic and therapeutic advances over the past decades, PVE continues to be characterized by high rates of relapse, morbidity, and mortality.

Because of the increase in prosthetic valve implantations performed for degenerative valvular disease in elderly patients and longer survival of patients with prosthetic valves, the number of patients at risk of PVE will continue to increase in the developed world.

PROSTHETIC VALVES

During the first half of 20th century, the management of patients with heart diseases saw dramatic breakthroughs with many medical innovations such as cardiac catheterization, invention of the heart-lung machine, and discovery of heparin.[1] During a person's lifetime, the four heart valves have to open and close approximately 2.5 billion times to maintain efficient unidirectional blood flow. As the average life span increases, the risk of structural degeneration of heart valves escalates. For patients with valvular heart disease, surgical interventions such as valvuloplasty and surgical decalcification procedures had poor results, and the need for valve replacement was realized. Valve replacement operations have dramatically improved survival and quality of life among patients with valvular heart disease. In the developed world, age-related degenerative valve disease now is the most common indication for valve replacement.[2–4]

An ideal prosthetic valve is one that can be placed in the heart, is compatible with cardiac physiology, and is chemically inert, nonimmunogenic, nonthrombogenic, and durable for decades.

Mechanical Valves

The initial step in the development of prosthetic valves occurred in 1954, when Hufnagel reported surgical correction of aortic insufficiency with a polymethyl methacrylate aortic ball valve that was implanted in the descending aorta with use of proximal and distal fixation rings.[5] Despite initial success, this surgical intervention was limited by embolization, thrombosis of valve, significant mechanical noise, and lower reduction in cardiac workload. Albert Starr and Dwight Harken developed a mechanical caged-ball valve made of a stainless steel cage, a knitted Teflon cloth fixation ring, and a heat-cured Silastic ball. On September 21, 1960, the first successful orthotropic valve placement was done by

Starr in the mitral position and subsequently by Harken in the aortic position.[6–8] The main problem with mechanical valves that remains to date is the high rate of thromboembolic complications. Starr, along with mechanical engineer M. Lowell Edwards, worked to reduce thromboembolic complications by pursuing design modifications such as reducing metal exposure area, coating surfaces with heparin, and altering the fabric to induce growth of the endothelium.[1] These design changes over time reduced the rate of thromboembolic episodes but did not eliminate the need for lifelong anticoagulation. Additional limitations of mechanical caged-ball valves are the substantial energy requirement and myocardial oxygen consumption required to open the ball valve and maintain cardiac output. In the late 1960s, mechanical valves with tilted disks with lower resistance to open the valve were introduced. A mechanical valve with a bileaflet mechanism was introduced in 1977 and has the advantage of unimpeded central flow of blood when the valve is in open positions. Mechanical valves now come in three designs: ball cage, tilted disk, and bileaflet.

Bioprosthetic Valves

The successful implantation of a cadaveric valve in the subcoronary aortic position by Donald Ross in 1962 was promising.[9] Subsequently, Binet and colleagues reported that five patients who received mercurochrome-formalin–preserved heterografts survived without anticoagulants. Heterograft valves from pigs and cows resemble human valves in size and structure. However, the enthusiasm for the hemodynamic and biologic advantages of biologic tissue valves was dampened by their poor midterm durability. Carpentier sought to improve durability while maintaining low thrombogenicity by combining biologic and mechanical structures, advancing a concept of bioprosthesis.[10] The durability of tissue valve was improved by chemical treatment of the porcine heart valve—washing the valve in Hank's solution and using an oxidizing agent to reduce antigenic components and glutaraldehyde treatment to stabilize collagen by establishing cross-links. The chemically treated porcine valve was then supported by a fabric-covered metal frame and a sewing ring, creating a hybrid bioprosthesis. Further advances included introduction of tissue substitutes such as bovine pericardium and new fixation strategies designed to maintain the molecular structure of tissue valves while limiting mineralization. Despite these significant advances, bioprostheses are prone to late structural degeneration and failure.[11,12]

Transcatheter Expandable Valves

Transcatheter aortic valve implantation (TAVI) has revolutionized the field during the past decade by combining the technologic advances in stent design, balloon angioplasty, interventional cardiology, and noninvasive real-time cardiac imaging.[13,14] In this technique, a bioprosthetic valve is delivered to the aortic position by means of a percutaneous catheter and then secured in place within the diseased valve with an expandable stent. A transfemoral approach is most common, but transapical and transsubclavian approaches may be considered in patients with difficult femoral access. Since US Food and Drug Administration (FDA) approval was granted in 2012, more than 50,000 patients have benefited from this procedure in the United States.[13] The two transcatheter aortic valves that are currently commercially available are (1) the Edwards SAPIEN valve, which is made of bovine pericardial trileaflets mounted on a balloon-expandable stainless steel stent, and (2) the Medtronic CoreValve, which is composed of a porcine pericardial valve with a self-expandable nitinol stent. TAVI was initially approved for patients with severe symptomatic aortic stenosis considered to be at high or greater risk of death or serious complications from open heart surgery.[15] In 2016 the indication was expanded to patients at intermediate risk.[16] The pivotal TAVI trials have shown promise in high-risk patients, with reduction in the 1-year mortality rate and improvements in quality-of-life measures.[17–20] Severe complications include cerebral embolism, need for a pacemaker after TAVI, infection, and aortic dissection or rupture.[17,19] Active endocarditis is considered an absolute contraindication for TAVI. A major limitation of first-generation TAVI is that the valve cannot be repositioned or retrieved once deployed. Suboptimal positioning of the valve may result in regurgitation, occlusion of coronary ostia, or conduction abnormalities.[13] Hopefully, the future generation valves can overcome this limitation and improve the success rate and safety profile of this technique.[21,22]

Transcatheter mitral and pulmonary valves have been introduced more recently.[23,24] In 2017 the FDA approved an expanded indication for the SAPIEN 3 valve for patients with symptomatic heart disease due to failure of a previously placed bioprosthetic aortic or mitral valve in whom risk of death or severe complications prohibits repeat valve replacement surgery.

EPIDEMIOLOGY

The incidence of PVE ranges from 1% to 6% of prosthetic valve implantations, or 0.3% to 0.6% per patient-year.[25–27] PVE accounts for 16% to 33% of all definite cases of infective endocarditis (IE), according to data from retrospective studies of single and multicenter tertiary care units in developed countries[27–34] and from prospective national,[35–37] European,[33] and international multicenter observational studies.[38] Risk factors associated with the development of PVE include male sex, previous native valve endocarditis (NVE), and long cardiopulmonary bypass time for prosthetic valve placement.[39–41] The cumulative risk of developing PVE is highest within the initial 12 months after replacement surgery, with a peak during the first 2 months.[38,41–45]

The prosthetic valve and annulus are vulnerable to secondary microbial seeding during the early postimplantation period when they lack an endothelial lining. The mean age of patients with PVE is 65 years; the range is 50 to 74 years.[35,38] The risk of PVE is higher in patients who undergo valve replacement surgery during active IE, especially in the setting of an unknown pathogen or insufficient antibiotic treatment.[32,40,43,45–47]

The mechanical prostheses appear to have a slightly higher risk during the first 3 months after operation, and the bioprosthetic valves appear to have a slightly higher risk after 12 months after operation, likely as a result of degenerative changes in the bioprosthetic leaflets.[40,41,48] The cumulative risk of PVE appears similar between mechanical and bioprosthetic valves.[41–43,48,49] However, a very large observational study of 38,500 elderly patients (age ≥65 years) demonstrated that those with bioprosthetic aortic valves had a higher risk of IE than did patients with mechanical aortic valves at 12-year follow-up.[50] The frequency of PVE is similar for both aortic and mitral prosthetic valves during the first year after operation.[41–43,47,51,52]

Prosthetic Valve Endocarditis After Transcatheter Valve Replacement

The incidence of early PVE after TAVI has ranged from 0.5% to 3.1%, similar to the incidence of PVE after open heart surgical valve replacements.[53–56] One proposed source of endothelial damage favoring subsequent endocarditis is the presence of residual aortic regurgitation, a major limitation of the first generation of transcatheter aortic valves.[53] Indeed, moderate-to-severe residual aortic regurgitation was identified as a characteristic associated with PVE in a review of 250 patients with PVE who underwent TAVI (22.4% vs. 14.7%; hazard ratio [HR], 2.05; 95% confidence interval [CI], 1.28–3.28).[56] A systematic review of 60 patients with PVE noted that most were men and had a higher risk profile with multiple comorbidities, such as diabetes mellitus, renal failure, and immunocompromise. Most patients with PVE were diagnosed between 3 and 9 months after implantation, with a median time of 5 months. Enterococci and *Staphylococcus aureus* were the predominant pathogens, followed by gram-negative organisms and fungi. Approximately half of the patients with PVE did not have a clear source or portal of entry of the pathogen. The most common presenting symptoms were fever and heart failure, followed by septic embolic lesions. Approximately 58% of patients with PVE had vegetations demonstrated with transesophageal echocardiography (TEE) and a significantly higher proportion of patients had perivalvular complications, such as abscesses (47%), fistulas (9%), and involvement of other valves (22%), comparable to rates of such complications in historical cohorts of PVE after open heart surgery.[53] Despite the high frequency of perivalvular complications, only 41% of patients underwent surgery, likely explained by the high-risk profile of patients who underwent surgical intervention, and in-hospital mortality neared 34%.[53]

MICROBIOLOGY

The microbiology of PVE depends on (1) time of onset of PVE (early vs. late PVE) and (2) site of acquisition (community-acquired vs. health care–associated PVE).

Early- and Late-Onset Prosthetic Valve Endocarditis

PVE has been categorized as early-onset PVE and late-onset PVE on the basis of the time period between prosthetic valve replacement and the manifestation of endocarditis. The microbiologic features of early- and late-onset PVE are different. Early-onset PVE has been due to microorganisms acquired intraoperatively or during the immediate postoperative period. *S. aureus* and coagulase-negative staphylococci (CoNS) are the most common pathogens in early PVE, followed in incidence by diphtheroids, fungi, and nosocomial aerobic gram-negative bacilli.[34,38,57–62] In the past, the time limit was arbitrarily chosen as 60 days, to differentiate perioperative infections from late community-acquired infections.[31,38,46] However, less virulent organisms acquired perioperatively may manifest many months after surgery. The peak incidence of CoNS endocarditis is between 60 and 365 days after valve implantation.[38] Hence, 1 year appears to be a better reference point to distinguish between early and late PVE. There has been a sharp decline in the proportion of early PVE, from 60% of all PVE cases in studies published in the 1970s[59,60,62] to about 10% to 20% in more recent studies,[38,58] without significantly affecting the overall rate of PVE.[26,38,41,61] The reduced early PVE rate is the consequence of better infection prevention and control practices, appropriate use of antimicrobial prophylaxis, improvements in design of prosthetic valves, and better surgical techniques.

Late PVE is usually considered to have been acquired in the community, unrelated to surgery or the perioperative period, with microbiologic findings resembling those of NVE. However, as a result of changes in the health care delivery system and the longer survival of patients with prosthetic valves, health care–associated PVE cases are increasing in the late period.[34] In some regions of the world, staphylococci have surpassed streptococci as the most frequent causative organisms of late PVE. In some hospitals, enterococci have surpassed viridans-group streptococci and thus are the third most common etiologic agent of late PVE.[34,38,58]

Health Care–Associated Prosthetic Valve Endocarditis

Health care–associated infections have become the most important risk factor for development of PVE in recent years.[34,38,63–65] Health care–associated PVE constituted about 37% of all cases in a prospective, multinational cohort.[38] *S. aureus* is now the leading causative organism of PVE, with increases in frequency of methicillin-resistant strains across geographic regions.[38] Nosocomial bacteremia and fungemia are high-risk factors for subsequent development of PVE.[63,66–68] Major risk factors for health care–associated PVE include presence of intravascular devices and hemodialysis. About 70% of health care–associated PVE cases are diagnosed within the first year after prosthetic valve implantation, and more than 60% occur beyond 60 days after operation.[38] Many aerobic gram-negative bacilli can cause health care–associated PVE, and multidrug resistance is common. Among this group, *Pseudomonas, Serratia, Acinetobacter,* and *Stenotrophomonas* spp. predominate. Fungal PVE due to *Candida* spp. can occur with health care exposure. *Candida albicans* and *Candida parapsilosis* are the most frequently isolated fungal pathogens of PVE.[69–72] Other non-*albicans Candida* spp. such as *Candida glabrata* and *Candida krusei* can cause PVE.[70]

Community-Acquired Prosthetic Valve Endocarditis

Most community-acquired PVE is caused by enterococci, viridans-group streptococci, and fastidious organisms including the HACEK group (*Haemophilus parainfluenzae, Aggregatibacter aphrophilus*, *Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens,* and *Kingella* spp.),[35,37,73–76] a similar constellation to those causing NVE in the community.

Late-onset PVE has been rarely caused by *Mycobacterium* spp., *Coxiella, Brucella, Tropheryma, Bartonella, Legionella* spp., *Enterovirus,* and *Aspergillus* spp. but can be difficult to identify as causal organisms. Fungal stains of the resected tissue can show characteristic fungal hyphal elements. Other rare fungal pathogens include *Scopulariopsis, Cryptococcus neoformans,* and *Histoplasma capsulatum.*[77–82] Rare cases of PVE with *Listeria,*[83–85] *Salmonella,*[86] and other gram-negative pathogens have been reported.

Outbreaks of Nontuberculous Mycobacterial Infections

Rapidly growing nontuberculous mycobacteria (NTM; *Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium wolinskyi*) have been associated with several outbreaks of surgical site infections after heart surgery.[87–90] Potential sources include prosthetic valve implant contamination during the manufacturing process, contaminated water used to cool the cardioplegia solution, and contaminated heater-cooler units used during open heart surgery.[91] Examples of potential contamination of implants during the manufacturing process include an outbreak of PVE due to *M. chelonae* and an outbreak of *M. fortuitum* infection involving patches used for repairing septal defects.[88,89]

A prolonged outbreak of infection was identified to be due to *Mycobacterium chimaera*, a member of the *Mycobacterium avium* complex, after open heart surgery. The outbreak involved multiple hospitals in several countries and has been traced to heater-cooler units from a single manufacturer, likely contaminated during the manufacturing process.[91] Many patients with *M. chimaera* PVE present several months to years after operation (median, 21 months), and high clinical suspicion is important in the diagnosis of infection.[92] This outbreak was identified after the local infection control team was alerted to two cases of unusually invasive *M. chimaera* infection, which prompted a subsequent outbreak investigation and was critical in the ultimate recognition of a global infection-control problem.

NTM are resistant to frequently used disinfectants such as chlorine and ozone.[93] They also have the propensity to form biofilms, contributing to their persistence in the hospital and in home water systems. Colonization of the hospital environment, especially the water systems, with NTM is common.[94] Aerosolization of these bacteria from the contaminated heater-cooler units used during the open heart surgical procedure was hypothesized to be the likely mode of transmission in the recent outbreak.[91]

PATHOGENESIS

PVE can occur as a result of direct microbial contamination of a prosthetic valve at the time of surgery or as a consequence of secondary hematogenous seeding from a distant infectious focus. The prosthetic valve and perivalvular tissue are vulnerable for secondary microbial seeding during the early postimplantation period, when they lack protective endothelial lining.

The pathogenesis of PVE consists of several steps including (1) microbial adherence to the prosthetic valve, damaged endothelium, platelet-fibrin aggregate, and/or periprosthetic tissue; (2) recruitment and activation of monocytes and platelets and activation of extrinsic coagulation cascade, resulting in an infected coagulum called a vegetation, the characteristic lesion of endocarditis; (3) persistence and growth of the microorganism within cardiac lesions, leading to local tissue destruction and invasion; and (4) dislodgement of the vegetations, which may occur and result in septic emboli in distant organs such as the brain, skin, spleen, and kidneys or in the musculoskeletal system.[95,96]

Microbial Adherence

Microbial adherence is a critical step in the pathogenesis of endocarditis and involves complex interactions between microbial surface proteins and host extracellular matrix molecules. The endothelial lining of the heart is usually resistant to microbial infection. However, during the early postimplantation period, the prosthetic valve, cardiac annulus, periannular tissue, sewing ring, and sutures are not endothelialized. The foreign material is often coated with extracellular host matrix molecules (e.g., fibrinogen, fibrin, fibronectin, collagen, elastin, plasma proteins, platelet proteins) that can serve as ligands for microorganisms.[97] Microorganisms can also adhere to and infect the sterile platelet-fibrin aggregate formed after injury or inflammation of the endothelium. The aging bioprosthesis, sutures, and sewing cuff fabric of a valve prosthesis are thrombogenic and favor deposition of fibrinogen-fibrin, fibronectin, plasma proteins, and platelets.

The microbial surface proteins that can bind to the host extracellular matrix proteins are called "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs).[98] These surface components play a key role in the initiation of endovascular infections, bone and joint infections, and prosthetic device infections. The usual pathogens of PVE (*S. aureus,* CoNS, streptococci) possess abundant MSCRAMMs. CoNS possess several adhesins, including autolysin/adhesins AtlE and Aae,[99,100] the fibrinogen-binding protein Fbe/SdrG,[101,102] the giant 1-MDa fibrinogen-binding protein Embp, and lipase GehD.[103] Additional protein and polysaccharide components such as autolysin AtlE are involved in primary attachment to polymers. CoNS have emerged as important pathogens in modern clinical practice, primarily because of their ability to adhere to biomaterials and form a stable biofilm.[104–106] Biofilm is a slimy, slippery coat formed by a structured community of bacterial cells enclosed in a self-produced extracellular polymeric matrix adherent to solid surfaces.[107,108]

S. aureus has many surface proteins and nonprotein adhesins[108] that play a crucial role in the pathogenesis of IE. These include clumping factor A (ClfA), fibrinogen-binding adhesins, and the bifunctional fibrinogen/fibronectin-binding protein A (FnBPA).[109–111] The microbial surface proteins of streptococci that have been studied in experimental models of IE include glucans *(Streptococcus sanguis, Streptococcus mutans, Streptococcus gordonii)*[112]; Fim A *(Streptococcus parasanguis)*[113]; Ace, an adhesin for collagen type IV, collagen type I, and laminin; endocarditis; and Hsa, a sialic acid–binding protein *(S. gordonii).*[114] Enterococci have biofilm-associated proteins *(Enterococcus faecalis),*[115,116] Acm and Scm, and collagen adhesins *(Enterococcus faecium).*[117]

Formation and Growth of Vegetations

The microbial growth within a platelet-fibrin aggregate leads to activation of the extrinsic coagulation cascade and recruitment of monocytes and platelets that result in the formation of vegetation. The adherent bacteria activate the extrinsic coagulation pathway by triggering release of tissue factor from monocytes that adhere to early vegetations[118,119] and from endothelial cells surrounding the infected valves.[120–122] They also influence recruitment and activation of monocytes.[123–125] For example, the FnBPA proteins of staphylococci interact with the endothelial cells and help in recruitment of monocytes by triggering the expression of intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1), interleukin (IL)-6, IL-8, monocyte chemotactic protein (MCP-1), and tissue factor.

Microorganisms also engage and activate platelets either directly or through bridging molecules. For example, *S. aureus* induces platelet activation by several surface proteins. ClfA/clumping factor B (ClfB) and FnBPA/fibronectin-binding protein B (FnBPB) are the major platelet-activating modulins.[126–128] *S. sanguis* activates platelets by either direct interaction between the serine-rich glycoprotein A (SrpA) and the glycoprotein Ib receptor (von Willebrand factor [vWF])[129] or specific antibody and complement assembly that link the surface proteins to platelet FcγRIIA and complement receptors.[130] Strains of *S. gordonii* stimulate platelet aggregation directly by binding to glycoproteins Ib and IIb through the serine-rich surface glycoproteins GspB and the sialic acid–binding adhesins (Hsa).[131,132]

These processes result in an enhanced procoagulant and inflammatory activity in the vicinity of the infection. The thrombus serves as a focus for adherence of additional bacteria and platelets, leading to growth of the vegetation.

Persistence and Growth of Microorganisms

Microorganisms are able to persist and grow within vegetations by using many mechanisms to evade the host defense system. The usual pathogens of IE have reduced susceptibility to platelet-microbicidal proteins in vitro.[133–135] ClfA of *S. aureus* inhibits phagocytosis by human polymorphonuclear leukocytes in the absence of fibrinogen.[136] Fibrin-adherent streptococci are not engulfed by monocytes.[137] *S. aureus* can internalize into intact endothelial cells through a fibronectin bridge between FnBPA and the endothelial $\alpha_5\beta_1$ integrins (fibronectin receptors). This internalization of *S. aureus* can lead to persistent or recurrent infection by avoiding host defense and membrane-active antimicrobial agents, such as β-lactams and glycopeptides.

The biofilm over the prosthetic valve also provides a protective environment for the bacteria. The sessile bacteria within the biofilm are less susceptible to the host immune system and antibiotics than the free-floating (planktonic) bacteria. The polymeric matrix acts as a diffusion barrier to retard the diffusion of antibiotics and reactive oxidants of phagocytic cells. Bacteria within the biofilm exhibit an altered phenotype with different patterns of growth, gene expression, and protein production. Nutrient-deficient bacteria in the biofilm switch to a slow-growing metabolically quiescent persister phenotype, which is less susceptible to antimicrobial agents.[137]

Tissue Destruction and Invasion

Microorganisms produce various toxins and tissue-degrading enzymes that result in invasion and tissue destruction. The extent and rapidity of tissue destruction depend on the virulence of the microorganisms. *S. aureus,* the most common pathogen of PVE, is a virulent organism capable of inducing significant tissue destruction in a short period of time. In *S. aureus,* the secretion of tissue-degrading enzymes and toxins is coordinated in a growth phase–dependent manner by global regulator loci such as accessory gene regulator *(Agr),* the stress response regulon (SigB), and staphylococcal accessory regulator *(SarA).*[109,138,139]

PATHOLOGY

The clinicopathologic findings of PVE usually differ between mechanical and bioprosthetic valves. Mechanical heart valves are primarily made of metal or carbon alloys and are classified according to their structure as caged-ball, single-tilting disk, or bileaflet-tilting disk valves.[140] Metal or carbon alloys are usually not well suited for microbial adherence. The infection of mechanical prostheses usually starts at the interface between the sewing cuff and the native tissue. The perivalvular tissue invasion can result in loosening of the sutures, causing periprosthetic leaks, or in ring abscesses (Fig. 81.1). Annular abscess or paravalvular leak was more common in mechanical PVE than in bioprosthetic PVE.[141] In a retrospective multicenter study at 16 tertiary referral hospitals, 148 (17%) of 872 patients with PVE had perivalvular complications of the aortic ring, including aortocavitary fistulas in 19% and nonruptured abscess in 81%.[142] The ring abscess may dislodge the prosthesis from its anchorage (Fig. 81.2) and give rise to the echocardiographic appearance of a "dancing" prosthesis. Acute ventricular decompensation and congestive heart failure (CHF), due to either valve obstruction or incompetence, are important complications of PVE requiring surgery. In one study, 45% of all patients with PVE developed prosthetic dehiscence with moderate or severe valve regurgitation.[142] Aortic PVE may also result in third-degree heart block if the infectious process extends to the conducting system of the heart. Ruptured ring abscesses may form fistulous tracks into cardiac chambers or into the intraventricular septum and lead to intracardiac shunting. The aortic PVE may result in aneurysms of the sinus of Valsalva by extending to the aortic root or aneurysms of the anterior leaflet of the mitral valve by extending through



FIG. 81.1 Aortic prosthetic valve endocarditis with valvular stenosis and paravalvular ring abscess. *(Courtesy Dr. William D. Edwards, Mayo Clinic.)*

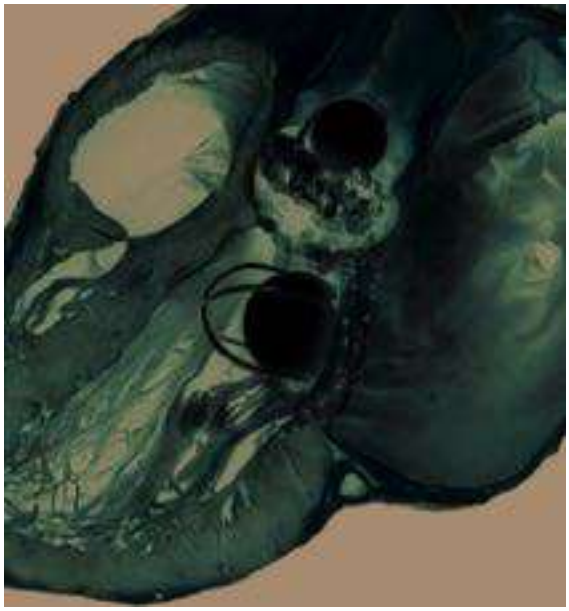

FIG. 81.2 Mechanical aortic prosthetic valve endocarditis with dehiscence and paravalvular leak. *(Courtesy Dr. William D. Edwards, Mayo Clinic.)*



FIG. 81.3 Aortic prosthetic valve endocarditis extending to involve other native valves. *(Courtesy Dr. William D. Edwards, Mayo Clinic.)*

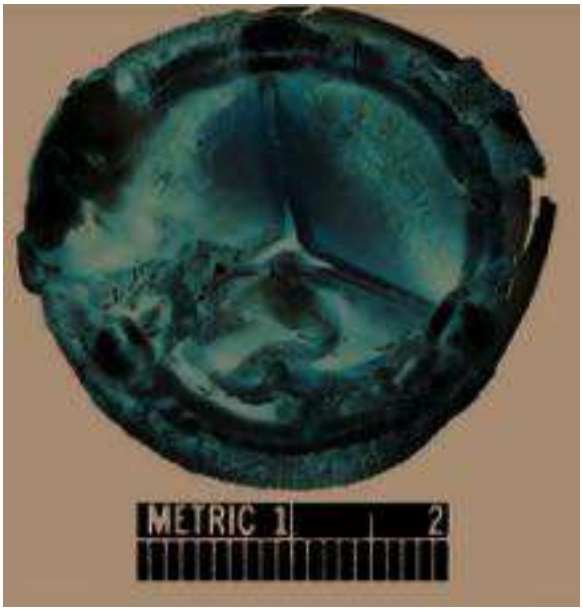

FIG. 81.4 Aortic bioprosthetic endocarditis involving the cusps. *(Courtesy Dr. William D. Edwards, Mayo Clinic.)*

mitral-aortic fibrous continuity. The infection may also extend to involve the other valves, resulting in multivalvular endocarditis (Fig. 81.3).

Bioprosthetic valves are homografts (preserved human aortic valves) or heterografts (bovine pericardial or porcine valve tissue mounted on a metal support), and the infection is usually restricted to the cusps (Fig. 81.4). The growth of thrombotic vegetations in the biosynthetic material can lead to cusp rupture, perforation, leak, and vegetations. However, if the sewing cuff is involved in infection, the pathologic process is similar to that of mechanical prosthetic valve infection.

The sizes and types of vegetations appear to be correlated with the virulence of the causative microorganism. *S. aureus* usually results in smaller vegetations but causes significant destruction and invasion of the tissue. Viridans-group streptococci have been associated with larger vegetations with slower, milder destruction of tissue. Fungi form large, bulky vegetations.[142] Large vegetations are also associated with the HACEK group of organisms.

CLINICAL PRESENTATION

The clinical presentation of PVE is variable, determined by the virulence of the pathogen and the time of presentation. Common clinical presentations include fever, new or changing murmur, heart block, congestive heart failure (CHF), and embolic events.[143] Fever is the most common symptom and sign of PVE and is present in 73% to 92% of patients. However, fever is often absent in patients with renal failure, elderly patients, and those taking antibiotic or antipyretic medications. New or changing murmur or CHF may be indicative of complications of PVE such as prosthetic valve dehiscence, fistulas, or perivalvular abscess.[144–147] New ventricular dysrhythmias or conduction abnormalities may arise if the infectious process (perivalvular abscess) extends into the electrical conduction system of the heart.[148,149] Perivalvular extension of the aortic PVE into the intraventricular septum can disrupt the proximal ventricular conduction system and result in complete heart block. Embolic stroke or brain abscess, or both, may be the initial presentation of PVE. In patients with left-sided endocarditis, both symptomatic (35%) and clinically silent (30%) cerebrovascular complications are common.[150] Mycotic aneurysms involving the cerebral vasculature are uncommon, but serious complications of PVE can result in intracranial hemorrhage.[151,152] Secondary metastatic abscess can develop in the musculoskeletal system (psoas abscess, vertebral osteomyelitis, and diskitis), kidneys, spleen, joints, and so on. Right-sided endocarditis involving the tricuspid or pulmonic prosthesis may result in septic pulmonary emboli. Glomerulonephritis or arthritis can be a manifestation of a systemic immune response but is uncommon in acute presentations.

DIAGNOSIS

Classic oslerian manifestations may often be absent in acute PVE because patients present during the early stage of the disease before the evolution of immunologic and embolic manifestations. The diagnosis of PVE can be challenging because atypical presentations are common in the elderly population with prosthetic valves. A high index of suspicion and comprehensive clinical, microbiologic, and echocardiographic evaluations are important for prompt diagnosis of PVE and its complications. Modified Duke criteria should be used as a clinical guide to evaluate all patients suspected to have PVE.[153,154] Since the Duke criteria were initially established for epidemiologic and clinical research purposes rather than to assist in clinical practice, clinical judgment should be used when they are applied in the management of patients.

Echocardiography

TEE is recommended in all cases of suspected PVE.[154] One of the two major criteria in the modified Duke diagnostic schema is echocardiographic evidence of oscillating intracardiac mass on the valve or

supporting structures, new partial dehiscence of prosthetic valve, or intracardiac abscess.[153] The utility of transthoracic echocardiography (TTE) is limited in the evaluation of patients with suspected PVE for two reasons.[155] First, reverberations and other artifacts created by the prosthesis hamper the quality of the TTE images. Second, TTE is not sensitive enough to evaluate for perivalvular complications, which is critical in the management of PVE. TEE has a higher sensitivity of 86% to 92% to detect vegetations when compared with TTE (17%–36%).[156–159] TEE is also superior to TTE for the detection of complications such as perforations, prosthetic valve dehiscence, perivalvular abscesses, and fistulas.[160] Thus, TEE is important to both establish the diagnosis of PVE and identify high-risk patients who may require early surgery.

False-negative TEE may occur in the early course of illness if the vegetations or perivalvular abscesses are too small to be detected.[161] If the clinical suspicion persists despite negative TEE, TEE should be repeated after 7 days, although again it can be falsely negative as a result of the intervening therapy. The interpretation of TEE is sometimes difficult because differentiation among thrombus, vegetation, or degenerative strand is not always obvious.

Complications of PVE such as valve dehiscence, perivalvular abscess, fistulas, and pseudoaneurysm may evolve over time despite negative initial TEE. Persistent fever despite appropriate antibiotic treatment and/or new conduction abnormalities should raise the suspicion for intracardiac perivalvular abscess. New or worsening heart failure may suggest mechanical valve dehiscence or perforation or rupture of the bioprosthetic valve cusp. TEE should be repeated promptly whenever there is a change in clinical condition that raises the suspicion of PVE complications. TEE may depict pseudoaneurysms as a pulsatile, echocardiographic-free sac around the prosthesis communicating with a cardiac chamber (Fig. 81.5).

Nuclear Medicine Imaging Techniques

Echocardiography is operator dependent and may be falsely negative if it is performed too early in the pathogenesis of PVE when vegetation and/or structural valve damage are not yet apparent.[162] Up to 30% of patients who undergo echocardiography because of clinical suspicion of PVE have inconclusive findings. To overcome this limitation, several nuclear medicine imaging procedures have been examined over the past few years, including labeled leukocyte scintigraphy, single-photon emission computed tomography (SPECT), and fluorine-18 fluorodeoxyglucose positron emission tomography (PET) with either computed tomography (^{18}F-FDG-PET/CT) or computed tomography angiography (CTA)[163] (Fig. 81.6).

Labeled Leukocyte Scintigraphy

Inflammatory cells migrate avidly to the site of infection by chemotaxis. Radiolabeled white blood cell (WBC) scans use this phenomenon to localize the site of infection by planar imaging. Labeled leukocyte scintigraphy techniques, such as indium 111 (^{111}In), gallium (^{67}Ga) with SPECT views, and technetium 99m (^{99m}Tc) hexamethyl propylene amine oxidase (HMPAO) with SPECT/CT acquisitions, have been reported to be helpful in identifying PVE or perivalvular abscesses even among patients with negative echocardiographic results.[164–168] One study showed the added value of ^{99m}Tc HMPAO with SPECT/CT in the management of IE by improving the performance of the modified Duke criteria.[167] ^{99m}Tc-labeled monoclonal anti–human granulocyte antibody–labeled leukocytes for diagnosis of PVE have been used in a small case-control study.[169] Limitations of WBC scans include poor spatial resolution and variable sensitivity affected by viability of leukocytes after the in vitro labeling process and rate of migration of leukocytes to the site of infection. The WBC labeling process is also time consuming, labor intensive, expensive, and slow; the imaging is performed 24 hours or later after injection of the radiolabeled leukocytes.[163]

Positron Emission Tomography/Computer Tomography

^{18}F-FDG-PET/CT combines the measurement of metabolic activity of tissue and anatomic evaluation of CT to achieve a diagnosis of PVE. Advantages of ^{18}F-FDG-PET/CT over WBC scans include high spatial and target-to-background contrast resolution without any requirement for labor-intensive in vitro WBC labeling processes. ^{18}F-FDG-PET/CT relies on the phenomenon that glucose uptake transporters (GLUT1) are overexpressed in the activated inflammatory cells (neutrophils, macrophages, lymphocytes) at the site of infection and hence accumulate FDG in high concentration.[170–172] In a prospective study of 72 patients with suspected PVE, ^{18}F-FDG-PET/CT had a sensitivity of 73% and specificity of 80% and it identified all cases of PVE in which pathologic diagnosis was confirmed after valve surgery. If ^{18}F-FDG-PET/CT is included as a major criterion in the modified Duke criteria, the sensitivity of the diagnostic scheme might rise to 97% from 70% by reclassification a number of "possible" cases to "definite" PVE.[173]

The addition of CTA to ^{18}F-FDG-PET has been proposed as a better imaging modality than nongated CT/^{18}F-FDG-PET to diagnose PVE and perivalvular complications such as mycotic aneurysm, myocardial abscess, fistula, and thrombosis, and for preoperative evaluation of coronary arteries.[174–176] ^{18}F-FDG-PET/CTA can help in the surgical decision process by identifying complications that may necessitate urgent surgical intervention.[177] However, well-controlled comparisons of diagnostic accuracy are not currently available.

Limitations of Positron Emission Tomography/Computer Tomography

Many unanswered questions remain regarding ^{18}F-FDG-PET/CT imaging procedures. The standard protocol employed in most nuclear medicine



FIG. 81.5 Transesophageal echocardiography image showing pseudoaneurysm. *(Courtesy Dr. Nandan Anavekar, Mayo Clinic.)*



FIG. 81.6 Multislice computed tomography scan showing perivalvular abscess. *(Courtesy Dr. Nandan Anavekar, Mayo Clinic.)*

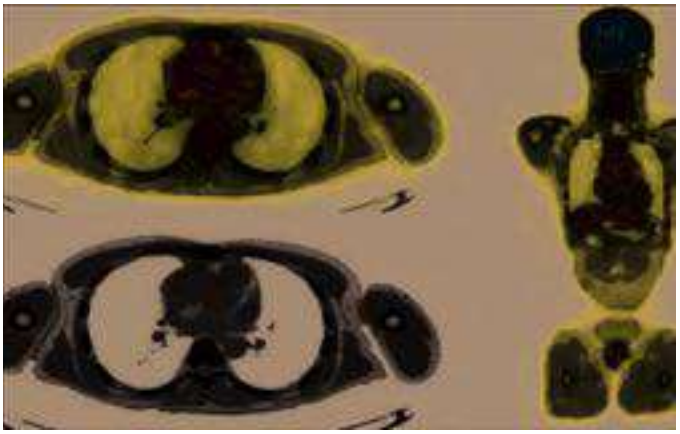

FIG. 81.7 Fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) scan positive for prosthetic aortic root endocarditis. ^{18}F-FDG-PET/CT relies on the phenomenon that glucose uptake transporters (GLUT1) are overexpressed in the activated inflammatory cells (neutrophils, macrophages, lymphocytes) at the site of infection and hence accumulate FDG in high concentration. This figure shows focal and heterogeneous FDG uptake around the prosthetic aortic root that appears to signify infection. *(Courtesy Dr. Maryam Mahmood, Mayo Clinic.)*

centers is designed to detect metastatic lesions, which have higher metabolic activity than infected tissue. Some authors have suggested that modifications in the protocol (delayed image acquisition at 2–3 hours after injection of glucose rather than at 1 hour) will increase the sensitivity of ^{18}F-FDG-PET/CT through better detection of glucose uptake by monocytes and macrophages at the level of infection.[178] The pattern and distribution of FDG uptake (focal, heterogeneous rather than diffuse) appear to signify infection more than the intensity of FDG uptake (maximum standardized uptake value [SUV_{max}]), and there is no established SUV cutoff value available (Fig. 81.7). A major concern with ^{18}F-FDG-PET/CT is the potential for false-positive results during the early postoperative period, because there is significant functional glucose uptake in the perivalvular tissue.[163,179,180] There are few available data about the normal evolution of FDG uptake in the perivalvular tissue in the early postoperative period. Use of BioGlue to seal aortic graft anastomoses has produced significantly high FDG uptake.[181] The inflammatory reaction and metabolic activity after cardiac surgery appear to be idiosyncratic, and therefore the FDG uptake pattern in the early postoperative period may not be generalizable. Current guidelines recommend deferring ^{18}F-FDG-PET/CT for up to 3 months after cardiac surgery. Physiologic myocardial FDG uptake and cardiac motion could interfere with results. Sensitivity of ^{18}F-FDG-PET/CT appears to be lower with small vegetation size, and antibiotic treatment may reduce inflammatory reaction, resulting in false-negative results.[182] Available studies of ^{18}F-FDG-PET/CT in the evaluation of PVE are limited by lack of controls,[173] and well-designed prospective studies are needed to further delineate its role in the management of patients with suspected PVE. Standardization of dietary protocols, timing of image acquisition, image processing protocols, and image interpretation criteria are important and needed. The current European guidelines for diagnosis of IE have included ^{18}F-FDG-PET/CTA in the diagnostic algorithm.[183] It appears to be useful especially among patients in whom clinical suspicion of PVE is high despite normal or inconclusive echocardiographic results. Among patients with "possible" PVE according to modified Duke criteria, ^{18}F-FDG-PET/CTA can help to reclassify them conclusively to either "rejected" or "definite" PVE.

Nuclear medicine imaging techniques examine the whole body. ^{18}F-FDG-PET/CT can be valuable in identifying subclinical extracardiac metastatic infectious lesions such as vertebral osteomyelitis, psoas abscess, pyomyositis, splenic abscess, and septic arthritis, with the exception of septic brain lesions.[184,185]

Identification of the Pathogen

Blood Cultures

Blood cultures remain the most valuable test in establishing the etiologic diagnosis of PVE. In clinically stable patients, at least three sets of blood cultures should be collected before the initiation of antibiotics. Blood cultures can be obtained at any time and are not necessarily timed with fever or chills because the bacteremia in endocarditis is low grade and continuous.[186,187] Growth of the microorganism in blood culture allows one to perform antimicrobial susceptibility testing, which is crucial in the selection of the most effective antibiotic regimen.

Blood cultures are positive in about 78% to 91% of patients with PVE.[32,38,57] Advances in bacterial culture techniques, with prolonged incubation times, presence of carbon dioxide, and use of enriched culture media have improved the yield of blood cultures. However, the prevalence of blood "culture-negative" PVE is still significant. Recent antibiotic administration is the most common cause of blood "culture-negative" PVE.[38] Routine blood cultures may also fail to detect fastidious microorganisms such as HACEK, *Bartonella, Brucella, Abiotrophia, Granulicatella* spp., and noncultivable microorganisms such as *Chlamydia* spp., *Coxiella burnetii, Legionella* spp., *Mycobacterium* spp., *Mycoplasma pneumoniae, Tropheryma whipplei,* and fungi.[188]

Valve Culture

If a patient undergoes surgery, surgical specimens, including vegetations, tissue fragments of bioprosthesis, periprosthetic tissues and abscesses, and embolic fragments, should be sent for histopathologic evaluation and cultures. Cultures of the valves are often negative owing to prior antibiotic administration.[189,190] Valve culture results should be interpreted with caution because contamination during handling from surgical resection to laboratory processing is possible.

Serologic Methods

Serologic methods can be used to complement culture-based methods to identify a pathogen. Modified Duke criteria have included specific serologic data as surrogate markers for positive blood cultures to establish an etiologic diagnosis in "culture-negative" endocarditis. An anti–phase I immunoglobulin G antibody titer of greater than or equal to 1:800 by microimmunofluoresence to *C. burnetii* is a major criterion in the modified Duke criteria for Q fever endocarditis.[154] Serologic tests are available for *Brucella melitensis, Bartonella* spp., *Legionella* spp., *M. pneumoniae,* and *Chlamydia* spp., and an antigen assay for *Aspergillus* spp. may be useful.[191]

Broad-Range Polymerase Chain Reaction Based on the Bacterial 16S rRNA Gene

Approximately 9% to 13% of patients with IE have negative blood cultures, and the rate is higher among patients who had received prior antibiotic therapy.[35,192] Without an identified causative pathogen, it is difficult to select an optimal antimicrobial regimen, often resulting in an empirical broad-spectrum combination regimen that may be suboptimal with a lower success rate while potentially increasing the risk of drug adverse events. Molecular diagnostic methods such as 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR) and sequencing may help to identify the pathogen among the culture-negative IE group.[193] The 16S rRNA genes comprise both highly conserved and variable regions. With use of broad-range PCR primers to amplify the conserved region of 16S rRNA genes, it is theoretically possible to identify a majority of bacterial pathogens.[194] PCR amplification of conserved sequences in the panfungal small subunit rRNA internal transcribed spacer sequence can be used to identify fungal microorganisms.[195] To enable identification of microorganisms at the subspecies level, the highly variable ribosomal intergenic sequences can be targeted for PCR amplification. In addition, this amplification can be extremely useful in defining whether a pathogen has genes that encode for antimicrobial resistance; this would be helpful, for example, in the case of identification of a *Staphylococcus* and whether it is oxacillin resistant. Other genes such as the 23S rRNA, 16S-23S intergenic spacer, and rpoB have been successfully used.[194]

PCR-based techniques have been used to detect microorganisms in blood specimens in addition to resected heart valve tissue.[196–200] Microbial

DNA can be present in valve tissue for several weeks after initiation of an appropriate antimicrobial agent.[198,201]

Studies evaluating user-developed 16S rRNA gene assays have reported a wide range of performance with varying sensitivities (range, 41.2%–100%) and specificities (range, 61.5%–100%) among patients with definite IE.[202] Two commercial assays are available but have been evaluated in only a small number of patients.[203,204] The sensitivity of broad-range 16S rRNA gene PCR assays has been lower among patients with prosthetic valve IE compared with those with native valve IE.[189,192,202] Limitations of molecular methods include potential contamination and cross-reaction of primers within the 16S rRNA gene with human DNA in clinical samples.[202,205] PCR-based methods also do not differentiate between viable and dead microorganisms.

Molecular techniques cannot replace culture-based methods but are additional tools that can be used to improve the etiologic diagnosis of IE. Currently, culture is indispensable for antimicrobial susceptibility testing.

Histology

Histologic examination of resected perivalvular and valvular tissues remains the gold standard for the diagnosis of PVE. Histologic criteria of PVE include demonstration of microorganisms in the resected specimens and pathologic lesions such as vegetations and inflammatory infiltrates, with or without annular abscess.[206] In situ visualization of causative agents in the resected valve specimens can be done with immunohistochemical methods. Autoimmunohistochemistry is an immunohistochemical method in which a patient's own serum is used as a source of antibodies that can be used to detect microorganisms in heart valve specimens.[207] Fluorescence in situ hybridization using fluorescent peptide nucleic acid (PNA) probes can penetrate into gram-positive bacteria within the resected valve tissue and can help to visualize the organism in situ.[208] If histologic examination fails to demonstrate microorganisms and vegetations, computer-assisted quantitative analysis of digitized microscopic images of the inflammatory pattern may assist in differentiating infective and noninfective inflammatory valve processes. Excessive neovascularization and a pattern composed of $CD15^+$ leukocytes exceeding 2% of the mechanical valve surface and exceeding 1.5% of the total bioprosthetic valve surface are highly predictive (90% and 98%, respectively) and specific (90% and 94%, respectively) for an acute infection.[206,209]

The management of PVE consists of effective pathogen-specific antimicrobial therapy and timely surgical intervention, when indicated. TEE is used to assess prosthetic valve function and vegetation size and to identify perivalvular complications that may require surgical intervention. It should be repeated during antimicrobial therapy when clinically indicated to monitor for development of complications of PVE. As discussed earlier, ^{18}F-FDG-PET/CTA can help in the surgical decision process by identifying perivalvular complications that may necessitate urgent surgical intervention.[177]

All patients suspected of having PVE should be hospitalized initially for close clinical monitoring. As previously discussed, pathogen identification, usually by isolation from blood cultures, is critical in defining an optimal treatment regimen. Because the infection is life-threatening, it is reasonable to initiate empirical antimicrobial therapy targeting the most likely pathogens after obtaining at least three sets of blood cultures. It is suggested that the empirical antimicrobial regimen be chosen to target the most likely pathogens of PVE, considering the time of onset of PVE and the likely site of acquisition.

ANTIMICROBIAL THERAPY

In vitro susceptibility testing should be used to define the most effective, pathogen-specific antimicrobial regimen. A microbicidal regimen is preferred for the treatment of PVE. At least two sets of blood cultures should be drawn every 24 to 48 hours until clearance of bloodstream infection is documented.[154] No randomized controlled trials have been conducted to establish the optimal duration of antimicrobial treatment. The duration of antimicrobial therapy is a minimum of 6 weeks and should be counted from the day of first negative blood cultures in culture-positive PVE.[154,183] If the resected valve or tissue culture or both are positive, it is reasonable to administer an entire course of antimicrobial therapy. In regimens that include multiple agents, they should be administered in temporal proximity to achieve maximal synergistic microbicidal effect.

Staphylococcal Prosthetic Valve Endocarditis

The antimicrobial regimens recommended for PVE due to *S. aureus* and CoNS are identical—based mainly on in vitro susceptibility testing (Table 81.1). A triple-drug combination antimicrobial regimen is recommended for the optimal treatment of staphylococcal PVE. The principal drug is a cell wall–active agent, chosen on the basis of methicillin susceptibility.[154] Vancomycin is the principal drug of choice for methicillin-resistant organisms, whereas semisynthetic penicillin (nafcillin, oxacillin) should be chosen for methicillin-susceptible organisms.[154] Cefazolin is used in patients who have a well-defined history of nonanaphylactoid reaction to penicillins. The second drug in combination therapy is rifampin, which is important in the treatment of staphylococcal foreign-body–related infections. In vitro studies, animal models of staphylococcal prosthetic device infections, and clinical studies have provided evidence supporting the role of rifampin in eradicating staphylococci adherent to prosthetic devices.[210–214] Rifampin resistance can easily develop owing to mutation of the ribosomal gene responsible for rifampin site of action.[212] The probability of this mutation and hence selection of rifampin-resistant strains is high when a large, highly dense, rapidly dividing bacterial population is exposed to ineffective rifampin-containing regimens. Hence, it is reasonable to initiate rifampin only after effective two-drug combination antistaphylococcal therapy has been administered for at least 2 days. If the *Staphylococcus* is not susceptible to two other drugs, a single agent can be used for 3 to 5 days before initiation of rifampin. The goal of this strategy is to reduce the number of organisms and hence lower the risk of selection of rifampin-resistant subpopulation. The third drug in the combination regimen is either an aminoglycoside or a fluoroquinolone. Gentamicin is recommended for

TABLE 81.1 Therapy for Prosthetic Valve Endocarditis Caused by Staphylococci, Suggested by the American Heart Association

REGIMEN	DOSAGE AND ROUTE	DURATION
Methicillin-Susceptible Staphylococci		
Nafcillin or oxacillin[a] *Plus*	2 g IV q4h	≥6 wk
Rifampin[b] *Plus*	300 mg PO or IV q8h	≥6 wk
Gentamicin[c]	3 mg/kg IV/IM q24h in 2 or 3 equally divided doses	2 wk
Methicillin-Resistant Staphylococci		
Vancomycin[d] *Plus*	15 mg/kg IV q12h	≥6 wk
Rifampin[b] *Plus*	300 mg PO or IV q8h	≥6 wk
Gentamicin[c]	3 mg/kg IV/IM in 2 or 3 equally divided doses	2 wk

[a]Cefazolin 2 g IV q8h can be used in non–immediate-type allergic reaction to penicillin. Consider skin testing for patients with history of immediate-type allergy to penicillin. Vancomycin is recommended only in patients unable to tolerate penicillins and cephalosporins.
[b]It is recommended to initiate rifampin therapy only after susceptibility results are known and ideally after 2 days of effective combination therapy, in an attempt to reduce the risk of emergence of rifampin resistance.
[c]Gentamicin should be administered in close proximity to vancomycin, nafcillin, or oxacillin to maximize synergy. Renal function and serum gentamicin concentrations should be closely monitored. Goal trough level is <1 μg/mL and peak level (1 h postdose) is 3–4 μg/mL.
[d]Vancomycin dosage should be adjusted to a trough level of 10–20 μg/mL. Dosages recommended are for patients with normal renal function.
From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation. *2015;132:1435–1486.*

the initial 2 weeks of therapy, although there are only limited clinical data to support this addition. In the case of gentamicin resistance, another aminoglycoside can be substituted if the organism is susceptible. If the organism is resistant to all aminoglycosides or if the patient is intolerant to aminoglycosides, a fluoroquinolone may be used as a substitute if the strain is susceptible.[212,213,215]

In vitro susceptibility testing should be repeated when there is failure of an antimicrobial regimen. Clinical failure with vancomycin therapy for methicillin-resistant *S. aureus* (MRSA) PVE can occur as a result of heterogeneous-type vancomycin-intermediate *S. aureus* (VISA) strains, and the optimal antimicrobial therapy in such cases is unknown.[216] Higher vancomycin minimal inhibitory concentrations (MICs) have been associated with worse clinical outcomes in both patients with MRSA infections treated with vancomycin and those with methicillin-susceptible *S. aureus* infections treated with β-lactams.[217,218] Higher vancomycin MICs may be surrogate markers for undefined host- or pathogen-specific factors that contribute to worse clinical outcome in some patients. Clinical experience is limited in the treatment of PVE with newer agents such as high-dose daptomycin and linezolid.[219–221] The role of ceftaroline or a combination regimen of daptomycin and ceftaroline in the treatment of staphylococcal PVE has yet to be defined. Staphylococcal PVE is often complicated by perivalvular extension or prosthetic valve dysfunction, necessitating surgical intervention for optimal management.

Streptococcal Prosthetic Valve Endocarditis

The antimicrobial regimens recommended by the American Heart Association (AHA) for the treatment of PVE caused by viridans-group streptococci, *Streptococcus gallolyticus,* or other penicillin-susceptible streptococci are listed in Table 81.2. The primary drug for treatment of streptococcal PVE is either penicillin or ceftriaxone, given for a minimum of 6 weeks. For PVE caused by highly penicillin-susceptible streptococci (MIC ≤0.12 μg/mL), the addition of gentamicin for the initial 2 weeks is optional because the combination regimen has not been shown to have superior cure rates compared with β-lactam monotherapy.[154] If the strain does not have high-level gentamicin resistance, intravenous (IV) once-daily gentamicin may be administered for the initial 2 weeks in patients who can tolerate aminoglycosides. For patients with PVE caused by streptococci with relative or high-level penicillin resistance (MIC >0.12 μg/mL), *Gemella, Abiotrophia,* or *Granulicatella* spp., a combination of penicillin or ceftriaxone with gentamicin is recommended for a minimum duration of 6 weeks. It is suggested to continue gentamicin for 6 weeks if the patient can tolerate it without significant nephrotoxicity. Monotherapy with vancomycin for a minimum of 6 weeks is recommended only for patients who cannot tolerate both penicillin and ceftriaxone.

PVE caused by *Streptococcus pneumoniae, Streptococcus pyogenes,* and group B, C, F, and G β-hemolytic streptococci are uncommon. In patients with PVE caused by penicillin-susceptible *S. pneumoniae,* 6 weeks of IV penicillin, cefazolin, or ceftriaxone is recommended. For PVE caused by *S. pneumoniae* with high penicillin resistance (MIC ≥2 μg/mL), 6 weeks of IV therapy—high-dose penicillin, cefotaxime, or ceftriaxone—is recommended. Cefotaxime or ceftriaxone is preferred if *S. pneumoniae* PVE is complicated with meningitis, because these agents have higher penetration into cerebrospinal fluid. In *S. pneumoniae* PVE with resistance to cefotaxime (MIC >2 μg/mL), IV vancomycin and rifampin can be added to cefotaxime or ceftriaxone.

TABLE 81.2 Therapy of Prosthetic Valve Endocarditis Caused by Streptococci as Suggested by the American Heart Association

REGIMEN	DOSAGE AND ROUTE	DURATION	COMMENTS
Penicillin-Susceptible (MIC ≤0.12 μg/mL) Strain of Viridans-Group Streptococci, *Streptococcus gallolyticus (bovis)*			
Either			Gentamicin is optional. Combination of penicillin or ceftriaxone with gentamicin has not been shown to be superior to monotherapy. Gentamicin should not be administered if creatinine clearance is <30 mL/min.
Aqueous crystalline penicillin G	24 million units/24 h IV, either continuously or in 4–6 equally divided doses	6 wk	
Or			
Ceftriaxone	2 g IV/IM q24h	6 wk	
With or without			
Gentamicin	3 mg/kg IM or IV single daily dose	2 wk	
Relatively or Fully Resistant to Penicillin (MIC >0.12 μg/mL) Strain of Viridans-Group Streptococci and *Streptococcus gallolyticus (bovis)*			
Either			Ampicillin 2 g IV q4h is a reasonable option if there is shortage of penicillin. See below for patients with β-lactam allergy.[a]
Aqueous crystalline penicillin G	24 million units/24 h IV, either continuously or in 4–6 equally divided doses	6 wk	
Or			
Ceftriaxone	2 g IV/IM q24h	6 wk	
Plus			
Gentamicin	3 mg/kg IM or IV single daily dose	6 wk	

[a]Monotherapy with IV vancomycin 15 mg/kg q12h for 6 wk is recommended only for patients unable to tolerate penicillin and ceftriaxone.
MIC, Minimal inhibitory concentration.
Dosages recommended are for patients with normal renal function.
From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation. *2015;132:1435–1486.*

Enterococcal Prosthetic Valve Endocarditis

The regimens recommended by the AHA for the treatment of enterococcal PVE are listed in Table 81.3. In contrast to streptococci, enterococci are relatively resistant to treatment with penicillin, ampicillin, or vancomycin, and monotherapy is not bactericidal. The synergistic effect achieved by combination of a cell wall agent (penicillin, ampicillin, or vancomycin) with an aminoglycoside is bactericidal against susceptible strains of enterococci.[222]

Enterococci are relatively impermeable to aminoglycoside, but cell wall–active agents increase this permeability, producing synergistic bactericidal effects. For synergism, the strain should be susceptible to the cell wall–active agent and not have high-level resistance to the aminoglycoside. High-level resistance is defined as growth at high concentrations of aminoglycoside (500 μg/mL of gentamicin or 1000 μg/mL of streptomycin).[154] All enterococci should be tested for susceptibility to cell wall–active agents (penicillin and vancomycin), and for high-level aminoglycoside resistance (both gentamicin and streptomycin). For susceptible strains, the combination of penicillin or ampicillin with gentamicin for 6 weeks has been the preferred regimen for several decades. However, aminoglycoside-containing regimens have higher risk of nephrotoxicity, especially in elderly patients with enterococcal PVE who also have multiple comorbidities. Guidelines have included a dual β-lactam option with ampicillin plus ceftriaxone (2 g IV every 12 hours) as a treatment regimen that should be administered for 6 weeks. If an aminoglycoside-containing regimen is chosen, the aminoglycoside should be given in multiple divided doses each day. Once-daily aminoglycoside administration has not been demonstrated to be as effective as multiple-daily administration in enterococcal endocarditis. Hence, in contrast to streptococcal PVE, once-daily administration is not recommended for enterococcal PVE. For patients with normal renal function, gentamicin should be administered every 8 hours.[223] The serum concentrations should be monitored closely, especially in patients with reduced renal function. The dosage should be adjusted to achieve a 1-hour serum concentration of approximately 3 μg/mL and a serum trough concentration of less than 1 μg/mL. A higher dosage of gentamicin increases the risk of nephrotoxicity without providing any additional enhanced bactericidal effect on enterococci.[224] About one-third of high-level gentamicin-resistant strains are susceptible to high levels of streptomycin and can be used for achieving synergy with cell wall–active agents.[224,225] Streptomycin is not favored owing to the higher risk of ototoxicity and the extremely limited availability of serum drug

TABLE 81.3 Therapy of Prosthetic Valve Endocarditis Caused by Enterococci as Suggested by the American Heart Association

REGIMEN	DOSAGE AND ROUTE	DURATION	COMMENTS
Enterococci Susceptible to Penicillin, Gentamicin, and Vancomycin			
Either ampicillin	2 g IV q4h	6 wk	Recommended for patients with renal clearance >50 mL/min. Vancomycin 15 mg/kg IV q12h can be substituted for ampicillin or penicillin only in patients unable to tolerate penicillin and ampicillin.
Or Aqueous crystalline penicillin G	18–30 million units/24 h, either continuously or in 6 equally divided doses	6 wk	
Plus Gentamicin[a]	1 mg/kg IV or IM q8h	6 wk	
Or Double β-Lactam Regimen—Ampicillin	2 g IV q4h	6 wk	Double β-lactam regimen is recommended for patients with renal clearance <50 mL/min.
Plus Ceftriaxone	2 g IV q12h	6 wk	
Enterococci Resistant to Penicillin but Susceptible to Gentamicin and Vancomycin *β-Lactamase–Producing Strain*			
Ampicillin-sulbactam	3 g IV q6h	6 wk	β-Lactamase–producing strains of enterococci are extremely rare. Confirmation will be necessary before selecting ampicillin-sulbactam as part of the treatment regimen. Vancomycin 15 mg/kg IV can be substituted for ampicillin-sulbactam in patients unable to tolerate ampicillin-sulbactam.
Plus Gentamicin[a]	1 mg/kg IV or IM q8h	6 wk	
Intrinsic Penicillin Resistance			
Vancomycin	15 mg/kg IV q12h	6 wk	Monitor renal function closely.
Plus Gentamicin[a]	1 mg/kg IV or IM q8h	6 wk	
Enterococci Resistant to Penicillin, Aminoglycoside, and Vancomycin			
Linezolid	600 mg IV or PO q12h	≥6 wk	Monitor for myelosuppression, neuropathy, and drug interactions.
Or Daptomycin	10–12 mg/kg IV q24h	≥6 wk	Severe myalgia may occur.
Enterococci Susceptible to Penicillin, Resistant to Aminoglycosides, or Gentamicin Resistant and Susceptible to Streptomycin			
Ceftriaxone sodium	2 g IV/IM q12h	≥6 wk	Streptomycin may be used along with ampicillin if renal clearance >50 mL/min, normal eighth cranial nerve function, and availability of rapid streptomycin serum concentration testing.
Plus Ampicillin sodium	2 g IV q4h	≥6 wk	

[a]Gentamicin should be given in 3 divided doses rather than a single daily dose.
Dosages recommended are for patients with normal renal function.
From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation. *2015;132:1435–1486.*

assay–monitoring capacity. Streptomycin should be avoided if renal clearance is less than 50 mL/min. Streptomycin should be administered every 12 hours in patients with normal renal function. The dosage should be adjusted to achieve a 1-hour serum concentration of 20 to 30 μg/mL and a trough concentration of less than 10 μg/mL.[224]

Double β-lactam combinations (ceftriaxone or cefotaxime or imipenem plus ampicillin) have been shown to have synergistic bactericidal activity against *E. faecalis* strains and have been considered as an alternative regimen for strains with high-level resistance to gentamicin and streptomycin but susceptible to penicillin.[226] An observational, nonrandomized, multicenter cohort study compared ampicillin plus ceftriaxone combination with ampicillin plus gentamicin for treatment of IE caused by *E. faecalis,* including 89 cases of *E. faecalis* PVE. The study did not find any difference in mortality, treatment failure, or relapse between the two groups, but the rate of interruption of antibiotic treatment due to adverse events (renal failure, elevation in creatinine) was higher in patients receiving ampicillin plus gentamicin. The authors concluded that the double β-lactam combination (ampicillin plus ceftriaxone) can be used safely to treat IE caused by both susceptible and high-level aminoglycoside-resistant strains of *E. faecalis.*[227]

For PVE caused by enterococci that are intrinsically resistant to penicillin but susceptible to aminoglycoside and vancomycin, a combination regimen of vancomycin with aminoglycoside for 6 weeks is suggested. Renal function should be closely monitored because this combination has a higher risk of renal toxicity. In extremely rare strains of *E. faecalis,* penicillin resistance may be due to inducible β-lactamase production rather than alteration in penicillin-binding proteins. For these β-lactamase–producing strains, ampicillin-sulbactam is the preferred cell wall–active agent, and vancomycin is recommended only for those who cannot tolerate ampicillin-sulbactam.[154]

Therapeutic options are limited in patients with PVE caused by enterococci that are resistant to penicillin, aminoglycoside, and vancomycin. Only case reports of treatment success with linezolid, quinupristin-dalfopristin, and daptomycin have been reported.[228–230] Combination therapy of ampicillin with daptomycin even in cases of ampicillin resistance might be a potential salvage regimen in patients unable to undergo surgery.[231,232] In patients with PVE caused by strains for which there is no effective bactericidal regimen, surgical intervention with valve replacement followed by a long (at least 8-week) course of postoperative antimicrobial therapy may be considered.

HACEK Prosthetic Valve Endocarditis

The regimens recommended by the AHA for treatment of HACEK PVE are listed in Table 81.4. Ceftriaxone or ampicillin-sulbactam is recommended as the preferred initial agent until susceptibility data are available because strains resistant to ampicillin due to β-lactamase production are increasing in frequency.[154] Fluoroquinolones have activity against HACEK organisms in vitro, but clinical efficacy data are limited. Antimicrobial therapy alone usually results in successful treatment of HACEK PVE if there is no perivalvular complication or valve dysfunction.[233]

Diphtheroid Prosthetic Valve Endocarditis

Diphtheroids are members of *Corynebacterium* species that are usually nonpathogenic commensals of the skin and mucous membranes (other

TABLE 81.4 Therapy of Prosthetic Valve Endocarditis Caused by HACEK as Suggested by the American Heart Association

REGIMEN	DOSAGE AND ROUTE	DURATION
Ceftriaxone *Or*	2 g q24h	6 wk
Ampicillin	2 g IV q4h	6 wk
Ciprofloxacin[a]	500 mg PO or 400 mg IV q12h	6 wk

HACEK, Haemophilus parainfluenzae, Aggregatibacter aphrophilus, Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, and *Kingella* spp.
[a]Ciprofloxacin is recommended only for patients unable to tolerate cephalosporin and ampicillin-sulbactam.
Dosages recommended are for patients with normal renal function.

than *Corynebacterium diphtheriae*) but have been known to cause PVE. Combination of penicillin or ampicillin with gentamicin for a minimum of 6 weeks is the recommended regimen for PVE caused by strains of diphtheroids (*Corynebacterium* spp.) that are susceptible to both penicillin and gentamicin. If the strain is resistant to gentamicin or penicillin, bactericidal synergism is not possible.[234] In patients with PVE caused by diphtheroid strains that are resistant to either penicillin or gentamicin and in patients who cannot tolerate penicillin, monotherapy with vancomycin for at least 6 weeks is recommended.

Enteric Gram-Negative Bacillary Prosthetic Valve Endocarditis

For PVE caused by enteric gram-negative bacilli, the optimal antibacterial regimen should be selected based on in vitro susceptibility tests.[154,183] Whenever possible, the combination of two effective agents should be administered for at least 6 weeks. In patients with PVE caused by multidrug-resistant gram-negative bacilli or *Pseudomonas* spp., surgical débridement and replacement of the infected valve followed by antimicrobial therapy is recommended.[235]

Fungal Prosthetic Valve Endocarditis

Candida spp. is the predominant pathogen that causes fungal PVE. Fungal PVE is associated with high rates of treatment failure, relapse, and mortality. Large, dense vegetations and frequent embolic events are characteristic features. Medical therapy alone is not sufficient to cure fungal PVE. Early surgical intervention is recommended by most authorities to improve survival and cure rate of fungal PVE.[70,154] Fungal PVE can recur even after this aggressive combined surgical-medical intervention, and prolonged, possibly lifelong suppressive therapy with oral antifungal agents is recommended by some experts.[70,81,236–239] If surgical intervention is not feasible, lifelong suppressive therapy is suggested. The antifungal regimen should be based on the fungal species and in vitro susceptibility data. Amphotericin B has been the drug of choice for fungal PVE for a long time. Lipid formulations of amphotericin have less nephrotoxicity and can be used. Echinocandins have been used in patients who cannot tolerate an amphotericin B formulation.[70] In a few case reports, combination antifungal therapy (e.g., caspofungin plus fluconazole, amphotericin B plus fluconazole) successfully cured candidal PVE without surgical intervention.[238,240,241]

Prosthetic Valve Endocarditis Due to Nontuberculous Mycobacteria

Rapidly growing NTM *(M. fortuitum, M. chelonae, Mycobacterium abscessus)* may cause PVE as a result of nosocomial acquisition and should be suspected in culture-negative PVE because blood cultures may be negative in as many as 40% of patients with NTM PVE.[92,242,243] The treatment of PVE due to NTM is poorly defined and often involves early surgical replacement of the infected prosthetic valve followed by several months of combination antibiotic therapy.[244–246] Antimicrobial regimens should be chosen based on identification and in vitro susceptibility testing of NTM isolates, despite the well-known limitations.[247] Combination of at least two active drugs (amikacin, carbapenems, macrolides, and fluoroquinolones) is suggested, and optimal duration of treatment is unknown. Relapse of NTM bacteremia can occur after discontinuation of antibiotics, prompting the suggestion of lifelong suppression by some authors.[245]

Culture-Negative Prosthetic Valve Endocarditis

The treatment regimen for culture-negative PVE should cover the most likely pathogens, considering prior antibiotic use, time of onset of PVE, and clinical and local epidemiologic clues. For early-onset PVE (<1 year after prosthetic valve surgery), the recommended empirical regimen includes vancomycin, gentamicin, cefepime, and rifampin, aiming to cover staphylococci, enterococci, and aerobic gram-negative bacilli including *Pseudomonas* spp.[154] Late onset of PVE (>1 year after prosthetic valve surgery) is usually caused by staphylococci, viridans-group streptococci, or enterococci. For late-onset PVE, AHA and Infectious Diseases Society of America guidelines suggest a combination of vancomycin and ceftriaxone.[154] In cases of suspected *Bartonella* PVE, empirical treatment with ceftriaxone, gentamicin, and doxycycline is recommended. Uncommon pathogens such as *Bartonella* spp., *Brucella* spp., *C. burnetii, Chlamydia* spp., *Legionella* spp., *T. whipplei,* NTM, and fungi should also be considered on the basis of clinical and epidemiologic clues (Table 81.5).[205,248,249] If there is no clinical improvement with an empirical regimen, surgical intervention for resection of the infected valve and obtaining samples for microbiologic and histopathologic evaluation should be considered.

SURGICAL INTERVENTION

Indications for Surgical Intervention

Current guidelines recommend surgical intervention in patients with hemodynamic instability, heart failure, and significant valve dysfunction or perivalvular complications.[154,250] Among those PVE patients with persistent bloodstream infection with antimicrobially resistant pathogens or those with a persistent large vegetation (>1 cm) after an embolic event, surgical intervention has been suggested as an option. Approximately 42% to 49% of patients with PVE undergo surgical interventions.[38,251] No randomized controlled trial has been conducted to evaluate the optimal management of PVE, so the recommended indications for surgical intervention in patients with PVE are based on data from observational studies, cohort studies, and expert opinion.[252] Some authorities recommend surgical intervention for all patients with mechanical PVE, and surgical intervention has been noted to be associated with higher survival rates than historical controls in some studies.[252–254] In an international prospective database of 1025 patients with PVE, 490 (47.8%) underwent surgical intervention during their index hospitalization, and early surgery was associated with lower in-hospital mortality in the unadjusted analysis and after controlling for selection bias.[255] However, analysis after adjustment for survivor bias did not show benefit with surgical intervention in in-hospital and 1-year mortality rates compared with rates in patients who were managed with medical therapy alone.[255] A systematic review and meta-analysis of 32 studies (retrospective or observational) analyzed 2636 patients with definite PVE to compare medical and surgical management.[256] The surgical intervention group included 1320 patients, was significantly younger, and had a higher number of men and patients with heart failure, prosthetic valve vegetation and perivalvular complications. The medical therapy group included 1316 patients and had a significantly higher number of patients with PVE due to *S. aureus.* Aortic PVE was the most common entity, and there was no significant difference between the two groups in incidence of early IE, fungal endocarditis, or embolic events. The mean time from diagnosis of PVE to surgical intervention was 16 days; follow-up data were available in 14 studies, with a mean duration of 22 months. In the pooled analysis, lower 30-day mortality (25% vs. 34%; relative risk [RR], 0.73) and higher survival at follow-up (69% vs. 58%; RR, 1.27) were noted in the surgical intervention group, mainly driven by data from the more recent studies (published after year 2000). There was no significant difference in the relapse rate of PVE between the two groups.[256] It should be noted that the surgical group had a higher prevalence of heart failure and paravalvular complications for which surgical intervention is recommended in the current guidelines. The results of the meta-analysis appear to support those

TABLE 81.5 Epidemiological Clues to Consider in Selecting Empirical Regimen for Culture-Negative Prosthetic Valve Endocarditis

EPIDEMIOLOGIC CLUE	ORGANISMS
Early-onset (≤1 year) PVE	Coagulase-negative staphylococci (CoNS), *Staphylococcus aureus,* aerobic gram-negative bacilli, fungi, *Corynebacterium* species, *Legionella* species
Late-onset (>1 year) PVE	CoNS, *S. aureus,* viridans-group streptococci, *Enterococcus* species, fungi, *Corynebacterium* species
Intravenous drug use	*S. aureus*, CoNS, β-hemolytic streptococci, fungi, gram-negative bacilli including *Pseudomonas aeruginosa*
Indwelling vascular device	*S. aureus*, CoNS, gram-negative bacilli, *Corynebacterium* species
Genitourinary disorders, infection, and manipulation	*Enterococcus* species, Group B streptococci, gram-negative bacilli, *Neisseria gonorrhea*
Chronic skin disorders	*S aureus*, β-hemolytic streptococci
Poor dental health, dental procedures	Viridans-group streptococci, nutritionally variant streptococci, *Abiotrophia defectiva Granulicatella* species, *Gemella* species, HACEK organisms
Alcoholism, cirrhosis	*Bartonella* species, *Aeromonas* species, *Listeria* species, *Streptococcus pneumoniae,* β-hemolytic streptococci
Diabetes mellitus	*S. aureus,* β-hemolytic streptococci, *S. pneumoniae*
Burn	*S. aureus,* gram-negative bacilli including *P. aeruginosa*, fungi
Dog or cat exposure	*Bartonella* species, *Pasteurella* species, *Capnocytophaga* species
Exposure to contaminated milk or infected farm animals	*Brucella* species, *Coxiella burnetii, Erysipelothrix* species
Homelessness, body lice	*Bartonella* species
AIDS	*Salmonella* species, *S. pneumoniae, S. aureus*
Pneumonia, meningitis	*S. pneumoniae*
Solid organ transplantation	*S. aureus, Aspergillus fumigatus, Enterococcus* species, *Candida* species
Gastrointestinal lesions	*Streptococcus gallolyticus (bovis), Enterococcus* species, *Clostridium septicum*

HACEK, Haemophilus parainfluenzae, Aggregatibacter aphrophilus, Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, and *Kingella* spp.
Modified from Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: Diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation. *2015;132:1435–1436.*

TABLE 81.6 Indications for Consideration of Surgical Intervention

- Severe heart failure; mild-moderate heart failure unresponsive to medical therapy
- Valvular dehiscence, obstruction, or leaflet perforation
- Perivalvular extension of infection leading to myocardial abscess, fistula, or shunt
- New severe conduction defects suggestive of perivalvular extension
- Multiple (>1) systemic emboli despite appropriate antimicrobial therapy
- Uncontrolled infection—persistent fever and positive blood cultures for >5 days
- Fungus, *Pseudomonas* spp., or other multidrug-resistant organisms

recommendations. It remains unclear whether patients with persistent bloodstream infection (>7 days) or a large vegetation (>1 cm) without other complications would benefit from early valve surgery. The decision for surgical intervention should be individualized for each patient after careful risk-benefit analysis (Table 81.6).

Severe Heart Failure Due to Prosthetic Valve Dysfunction

Severe heart failure can be due to prosthetic valve dehiscence, cusp leak, perivalvular leak, intracardiac shunt, or valvular stenosis or obstruction by large vegetations. TEE is useful to assess the etiology of heart failure. Heart failure is independently associated with higher mortality, and early surgical intervention improves in-hospital and 1-year survival rates.[257] Medical management may be attempted for minimal or mild heart failure symptoms due to ventricular dysfunction. Urgent surgical intervention should be strongly considered for patients with severe heart failure, valve dehiscence, and perivalvular leak or for those whose condition did not improve with medical management. Patients with moderate-to-severe heart failure due to valve dehiscence rarely survive for more than a few months without surgical intervention.[148,253,254] Surgical intervention should not be delayed to prolong the preoperative antimicrobial regimen in an attempt to reduce the rate of postoperative infection because this strategy increases operative mortality and also does not reduce the rate of postoperative infection.[258–260] Patients with moderate-to-severe heart failure due to prosthetic valvular obstruction, insufficiency, or valvular perforation should undergo urgent surgical intervention.

Perivalvular Extension of Infection

Perivalvular extension of the infection is common in early PVE, staphylococcal PVE, infection of the mechanical valves, and aortic prosthesis.[38,141,261] TEE is the echocardiographic modality of choice to evaluate for perivalvular complications such as perivalvular abscess, pseudoaneurysm, shunt, and fistulas. TTE does not adequately evaluate for perivalvular complications. ^{18}F-FDG-PET/CT shows promise in identification of perivalvular complications of PVE. Even if the initial TEE is negative for perivalvular invasive infection, patients should be monitored carefully during antimicrobial therapy for development of any new clinical signs suggestive of perivalvular complications. Persistent fever or bacteremia, or both, despite appropriate antimicrobial therapy, and new-onset conduction block are suggestive of perivalvular invasive infection, and TEE should be repeated promptly. Early surgical intervention is indicated in patients with perivalvular invasive infection because these patients are associated with higher mortality and rarely cured with antibiotics alone.[262–265]

High-Grade Pathogens

Patients with early-onset PVE caused by *S. aureus, Staphylococcus lugdunensis,* or CoNS have a higher prevalence of perivalvular complications, which are associated with a high mortality rate with medical therapy alone.[27,266–268] Hence, many authorities have recommended early surgical intervention in these patients.[269–271] PVE due to *S. aureus* is associated with poorer outcomes, and some experts have recommended early valve surgery in all patients, even those without perivalvular complications or heart failure due to structural valve damage.[252,270,272] However, this topic is still debatable, and one study concluded that early valve surgery is indicated only for those with perivalvular complications or heart failure.[266] Chirouze and colleagues analyzed 168 patients with definite PVE due to *S. aureus* in the prospectively collected international endocarditis database.[273] As previously well documented, *S. aureus* PVE was associated with higher 1-year mortality compared with PVE due to other organisms (48.2% vs, 32.9%; $P = .003$), and a higher frequency of stroke and prosthetic dehiscence. After adjusting for propensity factors, prognostic comorbidity metrics, and survival bias, there was no benefit in in-hospital or 1-year mortality with early valve surgery.[273] Limitations of study size and the observational nature of the study precluded any identification of a subgroup of *S. aureus* PVE patients who might benefit from early valve surgery. With the available data, it is reasonable to recommend that all patients with *S. aureus* PVE be evaluated carefully for valvular damage, perivalvular complications, and heart failure, and a decision for early valve surgery should be made by a multidisciplinary team after carefully weighing

the patient's individual characteristics. Early valve surgery may not be essential for all patients with PVE due to *S. aureus*; medical management alone may suffice for patients without heart failure or intracardiac complications. In a subgroup analysis of a meta-analysis that included five studies of patients with *S. aureus* PVE, surgical intervention was shown to be beneficial with lower 30-day mortality (31% vs. 48%; RR, 0.65) and higher survival at follow-up (78% vs. 29%; RR, 2.58).[256] If the patient is clinically stable and the initial TEE evaluation is negative for perivalvular complications, medical management can be attempted with careful clinical monitoring. Serial TEE and close follow-up after completion of antimicrobial therapy should be done to look for signs of relapse of infection and/or valve dehiscence. Patients with *Pseudomonas* PVE also require surgical intervention if medical therapy fails. Surgical intervention is indicated when an effective bactericidal antimicrobial regimen is unavailable for patients with PVE caused by multidrug-resistant gram-negative bacilli or vancomycin-resistant or aminoglycoside-resistant enterococci. Fungal PVE caused by *Candida* or *Aspergillus* spp. is rarely cured by antimicrobial treatment alone, and surgery is usually indicated.[70,274] Many experts recommend surgery for PVE caused by *Coxiella* and *Brucella* spp.

Late-onset PVE caused by community-acquired pathogens, including viridans-group streptococci, other streptococci, enterococci, CoNS, and HACEK group microorganisms, has a more indolent clinical presentation, usually without perivalvular extension, and can often be managed with antimicrobial therapy alone.[149,262,264] Surgery is also indicated in relapse of PVE, especially if caused by staphylococci or gram-negative bacilli.

Multiple Emboli

In patients with IE, larger vegetations (>10 mm) and those on the anterior mitral valve are noted to be associated with a higher frequency of cerebral embolism.[275,276] In one study, the risk of embolization was noted to rise with larger vegetation size, particularly in staphylococcal and mitral valve endocarditis.[277] Increase in size of vegetation during appropriate antibiotic therapy was associated with a higher risk of an embolic event.[277] In a contemporary, multinational, prospective registry, about 34% of patients with PVE developed stroke.[38] In NVE, the risk of embolization declines rapidly with antimicrobial therapy and reaches a low-risk state by 2 weeks.[276] A study concluded that early surgical intervention in patients with NVE and a large vegetation (>10 mm) is associated with a lower rate (3% vs. 28%) of composite end point of all-cause death, embolic events, or recurrence of IE at 6 months.[278] However, surgery to remove a large vegetation to prevent stroke is still controversial and not recommended when no other indication for surgery is present. Similar to NVE, recurrent emboli and persistent vegetation after embolism despite effective antimicrobial therapy are indications for surgery in PVE.[262,279]

Timing of Surgery

The optimal timing of surgical intervention is not clearly defined, and the question is not answered by the available data. The surgical mortality rate increases with the degree of heart failure, hemodynamic instability, and renal and other organ dysfunction.[258,279] If a patient has severe heart failure and hemodynamic instability owing to prosthetic valve dehiscence, perivalvular leak, valvular obstruction, or fistula, surgical intervention should be done promptly. Delaying surgery for additional days of antibiotics worsens the survival rate and does not reduce the postoperative reinfection rate of the new prosthetic valve. The rate of postoperative PVE is low, even if the blood cultures are positive on the day of surgical débridement and placement of the new prosthesis.[280] In patients who do not require urgent surgical intervention, efforts should be made to medically optimize their cardiopulmonary status before surgery. Surgery should be performed once comorbid conditions are stabilized. If the comorbid conditions do not improve promptly, surgery should not be delayed.

Timing of Surgery in Patients With Central Nervous System Embolism

Neurologic complications may worsen owing to systemic heparinization and hypotension associated with cardiopulmonary bypass.[281] Embolic stroke can turn into hemorrhagic stroke during cardiopulmonary bypass and heparinization, resulting in high morbidity and mortality. The frequency of neurologic complication is high (40%) if surgery is performed within 1 week after embolic stroke. The risk diminishes to 10% if surgery is performed 10 to 15 days after stroke.[253] Hence, surgery should be delayed for 3 to 4 weeks after a central nervous system (CNS) embolic event if the patient is hemodynamically stable. However, if the patient develops severe heart failure, hemodynamic instability, or multiple emboli, surgery should be performed without delay.

Surgical Treatment

The goals of surgical intervention are to eliminate intracardiac foci of infection with radical débridement[282–285] and to restore hemodynamic stability by placement of a new prosthesis. Extensive débridement and resection of all infected valvular and perivalvular tissues are important to reduce the risk of reinfection of the newly implanted prosthesis. Major reconstruction of aortic and/or mitral annuli and left ventricular outflow tract (LVOT) may be needed after extensive débridement. Biologic tissues such as autologous pericardium, glutaraldehyde-fixed bovine pericardium, and pulmonary or aortic autograft have been preferred for use in the reconstruction. Abscess cavities are closed with a pericardial patch or filled with gelatin-resorcin-formol or gentamicin-saturated fibrin glue.[286] In complicated aortic PVE with abscesses, reconstruction of the aortic root and LVOT is usually done with a cryopreserved aortic allograft.[263,287] It is useful in patients with complicated PVE requiring extensive débridement because the allograft can be molded to replace valve and débrided tissue, restore continuity, and produce excellent hemodynamic performance.[287] The risk of postoperative infection is also lower with an aortic allograft because it is a biologic material without a prosthetic sewing ring and thus likely more resistant to infection than a mechanical prosthesis or Dacron graft.[286] The main disadvantage of a cryopreserved aortic allograft is its time-limited durability. Some have reported development of significant aortic valve regurgitation more than 5 years after implantation. Stentless bioprostheses, such as the Toronto stentless porcine valve (St. Jude, Minneapolis, MN) and Freestyle valve (Medtronic, Minneapolis, MN), are increasingly used as substitutes for aortic allografts in aortic PVE with perivalvular abscesses, with good results. Mechanical and bioprosthetic valves have also been used in aortic PVE after extensive débridement. The rate of recurrent endocarditis was 2.5% with no statistical difference between mechanical and bioprosthetic valves in one survey.[288]

Both mechanical and bioprosthetic valves have been used as replacements in mitral PVE with no difference in recurrent infection or long-term event-free survival rates.[288,289] Mitral homografts do not have synthetic material and hence may be more resistant to postoperative infection in the setting of mitral PVE.[286]

Outcome of Patients Treated Surgically for Prosthetic Valve Endocarditis

The reported mortality rate for surgery in PVE is 4% to 30%.[284,285,290,291] Earlier surgical intervention in complicated PVE, facilitated by early identification of valve dehiscence and perivalvular abscess through use of TEE, improved surgical techniques, use of cryopreserved aortic grafts, more effective combination antimicrobial therapy, and advancements in postoperative critical care, has resulted in better PVE survival rates. The overall long-term (>10-year) survival rate has been reported to be 41% to 73%, which is significantly better than the expected 100% mortality rate in patients with complicated PVE treated medically.[284,286] The overall rate of recurrent PVE (relapse or reinfection) after surgery is 6% to 15%.[292] The cryopreserved aortic allografts have a lower incidence of early postoperative infection and reinfection compared with mechanical valves or xenografts.[263,293] About 18% to 26% of patients who undergo new valve implantation require reoperation because of either sterile prosthetic valve dysfunction or recurrent PVE.[290,292,294]

Duration of Antimicrobial Therapy Postoperatively

Dead microorganisms and evidence of active inflammation (presence of polymorphonuclear leukocytes [PMLs]) may persist for weeks to months after microbiologic cure.[295] Positive Gram stain or PCR results may be due to nonviable microorganisms. If the culture of the resected

valve or perivalvular tissue is positive, the consensus is that patients might benefit from a full course of appropriate antimicrobial therapy after surgery, discounting the preoperative antibiotic course. If the surgical cultures are negative, then the recommended duration of antimicrobial therapy may include the preoperative antibiotic course (counting from the day of the first negative blood culture). Lifelong oral suppressive antifungal therapy is recommended for patients with fungal PVE after a full course of IV antifungal therapy.[274]

ANTICOAGULATION THERAPY

Chronic anticoagulation is required for patients with mechanical prostheses in order to prevent thromboembolic events.[296] Anticoagulation may be continued with close clinical monitoring in the setting of mechanical PVE because there is substantial risk of thromboembolism if discontinued.[297] In a recent analysis of eight studies evaluating the role of anticoagulation in mechanical PVE, the aggregate risk of thromboembolism was noted to be significantly higher in those patients without anticoagulation (42% vs. 11%; $P < .0001$), suggesting that anticoagulation reduced the risk of thromboembolism without any significant increase in the risk of cerebral hemorrhage.[298] If a patient develops a CNS septic embolic event, anticoagulation should be reversed because there is increased risk of hemorrhagic transformation of the embolic infarct and subsequent extension of hemorrhage. Once the patient is clinically stable and there is no further extension of the cerebral hemorrhage, anticoagulation might be resumed cautiously with IV unfractionated heparin or an antiplatelet drug. Patients with a bioprosthetic valve do not require chronic anticoagulation therapy. In the setting of bioprosthetic PVE, anticoagulation does not have additional benefit in preventing septic emboli and it should not be initiated unless there are other independent medical indications for anticoagulation therapy.

In *S. aureus* left-sided PVE, anticoagulation therapy is associated with increased risk of death due to neurologic complications.[299] Many experts suggest discontinuation of all anticoagulation in those who experience a stroke until they have received at least 2 weeks of antibiotic therapy.[154]

Prior long-term administration of antiplatelet and/or statin drugs before the onset of IE may reduce symptomatic embolic events.[300–302] However, in a randomized, double-blinded, placebo-controlled trial, aspirin initiated after the diagnosis of IE did not reduce risk of embolic events and was likely associated with increased risk of bleeding.[303] Therefore, initiation of aspirin or statin in established IE to prevent thromboembolic events is currently not recommended.

PREVENTION

PVE is an uncommon but life-threatening infection with a high mortality rate despite appropriate medical and surgical interventions. It is essential to make every effort to prevent this devastating complication of cardiac valve replacement surgery. The prevention strategies should take into consideration the mode of acquisition of the infection and the likely pathogens involved.

Early-onset PVE is acquired likely as a result of perioperative contamination of the prosthetic valve or secondary seeding from a distant focus of infection such as an IV catheter or wound infection. The main tools to prevent early-onset PVE are perioperative antibiotic surgical site infection prophylaxis, infection-control measures, and improvements in surgical techniques. Perioperative antimicrobial prophylaxis should be administered intravenously within 1 hour before operation and repeated if the procedure is prolonged, in order to ensure maximal tissue drug levels during the entire procedure. Prophylaxis should be discontinued within 48 hours to reduce emergence of antimicrobial resistance and drug toxicity.[304–306] The standard perioperative prophylactic regimen is a first-generation cephalosporin (targeting *S. aureus* and CoNS), but it might be individualized on the basis of the local hospital susceptibility pattern and the patient's colonizing microbiota. The addition of vancomycin to cefazolin should be considered in certain situations: high prevalence of methicillin-resistant staphylococci in the hospital; patients with high risk for MRSA infection (elderly, diabetic); and patients with known MRSA colonization. It is also used in patients who cannot tolerate cephalosporins.[306,307] Some studies have suggested that preoperative screening for MRSA carriers and intranasal mupirocin may be effective in preventing postoperative MRSA surgical site infections.[308,309] The usefulness of preoperative chlorhexidine bathing is still unresolved.[310] Institutional infection-control practices should be updated regularly on the basis of new evidence and guidelines from national authorities. Early PVE usually starts in the sewing cuff–tissue interface, and impregnation of the prosthetic valve sewing cuffs with antimicrobial agents has been suggested as a preventive strategy.[311–314] In 1998 St. Jude Medical introduced prosthetic valves with silver-impregnated sewing cuffs (Silzone), designed to inhibit microbial attachment and colonization. But the product was withdrawn from the market when a significantly higher incidence of paravalvular leakage was noted in a large multicenter prospective randomized trial.[315] Increased risk of embolism during the early period was also observed in patients with Silzone valves in the mitral position.[316] It has been postulated that silver coating inhibited fibroblast growth, endothelialization of the prosthetic sewing ring, and healing, resulting in annular tissue necrosis, dehiscence, and paravalvular leakage.[316]

Late-onset PVE acquired in the community is usually caused by endogenous microbiota such as viridans-group streptococci and enterococci. Patients with prosthetic valves are considered to be a high-risk group.[317] Although there has been no randomized, placebo-controlled trial to define the efficacy and safety of antibiotic prophylaxis, there are recommendations for using it for dental procedures that involve manipulation of the gingival or periapical region of teeth or perforation of oral mucosa and during procedures on the respiratory tract, infected skin or skin structures, and musculoskeletal tissue.[317] Patients should undergo dental evaluation before valve surgery and be educated that good oral hygiene is important to prevent late-onset community-acquired PVE caused by oral pathogens. The proportion of late-onset PVE attributed to health care exposure is increasing; therefore, strict infection-control practices and careful evaluation of the need for central venous catheter use or continuation are important in its prevention.

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The complete reference list is available online at Expert Consult.

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82 Infections of Nonvalvular Cardiovascular Devices

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SHORT VIEW SUMMARY

CARDIOVASCULAR IMPLANTABLE ELECTRONIC DEVICE (CIED) INFECTIONS

- The infection rate of CIED infection is rising disproportionate to the rate of implantation.
- Most early-onset (within 6 months of implantation) CIED pocket infections are caused by device contamination at the time of implantation, whereas hematogenous seeding is a frequent source of late-onset (after 6 months of implantation) CIED lead infections.
- Hematogenous seeding of CIED leads is rare with gram-negative bacteremia.
- Blood cultures should be obtained in all patients at initial presentation. If blood cultures are positive, then transesophageal echocardiography should be obtained to evaluate for lead infection or valvular endocarditis.
- Complete removal of the CIED system is necessary for eradication of device infection.
- A replacement device can be implanted once blood cultures are negative for 72 hours and perhaps earlier in cases in which admission blood cultures are negative. A 2-week delay in implantation is recommended for patients with valvular endocarditis.
- All patients should receive perioperative antibiotics for CIED placement/revision as primary prevention of CIED infection.

LEFT VENTRICULAR ASSIST DEVICE (LVAD) INFECTIONS

- Besides being a bridge to transplantation, LVADs are increasingly being used as destination therapy for patients with end-stage heart failure who are not transplantation candidates.
- Driveline exit site infections are the most common type of LVAD-specific infections, followed by LVAD-related bloodstream infection and endocarditis.
- *Staphylococcus aureus* and coagulase-negative staphylococci are responsible for the majority of LVAD-specific infections.
- Most LVAD infections are managed with antimicrobial therapy alone because device removal is not an option in most cases.
- Suppressive antibiotics are used lifelong or until the infected LVAD is removed.
- Immobilizing the driveline at the skin exit site to prevent repeated skin trauma reduces the risk of local device infections.

PROSTHETIC VASCULAR GRAFT INFECTIONS (PVGIs)

- Staphylococcal species are the predominant pathogens in PVGIs.
- Computed tomographic (CT) scanning is the best tool in the diagnostic evaluation of graft infection. CT findings of perigraft fluid accumulation, fat stranding or gas bubbles, lack of a fat plane between graft and bowel, and anastomotic aneurysms are all suggestive of graft infection.
- Surgical management of an infected graft includes complete excision of infected graft material, débridement of all infected and devitalized tissues, and revascularization of distal tissues.
- If complete excision of an infected graft is not feasible, patients should be prescribed lifelong oral suppressive antibiotics after an initial 4-week course of parenteral antimicrobial therapy.

The rapid evolution of technology, coupled with an aging population with multiple comorbid conditions, has led to the development of several new implantable devices that help to improve or sustain life. However, despite improvements in device manufacturing and availability of experienced operators implanting these devices, infection has remained a major complication of implantable cardiovascular devices. Risk of infection varies greatly (Table 82.1) and depends on the type and size of device, site of implantation, surgical technique used for device implantation, and host factors. Infections that complicate nonvalvular cardiovascular devices are addressed in this chapter. Intravascular catheter-related infections are reviewed elsewhere (see Chapter 300).

CARDIOVASCULAR IMPLANTABLE ELECTRONIC DEVICES

Permanent pacemaker (PPM), implantable cardioverter-defibrillator (ICD), and cardiac resynchronization therapy (CRT) devices are the three most commonly used cardiovascular implantable electronic devices (CIEDs). Sternotomy or thoracotomy was required for epicardial lead placement for the first generation of CIEDs. In contrast, most devices today are implanted percutaneously using transvenous leads. This change has led to a marked reduction in implantation-related morbidity and avoidance of potentially life-threatening infectious complications of major cardiothoracic surgical interventions. However, infection remains a major complication of CIED implantation and is associated with significant morbidity, mortality, and financial cost.[1,2,3]

Epidemiology of Cardiovascular Implantable Electronic Device Infections

Reported rates of CIED infection have ranged from 0.13% to 19.9%.[4,5] Over the past 2 decades, the rate of CIED implantation has rapidly increased in the United States. In a survey of Nationwide Inpatient Sample (NIS) discharge records from 1993–2008,[3] the incidence of CIED implantation increased an average of 4.7% annually during the study period. Although the rate of CIED infection remained stable until 2004, it increased significantly from 1.53% in 2004 to 2.41% in 2008. This increase in the rate of device infections coincided with an increase in the number of comorbidities in device recipients. Moreover, this infection rate resulted in significant increases in in-hospital mortality and cost of care.[6] In a separate analysis of a Medicare population,[2] patients with CIED infection remained at higher risk of death, even after hospital discharge, compared with device recipients without infection.

Pathogenesis of Cardiovascular Implantable Electronic Device Infections

Microbial contamination of a device generator or leads with skin microbiota at the time of implantation is the most likely mechanism for most early-onset (within 6 months of device implantation) CIED infections.[7] This likely explains the preponderance of staphylococci as causative pathogens. Colonization and subsequent infection of CIED is facilitated by an ability of these organisms to adhere to device surfaces and produce biofilm.[8–10] This interferes with the host immune system's

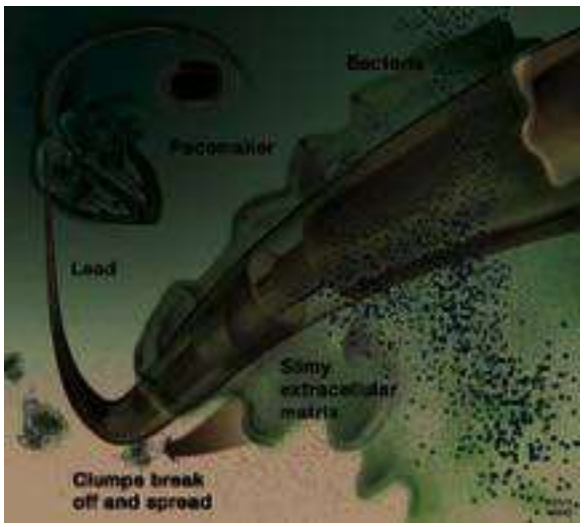

FIG. 82.1 Microbial biofilm formation on pacemaker lead. Biofilm forms on implants when bacteria stick to their surfaces. They multiply into colonies and form a protective slime layer that allows them to move and collect nutrients while staying safe from antimicrobial agents and host immune system. *(Copyright Mayo Clinic.)*

TABLE 82.2 Factors Associated With Increased Risk of Cardiovascular Implantable Electronic Device (CIED) Infections

Host Factors
Older age
Comorbid conditions (diabetes mellitus, heart failure, renal failure, malignancy)
Presence of a tunneled central venous catheter (such as hemodialysis catheter)
Chronic anticoagulation
Long-term corticosteroid therapy
Bloodstream infection (especially *Staphylococcus aureus*) from a distant focus
Device-Related Factors
History of multiple device-related procedures
History of CIED infection
Presence of >2 electrode leads
Epicardial leads
Abandoned leads
Recent device manipulation (i.e., generator exchange or lead revision)
Procedure-Related Factors
Fever within 24 hours of implantation
Temporary pacing before permanent device placement
No antibiotic prophylaxis before device implantation
Operator inexperience
Postoperative hematoma or other complications at generator pocket

TABLE 82.1 Nonvalvular Cardiovascular Device–Related Infections

TYPE OF DEVICE	RATE OF INFECTION (%)
Intracardiac	
Permanent pacemaker	0.13–19.9
Implantable cardioverter-defibrillator	0.3–3.2
Left ventricular assist devices	13–80
Ventriculoatrial shunts	2.4–9.4
Pledgets (cardiac suture line)	Rare
Patent ductus arteriosus occlusion devices	Rare
Atrial septal defect closure devices	Rare
Conduits	Rare
Patches	Rare
Arterial	
Peripheral vascular stents	0.05–4.0
Vascular grafts, including hemodialysis	1–6
Intraaortic balloon pumps	≤5–27
Angioplasty/angiography-related bacteremias	<1
Vascular closure devices	0.0–5.1
Coronary artery stents	Rare
Patches	1.8
Venous	
Vena caval filters	Rare

ability to eliminate infection due to neutrophil dysfunction, poor penetration of antibiotics into the biofilm, and downregulation of metabolic activity in infecting organisms that makes them less susceptible to some antimicrobials, necessitating device removal for the best chance of infection cure (Fig. 82.1). Once a generator or pocket is colonized, bacteria can migrate along the electrode leads and manifest as tunnel infection, bacteremia, or infected vegetations on electrode leads or cardiac valves. However, hematogenous seeding of CIED leads is the predominant mechanism for the majority of late-onset (after 6 months of implantation) device infections, especially when there is no clinical evidence of pocket infection. Up to a third of patients with bloodstream infection (BSI) due to gram-positive cocci, especially *Staphylococcus aureus,* in the setting of CIED may have an underlying device infection.[11,12,13,14] Hematogenous seeding of device leads, however, is exceedingly rare in patients with gram-negative bacteremia that originates from a distant source.[15]

Risk Factors for Cardiovascular Implantable Electronic Device Infections

Several host- and procedure-related factors predispose to an increased risk of CIED infection (Table 82.2).[16–23] These factors predispose to device infection by increasing the risk of generator or lead contamination at the time of implantation and/or delay in wound healing at a generator pocket site or increased risk of BSI from a distant source with hematogenous seeding of device leads.

In a case-control study,[16] presence of a permanent central venous catheter, prolonged corticosteroid use, a history of CIED infection, presence of more than two electrode leads, and a history of multiple device-related procedures were associated with increased risk of pacemaker infection. Other factors associated with higher risk of CIED infection include operator inexperience,[19] use of epicardial leads, postoperative complications at the generator pocket (delayed wound healing, hematoma formation), diabetes mellitus, renal failure, and heart failure.[17,21]

Risk factors associated with CIED infection were evaluated in another multicenter, prospective investigation[18] that included 6319 CIED recipients (5866 PPMs and 453 ICDs). Risk of device infection was significantly higher in patients who had fever within 24 hours of CIED implantation (adjusted odds ratio [aOR], 5.83), presence of a temporary pacing wire before device implantation (aOR, 2.46), and those who underwent an early reintervention (aOR, 15.04) for hematoma evacuation or lead revision. De novo device implantation (aOR, 0.46) and antibiotic prophylaxis (aOR, 0.4) both had protective effects.

Microbiology of Cardiovascular Implantable Electronic Device Infection

Staphylococci are the predominant pathogens in CIED infections.[24,25] In one study from Mayo Clinic,[4] coagulase-negative staphylococci (CoNS) (42%), followed by *S. aureus* (29%), were responsible for more than two-thirds of cases of device infection (Fig. 82.2). Early device infections (within 2 weeks of implantation) are primarily caused by *S. aureus.* Prevalence of methicillin-resistant *S. aureus* (MRSA) is variable depending on the geographic location of reporting institutions. In the previously

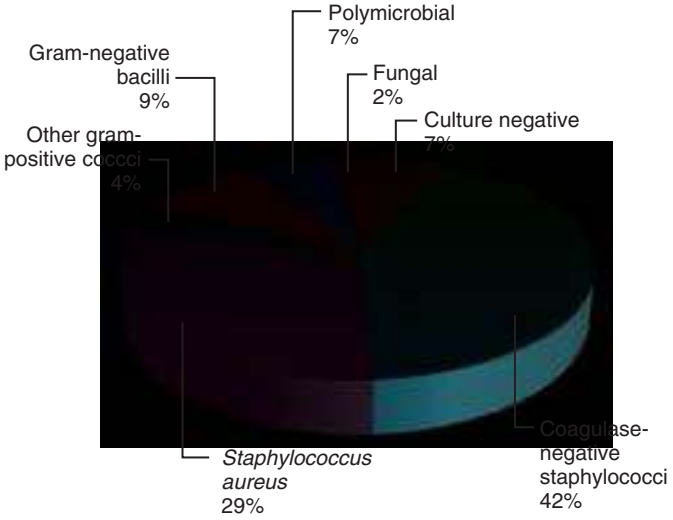

FIG. 82.2 Microbiology of cardiovascular implantable electronic device infections. *(Modified from Sohail MR, Uslan DZ, Khan AH, et al. Management and outcome of permanent pacemaker and implantable cardioverter defibrillator infections.* J Am Coll Cardiol. *2007;49:1851–1859.)*

referenced study[4] gram-negative bacteria; other gram-positive cocci, including enterococci, streptococci, and micrococci; and fungi (*Candida* spp. and *Aspergillus fumigatus*) were isolated in 9%, 4%, and 2% of cases, respectively. Polymicrobial infection may be present in up to 7% of cases and tends to be more common in patients with diabetes mellitus and those receiving corticosteroids.[26] Pocket site and blood cultures may be negative in patients who have previously received antibiotics or have low-virulence organisms associated with biofilm formation. Sonication techniques to disrupt biofilms can be a useful tool to increase culture yields in specimens suspected of harboring CIED infection.[27] In one study at the Mayo Clinic substantial bacterial growth was observed in 54% of sonicate fluids, significantly greater than the sensitivities of pocket swab (20%), device swab (9%), or tissue (9%) cultures.[28]

Clinical Manifestations of Cardiovascular Implantable Electronic Device Infection

CIED infection typically manifests in three distinct ways.[4] The most common presentation is pocket site infection (>60%). Patients generally present with localized inflammatory changes at the generator pocket site, including erythema, pain, swelling, warmth, drainage, or dehiscence of overlying skin. Systemic signs of sepsis or positive blood cultures are present in less than one-half of these cases. The second presentation is occult bacteremia or fungemia and no local changes at the pocket site. The third presentation is CIED-related endocarditis (lead or valvular vegetations, or both) in 10% to 23% of cases.[29,30] When vegetations are visualized on cardiac structures, the tricuspid valve is the most common site of infection (up to 25% of cases with CIED-related endocarditis).[30] However, vegetations can develop on the pulmonic or left-sided valves, especially in the setting of *S. aureus* bacteremia (SAB). Patients with SAB may have radiographic findings of multiple focal pulmonary infiltrates, abscesses, or cavitations due to septic emboli in as many as 40% of the cases.[31,32] Patients with CIED-related endocarditis have a higher mortality (14%–25%) compared with those without it (<5%), even with percutaneous lead extraction.[30,33] A small proportion (<5%) of the patients can present with erosion of a device lead or generator in the absence of inflammatory signs at the pocket and negative blood cultures.[4] Whether erosion is caused by low-grade infection or purely by mechanical factors may not be entirely clear in all patients.

Diagnosis of Cardiovascular Implantable Electronic Device (CIED) Infection

A diagnosis of CIED infection is easily discernible when pocket site inflammatory changes are present or when device erosion is evident. However, a diagnosis may be difficult to confirm in cases in which positive blood cultures are the sole manifestation of underlying CIED infection. It is critical that blood cultures be obtained in all patients with suspected CIED infection before starting any empirical antibiotics. If drainage is present, swab specimens should be submitted for cultures. Abnormal laboratory findings (leukocytosis, anemia, high erythrocyte sedimentation rate, or C-reactive protein) are present in 50% or less of cases, and the absence of these findings should not dissuade clinicians from considering a possibility of CIED infection in the appropriate setting.

Positive blood cultures, even in the absence of other manifestations of device infection, should prompt a serious consideration for underlying CIED infection. In patients with SAB, concomitant CIED infection is present in 30% to 50% of the patients.[14,34] Risk is especially high within the first year after device implantation. Of note, local signs or symptoms of pocket or tunnel infection may be absent in half of these cases. Patients with BSI due to CoNS or enterococci are also at significant risk of hematogenous seeding of CIED leads.[35] Therefore we recommend thorough evaluation for underlying CIED lead infection in these patients with transesophageal echocardiography (TEE) (Fig. 82.3). Although the presence of clots on CIED leads is considered evidence of lead infection, a major limitation of TEE is that bland (noninfected) clots on leads occur, and TEE is unable to distinguish bland clots from infected lead vegetations. In patients with no evidence of CIED infection (after a thorough evaluation that includes TEE and possibly positron emission tomography [PET]/computed tomography [CT]) at the time of gram-positive bacteremia, and for whom a decision is made to treat conservatively, there should be close follow-up over the next 12 weeks to monitor for relapsing bacteremia. Surveillance blood cultures after completing 2 to 4 weeks of appropriate parenteral antibiotics should be considered. Overall, risk of hematogenous seeding of CIED leads with gram-negative bacilli is low.[36] However, in a recent investigation[37] from Duke University that included patients with CIEDs, prosthetic heart valves, and left ventricular assist devices (LVADs), patients with BSI due to *Pseudomonas aeruginosa* or *Serratia marcescens* had higher risk of hematogenous seeding of cardiac devices compared with bacteremia due to other species of gram-negative bacilli.

Indium-labeled leukocyte or gallium scanning may be helpful in differentiating an infected CIED pocket from a noninfected pocket site. In one study sensitivity of technetium 99–labeled white blood cell (WBC) single-photon emission computed tomography/CT (SPECT-CT) was 94% for both detection and localization of CIED-associated infection. ^{99}Tc-labeled WBC scintigraphy not only enabled the confirmation of CIED infection but was also helpful in defining of the extent of device involvement and detection of associated complications. Moreover, ^{99}Tc-labeled WBC scintigraphy[38] reliably excluded device-associated infection during a febrile episode and sepsis, with 95% negative predictive value.[39]

PET scan combined with CT (PET-CT) is an emerging modality for confirmation of CIED infection in select cases. In one study[40] 32 of 42 patients with suspected CIED infection had a positive PET-CT. Twenty-four of these patients with positive findings on PET-CT underwent extraction with an excellent correlation with CIED infection. Ten patients, who had negative PET-CT imaging and were managed conservatively with antibiotics alone and without device removal, had no signs of infection relapse after an average follow-up duration of 13 months. In a recent meta-analysis[41] of 14 studies involving 492 patients, the pooled sensitivity of PET-CT for diagnosis of CIED infection was 83%, and the pooled specificity was 89%. PET-CT demonstrated a higher sensitivity of 96% and specificity of 97% for diagnosis of generator pocket infections. Diagnostic accuracy for lead infections or CIED-IE was lower with pooled sensitivity of 76% and specificity of 83%. Based on these data, PET-CT is a good adjunctive tool for the diagnosis of CIED infection in cases where clinical findings at the generator pocket site are not compelling, and TEE images in cases of suspected lead infection are inconclusive (Fig. 82.4). In addition to diagnosis of CIED infection, PET-CT can identify distant foci of infection or malignancy that may be clinically silent.

Lead tip cultures are frequently used to confirm the diagnosis of CIED-related infective endocarditis.[31,32,42] However, most transvenous leads are extracted percutaneously in current practice, and lead tips may be contaminated during removal through the incision of an infected

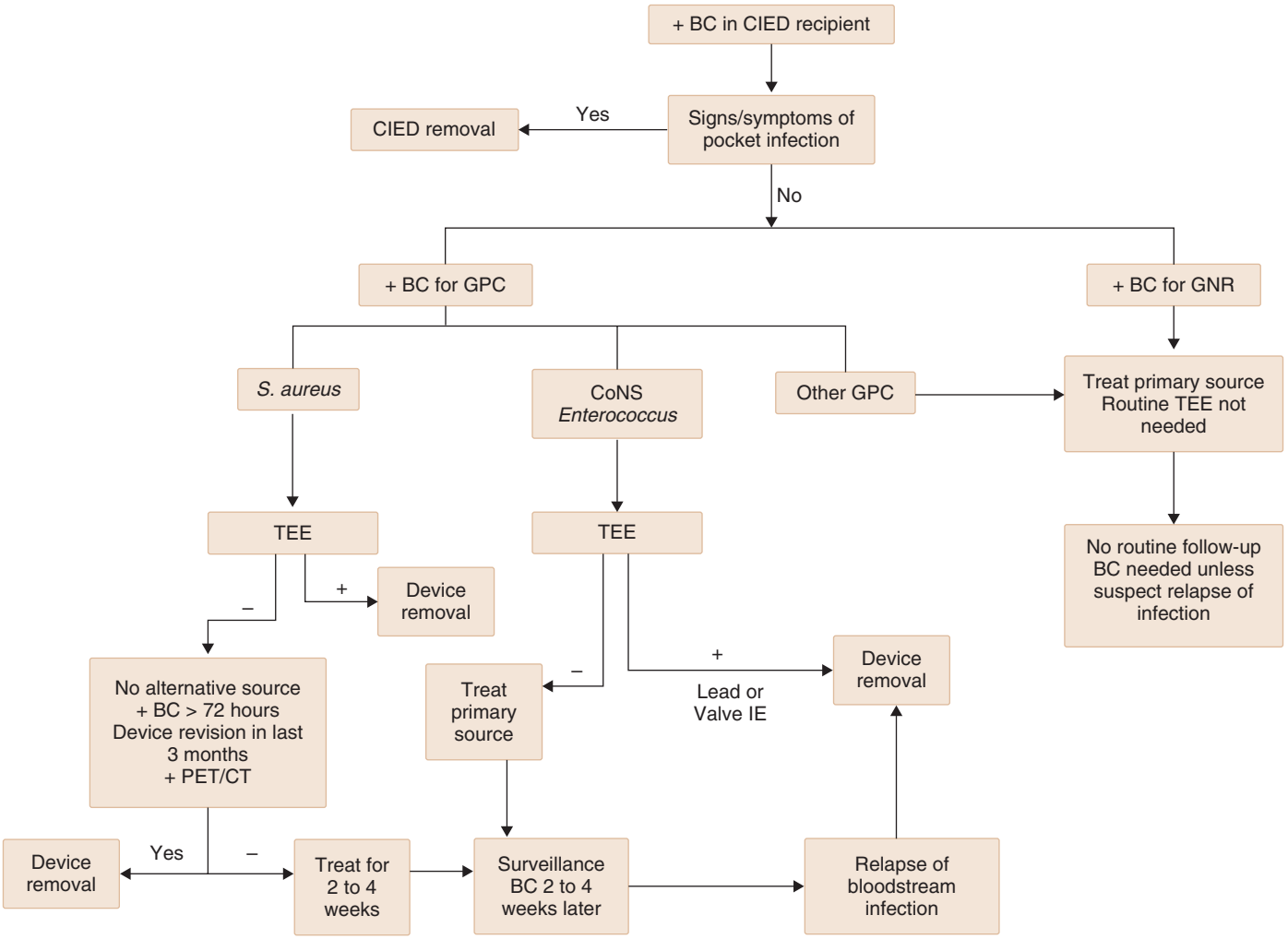

FIG. 82.3 Management of bacteremia in patients with a cardiovascular implantable electronic devices. *BC,* Blood culture; *CIED,* cardiovascular implantable electronic device; *CoNS,* coagulase-negative staphylococci; *CT,* computed tomography; *GNR,* gram-negative rods; *GPC,* gram-positive cocci; *IE,* infective endocarditis; *PET,* positron emission tomography; *TEE,* transesophageal echocardiogram. *(Modified from DeSimone DC, Sohail MR. Management of bacteremia in patients living with cardiovascular implantable electronic devices.* Heart Rhythm. *2016;13:2247–2252.)*

pocket.[30] Lead tip cultures are more reliable if they are extracted using a sterile technique (via a protected sleeve) or if removed from a different incision than that used at the generator pocket site.

Role of Echocardiography in Diagnosis of Cardiovascular Implantable Electronic Device Infection

In addition to identifying possible vegetations on CIED leads (with limitations as discussed earlier), echocardiography is critical in the diagnosis of other complications associated with CIED infection, such as valvular vegetations, perivalvular extension of infection, and abscess formation. The diagnostic superiority of TEE over transthoracic echocardiography (TTE) has been substantiated in multiple studies, with the reported sensitivity of TEE at >95% compared with <50% for TTE.[10,30,43,44] TEE is also helpful in assisting in decision making regarding the most appropriate extraction strategy by providing information regarding vegetation size and attachment of device leads to surrounding venous or cardiac structures.

A limitation, however, is that echocardiography cannot distinguish noninfected lead clots, which can be seen in 1.5% to 48% of noninfected patients, from infected vegetations on leads, depending on the type of tool used to identify the clots. In practice, patients who present with BSI and have a mass attached to a device lead on TEE are presumed to have CIED infection. PET-CT may be marginally helpful in these cases to confirm or exclude a "true" lead infection.

Complications of Cardiovascular Implantable Electronic Device Infection

Complications of CIED infection may include valvular endocarditis (mostly right sided), septic arthritis, spine infection (diskitis, vertebral osteomyelitis, or epidural abscess),[45] sternal wound infection, metastatic abscesses (lung, liver, spleen, brain, renal), and thrombosis of a subclavian vein or superior vena cava. In patients with metastatic abscesses or osteomyelitis it may be difficult to decipher whether an ectopic site is the source of bacteremia with hematogenous seeding of a cardiac device or vice versa.

Management of Cardiovascular Implantable Electronic Device Infection

The primary focus of treatment should be complete removal of the CIED system. Although no prospective, randomized trials have been conducted to evaluate the role of medical (antimicrobial) therapy alone versus a combined medical-surgical treatment approach, data from several retrospective analyses show a clear advantage of complete device removal.[4,26,46,47] For example, the reported mortality rate of CIED-related endocarditis ranges from 31% to 66% if the infected device is retained, compared with 18% or less in patients managed with a combined approach of complete device removal and parenteral antibiotics.[30,31,32] Patients with partial device removal (generator only) or those treated conservatively with antibiotics alone have a higher risk of treatment failure or relapse.[4,26,48] In one investigation of 416 patients with CIED

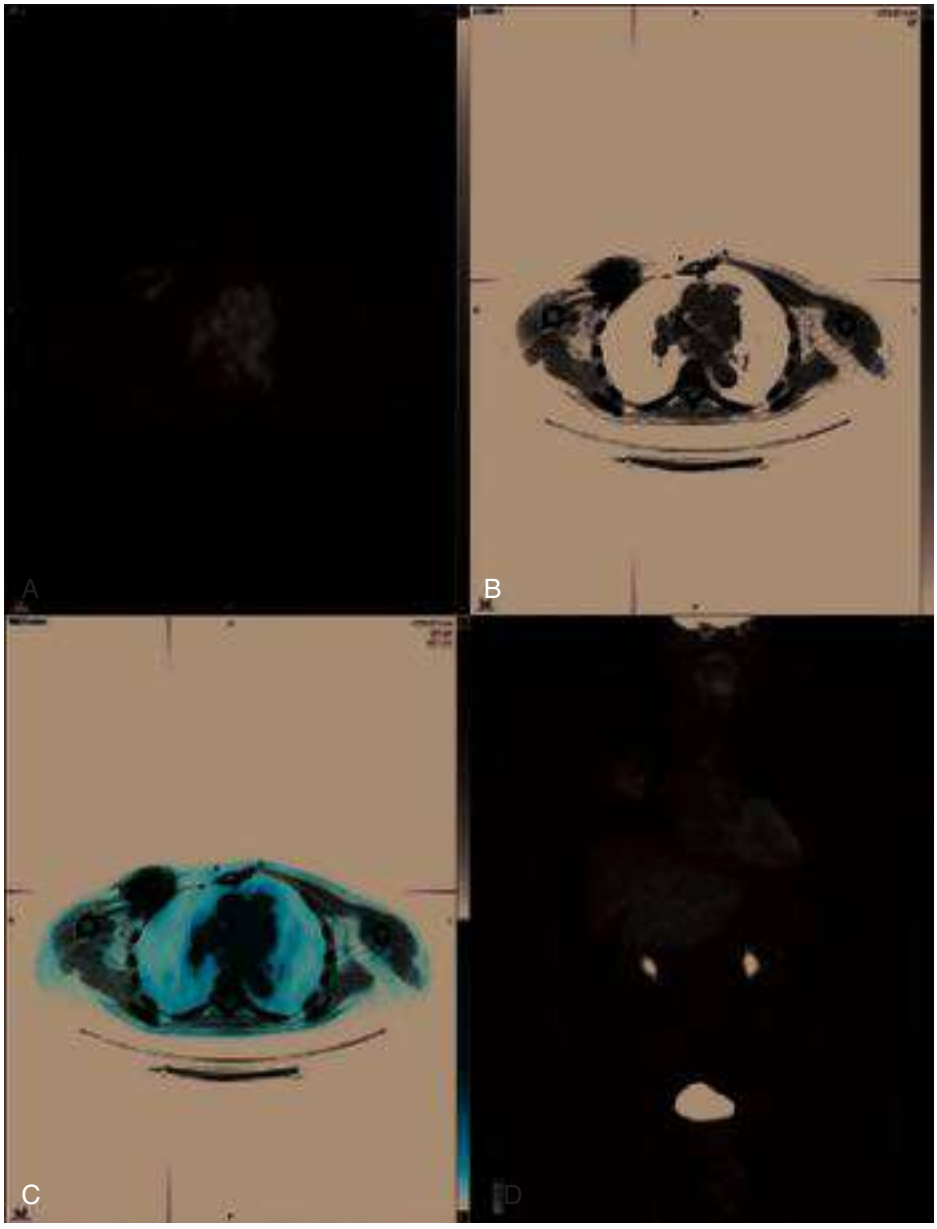

FIG. 82.4 Increased fluorodeoxyglucose activity at cardiovascular implantable electronic device generator pocket consistent with device infection. (A) Axial positron emission tomography. (B) Axial computed tomography. (C) Maximum intensity projection image. (D) Fused images. *(Copyright Mayo Clinic.)*

infection, antimicrobial therapy alone without device removal was associated with a sevenfold increase in 30-day mortality.[49] Moreover, immediate device removal, when compared with delayed extraction or no device removal, was associated with a threefold decrease in 1-year mortality in this analysis.

When planning device removal, three factors must be addressed. First, removal of a lead that is embedded in cardiac tissue can be difficult and potentially dangerous. Complications include tamponade due to tearing or perforation of the myocardial wall, laceration of the superior vena cava or tricuspid valve, hemothorax, fracture of lead fragment requiring surgical intervention, and life-threatening arrhythmias.[4] However, newer techniques, including the use of a locking stylet, photoablation of fibrous attachments with a laser sheath, and minimally invasive video-assisted thoracoscopic techniques, are less invasive and safer methods of device removal compared with open thoracotomy.[4,10]

Second, in cases where larger (>10 mm in diameter) vegetations are attached to infected leads, embolic showering of the pulmonary vasculature with percutaneous lead extraction is a concern. However, data from several studies[4,30,32,43,50] indicate that risk of clinically significant pulmonary emboli and death with percutaneous extraction is low and does not warrant routine surgical removal of leads via thoracotomy in this patient population. Routine screening for subclinical pulmonary emboli using CT or ventilation-perfusion scan is also not recommended.[30] Risks of thoracotomy and need for cardiac bypass (in mostly an older patient population) should be considered before deciding whether a percutaneous or a surgical approach should be used.

Third, the need for ongoing CIED support should be assessed before implanting a new device. The percentage of patients who do not require replacement of a PPM has ranged from 13% to 52%.[4,26,31,46] In patients who are dependent on a device, a temporary system can be placed, whereas BSI is cleared by antimicrobial therapy before implantation of a new device. However, use of temporary wires may increase the risk of new device infection.[18]

Figs. 82.5 and 82.6 summarize the management approach of CIED infection.[51,52] We recommend a two-step exchange in most patients (i.e., complete device removal, followed by replacement CIED, if needed, in a subsequent intervention). Clearance of BSI, if present, and adequate control of infection at the generator pocket site should be achieved

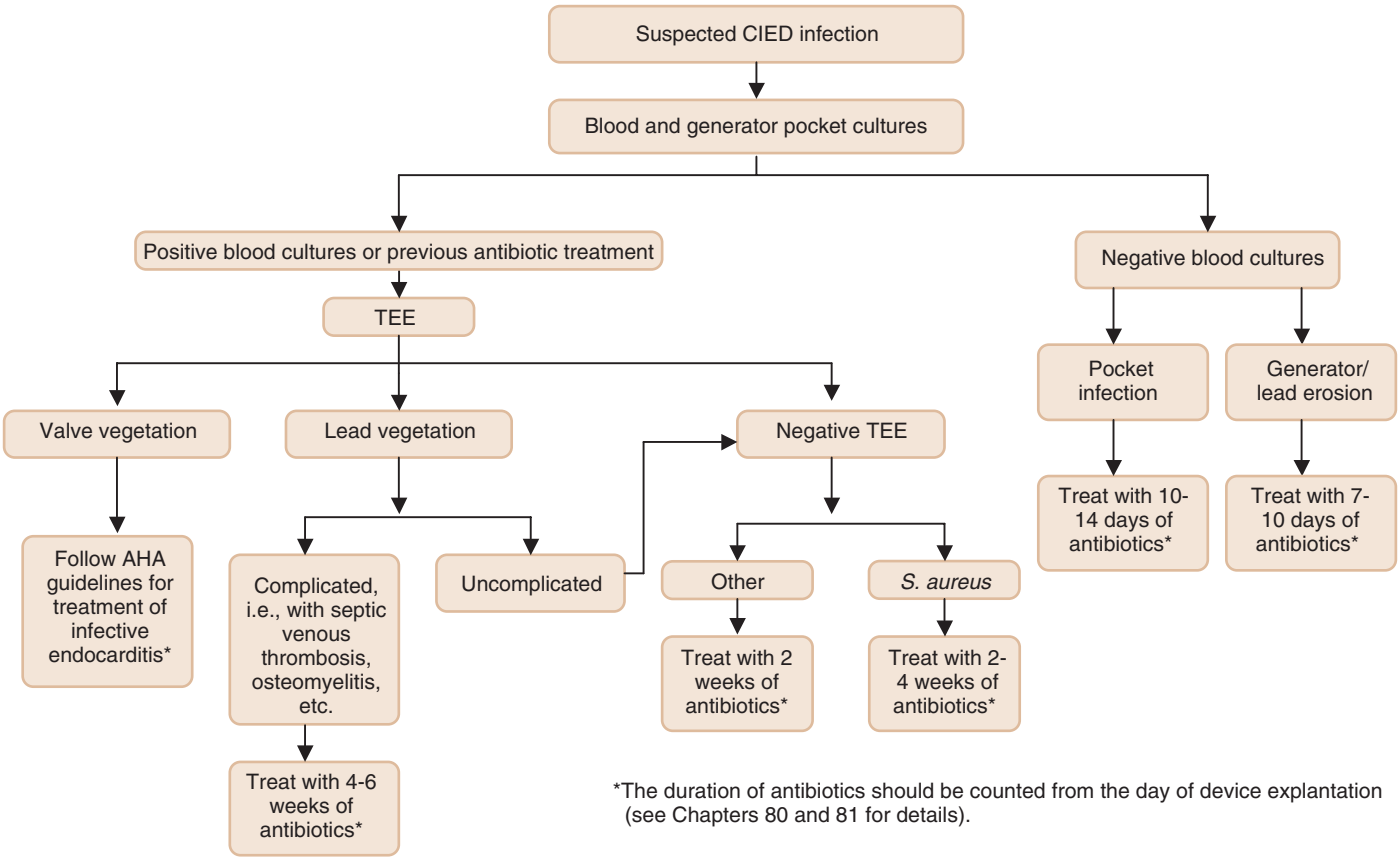

FIG. 82.5 Approach to the management of adults with cardiovascular implantable electronic device *(CIED)* infection. This algorithm applies only to the patients who are managed with complete device removal. *AHA,* American Heart Association; *S. aureus, Staphylococcus aureus; TEE,* transesophageal echocardiogram. *(Modified from Sohail MR, Uslan DZ, Khan AH, et al. Management and outcome of permanent pacemaker and implantable cardioverter defibrillator infections.* J Am Coll Cardiol. *2007;49:1851–1859.)*

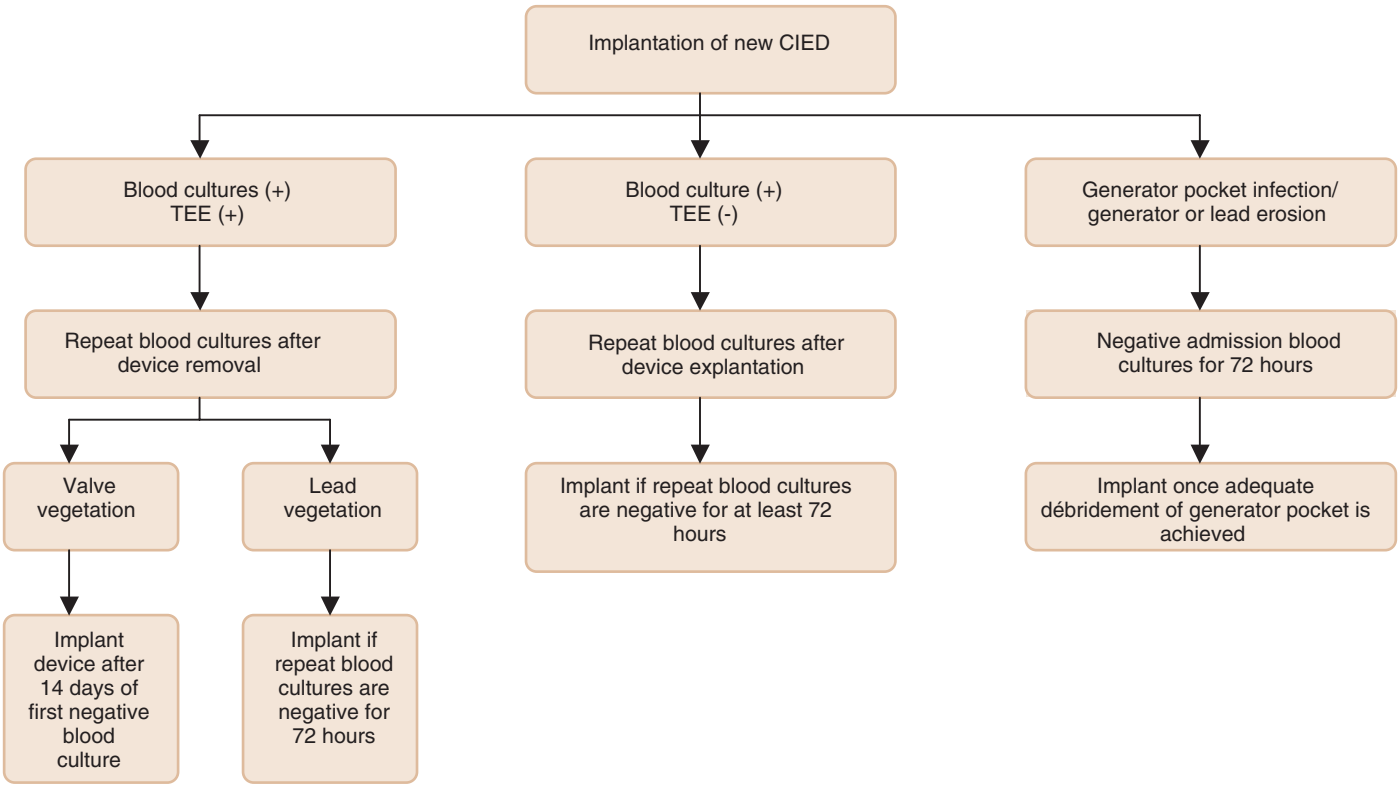

FIG. 82.6 Guidelines for implantation of a new device in patients with cardiovascular implantable electronic device *(CIED)* infection. *TEE,* Transesophageal echocardiogram. *(Modified from Sohail MR, Uslan DZ, Khan AH, et al. Management and outcome of permanent pacemaker and implantable cardioverter defibrillator infections.* J Am Coll Cardiol. *2007;49:1851–1859.)*

before placement of a new device (see Fig. 82.6). For patients who have BSI at initial presentation, blood cultures should be repeated after device explantation. Patients who have persistently positive blood cultures after device removal should be treated for at least 4 weeks with antibiotics even if a TEE is negative for valve vegetations. For patients who present with generator or lead erosion only, in the absence of clinical evidence of pocket infection, the replacement CIED may be placed immediately after removal of the original device (one-step exchange). The new device should be placed on the contralateral side in patients who have clinical or intraoperative findings consistent with generator pocket infection. Cure rates of more than 95% can be achieved.[4]

The choice and duration of antimicrobial therapy are dictated by pathogen, in vitro antimicrobial susceptibility results, and the presence or absence of concomitant valvular endocarditis. Empirical antibiotics should include coverage for MRSA and CoNS. Intravenous administration of antimicrobials is recommended. If an infected CIED cannot be removed, long-term oral suppressive antibiotics[53,54] (for the life of the CIED) should be administered after an appropriate course of initial induction therapy (see Fig. 82.2).

Prevention

Meticulous attention to aseptic technique is key to preventing microbiologic contamination of CIED generators and leads at the time of implantation. Several clinical studies have demonstrated the efficacy of antibiotic prophylaxis before device implantation.[16,18,55] In a recent large, prospective, randomized, double-blinded, placebo-controlled clinical trial,[56] patients undergoing CIED implantation or generator replacement were randomized to either receive a single dose of cefazolin or placebo for perioperative prophylaxis. In multivariable analysis a lack of antibiotic prophylaxis was an independent predictor of CIED infection ($P = .037$). For patients who are allergic to β-lactam antibiotics or are colonized with MRSA, vancomycin is an acceptable alternative. There are no published data to support the routine use of secondary prophylaxis for patients undergoing dental, respiratory, gastrointestinal (GI), or genitourinary procedures, and the practice is discouraged.[10]

Two recent large clinical trials (PADIT and WRAP-IT) assessed the role of additional interventions to prevent CIED infection compared to current standard of care. In the PADIT trial,[57] incremental approach included preoperative intravenous antibiotics (cefazolin plus vancomycin), intraoperative antimicrobial wash (bacitracin), and postoperative oral antibiotic (cephalexin or cefadroxil) therapy in patients deemed high-risk for CIED infection. However, these additional interventions did not result in a lower rate of CIED infection compared to a single dose of intravenous cefazolin administered preoperatively. WRAP-IT[58] was a randomized clinical trial that compared the use of an antibiotic-impregnated (minocycline and rifampin) envelope to standard of care in CIED recipients deemed at high risk of infection. Use of envelope was associated with 40% relative risk reduction in major CIED infections.

LEFT VENTRICULAR ASSIST DEVICES

Historically, LVADs were used to provide temporary support for patients with end-stage heart failure while awaiting heart transplantation (bridge to transplantation). However, with an increasing prevalence of advanced heart failure and a limited supply of donor organs, LVADs are increasingly being used as long-term myocardial surrogate therapy ("destination therapy"), especially in patients who are ineligible for a heart transplant.[59] With this changing trend, interest is shifting from short-term complications to long-term infection rates and survival. Infection is a major complication of LVAD therapy and was responsible for up to 20% of deaths in one cohort of LVAD recipients.[60]

LVADs are mechanical pumps that are implanted in the thoracic cavity to assist the pumping action of a failing heart. These devices are connected to the heart via two cannulas, pulling blood from the left ventricle and pumping into the aorta (Fig. 82.7). Most LVADs used in contemporary practice (e.g., HeartMate II and Jarvik 2000) are designed as axial flow pumps to provide continuous flow.[61] These devices do not require a mechanical valve, are smaller in size (and therefore less prone to infection), and last longer. These devices are connected to an external battery pack with a driveline. Increasingly, LVADs, which use electromagnetically or hydrodynamically levitated continuous-flow centrifugal pumps (e.g., HeartWare HVAD, Duraheart, Los Angeles) are being implanted in clinical practice. These devices have even simpler mechanics with fewer moving parts.

LVAD therapy is associated with significant infection risk. In the REMATCH (Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure) trial,[59] which examined the role of long-term LVAD use as destination therapy, infection and device failure were predominant factors that led to a limited 2-year survival rate of 23%. Within 3 months of LVAD placement, LVAD infection complicated 28% of cases. LVADs that use continuous-flow pumps are at lower risk of infection compared with devices with pulsatile flow pumps.[60,62]

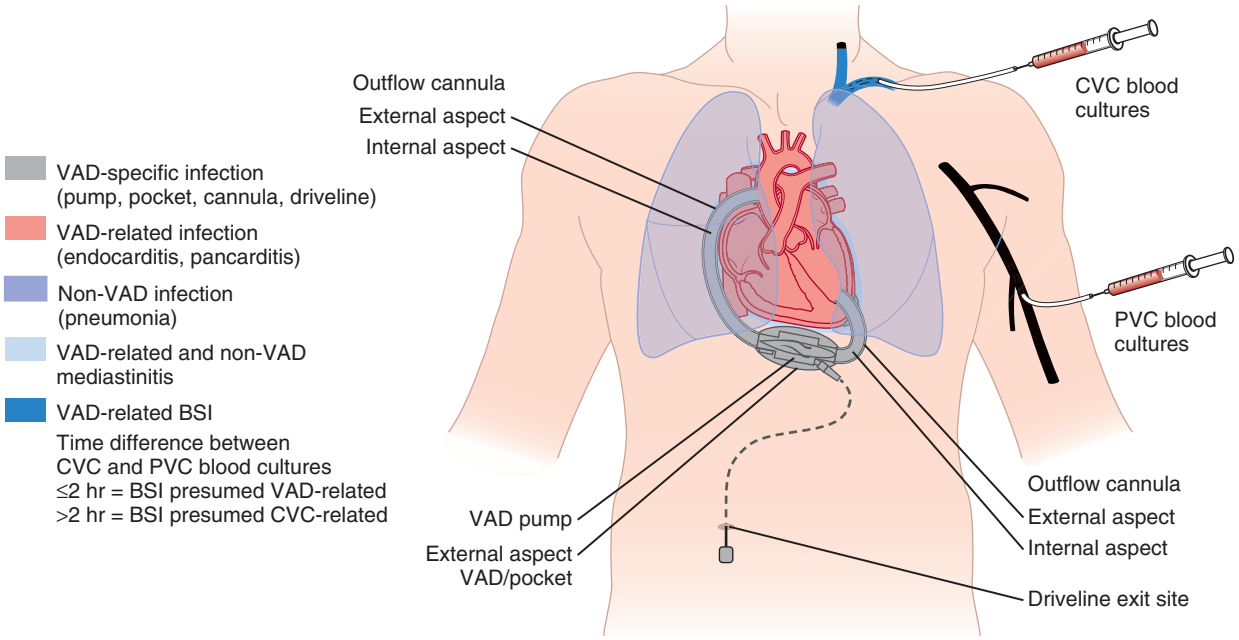

FIG. 82.7 Classification of left ventricular assist device *(VAD)*-associated infections. *BSI,* Bloodstream infection; *CVC,* central venous catheter; *PVC,* peripheral venous catheter. *(Modified from Hannan MM, Husain S, Mattner F, et al. Working formulation for the standardization of definitions of infections in patients using ventricular assist devices.* J Heart Lung Transplant. *2011;30:375–384.)*

Epidemiology of Left Ventricular Assist Device Infections

The risk of infection varies based on duration of LVAD support, with higher rates of infection reported in the destination therapy group compared with patients where LAVD is used as a bridge to transplantation.[64] In one review of LVAD-related complications the incidence of infection was two times higher in patients with an LVAD in place for more than 60 days compared with those with an LVAD for less than 30 days.[65]

The reported rates of infection in LVAD recipients vary from 13% to 80%[14] and depend on multiple factors, including underlying comorbidities, type of device, duration of LVAD support, and study definition of LVAD infection. In a cohort study[66] that included patients with HeartMate devices, the overall rate of surgical site infection was 44 per 100 LVAD implantations or 6.2 infections per 1000 device days. Seven of the patients had superficial infection that involved the driveline incision (19.4 infections/100 LVAD implantations), three patients had sternal infections (8.2 infections/100 LVAD implantations), and six had organ space infections that involved either the LVAD or the preperitoneal pocket that contained the pump (16.7 infections/100 LVAD implantations). Two of the patients also developed mediastinitis.

Risk of LVAD-related BSI was calculated in a retrospective study from the Cleveland Clinic.[67] An LVAD-related BSI was defined as one in which the same pathogen was cultured from the device and the blood and no other source of BSI was identified. Overall, 140 nosocomial BSIs occurred in 104 (49%) of 214 patients (7.9 BSIs/1000 LVAD days); 53 (38%) episodes were device related. Patients with LVAD-related BSI had a higher mortality rate of 58% compared with 42% in patients with LVAD support but no BSI ($P = .07$) in this investigation.

Pathogenesis of Left Ventricular Assist Device Infections

The impact of LVAD implantation on the immune system is speculated to be one factor that predisposes device recipients to infectious complications.[68,69] LVAD implantation can lead to increased susceptibility of circulating CD4 T cells to activation-induced apoptosis, resulting in a progressive decrease in CD4 T-cell count and impaired cell-mediated immunity. This has been postulated that LVAD recipients are at increased risk of infection, including those due to opportunistic pathogens. In one clinical survey[69] LVAD recipients were at increased risk of developing *Candida* infections compared with control subjects (28% vs. 3%; $P = .003$). Dysregulation of the humoral arm of the immune system has also been reported.

Risk Factors for Left Ventricular Assist Device Infections

Duration of LVAD support[64] and documented trauma at the driveline exit site are well-known risk factors associated with device infection.[70] Data from INTERMACS (Interagency Registry for Mechanically Assisted Circulatory Support), the largest registry of patients who received mechanical circulatory assist devices, suggest that continuous-flow devices have a lower rate of infectious complications compared with LVADs with pulsatile pumps. Risk factors for LVAD-related infection are summarized in Table 82.3.

TABLE 82.3 Risk Factors for Left Ventricular Assist Device Infection

Length and extent of surgery required for device implantation
Reoperation or revision
Hematoma at device pocket site
Persistent local trauma or poor wound healing at driveline exit site
Poor nutritional status of the host
Comorbid conditions (chronic heart failure, renal failure, diabetes mellitus, obesity)
Prolonged hospitalization
Length of time left ventricular assist device is in place
Foreign bodies in other locations (endotracheal tubes, urinary catheters, central venous or arterial catheters, drainage tubes, cardiovascular implantable electronic device)
Immune system dysregulation due to underlying disease or immunosuppressive drugs

Clinical Manifestations of Left Ventricular Assist Device Infections

Infectious complications related to LVADs can be broadly categorized into three types (Fig. 82.7): (1) LVAD-specific infections, infections that are related directly to the device hardware and include pump and cannula infections, pocket infections, and percutaneous driveline infections; (2) LVAD-related infections, which, although they can also occur in patients without LVADs, the presence of an LVAD warrants special management considerations, including valvular endocarditis, BSIs, and mediastinitis; and (3) non-LVAD infections, that is, those not related to LVAD placement and that may include pneumonia, urinary tract infection, and other remote infections.

The most common presentation of LVAD-specific infection is driveline exit site infection. Local inflammatory changes and purulent drainage are frequently seen. Device pocket infections usually present with frank inflammatory changes in the skin and soft tissues overlying a device pocket. Infection of the valves or other blood-contacting surfaces of the LVAD occurs less often. Clinical manifestations of this form of endocardial infection mirror the manifestations of native or prosthetic valve endocarditis. Fever and other signs of systemic inflammatory response may be apparent. Systemic or pulmonary (for devices that assist the right ventricle) embolic phenomena can be seen. Mechanical dysfunction of the LVAD can occur if an obstructing pannus of infection develops within the internal lumen of the inflow or outflow cannulas that are anastomosed into the apex of the left ventricle and ascending aorta, leading to clinical worsening of cardiac function.

Microbiology of Left Ventricular Assist Device Infections

S. aureus and CoNS account for greater than 50% of cases of LVAD-related infections. Enterococcal species, *Enterobacter* spp., and *P. aeruginosa* are other commonly isolated bacterial pathogens in LVAD-related infections.[67,71,72] Because of nosocomial acquisition of these infections, antimicrobial resistance is a common occurrence. Although colonization with *Candida* spp. occurs in up to 39% of patients with LVADs, clinical infection is less frequent.[73] Use of broad-spectrum antibiotics increases this risk. Eradication of infection is difficult due to microbial biofilm formation on prosthetic surfaces.

Management of Left Ventricular Assist Device Infections

Our approach to diagnosis and management of LVAD infection is summarized in Fig. 82.8.[72] Débridement of an infected driveline exit site or device pocket may be helpful to control localized infection in some patients. Vacuum-assisted closure systems can help to facilitate wound healing.[74] For patients with LVAD-related BSI, chronic suppressive antimicrobial therapy, after an induction course, is recommended. Suppressive antibiotics should be continued unless the infected LVAD is removed or the patient undergoes cardiac transplantation. However, earlier LVAD removal is required in some cases to control infection. Of note, LVAD-related infection is not a contraindication to cardiac transplantation.[10,71] In fact, for some patients cardiac transplantation is necessary not only for advanced heart failure but also for removal of the infected device to achieve control of ongoing infection.

Earlier reports suggested that patients with pretransplantation LVAD infection have similar short- (<6 months) and long-term (as long as 3 years) survival rates after cardiac transplantation, compared with noninfected controls.[67,75] However, a subsequent investigation[71] showed a prolonged length of hospitalization and increased early mortality in heart transplant recipients with pretransplantation LVAD-related infection. However, long-term survival rates were comparable between the two groups.

Optimal duration of antimicrobial therapy for LVAD-related BSI in patients who are awaiting heart transplantation is unclear. In one investigation of 76 patients with LVADs,[71] continuous antimicrobial treatment, from the time of initial diagnosis of BSI until transplantation or death, was associated with a lower rate of relapse compared with a limited antibiotic course of 2 to 6 weeks ($P = .003$). Relapses were diagnosed at a median of 12 days (range, 2–89 days) after discontinuation of antimicrobial therapy. All patients who had LVAD-related infection

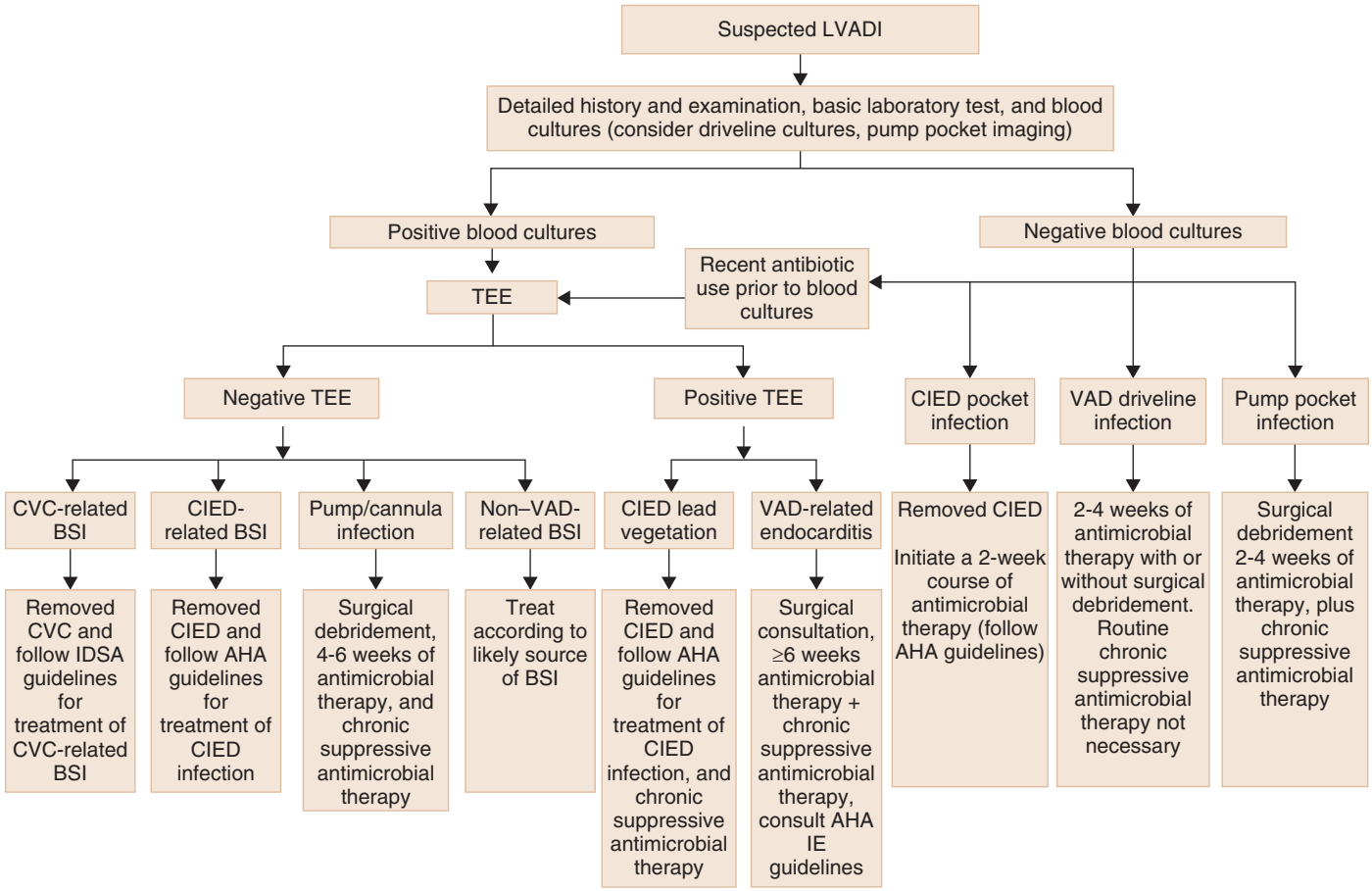

FIG. 82.8 Mayo Clinic guidelines for the management of left ventricular assist device–associated infections *(LVADIs).* These are general guidelines only, and LVADI infection management should be individualized based on clinical presentation and host factors. *AHA,* American Heart Association; *BSI,* bloodstream infection; *CIED,* cardiovascular implantable electronic device; *CVC,* central venous catheter; *IDSA,* Infectious Diseases Society of America; *TEE,* transesophageal echocardiogram; *VAD,* ventricular assist device. *(Modified from Nienaber JJC, Kusne S, Riaz T, et al. Clinical manifestations and management of left ventricular assist device-associated infections.* Clin Infect Dis. *2013;57:1438–1448.)*

due to *S. aureus* and were treated with limited courses of antibiotics experienced relapse. There was no difference in the two groups in terms of posttransplantation mortality, length of hospitalization, or 1-year survival.

Prevention

Several strategies have been advocated to reduce the rate of LVAD-related infections.[76] These include meticulous attention to aseptic techniques at the time of device implantation, use of chlorhexidine for skin preparation, perioperative intranasal mupirocin to diminish *S. aureus* colonization, elimination of percutaneous drivelines with totally implantable LVADs,[77] longer tunneling of the driveline, and immobilization of the driveline exit site using an abdominal binder. Since publication of the REMATCH trial,[59] a majority of LVAD implanters have been using a broad-spectrum antimicrobial regimen, including a β-lactam plus a fluoroquinolone plus an antifungal agent, for surgical site infection prophylaxis. However, a recent investigation from the Mayo Clinic[78] suggested that there was no obvious benefit of using a multidrug regimen as it did not impact infection-free survival or all-cause mortality compared with single-drug regimen. Based on these data, we recommend using a simplified regimen of cefazolin plus vancomycin for routine surgical site infection prophylaxis. Additional agents, such as an antifungal and an agent with gram-negative bacillary coverage, may be considered in patients with preexisting infections before LVAD implant, prolonged hospitalization, and colonization with multidrug-resistant (MDR) organisms.

PROSTHETIC VASCULAR GRAFTS

Despite technical advances in graft design, infection remains a significant complication associated with use of prosthetic vascular grafts and can lead to loss of limb or life. Infections involving the prosthetic vascular grafts can manifest in three distinct ways: (1) perigraft infection or abscess formation, (2) graft exposure due to disruption of the superficial soft tissue layers overlying the prosthesis, and (3) graft erosion or fistula formation involving a mucosal surface. However, these three presentations of graft infection are not mutually exclusive. Moreover, there is a dramatic increase in the use of endovascular grafts and stent grafts, which are at less risk of infection and are addressed later in this chapter.

Epidemiology of Prosthetic Vascular Graft Infection

The rate of prosthetic vascular graft infection (PVGI) varies and depends on the anatomic location of the prosthesis. For aortic grafts limited to the abdomen, graft-related infections occur in 1% or less of recipients. The rate of infection is higher (1.5%–2%) for aortic grafts that extend to the femoral location. Infrainguinal vascular grafts that originate in the groin are at the highest (6%) risk of complicating infection.[10,79,80,81] Reported morbidity (mostly amputations) and mortality rates for peripheral vascular grafts are 41% and 17%, respectively.[82] Prosthetic aortic graft infections have a higher mortality rate (24%–75%). The average 5-year survival rate for aortic graft infections is only 50%.[79,83]

Pathogenesis of Prosthetic Vascular Graft Infection

Several distinct routes of microbial contamination are recognized in vascular graft infections. Microbial seeding of a graft at the time of implantation or in the immediate postoperative period accounts for most of the graft infections. Vascular graft contamination can also occur if there is infection in a contiguous anatomic area, such as bacterial

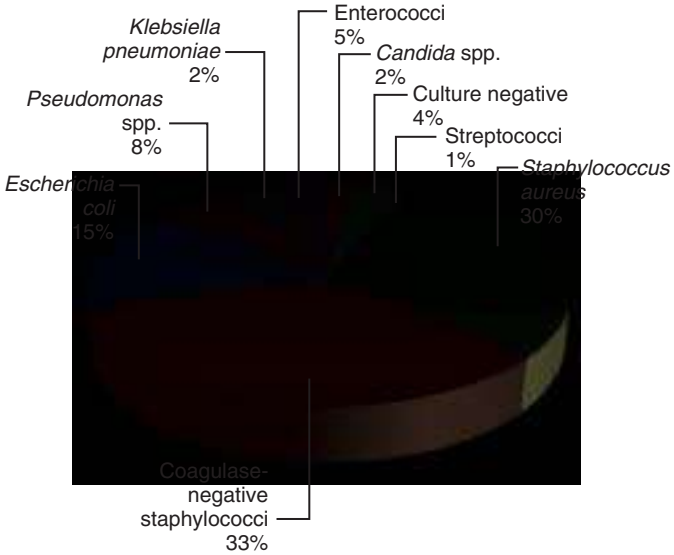

FIG. 82.9 Microbiology of prosthetic vascular graft infections (intraoperative culture results from 119 patients, 68 with aortoiliofemoral and 51 with extracavitary graft infections). *(Data from Bandyk DF, Novotney ML, Back MR, et al. Expanded application of in situ replacement for prosthetic graft infection.* J Vasc Surg. *2001;34:411–420.)*

TABLE 82.4 Risk Factors for Prosthetic Vascular Graft Infection

Emergent surgery
Lack of appropriate antimicrobial prophylaxis in the perioperative period
Groin incision
Bloodstream infection during index hospitalization
History of multiple invasive interventions before or after graft placement
Poor wound healing/infection at surgical site
Contiguous infection in the graft area
Comorbid conditions (diabetes mellitus, chronic renal insufficiency, obesity, immunocompromised host)

colonization of a thrombus in an aneurysmal sac or in atherosclerotic plaques.[83,84] Superficial surgical site infection can also lead to graft infection. Subsequent manipulation of an implanted graft by either surgical or percutaneous procedures can predispose to graft infection. Finally, BSI originating from an anatomically remote site of infection can predispose to hematogenous seeding of vascular grafts. The risk of hematogenous seeding is highest in the early postoperative period and decreases over time because of partial endothelialization of the graft.[84]

Risk Factors for Prosthetic Vascular Graft Infection

Purported risk factors for PVGI are summarized in Table 82.4.[10,79,85–87,88] In a large statewide dataset that included 14,000 patients who underwent aortic aneurysm repair,[88] BSI during the index hospitalization for repair was associated (OR, 4.2) with increased risk of aortic graft infection. In another case-control study[86] that included cases of aortic and peripheral graft infections collected over a 21-year period, groin incision (OR, 4.1) and wound infection (OR, 5.1) were risk factors for PVGI in a multivariable analysis. In one study that included 496 cases with prosthetic femoral artery grafts,[89] redo bypass (hazard ratio [HR], 5.8), active infection at the time of bypass (HR, 5.2), female gender (HR, 4.5), and diabetes mellitus (HR, 4.6) were associated with increased odds of PVGI in multivariable analysis.

Microbiology of Prosthetic Vascular Graft Infection

Staphylococcal species continue to be the predominant pathogens responsible for PVGI (Fig. 82.9). However, less virulent organisms that are normal skin flora account for an increasing portion of PVGI.[86] Pathogens included in this category are CoNS, *Corynebacterium* spp., and *Cutibacterium* (formerly *Propionibacterium*) *acnes.* PVGI caused by these organisms tend to be delayed and indolent in onset. In contrast, *S. aureus*–related graft infections occur early after graft implantation. In a prospective cohort study that included 85 patients with PVGI, *S. aureus* infection was more frequent in limb grafts compared with aortic grafts.[82] Enterococcal species and anaerobic bacteria, often as part of polymicrobial infection, have also been identified as pathogens of PVGI.[86] *Candida* spp. are an infrequent but difficult-to-treat cause of PVGI.

Clinical Manifestations of Prosthetic Vascular Graft Infection

Timing of onset and clinical presentation of PVGI vary based on location of the graft and virulence of the causative pathogen. Considering that intraoperative or immediate postoperative vascular graft contamination is the mechanism accounting for infection in most cases, clinical evidence of infection should be evident within 1 to 2 months after graft implantation. Although this is generally true for extracavitary (extremity grafts, especially infrainguinal) PVGI, intracavitary (abdominal graft) infections may present several years after placement.

In patients who present with early graft infection (within 3 months of implantation), local inflammatory findings indicating surgical site infection is present. Even when these findings appear superficial, underlying graft infection is nevertheless a consideration. Local complications associated with graft infection may include abscess or sinus tract formation, hemorrhage, graft occlusion, pseudoaneurysm formation, graft exposure, and poor tissue incorporation. Septic emboli and distal tissue ischemia can also occur. Systemic signs of sepsis can accompany the local findings, particularly when more aggressive pathogens, such as *S. aureus,* are present. Fever, chills, and leukocytosis are generally present in the setting of bacteremia or fungemia.

Late graft infection is characterized less often by systemic toxicity. The local stigmata of graft healing complications prevail. These include cutaneous sinus tracts, lack of graft incorporation by surrounding tissue, anastomotic aneurysm, and graft-enteric erosions or the development of fistulas.[90] The formation of cutaneous sinus tracts is an obvious clue that underlying graft infection is present. Poor graft incorporation may not be apparent until intraoperative inspection. The clinical presentation of pseudoaneurysms is variable, ranging from little to no inflammatory response to local pain at the aneurysm site with or without a palpable mass. For one-third of patients[90] the clinical presentation of anastomotic pseudoaneurysm is emergent in regard to threat of life (hemorrhagic shock) or limb (distal ischemia due to graft thrombosis or embolization).

PVGI-related enteric erosions and fistulas may present years after graft placement. They are diagnosed in less than 5% of patients who undergo aortic graft placement. These patients can present with systemic (sepsis) and local (abdominal pain) complaints. The aortic graft most often erodes the third or fourth portion of the duodenum. GI tract bleeding, which ranges from subtle to massive, can present as hematemesis, hematochezia, or melena. Polymicrobial infection, consisting of enteric flora, usually is confirmed with blood and graft and perigraft tissue cultures. A prompt diagnosis of this condition is lifesaving because mortality is universal if the complication is left untreated.

Diagnosis of Prosthetic Vascular Graft Infection

A practical approach to diagnostic workup of prosthetic graft infections is outlined in Fig. 82.10. Even when physical examination findings are suggestive of graft infection, diagnostic imaging is usually performed to confirm the diagnosis and to define extent of infection. Blood cultures may be negative if infection is limited to the extraluminal graft surface. A comparison of various imaging modalities is provided in Table 82.5.[83,85,91] CT is the best tool in the diagnostic evaluation of graft infection. Findings on a CT scan that support vascular graft infection include the presence of perigraft fluid not attributable to recent (≤3 months) graft implantation, increasing perigraft fluid in the early postoperative period, perigraft fluid with fat stranding or gas bubbles, lack of a fat plane between graft and bowel, and anastomotic aneurysms.[79] For less virulent pathogens, particularly CoNS, the inflammatory response may be limited, and the perigraft findings on CT may be minimal to nonexistent. The sensitivity of CT is decreased considerably in patients

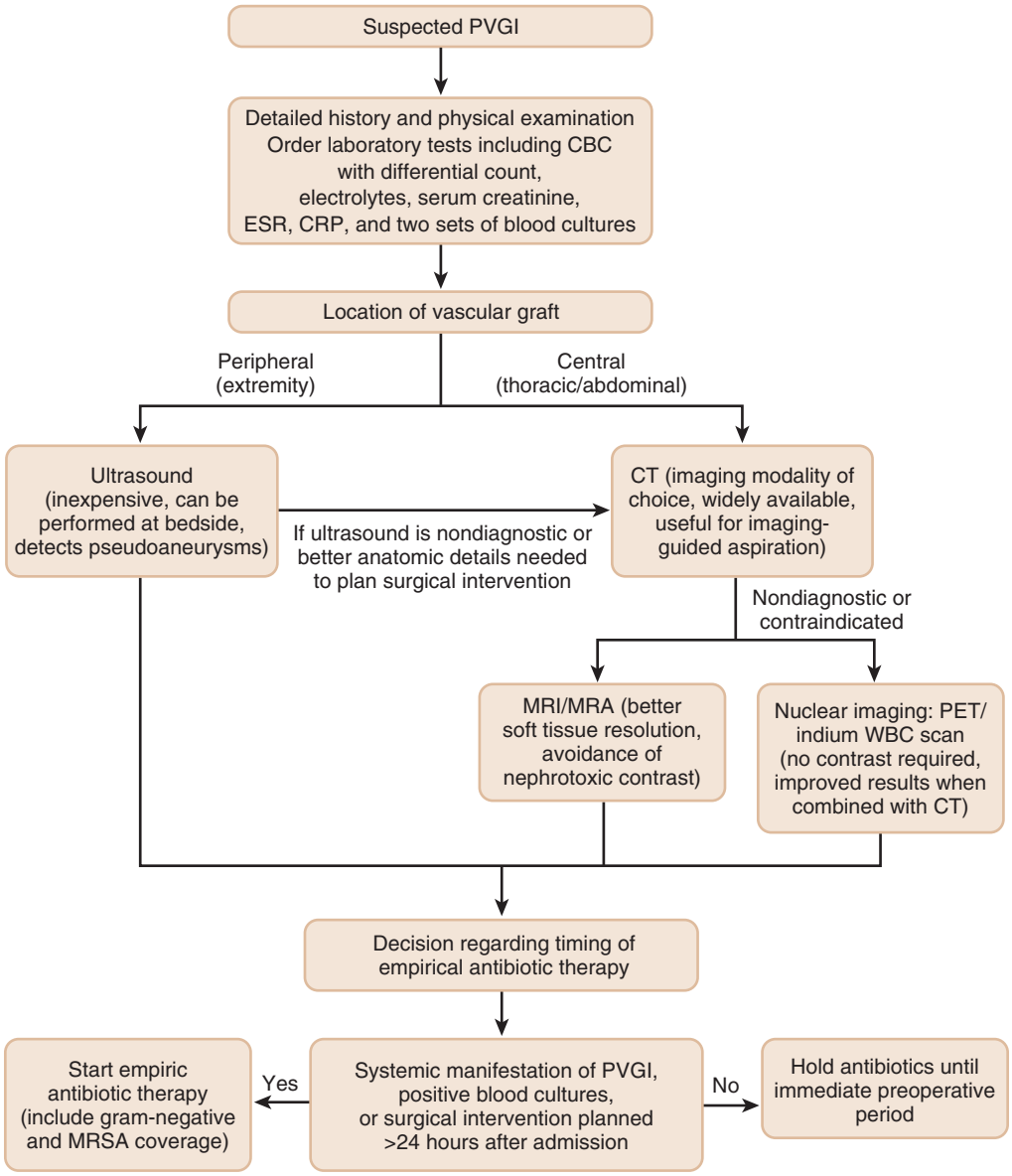

FIG. 82.10 Diagnostic evaluation and initial management of prosthetic vascular graft infections. *CBC,* Complete blood count; *CRP,* C-reactive protein; *CT,* computed tomography; *ESR,* erythrocyte sedimentation rate; *MRI/MRA,* magnetic resonance imaging/magnetic resonance angiography; *MRSA,* methicillin-resistant *Staphylococcus aureus; PET,* positron emission tomography; *PVGI,* prosthetic vascular graft infection; *WBC,* white blood cell. *(Modified from Nagpal A, Sohail MR. Prosthetic vascular graft infections: a contemporary approach to diagnosis and management.* Curr Infect Dis Rep. *2011;13:317–323.)*

TABLE 82.5 Comparison of Imaging Techniques for Diagnosis of Vascular Graft Infection

COMPUTED TOMOGRAPHY (CT)	MAGNETIC RESONANCE IMAGING (MRI)	ULTRASONOGRAPHY	NUCLEAR MEDICINE IMAGING (INDIUM-LABELED WBC SCAN AND GALLIUM SCAN)
Sensitivity: 95% Specificity: 85% Imaging modality of choice Images may be degraded in the presence of metallic hardware in spine or extremities Administration of potentially nephrotoxic contrast dye	Sensitivity: 68%–85% Specificity: 97%–100% Useful when CT findings are nondiagnostic Better soft tissue resolution More expensive than CT scan Use of nephrotoxic contrast agent is avoided Small risk of nephrogenic fibrosing dermopathy with use of gadolinium in patients with preexisting renal insufficiency	Useful for peripheral vascular grafts to detect pseudoaneurysms Least expensive imaging modality Can be performed at bedside No need for contrast administration	Sensitivity: 73% Specificity: 87% False-positive results in early postoperative period Decreased sensitivity with previous antimicrobial therapy Useful when CT or MRI findings are inconclusive

WBC, White blood cell.

with more indolent infections. In cases where a pseudoaneurysm is not detected on CT, imaging-guided percutaneous aspiration of perigraft fluid should be considered. Fluid should be sent for cytology and microbiologic analysis, including bacterial, fungal, and mycobacterial cultures.

Some of the previously mentioned CT findings of infection are normally seen in the early postoperative period. Perigraft fluid can persist for 3 months after graft implantation. Perigraft air related to graft placement is usually absorbed within 1 week of surgery, although it can persist for as long as 7 weeks.[92]

Magnetic resonance imaging (MRI) may be helpful when CT findings are inconclusive.[80,91,93] MRI is better in visualization of subtle perigraft inflammatory changes than CT. Moreover, MRI is often able to differentiate subacute or chronic hematoma from inflammatory changes in the perigraft area. In one investigation of intracavitary vascular graft infections,[91] MRI had an excellent positive predictive value (95%), but the negative predictive value was low (80%). Indium-labeled WBC scanning had a positive predictive value of 80% and a negative predictive value of 82% compared with MRI in the same investigation. On the basis of these data, MRI is a preferred modality over indium-labeled WBC scanning for the diagnosis of aortic graft infections. However, results are not mutually exclusive, and a combination of imaging techniques may be helpful in difficult-to-diagnose cases. Software-based fusion imaging that combines data obtained from CT angiography (CTA) and WBC scintigraphy may provide a better diagnostic yield compared with a single-imaging modality. In a recent investigation[94] software-based fusion imaging of both modalities resulted in improved sensitivity (94%), specificity (50%), positive predictive value (88%), and negative predictive value (67%).

Endoscopic examination of the GI tract is helpful in cases where a graft enteric erosion or fistula is the suspected source of GI bleeding. Angiography is generally not necessary to diagnose PVGI. However, it may be used to define complex vascular anatomy for planning revascularization.

PET, combined with CT scan (PET-CT), is another emerging modality for the diagnosis of PVGI.[95] In a study that included 39 subjects with graft infection,[39] combined PET-CT had 93% sensitivity and 91% specificity in the diagnosis of vascular graft infection. Positive and negative predictive values were 88% and 96%, respectively. In a more recent prospective study,[96] fluorodeoxyglucose (FDG)-PET had high sensitivity of 93%, specificity of 70%, positive predictive value of 82%, and negative predictive value of 88%. FDG-PET also had excellent interobserver consistency and provided a better diagnostic accuracy than CT for the diagnosis of PVGI.

Management of Prosthetic Vascular Graft Infection

Optimal management of PVGIs is challenging and requires a multidisciplinary team approach with involvement of both medical and surgical subspecialties. Four general principles are followed in the treatment of PVGIs (Table 82.6).[97] In 2016 the American Heart Association issued a scientific statement outlining recommendations for diagnosis and management of vascular graft infections.[98]

TABLE 82.6 General Principles for Management of Prosthetic Vascular Graft Infection

Complete excision of the infected graft material
Extensive débridement of all infected, devitalized tissues in the perigraft area
Revascularization of distal tissues
Microbiologic identification of causative pathogen, followed by appropriate systemic antimicrobial agents for >6 weeks (depending on clinical response and repeat imaging)
Bactericidal antibiotics preferred and combinations often used

In general, complete excision of infected graft and débridement of infected perigraft tissue is necessary to achieve cure of infection. Extraanatomic bypass revascularization and in situ reconstruction are the two main revascularization strategies after resection of infected graft material. The key advantages and disadvantages of both options are summarized in Table 82.7. In cases where extraanatomic bypass is the preferred method of revascularization, the extraanatomic bypass is done first, followed by infected graft excision and local débridement during either the same anesthesia (sequenced approach) or a second surgery at a later date (staged approach).[99] In situ reconstruction through uninfected tissue planes is the second method of revascularization.[100] Three types of grafts are available for use and include antibiotic-bonded prosthetic grafts, fresh and cryopreserved arterial allografts, and autogenous vein grafts. The advantages of the allografts and autogenous vein grafts include the decreased likelihood of new graft infection because native tissue is placed in situ rather than prosthetic material.[85] Autogenous vein grafts also have higher long-term patency rates.[101] However, the disadvantage is the prolongation of the operative time required for vein harvesting.

By tradition, extraanatomic bypass has been touted as the gold standard for the surgical management of PVGI because of the theoretically decreased risk of reinfection by avoiding placement of a new graft in a previously infected tissue bed. However, in a systematic review and meta-analysis,[102] pooled estimates of mean event rates for all outcomes (reinfection, amputation, conduit failure, mortality) favored the use of in situ reconstruction with autogenous veins, rifampin-bonded prostheses, or cryopreserved allografts over extraanatomic bypass revascularization.

Regardless of surgical approach, multiple intraoperative specimens of graft material and perigraft inflammatory tissues should be submitted for bacterial, fungal, and mycobacterial stains and cultures to maximize the yield. Empirical antibiotics should include coverage for CoNS and MRSA.

Conservative management of prosthetic graft infections is associated with high mortality, and this approach should be reserved for a specific situation.[103,104] In cases in which complete excision of the infected graft is not feasible (poor surgical candidate because of multiple comorbid conditions or limited revascularization options), lifelong (for the "life of the graft") suppressive antimicrobial therapy is recommended.

For management purposes PVGI can be classified into intracavitary and extracavitary graft infections. This classification is helpful in guiding surgical management of infected grafts and duration of antimicrobial therapy.

Surgical Management of Extracavitary Prosthetic Vascular Graft Infection

In cases of early (<2 months postoperatively) PVGI, where infection is not complicated by active bleeding and the anastomotic site is not involved, graft preservation rather than graft excision and reconstruction, is a reasonable consideration. However, for late-onset infection (after 2 months postoperatively), graft excision and reconstruction should be considered instead of graft preservation. In cases where infection involves the graft anastomotic site or infection is caused by MRSA, *Pseudomonas*, or MDR microorganisms, or for patients for whom graft

TABLE 82.7 Comparison of In Situ Reconstruction Versus Extraanatomic Bypass Revascularization for Management of Prosthetic Vascular Graft Infection

IN SITU RECONSTRUCTION		EXTRAANATOMIC BYPASS REVASCULARIZATION	
ADVANTAGES	**DISADVANTAGES**	**ADVANTAGES**	**DISADVANTAGES**
Reduced rate of lower extremity amputation Elimination of the need for a second operation Avoidance of creating an aortic stump that could blow out in future Lack of anatomic limitations to placement Shorter operating time when rifampin-bonded prosthetic grafts or arterial allografts are used Reinfection rate: 10% with prosthetic graft reconstruction vs. 3% with autogenous vein replacement	Theoretical risk of increasing chances of new graft infection by placing new prosthetic material or allograft in a previously infected tissue bed	Theoretical reduced risk of new graft infection by avoiding placement of a new prosthetic material or allograft in a previously infected tissue bed	Need for a second procedure if staged operative strategy is used Reduced limb salvage rates (20%–30% lower extremity amputation rate) Aortic stump blowout (20%) 20% reinfection rate

preservation or in situ reconstruction has failed, it is reasonable to perform extraanatomic revascularization, followed by graft excision instead of graft preservation or in situ reconstruction. For patients in whom PVGI is complicated by sinus tract formation or active bleeding, extraanatomic revascularization followed by graft excision is preferred.

Antimicrobial Management of Extracavitary Prosthetic Vascular Graft Infection

In cases where infection is limited to skin or soft tissues without CT findings to suggest graft involvement, a 2- to 4-week trial of antimicrobial therapy with or without surgical débridement is reasonable. For more advanced infection (sinus tract or fistula formation, anastomotic site involvement, or systemic infection), a 4- to 6-week course of parenteral induction antimicrobial therapy, followed by 6 months of oral antimicrobial therapy, is recommended.

Chronic (lifelong) suppressive antimicrobial therapy may be considered for infections caused by MRSA, *Pseudomonas*, MDR organisms, *Candida*, or other fungal species; for those patients who have undergone emergency or multiple surgeries; for patients with graft preservation or in situ reconstruction with extensive perigraft infection; or for patients who are poor candidates for reoperation. The choice of antibiotic depends on in vitro susceptibility testing. Suppressive therapy is usually with oral cephalosporins, penicillin derivatives, tetracyclines, or trimethoprim-sulfamethoxazole. Patients who are conservatively managed should have ultrasound examination every 3 to 6 months for 2 years, followed by lifelong ultrasound examination every 6 to 12 months.

Surgical Management of Intraabdominal Prosthetic Vascular Graft Infection

In general, graft excision and in situ reconstruction with cryopreserved, arterial allograft, or venous autograft or rifampin-bonded synthetic graft is recommended. However, in patients with infection caused by MRSA, *Pseudomonas*, or MDR microorganisms or those with extensive intraabdominal abscess or perigraft purulence, extraanatomic bypass revascularization, followed by graft excision, may be considered.

Antimicrobial Management of Intraabdominal Prosthetic Vascular Graft Infection

A 6-week course of parenteral antimicrobial therapy after surgery is recommended. An additional 3 to 6 months of oral antimicrobial therapy is recommended for cases managed with in situ reconstruction. Lifelong suppressive antimicrobial therapy may be considered in patients with extensive perigraft infection or infection caused by MRSA, *Pseudomonas*, or MDR microorganisms.

Management of Intrathoracic Prosthetic Vascular Graft Infection

In situ repair using cryopreserved arterial allografts, followed by 4 to 6 weeks of parenteral antimicrobial therapy, is recommended. In patients with a high risk of morbidity and mortality, those who cannot tolerate extensive reconstructive surgery, or those with in situ repair using a synthetic graft, lifelong suppressive antimicrobial therapy may be considered.

Prevention

Meticulous attention to aseptic procedures during vascular graft placement is the key to limiting the risk of subsequent graft infection. Administration of perioperative antibiotics is associated with a reduced risk of subsequent graft infection.[87] However, there is no benefit of extending antibiotic prophylaxis beyond 24 hours from graft placement.

HEMODIALYSIS PROSTHETIC VASCULAR GRAFTS

Infection is the second leading cause of death among patients with end-stage renal disease[105] and is often caused by *S. aureus*. Due to extensive health care system exposure and frequent administration of antibiotics in this high-risk population, MDR strains of *S. aureus*, enterococci, and gram-negative bacilli have emerged as a serious problem among these patients.

TABLE 82.8 Risk Factors for Polytetrafluoroethylene Graft Infection

Need for repetitive percutaneous cannulation
Breaks in sterile technique during cannulation
Poor patient hygiene
Prolonged postdialysis bleeding from the graft
Perigraft hematoma formation
Surgical manipulation of graft
Severe pruritus and scratching over needle sites
Human immunodeficiency virus coinfection
Lower extremity (thigh) graft
Bacteremia or fungemia caused by an ectopic site of infection

Epidemiology

Vascular access site infections constitute a major part of infection-related morbidity and mortality in long-term hemodialysis patients. Use of the prosthetic arteriovascular grafts composed of polytetrafluoroethylene (PTFE) is a major contributing factor for access site infections and associated morbidity and mortality.[106,107] These grafts are at higher risk of infection, up to four times higher incidence, compared with the native arteriovenous (AV) fistulas. Therefore PTFE grafts should only be used in cases in which surgical creation of a native AV fistula is not anatomically feasible. The main benefit of vascular grafts is that they do not require maturation, as AV fistulas do, and they can be used for hemodialysis within days of placement. The risk of infection with either tunneled or temporary hemodialysis catheters is highest. In one prospective survey[105] the risks of infection (per 1000 dialysis sessions) in patients with AV grafts (2), tunneled catheters (12.2), or temporary catheters (29.2) were much higher than the risks associated with the use of AV fistulas (0.9).

Risk factors for infection of PTFE grafts are listed in Table 82.8.[107,108]

Clinical Manifestations

Most patients with graft infection present with local inflammatory findings at the graft site. *S. aureus* (53%) and CoNS (20.3%) are the most commonly isolated pathogens.[10] However, occult BSI without local graft inflammatory changes can also be a manifestation of graft infection. An occult presentation is more common in patients with old, nonfunctioning, and thrombosed grafts.[109] Indium-labeled WBC scanning may be helpful in detecting these clinically silent graft infections. In one investigation[110] of occult infection involving such old or thrombosed grafts, the indium-labeled WBC scan showed 100% sensitivity and 75% specificity in detecting graft infection.

Management

Preservation of the vascular access site is a major consideration when making management decisions regarding PTFE graft infections because other potential venous access sites have been frequently exhausted in these patients. Parenteral antimicrobial therapy combined with local débridement is curative in selected cases of early graft infection or exposed vascular graft,[111] and removal of the entire graft is not always necessary. If conservative management is attempted the remaining graft segment should have adequate tissue coverage and close follow-up to ensure resolution of infection is necessary. However, presence of purulence or abscess in the immediate graft area or aneurysmal graft formation mandates graft removal. Old, thrombosed, nonfunctioning grafts should be resected when infection at this nidus is confirmed.

Three to 4 weeks of parenteral antimicrobial therapy, directed by in vitro susceptibility testing, is recommended.[112] In patients with SAB, transesophageal echocardiography should be performed to exclude infective endocarditis.

Prevention

Vascular access site infection is a leading cause of morbidity and mortality in hemodialysis patients. The total annual cost of graft infection-related morbidity in the United States has been projected to be more than $1 billion.[113] The best method to prevent a PTFE graft-related infection is to avoid its use. The National Kidney Foundation[105] and the Fistula

First Project[114] recommend placement of an AV fistula instead of PTFE grafting when anatomically feasible. Cryopreserved vein allografts may be an alternative in some scenarios.[115] Their patency rates are equivalent to that of expanded PTFE, and rates of reinfection are generally low.

Use of intranasal mupirocin to eradicate *S. aureus* colonization may help to reduce the risk of *S. aureus* bacteremia.[116] Prophylactic antibiotics should be administered preoperatively at the time of graft placement and before subsequent surgical graft manipulation, if required. In addition, adherence to infection control practices at the time of graft cannulation is mandatory.

INVASIVE NONSURGICAL CARDIOLOGIC PROCEDURES

The array of invasive nonsurgical cardiologic procedures continues to expand. Three of the more common procedures include percutaneous coronary intervention (PCI), diagnostic cardiac catheterization, and electrophysiologic studies. Most PCI procedures involve stent placement. Although coronary stent infection is exceedingly rare, BSI complicating the procedure has been the topic of numerous reports.[10] Local (groin) and metastatic infectious complications have been highlighted in other citations.

BSI after invasive nonsurgical cardiologic procedures is the most common infectious complication. It can be a manifestation of local vascular or soft tissue infection or a cause of metastatic infection that can result in a variety of syndromes, including infective endocarditis, septic arthritis, and epidural abscess formation.

Epidemiology

The incidence of BSI among patients who undergo invasive nonsurgical cardiologic procedures is low.[117,118] Risk of BSI depends on the duration of the procedure, multiple skin punctures performed, use of multiple balloons, and obesity. The incidence of early (≤36 hours postprocedure) BSI in patients who underwent PCI in a large prospective study was 0.61%.[118] In another study[119] 22,006 procedures that included cases of percutaneous transluminal coronary angioplasty, diagnostic cardiac catheterization, and electrophysiologic studies were reviewed. The overall incidence of BSI during the first 72 hours postprocedure was 0.11%. For PCI the BSI incidence was 0.24%. BSI was detected a median of 1.7 days after the procedure.

In one prospective study that included patients who underwent complex PCI[120] (requiring use of multiple devices in arterial circulation), blood cultures were obtained in all patients immediately and 12 hours after the procedure. Of the 147 patients included in this investigation, 26 (17.7%) had positive blood cultures immediately after PCI, whereas an additional 12% of patients yielded positive blood cultures in the next 12 hours, with femoral sheaths still in place. CoNS were the most commonly isolated organisms. However, none of these episodes of transient BSI was associated with clinical sequelae.

Risk Factors

The purported risk factors for the development of BSI after invasive nonsurgical cardiologic procedures are summarized in Table 82.9.[10] In one investigation[121] independent risk factors for PCI-related bacteremia included duration of the procedure (OR, 2.9), number of catheterizations at the same site (OR, 4.0), difficult vascular access (OR, 14.9), arterial sheath in place for more than 1 day (OR, 6.8), and heart failure (OR, 43.2). In another study[119] the median length of hospital stay was 21 days and 6 days for patients with and without procedure-related BSI, respectively. The overall mortality rate was several-fold higher (8%) among patients who developed postprocedure BSI compared with those who did not (0.009%).

TABLE 82.9 Risk Factors Associated With Bloodstream Infection After Invasive Nonsurgical Cardiologic Procedures

Age >60 years
Duration of the procedure
Repeated punctures at an ipsilateral femoral artery
Difficult vascular access
Inguinal hematoma
Arterial sheath in place for >24 hours
Brachial artery access via a cutdown approach
Heart failure

Clinical Manifestations

Fever and chills are the predominant symptoms of procedure-related BSI. However, fever and chills can occur during or soon after catheterization procedures secondary to noninfectious reasons, such as introduction of foreign proteins or endotoxin. Blood cultures should be obtained to confirm the underlying cause of fever. Local findings of infection in the femoral area are present in patients with femoral artery endarteritis, soft tissue infection, or pseudoaneurysm formation.[10]

Microbiology

S. aureus has been the major pathogen in most case series and characteristically causes the most serious infections. CoNS and enterococci are less commonly reported. In only one case series[119] gram-negative bacilli outnumbered gram-positive isolates as the cause of complicating bacteremia.

Treatment

Management of these cases is similar to that of other catheter-related BSIs.[122]

Prevention

Prevention efforts should be focused on use of meticulous sterile technique and avoidance of the risk factors cited earlier (see Table 82.9). These include avoidance of vascular access by puncturing endovascular grafts; contralateral femoral artery puncture for repeat procedures, especially if a closure device was used during the first procedure; and minimizing duration of indwelling sheaths.[10]

CORONARY ARTERY STENTS

Despite widespread use of coronary stents and the demonstrated risk of transient bacteremia associated with PCI,[123] infection of these stents has been rarely reported. Drug-eluting stents, in theory, may have a higher risk of infection because of impaired local immune response and delayed endothelialization.[124,125,126,127] Among the published cases,[124,125,128] men have predominated, perhaps due to higher rate of atherosclerosis that requires PCI. Symptoms of infection, with rare exception,[125,128] begin within days to less than 1 month after stent placement. Fever, chills, and chest pain are the most frequently reported symptoms. The large majority of infections are caused by *S. aureus*, followed by *P. aeruginosa* and CoNS. Blood cultures are positive in most patients with coronary artery stent infection. Complications may include myocardial abscess, pericardial empyema, aneurysm and pseudoaneurysm formation, coronary artery perforation, and myocardial infarction. Coronary arteriography or cardiac magnetic resonance angiography (MRA) may be required to evaluate for evidence of stent infection if echocardiography and CT are nondiagnostic.

Early-onset stent infections (detected within 10 days of stent placement), are mostly caused by *S. aureus* and should be managed aggressively. If no local complications are detected on imaging, conservative management with antimicrobial therapy and close follow-up may be an option in selected cases.[128] However, late-onset infections (occurring after 10 days of stent placement) or major complications necessitate combined medical and surgical intervention. Surgery includes removal of stent, if possible, and abscess drainage or perforation repair when indicated. The overall mortality rate of coronary artery stent infection among cases reported to date is 46.1% (6/13).[124,125]

PERIPHERAL VASCULAR STENTS

Peripheral vascular stents are being used increasingly for nonsurgical treatment of atherosclerosis complications. Complications of endovascular stent placement include vascular injury, thromboembolism, local delivery site/stent deployment irregularities, and hemorrhage.[129] Infection related to these devices is rare, with an estimated risk being less than 1 in 10,000 cases.[130]

TABLE 82.10 Risk Factors Associated With Peripheral Vascular Stent Infection

Prolonged procedural time
Prolonged use of an indwelling catheter or sheath
Reuse of the same sheath after 24 hours (for thrombolytic therapy)
Local hematoma formation
Multiple interventions on the same or an adjacent site
Use of the same femoral artery for vascular access within 1 week of previous catheterization

Epidemiology

There are limited published data regarding peripheral vascular stent infections. In one review of 65 cases of aortoiliac stent graft infection,[131] 50 had stents placed in the aorta for aneurysm management. In this report estimated prevalence of infection was 0.43% (range, 0.05%–4%). A majority (80%) of patients were male and had multiple comorbid conditions. Almost two-thirds of patients presented more than 4 months after stent graft placement. Clinical manifestations varied and ranged from indolent signs and symptoms to septic embolization and hemorrhagic shock. Aortoenteric fistulas were described in 20 (31%) cases. However, this high rate of fistula formation could be due to case selection and referral biases because only large referral medical centers were included. *S. aureus* was the most commonly isolated pathogen (24 cases, 54.5%).

Proposed risk factors for vascular stent infection are listed in Table 82.10.[10,129,132] However, due to limited number of cases, no risk factors have been validated in a multivariable analysis.

Diagnosis

Blood and operative specimens should be submitted for culture in all cases. CT and angiography can aid in the diagnosis of stent-related infection. The presence of fluid and inflammatory reaction around the stent on a CT scan is suggestive of stent infection. Patients with aortoenteric fistulas typically present with abdominal pain or GI bleeding, or both. Upper GI endoscopy and CTA have been crucial in establishing a diagnosis of this life-threatening complication.

Management and Outcome

The preferred management approach is excision of an infected stent and involved vessel, with extraanatomic revascularization in combination with parenteral antibiotics. In the previously cited review[131] surgical intervention with stent graft excision was done in 50 (82%) of 61 cases in which treatment modalities were described. Reported mortality rate was 14%. Empirical antibiotics should include coverage for *S. aureus,* the most common pathogen isolated.[10,132] Other less frequently reported pathogens include CoNS, *P. aeruginosa,* β-hemolytic streptococci, and, rarely, fungi.[133]

A conservative management strategy using antibiotics alone, without infected stent removal, has been used in a limited number of cases[130,131,132] and is associated with higher failure and mortality rates. This approach should be reserved for patients whose risk of surgery outweighs the potential benefits. In the review cited earlier,[131] conservative management had a higher mortality rate (36%) compared with a combined medical and surgical approach (14% mortality rate; $P = .086$).

Peripheral vascular stent infection can be complicated by pseudoaneurysm formation, arterial necrosis, abscess formation, septic peripheral emboli (requiring limb or digit amputation), and severe sepsis with multiorgan failure. Aortoenteric fistula may develop in patients undergoing endovascular stent-graft repair of abdominal aortic aneurysms.[134] These fistulas generally involve the third or fourth portion of the duodenum. Despite aggressive combined medical and surgical intervention, reported mortality rate has been as high as 40%.[135]

Prevention

Proper sterilization of the insertion site and adherence to other infection control procedures is critically important. Current practice guidelines recommend primary antibiotic prophylaxis at the time of stent deployment. However, secondary prophylaxis for dental, GI, and genitourinary procedures is not recommended.[5]

VASCULAR CLOSURE DEVICES

Percutaneous vascular closure devices (PVCDs) are used for hemostasis of a femoral artery puncture site after catheterization. They increase patient comfort and decrease time to hemostasis and ambulation compared with manual or mechanical compression.

PVCDs can be broadly categorized into two types: those that use various mechanisms of collagen or other substance deposition at an arterial puncture site and others that use a suture to close the arteriotomy site. Complications associated with use of these devices include hematoma, pseudoaneurysm formation, limb ischemia, AV fistula, or access site infection complicated by femoral endarteritis.[136]

Epidemiology and Clinical Manifestations

The reported infectious complication rate ranges from 0.0% to 5.1%. Hematoma formation at the puncture site and the presence of foreign material likely serve as a nidus for infection. In one review of 52 cases of PVCD infections[137] the median incubation time from device insertion to presentation with PVCD-related infection was 8 days (range, 2–29 days). The majority of patients presented with local inflammatory findings at the device deployment site, including pain, erythema, swelling, and drainage. Although fever was reported in only 38% of the cases, blood cultures were positive in the majority (86%) of patients. Infection associated with PVCDs results in localized endarteritis at the insertion site, a risk factor for development of mycotic pseudoaneurysm. In the previously cited review[137] mycotic pseudoaneurysm was diagnosed in 42% of the cases with PVCD infection. Ultrasonography should be performed in suspected cases to confirm the diagnosis.

Microbiology

S. aureus is the single most commonly (up to 75% of the cases) isolated pathogen, followed by gram-negative rods (13%) and CoNS (5%).[137] Other less common pathogens include *Peptostreptococcus, Enterococcus* spp., and *Corynebacterium* spp., frequently in the setting of polymicrobial infection (14% of cases).

Management

Treatment of PVCD-related infection requires surgical débridement in almost all cases, followed by reconstructive surgery in more than 50% of cases.[136,137] Cure rates as high as 90% can be achieved with combined medical and surgical intervention. The reported mortality rate is 6%, and the majority of deaths are due to *S. aureus* sepsis. Choice of antibiotics depends on results of blood and local wound/tissue cultures. Empirical therapy should cover MRSA and gram-negative rods while awaiting culture data. Two to 4 weeks of parenteral therapy is recommended in the majority of cases depending on the causative pathogen (longer therapy recommended for *S. aureus* infections) and clinical response.

Prevention of Percutaneous Vascular Closure Device–Related Infection

Preventive efforts should focus on the use of strict aseptic techniques. There are no clinical trial data on the utility of antibiotic administration at the time of PVCD placement. However, primary prophylaxis has been considered in patients with diabetes mellitus or in those in whom a prosthetic vascular graft puncture site is closed with one of these devices.[10,137] Secondary prophylaxis (e.g., dental procedures) is not recommended.[138]

INTRAAORTIC BALLOON PUMPS

Intraaortic balloon pumps (IABPs) are frequently used for short-term circulatory support in current practice. IABPs are used in a variety of clinical settings, including refractory cardiogenic shock, severe heart failure with reduced ejection fraction, difficulty weaning from cardiopulmonary bypass, refractory myocardial ischemia, and preoperative support in the presence of severe left ventricular dysfunction.[139] Early generations of the IABPs required surgical insertion and removal. However, balloon pumps used in current practice are inserted and removed percutaneously and have lower complication rates compared with early models.

TABLE 82.11 Risk Factors Associated With Intraaortic Balloon Pumps

Emergency insertion in the intensive care unit
Improper preparation of the femoral insertion site
Obesity
Surgical insertion of intraaortic balloon counterpulsation catheters
Duration of intraaortic balloon pump therapy
Presence of additional intravascular monitoring devices
Contaminated water reservoir

Infection is an uncommon but well-recognized complication of IABP insertion. Most infections originate at the insertion site but may track along the pump, resulting in BSI and sepsis. Reported rates of local wound infections and BSI are 5% and 2.2%, respectively.[10] The risk of infection increases with the duration of IABP support.[140] Longer duration of IABP support is associated with higher risk of BSI. In a multivariable analysis risk of IABP-related septicemia increased by an OR of 1.5 for each additional pump day.[141] In another analysis that included 733 patients with IABP insertion by surgical and percutaneous methods,[142] risk of infection was higher (26%) when IABPs were inserted while the patients were in intensive care units, especially in emergency settings, compared with elective placement performed in operating rooms or cardiac catheterization laboratories (12% and 17% infection rate, respectively). Risk factors associated with IABP-related infection are summarized in Table 82.11. Outbreaks of *Pseudomonas cepacia* and *S. marcescens* BSI due to contaminated IABP reservoirs have also been reported.[143,144]

A diagnosis of IABP-related BSI requires isolation of the same organism from blood and local wound cultures. However, most patients are treated presumptively on the basis of local inflammatory findings at the insertion site or positive blood cultures. Local wound débridement and parenteral antibiotics are required in most cases when early removal of IABP is not feasible. The duration of antibiotics is similar to other central catheter-related BSIs. Because of nosocomial acquisition of infection, empirical antibiotics should include coverage of methicillin-resistant staphylococci and MDR gram-negative bacteria while awaiting results of cultures and in vitro susceptibility testing. In a prospective analysis of 60 patients by Crystal and colleagues,[145] IABP-related BSI was not associated with increased mortality.

Preventive efforts should be focused on sterile insertion techniques and adherence to infection control procedures during routine care of the insertion site. Routine use of antibiotic prophylaxis around the time of insertion is not recommended.[10]

VENA CAVA FILTERS

Inferior vena cava (IVC) filters have been in use since the early 1970s, when the Greenfield filter was introduced,[146] and are generally inserted percutaneously via a femoral or jugular approach and positioned infrarenally in most patients. IVC filters are extensively used to prevent pulmonary embolism in patients with thromboembolic disease and a contraindication for or previous failure of anticoagulation.[147]

Although venous thrombosis at the filter site has been frequently reported (20%–40% of the cases), infection of an IVC filter remains a rare complication, with only few suspected cases published to date.[53,148–151] Clinical onset of infection occurred within 10 days of placement in all cases. Infection was confirmed by culture of the explanted device in four cases.[149–151] Blood cultures were positive in five of the six cases, and staphylococcal species were recovered in five patients. Hematogenous seeding of IVC filter with *Candida glabrata* was reported in one case,[151] where patient developed central line–related *C. glabrata* BSI after abdominal surgery for necrotizing pancreatitis. Ultimately, the IVC was removed, and *C. glabrata* was recovered from device cultures. Cure was achieved with device removal and 6 weeks of antifungal therapy.

Removal of an infected IVC filter, followed by 4 weeks of parenteral antimicrobial therapy, is recommended to eradicate infection.[149–151] If the infected IVC filter cannot be removed, long-term antibiotic suppressive therapy is recommended.[53]

DACRON CAROTID PATCHES

Synthetic carotid patches, consisting of either Dacron or PTFE, increase the safety and durability of carotid endarterectomy compared with primary arteriotomy closure. Their use is also associated with reduced risks of stroke, internal carotid thrombosis, and recurrent stenosis as a complication of carotid endarterectomy. Unlike autologous vein patches (mostly harvested from the greater saphenous vein and occasionally from the external jugular vein or common facial vein), synthetic carotid patch do not require a groin incision (to harvest saphenous vein) and do not have the lack of predictability of diameter size that characterizes vein grafts.

Synthetic carotid patch infection is rare.[152,153,154–157,158] The reported rate of infection ranges from 0.26% to 1.76%. The purported risk factors include early postoperative wound complications (hematoma or superficial surgical site infection) and comorbid conditions such as diabetes mellitus.[154,157] Perioperative prophylactic antibiotics are routinely administered for carotid patch placement surgery.[152,154–157,158]

Clinical Manifestations

The time from patch placement to clinical presentation with infection varies, ranging from 10 days to 86 months. However, up to 50% of patients present within 3 months of surgical intervention.[152,154–157,158] Clinical presentation includes fever and localized inflammatory findings at the surgical site, including erythema, warmth, draining sinus, abscess, and pseudoaneurysm formation. Ultrasonography can be helpful in diagnosing pseudoaneurysms. Septic emboli resulting in stroke have also been reported. CT or MRI/MRA may aid in the diagnosis of difficult cases in which florid local manifestations of infection are absent.

S. aureus and *Streptococcus* spp. are the most commonly isolated pathogens in early-onset (<6 months) Dacron carotid patch infections.[152,154–157,158] However, gram-negative bacteria, including *Pseudomonas, Proteus,* and *Bacteroides,* have been occasionally reported. A majority of late-onset (>6 months) carotid patch infections are caused by CoNS.

Management

Resection of the infected foreign material combined with parenteral antibiotics is necessary to eradicate infection. Autologous vein patches or interposition vein grafts are used for reconstruction thereafter. Replacement of an infected prosthetic patch with another Dacron graft should be avoided because it leads to a high rate of reinfection (up to 50% in one series).[155] A 2- to 4-week course of parenteral antibiotics is recommended to treat these infections.

CARDIAC SUTURE LINE INFECTIONS

Left ventriculotomy is performed for several reasons, including resection of aneurysms or scars, antiarrhythmic surgery, and placement of ventricular assist devices. A variety of devices are used to buttress sutures at the line of incision, including autologous or resorbable strips and Teflon pledgets or patches. Despite the high frequency of left ventriculotomy with myocardial suture line support device placement, infection of these devices is rare.[159,160,161] Two published case series[159,160] described 29 patients with cardiac suture line infection. However, this likely represents underreporting because of the difficulty in making a diagnosis due to often prolonged interval (average duration, 16 months) between ventriculotomy and onset of infection stigmata[159,161] and nonspecific clinical presentation. The diagnosis of suture support device infection may not be made until surgical intervention or postmortem examination.

Clinical Manifestations

Three clinical presentations have been appreciated: (1) chest wall or epigastric soft tissue infection, (2) bronchopulmonary infection, and (3) endocardial infection with bacteremia or fungemia. Chest wall or epigastric involvement is seen most commonly and presents as a chronic draining sinus (cardiocutaneous fistula),[161] a subcutaneous mass, or local pain. A syndrome of endocardial infection is the next most common presentation and typically manifests with BSI. If the underlying diagnosis is not appreciated, relapsing bacteremia or fungemia can occur after discontinuation of antimicrobial treatment. Eradication of infection is not achieved until débridement is performed. Bronchopulmonary

presentations (cardiobronchial fistulas) are less common. In this scenario patients present with recurrent hemoptysis, purulent sputum production, bronchiectasis, and pneumonia with or without empyema. Some patients may present with a combination of features that reflects more than one syndrome presentation. Left ventricular false aneurysms commonly develop.[159,160]

Diagnostic evaluation in suspected cases may include echocardiography, CT, and/or left ventricular angiography. Although staphylococcal species account for the majority of cardiac suture line infections, a variety of other organisms, including other skin flora, have been identified.

MANAGEMENT OF CARDIAC SUTURE LINE INFECTIONS

Optimal treatment includes early surgical débridement of infected native and foreign tissues, along with pathogen-specific antimicrobial therapy. Of 29 patients in two case series,[159,160] 23 (79.3%) survived with combined surgical débridement and antimicrobial therapy. In 4 of 6 patients who died, no débridement of the infected ventricular suture line was performed.[159] Preventive strategies include the use of absorbable sutures or autologous pericardium to support a suture line at the time of initial surgery, in addition to strict adherence to infection control procedures.

CLOSURE DEVICE TREATMENT OF PATENT DUCTUS ARTERIOSUS, ATRIAL SEPTAL DEFECT, AND VENTRICULAR SEPTAL DEFECT

Therapeutic cardiac catheterization with placement of closure devices for a variety of congenital defects is being used increasingly with success and avoiding the risks of surgery.[162] Several different devices are in use, and others are under investigation. Overall, complications related to device placement are infrequent, and infectious sequelae have been rare, with scant published reports.[163,164] It can be difficult to determine the interval between device placement and the onset of occlusion device-related infection. Nevertheless, in two reports[163,164] infection occurred less than 3 months after device placement. Pathogens were identified in both cases and included *S. aureus* in one[163] and *Bacillus pumilus*[164] in the other. In both cases device-related vegetations were demonstrated on TTE. After surgical excision of the devices and débridement of infected tissues, followed by administration of parenteral antibiotics over 6 weeks, both infections were eradicated.

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The complete reference list is available online at Expert Consult.

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83 Prevention of Infective Endocarditis

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SHORT VIEW SUMMARY

Definition of the Issue

- Infective endocarditis (IE) continues to cause serious morbidity and mortality; therefore prevention of IE is a priority.

Epidemiology

- The incidence of IE is low, estimated at 3 to 9 cases per 100,000 individuals annually in industrialized countries. However, incidence varies widely according to different patient characteristics, including the prevalence of predisposing cardiac conditions in the population and types of exposures that may give rise to transient bacteremia.
- Contributing factors include aging populations; the steadily increasing number of medical procedures, implants, and prostheses; and the rising frequency of health care–associated infections, especially catheter-related bloodstream infections.

Microbiology

- Not all bacteria are equally capable of adhering to damaged endothelium, as illustrated by the fact that the leading causes of IE are gram-positive bacteria: staphylococci, streptococci, and enterococci.
- These common gram-positive microbes, originating from the oropharynx, the skin, or the gastrointestinal and urinary tracts, all carry surface adhesins that mediate attachment to valvular extracellular host matrix proteins.
- Transient bacteremia due to gram-positive cocci can be induced by common dental or medical procedures, but there also are spontaneous low-grade, asymptomatic bacteremias that originate daily from the oropharynx. Invasive dental procedures may contribute, albeit infrequently, to the development of IE in adults, especially those with prosthetic heart valves.

Interventions to Prevent Infective Endocarditis

- For many years, various prophylactic strategies and guidelines based on antibiotic use have been proposed for patients with cardiac conditions predisposing to IE who undergo invasive procedures that may cause bacteremia. However, because no randomized clinical trials have been performed, proof of efficacy remains lacking. In addition, cost-effectiveness analyses have found conflicting results.
- Consequently, recent guidelines have shifted toward a dramatic reduction in indications for antibiotic prophylaxis of IE. The current approach is to limit antibiotic prophylaxis to individuals at highest risk of IE and highest risk of poor outcome of IE. These categories include patients with a history of IE, a mechanical or a biologic prosthetic valve, or a surgically constructed systemic or pulmonary shunt or conduit.
- For this limited group of high-risk patients, recommended antibiotic prophylaxis regimens remain based on administration of amoxicillin or ampicillin in a single dose administered 30 to 60 minutes before the scheduled procedure in patients with no allergy to penicillins. Patients with allergy to penicillin or ampicillin should receive clindamycin in a single dose of 600 mg in adults and 20 mg/kg in children, orally or intravenously.
- Several studies have now examined the effect of restricting antibiotic prophylaxis on the incidence of IE, with conflicting results. These studies were all observational and therefore cannot establish with certainty a causal link between restriction of antibiotic prophylaxis and increased incidence of IE. Various confounders could account for an increased incidence of IE irrespective of any change in guidelines for antibiotic prophylaxis.
- Beyond antibiotic prophylaxis, other interventions should be considered for prevention of IE. These include maintenance of good general and oral hygiene; prevention of health care–acquired bacteremias; and specific interventions to prevent IE secondary to the implantation of prosthetic valves, cardiac implanted electronic devices, and cardiac assist devices.

Prevention of infective endocarditis (IE) is an important clinical issue because this disease continues to cause serious morbidity and mortality. In developed countries, the annual number of cases has risen appreciably over the past years.[1,2] The incidence of IE in the United States was reported in 2013 to be approximately 12 cases per 100,000 persons per year, more than double previous estimates.[2] Advances in diagnosis and treatment clearly have improved outcomes in some subgroups, yet the overall long-term mortality of IE is only modestly better than it was 50 years ago.[3] Although the causative organism usually can be eradicated with antibiotics, such "microbiologic cure" often does not prevent permanent cardiac valvular damage and other sequelae, such as recurrent IE, which can impair quality of life, necessitate valve surgery, and reduce life span. In recent large series, 75% to 85% of patients survived the active phase of endocardial infection,[2,4] but survival rates at 1 year and longer are notably lower.[5,6] For example, overall survival at 4 years in one series was only 67%.[6] Furthermore, the increasing prevalence of antibiotic resistance among the common gram-positive cocci that cause most cases of endocarditis makes treatment more difficult.[7]

Thus, effective measures to prevent IE are clearly desirable. The most obvious potential intervention is administration of antibiotics, but despite years of study, this long-standing practice has never been proven to be effective in humans.[8] For this reason, the most recent practice guidelines recommend eliminating attempted antibiotic prophylaxis for IE in most settings, or even entirely.[9,10,11]

PATHOGENESIS OF INFECTIVE ENDOCARDITIS IN THE CONTEXT OF PREVENTION

The pathogenesis of IE is reviewed in detail in Chapter 80. The theoretical foundation for interventions intended to prevent IE is based on the following elements of pathogenesis. It is accepted that normal valve endothelium is naturally resistant to colonization by bacteria. In the conventional model of native valve endocarditis, the development of endocarditis results from colonization of previously damaged valve endothelium by bacteria that circulate in the bloodstream and have specific adherence properties. Endothelial damage may result from so-called jet lesions due to turbulent blood flow, or may be provoked by electrodes, catheters, or repeated intravenous injections of particulate matter in intravenous drug users. Chronic inflammation from rheumatic carditis and degenerative valve lesions in the elderly[12] also predispose to IE. Lesions of the valvular endothelium result in the exposure of underlying extracellular matrix proteins, the production of tissue factor,

and the deposition of fibrin and platelets as part of the attempted repair process. The resulting initial nidus of nonbacterial thrombotic endocarditis offers optimal conditions for adherence to some species of circulating bacteria.[13] Not all bacteria are equally capable of adhering to damaged endothelium, as illustrated by the fact that the leading causes of IE are gram-positive bacteria: staphylococci, streptococci, and enterococci.[3,14] These common gram-positive microbes, originating from the oropharynx, the skin, or the gastrointestinal tract, all carry surface adhesins that mediate attachment to extracellular host matrix proteins. In contrast, IE due to gram-negative bacteria is relatively rare.

Low-grade, asymptomatic bacteremia due to gram-positive cocci from the oropharynx occurs commonly, often daily.[15–17] Transient bacteremia can also be induced by common dental or medical procedures.[15,18,19] Studies have confirmed that invasive dental procedures may contribute, albeit infrequently, to the development of IE in adults,[20] especially in those with prosthetic heart valves.[21] Furthermore, health care–associated bacteremias due to gram-positive cocci, especially staphylococci, are common and have become an increasingly important risk factor for IE.[2,3]

ESTIMATION OF THE RISK FOR INFECTIVE ENDOCARDITIS

The incidence of IE is low, estimated at three to nine cases per 100,000 individuals annually in industrialized countries.[3,22–25] However, this incidence depends on the prevalence of predisposing cardiac conditions (PCCs) in the population and the number of factors that may give rise to transient bacteremia. The prevalence of PCCs for which prophylaxis is generally recommended is much higher than formerly thought. Previously unknown PCCs detected with systematic echocardiographic surveys are now estimated to exist in 2.5% (95% CI, 2.2%–2.7%) of the general population of the United States.[26] The prevalence of PCCs increases with age, to 13.3% (95% CI, 11.7%–15.0%) in individuals older than 75 years. The prevalence of previously known PCCs has been estimated to be 1.8% in the United States in the general population[26] and 3.3% in the 25- to 84-year-old French population.[27] Furthermore, it has been estimated that approximately 2.1 at-risk dental procedures are performed per patient per year.[27] The risk of developing IE in patients with PCCs after an at-risk dental "unprotected procedure" appears to be extremely low. A French study estimated the risk to be 1 in 54,300 in adults with native valve PCC, and 1 in 10,700 in adults with prosthetic valves.[27] The risks reported in a US study were even lower (one case of IE per 14 million dental procedures).[28]

Procedures at Risk for Causing Infective Endocarditis

The identification of procedures at risk for causing IE includes a consideration of several factors: (1) the number of different species of IE-inducing pathogens that are colonizing the site involved in an invasive procedure and that might enter the bloodstream, (2) the amount of bleeding induced by the procedure, (3) the frequency of positive blood cultures after a given procedure (Table 83.1), (4) the magnitude of the bacteremia after the procedure (inoculum size), (5) the duration of the bacteremia, and (6) the number of cases of IE that have been reported after any given procedure. However, there is wide variation in the reported frequencies of all these factors. For example, bacteremia is noted in 10% to 95% of patients after tooth extraction, a wide range that probably reflects the heterogeneity of these dental procedures, the varying dental status and immunity of the host, and the detection methods used.[29–33] Given the heterogeneity of these data, it is difficult to clearly establish definitive criteria for risk factors. A better knowledge of the factors predisposing to IE is necessary. Given that transient asymptomatic bacteremias that occur after tooth brushing, flossing, or chewing are common in everyday life, and given the high prevalence of PCCs in the general population, the incidence of IE is astonishingly low. Genetic host susceptibilities allowing or promoting attachment of microorganisms to valvular endothelium probably exist; their identification could allow prophylactic measures to be focused on those patients at highest risk.

On the other hand, the rate of bacteremia after at-risk procedures has been used as a surrogate measure of the risk for IE, and for selecting procedures requiring antibiotic prophylaxis. However, there is no evidence-based method to decide which procedures should be covered by antibiotic prophylaxis because there is no proof that the incidence, the magnitude, or the duration of bacteremia after a procedure increases the risk of IE. Therefore, use of these factors to identify procedures that warrant prophylaxis is somewhat artificial; nevertheless, it is the method usually used. This has resulted in complex and detailed lists of dental and other procedures for which prophylaxis is or is not recommended. The 2006 British Society for Antimicrobial Chemotherapy (BSAC) broke with this practice and introduced the notion of a general classification of "dental procedures involving dento-gingival manipulation" for which antibiotic prophylaxis is indicated.[34] Current guidelines that recommend antibiotic prophylaxis before certain dental procedures specify that antibiotic prophylaxis should be given for "dental procedures requiring the manipulation of the gingival or periapical region or the teeth or perforation of the oral mucosa (except for local anesthesia)."[10,35]

TABLE 83.1 Ranges of Positive Blood Samples After Various Oral Procedures

REFERENCES	ORAL PROCEDURES	POSITIVE BLOOD CULTURES (RANGE), %
172–177	Mastication	17–51
	Toothbrushing or irrigation	0–50
	Flossing	20–60
	Dental examination	17
173, 177–179	Dental polishing	24
	Intraligamentary local anesthetic injection	97
	Matrix band placement	32
	Rubber dam placement	30
	Slow drill	12
	Fast drill	4
29–33, 172, 180, 181	Single dental extraction	18–94
	Multiple dental extraction	10–85
172	Scaling	17–70
172, 182	Periodontal surgery	32–88
172, 182	Endodontic instrumentation	20–42
183	Postoperative suture removal	5
182, 183	Endodontic treatment	42

Preexisting Cardiac Conditions

The risk of occurrence of IE in any given case of PCC is most often indirectly estimated by comparing the frequency of different cardiac diseases in patients with IE versus its frequency in the general population (Table 83.2).[36] It has also been estimated by monitoring cohorts of cardiac patients and calculating the incidence of IE cases in this population, thus determining the lifetime risk of acquisition of IE associated with a specific PCC.[37–41,42,43,44] Recent reports have also used administrative databases to estimate this incidence.[21,45,46]

To determine which patients should receive prophylaxis, it is necessary to choose a threshold above which the risk for IE is considered to be significant. According to this gradation of risk, patients with a history of IE, a mechanical or biologic prosthetic valve, or a surgically constructed systemic or pulmonary shunt or conduit are the patients with the highest incidence of IE and therefore considered at highest risk of developing IE during their lifetime. This risk gradation is now widely accepted.[34,47,48]

The prognosis if IE were to occur in a given case of PCC must also be taken into account when choosing which PCCs should be covered. IE on prosthetic valves and in patients with a previous history of IE have poorer outcomes and higher risk of death. Other characteristics of patients' general health background also have been associated with in-hospital death during IE: old age, diabetes mellitus (primarily insulin-dependent diabetes), immunodeficiency, dialysis, and hepatic insufficiency.[49–52] These considerations were used in most guidelines to identify patients with worse prognosis should they develop IE (Table 83.3). This led US guidelines to recommend antibiotic prophylaxis for

TABLE 83.2 Estimates of Rates and Relative Risk for Infective Endocarditis (IE) Associated With Demographics and Selected Preexisting Cardiac Conditions

RISK FACTOR	RATE OF IE PER 10^5 PERSON-YEARS	RELATIVE RISK
None (reference group)	3–12	1.0
Mitral valve prolapse without murmur	3–12	1
Male gender	8–25	2.5
Age >65 yr	30–120	9
Mitral valve prolapse with murmur, thickening, and/or redundancy	45–180	15
Any cardiac valvular abnormality	50–200	17
Aortic stenosis	130–520	45
Ventricular septal defect (uncorrected)	150–800	50
Ventricular septal defect (corrected)	30–120	10
Congenital heart disease (many types)	15–600	5–55
Chronic rheumatic heart disease	100–400	35
Previous infective endocarditis	110–440	37
Prosthetic cardiac valve	225–900	75

Note that these are approximations based on multiple studies and reviews. These estimates could change with time as new studies are conducted, and they could be subject to significant error. Interpretation of these data should be considered in this context.
Modified from references 2, 36, 141, and 184 and J.M. Steckelberg (personal communication, 2009).

TABLE 83.3 Predisposing Cardiac Conditions According to the Risk to Develop Endocarditis

High-Risk Predisposing Cardiac Conditions

Previous infective endocarditis
Mechanical or biologic prosthetic valves
Surgically constructed systemic or pulmonary shunt or conduit
Complex cyanotic congenital heart disease (single ventricle states, transposition of the great arteries, tetralogy of Fallot)

Moderate-Risk Predisposing Cardiac Conditions

Most other congenital cardiac malformations (except isolated secundum atrial septal defect with no increased risk)
Acquired valvular dysfunction (e.g., rheumatic heart disease)
Hypertrophic cardiomyopathy
Mitral valve prolapse with valvular regurgitation and/or thickened leaflets

IE in cardiac transplant recipients with valve regurgitation due to a structurally abnormal valve.

Indirect Evidence Regarding the Risk for Infective Endocarditis Posed by Dental Procedures and the Efficacy of Prophylaxis

Because the implementation of a very large randomized clinical trial to assess the effectiveness of antibiotic prophylaxis has been regarded as unfeasible to date, indirect evidence has been sought.

Cohort Studies

There are few cohort studies in the literature that estimated the risk of IE after invasive procedures, because the number of patients needed to be enrolled to make such a study possible is very high. Furthermore, the time window during which an IE case could be reasonably considered as the consequence of an invasive procedure is unknown, given the fact that the incubation period between the microbial colonization of the endocardium and the first symptoms is not known precisely, but is likely quite short.[53] In two recent studies based on the analysis of administrative databases and considering the 3 months after dental care as the incubation period, authors did not find a statistically significant reduction of IE incidence in patients receiving antibiotic prophylaxis before dental procedures.[21]

Case-Control Studies

The role of dental procedures in the genesis of IE can also be evaluated indirectly in large series of IE or in case-control studies. In both cases, only a small minority of IE cases due to oral streptococci are preceded by an invasive dental procedure: 2.7% to 5% of cases in several epidemiologic series.[20,21,27,36,54] However, even in the case of a close temporal relationship between a dental procedure and the development of IE, it is not possible to determine with certainty whether the bacteremia that caused IE was induced by the procedure, or caused by dental disease predating the procedure, or due to a spontaneous daily bacteremia. This very low risk level probably explains why some case-control studies failed to identify any clear relationship between the onset of IE and preceding "at-risk" dental procedures, whereas others did; however, none have definitively proven the efficacy of antibiotic prophylaxis.[20,36,54–56] In a recent case-control study, authors reported using a population-attributable risk analysis that indicated that dental procedures within the preceding 3 months accounted for less than 20% of streptococcal IE cases, whereas incorrect and/or lack of oral hygiene habits accounted for two-thirds of them.[20]

Using a case-crossover design in a French administrative database, Tubiana and colleagues reported that the number of invasive dental procedures within the 3 months preceding oral streptococcal IE was significantly higher than during the same preceding control period in individuals with prosthetic cardiac valves (5.1% vs. 3.2%; odds ratio [OR], 1.66; 95% confidence interval [CI], 1.05–2.63).[21] Despite including 137,000 patients with prosthetic valves, the study was not sufficiently powered to prove the efficacy of antibiotic prophylaxis. A similar result has also been reported by others.[46]

In Vitro Antibiotic Susceptibility Studies

It is well known that the frequency and degree of antibiotic resistance among common bacterial pathogens has increased extensively worldwide and continues to increase. This topic is reviewed in Chapter 18 and will not be recapitulated here. However, it is obvious that increased antibiotic resistance among bacteria causing IE would almost certainly further impair the efficacy (if any) of attempted prevention of IE with antibiotics.

Experimental Infective Endocarditis

Studies in animal models have provided an important secondary source of information on the mechanisms and efficacy of prevention of IE. When a polyethylene catheter was passed into the right or left heart chamber of living rabbits, small sterile vegetations composed of platelets and fibrin developed at points of contact between the catheter and endocardium.[57] When staphylococci were placed in the lumen of the catheter, staphylococcal endocarditis resulted. Modifying this experimental model for IE by injecting organisms intravenously after intracardiac catheter placement has provided a convenient in vivo system for examining the efficacy of various antibiotic regimens for the prophylaxis of endocarditis.[58–62] A similar model in rats has also been used extensively to investigate antibiotic prophylaxis.[63,64] Under experimental conditions, the time of onset of IE is known exactly. Another important advantage is that the incidence of infection in untreated animals can be adjusted easily by altering the size of the inoculum; therefore the problem of very low infection rates can be overcome by choosing an inoculum of the test organism that is large enough to infect many or all of the experimental animals. Significant differences among antibiotic regimens being tested for prevention or treatment of IE can then be demonstrated with use of manageable numbers of animals in each group.[64–73]

Later experiments showed that a very-low-inoculum bacteremia, if sufficiently prolonged, also often caused IE in predisposed rats.[74] These

experimental conditions resemble the frequent low-level bacteremias originating from the oral cavity that are known to occur in the normal daily life of humans. They demonstrate that the area under the curve can be as important as the peak level of bacteremia in causing IE.

Early experiments using high inocula compared the efficacy of various antibiotic regimens against viridans-group streptococci in these model systems, proving that penicillin in a high sustained dose could prevent IE; that a synergistic combination of a penicillin plus an aminoglycoside was even more effective in preventing IE caused by viridans-group streptococci or enterococci; and that vancomycin provided an effective alternative to regimens that included penicillins.[58,60,61,69] Other antibiotics proven capable of preventing IE under controlled experimental conditions include ampicillin, amoxicillin,[66,73,75] ampicillin-sulbactam,[71] erythromycin,[73] clindamycin,[70] rifampin,[76] and azithromycin or clarithromycin.[72] In early animal studies, bacteriostatic antibiotics such as tetracycline usually failed to prevent IE, but further experiments have modified the view that bactericidal activity is essential for prophylaxis.[77] Streptomycin proved surprisingly effective in the prevention of experimental IE for some strains of enterococci, even though the concentration of streptomycin in serum was far too low to kill them.[58] Subinhibitory concentrations of certain antibiotics, especially vancomycin, can inhibit the adherence of streptococci to fibrin surfaces in vitro. Other experiments have demonstrated successful prophylaxis for various streptococci with sublethal doses of vancomycin, clindamycin, erythromycin, and even a tetracycline.[69,70,73] Penicillin was less effective in the prevention of experimental streptococcal endocarditis if the strain was tolerant to penicillin (i.e., inhibited but not killed).[78] However, penicillin retained some prophylactic activity even if the strain was so tolerant that bactericidal concentrations of penicillin could not be achieved in serum. These findings all suggest that prevention of IE in animals can sometimes be achieved by antibiotic effects that fall short of total bacterial killing, perhaps by an alteration of surface structures that mediate adherence to fibrin or by other unknown mechanisms. Thus, bactericidal action may be sufficient but not necessary for prevention of endocarditis.

The experimental observation that successful prophylaxis with amoxicillin can be reversed by administration of penicillinase shortly after colonization of the vegetation shows that neither killing of organisms in the bloodstream nor prevention of their adherence to the endocardium is essential for successful antibiotic prophylaxis.[64]

The implications of these extensive experimental findings for humans are uncertain. To place the experimental data in perspective, it should be emphasized that direct extrapolation of findings in animals to humans may not be meaningful. Although experiments in living animals provide a closer simulation of human endocarditis than any in vitro system, there are two important differences. First, a foreign body was present throughout many of these experiments because an intracardiac catheter often was left in place. The presence of a foreign body markedly lowers the inoculum required to initiate infection, and then makes that infection harder to eradicate. Therefore animal models probably simulate patients with prosthetic valves or pacemakers more closely than patients with congenital or rheumatic valvular disease. Second, in many of the experiments, a high inoculum was chosen deliberately to make statistical comparisons possible with a relatively small number of animals. Because both the presence of a foreign body and the use of high inoculum tend to make prevention harder to achieve, it is entirely possible that an antibiotic regimen that failed in animals might succeed in humans. Conversely, any regimen that proved effective under these challenging experimental conditions would be likely to provide a wide margin of effectiveness in clinical use. In summary, studies of prevention in animals have proven beyond doubt that antibiotics can prevent IE in vivo. They have provided insights into the mechanisms whereby different antibiotics and dosage regimens achieve prevention of IE, and they have provided a convenient in vivo method for ranking prophylactic antibiotic regimens in order of efficacy. For example, they support the conclusion that optimal bactericidal regimens, such as a penicillin plus an aminoglycoside or vancomycin, should provide a wider margin of effectiveness than a lower-ranking regimen, such as erythromycin or tetracycline.[60] However, they cannot prove whether any particular antibiotic regimen will or will not prevent endocarditis in humans.

Recent experiments using prolonged, low-inoculum bacteremias in predisposed rats showed that some antiplatelet and antithrombin drugs can prevent IE due to gram-positive cocci.[79] This suggests that long-term antiaggregant and/or anticoagulant therapy, which is already routinely used for some cardiovascular diseases, might also prevent IE.

Going forward, experimental models should continue to be useful for the initial evaluation of novel approaches to prevent IE—for example, new antibiotics, new anticoagulants and antiaggregants, new vaccines, and new interventions to prevent biofilm formation.

POTENTIAL INTERVENTIONS TO PREVENT ENDOCARDITIS

Correction of Predisposing Cardiac Conditions

Adult congenital heart diseases (ACHDs) are well-known predisposing factors for IE. Some congenital abnormalities that predispose to IE may be corrected. Surgical advancements have benefited many patients with ACHD and consequently reduced their risk of IE; on the other hand, prosthetic material inserted at surgery may constitute a nidus that favors attachment of circulating bacteria. In this population, IE incidence has been reported to be 133 cases per 100,000 person-years.[42] Valve-containing prosthetics were independently associated with greater short- and long-term risk after implantation (0–6 months—hazard ratio [HR], 17.29 [95% CI, 7.34–40.70]; 6–12 months—HR, 15.91 [95% CI, 6.76–37.45]; beyond 12 months—HR, 5.26 [95% CI, 3.52–7.86]).[42] Non–valve-containing prosthetics, including valve repair, appear to predispose to IE only in the first 6 months after implantation (HR, 3.34 [95% CI, 1.33–8.41]), but not thereafter.[42] These results are consistent with the findings of other studies that also showed that the incidence of IE in ACHD was lower than in patients with a history of IE or in those with a prosthetic heart valve [42,43,80]

Minimization of Portals of Entry for Microorganisms

Most cases of IE are caused by gram-positive cocci that originate from the skin, the oral cavity, or the gastrointestinal or urinary tracts. Whereas the oral cavity is the portal of entry for oral streptococcal bacteremia, improvement in general oral hygiene is probably responsible in part for the decrease in streptococcal IE in industrialized countries.[81] Invasive dental procedures have been reported to cause higher rates of bacteremia in individuals with poor oral hygiene who undergo tooth extraction.[82] The rate of IE patients with poor orodental status is higher, even in those with IE due to nonoral microorganisms.[20] Conversely, tooth brushing three times daily or more after meals in the absence of invasive dental hygiene such as use of toothpicks, dental water jets, flossing, and interdental brushes has been associated with a decreased risk of streptococcal IE.[20] Given the low rate of IE after invasive dental procedures, improving oral hygiene and eliminating incorrect oral hygiene habits may be the most efficient interventions to reduce the incidence of streptococcal IE.

Cutaneous portals of entry have increased in number as medical progress has increased the number of percutaneous procedures. Venous or arterial catheters are a major source for staphylococcal bacteraemia.[83] *Staphylococcus aureus* bacteremia is associated with all types of catheters, short term or long term, surgically inserted or peripheral, tunneled or nontunneled. The incidence of bloodstream infections as a complication of intravascular catheter use varies considerably with the type of catheter, the frequency of catheter manipulation, and patient-related factors. The highest risk, found by Maki and colleagues in a meta-analysis of 200 prospective studies, was associated with short-term noncuffed and nonmedicated central venous catheters (2.7 per 1000 catheter-days). For long-term cuffed and tunneled hemodialysis catheters, the incidence rate was 1.6 intravascular device–related bloodstream infections per 1000 intravascular device–days.[84] Chronic intravenous access was a common predisposing factor in the International Collaboration on Endocarditis (ICE)–Prospective Cohort Study (PCS), around 10% of IE cases, with evidence of geographic differences. For European countries involved in ICE-PCS, chronic intravenous access accounted for 5% of the predisposing conditions; it accounted for 25% in North America.[14] The infrequent use of native arteriovenous fistulas in the United States

as compared with their more frequent use in Europe (24% vs. 80%) may explain this difference.[85]

Aging of the population is associated with a higher prevalence of colonic tumors, which are a proven portal of entry of *Streptococcus bovis* IE, and to a lesser extent enterococcal IE.[86] The search for portals of entry and their eradication is warranted in IE patients to reduce the rate of recurrent IE episodes.[87]

Prevention of Health Care–Associated Infections

Health care–associated infective endocarditis (HCAIE) today accounts for an increasing proportion of IE cases—currently approximately one-third[3]—and therefore requires specific strategies for prevention. The affected population has medical conditions, interventions, and treatments that may cause bacteremia, and most patients have either degenerative valve disease or no intrinsic cardiac risk factors. The most frequent risk factors for HCAIE are hemodialysis, cancer, diabetes mellitus, and the presence of a cardiovascular implantable electronic device (CIED), but there are many others.[3,88] *S. aureus* is the causative organism in approximately one-third of cases.[3,88] The in-hospital mortality in patients with HCAIE is significantly higher than in those with community-acquired infection (31.1% vs. 20.3%; $P < .01$).[3]

S. aureus is the second most frequent microorganism causing bacteremia in industrialized countries and the first or second most frequent to cause IE, depending on country.[14] Asymptomatic carriage of *S. aureus* has been associated with the occurrence of bacteremia. In the United States, 10% to 20% of the population carries *S. aureus* persistently, and others carry it transiently.[89] Interventions to change practice and improve adherence to preventive procedures such as hand hygiene, barrier precautions, and antisepsis have already significantly reduced rates of central line–associated bloodstream infections.[90]

Administration of Systemic Antibiotics

For many years, various prophylactic strategies and guidelines based on antibiotic use have been proposed for patients with cardiac conditions that predispose to IE who undergo invasive procedures that may cause bacteremia.[10,35] Because no randomized clinical trials have been performed, proof of efficacy remains lacking,[36,55,91] and consequently more recent guidelines have shifted toward a dramatic reduction in indications for antibiotic prophylaxis of IE.[11,35,47,92] An additional reason to reduce or even abandon IE prophylaxis is the demonstration that "everyday low-level bacteremias" that occur after tooth brushing, flossing, or chewing likely far outweigh post–dental procedure bacteremias as a risk for IE.[8,93–96] The capability for a sustained low-level bacteremia—as a surrogate for everyday-life bacteremia—to induce IE has been confirmed experimentally, with 70% to 100% of animals developing IE, depending on the species of microorganism and the inoculum size.[74] On the other hand, when considering the high number of patients with PCCs for IE (2.5% of the general US population, 1.7% of the French population, and 7% of people older than 60 years) who are exposed to daily repeated bacteremia capable of inducing IE, the rarity of IE is striking.[27,95]

As the effectiveness of preventive antibiotic treatment remains to be proven, recommendations originating from different countries diverge widely with regard to the patient population and circumstances in which antibiotic prophylaxis should be administered. Since 2008, antibiotic prophylaxis is no longer recommended in the United Kingdom and Sweden.[97] The 2007 US[9] and the 2009/2015 European guidelines[10,92] recommend antibiotic prophylaxis only in patients with prosthetic heart valves, a history of IE, or congenital cyanotic heart disease who are undergoing invasive dental procedures.

EVOLUTION OF CLINICAL PRACTICE IN PREVENTION OF INFECTIVE ENDOCARDITIS

Historical Trends Over 7 Decades

Early strategies to prevent IE focused on bacteremias that were known to result from dental extractions. Initial trials showed that penicillin reduced the incidence of bacteremia after dental extraction. This led the American Heart Association (AHA) to recommend penicillin prophylaxis for patients with rheumatic heart disease and congenital heart disease in 1954. Between 1954 and 2002, AHA guidelines on prevention of IE were periodically updated, and the range of indications for antibiotic prophylaxis was extended beyond dental procedures to procedures involving the urinary and gastrointestinal tract, if such procedures were known to induce bacteremias. This came to a halt in 2002, when the French IE prophylaxis guidelines dramatically reduced the indications for prophylaxis, limiting them to patients at high risk of IE.[47] Between 2007 and 2009, guidelines in the United States and Europe also restricted the indications for antibiotic prophylaxis of IE.[9,92] Several facts explain this evolution of practice. First, no randomized controlled trial of antibiotic prophylaxis had ever been conducted to confirm the efficacy and assess the risk-benefit ratio of this intervention. Second, substantial evidence was presented that transient bacteremia is common with normal daily activities such as tooth brushing, flossing, and chewing food, which in all likelihood cumulatively contribute more to the risk of IE than brief dental procedures.[35,74,98] Third, the efficacy of antibiotic prophylaxis has been questioned in case-control studies.[36,55,91] Fourth, although rare, side effects including severe and sometimes fatal anaphylaxis can result from antibiotic prophylaxis.[99–102] Finally, widespread antibiotic use contributes to the emergence of antibiotic resistance and affects the microbiome, which may in turn increase the risk of diseases such as diabetes.[103] In an extreme step, the UK National Institute for Health and Care Excellence (NICE) recommended in 2008 that antibiotic prophylaxis be abandoned in all patients for all procedures.[97] It should be emphasized that these changes are not based on any new evidence that antibiotic prophylaxis does not work. Nor are they based on any new evidence that dental and medical procedures do not, on rare occasions, cause IE. The NICE guidelines were based on three primary considerations: First, very few cases of IE are caused by medical or dental procedures[36,54,104]; second, there are no evidence-based data demonstrating efficacy of antibiotic prophylaxis in humans; and third, cost-effectiveness and cost-benefit calculations have not demonstrated convincing benefits.[105,106]

Several studies have now examined the effect of restricting antibiotic prophylaxis on the incidence of IE. In France, where antibiotic prophylaxis was restricted to high-risk subjects in 2002, successive surveys conducted in 1991, 1999, and 2008 showed that the annual incidence of IE was stable at 35, 33, and 32 cases per million, respectively, suggesting no significant change after restriction of antibiotic prophylaxis. Of note, the number of cases caused by oral streptococci was also stable.[107] In the United States, using data from the Rochester Epidemiology Project, De Simone and colleagues found no change in the incidence of IE due to oral streptococci before and after the 2007 guideline change.[108,109,110] In contrast, two nationwide epidemiologic studies from the United States and the United Kingdom brought conflicting results. Using the Nationwide Inpatient Sample, Pant and colleagues identified a statistically significant increase in the incidence of IE caused by streptococci, whereas there was no significant change in the incidence of staphylococcal IE.[111] However, this study did not perform change point analysis to confirm that the incidence change coincided with the guideline change in 2007, and did not have access to antibiotic prophylaxis prescribing data to confirm that this had declined. In addition, enterococci were included in the streptococcal category, so the apparent increase in streptococcal IE might be due in part to rising rates of enterococcal IE.[112]

In the United Kingdom, the first analysis after the 2008 NICE guidelines showed no rising incidence of IE.[1] In 2015, though, an extended analysis from the same group showed that antibiotic prophylaxis dropped from 10,900 prescriptions per month to 2236 prescriptions per month after NICE guidelines were introduced, while in parallel the number of IE cases increased by 0.11 cases per 1 million persons per month, which translates to an excess of about 35 cases per month. Statistical analysis identified June 2008, which was 3 months after implementation of the new guidelines recommending nonuse of antibiotic prophylaxis, as the change point. However, the accuracy of the calculation of the change point date has been criticized,[113] and it was not possible to confirm that these cases were mostly due to oral streptococci because microbiologic data were unavailable.[114] Another study that used the Canadian Institute for Health Information Discharge Abstract Database to identify all hospitalizations between April 2002 and March 2013 in which IE was a primary diagnosis showed that although the incidence

TABLE 83.4 2014 American Heart Association (AHA) and 2015 European Society of Cardiology (ESC) Guidelines for Antibiotic Prophylaxis of Infective Endocarditis (IE)

INDICATION	AHA GUIDELINES 2014	ESC GUIDELINES 2015
Patient population	1. Patients with prosthetic cardiac valves 2. Patients with previous IE 3. Cardiac transplant recipients with valve regurgitation due to a structurally abnormal valve 4. Patients with congenital heart disease (CHD), including: a. Unrepaired cyanotic CHD, including palliative shunts and conduits; or b. Completely repaired CHD repaired with prosthetic material or device, whether placed by surgery or catheter intervention, during the first 6 months after the procedure; or c. Repaired CHD with residual defects at the site or adjacent to the site of a prosthetic patch or prosthetic device	1. Patients with any prosthetic valve, including a transcatheter valve, or those in whom any prosthetic material was used for cardiac valve repair 2. Patients with a previous episode of IE 3. Patients with congenital heart disease (CHD): a. Any type of cyanotic CHD b. Any type of CHD repaired with a prosthetic material, whether placed surgically or by percutaneous techniques, up to 6 months after the procedure or lifelong if residual shunt or valvular regurgitation remains
Procedure	Dental procedures that involve manipulation of gingival tissue, manipulation of the periapical region of teeth, or perforation of the oral mucosa	Dental procedures requiring manipulation of the gingival or periapical region of the teeth or perforation of the oral mucosa; the latter does not include local anesthetic injections in noninfected tissues

of IE hospitalizations increased throughout the time period, and slightly more after the AHA guidelines were released in 2007, these guidelines had no significant impact on the incidence of IE hospitalizations.[115] By contrast, a nationwide retrospective trend study conducted in the Netherlands using the national health care insurance database to assess the potential impact of the European Society of Cardiology (ESC) guidelines of 2009 by analyzing changes in the incidence of IE between 2005 and 2011 showed that the incidence of IE increased significantly above the projected historical trend after 2009, especially for the subgroup of streptococcal IE.[116]

Two studies also evaluated the impact of the 2007 AHA guidelines on the overall incidence of IE in children in the United States.[117,118] Both failed to show any impact of the new guidelines, although in one study a significant trend toward increased incidence of IE was observed for oral streptococcal IE in the 10- to 17-year-old group.[118]

Current Guidelines

The data summarized earlier are observational and therefore cannot establish with certainty that there is any causal link between restriction of antibiotic prophylaxis and incidence of IE. Various confounders could account for an increased incidence of IE irrespective of any change in guidelines for antibiotic prophylaxis. The change could be caused by an increased number of individuals at high risk of IE, resulting from population aging, increased numbers of patients with implanted intracardiac devices, or increased prevalence of diabetes mellitus or chronic dialysis.[119] Although a randomized clinical trial aimed at evaluating the efficacy of antibiotic prophylaxis of IE is highly desirable, it is also unlikely to be conducted in the near future, if ever. Therefore the current approach, reflected by both current AHA[120] and ESC[10] guidelines, is to limit antibiotic prophylaxis to individuals at highest risk of IE and highest risk of poor outcome of IE. These guidelines are summarized in Table 83.4. Prophylaxis for gastrointestinal, genitourinary, or orthopedic procedures specifically to prevent IE is no longer recommended, although it might be given to prevent other infections. Dental prophylaxis is recommended only for procedures associated with a high rate of bacteremia in that subgroup of subjects with highest risk of developing IE. This conservative pragmatic approach balances the risks and benefits of individual and population antibiotic use.

Timing, Dosage and Duration of Antibiotic Prophylaxis

Recommended antibiotic prophylaxis regimens for high-risk dental procedures in high-risk patients have not changed recently, still being based on administration of either amoxicillin or ampicillin in a single dose of 2 g in adults and 50 mg/kg in children, administered orally or intravenously 30 to 60 minutes before the scheduled procedure in patients with no allergy to penicillin or ampicillin. Patients with allergy to penicillin or ampicillin should receive clindamycin in a single dose of 600 mg in adults and 20 mg/kg in children, orally or intravenously (Table 83.5).[10]

TABLE 83.5 Regimens for Antibiotic Prophylaxis of Infective Endocarditis During Dental Procedures

CLINICAL SITUATION	ANTIBIOTIC	DOSE FOR ADULTS[b]	DOSAGE FOR CHILDREN[b,c]
Standard oral regimen—for most patients	Amoxicillin	2.0 g PO	50 mg/kg PO
If unable to take oral medication	Ampicillin	2.0 g IM or IV	50 mg/kg IM or IV
If allergic to penicillins	Clindamycin	600 mg PO	20 mg/kg PO
If allergic to penicillins and unable to take oral medication	Clindamycin	600 mg IM or IV	20 mg/kg IM or IV

[a]Only for patients with highest risk of adverse outcome of infective endocarditis.
[b]Single dose, 30 to 60 minutes before procedure.
[c]Not to exceed adult dose.

COST-BENEFIT AND COST-EFFECTIVENESS ANALYSES

Cost-benefit and cost-effectiveness analyses must be based on the assumption that prophylaxis is efficacious even though, as previously stated, this has not been proven.[121] Current cost-benefit and cost-effectiveness analyses have been based on data coming from studies with low levels of evidence and have found conflicting results.[105,106,122,123] Given the low price of amoxicillin, ampicillin, or clindamycin in prophylactic doses, and the high morbidity and mortality burden of IE, even a low efficacy of prevention would be cost-effective, provided that the cost of potential antibiotic adverse effects is also low. Data have indicated a very low incidence of adverse reactions to amoxicillin: 0 and 22.6 fatal and nonfatal adverse drug reaction per million prescriptions, respectively,[106] which is much lower than the previously reported rate of 10 to 30 deaths per million prescriptions.[124–126] This would favor the likelihood that antibiotic prophylaxis for high-risk individuals is cost-effective and even cost-saving.[106] However, the possible impact of prophylactic antibiotics on the acquisition of resistant bacteria among the commensal microbiota is uncertain and therefore has not thus far been taken into account in cost-benefit and cost-effectiveness analyses of antibiotic prophylaxis.

SPECIFIC ISSUES IN PREVENTION OF INFECTIVE ENDOCARDITIS

Prevention of Health Care–Associated Infective Endocarditis

In addition to uncertainty regarding the efficacy of antibiotic prophylaxis of IE of dental origin, it should be taken into account that the

epidemiologic profile of IE has changed substantially since the 1960s, with a striking decrease in the proportion of IE due to oral streptococci. Currently, IE occurs most often in relatively elderly patients, with no known predisposing condition in 50% of the cases, and is most often caused by staphylococci—both *S. aureus* and coagulase-negative staphylococci—as a consequence of health care–associated bacteremias.[3,88] Most of these patients with HCAIE have underlying conditions such as diabetes mellitus, chronic hemodialysis, or intracardiac devices.

Efforts should therefore be made to prevent HCAIE, so prevention of health care–acquired bacteremia is a logical target. Reducing the rate of central line–associated bloodstream infections can be achieved with practice-changing interventions.[90,102] Bundled interventions to reduce catheter-related bloodstream infection in high-risk patients, such as those on hemodialysis, could translate to a significant reduction in the incidence of IE.[127]

Prevention of Infective Endocarditis Due to the Implantation of Cardiac Implantable Electronic Devices

Because the incidence of IE in patients with permanent pacemakers is about 10 times higher than in the general population,[128] and even higher in patients with defibrillators,[129] attention should also be paid to the prevention of IE associated with CIEDs, which include permanent pacemakers and implantable defibrillators. The efficacy of antibiotic prophylaxis with cefazolin given before the implantation of a CIED has been demonstrated in a large, randomized, double-blind, placebo-controlled trial that showed a significant difference in favor of the cefazolin arm (cefazolin—2 infections in 314 patients, 0.63%; placebo—11 infections in 335 patients, 3.28%; relative risk [RR], 0.19; $P = .016$).[130] Current guidelines from the AHA,[131] BSAC,[132] and Heart Rhythm Society (HRS)[133] recommend that an antibiotic that has demonstrated in vitro effectiveness against staphylococci be administered before CIED implantation. Either cefazolin or vancomycin can be used, the latter being preferred only in areas with high rates of oxacillin resistance among staphylococci and/or in patients allergic to cephalosporins. Additional preventive measures including a search for and treatment of occult or smoldering infection; intravenous antibiotic prophylaxis; intensive aseptic or antiseptic implantation techniques using double gloving, thorough skin preparation, and antiseptic irrigation of the wound and the inside pocket; skin closure with absorbable sutures; and a postoperative course of antibiotics may even further reduce the incidence of infections in CIED implants.[134] To determine whether such additional measures would further reduce the risk of CIED infection, the Prevention of Arrhythmia Device Infection Trial enrolled more than 12,500 patients who underwent generator change, system upgrade, or new CIED implantation and is now in the follow-up stage.[135]

Prevention of Infective Endocarditis Due to Valvuloplasty and Valve Replacement

Early-onset IE after valve surgery is caused by various microorganisms, including staphylococci, gram-negative bacteria, and rarely fungi (see Chapter 81). No single antibiotic regimen is effective against all these organisms, and the use of broad-spectrum antibiotics may itself predispose to superinfection with resistant organisms. Therefore, antibiotic prophylaxis of IE resulting from valve surgery should be limited to a short course of an antistaphylococcal agent, such as a cephalosporin or vancomycin, given with the primary objective of preventing wound infections and the secondary objective of potentially preventing IE.[9,136]

Postoperative bloodstream infections and prosthetic valve IE due to *Mycobacterium chimaera* were first reported in 2013 in Switzerland,[137] and later reported worldwide.[138] Investigations demonstrated that these were due to contaminated heater-cooler units used for intraoperative extracorporeal circulation.[138] Prevention of these life-threatening infections is based on strict adherence to monitoring and decontamination procedures.[139,140]

Prevention of Infective Endocarditis in Patients With Prosthetic Heart Valves

Extensive clinical experience has established that patients with prosthetic heart valves are at high risk for IE (see Chapters 81 and 82 and Table 83.2).[36,141] The high mortality and morbidity associated with prosthetic valve endocarditis and removal and replacement of infected prosthetic valves make its prevention a priority. Although the incidence of IE after cardiac surgery has decreased significantly in recent years, the risk of early-onset IE (defined as occurring within 60 days after valve replacement) remains approximately 0.3% to 0.5% and thereafter approximates 0.5% to 1% per year (see Chapter 82). It is important to recognize this risk and to take all possible steps to minimize it. Before elective valve replacement, the dental health of every patient should be evaluated and any necessary dental work completed. Postoperatively, the patient should maintain good oral hygiene. Consultation between the patient's dentist and physician is important to ensure appropriate antibiotic prophylaxis for routine dental procedures. Strict attention should be paid to prevention and follow-up of health care–associated infections, especially catheter-related bloodstream infections. In addition, personal "endocarditis prevention cards" have been made available in some countries to be given to patients with prosthetic valves.[47]

Late-onset prosthetic valve endocarditis, defined as onset 60 days or more after the operation, is most likely to be caused by organisms originating from the oral cavity or the skin and reaching the valve via the bloodstream, as for native valve endocarditis. Antibiotic prophylaxis of IE in the setting of dental procedures should therefore be directed primarily against streptococci.

Cardiac Assist Devices and Artificial Hearts

Cardiac assist devices and artificial hearts pose a high risk of infection, which increases with duration of use and often has catastrophic consequences; more than 50% of patients develop a device-related infection in the weeks after implantation, and mortality caused by these infections is also greater than 50%.[142] Multiple strategies to prevent infections have been used and/or recommended. Unfortunately, the paucity of clinical studies of such strategies makes assessment of their efficacy difficult.[143] Systemic antibiotics are routinely given before and up to 48 to 72 hours postoperatively.[144] There is no standard regimen for antibiotic prophylaxis. Vancomycin, levofloxacin, and rifampicin were used in the original REMATCH study.[145] Different antibiotic regimens have subsequently been used. In a survey of centers performing ventricular assist device (VAD) surgery, most centers used a three- to four-drug regimen that included vancomycin, rifampicin, fluconazole, and an antibiotic active against gram-negative bacteria.[146]

Cardiac Transplantation

Although cardiac transplantation in itself is not an indication for IE prophylaxis, patients with valvulopathy after transplantation are at increased risk for IE and also at high risk for an adverse outcome if IE develops, with mortality as high as 80%. Therefore the AHA recommends that such patients receive antibiotic prophylaxis when an invasive dental procedure at risk for causing IE is performed.[9]

Medical Diagnostic Procedures

Common diagnostic procedures, such as bronchoscopy, gastrointestinal endoscopy, imaging studies with contrast enhancement, biopsies, and transesophageal echocardiography, may occasionally induce bacteremia but carry minimal risk for causing IE.[147] Therefore administration of antibiotics to prevent IE during such procedures is no longer recommended.[9,10,92]

Invasive Procedures Involving Infected or Nonsterile Sites

Incisions, biopsies, or other invasive procedures involving skin, soft tissues, or other sites in the presence of active bacterial infection can cause bacteremia and, rarely, IE. Examples include instrumentation of the urinary tract during infection, device infections, and dermatologic procedures. Many such patients would be treated with antibiotics anyway, irrespective of any consideration of prevention of IE. In such a setting, it appears reasonable to choose an antibiotic regimen that could prevent IE in those susceptible patients with highest risk of adverse outcome from IE (see Table 83.3). If so, the choice of antibiotic would be dictated by the bacterial species most likely to be involved—*S.*

aureus for skin and soft tissue infections and enterococci for urinary tract infections.

Body piercing and tattooing are a cause for concern, particularly for individuals with increased susceptibility for the acquisition of IE. Case reports of IE after piercing and tattooing are increasing, particularly when piercing involves the tongue.[148,149] Patients should be informed about the hazards of piercing and tattooing, and these procedures should be discouraged not only in highest-risk patients, but also in those with native valve disease. If undertaken, procedures should be performed under sterile conditions, although antibiotic prophylaxis is not recommended.[10]

Prophylaxis of Infective Endocarditis for Children

Antibiotic prophylaxis of IE is currently recommended by the AHA only for those children with underlying conditions that are more likely to have an adverse outcome of IE, just as in other age groups (see Table 83.3). The AHA guidelines of 2014 have been endorsed by the American Academy of Pediatric Dentistry,[150] which issued new guidelines in 2017. These are essentially similar with regard to indications and antibiotic regimens to be used for antibiotic prophylaxis of IE.[151] If used, appropriate adjustment of antibiotic dosage for children is required (see Table 83.5).[151]

Anticoagulant Therapy

The initial nonbacterial thrombotic lesions that can develop on the surface of an endothelial lesion may also be targets for prophylaxis of IE, using antiplatelet and/or antithrombin agents. It has been shown in an animal model that a combination of aspirin plus ticlopidine, as well as abciximab, prevented IE due to *Streptococcus gordonii* and *S. aureus*. Dabigatran also showed efficacy against IE due to *S. aureus* but not to *S. gordonii,* but acenocoumarol was ineffective.[79] Aspirin plus ticlopidine protected rats from IE due to *Enterococcus faecalis* and *Streptococcus gallolyticus.*[152] Antiplatelet and direct antithrombin agents may therefore be useful in the prophylaxis of IE in humans, and their efficacy should be further evaluated.[153] In particular, the potential dual benefit of dabigatran should be evaluated for patients with prosthetic valves, who require lifelong anticoagulation and in whom *S. aureus* IE is associated with high mortality. Although the concept seems potentially promising, to date no guidelines have recommended antithrombotic interventions to prevent IE.

Medicolegal Liability Issues

In the past, issues related to prophylaxis of endocarditis have frequently led to allegations of professional negligence, especially in the United States and the United Kingdom.[154,155] The most common allegation concerns failure to provide antibiotic prophylaxis to a patient with a PCC who develops IE after a dental procedure. However, it is impossible to prove that any single procedure known to cause bacteremia was the proximate cause in a case of IE, or that the failure of a physician or dentist to administer antibiotics was the proximate cause of acquisition of IE in an individual patient. Another common issue arises from the lack of definitive data on the upper limit for the incubation period of IE,[53] although a review of case reports indicates that the incubation period for streptococcal IE does not exceed 2 weeks in most cases.[53]

Recently, two widows in the United Kingdom whose husbands died from IE after dental scaling petitioned NICE, which then significantly altered its guidelines by adding a single word to the main recommendation, which now reads "Antibiotic prophylaxis against IE is not recommended *routinely* for people undergoing dental procedures."[156] The addition of the word *routinely* clearly implies that in individual cases, antibiotic prophylaxis may be appropriate. This subtle modification also places a new burden on UK clinicians, who now must identify patients at increased risk of IE, explain the risk and the ways in which it can be reduced, including antibiotic prophylaxis, and then help them decide whether they want antibiotic prophylaxis or not.[157]

A reasonable standard of care requires that health care professionals dealing with this question be aware of the risk factors for IE, and of current practice guidelines. They should inform susceptible patients of the risk—albeit small—that they may develop IE, and of the measures that they and their health care providers can take to reduce this risk, as described in this chapter. In view of the recent guidelines that strictly limit or eliminate antibiotic prophylaxis for IE, an informed decision by a health care professional not to prescribe antibiotic prophylaxis should not be construed as negligence, whatever the clinical outcome.

NEW HORIZONS FOR ANTIBIOTIC PROPHYLAXIS OF INFECTIVE ENDOCARDITIS

Oral Hygiene

It has been shown that poor oral hygiene is associated with a higher risk of bacteremia due to IE-causing organisms.[82] Therefore, conventional wisdom has promoted high oral hygiene standards as a way to prevent IE in at-risk patients. It is known that tooth brushing induces bacteremia[98] and may be associated with an increased risk of oral streptococcal IE. Nevertheless, it seems likely that in the long term, regular daily tooth brushing would be better than no tooth brushing in minimizing the risk of IE. Likewise, other dental hygiene procedures such as scaling and flossing have been shown to induce bacteremia, but little is known regarding the risk of IE associated with these two procedures. This risk has been estimated through an assessor-blinded case-control study in which cases and controls had definite IE caused either by oral streptococci or by nonoral pathogens, respectively. This study showed that in adjusted analysis, cases were more likely than controls to use toothpicks, dental water jets, or interdental and/or dental brushes, and to have had dental procedures during the prior 3 months, whereas they were less likely to brush their teeth after meals.[20]

Because oral health is of particular importance in patients with heart valve abnormalities owing to the risk of IE, a multidisciplinary working group reviewed the literature to propose individualized approaches for the evaluation and management of orodental status in patients with valvular disease at risk of IE. The main consequence of such management is the decrease in the number of contraindicated dental procedures. Conservative dental procedures can be performed before elective valvular interventions and during follow-up, even in patients at high risk of IE. Implant placement is no longer contraindicated in patients at high risk of IE, but the decision should take into account general and local factors associated with a risk of infectious complications and implant failure.[158]

"Big Data" as a Vehicle to Assess the Usefulness of Infective Endocarditis Prevention Measures

Recently a nationwide population-based cohort study using the French national health insurance administrative data linked with the national hospital discharge database assessed the relationship between invasive dental procedures and IE due to oral streptococci among people with prosthetic heart valves. The cohort included 138,876 adults with prosthetic heart valves (285,034 person-years), 69,303 of whom (49.9%) underwent at least one dental procedure. Among the 396,615 dental procedures performed, 103,463 (26.0%) were invasive and therefore presented a possible indication for antibiotic prophylaxis, which was performed in 52,280 (50.5%). With a median follow-up of 1.7 years, 267 people developed IE due to oral streptococci (incidence rate, 93.7 per 100,000 person-years; 95% CI, 82.4–104.9). Compared with nonexposure periods, no significantly increased rate of oral streptococcal IE endocarditis was observed during the 3 months after an invasive dental procedure (RR, 1.25; 95% CI, 0.82–1.82; $P = .26$) and after an invasive dental procedure without antibiotic prophylaxis (RR, 1.57; 95% CI, 0.90–2.53; $P = .08$).[21] Beyond the main conclusion—that the risk of IE after invasive dental procedures is low even in patients with prosthetic heart valves and could therefore be reduced only marginally by antibiotic prophylaxis—this study highlighted the potential opportunity to use existing "big data" to conduct a randomized, registry-based trial to assess the efficacy and usefulness of antibiotic prophylaxis of IE in humans, in a feasible and affordable fashion. Such registry-based randomized clinical trials on other issues have been conducted successfully in recent years, providing answers that would not have been obtained otherwise.[159,160,161,162]

Novel Approaches

Given the difficulty of curing device-related IE caused by biofilm-forming strains of multidrug-resistant *S. aureus,* novel strategies must be identified,

both for preventing and for treating this life-threatening condition. For prevention, targeting the inhibition of bacterial adhesion to both living and inert surfaces at the initial stage, thus reducing the chances of further development and establishment of biofilms, might be possible. Inhibition of bacterial adhesion at the time of intracardiac device insertion could be key and may be achieved through use of implants coated with various adhesion inhibitors. To date antibiotic-, silver ion–, or silver nanoparticle–coated implants, which can inhibit biofilm formation in vitro, have failed to prove to be both effective and well tolerated in humans. Indeed, enthusiasm for antibacterial coatings has been tempered by experience with the Silzone valve (St. Jude Medical, St. Paul, MN), which had a silver-coated sewing ring but had to be recalled within 3 years of its release in 1997 owing to an increased risk of thrombosis and paravalvular leaks.[163,164] However, novel strategies to target adherence are currently being evaluated.[165]

Future prevention strategies may also include vaccines. Human studies using vaccines targeting causes of IE have been aimed at *S. aureus, Pseudomonas aeruginosa,* and group B streptococci. Two candidate *S. aureus* vaccines evaluated in phase III clinical trials failed to demonstrate efficacy, with one failing to reach an efficacy end point of prevention of *S. aureus* bacteremia in patients undergoing hemodialysis,[166] and another leading to increased mortality in patients undergoing median sternotomy who developed staphylococcal infection.[167] Despite these negative results, other candidate *S. aureus* vaccines are currently being evaluated in humans. A new composite vaccine targeting five components of *S. aureus* has been shown to be highly protective in mouse models,[168] as has a recombinant *Lactococcus lactis* expressing ClfA or FnbpA in rats.[169] The rapid and robust human functional immune responses induced by other candidate vaccines support their further development for the prevention of *S. aureus* disease in adults.[170,171]

CONCLUSION

Although many relevant factors have changed or evolved over time, prevention of IE remains important today. Because no randomized clinical trial to confirm the efficacy and safety of antibiotic prophylaxis has ever been conducted, it is likely that the debate around indications for antibiotic prophylaxis of IE will continue for years to come. In the meantime, it is reasonable to recommend antibiotic prophylaxis for patients at high risk of IE and its complications before they undergo high-risk dental procedures. All patients at risk should maintain good oral hygiene. Prevention of HCAIE should also be targeted, through prevention of health care–associated bacteremias and antibiotic prophylaxis before the implantation of cardiac implantable electronic devices. Prevention of *S. aureus* IE has become a priority; in the future this might be achieved with a vaccine, and candidate *S. aureus* vaccines are currently being evaluated in humans.

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The complete reference list is available online at Expert Consult.

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84 Myocarditis and Pericarditis

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SHORT VIEW SUMMARY

MYOCARDITIS

- Inflammation of the myocardium, which is clinically manifested by chest pain, arrhythmias, and congestive heart failure (CHF), individually or in combination

Etiologic Agents

- Most commonly associated with viral infections, particularly enteroviruses, adenoviruses, human herpesvirus 6, and dengue viruses (see Table 84.1)
- Occasionally caused by bacteria, as a result of bacteremia, direct extension from a contiguous focus, or a bacterial toxin
- Caused by *Trypanosoma cruzi,* the cause of Chagas' disease, which is prevalent in South and Central America

Diagnosis

- In the setting of clinical suspicion, elevation of cardiac enzymes, electrocardiographic changes (nonspecific), echocardiography, and cardiac magnetic resonance imaging are helpful.
- Evidence of coincident viral infection, by culture, detection of viral DNA or RNA in peripheral samples or serology, is circumstantial.
- Endomyocardial biopsy can provide a definitive diagnosis, but sampling errors limit its utility.

Treatment

- Supportive care and management of CHF are essential.
- The benefit of immunosuppressive therapy is not established.
- The efficacy of intravenous immunoglobulin therapy is also not established.

PERICARDITIS

- Inflammation of the pericardium is clinically manifested by chest pain, pericardial friction rub, and pericardial effusion. These may be present individually or in combination. Monitoring for cardiac tamponade is important.

Etiologic Agents

- Enteroviruses are most common, but other viruses may sometimes be responsible (see Table 84.3).
- Bacteria rarely may cause purulent pericarditis, usually as a complication of pneumonia.
- *Mycobacterium tuberculosis* can cause pericarditis, usually as a complication of pulmonary tuberculosis. This is a major problem in Africa in association with acquired immunodeficiency syndrome.

Diagnosis

- Etiology is often undetermined in individual cases.
- Pericardiocentesis may yield an etiologic agent but is negative in the majority of patients.
- Evidence of viral infection by detection in peripheral samples or by serology provides only circumstantial evidence of possible etiology.
- Percutaneous pericardial biopsy or pericardiotomy with biopsy and drainage increase the diagnostic yield.

Treatment

- For presumed viral or idiopathic pericarditis, analgesic treatment with nonsteroidal antiinflammatory drugs (NSAIDs), together with bed rest, is often successful in relieving symptoms. NSAIDs are generally continued for 1 to 2 weeks or until symptoms resolve.
- Colchicine given with NSAIDs has been found to improve rate of recovery and reduce recurrence.
- Purulent pericarditis usually requires drainage in addition to appropriate antibiotics.
- Tuberculous pericarditis requires appropriate antituberculous therapy (see Chapter 249); concomitant administration of corticosteroids reduces the development of constrictive pericarditis and the need for repeated pericardiocentesis.

Inflammatory processes affecting the heart frequently involve both the myocardium (myocarditis) and the pericardium (pericarditis). However, involvement of one or the other usually predominates, and the syndromes of myocarditis and pericarditis are sufficiently distinct in clinical presentation, etiology, and pathophysiology to warrant separate consideration.

MYOCARDITIS

Myocarditis, literally "inflammation of the myocardium," is a protean disease with a wide variety of infectious (Table 84.1) and noninfectious (Table 84.2) causes. Postmortem examinations reveal evidence of previously unsuspected myocarditis in 1% to 4% of unselected cases,[1–3] with a higher incidence in young persons who have died suddenly.[4–7] The advent of modern molecular diagnostics has revealed that there are forms of infectious viral heart disease that do not appear to be associated with a typical inflammatory infiltrate.[8] Hence the term "myocarditis" may not literally describe the full gamut of infectious diseases of the myocardium. However, in clinical practice and in this chapter the term *myocarditis* is used to describe a range of infectious and inflammatory heart diseases that affect cardiac muscle and function, either directly or indirectly. This chapter focuses on infectious causes of myocarditis, recognizing that the etiologic agent remains unidentified in a significant percentage of patients.

The diagnosis of infectious myocarditis is usually considered when unexplained heart failure, chest pain, or arrhythmias occur after an upper respiratory tract infection or another presumed viral infection, or in the setting of a systemic febrile illness.[9] In many cases, however, the antecedent systemic illness is mild or long forgotten. In addition, myocarditis has been found histologically in 10% to 20% of cases of idiopathic dilated cardiomyopathy (DCM).[10–12] Because of the wide spectrum of clinical presentations, the frequent occurrence of asymptomatic cases, the paucity of noninvasive biologic markers, the limited sensitivity of histopathologic diagnosis, and problems with the laboratory identification of etiologic agents, the true incidence of myocarditis and infection-mediated cardiomyopathy may be significantly underestimated.[13,14]

In myocarditis the inflammatory process may affect myocytes, vascular elements, the conduction system, autonomic nerves, or the interstitium. In addition, it is not unusual for both the pericardium and myocardium to be affected at the same time. One or more of at least four mechanisms appears to be involved in the pathogenesis of myocarditis: (1) direct damage to cardiomyocytes by an infectious agent; (2) damage to myocytes

TABLE 84.1 Infectious Causes of Myocarditis

Viruses

Coxsackie B viruses[12,26,29,30,34–37,220,243,247,287–300,302–304,308,389,391,392,527–529]
Coxsackie A viruses[26,29,30,34,37,244,251,287,290,308,390]
Adenoviruses[8,27,28,248,249,251,287,530–533]
Echoviruses[26,287,534–536]
Nonpolio enteroviruses[8,12,26,37,287–289,291,292,302,369,528,530,537,538]
Parvovirus B19[28,32,41,539–543]
Polioviruses[24,544,545]
Rabies virus[546–548]
Mumps virus[19,549–551]
Rubeola (measles) virus[21–23]
Influenza A and B viruses[20,552–555]
Rubella virus[556,557]
Dengue viruses[44,558,559]
Chikungunya virus[559]
Yellow fever virus[560]
Argentine hemorrhagic fever virus (Junin virus)[560,561]
Bolivian hemorrhagic fever virus (Machupo virus)[561]
Lymphocytic choriomeningitis virus[562]
Lassa fever virus[563–565]
Varicella-zoster virus[566–574]
Human cytomegalovirus[26,59,297,575–580]
Epstein-Barr virus[8,581–588]
Human herpesvirus 6[589,590]
Herpes simplex virus[26,250,591,592]
Variola virus[593]
Vaccinia virus[594–598]
Hepatitis B virus[599–601]
Hepatitis C virus[602–604]
Respiratory syncytial virus[26,605,606]
Human immunodeficiency virus[52–54,59–61,65,66,69–72,166,477,607]

Bacteria and Rickettsiae

Brucella[91,392]
Campylobacter[88–90]
Corynebacterium diphtheriae[75–77]
Clostridium perfringens[78,79]
Francisella tularensis[92,93]
Neisseria meningitidis[82–84]
Salmonella[85–87]
Shigella[608]
Streptococcus pyogenes[94,95]
Staphylococcus aureus[96]
Listeria monocytogenes[98,99]
Vibrio cholerae[609]
Mycobacterium tuberculosis[101,610,611]
Legionella pneumophila[102]
Mycoplasma pneumoniae[103–107]
Chlamydia psittaci[108,109]
Chlamydia pneumoniae[108,110,111]
Rickettsia rickettsii[81,112]
Rickettsia prowazekii[81,113]
Rickettsia (Orientia) tsutsugamushi[81,114,115]
Coxiella burnetii[612,613]
Ehrlichia[614,615]
Borrelia burgdorferi[118–127]
Tropheryma whippelii[616]
Ureaplasma spp.[617]

Fungi

Aspergillus[150–155]
Candida spp.[150–152,156,157]
Blastomyces[465]
Coccidioides immitis[151,461]
Cryptococcus neoformans[151,152,158,159]
Histoplasma capsulatum[457]

Parasites[618]

Trypanosoma cruzi[128,129,135–137,223,224,619,620]
Trypanosoma gambiense[139]
Trypanosoma rhodesiense[139]
Trichinella spiralis[140–142]
Toxoplasma gondii[143–149]
Toxocara canis[621,622]

TABLE 84.2 Noninfectious Causes of Myocarditis

Checkpoint inhibitors[306]
Collagen vascular disease[285,623]
- Systemic lupus erythematosus[624]
- Systemic sclerosis[625–627]
- Rheumatoid arthritis[628,629]
- Dermatomyositis/polymyositis[630,631]
- Still's disease[632]

Thyrotoxicosis[285]
Thrombotic thrombocytopenic purpura[633]
Pheochromocytoma[623]
Peripartum[634]
Radiation induced[635–637]
Drug induced (directly toxic)[285,623,638]
- Cocaine
- Alcohol
- Emetine
- Catecholamines
- Arsenic
- Lead
- Cyclophosphamide
- Daunorubicin
- Adriamycin

Drug induced (hypersensitivity)[623,639]
- Methyldopa
- Sulfonamides
- Tetracycline

Scorpion, wasp, and spider stings[623,640]
Etiologic agents not yet identified
- Kawasaki disease[641]
- Giant cell myocarditis[642]
- Sarcoid[643,644]

because of a generalized cytokine or cell-mediated immune response; (3) cytotoxicity caused by viral antigen-specific immune reactions directed against infected cells; or (4) cytotoxicity caused by a circulating toxin. Damage to the vascular endothelium may also result in indirect myocardial injury.[15,16] Research has revealed the complexity of the mechanisms involved in cardiac myocyte injury, especially injury caused by enteroviruses, and has also suggested targets for intervention (Fig. 84.1).[17,18]

Etiologic Agents

Almost every infectious agent is capable of causing myocarditis (see Table 84.1), but viruses are the most important infectious causes of myocarditis in the United States and Western Europe, and many cases of idiopathic myocarditis are assumed to be viral in origin. Long before the era of modern virology, pericardial and myocardial involvement was recognized during outbreaks of mumps,[19] influenza,[20] measles,[21–23] poliomyelitis,[24] and enterovirus-associated pleurodynia.[25] Adenoviruses,[8,26–28] enteroviruses,[29,30] and parvovirus B19[8,26,31–33] have been implicated as major causes of myocarditis. Among the enteroviruses, some of the most commonly identified are group B coxsackieviruses,[12,34–37] and their presence in biopsy samples of patients with myocarditis has been shown by direct immunostaining.[38]

A study using real-time polymerase chain reaction (PCR) and reverse-transcription PCR (RT-PCR) assays to identify viruses in myocardial tissue from 624 children and adults with acute myocarditis and 149 with DCM reported adenoviruses in 23% of the patients with acute myocarditis and 12% of the patients with DCM. Enterovirus genomes were detected in 14% of the patients with acute myocarditis and in 8% of the patients with DCM. In addition, human cytomegalovirus (HCMV), parvovirus B19 (PVB19), influenza A virus, Epstein-Barr virus (EBV), herpes simplex virus (HSV), and respiratory syncytial virus were each identified in a small number of the patients with acute myocarditis but not in any with DCM.[26] A similar study of myocardial tissue from 245 adults with "idiopathic" DCM identified viral genomes in 67.4%, PVB19 in 51.4%, human herpesvirus 6 (HHV-6) in 21.6%, enteroviruses in 9.4%, adenoviruses in 1.6%, EBV in 2%, and HCMV in 0.8%; 27.3% of the positive samples had multiple agents.[8] In a similar study of cardiac tissue obtained by endomyocardial biopsy (EMB) from 24 consecutive patients with acute myocarditis with chest pain in whom myocardial infarction was excluded, 12 (50%) were positive for PVB19, 3 for an enterovirus, and 2 for an adenovirus.[32] Four of the 6 patients in whom coronary spasm could be induced were PVB19 positive. Only 1 of the 24 (1 of 3 that were enterovirus positive) had histopathologic evidence of myocarditis according to the Dallas Criteria (see later).

The marked increase in the detection of PVB19 and HHV-6 in patients with acute myocarditis or DCM during the past decades is noteworthy.[8,26,28,32] However, given the high prevalence of PVB19 and HHV-6

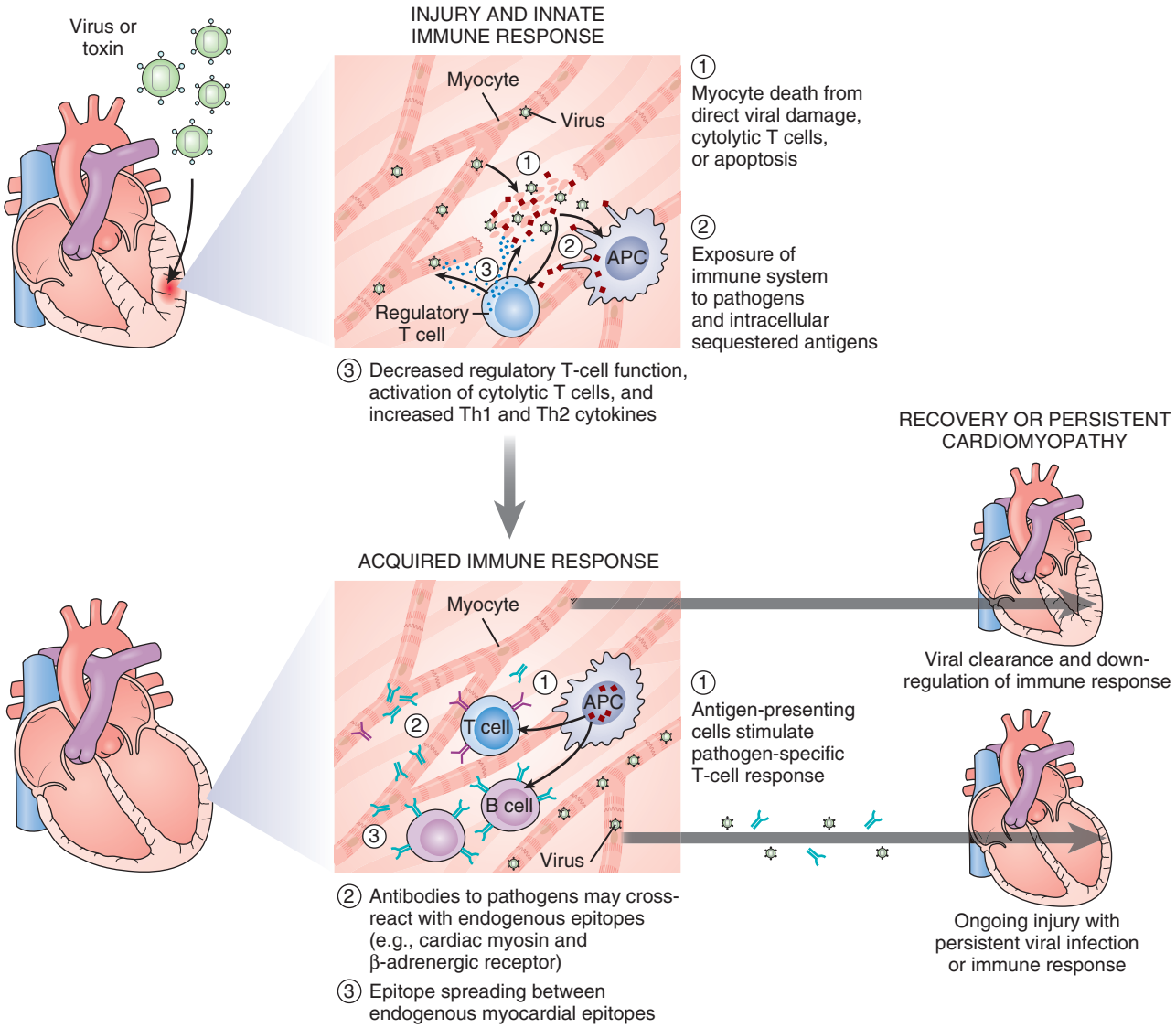

FIG. 84.1 Summary of the cellular and molecular pathogenesis of viral and autoimmune myocarditis, derived primarily from animal models. Three stages are summarized. Acute injury from direct viral infection and innate immune reaction leads to myocardial damage. Specific immunity mediated by T lymphocytes and antibodies is directed against the pathogen and potential endogenous heart epitopes. The pathogen may be cleared, and the immune reaction may decrease with improvement in cardiac function and few sequelae. In some patients persistent virus and inflammation contribute to chronic cardiomyopathy. *APC,* Antigen-presenting cell. *(Modified from Cooper LT. Myocarditis.* N Engl J Med. *2009;360:1526–1538.)*

in the general population, their pathogenic role remains to be proven. For example, in one study PVB19 was detectable by immunohistologic analysis in 65% of patients with acute myocarditis, 35% of patients with DCM, and 8% of noninflamed control hearts. PVB19 load was then assessed by genome copy numbers in the samples that were positive for PVB19 on immunohistologic analysis. PVB19 load was higher in patients with acute myocarditis, followed by those with DCM, and lowest in the patients with normal hearts without inflammation.[39] Viral RNA replicative intermediates were only detected in patients with inflamed hearts. It is important to note that in this study and others, PVB19 was found in endothelial cells and not myocardial cells. Verdonschot and colleagues[40] recently provided a comprehensive review of the relevance of cardiac PVB19 in myocarditis and dilated cardiomyopathy. Recognizing the controversy regarding the exact role of PVB19 in myocarditis, they conclude that PVB19 may be etiologically relevant when the viral load is high and PVB19 is actively replicating and in the presence of other cardiotropic viruses. An alternative possibility is that the presence of PVB19 may be a marker of inflammation. It is important to recognize that, especially with highly prevalent viruses, such as PVB19, HHV-6, and even HCMV and EBV, viral genomes detected in myocardial tissue by sensitive assays may have been carried into the myocardium by infiltrating inflammatory cells and have played no role in the etiology of the myocarditis or DCM.

There is also considerable temporal and geographic variation in the prevalence of infections caused by various adenoviruses and enteroviruses, as well as marked seasonal variation in the prevalence of infections caused by these and other viruses. Moreover, the prevalence of infections caused by some viruses, for example, adenoviruses and PVB19, is greater in children than in adults.[12,26,27,41] In consequence, the relative frequency with which different viruses are identified in acute myocarditis and DCM can be expected to vary from study to study and over time.

Dengue is the most prevalent mosquito-borne virus infection worldwide. It is caused by one of four distinct but antigenically related members of the Flaviviridae family, designated dengue virus serotypes 1 through 4.[42,43] The female *Aedes aegyptus* is the primary vector of dengue; *Aedes albopictus,* a secondary vector in Asia that is tolerant to temperatures below freezing, has spread to North America and Europe.[42] Dengue is endemic in more than 100 countries on every continent except Antarctica; approximately half the world's population is at risk of dengue virus infection, and more than a million cases occur each

year. Since 2010, local transmission has been reported in several countries in Europe and in China, and the disease is now endemic in Florida and the southern United States along the Mexican border. In 2012, more than 1.6 million cases of dengue were reported in the Americas.[42]

Dengue has been reported to cause a variety of cardiac manifestations, ranging from transient arrhythmias to cardiogenic shock and fatal myocarditis.[44–47] Cardiac myocyte necrosis has been observed as a result of direct dengue virus infection, and vascular endothelial cells also appear to be susceptible to infection.[48–50] Some dengue virus infections mimic acute myocardial infarction.[51]

Human immunodeficiency virus (HIV) infection, in advanced stages with high virus titers, is associated with a high incidence of cardiomyopathy. An incidence of cardiomyopathy of more than 50% has been documented in HIV-infected patients during their lifetime,[52–58] and evaluations of patients with acquired immunodeficiency syndrome (AIDS) have shown that cardiac dysfunction is highest in those with the most advanced disease.[59–61] However, many of these observations were made before implementation of antiretroviral therapy (ART). With the improved survival of HIV-infected patients associated with ART, the incidence of heart disease has increased, reflecting a higher incidence of coronary artery rather than myocardial disease. As might be expected, in areas such as sub-Saharan Africa, where ART is not widely administered, there is a significantly higher incidence of cardiomyopathy and myocarditis than that seen in developed parts of the world.[62,63] In developed countries in patients taking antiretrovirals, diastolic dysfunction is more common than systolic dysfunction.[64]

Although it is well accepted that HIV-infected patients can develop cardiomyopathy, the pathogenesis is not clear. Myocytes lack CD4 receptors, and despite the fact that HIV has been cultured from EMB samples[65–67] and identified by Southern blot analysis,[66,68] data are not convincing that HIV directly infects cardiac myocytes to induce cardiac dysfunction. The isolation of other known cardiotropic viruses from AIDS patients is frequently reported.[61,67,69,70] Malnutrition may also be a factor contributing to cardiac dysfunction in AIDS, and malnutrition and wasting are important independent predictors of cardiac morbidity and mortality.[71,72] Autoantibodies against myocardial proteins have been identified in some AIDS patients with cardiac dysfunction.[58] Immune dysregulation and cytokine production by infected monocytes and macrophages may be responsible for some cardiac pathology.[73,74]

Other viruses that have been implicated as causes of myocarditis or myopericarditis are listed in Table 84.1.

Nonviral Pathogens That Cause Myocarditis

Many nonviral pathogens cause myocarditis. For example, myocarditis is the most common cause of death in diphtheria[75–77]; the toxin produced by *Corynebacterium diphtheriae* severely damages the myocardium and conduction system. The cardiac damage seen in patients with *Clostridium perfringens* infection may be the result of toxin, metastatic abscess formation, or both.[78,79] The immunologically mediated carditis associated with acute rheumatic fever[80] is discussed in Chapter 198.

Invasion of the bloodstream by any bacterial pathogen may result in metastatic foci in the myocardium,[81] and myocarditis has been recognized in the course of meningococcemia,[82–84] salmonellosis,[85–87] *Campylobacter* infection,[88–90] brucellosis,[91] tularemia,[92,93] and bacteremias caused by streptococci,[94,95] staphylococci,[96,97] and *Listeria monocytogenes.*[98–100] More commonly, bacteria invade the myocardium as a complication of endocarditis by contiguous spread from infected valvular tissue or via septic embolization of the coronary arteries.[81,96,97]

Tuberculous myocarditis can occur without systemic symptoms and can be responsible for sudden cardiac death, especially in young people.[101] However, most tuberculous infections of the heart involve the pericardium (see later).

Myocarditis is a rare complication during *Legionella* infection.[102] It has also been observed in the course of *Mycoplasma pneumoniae,*[103–107] *Chlamydia psittaci*,[108,109] and *Chlamydia pneumoniae*[108,110,111] infections, and is commonly seen in rickettsial infections,[81,112–115] especially scrub typhus.[81,114,115] Approximately 10% to 16% of patients with Lyme disease develop cardiac abnormalities, most commonly conduction system disturbances (for review, see references 117–119). A recent pathology series showed direct myocardial involvement with *Borrelia burgdorferi.*[116] Lyme carditis usually resolves completely with no long-term sequelae, although cases of fulminant myocarditis have been reported.[117–127]

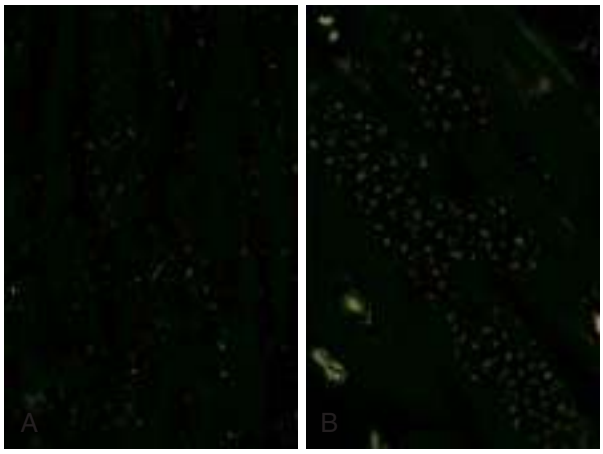

FIG. 84.2 *Trypanosoma cruzi* amastigotes in cardiac muscle stained with hematoxylin and eosin. Low-power magnification (A) and high-power magnification (B). Amastigotes are observed within cardiac myocytes. (Courtesy John T. Sullivan, University of San Francisco.)

In South America a principal agent responsible for myocarditis and heart failure is *Trypanosoma cruzi,* the protozoan that causes Chagas' disease (Fig. 84.2). This is discussed in greater detail in Chapter 276. Chagas' disease is a common cause of DCM worldwide, but the incidence in endemic areas is decreasing substantially.[128–131] In the 1980s it was estimated that there were 17 million people who were infected; these estimates had fallen to 5.7 million people by 2010.[128,131,132] Although the incidence is decreasing in endemic areas, there has been an increase in developed, nonendemic areas due to immigration.[128] The triatome vector that transmits the parasite is found in Texas and, although rare, autochthonous vector-borne transmission of *T. cruzi* in Texas has been reported.[133,134] Initial infection by *T. cruzi* is often asymptomatic or accompanied by a low-grade febrile illness. However, a subset of patients develop an acute illness complicated by myocarditis.[129,135,136] Myocarditis is the principal manifestation of chronic Chagas' disease, which occurs in approximately 30% to 40% of infected individuals. These patients typically have cardiomegaly with congestive heart failure (CHF) and a high incidence of thinning and bulging of the ventricular apex. The cardiomyopathy is often associated with conduction disturbances.[128,136–138] *Trypanosoma gambiense* and *Trypanosoma rhodesiense,* the agents of African trypanosomiasis, may also affect the heart with similar results, but central nervous system findings usually predominate in infections with those agents.[139]

Myocarditis is also observed in trichinosis[140–142] and is responsible for the occasional deaths that occur in severe infections. Myocarditis is common in disseminated toxoplasmosis,[143–149] and systemic aspergillosis and candidiasis may also involve the heart.[150–157]

In immunocompromised patients, myocarditis occurs as a consequence of a number of disseminated infections. Cryptococcal, *Toxoplasma,* and *Aspergillus* myocarditis have been reported in patients with AIDS.[145–147,155,158,159]

Pathology and Pathogenesis

Most of our knowledge about the pathogenesis of viral myocarditis comes from studies of coxsackievirus B3 (CVB3) and encephalomyocarditis virus infection in murine models. Susceptibility to CVB3-induced myocarditis in mice is age dependent and genetically determined, and mechanisms of injury vary in different mouse strains.[160–164] Myocardial pathology also depends on the infectious agent, the pathogenic mechanism involved, and the duration of the process. Fung and colleagues[165] describe the broad spectrum of myocarditis and evolving knowledge of the pathogenic role of viruses and other mechanisms of disease, in an ongoing effort to identify and evaluate new therapeutic interventions.

Early in many viral infections in humans, scattered hypereosinophilic myofibers, widespread edema, and only a few inflammatory cells may

be present. Later, loss of striation, nuclear degeneration, and fragmentation of myofibers can be seen. Degenerating or partially necrotic myofibers are usually surrounded by natural killer (NK) cells and macrophages, and later by T lymphocytes. The types of lymphocytes and macrophages that are present vary depending on the etiologic agent and the stage of infection.[166–169] Polymorphonuclear leukocytes are occasionally seen.[4] Return to normal cardiac function usually precedes resolution of the histologic abnormalities.[170] The acute process may resolve completely; healing and chronicity are reflected by the development of interstitial fibrosis and loss of myofibers.[171]

Coxsackieviruses and adenoviruses both use the coxsackievirus and adenovirus receptor (CAR) for cell entry.[172] Cardiac-specific deletion of CAR protects mice from development of contractile dysfunction and inflammation in the heart on infection with CVB3.[173,174] Immunohistochemical analysis revealed higher levels of expression of CAR in the hearts of patients with DCM, although no cause-and-effect relationship between CAR expression and DCM was established.[175]

Direct viral cytotoxicity plays a role in myocardial damage in mice and humans,[3,4,176,177] and prolonged viral persistence in the myocardium is associated with development of DCM. This notion was further supported by a clinical study of patients with biopsy-proven viral myocarditis in which prolonged viral persistence was associated with persistent immune cell infiltration in the myocardium and worsening of cardiac function on follow-up echocardiographic examination.[178]

At the molecular level, cytotoxicity by CVB3 can be partially explained by its production of protease 2A. This viral protease, the normal function of which is to cleave the viral polyprotein, also cleaves the subsarcolemmal protein dystrophin at a specific site. This destabilizes the dystrophin-glycoprotein complex, thereby facilitating viral propagation in the heart and causing cardiac myocyte dysfunction.[179–182] Knockin mutation of a protease 2A cleavage site in dystrophin that inhibits protease 2A cleavage decreases viral titer, disruption of the cell membrane, and severity of myocarditis.[183] Enteroviral proteases can cleave other host proteins that may have a direct effect on virus-mediated pathogenesis.[165] It has also been demonstrated that inducible, transgenic expression of CVB protease 2A alone in the myocardium can induce a cardiomyopathy that is associated with disruption of the sarcolemmal membrane.[184] It is also possible that loss of dystrophin in patients with Duchenne muscular dystrophy leads to increased susceptibility to myocarditis and, subsequently, to cardiomyopathy. There are studies showing that magnetic resonance imaging (MRI) or histologic evidence of myocardial inflammation in patients with Duchenne muscular dystrophy portends a higher risk of developing heart failure.[185] Recently, it has been shown in patients with acute myocarditis that there is a higher incidence of homozygous or compound heterozygous mutations in genes that have been implicated in cardiomyopathy, including several cytoskeletal genes, which provides additional insight into the mechanisms associated with increased susceptibility to myocarditis in humans.[186]

The pathogenesis of any viral infection is tightly linked to host immune responses. On one hand, inability to mount effective immune responses to myocardial infection can lead to increased viral replication, viral persistence, chronic infection, or a combination of these that may lead to DCM.[178] On the other hand, excessive activation of the immune system can cause immunopathologic myocardial damage and result in cardiomyopathy.

Host response to a pathogen is characterized by an early innate immune response, which is followed by an adaptive, antigen-specific immune response that includes antibodies and antigen-specific lymphocytes. Because the innate immune response is not antigen specific, it occurs more quickly and can be characterized by activation of a cytokine response and infiltration of NK cells. The innate immune response involves the activity of Toll-like receptors (TLRs), interferons (IFNs), Janus kinase–signal transducer and activator of transcription signaling pathway (JAK-STAT), and interleukins (ILs).[165]

The members of TLR family, also known as sentinels of the immune system, are implicated in the development of viral myocarditis. Mice lacking TLR3 have significantly higher mortality after infection with enteroviruses.[187] Genetic analysis of 57 patients with biopsy-proven myocarditis or DCM with biopsies positive for enterovirus RNA revealed a higher frequency of certain TLR3 mutations when compared with healthy matched control subjects.[188] Infection of stable cell lines expressing mutant TLR3 had minimal effect on induction of type I IFN when compared with the cells expressing wild-type TLR3. However, viral titers in supernatants from infected mutant TLR3-expressing cells were found to be significantly higher than in supernatants from cells expressing wild-type TLR3.

Several rodent experiments have highlighted the role of IFNs as the first line of defense against cardiotropic viruses.[189,190] Indirect evidence has also suggested that deficiency in IFN production predisposes to development of DCM after infection with cardiotropic viruses. One study found that stimulation by staphylococcal enterotoxin B elicited a smaller subset of IFN-γ–producing peripheral CD4 lymphocytes in patients with DCM when compared with healthy donors.[191]

IL-1, tumor necrosis factor (TNF), and bacterial lipopolysaccharide all enhance autoimmune injury and cause resistant mice to become susceptible to viral myocarditis.[192] Data demonstrating enhanced expression of intracellular adhesion molecule (ICAM)-1 in murine myocytes, as well as both type I and type II major histocompatibility complex (MHC) antigens in human myocarditis, also support the theory that autoimmunity can play a role in the development of viral myocarditis.[193–196]

Adaptive immunity comes into play at later stages of viral myocarditis, late in the first week and during the second week after infection. Activated lymphocytes help eliminate infected cardiomyocytes but can also cause "an innocent bystander effect" with excessive tissue damage, leading to cardiomyopathy. Cytotoxic T cells recognize both infected and uninfected myocytes, and their presence correlates with the degree of myocardial damage.[197–200] Variants of CVB3 that do not evoke cytotoxic T cells directed against both infected and uninfected myocytes fail to cause myocarditis, even though they are similar to myocarditic strains in their ability to replicate and stimulate neutralizing antibodies.[201] Mice lacking *p56lck*, a tyrosine kinase essential for T-cell activation, are protected against CVB3 myocarditis. After acquiring CVB3 infection, animals heterozygous for *p56lck* developed histopathologic changes consistent with DCM, whereas *p56lck*-deficient mice maintained normal cardiac structure.[202] However, it should be noted that in mice with severe combined immunodeficiency that lack mature B and T cells, there is an increase in CVB3-induced cardiomyocyte injury.[176] IL-17–producing Th17 cells are also implicated in the development of myocarditis.[203,204] A survival benefit and decreased inflammatory cell infiltration have been observed in the myocardium of mice that received allogeneic transplantation of regulatory T cells that limit CD4 activation and prevent inflammation before infection with CVB3.[205]

Mice and humans infected with CVB3 have been shown to develop autoimmune heart-reactive antibodies,[206–208] which may contribute to myocyte destruction.[162,209–213] Zhang and colleagues[214] demonstrated that a live-attenuated variant of CVB3 was able to protect mice against infection with the cardiovirulent wild-type strain. The attenuated virus was able to induce neutralizing antibodies, but, in contrast to wild-type virus, the attenuated virus failed to induce significant levels of antibody against cardiac myosin. In mice, susceptibility to damage from antimyosin antibodies is strain specific and appears to involve a genetically determined difference in target-organ sensitivity.[215] Antibodies to cardiac myosin that cross-react with β1-adrenergic receptors can contribute to cardiomyocyte apoptosis.[216,217] Of note, Huber and associates[218] have described three monoclonal antibodies to group A *Streptococcus* that also bind to various cardiac antigens and neutralize a myocarditic strain of CVB3.

Studies in mice have demonstrated increased virulence of CVB3 in selenium-deficient mice, which suggests that "oxidative stress" may contribute to myocardial damage.[219] The identification of CVB viruses in the myocardium in patients with Keshan syndrome, a form of myocarditis prevalent in a selenium-deficient region of China,[220] suggests that this murine model may have direct relevance to myocarditis in humans. Nonsteroidal antiinflammatory agents may also have deleterious effects,[221] perhaps through inhibition of IFN production.[222]

The immune response has been shown to have a significant role in Chagas' disease. In acute Chagas' disease pathologic examination often reveals parasites within cardiac myocytes. When rupture of the cysts occurs, there is a marked inflammatory infiltrate consisting of lymphocytes, plasma cells, macrophages, and some eosinophils.[135,151] In chronic

Chagas' disease the heart is often enlarged and flabby. Aneurysm formation may be present at the apex. The conduction system is often involved, and this is reflected by a high frequency of rhythm disturbances. Microscopic examination reveals focal mononuclear cell infiltrates and fibrosis.[135,151,223,224] At this stage parasites can be identified in only 25% of patients.[224] Epitopes shared by *T. cruzi* and cardiac myocytes and recognized by cytotoxic T cells may play an important role in the progression of myocarditis late in the disease.[129,225]

Myocardial microabscesses, affecting both myocytes and the conducting system, may occur in the course of systemic bacterial infections with organisms such as *Staphylococcus aureus,* but heart failure is rarely a direct consequence of such lesions.[96,97] In experimental animals *Borrelia burgdorferi* has a predilection for connective tissue in the heart base, and disease severity correlates with the number of spirochetes found.[226,227] Mouse models suggest that infection with *B. burgdorferi* has a different pathogenesis than enterovirus myocarditis; injury is independent of class II MHC–T-cell interactions and results from the response of macrophages to the spirochetes.[228]

Rickettsia and most fungi produce vasculitic lesions with surrounding inflammation. Damage to myocytes may be caused by the adjacent inflammatory process or may reflect anoxia caused by occlusion of small blood vessels.

Diphtheria toxin inhibits cellular protein synthesis. This results in hyaline degeneration and necrosis of myocardial fibers with a secondary inflammatory response.[4,76]

Clinical Manifestations

Patients with myocarditis may be asymptomatic with subacute infection, or they may have a rapidly progressive fatal disease[14,229–231] with a full range of presentations in between.[232] Myocarditis is often classified as fulminant, acute, or chronic.

The diagnosis of infectious myocarditis is usually considered when a person develops unexplained heart failure, chest pain or arrhythmias, or when cardiac abnormalities occur in the course of a recognized systemic infection. Although the diagnosis is made more commonly in pediatric and young adult populations, it is not exclusive to these groups. Fever, malaise, arthralgias, or upper respiratory tract symptoms may precede or accompany coxsackievirus myocarditis,[233–235] but these symptoms are not specific nor are they required to make the diagnosis.[13,236] Supraventricular tachycardia and ventricular extra systoles are common, and, on occasion, atrioventricular block may occur.[237,238] Arrhythmias may provide early evidence of involvement of the conduction system and may be responsible for the occurrence of sudden death in patients with myocarditis. Myocarditis may mimic acute myocardial infarction,[239–242] but care should be taken not to mistake myocardial infarction occurring in a patient with infection for myocarditis.[243] In fulminant and acute myocarditis there may be a low-level elevation of cardiac enzymes that remains elevated for several days.[244,245] Symptomatic pericarditis may or may not also be present.

Diagnosis

Recognizing myocarditis in clinical practice is particularly challenging due to the absence of a sensitive and specific gold standard for diagnosis.[13,14,231,236,246] Therefore it has been difficult to precisely assess the diagnostic value of different clinicopathologic symptoms and signs. Consequently, the diagnosis of myocarditis requires a high index of suspicion. In infants, myocarditis is often just one manifestation of a widespread fulminant systemic infection. Involvement of the lungs, liver, and central nervous system; disseminated intravascular coagulation; and circulatory collapse may obscure the clinical signs of cardiac disease.[247–250] Recognition of myocarditis can also present difficulties in older children and adults when it occurs as part of an overwhelming systemic infection. When sought, however, signs of cardiac dysfunction may be apparent. As stated previously, the diagnosis of myocarditis is generally entertained when a patient presents with new-onset heart failure or with more nonspecific symptoms, such as unexplained chest pain or arrhythmias.

Elevation in biomarkers of necrosis may occur, but such elevation is neither highly sensitive nor specific without additional information. Elevations of cardiac troponins occur more frequently than do elevations of the muscle-brain (MB) fraction of creatine kinase (CK-MB), although the CK-MB is often elevated in the serum of patients with acute myocarditis and significant ST-segment elevation.[251,252] In one study only 34% (18/53) of those with biopsy-proven myocarditis had increased serum troponin levels, as did 11% (4/5) of patients evaluated for possible myocarditis in which the biopsy was negative for myocarditis.[245] A more recent analysis of patients with a troponin-positive episode of chest pain and unobstructed coronary arteries was evaluated with cardiac MRI. The most common underlying cause for the troponin elevation was myocarditis (50%).[253] Another study looked at young adults between ages 18 and 40 years who presented with chest pain and elevated troponin: 59.2% were diagnosed with myocarditis, whereas 37.5% had myocardial infarction.[254] In a pediatric population elevated troponin in the first 72 hours after hospitalization for viral myocarditis was associated with the need for extracorporeal membrane oxygenation.[255] The sensitivity of the newest high-sensitivity troponin assays for myocarditis remains to be determined. Various heart-reactive antibodies have been detected in patients with myocarditis, and their persistence at high titer appears to be a poor prognostic sign.

Various electrocardiographic changes may be present with myocarditis, although they are nonspecific unless there is concomitant pericarditis. The changes that may be seen include nonspecific ST-segment and T-wave abnormalities, sinus tachycardia, ventricular and supraventricular arrhythmias, and atrioventricular or intraventricular conduction disturbances. The degree of ST-segment elevation and the extent and duration of later T-wave inversion have been reported to correlate with myocardial enzyme release and therefore with the amount of cell necrosis.[234,235,237,252,256] The presence of Q waves in addition to ST-segment elevation may herald a rapidly fatal course, and abnormal QRS complexes and left bundle branch block have been correlated with an increased risk of sudden cardiac death and the need for cardiac transplantation.[257,258]

Echocardiography is a key tool in the diagnosis and management of myocarditis, even though many of its findings are nonspecific. It allows careful assessment of ventricular chamber size and function and helps exclude other causes of cardiomyopathy, such as valvular disease and hypertrophic cardiomyopathy. It is also useful for detecting pericardial effusion, ventricular thrombus, aneurysm, and right ventricular involvement.[259] Contractility in acute myocarditis may range from normal to severely compromised, and, because the dysfunction is relatively acute, the ventricular chamber may not be greatly enlarged. Thus fulminant myocarditis is associated with a severe decrease in cardiac function but relatively normal ventricular diastolic dimension and, sometimes, even increased septal thickness due to inflammation and edema.[260] In general, ventricular dysfunction associated with myocarditis is global; however, myocardial involvement can be focal in nature and can present with regional wall motion abnormalities that are difficult to differentiate from myocardial ischemia. In a small study of patients with biopsy-proven myocarditis, echocardiographic evidence of right ventricular dysfunction was the strongest predictor of death or the need for cardiac transplantation.[261]

Repeated echocardiographic examinations may be used to monitor the resolution or progression of myocarditis. Persistent wall motion abnormalities and ventricular dilation suggest the development of DCM.

Cardiac magnetic resonance (CMR) imaging has become a valuable tool for the noninvasive diagnosis of myocarditis. Several techniques have been developed to improve the sensitivity and specificity of CMR imaging for the diagnosis of myocarditis. T2-weighted CMR imaging allows visualization of myocardial edema.[262,263] Early T1-weighted gadolinium enhancement (EGE) can detect hyperemia and myocardial inflammation.[262,264–266] Late gadolinium enhancement (LGE) can identify areas of necrosis and fibrosis, and it has been found to be a useful technique for diagnosing this type of injury in myocarditis.[262,267–270]

In the case of myocarditis LGE is usually observed in subepicardial regions or the midwall of the left ventricle. Subendocardial sparing distinguishes myocarditic patterns of LGE from ischemic injury. In myocarditis LGE tends to be more extensive and is frequently distributed in noncontiguous patches that often involve the lateral wall (Fig. 84.3).[267,271] In a recent study the presence of LGE was strongly predictive of cardiac mortality.[272] Proposed "Lake Louise Criteria" for CMR

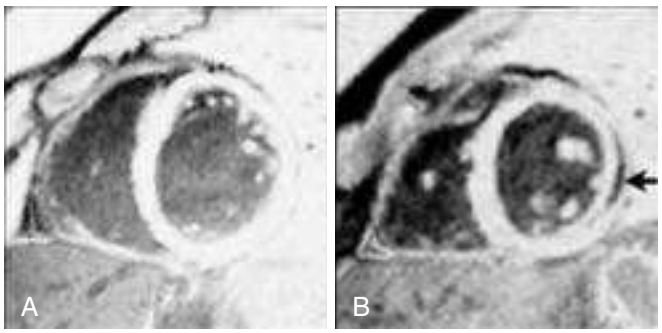

FIG. 84.3 Late gadolinium enhancement magnetic resonance images of the heart. (A) Normal myocardium. (B) Regional, subepicardial enhancement of the lateral wall *(arrow). (Modified from Friedrich MG, Sechtum U, Schulz-Menger A, et al. Cardiovascular magnetic resonance in myocarditis.* J Am Coll Cardiol. *2009;53:1475–1487.)*

image–based diagnosis of myocarditis recommend using a combination of all three MRI sequences (T2-weighted images, EGE, and LGE) in evaluation of patients with suspected myocarditis.[9]

On the basis of these criteria in the setting of clinically suspected acute myocarditis, at least two of the following should be present to confirm the diagnosis:

- Regional or global myocardial signal intensity increase in T2-weighted images, indicating tissue edema
- Increased global myocardial EGE in T1-weighted images
- Presence of at least one focal lesion with nonischemic regional distribution of LGE.

Lurz and colleagues[273,274] assessed the sensitivity and specificity of these criteria by comparing CMR imaging with EMB in patients with suspected acute myocarditis (symptom duration <14 days) and chronic myocarditis (symptom duration >14 days). They found that CMR imaging diagnostic performance is good in patients with suspected acute myocarditis (sensitivity, 81%; specificity, 71%; and diagnostic accuracy, 79%). These indicators are even better in patients with "infarct-like myocarditis" (elevated troponin levels, chest pain, and ST-segment elevation), with sensitivity, specificity, and diagnostic accuracy of 86%, 75%, and 84%, respectively. In contrast, diagnostic performance of CMR imaging in suspected chronic myocarditis was found to be unsatisfactory.[273]

More recently, the MyoRacer-Trial[274] assessed the diagnostic performance of comprehensive CMR imaging in 129 patients with suspected myocarditis, with biventricular endomyocardial biopsy serving as the gold standard. As defined by the area under the concentration-time curve (AUC), T1 (0.82) and T2 mapping (0.81) performed well for patients with acute symptoms ($N = 61$) and were superior to the older Lake Louise criteria (AUC, 0.56). For those with chronic symptoms ($N = 68$), T2 mapping alone yielded an acceptable AUC (0.77). Luetkens and colleagues[275] tested enhanced CMR techniques to improve the diagnostic yield for acute myocarditis. Imaging was performed in 34 patients and 50 control subjects. Clinical validation rather than endomyocardial biopsy was used for diagnosis. AUCs for native T1 (0.92–0.95) and T2 relaxation times (0.92) were excellent and when combined exceeded that of classic Lake Louise criteria (AUC, 0.99 vs. 0.90, $P = .008$). These results also support quantitative CMR parameters, especially using native mapping techniques, as a means of advancing the noninvasive diagnosis of myocarditis.[275]

EMB continues to be an important technique for the diagnosis of myocarditis. In 1986 a panel defined what are known as the "Dallas criteria" for making the diagnosis of myocarditis from EMB samples.[276] These criteria require an inflammatory infiltrate and associated myocyte necrosis or damage not characteristic of an ischemic event (Fig. 84.4). Borderline myocarditis is diagnosed in the presence of a less intense inflammatory infiltrate and does not require light microscopic evidence of myocyte necrosis. Both sampling error and variability in interpretation limit the usefulness of the Dallas criteria in clinical practice.[246] For example, postmortem analysis of hearts from patients who died of



FIG. 84.4 Coxsackievirus myocarditis with extensive infiltration of mononuclear cells and myocyte necrosis. *(Modified from Badorff C, Knowlton KU. Dystrophin disruption in enterovirus-induced myocarditis and dilated cardiomyopathy: from bench to bedside.* Med Microbiol Immunol. *2004;193:121–126.)*

myocarditis demonstrated that a single biopsy met Dallas criteria in only 25% of hearts, and five biopsies only made the diagnosis in approximately two-thirds of the hearts,[277,278] which is a reflection of the focal nature of the inflammatory response. Alternative criteria based on cell-specific immunoperoxidase stains for surface antigens on immune cells offer higher sensitivity and may have prognostic value.[196,279,280]

Although there are regional differences in the use of EMB and differences of opinion regarding its usefulness, EMB continues to have a role in establishing the diagnosis of myocarditis in new-onset heart failure.[281] Guidelines published under the auspices of the major cardiovascular and heart failure societies in 2007 recommend EMB for the following:

1. "Unexplained, new-onset heart failure of less than 2 weeks' duration associated with a normal-sized or dilated left ventricle in addition to hemodynamic compromise.
2. Unexplained new-onset heart failure of 2 weeks' to 3 months' duration associated with a dilated left ventricle and new ventricular arrhythmias, Mobitz type II second- or third-degree atrioventricular (AV) heart block, or failure to respond to usual care within 1 to 2 weeks."[281]

The European community has favored more liberal recommendations regarding the indications for EMB. They recommend EMB for essentially all patients suspected of having myocarditis.[282] Complications from right ventricular EMB are generally minor and can occur in up to 6% of cases. Serious adverse consequences (i.e., death, tamponade, perforation) occur in less than 1% if the operator is experienced.[268,283–285] In a study of 4221 patients who underwent EMB from 1983–2013, the overall complication rate was 0.33% for left ventricular EMB (LVEMB) and 0.45% for right ventricular EMB (RVEMB) and decreased over time, with LVEMB having a comparable or better diagnostic yield.[286]

Isolation of a virus that has been shown to be strongly associated with myocarditis or cardiomyopathy from the myocardium or demonstration of viral proteins or nucleic acids in the myocardium provides supportive evidence that virus infection is the cause of the cardiomyopathy. However, except for neonatal myocarditis or myocarditis occurring in immunocompromised patients or in some cases of fulminant myocarditis, viruses are rarely isolated from cardiac tissue.[14,26,34,231,287] To date, diagnosis of viral myocarditis has generally been based on the isolation of virus from another site (e.g., stool), the demonstration of a fourfold or greater rise in antibody titer from acute to convalescent sera, or the demonstration of a high titer of virus-specific immunoglobulin

M antibody in serum.[a] At best, such data provide only circumstantial evidence of causation of myocarditis and must be interpreted with caution because of the prevalence of asymptomatic infections by the same agents that cause myocarditis. In a prospective study 26% of patients without myocarditis had serologic evidence of infection with agents known to cause myocarditis.[237] These traditional methods have failed to provide a specific diagnosis in most patients with myocarditis of presumed viral origin.

The use of molecular techniques for the detection, amplification, and identification of viral nucleic acids in cardiac tissue obtained by EMB is now providing interesting, but somewhat confusing, data on the viral etiology of myocarditis and DCM.[b] Cloned DNA fragments complementary to different regions of the enterovirus genome have served as either type-specific or broadly cross-reactive hybridization probes, and PCR primers capable of detecting or amplifying nucleic acid sequences of a specific virus (e.g., CVB3) or most enteroviruses or adenoviruses permit the detection of viral genomes with unprecedented sensitivity. In situ hybridization, although somewhat less sensitive than PCR, has the advantage of identifying the specific cells that are infected, whereas PCR can increase the sensitivity of the assay to between 1 and 10 viral genomes per milligram of tissue. Overall, enterovirus RNA has been detected by these techniques in approximately 25% of specimens from patients with myocarditis and in about 15% of specimens from patients with DCM. Similar figures have been reported for adenovirus DNA.[8,12,28]

Results from individual studies have varied markedly, however. A large proportion of cases in which coxsackieviruses were implicated serologically did not give positive results by in situ hybridization.[290,295] Furthermore, the cells that contained enterovirus RNA were often not in areas of myocarditis,[290] and in some studies enterovirus RNA was also detected by PCR in cardiac tissue from control patients with a variety of other conditions but not from noncardiac control tissues.[292] These results raise a number of difficult questions, including the specificity of the assays, the sensitivity and validity of the histopathologic assessments, and the pathogenic significance of enterovirus RNA in the absence of inflammation or necrosis. The interpretation of such results is even more problematic when the viruses in question are members of the Herpesviridae or Parvoviridae families, which regularly persist in normal persons and which may even be present in the blood.[297–300] However, the presence of coxsackie B virus genomes within cardiac myocytes that can induce DCM in the absence of both virus replication and immune activation suggests that the mere presence of enterovirus RNA in the heart may have pathogenic significance.[301] In some studies the presence of enterovirus RNA in patients with myocarditis or DCM has been associated with a favorable prognosis,[302] but in others, patients with demonstrated enterovirus RNA have had a poor outcome.[296,303] Coupling PCR with mass spectrometry analysis has recently provided a method to rapidly detect and semiquantify common viruses in cardiac tissue and is capable of identifying 84 human viruses in one assay.[304]

The limitations of current diagnostic strategies underline the importance of identifying novel biomarkers for the diagnosis of viral myocarditis. Several contemporary observational patient registries of nonischemic cardiomyopathies have been assembled or proposed. Their purpose is to carefully characterize patient phenotypes, bank blood and tissue samples, and realize the potential to link clinical, DNA, and "-omic" markers to long-term outcomes. Information from several of these complementary efforts should lead to improved diagnosis, prognosis, and treatment of nonischemic cardiomyopathies, including myocarditis and other inflammatory cardiomyopathies.[305]

A wide variety of noninfectious diseases and agents may mimic infectious myocarditis and produce identical clinical syndromes (see Table 84.2). Of importance, fulminant and acute myocarditis have been associated with the use of novel checkpoint inhibitors as chemotherapeutic agents in patients with malignancies.[306,307]

Treatment

Because there is a lack of specific therapies for myocarditis of various etiologies, the mainstay of therapy for myocarditis is supportive care and standard management of heart failure (HF). With that treatment many patients with viral myocarditis recover.[308–310]

Management of HF secondary to myocarditis includes agents that inhibit angiotensin signaling, such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, chronic β-blocker therapy, aldosterone antagonists and diuretics for volume overload, as well as device-based therapy when indicated. However, it should be noted that the effectiveness of these therapies has not been proven in patients with myocarditis, and recommendations are derived from therapeutic recommendations for CHF in general. For this reason animal models of myocarditis provide valuable insight. In mice infected with either CVB3 or encephalomyocarditis virus, treatment with captopril, an angiotensin-converting enzyme inhibitor used in the treatment of heart failure from a variety of causes, reduced inflammation and enhanced survival. A nonspecific β-blocker has also been shown to be beneficial in mouse myocarditis.[311] However, digitalis worsened murine myocarditis.[312] Captopril has also been shown to ameliorate myocarditis in a mouse model of Chagas' disease.[313] Calcium channel blockers have improved murine myocarditis, but the application of these therapies is not well established in patients.[314–316]

Fulminant myocarditis is defined by the presence of cardiogenic shock and therefore requires aggressive therapy aimed at hemodynamic support. This usually includes inotropic agents to maintain cardiac output and blood pressure and, in extreme cases, support with a ventricular assist device. Aggressive support is crucial because long-term survival and recovery from this disease is reported to be greater than 90%, despite its severity on presentation.[229,317] Transplantation may be required if improvement does not occur with circulatory support by a ventricular assist device. However, sufficient time should be given to permit spontaneous recovery before considering cardiac transplantation because the long-term prognosis is generally good.[294,309,317–319]

Given the presence of a marked inflammatory infiltrate in the heart in most cases of myocarditis, considerable attention has focused on therapies aimed at inhibiting the immune system. Early in experimental CVB3 infection in mouse models, indomethacin, salicylates, and ibuprofen all increase viral replication and mortality.[221,320–322] No controlled data regarding their use in patients with myocarditis are available.

Administration of glucocorticoids during the acute phase of viral myocarditis is not established, and the deleterious effects of these drugs have been clearly demonstrated in the acute phase of coxsackievirus infection in mice.[323,324] In some uncontrolled trials patients with myocarditis proved by EMB or with positive gallium scans and who were given immunosuppressive agents showed improvement,[325–328] but, with the exception of a methodologically flawed trial in children,[329] controlled trials have failed to show benefit from treatment with prednisone alone or in combination with cyclosporine.[330–333] The largest controlled trial to date, which compared conventional therapy for CHF with conventional therapy plus prednisone and cyclosporine, failed to show benefit from immunosuppression.[330] A subset analysis of immunologic data from this study suggests that an early immune response may improve outcome, whereas an inadequate early immune response may predispose to viral persistence and chronic immunologic damage.[330]

As knowledge of the pathogenesis of viral myocarditis increases and our ability to rapidly identify the etiologic agents improves, subgroups that might benefit from immunosuppressive therapy may emerge. An example is the clearly beneficial effect of immunosuppression on giant cell myocarditis.[334] In the first randomized, placebo-controlled trial in patients with virus-negative myocarditis, in which EMBs were analyzed for the presence of inflammation and molecular analyses excluded virus infection, combination therapy with prednisone and azathioprine improved LV dimensions and ejection fraction compared with placebo.[335]

Data from mice and uncontrolled studies in humans suggest a beneficial role of immunoglobulins containing antibodies to the causative virus in the treatment of viral myocarditis and DCM. In mouse models of myocarditis induced by CVB3 and encephalomyocarditis virus, immunoglobulin administration suppressed myocardial damage and increased survival in association with a reduction in levels of proinflammatory cytokines and adhesion molecules, including TNF, IFN-γ, IL-6, and ICAM-1.[336,337] Maisch and colleagues[338] demonstrated improvement of hemodynamic function and the eradication of virus after hyperimmune

[a]References 19, 21–23, 25, 29, 30, 34, 35, 81, 233, 288.
[b]References 1, 8, 12, 13, 20, 26, 27, 251, 282, 287–296.

globulin treatment of patients with CMV myocarditis. An uncontrolled study in patients with idiopathic cardiomyopathy and an EMB-proven high PVB19 viral load demonstrated mild improvement in LV ejection fraction with intravenous immune globulin (IVIG) therapy.[339] Another small uncontrolled trial showed significantly improved ejection fractions in 10 patients with new-onset DCM or myocarditis who had New York Heart Association (NYHA) class III/IV heart failure with immunoglobulin administration.[340] However, a placebo-controlled study in adults with acute-onset DCM failed to demonstrate any benefit of treatment with IVIG.[341] Because there are only limited data from randomized trials of immunoglobulin treatment of myocarditis, some experts have concluded that "there are insufficient data from methodologically strong studies to recommend routine use of IVIG for acute myocarditis."[342]

Promising data suggest the efficacy of IFN treatment of viral myocarditis. Studies in mice[343,344] and anecdotal reports in patients[345–347] suggest potential benefit, although a small, single-center, matched-cohort trial demonstrated no significant difference in results between patients receiving IFN and control subjects.[348] A randomized, partially blinded phase II trial of IFN-β1b in patients with biopsy-proven chronic viral cardiomyopathy (CVC) showed that "IFN-β1b treatment resulted in virus clearance or a reduction in virus load, with favorable effects on quality of life, NYHA functional class, and patient global self-assessment in patients with CVC." The therapy was reported as safe. The patients' EMBs were all positive for enterovirus, adenovirus, or parvovirus B19.[349] A subsequent, small study reported that IFN-β therapy improved survival and clearance of enterovirus in patients who were PCR positive for enterovirus on EMB.[350]

Additional data suggest that immunoadsorption therapy might be beneficial in patients with myocarditis and inflammatory cardiomyopathy. Controlled randomized studies demonstrated improved cardiac index in patients with DCM, with elimination of anticardiac antibodies by immunoadsorption.[351,352] In an uncontrolled trial improvement in exercise capacity and decrease in B-type natriuretic peptide levels were reported in patients with DCM after immunoadsorption therapy.[353,354] Bulut and colleagues[355] reported an increase in LV ejection fraction and a decrease in LV end-diastolic dimension after this therapy. These data are intriguing, although larger randomized, controlled clinical trials are necessary to prove the benefit of immunoadsorption therapy. Currently, there is an ongoing trial (NCT00558584) enrolling 200 patients with DCM to assess the effect of immunoadsorption on LV ejection fraction.

Ganciclovir has been used successfully in the treatment of severe CMV infections, including myocarditis.[356] No antiviral drugs with efficacy against the enteroviruses are commercially available at this time. Pleconaril, an experimental agent active against most members of the Picornaviridae family, including enteroviruses, has shown some benefit in clinical trials (see Chapter 48) but was not approved due to side effects.[357–359] Pleconaril integrates into the picornavirus capsid and prevents the virus from attaching to cellular receptors and uncoating to release its RNA into the cell cytoplasm. WIN54954, an earlier compound in the same family, had potent antiviral effects in mouse models of CVB3 myocarditis,[360–363] but adverse effects limited its development.

Heymans and colleagues[232] have provided an excellent recent perspective on the current status of and new approaches to myocarditis and inflammatory cardiomyopathy.

Inhibition of the IL-1β pathway has garnered significant recent attention, with impressive therapeutic outcomes in two other inflammatory cardiovascular conditions: coronary artery disease (Canakinumab Antiinflammatory Thrombosis Outcomes Study [CANTOS])[364] and pericarditis (Anakinra-Treatment of Recurrent Idiopathic Pericarditis [AIRTRIP trial]).[365] Evidence suggests that this pathway also is involved in acute myocarditis. The French ACTION Study Group recently initiated a double-blind, randomized phase IIb clinical trial of anakinra (a recombinant IL-1 receptor antagonist), 100 mg, or placebo, given subcutaneously once daily until hospital discharge (maximum, 14 days), in addition to standard care, for acute myocarditis with follow-up over 6 months. Enrollment target is 120 patients, with a start date of May 30, 2017 and an estimated completion date of June 15, 2021.[366]

Exercise has been shown to increase mortality in murine models of CVB3 myocarditis and to worsen wall motion abnormalities in humans with myocarditis.[29,233,367,368] Thus bed rest is generally recommended in cases of acute myocarditis.

Prevention

The most effective means of preventing myocarditis is to eliminate its causes. To the extent that they have eliminated the diseases, vaccines against diphtheria, poliomyelitis, variola, measles, mumps, rubella, varicella, hepatitis virus A and B, influenza A and B, *Haemophilus influenzae* type b, *Streptococcus pneumoniae,* and *Neisseria meningitidis* have eliminated myocarditis caused by these infectious agents. The fact that most cases of myocarditis and DCM in North America and Europe are caused by a limited group of viral pathogens, namely CVB, adenoviruses, and perhaps parvovirus B19, should make it possible to markedly reduce the incidence of myocarditis and DCM in these regions. A safe and effective vaccine against the coxsackie B viruses could be produced with the same technique currently used to produce inactivated polio vaccine. A safe and effective live-oral adenovirus vaccine is currently administered to US military recruits, and a multivalent adenovirus vaccine capable of markedly reducing the incidence of myocarditis and DCM caused by adenoviruses is feasible. Although no vaccine is currently available for parvovirus B19, vaccines have been produced against animal parvoviruses, and the technology is readily available. The major impediments are cost and the relatively small fraction of infections by these agents that result in myocarditis or DCM. However, DCM occurs with an estimated annual incidence of 2 to 8 cases per 100,000, causes significant morbidity and mortality, and is the major reason for cardiac transplantation in the United States and Europe. This should provide sufficient rationale for considering vaccine development.

In addition to vaccination, proper nutrition with adequate selenium is thought to have had a beneficial effect, at least in the Keshan province in China, where selenium deficiency is prevalent.[219,369] It is notable that myocarditis and pericarditis is rare after live vaccination in adults.[370]

PERICARDITIS

Pericarditis (inflammation of the pericardium) may be caused by any of a wide variety of infectious and noninfectious processes. Pericarditis is generally classified as acute, recurrent, or chronic. This chapter focuses primarily on the etiologies of pericarditis, with less emphasis on these different presentations because the different infectious etiologies can contribute to all classifications.[371] Pericarditis may be clinically silent or may result in severe hemodynamic compromise and death. In 1892 Sir William Osler called attention to the frequency with which pericarditis was overlooked by the practitioner,[372] and series indicate that this is still true today.[373–384] Advances in medicine, including antibiotic therapy, cardiac surgery, hemodialysis, cancer chemotherapy, and organ transplantation, as well as the current epidemic of HIV infection and AIDS, have altered the etiologic spectrum of pericarditis over the course of the 20th century and into the 21st. Idiopathic and viral pericarditis now predominate and usually result in a benign, self-limited disease. Purulent bacterial pericarditis and tuberculous pericarditis are now less common, but they still cause significant morbidity and mortality and present a formidable diagnostic challenge. Imazio and colleagues[385] reviewed the evaluation and treatment of pericarditis with a focus on recent therapeutic strategies.

Etiologic Agents

Because of the difficulty in establishing a specific diagnosis, the cause of acute, self-limited pericarditis is never determined in most cases, and the disease is classified as idiopathic. In some series idiopathic pericarditis accounted for 40% to 86% of patients hospitalized with acute pericarditis.[376–382,386] Idiopathic pericarditis continues to account for a large percentage of cases, even when histology, cytology, viral PCR, and bacterial and fungal sequencing are performed on pericardial specimens.[387] There are no clinical or epidemiologic features that distinguish idiopathic pericarditis from acute pericarditis of proven viral origin. Thus, it is likely that viral infections are responsible for many, if not most, cases of acute pericarditis presently classified as idiopathic.

Most viruses infecting the heart can affect both the myocardium and the pericardium (see previous discussion). Of the many viruses associated with heart disease, the enteroviruses, especially the coxsackieviruses, are

most frequently implicated in pericarditis.[35,381,388–390] The association of myopericarditis with coxsackieviruses was first demonstrated in neonates with overwhelming, fatal systemic infections.[391] Pericarditis has also been recognized in the setting of epidemic coxsackievirus infections.[392] A century ago cases of acute benign pericarditis were recognized during epidemics of Bornholm disease (epidemic pleurodynia), and it was postulated that the etiologic agent of the two diseases was the same.[25,392] Subsequently, the group B coxsackieviruses were shown to be the principal cause of epidemic pleurodynia, and their etiologic role in the associated cases of pericarditis was well established.[392]

Coxsackieviruses have only rarely been isolated from pericardial fluid,[383,384,390] and, as with myocarditis, most diagnoses have been based on the isolation of virus from other body sites (e.g., stool) or the demonstration of a fourfold or greater rise in antibody titer after the acute illness, or both.[383,384,390,393–396] A number of other viruses have also been shown to cause pericarditis, but symptomatic involvement of the pericardium is uncommon. When it occurs, it is often a manifestation of severe disseminated infection. Viruses known to cause pericarditis are listed in Table 84.3.

A wide variety of bacteria can cause pericarditis. In the preantibiotic era, purulent pericarditis occurred primarily as a complication of pneumonia in previously healthy children and adults.[373,375–377,397,398] Of the 425 cases of purulent pericarditis reported in 1961 by Boyle and colleagues,[397] 43% were associated with pleuropulmonary infections. *S. pneumoniae* and *S. aureus* accounted for more than half of the cases. With the advent of antibiotics the incidence of purulent pericarditis decreased markedly. Although staphylococci and streptococci are still etiologic in a substantial number of cases,[374,399–407] the incidence of pneumococcal pericarditis has declined substantially, and gram-negative bacilli have assumed a much more important role.[373–375,398,408–416] Patients with purulent pericarditis are now often older and have an underlying predisposing condition.[373–377,398] Recent reports emphasized the importance of anaerobic bacteria, such as *Actinomyces, Prevotella, Fusobacteria, Peptostreptococcus*, and *Propionibacterium,* in pericarditis complicating esophageal perforation and mediastinitis from head and neck infections,[417–422] but anaerobes can also seed the pericardium via the bloodstream.[419–421] Purulent pericarditis may occur as a complication of meningococcal meningitis or fulminant meningococcemia, but *N. meningitidis,* especially serogroup C, also causes primary pericarditis.[423–425] A reactive, culture-negative pericarditis, presumably of immune origin, may also occur after successful treatment of meningococcal infection at another site.[423,424] *Neisseria gonorrhoeae* may also cause pericarditis, which may be either purulent or reactive.[426,427] *M. pneumoniae* can cause pericarditis; although it is uncommon, this manifestation has been observed in almost 1% of patients hospitalized with *M. pneumoniae* infection.[106,107,428,429] *Legionella pneumophila* has been isolated from pericardial fluid,[430–432] and pericarditis has occurred in association with pneumonia[430–434] and endocarditis.[435] Pericarditis has been observed in children and adults with Lyme disease, but myocarditis is a more common manifestation of *B. burgdorferi* infection.[119,436]

Bacterial infections account for proportionately more pericarditis in children; *S. aureus, H. influenzae,* and *N. meningitidis* are the most common etiologic agents.[437–440] Childhood immunization with *H. influenzae* type b conjugate vaccine has markedly reduced the frequency of *H. influenzae* type b infection in children, and this should result in a comparable reduction in the incidence of *H. influenzae* type b pericarditis.

Acute or chronic pericarditis is reported to occur in approximately 1% of patients with pulmonary tuberculosis.[441] Before the AIDS epidemic, because of the declining incidence of primary tuberculosis and the use of effective chemotherapy, *Mycobacterium tuberculosis* accounted for less than 5% of cases of acute pericarditis in Europe and North America.[441–445] In contrast, tuberculous pericarditis is a major cause of heart disease in Africa[62,231,446–448] and in patients with AIDS.[449–451] Diagnosis is difficult and mortality remains high.[442,443] *M. tuberculosis* continues to be an important treatable cause of chronic pericardial effusion and constrictive pericarditis (see Chapter 249).[444,446–448,452–456]

Primary pathogenic fungi are infrequently recognized as a cause of pericarditis. However, in large outbreaks pericarditis occurred in 6% of patients with acute symptomatic histoplasmosis.[457,458] In most it appeared to represent a sterile inflammatory response to infection in adjacent mediastinal lymph nodes and resolved spontaneously without specific therapy. In disseminated histoplasmosis the pericardium itself may be infected with *Histoplasma capsulatum*.[457,458] Pericarditis is rarely recognized in acute coccidioidomycosis.[459] Spontaneously resolving cases resembling those seen in acute histoplasmosis have been described,[460] but most reported cases have occurred in the setting of disseminated coccidioidomycosis and represent *Coccidioides immitis* infection of the heart.[461–464] Pericarditis caused by *Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans,* and other fungi occurs as a consequence of disseminated infection, by direct extension or after surgery, primarily in severely debilitated and immunocompromised patients, especially those with prolonged neutropenia who are receiving multiple courses of antibiotics.[150–152,398,465–474]

Before the introduction of potent antiretroviral therapy, pericarditis was the most frequent cardiac manifestation of HIV and AIDS. Pericardial effusions, most of which are asymptomatic, occur in 10% to 50% of

TABLE 84.3 Infectious Causes of Pericarditis

Viruses

Coxsackie A viruses[30,308]
Coxsackie B viruses[12,27,35,247,388,389,391,392,528,529,538]
Echoviruses[534–536]
Nonpolio enteroviruses
Adenoviruses[482]
Mumps virus[19,549,645]
Influenza A and B viruses[20,552–555,646]
Lymphocytic choriomeningitis virus[562]
Lassa fever virus[563–565]
Varicella-zoster virus[647]
Human cytomegalovirus[648–652]
Epstein-Barr virus[581,587]
Herpes simplex virus[653,654]
Human herpesvirus 6[655]
Variola (smallpox) virus[593]
Vaccinia virus[594–596,656]
Hepatitis B virus[600]
Human immunodeficiency virus[52,54–57,59,60,70,71]

Bacteria and Rickettsia

Streptococcus pneumoniae[397–400]
Other streptococcal species[94,401–405,657,658]
Staphylococcus aureus[96,97,373,397,398,406,407]
Neisseria meningitidis[83,84,423–425]
Neisseria gonorrhoeae[426,427]
Haemophilus influenzae[437–440]
Salmonella[85–87,408,409]
Yersinia[410]
Francisella tularensis[411,412,659]
Pseudomonas[413]
Campylobacter[414,415]
Brucella[416]
Listeria monocytogenes[99,660]
Nocardia[661]
Actinomyces[417,418]
Other anaerobic bacteria[419–422]
Corynebacterium diphtheriae[662]
Mycobacterium tuberculosis[442–453]
Nontuberculous mycobacteria[477–480,663]
Legionella pneumophila[430–435]
Mycoplasma pneumoniae[106,107,428,664,665]
Coxiella burnetii[666]
Chlamydia[108]
Borrelia burgdorferi[118,119,122,125,436]

Fungi

Aspergillus[150–152,466,667–669]
Candida spp.[150–152,467]
Blastomyces[465]
Coccidioides immitis[461,468,469]
Cryptococcus neoformans[151,152]
Histoplasma capsulatum[457]

Parasites[618]

Entamoeba histolytica[670–672]
Toxoplasma gondii[673,674]
Toxocara canis[675]
Schistosoma[676]

patients with AIDS,[52,59,451,475] but in a recent study of 802 HIV-infected outpatients, 85% of whom were receiving antiretroviral therapy, only 2 had effusions on echocardiography.[476]

Although most pericardial effusions are idiopathic, two-thirds of the cases with tamponade are caused by infection or neoplasm; *M. tuberculosis* and nontuberculous mycobacteria, particularly *Mycobacterium avium-intracellulare* complex and *Mycobacterium kansasii,* are responsible for 30% to 50%, and a wide variety of viral, bacterial, and fungal agents have also been isolated from the pericardial fluid.[52,59,451,475,477–484] Malignant effusions have also been observed, primarily EBV-associated lymphomas and Kaposi sarcoma.[c] As with myocarditis, pericardial effusions in HIV-infected patients are associated with advanced stages of infection.[451,481,482,484] The rare parasitic causes of pericarditis are referenced in Table 84.3.

Pathology, Pathogenesis, and Pathophysiology

The pericardium has two opposing mesothelial surfaces. The parietal pericardium forms a flask-shaped sac that encloses the heart and origins of the great vessels. It consists of a 1-mm-thick layer of dense collagen lined by a single layer of mesothelial cells that are covered by microvilli. The mesothelial cell layer is reflected onto the epicardial surface of the heart to form the visceral pericardium. The parietal pericardium has firm attachments to the sternum, diaphragm, and adventitia of the great vessels. The function of the normal pericardium has been a matter of considerable investigation and speculation.[485] It normally contains 15 to 50 mL of clear fluid, which may act as a lubricant. The pericardium reacts to acute injury by exuding fluid, fibrin, and cells in various combinations.[454,482,485,486] Acute pericarditis may resolve completely or progress to fibrous thickening, with or without constriction.

Cardiotropic viruses usually spread to the myocardium and pericardium hematogenously. Inflammation occurs in both visceral and parietal portions; effusion may develop and may be serous, serofibrinous, or serosanguineous. Concomitant myocarditis may or may not be evident. Although most patients with viral pericarditis recover completely, some have repeated disabling recurrences.[377,486–489] The pathophysiology of these recurrences has not been established, but it probably involves immunologic mechanisms and not recurrent or persistent virus replication. Acute pericarditis can cause a pericardial effusion that, in a small percentage of patients, causes pericardial tamponade. Rarely, viral pericarditis leads to constriction as a late complication.[396]

Bacterial pericarditis results from (1) spread from a contiguous focus of infection within the chest, either de novo or after surgery or trauma; (2) spread from a focus of infection within the heart, most commonly from endocarditis; (3) hematogenous infection; or (4) direct inoculation as a result of penetrating injury or cardiothoracic surgery. The incidence of purulent pericarditis arising from a contiguous pneumonia has steadily decreased and now usually occurs only when there has been significant delay in antibiotic therapy.[373,375,398,399] Pericarditis after cardiothoracic or esophageal surgery often occurs in patients with sternal wound infections or mediastinitis[373,398] and may be overlooked. Mortality is high. Pericarditis not infrequently accompanies fatal endocarditis,[96,97,490] especially that caused by *S. aureus*.[96] It often results from extension of a perivalvular abscess into the pericardium.[96,97,490] However, pericardial effusions in endocarditis may also be hemorrhagic or sympathetic and sterile.[490] The presence of preexisting nonbacterial pericardial effusion may predispose to the development of purulent pericarditis in bacteremic patients. Although the pericardial fluid may initially be clear,[397,398] it is usually grossly purulent and may be loculated by the time the disease is clinically apparent. Subsequent organization with adhesions, obliteration of the pericardial space, and calcification may occur and result in constrictive pericarditis.

Tuberculous pericarditis may develop from a hematogenous focus present from the time of primary infection; as a result of lymphatic spread from peritracheal, peribronchial, or mediastinal lymph nodes; or by contiguous spread from a focus of infection in lung or pleura. Four pathologic stages in tuberculous pericarditis have been described.[443,453] In the first stage there is diffuse fibrin deposition and granulomas with viable mycobacteria (Fig. 84.5). Second, a serous or serosanguineous pericardial effusion then develops, usually quite slowly and often without symptoms. Lymphocytes, monocytes, and plasma cells replace the polymorphonuclear cells that are present early in infection. In the third stage the effusion is absorbed, the pericardium thickens, granulomas proliferate, and a thick coat of fibrin is deposited on the parietal pericardium. Acid-fast bacilli become difficult to find as dense fibrous tissue and collagen are deposited. In stage 4, which is associated with constriction, the pericardial space is obliterated by dense adhesions, the parietal pericardium is markedly thickened, and many granulomas are replaced by fibrous tissue. This is often followed by the accumulation of cholesterol crystals and calcification. Constrictive pericarditis may develop in up to 50% of patients with tuberculous pericarditis despite the use of antituberculous chemotherapy.[d] Although the incidence of tuberculosis has declined, it remains an important cause of constrictive pericarditis, especially in developing countries.[446–448,454]

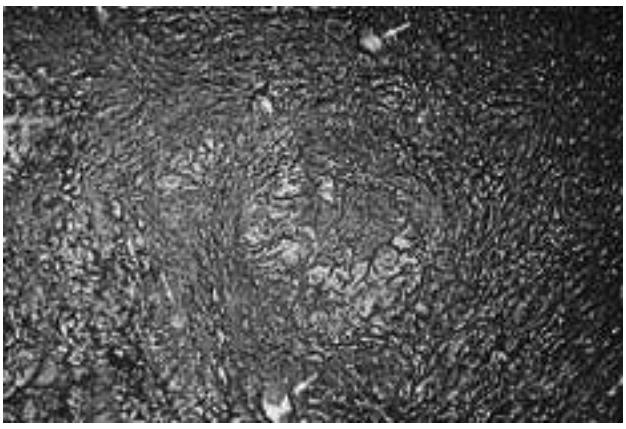

FIG. 84.5 Tuberculous pericarditis, with a typical granuloma in the pericardium. There is central necrosis with aggregates of epithelioid cells at the periphery. Several multinucleated giant cells *(arrows)* are present (original magnification, ×40). *(From Bloor CM. Pericarditis and myocarditis. In: Bloor CM, ed.* Cardiac Pathology. *Philadelphia: JB Lippincott; 1978:265–295.)*

The host inflammatory response is important in the pathogenesis of pericarditis. Xu and colleagues[492] recently updated the scientific understanding of pericarditis, distinguishing between autoinflammatory and autoimmune pericarditis. Activation of the inflammasome underlies autoinflammatory pericarditis. Viruses represent a common activator of the inflammasome, with activation triggering the release of cytokines, including IL-1, which recruit neutrophils and macrophages. Treatment with IL-1β antagonists, such as colchicine and anakinra, have proven to be clinically effective. In autoimmune pericarditis, pericardial inflammatory activity correlates with the severity of the underlying systemic autoimmune disease. Autoimmune pericarditis is characterized by a type I IFN signature. With either type of pericarditis, pericardial delayed hyperenhancement on MRI suggests ongoing inflammation and represents a target for treatment.

Regardless of the cause, if fluid accumulates rapidly in the pericardium and intrapericardial pressure rises, cardiac tamponade may result. Tamponade implies a progressive limitation of ventricular diastolic filling, with resultant reduction in stroke volume and cardiac output. In a series of medical patients with early cardiac tamponade, the cause was infectious in 12.5%, noninfectious in 74%, and undetermined in the remainder.[493] However, the proportion of cases that are idiopathic, associated with infection, or associated with other causes (e.g., cardiac surgery) depends upon the population studied.[374,378,393,450,451]

Clinical Manifestations

The presentation of acute pericarditis varies depending on the cause. However, chest pain, pericardial friction rub, typical electrocardiographic (ECG) changes, and pericardial effusion are the most typical clinical manifestations.[494] Even though the clinical suspicion of pericarditis may

[c]References 52, 59, 451, 475, 481, 482, 484.

[d]References 442–444, 446, 448, 452–454, 456, 491.

be high, it is important to consider the possibility of other causes of chest pain, including coronary artery disease. As the sensitivity of biomarkers of myocardial injury increases, it is likely that a greater proportion of patients with manifestations of pericarditis will also have evidence of myopericarditis as manifested by elevations in troponin I or T.

In viral or idiopathic pericarditis, chest pain is an important feature. This pain is often retrosternal, radiating to the shoulder and neck, and typically is aggravated by breathing, swallowing, and lying supine. In Smith's review of CVB heart disease in adults,[389] 67% of patients had chest pain. Fever was present in 59%. A concurrent or prodromal flulike illness with malaise, arthralgias, myalgias, and occasionally cough with sputum was present in 36%.

Bacterial pericarditis usually develops during the course of a severe systemic infection.[373–375,398] The patient is usually acutely ill, fever is almost always present, and dyspnea is common. However, chest pain is reported by only one-third of patients with purulent pericarditis, and a pericardial friction rub, pathognomonic of pericarditis, is likewise present in only about one-third.[373–375] The symptoms and signs of pericarditis that are present (i.e., fever, dyspnea, and tachycardia) are often attributed to the underlying disease. In consequence, purulent pericarditis is recognized in many patients only at necropsy or after severe hemodynamic compromise has developed.[373–375,482]

Tuberculous pericarditis most often has an insidious onset. Chest pain is present in 39% to 76%,[443,452,454,456] but it may be vague in nature. Weight loss, night sweats, cough, and dyspnea are common. Pericardial effusions in HIV-infected patients are usually asymptomatic, or they may be overlooked in those with severe intercurrent illness.[451,481,484]

The classic physical finding in acute pericarditis is the three-component pericardial friction rub, which reflects cardiac motion during atrial systole, ventricular systole, and rapid ventricular filling in early diastole. This three-component rub was present in 50% of patients with acute pericarditis reported by Spodick.[495] The ventricular systolic component is often the loudest and most frequently appreciated. Rubs are often evanescent and may vary in quality; they are characteristically high pitched, scratching, or grating. In the presence of significant pericardial effusion there may be jugular venous distention—the most common physical finding in acute cardiac tamponade. Enlargement of the cardiac silhouette usually does not occur until at least 250 mL of fluid have accumulated in the pericardial space[482]; if fluid accumulates rapidly, tamponade may occur without detectable enlargement of the cardiac silhouette. A pulsus paradoxus of more than 10 mm Hg, an elevated jugular venous pulse, and a prominent *x* descent with loss of the *y* descent in the jugular venous pressure may be present. Dyspnea is common, but signs of left-sided heart failure are usually absent in cardiac tamponade, and clear lung fields may help to differentiate tamponade from cardiogenic shock.

Constrictive pericarditis, with or without pericardial effusion, is observed in 10% to 20% of patients who present with clinical tamponade.[378] In recent series[378,496] half of the cases of constrictive pericarditis were idiopathic, and most of the others were associated with previous cardiac surgery, thoracic radiation, neoplasms, or tuberculosis. In a series of 26 patients with surgically proven constrictive pericarditis, 18% were found to have no increase in pericardial thickness.[496]

Although the pericardium produces no electrical activity, the ECG is abnormal in 90% of patients with acute pericarditis,[482,486,497] reflecting diffuse sub epicardial inflammation. Characteristic electrocardiographic changes are seen in approximately 50% of patients.[486,497] Early in pericarditis, ST-segment elevation without change in QRS morphology typically occurs in multiple leads. Several days later the ST segment returns to baseline, and there is T-wave flattening. During these early stages there may also be depression of the PR segment. In contrast to myocardial infarction the T-wave inversions in pericarditis usually do not occur until after the ST segment has returned to baseline. These T-wave inversions may last for weeks or months. Large pericardial effusions may be associated with reduced QRS voltage and electrical alternans. Sinus tachycardia is common, but the presence of other arrhythmias suggests preexisting underlying heart disease or significant myocardial involvement.[498]

Echocardiography has proved to be an extremely useful tool for diagnosis of pericardial effusion. The size of the effusion can be roughly quantitated, and early hemodynamic compromise can often be detected.[482] In addition, the echocardiogram can determine whether the ventricular function is compromised, as might occur in myopericarditis. Computed tomography has been useful in demonstrating pericardial thickening and, in some cases, in differentiating an uncomplicated transudate from a high-density exudate.[499,500] In addition, MRI techniques can easily detect pericardial fluid, pericardial thickening, and abnormal ventricular septal motion,[501,502] but they have no particular advantage over echocardiography.

TABLE 84.4 Major Noninfectious Causes of Acute Pericarditis

- Acute myocardial infarction[482,677–679]
- Cardiac injury[482]
  - Trauma (penetrating or blunt)[680]
  - Myocardial infarction (Dressler) syndrome[681]
  - Postpericardiotomy[512,680,682]
- Uremia[683–686]
- Neoplasia[482]
  - Primary
  - Metastatic
- Irradiation[482,635–637]
- Dissecting aortic aneurysm[687]
- Sarcoid[482,644]
- Collagen vascular diseases[482,631]
  - Systemic lupus erythematosus[624]
  - Systemic sclerosis/scleroderma[626,627]
  - Rheumatoid arthritis[628,629,688]
  - Rheumatic fever[482]
- Immunoglobulin G4–related disease[689]
- Inflammatory bowel disease[690]
- Myxedema[691]
- Drug induced[482]
  - Procainamide
  - Hydralazine

Diagnosis

A wide variety of agents and diseases can cause pericarditis and pericardial effusion (see Tables 84.3 and 84.4). Low-grade fever is common in many. A careful history, knowledge of the clinical setting in which the pericarditis occurs, and a search for clues outside the cardiovascular system are helpful in establishing a diagnosis. In a young person without underlying illness who presents with acute pericardial pain, the most likely diagnosis is viral or idiopathic pericarditis. However, establishing a specific viral diagnosis is difficult, costly, and often possible only in retrospect. Virus isolation can be attempted from throat and stool, and acute and convalescent sera can be tested for antibodies to potential pathogens (e.g., coxsackie B viruses and any other enteroviruses prevalent locally at the time), but these approaches frequently fail to yield a specific diagnosis. Viruses are rarely isolated from pericardial fluid, even in patients in whom the diagnosis of viral myocarditis is highly probable. The application of PCR technology using pathogen-specific primers and probes[503] for the detection of the genomes of pathogens in pericardial fluid, pericardial biopsy specimens, and, perhaps, peripheral blood could increase the proportion of patients with pericarditis in whom an etiologic diagnosis is established. However, the etiology of the pericarditis remains undetermined in the great majority of patients.

If the clinical suspicion of viral or idiopathic pericarditis is strong in an otherwise healthy patient with uncomplicated pericarditis, pericardiocentesis or other invasive procedures add little diagnostically[445] and carry a small but definite risk.[504] After excluding patients with postpericardiotomy syndrome, myocardial infarction, renal failure, known neoplastic disease, trauma, and irradiation, Soler-Soler and colleagues[380] prospectively evaluated 256 immunocompetent patients with primary acute pericardial disease. After thorough diagnostic evaluation, 221 (86%) were thought to have acute idiopathic pericarditis. Unsuspected neoplastic pericarditis was found in 12 (5%), tuberculosis in 11 (4%), and collagen vascular disease in 4 patients. Purulent pericarditis and viral pericarditis were each found in 3 patients, and *Toxoplasma gondii* infection was found in 4. The diagnostic yield was substantial when pericardiocentesis or pericardiectomy with biopsy was done to relieve cardiac tamponade (28% and 54%, respectively) but led to a specific etiology in only 5% and 4%, respectively, when these

procedures were done solely for the purpose of diagnosis. The authors concluded that the presence of a pericardial effusion per se is not an indication for an invasive procedure; in patients with pericardial effusion that has persisted for longer than 3 weeks, an invasive procedure may be indicated. In a similar study of patients with large (>20-mm echo-free space in diastole) pericardial effusions without tamponade physiology or suspected purulent pericarditis, the diagnostic yield for pericardiocentesis or surgery was only 7%.[505]

Among those observed expectantly without a drainage procedure, no patient developed tamponade or died as a result of pericardial disease; moderate or large effusions persisted in only 2 of 45 patients.[505] Similarly, small asymptomatic pericardial effusions in patients with HIV infection do not routinely require diagnostic evaluation.[451] In a study of 13 patients with AIDS who underwent surgical pericardial drainage, Kaposi sarcoma was found in 3 (all with preexisting extracardiac lesions), but no other specific causes were identified.[506] However, in patients who are symptomatic, about two-thirds of cases are caused by potentially treatable infections or neoplasms.[451]

When an invasive procedure is necessary for diagnosis, pericardiotomy with biopsy and drainage is preferable to pericardiocentesis because of greater diagnostic yield and fewer complications.[482] Pericardiocentesis alone establishes a specific etiologic diagnosis in 20% to 25% of cases; the availability of both fluid and tissue improves this yield to 54%.[445,456,505] The technique of percutaneous pericardial biopsy is an alternative to surgery for obtaining tissue, and it appears to be safe and effective in patients with a thickened pericardium.[507,508] Noninfectious diseases predominate as causes of significant pericardial effusion and cardiac tamponade,[e] but bacterial and tuberculous effusions are more likely to have serious hemodynamic consequences.[373,374,451,504] Untreated purulent pericarditis is usually rapidly fatal.[373–375,397,398,454] In acutely ill patients in whom purulent pericarditis is suspected, the diagnosis should be pursued quickly and aggressively.

Treatment

Bed rest, symptomatic therapy for pain, and careful monitoring for the development of hemodynamic compromise have been the mainstays of treatment for presumed viral or idiopathic pericarditis. Nonsteroidal antiinflammatory agents are often successful in relieving symptoms in acute pericarditis. Therapy is generally continued for 1 to 2 weeks or longer if symptoms fail to resolve. Discontinuation of therapy is generally tapered to avoid recurrence.[509,510] In addition to these therapies, colchicine has become a key part of pericarditis therapy.[385] A 2013 multicenter, double-blind, placebo-controlled trial compared placebo versus the administration of colchicine during a first attack of acute pericarditis. The dose was 0.5 mg twice daily for patients weighing more than 70 kg and once a day for those less than 70 kg. Usual therapy with aspirin or ibuprofen was included in both arms. The study found that the primary outcome of persistent or recurrent pericarditis occurred in 37.5% of the placebo group compared with 16.7% in the treated group. Colchicine also reduced the rate of symptom persistence at 72 hours, reduced the rate of hospitalization, and increased the rate of remission at 1 week.[511] Two subsequent meta-analyses confirmed the effectiveness of colchicine as adjunctive therapy to NSAIDs in reducing the number of recurrences of pericarditis.[512,513] Because myocarditis often accompanies viral pericarditis and steroids enhance myocardial injury during active virus replication, we believe that steroids should be avoided during the acute pericarditis and may predispose to recurrent episodes.[513] Steroids and other immunosuppressive agents have been used to treat debilitating recurrences of idiopathic pericarditis when other choices are limited, but controlled trials are lacking and serious adverse effects with these regimens are more common.[514,515]

Recurrent pericarditis still presents an ongoing challenge. The AIRTRIP trial used a double-blind, placebo-controlled, randomized withdrawal design to test the therapeutic potential of anakinra. Twenty-one patients who initially responded to a 2-month course of anakinra were randomized to continue daily subcutaneous drug ($n = 11$) or switch to placebo ($n = 10$) for 6 months or until a recurrence of pericarditis. Pericarditis recurred in 9 of 10 placebo patients and 2 of 11 patients continued on anakinra ($P < .001$). Injection site rashes were the most common adverse effect. The promise of IL-1 β blockade in recurrent or persistent pericarditis deserves further assessment in larger and longer-term studies.[365]

Surgical drainage of the pericardium, in addition to appropriate antibiotic therapy, is essential in almost all patients with purulent pericarditis.[397–399,420] Initial pericardiocentesis may be lifesaving, but fluid often reaccumulates and constriction can develop rapidly.[438,444–448] There is little rationale for irrigating the pericardium with antibiotics because penetration of antibiotics from blood is excellent.[516] With early diagnosis and aggressive therapy, *H. influenzae* pericarditis in young patients has a good prognosis.[438] However, overall mortality in bacterial pericarditis remains high (30%), especially when it develops after surgery or occurs in the course of endocarditis.[96,97,397–399,490,517] A retrospective review has suggested that fibrinolysis may have a role in preventing the late complications of purulent bacterial pericarditis as a less invasive alternative to pericardiectomy.[518]

Antituberculous therapy has reduced the mortality of tuberculous pericarditis substantially.[514] However, constrictive pericarditis may develop in 20% to 50% of patients despite appropriate treatment,[442–444,446–448,452,453,491] and patients with the clinical features of tamponade at presentation are at increased risk.[519] The addition of steroids to reduce inflammation and possibly avoid late constriction is favored by many, including the authors.[f] The use of corticosteroids, in addition to antituberculous therapy, is supported by the results of two large, controlled trials in Transkei reported by Strang and associates.[448,455] In a third large randomized trial in which two-thirds of the participants were HIV infected, the use of prednisolone did not have a significant effect on the combined end point of death from all causes, cardiac tamponade requiring pericardiocentesis, or constrictive pericarditis, but it did reduce the incidence of pericardial constriction and hospitalization.[522] In patients with tuberculous pericardial effusions the addition of prednisolone to a four-drug antituberculosis regimen reduced the risk of death, the need for repeat pericardiocentesis, and the need for open surgical drainage because of rapid reaccumulation of pericardial fluid; constrictive pericarditis developed in 8% of steroid recipients and 12% of controls.[455] Prednisolone was given in a dose of 60 mg daily for 4 weeks, followed by 30 mg daily for 4 weeks, 15 mg daily for 2 weeks, and 5 mg daily for 1 week in patients ≥15 years of age. In a similar trial in patients with active tuberculous constrictive pericarditis, the addition of prednisolone increased the rate of clinical improvement.[448] In 10 patients treated initially with 120 mg of prednisone daily, dramatic improvement occurred over a 1-week period, and the author suggested that higher initial doses might allow a shorter duration of treatment. Early surgical intervention is advocated in patients with hemodynamic compromise from recurrent effusion or progressive pericardial thickening.[456,491]

In a retrospective study of children with tuberculous pericarditis reported from Africa, 5 (40%) of 12 patients with evidence of constriction at presentation required pericardiectomy.[523] These authors recommended observation for a period of 6 weeks to 3 months after the initiation of treatment, with pericardiectomy reserved for those who fail to improve or who deteriorate. Pericardiectomy frequently results in striking hemodynamic improvement, but if diagnosis and therapy have been delayed, myocardial function may be affected, leading to less satisfactory results.[524,525] In some patients resolution of venous congestion requires several months.[523] Patients with calcific tuberculous pericarditis have a poorer prognosis than those who undergo operation earlier in the course of their disease.[523,525] Early pericardial drainage and intrapericardial fibrinolysis by means of the instillation of streptokinase have been advocated to facilitate drainage, prevent loculation, and reduce the risk of constrictive pericarditis.[526] However, this approach has not yet been evaluated with appropriate clinical trials.

[e]References 378, 445, 482, 484, 496, 505.

[f]References 442, 448, 455, 456, 520, 521.

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The complete reference list is available online at Expert Consult.

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85 Mediastinitis

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SHORT VIEW SUMMARY

Definition
- Mediastinitis is an infection involving the structures of the mediastinum (see Fig. 85.1).

Epidemiology
- Acute mediastinitis usually occurs via one of three routes: esophageal perforation, extension of a head and neck infection (descending necrotizing mediastinitis), and post–cardiac surgery (see Table 85.1).
- Post–cardiac surgery mediastinitis occurs in 1% to 2% of cardiac surgery cases, and important risk factors for mediastinitis include obesity, diabetes, *Staphylococcus aureus* colonization, use of bilateral internal thoracic arteries for coronary artery bypass, need for reexploration, and receipt of multiple blood products.
- Chronic/fibrosing mediastinitis is not technically an infection but is due to an excessive immune reaction to *Histoplasma* antigens in mediastinal lymph nodes, which results in fibrosis and narrowing of mediastinal structures.

Microbiology
- Oral microbiota, including streptococci, gram-negative bacilli, and anaerobes, predominate in mediastinitis due to esophageal perforation and descending head and neck infections.
- Poststernotomy mediastinitis is most frequently due to gram-positive cocci, especially staphylococci, whereas gram-negative bacilli and *Candida* are less common.

Diagnosis
- Esophageal rupture is usually manifested by chest pain, shortness of breath, and odynophagia.
- Signs of the initiating head and neck infection (swelling, pain, erythema) predominate in descending necrotizing mediastinitis.
- Post–cardiac surgery mediastinitis findings may range from subtle to fulminant but usually present within 2 weeks of surgery. Fever, wound drainage, cellulitis, and chest pain are often present.
- Laboratory findings may include leukocytosis and a rising procalcitonin in postmedian sternotomy patients.
- Fibrosing mediastinitis manifestations include cough, dyspnea, chest pain, and exercise intolerance but depend on the structures occluded.
- Computed tomography is the preferred diagnostic imaging for all forms of mediastinitis.

Therapy
- Broad-spectrum antimicrobials targeted to expected pathogens are essential.
- Aggressive surgical intervention is essential in all forms of infectious mediastinitis, with débridement of any infected or necrotic tissues.
- Esophageal perforation, if recognized early, is increasingly managed with stenting and broad-spectrum antibiotics, but many cases still require surgical repair.
- Post–cardiac surgery mediastinitis requires sternal and mediastinal débridement.
- Multiple surgical techniques for surgical treatment of poststernotomy mediastinitis can be used, with increasing use of negative pressure wound therapy and soft tissue flaps for reconstruction.
- The role, if any, for antifungal therapy in fibrosing mediastinitis is not established.
- In fibrosing mediastinitis, stenoses of vessels and other structures can be managed by surgical bypass or stenting.

Prevention
- Prevention of post–cardiac surgery is a major focus of quality measures and guidelines.
- Key factors associated with decreased post–cardiac surgery infection rates include appropriate antimicrobial prophylaxis (administration of an appropriate agent within 1 hour before surgery and for no longer than 24–48 hours), control of postoperative blood glucose, and perioperative use of nasal mupirocin in patients known to be colonized with *S. aureus*.

Mediastinitis can be organized into acute or chronic forms with etiologies, clinical presentations, and treatments that are strikingly different. Acute mediastinitis is an uncommon but potentially devastating infection involving the structures of the mediastinum. Before the development of sophisticated techniques in cardiovascular and thoracic surgery, most cases resulted from esophageal perforation or contiguous spread from oropharyngeal foci. Mediastinitis now occurs most frequently as a postoperative infection after median sternotomy. Regardless of the pathogenesis of infection, a high index of suspicion must be maintained for this infection so that aggressive, potentially lifesaving measures can be promptly initiated. Chronic mediastinitis, also known as *fibrosing, sclerosing,* or *granulomatous mediastinitis,* is a rare disorder often caused by *Histoplasma capsulatum.*

ANATOMIC CONSIDERATIONS

Detailed descriptions of mediastinal anatomy are available[1,2]; a few fundamental points are emphasized in this chapter. The mediastinum is the region within the thorax between the pleural sacs (Fig. 85.1). It extends from the diaphragm inferiorly to the superior aperture of the thorax. The sternum and costal cartilages make up the anterior boundary, and the 12 thoracic vertebral bodies border the mediastinum posteriorly. The mediastinum is arbitrarily divided into four subdivisions: superior, posterior, anterior, and middle. Structures within the mediastinum include the heart and great vessels, esophagus, distal portion of the trachea and mainstem bronchi, vagus and phrenic nerves, thymic remnants, and thoracic duct. These structures are surrounded by adipose tissue, loose connective tissue, and lymph nodes.

The mediastinum communicates with the structures of the head and neck via several fascial planes and potential spaces (see Chapter 64). The three major routes by which head and neck infections spread to the mediastinum are (1) the pretracheal space, (2) the long fascial planes of the posterior neck, and (3) the viscerovascular or lateral pharyngeal space. The long fascial planes of the posterior neck extend from the base of the skull to the diaphragm and include the retropharyngeal or retrovisceral space, the prevertebral space, and the danger space. In the preantibiotic era, Pearse[1] attempted to delineate the relative contribution of each route to the pathogenesis of mediastinitis and found the retropharyngeal space to be involved in 71% of cases, followed

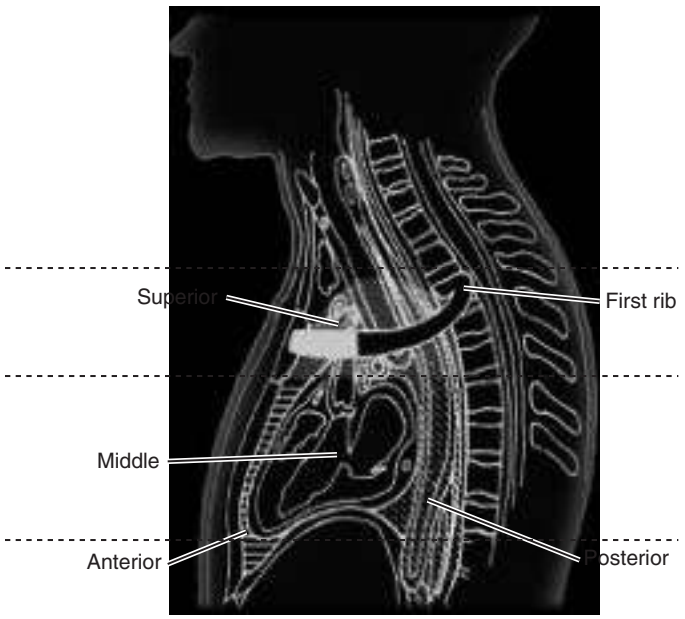

FIG. 85.1 **Anatomic boundaries and divisions of the mediastinum.**

by the lateral pharyngeal space in 21% and the pretracheal space in 8%.

ACUTE MEDIASTINITIS

Epidemiology and Pathogenesis

Primary infection of the mediastinum is rare. Essentially all cases of mediastinitis are due to the spread of infection from other sites or direct inoculation resulting from trauma or surgery. Table 85.1 summarizes the causes of mediastinitis; causes can be grouped into the following four categories: esophageal perforation, head and neck infection, infection originating at another site, and cardiothoracic surgery. The pathogenesis, clinical manifestations, and treatment vary according to the underlying cause of mediastinitis. Spontaneous pneumomediastinum is caused by alveolar rupture with air moving to the mediastinum, and is generally a benign condition not requiring antibiotics, surgical intervention, or often even hospitalization.[3]

Mediastinitis Secondary to Esophageal Perforation

Before the development of cardiac surgery, perforation of the esophagus was the leading cause of mediastinitis, followed by suppurative infections of the oropharynx.[1] In 1724 Boerhaave described the first case of mediastinitis caused by spontaneous rupture of the esophagus in a Dutch admiral who self-induced emesis.[4,5] This entity, known as *Boerhaave syndrome,* has continued to account for approximately 15% to 30% of cases of esophageal perforation.[6,7] Currently, esophageal perforation is most frequently due to iatrogenic injury.[6,7] Flexible fiberoptic endoscopy of the upper gastrointestinal tract is complicated by esophageal perforation in 0.074% to 0.4% of procedures.[7,8] The frequency of this complication increases with prior radiotherapy of the mediastinum and with interventions such as sclerotherapy, dilation, or esophageal stenting.[7,8] Swallowed foreign bodies, esophageal carcinoma, and nonsurgical trauma, such as transesophageal echocardiography, may also result in perforation of the esophagus and subsequent mediastinitis.[7,9,10]

The majority of esophageal perforations occur in the thoracic esophagus and are iatrogenic or spontaneous, whereas foreign-body injury is found predominantly in the cervical esophagus.[7,9,10] Depending on the site of esophageal perforation, mediastinitis may result from direct spillage of esophageal contents into the posterior mediastinum or the migration of contents, via the fascial planes of the neck, into the mediastinum. Spread of infection from the neck into the mediastinum is influenced by respiratory dynamics, in which the negative intrathoracic pressure generated during respiration tends to draw the infection into the mediastinum. A necrotizing chemical mediastinitis ensues, followed by a polymicrobial bacterial mediastinitis, which is often synergistic and necrotizing.

TABLE 85.1 Causes of Acute Mediastinitis

Esophageal Perforation
Iatrogenic
Esophagogastroduodenoscopy, esophageal dilation, esophageal variceal sclerotherapy, nasogastric tube, Sengstaken-Blakemore tube, endotracheal intubation, esophageal surgery including endoscopic resection, paraesophageal surgery, transesophageal echocardiography, transbronchial biopsy, anterior stabilization of cervical vertebral bodies, catheter ablation of atrial fibrillation
Swallowed Foreign Bodies
Bones, coins, can pull-tabs, drug-filled condoms, swords, ballpoint pens, button batteries
Trauma
Penetrating—gunshot wound, knife wound
Blunt—steering wheel injury, seatbelt injury, cardiopulmonary resuscitation, whiplash injury, barotrauma
Spontaneous or Other
Emesis, cricoid pressure during anesthesia induction, heavy lifting, defecation, parturition, carcinoma, ingestion of caustic or corrosive liquids
Head and Neck Infections
Odontogenic, Ludwig angina, pharyngitis, tonsillitis, parotitis, epiglottitis, Lemierre syndrome
Infection Originating at Another Site
Pneumonia; pleural space infection or empyema; subphrenic abscess; pancreatitis; cellulitis or soft tissue infection of the chest wall; osteomyelitis of sternum, clavicle, ribs, or vertebrae; hematogenous spread from distant foci
Lymph nodes: necrosis and hemorrhage (anthrax) or caseous necrosis (tuberculosis)
Cardiothoracic Surgery (Median Sternotomy)
Coronary artery bypass grafting, cardiac valve replacement, repair of congenital heart defect, heart transplantation, heart-lung transplantation, cardiac assist devices, extracorporeal membrane oxygenation, other types of cardiothoracic surgery

Mediastinitis Secondary to Head and Neck Infections or From Other Sites

Mediastinitis secondary to pharyngeal and odontogenic infections is often called "descending necrotizing mediastinitis or fasciitis," and before antibiotics were widely available, it accounted for 10% to 31% of mediastinitis cases.[1,11] Due to effective antimicrobials, these infections are now rare causes of mediastinitis. Middle-aged men predominate in most case series, with generally less than half of cases of descending necrotizing mediastinitis resulting from oral infections.[12] The remainder are due to pharyngeal or other cervical infections. The prototypic odontogenic infection leading to mediastinitis is Ludwig angina, which usually stems from an infection of the second or third mandibular molars spreading to involve the sublingual and submandibular spaces (see Chapter 64). From these spaces the infection can spread via the lateral pharyngeal space to involve the retropharyngeal space or carotid sheath and track into the mediastinum. During the antibiotic era, approximately 3.5% of cases of Ludwig angina have been complicated by mediastinitis.[13]

Mediastinitis resulting from infections involving the lateral pharyngeal space may originate from various sources, including the teeth, parotid glands, or tonsils, or, rarely, from otitis, mastoiditis, or epiglottitis.[12,14,15,16] Infections of the retropharyngeal space generally arise from perforation of the esophagus or by extension of pharyngitis, epiglottitis, or tonsillitis. These infections can easily spread into the superior mediastinum via the long fascial planes of the neck or, if the danger space is involved, into the posterior mediastinum. Not all patients with necrotizing

TABLE 85.2 Risk Factors for Surgical Site Infection/Mediastinitis Post–Cardiac Surgery

PREOPERATIVE RISK FACTORS	OPERATIVE RISK FACTORS	POSTOPERATIVE RISK FACTORS
Increasing age	Emergent surgery	Need for reexploration
Diabetes	Heart transplant	Prolonged ICU stay
Staphylococcus aureus nasal colonization	Increasing complexity of surgery	Need for mechanical ventilation >48 h
COPD	Use of internal thoracic arteries in CABG	Lack of perioperative glucose control
Peripheral vascular disease	Prolonged operative time	Placement of tracheostomy
Class 3–4 angina	Hair removal by razor, not clippers	Postoperative myocardial infarction
Renal failure requiring hemodialysis	Inappropriate timing of antibiotics	Receipt of multiple blood products
History of endocarditis	Prolonged time on cardiopulmonary bypass	
Cigarette smoking	High core temperature during bypass (>38°C)	
Low cardiac output		
Concurrent infection		
Prolonged preoperative hospitalization		
Preoperative use of a ventricular assist device		

CABG, Coronary artery bypass grafting; *COPD,* chronic obstructive pulmonary disease; *ICU,* intensive care unit.

infections of the face and neck will progress to mediastinitis. Of 286 deep neck infections at a single center over 11 years, only 6% progressed to involve the mediastinum.[17] Petitpas and colleagues[18] evaluated risk factors for the development of mediastinitis and found that a pharyngeal source, administration of oral glucocorticoids, and the presence of gas in the tissue were associated with the development of mediastinitis.

Rarely, mediastinitis is due to spread from other adjacent infections. The pretracheal space descends into the anterior mediastinum and most often is involved in mediastinitis complicating procedures involving the thyroid and trachea.[1] Cases have been described secondary to the following conditions: pneumonia; pleural space infection; osteomyelitis of the ribs, clavicle, sternum, or vertebrae; subphrenic abscess; pancreatitis; cellulitis; and hematogenous seeding from distant foci.

Mediastinitis Secondary to Cardiothoracic Surgery

Cardiothoracic operations are among the most common surgical procedures performed in larger hospitals, and thus mediastinitis has become a predominantly postsurgical infection. Many studies have documented the incidence and risk factors for the development of mediastinitis after cardiothoracic surgery. In 1984 Sarr and colleagues[19] reviewed the available literature and found the incidence of mediastinitis to be 0.4% to 5% among patients undergoing median sternotomy. Since that time, numerous studies have been published, with incidence rates of 0.5% to 4.4%, with more recent studies showing incidence rates generally <2%.[20–22,23,24,25,26] Some selected trials evaluating rates of infection after cardiac surgery deserve mention as they suggest rates of infection may be declining. An analysis of 133,045 coronary artery bypass grafting (CABG) procedures reported to the National Healthcare Safety Network (NHSN) between 2006 and 2008 found that 1.3% developed a deep tissue or organ space infection, although not all of these infections involved the mediastinum.[27] The Society of Thoracic Surgeons National Cardiac Database, a voluntary quality reporting database that reportedly captures >95% of cardiac surgery cases, reported major infections occurred in 3.51% of more than 330,000 procedures in 2002–03, of which 25.1% was attributed to mediastinitis, with an overall incidence of mediastinitis of roughly 0.9%.[28] Data from this same database in 2016, including more than 217,000 procedures, demonstrated deep sternal wound infection/mediastinitis rates of 0.3%.[29] In outbreak settings the incidence of mediastinitis can be much higher, with rates of infection up to 23%.[30,31]

Risk Factors for Mediastinitis

Numerous factors that increase the risk of mediastinitis in cardiothoracic surgery have been identified. Most studies examining these risk factors are retrospective case-control studies and are limited by the problems inherent in retrospective surveys. Risk factors generally can be divided into the following categories: preoperative, intraoperative, and postoperative (Table 85.2). Risk factors that have been identified preoperatively include increasing age, diabetes mellitus, obesity, previous sternotomy, chronic obstructive pulmonary disease, peripheral vascular disease, class 3 or 4 angina, renal failure requiring hemodialysis, history of endocarditis, cigarette smoking, low cardiac output states, preoperative *Staphylococcus aureus* colonization, hair removal with razor versus removal with electric clippers, and prolonged preoperative hospitalization.[a] The contribution of each individual risk factor is difficult to ascertain, but obesity is one of the most consistent and strong predictors of mediastinitis. A body mass index of greater than 30 increases the risk of developing mediastinitis 2.2- to 6.5-fold.[b] The increased risk is likely multifactorial and may involve increased technical difficulties during surgery, prolonged operative time, increased bleeding, inadequate preoperative antibiotics, and increased risk of sternal dehiscence.[36,40] The contribution of gender to the risk of mediastinitis is unclear. A study of more than 18,000 CABG procedures performed in Norway found male gender to be associated with a 7.9-fold increase in mediastinitis risk, but analysis of NHSN data including more than 133,000 CABG procedures found female gender increased the risk of complex sternal wound infection, although only slightly (odds ratio [OR], 1.7).[26,27] The reasons for these differences are unclear.

Intraoperative and surgical risk factors identified include emergent surgery, heart transplant surgery, the complexity of surgery, use of one or both internal thoracic arteries in CABG, prolonged operative time, prolonged time on cardiopulmonary bypass, high core temperatures (>38°C) during cardiopulmonary bypass, indiscriminate use of electrocautery, and inappropriate timing of antibiotic prophylaxis.[c] The use of bone wax on sternal edges to decrease bleeding has been implicated as a risk factor for mediastinitis based on animal data, but a prospective, randomized study involving 400 patients undergoing CABG found no significant increase in mediastinitis associated with its use.[43,44] Of note, no clinical benefit was derived from its use either. The use of off-pump CABG surgery does not alter the incidence of mediastinitis, although minimally invasive procedures may have lower infection rates.[45,46] Postoperative risk factors that place patients at greater risk for mediastinitis include the need for reexploration to control bleeding, a prolonged length of stay in the intensive care unit, prolonged mechanical ventilation (>48 hours), tracheostomy placement, lack of perioperative and postoperative glucose control, postoperative myocardial infarction, and blood product transfusion.[d]

Despite much research, there is not universal agreement regarding any of these risk factors and their relative contribution. For example, despite nearly 40 years of surgical experience, debate still exists on whether the use of internal thoracic artery (ITA) grafts in CABG predisposes patients to mediastinitis. Use of the ITA for revascularization is preferred because graft patency rates and long-term survival are significantly higher than saphenous venous grafts.[49,50] However, in 1972, on the basis of anatomic studies of sternal blood supply, Arnold[51] suggested that the use of the ITA in CABG procedures might lead to significant sternal ischemia and predispose patients to sternal osteomyelitis and mediastinitis. This hypothesis has been supported by several laboratory and numerous clinical studies.[e] A review of studies published between 1997 and 2007 found the use of bilateral ITA grafts increased the risk of mediastinitis significantly, especially in diabetic patients.[53] A large randomized trial found bilateral ITA grafting was associated with twice the rate of sternal wound complications of unilateral grafting.[54] It should be noted that a number of other investigators have not observed any increase in sternal wound infections in patients undergoing CABG when one or both ITA grafts were used.[f] The surgical technique of skeletonized bilateral internal mammary artery grafting may abrogate

[a]References 20, 21, **23**, **24**, 25, **26**, **28**, **32**, 33, **34**, 35, **36**, 37, 38.
[b]References **23**, **24**, 25, **26**, **28**, **32**, **36**, 39.
[c]References 20, **23**, **24**, 25, **28**, **32**, **34**, 37, 38, 41, 42, **43**.
[d]References 20, 21, **23**, **24**, **26**, **32**, 39, 47, 48.
[e]References 20, 21, **24**, 25, **34**, **52**, 53.
[f]References **26**, **28**, **36**, 37–39, 55, **56**.

the increased risk of mediastinitis and could be a potential explanation for variability in findings.[53]

An increasing body of evidence has associated the receipt of blood products, particularly packed red blood cells (RBCs), with increased rates of postoperative bacterial infections, including mediastinitis. More than 20 years ago, Loop and colleagues[20] found the risk of mediastinitis increased with the number of units of blood transfused postoperatively, and more recently, Risnes and colleagues[26] noted a similar association between transfusion of multiple units of blood and mediastinitis. The authors of a systematic review of seven studies, including more than 32,000 cardiac surgery cases, concluded that RBC transfusion is associated with an increase in mediastinitis risk.[57] It appears this transfusion risk may be dose dependent and does not seem to apply to other blood products, such as fresh-frozen plasma (FFP) or platelets.[58,59] It is hypothesized that transfusion induces a downregulation of the immune response, predisposing to infection. On the basis of these data, routine transfusion at arbitrary hemoglobin levels should be avoided, and strategies to minimize transfusion should be used, such as the use of intraoperative cell saver and provision of FFP and platelets when bleeding occurs.

The use of mechanical support devices, including left ventricular assist devices and intraaortic balloon pumps, either before or after cardiac surgery, has been associated with an increase in the risk of mediastinitis.[28,34,60] Extracorporeal membrane oxygenation (ECMO) has increasingly been used for respiratory support either alone or in the cardiac surgery postoperative period, with large-vessel cannulation either in the femoral area or directly into the thoracic cavity. ECMO use after cardiac surgery has been noted to be a risk factor for mediastinitis in at least one study, and in a survey of 220 patients on ECMO, 64% developed a nosocomial infection with mediastinitis occurring in 11%.[25,61]

Patients undergoing heart transplantation are at higher risk for development of mediastinitis, with incidences of 2.1% to 8.7%.[62,63,64,65,66] Data on risk factors for mediastinitis in heart transplant patients are much more limited but include similar factors, such as obesity and prolonged cardiopulmonary bypass.[66,67] One risk factor particularly prevalent in heart transplant patients is the presurgical use of mechanical support devices, such as left ventricular assist devices or artificial hearts, which appear to increase the risk of mediastinitis.[64,65,68] There is debate regarding the effect of specific immunosuppressive agents on sternal wound infection and mediastinitis risk. Although Kuppahally and colleagues[67] found use of sirolimus to be a risk factor for posttransplant wound complications, including mediastinitis, Zuckermann,[66] in an analysis of three studies of everolimus, including more than 1000 heart transplants, did not find an increase in incisional complications when compared with other immunosuppressive regimens. Donor-to-recipient transmission of bacteria rarely has been observed to result in mediastinitis.[69]

Rates of mediastinitis in children undergoing cardiac surgery are generally lower than adults and range from 0.09% to 1.4%,[70,71–73] with the largest survey of 30,000 procedures in the United States noting mediastinitis in 0.3% of cases.[71] Although pediatric patients typically lack the chronic medical conditions predisposing adults to infection, some similar risk factors have been identified, including American Society of Anesthesiologists physical status classification class, previous cardiac procedure, preoperative hospitalization or ventilator use, complexity of surgery, duration of procedure, and receipt of multiple postoperative transfusions, which have all been associated with either increased risk of surgical site infection (SSI) or mediastinitis.[71,72,74,75] Risk factors unique to children include very young age (<90 days) and the presence of a genetic syndrome.[71–73,75] In pediatric patients, delayed sternal closure may predispose to development of mediastinitis, particularly due to gram-negative pathogens.[76,77]

Pathogenesis

It is believed that the pathogenesis of post–cardiac surgery mediastinitis is primarily due to the inoculation of organisms from the patient's endogenous bacterial microbiota or the surgical field into the operative site. Bacteria are able to propagate in the protected avascular area of the surgical wound and cause infection. The identification of risk factors, such as the length of time of surgery, the complexity of surgery, and the need for reexploration, all of which increase the likelihood of contamination, support this hypothesis, as does the observation of lower rates of wound infection and mediastinitis in patients undergoing cardiac surgery with minimally invasive techniques.[21,46] Outbreaks of mediastinitis that have been epidemiologically linked to sources such as bacteria from a particular surgeon's hands or nares also lend support to the concept of direct inoculation of the wound.[78]

The specific source of the bacterial contamination is usually the patient's own endogenous microbiota, particularly if that patient is colonized with *S. aureus.* In 1959 Weinstein noted patients colonized with *S. aureus* had a fourfold higher SSI rate and through phage typing was able to demonstrate that *S. aureus* isolates causing SSI were usually identical to those isolated from the patient's nares preoperatively.[79] Later, Jakob and colleagues[r] used pulsed-field gel electrophoresis (PFGE) to show *S. aureus* mediastinitis isolates were identical to those that had been obtained preoperatively from the nares of patients. Through culturing the nares of medical personnel, they were also able to show that health care workers were rarely the source of *S. aureus,* although two vein–harvest site infection isolates were identical to an isolate from a ward nurse.

Pathogenic mechanisms and ability to cause disease may vary depending on the infecting organism. Certain strains of coagulase-negative staphylococci may be particularly adapted to causing mediastinitis in the postoperative period. Archer and Armstrong[80] showed that patients are colonized by small numbers of antibiotic-resistant, coagulase-negative staphylococci, which become the predominant species when subjected to the selective pressure of prophylactic antibiotics. In addition, Olsson and colleagues[81] noted coagulase-negative staphylococci isolated from deep sternal wounds were more likely to produce biofilm. Mechanism of disease may differ even between methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA). A multivariate analysis of postoperative mediastinitis found differing risk factors for infection caused by each organism, with obesity being the only risk factor for MSSA mediastinitis, whereas being female, age older than 70 years, and having diabetes were risk factors for MRSA infection.[82] Preoperative nasal colonization with *S. aureus* has been associated with the development of mediastinitis.[35] A more recent analysis using PFGE of nasal and clinical isolates of *S. aureus* from patients with post–cardiac surgery mediastinitis found MSSA strains isolated from the mediastinum were identical to nasal strains in seven of nine cases.[83] No nasal MRSA isolates were isolated from the patients with mediastinitis resulting from MRSA, and all mediastinal MRSA isolates were highly genetically related, suggesting a possible nosocomial source.

Various immunosuppressive effects of cardiopulmonary bypass that may contribute to the pathogenesis of mediastinitis after surgery have been elucidated, although no difference in infectious complications, including mediastinitis, was observed when comparing CABG with and without the use of a cardiopulmonary bypass pump.[22,84] The importance of environmental and postoperative factors has also been emphasized by outbreaks of mediastinitis that have been linked to various sources, including contaminated tap water, air with increased concentrations of *Aspergillus fumigatus* spores, use of heater-cooler units colonized with nontuberculous mycobacteria, and poor hand-washing technique during postoperative care.[30,31,85,86,87] Controlled prospective studies are necessary to define better the factors that influence post–cardiac surgery mediastinitis.

Bacteriology

The bacteriology of mediastinitis complicating cardiovascular surgery is strikingly different from mediastinitis secondary to head and neck infections or esophageal perforations (Table 85.3). Mediastinitis secondary to cardiothoracic surgery is primarily caused by gram-positive cocci and less often by gram-negative bacilli. The relative contribution of *S. aureus* and MRSA varies by series, with two recent reviews of more than 500 cases of mediastinitis implicating *S. aureus* in roughly 60%,[88,89] whereas other reviews have found coagulase-negative staphylococci to be the most frequently isolated organisms.[90,91] Improved microbiologic techniques have increasingly implicated *Cutibacterium acnes* as an etiologic agent, but the role of this organism in mediastinitis requires further study.[90,91] *Candida* is responsible for a growing number of cases

TABLE 85.3 Microbiology of Mediastinitis

Organisms Frequently Recovered in Mediastinitis Secondary to Infection of the Head and Neck or Esophageal Perforation

Anaerobic

Gram-positive cocci—*Peptostreptococcus* spp.
Gram-positive bacilli—*Actinomyces, Eubacterium, Lactobacillus*
Gram-negative cocci—*Veillonella*
Gram-negative bacilli—*Bacteroides* spp., *Fusobacterium* spp., *Prevotella* spp., *Porphyromonas* spp.

Aerobic or Facultative

Gram-positive cocci—*Streptococcus* spp., *Staphylococcus* spp.
Gram-positive bacilli—*Corynebacterium*
Gram-negative cocci—*Moraxella*
Gram-negative bacilli—Enterobacteriaceae, *Pseudomonas* spp., *Eikenella corrodens*
Fungi—*Candida albicans*

Representative Organisms Recovered in Mediastinitis Secondary to Cardiothoracic Surgery, With Representative Rate and Range

Gram-Positive Cocci

Staphylococcus aureus, 25% (7.1%–66.7%)
Staphylococcus epidermidis, 30% (6%–45.5%)
Enterococcus spp., 10% (8%–18.8%)
Streptococcus spp., 2% (0%–18.2%)

Gram-Negative Bacilli

Escherichia coli, 5% (0%–12.5%)
Enterobacter spp., 10% (4%–21.4%)
Klebsiella spp., 3% (0%–21.1%)
Proteus spp., 2% (0%–7.1%)
Other Enterobacteriaceae, 2% (0%–20%)
Pseudomonas spp., 2% (0%–54%)

Fungi

C. albicans, <2% (0%–20.5%)
Polymicrobial, 10% (0%–40%)

Others Occasionally Reported

Acinetobacter, Salmonella spp., *Legionella* spp., *Bacteroides fragilis, Corynebacterium* spp., *Burkholderia cepacia, Mycoplasma hominis, Candida tropicalis, Aspergillus* spp., *Nocardia* spp., *Kluyvera, Gordonia sputi, Mycobacterium fortuitum, Mycobacterium chelonae, Rhodococcus bronchialis*

Other Unusual Causes of Mediastinitis

Anthrax, brucellosis, actinomycosis, paragonimiasis, *Streptococcus pneumoniae*

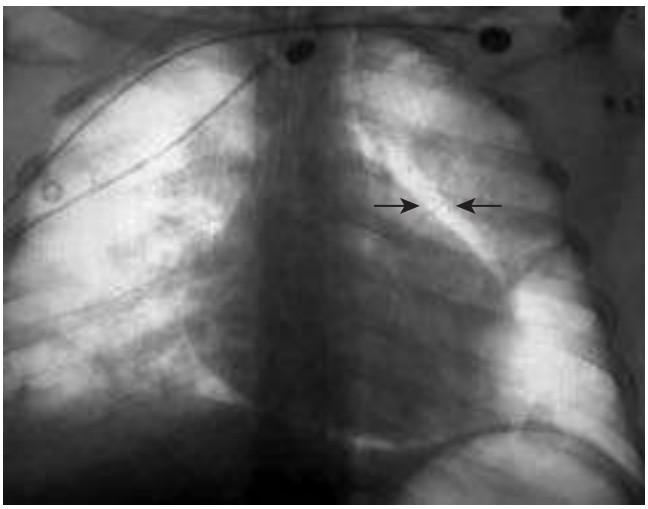

FIG. 85.2 Mediastinitis. Chest radiograph shows large pneumomediastinum and pneumopericardium *(arrows)* in a patient with mediastinitis.

of mediastinitis after cardiothoracic surgery and may be associated with increased mortality.[92,93]

The bacteriology of mediastinitis due to esophageal perforation and extension of head and neck infections is frequently polymicrobial. Synergistic infection comprising both oral anaerobes and gram-negative bacilli is usually present. The most frequently isolated organisms include viridans group streptococci; staphylococci, including *S. aureus*; peptostreptococci; *Bacteroides* spp.; *Prevotella* spp.; and *Fusobacterium.* The relative frequency with which these organisms are isolated varies because of the difficulty in obtaining reliable anaerobic culture data.[94]

Clinical Manifestations and Diagnosis

The clinical manifestations of mediastinitis differ according to the underlying cause of disease. When mediastinitis occurs due to extension of an odontogenic or pharyngeal infection, the symptoms and signs of the primary infections predominate, such as pain, odynophagia, skin erythema, fever, and swelling of the affected site. Esophageal perforation may be clinically obvious or inapparent depending on the nature of the injury. Early in the course of mediastinitis, the signs and symptoms may be subtle, but as the condition progresses, patients note increasing chest pain, respiratory distress, and odynophagia. Chest pain is often the most prominent symptom and may localize depending on the portion of the mediastinum involved. In anterior mediastinitis, pain is often located in the cervical or substernal region. Pain from posterior mediastinitis may localize to the epigastric area with radiation to the interscapular region.[11] Patients with Boerhaave syndrome (transmural perforation of the esophagus) typically present with excruciating anterior chest pain with a history or severe vomiting or retching. Pleural effusion is a common complication and may manifest as pleuritic chest pain. Retroperitoneal extension may be accompanied by acute abdominal signs and may prompt unnecessary exploratory laparotomy.

Examination frequently reveals fever; tachycardia, crepitus, and edema of the chest or neck may also be present. *Hamman sign,* a crunching, rasping sound heard over the precordium synchronous with the cardiac rhythm, caused by emphysema of the mediastinum, may be audible in 50% of patients with pneumomediastinum.[95] The heart sounds may be distant and dull. In the later stages of mediastinitis, signs of bacteremia and sepsis may predominate. The early diagnosis of mediastinitis in an infant or neonate can be particularly challenging. A peculiar, interrupted, staccato type of inspiration has been described in many patients.[96] The signs and symptoms of mediastinitis in older children are similar to those observed in adults.[97]

Laboratory testing usually reveals leukocytosis along with other signs of infection and potentially organ dysfunction. Radiographically, plain films of the chest may reveal mediastinal widening, air-fluid levels, and subcutaneous or mediastinal emphysema (Fig. 85.2).[13] The lateral chest radiograph may be useful in showing superior mediastinal gas not evident on upright films. Complications of mediastinitis, such as pleural effusion or pneumoperitoneum, may also be evident.

Computed tomography (CT) is essential for diagnosis of mediastinitis in patients with odontogenic and pharyngeal infections. CT imaging of both the neck and chest are necessary to define the extent of the infection and determine if mediastinal involvement has occurred as this often alters the surgical approach. Typical CT findings include evidence of the primary infection along with mediastinal encapsulated fluid collections, air in soft tissue planes, pleural effusions, abscesses with air bubbles, and increased density of mediastinal fat with loss of typical tissue planes.[12,98,99] Transesophageal endosonography has been used to guide needle aspiration of the posterior mediastinum, particularly in cases in which the CT scan is inconclusive.[100] Technetium-labeled white blood cell scans have been reported to be helpful in the diagnosis of mediastinitis in specialized circumstances when CT scanning was not readily available.[101] Magnetic resonance imaging (MRI) has rarely been used, and its utility is unknown.

Chest radiographs in patients with esophageal perforation reveal significant abnormalities in about 90% of patients, although establishing the presence of a perforation can be difficult, and multiple modalities of imaging and direct visualization using endoscopy are often necessary to confirm the diagnosis.[102] Contrast esophagography using water-soluble contrast agents is recommended as the initial imaging examination and may reveal extravasation of dye. Although dilute barium provides better definition of anatomy and detection of subtle defects and perforations,

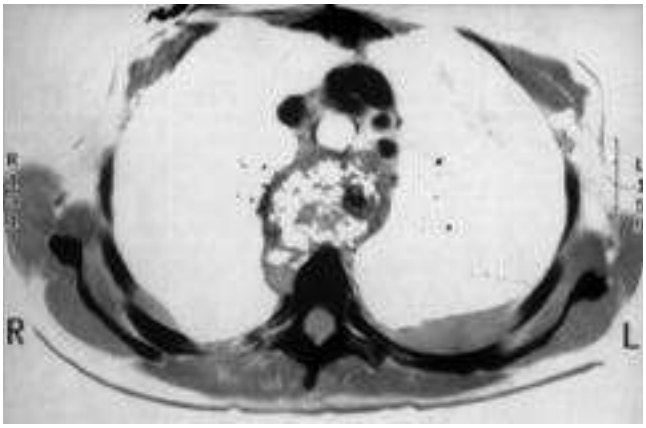

FIG. 85.3 Mediastinitis. Computed tomography scan of the chest at the level of the sixth thoracic vertebra shows large abscess within posterior mediastinum and left-sided pleural effusion. The patient had experienced perforation of the esophagus owing to a swallowed foreign body. *(From Rupp ME. Mediastinitis. In: Fishman AP, Elias JA, Fishman JA, et al, eds.* Fishman's Pulmonary Diseases and Disorders. *3rd ed. New York: McGraw-Hill; 1998:2036–2043.)*

it should generally be avoided because extravasated barium evokes significant inflammation.[7] CT is often helpful in both confirming the diagnosis and defining the extent of mediastinal contamination (Fig. 85.3), and some authors recommend the combination of both an esophagram and CT in all patients.[6,7,10] Upper endoscopy has been used both diagnostically to detect and evaluate the extent of injury and therapeutically to repair small injuries or remove foreign bodies.[10,103,104]

Mediastinitis Caused by *Bacillus anthracis*

The intentional release of *Bacillus anthracis* via the US Postal Service generated great interest in the recognition, management, and prevention of inhalational anthrax (also see Chapter 207). Inhalational anthrax exposure results in a hemorrhagic mediastinitis.[105] After inhalation, the 1- to 5-µm spores of *B. anthracis* are ingested by macrophages and transported to hilar and mediastinal nodes, where they germinate, propagate, and release toxins that lead to hemorrhage, edema, and necrosis. Approximately 90% of patients with inhalational anthrax exhibit mediastinal adenopathy or widening on radiographic studies.[105] The finding of mediastinal adenopathy or widening in a febrile patient should trigger strong suspicion for inhalational anthrax and institution of appropriate diagnostic and therapeutic measures.

Cardiac Surgery–Related Mediastinitis

Post–cardiothoracic surgery mediastinitis typically becomes clinically evident within 2 to 4 weeks after surgery, although rare instances occurring more than 1 year after surgery have been reported.[g] Infection resulting from gram-negative organisms may manifest earlier after surgery, typically within 2 weeks.[89] A review of 197 cases of mediastinitis found septic shock was more common in early-onset mediastinitis, as were enterococci and gram-negative pathogens, whereas *S. aureus* was the dominant pathogen after 14 days.[89] Usually, *S. aureus* mediastinitis is readily diagnosed on clinical grounds, whereas less virulent coagulase-negative staphylococci often have much subtler findings and may be initially misdiagnosed as uninfected in up to 30% of cases.[90]

In post–cardiac surgery patients the presentation of mediastinitis may be fulminant or subtle. Clinicians must have a high sensitivity for this infection in postoperative patients because they can present without localizing signs or symptoms, and the signs of sepsis may be the only evidence of infection.[19,89] Fever is the most common presenting symptom and may be accompanied by localized signs of infection, such as erythema, cellulitis, or purulent discharge from the mediastinal wound.[h] Patients may experience greater-than-normal postoperative pain, which can be pleuritic in nature. Sternal instability, dehiscence, and wound drainage are frequently noted and may be the only sign of infection in patients.[91] Dysphagia, the observation of bubbles emanating from the sternal wound, and chest wall emphysema are uncommon. Poststernotomy mediastinitis manifesting as a deep neck abscess without abnormal findings on chest examination has been reported, as has the development of septic shock and bacteremia without obvious signs of sternal infection.[89,107]

Laboratory tests usually show a moderate leukocytosis with a leftward shift of the white blood cell differential count. Biologic markers such as C-reactive protein and procalcitonin are typically elevated. Procalcitonin has been shown to be superior to C-reactive protein in diagnosing infection in patients having undergone cardiopulmonary bypass but has not been specifically studied in mediastinitis.[108] The presentation of mediastinitis in transplant patients may be atypical and lack the usual clinical findings of fever and elevated white blood cell count.[64] Procalcitonin has been found to be particularly useful in differentiating infection from rejection in transplant patients and may play an adjunctive role in the diagnosis of mediastinitis in this patient population.[109] Blood cultures are of variable usefulness in diagnosing mediastinitis. Patients with mediastinitis caused by *S. aureus* and gram-negative bacteria are frequently bacteremic. Post–cardiac surgery patients with bacteremia in which the source is not evident should be evaluated for possible mediastinitis because one author found the presence of *S. aureus* bacteremia in the first 60 days after median sternotomy had a 90% positive predictive value for mediastinitis.[110] Blood cultures are less useful in diagnosing mediastinitis caused by coagulase-negative staphylococci with rates of bacteremia typically less than 10%.[91]

Radiographically, mediastinal widening is a rare finding on plain chest films, and routine radiographs are usually of little value in the diagnosis of mediastinitis after cardiothoracic surgery (due to postsurgical abnormalities).[19,111] CT is usually the first diagnostic step when concern for postoperative mediastinitis exists, particularly when there is a need to differentiate superficial wound infections from deeper retrosternal processes. Characteristic findings include soft tissue swelling, pleural or pericardial effusions, fluid or air collections (or both), and sternal erosion.[111] Normal postoperative collections of fluid and gas are sometimes difficult to differentiate from early signs of mediastinitis, especially in the early postoperative period (<2 weeks).[111] The ability of CT to diagnose mediastinal infection was evaluated in 203 patients with mediastinitis and was found to have an overall sensitivity and specificity of 87.6% and 90.5%, respectively, and was particularly sensitive for detection of sternal osteomyelitis.[112] Dressler syndrome can be difficult to distinguish from mediastinitis after cardiac surgery by CT. The diagnostic value of nuclear scans has been espoused by several investigators and may be particularly useful when other imaging is equivocal.[113,114]

Browdie and colleagues[115] evaluated the relative value of CT, indium-111–labeled leukocyte scanning, and epicardial pacer wire cultures in 24 patients undergoing evaluation for possible mediastinitis and found that epicardial pacer wire cultures were 100% sensitive and 92% specific. Other authors have disagreed with the usefulness of epicardial pacer wire cultures. Two large studies, including more than 2000 patients, found routine pacer wire culture to be associated with an unacceptably high rate of false-positive findings.[116,117] In these two investigations the sensitivity and specificity of routine epicardial wire cultures ranged from 52.1% to 75% and 72.1% to 83.4%, respectively. Positive predictive values were 8.2% to 11.6%, and negative predictive values were 96.9% to 99.1%, suggesting that routine epicardial pacer wire cultures should not be obtained, but in cases where mediastinitis is a concern, they may have a role.[116,117] The usefulness of MRI and positron emission tomography (PET) scanning are not well defined, and MRI may be contraindicated when ferromagnetic metals are present in sternal wires, artificial heart valves, cardiac pacemakers, or vascular clips.

Several investigators have found mediastinal needle aspiration to be useful in the diagnosis of mediastinitis.[19,118] This method seems to be particularly useful for early diagnosis of mediastinitis before clinical symptoms make the diagnosis obvious, and it has been associated with improved clinical outcomes.[118]

[g]References 19, **23**, **32**, 37, **89**, 91, 106.
[h]References 19, 91.

Therapy

Therapy that includes medical and surgical techniques should be promptly initiated when mediastinitis is diagnosed along with aggressive nutritional and supportive care.

Mediastinitis Due to Esophageal Perforation

The principles of managing mediastinitis due to esophageal perforation include restoration of esophageal continuity, control of mediastinal contamination, and treatment with antimicrobials. Barrett[119] is credited with documenting in 1946 the first successful treatment of mediastinitis resulting from esophageal perforation. Since then, experts have usually recommended immediate aggressive surgical drainage, débridement of any devitalized tissue, and repair of the perforation.[7,9,120] A variety of surgical techniques may be used in the treatment of esophageal perforation, ranging from primary repair, which is preferred when feasible, to esophageal exclusion or diversion, to complete esophagectomy, which is usually reserved for patients with significant esophageal pathology such as malignancy.[6,104,121]

Over the past two decades, there have been aggressive efforts to identify those patients who may benefit from a "nonoperative" management strategy. Recent literature suggests that preferred algorithmic approaches take into account the location, severity, and control of the esophageal leak, along with the presence of tumor and severity of illness, to determine the best strategy for esophageal repair.[104,122] Nonoperative management is usually preferred in well-contained disruptions of the esophagus with minimal symptoms and no evidence of clinical toxicity or those with perforations identified before major mediastinal contamination occurs.[6,10,104,121,122] Although termed a "nonoperative" strategy, many patients have feeding tubes surgically implanted, and CT-guided drainage of any abscesses is essential for directing antimicrobial therapy and resolving infection.

A major advancement in treatment of esophageal perforation has been the development of endoscopic therapies, such as retrievable esophageal stents and endoscopic clips. Clipping may be particularly useful with small perforations due to foreign bodies.[123,124] Deployment of expandable esophageal stents to treat esophageal rupture has been increasingly common, with 30% of patients with benign esophageal ruptures managed using stents in 2014, compared with 7% in 2007.[125] Recent data suggest stenting may be associated with improvements in outcomes. A systematic review of 267 esophageal perforations managed by esophageal stenting noted perforation healing was achieved in 85% of patients with an overall mortality of 11%.[126] A propensity-matched study comparing stenting with primary surgical repair for iatrogenic rupture found similar rates of continued leak between the two procedures, but stenting was associated with decreased mortality (43% vs. 4%), shorter length of stay, and earlier oral intake.[127] It should be noted that many patients who underwent stenting required drainage of extraluminal fluid collections, often by video-assisted thorascopic surgery (VATS). Children with esophageal perforation are usually successfully managed with conservative treatment with broad-spectrum antibiotics, nasopharyngeal aspiration, parenteral nutritional support, and drainage of any fluid collections.[128,129]

Mediastinitis Due to Head and Neck Infection

As in esophageal perforation-induced mediastinitis, cases secondary to descending odontogenic or pharyngeal infection require prompt and aggressive surgical intervention. There is some debate in the literature regarding the preferred surgical approach, but all authors agree that adequate surgical drainage and débridement of all infected areas is of primary importance. Some authors have suggested that a transthoracic approach is necessary in all cases, noting that transcervical drainage may be inadequate.[16] An analysis of 69 cases found significantly higher mortality in patients managed with only drainage via the neck (47% vs. 19%).[98] Recent evidence suggests that the surgical approach should be based on the extent of the infection. When infection is confined to the superior mediastinum, management using cervicotomy with transcervical mediastinal drainage is likely adequate.[15,130,131,132] If mediastinitis has spread below the carina, the chest cavity should be entered using either sternotomy, posterior thoracotomy, or a combination of transcervical and subxiphoid drainage, depending on the location and extent of the infection.[15,132,133] Successful management of descending necrotizing mediastinitis using VATS has been reported as well.[134] Sumi and coworkers[135] described a nonoperative approach of aggressive CT-guided drainage catheters of both the neck and thoracic cavity in 14 patients. No patients required subsequent surgery, and only one patient died. Further research is necessary before this approach can be routinely advocated.

Although the importance of supportive therapy and surgical intervention cannot be overemphasized in both these forms of mediastinitis, the administration of appropriate antimicrobials is an essential component of therapy. Empirical regimens should be chosen based on the underlying cause and cover the major pathogens listed in Table 85.3. Penicillin G has traditionally been the antibiotic of choice in the treatment of anaerobic infections originating above the diaphragm and continues to exhibit excellent activity against most oral anaerobic bacteria. However, oral anaerobes such as *Prevotella* and *Porphyromonas* spp. (formerly *Bacteroides* spp.) are increasingly resistant to penicillin G, with agents such as broad-spectrum β-lactam/β-lactamase combinations, metronidazole, clindamycin, or carbapenems generally used.

Gram-negative enteric bacilli are also often involved in mediastinitis and should be accounted for when choosing an initial empirical regimen. Thus regimens including a β-lactam/β-lactamase inhibitor, a broad-spectrum cephalosporin plus metronidazole or clindamycin, or a carbapenem are reasonable empirical options. Coverage for MRSA is rarely indicated, as only approximately 5% of infections are caused by *S. aureus*, and this is mostly MSSA. Antibiotic therapy should be specifically tailored to the infecting organisms after definitive culture results are available, but treatment directed against anaerobic oropharyngeal organisms should be continued because of the difficulty in obtaining reliable anaerobic cultures.[94] The duration of therapy, which may range from weeks to months, is determined by the virulence of the bacteria, host factors, adequacy of drainage and the patient's response to therapy.

Cardiac Surgery–Related Mediastinitis

The treatment of mediastinitis secondary to cardiac surgery requires aggressive surgical drainage and débridement, including removal of any infected or necrotic tissue and bone along with sternal wires and any other foreign material. Although a few cases have been successfully treated by percutaneous catheter drainage alone, this is not preferred.[136] Two general approaches have been used in the surgical management of mediastinitis after cardiac surgery: open techniques and closed techniques. Open techniques involve débridement of infected tissue and open packing of the wound with delayed closure. Disadvantages of open procedures include respiratory insufficiency secondary to a lack of mechanical support for the thorax, resulting in prolonged mechanical ventilation, delayed healing and closure of the surgical wound, and hemorrhage from exposed vessels.

Rather than using open packing, many surgeons now use vacuum-assisted closure (VAC) therapy, also called *negative pressure wound therapy* (NPWT), either as a primary closure technique or as a bridge between débridement and definitive flap closure. Multiple studies comparing the use of NPWT with conventional treatment have shown the use of NPWT is associated with fewer dressing changes needed, earlier sternal rewiring, decreased need for soft tissue flaps, more rapid sterilization of the mediastinum, more rapid C-reactive protein decline, shorter in-hospital stays, reduced sternal reinfection rates, and decreased costs compared with usual care.[137,138,139,140] At least three studies have found NPWT to be associated with decreased mortality compared with historical management of patients using conventional surgical techniques.[24,139,140] A meta-analysis of 22 nonrandomized trials of NPWT for deep sternal wound infection found it was associated with significantly lower mortality (risk ratio [RR], 0.40; 95% CI, 0.28 to 0.57) and recurrence (RR, 0.34; 95% CI, 0.19 to 0.59).[141] Although these findings strongly suggest VAC therapy may be superior to other forms of surgical management, randomized trials comparing the use of VAC devices with other treatment modalities have not been performed. Although expert guidelines suggest that NPWT be used whenever delayed sternal wound closure is anticipated, a systematic review of the literature concluded that although NPWT was "efficacious and viable," the evidence supporting it was still "weak," and randomized controlled trial data were necessary.[141,142]

The closed method of surgical management involves débridement of affected tissue, followed by immediate closure of the sternum. If residual infection remains after closure, postoperative irrigation through drainage tubes or Redon catheters within the mediastinum may be performed, although this has become less common.[i] Sternal closure may be accomplished through direct closure of the wound, with or without sternal rewiring, or by the advancement of a soft tissue flap, usually from the pectoralis muscle. Some authors have reported high rates of success using these techniques, with cure rates greater than 90%.[143,144] A variety of soft tissue flaps may be used to reconstruct the chest wall, but no comparative data are available to guide selection. The most commonly used flap is the pectoral, but rectus abdominus transposition can be used, and omental tissue flaps have been successful in patients who have failed other treatment modalities.[146–150] A single report of the successful use of an anterolateral thigh, free myocutaneous flap has been published.[151] Tissue flaps can be used in primary closure of sternal wounds or after a period of open management with either traditional dressing changes or, more commonly, the use of NPWT as a bridge to flap. The preferred surgical management of mediastinitis is currently unknown, nor is the ideal timing of sternal closure, because some studies suggest improved outcomes with early sternal closure, whereas others report high rates of local recurrence.[106,152]

Irrigants used in mediastinitis have included various antimicrobial and antiseptic solutions, including antiseptics such as povidone-iodine or Dakin solution, various antibiotics, and saline. These agents have been associated with various complications, including the emergence of resistant organisms, pericardial and tissue toxicity, and systemic absorption and toxicity.[153,154] The commonly used irrigant povidone-iodine has been associated with iodine toxicity, renal failure, metabolic acidosis, and seizures and should generally be avoided.[145,155]

The use of parenteral antibiotics remains a cornerstone of therapy, although alone they are insufficient. Empirical therapy should be directed against staphylococci, including methicillin-resistant staphylococci and gram-negative aerobic bacilli. When definitive culture results are available, antimicrobials should be targeted to the pathogens isolated. As with mediastinitis secondary to infection of the head and neck, the duration of therapy is determined by multiple factors, including extent of infection, adequacy of drainage/débridement, presence of bone involvement, retention of prosthetic material, and pathogen isolated. Whether patients with mediastinitis caused by methicillin-resistant staphylococci are more likely to have treatment failure is a subject of debate. MRSA SSIs have been associated with increased mortality and treatment costs.[156] Some studies have associated MRSA poststernotomy mediastinitis with prolonged time to sterilization of the mediastinum, increased treatment failure, and increased mortality compared with MSSA.[106,157,158] Other authors, after controlling for comorbidities, choice of empirical initial antimicrobial regimen, and surgical treatment modality, found the mortality of MRSA poststernotomy mediastinitis to be quite high but not significantly different from other organisms.[158,159]

There has been significant interest in alternative antiseptics, and studies have suggested that in recalcitrant cases packing of the wound with granulated sugar or honey can successfully eradicate sternal infection.[160,161] The exact mechanism by which these substances promote wound healing has not been proven but is hypothesized to be a combination of promotion of wound healing with inhibition of bacterial growth through changes in tissue water content, changes in osmolality, and direct antibacterial growth inhibition.[162] Although there is still interest in these alternatives, NPWT has supplanted much of their use in mediastinal wounds.[163]

Prevention of Mediastinitis After Cardiac Surgery

Because of the devastating nature of poststernotomy mediastinitis, prevention is crucial. Numerous guidelines have been published regarding prevention of SSI and mediastinitis in the post–cardiac surgery setting.[145,164,165,166] Chapter 313 contains a detailed description of the prevention of SSIs, but a few items specific to cardiac surgery warrant mention. The Surgical Care Improvement Project (SCIP), which began in 2004, focuses on decreasing SSIs through implementation of evidence-based practices. Although SCIP measures are focused primarily on antimicrobial prophylaxis, control of 6 a.m. postoperative blood glucose in CABG surgery is also included. Several studies have shown that the control of postoperative blood glucose levels to less than 200 mg/dL, particularly using intravenous insulin protocols, significantly decreases the risk of deep sternal wound infection even in nondiabetic patients.[167,168,169] This effect has been quite significant, with a 50% or greater decrease in infection rates noted.

Antibiotic prophylaxis has become common in cardiac procedures despite the lack of placebo-controlled studies documenting its efficacy. Many questions exist about the optimal agent, dose, timing, and duration for antimicrobial prophylaxis. Guidelines from the Society of Thoracic Surgeons and by the American Society of Health System Pharmacists, Infectious Diseases Society of America, Surgical Infection Society, and Society for Healthcare Epidemiology of America have attempted to synthesize the data.[164,170,171] There is general agreement that prophylactic antimicrobials should be provided to achieve maximal tissue levels at the time of incision by infusing them within 1 hour of incision.[164] Very large patients may need higher doses of antimicrobials to achieve adequate concentrations, and higher doses should be used in larger patients. Another important aspect of antimicrobial prophylaxis is maintaining adequate tissue levels and agents with short half-lives, such as cefazolin, which should be redosed if the procedure duration exceeds two half-lives of the agent (typically 4 hours with cefazolin). Cefazolin has generally been regarded as the drug of choice for prophylaxis, with other early-generation cephalosporins, such as cefuroxime, generally considered to be equivalent.[164,171,172] The role of vancomycin as a routine prophylactic agent in cardiovascular surgery is controversial. The advantages of vancomycin, such as coverage of resistant pathogens, such as MRSA and enterococcal species, must be weighed against its disadvantages, including long infusion times, possible histamine release syndrome, and risk of development of vancomycin resistance in enterococci and staphylococci. In a comparison of vancomycin, cefazolin, and cefamandole, Maki and associates[173] showed a significant reduction in postoperative wound infections in patients receiving vancomycin prophylaxis. This finding has been contradicted by two meta-analyses and a systematic review, all of which found vancomycin to be no better than β-lactam antibiotics for the prevention of deep sternal wound infections, and in fact, vancomycin use was associated with increased rates of sternal wound infection in one analysis.[174,175,176] Another disadvantage of vancomycin use as a single agent for prophylaxis is a lack of coverage for gram-negative organisms. It is unclear if the addition of an agent with gram-negative activity, such as an aminoglycoside, fluoroquinolone, or cephalosporin, is necessary, but their use may be prudent when there is concern regarding these pathogens.[164,171] On the basis of these data, vancomycin should be reserved for prophylaxis in patients who cannot receive β-lactams due to severe allergy or who are at high risk for, or known to be colonized with MRSA.

Studies evaluating the duration of antimicrobial prophylaxis have found no clinical benefit to exceeding 48 hours of use, and use of prophylactic antibiotics beyond this time period has been associated with the emergence of resistant organisms.[164,170,175,177] The optimal duration of surgical prophylaxis is unclear as there are conflicting data regarding duration. Two meta-analyses suggested >24 hours of antibiotics was superior to <24 hours in preventing deep sternal wound infections, and current expert guidelines recommend prophylactic antibiotics be continued no longer than 48 hours postoperatively.[145,164,170,175,178] In contrast to this, recent Centers for Disease Control and Prevention guidelines recommend that surgical antimicrobial prophylaxis be discontinued at the time of wound closure in all procedures, including cardiac surgery.[179]

The prominent role of *S. aureus* in mediastinitis has made it a frequent target for prevention. The use of intranasal mupirocin to prevent SSIs through elimination of *S. aureus* nasal colonization has been supported by multiple trials, and application before or at the time of surgery is recommended.[164,171,180,181,182] This intervention should be targeted to patients known to be colonized with any form of *S. aureus* and not limited to those colonized only with MRSA.[181,182] If *S. aureus* colonization

[i]References 19, 44, **88**, **143**, 144, **145**.

status is unknown, it is reasonable to apply universally. Vaccines against *S. aureus* with the goal of preventing infections such as postsurgical mediastinitis are currently under investigation. Unfortunately, a large randomized trial in cardiac surgery patients found that although immunogenic, vaccines did not reduce *S. aureus* infections or SSI and increased mortality in those who developed an *S. aureus* infection.[183]

The use of topical antibiotics, such as gentamicin or vancomycin, applied to sternal wounds is controversial. Routine application of vancomycin paste to the cut sternal edges on opening and closing was recommended in one expert guideline[145] based on retrospective studies and a single randomized trial.[184,185] Not all retrospective studies support its use, and a recent meta-analysis, although supporting topical use, suggested publication bias might be present, making its routine adoption questionable.[186,187] Recent interest has also focused on gentamicin-collagen sponges implanted just before wound closure. Four randomized controlled studies have been performed, but only 1 study showed a significant decrease in deep sternal wound infections.[188,189,190,191] When the findings of these 4 studies were combined using meta-analysis, rates of deep sternal wound infections were lower with use of gentamicin sponges (OR, 0.62; 95% CI, 0.39 to 0.97).[192] Another meta-analysis that also included 10 observational studies found a similar reduction in the rate of deep sternal wound infections.[193] Despite these findings, more data are necessary before these devices are routinely implemented because the only US randomized trial found no impact on SSI rates.[190]

Finally, NPWT for prevention of infection in closed and noninfected wounds is seeing increased use. Small retrospective analyses have suggested that the use of NPWT for 5 to 7 days immediately after surgery may decrease infection rates in those who are at high risk (obese, bilateral ITA use, etc.).[194,195] Randomized trials are needed before this can be routinely recommended.

Complications and Prognosis

Complications of mediastinitis are due to local spread of infection and the systemic results of severe infection. Patients with descending necrotizing mediastinitis may require tracheostomy for airway protection, and prolonged mechanical ventilation is not uncommon.[12,16] Extension of the infection into contiguous structures and spaces can occur, including the pericardial space, resulting in pericardial effusion and tamponade; the pleural space; costochondral cartilages; and the peritoneum, resulting in peritonitis. Other major complications of mediastinitis, particularly after cardiac surgery include sternal osteomyelitis and sternal dehiscence or fracture.

An uncommon complication of mediastinitis after median sternotomy is an indolent costochondritis of one or more ribs, presenting as a localized swelling, erythema and pain. Débridement of necrotic cartilage maybe needed for cure.

Before the development of modern surgery and antibiotics, mediastinitis, from esophageal perforation, was regarded as uniformly fatal. With Barrett's first successful surgical repair of the esophagus in 1946,[119] morbidity and mortality decreased significantly but remains high. A national study in the United Kingdom between 2001 and 2012 showed that 30-day mortality decreased from 37% to 25%.[196] More recent studies using rapid diagnosis and a mix of surgical and nonsurgical treatments, including esophageal stenting, have demonstrated further declines in mortality (8%–12%), although outcomes vary depending on the nature of the rupture and management strategy used.[j] It is difficult to separate the influence that the nature of the injury and management strategy have on outcomes as they are often intertwined. General predictors of mortality in esophageal perforation include the presence of comorbidity, respiratory failure or signs of sepsis at diagnosis, malignant perforation, and a delay of more than 24 hours in initiation of therapy.[k] Rapidly recognized iatrogenic injuries managed by stenting may have mortality rates that are less than 5%.[10,104,198] The mortality associated with mediastinitis from head and neck infections, although still high, has declined with recent case series documenting mortality rates of 11% to 16%.[15,16,132,133] Specific prognostic factors in this form of mediastinitis have not been identified, but delays in diagnosis, severity of illness at presentation, and extension of the infection into the lower mediastinum may portend a worse outcome.[132,133,199]

Older studies in poststernotomy mediastinitis patients reported mortality rates of 30% to 50%, but more recent studies have described lower early mortality rates, often less than 10%, with some series reporting rates less than 5%.[l] This improvement in mortality rates is likely multifactorial, although a meta-analysis of NPWT suggested that it may be one of the major factors contributing to this improvement.[141] Although post–cardiac surgery mediastinitis is associated with an increase in postoperative mortality, there is debate in the literature regarding the long-term impact of this complication on mortality. Cayci and colleagues[201] found the development of deep sternal wound infection, after an initial increase in early mortality, did not alter long-term survival when preoperative, intraoperative, and postoperative risk factors were accounted for. Contrasting this, in 18,532 CABG procedures with a median of 10.4 years of follow-up, Risnes and coworkers[26] found mediastinitis was not associated with an increase in 30-day mortality but did result in a 59% increase in long-term mortality. An important factor in determining outcome is the length of time between diagnosis and initiation of definitive surgical and antimicrobial therapy, and delays are associated with worse outcomes.[106] Other prognostic indicators associated with increased mortality include advanced age, presence of significant underlying medical comorbidities, bacteremia, early-onset mediastinitis, mediastinal infection due to *Candida* spp. or MRSA, need for prolonged mechanical ventilation, need for intraaortic balloon pump, and cytomegalovirus shedding. [m]

In examining the economic ramifications of mediastinitis, Loop and coworkers[20] found the hospital charges of patients undergoing CABG who experienced mediastinitis were 280% greater than patients with uncomplicated bypass surgery, and the median length of stay ranged from 38 to 51 days. Other studies have reported that poststernotomy mediastinitis results in excess hospital stays of 12 to 30 days and a twofold to threefold increase in cost of care.[32,37,43,204] Because many of these infections are regarded as "preventable," beginning in 2008 the US Centers for Medicaid and Medicare Services stopped reimbursing hospitals for additional costs associated with postoperative mediastinitis. Data from the first 2 years after policy implementation showed alterations in coding but no measurable reduction in the rates of postoperative mediastinitis.[205]

CHRONIC/FIBROSING MEDIASTINITIS

Definition and Etiology

The terms *fibrosing, sclerosing,* and *granulomatous mediastinitis* refer to a chronic form of mediastinitis characterized by an invasive and compressive inflammatory mediastinal infiltrate. The first report of this entity, which may cause 10% of all primary mediastinal masses,[206] was a description by Ulmont in 1855.[207] Although the underlying pathophysiology of fibrosing mediastinitis has remained obscure, many authorities believe the majority of cases are secondary to infection with *H. capsulatum*. With careful analysis, 73% of cases previously characterized as nonspecific granulomatous mediastinitis were reclassified as secondary to *H. capsulatum* infection by restaining of the tissue with fungal stains and a thorough review of the pathologic sections.[208,209] A recent case series found 84% of patients with fibrosing mediastinitis had serologic, pathologic, or radiologic findings consistent with histoplasmosis.[210] Other infectious etiologies have been described, including, most frequently, tuberculosis,[211] and, more rarely, actinomycosis,[212] nocardiosis,[213] blastomycosis, coccidioidomycosis,[214] aspergillosis,[214] *Conidiobolus, Rhizopus* spp. and other causes of mucormycosis,[215,216] and *Wuchereria bancrofti* infection.[217] Older literature often lists syphilis as a prominent cause of granulomatous mediastinitis; however, this was based on seropositivity without other supporting evidence. Other conditions that closely mimic this entity include sarcoidosis, silicosis, lymphoma, mesothelioma, Behçet disease, mediastinal fibrosis associated with radiation, Riedel struma, and sclerosing cholangitis.[214,218–221] Some cases of fibrosing mediastinitis are likely a manifestation of other unusual

[j]References **7**, **10**, 103, **122**, 126, **127**.
[k]References **7**, **9**, **104**, **121**, 194, 197.
[l]References 20, **24**, 146, **163**, 200.
[m]References **88**, **89**, 93, 139, 157, 202, 203.

systemic fibroinflammatory disorders, including idiopathic retroperitoneal fibrosis, immunoglobulin (Ig)G4-related disease, or even mast cell activation syndrome.[222–224]

Manifestations and Diagnosis

Many patients with fibrosing mediastinitis are asymptomatic and come to medical attention when chest imaging incidentally reveals a mediastinal mass. When symptoms occur, they are usually related to invasion or obstruction of structures within or adjacent to the mediastinum (Table 85.4). The most common complaints at presentation are cough, dyspnea, chest pain, exercise intolerance, and hemoptysis.[210] Fibrosing mediastinitis is the most common nonmalignant cause of superior vena cava syndrome,[225] and patients typically present with plethora and edema of the face, neck, and upper torso; neck vein distention; headache; and visual disturbances. Obstruction of the pulmonary arteries results in cough, dyspnea, and symptoms consistent with right-sided heart failure. Pulmonary infarction, although rare, has been reported to occur in patients with fibrosing mediastinitis.[226] Pulmonary venous obstruction causes patients to experience a "pseudo–mitral stenosis syndrome" with exertional dyspnea, easy fatigability, and occasional hemoptysis. Patients with airway obstruction may present with cough, wheezing, dyspnea, hemoptysis, or recurrent episodes of bacterial bronchitis or pneumonia. Patients complaining of dysphagia may have esophageal obstruction secondary to posterior extension.

Findings on chest radiograph may be subtle, but most patients have mediastinal widening; other common radiographic findings include hilar mass, mediastinal calcification, and evidence of superior vena cava obstruction. CT is the preferred modality for evaluating fibrosing mediastinitis, and findings typically reveal one of two patterns. The most common finding is a focal, infiltrating mediastinal mass within the superior mediastinum, often with calcifications, which is most commonly on the right side.[210,227,228] Many authors believe this pattern is secondary to histoplasmosis or other infectious etiologies. The second pattern is characterized by diffuse mediastinal infiltration without prominent calcifications and may be more typical of noninfectious etiologies. CT is also useful for defining the extent of compression of mediastinal structures, with the exception of the esophagus, which is better imaged using an esophagram. Two large case series found vascular narrowing (39%–48%), especially the superior vena cava (20%–39%), to be the most commonly obstructed structures, followed by bronchial (27%–33%) and esophageal (3%–9%).[210,227] Magnetic resonance angiography offers little advantage over CT but along with pulmonary arteriography may be helpful in the assessment of the pulmonary vascular integrity.[229,230] Ventilation-perfusion lung scans often reveal large perfusion defects secondary to obstruction of the pulmonary vessels. PET scans, although not routinely indicated in fibrosing mediastinitis, are often used for evaluation of mediastinal mass lesions and may show increased metabolic activity suspicious for a malignancy.[210,230]

The diagnosis of fibrosing mediastinitis usually requires pathologic examination. Adequate tissue sampling is crucial in ruling out causes such as nodular sclerosing Hodgkin disease and sclerosing variants of non-Hodgkin lymphomas. The histologic appearance of fibrosing mediastinitis reflects a continuum of findings that range from predominantly granulomatous lesions with significant inflammation to minimally cellular fibrosing masses without inflammation.[210,221] Lesions noted may include caseating granulomas but typically are extensive paucicellular, densely hyalinized, collagenous tissue infiltrating and obliterating mediastinal adipose tissue, and structures with varying amounts of lymphocytes and mononuclear cells. Specific stains for fungi may reveal organisms consistent with *Histoplasma,* but cultures are usually negative.[206]

TABLE 85.4 Complications and Manifestations of Sclerosing Mediastinitis

Pulmonary venous or arterial obstruction
Superior vena cava obstruction
Inferior vena cava obstruction
Esophageal obstruction
Esophagobronchial obstruction
Tracheobronchial obstruction
Pulmonary venous or arterial obstruction
Pulmonary hypertension
Pulmonary infarction
Cor pulmonale
Thoracic duct obstruction
Constructive pericarditis
Coronary artery stenosis
Mediastinal nerve entrapment
Recurrent laryngeal nerve palsy

Pathogenesis

Fibrosing mediastinitis likely reflects a common immunologic end point of a heterogeneous group of inciting factors, and multiple mechanisms have been proposed to explain this condition. The findings of distinct radiographic and pathologic patterns of mediastinal disease suggest there may be multiple pathophysiologic mechanisms, although the most common mechanism is likely an immune-mediated hypersensitivity to *H. capsulatum* antigens possibly set off by the rupture of a caseous lymph node into the mediastinum.[206,210,227,228] A second similar hypothesis is the development of a delayed hypersensitivity reaction caused by the spread of soluble *Histoplasma* antigens into the mediastinum.[228] An alternative explanation proposes that fibrosing mediastinitis represents an abnormality of collagen production and organization similar to that of idiopathic retroperitoneal fibrosis, Riedel struma, or IgG4-related disorders leading to diffuse fibrosis without calcifications.[223,227,228] Either explanation is plausible, and the presence of a genetic predisposition and overlap between pathogenic mechanisms is possible. Supporting the role of genetic predisposition, a recent case-control study found histoplasmosis-associated fibrosing mediastinitis to be associated with human leukocyte antigen DQB1*04:02.[231]

Treatment

No controlled trials of medical or surgical therapy have been conducted in fibrosing mediastinitis. Patients without symptoms at the time of diagnosis may be safely managed with close monitoring, as demonstrated by 17 asymptomatic patients who were followed for a median of 68 months with none showing evidence of disease progression.[210] Despite the frequent finding of fungal organisms, little evidence supports the presence of an active infection at the time of diagnosis, and although antifungal therapies are frequently used (23 of 80 patients in one case series), the evidence supporting their efficacy is based on case reports of success only.[210,228,232] Surgical attempts to relieve vascular obstruction at the hilum can impede collateral flow around an obstructed superior vena cava. Case reports of success with corticosteroids have appeared, but their role in the treatment of fibrosing mediastinitis is minimal and likely limited to those where fibrosing mediastinitis is due to some other initiating condition, such as sarcoid or retroperitoneal fibrosis.[210,233] Despite a lack of efficacy, antifungal and antiinflammatory therapies are often used because no clearly efficacious treatment options exist. When medical therapy is provided, imaging and symptoms should be monitored, and patients without a clearly documented response should have therapy discontinued because it may result in toxicity and side effects. In small case series the selective estrogen receptor modulator tamoxifen was shown to improve idiopathic retroperitoneal fibrosis, leading some to use it in fibrosing mediastinitis with rare reports of success.[234,235] Tamoxifen's role as a treatment is unknown and may be limited to cases related to retroperitoneal fibrosis because estrogen and progesterone receptors have not been demonstrated to be present on cells mediating fibrosing mediastinitis.[221]

It is difficult to make recommendations regarding the timing or usefulness of surgical intervention due to the variable natural history of this disease because some patients progress to compression of vital structures, and others seem to have self-limited disease. Some have suggested that early surgical intervention with removal of granulomatous tissue may prevent the development of subsequent end-stage fibrosis, but the literature supporting this is scanty.[236] Due to the difficulty of the procedure and relatively high complication rates, surgical and nonsurgical interventions are generally reserved for patients who experience obstruction or invasion of mediastinal structures, leading to significant symptoms or cardiac complications. Despite the technical difficulties of surgical interventions, centers with experience have

published success rates of greater than 90%.[210] Recent advances in nonsurgical interventions have been used to relieve obstructions of the superior vena cava, pulmonary vessels, and bronchi. Although published success rates of relief of obstruction are somewhat lower than surgery (78%–80%), nonsurgical interventions avoid the significant morbidity associated with surgical interventions.[210,237] However, even nonsurgical interventions have been associated with high complication rates in patients with severe disease and have not been associated with improved clinical outcomes.[238] Regardless of the type of intervention, the progressive nature of the disease often means patients require multiple and progressive interventions over time. Although some patients with fibrosing mediastinitis will succumb to the disease, the overall prognosis is unknown, and outcomes may depend on the structures obstructed. Peikert and colleagues[210] used the Social Security Death Index database to follow up 80 patients with this disease for a median of 68 months (0–401 months) and found that, although 5 had died, only 2 deaths were specifically attributed to fibrosing mediastinitis. In contrast, 4 of 8 patients with pulmonary vein stenosis requiring intervention died within 4 weeks of their intervention, despite hemodynamic and symptomatic improvement.[238] There is some suggestion that bilateral mediastinal involvement, particularly when it involves the pulmonary veins, may be associated with increased mortality.[237,238]

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The complete reference list is available online at Expert Consult.

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86 Approach to the Patient With Central Nervous System Infection

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The central nervous system (CNS) may be infected by various agents, including viruses, bacteria, fungi, protozoa, and helminthes. In addition, numerous noninfectious etiologies may account for syndromes that mimic CNS infections. These include neoplastic diseases, intracranial tumors and cysts, medications, collagen vascular disorders, autoimmune disorders and other systemic illnesses, and conditions arising after various procedures that invade the CNS. The clinical presentation of a CNS infection may be acute, subacute, or chronic, depending on the virulence of the infecting agent and the location of the infection. Because CNS infections occur within the confines of the cranium or spinal column, they may be associated with significant morbidity and mortality, often necessitating emergent administration of antimicrobial therapy, and even neurosurgical interventions, to improve outcome. This chapter reviews the general approach to infections of the CNS, which are discussed in further detail in subsequent chapters of this section.

CLINICAL MANIFESTATIONS

The clinical presentation of a specific CNS infection depends on the pathogenesis of spread of the infection to the CNS, the virulence of the etiologic agent, and the area of CNS involvement. Most patients with CNS infections present with the clinical manifestations of fever, headache, altered mental status, or focal neurologic deficits. These findings are nonspecific, however, and not all patients with CNS infections develop all of these clinical manifestations. The likelihood of any specific clinical finding depends on the CNS syndrome caused by the infectious agent. These are reviewed subsequently.

Meningitis

Patients with acute meningitis most often present with fever, headache, meningismus, and altered mental status (see Chapter 87).[1,2] A typical adult patient with acute bacterial meningitis usually seeks medical attention within a few hours to several days after the onset of illness. The presentation may vary, depending on the age of the patient and the presence of various underlying conditions, for instance, head trauma, recent neurosurgery, presence of a cerebrospinal fluid (CSF) shunt (see Chapter 92), and immunocompromised state. The presentation may also vary depending on the microorganism causing meningitis. The most common etiologic agents of acute meningitis are unknown. When a cause is identified, viruses (most often enteroviruses; children > adults), West Nile virus (WNV), and herpes simplex virus (HSV) type 2 (adults), but also human immunodeficiency virus (HIV), varicella-zoster virus (VZV), less likely mumps virus, and bacteria (e.g., *Streptococcus pneumoniae, Neisseria meningitidis*, and *Listeria monocytogenes*).[2] Less commonly, parasites (e.g., *Naegleria fowleri* and *Angiostrongylus cantonensis)* may cause acute meningitis.

In contrast, patients with subacute (>5 days but <30 days of symptoms) or chronic meningitis (>30 days of symptoms) typically present over weeks to months, or even years (see Chapter 88).[1,2] These patients are more likely to be immunosuppressed, have abnormal neurologic findings, and to have hypoglycorrhachia with less pronounced CSF pleocytosis.[2] The most common etiology is idiopathic, but tuberculous meningitis and fungal meningitis (e.g., caused by *Cryptococcus neoformans, Histoplasma* spp., and *Coccidioides* spp.) are important considerations.[1,2] The clinical presentation of cryptococcal meningitis is different in patients with and without acquired immunodeficiency syndrome (AIDS). In non-AIDS patients cryptococcal meningitis is typically a subacute process, in which patients have days to weeks of symptoms characterized by headache, fever, meningismus, and personality changes. In AIDS patients the presentation is subtler with minimal, if any, symptoms; these patients may present with only headache and lethargy, with meningeal findings seen in only a few cases. Other fungi, such as *Candida* and *Aspergillus*, are unusual causes of meningitis, although cases of *Exserohilum rostratum* meningitis were reported in association with epidural or paraspinal glucocorticoid injections of preservative-free methylprednisolone from a single compounding pharmacy.[3]

Encephalitis

The syndrome of acute encephalitis is manifested by parenchymal brain inflammation that causes neurologic dysfunction (see Chapter 89).[4,5] An international consortium recently defined encephalitis with a combination of major and minor criteria. The major criteria are altered mental status (defined as a decreased, altered level of consciousness, lethargy, or personality change) lasting ≥24 hours without an alternative diagnosis and is a requirement for the diagnosis. The six minor criteria are (1) documented fever ≥38°C (100.4°F) within 72 hours before or after presentation, (2) seizures not attributable to a preexisting seizure disorder, (3) new-onset focal neurologic findings, (4) CSF white blood cells (WBCs) ≥5/mm^3, (5) new- or acute-onset neuroimaging abnormalities consistent with encephalitis, and (6) abnormalities on electroencephalography consistent with encephalitis and not secondary to other etiologies. The presence of two minor criteria indicate possible encephalitis, and greater than or equal to three indicate probable or confirmed encephalitis, if the etiologic agent is confirmed by brain biopsy, serologies, polymerase chain reaction (PCR), or antibodies in patients with autoimmune encephalitis. A clinical overlap between encephalitis and encephalopathy may exist, the latter referring to a clinical state of altered mental status that can manifest as confusion, disorientation, or other cognitive impairment, with or without evidence of brain tissue inflammation; encephalopathy can be triggered by a number of metabolic or toxic conditions but occasionally occurs in response to certain infectious agents, such as *Bartonella henselae* and influenza virus.[6]

Of all the pathogens reported to cause encephalitis, most are viruses that may be associated with specific clinical and neuroimaging findings that suggest their diagnosis.[4,5] Unilateral temporal lobe encephalitis is classically caused by HSV, leading to clinical manifestations characterized by personality changes, altered mentation, a decreasing level of consciousness, seizures, and focal neurologic findings (e.g., dysphasia, weakness, and paresthesias). Bilateral temporal lobe involvement or lesions outside the temporal lobe, insula, or cingulate are less likely caused by HSV.[7]

Other herpesviruses that cause encephalitis during any season include VZV, cytomegalovirus (CMV), and human herpesvirus 6 and are usually seen more frequently in immunosuppressed individuals. Arboviruses (e.g., West Nile, eastern equine, St. Louis, La Crosse, and Japanese encephalitis viruses) and respiratory viruses can present with a thalamic and basal ganglia encephalitis presenting with tremors, including Parkinsonism features.[8] Patients with WNV infection typically present between June and October, whereas respiratory viruses are usually present in children during the winter season. HIV can present as an encephalitis in patients not receiving antiretroviral therapy or can present as a CD8 encephalitis in those with immune reconstitution while on antiretroviral therapy.[9] Rabies virus is still a frequent cause of encephalitis in Asia (India especially) and in Africa. Enteroviruses are rare causes of encephalitis.[4,5]

Nonviral causes of encephalitis include *Mycobacterium tuberculosis*, *L. monocytogenes, Rickettsia, Ehrlichia* spp., *Bartonella* spp., *Mycoplasma pneumoniae*, and *Toxoplasma gondii* (more often seen in transplant patients with *Toxoplasma* encephalitis).[4] Several free-living amebae (i.e., *N. fowleri*, *Acanthamoeba* spp., and *Balamuthia mandrillaris*) may cause a fatal encephalitis, usually during the summer months.[4] Other epidemiologic clues that may be helpful in directing the investigation for an etiologic agent in patients with encephalitis include geographic locale, prevalence of disease in the local community, travel history, recreational activities, occupational exposure, insect contact, animal contact, vaccination history, and immune status of the patient.[4,5] In many cases of encephalitis (32%–75%) the etiology remains unknown, however, despite extensive diagnostic testing.[4,5,10] In addition, it is important to distinguish between infectious encephalitis and autoimmune encephalitis (antibody-mediated, postinfectious, or postimmunization acute disseminated encephalomyelitis). These latter syndromes are presumed to be mediated by an immunologic response to an antecedent antigenic stimulus provided by the infecting microorganism or immunization.[4,10] Anti–*N*-methyl-D-aspartate receptor (NMDAR) encephalitis[11,12] is the most common cause of antibody-associated encephalitis and is typically seen in young females with an associated ovarian teratoma. Anti-NMDAR encephalitis has now also been associated with both HSV and VZV infections.[13]

Focal Central Nervous System Syndromes

The clinical presentation in patients with focal CNS lesions (e.g., brain abscess, subdural empyema, and epidural abscess) depends on the route of spread of the infection to the CNS, location of the lesion, and severity of increased intracranial pressure (ICP) (see Chapters 90 and 91). Patients develop brain abscesses via contiguous spread (e.g., from sinusitis, otitis media, or mastoiditis), hematogenous dissemination (e.g., from the lung or in patients with infective endocarditis), or trauma. Initial clinical manifestations include headache, nausea, vomiting, and focal neurologic findings. Although patients may be brought to medical attention after development of a seizure or alteration in consciousness, fever is usually seen in less than 50% of patients. In patients with fungal brain abscess caused by *Aspergillus* spp. or the Mucorales, the clinical presentation may be that of a stroke syndrome because of the propensity of these organisms to invade blood vessels. In patients with focal infections that compress the spinal cord, such as epidural abscess or subdural empyema, the sequence of back pain, radiculopathy, and motor and sensory findings may eventually progress to complete paralysis, a clinical manifestation that is irreversible unless there is emergency intervention (see Chapter 91).

LUMBAR PUNCTURE

Lumbar puncture, with removal and analysis of CSF, is an essential procedure in the diagnosis of meningitis and encephalitis.[1,14] In the performance of a lumbar puncture the patient and the needle should be properly positioned to obtain adequate amounts of CSF; the patient is placed in either the lateral recumbent position on a firm surface with the back at the edge of the table and perpendicular to the table surface or sitting with the back curved.[14] The patient is first properly positioned, then the clinician wears sterile gloves and a mask and cleans the puncture site with antiseptic solution. Once the iodine solution has dried, 2 mL of 1% lidocaine without epinephrine is injected between the third and fourth lumbar vertebrae; once numb, a spinal needle (preferably 20 gauge or less) is inserted in the midline perpendicular to the back. After needle insertion, frequent removal of the stylet can determine whether the subarachnoid space has been entered. At that point a "pop" is felt, indicating penetration of the needle across the ligamentum flavum.[15]

Numerous complications have been associated with performance of lumbar puncture,[1,15] ranging from mild discomfort with insertion of the spinal needle to life-threatening conditions. The most common complication after lumbar puncture is headache, which is generally observed in 10% to 25% of patients; the headache is characteristically absent when the patient is recumbent and appears rapidly when the patient stands. The headache is believed to be secondary to low CSF pressure as a result of continued leakage of CSF at the site of the lumbar puncture. The risk of headache may be reduced by using smaller-gauge needles (20 gauge or less) or by placing the patient in the prone position for several hours after the procedure, although it is unclear whether the latter maneuver is effective in reducing the likelihood of headache after lumbar puncture. A recommendation from the American Academy of Neurology supports the use of atraumatic (Sprotte or Pajunk) needles, rather than the standard (Quincke) needle, to reduce the risk of postlumbar puncture headache.[15] Reinsertion of the stylet before needle removal has also been shown to decrease the risk of headache.[15] The headache usually resolves within hours to days after the procedure. Persistent headache can be treated by use of a "blood patch," in which some of the patient's own venous blood is injected outside the meninges at the site of the lumbar puncture; this procedure seals the site of CSF leakage.[15] In a recent systematic review and meta-analysis, use of atraumatic needles was associated with a decrease in the incidence of postdural puncture headache, need for intravenous fluid or controlled analgesia, need for epidural blood patch, any headache, nerve root irritation, and hearing disturbance, and it had similar efficacy to conventional needles.[16] This study suggests that atraumatic needles should be used in patients requiring lumbar puncture.[17]

Infection is a very rare complication after lumbar puncture (1/50,000 procedures), even in patients with concomitant bacteremia.[1,15] Although there have been conflicting studies on the risk of subsequent meningitis in patients who are bacteremic at the time of lumbar puncture, the importance of performing a diagnostic lumbar puncture in the appropriate clinical setting greatly outweighs any minor risk that the procedure itself might induce meningitis in a bacteremic patient. Lumbar puncture should not be performed in patients with established local infection in the lumbar space (e.g., spinal epidural abscess, spinal subdural empyema, or superficial or deep paraspinal infection); in these cases, CSF analysis should be obtained under fluoroscopic guidance via high cervical or cisternal puncture.[15]

Local bleeding is a more common complication after lumbar puncture[1,15]; up to 20% of patients have a so-called traumatic tap.[1,15] Bleeding may occur from inadvertent puncture of the venous plexuses located dorsally and ventrally to the spinal dura or secondary to injury to vessels that accompany the cauda equina. This local bleeding rarely does harm to the patient, although patients with coagulation disturbances or who are receiving anticoagulants may develop continued bleeding with the development of spinal subdural or epidural hematomas, which may compress the cauda equina and produce permanent neurologic injury. This complication is extremely rare, even in patients with coagulopathies, with only 35 cases described in the literature over the last 42 years.[18]

The most feared complication after lumbar puncture is brain herniation, which may occur in a patient with an elevation of ICP.[1,15] In patients who undergo lumbar puncture there is normally a mild, transient reduction of lumbar CSF pressure that is rapidly communicated throughout the subarachnoid space. In patients with bacterial meningitis and suspected severe intracranial hypertension or impending herniation, a 22- or 25-gauge spinal needle should be used, with careful observation for several hours after removal of CSF; monitoring and treatment of increased ICP may need to be considered (see later). In patients who have an intracranial space-occupying lesion, particularly one located in the posterior fossa, there is already a relative pressure gradient (with

downward displacement of the cerebrum and brainstem) that can be increased by lumbar puncture and precipitate brain herniation.

Certain patients should undergo neuroimaging studies (i.e., computed tomography [CT] or magnetic resonance imaging [MRI]) before lumbar puncture if there is a suspicion that their neurologic presentation may be secondary to an intracranial mass lesion with accompanying mass effect. The Infectious Diseases of America (IDSA) guidelines recommend that patients with suspected meningitis with the following characteristics should get a cranial imaging study before the lumbar puncture: immunocompromised state (HIV infection or AIDS, receiving immunosuppressive therapy, or after transplantation), history of CNS disease (mass lesion, stroke, or focal infection), new-onset seizure, abnormal level of consciousness, papilledema, or focal neurologic deficit (including dilated, nonreactive pupil; abnormalities of ocular motility; abnormal visual fields; gaze palsy; or arm or leg drift).[19] However, adherence to the IDSA guidelines is low (40%), with no clinical benefit in those patients undergoing cranial imaging without an indication.[20] Other guidelines (from the United Kingdom [UK], Europe, and Sweden) are more restrictive, with some guidelines (UK and Sweden) not recommending cranial imaging in immunosuppressed individuals but recommending cranial imaging only with focal neurologic deficits or finding a Glasgow Coma Scale score <12 in the UK or <10 in Europe and cerebral herniation signs in Sweden.[21,22,23] It has been suggested, however, that a normal CT scan does not always mean performance of a lumbar puncture is safe. Certain clinical signs of impending herniation, such as a deteriorating level of consciousness (particularly a Glasgow Coma Scale score ≤11), brainstem signs (including pupillary changes, posturing, or irregular respirations), or a very recent seizure, may be predictive of patients in whom lumbar puncture should be delayed.[24]

Opening Pressure and Appearance

In the evaluation of CNS infections via lumbar puncture, numerous parameters should be assessed.[1,15] CSF opening pressure is measured with an air-water manometer; in adults placed in the lateral decubitus position, the normal CSF opening pressure ranges from 50 to 195 mm H_2O; values <150 mm H_2O are clearly normal, and those >200 mm H_2O are abnormal. Pressure measured with the patient prone, as is done for fluoroscopic lumbar puncture, should be calculated as the distance from the CSF meniscus to the location of the patient's right ventricle. The CSF specimen should be sent to the laboratory for analysis (Table 86.1). The CSF is normally clear and colorless but may appear cloudy or turbid in patients with increased concentrations of WBCs (>200/mm^3), red blood cells (RBCs, >400/mm^3), bacteria (>10^5 colony-forming units/mL), or protein. In patients with a traumatic tap an initially bloody CSF (present when there are at least 6000 RBCs/mm^3) should clear as flow of CSF continues.

Xanthochromia, a yellow or yellow-orange supernatant of centrifuged CSF, is usually a result of RBC lysis and the presence of oxyhemoglobin, methemoglobin, and bilirubin; it characteristically appears 2 to 4 hours after RBCs have entered the subarachnoid space, although it has occasionally been seen for 12 hours.[1,15] The presence of xanthochromia distinguishes CSF that is bloody secondary to subarachnoid hemorrhage from CSF that is bloody secondary to a traumatic lumbar puncture, in which the centrifuged supernatant is clear. Xanthochromia may also be observed with very high CSF protein concentrations that can cause the CSF to clot (Froin syndrome, due to meningitis or spinal block) or as a consequence of systemic hyperbilirubinemia (>10–15 mg/dL).[15] In patients with meningitis, specific CSF results may vary depending on the infectious agent (Table 86.2). For additional information on CSF results for specific etiologic agents of meningitis and encephalitis, see the subsequent chapters in this section. Details of some CSF parameters are reviewed as follows.

Cell Count

The normal CSF WBC count in children and adults is 0 to 5/mm^3. CSF WBC counts in term neonates may be 32/mm^3 (mean, 8–9/mm^3), although by 1 month of age normal CSF has less than 10 WBCs/mm^3. Elevated CSF concentrations of WBCs are seen in patients with meningitis, encephalitis, and parameningeal foci of infection (e.g., space-occupying lesions).[1,15] False-positive elevations of CSF WBC counts can be found after traumatic lumbar puncture or in patients with intracerebral

TABLE 86.1 Tests of Cerebrospinal Fluid in Patients With Suspected Central Nervous System Infection

Routine Tests
White blood cell count with differential
Red blood cell count[a]
Glucose concentration[b]
Protein concentration
Gram stain
Bacterial culture
Selected Specific Tests Based on Clinical Suspicion
Viral culture[c]
Smears and culture for acid-fast bacilli
Venereal Disease Research Laboratory
India ink preparation
Cryptococcal polysaccharide antigen
Fungal culture
Antibody tests (IgM or IgG, or both)[d]
Nucleic acid amplification tests (e.g., polymerase chain reaction)[e]
Cytology[f]
Flow cytometry

[a]Should be checked in the first and last tubes; in patients with a traumatic tap, there should be a decrease in the number of red blood cells (RBCs) with continued flow of cerebrospinal fluid (CSF). See text for the formula for determining whether the numbers of CSF RBCs and white blood cells are consistent with a traumatic tap.
[b]Compare with serum glucose drawn just before lumbar puncture.
[c]Yield of viral culture may be low (see Chapters 87 and 89).
[d]May be useful for specific causes of meningitis and encephalitis (see Chapters 87, 88, and 89).
[e]Most useful for specific viral causes of encephalitis and causes of chronic meningitis; see text and Chapters 88 and 89 for details.
[f]In patients with suspected malignancy.

TABLE 86.2 Typical Cerebrospinal Fluid Findings in Patients With Selected Infectious Causes of Meningitis

CAUSE OF MENINGITIS	WHITE BLOOD CELL COUNT (cells/mm^3)	PRIMARY CELL TYPE	GLUCOSE (mg/dL)	PROTEIN (mg/dL)
Viral	50–1000	Mononuclear[a]	>45	<200
Bacterial	1000–5000[b]	Neutrophilic[c]	<40[d]	100–500
Tuberculous	50–300	Mononuclear[e]	<45	50–300
Cryptococcal	20–500[f]	Mononuclear	<40	>45

[a]May be neutrophilic early in presentation (see Chapter 87).
[b]Range from <100 to >10,000 cells/mm^3.
[c]About 10% of patients have cerebrospinal fluid (CSF) lymphocyte predominance.
[d]Should always be compared with a simultaneous serum glucose; ratio of CSF to serum glucose is ≤0.4 in most cases (see Chapter 87).
[e]May see a "therapeutic paradox," in which a mononuclear predominance becomes neutrophilic during antituberculous therapy.
[f]Greater than 75% of patients with acquired immunodeficiency syndrome have <20 cells/mm^3.

or subarachnoid hemorrhage in which RBCs and WBCs are introduced into the subarachnoid space (e.g., chemical meningitis). In these instances the following formula should be used as a correction factor for the true WBC count in the presence of CSF RBCs[1]:

$$\text{Adjusted WBCs in CSF} = \text{Actual WBCs in CSF} - \frac{\text{WBC in blood} \times \text{RBCs in CSF}}{\text{RBCs in blood}}$$

In the above equation, the amount being subtracted is the predicted CSF WBC count that would occur if all the CSF WBCs were the result of blood contamination.[1]

Generalized seizures may also induce a transient CSF pleocytosis that is primarily neutrophilic, although the total WBC count should not exceed 80/mm^3. Pleocytosis should not be ascribed to seizure activity alone, however, unless the fluid is clear and colorless, the opening pressure and CSF glucose are normal, the CSF Gram stain is negative, and the patient has no clinical evidence of CNS infection.[1] In patients with CSF pleocytosis, differential counts should be performed; a neutrophilic, mononuclear, or eosinophilic predominance may be suggestive of certain etiologies in the right clinical setting (see Table 86.2 and Chapters 88 and 89). Although CSF pleocytosis is suggestive of inflammation of the brain and/or meninges, the absence of CSF pleocytosis does not exclude the diagnosis of encephalitis.[25,26]

Glucose and Protein

CSF hypoglycorrhachia is seen in many CNS infections. The pathogenesis of CSF hypoglycorrhachia is multifactorial and may include an increased rate of macrovesicular glucose transport across arachnoid villi, increased glycolysis by leukocytes and bacteria, increased metabolic rate of the brain and spinal cord, or inhibition of glucose entry into the subarachnoid space caused by alterations in the membrane carrier system responsible for glucose transfer from blood to CSF.[15,27] The actual CSF glucose concentration may be falsely low in the presence of hypoglycemia, or may be incorrectly interpreted as normal when the patient is hyperglycemic (e.g., in diabetic patients). The CSF glucose should always be compared with a simultaneous serum glucose that is drawn before lumbar puncture; the normal lumbar CSF-to–serum glucose ratio is approximately 0.6, and ratios <0.5 should be considered abnormal.[15] Unfortunately, only 13% of patients with community-acquired meningitis have a simultaneous serum and CSF glucose performed.[27] An absolute CSF glucose <45 mg/dL can be used to define hypoglycorrhachia, as hypoglycemia and hyperglycemia are rarely seen in patients with meningitis.[27]

The CSF protein concentration is also elevated in numerous CNS infections, presumably because of disruption of the blood-brain barrier, manifested morphologically by separation of intercellular tight junctions and increased numbers of pinocytotic vesicles in microvascular endothelial cells.[1] Lumbar CSF protein concentrations >50 mg/dL and ventricular CSF concentrations >15 mg/dL are considered abnormal. A CSF protein >100 mg/dL is a risk factor for an urgent treatable cause of meningitis in adults, and a CSF protein <100 mg/dL is a predictor for enteroviral meningitis in children.[28,29] An elevated CSF protein may also be observed in various infectious and noninfectious disorders of the CNS. In patients with a traumatic lumbar puncture the true CSF protein concentration is determined by subtracting 1 mg/dL of protein for every 1000 RBCs/mm^3, although these determinations must be made in the same CSF tube.[15]

Other Cerebrospinal Fluid Tests

Additional studies of CSF may be useful in determining a specific etiologic diagnosis in patients with meningitis and encephalitis.[1] Cultures are important in attempts to identify a specific etiologic agent, but, depending on the pathogen, larger CSF volumes may be needed to increase the yield of a positive culture (e.g., for *M. tuberculosis* or fungi), and CSF cultures may require prolonged incubation and special techniques for isolation of specific pathogens (see Chapters 87–89). In some cases simple stains of CSF specimens may establish the etiologic diagnosis.

In patients with suspected bacterial meningitis, CSF Gram stain may provide a clue as to the etiologic diagnosis (see Chapter 87).[1] The CSF Gram stain is positive in 60% to 90% of patients with acute bacterial meningitis, although this sensitivity varies depending on the concentration of microorganisms in the CSF and the specific causative bacteria. Not all specialized stains of CSF to identify specific microorganisms are as sensitive as the Gram stain, however. In patients with tuberculous meningitis, less than 15% to 25% of specimens are smear-positive by acid-fast stain and 20% of patients with tuberculous meningitis have persistently negative CSF cultures. In patients with spirochetal meningitis (i.e., caused by *Treponema pallidum* or *Borrelia burgdorferi)* and *Toxoplasma* encephalitis, there are no effective specialized stains that identify these organisms in CSF; in these cases serum or CSF antibody studies are most often used to aid in the diagnosis (see Chapters 88 and 89).

CSF India ink examination is positive in 50% to 75% of patients with cryptococcal meningitis, and the yield increases to 88% in patients with AIDS. The most important CSF test in the diagnosis of cryptococcal meningitis is the CSF test for detection of cryptococcal polysaccharide antigen. The presence of virus-specific immunoglobulin M (IgM) in CSF is usually indicative of CNS disease because IgM antibodies do not readily diffuse across the blood-brain barrier; detection of IgM antibodies from patients with presumed flavivirus encephalitis is considered diagnostic of neuroinvasive disease.[4]

Nucleic acid amplification tests, such as PCR, of CSF are highly sensitive and specific for the diagnosis of many CNS infections and may be useful in providing a rapid diagnosis.[4,5,15] In patients with herpes simplex encephalitis CSF PCR is 96% to 98% sensitive and 95% to 99% specific in adult patients[30]; CSF PCR results are positive early in the disease course and remain positive during the first week of therapy, although false-negative results may occur if hemoglobin or other inhibitors are present in CSF. An initially negative CSF PCR result for HSV may become positive if the test is repeated 1 to 3 days after the initiation of treatment[4,31]; in undiagnosed cases in which patients have clinical or neuroimaging features suggestive of HSV encephalitis, consideration should be given to repeating the PCR for HSV 3 to 7 days later on a second CSF specimen.[4] Diagnosis of CMV encephalitis by PCR has a high sensitivity and specificity for the diagnosis of CNS involvement[32]; the absence of CMV DNA by PCR has a high negative predictive value. Nucleic acid amplification with reverse-transcriptase PCR has also been found to be highly sensitive (86%–100%) and specific (92%–100%) for the diagnosis of enteroviral infections of the CNS,[15] and it has high specificity for the diagnosis of other viral causes of CNS infections, such as VZV and JC virus.[15,29]

The clinical utility of PCR in patients with bacterial meningitis has been investigated with the use of a broad range of bacterial primers (i.e., broad-based PCR).[15,33] In one study[33] the broad range was highly sensitive and was able to detect pathogens up to 1 week after antibiotic therapy. A rapid (<1 hour) multiplex PCR that detects, with high sensitivity and specificity, 14 common causes of meningitis and encephalitis is now widely available.[34] This panel includes testing for six bacteria (*S. pneumoniae, N. meningitidis, Streptococcus agalactiae, Haemophilus influenzae, L. monocytogenes, Escherichia coli* K1), seven viruses (HSV-1 and HSV-2, human herpesvirus 6, CMV, enterovirus, parechovirus, VZV), and two fungi (*Cryptococcus gattii/neoformans*). Use of PCR to detect the fragments of mycobacterial DNA in CSF has also been used for rapid diagnosis of tuberculous meningitis.[35] The sensitivity for the different *Mycobacterium tuberculosis* (MTB) PCR technologies ranges from 55.8% to 87.6%, with recent nested MTB PCR showing sensitivity and specificity up to 89% and 100%, respectively.[36] In addition, in a recent study in 129 HIV-infected adults with suspected tuberculous meningitis, use of Xpert Ultra, a new, fully automated, nested real-time PCR assay for the GeneXpert (Cepheid; Sunnyvale, CA) platform, revealed that the Xpert Ultra sensitivity was 70% for probable or definite tuberculous meningitis compared with 43% for Xpert and 43% for culture.[37] At present, however, with the currently available MTB PCRs a negative CSF PCR result cannot be used to exclude the diagnosis of tuberculous meningitis. Although these molecular diagnostic methods have utility in the identification of the causative microorganism, they are often not available in resource-poor settings.[35] More information on the use of specialized CSF tests to establish an etiologic diagnosis

and use of other adjunctive tests in patients with meningitis and encephalitis can be found in Chapters 87 to 89.

NEUROIMAGING STUDIES

The use of neuroimaging studies, specifically CT and MRI, has been invaluable in localizing infectious processes within the CNS and in assessing response to therapy.[38,39] With CT scanning, areas of bone and blood appear as areas of high signal intensity compared with brain and CSF, which appear as areas of low signal intensity. After intravenous (IV) administration of an iodinated contrast agent, increased intensity (i.e., contrast enhancement) is seen in areas of blood-brain barrier breakdown, increasing the sensitivity for the diagnosis of certain abnormalities (e.g., brain abscesses). MRI directly generates images in all three planes (axial, coronal, and sagittal) for optimal evaluation of brain morphology and pathology; MRI can also provide information about blood flow within arteries and veins and shows enhancement of specific lesions after IV administration of gadolinium diethylenetriaminepentaacetic acid. Given the higher sensitivity of MRI compared with CT, MRI has become the preferred neuroimaging modality in the evaluation of virtually all patients with suspected CNS infection. In addition to conventional MRI sequences, recent advances have added further sensitivity and specificity to the diagnosis of brain infection.[38] These include diffusion-weighted imaging (DWI), diffusion tensor imaging, susceptibility-weighted imaging (SWI), perfusion-weighted imaging (PWI), and ^{1}H magnetic resonance spectroscopy. However, the availability of MRI, particularly with high-magnetic field strength, is more limited, especially in the developing world. These modalities are reviewed in further detail in subsequent chapters of this section, based on specific clinical syndromes, but some examples are given here.

The most important and specific neuroimaging technique in patients with HSV encephalitis is MRI with contrast enhancement, which shows lesions earlier than does CT[4]; MRI may also be useful in other causes of viral encephalitis (e.g., basal ganglia involvement in patients with WNV encephalitis) (see Chapter 89). DWI is superior to conventional MRI for the detection of early signal abnormalities in viral encephalitis caused by HSV, enterovirus 71, and WNV.[38,39,40] Neuroimaging studies are less important in the diagnosis of patients with bacterial meningitis but may be useful in patients who are not responding as expected, that is, have persistent or prolonged fever, clinical evidence of increased ICP, focal neurologic findings, new or recurrent seizures, enlarging head circumference (in neonates), persistent neurologic dysfunction, or persistently abnormal CSF parameters or cultures.[1] MR angiography and PWI in patients with bacterial meningitis may show vascular complications, such as vasculitis, including focal stenosis and irregularity of the major intracranial arteries as well as infectious dural sinus thrombosis[38,39]; SWI may visualize hemorrhages within the infarcted regions with high sensitivity.

MRI is more sensitive than CT in the detection of focal CNS infections and has the advantages of early detection of cerebritis, cerebral edema with greater contrast between edema and adjacent brain tissue, more conspicuous spread of inflammation into the ventricles and subarachnoid space, and earlier detection of satellite lesions (see Chapter 90). Contrast enhancement with gadolinium provides the added advantage of clear differentiation of the central abscess, surrounding enhancing rim, and cerebral edema surrounding the abscess. On T2-weighted images, the zone of edema that surrounds the abscess is one of high signal intensity. The key to distinguishing abscess from other cystic intracranial processes (e.g., necrotic tumors) is DWI[40]; pus in the abscess has restricted diffusion and appears DWI hyperintense. In AIDS patients with positive immunoglobulin G (IgG) antibody titers to *T. gondii*, the detection of multiple ring-enhancing lesions by CT with contrast enhancement or MRI is enough evidence to initiate a trial of anti-*Toxoplasma* chemotherapy[38] (see Chapter 90); CT or MRI is repeated in 10 to 14 days to document a clinical response. Advances in neuroimaging technology may refine further the approach to patients with CNS infections.

MANAGEMENT

Antimicrobial Therapy

Many factors influence the choice of a specific antimicrobial agent in the therapy of CNS infections. These factors have been best studied in experimental animal models of infections to evaluate use of antibacterial agents in the treatment of bacterial meningitis.[1,41] One important factor is the penetration of the antimicrobial agent into the CSF, to attain adequate drug concentrations to kill the meningeal pathogen rapidly and effectively. Drug penetration into CSF depends on the status of the blood-brain barrier, which is disrupted in the presence of meningeal inflammation. Some agents (e.g., corticosteroids) may reduce meningeal inflammation and reduce blood-brain barrier penetration of antimicrobial agents even though appropriate CSF vancomycin concentrations were documented in patients treated with adjunctive dexamethasone in one study.[42] Entry of antimicrobial agents into CSF is also enhanced by drugs that have a low molecular weight, low degree of ionization at physiologic pH, high lipid solubility, and low degree of protein binding.[1,41]

When the antimicrobial agent penetrates the CSF, it must exhibit rapid bactericidal activity against the infecting pathogen because bacterial meningitis is an infection in an area of impaired host resistance. A final factor that contributes to response to antimicrobial therapy is pharmacodynamics, which is concerned with the time course of antimicrobial activity at the site of infection; these factors and whether the specific agent kills by concentration-dependent or time-dependent killing are important to determine the dosing regimen for optimal effectiveness. Selection of empirical antimicrobial therapy must also be based on likely in vitro susceptibility.[1,19,41] In addition, intraventricular or intrathecal therapy may be required for eradication of resistant pathogens in patients with health care–associated ventriculitis and meningitis.[43] These principles are reviewed in further detail in Chapters 87 and 92.

Many of the principles for treatment of bacterial meningitis may also apply to treatment of other nonbacterial etiologies of meningitis and encephalitis. In addition, experimental and clinical studies have examined the penetration of specific antimicrobial agents into brain abscesses, which may be important in the antimicrobial therapy of these infections. In the therapy of focal intracranial infections, clinicians often rely on agents that are known to have efficacy in experimental animal models, which includes extrapolating data from animal models of meningitis, and based on anecdotal case series or case reports. This approach is reasonable because these infections are uncommon, and randomized trials of specific agents are not likely to be performed.

Surgical Therapy

Many patients with focal CNS infections require surgical therapy for optimal management (see Chapters 90 and 91).[44] Drainage of brain abscesses, subdural empyema, and epidural abscess is crucial to establishing a microbiologic diagnosis and to ensuring a good outcome by relieving the increased ICP and neurologic findings that are associated with these disorders. Aspiration can be performed via guidance with neuroimaging modalities (i.e., CT or MRI), which allows the surgeon rapid, accurate, and safe access to virtually any intracranial or parameningeal focus. Open craniotomy may be required for extensive intracranial infections that cannot be adequately drained via bur-hole aspiration (e.g., in patients with cranial subdural empyema). Although some patients with focal CNS infections can be treated with antimicrobial therapy alone, these patients must be selected carefully and followed to ensure that neurologic dysfunction does not develop or progress.

Adjunctive Therapy

The morbidity and mortality in patients with CNS infections remain unacceptably high. Because CNS infections can be complicated by cerebral edema and increased ICP (with the potential for brain herniation), various adjunctive strategies have been examined for their efficacy in CNS infections. Among the best-studied agents are corticosteroids. Adjunctive dexamethasone, given concomitant with or just before the first antimicrobial dose, improves outcomes in infants and children with *H. influenzae* type b, and perhaps pneumococcal meningitis, and in adults with pneumococcal meningitis (see Chapter 87).[1,19,44] Adjunctive corticosteroids may not be beneficial, however, in patients with bacterial meningitis in the developing world, most likely because of their delayed presentation.[45] Corticosteroids may also be beneficial in patients with focal intracranial infections and cerebral edema associated with significant mass effect.[44] Steroids may be beneficial in reduction of edema, leading

to improvement of symptoms and signs of neurologic dysfunction until more definitive interventions can be performed.

Other adjunctive modalities in treatment of CNS infections are directed toward reduction of increased ICP.[44,46] ICP can be reduced by decreasing total intracranial volume (by withdrawal of CSF via ventricular drainage), reduction of cerebral tissue volume (by osmotic dehydration), removal or decompression of an intracranial mass, or a decompressive craniectomy in refractory cases.[46] Although it should be noted that randomized studies of various strategies to lower ICP in patients with CNS infections have not been performed, some maneuvers may be beneficial.[45,46] Head elevation of 30 degrees is considered standard, and other factors that might increase ICP, such as pain, bladder distention, and agitation, should be avoided. Hyperventilation (to maintain the partial pressure of carbon dioxide in arterial blood at 27–30 mm Hg), which causes vasoconstriction and reduction in intracerebral volume, may also be used to reduce ICP, although it should be used only as a short-term intervention to quickly reduce ICP when it is dangerously elevated because it may induce cerebral ischemia (see Chapter 87). Hyperosmolar agents decrease ICP by making the intravascular space hyperosmolar relative to the brain, permitting movement of water from the brain tissue to the intravascular compartment. However, a controlled trial from Malawi of adjunctive glycerol in adult patients with bacterial meningitis showed that it was harmful and increased mortality.[47] Similarly, a clinical trial of hypothermia (to reduce ICP) in bacterial meningitis showed a trend toward worse clinical outcomes.[48] In patients who continue to have elevated ICP despite the aforementioned interventions, high-dose barbiturate therapy may be considered, although this treatment modality is associated with multiple complications, including myocardial depression and hypotension. One observational study, which reported findings on the use of measurements of continuous ICP and cerebral perfusion pressure in the treatment of patients with severe bacterial meningitis, indicated that the mean ICP was significantly higher, and the cerebral perfusion pressure was markedly decreased, in patients who did not survive despite appropriate treatment.[49]

Seizures may occur in patients with CNS infections and must be managed quickly and aggressively to avoid permanent anoxic ischemic changes.[1,45,46] Status epilepticus that is continuous for 90 minutes or more can lead to permanent neurologic injury. Short-acting anticonvulsants with a rapid onset of action (e.g., lorazepam or diazepam) should be given, followed by a long-acting agent, such as phenytoin. If these maneuvers fail to terminate the seizure, the patient should be intubated, mechanically ventilated, and treated with phenobarbital.

Fluid management is crucial in patients with CNS infections.[1,50] Some patients may be volume depleted as a result of decreased oral intake, vomiting, and diarrhea. In these patients IV fluids are required to maintain systemic and cerebral perfusion. Hyponatremia (serum sodium <135 mEq/L) may occur in patients with CNS infections, attributed to the syndrome of inappropriate secretion of antidiuretic hormone. There is some evidence to support the use of IV maintenance fluids in the first 48 hours in patients with bacterial meningitis[50]; although there is insufficient evidence to guide this practice, adult patients with meningitis should be treated with the goal of attaining a normovolemic state.[1,50]

Aggressive treatment of these complications in the critical care setting has improved the neurologic outcome of patients with CNS infections. The definitive treatment of specific infectious syndromes and the etiologic agents that cause them are reviewed in detail in the following chapters of this section.

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87 Acute Meningitis

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SHORT VIEW SUMMARY

Definition

- Meningitis, or inflammation of the meninges, is identified through an abnormal number of white blood cells in cerebrospinal fluid (CSF). Acute meningitis is clinically defined as a syndrome characterized by the onset of meningeal symptoms over the course of hours to up to several days.

Epidemiology

- Estimates from the Centers for Disease Control and Prevention (CDC) indicate that 10 to 15 million symptomatic enteroviral infections occur annually in the United States, which includes 30,000 to 75,000 cases of meningitis.
- In a surveillance study among residents in eight surveillance areas representing 17.4 million persons from 1998 to 2007, the impact of the heptavalent pneumococcal conjugate vaccine was appreciated, in which the incidence of bacterial meningitis caused by vaccine serotypes decreased from 0.61 cases per 100,000 population in 1998 to 1999 to 0.05 cases per 100,000 population in 2006 to 2007.
- In patients 16 years old or older, the relative frequency of isolation of meningeal pathogens in patients with community-acquired bacterial meningitis is somewhat different than in infants and children, with most cases caused by *Streptococcus pneumoniae, Neisseria meningitidis,* and *Listeria monocytogenes.*
- Sub-Saharan Africa has the highest incidence of meningitis due to epidemic meningococcal disease; in the past decade, serogroup A vaccine (MenAfriVac) has been successfully introduced in several countries and prevented new outbreaks.
- New epidemics of serogroup W and C meningococcal disease have occurred in sub-Saharan Africa, replacing serogroup A epidemics, whereas in European countries an increased incidence of cases caused by serogroup W has been described.

Microbiology

- Enteroviruses, currently the leading recognizable cause of aseptic meningitis syndrome, account for 85% to 95% of all cases in which a pathogen is identified.
- DNA of herpes simplex virus (HSV) has been detected in the CSF in published cases of Mollaret meningitis (now termed *recurrent benign lymphocytic meningitis*), almost all being HSV-2.
- A profound reduction has been seen in the incidence of invasive infections (including bacterial meningitis) caused by *Haemophilus influenzae* type b in the United States and western Europe; this decrease in infection is attributed, in part, to the widespread use of conjugate vaccines against *H. influenzae* type b that have been licensed for routine use in all children beginning at 2 months of age.
- From 1998 to 2007, a total of 2262 cases of meningococcal disease were reported to the Active Bacterial Core surveillance sites, with an annual incidence of 0.53 cases per 100,000 population; the incidence decreased from 0.92 cases per 100,000 population in 1998 to 0.33 cases per 100,000 population in 2007 before the introduction of the quadrivalent meningococcal conjugate vaccine.
- Patients with pneumococcal meningitis often have contiguous or distant foci of pneumococcal infection, such as pneumonia, otitis media, mastoiditis, sinusitis, and endocarditis; serious infection may be observed in patients with various underlying conditions (e.g., splenectomy or asplenic states, multiple myeloma, hypogammaglobulinemia, alcoholism, malnutrition, chronic liver or renal disease, malignancy, and diabetes mellitus).
- Outbreaks of *Listeria* infection have been associated with the consumption of contaminated coleslaw, raw vegetables, milk, and cheese, with sporadic cases traced to contaminated turkey franks, alfalfa tablets, cantaloupe, diced celery, hog head cheese (a meat jelly made from hog heads and feet), and processed meats, thus pointing to the intestinal tract as the usual portal of entry.
- Antibiotic prophylaxis has decreased neonatal early-onset disease caused by group B streptococci but with a concomitant rise in late-onset disease cases.
- Aerobic gram-negative bacilli (e.g., *Klebsiella* spp., *Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter* spp., *Salmonella* spp.) have become increasingly important as etiologic agents in patients with bacterial meningitis; these agents may be isolated from the CSF of patients after head trauma or neurosurgical procedures and may also be found in neonates, older adults, immunosuppressed patients, and patients with gram-negative sepsis.

Diagnosis

- Nucleic acid amplification tests, such as polymerase chain reaction (PCR) assay, are the most promising alternatives to viral culture for the diagnosis of enteroviral meningitis.
- CSF culture is the gold standard in diagnosis of bacterial meningitis and is positive in 80% to 90% of patients with community-acquired disease if CSF is obtained before the start of antimicrobial therapy.
- Broad-based bacterial PCR can be used to detect the most common microorganisms of bacterial meningitis in only one test and has adequate sensitivity and excellent specificity. Such tests can be performed within 2 hours in most industrialized countries, but they are scarce in resource-poor countries.
- The diagnostic accuracy of CSF lactate is better than that of the CSF white blood cell count, glucose, and protein in the differentiation of bacterial from aseptic meningitis, with sensitivities of 93% and 97% and specificities of 96% and 94%.

Therapy

- The initial management of a patient with presumed bacterial meningitis includes performance of a lumbar puncture to determine whether the CSF formula is consistent with that diagnosis.
- If purulent meningitis is present, institution of antimicrobial therapy should be based on the results of Gram staining; however, if no etiologic agent can be identified by this means or if performance of the lumbar puncture is delayed, institution of empirical antimicrobial therapy should be based on the patient's age and underlying disease status.
- Certain patients with bacterial meningitis should also be treated with adjunctive dexamethasone. In a Cochrane Database systematic review of 24 studies involving 4041 participants, adjunctive dexamethasone did not reduce overall mortality, but there was a trend toward lower mortality in adults; corticosteroids were associated with lower rates of severe hearing loss, any hearing loss, and neurologic sequelae, although these benefits were seen only in studies from high-income countries.
- Penicillin can never be recommended as empirical therapy in patients with suspected pneumococcal meningitis; as an empirical regimen, the combination of vancomycin plus a third-generation cephalosporin (either

SHORT VIEW SUMMARY—cont'd

cefotaxime or ceftriaxone) is recommended, especially in countries with a ceftriaxone resistance rate >1%.
- Empirical treatment of gram-negative meningitis could begin with ceftazidime, cefepime, or meropenem. If the organism is later found to be resistant to these cephalosporins and the carbapenems, colistin (usually formulated as colistimethate sodium) or polymyxin B should be substituted for meropenem and may also need to be administered by the intraventricular or intrathecal route.

Prevention
- Chemoprophylaxis is necessary for close contacts of patients with invasive meningococcal disease; the CDC currently recommends the administration of rifampin, ciprofloxacin, or ceftriaxone, which are all 90% to 95% effective at eradicating nasopharyngeal carriage, although cases of ciprofloxacin-resistant *N. meningitidis* have been reported in North Dakota and Minnesota, leading the CDC to no longer recommend ciprofloxacin for meningococcal chemoprophylaxis in selected counties of these states.
- Vaccination to prevent infection with specific meningeal pathogens is a very useful measure for decreasing the incidence of bacterial meningitis.
- For *H. influenzae* type b, the availability of conjugate vaccines has decreased the number of cases of *H. influenzae* type b meningitis by more than 90%.
- The first meningococcal conjugate vaccine (meningococcal polysaccharide-diphtheria toxoid conjugate vaccine containing serogroups A, C, W, and Y polysaccharides) was licensed for use in the United States for routine vaccination of all persons aged 11 to 18 years with one dose. In updated guidelines, a booster dose is now recommended at age 16 years and a two-dose primary series is administered 2 months apart for persons aged 2 through 54 years with persistent complement component deficiency or functional or anatomic asplenia and for adolescents with human immunodeficiency virus infection. Other persons who are at risk for meningococcal disease (e.g., microbiologists or travelers to an epidemic or highly endemic country) should receive a single dose. Two protein vaccines are marketed for short term protection against serogroup B menngococcal infection.
- The Advisory Committee on Immunization Practices now recommends use of the 13-valent pneumococcal conjugate vaccine to prevent pneumococcal disease in infants and children aged younger than 6 years; this vaccine has activity against the serotypes that were present in the heptavalent vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F) along with six additional serotypes (1, 3, 5, 6A, 7F, and 19A).

DEFINING ACUTE MENINGITIS

Meningitis, or inflammation of the meninges, is typically identified by means of a cerebrospinal fluid (CSF) pleocytosis of more than five white blood cells (WBCs) in the CSF, even though approximately up to 8% of patients with central nervous system (CNS) infections can present with absence of CSF pleocytosis.[1] Acute meningitis (duration of symptoms of less than 5 days) accounts for 75% of all community-acquired meningitis cases and is most commonly caused by unknown pathogens, as well as viral and bacterial organisms.[2] In contrast, patients with subacute meningitis (duration of 5 days or more) are more likely to have comorbidities, be immunosuppressed, have fungal etiologies, or present with abnormal neurologic examination findings or with hypoglycorrhachia (CSF glucose <45 mg/dL).[2] It is very important to distinguish acute or subacute meningitis syndromes from chronic meningitis (see Chapter 88) and encephalitis (see Chapter 89). Chronic meningitis (>4 weeks' duration of symptoms and signs and CSF abnormalities) has a broad differential diagnosis that includes conditions such as fungal infection, mycobacterial infection, neurobrucellosis, neurosyphilis, sarcoidosis, autoimmune disorders, paraneoplastic disorders, and vasculitis.[3] Encephalitis is a community-acquired illness with altered mental status for more than 24 hours with at least two (possible encephalitis) or three (probable encephalitis) of the following criteria: fever, new-onset seizure, new-onset focal neurologic finding, CSF pleocytosis, and abnormal findings at magnetic resonance imaging (MRI) of the brain or electroencephalogram (EEG) consistent with encephalitis.[4] The distinction among all of these syndromes is clinically useful in guiding management, but there can be significant overlap among the different causes; for example, West Nile virus (WNV) and herpes simplex virus (HSV) can lead to either an acute meningitis or an encephalitis presentation.

The acute meningitis syndrome may arise from a wide variety of infectious and noninfectious causes (Table 87.1). Diseases in which meningeal symptoms occur but are not predominant are excluded from the table. Many of the causes of chronic meningitis, which can manifest acutely, have been omitted but are listed in Chapter 88. Here, we review the common infectious causes of acute meningitis, with particular emphasis on epidemiology and etiology, pathogenesis and pathophysiology, clinical manifestations, diagnosis, management, and prevention.

EPIDEMIOLOGY AND ETIOLOGY

Viral Meningitis

Viruses are the most commonly identified cause of the *aseptic meningitis syndrome,* a term used for a community-acquired illness with CSF pleocytosis, a negative CSF or blood culture, normal neurologic examination findings, and a benign clinical outcome.[5] The most common viral etiologic agents that cause the acute aseptic meningitis syndrome are enteroviruses (EVs), herpesviruses, and arboviruses such as WNV.[5] These and other less common viruses are discussed in the following paragraphs.

Enteroviruses

EVs are currently the leading recognizable cause of aseptic meningitis syndrome, accounting for 48% to 95% of all cases in which a causative virus is identified.[5–7] Enteroviral infections are underreported to the Centers for Disease Control and Prevention (CDC) because surveillance is passive and because enteroviral infections are underdiagnosed, especially in adults, in whom a CSF EV polymerase chain reaction (PCR) assay is performed in only 15%.[5–7] A total of 118 types of EVs and 16 types of human parechoviruses (HPeVs) (both small viruses of the Picornaviridae family) have been described as causes of viral meningitis in the United States.[7] In addition to aseptic meningitis, these viruses can sometimes also cause acute flaccid paralysis (AFP), encephalitis, myocarditis, and sepsis, with worse clinical presentations most commonly seen in neonates or infants.[7] EVs have a worldwide distribution and, in temperate climates, have a summer/fall seasonality, although in tropical and subtropical areas they can occur perenially.[6] Transmission is mainly via the fecal-oral route, but it has also been documented via respiratory droplets.[6] From 2009 to 2013, based on data from the CDC laboratory-based surveillance system (the National Enterovirus Surveillance System), the majority of cases occurred from April to November and the two most common viruses identified were coxsackievirus A6, and HPeV type 3, accounting for a total of 24.6% of total serotypes detected.[7] Recently, EV D68 was implicated as a possible cause of AFP in the United States, as 43% of cases had EV D68 isolated from respiratory specimens with PCR.[8] A total of 202 cases of AFP (patients' median age ranging between 9 and 11 years) were described in the United States from 2012 to 2015, with the majority of patients having neurologic deficits at 1-year follow-up. In addition, EV 71 can also cause AFP, in addition to aseptic meningitis and a brainstem encephalitis, which may manifest with shock and pulmonary edema.[9] EV 71 has been implicated in large cyclic epidemics in Southeast Asia, but cases have now been reported worldwide.[10,11] From 2005 to 2008, EV 71 was detected in 29 patients in Denmark,[11] with the majority of them presenting with meningitis. Outbreaks of enteroviral meningitis have also been reported.[12–15] One outbreak involved 29 travelers in a school-organized trip to Mexico[12]; most cases were caused by echovirus 30 and coxsackievirus A1. Other reported enteroviral meningitis outbreaks have

TABLE 87.1 Differential Diagnosis of Acute Meningitis

Major Infectious Causes

Viruses

Nonpolio enteroviruses[a]
Arboviruses[b]
Herpesviruses[c]
Lymphocytic choriomeningitis virus
Human immunodeficiency virus
Adenovirus
Parainfluenza virus types 2 and 3

Rickettsiae

Rickettsia rickettsii
Rickettsia conorii
Rickettsia prowazekii
Rickettsia typhi
Orientia tsutsugamushi
Ehrlichia and *Anaplasma* spp.

Bacteria

Haemophilus influenzae
Neisseria meningitidis
Streptococcus pneumoniae
Listeria monocytogenes
Escherichia coli
Streptococcus agalactiae
Cutibacterium (formerly *Propionibacterium*) *acnes*
Staphylococcus aureus
Staphylococcus epidermidis
Enterococcus spp.
Klebsiella pneumoniae
Pseudomonas aeruginosa
Salmonella spp.
Acinetobacter spp.
Viridans streptococci (e.g., *Streptococcus salivarius*)
Streptococcus gallolyticus
Fusobacterium necrophorum
Stenotrophomonas maltophilia
Streptococcus pyogenes
Streptococcus suis
Pasteurella multocida
Capnocytophaga canimorsus
Nocardia spp.
Mycobacterium tuberculosis

Spirochetes

Treponema pallidum (syphilis)
Borrelia burgdorferi (Lyme disease)
Borrelia miyamotoi
Leptospira spp.

Protozoa and Helminths

Naegleria fowleri
Angiostrongylus cantonensis
Baylisascaris procyonis
Taenia solium
Toxocara spp.
Strongyloides stercoralis (hyperinfection syndrome)

Other Infectious Syndromes

Parameningeal foci of infection[d]
Infective endocarditis
Viral postinfectious syndromes
Postvaccination[e]

Noninfectious Causes and Diseases of Unknown Etiology

Intracranial Tumors and Cysts

Craniopharyngioma
Dermoid or epidermoid cyst
Teratoma

Medications

Antimicrobial agents[f]
Nonsteroidal antiinflammatory agents[g]
Muromonab-CD3 (OKT3)
Azathioprine
Cytarabine (high dose)
Carbamazepine[h]
Immune globulin
Ranitidine
Phenazopyridine

Systemic Illnesses

Systemic lupus erythematosus
Behçet disease
Sarcoidosis
Vogt-Koyanagi-Harada syndrome

Procedure Related

After neurosurgery
Spinal anesthesia
Intrathecal injections[i]
Chymopapain injection

Miscellaneous

Seizures
Migraine or migraine-like syndromes

[a]Primarily echoviruses and coxsackieviruses.
[b]In the United States, the major etiologic agents are the mosquito-borne California, St. Louis, eastern equine, and West Nile viruses and the tick-borne Colorado tick fever.
[c]Primarily herpes simplex virus type 2 but also herpes simplex virus type 1, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, and human herpesvirus 6.
[d]Brain abscess, sinusitis, otitis, mastoiditis, subdural empyema, epidural abscess, venous sinus thrombophlebitis, pituitary abscess, cranial osteomyelitis.
[e]Mumps, measles, polio, pertussis, rabies, vaccinia.
[f]Trimethoprim, sulfamethoxazole, trimethoprim-sulfamethoxazole, ciprofloxacin, penicillin, isoniazid, metronidazole, cephalosporins, pyrazinamide.
[g]Ibuprofen, sulindac, naproxen, tolmetin, diclofenac, ketoprofen.
[h]In patients with connective tissue diseases.
[i]Air, isotopes, antimicrobial agents, antineoplastic agents, corticosteroids, radiographic contrast media.

been caused by coxsackievirus B3[13] and echovirus types 18 and 30.[14,15]

Infants and young children are the primary victims of enteroviral meningitis because they are the most susceptible host population within the community. In a study of 156 enteroviral infections in children, risk factors for severe disease were absence of oral lesions, seizures, and lethargy, with the majority of the severe cases caused by EV 71 and coxsackievirus A16.[16] EVs are also a common cause of aseptic meningitis in adults, but these patients invariably have good clinical outcomes.[5] A few cases of enteroviral meningoencephalitis have also been seen in patients treated with the chimeric anti-CD20 monoclonal antibody rituximab.[17,18]

Herpesviruses

Herpesviruses include HSV types 1 and 2, varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpesvirus (HHV) types 6, 7, and 8. Although neurologic complications are known to occur with some of these viruses, complications associated with HSV are of the greatest significance. In a study of 404 adults with aseptic meningitis, HSV was the most commonly identified viral pathogen even though only 39% of patients had a CSF HSV PCR assay performed.[5] In patients beyond the neonatal period, it is critical to differentiate between HSV encephalitis (usually caused by HSV type 1), a potentially fatal infection, and HSV meningitis (most commonly caused by HSV type 2), a self-limited syndrome. The syndrome of HSV-2 aseptic meningitis is most commonly associated with primary genital infection and has a benign clinical outcome that does not appear to be impacted by antiviral therapy.[19] HSV-2 is also the most common cause of Mollaret meningitis (now termed *recurrent benign lymphocytic meningitis*), although a few cases associated with HSV-1 and EBV have been reported.[20] The majority of patients are female, have no history of genital HSV, and have no active lesions at presentation.[20] A double-blind,

randomized clinical trial of valacyclovir suppression showed no impact on decreasing recurrent rates in patients with HSV-2 meningitis.[21] Acute aseptic meningitis has also been associated with VZV in patients with or without typical skin lesions,[19] the latter known as *zoster sine herpete.* VZV is most likely an underdiagnosed treatable cause, because only 1.2% of patients with aseptic meningitis undergo a CSF VZV PCR assay.[5] A study in which a multiplex PCR assay was used documented that HHV-6 was more commonly detected than HSV-1 or HSV-2 in adults and children with meningitis and encephalitis.[22] The proportion of these HHV-6 cases that represent a true infection, versus reactivation or chromosomal integration, remains to be determined.[23] CMV and EBV may cause aseptic meningitis in association with a mononucleosis syndrome, particularly in an immunocompetent host.[24]

Arboviruses

Arboviruses (*ar*thropod-*bo*rne virus) include several families of viruses that are transmitted by mosquitos, ticks or sand flies.[25] The most common arthropod-transmitted cause of aseptic meningitis in the United States is WNV, a flavivirus. WNV infection is most commonly asymptomatic, with approximately 20% of patients having a febrile illness and 1% presenting with neuroinvasive disease.[26] Neuroinvasive disease may present as an aseptic meningitis, encephalitis or an AFP/myelitis, but may be underdiagnosed because only approximately one-third of adults and children with meningitis or encephalitis are tested.[27] There is no vaccine or therapy for WNV infection.

Less common arboviruses in the United States that can cause aseptic meningitis are two mosquito-borne illnesses—St. Louis encephalitis (a flavivirus) and the California encephalitis group of viruses (e.g., La Crosse, Jamestown Canyon, and snowshoe hare viruses, which are bunyaviruses)—and two tick-borne illnesses—Powassan virus in the northern central and eastern United States, and coltivirus (agent of Colorado tick fever) in the mountainous and western regions of the United States and Canada.[25] In 2015 the CDC reported a total of 2175 cases of WNV, followed by La Crosse (55), St. Louis encephalitis (23), Jamestown Canyon (11), Powassan (7), and eastern equine encephalitis (6).[28] In Europe, tick-borne encephalitis can be associated with a complex syndrome of meningoencephaloradiculitis (MER), which is associated with a relatively high risk of severe disease (requirement for intensive care and mechanical ventilation). Age, male sex, and preexisting diabetes mellitus were predictive of the more severe MER.[29] Toscana virus has emerged as one of the most common causes of meningitis or encephalitis during the summer in the Mediterranean countries[30]; it is transmitted by sand flies and is a bunyavirus.

Other Viruses

Lymphocytic choriomeningitis virus (LCMV) can cause aseptic meningitis[24]; this virus is now rarely reported as an etiologic agent.[5] A seroprevalence of 5% for LCMV was seen in 400 patients with neurologic infections, but there were no documented cases by PCR assay.[31] LCMV is transmitted to humans by contact with rodents (e.g., hamsters, rats, mice) or their excreta[24]; the greatest risk for infection is in laboratory workers, pet owners, and persons living in impoverished and unhygienic situations. Recent outbreaks have been reported in rodent breeding factories or infected households.[32,33] No evidence of human-to-human transmission has been reported.

In an unimmunized population, mumps can cause aseptic meningitis and encephalitis.[24] With the introduction of the measles-mumps-rubella (MMR) vaccine, the incidence of mumps-associated meningitis has dramatically decreased, now accounting for <1% of all cases of meningitis and encephalitis in the United Kingdom and the United States.[34,35]

Human immunodeficiency virus (HIV) can cause aseptic meningitis during HIV seroconversion; the clinical presentation is a mononucleosis-like picture.[24] HIV may also cause an encephalitis presentation in those with acquired immunodeficiency syndrome (AIDS) who are not receiving antiretroviral therapy (ART) (known as AIDS encephalopathy or HIV encephalitis) or in those patients on ART with CSF viral escape (detectable viral load in the CSF with undetectable or low-level viremia).[36] This latter form is named CD8 encephalitis and can be treated with steroids and by optimizing ART. A recent large study of community-acquired meningitis in the United States showed that 25% of patients tested had HIV coinfection.[37]

Japanese encephalitis is a vaccine-preventable infection that continues to cause both meningitis and encephalitis in countries where routine vaccination is not available.[38] Dengue, chikungunya, and Zika viruses are emerging causes of meningitis or encephalitis in several parts of the world.[39,40] The epidemic of Ebola virus disease in West Africa has revealed unusual characteristics of the disease not previously described, including viral relapse with acute meningitis, with high concentrations of virus in CSF. Antiviral therapy with an experimental agent and adjuvant corticosteroids led to resolution of the disease.[41] Other newly identified causes of meningitis include astrovirus MLB2, usually a gastrointestinal virus, and Cache Valley virus in an immunosuppressed individual.[42,43]

Bacterial Meningitis

Overview of Bacterial Meningitis

Bacterial meningitis is an important disease worldwide, with the major burden of disease in sub-Saharan Africa.[44,45] The Global Burden of Disease Study showed that meningitis caused 318,000 deaths each year worldwide (4.5 per 100,000 persons), resulting in 20,383 years of life lost in 2016.[46] The incidence rates vary per country, with most recent rates varying from 0.7 to 0.9 per 100,000 per year in the United States and European countries, whereas studies from Africa describe incidence rates varying from 10 to 40 per 100,000 per year.[47]

Studies on the epidemiology of community-acquired bacterial meningitis in the United Status showed that the disease changed substantially over the past 40 years (Table 87.2).[48–51] The overall annual attack rate for bacterial meningitis identified in a surveillance study of 27 states in the United States from 1978 through 1981 was approximately 3.0 cases per 100,000 population, with variability based on age, race, and sex.[48] The three most common meningeal pathogens were shown to be *Haemophilus influenzae, Neisseria meningitidis,* and *Streptococcus pneumoniae,* accounting for more than 80% of cases. A 1995 laboratory surveillance study serving acute-care hospitals in 22 counties of four states (>10 million population) showed that the incidence of bacterial meningitis decreased dramatically.[50] This decrease was a result of a vaccine-related decline in meningitis caused by *H. influenzae* type b

TABLE 87.2 Etiology of Bacterial Meningitis in the United States

	PERCENTAGE OF TOTAL CASES			
ORGANISM	**1978–1981[39]**	**1986[40]**	**1995[41]**	**2003–2007[42]**
Haemophilus influenzae	48	45	7	7
Neisseria meningitidis	20	14	25	14
Streptococcus pneumoniae	13	18	47	58
Streptococcus agalactiae	3	6	12	18
Listeria monocytogenes	2	3	8	3
Other	8	14	—	—
Unknown	6	—	—	—

(from 2.9 cases per 100,000 population in 1986 to 0.2 cases per 100,000 population in 1995).[49,50] In another surveillance study among residents in eight surveillance areas representing 17.4 million persons from 1998 to 2007, the impact of the heptavalent pneumococcal conjugate vaccine was appreciated; the incidence of meningitis caused by vaccine serotypes decreased from 0.61 cases per 100,000 population in 1998 to 1999 to 0.05 cases per 100,000 population in 2006 to 2007, although the number of cases of bacterial meningitis caused by nonvaccine serotypes increased by 61%.[50] The mean age of all patients with meningitis increased from 30.3 years in 1998 to 1999 to 41.9 years in 2006 to 2007. However, despite the declining incidence of bacterial meningitis in the United States, the overall case-fatality rates did not change significantly (15.7% in 1998 to 1999 compared with 14.3% in 2006 to 2007; $P = .50$).

Epidemiology of bacterial meningitis differs according to age group, with higher incidences in the very young (neonates) and the elderly.[52] In neonates, the majority of meningitis cases are caused by *Streptococcus agalactiae* (or group B streptococci) and *Escherichia coli*.[53] Whereas *Listeria monocytogenes* has traditionally been considered an important pathogen in neonates, 21st century epidemiologic studies from the United Kingdom and the Netherlands show that it is now an uncommon pathogen.[54,55] In children beyond the neonatal age, *S. pneumoniae* and *N. meningitidis* cause up to 90% of cases (Table 87.3).

In patients 16 years old or older, most cases are caused by *S. pneumoniae, N. meningitidis,* and *L. monocytogenes* (see Table 87.3).[47–51] Risk factors for meningitis in adults consist of splenectomy or splenic dysfunction, alcoholism, HIV, diabetes, cancer, use of immunosuppressive medications, solid organ transplantation, and bone marrow transplantation.[56–59] In selected groups, the increased risk of infection should warrant additional vaccination—for instance, in patients with splenectomy. Elderly patients and patients in an immunocompromised state are at higher risk of *L. monocytogenes* meningitis, which should therefore be covered in the empirical antibiotic treatment in anyone older than 50 years and in those in an immunocompromised state, irrespective of age.[52,53] Risk factors for death on admission among adults with community-acquired meningitis include older age, absence of otitis or sinusitis, alcoholism, tachycardia, lower score on the Glasgow Coma Scale, cranial nerve palsy, a CSF WBC count of less than 1000 cells per microliter, a positive blood culture, and a high serum C-reactive protein (CRP) concentration.[60]

Geographic differences in bacterial meningitis epidemiology are substantial (Table 87.4).[50–53] The majority of cases worldwide occur in sub-Saharan Africa, a region often referred to as the "meningitis belt." Epidemic meningococcal disease causes peaks in meningococcal meningitis incidence up to 100 per 100,000 population.[61] A study from Burkina Faso showed that despite the epidemics of meningococcal disease, 53% of cases were caused by *S. pneumoniae*.[62] In regions with high rates of HIV infection, bacterial meningitis due to nontyphoidal *Salmonella* species causes a substantial proportion of cases.[63,64] In Southeast Asia, the most common pathogen is *Streptococcus suis*, which is found in 25% to 30% of cases[65]; risk factors are close contact with pigs due to pig farming or the pork-processing industry, and alcoholism.[66]

Outcome also differs geographically, with mortality rates ranging from 54% in Malawi to 6% in Germany.[67,68] Mortality from neonatal meningitis in developing countries is estimated to be 40% to 58%, compared with only 10% in the developed world.[69]

Meningitis acquired in the hospital, whether after neurosurgical intervention, due to neurosurgical implants (e.g., CSF drains, deep brain stimulators), or during admission for other diseases, is also a significant problem. A 2017 guideline by the Infectious Diseases Society of America (IDSA) renamed this as *health care–associated ventriculitis and meningitis,* replacing the previously used term *nosocomial meningitis.*[70] In a review of 493 episodes of bacterial meningitis in adults 16 years old or older at the Massachusetts General Hospital from 1962 through 1988, 40% of episodes were found to be health care associated, with most cases (38%) caused by gram-negative bacilli.[71] The overall case-fatality rate for patients with health care–associated meningitis was 35% and did not vary significantly over the 27 years of the study. In a 2012 study from Korea of 91 adult patients with health care–associated meningitis, coagulase-negative staphylococci (41% of cases) and *Acinetobacter* species (33% of cases) were the most common pathogens, with 86% of patients having infection related to an external ventricular drain.[72] More recently

TABLE 87.3 Etiology of Bacterial Meningitis in Patients 16 Years of Age and Older

	PERCENTAGE OF TOTAL CASES			
ORGANISM	UNITED STATES (1962–1988)[44]	ICELAND (1975–1994)[43]	CANADA (1985–1995)[45]	UNITED STATES (1970–1998)[46]
Haemophilus influenzae	4	5	8	4
Neisseria meningitidis	14	56	2	14
Streptococcus pneumoniae	38	20	53	48
Listeria monocytogenes	11	6	25	7
Other[a]	20	—	12	27
Unknown	13	8	—	—

[a]Includes gram-negative bacilli, streptococci, enterococci, *Staphylococcus aureus,* anaerobes, and diphtheroids.

TABLE 87.4 Etiology of Bacterial Meningitis in Selected Series Outside the United States

	PERCENTAGE OF TOTAL CASES			
ORGANISM	UNITED KINGDOM (1980–1984)[50]	DAKAR, SENEGAL (1970–1979)[51]	SALVADOR, BRAZIL (1973–1982)[52]	MEXICO (1993–2003)[53]
Haemophilus influenzae	29	20	23	50
Neisseria meningitidis	25	11	22	2
Streptococcus pneumoniae	20	29	27	31
Streptococcus agalactiae	7	4	—	—
Listeria monocytogenes	2	<0.5	—	—
Other	16	9	20	6
Unknown	—	26	18	11

in 2016, a study of 215 adults and children with health care–associated meningitis demonstrated that 78% had adverse clinical outcomes; significant predictors were age >45 years, abnormal neurologic examination findings, and mechanical ventilation.[73]

In patients who survive their episode of bacterial meningitis, significant sequelae have been reported, with the risks for long-term disabling sequelae highest in low-income countries where the burden of bacterial meningitis is greatest. In a review of 132 studies including 18,183 survivors of acute bacterial meningitis, the risk for a major sequela (cognitive deficit, bilateral hearing loss, motor deficit, seizures, visual impairment, hydrocephalus) was greatest in Africa (25.1%) and southeast Asia (21.6%) when compared with Europe (9.4%)[74]; the risk for at least one major sequela was also pathogen dependent—24.7% in survivors of pneumococcal meningitis compared with 9.5% with *H. influenzae* type b meningitis and 7.2% with *N. meningitidis* meningitis. In another literature review of 1433 children who were survivors of bacterial meningitis, 705 were reported to have one or more long-term sequelae, the majority of which were behavioral or intellectual disorders or both.[75] In a prospective study, cognitive impairment was noted in 32% of adults after bacterial meningitis and mainly consisted of cognitive slowness.[76]

The likely etiologic agents of bacterial meningitis vary according to the age and underlying disease status of the patient (Table 87.5). The epidemiology and etiology of specific meningeal pathogens are reviewed next.

Haemophilus influenzae

H. influenzae was isolated in 45% to 48% of all cases of bacterial meningitis in the United States before introduction of the *H. influenzae* type b vaccine[48,49]; this organism is now isolated in only 3% to 7% of cases.[50,51,60] The overall mortality rate is 3% to 7%.[48–51,77] Most episodes of meningitis previously occurred in infants and children younger than 6 years (peak incidence of 6 to 12 months), with 90% of cases caused by capsular type b strains. Isolation of this organism in older children and adults should suggest the presence of certain underlying conditions, including sinusitis, otitis media, epiglottitis, pneumonia, diabetes mellitus, alcoholism, splenectomy or asplenic states, head trauma with CSF leak, and immune deficiency (e.g., hypogammaglobulinemia).[77–80] A profound reduction has been seen in the incidence of invasive infections (including bacterial meningitis) caused by *H. influenzae* type b in the United States and western Europe.[81–84] This decrease in infection is mainly attributed to the widespread use of conjugate vaccines against *H. influenzae* type b that have been licensed for routine use in all children beginning at 2 months of age. The number of cases of *H. influenzae* type b meningitis since the introduction of vaccination has decreased more than 90%, although because of vaccine expense, in developing countries the results are not as dramatic. Although the incidence in children has dramatically declined, the incidence of invasive *H. influenzae* disease in adults is more complex. In one population-based study of the epidemiology and outcome caused by typeable and nontypeable *H. influenzae* among adults in Utah during 1998 to 2008, there was an increase in incidence over the study period from 0.14 per 100,000 person-years in 1998 to 1.61 per 100,000 person-years in 2008 (subjects >65 years of age accounted for 51% of the cases and 67% of the deaths).[85]

TABLE 87.5 Relationship Between Common Bacterial Pathogens and Factors Predisposing to Meningitis

PREDISPOSING FACTOR	BACTERIAL PATHOGENS
Age	
<1 mo	*Streptococcus agalactiae, Escherichia coli, Listeria monocytogenes*
1–23 mo	*S. agalactiae, E. coli, Haemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitidis*
2–50 yr	*S. pneumoniae, N. meningitidis*
>50 yr	*S. pneumoniae, N. meningitidis, L. monocytogenes*, aerobic gram-negative bacilli
Immunocompromised state	*S. pneumoniae, N. meningitidis, L. monocytogenes*, aerobic gram-negative bacilli (including *Pseudomonas aeruginosa)*
Basilar skull fracture	*S. pneumoniae; H. influenzae;* group A streptococci
Head trauma; after neurosurgery	*Staphylococcus aureus*, coagulase-negative staphylococci (especially *Staphylococcus epidermidis*), aerobic gram-negative bacilli (including *P. aeruginosa*)

Neisseria meningitidis

N. meningitidis most commonly causes meningitis in children and young adults and is associated with an overall mortality rate of 3% to 13% in the United States.[48–51] More than 98% of cases of invasive meningococcal disease are sporadic.[86] Meningococci of serogroups B, C, and Y account for most of the endemic disease in the United States; disease caused by serogroups A and W seldom occurs in the United States. Annual outbreaks of meningococcal meningitis occur in the sub-Saharan meningitis belt during the dry season (December to June); cases tend to peak in late April and early May, when the dry desert wind (harmattan) has ceased and temperatures are high throughout the day, and terminate abruptly with the onset of the rainy season. During an active and ongoing, laboratory-based, population-based surveillance for meningococcal disease in the United States from 1992 to 1996, serogroup C caused 35%, serogroup B caused 32%, and serogroup Y caused 26% of cases.[87] Disease caused by serogroups A and C may occur in epidemics; group Y strains may be associated with pneumonia. From 1998 to 2007, a total of 2262 cases of meningococcal disease were reported to the Active Bacterial Core surveillance sites, with an annual incidence of 0.53 cases per 100,000 population.[51] The incidence decreased from 0.92 case per 100,000 population in 1998 to 0.33 cases per 100,000 population in 2007, with this historic low occurring before the introduction of the quadrivalent meningococcal conjugate vaccine. Infants younger than 1 year of age had the highest incidence (5.38 cases per 100,000 population), although the distribution of serogroups is also different in this population (serogroup B, 3.08 per 100,000 population; serogroup C, 0.53 per 100,000 population; and serogroup Y, 1.50 per 100,000 population). During the outbreak of meningococcal disease coinciding with the Hajj pilgrimage in March 2000, the attack rate of serogroup W disease was 25 cases per 100,000 pilgrims[88]; all outbreak-associated isolates of serogroup W were members of a single clone of the hypervirulent ET-37 complex, which occurred as the result of expansion of a clone that had been in circulation since 1970.[89] A high incidence of serogroup X cases was reported in Niger,[90] representing 51% of 1139 confirmed cases of meningococcal meningitis in 2006; serogroup X disease also emerged in Togo and Burkina Faso during 2006 to 2010.[91] A tenfold increase in incidence of serogroup W meningococcal disease was observed in the Netherlands after 2015, which was similar to a hyperendemic period of serogroup W disease in the United Kingdom occurring after 2009.[92,93] After replacement of the serogroup C vaccine with the ACWY vaccine in the United Kingdom, the incidence decreased by 69%.[94] Since 2010, large-scale vaccination campaigns against serogroup A meningococci have started in sub-Saharan Africa, resulting in a sharp decrease in epidemic meningococcal meningitis.[95] This almost completely prevented serogroup A disease and reduced the incidence of meningococcal disease by 57%.[95] However, since 2015, epidemics of serogroup C meningococcal have replaced those caused by serogroup A, with comparable incidence rates as were observed before vaccination. Continuous surveillance and quickly adaptable vaccination strategies are of vital importance to counter these epidemics.

A 2017 study of surveillance data from 25 European countries assessed almost 50,000 cases of meningococcal disease from the period of 2004 to 2014 and showed a variable incidence, ranging from 0.3 to 2.9 cases per 100 000 per year.[96] Overall, 74% of cases were caused by serogroup B, and serogroup C was the second most common, identified in 14% of cases.[96] In countries in which the serogroup C meningococcal conjugate vaccine (MenC) was implemented, a sharp decrease in serogroup C meningococcal disease was observed, whereas the incidence remained

similar in countries in which the vaccine was not introduced. An 8% annual decrease of serogroup B meningococcal disease was observed over the observation period, even though no vaccination had been introduced.

Respiratory tract infections, with viruses such as influenza virus, may play a role in the pathogenesis of invasive meningococcal disease.[79] Nasopharyngeal carriage of *N. meningitidis* is an important factor that leads to the development of invasive disease.[80] Patients with deficiencies in the terminal complement components (C5, C6, C7, C8, and perhaps C9), the so-called membrane attack complex, have a markedly increased incidence of neisserial infection,[81–83] including that caused by *N. meningitidis,* although mortality rates in patients with meningococcal disease are lower than those in patients with an intact complement system (3% vs. 19% in the general population). An increased risk for invasive meningococcal disease has also been described in a Dutch family with dysfunctional properdin,[84] which suggests a potential role for the alternate pathway in complement-mediated resistance against meningococci. Because meningococcal meningitis occurs in approximately 39% of persons with late complement component deficiencies and 6% of those with properdin deficiencies, it has been suggested that a screening test for complement function (i.e., CH_{50}) should be performed for patients who have invasive meningococcal infections, with consideration of direct assessment of terminal complement components and properdin proteins.[85] However, this is most appropriate for patients with recurrent neisserial infections. It has also been recently reported that unusual meningococcal strains with low levels of virulence similar to carriage strains are most frequently responsible for invasive meningococcal disease in patients with terminal complement pathway deficiency.[97] In addition, there is an increased risk of having meningococcal disease in men who have sex with men (MSM); the relative risk (RR) is 4 in MSM, and 10 if they are also HIV infected.[98] Most recently, there has been an increased risk in patients who are receiving eculizumab (1000- to 2000-fold increased risk).[99]

A vaccine for serogroup B meningococcal disease has been developed and was shown to be immunogenic in a randomized controlled trial.[100] After introduction of the vaccine in the United Kingdom in 2015, an incidence reduction of 50% was observed.[101] Based on the meningococcal typing system, it was predicted that two-thirds of serogroup B meningococci in the United Kingdom are covered by the vaccine, with rates varying between 60% and 80% in other European countries.[102,103]

Streptococcus pneumoniae

S. pneumoniae, the most frequently observed etiologic agent of bacterial meningitis in the United States, now accounts for 58% of the total cases.[51] Epidemiologic changes have been observed since introduction of the 7-valent pneumococcal conjugate vaccine (PCV7).[104] The incidence of pneumococcal meningitis decreased in children first, and later also in adults. The decrease was the result of a reduction of cases due to serotypes included in the vaccine, but at the same time an increased incidence in nonvaccine serotypes occurred, limiting the effect of the vaccine's introduction.[104] After this increase, vaccines covering additional serotypes (PCV10 and PCV13) have replaced PCV7. A 2016 study from France assessing 5166 pneumococcal isolates from meningitis cases showed that the effect of the introduction of PCV7 and PCV13 was modest.[47,105] In Ghana, introduction of PCV13 did not prevent a serotype 1 outbreak in 2015 and 2016, with incidence rates of 363 per 100,000 persons.[106] Overall a modest reduction in incidence of pneumococcal meningitis has been observed with frequent serotype replacement. In invasive pneumococcal disease other than meningitis, the effect of vaccination was substantially better, which was explained by different tropism for the meninges of the serotypes.[107]

The mortality rate of pneumococcal meningitis ranges from 18% to 26% in the United States.[48–51] In one study of 352 episodes of community-acquired pneumococcal meningitis in adults, 245 (70%) were associated with an underlying disorder and the overall in-hospital mortality rate was 30%[108]; independent predictors of unfavorable outcome were a low score on the Glasgow Coma Scale, cranial nerve palsies, elevated erythrocyte sedimentation rate, a CSF WBC count of less than 1000/mm,[3] and a high CSF protein concentration on admission. There was a high rate of neurologic sequelae in survivors; death in patients younger than 60 years was more often caused by neurologic complications, and in patients 60 years or older it was more likely secondary to systemic complications. Of the more than 90 known pneumococcal serotypes, 18 are responsible for 82% of the cases of bacteremic pneumococcal pneumonia, with a close correlation between bacteremic subtypes and those implicated in meningitis. Patients often have contiguous or distant foci of pneumococcal infection, such as pneumonia, otitis media, mastoiditis, sinusitis, and endocarditis. Serious infection may be observed in patients with various underlying conditions (e.g., splenectomy or asplenic states, multiple myeloma, hypogammaglobulinemia, alcoholism, HIV chronic liver or renal disease, malignancy, and diabetes mellitus).[109–112] Remote head injury and CSF leak are important predisposing factors for recurrent bacterial meningitis.[113] The pneumococcus is the most common etiologic agent of meningitis in patients who have sustained a basilar skull fracture with CSF leak.[114]

Listeria monocytogenes

L. monocytogenes causes 2% to 8% of cases of bacterial meningitis in the United States and carries a mortality rate of 15% to 29%.[48–51] Serotypes 1/2b and 4b have been implicated in up to 80% of meningitis cases caused by this organism. Epidemiologic data show that the incidence of *L. monocytogenes* meningitis is highest in neonates and elderly, with incidences up to 0.61 per 100,000 in neonates and 0.53 per 100,000 in the elderly.[54] A study from the Netherlands showed that the incidence of neonatal *Listeria* meningitis had decreased in the previous 25 years, potentially because of increased awareness of food restrictions for pregnant women. The rate of unfavorable outcome among adults with *Listeria* meningitis was found to increase over a 14-year period from 27% to 61%, with the emerging *L. monocytogenes* sequence type 6 identified as the main factor leading to a poorer prognosis.[115] A follow-up whole-genome sequencing study of these *Listeria* strains identified a plasmid containing the benzalkonium chloride tolerance gene that was associated with decreased susceptibility to disinfectants commonly used in the food-processing industry. Strains containing the plasmid had increased minimal inhibitory concentrations (MICs) to amoxicillin and gentamicin, which are commonly used in treatment of *L. monocytogenes* infections.[116]

Listeria has been isolated from dust, soil, water, sewage, and decaying vegetable matter (including animal feed and silage). Listerial infection is most common in infants younger than 1 month (up to 10% of cases), adults older than 60 years, alcoholics, cancer patients, those receiving corticosteroid therapy, and immunosuppressed adults (e.g., renal transplant recipients).[117–119] Other predisposing conditions include diabetes mellitus, liver disease, chronic renal disease, collagen vascular diseases, pregnancy, and conditions associated with iron overload. Although colonization rates are low, pregnant women (who account for 25% of all cases of listeriosis) may harbor the organism asymptomatically in their genital tract and rectum and transmit the infection to their infants. Adults younger than 50 years with *Listeria* meningitis should be screened for HIV infection.[120] Meningitis can also occur in immunocompetent children and adults.[121,122] Outbreaks of *Listeria* infection have been associated with the consumption of contaminated coleslaw, raw vegetables, milk, and cheese, with sporadic cases traced to contaminated turkey franks, alfalfa tablets, cantaloupe, diced celery, hog head cheese (a meat jelly made from hog heads and feet), and processed meats, thus pointing to the intestinal tract as the usual portal of entry.[117–119,123–126]

Streptococcus agalactiae (Group B *Streptococcus*)

Group B streptococci are a common cause of meningitis in neonates,[127] with 52% of all cases in the United States reported during the first month of life.[51] In a review of 444 cases of neonatal bacterial meningitis over a 7-year period, group B streptococci were the most common cause in early-onset (occurring between birth and day 4 of life) and late-onset (occurring between days 5 and 28 of life) disease, responsible for 77% and 50% of cases, respectively.[128] In the United States, the overall mortality rate ranges from 7% to 27%.[48–51] Survivors of group B streptococcal meningitis also have substantial long-term morbidity,[129] indicating the need for ongoing developmental follow-up and the development

of preventive strategies (see later discussion). Group B streptococci have been isolated from vaginal or rectal cultures of 15% to 35% of asymptomatic pregnant women[130]; colonization rates do not vary during pregnancy, and carriage may be chronic (40%), transient, or intermittent. The risk for transmission from mother to infant is increased when the inoculum of organisms and the number of sites of maternal colonization are increased; the route of delivery does not influence transmission. Intrapartum antibiotic prophylaxis was introduced in several high-income countries in the 1990s.[131] A study of 848 cases in newborns and infants in France documented that antibiotic prophylaxis was associated with a decrease in early-onset cases (0–6 days old) but with a concomitant increase in late-onset cases (7–89 days).[131] Most of the cases were caused by subtype III organisms (83.9%). Group B streptococci can also cause meningitis in adults.[132–135] Risk factors in adults include age older than 60 years, diabetes mellitus, pregnancy or the postpartum state, cardiac disease, collagen vascular diseases, malignancy, alcoholism, hepatic failure, renal failure, previous stroke, neurogenic bladder, decubitus ulcers, and corticosteroid therapy; in one review of group B streptococcal meningitis in adults, no underlying illnesses were found in 43% of patients.[133]

Aerobic Gram-Negative Bacilli

Aerobic gram-negative bacilli (e.g., *Klebsiella* spp., *E. coli, Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter* spp., *Salmonella* spp.) have become increasingly important as etiologic agents in patients with bacterial meningitis.[136–139] These agents may be isolated from the CSF of patients after head trauma or neurosurgical procedures[140–143] and may also be found in neonates, older adults, immunosuppressed patients, and patients with gram-negative sepsis. Some cases have been associated with disseminated strongyloidiasis in hyperinfection syndrome, a condition in which meningitis caused by enteric bacteria occurs secondary to seeding of the meninges during persistent or recurrent bacteremias associated with the migration of infective larvae.[144] Alternatively, the larvae may carry enteric organisms on their surfaces or within their own gastrointestinal tracts as they exit the intestine and subsequently invade the meninges. In patients with *E. coli* meningitis, 75% of cases are caused by strains possessing the K1 antigen.[127] Almost half of pregnant women have this organism isolated on rectal culture, and as many as 75% of their infants will be colonized during the first days of life; horizontal transmission from nursery staff members or other infants has also been reported.

Staphylococci

Meningitis caused by *Staphylococcus aureus* is usually found in early postneurosurgical or posttrauma patients and in those with CSF shunts; other underlying conditions include endocarditis, diabetes mellitus, alcoholism, chronic renal failure requiring hemodialysis, injection drug use, and malignancies.[145–148] In both adults and children, *S. aureus* is seen more frequently in patients with health care–associated ventriculitis and meningitis.[146,147] A study of 899 cases of nosocomial meningitis from Turkey showed that *S. aureus* accounted for 19% of cases.[148] Other sources of community-acquired *S. aureus* meningitis include patients with sinusitis, osteomyelitis, and pneumonia. Hospital-acquired cases are often caused by methicillin-resistant strains,[146] but recent a study documented that methicillin-susceptible strains caused two-thirds of pediatric cases.[147] In a multicenter review of 86 cases of methicillin-resistant *S. aureus* (MRSA) meningitis in adults,[146] the infection was nosocomial in 93% of cases; in those patients with postoperative meningitis, the most common predisposing conditions were the presence of CSF devices, neurosurgery, CSF leaks, and head trauma. Mortality rates have ranged from 14% to 77% in various series. In contrast, a pediatric study of 70 cases of *S. aureus* meningitis (75% were seen in patients with health care–associated ventriculitis and meningitis) found a low mortality rate (1.4%).[147] *Staphylococcus epidermidis* is the most common cause of meningitis in patients with CSF shunts (see Chapter 92).

Other Bacteria

A review of 28 cases of nocardial meningitis revealed predisposing conditions in approximately 75% of patients,[149] including immunosuppressive drug therapy, malignancy, head trauma, CNS procedures, chronic granulomatous disease, and sarcoidosis. Anaerobic meningitis is unusual and is generally associated with contiguous foci of infection (e.g., otitis, sinusitis, pharyngitis, brain abscess, head and neck malignancy, recent head and neck surgery or wound infection, and CNS trauma and neurosurgery)[150–153]; in many cases, more than one organism may be recovered. A study has advocated routine testing for anaerobes in patients with meningitis.[154] Enterococci are unusual etiologic agents of bacterial meningitis; most adult patients have underlying illnesses with CNS devices.[155] Disease caused by vancomycin-resistant *Enterococcus faecium* (VRE) is associated with a high mortality rate (18%–25%).[155,156] Despite the frequency with which the viridans-group streptococci cause bacteremia, they are unusual causes of meningitis (0.3%–5% of culture-proven cases).[157] *Streptococcus salivarius* meningitis has been reported after spinal anesthesia[158,159] and myelogram procedures,[160] supporting the importance of appropriate infection control practices (i.e., masks, proper aseptic technique, and safe injection practice) in those who perform spinal procedures. Group A streptococcal meningitis is also unusual, generally found in association with pharyngitis, otitis media, and sinusitis.[161] Group C streptococcal species (*Streptococcus equi* subsp. *zooepidemicus*) are rare causes of meningitis but may occur in humans after contact with domestic animals (especially horses) or their unpasteurized products.[162,163] *S. suis* is the most frequent cause of bacterial meningitis in southern Vietnam and is associated with significant morbidity involving hearing loss[164]; the pig is the natural reservoir of this microorganism and the main source of human infection. Risk factors for *S. suis* meningitis include eating "high-risk" dishes (e.g., undercooked pig blood or pig intestine) popular in parts of Asia, occupational exposure to pigs, and exposures to pigs or pork in the presence of skin injuries.[165] Diphtheroids, particularly *Propionibacterium* (now *Cutibacterium*) *acnes,* have become important etiologic agents of meningitis in patients with CNS shunt infections (see Chapter 92). With continued increases in numbers of immunocompromised patients and use of invasive diagnostic or therapeutic devices within the CNS, other (more unusual) bacterial pathogens may be reported as etiologic agents of acute meningitis.

Spirochetal Meningitis

Treponema pallidum

T. pallidum disseminates to the CNS during early infection.[166] The organism can be isolated by animal inoculation from the CSF of patients with primary syphilis, and CSF laboratory abnormalities are detected in 5% to 9% of patients with seronegative primary syphilis. The actual rate of invasion of the CNS during these early stages is likely to be considerably higher, however. Clinical neurosyphilis can be divided into four distinct syndromes[166–168]: syphilitic meningitis, meningovascular syphilis, parenchymatous neurosyphilis, and gummatous neurosyphilis. Some overlap may be seen in the clinical and laboratory findings of these syndromes. The incidence of syphilitic meningitis is greatest in the first 2 years after infection and is estimated to occur in only 0.3% to 2.4% of untreated syphilis cases. In contrast, meningovascular syphilis is found in 10% to 12% of individuals with CNS involvement[169] and occurs months to years after syphilis acquisition (peak incidence, approximately 7 years). Parenchymatous neurosyphilis has two variants: general paresis and tabes dorsalis. Both are relatively rare today and do not become apparent until 10 to 20 years after the acquisition of infection. Gummas are late manifestations of tertiary syphilis and may occur anywhere; gummatous neurosyphilis is rare. The overall incidence of neurosyphilis has increased, with many of the cases reported in patients with HIV infection.[167–169] In one report, 90% of patients with HIV with early syphilis undergoing lumbar puncture had evidence of CNS infection.[168]

Borrelia burgdorferi

The nervous system is eventually involved clinically in at least 10% to 15% of patients with Lyme disease, either while erythema migrans is still present or 1 to 6 months later.[170–172]

A 1992 study used PCR assay to detect spirochetal DNA in CSF samples from 8 of 12 patients with acute (<2 weeks) disseminated Lyme borreliosis,[172] which indicates that *B. burgdorferi* usually invades the CNS early in infection.

Protozoal and Helminthic Meningitis

Amebas

Despite the hundreds of species of free-living amoebas that are known, only a few have been reported to infect humans.[169–173] The most important are in the genera *Naegleria, Acanthamoeba,* and *Balamuthia. Naegleria fowleri,* the main protozoan causing primary amebic meningoencephalitis (PAM) in humans, has been recovered from lakes, puddles, pools, ponds, rivers, sewage sludge, tap water, air conditioner drains, and soil. Sporadic cases of PAM occur when persons, usually children and young adults, swim or play in water containing the amebas or when swimming pools or water supplies have become contaminated, often through failure of chlorination. In one review of 111 cases reported in the United States from 1962 to 2008, cases were reported in most southern states and occurred primarily in previously healthy young males exposed to warm recreational waters, especially lakes and ponds, in warm weather locations during summer months.[172] Activities that increase insertion of water into the nasal cavities (e.g., water skiing and wakeboarding) may increase the risk for developing PAM.[173] Asymptomatic carriage by humans can also occur. Several cases have been reported in HIV-infected patients, all with advanced HIV disease at the time of amebic infection.[174]

Angiostrongylus cantonensis

Infection of humans by larvae of the nematode *A. cantonensis* can lead to the development of an eosinophilic meningitis, and it is the most common cause of eosinophilic meningitis outside Europe and North America.[175–181] Humans become infected by eating infected intermediate hosts (i.e., mollusks, such as snails and slugs) or paratenic (i.e., freshwater prawns, crabs, frogs, and planaria) hosts or by eating food such as leafy green vegetables contaminated by these hosts. The larvae invade the brain either directly from the bloodstream or after migrating through other organs before reaching the spinal cord and brain. Once in the CNS, the larvae mature into adult worms that migrate through the brain. *A. cantonensis* is widespread, and human infection is fairly common and reported from many parts of the world (e.g., Thailand, India, Malaysia, Vietnam, Indonesia, Papua New Guinea, Taiwan, China and the Pacific Islands, including Hawaii). The parasites may spread to many countries by rats moving freely from port to port on ships; the rat infection rate in urban Bangkok has reached about 40%. A large outbreak of *A. cantonensis* meningitis was described in North America in travelers who had visited the Caribbean[181]; in this outbreak, the lettuce in a Caesar salad shared by the case patients was the most likely mode of transmission. After this outbreak, parasitologic surveys documented *A. cantonensis* in the Jamaican rat and land snail populations.[182] Tourists and visitors to endemic areas are at risk for becoming infected with *A. cantonensis.*

Other infectious causes of eosinophilic meningitis include *Gnathostoma* species, *Baylisascaris procyonis, Toxocara* species, and *Taenia solium.*[170] These are discussed in detail in other chapters of this book.

PATHOGENESIS AND PATHOPHYSIOLOGY

Viral Meningitis

Initiation of Infection

After the colonization of selected mucosal surfaces in the body by various viruses, the host possesses numerous barriers to prevent viral entry.[182] For example, the respiratory tract contains a thin film of mucus and a mucociliary escalator that moves viral particles away from the lower respiratory tract; even if this barrier is crossed, alveolar macrophages are actively phagocytic for viral particles. Gastric acidity inactivates most swallowed viruses, and gastrointestinal enzymes and bile also disrupt viral envelopes, capsid proteins, and lipoprotein membranes; however, some nonenveloped, acid-resistant viruses (e.g., EVs, adenoviruses, reoviruses, parvoviruses) are adapted for replication in the gastrointestinal tract. When the host has had previous contact with the viral agent, the mucosa of the gastrointestinal and respiratory tracts may be coated with secretory immunoglobulin A (IgA), which neutralizes the virus and prevents attachment and subsequent cell penetration. If certain viruses are able to escape initial host defense mechanisms, they may replicate and disseminate with the potential for CNS invasion.

Viremia and Central Nervous System Invasion

After hematogenous dissemination of the virus, CNS infection may occur. Most neurotropic viruses first multiply at extraneural sites (initially at the portal of entry), establish viremia, and then cross the blood-brain barrier (BBB) to invade the CNS.[182,183] For example, EVs initially multiply in the peritonsillar lymphatics, Peyer patches, lamina propria of the intestine, and vascular and endothelial cells, depending on the particular agent. M cells may mediate virus penetration from the gut lumen to lymphoid cells. From this initial site, the virus then disseminates to vascular tissue (e.g., liver, spleen, and muscle), where further multiplication augments the viremia. After viremia, viral particles are normally cleared by the reticuloendothelial system, with the speed of removal directly related to virus size (i.e., large viruses are cleared more promptly from the bloodstream). Viruses may also elude host clearance by associating with certain cells. Some viruses (e.g., measles, herpes, mumps) grow in human leukocytes, which protects them from phagocytosis by the reticuloendothelial system, neutralization by circulating antibody, and inactivation by nonspecific serum inhibitors.

CNS invasion by viruses may occur via several mechanisms. Most viruses invade directly across cerebral capillary endothelial cells, the major site of the BBB. Some viruses directly infect cerebral microvascular endothelial cells before infection of adjacent glia and neurons,[182,183] whereas others initially infect glia without evidence of endothelial cell infection. Still other viruses may be carried between cerebral endothelial cells in infected leukocytes after BBB disruption. Another site of virus entry is the choroid plexus epithelium. Studies of mumps virus in hamsters have shown a sequence of infection from the choroid plexus to the ependyma to parenchymal cells; viral nucleocapsids have been found in the choroid plexus and ependymal cells of humans with mumps meningitis. Viruses may reach the CNS by spread along olfactory nerves.[182,183] In an experimental hamster model, intranasal inoculation of HSV and togaviruses led to early infection of the olfactory bulb, which could be inhibited by cutting the olfactory tracts or chemically treating the olfactory mucosa. Peripheral nerve spread by viruses may also lead to CNS invasion.

Virus Spread Within the Central Nervous System

Regardless of the mechanism of CNS invasion, the production of disease requires viral attachment to and penetration of susceptible cells, spread within the nervous system, and induction of cellular changes. Viral entry into the subarachnoid space via the choroid plexus leads to dispersion of virus within CSF in contact with meningeal and ependymal cells[182]; sequential spread of virus may then occur in a contiguous fashion to glia and neurons. Other viruses spread through extracellular gaps between cells and CNS processes (e.g., dendrites, axons, or glia), or they transit along the extensive axonal and dendritic ramifications of neurons by way of the glia, or they are carried by mobile leukocytes in the inflammatory response. Experimental evidence supports each mode of transit, and all may be involved to various degrees in different viral infections.

Once viral infection of the CNS occurs, inflammatory cells usually accumulate, although the mechanisms leading to recruitment of inflammatory cells and their role in viral CNS infections are only partially understood.[182,183] It appears that the initial inflammatory response is immunologically specific and consists of a population of lymphocytes sensitized by the virus. However, an inflammatory response may fail to develop in other viral CNS infections, and this may depend on host age rather than the virus itself. Sensitized lymphocytes probably respond to a virus-specific protein that diffuses or is transported to the luminal surface of the endothelium, with subsequent passage through endothelial cells and release of inflammatory cytokines.

After development of a CSF inflammatory response, alterations in the BBB permit the traversal into CSF of serum proteins, including immunoglobulins. In addition, local CNS immunoglobulin synthesis occurs as B cells enter the CSF and differentiate into plasma cells. Intracerebral synthesis of immunoglobulins also is reflected by an increase in the CSF-to-serum ratios of specific immunoglobulins that persist for several weeks after infection.[182] The production of oligoclonal IgG

proteins within the CNS has been demonstrated in patients with meningitis caused by mumps virus, VZV, and HIV.[21,26,184,185] Furthermore, elevated CSF concentrations of oligoclonal IgG may persist for up to 1 year in patients with mumps meningitis,[21] which suggests the possibility of viral persistence and ongoing antigenic stimulation.

An intact host immune response appears to be important for clearance of virus from the CNS[182,183]; T-cell responses appear to be more important than B-cell responses. Failure of an immune response to develop may be a result of immunologic tolerance, host immune defects, or the ability of the virus to escape immune surveillance. Chronic infections with VZV, CMV, adenovirus, and measles virus have developed in patients with depressed cell-mediated immunity.

The CNS may also form anatomic viral reservoirs for persistent viral infection. Zika virus (ZIKV), for example, can persist in CSF of infected rhesus monkeys for weeks after virus has been cleared from peripheral blood, urine, and mucosal secretions. ZIKV-specific neutralizing antibodies were correlated with rapid clearance of virus in peripheral blood but remained undetectable in CSF for the duration of the study.[186]

Bacterial Meningitis

Numerous investigations over the past 40 to 50 years have elucidated many of the pathogenic and pathophysiologic mechanisms operable in bacterial meningitis.[187–194] Fig. 87.1 shows a simplistic hypothetical scheme of these mechanisms.

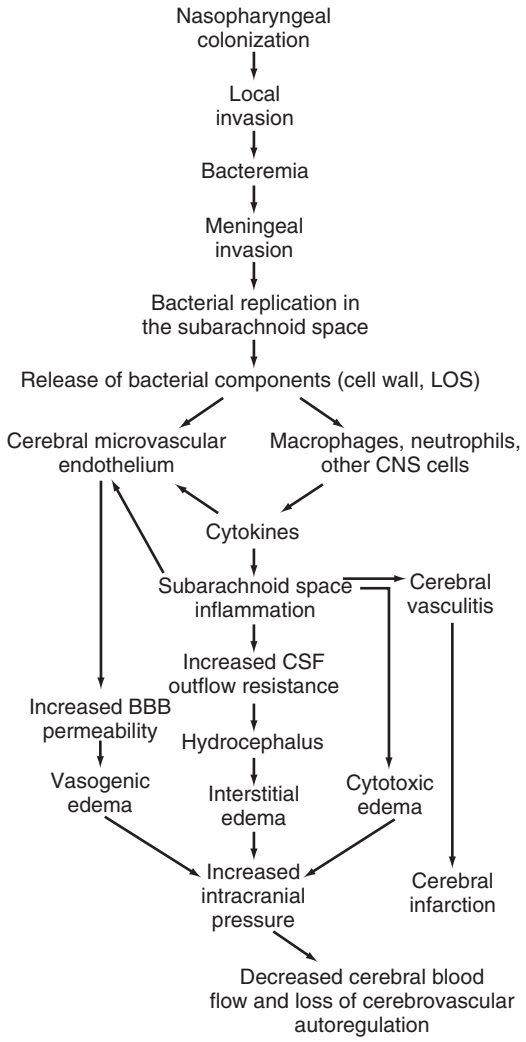

FIG. 87.1 Simplified scheme of the pathogenesis and pathophysiology of bacterial meningitis. *BBB,* Blood-brain barrier; *CNS,* central nervous system; *CSF,* cerebrospinal fluid; *LOS,* lipooligosaccharide. *(From Tunkel AR, Scheld WM. Pathogenesis and pathophysiology of bacterial meningitis.* Clin Microbiol Rev. *1993;6:118–136.)*

Mucosal Colonization and Systemic Invasion

The early pathogenic events that result in bacterial meningitis depend on an interplay between specific virulence factors and host defense mechanisms (Table 87.6). The initiation of infection with meningeal pathogens usually begins with host acquisition of a new organism by nasopharyngeal colonization.[189,195] Many of the major meningeal pathogens possess surface characteristics that enhance mucosal colonization. For example, the fimbriae (or pili) of *N. meningitidis* mediate adherence of this organism to nasopharyngeal epithelial cells; these fimbriated strains accounted for 80% of primary meningococcal isolates from nasopharyngeal carriers and from the CSF of patients with meningococcal meningitis. The fimbriae appear morphologically as aggregated bundles or single filaments. Once meningococci attach to nonciliated nasopharyngeal epithelial cells via a specific cell surface receptor (most likely CD46), they are transported across these cells within a phagocytic vacuole; this process appears to be essential for the subsequent development of invasive meningococcal disease. Studies have provided more information on the pathogenesis of meningococcal invasion. After attachment to the host endothelial cell, *N. meningitidis* induces formation of protrusions in the plasma membrane of host cells that aggregate bacteria into microcolonies and facilitate fimbriae-mediated contact between bacteria and between bacteria and host cells; after attachment and aggregation, organisms detach from the aggregates to systematically invade the host by means of a transcellular pathway that crosses the respiratory epithelium.[196] The detachment occurs with expression of pilin phosphotransferase, the enzyme that transfers phosphoglycerol onto pilin, altering the charge on the pilin structure and destabilizing pili bundles, thereby reducing bacterial aggregation and promoting detachment from the cell surface.[197]

Fimbriae have also been implicated in the attachment of *H. influenzae* to upper respiratory tract epithelial cells,[189] although fimbriae have not been found on isolates from the CSF or blood of patients with invasive disease. This observation suggests that although fimbriae play an initial role in adherence of *H. influenzae* within the nasopharynx, their presence is not necessary for the development of invasive disease, including meningitis. In addition, acquisition of and colonization by *H. influenzae* type b may be promoted after respiratory tract infection by viral agents such as the influenza A/Victoria virus and respiratory syncytial virus, although the precise role of a preceding upper respiratory viral infection in the enhancement of nasopharyngeal colonization by *H. influenzae*

TABLE 87.6 Selected Factors Involved in the Pathogenesis of Bacterial Meningitis

PATHOGENIC EVENT	BACTERIAL FACTORS	HOST FACTORS
Mucosal colonization	Fimbriae, polysaccharide capsule, IgA protease production, bacteriocins	Mucosal epithelium, secretory IgA, ciliary activity, anticapsular antibodies, pilin phosphotransferase
Intravascular survival	Polysaccharide capsule	Complement activation, organism-specific antibodies, TLR-9 single nucleotide polymorphisms, migration inhibitory factor single nucleotide polymorphisms
Meningeal invasion	Fimbriae, association with monocytes, *ibe10,* OmpA, extracellular loops of OmpA, platelet-activating factor receptor, pneumococcal choline-binding protein A, lipoteichoic acid, listeriolysin O, choline-binding protein CbpA, RrgA	Blood-brain barrier, cytotoxic necrotizing factor-1, cysteinyl leukotrienes, biopterin, cytosolic phospholipase $A_2\alpha$, β_2-adrenoceptor
Survival in the subarachnoid space	Polysaccharide capsule	Poor opsonic activity

type b is controversial. After nasopharyngeal colonization, invasion into the bloodstream by *H. influenzae* appears to occur via a breakdown in the tight junctions between epithelial cells, thereby leading to invasion by an intercellular mechanism.[195]

Surface encapsulation may also be an important virulence factor for nasopharyngeal colonization and systemic invasion of meningeal pathogens. In an experimental infant rat model, it was demonstrated that although all encapsulated strains of *H. influenzae* had the potential for systemic invasion after intraperitoneal inoculation, type b strains were the most virulent and were the only capsular types capable of systemic invasion after intranasal inoculation. Indeed, antibodies to type b capsule, which are almost uniformly detected in humans by the age of 4 years even in the absence of known exposure to *H. influenzae* type b, are protective against invasive disease. Polysaccharide capsule may also be an important virulence factor for the development of invasive disease by *S. pneumoniae.* In addition, in vivo capsular transformation events may equip pneumococcal strains with highly virulent blood-invasive phenotypes, thereby increasing the seriousness of pneumococcal infection, especially that caused by multidrug-resistant strains.[198]

Adherence of microorganisms to mucosal surfaces may be inhibited by natural antibodies found in mucosal secretions, such as IgA. However, it appears that the presence of high concentrations of circulating IgA antibodies to *N. meningitidis* may permit the development or progression of invasive disease by preferentially binding to the organism and blocking the beneficial effects of IgG and IgM antibodies.[187] In addition, species of many pathogenic bacteria (e.g., *Neisseria, Haemophilus, Streptococcus*) produce IgA1 proteases that cleave IgA in the hinge region and facilitate the adherence of bacterial strains to mucosal surfaces through local destruction of IgA. However, the exact role of IgA protease production in this pathogenic sequence remains unclear. The presence of anticapsular polysaccharide antibodies may also be effective in decreasing nasopharyngeal carriage of meningeal pathogens. In an intralitter transmission model in which infant rats were intranasally inoculated with *S. pneumoniae* and placed in a cage with other infant rats, pretreatment of uninoculated rats with systemic IgG antibodies to pneumococcal polysaccharide reduced the intralitter transmission of *S. pneumoniae*, which suggests that IgG antibodies to pneumococcal polysaccharide may be sufficient to reduce pneumococcal nasopharyngeal carriage in humans.

Intravascular Survival

Once bacteria cross the mucosal barrier and gain access to the bloodstream, they must overcome additional host defense mechanisms to survive. The presence of bacterial capsule, by effectively inhibiting neutrophil phagocytosis and resisting classical complement-mediated bactericidal activity, may enhance bloodstream survival of the organism, thereby facilitating intravascular replication.[188] The most common meningeal pathogens *(H. influenzae, N. meningitidis, S. pneumoniae, E. coli, S. agalactiae)* are all encapsulated. In addition, certain capsular types are disproportionately associated with the development of meningitis. For example, about 84% of cases of neonatal meningitis caused by *E. coli* are caused by strains bearing the K1 antigen; in the absence of specific host antibody to the K1 capsule, these organisms are profoundly resistant to phagocytosis. Presence of the K1 capsule and a high degree of bacteremia are key determinants in the development of *E. coli* meningitis.[199] It has been shown that *E. coli* strain C5, which causes neonatal meningitis, harbors a pathogenicity island designated PAI I_{C5} that contributes to the pathogenicity of *E. coli* meningitis by causing a high-grade bacteremia,[200] although further studies are needed to determine the importance of this factor in the development of *E. coli* meningitis.

The host possesses several defense mechanisms to counteract the antiphagocytic effects of the bacterial capsule.[189] For example, activation of the alternate complement pathway by the capsular polysaccharide of *S. pneumoniae* results in the cleavage of C3 with attachment of C3 to the bacterial surface.[189] This series of events facilitates opsonization, phagocytosis, and intravascular clearance of the organism. *H. influenzae* type b also activates the complement cascade. Experimental studies in a rat model have shown that after intravenous or intraperitoneal challenge with *H. influenzae* of varying serotypes (a, b, c, or d), a greater incidence and magnitude of bacteremia developed in rats depleted of C3. Although the incidence of bacteremia caused by type b organisms increased from 63% to 95% in complement-depleted rats, the incidence and severity of meningitis were unaffected by complement depletion. In an experimental study of pneumococcal meningitis in mice and rats, complement inhibition by C1 inhibitor was associated with reduced clinical illness, a less-pronounced inflammatory infiltrate around the meninges, and lower brain concentrations of proinflammatory cytokines and chemokines.[201]

Complement system activation is also an essential host defense mechanism against invasive disease caused by *N. meningitidis.* Patients with deficiencies in the membrane attack complex are particularly prone to neisserial infections, although usually with a more favorable outcome when appropriate therapy is instituted.[81] The reasons for the worse outcome in patients with an intact complement system are unclear, although a qualitative relationship can be shown among the concentration of circulating meningococcal lipooligosaccharide, a fatal outcome, and the degree of complement activation.[189]

The main cascade pathways activated after bacterial invasion of the bloodstream are the complement system, the inflammatory response, and the coagulation and fibrinolytic pathways, all of which are able to interact with one another. Genetic polymorphisms are among the components of these pathways (e.g., complement deficiencies and defects in sensing or opsonophagocytic pathways), and these are involved in determining the susceptibility to infection, along with the severity of disease and outcome.[202] Cytokines coordinate a wide variety of inflammatory reactions and have important roles in the initiation, maintenance, and termination of these reactions. Prominent proinflammatory cytokines include tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6. Essential parts of the inflammatory response include activation of coagulation and fibrin deposition, which shifts the hemostatic balance toward thrombosis.[203]

N. meningitidis can directly bind to factor H (fH), which is the main regulator of alternative complement activation, through surface molecules, including fH-binding protein (fHbp), neisserial surface protein A (NspA), and porin B.[102,103,203] The increase in the environmental temperature that occurs as the bacteria change habitat from the nasopharynx to the bloodstream has been identified as a "danger signal" for *N. meningitidis,* which prompts an upregulation of capsular biosynthesis and fHbp expression, thus enhancing its capacity to withstand complement attack.[203]

Meningeal Invasion

The mechanism by which meningeal pathogens gain access to the CNS has been the subject of intensive study; bacteria may potentially enter via intercellular (between cells) or transcellular (through cells) routes, leukocyte-facilitated mechanisms, or nonhematogenous routes by retrograde transport within cranial nerves.[204] The BBB is formed by microvascular endothelial cells and restricts invasion by bloodborne pathogens. Cerebral capillaries, as opposed to other systemic capillaries, have adjacent endothelial cells fused together by tight junctions that prevent intercellular transport, the virtual absence of pinocytotic vesicles, and a large quantity of mitochondria. Nevertheless, recent insight shows that pathogens can cross the BBB transcellularly, paracellularly, in infected phagocytes (i.e., via a "Trojan-horse" mechanism), or through a combination of these routes.[205] Some of these mechanisms are discussed in detail later.

The development of a sustained, high-grade bacteremia has been suggested as one important factor that leads to meningeal invasion.[189] In one study, culture-positive meningitis was produced in an experimental infant rat model only after an intense bacteremia had been present for at least 6 hours. In an experimental rat model of pneumococcal meningitis, attenuation of the bacteremic component of pneumococcal meningitis improved clinical symptoms and significantly reduced ventricular expansion and breakdown of the BBB,[206] suggesting that systemic infection contributes to the pathophysiology of bacterial meningitis. However, sustained bacteremia cannot be the sole factor responsible for meningeal invasion, because many other organisms (e.g., viridans-group streptococci) that produce high-grade bacteremia during infective endocarditis rarely produce meningitis. Studies have shown that a high degree of bacteremia is necessary, but not sufficient, for the

development of meningitis and that microbial binding to brain microvascular cells is needed, at least in vitro.[205]

The site of CNS invasion by meningeal pathogens is also unclear. Early studies suggested that invasion from the bloodstream was via the dural venous sinus system, whereas other experiments suggested that the site of invasion was above the cribriform plate or via the choroid plexus (because of their exceptionally high rate of blood flow of approximately 200 mL/g/min).[189] Experimental studies, however, have demonstrated that receptors for some meningeal pathogens are present on cells of the choroid plexus and cerebral capillaries. In cryostat sections of infant rat brain cortical slices, *E. coli* strains possessing S fimbriae have been shown to bind specifically to the luminal surfaces of the vascular endothelium and the epithelium lining the choroid plexus and brain ventricles. Phase variation to the nonfimbriated form may then be necessary for these bacteria to invade the CNS. To understand the cellular mechanisms important for meningeal invasion, the invasion of *E. coli* into endothelial and epithelial cell cultures was studied. It appeared that microtubule-dependent and/or microfilament-dependent pathways, which rearrange the cell cytoskeleton, may be important for bacterial uptake and crossing of the BBB.[207] *N. meningitidis* was also shown to adhere in vivo to the endothelium of both the choroid plexus and the meninges in a fatal case of meningococcemia[208]; isolates obtained from the CSF expressed significantly more PilC protein than did the blood isolates, which suggests that PilC plays an important role for this organism to cross the BBB. Despite these studies, the importance of adherence of meningeal pathogens to sites within the CNS requires further investigation.

Bacterial Survival Within the Subarachnoid Space

Once meningeal pathogens enter the subarachnoid space, host defense mechanisms are generally inadequate to control the infection.[205] CSF concentrations of complement components are absent or minimal; meningeal inflammation leads to increased, but low, CSF complement concentrations. This relative complement deficiency may be of critical importance because specific antibody or complement, or both, are essential for opsonization of encapsulated meningeal pathogens and efficient phagocytosis. Observations in experimental animal models and in patients with meningitis have revealed absent or barely detectable opsonic and bactericidal activity. The explanation for this low level of complement components during bacterial meningitis is unclear. It has been suggested that degradation of complement components crossing the BBB by leukocyte proteases may result in inefficient opsonic activity at the site of infection. Indeed, in an experimental rabbit model of pneumococcal meningitis, the intracisternal inoculation of a nonspecific protease inhibitor (phenylmethylsulfonyl fluoride) led to a decline in pneumococcal concentrations in CSF when compared with saline-inoculated controls.

Immunoglobulin concentrations are also low in normal CSF (blood-to-CSF ratio of IgG of about 800:1), and although concentrations increase during bacterial meningitis, they remain low in comparison with simultaneous serum concentrations.[189] In an experimental rabbit model, the intravenous administration of a bactericidal monoclonal antibody against the polyribosylribitol phosphate of *H. influenzae* type b produced high serum antibody concentrations, but BBB permeability was poor (5.5% or less) even in the presence of meningeal inflammation, which suggests that systemic administration of type-specific antibodies alone is likely to be suboptimal in therapy for bacterial meningitis.

Bacterial meningitis is characterized by the development of a neutrophilic pleocytosis within the CSF, although the precise mechanism of leukocyte traversal across the BBB is undefined.[189] The complement component C5a has been suggested to be one chemotactic component, with chemotactic activity appearing 2 to 4 hours before neutrophil influx into CSF. In an experimental rabbit model, the intracisternal inoculation of C5a led to an influx of leukocytes into CSF 1 hour after inoculation, a response that was attenuated by coadministration of prostaglandin E_2 (PGE_2) in a dose- and time-dependent manner, which suggests a direct antiinflammatory action of PGE_2 on C5a-induced CSF pleocytosis during bacterial meningitis. CSF C5 fragment concentrations in patients with bacterial meningitis have been correlated with several indicators of poor prognosis; a common nonsynonymous C5 SNP (rs17611) is associated with unfavorable outcome.[209] Consistent with these human data, C5a receptor–deficient mice with pneumococcal meningitis had lower CSF WBC counts and decreased brain damage compared with wild-type mice. Elevated CSF concentrations of two alternate pathway complement activation proteins, C3 and factor B, have also been found in mice and in patients with bacterial meningitis. In the mouse model of *Listeria* meningitis, intrathecal synthesis of C3 and factor B occurred during the course of the disease.

Despite this early influx of leukocytes in bacterial meningitis, host defense in CSF remains suboptimal because of the lack of functional opsonic and bactericidal activity. With inefficient phagocytosis, bacteria can multiply to huge concentrations in the CSF during meningitis.

Induction of Subarachnoid Space Inflammation

The induction of a marked subarachnoid space inflammatory response by meningeal pathogens contributes to many of the pathophysiologic consequences of bacterial meningitis and therefore to significant morbidity and mortality from this disorder. The invasion of leukocytes may contribute to the deleterious effects of inflammation within the brain with subsequent development of neuronal damage.[210] Experimental studies have focused on the virulence factors of meningeal pathogens and the specific inflammatory mediators that they induce (Table 87.7), in order to learn more about the mechanisms responsible for subarachnoid space inflammation. Replication and lysis of bacteria in the subarachnoid space lead to release of bacterial virulence components (e.g., peptidoglycan from gram-positive bacteria and lipooligosaccharide from gram-negative bacteria), which triggers the inflammatory response and production and release of inflammatory cytokines and chemokines.[211]

Alterations of the Blood-Brain Barrier

Bacterial meningitis has been shown to increase permeability across the BBB, which is composed of the arachnoid membrane, choroid plexus epithelium, and cerebral microvascular endothelium; the cerebral microvascular endothelium has been the site of intensive study in bacterial meningitis.[188,189] An adult experimental rat model was used to examine the propensity for meningeal pathogens to induce functional and morphologic alterations of the BBB. After intracisternal inoculation of *E. coli, S. pneumoniae,* or *H. influenzae* type b, alterations of the BBB were found with all three pathogens and manifested morphologically with an early and sustained increase in pinocytotic vesicle formation

TABLE 87.7 Potential Factors Contributing to Subarachnoid Space Inflammation

Bacterial Factors
Cell wall components
Lipooligosaccharide
Outer membrane vesicles
Peptidoglycan
Host Factors
Prostaglandins (PGE_2, prostacyclin)
Interleukins (IL-1β, IL-6, IL-8, IL-12, IL-16)
Interferon-γ
Growth-related gene product-α
Tumor necrosis factor-α
Platelet-activating factor
Transforming growth factor-1β
Macrophage inflammatory proteins 1 and 2
Leukocyte integrins (CD18)
Leukocyte selectins
Endothelial leukocyte adhesion molecule 1
Intercellular adhesion molecule 1 (ICAM-1)
Toll-like receptors (TLR2 and TLR4)
Complement components (C5a, C5b-9)
Substance P
Myeloid differentiation primary response protein 88 (MyD88)
Nuclear factor kappa B
Reactive nitrogen intermediates
Peroxynitrite

and progressive separation of intercellular tight junctions. These morphologic changes correlated with the functional penetration of albumin, a molecule normally excluded by an intact BBB, into the CSF. Intracisternal inoculation of an unencapsulated strain of *H. influenzae* caused an increase in pinocytotic vesicle formation without separation of intercellular tight junctions, which suggests that encapsulation of *H. influenzae* was not essential for BBB injury but facilitated its progression by avoidance of host defense mechanisms. The increased BBB permeability was observed in both normal and leukopenic animals, although permeability was augmented by the presence of leukocytes. The site of BBB injury was subsequently examined by in situ tracer perfusion and immunolabeling procedures to identify the topography and microvascular exit pathways of bovine serum albumin. Exit of both perfused colloidal gold bovine serum albumin and immunodetectable bovine serum albumin was through open intercellular junctions of venules in the pia-arachnoid, thus specifically and topographically localizing the BBB injury in bacterial meningitis to the meningeal venules.

Although most adjunctive strategies aim to attenuate the severity of CSF inflammation, findings in clinical and experimental studies of bacterial meningitis suggest that the inflammatory response might also have protective effects. A low CSF leukocyte count has been associated with an increased risk for adverse outcome in adults with pneumococcal meningitis. In addition, inhibition of different mediators implicated in the inflammatory response has led to aggravation of the disease and its complications in experimental pneumococcal meningitis. Although mice deficient in MyD88, a key adapter protein in Toll-like receptor (TLR)–mediated signaling pathways, showed diminished CSF inflammation, MyD88 deficiency was associated with poor outcome because of severe pneumococcal bacteremia.[212]

Cerebral Edema and Increased Intracranial Pressure

Cerebral edema is the major element contributing to increased intracranial pressure during bacterial meningitis and may result in life-threatening cerebral herniation and other complications.[189] The origin of the cerebral edema may be vasogenic, cytotoxic, or interstitial, or any combination of the three; all three elements probably contribute to cerebral edema during bacterial meningitis. Vasogenic cerebral edema is primarily a consequence of increased BBB permeability; cytotoxic cerebral edema results from swelling of the cellular elements of the brain, most likely through release of toxic factors from neutrophils, bacteria, or both; and interstitial cerebral edema reflects obstruction of the flow of normal CSF as in hydrocephalus. The last factor has been examined in an experimental rabbit model of pneumococcal or *E. coli* meningitis in which the CSF outflow resistance (defined as factors that inhibit the flow of CSF from the subarachnoid space to the major dural sinuses) was markedly elevated and remained elevated for as long as 2 weeks despite rapid CSF sterilization with penicillin therapy. In bacterial meningitis, cytotoxic edema appears to be facilitated by aquaporin-4, which facilitates water movement into brain astroglia and water movement out of the brain in vasogenic edema.[213]

These concepts have been examined in greater detail in an experimental animal model of pneumococcal meningitis in which brain water content (indicative of cerebral edema if elevated), CSF lactate concentrations, and CSF pressure were measured.[189] All three parameters were elevated in infected animals. Although treatment with ampicillin rapidly sterilized the CSF and normalized the brain water content and CSF pressure, the CSF lactate concentration remained elevated. The bacterial virulence factor responsible for the production of brain edema was subsequently examined in an experimental animal model of *E. coli* meningitis in which therapy with cefotaxime, but not chloramphenicol, induced a marked rise in CSF endotoxin concentrations that was associated with increased brain water content. The peptidoglycan of the *H. influenzae* cell wall also induced cerebral edema without perturbing the other parameters of inflammation (e.g., increased BBB permeability), which suggests that peptidoglycan induces cytotoxic rather than vasogenic cerebral edema. Neutrophils appeared to contribute to the development of cerebral edema if adequately stimulated, although the parameters of increased intracranial pressure and increased CSF concentrations of lactate and protein were unrelated to the presence of neutrophils. However, this area remains controversial because neutrophils are required for the increased BBB permeability seen in response to the intracisternal inoculation of bacterial virulence factors and inflammatory mediators.

Variability among bacterial strains may also be an important determinant in production of the subarachnoid space inflammatory response and brain edema in bacterial meningitis. Intracisternal inoculation of three different pneumococcal isolates resulted in pronounced differences in the pathophysiologic profiles 24 hours after challenge.[189] When pneumococcal cell wall fragments were inoculated intracisternally, the chemical composition of the fragments, specifically the degree of teichoication, influenced the induction of brain edema. In a subsequent study in an experimental rabbit model, serotype-specific characteristics of pneumococci were found to play a major role in the subarachnoid space inflammatory process, although significant differences in brain water content were observed only with one of the serotypes tested. It is unclear, however, whether these differences affect the clinical expression of disease in patients with bacterial meningitis.

The infusion of hypertonic mannitol to treat increased intracranial pressure has been evaluated in a rabbit model of *H. influenzae* type b meningitis.[189] In all animals, mannitol consistently reduced intracisternal pressure, although the magnitude of reduction was greater in infected animals, and brain water content was no different in mannitol-treated animals than in untreated ones. In contrast, in an experimental rat model of pneumococcal meningitis, mannitol modulated changes in cerebral blood flow, intracranial pressure, and brain water content, perhaps by a mechanism of scavenging hydroxyl radicals, which have been shown to be involved in the pathogenesis and pathophysiology of cerebral ischemia and neuronal injury in bacterial meningitis (see later discussion). In an experimental rabbit model of bacterial meningitis, use of adjunctive 3% hypertonic saline significantly elevated mean arterial pressure, reduced intracranial pressure, greatly improved cerebral perfusion pressure, inhibited brain aquaporin 4 expression, reduced cerebral edema, and attenuated brain damage with a superior effect over 20% mannitol.[214] Use of adjunctive glycerol in infant rats and adult mice with pneumococcal meningitis was not found to be beneficial.[215]

Alterations in Cerebral Blood Flow

Bacterial meningitis exerts profound effects on blood vessels that course through the subarachnoid space,[189] and the resulting vasculitis leads to narrowing or thrombosis of cerebral blood vessels and the propensity for ischemia and/or infarction of underlying brain. In combination with increased intracranial pressure, these changes may result in altered cerebral blood flow in patients with bacterial meningitis. An infant rhesus monkey model of *H. influenzae* meningitis demonstrated that cerebral cortical hypoperfusion occurs during meningitis and causes relative cerebral anoxia. Cerebrovascular autoregulation, in which cerebral blood flow is unchanged despite alterations of blood pressure over a wide range, is also lost during experimental bacterial meningitis. Furthermore, studies in an experimental rabbit model of pneumococcal meningitis demonstrated that animals given a lower intravenous fluid regimen (50 mL/kg/24 h) of normal saline had a lower mean arterial pressure, lower cerebral blood flow, and a higher concentration of CSF lactate than did animals that received a higher fluid regimen (150 mL/kg/24 h). In the first 4 to 6 hours of antibiotic administration, rabbits receiving lower fluid regimens had a significant decrease in mean arterial pressure and cerebral blood flow and a significant increase in CSF lactate concentrations when compared with rabbits receiving higher fluid regimens. These results, in combination with other experimental studies in which an increase in cerebral blood flow was noted within the first few hours of intracisternal inoculation of either live pneumococci or pneumococcal cell wall fragments, suggest that maintenance of adequate intravascular volume and minimization of stimuli that increase systemic blood pressure may be important in the treatment of bacterial meningitis. These findings may also be of potential clinical relevance inasmuch as inadvertent increases in mean arterial pressure directly increase cerebral blood flow and intracranial pressure, and depletion of intravascular volume with decreases in mean arterial pressure can cause parallel decreases in cerebral blood flow and a reduction in substrate delivery

to the brain. Therefore the brain is at risk from either hypoperfusion or hyperperfusion. As demonstrated by near infrared spectroscopy in conjunction with measurement of cerebral blood flow in an experimental rabbit model of pneumococcal meningitis, infected animals had a relative increase in the deoxygenated hemoglobin fraction and a decrease in the oxygenated hemoglobin fraction, thus supporting the possibility of cerebral venous engorgement in bacterial meningitis, which may contribute to intracranial hypertension in this disorder.

Cerebral blood flow has been measured in patients with bacterial meningitis. During the early phases of the disease, there is an increase in blood flow, whereas in advanced disease, cerebral blood flow is reduced.[216] In an early study,[189] measurement of cerebral blood flow (by the xenon 133 intraarterial injection method) revealed a 30% to 40% reduction in average total blood flow in five patients with pneumococcal meningitis (mean age, 54 years), but not in five patients with meningococcal meningitis (mean age, 20 years). An inverse relationship between cerebral blood flow and intracranial pressure has been observed in infants with bacterial meningitis; among eight patients, alterations were noted only in the four older infants (age range, 3–10 months) and not in the four neonates (age range, 5–30 days) in whom no changes in cerebral blood flow velocity were detected. In another study of 17 children (aged 8 days to 6 years) with bacterial meningitis, transcranial Doppler ultrasound monitoring demonstrated an improvement in cerebral blood flow velocity with resolution of meningitis. This observation suggests that in the early phase of bacterial meningitis, increased cerebrovascular resistance may contribute to a relative impairment in cerebral perfusion.

In a subsequent study in 20 children seriously ill with bacterial meningitis, total and regional cerebral blood flow measured with stable xenon computed tomography (CT) revealed a global decrease in flow and even more regional variability.[189] Although autoregulation of cerebral blood flow was preserved in the patients studied, hyperventilation reduced flow below the ischemic threshold, which raises important concerns about the routine use of hyperventilation in the management of increased intracranial pressure in patients with bacterial meningitis. In contrast, in another study of 9 patients with bacterial meningitis, 8 of whom had impaired cerebral blood flow autoregulation, autoregulation was partially or completely restored in 6 of 8 patients by short-term hyperventilation[217]; this recovery may protect the brain from fluctuations in perfusion pressure. In another study of 86 adult patients with bacterial meningitis, cerebral angiography was performed in 27 patients who had focal deficits (clinically, on cranial CT, or both) and who had persistent coma without an explained cause despite 3 days of antimicrobial therapy.[218] Thirteen of the patients who underwent angiography had alterations in their blood vessel system; the prognosis in these patients was poor. However, definitive changes in cerebral blood flow during bacterial meningitis are controversial and may vary with the stage of disease. These blood flow alterations may lead to regional hypoxia, increased lactate concentrations in the brain secondary to utilization of glucose by anaerobic glycolysis, and CSF acidosis, which may be a precursor to encephalopathy.[189]

CLINICAL MANIFESTATIONS

Viral Meningitis

Enteroviruses

The clinical manifestations of enteroviral meningitis depend on host age and immune status.[24,219,220] In neonates with proven enteroviral meningitis, fever is a ubiquitous finding and is usually accompanied by any combination of vomiting, anorexia, rash, and upper respiratory tract symptoms and signs. Neurologic involvement may be associated with nuchal rigidity and a bulging anterior fontanelle, although infants younger than 1 year are less likely to demonstrate meningeal signs. Mental status may be altered, but focal neurologic signs are uncommon.[220,221] A more severe form of meningoencephalitis may be seen in neonates, who appear to be at greatest risk for morbidity and mortality, particularly when symptoms and signs develop during the first day of life (after presumed transplacental transmission of the virus). With disease progression, a sepsis-like syndrome characterized by multiorgan involvement (e.g., hepatic necrosis, myocarditis, and necrotizing enterocolitis), disseminated intravascular coagulation, and cardiovascular collapse may develop. Findings in the CNS include seizures and focal neurologic signs. Lack of humoral antibody may contribute to the severity of neonatal infection.

The findings in neonates contrast to the clinical findings of enteroviral meningitis beyond the neonatal period (>2 weeks), in which severe disease and poor outcome are rare.[24,222] Infants usually present with fever, irritability, feeding difficulties, and rash; the majority of them have a good clinical outcome.[222] Approximately one-third of patients have stiff neck, and less than 2% of patients present with altered mental status.[30] A study of 187 children with meningitis identified the following variables to be associated with a positive CSF EV PCR assay result: May to November presentation, a CSF protein concentration <100 mg/dL, and normal findings on neurologic examination.[223] If ≤1 variable was present, the probability of having EV was 0%. This model could aid in deciding when to test patients with meningitis with an CSF EV PCR.

Headache is nearly always present in adults; photophobia is seen in approximately one-third of patients with EV meningitis.[30] Nonspecific symptoms and signs include vomiting, anorexia, rash, diarrhea, cough, upper respiratory tract findings (especially pharyngitis), and myalgias. Other clues to the presence of enteroviral disease include the time of year (more prevalent in the summer and autumn months), known epidemic disease in the community, exanthems, myopericarditis, conjunctivitis, and specifically recognizable enteroviral syndromes such as pleurodynia, herpangina, and hand-foot-and-mouth disease.[220] In addition, specific clinical stigmata may be associated with certain enteroviral serotypes.[220] For example, echovirus 9 is associated with scattered maculopapular rashes. Herpangina, in particular the finding of painful vesicles on the posterior oropharynx, is associated with coxsackievirus A; the presence of pericarditis or pleurisy may identify coxsackievirus B. The duration of illness in enteroviral meningitis is usually less than 1 week, with many patients reporting improvement after lumbar puncture, presumably from reduction in intracranial pressure. In contrast, during an outbreak of EV 71 infection in Taiwan in patients 3 months to 8.2 years of age, the chief neurologic complaint was rhombencephalitis (seen in 90% of children), which carried a case-fatality rate of 14%.[224] In another outbreak in young children with EV 71 infection in Perth, Western Australia,[225] neurologic syndromes included aseptic meningitis, Guillain-Barré syndrome, acute transverse myelitis, acute cerebellar ataxia, opsomyoclonus syndrome, benign intracranial hypertension, and febrile convulsions; this contrasts to previous epidemics, in which encephalitis was the most frequent syndrome of neurologic disease. In one prospective clinical study, brainstem encephalitis (which included signs such as myoclonic jerks, tremor or ataxia, cranial nerve palsies evident from eye movement disorders, facial weakness, and bulbar palsy) was the most frequent (58% of neurologic manifestations), followed by aseptic meningitis (36% of neurologic manifestations).[226]

A unique clinical situation is seen in children and adults with absent or deficient humoral immunity that impairs clearance of EVs. In persons who are agammaglobulinemic, a chronic enteroviral meningitis or meningoencephalitis may develop and last several years, often with a fatal outcome[219,220]; this syndrome has been designated chronic enteroviral meningoencephalitis in agammaglobulinemia (CEMA).[227] CEMA can manifest with headache, seizures, hearing loss, lethargy or coma, weakness, ataxia, paresthesias, loss of cognitive skills, and quadriplegia and can be fatal. Extraneurologic manifestations may include a chronic skin rash, fever, arthralgias, hepatitis, myositis, peripheral edema, endocrinopathies, retinopathy, and myocarditis.

Herpesviruses

Meningitis associated with HSV-2 infection is usually characterized by stiff neck, headache, and fever.[19,24] HSV-2 is the main cause of recurrent benign lymphocytic meningitis (Mollaret meningitis), which can develop in up to 10 recurrent episodes of meningitis that last 2 to 5 days followed by spontaneous recovery.[20] In sharp contrast to encephalitis, acyclovir has not been shown to improve outcomes in meningitis, a benign illness. The majority of patients are women in their fourth decade of life who have no active or history of genital herpes. HSV-1 and EBV can also occasionally cause recurrent meningitis.[20] VZV is the second most common herpesvirus causing aseptic meningitis, with the majority of cases occurring without the typical vesicular rash (zoster sine herpete).[228]

An acute presentation with a mononucleosis-like picture with rash, pharyngitis, lymphadenopathy, and splenomegaly should suggest EBV infection.[24]

West Nile virus

Neuroinvasive disease develops in approximately 1% of patients with WNV infections during the summer months in the United States.[27] Patients can present with meningitis, encephalitis, or AFP; up to 50% of patients with encephalitis have concomitant chorioretinitis.[229] Patients with meningitis typically present with fever, headache, nausea, vomiting, stiff neck, photophobia, and occasionally a maculopapular rash.[230] In addition, patients may have persistent headaches, abnormal neurologic examination findings, neurocognitive impairment, and chronic fatigue years after infection.[231,232]

Mumps Virus

In patients with mumps, CNS symptoms usually follow the onset of parotitis, when present, by about 5 days. The most frequent clinical manifestation of mumps CNS infection is the triad of fever, vomiting, and headache (Table 87.8).[233] The fever is usually high and lasts for 72 to 96 hours. Salivary gland enlargement is present in only about 50% of patients. Other findings include neck stiffness, lethargy or somnolence, and abdominal pain. Most patients have signs of meningitis but no evidence of cortical dysfunction. Defervescence is usually accompanied by clinical recovery, and in uncomplicated cases the total duration of illness is 7 to 10 days. Rarely, mumps may cause encephalitis, seizures, polyradiculitis, polyneuritis, cranial nerve palsies, myelitis, Guillain-Barré syndrome, and death.

Bacterial Meningitis

Patients with bacterial meningitis classically present with fever, headache, meningismus, and signs of cerebral dysfunction (i.e., confusion, delirium, or a declining level of consciousness ranging from lethargy to coma) (Table 87.9).[60] However, clinical presentation may vary based on age and underlying disease status and as a result of infection by specific bacterial pathogens.

TABLE 87.8 Presenting Symptoms and Signs in Patients With Central Nervous System Mumps

SYMPTOM OR SIGN	RELATIVE FREQUENCY (%)
Fever	88–100
Vomiting	68–94
Headache	47–88
Salivary gland swelling	47–62
Meningismus	43–93
Lethargy	28–69
Abdominal pain	14–23
Seizures	14–18

Modified from Gnann JW Jr. Meningitis and encephalitis caused by mumps virus. In: Scheld WM, Whitley RJ, Marra CM, eds. Infections of the Central Nervous System. *4th ed. Philadelphia: Lippincott Williams & Wilkins; 2014.*

TABLE 87.9 Presenting Symptoms and Signs in Patients With Bacterial Meningitis

SYMPTOM OR SIGN	RELATIVE FREQUENCY (%)
Headache	≥85
Fever	≥80
Meningismus	≥80
Altered sensorium	≥75
Vomiting	Approximately 35
Seizures	Approximately 30
Focal neurologic findings	10–35
Papilledema	<5

Neonates, Infants, and Children

The symptoms and signs of acute bacterial meningitis in neonates, infants, and children depend on the age of the child, duration of illness, and host response to infection[64,234]; the clinical manifestations can be subtle, variable, nonspecific, and even absent. For example, neonates with bacterial meningitis usually do not have meningismus.[235] Clinical clues to the presence of meningitis in neonates are temperature instability (hypothermia or hyperthermia), listlessness, high-pitched crying, fretfulness, lethargy, refusal to feed, weak suck, irritability, jaundice, vomiting, diarrhea, or respiratory distress. A change in the child's affect or state of alertness is one of the most important signs of meningitis. A bulging fontanelle (seen in one-third of cases in neonates) usually occurs late during the course of illness; seizures are observed in 40% of neonates with bacterial meningitis. In children 1 to 4 years of age, fever, vomiting, and nuchal rigidity are the most common initial symptoms.[234]

In a systematic review of 10 studies of prospective data on clinical manifestations suggestive of acute meningitis in children, bulging fontanelle, neck stiffness, seizures, and reduced food intake raised concerns for the presence of meningitis.[236] In children, the absence of meningeal signs and presence of an abnormal cry independently lowered the likelihood of meningitis, although the absence of fever did not exclude the diagnosis. In one review of children aged 2 months to 15 years who presented with suspected meningitis, the classic clinical signs had limited value in establishing the diagnosis.[237] Clinical examination revealed nuchal rigidity in 65% of those with meningitis; Brudzinski and Kernig signs (see later discussion) were elicited in 51% and 27% of those with meningitis, respectively. Accordingly, physicians should have a low threshold for performing lumbar puncture in patients at high risk for bacterial meningitis, given the serious nature of this disease.

Adults

In a review of 493 cases of acute bacterial meningitis in adults,[71] the triad of fever, nuchal rigidity, and change in mental status was found in only two-thirds of patients, but all had at least one of these findings. In a study of 1412 episodes of community-acquired bacterial meningitis in the Netherlands, the triad of fever, neck stiffness, and altered mental status was present in only 41% of episodes,[60] with the most common clinical manifestations being headache (83%), neck stiffness (74%), fever (74%), altered mental status (71%), and nausea (62%). The meningismus may be subtle, marked, or accompanied by the Kernig or Brudzinski sign or both.[238] The Kernig sign is elicited with the patient supine, with the thigh flexed on the abdomen and the knee flexed. The leg is then passively extended, and in the presence of meningeal inflammation, the patient resists leg extension. This technique differs somewhat from the maneuver as first described by Kernig, in which the patient was initially seated. Several signs were described by Brudzinski, although the best known is the nape-of-the-neck sign, in which passive flexion of the neck results in flexion of the hips and knees. However, in a prospective study of 297 adults with suspected meningitis, the diagnostic accuracy of meningeal signs was poor; both Kernig and Brudzinki signs had a 5% sensitivity, and nuchal rigidity a 30% sensitivity,[239] indicating that they did not accurately distinguish patients with meningitis from those without meningitis. Therefore the absence of these findings does not rule out the diagnosis of bacterial meningitis. Cranial nerve palsies (especially those involving cranial nerves III, IV, VI, and VII) and focal neurologic deficits (aphasia, hemiparesis, monoparesis) are seen in 9% and 22% of cases, respectively.[47] Cranial nerve palsies probably develop as the nerve becomes enveloped by exudate in the arachnoid sheath surrounding the nerve, or they may be a sign of increased intracranial pressure. Seizures occur in 14% of patients.[47] Focal neurologic deficits and seizures arise from cortical and subcortical ischemia, which results from inflammation and thrombosis of blood vessels, often within the subarachnoid space. In a study of 696 patients with community-acquired bacterial meningitis, cerebral infarction occurred in 174 (25%) episodes and was seen in 128 (36%) of 352 patients with pneumococcal meningitis[240]; an unfavorable outcome occurred in 62% of patients with cerebral

infarction. Diffuse cerebral disseminated intravascular coagulation may be another explanation for cerebral infarction complicating pneumococcal meningitis.[241] Papilledema is seen in less than 5% of cases early in infection, and its presence should suggest an alternative diagnosis. Hydrocephalus complicates 5% of episodes of community-acquired bacterial meningitis in adults and is associated with a high case-fatality rate.[242] With disease progression, signs of increased intracranial pressure may develop, including coma, hypertension, bradycardia, and palsy of cranial nerve III. Endocarditis complicates 2% of episodes of community-acquired bacterial meningitis in adults and is also associated with a high case-fatality rate[243]; clues suggesting the diagnosis of endocarditis in patients with bacterial meningitis are cardiac murmurs, persistent or recurrent fever, a history of heart valve disease, and *S. aureus* as the causative pathogen.

To further characterize the accuracy and precision of the clinical examination in adult patients with acute meningitis, 845 episodes of acute meningitis (confirmed with lumbar puncture or autopsy) in patients aged 16 to 95 years were reviewed[244]; the majority of patients in this review had acute bacterial meningitis, although 62 had tuberculous or "aseptic" meningitis. The results demonstrated that individual items of the clinical history (i.e., headache, nausea, and vomiting) had a low accuracy for the diagnosis of acute meningitis in adults. However, on review of the accuracy of physical examination findings, the absence of fever, neck stiffness, and altered mental status effectively eliminated the likelihood of acute meningitis; the sensitivity was 99% to 100% for the presence of one of these findings in the diagnosis of acute meningitis.

Older Adults

The clinical presentation in older adults differs significantly from that younger adults and represents a diagnostic challenge that can delay appropriate therapy.[245–247] In a study of 619 adults with community-acquired meningitis, elderly patients were less likely to complain of headache, stiff neck, photophobia, and nausea, but more likely to present with fever or with abnormal neurologic examination findings.[246] Elderly patients also had higher rates of abnormal cranial imaging findings and worse clinical outcomes. In another study of 696 adults with community-acquired bacterial meningitis, older adults were also less likely to complain of headache and stiff neck, but more likely to have sepsis and abnormal neurologic examination findings.[247] In a study of 185 patients 65 years of age and older, the diagnosis of community-acquired bacterial meningitis was more difficult because of the absence of characteristic meningeal signs[233]; compared with adult patients younger than 65 years, older patients showed greater neurologic severity, with a high number presenting with coma on admission, seizures, and hemiparesis. In a study of 160 adults with health care–associated ventriculitis or meningitis, older patients had more comorbidities, altered mental status, and worse CSF abnormalities (higher CSF pleocytosis and protein, and lower CSF glucose concentrations).[248]

Underlying Conditions

In patients with head trauma, the symptoms and signs of meningitis may be present as a result of the underlying injury and not meningitis.[70] In patients who have sustained a basilar skull fracture in which a dural fistula is produced between the subarachnoid space and the nasal cavity, paranasal sinuses, or middle ear, a common finding is rhinorrhea or otorrhea secondary to a CSF leak[70]; in these patients, meningitis may be recurrent and is most commonly caused by *S. pneumoniae.* Fortunately, the incidence of posttraumatic CSF leaks in patients with head trauma is low (0.1%–0.2%)[249]

The diagnosis of bacterial meningitis in neutropenic patients requires a high index of suspicion, because symptoms and signs may initially be subtle because of the impaired ability of the patient to mount a subarachnoid space inflammatory response.[189] In all of these subgroups of patients, altered or changed mental status should not be ascribed to other causes until bacterial meningitis has been excluded with CSF examination.

Pathogen-Specific Findings

A specific etiologic diagnosis in patients with bacterial meningitis may be suggested by certain symptoms or signs.[189] About 50% of patients with meningococcemia, with or without meningitis, present with a prominent rash located principally on the extremities. Early in the course of illness, the rash is typically erythematous and macular, but it quickly evolves into a petechial phase with further coalescence into a purpuric form. The rash often matures rapidly, with new petechial lesions appearing during the physical examination. In one review of the clinical manifestations of 255 patients with acute meningococcal meningitis,[250] a petechial rash was observed in three-fourths of the patients; the rash was more commonly seen in children and adults younger than 30 years (81%) than in patients 30 years and older (62%). However, others have observed a rash to be present in only up to 26% of cases and (if present) to be more likely to be scanty or more atypical than that seen in patients with meningococcal septicemia.[251] A similar rash may also be seen in splenectomized patients with rapidly overwhelming sepsis caused by *S. pneumoniae, H. influenzae* type b, or *Capnocytophaga canimorsus* (seen after a dog bite).

Patients with *L. monocytogenes* meningitis have an increased tendency to have seizures and focal deficits early in the course of infection, and some patients may present with ataxia, cranial nerve palsies, or nystagmus secondary to rhombencephalitis[117–119]; however, patients with *Listeria* meningitis may not present with any focal signs. In a large review of CNS infections caused by *L. monocytogenes,*[118] the most frequent findings were fever, headache, and altered sensorium but 42% had no meningeal signs. In a nationwide French study, a total of 818 cases of listeriosis were analyzed of which 252 (31%) were neurolisteriosis.[252] The majority of patients with neurolisteriosis presented with encephalitis (87%), with brainstem involvement in 17%. The most common findings were nuchal rigidity (65%), aphasia (19%), seizures (18%), and focal limb weakness (12%). Finally, another large study of 375 patients with neurolisteriosis in the Netherlands documented that mortality has remained high, especially in older patients and those with concomitant bacteremia.[54]

Spirochetal Meningitis

Treponema pallidum

The clinical manifestations of neurosyphilis that have been described are based on studies compiled before the availability of penicillin, and it is not known whether the clinical findings of symptomatic neurosyphilis have been modified in the antibiotic era or by associated HIV infection.[166] Although the clinical manifestations of neurosyphilis are numerous, only patients with acute meningitis and meningovascular syphilis are discussed here.

Patients with syphilitic meningitis usually present in a manner similar to that of patients with other forms of aseptic meningitis—that is, with complaints of headache, nausea, and vomiting. In one series, these complaints were present in 91% of patients.[237] Meningismus occurred in 59% and fever in less than half of the patients with syphilitic meningitis. Seizures occurred in 17% of patients, whereas cranial nerve palsies were found in 45% of cases (most commonly cranial nerves VII and VIII, followed by II, III, VI, and V). Focal abnormalities such as hemiplegia, aphasia, and mental status changes were seen less commonly. Syphilitic meningitis rarely affects the spinal cord.

Meningovascular syphilis is clinically distinguished from syphilitic meningitis temporally and on the basis of focal neurologic findings as a result of focal syphilitic arteritis, which almost always occurs in association with meningeal inflammation.[166] Most patients experience weeks to months of episodic prodromal symptoms and signs, including headache or vertiginous episodes, personality changes (e.g., apathy or inattention), behavioral changes (e.g., irritability or memory impairment), insomnia, or seizures. Focal deficits, which reflect episodes of ischemia in regions of the brain with involved blood vessels (usually in the distribution of the middle cerebral artery), may also occur; if untreated, these deficits may progress to a stroke syndrome with attendant irreversible neurologic deficits.

Coinfection with HIV may modify the clinical spectrum of syphilis. Case reports and small series have suggested that patients with HIV infection are more likely to progress to develop neurosyphilis and have accelerated disease courses.[167–169] However, few clinical data currently support these hypotheses. In one study of HIV-infected and HIV-uninfected patients with syphilis at sexually transmitted disease clinics

in Baltimore,[253] no significant differences were observed in clinical stage or in disease progression.

Neuroborreliosis

Neuroborreliosis due to *B. burgdorferi, Borrelia garinii,* or *Borrelia afzelii* is identified in approximately 15% of infected individuals.[254] Meningitis is the most common neurologic manifestation of acute disseminated Lyme disease, usually following erythema migrans by 2 to 10 weeks[170]; however, only about 40% (range, 10%–90%) of cases of Lyme meningitis are preceded by this characteristic rash.[170,171] Headache is the single most common symptom (30%–90% of patients) in Lyme meningitis, whereas neck stiffness is seen in only 10% to 20% of cases. Photophobia, nausea, and vomiting are intermediate in frequency between headache and neck stiffness. About two-thirds of patients have accompanying systemic symptoms, including malaise, fatigue, myalgias, fever, arthralgias, and involuntary weight loss. In untreated cases, the duration of symptoms ranges from 1 to 9 months. Patients typically experience recurrent attacks of meningeal symptoms lasting several weeks and alternating with similar periods of milder symptoms.[5,66,170,171,254]

About half of patients with Lyme meningitis have mild cerebral symptoms consisting most commonly of somnolence, emotional lability, depression, impaired memory and concentration, and behavioral symptoms.[170,254] These symptoms may fluctuate in severity in untreated patients before resolution. Transverse myelitis, spastic paraparesis or quadriparesis, disturbances of micturition, and Babinski sign are also reported during this stage. Approximately 50% of patients also have cranial neuropathies. Facial nerve palsy is the most common (80%–90%) of the cranial nerve palsies overall and occurs with rapid onset (often in 1–2 days), frequently accompanied by slight ipsilateral facial numbness or tingling or ipsilateral ear or jaw pain. The facial palsy is bilateral in 30% to 70% of cases, although the two sides are affected asynchronously in most cases. Other cranial nerves affected less commonly are cranial nerves II and III, the sensory portion of V, VI, and the acoustic portion of VIII. Recovery usually takes place within 2 months. In a large study of 431 patients with neuroborreliosis in Denmark, residual symptoms were seen in 28% and were associated with a delay in therapy.[254]

Protozoal and Helminthic Meningitis

Amebas

PAM occurs in two forms.[169–171] The acute form (incubation period, 3–8 days) is characterized by the sudden onset of high fever, photophobia, bifrontal or bitemporal headache, nuchal rigidity, and progression to stupor or coma and is usually indistinguishable from acute bacterial meningitis, although focal neurologic signs and seizures are more common in PAM. A review of 138 cases of PAM in the United States from 1962 to 2015 showed that the mortality rate was 97%; a few recent cases have been described in lakes in Minnesota (a result of global warming) and related to tap water.[255] Because of early involvement of the olfactory area, early symptoms of abnormal smell or taste may be reported. Confusion, irritability, and restlessness progress to delirium, stupor, and, finally, coma. Death in untreated patients generally occurs within 2 to 3 days from the onset of symptoms. The rapid progression of infection presents challenges to identify *N. fowleri* and initiate therapy before the onset of severe symptoms and death.[172] Therefore the key to early diagnosis is physician awareness and clinical suspicion.[256,257]

In contrast, the subacute or chronic form of PAM manifests more insidiously with low-grade fever, headache, and focal signs (e.g., hemiparesis, aphasia, cranial nerve palsies, visual field disturbances, diplopia, ataxia, seizures)[169,170]; the olfactory bulbs are usually spared. Deterioration occurs over a period of 2 to 4 weeks until death. However, longer durations of illness have also been reported (range, 5–18 months).

Angiostrongylus cantonensis

Symptoms of meningitis begin 6 to 30 days (typically 1–2 weeks) after the ingestion of raw mollusks or other sources of the parasite.[176–178] Findings include severe headache (90%), stiff neck (56%), paresthesias (54%), and vomiting (56%). Moderate fever is present in about half of the cases. In a recent outbreak in 12 people with a common foodborne exposure in Jamaica, the mean incubation period was 11 days (range, 6–31 days).[180] Headache was the most frequent symptom. Visual disturbances or photophobia were seen in 92% of patients, and nuchal rigidity or neck pain and fatigue were present in 80% of cases. Hyperesthesias or paresthesias were present in 75% of patients in a patchy distribution of the extremities or trunk and did not correspond to any dermatomal pattern. In a review of 18 cases in Hawaii from 2001 to 2005, 94% of patients had headache and 65% had sensory symptoms consisting of paresthesias, hyperesthesias, and/or numbness; those symptoms lasted a median of 17 and 55 days, respectively.[258] Disease usually resolves spontaneously after 1 to 2 weeks, but headaches and paresthesias can persist for weeks to months.[178]

DIAGNOSIS

The characteristics of diagnostic tests for categories of pathogens in patients with suspected meningitis are described here. It is important to note that many patients with community-acquired meningitis present with a negative Gram stain, largely because aseptic meningitis is so common.[2] The spectrum of causes in these patients is broad and, besides CSF culture for bacterial pathogens, molecular diagnostics using PCR has been developed to identify bacteria, viruses, and fungi. As individual PCRs for each pathogen in the differential diagnosis may be time consuming and expensive, several biotech companies have developed multiplex PCR panels with rapid turnover times. Developed multiplex assays include the BioFire FilmArray, Fast track, Seegene, and Taqman Array Card. The most studied and US Food and Drug Administration (FDA)-approved platform is the BioFire Meningitis/Encephalitis panel that detects six bacteria (*S. pneumoniae, N. meningitidis, S. agalactiae, H. influenzae, L. monocytogenes,* and *E. coli* K1), seven viruses (HSV-1 and HSV-2, HHV-6, CMV, EV, parechovirus, and VZV), and two fungi (*Cryptococcus gattii*/*Cryptococcus neoformans*); the test uses 0.2 mL of CSF and can provide results in 1 hour.[22,259–261] A strategy in which the panel was used in patients with meningitis with a negative Gram stain resulted in an increase of 22.9% in diagnoses rendered, mostly commonly viral pathogens, but also two cases with *S. pneumoniae* and a case of *C. gattii*/*C. neoformans*. However, 15.2% (5 of 33) of FA ME–negative isolates were positive according to standard assays (four cases of WNV and a case of *Histoplasma capsulatum*, pathogens not included in the panel).[259] In a retrospective analysis of CSF from HIV patients with cryptococcosis in Uganda, the test was considered useful in distinguishing culture-positive relapse from culture-negative immune reconstitution syndrome.[262] A multicenter prospective study of 1560 patients tested with the panel showed a high sensitivity and specificity for the 14 pathogens in the panel.[22] Of all of the assays, the most comprehensive one is the TaqMan Array Card, which includes 21 pathogens: 2 parasites (*Balamuthia mandrillaris* and *Acanthamoeba*), 6 bacteria (*S. pneumoniae, H. influenzae, N. meningitidis, Mycoplasma pneumoniae, Mycobacterium tuberculosis,* and *Bartonella*), and 13 viruses (parechovirus, dengue virus, Nipah virus, VZV, mumps virus, measles virus, lyssavirus, HSV-1 and HSV-2, EBV, EV, CMV, and chikungunya virus). These panels are probably very useful for ruling disease in, but are likely to be of limited value when negative. None of the panels so far have been studied in a randomized controlled trial to show effectiveness or in a prospective cost-effectiveness study.

Viral Meningitis

Cerebrospinal Fluid Examination

CSF pleocytosis is almost always present in patients with enteroviral meningitis, although some EVs have been isolated from young infants with clinical evidence of meningitis but no CSF WBCs.[220,260] A study of 390 patients with enteroviral meningitis showed that 16% to 18% of children and 68% to 77% of neonates had no CSF pleocytosis; younger age, lower serum WBC counts, and shorter duration of symptoms prior to the lumbar puncture were predictors for lack of CSF pleocytosis.[260] The cell count is usually 100 to 1000/mm,[3] although counts in the several thousands have also been reported.[220] EV can manifest with a neutrophilic pleocytosis in 39% of patients.[261] If a repeat lumbar puncture is done more than 8 hours later, the CSF WBC differential may change to a lymphocytic pleocytosis,[265] but this practice is done currently in only 0.5% of patients with a viral CNS infections.[261] However, in a recent retrospective chart review of 158 cases of meningitis (138 aseptic and 20 bacterial), 51% of the 53 patients with aseptic meningitis and duration

of symptoms of more than 24 hours had a neutrophil predominance in CSF,[266] suggesting that a CSF neutrophil predominance is not useful as a sole criterion in distinguishing between aseptic and bacterial meningitis. In addition, in one study of 65 infants younger than 3 months of age with enteroviral meningitis, 60% had no CSF pleocytosis.[267] This was also observed in another study of 60 patients, 23 of whom had no CSF pleocytosis[268]; those who lacked CSF pleocytosis were younger, had experienced more drowsiness, had lower peripheral WBC counts, and had a higher serum CRP concentration. Elevated CSF protein and decreased CSF glucose concentrations, if present, are usually mild, although extreme degrees of both have been reported. A specific virologic diagnosis of enteroviral meningitis depends on isolation of the virus from the CSF in tissue culture,[269] although the sensitivity for enteroviral serotypes is only 65% to 75%, largely a result of the inability to grow many coxsackievirus A serotypes, which require suckling mouse inoculations.[220,269] The difficulty in isolation of EVs from CSF may also relate to the low titers of EV in CSF (as low as a median tissue culture infective dose of 10 to 10^3/mL of CSF) and that no single cell line is optimal for the detection of all members of the genus.[220] Furthermore, the time required for identifying an EV from CSF using cell cultures is too long to be of clinical usefulness in establishing the diagnosis; the mean time for CSF EVs to grow is 3.7 to 8.2 days. In one study of viral cultures on 22,394 CSF samples, virus was recovered from only 5.7% of samples, most of which (98.4%) were EVs,[270] indicating that CSF viral cultures are insensitive for the diagnosis of viral meningitis. Although isolation of a nonpolio EV from the throat or rectum of a patient with aseptic meningitis is suggestive of an etiologic diagnosis, the mean shedding periods from those sites after infection are 1 week and several weeks, respectively. In addition, viral shedding can occur in 7.5% of healthy controls during EV epidemics.[220] Therefore shedding from a past infection cannot be ruled out. Furthermore, a study found that non-CSF viral cultures were not helpful in predicting enteroviral CNS infection, inasmuch as EVs were isolated at the same frequency from non-CSF sites in infants in whom EVs were cultured from CSF as in hospitalized infants with an acute illness whose CSF was negative. Follow-up acute and convalescent serologic testing for the specific isolated strain may confirm the etiologic diagnosis.[220] MRI of patients with rhombencephalitis has demonstrated high-intensity lesions in the brainstem, most commonly located in the tegmentum.[220]

Rapid diagnosis of EV infection through immunoassay techniques has been hampered by the lack of a common antigen among the various serotypes and the low concentrations of virus in body fluids.[17,220] Nucleic acid amplification tests, such as the PCR assay, have mostly replaced viral culture for the diagnosis of enteroviral meningitis. All the primers are directed at highly conserved regions of the 5′-noncoding region of the viral genome and designed for reverse transcription combined with PCR. Enteroviral reverse-transcriptase polymerase chain reaction (RT-PCR) has been tested in clinical settings by numerous investigators and found to be more sensitive than culture for the detection of the EV; the sensitivity has ranged from 86% to 100% and specificity from 92% to 100% for the diagnosis of enteroviral meningitis.[271–273] The Xpert enterovirus assay for detection of enteroviral RNA had a sensitivity of 94.7%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 98.2% for the diagnosis of enteroviral meningitis.[274] In addition, the time to identification of the EV with RT-PCR is significantly reduced (hours to a day) compared with cell culture, which may lead to shortened patient hospitalization, less use of antimicrobial agents for treatment of presumptive bacterial meningitis, and reduction of the need for ancillary diagnostic tests.[275] In one study in children with enteroviral meningitis with use of rapid enteroviral molecular tests (results are available within 3–24 hours), the median duration of hospitalization and duration of antimicrobial therapy were reduced to 2 days and 1 day, respectively.[276] EV-specific PCR tests do not detect HPeV, so testing for HPeV-specific PCR is required to detect these agents as a cause of viral meningitis.[22] In one study, HPeV-3 was detected in the CSF with PCR.[277] HPeV testing as part of the BioFire FA ME panel has shown excellent sensitivity.[22]

Patients with mumps meningitis almost always have CSF pleocytosis (usually <500/mm^3), primarily of mononuclear cells (>80% lymphocytes in 80%–90% of patients); the pleocytosis may persist for weeks. CSF protein concentrations are reported in some series to be normal in more than half of patients with mumps meningitis.[233] The CSF glucose content is normal in most patients, but it may be depressed in up to 25% of cases. Complement fixation and hemagglutination inhibition on serum specimens are the most reliable serologic tests for the diagnosis of mumps. Testing of paired acute and convalescent sera should demonstrate a diagnostic fourfold rise in mumps antibody titer. Mumps virus can be isolated from saliva from virtually all patients with mumps parotitis and can also be recovered in urine for up to 2 weeks after the onset of illness. Mumps virus can be grown from CSF in tissue culture for at least 1 week after the onset of disease, but the sensitivity of this technique is highly variable (30%–50% if collected from CSF early during the course of mumps CNS infection).[233] Application of molecular diagnostic techniques such as PCR assay (e.g., TaqMan array) may make enable the diagnosis of mumps to be made more quickly and more reliably in the future.[264]

Patients with HSV-2 meningitis also present most commonly with a lymphocytic meningitis (<500/mm^3) and a normal glucose content but can present with a mild hypoglycorrhachia (30–45 mg/dL) or with a neutrophilic pleocytosis.[19,261,278] CSF protein concentrations have been reported to be higher than in patients with enteroviral meningitis.[30] PCR has become the standard method of diagnosis for all herpesviruses (HSV-1, HSV-2, HHV-6, CMV, EBV, VZV). HSV-2 has now been identified as the main cause of Mollaret meningitis (i.e., recurrent benign lymphocytic meningitis) in patients without symptoms or signs of genital infection.[19,20] VZV PCR assay has also confirmed several cases of herpes zoster meningitis even without the typical vesicular rash (zoster sine herpete).[19,228]

Bacterial Meningitis

Cerebrospinal Fluid Examination

The diagnosis of bacterial meningitis rests on CSF examination by lumbar puncture[279–282]; typical CSF findings in acute bacterial meningitis are shown in Table 87.10. In virtually all cases, the opening pressure is elevated, with values over 600 mm H_2O suggesting the presence of cerebral edema, intracranial suppurative foci, or communicating hydrocephalus. The WBC count is elevated in untreated bacterial meningitis, usually 1000 to 5000/mm^3 (range, <100 to >10,000/mm^3); more than 90% of patients present with a CSF WBC count greater than 100/mm.^{3} Neutrophils usually predominate, although approximately 10% of patients with acute bacterial meningitis present with a predominance of lymphocytes in CSF; such predominance is more common in neonatal gram-negative bacillary meningitis and meningitis caused by *L. monocytogenes* (about 30% of cases). A study of 175 children with culture-proven bacterial meningitis showed that 16.2% of patients had a very low CSF white cell count (0–20/mm^3) and it was a predictor for a poor prognosis.[283] In adults, the absence of a CSF pleocytosis in pneumococcal meningitis is extremely rare (0.2%) but can account for almost 10% of all cases of meningococcal meningitis.[284,285] Therefore a Gram stain and culture should be performed on all CSF specimens even if the WBC count is normal. A CSF glucose concentration decreased to less than 40 mg/dL is found in about 60% of patients, and a

TABLE 87.10 Typical Cerebrospinal Fluid Findings in Patients With Bacterial Meningitis

CSF PARAMETER	TYPICAL FINDING
Opening pressure	200–500 mm H_2O
White blood cell count	1000–5000/mm^3 (range, <100 to >10,000)
Percentage of neutrophils	≥80%
Protein	100–500 mg/dL
Glucose	≤40 mg/dL
CSF-to-serum glucose ratio	≤0.4
Gram stain	Positive in 60%–90%
Culture	Positive in 70%–85%

CSF, Cerebrospinal fluid.

CSF-to-serum glucose ratio less than 0.25 occurs in 967 of 1412 (68%) patients.[60] CSF protein concentrations are elevated in virtually all patients, presumably because of disruption of the BBB. However, a normal CSF WBC count and protein concentration may be rarely seen in specimens obtained at the onset of meningitis, in some cases of neonatal meningitis, and in severely immunocompromised patients.

Gram stain examination of CSF permits rapid, accurate identification of the causative microorganism with sensitivity ranging from 20% in health care–associated ventriculitis and meningitis to 60% to 90% in patients with community-acquired bacterial meningitis, with a specificity of nearly 100%.[60,73] The likelihood of detecting the organism by Gram stain correlates with the concentration of bacteria in CSF; concentrations of 10^3 or fewer colony-forming units/mL are associated with positive Gram stains about 25% of the time, whereas CSF concentrations of bacteria of 10^5 or greater lead to positive microscopy results in up to 97% of cases.[267] The clinical usefulness of the Gram stain also depends on the bacterial pathogen.[260] Bacteria have been observed in 90% of cases of meningitis caused by *S. pneumoniae,* 86% of cases caused by *H. influenzae,* 75% of cases caused by *N. meningitidis,* and 50% of cases caused by gram-negative bacilli; the CSF Gram stain is positive in 24% to 32% of patients with *L. monocytogenes* meningitis.[236,252] CSF culture is the gold standard in diagnosis and is positive in 80% to 90% of patients with community-acquired bacterial meningitis if CSF is obtained before the start of antimicrobial therapy.[286] In a nationwide French study of 252 patients with neurolisteriosis, the diagnosis was established through CSF culture in 84%; in the other 16%, diagnosis was documented either with CSF PCR or with positive blood culture.[252] In health care–associated ventriculitis and meningitis, only 50% of patients have a positive CSF culture, most likely because of the frequent use of empirical antibiotic therapy before CSF studies are performed.[73] The probability of identifying the organism decreases significantly in patients who have received prior antimicrobial therapy (40%–60% and <50% positivity on Gram stain and culture, respectively), which resulted in the recommendation in the UK guidelines that a PCR assay be routinely performed for the two most common meningeal pathogens (*S. pneumoniae* and *N. meningitidis*) in patients presenting with meningitis.[287] In studies of infants and children with bacterial meningitis, initially positive CSF cultures became sterile in 90% to 100% of patients within 24 to 36 hours of administration of "appropriate" antimicrobial therapy.[288] Furthermore, it has been suggested that CSF sterilization may occur more rapidly after initiation of parenteral antimicrobial therapy than previously suggested, with complete sterilization of CSF containing meningococci within 2 hours, and the beginning of sterilization of pneumococci by 4 hours into therapy.[289] However, in most infants and children with bacterial meningitis who have received prior antimicrobial therapy, no significant differences in the CSF indices occur, although two studies revealed significantly lower CSF protein concentrations and rates of Gram stain positivity.[290]

Several rapid diagnostic tests have been developed to aid in the etiologic diagnosis of bacterial meningitis.[61,290] Latex agglutination techniques detect the antigens of *H. influenzae* type b, *S. pneumoniae, N. meningitidis, E. coli* K1, and the group B streptococci. However, many of the test kits do not include tests for group B meningococci, and other kits are probably poor because of the limited immunogenicity of group B meningococcal polysaccharide. The routine use of CSF bacterial antigen tests for the etiologic diagnosis of bacterial meningitis has been questioned.[290] In one study of 901 CSF bacterial antigen tests performed over a 37-month period, no modification of therapy occurred in 22 of 26 patients with positive results. False-positive results have occasionally resulted in unnecessary treatment and prolonged hospitalization.[291] Another study of 344 CSF specimens submitted for bacterial antigen assays found that 10 specimens represented true infections (by culture criteria), for a sensitivity and specificity of 70% and 99.4%, respectively[291]; a positive CSF antigen test did not affect clinical therapy or hospital course. Furthermore, in patients with culture-negative meningitis, CSF latex agglutination had a sensitivity of only 7% in one study.[292] Given that bacterial antigen testing does not appear to modify the decision to administer antimicrobial therapy and that false-positive results have been reported, routine use of this modality for rapid determination of the bacterial etiology of meningitis is not recommended, although it might be considered for patients who have been pretreated with antimicrobial therapy and when CSF Gram stain and culture results are negative.[293] An immunochromatographic test for detection of *S. pneumoniae* in CSF was found to be 100% sensitive and specific for diagnosing pyogenic pneumococcal meningitis,[294] although more studies are needed to demonstrate the usefulness of this test in the diagnosis of pneumococcal meningitis; the overall sensitivity of the test is 95% to 100%.[295] In another trial, this immunochromatographic test provided substantial benefit over latex agglutination tests and was positive in 99% of culture-positive cases of pneumococcal meningitis and negative in 99% of culture-confirmed cases caused by other pathogens.[296]

PCR assay has been used to amplify DNA from patients with meningitis caused by several meningeal pathogens and is now advocated in the United Kingdom guidelines.[287,297,298]

The clinical usefulness of PCR assay for the diagnosis of meningitis has been assessed with the use of a broad range of bacterial primers. The test characteristics for broad-based bacterial PCR demonstrated a sensitivity of 100%, a specificity of 98.2%, a positive predictive value of 98.2%, and a negative predictive value of 100%.[299] In another study with use of a multiplex PCR assay for detection of *N. meningitidis, S. pneumoniae,* and *H. influenzae* type b, the overall specificity and positive predictive value were 100% and the negative predictive value was 99.1% to 99.5%.[300] Multiplex assays for detecting genes of meningeal pathogens were 100% specific for detecting target organisms or serogroups, and the lower limit of detection was similar to that for the singleplex assays.[301] In another study, the sensitivity of broad-range PCR was higher than that of culture (59% vs. 43%), whereas the specificity was 97% for both methods of diagnosis.[302] Therefore broad-based bacterial PCR assay can be used to detect the most common microorganisms in only one test and has adequate sensitivity and excellent specificity[282]; these tests can be done within 2 hours in most industrialized countries, although they are scarce in resource-poor countries. PCR assay may be particularly useful in patients with bacterial meningitis who have received prior antimicrobial therapy and are more likely to have negative CSF cultures.[303] The test is rapid, is able to detect low numbers of bacteria, and detects nonviable etiologic agents in patients who have received prior antimicrobial therapy. The sensitivity and specificity of PCR assay in CSF for the diagnosis of pneumococcal meningitis are 92% to 100% and 100%, respectively.[295] Real-time PCR assay has also been used for the diagnosis of *L. monocytogenes* meningoencephalitis and is also included in the BioFire FA ME panel.[252,259,304] Problems with false-positive results arise with use of the PCR test, although further refinements in this technique may lead to its usefulness in the diagnosis of bacterial meningitis, particularly when CSF Gram stain and cultures are negative. Another potential application of the PCR assay is rapid detection of the in vitro susceptibility of meningeal pathogens to specific antimicrobial agents. In one report, a novel real-time PCR-hybridization assay was developed for the rapid detection of penicillin susceptibility in *S. pneumoniae*; when applied to 24 pneumococcal DNA-positive CSF extracts, penicillin-sensitive *S. pneumoniae* was detected in all instances.[305] Further studies may establish the usefulness of this rapid technique in allowing clinicians to decide on the use of specific antimicrobial therapy in patients with bacterial meningitis or to use or avoid adjunctive dexamethasone (see later discussion).[306]

Differentiation of Bacterial From Viral Meningitis

In patients without a positive CSF Gram stain or culture, the diagnosis of acute bacterial meningitis is often difficult to establish or reject. The most common etiologies for meningitis in the United States in both adults and children are viral; the majority of these patients receive empirical antibiotic therapy.[35,308] A combination of clinical manifestations, with or without test results, has been assessed to develop models in an attempt to accurately predict the likelihood of bacterial meningitis compared with other potential causes (most often viruses).[282] In one analysis of the records of 422 immunocompetent patients older than 1 month of age with acute bacterial or viral meningitis, it was found that CSF glucose concentration less than 34 mg/dL, a CSF–to–blood glucose ratio less than 0.23, a CSF protein concentration greater than 220 mg/dL, more than 2000 leukocytes/mm^3 of CSF, and more than 1180 neutrophils/mm^3

of CSF were individual predictors of bacterial rather than viral meningitis, with 99% certainty or better.[309] This model has been validated in several retrospective studies.[310] Many other prediction models have been developed and studied.[282] In a meta-analysis of bacterial meningitis score validation studies in which 5312 patients were identified from eight studies, 4896 (92%) had sufficient clinical data for calculation of the bacterial meningitis score, which identified children with CSF pleocytosis who were at very low risk for bacterial meningitis (low-risk features were negative CSF Gram stain, CSF absolute neutrophil count <1000 cell/mm,[3] CSF protein <80 mg/dL, and peripheral absolute neutrophil count <10,000 cells/mm^3).[311] Not surprisingly, one of the most important predictors for bacterial meningitis in this scoring system is a positive Gram stain, wherein the diagnosis is not a dilemma to clinicians. This scoring system has a combined sensitivity of 99.3%, with specificity of 62.1% and negative predictive value of 99.7%, indicating that this scoring system could be used to assist clinical decision making for the management of children with CSF pleocytosis. Despite the positive results of this meta-analysis and other studies, clinical judgment should continue to be used in decisions about the need for administration of empirical therapy in patients with suspected bacterial meningitis.[282] A study of 960 adults derived and validated a risk score in patients with meningitis and a negative Gram stain that identified a "zero risk" subgroup for any urgent treatable etiology (e.g., bacterial meningitis, herpes simplex encephalitis, fungal etiology) with 100% sensitivity.[312] Use of these models should be limited to the age cohort in which they were developed, but they could help clinicians in making a management decision.

Elevated CSF lactate concentrations may also be useful in differentiating bacterial from nonbacterial meningitis in patients who have not received prior antimicrobial therapy.[313–315] Two meta-analyses, one including 25 studies with 1692 patients (adults and children)[313] and the other including 31 studies with 1885 patients,[314] concluded that the diagnostic accuracy of CSF lactate is better than that of the CSF WBC count, glucose concentration, and protein level in the differentiation of bacterial from aseptic meningitis; sensitivities of 93% and 97% and specificities of 96% and 94%, respectively, were seen. In another study of adult patients with negative direct CSF examination, the most highly discernible parameter for the differential diagnosis of bacterial meningitis proved to be the CSF lactate value, with a sensitivity of 94%, specificity of 92%, negative predictive value of 99%, and positive predictive value of 82% at a cutoff of 3.8 mmol/L.[316] However, in patients who received antimicrobial therapy prior to lumbar puncture, CSF lactate concentrations had a substantially lower sensitivity of 49% compared with 98% in those not receiving antimicrobial pretreatment,[314] such that the usefulness of the CSF lactate value in patients pretreated with antimicrobial therapy is probably limited.

Several proteins have been examined for their usefulness in the diagnosis of acute bacterial meningitis. Specifically, CRP, detected either in serum or CSF, and serum procalcitonin concentrations have been elevated in patients with acute bacterial meningitis and may be useful in discriminating between bacterial and viral meningitis. In one study, serum CRP enabled Gram stain–negative bacterial meningitis to be distinguished from viral meningitis on admission with a sensitivity of 96%, a specificity of 93%, and a negative predictive value of 99%.[317] In another study, a serum procalcitonin concentration of more than 0.2 ng/mL had a sensitivity and specificity of up to 100% in the diagnosis of bacterial meningitis,[318] although false-negative results have been reported.[319] In another study, serum procalcitonin, at a cutoff of 0.28 ng/mL, had a sensitivity of 95%, specificity of 100%, negative predictive value of 100%, and positive predictive value of 97% in the diagnosis of bacterial meningitis.[316] In patients with meningitis in whom the CSF Gram stain is negative and analysis of other parameters is inconclusive, serum concentrations of CRP or procalcitonin that are normal or below the limit of detection have a high negative predictive value in the diagnosis of bacterial meningitis, so these patients (i.e., with a presumptive diagnosis of viral meningitis) can be carefully observed without initiation of antimicrobial therapy.[293,320] Finally, both CRP and procalcitonin have been used to assess the prognosis of patients with bacterial meningitis.[321,322]

Other markers that have been studied as markers for acute bacterial meningitis in children and adults include CSF concentrations of cortisol, heparin-binding protein, soluble triggering receptor expressed on myeloid cells 1 (TREM-1), IL-6, IL-12, IL-1β, TNF-α, complement factor B, and C3.[282] Most of these studies included low numbers of patients, limiting their generalizability. In one study, heparin-binding protein had a sensitivity of 100% and specificity of 99.2% in the differentiation of bacterial from aseptic meningitis.[323]

Radiography

Cranial CT or MRI does not aid in the diagnosis of acute bacterial meningitis and may be normal early in the course of infection. However, one of these modalities should be considered during the course of illness in patients who have persistent or prolonged fever, clinical evidence of increased intracranial pressure, focal neurologic findings or seizures, enlarging head circumference (in neonates), persistent neurologic dysfunction, or persistently abnormal CSF parameters or cultures (Fig. 87.2).[290] Gadolinium-enhanced MRI is the most sensitive modality for these complications, particularly with regard to infarction, especially when seen on diffusion-weighted imaging, and ventriculitis.[324] Magnetic resonance angiography and perfusion-weighted imaging may show vascular complications, including focal stenosis and irregularity of major intracranial arteries. However, cranial CT may underestimate the possibility of increased intracranial pressure in patients with pneumococcal meningitis,[325] so intracranial pressure monitoring should be considered in patients with prolonged coma. Cranial CT or MRI has been recommended at the end of antimicrobial therapy in neonates in order to be certain that no intracranial complications have occurred.[326] In one review of 107 children with bacterial meningitis who underwent CT,[327] one or more abnormalities were found in 52% of cases, although most findings did not require specific intervention. This lack of clinical utility was also seen in a study of 614 adults with community-acquired meningitis in which a head CT scan was done in 549 (89%) of patients; 15 patients (2.7%) had major intracranial findings that influenced clinical management in only 8 (1.5%) patients.[328]

Radiographic studies may be useful in the subset of patients with meningitis as a result of a basilar skull fracture with CSF leak.[114] CT may detect air-fluid levels, opacification of the paranasal sinuses, or intracranial air; CT with sagittal reconstruction can also be used to document or localize fracture sites. Radioisotope cisternography with cottonoid pledgets placed at the outlet of the sinuses within the nasal passage can be used to document a CSF leak, although high-resolution CT with water-soluble contrast enhancement of the CSF (metrizamide cisternography) is the best test for defining the site of leakage.

Spirochetal Meningitis

Treponema pallidum

For the diagnosis of CNS involvement in patients with syphilis, no single routine laboratory test is definitive. CSF cellular and protein abnormalities have been reported to occur in 10% to 20% of patients with primary syphilis, 30% to 70% of patients with secondary syphilis, and 10% to 30% of patients with latent syphilis.[166] CSF abnormalities are common in patients with syphilitic meningitis, but they are nonspecific. Findings include a mononuclear pleocytosis (>10 cells/mm^3 in most patients), elevated CSF protein concentrations (78% of patients), and mild decreases in CSF glucose concentrations (<50 mg/dL in 55% of patients).[237] A lack of CSF pleocytosis can also be seen in 22% of patients with documented neurosyphilis.[1] Oligoclonal bands and intrathecally produced antitreponemal antibodies are frequently present. Recovery of *T. pallidum* from CSF specimens through animal inoculation is difficult, expensive, time-consuming, and not routinely performed.[166]

The initial test for syphilis has become the enzyme-linked immunosorbent assay (ELISA) IgG, using recombinant proteins of *Treponema pallidum*. This test is considered sensitive but not specific. A positive serum ELISA is reflexed to a nontreponemal test, which is less sensitive but more specific, such as the rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL) test. If the result of this test is negative, a treponemal test is used for confirmation, such as the fluorescent treponemal antibody absorption (FTA-ABS) test, *T. pallidum* particle agglutination assay (TPPA), or chemiluminescense immunoassay (see Chapter 237).[166,237] However, serologic testing of CSF in patients with syphilis is problematic. For example, CSF collected by means of lumbar puncture is subject to blood contamination in about 10% of

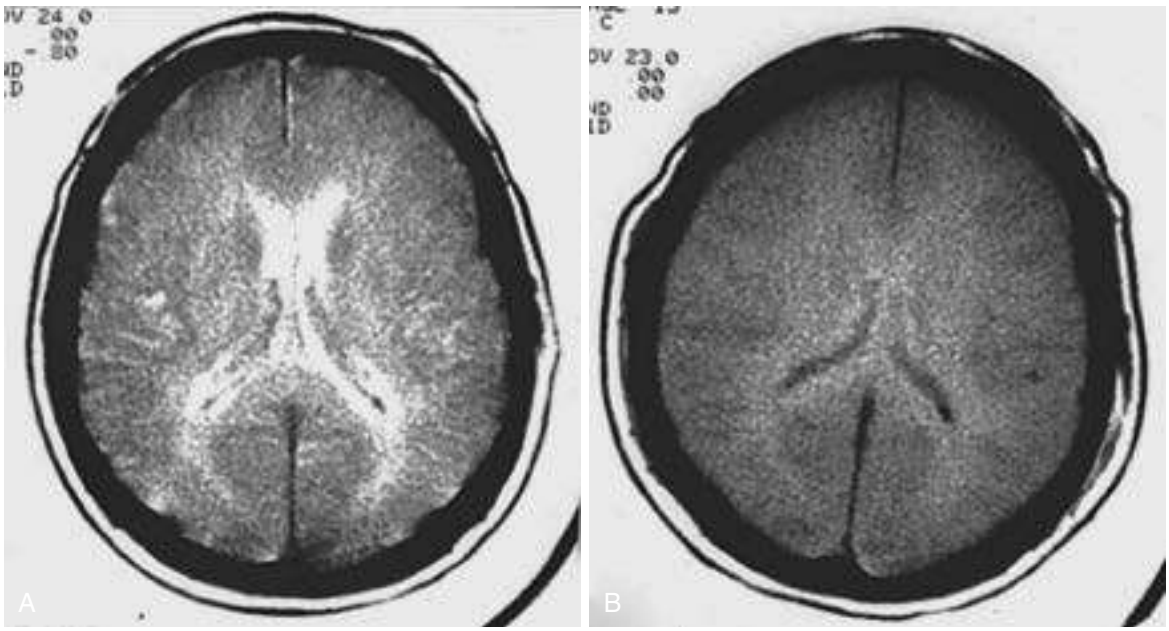

FIG. 87.2 Computed tomography (CT) scans of the head in a patient with pneumococcal meningitis. (A) CT scan on presentation reveals moderate cortical atrophy. (B) CT scan 3 days later reveals diffuse swelling of the cerebral hemispheres bilaterally, with effacement of the ventricular system.

patients, which may lead to contamination of CSF and therefore a false-positive serologic test result[166,329]; the likelihood of a false-positive test result depends on the relative amount of contamination, the antibody titer in blood, and the sensitivity of the test. For patients with a serum VDRL value of 1:256 or less, sufficient blood contamination to be visible to the naked eye is required for the result of the CSF VDRL test to be falsely positive. Although the specificity of the CSF VDRL test for the diagnosis of neurosyphilis is high, the sensitivity is low (reactive tests in only 30%–70% of patients).[166] Therefore a reactive CSF VDRL test in the absence of blood contamination is sufficient to diagnose neurosyphilis; a nonreactive result does not exclude the diagnosis. The CSF FTA-ABS test has also been examined as a possible diagnostic test for neurosyphilis.[166,329] A nonreactive test effectively rules out the likelihood of neurosyphilis, but the specificity of the test is much less than that of the CSF VDRL test because of the possibility of leakage of small amounts of antibody from the serum into CSF. Furthermore, no compelling data confirm the significance of a reactive CSF FTA-ABS as being useful for the diagnosis of neurosyphilis.[166] Other serologic tests that are currently being evaluated are the CSF-TPPA and the CSF *T. pallidum* hemagglutination assay (CSF-TPHA).[330] PCR has also been used to detect *T. pallidum* DNA in CSF samples in patients with neurosyphilis, with sensitivities of 69.7% to 75.8% and specificities of 86.8% to 92.3%.[331] Because of the low sensitivity of the CSF VDRL test, and until further studies demonstrate the usefulness of rapid diagnostic techniques, the diagnosis of neurosyphilis is based on elevated CSF concentrations of WBCs, protein, or both in the appropriate clinical and serologic setting.[166]

Borrelia burgdorferi

Typical CSF changes in patients with Lyme meningitis are a pleocytosis (usually <500 cells/mm^3 but up to 3500 cells/mm^3), with more than 90% lymphocytes in 75% of cases[170]; plasma cells may also be present. CSF protein concentrations are usually elevated (up to 620 mg/dL), and the CSF glucose concentration is usually normal, although it can be low in patients with illness of long duration. Oligoclonal banding may be present, with the bands reactive to *B. burgdorferi* antigens. Because viral meningitis is an important differential diagnosis in endemic areas, a clinical prediction rule has been used to help clinicians differentiate these two conditions. The "Rule of 7s" classifies children at low risk for Lyme meningitis when each of the following three criteria are met: <7 days of headache, <70% CSF mononuclear cells, and absence of seventh or other cranial nerve palsy.[332]

The best currently available laboratory test for the diagnosis of Lyme disease is demonstration of specific serum IgG antibody to *B. burgdorferi* by ELISA, followed by at least five bands on a Western blot. This positive test result in a patient with a compatible neurologic abnormality is strong evidence for the diagnosis.[170,333] However, these tests are not standardized, and marked variability is seen among laboratories.[254] Most laboratories now use an ELISA with sonicated *B. burgdorferi* as the antigen, although a few still use the immunofluorescence technique. By the time that subacute disseminated (i.e., stage II) disease has developed in most patients, serum concentrations of IgG antibody to *B. burgdorferi* are elevated. False-positive reactions have been reported in patients with rheumatoid arthritis, Rocky Mountain spotted fever, infectious mononucleosis, tuberculous meningitis, leptospirosis, yaws, syphilis, and relapsing fever,[170] although high titers of cross-reacting IgG antibodies have been detected only in patients with syphilis or relapsing fever. False-negative results may be obtained from an unreliable assay, early infection, or early antibiotic use, which may blunt the normal humoral immune response. It is currently recommended that when the pretest probability of Lyme disease is 0.20 to 0.80, sequential testing with ELISA and Western blot is the most accurate method for ruling in or out the possibility of Lyme disease.[334,335] Interpretation of IgM Western blot to *B. burgdorferi* is more problematic because of false positives. The test should never be ordered without a positive IgM ELISA and only if symptoms have been present for less than 4 weeks. Persistence of IgM antibody for months or even years limits interpretation as a recent infection. The sensitivity of two-tier testing in one study of patients with later manifestations of Lyme disease was 100% and the specificity was 99%[336]; all patients had positive C6 peptide ELISA results. Omission of the first-tier ELISA or interpretation of the immunoblot with criteria that are not evidence based will potentially decrease the specificity of testing and are contributing factors to misdiagnosis[337]; a concern with IgM immunoblots in clinical practice has been many false-positive results caused by overreading of nonspecific weak bands. Only <1% of peripheral IgG filters into the CSF with an intact BBB, allowing for the ability to compare CSF and serum antibody concentrations and documenting intrathecal antibody (ITAb) production.[170] This method appears to identify virtually all patients with Lyme meningitis and in the European guidelines is required for definitive diagnosis of neuroborreliosis.[338] Intrathecal synthesis of antibodies can persist for several months to up to 10 years after successful diagnosis and therapy.[337] In addition, ITAb also has issues with false positives and

cross-reactivity. One study of 123 patients with anti-*Borrelia* antibody in CSF in France found that another cause was responsible for symptoms in 60% of patients,[339] although determination of the antibody index had a sensitivity of 75% and specificity of 97% for diagnosis. In addition, The C6-peptide ELISA in CSF has also been evaluated in the diagnosis of Lyme neuroborreliosis with a sensitivity of 95% and a specificity of 83%.[340] More recent studies measuring antibodies to C6 have suggested that this is at least as accurate as whole-cell sonicate ELISAs, and may provide a substitute for either the ELISA or Western blot in the two-tier approach.[341,342]

The technique of PCR on CSF samples has also been successfully used to identify *B. burgdorferi* DNA in patients with Lyme neuroborreliosis[170] (see Chapter 241), although PCR must still be considered experimental in the diagnosis of CNS Lyme disease. CSF PCR assay has a low sensitivity but may be useful in very early Lyme neuroborreliosis in those with a negative antibody index or in patients with immunodeficiency.[338] A negative CSF PCR assay does not exclude the diagnosis.[340] Studies have recently suggested that the B-cell attracting chemokine CXCL13 is reliably increased in the CSF of patients with early Lyme neuroborreliosis. One study showed a sensitivity of 94.1% and specificity of 96.1%, which were higher than the sensitivity and equal to the specificity of the antibody index (85.7% and 96.1%, respectively),[343] although there is not enough evidence to recommend CSF measurement of CXCL13 as a routine diagnostic tool.[338]

Radiologic studies may also be useful in patients with CNS manifestations of Lyme disease. CT has shown both enhancing and nonenhancing low-density lesions, mass effect, and cerebral demyelination. MRI may reveal punctate hyperresonant areas without mass effect within the cerebral white matter (Fig. 87.3).

Protozoal and Helminthic Meningitis

Amebas

The CSF formula in patients with the acute form of PAM resembles bacterial meningitis with high opening pressures, a high degree of pleocytosis (median CSF WBCs, 2400 cells/mm^3) with neutrophilia, severe hypoglycorrhachia (median CSF glucose, 23 mg/dL), and high CSF protein concentrations (median, 365 mg/dL).[169–171,344] Patients also may present with a hemorrhagic CSF (median CSF red blood cell count, 265 cells/mm^3), most likely due to necrotizing encephalitis.[169] Gram stain is always negative, but CSF smears should be stained with Giemsa or Wright stains to possibly identify the trophozoite. Examination of fresh, warm specimens of CSF can reveal the ameboid movements of motile trophozoites, or the organism can be identified with PCR at the CDC.[257] In the largest study done to date, a premortem diagnosis was documented in only 27% of 142 patients with PAM.[255] Establishing the diagnosis is very important because miltefosine has been used successfully in some patients.[256] Trophozoites can be demonstrated with light or electron microscopy of brain tissue in autopsy specimens.

Patients with the subacute or chronic form of the illness caused by *Acanthamoeba* species have a less florid CSF inflammatory response with a predominant mononuclear leukocytosis. The CSF protein concentration is elevated, and the glucose content is often normal or reduced.[257] Amebas may be seen in the CSF and the diagnosis confirmed with a CSF *Acanthamoeba* PCR assay performed at the CDC. Patients may present with a chronic meningitis with hydrocephalus resembling tuberculous meningitis.[257] The value of serologic tests is variable.[169] Serum immunofluorescence, amebic immobilization titers, and complement-fixing antibodies support the diagnosis, although demonstration of rising titers is necessary to establish the diagnosis because some healthy persons have circulating antibodies.

Angiostrongylus cantonensis

The combination of a history of ingestion of food suspected to be affected, moderate-to-high peripheral eosinophilia, and CSF eosinophilia leads to the suspicion of angiostrongyloidiasis.[71,175,179] The CSF leukocytosis is moderate, with 16% to 72% eosinophils and an increased protein concentration, but the glucose concentration is normal. However, eosinophilia is not always present in the peripheral blood or in CSF on initial evaluation.[176] In the Jamaican outbreak, eosinophilia was not present in nearly half of the patients at the time of hospital admission, and was initially present in 56% and 44% of CSF and peripheral blood specimens, respectively.[180] Larvae are occasionally found on microscopy of CSF and are seen more often in pediatric patients than in adults. Serologic studies for *A. cantonensis* are not widely available and are not available in a timely manner for making clinical decisions.[176] In one study, serum antibodies were detected in 30 of 31 patients tested, with CSF antibodies positive in 17 of 30 patients.[303] The detection of circulating antigen in serum or CSF may also provide rapid confirmation of infection.[345] PCR for *A. cantonensis* has been used in patients with eosinophilic meningitis in Laos, with promising results.[346]

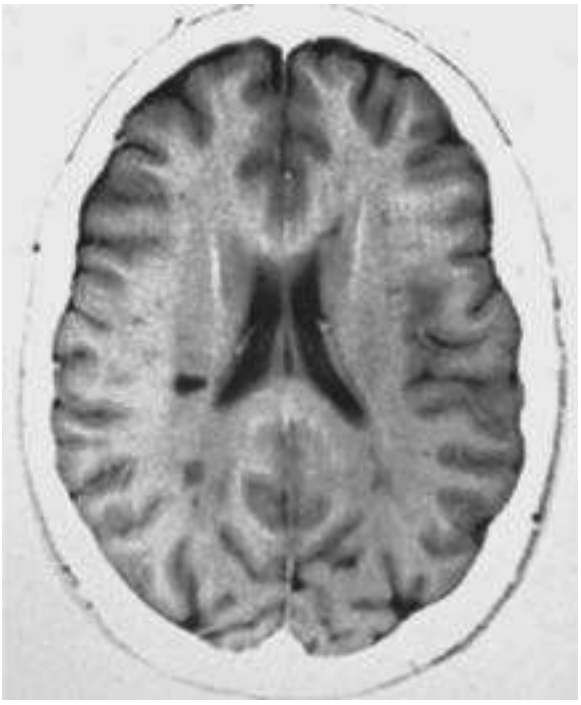

FIG. 87.3 **T2-weighted magnetic resonance image in a patient with Lyme disease reveals areas of increased signal intensity in the cerebral white matter.**

INITIAL MANAGEMENT OF PATIENTS WITH ACUTE MENINGITIS

Who Should Have a Lumbar Puncture?

The initial management of a patient with presumed bacterial meningitis includes performance of a lumbar puncture to determine whether the CSF formula is consistent with that diagnosis (Fig. 87.4).[189,293] If purulent meningitis is present, institution of antimicrobial therapy should be based on the results of Gram staining (Table 87.11). However, if no etiologic agent can be identified by this means or if performance of the lumbar puncture is delayed, institution of empirical antimicrobial therapy, after obtaining blood cultures, should be based on the patient's age and underlying disease status (Table 87.12). Although no prospective data are available on the timing of administration of antimicrobial therapy in patients with bacterial meningitis, a retrospective cohort study in patients with community-acquired bacterial meningitis demonstrated that a delay in initiation of antimicrobial therapy after patient arrival in the emergency department was associated with an adverse clinical outcome when the patient's condition advanced to a high stage of prognostic severity,[347] thus supporting the assumption that treatment of bacterial meningitis before it advances to a high level of clinical severity improves clinical outcome. This concept has also been supported by several retrospective studies, all showing an increase in adverse outcomes with delays of antibiotic therapy; the majority of these studies show an increase in mortality with >6 hours delay.[348–352]

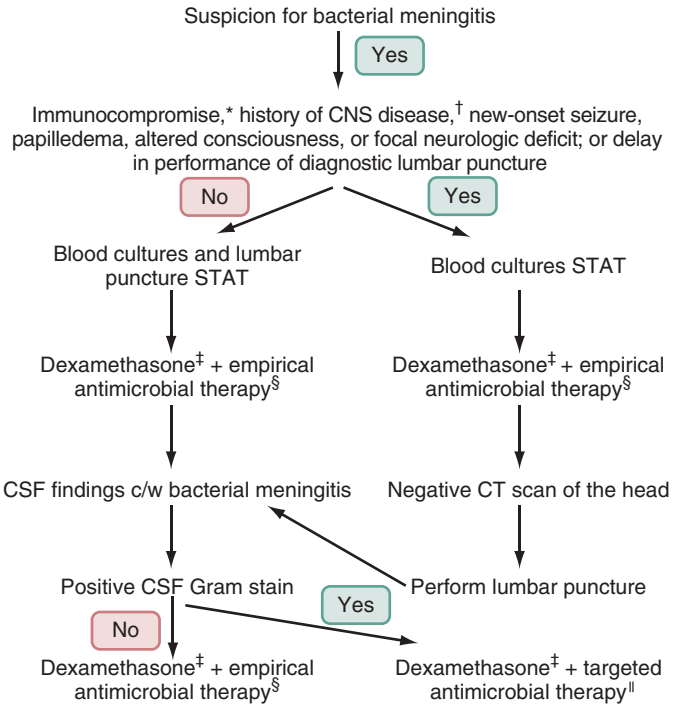

*HIV infection or AIDS, receiving immunosuppressive therapy, or after transplantation.
†Mass lesion, stroke, or focal infection.
‡See text for specific recommendations for use of adjunctive dexamethasone in adults with bacterial meningitis. Dexamethasone and antimicrobial therapy should be administered immediately after CSF sample is obtained.
§See Table 87-12.
‖See Table 87-11. Many authorities would continue empirical antimicrobial therapy (see Table 87-12) pending organism identification.

FIG. 87.4 Management algorithm for adults with suspected bacterial meningitis. *AIDS,* Acquired immunodeficiency syndrome; *CNS,* central nervous system; *CSF,* cerebrospinal fluid; *CT,* computed tomography; *HIV,* human immunodeficiency virus; *STAT,* statim (immediately). *(From Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis.* Clin Infect Dis. *2004;39:1267–1284.)*

TABLE 87.11 Recommended Antimicrobial Therapy for Acute Bacterial Meningitis

MICROORGANISM[a]	ANTIMICROBIAL THERAPY
Haemophilus influenzae type b	Third-generation cephalosporin[b]
Neisseria meningitidis	Third-generation cephalosporin[b]
Streptococcus pneumoniae	Vancomycin plus a third-generation cephalosporin[b,c]
Listeria monocytogenes	Ampicillin or penicillin G[d]

[a]Pathogen presumptively identified by positive Gram stain.
[b]Cefotaxime or ceftriaxone.
[c]Some experts would add rifampin if dexamethasone is also given.
[d]Addition of an aminoglycoside should be considered.
Modified from Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. *2004;39:1267–1284.*

Who Should Undergo Cranial Computed Tomography Before Lumbar Puncture

Obtaining a screening head CT scan before a lumbar puncture to rule out an intracranial mass has become routine practice in the emergency department.[352a] This is done owing to the fear of potentially placing the patient at risk for brain herniation. However, clear evidence that lumbar puncture causes brain herniation is lacking, because the natural course of the disease may itself result in herniation. In patients who present with focal neurologic findings, or who have papilledema and suspected bacterial meningitis, CT of the head is recommended before lumbar puncture in order to rule out the presence of brain shift (as a result of an intracranial mass lesion or generalized brain edema) for the potential risk for herniation,[189,293] despite the fact that up to 43% of patients with bacterial meningitis who had brain herniation had a normal CT scan.[353] Furthermore, the time involved in waiting to perform CT significantly delays the initiation of antimicrobial therapy, with the potential for increased morbidity and mortality in patients with bacterial meningitis. Therefore emergency empirical antimicrobial therapy and adjunctive dexamethasone therapy, after specimens for culture have been obtained, should be initiated before sending the patient for CT.[293] Although CSF cultures may be sterile after the initiation of antimicrobial therapy, pretreatment blood cultures and the CSF formula or Gram stain will provide evidence for or against a diagnosis of bacterial meningitis. In one retrospective review of 177 patients (39 of whom had received prior antimicrobial therapy) with CSF culture-proven bacterial meningitis,[354] the combination of blood culture and CSF Gram stain, with or without latex agglutination, identified the causative bacterium in 92% of patients. In 2004, the IDSA guidelines recommended that the following adult patients should undergo CT prior to lumbar puncture: those with immunocompromised state, history of CNS disease, new-onset seizure, papilledema, abnormal level of consciousness, or focal neurologic deficit.[293] The Swedish guidelines removed altered mental status as an indication for cranial imaging, reporting that this change in practice was associated with earlier treatment and improved outcomes.[355,356] In 2016, both the United Kingdom and the European guidelines further restricted the recommendations to include only focal neurologic findings (except cranial nerve palsy in the European guidelines, new-onset seizures in the European guidelines, and uncontrolled seizure in the UK guidelines) and a lower Glasgow Coma Scale score (≤12 in UK and <10 in European guidelines).[53,287] In addition, the European guidelines and the IDSA guidelines recommend that immunosuppressed patients undergo cranial imaging.[53,293] The Swedish guidelines are the most selective; in these guidelines, a CT is recommended only if there is a suspicion of cerebral mass or abscess (>4 days of cerebral symptoms or neurologic deficit except cranial nerve palsy) or if there are signs of herniation.[355] A study of 815 adults with bacterial meningitis showed a decrease in mortality if there was adherence to the Swedish guidelines in contrast to the IDSA or European guidelines.[356] Compliance in clinical practice with all these guidelines remains low, at about 50% in several recent studies.[328,356–358]

TABLE 87.12 Empirical Therapy for Purulent Meningitis

PREDISPOSING FACTOR	ANTIMICROBIAL THERAPY
Age	
<1 mo	Ampicillin plus cefotaxime; or ampicillin plus an aminoglycoside
1–23 mo	Vancomycin plus a third-generation cephalosporin[a,b]
2–50 yr	Vancomycin plus a third-generation cephalosporin[a,b,c]
>50 yr	Vancomycin plus ampicillin plus a third-generation cephalosporin[a]
Immunocompromised state	Vancomycin plus ampicillin plus either cefepime or meropenem
Basilar skull fracture	Vancomycin plus a third-generation cephalosporin[a]
Head trauma; after neurosurgery	Vancomycin plus either ceftazidime, cefepime, or meropenem

[a]Cefotaxime or ceftriaxone.
[b]Some experts would add rifampin if dexamethasone is also given.
[c]Add ampicillin if meningitis caused by *Listeria monocytogenes* is suspected.
Modified from Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. *2004;39:1267–1284.*

Empirical Therapy of Community-Acquired Acute Bacterial Meningitis in Adults

Without an organism on Gram stain, a positive culture from blood or CSF, or a positive PCR assay result, the recommended treatment for

community-acquired bacterial meningitis in adults is cefotaxime 8 to 12 g/day divided into every 4- or 6-hour doses or ceftriaxone 4 g/day divided into doses every 12 hours. Vancomycin 35 to 45 mg/kg/day divided into doses every 8 or 12 hours is used for the possibility of *S. pneumoniae* with reduced susceptibility to ceftriaxone (MIC ≥2 μg/mL). If *L. monocytogenes* is a concern in patients who are immunosuppressed or older than 50 years, ampicillin can be given as 12 g/day divided into 4- hour intervals. Dexamethasone remains controversial but is recommended to be given at least 20 to 30 minutes before the first dose of antibiotic, beginning with 0.15 mg/kg every 6 hours. Once the first dose of antibiotic has been given, starting dexamethasone is no longer advocated by the 2004 IDSA guidelines, but it is recommended based on expert opinion up to 4 hours after starting antibiotics in the 2016 European guidelines and up to 12 hours in the 2016 UK guidelines. A duration of antimicrobial therapy of at least 14 days is recommended when the pathogen remains unknown.

Selecting the Right Antimicrobial

Once the infecting meningeal pathogen has been isolated and susceptibility testing results are known, antimicrobial therapy can be modified for optimal treatment (Table 87.13).[189,267,304] Recommended dosages of antimicrobial agents for adults with infections of the CNS are shown in Table 87.14, and those for neonates, infants, and children are presented in Table 87.15.

Adjunctive Corticosteroids

Experimental Data

Certain patients should receive adjunctive dexamethasone therapy when presenting with suspected or proven bacterial meningitis.[286,287,293] As stated earlier (see "Pathogenesis and Pathophysiology"), generation of pneumococcal cell wall components in an experimental animal model of pneumococcal meningitis after treatment with bacteriolytic antibiotics may contribute to the inflammatory response in the subarachnoid space.

TABLE 87.13 Specific Antimicrobial Therapy for Acute Meningitis

MICROORGANISM	STANDARD THERAPY	ALTERNATIVE THERAPIES[a]
Bacteria		
Haemophilus influenzae		
β-Lactamase negative	Ampicillin	Ceftriaxone or cefotaxime or cefepime or chloramphenicol or aztreonam or a fluoroquinolone[b]
β-Lactamase positive	Ceftriaxone or cefotaxime	Cefepime or chloramphenicol or aztreonam or a fluoroquinolone[b]
Neisseria meningitidis		
Penicillin MIC <0.1 μg/mL	Penicillin G or ampicillin	Ceftriaxone or cefotaxime or chloramphenicol
Penicillin MIC 0.1–1.0 μg/mL	Ceftriaxone or cefotaxime	Chloramphenicol or a fluoroquinolone[b] or meropenem
Streptococcus pneumoniae		
Penicillin MIC ≤0.06 μg/mL	Penicillin G or ampicillin	Ceftriaxone or cefotaxime or chloramphenicol
Penicillin MIC ≥0.12 μg/mL		
Ceftriaxone or cefotaxime MIC <1.0 μg/mL	Ceftriaxone or cefotaxime	Meropenem or cefepime
Ceftriaxone or cefotaxime MIC ≥1.0 μg/mL	Vancomycin[c] plus ceftriaxone or cefotaxime	Vancomycin plus moxifloxacin[d]
Enterobacteriaceae[e]	Ceftriaxone or cefotaxime	Aztreonam or a fluoroquinolone[b] or trimethoprim-sulfamethoxazole or meropenem or ampicillin
Pseudomonas aeruginosa	Ceftazidime or cefepime	Aztreonam or a fluoroquinolone[b] or meropenem
Acinetobacter baumannii[e]	Meropenem	Colistin (usually formulated as colistimethate sodium)[g] or polymyxin B[g]
Listeria monocytogenes	Ampicillin or penicillin G[f]	Trimethoprim-sulfamethoxazole
Streptococcus agalactiae	Ampicillin or penicillin G[f]	Ceftriaxone or cefotaxime or vancomycin
Staphylococcus aureus		
Methicillin-sensitive	Nafcillin or oxacillin	Vancomycin or linezolid or daptomycin
Methicillin-resistant	Vancomycin[c]	Trimethoprim-sulfamethoxazole or linezolid or daptomycin or ceftaroline
Staphylococcus epidermidis	Vancomycin[c]	Linezolid
Spirochetes		
Treponema pallidum	Penicillin G	Ceftriaxone[h]
Borrelia burgdorferi	Ceftriaxone or cefotaxime	Penicillin G or doxycycline
Protozoa		
Naegleria fowleri	Amphotericin B[i] plus rifampin plus azithromycin plus fluconazole plus miltefosine[j]	—

[a]There may not be clinical data to support all recommendations for alternative antimicrobial agents in patients with bacterial meningitis, but specific agents are suggested based on cerebrospinal fluid (CSF) penetration in experimental animal models and in vitro activity against the offending pathogen, and from isolated case reports documenting successful outcome.
[b]Clinical data are limited on the use of fluoroquinolones for therapy for gram-negative meningitis but may be considered in patients not responding to standard therapy or when disease is caused by resistant organisms.
[c]Addition of rifampin may be considered; see text for indications.
[d]Would recommend moxifloxacin, given its excellent CSF penetration and in vitro activity against *S. pneumoniae,* although there are no clinical data to support its usefulness in patients with pneumococcal meningitis; if used, many authorities would combine moxifloxacin with vancomycin or a third-generation cephalosporin such as cefotaxime or ceftriaxone.
[e]Choice of a specific antimicrobial agent must be guided by in vitro susceptibility testing.
[f]Addition of an aminoglycoside should be considered.
[g]Might also need to be administered by the intraventricular or intrathecal routes.
[h]Alternative agent for use in patients allergic to penicillin. Not well studied. See Chapter 239.
[i]Intravenous and intrathecal administration.
[j]Available by contacting the Centers for Disease Control and Prevention (770-488-7100).
MIC, Minimal inhibitory concentration.
Modified from van de Beek D, Brouwer MC, Thwaites GE, et al. Advances in the treatment of bacterial meningitis. Lancet. *2012;380:1693–1702.*

TABLE 87.14 Recommended Dosages of Antimicrobial Agents for Bacterial Meningitis in Adults With Normal Renal and Hepatic Function

ANTIMICROBIAL AGENT	TOTAL DAILY DOSE	DOSING INTERVAL (h)
Amikacin[a]	15 mg/kg	8
Ampicillin	12 g	4
Aztreonam	6–8 g	6–8
Cefepime	6 g	8
Cefotaxime	8–12 g	4–6
Ceftaroline	1800 mg	8
Ceftazidime	6 g	8
Ceftriaxone	4 g	12–24
Chloramphenicol[b]	4–6 g	6
Ciprofloxacin	800–1200 mg	8–12
Daptomycin	6–10 mg/kg	24
Gentamicin[a]	5 mg/kg	8
Linezolid	1200 mg	12
Meropenem	6 g	8
Moxifloxacin[c]	400 mg	24
Nafcillin	12 g	4
Oxacillin	12 g	4
Penicillin G	24 million units	4
Rifampin	600 mg	12–24
Tobramycin[a]	5 mg/kg	8
Trimethoprim-sulfamethoxazole[d]	10–20 mg/kg	6–12
Vancomycin[e]	30–45 mg/kg	8–12

[a]Need to monitor peak and trough serum concentrations.
[b]Higher dose recommended for pneumococcal meningitis.
[c]No data on optimal dosage for patients with bacterial meningitis.
[d]Dosage based on trimethoprim component; many experts would use a dose of 5 mg/kg every 8 hours.
[e]Maintain serum trough concentrations of 15–20 μg/mL; one study recommended continuous infusion of 60 mg/kg/day (see text for details).
Modified from Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. *2004;39:1267–1284.*

The inflammatory response induced by either live pneumococci or pneumococcal cell wall was reduced by agents (e.g., methylprednisolone, oxindanac) that inhibit the cyclooxygenase pathway of arachidonic acid metabolism, and a correlation was noted between CSF concentrations of the arachidonic acid metabolite PGE_2 and CSF leukocytes. In another study, administration of the antiinflammatory agent indomethacin decreased both brain water content and CSF concentrations of PGE_2 during experimental pneumococcal meningitis, although intracranial pressure was not reduced. In addition, the administration of either dexamethasone or oxindanac lessened the massive influx of serum albumin and other proteins of high and low molecular weight into the CSF during the early stages of experimental pneumococcal meningitis. Several corticosteroid agents have also been examined in experimental animal models of meningitis. Early studies revealed that methylprednisolone administration led to a significant reduction in the mass of leukocytes within the meninges of rabbits with pneumococcal meningitis. Another study demonstrated that CSF outflow resistance was reduced by methylprednisolone therapy and to a greater extent than in untreated or penicillin-treated rabbits with pneumococcal meningitis. In further studies that examined the effects of corticosteroids (methylprednisolone or dexamethasone) on brain water content, CSF pressure, and CSF lactate in rabbits with pneumococcal meningitis, it was found that both agents completely reversed the development of brain edema but that only dexamethasone led to a reduction in CSF pressure and lactate; however, neither agent was superior to therapy with ampicillin alone in reducing cerebral edema or intracranial pressure, and no comparison was made between ampicillin alone and the combination of ampicillin plus corticosteroids, a comparison that would have been relevant to the potential clinical usefulness of adjunctive corticosteroid therapy in bacterial meningitis. A subsequent study did examine treatment with ceftriaxone versus ceftriaxone plus dexamethasone in an experimental rabbit model of *H. influenzae* meningitis. Although combination therapy consistently reduced the brain water content, CSF pressure, and CSF lactate to a greater degree than ceftriaxone alone, the differences were not statistically significant. The authors suggested, however, that adjunctive dexamethasone might be more beneficial if administered early or even before antibiotic-induced bacterial lysis and release of microbial products. In a subsequent analysis using the experimental rabbit model of *H. influenzae* type b meningitis, ceftriaxone administration led to a significant increase in CSF endotoxin concentrations 2 hours after administration, which was followed by a rise in CSF TNF concentrations. Simultaneous administration of dexamethasone and ceftriaxone did not affect the release of endotoxin into CSF, but it markedly attenuated CSF concentrations of TNF measured 8 hours later. Adjunctive dexamethasone therapy also resulted in a significant decrease in CSF leukocytosis and a trend toward earlier improvement in CSF concentrations of glucose, lactate, and protein. These parameters improved without any apparent decrease in the rate of bacterial killing within the CSF in vivo.

Clinical Studies of Corticosteroids in Infants and Children

On the basis of these observations, numerous clinical trials were undertaken to determine the effects of adjunctive dexamethasone on outcome in patients with bacterial meningitis.[359–389] One meta-analysis of these clinical studies confirmed the benefit of adjunctive dexamethasone (0.15 mg/kg every 6 hours for 2–4 days) for *H. influenzae* type b meningitis and, if begun with or before parenteral antimicrobial therapy, suggests benefit for pneumococcal meningitis in childhood.[380] Evidence of clinical benefit was strongest for improved hearing outcomes. In contrast, a retrospective, nonrandomized study of children with pneumococcal meningitis, published after the meta-analysis, demonstrated that the use of adjunctive dexamethasone was not associated with a beneficial effect,[371] although the dexamethasone was administered before or within 1 hour of the first dose of antibiotic and the children in the dexamethasone group had a higher severity of illness. In addition, in a published double-blind, placebo-controlled trial of adjunctive dexamethasone in Malawi,[375] the overall number of deaths in the two treatment groups was similar (31% in each group), as was the frequency of neurologic sequelae. The factors that may have accounted for these results include the fact that Malawian children had severe disease associated with undernutrition and HIV infection and the fact that they were presented for medical attention after a delay, which resulted in very high case-fatality rates and significant long-term morbidity.[381] Corticosteroids do not reverse CNS damage that has already resulted from the pathophysiologic consequences of bacterial meningitis (e.g., cerebral edema and increased intracranial pressure). However, even in children with bacterial meningitis in the developing world, use of adjunctive dexamethasone should be considered, because no adverse effects were attributable to its administration in this trial, and its use may benefit some of the children with this devastating disorder. In a retrospective cohort study of 2780 children from 27 tertiary care children's hospitals discharged with bacterial meningitis as their primary diagnosis, adjuvant corticosteroid therapy was not associated with time to death or time to hospital discharge.[377] However, the study was limited by the low mortality rate of bacterial meningitis in children, the number of patients included in each subgroup was small, the dose or timing of corticosteroid administration could not be ascertained, and potential benefits in terms of morbidity were not assessed.

Clinical Studies of Corticosteroids in Adults

In adult patients with bacterial meningitis, use of adjunctive dexamethasone is generally recommended.[286,287] In a prospective, randomized, double-blind trial in 301 adults with bacterial meningitis,[376] adjunctive dexamethasone was associated with a reduction in the proportion of

TABLE 87.15 Recommended Dosages of Antimicrobial Agents for Bacterial Meningitis in Neonates, and Infants and Children With Normal Renal and Hepatic Function

	TOTAL DAILY DOSE (DOSING INTERVAL IN HOURS)		
ANTIMICROBIAL AGENT	**NEONATES (BIRTH–7 DAYS OLD)[A]**	**NEONATES (8–28 DAYS OLD)[A]**	**INFANTS AND CHILDREN**
Amikacin[b]	15–20 mg/kg (12)	30 mg/kg (8)	20–30 mg/kg (8)
Ampicillin	150 mg/kg (8)	200 mg/kg (6–8)	300 mg/kg (6)
Cefepime	—	—	150 mg/kg (8)
Cefotaxime	100–150 mg/kg (8–12)	150–200 mg/kg (6–8)	225–300 mg/kg (6–8)
Ceftazidime	100–150 mg/kg (8–12)	150 mg/kg (8)	150 mg/kg (8)
Ceftriaxone	—	—	80–100 mg/kg (12–24)
Chloramphenicol	25 mg/kg (24)	50 mg/kg (12–24)	75–100 mg/kg (6)
Gentamicin[b]	5 mg/kg (12)	7.5 mg/kg (8)	7.5 mg/kg (8)
Meropenem	—	—	120 mg/kg (8)
Nafcillin	75 mg/kg (8–12)	100–150 mg/kg (6–8)	200 mg/kg (6)
Oxacillin	75 mg/kg (8–12)	150–200 mg/kg (6–8)	200 mg/kg (6)
Penicillin G	0.15 mU/kg (8–12)	0.2 mU/kg (6–8)	0.3 mU/kg (4–6)
Rifampin[c]	—	10–20 mg/kg (12)	10–20 mg/kg (12–24)
Tobramycin[b]	5 mg/kg (12)	7.5 mg/kg (8)	7.5 mg/kg (8)
Trimethoprim-sulfamethoxazole[d]	—	—	10–20 mg/kg (6–12)
Vancomycin[e]	20-30 mg/kg (8–12)	30–45 mg/kg (6–8)	60 mg/kg (6)

[a]Smaller dosages and longer intervals of administration may be advisable for very-low-birth-weight neonates (<2000 g).
[b]Need to monitor peak and trough serum concentrations.
[c]Maximum daily dose of 600 mg.
[d]Dosage based on trimethoprim component; many experts would use a dose of 5 mg/kg every 8 hours.
[e]Maintain serum trough concentrations of 15–20 µg/mL.
Modified from Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. *2004;39:1267–1284.*

patients who had unfavorable outcomes (15% vs. 25%, $P = .03$) and in the proportion of patients who died (7% vs. 15%, $P = .04$). Neurologic sequelae were not reduced, although neurologic sequelae were seen predominantly in the most severely ill patients and the proportion of severely ill patients who survived to be tested was larger in the dexamethasone group. The benefits were most striking in the subgroup of patients with pneumococcal meningitis (unfavorable outcome in 26% of those receiving dexamethasone vs. 52% in those receiving placebo, $P = .006$; and death in 14% of those receiving dexamethasone vs. 34% in those receiving placebo, $P = .02$) and in those with moderate-to-severe disease as assessed by the admission Glasgow Coma Scale score. On the basis of these data and the apparent absence of serious adverse outcomes in the patients who received dexamethasone, the routine use of adjunctive dexamethasone (given concomitantly with or just prior to the first dose of an antimicrobial agent for maximal attenuation of the subarachnoid space inflammatory response) is warranted in most adults with pneumococcal meningitis.[382] Adjunctive dexamethasone should not be used in patients who have already received antimicrobial therapy; if the meningitis is subsequently found not to be caused by *S. pneumoniae,* dexamethasone should be discontinued, although some experts recommend use of adjunctive dexamethasone regardless of microbial etiology. In patients with meningococcal meningitis who received adjunctive dexamethasone, there was no improvement in rates of unfavorable outcome, although its use was not associated with harm.[383] Despite these positive benefits in terms of morbidity and mortality, there were some concerns regarding cognitive long-term outcome in patients treated with dexamethasone. However, a follow-up study of 87 eligible patients in which 46 were treated with adjunctive dexamethasone and 41 with placebo, neuropsychologic evaluation showed no significant differences between patients treated with dexamethasone or placebo.[384] In an evaluation of 357 episodes of pneumococcal meningitis from 2006 to 2009 in the Netherlands since implementation of adjunctive dexamethasone on a large-scale basis, the prognosis improved, with mortality rates decreasing from 30% to 20%.[385] A significant reduction in the mortality in pneumococcal meningitis has also been observed in the United States that is temporarily related to the introduction of adjunctive dexamethasone.[390] Approximately 1% to 4% of adults with community-acquired bacterial meningitis develop a delayed cerebral thrombosis after they have had an excellent initial recovery from their episode of meningitis.[386,387] A recent study showed a potential association with the use of adjunctive steroids, but further studies are needed.[391]

Despite the positive benefits of adjunctive dexamethasone in adults with bacterial meningitis described previously, the routine use of adjunctive dexamethasone in patients with bacterial meningitis in the developing world has been controversial. In one randomized, double-blind, placebo-controlled study in adolescents and adults in Vietnam with confirmed bacterial meningitis,[65] patients who received adjunctive dexamethasone experienced a significant reduction in the risk for death at 1 month (RR, 0.43) and the risk for death or disability at 6 months (RR, 0.56); the highest proportion of cases in this study were caused by *S. suis,* followed by *S. pneumoniae.* In contrast, in a randomized, double-blind, placebo-controlled study from Malawi, there were no significant differences in mortality at 40 days in the intention-to-treat analysis (56% in the dexamethasone group vs. 53% in the placebo group) or when the analysis was restricted to patients with proven pneumococcal meningitis (53% in the dexamethasone group vs. 50% in the placebo group).[379] However, in this trial, almost 90% of the patients were infected with HIV and most likely had advanced disease; delayed presentation was also associated with a poorer outcome, although adjusting for this factor in the analysis had no effect. These data suggest that adjunctive dexamethasone is not beneficial in resource-poor countries where a substantial number of patients are infected with HIV.[388] In a Cochrane Database systematic meta-analysis of 24 studies involving 4041 participants, adjunctive dexamethasone did not reduce overall mortality, but there was a trend toward lower mortality in adults; corticosteroids were associated with lower rates of severe hearing loss, any hearing loss, and neurologic sequelae, although these benefits were seen only in studies from high-income countries.[389] In a subgroup analysis based on causative microorganism, corticosteroids reduced severe hearing loss in patients

with *H. influenzae* meningitis and mortality in patients with *S. pneumoniae* meningitis.

The use of adjunctive dexamethasone is of particular concern in patients with pneumococcal meningitis caused by highly penicillin- and cephalosporin-resistant strains, in which case patients may require antimicrobial therapy with vancomycin.[286,293] A diminished CSF inflammatory response after dexamethasone administration might significantly reduce vancomycin penetration into CSF and delay CSF sterilization, as shown in an experimental rabbit model of penicillin- and cephalosporin-resistant pneumococcal meningitis. This result was confirmed in another rabbit model of pneumococcal meningitis in which significantly lower CSF vancomycin concentrations and differences in bacterial killing were found in the dexamethasone-treated rabbits. However, CSF vancomycin penetration was not reduced by dexamethasone in a study in children[392]; and in another study in which a continuous infusion of vancomycin was used (60 mg/kg/day), adequate CSF concentrations (7.9 μg/mL) were achieved despite the concomitant administration of adjunctive dexamethasone.[393] CSF concentrations of ceftriaxone are not significantly altered in animals or patients treated with adjunctive dexamethasone.[394,395]

Dexamethasone is the only adjunctive therapy that has been advocated by the IDSA, UK guidelines, and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines in patients with bacterial meningitis.[53,287,293] Available evidence suggests that, in high-income countries, dexamethasone treatment should be started with or even before the first dose of antibiotics and continued for 4 days in both children and adults (although with different dose regimens). Guidelines recommend suspending dexamethasone treatment if the bacterial meningitis diagnosis is not confirmed or if the causative pathogen is other than *H. influenzae* or *S. pneumoniae* (although some experts advise dexamethasone to be continued irrespective of the meningeal pathogen).[53,287]

ANTIMICROBIAL THERAPY

Viral Meningitis

Specific antiviral chemotherapy for the EVs is not currently available; treatment is supportive. Pleconaril, an agent that has been evaluated in enteroviral infections,[396,397] is a novel compound that integrates into the hydrophobic pocket of picornaviruses (including EVs) and prevents viral replication by inhibiting viral uncoating and blocking viral attachment to host cell receptors. It is orally bioavailable in all age groups, and CNS concentrations are four times higher than those in serum, making it a possible agent for the treatment of enteroviral neurologic infection (see Chapter 48).[396] In a preliminary report on the outcome of potentially life-threatening EV infections in patients treated with pleconaril by compassionate-use protocol,[397] the drug had beneficial effects on the clinical, virologic, laboratory, and radiologic parameters in patients with EV infections. Of the 16 patients with CEMA, 12 improved on pleconaril and 4 were unchanged and stable. In placebo-controlled clinical studies of pleconaril treatment of adults and children with non–life-threatening enteroviral meningitis, clinical benefits (i.e., faster resolution of headache and return to work or school) and virologic benefits, and a favorable safety profile, were documented.[219] In a double-blind, placebo-controlled trial in infants (<12 months) with enteroviral meningitis, efficacy of pleconaril was not demonstrated, which may have been a result of the low yield of serial viral cultures, relatively short and benign clinical courses, and the small number of subjects enrolled.[398] In another randomized, multicenter, double-blind placebo-controlled trial of 607 patients with enteroviral meningitis, pleconaril shortened the course of illness compared with placebo-treated patients, especially early in the disease course.[399] Benefits were only modestly achieved in the subgroup of patients with more severe disease. Pleconaril is not active against EV 71.[396] Despite potential for its use in neonates with enteroviral sepsis,[396] its use in enteroviral meningitis is debated, given that most patients with enteroviral meningitis recover quickly and with minimal sequelae. Furthermore, pleconaril has not achieved approval from the FDA because it induces CYP3A enzyme activity and has the potential for drug interactions, such that the sponsor did not pursue approval.

Recovery of patients with HSV-2 meningitis is usually complete without neurologic sequelae. It is not clear whether antiviral treatment alters the course of mild meningitis.[359,363] In one retrospective observational study, neurologic outcomes were significantly improved in immunocompromised patients with HSV meningitis who were treated with antiviral therapy, but not in immunocompetent patients, suggesting that immunocompromised patients with HSV meningitis may benefit from antiviral therapy[400]; however, more data are needed. A recent retrospective study showed that all patients with herpes simplex meningitis had good clinical outcomes irrespective of antiviral therapy.[19] It is also unclear whether treatment with prophylactic antiviral therapy is necessary in patients with recurrent HSV-2 meningitis. In one double-blind randomized controlled trial, patients with acute primary or recurrent HSV-2 meningitis were assigned to placebo or valacyclovir suppressive therapy after acute treatment with valacyclovir for 1 week; suppressive therapy was not shown to prohibit recurrent meningitis and was associated with a higher frequency of meningitis after cessation of active drug.[21] Therefore suppressive therapy cannot be currently recommended in patients with recurrent episodes of HSV-2 meningitis pending further study.

Bacterial Meningitis

Principles of Therapy

Many factors influence the choice of an antimicrobial agent in the treatment of bacterial meningitis. Use of animal models of infection has permitted quantification of the relative penetration of drug into CSF, the effects of meningitis on this entry parameter, and the relative bactericidal efficacy (defined as the rate of bacterial eradication) within purulent CSF.[290,401,402]

The first factor relates to penetration of the antimicrobial agent into CSF, which depends, to a great extent, on the status of the BBB.[290,401] For example, β-lactam antibiotics such as penicillin penetrate into CSF poorly (about 0.5%–2.0% of peak serum concentrations) when the BBB is normal. In the presence of meningeal inflammation, CSF penetration of the antibiotic is enhanced because of increased permeability across the BBB, perhaps as a result of separation of intercellular tight junctions and increased numbers of pinocytotic vesicles in cerebral microvascular endothelial cells. Antimicrobial entry decreases as inflammation subsides, indicating that maximal parenteral doses of antimicrobial agents should be continued throughout the course of therapy to maintain adequate CSF concentrations. Antibiotic entry into CSF is also enhanced by drugs with a high lipid solubility, low molecular weight, low degree of protein binding in serum, and low degree of ionization at physiologic pH.

The second factor is the bactericidal activity of the antimicrobial agent within purulent CSF.[290,401,402] Because of the accumulation of lactate in CSF during bacterial meningitis, the pH of CSF is decreased, thereby inhibiting the bactericidal activity of the aminoglycosides; this is likely to have contributed to the poor response observed with the aminoglycosides in the therapy of meningitis in experimental animal models and in patients. Elevated CSF protein concentrations may decrease the efficacy of antimicrobial agents that are highly protein bound because free drug is needed for the antibacterial effect. Drug that penetrates the CSF may be removed by an active transport system that exists in the choroid plexus (as for the penicillins and cephalosporins), or it may be converted to an inactive metabolite. In addition, other drugs may influence antibiotic activity within purulent CSF. In experimental animal models of meningitis, antagonism has been shown when a bactericidal agent is coadministered with a bacteriostatic antibiotic (e.g., chloramphenicol plus gentamicin). However, in other instances the combination of antibiotics may be synergistic, as in the combination of penicillin or ampicillin with gentamicin in *L. monocytogenes* meningitis and that of ampicillin plus gentamicin against *S. agalactiae*.

A third factor concerns the importance of bactericidal activity in CSF for optimal therapy inasmuch as bacterial meningitis represents an infection in an area of impaired host defense. Multiple studies in experimental animal models have shown that rapid bacterial killing is observed in vivo only when CSF concentrations of β-lactams or aminoglycosides exceed the minimal bactericidal concentration (MBC) by about 10- to 20-fold.[290,401] The importance of rapid bacterial killing has also been examined in patients with bacterial meningitis. One study that compared outcomes in infants and children with bacterial meningitis

who had negative or positive CSF cultures 18 to 36 hours after the initiation of antimicrobial therapy revealed an increased rate of neurologic complications (i.e., ataxia, hemiparesis, developmental delay, moderate-to-severe hearing impairment) in the group in which the causative organism could still be recovered after this interval.

A final factor that may contribute to response to antimicrobial therapy in bacterial meningitis is pharmacodynamics,[290,401–404] which is concerned with the time course of antimicrobial therapy at the site of infection and is important in determining a dosing regimen for optimal effectiveness. In time-dependent antimicrobial activity, as observed with β-lactam agents, the bactericidal activity of an antimicrobial agent depends on the time for which its concentration exceeds the MIC as a proportion of the dosing interval. As stated earlier, CSF concentrations of β-lactams need to exceed the MBC by at least 10- to 20-fold for the maximal bactericidal effect to be obtained, although peak CSF antimicrobial concentrations and the time for which the antimicrobial concentrations exceed the MBC are interrelated and time greater than MBC increased in parallel with peak CSF concentrations. This explains why the bactericidal effect did not improve with larger antimicrobial doses. The second pattern of antimicrobial activity is concentration dependent, and it is characterized by killing over a wide range of antimicrobial concentrations and a prolonged recovery period (i.e., postantibiotic effect) after drug concentrations fall below the MIC; this is seen with the aminoglycosides and fluoroquinolones. Although single-dose gentamicin therapy was as effective as divided-dosage regimens in an experimental animal model despite different times for which CSF gentamicin concentrations exceeded the MBC, the applicability of these findings to humans needs to be established. The pharmacodynamic characteristics of the fluoroquinolones are very similar to those of the aminoglycosides, although features of both time dependency and concentration dependency have been demonstrated with the fluoroquinolones in animal models of meningitis. There have also been investigations to determine whether continuous infusion of antimicrobial agents improves outcome in patients with bacterial meningitis. In a study of 723 African children with bacterial meningitis randomly assigned to receive boluses or continuous infusion of cefotaxime for the first 24 hours of therapy, 272 children died, but the mode of administration did not significantly change the proportion of children who died or were severely disabled at the time of hospital discharge[405]; however, in a planned subgroup analysis, children with pneumococcal meningitis given continuous cefotaxime infusion were significantly less likely to die or have sequelae.

Specific Antimicrobial Therapy

Haemophilus influenzae

Therapy for meningitis caused by *H. influenzae* type b has been markedly altered by the emergence of β-lactamase–producing strains.[290] Resistance of *H. influenzae* to chloramphenicol has also been described, although more commonly in areas such as Spain (>50% of isolates) than the United States (<1% of isolates). In Brazil, the prevalence of β-lactamase–producing strains of *H. influenzae* was 18.4% (range, 6.6%–57.7%), with almost 17% of strains also resistant to chloramphenicol.[406] Chloramphenicol resistance is of particular concern in developing countries of the world, where it is often used as first-line treatment for suspected bacterial meningitis.[290] In an observational study with a retrospective control group conducted in Papua New Guinea, where chloramphenicol was used as empirical treatment followed by ceftriaxone when in vitro resistance to chloramphenicol was found in *H. influenzae,* there was invariably a very poor outcome (i.e., death or severe neurologic sequelae) in patients with chloramphenicol-resistant disease (71% vs. 9% when chloramphenicol was used as first-line therapy).[407] Even in patients with chloramphenicol-sensitive isolates, a prospective study found chloramphenicol to be bacteriologically and clinically inferior to ampicillin, ceftriaxone, or cefotaxime in the treatment of childhood bacterial meningitis caused predominantly by *H. influenzae* type b.[408] Furthermore, the use of chloramphenicol can be problematic because of its unpredictable metabolism in young infants and its pharmacologic interactions with other concomitantly administered drugs, such as phenobarbital, rifampin, phenytoin, and acetaminophen, which increase the likelihood of toxicity.[189]

Several studies have documented the efficacy of third-generation cephalosporins (particularly cefotaxime or ceftriaxone) to be similar to that of the combination of ampicillin plus chloramphenicol for bacterial meningitis.[290] Based on these findings, the third-generation cephalosporins are recommended as empirical antimicrobial therapy for children with bacterial meningitis. The second-generation cephalosporins should not be used for therapy for bacterial meningitis. A prospective randomized study comparing ceftriaxone with cefuroxime for the treatment of childhood bacterial meningitis documented more rapid CSF sterilization (2% vs. 12% of CSF cultures positive at 18–36 hours, $P = .11$) and a lower incidence of hearing impairment (4% vs. 17%, $P = .05$) in the patients receiving ceftriaxone.[409] Cefepime has been studied in the treatment of bacterial meningitis.[410] Cefepime has in vitro activity and cure rates similar to those of cefotaxime and ceftriaxone in patients with meningitis caused by *H. influenzae, N. meningitidis,* and *S. pneumoniae*; it also has greater in vitro activity against *Enterobacter* species and *P. aeruginosa.* In a prospective randomized comparison of cefepime and cefotaxime for the treatment of bacterial meningitis in infants and children,[411] cefepime was found to be safe and therapeutically equivalent to cefotaxime and can be considered a suitable therapeutic alternative for the treatment of patients with this disease.

Neisseria meningitidis

Penicillin G and ampicillin are the antimicrobial agents of choice for meningitis caused by *N. meningitidis.*[290] However, these recommendations may need to be modified in the future because of trends in the antimicrobial susceptibility of meningococci. Meningococcal strains that are of intermediate susceptibility to penicillin G and have an MIC range of 0.1 to 1.0 μg/mL have been reported from several areas (particularly Spain). For example, of 3264 strains of *N. meningitidis* isolated from blood and CSF in Spain during 1978 to 1985,[412] only one resistant isolate was observed, whereas 9 (5%) of 168 invasive isolates of intermediate susceptibility to penicillin G were found in the first 6 months of 1986; this figure reached 20% in 1989. This decreased susceptibility was reported to be mediated by a reduced affinity of the antibiotic for penicillin-binding proteins 2 and 3. Decreased meningococcal susceptibility to penicillin has also been reported in Greece, Switzerland, Romania, France, Belgium, the United Kingdom, Malawi, South Africa, Canada, Croatia, and Turkey.[290,412–416] High-level penicillin resistance resulting from β-lactamase production has also been reported, and the MICs for these strains may be as high as 256 μg/mL.[416] Furthermore, high-level chloramphenicol resistance (MIC ≥64 μg/mL) has been described as resulting from the presence of the *catP* gene on a truncated transposon that has lost mobility because of internal deletions[417]; transmission of genetic material between strains of *N. meningitidis* probably played an important role in dissemination of the gene.

In the United States, meningococcal strains with reduced susceptibility to penicillin have also been described.[418–421] In a population-based surveillance study of invasive meningococcal disease in selected areas of the United States, 3 of 100 isolates had penicillin MICs of 0.125 μg/mL.[418] In another active, population-based surveillance in seven geographically dispersed areas of the United States during 1997,[421] 3 of 90 isolates were of intermediate susceptibility to penicillin, with MICs of 0.12 μg/mL, whereas 49 of the remaining 87 isolates had MICs of 0.06 μg/mL. In Ontario, Canada, the prevalence of invasive meningococcal disease caused by strains with decreased in vitro susceptibility to penicillin was much higher (21.7%) in 2006,[422] although it did not change in frequency between 2000 and 2006. These data indicate the importance of continued surveillance for these strains.

The clinical significance of these isolates is unclear at present because many patients with meningitis caused by these meningococci have recovered with standard penicillin therapy. However, isolated reports of treatment failure have been described.[423,424] Furthermore, in a study from Spain, reduced susceptibility of *N. meningitidis* to penicillin was seen in 34% of 213 children with meningococcal meningitis[425]; in this report, reduced penicillin susceptibility was more frequent in strains responsible for death or sequelae (60% vs. 32%, $P = .04$). On the basis of these data, some authorities would treat patients who have meningococcal meningitis with a third-generation cephalosporin (either cefotaxime or ceftriaxone), and these agents are likely to emerge as

first-line treatment in the future. Susceptibility testing of the isolate should be performed for patients who fail to respond appropriately.

Streptococcus pneumoniae

Therapy for meningitis caused by pneumococci has recently been modified according to current pneumococcal susceptibility patterns.[282,290,293] In the past, pneumococci were uniformly susceptible to penicillin, with MICs of 0.06 μg/mL or less. Numerous reports from throughout the world then documented strains of pneumococci that are of intermediate susceptibility to penicillin (MIC range, 0.1–1.0 μg/mL), and strains that were highly resistant to penicillin (MIC, 2.0 μg/mL or higher). The Clinical and Laboratory Standards Institute has redefined the in vitro susceptibility breakpoints for pneumococcal isolates from patients with meningitis as either susceptible or resistant, with intravenous penicillin breakpoints of 0.06 μg/mL or lower and 0.12 μg/mL or greater, respectively.[426] The mechanism of this resistance is an alteration in the structure and molecular size of penicillin-binding proteins. Resistance has been reported in several different pneumococcal serotypes, although the overwhelming majority of resistant strains are serotypes 6, 14, 19, and 23; most of the multidrug-resistant strains isolated in the United States disseminated from a multiresistant serotype 23F clone of *S. pneumoniae* that was isolated in Spain as early as 1978. In Brazil, penicillin resistance was mainly detected in isolates of serotypes 14 (61%), 23F (16%), 6B (10%), and 19F (3%).[427] Results of recent surveillance studies in the United States show that the prevalence of penicillin-nonsusceptible *S. pneumoniae* ranges from 25% to more than 50%[428]; rates are as high as 60% in some parts of Latin America and as high as 80% in some countries in Asia. Factors reported to predispose to resistance include the patient's age (<10 or >50 years), immunosuppression, prolonged hospital stay, children in daycare settings, infection by serotypes 14 and 23, and frequent, prolonged, or prophylactic use of antimicrobial therapy. However, penicillin-nonsusceptible strains have been isolated even when no risk factors or comorbidities are identified.[429]

Several alternative agents for the treatment of meningitis caused by penicillin-resistant pneumococci have been evaluated by in vitro susceptibility testing, in animal models, and in patients.[282,290,293] Here, we will restrict our discussion to agents that have been examined in clinical trials, unless animal model data have influenced treatment recommendations. Chloramphenicol is one agent that has been studied for the treatment of pneumococcal meningitis. However, clinical failures with chloramphenicol have been reported in patients with penicillin-resistant isolates, probably because of the poor bactericidal activity of chloramphenicol against these strains; 20 of 25 children had an unsatisfactory outcome (i.e., death, serious neurologic deficit, poor clinical response) in one study.[430] Despite susceptibility on disk testing, chloramphenicol MBCs of the penicillin-resistant pneumococcal isolates were significantly higher than those for the penicillin-sensitive isolates, with subsequent subtherapeutic bactericidal activity and treatment failure. Chloramphenicol resistance was also found in 27% of pneumococcal isolates in Malawi during 2004 to 2006[431] and in 43% of isolates in Papua New Guinea.[432]

Third-generation cephalosporins have been considered the treatment of choice in pneumococcal meningitis caused by strains that are of intermediate susceptibility to penicillin.[290,293] Cefotaxime and ceftriaxone are the third-generation agents of choice; ceftizoxime is not recommended because its MIC to resistant pneumococci tends to be higher than that of either cefotaxime or ceftriaxone. However, some reports of meningitis treatment failure with the third-generation cephalosporins have appeared, and pneumococcal strains have emerged that are resistant to these agents (MIC ≥2 μg/mL).). In 2011–2012, 8.9% of 1190 *S. pneumoniae* isolates from the United States were ceftriaxone resistant.[433] When the MIC to the third-generation cephalosporin is 1 μg/mL or less, some patients have been treated successfully with either high-dose cefotaxime or ceftriaxone alone, although one study found that high-dose cefotaxime did not have reliably sufficient CSF bactericidal activity against cephalosporin-resistant pneumococci.

Vancomycin has been evaluated in 11 adult patients with meningitis caused by pneumococcal strains that are of intermediate susceptibility to penicillin.[434] This therapy was associated with clinical failure in 4 patients; no failures occurred in 14 subsequent patients treated with ceftriaxone. In 2 of the patients in whom therapy failed, CSF vancomycin concentrations were undetectable at 48 hours, and in a third patient, symptoms recurred on the eighth day of antimicrobial therapy. The concomitant administration of dexamethasone and the subsequent decreased inflammation and poor entry of vancomycin into CSF may have contributed to this negative outcome; this explanation has been supported in an experimental rabbit model of pneumococcal meningitis. These data support the concept that vancomycin should not be used alone for the treatment of pneumococcal meningitis.[435] Of additional concern is the description of *S. pneumoniae* strains that are tolerant to vancomycin.[436,437] A vancomycin- and cephalosporin-tolerant strain of *S. pneumoniae* was isolated from the CSF of a patient with meningitis who then developed recrudescence of meningitis despite therapy with vancomycin plus a third-generation cephalosporin[438]; these data may have important implications in the use of vancomycin for pneumococcal meningitis. Appropriate CSF concentrations of vancomycin, however, may be attained even when patients are receiving adjunctive dexamethasone as long as appropriate dosages of vancomycin are used. In a study of 14 patients, intravenous administration of vancomycin (at a continuous infusion of 60 mg/kg/day, after a 15-mg/kg loading dose) led to mean serum and CSF vancomycin concentrations of 25.5 μg/mL and 7.9 μg/mL, respectively.[393] These data indicate that appropriate CSF concentrations can be attained when appropriate doses are used. Trough serum vancomycin concentrations of 15 to 20 μg/mL are recommended.[440]

In view of the aforementioned data, penicillin can never be recommended as empirical therapy in patients with suspected pneumococcal meningitis. As an empirical regimen, the combination of vancomycin plus a third-generation cephalosporin (either cefotaxime or ceftriaxone) is recommended,[290,293,406,407] although UK and ESCMID guidelines recommend use of a third-generation cephalosporin alone in countries where the prevalence of cephalosporin-resistant pneumococcus is <1%.[53,287,441] The combination of vancomycin plus a third-generation cephalosporin was synergistic in a rabbit model of penicillin-resistant pneumococcal meningitis and was synergistic, or at least additive, in the CSF of children with meningitis. The addition of rifampin to vancomycin with or without a third-generation cephalosporin has been recommended by some authorities, although clinical data are lacking; rifampin should be added only if the organism is demonstrated to be susceptible, the expected clinical or bacteriologic response is delayed, or the pneumococcal isolate has a cefotaxime or ceftriaxone MIC greater than 4.0 μg/mL. However, in a retrospective study of 109 children with pneumococcal meningitis who received empirical vancomycin in combination with cefotaxime or ceftriaxone, patients with hearing loss had a significantly shorter median vancomycin start time than those with normal hearing (<1 hour vs. 4 hours).[442] In a multiple logistic regression analysis, hearing loss was independently associated with a vancomycin start time of 2 hours or less, suggesting that the first vancomycin dose should be given 2 hours or more after administration of the cephalosporin for suspected or confirmed bacterial pneumococcal meningitis, although more studies are needed. Any patient who is not improving as expected or who has a pneumococcal isolate for which the cefotaxime or ceftriaxone MIC is greater than 2.0 μg/mL should undergo a repeat lumbar puncture to document sterility of CSF after 36 to 48 hours of therapy; this may be especially important for patients who are receiving adjunctive dexamethasone therapy. In patients not responding, intrathecal or intraventricular vancomycin also remains a reasonable option.[293] Once susceptibility studies of the isolated pneumococcus are performed, antimicrobial therapy can be modified for optimal treatment (see Table 87.13).

With continued emergence of penicillin- and cephalosporin-resistant strains of *S. pneumoniae,* other antimicrobial agents have been evaluated for their efficacy in pneumococcal meningitis.[290,293] Meropenem, a carbapenem with a broad spectrum of in vitro activity, including activity against penicillin-resistant pneumococci, has been approved by the FDA for the treatment of bacterial meningitis in children 3 months and older. Meropenem has been studied for the treatment of meningitis in both adults and children in several clinical trials, with microbiologic and clinical outcomes similar to those with either cefotaxime or ceftriaxone. Meropenem was also used successfully in one patient with multidrug-resistant pneumococcal meningitis. In one prospective study of 258

children with bacterial meningitis, patients were randomly assigned to receive either meropenem or cefotaxime; there were no significant differences in outcome, with clinical cure (with or without sequelae) in 97% and 96% of patients treated with meropenem and cefotaxime, respectively.[443] However, in a study of 20 cefotaxime-resistant *S. pneumoniae* isolates,[444] 4 were of intermediate susceptibility and 13 were resistant to meropenem, suggesting that meropenem may not be a useful alternative agent for the treatment of pneumococcal isolates that are highly resistant to penicillin and cephalosporins.[293] Further studies are needed to determine the efficacy of meropenem in pneumococcal meningitis caused by penicillin- and cephalosporin-resistant strains, although isolated case reports have shown efficacy.

The fluoroquinolones have previously lacked sufficient in vitro activity against *S. pneumoniae* to warrant their investigation in the treatment of CNS infections. However, newer agents (moxifloxacin, gemifloxacin, gatifloxacin, garenoxacin) have shown excellent in vitro activity and have been evaluated in experimental animal models of infection.[293,445] Trovafloxacin has been compared with ceftriaxone with or without vancomycin in a multicenter, randomized, comparative trial enrolling 311 children from 11 countries; *S. pneumoniae* was isolated in 27% of cases.[445] The overall efficacy of both treatment groups was comparable in terms of CSF sterilization (94% in the trovafloxacin group and 96% in the comparator) and clinical success at the end of treatment (75% in the trovafloxacin group and 82% for the comparator). Although trovafloxacin is no longer used because of concerns about liver toxicity, these data suggest the potential usefulness of the newer fluoroquinolones in the treatment of bacterial meningitis. However, further clinical trials are needed before these agents can be recommended. Of concern are the reports of decreased pneumococcal susceptibility to the fluoroquinolones, the development of fluoroquinolone-resistant *S. pneumoniae* associated with levofloxacin therapy,[446] and the report of a case of fatal meningitis caused by a levofloxacin-resistant strain of *S. pneumoniae*[447]; these data highlight the need to monitor the in vitro susceptibility of pneumococci to these agents. Nevertheless, a combination regimen of a third-generation cephalosporin plus a newer-generation fluoroquinolone may emerge as the treatment option of choice for pneumococcal meningitis in the future.

Listeria monocytogenes

Despite their broad range of in vitro activity, third-generation cephalosporins are inactive in meningitis caused by *L. monocytogenes.* For patients with *Listeria* meningitis, therapy should consist of ampicillin or penicillin G[117–119,290,293]; the addition of an aminoglycoside should be considered in proven infection because of in vitro synergy and enhanced killing in vivo, as documented in a variety of animal models of *Listeria* infection. Nevertheless, it is important to emphasize that a controlled clinical trial comparing ampicillin alone with ampicillin plus gentamicin has never been performed in humans with listeriosis, although many authorities recommend the addition of an aminoglycoside to ampicillin for at least the first week of therapy for CNS infection.[119] In contrast, in a cohort of 118 patients with listeriosis, the aminoglycoside-treated group had increased rates of kidney injury and mortality.[448] In addition, in a retrospective review of patients with listeriosis (58% with primary bacteremia and 42% with meningitis), differences in mortality were not seen in those treated with ampicillin or with the combination of ampicillin and gentamicin.[449] In contrast, in a national prospective observational cohort study, those receiving amoxicillin-gentamicin combination therapy had an improved survival.[252] However, more data are needed.[450] An alternative agent in a penicillin-allergic patient is trimethoprim-sulfamethoxazole, which is bactericidal against *Listeria* in vitro. In one retrospective series, therapy with trimethoprim-sulfamethoxazole plus ampicillin was associated with a lower failure rate and fewer neurologic sequelae than the combination of ampicillin plus an aminoglycoside,[450] although more data are needed before this combination can be recommended. Oral therapy with trimethoprim-sulfamethoxazole has been used in some patients with *Listeria* meningitis and may be considered in those who demonstrate a rapid clinical response to intravenous therapy and in whom good adherence is expected.[451] Although chloramphenicol has varying activity against *Listeria* in vitro, its use has been associated with an unacceptably high failure rate in patients with *Listeria* meningitis. Vancomycin is also unsatisfactory for *Listeria* meningitis despite favorable in vitro susceptibility results. However, intraventricular administration of vancomycin was successful in one case of recurrent *L. monocytogenes* meningitis. Rifampin is bacteriostatic against *L. monocytogenes* in vitro and was no better than penicillin alone when evaluated in the experimental rabbit model of meningitis. Meropenem is active in vitro and in experimental animal models of *L. monocytogenes* meningitis and may be a useful alternative if found to be clinically efficacious. The fluoroquinolones and linezolid have also demonstrated good in vitro activity against *L. monocytogenes,* although there is limited clinical experience to recommend these agents in patients with *Listeria* meningitis.[449]

Streptococcus agalactiae

Standard therapy for neonatal meningitis caused by group B streptococci is the combination of ampicillin plus an aminoglycoside,[133] which is also recommended for adult patients with meningitis caused by this organism.[134] This combination is recommended because of documented in vitro synergy and reports detailing the presence of penicillin-tolerant strains. Alternative agents are the third-generation cephalosporins; vancomycin is reserved for patients who are allergic to penicillin.

Aerobic Gram-Negative Bacilli

Treatment of bacterial meningitis caused by aerobic gram-negative bacilli has been revolutionized by the availability of third-generation cephalosporins.[290,293] Previous mortality rates with standard regimens (usually an aminoglycoside with or without chloramphenicol) ranged from 40% to 90% versus cure rates of 78% to 94% with the third-generation cephalosporins. Cefotaxime is preferred over ceftriaxone as the third-generation cephalosporin for use in neonates because it has been used more extensively and is not excreted in bile, which may have an inhibitory effect on the bacterial flora of the intestinal tract.[290] Ceftriaxone also has increased protein binding. One particular third-generation cephalosporin, ceftazidime, has enhanced in vitro activity against *P. aeruginosa* and resulted in the cure of 19 of 24 patients in one study of *P. aeruginosa* meningitis when administered alone or in combination with an aminoglycoside.[452] In another study of 10 pediatric patients with *Pseudomonas* meningitis, 7 patients were cured clinically and 9 were cured bacteriologically when treated with ceftazidime-containing regimens.[453]

Concomitant intrathecal or intraventricular aminoglycoside therapy should be considered in patients with gram-negative meningitis who are not responding to conventional parenteral therapy. However, this mode of administration is rarely needed at present and was associated with a higher mortality rate than systemic therapy alone in infants with gram-negative meningitis and ventriculitis.[454]

Given the emergence of strains of gram-negative bacilli that are resistant to the third-generation cephalosporins,[420] the use of other intravenous agents, with or without intraventricular or intrathecal antimicrobials, may need to be considered, and several have been used in patients with meningitis caused by aerobic gram-negative bacilli.[290,293] Aztreonam attains excellent CSF concentrations and has been shown to be efficacious in the treatment of gram-negative meningitis. Imipenem was found to be efficacious in one case of *Acinetobacter* meningitis and in eradication of bacteria from CSF in a study of 21 children with bacterial meningitis (most cases caused by *H. influenzae* type b and *N. meningitidis*), although a high rate of seizure activity (33%) limits its usefulness in the treatment of bacterial meningitis. High-dose meropenem (2 g every 8 hours) given for 18 weeks was successful in a lymphoma patient with *P. aeruginosa* meningitis in whom therapy with ceftazidime plus gentamicin had failed, and in a patient with posttraumatic meningitis caused by *P. aeruginosa.* Cefepime was successful in a patient with postoperative meningitis caused by *Enterobacter aerogenes.*[455] Ceftazidime-avibactam plus intraventricular gentamicin was successfully used in a patient with carbapenem-resistant *Klebsiella pneumoniae* meningitis,[456] and the intravenous and intracerebroventricular administration of tigecycline was safe and effective in another case of multidrug-resistant *K. pneumoniae* meningitis.[457] For empirical treatment of *Acinetobacter* meningitis, intravenous use of meropenem, with or without an aminoglycoside administered through an intraventricular or intrathecal route, has been recommended[458]; if the organism is later found to be resistant to carbapenems, colistin (usually formulated as colistimethate sodium) or polymyxin B should

be substituted for meropenem and may also need to be administered through an intraventricular or intrathecal route.[459] Colistin (5 mg/kg/day as colistin base or 13.3 mg/kg/day as colistimethate) was successfully administered intravenously to treat a patient with meningitis caused by multidrug-resistant *Acinetobacter baumannii*[460]; it was also efficacious when given intrathecally in other cases of meningitis caused by this same multidrug-resistant organism,[461,462] and intrathecally administered polymyxin E has also been used in a patient with *Acinetobacter* meningitis.[463] In a summary of treatment of multidrug-resistant *A. baumannii,* a total of 14 patients were treated for CNS infection (ventriculitis or meningitis) with colistin given intravenously and/or either intrathecally or intraventricularly[464]; sterilization was achieved in all cases and cure was reported in 13 of 14 cases. In the presence of meningitis, CSF concentrations of colistin were shown to be 0.5 μg/mL (34%–67% of serum concentrations).[465] Two cases of *A. baumannii* meningitis were also successfully treated with tigecycline.[466] The fluoroquinolones (e.g., ciprofloxacin, pefloxacin) have been used successfully in some patients with gram-negative meningitis.[467] In one case series of 12 neonates and infants with nosocomial meningitis (in which 6 cases were attributed to gram-negative bacilli), 10 patients were cured and in 7 children no neurologic sequelae appeared after a 2- to 4-year follow-up.[468] Another patient who developed *P. aeruginosa* meningitis after a laminectomy was cured with the addition of high-dose ciprofloxacin (400 mg IV every 8 hours) to the previous regimen of ceftazidime and gentamicin,[469] and a preterm infant with *Stenotrophomonas maltophilia* meningitis was successfully treated with ciprofloxacin.[469] The limited published literature on use of the fluoroquinolones suggests that the primary area of usefulness of these agents is for the treatment of multidrug-resistant gram-negative organisms (e.g., *P. aeruginosa*) or when the response to conventional β-lactam therapy is slow (e.g., meningitis caused by *Salmonella* species).[290,293] Data are currently insufficient to recommend any fluoroquinolone for empirical therapy in any patient with community-acquired bacterial meningitis.

Staphylococci

S. aureus should be treated with nafcillin or oxacillin,[470,471] and vancomycin should be reserved for patients allergic to penicillin or when methicillin-resistant organisms are suspected or isolated. However, given the likelihood of MRSA in patients with *S. aureus* meningitis, vancomycin should be used as empirical therapy pending results of in vitro susceptibility testing.[471] The addition of rifampin or trimethoprim-sulfamethoxazole should be considered in patients not responding to therapy and if the organism is susceptible. Linezolid has been used successfully in some patients with MRSA CNS infections.[472,473] Daptomycin has been shown to have similar antibacterial activity to vancomycin in an experimental model of MRSA meningitis,[474] and daptomycin plus rifampin has been successfully used in patients with MRSA meningitis.[475–477] Ceftaroline has also been successfully used in two patients with MRSA meningitis,[478] one with a ventriculoperitoneal shunt infection[479] and the other with MRSA bacteremia and meningitis who was also treated with rifampicin during the initial 2 weeks of therapy.[480] Meningitis caused by coagulase-negative staphylococci, the most commonly encountered organisms in CSF shunt infections, should be treated with vancomycin; rifampin should be added if the patient fails to improve. Removal of the shunt is often necessary to optimize therapy (see Chapter 92). In one review of 14 cases, removal of the neurosurgical devices and intravenous vancomycin therapy led to survival in 12 of 14 patients.[481]

Other Bacteria

Salmonella meningitis should be treated with a third-generation cephalosporin, with or without a fluoroquinolone, because conventional antimicrobial agents (e.g., chloramphenicol, ampicillin, co-trimoxazole) have an unacceptable cure rate and relapse rate and high associated mortality[63]; the fluoroquinolones have a cure rate of approximately 89%, and the third-generation cephalosporins have a cure rate of about 85%. Ampicillin plus gentamicin should be used for enterococcal meningitis caused by susceptible strains; given its success in enterococcal endocarditis (see Chapter 80), the combination of ampicillin plus ceftriaxone may be a reasonable approach to therapy in patients with enterococcal meningitis. Vancomycin, or ampicillin-sulbactam, is substituted for ampicillin for β-lactamase–producing strains. In patients with meningitis caused by VRE, several case reports have shown successful treatment with intravenous linezolid[482,483]; given the failures associated with other alternative agents, use of linezolid is reasonable in meningitis caused by this multidrug-resistant organism. One patient with VRE meningitis was successfully treated with intravenously and intraventricularly administered chloramphenicol.[484] Other isolated case reports have demonstrated success with use of intraventricular administration of daptomycin combined with intravenous linezolid[485] and with intravenous combination daptomycin and gentamicin or linezolid.[486]

Duration of Therapy

The duration of therapy for bacterial meningitis has been based more on tradition than on scientific evidence.[290,293,407] The duration of antimicrobial therapy in patients with bacterial meningitis has been 10 to 14 days for cases of nonmeningococcal meningitis. Several studies comparing 7 with 10 days of treatment in infants and children with *H. influenzae* type b meningitis, however, have documented that 7 days of therapy is safe and effective, although therapy must be individualized and some patients may require longer courses. Meningococcal meningitis can be treated for 7 days with intravenous penicillin, and some authors have also suggested that 4 days of therapy is adequate; this study requires confirmation because only 50 patients were studied and no control group was included. A single dose, or even two to three doses, of long-acting penicillin or chloramphenicol has been used successfully in developing countries to treat meningococcal meningitis, although this therapy is not considered standard. In one randomized trial of 4 versus 7 days of ceftriaxone therapy in children with bacterial meningitis who had an initial rapid recovery, no significant differences in outcome were observed in the two groups at completion of therapy or at a follow-up of 1 to 3 months after discharge. In another trial, the clinical outcome of patients treated for 7 days with ceftriaxone was similar to 10-day therapy for acute bacterial meningitis in children in developing countries and was associated with a lower incidence of nosocomial infection and earlier hospital discharge. In a double-blind randomized trial of 5 or 10 days of therapy with ceftriaxone for bacterial meningitis in children beyond the neonatal period, it was determined that ceftriaxone could be discontinued in those patients who were stable after 5 days of treatment,[487] although the uncertainties around organism-specific data (especially for *S. pneumoniae*) and the need for clinical judgment at day 5 should lead to caution in reducing treatment duration.[488] In adults with meningitis caused by enteric gram-negative bacilli, treatment regimens should be continued for 3 weeks because of the high rate of relapse in patients treated with shorter courses of therapy. Ten to 14 days is recommended for the treatment of meningitis caused by *S. pneumoniae* and 14 to 21 days for group B streptococci. Patients with *L. monocytogenes* meningitis should be treated for at least 21 days.[53,290,293]

Outpatient antimicrobial therapy may also be appropriate for certain patients with bacterial meningitis. The following criteria have been suggested to guide outpatient antimicrobial therapy[290,293]: inpatient therapy for at least 6 days; no fever for at least 24 to 48 hours before initiation of outpatient therapy; no significant neurologic dysfunction, focal findings, or seizure activity; clinically stable or improving condition; intake of all fluids by mouth; first dose of outpatient antimicrobial therapy given under medical supervision and without reaction; access to home health nursing for antimicrobial administration; reliable intravenous line and infusion device, if needed; daily examination by a physician and an established plan for physician visits, nurse visits, laboratory monitoring, and emergencies; patient and/or family compliance with the program; and a safe environment with access to a telephone, utilities, food, and a refrigerator. In addition, completion of antimicrobial therapy in a skilled nursing facility may be appropriate for selected patients who need continued care but do not require acute hospitalization.

Spirochetal Meningitis

Treponema pallidum

In patients with syphilis with CSF abnormalities but without clinically apparent disease, the goals of therapy are to prevent progression to symptomatic disease and ameliorate the laboratory abnormalities believed to indicate disease activity.[489] For patients with clinical neurosyphilis

syndromes, the goal may be to reverse clinical symptoms and signs or to arrest disease progression. In patients with syphilitic meningitis whose clinical picture is that of meningeal inflammation as a result of the acute inflammatory response, clinical findings other than cranial nerve abnormalities usually resolve without therapy. In patients with meningovascular syphilis, the prognosis after therapy is quite good, except perhaps in patients with larger, clinically apparent neurologic deficits before therapy; therapy in this situation may halt progression and prevent further ischemic events caused by neurosyphilis.

The drug of choice for the treatment of neurosyphilis is penicillin G (see Table 87.13), although considerable controversy remains regarding the most appropriate total dose and the formulation and duration of therapy.[329] Therapy with benzathine penicillin (2.4 million units intramuscularly) does not reliably produce CSF penicillin concentrations above 0.018 μg/mL and should not be used for the treatment of neurosyphilis. Furthermore, in a small but poorly defined proportion of patients with syphilis treated with benzathine penicillin, therapy fails, defined as persistent CSF abnormalities with clinically apparent neurosyphilis. However, in many patients treated with benzathine penicillin, their CSF abnormalities resolve and their condition does not progress, which suggests that factors other than CSF concentrations of penicillin play a role in response to therapy.

The preferred antimicrobial regimen for the treatment of CNS syphilis is intravenous aqueous crystalline penicillin G at a dose of 18 to 24 million units daily in divided doses every 4 hours or by continuous infusion for 10 to 14 days.[329] Alternatively, procaine penicillin, 2.4 million units intramuscularly daily, plus probenecid, 500 mg orally four times daily, both for 10 to 14 days, can be used. Some experts also recommend follow-up therapy with one injection of benzathine penicillin G (2.4 million units intramuscularly once per week for 3 weeks), although no data support this recommendation. No large studies have been performed to evaluate alternative antimicrobial agents for neurosyphilis. On the basis of case reports, clinical experience, and extrapolations from experimental animal studies, the tetracyclines, chloramphenicol, and ceftriaxone have all been described to be of potential clinical usefulness in penicillin-allergic patients. One experimental study, however, suggested that ceftriaxone may not be adequate therapy for neurosyphilis.[490] Furthermore, a study of 43 HIV-infected patients with latent syphilis or neurosyphilis treated with ceftriaxone (1 or 2 g daily for 10–14 days) had a 23% failure rate,[459] similar to that seen in 13 HIV-infected patients with latent syphilis or neurosyphilis treated with benzathine penicillin (30%). Despite these concerns, ceftriaxone (2 g either intramuscularly or intravenously for 10–14 days) can be used as an alternative agent for treatment of neurosyphilis in patients allergic to penicillin.[491] In HIV-infected patients with neurosyphilis, careful monitoring for response to therapy is needed.[492] Follow-up lumbar puncture should be performed every 6 months in all patients until the CSF changes have normalized. However, a report demonstrated that in most patients with neurosyphilis, normalization of the serum RPR in patients treated for neurosyphilis correctly predicted the success of therapy and normalization of CSF parameters after treatment,[493] suggesting that repeat CSF analysis can be avoided. The accuracy is lower among HIV-infected patients not receiving ART than in those receiving ART. Several reports of failures in HIV-infected patients receiving standard therapy for neurosyphilis have appeared; these failures probably occurred because the patient's immunologic response has an important role in controlling the infection even in the presence of "adequate" antimicrobial therapy.

Borrelia burgdorferi

Parenteral antimicrobial therapy is usually needed to treat the neurologic manifestations of Lyme disease, including meningitis (see Table 87.13).[494,495] Initial studies used high-dose (15–20 million units daily) intravenous penicillin G for 10 to 14 days, although one author found the benefits limited to patients treated within 5 weeks of the onset of neurologic symptoms. The meningeal and systemic reactions tend to improve within days, whereas radicular pain and motor deficits improve over many weeks. CNS abnormalities are arrested by treatment and may slowly improve, but some residual deficit is common. Some patients have also responded to treatment with oral or intravenous administration of doxycycline, which has been found to be as efficacious as penicillin in several studies.[495] Patients who have not responded to intravenous penicillin have responded to intravenous therapy with cefotaxime, ceftriaxone, or chloramphenicol. In one prospective randomized trial, ceftriaxone was superior to penicillin in therapy for late Lyme borreliosis. The current recommendation is to treat most patients with Lyme meningitis with intravenously administered ceftriaxone at a dosage of 2 g daily for 14 days (range, 10–28 days)[494]; the literature contains no agreement on the duration of therapy or on the minimal adequate dose of the antimicrobial agent. No evidence supports treatment durations longer than 4 weeks. However, no regimen has proved to be universally effective. Although one report has indicated that high-dose oral doxycycline may produce inhibitory concentrations against *B. burgdorferi* in CSF, parenteral regimens are generally necessary for CNS infection. In contrast, in a randomized, double-blind trial including 118 patients with neuroborreliosis, use of a 2-week regimen of oral doxycycline was not inferior to parenteral ceftriaxone[495]; the patients in this trial generally had mild neurologic symptoms, suggesting that oral treatment with doxycycline might be sufficient in patients with mild symptoms.

Protozoal and Helminthic Meningitis

Amebas

Many antimicrobial agents, including amphotericin B, the tetracyclines, the imidazoles, artemisinin compounds, and rifampin, have in vitro activity against free-living amebas; phenothiazines are amebicidal only at high concentrations. Amphotericin B is rapidly amebicidal against *N. fowleri* in vitro, but it is much less active against *Acanthamoeba*. Rare patients have survived after therapy for PAM,[496–498] and all received amphotericin B along with various other antimicrobial agents. One documented survivor received amphotericin B and miconazole intravenously and intrathecally and rifampin, sulfisoxazole, and dexamethasone. Another received amphotericin B, rifampicin, and ornidazole. However, no clearly effective regimen has been established. An effective regimen used in recent cases has consisted of amphotericin B (intravenous and intrathecal administration), azithromycin, fluconazole, rifampin, and miltefosine, which is available from the CDC; the addition of dexamethasone, hyperosmolar therapy (with mannitol and 3% saline), moderate hyperventilation, and induced hypothermia was also used to aggressively treat cerebral edema and resulting increased intracranial pressure.[169] The addition of miltefosine has led to survival in some patients,[257,498–502] although failures have been reported.[502] Therapy is usually continued for 2 to 4 weeks if the clinical response is good and no complications occur.

Angiostrongylus cantonensis

Treatment of symptoms such as headache, nausea, and vomiting with analgesics and rehydration is indicated for eosinophilic meningitis caused by *A. cantonensis*.[175–179] Most patients recover within 1 to 2 weeks. Treatment with specific anthelmintic agents is controversial; exacerbation of neurologic symptoms after larval death is a theoretical complication of anthelmintic therapy. The benzimidazoles have been tried in humans without definite benefit. Thiabendazole cleared *A. cantonensis* from rats in one study, and it has been used in the early stages of migration of the larvae of *A. cantonensis,* but the drug fails as soon as the worm reaches the CNS. Clinicians in Taiwan routinely treat eosinophilic meningitis with anthelmintics[176]; agents used include mebendazole, levamisole, and albendazole. However, no randomized study of use of anthelmintic agents for eosinophilic meningitis has been reported, and there are insufficient data to recommend their use.[178]

ADJUNCTIVE THERAPY

Viral Meningitis

Adjunctive measures have been used in seriously ill patients with enteroviral meningitis. Because enteroviral clearance from the host is antibody mediated, exogenously administered antibody has been examined.[219,220] Administration of immune globulin through multiple routes (including directly into the CNS) has led to stabilization or improvement of agammaglobulinemic patients with chronic enteroviral meningitis or meningoencephalitis, but results have varied. Neonates with overwhelming enteroviral sepsis and meningitis have received intravenous immune globulin (IVIG), maternal plasma, and exchange

transfusions with occasional success. In a single randomized trial of IVIG plus standard therapy versus standard therapy alone in neonates suspected of having enteroviral infection during the first 2 weeks of life, IVIG failed to reduce viremia or lead to significant outcome differences, although the study enrolled too few patients for a definitive conclusion[220]; 75% of these patients had clinical or laboratory evidence of meningitis. IVIG has been routinely used for the treatment of disease caused by EV 71, although uncertainty remains as to whether this therapy is effective.[9]

Bacterial Meningitis

Despite the availability of effective bactericidal antimicrobial agents in the treatment of bacterial meningitis, morbidity and mortality from this disorder remain unacceptably high. The complexity of the management of bacterial meningitis, which includes a number of important complications, such as meningoencephalitis, systemic compromise, stroke, and raised intracranial pressure, has led to examination of various adjunctive strategies to improve outcome.[503] These have included antiinflammatory agents and methods to reduce increased intracranial pressure. Management in an intensive care unit (ICU) is recommended in patients with bacterial meningitis in order to recognize changes in the patient's consciousness, monitor for development of new neurologic signs, monitor for seizures, treat severe agitation, and manage complications (e.g., septic shock, hyponatremia, and increased intracranial pressure).[503] In one study of patients with pneumococcal meningitis, mortality was reduced to 5.5% (from 24% in a previous analysis), likely a result of routine use of adjunctive corticosteroids and other interventions performed in the ICU,[504] although the direct impact of a single intervention remains unclear.

Antiinflammatory Agents

Because the subarachnoid space inflammatory response is a major factor contributing to morbidity and mortality, investigators have examined whether attenuation of this response would improve outcome in bacterial meningitis. Adjunctive dexamethasone has been the agent most extensively studied in experimental animal models and in patients, and it should now be used in the initial approach to most patients with acute bacterial meningitis (see earlier section, "Initial Management of Patients With Acute Meningitis," for a description of experimental studies and clinical trials of adjunctive dexamethasone in bacterial meningitis).

Other agents that reduce subarachnoid space inflammation have also been examined as possible adjuncts in the treatment of bacterial meningitis. Pentoxifylline (a phosphodiesterase inhibitor that decreases endotoxin-induced TNF-α production, attenuates the inflammatory action of IL-1 and TNF on leukocyte function, and blocks the lipooligosaccharide–induced release of TNF and IL-1 from microglial cell cultures) has been examined in an experimental rabbit model of *H. influenzae* type b meningitis.[505] Administration of pentoxifylline 20 minutes before intracisternal challenge with *H. influenzae* type b lipooligosaccharide significantly reduced CSF concentrations of leukocytes, protein, and lactate. However, dexamethasone was superior to pentoxifylline in modulation of these CSF inflammatory changes, and no appreciable synergism was observed when both agents were administered. Thalidomide, which also inhibits TNF-α production, was studied in an experimental rabbit model of meningitis. Thalidomide reduced TNF-α production after intracisternal challenge with either *H. influenzae* type b or *S. pneumoniae,* although it had a relatively greater effect on the inflammatory response to *S. pneumoniae.*[506] Adjunctive treatment with a C5-specific monoclonal antibody (C5-Ab) was studied in an experimental pneumococcal mouse model. Neutralization experiments showed that adjunctive treatment with C5-Ab improved outcome in mice with pneumococcal meningitis. The observed effect of C5-Ab was superior to that of adjunctive dexamethasone.[503]

Other studies have examined the effects of a monoclonal antibody (IB4) directed against the CD18 family of receptors on leukocytes to reduce CSF inflammation. Intravenous inoculation of IB4 blocked the accumulation of leukocytes in CSF despite intracisternal challenge with *H. influenzae* type b, *N. meningitidis,* pneumococcal cell wall, or lipooligosaccharide.[507] Furthermore, the monoclonal antibody was effective in preventing the development of cerebral edema and death in animals challenged with lethal doses of *S. pneumoniae.* In a study using an experimental rabbit model of *H. influenzae* type b meningitis,[508] the concomitant administration of dexamethasone and IB4 led to a marked attenuation of all indices of meningeal inflammation and a reduction in brain water content when compared with the results obtained in untreated animals or when each agent was used alone.

Bactericidal/permeability-increasing protein, which is present in the azurophilic granules of neutrophils and binds to and neutralizes the biologic activity of the lipid A portion of lipooligosaccharide, has also been studied for its effects on CSF inflammation in bacterial meningitis. In an experimental rabbit model, the intracisternal inoculation of recombinant bactericidal/permeability-increasing protein significantly reduced CSF inflammation in response to meningococcal endotoxin.[509] This effect was not seen after systemic administration, probably because of failure of this protein to cross the BBB.

Although experimental results with these agents have shown a benefit in reduction of CSF inflammation in animals with bacterial meningitis, none has been studied in clinical trials. The main goal for new therapies will be based on studies that assess attenuation of the inflammatory response and studies of other agents that regulate damage mediated by reactive oxygen species and reactive nitrogen species, caspase inhibition, complement system activation, vascular integrity, or release of danger-associated molecular patterns that sustain the immune reaction and drive forward brain damage even after CSF sterilization[510–512] Pending randomized clinical trials of these therapies, likely in combination with antimicrobial therapy and dexamethasone, their use in patients cannot be recommended at present.

Another promising strategy has been to use nonbacteriolytic antibiotics to attenuate the inflammatory response generated after antimicrobial-induced lysis. In a pilot study in children (3 months to 12 years of age) with bacterial meningitis, patients were randomly assigned to receive one dose of rifampin 30 minutes before administration of ceftriaxone compared with use of ceftriaxone alone.[511] Children in the rifampin pretreatment group had significantly lower CSF concentrations of TNF-α, S100B, and neuron specific enolase; rifampin-pretreated patients also had reduced morbidity and neurologic sequelae, although the differences were not statistically significant. However, given the small and heterogeneous nature of these groups, more data are needed.

Reduction of Intracranial Pressure

For patients with bacterial meningitis who have signs of increased intracranial pressure (e.g., altered level of consciousness; dilated, poorly reactive, or nonreactive pupils; ocular movement disorders) and who are stuporous or comatose, it has been suggested that intracranial pressure–guided treatment could be beneficial.[513] However, it is unclear which patients may benefit from such an approach, and interventions to decrease the intracranial pressure may be harmful as well. A study in 1412 episodes of bacterial meningitis showed that half of the patients had a very CSF high openings pressure at the lumbar puncture (>40 cm H_2O/>29 mm Hg), of whom the majority had a favorable outcome.[60] Studies advocating reduction in intracranial pressure have advised to reduce the pressure below 20 mm Hg. In one study of 15 patients with bacterial meningitis in whom intracranial pressure was measured,[514] intracranial pressure was successfully lowered in most patients by a broad range of measures and using unconventional volume-targeted ("Lund concept") intracranial pressure management, which consisted of sedation, corticosteroids, normal fluid and electrolyte homeostasis, blood transfusion, albumin infusion, decrease of mean arterial pressure, treatment with a prostacyclin analogue, and eventually thiopental, ventriculostomy, and dihydroergotamine. In nonsurvivors, mean intracranial pressure was significantly higher and cerebral perfusion pressure was markedly lower than in survivors despite treatment; however, this was not a comparative study and the results should be interpreted with caution.

Several methods are available to reduce intracranial pressure,[290,513] including elevation of the head of the bed to 30 degrees to maximize venous drainage with minimal compromise of cerebral perfusion; hyperventilation to maintain the $Paco_2$ between 27 and 30 mm Hg, which causes cerebral vasoconstriction and a reduction in cerebral blood volume; use of hyperosmolar agents (e.g., mannitol) to make the intravascular space hyperosmolar to the brain and permit movement

of water from brain tissue into the intravascular compartment; and corticosteroids. However, some experts have questioned the routine use of hyperventilation to reduce intracranial pressure in patients with bacterial meningitis. In infants and children with bacterial meningitis who have initially normal CT scans of the head, hyperventilation can safely reduce elevated intracranial pressure because it is unlikely that cerebral blood flow would be reduced to ischemic thresholds. However, in children with cerebral edema evident on head CT, cerebral blood flow is more likely to be normal or reduced. Although hyperventilation might decrease intracranial pressure, it would do so at the cost of a significant reduction in cerebral blood flow, possibly approaching ischemic thresholds. These patients may benefit more from the early use of diuretics, osmotically dehydrating agents (provided that intravascular volume is protected), and corticosteroids; however, controlled trials exploring these issues have yet to be performed.

Glycerol, an osmotic dehydrating agent that can be given orally, has been evaluated in a trial of 122 infants and children with bacterial meningitis.[515] Patients in this study were randomized to receive adjunctive intravenous dexamethasone, oral glycerol, dexamethasone plus glycerol, or neither. Seven percent of the glycerol-treated patients and 19% of those not given glycerol had audiologic or neurologic sequelae (P = .052). A more recent clinical trial suggested that oral glycerol (6 g/kg/day in four divided doses) prevented severe neurologic sequelae in children with bacterial meningitis,[516] although methodologic questions have been raised about this study.[517] In a study of 383 children with bacterial meningitis randomly assigned to receive dexamethasone, dexamethasone plus glycerol, glycerol, or placebo, neither dexamethasone nor glycerol prevented hearing loss[518]; presenting status and young age were the factors that predicted hearing impairment. In addition, a randomized controlled trial of 265 Malawian adults with bacterial meningitis showed that adjuvant glycerol was harmful and increased mortality[519]; therefore adjunctive glycerol cannot be recommended in adults with bacterial meningitis in resource-poor settings with a high HIV prevalence.[520] Further placebo-controlled, blinded studies are required before glycerol can be routinely recommended in patients with bacterial meningitis.

Induced hypothermia, used for treatment of cerebral hypoxemia after cardiac arrest and in animal models, has also been shown to reduce intracranial hypertension in meningitis.[488] Despite some observational studies showing benefit, a randomized controlled trial was stopped early because of an increased risk of death in patients in the intervention group.[521]

Patients who continue to have elevated intracranial pressures despite the aforementioned measures may be treated with high-dose barbiturate therapy,[290,522] which decreases cerebral metabolic demands and cerebral blood flow. Barbiturates can also cause vasoconstriction in normal tissue, thereby shunting blood to ischemic tissue and protecting the brain from ischemic insult. During the administration of pentobarbital, the patient is monitored to measure decreases in intracranial pressure or the dose can be titrated to the development of a burst suppression pattern on the EEG. Cardiac parameters also need to be monitored (by placement of a Swan-Ganz catheter) because of the risk for cardiac toxicity (e.g., decreased cardiac output, decreased contractile force, arrhythmias) with high-dose barbiturate therapy. This mode of treatment of meningitis and elevated intracranial pressure is of unproven benefit, however, and must be considered experimental.

In patients who develop hydrocephalus, repeated lumbar punctures or placement of a lumbar drain can reduce intracranial pressure,[523] although invasive procedures should be withheld in patients with mild enlargement of the ventricular system and without clinical deterioration.

Surgery

Surgical intervention may be required in some patients with bacterial meningitis. Patients who have sustained a basilar skull fracture with CSF leak may have persistent dural defects that can lead to recurrent episodes of bacterial meningitis.[20] Many leaks will cease spontaneously, but surgery is indicated for leaks that persist for several weeks or in patients who present with delayed or recurrent infection. Surgery is not indicated in the acute phase (before 7 days) of leakage[70]; no difference in outcome is seen when patients with acutely repaired leaks are compared with those whose leaks stop spontaneously within 7 days. Surgical intervention may also be required in patients in whom recurrent meningitis develops from congenital or acquired cranial defects and dermal sinuses.

Helminthic Meningitis

In seriously ill patients with *A. cantonensis* meningitis, the major treatment objective is control of intracranial pressure; repeated lumbar punctures can produce dramatic, although transient, clinical improvement.[523–527] Anecdotal case series have shown benefits of corticosteroids, although one large observational study in Thailand concluded that prednisone was not effective. However, in another placebo-controlled study from Thailand, a 2-week course of prednisolone (60 mg/day) was effective and safe in patients with eosinophilic meningitis (most cases presumptively caused by *A. cantonensis*).[524] Those patients who received corticosteroids demonstrated improved rates of headache resolution, a more rapid improvement in symptoms, and less need for repeat lumbar punctures. A Cochrane Database systematic review concluded that corticosteroids did significantly help relieve headache in patients with eosinophilic meningitis.[525] Although the role of anthelmintic therapy is controversial, some studies have evaluated combined therapy with corticosteroids and anthelmintics (albendazole and mebendazole) and have suggested a potential benefit.[526,527] More studies are needed.

PREVENTION

Bacterial Meningitis

Haemophilus influenzae

Chemoprophylaxis

It has become clear that the spread of several types of bacterial meningitis can be prevented with prophylaxis of contacts of cases with antimicrobial agents. Several studies have documented the transmission of *H. influenzae* type b from patients with meningitis to household contacts.[290] The risk is markedly age dependent, highest for children younger than 2 years. Most secondary cases (75%) occur within 6 days of onset of the index case, although untreated household contacts remain at increased risk for *H. influenzae* type b disease for at least 1 month after onset in the index patient. Daycare outside the home is considered another risk factor for transmission.[528] Secondary disease is more likely to develop in children younger than 2 years. Controversy regarding the magnitude of the risk to children in daycare settings, however, has led to disagreement concerning the recommendation for chemoprophylaxis of children in these facilities.

The rationale for the use of chemoprophylaxis for prevention of secondary disease is eradication of nasopharyngeal colonization of *H. influenzae* type b, thereby preventing transmission to young, susceptible contacts and the development of invasive disease in those already colonized. The recommended chemoprophylactic agent of choice is rifampin (20 mg/kg, maximum 600 mg, daily for 4 days) for all individuals, including adults, in households with at least one unvaccinated or incompletely vaccinated child younger than 48 months (see Chapter 225).[290] One study suggested that 2 days of rifampin therapy was as efficacious as 4 days of treatment, although further study is required before a recommendation to shorten the duration of prophylaxis can be made. The index patient may also need to receive rifampin prophylaxis. Ampicillin and chloramphenicol, unlike ceftriaxone and cefotaxime, do not effectively eliminate nasopharyngeal colonization. Rifampin is not recommended for pregnant women who are contacts of infected infants, because the risk of rifampin to the fetus has not been established. Chemoprophylaxis is not currently recommended for daycare contacts 2 years old or older unless two or more cases occur in the daycare center within a 60-day period. For children younger than 2 years, the CDC recommends prophylaxis for daycare contacts whereas the American Academy of Pediatrics does not in most cases. The question of whether to administer prophylaxis in this setting needs to be individualized and should be considered more strongly in daycare centers that resemble households, where children have prolonged contact.

Neisseria meningitidis

Chemoprophylaxis

Chemoprophylaxis is also necessary for close contacts of patients with invasive meningococcal disease[290]; up to 10% of patients with

meningococcal meningitis have had contact with an individual with another known case. The definition of "close contact" has not been clearly elucidated but generally refers to persons who have had prolonged (≥8 hours) contact while in close proximity (≤3 feet) to the patient or who have been directly exposed to the patient's oral secretions (e.g., through kissing, mouth-to-mouth resuscitation, endotracheal intubation, or endotracheal tube management) within 1 week before the onset of the patient's symptoms until 24 hours after initiation of appropriate antimicrobial therapy.[528,529] The estimated prevalence of meningococcal carriage in the United States is 5% to 10% under nonepidemic conditions. In closed populations such as military recruits, carriage rates can reach levels of 40% to 90%. Carriage may last for a long time (at least months) in about 25% of carriers, is intermittent in one-third, and is transient or infrequent in the remaining 40%.[528,529] Household contacts exposed to an individual with meningococcal disease have a 500- to 800-fold increased risk for invasive disease.[290] College students, especially those living in dormitories, are at moderately increased risk for meningococcal disease compared with other persons of their age.[530] Secondary systemic meningococcal disease often develops within 5 days of recognition of the index case, with 70% to 80% of secondary cases occurring within 14 days of the primary case; in one report, 9 of 17 (53%) secondary cases occurred 5 to 39 weeks after the primary case. Transmission of *N. meningitidis* has also been documented in a campus bar, a dance club, and a sports club.

Chemoprophylaxis is recommended for close contacts of the index patient, defined as household contacts, daycare center members, and anyone directly exposed to the patient's oral secretions; chemoprophylaxis should also be administered to close contacts who have received vaccination with the meningococcal conjugate vaccine (see later discussion), because the vaccine does not confer protection against all meningococcal serogroups.[86] Meta-analysis of data from high-resource settings has shown that prophylaxis of household contacts was associated with an 84% reduction in the risk of meningitis.[530] For travelers, chemoprophylaxis should be considered for any passenger who had direct contact with respiratory secretions from an index patient or anyone seated directly next to an index patient in a prolonged flight (lasting 8 hours or longer). Chemoprophylaxis is not recommended for school, work, or transportation contacts. Chemoprophylaxis may also need to be administered to the index patient before hospital discharge because certain antimicrobial agents (e.g., high-dose penicillin or chloramphenicol) do not reliably eradicate meningococci from the nasopharynx of colonized patients. Chemoprophylaxis should be administered as soon as possible (ideally within 24 hours) after the case is identified; administration 14 or more days after the onset of illness in the index patient is probably of limited value.

The optimal chemoprophylactic agent to prevent invasive meningococcal disease is controversial.[290] The CDC currently recommends the administration of rifampin, ciprofloxacin, or ceftriaxone, which are all 90% to 95% effective at eradicating nasopharyngeal carriage.[86] Rifampin is given at 12-hour intervals for 2 days in the following doses: adults, 600 mg; children 1 month or older, 10 mg/kg; and infants younger than 1 month, 5 mg/kg. However, rifampin has several shortcomings, including adverse events, necessity for multiple doses over a 2-day period, and emergence of resistant organisms (up to 10%–27% of isolates), which may then cause invasive disease. In the search for alternative agents, ceftriaxone (250 mg IV in adults and 125 mg IV in children) eliminated the serogroup A carrier state in 97% of patients in one study for up to 2 weeks, although parenteral administration is required. Additional studies have demonstrated a single oral dose of ciprofloxacin (500 mg in adults) to be very effective in elimination of the nasopharyngeal carriage of *N. meningitidis*. Ciprofloxacin concentrations in nasal secretions have been shown to exceed the MIC_{90} for meningococci. Ciprofloxacin may well supplant rifampin for chemoprophylaxis in adults. Ciprofloxacin is not recommended for use in children because of concern regarding cartilage damage. However, three cases of ciprofloxacin-resistant *N. meningitidis* have been reported in North Dakota and Minnesota, leading the CDC to no longer recommend ciprofloxacin for meningococcal chemoprophylaxis in selected counties of these states.[531] Decreased in vitro susceptibility to fluoroquinolones has also been reported in South Africa,[532] indicating the necessity for continued surveillance. In pregnant patients, ceftriaxone is probably the safest alternative agent for chemoprophylaxis. Azithromycin (500 mg orally once) was also shown to be as effective as a four-dose regimen of rifampin in the eradication of meningococci from the nasopharynx.[533] Azithromycin is safe, easy to administer, and also available in a suspension form, but further evaluation is needed. Widespread chemoprophylaxis to low-risk contacts should be discouraged because of the concern over emergence of resistant organisms and possible future limitations on this approach.

Streptococcus pneumoniae

Chemoprophylaxis

The risk for secondary pneumococcal disease in contacts of infected patients has not been defined, although outbreaks have been described in closed populations such as gold miners, military recruits, and jail inmates.[290] In one outbreak in a daycare center,[534] treatment of 97% of the daycare center children and staff with rifampin (10 mg/kg twice daily for 2 days) resulted in a 70% reduction (i.e., only partial eradication) of positive nasopharyngeal cultures for *S. pneumoniae* but did not prevent new acquisition of this organism by three children and one family member. Further studies are needed before chemoprophylaxis is recommended for contacts of patients with pneumococcal meningitis. Some authors do recommend prophylaxis with oral penicillin in patients with sickle cell disease; therapy in such patients has been shown to reduce the incidence of pneumococcal bacteremia by 84%.[290]

Streptococcus agalactiae

Chemoprophylaxis

Administration of ampicillin during labor to mothers with prenatal vaginal or rectal group B streptococcal colonization and obstetric risk factors (e.g., premature labor, prolonged rupture of membranes, or intrapartum fever) has been associated with reduced rates of colonization and early-onset streptococcal sepsis in the neonate.[535] The CDC has established guidelines for the prevention of group B streptococcal infection by chemoprophylaxis.[536] Indications are a previous infant with invasive group B streptococcal disease, group B streptococcus bacteriuria during any trimester of the current pregnancy (unless a cesarean delivery is performed before onset of labor in a woman with intact amniotic membranes), or positive group B streptococcus vaginal-rectal screening culture in late gestation (optimally at 35–37 weeks' gestation). If the group B streptococcus status at the onset of labor is unknown, intrapartum antimicrobial prophylaxis should be administered for delivery at less than 37 weeks' gestation, amniotic membrane rupture greater than or equal to 18 hours, intrapartum temperature greater than or equal to 38.0°C (100.4°F), or an intrapartum nucleic acid amplification test positive for group B streptococcus. Chemoprophylaxis should consist of intrapartum intravenous penicillin G (5 million units initially, and then 2.5–3 million units every 4 hours) or alternatively intravenous ampicillin (2 g initially, and then 1–2 g every 4 hours) until delivery. Intravenous cefazolin can be used in patients with penicillin allergy, although intravenous clindamycin should be used in those with severe penicillin allergy (i.e., anaphylaxis, angioedema, respiratory distress, or urticaria) when the isolate is susceptible to clindamycin and erythromycin; if the isolate is not susceptible to clindamycin and erythromycin, vancomycin is recommended.

Basilar Skull Fracture

A number of studies have used prophylactic antibiotics in patients with basilar skull fractures and CSF leak on the premise that in patients with a dural defect the CSF is exposed to pathogenic organisms from the nasopharynx, nasal or mastoid sinuses, or external auditory canal.[537,538] Interpretation and comparison of the various studies examining this question are confounded by multiple variables, including patient selection, choice of antimicrobial agents, and definition of infection. No prospective controlled trials have examined the efficacy of prophylactic antimicrobial agents in these patients, although a meta-analysis suggested that antibiotic prophylaxis did not prevent meningitis in patients with basilar skull fracture.[537] These data have also been analyzed in a Cochrane Database systematic review[538] in which prophylactic antibiotic use in patients with basilar skull fracture, whether or not there was evidence of CSF leak, was not supported. However, published studies have biases and

randomized controlled trials are needed. Antibiotic use does not appear to change the incidence of posttraumatic bacterial meningitis and may result in the selection and growth of resistant organisms.

Haemophilus influenzae

Immunoprophylaxis

Vaccination to prevent infection with specific meningeal pathogens is a very useful measure for decreasing the incidence of bacterial meningitis. For *H. influenzae* type b, the availability of conjugate vaccines has decreased the number of cases of *H. influenzae* type b meningitis more than 90% in recent years.[41,62,63] Three *H. influenzae* type b conjugate vaccines are now licensed for infant immunization. The Advisory Committee on Immunization Practices (ACIP) has recommended vaccine doses at 2, 4, and 6 months of age[539]; if PRP-OMP (PedvaxHIB; Merck & Co., Whitehouse Station, NJ) is administered at 2 and 4 months, a dose at 6 months is not required. A booster dose of vaccine is also administered at 12 to 15 months of age, no matter which vaccine was administered for the primary series. In addition to preventing invasive disease caused by *H. influenzae* type b, conjugate vaccines are effective in reducing nasopharyngeal colonization and therefore may confer protection to populations not targeted for immunization through herd immunity. However, this reduction may open ecologic niches for non–type b strains of *H. influenzae.* In a surveillance study conducted in Brazil before and after the introduction of *H. influenzae* type b conjugate vaccine immunization, the incidence of *H. influenzae* type b meningitis decreased by 69%, but the incidence of *H. influenzae* type a meningitis increased eightfold,[540] highlighting the importance of maintaining surveillance to monitor potential increases in disease due to serotype replacement. Of additional concern is the report of cases of invasive *H. influenzae* type b disease in children previously vaccinated against this disease in Nottingham, United Kingdom.[541] There are several possible reasons for this increase, most notably that the vaccination schedule used in the United Kingdom is to vaccinate at 2, 3, and 4 months of age; the absence of a booster dose may have led to this increase in invasive disease.[542] In the developing world, declining rates of *H. influenzae* type b meningitis have also been reported since introduction of the *H. influenzae* type b conjugate vaccines,[543] with effectiveness of 88% to 94%.[544–546]

Neisseria meningitidis

Immunoprophylaxis

On the basis of the success of conjugate *H. influenzae* type b conjugate vaccines (see earlier discussion), conjugate vaccines for use against disease caused by serogroups A and C meningococci have been developed. The United Kingdom became the world's first country to implement vaccination with a monovalent serogroup C conjugate meningococcal vaccine, in which children are vaccinated at 2, 3, and 4 months of age.[547] The efficacy of the meningococcal serogroup C conjugate vaccine in adolescents and toddlers was evaluated after the first 9 months after vaccine introduction in a catch-up program in which toddlers and adolescents received a single dose of CRM_{197}-meningococcal C vaccine.[548] The short-term vaccine efficacies in toddlers and adolescents were 92% and 97%, respectively. In an update of the epidemiologic effect of the first 18 months of the UK meningococcal C conjugate vaccine program, the overall reduction of cases of serogroup C disease from 1998–1999 to 2000–2001 was 81%,[549] with some variability based on age group. In another case-control study in teenagers in the United Kingdom to assess vaccine efficacy, the protective effectiveness of the vaccine was 93%.[550] Furthermore, carriage of meningococci was reduced by 66% in students aged 15 to 17 years,[551] demonstrating that meningococcal C conjugate vaccines protect against carriage of meningococci that express serogroup C polysaccharide capsules. Although concerns have been raised that use of the serogroup C meningococcal conjugate vaccine would result in an increase in meningococcal disease caused by non–serogroup C isolates, this has not been realized.[552] The reduction in serogroup C carriage lasted for at least 2 years in one multicenter survey of carriage since vaccine introduction, also with no evidence of serogroup replacement.[553]

The first meningococcal conjugate vaccine (meningococcal polysaccharide–diphtheria toxoid conjugate vaccine containing serogroups A, C, W, and Y polysaccharides) was licensed for use in the United States in January 2005 for routine administration, starting at age 11 to 12 years with catch-up vaccination for 15-year-old adolescents and those entering high school.[554] These recommendations were later revised to include routine vaccination of all persons aged 11 to 18 years with one dose.[555] In updated guidelines, a booster dose is now recommended at age 16 years, and a two-dose primary series is administered 2 months apart for persons aged 2 through 54 years with persistent complement component deficiency or functional or anatomic asplenia and for adolescents with HIV infection[556]; other persons at risk for meningococcal disease (e.g., microbiologists or travelers to an epidemic or highly endemic country) should receive a single dose. A two-dose primary series is also recommended for any child 9 to 23 months of age at increased risk for invasive meningococcal disease.[557] In adolescents, protective antibodies will likely persist as long as and probably longer than after administration of the meningococcal polysaccharide vaccine.[558,559] The vaccine may also provide herd immunity by decreasing nasopharyngeal colonization.[560] Immunization of infants with a novel tetravalent CRM_{197}-conjugated meningococcal vaccine was well tolerated and immunogenic,[558] suggesting its usefulness in this patient population.

After decades of research toward this end, two vaccines for the prevention of meningococcal serogroup B disease were recently approved for use in the United States.[529,561–564] The first of these, Trumenba (Pfizer, New York, NY), was approved for use in 2014 for individuals aged 10 through 25 years, administered in three intramuscular injections on a 0-, 2-, and 6-month schedule. The second, Bexsero (Novartis, New York, NY), approved in 2015 also for persons aged 10 through 25 years, is administered in two intramuscular injections at least 1 month apart. Both are associated with high rates of local and systemic adverse reactions (i.e., injection site pain, myalgias, fatigue, and headaches). The multicomponent meningococcal B vaccine has been shown to achieve satisfactory immune response in children and adolescents within 30 days of vaccination.[566] The ACIP recommendation is that adolescents and young adults, ages 16 to 23 years, *may* be vaccinated for short-term protection against most strains of *N. meningitidis* serogroup B (Category B) but that the risk of that infection in the United States is currently low (see Chapter 211 for more information).[566]

Streptococcus pneumoniae

Immunoprophylaxis

Use of the current 23-valent pneumococcal vaccine in the prevention of pneumococcal meningitis has never been proved, although it may be assumed that the overall efficacy of the vaccine is about 50% against pneumococcal meningitis (with a wide 95% confidence interval [CI]); in addition, no data have proved efficacy in infants and very young children.[189] One study examined the efficacy of a heptavalent conjugate pneumococcal vaccine in 37,868 infants and children and demonstrated an efficacy of 97.4% in the prevention of invasive pneumococcal disease in fully vaccinated children,[567] leading to the recommendation to vaccinate all infants younger than 2 years of age with the currently licensed heptavalent pneumococcal conjugate vaccine (Prevnar; Wyeth Pharmaceuticals, Philadelphia, PA)[568]; the vaccine is administered in four doses at 2, 4, 6, and 12 to 15 months of age.[569] In a study conducted in Israel, administration of a 9-valent pneumococcal conjugate vaccine to toddlers attending daycare centers led to a reduction in nasopharyngeal carriage of *S. pneumoniae,*[569] suggesting that widespread vaccination may result in marked herd immunity. In a study of population-based data from the Active Bacterial Core surveillance system of the CDC on trends in the rate of invasive pneumococcal disease after licensure of the heptavalent pneumococcal vaccine, there was a decline in the rate of invasive pneumococcal disease, with the largest decline (69%) in children younger than 2 years.[571] Disease also fell in adults (32% lower for those 20–39 years of age, 8% lower for those 40–64 years of age, and 18% lower for those 65 years and older) and in persons with invasive disease caused by pneumococcal strains that were not susceptible to penicillin (35% reduction). These findings were verified in other studies that reported a decrease in the incidence of invasive pneumococcal disease[572–574]; the rate of antimicrobial resistant pneumococcus decreased with use of the conjugate vaccine. Significant decreases in the case-fatality and mortality rates for invasive pneumococcal disease have also been

reported.[575] However, questions have arisen regarding an increase in disease caused by serotypes not in the vaccine and the fact that vaccine efficacy has not yet been studied in the developing world.[576] Since the introduction of the pneumococcal conjugate vaccine in the United States, pneumococcal meningitis rates have decreased by 33% from 0.8 to 0.55 cases per 100,000 population, with the decrease greatest in children younger than 5 years[577]; hospitalization for pneumococcal meningitis has also decreased in both children and adults. Declines in the incidence of pneumococcal meningitis have been reported in Spain without evidence of an increase in disease caused by serotype replacement.[578] However, since introduction of the heptavalent pneumococcal conjugate vaccine, there has been an emergence in cases of invasive pneumococcal disease caused by serotypes that are not in the vaccine.[104,578–580] In a CDC review, the overall incidence of invasive pneumococcal disease among children younger than 5 years declined from 98.7 cases per 100,000 population in 1998–1999 to 23.4 cases per 100,000 population in 2005,[580] although there was an increase in cases caused by serotype 19A (from 2.6 to 9.3 cases per 100,000 population). This increase in nonvaccine serotypes has been small compared with the decline in disease caused by serotypes in the vaccine. Although rates of pneumococcal meningitis have been reported to decrease among children and adults since introduction of the heptavalent pneumococcal conjugate vaccine (1.13 cases to 0.79 cases per 100,000 persons between 1998–1999 and 2004–2005), the increase in meningitis caused by serotypes not in the vaccine (specifically serotypes 19A, 22F, and 35B) is of concern and has included some strains that are not susceptible to many antibiotics.[104] There was also a 59% reduction in pneumococcal meningitis in children younger than 14 years of age in Uruguay after introduction of heptavalent vaccine into the routine vaccination schedule.[582] However, it should be noted that serotype replacement has occurred after introduction of the heptavalent pneumococcal conjugate vaccine,[583,584] with invasive pneumococcal disease seen in most populations. The ACIP now recommends use of the 13-valent pneumococcal conjugate vaccine to prevent pneumococcal disease in infants and young children aged younger than 6 years[585]; this vaccine has activity against the serotypes that were present in the heptavalent vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F) along with six additional serotypes (1, 3, 5, 6A, 7F, and 19A). Adults 19 years of age and older and with immunocompromising conditions should receive the 13-valent pneumococcal conjugate vaccine followed by the 23-valent pneumococcal polysaccharide vaccine at least 8 weeks later.[585,586] Most recently, the ACIP has recommended that all adults aged 65 years and older should also receive both the 13-valent pneumococcal conjugate vaccine and the 23-valent pneumococcal polysaccharide vaccine.[587]

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88 Chronic Meningitis

John E. Bennett and Susan E. Hoover

SHORT VIEW SUMMARY

Definition
- Chronic meningitis, defined here as at least 4 weeks of symptoms with signs of inflammation in the cerebrospinal fluid (CSF), must be distinguished from recurrent aseptic meningitis, chronic myeloradiculitis, and chronic encephalitis.

Etiology
- Major causes are fungal infections, tuberculosis, syphilis, and malignancy (see Table 88.2)

Diagnosis
- Major diagnostic tools are gadolinium-enhanced magnetic resonance imaging (MRI) and CSF studies (see Table 88.1). Surgical biopsy has a low yield.
- Polymerase chain reaction assay has taken on an increasing role, including its use in the diagnosis of Whipple disease, chronic enteroviral meningitis, lymphoma, and tuberculosis.

Therapy
- Empirical therapy for suspected tuberculous meningitis is often used because of disease severity, but addition of prednisone may cause deterioration of unsuspected fungal meningitis. Worsening of infection may not be detected initially because corticosteroids can temporarily improve hypoglycorrhachia, fever, and cerebral edema on T2-weighted MRI. The same issue arises with corticosteroid treatment of suspected sarcoidosis or autoimmune meningitis. Only when subsequent, potentially irreversible deterioration occurs despite corticosteroids is an infectious cause found. The benefit of corticosteroids in empirical regimens is usually outweighed by the harm.

This chapter focuses on the differential diagnosis of chronic meningitis. The reader is referred to the chapters on individual infections for discussions of their management.

DISTINCTION FROM OTHER CHRONIC CENTRAL NERVOUS SYSTEM INFECTIONS

Indolent infections in the spinal cord and brain manifest quite differently from infections in the meninges and are discussed briefly before addressing the topic of chronic meningitis.

MYELITIS, MYELORADICULITIS, AND POLYRADICULITIS

Although "myelo" refers to spinal cord and "radiculo" refers to the nerve roots, infection of the spinal cord generally results in a pathologic process of the nerve roots and vice versa. The clinical presentation may be acute or chronic. Examples of acute presentations are transverse myelitis, Guillain-Barré syndrome, poliomyelitis, and infections due to West Nile virus and coxsackievirus. Chronic myeloradiculitis can result from syphilis (tabes dorsalis),[1] human T-cell lymphotropic virus type 1 (tropical spastic paraparesis),[2] cytomegaloviral polyradiculopathy in patients with acquired immunodeficiency syndrome (AIDS),[3] herpes simplex virus infection, human immunodeficiency virus type 1 (HIV-1) infection, and schistosomiasis (usually caused by *Schistosoma haematobium*).[4] Meningeal carcinomatosis, spinal cord tumors, and spinal epidermoid cysts may manifest as chronic myeloradiculitis. Patients with cat-scratch encephalomyelitis may present subacutely with spinal cord signs.[5] Myeloradiculitis may be a prominent feature of tuberculous meningitis, but the severe systemic features of disseminated tuberculosis easily distinguish this entity from other causes of chronic myeloradiculitis. Initial symptoms of chronic myeloradiculitis commonly include decreased muscular strength in the extremities, loss of bladder control, and, less commonly, back pain or shooting pains in the torso or extremities. Examination may reveal decreased deep tendon reflexes, loss of proprioception in the extremities, decreased rectal sphincter tone, or decreased perception of pain or light touch in the extremities. Symptoms and signs are most marked in the lower extremities. Mild-to-moderate cerebrospinal fluid (CSF) pleocytosis and increased CSF protein level are usual findings, but hypoglycorrhachia is not found. Gadolinium-enhanced magnetic resonance imaging (MRI) may show meningeal enhancement along the cord or cauda equina. Nerves in the cauda equina may appear clumped on axial views of MRI. Epidural or intradural abscess and malignant lesions causing spinal cord symptoms can be distinguished from myeloradiculitis on MRI.

Cauda equina syndrome is a constellation of neurologic symptoms and signs due to myeloradiculitis in the conus medullaris, including urinary incontinence, residual bladder urine, unilateral or bilateral leg or sciatic pain, leg weakness, saddle anesthesia, sexual dysfunction, and loss of ankle tendon reflexes. Some patients receiving *Exserohilum*-contaminated methylprednisolone injections developed cauda equina syndrome as infection crossed the dura mater to form abscesses in the cauda equina.[6]

ENCEPHALITIS

Patients with encephalitis (see Chapter 89) most often present with an acute disease, although infection may be diphasic, and sequelae may persist long after the acute infection has resolved. Acute disseminated encephalomyelitis manifests typically 1 to 3 weeks after a viral infection with a monophasic, rapidly progressing illness—usually with fever, headache, somnolence, and seizures. Cranial nerve palsies and other focal neurologic signs may appear. Indolent onset is usual in some causes of chronic encephalitis such as syphilis (general paresis), subacute sclerosing panencephalitis (measles virus), Lyme disease *(Borrelia burgdorferi)*, African trypanosomiasis, Whipple disease *(Tropheryma whipplei)*, bartonellosis (cat-scratch disease), autoimmune encephalopathy with anti–*N*-methyl-D-aspartate receptor antibody, HIV-1 infection, and progressive multifocal leukoencephalopathy (polyomavirus). Rabies is delayed in onset but rapidly becomes more severe once symptoms develop. Although chronic encephalitis symptoms overlap symptoms of chronic meningitis, dementia and personality change are early and prominent signs in chronic encephalitis. In contrast to acute presentations

of viral encephalitis with fever and headache, patients with chronic encephalitis may have little fever or headache. Depending on the etiology, seizures and focal signs may occur. CSF pleocytosis and increased protein are usual, but hypoglycorrhachia is rare.

BRAIN ABSCESS

Brain abscess (see Chapter 90) may cause intense headache, but focal signs and seizures soon predominate. Similar to chronic meningitis, fever is not a predominant symptom in brain abscess. Gadolinium-enhanced MRI is the most sensitive imaging technique for focal intracerebral lesions but does not reliably distinguish abscesses from tumors or intracerebral granulomas such as those due to toxoplasmosis, tuberculosis, cryptococcosis, or histoplasmosis. Cystic lesions and intracerebral calcifications of neurocysticercosis are well visualized on computed tomography. Fluorodeoxyglucose-labeled positron emission tomography can be used alone or with computed tomography to assess metabolic activity of the lesion, but distinction between infectious and malignant lesions is not simple.

Recurrent aseptic meningitis should be distinguished from chronic meningitis and should suggest herpes simplex virus (Mollaret meningitis), repeated episodes of drug-induced meningitis (e.g., from nonsteroidal antiinflammatory drugs or intravenous immune globulin), or epidermoid cysts and craniopharyngiomas intermittently discharging keratinaceous debris into the CSF.

CHRONIC MENINGITIS

Clinical Manifestations

For the purposes of this chapter, patients with chronic meningitis have the indolent onset of symptoms compatible with chronic central nervous system (CNS) infection for at least 4 weeks and have signs of chronic inflammation in the CSF.[7–9] Chronic meningitis must be distinguished from recurrent aseptic meningitis or persistent sequelae of encephalitis. A careful history from the patient or family member may be needed to date the onset of symptoms and to distinguish chronic from recurrent meningitis. What may have seemed like a sudden onset may, on further questioning, have been the culmination of a much longer process. Symptoms of chronic meningitis can wax and wane over weeks and months. An abnormal result of CSF testing may not have been repeated to see if improved symptoms were accompanied by an improvement in CSF abnormalities. Early symptoms of chronic meningitis include headache, nausea, and decreased memory and comprehension. When hydrocephalus complicates indolent meningitis, dementia can be a prominent finding, which is better related by family members than by the patient. Later symptoms of chronic meningitis include decreased vision, double vision, cranial nerve palsies, unsteady gait, emesis, and confusion.

Diagnosis

Physical Examination

Patients with chronic meningitis may have a normal physical examination, including absence of fever. Neurologic examination is the most frequent abnormality, with decreased recent and remote memory, confusion, apathy, papilledema, and cranial nerve palsies, particularly sixth nerve palsy and deafness. As cerebral edema causes brainstem compression, upper motor neuron signs, increased deep tendon reflexes, ankle clonus, a positive Babinski sign, and Cheyne-Stokes respiration may be noted. Other than cranial nerve palsies, neurologic signs are decidedly symmetrical. Resting tremor, rigidity, and decreased mental acuity occasionally suggest Parkinson disease.

Skin lesions may be an important source of biopsy material in sarcoidosis, cryptococcosis, coccidioidomycosis, blastomycosis, or sporotrichosis. Vitiligo and poliosis may suggest Vogt-Koyanagi-Harada syndrome. This syndrome may manifest as aseptic meningitis, although the most common manifestation is bilateral posterior uveitis. Lymphadenopathy may point toward sarcoidosis, lymphoma, or hematogenously disseminated tuberculosis or histoplasmosis. A dilated funduscopic examination may reveal retinal lesions of Behçet syndrome, Vogt-Koyanagi-Harada syndrome, sarcoidosis, tuberculosis, coccidioidomycosis, or cryptococcosis. The Argyll Robertson pupil sign is highly suggestive of neurosyphilis.

History

Exposure history can be helpful in that coccidioidomycosis is rare in patients who have never been in the southwestern United States or northern Mexico (see later discussion). The endemic area for histoplasmosis is broad, although in the United States it is uncommon in the Pacific Northwest and Rocky Mountain states. Immigrants from countries where tuberculosis is prevalent are at increased risk for reactivation, as are patients who have lived in a household where someone had tuberculosis. Neurocysticercosis occurs in residents of endemic countries but rarely in travelers to these regions. Lyme borreliosis is endemic in the northeastern United States. A history of a typical lesion of erythema migrans may suggest the diagnosis of Lyme disease. Travelers outside the United States may have been exposed to *Angiostrongylus* or *Brucella.*

A patient's occupation is rarely helpful diagnostically. Age is important, in that cryptococcosis is uncommon in the first decade of life. A history of risk factors for sexually transmitted disease should suggest the possibility of neurosyphilis or HIV-associated infections. Underlying disease is important in that AIDS and corticosteroid therapy are major predisposing factors to cryptococcal and *Acanthamoeba* meningitis.

Imaging

Contrast-enhanced MRI is the preferred test for imaging the brain and may show intracranial mass lesions, hydrocephalus (Fig. 88.1), pachymeningitis, or parameningeal foci such as mold sinusitis or actinomycosis of the paranasal sinuses or middle ear. Increased intracranial pressure may be deduced from flattening of the sulci over the cerebral convexities, particularly in younger patients without preexisting atrophy. Increased width of the optic sheath is sometimes evident in patients with cerebral edema or papilledema. Granuloma may be seen within the brain of patients with tuberculosis (Fig. 88.2)[10] or cryptococcal or *Histoplasma* meningitis. Patients with blastomycosis or nocardiosis usually have one or more brain abscesses in addition to chronic meningitis. Spillage of the contents of an intracranial epidermoid cyst or craniopharyngioma may occur spontaneously or result from surgery. Rarely, repeated rupture can lead to chronic granulomatous meningitis.[11] Epidermoid cysts are readily seen on MRI but must be distinguished from cystic tumors and cysticercosis.[12]

Laboratory Findings

Cerebrospinal Fluid

The opening pressure is usually elevated beyond 120 mm of fluid when measured with the patient in the lateral decubitus position. CSF glucose, protein, cell count, and differential should be measured in all

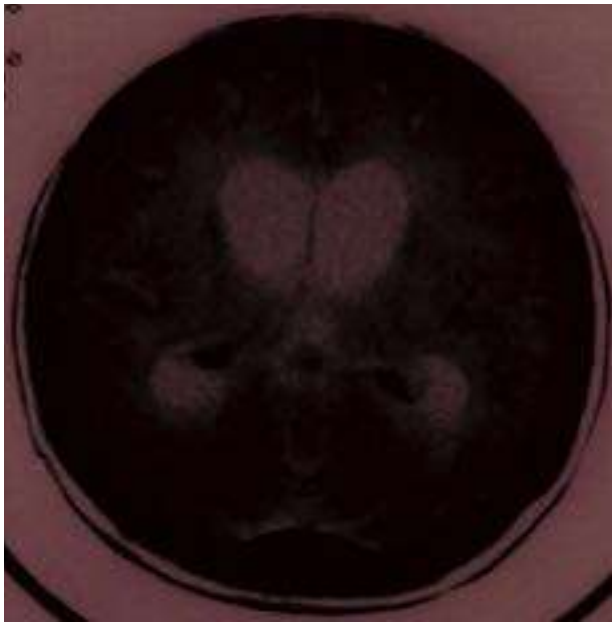

FIG. 88.1 Computed tomography scan showing enlarged lateral cerebral ventricles from cryptococcal meningitis.

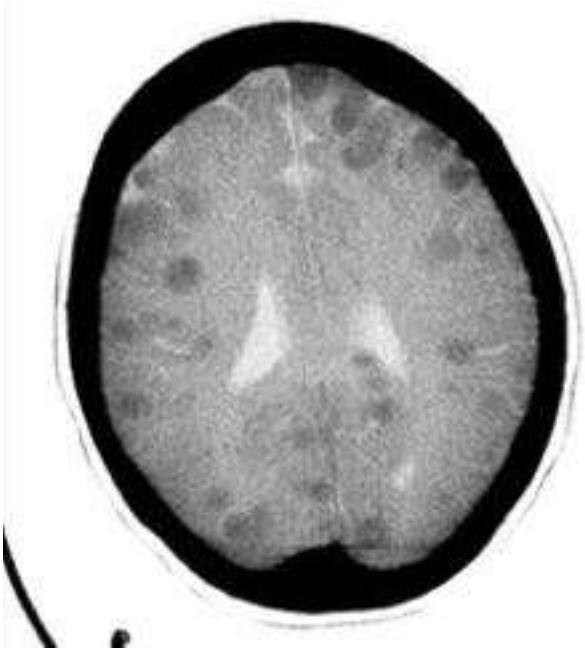

FIG. 88.2 Computed tomography scan of a patient with tuberculous meningitis and numerous intracerebral granulomas.

cases of suspected chronic meningitis. Care should be taken in ordering additional tests to prioritize them by the clinical likelihood of a particular diagnosis, as the amount of CSF may be limiting. Glucose concentrations below 40 mg/dL are a valuable indication of fungal or mycobacterial chronic meningitis and are uncommon in syphilis, Lyme disease, parameningeal infections, or most noninfectious causes. Patients with the lowest CSF glucose level generally have the greatest increase in cell count and protein levels, but there is an exception to this rule. Profound hypoglycorrhachia with nearly normal cell count and protein levels should suggest carcinomatous meningitis. In infectious causes of chronic meningitis, a lymphocytic pleocytosis is usual, but the cell count and cell type are variable, with a neutrophilic predominance not being rare. Culture of an adequate volume of CSF is imperative for optimal recovery of fungi and *Mycobacterium tuberculosis*. Culture of at least 3 to 5 mL for fungi and an equivalent amount for mycobacteria is recommended. Microbiology laboratory technicians will need to be asked to culture the entire volume or may culture their usual volume, usually 1 mL or less, and save the remaining volume in the refrigerator.

Sampling the ventricular CSF is less helpful in chronic meningitis than sampling lumbar fluid. Ventricular fluid is often surprisingly normal, even in the absence of obstructive hydrocephalus and in the presence of very abnormal lumbar fluid. Postneurosurgical infections with a ventriculoperitoneal shunt or a foreign body in the surgery site typically produce negative cultures of lumbar fluid, although cultures taken from an infected ventriculoperitoneal shunt or at neurosurgery in the area of the infection may be positive.

Other potentially helpful tests are listed in Table 88.1. Lymphomatous meningitis may be diagnosed by polymerase chain reaction (PCR) assay of CSF in patients with nondiagnostic cytology results by showing a clonal population of lymphocytes having the same immunoglobulin or T-cell receptor genes.[13] Flow cytometry of CSF can also be used to detect a malignant monoclonal lymphocyte population and lymphoblasts.[14]

Peripheral Blood

Serum serology can be helpful in the diagnosis of coccidioidomycosis, brucellosis (agglutinin only), Lyme disease, and syphilis. In patients with a clinical syndrome compatible with neurosyphilis but a negative Venereal Disease Research Laboratory (VDRL) or rapid plasmin reagin test result, a treponemal test should be requested. Positive treponemal tests preferably should be confirmed by a different treponemal test because false-positive reactions occur. Serum cryptococcal and *Histoplasma* antigen tests can be done, although urine can also be tested for *Histoplasma* antigen.

TABLE 88.1 Diagnostic Tests for Chronic Meningitis

Cerebrospinal Fluid Tests
Glucose, protein, and cell count and differential (including eosinophils)
India ink on centrifuged sediment
Fungal culture of 3–5 mL of cerebrospinal fluid
Cytopathology for malignant cells, including polymerase chain reaction assay or flow cytometry for monoclonal B cells
Periodic acid–Schiff stain of cytopathologic specimen for Whipple disease
VDRL test for syphilis
Cryptococcal antigen
Histoplasma antigen
Aspergillus galactomannan antigen
Coccidioides antibody (complement fixation or immunodiffusion)
Nucleic acid tests for *Mycobacterium tuberculosis,* Whipple disease, enterovirus infection, lymphoma
Culture for enterovirus, *Acanthamoeba*
Serum Tests
Rapid plasma reagin test or antitreponemal antibody test
Antibody to *Coccidioides, Histoplasma, Toxoplasma, Brucella*
Histoplasma antigen

RPR, Rapid plasma reagin; *VDRL,* Venereal Disease Research Laboratory.

Brain Biopsy

Biopsy of meninges and cerebral cortex has had a low yield in chronic meningitis. The highest yield has been biopsy of lesions that were enhancing on MRI and located in the posterior fossa or pterional area, rather than over the cerebral convexities.[15,16] Malignancies constitute the majority of diagnoses. An exception is immunoglobulin G4 (IgG4)–related hypertrophic pachymeningitis, in which biopsy of the thickened dura mater is the most reliable diagnostic method.

Differential Diagnosis

Cryptococcus Meningitis

Cryptococcus neoformans and *Cryptococcus gattii* (see Chapter 262) are the most common causes of fungal meningitis (Table 88.2). Most patients are immunosuppressed, but a previously healthy patient with cryptococcosis has fewer organisms in the CSF and may present more of a diagnostic dilemma. In the latter group, symptoms can be indolent, with headaches present for weeks or months. Dementia may attract attention only after a vehicular accident or if inattention to business or personal matters creates a crisis. Skin lesions precede meningeal symptoms in about 10% of patients and are useful for diagnosis. MRI is most often normal unless hydrocephalus has supervened (see Fig. 88.1). CSF cultures of patients with AIDS with cryptococcal meningitis become positive in a few days, but CSF cultures from indolently ill patients may require a 2-week incubation and culture of more than 1 mL of CSF. CSF antigen is more often positive than serum antigen, with the CSF antigen being negative only in the most indolent patients (i.e., patients with profound hypoglycorrhachia, high protein level, and high cell count).

Coccidioides Meningitis

Principal areas of exposure for coccidioidal meningitis (see Chapter 265)[17–19] are central and southern Arizona and the Central Valley of California, although the endemic area is much broader. Spotty endemic areas exist in contiguous parts of the Southwest. Extremely brief exposures of visitors from outside the endemic area are sufficient to cause infection. The initial infection may be asymptomatic or a community-acquired pneumonia. Previously healthy people with meningitis may present with the indolent onset of headache, which has been present for weeks or months at the time of diagnosis. The pulmonary portal, prominent earlier, may be visible only as hilar adenopathy or a small pulmonary infiltrate at the time symptoms of meningitis appear. The patient may recall the pneumonia as a period of fever and cough occurring 2 to 4 weeks after exposure. Immunosuppressed patients, including patients with AIDS, are more likely to present with a systemic illness, including

TABLE 88.2 Differential Diagnosis of Chronic Meningitis

Mycoses

Cryptococcus (cryptococcosis)
Coccidioides (coccidioidomycosis)
Histoplasma (histoplasmosis)
Candida (candidiasis)
Sporothrix (sporotrichosis [rare])
Blastomyces (blastomycosis [rare])
Other molds (rare): *Scedosporium, Aspergillus, Cladophialophora,* and other dark-walled molds

Bacteria

Mycobacterium tuberculosis (tuberculosis)
Treponema pallidum (syphilis)
Borrelia burgdorferi (Lyme disease)
Tropheryma whipplei (Whipple disease)
Actinomyces (actinomycosis [parameningeal, rare])
Nocardia (nocardiosis [with brain abscess])
Brucella (brucellosis [rare])

Parasites

Acanthamoeba (acanthamebiasis)
Balamuthia (balamuthiasis)
Taenia solium (cysticercosis)
Angiostrongylus cantonensis (angiostrongyliasis)

Viruses

Echovirus (meningoencephalitis)

Postneurosurgical Causes

Infected cerebrospinal fluid shunt
Infected prosthetic material

Tumors

Diffuse gliomatosis
Metastatic meningeal malignancy, including lymphomatous meningitis

Other Causes

Sarcoidosis
Vogt-Koyanagi-Harada syndrome
Behçet syndrome
IgG4-related hypertrophic pachymeningitis

fever, headache, profound malaise, and lesions in the bone or skin. The chest radiograph of immunosuppressed patients may show single or diffuse infiltrates. Low-grade eosinophilia in the blood and more commonly in the CSF is a valuable sign, although Wright-Giemsa staining of the CSF may be needed to detect eosinophilia. The single best test is not culture or smear of CSF, either of which is uncommonly positive, but rather a positive antibody test done in a reliable reference laboratory. The complement fixation test on CSF is specific and sensitive. CSF antibody detected by an immunodiffusion test with a band of identity is a useful screen because it is sensitive, but it is less specific than the complement fixation test. None of the commercially available enzyme-linked immunosorbent assays has sufficient specificity or sensitivity in CSF to be useful. Serum complement fixation and enzyme immunoassay tests are usually positive but may remain positive after remote infection, a so-called serologic scar.

Histoplasma Meningitis

An extremely indolent, chronic meningitis can be the presenting sign of *Histoplasma* meningitis (see Chapter 263) in previously healthy patients. A more subacute meningitis may be part of more systemic illness, particularly in immunosuppressed patients. Patients at risk for dissemination to the brain and other organs include patients with AIDS, solid-organ transplant recipients, and patients taking adrenal corticosteroids or tumor necrosis factor-α inhibitors.[20] Signs of dissemination outside the neuraxis, if present, are extremely variable including Addison disease, chronic granulomatous hepatitis, endocarditis, or pancytopenia. Patients do not give a history suggesting acute pulmonary histoplasmosis before the diagnosis of meningitis. Accompanying pulmonary symptoms are minimal or absent. MRI may show an intracerebral granuloma in some patients. *Histoplasma* antigen or antibody may be detectable in the CSF. CSF cultures are usually negative, although a high-volume culture (at least 5 mL) held for 6 weeks is occasionally successful. A serum or urine antigen test is usually negative unless dissemination is suggested by nonneural findings. Empirical use of corticosteroids can worsen infection and inadvertently make diagnosis more detectable.[21]

Candida Meningitis

Candida (see Chapter 256) is commonly found in multiple small brain abscesses in patients dying of disseminated candidiasis, but presentation as chronic meningitis is rare. Most such patients are very-low-birth-weight neonates or postneurosurgical or immunosuppressed patients.[22,23] Patients with a homozygous *CARD9* mutation are predisposed to *Candida* meningitis.[24] Neonates with *Candida* meningitis have typically had long stays in a neonatal intensive care unit and required prolonged use of intravascular catheters. Others are infants with severe congenital abnormalities of the intestine or urinary tract that required complicated surgical repair. *Candida* enters the bloodstream through an intravascular catheter, from complications of intestinal surgery, or from an obstructed urinary tract. Difficulty assessing the neurologic status of these neonates and the absence of fever cause delays in diagnosis. Hydrocephalus may already be present when the diagnosis of *Candida* meningitis is made. CSF is markedly abnormal, but culture has such few organisms that contamination of the CSF culture may be suspected. Cases of CSF shunts infected with *Candida* may manifest as partial or complete blockage of the shunt, usually in children with multiple shunt revisions or skin ulcers over the shunt valve or tubing. Patients with hematologic malignancies may develop meningitis as a part of disseminated candidiasis. Meningitis may be recognized only after antifungal therapy and return of marrow function has allowed control of the other manifestations of disseminated candidiasis.

Sporothrix Meningitis

The published cases of sporotrichosis meningitis (see Chapter 259) have presented formidable diagnostic difficulties because the organism has proven very difficult to recover in CSF culture.[25] A few patients have had disseminated skin lesions that provided a site for biopsy. Infection has occurred in previously healthy patients and in immunosuppressed patients.

Blastomyces Meningitis

Patients with blastomycosis (see Chapter 264) present with one or more abscesses in the brain or spinal cord. When a brain abscess ruptures into the ventricular system, a rapidly progressive purulent meningitis results with a strong tendency to obstruct the aqueduct of Sylvius, accelerating the progression to coma. Skin, bone, or lung lesions of blastomycosis are present in most patients and permit diagnosis.

Phaeohyphomycotic Meningitis

Dark-walled molds have a predisposition for spread from the lung to the brain, causing a brain abscess, phaeohyphomycotic meningitis, or both (see Chapter 268). CSF cultures are often sterile, but direct detection of fungal DNA may have a higher yield.[26] Brain biopsy is sometimes required for diagnosis.[27,28] Most patients are previously healthy and have no visible pulmonary portal or other disease outside the CNS. Both of the outbreaks of epidural abscess and meningitis due to contaminated epidural methylprednisolone injections were due to phaeohyphomycetes: *Exophiala* and *Exserohilum.*[6,27,29] In the latter cases, extension to the brain led to vascular invasion and stroke.[30]

Other Molds Causing Meningitis

Scedosporium prolificans and *Scedosporium apiospermum* infections have a predisposition for hematogenous spread to the CNS, usually manifesting as brain abscess but occasionally as chronic meningitis. Immunosuppression is present in most patients, although intravenous drug abusers may also develop the infection. *Aspergillus* may enter the CSF as an extension of sphenoid or ethmoid sinusitis, as mastoiditis, or rarely as a complication of neurosurgery and manifest as chronic meningitis.[31–33] Immunosuppressed patients with aspergillosis and mucormycosis in

the CNS most commonly present with angioinvasion with hemorrhagic infarction, not chronic meningitis.

Mycobacterium tuberculosis Meningitis

Tuberculous meningitis (see Chapter 249) is probably the most common cause of chronic meningitis, and because diagnosis can be difficult, empirical treatment for *Mycobacterium tuberculosis* may be started when all other diagnostic measures fail (see later discussion). Children with hematogenously disseminated tuberculosis are prone to a more rapid course than is typical for adults, with symptoms present for only 2 to 4 weeks before diagnosis. Fever, miliary lesions in the lung and retina, marrow suppression, and hepatosplenomegaly may herald the systemic disease, whereas headache followed by confusion and coma portend a poor prognosis from the CNS disease. Disease in previously healthy adults tends to be more indolent with fewer signs of disseminated tuberculosis other than fever, weight loss, night sweats, and malaise. Chest radiography is abnormal in only about half of patients. Diagnosis is suspected from country of origin or household exposure to tuberculosis, both typically occurring years before symptoms are evident. Tuberculin skin test and interferon-γ release tests may be negative and neither support nor exclude the diagnosis. MRI may be normal or show small lesions in the cortical sulci (see Fig. 88.2) or may show hydrocephalus. Hypoglycorrhachia is usual, as are pleocytosis of up to a few hundred cells and an elevated protein level. The adenosine deaminase level may be elevated in the CSF, but this finding is not more helpful than a low CSF glucose level. Smear rarely demonstrates acid-fast bacilli. Culture in automated broth culture techniques is positive in about half of the cases but requires about 2 weeks of incubation, which is often too long a period to assist early therapeutic decisions. The old literature referred to taking the clot that forms in the CSF from patients with tuberculosis as it stands, the so-called pellicle, and using those clumped strands of cells and clot from several milliliters of CSF for smear and culture. Although no modern laboratory would consider using such a time-consuming technique, the popularity of that technique speaks to the small inoculum of organisms in the CSF and the need for culturing more than minimal volumes of CSF. Nucleic acid–based tests such as the *Mycobacterium tuberculosis* Direct Test (Hologic Gen-Probe, San Diego, CA) and Xpert MTB/RIF (Cepheid, Sunnyvale, CA) (see Chapter 16) are approved for use on sputum samples but not CSF. Although highly specific, these tests lack sensitivity when performed on CSF.[34,35] The Xpert MTB/RIF Ultra test provided superior results in the diagnosis of tuberculous meningitis in patients with HIV in Uganda.[36] This study also found that culturing more than 6 mL of CSF significantly improved yield. Although many referral laboratories offer PCR diagnosis, the lack of standardization makes interpretation difficult. The issue of empirical therapy for tuberculous meningitis is discussed later in this chapter.

Treponema pallidum Meningitis

CNS manifestations of syphilis (see Chapter 237) are not easily divided into categories. Secondary syphilis causes aseptic meningitis, but illness resolves either spontaneously or with therapy so that chronicity at this stage of infection is uncommon. Meningovascular syphilis is a later complication that occurs 2 to 10 years after infection as an ischemic stroke in a young person. Presenting symptoms of parenchymal disease are gumma formation or general paresis 15 to 20 years after infection or tabes dorsalis even later. General paresis has prominent encephalopathic signs and may have ocular signs as well. Tabes dorsalis has prominent spinal cord signs and symptoms with radicular pain ("lightning pains") that sets it apart from the usual patient with chronic meningitis, in whom spinal cord symptoms are uncommon. Patients with neurosyphilis may present simply with chronic meningitis with a CSF pleocytosis and elevated protein levels but normal glucose level. Some of these patients have a waxing and waning course, perhaps beginning when they had secondary syphilis. In a patient with compatible neurologic signs or symptoms and an abnormal CSF, a positive VDRL on CSF is diagnostic of neurosyphilis, but the test is insensitive. In the appropriate setting, a fluorescent treponemal antibody absorption test on CSF may be indicated. A significant minority of patients with neurosyphilis have negative nontreponemal tests, such as VDRL or rapid plasmin reagin on serum, in which case a treponemal antibody test should be considered.[37]

Borrelia burgdorferi

CNS disease (Lyme borreliosis) can occur weeks to months after infection with *Borrelia burgdorferi* (see Chapter 241). Cranial nerve palsies are common, in contrast to syphilitic meningitis. Cognitive decline and ataxia may occur because of encephalitis. Seizures, ataxia, painful radiculopathy, or, rarely, meningitis may also occur. The CSF shows a modest pleocytosis, slight elevation of protein levels, and normal glucose level. Enzyme immunoassay and Western blot for IgG antibody in CSF are helpful but probably insensitive. PCR detection in CSF also appears to be of low sensitivity.[38,39]

Tropheryma whipplei Meningitis

Whipple disease of the CNS most often manifests as the indolent onset of dementia with memory loss or personality change (see Chapter 210). Other frequent clinical signs include ophthalmoplegia, nystagmus, or myoclonus. Elevated CSF protein and mixed cellularity pleocytosis are found in half of the cases.[40] Periodic acid–Schiff–positive inclusions of macrophages in a cytospin of CSF may be seen. The PCR assay of CSF is the single best test. Duodenal biopsy for periodic acid–Schiff–positive inclusions in submucosal macrophages may be useful even in the absence of diarrhea.[40]

Nocardia and *Actinomyces* Meningitis

Nocardiosis (see Chapter 253) has a strong tendency for hematogenous spread from the lungs to the brain. An acute or subacute meningitis can result from rupture of an abscess into the CSF. Actinomycosis (see Chapter 254) can cross the dura mater from a contiguous infection in the paranasal sinus or middle ear and cause an indolent meningitis. The organisms can rarely be isolated from the CSF. Actinomycotic meningitis predominantly results from a parameningeal focus.

Brucella Meningitis

Although brucellosis (see Chapter 226) is now rare in the United States, it remains a common disease in many countries. Ingestion of unpasteurized dairy products such as soft cheeses from other countries is a common source of infection in the United States. Illness is usually acute but may be relapsing or occasionally chronic. A few patients with chronic meningitis have been reported, but positive CSF cultures have been unusual. Serum *Brucella* agglutinins and an exposure history are helpful to screen patients. Serologic studies for brucellosis other than agglutinin-based tests are not recommended.

Amebic Granulomatous Meningitis

In contrast to *Naegleria,* which causes acute meningitis, cerebral manifestations of *Acanthamoeba* (see Chapter 273) infections evolve over a few weeks, with decreased mental status, seizures, fever, headache, meningitis, visual disturbance, and ataxia, with hemiparesis as a later manifestation. Most reported patients have suppressed immune function, such as patients with AIDS or stem cell transplant recipients. Others have been chronically ill and debilitated.

Balamuthia mandrillaris (see Chapter 273) can infect both immunocompetent and immunocompromised hosts. The course can be indolent with fever, headache, and seizures. Mortality is high due to difficulty in diagnosis, which is often made by immunohistochemistry or PCR at autopsy. There are no well-established treatments.

Angiostrongylus cantonensis Meningitis

Most cases of meningitis caused by *Angiostrongylus cantonensis* (see Chapter 290) in the United States have been in travelers recently returned from the South Pacific, Hawaii, or the Caribbean who acquired infection from eating uncooked produce.[41] Patients present with an acute eosinophilic meningitis, although symptoms can linger for several weeks.

Taenia solium Meningitis

In patients with neurocysticercosis (see Chapter 289), the cystic lesions can occur anywhere in the neuraxis, usually causing seizures, but patients occasionally present with chronic meningitis. A cyst in the third ventricle can obstruct the aqueduct of Sylvius, and patients can present with hydrocephalus.

Enteroviral Meningitis

Enteroviruses (see Chapter 172) cause acute aseptic meningitis or myelitis in children and young adults. Patients with X-linked agammaglobulinemia or with large B-cell lymphoma treated with rituximab may acquire chronic enteroviral meningoencephalitis.[42,43] Diagnosis can be made by culture or real-time PCR assay of CSF.

Postneurosurgical Meningitis

Patients may present with persistent low-grade fever, headache, and low-grade CSF pleocytosis after neurosurgery. These same findings in the immediate postoperative period are difficult to distinguish from the usual postoperative findings. Persistence of these findings, particularly if a plastic implant was used, increases the likelihood of infection with a low-grade pathogen, most often *Staphylococcus epidermidis*. Recovery of the organism from lumbar CSF is rare, but the organism may be found on implanted material when removed at neurosurgery. *S. epidermidis* is difficult to eradicate from implanted intracranial material because of the relatively poor penetration of vancomycin into the CSF and likely because of the organism's ability to form a biofilm on foreign bodies. Linezolid has better penetration into the CSF, but removal of the material may be required for cure despite temporary improvement with systemic antibiotics. Infected ventricular or lumbar CSF shunts share many of these same problems and are discussed in Chapter 92.

Sarcoidosis Meningitis

Chronic meningitis due to sarcoidosis is a diagnosis of exclusion, with many patients reported to have the diagnosis even in the absence of extraneural signs of sarcoidosis. Cranial nerve palsies are the most common presenting sign.[44,45] Biopsy of a skin lesion, lung, or enlarged lymph node may show noncaseating granuloma, supporting the diagnosis. Treatment with corticosteroids may reveal a fungal or other infectious cause of meningitis.

Vogt-Koyanagi-Harada Syndrome Meningitis

Vogt-Koyanagi-Harada syndrome is an autoimmune disease targeting melanocytes. Manifestations include bilateral eye disease, chronic meningitis, and, later in the disease course, skin findings. Ocular findings are diffuse choroiditis or chronic iridocyclitis. CNS findings include headache, nausea, neck stiffness, tinnitus, and CSF pleocytosis. Alopecia, vitiligo, or poliosis occurs late in the disease.[46]

Behçet Syndrome Meningitis

Behçet syndrome technically does not belong on this list because it does not cause chronic meningitis. It causes recurrent episodes of aseptic meningitis and in some cases progressive parenchymal involvement. Diagnosis is made by the presence of recurrent oral ulcerations and at least one of the following: recurrent genital ulcers, eye lesions, skin lesions, or a positive pathergy test.[47,48]

Neoplastic Meningitis

Dissemination of cancer to the leptomeninges is most commonly from breast cancer, non-Hodgkin lymphoma, non–small cell lung carcinoma, acute leukemia, or melanoma. Presenting symptoms may be cranial nerve palsy, spinal cord symptoms, or headache. Diagnosis is usually known from a prior diagnosis of a malignancy elsewhere or by CSF cytology. Hypoglycorrhachia may be profound, but pleocytosis and elevated protein levels are modest at most. Rarely, leptomeningeal gliomatosis can originate from a primary supratentorial lesion too small to be detected by MRI and not detected by CSF cytology.[49] Lymphomatous meningitis may not be readily detectable by cytospin cytology, so demonstration of lymphocyte clonality by PCR assay or flow cytometry may be required. Meningeal biopsy over an area of enhancement on MRI may be necessary for diagnosis. Although the lymphoma may be known from other sites in the body, distinguishing lymphoma in the meninges from cryptococcal meningitis or other infectious causes is vital.

IgG4-Related Hypertrophic Pachymeningitis

IgG4-related disease is a chronic, indolent fibroinflammatory disease that can affect any organ. In the CNS, fibrous thickening of the dura mater can be focal or diffuse, involving the cranial dura mater, spinal cord, or both.[50] Cranial lesions resemble idiopathic hypertrophic pachymeningitis, manifesting as progressive cranial nerve palsies, headache, or vestibular symptoms. CSF may show mild abnormalities of cell count and protein. Focal or diffuse thickening of the dura mater is seen on MRI. Biopsy of the dura in patients with hypertrophic pachymeningitis associated with IgG4-related disease shows storiform fibrosis with an increased number of plasma cells that are IgG4-positive on immunostaining (IgG4+/IgG+ plasma cell/ratio). Relative concentrations of IgG4 in serum and CSF (IgG4 index) are increased.[51] Treatment is usually with corticosteroids or other immunosuppressive agents.

EMPIRICAL THERAPY FOR PRESUMED TUBERCULOUS MENINGITIS

Delay in diagnosis of tuberculous meningitis is strongly associated with neurologic damage and death. Patients with high fever and rapid decline in consciousness may be candidates for empirical therapy for tuberculous meningitis with a four-drug regimen (see Chapters 39, 87, and 249). Immigrants from countries with a high incidence of tuberculosis and patients with a history of tuberculosis in a household member are at especially high risk. Before therapy, several milliliters of CSF should be cultured for mycobacteria, and the centrifuged sediment should be examined by fluorochrome stain for acid-fast bacilli. If there are lung lesions, sputum should be smeared and cultured for acid-fast bacteria as well. Acid-fast stains should be done, and nucleic acid amplification testing of CSF should be requested. Repeat weekly lumbar punctures during therapy should show an improvement in hypoglycorrhachia, if present, in the second or third week, along with clinical improvement. Pleocytosis and elevated CSF protein levels are more variable and slower to respond. Administration of corticosteroids should not be routine during empirical therapy for tuberculous meningitis because corticosteroids can cloud the clinical response by nonspecifically decreasing fever, decreasing cerebral edema on MRI, and improving CSF pleocytosis and hypoglycorrhachia. If meningitis is due to a fungus, infection may increase and be undetected initially because of the antiinflammatory effects of corticosteroids.

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89 Encephalitis

J. David Beckham and Kenneth L. Tyler

SHORT VIEW SUMMARY

Definition
- Encephalitis is an inflammatory process involving the brain parenchyma associated with clinical or laboratory evidence of neurologic dysfunction.

Epidemiology
- It occurs most frequently in infants younger than 1 year and in elderly patients older than 65, with intermediate incidence in individuals between these age extremes.

Etiology
- Up to 60% of encephalitis cases result from an unidentified etiologic agent.
- Viruses, bacteria, and autoimmune inflammation cause the majority of known encephalitis cases.

Diagnosis
- A compatible febrile syndrome with evidence of central nervous system impairment exists (see Table 89.1).
- Standard cerebral spinal fluid (CSF) analysis and neuroimaging with magnetic resonance imaging are preferred.
- Specific CSF and/or serum studies for defined etiologies of encephalitis are warranted (see Table 89.3).

Therapy
- Early empirical use of high-dose acyclovir is warranted to treat possible herpes simplex encephalitis pending diagnostic studies.
- Antiviral therapy is indicated for the treatment of other herpesviruses that cause encephalitis.
- Empirical antiviral therapy is recommended for suspected encephalitis associated with influenza.
- There is currently no therapy of known benefit for patients with encephalitis due to arthropod-borne viruses.

Prevention
- Routine vaccination for common pathogens and vaccination for Japanese encephalitis virus in selected travelers may prevent some cases of encephalitis.
- Procedures to decrease exposure to mosquito bites may decrease the risk for arthropod-borne virus–related cases of encephalitis.

Viral invasion of the central nervous system (CNS) can result in various clinical syndromes, including encephalitis, meningitis, myelitis, and neuritis.[1] *Encephalitis* is defined as an inflammatory process of the brain parenchyma associated with clinical or laboratory evidence of neurologic dysfunction. The infectious causes of encephalitis, predominantly viruses, are the focus of this chapter. Noninfectious forms of encephalitis may result from inflammatory processes associated with autoimmune, paraneoplastic, neoplastic, or collagen vascular diseases and are discussed only as they affect the differential diagnosis. Focal suppurative infections of the CNS including cerebritis, brain abscess, and empyema are discussed in Chapters 90 and 91.

Patients with suspected encephalitis often have prolonged hospitalizations, may require a multitude of expensive diagnostic tests, and frequently have poor outcomes including disability and death.[2] There were an estimated 263,352 encephalitis-associated hospitalizations in the United States from 1998 to 2010, with an overall mortality rate of approximately 6%.[3] Total medically related charges for encephalitis-associated hospitalizations were an estimated $2 billion per year in 2008–2010.[4] A diagnosis of encephalitis was most frequent in infants younger than 1 year (11.1 per 100,000) and elderly patients older than 65 years (13.2 per 100,000), with an intermediate incidence of 4.0 to 8.4 per 100,000 individuals between these age extremes.[4]

In patients with encephalitis, the success in finding a specific defined pathogen varies widely and is influenced by factors such as season of the year, geographic location, vector exposure, patient age, patient immune status, and case definition. In general, approximately half of the patients diagnosed with encephalitis have no defined etiology identified despite an extensive diagnostic workup.[1,2,4] Using the Nationwide Inpatient Sample for 1998 through 2010, one study found that 50% of hospitalizations due to encephalitis had no specified etiology.[3]

In a retrospective series reviewing etiologies of "neuroinfectious diseases" in hospitalized patients from an academic tertiary referral center, a specific pathogen was identified in 62.5% of patients who tested negative for human immunodeficiency virus (HIV) and 89% of HIV-positive patients.[2] The most common identified pathogens and conditions in HIV-negative patients were viruses (38%), bacteria (33%), Lyme disease (7%), fungi (7%), syphilis (5%), mycobacteria (5%), prions (3%), and *Pneumocystis jirovecii* (2%). Among HIV-positive patients, the common pathogens included viruses (46%), fungi (32%), and *Toxoplasma* (11%), with bacteria, tuberculosis, and syphilis accounting for the remaining infections (11%).[2] Among the 22 cases of viral encephalitis, the most commonly identified viruses in HIV-negative patients were varicella-zoster virus (VZV; 36%), herpes simplex virus (HSV; 32%), cytomegalovirus (CMV; 9%), West Nile virus (WNV; 9%), enteroviruses (9%), and hepatitis C virus (5%).[2] In HIV-positive patients, the viral etiologies included HIV encephalopathy (54%), JC virus–induced progressive multifocal leukoencephalopathy (PML; 31%), VZV (7.5%), and CMV (7.5%).

The California Encephalitis Project (CEP) searched for etiologies of encephalitis in immunocompetent hospitalized patients who met defined criteria for encephalitis, including altered consciousness for 24 hours or more and at least one of the following characteristics: fever, seizures, focal neurologic findings, cerebrospinal fluid (CSF) pleocytosis, or consistent electroencephalogram (EEG) or neuroimaging findings.[1,5] A confirmed or probable cause was found in only 16% of cases; these causes included viruses (69%), bacteria (20%), prions (7%), parasites (3%), and fungi (1%). The most commonly identified viruses included enteroviruses (25%), HSV-1 (24%), VZV (14%), WNV (11%), and Epstein-Barr virus (EBV) (10%), with other agents accounting for 16%.[5] In a prospective study in England, 42% of patients had encephalitis due to an infection; HSV(19%) and VZV(5%) were the most commonly identified infectious agents.[6] Acute immune-mediated encephalitis (21%) was a major cause of encephalitis, and unknown causes (37%) of encephalitis were still common.

CLINICAL SYNDROMES

Clinicians frequently encounter patients with fever and signs or symptoms of CNS disease in whom infectious encephalitis is part of a larger differential diagnosis. This clinical presentation is nonspecific with a broad differential diagnosis including diseases as diverse as viral

encephalitis; viral meningitis; bacterial meningitis; focal suppurative infections (brain abscess, subdural or epidural empyema, and cerebral venous sinus thrombophlebitis); acute disseminated encephalomyelitis (ADEM); autoimmune encephalitis; and encephalopathy. The challenge to the clinician is to differentiate among these clinical entities quickly and efficiently, because appropriate diagnosis and therapy can have an important effect on neurologic outcomes and mortality.

When evaluating a patient with possible encephalitis, it is important to distinguish among (1) infectious processes; (2) encephalopathy; and (3) autoimmune processes such as autoimmune encephalitis (Fig. 89.1).[7] *Encephalopathy* refers to diffuse cerebral dysfunction without associated inflammation of brain tissue.[8] Although there are multiple causes of encephalopathy, the most commonly encountered causes include toxins (e.g., alcohol, licit or illicit drugs) and metabolic dysfunction (e.g., hypoxia, hypoglycemia or hyperglycemia, electrolyte disorders, renal or hepatic failure).[8] Table 89.1 lists common factors that can be used to differentiate encephalitis from encephalopathy. Fever, headache, peripheral leukocytosis, and CSF pleocytosis all favor encephalitis. Although some encephalopathies may produce focal neurologic signs, these are more typical in encephalitis, as are focal seizures. Neuroimaging studies are generally normal in patients with encephalopathy, and EEG abnormalities are generally limited to diffuse slowing and the occasional appearance of triphasic waves. Conversely, patients with encephalitis frequently have neuroimaging abnormalities and may show focal EEG

TABLE 89.1 Comparison of Encephalitis and Encephalopathy

	ENCEPHALITIS	ENCEPHALOPATHY
Clinical Features		
Fever	Common	Uncommon
Headache	Common	Uncommon
Depressed mental status	May fluctuate	Steady decline in mental status
Focal neurologic signs	Common	Uncommon
Seizures	Common	Uncommon
Types of seizures	Generalized or focal	Generalized
Laboratory Results		
Complete blood count	Leukocytosis common	Leukocytosis uncommon
Cerebrospinal fluid	Pleocytosis common	Pleocytosis uncommon
Electroencephalogram	Diffuse slowing and occasional focal abnormalities or periodic patterns	Diffuse slowing
Magnetic resonance imaging	May have focal abnormalities	No focal abnormalities

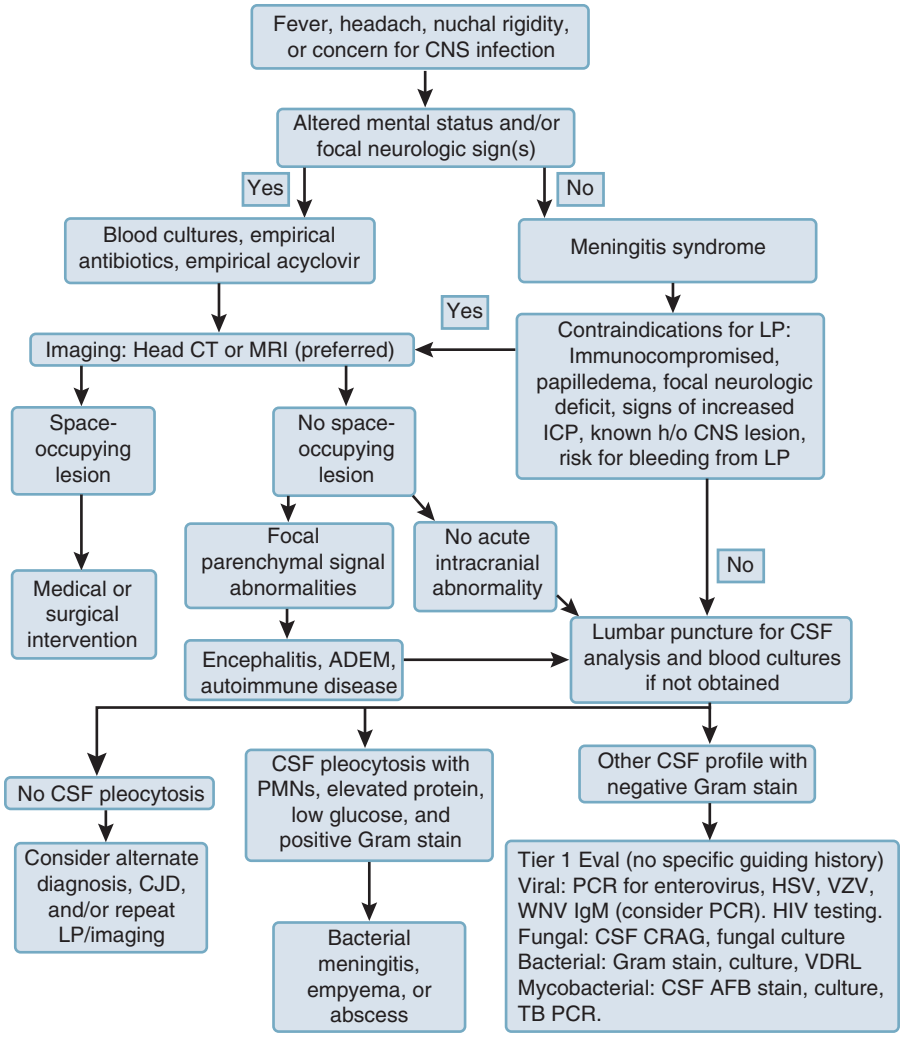

FIG. 89.1 Approach to the patient with possible central nervous system *(CNS)* infection. *ADEM,* Acute disseminated encephalomyelitis; *AFB,* acid-fast bacilli; *CJD,* Creutzfeldt-Jakob disease; *CSF,* cerebrospinal fluid; *CRAG,* cryptococcal antigen; *CT,* computed tomography; *HIV,* human immunodeficiency virus; *HSV,* herpes simplex virus; *ICP,* intracranial pressure; *IgM,* immunoglobulin M; *LP,* lumbar puncture; *MRI,* magnetic resonance imaging; *PCR,* polymerase chain reaction; *PMN,* polymorphonuclear leukocyte; *TB,* tuberculosis; *VDRL,* Venereal Disease Research Laboratory; *VZV,* varicella zoster virus; *WNV,* West Nile virus. Tier 1 evaluation described. See text for subsequent evaluation based on exposures.

abnormalities or specific patterns of dysfunction that are rarely seen in encephalopathy (see later).

AUTOIMMUNE ENCEPHALITIS

Antibody-mediated autoimmune encephalitis is an important cause of encephalitis and should be considered in the differential diagnosis of patients with encephalitis. In addition to traditional paraneoplastic autoimmune etiologies of encephalitis (e.g., anti-Ma, anti-Yo, anti-Hu), neuronal antigen targeting by antibodies in the CNS is increasingly identified as an important cause of encephalitis and was recently reviewed.[9] About 70% of patients develop prodromal symptoms including headache, fever, nausea, vomiting, diarrhea, or upper respiratory tract symptoms. Within a few days to 2 weeks, patients can develop psychological symptoms including anxiety, insomnia, delusions, mania, or paranoia. Short-term memory loss is common but may be underrecognized owing to rapid disintegration of language and reduction in verbal output, which can progress to frank mutism. In more advanced stages of disease, patients develop decreased responsiveness, autonomic instability, dyskinesias, dystonia, rigidity, and opisthotonic postures.

In an analysis of patients younger than 30 years and enrolled in the CEP, anti–*N*-methyl-D-aspartate (NMDA) receptor encephalitis and viral encephalitis were noted to be equally important etiologies of encephalitis.[10] CSF in patients with anti–NMDA receptor encephalitis was characterized by a mild pleocytosis (median white blood cell count, 23 cells/mL) in the CSF with normal protein and glucose levels. The findings of neuroimaging with magnetic resonance imaging (MRI) were often abnormal but nonspecific, with no characteristic pattern or defining imaging results. When brain MRI findings were abnormal (50%), changes included T2 and fluid-attenuated inversion recovery (FLAIR) signal hyperintensity in the hippocampi, cerebellar or cerebral cortex, frontobasal and insular regions, basal ganglia, brainstem, and rarely the spinal cord. EEGs in patients with anti-NMDA receptor encephalitis were often abnormal, most commonly due to diffuse or focal slowing patterns with occasional superimposed epileptic activity.

Autoimmune encephalitis is diagnosed by demonstrating the presence of specific antineuronal antibodies in the serum and/or the CSF in a patient with characteristic features of autoimmune encephalitis and negative findings on evaluation for infectious etiologies. Guidelines have been established to facilitate diagnosis of autoimmune limbic encephalitis in the absence of detectable autoantibodies.[9] Studies have also shown that viral causes of encephalitis such as HSV may be associated with production of autoreactive antibodies.[11] It is important to remember that the diagnoses of autoimmune encephalitis and viral encephalitis are not mutually exclusive; patients with HSV and other viral encephalitides may also have or develop autoantibodies and patients in whom autoantibodies are detected may also have an associated viral encephalitis. The list of autoantibodies associated with autoimmune encephalitis is rapidly expanding; the most commonly identified antibodies are those against the NMDA receptor; leucine-rich, glioma-inactivated 1 (LGI-1) protein; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPAR); and γ-aminobutyric acid B receptor ($GABA_BR1$). Although patients with autoimmune encephalitis generally have antibodies present in serum, in the case of anti-NMDA receptor encephalitis, CSF autoantibodies may be present in the absence of serum antibodies.

Treatment of autoimmune encephalitis is largely based on immunosuppressive regimens. Response to treatment for autoimmune encephalitis is variable, and relapse can occur in up to 24% of patients.[12] In female patients with NMDA receptor encephalitis, evaluation for an underlying ovarian teratoma and removal, if a tumor is found, is indicated. First-line immunotherapy consists of corticosteroids, intravenous immunoglobulins (IVIGs), or plasma exchange. In patients without a tumor, delayed diagnosis, or poor response to first-line therapy, second-line immunotherapy with rituximab, cyclophosphamide, or both is indicated. Despite the difficulty in treating these patients, the mortality rate is low, yet long-term neurologic outcomes are variable with potential for severe, fixed neurologic impairment.

It is also important to consider postinfectious encephalomyelitis or ADEM when evaluating patients with possible encephalitis. ADEM is an inflammatory demyelinating disease of the CNS that follows an infection or vaccination, typically after a delay of 1 to 4 weeks.[13] Most cases are monophasic and associated with MRI findings of diffuse or multifocal subcortical and central white matter lesions with increased signal on T2-weighted and FLAIR sequences.[14,15] A preceding infection or vaccination is found in about two-thirds of pediatric cases and half of adult cases.[14,16] Studies have stressed the importance of antecedent infections caused by influenza A and B, hepatitis viruses, nonspecific flu-like upper respiratory tract infections, and nonviral infectious agents including *Mycoplasma*.[16] Postvaccination cases of ADEM are rare with the vaccines in general use now.

GENERAL CLINICAL APPROACH

When an infection of the CNS is considered in the differential diagnosis, the evaluation should progress efficiently to rule out important reversible causes of disease (see Fig. 89.1). Diagnostic algorithms have been developed to help standardize the clinical approach to these complex patients.[8] In cases of bacterial meningitis and herpes simplex encephalitis (HSE), studies have shown that delay in therapy increases morbidity and mortality.[17–19] When evaluating a patient with possible encephalitis, the initial diagnostic goals are to obtain CSF for appropriate studies and to perform neuroimaging, preferably MRI, unless contraindicated. In patients who are older than 60; are immunocompromised; have a history of known CNS disease; or have recent seizures, altered consciousness, focal neurologic deficits, or other signs suggestive of increased intracranial pressure (ICP), neuroimaging should be completed before lumbar puncture.[18,20] In patients with suspected bacterial meningitis, empirical antibiotic therapy should be initiated after blood cultures are performed if lumbar puncture is delayed for neuroimaging (see Chapter 87).[18] Empirical acyclovir therapy should be initiated in patients with suspected encephalitis, pending results of diagnostic studies.[7]

MRI is more sensitive and provides more detailed diagnostic information than computed tomography (CT) in patients with suspected CNS infections, including encephalitis, and is the procedure of choice.[21,22] Neuroimaging studies can provide valuable clues that can assist in specific diagnosis of encephalitis. The most common abnormalities include areas of increased signal on T2 and FLAIR sequences. Diffusion-weighted imaging sequences may show abnormalities before, or that are more extensive than those seen in, T2 and FLAIR sequences.[21–26]

CSF examination is part of basic testing in all patients with suspected encephalitis or meningitis.[7,18] The most common CSF profile in patients with viral encephalitis is a lymphocytic pleocytosis with a normal glucose concentration and an elevated protein concentration. This pattern is similar to that seen in most cases of autoimmune encephalitis, ADEM, and several other causes of noninfectious encephalitis. The presence of neutrophils rather than lymphocytes as the predominant cell type occurs frequently in patients with WNV neuroinvasive disease or tick-borne encephalitis virus and can occur with eastern equine encephalitis and some enteroviruses (see specific virus sections for details).

Suggested CSF diagnostic studies in immunocompetent adults in the United States with suspected encephalitis include a CSF opening pressure, cell count and differential, protein and glucose concentrations, Gram stain, and bacterial cultures (see Fig. 89.1, tier 1 studies). Initial viral studies of the CSF should include polymerase chain reaction (PCR) studies for HSV, VZV, and enteroviruses, and WNV immunoglobulin M (IgM) serology (in the appropriate season). Routine testing should also include HIV testing, CSF Venereal Disease Research Laboratory (VDRL) test with serum treponemal assessment, and cryptococcal antigen testing. Additional diagnostic tests, or tier 2 tests, are guided by and prioritized according to the clinical and epidemiologic clues obtained during evaluation of the patient (Table 89.2). Diagnostic algorithms for patients with encephalitis have been proposed.[8]

In immunocompromised patients, CSF PCR for CMV, human herpesvirus type 6 (HHV-6), and in specific settings JC virus can be included in the initial evaluation depending on the underlying risk factors. A second tier of CSF diagnostic studies in immunocompetent patients might include PCR studies of the CSF for HIV, *Mycoplasma pneumoniae,* and *Mycobacterium tuberculosis*; IgM serologies for specific viruses such as WNV, Powassan virus, La Crosse virus (California encephalitis), St. Louis encephalitis virus, and eastern equine encephalitis virus, and Lyme disease (as geographically appropriate); acid-fast bacillus smear and culture; tuberculosis PCR; fungal cultures; *Coccidioides*

TABLE 89.2 Possible Etiologic Agents of Encephalitis Based on Epidemiology and Risk Factors

EPIDEMIOLOGY OR RISK FACTOR	POSSIBLE INFECTIOUS AGENTS
Travel	
Caribbean islands, Central America, South America	Rabies virus; eastern equine encephalitis virus; western equine encephalitis virus; Venezuelan equine encephalitis virus; St. Louis encephalitis virus; Rocio virus; Zika virus; chikungunya virus; Dengue virus
Australia	Murray Valley encephalitis virus; Japanese encephalitis virus; Hendra virus
Southeast Asia, China	Japanese encephalitis virus; tick-borne encephalitis; Nipah virus; Me Tri virus; Semliki Forest virus
India, Nepal	Rabies virus; Japanese encephalitis virus; chikungunya virus
Africa	Rabies virus; WNV; Rift Valley fever virus
Middle East	WNV
Europe	WNV; tick-borne encephalitis; louping ill virus; Toscana virus
Russia	Tick-borne encephalitis; Powassan virus
Insect Contact	
Mosquitoes	WNV; Zika virus; Eastern equine encephalitis virus; Venezuelan equine encephalitis virus; St. Louis encephalitis virus; Murray Valley encephalitis virus; Japanese encephalitis virus; California encephalitis group; chikungunya virus
Ticks	Tick-borne encephalitis; Powassan virus; louping ill virus
Animal Contact	
Old World monkeys	Herpesvirus B
Birds	WNV; eastern equine encephalitis virus; Venezuelan equine encephalitis virus; St. Louis encephalitis virus; Murray Valley encephalitis virus; Japanese encephalitis virus
Rodents	Eastern equine encephalitis virus (South America); Venezuelan equine encephalitis virus; tick-borne encephalitis; Powassan virus; La Crosse virus (chipmunks and squirrels); LCMV; monkeypox
Horses	Eastern equine encephalitis virus; western equine encephalitis virus; Venezuelan equine encephalitis virus; Hendra virus
Swine	Japanese encephalitis virus; Nipah virus
Dogs	Rabies virus
Bats	Rabies virus; Nipah virus
Raccoons	Rabies virus
Skunks	Rabies virus
Sheep and goats	Louping ill virus
Human Contact	
Person-to-person transmission	HSV (neonatal); VZV; Venezuelan equine encephalitis virus (rare); poliovirus; enteroviruses; measles virus; mumps virus; rubella virus; EBV; HHV-6; herpesvirus B; WNV (transfusion, transplantation, breast-feeding); HIV; rabies virus (transplantation); influenza virus
Season	
Late summer, early fall	All agents transmitted by mosquitoes and ticks (see above); enteroviruses
Winter	Influenza virus; LCMV
Recreational Activities	
Sexual contact	HIV; Zika virus
Swimming	Enteroviruses
Camping, hunting	All agents transmitted by mosquitoes and ticks (see above)
Spelunking	Rabies virus
Occupation	
Physicians and health care workers	VZV; HIV; influenza virus
Veterinarians	Rabies virus
Laboratory workers	WNV; HIV
Workers exposed to Old World primates	Herpesvirus B
Workers exposed to horses	Hendra virus
Ingestions	
Unpasteurized milk	Tick-borne encephalitis
Age	
Neonates	HSV-2; CMV; rubella virus; Zika virus
Infants and children	Eastern equine encephalitis virus; Murray Valley encephalitis virus (rapid in infants); influenza virus; La Crosse virus
Elderly	Eastern equine encephalitis virus; St. Louis encephalitis virus; WNV

Continued

TABLE 89.2 Possible Etiologic Agents of Encephalitis Based on Epidemiology and Risk Factors—cont'd

EPIDEMIOLOGY OR RISK FACTOR	POSSIBLE INFECTIOUS AGENTS
Other	
Unvaccinated	VZV; Japanese encephalitis virus; poliovirus; measles virus; mumps virus; rubella virus
Recent vaccination	ADEM
Transfusion and transplantation	CMV; EBV; WNV; HIV; tick-borne encephalitis virus; rabies virus; LCMV
Immunocompromised	VZV; CMV; HHV-6; WNV; HIV; JC virus
Agammaglobulinemia	Enteroviruses

ADEM, Acute disseminated encephalomyelitis; *CMV,* cytomegalovirus; *EBV,* Epstein-Barr virus; *HHV,* human herpesvirus; *HIV,* human immunodeficiency virus; *HSV,* herpes simplex virus; *LCMV,* lymphocytic choriomeningitis virus; *VZV,* varicella-zoster virus; *WNV,* West Nile virus.
Modified from Tunkel AR, Glaser CA, Bloch KC, et al. The management of encephalitis: clinical practice guidelines by the Infectious Disease Society of America. Clin Infect Dis. *2008;47:303–327.*

complement fixation titer; and antibody titer. Other studies to consider based on epidemiologic risk profiles include PCR studies of the CSF for *Rickettsia, Ehrlichia,* and *Anaplasma*; CSF *Histoplasma* antigen; and CSF enzyme-linked immunosorbent assay (ELISA) and Western blot for *Borrelia burgdorferi*. Such tests are appropriate in areas where these agents are endemic or with potential exposure history.

CSF studies should be supplemented with serum diagnostic studies. Common tests include HIV serology and plasma HIV RNA viral load; EBV serologies (IgG and IgM antibodies against viral capsid antigen and IgG antibodies against early and nuclear antigens); *Mycoplasma* serologies; syphilis serology; *Borrelia* serologies[a]; *Rickettsia, Ehrlichia,*[a] and *Anaplasma* serologies[a]; serum cryptococcal antigen[a]; serum antibodies against *Coccidioides*[a]; and urine *Histoplasma* antigen.[a]

An EEG can be helpful in evaluating the type and frequency of seizures and the presence of nonconvulsive status epilepticus. The degree and severity of slowing is a sensitive indicator of the presence and severity of metabolic encephalopathies. Specific EEG patterns may suggest an increased likelihood of specific diagnoses (see "Diagnostic Tests").

Clinical Manifestations

The clinical presentation of patients with viral encephalitis can be heterogeneous, necessitating a high index of suspicion in any patient with signs of infection and CNS dysfunction. The syndrome of acute viral encephalitis is characterized by fever, headache, and altered mental status.[7] These may be combined with focal neurologic deficits corresponding to areas of the CNS that are infected and injured, which vary with specific pathogens. This constellation of signs and symptoms is not unique for encephalitis and can occur in various other diseases, including viral and bacterial meningitis, brain abscess, cerebritis, subdural and epidural empyema, and septic cerebral venous or sinus thrombosis. The frequency of specific signs and symptoms varies among different types of encephalitis and is discussed more completely in the sections on individual viral pathogens.

The presence of specific sets of neurologic signs and symptoms by themselves does not allow unequivocal diagnosis of a specific cause of encephalitis. Findings of neurologic examination can increase the likelihood of some etiologic agents and reduce the likelihood of other pathogens, facilitating and guiding confirmatory diagnostic testing. The most common focal neurologic signs associated with encephalitis include hemiparesis, aphasia, ataxia, cranial nerve (CN) palsies, myoclonus, and seizures. Other important neurologic findings include loss of temperature and vasomotor control because of autonomic dysfunction and either diabetes insipidus or the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) resulting from hypothalamic dysfunction.

Signs of parkinsonism (bradykinesia, rigidity, rest tremor) may suggest infection with a flavivirus (e.g., WNV, St. Louis encephalitis virus, Japanese encephalitis virus [JEV]), and frontotemporal signs such as aphasia, memory impairment, and personality changes may suggest limbic encephalitis from HSV, HHV-6 infection, a paraneoplastic etiology, or autoimmune limbic encephalitis. Seizures occur most frequently in encephalitides that involve the cortex (e.g., HSV encephalitis) and less commonly with flavivirus infections (e.g., WNV, St. Louis encephalitis virus, JEV) that involve predominantly deep gray matter structures such as the basal ganglia and thalamus. Brainstem involvement can occur with HSV, enterovirus 71 infections, and flaviviruses (e.g., WNV), whereas cerebellitis is associated with EBV, VZV, mumps, and flavivirus (e.g., WNV, St. Louis encephalitis virus) infections. Flaviviruses (e.g., WNV, JEV), enteroviruses D-68 and 71, and poliovirus can cause disease in the anterior horn of the spinal cord, resulting in a poliomyelitis and the clinical syndrome of acute flaccid paralysis.

The frequency of CN palsies varies greatly in patients with encephalitis. CN palsies are common in several nonviral infections that can mimic viral encephalitis (e.g., Lyme disease, syphilis, tuberculosis, fungal infections) and most commonly produce peripheral facial palsy (CN VII), vestibular or cochlear dysfunction (CN VIII), or paralysis of extraocular movements (CN III, IV, or VI). CN palsies are generally infrequent in viral encephalitis, unless there is concomitant brainstem involvement. However, WNV infection and VZV vasculopathy have been associated with palsies of various CNs (see specific sections).

The epidemiologic features of different neurotropic viruses vary widely and often help guide diagnostic testing (see Table 89.2). Immunocompromised patients are at increased risk for neuroinvasive viral infections resulting from VZV, CMV, HHV-6, JC virus (PML), and WNV. Similarly, older individuals have an increased risk of neuroinvasive disease after infection with many arboviruses (*ar*thropod-*b*orne *viruses*), including WNV, St. Louis encephalitis virus, and eastern equine encephalitis virus, and have an increased incidence of HSV encephalitis. Prenatal infection with subsequent pregnancy loss or devastating postnatal complications including microcephaly, intracranial calcifications, ventriculomegaly, and developmental abnormalities frequently occurs after infection with CMV and Zika virus (ZIKV).

Seasonal incidence is characteristic of all arbovirus infections in the United States. Most arboviruses are transmitted by a mosquito vector during summer and early fall months in patients living in areas of epizootic transmission of virus. By contrast, infection with some viruses, including lymphocytic choriomeningitis virus (LCMV) and influenza, are more frequent during late fall and winter months. Many arboviruses are geographically restricted, and residence or a recent travel history can provide important clues to potential exposures. WNV is now endemic throughout the United States and causes sporadic seasonal epidemics (www.cdc.gov/ncidod/dvbid/westnile/). Other arboviruses are also geographically restricted in the United States, and Powassan virus has emerged as an increasingly common cause of encephalitis (Fig. 89.2). A history of travel to specific foreign countries where other arboviruses are endemic should be specifically sought, and diagnostic studies should be adjusted appropriately.

The general physical examination also provides important clues that assist in the diagnosis of encephalitis. Skin rashes are common and characteristic for VZV, WNV, ZIKV, some enteroviruses, measles, and rubella and may occur with various nonviral infections, including Lyme

[a]Based on geographic setting and exposure history.

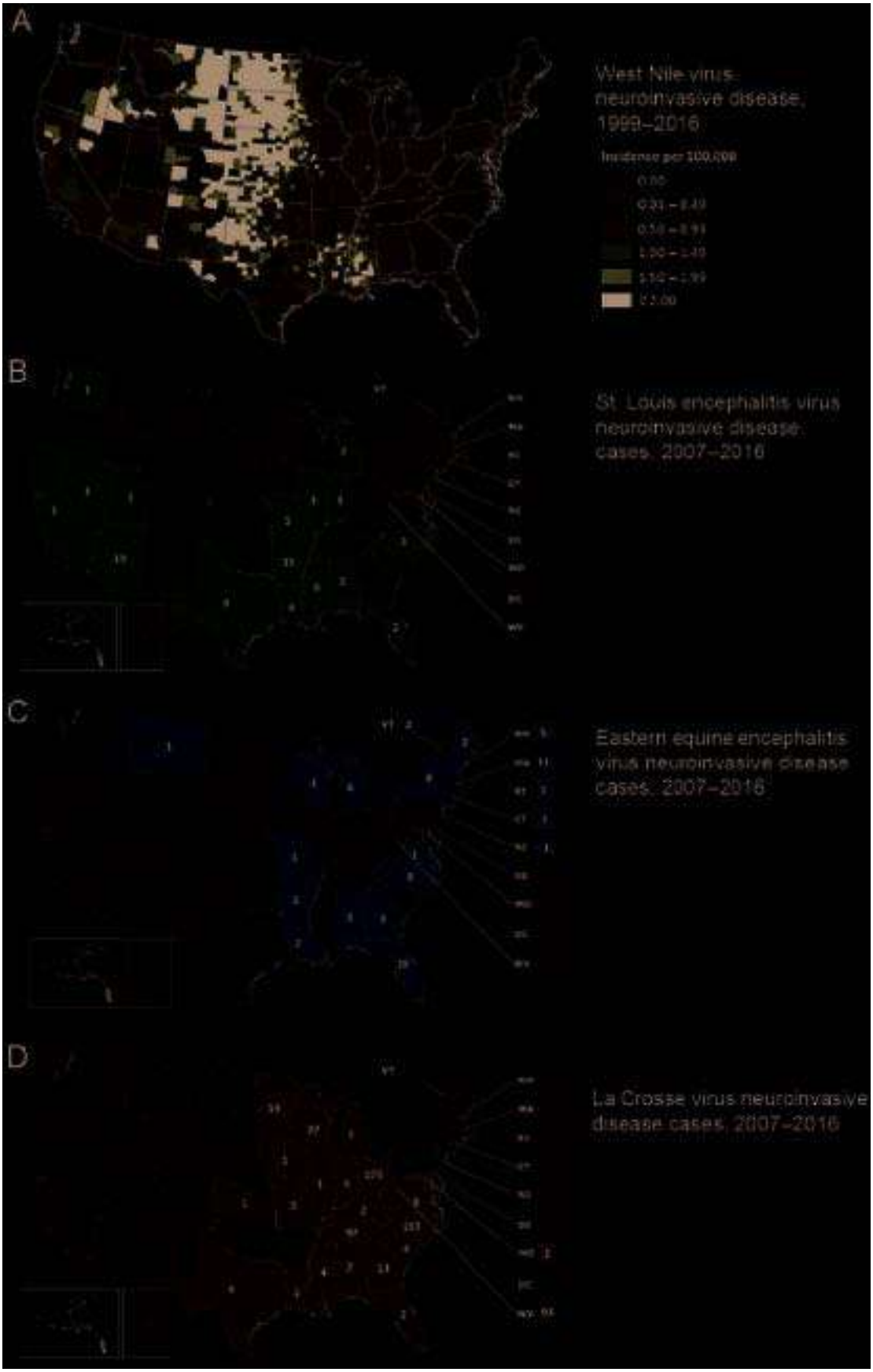

FIG. 89.2 **Incidence of select arboviruses in the United States.** (A) Annual incidence of West Nile virus neuroinvasive cases by county from 1999 to 2016. Number of neuroinvasive cases of (B) St. Louis encephalitis virus, (C) eastern equine encephalitis virus, and (D) La Cross virus reported to the Centers for Disease Control and Prevention from 2007 to 2016. *(Modified from Centers for Disease Control and Prevention.* www.cdc.gov/westnile, www.cdc.gov/sle, www.cdc.gov/easternequineencephalitis, www.cdc.gov/lac.*)*

disease, syphilis, mycoplasmosis, rickettsiosis, anaplasmosis, and ehrlichiosis. Oral mucosal ulcerations may suggest herpangina and an enterovirus. Ocular findings including chorioretinitis occur with WNV, CMV, and *Bartonella* infection (cat-scratch disease). Parotitis, orchitis, and oophoritis can occur with mumps and LCMV infections.

Diagnostic Tests

When a patient develops encephalitis, specific diagnosis is usually based on CSF studies. Diagnostic studies obtained from samples outside the CNS, including serum serologies, serum nucleic acid amplification tests, serum or urine antigen tests, and cultures of body fluids (blood, nasopharyngeal swab, sputum, and urine), may be useful in establishing the presence of a particular infectious process. Despite the presence of a systemic infection, additional testing may be required to establish a definitive cause of CNS disease. General diagnostic studies such as a complete blood count and markers of inflammation (e.g., erythrocyte sedimentation rate, C-reactive protein) may support the presence of an infectious or inflammatory process but do not help determine a specific etiology. Chest radiography may suggest an infectious cause, such as influenza, mycoplasmosis, tuberculosis, or coccidioidomycosis. It may also suggest the presence of cancer or other noninfectious causes of encephalitis.

Neuroimaging

Unless specifically contraindicated, all patients with suspected encephalitis should undergo MRI. The sensitivity and specificity of different patterns of neuroimaging abnormalities for the diagnosis of specific forms of encephalitis have not been formally defined. Different forms of encephalitis may produce distinctive MRI patterns that can provide clues suggestive of particular agents, which can guide more definitive confirmatory tests.

Temporal lobe and limbic abnormalities are seen in HSV, HHV-6, and autoimmune encephalitis, whereas subependymal enhancement occurs with CMV ventriculitis. MRI often reveals multifocal hemorrhagic infarctions and demyelinating lesions in cases of VZV vasculopathy.[27] Predominant demyelination on MRI studies suggests PML caused by JC virus infection, ADEM, or encephalitis mimics such as multiple sclerosis or other noninfectious CNS inflammatory diseases. PML lesions typically do not enhance or do so faintly at lesion margins, whereas MRI lesions associated with ADEM typically uniformly enhance acutely and become nonenhancing over time.

In HSV encephalitis, MRI is significantly more sensitive than CT, revealing temporal lobe abnormalities in 90% of PCR-proven HSV cases (Fig. 89.3).[28] In one study, 79% of patients with HSV encephalitis had abnormalities on their initial CT scan, but 100% of the 17 patients whose CT scan was initially normal and who had repeat scans had abnormalities an average of 5 days later.[28] MRI abnormalities of the temporal lobe were shown in 89% of patients with HSV; 36% also had frontal lobe involvement. Only 9% of adult patients with HSV encephalitis had MRI with abnormalities exclusively outside the frontotemporal area, although patterns in infants and children may be more variable. In 57% of HSV encephalitis patients, the abnormalities were predominantly unilateral.[17] Characteristic MRI changes occur early in the course of HSV encephalitis and include high–signal intensity lesions on T2-weighted and FLAIR images involving the medial and inferior temporal lobes with extension into the insula. MRI abnormalities can also include the orbitofrontal gyri and inferomedial frontal lobes.[29,30] Diffusion-weighted imaging abnormalities may antedate and be more extensive than abnormalities seen on T2 and FLAIR sequences.[21–26]

CT scans are almost invariably normal early in WNV infection. MRI abnormalities are less frequent in cases of WNV than in cases of HSV encephalitis. In two series involving 34 patients, initial MRI studies were normal in approximately one-third of patients. MRI abnormalities associated with WNV encephalitis included areas of increased T2 and FLAIR and low T1 signal that involved the basal ganglia, thalamus, and brainstem.[31,32] The MRI abnormalities in WNV infection are generally distinct from the abnormalities seen in HSV encephalitis, although in one series about 20% of patients with WNV encephalitis had abnormalities limited to the mesial temporal lobes.[32] Some patients with WNV infection have only meningeal enhancement or abnormalities seen only on diffusion-weighted images.

Patients with JEV and St. Louis encephalitis virus have MRI abnormalities similar to those reported for WNV. In one study, MRI was more sensitive than CT for JEV infections, showing abnormalities in more than 90% of adults and children.[33] The most commonly involved areas were the thalamus (88%), basal ganglia (41%–54%), and brainstem,[33,34] but some patients can have medial temporal lesions resembling those seen in HSV encephalitis.[35] Patients infected with enterovirus 71 may show increased T2 and FLAIR signal intensity in the midbrain, pons, and medulla. Congenital and neonatal ZIKV infection can result in a wide spectrum of abnormalities including microcephaly, intracranial calcifications, ventriculomegaly, and developmental abnormalities.

Cerebrospinal Fluid Analysis

Cerebrospinal Fluid Profile

CSF examination is an essential part of the diagnosis of encephalitis and should be performed in all patients unless absolutely contraindicated.[7] The most typical CSF profile in patients with viral encephalitis is a CSF pleocytosis with a predominance of lymphocytes, a normal glucose level, and an elevated protein level. In cases of flavivirus infection, particularly WNV, neutrophils may predominate,[36] a finding also associated with eastern equine encephalitis. In one large series, the median percentage of neutrophils in patients with WNV encephalitis was 45%, and 37% of cases had a neutrophil predominance.[36] By contrast, patients with HSV encephalitis typically have 5% to 24% neutrophils.[17,37]

The magnitude of CSF pleocytosis varies greatly in different forms of encephalitis. In the classic studies of biopsy-proven HSV encephalitis by the Collaborative Antiviral Study Group (CASG), patients had a median CSF white blood cell count of 130 cells/mm^3; 68% of patients had 50 to 500 cells/mm^3. Only 4% of patients had fewer than 5 cells/mm^3, and only 8% had more than 500 cells/mm^3.[38] Similar CSF profiles were noted in a study of HSV encephalitis confirmed with CSF PCR. In that study, no patient with HSV encephalitis had fewer than 5 cells/mm^3, and 69% of HSV encephalitis patients had 50 to 500 cells/mm^3.[28] Two later PCR-based studies reported that patients had a mean of 202 to 237 leukocytes/mm^3.[17,37] In a large series of cases with serologically proven WNV encephalitis, the mean CSF cell count was 227 cells/mm^3 (95% confidence interval [CI], 133–321).[36] Patients with mumps meningoencephalitis had higher mean cell counts compared with WNV patients, with a mean of 540 ± 460 cells/mm^3 and an average of 56% lymphocytes.[39]

Although pathologic evaluation of HSV encephalitis often shows the presence of hemorrhagic necrosis, CSF red blood cell counts do not differ significantly between patients with biopsy-proven HSV encephalitis and nonherpetic encephalitis.[38,40] Glucose concentrations are normal in more than 95% of patients with encephalitis caused by HSV and flaviviruses (WNV, St. Louis encephalitis virus) but may be low in some cases of CMV, mumps, and eastern equine encephalitis infection.[38,39,41,42]

Polymerase Chain Reaction and Antibody Studies

For some neuroinvasive viruses, PCR of the CSF has high sensitivity and specificity, making it the diagnostic study of choice for identifying a specific viral etiology.[43] HSV PCR of the CSF has a sensitivity of 98% and a specificity of 94%.[30,44] The sensitivity of PCR for detection of HSV encephalitis varies with the timing of the study. In the CEP, three patients with negative CSF HSV PCR test results performed within 72 hours of symptom onset had positive test results 4 to 7 days later.[45] By contrast, in a study using nested PCR to detect HSV DNA in CSF, 100% (18 of 18) of patients tested within 72 hours of onset of neurologic symptoms had amplifiable DNA.[46] CSF HSV PCR sensitivity declines as a function of duration of antiviral therapy; 98% of studies remain positive in patients treated for 7 days or less, followed by a decrease in sensitivity with ongoing treatment to 47% at 8 to 14 days and 21% after 15 days of antiviral treatment.[44] These results reflect the progressive decline in CSF viral load that occurs as a function of duration of acyclovir therapy. With use of quantitative PCR, acyclovir-treated patients had negative PCR results 19 ± 6 days (range, 9–28 days) after initiation of acyclovir

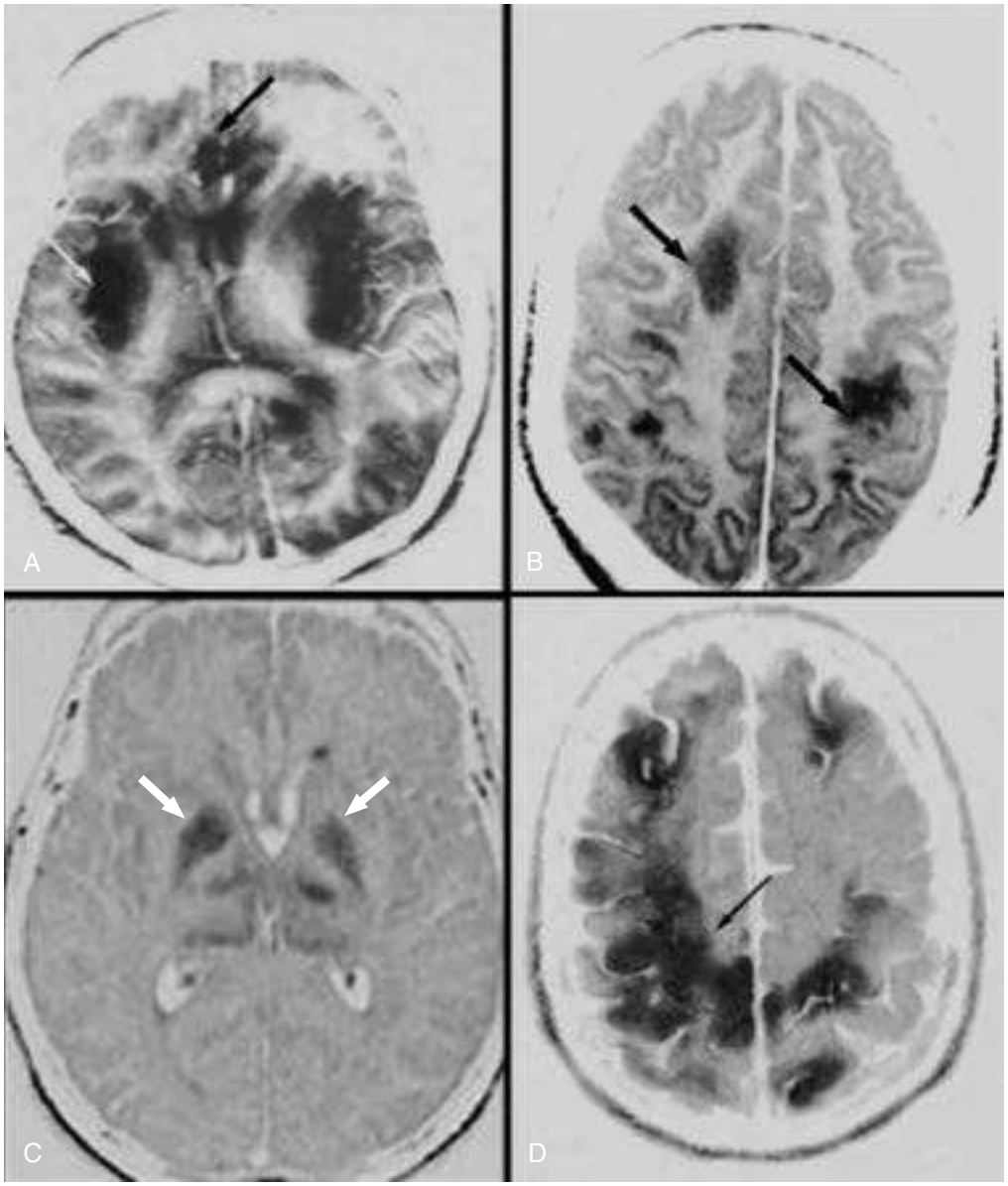

FIG. 89.3 Typical magnetic resonance imaging (MRI) changes associated with viral encephalitis. (A) Herpes simplex virus type 1 encephalitis with increased T2-weighted signal in bilateral temporal lobes. Increased signal does not extend beyond the insular cortex *(black arrow)* but does involve the cingulate gyrus *(white arrow).* (B), Varicella-zoster virus vasculopathy on proton-density MRI scan with multiple areas of infarction in both hemispheres *(arrows).* (C) West Nile virus encephalitis with increased signal on FLAIR MRI image of the basal ganglia *(arrows).* (D) Enterovirus encephalitis with increased signal intensity on FLAIR MRI image in both hemispheres, greater on the right, in the posterior cerebral hemisphere *(arrow). (Modified from Gilden DH, Mahalingam R, Cohrs RJ, Tyler KL. Herpesvirus infections of the nervous system.* Nat Clin Pract Neurol. *2007;3:83; and Debiasi RL, Tyler KL. West Nile virus meningoencephalitis.* Nat Clin Pract Neurol. *2006;2:264.)*

therapy.[47] Neither the initial CSF HSV viral load nor its maximal level predicts outcome[47,48]; however, one study found that patients with extremely high copy number (>100,000 copies HSV DNA per milliliter) were more likely to have a reduced level of consciousness, CT scan lesions, and poor outcomes compared with patients with less than 100,000 DNA copies/mL.[49]

The results of HSV PCR should be interpreted in the context of the timing of the study and the pretest probability of disease. In patients with a high pretest probability of HSV encephalitis (e.g., 60%), a positive PCR result increases the posttest probability to 99%, and a negative study result reduces it to 6%. In patients with a moderate pretest probability of HSV (e.g., 35%), the posttest probability for a positive test result is 98%, and probability for a negative test result decreases to 2%. In patients with low pretest probability of HSV encephalitis (e.g., 5%), the posttest probabilities are 84% for a positive test result and 0.2% for a negative test result.[50]

HSV-specific CSF antibody testing to detect intrathecal synthesis of antibody can complement PCR testing when specimens are obtained later in the course of illness. After the first week of disease, the sensitivity of CSF HSV PCR declines (see earlier), whereas the sensitivity of CSF antibody testing increases. In one series of patients with HSV encephalitis, none of 36 CSF samples taken later than 30 days after onset of neurologic symptoms had amplifiable DNA, but all had detectable HSV antibody.[46]

PCR detection of VZV DNA in the CSF has a specificity of greater than 95%, but sensitivity can decrease after 2 to 3 weeks following presentation. In cases of VZV CNS vasculopathy, CSF should also be tested for intrathecal synthesis of VZV-specific antibody (CSF–to–serum IgG ratio) because these studies are complementary to PCR.[51]

PCR for EBV or HHV-6 DNA in the CSF can be a valuable addition to the evaluation of a patient with viral encephalitis.[52,53] However, the

results must be interpreted with caution and correlated with the clinical presentation, imaging, and serology results because latently infected, HHV-6– or EBV-positive mononuclear cells in the CSF can produce false-positive results.

CSF PCR is extremely specific (>95%) for diagnosis of PML in immunocompromised patients with appropriate clinical and radiographic findings. The sensitivity of CSF JC virus PCR is quite variable in different settings (50%–80%). For example, patients with PML associated with use of immunomodulatory drugs such as natalizumab frequently have a low JC virus CSF copy number and those with HIV-associated PML a higher copy number, with assay sensitivity paralleling copy number. Higher CSF JC viral loads correlate with worse outcomes in patients with HIV-associated PML.[54] A positive JC virus serum antibody finding is of limited diagnostic use because approximately 55% to 85% of individuals are seropositive in serum by adult life, although the absence of detectable anti–JC virus antibodies may decrease the likelihood of PML in the differential diagnosis unless a patient is severely immunocompromised and unable to mount an antibody response. In some studies, increasing titers of CSF or serum JC virus antibodies have immediately preceded the onset of PML.

In the case of flaviviruses including WNV, CSF PCR is less sensitive (57%–70%) compared with detection of CSF IgM antibodies for diagnosis of neuroinvasive disease.[55] An exception may occur in immunocompromised patients, such as bone marrow and solid organ transplant recipients, who have a prolonged, high-titer viremia, and can have positive serum or CSF PCR studies, or both, when antibody tests are negative or seroconversion is delayed.[56,57]

In a study of blood donors with asymptomatic WNV infection detected with positive results of serum nucleic acid amplification testing, peak serum RNA levels occurred at 5 to 6 days after an infected mosquito bite, IgM seroconversion occurred 3.9 days after RNA detection (95% CI, 3.4–4.4), and IgG seroconversion occurred at 7.7 days after RNA detection (95% CI, 6.9–8.5).[58] CSF IgM WNV antibodies are diagnostic of acute WNV infection and neuroinvasive disease; their presence is indicative of intrathecal synthesis because IgM molecules cross the blood-brain barrier poorly owing to their large size. CSF IgM antibodies are found in approximately 80% of patients with WNV neuroinvasive disease within 1 week of onset of symptoms, increasing at a rate of about 10% per day after symptom onset.[59] It is unclear how long CSF WNV IgM antibodies persist. In one small study, 60% of patients with WNV encephalitis had persisting CSF IgM antibody for as long as 500 days.[60]

Future diagnostic approaches. Recent advances in nested PCR testing using film-array technology now allow for multiplex testing of patient samples against a panel of pathogens. Clinical laboratories are increasingly using multiplex PCR testing for multiple clinical samples including respiratory samples, stool samples, and CSF samples. A multicenter study of a multiplex diagnostic CSF panel found that the assay exhibited high specificity for all tested pathogens but exhibited variable sensitivity for several that ranged from 0% to 100%.[61] Some variability in sensitivity was due to low numbers of pathogens in the study groups. Also, inclusion of targets such as HHV-6 that can produce positive test results in situations that are not likely due to HHV-associated disease may create problems with interpretation. The current commercially available CSF arrays are more applicable for pediatric populations, and further development of these arrays will likely provide new diagnostic approaches for cases of adult encephalitis. In addition to multiplexed assays, next-generation sequencing (NGS) assays of CSF samples are being evaluated and applied to clinical samples.[62] As the cost for sequencing technology continues to decrease and processes are simplified, future diagnostic approaches may include routine metagenomic NGS for unbiased detection of pathogens. To date this technology has proven to be most useful in immunocompromised patients rather than as a global diagnostic tool. It is important to recognize that the major advantage of this approach is its "unbiased" nature—meaning its ability to enable detection of a wide variety of pathogens. Conversely, NGS may be less sensitive than individually targeted PCR assays for specific pathogens and requires meticulous attention to technique to avoid contamination from reagents or other ambient sources.

Electroencephalography

EEGs in patients with viral encephalitis are frequently abnormal, but the results only rarely provide a clue to a specific etiologic diagnosis. The most common abnormality is the presence of generalized slowing.[63] Focal EEG abnormalities, most commonly involving the temporal lobes, are seen in 75% to 80% of patients with HSV encephalitis.[28,38] Common abnormalities include the presence of frontotemporal slowing, temporal sharp or spike activity, and periodic lateralizing epileptiform discharges at a rate of 2 to 3 Hz.[38] None of these patterns is diagnostic of HSV encephalitis. Periodic lateralizing epileptiform discharges, previously considered characteristic of HSV encephalitis, can also be seen in other types of encephalitis (e.g., La Crosse/California virus encephalitis).[64] Appearance and resolution of EEG abnormalities do not correlate well with clinical presentation or resolution of disease.[30,65]

EEG abnormalities have been reported in approximately 60% to 90% of patients with WNV encephalitis. The most common finding is the presence of diffuse irregular slow waves, although the presence of anteriorly predominant slowing may suggest the diagnosis.[66] Triphasic slow waves, generally considered more characteristic of metabolic encephalopathies, have also been reported in WNV encephalitis.[67]

Other Diagnostic Studies

In general, CSF culture is of limited value in cases of viral encephalitis and is not recommended for routine clinical application.[7] Occasionally, acute and convalescent serum studies suggest a viral etiology and may be required in rare cases when CSF evaluation is impossible. Additional studies, such as PCR assay of gastrointestinal samples for enteroviruses, have been used as supportive diagnostic tests for viral encephalitis.[63] Other supportive tests include PCR or antigen detection of respiratory secretions for adenoviruses, parainfluenza, or influenza viruses. Fluid from skin vesicles should be examined with PCR for evidence of enteroviruses, VZV, or HSV. Brain biopsy is rarely used today because of the availability and accuracy of PCR and antibody tests. Brain biopsy still serves a diagnostically useful function, however, in unexplained cases of encephalitis associated with progressive neurologic deterioration.[7]

Management

The clinical status of patients with encephalitis can deteriorate rapidly; patients should be closely monitored in an intensive care unit or equivalent setting. Viral encephalitis caused by arboviruses or herpesviruses does not require patient isolation for infection control. Respiratory or contact isolation should be considered in cases of encephalitis of unknown etiology or in patients with possible bacterial meningitis or a skin rash. Universal precautions should be applied to handling all body fluids, including CSF, blood, saliva, respiratory secretions, stool, and urine because their potential infectivity varies with the inciting pathogen.

Patients with encephalitis may develop increased ICP. Patients with potential signs of increased ICP, such as decreased level of consciousness, papilledema, or cerebral edema, often require continuous ICP monitoring. Clinical trials studying the role of corticosteroids in encephalitis patients have been inconclusive, and further studies are necessary; however, in cases of encephalitis with increased ICP, corticosteroids may be used to treat cerebral edema.[63,68,69] Additional measures to reduce increased ICP acutely include hyperventilation and administration of intravenous (IV) mannitol.

Patients with certain types of encephalitis are likely to be at increased risk of seizures, and seizures can contribute to transiently increased ICP. There is no proven clinical value for primary prophylactic anticonvulsant therapy in patients with encephalitis. Patients with seizures are generally treated urgently with lorazepam or diazepam followed by maintenance therapy with IV fosphenytoin. Patients may require continuous EEG monitoring because clinical observation in obtunded patients may not reliably detect seizures.

Patients with encephalitis can experience autonomic dysfunction resulting in hypotension or cardiac arrhythmias and should undergo close monitoring of blood pressure and electrocardiogram until clinically stable. If the airway is compromised because of alterations in

consciousness, intubation should be considered to protect the airway and prevent aspiration. If clinically indicated, empirical antimicrobial agents should be initiated in patients with suspected bacterial infection or bacterial meningitis.

Few randomized controlled trials have assessed the efficacy of antiviral treatments or immunomodulatory therapy in patients with viral encephalitis, other than acyclovir therapy in patients with HSV encephalitis. Current recommended treatments for viral encephalitis are largely based on case reports or small clinical case series. (See the Herpesviruses section later for more information on acyclovir therapy.)

VIRAL ETIOLOGY OF ENCEPHALITIS

General features of the basic biology, pathogenesis, clinical features, epidemiology, diagnosis, and treatment of particular agents are discussed in the individual chapters devoted to these agents and have been reviewed.[70] Brief summaries of relevant issues related to encephalitis are provided in the subsequent sections, and additional encephalitis viruses not discussed in the text are summarized in Table 89.3. Table 89.4 presents the Infectious Diseases Society of America (IDSA)–US Public Health Service (USPHS) grading system for ranking recommendations.

TABLE 89.3 Other Important and Emerging Causes of Viral Encephalitis

VIRAL ETIOLOGY	EPIDEMIOLOGY	CLINICAL FEATURES	DIAGNOSIS	TREATMENT
Adenovirus	Children and immunocompromised patients	Associated pneumonia	PCR or culture of brain biopsy specimen or CSF	Supportive
Chikungunya virus	Epidemic setting; India and Southeast Asia; mosquito vector	Febrile syndrome with rash and arthralgias	CSF and serum IgM and PCR	Supportive
Hendra virus	Australia; fruit bat reservoir; humans infected by secretions of bats	Fever, drowsiness, seizures, and coma associated with a flulike prodrome	Contact Special Pathogens Branch at CDC	Supportive
HIV	Worldwide epidemic; recent high-risk behavior	Fever, headache-associated acute retroviral syndrome; commonly associated with HIV dementia	HIV serology testing and HIV quantitative PCR of CSF; MRI may reveal T2 or FLAIR hyperintense lesions in periventricular regions and centrum semiovale	Combination antiretroviral therapy
Influenza	Fall and winter seasonal predilection; worldwide distribution; rare complication in children	Associated febrile syndrome, myalgias, respiratory prodrome; may be associated with bilateral thalamic necrosis	Viral culture, antigen detection, and PCR in respiratory secretions	Oseltamivir (III-C); poor outcomes
Japanese encephalitis	Mosquito vector, swine and bird reservoirs; most common cause of epidemic viral encephalitis throughout Southeast Asia and Australia	Seizures and parkinsonian features common; acute flaccid paralysis; case-fatality rate of 20%–30%	Serum IgM or acute/convalescent IgG; CSF IgM or antigen; MRI can show T2 and FLAIR hyperintense lesions at basal ganglia, thalami, and midbrain	Supportive; formalin-inactivated mouse brain–derived vaccine available for prevention
JC virus	Cell-mediated immunodeficiencies (AIDS) and immunomodulating therapy (e.g., natalizumab)	Cognitive dysfunction, limb weakness, gait disturbance, visual loss, focal neurologic findings	CSF PCR (sensitivity 50%–70% for PML); MRI shows ≥1 nonenhancing, confluent subcortical white matter hyperintensity on T2 and FLAIR sequences	Combination antiretroviral therapy in AIDS patients or reversal of immunosuppression
Louping ill virus	Tick-borne disease; found in Ireland, Scotland, and England; associated with livestock	Usually mild febrile illness with associated confusion and stupor in some; deaths are rare	Serum IgM ELISA or a fourfold increase in IgG antibody in paired acute and convalescent sera	Supportive
LCMV (see Chapter 167)	Rodent-borne virus infects humans with exposure to infected urine, feces, saliva, or blood; severe disease in immunocompromised patients	Fever, headache, leukopenia, and thrombocytopenia; encephalitis characterized by personality changes, increased ICP, paraplegia, and cranial nerve and sensory dysfunction	CSF and serum IgM ELISA	Supportive
Me Tri virus	Mosquito-borne; Southeast Asia; transmitted among livestock	Fever, rash, seizures, lethargy, and meningismus	CSF PCR and IgM ELISA, serology	Supportive
Monkeypox	Prairie dog exposure	Vesiculopustular rash on head, extremities, palms, and soles; adenopathy; encephalitis is rare, with confusion, somnolence, and diminished reflexes	Skin biopsy, CSF, and serum IgM, serology; MRI showing T2 and FLAIR hyperintense lesions of the pons, thalamus, and subparietal cortex	Supportive care; consider cidofovir or vaccinia immune globulin (III-C)
Mumps virus	Unvaccinated	Previous parotitis followed by headaches, vomiting, seizures, altered consciousness, and sensorineural hearing loss	Fourfold IgG increase in paired acute and convalescent sera, culture of saliva, CSF culture and PCR	Supportive
Murray Valley encephalitis virus	Mosquito vector; bird reservoir; Australia and New Guinea	Rapid onset in infants with case-fatality rate of 15%–30%	IgG antibody testing with fourfold increase in paired acute and convalescent sera	Supportive
Nipah virus	Exposure to infected pigs; pteropid bat reservoir; exposure to infected bats or bat roosting sites; close contact to infected humans; South Asia	Fever, headache, altered consciousness, dizziness, vomiting, myoclonus, dystonia, areflexia, hypotonia; pneumonitis	Fourfold IgG increase in paired acute and convalescent sera; CSF culture; MRI may show T2 focal hyperintensity of subcortical and deep white matter of cerebral hemispheres; contact Special Pathogens Branch at CDC	Supportive; ribavirin (III-C)

Continued

TABLE 89.3 Other Important and Emerging Causes of Viral Encephalitis—cont'd

VIRAL ETIOLOGY	EPIDEMIOLOGY	CLINICAL FEATURES	DIAGNOSIS	TREATMENT
Powassan virus	Tick vector; rodent reservoir; New England states, Canada, and Asia	Case-fatality rate of 10%–15% and focal neurologic findings in >50% of patients	Serum and CSF IgM; IgG antibody fourfold increase in acute and convalescent paired sera	Supportive
Rift Valley fever virus	Sub-Saharan Africa, Egypt, Saudi Arabia, Yemen; mosquito vector and livestock reservoir; humans infected via exposure to infected animal secretions	1% of infected humans develop encephalitis with headache, meningismus, and altered consciousness	ELISA antigen detection or culture from serum and PCR; contact Special Pathogens Branch at CDC	Supportive
Rocio virus	Cause of epidemic encephalitis in Brazil; mosquito vector	Fever, headache, confusion, motor impairment, and cerebellar syndrome; sequelae include ataxia, dysphagia, incontinence, and memory problems	Fourfold IgG increase in acute and convalescent sera	Supportive
Rubella virus	Unvaccinated adults	Rash followed by headache, dizziness, behavioral changes, and seizures	CSF IgM; fourfold IgG increase in paired acute and convalescent sera	Supportive
Snowshoe hare virus	Mosquito-borne; North America; children predominantly affected by encephalitis	Fever, headache, confusion, and lethargy; low mortality and rare long-term neurologic sequelae	CSF and serum IgM ELISA or fourfold increase in IgG acute and convalescent sera	Supportive
Tick-borne encephalitis virus	Tick vector; rodent reservoir; unpasteurized milk; Eastern Russia, central Europe, Far East	Acute encephalitis; acute flaccid paralysis	Serum IgM or fourfold increase in IgG antibody in paired acute and convalescent sera	Supportive
Toscana virus	Sandfly vector; infection during summer months in Mediterranean countries	Fever, headache, meningismus, and meningoencephalitis with coma, lethargy, hydrocephalus, and hepatosplenomegaly	CSF PCR; serum and CSF IgM; fourfold increase in IgG acute and convalescent sera	Supportive
Vaccinia	Most cases are postinfectious; rare event after vaccination	Abrupt encephalopathy with focal neurologic signs 2–30 days postvaccination	CSF PCR or IgM	Supportive; corticosteroids if postvaccination (III-C); consider cidofovir or vaccinia immune globulin
Zika virus	Mosquito-borne, epidemic febrile rash	Fever, rash, congenital infection	PCR of serum and ZIKV-specific IgM	Supportive

AIDS, Acquired immunodeficiency syndrome; *CDC,* Centers for Disease Control and Prevention; *CSF,* cerebrospinal fluid; *ELISA,* enzyme-linked immunosorbent assay; *FLAIR,* fluid-attenuated inversion recovery; *HIV,* human immunodeficiency virus; *ICP,* intracranial pressure; *LCMV,* lymphocytic choriomeningitis virus; *MRI,* magnetic resonance imaging; *PCR,* polymerase chain reaction; *PML,* progressive multifocal leukoencephalopathy.
Modified from Tunkel AR, Glaser CA, Bloch KC, et al. The management of encephalitis: clinical practice guidelines by the Infectious Disease Society of America. Clin Infect Dis. *2008;47:303–327.*

TABLE 89.4 Infectious Diseases Society of America–US Public Health Service Grading System for Ranking Recommendations

CATEGORY, GRADE	DEFINITION
Strength of Recommendation	
A	Good evidence to support recommendation for use
B	Moderate evidence to support recommendation for use
C	Poor evidence to support recommendation for use
Quality of Evidence	
I	Evidence from ≥1 randomized, controlled trial
II	Evidence from ≥1 well-designed clinical trial, without randomization; from cohort or case-control analytical studies (preferably from >1 center); from multiple time series; or from dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

Modified from Tunkel AR, Glaser CA, Bloch KC, et al. The management of encephalitis: clinical practice guidelines by the Infectious Disease Society of America. Clin Infect Dis. *2008;47:303–327.*

Herpesviruses

The Herpesviridae are ubiquitous viruses found in multiple animal species. Herpesviruses that cause neuroinvasive disease in humans include HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8, and simian herpesvirus B.

Herpes Simplex Virus Types 1 and 2

Herpes Simplex Virus Pathogenesis

The exact mechanism by which HSV-1 and HSV-2 invade the CNS is unknown. Primary HSV-1 infection frequently causes gingivostomatitis, which is followed by axoplasmic transport of virus to the trigeminal sensory ganglion, where latency can be detected in virtually all seropositive individuals.[30,71] Trigeminal ganglion reactivation of HSV-1 usually results in retrograde axonal transport and the development of herpes labialis, but rarely reactivation may result in spread via the tentorial nerves to the anterior and medial cranial fossa.[72] It has been suggested, on the basis of animal inoculation studies, that the olfactory tract could also provide a potential route of neuroinvasion for HSV-1. In the case of primary infection, virus could invade the olfactory bulbs through the nose and spread via olfactory pathways to the orbitofrontal and medial temporal lobes.[30] At autopsy, approximately one-third of HSV-seropositive individuals with no known neurologic illness have PCR-amplifiable HSV DNA in the CNS, including the olfactory bulbs, hippocampus, and brainstem.[73] If this represents latent virus, which has not yet been convincingly established, reactivation from CNS sites could provide another mechanism for development of HSV encephalitis.

Cell death and tissue injury in HSV encephalitis likely result from direct viral killing of neurons and immune-mediated mechanisms of cell death. Several studies have shown that apoptosis plays a role in

neuronal cell death in human HSV encephalitis.[74,75] Studies in humans and mice have shown that the host's immune response may contribute to HSV-induced neuronal injury and death during encephalitis.[76–78] Genetic defects in TLR3-interferon and interferon responses have been shown to predispose to HSV encephalitis in several studies and may be particularly important in individuals with recurrent disease or in families with multiple cases of herpetic encephalitis.

HSV is the most common cause of sporadic encephalitis in the United States, with an incidence of hospitalization ranging from 1 to 1.2 per 100,000 population per year; it accounted for approximately 3300 hospitalizations per year from 1998 to 2010.[4] A recent multinational study in immunocompetent adults found that HSV-1 accounted for 92% of HSV encephalitis cases, with the remainder caused by HSV-2.[79] In patients with HSV in the CNS without evidence of encephalitis, largely meningitis cases, HSV-1 accounted for 45% of cases and HSV-2 accounted for the remainder. Although HSV encephalitis is the most commonly identified specific cause of viral encephalitis, it still accounts for only approximately 10% of all encephalitis cases.[30,80,81] HSV encephalitis has no seasonal or gender predilection. Cases occur in a bimodal age distribution with approximately one-third of individuals affected at younger than 20 years, and half of cases occur in individuals older than 50 years.[30]

Herpes Simplex Virus Encephalitis: Clinical Presentation

Patients with HSV encephalitis present with fever (90%–100%), altered consciousness (97%–100%), and headache (70%–81%).[17,38,82] Other common clinical features of HSV encephalitis include disorientation (70%–76%), seizures (40%–68%), behavior or personality change (41%–87%), memory disturbance (24%–45%), motor deficit (30%–40%), and speech disturbances (31%–59%).[17,28,38,40] A more recent multinational study of patients with HSV neuroinvasive disease found that fever (83%), change in consciousness (80.2%), headache (59.9%) and disorientation (58.3%) were the most common symptoms associated with encephalitis.[79] Mild or otherwise atypical presentations of HSV encephalitis have been reported in 14% to 17% of PCR-proven cases. These patients typically have mild alteration in level of consciousness and an absence of focal features at neurologic examination (although focal features may be present on EEG or neuroimaging studies).[28,83,84] A "normal" initial neurologic examination has been reported in less than 6% of patients.[17] In the original CASG trial comparing vidarabine and acyclovir, 29% of patients had a Glasgow Coma Scale (GCS) score of 6 or less, 41% had a GCS score of 7 to 10, and 30% had a GCS score of 10 or greater.[40] In later studies using CSF HSV PCR rather than brain biopsy for diagnosis, the level of consciousness of patients has been higher because of earlier detection. In the study by Domingues and associates,[28] 78% of patients had a GCS score of 12 or greater, including 28% with a GCS score of 15.

Herpes Simplex Virus Encephalitis: Diagnosis

PCR of HSV DNA in the CSF is the procedure of choice for diagnosis of HSV encephalitis[44,46] (see earlier for full discussion). Compared with brain biopsy, CSF HSV PCR has a sensitivity of 98% and a specificity of 94%, and more recent PCR tests for HSV exhibit sensitivity >95% and specificity >99%.[44,79] PCR results need to be evaluated in light of the timing of test performance. False-negative PCR results may also occur in bloody CSF specimens as a result of the presence of porphyrin compounds derived from degradation of heme in erythrocytes or during early stages of presentation, notably the first 48 hours after symptom onset.[46]

The results of diagnostic tests including CSF examination, neuroimaging, and EEG in HSV encephalitis were discussed in earlier sections. As noted, 96% to 100% of patients have a CSF pleocytosis (>5 white blood cells/mm^3) with a lymphocytic predominance. The protein is usually elevated (mean and median, 80–85 mg/dL), and the glucose level is normal in 95% of cases.[17,28,40] Initial CT scan can be abnormal in 40% to 79% of patients.[17] The most commonly observed abnormalities have been found to occur in the temporal (53%) or temporal and frontal lobes (36%).[17] MRI is more sensitive than CT and reveals abnormalities of the temporal lobes in 89% of cases confirmed with CSF HSV PCR (see Fig. 89.3A).[28] EEG patterns are not specific for HSV encephalitis, but as noted earlier, focal EEG abnormalities have been reported in 81% of biopsy-proven HSV encephalitis cases[38] and temporal lobe abnormalities occur in 75% of PCR-proven cases.[28] A more recent study found that MRI was abnormal in 84% of HSV encephalitis patients, and 91% of patients had an abnormal EEG.[79]

Herpes Simplex Virus Encephalitis: Treatment and Outcomes

The treatment and factors influencing prognosis for HSV encephalitis were discussed earlier. IV acyclovir (10 mg/kg every 8 hours) is the drug of choice for treatment of HSV encephalitis. The recommended duration of acyclovir therapy is 14 to 21 days according to the IDSA guidelines, and the mean duration of therapy in many studies is 21 days.[7] In the original CASG trial of adenine arabinoside for treatment of HSV encephalitis, the mortality in the 10 placebo-treated patients was 70%. Of the 3 survivors, 1 had minor, 1 had moderate, and 1 had severe sequelae.[85] A randomized placebo-controlled phase III clinical trial comparing long-term oral valacyclovir with placebo after completion of standard IV acyclovir treatment for HSE found no benefit to ongoing oral antiviral therapy for HSE.[86] Similarly, there is no proven benefit of adjunctive steroid therapy for treatment of HSV encephalitis, although uncontrolled studies and some experimental animal models suggest possible benefit.

Guidelines from IDSA advise that acyclovir should be initiated in all patients with suspected encephalitis, pending results of diagnostic studies (level III-A recommendation; see Table 89.4).[7] The goal of empirical treatment before diagnosis is to increase the likelihood of initiating treatment at an earlier stage in patients who ultimately prove to have HSV encephalitis. There is no proven benefit of acyclovir therapy in patients with non-HSV encephalitis (see discussion of specific herpesviruses for virus-specific details). The benefit of "empirical" use of acyclovir in patients with suspected encephalitis, rather than in patients with established HSV encephalitis, has not yet been established in a randomized controlled clinical trial.[40] Several studies have now shown that initiation of acyclovir within 2 days of hospitalization is associated with more favorable outcomes in patients with HSV encephalitis.[17,87,88] Multiple factors contribute to unfavorable outcomes, including advanced age, comorbid conditions, symptom duration before hospitalization, severely depressed consciousness, extent of brain involvement at MRI, and duration of hospitalization.[17,87–89]

A retrospective study found that only 29% of patients meeting specified criteria for suspected encephalitis (fever, neuropsychiatric abnormality, CSF pleocytosis, and negative Gram stain) received empirical acyclovir in the emergency department (median time to administration, 1.5 hours; 95% CI, 0–31.1 hours). The remaining 71% received acyclovir after inpatient admission (median time, 16 hours; 95% CI, 7.5–44 hours).[90] Five of 24 patients studied were eventually diagnosed with HSV encephalitis; only 2 of the 5 diagnosed with HSV encephalitis received acyclovir in the emergency department. In a large French study, a mean delay of 2 days ± 2.7 occurred between hospital admission and initiation of acyclovir therapy, and a mean of 5.5 days ± 2.9 lapsed between reported onset of symptoms and initiation of treatment.[17] These studies suggest that delays in administration of empirical antiviral therapy are common.

Several factors influence the prognosis of HSV encephalitis, including patient age, level of consciousness, immune suppression, and duration of clinical encephalitis before initiation of acyclovir therapy (measured by day of symptom onset or day of hospitalization). In the CASG trials of vidarabine versus acyclovir, age was stratified into patients younger than 30 years and patients 30 years of age or older.[40] Mortality was 6% (1 of 18) in acyclovir-treated patients younger than age 30 years but increased to 36% (5 of 14) in the older age group. It was subsequently reported that mortality in acyclovir-treated patients increased in a stepwise fashion from 11% (<2 years old) to 22% (22–59 years old) to 62% (≥60 years old).[91]

Similarly, level of consciousness at presentation, as evaluated according to GCS score, influenced mortality. In the original trial comparing vidarabine and acyclovir, mortality was 25% (2 of 8) in patients with a GCS score less than 6 compared with 17% (4 of 24) in patients with

a GCS score greater than 6.[40] It was subsequently noted that mortality was 0% in acyclovir-treated patients with a GCS score greater than 10 compared with 25% in patients with GCS score of 10 or less.[91] Patients with a GCS score of 7 to 10 and early institution of acyclovir therapy have improved outcomes compared with patients with lower GCS scores or late institution of acyclovir therapy.[91] In one large study, patients treated more than 2 days after hospital admission had a 3.1-fold (95% CI, 1.1–9.1) greater likelihood of a poor outcome compared with patients treated earlier.[17] In the vidarabine and acyclovir comparative study, the mortality rate in patients treated within 4 days of onset of fever, headache, and focal neurologic findings was 0% compared with a mortality of 35% in patients with symptoms of disease for more than 4 days.[40] Additional factors reported to influence prognosis include the Simplified Applied Physiology Score (SAPS II),[17] presence of lesions on an initial CT scan, and immunocompromised state.[92] Studies have indicated that the absolute value of the HSV PCR viral load in the CSF has no prognostic implications.[93,94] The role of management of increased ICP in HSV encephalitis has not been studied systematically, but use of aggressive medical and surgical therapy has been reported to be beneficial.[95]

HSV-2 Central Nervous System Disease

When evaluating patients with HSV neuroinvasive infections, the clinician must remember that HSV can also cause recurrent meningitis and myelitis. Although HSV-1 is the most common cause of sporadic, viral encephalitis, HSV-2 is the most common cause of recurrent, benign lymphocytic meningitis.[96] Patients with recurrent HSV-2 meningitis are more likely to be female (69%) and have a mean age of 38 years.[96,97] Patients present with acute onset of headache, fever, photophobia, and meningismus. Episodes last for a mean of 6 days, and patients have an average of four recurrences.[96,97] CSF is remarkable for lymphocytic pleocytosis (range, 86–1800), mildly elevated protein (range, 60–258), and normal glucose.[98] Diagnosis is made with HSV PCR of the CSF and is positive in 85% of patients with recurrent meningitis.[97] Neuroimaging is normal in the majority of patients (83%), although some patients develop nonspecific changes (14%) or meningeal changes (3%).[98] Of patients with CSF that was positive for HSV with PCR, 95% of cases are caused by HSV-2. Although recurrent HSV meningitis is a benign, self-limiting illness, acyclovir was used in the past for suppression of recurrences.[96] However, a double-blind, randomized trial demonstrated that suppressive therapy with valacyclovir was equivalent to placebo in suppressing HSV meningitis recurrences.[99] After 1 year of study drug exposure, patients were followed for an additional year off study drug, and patients exposed to valacyclovir had a significantly elevated risk (hazard ratio, 3.29) of recurrent HSV meningitis compared with the placebo group.

Herpes Simplex Virus Myelitis

HSV-1 and HSV-2 cause myelitis, but HSV-2 most commonly causes myelitis in adults. Most cases manifest as a monophasic illness (about 20% are recurrent) associated with acute onset of paralysis in the legs or less commonly the arms, reduced or absent reflexes, hyperreflexia with extensor-plantar responses, decreased sensation, and decreased anal tone or urinary incontinence with sacral dermatome involvement.[100–102] Severe forms of HSV myelitis include acute necrotizing myelopathy, which occurs in patients with underlying disease such as HIV, diabetes, or malignancy. CSF examination shows lymphocytic pleocytosis, elevated protein, and normal glucose concentration.[100,101] MRI typically exhibits intramedullary fusiform or spindle-shaped areas of increased T2-weighted signal.[100,101] Diagnosis is made with HSV PCR in the CSF, but measuring intrathecal synthesis of HSV-specific immunoglobulins may be helpful in the diagnosis of recurrent cases.[101] There are no controlled trials of antiviral therapy for HSV myelitis; however, there are anecdotal reports of success with IV acyclovir (10 mg/kg every 8 hours) for at least 14 days followed by oral antiviral drugs (valacyclovir, 1 g three times daily) until symptoms resolve. Despite therapy, the prognosis is extremely variable. In one small study of nine patients with HSV myelitis, one-third (three patients) made a complete recovery, whereas the remaining patients had residual sequelae including paraplegia and tetraplegia.[101]

Varicella-Zoster Virus

Varicella-Zoster Virus Pathogenesis and Central Nervous System Disease

Primary VZV infection (chickenpox) occurs mainly in children 1 to 9 years old, and seroprevalence by adult life is greater than 95%. After primary infection, the virus establishes latency in dorsal root ganglia. Reactivation results in herpes zoster (shingles). VZV CNS infection (encephalitis or vasculopathy) can occur during primary infection or after viral reactivation from latency.

During primary infection with VZV, acute cerebellar ataxia develops in 1 in 400 children younger than 15 years old.[103] It is unknown whether this clinical presentation is due to direct viral infection in the CNS[104] or represents a postinfectious immune-mediated state.[105] The clinical presentation is characterized by onset of gait ataxia, tremor, vomiting, and headache occurring 1 to 3 weeks after onset of chickenpox. CSF shows a lymphocytic pleocytosis with elevated protein. Imaging findings are often normal, and patients tend to have a full recovery. A study of VZV encephalitis cases found that a variety of VZV genotypes still cause neuroinvasive disease despite introduction of varicella vaccine, and vaccine genotypes are rarely associated with neuroinvasive infection in the CNS.[106]

VZV infection can cause myelitis associated with shingles or, more rarely, in the absence of rash ("zoster sine herpete"). In immunocompetent patients, myelitis is focal and typically manifests as weakness in myotomes corresponding to the dermatomes of the associated shingles outbreak (segmental zoster paresis). Immunocompetent and immunocompromised patients may develop more severe zoster myelitis.[107] In these cases, myelitis follows the rash by a median of 12 days, with maximal deficit being reached an additional 10.5 days later. The most common neurologic manifestations are weakness (75%), usually involving the leg ipsilateral to the rash, and less commonly paraparesis or paraplegia. Sensory abnormalities are common (approximately 50%) and can include dysesthesias, paresthesias, loss of pain-temperature or position-vibration sense or less commonly a sensory level to all modalities, or Brown-Séquard syndrome. Sphincter disturbances occur in approximately 25%. Some patients may have recurrent episodes of myelitis.[108] About 75% of patients have a lymphocytic CSF pleocytosis with elevated protein (approximately 70%) and normal glucose (approximately 95%) levels. MRI may show increased T2 signal lesions in the cord in association with cord swelling.[108] Diagnosis can be confirmed with demonstration of VZV DNA in CSF with PCR, or intrathecal anti-VZV antibody synthesis (increased CSF–to–serum IgG ratio).[108]

Varicella-Zoster Virus Vasculopathy

Modern studies suggest that most cases of VZV "encephalitis" are actually caused by VZV CNS vasculopathy, which most commonly involves large vessels (granulomatous arteritis) in immunocompetent patients and small vessels in immunocompromised patients.[108–113] However, a case series found that only 4 of 20 patients with VZV encephalitis developed a vasculopathy, and 14 of the 20 patients had nonvascular, nonspecific, or normal neuroimaging results.[114] In elderly immunocompetent patients, large vessel vasculopathy follows an episode of trigeminal herpes zoster by a few weeks to months with an average time to onset of 4 months, resulting in a mortality rate of 25%.[115] Most cases are monophasic, but relapses can occur. Patients develop myriad symptoms and acute focal deficits that vary with the location of the lesions. MRI findings are abnormal in 97%, with multifocal hyperintense lesions on T2-weighted FLAIR images (see Fig. 89.3B).[115] Lesions are typically located in the white matter or at the gray-white matter junction. Typical CSF findings include lymphocytic pleocytosis (67%), elevated protein level, and normal glucose level.

Small vessel VZV vasculopathy classically occurs in immunocompromised patients, resulting in a clinical syndrome of altered mental status changes, headache, focal deficits, and seizures.[115] The typical rash of herpes zoster is often absent. Neuroimaging shows evidence of multifocal infarcts, and CSF analysis reveals mild pleocytosis and a normal-to-mild elevation in CSF protein. In a retrospective study of VZV vasculopathy, 70% of patients had abnormalities at magnetic resonance angiography or traditional angiography.[115] Fifty percent had

evidence of small vessel and large vessel involvement. Angiography reveals focal narrowing or occlusion of involved vessels.

Diagnosis of VZV vasculopathy can be made by demonstration of intrathecal synthesis of VZV-specific antibody or by demonstration of VZV DNA in CSF with PCR.[115] Because VZV can cause a vasculopathy, some studies suggest that serum VZV PCR assay is sensitive and can help support a potential diagnosis of VZV CNS vasculopathy. At autopsy, involved vessels show focal inflammation with multinucleated giant cells, VZV antigen in endothelial cells, and Cowdry type A inclusion bodies in infiltrating histiocytes.[116]

Varicella-Zoster Virus Meningitis

VZV can produce an aseptic meningitis that can occur in the absence of rash. The frequency of VZV meningitis is uncertain; however, in one large series it accounted for 8% of cases. Patients typically have a lymphocytic pleocytosis in the CSF with elevated protein and normal glucose.[117] Neuroimaging findings are usually normal. Diagnosis can be made by showing VZV DNA in CSF with PCR. It has been reported that VZV viral loads are lower in patients with meningitis (mean, 4000 copies/mL) compared with patients with encephalitis (mean, 72,000 copies/mL).[118] It has been suggested that the severity of VZV CNS disease may correlate with CSF viral load. Patients with fewer than 10,000 VZV DNA copies/mL have a milder course compared with patients with higher viral loads.[118]

Varicella-Zoster Virus Central Nervous System Disease Treatment

No randomized controlled clinical trials are available to guide therapy for VZV CNS infection, but treatment with high-dose IV acyclovir (10–15 mg/kg every 8 hours for 10–14 days) has been recommended for severe disease.[7,111,112] Some experts also recommend a short course of adjunctive corticosteroid therapy (e.g., prednisone, 60–80 mg/day for 3–5 days).[109,111] In a noncontrolled retrospective review of VZV vasculopathies, 66% of patients who received acyclovir alone and 75% who received acyclovir and steroids improved or stabilized.[115] IDSA guidelines[7] indicate that acyclovir therapy is recommended (category III-B), that ganciclovir can be considered an alternative (category III-C), and that adjunctive corticosteroids can be considered (category III-B).

Cytomegalovirus

CMV is a ubiquitous human virus, with a 90% to 100% antibody seroprevalence in adults. It is transmitted through various routes, including body fluids such as saliva and genital secretions, blood transfusions, and organ transplants. CMV transmission typically occurs during childhood or early adulthood owing to common transmission via body fluid exposure. CMV causes acute infection and reactivation disease from latent virus. Primary infection with CMV is often clinically silent but may produce a mononucleosis-like syndrome. Neurologic complications from CMV infection in adults include retinitis, encephalitis, polyradiculomyelopathy, and neuropathy, all of which occur predominantly in immunocompromised hosts. CMV is also an important cause of congenital infections (see Chapter 137).

CMV encephalitis most commonly occurs in patients with a compromised immune system because of acquired immunodeficiency syndrome (AIDS) or organ transplantation; however, CMV neuroinvasive infections can infrequently occur in immunocompetent patients.[119] In patients with AIDS, CMV encephalitis occurs when the $CD4^+$ cell count is less than 50 cells/mm^3. Patients may develop a nonspecific febrile encephalopathy with or without focal signs. Some patients present with acutely altered mental status, and others present with a slowly progressive ventriculoencephalitis with CN palsies. Findings at MRI of the brain in AIDS patients with CMV encephalitis are variable, but hyperintense signal in the periventricular white matter is often seen on T2-weighted images and ependymal enhancement on contrast-enhanced T1-weighted images.[120] The CSF in patients with CMV encephalitis is nonspecific with mild lymphocytic pleocytosis and mild elevation in CSF protein level. In patients with CMV polyradiculopathy, the CSF profile is distinctive, with neutrophilic pleocytosis, low glucose, and elevated protein. Diagnosis is confirmed through amplification of CMV DNA from CSF by PCR. CMV PCR in the CSF of AIDS patients with CMV encephalitis has a reported sensitivity of 62% to 100% and specificity of 89% to 100%.[121]

Patients with HIV-associated CMV ventriculoencephalitis have a median survival of 42 days.[122] In a prospective study of 146 HIV patients with neurologic symptoms, the median survival was 50 days in patients with a positive CMV CSF PCR result compared with 205 days in patients with a negative CMV CSF PCR result ($P < .001$).[123]

Recommended initial treatment for CMV neuroinvasive disease includes the combination of ganciclovir (5 mg/kg IV every 12 hours) and foscarnet (60 mg/kg IV every 8 hours or 90 mg/kg IV every 12 hours) (category III-B).[7] Initial therapy is generally maintained for 2 to 3 weeks and is often followed by lower-dose maintenance therapy. Efforts should be made to reduce the degree of host immunosuppression whenever possible.

Epstein-Barr Virus

Primary EBV infection can be asymptomatic or can result in a mononucleosis syndrome characterized by cervical lymphadenopathy, exudative pharyngitis, and splenomegaly. Significant CNS disease occurs in less than 1% of acute EBV infectious mononucleosis cases and can manifest with myriad CNS syndromes, including meningitis, encephalitis, transverse myelitis, and Guillain-Barré syndrome.[124] The frequency of EBV as a cause of encephalitis is unknown.

Patients with EBV encephalitis present with altered consciousness, seizures, and focal neurologic deficits. In a study of 21 children (<17 years old) with EBV CNS infection, only 1 patient had concomitant infectious mononucleosis.[124] Other patients had a nonspecific presentation with fever (81%), headache (66%), and seizures (48%). CSF shows a pleocytosis (mean, 58 cells/mm^3) with mildly elevated protein (mean, 48 mg/dL) and normal glucose levels. In one series, 60% of patients had a neutrophil predominance.[124] About 80% of patients have MRI abnormalities, which can take the form of increased signal on T2 and FLAIR sequences that most commonly involve the basal ganglia and thalamus, temporal lobes, and subcortical white matter. In some patients, lesions are consistent with the lesions seen in ADEM or acute hemorrhagic leukoencephalitis. EEG abnormalities occur in approximately 75% of patients, most commonly in the form of generalized slowing, although patients may also have focal slowing, frequent intermittent rhythmic delta-wave activity, or epileptiform discharges.

Diagnosis of EBV CNS disease includes serology testing including IgM and IgG antibodies to viral capsid antigens and antibodies against Epstein-Barr early antigen (EA) and Epstein-Barr nuclear antigen (EBNA), and CSF PCR testing. Diagnosis of neuroinvasive disease is typically made with EBV PCR of the CSF or demonstration of intrathecal antibody synthesis (CSF EBV viral capsid antigen IgM or elevated EBV viral capsid antigen IgG CSF-to-serum ratio). PCR testing in the CSF needs to be interpreted with caution because false-positive results due to presence of EBV DNA in CSF mononuclear cells resulting from other conditions may occur. A study of patients with EBV DNA in the CSF at PCR found evidence of EBV encephalitis in patients with stem cell transplantation, but positive EBV DNA in the CSF of other patients did not appear to be causally related and was often associated with another infection in the CNS.[125] EBV serology consistent with acute infection (e.g., EBV viral capsid antigen IgM in the absence of IgG, elevated IgG antibody titers against early antigen but not nuclear antigen) supports, but does not definitively establish, the presence of EBV CNS disease. A study of 28 patients with CNS EBV infection found that patients with encephalitis had a high EBV PCR copy number (4.2 $\log_{10}$ copies/mL) and high leukocyte counts (143 cells/mm^3) in the CSF.[52]

There are no controlled trials evaluating therapy in patients with EBV CNS infection; however, some case reports suggest that ganciclovir treatment improved outcomes in patients with EBV meningoencephalitis.[126] Steroids have shown benefit in uncontrolled trials in some patients, including patients with evidence of increased ICP (category III-C).[7] Mortality of EBV encephalitis is 10% or less. Prognosis for survivors is excellent; 90% return to normal function, and the remaining 10% have only mild residual deficits.[124]

Human Herpesvirus 6

HHV-6 is a ubiquitous viral infection of lymphocytes that causes a spectrum of disease on primary infection, including exanthem subitum (roseola) in infants, febrile seizures, and lymphadenopathy syndromes. Virus then becomes latent and can periodically reactivate to cause disease.

HHV-6 is increasingly recognized as a cause of encephalitis in immunocompromised adults,[127,128] particularly patients with allogeneic bone marrow transplants (hematopoietic stem cell transplants).[129,130] The predominant amount of data in hematopoietic stem cell transplant patients, which consists of small case series, case reports, and small cohort studies, supports a diagnosis of HHV-6 encephalitis in hematopoietic stem cell transplant patients with encephalitis, without other causes of neurologic disease, and CSF positive for HHV-6 with PCR assay.[131] In hematopoietic stem cell transplant patients, HHV-6 causes limbic encephalitis, characterized by short-term memory loss, insomnia, and seizures.[132] MRI exhibits characteristic increased hyperintense signal on T2 images of the medial temporal lobe.[133] Diagnosis is made through demonstration of HHV-6 DNA in the CSF. In a case-control study, HHV-6 PCR from the CSF of 22 bone-marrow transplant patients with neurologic symptoms was compared with 107 immunocompromised controls without neurologic symptoms.[134] Because HHV-6 is latent in lymphocytes, interpretation of PCR-positive samples in blood and in the CSF can be complicated by confounding detection of latent HHV-6 DNA in lymphocytes. Thus interpretation of PCR results should be placed in the context of the disease presentation and the degree of host immune suppression.

HHV-6 is most commonly associated with focal encephalitis in bone marrow transplant patients. Estimates of its frequency have ranged from 6.5% in specimens from the CASG HSV encephalitis treatment trials[135] to 0.4% (4 per 1000) in the CEP.[127] Patients typically have CSF lymphocytic pleocytosis, elevated protein level, and normal glucose level. MRI may be normal or may show temporal lobe abnormalities. Diagnosis is typically made with CSF PCR or serum serology. Sensitivity and specificity of these tests are unknown. It has been suggested that high CSF viral loads support the diagnosis.[127] The possibility that latent HHV-6 DNA in mononuclear cells may result in false-positive PCR test results may complicate diagnosis.

Treatment of HHV-6 encephalitis is similar to that of CMV CNS infections. In general, HHV-6 is not responsive to acyclovir therapy but is responsive to ganciclovir and foscarnet. Although HHV-6 is sensitive to cidofovir therapy, this drug is not recommended for treatment of HHV-6 CNS disease because of the high risk of adverse events and uncertainty about its degree of CNS penetration. There are no controlled clinical trials studying antiviral therapy for HHV-6 limbic encephalitis. Case studies have suggested that therapy with ganciclovir or foscarnet can be successful in bone marrow transplant recipients.[136,137] Other reports have found that antiviral treatments do not prevent death and do not reduce CSF HHV-6 DNA viral load.[138,139] IDSA guidelines recommend use of ganciclovir or foscarnet in immunocompromised patients (category III-B) and indicate that these agents can be considered in immunocompetent patients, although data on efficacy are lacking (category III-C).[7]

Herpesvirus B

Herpesvirus B infects Old World monkeys and is the only nonhuman herpesvirus that also infects humans, resulting in a high mortality.[140] Monkeys of the genus *Macaca* (macaques) are lifelong viral carriers and asymptomatically shed virus. Humans develop disease after a monkey bite or scratch or mucosal contact with infected body fluid. Vesicles develop at the site of exposure, followed by onset of a flulike syndrome characterized by fever, chills, myalgias, and headache.[140] When the virus invades the CNS, the patient develops diplopia, ataxia, hyperesthesias, agitation, and ascending paralysis.

Diagnosis is made through wound or contact site culture and demonstration of an antibody response with acute and convalescent sera.[140] Culture must occur in a Biosafety Level 4 facility, so local health authorities should be notified if herpesvirus B is suspected. Because mortality approaches 100% after CNS symptoms develop, treatment should be initiated at exposure rather than delayed until onset of symptomatic disease.

There are no controlled clinical trials studying regimens for post-exposure prophylaxis (PEP), treatment of symptomatic individuals who have not yet developed CNS symptoms, or treatment of patients with CNS disease. PEP refers to administration of antiviral drugs in individuals who have been potentially exposed to virus but who are not known to be infected. The regimen recommended for PEP in asymptomatic individuals is oral valacyclovir (1 g orally three times a day for 14 days).[141] In patients with symptomatic disease who do not have CNS signs or symptoms, either acyclovir (12.5–15 mg/kg every 8 hours) or IV ganciclovir (5 mg/kg every 12 hours) is recommended. In individuals with CNS symptoms, it has been suggested that ganciclovir treatment is preferable.[141] Therapy is generally continued for a minimum of 14 days and is extended if symptoms have not resolved or cultures remain positive. When IV therapy is discontinued, patients should be monitored for viral shedding (cultures of conjunctivae and oral mucosa) initially at weekly intervals and later less frequently (one to two times a year). Lifelong suppressive therapy with oral valacyclovir is usually recommended.[141]

Vector-Borne Viral Infections

Vector-borne viral infections are important causes of viral encephalitis in the United States as a result of the endemic establishment of WNV circulation. With the emergence of ZIKV in the Americas, close monitoring for continued emergence and spread of new neuroinvasive arthropod-borne viruses needs to be a priority. For example, the upper Midwest and Northeast of the United States have seen a significant increase in Powassan virus infections. Powassan virus is a member of the genus *Flavivirus* and related to WNV. This virus is spread by *Ixodes* ticks and causes disease similar to WNV (see later). The most common vectors that transmit encephalitic viruses in the United States are mosquito species[142] and tick species.[143] These arthropods and the viruses they transmit constitute a common group of viruses called *ar*thropod-*bo*rne viruses, or *arbo*viruses. Viruses included in this group belong to four families: Togaviridae, Flaviviridae, Bunyaviridae, and Reoviridae; each specific arbovirus within a family is commonly transmitted by a specific species of mosquito or tick.

Encephalitic Arbovirus Pathogenesis

Many encephalitic arboviruses maintain an enzootic (animal) cycle that does not involve human infection. However, some arboviruses, such as ZIKV, can establish an urban or epizootic cycle of transmission that involves humans as an amplifying host. After a bite from an infected mosquito, the virus replicates in dendritic cells and macrophages in local tissue and lymph nodes, resulting in a primary viremia that disseminates virus to end organs including the CNS. The ability of an arbovirus to invade the CNS (neuroinvasiveness) is determined by multiple viral and host factors.[144] Proposed routes of arboviral CNS entry include penetration of the cerebral microvasculature after infection of endothelial cells, diapedesis of infected leukocytes, penetration of fenestrated capillaries in structures such as the choroid plexus, and transneuronal spread of virus.

When the virus has penetrated the blood-brain barrier, arboviruses can directly infect and cause death of neurons. Several studies have shown that apoptosis is an important mechanism of WNV neuron cell death and CNS injury.[145–147] Immune responses also contribute to clearance of virus and immunopathologically mediated neuronal cell death.

West Nile Virus (Also See Chapter 153)

WNV is an arbovirus in the family Flaviviridae, genus *Flavivirus,* along with other important human viral pathogens including dengue virus, ZIKV, yellow fever virus, JEV, and many others. WNV infection is endemic in the United States and is the second most common cause of viral encephalitis in the United States after HSV (see Fig. 89.2A). Since its emergence in New York City in 1999 and through 2016, approximately 50,000 cases of WNV infection have been reported in the United States, including 18,810 cases (42%) of neuroinvasive disease and 1765 (4%) deaths (www.cdc.gov/ncidod/dvbid/westnile). The

epidemics of WNV in 2003 and 2012 were the largest outbreaks of neuroinvasive viral infections ever reported in the Western Hemisphere.[148]

West Nile Virus Pathogenesis

WNV is transmitted via a mosquito bite from an infected *Culex* mosquito. Transmission also can occur after organ transplantation of WNV-infected organs,[149] breastfeeding,[150,151] and blood transfusions.[152] Most (80%) infections are asymptomatic. Approximately 20% of infected individuals develop an acute febrile flulike illness (West Nile fever) characterized by fever, headache, fatigue, anorexia, nausea, myalgia, and lymphadenopathy. A maculopapular rash involving the trunk and limbs occurs in 25% to 50%.[153] Less than 1% of WNV-infected individuals develop neuroinvasive disease, including meningitis, encephalitis, and acute flaccid paralysis.[148,154–156] It has been estimated that 30% to 40% of patients with neuroinvasive WNV infection develop meningitis, 50% to 60% develop encephalitis, and 5% to 10% develop acute flaccid paralysis.[157,158] Other reported syndromes include rhabdomyolysis,[159] chorioretinitis,[160] myositis,[161] and autonomic nerve dysfunction.[162]

Neuroinvasive disease most commonly occurs in older individuals (>60 years old). In one study, the odds ratio of developing encephalitis was 2.2 (95% CI, 1.6–3.1) in individuals older than 64 years.[163] Additional identified risk factors for encephalitis include hypertension and diabetes.[154,163,164] Immunocompromised patients, including organ transplant recipients, are at high risk of developing severe WNV disease. Specific genetic factors in humans shown to enhance susceptibility to serious WNV disease include single nucleotide polymorphisms in the oligoadenylate synthetase gene, which encodes an interferon inducible enzyme involved in antiviral innate immunity,[165] and a genetic deficiency of the chemokine receptor CCR5, which may inhibit trafficking of WNV-specific $CD8^+$ T cells into the CNS.[166]

West Nile Virus: Infection Clinical Features

WNV meningitis is characterized by the abrupt onset of fever, headache, meningeal signs, photophobia, and phonophobia. Patients have lymphocytic CSF pleocytosis with an average of 226 cells/mm^3, mildly elevated protein, and normal glucose. Neutrophils, rather than lymphocytes, predominate in the CSF in approximately 50% of patients with WNV meningitis.[36] Neuroimaging studies are unremarkable, and the EEG is usually normal.

WNV encephalitis is distinguished from meningitis by the presence of signs and symptoms of brain parenchymal involvement or abnormalities at neuroimaging or on EEG indicative of brain parenchymal involvement. Patients with WNV encephalitis present with fever (70%–100%), headache (50%–100%), and altered mental status (45%–100%). Common signs unusual in other forms of viral encephalitis that may suggest the diagnosis of WNV or flavivirus infection include tremor, parkinsonism, and myoclonus (20%–40%).[156,157] Weakness is common and may be generalized or of a lower motor neuron type associated with hypotonia and areflexia with preserved sensation. Cranial neuropathies, most commonly involving unilateral or bilateral peripheral facial palsy, occur in approximately 20%.

The prevalence of tremors ranges from 12% to nearly 100% in different studies.[155] It is often coarse, typically involves the arms, and has postural and kinetic components.[167] Parkinsonian features also occur with variable frequency and include signs such as bradykinesia, hypomimia, and postural instability.[155,167] Myoclonus can resemble that seen in prion diseases and usually involves the upper extremities and face. Cerebellar abnormalities including incoordination and gait ataxia occur in a variable percentage of cases.[154,157,164,168]

Patients with WNV infection have a normal complete blood count or mild leukocytosis.[3] The CSF findings in patients with encephalitis are almost identical to the findings with meningitis, including pleocytosis (mean, 227 cells/mm^3), elevated protein level, and normal glucose level. There is a predominance of neutrophils rather than lymphocytes in 37% of cases.[36] MRI is abnormal in approximately 50% to 70% of WNV encephalitis cases, which is somewhat less frequent than in HSV encephalitis, and the frequency depends on the timing of the studies and the imaging sequences used. CT considerably less sensitive than MRI, and CT scans are usually normal. When present, MRI abnormalities typically involve the thalamus, basal ganglia, and brainstem. Less commonly, they involve the deep white matter (see Fig. 89.3C).

WNV can produce a poliomyelitis-like acute flaccid paralysis that results from viral injury to motor neurons in the anterior horns of the spinal cord. Patients typically develop acute onset of asymmetrical limb paralysis associated with decreased or absent reflexes and preserved sensation. Weakness may be associated with respiratory impairment from diaphragm or intercostal muscle paralysis. Electrophysiology studies obtained acutely show reduction in amplitude or absence of compound muscle action potentials with relatively preserved sensory nerve action potentials. Electromyographic studies obtained 2 to 3 weeks after onset show characteristic features of denervation, including increased insertional activity and fasciculations. In contrast to Guillain-Barré syndrome, there is no evidence of significant demyelination (slowed conduction velocities or conduction block). In most, but not all, cases of WNV, acute flaccid paralysis is associated with clinical signs and symptoms of systemic infection, and the syndrome may occur in association with meningitis or encephalitis. Patients typically have CSF features similar to the features seen in meningoencephalitis. MRI of the spinal cord may show increased signal in the anterior horns on T2 and FLAIR sequences.

West Nile Virus Infection: Diagnosis

WNV neuroinvasive disease is usually diagnosed through demonstration of WNV-specific IgM in CSF with ELISA.[169] In some patients, CSF WNV IgM may persist for 1 year or longer, and it may be necessary to perform serial studies of serum and CSF IgG and IgM to definitively distinguish acute from remote infection. CSF PCR for WNV is highly specific but less sensitive than serologic studies. CSF PCR may be particularly useful early in infection, however, before antibody responses have fully evolved, and in immunocompromised individuals who may have delayed or absent seroconversion.[169] Antibodies reacting with WNV antigens in ELISA tests may occur as a result of heterologous cross-reactions induced by infection with or vaccination against other flaviviruses, including St. Louis encephalitis virus, yellow fever virus, and JEV. In some cases, it may be necessary to confirm ELISA results with plaque reduction neutralization assays. Neutralization antibody titers are typically highest against the inciting virus compared with cross-reacting species.

West Nile Virus: Prevention and Treatment

There is no specific therapy of proven benefit for WNV infection. Thus prevention of mosquito bites remains the best strategy for WNV infection. This includes avoiding outdoor exposure during dawn and dusk time periods when *Culex* mosquito activity increases, wearing long sleeves and pants, and using *N,N*-diethyl-meta-toluamide (DEET)-containing insect repellent on exposed skin when outdoors. A study was able to show that a live-attenuated chimeric WNV vaccine (rWN/DEN4delta30) was immunogenic and well tolerated in a cohort of flavivirus-naïve adults aged 50 to 65 years.[170] Targeted development of a vaccine for older adults may be a cost-effective approach to deploy in regions of high endemic WNV circulation or during new epidemics. Isolated case reports and small series have revealed both benefit and lack of effect from treatment with IVIG-containing high-titer anti-WNV antibodies (Omr-IgG-am) and with interferon-α. A phase I/II trial to evaluate the safety and efficacy of a humanized monoclonal antibody (MGAWN1) directed against an epitope on the WNV envelope glycoprotein was closed owing to low enrollment (NCT00515385). Isolated reports of corticosteroid use in patients with WNV acute flaccid paralysis and brainstem disease do not permit any conclusions about efficacy.[171]

West Nile Virus: Infection Outcome

Mortality from WNV neuroinvasive disease is approximately 12% and occurs almost exclusively in the subsets of patients with severe encephalitis or severe acute flaccid paralysis. The frequency and severity of sequelae are still not well understood.[172] Six months after the acute infection, 40% of patients with movement disorders such as myoclonus, parkinsonism, or tremors have residual symptoms, and 20% have ongoing symptoms at 18 months of follow-up.[172] More recent studies have also suggested that 50% of WNV encephalitis survivors report cognitive

problems, decreased motor speed, and diminished dexterity 3 months after the initial infection.[172] Long-term follow-up of patients with WNV infection found that about 31% of participants experienced more than 6 months of fatigue symptoms after infection, with an average duration of 5 years.[173] However, it is not clear how different the infected cohort is from prospective, age-matched community control subjects.

St. Louis Encephalitis

St. Louis encephalitis virus has been an important cause of arbovirus encephalitis in the United States since the 1930s and was the most important neuroinvasive flavivirus in North America until the emergence of WNV.[3] St. Louis encephalitis virus is found in a broad range from Canada and the United States to Central and South America (see Fig. 89.2B). Transmission to humans in the western United States is primarily via *Culex tarsalis*; in the eastern United States, it is through *Culex pipiens, Culex quinquefasciatus,* and *Culex nigripalpus.* The incidence rates for St. Louis encephalitis virus in the United States range from 0.003 to 0.752 per 100,000, with a median of 35 cases per year.[174] In 1975 the largest outbreak recorded in the United States involved 2800 cases in 31 states. In 2015 and 2016, 19 and 7 respective cases of St. Louis encephalitis virus infection, mainly in Arizona and the Mississippi river basin, were reported to the Centers for Disease Control and Prevention (CDC) (www.cdc.gov/ncidod/dvbid/sle/index.html). Thus St. Louis encephalitis continues to circulate in the continental United States.

St. Louis Encephalitis Virus Infection: Clinical Features

After the bite of an infected mosquito, an incubation period of 4 to 21 days precedes the onset of clinical symptoms. In adults, symptomatic infection occurs in 1 of 300 individuals exposed to virus.[3] These patients develop a flulike illness characterized by fever, myalgias, headaches, and other nonspecific symptoms including nausea, vomiting, cough, and sore throat. In patients younger than 20 years, 40% develop meningitis and 50% develop encephalitis.[175] In patients older than 60 years, more than 90% of patients develop encephalitis. Common manifestations of St. Louis encephalitis virus include reduced level of consciousness with lethargy, coma, tremors, myoclonic jerks, opsoclonus, nystagmus, and ataxia. Mortality of St. Louis encephalitis virus infections ranges from 4% to 27%,[175] seizures develop in 47% of patients, and acute flaccid paralysis has been associated with 6% of encephalitis cases.[176,177]

St. Louis Encephalitis Virus Infection: Diagnosis

MRI findings are often normal, but images may show high T2 signal intensity of the substantia nigra. In a case series of 11 patients, CSF studies revealed a lymphocytic pleocytosis in all patients (mean, 107 cells/mm^3; range, 5–446 cells/mm^3), elevated protein level (mean, 67 mg/dL) in 7 patients, and normal CSF glucose level.[177] EEG is almost invariably abnormal, with the most common finding being generalized slowing. Severely affected patients may have seizures or periodic lateralizing epileptiform discharges. General laboratory studies reveal a peripheral leukocytosis, hyponatremia, mild transaminitis, and sterile pyuria.[176] Diagnosis is based on demonstration of anti–St. Louis encephalitis virus IgM antibodies in the serum or CSF.[178] A fourfold increase in neutralizing antibody titers in the serum during the acute and convalescent phases of disease can also be used to establish a diagnosis. There is no therapy of proven efficacy for St. Louis encephalitis virus, although therapy with interferon-α2 may be considered (III-C).[7] An open-label, nonrandomized study of interferon-α2b suggested that therapy may improve outcome.[179]

Zika Virus (Also See Chapter 153)

ZIKV is an arbovirus in the flavivirus genus and shares many features with both dengue virus and WNV in terms of molecular structure and clinical features. Like dengue, ZIKV can be spread by *Aedes aegypti* mosquitos in an urban cycle that involves humans as an amplifying host. Like WNV, ZIKV is neuroinvasive, but ZIKV is the first flavivirus known to cause severe neurologic disease in human fetuses when a pregnant mother is acutely infected. ZIKV transmission in the Americas peaked in the summer and early fall of 2016. There have been 5590 reported symptomatic infections in the continental United States and 37,086 cases of symptomatic infection in US territories. This has resulted in possible ZIKV infections in 2311 and 4621 pregnant women in the US states and US territories, respectively (www.cdc.gov/zika/reporting).

Zika Virus Clinical Features

The pathogenesis in adults is likely similar to that of other flaviviruses described earlier and results in asymptomatic infection in up to 80% of adults exposed to infection.[180] In adult patients who develop disease, symptoms are characterized by fever, rash, myalgias, arthralgias, headache, and nonpurulent conjunctivitis that usually resolves in 7 days. Hospitalization and severe disease following acute ZIKV infection in adults are rare. During the ZIKV outbreak in Brazil, a prospective cohort of adult patients with neurologic complications was evaluated during a 3-month period of enrollment in the summer.[181] Of the 40 patients included in the study, 68% had Guillain-Barré syndrome, 13% had encephalitis, and 5% had transverse myelitis.

Despite relatively uncomplicated disease in adults, ZIKV complications can be clinically severe when pregnant women are acutely infected, resulting in infection in the fetus and congenital defects. Birth defects due to ZIKV are broadly categorized as congenital ZIKV syndrome and include chorioretinal atrophy and scarring, optic nerve abnormalities, microcephaly, developmental delay, arthrogryposis, hearing loss, and a variety of brain developmental abnormalities. Fetal abnormalities in the brain after ZIKV infection are directly related to virus infection of neuronal progenitor cells and astrocytes in the brain and other developing nervous system structures.

Zika Virus: Transmission

The major mode of ZIKV transmission remains mosquito-borne transmission from the bite of an infected *A. aegypti* mosquito. However, ZIKV is the first known arbovirus that can also be spread through sexual transmission. Because of the risk of sexual transmission, the CDC has published recommendations on prevention of ZIKV by sexual contact (www.cdc.gov/pregnancy/zika). ZIKV can also be spread through blood transfusion and organ transplantation, similar to WNV.

Zika Virus Infection: Diagnosis and Management

In suspected cases of ZIKV disease, diagnosis requires a compatible clinical syndrome and recent travel to an endemic area with ongoing ZIKV transmission.[180] Women who are pregnant and have traveled to endemic areas should be evaluated by health professionals with expertise in evaluation and monitoring of high-risk pregnancies associated with ZIKV. Suspected cases of ZIKV disease can be diagnosed through molecular detection of ZIKV RNA with reverse-transcriptase polymerase chain reaction (RT-PCR) testing in serum, CSF, and urine samples or serologic testing for ZIKV-specific IgM antibodies and ZIKV-specific neutralizing antibodies in serum or CSF. Serologic detection of ZIKV IgM and neutralizing antibodies is sensitive for infection after 1 week of symptoms but lacks specificity owing to antibody cross-reactivity among all related flaviviruses. However, ZIKV PCR testing is sensitive in the first week of symptoms and has a specificity of >95%.

There are no approved therapies for ZIKV infection. Prevention of infection is based on prevention of mosquito bites and avoidance of travel to an area of ongoing mosquito transmission of ZIKV. ZIKV may be shed for prolonged periods in semen, and sexual transmission of ZIKV has been described (see Chapter 153).

Multiple candidate ZIKV vaccines have shown efficacy in animal models, and have demonstrated immunogenicity in phase I clinical trials (see Chapter 153).

Eastern Equine Encephalitis Virus

Eastern equine encephalitis virus is an alphavirus that causes a sporadic, mosquito-borne viral infection endemic in the eastern United States and the Caribbean. There are four lineages of eastern equine encephalitis virus: group I causes most disease in humans, and groups IIA, IIB, and III cause primarily equine disease in Central and South America. Eastern equine encephalitis virus is maintained in an enzootic cycle with avian species after a bite from a mosquito vector, *Culiseta melanura*.[182] Birds serve as the primary reservoir host and amplifying hosts, and humans are incidentally infected by various mosquito bridging vectors, including

Culex and *Aedes* spp.[183] In the United States, most cases occur along the eastern seaboard. Cases are found sporadically along the Gulf Coast and upper Midwest, typically within 5 miles of swamplands or marshlands (see Fig. 89.2C). Approximately 1 in 30 individuals exposed to eastern equine encephalitis virus develops disease.[184] Eastern equine encephalitis virus causes sporadic infections in human populations during the summer months and occasional epidemic outbreaks.

Patients with eastern equine encephalitis virus develop nonspecific symptoms common to other causes of viral encephalitis, including fever, chills, malaise, and myalgias.[41] The prodrome is followed by either recovery without neurologic illness or the onset of encephalitis characterized by severe headache, confusion, nausea, and vomiting. Seizures, focal neurologic deficits such as CN palsies or focal weakness, and meningismus are common findings.[41] Brainstem involvement is common and is associated with gaze palsies, nystagmus, and pupillary abnormalities. Mortality is about 33% but increases to 50% in patients older than 60 years. Moderate-to-severe sequelae occur in one-third of survivors.[41]

In one study CT was reported to show findings suggestive of diffuse cerebral edema in 40% of cases.[185] Similar to the flaviviruses, in eastern equine encephalitis MRI abnormalities occur predominantly in the thalamus, basal ganglia, and brainstem.[41] EEG is typically diffusely slow, with some severely ill patients having burst suppression or diffuse high-voltage delta-wave slowing.[185] Laboratory studies may show a leukocytosis with a neutrophil predominance and hyponatremia in 60% of patients.[41] CSF typically shows a significant pleocytosis; two studies demonstrated a mean cell count of 370 leukocytes/mm^3 and 940 leukocytes/mm^3, respectively.[41,185] Two-thirds of eastern equine encephalitis virus cases display a neutrophil predominance, with a median neutrophil proportion of 70% of cells.[41,185] CSF protein is often elevated (median, 97 mg/dL), and 90% of patients have CSF glucose concentrations less than 60% of coincident serum values.[41,185] CSF red blood cells are common, reflecting the necrotic and hemorrhagic features of the encephalitis pathologically.

Diagnosis is typically made through demonstration of IgM antibodies in CSF with capture ELISA, demonstration of serum IgM antibodies, or a fourfold increase in IgG antibodies between acute and convalescent sera. No proven antiviral therapy exists for eastern equine encephalitis virus. Treatment is focused on supportive care and managing complications such as seizures and increased ICP. There is no commercial vaccine for eastern equine encephalitis virus, but standard precautions to prevent mosquito bites may help to prevent infection. In evaluating laboratory and imaging studies for prognostic value, one study found that CSF leukocytosis greater than 500 cells/mm^3 and hyponatremia less than 130 mEq/L were predictive of a poor outcome,[41] but a longer prodromal period was associated with a better prognosis. Overall mortality is approximately 30%, although higher mortality rates have been reported in older individuals. Sequelae are more common and generally more severe in children.[185]

Western Equine Encephalitis

Western equine encephalitis virus is an alphavirus that was originally isolated from the brains of horses in an epizootic outbreak in the San Joaquin Valley of California in 1930.[186] There have been 639 cases of western equine encephalitis virus in the United States since 1964 but none since 1994.[186a] A single case of probable western equine encephalitis was reported from Uruguay in 2009.[187]

Venezuelan Equine Encephalitis

Venezuelan equine encephalitis virus is an alphavirus that was originally isolated from the brains of dead horses.[188] Venezuelan equine encephalitis virus circulates between a mosquito vector, *Culex (Melanoconion),* and forest-dwelling small mammals and birds in Central and South America. It emerges during epizootic outbreaks to infect horses and humans via bridge vectors such as *Aedes taeniorhynchus.* Epidemics typically occur in northern South America but have extended as far north as Mexico and Texas.[189] In areas of sylvatic (forest) activity, seroprevalence can be 50%. During an outbreak of Venezuelan equine encephalitis virus in Venezuela and Colombia in 1995, approximately 3000 cases of neurologic disease were reported, resulting in 300 deaths.[190,191] In contrast to other arbovirus infections in humans, viremia in humans is sufficient to transmit virus to mosquitoes. Forty percent of patients with Venezuelan equine encephalitis virus have virus in the pharynx, suggesting that direct spread between humans may be possible, although this has never been shown.[190,192]

Symptomatic Venezuelan equine encephalitis virus results in neurologic disease in a few cases after a viral prodrome of fever, headache, photophobia, conjunctival injection, myalgia, arthralgia, nausea, and dizziness. Pharyngeal inflammation, painful cervical lymphadenopathy, somnolence, and tremulousness may occur.[193,194] CSF analysis reveals lymphocytic pleocytosis, elevated protein, and in some cases an elevated glucose ratio. There are few reports of neuroimaging studies. CT scans are usually normal. EEG typically shows diffuse slowing, although some cases have focal temporal slowing similar to that seen in HSV encephalitis. General laboratory abnormalities include leukopenia and elevated transaminase levels.[193]

Venezuelan equine encephalitis virus is diagnosed by detection of specific IgM antibody in the CSF or serum. Venezuelan equine encephalitis virus nucleic acid can also be amplified with PCR from the blood or pharynx on the eighth day of illness.[192] Fatality rates range from 0.2% to 1% of symptomatic Venezuelan equine encephalitis virus patients and 10% to 25% of patients with encephalitis. There is no antiviral therapy of proven benefit.

Chikungunya

Chikungunya virus (CHIKV) is an Old World alphavirus that reemerged as a cause of a major epidemic in Africa in 2004, then spread to the island of Réunion in the Indian Ocean in 2006 and to Southeast India itself. As of late July 2014, CHIKV had spread extensively into Caribbean islands and to multiple Central and South American countries (see Chapter 151). CHIKV generally causes an acute infection consisting of fever, rash, and polyarthritis that can be severe and prolonged (see Chapter 151). CNS involvement has been reported but appears to be uncommon.[226,227195–199]

The large outbreak of CHIKV infection in Réunion enabled a detailed study of CHIKV-associated encephalitis to be carried out.[200] CHIKV-associated encephalitis was defined by the presence of CHIKV RNA or anti-CHIKV IgM, along with clinical criteria of the International Encephalitis Consortium. Twenty-four cases of encephalitis were reported for a cumulative incidence rate of 8.6 per 100,000 persons. Incidence rates were highest in patients younger than 1 year and in those older than 65 years. The case-fatality rate of CHIKV-associated encephalitis was 16.6%, and the proportion of children diagnosed with persisting disabilities was between 30% and 45%. Poor prognoses (death or sequelae) were more frequent in adults (53.6%) than in children (18.2%).[200]

California Encephalitis Group

California encephalitis virus, La Crosse virus, Jamestown Canyon virus, and Tahyna virus are the major causes of encephalitis in the California encephalitis group within the family of Bunyaviridae and genus *Bunyavirus.* Of these viruses, La Crosse virus, California encephalitis virus, and Jamestown Canyon virus are causes of disease in the United States, and Tahyna virus is predominantly a cause of encephalitis in Russia. La Crosse virus is the most common cause of disease in the California encephalitis group. It was originally described in 1965 after a postmortem examination of a child who died of encephalitis in La Crosse, Wisconsin.[201] La Crosse virus is transmitted in an enzootic pattern between squirrels and chipmunks by the mosquito *Aedes triseriatus* in areas of the Mississippi and Ohio river basins (see Fig. 89.2D).[202,203] Recent outbreaks of La Crosse virus have demonstrated a shift in incidence to the Appalachian region and West Virginia.[204] Human exposure is often associated with camping or other recreational activities in wooded areas in endemic regions.

California encephalitis virus was originally isolated in 1941,[205] but it is rare. Most human cases occur in the western United States and Canada. The ratio of asymptomatic to symptomatic infections is 1000 : 1.[206]

Although La Crosse and California encephalitis viruses cause most disease in children, Jamestown Canyon virus affects predominantly elderly individuals in regions of the northern United States, with

seroprevalence in some areas reaching 10%.[207] A report of a Jamestown Canyon virus infection in Montana underscores continued low-level transmission in the northern continental United States.[208] La Crosse virus causes infection predominantly in children (mean age, 7.5 years). Symptoms of encephalitis include fever, headache, vomiting in 70%, seizures in 46%, and altered mental status in 42%.[206] Focal neurologic signs include hemiparesis, aphasia, dysarthria, and chorea. About 10% of patients develop increased ICP, and rarely cerebral herniation can occur.[206] Jamestown Canyon virus has similar clinical features.

CSF analysis reveals a lymphocytic pleocytosis of 600 cells/mm^3, normal glucose level, and an increased protein level in 30% of patients.[206] Peripheral leukocytosis and hyponatremia secondary to SIADH are common. IgM detection in the CSF or a fourfold increase in paired sera for IgG is considered diagnostic for infection.

No antiviral therapy currently exists for the California encephalitis group of viruses, and no vaccine is available. Mortality from La Crosse encephalitis is approximately 1% to 3%, and most survivors return to normal function.[204,206] Ribavirin treatment for La Crosse encephalitis in children is not recommended owing to problems with pharmacokinetics, toxicity at higher doses, and penetration into the CNS.[209] Predictors of a poor outcome include hyponatremia, persistently elevated body temperature, and a GCS score less than 13.[205,210]

Japanese Encephalitis

JEV was initially isolated in Japan in 1935. JEV causes infections across all of Asia, the western Pacific region, and parts of Australia. JEV is the most important cause of viral encephalitis in the world, causing 68,000 cases and 13,000 to 20,000 deaths annually.[211] The virus is transmitted naturally in an enzootic cycle among birds, pigs, and other vertebrate hosts by the *Culex tritaeniorhynchus* mosquito and other *Culex* spp. Similar to other flaviviruses, humans are incidental hosts that become infected when encountering the enzootic cycle. In rural areas where JEV is endemic, it is predominantly a disease of children, in whom seroprevalence approaches 100%, but only 1 in 300 exposures results in clinical disease.[211] Several reports also highlight the importance of JEV in returning travelers from endemic regions.[212,213]

After a 1- to 2-week incubation period, symptomatic patients develop a nonspecific febrile illness followed by neurologic symptoms of altered consciousness and seizures in 85% of children and 10% of adults.[214,215] Subtle focal seizures, such as twitching of an eyebrow or finger, can be important clinical findings in JEV infection.[211] Similar to other flaviviruses, in patients with JEV, movement disorders occur in 25% and are characterized by parkinsonism, dystonia, jaw dystonias, opisthotonus, choreoathetosis, orofacial dyskinesia, myoclonic movements, and opsoclonus-myoclonus.[211,215,216] Other focal neurologic findings include CN palsies, upper motor neuron weakness, and a lower motor neuron syndrome of acute flaccid paralysis.

CSF shows an elevated opening pressure in 50% of patients. The characteristic CSF profile is lymphocytic pleocytosis, normal glucose level, and mildly elevated protein level. MRI is often normal but may reveal high T2-weighted signal intensity in the thalami, basal ganglia, midbrain, brainstem, and occasionally spinal cord.[217,218] Diagnosis is confirmed with IgM capture ELISA in the CSF or with amplification of viral nucleic acid from CSF or brain tissue by PCR.[219–222] The sensitivity of commercial IgM ELISAs in CSF ranges from 65% to 69%, with specificities of 89% to 100%.[223]

The mortality rate in hospitalized patients ranges from 20% to 30%, and 50% of survivors have severe neurologic sequelae. Poor prognostic signs include altered level of consciousness, multiple seizures, increased ICP, isolation of virus in the CSF, and low titer of JEV–specific immunoglobulins in the CSF.[211–215,224] In a study of 118 cases of JEV in Malaysia from 1997 to 2005, the mortality rate was 8%, and 57% of survivors experienced moderate-to-severe long-term neurologic sequelae.[225] Predictors of long-term poor outcome included a GCS score of 8 or lower or two or more witnessed seizures.

No therapy for JEV has been proven efficacious in randomized controlled clinical trials. Prevention is the primary strategy to control JEV infection. Personal protection with insect repellents, mosquito avoidance, and mosquito population control programs are important approaches. The most effective prevention to date is vaccination. Two JEV vaccines are licensed in the United States. A mouse brain–derived, formalin-inactivated vaccine (JE-MB) showed efficacy in a randomized double-blind trial in Thailand and is approved in the United States for use in individuals older than 1 year. Production of JE-MB was discontinued in 2006, and remaining vaccine is reserved for use in children 1 to 16 years of age[227] (see Chapter 153). An inactivated Vero cell culture–derived vaccine (JE-VC) was approved for use in the United States in 2009 for individuals 17 years of age and older.[227] A live-attenuated vaccine developed in hamster kidney cells (SA14-14-2) is used in China, and several other vaccines are either currently in use (chimeric JEV–yellow fever 17D vaccine) or in development.[174,228]

Influenza

Influenza A is known to be associated with immune-mediated neurologic complications such as Guillain-Barré syndrome or transverse myelitis. The overall role of influenza as a cause of CNS disease has been difficult to ascertain. Less commonly, influenza has been associated with encephalitis in children and young adults. During the influenza A (H1N1) pandemic in 2009, influenza was associated with encephalitis and neurologic complications. In a study of 506 children hospitalized with H1N1, 1.4% ($n = 7$) of patients presented with encephalitis, and 5 of the patients with encephalitis had a preexisting medical condition.[229] When seizures are included, the incidence of neurologic complications associated with H1N1 influenza increases to 9.7%. Severe influenza in children and young adults with underlying neurologic or neurodevelopmental conditions has occurred in smaller outbreaks.[230]

Patients often present with typical influenza symptoms during the winter and spring months. Symptoms include fever; respiratory abnormalities (cough, coryza, wheezing, labored breathing); nausea; and diarrhea. Patients with severe influenza develop increased work of breathing, and neurologic involvement can be characterized by altered mental status, seizures, or focal neurologic signs. The diagnosis is commonly made on the basis of the association of the rapid influenza diagnostic test (RIDT) or positive RT-PCR studies on respiratory samples. CSF may show evidence of a pleocytosis with normal glucose and protein levels, but PCR studies for influenza are often negative.[231] It is unclear whether CNS complications associated with influenza are a result of direct neuroinvasion or secondary to immune-mediated mechanisms.[231]

Although the efficacy of antiviral therapy in neurologic complications associated with influenza has not been established in clinical trials, it would be prudent to administer antiviral therapy as early as possible, and ideally within 48 hours of symptom onset.[230] Antiviral therapy for influenza should be started regardless of vaccination status and is often initiated with other empirical antibiotic therapy (such as acyclovir), pending results of the clinical investigation. As therapy for influenza, oseltamivir (75 mg by mouth twice daily for patients > 40 kg) has been recommended for at least 10 days (https://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm) (also see Chapter 165).

Colorado Tick Fever Virus (See Chapter 149)

Colorado tick fever virus belongs to the family Reoviridae. It is transmitted by the wood tick *Dermacentor andersoni* mainly in the western mountain regions of the United States and Canada above 4000 feet in elevation.[232] An enzootic infection cycle exists between small mammals, such as ground squirrels, marmots, or chipmunks, and *D. andersoni*. When infected, the tick is infected for life (transstadially) throughout the three main stages of development—larval, nymphal, and adult. Humans are exposed to the habitat of *D. andersoni* during summer months and are dead-end hosts.

A history of tick bite is obtained in approximately 90% of patients, and the incubation period is 0 to 14 days (mean, 3 days).[232] Patients develop abrupt onset of fever, chills, generalized myalgias, severe headache, hyperesthetic skin, and severe malaise. Gastrointestinal symptoms such as nausea, vomiting, or diarrhea may be present. About 15% of patients develop a maculopapular or petechial rash. Other physical findings include pharyngitis, mild lymphadenopathy, or mild splenomegaly. In 50% of patients, fever resolves after 2 days and then recurs in the biphasic or "saddleback" pattern.[232] After convalescence, patients older than age 30 may have persistent fatigue for 3 weeks.

Complications such as encephalitis or meningitis predominantly occur in children in about 5% to 10% of the cases. Colorado tick fever virus is rarely fatal.[233] In these patients, neurologic signs and symptoms include nuchal rigidity, photophobia, and mild altered mental status.

The diagnosis of Colorado tick fever virus infection is usually made through IgM capture ELISA, neutralization, or complement fixation.[234] There is no established treatment for Colorado tick fever virus. Most patients recover, although rare deaths have been reported as a result of intravascular coagulopathy after Colorado tick fever virus infection.[233]

Enteroviruses

Enteroviruses comprise more than 70 different viruses in the Picornaviridae family. They can be grouped into polioviruses, coxsackieviruses A and B, echoviruses, and numbered enteroviruses. Enteroviruses cause more than 80% of meningitis cases and also cause more severe neurologic diseases such as encephalitis. Enterovirus infections in the CNS have been reviewed[235] (see Chapters 170–172).

Poliovirus

In 1988 the World Health Assembly started the Global Polio Eradication Initiative, resulting in a decrease from more than 350,000 cases of poliovirus in 1988 to 223 cases in 2012. In 2017 there were only 20 cases of wild-type polio (all type 1) reported worldwide (12 in Afghanistan and 8 in Pakistan); 86 cases of polio associated with circulating vaccine-derived poliovirus strains occurred in 2017 (74 in Syria and 12 in Congo). Poliovirus is transmitted by fecal-to-oral contact and by pharyngeal spread during epidemics. Symptomatic poliovirus infection results in meningitis in 8% of cases and paralytic disease in 1% of all cases. After an incubation period of 1 to 2 weeks, patients develop fever, headache, meningismus, altered mental status, and seizures (in children and infants). Asymmetrical flaccid paralysis, diaphragm paralysis, and CN palsies may follow.

CSF evaluation reveals polymorphonuclear cells early followed by a shift to lymphocytes after several days, elevated protein level (100–300 mg/dL), and normal glucose level. Diagnosis is made with CSF RT-PCR. Treatment for poliovirus infection is supportive. The last large outbreak of poliovirus in the Western Hemisphere occurred in 2000, when 19 cases in the Dominican Republic were reported; the cases were due to a circulating recombinant poliovirus derived from the polio type 1 strain in the oral polio vaccine, rather than wild-type virus. As of 2000, the Advisory Committee on Immunization Practices has recommended the use of inactivated polio vaccine in the United States for the entire primary immunization series (see Chapter 171). Trivalent, live-attenuated (Sabin) vaccine is still used in endemic areas of poliovirus infection.

Nonpoliovirus Enteroviruses

Nonpolio enteroviruses cause a wide spectrum of disease in the CNS, including aseptic meningitis, encephalitis, acute poliomyelitis, acute cerebellar ataxia, optic neuritis, and cranial neuritis (see Chapter 172). In neonates, encephalitis is a complication of an overwhelming sepsis-like syndrome with 10% mortality. Enteroviruses may cause encephalitis, particularly in patients with hypogammaglobulinemia and neonates. In an analysis of the CEP, enteroviruses were responsible for 4.6% of encephalitis cases.[236] This is likely an underestimate, however, based on the referral bias inherent in the design of the study. Of the 73 patients diagnosed with confirmed enterovirus encephalitis, 23% were adults who often presented with mild symptoms and altered consciousness (46%), including rare cases of coma (5%) or personality changes (5%).[236] In 2014, enterovirus D-68 emerged as a major cause of acute upper respiratory infections in children. This outbreak was also associated with a dramatic upsurge in cases of polio-like acute flaccid myelitis (AFM) in children[237] resulting in 120 CDC-confirmed cases in children from 34 states. AFM appears to be following an alternating-year pattern; in 2015, cases returned to baseline levels (21 cases, 16 states), only to rise again in 2016 when 144 cases were reported from 37 states and then fall in 2017 (17 cases). Although the role of enterovirus D-68 in the upsurge in AFM cases has not been definitively proven, accumulating epidemiologic and virologic evidence increasingly suggests a potential causal role.

Enterovirus encephalitis occurs during the summer months from June to October. MRI has a variable sensitivity for enteroviral encephalitis; some studies can show increased T2-weighted signal intensity of both cerebral hemispheres with associated edema (see Fig. 89.3D). In the CEP, cases of enterovirus encephalitis were diagnosed with RT-PCR of the CSF, and serotyping was available for 20 cases, with echovirus 30 being the most common strain isolated, followed by enterovirus 71, echovirus 18, and coxsackieviruses.[236] Enterovirus 71 can produce more severe disease, with cases of focal and generalized encephalitis reported.[238,239] Cases of enterovirus 71 encephalitis have been associated with a syndrome of shock, pulmonary edema, and extensive brainstem injury (rhombencephalitis) linked to neurogenic pulmonary edema in some cases. In the CEP, two of the four deaths from enteroviruses were due to enterovirus 71, and the other two enteroviruses were not serotyped.[236]

Currently, no specific antiviral therapy is approved for the treatment of enterovirus infections. Various serotypes have been associated with variable sensitivity to pleconaril, an experimental drug.[240] However, clinical outcome data are insufficient to recommend its use in patients with enterovirus encephalitis, and its development has been halted.

Rabies Virus

Rabies virus belongs to a family of RNA viruses called Rhabdoviridae, genus *Lyssavirus*. It is most commonly transmitted to humans through the saliva of an infected animal after a bite (see Chapter 163). In 2012 there was no evidence of dog-to-dog transmission of rabies in the United States and Puerto Rico, and no human infections have been attributed to a dog bite in the United States.[241] Dogs that become infected in the United States are infected with rabies from wildlife. Approximately 93% of the rabies detected in the United States is found in animal wildlife, such as raccoons, bats, skunks, and foxes.[241] In contrast, canine rabies is enzootic in the dog population in many developing countries, and travelers to these countries can be at risk for exposure to rabies from domestic and feral dogs.[242,243] In Bali, 104 cases of human rabies were reviewed and 92% had a history of a dog bite.[243] In the United States, human cases of rabies are rare. Most human cases of rabies have followed bat exposure[244]; however, one patient who died of undiagnosed rabies in 2004 was subsequently found to have rabies only after organs were transplanted from this patient into four individuals, resulting in clinical rabies and death of all recipients.[245]

After a bite wound, rabies virus from infected saliva invades motor and sensory nerves. Neurologic disease is preceded by an incubation period that ranges from 7 days to more than 6 years, with a median incubation of 1 to 2 months.[246,247] The virus moves centripetally from the periphery to the dorsal root ganglia and the spinal cord using fast axoplasmic transport,[248–250] resulting in nerve dysfunction manifesting as a prodrome of neuropathic pain. When rabies has invaded the nerve cell body in the spinal cord, acute neurologic symptoms evolve into encephalitis.

Patients present with a syndrome of hyperactivity (furious rabies) in 80% of the cases, characterized by hydrophobia and aerophobia owing to spasms of the pharyngeal and nuchal muscles triggered by swallow attempts or other types of stimuli.[251] These spasms increase in frequency and are followed by agitation, hallucinations, autonomic hyperactivity, and seizures. Body temperature may be as high as 107°F. Paralytic (dumb rabies) presentations constitute 20% of cases and are characterized by paresthesias, weakness, and flaccid paralysis in the bitten limb. Diagnosis should be considered in any patient with altered consciousness and an exposure history or travel to an endemic area. Diagnosis is made with immunohistochemical staining for rabies antigen from a skin biopsy specimen in the region of the hairline at the neck.[251] Recent development of a reverse-transcription heminested PCR test on skin biopsy specimens demonstrated a sensitivity of greater than or equal to 98% and a specificity of 100%.[252] Other diagnostic methods include examination of corneal smears for rabies antigen and intracerebral inoculation of mice with patient saliva. Detection of rabies virus neutralizing antibodies in the CSF or serum is diagnostic in unimmunized patients, but antibody detection tests can lack sensitivity.

Infected patients should be isolated because body fluids can contain high titers of active virus. Treatment of rabies virus infection is largely supportive and results in a uniformly fatal outcome. One case of survival after rabies virus infection was reported in a 15-year-old girl after treatment with coma induction and EEG burst suppression using midazolam and ketamine supplemented with barbiturates and benzodiazepines.[253] Antiviral therapy included IV ribavirin (33 mg/kg load and then 16 mg/kg every 6 hours) and enteral amantadine (200 mg/day).[253] Despite this case of survival with treatment of rabies virus infection, several failures have been reported.[254]

The mainstay of rabies treatment is PEP. Wounds should be cleansed with soap and water followed by povidone-iodine. Human diploid cell rabies vaccine, rabies vaccine adsorbed, or purified chick embryo vaccine should be administered intramuscularly.[255] In 2010, new CDC recommendations for rabies PEP included vaccination on days 0, 3, 7, and 14 but no dose at day 28 as previously instructed.[256] Previously vaccinated individuals are still given vaccine on days 0 and 3. In addition to vaccination, PEP includes human rabies immunoglobulin, 20 IU/kg, administered as soon as possible after the exposure, up to 7 days after the first dose of vaccine. As much of the dose as possible should be administered at the bite wound site, and any remaining dose should be administered intramuscularly in the same limb, but distant to the vaccination site. Using the CDC- and World Health Organization–recommended rabies PEP regimen, a study of 110 rabies-exposed travelers found that only 14% received human rabies immunoglobulin and that antibody levels afterward were less than 0.5 IU/mL in 6.7% of travelers who received four doses of vaccine.[257] Thus follow-up antibody titers at day 21 after rabies PEP vaccination may be useful.[257] If the animal that caused the bite wound is available, healthy animals should be quarantined and observed for clinical illness over a 10-day period. If the animal is ill, it should be euthanized and the brain should be analyzed by the local health department for evidence of rabies infection.

Measles Virus

Measles virus remains an important cause of morbidity and mortality throughout the world (see Chapter 160).[258] In approximately 0.1% of cases, measles virus can cause an acute postinfectious measles encephalitis resulting in a 20% mortality.[246] Measles virus can also cause a chronic infection in neurons that results in subacute sclerosing panencephalitis (SSPE) or measles inclusion body encephalitis.[246,259] Measles inclusion body encephalitis is a rare and uniformly fatal condition that causes disease in young patients with defective cellular immunity.[259,260] SSPE is a slowly progressive disorder characterized by seizures, deterioration of cognitive and motor function, myoclonic movements, and death. It occurs 5 to 15 years after initial measles infection, which usually occurs in children younger than 2 years.[261] Immunization has reduced the incidence of SSPE more than 90% in developed countries; however, the rate of SSPE is 21 per 1 million population in India.[261,262] When SSPE occurs in adults, the mean age is 25 years and most patients have an undocumented or negative vaccination history for measles.

Initial symptoms may be subtle and include intellectual deterioration and behavioral changes.[261] These symptoms progress to myoclonic jerks, ataxia, dystonia, and generalized or partial seizures.[261,262] Ocular and visual symptoms occur in 10% to 50% of patients and precede neurologic disease by several years. Ocular symptoms include cortical blindness, chorioretinitis, and optic atrophy.[263,264] CSF examination findings are usually normal with a markedly increased immunoglobulin level. Neuroimaging findings are generally limited, but MRI may reveal high signal intensity on T2-weighted images in the white matter, particularly the occipital subcortical white matter, with near-uniform sparing of gray matter.

SSPE has a rapidly progressive course in adults.[265] A small, single uncontrolled trial suggested that treatment with oral inosine pranobex (Isoprinosine) and interferon-α may be effective for adult-onset SSPE,[266] but in general no adequate therapy is currently available for the treatment of SSPE.

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The complete reference list is available online at Expert Consult.

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90 Brain Abscess

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SHORT VIEW SUMMARY

Definition

- Brain abscess is a focal, intracerebral infection that begins as a localized area of cerebritis and develops into a collection of pus surrounded by a well-vascularized capsule.

Epidemiology

- Brain abscess accounts for approximately 0.4 to 0.9 cases per 100,000 people per year, with higher rates in immunocompromised patients.
- In most pediatric and adult series, a male predominance exists (a ratio of 2:1 to 3:1) with a median age of 30 to 40 years, although age distribution varies depending on the predisposing condition leading to formation of brain abscess.
- The incidence of brain abscess is also affected by the general health of the population. In one study of 973 patients from one tertiary hospital in South Africa from 1983 to 2002, the incidence declined during the study period as a result of improvements in socioeconomic standards and availability of health care services.
- The incidence of otogenic abscesses has decreased, whereas the incidence of posttraumatic and postoperative brain abscesses has increased.

Microbiology

- Streptococci (aerobic, anaerobic, and microaerophilic) are the bacteria most commonly cultured from patients with bacterial brain abscess, and they are frequently isolated in mixed infections (30%–60% of cases).
- *Staphylococcus aureus* accounts for 10%–20% of isolates, usually in patients with cranial trauma or infective endocarditis, and it is often isolated in pure culture; cases caused by community-associated methicillin-resistant *S. aureus* strains have been reported.
- Attention to proper culture techniques has increased the isolation of anaerobes from brain abscesses with *Bacteroides* and *Prevotella* spp., isolated in 20%–40% of patients, often in mixed culture.
- Enteric gram-negative bacilli (e.g., *Proteus* spp., *Escherichia coli, Klebsiella* spp., and *Pseudomonas* spp.) are isolated in 23%–33% of patients, often in patients with otitic foci of infection, patients with septicemia, patients who have had neurosurgical procedures, or patients who are immunocompromised.
- Immunocompromised patients with brain abscess have a distinct microbiology: toxoplasmosis and tuberculosis in patients with advanced human immunodeficiency virus (HIV) infection; *Aspergillus* and *Candida* in patients with hematologic malignancies; and *Listeria, Nocardia,* various fungi, and *Toxoplasma* in solid-organ transplant recipients and other patients with compromised cellular immunity.
- Nocardial brain abscess may occur as an isolated central nervous system (CNS) lesion or as part of a disseminated infection in association with pulmonary or cutaneous disease.

Diagnosis

- Magnetic resonance imaging is the imaging modality of choice in the evaluation of a patient suspected to have this disorder.
- The combined use of proton magnetic resonance spectroscopy, diffusion-weighted imaging, and diffusion tensor imaging has been shown to improve the specificity of diagnosis of focal ring–enhancing lesions and differentiate brain abscess from other cystic lesions of the brain including tumors.
- A major advance in the use of computed tomography (CT) in patients with suspected brain abscess is the ability to perform stereotactic CT-guided aspiration to facilitate microbiologic diagnosis and to guide antimicrobial therapy; at the time of aspiration, specimens should be sent for Gram stain, routine aerobic and anaerobic cultures, and cultures for mycobacteria and fungi.

Therapy

- When abscess material has been obtained for microbiologic and histopathologic studies, empirical antimicrobial therapy should be initiated on the basis of the patient's predisposing conditions and the presumed pathogenesis of abscess formation (see Table 90.3).
- In HIV-infected patients with CNS mass lesions, the initial approach to management is different because of the high likelihood of the diagnosis of toxoplasmic encephalitis.
- Antimicrobial therapy with high-dose intravenous agents has traditionally been administered for 6 to 8 weeks in patients with bacterial brain abscesses.
- Surgical management is required in most patients with bacterial brain abscess for optimal therapy. The best candidates for medical therapy appear to be patients with a small abscess (≤2.5 cm), with good clinical condition (Glasgow Coma Scale score >12), and with a well-known etiology.
- Surgical aspiration or removal of all abscesses larger than 2.5 cm in diameter combined with a 6-week or longer course of intravenous antimicrobial therapy, along with response on follow-up neuroimaging, should result in a cure rate of more than 90% in patients with bacterial brain abscess.
- The optimal therapy for fungal brain abscess usually requires a combined medical and surgical approach; immunocompromised patients have a high mortality rate despite surgery and antifungal therapy.

Brain abscess is a focal, intracerebral infection that begins as a localized area of cerebritis and develops into a collection of pus surrounded by a well-vascularized capsule.[1] Brain abscess was an almost uniformly fatal disease before the late 1800s, when surgical techniques (i.e., drainage) led to cure in selected patients. Further advances in the management of brain abscess were made after the introduction of antimicrobial therapy and stereotactic brain biopsy and aspiration techniques. In this chapter, the common bacterial, fungal, and protozoal causes of brain abscess are reviewed, and the clinical presentation, diagnosis, and approach to management are emphasized.

EPIDEMIOLOGY AND ETIOLOGY

Brain abscess is one of the most serious complications of head and neck infections. Estimates reveal the incidence of brain abscess to be approximately 0.4 to 0.9 cases per 100,000 population, with higher rates in immunocompromised patients.[2,3] In a 14-year epidemiologic review of 6027 cases in Taiwan, the overall incidence was 1.8 cases per 100,000 person-years and increased with age (0.58 per 100,000 person-years in individuals 0–14 years of age and 4.67 per 100,000 person-years in individuals older than 60 years).[4] In most pediatric and adult series, a male predominance exists (ratio of 2:1 to 3:1) with a median age of

30 to 40 years, although the age distribution varies depending on the predisposing condition leading to the formation of brain abscess. When the abscess is related to a focus in the paranasal sinuses, most patients are 10 to 30 years of age; when the abscess is from an otitic focus, patients are younger than 20 or older than 40 years.[2] The incidence of brain abscess is also affected by the general health of the population. In one study of 973 patients from one tertiary hospital in South Africa from 1983 to 2002,[5] the incidence declined during the study period as a result of improvements in socioeconomic standards and availability of health care services. In that series, the mean age was 24 years, and three-fourths of patients were men; predisposing conditions were otorhinogenic (38.6%), traumatic (32.8%), pulmonary (7%), cryptogenic (4.6%), postsurgical (3.2%), meningitic (2.8%), cardiac (2.7%), and other (8.6%). Overall, about 25% of cases of brain abscess occur in children, most in the 4- to 7-year age group; they usually originate from an otitic focus or in patients with cyanotic congenital heart disease. However, in a series of 27 children with bacterial brain abscess, sinusitis, meningitis, and traumatic brain injury were the most common predisposing conditions.[6] Brain abscess is extremely rare in patients younger than 2 years. Brain abscess is a rare complication after cranial operations, seen in 0.2% of 1587 operations in one report.[7] The predisposing conditions for brain abscess have changed in recent years; over the past 10 to 15 years, the incidence of otogenic abscesses decreased, whereas the incidence of posttraumatic and postoperative brain abscesses increased.[8] In more recent series, there has been an increase in the number of cases seen in immunocompromised hosts. In these patients, hematogenous dissemination is the more common pathogenesis rather than extension from a contiguous focus of infection.

The outcome in patients with brain abscess in terms of both mortality and long-term sequelae has steadily improved.[3,9] Recent mortality rates have ranged from 0% to 24%,[2] with improvement likely attributed to the availability of more effective antimicrobial regimens (e.g., the addition of metronidazole), new surgical techniques, and, most importantly, the availability of computed tomography (CT). Data from the University of California in San Francisco showed a decrease in the overall mortality rate from 44% during the 3 years before availability of CT to 0% for the 3 years after the introduction of CT in 1977,[10] principally related to early diagnosis and an accurate method of postoperative follow-up with CT. In a retrospective analysis of 620 cases of brain abscess from China over a 62-year period,[11] mortality decreased from 22.8% in 1952 to 6.3% in 2014, likely due to improvements in neurosurgical techniques, cranial imaging, and antimicrobial regimens. However, in more general series, mortality ranged from 8% to 25%.[5,12,13–18]

The incidence of neurologic sequelae in patients who survive a brain abscess ranges from 20% to 70%. In one study of factors influencing the outcome in 39 cases of bacterial brain abscess, the prognosis was primarily determined by the rapidity of progression of the disease before hospitalization and the patient's mental status on admission.[12] Poor prognostic factors have included poor Glasgow Coma Scale score and the presence of underlying diseases (e.g., immunodeficiency, malignancy, hematologic disorders) and other comorbid conditions. In one study of 142 patients with brain abscess, Glasgow Coma Scale score greater than 12 and no evidence of sepsis were associated with a more favorable outcome.[16] In other series, outcome has been related to the approach to management. In one review of 80 surgically treated brain abscesses in 59 patients,[19] immunosuppression, hematogenous spread, and advanced age were predictors of poor outcome. In another series of 973 patients with brain abscess from South Africa,[5] 97% of whom underwent surgical drainage, predictors of mortality included cerebral infarction, ventriculitis, coma, hydrocephalus, dilated pupils, bilateral abscesses, multiple abscesses, human immunodeficiency virus (HIV) coinfection, papilledema, and neurologic deterioration. In a 22-year retrospective study of 31 patients who underwent nonoperative management of bacterial brain abscess, the overall mortality rate was 48%; the Glasgow Coma Scale score at presentation, septic shock, and neck stiffness were risk factors for poor outcome.[20] Early recognition of predisposing conditions is important for improving the outcome in brain abscess. In patients with bacterial brain abscess complicated by intraventricular rupture, mortality rates are much higher, ranging from 27% to 85%.[21]

TABLE 90.1 Predisposing Conditions and Microbiology of Brain Abscess

PREDISPOSING CONDITION	USUAL MICROBIAL ISOLATES
Otitis media or mastoiditis	Streptococci (anaerobic or aerobic), *Bacteroides* and *Prevotella* spp., Enterobacteriaceae
Sinusitis (frontoethmoid or sphenoid)	Streptococci, *Bacteroides* spp., Enterobacteriaceae, *Staphylococcus aureus, Haemophilus* spp.
Dental infection	Mixed *Fusobacterium, Prevotella, Actinomyces,* and *Bacteroides* spp., streptococci
Penetrating trauma or postneurosurgical	*S. aureus,* streptococci, Enterobacteriaceae, *Clostridium* spp.
Lung abscess, empyema, bronchiectasis	*Fusobacterium, Actinomyces, Bacteroides,* and *Prevotella* spp., streptococci, *Nocardia* spp.
Bacterial endocarditis	*S. aureus*, streptococci
Congenital heart disease	Streptococci, *Haemophilus* spp.
Neutropenia	Aerobic gram-negative bacilli, *Aspergillus* spp., Mucorales, *Candida* spp., *Scedosporium* spp.
Transplantation	*Aspergillus* spp., *Candida* spp., Mucorales, *Scedosporium* spp., Enterobacteriaceae, *Listeria monocytogenes*, *Nocardia* spp., *Toxoplasma gondii, Mycobacterium tuberculosis*
HIV infection	*T. gondii, Nocardia* spp., *Mycobacterium* spp., *Listeria monocytogenes, Cryptococcus neoformans*

When the microorganisms likely to be responsible for causing brain abscesses are evaluated, the isolation frequency depends on the predisposing condition (Table 90.1).[2,14,22] Improved microbiologic culture techniques, particularly for anaerobes, have had a significant impact on the awareness of microorganisms that are found in brain abscesses. In addition, immunocompromised patients with brain abscess have a distinct microbiology.[2,3] This includes *Toxoplasma* and *Mycobacterium tuberculosis* in patients with advanced HIV infection; *Aspergillus* and *Candida* in patients with hematologic malignancies; and *Listeria, Nocardia,* various fungi, and *Toxoplasma* in solid-organ transplant recipients and other patients with compromised cellular immunity.[23] The common bacteria, fungi, protozoa, and helminths that can produce brain abscess are reviewed next.

Bacterial Brain Abscess

Streptococci (aerobic, anaerobic, and microaerophilic) are the bacteria most commonly cultured from patients with bacterial brain abscess, and they are frequently isolated in mixed infections (30%–60% of cases).[2,14] These bacteria, especially the *Streptococcus anginosus (milleri)* group *(Streptococcus anginosus, Streptococcus constellatus,* and *Streptococcus intermedius)*, normally reside in the oral cavity, appendix, and female genital tract, and they have a proclivity for abscess formation (see Chapter 203). Although streptococcal brain abscesses are seen most often in patients with oropharyngeal infections or infective endocarditis, they are also isolated after neurosurgical or other medical procedures.[24]

Staphylococcus aureus generally accounts for 10% to 20% of isolates, usually in patients with cranial trauma or infective endocarditis, and it is often isolated in pure culture; cases caused by community-associated methicillin-resistant *S. aureus* strains have been reported.[25] The attention to proper culture techniques has increased the isolation of anaerobes from brain abscesses with *Bacteroides* and *Prevotella* spp., isolated in 20% to 40% of patients, often in mixed culture.[2,3] Enteric gram-negative bacilli (e.g., *Proteus* spp., *Escherichia coli, Klebsiella* spp., and *Pseudomonas* spp.) are isolated in approximately 23% to 33% of cases, often in patients

with otitic foci of infection, patients with septicemia, patients who have had neurosurgical procedures, or patients who are immunocompromised. At one center, *Klebsiella* was the most prevalent pathogen (usually associated with hematogenous dissemination or postneurosurgical states),[26,27] followed by *Proteus* and *Enterobacter* spp.[28] Multiple organisms are cultured in 14% to 28% of cases in patients with positive culture results.[2,13–16] The incidence of negative cultures has ranged from 0% to 43% in selected series,[2,13–17,29,30] with the frequency often correlating with prior use of antimicrobial therapy.

In one review and meta-analysis of 123 studies including 9699 patients with brain abscess, streptococci were the most commonly cultured bacteria (34% of cases), followed by staphylococci (18% of cases) and enteric gram-negative bacteria (15% of cases).[31] Almost one-fourth of cases were polymicrobial. Of note, 32% of cases had negative cultures, possibly reflecting prior use of antimicrobial therapy and limitations of diverse diagnostic microbial methodology over the 8 decades of the meta-analysis. As whole-genome sequencing and targeted-genome sequencing move into diagnostic microbiology laboratories, a larger range of microbial pathogens in brain abscesses is going to be encountered, including more fastidious organisms (see below).[32] How that will impact therapy is yet to be determined.

Other bacterial pathogens may be isolated from brain abscesses in selected patients or from immunocompromised patients. Although *Haemophilus influenzae, Streptococcus pneumoniae,* and *Listeria monocytogenes* are common etiologic agents of bacterial meningitis, they are rarely isolated from patients with pyogenic brain abscesses (<1% of cases).[33–36] However, these organisms may be seen in patients with bacterial meningitis complicated by cerebritis resulting in abscess formation during the clinical course.[37] Brain abscess accounts for about 10% of central nervous system (CNS) infections caused by *L. monocytogenes.*[35,38] *Listeria* brain abscess is usually seen in immunocompromised patients. In a review of 39 cases of *Listeria* brain abscess, 85% of the patients had significant underlying conditions (including leukemia, lymphoma, HIV infection, and various conditions requiring corticosteroids or other immunosuppression), and disease was often associated with concomitant meningitis (38% of cases) and bacteremia (86% of cases).[36] In contrast, although meningitis caused by facultative gram-negative organisms (e.g., *Citrobacter koseri [Citrobacter diversus], Proteus* spp., *Serratia marcescens, Enterobacter* spp.) is infrequent, it is associated with concomitant brain abscess in more than 75% of cases[1,39–41]; children with bacteremia or meningitis caused by these organisms should be evaluated for the possibility of brain abscess.

Salmonella spp. have rarely been reported to cause brain abscess, usually after bacteremia in the presence of some compromise of the reticuloendothelial system.[42] Cerebral abscesses may also be a complication of infection with *Burkholderia pseudomallei.*[43] Actinomycosis of the CNS may manifest as brain abscess, usually secondary to hematogenous spread from a primary infection in the lung, abdomen, or pelvis, although contiguous spread from foci of infection in the ears, paranasal sinuses, or cervicofacial regions may occur; actinomycotic brain abscess should be considered in patients with head trauma, previous surgery, and otorhinolaryngeal infections who present with a long duration of neurologic symptoms and no fever.[44] Nocardial brain abscess may occur as an isolated CNS lesion or as part of a disseminated infection in association with pulmonary or cutaneous disease.[1] This organism is most often isolated in patients with defects in cell-mediated immunity (in patients receiving corticosteroid therapy, in organ transplant recipients, in patients infected with HIV, and in patients with neoplastic disease),[45,46] although 50% of patients with nocardiosis have no readily identifiable underlying immune compromise. In most series of organ transplant recipients with *Nocardia* infection,[46–48] use of trimethoprim-sulfamethoxazole at the dose administered for prophylaxis against *Pneumocystis jirovecii* was not shown to offer adequate protection against nocardiosis. Cases of nocardial brain abscess have also been seen in pregnant patients.[49]

Mycobacteria may also cause focal CNS lesions.[1,2] *M. tuberculosis* may cause tuberculomas (single or multiple, characterized by granulomatous inflammation with caseating necrosis) or "true" brain abscesses, which are similar to pyogenic abscesses (i.e., an encapsulated collection of pus, instead of granulomatous inflammation) and require drainage[50,51]; although tuberculomas are more common, some publications fail to make the distinction between these two entities.[52] Cases have been reported in patients with HIV infection and after solid-organ transplantation,[53–55] although tuberculous brain abscess can be seen in immunocompetent and immunocompromised hosts.[56] In a series of 715 cases of brain abscess in India from 1999 to 2006, 60% of patients had infection with *M. tuberculosis*[8]; this contrasts with a meta-analysis that found only 42 cases of tuberculosis among 9699 cases of brain abscess.[31] A prospective case series from India of 93 patients with intracranial tuberculosis found tuberculoma to be 10 times more common than abscess[57]; in this series, only one-third of patients had abnormalities on chest radiograph. Nontuberculous mycobacteria are found much less frequently, although there are well-documented cases of *Mycobacterium kansasii* brain abscess in patients with AIDS.[58,59] Cases of brain abscess caused by *Mycobacterium avium* complex have also been described in HIV-infected patients,[60,61] sometimes appearing as part of immune reconstitution inflammatory syndrome.[62]

Fungal Brain Abscess

The incidence of fungal brain abscess has increased as a result of the prevalent administration of immunosuppressive agents, broad-spectrum antimicrobial therapy, and corticosteroids.[63,64,65] However, some fungi exhibit neurotropism and can infect apparently immunocompetent hosts.[66] The risk of CNS infections in immunocompromised hosts is often defined by the immunocompromised state. Neutropenia and hematopoietic stem cell transplantation predispose mainly to *Candida, Aspergillus,* and other opportunistic molds such as the Mucorales, *Scedosporium,* and *Paecilomyces*. Solid-organ transplantation predisposes to *Candida* and *Aspergillus* as well as dematiaceous molds. HIV predisposes to *Cryptococcus*. Finally, some primary immunodeficiencies may have specific susceptibilities (e.g., chronic granulomatous disease for *Aspergillus* and *Paecilomyces lilacinus [Purpureocillium lilacinum]* and CARD9 deficiency for CNS candidiasis).

The diagnosis of fungal brain abscess is often unexpected, and many cases are not discovered until autopsy. In autopsy studies, *Candida* spp. have emerged as the most prevalent etiologic agents; neuropathologic lesions include microabscesses, macroabscesses, noncaseating granulomas, and diffuse glial nodules. Risk factors for invasive *Candida* infection include the use of corticosteroids, broad-spectrum antimicrobial therapy, and hyperalimentation. Disease is also seen in premature infants; in patients with malignancy, neutropenia, chronic granulomatous disease, diabetes mellitus, or thermal injuries; and in patients with a central venous catheter in place.[65,67] However, several other pathogenic fungi should be considered in the differential diagnosis of fungal brain abscess, particularly in immunosuppressed patients.

Cases of intracranial infection caused by *Aspergillus* spp. have been reported worldwide, with most cases occurring in adults. Cerebral aspergillosis is reported in 10% to 20% of all cases of invasive aspergillosis; the brain is rarely the only site of infection.[68] The lungs are the usual site of primary infection, and intracranial seeding occurs during dissemination of the organism or by direct extension from an area anatomically adjacent to the brain (e.g., the paranasal sinuses).[65] Most cases of invasive aspergillosis are found in neutropenic patients who have an underlying hematologic malignancy. Other risk groups include patients with hepatic disease, Cushing syndrome, diabetes mellitus, chronic granulomatous disease, or HIV infection; injection drug users; postcraniotomy patients; organ transplant recipients; recipients of allogeneic bone marrow and stem cell transplants; and patients receiving long-term corticosteroid therapy.[63,68,69] *Aspergillus* brain abscess has been reported in patients with primary CNS lymphoma receiving the tyrosine kinase inhibitor ibrutinib,[70] but this potential risk factor awaits confirmation.

Mucormycosis (zygomycosis) is one of the most acute, fulminant fungal infections known. Many predisposing conditions to mucormycosis have been described including diabetes mellitus (70% of cases) usually in association with acidosis, acidemia from profound systemic illnesses (e.g., sepsis, severe dehydration, severe diarrhea, chronic renal failure), hematologic neoplasms, renal transplantation, injection drug use, and the use of deferoxamine.[65,71,72] Mucormycosis has emerged as an important invasive fungal infection in solid-organ and hematopoietic stem cell

transplantation recipients[73]; 10% to 15% of hematopoietic stem cell transplant recipients with mucormycosis have CNS involvement, most frequently in the context of dissemination. Less than 5% of cases involve normal hosts. CNS disease may result from direct extension of the rhinocerebral form of mucormycosis, by open head trauma or by hematogenous dissemination. The order Mucorales includes many species that have caused brain lesions (see Chapter 258), with *Rhizopus arrhizus (Rhizopus oryzae)* being one of the most common.

Infection with *Scedosporium* spp. *(Scedosporium apiospermum* and *Scedosporium prolificans* [now *Lomentospora prolificans*]*)* may cause CNS disease in normal and immunocompromised hosts (e.g., patients with neutropenia or cellular immunodeficiency).[65,74–78] *S. apiospermum* is the asexual form of *Pseudallescheria boydii* and may enter the CNS by direct trauma, by hematogenous dissemination from a pulmonary route, by direct extension from infected sinuses, or perhaps by way of an intravenous catheter. One case was observed in a patient who underwent extracorporeal membrane oxygenation.[79] Brain abscess is the usual CNS manifestation, although meningitis and ventriculitis have also been reported. There is an association between near drowning in polluted water and subsequent illness, resulting from the presence of the pathogen in contaminated water and manure.

Many of the etiologic agents of fungal meningitis may also cause brain abscess (e.g., *Cryptococcus neoformans, Coccidioides* spp., *Histoplasma capsulatum,* and *Blastomyces dermatitidis* as well as *Fusarium* spp.)[65,80]; the epidemiologic and etiologic characteristics of these organisms are described in other chapters of this book. Many of the melanized, or dematiaceous, fungi have also been reported to cause brain abscess including *Cladophialophora bantiana, Bipolaris hawaiiensis, Bipolaris spicifera, Exophiala dermatitidis (Wangiella dermatitidis), Ochroconis gallopava (Dactylaria constricta* var. *gallopava), Chaetomium strumarium, Curvularia pallescens, Rhinocladiella mackenziei, Fonsecaea monophora,* and *Acrophialophora fusispora*.[65,81–85] More than half of published cases were in patients with no risk factors or immunodeficiency; no specific exposures were associated with onset of infection, although most cases seem to occur in rural areas.[83]

Protozoal and Helminthic Brain Abscess

Several protozoa and helminths have been reported to produce brain abscess[1,2,86,87,88,89] including *Trypanosoma cruzi, Entamoeba histolytica, Schistosoma* spp., and *Paragonimus* spp. In addition, free-living amebas (*Naegleria* spp., *Acanthamoeba* spp., and *Balamuthia* spp.) are preferentially neurotropic. Neurocysticercosis, caused by the larval form of *Taenia solium,* is a major cause of brain lesions in the developing world.[90,91] *Echinococcus granulosus* and *Echinococcus multilocularis* can rarely involve the brain and manifest as ring-enhancing lesions. The epidemiologic features and approach to diagnosis and management of these and other protozoa and helminths are discussed in other chapters of this book.

Toxoplasma gondii is the most common protozoal cause of brain abscess.[88,92] The incidence of human infection caused by *T. gondii* depends on dietary habits (especially the amount of meat consumed and whether eaten rare, raw, or well done), the number of stray cats living in close proximity to humans (as infected cats may shed millions of oocysts in their feces), climatic conditions (moderate temperatures and high humidity favor oocyst survival in soil), and overall level of sanitation and hygiene. *T. gondii* infection of the CNS appears in various syndromes but is usually associated with the development of intracerebral mass lesions or encephalitis in immunocompromised hosts, although cases of CNS toxoplasmosis have been rarely described in presumptively immunocompetent persons.

The number of cases of CNS toxoplasmosis has increased dramatically since 1981, specifically in patients with HIV infection, most commonly in patients with $CD4^+$ lymphocyte counts <50/cells mm^3.[88,92–95] Studies before the advent of antiretroviral therapy estimated that 5% to 47% of patients latently infected with *T. gondii* would develop CNS disease.[96] The use of trimethoprim-sulfamethoxazole prophylaxis of *P. jirovecii* pneumonia and antiretroviral therapy has substantially decreased the incidence of *Toxoplasma* encephalitis.[97,98]

CNS toxoplasmosis can be seen after allogeneic hematopoietic stem cell transplantation,[88,92,99] particularly in patients with increased immunosuppression because of graft-versus-host disease or T-cell depletion and in recipients of cord blood. Solid-organ transplant recipients are also at risk. Disease in organ transplant recipients not only occurs secondary to reactivation but may also occur after the transfer of infected cysts in the allograft, most commonly in heart transplant recipients. CNS toxoplasmosis is less common in patients with other immunocompromised states.

PATHOGENESIS AND PATHOPHYSIOLOGY

Pathogenesis

Microorganisms can reach the brain by several different mechanisms (see Table 90.1).[1,2,13–18,29,33] The most common pathogenic mechanism of brain abscess formation is spread from a contiguous focus of infection, most often in the middle ear, mastoid cells, or paranasal sinuses. Brain abscess occurring secondary to otitis media is usually localized to the temporal lobe or the cerebellum. Compared with earlier reports, more recent series have shown a decrease in the number of cases secondary to otitis media and an increase in cases after neurosurgery and trauma.[18] If antimicrobial therapy of otitis is neglected, however, there is an increased risk for intracranial complications. Paranasal sinusitis continues to be an important condition predisposing to brain abscess. The frontal lobe is the predominant abscess site, although when brain abscess complicates sphenoid sinusitis, the temporal lobe or sella turcica is usually involved. Dental infections are a less common cause of brain abscess[100,101]; infections of molar teeth seem most often to be the inciting factor. The frontal lobe is the usual site of the abscess after dental infection, but temporal lobe extension has also been reported.

A second mechanism of brain abscess formation is hematogenous dissemination to the brain from a distant focus of infection. These abscesses are usually multiple and multiloculated, and they have a higher mortality rate than abscesses that arise secondary to contiguous foci of infection.[2] The most common sources of initial infection in adults are chronic pyogenic lung diseases, especially lung abscess, bronchiectasis, empyema, and cystic fibrosis. Brain abscess may also occur hematogenously from wound and skin infections, osteomyelitis, pelvic infection, cholecystitis, and other intraabdominal infections. Another predisposing factor leading to hematogenously acquired brain abscess is cyanotic congenital heart disease,[102–104] which accounts for 5% to 15% of all brain abscess cases, with higher percentages in some pediatric series. These are most commonly seen in patients with tetralogy of Fallot or transposition of the great vessels. Brain abscess is rare after bacterial endocarditis (<5% of cases in most series),[105,106] despite the presence of continuous bacteremia; one recent study found brain abscess in 14 of 198 critically ill patients with infective endocarditis.[107]

Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome) is a predisposing factor for brain abscess (occurring in about 1% of patients) and is almost always observed in patients with coexisting pulmonary arteriovenous malformations, perhaps by allowing septic emboli to cross the pulmonary circulation without capillary filtration or by bacterial seeding of an ischemic portion of the brain after paradoxical sterile emboli.[108–111] The risk for developing brain abscesses in patients with hereditary hemorrhagic telangiectasia ranges from 5% to 9% and is 1000 times greater than in the general population. In a study of 445 consecutive patients with CT-confirmed pulmonary arteriovenous malformations (including 403 with hereditary hemorrhagic telangiectasia), 37 (8.3%) had a cerebral abscess[112]; 29 of the 37 patients had no pulmonary arteriovenous malformation diagnosed before the abscess, suggesting that these abnormalities may account for a significant proportion of cases of cerebral abscess in immunocompetent patients. Brain abscesses have also developed after esophageal dilation and after sclerosing therapy for esophageal varices.[113–115]

Trauma is a third pathogenic mechanism in the development of brain abscess. Brain abscess occurs secondary to an open cranial fracture with dural breach or as a result of neurosurgery or a foreign-body injury.[116] Four cases of brain abscess were reported in patients with malignant gliomas after craniotomy. These patients were treated with carmustine wafers (Gliadel wafers),[117] which are placed locally in the tumor bed and release carmustine after they undergo hydrolysis. The wafers may serve as a nidus for subsequent infection.

The incidence of brain abscess formation after head trauma ranges from 3% to 17% in military populations, where it is usually secondary to retained bone fragments or contamination of initially "sterile" missile sites with bacteria from skin, clothes, and the environment.[118] In a study of 160 war missile penetrating craniocerebral injuries in Croatia, in which 21 skull base injuries were treated surgically,[119] the authors did not attempt to remove all retained metallic or bone fragments but only the accessible ones. Retained foreign bodies did not seem to increase the infection rate except in cases with an in-driven cluster of bone fragments or cerebrospinal fluid (CSF) leak; three cases of brain abscess were seen, for which repeat surgery was required. These findings were confirmed in another retrospective study from Croatia in 88 patients with missile brain wounds in which only accessible bone and metallic fragments were removed during intracranial débridement[120]; there were nine cases of brain abscess, and retained fragments were not responsible for an increased rate of infection. In another study of 43 patients who survived low-velocity missile injuries of the brain during military conflicts and who had retained intracranial fragments,[121] suppurative sequelae were seen in 6 patients, and 2 of these patients progressed to brain abscess. Traumatic predisposing conditions to brain abscess in the civilian population (incidence of 2.5%–10.9% after trauma) include compound depressed skull fractures; dog bites; rooster pecking; horse bites; tongue piercing; and, especially in children, injury from lawn darts and pencil tips.[122–125] Nosocomial brain abscess has also been reported after halo pin insertion,[126] after electrode insertion to localize seizure foci,[127] and after placement of deep brain stimulation hardware[128] and intracranial pressure monitors.[129]

Brain abscess is cryptogenic in 10% to 35% of patients.[2,22] Patent foramen ovale has been suggested as a possible predisposing condition in patients with cryptogenic brain abscess,[130] although this report of two patients did not establish patent foramen ovale as a definitive risk factor.

Natural History of Infection

Several animal models have been used to examine the pathophysiologic consequences and temporal course of brain abscesses after the initiation of infection. A canine model was used to define the pathologic stages of brain abscess formation after the inoculation of α-hemolytic streptococci.[131] On the basis of detailed histologic evaluation, four stages of brain abscess evolution were defined: early cerebritis (days 1–3), late cerebritis (days 4–9), early capsule formation (days 10–13), and late capsule formation (day 14 and later after initial inoculation). Although these stages are arbitrary, they are useful in classification and in comparing organisms with regard to their virulence in the production of brain abscess. The early cerebritis stage is characterized by an acute inflammatory infiltrate with visible bacteria on Gram stain and marked edema surrounding the lesion. The center of the lesion becomes necrotic during the late cerebritis stage, and macrophages and fibroblasts begin to invade the periphery. With early capsule formation, the necrotic center begins to decrease in size, with simultaneous development of a collagenous capsule that is less prominent on the ventricular side of the lesion; cerebral edema also starts to regress during this stage. In this canine model, the collagen capsule was complete circumferentially by the end of the second week and then increased in density and thickness.

Similar neuropathologic findings have been observed in a model of experimental anaerobic brain abscess,[132] although capsule formation could not be divided into early and late stages because of delayed encapsulation. A subsequent study revealed that *S. aureus* was more virulent than the α-hemolytic streptococci in brain abscess formation[133]; the amount of necrosis, the total area of involvement after staphylococcal challenge, the course of infection as it progressed toward resolution, the time for the abscess to reach a stable size, and the time to contain the necrotic region with a collagenous capsule all were greater after inoculation of *S. aureus*. Capsule formation was less prominent on the ventricular than on the cortical surface in these studies,[131–133] perhaps because differences in vascularity between cortical gray matter and white matter allowed greater fibroblast proliferation on the cortical side of the abscess; this may explain the tendency for brain abscesses to rupture into the ventricular system, rather than into the subarachnoid space.

An alternative hypothesis was supported in an experimental rat model after inoculation of *E. coli*.[134] It was suggested in this study that brain abscesses tended to rupture intraventricularly because the infectious process is directed along the major white matter tracts (areas of low tissue resistance) rather than as a result of asymmetrical collagen deposition. The importance of virulence factor production in development of brain abscess was shown by the inability of heat-inactivated *S. aureus* to induce proinflammatory cytokine or chemokine expression in an experimental murine model[135]; α-toxin was identified as a key virulence factor for survival of *S. aureus* in the brain and the subsequent development of brain abscess.

CLINICAL PRESENTATION

The clinical course of brain abscess ranges from indolent to fulminant.[a] Most clinical manifestations (Table 90.2) are not due to the systemic signs of infection, but rather to the size and location of a space-occupying lesion within the brain and the virulence of the infecting microorganism. Headache is the most common presenting symptom and is observed in 70% to 75% of patients. The headache may be moderate to severe and hemicranial or generalized, but it lacks particularly distinguishing features, accounting for frequent delays in diagnosis. Sudden worsening of the headache, accompanied by a new onset of meningismus, may signify rupture of the abscess into the ventricular space[136]; this complication is often associated with a high mortality rate (85% in some series). The classic triad of headache, fever, and focal neurologic deficit is present in only about 20% of patients with brain abscess on admission.[137]

In a study of 33 consecutive patients with intraventricular rupture of brain abscess, severe headaches and signs of meningeal irritation were prominent before rupture, with a rapidly deteriorating clinical condition developing within 10 days after the signs of meningeal irritation[138]; CT performed before rupture showed localized enhancement of the ventricular wall adjacent to the abscess, most likely because capsule formation was more complete on the cortical side than on the ventricular side of the abscess. In another study, intraventricular rupture was more likely if the abscess was deep seated, multiloculated, and in close proximity to the ventricular wall[21]; a reduction of 1 mm in the distance between the ventricle and the abscess increased the rupture rate by 10%. In addition, the clinical presentation of brain abscess in an immunocompromised patient may be masked by the diminished inflammatory response.[63,64]

The location of the brain abscess defines the clinical presentation.[2,13–18,29,33] Patients with a frontal lobe abscess often present with headache, drowsiness, inattention, deterioration of mental status, hemiparesis with unilateral motor signs, and a motor speech disorder. The clinical presentation of cerebellar abscesses includes ataxia, nystagmus, vomiting, and dysmetria. Temporal lobe abscesses may cause ipsilateral headache and aphasia if the lesion is in the dominant

TABLE 90.2 Common Symptoms and Signs in Brain Abscess

SYMPTOM OR SIGN	FREQUENCY (%)
Headache	49–97
Mental status changes	28–91
Focal neurologic deficits	20–66
Fever	32–79
Triad of headache, fever, and focal deficit	<50
Seizures	13–35
Nausea and vomiting	27–85
Nuchal rigidity	5–52
Papilledema	9–51

[a]The clinical presentation varies depending on size and location of the abscess.
Data from references 1, 2, 5, 12, 22, 29, and 33.

[a]References 1–3, 12–18, 21, 22, 29, 31, 33.

hemisphere; a visual field defect (e.g., an upper homonymous quadrantanopia) may be the only presenting sign of a temporal lobe abscess. Abscesses of the brainstem usually manifest with facial weakness, fever, headache, hemiparesis, dysphagia, and vomiting.[139,140] The classic findings of a well-defined brainstem syndrome are frequently lacking in patients with brainstem abscesses because the abscess is likely to extend longitudinally along fiber tracts, rather than expanding transversely.[1]

Certain pathogens may lead to the development of specific clinical characteristics after CNS infection. In patients with nocardial brain abscess, the presentation is generally nonspecific, with fever, headache, and focal deficits determined by the site and size of the lesion.[45,46,141] The clinical suspicion of nocardial brain abscess may be increased by the presence of pulmonary, skin, or muscle lesions, which are present concurrently in many, but not all, cases. All patients with pulmonary nocardiosis should undergo evaluation to exclude CNS disease. In HIV-infected patients with tuberculous brain abscess, seizures, headache, altered consciousness, and hemiparesis are prominent presenting symptoms.[55]

Patients with *Aspergillus* brain abscess most commonly manifest signs of a stroke syndrome (secondary to ischemia or intracerebral hemorrhage or both) referable to the involved area of brain.[65] Headache, encephalopathy, and seizures may also occur. Fever is an inconsistent feature, and signs of meningeal irritation are rare. Patients who are severely immunocompromised usually present with nonspecific findings (i.e., alteration in mental status or seizures or both) shortly before death, whereas patients who are less immunocompromised are more likely to have headache and focal neurologic deficits.[68] Patients with *Aspergillus* brain abscess commonly have evidence of aspergillosis involving other organ systems.

Rhinocerebral mucormycosis initially manifests with symptoms referable to the eyes or sinuses including headache (often unilateral), facial pain, diplopia, lacrimation, and nasal stuffiness or epistaxis[65,72]; fever is usual. As the infection spreads to contiguous structures, necrotic lesions appear in the turbinates, nose, paranasal skin, or hard palate. Chemosis, proptosis, and external ophthalmoplegia may occur. Cranial nerve abnormalities are common (including cranial nerves II–VII, IX, and X), and blindness may occur as a result of invasion of the cavernous sinus, ophthalmic artery, and orbit. Thrombosis is a striking feature of this disease because the organism has a proclivity for blood vessel invasion. Focal neurologic deficits such as hemiparesis, seizures, or monocular blindness suggest far-advanced disease. With further progression, invasion of the internal carotid artery in the cavernous sinus can occur, accompanied by metastatic lesions in the frontoparietal cortex and deepening coma. Among patients with nonrhinocerebral brain abscess caused by Mucorales, fever, headache, or focal neurologic deficits were present in more than half of the patients. In one review of 22 cases,[71] 50% of the patients were injection drug users, and the basal ganglia were the most commonly involved CNS site (83% of patients).

Brain abscess caused by *S. apiospermum* tends to occur in immunocompromised patients or in patients 15 to 30 days after an episode of near drowning.[75,76] Brain abscesses can be located in the cerebrum, cerebellum, or brainstem; clinical presentations include seizures, altered consciousness, headache, meningeal irritation, focal neurologic deficits, abnormal behavior, and aphasia. The clinical manifestations of CNS disease caused by *Cryptococcus, Histoplasma, Coccidioides, Candida,* and other fungal pathogens depend on the intracranial location of the abscess. In one review, nearly one-third of bone marrow transplant recipients with brain abscess caused by *Candida* spp. had no signs or symptoms[64]; these infections were commonly diagnosed at postmortem examination.

The clinical manifestations of CNS toxoplasmosis in immunocompromised patients are variable, ranging from an insidious process evolving over several weeks to acute onset with a confusional state; the initial symptoms and signs may be focal or nonfocal or both.[88,92] *T. gondii* has a predilection to localize in the basal ganglia and brainstem, producing extrapyramidal symptoms resembling those of Parkinson disease. Generally, patients who present with nonfocal abnormalities develop signs of focal neurologic disease as the infection progresses, although some patients develop a diffuse, rapidly fatal encephalopathic process. Nonfocal evidence of neurologic dysfunction may predominate including generalized weakness, headache, confusion, lethargy, alteration of mental status, personality changes, and coma. CNS toxoplasmosis may also manifest differently depending on the risk group. Infection in transplant recipients is often diffuse, disseminated disease. Localizing neurologic signs tend to occur late in the course of infection or not at all in transplant recipients. In patients with underlying malignancies (e.g., Hodgkin disease), the presentation of toxoplasmic encephalitis is evenly distributed between focal and nonfocal manifestations of encephalitis.

Patients with AIDS and toxoplasmic encephalitis often present subacutely with nonspecific symptoms such as neuropsychiatric complaints, headache, disorientation, confusion, and lethargy progressing over 2 to 8 weeks; associated fever and weight loss are also common.[92–94,96] Patients develop clinical evidence of focal CNS mass lesions with ataxia, aphasia, hemiparesis, visual field loss, and vomiting or a more generalized encephalitis with increasing confusion, dementia, and stupor; seizures are common and may be the presenting clinical manifestation of CNS toxoplasmosis in patients with AIDS.

DIAGNOSIS

CT has revolutionized the diagnosis of brain abscess. Before the advent of CT, delays in diagnosis contributed significantly to the high morbidity and mortality in patients with brain abscess. The characteristic CT appearance of brain abscess is a hypodense center with a peripheral uniform ring enhancement after the injection of contrast material; this is surrounded by a variable hypodense area of brain edema (Fig. 90.1).[142] Other CT findings include nodular enhancement and areas of low attenuation without enhancement; the latter is observed during the early cerebritis stage before abscess formation. As the abscess progresses, contrast enhancement is observed. When the abscess becomes encapsulated in the later stages, contrast material no longer differentiates the lucent center, and the CT appearance is similar to the early cerebritis stage. CT is also useful for following the course of brain abscess, although after aspiration, improvement in the CT appearance may not be seen for 5 weeks or longer.

Magnetic resonance imaging (MRI) is now the first imaging choice in the evaluation of a patient with suspected brain abscess. MRI is more sensitive than CT and offers significant advantages in the early detection of cerebritis, including greater contrast between cerebral edema and adjacent brain, more conspicuous spread of inflammation into the ventricles and subarachnoid space, and earlier detection of satellite lesions (Fig. 90.2).[142] On T1-weighted images, the abscess capsule often appears as a discrete rim that is isointense to mildly hyperintense. Contrast enhancement with the paramagnetic agent gadolinium diethylenetriamine pentaacetic acid provides the added advantage of clearly differentiating the central abscess, the surrounding enhancing rim, and the cerebral edema surrounding the abscess.

On T1-weighted images, enhancement of the abscess capsule occurs. On T2-weighted images, the zone of edema that surrounds the abscess is one of marked high signal intensity; the capsule now appears as a well-defined hypointense rim at the margin of the abscess. Therapy with corticosteroids can decrease enhancement seen with CT and MRI. The addition of functional MRI sequences provides more information. The combined use of fluid-attenuated inversion recovery imaging, proton magnetic resonance spectroscopy (MRS), diffusion-weighted imaging (DWI) with measurement of an apparent diffusion coefficient, and diffusion tensor imaging has been shown to improve the specificity of the diagnosis of focal ring–enhancing lesions and to differentiate brain abscess from other cystic lesions of the brain including tumors.[2,143,144] Although the sensitivity and specificity of these techniques is not 100%, restriction of diffusion (hyperintense on DWI, low values on apparent diffusion coefficient map) is characteristic of pyogenic abscesses (Fig. 90.3).[145] Abscess is distinguished from rim-enhancing tumors by demonstrating amino acids within the contents of the cysts, a finding that is essentially diagnostic of the presence of activated polymorphonuclear leukocytes. Proton MRS can differentiate necrotic or cystic tumors and cerebral abscesses; in combination with DWI, it can significantly increase the diagnostic accuracy of conventional MRI with a sensitivity of 72% to 96% and a specificity of 86% to 96%.[144] Succinate and acetate peaks on proton MRS, observed only in anaerobic infections

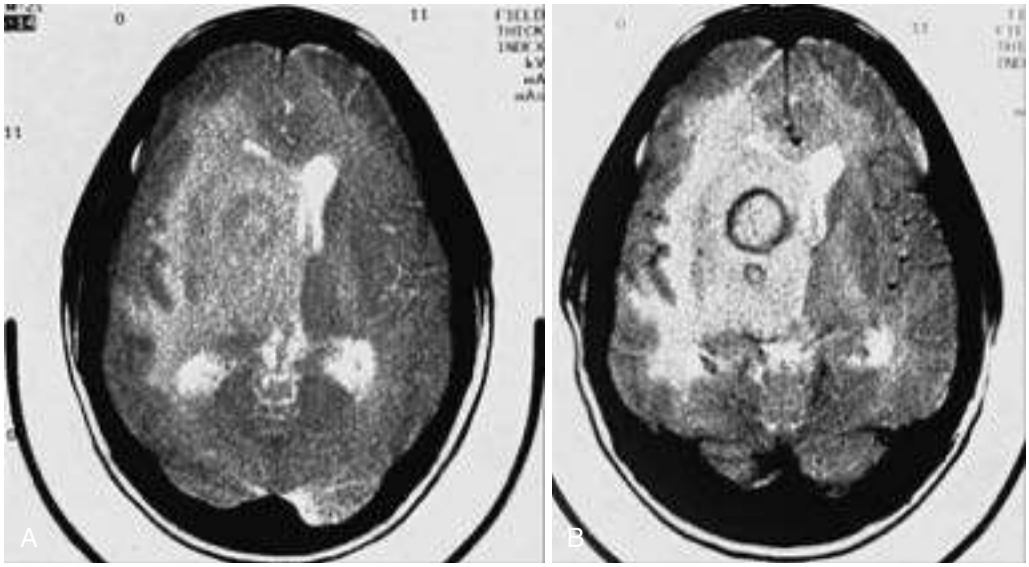

FIG. 90.1 Computed tomography (CT) of the head reveals a large rounded area of low attenuation in the right lentiform nucleus, compression of the horn of the right lateral ventricle, and a shift to the left with vasogenic edema. (A) Unenhanced CT scan reveals increased signal within the center of the area of low attenuation. (B) After intravenous administration of a contrast agent, there is ring enhancement of the abscess and evidence of a smaller satellite lesion.

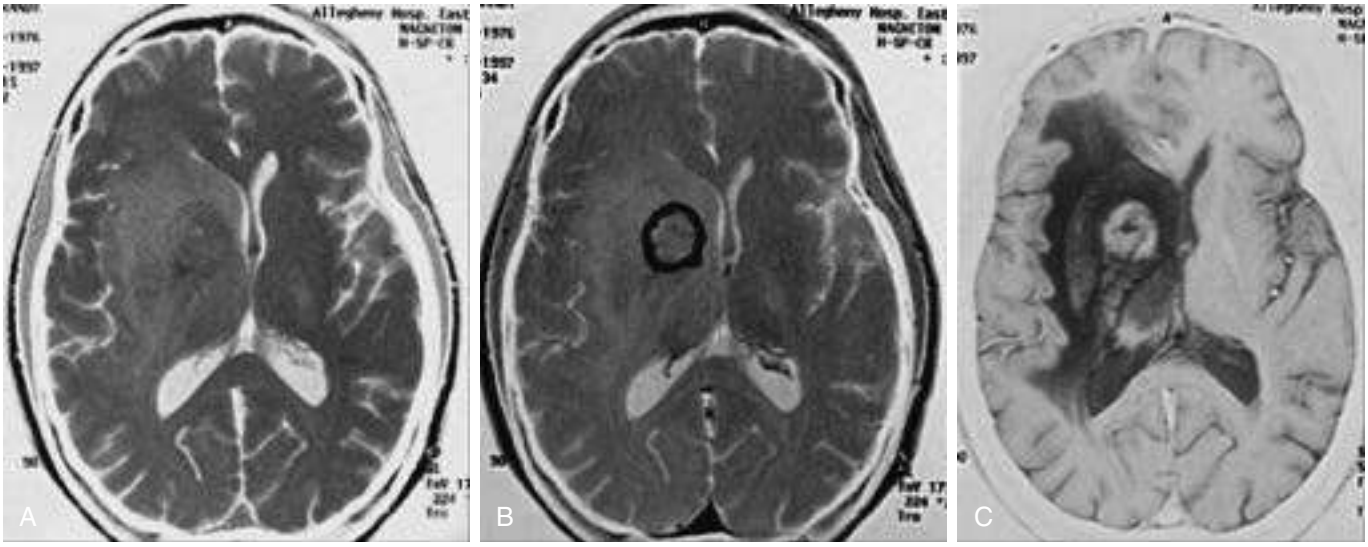

FIG. 90.2 Magnetic resonance imaging of the brain (same patient as shown in Fig. 90.1) reveals 2-cm, round, ring-enhancing lesion in right lentiform nucleus with associated vasogenic edema and midline shift to the left. (A) T1-weighted image reveals ill-defined area of low attenuation. (B) T1-weighted image after administration of gadolinium reveals ring enhancement of the abscess. (C) T2-weighted image shows hypointensity of rim of abscess with large area of high signal intensity consistent with cerebral edema.

due to glycolysis and subsequent fermentation, can be used to further differentiate anaerobic from aerobic metabolism.[144] Although the presence of amino acids in proton MRS is a sensitive marker of pyogenic abscess, its absence does not exclude a pyogenic etiology.[146]

CT and MRI are also quite sensitive in defining lesions in patients with fungal brain abscess; these modalities are not specific, although some exceptions do exist. The finding of a cerebral infarct in a patient with risk factors for invasive aspergillosis should suggest that diagnosis.[65] The areas of infarction typically develop into either single or multiple abscesses involving the cerebrum (usually frontal or temporal lobes) or cerebellum. In immunosuppressed patients with CNS aspergillosis, there is little or no contrast enhancement with MRI.[69] In patients with rhinocerebral mucormycosis, CT and MRI may show characteristic changes including sinus opacification, erosion of bone, and obliteration of deep fascial planes. Frontal lobe involvement in mucormycosis may show little or no ring enhancement; the lack of contrast enhancement is a poor prognostic sign because it indicates failure of host defense mechanisms to isolate or encapsulate the offending organism. Cavernous sinus involvement may be seen on MRI.

CT and MRI are extremely useful in the diagnosis of CNS toxoplasmosis.[92,93] The characteristic CT appearance (seen in 90% of patients) is rounded isodense or hypodense lesions with ring enhancement after the administration of contrast material; homogeneous enhancement or no enhancement can also be seen. The eccentric target sign in a T1 postcontrast image is particularly suggestive of toxoplasmosis. There are multiple lesions in 70% to 80% of cases, often involving the corticomedullary junction and the basal ganglia, although any part of the CNS may be involved. Marked edema and a mass effect are also frequently observed. A double-dose, delayed-contrast study may be a more sensitive method for delineating the true extent of disease. CT usually underestimates the number of lesions documented pathologically at autopsy. MRI has a greater sensitivity than CT and has detected lesions in patients

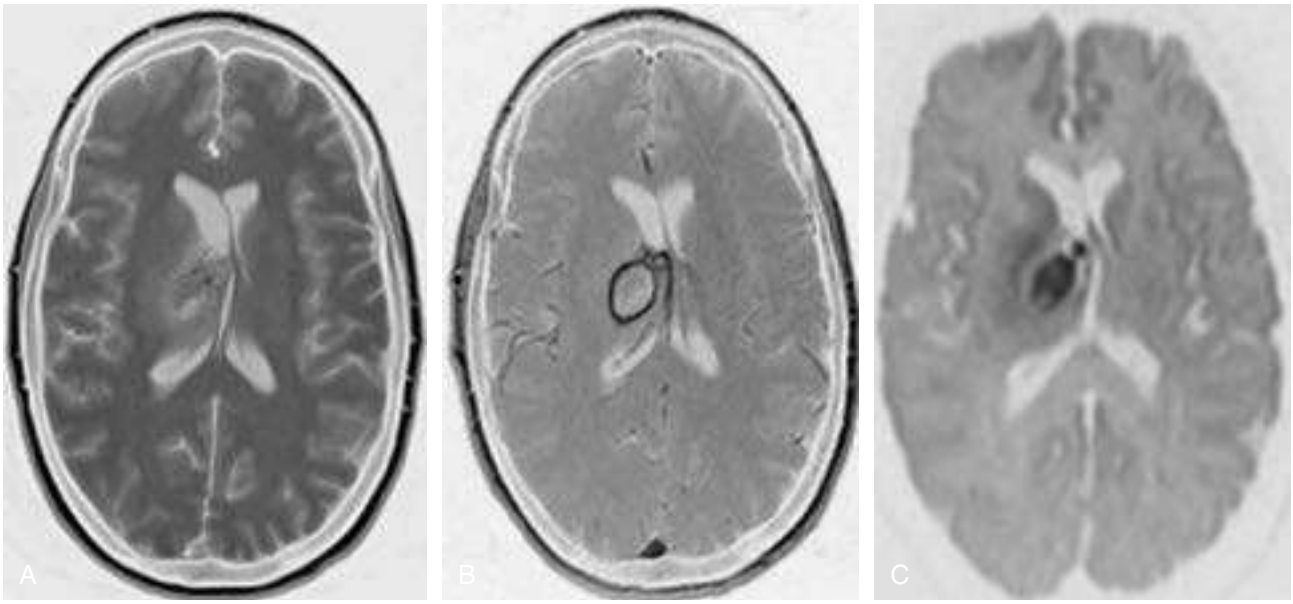

FIG. 90.3 Magnetic resonance imaging of the brain reveals a 3 × 1.7 cm bilobed peripherally enhancing, centrally restricted brain abscess located within the right thalamus. (A) T1-weighted image. (B) T1-weighted image after administration of gadolinium reveals ring enhancement of the abscess. (C) Diffusion-weighted imaging reveals marked hypersignal, a finding consistent with pyogenic brain abscess.

with active toxoplasmic encephalitis whose CT scans were normal. MRI should be performed in patients with AIDS and neurologic symptoms in whom CT shows no abnormality (or only cerebral atrophy). CT and MRI also may be useful in following the response to therapy because most patients show radiographic evidence of improvement within 10 to 14 days of initiation of anti-*Toxoplasma* therapy.[95,147]

The characteristic radiographic appearance of CNS toxoplasmosis in patients with AIDS, combined with its high prevalence, has been used to postpone the need for brain biopsy to establish a definitive diagnosis. The differential diagnosis of localized intraparenchymal brain lesions in patients with AIDS primarily also includes CNS lymphoma; progressive multifocal leukoencephalopathy; and, less commonly, tuberculosis, cryptococcoma, chagoma, and pyogenic brain abscess, the last mentioned usually in injection drug users or in the presence of another focus of infection. In patients with AIDS, because toxoplasmic encephalitis occurs as a result of a recrudescence of a latent infection,[93,94] the presence of anti-*Toxoplasma* immunoglobulin G (IgG) antibody can almost uniformly be shown before the development of the encephalitis. More than 97% of patients with AIDS and toxoplasmic encephalitis have serum IgG antibody titers against *T. gondii* ranging from 1 : 8 to more than 1 : 1024[92]; the predictive value of a positive serologic result in patients with characteristic abnormalities on radiographic studies may be 80% in the United States.[93,148] In contrast, in a retrospective review of 115 patients with AIDS and CNS toxoplasmosis at San Francisco General Hospital between 1981 and 1990, 4 of 18 patients with pathologically confirmed disease had undetectable anti-*Toxoplasma* IgG antibody by an indirect immunofluorescence assay.[95] Despite these conflicting data, many physicians in the United States initiate a therapeutic trial of anti-*Toxoplasma* chemotherapy in a patient with AIDS who is seropositive for *T. gondii* and has characteristic neuroradiographic abnormalities.[149] This is generally a valid approach in patients with AIDS and presumed CNS toxoplasmosis (see Chapter 278). Follow-up imaging in 2 weeks is obtained to decide whether a biopsy is necessary. Several meta-analyses suggest that brain biopsy is safe and effective and frequently results in a change in management in patients with AIDS and intracranial lesions when imaging studies are not characteristic for a specific diagnosis.[150,151]

CSF polymerase chain reaction (PCR) assay has also been used as a diagnostic test for toxoplasmic encephalitis; CSF PCR assay has a specificity of 96% to 100% but a sensitivity of only about 50% in the diagnosis of CNS toxoplasmosis.[96] Although the results from different reported studies vary, it seems clear that a positive PCR result in blood or CSF is very strong evidence in favor of the diagnosis of toxoplasmic encephalitis, whereas a negative result does not exclude the diagnosis.[152–154]

A major advance in the use of CT in patients with suspected brain abscess is the ability to perform stereotactic CT-guided aspiration to facilitate microbiologic diagnosis and to guide antimicrobial therapy. Ultrasound-guided aspiration, via transdural insonation, has also been performed through a single bur hole and showed excellent abscess visualization in 10 patients in one study,[155] although aspiration by CT guidance is generally the preferred method. Aspiration during the cerebritis stage may be complicated by hemorrhage.

At the time of aspiration, specimens should be sent for Gram stain, routine aerobic and anaerobic cultures, and cultures for mycobacteria and fungi. In patients with a likely bacterial brain abscess, 16S ribosomal RNA gene sequencing and amplification may serve as an important adjunctive tool in patients with negative culture results but who have histopathologic and Gram stain findings suggestive of a bacterial abscess[156–159]; this technique may provide a definitive etiologic diagnosis allowing for targeted antimicrobial therapy, although more studies are needed. In another study, use of multiple 16S ribosomal DNA sequencing was found to dramatically increase the number of infectious agents identified in cerebral abscesses.[160] These data were confirmed in a subsequent study in which metagenomic analysis from 71 patients identified 71 taxa that had not been previously identified, including 37 that are yet uncultured[161]; the major source of these agents was the sinuses and dental flora, and they were found in polymicrobial specimens. However, the importance of many of these pathogens in patients with brain abscess is unclear.[162] In addition, archaea-targeted PCR sequencing and metagenomics has demonstrated that methanogens (i.e., antibiotic-resistant anaerobic archaea that escape routine detection in clinical microbiology laboratories) belong to the anaerobic community responsible for brain abscess,[163] suggesting that medical treatment of brain abscess should incorporate agents (e.g., imidazole derivatives) active against these microorganisms, although more data are needed. Special stains such as acid-fast stains for mycobacteria, modified acid-fast stains for *Nocardia,* and other special stains (e.g., mucicarmine or Fontana-Masson for *Cryptococcus,* methenamine silver for fungi) should also be used to aid in making an etiologic diagnosis.

Aspergillus spp. manifest as septate hyphae in tissue sections with acute-angle, dichotomous branching. Biopsy specimens of cerebral lesions in mucormycosis usually show broad, irregular diameter hyphae; right-angle branching; and a lack of septa. *S. apiospermum, L. prolificans,*

and *Fusarium* hyphae cannot be distinguished reliably from *Aspergillus* spp. Hyphae of melanized, or dematiaceous, fungi in the brain may be brownish on hematoxylin and eosin stain and usually brown on Fontana-Masson stain and tend to have swollen and constricted areas in the hyphae, but they are not reliably distinguished from other molds. In patients with CNS toxoplasmosis,[92] diagnosis may require specialized immunohistochemical techniques (peroxidase-antiperoxidase) to detect the organisms or its antigens. Pseudocysts and tachyzoites, which are easily identifiable by histopathologic stains, may not be found in the center of the necrotic lesion and are best identified at the periphery of the lesion or within normal brain tissue. The immunofluorescence technique that uses monoclonal anti-*Toxoplasma* antibodies on brain touch preparations is a sensitive test for rapid diagnosis.

INITIAL MANAGEMENT OF THE PATIENT WITH BRAIN ABSCESS

The initial approach to a patient with a suspected brain abscess is a multidisciplinary one and should include evaluation by a neuroradiologist, a neurosurgeon, and an infectious diseases specialist using the following steps[1,2,31,137,164]:

1. MRI or contrast CT should be performed to verify the suspicion of brain abscess; if confirmed, plans for aspiration or surgery should be made (see below).
2. Obtain ancillary studies in an attempt to establish an etiologic diagnosis. These include blood cultures, which may have a yield of up to 28%; CSF analysis (if neuroimaging findings do not indicate risk of brain herniation with lumbar puncture), which may have utility for some conditions in the differential diagnosis, particularly in immunocompromised patients; and CT of the chest, especially in immunocompromised patients in whom there may also be involvement with the same pathogen (e.g., *Nocardia* or *Aspergillus*).
3. If single or multiple ring-enhancing lesions are found, the patient should undergo surgery. All lesions greater than 2.5 cm in diameter should be excised or stereotactically aspirated, and specimens should be sent to the microbiology and pathology laboratories (discussed earlier). For abscesses in the early cerebritis stage, or when the abscesses are 2.5 cm in diameter or less, the largest lesion should be aspirated for diagnosis and identification of the organism.
4. When abscess material has been obtained for microbiologic and histopathologic studies, empirical antimicrobial therapy should be initiated on the basis of the patient's predisposing conditions and the presumed pathogenesis of abscess formation (Table 90.3). If a primary source of infection outside the CNS is recognized as potentially having led to formation of the brain abscess, empirical antimicrobial therapy can be begun on the basis of microbiologic studies from the other source (e.g., positive blood cultures in a patient with infective endocarditis).
5. Therapy with corticosteroids should be initiated in patients with significant edema and an associated mass effect that is causing increased intracranial pressure or a predisposition to transtentorial herniation. Phenytoin should be considered to prevent seizures during early stages of therapy.

The empirical approach to antimicrobial therapy for bacterial brain abscess should take into account the frequency of isolation of certain organisms.[2] Because of the high rate of isolation of streptococci (particularly the *S. anginosus [milleri]* group) from brain abscesses of various causes, high-dose intravenous penicillin G or another drug (e.g., a third-generation cephalosporin, either cefotaxime or ceftriaxone) active against this organism should be included in the initial therapeutic regimen. Penicillin G is also active against most anaerobic species with the notable exception of *Bacteroides fragilis,* which may be isolated in a high percentage of cases of brain abscess; metronidazole should be included in the initial regimen when this organism is suspected. Metronidazole has bactericidal activity against *B. fragilis* and *Prevotella melaninogenica* and attains high concentrations in brain abscess pus, and its entry into cerebral abscesses is not affected by concomitant corticosteroid therapy.[165] The combination of metronidazole plus a third-generation cephalosporin is a common regimen used in more recent series.[18]

TABLE 90.3 Empirical Antimicrobial Therapy for Bacterial Brain Abscess

PREDISPOSING CONDITION	ANTIMICROBIAL REGIMEN
Otitis media or mastoiditis	Metronidazole + third-generation cephalosporin[a]
Sinusitis (frontoethmoid or sphenoid)	Metronidazole + third-generation cephalosporin[a,b]
Dental infection	Metronidazole + third-generation cephalosporin[a]
Penetrating trauma or postneurosurgical	Vancomycin + third-generation or fourth-generation cephalosporin[a,c]
Lung abscess, empyema, bronchiectasis	Third-generation cephalosporin[a] + metronidazole + sulfonamide[d]
Bacterial endocarditis	Vancomycin[e]
Congenital heart disease	Third-generation cephalosporin[a]
Unknown	Vancomycin + metronidazole + third-generation or fourth-generation cephalosporin[a,c]

[a]Cefotaxime or ceftriaxone; the fourth-generation cephalosporin cefepime may also be used.
[b]Add vancomycin when infection caused by methicillin-resistant *Staphylococcus aureus* is suspected.
[c]Use ceftazidime or cefepime as the cephalosporin if *Pseudomonas aeruginosa* is suspected.
[d]Trimethoprim-sulfamethoxazole; include if *Nocardia* spp. is suspected.
[e]Additional agents should be added based on other likely microbiologic etiologies.

When *S. aureus* is considered a likely pathogen (e.g., after cranial trauma or after neurosurgery), vancomycin should be used, but therapy should be changed to nafcillin if methicillin-sensitive organisms are isolated.[166] Because an increase in community-acquired methicillin-resistant *S. aureus* has been observed in recent years, vancomycin should be used empirically when *S. aureus* brain abscess is suspected or proven[25,167] until in vitro susceptibility testing is performed. Use of ceftaroline in brain abscess remains undefined. If *P. aeruginosa* is a likely infecting pathogen, ceftazidime or cefepime is the agent of choice. In patients with a bacterial brain abscess of unclear pathogenesis, empirical therapy with vancomycin, metronidazole, and a third-generation or fourth-generation cephalosporin (cefotaxime or ceftriaxone, or ceftazidime or cefepime if *P. aeruginosa* is suspected) is recommended pending culture results.

The initial empirical antimicrobial approach to immunocompromised patients is not evidence based but focuses on pathogens historically associated with specific immune defects of the patient. In transplant recipients (both solid-organ and hematopoietic stem cell transplants), *Nocardia* and fungal infections are additional considerations, so the combination of high-dose meropenem (which will cover most *Nocardia,* anaerobes, and gram-negative bacilli, including *Pseudomonas,* and possibly *Listeria*) plus voriconazole and vancomycin (until infection with methicillin-resistant *S. aureus* has been excluded) would be chosen by many experts. In patients with hematologic malignancies in whom neutropenia is the main risk factor, appropriate coverage for fungal infections is needed, and combination therapy with voriconazole and an amphotericin B preparation may be considered; the antifungal prophylaxis that the patient was receiving (if any) should be taken into consideration when choosing an empirical antifungal regimen. A very unusual but characteristic distinct syndrome of bacteremia, meningitis, and brain abscess caused by *Bacillus cereus* have been described in patients receiving high-dose chemotherapy for acute leukemia, sometimes with thrombosis and hemorrhage,[168–170] and addition of vancomycin should be considered in the empirical regimen in the appropriate clinical setting.

In HIV-infected patients with CNS mass lesions, the initial approach to management is different because of the high likelihood of the diagnosis of toxoplasmic encephalitis.[93,171] For patients with large lesions showing

a mass effect and threatening impending herniation, open biopsy with decompression is the standard. In HIV-infected patients with multiple ring-enhancing lesions on contrast-enhanced CT or MRI and positive anti-*Toxoplasma* IgG serologic tests, empirical therapy for toxoplasmic encephalitis should be initiated (Table 90.4); clinical and radiographic improvement should be observed within 10 to 14 days in 95% of patients with toxoplasmic encephalitis (see above).[96,147] A strategy for the management of suspected *Toxoplasma* CNS lesions in HIV-infected patients is presented in Chapter 278.

THERAPY

When the infecting pathogen is isolated, antimicrobial agents can be modified for optimal therapy. Recommendations for standard therapy, with alternative agents, are provided in Table 90.4. Table 90.5 lists dosages of these agents used for CNS infections. In this section the principles of antimicrobial use and surgical therapy for bacterial and fungal brain abscesses are reviewed. The therapeutic approach to toxoplasmic encephalitis is discussed in Chapter 278.

Bacterial Brain Abscess

Antimicrobial Therapy

No randomized controlled trials have evaluated the efficacy of different antimicrobial agents in the treatment of bacterial brain abscess. The antimicrobial agents used to treat bacterial brain abscess should be able to penetrate into the abscess cavity and should have in vitro activity against the pathogens isolated.[1,2] The few studies that have addressed the penetration of antimicrobial agents into brain abscess fluid have included limited numbers of patients. Concentrations of penicillin G have been measured in brain abscess pus but were detected consistently only if the daily dosage in adults exceeded 24 million units; in some cases, penicillin G may be inactivated in pus, with the result that bacteria can still be cultured despite adequate penicillin concentrations. Limited data are available on the penetration of the semisynthetic penicillins (e.g., nafcillin, oxacillin) into brain abscesses, although some studies suggest that concentrations of these drugs in brain abscess fluid are variable.

Metronidazole has excellent in vitro activity against strict anaerobes, making it an important agent for the treatment of patients with brain abscess.[165] Its excellent pharmacokinetic profile (i.e., good oral absorption and penetration into brain abscess cavities) has made metronidazole a more attractive antianaerobic agent than chloramphenicol for therapy of brain abscess. However, metronidazole must always be used in combination with an antimicrobial agent effective against streptococci because polymicrobial infections are common in patients with brain abscesses. Vancomycin has also been shown to have excellent concentrations in brain abscess fluid after prolonged therapy. In one study,

TABLE 90.4 Antimicrobial Therapy for Brain Abscess

ORGANISM	STANDARD THERAPY	ALTERNATIVE THERAPIES
Bacteria		
Actinomyces spp.[b]	Penicillin G	Clindamycin
Bacteroides fragilis[b]	Metronidazole	Clindamycin
Enterobacteriaceae[b]	Third-generation cephalosporin[c]	Aztreonam, trimethoprim-sulfamethoxazole, fluoroquinolone, meropenem
Fusobacterium spp.[b]	Metronidazole	Clindamycin, meropenem
Haemophilus spp.[b]	Third-generation cephalosporin[c]	Aztreonam, trimethoprim-sulfamethoxazole
Listeria monocytogenes	Ampicillin or penicillin G[d]	Trimethoprim-sulfamethoxazole
Mycobacterium tuberculosis	Isoniazid + rifampin + pyrazinamide + ethambutol	
Nocardia spp.	Trimethoprim-sulfamethoxazole or sulfadiazine	Minocycline, imipenem, meropenem, third-generation cephalosporin,[c] amikacin, linezolid
Prevotella melaninogenica[b]	Metronidazole	Clindamycin, meropenem
Pseudomonas aeruginosa	Ceftazidime or cefepime	Aztreonam, fluoroquinolone, meropenem
Staphylococcus aureus Methicillin-sensitive Methicillin-resistant	 Nafcillin or oxacillin Vancomycin	 Vancomycin Trimethoprim-sulfamethoxazole
Streptococcus anginosus (milleri) group, other streptococci[b]	Penicillin G	Third-generation cephalosporin,[c] vancomycin
Fungi		
Aspergillus spp.	Voriconazole	Liposomal amphotericin B, amphotericin B lipid complex, amphotericin B deoxycholate, itraconazole,[e] posaconazole[e]
Candida spp.	Liposomal amphotericin B,[f] amphotericin B lipid complex,[f] amphotericin B deoxycholate[f]	Fluconazole, voriconazole
Cryptococcus neoformans	Amphotericin B deoxycholate,[f] liposomal amphotericin B,[f] amphotericin B lipid complex[f]	Fluconazole
Mucorales	Liposomal amphotericin B, amphotericin B lipid complex, amphotericin B deoxycholate	Posaconazole,[e] isavuconazole[e]
Scedosporium apiospermum	Voriconazole	Itraconazole,[e] posaconazole[e]
Protozoa		
Toxoplasma gondii	Pyrimethamine + sulfadiazine	Pyrimethamine + clindamycin; trimethoprim-sulfamethoxazole; pyrimethamine + azithromycin, clarithromycin, atovaquone, or dapsone

[a]Choice of specific antimicrobial agents for standard therapy, or consideration of alternative therapies, should be based on in vitro susceptibility testing for pathogens for which testing can be performed.
[b]Depending on the pathogenesis of bacterial brain abscess (see text), these bacteria may be isolated as part of a mixed infection.
[c]Cefotaxime or ceftriaxone.
[d]Addition of an aminoglycoside should be considered.
[e]Consider for use in salvage therapy in nonresponding patients or in patients intolerant of amphotericin B–based therapies.
[f]Addition of flucytosine may be considered.

TABLE 90.5 Recommended Dosages of Antimicrobial Agents for Central Nervous System Infections in Adults

ANTIMICROBIAL AGENT	TOTAL DAILY DOSAGE	DOSING INTERVAL (h)
Amikacin[b]	15 mg/kg	8
Amphotericin B deoxycholate	0.6–1.0 mg/kg[c]	24
Amphotericin B lipid complex	5 mg/kg	24
Ampicillin	12 g	4
Atovaquone[d]	3000 mg	6
Azithromycin	1200–1500 mg	24
Aztreonam	6–8 g	6–8
Cefepime	6 g	8
Cefotaxime	8–12 g	4–6
Ceftazidime	6 g	8
Ceftriaxone	4 g	12
Ciprofloxacin	800–1200 mg	8–12
Clindamycin	2400–4800 mg	6
Dapsone[d]	100 mg	24
Ethambutol[d]	15 mg/kg	24
Fluconazole	400–800 mg	24
Flucytosine[d,e]	100 mg/kg	6
Gentamicin[b]	5 mg/kg	8
Isavuconazole[f]	200 mg	24
Isoniazid[d]	300 mg	24
Itraconazole	400–800 mg	12
Linezolid	1200 mg	12
Liposomal amphotericin B	5–7.5 mg/kg	24
Meropenem	6 g	8
Metronidazole	30 mg/kg	6
Nafcillin	12 g	4
Oxacillin	12 g	4
Penicillin	24 million U	4
Posaconazole[g]	300 mg	24
Pyrazinamide[d]	15–30 mg/kg	24
Pyrimethamine[d,h]	25–100 mg	24
Rifampin[d]	600 mg	24
Sulfadiazine[d]	4–6 g	6
Tobramycin[b]	5 mg/kg	8
Trimethoprim-sulfamethoxazole[i]	10–20 mg/kg	6–12
Vancomycin[j]	30–45 mg/kg	8–12
Voriconazole[k]	8 mg/kg	12

[a]Patients with normal renal and hepatic function; unless indicated, intravenous mode of administration used.
[b]Need to monitor peak and trough serum concentrations.
[c]Dosages up to 1.5 mg/kg/day may be used for aspergillosis or mucormycosis.
[d]Dosage for oral administration.
[e]Maintain serum concentrations of 50–100 µg/mL.
[f]Isavuconazole is available commercially as isavuconazonium sulfate, the prodrug of isavuconazole. The drug requires IV loading dose of 372 mg of isavuconazonium (equivalent to 200 mg of isavuconazole) every 8 hours for 6 doses, followed by dosing every 24 hours.
[g]IV loading dose of 300 mg every 12 hours for 2 doses. There are limited clinical data on the use of newer formulations (i.e., IV and delayed-release tablets) of posaconazole in the management of fungal central nervous system infections, but these formulations have improved pharmacokinetic properties and are recommended. The oral suspension of posaconazole (200 mg every 6 hours initially, then 400 mg every 12 hours) has been successfully used in isolated cases.
[h]Higher dosages used in patients with AIDS and toxoplasmic encephalitis; load with 100–200 mg.
[i]Dosage based on trimethoprim component; higher dose used for *Nocardia* brain abscess.
[j]Adjust dosage based on trough serum concentration; maintain at 15–20 µg/mL.
[k]IV loading dose of 6 mg/kg IV every 12 hours for two doses; maintain serum trough concentrations of 2–5 µg/mL.

simultaneous measurement of vancomycin concentrations in serum and brain abscess fluid was obtained 1 hour after a 500-mg dose[166]; vancomycin concentrations obtained before and during operative removal of the brain abscess were 15 µg/mL and 18 µg/mL, respectively, with a simultaneous serum vancomycin concentration of 21 µg/mL.

The third-generation cephalosporins are attractive agents for the treatment of brain abscess because of their good CNS penetration and excellent in vitro activity against many of the pathogens isolated from bacterial brain abscesses. When cefotaxime was given in higher doses than usually recommended (3 g every 8 hours), brain abscess concentrations of cefotaxime and its active metabolite, desacetylcefotaxime, were greater than the minimal inhibitory concentrations of most gram-positive and gram-negative organisms against which cefotaxime is used systemically. When combined with metronidazole and used in conjunction with surgical excision, high doses of cefotaxime also have been effective clinically in the treatment of brain abscess.[172] Ceftriaxone and ceftazidime have been used in the treatment of brain abscess,[173,174] although only a few patients have been studied. Ampicillin-sulbactam has also been shown to successfully treat brain abscesses[175]; intracavitary concentrations were variable but adequate in most cases.

Imipenem has been used successfully to treat pyogenic and nocardial brain abscesses,[176,177] although the use of imipenem has been associated with an increased risk for seizures, limiting its usefulness in patients with CNS mass lesions. Meropenem, a carbapenem antimicrobial agent similar to imipenem, was successful in one case of an *Enterobacter cloacae* brain abscess,[178] suggesting that this agent may be useful in cases of brain abscess, especially when caused by resistant pathogens. Usage of doripenem in brain abscess is as yet undefined. In an 11-year retrospective, nonrandomized study comparing intravenous cefotaxime and metronidazole with intravenous meropenem or imipenem monotherapy, treatment with meropenem was associated with a significantly lower mortality rate and a lower seizure rate compared with imipenem.[179] The fluoroquinolones have good CNS penetration and have been used anecdotally in the treatment of patients with brain abscess,[180] although further data are needed to determine the efficacy of the fluoroquinolones for the treatment of brain abscess.

Antimicrobial therapy with high-dose intravenous agents has traditionally been administered for 6 to 8 weeks in patients with bacterial brain abscesses.[1,2,22] This is often followed by oral antimicrobial therapy for 2 to 3 months if an appropriate agent or agents are available, although the efficacy and necessity of this approach have not been established. Biweekly neuroimaging for up to 3 months is also suggested to monitor for abscess reexpansion or failure to resolve.[22,164] The combination of surgical aspiration or removal of all abscesses larger than 2.5 cm in diameter with a 6-week or longer course of intravenous antimicrobial therapy along with improvement on follow-up neuroimaging should result in a cure rate of more than 90%.[8] Patients should be followed with neuroimaging until the abscess has completely resolved; if the abscess enlarges after 2 weeks of antimicrobial therapy or fails to resolve after 3 to 4 weeks, further surgical aspiration or excision should be performed.

Shorter courses (3–4 weeks) of antimicrobial therapy may be adequate for patients who have undergone surgical excision of the abscess. Surgical therapy (i.e., excision or aspiration) is often required for the optimal management of brain abscess (discussed later), although certain subsets of patients may be treated with antimicrobial therapy alone.[140,181–183] These subsets include patients with medical conditions that increase the risk of surgery, multiple abscesses, abscesses in a deep or dominant location, concomitant meningitis or ependymitis, early abscess reduction with clinical improvement after antimicrobial therapy, and abscess size smaller than 3 cm. In one series, no abscess larger than 2.5 cm resolved without surgical therapy.[181] The best candidates for medical therapy appear to be patients with a small abscess (≤2.5 cm), in good clinical condition (Glasgow Coma Scale score >12), and with a well-known etiology.[184] However, surgery should be considered in patients in whom the clinical condition is worsening or in patients without clinical or radiologic improvement within 1 to 2 weeks.

Patients treated with antimicrobial therapy alone may require prolonged (up to 12 weeks) courses of parenteral treatment and must receive careful clinical and radiographic follow-up. The Infection in Neurosurgery Working Party of the British Society for Antimicrobial Chemotherapy recommends intravenous therapy for 1 to 2 weeks for bacterial brain abscess, after which time, depending on the clinical response, change to an appropriate oral regimen can be considered.[185] This approach has been used in several series,[18,186,187] although it cannot be considered standard therapy in most patients with bacterial brain abscess. The ability of MRI and DWI to track resolution of the abscess has proved very useful in identifying patients with bacterial brain abscess who may need only 2 weeks of postoperative intravenous antibiotics.[188]

When a brain abscess caused by *Nocardia* is suspected or proved, the sulfonamides, with or without trimethoprim, are recommended as first-line therapy,[46,189,190] although treatment failures have been reported.[191] Alternative agents include minocycline, amikacin, imipenem, third-generation cephalosporins, and linezolid, which are among the most active agents against *Nocardia* in vitro.[192–194] However, the taxonomy of *Nocardia* has changed considerably over the past decade, so it is recommended to use molecular methods for identification, and in vitro susceptibility testing should ideally be performed by a reference laboratory.[195] Also, in vitro activity does not always correlate with clinical efficacy. Combination therapies have been studied,[196–200] and combination regimens containing third-generation cephalosporins or imipenem along with a sulfonamide or amikacin should be considered for immunocompromised patients or patients in whom therapy fails.[1,201] Of note, 100% of *Nocardia* isolates are susceptible to linezolid. In addition, linezolid was shown to be efficacious in two patients with multiple *Nocardia* brain abscesses,[202] although therapy was changed in one patient as a result of an adverse effect from linezolid. One patient was successfully treated with unexpectedly low doses of linezolid along with therapeutic drug monitoring for optimization of drug exposure.[203] Tedizolid is also active in vitro against all isolates of *Nocardia*[204]; it may be less myelosuppressive than linezolid and has been successfully used for 6 months in a patient with *Nocardia farcinica* brain abscess without toxicity,[205] although more data are needed, as the CNS penetration of tedizolid is less than linezolid. Brain abscess caused by *N. farcinica*, a species highly resistant to various antimicrobial agents, has also been successfully treated with moxifloxacin either alone or in combination with another agent.[206–208] The European experience with *Nocardia* infections after solid-organ transplantation suggests that 40% of cases of CNS involvement may be asymptomatic, confirms the increased virulence of *N. farcinica*, and fails to show any advantage of combination therapy over monotherapy.[48,209] The duration of antimicrobial therapy for nocardial brain abscess ranges from 3 to 12 months.[46,210] Therapy in immunosuppressed patients should probably be continued for 1 year,[141] with careful follow-up to monitor for relapse.

The therapy for tuberculous brain abscess is similar to that for tuberculosis in other locations. However, treatment is more complex for patients with tuberculous brain abscess caused by multidrug-resistant and extremely drug-resistant strains. Because published reports on tuberculous brain abscess consist mainly of case reports and case series, overall mortality rates are not well defined.[55]

Surgical Therapy

Most patients with bacterial brain abscess require surgical management for optimal therapy. The two procedures available are aspiration of the abscess after bur hole placement and complete excision after craniotomy.[22,30,33,211] No prospective trial comparing the two procedures has ever been performed, although in a series of 142 patients with brain abscess, no significant differences in outcome were observed in patients treated with excision, craniotomy with drainage, or stereotactic drainage.[17] However, in one retrospective review of 47 studies from the years 1990–2008, patients who underwent aspiration had a mortality rate of 6.6% compared with 12.7% in patients who underwent surgical excision.[212] In contrast, a meta-analysis of more recent series found that for encapsulated abscesses in noneloquent areas of the brain, excision resulted in improved neurologic status at 1 month, lower reoperation rate, and shortened duration of hospital stay and antimicrobial duration.[213] The choice of procedure must be individualized for each patient. Aspiration may be performed by a stereotactic procedure using CT or MRI guidance, which affords the surgeon rapid, accurate, and safe access to virtually any intracranial point, including areas located in deep critical regions of the CNS (e.g., brainstem, cerebellum, or diencephalic structures adjacent to the ventricles)[174,214–218]; aspiration can also be used for swift relief of increased intracranial pressure.

Complete excision by craniotomy is now infrequently performed because of the development of aspiration and closed drainage techniques described previously, but it may be required in patients with multiloculated abscesses (for whom aspiration techniques have failed), abscesses containing gas, or abscesses that fail to resolve. Posttraumatic abscesses containing foreign bodies or retained bone fragments usually require excision to prevent recurrence, as do abscesses resulting from fistulous communications and abscesses localized to one lobe but contiguous to a primary focus.[22] Excision of abscesses in the cerebellar area has also been recommended, although in recent years bur hole aspiration has emerged as a satisfactory method of drainage[8]; drainage is important because precipitous neurologic deterioration can occur in the relatively smaller volume of the posterior fossa in the presence of disproportionate edema, especially in children.[219] In one retrospective review, there was a lower mortality in the excision group.[220] Patients with cerebellar abscesses that demonstrate mass effect, effacement, or displacement of the fourth ventricle in the setting of overt or incipient hydrocephalus should also have CSF diversion with placement of an external ventricular drain[5,8]; the presence of a periventricular lucency (transependymal flow) is an absolute indication for immediate ventricular drainage regardless of the level of consciousness.

Craniotomy with total excision is difficult in cases of nocardial brain abscess because these abscesses are usually multiloculated.[141] In one review of 11 patients with nocardial brain abscess,[221] aspiration alone (which was repeated as clinically indicated) was a safe, efficacious treatment for 9 patients. In another series of three patients with nocardial brain abscess, cure was achieved only after neurosurgical enucleation,[222] suggesting that an aggressive approach should be used. In patients with tuberculous brain abscess, an early surgical procedure is mandatory to establish the diagnosis and improve the efficacy of antituberculous therapy[56]; procedures include stereotactic-guided aspiration, simple puncture, continuous drainage, and repeated aspiration through bur holes. In patients with intraventricular rupture of a purulent brain abscess who have dilated ventricles and ventriculitis, ventricular drainage combined with the administration of appropriate intravenous or intrathecal antimicrobial agents, or both, is recommended[21,138]; urgent craniotomy and abscess drainage with or without lavage of the ventricular system has also been suggested, although the optimal approach has not been clarified.

Fungal Brain Abscess

The optimal therapy for fungal brain abscess usually requires a combined medical and surgical approach; surgery involves either excision or drainage of the abscess. Therapy for fungal brain abscess in immunocompromised patients carries a high mortality rate, however, despite surgery and antifungal therapy.[223] Nevertheless, early recognition of this infection can lead to a successful outcome, especially if leukocyte counts return to normal or if the dosages of immunosuppressive agents can be reduced.[1] The mainstay of medical therapy for candidal brain

abscess is an amphotericin B preparation plus 5-flucytosine.[65,224] The lipid formulations of amphotericin B are preferred for CNS infections, with fluconazole suggested for step-down therapy.[225] The efficacy of fluconazole in the treatment of *Candida* brain abscess has not been evaluated, although one case report in a premature infant with *Candida albicans* brain abscess showed a decrease in abscess size after the addition of fluconazole to amphotericin B plus 5-flucytosine.[226] Echinocandins do not cross the blood-brain barrier, and cases of brain abscesses have developed during treatment of *Candida* endocarditis with caspofungin.[227]

The antifungal therapy of choice for *Aspergillus* brain abscess was previously amphotericin B deoxycholate (0.8–1.25 mg/kg/day); doses up to 1.5 mg/kg/day have been used, depending on the clinical response.[228] However, its efficacy was notoriously low.[229] Voriconazole is now the drug of choice in patients with *Aspergillus* brain abscess.[223,230] In one review of voriconazole in the treatment of invasive aspergillosis that included 19 patients with cerebral disease, 3 (16%) patients had a partial response to treatment,[231] although the response rate in more recent studies is approximately 35%.[69,230] Combination therapy (voriconazole, combined with either caspofungin or liposomal amphotericin B) has also been advocated.[232–234] Posaconazole has poor penetration into the CNS, although there are reports of efficacy in CNS aspergillosis.[235] There are no current reports of CNS aspergillosis treated with isavuconazole.

Mucormycosis also should be treated with amphotericin B deoxycholate or one of its lipid formulations, along with correction of underlying metabolic derangements and aggressive surgical débridement.[65,72,223,236,237] Some data have suggested that the lipid formulations of amphotericin B resulted in higher recovery rates than amphotericin B deoxycholate in patients with mucormycosis and hematologic diseases or solid-organ transplantation, leading to a recommendation for use of a lipid formulation, usually liposomal amphotericin B, as first-line therapy.[73] The role of surgery in the treatment of cerebral mucormycosis cannot be overemphasized. Because of their propensity for invading blood vessels, the Mucorales cause extensive tissue infarction, impairing the delivery of antifungal agents to the site of infection. Surgery is often the only modality that may effectively eliminate the invading microorganisms. Amphotericin B has also been applied topically in the orbital cavities in patients with rhinocerebral mucormycosis,[237] although it is unclear if this is beneficial. Posaconazole or isavuconazole may be considered as follow-up therapy in patients who have already responded to amphotericin B–based therapies.[238] Given the relatively low penetration of posaconazole into the CNS, we recommend use of delayed-release tablets or the intravenous formulation as well as measuring serum concentrations of this agent, although there are limited clinical data on the use of these new formulations in the treatment of fungal CNS infections. Hyperbaric oxygen therapy has been reported to be a useful adjunct in cerebral mucormycosis,[239,240] although no prospective, controlled trials have been done to assess its efficacy adequately.

For *Scedosporium* brain abscess, surgical drainage is the cornerstone of effective therapy.[75] The organism shows in vitro resistance to amphotericin B. Based on clinical experience and the absence of good alternative agents, voriconazole is now the antifungal agent of choice for treatment of *S. apiospermum* brain abscess.[241] In one review of the use of voriconazole, which included 21 patients with CNS disease caused by *Scedosporium*, 43% had a therapeutic response.[242] There is a case report of successful use of posaconazole in the treatment of *S. apiospermum* brain abscess.[243] *L. prolificans (S. prolificans)* is a mold usually resistant to all antifungal agents; the combination of voriconazole and terbinafine was successful in one patient with chronic granulomatous disease and a brain abscess caused by this pathogen.[244]

Some dematiaceous fungi are often isolated from brain abscesses in immunocompromised and immunocompetent patients. Some, such as *Cladophialophora bantiana, Rhinocladiella mackenziei,* and *Exophiala dermatitidis,* seem to have a special tropism for the CNS. Complete excision of brain abscess caused by these fungi whenever feasible is associated with better outcomes than aspiration or partial excision.[83] However, the outcome remains poor, with an overall mortality of greater than 70%. Because these infections are uncommon, there are not good data on the antifungal agent of choice. Voriconazole, posaconazole, and itraconazole usually have in vitro activity, and the combination of amphotericin B and flucytosine shows synergism in vitro.[245] Of the azole antifungal agents, most experience is with itraconazole, but there are reported cases of successful treatment with posaconazole.[246,247] The reports of successful outcomes of these infections usually involve surgical incision and drainage (sometimes repeatedly) and a combination of antifungal agents.[66,248]

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The complete reference list is available online at Expert Consult.

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91 Subdural Empyema, Epidural Abscess, and Suppurative Intracranial Thrombophlebitis

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SHORT VIEW SUMMARY

Definition
- *Subdural empyema* refers to a collection of pus between the dura and arachnoid.
- *Epidural abscess* is a localized collection of pus between the dura mater and overlying skull or vertebral column.
- *Suppurative intracranial thrombophlebitis* includes dural venous sinus thrombosis and suppuration, most commonly septic cavernous sinus thrombosis.

Epidemiology
- The most common conditions predisposing to cranial subdural empyema are otorhinologic infections, especially of the paranasal sinuses, which are affected in 40% to 80% of cases. Spinal subdural empyema originates hematogenously.
- About 0.2 to 2 per 10,000 hospitalized patients have spinal epidural abscess, with most cases usually secondary to extension from vertebral osteomyelitis. More recent series reveal an incidence of up to 8.0 cases per 10,000 admissions, with patients more likely to have experienced at least one previous health care visit or to have received antimicrobials within 30 days of admission; to have comorbidities of injection drug use, alcohol abuse, and obesity; and to harbor coinfection at a noncontiguous site.
- Septic cavernous thrombosis usually originates in sphenoid and ethmoid sinusitis.

Microbiology
- A number of bacterial species have been isolated in patients with cranial subdural empyema, including aerobic streptococci, staphylococci, aerobic gram-negative bacilli, and anaerobic streptococci and other anaerobes.
- *Staphylococcus aureus* is the most common etiologic agent in patients with spinal epidural abscess.
- Staphylococci and streptococci are the usual bacteria causing septic cavernous sinus thrombosis.

Diagnosis
- Epidural spinal abscess should be suspected in patients with back pain, fever, and pain on percussion over the spine. Weakness or other long-tract signs are present initially in half of the cases. Magnetic resonance imaging (MRI) with gadolinium enhancement is the diagnostic procedure of choice in patients with subdural empyema and epidural abscess.
- Septic cavernous sinus thrombosis should be suspected in patients with headache, fever, and diplopia. Unilateral facial pain, decreased vision, or ptosis may also be seen. The noninvasive diagnostic procedure of choice in patients with suppurative intracranial thrombophlebitis is MRI and magnetic resonance venography; computed tomography venography is done in patients unable to undergo MRI.

Therapy
- Subdural empyema is a medical and surgical emergency. The goals of surgery are to achieve adequate decompression of the brain and completely evacuate the empyema; based on retrospective outcome data, craniotomy is the surgical procedure of choice.
- The principles of therapy for spinal epidural abscess are prompt laminectomy and surgical decompression in patients with neurologic dysfunction, drainage of the abscess, and 4 to 8 weeks of intravenous antimicrobial therapy, selected on the basis of culture and susceptibility testing. Antimicrobial therapy alone can be considered in patients with localized pain and radicular symptoms without long-tract findings, but frequent neurologic examinations and serial MRI studies should be performed to demonstrate resolution of the abscess. The risk of this approach is that rapid progression in the absence of surgery can cause permanent paraplegia.
- Empirical antimicrobial therapy for suppurative intracranial thrombophlebitis is usually vancomycin, metronidazole, and a third- or fourth-generation cephalosporin; anticoagulation should also be used in patients with septic cavernous sinus thrombosis unless there are contraindications.

SUBDURAL EMPYEMA

Epidemiology and Etiology

Subdural empyema refers to a collection of pus in the space between the dura and arachnoid. It accounts for 15% to 20% of all localized intracranial infections. Before the advent of antimicrobial therapy, the disease was essentially fatal (almost 100% mortality), but with current methods of diagnosis and treatment, mortality rates are approximately 10% to 20%.[1–5] The most common conditions predisposing to cranial subdural empyema are otorhinologic infections, especially of the paranasal sinuses, which are affected in 40% to 80% of patients[1,2,4–7]; this percentage may even be higher in children.[8–10] In contrast, in one 20-year review of 31 children with subdural empyema, only about 10% had a prior otorhinolaryngeal infection; about 20% had developed subdural empyema after head trauma or neurosurgery.[11] However, in another systematic review of intracranial complications of pediatric sinusitis that included 16 studies involving 180 patients,[12] the most common intracranial complication was subdural empyema, accounting for 49% of intracranial complications. The pathogenesis of cranial subdural empyema involves spread of the infection to the subdural space via valveless emissary veins in association with thrombophlebitis or via extension of osteomyelitis of the skull with accompanying epidural abscess. Once the infection reaches the subdural space, it can spread without interruption over the convexities of the brain. The mastoid and middle ear are the source in 10% to 20% of patients, especially in geographic areas where cases of otitis media are not treated promptly with antimicrobial therapy. Other predisposing conditions in patients with subdural empyema include skull trauma, neurosurgical procedures, and infection of a preexisting subdural hematoma[4,5,13–16] which may occur as a result of hematogenous dissemination from a distant infection.[17] The infection is metastatic in about 5% of cases, principally from a pulmonary source. Rare predisposing factors include cranial traction devices, nasal surgery, ethmoidectomy, and nasal polypectomy,[4] and the presence of multimodal intracranial monitoring devices.[18] In one series of patients with infratentorial empyema,[19] cases tended to cluster in the spring and summer months, with disease seen more commonly in males than females (65% vs. 35%). In infants with subdural empyema, meningitis is an important predisposing condition.[20] Subdural empyema occurs in about 2% to 10% of infants with bacterial meningitis,[5] presumably secondary to infection of an initially sterile subdural effusion. However, *Haemophilus influenzae* type b immunization has decreased the incidence of bacterial meningitis in

infants and children (see Chapter 87), and cranial subdural empyema is now more commonly seen in teens and young adults.[21]

A number of bacterial species have been isolated in patients with cranial subdural empyema,[3–6,22] including aerobic streptococci (25%–45% of cases), staphylococci (10%–15% of cases), aerobic gram-negative bacilli (3%–10% of cases), and anaerobic streptococci and other anaerobes (33% to as much as 100% in some small series in which careful culturing was performed). Polymicrobial infections are common. These organisms make up the microbial flora that is frequently isolated from patients with chronic sinusitis or cranial abscess. Postoperative and posttraumatic infections are more commonly caused by staphylococci and aerobic gram-negative bacilli. Unusual pathogens include *Salmonella* species[23,24] and *Cutibacterium (Propionibacterium) acnes*.[25–27] The latter microorganism has been isolated after trauma, neurosurgical procedures, or use of prosthetic material such as dural allografts, a material no longer used. In two series, 17%[5] and 21%[4] of samples taken from patients with cranial subdural empyema were sterile, although operative cultures have been reported to be sterile in 7% to 53% of cases.[7] In patients with bacterial meningitis who develop focal neurologic deficits, the diagnosis of cranial subdural empyema should be considered, particularly in those with group A streptococcal meningitis or with preceding otitis or sinusitis.[28] Subdural empyema is a rare complication of meningococcal meningitis, primarily affecting infants or very young children.[29] Cranial subdural empyema caused by *Mycobacterium tuberculosis*[30,31] and *Candida* species[32] has also been reported.

Spinal subdural empyema is a rare condition that usually occurs secondary to metastatic infection from a distant site,[33,34] although cases have been reported after epidural steroid injection.[35] The most frequent microbial isolate is *Staphylococcus aureus,* with streptococci, coagulase-negative staphylococci, and gram-negative bacilli found less frequently.[36]

Clinical Features

The clinical presentation of cranial subdural empyema can be rapidly progressive, with symptoms and signs secondary to the presence of increased intracranial pressure, meningeal irritation, or focal cortical inflammation.[1–5,7,36] This acute presentation of subdural empyema is seen most often in patients with contiguous spread of infection; the diagnosis should be considered in patients with acute bacterial sinusitis in combination with severe intractable headache, varying degrees of altered level of consciousness, focal neurologic deficits, signs of meningeal irritation, or a combination of these.[37,38] Fever higher than 39°C is present in most cases. Headache, which may be localized to the infected sinus or ear, is a prominent complaint and becomes generalized as the infection progresses. Vomiting is common as intracranial pressure increases. Altered mental status (i.e., drowsiness and disorientation), which progresses to obtundation or coma if the infection is not treated, is seen in more than two-thirds of patients. However, the triad of fever, headache, and altered mental status may be less commonly seen in children than in adults.[10] Focal neurologic signs appear in 24 to 48 hours and progress rapidly, with eventual involvement of the entire cerebral hemisphere. The most common focal signs are hemiparesis and hemiplegia; ocular palsies, dysphasia, homonymous hemianopsia, dilated pupils, and cerebellar signs have all been reported. However, in one study, no focal signs were observed in 41% of 699 patients with cranial subdural empyema.[5] One-third of patients in this series also presented with subgaleal abscesses, suggesting that the infection had spread from the calvaria across the dura. Seizures (either focal or generalized) are seen in 25% to 80% of cases.[7] Signs of meningeal irritation are present in approximately 80% of patients, although fewer have the Kernig or Brudzinski sign. In untreated patients, there is rapid neurologic deterioration with signs of increased intracranial pressure and cerebral herniation, although papilledema develops in less than 50% of cases. This fulminant presentation, however, may not be seen in patients who have subdural empyema after cranial surgery or trauma, those who have received prior antimicrobial therapy, those with infected subdural hematomas, or those with infections metastatic to the subdural space. In one review of 55 patients with traumatic cranial subdural empyema,[16] headache (84% of cases), fever (69% of cases), and neck stiffness (65% of cases) were the most common clinical features, and the mean time from initial trauma to presentation was 19 days (range, 4–60 days). In patients with infratentorial empyema, clinical features are usually nonspecific, with the triad of fever, headache, and vomiting being the most common symptoms[19]; cerebellar signs, which could localize the lesion to the posterior fossa, were elicited in only 40% of cases. In subdural empyema of infancy (which occurs in patients with bacterial meningitis), persistent fever, declining neurologic status, seizures, or some combination of these is most frequently observed.[21]

The clinical presentation of spinal subdural empyema is usually one of radicular pain and symptoms of spinal cord compression, which may occur at multiple levels.[34,36,39] The clinical presentation is difficult to distinguish from that of spinal epidural abscess, although tenderness on palpation is said to be a feature of epidural abscess and not subdural empyema (see later discussion).

Diagnosis

The diagnosis of cranial subdural empyema should be considered for any patient who presents with meningeal signs and a focal neurologic deficit.[1–4,5] A lumbar puncture with cerebrospinal fluid (CSF) analysis is contraindicated because of the risk of cerebral herniation. In one large series,[5] 280 patients with cranial subdural empyema underwent lumbar puncture; 33 patients were thought to have experienced neurologic deterioration as a direct result of this procedure, with 3 unnecessary deaths. In patients in whom a lumbar puncture has been done, CSF findings have been nonspecific and have included an elevated opening pressure; neutrophilic pleocytosis (although in up to 40% of patients there may be a mononuclear pleocytosis); and an increased CSF protein concentration.[36] CSF Gram stain and cultures are negative unless the course is complicated by the presence of bacterial meningitis. Cranial radiographs may show evidence of concurrent sinusitis or osteomyelitis, but they are not useful in establishing the diagnosis of subdural empyema.

Diagnostic procedures for cranial subdural empyema include either computed tomography (CT) with contrast or magnetic resonance imaging (MRI).[1,7,34,36] The typical CT appearance is that of a crescentic or elliptic area of hypodensity below the cranial vault or adjacent to the falx cerebri; loculations may also be seen. Depending on the extent of disease, there may be an associated mass effect with displacement of midline structures. After the administration of intravenous contrast, a fine, intense line of enhancement can be visualized between the subdural collection and the cerebral cortex. MRI provides better clarity of morphologic detail and may reveal the presence of a subdural empyema not seen on CT; it is particularly helpful in detecting subdural empyemas located at the base of the brain, along the falx cerebri, or in the posterior fossa. MRI can also be used to differentiate extraaxial empyemas from most sterile effusions and subdural hematomas. Diffusion-weighted images and apparent diffusion coefficient images add to the diagnostic value of MRI; in patients with subdural empyema, there is consistently restricted diffusion (high signal) with corresponding low signal on apparent diffusion coefficient images.[40] Based on these differences, MRI is now considered the diagnostic procedure of choice for cranial subdural empyema (Fig. 91.1). Both CT and MRI are also useful for demonstrating sinusitis and otitis. MRI is the diagnostic procedure of choice for spinal subdural empyema[36,41] because it is better than CT in defining the extent of the lesion.

Management and Outcome

Subdural empyema is a medical emergency, and its treatment optimally requires a combined medical and surgical approach. Surgery (i.e., drainage) is necessary because antimicrobial agents alone do not reliably sterilize the empyema. Cultures (aerobic and anaerobic) of purulent material are necessary to guide the use of specific antimicrobial agents, and surgical decompression is useful for controlling increased intracranial pressure. Once purulent material has been aspirated via craniotomy or, less optimally, by burr hole placement, antimicrobial therapy should be initiated based on Gram stain results and on the likely predisposing factor that led to the development of the subdural empyema. If *S. aureus* is a suspected pathogen, vancomycin should be used empirically but changed to nafcillin if the organism is found to be methicillin susceptible and the patient is not allergic to penicillin. Linezolid has been successfully

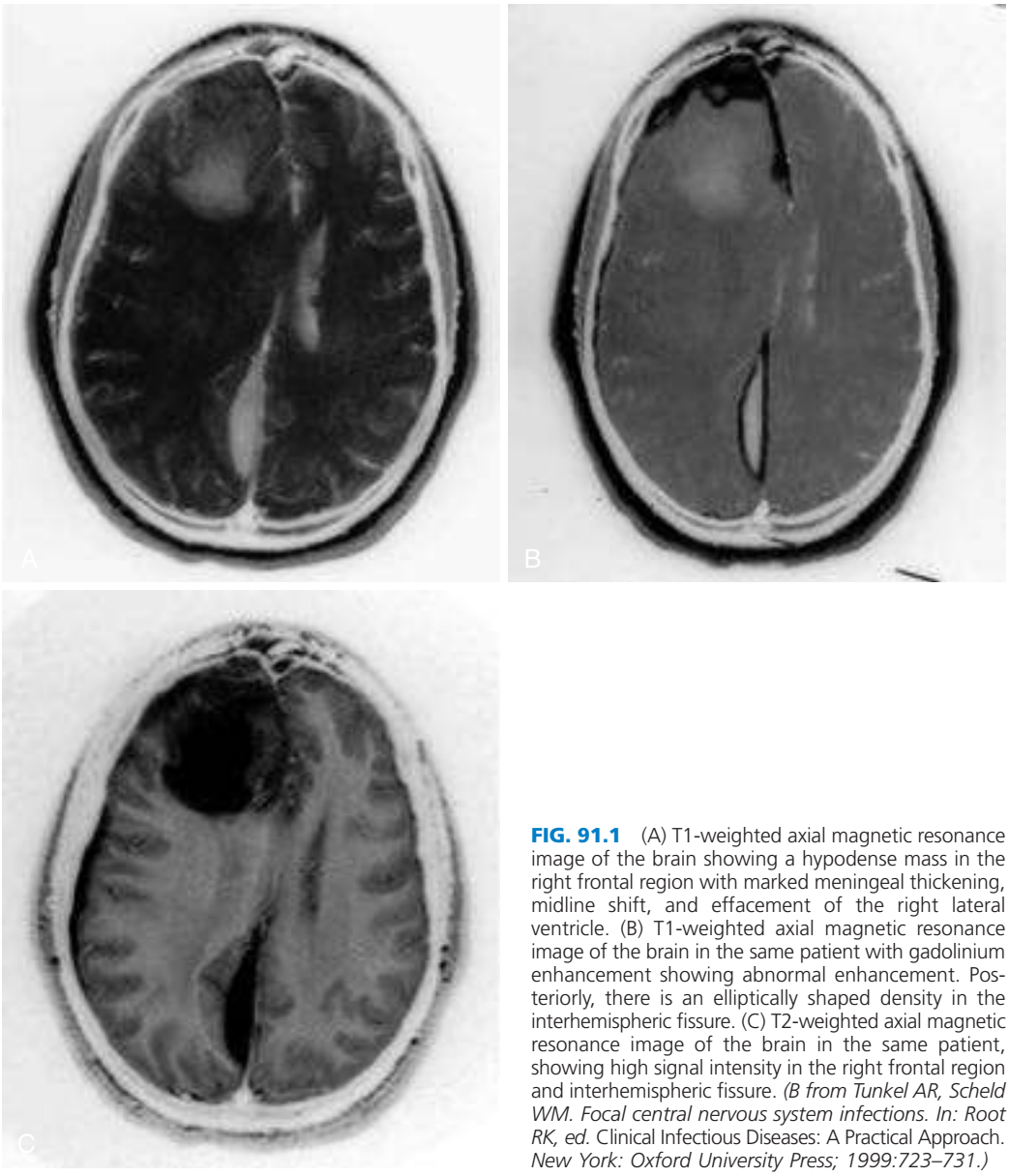

FIG. 91.1 (A) T1-weighted axial magnetic resonance image of the brain showing a hypodense mass in the right frontal region with marked meningeal thickening, midline shift, and effacement of the right lateral ventricle. (B) T1-weighted axial magnetic resonance image of the brain in the same patient with gadolinium enhancement showing abnormal enhancement. Posteriorly, there is an elliptically shaped density in the interhemispheric fissure. (C) T2-weighted axial magnetic resonance image of the brain in the same patient, showing high signal intensity in the right frontal region and interhemispheric fissure. *(B from Tunkel AR, Scheld WM. Focal central nervous system infections. In: Root RK, ed.* Clinical Infectious Diseases: A Practical Approach. *New York: Oxford University Press; 1999:723–731.)*

used in isolated cases of subdural empyema caused by streptococci[42] and can be considered for patients with subdural empyema caused by gram-positive bacteria in whom conventional therapy fails. Metronidazole is recommended if anaerobes are suspected. For infection likely caused by aerobic gram-negative bacilli, empirical therapy with cefepime, ceftazidime, or meropenem is appropriate, pending microorganism identification and in vitro susceptibility testing. Metronidazole is not necessary for antianaerobic activity if meropenem is used. Dosage information for these agents can be found in Chapter 90. Depending on the clinical response, parenteral antimicrobial therapy should be continued for 3 to 4 weeks after drainage, although there are no firm data to support a specific duration of antimicrobial therapy in patients with subdural empyema; longer periods of intravenous and perhaps oral therapy may be required if the patient has accompanying osteomyelitis. There have been anecdotal cases in which cranial subdural empyema was treated with antimicrobial therapy alone. This approach may be appropriate in patients with minimal or no impairment of consciousness, no major neurologic deficit, limited extension of the empyema without midline shift, and early improvement with antimicrobial therapy alone.[4,21] However, these selected patients need careful clinical and radiographic monitoring and probably longer courses of antimicrobial therapy.

The goals of surgical therapy in cranial subdural empyema are to achieve adequate decompression of the brain and to completely evacuate the empyema. The optimal surgical approach in patients with cranial subdural empyema (drainage via craniotomy or burr holes) is controversial.[1,21,36,43] Some studies have demonstrated a lower mortality rate in patients who have undergone craniotomy, although it may be that a larger number of patients treated with burr hole drainage were more ill and had a greater surgical risk. Use of burr holes with irrigation may be more efficacious for drainage in the early stages of subdural empyema when the pus is liquid,[44,45] allowing easier aspiration. If drainage is accomplished via burr holes, multiple burr holes may be necessary to allow extensive irrigation. When the pus becomes thickened and loculated as the disease progresses, these patients should undergo craniotomy. However, craniotomy may be essential for posterior fossa subdural empyema or if drainage is inadequate after burr holes.

If craniotomy is performed, wide exposure is necessary to allow adequate exploration of all areas where the empyema is suspected (Fig. 91.2). The neurosurgeon may also elect to leave drains or catheters in the subdural space, although this may increase the subsequent risk of nosocomial infections. In one report,[46] the efficacy of craniotomy versus CT-guided burr hole or craniectomy drainage was analyzed during the periods 1983 to 1987 (189 patients) and 1988 to 1997 (509 patients).



FIG. 91.2 Operative photograph of a patient with cranial subdural empyema revealing extensive purulent material over the cerebrum. *(Courtesy Alan R. Turtz, MD, Cooper University Hospital, Camden, NJ.)*

At operation, the empyema collections were sometimes found to be more loculated, tenacious, and extensive than indicated by neuroimaging studies. Based on this experience, craniotomy became the preferred method of drainage for these authors beginning in 1988. A significant improvement in outcome was demonstrated between the 1983 to 1987 study period (good outcome in 71.4% of patients) and the 1988 to 1997 period (good outcome in 86.1%; $P = .001$). Analysis of the entire database (1983 through 1997) revealed that the mortality rates were higher for patients treated only with drainage via burr holes (23.3%) compared with those who underwent craniectomies (11.5%) or craniotomies (8.4%). Patients with multiple burr holes or craniectomy drainage not only required more frequent operations to drain recurrent or remaining pus but also exhibited higher mortality rates and poorer outcomes. The authors recommended limited procedures (i.e., burr hole drainage or craniectomy) only for patients with septic shock, patients with localized parafalcine collections, and children with subdural empyemas secondary to meningitis (because there is usually no brain swelling and the pus is thin). These data were confirmed in another study of 90 patients with intracranial subdural empyema in which patients with a cranial bone opening procedure had a better result in terms of clinical improvement and clearance of the empyema on CT images[47]; reoperation was more frequent among patients who had undergone burr hole surgery. Regardless of the initial surgical approach, several studies have shown that a number of patients require reoperation; in one study, reoperation was required in half of those patients treated with burr hole drainage and one-fifth of those initially treated with craniotomy.[48] Surgical correction of the antecedent otorhinologic infection may also be required.

In the modern era, survival rates among patients with cranial subdural empyema are greater than 90% for those who are awake and alert at presentation but less than 50% for those who are unresponsive to pain at presentation.[36] Furthermore, 10% to 44% of patients may experience permanent neurologic deficits.[4,21] Cranial subdural empyemas may also be complicated by septic venous thrombosis, localized cerebritis, and cerebral abscesses. In a review that included 65 patients with supratentorial empyema and 27 patients with infratentorial empyema,[49] mortality rates were 10.8% and 3.7%, respectively; the lower mortality in those with infratentorial empyema supports the importance of early surgery, effective management of hydrocephalus, and adequate antimicrobial therapy.[19]

In patients with spinal subdural empyema, empirical antimicrobial therapy should be directed against staphylococci, streptococci, and aerobic gram-negative bacilli,[36] with adjustments based on culture results. Laminectomy is also necessary for drainage of the infection.

EPIDURAL ABSCESS

Epidemiology and Etiology

Epidural abscess is a localized collection of pus between the dura mater and the overlying skull or vertebral column. Because cranial epidural abscess can cross the cranial dura along emissary veins, an accompanying subdural empyema is often present.[1] Therefore the bacterial etiology and pathogenesis of cranial epidural abscess are usually identical to those described earlier for cranial subdural empyema, with the initial focus usually in the paranasal sinuses, middle ear, or mastoid cells; 60% to 90% of cases are associated with sinusitis and otitis, and complications are more common in children, adolescents, and young adults.[50] Cranial epidural abscess may also occur after trauma, fetal scalp monitoring, halo pin penetration, and recent intracranial, transnasal, or transmastoid surgical procedures.[15,20,50,51]

About 0.2 to 2 per 10,000 hospitalized patients have been previously reported to have spinal epidural abscess, with apparent increases in incidence likely from rises in the number of injection drug users and use of therapeutic spinal interventions.[50] In a recent study of 162 cases of spinal epidural abscess in adults, the incidence increased from 2.5 to 8.0 cases per 10,000 admissions from 2005 to 2015.[52] Compared with 88 control subjects, patients were significantly more likely to have experienced at least one previous health care visit or to have received antimicrobials within 30 days of admission; to have comorbidities of injection drug use, alcohol abuse, and obesity; and to harbor coinfection at a noncontiguous site. However, in another retrospective review of 101 cases from 2004 to 2014, the incidence was 5.1 cases per 10,000 admissions, with no significant change during the study period[53]; an identifiable underlying risk factor was identified in 84% of patients, most likely diabetes mellitus and injection drug use. Spinal epidural abscess usually occurs secondary to hematogenous dissemination from foci elsewhere in the body to the epidural space or by extension from local infection.[54–67] Most patients have one or more predisposing conditions, a spinal abnormality or intervention, or a local or systemic source of infection.[65] Bacteremia may be an important predisposing factor because the incidence of spinal epidural abscess is increased in patients who use injection drugs[68,69] and in those who have intravenous catheters or infective endocarditis. Diabetes mellitus also appears to be an important risk factor; it has been identified in up to 50% of patients.[57,59,62,70] Other predisposing factors include immunosuppressive therapy, human immunodeficiency virus infection, malignancy, renal insufficiency, and alcoholism. Hematogenous spread occurs in 25% to 50% of cases, secondary to infections of the skin (e.g., furuncles, cellulitis, infected acne), urinary tract infections, periodontal abscesses, pharyngitis, pneumonia, or mastoiditis. Mild blunt spinal trauma (a history elicited from 15%–35% of patients) may provide a devitalized site that is susceptible to transient bacteremia, although it is unclear whether this represents a true risk factor for the subsequent development of spinal epidural abscess. A primary source of infection is not identified in 20% to 40% of patients.[54–57,66] Infection of the epidural space has also been reported after penetrating injuries, extension of decubitus ulcers or paraspinal abscesses, back surgery, lumbar puncture, CT-guided needle biopsies, and administration of epidural anesthesia or analgesia.[70–75]

Many cranial epidural abscesses are polymicrobial in origin and include anaerobic gram-positive cocci, staphylococci, streptococci, and anaerobic gram-negative bacilli.[50] Anaerobic organisms are identified in cultures from many sinusitis-associated intracranial epidural abscesses.

The infecting microorganism in most (50%–90%) of the patients with spinal epidural abscess is *S. aureus*[54–67,70,76–78]; the proportion of cases caused by a methicillin-resistant strain has increased in recent years,[65] representing 41% of cases in one series[78] and 31% and 27% of *S. aureus* isolates in other studies.[52,53] Other isolates include aerobic and anaerobic streptococci (8%–17% of cases) and aerobic gram-negative bacilli (10%–17% of cases), especially *Escherichia coli* and *Pseudomonas aeruginosa*; *Pseudomonas* spp. are more commonly isolated from injection drug users.[50] In recent case series, investigators have noted an increased incidence of aerobic gram-negative bacilli, streptococci, and anaerobes. In one series from Taiwan, 28.5% of 42 cases of adult spinal epidural abscess were caused by gram-negative bacilli.[79] More than one microorganism is isolated in about 5% to 10% of cases.[54,57,58,69] Isolation of coagulase-negative staphylococci is associated with spinal procedures such as placement of catheters for analgesia, glucocorticoid injections, and surgery.[65] In addition to numerous case reports of patients with spinal epidural abscess caused by other bacteria, epidural infection can also be caused by *Nocardia* and *Actinomyces* spp.[80–82] and *M. tuberculosis*[83];

M. tuberculosis is a more common cause of spinal epidural abscess in regions with rising numbers of immunocompromised patients.[50] Fungi have also been isolated in patients with spinal epidural abscess.[58,84–86] Cases of *Exserohilum rostratum* epidural abscess were reported in association with contaminated epidural or paraspinal glucocorticoid injections of preservative-free methylprednisolone from a single compounding pharmacy (see Chapter 268).[87] Meningitis occurred in some cases either through the contaminated injection into the meninges or transdural spread from an epidural infection.

Clinical Features

The clinical presentation in patients with cranial epidural abscess may be insidious and overshadowed by the primary focus of infection (i.e., sinusitis or otitis media).[1,21,58] Fever and headache are the usual complaints, but the patient may otherwise feel well (leading to a delay in diagnosis) unless the clinical course is complicated (e.g., by development of subdural empyema, brain abscess, or meningitis). This insidious presentation occurs because the dura is closely opposed to the inner surface of the cranium, so the abscess usually enlarges too slowly to produce a sudden onset of major neurologic deficits (in contrast to the presentation in patients with cranial subdural empyema) unless there is deeper intracranial extension. Eventually, focal neurologic signs and seizures (either focal or generalized) may develop. About 45% of patients also have periorbital cellulitis or frontal edema.[50] In the untreated patient, papilledema and other signs of increased intracranial pressure develop as the abscess enlarges. Otitis media and mastoiditis can spread along the temporal bone to the petrous pyramid (petrositis) and cause a cranial epidural abscess, resulting in Gradenigo syndrome, which is characterized by involvement of cranial nerves V and VI, with unilateral facial pain and weakness of the lateral rectus muscle.[88]

The clinical findings in patients with spinal epidural abscess may develop within hours to days (usually after hematogenous seeding), or the course may be more chronic, over weeks to months (usually in association with vertebral osteomyelitis or another contiguous focus of infection).[54–66,89] The presentation in most patients with spinal epidural abscess progresses through the following four clinical stages: (1) backache and focal vertebral pain, with tenderness on percussion; (2) nerve root pain, manifesting with radiculopathy, paresthesias, or both; (3) spinal cord dysfunction, characterized by defects of motor, sensory, or sphincter function; and (4) paraplegia. It is important to note that the exact mechanism by which the epidural abscess causes neurologic injury is not totally clear. The widespread belief is that it is a combination of direct mechanical compression by an expanding abscess, with or without associated vascular damage that may be a consequence of compression of the intrinsic circulation of the spinal cord or local arteritis from the infection.[39,65,90,91] Pain is the most consistent symptom (70%–90% of cases) and is usually accompanied by local tenderness at the affected level. Fever is reported in 60% to 70% of patients, although it was noted in only 32% of patients in one study.[59] The specific neurologic signs depend on the level of spinal cord involvement. Because the neurologic manifestations are usually reversible before complete paralysis occurs, emergency imaging studies and intervention are necessary if the diagnosis is being considered (see later discussion). The classic triad of back pain, fever, and neurologic deficits is seen in the minority (8%–37%) of patients.[39,52,53,65,91] Patients with tuberculous spinal epidural abscess have a more insidious course than those with nontuberculous spinal epidural abscess owing to the slow proliferation of *M. tuberculosis* compared with the rapid proliferation of pathogens in nontuberculous disease.[50]

Diagnosis

MRI (with gadolinium enhancement) is the diagnostic procedure of choice for cranial epidural abscess. It demonstrates a superficial, circumscribed area of diminished intensity with pachymeningeal enhancement. The possibility of adjacent subdural empyema or other intracranial infection can also be assessed.[1,58] CT scanning may be used to image bone or if MRI is not available. MRI is superior to CT in the identification and delineation of the collections; gadolinium enhancement helps in diagnosis by showing a thickened dural surface, which helps to differentiate epidural abscesses from sterile effusions and hematomas, which may occur in patients after trauma or cranial surgery. Cranial epidural abscess can cross the midline of the brain, which helps to differentiate it from subdural empyema, and most do not involve the subjacent brain parenchyma.[50]

MRI is also the diagnostic procedure of choice in cases of suspected spinal epidural abscess (Figs. 91.3 and 91.4).[58–60,62,65,66,76] MRI is recommended over CT because it can better depict the spinal cord and epidural space in both sagittal and transverse sections and can also be used to identify accompanying osteomyelitis, intramedullary spinal cord lesions, and diskitis. The epidural mass may be isointense or hypointense on T1-weighted images and hyperintense on T2-weighted images. Gadolinium enhancement typically demonstrates linear enhancement surrounding nonenhancing purulent or necrotic matter. The enhancement may be either homogeneous or heterogeneous in the stage of epidural

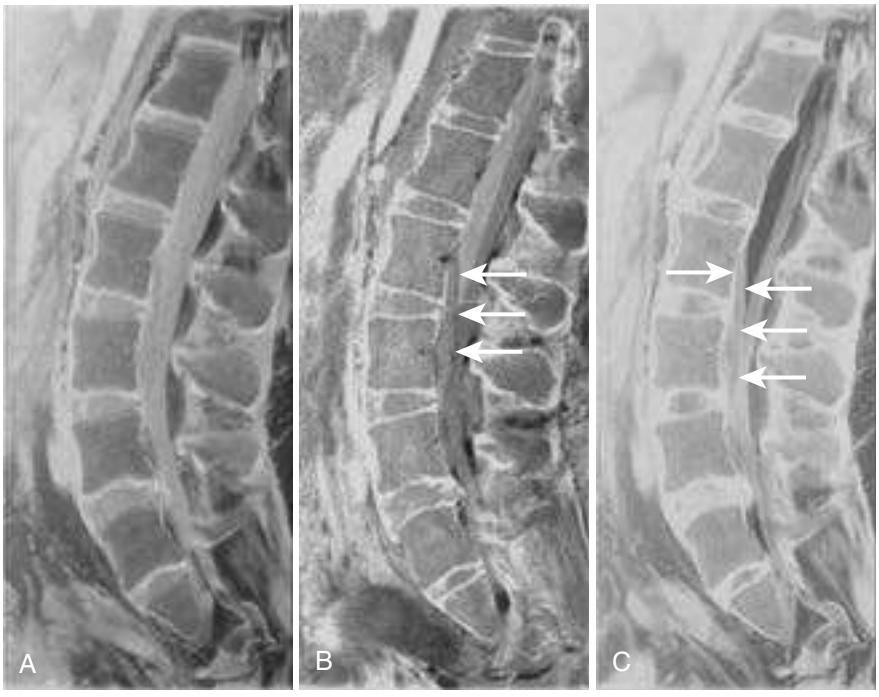

FIG. 91.3 Precontrast (A) and postcontrast (B) T1-weighted sagittal images demonstrating rim-enhancing soft tissue in the ventral and dural epidural space *(arrows),* consistent with epidural abscess. (C) T2-weighted sagittal image demonstrating compression of the thecal sac *(arrows). (Courtesy Stanley Lu, MD, Monmouth Medical Center, Long Branch, NJ.)*



FIG. 91.4 Magnetic resonance image of spine with arrow pointing to fungal epidural abscess after injection of contaminated corticosteroid.

plexus engorgement or phlegmon or peripherally with central fluid signal in the mature stage of a centrally pus-filled abscess.[92] MRI can also enable ready assessment of the response to therapy (see later discussion). CT myelography may be performed if MRI is unavailable or contraindicated.

Management and Outcome

The management of cranial epidural abscess requires a combined medical and surgical approach. Empirical antimicrobial therapy, after cultures have been obtained, for cranial epidural abscess is similar to that for cranial subdural empyema (see earlier discussion); therapy is usually continued for 3 to 6 weeks after surgical drainage or longer (6–8 weeks) if osteomyelitis is present.[21] Surgical drainage is also required; craniotomy or craniectomy is generally preferred over burr hole placement or aspiration of purulent material through the scalp.[1,15,21]

The principles of therapy for spinal epidural abscess are prompt surgical decompression, drainage of the abscess, and long-term antimicrobial therapy.[50,54–66] Empirical antimicrobial therapy for spinal epidural abscess, initiated after cultures have been obtained, must include a first-line antistaphylococcal agent (i.e., vancomycin pending organism identification and in vitro susceptibility testing) plus coverage for aerobic gram-negative bacilli (e.g., cefepime, ceftazidime, meropenem), especially for any patient with a history of spinal procedure or injection drug use. Once the infecting pathogen has been identified (through cultures of surgical drainage fluid, CT-guided aspiration specimens, or blood), antimicrobial therapy can be modified based on the results of in vitro susceptibility testing. Duration of antimicrobial therapy is usually 4 to 6 weeks; 6 to 8 weeks are recommended in patients with contiguous osteomyelitis because one study suggested that relapse was less likely in patients who received at least 8 weeks of antimicrobial therapy[93]; in some centers, further oral antimicrobial courses of 1 to 2 months are given.[50] Patients with tuberculous spondylitis and spinal epidural abscess must receive antituberculous therapy; a 12-month course has been recommended for patients with spinal epidural abscess.[50]

Patients with spinal epidural abscess and neurologic dysfunction require surgical drainage with decompression and drainage performed as a surgical emergency to minimize the likelihood of permanent neurologic sequelae.[54–66] Depending on location and extent of the epidural abscess, the surgical approaches include laminectomy, hemilaminectomy, or interlaminar fenestration.[66] Primary microsurgery with decompression at the site of maximal cord compression, with abscess and/or granulation tissue removal, has also been used successfully.[94] CT-guided aspiration can be used for culture if emergent surgery is delayed but is ineffective for drainage because of loculations in the pus. Endoscopic drainage has been used in a few cases. There have been no randomized, prospective trials that have compared the efficacy of antimicrobial therapy plus surgery with that of antimicrobial therapy alone in patients with spinal epidural abscess. However, in one literature review of 38 patients with spinal epidural abscess treated with antimicrobial therapy alone,[95] 23 patients recovered, 2 died, 1 worsened, and 12 remained the same or improved. In contrast, a retrospective analysis of 57 cases of spinal epidural abscess revealed that patients could be treated safely and effectively with prolonged intravenous antimicrobial therapy alone or in combination with percutaneous needle aspiration for culture irrespective of the neurologic abnormality at the time of presentation,[96] although the numbers of patients in each of the outcome subgroups were small. In another retrospective study of 52 patients with no evidence of systemic sepsis and with neurologic examination findings that were normal or stable (defined as radiculopathy or signs of partial cord compression present for more than 72 hours before admission and without deterioration),[97] 24 of 29 patients treated with medical therapy alone had a good or excellent neurologic outcome. Based on these studies, it would appear that antimicrobial therapy alone can be considered in patients who have localized pain or radicular symptoms without long-tract findings. However, these patients require frequent neurologic examinations and serial MRI studies to demonstrate resolution of the abscess. In addition, delayed surgery runs a high risk of rapid progression to permanent paraplegia,[91,98,99] and neurologic deterioration has been demonstrated in a substantial number of patients even while they were receiving appropriate antimicrobial therapy.[50,55,57,65,95] A number of studies have demonstrated that in many patients initially treated with only antimicrobial therapy, medical management failed and surgical intervention was required (ranging from 10% to 75%),[100–103] and some studies have raised concerns that patients treated initially with medical therapy and who later underwent surgery were weaker when compared with their findings at initial presentation.[100,101,104]

Emergency surgical decompression remains the procedure of choice and certainly should be performed in any patient with increasing neurologic deficit, persistent severe pain, or increasing fever or peripheral white blood cell count.[105] Surgery is not likely to be a viable therapeutic option in patients who have experienced complete paralysis for longer than 24 to 36 hours,[57,65] although some experts would perform surgical therapy in patients if complete paralysis has lasted less than 72 hours,[61] and surgical therapy may be required to treat the epidural infection and control sepsis. Nonsurgical management may also be appropriate for patients with panspinal infection or in those with a high surgical risk.[65] In one retrospective series of 104 patients with a diagnosis of spinal epidural abscess over a 10-year period, there were no statistically significant differences between the groups managed with or without surgery, although 30.6% of patients with a dorsal spinal epidural abscess were paraplegic or quadriplegic compared with only 7.3% of those with a ventral spinal epidural abscess ($P = .003$),[106] suggesting that the anatomy of the spinal epidural abscess might play a role in determining the treatment plan.[99]

In patients with cranial epidural abscess, factors associated with good outcomes are young age, absence of encephalopathy or severe neurologic deficit at presentation, and lack of comorbid conditions.[50] Poor prognosis is linked to herniation, delayed neuroimaging, and low clinical suspicion with early development of encephalopathy.

Mortality rates for spinal epidural abscess ranged from 5% to 32% in recent series.[a] Irreversible paralysis continues to affect 4% to 22% of patients.[53,65] The final neurologic outcome in patients with spinal epidural abscess is related to the severity of neurologic impairment before initiation of appropriate therapy.[57–60,65,66,70,107] Complete recovery with return of full neurologic function is most likely if neurologic signs are present for less than 24 hours before initiation of therapy; complete recovery is less likely if symptoms persist longer than 36 to 48 hours. A worse

[a]References 52, 53, 56, 57, 59, 60, 62, 69, 94.

outcome has been observed in patients with multiple medical problems, increasing age (older than 50 years), previous spinal surgery, cervical or thoracic abscess location, sepsis at presentation, leukocytosis (>14,000/mm^3 at admission), thrombocytopenia (<100,000/mm^3), elevated serum C-reactive protein (CRP) concentrations during the second postoperative week, isolation of methicillin-resistant staphylococci, and degree of thecal sac compression (>50% spinal canal involvement).[50,59,70,78] In one review of spinal epidural abscess in patients on hemodialysis, there was a higher mortality rate and patients were less likely to improve neurologically when compared with nonhemodialysis patients.[108] In a study of spinal epidural abscess patients undergoing surgical drainage along with antimicrobial therapy, leukocytosis, elevated CRP, poor glycemic control, and motor deficits at the time of operation were all found to have a strong influence on motor function improvement after surgical treatment.[109] The final neurologic outcome and functional capacity should be assessed at least 1 year after treatment because, until then, patients may continue to regain some neurologic function.[65]

SUPPURATIVE INTRACRANIAL THROMBOPHLEBITIS

Epidemiology and Etiology

Suppurative intracranial thrombophlebitis includes both venous thrombosis and suppuration. It may begin within veins or venous sinuses, or it may occur after infection of the paranasal sinuses, middle ear, mastoid, face, or oropharynx.[110–112] Additional vessels may be involved by propagation or discontinuous spread. Suppurative thrombophlebitis also occurs in association with epidural abscess, subdural empyema, or bacterial meningitis. Occasionally, there is metastatic spread from a distant site of infection.

The development of septic intracranial venous sinus thrombosis depends on the close proximity of various structures to the dural venous sinuses. The usual predisposing condition for septic cavernous sinus thrombosis is paranasal sinusitis (especially of the ethmoid or sphenoid sinuses).[110,111,113,114] Infections of the face and mouth are less commonly the primary sources of infection in the antimicrobial era. Otitis media and mastoiditis are infections that are associated with lateral (transverse) sinus thrombosis[110,115–119] and infection of the superior and inferior petrosal sinuses; the lateral sinus is a more common site of thrombosis in children.[120] Infections of the face, scalp, subdural space, epidural space, and meninges are associated with septic thrombophlebitis of the superior sagittal sinus. In all these infections, the likely microorganisms depend on the associated primary condition. The most important infecting microorganism in septic cavernous sinus thrombosis is *S. aureus,* which is isolated in 60% to 70% of patients.[110,113,114] Streptococci, especially the *Streptococcus anginosus* group *(Streptococcus milleri)*, are also important agents.[121,122] However, the likely infecting bacterial pathogens depend on the pathogenesis of infection: staphylococci, streptococci, gram-negative bacilli, and anaerobes if the antecedent condition is sinusitis, and predominantly *S. aureus* in the case of facial infections. *S. aureus,* streptococci, and *E. coli* are commonly reported as pathogens in patients with lateral sinus thrombosis,[110,116] although in one study, *Fusobacterium necrophorum* was the microorganism isolated in four of six children with lateral sinus thrombosis.[117] In another study of patients with lateral sinus thrombosis secondary to chronic ear infection,[116] all patients had mixed infections, and *Bacteroides fragilis* was isolated in five of six patients. Lateral sinus thrombosis complicating mastoiditis continues to be a problem in the postantibiotic era.[118] Venous sinus thrombosis may also be seen in patients with rhinocerebral mucormycosis[123] or invasive aspergillosis.[124]

Clinical Features

The clinical findings in patients with suppurative intracranial thrombophlebitis depend on the sinus involved and the presence of concomitant central nervous system infection (e.g., meningitis, brain abscess, subdural empyema).[110,111] The two cavernous sinuses are positioned on either side of the sella turcica; they are connected by anterior and posterior intercavernous sinuses that encircle the pituitary gland.[113,114] The horizontal segment of the internal carotid artery and cranial nerve VI run through the lumen, and cranial nerves III and IV and the ophthalmic and maxillary branches of cranial nerve V run through the outside layers of the lateral walls of the cavernous sinuses (Fig. 91.5). The clinical manifestations may relate to damage to the nerves that traverse the cavernous sinuses. The most common symptoms in patients with septic cavernous sinus thrombosis are headache, fever, and diplopia[110,122]; headache is more common if the antecedent condition is sinusitis rather than a facial infection. Other symptoms include blurred vision, facial pain, and ptosis.[121,122] Signs include fever (present in >90% of patients), proptosis, chemosis, periorbital edema, and weakness of the extraocular muscles (secondary to involvement of cranial nerves III, IV, and VI).[110,111,113,114] Because cranial nerve VI is the only cranial nerve that traverses the interior of the cavernous sinus, a lateral gaze palsy may be an early neurologic finding. Papilledema or venous engorgement is observed in 65% of patients, and a change in mental status in 55%. Meningismus is present in up to 40% of patients, usually secondary to retrograde spread of the thrombophlebitis. Less than 50% of patients have dilated or sluggishly reactive pupils, decreased visual acuity (which may progress to blindness), and dysfunction of cranial nerve V. As infection spreads to the opposite cavernous sinus through the intercavernous sinuses, findings are duplicated in the opposite eye, usually within 24 to 48 hours after the initial unilateral periorbital edema. Carotid artery inflammation and cortical vein thrombophlebitis are particularly serious complications of septic cavernous sinus thrombosis because they carry the risk of cerebral hemorrhagic infarction.[110,114]

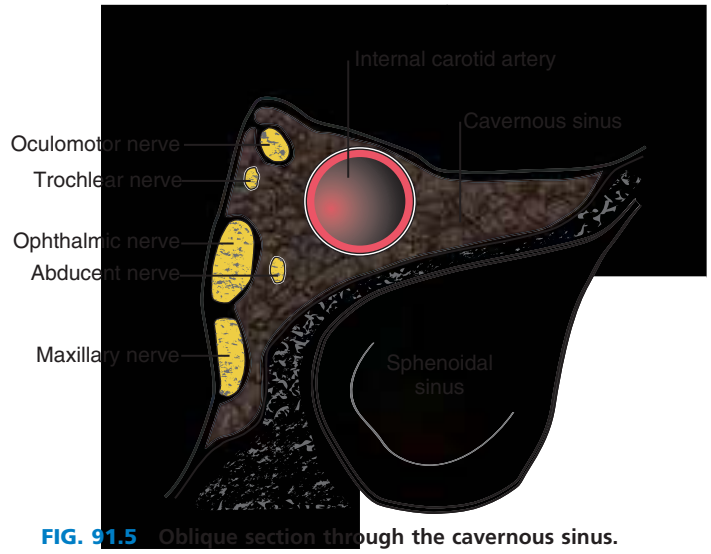

FIG. 91.5 Oblique section through the cavernous sinus.

Persons with septic cavernous sinus thrombosis may present with either acute or chronic illness.[110,111] In the acute presentation (usually secondary to facial infection), the time between primary infection and cavernous sinus thrombosis is short (usually <1 week), and the patient presents in a significantly toxic state, with rapid development of the signs and symptoms described earlier; there is also rapid progression to bilateral eye signs. In contrast, in the more indolent form of cavernous sinus thrombosis, which usually occurs secondary to dental infection, otitis media, or paranasal sinusitis, the orbital manifestations are often unimpressive and involvement of the contralateral eye is a late and inconsistent finding.

The presentation in patients with septic lateral sinus thrombosis is typically more gradual, and patients complain predominantly of headache (>80% of cases); photophobia, earache, vomiting, and vertigo may also occur because otitis media is a common predisposing condition.[110,116–118,125,126] Fever and abnormal ear findings are observed in most patients (79% and 98%, respectively), and palsy of cranial nerve VI, facial pain and altered facial sensation, papilledema, mastoid tenderness, and nuchal rigidity may also be present. Posterior auricular swelling and pain (Griesinger sign) are present in almost 50% of cases; a common complication is otitic hydrocephalus. In one study of 13 patients, the majority exhibited cranial neuropathies and raised intracranial pressure.[127]

The triad of suppurative otitis, cranial nerve VI palsy, and cranial nerve V irritation resulting in retro-orbital and temporoparietal pain (Gradenigo syndrome), which suggests the presence of inflammation along the petrous part of the temporal bone, should also raise the suspicion of lateral (transverse and sigmoid) sinus thrombosis.[128] Inflammation can spread from the petrous bone to the cavernous sinus.

Thrombosis of the superior sagittal sinus produces an abnormal mental status, motor deficits, nuchal rigidity, and papilledema.[110] Seizures occur in more than half of these patients. The majority of cases occur in the setting of bacterial meningitis, in which the onset may be fulminant. Patients with sinusitis as a predisposing condition tend to have a subacute onset of symptoms.

Diagnosis

Laboratory studies are usually nonspecific in suppurative intracranial thrombophlebitis.[110,111] Lumbar puncture demonstrates a mild pleocytosis (mononuclear, neutrophilic, or mixed) and an elevated CSF protein concentration (consistent with a parameningeal focus of infection), although there may be findings of frank meningitis in patients with septic thrombosis of the superior sagittal sinus, and the causative microorganism may be isolated on culture of CSF. Blood cultures may be positive, especially in patients with a rapidly progressive course. Chest radiographs may reveal evidence of septic pulmonary emboli after propagation of the thrombus into the inferior petrosal sinus and jugular vein. Sinus radiographs may document involvement of the paranasal sinuses, although conventional radiographs are inferior to CT or MRI in the detection of sphenoid sinusitis.

The noninvasive diagnostic procedures of choice for suppurative intracranial thrombophlebitis are MRI (Figs. 91.6 to 91.8) and magnetic resonance venography.[36,128,129] In septic cavernous sinus thrombosis, the lateral wall of the cavernous sinus bulges laterally, a subdural empyema may be adjacent laterally, the superior ophthalmic vein may be distended and thrombosed, the carotid artery is narrowed in the siphon, and cerebral thrombosis from emboli may be found. The flow void signal, a dark area on gadolinium-enhanced T1 weighting, is absent in a thrombosed vessel (see Fig. 91.6). Magnetic resonance venography is extremely useful in showing detailed venous anatomy and demonstrating an area of decreased or absent signal in the area of the thrombus.[128] In patients unable to undergo MRI, CT venography appears to have a degree of sensitivity and specificity equaling that of magnetic resonance venography.[128,129] High-resolution CT (with and without intravenous contrast) with a slice thickness of 3 mm or less may also show findings of venous sinus thrombosis.[130] In patients with septic cavernous sinus thrombosis, CT usually reveals unilateral or bilateral multiple irregular filling defects in the enhancing cavernous sinus, with or without orbital inflammatory change, or enlargement or expansion of the cavernous sinus with lateral wall flattening or convexity rather than the normal concavity. An additional benefit of both MRI and CT is the ability to fully evaluate the paranasal sinuses and to provide information concerning subdural and epidural infection, cerebral infarction, cerebritis, hemorrhage, and cerebral edema.

Management and Outcome

Selection of appropriate antimicrobial therapy for suppurative intracranial thrombophlebitis depends on the antecedent clinical condition; the likely microorganisms are similar to those observed in cranial subdural empyema and cranial epidural abscess (see earlier discussions). If the antecedent clinical condition is paranasal sinusitis, empirical antimicrobial therapy should be directed toward staphylococci, streptococci, aerobic gram-negative bacilli, and anaerobes. In septic cavernous sinus thrombosis, an antistaphylococcal agent should always be included because of the high likelihood of isolation of *S. aureus*. Vancomycin is recommended empirically, pending results of in vitro susceptibility testing. An appropriate empirical regimen is vancomycin, metronidazole, and a third- or fourth-generation cephalosporin, pending culture results and in vitro susceptibility testing. Intravenous antimicrobial therapy is usually continued for at least 3 to 4 weeks, but the duration needs to be individualized depending on the clinical response. The role of corticosteroids is not well established in the management of septic cerebral venous thrombosis; although they may help in decreasing inflammation and swelling, this use is not well supported by evidence.[129]

Surgery may also be necessary for optimal treatment of septic intracranial thrombophlebitis.[110,111,113,114] Surgical therapy for infected sinuses is necessary if antimicrobial therapy alone is ineffective; this is especially important in patients with cavernous sinus thrombosis secondary to sphenoid sinusitis. In fact, some authors have recommended operative intervention for all patients who develop cavernous sinus thrombosis as a complication of sinusitis. Mastoid surgery is an important adjunct in patients with septic lateral sinus thrombosis,

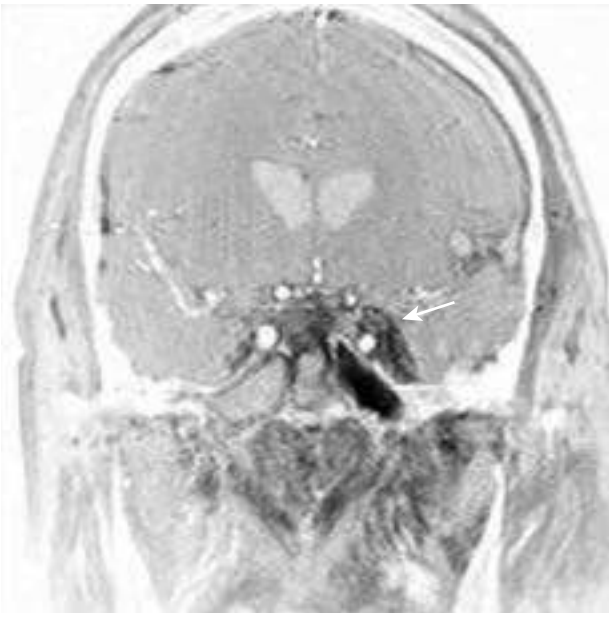

FIG. 91.6 Magnetic resonance image of cavernous sinus thrombosis. Arrow points to contrast-enhanced cavernous sinus with a dark circle of a flowing carotid artery (flow void signal). The dark white area below is the inflamed sphenoid sinusitis, which caused the cavernous sinus thrombosis.



FIG. 91.7 Magnetic resonance image of superior sagittal sinus thrombosis. There is increased signal with the vessel on the T2-weighted image *(arrowheads)*, indicating the presence of thrombus. *(Courtesy Dr. Wayne Davis, University of Utah.)*

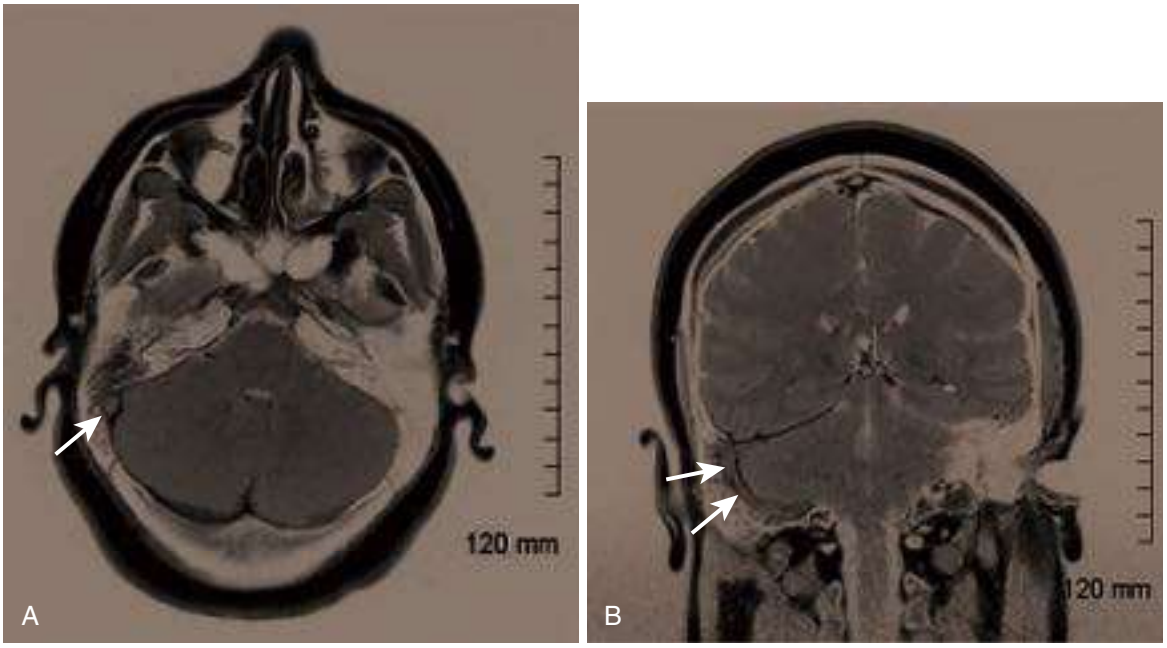

FIG. 91.8 Gadolinium-enhanced T1-weighted images in the axial (A) and coronal (B) planes demonstrating extensive filling defects of the right sigmoid sinus *(arrows)* representing thrombosis and associated with adjacent right mastoiditis. *(Courtesy Stanley Lu, MD, Monmouth Medical Center, Long Branch, NJ.)*

although the extent of mastoid surgery and the management of the thrombosed sinus remain controversial.[118,125,126,131–134] Internal jugular vein ligation has been used for lateral sinus vein thrombosis, more commonly in the preantibiotic era,[116,131,132] but the efficacy of this procedure is poorly defined and it is not part of routine management. Some authors have recommended ligation of the internal jugular vein for patients who develop septic embolization despite antimicrobial therapy and surgical drainage.[117,133,135] Surgical therapy may also be required for other infections (e.g., dental abscesses, complicating brain abscess, subdural empyema).

Although anticoagulation is recommended in patients with aseptic cerebral venous thrombosis,[136–138] the use of anticoagulation in suppurative intracranial thrombophlebitis is controversial. There is support in the literature for the use of anticoagulation (i.e., low-molecular-weight heparin) to prevent the spread of the thrombus from the cavernous sinus to other dural venous sinuses and cerebral veins.[110,111,113,114] Retrospective evidence indicates that anticoagulation, in combination with antimicrobial therapy, reduces mortality and is most beneficial if given early (within 7 days after hospitalization) in the treatment of cavernous sinus thrombosis.[110,139] In a recent review of patients with cavernous sinus thrombosis, anticoagulation was mentioned in the management of 41 of 88 patients[140]; compared with patients who did not undergo anticoagulatation, a considerably greater number of patients made a full recovery (53.6% vs. 32%) and fewer patients died (12% vs. 28%), although there was no difference in morbidity. However, it must be recognized that anticoagulation carries the risk of intracranial hemorrhage from sites of cortical venous infarction or from sites on the intracavernous walls of the carotid artery. Some authors have not recommended anticoagulation in patients with septic lateral sinus or superior sagittal sinus thrombosis because of the high number of venous hemorrhagic infarcts observed postmortem[110]; most of these infections can also be controlled with antimicrobial therapy and surgery, and the use of anticoagulation has not been shown to be beneficial.[131,132] However, in one study of 7 patients with lateral sinus thrombosis, 6 of whom received anticoagulation for an average of 24 months, only 1 had a complication of epistaxis, which resolved with pressure.[126] In another study of patients with lateral sinus thrombosis, anticoagulants were used in 4 patients with thrombus progression into the internal jugular vein and transverse sinuses without evidence of complications.[119] Despite these findings, most authors agree that there is no place for anticoagulation in the management of septic lateral sinus thrombosis.[133] In the absence of prospective data, anticoagulation should be considered in the treatment of septic cavernous sinus thrombosis unless there are contraindications or documented hemorrhagic intracranial complications on neuroimaging studies. Some practitioners use anticoagulation in cavernous sinus thrombosis only as an adjunct to antimicrobial therapy in patients with a deteriorating clinical condition.[114] The duration of anticoagulation has varied from 2 weeks to several months; some have continued anticoagulation until complete thrombus resolution has been documented on neuroimaging studies.

Before the availability of antimicrobial therapy, suppurative intracranial thrombophlebitis carried a mortality rate of 80% to 100%. Since 1940, mortality rates have ranged from 0% to 16% in patients with septic lateral sinus thrombosis[116] and from 13% to 30% in patients with septic cavernous sinus thrombosis,[110,113] although in one series of 14 pediatric patients with septic cavernous sinus thrombosis, 79% died despite parenteral antimicrobial therapy.[141] Mortality in septic sagittal sinus thrombosis is much higher, reported as 78% in one review.[110] Among patients who survive their episode of septic cavernous sinus thrombosis, up to 50% are left with long-term cranial nerve deficits,[110,114] although they may continue to improve for several months after completion of therapy. Partial or complete visual loss is seen in 7% to 22% of patients. Patients should be observed for several months after completion of antimicrobial therapy because isolated relapses have been documented within 6 weeks after apparent clinical resolution, and intracranial abscesses have been demonstrated as long as 8 months later.[114]

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The complete reference list is available online at Expert Consult.

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92 Cerebrospinal Fluid Shunt and Drain Infections

Adarsh Bhimraj and Allan R. Tunkel

SHORT VIEW SUMMARY

Definition

- Ventriculoperitoneal (VP) shunt infections can be either superficial, involving the skin and soft tissue adjacent to the shunt valve, reservoir, or tubing, or it can be a deeper infection, involving the cerebral ventricles proximally or the peritoneum distally.
- Cerebrospinal fluid (CSF) drain infections can be subcutaneous tunnel infections, catheter exit site infections, or ventriculitis.

Epidemiology

- The case incidence of CSF shunt infection (i.e., the occurrence of infection in any given patient) has ranged from 5% to 41%, although usually it is 4% to 17%.
- The operative incidence (i.e., the occurrence of infection per procedure) has ranged from 2.8% to 14%, although usually it is less than 6%.
- In patients with external ventricular drains, the incidence of ventriculitis ranges from 10.6 to 11.4 per 1000 catheter-days.

Microbiology

- Staphylococcal species account for the majority of isolates in patients with CSF shunt infections, with *Staphylococcus epidermidis* most frequently isolated (47%–64% of infections), followed by *Staphylococcus aureus* (12%–29% of infections).
- The most common isolated gram-negative species are *Escherichia coli, Klebsiella, Proteus*, and *Pseudomonas*; cases of *Acinetobacter* meningitis have also been reported in patients with VP shunts.
- In recent years an increasing prevalence of diphtheroids, principally *Cutibacterium acnes* (formerly *Propionibacterium acnes*) has been found in CSF shunt infections.

Diagnosis

- The diagnosis is established by either direct culture of the CSF obtained by shunt aspiration or by culture of the proximal shunt components if the shunt is explanted.
- CSF culture from the shunt, reservoir, or drain is the most important test to establish the diagnosis of infection; CSF aerobic and anaerobic cultures should be obtained and held for 7 to 10 days to detect fastidious organisms.
- In patients with lumbar drains or externalized ventricular drains, definite infection is defined as a positive CSF culture (obtained from the ventricular or lumbar catheter) associated with CSF pleocytosis.

Therapy

- The principles of antimicrobial therapy for CSF shunt infections are generally the same as those for acute bacterial meningitis; the agent selected must penetrate the central nervous system and attain adequate CSF concentrations.
- Empirical therapy with intravenous (IV) vancomycin plus either cefepime, ceftazidime, or meropenem is appropriate.
- Optimal therapy of an infected CSF shunt is an initial IV antimicrobial, followed by removal of all components of the infected shunt, insertion of a fresh ventricular catheter, and a period of external drainage with continued systemic antibiotics until the drain culture is negative. Later the drain is removed and a new shunt is placed.
- Direct instillation of antimicrobial agents into the lateral ventricle or, in the case of lumbar shunts, into the lumbar thecal sac may be necessary in patients with shunt infections that are difficult to eradicate with IV antimicrobial therapy and shunt removal or when the patient is unable to undergo shunt replacement. Antimicrobials in the absence of shunt removal are seldom effective.

Prevention

- There is evidence to support the use of periprocedural prophylactic antimicrobial administration for patients undergoing CSF shunt insertion and placement of external ventricular drains.
- Use of antimicrobial-impregnated CSF shunts and CSF drains appears to be safe and possibly effective in prevention of ventriculitis. Detailed operating room procedures have been advocated to decrease postoperative infections.
- Use of a standardized protocol and reducing variation by adherence to a common protocol are effective at reducing CSF shunt and CSF drain infection rates.

In the United States hydrocephalus accounts for approximately 70,000 annual hospital admissions[1] and is treated by diverting the cerebrospinal fluid (CSF) either to another body cavity (via CSF shunt) or externally (via CSF drain). CSF shunts are considered to be permanent catheters in which the proximal end of the shunt is in the cerebral ventricle, an intracranial cyst, or the lumbar subarachnoid space; the distal end usually terminates in the peritoneal, pleural, or vascular space (Fig. 92.1). Most neurosurgeons prefer the peritoneal cavity as the shunt terminus (i.e., a ventriculoperitoneal [VP] shunt) rather than the vascular space (ventriculoatrial shunt) because VP shunts require fewer revisions, are easier to place and revise, and have fewer serious complications.[2] Part of the system (as a separate integrated component) is a pressure-regulating valve that is usually placed just outside the skull or as an integral part of the distal tubing.[4] Reservoirs for intermittent percutaneous access can also be added to the system or incorporated into the valve assembly. Additional hardware includes antisiphon valves and various connectors, allowing interconnection of more than one catheter or device. Programmable valves that can change the opening pressure by means of an external magnet are often used but may need to be reset after a magnetic resonance imaging (MRI) scan.

CSF drains are temporary catheters that divert the fluid externally.[2,5] The proximal end is either in the cerebral ventricle (ventricular drain) (see Fig. 92.1) or in the lumbar subarachnoid space (lumbar drain). Ventricular drains are also useful for the temporary management of patients with elevated intracranial pressure secondary to acute hydrocephalus caused by intracranial hemorrhage, neoplasms obstructing the CSF circulation, or trauma. Ventricular drains are usually tunneled, in which a subcutaneous tract is created between the bur hole and the catheter exit site. Lumbar drains are used in patients with CSF leaks after neurosurgery. The distal end of the catheter is connected to a collecting system, which has a drip chamber, sampling and injection ports (used to obtain CSF and inject medications), and a collection bag.[2,5] The drip chamber is positioned so that the drop-forming end is a certain distance, such as 10 cm, above the right atrium or, in recumbent

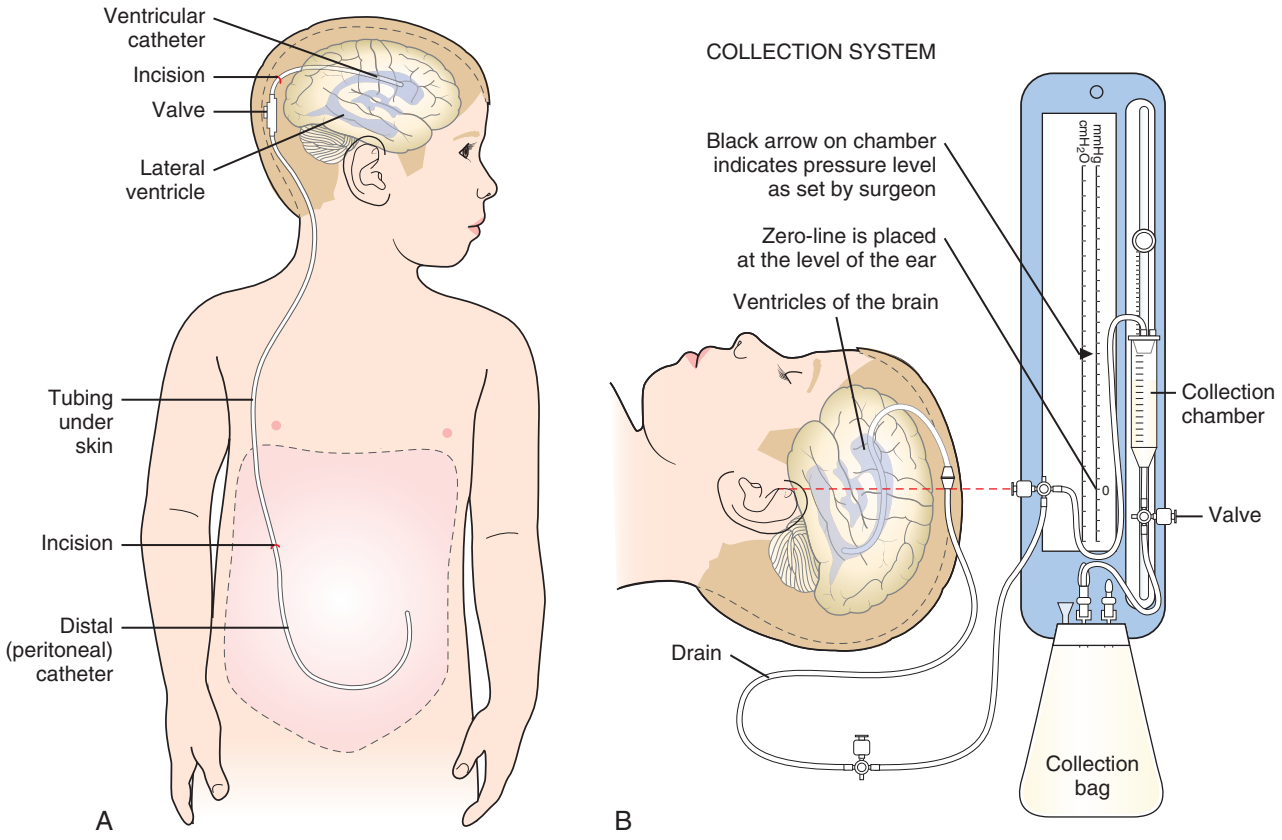

FIG. 92.1 (A) Ventricular shunt for hydrocephalus. (B) Typical arrangement of external ventricular drain.

patients, above the external auditory canal (see Fig. 92.1). Overdraining can result in a subdural effusion, possibly rupturing a small blood vessel crossing the pia arachnoid.

Both CSF shunts and CSF drains are prone to infection. VP shunt infections can be either superficial, involving the skin and soft tissue adjacent to the shunt valve or reservoir, or can be a deeper infection involving the cerebral ventricles proximally or the peritoneum distally. Ulceration of skin over the shunt valve can introduce air or organisms into the lateral ventricle. CSF drain infections can be tunnel infections, catheter exit site infections, or ventriculitis. In the following sections, we review the epidemiology, etiology, clinical features, and management approach to CSF shunt and CSF drain infections.

EPIDEMIOLOGY

The case incidence of CSF shunt infection (i.e., the occurrence of infection in any given patient) has ranged from 5% to 41% in various series,[2] although the incidence is usually in the range of 4% to 17%.[6–13] The operative incidence (i.e., the occurrence of infection per procedure) has ranged from 2.8% to 14%,[2] although most series have generally reported operative infection rates of less than 6%.[14–16] In four prospective, randomized trials in pediatric patients with CSF shunt placement or shunt revision, infection rates ranged from 8% to 11%.[17–20] In another large retrospective review of 1173 children shunted for hydrocephalus from 1985 to 2004, 158 patients presented with a total of 190 episodes of shunt infection, for a case incidence of 13.6% and an operative incidence of 5.9%.[12] In a study by the Canadian Nosocomial Infection Surveillance Program in patients with CSF shunt-associated infections, the infection rate was 4.1% during the first year after placement.[21] In another large study of cases reported to the Pediatric Health Information System longitudinal administrative database from 41 children's hospitals, which included 7071 children with uncomplicated CSF shunt placements between January 1, 2001 and December 31, 2005, the unadjusted 24-month CSF shunt infection rate was 11.7% per patient and 7.2% per procedure.[22]

TABLE 92.1 Factors Associated With an Increased Risk of Cerebrospinal Fluid Shunt Infection

Premature birth
Younger age
Previous shunt infection
Cause of hydrocephalus[b]
Experience of the neurosurgeon
Number of people traversing the operating theater
Exposure to perforated surgical gloves[c]
Intraoperative use of the neuroendoscope
Length of the shunt procedure
Insertion of catheter below T7 in ventriculoatrial shunting[d]
Patient skin preparation
Shaving of skin
Exposure of large areas of patient's skin during the procedure
Shunt revision[e]

[a]Not all studies have found all these factors to be associated with an increased risk of cerebrospinal fluid shunt infection.
[b]Purulent meningitis, hemorrhage, myelomeningocele.
[c]Double gloving when handling implantable devices may reduce the risk of infection.
[d]Presence of foreign-body irritation on the tricuspid valve, with thrombus formation and subsequent infection during bacteremia.
[e]The risk may be especially high in patients undergoing three or more revisions.

A number of factors have been reported to be associated with an increased risk of CSF shunt infection (Table 92.1). Children are more likely than adults to acquire shunt infection. In one study that included 7071 children,[22] factors associated with infection were young age, female sex, African-American race, public insurance, etiology of intraventricular hemorrhage, respiratory complex clinical condition, subsequent revision procedure, hospital volume, and surgeon case volume. A higher case incidence rate may be related to the infection rate with succeeding shunt revisions.[23] The infection rate may be especially high in those undergoing three or more revisions,[24] although that has not been observed in all studies.[9] In patients undergoing revision after treatment for an

infected CSF shunt, the operative incidence of infection is considerably higher (12%–26%).[25,26] The same microorganism has been cultured at least one-half to two-thirds of the time, suggesting that the initial infection was not adequately treated. Premature birth, previous shunt infection, and intraoperative use of the neuroendoscope were identified as independent risk factors in a recent study of patients with VP shunt infections.[27] In a study of 675 children who had an initial CSF shunt infection (treated by shunt removal/new shunt placement, externalization, nonsurgical management, or removal with no shunt placement), the 6-month reinfection rate was 14.8%, and the median time to infection was 21 days[28]; reinfection was associated with nonsurgical management.

In a large meta-analysis of 35 studies of patients with CSF drains, which yielded 752 infections from 66,706 catheter-days of observation, the overall pooled incidence of external ventricular drain-related CSF infection was 11.4 per 1000 catheter-days (95% confidence interval [CI], 9.3 to 13.5); for high-quality studies, the incidence was 10.6 per 1000 catheter-days (95% CI, 8.3 to 13).[29] Factors associated with an increased risk of infection are intraventricular hemorrhage, subarachnoid hemorrhage, cranial fracture with CSF leak, catheter irrigation, craniotomy, and duration of catheterization. Although controversy exists regarding the relationship between the duration of catheterization and risk of infection, most studies consider extended catheter duration, usually exceeding 5 days, to be an important risk factor for subsequent infection.[5] In a recent prospective multicenter cohort study of external ventricular drain (EVD) insertions in the United Kingdom and Ireland, the 30-day EVD infection rate was 9.3%; a regression analysis in the same study showed that duration of EVD placement and frequent CSF sampling were independently associated with an increased risk of ventriculitis.[30]

ETIOLOGY

The etiologic agents causing both CSF shunt and CSF drain infections are fairly similar; the most common microorganisms are those that colonize the scalp and skin of the back, or those that are present in the health care environment. The etiologic agents identified in CSF shunt infections are shown in Table 92.2.[2,13,31,32] Staphylococcal species account for the majority of isolates in patients with CSF shunt infections, with *Staphylococcus epidermidis* most frequently isolated (47%–64% of infections), followed by *Staphylococcus aureus* (12%–29% of infections). The most common isolated gram-negative microorganisms are *Escherichia coli, Klebsiella, Proteus*, and *Pseudomonas*; cases of *Acinetobacter* meningitis have also been reported in patients with VP shunts.[33–35] The usual etiologic agents of community-acquired bacterial meningitis (i.e., *Haemophilus influenzae, Streptococcus pneumoniae*, and *Neisseria meningitidis)* are only isolated in approximately 5% of CSF shunt infections. In 10% to 15% of cases more than one microorganism is isolated, usually including a *Staphylococcus* strain or *E. coli.* In recent years an increasing prevalence of diphtheroids, particularly *Cutibacterium acnes* (formerly *Propionibacterium acnes)* has been found in CSF shunt infections. However, it may be that the incidence is not increasing but, rather, that the etiologic diagnosis was not made because isolation of these organisms was not considered to be significant, or adequate culture techniques (i.e., anaerobic culture and prolonged incubation) were not used.[32,36] Fungal shunt infections are rare, although the frequency of *Candida* shunt infections has increased in recent years (ranging from 6%–17% in one review).[37] Fungal shunt infections may be seen in patients receiving broad-spectrum antimicrobial therapy, corticosteroids, and hyperalimentation; in those with indwelling intravascular catheters; or in those who are immunocompromised. A previous ventricular shunt may also increase the risk of subsequent *Candida* meningitis.[38]

Different microorganisms may be isolated depending on the type of shunt. Shunts terminating in the peritoneal cavity may have a greater risk of infection with gram-negative organisms[23,39]; mixed infections may be seen when the catheter has perforated a hollow viscus.[40,41] Although some investigators have found an increased prevalence of gram-negative shunt infections in patients with a peritoneal termination,[42] others have found no difference in the bacterial spectrum in those with VP or ventriculoatrial shunts.[43]

In patients with VP shunt-related peritonitis and pseudocysts, the pathogenic organisms are mostly gram positives, especially *Staphylococcus* spp., and this might be from transcutaneous spread along the distal catheter, rather than from gut perforation[44,45] (Fig. 92.2).

Lumboperitoneal shunts have distributions of infecting organisms similar to those originating in the ventricles.[42,43] Gram-positive cocci account for the majority of cases of ventriculitis associated with CSF drains, but infections caused by gram-negative bacilli, gram-positive bacilli, fungi, and antimicrobial-resistant bacteria have also been described.[5,46] At certain institutions the incidence of gram-negative CSF drain ventriculitis was higher. In an Australian study gram-negative infections accounted for 71% of the cases, with 48% caused by *Acinetobacter* spp.[47] Similarly, in a study from Singapore, 50% of the infections were caused by *Acinetobacter* spp.[48] These studies emphasize the importance of considering local or institutional microbiology and antibiograms in choosing empirical antimicrobial therapy.

PATHOGENESIS

There are four mechanisms by which CSF shunts may become infected.[2] The first mechanism is retrograde infection from the distal end of the shunt from a peritonitis, although this is rare. For example, bowel perforation can lead to distal catheter contamination. Retrograde infection is the most likely mechanism of infection of CSF drains. Microorganisms may enter the device by tracking from the exit site alongside the device, gaining access to the fluid column that drains CSF, or they may be introduced from flushing the tubing to maintain tubular function.

The second mechanism is through the skin, such as inserting a needle into the reservoir or the shunt to culture the CSF or assess patency, injecting drug into a ventricular reservoir, and after breakdown

TABLE 92.2 Bacterial Etiologic Agents in Cerebrospinal Fluid Shunt Infections

ETIOLOGIC AGENT	INCIDENCE (%)
Staphylococci[a]	55–95
Gram-negative bacteria	6–20
Streptococci	8–10
Diphtheroids[b]	1–14
Anaerobes	6
Mixed cultures	10–15

[a]The majority are caused by coagulase-negative staphylococci.
[b]Including *Cutibacterium acnes,* formerly *Propionibacterium acnes.*

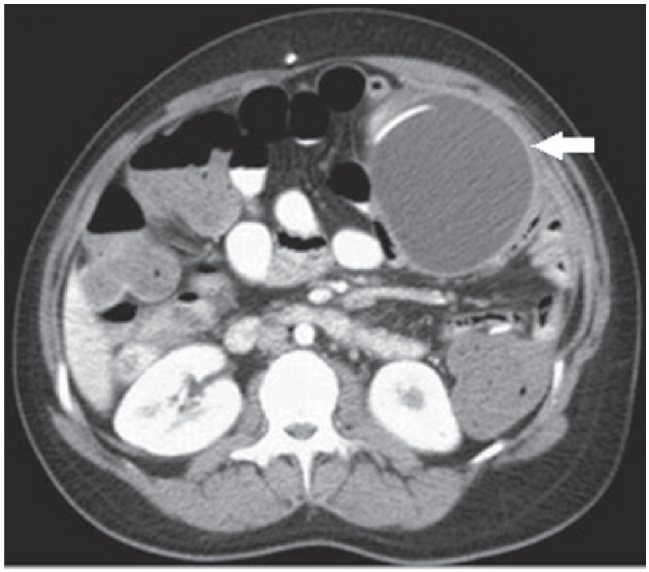

FIG. 92.2 Infected ventriculoperitoneal shunt–related pseudocyst (white arrow points to pseudocyst). *(From Gayer G, Lubner MG, Bhalla S, Pickhardt PJ. Imaging of abdominal and pelvic surgical and postprocedural foreign bodies.* Radiol Clin North Am. *2014;52:991–1027.)*

of the skin overlying the shunt.[2] The infection may be surgically acquired (i.e., when the incision fails to heal) or because the patient scratches the open wound, often seen in infants and small children. In premature infants with thin skin or in debilitated or immobile patients, a decubitus ulcer may develop over the shunt. Infection of tissues near the shunt site may also lead to direct inoculation of microorganisms.

A third mechanism is hematogenous seeding. Patients with ventriculoatrial shunts who have a foreign body (i.e., the catheter) in the vascular system are at continued risk of infection from bacteremia (with a retrograde infection). The susceptibility of nonvascular shunts to an infection that is spread hematogenously is less clear, although the likelihood is very low.

The fourth and most frequent mechanism is colonization of the shunt at the time of surgery.[2] This mechanism is suggested by the timing of most shunt infections, usually within the first month of operation, and by the microorganisms that are isolated. In one study in adults with CSF shunt-associated infections, 62% occurred within the first month after shunt surgery, and 72% were acquired intraoperatively.[13] At the time of implantation, direct exposure and handling of the shunt can allow bacterial contamination. One prospective observational study identified holes in the surgical gloves, combined with digital shunt handling by the surgical team, as a possible risk factor.[25] Instituting the practice of double gloving led to a reduction in shunt infection rates compared with historical controls.[49] However, in multiple studies using surveillance culture techniques, less than 50% of the microorganisms cultured from the wound or infected shunt could be traced directly to the patient,[2,40,50] suggesting that there are other factors that affect colonization and subsequent CSF shunt infection. Another study found that, in 13 shunt infections that occurred out of 108 shunt placements, only one CSF shunt infection had an isolate that was similar to the perioperative surgical site culture,[51] suggesting that contamination at the time of surgery may be an uncommon cause of shunt infection.

CLINICAL FEATURES

The clinical features of CSF shunt infection can be quite variable and depend on the pathogenesis of infection, organism virulence, and type of shunt.[2,13,24,42,43] Unlike the organisms that cause community-acquired bacterial meningitis, those causing CSF catheter-associated ventriculitis, such as coagulase-negative staphylococci and *C. acnes*, are indolent, evoke minimal inflammation, and are usually pathogenic in the presence of prosthetic material. Often, there may only be minimal ventriculitis without meningeal involvement, or only mechanical blockage as a result of biofilm formation in or on the catheter.[52,53] Therefore the clinical symptoms of meningitis may be absent and the clinical presentation more subtle, with a longer duration of symptoms. The most frequent symptoms in patients with CSF shunt infection are headache, nausea, lethargy, and change in mental status, seen in as many as 65% of infected patients; these symptoms occur as a result of shunt malfunction secondary to the infection. Fever is reported in as few as 14% to as many as 92% of cases, so the absence of fever cannot exclude the possibility of infection, although fever is typically present in the majority of patients. Pain, often related to infection at the peritoneal or pleural endings of the shunt, may be absent in as many as 60% of infections.

Symptoms and signs of a CSF shunt infection may be referable to either the proximal or distal portion of the shunt. Infection beginning in the proximal portion of the shunt (i.e., the catheter within the CSF space) results in meningitis or ventriculitis in approximately 30% of cases and may cause shunt obstruction or decreased function.[2,23,43] Rarely, intracranial empyemas and abscesses may occur secondary to a poorly treated infection, in the presence of retained hardware, or in patients with external monitors.

Symptoms of infection referable to the distal portion of the shunt are more specific to terminus location.[2,23] Infected shunts that terminate in the pleural or peritoneal space may lead to an inflammatory response in the absorbing tissue (i.e., pleuritis or peritonitis). In patients with infected VP shunts, symptoms of peritonitis appear as the peritoneal inflammation becomes more severe, and fever, anorexia, and other signs and symptoms of an acute abdomen develop. With low-virulence organisms, localizing signs of peritonitis may be confined to abdominal tenderness and/or guarding. In the peritoneal cavity host defense mechanisms attempt to limit the infection, often resulting in the encystment of the shunt catheter, fluid buildup within the pseudocyst, and loculation of pockets of fluid within the abdomen. These fluid collections can grow quite large because deposited CSF is not absorbed within the pseudocyst. Partial or complete shunt obstruction may result.

Infected ventriculoatrial shunts lead to bacteremia, which may occur secondary to infected CSF directly entering the bloodstream, resulting from an infected thrombus or atrial mural vegetation at the end of the vascular catheter, or represent true bacterial endocarditis. However, the clinical presentation of an infected vascular shunt is usually nonspecific, with fever and lethargy often seen. One unique complication of a chronic vascular shunt is shunt nephritis,[2,10,23,43] which is observed in 4% to 14% of patients with infected ventriculoatrial shunts. The majority of isolated bacteria in patients with shunt nephritis are usually coagulase-negative staphylococci, although diphtheroids and other pathogens have been isolated. The pathogenesis of shunt nephritis is similar to that of subacute bacterial endocarditis, with deposition of immunoglobulin M and G antigen-antibody complexes in the renal glomeruli. The complement system is activated, with subsequent depletion of circulating complement factors C3 and C4. Failure to detect this condition can lead to permanent renal failure. Treatment usually, but not always, leads to resolution of the renal dysfunction.

Some shunt infections, however, are insidious and cause few or no symptoms, perhaps only an intermittent low-grade fever or general malaise. The patient may present with an unexplained occlusion of an open-ended peritoneal catheter or failure of peritoneal CSF absorption. Therefore the clinician must consider the possibility of CSF shunt infection in these patients and institute an appropriate diagnostic workup (see later).

Lumbar or ventricular drains become infected from organisms introduced through the drainage system or through the skin site.[2,54,55] Infections are more frequent with CSF drains than CSF shunts and may be caused by hospital flora. The change in mental status that occurs in patients in whom meningitis or ventriculitis develops may be difficult to distinguish from the impaired level of consciousness that is a manifestation of the patient's underlying disease. In patients with CSF drain-related ventriculitis, symptoms and signs are not very useful. The underlying reason for drain placement, such as subarachnoid hemorrhage or tumor, can also cause a similar neurologic presentation, and these patients are often unresponsive in the intensive care unit and unable to report symptoms. Fever that occurs in these patients may also be from other sources of infection.

DIAGNOSIS

The diagnosis of CSF shunt and CSF drain infections is difficult because changes in CSF parameters may be subtle, especially with indolent organisms,[2,13] thus making it hard to determine if the abnormalities are related to infection or secondary to underlying reason for catheter placement, such as hydrocephalous from intracranial hemorrhage or a result of neurosurgery itself.[56,57] In patients with suspected CSF shunt ventriculitis the diagnosis is established by either direct culture of the CSF obtained by shunt aspiration or by culture of the proximal shunt components if the shunt is explanted.[2,43] To obtain fluid from a ventricular shunt, there must be a reservoir that can be tapped. These reservoirs are typically located in an easily accessible subcutaneous location. After sterile preparation the reservoir is percutaneously punctured; most shunts should not be punctured with anything larger than a 25-gauge needle. Introduction of infection is a possible, albeit low, risk of this procedure. In a pediatric study with 266 children who underwent 542 shunt aspirations, there was no evidence of shunt infection; one patient developed an infection after a shunt tap, but there was redness over the shunt tract at the time of the aspiration.[58]

In patients with infected ventricular shunts, the microbiologic yield is greater when CSF is obtained from a shunt aspiration than from a lumbar puncture.[13] Direct aspiration of the shunt yielded a positive culture in 91% to 92% of cases, whereas CSF culture from a lumbar puncture was positive in only 45% to 67% of cases.[13,59]

Once obtained, CSF should be sent for cell count with differential, chemistries (glucose and protein), Gram stain, and aerobic and anaerobic cultures.[2] Although high white blood cell (WBC) counts correlate with

the presence of infection, infection may be present even in patients with normal CSF WBC counts. CSF WBC counts and lactate concentrations were normal in approximately 20% of episodes in one study of adults with shunt-associated infection.[13] CSF eosinophilia (>8% of the differential count) has been associated with an indolent infection.[60] The cell count may be obscured by recent neurosurgery or intraventricular hemorrhage, which can cause an inflammatory reaction, so-called chemical meningitis.[56] Attention should also be paid to the site of CSF sampling because the CSF WBC count in samples obtained by shunt aspiration or from ventricular fluid tends to be lower than when CSF is obtained after lumbar puncture.[13] A negative result of a CSF Gram stain does not exclude the likelihood of infection.

CSF culture from the shunt reservoir is the most important test to establish the diagnosis of infection.[32] The culture will usually be positive in patients with an infected device even when there is no pleocytosis or alteration in CSF chemistries. CSF cultures may require several days to weeks of incubation before they can be called negative, especially for slowly growing organisms such as *C. acnes*, or the results may be confounded in patients who have received previous antimicrobial therapy. On occasion, shunts may be tapped for evaluation of function in patients with no clinical evidence of infection and may be found to be culture positive. In this situation contamination may be responsible for the positive culture, but true infection must be strongly considered. The shunt should be retapped, and a positive culture with the same microorganism is usually indicative of true infection.[2]

In patients with external ventricular drains and lumbar drains, the diagnosis of ventriculitis is more difficult than in patients with CSF shunts, as chemical meningitis and contamination of cultures during CSF collection is more frequent. The diagnostic conundrum relates to the degree of CSF pleocytosis or CSF hypoglycorrhachia that is consistent with an infectious ventriculitis and not a chemical meningitis resulting from hemorrhage or neurosurgery, as there is a considerable overlap in CSF parameters between these two entities. Although CSF lactate has been suggested as one parameter that may be a useful indicator of bacterial meningitis in CSF obtained via lumbar puncture, a recent study demonstrated that CSF lactate alone is not a reliable indicator of bacterial ventriculitis in patients with external ventricular drains.[61] In contrast, another study found CSF lactate to be a good marker for external drain-related infections.[62] More data are needed.

Another conundrum relates to the finding of a single positive CSF culture, which may be a contaminant or a true pathogen; abnormal CSF WBC counts and CSF glucose concentrations are often seen in these patients with chemical meningitis. The published literature on CSF drain-related ventriculitis is also plagued with the lack of clear diagnostic criteria, with a multitude of heterogeneous definitions. One study found 16 unique definitions in the published literature; when the definitions were applied to the test cohort, the frequency of infection ranged from 22% to 94% (median, 61% with an interquartile range of 56%–74%).[63] With that caveat, a definite infection can be defined as a positive CSF culture for a pathogenic organism (obtained from the ventricular or lumbar catheter) associated with significant CSF pleocytosis or CSF hypoglycorrhachia.[5,54,64] Progressively decreasing CSF glucose and increasing CSF pleocytosis on serial CSF analyses, in the absence of positive CSF cultures or positive Gram stain, characterizes a suspected infection in the absence of another etiology. A contaminating microorganism is defined as an isolated positive CSF culture and/or positive Gram stain with a normal CSF cell count, CSF glucose, and CSF protein. Normal protein in the ventricular CSF is less than 20 mg/dL, lower than in lumbar CSF.

In the absence of clinical evidence of a CSF shunt infection, routine cultures of shunt components, when shunts are removed for other indications, should not be done.[2] In one study of 174 shunt revisions, 19 patients had positive shunt component cultures without signs of infection (i.e., asymptomatic bacteriologic shunt contamination), only one of which was treated with antimicrobial therapy.[65] There was no increase in the risk of shunt malfunction in this group when compared with other patients with CSF shunts in the database at their institution.

Polymerase chain reaction (PCR) assay has been evaluated to detect the presence of bacterial DNA in CSF from patients with external ventricular drains and VP shunts. In one study that used PCR to detect gram-positive bacteria in 86 specimens, 42 were culture negative but PCR positive.[66] There were no positive culture results in patients with a negative CSF PCR, suggesting that a negative result is predictive of the absence of infection. More studies are needed, however, before routine use of PCR can be recommended in this setting.[2]

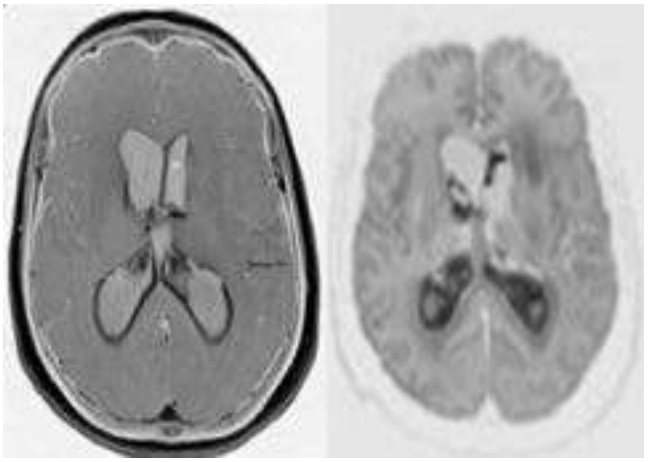

FIG. 92.3 Intraventricular empyema. *Left*, T1 post-contrast image showing ependymal enhancement of the occipital horns of lateral ventricles. *Right*, Areas of restricted diffusion in both the occipital horns of the lateral ventricles suggestive of purulent material.

In patients with ventriculoatrial shunts, blood cultures should also be performed because bacteremia is invariably present in patients with infected ventriculoatrial shunts (positive blood cultures in >90% of cases).[2] This contrasts with infections in other types of CSF shunts in which the incidence of negative blood cultures approaches 80%.[43]

The diagnosis of CSF shunt infection may be more difficult to establish when the distal portion of the VP shunt is infected.[2] The shunt tap may be normal with negative cultures if a retrograde infection has not yet developed in the patient. VP shunts with distal occlusion but no symptoms or signs of infection should be investigated for possible infection at the time of revision. A computed tomography or ultrasound scan of the abdomen may identify CSF loculations at the shunt terminus (see Fig. 92.2). Although some free fluid in the pleural or peritoneal cavities is normal, it should not be confused with the larger volumes and cysts seen with infection at the shunt terminus.

Neuroimaging studies are not useful in identifying CSF shunt infection but may show evidence of shunt malfunction and should be performed to evaluate ventricular size before initiating surgical treatment.[2] In acute shunt obstruction, extravasation of CSF from the lateral cerebral ventricles into contiguous cerebral cortex may occur. If there has been prior neurosurgery, imaging may detect the presence of potentially contaminated extraneous catheters from previous surgical procedures in either the brain or abdomen. Rarely, a subdural empyema or brain abscess may be the first indication of a shunt infection. In CSF drain-related infections that are refractory to intravenous (IV) and intraventricular therapy, MRI of the brain, with and without contrast and with diffusion-weighted imaging, is helpful in detecting intraventricular empyema, which often needs surgical drainage in addition to antimicrobial therapy (Fig. 92.3).[67,68]

THERAPY

Numerous methods for treating CSF shunt infections have been reported, but no randomized, prospective studies have been done to evaluate different antimicrobial therapies.[2,69] The therapeutic approach to an infected CSF shunt must take into account the need for a functioning device during or after treatment. For example, in patients with noncommunicating hydrocephalus and persistent infection, shunt removal and an endoscopic third ventriculostomy have been advocated in children[70]; even if the endoscopic third ventriculostomy fails, the VP shunt inserted after failure appears to have better longevity than if the VP shunt is inserted without first performing the ventriculostomy.[71] Factors to be considered in the therapy of an infected CSF shunt include the selection

of antimicrobial therapy, timing of hardware removal, timing of shunt replacement, and duration of antimicrobial therapy. These therapeutic approaches, however, have not been standardized in published studies, and there is wide variation in clinical practice.[72]

Antimicrobial Therapy

The principles of antimicrobial therapy for CSF shunt infections are generally the same as those for acute bacterial meningitis (see Chapter 87 for antimicrobial choice and dosing recommendations)[2,73]; the agent selected must penetrate the central nervous system and attain adequate CSF concentrations. However, because the organisms that cause CSF shunt infections often form biofilms, into which antimicrobial agents do not penetrate well, drug therapy may be problematic for patients when the catheter is not removed. If CSF pleocytosis is present, antimicrobial therapy should be initiated after appropriate cultures are obtained but before culture results are available. The most likely microorganisms associated with CSF shunt and CSF drain infections are coagulase-negative staphylococci (especially *S. epidermidis*), *S. aureus, C. acnes*, and gram-negative bacilli (including *Pseudomonas aeruginosa*). Empirical therapy with IV vancomycin plus either cefepime, ceftazidime, or meropenem is appropriate.[2,73] The empirical choice to treat a presumptive gram-negative pathogen should be based on the local antimicrobial resistance patterns of these pathogens. Therapy can then be modified once an organism is isolated and in vitro susceptibility results are available. If staphylococci are isolated and the organism is methicillin susceptible, therapy should be changed to either nafcillin or oxacillin. The addition of rifampin to an antistaphylococcal agent may augment treatment,[74] especially if the infected catheter is retained. One patient with an *S. epidermidis* VP shunt infection[75] and another with an *Enterococcus faecalis* VP shunt infection[76] were cured with shunt removal and IV linezolid, although linezolid cannot be considered as first-line therapy for this infection. Daptomycin, combined with rifampin, has also been successfully used in patients with infections of CSF shunts caused by gram-positive pathogens.[77] In one study of six neurosurgical patients with indwelling external CSF shunts and suspected meningitis or ventriculitis,[78] a single dose of daptomycin (10 mg/kg) led to an overall CSF penetration of 0.8%; when corrected for protein binding, the overall CSF penetration was 11.5%. An IV amphotericin B preparation (usually liposomal amphotericin B), often combined with 5-flucytosine, is recommended for *Candida* shunt infection[2]; once the patient shows clinical improvement, a change of therapy to fluconazole may be considered if the isolated species is susceptible.

Intraventricular and Intrathecal Antimicrobial Therapy

Direct instillation of antimicrobials into the lateral ventricles (via a ventricular drain or a ventricular access device such as an Ommaya reservoir) or the lumbar subarachnoid space (via a lumbar drain) may be necessary in patients with shunt or drain infections that are difficult to eradicate with IV antimicrobial therapy or when the patient is unable to undergo the surgical components of therapy (see later).[2,79] This route of administration bypasses the blood-CSF barrier, with controlled delivery of the antimicrobial agent to the site of infection. No antimicrobial agent has been approved by the US Food and Drug Administration for intraventricular use. However, intraventricular antimicrobial therapy has been recommended as an option for patients in whom the infection responds poorly to systemic antimicrobial therapy alone.[2] A recent systematic review sponsored by the American Association of Neurological Surgeons/Congress of Neurological Surgeons noted insufficient evidence to recommend their use in pediatric shunt infections,[80] although other studies suggest benefit. There have been several studies on the pharmacokinetics (PK), safety, and efficacy of intraventricular antimicrobial therapy, especially in adults[81–87]; CSF sterility and normalization of CSF parameters were achieved sooner with intraventricular and IV use when compared with IV use alone. A randomized trial in 10 patients with staphylococcal ventriculitis showed that much higher CSF concentrations were achieved with intraventricular vancomycin when compared with IV therapy.[88] In another study of 34 patients with persistently positive CSF cultures despite antimicrobial treatment, those who received intraventricular or lumbar intrathecal antimicrobials achieved CSF sterilization within 24 hours in 50% of patients and within 48 hours in an additional 18% of patients[89]; only 3 patients had adverse effects, all of which were clinically insignificant. The clinical outcome of patients, as assessed by the modified Rankin Scale, improved in 50% and stayed unchanged in 29%. In another study on infections in patients with CSF diversion devices, 25 patients received intraventricular and systemic antimicrobials, and 23 received systemic antimicrobials alone; the mean times to CSF sterilization and normalization of CSF microscopy were significantly shorter for the intraventricular group ($P < .05$ and $P < .005$, respectively), as was duration of hospital stay ($P < .002$) and required length of systemic antimicrobial therapy ($P < .001$).[90] However, use of intraventricular antimicrobial agents was not recommended in infants based on data in a Cochrane Review[91]; one clinical trial found a three times higher relative risk of mortality for infants with gram-negative meningitis who were treated with intraventricular gentamicin and IV antimicrobials when compared with IV therapy alone, although one-half of the infants in the intraventricular gentamicin group had received only one dose, raising doubts about the exact cause of death.

Antimicrobial agents administered by the intraventricular or intrathecal route should be preservative free. When administered through a ventricular drain, the drain should be clamped for 15 to 60 minutes to allow the antimicrobial solution to equilibrate in the CSF before opening the drain.[92] In treating VP shunt ventriculitis, administration of the antimicrobials through the shunt reservoir may also result in the agent draining into the peritoneal cavity; to avoid this issue, antimicrobials can be administered via a ventricular access device separate from the shunt reservoir.[93]

There are limited studies published on antimicrobial CSF PK/pharmacodynamics (PD); that is, the relationship between intraventricular and intrathecal antimicrobial dose and frequency of administration with CSF concentrations or CSF sterilization and normalization of CSF inflammatory parameters. There is a dire need for such studies, but use of intraventricular therapy should generally be based on sound general PK/PD principles. At this time, intraventricular therapy should be reserved for organisms with high minimum inhibitory concentrations (MICs) in which use of IV antimicrobials cannot achieve the desired CSF PK/PD targets based on pathogen and MIC or in patients with clinical failure with use of IV antimicrobials (defined as persistent CSF cultures on IV therapy). The recommended doses of antimicrobial agents for intraventricular use, based on limited studies, has been outlined in Table 92.3, with adjustments of dose and dosing interval based on the ability of the agent to achieve adequate CSF concentrations.[2,73] Determining the correct dosing regimen is challenging as the CSF concentrations obtained for the same intraventricular dose in PK studies have been highly variable, probably because of the differences among patients in either the volume of distribution, ventricular size, or variable CSF clearance as a result of CSF drainage.[81–86,91] A consensus guideline by the British Society for Antimicrobial Chemotherapy Working Party on Infections in Neurosurgery has recommended that the initial dose of an intraventricular antimicrobial be based on ventricular volume.[94] The recommended dose of vancomycin is 5 mg in patients with slit ventricles, 10 mg in patients with normal-sized ventricles, and 15 to 20 mg in patients with enlarged ventricles; using the same rationale, the initial dosing of an aminoglycoside can also be tailored to ventricular size. The same working party recommended that the frequency of dosing be based on the daily volume of CSF drainage: once-daily dosing if CSF drainage is greater than 100 mL/day, every other day if the drainage is 50 to 100 mL/day, and every third day if drainage is less than 50 mL/day. This approach, though pragmatic, has not been empirically validated in clinical studies. Another approach is to base dosing on monitoring of CSF drug concentrations. However, there are very few studies that have evaluated CSF therapeutic drug monitoring and, given the variable CSF clearance of an antimicrobial agent, it is difficult to determine when to obtain CSF to measure peak and trough drug concentrations. A CSF drug concentration can be obtained 24 hours after administration of the first dose that can be presumed to be the trough CSF concentration. The trough CSF concentration divided by the minimal inhibitory concentration of the agent for the isolated bacterial pathogen is termed the *inhibitory quotient*, which should exceed 10 to 20 for consistent CSF sterilization.[2,73] Although not standardized, this approach is

TABLE 92.3 Antimicrobial Agents Administered by the Intraventricular Route

ANTIMICROBIAL AGENT	DAILY INTRAVENTRICULAR DOSE
Vancomycin	5–20 mg[b]
Gentamicin	1–8 mg[c]
Tobramycin	5–20 mg
Amikacin	5–50 mg[d]
Polymyxin B	5 mg[e]
Colistin[f]	10 mg
Teicoplanin	5–40 mg[g]
Quinupristin/dalfopristin	2–5 mg
Daptomycin	2–5 mg[h]
Amphotericin B	0.01–0.5 mg in 2 mL of 5% dextrose in water[i]

[a]There are no specific data that define the exact dose of intraventricular antimicrobial agents that should be used in cerebrospinal fluid shunt and drain infections; see text for details of specific studies.
[b]Most studies have used a 10- or 20-mg dose.
[c]Four to 8 mg in adults and 1–2 mg in infants and children.
[d]Thirty milligrams daily is the usual intraventricular dose.
[e]Dose of 2 mg/day in children.
[f]Formulated as colistimethate sodium.
[g]Five to 20 mg every 24–48 hours used in one study.[98]
[h]One study used 10 mg every day for 2 days and 10 mg every 48 hours[99]; another study used 5 or 10 mg every 72 hours.[100]
[i]Dose for *Candida* shunt infection. Not usually necessary but may be needed if removal of the device is too risky or the patient has not responded to systemic antifungal therapy.

reasonable to ensure that adequate CSF concentrations of these agents are obtained.

Although there are methodologic limitations in published studies, intraventricular vancomycin was shown to be safe and efficacious in a recent systematic review in adults.[83] Indeed, combined intraventricular and IV use may improve CSF vancomycin concentrations without side effects.[95] Intraventricular aminoglycosides were also shown to be effective[81,85]; in one study, there were no relapses when intraventricular gentamicin was combined with IV meropenem in patients with neurosurgical gram-negative bacillary ventriculitis and meningitis.[85] In another study of treatment of 34 consecutive shunt infections in 30 children, high-dose intraventricular antimicrobial therapy sterilized CSF cultures in 100% of 26 children who were treated for 3 days or more.[96] IV and intraventricular quinupristin/dalfopristin has been used successfully to treat a patient with ventriculostomy-related meningitis caused by vancomycin-resistant *Enterococcus faecium*.[97] Teicoplanin, a glycopeptide antimicrobial agent not currently licensed in the United States, was also found to be successful after intraventricular administration in seven patients with staphylococcal neurosurgical shunt infections.[98] Intraventricular daptomycin was successfully used in individual case reports in patients with CSF shunt and CSF drain infections caused by methicillin-resistant coagulase-negative staphylococci and resistant enterococci.[99–102] Intraventricular colistin, usually formulated as colistimethate sodium, and polymyxin B have been used in the treatment of gram-negative ventriculitis and meningitis,[79,103–105] but these agents should be reserved for patients with infections caused by multidrug-resistant gram-negative bacteria or in those failing therapy with standard IV agents. Colistimethate is biologically inactive before hydrolytic cleavage into free colistin, unlike polymyxin B, so the latter is preferable. Penicillins and cephalosporins should not be given by the intrathecal route because they have been associated with significant neurotoxicity, especially seizures.[79]

Shunt Removal

The approaches to the treatment of CSF shunt infections in the published literature have included antimicrobial therapy with or without shunt removal.[2,23,42,43,106] There has only been one prospective, randomized trial of three different approaches to management of infected CSF shunts in 30 children, all of whom received antimicrobial therapy[107]; not removing the shunt led to a 30% cure, one-stage shunt replacement led to a 90% cure, and two-stage shunt replacement led to a cure in 100% of patients. In early attempts to treat CSF shunt infections, IV and/or intraventricular antimicrobial agents were used exclusively to avoid additional operations and to maintain CSF diversion during treatment. Success with this approach, however, was low (34%–36%) and carried a high mortality rate.[39,108] In addition, the instillation of antimicrobial agents into CSF often required a lengthy hospitalization, and the frequency of an adverse outcome was unacceptably high. The ability of many of these organisms to adhere to prostheses and survive antimicrobial therapy likely precluded optimal treatment in situ. However, in one observational study of treatment with systemic and intraventricular antimicrobial agents (instilled via a separate ventricular access device), 84% of 43 patients were cured, with a 92% success rate for infections caused by bacteria other than *S. aureus*,[93] suggesting that conservative management may be appropriate for selected patients with CSF shunt infections caused by less virulent microorganisms, such as coagulase-negative staphylococci. Similar results have been achieved in patients with CSF shunt infections caused by *C. acnes*.

Combining the removal of shunt hardware with immediate shunt replacement and IV antimicrobial therapy cures approximately 65% to 75% of patients with shunt infections,[39,108] although the failure and reinfection rates still remain quite significant with this approach. The other option is shunt removal with delayed replacement (to treat the infection with antimicrobial therapy in the absence of any foreign devices), although this approach leaves untreated the reason for the initial shunt placement.[109]

The most successful approach to treatment of a CSF shunt infection is systemic antimicrobial use with removal of all components of the infected shunt, followed by insertion of a ventricular drain. When the drainage cultures are negative, the drain is removed and a new CSF shunt placed (see later).[2,72] The ventriculitis of shunt infections appears to clear more quickly with external drainage. The presence of a drainage catheter also allows monitoring of CSF parameters, including cultures, and allows administration of intraventricular antimicrobial therapy, if necessary. Ventricular drainage also allows continued treatment of the underlying hydrocephalus and avoids the complications associated with only shunt removal. With this approach, treatment success is usually greater than 85%.[2,31,39,108] In one recent retrospective, observational study of 86 episodes in patients with VP shunt infections and who were 12 years or older, 6 episodes were treated with only antibiotics, and 80 episodes were treated with strategies that combined antimicrobial therapy with surgical treatment—either two-stage shunt replacement, one-stage shunt replacement, or shunt removal without replacement.[110] Administration of antibiotics with a two-stage shunt replacement was the most successful strategy, with a cure rate of 89% and CSF sterilization rate of 95%, indicating that this approach remains the optimal approach to patients with VP shunt infection.

The greatest risk of the external ventricular drain is that of secondary infection.[2,5,111] A longer duration of the drain appears to increase the risk, although prophylactic catheter exchange performed every 5 days does not significantly decrease the likelihood of CSF infection.[112,113] In one randomized, controlled trial with 103 patients who required external ventricular drains for more than 5 days,[113] the CSF infection rate was 7.8% for the group that underwent catheter exchange every 5 days and 3.8% for the no-change group ($P = .5$), suggesting that a single external ventricular drain can be used for as long as clinically indicated unless a change is necessary because of CSF infection or catheter malfunction. Attention to maintaining a sterile, closed system with avoidance of injections into the system and surveillance of the draining CSF will usually keep this risk to less than 5%. In the treatment of infection caused by an external ventricular drain, removal of the drain is an important adjunctive measure.[2]

Duration of Antimicrobial Therapy and Shunt Reimplantation

The duration of antimicrobial therapy for CSF shunt infections is not completely defined and is dependent on the isolated microorganism, the extent of infection as defined by cultures obtained after

externalization, and occasionally CSF findings.[2] Once the infected CSF shunt has been removed, the optimal timing of shunt reimplantation has not been studied. Early placement may increase the risk of relapse, but a delay in reimplantation may increase the risk of secondary infection of the external ventricular drain. The timing of reimplantation should be individualized based on the isolated organism, severity of ventriculitis, and improvement of CSF parameters and CSF sterilization in response to antimicrobial therapy. In patients with shunt infection caused by coagulase-negative staphylococci and with normal CSF findings, the presence of negative CSF cultures for 48 hours after externalization generally confirms that removal of the hardware effected a cure and the patient can be reshunted on the third day after removal. If a coagulase-negative *Staphylococcus* was isolated in association with CSF abnormalities (i.e., CSF pleocytosis, abnormal chemistries), a true infection was likely present. If repeat cultures are negative, 7 days of antimicrobial therapy are usually recommended before reshunting, but if repeat cultures are positive, antimicrobial treatment is continued until CSF cultures remain negative for 7 to 10 consecutive days before a new CSF shunt is placed. This approach is also recommended for infection caused by *C. acnes*.[32] For shunt infections caused by *S. aureus* or gram-negative bacilli, 10 days of antimicrobial therapy with negative cultures are recommended before reshunting.[2,73,114] Some experts also suggest that consideration be given to a 3-day period off antimicrobial therapy to verify clearing of the infection before shunt reimplantation, although this observation period is generally not recommended.[2,73] It is important to note, however, that these recommendations have not been rigorously studied, and some patients may require a longer duration of antimicrobial therapy before a new CSF shunt is placed. Furthermore, significant variations have been observed in the duration of antimicrobial therapy in patients with CSF shunt infections.[26,72] Careful follow-up after reimplantation is also critical to ensure that the patient has been cured. Regardless of the manner of treatment, CSF shunt infection can recur. In one study[26] the recurrence rate was 26%, with two-thirds of cases caused by the same microorganism; the recurrence rate in patients with *S. epidermidis* shunt infection was 29%. A major risk factor for recurrence was a history of shunt infection within the preceding 6 months. In one recent study in children, risk factors for reinfection were complex shunts (i.e., multiple shunts placed or any single shunt with multiple catheters together), an atrial shunt, any complication after the first infection (e.g., shunt malfunction, hemorrhage, or CSF leak), or intermittent negative cultures (defined as positive CSF cultures that clear and then return over the course of treatment).[115]

Surgical Management of Ventriculoperitoneal Shunt–Related Peritoneal Pseudocyst

Abdominal peritoneal pseudocyst at the distal terminus of the VP shunt is a rare complication, with very limited studies on its management.[45,116,117] Based on limited studies, we recommend externalization of the distal catheter and, if the pseudocyst is still present, drainage of the pseudocyst and removal of at least the distal components of the VP shunt tubing and reimplantation with repositioning of the distal catheter in a different location.

Surgical Management of Intraventricular Empyema

Intraventricular empyema is more commonly seen in patients with gram-negative ventriculitis, usually does not resolve with only IV and/or intraventricular therapy, and often requires surgical drainage. Open craniotomy can be performed for drainage, but less invasive neuroendoscopic approaches have been shown to be safe and successful in small studies.[67,68]

PREVENTION

There have been few systematic studies on specific surgical techniques to minimize the possibility of infection in patients undergoing CSF shunt placement. Much of what is recommended is based on the described risk factors for shunt infection (see Table 92.1).[2] In one prospective, randomized controlled trial in 61 patients undergoing 84 shunt procedures, the shunt infection rate was reduced in those getting antimicrobial sutures (4.3% vs. 21%; $P = .038$),[118] although there was a high rate of infection in the control group. There is some weak evidence that double gloving may decrease CSF shunt infection rates,[49] and good surgical techniques[119] and adherence to infection control measures are important. In patients with external ventricular drains, adherence to a checklist for insertion that included hand hygiene, appropriate skin preparation with povidone iodine, allowing the skin preparation to completely dry before insertion, use of all five maximal sterile barriers (sterile gloves, sterile gown, cap, mask, and large sterile drape), and adherence to the policy for external ventricular drain maintenance led to a decline in infection rates from 16 per 1000 external ventricular drain catheter days to 4.5 per 1000 catheter days[120]; this infection rate further decreased to 1.3 per 1000 catheter days. Initiation of this protocol eventually resulted in no infections over a 25-month period. Other studied interventions are detailed in the following sections.

Antimicrobial Prophylaxis

There is evidence to support the use of periprocedural prophylactic antimicrobial administration for patients undergoing CSF shunt insertion and placement of external ventricular drains.[2] Although no prospective, randomized trials of periprocedural prophylactic antibiotics for CSF shunt placement have been adequately powered to clearly establish efficacy, several meta-analyses have concluded that this approach decreases infection rates by approximately 50%.[121,122] A Cochrane Database Review indicated that the odds ratio for decreased infection was 0.52 (95% CI, 0.36 to 0.74).[123] The antimicrobial agent should be given before incision to achieve adequate tissue concentrations and continued for as long as 24 hours postoperatively.

Although the use of periprocedural prophylactic antibiotics for placement of external ventricular drains is also generally accepted, the use of prophylactic prolonged systemic antimicrobials for the duration of external CSF drainage is more controversial.[2] One study noted that the infection rate was 3.8% in those who received prophylactic antibiotics for the duration of placement of the external ventricular drain and 4.0% for those who received only periprocedural antibiotics,[124] suggesting that prophylactic antibiotics throughout drainage did not significantly decrease the rate of ventriculitis and might select for emergence of resistant organisms. In contrast, another study demonstrated the benefit of prophylactic antibiotics (2.6% CSF infection rate vs. 10.6% in those who only received periprocedural antibiotics; $P = .001$),[125] although the infections in those receiving prophylactic antimicrobials were caused by more drug-resistant virulent pathogens and the mortality rate was higher (66% vs. 41%). In a pooled estimate of nine studies, the CSF infection rate was 8.1% in those who received periprocedural antibiotics and 5.3% in those who received antibiotics for the duration of external drainage,[5] leading to a recommendation to maintain prophylactic antibiotics in all patients while the external ventricular drain is in place, although this is not the practice in all centers. In a more recent systematic review that pooled data from two randomized controlled trials and four observational studies,[126] there was a reduced relative risk (RR) of 0.45 with use of prophylactic prolonged systemic antimicrobials; although there were significant methodologic limitations and heterogenicity in the pooled studies, the definitions of ventriculitis were variable, the type and dose of antimicrobials were different, adverse effects were not well studied, and most of the studies were retrospective and prone to bias. In light of these findings, and also based on the availability of a safer efficacious alternative (i.e., antimicrobial-impregnated catheters; see later), we do not favor use of prophylactic prolonged systemic antimicrobials for prevention of infection in patients with external ventricular drains.

Antimicrobial-Impregnated Catheters

Antimicrobial-impregnated catheters for external ventricular drains and CSF shunts have been under development for several decades and more recently have been introduced into clinical practice. They are typically impregnated with either minocycline or clindamycin, combined with rifampin. In one prospective, randomized trial of 110 patients who underwent placement of CSF shunts impregnated with clindamycin and rifampin, there was a decrease in infection rate from 16.6% to 6%,[127] although the study was hampered by a small number of patients

TABLE 92.4 Individual Components of a Standardized Protocol for Cerebrospinal Fluid Shunt Insertion

Sign on operating room door to minimize traffic[a]
Patient position of operative site away from door[a]
Antimicrobial agents received before incision
Hair clipped, not shaved[a]
ChloraPrep (chlorhexidine) applied by or approved by attending physician
Wait 3 minutes to allow ChloraPrep to dry
Proper hand-washing technique by all team members
Double gloving by all team members
Ioban use (a sterile, adhesive, antimicrobial plastic surgical drape through which the incision can be made)
Vancomycin/gentamicin injection into shunt reservoir[a]
Dressing

[a]Features not included in revised protocol, which included antimicrobial-impregnated catheters (see text for details).[138]

From Kestle JRW, Riva-Cambrin J, Wellons JC 3rd, et al. A standardized protocol to reduce cerebrospinal fluid shunt infection: the Hydrocephalus Clinical Research Network Quality Improvement Initiative. J Neurosurg Pediatr. *2011;8:22–29.*

TABLE 92.5 External Ventricular Drain Infection Control Protocol

External Ventricular Drain Placement

- Physician washes/cleanses hands with alcohol-based solution
- Broad clipping of hair with sufficient room to place a medium-sized adherent transparent dressing film using coarse and fine clippers
- Single dose of antibiotic (e.g., cefazolin) is given before incision
- No prophylactic antibiotics are given after the single pre-incision dose
- Chlorhexidine skin preparation before draping
- Full sterile draping of patient's head and body
- Physician wears cap, mask, sterile gown, and sterile gloves
- All staff in room wear cap and mask
- Repeat chlorhexidine skin preparation after draping
- Rifampin/minocycline-coated ventricular catheter is placed
- Catheter is tunneled approximately 3–5 cm from insertion site

Dressing

- Catheter is secured in question-mark pattern using surgical staples
- Benzoin tincture is applied to skin and allowed to fully dry
- Chlorhexidine-eluting patch is applied over catheter exit site
- Medium-sized adherent transparent dressing film is applied
- Secure borders of dressing film with sterile adhesive strips
- No routine dressing changes; change for dressing compromise only

External Ventricular Drain Manipulation

- All staff in room wear cap and mask
- Three-way stopcock of access port is turned to 45 degrees ("off" to all directions)
- Access port and surrounding tubing are submerged in isopropyl alcohol
- Physician dons cap, mask, sterile gown, and sterile gloves
- Access port and surrounding tubing are cleaned with chlorhexidine
- Port cap is removed and discarded
- Luer fitting inside of port is cleaned (multiple times) with chlorhexidine
- Luer fitting inside of port is rinsed (multiple times) with sterile, preservative-free normal saline
- Cerebrospinal fluid draw or flush is performed with strict sterile technique
- New sterile port cap is applied

From Flint AC, Rao V, Renda NC, et al. A simple protocol to prevent external ventricular drain infections. Neurosurgery. *2013;72:993–999.*

and a high infection rate in the control group. Other noncontrolled studies in patients with CSF shunts have led to mixed results, some supporting[128,129] and others disputing[130,131] these findings. In a meta-analysis of pooled data from 12 studies comparing antimicrobial-impregnated with non–antimicrobial-impregnated VP shunts, there was a statistically significant decrease in infections in patients who had received antimicrobial-impregnated shunts (RR, 0.37; $P < .0001$).[132] In another systematic literature review of 5613 shunt procedures, use of antimicrobial-impregnated shunt catheters was associated with a decreased risk of shunt infection (3.3% vs. 7.2%; $P < .00001$),[133] with significant differences in both children and adults; use did not appear to be associated with emergence of antimicrobial-resistant infections. Use of antimicrobial-impregnated shunts has not only reduced the incidence of CSF shunt infections but also resulted in significant hospital cost savings.[134] In one study that compared the effectiveness of antimicrobial-impregnated shunt catheters in 12,589 consecutive cases from 287 hospital systems in pediatric and adult patients with hydrocephalus, there was a significant reduction in infection in both pediatric patients (2.6% vs. 7.1%; $P < .01$) and adults (2.2% vs. 3.6%; $P = .02$) who received antimicrobial-impregnated catheters, a reduction that was demonstrated regardless of hospital size, annual shunt procedure volume, hospital location, or patient risk factors.[135]

Similar results have been noted in patients who received antimicrobial-impregnated external ventricular drains. A prospective, randomized trial of 306 patients who had placement of external ventricular drains impregnated with minocycline and rifampin showed a decrease in the CSF infection rate from 9.4% to 1.3%.[136] Pooled data from five studies in one meta-analysis showed a statistically significant benefit for antimicrobial-impregnated external ventricular drains (RR, 0.31; $P = .009$).[132] Therefore use of antimicrobial-impregnated CSF shunts and CSF drains appears to be safe and effective in prevention of ventriculitis,[2] although prospective, randomized controlled trials are needed to firmly confirm their benefits.

Combined Interventions

Many of the studies on prevention of CSF shunt infections detailed earlier have examined single interventions to determine effects on infection rates. However, use of "practice bundles" may also be valuable in development of standardized protocols for insertion of CSF shunts.[2] The Hydrocephalus Clinical Research Network undertook an initiative in which centers agreed to develop an 11-step protocol to try to reduce CSF shunt infection rates (Table 92.4); this was a collaboration of pediatric neurosurgical centers and included all children getting shunts or revisions.[137] The initiative involved 21 surgeons and included 1571 procedures in 1004 children. Overall protocol compliance was about 75%, and another 20% followed 10 of the 11 steps. The Network infection rate decreased from 8.8% before the protocol to 5.7% while using the protocol (RR reduction, 36%; $P = .0028$), indicating that use of a standardized protocol and reducing variation by adherence to a common protocol are effective at reducing CSF shunt infection rates. Only proper hand-washing technique by all team members emerged as an independent predictor of decreased infection rates; factors associated with increased infection were use of BioGlide catheters and use of antiseptic cream by any members of the surgical team. The Hydrocephalus Clinical Research Network has subsequently published results of a revised procedure, with results from January 2012 through September 30, 2013.[138] To simplify the protocol, injection of vancomycin and gentamicin into the shunt reservoir was eliminated, as was orientation of the surgical field in respect to the operating room door, placing a sign on the operating room door, or requiring hair clipping rather than shaving. Antibiotic-impregnated catheters were used for the shunts, unlike the prior protocol. A total of 1935 procedures were performed on 1670 patients in eight centers; revisions constituted 1193 (62%) of these procedures. The infection rate was 6.0%, similar to 5.7% with the prior protocol. The role of antibiotic impregnated catheters in the new protocol could not be determined.

Similarly, in patients requiring placement of an external ventricular drain after a simple infection control protocol (Table 92.5) reduced ventriculitis rates from 6.3% in the baseline period to 0.8% in the first 3 years of the protocol period.[139] In a 4-year follow-up the authors reported a further decrease in the ventriculitis rate to 0%.[140]

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The complete reference list is available online at Expert Consult.

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93 Cellulitis, Necrotizing Fasciitis, and Subcutaneous Tissue Infections

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SHORT VIEW SUMMARY

Definition
- Skin and soft tissue infections are characterized by location, depth of infection, etiologic agent, and clinical setting and may result from either primary cutaneous inoculation or, less commonly, hematogenous seeding.
- *Impetigo* is a superficial crusting and at times bullous infection of the skin; localized progression into the dermis leads to ecthyma.
- *Folliculitis* is a localized infection of hair follicles, which can extend into subcutaneous tissue, resulting in furuncles. These, in turn, may coalesce, leading to carbuncle formation.
- *Erysipelas* is a rapidly progressive infection of the superficial dermis, with sharp erythematous borders; *cellulitis* reflects deeper dermal involvement.
- Necrotizing skin and soft tissue infections, including *necrotizing fasciitis,* are rare life-threatening infections, occur in a variety of clinical settings, and require prompt diagnosis and surgical intervention.

Epidemiology
- Significant skin and soft tissue infection may occur throughout the age spectrum. Minor local trauma as the initial pathogenic event is a common feature of these processes. Although invasive infections may occur in previously healthy individuals, a variety of systemic risk factors predispose individuals to these infections.

Microbiology
- Hemolytic streptococci and *Staphylococcus aureus,* including methicillin-resistant *S. aureus* (MRSA), are the most common causes of superficial cutaneous infection.
- Mixed infection of facultative gram-negative bacilli, anaerobes, and gram-positive organisms most frequently cause necrotizing infection, but group A streptococci, clostridia, and *Vibrio* species (as well as other facultative gram negatives) can also cause monomicrobial necrotizing fasciitis.
- A broad differential diagnosis must be considered in confronting infections in immunocompromised patients.

Diagnosis
- Most cutaneous infections are not associated with bacteremia, and diagnosis and empirical therapy are based on physical findings and clinical setting.
- In immunocompromised hosts, tissue biopsy for microbiologic and histologic study is critical to plan definitive therapy.

Therapy
- Oral therapy targeted against gram-positive pathogens is appropriate for mild disease, with close follow-up and revision of therapy for inadequate response. Parenteral therapy targeted more broadly against gram-positive organisms (including *S. aureus* and MRSA), gram-negative pathogens, and anaerobes is required in many settings of severe infection, particularly in compromised hosts.
- Necrotizing infections require urgent surgical débridement.
- Infections associated with toxic shock syndrome are treated with protein synthesis inhibitors such as clindamycin as an adjunctive measure to antibiotic treatment.

Prevention
- Good hygienic practices and attention to early therapy for superficial processes such as dermatophyte infection reduce the risk for cutaneous and soft tissue infection.
- Individuals with recurrent cellulitis may benefit from chronic antibiotic suppression.

CELLULITIS AND SUPERFICIAL INFECTIONS

Bacterial and mycotic infections, exclusive of those caused by the common dermatophytes, are discussed in this chapter. Classification of cutaneous infections on morphologic and clinical grounds can be very helpful in providing initial clues regarding the most likely responsible infectious agents (Table 93.1).

Primary Pyodermas

Impetigo

Impetigo is an initially vesicular, later crusted, superficial infection of the skin. Most cases occur in children. The annual incidence of impetigo is 1% to 2%, with annual summer epidemics.[1] There are both geographic and yearly variations in incidence related to the clonal spread of virulent or resistant pathogens.[2] Previously, group A streptococcus was the principal cause of impetigo and was isolated from about 80% of cases, either alone or mixed with *Staphylococcus aureus.*[3] In the past 20 years, group A streptococcus has been found less commonly (20%–30%) in impetigo and has been supplanted by *S. aureus,*[1–4] with a growing frequency of methicillin-resistant *S. aureus* (MRSA) isolates, although the relative frequency of these pathogens (alone or in mixed infection) varies among different populations.[5] Conceivably, the role of staphylococci may be somewhat overestimated because these organisms are common secondary invaders, and some strains produce bacteriocins that may impair the recovery of group A streptococci.

Pathologic Characteristics and Pathogenesis

Histopathologically, impetigo consists of superficial, intraepidermal, unilocular vesicopustules. In epidemiologic studies, group A streptococcal acquisition on normal skin antedates the appearance of impetigo by about 10 days.[3] During that time, minor trauma (e.g., insect bite, abrasion) or

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TABLE 93.1 Classification of Bacterial and Mycotic Infections of the Skin

TYPE OF LESION	CAUSATIVE AGENTS
Primary Pyodermas	
Impetigo, bullous impetigo	*Staphylococcus aureus,* group A streptococci
Folliculitis	*S. aureus, Candida, Pseudomonas aeruginosa, Malassezia furfur, Pityrosporum ovale*
Furuncles and carbuncles	*S. aureus*
Paronychia	*S. aureus,* group A streptococci, *Candida, P. aeruginosa*
Ecthyma	Group A streptococci
Chancriform lesions	*Treponema pallidum, Haemophilus ducreyi, Sporothrix, Bacillus anthracis, Francisella tularensis, Mycobacterium ulcerans, Mycobacterium marinum*
Erysipelas	Group A streptococci
Cellulitis	Group A or other streptococci, *S. aureus;* rarely, various other organisms
Membranous ulcers, including cutaneous diphtheria	*Corynebacterium diphtheriae*
Erythrasma	*Corynebacterium minutissimum*
Scarlet Fever Syndromes	
Scalded skin syndrome	*S. aureus* (phage group II)
Scarlet fever	Group A streptococci, rarely *S. aureus*
Toxic shock syndrome	*S. aureus* (pyogenic toxin-producing strains), *S. pyogenes*
Infectious Gangrene and Gangrenous Cellulitis	
Streptococcal gangrene and necrotizing fasciitis	Group A streptococci, mixed infections with Enterobacteriaceae and anaerobes
Progressive bacterial synergistic gangrene	Anaerobic streptococci plus a second organism (*S. aureus, Proteus*)
Gangrenous balanitis and perineal phlegmon	Group A streptococci, mixed infections with enteric bacteria (e.g., *Escherichia coli, Klebsiella*) and anaerobes
Gas gangrene, crepitant cellulitis	*Clostridium perfringens* and other clostridial species; *Bacteroides*, peptostreptococci, *Klebsiella, E. coli*
Gangrenous cellulitis in immunosuppressed patients	*Pseudomonas, Aspergillus,* agents of mucormycosis
Secondary Bacterial Infections Complicating Preexisting Skin Lesions	
Burns	*P. aeruginosa, Enterobacter,* various other gram-negative bacilli, various streptococci, *S. aureus, Candida, Aspergillus*
Eczematous dermatitis and exfoliative erythrodermas	*S. aureus,* group A streptococci
Chronic ulcers (varicose, decubitus)	*S. aureus,* streptococci, coliform bacteria, *P. aeruginosa,* peptostreptococci, enterococci, *Bacteroides, C. perfringens*
Dermatophytosis	*S. aureus,* group A streptococci
Traumatic lesions (e.g., abrasions, animal bites, insect bites)	*Pasteurella multocida, C. diphtheriae, S. aureus,* group A streptococci
Vesicular or bullous eruptions (varicella, pemphigus)	*S. aureus,* group A streptococci
Acne conglobata	*Propionibacterium acnes*
Hidradenitis suppurativa	*S. aureus, Proteus* and other coliforms, streptococci, peptostreptococci, *P. aeruginosa, Bacteroides*
Intertrigo	*S. aureus,* coliforms, *Candida*
Pilonidal and sebaceous cysts	Peptostreptococci, *Bacteroides,* coliforms, *S. aureus*
Pyoderma gangrenosa	*S. aureus,* peptostreptococci, *Proteus* and other coliforms, *P. aeruginosa*
Cutaneous Involvement in Systemic Bacterial and Mycotic Infections	
Bacteremias	*S. aureus,* group A streptococci (also other groups such as D), *Neisseria meningitidis, Neisseria gonorrhoeae, P. aeruginosa, Salmonella typhi, Haemophilus influenzae*
Infective endocarditis	Viridans-group streptococci, *S. aureus,* group D streptococci, and others
Fungemias	*Candida, Cryptococcus, Blastomyces dermatitidis, Fusarium*
Listeriosis	*Listeria monocytogenes*
Leptospirosis (Weil syndrome and pretibial fever)	*Leptospira interrogans* serotypes
Rat-bite fever	*Streptobacillus moniliformis, Spirillum minus*
Melioidosis	*Burkholderia pseudomallei*
Glanders	*Burkholderia mallei*
Carrión disease (verruga peruana)	*Bartonella bacilliformis*

Continued

TABLE 93.1 Classification of Bacterial and Mycotic Infections of the Skin—cont'd

TYPE OF LESION	CAUSATIVE AGENTS
Parainfectious and Postinfectious Nonsuppurative Complications	
Purpura fulminans (manifestation of disseminated intravascular coagulation)	Group A streptococci, *N. meningitidis, S. aureus,* pneumococci
Erythema nodosum	Group A streptococci, *Mycobacterium tuberculosis, Mycobacterium leprae, Coccidioides immitis, Leptospira autumnalis, Yersinia enterocolitica, Legionella pneumophila*
Erythema multiforme–like lesions (rarely), guttate psoriasis	Group A streptococci
Other Lesions	
Nodular lesions	*Candida, Sporothrix, S. aureus* (botryomycosis), *M. marinum, Nocardia brasiliensis, Leishmania brasiliensis*
Hyperplastic (pseudoepitheliomatous) and proliferative lesions (e.g., mycetomas)	*Nocardia, Pseudallescheria boydii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Phialophora, Cladosporium*
Vascular papules/nodules (bacillary angiomatosis, epithelioid angiomatosis)	*Bartonella henselae, Bartonella quintana*
Annular erythema (erythema chronicum migrans)	*Borrelia burgdorferi*

primary dermatoses predispose to the development of infected lesions. Impetigo is most common during hot, humid summer weather. Two to 3 weeks after skin acquisition of streptococci, pharyngeal colonization by the same organism occurs in about 30% of children with skin lesions. (The sporadic cases of facial impetigo occurring in cooler climates probably result from contiguous spread from an initial nasopharyngeal infection, and the serotypes involved are those commonly causing pharyngeal disease.) In staphylococcal impetigo (in which *S. aureus* is the only pathogen), skin infection follows nasal colonization.[5,6] Among returning travelers, impetigo is commonly associated with antecedent insect bites.[7]

Nonbullous impetigo caused by group A streptococcus (*Streptococcus pyogenes*) begins when the corneal layer of the epidermis is disrupted and the bacteria gain access to highly differentiated subcorneal keratinocytes. Impetigo strains, but not pharyngeal strains, of *S. pyogenes* preferentially bind to differentiated keratinocytes,[8] mediated by *S. pyogenes* M protein.[9] A number of additional surface proteins serve as adhesins and promoters of internalization within epithelial cells,[10] and their preferential expression accounts for the selective tropism observed among different *S. pyogenes* strains.[11,12] Staphylococcal infection usually develops after nasal or skin colonization. Bacteriocin production may inhibit competing commensal flora, and several virulence factors have been proposed to enhance adhesion to epithelial cells and to underlying matrix proteins.[13] Surprisingly, the production of exfoliative toxins A and B has been associated with both nonbullous and bullous impetigo; Panton-Valentine leukocidin is generally absent among staphylococcal impetigo isolates.[5] Impetigo is a highly communicable infection. Spread in families (particularly among preschool-age children) is facilitated by crowding and poor hygiene.

Clinical Manifestations

Streptococcal impetigo begins on exposed areas as small vesicles, sometimes with narrow inflammatory halos, that rapidly pustulate and readily rupture. The purulent discharge dries and forms the characteristic thick, golden-yellow, "stuck-on" crusts. Pruritus is common, and scratching of lesions can spread infection. Occasionally, large crusts are produced by coalescence of smaller pustules. The lesions remain superficial and do not ulcerate or infiltrate the dermis; mild regional lymphadenopathy is common. Healing generally occurs without scarring. The lesions are painless, and constitutional manifestations are minimal.

Laboratory Findings

Gram-stained smears of vesicles show gram-positive cocci. Culture of exudate beneath an unroofed crust reveals *S. aureus,* group A streptococci, or a mixture of streptococci and *S. aureus.* The anti–streptolysin O titer after streptococcal impetigo is scant, probably related to inhibition of streptolysin O by skin lipids at the infection site. In contrast, the anti–desoxyribonuclease B response readily occurs after streptococcal impetigo (with elevated titers in 90% of patients with nephritis complicating streptococcal skin infections).[14]

Etiologic Agents

The group A streptococci responsible for impetigo usually belong to different M serotypes from those of strains that produce pharyngitis. M surface proteins, which are key virulence factors, are encoded in *emm* genes. Five major *emm* chromosomal patterns (groups A through E) have been described based on nucleotide sequences encoding part of the peptidoglycan-spanning M protein domain.[15] Almost all group A streptococcal impetigo isolates belong to *emm* chromosomal patterns D and, to a lesser extent, E, whereas most isolates from patients with uncomplicated pharyngitis or acute rheumatic fever belong to *emm* chromosomal pattern group A, B, or C. Groups C and G streptococci may rarely cause impetigo; group B streptococci have been associated with impetigo in neonates. The rising incidence of MRSA impetigo has been associated with both hospital- and community-acquired strains.[5]

Differential Diagnosis

Although the initial vesicular lesions may resemble early varicella, the crusts of impetigo are darker brown and harder. The central clearing of a confluent cluster of lesions of impetigo may suggest tinea circinata but can be distinguished by the thick crusts, which are not formed in the fungus infection. When the vesicles of herpes simplex become turbid, they may resemble those of impetigo. Distinguishing between herpes simplex and impetigo is important because irritation from topical therapy may exacerbate primary herpes simplex lesions. Acute palmoplantar pustulosis, a sterile, idiopathic, self-limited pustular eruption on the palms and soles that sometimes occurs after pharyngitis, may initially resemble impetigo.[16] Localized acute pustular psoriasis may also be mistaken for impetigo. Primary cutaneous listeriosis, an occupational disease of veterinarians and farmers involved in calving, is characterized by papulovesicular and pustular lesions on the forearms that may resemble those of impetigo.[17] Atopic or contact dermatitis, discoid lupus erythematosus, and infestations such as scabies may mimic impetigo or develop secondary impetiginization.[18]

Presumptive Therapy

Penicillin was the classic drug of choice for the treatment of impetigo, because of the predominant role of group A streptococci and the subsequent risk for acute glomerulonephritis. Because mixed streptococcal-staphylococcal or staphylococcal impetigo is clinically indistinguishable from streptococcal impetigo, and because *S. aureus,* either alone or in concert with *S. pyogenes,* currently is the predominant cause of impetigo, primary therapy must be revised accordingly. Penicillinase-resistant oral penicillins (e.g., dicloxacillin or amoxicillin-clavulanate) or cephalosporins (e.g., cephalexin, cefadroxil) are generally effective for treating methicillin-sensitive strains of *S. aureus* as well as *S. pyogenes.* The relative incidence of MRSA (vs. methicillin-susceptible *S. aureus* [MSSA]) causing impetigo is not well established, and likely differs among different populations. Erythromycin or newer macrolides generally are reserved for β-lactam–allergic patients. The efficacy of

macrolides may be reduced in areas in which erythromycin-resistant staphylococci and streptococci are prevalent. Local care (removal of crusts by soaking with soap and water) is helpful. Patients with extensive disease should undergo wound culture and reevaluation after an initial trial of oral therapy. The presence of MRSA must be strongly considered if there is a poor initial response to therapy, even if no culture data are available, and empirical therapy must be broadened (see later). Co-trimoxazole has excellent activity against community-acquired MRSA, and in vitro data support its possible use against *S. pyogenes* as well, although clinical data are limited.[19] A recent review suggests that this agent is useful both for presumed *S. aureus* and *S. pyogenes* skin infections.[20] Clindamycin and macrolides have variable efficacy against many community-acquired MRSA isolates and often vary in distinct populations. Topical antibiotic therapy may be considered when treating limited impetigo, including that caused by MRSA.

Topical mupirocin ointment in a polyethylene glycol base is as effective as oral erythromycin for the treatment of impetigo[21] and is more effective when treating erythromycin-resistant *S. aureus.*[22] In the past decade, the incidence of mupirocin resistance among *S. aureus* isolates has been rising slowly but steadily.[23] A second topical antibiotic, retapamulin ointment, is approved for the treatment of impetigo. This is the first of a novel class of bacterial protein synthesis inhibitors (pleuromutilins) and is effective against *S. aureus* and *S. pyogenes* independent of other antibiotic susceptibilities.[24] Fusidic acid cream is available in Europe and is effective in treating childhood impetigo (primarily *S. aureus*), but rising resistance rates[2] and policies to restrict its applicability for systemic infection have limited its use. Gentle application of topical agents is important to minimize tissue maceration and spread of infection. Systemic therapy is preferred when treating widespread impetigo. Close follow-up of patients is important because of rising rates of resistance to these topical and systemic agents.

Mupirocin has also been used topically to eradicate MRSA from secondarily infected skin lesions and from colonized patients. However, because resistance in *S. aureus* strains has emerged sooner than anticipated after the introduction of mupirocin,[22] particularly when long-term therapy was used, prolonged administration should probably be avoided.

Bullous Impetigo

Clinical Manifestations

The bullous form of impetigo is caused by *S. aureus* of phage group II (usually type 71); it occurs principally in neonates and young children and accounts for about 10% of all cases of impetigo. The lesions begin as vesicles that turn into flaccid bullae initially containing clear yellow fluid. No erythematous areola is noted, and the Nikolsky sign is absent. The bullae quickly rupture, leaving a moist red surface, and then form thin, varnish-like light brown crusts. Bullous impetigo, like the staphylococcal scalded skin syndrome (SSSS) and the staphylococcal scarlatiniform syndrome, represents a cutaneous response to the two extracellular exfoliative toxins (ETA and ETB) produced by phage group II *S. aureus. S. aureus* isolates from patients with bullous impetigo uniformly carry these exfoliative toxins.[5,25] ETA is chromosomally encoded, and the heat-labile ETB is plasmid encoded. Both are glutamate-specific serine proteases that bind to and cleave desmoglein-1, a desmosomal transmembrane glycoprotein necessary for epidermal cell adhesion.[25,26] The toxins may act locally at the site of cutaneous infection to produce bullous impetigo or may spread systemically to cause generalized blistering when produced at a site of cutaneous *S. aureus* infection. Staphylococci are regularly isolated from the skin lesions of bullous impetigo. Streptococcal superinfection rarely complicates bullous impetigo, probably because type 71 strains of *S. aureus* produce a bacteriocin that inhibits streptococci. Fever and constitutional symptoms are uncommon, and healing occurs without scarring. Mild infections are often missed[27] and have even been misdiagnosed as nonaccidental scalds in young children.[28] Rarely, bullous impetigo has been attributed to group A streptococcal infection.

Presumptive Therapy

Extensive bullous impetigo caused by MSSA generally responds to treatment with a penicillinase-resistant penicillin agent (e.g., dicloxacillin, 25–50 mg/kg daily in divided doses orally every 6 hours for a child; or amoxicillin-clavulanic acid), cephalosporin (e.g., cephalexin, 25–50 mg/kg daily in divided doses orally every 8–12 hours for a child), or erythromycin or clindamycin for a penicillin-allergic patient. When MRSA is widespread in the community, culture and initial oral therapy with co-trimoxazole, clindamycin, or linezolid may be considered for mild-to-moderate disease, with initial intravenously administered vancomycin reserved for patients with widespread disease.

Staphylococcal Scalded Skin Syndrome

SSSS is the most severe and systemic manifestation of infection with *S. aureus* strains producing an exfoliative exotoxin; it is characterized by widespread bullae and exfoliation.[26,29] Pemphigus neonatorum (Ritter disease) is SSSS in the neonate. The more general term *toxic epidermal necrolysis* is often used to encompass both SSSS and a morphologically similar syndrome of various causes (drug reactions, viral illnesses; see Chapter 194). SSSS has been associated with both MSSA and MRSA.

Clinical Manifestations

SSSS usually occurs in younger children but can rarely develop in adults. Epidemics have occurred in neonatal nurseries.[30] SSSS begins abruptly (sometimes a few days after a recognized staphylococcal infection) with fever, skin tenderness, and a scarlatiniform rash. The Nikolsky sign can be demonstrated. Large, flaccid, clear bullae form and promptly rupture, resulting in the separation of sheets of skin. New bullae appear over a period of 2 to 3 days. Exfoliation exposes large areas of bright red skin surface (Fig. 93.1). Denuded surfaces are generally devoid of *S. aureus,* since exfoliative toxins are disseminated hematogenously from a primary infection. Indeed, *S. aureus* may be recovered from a distant site of infection or colonization. This is in contrast to the ready recovery of organisms from sites of localized bullous impetigo, where toxin is elaborated at the site of the lesion. In settings in which the diagnosis



FIG. 93.1 Staphylococcal scalded skin syndrome in a young infant. Exfoliation has occurred on the face, chest, and groin, exposing areas of bright red skin surface.

of SSSS is uncertain, a biopsy obtained from an area of desquamation will demonstrate bland intraepidermal cleavage at the granular layer, which is distinct from the subepidermal separation observed in bullous disorders and toxic epidermal necrolysis. With appropriate fluid replacement and antimicrobial therapy, the skin lesions heal within 2 weeks, in contrast to drug-induced toxic epidermal necrolysis, in which recovery is more prolonged because the entire epidermis must be replaced and scarring is more frequent. The mortality rate from SSSS in children is less than 3% but is often higher in adults, many of whom have underlying immunodeficiency, renal failure, or other significant comorbidities.

Presumptive Therapy

Intravenous vancomycin is indicated for the initial treatment of SSSS because of the widespread staphylococcal infection and rapid progression of the skin lesions, particularly in settings such as a neonatal nursery and in communities with high rates of MRSA disease. Once MSSA is identified, intravenous nafcillin (100 mg/kg/day for neonates, 100–200 mg/kg/day for older children) is appropriate. Topical treatment consists of cool saline compresses. Systemic corticosteroids alone should not be used in the treatment of SSSS, although they may be indicated in therapy for drug-induced toxic epidermal necrolysis.

Staphylococcal Scarlet Fever

Staphylococcal scarlet fever is fundamentally a forme fruste of SSSS that does not progress beyond the initial stage of a generalized erythematous eruption. However, *S. aureus* enterotoxins (A through D) and toxic shock syndrome toxin 1 (TSST-1) are more frequently associated with staphylococcal scarlet fever than are ETA and ETB.[31] The rash is indistinguishable from that of scarlet fever, and Pastia lines can develop. However, pharyngitis is not usually present and an enanthem does not develop. Desquamation, beginning on the face and involving most of the body, occurs 2 to 5 days after onset of the scarlatiniform rash. Antibiotic treatment (penicillinase-resistant penicillins or alternative therapy effective against MRSA) is indicated.

Toxic Shock Syndrome

Toxic shock syndrome is another acute febrile illness with a generalized scarlatiniform eruption associated with *S. aureus* infection.[32] Other elements of the syndrome include (1) hypotension (shock), (2) functional abnormalities of three or more organ systems, and (3) desquamation in the evolution of the skin lesions[29,33] associated with the elaboration of TSST-1 or one of several staphylococcal enterotoxins. These exotoxins act as superantigens that activate large numbers of T cells, triggering systemic inflammation, distinct from the direct-acting exfoliative toxins responsible for bullous impetigo and SSSS (see Chapter 194).

Folliculitis

Folliculitis is a pyoderma located within hair follicles and the apocrine regions. The lesions consist of small (2–5 mm), erythematous, sometimes pruritic papules often topped by a central pustule and a fine surrounding collar of desquamation.[34] Sycosis barbae is a distinctive form of deep folliculitis, often chronic, that occurs on bearded areas. *S. aureus* is the usual cause of folliculitis. *Pseudomonas aeruginosa* (most often serotype O11) has been responsible for folliculitis acquired from swimming pools and whirlpools contaminated with large numbers of these organisms[35]; *Aeromonas* folliculitis has been reported rarely in similar settings. This type of skin infection produces pruritic, sometimes tender, papulourticarial lesions (appearing within 48 hours after exposure) that eventuate in pustule formation. Lesions in different stages of development (macules, papules, papulopustules) are present simultaneously. Preferred sites include the buttocks, hips, and axillae, particularly areas in contact with bathing suits; the palms and soles are generally spared. Otitis externa is also a common manifestation. Healing occurs spontaneously within 5 days, by drainage or regression. Rarely, scarring develops when a pustule progresses to furuncle formation. If folliculitis is acquired in a whirlpool, the lesions are sharply limited to the trunk below the upper part of the chest or neck. Inadequate chlorine levels in whirlpools, hot tubs, and swimming pools have been responsible for many of the outbreaks reported. In granulocytopenic and immunosuppressed hospitalized patients, *P. aeruginosa* O-11 from tap water used for washing was implicated in folliculitis that rapidly progressed to ecthyma gangrenosum.[36] Immersion is not required to develop *Pseudomonas* folliculitis; it has been reported in a toddler exposed to a contaminated washcloth and bath mat.[37] Folliculitis, often perioral and perinasal, caused by Enterobacteriaceae, can occur as a complication in patients with acne and rosacea, usually during prolonged courses of oral antibiotic therapy.[38] *P. aeruginosa* can also cause superinfection in patients with acne and rosacea after prolonged broad-spectrum antibiotic therapy.

Candida may cause folliculitis, typically producing pruritic satellite lesions surrounding areas of intertriginous candidiasis, commonly in infants or rarely in patients receiving prolonged antibiotic or corticosteroid therapy.[39] *Malassezia furfur,* a common skin saprophyte, or occasionally other dermatophytes, also produces a folliculitis with pruritic erythematous papules and papulopustules on the trunk, upper extremities, and face, particularly in the setting of diabetes mellitus, corticosteroid administration, or granulocytopenia,[39–41] and may be confused with acne vulgaris.[42] These lesions, particularly the early papulonodular ones, may suggest those of systemic candidiasis, a diagnosis that may seem to be supported by the presence of budding yeast forms on Gram-stained material from unroofed lesions. Unlike *Candida, M. furfur* requires lipid-supplemented media for primary isolation.

Additional nonbacterial folliculitis syndromes have been associated with herpes simplex, particularly in the presentation of severe sycosis barbae,[43] which may or may not be associated with secondary staphylococcal infection. Folliculitis, localized or disseminated, may occur after smallpox vaccination but does not represent progressive cutaneous viral infection.[44] Folliculitis has been associated with *Demodex* mite infestation[45] and rarely with parasitic disease.

Eosinophilic pustular folliculitis, a rare pruritic dermatosis characterized by recurrent crops of follicular papules and pustules with eosinophilic infiltration of perifollicular dermis, occurs particularly in the setting of acquired immunodeficiency syndrome (AIDS). It resembles bacterial or mycotic folliculitis but is a sterile process confirmed by biopsy.[46] Amicrobial pustulosis, another noninfectious folliculitis syndrome, may develop in normal hosts.[47] Folliculitis may develop after the use of tyrosine kinase inhibitors and immunomodulatory agents, including infliximab.[48]

Local measures such as saline compresses and topical antibacterial agents (e.g., mupirocin) or antifungal agents (e.g., clotrimazole) are usually sufficient to control the infection.[49] Severe or widespread disease (e.g., pseudomonal infection) may respond to oral fluoroquinolone therapy. Severe or refractory lesions should be cultured and considered for biopsy to assess uncommon causes of infection or noninfectious processes.

Furuncles and Carbuncles

Definition and Pathologic Characteristics

A furuncle (boil) is a deep inflammatory nodule extending into subcutaneous tissue that develops from preceding folliculitis. A carbuncle is a more extensive coalescent process involving multiple follicles that extends into the subcutaneous fat in areas covered by thick, inelastic skin. In the latter, multiple abscesses separated by connective tissue septa develop and drain to the surface along hair follicles.[21] *S. aureus* is almost invariably the causative agent (see Chapter 194), although occasionally coagulase-negative staphylococci can be recovered as the sole pathogen, particularly in elderly and immunocompromised patients.[50]

Clinical Manifestations

Furuncles occur in skin areas that are subject to friction and perspiration and contain hair follicles (especially the neck, face, axillae, and buttocks). Predisposing factors include obesity, blood dyscrasias, treatment with corticosteroids, defects in neutrophil function or number, and probably diabetes mellitus. MRSA as a cause of recurrent furunculosis is increasingly recognized.[51] A furuncle begins as a firm, tender, red nodule that soon becomes painful and fluctuant. Spontaneous drainage of pus commonly occurs, and the lesion subsides. A carbuncle is a larger, deeper, indurated, more serious lesion, usually located at the nape of the neck, on the back, or on the thighs. Fever and malaise are frequently

present, and some patients are acutely ill. As the lesion progresses, drainage occurs externally along the course of multiple hair follicles. A leukocytosis occurs, particularly if the lesion contains a large amount of undrained pus or if complicating cellulitis or bacteremia is present.

Bloodstream invasion can occur unpredictably (and is sometimes precipitated by manipulation of the lesions) and can result in endocarditis, osteomyelitis or other metastatic foci, and septic shock.[52] Lesions about the upper lip and nose present the special problem of possible spread of infection via the facial and angular emissary veins to the cavernous sinus.

Presumptive Therapy

Most furuncles are satisfactorily treated by the application of moist heat, which promotes localization and drainage of the process. Larger lesions may be drained to accelerate resolution. A carbuncle, a furuncle with surrounding cellulitis or fever, or a furuncle located about the midface generally requires systemic antibiotic therapy. Although conventional antistaphylococcal β-lactam antibiotics (e.g., dicloxacillin or cephalexin, 500 mg orally every 6–8 hours for an adult) are effective against MSSA, possible MRSA infection requires coverage with a suitable agent such as co-trimoxazole, often at generous dosage (e.g., 2 double-strength tablets twice daily). In a penicillin-allergic adult, clindamycin (300 mg orally every 6–8 hours), macrolides, or co-trimoxazole are alternatives, but in many communities there is significant resistance to macrolides and clindamycin. If the lesions are large and fluctuant, surgical drainage is indicated. Antibiotic treatment should be continued until evidence of acute inflammation has subsided. Patients with moderate to severe disease are best treated with initial parenteral therapy (e.g., vancomycin, linezolid, daptomycin, or ceftaroline; see Chapter 194).

Management of recurrent furunculosis presents a troublesome problem. This disease is not associated with a specific staphylococcal clonotype, although Panton-Valentine leukocidin expression appears to be a major virulence factor for the development of furuncles regardless of methicillin resistance status.[53] Most patients have no definable underlying defects in host defenses, although host abnormalities (e.g., granulocyte dysfunction, mannose-binding lectin deficiency) have been reported rarely[54,55]; *S. aureus* colonization (especially of the nares), recurrent skin trauma, and suboptimal hygiene are frequent contributing factors. Prophylaxis of recurrent episodes involves several measures:

1. *Antibiotic treatment.* Systemic antibiotic treatment as described should be administered for the most recent episode. Prolonged treatment (2 months) is no more effective than a 10- to 14-day course in preventing recurrences.
2. *General skin care.* Antibacterial soap and water should be used to reduce the number of *S. aureus* organisms on the body surface, and careful hand washing should be performed after contact with lesions. A separate towel and washcloth (carefully washed in hot water before reuse) should be reserved for the patient. Chlorhexidine solution (4%), an antimicrobial skin cleanser, hexachlorophene, or dilute bleach (0.005%; ½ cup in a 40-gallon tub)[56] may be used to decrease further staphylococcal skin colonization. Hexachlorophene is contraindicated for use in neonates and infants because of potential neurotoxicity.
3. *Care of clothing.* Sheets and underclothing should be laundered at high temperatures and changed daily.
4. *Care of dressings.* Draining lesions should be covered at all times with sterile dressings to prevent autoinoculation, and the dressings should be wrapped and promptly disposed of after removal.

Further measures aimed at elimination of nasal carriage and subsequent shedding of *S. aureus* (MSSA or MRSA) onto the skin may be warranted in the management of refractory cases. Intranasal application of a 2% mupirocin calcium ointment in a soft paraffin base twice daily for 5 days can eliminate short-term *S. aureus* carriage, with longer-term eradication success rates of approximately 60%.[57] Mupirocin resistance is very low in unselected community-acquired staphylococcal infections but may be substantial in selected populations.[58] Although oral rifampin has been used to eliminate staphylococcal nasal carriage and interrupt a cycle of recurrent furunculosis in selected patients, such monotherapy can lead to the rapid selection of rifampin-resistant strains. Intensive combined topical and systemic decolonization strategies (topical chlorhexidine, nasal mupirocin, and dual oral therapy with rifampin and doxycycline to prevent rifampin resistance) eradicated carriage in 74% of older adult patients colonized with MRSA.[58] Chlorhexidine resistance is uncommon but has been associated with the presence of staphylococcal plasmid-encoded efflux pumps and may contribute to decolonization failure.[59] Various staphylococcal vaccines have not proved effective in preventing recurrent furunculosis. Patients with recurrent furunculosis despite conventional treatment should undergo biopsy to assess possible uncommon pathogens; extensive furuncles caused by rapidly growing mycobacteria were identified in clients of a nail salon that had whirlpool footbaths.[60]

Ecthyma

The lesions of ecthyma begin in a fashion similar to those of impetigo but penetrate through the epidermis. Group A streptococci produce the lesions de novo or secondarily infect preexisting superficial lesions (e.g., insect bites, excoriations), with both mechanisms resulting in the same clinical picture.[61] Ecthyma lesions most frequently occur on the lower extremities, particularly in children and older adults. They consist of punched-out ulcers covered by greenish-yellow crusts that extend deeply into the dermis and are surrounded by raised violaceous margins. Treatment is the same as for impetigo. These group A streptococcal infections may lead to deeper parenchymal infection and also may trigger poststreptococcal glomerulonephritis.[61] Very extensive involvement with complicating bacteremia was reported in a patient with AIDS.[62] Invasive infections caused primarily by *P. aeruginosa* (ecthyma gangrenosum) may resemble streptococcal ecthyma initially but generally occur in compromised hosts and have a more aggressive course with frequent bacteremia (see later discussion); this pattern has been observed with a variety of pathogens and has sometimes developed in apparently normal hosts.[63]

Chancriform Lesions: Anthrax

A variety of infections, often with systemic consequences, are characterized by an initial chancriform lesion (see Table 93.1). Of the nonvenereal infections, anthrax has one of the most prominent chancriform lesions. (See Chapter 207 for a detailed discussion of anthrax.)

Pathogenesis

In the 20 years before 2001, naturally acquired anthrax infections, occurring in those working with raw imported wool, animal skins, and similar products contaminated with spores of *Bacillus anthracis,* were rare (<1 case/year) in the United States. Routine safety measures for such employees have almost eliminated anthrax from this group; sporadic cases still occur in transient workers in factories (e.g., ventilation repairmen) and in those who directly import wool for their own weaving. Most infections occur on the face, neck, or arms in an area with a minor abrasion. Anthrax has been associated with injection of heroin, particularly in the United Kingdom and other Western European countries, with characteristically deeper skin lesions.[64] Rarely, pulmonary infection occurs after inhalation of *B. anthracis* or intestinal anthrax results from ingestion of the organism (e.g., after playing animal-skin drums[65]). A bioterrorism-associated anthrax outbreak occurred suddenly in the United States in 2001, when *B. anthracis* spores were mailed in letters to Washington, DC, New York City, and Florida locations.[66] Twenty-two cases of anthrax ensued, 11 of the cutaneous form and 11 of the inhalational form.

Clinical Manifestations

After an incubation period of 1 to 8 days, a painless, sometimes pruritic, papule develops on an exposed area. The lesion enlarges, vesiculates (malignant pustule), and becomes surrounded by a wide zone of brawny, erythematous, gelatinous, nonpitting edema[67,68] as a result of local bacterial proliferation and toxin production.[69] The edema may become massive in young infants and in individuals with lesions on the face or neck and may suggest the diagnosis of cellulitis. Malaise and low-grade fever are present. As the lesion evolves, the initial vesicle enlarges and becomes hemorrhagic, necrotic, and covered by an eschar of variable



FIG. 93.2 Chancriform lesion of anthrax on the forehead. Note the prominent surrounding zone of gelatinous edema that is evident around the right eyelids. *(Courtesy Dr. Louis Weinstein, Boston.)*

TABLE 93.2 Initial Oral Antimicrobial Therapy for Cutaneous Anthrax When Risk of Bioterrorism Exists

DRUG	DOSAGE	DURATION
Adults		
Ciprofloxacin	500 mg q12h	60 days[b,c]
or		
Doxycycline	100 mg q12h	
Children		
Ciprofloxacin	15 mg/kg q12h (not to exceed 1 g/d)	60 days[b,c]
or		
Doxycycline[d]		
>8 yr and >45 kg	100 mg q12h	
>8 yr and ≤45 kg	2.2 mg/kg q12h	
≤8 yr	2.2 mg/kg q12h	

[a]See also Chapter 207.
[b]Previously, treatment recommended for cutaneous anthrax was for 7 to 10 days; this was increased to 60 days in the setting of bioterrorism risks because of the likelihood of concomitant aerosol exposure.
[c]Amoxicillin, 500 mg orally q8h for an adult (or 80 mg/kg/d, divided q8h, for a child), is an option for completion of treatment after clinical improvement.
[d]Use of tetracyclines is warranted in children because of the seriousness of the infection.
Modified from Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep. *2001;50:909–919.*

dimensions (Fig. 93.2). Frequently, regional lymphadenopathy is present. Uncommonly, lymphangitis occurs from the initial process or from secondary infection. At all stages, the lesion remains painless. If untreated, bacteremic dissemination of infection from a skin site may occur, accompanied by high fever and hypotension. Meningitis may complicate bacteremic infection or primary pulmonary anthrax.

The epidemiologic background and the striking appearance of extensive gelatinous edema serve to distinguish anthrax from other types of chancriform lesions. A staphylococcal pustule or carbuncle with a necrotic eschar may be mistaken for early anthrax. However, the former is very painful and tender, and the causative agent can usually be demonstrated on a Gram-stained smear of material from the lesion. Other lesions mimicking the eschar of anthrax include ecthyma (usually lacking edema), ecthyma gangrenosum (usually in neutropenic patients with *P. aeruginosa* bacteremia), brown recluse spider bite (primarily in rural areas in southern and southwestern states and painful during incipient necrosis), orf (after exposure to sheep and with scab but without large eschar or gelatinous edema), and other infection-associated eschars at sites of tick or mite bites (e.g., from tularemia, rickettsial spotted fevers, scrub typhus).

Presumptive Therapy

Incision and débridement should be avoided because they may increase the likelihood of bacteremia, but skin punch biopsy after initiation of antimicrobial therapy may be necessary to establish the diagnosis by culture, immunohistochemical staining, or polymerase chain reaction (PCR) testing for *B. anthracis.* Almost all naturally occurring strains are susceptible to penicillin, and it has been the drug of choice for decades. With the concern that bioterrorism attack strains might be weaponized to be resistant to penicillins (and other commonly used antimicrobial agents), the initial treatment of cutaneous anthrax with oral ciprofloxacin or doxycycline has been recommended (Table 93.2).[70] Susceptibility studies of *B. anthracis* strains (both naturally occurring strains and those from the 2001 bioterrorism attacks) demonstrate susceptibility to ciprofloxacin, tetracyclines, clindamycin, imipenem, rifampin, chloramphenicol, aminoglycosides, cefazolin, vancomycin, macrolides, and linezolid. Obiltoxaximab, a monoclonal antibody directed against the protective antigen of *B. anthracis,* has been licensed as an adjunctive agent for the prophylaxis and therapy of inhalational anthrax.[71]

Erysipelas

Erysipelas is a distinctive type of superficial cellulitis of the skin, with prominent lymphatic involvement. It is generally attributed to infection by group A streptococci (uncommonly, by group C or G streptococci and rarely by group B streptococci, particularly in neonates, or *S. aureus*).[72] Admittedly, the causative pathogen is difficult to identify in nonbacteremic cases. Recent reviews of bacteremic patients with erysipelas recovered pathogens in 5% to 9% of cases, with 30% to 50% caused by group A streptococci, up to 40% due to other β-hemolytic streptococci, and roughly 10% to *S. aureus* or gram-negative pathogens.[73,74]

Clinical Manifestations

Erysipelas is more common in infants, young children, and older adults. Formerly, the face was most commonly involved, and an antecedent streptococcal respiratory tract infection preceded cutaneous involvement in about one-third of patients. Now, 70% to 80% of erysipelas lesions involve the lower extremities and 5% to 20% are on the face.[74,75] Portals of entry may be skin ulcers, local trauma or abrasions, psoriatic or eczematous lesions, or fungal infections, but often the skin of the involved area is grossly intact. In the neonate, erysipelas may develop from an infection of the umbilical stump. Predisposing factors include subclinical[76] or extensive lymphedema, venous stasis, obesity, paraparesis, diabetes mellitus, alcohol abuse, and nephrotic syndrome. Erysipelas tends to occur in areas of preexisting lymphatic obstruction or edema (e.g., after a radical mastectomy). In addition, because erysipelas itself produces lymphatic obstruction, it tends to recur in an area of earlier infection. Over a 3-year period, the recurrence rate is about 30%,[74,75] predominantly in individuals with venous insufficiency or lymphedema. Asymptomatic anal colonization with group A or G streptococci[77] or vaginal carriage of group B streptococci[78] may serve as a reservoir in individuals with relapsing erysipelas (or cellulitis).

Erysipelas is a painful lesion with a bright red, edematous, indurated (peau d'orange) appearance and an advancing, raised border that is sharply demarcated from the adjacent normal skin (Fig. 93.3). Fever is a feature. Facial erysipelas commonly extends across the bridge of the nose with bilateral involvement of the cheeks. Uncomplicated erysipelas remains confined primarily to the lymphatics and the dermis. Bullous erysipelas is a complication of severe disease that is observed in about



FIG. 93.3 Facial erysipelas involving both cheeks and bridge of nose. The sharp demarcation between the bright red area of erythema and the normal surrounding skin is evident. *(From Fitzpatrick TB, Eisen AZ, Wolff K, et al, eds.* Dermatology in General Medicine. *New York: McGraw-Hill; 1971.)*

5% of cases. The bullae are flaccid and intraepidermal, and cultures of blister fluid are often sterile before erosion occurs[79]; in some cases, *S. pyogenes* or *S. aureus* or both have been recovered, including MRSA strains.[80] Occasionally, the infection extends more deeply and produces cellulitis, subcutaneous abscess, and necrotizing fasciitis.

Leukocytosis is common. Group A streptococci usually cannot be cultured from the surface of the skin lesion, and only rarely can they be isolated from punch biopsy or tissue fluid aspirated from the advancing edge of the lesion. In cases of erysipelas complicating infected ulcers, group A and other β-hemolytic streptococci have been isolated from the ulcerated area in up to 30% of patients.

Differential Diagnosis

The diagnosis is made on the basis of the appearance of the lesion and the clinical setting. Early herpes zoster involving the second division of cranial nerve V may resemble unilateral facial erysipelas but can be distinguished by the pain and hyperesthesia preceding the skin lesions. Occasionally, contact dermatitis or giant urticaria may look like erysipelas but can be distinguished by the absence of fever and the presence of pruritus. Lesions closely resembling erysipelas, but apparently not caused by streptococcal infection, may occur repeatedly in patients with familial Mediterranean fever.[81] Diffuse inflammatory carcinoma of the breast may mimic low-grade erysipelas. Erythema chronicum migrans, the cutaneous lesion of Lyme disease, resembles erysipelas but is not painful, progresses much more slowly, may demonstrate central clearing, and has less associated fever. An erysipelas-like skin lesion was reported in several patients with hypogammaglobulinemia and *Campylobacter jejuni* bacteremia.[82]

Presumptive Therapy

Mild early cases of erysipelas in an adult may be treated with oral penicillin V (500 mg every 6 hours) or initial intramuscular procaine penicillin (600,000 units once or twice daily). Erythromycin (250–500 mg orally every 6 hours) or other macrolides are suitable alternatives in allergic patients; in some areas *S. pyogenes* demonstrates frequent macrolide resistance and clindamycin may be more appropriate, although clindamycin resistance among group A streptococci is rising (~15%).[83]

For more extensive erysipelas, patients should be hospitalized and receive parenteral aqueous penicillin G (2 million units every 6 hours), a first-generation cephalosporin, or ampicillin-sulbactam therapy. Although typical erysipelas can be readily distinguished from cellulitis, which can be of staphylococcal or streptococcal origin, the differentiation occasionally may not be clear-cut. Under such conditions, antistaphylococcal therapy (nafcillin, oxacillin, ampicillin-sulbactam, or a first-generation cephalosporin) should be considered. In an acutely ill patient or a patient with significant bullous erysipelas, intravenous administration of vancomycin is indicated to treat possible MRSA[80] or nonsusceptible streptococci pending blood culture results. Chronic antibiotic suppression (oral penicillin or parenteral benzathine penicillin) may reduce the recurrence rate of erysipelas or lower extremity cellulitis.[84–86]

Cellulitis

Cellulitis is an acute spreading infection of the skin that extends deeper than erysipelas and involves the subcutaneous tissues. Group A streptococcus, other β-hemolytic streptococci, and *S. aureus* are the most common causative agents.

Clinical Manifestations

Previous trauma (laceration, abrasion, puncture wound), often minor (shaving or athletic abrasion), or an underlying skin lesion (furuncle, ulcer, dermatophyte infection) predisposes to the development of cellulitis. Occasionally, secondary cellulitis results from bloodborne spread of infection to the skin and subcutaneous tissues; rarely, it is caused by direct spread from subjacent infections (subcutaneous abscesses, fistulas from osteomyelitis). Within several days after the inciting trauma, local tenderness, pain, and erythema develop and rapidly intensify. Malaise, fever, and chills develop. The involved area is often extensive, with marked erythema, warmth, and swelling. In contrast to erysipelas, the borders of a cellulitic area are not elevated and sharply demarcated; patchy involvement with skip areas may occur. Regional lymphadenopathy is common, lymphangitic streaking proximal to the cellulitic area is occasionally present, and bacteremia can occur. Local abscesses may develop, and small patches of overlying skin may subsequently undergo necrosis. A nidus of suppuration with surrounding cellulitis is a hallmark of staphylococcal cellulitis, although this diagnostic finding is not always present. Superinfection with gram-negative bacilli may supervene.

Cellulitis caused by group A streptococci may occur as a postoperative wound infection. Although it is uncommon today, it is particularly noteworthy because of the rapidity with which it can spread and invade the bloodstream. Such infection may be manifested within 6 to 48 hours after surgery (comparable to the short incubation period of postoperative clostridial myonecrosis), earlier than the usual postoperative staphylococcal infection, which is not evident for at least several days after surgery. Hypotension, often associated with bacteremia, may be the initial sign of infection, before significant incisional erythema is evident. A thin serous discharge may be expressed on compression of the wound margins, and streptococci can be identified on a Gram-stained smear.

Cellulitis is a serious disease because of the propensity of infection to spread via the lymphatics and bloodstream. Cellulitis of the lower extremities, particularly in older patients, may be complicated by thrombophlebitis. In patients with chronic dependent edema, cellulitis may spread extremely rapidly.

A form of cellulitis that is distinctive by virtue of its clinical setting occurs in the lower extremities of patients whose saphenous veins have been harvested for coronary artery bypass surgery.[87] Occasionally, an associated lymphangitis is present. In some patients, episodes of cellulitis are recurrent. Systemic manifestations such as chills, high fever, and toxicity are prominent. The area of cellulitis extends along the course of the saphenous venectomy, with marked edema, erythema, and tenderness. Occasionally, the involved areas are somewhat similar to those observed in erysipelas (pseudoerysipelas). Although a bacterial cause is not defined in most cases, isolates available from involved skin or blood implicate non–group A β-hemolytic streptococci (groups C, G, and B) as major causes. The portal of entry of the infection is often an associated area of tinea pedis. The combination of compromised lymphatic drainage and minor venous insufficiency after saphenous venectomy may result in lower leg edema, a favorable setting for cellulitis. Endoscopic

TABLE 93.3 Anatomic Variants of or Predispositions to Cellulitis

ANATOMIC VARIANT OR PREDISPOSITION	LOCATION	LIKELY BACTERIAL CAUSE
Periorbital cellulitis	Periorbital	*Staphylococcus aureus, Streptococcus pneumoniae,* group A streptococci
Buccal cellulitis	Cheek	*Haemophilus influenzae* type b
Cellulitis complicating body piercing	Ear, nose, umbilicus	*S. aureus,* group A streptococci
After mastectomy (with axillary node dissection)[77]	Ipsilateral upper extremity	Non–group A β-hemolytic streptococci
After lumpectomy (with limited axillary node dissection, breast irradiation)[78]	Ipsilateral breast	Non–group A β-hemolytic streptococci
After saphenous vein harvest for coronary artery bypass	Ipsilateral leg	Group A or non–group A β-hemolytic streptococci
After radical pelvic surgery, radiation therapy	Vulva, inguinal areas, legs	Group B and group G streptococci
After liposuction	Thigh, abdominal wall	Group A streptococci, peptostreptococci
Postoperative (very early) wound infection	Abdomen, chest, hip	Group A streptococci
Injection drug use ("skin popping")	Extremities, neck	*S. aureus,* streptococci (groups A, C, F, G)[a]
Perianal cellulitis	Perineum	Group A streptococcus

[a]Other bacteria to consider based on isolation from skin or abscesses in this setting include *Enterococcus faecalis,* viridans-group streptococci, coagulase-negative staphylococci, anaerobes (including *Bacteroides* and *Clostridium* spp.), and Enterobacteriaceae.

venous harvesting appears to have a lower risk for postoperative lower extremity cellulitis than open venectomy.[88,89] The inflammation from an initial episode of cellulitis, erysipelas, or lymphangitis obstructs lymphatic drainage, enhancing the predisposition to further episodes of infection. Other variants of postoperative cellulitis or predispositions to cellulitis have been described (Table 93.3).[90–92]

Recurrent cellulitis is a common and vexing problem and has been observed in 22% to 49% of patients with cellulitis.[93,94] A variety of predisposing local factors have been reported, including chronic edema, lymphedema, venous insufficiency, deep venous thrombosis, peripheral vascular disease, tinea pedis, and saphenous vein surgery, as well as obesity, which contributes to venous stasis and lymphedema.[93,95] In addition to providing increased quantities of interstitial protein-rich fluid, lymphedema affects immune cell trafficking and innate immune processes, which may contribute to the risk of recurrent cellulitis.[96,97] Microsurgical lymphovenous anastomosis has been studied to diminish lymphedema and appears to reduce the rate of recurrent cellulitis.[98] Recurrent episodes of cellulitis or pseudoerysipelas caused by groups B and G streptococci have also occurred in patients with lower extremity lymphedema secondary to radical pelvic surgery, radiation therapy, or neoplastic involvement of pelvic lymph nodes.[90] Typically, the cellulitis involves the vulva, inguinal areas, and both lower extremities. In this setting, recurrent episodes have occurred in association with recent coitus.[99] An uncommon but distinctive form of streptococcal cellulitis, perianal group A streptococcal cellulitis, occurs principally in children.[100,101] The clinical features consist of perianal pruritus, purulent secretions, intense perianal erythema, pain on defecation, blood-streaked stools from anal fissures, and chronicity (months) if not treated with penicillin. Relapse is relatively common because perianal colonization often persists despite appropriate systemic oral therapy and eradication attempts with clindamycin or rifampin are not consistently effective.

Depending on the clinical setting, cellulitis caused by a number of nonstreptococcal pathogens may be seen.[102] Before routine immunization of infants with the conjugated protein polysaccharide *Haemophilus influenzae* type b vaccine, buccal cellulitis—originating in the upper respiratory tract, often demonstrating a bluish (blue dome) appearance, and caused by *H. influenzae* type b—accounted for up to 25% of cases of facial cellulitis in infants in the 3- to 24-month age group.[102] Complicating bacteremia was frequently present. Rarely, pneumococcal facial cellulitis acquired through the bacteremic route presents in children[103] and in adults with a variety of underlying systemic risk factors.[104] Soft tissue infections by the pneumococcus can bear a striking resemblance to streptococcal erysipelas. A rare but particularly troublesome, chronic, and progressive form of cellulitis, known as dissecting cellulitis of the scalp or perifolliculitis capitis, is probably similar to hidradenitis suppurativa and acne conglobata in pathogenesis, and often occurs in conjunction with these inflammatory disorders.[105] The clinical features consist of recurrent painful, fluctuant dermal and subcutaneous nodules accompanied by purulent drainage from burrowing interconnecting abscesses, scarring, and alopecia. *S. aureus* is most commonly isolated. Effective treatment has involved wide excision and skin grafting. A variety of antiinflammatory regimens, including isotretinoin, dapsone, and infliximab, have been reported to be successful in case reports in the literature.

A polymorphonuclear leukocytosis is usually present, regardless of the bacterial cause of cellulitis. Although culture of needle aspirates from areas of cellulitis is not indicated ordinarily because of generally low sensitivity rates, such studies provide the best information on likely pathogens,[106] as noted in a recent systematic review demonstrating that such direct sampling identified a pathogen in only 16% of 808 patients. Together, *S. aureus* (~50%) and group A streptococci (~25%) represented over 75% of recovered pathogens. The remaining identified pathogens included group B and viridans-group streptococci, *Enterococcus faecalis,* gram-negative bacilli (Enterobacteriaceae, *H. influenzae, Pasteurella multocida, P. aeruginosa,* and *Acinetobacter* spp.), and rarely *Candida albicans.*[106] Cultures of ulcers and abrasions contiguous with areas of cellulitis have shown similar gram-positive pathogens (*S. aureus* or group A streptococci, or both). It is reasonable to consider diagnostic aspiration only if unusual pathogens are suspected (e.g., in immunocompromised patients), fluctuant areas are detected, or initial antimicrobial therapy has been unsuccessful.

A broader spectrum of pathogens has been isolated from deep wounds or débrided tissue in diabetic patients with limb-threatening infections (including cellulitis).[107] These include gram-positive aerobes in the majority of patients (*S. aureus,* including methicillin-resistant *S. aureus; Enterococcus* spp.; various streptococcal species), with common recovery as well of both gram-negative aerobes (Enterobacteriaceae, *Acinetobacter, P. aeruginosa*) and anaerobes (*Bacteroides, Peptococcus*). In cellulitis complicating decubitus ulcers, this broad range of microorganisms also should be considered as potential pathogens. If this complication develops in a hospitalized patient, resistant nosocomial pathogens should be considered when deciding on empirical antibiotic coverage.[107] *Helicobacter cinaedi* cellulitis and bacteremia have been reported in immunocompetent patients recovering from orthopedic surgery[108] and in immunocompromised hosts.[109]

Blood cultures are positive in only 2% to 8% of patients with community-acquired cellulitis,[73,93] although, recently, positive blood cultures were reported in 18.4% of patients in a large prospective multicenter analysis conducted in Spain.[94] Roughly three-fourths of isolates are group A or other β-hemolytic streptococci (particularly group C or G), or *S. aureus,* and the remainder are diverse gram-negative pathogens, including *H. influenzae, P. multocida, P. aeruginosa,* Enterobacteriaceae, or *Vibrio vulnificus.* Blood cultures appear to be positive more frequently with cellulitis superimposed on lymphedema.[110]

Environmental exposures are often important risk factors for the development of cellulitis. *Erysipelothrix rhusiopathiae* is the causative agent of erysipeloid, a somewhat indolent cellulitis occurring principally in persons who handle saltwater fish, shellfish, poultry, meat, and hides[111] (see Chapter 209). The infection, which usually occurs in the summer,

is introduced through an abrasion on the hands. A painful violaceous area appears within 1 week after the injury. As the process spreads peripherally with distinct raised borders, the central portion of the lesion clears. Ulceration is not a feature. Occasionally, an adjacent joint is involved; rarely, bacteremia and endocarditis may follow. The causative organism is not usually observed in Gram-stained drainage from the lesion but may be isolated on culture of a biopsy specimen taken from the advancing margin of the lesion. The development of a typical lesion in a person handling fish or meat products suggests the diagnosis. Other forms of bacterial cellulitis or erysipelas may resemble erysipeloid, particularly if the lesion is on the hand and evolves gradually. A somewhat similar lesion of unknown origin, called seal finger, occurs in aquarium workers and veterinarians secondary to seal bites or trauma sustained in caring for these animals. Although penicillin is the antibiotic of choice for the treatment of erysipeloid, it appears that seal finger responds to tetracycline.[112] In support of this practice, a novel *Mycoplasma* species has been identified by 16S ribosomal RNA PCR from a patient with seal finger and subsequent disseminated infection.[113] *Aeromonas hydrophila,* a gram-negative bacillus found particularly in lakes, rivers, and soil, may produce an acute cellulitis after introduction of the organism through a laceration acquired during swimming in fresh water. These infections occur most often in spring and summer.[114,115]

Cellulitis, bullous lesions, or necrotic ulcers may complicate infection of a traumatic wound sustained in salt water (or brackish inland waters)[116] or from contact with raw seafood. Such infections, caused by *Vibrio* species (primarily *V. vulnificus* but also occasionally *Vibrio alginolyticus,* non–serogroup O1 *Vibrio cholerae,* and *Vibrio parahaemolyticus*),[115] can result in bacteremia and progress to necrosis, which requires extensive surgical débridement.[117] A rapidly progressive primary septicemia caused by *V. vulnificus* may occur after entry of the organism through the gastrointestinal tract (e.g., consumption of raw oysters) rather than through abraded skin. Cellulitis with hemorrhagic skin bullae often occurs rapidly after the bacteremia. Particularly at risk for the septicemic form of disease are patients with alcoholic cirrhosis, hemochromatosis, or thalassemia—presumably as a result of enhanced growth of *V. vulnificus* mediated by these processes associated with enhanced iron storage. The systemic *Vibrio* infections have mortality rates of 20% to 40%.[118,119] These vibrios are generally susceptible in vitro to tetracyclines, chloramphenicol, aminoglycosides, and third-generation cephalosporins. Tetracyclines have been considered first-line treatment of *V. vulnificus* infections, with cefotaxime and ciprofloxacin as alternatives.[102] Combination therapy with doxycycline and a third-generation cephalosporin is frequently recommended.[115] A variety of bacteria (*Serratia, Proteus,* other Enterobacteriaceae, *Campylobacter* [both *C. fetus* and *C. jejuni*], and *H. cinaedi*[120]) and fungi (*Cryptococcus neoformans,*[121] *Fusarium* spp.[122]) that rarely cause cellulitis in healthy individuals may produce bloodborne cellulitis in an immunocompromised or granulocytopenic patient. *Legionella* species have very rarely produced cellulitis (*L. pneumophila* in association with pneumonia[123] and *L. micdadei* in a renal transplant recipient[124]). Spontaneous *Escherichia coli* cellulitis occurs in children with symptomatic nephrotic syndrome[125] and in neutropenic patients with underlying hematologic malignancy, often with accompanying septic shock.[126]

A variety of processes that resemble cellulitis in appearance should be distinguished from it.[93,127,128] These include infections as well as inflammatory and neoplastic entities (Table 93.4).

Envenomation after puncture wounds by the spines of a stonefish (indigenous to shallow waters of the South Pacific) produces local edema and erythema that may suggest acute bacterial cellulitis acquired in seawater.[129] This reaction may be accompanied by serious systemic toxicity, including acute pulmonary edema. Numerous other marine species can provoke cutaneous reactions with prominent vesicular formation or urticaria that may mimic cutaneous infection.[130]

Familial Mediterranean fever is seen in Sephardic Jews and in those from the Middle East. Patients have a history of previous bouts of fever, sometimes accompanied by erysipelas- or cellulitis-like, noninfectious episodes of localized erythema and often by crises of abdominal pain.[81,131]

Sweet syndrome consists of the acute development of tender erythematous pseudovesiculated plaques, fever, and neutrophilic leukocytosis, often associated with malignancy or a variety of medications.[132,133] If lesions occur on the face, they may suggest erysipelas or periorbital cellulitis.[134]

Kawasaki disease occurs in infancy or childhood and is characterized by fever, conjunctivitis, acute cervical lymphadenopathy, and a polymorphic eruption. Facial rash and conjunctivitis may suggest periorbital cellulitis,[135] and trunk and especially perineal involvement may suggest cellulitis. The constellation of clinical and laboratory findings typically supports the diagnosis of Kawasaki disease.

The appearance and clinical features of Wells syndrome (eosinophilic cellulitis) consist of urticaria-like, moderately erythematous, edematous lesions that develop rapidly and are often accompanied by fever. It can be distinguished from the usual bacterial cellulitis by its minimal tenderness, lack of local heat, and failure to respond to antibiotics. Biopsy of the early lesion shows marked infiltration of the dermis with eosinophils. The lesions resolve in several weeks but frequently recur and respond to corticosteroid therapy.[136] A variety of empirical therapies have been employed, but randomized clinical trials are lacking.[137]

Lymphatic cutaneous metastases from neoplasms, particularly adenocarcinoma, may produce a localized, edematous, erythematous lesion resembling cellulitis. First described as inflammatory carcinoma of the breast, carcinoma erysipeloides involves the skin overlying the site of the primary tumor or at sites of distant metastases. Progression is slower than that of cellulitis, and fever is not a feature.[138] Very rarely, lymphomatous involvement of subpectoral or retromammary nodes may produce an erythematous lymphedema of the breast, suggesting subacute cellulitis or inflammatory carcinoma of the breast.

TABLE 93.4 Infectious and Noninfectious Processes to Be Distinguished From Cellulitis

Infections
Herpes simplex
Herpes zoster
Necrotizing fasciitis types I and II
Anaerobic myonecrosis (gas gangrene)
Cutaneous anthrax with prominent surrounding gelatinous edema
Prominent response to vaccination with vaccinia
Erythema chronicum migrans lesion of Lyme disease
Inflammatory and Neoplastic Processes
Hematoma
Venous stasis dermatitis
Calciphylaxis
Insect bite (hypersensitivity response)
Fixed drug reaction
Envenomation from spines of stonefish
Acute gout
Deep venous thrombophlebitis of lower extremity
Familial Mediterranean fever–associated cellulitis-like erythema
Pyoderma gangrenosa (particularly lesions starting in subcutaneous fat as acute panniculitis)
Sweet syndrome (acute febrile neutrophilic dermatosis)
Kawasaki disease
Wells syndrome (eosinophilic cellulitis/fasciitis)
Carcinoma erysipeloides

Modified from Swartz MN. Clinical practice: cellulitis. N Engl J Med. *2004;350:904–912. Copyright ©2004 Massachusetts Medical Society.*

Presumptive Therapy

Concern regarding MRSA infection has complicated routine empirical cellulitis therapy.[21,93,139,140] Traditionally, β-lactam antibiotics active against penicillinase-producing *S. aureus* were administered for the initial treatment of cellulitis because the great majority of cases were caused by streptococci or MSSA; cephalosporins were sometimes used for lower extremity infection, particularly when comorbid conditions (diabetes mellitus, peripheral vascular disease) or trauma was present. Streptococcal cellulitis typically has a more acute presentation and progression than staphylococcal disease, and β-lactam therapy can be initiated in this setting. More indolent cellulitis or infection associated with a focal abscess, pustule, or eschar is more suggestive of staphylococcal disease and should be addressed initially with therapy effective against MRSA.[141] The Infectious Diseases Society of America practice guidelines distinguish

between purulent and nonpurulent cellulitis; empirical therapy for the former should be targeted toward MRSA, whereas empirical therapy for the latter may still include a penicillinase-resistant β-lactam to treat hemolytic streptococci and MSSA.[140] After initiating empirical therapy, it is crucial to monitor patients closely and to revise therapy if there is a poor response to initial treatment.

Intravenous antibiotic therapy is essential if the lesion is rapidly spreading, the systemic response is prominent, or there are significant comorbidities (i.e., asplenia, neutropenia, immunocompromise, cirrhosis, cardiac or renal failure, local trauma, or preexisting edema). Cefazolin (1.0–1.5 g IV every 8 hours) or nafcillin (2.0 g IV every 4–6 hours) is a reasonable choice for an average-risk adult with moderately severe nonpurulent cellulitis. When addressing severe cellulitis or moderate disease in high-risk individuals as well as in penicillin-allergic individuals, vancomycin (1.0 g IV every 12 hours) or linezolid (0.6 g IV every 12 hours) is indicated. Once-daily intravenously administered daptomycin is also an option for treatment of MRSA staphylococcal soft tissue infections; the "fifth-generation" cephalosporin ceftaroline may evolve to become a more common, cost-effective second-line therapy against MRSA skin infections.[142] Several newly approved agents with broad activity against gram-positive pathogens and unique pharmacokinetic features are also available, including the lipoglycopeptide agents dalbavancin, telavancin, and oritavancin, and the second-generation oxazolidinone agent tedizolid. Their cost-effective role in the management of patients with cellulitis and other skin infections is not established.[143] After nafcillin therapy for nonpurulent cellulitis, dicloxacillin or cephalexin (0.5 g every 6–8 hours) or a longer-acting agent (e.g., cefadroxil 0.5–1.0 g once or twice daily) may be given once fever abates and skin lesions begin to resolve. Follow-up oral therapy for adult patients with MRSA infection requires linezolid (0.6 g every 12 hours); clindamycin may be used if MRSA is recovered from purulent material and sensitivity is confirmed in vitro.

In many communities, MRSA isolates have significant rates of clindamycin resistance. If the cellulitis is early and mild and no significant comorbidities are present, initial therapy with the previously noted oral follow-up antimicrobial agents may be used initially. Although co-trimoxazole is not approved by the US Food and Drug Administration for the treatment of MRSA skin and soft tissue infections, it is widely utilized to treat proven and presumed staphylococcal (especially MRSA) skin infections.[144] A recent prospective trial assessing the benefit of oral co-trimoxazole in addition to cephalexin for the treatment of outpatient cellulitis showed no added benefit, presumably reflecting the paucity of MRSA as the etiology of these infections.[145] A recently conducted study investigated treatment of 524 patients with cellulitis or skin abscesses or both, treated with either clindamycin or co-trimoxazole. The proportion of patients cured was similar in both groups (in evaluable patients, 89.5% in the clindamycin group and 88.2% in the co-trimoxazole group),[146] although the incidence of clindamycin resistance among the methicillin-resistant staphylococcal isolates was low (4.5%). The comparability of these agents might possibly differ in areas where there is a significantly higher rate of resistance to either agent. There were nonsignificant differences favoring co-trimoxazole in patients who had abscesses, and favoring clindamycin in those who had only cellulitis.[147] A recently conducted study compared treatment of uncomplicated skin abscesses undergoing drainage with supplemental trimethoprim-sulfamethoxazole (320/1600 mg twice daily) for 7 days or placebo, and achieved clinical cure of abscesses in 80.5% of trimethoprim-sulfamethoxazole recipients compared with 73.6% of placebo recipients ($P < .001$).[148] The majority of isolates that were obtained were MRSA (43.5%) or MSSA (15.9%).

Cellulitis in the setting of a diabetic foot infection may involve a much wider spectrum of potential pathogens and warrants broader antimicrobial coverage, such as ampicillin-sulbactam (3.0 g IV every 6 hours in adults), imipenem-cilastatin or meropenem, or other antimicrobial combinations targeting anaerobes as well as gram-positive and gram-negative aerobes.[107] Vancomycin coverage against MRSA is frequently administered as well, particularly in patients who have had prior MRSA infection, reside in areas of high MRSA incidence, or have severe infection.[107]

Specially tailored initial antimicrobial therapy is warranted if additional bacterial species are likely to be involved in cellulitis after unusual exposures. These include human or animal bites, for which initial therapy might involve ampicillin-sulbactam given intravenously or amoxicillin-clavulanate orally (500 mg every 8 hours or 875 mg every 12 hours in an adult). In the setting of cellulitis after an abrasion or laceration occurring with saltwater exposure, in which *V. vulnificus* might be the pathogen, treatment with doxycycline (200 mg IV daily in two divided doses) along with an antimicrobial agent targeted to pathogens that are more common is appropriate. Similarly, in the setting of cellulitis after an abrasion or laceration occurring with freshwater exposure, in which *A. hydrophila* might be involved, initial treatment with ciprofloxacin (400 mg IV or 500 mg orally every 12 hours) along with an antimicrobial agent targeted to more common pathogens is indicated; alternatively, a combination of ceftazidime plus gentamicin may be used.

Initial local care of cellulitis includes immobilization and elevation of the involved limb to reduce swelling and application of a cool, sterile saline dressing to remove purulent exudate from any associated ulcer or infected abrasion and to decrease local pain. Patients who have cellulitis at the saphenous site after coronary artery bypass surgery and fungal infection in the interdigital spaces should be treated topically for the latter with miconazole, clotrimazole, or terbinafine. The initial antibiotic (e.g., cefazolin) should be given by the intravenous route for 3 to 5 days, until fever abates and skin findings begin to resolve, to ensure prompt resolution before switching to other routes of therapy. Attention to the problem of tinea pedis before bypass surgery can prevent this form of cellulitis. Similar prompt attention to pedal epidermophytosis in patients who have had one such episode of cellulitis can obviate subsequent episodes.

Recurrent episodes of cellulitis usually occur in patients with peripheral edema. The use of support stockings and good skin hygiene can reduce its frequency or eliminate recurrences. Patients with lymphedema may benefit from regular lymphatic pneumatic compression treatments to improve chronic lymphedema and reduce the frequency of recurrent infections.[149] In the occasional patient who continues to have frequent episodes of cellulitis or erysipelas despite such measures, prophylactic monthly benzathine penicillin, oral penicillin V (250–500 mg twice daily),[85,86,150] or erythromycin (250 mg orally once or twice daily) for the penicillin-allergic patient may be indicated.[21]

Membranous Ulcers

Infected ulcers of varied or mixed bacterial origin may be covered at their base by a layer of necrotic debris resembling a membrane. The latter is not usually strongly adherent and can be removed without much difficulty. In addition, such a lesion generally has abundant purulent drainage, attributable to infection with pyogenic bacteria. Membrane-covered lesions (both superficial and deep ulcers) are also produced by cutaneous infection with *Corynebacterium diphtheriae* (see next section).

Cutaneous Diphtheria

Cutaneous diphtheria (see Chapter 204) is uncommon in developed countries; most cases occur in unimmunized persons in overcrowded, underdeveloped parts of the world (particularly in tropical areas), in republics of the former Soviet Union, and among refugee populations[151,152] and are associated with skin trauma, including insect bites, poor hygiene, and inadequate immunization. Endemic toxigenic *C. diphtheriae* has been documented among Northern Plains Native Americans and among the urban destitute in Vancouver, British Columbia.[153] In addition, cutaneous diphtheria has been imported by travelers returning from areas of endemic diphtheria, including the republics of the former Soviet Union. Toxigenic *Corynebacterium ulcerans* can also produce cutaneous diphtheria,[154] and its incidence is rising in much of Western Europe.

Clinical Manifestations

Three types of cutaneous lesions have been described in cutaneous diphtheria: (1) wound diphtheria—secondary *C. diphtheriae* infection of a preexisting wound, which becomes partially covered by a membrane and encircled by a zone of erythema; (2) primary cutaneous diphtheria—a disease of the tropics that begins as a single pustule or as several pustules, usually on a lower extremity, and progresses to form a punched-out ulcer covered by a gray-brown membrane; and (3) superinfection of

eczematized skin lesions—a superficial membranous infection. *C. diphtheriae* has also been isolated from lesions resembling impetigo, ecthyma, and infected insect bites, where they may represent true infections or merely a cutaneous carrier state.[155] Chronic, nonhealing skin ulcers in intravenous drug users that are caused by trauma and are infected with *S. aureus* or various types of streptococci have been found on occasion to be superinfected with nontoxigenic strains of *C. diphtheriae*.[156] Cutaneous diphtheria may be as contagious as the respiratory form of the disease among schoolchildren.

Occasionally, membranous pharyngitis accompanies cutaneous diphtheria. However, 20% to 40% of patients with cutaneous diphtheria carry *C. diphtheriae* in their upper respiratory tract.[155] Myocarditis is extremely rare as a complication of cutaneous diphtheria, but cranial nerve palsies and Guillain-Barré syndrome occur in 3% to 5% of patients with membranous diphtheritic skin ulcers.

Laboratory Findings

Characteristic beaded, metachromatically staining bacilli can be found in methylene blue–stained smears of the edge of the membrane, but the diagnosis can be established only by isolation of *C. diphtheriae* from a suggestive skin lesion. Selective media (cystine tellurite blood agar or fresh Tinsdale medium) are necessary for isolation to inhibit other bacteria in skin ulcers; a specific request for *C. diphtheriae* culture generally is required. Rapid speciation by mass spectroscopy is performed in reference laboratories. In addition to isolation of the organism, toxigenicity should be demonstrated by an Elek plate (agar diffusion precipitin reaction), by diphtherial toxin gene analysis by PCR assay, or by guinea pig inoculation (dermonecrosis).[157]

Differential Diagnosis

Pyogenic infection of ulcerated traumatic lesions is usually purulent, and the lesions are not covered by a membrane. Cutaneous fungal infections have more proliferative and irregular margins. The early stages of primary cutaneous diphtheria and secondary infection of insect bites and abrasions with *C. diphtheriae* may closely resemble those of impetigo. Cutaneous diphtheria may be misdiagnosed as pyoderma gangrenosum.

Presumptive Therapy

If a presumptive diagnosis of ulcerative cutaneous diphtheria is made on clinical grounds and on the basis of preliminary bacteriologic findings, antitoxin (20,000–40,000 units IM or IV) is administered after testing for sensitivity to horse serum. Antibiotic administration (erythromycin, 2.0 g/day orally; or procaine penicillin, 1.2–2.4 million units/day IM for 7–10 days in an adult) also assists in elimination of the convalescent carrier state. Removal of necrotic debris aids in healing of the lesions.

Infectious Gangrene (Gangrenous Cellulitis)

Infectious gangrene is a rapidly progressive cellulitis with extensive necrosis of subcutaneous tissues and the overlying skin. Several clinically distinct syndromes are recognized, depending on the specific causative organism, anatomic location of the infection, and predisposing conditions. Such clinical entities include the following:

1. Necrotizing fasciitis: type I, or polymicrobial, often including Enterobacteriaceae and anaerobes; type II, or streptococcal gangrene caused by *S. pyogenes*; type III, caused by *Vibrio* or other marine pathogens
2. Gas gangrene (clostridial myonecrosis) and anaerobic cellulitis
3. Progressive bacterial synergistic gangrene
4. Synergistic necrotizing cellulitis, perineal phlegmon, and gangrenous balanitis
5. Gangrenous cellulitis in an immunosuppressed patient
6. Very localized areas of skin necrosis complicating conventional cellulitis

Pathologic Characteristics and Pathogenesis

The pathologic changes of gangrenous cellulitis are those of necrosis and some hemorrhage in the skin and subcutaneous tissues. In most types of gangrenous cellulitis, an abundant polymorphonuclear leukocytic exudate is present, but in clostridial myonecrosis, the exudate is thin and consists of fluid, fibrin, and gas but few leukocytes. Fibrin thrombi are frequently present in small arteries and veins of the dermis and subcutaneous fat, particularly in streptococcal gangrene.[158] In most cases, gangrenous cellulitis develops after introduction of the infecting organism at the infected site. It may also result from extension of infection from a deeper focus to involve the subcutaneous tissues and skin, as in clostridial myonecrosis after intestinal surgery or in perineal phlegmon after dissection of infection from a perirectal abscess. Occasionally, gangrenous cellulitis begins at a site of metastatic infection in the course of a bacteremia (e.g., clostridial myonecrosis caused by *Clostridium septicum* at a peripheral site secondary to spread from an associated colonic neoplasm, or *Pseudomonas* gangrenous cellulitis).

Clinical Manifestations

Streptococcal gangrene. Streptococcal gangrene is a rare form of cutaneous and subcutaneous gangrene caused by group A (or C or G) streptococci, involving the superficial fascia but generally sparing the deep fascial (muscle fascia) layer, that usually develops at a site of trauma on an extremity but may occur in the absence of an obvious portal of entry. Like necrotizing fasciitis type II (see "Necrotizing Fasciitis" later), surgical exploration and thorough débridement are critical. The lesion begins as a local, exquisitely painful area of erythema and edema. The extent of this aggressive process initially is often underestimated, because it spreads widely in the deep subcutaneous tissue, with relative sparing of overlying skin.[159] The streptococcal toxic shock syndrome may evolve rapidly and be the most prominent feature.[160] If untreated, the skin becomes dusky over the next 1 to 3 days. Bullae containing yellowish to red-black fluid develop and rupture.[161] The lesion evolves into a sharply demarcated area covered by necrotic eschar and surrounded by a border of erythema. The process at this point resembles a third-degree burn, for which it could be mistaken if a history were not available. Lymphangitis is rarely evident. Extensive necrotic sloughing can result because of deep penetration of the infection along fascial planes. Bacteremia, metastatic abscesses, and death may result from this life-threatening illness if appropriate combined antibiotic therapy and surgical exploration are not initiated promptly. Secondary thrombophlebitis may be a complication if the lower extremities are involved. Streptococci can usually be cultured from the early bullous lesions and frequently from blood.

Progressive bacterial synergistic gangrene. This distinctive lesion usually occurs after infection at an abdominal operative wound site (frequently when wire sutures have been used) or abutting an ileostomy or colostomy, a fistulous tract, or a chronic ulcer on an extremity.[162] It begins as a local tender area of swelling and erythema that subsequently ulcerates. The painful shaggy ulcer gradually enlarges and is characteristically encircled by a margin of gangrenous skin (Fig. 93.4). Surrounding the latter is a violaceous zone that fades into an outer, pink, edematous border area. If untreated, the process extends slowly but relentlessly, ultimately producing an enormous ulceration.

Microaerophilic or anaerobic streptococci can be recovered from aspirates of the advancing margin of the lesion, and *S. aureus* (or occasionally *Proteus* or other gram-negative bacilli) are present in the ulcerated area. Meleney[162] reproduced similar lesions by experimentally injecting both microaerophilic streptococci and *S. aureus* (but not either alone) into the skin of animals. Similar lesions can be seen rarely with amebic (*Entamoeba histolytica*) cutaneous gangrene at abdominal or thoracic operative wound sites. These should be considered in appropriate settings[163,164] so that appropriate measures (e.g., stool examination for amebas, serologic tests, periodic acid–Schiff stain of scrapings, or biopsy of the lesion) can be undertaken to exclude this diagnosis.

Gas gangrene, anaerobic cellulitis, and other forms of crepitant cellulitis. See Chapters 94 and 246 and the section "Subcutaneous Tissue Infections and Abscesses" later in this chapter.

Gangrenous cellulitis in immunocompromised hosts. The causes of cellulitis in an immunocompromised host include agents that produce such infections in healthy individuals and other organisms not ordinarily regarded as causes of cellulitis, including gram-negative bacilli and fungi. *Pseudomonas* bacteremia may produce gangrenous cellulitis (see later section "Cutaneous Involvement in Systemic Bacterial and Mycotic Infections") in immunocompromised hosts, patients with thermal burns, and others. Mucormycotic gangrenous cellulitis may be engrafted on



FIG. 93.4 Progressive bacterial synergistic gangrene of abdominal wall. Ulcerated areas developed about wire stay sutures, which were removed. *(From Bornstein DL, Weinberg AN, Swartz MN, et al. Anaerobic infections: review of current experience.* Medicine [Baltimore]. *1964;43:207–232.)*

an extensive burn wound, or it may rarely develop in patients with diabetes mellitus, in individuals infected with human immunodeficiency virus (HIV), or in those receiving immunosuppressive therapy.[165] Local factors (e.g., open fracture sites, ileostomy stomas, fistulous tracts) also play a predisposing role in this type of infection. Spores of *Rhizopus* species (members of the Mucoraceae family) contaminating Tensoplast tape used for occlusive dressings have resulted in progressive local and disseminated infection in immunosuppressed patients.[166] The infection may exhibit an indolent course, with minimal fever and a slowly enlarging black ulcer, or it may follow a rapidly progressive febrile course. The characteristic lesion consists of a central anesthetic, black, necrotic area, with a surrounding raised zone of violaceous cellulitis and edema.[167] Superficial vesicles and blistering may occur in the gangrenous area. Skin infection usually does not result from an initial pulmonary or rhinocerebral focus, and hematogenous dissemination is not ordinarily demonstrable. Cultures of the necrotic skin or aspirates from the advancing margin usually do not reveal the fungus. Identification of the cause is best obtained from biopsy specimens—fungal wet mount of crushed tissue, tissue sections stained with hematoxylin and eosin (showing tissue and vascular invasion by characteristic broad hyphae), and culture. Necrotizing angioinvasive cellulitis caused by the zygomycete *Apophysomyces elegans* sporadically occurs in a small number of nonimmunocompromised patients after traumatic injuries potentially contaminated with soil.[168] Similarly, gangrenous skin lesions may also occur with disseminated aspergillosis in immunocompromised hosts and burn patients.

Prominent necrosis of skin and subcutaneous fat occurs rarely in patients who have chronic renal failure (with secondary hyperparathyroidism), in those receiving chronic dialysis therapy, in patients with extensive calcification of the small arteries of subcutaneous tissue, and in those in whom the calcium phosphate product is markedly elevated. Previously known as calciphylaxis, the process that results in acute local calcification is now termed *calcific uremic arteriolopathy.*[169,170] Precipitating factors for this process are poorly defined but include local trauma, hyperphosphatemia, diabetes mellitus, and systemic infection. The skin lesions begin as dark red, irregular areas resembling livedo reticularis. They become plaquelike or nodular, are painful, and rapidly increase in size but remain well demarcated. They progress to gangrenous necrosis with eschar formation. Secondary infection of necrotic areas may follow. Histologically, involved areas show extensive vascular calcification, calcinosis cutis, and ischemic skin necrosis. Bacteremia originating elsewhere may contribute to the local ischemic process through further lesional thromboses mediated by disseminated intravascular coagulopathy. These necrotic skin ulcers in patients with chronic renal failure resemble those of infective gangrenous cellulitis, particularly if they become secondarily infected.

Differential Diagnosis

The bite of the brown recluse house spider can produce a necrotizing skin lesion that resembles infectious gangrenous cellulitis. The occurrence of fever and chills 24 to 48 hours after the bite enhances the mimicry. In many cases, presumed spider bites are actually manifestations of primary MRSA cutaneous lesions, with focal skin necrosis complicating furunculosis and surrounding cellulitis.[171] The differential diagnosis of infectious gangrene and gangrenous cellulitis is presented in Table 93.5.

Presumptive Therapy

Treatment of streptococcal gangrene consists of immediate surgical exploration with longitudinal incisions extending to the deep fascia and beyond the involved gangrenous and undermined areas.[161] Distinction between streptococcal gangrenous cellulitis and streptococcal necrotizing fasciitis may be difficult before surgical exploration. Initial resuscitation measures with intravenous fluids and pressor support are essential in the presence of hypotension caused by accompanying streptococcal bacteremia or the streptococcal toxic shock syndrome. Areas of cutaneous necrosis are widely excised, and, if present, nonviable fascia is débrided. Reexploration is commonly performed within 24 hours. Antibiotic therapy consists of high-dose intravenous aqueous penicillin G (3–4 million units every 4 hours) in the setting of normal renal function. In the treatment of both streptococcal gangrene and streptococcal toxic shock, the addition of clindamycin to penicillin is recommended because it has been shown to reduce the early release of streptococcal pyrogenic exotoxin A in vitro.[21,172]

If there is any question regarding the causative agent (e.g., possibly *S. aureus* rather than group A streptococcus), vancomycin should be started empirically until MRSA can be excluded. Necrotizing fasciitis resulting from mixed anaerobes and facultative organisms (synergistic necrotizing cellulitis) can usually be suspected at the outset from the foul odor, frequent soft tissue gas, and appearance of the exudate on a Gram-stained smear. After surgery, the wound is treated with elevation and moist dressings. Skin grafting is usually required later.

Progressive bacterial synergistic gangrene can be difficult to treat. Wide excision of all necrotic tissue (extending into normal tissue), combined with broad-spectrum antibiotic treatment, is usually required. The initial use of a β-lactam–β-lactamase inhibitor combination (e.g., piperacillin-tazobactam) or a carbapenem agent (e.g., imipenem-cilastatin or meropenem), combined with vancomycin or another agent effective against MRSA, may be a reasonable empirical strategy until microbiologic data can be used to refine therapy.

Erythrasma

Erythrasma is a common superficial bacterial infection of the skin, caused by *Corynebacterium minutissimum,* a species that can be grown aerobically. It is characterized by slowly spreading, pruritic, reddish-brown macular patches, usually located in the genitocrural area, with sparing of intertriginous creases.[173] The lesions are finely scaled and finely wrinkled, and are more common in men and in obese individuals with diabetes mellitus. The disease may be asymptomatic or may undergo periodic exacerbations. Gram-stained imprints of the skin surface show large numbers of small gram-positive bacilli. Examination of the lesions under a Wood lamp reveals a distinctive coral red fluorescence.

The principal superficial skin infections to be considered in the differential diagnosis are tinea versicolor lesions on the trunk, tinea cruris (a deeper, more inflammatory, and more rapidly progressive process), and possibly candidiasis. Oral erythromycin (1.0 g/day for 5–7 days) has been efficacious in the past, with clearing of lesions within a few weeks. Topical treatment with 2% erythromycin gel[173] or an aqueous

TABLE 93.5 Differential Diagnosis of Infectious Gangrene and Gangrenous Cellulitis

	PROGRESSIVE BACTERIAL SYNERGISTIC GANGRENE	SYNERGISTIC NECROTIZING CELLULITIS[a]	STREPTOCOCCAL GANGRENE[b]	CLOSTRIDIAL MYONECROSIS (GAS GANGRENE)	NECROTIZING CUTANEOUS MUCORMYCOSIS	BACTEREMIC *PSEUDOMONAS* GANGRENOUS CELLULITIS	PYODERMA GANGRENOSUM
Predisposing Conditions	Surgery, draining sinus	Diabetes common	Occasionally diabetes or myxedema, after abdominal surgery	Local trauma	Diabetes, corticosteroid therapy	Burns, immunosuppression	Ulcerative colitis, rheumatoid arthritis
Pain	Prominent	Prominent	Prominent	Prominent	Minimal	Mild	Moderate
Systemic Toxicity	Minimal	Marked	Marked	Very marked	Variable	Marked	Minimal
Course	Slow	Rapid	Very rapid	Extremely rapid	Rapid	Rapid	Slow
Fever	Minimal or absent	Moderate	High	Moderate or high	Low grade	High	Low grade
Anesthesia of Lesion	–	–	±	–	+	±	–
Crepitus	–	Often present	–	+	–	–	–
Appearance of Involved Area	Central shaggy, necrotic ulcer surrounded by dusky margin and erythematous periphery	Crepitant cellulitis; thick, copious, foul-smelling "dishwater" drainage from scattered areas of skin necrosis	Necrosis of subcutaneous tissue and fascia with black, necrotic, "burned" appearance of overlying skin	Marked swelling, yellow-bronze discoloration of skin, brown bullae, green-black patches of necrosis, serosanguineous discharge	Usually a central black, necrotic area with a purple raised margin; may also appear as just a black ulcer	A sharply demarcated, necrotic area with black eschar and surrounding erythema resembling a decubitus ulcer; may evolve from initial hemorrhagic bulla	Begin as bullae, pustules, or erythematous nodules that ulcerate deeply; often multiple, large, and coalescing; usually on lower extremities or abdomen
Cause	Microaerophilic streptococci plus *Staphylococcus aureus* (or sometimes *Proteus*)	Usually a mixture of organisms (e.g., *Bacteroides*, peptostreptococci, *Escherichia coli*)	Primarily group A streptococci; if secondary to abdominal surgery, enteric bacteria also involved	*Clostridium perfringens* (occasionally other clostridia)	*Rhizopus, Mucor, Absidia*	*Pseudomonas aeruginosa*	Not an infection primarily; may be confused with such lesions resulting from secondary colonization by Enterobacteriaceae, microaerophilic streptococci, *P. aeruginosa,* or *S. aureus*

[a]Similar to necrotizing fasciitis type I.
[b]Necrotizing fasciitis type II.
–, Absent; +, present; ±, occasionally present.
Modified from Swartz MN. Clinical practice: cellulitis. N Engl J Med. *2004;350:904–912. Copyright ©2004 Massachusetts Medical Society.*

solution of 2% clindamycin hydrochloride can also be effective. More recently, macrolide resistance has been reported, and tetracycline or amoxicillin-clavulanate therapy has been proposed.[174] *C. minutissimum* is also the causative agent of pitted keratolysis, a superficial process consisting of crateriform pits up to 7 mm in width occurring on the pressure-bearing areas of the sole and on the ventral aspect of the toes.[175] The lesions are malodorous and are associated with hyperhidrosis.

Secondary Bacterial Infections Complicating Preexisting Skin Lesions

A variety of skin lesions (e.g., burns, eczematous dermatitides, and traumatic lesions) may become secondarily infected (see Table 93.1). Such infected lesions usually do not exhibit distinctive morphologic characteristics based on the infecting organism. Rather, the appearance of the lesions is determined to a large measure by the nature of the preexisting injury or dermatosis, such as dermatophytosis and acne conglobata, which are often treated primarily by dermatologists. Several of the other secondarily infected dermatoses have some distinctive clinical and bacteriologic features and merit brief consideration.

Diabetic Foot and Other Chronic Superficial Skin Ulcers

Decubitus Ulcers

Various aerobic, facultative, and anaerobic organisms (e.g., staphylococci, streptococci, Enterobacteriaceae, *Pseudomonas,* enterococci, peptostreptococci, *Clostridia*, and *Bacteroides* spp.) colonize and secondarily infect decubitus ulcers.[176] The extensive undermining and tissue necrosis of these ulcers and their location, frequently in proximity to the anus, provide the opportunity for invasion by anaerobes. Such lesions have been associated with bacteremias caused by *Bacteroides fragilis, Peptostreptococcus* species, *S. aureus, Enterococcus,* and various streptococci and facultative gram-negative bacilli. Cultures of the ulcer base (preferably by biopsy)[176] are sometimes helpful in guiding the selection of antibiotic therapy when there is apparent cellulitis, progressive ulceration, or systemic signs of infection. When systemic therapy is needed, broad-spectrum coverage, including anaerobic activity, is initiated and modified if necessary by subsequent culture data.

Diabetic Ulcers

Chronic foot infections in patients with diabetes mellitus are common and difficult problems.[107,177,178] They usually complicate the initially uninfected ulcerations that follow minor trauma in patients with peripheral neuropathy, chronic neuropathic ulcers, or arterial vascular insufficiency and take the form of cellulitis, soft tissue necrosis, or osteomyelitis with a draining sinus. These infections are classified depending on the extent of infection, as follows[107]:

1. Mild—if present, less than 2 cm beyond the ulcer margins
2. Moderate—more extensive or invasive infection, or both, associated with necrosis (gangrene), abscess, and deep soft tissue or skeletal involvement or both
3. Severe—the presence of systemic complications, such as fever, hypotension, and acidosis

S. aureus and β-hemolytic streptococci are frequently responsible for cellulitis or mildly infected ulcers. More chronic lesions and those previously treated with antibiotics may also contain Enterobacteriaceae or nonenteric gram-negative bacilli such as *P. aeruginosa,* or both. Malodorous wounds often indicate the presence of anaerobic pathogens. Chronic refractory ulcers, especially if associated with gangrene, are infected with a wide variety of microorganisms, including the previously mentioned pathogens as well as enterococci, diphtheroids, anaerobes, and even fungi.[179,180] Deep tissue cultures obtained through local débridement provide the most reliable bacteriologic information in diabetic foot infections. If they are not available, cultures and Gram-stained smears of material obtained from curettage of the base of the ulcer or from a purulent exudate may provide information needed to guide antimicrobial therapy. Gas present in surrounding tissues on radiologic examination may represent air introduced through the ulcer or gas generated in soft tissues by the infecting anaerobic or coliform organisms.

Antibiotic treatment of infected diabetic foot ulcers is based on meaningful bacteriologic data, if available. Initial antimicrobial treatment in a previously untreated patient with a non–limb-threatening infection is focused primarily on staphylococci and streptococci. For mild infections that can be treated at home, oral clindamycin, cephalexin, dicloxacillin, or amoxicillin-clavulanate for 2 weeks has been traditional first-line therapy. If superficial ulcers are complicated by cellulitis warranting parenteral antibiotics, intravenous cefuroxime, cefoxitin, or ampicillin-sulbactam has been recommended.[176] The incidence of MRSA has ranged between 10% and 30% in recent studies of infected diabetic foot ulcers,[180] thus requiring early culture attempts and close surveillance of patients initially treated with β-lactam agents or clindamycin. Initial antimicrobial treatment of severe or limb-threatening infections involves the use of broad-spectrum polymicrobial antibiotics against *S. aureus* (including MRSA), group B streptococci, other streptococci, Enterobacteriaceae, anaerobic gram-positive cocci, and *Bacteroides* spp. (including *B. fragilis*). Fluoroquinolone monotherapy is problematic because of possible *Bacteroides* species and other anaerobes in these infections, as well as the rising resistance observed among *S. aureus* strains and gram-negative pathogens. Currently, a variety of regimens is advocated for initial empirical therapy for limb-threatening infections[107,177]: ampicillin-sulbactam or a carbapenem, piperacillin-tazobactam, clindamycin plus a third-generation cephalosporin, and clindamycin plus a fluoroquinolone. Vancomycin is generally included as initial empirical therapy in patients with severe infection, known recent prior MRSA carriage or hospitalization or both, and residence in areas of increased MRSA prevalence. Aminoglycosides are less attractive in this setting because of the advanced age of patients and frequent associated diabetic nephropathy.

Initial surgical management includes unroofing encrusted areas, probing the wound to determine the extent of tissue destruction and possible fascial or bone involvement or both, and débriding necrotic tissue. Determining the vascular status and the extent of limb ischemia is necessary when formulating a strategic wound care program. Edema should be reduced by bed rest, elevation, and diuretic therapy, as indicated. Control of diabetes is of considerable importance. Open ulcers should be gently packed two or three times daily with sterile gauze moistened with normal saline or quarter-strength povidone-iodine (Betadine); additional wound care measures such as negative pressure dressings and topical antibiotic therapy are available, but assessments of comparative efficacy are generally lacking.[181] Surgical débridement and drainage should be carried out promptly in patients with deep ulcers extending to subcutaneous tissue or if deep tissue necrosis or suppuration is present.[107,177]

Occupation-Related Ulcers

Contaminated traumatic wounds often involve loss of skin and subcutaneous tissue, with ensuing cellulitis and deeper infections. The variety of work-associated injuries and exposures leads to a wide range of possible occupationally related infections, particularly among agricultural, aquatic, and meatpacking workers (especially those engaged in poultry and pork processing), where lacerations and degloving injuries are readily contaminated.[182–184] The bacteriology of initial wounds sustained by agricultural workers in recent series demonstrates a broad range of pathogens, including gram-negative bacilli (particularly *Enterobacter* spp. and *Stenotrophomonas maltophilia*) as well as both coagulase-positive and coagulase-negative staphylococci.[185] An often occupationally related form of cellulitis caused by *Streptococcus iniae* has occurred in those exposed to aquacultured fish, primarily tilapia (e.g., fish farmers, fish cutters, cooks).[186] *S. iniae* is a fish pathogen that has caused outbreaks of invasive disease with high mortality among tilapia in aquaculture farms or merely colonization of the surface of fish. Human infection occurs after a puncture wound of the hand by a fish bone or knife while handling or preparing fish. Cellulitis develops rapidly (within 6–24 hours after the injury). Fever, lymphangitis originating from the site of injury, and bacteremia are frequently observed. Metastatic infections such as septic arthritis, meningitis, and endocarditis may occur. Bulla formation and skin necrosis are not seen. Penicillin G is the treatment of choice, but *S. iniae* is also susceptible in vitro to cefazolin and ceftriaxone. Commercial and sport fishing or recreational activity in fresh water may result in puncture wounds or lacerations from the venomous spines of catfish.[187,188] Such wounds can be associated with local envenomation reactions and may rapidly become secondarily infected, particularly by

gram-negative bacilli found in ponds and lakes (especially *A. hydrophila,* enteric gram-negative rods such as *Edwardsiella tarda, Klebsiella,* and *E. coli*) or *Vibrio* species or *Mycobacterium marinum* in coastal waters.[189]

Posttraumatic Opportunistic Skin Infections in Immunocompromised Patients

Unusual pathogens may invade the skin of immunocompromised patients after local, often minor, lacerations or abrasions. Bacterial pathogens associated with opportunistic primary cutaneous infections include relatively commonly encountered pathogens such as *P. aeruginosa* and *Stenotrophomonas*,[190] as well as *Bacillus cereus, Nocardia,* and mycobacteria (e.g., *M. marinum, Mycobacterium haemophilum*). Primary cutaneous *B. cereus* infection can occur in neutropenic patients[191] and has been reported in a patient with severe combined immunodeficiency disease. *B. cereus* has also caused epidemic extremity infections among healthy jungle trekkers in Costa Rica after repetitive local trauma[191] and superficial scalp infection in healthy individuals after military haircuts.[192] The lesions are vesicular or pustular and usually occur on the hand or on an extremity during warm weather. Bacteremia is not a feature. Intravenously administered vancomycin is the preferred treatment.[191] Rarely, scattered papular and nodular lesions in patients with AIDS show on biopsy an abscess containing a granule consisting of basophilic-staining cocci surrounded by eosinophilic material (Splendore-Hoeppli phenomenon).[193] This particle superficially resembles a sulfur granule of actinomycosis or a mycetoma, but it is the lesion of botryomycosis and is caused by *S. aureus*.[194] Botryomycosis also occurs in immunocompetent patients; a foreign body may play a role in initiating or perpetuating the lesion, which has the gross appearance of a small infected sebaceous cyst or may resemble prurigo nodularis. Several cases of botryomycosis have occurred in patients with the hyperimmunoglobulin E syndrome associated with recurrent staphylococcal infections.

Endemic dimorphic mycoses and yeasts (e.g., blastomycosis, coccidioidomycosis, histoplasmosis, and cryptococcosis) cause skin lesions by hematogenous spread following primary pulmonary infection.[195] Most mold infections in this population are nodular or ulcerative and are acquired by traumatic inoculation (*Fusarium, Scedosporium* spp.[196]; dark-walled fungi and additional molds[197]), as are skin infections from *Prototheca*[198] (see later) and *Sporothrix schenckii. Sporothrix* and *M. marinum* infections commonly progress to nodular lymphangitis in both normal and compromised hosts; in contrast, *Nocardia* nodular lymphangitis has been recognized only in immunocompromised hosts.[199] In immunocompromised hosts, cutaneous fungal disease may reflect local inoculation but secondary spread from a pulmonary or other source can develop; disseminated infection is often present at the time cutaneous lesions are investigated.[200,201] Primary cutaneous invasive aspergillosis has been recognized with increased frequency, often in the absence of known local trauma. It appears as enlarging nodules that may undergo local necrosis and ulceration.[202] Cutaneous protothecosis (*Prototheca wickerhamii*) occurs occasionally in patients with AIDS or other immunosuppressed states,[198] and rarely in nonimmunosuppressed patients who have sustained trauma, water exposure, or local corticosteroid injections. Excision therapy or the use of amphotericin B or azole antifungal agents has been successful. A typical dermatophyte, *Trichophyton rubrum,* which ordinarily produces only superficial skin infections, may invade the deeper subcutaneous tissues of immunosuppressed hosts and produce multiple nodular or fluctuant masses; it responds to azole therapy (itraconazole or posaconazole) or terbinafine.[203]

Bacillary Angiomatosis in Patients With Acquired Immunodeficiency Syndrome

Bacillary angiomatosis (epithelioid angiomatosis) is a form of bartonellosis that involves primarily the skin but also visceral organs in patients with AIDS, occasionally in patients with other forms of immunosuppression (e.g., solid-organ transplantation), and rarely in immunocompetent individuals (e.g., at the site of healing second-degree burns; see Chapter 234).[204,205]

The lesions begin as tiny red papules that enlarge to become exophytic or pedunculated nodules, occasionally reaching 1 cm or more in diameter. Often they are dome shaped, vascular lesions, and they may possess slight surrounding erythema or a collarette of scale or both. Deeper nodules in the dermis or subcutaneous tissue are flesh colored, with a somewhat rubbery to firm consistency, and may be movable or fixed to underlying structures. The lesions bleed readily if incised. Only a few lesions may be present, or they may be quite abundant, covering the body. Oral, nasal, conjunctival, genital, and anal mucosal lesions occur. Visceral involvement takes the form of bacillary peliosis hepatis, with hypodense lesions of the liver and spleen demonstrable on abdominal computed tomography (CT). Many patients with bacillary angiomatosis have a history of cat contact or cat scratches.

The lesions of bacillary angiomatosis grossly resemble those of Kaposi sarcoma, pyogenic granuloma, hemangioma, subcutaneous tumors, or verruga peruana (eruptive phase of bartonellosis in Peru and Ecuador) and require biopsy for definitive diagnosis. Histologically, bacillary angiomatosis consists of a circumscribed, lobular proliferation of capillaries lined with prominent large endothelial cells, an inflammatory infiltrate with neutrophils, and, characteristically, aggregates of bacillary bodies that are demonstrable on Warthin-Starry silver stain. The role of *Bartonella* species as causative agents in this syndrome was demonstrated by bacterial 16S ribosomal gene analysis in infected tissue and led to the development of serologic diagnostic tests. *Bartonella henselae* and *Bartonella quintana* cause bacillary angiomatosis with equal frequency.[205]

The diagnosis of bacillary angiomatosis is made by the clinical appearance of the lesions in a patient with HIV infection and confirmed on biopsy (Warthin-Starry silver stain). Because the skin lesions are often extensive and systemic manifestations (e.g., fever, peliosis hepatis) can be features, antimicrobial treatment is indicated. Prolonged macrolide therapy is given for 3 months; doxycycline has been used in macrolide-intolerant patients, and rifampin may be added as a second agent in severely ill patients. Dramatic exacerbation of bacillary angiomatosis may rarely complicate the immune reconstitution inflammatory syndrome in treated HIV-infected patients.[206]

Hidradenitis Suppurativa

Hidradenitis suppurativa is a rather common and extremely troublesome, chronic, suppurative, cicatricial disease of apocrine glands in the axillary, genital, and perianal areas; it occurs in up to 1% of some European populations,[207] but the estimated US prevalence is only 0.05%.[208] The primary lesion appears to be unexplained keratinous plugging of the apocrine gland ducts, with early perifollicular lymphocyte infiltration and subsequent dilatation and eventual rupture of the gland with surrounding tissue inflammation,[207,209] although in some patients apocrine inflammation is dominant and, in others, keratinized epithelium is the focus of early inflammation.[209] It has been associated with certain autoimmune disorders (e.g., thyroid disease, inflammatory bowel disease, Sjögren syndrome), as well as with common conditions[210] including obesity, smoking, the metabolic syndrome,[211] and diabetes mellitus.[212] Lesional biopsies demonstrate broad suppression of the dermal innate immune response.[213] The initial lesions are tender, reddish-purple nodules that slowly become fluctuant and drain. Irregular sinus tracts are formed with repeated crops of lesions, and ultimately the involved areas show a mixture of burrowing, draining tracts and cicatricial scarring. In some patients, hidradenitis suppurativa is associated with acne conglobata or dissecting cellulitis of the scalp. In such patients, a distinctive spondyloarthropathy may occur.[214] Squamous cell carcinoma is a rare complication of hidradenitis suppurativa lesions.

Although not initially infected, the lesions frequently become infected secondarily.[215] Staphylococci, nonhemolytic streptococci, *Streptococcus anginosus* group, *E. coli, Proteus,* and *Pseudomonas* are often isolated from established draining lesions; anaerobic organisms (e.g., *Bacteroides,* anaerobic gram-positive cocci), suggested by the foul odor associated with these lesions, have also been isolated. The chronicity and localization of these lesions establishe the diagnosis of hidradenitis suppurativa, but other focal inflammatory processes should be considered in selected cases (e.g., furuncle, carbuncle, infected dermoid cyst, granuloma inguinale, lymphogranuloma venereum, infected pilonidal cyst).[215]

Treatment of hidradenitis suppurativa is difficult, particularly when the process is chronic and extensive, because of the multiple deep-seated sites of secondary infection that are inaccessible to antibiotics.[210,216] Mild disease, with isolated nonscarring nodules, responds to topical

clindamycin therapy or resorcinol. Intralesional triamcinolone is often effective to address acute flares. Moderate disease can be controlled with oral tetracycline or doxycycline monotherapy or with combination clindamycin and rifampin, although the antiinflammatory properties of these agents may account for their efficacy.[216] Disease flares associated with secondary infection may respond to oral antimicrobial therapy (based on Gram-stained smears and culture results), recognizing that resistance to maintenance topical or oral therapy is commonly present. Local moist heat to help establish drainage may also be helpful in treating the initial phases of infection. Surgical drainage is required for the management of large abscesses. In severe resistant cases exhibiting chronicity and scarring, unroofing of sinus tracts and marsupialization or radical excision of most of the involved area, followed by skin grafting, may become necessary.[210] Carbon dioxide or neodymium:yttrium-aluminum-garnet laser therapy has been effective in selected patients and avoids excisional procedures[215]; photodynamic therapy is also being investigated. Adjunctive measures including metformin, dapsone, and colchicine appear promising based on limited clinical data. Immunosuppressive therapies, including cyclosporine and tumor necrosis factor inhibitors such as infliximab and adalimumab, for the management of this chronic inflammatory condition are employed more regularly,[210,216] although clinical experience remains limited.[217]

Infected Epidermal Cysts

Epidermal cysts are closed sacs lined with proliferating epidermal cells located about the head, trunk, extremities, and vulvovaginal and scrotal areas. Lacking communication with the skin surface, they can become infected and result in abscess formation. *S. aureus* (frequently present as the sole aerobic organism) and various streptococci are the principal aerobic or facultative isolates from these abscesses. *Peptostreptococcus* and *Bacteroides* species, the primary anaerobic isolates, are often present in polymicrobial mixtures in cyst abscesses about the head, perineum, and vulvovaginal area.[218] Treatment consists principally of surgical drainage, and initial antimicrobial therapy (clindamycin, cefoxitin, or amoxicillin-clavulanate), if needed, is aimed at *S. aureus* and the probable anaerobes pending results of Gram-stained smears and cultures. Although generally localized, rupture of an infected sebaceous cyst has led to adjacent necrotizing infection.[219]

Self-Induced Skin Infections

Rarely, persisting unexplained skin ulcers are self-induced. Their colonization with a variety of gram-negative and gram-positive bacteria is inevitable. However, the continuing ulceration is the result of repeated, self-induced trauma rather than bacterial infection per se, a form of dermatitis artefacta.[220,221] Facial lesions as well as truncal and limb involvement can be seen. Very rarely, unexplained continuing or recurrent polymicrobial cellulitis or a subcutaneous abscess (particularly caused by mixed oral or intestinal flora) is the result of injection of foreign material containing saliva or other contaminated fluids into subcutaneous tissue. Examination of biopsy specimens from the involved area by polarizing microscopy may reveal the presence of birefringent foreign bodies, which suggest the true diagnosis.

Cutaneous Involvement in Systemic Bacterial and Mycotic Infections

Cutaneous manifestations may be a prominent and often diagnostic feature of various bacteremias, fungemias, and systemic bacterial infections (see Table 93.1).[222] In leptospirosis, rat-bite fever, and listeriosis, cutaneous manifestations are a small part of the total clinical picture; these conditions are considered in the chapters dealing with the responsible organisms. In some systemic infections, cutaneous manifestations are noninfectious complications of the illness (e.g., erythema nodosum, erythema multiforme, guttate psoriasis, purpura fulminans).

Bacteremias

Staphylococcus aureus

The occurrence of skin lesions (pustules, subcutaneous abscesses, purulent purpura) in the course of bacteremia or endocarditis secondary to *S. aureus* can provide an early clue to the nature of the infecting organism. The most distinctive of these lesions is purulent purpura, a small area of purpura with a white purulent center. Aspiration of the contents of the central portion reveals staphylococci and polymorphonuclear leukocytes and are typically culture positive. Rarely, scattered tender subcutaneous nodules may develop during *S. aureus* bacteremia.

Pseudomonas aeruginosa

Five types of skin lesion have been described in the course of *Pseudomonas* septicemia:

1. *Vesicles and bullae.* These lesions occur as isolated bullae or occasionally in small clusters anywhere on the skin surface. They rapidly become hemorrhagic and have a narrow encircling zone of dusky erythema. Occasionally, in infants, the lesions are surrounded by large, erythematous halos resembling insect bites or erythema multiforme.
2. *Ecthyma gangrenosum.* This lesion is a round, indurated, ulcerated, painless area with a central gray-black eschar and a surrounding narrow zone of erythema. These lesions may develop de novo, or they may evolve from an initial bullous lesion.
3. *Subcutaneous nodules.* Solitary or multiple, minimally fluctuant, subcutaneous nodules are uncommon features of *Pseudomonas* bacteremia, seen primarily in immunocompromised hosts.[223] Similar nodules in this population may have a wide range of other infectious causes—*S. aureus* and botryomycosis, mycobacterial infections, candidiasis and other deep mycoses, protothecosis, *Acanthamoeba* infection, and bacillary angiomatosis.
4. *Gangrenous cellulitis.* Gangrenous cellulitis is a superficial, sharply demarcated necrotic area that may resemble a decubitus ulcer or an area of cellulitis with edema and some necrosis of the overlying skin.
5. *Macular or maculopapular lesions.* These lesions are small, oval, erythematous macules located predominantly over the trunk that resemble the rose spots of typhoid fever. Such lesions have been reported, particularly in the tropics, in association with fever and diarrhea in the syndrome described as Shanghai fever.

These metastatic lesions typically contain numerous gram-negative bacilli but relatively few polymorphonuclear leukocytes. The development of such lesions in a febrile leukemic patient undergoing induction chemotherapy or on uninvolved skin areas of a patient with extensive thermal burns should strongly suggest the presence of *Pseudomonas* bacteremia. Presumptive antibiotic management should be aimed at *P. aeruginosa* and include a combination of an antipseudomonal β-lactam (cefepime, ceftazidime, piperacillin-tazobactam, imipenem, or meropenem) with tobramycin or a fluoroquinolone. Ecthyma gangrenosum has been associated with bacteremia caused by other gram-negative bacilli or with disseminated candidiasis at increasing frequency,[63] or it may develop as a primary necrotizing cutaneous infection in the absence of prior bacteremia (e.g., reflecting progression of *Pseudomonas* folliculitis in an immunocompromised patient).[224,225] Secondary bacteremia may develop, particularly in neutropenic patients.

Neisseria meningitidis

The skin lesions of acute meningococcemia consist of erythematous macules (initially), petechiae, purpura, and ecchymoses located on the extremities and trunk. Extensive gun-metal gray, hemorrhagic, necrotic patches can develop by confluence of petechial and purpuric lesions in fulminant meningococcemia. Symmetrical peripheral gangrene and purpura fulminans occur with prominent disseminated intravascular coagulation. Occasionally, gram-negative diplococci can be observed on smears of plasma obtained from the skin lesions of patients with acute meningococcemia.

Skin lesions are an important feature of the unusual syndrome of chronic meningococcemia, characterized by recurrent cycles of fever, arthralgia, and rash over a period of 2 to 3 months.[226,227] The rash appears in crops, each consisting of a small number of individual lesions during febrile episodes. The lesions are generally located on the extremities, particularly about joints. They may consist of erythematous maculopapules, petechiae, petechiae with vesiculopustular centers, petechiae

with small areolas of pale erythema, suggillations, or tender erythema nodosum–like nodules. Biopsy specimens of the lesions reveal the histologic picture of leukocytoclastic angiitis, a finding that may erroneously direct attention toward the diagnosis of a small vessel hypersensitivity vasculitis and away from that of vasculitis secondary to systemic infection. Biopsy of nonpurpuric erythematous areas demonstrates meningococcal adherence to the endothelium with disruption of intercellular junctions and extravasation of bacteria to the extravascular space.[228] Microbiologic confirmation of the diagnosis is challenging because of the low rate of positive cultures from blood or skin biopsy specimens; occasionally, biopsy specimens display gram-negative diplococci on Gram stain, but real-time PCR analysis of biopsy tissue can confirm the presence of *N. meningitidis*.[228]

Neisseria gonorrhoeae

The skin lesions of gonococcemia consist of a mixture of pustules surrounded by a thin zone of purpura, macules, papules, purpuric vesicles and bullae, and purpuric infarcts. The lesions are few, scattered over the distal ends of extremities in particular, and frequently painful. They are part of the gonococcemic dermatitis-arthritis syndrome.[229,230] *N. gonorrhoeae* is isolated from fewer than 5% of skin lesions, but gonococcal antigens may be identified by immunofluorescence staining or PCR of skin lesions. Cultures of the pharynx, genital sites, and joint fluid (if present) may confirm the diagnosis. In addition to arthralgias and frank arthritis, tenosynovitis may be a conspicuous feature. *N. meningitidis* can present with features closely mimicking disseminated *N. gonorrhoeae* infection.[231] Paradoxically, disseminated gonococcal infection among patients receiving the monoclonal complement inhibitor eculizumab often lacks skin lesions.[232]

Salmonella enterica Serovar Typhi

Rose spots frequently appear 7 to 10 days into the febrile course of untreated typhoid fever. The lesions are slightly raised, small (1- to 3-mm), pink papules that tend to occur in crops of 10 to 20 lesions. They are found most commonly on the upper part of the abdomen, the lower part of the chest, and the back. Rose spots are less frequently found in enteric fever caused by *Salmonella* species other than *Salmonella enterica* serovar Typhi. This organism can sometimes be found on Gram-stained preparations from the papules and isolated on culture; early antibiotic treatment (e.g., with ciprofloxacin or ceftriaxone) prevents the appearance of these skin lesions.

Haemophilus influenzae

Cellulitis involving the face, neck, or upper extremities occasionally occurs with bacteremic *H. influenzae* type b infection in children, particularly those younger than age 3 years. Although commonly described as having a peculiar purple-red or blue-red (violaceous) hue, the lesion most often is erythematous, indurated, and indistinguishable from cellulitis caused by streptococci or staphylococci. The site of primary infection is the pharynx, middle ear, or elsewhere in the upper respiratory tract. Direct invasion across traumatized buccal mucous membranes by *H. influenzae* type b colonizing the respiratory tract has been suggested as the pathogenesis of most cases of buccal cellulitis in children.[233] This infection is life threatening and acute; bacteremia (sometimes complicated by meningitis) occurs in about 80% of cases. *H. influenzae* type b cellulitis in children has almost disappeared in developed countries since widespread immunization with conjugate vaccines was introduced nearly 3 decades ago. A few cases have been reported in adults with epiglottitis or other forms of upper respiratory tract disease caused by *H. influenzae*.[234] Although adult *H. influenzae* cellulitis generally involves cervical or thoracic areas, bacteremic lower extremity *H. influenzae* type b cellulitis has been reported in normal as well as in immunocompromised adults older than age 60 years. In view of the significant rate of β-lactamase production among clinical strains of *H. influenzae* type b, initial therapy should include a third-generation cephalosporin.

Helicobacter cinaedi

An indolent syndrome of primary *H. cinaedi* bacteremia (generally without antecedent gastroenteritis) with fever and at times recurrent multifocal cellulitis has been described in immunocompromised hosts.[109,235,236] *H. cinaedi* bacteremia and cellulitis have also been observed in normal hosts, such as patients recovering from orthopedic surgery, in the course of an apparent nosocomial outbreak.[108] The cellulitis in some patients is described as having an atypical appearance—salmon pink, red-brown, or copper-colored, and lacking the expected local heat; some patients have more discrete indurated plaquelike lesions. Rarely, local pyoderma gangrenosum–like cutaneous infection without associated bacteremia may occur.[109,162] An appreciation of the rash is valuable since growth in blood cultures is often slow. *H. cinaedi* is susceptible to ceftriaxone, imipenem, tetracyclines, aminoglycosides, rifampin, and often ciprofloxacin. Prolonged therapy (2–6 weeks) is usually required to ensure resolution of symptoms and prevent recurrence.

Infective Endocarditis

The cutaneous lesions of subacute bacterial endocarditis consist of petechiae, subungual splinter hemorrhages, Osler nodes, and Janeway lesions.[237] Petechiae tend to occur in small crops, particularly in the conjunctivae, on the palate, and on the upper part of the chest and extremities, and they are the most common skin lesions of endocarditis (see Fig. 80.2). Rarely, petechiae are extremely numerous, particularly on the lower extremities, and suggest a primary vasculitis. Osler nodes are split pea–sized, erythematous, tender nodules located principally on the pads of the fingers and toes. They are few in number at any given time and occur in about 15% of patients with subacute bacterial endocarditis. The lesions are usually transient and clear in 1 to 2 days. Similar lesions may also occur in acute endocarditis (e.g., secondary to *S. aureus*). Histologic examination of such lesions in several cases of acute endocarditis has suggested septic embolization in their pathogenesis.[238] The genesis of Osler nodes in subacute bacterial endocarditis may have a different basis, perhaps sterile embolization or an allergic vasculitis. Janeway lesions (see Fig. 80.3) are painless, small, erythematous macules or minimally nodular hemorrhages in the palms or soles that occur in acute or subacute endocarditis—more commonly in the former, particularly if *S. aureus* is the cause, in which case they occur in 6% of patients.[239] Histologic findings in a case of *S. aureus* endocarditis have indicated that Janeway lesions are caused by septic microembolization.[238] Gangrenous or hemorrhagic lesions resembling Janeway lesions have been observed in marantic endocarditis[240] and in a patient with end-stage renal failure and dystrophic cardiac valvular calcification.[241]

Fungemias: *Candida albicans* and Other Yeasts

Systemic candidiasis developing in settings such as leukemia, immunosuppression, extensive antibiotic therapy, hyperalimentation, heroin addiction, or cardiac surgery may be difficult to diagnose clinically until the organism is isolated from routine blood cultures. The portal for disseminated candidiasis (or aspergillosis) may be an area of skin injured in the course of intravenous therapy (or trauma induced by adhesive tape or extravasation of intravenous fluid). Examination of the optic fundi for possible candidal endophthalmitis is sometimes helpful in making an early diagnosis of candidal fungemia while awaiting isolation of the organism from blood cultures. In neutropenic patients, the appearance of multiple, discrete (2- to 5-mm), pink maculopapules (sometimes with pale centers) on the trunk or extremities can suggest the diagnosis.[242] In some of these patients, severe diffuse muscle tenderness is present and muscle biopsy specimens have shown necrosis with yeast and pseudohyphal forms.[243] This is not entirely specific for candidiasis, because a similar presentation was seen with *Scedosporium inflatum*.[244] Disseminated cryptococcosis can also be associated with superficial (cellulitic) and deep soft tissue infection.[245] Occasionally, subcutaneous candidal abscesses may develop in the course of fungemia. Aspiration of such abscesses reveals yeasts on stained smear. Punch biopsy specimens of the maculopapular lesions provide a more accurate diagnosis than simple culture because histologic sections can reveal yeast cells in blood vessels and pseudohyphae in adjacent soft tissue. Isolation of *Candida* from an unroofed lesion may represent only surface colonization or may be consistent with *Candida* folliculitis rather than disseminated candidiasis. *M. furfur* folliculitis can be mistaken for hematogenous *Candida* lesions by appearance and histopathology.

SUBCUTANEOUS TISSUE INFECTIONS AND ABSCESSES

Exact categorization of some bacterial infections of the soft tissues (e.g., skin, subcutaneous tissue, fascia, skeletal muscle) may be difficult. Although differences between a superficial pyoderma and a necrotizing myositis such as gas gangrene are readily apparent, distinctions between many other types of soft tissue infection are sometimes blurred. Classification is usually based on features such as the anatomic structure involved, infecting organisms, and the clinical picture. Some infections may involve several components of the soft tissue, and multiple bacterial species may produce infections with the same clinical appearance.

To further compound the problem of classification, a variety of designations have been given to closely related or almost identical processes. For example, streptococcal gangrene has also been referred to as necrotizing fasciitis, but over time it became apparent that it was sometimes caused by bacteria other than group A streptococci.[21] Therefore streptococcal gangrene can be considered the major subset of necrotizing fasciitis. For convenience, because a major feature of its manifestation is cutaneous gangrene, streptococcal gangrene was considered in the preceding section with cellulitis and infectious cutaneous gangrene. Necrotizing fasciitis is reconsidered in this section on subcutaneous tissue infections, particularly in relation to its nonstreptococcal causes. Another nomenclature problem arises from infections that involve multiple soft tissue strata and that can be caused by a variety of bacterial species. For example, the condition known as synergistic necrotizing cellulitis has also been described as gram-negative anaerobic cutaneous gangrene and as synergistic nonclostridial anaerobic myonecrosis.[246,247] Because of the prominence of subcutaneous tissue involvement in this condition, it is considered primarily in this part of the chapter, although it could be considered almost as readily in the first part (see under "Cellulitis and Superficial Infections") or in the chapter on myositis (see Chapter 94). These classic and somewhat overlapping terms seem to be receding from clinical use, as the more general term *necrotizing soft tissue infection* gains popularity.[248]

Clostridial Anaerobic Cellulitis

Clostridial anaerobic cellulitis is a necrotizing clostridial infection of devitalized subcutaneous tissue. Deep fascia is not appreciably involved, and ordinarily no associated myositis is present. Gas formation is common and often extensive. Anaerobic cellulitis is several times more common than gas gangrene in war wounds.

Pathologic Characteristics and Pathogenesis

Clostridial species, usually *Clostridium perfringens,* are introduced into subcutaneous tissue through a dirty or inadequately débrided traumatic wound, through contamination at surgery, or from a preexisting localized infection. The last is frequently located in the perineum, abdominal wall, buttocks, or lower extremities, areas that are readily contaminated with fecal flora. The presence of foreign debris and necrotic tissue in the depths of a wound provides a suitable anaerobic milieu for clostridial proliferation. Very rarely, clostridial anaerobic cellulitis develops not after primary cutaneous injury but rather as a consequence of primary *C. septicum* bacteremia in the setting of leukemia and granulocytopenia.[249] Intestinal erosions are the presumed initial portals of entry. This type of *C. septicum* cellulitis must be distinguished from the even more life-threatening bacteremic *C. septicum* myonecrosis, which is often associated with a cryptic underlying colonic neoplasm (see Chapter 94).

Clinical Manifestations

The incubation period is several days, longer than the 1 to 2 days for clostridial myonecrosis. The onset is gradual, but the process may subsequently spread rapidly.[250] Local pain, tissue swelling, and systemic toxicity are not prominent features, and the relative mildness of the process helps distinguish it from true gas gangrene. The dark blebs and bronzing of the skin seen in gas gangrene are not usually features of clostridial cellulitis. Thin, dark, sometimes foul-smelling drainage from the wound (often containing fat globules) is characteristic, as is extensive tissue gas formation, which is more prominent than that observed in clostridial myonecrosis. Frank crepitus is present in the involved area and may extend widely, even beyond the limits of the active infection. *C. perfringens* cellulitis has been recurrent in the setting of retained foreign bodies.[251]

Gram-stained smears of drainage material show numerous blunt-ended, thick, gram-positive bacilli and variable numbers of polymorphonuclear leukocytes. Soft tissue radiographs show abundant gas, but not usually in the feathery linear pattern in muscles observed in clostridial myonecrosis.

Etiologic Agents

C. perfringens is the most common clostridial species responsible for this infection, but *C. septicum* and other species have been isolated. Sometimes, clostridia are present in mixed culture with facultative organisms.

Differential Diagnosis

If wound crepitus is observed, a variety of possibilities must be considered in the differential diagnosis (Table 93.6). The first is clostridial myonecrosis (gas gangrene) because of the fulminant, life-threatening nature of the infection and the requirement for emergency surgery. At the same time, distinguishing between clostridial gas gangrene and anaerobic cellulitis is essential to avoid performing unnecessarily extensive surgery. Ultimately, the two processes are differentiated in the operating room, when the wound is laid open and the viability and appearance of the muscle are observed. The muscle is normal (pink) in clostridial cellulitis but distinctly abnormal in clostridial myonecrosis: it is discolored, fails to contract on stimulation, and does not bleed from a cut surface (see Chapter 94).

Presumptive Therapy

Surgical exploration is essential to determine the presence of any muscle involvement. If no myonecrosis is found, treatment should be limited to débridement of necrotic tissue, removal of any foreign material, and drainage of pus after the wound is opened widely. Initial antimicrobial management of clostridial cellulitis requires broad-spectrum antibiotic therapy until surgical exploration has been carried out and Gram-stained smears of material from the lesion have been evaluated. Empirical therapy must cover clostridial infection (myonecrosis or anaerobic cellulitis) and necrotizing polymicrobial infection. Intravenous penicillin (2–3 million units every 3 hours or 3–4 million units every 4 hours) or ampicillin (2 g every 4 hours), plus intravenous clindamycin (0.6 g every 6–8 hours) or metronidazole (1 g loading dose followed by 0.5 g every 6 hours), provides a two-drug combination for treatment of the anaerobic organisms likely to be involved; clindamycin resistance has been reported rarely when used as monotherapy in penicillin-allergic patients and should be combined with metronidazole in these patients. Ampicillin-sulbactam (3 g every 6 hours) may also be used as initial therapy. Use of an additional antimicrobial agent (fluoroquinolone agent such as ciprofloxacin, third-generation cephalosporin, or aminoglycoside) aimed at aerobic gram-negative bacilli is based on evaluation of Gram-stained smears of exudate and tissue. Definitive selection of antimicrobial agents is subsequently based on the results of cultures and antimicrobial susceptibility tests.

Nonclostridial Anaerobic Cellulitis

A clinical picture very similar to that of clostridial anaerobic cellulitis can be produced by infection with various non–spore-forming anaerobic bacteria (e.g., *Bacteroides* species, peptostreptococci, peptococci, either alone or as mixed infections).[247] The anaerobic bacteria may be present along with facultative species (coliform bacilli, various streptococci, staphylococci) in a mixed infection. Gas-forming soft tissue infections have been produced by *E. coli, Klebsiella, Aeromonas,* and perhaps other facultative bacteria.[252]

Because the clinical features and setting are similar to those of clostridial anaerobic cellulitis, the same initial broad-spectrum antimicrobial therapy (see earlier discussion) is appropriate to cover the mixed bacterial nature of the infection. Ampicillin-sulbactam or piperacillin-tazobactam may also be used as initial therapy in conjunction with a fluoroquinolone or aminoglycoside; such double coverage broadens activity against potentially resistant gram-negative pathogens. Monotherapy with a penem agent such as meropenem may be considered.

TABLE 93.6 Differential Diagnosis of Crepitant Soft Tissue Wounds

	CLOSTRIDIAL	NONCLOSTRIDIAL ANAEROBIC CELLULITIS	CLOSTRIDIAL MYONECROSIS (GAS GANGRENE)	ANAEROBIC STREPTOCOCCAL MYOSITIS	NECROTIZING FASCIITIS (TYPE I)[b]	INFECTED VASCULAR GANGRENE	SYNERGISTIC NECROTIZING CELLULITIS[c]	NONINFECTIOUS CAUSES OF GAS IN TISSUES
Predisposing Conditions	Local trauma or surgery	Diabetes mellitus, preexisting localized infection	Local trauma or surgery	Local trauma	Diabetes mellitus, abdominal surgery, perineal infection	Peripheral arterial insufficiency	Diabetes mellitus, cardiorenal disease, obesity, perirectal infection	Mechanical effects of penetrating trauma, injuries involving the use of compressed air, entrapment of air under loosely sutured wounds or under ulcers, irrigation of wounds with hydrogen peroxide, IV catheter placement, dissection of air from tracheostomy, or spontaneous mediastinal emphysema
Incubation Period	Usually >3 d	Several days	1–2 d	3–4 d	1–4 d	>5 d	3–14 d	Less than 1 h
Onset	Gradual	Gradual or rapid	Acute	Not as rapid as gas gangrene	Acute	Gradual	Acute	Usually present immediately after trauma or manipulation; may not be recognized until examination several hours later
Pain	Mild	Mild	Marked	Marked; occurs late	Moderate or severe	Variable	Severe	Mild
Swelling	Moderate	Moderate	Marked	Moderate	Marked	Moderate or marked	Moderate or marked	Slight or absent
Skin Appearance	Minimal discoloration	Minimal discoloration	Yellow-bronze, dark bullae, green-black patches of necrosis	Erythema	Erythematous cellulitis, areas of skin necrosis	Discolored or black	Scattered areas of skin necrosis	Only those resulting from initiating trauma
Exudate	Thin, dark	Dark pus	Serosanguineous	Abundant, seropurulent	Seropurulent	None	"Dishwater" pus	None
Gas	++++	++++	++	±	++	+++	++	Variable but present; does not extend
Odor	Sometimes foul	Foul	Variable, slightly foul or peculiarly sweet	Slight, "sour"	Foul	Foul	Foul	None
Systemic Toxicity	Minimal	Moderate	Marked	Only late in course	Moderate or marked	Minimal	Marked	None
Muscle Involvement	None	None	++++	+++	None	Dead	++	None

[a]In addition to the causes of crepitant infections listed in this table, *A. hydrophila* myositis may be associated with gas in soft tissues.
[b]The term *necrotizing fasciitis* is used here to designate forms of this syndrome other than streptococcal gangrene.
[c]Synergistic necrotizing cellulitis is essentially the same process as type I necrotizing fasciitis. Because the former occasionally tends to involve muscle, it is given a separate designation here; however, the two processes are clinically indistinguishable in most cases.
IV, Intravenous; ±, rarely present; ++, present to mild extent; +++, present to moderate extent; ++++, extensive.
Modified from Finegold SM. Anaerobic Bacteria in Human Disease. *New York: Academic Press; 1977.*

Evaluation of Gram-stained smears of exudate aspirated from the lesion supplies a more focused basis for initial antimicrobial therapy. Subsequent results of cultures and susceptibility testing of aspirates or tissue removed at surgical exploration provide the information needed for narrowing (or extending) antimicrobial therapy. The surgical approach used is the same as for the treatment of clostridial anaerobic cellulitis.

Necrotizing Fasciitis

The term *necrotizing fasciitis* encompasses at least three distinct bacteriologic entities.[21,253,254] In type I (polymicrobial) necrotizing fasciitis, at least one anaerobic species (most commonly *Bacteroides* or *Peptostreptococcus*) is isolated in combination with one or more facultative anaerobic species, such as streptococci (other than group A) and members of the Enterobacteriaceae (e.g., *E. coli, Enterobacter, Klebsiella, Proteus*). An obligate aerobe such as *P. aeruginosa* is only rarely a component of such a mixed infection. Cases in which only anaerobes are present appear to be rare. In type II disease, generally attributed to monomicrobial infection and most commonly due to group A streptococci (also known as hemolytic streptococcal gangrene), group A streptococci are isolated alone or in combination with other species, most commonly *S. aureus.* Recognition of type III necrotizing fasciitis due to infection solely by marine gram-negative pathogens such as *Vibrio* species or *Aeromonas hydrophila* has been proposed.[253,254]

Classification of necrotizing fasciitis syndromes continues to evolve. Monomicrobial gram negative infections due to Klebsiella[255] and other Enterobacteriaceae[256] have been reported predominantly in Asian populations, particularly among diabetics.[256] Monomicrobial necrotizing fasciitis due to miscellaneous gram-negative infections such as *P. multocida* and *H. influenzae,* have also been reported.[257] In addition, fungal necrotizing fasciitis due to both yeasts and true molds have been documented and proposed as representing a fourth type of necrotizing fasciitis.[257] Streptococcal gangrene was considered in the first part of this chapter as a form of gangrenous cellulitis. In this section, specific comments about streptococcal gangrene are limited to the expanded setting in which the disease can appear and the changes in clinical features noted with the recent apparent increase in bacteremic and severe invasive group A streptococcal infections and their association with the streptococcal toxic shock–like syndrome.[4,159,160]

Hemolytic streptococcal gangrene occurs after minor trauma, stab wounds, or surgery, particularly in the context of diabetes and peripheral vascular disease, but cirrhosis and corticosteroid therapy have also been predisposing factors.[159] In outbreaks of streptococcal toxic shock–like syndrome, chills, fever (or profound hypothermia and shock), confusion, vomiting, diarrhea, tachycardia, hypotension, and multiorgan failure are prominent features. Necrotizing fasciitis is present in about half of cases of streptococcal toxic shock–like syndrome.[4,160] Streptococcal strains of M protein types 1, 3, 12, and 28 are most commonly involved and, in the United States, they usually elaborate pyrogenic exotoxin A. The role of nonsteroidal antiinflammatory drugs as a risk factor for developing necrotizing fasciitis or increasing the rate of complications in this setting continues to be debated.[258,259] These agents may simply delay timely diagnosis by reducing pain and local inflammatory findings, or may act as an important accelerator of necrotizing infection by reducing prostaglandin production and increasing the production of proinflammatory cytokines.[259] Leukocytosis, thrombocytopenia, azotemia, and increased serum levels of creatine phosphokinase are commonly present. Increasing creatine phosphokinase levels may serve as an indication of progression of streptococcal cellulitis to necrotizing fasciitis and myositis. Unlike many earlier cases of hemolytic streptococcal gangrene, which affected older individuals with underlying diseases, more recently streptococcal toxic shock syndrome has occurred primarily in young, previously healthy adults after minor trauma.[260] In 70% of patients, soft tissue findings progressed to hemolytic streptococcal gangrene with the development of vesicles, violaceous bullae, and necrosis of subcutaneous tissues typical of necrotizing fasciitis (or myositis) requiring surgical débridement.[260] The mortality rate was about 30%. In young children, the skin lesions of varicella can be superinfected with group A streptococci and become a major risk factor for group A streptococcal necrotizing fasciitis.[261]

In patients with severe, invasive group A streptococcal infection, a primary site of infection generally involving skin and soft tissue is identifiable in roughly 75% of cases, with necrotizing fasciitis present in most of these patients.[160] There is a high rate of streptococcal bacteremia. The pathogenesis of streptococcal toxic shock appears to involve microbial and host factors. The predominant group A streptococci isolated in several outbreaks have expressed the common M protein type M1 or M3 (although other M types are also associated with this syndrome) and are classified in the tissue-tropic *emm* families D and E; they possess pyrogenic exotoxin gene *speA* or *speC* and express pyrogenic exotoxins in vitro.[262] Genome microarray analysis has demonstrated that M1 and M3 strains carry several additional unique virulence factors that may be responsible for the apparent association of these strains with invasive infection.[263] Host susceptibility to streptococcal toxic shock syndrome may be related to an absence of suitable protective antibodies against the M protein of invading streptococci as well as against the pyrogenic exotoxins. Such seronegative individuals would be expected to be at heightened risk for invasive streptococcal infection and toxic shock syndrome,[160] but a recent multicenter retrospective cohort study of pressor-dependent patients with necrotizing fasciitis, involving over 4000 patients at 121 centers, found that intravenous immune globulin therapy was actually administered in only 4% of patients and had no effect on survival or hospital length of stay.[264] Because the streptococcal pyrogenic exotoxins act as superantigens, which activate T cells by binding to human leukocyte antigen (HLA) class II molecules, protection against or heightened susceptibility to streptococcal toxic shock may correlate with certain HLA-DQ polymorphisms.[265] The more general features of necrotizing fasciitis are now considered.

Clinical Manifestations

Necrotizing fasciitis is an uncommon severe infection involving the subcutaneous soft tissues, particularly the superficial (and often the deep) fascia. It is usually an acute process but rarely may follow a subacute progressive course. Necrotizing fasciitis can affect any part of the body but is most common on the extremities, particularly the legs. Extremity involvement is particularly common with type II (group A streptococcal) necrotizing fasciitis; abdominal wall, perianal and groin areas, and postoperative wounds are more common with type I (polymicrobial) infections.[253,254] The portal of entry is usually a site of trauma, often minor (e.g., laceration, abrasion, burn, insect bite); a laparotomy performed in the presence of peritoneal soiling[266] (e.g., penetrating abdominal trauma or perforated viscus) or another surgical procedure (e.g., hemorrhoidectomy, vasectomy); perirectal abscess; decubitus ulcer; or intestinal perforation. The last may be secondary to occult diverticulitis,[267] rectosigmoid neoplasm, perforated appendicitis, or a foreign body such as a chicken bone or toothpick. Necrotizing fasciitis from such intestinal sources may present in the lower extremity (extension along the psoas muscle),[267] as well as in the groin or abdominal wall (via a colocutaneous fistula). Particular clinical settings in which necrotizing fasciitis may develop include diabetes mellitus,[253] alcoholism, and parenteral drug abuse,[268] often following "skin popping."[268,269]

In the neonate, necrotizing fasciitis can be a serious complication of omphalitis. Initial swelling and erythema about the umbilicus can progress over several hours to several days and result in purplish discoloration and periumbilical necrosis.[270] Involvement of the anterior abdominal wall frequently extends to the flanks and even onto the chest wall.

The affected area is initially erythematous, swollen, without sharp margins, hot, shiny, exquisitely tender, and painful.[253,254,271] Lymphangitis and lymphadenitis are infrequent. The process progresses rapidly over hours to days, with sequential skin color changes from red-purple to patches of blue-gray. Within 3 to 5 days after onset, skin breakdown with bullae (containing thick pink or purple fluid) and frank cutaneous gangrene (resembling a thermal burn) can be seen. By this time, the involved area is no longer tender but rather has become anesthetic secondary to thrombosis of small blood vessels and destruction of superficial nerves located in the necrotic, undermined subcutaneous tissue. The onset of anesthesia may antedate the appearance of skin necrosis and provide a clue that the process is necrotizing fasciitis and not a simple cellulitis. Marked swelling and edema may produce a

compartment syndrome especially with extremity involvement, with complicating extensive myonecrosis requiring prompt fasciotomy. Measurement of compartment pressure may aid the evaluation in early situations in which marked pain and swelling are present without concomitant skin changes that would indicate the diagnosis.[4] Subcutaneous gas is often present in the polymicrobial form of necrotizing fasciitis, particularly in patients with diabetes mellitus. Systemic toxicity is prominent, and the temperature is often, but not universally, elevated in the range of 38.9°C to 40.5°C (102°F–105°F). Immunosuppressed individuals may lack fever or systemic toxicity, making the diagnosis particularly challenging. On probing the lesion with a hemostat through a limited incision, the instrument easily passes along a plane just superficial to the deep fascia. Such easy passage would not occur with ordinary cellulitis.

Leukocytosis is commonly present; hyponatremia and azotemia underscore the likelihood of necrotizing infection.[272] Gram-stained smears of exudate usually reveal a mixture of organisms or, in the case of streptococcal gangrene, chains of gram-positive cocci. Rarely, a single population of gram-negative rods can be seen, suggesting type III necrotizing fasciitis. In one case, we observed numerous long, gram-positive bacilli with subterminal spores (along with gram-negative bacilli) in the foul-smelling, purulent exudate of a patient with crepitant necrotizing fasciitis after a lower leg amputation for peripheral vascular disease. The presence of numerous spores in the wound exudate indicated that the gram-positive bacilli were unlikely to be *C. perfringens.* Before surgery, the patient had *Clostridioides difficile* (formerly *Clostridium difficile*) enterocolitis, and *C. difficile* was isolated along with several members of Enterobacteriaceae from the wound drainage material.

Blood cultures are frequently positive. Hypocalcemia (without tetany) may occur if necrosis of subcutaneous fat is extensive.

Fournier gangrene. A form of necrotizing fasciitis occurring about the male genitalia and the perineum of both sexes is known as Fournier gangrene[21,273,274] and also as idiopathic gangrene of the scrotum, streptococcal scrotal gangrene, and perineal phlegmon. It may be confined to the penis or scrotum, or it may extend to involve the perineum, penis, and abdominal wall. Although typically seen in individuals over the age of 50, it may occur in children and young adults.[275] Systemic predisposing factors include diabetes mellitus, obesity, smoking, vascular disease, immunocompromised states (including HIV infection), local trauma, paraphimosis, periurethral extravasation of urine, perirectal or perianal infection,[276] and surgery in the area (circumcision, herniorrhaphy, Bartholin abscess). In cases originating in the genitalia, the infecting bacteria probably penetrate the urethra and paraurethral glands. Once infection penetrates Buck fascia of the penis, it can spread along the contiguous fascias of the scrotum and penis (dartos fascia), Colles fascia of the perineum, and Scarpa fascia of the anterior abdominal wall.[277,278] Anaerobic bacteria play an important role and contribute to the typical foul odor associated with this form of necrotizing fasciitis. Mixed cultures containing facultative organisms (*E. coli, Klebsiella,* other Enterobacteriaceae, enterococci), along with anaerobes *(Bacteroides, Fusobacterium, Clostridium,* anaerobic or microaerophilic streptococci), and occasionally *C. albicans,*[277] have been obtained from studied cases.[273,279] *S. aureus* and various β-hemolytic streptococci occur in roughly one-third of patients[279]; rarely, group A streptococcal gangrene evolving from streptococcal balanitis can also involve the male genital area. Multidrug-resistant organisms are increasingly common, including *Acetinobacter* in some series, underscoring the importance of initial broad-spectrum antibiotic therapy.[280]

The infection commonly starts as cellulitis adjacent to the portal of entry. Early in the course of Fournier gangrene, the involved area is swollen, erythematous, and tender as the infection begins to involve the deep fascia. Pain is prominent; fever and systemic toxicity are marked. Swelling and crepitus of the scrotum quickly increase, and dark purple areas develop and progress to extensive scrotal gangrene. If the abdominal wall becomes involved in an obese patient with diabetes, the process can spread extremely rapidly.

Other special anatomic forms of necrotizing fasciitis. Necrotizing fasciitis of the face and eyelids,[281] neck,[282,283] and lips is uncommon but life threatening. It is most often caused by group A streptococci, alone or with *S. aureus,* and represents streptococcal gangrene; occasionally, it represents mixed infections of group A streptococcus with Enterobacteriaceae or oral *Bacteroides* species. Although necrotizing fasciitis of the head and neck is often considered a single entity, in fact it represents two conditions etiologically.[284] Craniofacial necrotizing fasciitis is commonly caused by group A streptococci or *S. aureus,* whereas cervical necrotizing fasciitis often represents a polymicrobial process (e.g., group A streptococci, various other streptococcal species, *Bacteroides* or *Peptostreptococcus* spp.). In mixed infections, crepitus may be a feature, as may necrosis of the epidermis and superficial fascia. Trauma is the usual precipitating cause of necrotizing fasciitis of the periorbital areas and face; dental, oral (including sialoadenitis), and pharyngeal infections predispose to cervical necrotizing fasciitis. Differentiation of the latter from cervical soft tissue infection of odontogenic origin can be difficult, but rapid spread of infection to other areas of the neck, severe pain, and systemic symptoms along with subcutaneous crepitus suggest the diagnosis of necrotizing fasciitis. If crepitus is not palpable, soft tissue radiography or prompt CT may help in the diagnosis by demonstrating subcutaneous gas. The mortality associated with cervical necrotizing fasciitis is about four times as high as that from craniofacial necrotizing fasciitis.[284]

Other microbial causes of necrotizing fasciitis. Various pathogens have been occasionally recovered from wounds and often from blood cultures of patients with necrotizing fasciitis. Gram-negative necrotizing fasciitis due to *A. hydrophila* has been reported after open trauma. Immunocompromised hosts, including neutropenic children receiving cancer chemotherapy, are at risk for necrotizing fasciitis caused by *P. aeruginosa* or Enterobacteriaceae.[285] The acute cellulitis caused by *V. vulnificus* and other *Vibrio* species (described earlier) may extend to the superficial and deep fascia and produce necrotizing fasciitis. Similarly, MRSA may cause necrotizing fasciitis, usually in HIV-infected or other immunocompromised hosts.[286] Very rare causes of necrotizing fasciitis include nasopharyngeal pathogens such as *S. pneumoniae* and *H. influenzae* type b, food-associated pathogens (*Listeria monocytogenes, Salmonella* spp.), and environmental species such as *A. hydrophila, Elizabethkingia meningoseptica, Chryseobacterium* species, *Ochrobactrum anthropi*, and *Serratia marcescens.* The uncommonly encountered necrotizing fasciitis caused by *S. marcescens* has been the subject of case reviews that have emphasized its high mortality.[287,288]

Differential Diagnosis

Table 93.6 details the differential diagnosis of necrotizing fasciitis. Necrotizing neutrophilic dermatoses, including progressive pyoderma gangrenosum and necrotizing Sweet syndrome, can present with systemic features including shock, and can mimic necrotizing fasciitis.[289]

Prompt diagnosis is of paramount importance because of the rapidity with which the process can progress. The reported mortality rate of necrotizing fasciitis has approached 35% overall and is somewhat less (15%) for Fournier gangrene[273,274]; current analyses demonstrate improved survival overall (~15%).[290,291] Prompt diagnosis is generally associated with enhanced survival. Early clinical differentiation of necrotizing fasciitis from cellulitis can be difficult because the initial signs—including pain, edema, and erythema—are not distinctive, particularly when deep trunk or retroperitoneal sites are involved.. However, the presence of severe pain or marked systemic toxicity out of proportion to the local findings should alert the physician to this possibility. Although CT and magnetic resonance imaging (MRI) can demonstrate subcutaneous and fascial edema, as well as tissue gas, in patients with necrotizing fasciitis and can distinguish this process from cellulitis, delay of definitive surgical treatment, such as can occur with the use of MRI, should be avoided.[292,293] Ultrasonography and CT are more rapidly performed and have occasionally been helpful for assessing possible Fournier gangrene when local scrotal inflammatory findings are present but cutaneous necrotizing infection or crepitus is not apparent.[294,295] However, in patients in whom the diagnosis is clearly suspected, the most expeditious route to diagnosis is through surgical exploration or biopsy, without introducing delay for imaging studies. Characteristic abnormalities are readily seen upon exposing the deep subcutaneous tissues, with discolored and edematous tissue, local necrosis, and a characteristic thin exudate. Blunt dissection readily separates the tissues planes due to local spread and undermining of the adjacent tissue.[248]

Imaging studies can be most useful early in the process, when pain and swelling are evident but cutaneous changes are absent and the diagnosis is uncertain. Imaging studies may also be helpful for monitoring clinical progress after surgical débridement, when further surgery may need to be considered. Additionally, imaging studies are useful in the diagnosis and management of necrotizing fasciitis in areas of the body in which the process is more inaccessible (e.g., retroperitoneal involvement[296]) or may readily spread to other tissue compartments (e.g., cervical fasciitis spreading to the mediastinum, pleura, and pericardium). Several imaging findings (e.g., fascial thickening) are actually nonspecific, and distinguishing between a surgical emergency and an inflammatory fasciitis syndrome is based on the dramatic clinical features.[297] Frozen-section examination of biopsy specimens (including dermis, infected subcutaneous tissue, fascia, and underlying muscle) has been found to be helpful for early diagnosis.[298]

Presumptive Therapy

Once the diagnosis is made, immediate surgical débridement is essential. In patients in whom the diagnosis is clearly suspected on clinical grounds (deep pain with patchy areas of surface hypoesthesia, crepitation, or bullae and skin necrosis), direct operative intervention is indicated. Extensive incisions should be made through the skin and subcutaneous tissues and should go beyond the area of apparent involvement until normal fascia is found. Necrotic fat and fascia should be excised, and the wound should be left open. A second-look procedure is frequently necessary 24 hours later to ensure adequacy of the initial débridement.[253,254] In the case of Fournier gangrene, orchiectomy is not usually necessary because the testes have their own blood supply originating from the retroperitoneum, independent of the compromised fascial and cutaneous circulation of the scrotum. Initial antimicrobial therapy is based on the evidence for prominent roles of anaerobic bacteria, Enterobacteriaceae, and various streptococci in this process and on the specific findings on Gram-stained smears. The relatively frequent presence of *S. aureus* (approximately 20%) further complicates initial antibiotic management. Antibiotics used before bacteriologic data are obtained include combinations of vancomycin, cefepime, gentamicin, and metronidazole; vancomycin, piperacillin-tazobactam, and gentamicin; and vancomycin with imipenem or meropenem. Patients intolerant of vancomycin should receive linezolid or daptomycin until assessment of resistant gram-positive pathogens is completed. Aminoglycosides must be used cautiously and monitored closely, since there is a high rate of acute kidney injury in these patients. A fluoroquinolone such as ciprofloxacin is often administered rather than gentamicin owing to impaired renal function at presentation. For group A streptococcal necrotizing fasciitis, penicillin or ampicillin plus clindamycin is recommended.

Several ancillary therapies, none of which are substitutes for prompt surgical débridement or of proven efficacy, have been described. One is the use of intravenous immune globulin to treat the streptococcal toxic shock syndrome accompanying the treatment of group A streptococcal necrotizing fasciitis.[4,262,299] A recent large-scale retrospective cohort analysis demonstrated that intravenous immune globulin administration did not have a demonstrable effect on survival or length of hospital stay.[264] The other is the use of hyperbaric oxygen for the treatment of polymicrobial necrotizing fasciitis, particularly of the trunk.[272,300] A large Australian retrospective study demonstrated a statistically significant reduction in mortality among recipients of hyperbaric oxygen therapy, but many potential biases confound such analysis.[301] This modality is not widely available and should not delay urgent débridement and conventional therapy with appropriate antibiotics and intensive care supportive measures (also see Chapter 50).

Synergistic Necrotizing Cellulitis

Clinical Manifestations

Synergistic necrotizing cellulitis (gram-negative anaerobic cutaneous gangrene, necrotizing cutaneous myositis, synergistic nonclostridial anaerobic myonecrosis) is a variant of necrotizing fasciitis, with prominent involvement of skin and muscle and of subcutaneous tissue and fascia. Some cases of Fournier gangrene extending onto the abdominal wall are examples of this condition. Predisposing factors include diabetes mellitus, obesity, advanced age, and cardiorenal disease. Most infections are located on the lower extremities or near the perineum (e.g., originating in a perirectal abscess).[246] The lesion may first be manifested as small skin ulcers draining foul-smelling, reddish-brown (dishwater) pus. Circumscribed areas of blue-gray gangrene surround these draining sites, but the intervening skin appears normal despite necrosis of underlying subcutaneous tissue, fascia, and muscle. Local pain and tenderness are marked. Tissue gas is apparent in about 25% of patients. Systemic toxicity is a feature; about 50% of patients have bacteremia.

Etiologic Agents

Cultures consistently show mixtures of anaerobic bacteria (anaerobic streptococci, *Bacteroides,* or both) and facultative bacteria *(Klebsiella, Enterobacter, E. coli, Proteus),*[246] similar to other necrotizing soft tissue processes, although monomicrobial infection (e.g., due to *B. cereus*) has been reported.

Presumptive Therapy

Initial surgery involves incision and drainage, as with necrotizing fasciitis, but radical débridement is often necessary because of extensive involvement of deep fascia and muscle.[246] Amputation may be required. Antibiotic management is initially based on the results of Gram-stained smears of wound exudates, but it should include an antimicrobial agent effective against *Bacteroides* (see earlier discussion of presumptive therapy for type I necrotizing fasciitis).

Miscellaneous Infections Secondary to Trauma

Bite Infections

Infections secondary to bites are discussed in Chapter 315.[302–304]

Burn Infections

Infections secondary to burns are discussed in Chapter 314.

Injection Site Abscesses

Subcutaneous and intramuscular abscesses infrequently occur after therapeutic injections. *S. aureus,* facultative gram-negative bacilli, and anaerobic bacteria are usually implicated. Hematomas may represent sites of delayed infection. Gas gangrene has occurred after various injections, including insulin[305] and aqueous epinephrine. Opportunistic infections, including mucormycosis, have complicated insulin injections in immunocompromised hosts.[306] Subcutaneous and intramuscular abscesses caused by a variety of oral anaerobes and streptococci have occurred after skin popping or attempted intravenous injections by narcotic addicts.[247,307] Injection-related anthrax is being increasingly recognized in the United Kingdom.[64] In the case of subcutaneous abscesses secondary to intravenous drug abuse, vascular ultrasonography may be helpful regarding the need for excision of involved veins, which often contain pus (suppurative thrombophlebitis) or an infected thrombus,[307,308] in addition to débridement and drainage.

Factitial Disease (Self-Induced Abscesses)

Occasionally, subcutaneous abscesses and cellulitis are produced when a patient deliberately injects or inserts contaminated substances into the skin.[309,310] Such abscesses are often recurrent and may be of monomicrobial or polymicrobial origin, usually consisting of oral or fecal flora. Sterile abscesses may be induced by the introduction of foreign material without bacterial contamination. Such foreign material may be identified by examination of biopsy specimens with polarizing microscopy.

Subcutaneous Infections Originating in Contiguous Foci

Osteomyelitis

Occasionally, most commonly in a child, acute hematogenous osteomyelitis is manifested as a subcutaneous abscess (see also Chapter 104). Under these circumstances, a subperiosteal abscess has ruptured through intervening tissue into the subcutaneous tissue. *S. aureus* is the most common causative agent in such infections. It is important to recognize the nature of the process because of the different therapeutic

programs required for osteomyelitis and for a subcutaneous abscess of cutaneous origin. Involvement of subcutaneous tissue as a consequence of osteomyelitis may also occur in the form of a draining sinus associated with chronic osteomyelitis and sequestrum formation. Multiple draining sinuses may occur as a result of multiple foci of osteomyelitis in disseminated blastomycosis. Routine radiographs may demonstrate underlying osteomyelitis in relation to the observed subcutaneous abscess; in acute osteomyelitis, MRI will be diagnostic, even when conventional studies are nondiagnostic.

Actinomycosis

Subcutaneous abscesses frequently develop in the course of cervical, thoracic, or sometimes abdominal actinomycosis. Draining sinuses ultimately result (see Chapter 254).

Primary Pyodermas

On occasion, more superficial skin infections beginning as folliculitis, furunculosis, or cellulitis may progress into the deeper subcutaneous tissue and form a subcutaneous (sometimes cold) abscess. *S. aureus* is commonly the cause. Such progression occurring repeatedly might suggest certain underlying phagocytic cell defects, such as chronic granulomatous disease of childhood[311] or hyperimmunoglobulin E syndrome (Job syndrome).[312,312a] These disorders are discussed further in relation to suspected immunodeficiency states in Chapter 12.

In a cataloguing of the bacteriology of a large number of cutaneous abscesses (with unspecified individual predisposing causes), *S. aureus* was the single most common aerobic-facultative isolate, followed in frequency by groupable (A, B, C, D) and nongroupable streptococci.[218] Among anaerobic isolates, *Bacteroides* species (most commonly the *B. fragilis* group) were most frequent, followed by *Peptostreptococcus* and *Clostridium* species. These abscesses are commonly polymicrobial (mixed aerobic-anaerobic). As might be predicted, *S. aureus* is the principal isolate in infections (both abscesses and wounds) of the extremities and trunk, whereas anaerobes are more numerous than aerobic facultative species in such infections in the genital, perirectal, inguinal, and head and neck areas.

Subcutaneous Abscesses in the Course of Bacteremic Infections

Metastatic pyogenic infections can occur during the course of bacteremias or endocarditis caused by various common invasive organisms (e.g., *S. aureus*) in subcutaneous tissue, as well as other organs and tissues. These abscesses are tender and fluctuant. Rarely, multiple, firm, nodular subcutaneous lesions clinically resembling those of Weber-Christian disease occur in the course of a staphylococcal bacteremia. If promptly identified and treated, the process may be aborted before frank abscess formation occurs. Other pathogens that commonly cause bacteremia but are only rarely associated with metastatic subcutaneous abscesses include *Streptococcus pneumoniae*[313] and *V. vulnificus*.[314] Conversely, bacterial pathogens that are infrequently responsible for bacteremia (e.g., *Nocardia* spp.,[315] *Corynebacterium jeikeium*[316]) may also occasionally produce metastatic cutaneous abscesses in immunocompromised or debilitated individuals.

Mycetoma

See Chapter 261.

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The complete reference list is available online at Expert Consult.

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94 Myositis and Myonecrosis

Mark S. Pasternack

SHORT VIEW SUMMARY

Definition

- Myositis is an inflammatory and generally necrotizing process primarily due to hematogenous seeding of muscle with subsequent bacterial invasion. Direct inoculation of muscle as the result of penetrating trauma is also an important mechanism of infection (associated with clostridial myonecrosis). More generalized muscle inflammation may also accompany a variety of acute and chronic viral and parasitic disorders.

Epidemiology

- Pyomyositis occurs across the age spectrum in temperate regions and may occur in previously healthy as well as immunocompromised individuals; in warm climates infections in children predominate (i.e., tropical pyomyositis). Clostridial myonecrosis most commonly complicates penetrating trauma (e.g., vehicular accidents, war, natural disasters), especially in resource-limited settings.

Microbiology

- *Staphylococcus aureus* is the classic cause of pyomyositis, but similar illnesses have been associated with a wide variety of bacterial pathogens, particularly in compromised hosts. *Clostridium perfringens* myonecrosis complicates penetrating trauma, but nontraumatic clostridial myonecrosis may develop after hematogenous dissemination of more aerotolerant species (e.g., *Clostridium septicum, Clostridium sordellii*). Group A streptococci can also cause severe myonecrotic infection, which is a true medical emergency. Acute generalized muscle inflammation occurs after influenza and dengue virus infections, but a wide variety of viral pathogens have sporadically led to significant muscle injury and even severe rhabdomyolysis.

Diagnosis

- Consideration of these uncommon processes is the first step toward the proper diagnosis because the focal progressive pain of pyomyositis mimics a variety of infectious and noninfectious disorders. Blood cultures and (percutaneous) drainage based on the findings of cross-sectional imaging, with subsequent microbiologic characterization, confirm the diagnosis and guide therapy. Pyomyositis may accompany toxic shock, and investigation of a focal process responsible for fulminant systemic illness is essential. Gas production in muscle and soft tissue in the setting of a rapidly progressive illness occurs in clostridial myonecrosis and related infections; this is a surgical emergency and exploration for débridement of nonviable tissue and appropriate cultures is critical.

Therapy

- Ideally, after expedited imaging and drainage, empirical broad-spectrum antibacterial therapy effective against *Staphylococcus aureus,* including methicillin-resistant *S. aureus*, and gram-negative bacilli and anaerobes should be administered. The findings of associated toxic shock mandate the addition of a protein synthesis inhibitor (e.g., clindamycin). There is a lack of evidence supporting the use of intravenous immunoglobulin therapy in this setting. Narrow-spectrum therapy is appropriate after identification and sensitivity testing of the isolated pathogen. Patients presenting with the clinical findings of gas gangrene (clostridial myonecrosis) require immediate high-dose penicillin and clindamycin and urgent surgical exploration.

Prevention

- Because most episodes of pyomyositis develop after transient bacteremia, prevention is not practical. Prompt débridement of devitalized tissue after penetrating injury is highly effective at preventing clostridial myonecrosis.

Infection of skeletal muscle (infectious myositis) is uncommon. When it occurs, a wide range of organisms may be responsible: bacteria, mycobacteria, fungi, viruses, and parasitic agents.[1] Bacteria invade muscle either from contiguous sites of infection (e.g., skin and subcutaneous abscesses, penetrating wounds, decubitus ulcers, osteomyelitis) or by hematogenous spread from a distant focus. It is helpful to categorize infectious myositis on the basis of clinical manifestations. These may be very distinctive, as in clostridial gas gangrene, and suggest the specific etiologic agent, or they may be very nonspecific, as in the myalgias of viral infections and infective endocarditis (Table 94.1). In certain instances (e.g., psoas abscess) it is the anatomic location rather than the morphologic characteristics of the lesion or the nature of the infecting agent that distinguishes the particular type of muscle infection.

PYOMYOSITIS

Pyomyositis is an acute bacterial infection of skeletal muscle that is most commonly caused by *Staphylococcus aureus.* After seeding of muscle and initial phlegmon development, pus accumulates within muscles; the muscle infection is not usually due to primary infection of adjacent skin, soft tissue, or bone. Clinically, pyomyositis is characterized by fever, localized muscle pain and stiffness, swelling, and tenderness.

Pathogenesis and Pathologic Characteristics

Bacterial infections of muscle usually occur after a penetrating wound, prolonged vascular insufficiency in an extremity, or a contiguous infection. Bacteremic spread of infection to skeletal muscle is extremely uncommon. Among fatal cases of staphylococcal septicemia, abscesses in skeletal muscle are found in less than 1%.[2] Pyomyositis (primary muscle abscess) is a bacterial infection of muscle that occurs in the absence of a predisposing site of infection. *S. aureus* is the most common cause.[3,4] Blood cultures are positive in 5% to 35% of the cases in most series at the time of presentation; metastatic infections in tissue other than muscle are rare, although multifocal pyomyositis,[5,6] distant hematogenous osteomyelitis,[7] and the development of venous thrombosis and septic pulmonary emboli have been reported.[8] In individual patients with multifocal infections or associated endocarditis, it may be challenging to clarify whether sustained bacteremia was the primary process or the result of progressive pyomyositis.[9,10]

Most cases of pyomyositis occur in the tropics, hence the term *tropical pyomyositis.* Historically, it accounted for 1% to 4% of hospital admissions in some tropical areas,[11] although recently in northern India pyomyositis accounted for only 0.03% of admissions.[12] In more temperate areas, pyomyositis is very uncommon, with approximately 330 reported cases

TABLE 94.1 Classification of Infectious Myositis

TYPE OF PROCESS	CLINICAL PATTERN	SPECIFIC CAUSES
Pyogenic and predominantly localized (spreading by contiguity)	Pyomyositis	*Staphylococcus aureus* Group A streptococci (occasionally) Other gram-positive cocci (rarely) Group B, C, or G streptococci *Streptococcus pneumoniae* Gram-negative bacilli (rarely) Anaerobic bacteria (rarely) *Fusobacterium necrophorum* Clostridia Mycobacteria (rarely) *Mycobacterium tuberculosis* *Mycobacterium avium-intracellulare* Fungi (rarely) *Cryptococcus neoformans*
	Gas gangrene	*Clostridium perfringens;* on occasion other histotoxic clostridial species
	Nonclostridial (crepitant) myositis	
	Anaerobic streptococcal gangrene	*Peptostreptococcus* (plus group A streptococci or *S. aureus*)
	Group A streptococcal necrotizing myositis	Group A streptococci
	Synergistic nonclostridial anaerobic myonecrosis	Mixed infections: *Bacteroides* and other anaerobic non–spore-forming gram-negative bacilli; *Peptostreptococcus* and various streptococci; *Escherichia coli; Klebsiella; Enterobacter*
	Infected vascular gangrene	Same as for synergistic nonclostridial anaerobic myonecrosis
	Aeromonas hydrophila myonecrosis	*A. hydrophila*
	Psoas abscess	Gram-negative bacilli; *S. aureus;* mixed infections; *M. tuberculosis*
Nonpyogenic and predominantly generalized	Myalgias	Viral infections (e.g., influenza, dengue); infective endocarditis; bacteremias (e.g., meningococcemia); rickettsioses (e.g., Rocky Mountain spotted fever); toxoplasmosis
	Pleurodynia	Coxsackievirus B
	Myalgias with eosinophilia	
	Trichinosis	*Trichinella spiralis, Trichinella pseudospiralis*
	Cysticercosis (also subcutaneous nodules)	*Taenia solium*
	Muscle degeneration and destruction associated with infections elsewhere	
	Acute rhabdomyolysis	Influenza viruses, dengue virus, echoviruses, coxsackieviruses, Epstein-Barr viruses, *Legionella,* and others (see text)

in the United States between 1981 and 2002[4] and a reported incidence of 0.5 per 100,000 annually in Australia early in this century.[9] More recently the annual incidence in selected regions in Australia has risen to as high as 13.5 per 100,000.[12a] Pyomyositis occurs at all ages, in the tropics more frequently among children,[13] but in North America more often in adults and the elderly.[4,14] No convincing evidence relates pyomyositis causally to predisposing circumstances peculiar to the tropics (e.g., malaria, filariasis, arbovirus infection). However, an association between *Toxocara canis* infection (visceral larva migrans) and staphylococcal pyomyositis has been proposed.[15] Migration of the guinea worm *Dracunculus medinensis* in the deep connective tissues of the lower extremities may be complicated by staphylococcal abscesses, but these are located between muscle groups and are not the intramuscular abscesses typical of pyomyositis. Approximately 40% of cases in temperate climates lack any relevant underlying disease, but the remainder have possible predisposing risk factors, such as intravenous (IV) drug abuse and systemic conditions, including human immunodeficiency virus (HIV) infection, diabetes mellitus, alcoholic liver disease, corticosteroid therapy, hematologic malignancies (e.g., leukemia, lymphoma, or multiple myeloma), other hematologic processes (e.g., Felty syndrome, myelodysplasia, sickle cell disease, cyclic neutropenia, chronic granulomatous disease) and/or their cytotoxic therapies, and rheumatologic diseases (particularly rheumatoid arthritis and systemic lupus erythematosus).[4,14] The postpartum,[16] postabortion,[17] and postoperative states, as well as deep acupuncture,[18] are rare predisposing risk factors for the development of pyomyositis.

Pyomyositis has been reported repeatedly in patients with HIV infection, with or without acquired immunodeficiency syndrome (AIDS) (including one neonate); in the majority of these patients it was caused by *S. aureus.*[4,19] The predisposition to pyomyositis in AIDS patients probably relates to granulocyte dysfunction,[20] progressive cell-mediated immunodeficiency,[21] and possible muscle injury (e.g., HIV myopathy, zidovudine-associated mitochondrial myopathy, myositis from parasitic disease, *Mycobacterium avium* complex infection). Although *S. aureus* is the etiologic agent in the majority of HIV-associated cases, *Salmonella* accounts for as many as 10% and streptococci for 5% of pyomyositis episodes in this population[4,19]; pneumococci, enterococci, and granulomatous infections due to *Mycobacterium tuberculosis* and *Sporothrix schenckii* have also been reported. Pyomyositis has been reported in growing numbers of IV drug abusers (with or without HIV infection) caused primarily by *S. aureus* but also by streptococci, gram-negative bacilli, or multiple organisms (including anaerobes).[22] Pyomyositis is a rare complication of bacterial endocarditis and has been reported in an IV drug abuser who had left-sided *S. aureus* infection[9,23] and in a patient with subacute endocarditis due to *S. haemolyticus.*[10]

The presumed pathogenesis of (primary) pyomyositis is thought to reflect initial bacteremia, commonly asymptomatic and transient. Because muscle trauma (locus minoris resistentiae) is necessary to produce pyomyositis in experimental animals after IV injection of *S. aureus,*[24] a role for local mechanical injury has been hypothesized. Secondary pyomyositis reflects spread of infection from a contiguous source, typically a site of hematogenous osteomyelitis. The frequency of pyomyositis complicating staphylococcal osteomyelitis may be increased in community-acquired methicillin-resistant *S. aureus* (MRSA) strains expressing Panton-Valentine leukocidin[25] and, in general, molecular features of distinct *S. aureus* clones correlate with superficial or invasive clinical syndromes.[26]

Clinical Manifestations

In 20% to 50% of cases there has been recent blunt trauma to or vigorous exercise of the involved area or a local primary dermatologic process.[4,9] The clinical picture often involves three stages. In the first, or invasive, stage, the onset is subacute with variable fever, local swelling with or without erythema, mild pain, and minimal tenderness. Noninfectious processes such as torticollis or muscle sprain are often considered, particularly if fever is not prominent.[27] The area is indurated or has a

wooden consistency. This stage is often overlooked. Because the initial swelling is firm and pain is not striking, and/or involves deep muscles not easily assessed at the bedside, attention is directed away from an infectious cause. Aspiration, if attempted, yields no pus. The second, or suppurative, stage occurs 10 to 21 or more days later, and this is the time when most patients are diagnosed. The patient is febrile, and distinct muscle tenderness and swelling (conforming to the involved muscle) are present. The overlying skin is intact and warm, and erythema is commonly absent. At this point pus can be aspirated from the involved muscle. In the third stage, systemic manifestations of sepsis and local findings of erythema, exquisite tenderness, and fluctuance are striking. If untreated the infection can progress to metastatic abscesses, shock, renal failure, and death[28]; the overall mortality rate is approximately 5%[4] and is largely associated with the presence of severe sepsis and septic shock.[4] The progression of pyomyositis from the initial invasive stage, associated with muscle inflammation and swelling, to the suppurative stage, with focal abscess formation, was documented by serial imaging in a patient whose infection was initially managed with antibiotic therapy.[29]

On occasion, the onset is acute rather than subacute, with malaise, chills, and high fever. Rarely, the clinical picture is combined with that of toxic shock syndrome.[30,31] This is a particular risk of myositis caused by group A β-hemolytic streptococci (see later discussion). Because the muscle abscesses are contained by the overlying fascia, local erythema and warmth may be minimal and the severity of the process not appreciated until the infection extends to the subcutaneous tissues some days to weeks later. Regional lymphadenitis is not a feature. Usually only a single muscle group is involved, but multiple muscle abscesses may be present at presentation[32] and so prominent as to mimic critical illness myopathy[6] or develop after contiguous spread (e.g., from the psoas to the thigh adductors[33]). The most frequent sites of involvement are the large muscles of the lower extremities (e.g., quadriceps femoris, gluteus group) and the trunk muscles, but a variety of other muscles can be involved (Fig. 94.1) including the smaller hip rotators (obturator internus and externus, piriformis), cervical paraspinal muscles,[34] sternocleidomastoids, and even ocular muscles.[35] Involvement of the abdominal muscles is uncommon[7] but noteworthy because it may mimic an acute abdomen.[36] Even in fatal cases, the diagnosis may be overlooked and involvement of superficial muscles may be missed at autopsy.[28]

Leukocytosis is prominent; eosinophilia is common in patients with tropical pyomyositis (even in the presence of a prominent granulocytosis) and may simply reflect concomitant parasitic infestation. Serum muscle enzyme levels may be elevated, particularly with severe streptococcal disease, but they frequently are normal despite gross muscle destruction. However, marked rhabdomyolysis with myoglobinuria and acute renal failure, more commonly seen in viral myositis, was reported in a patient with pyomyositis.[37]

Etiologic Agents

S. aureus is responsible for 95% of tropical pyomyositis cases, whereas in temperate areas *S. aureus* is the cause of 66% to 70% of cases.[4,14] Group A streptococci account for 1% to 5% of the cases. Other gram-positive organisms uncommonly implicated in pyomyositis include various streptococci (groups B, C, and G), *Streptococcus pneumoniae,*[38] *Enterococcus faecalis,*[39] and *Streptococcus anginosus.*[40] Myositis due to viridans streptococci complicating the viridans streptococcal sepsis syndrome in acute myelogenous leukemia has been reported.[41] Pyomyositis due to a dual infection by the coagulase-negative staphylococci *Staphylococcus capitus* and *Staphylococcus saccharolyticus* was reported in a diabetic host.[34] Other very rare causes include Enterobacteriaceae[1] (*Escherichia coli* [particularly in neutropenic patients with hematologic malignancy[42]], *Klebsiella oxytoca, Klebsiella pneumoniae, Serratia marcescens, Morganella morganii, Citrobacter freundii, Enterobacter* spp., *Salmonella* spp.[43]), *Yersinia enterocolitica, Francisella novicida,*[44] *Neisseria gonorrhoeae, Haemophilus influenzae, Pseudomonas aeruginosa, Burkholderia cenocepacia,*[45] and *Aeromonas hydrophila.* Anaerobes (*Fusobacterium nucleatum, F. necrophorum,*[46] *Veillonella* spp., oral anaerobic streptococci,[47] *Capnocytophaga sputigena,*[48] actinomycetes, and *Clostridium septicum*) have been the cause in several cases. Pyomyositis may be caused by mixed pathogens, especially in patients with diabetes mellitus in whom, in addition to *S. aureus,* gram-negative or anaerobic pathogens, or both, were recovered in approximately 35% of episodes,[49] and dual infection by coagulase negative staphylococci was observed.[34] In the past *Burkholderia mallei* and *Burkholderia pseudomallei* have very rarely caused muscle abscesses in the septicemic or chronic suppurative forms of glanders and melioidosis, respectively. *Aspergillus fumigatus* has caused a localized muscle abscess in rare patients with myelodysplasia or AIDS and in patients who have received corticosteroids.

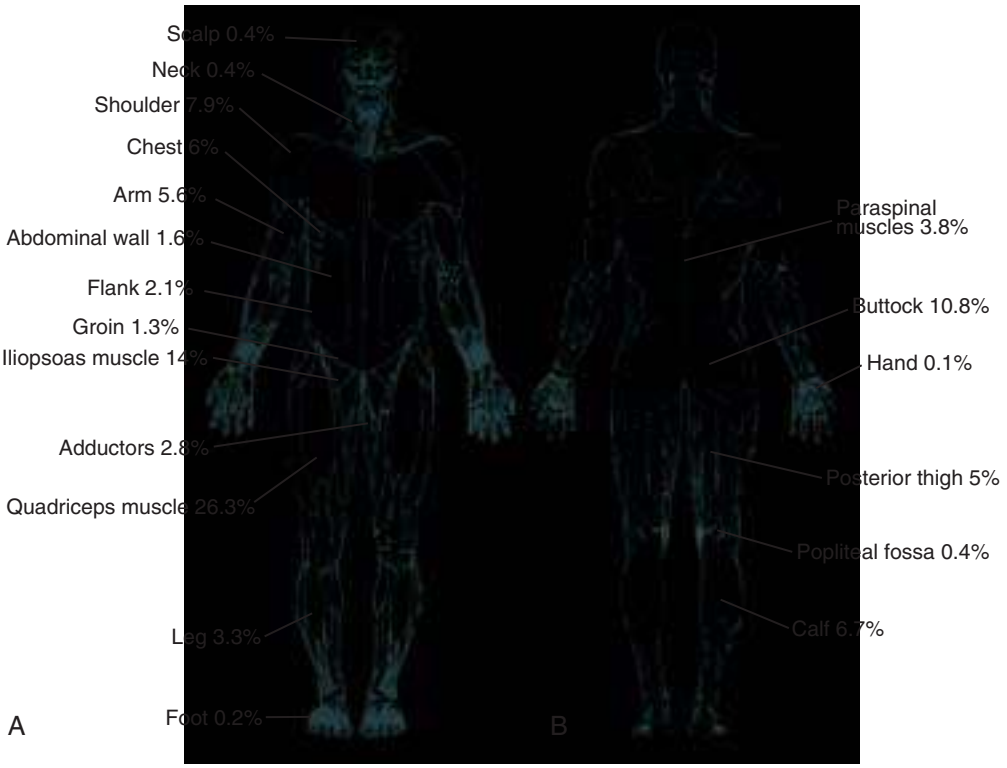

FIG. 94.1 Distribution of sites of pyomyositis. (A) Anterior view. (B) Posterior view. *(Modified from Bickels J, Ben-Sira L, Kessler A, et al. Primary pyomyositis.* J Bone Joint Surg Am. *2002;84:2277–2286.)*

Fusarium spp. has been reported to cause bilateral lower limb pyomyositis in a patient with relapsed acute myelogenous leukemia.[50] Pathogenic yeasts can cause myositis on rare occasions. Pyomyositis due to *Cryptococcus neoformans* was reported in an immunocompromised host,[51] and multifocal *Histoplasma capsulatum* nodular myositis was identified in an HIV-infected patient.[52] Hematogenously disseminated candidiasis in neutropenic patients can manifest as fever and muscle pain. The scattered *Candida* abscesses in the muscle are generally too small to localize by symptoms and physical examination, but myositis in these patients is often accompanied by small, tender, erythematous, hematogenously disseminated skin lesions. Punch biopsy of a skin lesion shows numerous *Candida* organisms in the dermis. Larger candidal lesions may be identified and managed using conventional imaging approaches.[53] Tuberculous pyomyositis is a rare form of extrapulmonary tuberculosis recognized in both competent[54] and immunocompromised[55] hosts and can present with or without features of disseminated disease.[54] Pyomyositis due to *M. avium* complex is occasionally diagnosed in HIV-infected patients, sometimes as part of the immune reconstitution syndrome,[56] and *Mycobacterium haemophilum* pyomyositis was observed in a renal transplant recipient.[57] Extrapulmonary *Pneumocystis jirovecii* infection is relatively uncommon in patients with AIDS, but in one patient it manifested as an intramuscular, localized, painful thigh swelling with typical granulomatous histopathology.[58]

Differential Diagnosis

Early in the course of pyomyositis, other diagnoses may be suspected, particularly in nontropical areas: fever of obscure origin (in the early phase, when localizing findings may be minimal or absent), osteomyelitis, septic arthritis, appendicitis or diverticulitis, muscle strain, contusion or hematoma, muscle rupture, and thrombophlebitis. Iliopsoas myositis has mimicked appendicitis[59]; obturator internus, iliacus, and rarely obturator externus pyomyositises have mimicked septic arthritis of the hip[60]; and pyriformis muscle pyomyositis has simulated an epidural abscess because of severe back and radiating (sciatic) leg pain.[61] Rarely, pyomyositis of the erector spinae[62] or piriformis[63] can extend to the intraspinal canal, leading to a true spinal epidural abscess. Pyomyositis involving deep pelvic muscles may be difficult to diagnose in patients with active joint disease, such as rheumatoid arthritis, in whom symptoms may readily be attributed to the primary disease. Muscle infarction is an uncommon condition that occurs most frequently in the quadriceps muscle in patients with poorly controlled diabetes mellitus with nephropathy, neuropathy, and hypertension. It may suggest pyomyositis because of the acute onset of pain and presence of tender local swelling, but, in contrast to pyomyositis, fever is absent.[64,65] In the patient with multiple sites of muscle involvement and eosinophilia (from incidental parasitic infestation), the picture may initially suggest trichinosis. Rupture of the muscle abscess through the fascia into subcutaneous tissues may suggest the diagnosis of cellulitis. The presence of a slowly enlarging, painful mass in an extremity of a patient with only low-grade fever may suggest the diagnosis of sarcoma. Pyomyositis of the pectoral muscle can pose a particular diagnostic problem because it must be distinguished not only from muscle rupture, hematoma, and sarcoma but also from cryptic abscessed subpectoral nodes complicating infection of the ipsilateral thumb or index finger. Streptococcal necrotizing fasciitis, like gangrenous streptococcal myositis, manifests initially as local pain disproportionate to any physical findings. Localized swelling, tenderness, and erythema ensue, but only in advanced stages are the characteristic violaceous skin changes, bullae, and frank skin necrosis seen overlying areas of fascial necrosis. Rapid frozen-section biopsy or surgical exploration may be needed to distinguish among these processes.

Diagnosis

Prompt imaging is essential in the evaluation of patients with focal soft tissue pain and fever because the possible need for surgical exploration must be assessed urgently. Plain radiographs can demonstrate focal soft tissue swelling, the presence of gas in the soft tissues, and any primary skeletal abnormalities (e.g., fracture, osteomyelitis, osteosarcoma). Ultrasonography is readily accessible and can demonstrate muscle enlargement in the initial stage of pyomyositis; it can also show the presence of focal abscess formation in the suppurative stage of disease, revealing hypoechoic areas with internal echoes,[66–68] especially if symptoms are localized in an extremity. Ultrasound assessment of the hip can detect the presence of a joint effusion, but it may be less sensitive in assessing the deep pelvic musculature. Ultrasonography is usually followed by cross-sectional imaging to gain greater anatomic detail and should not delay definitive imaging studies in acutely ill patients.

Magnetic resonance imaging (MRI) has proved invaluable in the assessment of patients with pyomyositis because it identifies focal muscle edema and localizes the presence of focal abscess formation with great precision.[69] MRI can demonstrate enlargement of involved muscles and a slight increase in signal intensity on T1-weighted images in the involved area, with a hypointense central area and a surrounding gadolinium-enhanced rim. It also shows a diffuse increase in signal intensity on T2-weighted images, with a central high-signal-intensity fluid collection surrounded by a low-intensity rim.[70] Computed tomography (CT) is a more readily available cross-sectional modality, often performed during emergency department evaluation that provides less anatomic detail at the inflammatory stage of myositis but is helpful when used to guide percutaneous drainage of an established muscle abscess. CT can reveal low-density areas with loss of muscle planes, central fluid collection, and a surrounding rim of contrast enhancement characteristic of pyomyositis.[66] Enlargement of the involved muscle is usually evident. Superimposed cellulitis may sometimes be evident on CT, namely, skin thickening, stranding of subcutaneous fat with blurring of fat and fascial planes, and subcutaneous venous distention. Radionuclide imaging using indium 111–labeled leukocytes, sometimes performed as dual SPECT (single-photon emission CT)/CT, provides functional and anatomic detail but has not been compared directly with MRI regarding predictive value or cost.[71]

Empirical Therapy

Drainage of all established abscesses is essential, and selection of open or percutaneous drainage via ultrasound or CT guidance depends on the abscess location, size, and complexity and the available expertise. Initial antibiotic therapy should consist of empirical IV vancomycin because of the preponderance of *S. aureus* isolates from these abscesses and the frequent and increasing incidence of MRSA. In compromised hosts or patients with severe disease, empirical high-dose broad-spectrum therapy effective against gram-negative pathogens and anaerobes should be added initially (e.g., β-lactam/β-lactam inhibitor combination such as piperacillin-tazobactam or a carbapenem such as meropenem). Early modification of initial antimicrobial therapy is based on interpretation of a Gram stain of pus and subsequent cultures and susceptibility testing. If a group A *Streptococcus* is isolated, treatment should be changed to high-dose penicillin G and clindamycin (see later discussion). Continued fever after surgical or percutaneous needle drainage of a muscle abscess while the patient is receiving appropriate antimicrobial therapy suggests the presence of other undrained suppurative foci; relapsing or recurrent pyomyositis is frequent among patients with advanced HIV infection.[4] Pyomyositis may be complicated by a compartment syndrome, particularly if it occurs in the forearm[72] or anterior tibial compartments,[73] and may require surgical drainage, fasciotomies, and débridement beyond simple percutaneous drainage.

The prognosis after definitive treatment of pyomyositis is excellent, unless staphylococcal or streptococcal infection is complicated by the presence of toxic shock syndrome.[30,31] The multiorgan failure associated with established toxic shock carries a disturbingly high mortality rate. In the absence of toxic shock, definitive drainage accompanied by prolonged effective antibiotic therapy usually leads to complete resolution of infection with little or no long-term morbidity, even in immunocompromised individuals. Delays in diagnosis and definitive drainage (when drainage is necessary) have led to muscle fibrosis, with the need for widespread excision, and subsequent functional disability.

GROUP A STREPTOCOCCAL NECROTIZING MYOSITIS

In addition to producing an occasional case of typical pyomyositis with abscess formation, on rare occasions group A streptococci cause a fulminant form of myositis, which is a true medical emergency, referred to as peracute streptococcal pyomyositis, streptococcal necrotizing myositis, streptococcal myonecrosis, or spontaneous streptococcal gangrenous

myositis.[74,75] Both necrotizing myositis and necrotizing fasciitis are frequently associated with group A streptococcal toxic shock syndrome.[75,76] The entire clinical course may occur in 2 to 3 days, with intense pain, boardlike swelling of the affected muscle, and fever. The overlying skin may be uninvolved, or it may become erythematous or violaceous and indurated and contain petechiae and bullae.[75] Most cases involve the extremities although the deep hip musculature and other sites are occasionally observed[77] and appear to develop spontaneously without antecedent pharyngitis or tonsillitis. Bacteremia and toxemia are prominent features and contribute to the very high mortality rate (80%–100%).[75] The rapid spread of infection in a closed compartment of muscles can markedly increase intramuscular pressure, resulting in further necrosis of muscle.[78] However, both processes can be simultaneously present in the same area. The compartment syndrome with group A streptococcal myositis (e.g., a tibial compartment syndrome) may develop in the absence of frank fascial and muscle necrosis with muscle bulging and increased pressure secondary to edema and serosanguineous exudate.[79] Progressive weakness of the compartment muscles, which are swollen and tender, severe pain on movement of the lower leg, and overlying cutaneous hyperesthesia underscore the need for urgent decompressive surgery.

Streptococcal necrotizing fasciitis may resemble streptococcal myositis clinically, although the presence of tense bullae and focal skin necrosis is more suggestive of the former; often both conditions are present.[80,81] MRI may disclose the predominantly involved structure, but urgent surgical exploration, always necessary in the setting of suspected toxic shock associated with focal pain and swelling, should provide a clear answer. Rarely, acute streptococcal myositis with toxic shock syndrome is caused by *Streptococcus dysgalactiae* subsp. *equisimilis,* which may be typed as Lancefield group A, C, or G.[81,82]

Laboratory findings include leukocytosis and an elevated serum creatine phosphokinase level, in marked distinction to nonstreptococcal forms of pyomyositis, in which little, if any, creatine phosphokinase elevation occurs. This medical emergency requires prompt clinical diagnosis with verification at surgery. Distinguishing group A streptococcal necrotizing myositis from streptococcal necrotizing fasciitis and spontaneous clostridial myonecrosis may be difficult clinically, but gas in the tissue suggests spontaneous clostridial myonecrosis. In any case, all of these necrotizing soft tissue infections require prompt surgical exploration. Ultrasonography, CT, or MRI usually reveals muscle swelling and fluid collection in muscle compartments. If prolonged delays are encountered in the pursuit of imaging studies, proceeding directly to surgical exploration with an initial limited surgical approach for diagnostic purposes is justified. Cross-sectional imaging is particularly helpful to address the specific site of muscle involvement in patients with hip pain and a compatible illness to guide optimal surgical exploration. Early aggressive surgical intervention with fasciotomy and débridement of necrotic tissue is indicated; in some instances, amputation is required. If the operative Gram stain suggests streptococcal infection, antibiotic therapy should consist of high doses of penicillin G (3 million units IV every 3 hours or 4 million units every 4 hours or adjusted appropriately for renal insufficiency), along with clindamycin (600 mg IV every 6–8 hours).[83] Clinical experience suggests that clindamycin has greater efficacy against group A streptococci in this life-threatening infection (Eagle effect) because of its greater activity against large bacterial populations in stationary phase growth, its more sustained postantibiotic effect, and its suppression of the production of toxins and other virulence factors through its inhibition of bacterial protein synthesis.[75] Unfortunately, the incidence of clindamycin resistance among group A streptococcal isolates has been rising and is now ≈15% in the United States.[84] Monotherapy with clindamycin should be avoided unless clindamycin susceptibility is confirmed by antibiotic susceptibility testing. The use of intravenous immunoglobulin G (IVIG) as an adjunct in the treatment of streptococcal toxic shock to neutralize streptococcal exotoxins and perhaps to modulate the host immune response has gained popularity based on retrospective studies and one small prospective, randomized trial,[85] but conclusive evidence supporting its use remains limited.[86] A large analysis of patient outcomes after presentation with necrotizing soft tissue infection and shock did not demonstrate a beneficial effect of IVIG therapy with regard to survival or length of hospital stay,[87] and consensus regarding its use remains elusive.[88]

GAS GANGRENE (CLOSTRIDIAL MYONECROSIS)

Gas gangrene is a rapidly progressive life-threatening infection of skeletal muscle caused by clostridia (principally *Clostridium perfringens*). It usually occurs after penetrating muscle injury and contamination (as in a dirty traumatic wound) or rarely postoperatively. Nontraumatic gas gangrene, usually caused by *C. septicum,* is a complication of bacteremia often arising from an occult gastrointestinal mucosal lesion, such as an adenocarcinoma, or as a complication of neutropenic colitis (also see Chapter 246).

Pathogenesis and Pathologic Characteristics

Gas gangrene occurs in settings that have in common muscle injury and contamination with soil or other foreign material containing spores of *C. perfringens* or other histotoxic clostridial species.[89] Classic scenarios include (1) accidental traumatic civilian injuries such as compound fracture; (2) penetrating war wounds[90]; (3) surgical wounds, particularly after bowel or biliary tract surgery[91] or septic abortion; and (4) arterial insufficiency in an extremity.[92] Rare cases of gas gangrene have occurred after parenteral injection of medication, including aqueous epinephrine[93]; subcutaneous insulin administration[94]; parenteral injection of methamphetamine or heroin,[95] and even fluoroscopically guided sacroiliac joint injection.[96] Fulminant gas gangrene has complicated routine venipuncture[92] or platelet infusions[97] in patients with granulocytopenia. *C. perfringens* organisms usually are present in large numbers as normal flora in human feces and therefore can endogenously contaminate skin surfaces. Despite a high frequency (as high as 88%) of clostridial contamination of major traumatic open wounds, the incidence of gas gangrene in this setting is only 1% to 2%,[98] emphasizing the importance of devitalized tissue and the presence of foreign bodies in the pathogenesis of gas gangrene. The minimal dose of *C. perfringens* needed to produce fatal gas gangrene in experimental animals is reduced by a factor of 10^6 if the organism is injected into devitalized muscle contaminated with sterile dirt rather than into normal muscle. The policy of prompt, thorough débridement and of leaving wounds open has decreased the incidence of gas gangrene in wartime injuries; only 22 cases among 139,000 combat casualties in Vietnam were reported.[99]

Gas gangrene may occasionally develop in the absence of an obvious external wound. This form of clostridial myonecrosis is designated spontaneous, nontraumatic gas gangrene. Its principal cause is *C. septicum,* a relatively aerotolerant species that spreads by the bacteremic route and is more capable of establishing infection without significant antecedent tissue injury than other clostridia. Intestinal tract abnormalities (colon cancer, diverticulitis, bowel infarction, necrotizing enterocolitis, volvulus) are the major predisposing conditions.[100] Colon cancer, often cryptic, is the most common of these, occurring in as many as 88% of patients with *C. septicum* bacteremia. Other predisposing disorders include leukemia, other causes of neutropenia, and diabetes mellitus. The primary source of infection is probably mucosal ulceration or perforation of the intestinal tract. The spread by the bacteremic route probably accounts for the bilateral multifocal involvement observed in a few patients with spontaneous gas gangrene.[101] However, it may also manifest in the buttocks or flanks after an intraabdominal catastrophe, with rapid extension of infection along the iliopsoas or other deep muscle groups. The progression of *C. septicum* spontaneous gas gangrene may be even more fulminant than that of traumatic *C. perfringens* gas gangrene; the mortality rate of the former is 67% to 100%, with most patients dying within 24 hours after onset.[100]

The involved muscle undergoes rapid disintegration. Initially it may exhibit only pallor, edema, and loss of elasticity. When examined at surgery, it fails to contract on stimulation and does not bleed from a cut surface. Later it becomes discolored (reddish purple, then greenish purple and gangrenous) and friable. Histologically, the muscle fibers show coagulation necrosis, cavities caused by gas production, and a loss of supporting connective tissue; numerous gram-positive bacilli are present. Few, if any, inflammatory cells are present. Among the more than 16 identified clostridial toxins,[102] evidence suggests that the α- and Θ-toxins of *C. perfringens* are major virulence factors that lead to myonecrosis and apparent lack of inflammation at the site of infection

through cytolysis.[103] Intravascular thrombosis due to the local effects of α-toxin on platelets and granulocytes appears to be responsible for the severe herald pain (which may be ischemic) and extensive myonecrosis.[104] In addition to these local effects, the α-toxin provokes systemic hypotension by directly suppressing myocardial contractility and triggering the release of endogenous inflammatory mediators.[105]

Clinical Manifestations

The usual incubation period between injury and the development of clostridial myonecrosis is 2 to 3 days, but it may be as short as 6 hours. The onset is acute. Pain is the earliest and most important symptom, although on occasion a sense of heaviness may be the only initial symptom. Pain rapidly increases in intensity, beyond what would generally be associated with the preceding injury or surgical procedure, and may become excruciating. The patient soon appears severely ill, pale, and sweaty. Hypotension, tachycardia, shock, and renal failure follow. The patient may be apathetic or may be apprehensive and restless but mentally clear. Delirium, stupor, and unconsciousness may supervene. Low-grade fever is frequently present, often with a temperature below 38.3°C (101°F); hypothermia is a poor prognostic sign and is usually associated with shock. Jaundice may become evident. The process may rapidly progress over a period of hours, with a fatal outcome if not treated aggressively.

Initially, tense edema and local tenderness may be the only local findings. Swollen muscle may herniate through an open wound. A serosanguineous, dirty-appearing discharge containing numerous organisms but few leukocytes escapes from the wound and has a peculiar foul odor. Gas bubbles may be visible in the discharge. Crepitus is usually present, but not prominent; sometimes it is completely obscured by very marked edema. The skin adjacent to the wound is initially swollen and white but rapidly takes on a yellowish or bronze discoloration (Fig. 94.2). Tense blebs containing thin, serosanguineous or dark fluid develop in the overlying skin, and areas of green-black cutaneous necrosis appear. In fulminant cases this progresses visibly over 2 to 4 hours, as indicated by advancing edema and crepitation.

Laboratory Findings

The hematocrit is usually decreased, despite progressive local edema and expected hemoconcentration, because of the lysolecithinase activity of clostridial α-toxin and acute hemolysis. Initial leukocytosis is common. *C. perfringens* bacteremia occurs in about 15% of patients with gas gangrene.[106] Intense bacteremia (with associated intravascular hemolysis) occurs more frequently after uterine infection.[107]

A Gram-stained smear of the wound exudate or an aspirate from a cutaneous bleb reveals many large, gram-positive bacilli with blunt ends but few polymorphonuclear leukocytes (see Chapter 246).[101] In almost all cases spores are not evident. The presence of subterminal spores suggests *C. septicum*. Not infrequently, scattered gram-negative bacilli are also present, particularly in grossly contaminated wounds. The growth of *C. perfringens* in culture can be extraordinarily rapid (generation time as little as 8 minutes), paralleling the rapid advance of the infection in devitalized tissue. Examination of liquid anaerobic cultures for gas production ("stormy fermentation") and subsequent Gram-stain examination as early as 6 hours after inoculation may provide an early presumptive diagnosis of the infecting species. Radiographs and CT scans of the involved areas show extensive and progressive gaseous dissection of muscle and fascial planes.



FIG. 94.2 Clostridial gas gangrene of the left upper extremity. There is prominent characteristic bronze discoloration of the skin extending over the shoulder. Crepitus could be palpated beyond the area of discoloration onto the back.

Etiologic Agents

C. perfringens is most commonly isolated from the lesions of gas gangrene (80%–95% of the cases).[90,99] *Clostridium novyi* is involved in 10% to 40% of the cases and *C. septicum* in 5% to 20%. Other clostridial species (e.g., *C. bifermentans, C. histolyticum, C. fallax, C. ramosum,* and *C. sordellii*) have been implicated on rare occasions (e.g., *C. sordellii* with home abortions).[89] In addition to clostridia, other organisms (e.g., *E. coli, Enterobacter* spp., enterococci) are sometimes isolated from the lesions of gas gangrene, reflecting the contaminated character of the initiating trauma or lesion.[106]

Differential Diagnosis

The major differential diagnostic considerations are other gas-forming infections of the soft tissues (clostridial anaerobic cellulitis, nonclostridial crepitant myositis, nonclostridial crepitant cellulitis). Clostridial anaerobic cellulitis (see Chapter 93) is more gradual in onset and progression, and the systemic manifestations of illness are much milder than in gas gangrene. Local pain is relatively mild, and the skin lesions of gas gangrene (bronzing, dark blebs) do not develop. Paradoxically, gas formation is often much more extensive in clostridial cellulitis than in gas gangrene. Clinically, it is often difficult to distinguish between early clostridial cellulitis and myonecrosis. Definitive evaluation requires examination in the operating room for the characteristic changes of myonecrosis described earlier. The clinical picture of nonclostridial crepitant cellulitis is very similar to that of clostridial cellulitis. Although contamination of a surgical or traumatic wound may be the source of infection in both types of cellulitis, nonclostridial crepitant cellulitis frequently develops in the setting of vascular insufficiency or perirectal infection. Bacteria isolated from nonclostridial crepitant cellulitis include facultative species (e.g., *E. coli, Klebsiella*, various streptococci) and anaerobic bacteria (e.g., *Bacteroides, Peptostreptococcus*). Commonly, these are present in mixed culture and can be seen on the Gram-stained smear of a wound aspirate.

Empirical Therapy

Treatment includes emergency surgical exploration, both to define the nature of the process (gas gangrene vs. crepitant cellulitis) by direct muscle examination at the site of infection and to perform appropriate débridement. Prompt and extensive surgery is the principal element in the treatment of gas gangrene. This includes excision of involved muscles (or amputation if necessary) and fasciotomies to decompress and drain the swollen fascial compartments. Antibiotic therapy is an important adjunct to surgical management. Penicillin G, the traditional antibiotic of choice, is administered in a dose of 2 million units IV every 2 hours or 3 million units IV every 3 hours (24 million units/day) (or adjusted for acute renal insufficiency) for an adult. Currently, combined penicillin and clindamycin (600 mg IV every 6–8 hours) is widely used in treatment. The addition of clindamycin is based on results of experimental studies of fulminant clostridial myonecrosis in mice, in which clindamycin, metronidazole, and tetracycline were each more effective than penicillin.[108] In vitro, the addition of penicillin to metronidazole antagonizes the activity of the latter; in contrast, the combination of penicillin with clindamycin provides slightly greater efficacy than clindamycin alone but significantly enhanced efficacy over that of penicillin alone.[109]

An additional antimicrobial agent (e.g., ciprofloxacin, a third- or fourth-generation cephalosporin, or a carbapenem agent) may be used initially if Gram-stained smears of the wound exudate show gram-negative bacilli and the predominant gram-positive bacilli. Patients who are highly penicillin allergic may be treated with clindamycin; plasmid-mediated resistance to tetracycline and erythromycin is now common

among clostridia. Although the majority of *C. perfringens* isolates are susceptible in vitro to cephalosporins, the second-generation agents cefotetan and cefoxitin appear to have more favorable minimal inhibitory concentrations than the third-generation agent ceftriaxone.[110] *C. perfringens* is highly susceptible in vitro to the carbapenems, metronidazole,[110] and linezolid, but experience with the use of these drugs in clostridial myonecrosis is limited.

The role of hyperbaric oxygen therapy is still debated (see Chapter 50).[111,112] Elevated partial pressures of oxygen are thought to reduce the rate of clostridial replication and to suppress toxin expression.[111] The rarity of clostridial myonecrosis and the limited availability of hyperbaric oxygen facilities has made prospective, controlled clinical trials impractical. Its use should never delay immediate surgical débridement if possible. Its most appropriate role at present seems to be in the management of extensive truncal involvement, for which complete surgical excision would be impossible (paraspinal sites) or mutilating. In a murine model of *C. perfringens* myonecrosis initiated with a high inoculum, clindamycin therapy was more effective than hyperbaric oxygen, and the addition of the latter provided no greater efficacy than clindamycin alone.[113] Initial hyperbaric oxygen therapy may decrease the extent of débridement that is necessary under these circumstances. The efficacy of IV-administered polyvalent gas gangrene antitoxin has never been established clinically, and it is no longer available. Comprehensive ancillary therapy in the intensive care unit (ICU) is essential in the management of gas gangrene, including attention to fluid and electrolyte replacement and maintenance of adequate hematocrit levels through transfusion.

NONCLOSTRIDIAL (CREPITANT) MYOSITIS

Nonclostridial (crepitant) myositis includes four relatively distinct entities that differ from gas gangrene in their clinical picture and bacteriologic characteristics: (1) anaerobic streptococcal myonecrosis, (2) synergistic nonclostridial anaerobic myonecrosis, (3) infected vascular gangrene, and (4) *A. hydrophila* myonecrosis.

Anaerobic Streptococcal Myonecrosis

Anaerobic streptococcal myonecrosis is an acute interstitial myositis that clinically resembles subacute clostridial gas gangrene. The initial manifestations are swelling and a copious seropurulent exudate occurring 3 to 4 days after an injury. Pain develops later, unlike the early occurrence of pain in gas gangrene. Tissue gas is present in muscle and fascial planes but is not extensive. The wound has an unpleasant sour odor. The involved muscles are discolored but do react to stimulation. In contrast to gas gangrene, early cutaneous erythema is prominent. If it is not adequately treated, the infection progresses, with the development of toxemia, frank gangrene, and shock.

Numerous streptococci and polymorphonuclear leukocytes are present in the exudate. The infection is usually mixed (anaerobic streptococci with group A streptococci or *S. aureus*). A mixed infection of muscle with both *Finegoldia magna* and *Bacillus subtilis* has been observed on several occasions in the setting of vascular injury. The clinical picture, along with the appearance of the Gram-stained smear, initially might suggest the diagnosis of clostridial myonecrosis.[114] Treatment involves the use of large doses of penicillin and initial antistaphylococcal therapy, such as vancomycin, if indicated by initial Gram stain along with surgical débridement.

Synergistic Nonclostridial Anaerobic Myonecrosis

Synergistic nonclostridial anaerobic myonecrosis, a severe infection seen particularly in patients with diabetes mellitus and those with neutropenia, is also known as synergistic necrotizing cellulitis (see Chapter 93). It involves skin, subcutaneous tissue, fascia, and muscle. The most extensive involvement is in the subcutaneous tissues and fascia; changes in overlying skin and underlying muscle are usually secondary. Although a mixture of anaerobic and facultative organisms is commonly recovered at surgical exploration, on rare occasions crepitant myonecrosis may be caused by *K. pneumoniae,*[115] *Enterobacter cloacae,*[116] or *Bacillus cereus*[117] unaccompanied by other organisms (aerobic or anaerobic) in high-risk patients. The clinical course is rapidly progressive, often leading to a fatal outcome despite emergency surgical exploration and débridement of necrotic tissue.

Infected Vascular Gangrene

Infected vascular gangrene is a mixed infection that develops in a group of muscles or in a limb that is devitalized as a result of arterial insufficiency, particularly in patients with diabetes mellitus. *Proteus* spp., *Bacteroides* spp., and anaerobic streptococci are among the bacteria found in such lesions. Gas formation and foul-smelling pus are prominent. The infection does not extend beyond the area of vascular gangrene to involve healthy muscle. *Bacillus cereus* infection has been associated with myonecrosis with slight crepitus after thrombosis of arterial grafts in addition to more aggressive posttraumatic infections.[117]

Aeromonas hydrophila Myonecrosis

Rapidly progressive myonecrosis caused by *A. hydrophila,* a facultatively anaerobic gram-negative bacillus, may occur after penetrating trauma in a freshwater environment or in association with fish or aquatic animals.[118,119] Although *Aeromonas* was associated with pyomyositis and a compartment syndrome in neutropenic patients,[120] spontaneous (nontraumatic) myonecrosis due to *Aeromonas* has not been reported in other settings. In a few instances myonecrosis has been accompanied by gas spreading extensively in soft tissue planes. The rapid onset (24–48 hours) and rapid progression after trauma resemble those of clostridial gas gangrene. The prominence of pain, marked edema, serosanguineous bullae, and toxicity, in addition to the presence of gas in fascial planes, adds to the similarity of these conditions, and elicitation of freshwater exposure supports the diagnosis of *Aeromonas* infection. Bacteremia is frequently present. Treatment consists of extensive surgical débridement and prompt initiation of antimicrobial therapy. Most isolates of *Aeromonas* are susceptible in vitro to gentamicin, tobramycin, carbapenems, and ciprofloxacin.[118] Third- and fourth-generation cephalosporins, trimethoprim-sulfamethoxazole, and aztreonam also appear to be active, although individual strains may express β-lactamases that selectively hydrolyze cephalosporins or carbapenems.

PSOAS ABSCESS

Infection of the psoas muscle takes the form of either an abscess or a phlegmon, similar to the progression seen in primary pyomyositis. Unlike pyomyositis of other sites, psoas infections in temperate regions most commonly develop after the spread of infection from an adjacent structure (secondary psoas abscess)[1]; in tropical areas primary psoas abscesses, which develop by the hematogenous route, dominate, and *S. aureus* is the most common cause in this setting.[1,121,122] In adult women, hematogenous psoas abscesses have been observed as a complication of spontaneous vaginal delivery.[16,123] A psoas abscess usually is confined within the psoas fascia, but, on occasion, because of anatomic relationships, infection extends to the buttock, hip, or upper thigh.[33] A psoas abscess may complicate pyogenic, tuberculous, or fungal vertebral osteomyelitis. Tuberculosis was formerly the principal cause of psoas abscesses; now they most commonly result from direct extension of intraabdominal infections (e.g., diverticulitis, appendicitis, Crohn disease),[121] rupture of a colonic adenocarcinoma,[124] or from vertebral infection.[122] On occasion, a psoas abscess results from extension of a perinephric abscess or from secondary infection of a retroperitoneal hematoma. The organisms involved in the spread of infection from an intestinal site are usually members of the aerobic and anaerobic bowel flora. *S. aureus* is the most common cause of psoas abscess secondary to vertebral osteomyelitis.

The iliacus muscle, applied to the ilium in the iliac fossa, forms a conjoined tendon with the lower portion of the psoas muscle. Osteomyelitis of the ilium or septic arthritis of the sacroiliac joint can penetrate the sheaths of either or both muscles in this location, producing an iliacus or psoas abscess.[125]

Clinical manifestations of a psoas abscess include fever, malaise, lower abdominal or back pain, or pain referred to the hip or knee. Initially a primary orthopedic injury is often suspected. A limp may be evident, and flexion deformity of the hip may develop from reflex spasm, suggesting septic arthritis of the hip. The psoas sign is evident. Often a tender mass can be palpated in the groin.

Radiographs may show a bulge produced by a psoas muscle abscess or the presence of gas within the psoas sheath. Calcification in a psoas abscess strongly suggests tuberculosis. CT is the most rapid and sensitive noninvasive imaging technique to assess the psoas and iliacus muscles,[126] although in the early stages of illness subtle asymmetrical psoas enlargement may be overlooked.[127] Ultrasonography is less reliable for detecting small lesions or a phlegmon. Radionuclide imaging is no longer widely used in this situation. CT may show diffuse enlargement of the psoas (phlegmon), a sharply circumscribed, low-density fluid collection (abscess) within the muscle, or the presence of gas within the muscle (indicative of abscess).[126] MRI of the pelvis can reveal enlarged psoas and iliacus muscles displaying grossly abnormal signal intensities.

Pyogenic psoas abscesses require drainage and initial empirical antibiotic therapy based on knowledge of the origin of the infection. CT is often quite valuable for abscess visualization and catheter drainage,[128] with direct surgical drainage reserved for unsuccessful interventional radiologic attempts and instances of inadequate catheter access. Although culture-negative psoas abscesses can be seen when drainage procedures follow an initial course of empirical antibiotic therapy, sterile pseudopsoas abscesses associated with erosive diskitis due to calcium pyrophosphate deposition have been reported.[129] If the process appears to be a phlegmon, repeat CT during the course of antibiotic therapy can confirm resolution of the anatomic changes and help determine the duration of antibiotic therapy.

OTHER SPECIFIC SITES OF MUSCLE ABSCESSES

Infective myositis or pyomyositis may occasionally occur in less common anatomic areas and mimic other more common infections; deep pelvic muscle infections are relatively more common in children. Iliacus pyomyositis[60] and pyomyositis of the adductor muscles or the obturator internus muscle[130] may mimic septic arthritis of the hip, pyriformis pyomyositis may suggest a spinal epidural abscess[61] or pelvic osteomyelitis,[131] and iliopsoas myositis[59] may mimic appendicitis. On occasion, the primary myositis may actually progress to involve adjacent joints, resulting in adjacent septic arthritis.

MYALGIAS

Myalgias are prominent features of a variety of infections, such as dengue, influenza, and Rocky Mountain spotted fever, and are often associated with mildly to moderately elevated levels of creatine phosphokinase.[132] Histologic changes include the presence of virions and variable patchy myonecrosis, often with a paucity of inflammation.[132,133] Clinically significant muscle weakness is occasionally present, often in association with severe rhabdomyolysis (see later discussion).[133–136] In addition to the focal myositis syndromes discussed earlier, a variety of pathogens are associated with acute diffuse muscle injury (culminating in rhabdomyolysis) or with chronic diffuse muscle injury, mimicking autoimmune polymyositis (see Table 94.1). The histopathologic similarities observed between autoimmune muscle injury and the polymyositis associated with certain infectious processes and the demonstration of pathogen-specific antigen recognition by infiltrating lymphocytes suggest that infection may trigger an autoimmune attack on myocytes in at least some instances.[137,138]

Influenza

Muscle aches are common early in the course of influenza.[139] On occasion, severe bilateral muscle pains in the lower limbs may develop in the recovery phase of influenza A or B, particularly in young children, which has been termed *acute benign myositis.*[140,141] Although influenza B is less common than influenza A, the rate of influenza B–associated myositis greatly exceeds that of influenza A.[134,135] Muscle tenderness is demonstrable, principally in the gastrocnemius and soleus muscles, and calf swelling may be present. Deep tendon reflexes and muscle strength are normal, but there is considerable difficulty in walking. The leg pains and muscle tenderness subside in less than 1 week. Increases in serum concentrations of aldolase and creatine phosphokinase, sometimes marked, usually peak within 2 weeks of symptom onset.[141] The specimens from the few biopsies performed have shown either nonspecific degenerative changes or muscle necrosis with polymorphonuclear leukocytic infiltration. Whether this myositis is generally caused by direct viral invasion or by some immunologic or other response is unknown. Direct viral replication within skeletal muscle has been demonstrated in fatal cases of influenza A.[142] Life-threatening rhabdomyolysis with extreme increases in creatine phosphokinase and myoglobin-induced acute renal failure are rarely seen after influenza A infection; the prognosis is favorable but may require fasciotomy for extremity compartment syndrome,[143] short-term dialysis,[136,143] or even extracorporeal membrane oxygenation if myocardial dysfunction is present. Isolated influenza myocarditis[144] and perimyocarditis, sometimes quite severe, with resultant cardiogenic shock[145,146] or pericardial tamponade,[147] can occur in the absence of generalized rhabdomyolysis (see Chapter 165).[148] Interestingly, influenza A–triggered Takotsubo ("stress") cardiomyopathy has been observed.[149]

Infective Endocarditis

Prominent myalgias occur in about 15% of patients with infective endocarditis.[150] They may be either diffuse or localized. The pathogenesis is not known, but in one instance muscle biopsy specimens showed a small focus of muscle fiber destruction and leukocytic infiltration consistent with embolization to a small artery. On rare occasions infective endocarditis may lead to frank pyomyositis,[10,23] pain and weakness with a dermatomyositis-like presentation,[151] or rhabdomyolysis.[152]

Toxoplasmosis

The major features of acute acquired disseminated toxoplasmosis are those of meningoencephalitis, myocarditis, pneumonitis, lymphadenitis, rash, and occasionally hepatitis (see Chapter 278). In rare instances, particularly (but not exclusively[153]) in immunocompromised hosts (e.g., HIV infection,[154] chronic immunosuppressive therapy after organ transplantation, CD4 lymphopenia[155]), polymyositis may be a prominent clinical manifestation resembling autoimmune polymyositis. Marked myalgias, muscle weakness and swelling, and fasciculations occur in such patients. Muscle biopsy specimens show interstitial myositis with destruction of muscle fibers, and pseudocysts of *Toxoplasma gondii* can be found in areas of muscle that are free of inflammatory reaction.[154] It appears that dysregulation of regulatory T cells plays an important role in the development of *Toxoplasma* skeletal muscle involvement.[156]

Other Causes

On occasion, the only clinical manifestations of initial infection with HIV type 1 (HIV-1) are those of polymyositis (myalgias, muscle weakness, and increased serum levels of muscle enzymes).[157] HIV-1 viral antigens can be found in $CD4^+$ T lymphocytes in areas of muscle fiber inflammation and necrosis.[158] During the subsequent course of HIV-1 infection, various forms of muscle disease may develop,[158] including generalized or localized myalgias; HIV myopathy (polymyositis); inclusion body and nemaline myopathies; muscle atrophy accompanying AIDS wasting syndrome or vasculitis; opportunistic infectious myositis, including syphilis[159]; and mitochondrial myopathy related to antiretroviral therapy (see Chapter 128). The clinical presentation of HIV-1 myopathy (inflammatory polymyositis) is that of progressive proximal muscle weakness. It can develop in all stages of the infection and does not correlate with CD4 count.[160] Increased levels of serum creatine phosphokinase and electromyographic changes assist in diagnosis. Muscle biopsy can help resolve this rather long differential diagnosis and guide specific therapy.[158] The inflammatory myopathy may represent primarily an HIV-associated autoimmune process and may respond clinically to prednisone and/or additional immunosuppressive therapies.[157,160]

Inflammatory myositis with a lymphoplasmacytic cellular response has been documented in patients with human T-cell lymphotropic virus-1 (HTLV-1)–associated polymyositis.[161] Muscle-infiltrating $CD8^+$ lymphocytes specific for viral and class I major histocompatibility determinants are present.[137,162] In many areas of highly endemic HTLV-1 infection the majority of patients with polymyositis are HTLV-1 seropositive.[161] There may be a direct toxic effect of HTLV-1 Tax-1 protein on myocytes, even in the absence of myocyte infection.[163] In addition, specific Tax-1 cytotoxic T-lymphocyte activity is present in HTLV-1–infected patients with muscle disease.[163] Inflammatory myositis is rarely

a major feature in Lyme disease[164] and, if present, is often a focal process in relation to adjacent joint or skin involvement. Rarely, a patient presenting with apparent lower extremity pyomyositis actually has Lyme arthritis complicated by a ruptured Baker cyst with a distal intermuscular fluid collection that is positive for *Borrelia burgdorferi* by polymerase chain reaction (PCR) testing. Spirochetes consistent with *B. burgdorferi* may be present on Dieterle silver stain of muscle biopsy specimens, or the presence of *Borrelia* may be confirmed by PCR analysis.[164] Most cases have been reported from Europe, reflecting differences between European and American isolates. Rarely, infection by *Sarcocystis,* an intracellular sporozoan parasite, has been observed in histologic sections of muscle of individuals with muscle pain or weakness, mainly outside the United States.[165] Nematode myositis due to the minute nematode *Haycocknema perplexum* has been observed in Australia.[166] In addition to toxoplasmosis, HIV-infected individuals have developed protozoan myositis due to a variety of microsporidia,[167] including *Trachipleistophora hominis* (see Chapter 268).[168]

PLEURODYNIA SYNDROMES

Epidemic pleurodynia is an acute, febrile disease caused by group B (or rarely by group A) coxsackieviruses that is characterized by the sudden onset of sharp chest pain over the lower ribs or sternum (see Chapter 172). Paroxysms of knifelike pain are precipitated by voluntary or respiratory movements. Muscle tenderness may be present. Abdominal pain may also be present in some patients; in others, abdominal pain may be the sole manifestation, simulating intraperitoneal processes.

Group B coxsackieviruses produce visceral lesions and some focal myositis in experimental animals. Myositis has not been demonstrated as a feature pathologically, either in fatal cases of severe neonatal coxsackievirus B infection or in the few biopsy specimens obtained from affected muscles of patients with epidemic pleurodynia, but it has been associated with rhabdomyolysis complicating mild exercise in the recovery phase of illness.[169] A variety of other enteroviruses rarely provoke rhabdomyolysis.[1] Focal myositis with localized swelling and perivascular mononuclear cell infiltration sparing myocytes was observed in a patient with coxsackievirus A21 infection.[170] Coxsackie B4 has been implicated as a cause of acute myocarditis mimicking influenzal myocarditis.[171]

MYALGIAS WITH EOSINOPHILIA (PARASITIC MYOSITIS)

Trichinosis

Trichinosis is acquired by ingestion of encysted larvae in insufficiently cooked pork or, less commonly, bear meat, wild boar meat, horse meat, or walrus meat. The prominent clinical manifestations of trichinosis include fever, myositis, periorbital edema, and eosinophilia. An initial intestinal phase (nausea, vomiting, nonbloody diarrhea) caused by larval release in the stomach, followed by larval maturation and copulation in the small intestine during the first week, is followed during the second week by release of progeny larvae, mucosal invasion, hematogenous dissemination, and invasion of skeletal muscle (see Chapter 287).[172] Serious complications in the form of myocarditis, meningoencephalitis, and pneumonitis can occur.[172] Myalgias, frequently accompanied by muscle swelling and weakness and occasionally associated with fasciculations, are present in most patients with the disease. Muscles commonly involved include the extraocular muscles, flexor muscles of the extremities, back muscles, and muscles used in chewing and swallowing. Periorbital edema, chemosis, and conjunctival hemorrhages are related to larval invasion of extraocular muscles. The inflammatory response in muscle produces increased serum levels of muscle enzymes and is associated with prominent eosinophilia.

Muscle biopsy specimens reveal encysted larval trichinae in necrotic muscle fibers, surrounded by inflammatory cells (predominantly eosinophils and neutrophils, but also lymphocytes). Severe skeletal muscle involvement reflects the burden of infection and possible host immunosuppression. Although granulomatous reactions have been observed in the heart and lungs in fatal cases, larval encystment does not take place in organs other than skeletal muscle.

Trichinella spiralis is the most common cause of human trichinosis, but multiple other species can infect humans.[172] Unlike *T. spiralis,* *Trichinella pseudospiralis* does not undergo encystment in skeletal muscle and leads to prolonged larval migration and clinical symptoms.

Diagnosis of trichinosis is made on the basis of the clinical picture, eosinophilia, elevated muscle enzymes, compatible serologic findings (enzyme-linked immunosorbent assay, immunoblotting, and, if needed, muscle biopsy), and appropriate epidemiologic investigation.[172] Benzimidazole compounds (thiabendazole, mebendazole, albendazole), which kill mature worms, are the most effective therapies early in the illness; albendazole may have the advantage of being better tolerated. Short courses of systemic corticosteroid therapy ameliorate clinical symptoms and are administered in severe cases.[172]

Trichinosis should be distinguished from the eosinophilia-myalgia syndrome, which results from the ingestion of certain tryptophan products and is characterized by prominent myalgias, fatigue, and eosinophilia, followed, in some instances, by the development of neurologic and scleroderma-like skin changes.[173]

Cysticercosis (*Cysticercus cellulosae* Myositis)

Human cysticercosis is rare in the United States but common in Latin America and Asia. It results from the ingestion and subsequent hatching of viable eggs of *Taenia solium* into the larval form (cysticercus) of the parasite (see Chapter 289). Eggs reach the upper intestinal tract in food contaminated by feces from a person parasitized by the adult worm. Autoinfection can occur through the fecal-oral route and possibly by reverse peristalsis transporting intestinal egg-laden proglottids back into the duodenum or stomach, where they hatch. From the stomach they are widely distributed to the skeletal muscle, subcutaneous tissues, heart, eye, and central nervous system.

Symptomatic involvement of muscle is uncommon. On occasion, the stage of invasion is characterized by fever, muscle tenderness, and eosinophilia, particularly with large inocula.[174] More characteristically, asymptomatic calcified cysts ("puffed rice" appearance) are detected in muscles on soft tissue radiographic films of patients with neurologic manifestations.

MUSCLE DEGENERATION ASSOCIATED WITH INFECTIONS AT OTHER SITES

Acute Rhabdomyolysis

Myoglobinuria occasionally occurs after an acute illness with symptoms suggesting an upper respiratory tract infection and has been associated with a variety of respiratory viral pathogens, including influenza viruses A and B, parainfluenza virus, adenovirus, and severe acute respiratory syndrome–coronavirus, as well as *Mycoplasma pneumoniae* and *Legionella pneumophila.* Rhabdomyolysis has also complicated systemic infections caused by HIV, Epstein-Barr virus, cytomegalovirus, human herpesvirus 6, measles virus, varicella virus, dengue virus, West Nile virus, rabies virus, and parvovirus B19. A variety of bacterial pathogens have led to rhabdomyolysis among critically ill patients with sepsis[175,176]; gram-positive pathogens, including *S. aureus* and *S. pneumoniae,* predominate, but *Salmonella* spp., *Neisseria meningitidis,* and Enterobacteriaceae or nonenteric pathogens, such as *P. aeruginosa* or *Acinetobacter baumannii,* may be responsible. Leptospirosis, brucellosis, and rickettsial infections can also trigger rhabdomyolysis. Diffuse muscle pains (especially in the extremities), weakness, swelling, and tenderness are prominent features, along with increased muscle enzyme (often striking), myoglobinuria, and even acute renal failure.

Muscle Proteolysis and Mediators of Fever in Patients With Sepsis

Muscle involvement in the form of myalgias and weakness is common in the course of systemic infections. Accelerated catabolism of skeletal muscle contributes to the marked weakness and muscle wasting that can be observed in systemic infections. This seems to be part of an acute-phase host response to sepsis and trauma likely triggered by a variety of mediators, including interleukin-1 (IL-1), tumor necrosis factor, interferon-α, and IL-6, as well as endogenously and exogenously administered glucocorticoids. Detailed studies of myocyte mitochondrial number, protein synthesis and expression, mitochondrial enzyme activity,

and messenger RNA levels (including microRNA) in muscle biopsy specimens from septic ICU patients with multiorgan system failure demonstrate a loss of functional mitochondria with sustained but dysregulated mitochondrial protein expression and enhanced proteolytic activity. Transcriptional analysis demonstrated that several critical intracellular pathways (oxidative stress, apoptosis, proteasome function, ion homeostasis, and kinase signaling) are perturbed in these patients.[177]

The intracellular events accompanying muscle catabolism involve prostaglandin E_2 synthesis, direct cleavage of actomyosin by caspase 3,[178] and subsequent degradation by proteasomes. Additional mechanisms also help regulate the process of muscle cachexia.[179] An important role of prostaglandin E_2 in the generation of myalgias and fever is consistent with the amelioration of these symptoms after administration of nonsteroidal antiinflammatory prostaglandin synthesis inhibitors.

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95 Lymphadenitis and Lymphangitis

Mark S. Pasternack

SHORT VIEW SUMMARY

Definition: Lymphadenitis
- Acute (suppurative) or chronic (often granulomatous) inflammation of the lymph nodes

Epidemiology
- Suppurative lymphadenitis most common among young children; granulomatous lymphadenitis may occur in individuals of all ages

Microbiology (see Table 95.1)
- Suppurative lymphadenitis is due primarily to *Staphylococcus aureus* (methicillin sensitive and methicillin resistant) and group A streptococci in normal hosts; a variety of pathogens may cause suppurative lymphadenitis in immunocompromised individuals.
- Granulomatous lymphadenitis is due primarily to nontuberculous mycobacteria in healthy children. *Mycobacterium tuberculosis* is the most important etiology in older patients, but it may be due to a variety of pathogens in compromised hosts.

Diagnosis
- Needle aspiration, incisional biopsy, or excisional biopsy, depending on the tempo and severity of the illness and possible immunodeficiency

Therapy
- Drainage of established abscesses and conventional antimicrobial therapy against gram-positive pathogens for acute suppurative lymphadenitis
- Targeted therapy based on microbiologic/histopathologic investigation for chronic granulomatous lymphadenitis

Prevention
- Early therapy of primary superficial infections and early therapy of acute adenitis episodes may reduce the risk of suppuration and need for surgical drainage.

Definition: Lymphangitis
- Lymphangitis is an inflammation of lymphatic channels, usually in subcutaneous tissues. It is caused by an acute process of bacterial origin or as a chronic process of mycotic, mycobacterial, or filarial etiology (see Table 95.2).

LYMPHADENITIS

Lymphadenitis is an acute or chronic inflammation of lymph nodes. It may be restricted to a solitary node or to a localized group of nodes draining an anatomic area (regional lymphadenitis), or the involvement can be generalized during a systemic infection. The gross features may reflect nonsuppurative, suppurative, necrotizing, or caseous inflammation, depending on the nature of the infecting microorganism, or noninfectious inflammatory or neoplastic processes. Fine-needle aspiration cytology is often a valuable minimally invasive diagnostic procedure, although it occasionally may be complicated by sinus tract formation in patients with granulomatous lymphadenitis.[1]

Pathogenesis and Pathologic Changes

Acute Lymphadenitis

Lymph nodes serve as filters, removing infectious agents from lymphatics draining areas of acute inflammation. The initial histologic response consists of swelling and hyperplasia of sinusoidal lining cells and the infiltration of leukocytes. Depending on the nature of the infecting organism, host defenses, and antimicrobial therapy, the process may or may not progress to abscess formation. With some microorganisms, a more distinctive pathologic picture may be seen: granulomas and caseation necrosis with infections due to *Mycobacterium tuberculosis* and nontuberculous mycobacteria; stellate abscesses surrounded by palisading epithelioid cells (granulomatous abscess)[2] with lymphogranuloma venereum (LGV), cat-scratch disease, tularemia, yersiniosis, fungal lymphadenitis, and chronic granulomatous disease; or reactive follicular hyperplasia with scattered clusters of epithelioid histiocytes characteristically blurring the margins of germinal centers, along with focal distention of subcapsular and trabecular sinuses by monocytoid B cells, in toxoplasmosis.[3] The necrotizing granulomatous lymphadenitis that occurs in tularemia can resemble that occurring in cat-scratch disease but often exhibits more granulomatous inflammation. *Yersinia* (*Yersinia pseudotuberculosis* or *Yersinia enterocolitica*) infection in mesenteric lymph nodes can also cause a necrotizing lymphadenitis. Necrotizing nongranulomatous lymphadenitis may be a feature of processes to which an infectious cause has not yet been ascribed: Kikuchi necrotizing lymphadenitis, Kawasaki disease, and systemic lupus erythematosus.[4] Necrotizing viral lymphadenitis has been documented to be due to herpes simplex virus, primarily in individuals with underlying hematologic disorders,[5] cowpox,[6] parvovirus B19,[7] and human herpesvirus 6 (HHV-6)[8] in healthy individuals, as well as in patients with systemic lupus erythematosus.

Chronic Lymphadenitis

Histologically, the response is proliferative with hyperplasia of reticuloendothelial cells, prominent germinal centers, and dilated lymph sinuses filled with mononuclear cells. This nonspecific picture can be seen with a variety of infections and may be observed initially on biopsy in a patient subsequently proven to have a lymphoproliferative disorder. Noncaseous granulomatous lymphadenitis is characteristic of sarcoidosis. More destructive granulomatous lymphadenitis with necrosis or caseation may occur in tuberculous or fungal lymphadenitis, particularly histoplasmosis.

Dermatopathic lymphadenitis (lipomelanotic reticuloendotheliosis) is a distinctive form of chronic lymphadenitis involving lymph nodes draining sites of chronic pruritic dermatitides. These enlarged nodes characteristically demonstrate accumulation of lipid and melanin in macrophages derived from the cutaneous inflammatory process. The hyperplastic appearance of such nodes may be so prominent as to suggest erroneously a lymphoproliferative disorder. Similarly, inflammatory pseudotumor can present with progressive cervical lymphadenopathy with systemic symptoms and thus mimic tuberculosis (TB) or lymphoma.

Clinical Manifestations

Acute Regional Lymphadenitis Due to Pyogenic Bacteria

Palpable lymph nodes do not always indicate serious or ongoing disease. Some degree of inguinal lymphadenopathy is relatively common, reflecting previous episodes of infection in the lower extremities (e.g., interdigital web infections secondary to dermatophytosis); similarly,

slight enlargement of cervical nodes may persist from previous pharyngeal or dental infections. Lymphadenopathy in certain anatomic areas (preauricular, posterior auricular, supraclavicular, deltoideopectoral, and pectoral) should always be viewed with greater suspicion because these nodes are not frequently enlarged as a result of local subclinical infections or minor trauma. Enlargement of superficial lymph nodes along the external jugular vein, as well as of nodes that drain the earlobe and the floor of the external acoustic meatus, may be associated with superficial infection accompanying recent ear piercing. Rarely, a firm mass in the tail or lateral aspect of the breast, suggestive of carcinoma, proves to be an enlarged lymph node in an unusual location caused by toxoplasmosis.[3]

Acute suppurative lymphadenitis is far more common in children than in adults. In the past 4 decades *Staphylococcus aureus* (both methicillin-susceptible and methicillin-resistant strains[9,10]) has superseded group A streptococci as the most frequent etiologic agent. The most common sites of involvement are, in descending order, submandibular (submaxillary), anterior and posterior cervical, inguinal, and axillary lymph nodes. The portal of entry for infection is frequently difficult to determine in children when cervical lymph nodes are involved. The recovery of a variety of anaerobic pathogens and/or α-hemolytic streptococci, including *Streptococcus intermedius*[11] in cervical lymph node aspirates from some patients, suggests that in addition to cutaneous foci, primary dental or pharyngeal infections may lead to suppurative lymphadenitis.[12,13]

On examination the involved area is swollen, and the node(s) are usually at least 3 cm in diameter and tender. Fever is commonly present. The node(s) may be firm or frankly fluctuant. The overlying skin is warm and often erythematous and edematous.

Syndromes Due to Suppurative Lymphadenitis at Specific Anatomic Sites

Cervical Lymphadenitis

Acute unilateral adenitis of pyogenic origin occurs most often in preschool-age children; bilateral disease occurs in less than 10% of cases.[10] Fever (38°C–39°C) is common, and local swelling is often present for some days before the patient is seen by a physician. Although in only a minority of cases is there a history of sore throat, group A streptococci historically have been the most common cause of suppurative cervical lymphadenitis in children. *S. aureus* or a combination of *S. aureus* and group A streptococci is often responsible for suppurative cervical lymphadenitis associated with pyodermas of the face and scalp. Improved anaerobic culture techniques have demonstrated a variety of oral anaerobes in percutaneous needle aspirates in up to 40% of patients, as either pure anaerobic infections or mixed infections with conventional gram-positive aerobes.[12,13] Acute torticollis may result from cervical lymphadenitis of either bacterial or viral (e.g., infectious mononucleosis) origin and may be the initial symptom that brings the child to the physician.[14] Cervical lymphadenitis associated with torticollis may also indicate the presence of associated retropharyngeal and/or parapharyngeal space infection.[15]

On examination there is prominent swelling of the neck or face owing to the enlargement of a single node or a matted collection of adjacent lymph nodes, which may be walnut sized. The mass is typically exquisitely tender and firm at presentation but may be fluctuant initially or develop fluctuance during the course of therapy. The swelling may be sufficiently marked to limit mouth opening or evoke trismus. A white blood cell count of 12,000 to 25,000/mm^3 is common. Drainable suppurative lymphadenitis may develop in up to 20% of acute cervical lymphadenitis episodes, particularly in younger children with unilateral disease and symptom duration over 48 hours.[16]

Acute bilateral cervical adenitis usually involves multiple nodes that are enlarged and somewhat tender in association with viral pharyngitis, infectious mononucleosis and related syndromes, HHV-6,[8,17] streptococcal pharyngitis, or periodontal infection. Such lymphadenopathy may progress to suppuration if the primary pyogenic process is untreated. Bilateral suppurative lymphadenitis may occur at increased frequency in immunocompromised hosts (e.g., chronic granulomatous disease).[18]

Acute Axillary Lymphadenitis

This process, when due to *Streptococcus pyogenes,* is characterized by an abrupt onset with chills, fever, marked axillary pain, and prominent edema of the shoulder, arm, axilla, supraclavicular fossa, and pectoral areas.[19] The site of initiating infection is usually a pustule or traumatic lesion on the hand or arm. The involved area, although edematous, does not have features of cellulitis, lymphangitis, or erysipelas. Ipsilateral pleural effusion may develop due to blockage of lymphatic vessels draining the parietal pleura into the involved lymph nodes at the junction of the internal jugular and subclavian veins. Thrombosis of the axillary and subclavian veins may be a complication. In countries where infants are routinely inoculated with Calmette-Guérin bacillus (BCG), acute axillary lymphadenitis ipsilateral to the BCG inoculation site is occasionally seen[2,20] and, if severe, may implicate significant underlying immunodeficiency.[21]

Subpectoral Lymphadenitis

An unusual course may be taken occasionally by infection (usually streptococcal but sometimes staphylococcal) of the thumb or the interdigital web between the thumb and index finger. Lymphatics from this area do not pass through the epitrochlear nodes but drain directly into the axillary nodes, which in turn communicate with the subpectoral nodes. If infection is not contained in the axillary nodes, subpectoral lymphadenitis develops and may progress to frank suppuration. Infection in this area may dissect downward and manifest as cellulitis over the lower chest and upper abdomen, suggesting an intraabdominal infection. On occasion, large subpectoral abscesses may suggest a tumor because the overlying pectoralis major obscures the local warmth and erythema commonly associated with infection. Rarely, contraction of the pectoral muscle (as on arm elevation) causes movement of the pectoral area swelling, suggesting avulsion of the inferior attachments of this muscle. The suppurating nodes may drain onto the chest wall. Pleural effusion may develop on the involved side.

Deep Neck Space Lymphadenitis

The development of progressive parapharyngeal or retropharyngeal infection complicating primary intraoral infection may become a life-threatening complication due to progressive inflammation, necrosis, or abscess formation, or a combination of these, compromising vital neck structures and the mediastinum (see Chapter 85). Computed tomography (CT) has been invaluable to localize these infections and guide surgical management, but in some cases, focal low-density abnormalities that do not yield drainable pus at exploration are identified.[22,23] In these cases focal parapharyngeal[23] or retropharyngeal lymphadenitis,[22] recognizable by its relatively small size or relatively posterior location in the parapharyngeal space, may account for these radiologic abnormalities and be amenable to nonsurgical management with antibiotic therapy and close follow-up. Acute cervical lymphadenitis may be present in association with these deep-space infections and may represent the presenting complaint, leading to initial evaluation.[15]

Acute Mediastinal Lymphadenitis

Acute suppurative mediastinal lymphadenitis is usually recognized in the course of managing bacterial mediastinitis, a fulminant process typically complicating progressive infections of the upper respiratory tract or perforation of the esophagus or bronchial tree as the result of trauma or surgery. Rarely, odontogenic foci of infection may lead to progressive mediastinal lymphadenitis without apparent progressive infection of the head and neck. Suppurative mediastinal lymphadenitis may result in the superior vena cava syndrome.[24] The superior vena cava syndrome has also been attributed to marked reactive lymphadenopathy in the setting of cystic fibrosis without frank mediastinal suppuration.[25] Prominent acute hemorrhagic thoracic lymphadenitis is a feature of inhalation anthrax, an aspect reemphasized in the 2001 bioterrorism-related anthrax outbreak.[26]

Acute Suppurative Epitrochlear Lymphadenitis

The epitrochlear nodes receive lymphatic drainage from the middle, ring, and little fingers and from the medial portion of the hand and the ulnar aspect of the forearm. Acute suppurative epitrochlear

lymphadenitis is uncommon and generally accompanies a primary pyoderma or a secondarily infected skin lesion. Unilateral tender swelling, erythema, and induration of the epitrochlear area develop and may subsequently spread along the medial aspect of the arm and forearm. There is often pain on elbow movement, moderate fever, and leukocytosis. The diagnosis is apparent when a discrete, tender nodular swelling can be palpated, but when the area is diffusely swollen and movement at the elbow is limited, the picture may suggest septic arthritis, osteomyelitis, subcutaneous abscess, or olecranon bursitis, and ultrasound may help to clarify the diagnosis.[27,28] Group A streptococci and *S. aureus* are implicated most commonly. In contrast, cat-scratch disease and TB involving the epitrochlear lymph nodes present in a more indolent fashion.

Suppurative Iliac Lymphadenitis

The iliac lymph nodes, located along the external and common iliac arteries in the anterior retroperitoneal space, receive lymphatic drainage from the lower abdominal wall and the superficial and deep inguinal nodes. Iliac lymphadenitis may develop secondary to infection of the lower extremities, lower abdominal wall, or perineum, or rarely may result from hematogenous infection. After infection develops, it appears to break through fascial compartments in the iliac fossa, and abscess formation ensues. Formerly, most cases occurred in children and young adults, but more recently there seems to be no age predilection. The suppurative lymphadenitis progresses to abscess formation in the space between the posterior peritoneum and the psoas and iliacus fascia.[29] Patients may present with an unexplained limp without systemic symptoms, followed by back and hip pain. Although extension of the thigh is painful, abduction and adduction of the hip evoke minimal discomfort. The symptomatology and clinical findings suggest possible septic arthritis and osteomyelitis. Only after some days or weeks does lower abdominal pain develop, and the patient becomes acutely ill with high fever and marked leukocytosis. Patients may demonstrate a tightly flexed hip, ipsilateral rectus muscle spasm, or possibly a tender posterolateral pelvic mass or a tender inguinal mass suggesting an incarcerated inguinal hernia. By this stage the abscess may be sizable and displace the sigmoid colon or the lower third of the ureter, or both. Right-sided symptoms suggest the diagnosis of *retrocecal* appendicitis with abscess, ischemic colitis, or cecal carcinoma, but the antecedent limp is an important clinical clue. TB of the spine with psoas abscess formation, pelvic inflammatory disease, and tumor of the thigh should also be considered.[30] Tuberculous (BCG) iliac lymphadenitis has also been reported after intravesical BCG instillation for the treatment of bladder carcinoma.[31] Cross-sectional imaging (CT or magnetic resonance imaging) can be helpful in defining an inflammatory collection abutting the psoas and iliacus muscles and narrowing the differential diagnosis.[32] *S. aureus* is the microorganism most commonly implicated, followed in frequency by streptococci. Rarely, noninfectious processes such as Kikuchi-Fujimoto disease may present with fever and prominent iliac lymphadenopathy mimicking suppurative iliac lymphadenitis.[33]

Acute Regional Lymphadenitis Due to Infecting Agents Other Than Pyogenic Bacteria

A variety of organisms other than the common pyogenic ones may produce localized lymphadenitis, in some cases going on to abscess and sinus tract formation. These infections are generally distinguishable by a prolonged and indolent course, the atypical anatomic areas involved, the lack of previous superficial pyogenic infection, and sometimes by additional clues in the history and epidemiology (e.g., cat scratch, previous TB, travel history, recent sexual exposures, clinical setting, underlying host immunodeficiency). Syphilitic cervical lymphadenopathy presents subacutely with painless firm prominent adenopathy and may be accompanied by an oral chancre, aiding in the clinical diagnosis, but may be an obscure diagnosis in the absence of oropharyngeal ulceration.[34] Clinical suspicion of syphilis should lead to serologic confirmation of the diagnosis, but excisional biopsy and demonstration of "starry sky" histologic features with prominent plasma cell infiltration, occlusive capillaritis, and endothelial swelling,[35] as well as the presence of spirochetes using Warthin-Starry staining,[36] may confirm the diagnosis when the prebiopsy probability is low. Advanced imaging techniques, such as ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET)-CT scanning, has identified *Coxiella burnetii* lymphadenitis as a focal infection or in association with deep parenchymal infection in patients diagnosed with chronic Q fever.[37,38] Cowpox can cause ulcerating lesions in unimmunized individuals that are associated with regional lymphadenopathy and may mimic ulceroglandular tularemia and (less likely) plague.[38a]

A broad spectrum of microorganisms must be considered as the cause of firm or fluctuant lymphadenitis when evaluating compromised hosts. Actinomycotic lymphadenitis may occur in normal and immunocompromised hosts.[39] Mycotic (*Candida albicans, Aspergillus* spp.) cervical lymphadenitis has occurred after oral mucositis in neutropenic children[40] and adults[41] with leukemia, but a rare case of bilateral cervical lymphadenopathy due to *Aspergillus fumigatus* was described in a young girl without underlying disease, neutropenia, or immunosuppression.[42] Cryptococcal lymphadenitis has also been observed in apparently normal hosts.[43] Acquired immunodeficiency due to the development of autoantibodies against interferon-γ (anti–IFN-γ) has been identified among some Asian and African individuals. This may underlie some cases of opportunistic infections, including nontuberculous mycobacterial lymphadenitis, in previously apparently normal hosts carrying selected human leukocyte antigen DR and DQ alleles.[44] Necrotizing cervical lymphadenitis due to *Histoplasma capsulatum* was observed in a woman of African descent with anti–IFN-γ antibodies.[45] In patients with suppurative lymphadenitis complicating chronic granulomatous disease, the microbial etiology is usually a catalase-positive pathogen.[46] In addition to the commonly involved *S. aureus,* these include members of the family Enterobacteriaceae *(Klebsiella, Serratia, Salmonella), Pseudomonas* or *Burkholderia cepacia*, *Aspergillus, Nocardia,* BCG,[47] *Chromobacterium violaceum, Rothia aeria,*[48] and Acetobacteraceae-related agents (*Granulobacter bethesdensis*[49] and *Acidomonas methanolica*).[18,50]

A wide variety of opportunistic pathogens may lead to peripheral lymphadenitis in patients with human immunodeficiency virus (HIV) infection, particularly with progressive immunodeficiency. These include *H. capsulatum, Cryptococcus neoformans,*[43,51] *Pneumocystis jirovecii,*[52] and *Bartonella henselae*[53] (bacillary angiomatosis), as well as TB[54,55,56,57] and nontuberculous mycobacteria[58] and syphilis.[59] Parenchymal disease and lymphadenitis may coexist, although lymphadenitis may present in isolation. Lymphadenitis arising from mixed infections involving more than one of these opportunistic pathogens can occur (e.g., cryptococci and *Histoplasma*),[60] and specimens (either fine-needle samples or excisional biopsies) should be assessed thoroughly, both histologically and microbiologically, for precise diagnoses. Conversely, generalized or prominent regional lymphadenopathy may be present with progressive HIV infection in the absence of opportunistic infection and respond to antiretroviral therapy. Fine-needle aspiration cytology can be diagnostic of HIV lymphadenitis and occasionally establishes the diagnosis if prior serologic screening has not been performed.[61] Infectious lymphadenitis due to opportunistic pathogens may complicate advanced untreated HIV infection[51] or may flare after initial control of infection and subsequent initiation of highly active antiretroviral therapy as part of the immune reconstitution inflammatory syndrome.[62] Previously unrecognized latent or successfully treated infections may become prominent after immune reconstitution, and a thorough diagnostic evaluation, frequently requiring lymph node biopsy for microbiologic diagnosis and histopathology, is necessary. *M. tuberculosis*[63] or *Mycobacterium avium-intracellulare* lymphadenitis[64] as part of disseminated mycobacterial infection is a well-recognized complication of immune reconstitution inflammatory syndrome with important therapeutic and infection control implications, particularly in resource-limited settings.[56,57] Cryptococcal lymphadenitis developing after clinically successful primary therapy in the setting of subsequent immune reconstitution is also well known.[65,66,67] In addition, unusual microorganisms have been responsible for lymphadenitis on rare occasions in immunocompetent persons. Such microorganisms include *Rhodococcus equi, Y. pseudotuberculosis,*[68] *Corynebacterium pseudotuberculosis,*[69] nocardiosis,[70] and *Coxiella burnetii.*[71]

Specific Types of Nonpyogenic Regional Lymphadenitis

Scrofula (Tuberculous Cervical Lymphadenitis)

TB cervical adenitis, formerly a common disease in children and young adults, is now infrequent in modern societies but is still common in resource-limited settings.[72,73,74] It is still occasionally seen in individuals who immigrated to this country from endemic regions or in residents of rural areas in this country and reflects breakdown of previous cervical node TB, acquired by either ingestion of infected milk (bovine TB)[75] or lymphohematogenous spread of infection from an initial pulmonary focus to these lymph nodes. Cervical lymphadenitis due to *M. tuberculosis* is seen in this country in adults of particular ethnic groups: Native Americans, Latinos, and recent immigrants from Haiti and Asia. In the United States and other industrialized societies, tuberculous cervical lymphadenitis (scrofula) is now uncommon and is much more frequently due to atypical mycobacteria. Although *Mycobacterium scrofulaceum* was once the predominant pathogen in this setting, *M. avium-intracellulare* complex is now the principal etiologic agent[76,77,78,79] in children and adults. In recent years a number of newly recognized, difficult-to-isolate, and uncommon mycobacterial species have been reported as occasionally causing childhood cervical lymphadenitis, including *Mycobacterium interjectum, Mycobacterium malmoense, Mycobacterium haemophilum, Mycobacterium xenopi, Mycobacterium szulgai, Mycobacterium lentiflavum, Mycobacterium heidelbergense, Mycobacterium bohemicum,* and more than a dozen others, often requiring gene sequencing for definitive diagnosis.[78]

M. tuberculosis infections, including cervical lymphadenitis and other forms of extrapulmonary disease, are frequent in the HIV-infected population.[80] *M. tuberculosis* lymphadenitis in the HIV-infected patient differs in several respects from the infection in HIV-negative patients: a higher frequency of fever, common concomitant parenchymal infection, frequent negative purified protein derivative (PPD) skin tests (anergy), and a higher frequency of positive smears for acid-fast bacilli (and greater numbers of organisms) on fine-needle aspirates or excised lymph nodes.[81] Deep tuberculous lymphadenitis (e.g., lymphadenitis in relation to the bile duct) may occur and present with unusual features such as obstructive jaundice.[82]

In parts of the world where BCG vaccination of infants is commonly practiced, subcutaneous abscesses and regional lymphadenitis are not uncommon complications, occurring 2 to 8 weeks after vaccination but usually resolving spontaneously. On occasion, regional (axillary, supraclavicular, or cervical) lymphadenitis progressively enlarges and goes on to caseating suppuration.[20,21,83] This was once considered quite rare, but newer strains of BCG vaccine have been associated with markedly higher rates of suppurative adenitis (1/1543 cases), potentially limiting its use as a preventive measure.[84,85]

The onset of scrofula is insidious; fever and other systemic manifestations are present in only a small minority of cases. Several nodes are frequently enlarged and matted together, and the resultant mass may develop a swollen fluctuant area, which brings the patient to medical attention. The process is usually painless. In most cases clinical evidence of TB elsewhere is absent. Localized erythema may develop over the involved nodes and be followed by progressive fluctuance and spontaneous drainage of caseous material onto the skin surface (scrofuloderma).

Ultrasonography or CT may demonstrate deeper regional adenopathy in addition to the clinically apparent lymphadenitis. The identification of the mycobacterial species involved is important because management of mycobacterial lymphadenitis depends critically on whether the causative agent is *M. tuberculosis* or a nontuberculous mycobacterium. Excisional biopsy has the highest rates of microbiologic diagnosis (up to ≈90%), but in regions with high rates of endemic *M. tuberculosis* infection, fine-needle aspiration recovers *M. tuberculosis* in greater than 60% of cases[73] and is less invasive. *M. tuberculosis* lymphadenitis generally responds to conventional 6-month therapy with or without lymph node excision. Of interest, paradoxical reactions resembling the immune reconstitution syndrome have been observed in HIV-negative hosts receiving therapy for tuberculous lymphadenitis, with an initial favorable response followed by subsequent fever and apparent progression.[86]

Nontuberculous mycobacteria, most commonly *M. avium-intracellulare* complex, is responsible for the vast majority of cervicofacial mycobacterial lymphadenitis.[87] This is an uncommon process (annual incidence ≈1–5/100,000 children), which occurs predominantly in young, otherwise healthy children. Disease is usually unilateral and localized to submandibular or anterior cervical nodes. There is indolent progression over 1 to 3 months with nodal enlargement, followed by the development of overlying erythema, and in untreated cases, eventual sinus tract formation and drainage develops.

The nontuberculous mycobacteria causing cervical lymphadenitis, although often sensitive to macrolide agents, are frequently largely resistant to conventional antituberculous chemotherapy, and surgical excision of the involved fluctuant node or nodes is indicated,[88–91] both for initial control of infection and for recovery of the pathogen for drug susceptibility testing. Adjunctive therapy with rifampin and clarithromycin is sometimes considered when treating *M. avium-intracellulare* lymphadenitis in normal hosts, especially when imaging by ultrasonography or CT demonstrates extensive deep lymph node involvement or involvement of submandibular nodes abutting the facial nerve, which precludes total excision. Multidrug therapy is required to treat these infections in compromised hosts, such as HIV patients in whom bacillemia is common and the infection is best considered a systemic illness requiring prolonged systemic therapy. Antituberculous therapy is usually not necessary for BCG nonsuppurative lymphadenitis, but if suppurative lymphadenitis develops, aspiration[83] or complete excision and multidrug antituberculous chemotherapy are indicated.[20]

Granulomatous Lymphadenitis Caused by Nondiphtheria Corynebacteria

Subacute or chronic relapsing lymphadenitis has been reported occasionally to be due to *Corynebacterium pseudotuberculosis (Corynebacterium ovis).* Most patients have lived in Australia[92] and have had extensive contact with animals, particularly sheep, in which *C. pseudotuberculosis* is a common cause of caseous lymphadenitis. The histologic picture is that of a suppurative or necrotizing granulomatous process. Treatment consists of prolonged antibiotic (erythromycin or penicillin) therapy combined with surgical drainage or excision of the involved nodes.

Oculoglandular (Parinaud) Syndrome

Preauricular lymphadenopathy can occur secondary to granulomatous nodular conjunctival infection caused by the introduction of certain pathogens to the external eye. Oculoglandular syndromes occur occasionally in tularemia, cat-scratch disease,[93,94] listeriosis, sporotrichosis, adenovirus infection, murine typhus,[95] and LGV. Epidemic keratoconjunctivitis due to adenoviruses is often associated with an enlarged preauricular lymph node as well.

Cat-Scratch Disease

Cat-scratch disease[93,96] is a slowly progressive and sometimes chronic form of regional lymphadenitis caused by *B. henselae* (see Chapter 234). Although cat-scratch disease is usually a benign and ultimately self-limited process, it may be complicated by acute encephalitis, hepatitis, osteomyelitis, neuroretinitis, arthritis, pleuritis, atypical pneumonia, hilar adenopathy, and thrombocytopenia.[96] *B. henselae* bacilli are slow growing, requiring 2 to 6 weeks of incubation in a moist environment (e.g., in a sealed gas-permeable plastic bag) under 5% carbon dioxide for growth on blood-agar plates (human blood is preferable to sheep or horse blood).

Approximately 90% of patients with cat-scratch disease give a history of contact with cats (most often kittens), and most have been scratched. A primary lesion (small papule or vesicle resembling an insect bite) develops at the site of the scratch 7 to 14 days after contact with the animal. This primary lesion lasts for several weeks to months and may be helpful in diagnosis, but it is not always present when the patient presents for evaluation. Lymphadenopathy develops within 1 to 3 weeks of the appearance of the skin papule.[96] There is no lymphangitis. The lymphadenopathy progresses to suppuration in 10% to 50% of cases, but the course is slower than that of suppurative lymphadenitis due to pyogenic bacteria, and most patients are only mildly ill. Regional lymphadenitis is the sole manifestation of cat-scratch disease in half of the cases. Individuals receiving anti–tumor necrosis therapies have been reported to experience stereotypic cat-scratch adenitis and not

disseminated bartonellosis.[97] Almost any peripheral lymph node may be involved, including isolated involvement of epitrochlear or mediastinal nodes, but the axillary nodes are most commonly affected. The nodes are tender, especially when there is frank suppuration. Low-grade fever is present in only a third of patients. Approximately 10% to 15% of patients have features of a more systemic illness: anorexia, headache, weight loss, and hepatosplenomegaly. Unusual clinical presentations include Parinaud oculoglandular syndrome (conjunctivitis from ocular inoculation associated with ipsilateral preauricular lymphadenopathy) in 4% of patients,[94] neuroretinitis, and, rarely, acute encephalitis/encephalopathy, usually with a sudden seizure as the initial neurologic manifestation, occurring several weeks after the first symptoms of cat-scratch disease. *B. henselae* infection has been confirmed by polymerase chain reaction (PCR) testing of biopsy tissue[98] within the first 6 weeks of lymphadenopathy and from conjunctival scraping in the setting of Parinaud oculoglandular syndrome.[93] Chronic generalized lymphadenopathy due to *Bartonella henselae* has been observed in HIV-infected patients in the absence of bacillary angiomatosis.[99] *B. henselae* infections can be confirmed serologically at the Centers for Disease Control and Prevention and other reference laboratories.

Mediastinal Lymphadenopathy

In contrast to acute or subacute peripheral regional lymphadenitis in which local signs and symptoms prompt medical attention, mediastinal lymphadenitis is sometimes detected only in the course of evaluation of nonlocalizing systemic symptoms, such as fever and weight loss, or primarily pulmonary symptoms, such as cough, chest pain, and sputum production. Distinctive radiologic features may support particular diagnoses. Symmetrical hilar adenopathy with clear lung fields or symmetrical interstitial fibrosis suggests sarcoidosis, particularly when accompanied by any of a variety of extrathoracic manifestations. Asymmetrical or unilateral hilar adenopathy, particularly when associated with ipsilateral chronic focal lung disease with calcification, fibrosis, and/or cavitation, suggests TB or fungal infection. Peripheral rim enhancement of mediastinal lymph nodes on contrast-enhanced CT imaging is characteristic of granulomatous lymphadenitis and implicates TB, histoplasmosis, or cryptococcosis.[100] The presence of mediastinal widening in association with subacute or chronic mediastinal lymphadenopathy strongly suggests histoplasmosis. *Cryptococcus* can cause mediastinal lymphadenitis or mediastinitis, or both, in immunocompromised hosts, mimicking lymphoma,[101] and in HIV patients as part of acquired immunodeficiency syndrome (AIDS), particularly in the immune reconstitution syndrome. Mediastinal lymphadenitis in organ transplant recipients may be caused by a variety of expected and unusual pathogens, including *Rhodococcus*.[102] The underlying health, travel and exposure history, extrapulmonary signs and symptoms, and ancillary laboratory tests (e.g., angiotensin-converting enzyme levels, anergy) may all contribute to a presumptive diagnosis. Ultimately most adults require lymph node biopsy or transbronchial lung biopsy for pathologic and microbiologic confirmation to address possible infection by uncommon pathogens or nodal involvement by noninfectious causes,[103] such as sarcoidosis,[104] inhalational disorders such as silicosis and berylliosis, drug reactions (e.g., phenytoin), primary amyloidosis, Kikuchi-Fujimoto disease,[105] Castleman disease, or malignancy. Necrotic, edematous hilar and mediastinal lymph nodes are early events in inhalation anthrax (see Chapter 207).

Inguinal Buboes of Sexually Transmitted Disease

Inguinal lymphadenopathy due to pyogenic infections or cat-scratch disease is usually unilateral. Prominent bilateral (or unilateral) adenopathy, particularly in men, is suggestive of several sexually transmitted diseases. The genital chancre of primary syphilis is usually accompanied by one or several discrete, firm, nonsuppurative, painless, enlarged nodes in one or both inguinal areas. Constitutional signs are lacking. The overlying skin is uninflamed. In secondary syphilis painless generalized lymphadenopathy usually precedes the characteristic cutaneous eruption.

LGV infections arise after the sexual transmission of selected *Chlamydia trachomatis* serovars. The asymptomatic primary genital lesion (painless papule, vesicle, or erosion) is usually transient. The initial manifestation of the disease after heterosexual transmission is usually the characteristic inguinal bubo, occurring 10 to 30 days after sexual exposure and 1 to 2 weeks after the primary lesion. The adenopathy is more commonly unilateral. Initially the node is tender, discrete, hard, and movable, but subsequently the inflammatory process involves multiple nodes in the area. Chills, fever, and constitutional symptoms are common at this stage. As a result of periadenitis, the nodes become fixed and matted into an oval or lobulated mass. The mass is adherent to the overlying skin, which is purplish. Foci of suppuration develop, with multiple fistulous tracts. A central lengthwise linear depression (so-called groove sign of LGV) is produced by involvement of nodes above and below the inguinal ligament. Although characteristic of LGV, the groove sign may rarely be produced by suppurative bacterial lymphadenitis or lymphomatous involvement of inguinal nodes. In the past decade there has been an epidemic of LGV in industrialized nations involving men who have sex with men; although proctitis and proctocolitis have been the dominant clinical features,[106] inguinal lymphadenitis has been present in approximately 10% of cases.[107] The diagnosis can be made serologically and clinically after a satisfactory response to doxycycline therapy.[108] Culture and molecular methods of diagnosis (e.g., PCR) are available in reference laboratories.[108]

Chancroid is usually accompanied by painful, tender, inguinal adenopathy. The primary lesion is a papule or pustule that progresses to form an extremely painful and tender but nonindurated ulceration with undermined edges, quite in contrast to a syphilitic chancre. Autoinoculation is common, with lesions on opposing or contiguous areas of the skin. The adenopathy of chancroid develops approximately 1 to 2 weeks after the primary lesion appears and, unlike in LGV, is present while the ulcer is still active. Systemic symptoms accompany chancroid only rarely. The chancroidal bubo is typically unilateral and composed of fused inguinal nodes and is more painful than that of LGV. Unilocular suppuration may develop, although in most patients the lymphadenitis subsides without suppuration. As with a variety of pathogens, individuals dually infected with HIV and *Haemophilus ducreyi* have more severe and extensive ulceroglandular disease.[109] A nonsexual form of *H. ducreyi* infection associated with chronic leg ulcers has been recognized in the Pacific region and in Africa.[110]

Primary genital herpes simplex infection in men and women is often associated with tender inguinal adenopathy, with evidence of focal necrosis, viral intranuclear cytopathic effects, and giant cell formation if involved lymph nodes are subjected to biopsy. Similar histologically proven, recurrent, localized and generalized herpetic lymphadenitis can occur in immunocompromised patients in the absence of overt mucocutaneous lesions.[5,111] The pseudobuboes of granuloma inguinale are produced by subcutaneous granulomatous infection rather than by suppurative lymphadenitis.

Inguinal Buboes of Other Than Sexually Transmitted Disease Origin

Inguinal or femoral buboes occur in bubonic plague because the flea bite initiating the infection is commonly on a lower extremity,[112] but involvement of other peripheral nodes can occur. The disease begins with fever, malaise, headache, and tender regional adenopathy after an incubation period of 2 to 6 days. Only rarely is a lesion (papule, pustule) at the insect bite site evident at the onset of illness. A large, matted collection of lymph nodes with surrounding edema quickly develops and may go on to suppuration and spontaneous drainage. If not treated promptly, the infection rapidly progresses to a septicemic or pneumonic phase. The diagnosis should be suspected in a febrile, acutely ill patient with a large cluster of extremely tender lymph nodes and a history of exposure to fleas, rodents, or rabbits in the western United States[113] (see Chapter 229). One must consider possible plague when hunters, backpackers, or rural workers from this region develop a compatible illness, even in the absence of definite exposures to these vectors. Tularemia may mimic the epidemiologic and clinical features of bubonic plague but is more likely to produce an ulceroglandular or pure glandular syndrome[114] (Table 95.1), with a primary lesion at the site of inoculation.[115] Cutaneous anthrax is another cause of ulceroglandular infection, but the marked local edema and eschar formation at the site of inoculation dominate the clinical picture.[26] Diagnostic procedures include blood

TABLE 95.1 Forms of Lymphadenitis

DISEASE	INFECTING ORGANISM	REGIONAL	REGIONAL WITH SUPPURATION (OR CASEATION)	INGUINAL BUBO FORMATION	ULCEROGLANDULAR	OCULOGLANDULAR	GENERAL
Bacterial							
Pyogenic	Group A or B streptococci, *Staphylococcus aureus*	+	+				
Scarlet fever	Group A streptococci	+	+				+
Diphtheria	*Corynebacterium diphtheriae*	+					
Fusospirochetal angina	*Prevotella melaninogenica*, peptostreptococci, etc.	+					
Scrofula	*Mycobacterium tuberculosis* *Mycobacterium scrofulaceum* *Mycobacterium avium-intracellulare*	+ + +	+ + +				
Miliary tuberculosis	*M. tuberculosis*	+					+
Brucellosis	*Brucella*	+					+
Leptospirosis	*Leptospira*	+					+
Syphilis	*Treponema pallidum*	+					+
Chancroid	*Haemophilus ducreyi*	+					
Plague	*Yersinia pestis*	+	+	+			
Tularemia	*Francisella tularensis*	+	+	+	+	+	
Rat-bite fever	*Streptobacillus moniliformis* *Spirillum minus*	+ +			+ +		
Anthrax	*Bacillus anthracis*	+	+		+		
Listeriosis	*Listeria monocytogenes*	+					
Melioidosis	*Burkholderia pseudomallei*	+	+	+			
Glanders	*Burkholderia mallei*	+	+	+			
Cat-scratch disease	*Bartonella henselae*	+	+	±	±	+	
Typhoid fever	*Salmonella typhi*	+					
Mycotic							
Histoplasmosis	*Histoplasma capsulatum* *H. capsulatum* var. *duboisii*	+ +					
Coccidioidomycosis	*Coccidioides immitis*	+					
Paracoccidioidomycosis	*Paracoccidioides brasiliensis*	+					
Cryptococcosis	*Cryptococcus neoformans*	+	+				
Rickettsiae							
Boutonneuse fever, etc.	*Rickettsia conorii*	+					
Scrub typhus	*Rickettsia tsutsugamushi*	+					
Rickettsialpox	*Rickettsia akari*	+					
Chlamydial							
Lymphogranuloma venereum	*Chlamydia trachomatis*	+	+	+			

Viral								
Measles	Measles virus							+
Rubella	Rubella virus							+
Infectious mononucleosis	Epstein-Barr virus							+
Cytomegalovirus mononucleosis	Cytomegalovirus							+
Dengue fever	Dengue virus							+
West Nile fever	West Nile virus							+
Lassa fever	Lassa fever virus							+
Oropharyngeal herpes infection	HSV type 1	+	±					+
Genital herpes infection	HSV type 2	+						
Primary HHV-6 infection	HHV-6	+						
Primary HHV-7 infection	HHV-7	+						
Cowpox	Cowpox virus	+	±					
Pharyngoconjunctival fever	Adenovirus (types 3 and 7)	+	+					
Epidemic keratoconjunctivitis	Adenovirus (types 8 and 19)	+						
AIDS, AIDS-related complex	Human immunodeficiency virus							+
Protozoan								
Kala-azar	*Leishmania donovani*							+
African trypanosomiasis	*Trypanosoma brucei*					+		+
Chagas disease	*Trypanosoma cruzi*						+	+
Toxoplasmosis	*Toxoplasma gondii*	+					+	+
Helminthic								
Filariasis	*Wuchereria bancrofti* *Brugia malayi*	+ +						+ +
Loiasis	*Loa loa*			+				
Onchocerciasis	*Onchocerca volvulus*			+				

+, Present; ±, rare; *AIDS,* acquired immunodeficiency syndrome; *HHV,* human herpesvirus; *HSV,* herpes simplex virus.

cultures, which yield uniformly positive results in the septicemic phase of plague, as well as cultures and stained smears of carefully obtained bubo aspirates. Appropriate treatment (see later) should be instituted immediately, before results of cultures become available, if bubonic plague is suspected. Miscellaneous causes of inguinal buboes include cat-scratch disease, group A streptococci superimposed on chronic lymphadenopathy in homosexual men,[116] and melioidosis.[117]

Generalized Lymphadenitis Associated With Systemic Infections

Widespread nonsuppurative lymphadenitis is a common feature of a variety of disseminated bloodstream infections and generally reflects the presence of the invading microorganism in the nodes. Generalized lymphadenopathy is a feature, for example, of secondary syphilis, HIV infection, infectious mononucleosis, acute toxoplasmosis, leptospirosis, and miliary TB, and, considered broadly, may occur in a variety of infections due to bacterial, rickettsial, chlamydial, spirochetal, viral, protozoal, and helminthic agents (see Table 95.1). Generalized lymphadenitis due to *Bartonella* infection has been observed in HIV-infected individuals,[99] sometimes associated with hemophagocytic lymphohistiocytosis.[118] Leishmanial lymphadenitis has been documented by fine-needle aspiration cytology in an HIV-infected patient.[119] In immunocompromised hosts, particularly after organ transplantation, Epstein-Barr virus infection may evolve into a lymphoproliferative disorder (posttransplantation lymphoproliferative disorder) that requires modulation of immunosuppressive therapy and possible rituximab administration[120,121] (see Chapter 138).

Recurrent Lymphadenitis

Periodic Fever, Aphthous Ulcers, Pharyngitis, and Adenitis Syndrome

There are several periodic fever syndromes that possess well-defined genetic defects or readily identifiable laboratory abnormalities in addition to distinctive patterns of illness. Lymph node involvement is generally not a prominent feature of these diseases. However, recurrent cervical lymphadenitis is a prominent feature of periodic fever, aphthous ulcers, pharyngitis, and adenitis syndrome (PFAPA)[122] (Marshall syndrome), a disease of unknown etiology but possibly associated with interleukin (IL)-1β receptor polymorphisms[123] or regulators of the NLRP3 (NOD-like receptor family, pyrin domain–containing 3) inflammasome, such as CARB-8.[124] This syndrome, which has its onset primarily, but not exclusively, in young children, is recognized by roughly monthly sudden bouts of high fever associated with oropharyngeal involvement (aphthous ulcers, pharyngitis) and cervical adenitis and has an incidence of roughly 2.3 per 10,000.[122] Adult onset of PFAPA shares the same clinical features as the more typical childhood-onset syndrome.[125] Febrile episodes are accompanied by leukocytosis and elevation of the erythrocyte sedimentation rate. Patients become entirely well after 4 to 5 days of fever and remain asymptomatic, with normal laboratory studies until the next bout of fever. The syndrome persists for an average of 4 to 5 years. The acute febrile symptomatic episodes can be promptly aborted by single doses of prednisone (1–2 mg/kg).[122] Colchicine, cimetidine, and IL-1 inhibitors such as anakinra may be effective second-line therapies.[126] The evidence that tonsillectomy induces permanent remission of symptoms in nearly all cases is increasingly robust, with remission rates greater than 95% commonly reported.[127] Excised tonsillar tissue from PFAPA patients show changes in nodal germinal center size, but this does not explain the efficacy of tonsillectomy in this population.[128]

Filarial Lymphangitis

Recurrent inguinal lymphadenitis, accompanied by lymphangitis, is a common problem in individuals with bancroftian filariasis. These acute inflammatory episodes (acute filarial lymphangitis) are thought to reflect the death of adult filariae with elicitation of a brisk host response but at times may represent bacterial infection that complicates adult worm-induced lymphectasia (see later).[129]

Etiologic Agents and Differential Diagnosis

It is helpful for purposes of the differential diagnosis to consider infective lymphadenitis in several categories (see Table 95.1): (1) regional lymphadenopathy, (2) regional lymphadenopathy with breakdown of nodes, (3) inguinal bubo formation, (4) ulceroglandular syndrome, (5) oculoglandular syndrome, and (6) generalized lymphadenopathy.

In distinguishing among the causes of fluctuant cervical lymphadenitis, the history and disease tempo may suggest a streptococcal (preceding tonsillitis) or staphylococcal process (recent facial or neck infection), or more indolent TB (previous exposure to TB) or cat-scratch disease (exposure to cat) as the causative disorder. In recent years an increasing fraction of reported suppurative cervical adenitis episodes is caused by *S. aureus; S. aureus* was recovered in approximately 40% to 50% of cases (two-thirds methicillin sensitive, one-third methicillin resistant), whereas group A streptococci were recovered in only approximately 15% to 20% of cases; 20% to 30% of cases were culture negative.[9,10] A subacute clinical course with little fever and a normal leukocyte count would be more consistent with cat-scratch disease or mycobacterial involvement. Sinus tract formation suggests infection due to *M. tuberculosis* or a nontuberculous mycobacterium. Gram-stained and Ziehl-Neelsen smears and culture (including cultures for mycobacteria) of material aspirated or drained from suppurating nodes provide a diagnosis in approximately two-thirds of such cases of cervical lymphadenitis. Further information may be provided by skin tests (PPD) or IFN-γ release assays,[130] or both; serologic tests (anti–streptolysin O and anti–DNase B antibody titers); histologic examination (e.g., caseation necrosis suggesting mycobacterial infection or bimorphic appearance suggesting cat-scratch disease); and molecular diagnostic tests, such as PCR (if available) of an excised node when culture of aspirated material is unrevealing. Surgical exploration affords both diagnosis and therapy of suppurative lymphadenitis. Fine-needle cytologic diagnosis is often effective for the diagnosis of *M. tuberculosis* infection, and in many centers with procedural and cytologic expertise, this procedure is initially used in the evaluation of subacute nonsuppurative lymphadenitis.[131] Simple incision and drainage are satisfactory for bacterial or *B. henselae* lymphadenitis. Complete excisional biopsy is preferred when exploring mycobacterial adenitis to reduce the risk of poor wound healing, unsightly scar formation, and relapse[88–91]; thus the presenting clinical features must guide surgical strategy.

A variety of noninfectious processes may resemble unilateral cervical lymphadenitis. Lymphoma may be suggested by the indolent course of cat-scratch disease. Kawasaki disease (acute febrile mucocutaneous lymph node syndrome), a vasculitic process of unknown etiology almost exclusively affecting infants and young children, is characterized by nonsuppurative cervical lymphadenopathy (also see Chapter 297).[132–134,135] The age of the patient, extended febrile course, conjunctival injection, polymorphic erythematous rash, and subsequent acral desquamation strongly suggest the diagnosis. In some patients unilateral lymphadenitis may precede mucocutaneous manifestations and make diagnosis more difficult by initially suggesting bacterial involvement.[136,137] A variety of additional clinical (uveitis, hydrops of the gallbladder) and laboratory findings (sterile pyuria, inflammatory synovitis, aseptic meningitis, hypoalbuminemia, thrombocytosis, elevated erythrocyte sedimentation rate, and C-reactive protein) may also be present and support the diagnosis.[132] Cases of Kawasaki disease, in both "classic" and "incomplete" forms, have been rarely reported in adults.[138]

An additional benign disorder of lymph nodes, histiocytic necrotizing lymphadenitis or Kikuchi-Fujimoto disease, was first reported in Japan 40 years ago but is now widely observed.[139,140,141,142] Clinical features consist of localized, sometimes tender cervical lymphadenopathy, often with an upper respiratory prodrome and associated in some patients with fever or rash. Most cases occur in women, commonly younger than 40 years. The involved nodes are usually rubbery or firm, discrete, and often modest in size, but occasionally they can exceed 4 cm in diameter.[139] Although 75% of patients have unilateral cervical adenopathy, bilateral disease and involvement of other nodal regions can occur. CT imaging frequently demonstrates involvement of multiple lymph nodes.[143] On occasion, patients present with generalized lymphadenopathy, and, rarely, hepatosplenomegaly occurs.[140] Less frequent symptoms include nausea, vomiting, weight loss, and night sweats, suggesting the diagnosis of lymphoma. Mild leukopenia is generally present; lymphocytosis may suggest infectious mononucleosis. The illness does not respond to antibiotics, but it usually resolves spontaneously within 1 or 2 months,[141] although recurrences are seen. Histologically, surgical biopsy specimens may be erroneously interpreted as lymphoma, but the principal findings

are those of focal or confluent nodules (composed of crescentic histiocytes, plasmacytoid monocytes, immunoblasts, and karyorrhectic debris) combined with patchy areas of coagulative necrosis. Neutrophils are few or absent. Diagnosis can be made in many cases by fine-needle aspiration cytology,[1,144] although the cytologic findings may overlap with other conditions, particularly TB, and specimens should be submitted for mycobacterial studies and cytologic examination.[145] Histologically, the lymph node appearance in systemic lupus erythematosus is similar to that in Kikuchi disease, but hematoxylin bodies are characteristic of the former. Serologic studies (antinuclear antibody and anti–double-stranded DNA antibody titers) help to distinguish between these processes. Although a viral origin is suspected based on the clinical features, serologic, ultrastructural, and molecular biologic studies have failed to identify consistently a specific agent.[139]

Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) is another benign process that produces extensive, painless lymphadenopathy in the cervical areas, primarily in adolescents and young adults with a mean age at onset of 20 years.[146] It represents a heterogenous disorder, with acquired mutations in cell activation pathways seen in some individuals.[147] It is often accompanied by fever, neutrophilic leukocytosis, and polyclonal hypergammaglobulinemia. Histologically, there is extensive proliferation of histiocyte-like cells within the sinuses of the involved lymph nodes.[146] The etiology is unknown, and the prognosis is favorable. Spontaneous remission may occur in half of all patients. Excision of lymph nodes associated with obstructive complications or administration of glucocorticoid therapy can be considered in selected patients.[147]

Kimura disease, a pruritic infiltrative eosinophilic process of soft tissue associated with hyperimmunoglobulin E, seen predominantly in men, often presents with painless regional lymphadenopathy, most commonly involving cervical lymph nodes, although generalized lymphadenopathy may develop. The soft tissue and laboratory findings (eosinophilia and elevated immunoglobulin [Ig]E) and cytologic abnormalities on fine-needle aspiration all contribute to confirm the diagnosis.[148,149]

Bronchial cleft cysts and cystic hygromas may be mistaken for cervical lymphadenitis, particularly if infected; thyroglossal duct cysts may suggest infected submental nodes. Infections of these congenital structures must be considered, particularly if a patient presents with a history of recurrent bacterial cervical adenitis. Lymphoepithelial cysts, indolent lesions of the salivary glands (usually the parotid) occurring in HIV-infected patients, may mimic preauricular lymphadenopathy or suggest Sjögren syndrome.[150] Submaxillary sialadenitis or salivary gland tumors may mimic submandibular lymphadenitis. Bimanual (intraoral and submandibular) palpation can be helpful in distinguishing between these processes.

Isolated inguinal lymphadenitis or bubo formation in the adult suggests venereal disease (syphilis, LGV, chancroid). Distinctive associated primary lesions are usually features of syphilis and chancroid but not of LGV, but in some individuals a syphilitic chancre may not be visible because of vaginal, cervical, or rectal inoculation. The inguinal adenopathy of primary syphilis consists of painless, firm, discrete, movable nodes without erythema of the overlying skin. The nodes do not suppurate, whereas spontaneous rupture of the buboes of LGV and chancroid may occur. The groove sign, reflecting lymphadenopathy proximal and distal to the inguinal ligament, is suggestive of LGV. The buboes of chancroid are characteristically painful. Axillary, cervical, and inguinal buboes may occur with plague and tularemia. In plague an inguinal location is common. The geographic locale and a history of animal exposure are important clues to the diagnosis. Inguinal and femoral nodes can be involved in cat-scratch disease, although much less frequently than axillary or cervical nodes, and this diagnosis should be considered if only limited toxicity or fever is present.

Generalized lymphadenopathy is frequently a manifestation of disseminated infection (see Table 95.1). Clues may be provided by the age of the patient and the presence of a characteristic rash (childhood exanthems, secondary syphilis), geographic factors (dengue, filariasis, localized *Leishmania* lymphadenitis[119,151] histoplasmosis), occupation and dietary history (brucellosis, toxoplasmosis); exposure to animals and their excreta (e.g., standing water [leptospirosis]), and the presence of atypical lymphocytes (infectious mononucleosis, cytomegalovirus [CMV] infection, acute toxoplasmosis, HIV). Diagnosis of toxoplasmic lymphadenitis in the immunocompetent patient is based primarily on serologic testing,[152,153] although sometimes a lymph node biopsy is performed because of initial concern for lymphoma. A negative result on the Sabin-Feldman dye test or on a comparable test for *Toxoplasma* IgG antibody (indirect immunofluorescence, passive hemagglutination, or enzyme-linked immunosorbent assay [ELISA]) practically excludes the diagnosis. Laboratory diagnosis of acute toxoplasmic lymphadenitis can be made by seroconversion from a negative to a positive result on IgG antibody testing, by demonstration of a fourfold increase in titers over 3 weeks, or by single Sabin-Feldman dye titers of 1:1024 or greater after 3 months of symptoms.[154] With toxoplasmosis the results of the ELISA are positive for IgM antibodies within the first 3 months of infection in most patients. A panel of serologic responses (dye test, IgM, IgA, IgE ELISA, and IgG avidity testing based on preferential binding to acetone versus formalin-fixed tachyzoites) helps to define recent infection and thus may avoid excisional biopsy.[152] High-avidity IgG responses suggest long-standing and likely prior incidental toxoplasmosis infection and encourage tissue biopsy for new-onset lymphadenopathy.[154] In endemic areas generalized nonsuppurative (and, rarely, suppurative) lymphadenopathy occurs in typhoid fever.[155]

Widespread suppurative infections of lymph nodes occur as a result of the microbicidal defect characteristic of neutrophils and monocytes in patients with chronic granulomatous disease. Recurrent infections (involving the skin, bones, lungs, liver, as well as lymph nodes) beginning in childhood and typically due to *S. aureus* and certain gram-negative bacilli (*Escherichia coli,* salmonellae, *Serratia marcescens*) or a variety of rare bacteria suggest the diagnosis. Focal suppurative lymphadenitis can also be seen in patients lacking early complement factors C1r or C3.

Widespread lymphadenopathy may be a feature of many noninfectious diseases, particularly infiltrative processes such as lymphoma and reticuloendothelioses. Miscellaneous noninfectious processes producing prominent peripheral lymphadenopathy include rheumatoid arthritis and adverse reactions after prolonged use of phenytoin. A rare form of regional lymphadenopathy, showing sinus histiocytosis (with the cells seen to contain metal granules or polyethylene) histologically, can be the result of wear-induced debris from an adjacent metallic prosthesis.[156,157] Similarly, enlarged regional lymph nodes (showing noncaseating granulomas containing silicone or histiocytic necrotizing lymphadenitis[158–160]) have been described draining areas in which a silicone mammary prosthesis[158,159] or a Silastic joint prosthesis[160] had been inserted previously. These lymph nodes may be ^{18}F-FDG avid by PET-CT scanning and erroneously suggest metastatic disease.[161]

Generalized Lymphadenopathy With Acquired Immunodeficiency Syndrome

HIV-infected patients may have generalized lymphadenopathy reflecting progressive primary HIV infection[61,162] (see Chapter 122). These immunosuppressed individuals are also at risk for lymph node involvement with opportunistic infection or neoplastic disease (particularly Kaposi sarcoma and a number of lymphoproliferative processes[162]). Commonly encountered opportunistic pathogens have included CMV, *M. avium-intracellulare, M. tuberculosis* (in high-incidence regions), rarely *Bartonella,* and/or fungal pathogens, such as *C. neoformans* or rarely *P. jirovecii*. Histoplasmosis may occur in endemic areas, and dual infections[60] or mixed infectious and neoplastic diseases are occasionally seen. Generalized mycobacterial lymphadenitis usually shows a few poorly formed or no granulomas and a prominent histiocytic reaction. Large clusters (globi) of acid-fast bacilli are present within the cytoplasm of histiocytes. Kaposi sarcoma in patients with AIDS may follow the pattern of generalized lymph node involvement and a fulminant course with mucosal and visceral lesions.[163] Multicentric Castleman disease and a variety of B- and T-cell lymphomas may present with lymphadenopathy in HIV-infected individuals, reflecting HIV-induced immunosuppression and resultant Epstein-Barr virus and HHV-8/Kaposi sarcoma–associated herpes virus–driven lymphocyte proliferation.[162]

Lymphadenopathy occurs in 50% to 75% of patients with primary HIV infection who present with an acute illness approximately 3 to 6 weeks after initial exposure to HIV.[164] These patients frequently experience a heterophile-negative mononucleosis-like syndrome consisting of fever, malaise, myalgias, headaches, sore throat, diarrhea,

leukopenia, thrombocytopenia, and a maculopapular rash. After the acute clinical illness subsides, lymphadenopathy may remain as persistent generalized lymphadenopathy, involving at least several extrainguinal sites, of at least 3 months' duration. This progressive lymphadenopathy early in the course of this infection may be the result of an active immune response against HIV in the affected lymph nodes.[165] The nodes are a prominent site of HIV replication and may regress somewhat in the first year or two after infection. The nodes are generally discrete and nontender; suppuration does not occur. In addition to characteristic cytologic findings,[61] immunohistochemical staining for p24 antigen expression will confirm HIV lymphadenitis in biopsy specimens of patients not thought to be at risk of HIV infection or who decline HIV testing.[166]

Presumptive Treatment of Lymphadenitis

Initial treatment of infective lymphadenitis requires some narrowing of the diagnostic possibilities (see Table 95.1). Localized pyogenic lymphadenitis generally responds well to early antibiotic treatment. When cervical lymphadenitis has clearly developed from a pharyngeal or periodontal portal, initial treatment of mild-to-moderate illness in nontoxic patients with penicillin is appropriate. Penicillin V (500 mg) administered orally every 6 hours or amoxicillin (500 mg) every 8 hours for 2 weeks in older children and adults is usually adequate. When the risk factors for cervical lymphadenitis are unclear, initial broader-spectrum coverage targeted for possible *S. aureus* infection is appropriate (see later). Macrolide therapy—azithromycin (5 mg/kg/day after a 10-mg/kg loading dose) or clindamycin (300 mg orally three times daily or 30 mg/kg/day in three divided doses for children)—is an alternative for patients allergic to penicillin, although resistance to macrolides among streptococci is known and may be striking in certain communities.[167,168] Nauseated patients with early or mild disease may receive initial parenteral therapy with daily ceftriaxone to ensure effective therapy before transitioning to oral therapy. In patients who are more acutely ill, high-dose penicillin G or broader-spectrum coverage, such as parenteral ampicillin-sulbactam or clindamycin (to cover possible β-lactamase–producing oral pathogens) administered parenterally, is indicated.

Pyogenic lymphadenitis complicating skin infections may be of staphylococcal or streptococcal etiology; gram-negative infections in competent hosts are extremely rare.[9,10] In communities with low rates of methicillin-resistant *S. aureus* (MRSA) infections, a penicillinase-resistant penicillin (e.g., dicloxacillin), a first-generation cephalosporin (e.g., cephalexin, given 0.5 g orally every 6 hours for the older child or an adult), or amoxicillin-clavulanic acid (875 mg orally twice daily) may be considered for nontoxic outpatients with mild-to-moderate infection. The current epidemic of community-acquired MRSA[9,10,169] has made empirical antibiotic management problematic. Trimethoprim-sulfamethoxazole (TMP-SMX) and doxycycline are generally effective against community-acquired MRSA isolates in vitro, but there are limited data regarding their clinical efficacy in this situation. Fluoroquinolones are no longer reliably efficacious against methicillin-susceptible *S. aureus* or MRSA in vitro,[9] and moreover, resistance can emerge readily when staphylococcal infections are treated with fluoroquinolones as monotherapy.[170] Macrolide resistance is common; clindamycin, although effective against clindamycin-susceptible MRSA strains, has variable resistance rates in different communities that may approach 50% in some settings, making linezolid, which is expensive and often unavailable, the only remaining reliable oral agent available. Thus patients will frequently require hospitalization and administration of vancomycin if they fail to respond to oral therapy. The recovery of a MRSA isolate by blood culture, node aspirate, or even nasal culture for susceptibility testing is thus helpful with regard to antibiotic therapy strategy.

In the more acutely ill patient, intravenous (IV) administration of vancomycin should be used. Failure to show improvement or progression to suppuration is an indication for imaging by ultrasonography or CT and consideration for percutaneous needle aspiration (for bacteriologic diagnosis and treatment) or surgical drainage. Many patients who present with acute suppurative lymphadenitis lack a history of focal infection; in these individuals antistaphylococcal therapy is appropriate. Surgical exploration before the evolution of frank suppuration is associated with difficult dissection and recovery of little, if any, purulent material. Hence exploration is usually reserved for clear-cut suppurative adenitis manifested by palpable fluctuance or the demonstration of necrotic nodes by cross-sectional imaging in the setting of continued fever and toxicity. In some centers needle aspiration is performed rather than open surgical drainage, with favorable clinical outcomes and avoidance of open surgery.[171] Recovery of MRSA at the time of a drainage procedure is invaluable to guide appropriate postoperative antibiotic therapy, particularly when considering a transition to oral therapy after initial improvement with parenteral therapy.

For cat-scratch disease, usually a self-limited process resolving in 2 to 4 months, treatment is principally symptomatic (see Chapter 234). If the nodes become fluctuant, aspiration is appropriate for both relief of pain and bacteriologic diagnosis. A limited number of strains of *B. henselae* from cases of cat-scratch disease that have been studied show in vitro susceptibility to a variety of antimicrobial agents, particularly to macrolides, ciprofloxacin, penicillins, and doxycycline.[172] In a retrospective review of antimicrobial treatment of several hundred patients with cat-scratch disease, clinical efficacy (58%–87%) was attributed to four drugs (in increasing order): TMP-SMX, gentamicin, ciprofloxacin, and rifampin.[173] Azithromycin has also been used to treat *B. henselae* lymphadenitis[174]; the intracellular concentration of this agent may offer a therapeutic advantage over other macrolides, but clinical trials comparing these agents are not available. In general the efficacy of antibiotic therapy in competent hosts seems limited[172]; therapy may be reasonable in patients with more severe or extranodal cat-scratch disease. Oral corticosteroids have been used in individuals with *B. henselae* lymphadenitis[175] or hepatosplenic infection refractory to antibiotic therapy. Bacillary angiomatosis and bacillary peliosis hepatitis, infections in patients with AIDS that are due to *B. henselae* or closely related Bartonellaceae spp., appear to respond to prolonged antimicrobial therapy with erythromycin, doxycycline, or rifampin.[172] Similarly, bartonellosis in solid-organ transplant recipients has responded to a variety of antibiotic regimens.[176]

If the diagnosis of bubonic plague is suspected, antibiotic treatment should be instituted promptly. Streptomycin (1 g intramuscularly [IM] every 12 hours in adults), alone or in combination with tetracycline (0.5 g orally every 6 hours in adults) or doxycycline (0.1 g orally every 12 hours in adults), is the preferred antibiotic therapy, and treatment is continued for 10 days. If streptomycin is not readily available, IV gentamicin should be considered, although clinical experience is more limited (see Chapter 229).[121,177]

LYMPHANGITIS

Lymphangitis is an inflammation of lymphatic channels, usually in the subcutaneous tissues. It occurs either as an acute process of bacterial origin or as a chronic process of mycotic, mycobacterial, or filarial etiology.

Pathologic Changes and Pathogenesis

The visible red streaking in acute lymphangitis stems from inflammation in the walls (and surrounding tissue spaces) of dilated lymphatic channels. Lymphatic obstruction often occurs on healing, sometimes resulting in persistent lymphedema. Cutaneous lymphatic sporotrichosis, a form of chronic lymphangitis, produces a combined suppurative and granulomatous response.

Clinical Findings

Acute Lymphangitis

Acute lymphangitis develops when a superficial dermal infection, commonly on an extremity, is not contained locally but spreads along lymphatic channels. Such infections are most often due to group A streptococci (and on occasion streptococci of other groups and, rarely, to *S. aureus* or *Pasteurella multocida* after a cat bite). Systemic manifestations may develop rapidly before evidence of infection becomes apparent at the site of inoculation of organisms, and they may be more prominent than might be anticipated based on local pain and erythema. Red linear streaks, a few millimeters to several centimeters in width, extend from the initial site of infection toward the regional lymph nodes, which are enlarged and tender. Peripheral edema of the involved extremity often

occurs. The time course of this type of infection can be accelerated from initial lesion to lymphangitis to complicating bacteremia in 24 to 48 hours. On occasion, there are recurrent episodes of lymphangitis, with the initial episode causing some degree of chronic lymphedema, in turn predisposing to another episode. Rarely, elephantiasis nostras verrucosa, a temperate-zone (nonfilarial) form of progressive lymphatic obstruction of a lower limb, can follow recurrent episodes of streptococcal lymphangitis. With each episode, further localized edema occurs, eventuating in grotesque enlargement of the extremity due to permanent solid edema, fibrosis of dermis and subcutaneous tissues, and verrucous pachydermia.[178] The peripheral white blood cell count is commonly increased. The etiologic agent sometimes can be identified on Gram-stained smears and cultures obtained from the initial lesion. Blood cultures also may reveal the causative organism.

Acute lymphangitis or lymphadenitis, usually involving the lower extremities, is a feature of filariasis due to *Wuchereria bancrofti* (and sometimes to *Brugia malayi*).[129,179] These mosquito-borne diseases are endemic to Africa, Southeast Asia and the Pacific, and tropical South America. The acute form of disease is characterized by recurrent episodes of headache, backache, lymphangitis, lymphadenitis, epididymitis, and orchitis. Bancroftian lymphangitis may involve the breast, with a clinical appearance suggesting carcinoma.[180] Fever is uncommon. The adult filariae reside in lymphatics and lymph nodes and discharge microfilariae into the bloodstream. With prolonged exposure in an endemic area, chronic lymphatic obstruction can develop with elephantiasis of the skin and scrotum.[180] In this setting recurrent episodes of lymphangitis (dermatolymphangioadenitis) may be the result of both the parasitic infestation and superimposed streptococcal infections (to which the chronic lymphedema predisposes).[129,181] The presence of microfilarial intracellular rickettsiae *(Wolbachia)* may help drive the ongoing host inflammatory response with resultant tissue injury.[179] Conventional serologic tests for bancroftian filariasis have limited specificity due to cross-reactivity with other filaria and even helminths. A rapid-format, immunochromatography filariasis card test is now widely used for the diagnosis of bancroftian filariasis.[182] Lymph node or lymphatic vessel biopsy may occasionally be necessary for diagnosis but is avoided if possible to minimize further lymphatic injury of the affected limb.

Chronic Granulomatous Lymphangitis

Unlike acute lymphangitis, chronic granulomatous lymphangitis is an indolent process associated with little pain or systemic evidence of infection and is most commonly due to sporotrichosis[183] (see Chapter 259), although a rather wide range of pathogens has been associated with this syndrome.[184] Minor trauma (e.g., from a barberry or rosebush thorn) inoculates *Sporothrix schenckii* (present on some plants and in gardeners' sphagnum moss) into the skin of a gardener's hand or finger. An erythematous subcutaneous nodule (often becoming fluctuant) or a chancriform ulcer subsequently develops at the site of inoculation. The lesion does not respond to local treatment or administration of common antibacterial agents. Slowly, multiple subcutaneous nodules appear and extend proximally along the course of regional lymphatics, which become thickened. Other infections producing a sporotrichoid pattern are described as nodular lymphangitis[185] and lymphocutaneous syndrome.[186]

Cutaneous infection ("swimming pool granuloma") with *Mycobacterium marinum,* an atypical mycobacterium that grows optimally at 25°C to 32°C and is found in swimming pools and fish tanks, produces a chronic nodular, verrucous, or ulcerative lesion at the site of an abrasion, usually about the knees or elbows. The lesion is usually solitary, but on occasion new lesions develop proximally, as in sporotrichosis. Multiple sporotrichoid lesions have occurred occasionally in infections due to *Nocardia brasiliensis* and in rare infections due to *Mycobacterium kansasii, Mycobacterium chelonae*, and *Nocardia asteroides.* Even rarer causes of nodular lymphangitis include leishmaniasis,[186] staphylococcal lymphangitis,[186,187] botryomycosis, and tularemia.[184,186] A rare but most troublesome process is localized granulomatous lymphangitis of the penile and scrotal skin with resulting chronic edema of the genital area. It may be associated with LGV, granuloma inguinale, Milroy disease, self-inflicted trauma, Melkersson-Rosenthal syndrome, or Crohn disease, or it may be idiopathic.[188] Penile edema can be an initial manifestation of Crohn disease or can develop as a late complication of well-established gastrointestinal disease.

Pseudolymphangitis

Exogenous liquid chemical agents that drain along the skin may produce localized linear inflammatory reactions and mimic superficial lymphangitis. When the provoking agent is directly toxic, the etiology of the rash is obvious. However, certain plant products can serve as photosensitizing agents, and the history of initial contact may not be recalled by the patient. In particular, lime rinds and juice contain several coumarin moieties that can provoke phytophotodermatitis.[189]

Etiologic Agents

In the United States acute lymphangitis is most commonly due to group A streptococci, and chronic lymphangitis is usually caused by *S. schenckii.* Other infectious agents occasionally produce lymphangitis (Table 95.2).

Differential Diagnosis

The combination of a peripheral infection or traumatic lesion and the acute onset of fever with proximal red linear streaks directed toward regional lymph nodes are diagnostic of acute lymphangitis. In the legs thrombophlebitis may produce linear areas of tender erythema, but the absence of an initiating lesion and tender regional adenopathy is helpful in distinguishing it from lymphangitis. It is important to note that, on occasion, severe lymphangitis may be complicated by the development of secondary thrombophlebitis. A history of rat bite and the subsequent development of lymphangitis suggest *Streptobacillus moniliformis* infection. Filariasis is a consideration when an appropriate geographic history is obtained. Sporotrichosis is considered when chronic ulcerative lymphangitis develops in a person working with plants, soil, or timbers. *M. marinum* is suggested as the etiologic agent when sporotrichoid lesions develop in a person who has been around brackish water environments, swimming pools, and fish tanks. In the absence of successful culture of draining purulent material, tissue biopsy for extensive

TABLE 95.2 Causes of Lymphangitis

CLINICAL FORM	ETIOLOGIC AGENT	RELATIVE FREQUENCY AS CAUSE OF LYMPHANGITIS
Acute	Group A streptococci	Common
	Staphylococcus aureus	Occasional
	Pasteurella multocida	Occasional
	Streptobacillus moniliformis (rat-bite fever)	Rare
	Wuchereria bancrofti; Brugia malayi (filariasis)	Rare (only in immigrants from endemic areas)
Chronic	*Sporothrix schenckii* (sporotrichosis)	Occasional
	Mycobacterium marinum (swimming pool granuloma)	Occasional
	Mycobacterium kansasii	Rare
	Nocardia brasiliensis	Rare
	W. bancrofti; B. malayi	Rare (only in immigrants from endemic areas)
	Nocardia asteroides	Very rare
	Mycobacterium chelonae	Very rare
	S. aureus (botryomycosis)	Very rare
	Leishmania brasiliensis or *Leishmania mexicana*	Very rare
	Francisella tularensis	Very rare

microbiologic (routine, fungal, mycobacterial), molecular (PCR), and histologic evaluation are important for confirming a specific etiology and initiating targeted therapy.

Presumptive Therapy

Penicillin therapy is the recommended initial treatment for acute lymphangitis. Prompt empirical therapy is crucial because disease progression may be quite rapid. Mild-to-moderate disease may be managed in an outpatient setting for nontoxic, reliable patients. In moderate cases an initial dose of IM ceftriaxone (1 g) may be administered, followed by high-dose oral therapy with penicillin V or amoxicillin (500 mg every 6 hours) if close monitoring is possible. Oral dicloxacillin or cephalexin (500 mg every 6 hours) is commonly given if there is concern regarding a possible staphylococcal etiology, but staphylococcal infections generally have a somewhat more indolent course, often with local suppuration at the primary site of infection with adjoining cellulitis, rather than rapidly progressive lymphangitis. If MRSA is prevalent in the community, consideration of methicillin-resistant staphylococcal infection should lead to appropriate therapy as discussed previously. More acutely ill patients, particularly those in whom bacteremia may have developed, should be hospitalized and given parenteral aqueous penicillin G (e.g., 2 million units every 4–6 hours) or ceftriaxone (2 g IV daily). If a possible staphylococcal etiology is suspected, vancomycin (1 g every 12 hours) should be initially administered to hospitalized patients.

The initial treatment of presumptive lymphocutaneous sporotrichosis is itraconazole; a saturated solution of potassium iodide is also effective (see Chapter 259).[183,190] If sporotrichoid *M. marinum* infection is suspected, the diagnosis should be confirmed by demonstration of acid-fast bacilli and isolation of the organism at 30°C on appropriate media. Localized swimming pool granulomas are often treated by limited surgical excision. Chemotherapy is reserved for more extensive and sporotrichoid forms of infection. Based on limited data, monotherapy with doxycycline or clarithromycin has been suggested; combination therapy (rifampin with ethambutol and TMP-SMX or doxycycline-clarithromycin) in more severe disease is recommended (see Chapter 252).[191,192] Chronic lymphedema, a complication of recurrent lymphangitis, has been treated primarily in the past with elevation and elastic hosiery. Newer and more successful methods to increase lymph drainage for problematic patients have recently been developed and include remedial exercises, manual massage, multilayered bandage wrapping, and intermittent pneumatic compression massage.[193] Individuals with frequent episodes of recurrent cellulitis occurring in the setting of chronic lymphedema may benefit from chronic suppressive therapy with penicillin (e.g., 250 mg orally twice daily).

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The complete reference list is available online at Expert Consult.

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J Gastrointestinal Infections and Food Poisoning

96 Syndromes of Enteric Infection

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SHORT VIEW SUMMARY

- Gastrointestinal (GI) infections are an important cause of morbidity and mortality worldwide.
- GI infections may manifest with acute vomiting, acute watery diarrhea, bloody diarrhea (dysentery), persistent diarrhea, or enteric fever. Most pathogens may cause more than one syndrome.
- GI infections in adults are usually uncomplicated. Complicated GI infections are associated with certain clinical features, epidemiologic risk factors, and host determinants.
- Although a microbiologic diagnosis is not necessary for most GI infections, diagnostic testing is important for the management of severe disease and may also have public health implications, particularly in recognizing outbreaks.
- Rehydration is the mainstay of treatment for most GI infections, and the role of antimicrobial and other therapies varies depending on the specific pathogen and clinical scenario.

Gastrointestinal (GI) infections have a range of clinical manifestations and can result from infection with viruses, bacteria, protozoa, or parasites. Symptoms of gastroenteritis relate to infection at the mucosal surface, to direct microbial invasion of the gut, and/or to the effect of microbial toxins on GI mucosal cells or on the central or enteric nervous systems. Systemic symptoms may occur in association with GI infection as a result of microbial dissemination via the bloodstream, the systemic effects of toxins produced in the GI tract, and/or the host inflammatory response to the infection. Enteric pathogens are frequently transmitted through contaminated food or water, and some pathogens with low infectious doses may spread from person to person. These pathogens may cause outbreaks of local and international significance and are among the leading causes of childhood morbidity and mortality, particularly in populations with limited access to safe water or adequate sanitation. Travel and the globalization of the food supply are important factors in the global dissemination of enteric infections.

Most GI infections due to common bacterial and viral causes are self-limited, with symptoms resolving in a normal host usually within 7 days. In such cases a specific microbiologic diagnosis is not necessary unless the disease is more severe or is part of an outbreak. An array of diagnostic and therapeutic strategies is available for GI infections, and these need to be applied in clinical scenarios in which they will yield the most benefit to the patient. The emergence of multidrug-resistant enteric pathogens, such as nontyphoidal *Salmonella, Shigella,* and *Vibrio cholerae,* and the increasing frequency and severity of nosocomial *Clostridioides difficile* (formerly *Clostridium difficile*) infection underscore the need for optimal, cost-effective approaches to the diagnosis, management, and control of enteric infections.

The term *gastroenteritis* is applied to syndromes of diarrhea or vomiting. *Diarrhea* is commonly defined as three or more loose stools in a 24-hour period[1]; it is considered *acute* when the duration is 14 days or less and *persistent* when the duration is 14 days or longer. Causative organisms differ significantly between gastroenteritis that is *community acquired* and that which is *nosocomial.* A broader spectrum of organisms may cause enteric infection in the immunocompromised host, and clinical manifestations may be more severe and more prolonged in such individuals.

OCCURRENCE AND SCOPE OF GASTROINTESTINAL INFECTIONS

Diarrhea is an important cause of morbidity and mortality worldwide, particularly in children living in areas without adequate access to safe water and sanitation. The Global Burden of Disease Study found that diarrhea was the ninth leading cause of death globally in 2015 and was responsible for 8.6% of deaths among children younger than 5 years.[2] The greatest impact of diarrheal illness is among children younger than 5 years in low- and middle-income countries, where diarrhea is the second most common cause of death beyond the neonatal period.[2,3] Death rates from diarrhea have declined over the past decade with improvements in sanitation, more widespread use of oral rehydration solution, and the introduction of rotavirus vaccination programs. Nevertheless, diarrheal disease continues to cause significant morbidity, with nearly 1.7 billion cases of childhood diarrheal disease globally every year.[4,5]

Diarrhea has a complex interplay with nutritional status among children. Undernutrition, particularly micronutrient deficiency, increases susceptibility to acute infectious diarrhea and predisposes to more prolonged episodes of diarrheal illness. Conversely, diarrheal disease may exacerbate nutritional deficiencies in several ways. Overall caloric demands are increased during acute diarrheal illness. Gut injury causes malabsorption, and anorexia and altered eating habits may also affect caloric intake. In parts of the world where body stores of vitamin A are low, children with acute or persistent diarrhea can quickly develop complications of vitamin A deficiency, including xerophthalmia.

Repeated episodes of diarrheal illness are associated with deficits in the physical and cognitive development of children. A multivariate analysis of longitudinal data from five developing countries found that the odds of stunting at 2 years increased with each diarrheal episode and with each day of diarrhea.[6] Early childhood diarrhea has also been associated with subsequent impairments in cognitive development and school performance.[7,8] Thus, the overall health impact of diarrheal disease

in children may be even greater than is suggested by incidence and mortality data alone.

OCCURRENCE AND SCOPE OF GASTROINTESTINAL INFECTIONS IN HIGH-INCOME COUNTRIES

Approximately 179 million cases of acute gastroenteritis occur in the United States each year.[9] Although gastroenteritis is most often an uncomplicated illness, outbreaks of acute gastroenteritis are, nonetheless, an important public health concern; 10,756 outbreaks of gastroenteritis due to presumed person-to-person spread or environmental contamination occurred in the United States in 2009–13.[9] Closed and semiclosed communities, including schools, residential facilities, and cruise ships, are important settings for such outbreaks. Norovirus, which is highly contagious and robust in surviving on surfaces, is the most common etiologic agent associated with outbreaks of acute gastroenteritis and the leading cause of medically attended acute gastroenteritis in the United States.[9–11] Other reported causes of outbreaks of acute gastroenteritis transmitted by person-to-person contact in the United States include *Shigella,* Shiga toxin–producing *Escherichia coli* (STEC, also known as enterohemorrhagic *E. coli*), and rotavirus. Historically, rotavirus has been the most common cause of acute gastroenteritis among infants and young children; pediatric rotavirus vaccination was broadly recommended in the United States in 2006,[12] and pediatric diarrheal hospitalizations and medical expenditures have declined sharply since then.[13,14]

Outbreaks of foodborne gastroenteritis are also common, even in high-income countries. The Centers for Disease Control and Prevention (CDC) estimates that there are 9.4 million illnesses, 59,961 hospitalizations, and 1351 deaths due to foodborne disease annually in the United States.[15] Among the most important pathogen-food combinations in terms of disease burden are *Campylobacter* (poultry), *Toxoplasma gondii* (pork), *Listeria monocytogenes* (delicatessen meats and dairy), and *Salmonella enterica* (poultry).[16] Norovirus may spread by consumption of contaminated food and water, as well as by airborne droplets of vomitus and fomite contamination.[17–20] Multinational epidemics can highlight the global significance and economic impact of foodborne GI infections. In 2011, a multinational outbreak of STEC O104:H4, transmitted via contaminated bean sprouts, was centered in northern Germany and affected nearly 4000 individuals, with 53 deaths.[21–23] The cost of this outbreak in material and human losses was estimated to be more than 2 billion US dollars.

Gastroenteritis is also a common nosocomial infection in the developed world, and the causative organisms are distinct from those of diarrhea in the community. *C. difficile* is the predominant cause of nosocomial diarrhea among adults in the United States,[24] but other bacterial pathogens, including *Klebsiella oxytoca*[25] and toxin-producing strains of *Clostridium perfringens,*[26] have also occasionally been associated with nosocomial diarrhea. Outbreaks of norovirus are common in health care settings, and illness can be more prolonged in hospitalized individuals.[27]

PATHOGENS ASSOCIATED WITH GASTROINTESTINAL INFECTIONS

GI infections can result from infection with viruses, bacteria, protozoa, or parasites (Table 96.1). Most of the agents of GI infection can cause more than one syndrome of enteric infection. Precise data regarding the incidence of enteric pathogens responsible for diarrheal illness are often limited. For example, the microbiologic etiology was unknown in 31% of outbreaks of acute gastroenteritis transmitted by person-to-person spread in the United States in 2009–13.[9] When active surveillance is performed, norovirus has been identified as the most common cause of medically attended acute GI illness in the United States.[28,29] The CDC's Foodborne Diseases Active Surveillance Network (FoodNet) monitors cases of nine laboratory-diagnosed enteric organisms reported from 10 US sites; in 2013–16, nontyphoidal *Salmonella*, *Campylobacter,* and *Shigella* were the three leading pathogens identified, with *Listeria* being associated with the highest number of deaths.[30]

The spectrum of causative pathogens in resource-limited areas is different than in high-income countries. Using a combination of culture-based and quantitative molecular diagnostic testing methods, the Global Enteric Multicenter Study (GEMS), a large case-control study of diarrhea in children in Africa and Asia, identified that most attributable cases of moderate-to-severe diarrhea were due to six pathogens: *Shigella* spp., rotavirus, adenovirus 40/41, enterotoxigenic *E. coli* (ETEC), *Cryptosporidium* spp., and *Campylobacter* spp.[31,32] In addition, in settings where there is a high burden of enteric disease in children, concomitant infection with multiple pathogens is common.[32,33] For example, Bangladeshi infants with diarrheal illness had an average 5.6 pathogens detected versus 4.3 pathogens detected in control samples.[33]

ENVIRONMENTAL RISK FACTORS FOR GASTROINTESTINAL INFECTION

Environmental factors play an important role in the epidemiology of gastroenteritis. Most important, exposure to contaminated food and/or water plays a key role in the transmission of most GI pathogens; lower-income countries that lack sanitation infrastructure and access to safe water consequently have the highest rates of diarrhea-associated morbidity and mortality.

International travel is one important risk factor for GI infection in developed countries. Traveler's diarrhea is the most common travel-related infectious illness and occurs in up to 40% of travelers to regions of Asia, Africa, and Latin America. Among US travelers, the majority of cases of traveler's diarrhea occur in individuals returning from Latin America and the Caribbean, but risk is greatest after travel to Africa.[34] A variety of organisms may cause traveler's diarrhea (see Table 96.1), although ETEC strains are the most common cause overall. *Campylobacter,* nontyphoidal *Salmonella,* and *Shigella* are the pathogens that are most commonly identified when microbiologic evaluation is performed.[34]

Consumption of contaminated food remains an important risk factor for the acquisition of enteric infection. Fruits, vegetable row crops, beef, sprouts, and seeded vegetables were the most commonly implicated foods in 120 multistate foodborne outbreaks in the United States from 2010–14.[31,35] Ill food handlers are also important sources for foodborne outbreaks, particularly due to norovirus and other pathogens easily transmitted by person-to-person contact. The US Food and Drug Administration Food Code requires no bare-hand contact with ready-to-eat foods, but a failure to maintain proper personal hygiene has been observed in as many as 76% of restaurants.[36] *Campylobacter, Salmonella,* and STEC are zoonotic infections, and consequently food prepared from infected animals may be contaminated when sold to consumers. In contrast, humans are the only natural host for *Shigella* and norovirus, so food may be contaminated during preparation or by an infected food service worker.

GI infections exhibit a seasonal pattern in both temperate and tropical climates. Enteric infections in temperate climates may occur during the winter when individuals tend to congregate together indoors. The wintertime predominance of norovirus infection is so marked that it has been called "winter-vomiting disease." Foodborne outbreaks occur more commonly during summer months.[37] In tropical areas, such as Bangladesh, there are distinct summer peaks of GI illness, and seasonal flooding may play a role in facilitating the transmission of organisms.[38]

Behavioral and environmental factors also play a role in the spread of nosocomial diarrhea. Spores of *C. difficile* often contaminate hospital environments and the hands of health care workers. These spores are resistant to alcohol-based disinfectants and can survive on environmental surfaces for as long as 6 months. Spores are resistant to gastric acid and can easily spread from one patient to another on the hands of health care workers or from contaminated surfaces in the room of an infected patient, even after they have been discharged.[24]

HOST FACTORS IN GASTROINTESTINAL INFECTION

Host factors are important in determining which individuals become ill after exposure to an enteric pathogen.

Age

The epidemiology and clinical manifestations of enteric infection vary with age, with young children and the elderly usually being susceptible

TABLE 96.1 Common Gastrointestinal Pathogens and Their Key Epidemiologic Features

ORGANISM	KEY EPIDEMIOLOGIC FEATURES
Viruses	
Adenovirus	Serotypes 40/41 among the leading causes of infantile gastroenteritis globally
Astrovirus	Outbreaks in closed populations
Norovirus	Most common cause of medically attended gastroenteritis in United States: winter vomiting illness; environmentally hardy
Rotavirus	Most common global cause of gastroenteritis in young children, particularly in settings without rotavirus vaccination
Sapovirus	Mainly affects infants and toddlers
Bacteria	
Aeromonas spp.	Widely distributed in aquatic environments; may cause diarrhea or extraintestinal infection
Bacillus cereus	Vomiting illness; rare fatal cases with hepatic necrosis; testing for toxin available
Campylobacter jejuni	Associated with poultry; common cause of traveler's diarrhea in Asia; associated with postinfectious arthritis and Guillain-Barré syndrome
Clostridioides difficile	Leading cause of mortality from gastrointestinal infection in United States
Clostridium botulinum	Vomiting illness due to preformed toxin ingestion; infant botulism due to germination of spores presents with progressive weakness
Enteroaggregative *Escherichia coli*	Persistent diarrhea in young children, associated with malnutrition
Enterohemorrhagic *E. coli* (STEC)	Associated with hemolytic-uremic syndrome in children
Enteroinvasive *E. coli*	Associated with dysentery
Enteropathogenic *E. coli*	Acute watery diarrhea
Enterotoxigenic *E. coli*	Most common cause of traveler's diarrhea
Listeria	Associated with raw dairy products; pregnancy complications with systemic illness
Nontyphoidal *Salmonella* spp.	Intestinal carriage can be prolonged
Plesiomonas shigelloides	May cause watery diarrhea, dysentery, or extraintestinal infection
Shigella spp.	Most common global cause of dysentery
Staphylococcus aureus	Vomiting due to preformed staphylococcal enterotoxin ingestion
Vibrio cholerae	Outbreaks of watery diarrhea associated with lack of sanitation and humanitarian crises
Vibrio parahaemolyticus	Associated with shellfish consumption
Yersinia enterocolitica	Zoonosis; able to grow in refrigerated food; associated with postinfectious polyarthritis
Yersinia pseudotuberculosis	Appendicitis-like syndrome
Protozoa	
Cryptosporidium hominis/parvum	Major cause of childhood diarrheal in children in low-income countries
Cyclospora cayetanensis	Opportunistic infection; associated with foodborne outbreaks
Cystoisospora belli	Tropical and subtropical areas; opportunistic infection
Entamoeba histolytica	May cause liver abscess
Giardia lamblia	Associated with drinking from contaminated streams
Microsporidium	Ubiquitous in the environment; opportunistic infection
Parasites	
Anisakis simplex	Vomiting illness after consuming raw fish

STEC, Shiga toxin–producing enterohemorrhagic *E. coli* (original, alternative definition of enterohemorrhagic *E. coli*).

to the most severe complications. Globally, children younger than 5 years suffer the greatest morbidity and mortality from GI infection. Participants in the GEMS in sub-Saharan Africa and South Asia experienced 20 episodes of moderate-to-severe diarrhea in the first 2 years of life.[39] The elevated risk for children in this age range can be attributed to the loss of maternal antibodies and the discontinuation of breastfeeding, which is highly protective against diarrheal disease.[40] High rates of antibiotic use in the first 2 years of life may also play a role in elevating this risk through alterations in the intestinal microbiome.[41] Breastfeeding protects against GI pathogens via a number of mechanisms. Human milk glycans function as soluble receptors that inhibit pathogens from adhering to their target receptors on the intestinal mucosa.[42] Breast milk also contains high levels of secretory immunoglobulin A (IgA), which plays a crucial role in mucosal immunity, and breastfeeding reduces exposure to contaminated foods and ensures adequate nutrition for infants. In large part because of its protective effects against GI infection, World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life if the mother is not infected with human immunodeficiency virus (HIV).[43]

Numerous GI pathogens have higher attack rates in young children. Rotavirus causes nearly uniform infection in the first or second year of life in unvaccinated populations. ETEC, enteropathogenic *E. coli* (EPEC), and STEC, *Shigella, Salmonella, Campylobacter jejuni,* and *Giardia lamblia* also have higher attack rates among children than adults. For *C. jejuni,* there is a distinct second peak in incidence in young adulthood that is not fully understood but may relate to food preparation habits.[44]

Older individuals are also more susceptible to complications of enteric infection.[45] In the United States the highest death rates for *Salmonella*

are among individuals age 65 years or older.[15,46,47] Advanced age is a risk factor for the development of and death from C. *difficile*–associated disease.[48] Critically ill elderly patients are 68% more likely to experience 30-day mortality from *C. difficile*–associated disease than younger individuals.[49] A dramatic progressive increase in the risk for invasive listeriosis occurs between 45 and 84 years of age.[50] Norovirus gastroenteritis is typically mild in healthy adults, but more severe outcomes can occur in the institutionalized elderly.[11] Rotavirus outbreaks may also occur among the elderly in residential facilities, with considerable morbidity.[51] These age-dependent changes in the incidence and severity of enteric disease may relate to immune senescence, alterations in the gut microbiota, or environmental and behavioral factors, including more frequent antibiotic use.

Gastric Acidity and Physical Barriers

The acidic pH of the stomach is an important barrier to enteric pathogens. Gastric acidity is found in nearly all vertebrates, and the preservation of this highly energy-consuming process across species reflects its biologic importance. Experimental studies have shown that bacteria instilled into the intact human stomach at a pH of 4.0 are killed within 15 minutes but remain viable in the achlorhydric stomach for at least 1 hour.[52] Many bacteria capable of causing GI disease, such as *Yersinia enterocolitica* and *Helicobacter pylori,* have developed an acid tolerance response that is activated under conditions of low pH to protect the organism from gastric acidity. Impaired acid secretion increases both the frequency and the severity of infection with a number of enteric pathogens.[53] Patients taking histamine type 2 (H2) blockers or proton pump inhibitors, which induce blockade of gastric acid, are more susceptible to infection with *V. cholerae,*[54] nontyphoidal *Salmonella,*[55] *C. jejuni,*[56] *Listeria,*[57] and some strains of *E. coli.*[53]

Intestinal motility is another important line of defense against invasion of the gut by microorganisms. Normal peristalsis helps to maintain the enteric microbiota and to clear pathogenic bacteria from the small intestine. A placebo-controlled trial in men with shigellosis demonstrated increased duration of fever and prolonged shedding of organisms in the stool among individuals treated with diphenoxylate hydrochloride with atropine (Lomotil).[58] Patients treated with opiates for mild *Salmonella* gastroenteritis may develop bacteremia, and case reports suggest that complications of *Campylobacter, Entamoeba histolytica,* and *C. difficile* enteritis are more common in individuals who have received antimotility agents or who have underlying gastric motility disorders.[59–61]

Intestinal Microbiome

The human intestinal tract contains a complex microbial community of approximately 100 trillion cells, which exceeds the number of human cells by a factor of 10. The intestinal microbiome plays an important role in maintaining human health, including influencing the development of innate and adaptive immunity, providing nutrients and vitamins, and maintaining epithelial integrity.[62,63] The intestinal microbiome also prevents colonization and infection by GI pathogens. Specifically, the intestinal microbiome may compete with pathogenic organisms for nutrients, for specific niches within the intestine, or for intestinal binding sites; it also may defend against pathogens by maintaining a low luminal pH or by producing compounds that are inhibitory to pathogens.

The intestinal microbiome varies by numerous factors, including age, location in the intestine, geographic region of the world, host genetic factors, nutritional status, and prior antimicrobial exposures, among others. A perturbation of the intestinal microbiota is also evident after enteric infection and its treatment. For instance, the fecal communities in patients with recurrent *C. difficile*–associated disease are markedly less diverse than those of control subjects or those with a first episode of *C. difficile*–associated disease.[64]

Immunocompromise

Immunocompromised individuals are at higher risk for acquiring GI infections and may have more severe disease if infected.[65] Immunocompromised individuals may also experience prolonged excretion of enteric pathogens, including *Salmonella, Campylobacter,* and *Shigella.* Organisms that cause symptomatic infection less commonly in healthy hosts, such as *Mycobacterium avium* complex, cytomegalovirus, microsporidia, and *Cyclospora cayetanensis,* may cause disease in immunocompromised hosts. *Strongyloides stercoralis* can cause hyperinfection and severe illness in immunocompromised patients.[66] More severe and prolonged norovirus infection occurs in individuals with congenital immunodeficiency, organ transplant, cancer chemotherapy, and infection with HIV.[67]

Genetic Determinants

The study of human genes that influence susceptibility to enteric infection is still in its infancy, but a number of notable associations have been made that shed light on the pathogenesis of intestinal infection and inflammation. For instance, the neutrophil chemotactic factor interleukin-8 (IL-8), produced by macrophages and epithelial cells, is central to the pathogenesis of several bacterial enteric infections. A common variant in the IL-8 promoter is associated with an increased risk for severe infection with *C. difficile*[68] and enteroaggregative *E. coli*[69] and an elevated risk for gastric cancer associated with *H. pylori* infection.[70] These associations suggest a particular role for an exaggerated inflammatory response in the pathogenesis of these diseases. Similarly, individuals with the O blood group are at an increased risk for severe *V. cholerae* infection,[71] as well as for diarrhea after norovirus infection.[72] Norovirus and rotavirus also recognize and bind to histo–blood group antigens, and variants in genes controlling the expression of histo–blood group antigens on gut surface epithelium are associated with risk of infection.[73,74] These associations underscore the important role of blood group glycoconjugates in binding directly to enteric pathogens or their toxins.

Nutritional Status

Underlying malnutrition is a major risk factor for diarrheal illness, particularly among children in the developing world.[75] The risk for diarrhea associated with malnutrition may be pathogen specific. In a prospective study of 289 Bangladeshi children between the ages of 2 and 5 years, the risk for diarrheal illness with ETEC, *E. histolytica,* and *Cryptosporidium* was significantly higher among malnourished children; this was not the case for multiple other enteric pathogens that were evaluated.[76] Malnourished children with diarrhea in Ghana were frequently coinfected with enteroaggregative *E. coli* and *Cryptosporidium.*[77]

MICROBIAL FACTORS IN GASTROINTESTINAL INFECTION

A number of microbial factors allow pathogenic microorganisms to colonize and cause infection of the human GI tract. In bacteria, genes required for colonization and infection are often carried on laterally transferred genetic elements, such as virulence plasmids, transposons, or bacteriophage, or on pathogenicity islands in the bacterial chromosome. Pathogenic bacteria produce and secrete a number of proteins that modulate their interaction with the host. In gram-negative bacterial pathogens, proteins must be secreted across both the inner and outer membranes; and for a subset of proteins that act within host cells, these proteins must also enter the host cell. A variety of protein secretion systems have been identified in gram-negative bacteria for these purposes, and these have been described as types I to VI. Proteins secreted by the type I, III, IV, and VI secretion systems are taken in a single step from inside the bacterial cell to the outside; in the type II and V systems, proteins are first transported across the inner membrane into the periplasmic space by the general secretion pathway shared by all bacteria and then exported across the outer membrane in a separate step by the additional protein secretion system. Type III, IV, and VI secretion systems deliver their secreted proteins from the bacterial cytoplasm across both bacterial membranes and the host cell membrane, directly into the host cytoplasm to affect host cell physiology. Among these protein secretion systems, type III protein secretion is one of the most commonly used by bacterial pathogens that cause GI infection.[78] Pathogens have a variety of strategies to survive and replicate in the GI tract, including nutrient acquisition, production of proteases that cleave the human adaptive immune protein IgA1, and the ability to be motile and to manifest chemotaxis toward intestinal epithelial surfaces. Many pathogens are capable of sensing environmental signals that indicate they are in the human GI tract and to use these signals

to trigger coordinated regulation of a set of virulence genes termed a *virulence regulon* to cause infection.[79,80]

Infection of the GI tract involves several steps in pathogenesis, including adherence/attachment to the GI mucosa, cellular invasion, production of exotoxins, changes in epithelial cell physiology, loss of brush border digestive enzymes, and/or cell death. The net result of these can be increased intestinal motility, net fluid secretion, influx of inflammatory cells, and/or intestinal hemorrhage, with the clinical manifestations of gastroenteritis.

Inoculum Size

The inoculum of microorganisms needed to produce infection can vary widely across organisms and between different hosts. The median infective dose (ID_{50}) represents the inoculum required to infect 50% of a population. The ID_{50} is influenced strongly by gastric acidity because many organisms are susceptible to killing by gastric acid. Some organisms have a very low ID_{50} because they are resistant to killing by gastric acid.[81,82] These organisms include *E. coli* and *Shigella,* the cyst forms of certain parasites, including *Cryptosporidium, G. lamblia,* and *E. histolytica,* and norovirus.[11] The ID_{50} of these pathogens may be as low as 10 to 100 organisms, cysts, or viral particles; such low infectious doses may facilitate direct person-to-person spread and influence the epidemiology of these infections.[83] Most agents that infect the GI tract have an intermediate ID_{50}, generally in the range of 10^3 to 10^8 microorganisms. Other organisms, such as *Yersinia,* have a higher ID_{50}, such as 10^{10} or more. Other factors that may influence the ID_{50} include intestinal motility, prior immunity to that pathogen, and the presence of normal intestinal microbiota. Notably, infection still occurs in a smaller subset of individuals exposed to considerably lower doses than the $ID_{50,}$ and this can result in a substantial burden of disease for enteric pathogens that are widely distributed.

Adherence/Attachment

Adherence to host cell surfaces is essential to the pathogenesis of diarrheal disease. Interaction of bacterial surface proteins with host cell glycolipid, glycoprotein, or protein receptors conveys tissue and species specificity to this binding.

Pili (or fimbriae) are one of the more common mechanisms used by bacteria to adhere to the GI mucosa.[84] These are surface appendages made up of repeated polymers of a protein called pilin. Other proteins may coat the tip of the structure and confer additional specificity of binding. Examples of fimbrial adhesins in diarrheal pathogens include the bundle-forming pilus (Bfp) of EPEC[85] and the colonization factor antigens of ETEC.[86] Some bacteria may also use nonfimbrial adhesins in the outer membrane to bind to epithelial surfaces or intercellular matrix materials, such as fibronectin. The Bfp of EPEC produces a pattern of localized adherence on GI mucosal cells; other pathogenic *E. coli* produce different patterns of adherence, such as the aggregative adherence of enteroaggregative *E. coli.*[87] This property can be mediated by multiple adherence factors.

Some bacterial pilus structures, such as the toxin-coregulated pilus (TcpA) of *V. cholerae,*[88] mediate cell-cell interactions between bacteria to create microcolonies on the GI surface, rather than binding to a specific intestinal receptor.

EPEC and STEC produce an intimate interaction with the surface of GI epithelial cells termed an *attaching-effacing lesion;* these adherent bacteria remain on the outside of the cell surface but are intimately attached through a coordinated series of steps that involves a type III protein secretion system.[89] All of the genes involved in inducing the attaching-effacing lesion in EPEC and STEC are encoded in a pathogenicity island termed the *locus of enterocyte effacement.*[90]

The VP1 protein on the surface of human norovirus strains binds to complex carbohydrates of the histo–blood group antigen system on GI epithelial cells to initiate infection of the cell.[11]

Invasion

Certain enteropathogenic bacteria invade into intestinal epithelial cells or traffic through them to the underlying submucosal space as part of the pathogenic process.[79] Epithelial invasion often occurs preferentially through specific microfold (M) cells in the intestinal mucosa, and this provides access to the submucosal space and the basolateral surface of the epithelial cells, from where bacteria can either enter the epithelial cell or be taken up by macrophages or interact with the submucosal lymphoid system. Many of the invasion strategies used by bacteria, including those used by *Salmonella, Shigella,* and *Yersinia,* involve a type III secretion system similar to that of EPEC and STEC.

For example, *Salmonella* uses a type III secretion system encoded on *Salmonella* pathogenicity island-1 to induce reorganization of host cell actin under the adherent bacterium, to ruffle the host cell membrane, and to cause engulfment and ingestion of the bacterium via macropinocytosis.[91] The organisms can then live and replicate in specialized phagosomal vacuoles that are able to prevent phagolysosomal fusion and acidification.[92]

Shigella accesses the basolateral surface of intestinal epithelial cells by uptake into and transcytosis of M cells, then uptake into epithelial cells, mediated by a type III protein secretion system encoded on a virulence plasmid.[93,94] Once within the intestinal epithelial cell, *Shigella* lyses the vacuolar membrane to escape into the cell cytoplasm. In the cytoplasm it replicates intracellularly and catalyzes polymerization of host cell actin at one pole of the bacterium, to move within cells and to spread directly from one epithelial cell to another.[95]

The host responses to attachment and intracellular invasion by enteric pathogens may lead to the release of proinflammatory cytokines, such as IL-8 and others, and these may mediate influx of inflammatory cells into the GI mucosa, as well as symptoms of disease.

Toxins

A number of pathogenic bacteria produce protein exotoxins (Table 96.2), and several of these have an A (enzymatically active)–B (binding) motif. These exotoxins bind to specific receptors on eukaryotic cells, are internalized and catalyze specific enzymatic activities within the cell, and lead to alterations in cell physiology with secretion of fluid and electrolytes (enterotoxins) or cell death (cytotoxins) or both. Other exotoxins (neurotoxins) of enteric bacterial pathogens act directly on the central or enteric nervous systems rather than the GI mucosal cells.[96]

Neurotoxins

Toxins of pathogenic bacteria in the GI tract with direct neurotoxicity are often ingested preformed. For instance, *Staphylococcus aureus* produces a potent enterotoxin that acts on the central autonomic nervous system, leading to an acute upper GI syndrome characterized primarily by vomiting.[97] A similar emetic neurotoxin is produced by *Bacillus cereus,* particularly when the organism grows in contaminated fried rice.[98] Symptoms of both of these toxin-mediated illnesses generally occur 1 to 6 hours after ingestion of the preformed toxin. *B. cereus* and *C. perfringens* can also produce enterotoxins that produce primarily a diarrheal illness with an incubation period of 8 to 16 hours.[26,99] Neurotoxins are also present in certain seafood, such as the agents of paralytic shellfish poisoning and ciguatera toxins.[100] Last, botulinum toxin, produced by *Clostridium botulinum* and other clostridial species,

TABLE 96.2 Enteric Bacterial Toxins

TOXIN TYPE	TOXIN-PRODUCING BACTERIA	TOXIN NAME, IF RELEVANT
Neurotoxin	*Staphylococcus aureus* *Bacillus cereus* *Clostridium botulinum*	Enterotoxin B Emetic toxin Botulinum toxin
Enterotoxin	*Vibrio cholerae* Enterotoxigenic *Escherichia coli* *Clostridium perfringens*	Cholera toxin Heat-labile toxin, heat-stable toxin Enterotoxin
Cytotoxin	*Shigella dysenteriae* type I Enterohemorrhagic *E. coli* *Vibrio parahaemolyticus* *Campylobacter jejuni* *Clostridioides difficile* *Bacteroides fragilis* *Clostridium perfringens*	Shiga toxin Shiga toxins 1 and 2 Thermostable direct hemolysin Cytolethal distending toxin Toxin A, toxin B *B. fragilis* toxin Alpha toxin

prevents release of acetylcholine from the presynaptic vesicle of the neuromuscular junction, leading to the clinical syndrome of botulism with paralysis.[101]

Enterotoxins

A number of pathogenic bacteria produce enterotoxins that act directly on GI mucosal cells to stimulate net fluid secretion. The prototypical example of this class is cholera toxin, which is an A_1-B_5 protein enterotoxin that is exported out of the bacterial cell by a type II protein secretion system.[102] The B pentamer binds to the enterocyte surface receptor GM1 monosialoganglioside. The A subunit is then nicked by a protease and reduced, and a portion of the A subunit enters the eukaryotic cell cytoplasm, where it catalyzes adenosine diphosphate ribosylation of an arginine residue on the Gsα subunit of adenylate cyclase, leading to increased intracellular cyclic adenosine monophosphate and net fluid secretion through the apical chloride channels of the epithelial cell.[103] The genes for the A and B subunits of cholera toxin are encoded together on a bacteriophage that inserts itself into the chromosome of pathogenic strains of *V. cholerae.*[104]

ETEC produces a heat-labile enterotoxin that is very similar to cholera toxin, both in protein sequence as well as in mechanism of action.[86] Strains of ETEC may also produce a heat-stable enterotoxin that is secreted extracellularly and binds and activates intestinal guanylate cyclase in the cell membrane of intestinal epithelial cells. Heat-stable enterotoxin acts as a homologue of the peptide guanylin, an endogenous peptide made by small intestinal villus cells that regulates normal intestinal secretion by stimulating intracellular cyclic guanosine monophosphate production.[105]

Cytotoxins

One example of a potent cytotoxin produced by enteric pathogens is the Shiga toxin family of proteins produced by *Shigella dysenteriae* type 1 and by strains of STEC; these are A_1-B_5 protein toxins.[106] The B pentamer binds toxin to a receptor on the cell surface, globotriaosylceramide (Gb3); the toxin is then internalized and undergoes retrograde transport to the endoplasmic reticulum and the nuclear membrane.[107] The A subunit is nicked by a protease, reduced, and enters the cytoplasm, where it cleaves a specific adenine residue from the 28S ribosomal RNA in the 60S ribosomal subunit, inhibiting protein synthesis and causing cell death.[108] This toxin family specifically targets endothelial cells that are rich in the Gb3 receptor, causing vascular damage, bloody diarrhea, and, in some cases, the hemolytic-uremic syndrome (HUS).[109]

A number of bacterial enteric pathogens, including *C. jejuni* and some strains of *E. coli,* produce a cytolethal distending toxin.[110] This toxin produces cell cycle arrest, leading to cytoplasmic distention and cell death.

Toxigenic strains of *C. difficile* encode two protein exotoxins—toxin A and toxin B; the genes encoding these two toxins are located near each other on a pathogenicity island.[111] Toxin A binds to a specific receptor on the brush border of the intestinal epithelium, a glycoprotein with an α-linked galactose; the intestinal receptor in humans for toxin B is less well characterized. After binding to the appropriate receptors, the toxins are internalized, where they act within the eukaryotic cell to covalently modify proteins in the Rho subfamily, a group of low-molecular-weight guanosine triphosphate–binding proteins involved in regulation of the actin cytoskeleton.[112] This is followed by disaggregation of polymerized actin, opening of tight junctions between cells, cell rounding, and subsequent cell death.

The protozoal parasite *E. histolytica* is able to lyse phagocytic cells after direct contact by release of a protozoal phospholipase A and pore-forming peptides.[113] The action of these toxins leads to direct cell death of responding phagocytic cells.

MAJOR CLINICAL SYNDROMES

GI infection may manifest as reasonably distinct clinical syndromes, including acute vomiting, acute watery diarrhea, profuse watery diarrhea, invasive or bloody diarrhea (dysentery), persistent diarrhea, and enteric fever (Table 96.3). This classification scheme is useful in considering the etiology, pathogenesis, and management of each type of illness. However, there is much overlap, and organisms that cause bloody diarrhea, for example, may also manifest as watery diarrhea.

Acute Vomiting

Acute vomiting illnesses are frequently caused by noroviruses or by bacterial food poisoning. Norovirus gastroenteritis is typically mild in healthy adults, but hospitalization and death from norovirus gastroenteritis are more common with genogroup II genotype 4 infection and in young children, the elderly, and the immunocompromised.[11,114] Bacterial food poisoning results from the ingestion of preformed toxins elaborated outside the host; these illnesses have short incubation periods (1–6 hours) and generally last less than 12 hours. Toxin-producing staphylococci can multiply at a wide range of temperatures; organisms consequently have the opportunity to reproduce in food that is left to cool slowly or that remains at room temperature after cooking. *B. cereus* can produce a food poisoning syndrome with a short incubation period (the emetic form, mediated by a staphylococcal type of neurotoxin) or a longer incubation period of 8 to 16 hours (the diarrheal form, mediated by an enterotoxin resembling *E. coli* heat-labile toxin). Microbiologic

TABLE 96.3 Clinical Syndromes Associated With Community-Acquired Gastrointestinal Infection

CLINICAL SYNDROME	SIGNS AND SYMPTOMS	PATHOGENIC MECHANISM	EXAMPLE PATHOGENS
Acute watery diarrhea[a]	Loose stools, often with mucus but not blood Occasional vomiting and anorexia Low-grade fever Malaise	Local infection in the gut	Norovirus genogroups I, II, and IV, enteric adenovirus types 40 and 41, rotavirus, enterotoxigenic *Escherichia coli,* enteropathogenic *E. coli, Cryptosporidium, Clostridium perfringens, Bacillus cereus*
Dysentery (acute bloody diarrhea)	Loose stools with gross blood and mucus Fever Abdominal cramps and, in some cases, tenesmus May be clinically toxic	Local invasion of the gut	*Shigella,* enteroinvasive *E. coli, Campylobacter jejuni, Entamoeba histolytica,* nontyphoidal *Salmonella, Yersinia enterocolitica, Aeromonas, Plesiomonas, Clostridioides difficile*
Profuse purging	Copious watery stools resembling "rice water" Low-grade fever Overt signs of dehydration	Toxin mediated	*Vibrio cholerae* O1 and O139, enterotoxigenic *E. coli*
Persistent diarrhea	Similar to acute diarrhea, but symptoms persist for at least 14 days	Local infection in the gut and/or immune compromise of host	*Giardia lamblia, Cryptosporidium hominis/parvum, Cystoisospora belli, Cyclospora cayetanensis,* enteropathogenic *E. coli,* enteroaggregative *E. coli*
Acute vomiting	Sudden onset of nausea and vomiting Little or no diarrhea	Local infection in the gut or intoxication	Norovirus, food poisoning due to *Staphylococcus aureus, Bacillus cereus*
Enteric fever	Fever Lymphadenopathy	Local invasion of the gut with systemic spread	*Salmonella enterica* serovar Typhi, *S. enterica* serovar Paratyphi A, B, or C

[a]Etiologic agents that can cause dysentery can also cause acute watery diarrhea.

methods are rarely used to confirm a specific cause of bacterial food poisoning, although it is possible to do so if necessary for an epidemiologic investigation.

Acute Watery Diarrhea

Most bacterial and nonbacterial enteric pathogens cause acute watery diarrhea, and consequently this syndrome is not characteristic of any single organism. Common causes include rotavirus in infants, particularly those who have not been vaccinated, ETEC in older children and adults, and norovirus. Norovirus is now the most common cause of acute GI illness in both children and adults in the United States.[10] Enteric adenoviruses 40 and 41 may cause acute watery diarrhea, particularly in children younger than 5 years.[115,116] *V. cholerae* infection should be considered when diarrheal purging is profuse, particularly with a characteristic rice-water appearance, or when otherwise healthy older children and adults die of watery diarrhea. The enterotoxin of *C. perfringens* causes moderately severe abdominal cramps and watery diarrhea; heat-resistant spores of *C. perfringens* can survive in inadequately cooked meat, poultry, or legumes.

In most cases of acute watery diarrhea a specific microbiologic diagnosis is not necessary. Treatment should be focused on fluid repletion, and antibiotics are not indicated except in situations in which they may shorten the duration of the illness. An example of this is epidemic *V. cholerae* infection, in which antibiotics for moderate or severe illness shorten symptoms and may play a role in optimizing case management.[117] It is also useful to distinguish cholera from other causes of acute watery diarrhea because cholera may affect large communities, necessitating initiation of a rapid response to improve the water supply and sanitation systems.[102]

Diarrhea With Fever

Invasive diarrhea, or dysentery, is suggested by the presence of blood or mucus or both in fecal matter and is most often the result of inflammation of the small bowel or colon in response to invasive bacterial infection. Fecal leukocytes are often detectable by direct microscopy. Fever usually accompanies invasive diarrhea, also resulting from the pronounced mucosal inflammatory response. Major causes of bloody diarrhea in the United States include *Shigella, C. jejuni,* nontyphoidal *Salmonella,* and STEC (often without fever). Other organisms that may cause dysentery include *Aeromonas, Plesiomonas,* noncholera vibrios, *Y. enterocolitica,* and *E. histolytica.*

HUS, characterized by acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia, may result from infection with STEC or with *S. dysenteriae* serotype 1. This syndrome has a mortality rate of 3% to 5% and is the leading cause of renal failure in childhood in the United States.[118] Disease results from the effects of Shiga toxin, absorbed systemically from the gut, on the renal endothelium. Certain antibacterial drugs increase the induction of phage-mediated Shiga toxin production and may increase the risk for development of HUS.

Persistent Diarrhea

Diarrhea lasting for more than 2 weeks is classified as persistent or chronic diarrhea. In some cases persistent diarrhea is associated with infection with enteroaggregative *E. coli* or parasitic infections, such as *Cryptosporidium hominis/parvum, Cystoisospora belli,* or *C. cayetanensis.* However, many cases of persistent diarrhea are triggered by a previous episode of acute gastroenteritis, and diarrhea is perpetuated by an inability to restore normal resorptive capacity after intestinal injury. Persistent diarrhea is associated with malnutrition and chronic enteropathy and should also raise suspicion for underlying illnesses, such as HIV infection; in HIV-infected persons, unexplained persistent diarrhea constitutes an acquired immunodeficiency syndrome–defining illness. Brainerd diarrhea is a persistent, severe, watery diarrheal illness that has been recognized in outbreaks, and that presumably occurs sporadically, but an etiologic agent has yet to be identified.[119]

Enteric Fever

Enteric fever is a febrile illness that follows systemic spread of *S. enterica* after local invasion of the gut.[120] Typhoid fever results from infection with *S. enterica* serovar Typhi, and paratyphoid fever results from infection with *S. enterica* serovar Paratyphi A, B, or C. All are characterized predominantly by persistent fever, but hepatosplenomegaly, abdominal pain, and neuropsychiatric symptoms may also occur. Perforation of the distal ileum, related to congested Peyer patches, is a potentially serious complication.

Complications of Acute Enteric Infection

Complications during an episode of acute gastroenteritis may manifest within the GI tract, such as the syndrome of pseudoappendicitis or exacerbation of underlying inflammatory bowel disease, or manifest outside the GI tract, owing to bacteremic spread of the organism or systemic action of a bacterial toxin, such as HUS (Table 96.4). Five to 10 percent of hospitalized children younger than 15 years with *Shigella* infection will experience a seizure.[121] Complications of gastroenteritis may also occur at some time after the acute illness, often the result of immunologic response to the infection. This latter category includes a postinfectious reactive arthritis seen in a small fraction of patients after infection with *Campylobacter, Shigella flexneri,* nontyphoidal *Salmonella, Yersinia,* and occasionally STEC; this syndrome occurs almost exclusively in individuals who are human leukocyte antigen–B27 positive.[122] Another example is the Guillain-Barré syndrome, seen after infection with *C. jejuni,* as a result of antibodies cross-reactive with GM1 ganglioside or with GQ1b (producing the Miller Fisher variant of Guillain-Barré syndrome).[123,124]

Asymptomatic Passage of Enteropathogens

Enteric pathogens may be excreted by individuals without symptoms of diarrhea, and there are host and microbial explanations for this phenomenon.[125] First, some enteric pathogens may be excreted asymptomatically for weeks after recovery from an acute diarrheal illness. Enteric pathogens associated with extended excretion after an episode

TABLE 96.4 Complications That May Accompany or Follow an Episode of Acute Diarrheal Illness

COMPLICATION	COMMONLY ASSOCIATED BACTERIAL AGENT(S)	COMMENT
Bacteremia	Nontyphoidal *Salmonella enterica, Campylobacter fetus, Shigella* spp.	Particular concern in HIV-infected individuals
Seizure	*Shigella* spp.	Particularly in children
Chronic diarrhea		Occurs in ≈1% of travelers with acute diarrhea Causes may include lactase deficiency, small bowel bacterial overgrowth, and malabsorption syndromes
Initial presentation or exacerbation of inflammatory bowel disease	All bacterial pathogens, including *Clostridioides difficile*	
Irritable bowel syndrome		May follow infection with many bacterial pathogens
Reactive arthritis (formerly Reiter syndrome)	*Shigella, Salmonella, Campylobacter, Yersinia,* STEC, *C. difficile*	Particularly likely after infection with invasive organisms and in patients who are HLA-B27 positive
Hemolytic-uremic syndrome	Shiga toxin–producing bacteria (*Shigella dysenteriae* type I and STEC)	Characterized by hemolytic anemia, thrombocytopenia, and renal failure
Guillain-Barré syndrome	*Campylobacter jejuni*	Cross-reactive antibodies to GM1 or GQ1b gangliosides

HIV, Human immunodeficiency virus; *HLA,* human leukocyte antigen; *STEC,* Shiga toxin–producing *E. coli.*

of acute diarrhea include nontyphoidal *Salmonella, C. jejuni,* norovirus, and, uncommonly, *Shigella.*[126] A host with preexisting immunity may also asymptomatically excrete an enteric pathogen after ingestion. Some individuals, owing to age or genotype, are inherently less susceptible to symptomatic or severe diarrheal infection with certain pathogens, as exemplified by the relationship between ABO blood group and *V. cholerae* and norovirus infection. Similarly, healthy infants frequently carry toxigenic strains of *C. difficile* without evidence of disease.[24]

DIAGNOSTIC APPROACH TO ENTERIC INFECTIONS

A thorough history and physical examination should be the first step in evaluating the patient with a GI illness. The history should focus on recent travel, specific items in the diet, animal and other epidemiologic exposures, recent and current medications, and underlying medical conditions, particularly immunosuppressive conditions. The nature of the symptoms (vomiting, type of diarrhea—watery, mucus, or blood—and the presence of fever or other systemic symptoms) should also be explored. The clinician should be aware of any relevant ongoing outbreaks in the community or in the patient's travel destinations. A directed physical examination is important and should focus on vital signs (particularly fever and orthostasis), volume status, abdominal tenderness, complications outside the GI tract, and the sensorium.

For purposes of directing the diagnostic evaluation, diarrheal illness can be divided into community-acquired/traveler's diarrhea, nosocomial diarrhea, and persistent diarrhea (Fig. 96.1). Most cases of community-acquired or traveler's diarrhea are mild and self-limited, and a specific microbiologic diagnosis usually is not necessary.[127] However, the presence of fever or blood in the stool should prompt evaluation of a fecal specimen for commonly associated pathogens, including *Salmonella, Shigella, Campylobacter,* STEC, and, in the case of appropriate epidemiologic exposure, *E. histolytica.* Testing for *C. difficile* should also be considered if there is a history of recent antibiotic use or if other testing is negative. Nosocomial diarrhea is a common complication in hospitalized patients. *C. difficile* is by far the most common infectious cause of nosocomial diarrhea, with 10% to 20% of patients becoming colonized with this organism during hospitalization.[24,128] Other causes of antibiotic-associated nosocomial diarrhea, including cytotoxic *K. oxytoca,* enterotoxin-producing *C. perfringens,* and potentially *S. aureus,* are rare and not readily diagnosed. Of importance, most cases of diarrhea in the hospitalized patient are noninfectious and are due to medications, enteral feeding, or underlying illness.[129] Persistent diarrhea, particularly in the immunocompromised host, should prompt consideration of a distinct array of organisms, including parasites (*Giardia, C. hominis/parvum, C. belli, C. cayetanensis,* microsporidia) and *M. avium* complex. In transplant patients, cytomegalovirus and GI viruses, such as rotavirus, adenovirus, and norovirus, should be considered, as well as parasites and bacteria that are typically associated with community-acquired diarrhea.

Most laboratories are able to culture for *Salmonella, Shigella,* and *Campylobacter* and to test for *C. difficile* and STEC. Consultation with the laboratory and use of special media may be required if other pathogens, such as *Yersinia* or *Vibrio,* are suspected. Culture-independent diagnostic tests for diarrheal pathogens are increasingly available but are limited by their lack of antibiotic susceptibility data.[130] The failure to obtain clinical isolates also hampers the ability of public health officials to perform surveillance activities and detect outbreaks.[30] The specimen of choice for laboratory testing is a loose stool that takes the shape of the container. Multiple stool specimens are rarely indicated for the detection of stool pathogens. In a study of 1256 adult patients who submitted more than one specimen, the enteric pathogen was detected

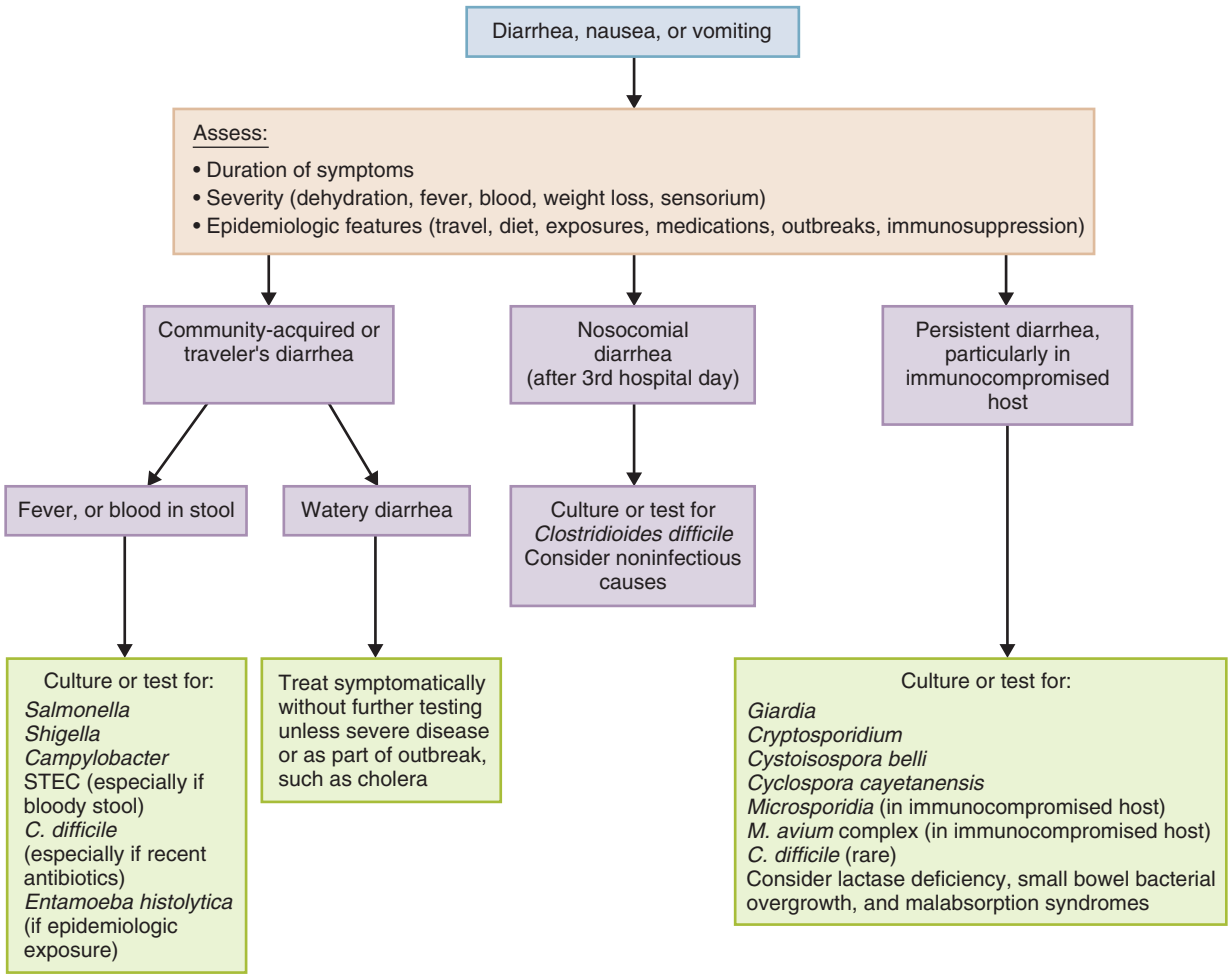

FIG. 96.1 Approach to diagnosis of infectious diarrhea. *STEC,* Shiga toxin–producing *Escherichia coli.*

in the first sample 87% to 94% of the time, with the second specimen increasing the detection rate to 98%.[131] In pediatric patients the first specimen detects 98% of the identifiable enteric pathogens.[132] Thus one sample for children and a second for selected adult patients are generally sufficient. Rectal swabs are less sensitive than stool specimens and are not recommended in adults, but in symptomatic pediatric patients, rectal swabs and stool culture are equivalent in the ability to detect fecal pathogens.[133,134] Given that most community-acquired gastroenteritis has an incubation period of less than 3 days, evaluation in a hospitalized patient after the third hospital day can be focused on nosocomial pathogens alone.[135]

THERAPY FOR ENTERIC INFECTIONS

Rehydration is the mainstay of treatment of GI infection. In the vast majority of cases dehydration can be effectively treated with oral rehydration salts (ORS).[136] A reduced-osmolarity ORS solution, containing 75 mEq/L of sodium and 75 mmol/L of glucose, is officially recommended by WHO and the United Nations Children's Fund for this purpose. A diet of easily digestible food is commonly recommended for those with diarrhea, and randomized studies support the continuation of feeding in children.[137] Adults with severe dehydration should receive intravenous fluids. Lactated Ringer solution or lactated Ringer solution with 5% dextrose is preferred, but normal saline can also be used. Disadvantages of normal saline are that it does not contain potassium to correct losses or a base to correct acidosis.

The role of antimicrobial therapy for acute bacterial gastroenteritis depends on the pathogen (Table 96.5). For some pathogens, antimicrobial therapy has been shown in randomized, controlled trials to reduce the duration of symptoms and complications, as for infection with *Shigella,*[138] *V. cholerae,*[102] and ETEC,[139] and antibiotics are recommended for moderate or severe illness. For other pathogens, such as *Campylobacter,* trials show only a modest reduction in duration of symptoms of 1.3 days, and antibiotics are recommended only for those with severe illness or risk factors for severe illness, such as the elderly, pregnant women, or the immunocompromised.[140] Antimicrobial therapy for patients with nontyphoidal *Salmonella* infection has no significant effect on the length of illness and may prolong carriage of the organism in the stool[141]; therefore antibiotics should not be given except in particularly severe illness, in patients older than 50 years (who are at risk for a mycotic aneurysm from bacteremic seeding of preexisting atherosclerotic plaque), in infants younger than 12 months (who are at risk for *Salmonella* meningitis), in individuals with cardiac or joint prostheses at risk for bacteremic seeding, in pregnant women near term, and in the immunocompromised. Last, therapy for STEC infection has no effect on the duration or severity of symptoms in most individuals, and there is a risk that certain antimicrobial agents may increase the risk for HUS through induction of Shiga toxin, particularly in children younger than 10 years. Therefore antimicrobial agents should generally be avoided for this infection, particularly for children.[142,143]

Antimicrobial resistance in enteric pathogens, particularly in the developing world, is increasingly common and complicates clinical treatment and the management of diarrheal outbreaks.[144,145] Multidrug resistance has been identified in nontyphoidal *Salmonella, Shigella* spp., and *V. cholerae.*[146–149] If antimicrobial treatment is indicated for a diarrheal disease, the choice of agent should be based on recent local susceptibility testing. Misuse and overuse of antibiotics in the treatment of diarrheal diseases has played an important role in the development of drug resistance. Use of antibiotics in farm animals has also contributed to antimicrobial resistance in human infection. Identical strains of antibiotic-resistant pathogens have been demonstrated in both infected humans and the farm animals that were the source of those infections.[150] This suggests that antibiotic use in farm animals meant for human consumption should be restricted to treatment of specific infections in those animals and not for growth promotion.

Antimotility agents may aggravate disease due to invasive organisms if not given with effective antibiotics, and they may increase absorption of Shiga toxin in STEC infection, increasing the risk for HUS. Antimotility agents should therefore be avoided in those with suspected or documented infection with STEC and should be given only along with antibiotics in patients with bloody diarrhea or fever.[151,152] Antimotility agents are considered safe for use in individuals with traveler's diarrhea.[153]

TABLE 96.5 Antimicrobial Therapy for Bacterial Gastroenteritis

USEFULNESS OF ANTIBIOTICS	EXAMPLE ORGANISMS
Antibiotics have demonstrated benefit in RCTs and are indicated for patients with moderate-to-severe disease.	*Shigella* spp., enteroinvasive *Escherichia coli,* enterotoxigenic *E. coli* *Vibrio cholerae, Aeromonas, Plesiomonas*
Antibiotics have demonstrated only modest benefit in RCTs and are indicated only for patients with severe or prolonged illness, or in abnormal hosts.	*Campylobacter jejuni*
Antibiotics have demonstrated no clear benefit in RCTs and may prolong excretion of organisms. Antibiotics should be used in selected circumstances (see text).	Nontyphoidal *Salmonella* spp.
Antibiotics have demonstrated no clear benefit in RCTs and in some studies, particularly in children, may increase risk for complications such as hemolytic-uremic syndrome.	Shiga toxin–producing *E. coli*

RCTs, Randomized controlled trials.

PREVENTION AND CONTROL OF ENTERIC INFECTIONS

Acute diarrheal diseases can be prevented with a variety of measures focused on limiting the spread of organisms within the community and from person to person. Because diarrheal diseases spread by a fecal-oral route, hand washing is considered a key barrier to the transmission of enteric pathogens. A systematic review and meta-analysis of studies from the developing world and from US and Australian child care settings estimated that hand washing with soap reduces the risk for diarrheal diseases by 42% to 47%.[154] Globally, more than 1 billion people lack access to improved drinking water supplies. Strategies to improve the microbial quality of drinking water can be applied at the source or in the household. Water-source strategies include protected wells, bore holes, or public tap stands; household strategies include improved water storage or approaches for treating water, such as chlorination, solar disinfection, filtration, or combined flocculation and disinfection. Although randomized studies are few, interventions to improve the microbial quality of drinking water are likely effective in preventing diarrhea.[155]

Many community-acquired bacterial GI infections are foodborne. Reduction of such illnesses depends on monitoring of food sources for bacterial contamination and removal from the market when such contamination is detected. Food should be appropriately refrigerated to prevent bacterial multiplication and cooked to recommended temperatures to ensure killing of any existing bacteria. Food preparers should wash hands after handling any uncooked food products, after cleaning surfaces where food is prepared, and after defecation. Ill food preparers should be kept out of work until symptoms of a diarrheal illness have resolved and, in some cases, stool cultures for the pathogen have become negative.

There are three GI infections for which vaccines have shown efficacy and are approved for use. A pentavalent rotavirus vaccine was recommended for routine use in infants in the United States in 2006, with three doses given at 2, 4, and 6 months, respectively.[12] A monovalent rotavirus vaccine was recommended as an alternative in 2008, with two doses given at 2 and 4 months. As described earlier, this vaccine has had a substantial impact on the morbidity associated with rotavirus infection in children in the United States.[13]

For cholera, there are two killed whole-cell oral cholera vaccines that are internationally licensed and prequalified by WHO. One is a monovalent *V. cholerae* O1 vaccine, supplemented with the B subunit of cholera toxin (manufactured as Dukoral [Valneva; Solna, Sweden]), and the second is a bivalent *V. cholerae* O1 and O139 vaccine without

supplemental B subunit (manufactured as either Shanchol or Euvichol [SBL Vaccines; Shantha Biotec, Hyderabad, India]). The creation of a WHO global cholera vaccine stockpile in 2013 derived from the lower-cost bivalent vaccine has led to increasing use of killed oral cholera vaccines globally, whereas the monovalent vaccine has been used primarily in travelers from high-income countries. A recent meta-analysis demonstrated that these vaccines have an effectiveness of 75% over 2 years when administered in a two-dose schedule but provide substantially less protection in children younger than 5 years.[20,102] In addition to these two killed whole-cell vaccines, a live-attenuated monovalent oral cholera vaccine, CVD 103-HgR (manufactured as Vaxchora [PaxVax; Redwood City, CA]), is licensed in the United States and currently recommended for adult travelers to cholera endemic areas.[156]

There are two current approaches to typhoid vaccination: an oral, live-attenuated Ty21a vaccine, which is given in three doses and produces approximately 50% protective efficacy over the subsequent 3 years, and a parenteral Vi (virulence) polysaccharide vaccine, which is given in one dose and produces approximately 60% protective efficacy over the subsequent 2 years.[157,158]

A number of vaccines for GI infections are being developed or may be available in the near future. A norovirus vaccine consisting of virus-like particles given intranasally has shown evidence of protective efficacy after subsequent challenge with wild-type norovirus.[159] An *S. enterica* serovar Typhi vaccine, in which the Vi polysaccharide is conjugated to a protein carrier, improves efficacy over the polysaccharide vaccine alone but is not yet manufactured or available for clinical use.[17]

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97 Esophagitis

Paul S. Graman

SHORT VIEW SUMMARY

Definition
- Inflammation of the esophagus, of noninfectious or infectious etiology

Epidemiology
- Gastroesophageal reflux disease is the most common cause. Esophageal infections occur predominantly in patients with impaired immunity, particularly those with acquired immunodeficiency syndrome or receiving cancer chemotherapy. Immunocompetent persons are occasionally affected.

Microbiology
- *Candida* spp., herpes simplex virus (HSV), and cytomegalovirus (CMV) are most common.

Diagnosis
- Endoscopy and biopsy for immunohistopathology and cultures; polymerase chain reaction for HSV

Therapy (see Table 97.2)
- *Candida:* fluconazole, itraconazole, amphotericin lipid formulations, voriconazole, echinocandins
- HSV: acyclovir or valacyclovir, famciclovir; foscarnet for acyclovir-resistant HSV
- CMV: ganciclovir, valganciclovir, or foscarnet
- Aphthous ulceration (in acquired immunodeficiency syndrome): prednisone, thalidomide

Prevention
- Recipients of allogeneic hematologic stem cell transplants who are neutropenic commonly receive antiviral and antifungal prophylaxis.

Esophagitis, or inflammation of the esophagus, is most often caused by noninfectious conditions, of which gastroesophageal reflux disease is the most common. Eosinophilic esophagitis, in which eosinophils infiltrate the mucosa, is increasingly recognized and associated with food allergy. Esophageal infection occurs predominantly in patients in whom immunity is impaired as a result of cancer chemotherapy, transplantation, or human immunodeficiency virus (HIV) infection and occasionally in persons who are otherwise healthy. *Candida albicans,* cytomegalovirus (CMV), and herpes simplex virus (HSV) are the leading etiologic agents of esophageal infection. Among patients with HIV infection, aphthous ulceration of the esophagus is a well-recognized entity. Acute HIV infection may also be a direct cause of esophageal ulceration.[1] Various other fungal, viral, mycobacterial, and parasitic agents have been shown to cause esophagitis on rare occasion. Pill-induced esophagitis resulting from local mucosal injury has been attributed to almost 100 different drugs, particularly if they are ingested without water or in the supine position; antibiotics and antiviral agents are implicated in 50% of such cases.[2,3] Infectious and noninfectious causes of esophagitis are listed in Table 97.1. Multiple concomitant causes of esophagitis are common in patients who are significantly immunosuppressed or critically ill.[4]

CLINICAL MANIFESTATIONS

Most patients with esophagitis present with odynophagia (pain on swallowing) or dysphagia, described as difficulty swallowing or a sense of obstruction that is substernal, epigastric, or in the throat. Liquids are often better tolerated than solids such as meats, which may worsen both odynophagia and dysphagia. Pain may be exacerbated by the ingestion of acidic liquids and by eructation. Ulcerative esophagitis is characterized primarily by odynophagia, which can be severe, at times to the point of limiting oral intake and resulting in weight loss and dehydration. Spontaneous substernal pain or burning sensation may also occur intermittently, unrelated to swallowing. Gastrointestinal (GI) bleeding is rarely the initial manifestation of esophagitis, but it does occur.[5] Among patients evaluated for nausea and vomiting or abdominal pain, endoscopically proven esophagitis may be present in the absence of specific esophageal symptoms. Weight loss and anemia are observed presenting signs of *Candida* esophagitis in older patients.[6] In one review, odynophagia or dysphagia was absent in 21% to 41% of patients with documented esophagitis caused by *Candida* spp., HSV, CMV, or, in rare cases, *Mycobacterium tuberculosis.*[7] Fever accompanied esophagitis in 20% of patients with CMV or mycobacterial infection but was less common among those with *Candida* or HSV infection. Nausea and vomiting were most common (42%) in patients with CMV esophagitis, which possibly reflects the fact that CMV infection is seldom confined to the esophagus. Oral lesions frequently provide clues to the diagnosis of esophagitis, particularly in patients with acquired immunodeficiency syndrome (AIDS). Oral thrush is seen in most patients with esophageal candidiasis and AIDS.[8] The finding of oropharyngeal candidiasis in a patient with esophageal symptoms and AIDS has a positive predictive value of 70% or greater for esophageal involvement.[4,9–11,12] Similarly, oropharyngeal vesicles or ulcerations may suggest, but do not prove, concomitant esophageal HSV or aphthous ulceration in a symptomatic patient.

SPECIFIC ETIOLOGIC AGENTS

Candida Esophagitis

C. albicans is the predominant pathogen of esophagitis. Non-*albicans* species of *Candida,* including *Candida tropicalis, Candida parapsilosis, Candida krusei,* and *Candida glabrata,* are implicated less often but may play a greater role as a result of selective pressure among patients who have received antifungal agents.[13,14] Small numbers of *C. albicans* are part of the normal oral microbiota; esophageal colonization may be present in up to 20% of the population,[15] particularly in patients treated with histamine type 2 blockers.[16] Colonization is the initial process in which superficial adherence and proliferation of organisms remain confined to the superficial mucosa, without penetration or inflammation of the epithelium. Colonization progresses to infection if systemic and local defenses are inadequate for preventing invasion into deeper epithelial layers; pseudohyphae are present at the advancing margin of tissue involvement. On endoscopic examination the esophagus appears hyperemic with discrete, yellow-white mucosal plaques that are firmly

TABLE 97.1 Etiology of Esophagitis

COMMON	RARE
Infectious	
Candidiasis	*Mycobacterium tuberculosis*[80,81]
Cytomegalovirus	*Mycobacterium avium complex*[9,76]
Herpes simplex virus	*Cryptococcus neoformans*[4,82]
HIV infection, acute	*Histoplasma capsulatum*[77] *Actinomyces*[78,79] *Saccharomyces cerevisiae* *Cryptosporidium* *Pneumocystis jirovecii*[74] Varicella-zoster virus[83,84] Epstein-Barr virus[75] Human papillomavirus
Noninfectious	
Gastroesophageal reflux	Ingestion of corrosives (e.g., lye)
Mucositis resulting from cancer chemotherapy Mucositis resulting from radiation therapy	Local mucositis from tablets or capsules[2,3] (e.g., doxycycline, tetracycline, clindamycin, penicillin, rifampin, zidovudine, dideoxycytidine [ddC, zalcitabine], nelfinavir)
Aphthous ulcers	
Eosinophilic esophagitis caused by food allergy	
Nonsteroidal antiinflammatory drugs	
Bone resorption inhibitors (e.g., alendronate)	

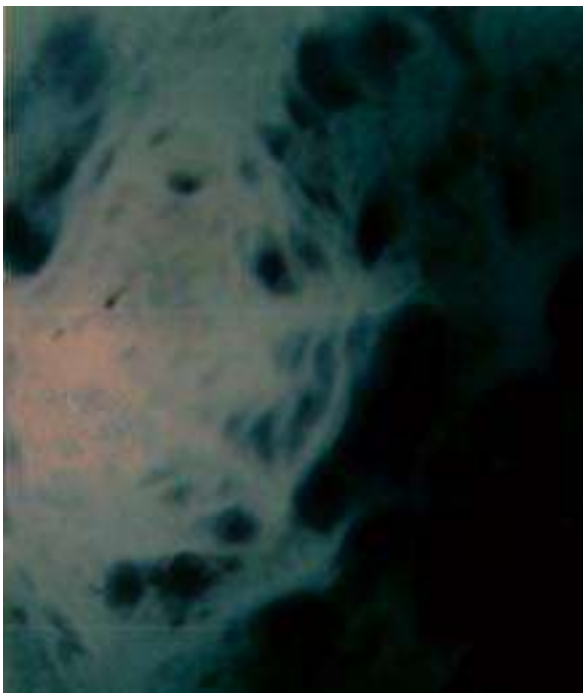

FIG. 97.1 Endoscopic appearance of esophageal candidiasis, with typical white plaques and nodules, in a patient with multiple myeloma. *(Courtesy Dr. Arthur DeCross, Rochester, NY.)*

adherent and, when removed, reveal an underlying rough and friable surface (Fig. 97.1). Lesions are most frequently located in the distal third of the esophagus. Disease may progress to involve large confluent plaques, ulceration, luminal narrowing, strictures, and necrosis.[2,17] Perforation of the esophagus is a rare complication of necrotizing esophagitis that may necessitate surgical intervention.[18]

Systemic host factors predisposing to esophageal candidiasis include acute[19] and advanced HIV infection, diabetes mellitus, leukemia and lymphoma, broad-spectrum antimicrobial therapy, antineoplastic therapy, corticosteroid therapy, and bone marrow or solid-organ transplantation. In addition to immune dysfunction, contributing local factors are those that impair esophageal motility (e.g., systemic sclerosis, achalasia, esophageal webs or rings, obstructing esophageal cancer) and conditions that result in mucosal injury (e.g., reflux, HSV esophagitis). Esophageal symptoms occur in up to half of all patients with AIDS,[9,20] and *Candida* accounts for 50% or greater of these cases.[4,12] Porro and colleagues[11] documented esophageal candidiasis in 48% of patients with AIDS who were admitted to a hospital, although 40% of those infected reported no esophageal symptoms. Transplant recipients, many of whom receive routine antifungal prophylaxis, appear less susceptible to *Candida* esophagitis, which developed in 5 (2.2%) of 224 renal transplant recipients,[21] 12 (2.2%) of 547 solid-organ transplant recipients,[22] and none of 304 cardiac transplant recipients.[23] Among symptomatic bone marrow transplant recipients, esophageal infection was diagnosed in 21 of 46 endoscopic examinations, but *Candida* accounted for only 5 of the 21 infections, the remainder being caused by CMV, HSV, or both.[24]

Diagnosis

Accurate diagnosis of esophageal candidiasis is established by endoscopy with directed brushings and biopsies. The characteristic gross appearance of candidiasis is suggestive but occasionally misleading, inasmuch as white exudative lesions may also be visualized with HSV or CMV infection or pill esophagitis. Brushings of exudative lesions and ulcer craters are obtained with a sheathed cytology brush, smeared onto slides, and submitted for calcofluor white, silver, or Gram stain. Biopsies of lesions and the edges of ulcers are submitted for histopathologic examination and for viral culture to identify CMV and HSV. Masses of yeast and pseudohyphae seen in tissue or brushings are diagnostic of *Candida* infection. Fungal cultures are not generally helpful except to identify the pathogen in cases of fungal esophagitis that are refractory to treatment. Blind brushing of the esophagus via a nasogastric tube has been advocated as an alternative to endoscopy in patients with HIV infection and suspected esophagitis; in one study, this technique was 96% sensitive and 87% specific for the diagnosis of candidiasis.[10]

Radiology

Radiologic contrast studies are of limited diagnostic value and are seldom performed in patients with esophagitis. Although focal or confluent plaques or a diffuse, "shaggy" appearance is characteristic of candidiasis on esophagography (Fig. 97.2), the examination result may be normal in some patients, and visualized abnormalities such as plaques, ulcerations, fistulas, or masses are often nonspecific; concurrent infections are likely to be missed.[5,25] Radiographic studies may be useful if endoscopy is unavailable. Computed tomographic scanning may demonstrate thickening of the esophageal wall in patients with esophagitis, but this finding is neither sensitive nor specific for infection.[26]

Cytomegalovirus Esophagitis

CMV esophagitis occurs most often in patients with AIDS or severe immunosuppression. CMV was an esophageal pathogen or copathogen in 33 (30%) of 110 patients with HIV infection and esophageal symptoms[4] and in 7 (33%) of 21 symptomatic recipients of bone marrow transplants.[24] Rare cases in immunocompetent hosts have been reported.[27] Symptoms are indistinguishable from those associated with *Candida* or HSV esophagitis. The endoscopic appearance of CMV esophagitis is typified by large (>10 cm^2), shallow, "punched-out" ulcers, solitary or multiple in number, located in the middle to distal part of the esophagus.[28] The ulcer margins are distinct, and the intervening mucosa appears relatively normal. Isolation of CMV in culture is not diagnostic because virus harbored in blood or saliva may contaminate esophageal specimens. Conversely, culture is not positive in all actual cases. Histopathologic examination is the most reliable diagnostic method when mucosal and submucosal biopsy samples are obtained from the ulcer edge and ulcer

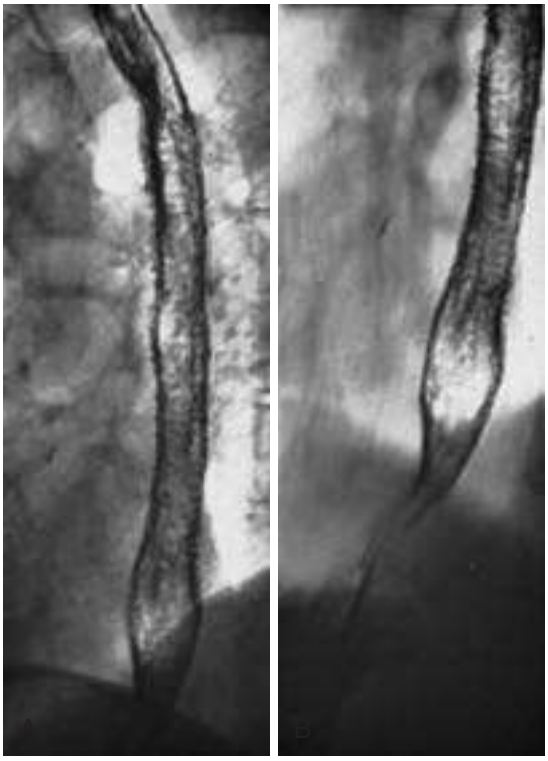

FIG. 97.2 (A and B) Barium contrast esophagograms of *Candida* esophagitis, revealing marked irregularity of the esophagus as a result of multiple plaques in a patient with acquired immunodeficiency syndrome and severe odynophagia. *(From Polis M. Esophagitis. In: Mandell GL, Bennett JE, Dolin R, eds.* Principles and Practice of Infectious Diseases. *4th ed. New York: Churchill Livingstone; 1995:962–965.)*

base; routine hematoxylin and eosin staining demonstrates enlarged endothelial cells or fibroblasts containing large, dense intranuclear inclusions.[28] Multiple biopsies increase the yield for diagnosis of CMV.[29] Immunohistochemical and direct fluorescent staining techniques, highly specific for CMV and HSV, are also useful in establishing these diagnoses. Coinfections of the esophagus are common in patients with CMV; concomitant candidiasis (73%) and HSV (12%) have been reported among these patients.[4]

Herpes Simplex Virus Esophagitis

HSV esophagitis is usually identified in patients with AIDS or other significant immunosuppressive conditions, although cases in healthy adults also occur. Of 23 patients with HSV esophagitis reported by McBane and Gross,[30] 7 (30%) had hematologic malignancies, 4 (17%) had received chemotherapy or irradiation for solid tumors, 8 (35%) had been treated with immunosuppressive agents for nonmalignant conditions, and 1 was otherwise healthy. HSV esophagitis accounts for 6% to 16% of HIV-infected patients with esophageal symptoms.[20] In a series of 21 bone marrow transplant recipients with esophagitis, HSV was identified in 48%.[24] Fifty-six cases of HSV esophagitis have been described in immunocompetent hosts, predominantly men, in whom the infection is typically self-limited.[31–33] Odynophagia, chest pain, fever, and dysphagia are the most common initial symptoms; clinically significant GI bleeding occurs in 5% to 10%.[33,34] Esophageal rupture is a rare complication. Oral, labial, or cutaneous HSV is clinically evident in only 19% to 38% of cases.[31,34]

Diagnosis

Typical lesions of HSV esophagitis appear endoscopically as multiple, small, superficial ulcers in the distal third of the esophagus; larger confluent ulcers, pseudomembranes, or diffusely denuded epithelium may be seen as the infection progresses (Fig. 97.3). "Volcano ulcers" may have raised margins around the central crater. Vesicles are rarely



FIG. 97.3 Herpes simplex esophagitis, characterized by numerous small ulcerations, in an immunocompetent adult with fever and odynophagia. *(Courtesy Dr. Charles Michalko, Rochester, NY.)*

visualized. Double-contrast esophagography demonstrates ulcerative irregularities that are not specific or diagnostic. Viral culture of brushing or biopsy samples is positive for type 1 HSV (HSV-1) in most, but not all, cases; HSV-2 has rarely been implicated.[34,35] Quantitative polymerase chain reaction (PCR) for HSV-1 in biopsy specimens is highly sensitive (95%) and reasonably specific (73%) compared with histopathology and immunohistochemistry and may aid in diagnosis.[36] Histologic examination may reveal characteristic ballooning degeneration, multinucleated giant cells, and prominent intranuclear Cowdry type-A inclusion bodies in 55% to 70% of biopsy specimens. Superficial candidal invasion of HSV ulcers is often seen on biopsy. Several cases of HSV esophagitis have been reported in immunocompetent individuals with preexisting eosinophilic esophagitis.[37]

Aphthous (Idiopathic) Ulceration of the Esophagus

Aphthous, or idiopathic, ulceration, an important variant of esophagitis in patients with advanced HIV infection, accounts for approximately 5% of patients with AIDS who have esophagitis.[4,9] No specific cause is evident in 40% of patients with AIDS and esophageal ulceration,[38] and painful ulcers are often present in the oropharynx as well.[39] Esophageal ulceration has also been described in patients with acute HIV infection who present initially with odynophagia, and some authorities believe that detection of HIV virions by electron microscopy in the margins of these ulcers may indicate a direct pathogenic role for HIV itself.[1] Others, having detected HIV nucleic acid by in situ hybridization or PCR in the esophageal mucosa of up to 80% of patients with AIDS and idiopathic ulceration or with specific etiologic diagnoses (*Candida*, CMV, or HSV esophagitis), have concluded that HIV is not a primary pathogen in ulcerative disease.[12,40] Unrecognized other infectious agents may contribute to the pathogenesis of these idiopathic ulcerations.

MANAGEMENT OF ESOPHAGEAL INFECTION

General Considerations in Therapy

Specific therapies for infectious causes of esophagitis are listed in Table 97.2. The approach to diagnosis and therapy in a particular patient often depends on the presence of underlying disease and the severity of immunosuppression (Table 97.3).

Patients receiving cancer chemotherapy may experience severe mucositis with odynophagia that is clinically indistinguishable from fungal or viral esophagitis. Oropharyngeal candidiasis is highly predictive of esophageal candidiasis in patients with cancer, particularly in patients with mucositis after chemotherapy.[41] Diagnostic endoscopy should be pursued whenever possible, but in practice the procedure is frequently deferred because of bleeding, severe pain, mucosal friability, or critical illness; empirical therapy for *Candida* and HSV infection is appropriate in the interim period, particularly if oral thrush or mucocutaneous HSV lesions are clinically evident or if cultures are positive. Patients with fever and neutropenia (<100 neutrophils/mm^3) are usually treated empirically with systemic antifungal agents (amphotericin B, lipid formulations of amphotericin, voriconazole, caspofungin, or other echinocandins) in doses sufficient to treat either esophageal or disseminated candidiasis. Intravenous (IV) acyclovir is often administered in this acute setting if HSV stomatitis or labialis is present or if esophageal symptoms are severe. Recipients of allogeneic bone marrow transplants who are neutropenic commonly receive antiviral prophylaxis until engraftment occurs. Esophageal infections in recipients of bone marrow transplants usually begin more than 40 days after transplantation, and neutrophil counts are usually within the normal range at that time; CMV and HSV infections are at least as common as *Candida* infections in this group of patients, and treatment should be guided by the results of endoscopic diagnosis.[24] Similarly, treatment of esophagitis in recipients of solid-organ transplants or in immunocompetent hosts should be guided by endoscopic appearance, culture data, and histopathologic findings. Tacrolimus and cyclosporine levels may be elevated and should be monitored in transplant recipients who are receiving concomitant fluconazole, voriconazole, or itraconazole.

TABLE 97.2 Treatment of Esophagitis

CAUSE	USUAL TREATMENT (ADULT DOSE)	ALTERNATIVE DRUGS
Candida	Fluconazole, 200–400 mg/day PO for 14–21 days; for patients unable to tolerate PO therapy, IV fluconazole, 400 mg/day or an echinocandin is recommended; maintenance suppressive therapy may be necessary in AIDS (fluconazole, 100–200 mg 3 times weekly PO)[46]	Itraconazole oral suspension, 200 mg/day PO Voriconazole,[64] 200 mg bid PO[a] Amphotericin B, 0.3–0.7 mg/kg/day IV for 7 days Caspofungin, 50 mg/day IV after 70–mg loading dose[a] Micafungin, 150 mg/day IV Anidulafungin, 200 mg/day IV[46]
Herpes simplex virus	Acyclovir, 5 mg/kg IV q8h for 7–14 days or 400 mg 5 times daily PO for 14–21 days *or* valacyclovir, 1 g PO tid for 14–21 days[b]; maintenance suppressive therapy may be necessary in AIDS	Famciclovir, 500 mg bid PO for 14–21 days (not for acyclovir-resistant infection) Foscarnet, 90 mg/kg q12h IV for 7–14 days (used for acyclovir-resistant infection)
Cytomegalovirus	Ganciclovir, 5 mg/kg IV q12h for 14–21 days; maintenance suppressive therapy usually is necessary in AIDS (ganciclovir, 5 mg/kg/day IV 7 days/wk or 6 mg/kg/day IV 5 days/wk)	Foscarnet, 90 mg/kg q12h IV for 14–21 days; suppression with foscarnet, 90–120 mg/kg/day IV Valganciclovir, 900 mg bid PO for treatment, and 900 mg qd for maintenance/suppression[b]
Aphthous ulceration (in AIDS)	Prednisone,[57] 40 mg/day PO for 14 days, then taper	Thalidomide,[58,85] 200 mg/day PO[b]

[a]Amphotericin, echinocandins, and voriconazole are indicated for severe or refractory esophageal candidiasis.
[b]Not approved by the US Food and Drug Administration for this indication.
AIDS, Acquired immunodeficiency syndrome; *bid,* twice daily; *IV,* intravenous.

Esophagitis in Patients With Acquired Immunodeficiency Syndrome

Before the introduction of antiretroviral therapy (ART), esophageal symptoms occurred in 40% to 50% of patients with AIDS at some point in the course of the disease and often had a significant effect on nutritional status and overall morbidity.[5,9,20] The incidence of esophagitis has declined dramatically since the advent of ART; however, cases of opportunistic infection of the esophagus continue to occur in patients in whom antiretroviral therapy fails because of drug resistance or noncompliance.[42–44,45] The frequencies of various causes are shown in Table 97.3, and treatment regimens are listed in Table 97.2. *Candida* esophagitis is the most common type; it is treated empirically with oral fluconazole[46] in symptomatic patients, particularly if oropharyngeal candidiasis is also observed. The presence of oral thrush is predictive of esophageal involvement in greater than 70% of such cases.[4,9–11,12,47] Itraconazole solution is comparable to fluconazole,[48] but itraconazole capsules are erratically absorbed and are less effective.[49] For patients who cannot tolerate oral therapy, IV fluconazole or an echinocandin is recommended.[46] Complete symptomatic response can be expected in greater than 80% of patients treated empirically, usually within 1 week. If the patient has no response to empirical azole therapy within 14 to 21 days, endoscopy should be performed to establish a diagnosis.[46,50] Among patients in whom empirical azole therapy for esophageal symptoms has failed, endoscopy demonstrates ulceration of the esophagus in 62% to 77%, attributable most often to CMV infection (32%–40%), aphthous disease (27%–32%), or HSV infection (5%–8%).[51,52] Early endoscopy is appropriate in patients with severe symptoms or GI bleeding.

A viral cause is identified in approximately one-third of cases, often in association with candidiasis. Empirical antiviral therapy for CMV or HSV is discouraged. Patients with CMV esophagitis confirmed by culture or histopathologic examination are treated with ganciclovir, 5 mg/kg IV every 12 hours. Although no trials have been conducted to test the efficacy of valganciclovir in CMV esophagitis, Centers for Disease Control and Prevention, National Institutes of Health, and Infectious Diseases Society of America guidelines state that patients may be switched to valganciclovir 900 mg orally every 12 hours once they can tolerate oral therapy.[53] Cases refractory to ganciclovir may

TABLE 97.3 Causes of Esophagitis in Symptomatic Patients With Underlying Disease

CAUSE	AIDS[4,9,12] (*N* = 183)	BONE MARROW TRANSPLANTATION[24] (*N* = 39)	SOLID-ORGAN TRANSPLANTATION[23,86] (*N* = 88)
Candida alone	38%	10%	3%
Candida + other	22%	3%	—
Cytomegalovirus	21%	26%	6%
Herpes simplex virus	11%	26%	7%
Aphthous ulceration	4%	—	—
Kaposi sarcoma	7%	—	—

[a]Patients on whom upper endoscopy was performed to evaluate odynophagia, dysphagia, epigastric pain, nausea and vomiting, or gastrointestinal bleeding.
AIDS, Acquired immunodeficiency syndrome.

respond to foscarnet, 90 mg/kg IV every 12 hours. A partial or complete response to induction therapy is observed in 75% to 85% of patients treated with either ganciclovir or foscarnet,[54–56] but relapses are common with or without maintenance therapy. Documented HSV esophagitis is usually treated with IV acyclovir initially in severe cases; therapy may be continued with oral acyclovir, valacyclovir, or famciclovir. Complete resolution is reported in 70% of patients treated with acyclovir, but relapse occurs in 15% of patients with HSV esophagitis within 4 months.[34] If acyclovir therapy has failed because of resistance, no response to ganciclovir is expected, and foscarnet should be given.

Aphthous ulceration of the esophagus has been ameliorated with a regimen of prednisone, 40 mg daily for 2 weeks, in greater than 90% of cases.[57] A placebo-controlled trial demonstrated complete healing of AIDS-associated oropharyngeal aphthous ulcers in 55% of 29 patients treated with thalidomide, 200 mg daily for 1 month, in comparison with only 7% of patients who received placebo.[58] In a similar randomized study of 24 HIV-infected patients with biopsy-confirmed aphthous ulceration of the esophagus, the same regimen of thalidomide resulted in complete healing at 4 weeks in 73% of treated subjects, in comparison with 23% of placebo recipients ($P = .033$).[59] In a series of 12 patients with idiopathic esophageal ulceration, 92% experienced complete resolution of symptoms with this treatment regimen.[60] Use of these therapies should be considered for patients with esophageal ulcerations not attributable to specific pathogens. Thalidomide in lower dosages of 100 mg three times per week was not effective in preventing recurrences of oral and esophageal aphthous ulcers in HIV-infected patients.[59] Zidovudine, zalcitabine (ddC), and nelfinavir capsules may also cause esophageal ulceration; patients should be advised to take these and all oral medications in the upright position and with sufficient water.

Approximately 5% of endoscopically proven cases of *Candida* esophagitis are refractory to fluconazole therapy because of either acquisition of a resistant strain or gradual emergence of resistance over time. Refractory candidiasis—most common in patients with advanced AIDS, a $CD4^+$ T-cell count lower than $50/mm^3$, and long-term exposure to azole antifungal agents—is an indicator of poor disease prognosis.[61] Patients unresponsive to fluconazole, 200 mg daily for 2 weeks, may respond to higher doses, at least transiently; 50% to 60% of such patients have responded to itraconazole oral solution, 200 mg daily.[61,62] Chronic prophylaxis with itraconazole is associated with reduced susceptibility to itraconazole and cross-resistance to fluconazole.[63] Oral voriconazole has demonstrated efficacy equivalent to that of fluconazole in treatment of esophageal candidiasis; voriconazole may be effective in management of fluconazole-refractory cases.[64] Oral posaconazole has yielded clinical responses in 74% of patients with esophageal candidiasis refractory to fluconazole or itraconazole.[65] The newer agent isavuconazole has also shown efficacy equivalent to fluconazole in treatment of uncomplicated esophageal candidiasis.[67] Echinocandins are usually effective but are associated with higher relapse rates than seen with fluconazole, and higher doses of echinocandins are recommended for esophagitis than for candidemia.[46] Caspofungin has proved as efficacious as fluconazole or amphotericin B, including good responses in cases refractory to fluconazole; however, response rates to either amphotericin or caspofungin are reduced in patients for whom fluconazole previously failed, probably because these patients have more severe immunosuppression.[68,69] Micafungin and anidulafungin have also proved as effective as fluconazole.[70,71] Emergence of resistance to echinocandins during echinocandin therapy has been reported.[72,73] Parenteral amphotericin has been reserved for patients in whom other therapies fail; doses of 15 to 20 mg daily are often sufficient. Relapse rates are higher with refractory candidiasis, and maintenance therapy is almost always required. ART is critical in such patients and must be optimized. To reduce the risk of refractory disease, continuous antifungal prophylaxis should be avoided in most patients with AIDS who have mucosal candidiasis unless recurrences are particularly frequent or severe.[61]

Various other pathogens have caused rare cases of esophagitis in patients with AIDS, including *Pneumocystis jirovecii,*[74] Epstein-Barr virus,[75] *Mycobacterium avium* complex,[9,76] *Histoplasma capsulatum,*[77] *Cryptococcus neoformans,*[4] *Cryptosporidium, Actinomyces,*[78,79] *Trichomonas,* human papillomavirus, and *Saccharomyces.* Therapy is the same as for infections with these agents at other sites. Kaposi sarcoma involving the esophagus has been documented in up to 7% of patients with AIDS who have esophageal symptoms.[4,9,12] As opportunistic infections have decreased in frequency, gastroesophageal reflux disease is relatively more common as a cause of esophagitis among patients with HIV infection.[45]

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The complete reference list is available online at Expert Consult.

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98 Diarrhea With Little or No Fever

Luther A. Bartelt and Richard L. Guerrant

SHORT VIEW SUMMARY

Definition

- A diverse group of pathogens cause acute and chronic forms of gastroenteritis that occur in both pediatric and adult patients. These diseases have the unifying characteristic of being predominantly clinically *noninflammatory* (typically without dysentery and with little or no fever) in nature.

Epidemiology

- Noninflammatory gastroenteritides are among the most common infections of humans. As a group, they are second in incidence only to viral upper respiratory infections.
- Most cases of these diseases are not tracked or reported, but they are estimated to affect tens of millions of people or more worldwide each year.
- There are baseline endemic sporadic and seasonal rates as well as epidemic outbreaks of most forms of these infections.
- The rates of infection and etiologic agents vary according to age, climate, and geography. In addition, there are differences in these parameters observed for place of acquisition (e.g., community-acquired vs. health care facility–acquired infections or travel-associated infections).

Microbiology

- Viruses including members of the rotavirus, norovirus, adenovirus, and astrovirus genera are the cause of most cases of noninflammatory gastroenteritis.
- Among the bacterial causes of this syndrome, certain pathogenic strains of *Escherichia coli* and some serotypes of cholera and noncholera *Vibrio* spp. are particularly noteworthy.
- Certain protozoan types of parasites can cause a predominantly noninflammatory type of gastroenteritis and include members of the *Cryptosporidium, Giardia, Cystoisospora,* and *Cyclospora* genera.
- Although many of the etiologic agents are similar, there are also some important differences between the endemic and epidemic causes of noninflammatory gastroenteritis in resource-limited countries compared with resource-abundant countries.
- Neonatal nursery–associated and nosocomial outbreaks of this syndrome differ from community outbreaks.
- The number of potential infectious agents is much greater in immunocompromised hosts compared with immunocompetent hosts.

Diagnosis

- The typical clinical syndrome consists of varying degrees of watery diarrhea with or without nausea and vomiting, often in combination with abdominal discomfort. Fevers when present are typically low-grade. Myalgias and arthralgias or other systemic symptoms can occur but are frequently absent.
- Most cases of this syndrome, regardless of etiology, are self-limited, and no specific pathogen is identified. The duration may be prolonged in immunocompromised and malnourished hosts.
- In certain instances such as epidemics, health care–associated outbreaks, foodborne cases, cases in immunocompromised hosts, and cases associated with international travel, an etiologic agent should be identified by culture, immunoassay, or molecular diagnostic assay.

Therapy

- Adequate replacement of fluids and electrolytes is the mainstay of treatment for all forms of gastroenteritis.
- In most cases, specific antimicrobial therapy is unnecessary. However, in severe forms or select populations or situations, specific antiviral, antibacterial, or antiparasitic treatment may be beneficial. When used, the choice of antimicrobial therapy should be guided by the identification of a specific pathogen.
- There are increasing reports of emerging multidrug resistance among Enterobacteriaceae (*Campylobacter, Shigella,* and *Salmonella* spp.) members of this group.

Prevention

- Adequate sanitation for the local water supply and food processing and distribution systems helps to prevent many forms of endemic, community-acquired noninflammatory gastroenteritis.
- Personal protective equipment such as gloves, hand hygiene with soap and water, and patient education are used in health care settings to reduce nosocomial spread.
- Although there has been considerable interest in developing effective vaccination or immunization schemes for many of these infectious agents, only vaccines for rotavirus are currently available for general use.

Gastroenteritis is one of the most common infectious disease syndromes. An estimated 2.4 billion episodes occur worldwide each year,[a] with children younger than 5 years old living in resource-limited settings accounting for nearly 1 billion of these infections and more than 500,000 deaths yearly.[b] Despite decreasing global mortality rates, the collective morbidities due to these infections in resource-limited settings remain significant.[5,6,7,9] Also, the emergence of *Clostridiodes difficile* (formerly *Clostridium difficile*) has been associated with increasing diarrhea-associated mortality in the United States and other resource-abundant countries. Despite considerable morbidity and mortality, most episodes of acute gastroenteritis are self-limited. In most cases there is no clinically recognizable inflammatory process (i.e., fever or dysentery),[11,12] suggesting viral, toxigenic bacterial, or noninvasive parasitic etiologies.[c] With the notable exceptions of profuse purging (*cholera gravis*) as a potentially fatal manifestation of *Vibrio cholerae* and diarrhea persisting for greater than 2 weeks due to parasitic causes, clinical symptoms rarely indicate a specific etiology in an afebrile patient. Fever can accompany many etiologies of gastroenteritis; the absence of fever or blood in the stool, or both, diminishes the likelihood of an invasive bacterial or amebic process and the urgency to treat (see Chapter 99). Local and seasonal prevalence, outbreaks, host factors (e.g., age and immune compromise), antibiotic use, and the environment (varying across industrialized and nonindustrialized, and community and health care environments), influence the likelihood of the attributable pathogen.[6,7,9,10] Fluid and electrolyte replacement appropriate to the severity of illness is the

[a]References 1, 2, 3, 4–6, 7, 8, 9, 10.
[b]References 3, 4, 5, 6, 8, 9.
[c]References 1, 2, 4, 5, 6, 7, 8, 9, 10.

mainstay of treatment. Pathogen-directed diagnostic tests should guide antimicrobial therapy when indicated.

EPIDEMIOLOGY OF ACUTE NONINFLAMMATORY DIARRHEA

Despite declining global incidence, a broad group of gastrointestinal pathogens remains that cause typically self-limited, watery diarrhea with or without nausea and vomiting, often accompanied by other abdominal complaints. Collectively, in 2015 these pathogens caused more than 1.3 million deaths globally, were the ninth leading cause of death for all ages, and were the fourth leading cause of death in children less than 5 years of age.[3] Pathogen-attributable burden varies geographically and throughout the human life span and is largely influenced by economic indicators and rotavirus vaccine availability.[3,9] The burden of gastrointestinal infections is overwhelmingly skewed toward young children in resource-limited countries, particularly children with malnutrition. Therefore diarrhea-related mortality in children less than 5 years old is almost exclusive to regions of sub-Saharan Africa and South Asia.[3] In contrast, pediatric gastroenteritis in the United States is seldom fatal. However, total diarrhea-attributable mortality across all ages in the United States increased by 36.8% from 2005 to 2015, largely due to the emergence of health care–associated and antibiotic-associated *C. difficile* diarrhea, which disproportionally affects older adults. In the United States there are an estimated 179 million outpatient visits and nearly 500,000 hospitalizations due to foodborne or waterborne gastroenteritis per year.[13]

COMMUNITY-ACQUIRED DIARRHEA

Acute Pediatric Diarrhea

The rapid decline of infant mortality rates during the 20th century coincided with increased availability of piped water and chlorination systems. In areas of the world that continue to have unsafe water and sanitation, gastroenteritis remains a major cause of infant mortality.[3] The highest attack rate of diarrhea occurs at the time of weaning (weanling diarrhea), usually between 6 and 24 months of age.[14,15] The increased susceptibility of a recently weaned infant is related to several factors.[16] First, human breast milk appears to convey a level of resistance to some gastrointestinal pathogens and may protect an infant who is still breastfeeding.[17–21] In contrast, in resource-limited settings, weaning and complementary foods are often prepared under conditions of poor hygiene and are frequently found to be contaminated with large numbers of potential diarrheal pathogens.[22,23] Children may also ingest soil contaminated with diarrheogenic bacteria.[24] Finally, undernutrition can influence pathogen-attributable diarrhea risk.[25–28]

Early childhood diarrhea, regardless of etiology, often manifests as acute, watery, noninflammatory diarrhea. Cases are sporadic, but there are seasonal patterns, often peaking in the summer months in resource-limited settings.[9] In a well-nourished infant, the disease is usually short-lived and resolves within 2 to 3 days with adequate hydration.[15,29] Diarrhea in a malnourished child tends to persist or to recur and is often much more severe.

Early childhood diarrhea has been most commonly associated with rotaviruses[29–31] and enterotoxigenic *Escherichia coli* (ETEC).[32,33,34] Use of molecular diagnostics in longitudinal and case-control studies has refined these pathogen-attributable estimates across multiple global regions.[5,6,9,35] Using quantitative polymerase chain reaction (PCR) methods, up to 90% of moderate-to-severe acute diarrheal episodes were pathogen-attributable in low-income–to–middle-income settings.[6] Rotavirus is the most common global etiology of severe acute watery diarrhea.[6,9,35] Other viruses, predominately norovirus GII and adenovirus types 40 and 41, and to a lesser extent sapovirus and astrovirus, are significant contributors, especially where rotavirus vaccine is established.[6,9,35] Cases due to *Shigella* spp. slightly outnumber cases caused by heat-stable enterotoxin-producing ETEC (ST-ETEC).[6,9,35] These studies have also revealed the emerging importance of previously difficult to detect or noncultivable pathogens such as *Campylobacter* spp. and the protozoan *Cryptosporidium*. In some settings, *Cryptosporidium* is second only to viral etiologies and, together with rotavirus and *Shigella* spp., may combine to account for more than 50% of diarrhea-attributable mortality in children less than 5 years of age.[5,35] Although these studies have clarified the major drivers of early childhood diarrhea at the population level, the clinical application of these tools for individual cases is more challenging. First, molecular diagnostics are seldom available. Second, 40% or more of diarrheal stools may harbor two or more pathogens simultaneously.[6]

Diarrhea Caused by Rotavirus

Rotaviruses are the most common cause of severe diarrhea in infants and young children (see Chapter 150).[35–38,39] Worldwide, rotaviruses cause more than 100 million cases of gastroenteritis and up to 240,000 deaths each year in children younger than 5 years old.[3,37,40] Of these rotavirus-induced deaths, more than 90% occur in children residing in low-income or middle-income countries.[40] Although most adults have antibody to rotaviruses that may be protective, unvaccinated children younger than 2 years are highly susceptible to rotavirus diarrhea.[37]

In the United States few deaths are attributable to rotavirus infection; however, before inclusion of rotavirus vaccine in childhood immunization programs, almost every child had been infected by the age of 5 years, and rotavirus-induced gastroenteritis caused about 400,000 physician encounters, almost 200,000 emergency department visits, and about 70,000 hospitalizations each year.[37] Since routine vaccination began in 2008, rotavirus-related diarrhea has declined by 62% to 71%, and hospitalization for all-cause gastroenteritis has declined by 31% to 33%.[41,42] Infection occurs more frequently in the winter or in cooler, dry months[29,30,43–50] than in the summer months.[9,51]

There are five major rotavirus serotypes, A through E, with group A strains further subdivided based on the combination of G (the VP7 glycoprotein) and P (the VP4 protease-cleaved protein) types.[37,38,52–56] Worldwide, five serotypes (G1 through G4 and G9) predominate.[37,38,52–54,57] A particular strain (the number in brackets indicates the genotype), P1B[4]G2, is associated with a more severe form of infection in children.[58,59] Such epidemiologic information was important in designing rotavirus vaccines. Before rotavirus vaccination in the United States, six rotavirus strains were most commonly found: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G9P[6].[54–57,60,61] In the postvaccine era, G3P[8] was predominant through 2011 and has since been replaced by G12P[8]. Vaccine-derived strains occur in 0.6% to 3.4% of cases.[62] Recent outbreaks of milder illness have been associated with more unusual serotypes, for example, strain G8P[8], which affected vaccinated and nonvaccinated individuals equally.[63]

Rotavirus diarrhea is usually mild, without high-grade fever, and is often associated with vomiting; however, clinical syndromes range from asymptomatic carriage to severe gastroenteritis with profound dehydration, shock, and death.[29,30,36,37,64] Serious complications of rotavirus gastroenteritis include necrotizing enterocolitis in neonates.[60,65,66] Severe cases are more common in resource-limited settings,[37,65] and among severely immunocompromised patients. Large symptomatic outbreaks of moderately severe rotavirus diarrhea can occur. Although outbreaks are geographically distinct, they are often caused by the same rotavirus strain.

The diagnosis of rotavirus diarrhea is established with the use of a variety of molecular assays designed to detect virus-specific antigens, antibodies, or nucleic acids in feces of infected patients.[67–70] Rotaviruses can also be cultured directly from stool samples, although this method is time-consuming, expensive, and limited primarily to research-based laboratories.

The human rotaviruses are nonenveloped RNA viruses of approximately 70 nm belonging to the Reoviridae family.[37,52,53] The major rotavirus-mediated effects on the host include disruption of intestinal epithelial cell brush-border enzymes, cytotoxicity, and alteration of the enteral nervous system.[38,39,71–73] Normal columnar epithelium at the villus tips is replaced by irregular cuboidal, cryptlike cells, leading to multiple defects in fluid and electrolyte regulation. Many of these effects are believed to be mediated by a calcium ion–dependent enterotoxin.

The loss of absorptive villus tip cells may be responsible for the fluid imbalance and nutritional impact of rotavirus infections. The degree of microvillus damage roughly parallels the severity of diarrhea and dehydration.[74] Certain patients with rotavirus gastroenteritis also exhibit extraintestinal symptoms in association with detectable antigenemia and viremia, supporting a systemic phase to this infection.[75–78]

Oral or intravenous rehydration is the cornerstone of therapy for rotavirus gastroenteritis. No definitive antiviral therapy is established for rotavirus infection; however, the antiparasitic drug nitazoxanide was shown in randomized, controlled studies to shorten the duration of illness in children and adults with symptomatic rotavirus diarrhea.[79,80] Adjuvant probiotic use may reduce repeated diarrhea episodes and promote intestinal function after rotavirus infection,[81] but the role of specific probiotics regarding duration and severity of illness across different populations requires further investigation.[82]

Because of the great global burden of childhood rotavirus gastroenteritis, significant multinational efforts have been directed toward the development of a safe and effective rotavirus vaccine. The first rotavirus vaccine approved for use in the United States was the rhesus-human reassortant rotavirus tetravalent vaccine (RotaShield; Wyeth-Lederle, Madison, NJ). It was approved and released in 1998 for use in infants. Despite high efficacy, the vaccine was removed less than 1 year later because of concerns about possible cases of intussusception in vaccinated infants.[83,84] After the withdrawal of the rhesus-human reassortant rotavirus tetravalent vaccine, two new rotavirus vaccines became licensed and approved for use in infants in the United States:[37,53,85,86] first, a live oral pentavalent rotavirus vaccine (RotaTeq; Merck Vaccines, Whitehouse Station, NJ) comprising bovine-human recombinant strains in 2006,[37,85,86,87] followed by a monovalent human rotavirus vaccine (Rotarix; GlaxoSmithKline, London, United Kingdom) derived from an attenuated P1A[8]G1 rotavirus strain in 2008.[37,85,86,87–89] Both are administered starting between 6 and 14 weeks of age in a three-dose (pentavalent rotavirus vaccine) or two-dose (human rotavirus vaccine) schedule and are highly efficacious. No significant increased risk of intestinal intussusception has been observed to date. Neither vaccine should be administered to infants with severe combined immunodeficiency syndrome[90]; however, despite a theoretical risk of transmission of vaccine-derived strains within a household, living with an immunosuppressed patient is not an absolute contraindication.[91]

Routine rotavirus vaccination has dramatically reduced rotavirus-diarrhea in the United States, and use of the rotavirus vaccine has been estimated to have saved $1.2 billion in annual health care costs associated with acute gastroenteritis.[42] Rotavirus vaccine is now available in more than 90 countries; however, vaccine efficacy varies geographically and is lower in resource-limited countries where the bulk of rotavirus infections still occur.[3] Among the potential reasons for decreased rotavirus efficacy is as yet poorly understood differences in mucosal immune responses in children in these settings, particularly children with evidence of undernutrition, and possibly differences in intestinal microbial ecology or the presence of high maternal antirotavirus immunoglobulin A, or both. A parenterally administered P2-VP8-P[8] subunit vaccine appears to be safe and immunogenic.[92]

Acute Nausea and Vomiting (Winter Vomiting Disease)

The syndrome of acute nausea and vomiting (intestinal flu, viral gastroenteritis, or what had been called *winter vomiting disease*) sometimes with low-grade fever commonly occurs in winter months in temperate climates.[93,94] Although this syndrome overlaps with rotavirus-associated infantile gastroenteritis, rotaviruses are relatively uncommon causes of winter vomiting disease in older children and adults. The Cleveland family studies of Dingle and coworkers[95] showed gastrointestinal illnesses were most common between the ages of 1 and 10 years, when approximately two illnesses occurred per person per year. Illnesses peaked from November through February, with June being the month of lowest frequency. Most illnesses lasted 1 to 3 days; 20% occurred with respiratory symptoms, and 20% involved only diarrhea.

Illnesses tended to occur in one of two patterns: (1) a mild afebrile illness with watery diarrhea or (2) a more severe febrile illness with vomiting, headache, and constitutional symptoms. Although etiologic agents were rarely identified, these two patterns of illness also developed among volunteers who ingested filtrates prepared from the feces of ill patients.[96–98] Studies done in Charlottesville, Virginia, confirmed this pattern of wintertime gastroenteritis including clustering in families, highest attack rates in children, and absence of identifiable etiologic agents in most cases despite the application of techniques for virologic and enterotoxin studies.[99,100]

Caliciviruses are now recognized as the etiologies of many cases of gastroenteritis occurring in winter months. The Caliciviridae family comprises four genera:[101,102] *Norovirus, Sapovirus, Lagovirus*, and *Vesivirus* (see Chapter 176). Viruses belonging to *Norovirus* (from *Nor*walk) and *Sapovirus* (from *Sap*poro) genera are currently the most common causative agents for viral gastroenteritis in humans.[101,102,103,104,105] Noroviruses and sapoviruses may predominate as causes of early childhood diarrhea in rotavirus-vaccinated populations.[6,7,35,106] Noroviruses, in particular, cause major outbreaks, especially in older children and adults (see Chapter 176). Immunocompromised patients can develop chronic norovirus infection lasting for months or years (see "Diarrhea in Immunocompromised Patients").[107]

Norovirus

Norovirus accounts for more than one-third of the outbreaks of nonbacterial gastroenteritis in the United States.[108,109] Initially these viruses were referred to as Norwalk-like viruses. Their individual names were originally derived from the site of origin of each particular outbreak and included Norwalk,[110,111,112] Hawaii,[113] Snow Mountain,[114] Taunton, and W agents.[115] Person-to-person spread is common, and secondary attack rates can be high.

Norovirus outbreaks have been observed in a wide variety of settings[116,117] including hospitals,[118] extended-care facilities,[119] child care centers,[120] cruise ships,[121] refugee centers associated with natural disasters (e.g., Hurricane Katrina[122]), and military combat areas including Afghanistan[123] and Iraq.[124] Several genotypes are known to infect humans[103,116,117]; in recent years, newly identified variant strains of norovirus genotype GII.4 have caused outbreaks worldwide[125–127] including in the United States, United Kingdom, Europe, New Zealand, and Australia.

The pathophysiologic features of norovirus gastroenteritis parallel features of rotavirus in several respects.[101,102,103,117] Each type is essentially noninflammatory and causes villus disruption and transient brush-border enzyme deficiencies in the upper small intestine, without any alteration in adenylate cyclase activity.[54,128,129] This enteropathy can be severe in patients with underlying immunocompromise, especially patients with common variable immunodeficiency.[130] The roles of enzyme deficiencies, malabsorption of xylose and lactose, and an increase in intestinal bacteria during norovirus illness remain unclear.[128,131]

Sapovirus

Global studies on etiology of pediatric diarrhea in low-income–to–middle-income countries have unmasked a greater burden of sapovirus diarrhea than previously recognized.[6,7] Sapovirus was the second most common all-cause etiology of diarrhea in the community setting,[7] and cumulative incidence may exceed 80% by 2 years of age.[132] Sapoviruses were initially called typical human caliciviruses or Sapporo-like viruses after the prototype strain found in an outbreak in Sapporo, Japan, in 1982. Similar to norovirus, diverse sapovirus genogroups and genotypes cause infections in humans. Repeated infections are typically due to different genotypes, suggesting immunity is genotype-specific.[132] Sapovirus symptoms overlap with norovirus symptoms. Similar to norovirus, sapovirus can cause outbreaks in long-term care facilities, but fewer laboratories specifically test for sapovirus. Sapovirus should be considered if the cause for an outbreak is otherwise unexplained.[133] Sapovirus also occurs as a coinfection with other pathogens.

Diarrhea Caused by Astrovirus, Adenovirus, and Other Possible Viral Etiologies

Several other groups of viral pathogens have been identified in endemic and epidemic gastroenteritis (Table 98.1) including[36,67,134–136] (1) other types of caliciviruses with characteristic, "chalice-like" surface; (2) astroviruses, with a five- or six-pointed starlike surface structure[137]; (3) enteric adenoviruses (especially serotypes 40 and 41)[6,138]; and (4) human enteric coronaviruses,[139,140] as well as other miscellaneous or less well-characterized viral agents. More detailed discussions of these agents may be found in Chapters 142, 155, 172, 174, 177, and 178.

TABLE 98.1 Viral Pathogens Causing Gastroenteritis

ESTABLISHED PATHOGENS	LIKELY AND EMERGING PATHOGENS
Adenoviruses (enteric types)	Bocaviruses
Astroviruses	Coronaviruses
Caliciviruses (including noroviruses and sapoviruses)	Enteroviruses (various)
Rotaviruses groups A–C	Picobirnaviruses, picornaviruses
Cytomegalovirus	Pestiviruses
	Toroviruses

Data from references 36, 60, 67, 104, 105, 116, 120, 135, 137, 149, 393, and 394.

Astroviruses are among the most common causes of pediatric viral gastroenteritis.[141–144] Astrovirus occasionally occurs in association with other enteric pathogens; in these cases the illness is more severe and protracted.[143] After rotavirus, adenovirus 40/41 was the most common viral etiology of moderate-to-severe acute diarrhea in children in low-income settings and the third most common cause overall.[6] In a case-control study in China, adenovirus serotype 3 was also associated with diarrhea, whereas asymptomatic detection of other serotypes (1, 2, 5, 6, 31, and 56) were more common in healthy children.[145]

Vomiting and diarrhea can also manifest during infections with respiratory viruses. Human coronaviruses are known respiratory pathogens that can be isolated from intestinal tissues and stools.[139,140,146] Enteric coronaviruses have clear pathology in animals (i.e., transmissible gastroenteritis in pigs), and all four of the human coronaviruses (HCoV-229E, HCoV-NL63, HCoV-HKU1, and HCoV-OC43) have been detected in cases of acute gastroenteritis. However, codetection with other common viruses such as rotavirus and norovirus is frequent, and the role of human coronavirus as a primary etiology of diarrhea is likely minor.[146] Up to 10% to 20% of children with seasonal influenza present with diarrhea, although this is uncommon in adults. Diarrhea may be more prominent with Asian avian influenza A (H5N1) infection.[147]

Bocaviruses,[148] pestiviruses,[149] toroviruses,[150,151] and picobirnaviruses[152] are becoming better recognized as pathogens in children and adults, but their roles in causing diarrheal illnesses are uncertain. Similarly, although enteroviruses may cause systemic illnesses, their role as diarrheal pathogens is minimal. Unbiased viral metagenomics approaches have further identified sequences of obscure viruses in diarrheal stools that require further clarification.[153] In addition, a hallmark characteristic of the 2014 Ebola virus disease epidemic caused by *Zaire ebolavirus* (EBOV) in West Africa was nausea and abdominal pain, with voluminous vomiting and diarrhea that led to life-threatening cholera-like gastrointestinal volume losses through unclear mechanisms.[154,155]

Nucleic acid–based assays (e.g., reverse-transcriptase PCR) of stool specimens, either pathogen-specific or as part of multipathogen panels, are the standard method of gastrointestinal virus detection. Rotavirus-specific and norovirus-specific reverse-transcriptase PCR is also available. In the typical outpatient clinical situation, norovirus-specific diagnostic tests are rarely performed because results of this assay do not alter management of the illness.

Cryptosporidiosis

Cryptosporidium is another cause of acute, noninflammatory diarrhea, second only to viruses in some countries.[6,9,156] Sporadic cryptosporidiosis typically occurs through direct exposure to either infected people or animals. Outbreaks occur via contaminated food, drinking water, or recreational water including swimming pools.[d] Of the more than 20 species of this tiny coccidian intracellular protozoal parasite, two, *C. parvum* and *C. hominis,* cause the majority of human disease.[161–165] Cryptosporidiosis is commonly asymptomatic or mild in normal hosts and usually results from waterborne outbreaks, often related to contaminated drinking water or public swimming pools.[161–165] However, infection can be severe in infants and toddlers, accounting for >200,000 deaths per year.[166] Malnourished children and immunocompromised hosts, especially patients with advanced human immunodeficiency virus (HIV) infection, can manifest severe diarrhea.[e] *Cryptosporidium*, either with or without diarrhea, in some children can also contribute to the development of malnutrition.[169]

Cryptosporidium causes a secretory form of diarrhea associated with dysregulated intestinal absorption that may be less responsive to oral rehydration than other diarrhea etiologies. In addition to gastroenteritis, biliary infection with cholangitis has been reported. The pathogenesis of cryptosporidiosis is incompletely understood; the organism primarily alters villus structure and function.[162,170,171] No specific enterotoxin has been identified. Diagnosis depends on identification of oocysts in stool specimens by standard light microscopy, immunofluorescence assays, or nucleic acid–based molecular tests.[165,172–174] In addition to supportive care, nitazoxanide has been shown to be moderately successful in various patients with cryptosporidiosis. Data are inconclusive regarding universally recommended duration of therapy, especially in immunocompromised patients.[175–177,178,179] Alternative therapeutic agents for immunosuppressed patients include combinations of paromomycin and azithromycin. Novel drugs such as bumped-kinase inhibitors and pyrazolopyridine KDU731 appeared highly effective in preclinical studies.[180,181] Despite resurging interest, vaccine development remains in early stages, in part because determinants of host immunity are poorly understood.

Diarrhea Due to Enteropathogenic Bacteria

Several diarrheogenic *E. coli* pathotypes (ETEC, enteroaggregative *E. coli* [EAEC], enteropathogenic *E. coli* [EPEC], enteroinvasive *E. coli* [EIEC], and Shiga toxin–producing *E. coli* [STEC]), *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., and both *V. cholerae* and non-cholera *Vibrio* spp.) can cause noninflammatory diarrhea. *Shigella* spp. and ETEC are the most common bacterial etiologies of acute gastroenteritis worldwide.[35] Either may be associated with low-grade fever.[6,9] The inflammatory manifestations of these bacterial gastroenteritides are discussed in greater detail in Chapter 99. Among typically noninflammatory bacterial etiologies, ETEC and *V. cholerae* account for 6% to 17% of diarrhea-attributable mortality in children younger than 5 years of age.[3]

The greatest risk for ETEC diarrhea is between age 12 and 24 months.[35] Antibody directed against the heat-labile enterotoxin (LT) of *E. coli* in human colostrum may account for lower burden of ETEC in infancy.[17,18,182] Diarrhea produced by LT shares the adenylate cyclase–activating mechanism with cholera toxin.[183,184,185,186,187] Certain strains of *Klebsiella, Citrobacter,* and *Aeromonas* produce an LT-like toxin and may cause a small percentage of diarrheal disease in children.[188]

EAEC, although frequently subclinical, is among the most commonly identified enteropathogens. EAEC infection is nearly universal in children in resource-limited settings. Despite no clear association with diarrhea, EAEC, even in the absence of diarrhea, is associated with growth impairment,[189] particularly when found as a coinfection with LT-ETEC, EPEC, and *Campylobacter* spp.[189,190] Similarly, independent of diarrhea, cumulative and diverse pathogen exposures are associated with growth impairment in the first 2 years of life; these findings highlight the substantial potential cost of early-life gastrointestinal infection in low-resource settings (see Chapter 102).[191,192]

Diarrhea in Adults

In resource-abundant settings, acute gastroenteritis in adults is most often caused by noroviruses.[f] Less common viruses in adults include rotaviruses,[62,195,196] adenoviruses, and coxsackieviruses. *C. difficile* and its potential for recurrence is of increasing concern in patients with recent antibiotic use (see Chapters 99 and 243).[197] Bacterial gastroenteritides such as nontyphoidal *Salmonella* spp. are important causes of foodborne diarrhea outbreaks, often with fever (see Chapter 99). In addition, several agents of food poisoning such as *Clostridium perfringens, Bacillus cereus,* and *Staphylococcus aureus* commonly cause diarrheal syndromes in adults (see Chapter 101).

[d]References 157, 158, 159, 160, 161, and 162.

[e]References 157, 159, 160, 161, 162, 166, 167, and 168.

[f]References 103, 111, 117, 127, 193, and 194.

In adults living in resource-limited settings, several pathogens are known to cause sporadic noninflammatory diarrhea, but none has had as much historical impact or notoriety as *V. cholerae,* causing cholera.[198,199] Cholera has long been endemic in South Asia. With the increased infection-to-case ratio of El Tor cholera, the current seventh pandemic swept most of the continents of the Eastern Hemisphere including Asia, Africa, and the Mediterranean portions of Europe in the 1960s.[200] Rare isolated cases occurred in the United States[201,202] and Europe related to contaminated mineral water[203] and to undercooked shellfish.[204,205] Beginning in Madras, India, in late 1992 and rapidly spreading to Calcutta and Bangladesh in 1993, a new strain of non-O1 *V. cholerae*—O139, called Bengal—caused epidemic cholera gravis.[206–210] Most recently, natural disasters and social conflict have preceded large outbreaks including the introduction of *V. cholerae* into previously cholera-free Haiti following the devastating earthquake in 2010 and more than 750,000 cases in conflict-stricken Yemen beginning in 2016 that is still ongoing.

Severe, watery diarrhea in a patient residing in or having recently traveled to an endemic area should raise suspicion of cholera. The disease can be so fulminant as to cause hypovolemic shock and death from the outpouring of fluid into the upper portion of the small bowel before the first diarrheal stool occurs.[211] As discussed in detail in Chapter 214, the entire dehydrating syndrome of cholera appears to be related to the activation of intestinal adenylate cyclase by the potent cholera enterotoxin.[183,184,212,213] To make the diagnosis bacteriologically, stool specimens should be cultured onto thiosulfate citrate bile salts sucrose agar. Of prime importance in therapy is fluid replacement, accomplished either intravenously with isotonic fluids or orally with glucose-electrolyte solutions.

Patients from whom *V. cholerae* cannot be isolated may have a cholera-like syndrome caused by certain strains of ETEC. In 1956, De and associates[214] demonstrated that *E. coli* isolated from adults and children with this syndrome caused fluid accumulation similar to that seen with *V. cholerae* infection in ligated rabbit ileal loops. In the early 1960s Trabulsi,[215] working in São Paulo, Brazil, reported similar findings with toxigenic *E. coli.* Various investigators then demonstrated that acute undifferentiated diarrhea in adults was caused by ETEC strains that usually were not of the classically recognized pathogenic serotypes.[216,217,218,219] The toxic material present in the culture filtrate of these *E. coli* strains was demonstrated to be heat labile and nondialyzable. Subsequent studies demonstrated two types of enterotoxin produced by ETEC—LT and heat-stable enterotoxin (ST).[220] LT activates adenylate cyclase[185,186,187] and is antigenically and mechanistically similar to cholera toxin in many ways. In contrast, ST activates guanylate cyclase,[221–223] has an earlier onset of action,[220] has greater tissue specificity,[223] and has a much lower molecular weight than LT.[224]

Several studies have shown that ETEC strains producing LT only, ST only, or LT plus ST are associated with episodes of diarrhea in adults.[8] Adults in resource-limited settings who carry LT-producing *E. coli* are often asymptomatic.[225,226] In contrast, ST-producing strains are significantly associated with diarrheal disease. Diarrhea due to ETEC is less common in the United States.[43,99]

Diarrhea in Immunocompromised Patients

Patients With HIV Infection

Patients with HIV infection often develop or present with diarrhea. With increased access to antiretroviral therapy (ART), the incidence of infectious diarrhea among HIV-infected patients decreased markedly.[227–229] However, among HIV-infected patients in the United States meeting criteria for acquired immunodeficiency syndrome (AIDS), 30% to 60% present with diarrhea,[157,228,230–233,234,235] and this figure can reach 95% in parts of Africa or Haiti.[234] In many patients with advanced HIV infection and immunocompromise, diarrhea becomes prolonged, causes severe malnutrition, predisposes to other types of serious infections, and can be life threatening. In addition, symptomatic management of chronic diarrhea in such patients poses major difficulties. The importance of the interactions of HIV with the components of the intestinal immune system in modulating systemic HIV infection has come to light in recent years.[236–238]

TABLE 98.2 Possible Enteric Pathogens in Patients With Acquired Immunodeficiency Syndrome

PATHOGEN	% WITH DIARRHEA (*n* = 181)	% WITH NO DIARRHEA (*n* = 28)
Cytomegalovirus	12–45	15
Cryptosporidium	14–30	0
Microsporidia	7.5–33	0
Entamoeba histolytica	0–15	0
Giardia lamblia	2–15	5
Salmonella spp.	0–15	0
Campylobacter spp.	2–11	8
Shigella spp.	5–10	0
Clostridiodes difficile (formerly *Clostridium difficile*) toxin	6–7	0
Vibrio parahaemolyticus	4	0
Mycobacterium spp.	2–25	0
Cystoisospora belli	2–6	0
Cyclospora	0–11	0
Blastocystis hominis	2–15	16
Candida albicans	6–53	24
Herpes simplex	5–18	40
Chlamydia trachomatis	11	13
Strongyloides	0–6	0
Intestinal spirochetes	11	11
One or more pathogens	55–86	39

Data from references 242–246, 248–252, and 255–266.

Although some investigators have reported an enteropathy without identifiable infectious pathogens[239,240] or with primary HIV infection of enterochromaffin cells in the bowel mucosal crypts and lamina propria,[241] others have reported one or more enteric pathogens in 55% to 85% of patients with AIDS and diarrhea.[235,242,243] As shown in Table 98.2, the leading agents found in these patients are *Cryptosporidium,* cytomegalovirus (CMV), microsporidia, *Entamoeba histolytica, Giardia lamblia, Salmonella, Campylobacter, Shigella, C. difficile, Vibrio parahaemolyticus,* and *Mycobacterium* spp.[242–246,247] *Cyclospora* and *Cystoisospora belli* also cause persistent diarrhea in patients with AIDS.[235,248–250] *Pneumocystis jirovecii* infection can occasionally involve the intestinal tract.[251] Men with HIV who have sex with men are at increased risk of *G. lamblia, E. histolytica, Campylobacter jejuni, Shigella, Chlamydia trachomatis, C. difficile*, (with proctitis) *Neisseria gonorrhoeae,* herpes simplex virus, or *Treponema pallidum.*[252]

Although eradicative treatment may be difficult, most patients respond to specific antimicrobial or antiparasitic therapy and ART, emphasizing the need to identify an etiologic agent whenever possible.[179,253] The antiviral agent ganciclovir often controls intestinal CMV,[254,255] and most bacterial and parasitic infections can be treated with the expectation of some improvement. *Cryptosporidium,* which infects 3% to 21% of patients with AIDS in the United States, can be found in 50% of patients with AIDS and diarrhea in Africa and Haiti.[157,158,159,160,234] *Cryptosporidium*-mediated biliary infection occurs in this patient population as well. Similarly, certain microsporidia organisms, such as *Encephalitozoon intestinalis* or *Enterocytozoon bieneusi,* can cause persistent and severe diarrhea in HIV-infected individuals. *Cryptosporidium* and microsporidial infections are associated with villus atrophy, crypt hyperplasia, increased intraepithelial lymphocytes, and D-xylose malabsorption.[244]

The same acid-fast stain that detects *Cryptosporidium* or *Mycobacterium* in fecal specimens may also reveal *C. belli* and *Cyclospora* in approximately 2% of patients with AIDS with diarrhea in the United States and in 15% of patients in Africa.[234,235,249,250,256] Nontyphoidal *Salmonella* infections occur with an estimated 20-fold increase in

frequency and with increased severity in patients with AIDS.[257–260] Enteric viruses also are associated with diarrhea in HIV-infected individuals. In one study, astrovirus, picobirnavirus, calicivirus, and adenovirus were found in 6% to 12% of HIV-positive patients with diarrhea.[261] In addition, ART regimens can cause diarrhea, often complicating the workup of an HIV-infected patient with persistent diarrhea.[229,262,263]

Several practical algorithmic approaches to the diagnosis and management of diarrhea in HIV-infected patients have been published.[231,232,235] These strategies favor the use of early, noninvasive stool studies and practical empirical treatment trials, followed by more invasive tests (e.g., endoscopy with biopsy) for patients with refractory or more severe presentations. ART has decreased the incidence of several important opportunistic infections including certain causes of gastroenteritis. Of particular note, there has been a dramatic decline in the incidence of tissue-invasive infections caused by CMV gastrointestinal disease.[255,264,265] An effort to judiciously adhere to safe food and water guidelines in higher-risk patients can significantly help in preventing many of these serious enteric infections.[266]

Patients With Solid-Organ Transplants and Others Receiving Immunosuppressants

Chemotherapy for malignancy, autoimmune disease, and especially antirejection medications following solid-organ transplantation (SOT) or hematopoietic cell transplantation (HCT) increase risk of serious enteric infections.[267,268] Frequent encounters with the health care system, prolonged hospitalizations, and antimicrobials contribute to high rates of *C. difficile* infection (CDI) in these populations. Transplantation of any organ is associated with increased risk of CDI. CDI occurs in 9% to 13% of patients following HCT and up to 20% following SOT.[268] Hospital-onset CDI after SOT can prolong hospitalization and is associated with allograft dysfunction.[269]

In addition to CDI, several other bacteria, viruses, and parasites can cause diarrhea after SOT or HCT. Viral infections are common in this population and can result in long-term shedding, particularly in cases of norovirus.[107] There is less seasonal variation in norovirus infections in immunocompromised hosts, and these infections manifest with more prolonged symptoms and viral shedding than infections in immunocompetent hosts. Up to one-third of these patients have diarrhea persisting for 2 weeks or longer, and shedding has been reported beyond a year.[270] Symptoms may occur at lower levels of viral genome copies per gram of stool. Prolonged viral shedding in these patients is associated with an increased diversity of genomic variants in an infected individual.[271] The pathogenicity of these divergent variants is unclear, and therefore the clinical significance of continued norovirus detection over prolonged periods of time in patients with mild symptoms is uncertain. Flattening of the villus border and loss of villin colocalizes with viral particles on duodenal biopsy specimens, but other histologic changes described in chronic norovirus infections in immunocompromised patients (edema, cellular debris, and metaplasia) are nonspecific and overlap with noninfectious etiologies of enteritis in these patients.[272] No specific therapy is available. Combinations of oral immunoglobulins, nitazoxanide,[273] and decreasing immunosuppressive therapy[274] may benefit some patients, but results are inconsistent.

CMV enteritis should be considered in the differential diagnosis of diarrhea following transplantation, especially in donor-recipient mismatch cases. CMV enteritis often occurs in the absence of peripheral viremia, and direct intestinal sampling is recommended to rule out this condition. Diarrhea may also be a presenting symptom of disseminated adenovirus in transplant recipients. Reduction of immunosuppression is part of management for any infectious diarrhea in this population. Ganciclovir is effective treatment for most cases of CMV enteritis; however, options for adenovirus are more limited. Brincidofovir, a prodrug of cidofovir, is conjugated to a lipid to enhance intracellular delivery with lower plasma concentrations and less renal toxicity than cidofovir. This drug has been prescribed on a compassionate use basis for disseminated adenovirus infection in this population, but efficacy data are lacking, and diarrhea itself may be a significant side effect of brincidofovir.

Cryptosporidiosis can also be severe in these patients and may mimic graft-versus-host disease on endoscopy and intestinal biopsy. The clinical presentation of these infectious etiologies overlaps with medication side effects, especially mycophenolate and chemotherapy, which cause direct epithelial cell injury.[269]

TRAVEL-ASSOCIATED DIARRHEA (TURISTA)

Whether it "arouses one from bed with a start at 4 a.m. for a record-breaking race to the bathroom to begin a staccato ballet"[275] or produces the poetry of the psalmist ("I am poured out like water … my heart like wax is melted in the midst of my bowels"[276]), traveler's diarrhea has a major impact each year on the 300 million to 500 million international travelers and probably on the distribution of well over $100 billion in international tourism receipts.[277,278]

Diarrhea is the most common and among the most disconcerting illnesses that threaten the traveler.[279,280–285] North American and northern European travelers appear to be at greatest risk when they travel to Latin America, southern Europe, Africa, or Asia.[286–290] Most symptoms of traveler's diarrhea begin 5 to 15 days after arrival, although a range from 3 to 31 days has been noted.[256,279,291–296] The illness is typically manifested by malaise, anorexia, and abdominal cramps followed by the sudden onset of watery diarrhea. Nausea and vomiting occur in 10% to 25% of cases. The diarrhea is usually noninflammatory, although a low-grade fever is present approximately one-third of the time. The duration is usually 1 to 5 days, but up to 50% of patients have illness that continues 5 to 10 days and sometimes beyond.

The attack rate for traveler's diarrhea varies from 5% to 50%, depending on the destination and duration of the trip. Destinations with increased risk for acquiring traveler's diarrhea include portions of Africa and Asia (including India), and certain areas of Central and South America.[279,296,297] The attack rate also appears to decrease with age after 25 years, an observation that may reflect different habits and exposures rather than inherent susceptibility.[275,289] Expatriate residents living in certain countries appear to be at some level of persistent risk for diarrhea of infectious causes; for instance, an attack rate of 49% per month is observed during the first 2 years of residence in Nepal.[298]

For many years, the etiology of turista was an enigma; only infrequently had parasites or bacteria, such as amebas, *Giardia, Salmonella,* or *Shigella,* been identified, and viral studies had failed to elucidate significant causes. The first suggestion that an infectious process was likely came from the effective reduction in the attack rate achieved by the use of prophylactic antimicrobial agents.[291,296,299,300] Studies by Kean[275] suggested that certain EPEC serotypes may be involved in up to one-third of the cases. The involvement of *E. coli* was further confirmed in an outbreak of traveler's diarrhea among British troops in Aden, where *E. coli* O148 was identified in 54% of the British troops with diarrhea.[292]

Later studies demonstrated ETEC in approximately 50% (range, 5%–75%) of cases of traveler's diarrhea in Latin America, Africa, and Asia (Table 98.3).[301–303] The attack rate ranged from 20% to 100% (median, 52%–54%) (see Table 98.3).[279,297,300,304] ETEC was almost never present before travel, and such organisms were acquired by only 12.6% of fellow travelers who did not become ill.[256,294,295] ETEC producing LT, ST, or both can cause traveler's diarrhea (Table 98.4). In some areas, EAEC is a major cause (see Chapter 218).[279,303]

A number of other microbial agents have also been identified in small subsets of patients with traveler's diarrhea. *Salmonella, Shigella, Campylobacter, Vibrio,* and *Aeromonas* spp. are increasingly recognized in travelers with diarrhea.[304,306–308] Pathogen-specific prevalence appears to vary by destination. *Campylobacter* was present in 25% to 35% of cases during travel to South Asia and 15% to 25% of cases related to travel in Southeast Asia, but accounted for less than 5% of cases from Africa or Latin America.[279] In US military personnel deployed to Southeast Asia, *Campylobacter* spp. were detected in 35% and nontyphoidal *Salmonella* in 23% of cases of diarrhea.[306] In Thailand, *Campylobacter* was the most common etiology (28%) of pathogen-attributable diarrhea cases.[308] *Shigella* accounted for 5% to 15% of cases from Latin America, Africa, and South Asia and was less frequent in travelers to Southeast Asia.[279] *Salmonella* infections were more common during travel to Africa and Southeast Asia than to South Asia or Latin America.[279] *V. cholerae* is rarely a problem for most US travelers, but infection can occur among military personnel or relief workers in endemic cholera locales.[289,308,309] Single-dose live lyophilized CVD 103-HgR (Vaxchora)

oral cholera vaccine is approved for travel to areas with active cholera transmission.

Rotavirus or calicivirus infections have been described in 0% to 36% of cases but were often found in association with either bacterial or parasitic pathogens.[310] Noroviruses in particular have been implicated in several large outbreaks of gastroenteritis or diarrhea on large cruise ships.[311–313] In many respects, however, cruise ship–associated outbreaks of gastroenteritis share more epidemiologic characteristics with institution-associated gastroenteritis than with classic traveler's diarrhea.

Intestinal protozoa also may be important causes of traveler's diarrhea. Agents such as *C. parvum, Cyclospora cayetanensis, G. lamblia,* and various microsporidia should be considered in the workup of subacute or chronic noninflammatory diarrhea in returned international travelers.[314–316] In the GeoSentinel Surveillance Network global database of 2902 unwell travelers seeking medical care, parasitic etiologies (predominantly *G. lamblia* [27%], *E. histolytica* [14%]) accounted for 65% of chronic diarrhea cases, whereas bacteria (*Campylobacter* [13%], *Shigella* [6%]) were identified in 31% and viruses (hepatitis A and hepatitis E) were identified in only 3%. *G. lamblia* was the most commonly identified pathogen regardless of travel destination[317] Other protozoal parasites such as *Blastocystis hominis* can be commonly identified in the stools of persons traveling to developing countries. However, it has been difficult to ascertain whether these organisms actually cause disease or are merely commensal.[318–320] A subset of patients have persistent diarrhea for which no infectious agent can be implicated. Chronic idiopathic diarrhea (see "Brainerd Diarrhea") has been reported to occur in a few small travel-related outbreaks[321–324] and may cause prolonged diarrhea in sporadic cases.

In contrast to the frequent identification of potential etiologic agents among travelers to tropical areas who develop diarrhea, travelers to temperate areas often develop a mild syndrome of diarrhea for which no infectious cause can be identified.[325] Environmentally distributed *Cryptosporidium* and *Giardia* and oocysts are hardy in temperate regions, have zoonotic reservoirs in these regions, and are relatively tolerant to chlorination. Travelers to certain areas such as Russia and national parks in the United States who drink unfiltered or unboiled water may therefore be susceptible to development of the more insidious watery diarrhea seen with giardiasis or cryptosporidiosis.[326–329] Strongyloidiasis acquired during travel may cause noninflammatory diarrhea, abdominal pain, and eosinophilia.[330]

Several other potentially serious infections manifested initially by diarrhea or abdominal pain may be acquired by travelers. Malaria may begin as gastroenteritis with nausea, vomiting, diarrhea, or abdominal pain in 30% to 50% of cases.[331] The physician should also remember to consider typhoid fever and other infections that may be manifested with a typhoidal pattern including plague, melioidosis, typhus, and arboviral hemorrhagic fevers.[331,332]

The desire to control the bothersome problem of diarrhea in travelers has led to a variety of medications used for management.[275] Commonly used remedies, such as diphenoxylate-atropine (Lomotil) and kaolin-pectin suspension, were found to have little or no efficacy in most studies.[333] Lomotil and other antimotility agents may actually worsen the illness in inflammatory processes such as shigellosis.[334] Bismuth subsalicylate (Pepto-Bismol) was shown to inhibit enterotoxin activity in experimental animal models,[335] and it has been recommended for symptomatic therapy as well as prophylaxis.[334,336,337] As in any type of diarrheal illness, the mainstay of therapy is adequate hydration with an oral glucose-electrolyte or sucrose-electrolyte solution.

Prevention of traveler's diarrhea should be directed toward reducing the consumption of infectious agents in food and water. Foods that are handled but not cooked (e.g., salads, raw vegetables) are high-risk foods.[279,280,281] Bottled, noncarbonated water cannot be considered safe because outbreaks of cholera[203] have been traced to bottled water, and typhoid fever[339,340] has been traced to other bottled beverages. Care in eating and drinking may reduce one's risk, even in highly endemic areas, to less than 15%.[338,341,342]

The efficacy of prophylactic antimicrobial agents has been documented in several studies.[291,296,343–345] The increased risk of acquiring a more severe infection (e.g., salmonellosis), the risk of drug side effects (e.g., photosensitivity in the tropics), and the emergence of drug-resistant organisms[347] should preclude the widespread use of antibiotic prophylaxis at this time.[348] Because treatment regimens that combine loperamide with an antibiotic are rapidly effective in controlling traveler's diarrhea (<10 hours), most experts consider prophylactic therapy only for travelers with special issues such as individuals with a high risk of infection and individuals for whom it is important to remain disease-free during the trip.[349,350]

Empirical self-treatment of traveler's diarrhea with a fluoroquinolone such as ciprofloxacin or levofloxacin for 1 to 3 days or a single dose of

TABLE 98.3 Etiology of Acute Traveler's Diarrhea in Three Locales

CHARACTERISTIC	LATIN AMERICA	AFRICA	ASIA
Duration of stay (days)	21 (2–42)	28 (28–35)	(28–42)
Attack rate (%)	52 (21–100)	54 (36–62)	(39–57)
Organism (%)			
No bacterial pathogen	28	—	35
Bacterial pathogen	72	—	80
Enterotoxigenic *Escherichia coli*	46 (28–72)	36 (31–75)	15 (5–25)
Enteroaggregative *Escherichia coli*	30 (25–35)	<5	17 (15–25)
Shigella	5 (0–30)	5 (0–15)	5 (4–15)
Salmonella	<5	10 (5–15)	10 (5–15)
Campylobacter jejuni	<5	<5	15 (2–35)
Vibrio parahaemolyticus	—	—	7 (1–13)
Rotavirus	23 (0–36)	10 (5–15)	5 (0–15)
Norovirus	10	—	3
Protozoa (*Giardia, Cryptosporidium, Entamoeba histolytica,* others)[b]	7	<5	11

[a]Values shown are median (range) from multiple studies.[278,279,300,303,305,352,444]
[b]Parasitic etiology may increase to 65% among returned travelers seeking medical care.[317]

TABLE 98.4 Frequency of Enterotoxigenic *Escherichia coli* in Association With Traveler's Diarrhea in Latin America, Africa, and Asia

FEATURE	REPORTED FREQUENCY (%) Gastroenterologists in Mexico[293]	Peace Corps Volunteers in Kenya[294]	Yale Glee Club in Latin America[295]	Japanese Travelers Returning to Tokyo From India, Southeast Asia, Orient[304]	Total
Illness attack rate	49% in 16 days	69% in 5 wk	74% in 1 mo	—	
Type of enterotoxin					
LT only	16	33	25	4.8	21
LT and ST	16	15	12.5	11.8	38
ST only	9.8	2	19	13.6	41
% of ill patients with ETEC	41 (21/51 cases)	52 (14/27 cases)	56 (9/16 cases)	32 (270/843 cases)	33.5

ETEC, Enterotoxigenic *Escherichia coli; LT,* heat-labile; *ST,* heat-stable.

1000 mg of azithromycin, can significantly reduce the duration and severity of the disease (see Chapters 318 and 319).[279,280,281,304,351–353] Therapy with trimethoprim-sulfamethoxazole had traditionally been suggested for children, but because of resistance, most experts currently recommend the use of a macrolide such as azithromycin.[279,280,281,354] Fluoroquinolone resistance has emerged among all bacterial etiologies. *Campylobacter* spp. resistant to quinolones is of special concern for travelers to many areas of the world, most notably Thailand and other parts of Southeast Asia.[355–358] Nearly 90% of *Campylobacter* diarrhea cases in US military personnel deployed to Southeast Asia were resistant to ciprofloxacin.[306] Resistance to quinolones has also increased among ETEC and EAEC strains, especially strains from India and sub-Saharan Africa. Extended-spectrum β-lactamase ETEC and EAEC strains expressing $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$ genes are emerging.[307] Fluoroquinolone drugs are contraindicated in pregnant women[279,280,281,350,352] and in children younger than 16 years. Also, US Food and Drug Administration black box warnings exist for arthropathy, neuropathy, tendinopathy, and central nervous system side effects associated with fluoroquinolones.[359] The adverse reactions associated with quinolones combined with increasing resistance have made azithromycin the preferred first choice for empirical self-treatment of traveler's diarrhea in many individuals. Resistance to azithromycin among ETEC and EAEC, however, is greater than 25% in parts of Southeast Asia, India, and Africa. Rifaximin, a nonabsorbable relative of rifampin, is useful for treatment of less severe cases of traveler's diarrhea in the absence of fever. Levofloxacin, azithromycin, and rifaximin in combination with loperamide have similar efficacies of 91% and 96% cure at 48 and 72 hours, respectively.[360] Because of ongoing changing resistance patterns among bacterial etiologies of traveler's diarrhea, pretravel counseling, empirical acute treatment, and posttravel diagnostic and treatment recommendations should consider up-to-date reported susceptibility patterns that are specific to geographic destinations, such as can be found at the Centers for Disease Control and Prevention website (cdc.gov/travel). The benefits of treatment also need to be weighed against risk of acquisition of colonization with extended-spectrum β-lactamase–producing Enterobacteriaceae during travel and increased susceptibility to pathogens after the antibiotic course is over but before the microbiome has recovered; antibiotics are not recommended for cases of mild traveler's diarrhea for these reasons.

DIARRHEA IN INSTITUTIONS

Institutions provide unique settings that promote the acquisition of certain enteric pathogens. As with diarrhea in patients with AIDS and traveler's diarrhea, many cases of institution-acquired diarrhea are noninflammatory. Given the rise *C. difficile*–associated disease (CDAD), new-onset diarrhea in patients in the hospital or long-term care facilities should prompt stool immunoassay or PCR assaying for the presence of *C. difficile* toxins.

Epidemic Nosocomial Diarrhea in Newborns

Epidemic nosocomial neonatal diarrhea has long been recognized as a potentially serious problem with mortality rates of 24% to 50%.[362,363] The unusual susceptibility of newborns to this syndrome may be explained by their underdeveloped intestinal microbiota or lack of specific immunity. Prematurity or congenital cardiac or pulmonary disease complicates this situation. The consequences of diarrhea in newborns are unusually severe, partially because they have poorly developed homeostatic mechanisms with limited water and electrolyte reserves. Crowded conditions in newborn nurseries promote nosocomial transmission.[364] In addition, delays in recognizing a nursery outbreak may occur because infants may not develop diarrhea until after they have been discharged from the hospital.

The onset of illness is often insidious, with the development of listlessness, irritability, and poor feeding over a period of 3 to 6 days.[363,365,366] Vomiting and fever are infrequent, and the stools tend to be watery; yellow-green in color; and usually without mucus, pus, or blood.[367,368] Early signs such as failure to gain weight or a slight weight loss and abdominal distention may be subtle. The disease may progress to more severe signs of dehydration and shock with depressed sensorium, drowsiness, coma, sunken eyes, circumoral cyanosis, and grayish discoloration of the skin. Shock without hyperpnea often occurs in this setting despite the development of severe acidosis. Poorly nourished infants may develop severe hypokalemia, hyponatremic dehydration, or paradoxical edema. Appropriate antibiotic therapy must be tailored to the specific sensitivity pattern of the organism isolated.[367]

The typical illness usually lasts 5 to 15 days, but persistence or relapse may occur over the course of several weeks after the initial onset of symptoms. Complications may include otitis media, pneumonia, bacteremia, peritonitis, and renal vein or cerebral sinus thrombosis. Several potentially life-threatening processes may mimic this infantile diarrhea syndrome. So-called parenteral diarrhea refers to the well-recognized but poorly understood tendency for systemic infections or localized infections elsewhere (e.g., otitis, meningitis) to be manifested clinically with diarrhea. Likewise, a strangulated hernia, intussusception, or torsion of an ovary or testis may be manifested by abdominal pain or diarrhea.

Epidemic diarrhea among hospitalized newborns has been commonly associated with certain enteropathogenic serotypes of *Escherichia coli* (EPEC). In many areas such as South Africa and southern Brazil, EPEC organisms are also among the most common causes of sporadic diarrhea in infants and young children, especially during the summer months.[369–371] Up to 20% of cases of endemic childhood diarrheal illness, even in temperate, more developed areas of the world such as England and Canada, are also known to be caused by EPEC organisms.[369,370]

The association of a certain strain of *E. coli* with infantile diarrhea was first demonstrated by slide agglutination by Bray and Beavan[372] in 1945 and reported in further detail in 1948. Excluding certain invasive serotypes (see Table 99.2 in Chapter 99), there are now at least 14 classically recognized EPEC *E. coli* serotypes including O111-K58 (α), O55-K59 (β), O127-K63, O128-K67, O26-B6, O86-K61, O119-K69, O125-K70, O126-K71, O20-B7, and O44-K74. Additional serotypes recognized as causes of epidemic infantile diarrhea include O114,[362,373,374] O142,[364,375] and O158.[376] The mechanism by which most EPEC organisms cause disease involves a complex set of attachment and effacement traits, as detailed in Chapter 96. Although most are not invasive and do not produce conventionally recognized LT or ST enterotoxins, these organisms are capable of causing diarrheal disease in human volunteers, from whom the organism can be reisolated and in whom an antibody response can be documented.[365,377]

In addition to EPEC organisms, certain enterohemorrhagic (EHEC) and enterotoxigenic (ETEC) strains of *E. coli* have caused outbreaks of diarrhea in infants. *E. coli* O157-H7 and other EHEC strains have been associated with a syndrome of hemorrhagic diarrhea with only minimal inflammation and mild severity. Most EHEC organisms secrete a Shiga-like toxin, and certain strains of EHEC have also been associated with outbreaks and sporadic cases of hemorrhagic diarrhea that are more severe and can include a form of hemolytic-uremic syndrome.[378–380,381–383]

Diarrheogenic *E. coli* serotypes are not the only bacterial causes of epidemic infantile diarrhea. For example, an outbreak of diarrheal illness was described in which multiple serotypes of different organisms (*E. coli, Klebsiella,* and *Citrobacter*) were found to be transiently enterotoxigenic.[384] This observation suggests the transmission of enterotoxigenic potential between susceptible strains, likely by certain plasmids[385] or by bacteriophages.[386] Another report of sporadic diarrhea in infants and children in Africa showed that several enteric organisms other than *E. coli* may produce an enterotoxin under certain conditions.[32] This finding is relevant to other situations as well, as typified by an outbreak of watery diarrhea occurring on a cruise ship and found to be caused by enterotoxigenic strains of *E. coli* as well as *Klebsiella* spp. and *Citrobacter* spp.[387] Many other well-known bacterial causes of diarrhea such as shigellosis[388] and epidemic salmonellosis may also spread readily in the newborn nursery.[389,390] Viral causes of epidemic infantile diarrhea include, but are not limited to, specific types of echoviruses,[391] coxsackieviruses,[392] adenoviruses,[393] and rotaviruses.[36,37,394–397]

Hospitals

Nosocomial diarrhea is among the most common nosocomial outbreaks reported to the Centers for Disease Control and Prevention.[398,399] Nosocomial diarrhea appears to be a significant factor predisposing to other nosocomial infections such as urinary tract infections.[400] Overall

rates range from 2.3 to 4.1 illnesses per 100 admissions on pediatric wards[399,401] and from 7.7 per 100 admissions to 41% of adults hospitalized in intensive care units.[399,402,403]

C. difficile remains the most common, most serious, and most costly infectious cause of nosocomial diarrhea in hospitalized patients[404–406] and ranks among the most commonly reported hospital-acquired pathogens.[407] In particular, CDAD is an important emerging nosocomial infection worldwide, especially among elderly hospitalized patients and patients occupying beds in surgical wards or intensive care units.[406,408–410] Most sporadic and outbreak cases of CDAD appear to be caused by exposure to contaminated environmental surfaces rather than direct contact with an index case.[411–413]

Other enteric pathogens are occasionally identified in outbreaks of nosocomial diarrhea. *Klebsiella oxytoca* can produce a toxin that inhibits DNA synthesis and, similar *C. difficile,* causes diarrhea and sometimes hemorrhagic colitis associated with recent antibiotic use. Enterotoxin-producing methicillin-resistant *S. aureus* has been implicated as a rare cause of antibiotic-associated diarrhea.[403] Salmonella is a common cause in reported outbreaks of nosocomial gastroenteritis and may rarely cause pseudomembranous colitis after antibiotic exposure. *Cryptosporidium* may be associated with cases of nosocomial diarrhea involving chronically ill, pediatric, and elderly patients as well as HIV-infected patients and other immunocompromised patients.[414] In young children and in immunocompromised hosts, viral agents (rotaviruses, adenoviruses, coxsackieviruses, and others) are often found.[268,401] In addition, there has been a newfound appreciation for the roles of certain viruses (e.g., norovirus, rotaviruses, adenoviruses, coxsackieviruses, and others) as causes of nosocomial diarrhea, especially in neonates, children, and patients in intensive care units.[335,403,415,416]

Long-Term Care Facilities

Diarrheal illnesses constitute a significant problem in extended-care facilities for elderly patients. A conservative estimate based on passively reported illness rates is that one-third of patients in long-term care facilities experience diarrhea each year.[417–419] CDAD remains a common and important cause of diarrhea in these facilities.[361,420,421] Sporadic cases and epidemic outbreaks of CDAD have been reported in many types of long-term care facilities. In other instances, viral causes of gastroenteritis or diarrhea have been identified in certain outbreaks occurring in these settings. The frequency of potentially transmissible enteric pathogens emphasizes the importance of careful hand washing in situations in which hygiene is often difficult.

Daycare Centers

Another special institutional setting in which hygiene is difficult and enteric infections are increasingly appreciated is daycare centers. Numerous outbreaks have been reported in association with viruses, bacteria, or parasites. The most common etiologic agents in infants and children younger than 2 years are rotaviruses, whereas older toddlers have been more likely to acquire *G. lamblia.*[422] Newer diagnostic tests based on immunoassays and reverse-transcriptase PCR have been used to detect additional agents such as astrovirus in many diarrhea outbreaks in daycare centers.[423,424] A clinical syndrome of prolonged noninflammatory diarrhea may be associated with *Cryptosporidium* in daycare centers.[425–427] Outbreaks of inflammatory diarrhea caused by *Shigella, Campylobacter,* and *C. difficile* have also been reported.[301,302]

TREATMENT OF ACUTE NONINFLAMMATORY DIARRHEA

Treatment of diarrhea from any cause in adults and children consists primarily of rehydration.[253] If glucose or sucrose accompanies oral isotonic fluid, the coupled absorption of sodium and water is often sufficient to replace fluid loss.[428] Bismuth subsalicylate may reduce enterotoxin action,[429] and if there is no significant febrile or inflammatory process, low doses of antimotility agents may offer some relief with minimal risk in adults. Some studies also suggest that novel analogues of glutamine may be beneficial in reducing the severity and extent of symptoms associated with certain forms of infectious diarrhea.[430] The potential utility of probiotic compounds for the treatment of various forms of diarrhea has also garnered attention. The current data are not sufficient to issue a general recommendation on the use of probiotics for the management of infectious diarrhea (see Chapter 3).[431–436]

There is interest in the roles of various micronutrients in the management of diarrheal disease, especially in young children residing in underdeveloped areas.[436,437] Several agents have been examined, but the strongest support has emerged relating to zinc supplementation. Several randomized, controlled international studies as well as a Cochrane Database Review found a beneficial effect of oral zinc supplementation in the prevention and management of a variety of infectious forms of diarrhea, especially in children.[438,439,440,441]

Chronic Noninflammatory Diarrhea

Syndromes of chronic noninflammatory diarrhea of infectious etiology include giardiasis, tropical sprue–like syndromes, syndromes of bacterial overgrowth, and *Cryptosporidium* or *C. belli* infection (especially in immunocompromised hosts).[234,241,442,443] In a patient with weight loss, malaise, and watery or fatty stools, giardiasis or some other cause of a malabsorption syndrome should be suspected. This syndrome may also be associated with hypocalcemia; with iron or folate deficiency anemia; or with deficiency of vitamin D, vitamin K, or protein.

Giardiasis is endemic throughout most the world but still may often go undiagnosed for weeks of illness.[161,163,444,445–447] Transmission is through water or food during outbreaks and by direct person-to-person contact. Clinical syndromes range in severity from asymptomatic infection to severe, persistent diarrhea associated with anorexia, weight loss, and malnutrition.[161] Current immunoassays and molecular tests have markedly improved diagnostic sensitivity.[161,163] Recommended therapy classically included metronidazole, with a reported 70% cure rate.[445] More recently, tinidazole and nitazoxanide have shown improved efficacy for treatment and are associated with far fewer side effects than standard courses of metronidazole (see Chapters 28 and 42).[448]

Other infectious agents of chronic noninflammatory diarrhea include *Cryptosporidium, C. belli,* and *Cyclospora.* Each of these agents can be identified by standard stool analyses using ova and parasite testing combined with specific immunoassay as needed. Of note, *Cyclospora* was identified as the etiologic agent causing a large, multistate outbreak of gastroenteritis in 2013 in the United States.[449] *Cyclospora* infection is treated with trimethoprim-sulfamethoxazole.[250,444]

Bacterial Overgrowth Syndromes

Many syndromes have been described in which impaired absorption was attributed to abnormal bacterial colonization in the upper small intestine.[443] Whether these organisms are pathogens or part of the normal intestinal microbiota abnormally distributed is unclear at the present time.

Normally the upper portion of the small bowel is relatively sparsely populated, with less than 10^5 organisms/mL; these are predominantly facultative gram-positive organisms (diphtheroids, streptococci, and lactobacilli).[450] The organisms most often incriminated in bacterial overgrowth syndromes in the small bowel are aerobic enteric coliforms (members of the family Enterobacteriaceae), anaerobic gram-negative fecal flora (*Bacteroides* and other genera), and miscellaneous other organisms such as *Plesiomonas shigelloides*.[451]

Bacterial colonization in the upper part of the small bowel may be associated with malabsorption or chronic diarrhea in the absence of significant histopathologic changes. Small bowel overgrowth is usually associated with a predisposing bowel abnormality, such as achlorhydria (from gastritis, pernicious anemia, or gastric surgery), blind-loop syndromes, cholangitis, impaired motility (scleroderma, diabetic neuropathy, vagotomy), surgery, strictures, diverticula, or radiation damage.[452,453] Protein, folate, or vitamin B_{12} deficiency may also render the bowel more susceptible to microbial colonization and injury.[450,454] An episode of acute infectious diarrhea may be the initiating event in the establishment of small bowel colonization and chronic diarrhea.[450,455,456] Lindenbaum and colleagues[457] described spruelike morphologic changes in the upper portion of the small bowel in association with increased numbers of bacteria and malabsorption among Peace Corps volunteers living in Pakistan.

Small intestinal bacterial overgrowth may causes malabsorption through bacterial binding (e.g., vitamin B_{12}) or utilization (e.g.,

carbohydrates) of nutrients, deconjugation of bile salts by bacteria such as enterococci and anaerobes,[458] or the toxic effects of bacterial products such as fatty acids or amines.[450] *E. coli* strains that lack other recognized virulence traits but colonize the bowel were shown to cause prolonged diarrhea in a rabbit model,[459] with an associated impairment in water and electrolyte absorption and disaccharidase activity.[460]

The approach to a patient with suspected bacterial overgrowth as a cause of malabsorption or chronic diarrhea should include quantitative aerobic and anaerobic cultures of the upper small bowel contents, obtained by intubation or string passage. Because the critical number of organisms appears to be approximately 10^5/mL, semiquantitative estimates from a Gram stain (analogous to the urine Gram stain) may also prove valuable. In addition, the ^{14}C-glycocholic acid breath test for bacterial deconjugation of bile salts has been shown to be helpful for diagnosis in some patients.[461]

Patients with symptomatic bacterial overgrowth are potential candidates for antibiotic therapy, especially if a predisposing condition such as achlorhydria, scleroderma, or diabetes is present. Depending on the results of quantitative cultures of upper small bowel aspirates, therapy may need to be directed against anaerobes as well as aerobic coliform organisms.[450,455]

Brainerd Diarrhea

Brainerd diarrhea is a clinical syndrome of chronic, self-limited, idiopathic diarrhea lasting for >4 weeks and up to several months.[462] Symptoms often occur with an acute onset, but an infectious etiology is not found, and other noninfectious causes are also absent. Stool frequency ranges from 5 to 25 bowel movements per day and fecal electrolyte and osmolality testing are consistent with secretory diarrhea.[462] Cases can be sporadic without evidence of household clustering; however, outbreaks including the first description of the entity in Brainerd, Minnesota, in 1983 also occur.[324] Travel and consumption of untreated water or unpasteurized milk have been associated with this entity, but infectious, chemical, or other toxins have not been identified as etiologies.[322,463] Although symptoms often are prolonged, recovery is nearly universal. Relapse or progression to chronic sequelae suggests an alternative diagnosis such as lymphocytic colitis, collagenous colitis, malignancy, or drug reactions.

Other Noninfectious Mimics of Gastroenteritis

Acute noninflammatory diarrhea may be a consequence of several noninfectious processes. Exposures to antibiotics and other medications are common causes of noninfectious diarrhea, and although diarrhea is typically mild, it can result in additional costs related to diarrhea workups and medication nonadherence, and in some cases necessitate a change in therapy. As with agents that effect an osmotic diuresis, nonabsorbable agents such as sorbitol may cause diarrhea if consumed in excess. Heavy metal poisoning (i.e., arsenic, tin, iron, cadmium, mercury, or lead) is often associated with diarrhea, probably as a result of toxic effects on the mucosal epithelium.

Endocrine causes of diarrhea that may share the adenylate cyclase–activating mechanism with enterotoxins include non–beta-islet cell tumors, medullary carcinoma of the thyroid, carcinoid tumors, and others that are associated with increased serum levels of prostaglandins or vasoactive intestinal polypeptide.[464] Patients with thyrotoxicosis or adrenal or parathyroid insufficiency may have diarrhea. Congenital and acquired enzyme deficiencies include lactase deficiency and pancreatic or biliary insufficiency, in which inadequately degraded or absorbed nutrients may promote an osmotic diarrhea. In a child who has diarrhea and edema, hypertension, or petechiae, hemolytic-uremic syndrome with or without enterohemorrhagic *E. coli* O157-H7 should be suspected. Patients with dermatitis herpetiformis may also have diarrhea that may respond to sulfone or sulfapyridine therapy or to a gluten-free diet.

Noninfectious causes should also be considered in the differential diagnosis of chronic noninflammatory diarrhea. Examples include congenital deficiency syndromes and food allergies, certain neoplastic and endocrine processes, and less well-understood functional disorders. Causative disorders to be considered in the first two categories are milk allergies, disaccharidase deficiencies, gluten enteropathy, acrodermatitis enteropathica, β-lipoprotein deficiency, familial hyperchloremic alkalosis (congenital chloridorrhea), Leiner disease, and Wiskott-Aldrich syndrome. Neoplastic and endocrine causes of diarrhea include carcinoid tumors, Werner syndrome (multiple endocrine adenomatosis), Zollinger-Ellison syndrome (gastrinoma), pancreatic cholera syndromes, medullary carcinoma of the thyroid, and thyrotoxicosis. Patients with partial mechanical bowel obstruction or pellagra may also have chronic diarrhea. Milder forms of inflammatory bowel disease and irritable bowel disease including postinfectious subtypes can also be associated with a variety of types of chronic diarrhea. Although a thorough search for an infectious cause of any form of chronic diarrhea is usually warranted, most often the specific diagnosis of one of these etiologies requires referral to a gastroenterologist.

ACKNOWLEDGMENT

The authors thank Dr. David Bobak for his contributions to previous versions of this chapter in prior editions of *Principles and Practice of Infectious Diseases.*

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99 Acute Dysentery Syndromes (Diarrhea With Fever)

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SHORT VIEW SUMMARY

Definition

- Diarrhea associated with fever is typically seen in acute and chronic inflammatory enteritides that are caused by several specific infectious agents.

Epidemiology and Clinical Signals and Symptoms

- Acute dysenteric syndromes can be caused by any of several diverse pathogens, depending on exposures. *Campylobacter, Shigella,* and *Salmonella* are often febrile illnesses (with or without bloody stools) acquired in settings of suboptimal sanitation or food handling, as can be enterohemorrhagic *Escherichia coli* (EHEC), but with less likelihood of fever. *Shigella* can be spread person-to-person by low inocula, and recent antibiotic or hospital exposure makes *Clostridioides difficile* (formerly *Clostridium difficile*) more likely.
- The bacterial enteric pathogens most associated with illnesses in children younger than 5 years in the United States are nontyphoidal *Salmonella*, followed by *Campylobacter* spp., *Yersinia enterocolitica,* and *E. coli* O157. In developing countries norovirus GII, rotavirus, *Campylobacter* spp., astrovirus, and *Cryptosporidium* spp. have the highest attributable burdens of diarrhea in the first year of life. In the second year of life the major pathogens were *Campylobacter* spp., norovirus GII, rotavirus, astrovirus, *Shigella* spp., and *Campylobacter* spp., and *Shigella* spp. were primarily associated with bloody diarrhea, and fever and vomiting were associated with rotavirus and vomiting with norovirus GII.
- Venereal exposure, particularly among men who have sex with men, may implicate gonococci, herpes simplex virus, *Chlamydia trachomatis*, or *Treponema pallidum* as a cause of proctitis, or *Campylobacter, Shigella, C. trachomatis* (lymphogranuloma venereum serotypes), or *C. difficile* as a cause of colitis.
- A history of antibiotic intake, colonic instrumentation, and/or recent admission to a health care facility would strongly suggest *C. difficile* infection (CDI).

Microbiology

- Genomic studies of *Shigella* spp. have indicated that *Shigella* and enteroinvasive *E. coli* are derived from multiple origins of *E. coli* and form a single pathovar.
- The cause of a recent outbreak of bloody diarrhea and severe hemolytic-uremic syndrome, unlike prior EHEC strains that had exhibited enteropathogenic *E. coli* traits of attachment and effacement, was a Shiga toxin–producing enteroaggregative *E. coli* strain.
- *Campylobacter* spp. have a small genome (1.6–2.0 megabytes) and can cause both intestinal and systemic infections.
- The primary virulence factors that are known to cause disease in CDI are the two large toxins: *C. difficile* toxin (Tcd)A, or toxin A (308 kilodaltons [kDa]), and TcdB, or toxin B (270 kDa), although TcdB may be more clinically relevant than TcdA.
- *Vibrio parahaemolyticus* has been recognized since 1950 in Japan and is a cause of seafood poisoning.
- *Salmonella* flagellin is regulated by the *fliC* gene, which is the major ligand for Toll-like receptor 5, nucleotide oligomerization domain-like receptors, and interleukin-1b–converting enzyme (ICE) protease-activating factor (IPAF) protein.

Diagnosis

- Any of the previously mentioned microorganisms may cause an acute dysentery syndrome with blood and pus in the stool.
- Examination for leukocytes or for fecal lactoferrin may suggest intestinal inflammation, even if blood is not present in the stool on gross examination.
- Recent approaches using multiple pathogen nucleic acid amplification tests for simultaneous detection of several enteropathogens hold promise, with high accuracy, sensitivity, and specificity and being potentially suited for surveillance or clinical purposes.

Therapy and Prevention

- Because there are many etiologic agents, the treatment and prevention rely on each specific cause of acute and chronic inflammatory enteritides, which are viewed in more detail in this chapter. Although *Shigella* and perhaps *Campylobacter* durations of infection and symptoms may be reduced by antimicrobials to which they are susceptible, antibiotics may also be indicated for systemic *Salmonella* infections for which foreign intravascular material or prostheses or severe atherosclerosis may pose increased risk. Some antimicrobials may worsen EHEC toxin production and illness severity.

DEFINITION

Diarrhea associated with fever typically suggests an acute inflammatory enteritis caused by one of several specific infectious agents. However, noninflammatory enteritides caused by viral or other agents may also present with diarrhea and fever. This chapter will address bacteria and amebic colitis as potential major causes of this syndrome. Viral and other parasitic etiologies will be addressed in another chapter. The acute inflammatory enteritides include several specific, distal, small bowel, and colonic infections, such as those caused by *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and enteroinvasive *Escherichia coli* (EIEC), as well as the syndromes of necrotizing enteritis (NE) and antibiotic-associated pseudomembranous enterocolitis (*Clostridioides difficile* [formerly *Clostridium difficile*]). Several other infectious agents cause chronic enteric inflammatory processes that may result in syndromes of abdominal pain, weight loss, diarrhea, or malabsorption. These involve disease processes such as gastrointestinal (GI) mycoses; mycobacterioses; bacterial infections, such as those caused by enteropathogenic *E. coli* (EPEC), EIEC, enterotoxigenic (enterohemorrhagic [EHEC]), and enteroaggregative *E. coli* (EAEC); and syphilis.

EPIDEMIOLOGY

Syndromes of acute dysentery with fecal blood and pus have been well recognized since the days of Hippocrates. Dysentery is defined as frequent small bowel movements accompanied by blood and mucus, with tenesmus or pain on defecation. This syndrome, with or without fever, implies an inflammatory invasion of the colonic mucosa resulting from bacterial, cytotoxic, or parasitic destruction.

The pathologic changes of inflammatory colitis range from a superficial intense exudative inflammatory process involving the colonic mucosa, as seen in infection by shigellae or invasive *E. coli* organisms,

to deeper, penetrating, flask-shaped ulcers with undermined edges, as seen in amebic dysentery. The pathogenesis of the inflammatory colitides may involve cytotoxic products of shigellae,[1] certain *E. coli* strains,[2] clostridia, or other organisms.

The epidemiologic patterns of acute dysenteric syndromes are influenced by the unusually low inoculum for infection required by organisms such as shigellae or amebas. As few as 100 shigellae or 10 cysts of enteric parasites, such as *Entamoeba coli* or *Giardia lamblia,* have been found to result in infection in adult volunteers.[3,4] In consequence, there is a substantial risk of person-to-person spread in daycare centers,[5] institutions, or other areas where nonhygienic conditions may allow direct fecal-oral spread. The cysts of parasites such as *Entamoeba histolytica* or *Balantidium coli* often resist chlorination and therefore may cause waterborne outbreaks of dysenteric illnesses. Saltwater or seafood exposure should lead to consideration of *Vibrio parahaemolyticus* as a potential cause of inflammatory colitis or of watery diarrhea, and farm or domestic animal exposure might lead to consideration of nontyphoid *Salmonella* spp., *Campylobacter jejuni* or *Campylobacter coli*, or *Yersinia enterocolitica.* A study in children younger than 5 years in the United States found nontyphoid *Salmonella*, followed by *Campylobacter, Y. enterocolitica,* and *E. coli* O157, were the most common bacterial enteric pathogens associated with illnesses.[6] When typhoid fever is present with diarrhea in an endemic area, the diarrhea is often inflammatory, with many fecal polymorphonuclear or mononuclear leukocytes seen on microscopic examination.[7] A recent multisite birth cohort study (Malnutrition and Enteric Disease Study [MAL-ED]) showed that norovirus GII, rotavirus, *Campylobacter* spp., astrovirus, and *Cryptosporidium* spp. exhibited the highest attributable burdens of diarrhea in the first year of life. In the second year of life the major pathogens were *Campylobacter* spp., norovirus GII, rotavirus, astrovirus, and *Shigella* spp.[8] *Campylobacter* spp. and EIEC were associated with dysentery. Rotavirus and *Shigella* spp. were associated with fever, whereas rotavirus and norovirus GII were most associated with vomiting. A history of travel to areas of poor sanitation may implicate any of the aforementioned pathogens. Finally, venereal exposure, particularly among men who have sex with men, may implicate gonococci, herpes simplex virus, *Chlamydia trachomatis* (including lymphogranuloma venereum serotypes), or *Treponema pallidum* as a cause of proctitis, or *Campylobacter, Shigella, E. histolytica*, or *C. difficile* as a cause of colitis.[9] A history of antibiotic intake and/or recent admission to a health care facility would strongly suggest *C. difficile* infection (CDI), although community-acquired infection has been recognized as well.

Although not grossly inflammatory, the still enigmatic Brainerd diarrhea syndrome, which has been associated with raw milk or unchlorinated water consumption in several outbreaks, may involve some proximal colonic lymphocytic infiltration.[10,11]

The potential causes of acute dysentery are listed in Table 99.1.

TABLE 99.1 Differential Diagnosis of Acute Bacterial Dysentery and Inflammatory Enterocolitis

Specific Infectious Processes

Bacillary dysentery (*Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Shigella boydii;* invasive *Escherichia coli*)
Campylobacteriosis *(Campylobacter jejuni)*
Amebic dysentery *(Entamoeba histolytica)*
Ciliary dysentery *(Balantidium coli)*
Vibriosis *(Vibrio parahaemolyticus)*
Salmonellosis (*Salmonella enterica* serovar Typhimurium)
Typhoid fever (*Salmonella enterica* serovar Typhi)
Enteric fever (*Salmonella enterica* serovar Choleraesuis, *Salmonella enterica* serovar Paratyphi)
Yersiniosis *(Yersinia enterocolitica)*

Proctitis

Gonococcal *(Neisseria gonorrhoeae)*
Herpetic (herpes simplex virus)
Chlamydial *(Chlamydia trachomatis)*
Syphilitic *(Treponema pallidum)*

Other Syndromes

Necrotizing enterocolitis of the newborn
Enteritis necroticans
Pseudomembranous enterocolitis or *Clostridioides difficile* (formerly *Clostridium difficile*) colitis without overt pseudomembranes (*C. difficile*)
Diverticulitis
Typhlitis

Chronic Inflammatory Processes

Enteropathogenic and enteroaggregative *E. coli*
Syphilis
Gastrointestinal tuberculosis
Gastrointestinal mycosis (including *Basidiobolus ranarum*)
Parasitic enteritis

Syndromes Without Known Infectious Cause

Idiopathic ulcerative colitis
Crohn disease
Radiation enteritis
Ischemic colitis
Allergic enteritis
Brainerd diarrhea

MICROBIOLOGY AND PATHOGENESIS OF THE MAJOR CAUSES OF ACUTE DYSENTERIC SYNDROMES

Shigella spp. and Enteroinvasive *Escherichia coli*

Bacillary dysentery is caused by *Shigella* spp. and EIEC. It is estimated that *Shigella* spp. (see Chapter 224) infect more than 200 million people and cause 650,000 deaths each year worldwide.[12] The four *Shigella* species are classified in subgroups A to D—*Shigella dysenteriae, Shigella flexneri, Shigella sonnei*, and *Shigella boydii*, respectively. Genomic studies of *Shigella* spp. have indicated that *Shigella* and EIEC are derived from multiple origins of *E. coli* and form a single pathovar.[13] *S. dysenteriae* is capable of elaborating a potent toxin with enterotoxic, cytotoxic, and neurotoxic properties (Shiga toxin).[13] *S. dysenteriae* is responsible for severe and epidemic disease, and *Shigella boydii* is mostly seen in the Indian subcontinent. *S. flexneri* and *S. sonnei* are the most common species around the world.[14] Shigellae are facultative intracellular pathogens and may cause acute bloody dysentery with high fever and systemic manifestations of malaise, headache, and abdominal pain. The incubation period ranges from 6 hours to 9 days but is usually less than 72 hours. *Shigella* spp. are the most common cause of bloody diarrhea in children, and the syndrome may be particularly severe in poorly nourished children.[14]

Despite the intense superficial destructive process in the colonic epithelium that typifies acute shigellosis, bacteremia and disseminated infection are relatively rare.[15] *S. flexneri* invades and causes inflammatory destruction of the human colonic epithelium. The cell and tissue invasion results from a type III secretion system that delivers effector protein into target eukaryotic cells.[16] Acute shigellosis by *S. dysenteriae* type 1 induces apoptotic cell death in rectal tissues associated with increased production of Fas/Fas ligand, perforin, caspase-1, and caspase-3 but reduced production of B-cell lymphoma 2 (Bcl-2) and interleukin-2 (IL-2).[17] *Shigella* infection, especially with *S. dysenteriae* type 1, is associated with enteric protein loss that ceases after appropriate antimicrobial therapy.[18] This protein loss may contribute to increased susceptibility to secondary infections or growth stunting.[19,20] A complication of severe shigellosis in childhood is a hemolytic-uremic syndrome (HUS), which may be associated with a leukemoid reaction, pseudomembranous colitis, circulating immune complexes, and circulating endotoxin, usually in the absence of demonstrable bacteremia.[21]

Intestinal obstruction, which occurs in about 3% of patients, is a poor prognostic sign, not infrequently associated with death or the development of HUS.[22] Other, more common extraintestinal manifestations of shigellosis are headache, meningismus, and occasionally seizures, especially in children.[23] These findings may be attributable to a neurotoxin that has been demonstrated with *S. dysenteriae* type 1[1,24] but may be seen with any *Shigella* infection. A serious arthritis similar to that seen in reactive arthritis has been described in up to 10% of patients 2 to 5 weeks after the dysenteric illness that characteristically occurs in patients

TABLE 99.2 Enteroinvasive *Escherichia coli* Serotypes

SEROTYPE	DIFCO SEROGROUP TEST[a]
O28	C
O29	—
O112	C
O124	B
O136	C (Trabulsi 193-T-64)
O143	—
O144	—
O152	(Trabulsi 185-T-64)

[a]Difco Laboratories, Detroit.
Modified from references 26, 28, 29, and 184.

with human leukocyte antigen (HLA)-B27.[25] Culture-positive conjunctivitis during acute shigellosis has also been described and may represent autoinoculation of the conjunctiva analogous to that induced in guinea pigs in the Sereny test.[26] Arthritis syndromes have also been described after inflammatory colitis with *Y. enterocolitica, Salmonella enteritidis*, or *C. difficile*, again in association with HLA-B27.[27]

Certain *E. coli* strains may produce a syndrome identical to that seen with acute shigellosis. The incubation period is usually 2 to 3 days after ingestion. Although invasive *E. coli* organisms appear to be limited to certain serotypes (Table 99.2),[26,28,29] to confirm the presence of such organisms, their invasive potential should be demonstrated in research studies with the guinea pig conjunctivitis (Sereny) test[30] or in HeLa cells,[31] or the 120- to 140-megadalton plasmid associated with invasiveness in *Shigella* and invasive *E. coli* should be identified.[32] Invasive *E. coli* organisms were responsible for a single widespread outbreak of dysentery associated with imported French Camembert cheese.[29,33] Although they have been identified as occasional causes of diarrhea in Brazil,[26] invasive *E. coli* organisms do not appear to be frequent causes of sporadic diarrhea in the United States. Because they are often slow to ferment lactose in the laboratory, invasive *E. coli* organisms may be initially mistaken for shigellae,[26,29,31] to which they are closely related. Invasive *E. coli* organisms are also usually lysine negative, often nonmotile,[34] and antigenically related to *Shigella*.[35]

Shiga Toxin–Producing *Escherichia coli*

A significant cause of bloody diarrhea and potentially fatal HUS is now recognized to be due to EHEC organisms (see Chapter 98) that produce Shiga-like toxin, although the frequency with which they cause inflammatory diarrhea is not clear.[2,36] These organisms have attracted particular attention because of widespread outbreaks in popular hamburger chain restaurants[37] and with uncooked produce, such as spinach,[38,39] and a widespread outbreak of bloody diarrhea and severe HUS, starting in Germany and extending across Europe, associated with eating imported Egyptian fenugreek sprouts in the early summer of 2011. The latter, unlike prior EHEC strains that had exhibited EPEC traits of attachment and effacement, was a Shiga toxin–producing EAEC strain.[40,41] Although it accounts for only 0.8% to 3.0% of all cases of diarrhea in the United States and Canada, EHEC (serotype O157) is estimated to account for 15% to 36% of cases of bloody diarrhea.[2] Most recognized EHEC strains are of serotype O157; others include O26:K60:H11, O103:H2, O91:H2, O145:H$^-$, O111:K58:H$^-$, O38:H21, O6:H$^-$, O5:H$^-$, O128, O139, O113:K75, O121, and O172.[2,28,42] EHEC organisms were the most commonly recognized cause of diarrhea (3%) among 5415 patients studied in Calgary in Alberta, Canada, where such cases showed a summer seasonal peak.[43] In addition to causing 15% to 36% of all cases of bloody diarrhea, including outbreaks of hemorrhagic colitis, EHEC is associated with 75% to 90% of cases of HUS in North America, a complication that develops in 8% of EHEC infections.[2]

The incubation period is usually 3 to 4 days after the ingestion of contaminated food or water. Cattle are the major reservoir of the organism. The consumption of inadequately cooked beef, raw milk, or other products contaminated by the intestinal contents of cattle, and occasionally of contaminated water, has been recognized as an important source of O157:H7 infection.[44] A waterborne outbreak of *E. coli* O157:H7 has been documented in upstate New York at a county fair.[45] The infection causes abdominal cramps and watery diarrhea, followed by bloody diarrhea.[46] In contrast to more inflammatory and invasive enteropathies (such as shigellosis or campylobacteriosis), fever is typically absent or minimal on presentation. The HUS and thrombocytopenia may occur 2 to 4 days after the onset of diarrhea, especially in children younger than 5 years and in older adults. EHEC O157:H7 forms characteristic actin pedestals on infected mammalian cells.[47] Previously, typical EHEC organisms shared EPEC traits of secreting their own translocated intimin receptor (Tir) molecule (via a type II secretion system) into the plasma membranes of host cells.[48] The attachment of the organism via its Tir receptor is required to trigger the assembly of actin into focused pedestals beneath bound bacteria. EHEC produces Stx1 or Stx2; the latter is more associated with HUS.[49] Another EHEC virulence factor, H7 flagellin, is also associated with activation of proinflammatory signals in human colonic epithelial cells in response to this noninvasive pathogen.[48] The German outbreak EHEC strain was an EAEC O104:H4 strain that produced Stx2a and was positive for several EAEC genes, including *aggR, aggA* (encoding for the biofilm-producing aggregative adherence fimbriae I [AAF/I]), *sigA, sepA, pic, aatA, aaiC,* and *aap,* but it was negative for the *eae* (*E. coli* attaching and effacing) gene and produced an extended-spectrum β-lactamase.[50,51]

Enteropathogenic and Enteroaggregative *Escherichia coli*

EPEC organisms that cause acute diarrhea in infants may rarely be associated with an insidious persistent or relapsing illness.[52] *E. coli* organisms in O groups 1, 2, 4, 7, and 75 that produce hemolysin and necrotoxin have been isolated from patients with ulcerative colitis. These toxic organisms are not present in healthy people or in patients with acute diarrheal syndromes.[53] In addition, EAEC organisms are recognized as a cause of persistent diarrhea in India, Brazil, and Mexico.[54–56] EAEC infections are an emerging problem (at least in terms of their recognition), and EPEC infections remain important in many parts of the world.[57–59] EAEC organisms have been associated with intestinal inflammation and malnutrition, even in the absence of diarrhea.[60,61] The effects of antibiotic treatment constitute a topic of current interest, and such therapy may be feasible.[62]

Campylobacter Enteritis

Campylobacter spp. have a small genome (1.6–2.0 megabytes) and may live in the GI tract of their natural zoonotic hosts for a long time and can cause intestinal and systemic infection in humans. The genome sequence from multiple *C. jejuni* and several other *Campylobacter* spp. have been published.[63–65] There currently are 16 *Campylobacter* spp. and 6 subspecies. *C. jejuni* and *Campylobacter coli* are the most prevalent species associated with enteric infections. *C. jejuni* is the most prevalent bacteria isolated from enteric infections in industrialized countries. *C. jejuni* and *C. coli* are highly prevalent in developing countries, especially in children younger than 2 years with enteric infection or in asymptomatic carriers. *C. jejuni* systemic infections (see Chapter 216) have been recognized for many years. Although the majority of *Campylobacter* bloodstream infections in humans are with *Campylobacter fetus* (old subspecies, *C. fetus* subsp. *intestinalis*),[66] *C. jejuni* commonly causes an enteric infection in all age groups. This organism was recognized many years ago as a cause of calf and swine dysentery. Commercially available techniques of fecal culture have enabled the culture of *C. jejuni* on highly selective media at 42°C from fecal specimens of patients with diarrhea.[67] *Campylobacter* enteritis results from oral transmission, and a variable but relatively small dose ($5–8 \times 10^2$ cells/mL) is sufficient to cause infection.[68] This organism causes a syndrome of severe abdominal pain, fever, and acute inflammatory enteritis that may range from watery diarrhea to severe dysentery with blood and pus in the stools.[69] Infection has also been associated with sequelae of reactive arthritis and the Guillain-Barré syndrome.[70] Cross-reacting antibodies recognizing both *C. jejuni* lipopolysaccharide and antigenic determinants of nerve gangliosides are speculated to contribute to the development of the

axonal nerve damage after *C. jejuni* infection. Reports from Belgium, England, and central Africa have revealed that in 5% to 14% of unselected cases of diarrhea, *C. jejuni* is present,[69,71] and outbreaks of *Campylobacter* enteritis have been associated with ingestion of contaminated water, raw milk, and uncooked meat and poultry. A review of common source outbreaks of *Campylobacter* infection in the United States between 1997 and 2008 showed that unlike sporadic illnesses, which are primarily attributable to poultry, dairy products were the vehicle most often associated with these outbreaks.[72] There are several genes involved in the adhesion, colonization, invasion, and toxin production by *Campylobacter* spp., and the specific balance between these genes and host susceptibility to *Campylobacter* enteritis remains uncertain.[63]

Clostridioides difficile Colitis

CDI is the most common cause of antibiotic-associated diarrhea. In the past decade the incidence and mortality rates of CDI increased because of the emergence of strains characterized as BI/NAP1/O27/III, which have heightened toxinogenesis.[73] Advanced age is a critical risk factor for severe disease, recurrence, and mortality,[74] although CDI is also now appearing in younger, lower-risk populations.[75] Disruption of normal intestinal microbiota from prior antibiotic use allows *C. difficile* colonization, germination of spores, and production of toxins. The primary virulence factors that are known to cause clinical disease in CDI are two large toxins: *C. difficile* toxin A (TcdA), or toxin A (308 kDa) and TcdB, or toxin B (270 kDa).[76] Both toxins are glucosyltransferases that inactivate Rho, Rac, and Cdc42 proteins and result in actin condensation and subsequent cytoskeletal changes, apoptosis, and cell death of target cells. Tcds also induce an intense inflammatory response characterized by infiltration of inflammatory cells, especially neutrophils; activation of submucosal neurons; secretion of cytokines; chemokines and arachidonic acid metabolites; and production of substance P and reactive oxygen intermediates. Some *C. difficile* strains, including the epidemic RTO27, produce binary toxin that remains poorly characterized but may have a role in host attachment and colonization. Clinical presentation ranges from asymptomatic colonization to severe disease with pseudomembranous colitis (Fig. 99.1) and septic shock. Increased fecal lactoferrin, IL-1β, and IL-8 were observed in patients with CDI.[77] Leukocytosis suggests severe infection, and leukemoid reactions have been described in colitis caused by *C. difficile.*[78]

Vibriosis

In addition to classic and El Tor *Vibrio cholerae* O1 and O139, non-O1 *V. cholerae* and several halophilic *Vibrio* spp. (see Chapters 214 and 215) are now recognized to cause diarrhea and occasional wound or bloodstream infection.[79] The most common and best characterized is *V. parahaemolyticus*, which has been recognized since 1950 in Japan and was identified as a *Vibrio* spp. in 1963. *V. parahaemolyticus* is a cause of seafood poisoning, with onset of signs and symptoms 9 to 25 hours after the ingestion of inadequately cooked fish or shellfish. This has been reported throughout the coastal areas of the United States, especially in warmer waters and on cruise ships and is the most common form of food poisoning in Japan, where raw seafood is commonly eaten as sushi.[80] Diarrhea may be explosive and watery or may be characterized by full-blown dysentery with blood and pus and superficial ulceration on proctoscopic examination.[81] The latter syndrome may be associated with cramps, nausea, vomiting, headache, and fever. The illness is usually self-limited, resolving within 3 to 4 days.

Other halophilic vibrios include *Vibrio alginolyticus, Vibrio fluvialis, Vibrio hollisae, Vibrio damsela*, and *Vibrio vulnificus*, which have been associated with enteric, wound, or systemic infections in humans.[79] *V. vulnificus* has been associated with life-threatening septicemia, often in persons with preexisting liver disease, occurring within 24 hours of ingesting raw oysters.[79,82]

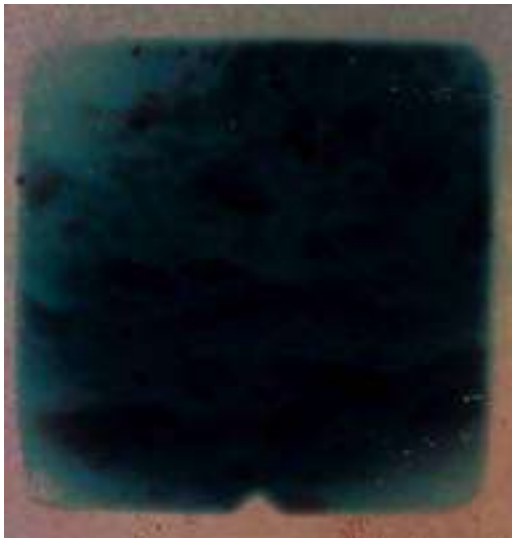

FIG. 99.1 Proctoscopic view of pseudomembranous colitis in a patient who received clindamycin. Note the 4- to 8-mm raised white plaques overlying an erythematous mucosa. *(From Tedesco FJ, Barton RW, Alpers DH. Clindamycin-associated colitis.* Ann Intern Med. *1974;81: 429–433.)*

Salmonellosis and Enteric Fever

Salmonella enterocolitis is characterized by fever, cramping, abdominal pain, and diarrhea that begin 8 to 48 hours after ingestion of an infective dose, usually with food, and usually lasts 3 to 5 days (see Chapter 223). The diarrheal stools of patients with salmonellosis often contain a moderate number of polymorphonuclear leukocytes, usually fewer than is typical of shigellosis.

Although *Salmonella* enteritis predominantly involves the lamina propria in the small bowel, several reports have noted *Salmonella enterica* serovar Typhimurium as a cause of colitis, with crypt abscesses and erosion and ulcerations of the colonic mucosa, which result in blood and pus in the stool.[83] Certain other strains of *Salmonella*—including *Salmonella enterica* serovar Choleraesuis and *Salmonella enterica* serovar Paratyphi, and especially *Salmonella enterica* serovar Typhi—tend to elicit a mononuclear response and cause the bacteremia that is characteristic of enteric fever. Animal models for *S.* Typhimurium continue to advance our understanding of the pathogenesis of intestinal and systemic *Salmonella* infections.[84] *Salmonella* flagellin is regulated by the *fliC* gene, which is the major ligand for Toll-like receptor 5 (TLR5), nucleotide oligomerization domain–like receptors, and interleukin-1b–converting enzyme (ICE) protease-activating factor (IPAF) protein. TLR5 is located in the plasma and phagosomal membranes, and the other two are located in the cytoplasm of the host's Peyer patch cells. Flagellin activates TLR5 and leads to the production of IL-12, whereas the activation of IPAF leads to the production of IL-1β and IL-18. Both pathways act synergistically to activate interferon-γ–producing Th1 cells. These proinflammatory cytokines play important roles in the induction of inflammation with *Salmonella* infections.

Typhoid fever may lead to an erosion of the blood vessels in Peyer patches that, without appropriate treatment, could result in gross blood in the feces in 10% to 20% of patients. Severe intestinal hemorrhage may complicate approximately 2% of cases late in the course of untreated typhoid fever. Such intestinal bleeding may precede perforation, another complication of typhoid fever.[85] However, typhoid fever is primarily a systemic illness, not a diarrheal disease (see Chapter 100).

Yersiniosis

Y. enterocolitica (see Chapter 229A) is another increasingly recognized enteric pathogen that may be responsible for an enteric fever–like illness, mesenteric adenitis (which may mimic acute appendicitis), or an inflammatory ileitis or ulcerative colitis syndrome with fecal neutrophils and mononuclear cells.[86] *Y. enterocolitica* and *Yersinia pseudotuberculosis* may cause similar symptoms and signs. The virulence of *Yersinia* is associated with the direct injection of proteins, such as *Yersinia* protein kinase A (YpkA) and *Yersinia* outer protein E (YopE), into eukaryotic cells.[87,88] *Yersinia* infection may also be associated with migratory polyarthritis, reactive arthritis, or erythema nodosum. A syndrome of acute diarrhea and vomiting is especially common in young children. The organism may cause disseminated abscesses in the liver and spleen[89]

or an inflammatory colitis.[90] The causative agent, a gram-negative member of the family Enterobacteriaceae, is in the same genus as the plague bacillus *Yersinia pestis* and is sometimes mistaken for *Proteus* on initial culture plates.

Amebiasis

Entamoeba histolyticum is one of the classic causes of dysenteric syndromes. Although the course and severity of the infection is variable, amebiasis is one infection that can cause sustained febrile diarrhea with stools becoming grossly bloody. For patients living in endemic areas or travelers who have been to such endemic areas and who have dysenteric symptoms, amebiasis should be considered in the differential diagnosis. For further details about amebiasis, please see Chapter 272.

DIAGNOSIS

Diarrhea with fever, blood in the stool, severe abdominal pain, or signs of sepsis necessitates evaluation for enteric pathogens to guide disease management.[91] Clinical and epidemiologic information direct the specific tests to be requested. Examination for fecal leukocytes often reveals sheets of polymorphonuclear leukocytes in clumps of mucus, even in the absence of gross blood in the stool specimen (Fig. 99.2).[92,93] Fewer pyknotic leukocytes are reported in amebic dysentery[94–96]; this may be attributable to the deeper undermining ulcers characteristic of intestinal amebiasis or to a contact-dependent cytolytic effect of the ameba on leukocytes. The diagnosis of shigellosis is made using bacterial cultures or polymerase chain reaction (PCR) assay for the invasiveness plasmid in organisms isolated from stool, rectal swab, or endoscopic biopsy specimens.[97] The use of fresh specimens promptly plated onto appropriate enteric culture media is very important to ensure the isolation of shigellae.[98]

Specialized techniques are required to isolate *Vibrio* (thiosulfate citrate bile salts [TCBS] agar),[99] *Yersinia* (cold enrichment),[100] or *C. jejuni*.[67] The identification of toxigenic *C. difficile* is done by immunoassay for either *C. difficile* toxin A or B, or cell culture cytotoxicity or PCR assay for *C. difficile* toxin B.[101–104]

The diagnosis of infection with EHEC depends on culturing stool on MacConkey culture medium, on which the original O157:H7 EHEC strain yields sorbitol-negative colonies.[105] The isolated *E. coli* is serotyped for somatic (O) and flagellar (H) antigens. Rapid methods for detection of Shiga toxins in the stool samples are now in use.[106,107] The pathogenic Kanagawa-positive strains from patients produce β-hemolysis on special (Wagatsuma) medium—in contrast to environmental isolates—and are best isolated as blue-green colonies (alkaline) on TCBS agar.[99] A PCR method provides a species-specific probe for *V. parahaemolyticus, V. alginolyticus, Vibrio anguillarum*, and a cluster of related species.[108] Cultivation may require cold enrichment techniques.[100] PCR methods for detection of pathogenic *Y. enterocolitica* are more sensitive than culture methods.[109]



FIG. 99.2 Methylene blue stain of fecal leukocytes from patient with colitis. This exudative response may be seen in any active colitis syndrome, including those caused by the inflammatory or invasive pathogens discussed in this chapter.

Recent approaches using multiple pathogen nucleic acid amplification tests, such as a laboratory-developed TaqMan Array Card (Life Technologies, Grand Island, NY) for simultaneous detection of several enteropathogens, hold promise, with high accuracy, sensitivity and specificity, as well as being potentially suited for surveillance or clinical purposes.[110] However, interpretation of results may be challenging in the presence of multiple pathogens and in determining which specific organisms are clinically relevant. Quantification of burden of infection may assist in the interpretation of results from these molecular assays.[111]

Amebic dysentery is traditionally diagnosed by direct examination of wet mounts of fresh fecal or proctoscopic specimens, which reveal *E. histolytica* trophozoites or cysts characterized by four or fewer delicate nuclei with central karyosomes. Additional patients may be diagnosed by biopsy, which may reveal periodic acid–Schiff stain (PAS)-positive trophozoites or cysts in the undermining ulcer in the lamina propria, or by a fecal or serum *E. histolytica*–specific antigen test, which distinguishes virulent *E. histolytica* from avirulent *Entamoeba dispar* infections.[112]

Sigmoidoscopic examination, especially with biopsy, may be useful in the diagnosis of a pseudomembranous enterocolitis or in the identification of parasites such as *E. histolytica* (with a special PAS stain) or *B. coli*. Amebic colitis is associated with discrete small ulcerations with undermined edges amid relatively normal mucosa. Acute shigellosis causes more widespread, shallow, 3- to 7-mm ulcers with a more intense inflammatory exudate. Barium studies are unnecessary and are relatively contraindicated for toxic patients with acute colitis.

Other considerations in the differential diagnosis of inflammatory colitis are pseudomembranous enterocolitis, which may be associated with antibiotic use, and the potentially rapidly progressive necrotizing enterocolitis syndromes (see Chapters 79 and 243). These diagnoses are suggested by the setting, clinical course, history, and findings on radiologic and proctoscopic examinations. Noninfectious syndromes that may be manifested as acute inflammatory enterocolitis include idiopathic ulcerative colitis and Crohn disease.

Finally, the presence of biomarkers of acute or prolonged intestinal inflammation, such as fecal myeloperoxidase and neopterin, may be associated with growth shortfalls in young children in developing areas.[113]

THERAPY

Therapy consists of careful supportive fluid management with specific antimicrobial therapy directed at a specific pathogen if suspected on the basis of the epidemiologic setting or microbiologic test results. Fluid resuscitation is the primary treatment of all acute diarrheal disease syndromes. Oral rehydration salt solution (World Health Organization formula) is recommended for oral rehydration therapy.[114] Presumptive therapy for the inflammatory colitides varies greatly with the different organisms and is influenced by the increasing resistance of enteric pathogens to antimicrobial therapy.[115] For example, an acute febrile dysenteric illness in a young child with daycare exposure or in an area in which shigellosis is common should be treated with an antimicrobial agent, such as one of the fluoroquinolones. Early administration of empirical antibiotic treatment is justified in children hospitalized for clinical dysentery.[116] Short-course (3 days) oral ciprofloxacin for *S. dysenteriae* type 1 dysentery in children has been effective.[117] If the *Shigella* organism is sensitive, prompt therapy can successfully stop the diarrhea, alleviate systemic symptoms, and reduce shedding of the organisms in the feces.[118–120] Because shigellae are increasingly resistant to multiple antibiotics,[121,122] the practitioner must be familiar with the local resistance pattern of shigellae to treat acute shigellosis appropriately when it is first suspected. Although the fluoroquinolones presently are not approved for use in children in the United States, available data suggest that a short course of therapy is safe.[123] Although ciprofloxacin may shorten the clinical course of *Salmonella* infections, it does not eradicate the organism.[124] Studies have shown increasing resistance of *Campylobacter* and even *Salmonella* to quinolones.[125,126] Azithromycin may reduce the duration of symptoms of *Campylobacter* or *Shigella* enteritis. Zinc 20 mg daily (10 mg of elemental zinc in 5 mL) given in two equally divided doses for 2 weeks showed a significant reduction in the duration of acute shigellosis, promoted better weight gain, and reduced diarrheal morbidity in malnourished children at 6 months

after infection.[127] Vitamin A has also been used to reduce severity and mortality from shigellosis.[128]

Antibiotic therapy has not been shown to be effective for disease caused by EHEC or EIEC. Some EHEC strains may increase their phage-mediated production of Shiga-like toxins when exposed to antibiotics to which they are sensitive, such as ampicillin, tetracycline, sulfamethoxazole-trimethoprim, or quinolones.[129–132]

Most patients with mild-to-moderate *C. jejuni* enterocolitis do not benefit from antibiotic treatment. Severely ill patients, especially those who are debilitated or immunocompromised, appear to benefit from antibiotic therapy, and erythromycin (or other macrolides) or ciprofloxacin are the drugs of choice. Increased fluoroquinolone resistance in *Campylobacter* spp. from humans and animals, the result of mutations in topoisomerase genes, has been well-documented.[133] Two studies now associate symptomatic and asymptomatic *Campylobacter* infections with reduced growth in Brazilian and Peruvian children.[134,135]

Vancomycin and fidaxomicin are the drugs of choice for nonsevere and severe disease caused by CDI (see Chapter 243 for further details).[136,137] Recurrent disease occurs in 20% to 65% of the patients, depending on the number of previous episodes of the disease.[138] Fidaxomicin, the only other US Food and Drug Administration–approved drug for CDI, is reported to be equivalent to vancomycin in treating acute CDI but superior in preventing relapses.[139] However, its advantage over vancomycin is lost in infections caused by the prevalent epidemic strain BI/NAP1/027. Alternative approaches to prevent recurrent disease include probiotics,[140] although their efficacy is marginal; fecal microbiota transplantation (FMT)[141]; or use of monoclonal antibodies.[142] FMT is increasingly being used to prevent recurrent disease or treat refractory CDI. More recently, the monoclonal antibody against TcdB has been shown to confer modest protection against recurrent CDI.[91] Probiotics, when administered in patients receiving antibiotics, may be beneficial for patients at high risk of acquiring CDI.[143]

Antibiotic treatment with tetracycline or doxycycline can diminish the duration and severity of GI symptoms caused by *V. parahaemolyticus*. To prevent *V. parahaemolyticus* infections, consumption of raw or undercooked shellfish and exposure of wounds to seawater should be avoided.[144]

Antibiotic therapy is not required for *Salmonella* enteritis, but patients who are severely ill or those with risk factors for developing extraintestinal spread of infection should be treated with fluoroquinolones or third-generation cephalosporins.

Yersinia infection is usually self-limiting; however, patients with severe illness or systemic infection are usually treated with tetracycline, chloramphenicol, or ciprofloxacin.

PREVENTION

Enteric pathogens are predominantly spread through contaminated food or water and sometimes via direct fecal-oral spread. Spread of infection occurs during ingestion of contaminated food or water or contact with a contaminated environment. Infection control measures include hand hygiene, proper food handling, and access to clean water. In some instances isolation and treatment of infected individuals are necessary to prevent spread of infection, for example, in cases of CDI.

Immunization of susceptible individuals is an important component of prevention. However, for bacterial enteric pathogens, vaccine is only available for typhoid fever and cholera. Typhoid vaccination is recommended for people traveling to endemic areas. Ty21a is a live-attenuated oral vaccine containing *S.* Typhi in capsule formulation. Another typhoid vaccine is the parenteral Vi-polysaccharide. For individuals traveling to areas with recent or ongoing cholera outbreaks, CVD 103-HgR, a live-attenuated oral vaccine, is available. There is a need to develop effective vaccines for most infectious agents causing diarrhea worldwide.

OTHER ACUTE INFECTIOUS/ INFLAMMATORY PROCESSES INVOLVING THE INTESTINAL TRACT

Gonococcal Proctitis

Neisseria gonorrhoeae (see Chapter 212) may be the cause of ulcerative proctitis, usually acquired by anal intercourse.[9] The resultant purulent proctitis is accompanied by an erythematous, friable mucous membrane in the rectal vault and occasional abscess or fistula formation. Although copious purulent discharge, tenesmus, and burning rectal pain may be noted, two-thirds of culture-positive patients with anorectal gonococcal infection are asymptomatic.[145] Additional diagnostic possibilities in cases of venereally acquired proctitis are syphilitic, herpetic, and chlamydial proctitis.[146]

Unusual Causes of Dysentery

Spirillar or spirochetal dysentery has been reported to occur in southern France and has been attributed to *Spirillum* spp. Although severe mucoid diarrhea or dysentery has been associated with intestinal spirochetes, their frequency and role in causing enteric disease are unclear. A DNA probe for the 16S ribosomal RNA of the agent of swine dysentery, *Treponema hyodysenteriae*, has been developed and may open new approaches to the recognition of similar infections in humans.[147]

Other unusual causes of colitis include brucellosis[148] and adenovirus infections.[149,150]

Necrotizing Enterocolitis in the Newborn

The syndrome of diffuse, fulminating, necrotizing colitis has been recognized in infants since the 1960s. This syndrome probably represents the same entity described as spontaneous intestinal perforation and peritonitis as early as 1838.[151] Although milder forms of the syndrome doubtless exist, the syndrome of necrotizing enterocolitis (NEC) is defined by the presence of air in the wall of the intestine, portal venous system, or peritoneal cavity, or by necrosis of the bowel wall with mucosal sloughing. This fulminant syndrome often leads to intestinal perforation, peritonitis, and bacteremia. It is a major cause of mortality in low-birth-weight infants (<1500 g) after the first week of life.[152] The diffuse necrotic changes that characterize this syndrome most often occur in the terminal ileum but may be seen in the colon or in the proximal portion of the GI tract.

The pathogenesis of NEC appears to involve mucosal injury that is most often of ischemic origin from hypoxemic or hypotensive episodes; these may occur in premature infants or in the presence of complicating factors, such as an umbilical vein exchange transfusion. Ischemia may also result from the effects of endotoxemia, followed by the effects of epinephrine, to which the vessels supplying the terminal ileum may be especially sensitive. Other factors predisposing to mucosal ischemia include asphyxia in association with hyaline membrane disease in premature infants or cyanotic heart disease. Increased intraluminal pressures may contribute to ischemia and pneumatosis, a process that may also play a role in previously normal infants who develop NEC after protracted periods of diarrhea. Some investigators have suggested a localized Shwartzman reaction to endotoxemia or gram-negative bacteria.[153] The absence of lysozyme (normally present in human breast milk) may allow overgrowth of gram-negative bacilli. EPEC serotype O111:B4 has also been associated with NEC.[154] Because of the association with the use of umbilical vein polyvinylchloride catheters and feeding tubes, the toxic effect of plasticizers leached from the polyvinylchloride materials has also been suggested.[155] Reports of outbreaks of NEC in newborn intensive care units (ICUs)[156,157] have led to a careful search for infectious agents, including viral, fungal, and bacterial pathogens.[158] Among bacteria, *Pseudomonas, Klebsiella*,[159] certain *E. coli* strains,[154,160] *Salmonella*,[161] and *Clostridium butyricum*[157] have been implicated. The roles of ischemia and bacteria have been suggested by Barlow and colleagues[162] with studies in an experimental rat model of NEC, in which breast milk was shown to be protective. On the basis of an acidic intraluminal pH (<5.0) and organic acids in human neonates with NEC,[163] increased numbers of lactose-fermenting *Klebsiella* organisms have been postulated to play a role in the pathogenesis.[164] Other investigators have suggested a role for platelet-activating factor and protection by superoxide dismutase or endogenous nitric oxide.[165,166] The role of the intestinal microbiota in the pathogenesis of NEC is being actively investigated.

Clinical features of this serious condition in newborn infants include apneic spells, vomiting, abdominal distention, and occasionally bloody diarrhea. Most infants are younger than 1 week, and there is an association with prematurity, maternal infections during delivery (e.g., amnionitis with prolonged postruptured membranes status), and exchange transfusion via the umbilical vein. There is no gender or seasonal predilection. The

disease often progresses rapidly to intestinal perforation, shock, septicemia, and pneumatosis intestinalis. Air may also be evident in the portal venous system or biliary tract on plain radiographs. This syndrome is associated with a mortality rate that is often in excess of 70%.

The diagnosis of NEC should be considered in any premature infant with altered GI function, abdominal distention, or apneic spells. Further investigation should include examination of the stool for occult blood and for the presence of reducing substances. Plain abdominal radiographs may reveal air in the bowel wall, peritoneal cavity, or portal venous system, and there may be bloody diarrhea late in the course of the disease. Management must be initiated early and aggressively for any infant suspected of having NEC. Umbilical catheters should be removed, oral feeding should be stopped, and nasogastric aspiration should be initiated. Intravenous fluid therapy is of paramount importance. Laparotomy and excision of the necrotic bowel are often necessary and should be done aggressively if there is any evidence of peritonitis or obstruction.[167]

Prevention of NEC includes avoidance of risk factors and careful infection control measures in newborn ICUs. Hypertonic elemental formulas have been implicated and should be avoided in high-risk patients. NEC rarely occurs in breastfed infants. Explanations for the advantage of human breast milk include the presence of lysozyme, antibodies, and cellular elements that may play a protective role against potential infectious agents. Although oral prophylactic nonabsorbable antibiotics have been suggested, serious questions remain about the use of prophylactic antibiotics, even in high-risk newborn infants weighing less than 1500 g. A few reports have shown that epidermal growth factor, which is found in high concentrations in breast milk, may have a cytoprotective effect, and probiotics may decrease or prevent neonatal necrotizing enterocolitis.[166,168]

Darmbrand, Pig-Bel, Necrotizing Enteritis in Adults (Enteritis Necroticans)

First described as *Darmbrand* (meaning fire bowels) in epidemics of enteritis necroticans in northern Germany in the immediate postwar period in the mid-1940s, a severe necrotizing jejunitis has also been recognized in epidemic and sporadic forms after pork feasting in the highlands of Papua New Guinea.[169] Pig-bel was the name given to the syndrome of abdominal discomfort that followed a large pork meal, commonly eaten after a large pig kill, which takes place every 3 to 10 years among the highland Melanesians of Papua New Guinea. Sporadic cases have been reported from other parts of the world, including the United States.[170]

The pathologic findings are those of acute, patchy, necrotizing disease of the small bowel in previously healthy people, which may proceed rapidly to segmental gangrene, with small amounts of gas in the mucosa, mesentery, or nodes.

Several theories of pathogenesis have been suggested, most of which involve the toxic products of *Clostridium perfringens* type C, including α- and β-toxins. Sporadic cases of NE have been noted in association with nutritional disorders, alcoholism, and malabsorption, and after pancreatic or gastric resection.[171] After gastric surgery, increased numbers of *C. perfringens* organisms and increased levels of α-toxin have been noted in the upper small bowel and stomach. Whether α- or β-toxins are capable of causing NE alone, or whether they initiate the invasion of the mucosa by other organisms, such as gram-negative rods, is currently unclear. An attractive hypothesis was suggested by Lawrence and Walker,[171] which could explain the association of NE with poor nutrition and episodic dietetic overindulgence. The low-protein diet of Papua New Guinea highlanders is associated with low levels of digestive proteases in the intestinal lumen, which can be shown to inactivate the β-toxin. The proteases can be further blocked by the oral intake of trypsin inhibitors, which are found in this population in such dietary staples as sweet potatoes. Proteases return with improved diet, as occurred in postwar Germany. This hypothesis has been confirmed in an animal model that required protease inhibitors for symptomatic infection.[172]

The clinical syndromes of NE range from anorexia, vomiting, severe abdominal pain, and bloody diarrhea to fulminant toxemia and shock. Acute complications that necessitate emergency surgery include paralytic ileus, bowel strangulation, and bowel perforation with peritonitis. These complications are common in the first 2 weeks of illness. Later complications that may also necessitate surgery include scarring, leading to stenosis; obstruction; malabsorption; and fistulas. NE occurs with greater frequency and greater severity in children younger than 10 years. In contrast to European control subjects who rarely have antibodies, 70% of the healthy adults in Papua New Guinea have demonstrable antibody to clostridial β-toxin.[169,171]

The syndrome is defined by the pathologic findings but should be suspected in patients who develop severe abdominal pain, bloody diarrhea, ileus, and toxemia. The course is often too fulminant for radiographic detection of air in the bowel wall to be of any diagnostic value.

Agents held responsible for causing NE include *C. perfringens* type C, once designated as type F in the older classification of *Clostridium welchii.* Most surgically resected bowel specimens from patients with NE contain *C. perfringens*, more than half of which are type C. Furthermore, 12 of 21 cases described had a significant change in serum β-antitoxin titer after illness with pig-bel in Papua New Guinea.[169] Although polyvalent gas gangrene antiserum was ineffective, administration of type C antiserum resulted in a 30% decrease in the need for surgery, and mortality was reduced from 43% to 19%.[169] Furthermore, active immunization against the β-toxin has also proved effective in preventing pig-bel.[173]

Some have suggested that type A *C. perfringens*, staphylococci, or even hepatitis virus may be responsible for NE. The syndrome of enteritis gravis has been described in association with infectious hepatitis, although no viral cause has been documented.

Considerations in the differential diagnosis of NE include acute shigellosis, acute food poisoning syndromes, antibiotic-associated pseudomembranous colitis, and acute ulcerative colitis. The absence of colonic involvement; the epidemiologic setting, especially in poorly nourished patients; and the rapid progression to toxemia and shock are strongly suggestive of NE.

Therapy for NE includes careful supportive care and bowel decompression. Fluid requirements may be substantially greater than what is indicated by fecal output. Resection of the involved bowel must be considered if there is a persistence of paralytic ileus, a rapid increase in signs of toxemia, localized or diffuse signs of peritonitis, persistent pain, or a palpable mass lesion. If subacute obstruction or malabsorption is suspected on the basis of weight loss, elective surgery may be required up to 6 months after the acute illness. Raw peanut or soybean diets should be avoided because they contain trypsin inhibitors. *C. perfringens* type C antiserum containing β-antitoxin or the active β-toxin vaccine should be available for use in areas where NE can be expected to occur.

CHRONIC INFECTIOUS/ INFLAMMATORY PROCESSES INVOLVING THE INTESTINAL TRACT

Chronic inflammatory enteritides are often indolent, slowly progressive infections. Often, there is a history of weeks or months of fever, abdominal pain, weight loss, or other systemic manifestations. Recurring or relapsing symptoms may be seen with *C. jejuni* or *Salmonella* gastroenteritis. In addition, 16% of cases of shigellosis may become prolonged, lasting for 3 weeks or longer.[174] Any diarrheal illness that extends beyond 2 weeks is considered severe and is associated with a high risk of nutritional morbidity in children who live in tropical developing areas.[175–177]

Syphilis

Syphilis (see Chapter 237) can also involve the GI tract, usually in the upper part of the small bowel or stomach. An acute erosive and infiltrative gastritis with motile spirochetes and a positive specific response on treponemal immunofluorescence testing has been reported in late secondary syphilis.[178] The initial complaints are upper abdominal pain, vomiting, and weight loss. More classic are the late GI manifestations of lues: pyloric obstruction, hourglass constriction, and linitis plastica of the stomach. Less commonly, gumma may be seen in the small bowel or colon.

Gastrointestinal Tuberculosis

Intestinal tuberculosis (TB), once considered common, had become a relatively rare disease but is now reemerging in association with

acquired immunodeficiency syndrome and with multidrug-resistant *Mycobacterium tuberculosis* (see Chapter 249).[179] Intestinal involvement with TB may be either primary, from ingestion of the organisms, or secondary, usually from a pulmonary source.

Primary intestinal TB without pulmonary disease often results in hypertrophic mucosal changes. Patients with acute miliary TB may also have GI involvement.[180] Patients with primary intestinal TB may present with abdominal pain, fever, and a tender, fixed, palpable mass in the ileocecal area. Primary hypertrophic intestinal TB continues to occur in the Middle East[181] and in India.[182] Intestinal involvement secondary to pulmonary TB may result from swallowing infected sputum or from biliary excretion of the organism from an infected liver. The frequency of secondary intestinal TB increases with far-advanced pulmonary disease. Hippocrates stated that "diarrhea attacking a person with phthisis is a mortal symptom."

TB may involve any part of the GI tract, but most ulcerative and hypertrophic types occur in the ileocecal region, where there is a predominance of submucosal lymphatic tissue. The most common features are fever and abdominal pain that is often relieved by defecation or vomiting. Weight loss is more common in secondary intestinal TB. Only one-third of the patients with GI TB have diarrhea. Diarrhea may be related to exacerbations of abdominal pain and occasionally occurs with extensive involvement of the small intestine, which may cause steatorrhea and a malabsorption syndrome. Although ulceration and mucous diarrhea are relatively common with secondary intestinal TB, hemorrhage and the presence of gross blood in the stool are distinctly uncommon, perhaps because of the associated obliterative endarteritis.

The diagnosis of GI TB may be very difficult radiologically and even histologically. It must be distinguished from regional enteritis, sarcoidosis, actinomycosis, ameboma, carcinoma, and periappendiceal abscess. In contrast to Crohn disease, GI TB rarely causes anal lesions, fistulas, or perforation. It is often associated with miliary nodules on the serosa, it rarely causes strictures longer than 3 cm, and it may cause circumferential transverse ulcers. TB may also cause fibrosis of the muscularis mucosa, pyloric metaplasia, and epithelial regeneration.[183] There may be minimal or no radiologic changes in the bowel mucosa. Small mucosal ulcerations may result in tiny calcified nodules in the mucosa in association with calcified mesenteric lymph nodes analogous to those seen in the pulmonary Ghon complex. The ileocecal region often reveals radiologic evidence of irritability and hypermotility, with hypersegmentation of the mucosal folds or poor filling of the ileocecal region detected by barium enema. On occasion, frank ulcerations can be noted on contrast studies, and late in the course there is scarring. The diagnosis requires a careful examination of involved tissue for acid-fast bacilli by using special stain and culture. Caseous necrosis is more frequently found in the mesenteric nodes than in intestinal tissue itself. Complications of intestinal TB include perforation, peritonitis, and obstruction from hypertrophy, scarring, or tuberculoma.

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The complete reference list is available online at Expert Consult.

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100 Typhoid Fever, Paratyphoid Fever, and Typhoidal Fevers

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SHORT VIEW SUMMARY

Definition

- Enteric fever, or "typhoid fever," is a nonspecific febrile illness caused by typhoidal *Salmonella* (*Salmonella enterica* serotypes Typhi and Paratyphi); the diagnosis should be considered in any patient with otherwise unexplained prolonged fever who has resided in or recently traveled in a typhoid-endemic setting.
- The term *typhoidal fever* is sometimes used more broadly to refer to a syndrome of persistent high-grade fevers, often with no localizing features. This chapter also covers the broader differential diagnosis and approach to patients with typhoidal fever, relapsing or prolonged fever, and fever accompanied by abdominal pain.

Epidemiology

- An estimated 12 to 24 million cases of enteric fever occur each year, largely in impoverished areas of Asia and Africa.
- Enteric fever is transmitted via the fecal-oral route, with contaminated municipal water supplies being commonly involved in transmission.
- Multidrug-resistant strains of the major causative agents of enteric fever (*S. enterica* serotypes Typhi and Paratyphi A) are now common worldwide.

Clinical Manifestations

- Common life-threatening complications of enteric fever include intestinal hemorrhage, perforation, encephalopathy, and shock.
- Untreated, patients with enteric fever may be febrile for 3 to 4 weeks or longer, with mortality rates exceeding 10%, and prolonged asthenia and fatigue are common among survivors.
- Chronic biliary carriage of typhoidal *Salmonella* may occur after resolution of the acute illness.

Diagnosis

- Current diagnostic tests for enteric fever are imperfect. Blood cultures are 30% to 70% sensitive; bone marrow cultures are more sensitive but are impractical; serologic assays lack both sensitivity and specificity, especially in enteric fever endemic areas; and nucleic acid amplification assays with sufficient sensitivity are not available.

Therapy

- Given the morbidity of typhoid fever, the risk of complications, and the lack of optimal diagnostic tests, the initiation of antibiotics for treating individuals with suspected enteric fever may be based on a presumptive diagnosis, particularly in resource-limited settings.
- The most commonly used agents for treating individuals with enteric fever are fluoroquinolones, azithromycin, and cefixime or ceftriaxone. Chloramphenicol, trimethoprim-sulfamethoxazole, and amoxicillin may be used to treat patients with susceptible strains.

Prevention

- An oral-attenuated typhoid vaccine and injectable polysaccharide vaccine are internationally commercially available and provide 50% to 75% protection for 5 and 2 years, respectively. A Vi-tetanus toxoid conjugate vaccine is now prequalified and recommended by the World Health Organization. No vaccine effective against paratyphoid A is currently commercially available.

ENTERIC FEVER (TYPHOID AND PARATYPHOID FEVER)

History

Historically termed *putrid fever* or *dothienteritis,* the name *typhoid* was coined in 1829 by Pierre Charles Alexander Louis. The name *typhoid* means "typhus-like" and reflects the difficulty in differentiating the illness from epidemic typhus, another common cause of prolonged fever in Europe during the 19th century. Louis used the term *typhoid* in a landmark report comparing the intestinal pathology in 50 patients who died of typhoid fever with 83 patients who died of noninfectious causes, and he described the characteristic inflammation of Peyer patches, intestinal ulceration, and mesenteric adenitis associated with typhoid fever.[1]

William Budd is credited with the recognition of the fecal-oral transmission of typhoid fever in 1839, based on his meticulous observations during a large epidemic in the Taw Valley of England, although these findings were not widely recognized until many years later.[2]

The etiologic agent of typhoid fever was identified in pathologic specimens by Karl Eberth, who referred to it as *Bacillus typhosus*; the organism was subsequently first cultured in 1884 by Georg Gaffky at the Berlin Institute for Infectious Diseases. The term *paratyphoid fever* was first used in 1896 by Raoul Bensaude and Emile Achard, who isolated what would subsequently be recognized as *Salmonella* Paratyphi B. The role of asymptomatic carriers in the transmission of infectious disease was first recognized by Robert Koch, a theory that he derived from his longitudinal observations of patients recovered from typhoid but with continued shedding of *B. typhosus*.[3] An understanding of the role of asymptomatic carriers in disease transmission was soon extended to other organisms and was an established mode of disease transmission at the time of George Soper's association of Mary Mallon (also known as Typhoid Mary) with repeated outbreaks of typhoid fever in households in New York, ultimately leading to her lifelong quarantine on North Brother Island.[4]

Widal developed the first serologic test for typhoid fever in 1896, an agglutination assay that detects the presence of antibodies against the O and H antigens of *Salmonella* Typhi.[5] Despite inherent and significant limitations (see "Diagnosis"), the Widal test remains widely used in many resource-limited settings. The first vaccines for typhoid fever were killed whole-cell vaccines developed in 1896, credit for which is shared by Almroth Wright and Richard Pfeiffer.[6–9] Despite advances in the development of a vaccine, typhoid fever remained a leading cause of morbidity and mortality in the early 19th century. A prominent example was a typhoid fever epidemic in the Spanish-American War, in which 20,000 American Army recruits contracted the disease and 1600 died. This defining event was linked to the first compulsory US military–wide vaccination program, in which a killed whole-cell, injectable typhoid vaccine developed by Frederick Russell was used.[10]

In the early 20th century, "typhomalaria" or "typhomalarial fever" remained an extremely common diagnosis, indicating that differentiating typhoid from malaria (and other causes of persistent fever) on clinical grounds was difficult. The difficulty distinguishing typhoid fever from typhus and other causes of fever proved clinically fortuitous. In 1948, Theodore Woodward and coworkers[11,12] reported the effect of chloramphenicol in two patients who were referred for a study of the efficacy of the drug in scrub typhus. Both were subsequently found to have *Salmonella* Typhi bacteremia, and both patients improved rapidly after antibiotic therapy.[11] Typhoid fever mortality has been reduced from 10% to 1% or lower with the use of antibiotics. However, successive pandemics of antibiotic-resistant organisms have occurred, including, most recently, the emergence of fluoroquinolone- and ceftriaxone-resistant strains of *Salmonella* Typhi. In 2001, the complete sequence of a *Salmonella* Typhi strain (CT18, isolated from a patient in Vietnam in 1993) was published by Parkhill and coworkers[13] at the Wellcome Trust Sanger Institute, ushering in a genomic era in our understanding of typhoid fever. Genomic analysis has opened up new insights into the history and epidemiology of typhoidal *Salmonella,* from zoonotic spillover[1] to previously unrecognized historical outbreaks[2] and recent global dissemination.[3]

Etiologic Agents of Enteric Fever

Nomenclature and Classification of Typhoidal *Salmonella enterica*

Salmonella Typhi and *Salmonella* Paratyphi A, B, and C are gram-negative bacilli that belong to the species *S. enterica* subspecies *enterica.* All *S. enterica* are categorized serologically by the Centers for Disease Control and Prevention (CDC) according to a modified Kauffman and White classification scheme, assigned based both on the O (the O polysaccharide) and H (flagellar) antigens.[14,15] Although assigning a serogroup based on O antigen agglutination tests is a common procedure in many clinical microbiology laboratories, the approach has limited clinical utility. Complete serotyping based on both the O and H antigen is most commonly performed in reference laboratories. In addition to serogrouping and serotyping, standard microbiologic growth and biochemical parameters are also routinely used in the clinical laboratory for the presumptive identification of *S. enterica.* Serologic and selected biochemical characteristics that are used in the identification of *Salmonella* Typhi and *Salmonella* Paratyphi A, B, and C in microbiology laboratories are summarized in Table 100.1.

Clinical Distinction Between Typhoidal and Nontyphoidal *Salmonella enterica*

Salmonella Typhi and *Salmonella* Paratyphi A and B are human-restricted pathogens. *Salmonella* Paratyphi C is pathogenic for animals and humans, and *Salmonella* Typhi and *Salmonella* Paratyphi are classified clinically as typhoidal *Salmonella* strains. This distinction separates these strains from all other pathogenic *S. enterica* serotypes, which are referred to collectively as nontyphoidal *Salmonella* (often abbreviated as NTS; see Chapter 223). Typhoidal strains cause enteric fever in all human hosts, whereas NTS strains are classically associated with inflammatory diarrhea in human hosts or preferentially cause invasive disease in young children and immunocompromised individuals, with particular associations with malnutrition, HIV, and malaria. However, the clinical distinction between typhoidal and NTS is not absolute. NTS may cause invasive infection that includes prolonged bacteremia (with or without pyogenic foci) that may mimic the systemic illness caused by typhoidal strains.[16] Although host factors influence susceptibility to invasive NTS infection, the likelihood of invasive infection is also dependent on characteristics of the bacterial strain. For example, the emergence of a dominant genotype associated with invasive NTS, *Salmonella* Typhimurium ST313, in sub-Saharan Africa, illustrates the clinical overlap between typhoidal and nontyphoidal *S. enterica.*[17]

Genomic Features

Escherichia coli and *S. enterica* diverged over 100 million years ago, and *S. enterica* is now a genetically heterogeneous species.[18] *Salmonella* Typhi is genetically homogeneous, meaning there is very limited genetic diversity across *Salmonella* Typhi isolates throughout the world. Based on standard rates of genetic drift, it is estimated that *Salmonella* Typhi emerged from a single-point origin within the last 50,000 years, coinciding with the time of human migrations out of Africa and into Asia.[19,20] The genetic material of *Salmonella* Typhi consists of a single chromosome that contains approximately 5×10^6 base pairs encoding approximately 4000 genes. *Salmonella* Typhi often harbors additional plasmids that may be cryptic (confer an unknown function) or involved in pathogenesis. The latter include IncHI1 plasmids that comprise the majority of resistance plasmids found in *Salmonella* Typhi and that remain stable in the bacteria without ongoing pressure from antibiotic selection.[21,22]

The genetic core of *S. enterica* consists of genes that are conserved across Enterobacteriaceae and is approximately 90% similar to *E. coli*. In contrast, the genetic core varies approximately 1% between *S. enterica*

TABLE 100.1 Classification and Selected Microbiologic Characteristics of Typhoidal *Salmonella*

SEROTYPE NAME		*SALMONELLA* TYPHI	*SALMONELLA* PARATYPHI A	*SALMONELLA* PARATYPHI B (SCHOTTMUELLERI)	*SALMONELLA* PARATYPHI C (HIRSCHFELDII)	COMMENTS
Serologic classification[a]	Serogroup	D	A	B	C	
	O antigen	**9**, 12	1, **2**, 12	1, **4**, (5), 12	6, 7	
	H antigen Phase 1 : 2	d: –	a: (1, 5)	b: (1, 2)	c: (1, 5)	Uncommon *Salmonella* Typhi H antigen variants include Hj:z66 found in Indonesia
	K antigen	Vi	–	–	Vi	
Selected biochemical characteristics[b]	Lactose fermentation	–	–	–	+	The majority of *Salmonella* Paratyphi C ferment lactose
	Sulfide (H_2S) production	+ (weak)	–	+	+	3% of *Salmonella* Typhi do not produce hydrogen sulfide
	Gas produced during glucose fermentation	–	+	+	+	
	Lysine decarboxylase	+	–	+	+	
	Ornithine decarboxylase	–	+	+	+	

[a]Strong antigenic determinants are listed in bold; weaker or sometimes absent ones are in parentheses.
[b]Isolates can be identified as *Salmonella* using traditional methods. A presumptive diagnosis of *Salmonella* Typhi can often be made for *Salmonella* isolates based on selected biochemical characteristics, in addition to agglutination with D and Vi antisera.
From World Health Organization (WHO), Department of Vaccines and Biologicals. Background Document: The Diagnosis, Treatment and Prevention of Typhoid Fever. *Geneva: WHO; 2003; and WHO, Department of Communicable Disease Surveillance and Response.* Salmonella *serotype Typhi. In:* WHO Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. *Geneva: WHO; 2003:103–118.*



FIG. 100.1 Global distribution of typhoid fever. *(Modified from Mogasale V, Maskery B, Ochiai RL, et al. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment.* Lancet Glob Health. *2014;2:e570–e580; and Kim JH, Mogasale V, Im J, Ramani E, Marks F. Updated estimates of typhoid fever burden in sub-Saharan Africa.* Lancet Glob Health. *2017;5:e969.)*

serovars. In addition to the genetic core, *Salmonella* Typhi contains pathogenicity islands—horizontally acquired genetic elements that are often associated with unique properties of each strain and are essential for specific phenotypes associated with pathogenesis of each serotype.[18] In *S. enterica,* these are specifically identified as *Salmonella* pathogenicity islands, or SPIs, and are designated with a number. *Salmonella* Typhi possesses more than 20 SPIs, many of which are found in other *S. enterica* serotypes. For example, SPI-7 is a 134-kb genetic island that possesses elements encoding the viaB locus, which is involved in production of the Vi capsule and evasion of the host immune response.[23] SPI-7 is widely conserved across *Salmonella* Typhi and is also found in *Salmonella* Paratyphi C.

One of the most remarkable genetic features of *Salmonella* Typhi is the accumulation of more than 200 (apparently) functionally inactive genes known as pseudogenes.[13] This adaptation may play a major role in the host restriction of *Salmonella* Typhi. The accumulation of a large number of pseudogenes is a common feature shared with *Salmonella* Paratyphi A, B, and C, which emerged independently of *Salmonella* Typhi (although subsequent recombination events between *Salmonella* Typhi and *Salmonella* Paratyphi A appear to have occurred),[24] yet are also adapted specifically to cause disseminated infection in humans. The same phenomenon has been observed in *Salmonella* Typhimurium ST313 in sub-Saharan Africa; ST313 has accumulated extensive gene deletions and (apparent) pseudogene formation (more than half of which are common to *Salmonella* Typhi and *Salmonella* Paratyphi A) compared with less invasive *Salmonella* Typhimurium strains.[25]

Epidemiology

Burden and Distribution

Measures of the burden of enteric fever are limited by the absence of surveillance in many regions and also by the limited sensitivity of current diagnostic tests. Recognizing these limitations, estimates of the number of cases of typhoid fever range from 12 to 27 million annually.[4] The incidence of typhoid fever and the mortality of the disease vary dramatically by region (Fig. 100.1). In South Asia and parts of sub-Saharan Africa, the incidence approaches 1000 cases per 100,000 person-years.[5,26] In parts of northern Africa, South America, and Southeast Asia, the incidence is much lower and ranges between 10 and 100 cases per 100,000 person-years. Disease is highly heterogeneous even within endemic countries, and the incidence tends to be considerably higher in urban than in rural areas. In the United States and many other high-resource settings, the disease occurs sporadically, with less than 1 case per 100,000 person-years, and occurs most often in travelers returning from endemic zones (see "Acquisition of Disease in Areas Where Enteric Fever Occurs Sporadically").

Salmonella Paratyphi A is by far the most common cause of paratyphoid fever and is responsible for an estimated 5 million cases of enteric fever annually.[6,27] The proportional burden of enteric fever caused by *Salmonella* Paratyphi A has increased substantially over the past 2 decades, and in some areas of South Asia, *Salmonella* Paratyphi A is responsible for more than 20% to 50% of cases of enteric fever.[6,28] In contrast, *Salmonella* Paratyphi B and C remain less common causes of enteric fever globally, with the proportional incidence of infections caused by these pathogens varying by location.

Severity

In the preantibiotic era, 10% to 15% of clinically recognized cases of typhoid fever were fatal, whereas other cases resolved over a 3- to 4-week course of an often severe and debilitating illness.[7] Prospective surveillance in the postantibiotic era has revealed that the majority of cases of enteric fever are uncomplicated and do not warrant hospitalization. Although the current mortality of typhoid fever is estimated at 0.5% to 1%, this estimate is based on few data.[8] Reported mortality rates in hospitalized patients with enteric fever in resource-limited settings vary widely by location, ranging from 0% to 15%, with a median of 2%.[29] In the United States, the fatality rate of reported cases is 0.2%.[30] In addition to fatal cases, in sub-Saharan Africa and other regions where enteric fever is highly endemic, intestinal perforation caused by enteric fever is a leading cause of peritonitis and acute abdominal conditions requiring surgery in children.[31]

Source of Infection

Salmonella Typhi and *Salmonella* Paratyphi A and B are human restricted. There are no known animal reservoirs of these pathogens that cause enteric fever, and the source of infection is organisms shed in the stool of infected humans. *Salmonella* Paratyphi C can be shed by humans and animals. In the preantibiotic era, patients recovering from enteric fever shed organisms in the stool during acute illness and typically for weeks during convalescence. In addition, a small portion of individuals convalescing from typhoid fever develop chronic asymptomatic carriage (defined as shedding of the bacteria in stool or urine for more than a year), which may persist for life.[32] In the preantibiotic era, up to 5% of typhoid fever survivors developed chronic asymptomatic bacterial shedding in the stool,[33,34] with gallbladder disease being the major risk factor for carriage.[35,36] Risk of carriage appears to be far greater in adults (3%–10%) than in young children (<1%).[9] Studies from Nepal, where

enteric fever is highly endemic, found that 3.5% of individuals undergoing elective cholecystectomy grew *Salmonella* Typhi or *Salmonella* Paratyphi A from biliary cultures.[37] Even higher rates were observed in Santiago, Chile in the early 1980s.[10] Urinary carriage may also occur, frequently in conjunction with urinary schistosomiasis.[38] Although chronic carriers are a source of transmission where enteric fever occurs sporadically,[30,39] their importance in the transmission of enteric fever in highly endemic areas is uncertain.[11]

Mode of Transmission

Infections with typhoidal *S. enterica* are most often acquired via ingestion of fecally contaminated water or food. *Salmonella* Typhi may persist for weeks after passage in water and may persist in a variety of contaminated food items—for example, dehydrated formula and iced beverages.[39–41] Volunteer studies may not optimally mimic natural exposure; however, the inoculum required to produce disease in 50% of adult volunteers (ID_{50}) is $10^{5\text{-}7}$ organisms, although fewer than 10^3 organisms may produce disease.[42,43]

Molecular epidemiologic studies based on high-resolution genotyping implicate waterborne transmission as the most likely route of transmission in the majority of cases of enteric fever.[44] A study in the Kathmandu valley of Nepal revealed that >80% of water collected from public taps contained typhoidal *Salmonella* DNA.[12] Both waterborne and foodborne transmission may contribute to large epidemics, and in some cases, massive epidemics have been associated with water contamination from a single source.[13,45] In contrast, direct person-to-person transmission of the etiologic agents of enteric fever appears less common, and more than 80% of cases of enteric fever occur in individuals with no known contact with an individual with symptomatic infection.[46] Even more remarkably, in households where multiple cases of enteric fever occur, only 20% of those infections shared a common bacterial genotype, suggesting that multiple infections within a household occur through a community-based exposure to multiple circulating genotypes, rather than through direct person-to-person transmission.[44] In highly endemic areas, consistent personal risk factors for infection with typhoidal *S. enterica* include drinking nonboiled water and eating food prepared outside the home, including street-vended foods and beverages.[14,47,48] In South Asia, peak transmission occurs during and immediately after the monsoon season (peaks in June through October),[15] although disease is present year-round.[49]

Acquisition of Disease in Areas Where Enteric Fever Occurs Sporadically

In areas where enteric fever occurs sporadically, most cases are imported through travel. In the United States, >85% of cases occur in travelers to endemic areas.[16,30,50] The rate of typhoid fever is estimated at 10 per 1 million travelers arriving from Asia but increases to 89 per 1 million travelers arriving from India. More than 80% of all travel-associated cases in the United States relate to travel to South Asia.[16] The risk of travel-related enteric fever is higher among travelers visiting friends and relatives.[51] Many of the remaining non–travel-associated cases in the United States are traceable to small, local foodborne outbreaks and/or a chronic carrier.[30] From approximately 1991 to 2010, more than half of foodborne outbreaks in the United States were linked to an asymptomatic carrier.[50,52] In 2010, an outbreak of *Salmonella* Typhi occurred that involved at least 12 cases across three western US states and was attributed to imported fruit pulp from Guatemala.[53] Also, in areas where enteric fever occurs sporadically, small clusters of cases of sexual transmission of *Salmonella* Typhi have been documented in men who have sex with men.[54] Infections and clinical cases have occurred among microbiology laboratory staff, and in at least once instance, secondary cases occurred among family members of laboratory personnel.[17]

Antibiotic Resistance and Emergence of Pandemic *Salmonella* Typhi

Chloramphenicol-resistant *Salmonella* Typhi was first reported in 1950, 2 years after the antibiotic was first used to treat patients with typhoid fever.[55] However, widespread dissemination of antibiotic-resistant strains did not occur until the 1970s. Multidrug-resistant (MDR) *Salmonella* Typhi carrying IncHI1 plasmid-mediated resistance to chloramphenicol, ampicillin, trimethoprim, and sulfonamides became common in the 1980s. Nalidixic acid–resistant strains of *Salmonella* Typhi and *Salmonella* Paratyphi A, with decreased susceptibility to fluoroquinolone antibiotics, emerged in the 1990s,[56] and now the vast majority of typhoidal *Salmonella* isolates from South Asia have reduced susceptibility or high-level resistance to fluoroquinolones.[18,19] At present, reduced susceptibility to ciprofloxacin is less common and more sporadic in sub-Saharan Africa.[20]

Although the lack of genetic diversity of *Salmonella* Typhi previously hindered efforts to characterize the global epidemiology of disease, the increasing use of high-density genotyping methods, including whole-genome sequencing, has circumvented these challenges and resulted in a better understanding of the forces driving the evolution and transmission of *Salmonella* Typhi. Although a general lack of diversity among *Salmonella* Typhi suggests a lack of strong selection pressure over millennia, the recent expansion of a single fluoroquinolone-resistant haplotype known as H58 into a pandemic strain found throughout Asia and Africa, and the emergence of several other lineages with diverse *gyrA* mutations, suggest that fluoroquinolones may be exerting a uniquely strong selection pressure that is shaping the global evolution of *Salmonella* Typhi.[20,57] More recently, *Salmonella* strains with resistance to third-generation cephalosporins (e.g., ceftriaxone) have been reported.[21–24]

Host Factors and Susceptibility to Infection

Historically, enteric fever was considered a disease that predominantly affected school-aged children and young adults. However, prospective community-based surveys in highly endemic areas have demonstrated that the incidence of *Salmonella* Typhi bacteremia is often highest in young children (<5 years old) and that this organism may be responsible for more than 75% of cases of occult bacteremia in these settings.[58] The higher incidence of enteric fever in older children among inpatients may reflect a predisposition to more severe disease in this older age group. In highly endemic areas, *Salmonella* Typhi bactericidal antibodies and anticapsular (Vi) antibodies increase significantly over the first decade of life.[59] In less endemic areas, the median age of patients with typhoid fever increases, suggesting that the age distribution of patients may be influenced by acquired immunity. However, protection from a single episode of infection is limited, as demonstrated by frequent relapse and recurrent infections among patients who have recovered from typhoid fever.[60]

Although invasive NTS infections classically occur in persons who are compromised by extremes of age, malnutrition, immunodeficiency, or genetic risk factors, there is not an overt association between host risk factors and susceptibility to enteric fever. Various candidate gene analyses have drawn associations between single-nucleotide variations in Toll-like receptor 4 (TLR4), cystic fibrosis transmembrane conductance regulator (CFTR), and specific human leukocyte antigen (HLA) types with susceptibility to typhoid fever in Vietnam[61,62]; however, no detailed genome-wide study of typhoid fever associations has yet been reported.

Pathogenesis

Typhoidal *Salmonella* infection is acquired through ingestion of bacilli, typically in contaminated water, drinks, or food. The ID_{50} is often cited to be $10^{5\text{-}7}$ organisms, but low inocula ($<10^3$ organisms) may also produce infections.[25] After passing through the intestinal epithelium, typhoidal *Salmonella* are phagocytosed by macrophages and proliferate in the submucosa before dissemination via the lymphatic system and bloodstream. This period coincides with the onset of systemic symptoms and, in some cases, end organ manifestations. It is during this phase that bacilli enter the hepatobiliary system and may establish carriage in the gallbladder. Disseminated infection is terminated by immune clearance or administration of antibiotics, but bacterial persistence in the bone marrow is often longer.

It is important to note that enteric fever and NTS infections differ in fundamental aspects. With NTS, infections are typically self-limited in immunocompetent hosts and manifest largely as an inflammatory gastroenteritis. In comparison, typhoidal serotypes are able to evade the normal host inflammatory response and cause prolonged bacteremia even in immunocompetent individuals, typically without overwhelming sepsis or pyogenic foci of infection. Specific bacterial adaptations used

by *Salmonella* Typhi to produce persistent bacteremia in the absence of an immediately overwhelming inflammatory response include strategies to breach the intestinal epithelium in the absence of inflammatory diarrhea, to evade detection by host pattern-recognition receptors,[63] to produce an AB5 bacterial toxin that acts through cell-to-cell paracrine signaling pathways,[64] and to persist and disseminate throughout the human host within phagocytic cells.[65] Much of our understanding of host-pathogen interactions in enteric fever is extrapolated from mouse models of enteric fever, which use Nramp1-deficient mice that develop disseminated infection from *Salmonella* Typhimurium. Additional models are under development.

Invasion

In contrast to NTS and other enteroinvasive pathogens, it is thought that soon after ingestion, typhoidal *Salmonella* pass though the intestinal epithelium through specialized microfold cells (M cells) that transport the bacteria across the basolateral membrane, where they are then phagocytosed by macrophages in the intestinal lymphoid tissue.[66] Such a stealth entry mechanism may explain why the invasion phase of *Salmonella* Typhi infection is usually asymptomatic and is accompanied by transient or mild diarrhea in only 10% to 20% of patients.

Latency and Dissemination

Typhoidal *Salmonella* deploy an array of virulence factors that enable them to persist and replicate in an intracellular compartment.[67,68] Important cloaking mechanisms of *Salmonella* Typhi include suppression of flagellar protein synthesis, thus masking a potent inducer of TLR5 responses,[69] and synthesis of the Vi capsule that presumably masks the detection of lipopolysaccharide (LPS) and other outer membrane components.[70] The genes that repress flagellin biosynthesis and are responsible for Vi biosynthesis are encoded on the viaB locus located on SPI7, which is absent in most NTS strains.[23] Induction of other bacterial defenses that are required for survival in the intracellular compartment are regulated by a two-component bacterial sensory-regulatory system, PhoP/PhoQ.[71,72] The sensor PhoQ detects signals present inside the host phagosome and activates the transcriptional regulator PhoP, which in turn controls the expression of a large number of downstream genes involved in modification of lipid A, resistance to antimicrobial peptides, and acidification of the phagosome.[73,74] Consistent with a role in pathogenesis, *phoPQ* mutants are nonvirulent in humans and have been evaluated as candidate live-attenuated vaccines.[75,76]

Quantitative blood cultures demonstrate that more than 60% of culturable circulating *Salmonella* Typhi organisms reside in the intracellular compartment.[77] Presumably, this allows for dissemination throughout reticuloendothelial tissues via the blood and lymphatic systems. The heaviest burden of infection is established in the reticuloendothelial system (intestinal lymphoid tissue, liver, spleen, and bone marrow) and gallbladder. Patients with typhoid fever most often have very low grade bacteremia, a feature that presents a formidable diagnostic challenge. The kinetics of quantitative bacterial cultures has been studied in Vietnam.[77] The median number of culturable bacteria is less than 1 colony-forming unit (CFU) per milliliter of whole blood in adults, and slightly higher (1.5 CFUs/mL) in children.[77] Slightly more culturable organisms are present in the bone marrow, with a median of 10 per milliliter of bone marrow.[78] However, over the course of illness, the number of culturable *Salmonella* Typhi organisms in the blood decreases (median of 2 CFUs/mL in the first week to 0.1 CFUs/mL in the second and subsequent weeks), and the number of culturable bacilli increases in the bone marrow (4.8 CFUs/mL in the first week to 158 CFUs/mL in the third and subsequent weeks). As a result, the proportion of bacteria in the bone marrow increases from 5:1 (marrow to blood) in the first week of illness to more than 150:1 in the third week of illness, reflecting relative clearance of bacteria from peripheral blood but persistence in the marrow compartment.[78] Pathologic examination of bone marrow may demonstrate monocytic infiltrates typical of typhoid fever (see "Intestinal and Other Local Pathology").

The initial period of replication and dissemination represents the incubation period of typhoidal *Salmonella* infection. This period typically lasts 1 to 2 weeks but can range widely (3–60 days), depending on the number of organisms ingested.[79] Ultimately, infection results in the sufficient secretion of inflammatory cytokines, including pyrogenic cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which result in fever. However, unlike what occurs during prototypical gram-negative bacteremia, septicemia with hypotension, neutrophilia, and disseminated intravascular coagulopathy (DIC) are extremely uncommon in patients with typhoid or enteric fever.

Intestinal and Other Local Pathology

Intestinal lymphoid tissue is a predominant site of localized inflammation and persistent bacterial replication in cases of severe enteric fever (Fig. 100.2).[80–83] Early in the course of illness, Peyer patches in the terminal ileum and draining mesenteric nodes are enlarged and contain infiltrates

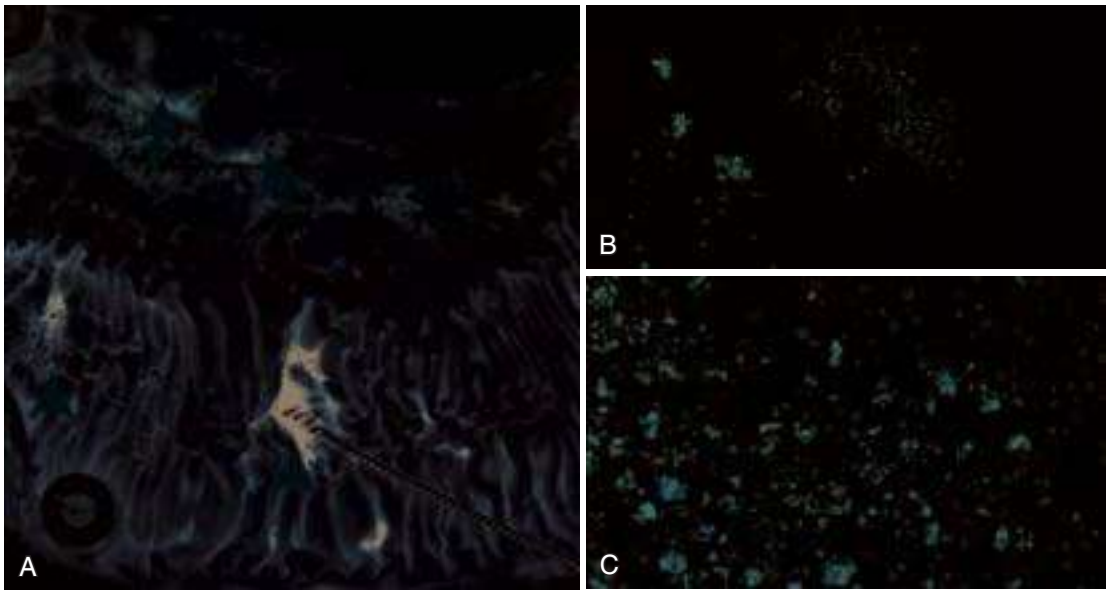

FIG. 100.2 Intestinal pathologic appearance of enteric fever. (A) The visualized mucosal surface of a surgically resected area demonstrates two perforations *(arrowheads)*; the visualized serosal surface demonstrates significant enlargement of lymph nodes *(arrows)*. (B and C) Lower- and higher-magnification views demonstrate immunohistochemical staining for the *Salmonella* O:9 antigen in a necrotic lymph node. *(From Neil KP, Sodha SV, Lukwago L, et al. A large outbreak of typhoid fever associated with a high rate of intestinal perforation in Kasese District, Uganda, 2008–2009.* Clin Infect Dis. *2012;54:1096.)*

consisting of mononuclear cells, including macrophages and lymphocytes.[80] As disease progresses, necrosis of intestinal lymphoid tissue occurs, with mixed inflammatory infiltrates, including neutrophils and ulceration and sloughing of overlying intestinal mucosa.[80] Immunohistochemical staining for the *Salmonella* Typhi O antigen reveals abundant organisms in necrotic areas.[81] The clinical manifestations of these changes include hemorrhage and/or intestinal perforation, two of the major life-threatening complications of enteric fever (Fig. 100.3). Other affected organs include the liver, with monocytic infiltrates and foci of parenchymal necrosis, and the spleen, with nodular monocytic infiltrates in the red pulp.[84] Similar monocytic infiltrates may occur in the gallbladder, and gallbladder perforation, which may mimic intestinal perforation, is a rare complication of typhoid fever.[85]

Relapse and Chronic Carriage

Untreated, typhoid fever may persist for up to 4 weeks, and relapse occurs in up to 10% of untreated cases, usually within 2 weeks after initial resolution of fever.[86] Relapse is usually due to the same strain as that associated with the original infection.[87] The gallbladder is the primary site of chronic carriage, although some asymptomatic carriers continue to shed the organism in stool even after cholecystectomy. The in vitro and clinical observations that *Salmonella* Typhi forms biofilms on cholesterol gallstones may explain the strong epidemiologic association between gallstones and carriage.[88,89] An association has been reproducibly observed between chronic carriage of *Salmonella* Typhi and risk of gallbladder carcinoma, but it is unknown whether carriage causes cancer or reflects underlying biliary epithelial surface abnormalities.[90,91]

Clinical Manifestations

The clinical manifestations of enteric fever are nonspecific. Fever is reported in the vast majority of clinically apparent cases, and many other symptoms are variably reported, including headache, cough, nausea, vomiting, constipation, and diarrhea.[8] Although paratyphoid fever was considered less severe than typhoid fever, more recent comparisons suggest that typhoid fever and paratyphoid fever caused by *Salmonella* Paratyphi A are generally indistinguishable on clinical grounds.[92] Most patients with enteric fever are diagnosed in the ambulatory setting, and up to 90% are treated as outpatients.[86] In contrast, classic descriptions of the features of enteric fever are derived from series of hospitalized patients with more severe disease.[93] Complications of enteric fever were generally thought to occur late in the course of disease, usually after 2 weeks of fever. However, experience has demonstrated that major complications of enteric fever, including intestinal perforation and encephalopathy, may occur within days of onset of fever.[81,82,94]

Uncomplicated Typhoid Fever

Clinical features of enteric fever diagnosed in the ambulatory setting are listed in Table 100.2. Fever without localizing signs or symptoms may be the sole manifestation of enteric fever. The onset of fever may be insidious, and fevers typically increase over the first week of illness.[95] A variety of other nonspecific flulike symptoms are common early in the course of enteric fever, and headache, anorexia, myalgias, and malaise may precede the onset of fever.[92,96] Mild confusion may be seen even in ambulatory patients, and a nonproductive cough is also a common feature of uncomplicated enteric fever. Abdominal complaints may include diarrhea, constipation, and abdominal pain. Invasive diarrhea, such as seen in NTS gastroenteritis, does not typically occur in enteric fever.

The physical findings of uncomplicated enteric fever are also nonspecific. Although relative bradycardia, or pulse-temperature dissociation, is a classic sign of enteric fever, it may not be a clinically useful predictor of enteric fever for individual patients, and is absent in the majority of patients.[8,97] Rose spots, the classic cutaneous manifestation of enteric fever, are 1- to 4-mm blanching pink macules, and are most often seen on the chest, back, and abdomen during the second week of fever. However, rose spots are uncommon in uncomplicated typhoid fever. Mild, nonlocalizing, abdominal tenderness may be present. Hepatomegaly and splenomegaly, if present, are usually modest. A white or yellowish-brown coating of the tongue that spares the tongue's edges is a common physical finding.[92]

Laboratory Findings in Uncomplicated Infection

Although abnormal white blood cell counts in peripheral blood are common in patients with bacteremia, the majority of patients with uncomplicated enteric fever do not have leukocytosis, neutrophilia, or increased immature neutrophils.[92] However, either leukocytosis or leukopenia may be present. Both the hematocrit and platelet counts are typically normal or slightly low. Elevated serum aspartate transaminase and alanine transaminase are very common in enteric fever; values two to three times above the upper limit of the normal range are typical.[98] On occasion, more severe hepatitis is observed, but very high levels of blood transaminases (>500 IU/L) should prompt concern for other causes, including viral hepatitis or drug toxicity.[98]

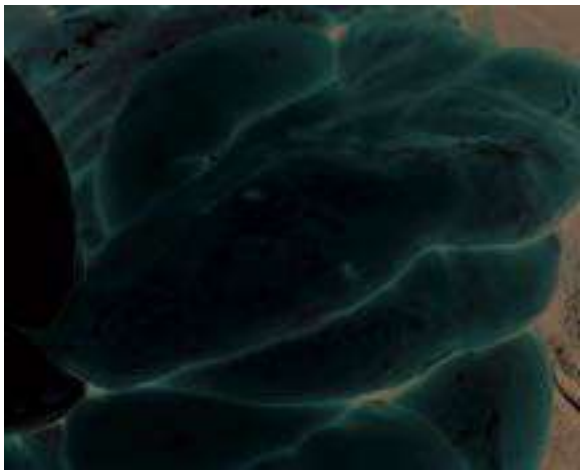

FIG. 100.3 Intraoperative photograph of intestinal perforation caused by *Salmonella* Typhi. Intestinal perforation is visible on the antimesenteric border of the small bowel, which is inflamed with patchy exudates on the serosal surface. *(Courtesy Dr. Pukar Maskey, Patan Hospital, Katmandu, Nepal. From Harris JB, Brooks WA. Typhoid and paratyphoid (enteric) fever. In: Magill AJ, Ryan ET, Hill DR, Solomon T, eds.* Hunter's Tropical Medicine and Emerging Infectious Diseases. *9th ed. Philadelphia: Saunders; 2013:568–576.)*

TABLE 100.2 Clinical Features of Typhoid and Paratyphoid Fever

	CLINICAL FEATURE	APPROXIMATE FREQUENCY[a]
Flulike symptoms	Fever	>95%
	Headache	80%
	Chills	40%
	Cough	30%
	Myalgia	20%
	Arthralgia	<5%
Abdominal symptoms	Anorexia	50%
	Abdominal pain	30%
	Diarrhea	20%
	Constipation	20%
Physical findings	Coated tongue	50%
	Hepatomegaly	10%
	Splenomegaly	10%
	Abdominal tenderness	5%
	Rash	<5%
	Generalized adenopathy	<5%

[a]The proportion of patients demonstrating these clinical features of enteric fever varies, depending on the time, the region, and the type of clinical population (hospitalized or ambulatory) assessed. Estimates are drawn from recent case series in an endemic area, with patients presenting for ambulatory or inpatient care.[92,96] *Modified from Magill AJ, Ryan ET, Hill DR, Solomon T, eds.* Hunter's Tropical Medicine and Emerging Infectious Diseases. *9th ed. Philadelphia: Saunders; 2013:568–576.*

Severe Illness

Classic descriptions of the features of enteric fever are drawn from observations of series of hospitalized patients in the preantibiotic era, in which mortality rates were consistently in the 10% to 15% range. The natural history of untreated disease included progressively increasing fevers over the first week of illness, followed by increasing abdominal complaints and rash over the second week of illness, followed by complications, including intestinal hemorrhage and perforation, or gradual resolution in the third and fourth weeks of illness.[93]

In the present antibiotic era, it remains true that because enteric fever progresses gradually, patients who are severely ill with enteric fever may have been febrile for longer than a week when they first come to clinical attention. Patients with severe enteric fever may appear toxic, and characteristically would have moderate abdominal pain or tenderness, and constipation or diarrhea. Physical findings in severe enteric fever include hepatomegaly and splenomegaly. Patients with severe enteric fever are more likely to have major complications listed in Table 100.3. Complications associated with increased mortality in severe typhoid fever include intestinal hemorrhage and perforation, severe encephalopathy, seizures, and pneumonia.[99]

Gastrointestinal Complications

Intestinal hemorrhage occurs in up to 10% of hospitalized patients with severe enteric fever and is usually self-limited.[86] Intestinal perforation, a major life-threatening complication of enteric fever, occurs in up to 3% of hospitalized patients.[100,101] These complications arise directly from invasion of *Salmonella* into Peyer patches in the small intestines. A series of patients from Vietnam with intestinal perforation resulting from enteric fever demonstrated that the median length of illness preceding perforation was 9 days from the onset of fever, although some cases occurred within the first week.[82] Perforation is suggested by clinical signs of peritonitis, including tachycardia, leukocytosis, neutrophilia, and abdominal pain with guarding and rebound tenderness. The clinical diagnosis of perforation requires a high index of suspicion because patients with severe enteric fever may have a toxic appearance and significant abdominal tenderness even before perforation. Radiographic evidence of pneumoperitoneum may be present in only 50% of cases.

TABLE 100.3 Complications of Typhoid and Paratyphoid Fever

SYSTEM	COMPLICATION	NOTES
Gastrointestinal	Hemorrhage	10%–15% in hospitalized patients
	Perforation	3% in hospitalized patients
Hepatobiliary	Jaundice	1%–3% in hospitalized patients
	Hepatitis	Usually subclinical (↑ ALT/AST)
	Acute cholecystitis	Rare; gallbladder may perforate
Neurologic	Mild encephalopathy	Confusion or apathy common
	Severe encephalopathy	Delirium, stupor, or coma
	Seizures	Common in children ≤5 yr
	Meningitis	Rare, primarily infants
	Guillain-Barré syndrome	Reported
Respiratory	Bronchitis	Dry cough is common
	Pneumonia	May be other concomitant bacterial infection (e.g., *Streptococcus pneumoniae*)
	Empyema	Rare reports
Cardiovascular	Myocarditis	Usually subclinical (ECG changes)
	Endocarditis	Rare reports
Hematologic	Anemia	Usually subclinical
	Disseminated intravascular coagulation	Usually subclinical (↑ PT/PTT)
Other	Musculoskeletal pyogenic infections	Osteomyelitis (particularly vertebral), psoas abscess and others reported
	Hemolytic-uremic syndrome	Reported
	Miscarriage	Reported

ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *ECG,* electrocardiogram; *PT,* prothrombin time; *PTT,* partial thromboplastin time.
Modified from Magill AJ, Ryan ET, Hill DR, Solomon T, eds. Hunter's Tropical Medicine and Emerging Infectious Diseases. *9th ed. Philadelphia: Saunders; 2013:568–576.*

Neurologic Complications

Various case series have documented a widely varying prevalence of diverse neuropsychiatric manifestations during enteric fever, ranging from encephalopathy, encephalomyelitis, transverse myelitis, meningitis, ataxia, and Guillain-Barré syndrome.[94,102] Although most of these manifestations are uncommon, patients with severe enteric fever often present with some component of encephalopathy, and a history of confusion or an apathetic affect are common.[86] Of note, typhoid was named for its clinical similarity to typhus, and the name *typhus* was from the Greek term for "smoky" or "cloudy," reflecting the neurologic features that often accompanied this febrile illness. More severe encephalopathy, manifesting with delirium, stupor, and coma, occurs in a smaller number of hospitalized patients and is associated with a high risk of mortality.[99,103,104] Osler described this as a "pseudo-wakeful state" of "muttering" delirium, known as "coma vigil," and noted that it portends poor outcomes. Even in cases of severe encephalopathy, microbiologic evaluation of cerebral spinal fluid is usually unrevealing, and pleocytosis, if present, usually reveals fewer than 35 cells/μL.[103,104] Encephalopathy during enteric fever may be most common among older children and young adults.[105] Seizures during enteric fever are most common in young children and are associated with increased mortality.[99] Meningitis caused by *Salmonella* Typhi is seen primarily in infants.[106]

Metastatic Pyogenic Complication

Enteric fever is a disseminated bacterial infection, but pyogenic abscess formation during enteric fever is notably infrequent, although *Salmonella* Paratyphi C is more likely to cause abscesses and focal infections than the other typhoidal strains. This contrasts with what occurs during invasive nontyphoidal salmonellosis, in which osteomyelitis, joint infection, abscess formation, and endovascular infection more frequently occur. Although uncommon, pyogenic complications during typhoid have been described and include empyema, osteomyelitis, muscle abscess (particularly involving the psoas), and endovascular infections and endocarditis.[107]

DIAGNOSIS

The laboratory diagnosis of enteric fever is often challenging because current culture-based, serologic, and molecular diagnostic tests for enteric fever fail to achieve an optimal combination of sensitivity and specificity.[108,109] For this reason, it is often appropriate to treat patients with suspected enteric fever with empirical antibiotic therapy. The diagnosis of enteric fever should be considered in any person with fever, especially in those with fever lasting longer than 3 days and who have had an exposure in the last 1 to 6 weeks to an area where enteric fever is endemic. In endemic areas, other clinical factors that are associated with a higher likelihood of enteric fever include a temperature greater than 39°C, ill appearance, young age (<5 years), and any abdominal complaints, including abdominal pain, diarrhea, or constipation.[58] As the duration of fever increases, the likelihood of enteric fever increases.[86] Despite this, it is important to recognize that the majority of individuals suspected of having enteric fever, even in endemic settings, do not have enteric fever but rather an alternative infection[18]; therefore, clinical suspicion of and empirical treatment for enteric fever should not halt the search for alternative causes of fever.

Culture-Based Diagnostics

Although a presumptive diagnosis of enteric fever may be sufficient grounds for initiating and continuing antimicrobial therapy, a definitive diagnosis of enteric fever is made only through the isolation of a typhoidal serotype of *S. enterica* (*Salmonella* Typhi or *Salmonella* Paratyphi A,

B, and C) from the blood, bone marrow, stool, urine, or other clinical specimen of a febrile patient. Isolation of the causative organism also allows antimicrobial resistance testing, which facilitates optimal management.

Blood culture is the most common method of diagnosis, where adequate microbiologic facilities exist. The sensitivity of blood culture varies from approximately 40% to 80%,[86,110,111] and a recent meta-analysis estimated an average sensitivity of 61%.[27] The low-grade bacteremia in enteric fever is an inherent limiting factor to the sensitivity of blood cultures, as is prior receipt of antibiotics.[77] To maximize the sensitivity of blood cultures, the World Health Organization (WHO) recommends that cultures be performed using 10 to 15 mL of blood from school-children and adults, and 2 to 4 mL of blood from toddlers and preschool-aged children.[79] The use of a nonselective broth media, as is standard for most blood cultures, is encouraged because it maximizes detection of a wide range of pathogens. The sole use of *Salmonella* selective or enriching media, such as ox bile medium, is discouraged.[79]

Although less sensitive than blood cultures, stool cultures are positive in more than 50% of children and 30% of adults with enteric fever, and they increase the diagnostic yield over blood cultures alone by approximately 5%.[112] To maximize detection, WHO recommends the use of greater than 1 g of stool and selenite enrichment broth.[79] Culturing of bone marrow has the highest sensitivity (80%–95%) of all the culture-based approaches, with culturing of a 1-mL aspirate of bone marrow having the sensitivity of a 15-mL peripheral blood culture in an adult.[77,78] Culturing of bone marrow is, however, not clinically practical for most patients with suspected enteric fever, although microbiologic and hematologic analysis of a bone marrow aspirate may be part of an evaluation of a patient with a prolonged fever of unknown origin when less invasive testing is unrevealing. Other specimens that may yield growth of *Salmonella* Typhi or *Salmonella* Paratyphi A, B, and C include urine, duodenal aspirates, and specimens from skin biopsy of rose spots. However, these approaches are not routinely recommended. Cultures made from intestinal biopsy specimens and peritoneal fluid of patients with perforation are rarely positive.[81,82]

Serologic Tests

The Widal test detects agglutinating antibodies against the O and H antigens of *Salmonella* Typhi. The Widal test was developed over a century ago and remains one of the world's most widely used diagnostic tests but suffers from significant limitations in its sensitivity, specificity, and reliability.[108] As with most serologic tests, a false-negative Widal test result may occur early in the course of illness, and a false-positive Widal test result may be caused by past infection or previous exposure to cross-reactive antigens or vaccination. There are no universal standards that define the cutoff dilution of agglutinating antibodies to indicate a positive Widal test result. The very low specificity of the assay (50%–70%) and the inability to discern active from previous infection or vaccination means that the assay should rarely, if ever, be used.

Other rapid serologic tests for enteric fever have been developed. Major commercial tests include the IDL Tubex and Typhidot assays. The IDL Tubex test (IDL Biotech AB, Bromma, Sweden) is a rapid immunochromatographic test that detects immunoglobulin M (IgM) antibodies to the O:9 antigen (the major antigenic determinant of *Salmonella* Typhi LPS). An advantage of this approach is that T-cell–independent IgM responses that target *S. enterica* O polysaccharide develop early in the clinical course of illness. Studies have suggested that the sensitivity of the IDL Tubex is approximately 70% to 80%, with a specificity of 80% to 90% when evaluated against standards involving patients with blood culture–proven typhoid fever versus other known causes of bacteremia.[26,113–116] The Typhidot assay (Malaysian Biodiagnostic Research, Bangi, Malaysia) that detects both IgM and IgG to a 50-kDa outer membrane protein antigen of *Salmonella* Typhi has similar performance characteristics.[113–115,117]

Several newer serologic approaches have been developed and evaluated in clinical studies. These include detection of anti-*Salmonella* IgA in secretions of peripheral blood lymphocytes[28,29,118] and new antigen targets for enzyme-linked immunosorbent assays (ELISAs).[30,31] These diagnostics have demonstrated greater accuracy in distinguishing typhoid from other febrile illnesses than currently available serologic tests, but these newer assays have not yet been commercialized.

Molecular Approaches

Molecular diagnostic approaches based on nucleic acid detection have met with only mixed and limited success, presumably reflecting the low-level bacteremia that has hindered culture-based methods, and the action of inhibitors in human blood; such assays are not currently commercially available.[32,108] Although some promising results with new approaches have been seen in small studies,[33] further clinical validation is needed to characterize their accuracy. The lack of adequate diagnostic testing for enteric fever means that this remains an active area of investigation.[108]

Screening for Chronic Carriage

Identifying chronic carriers of typhoidal *Salmonella* may be important for prevention of transmission, particularly in the context of enteric fever outbreaks in nonendemic settings in which the source of infection is unknown. The conventional approach to identify carriers involves collection of at least three stool specimens (separated by days to weeks) for microbiologic culture to isolate *Salmonella*. Although specificity is excellent, the sensitivity of this approach is estimated at 70% to 80%.[34] Screening by duodenal string capsules is an alternative which may improve this yield, although the string test is not currently commercially available.[35] There is an extensive body of literature on the use of Vi antibody testing to identify carriers, but the results have been mixed,[36,37] and using Vi as a carrier detection target will be further compromised as Vi-based typhoid conjugate vaccines become key components of global typhoid-control strategies.

Management

Appropriate antibiotic management (Table 100.4) reduces the mortality rate of enteric fever from 10% to 15% to less than 1%, and shortens the duration of fever from 3 to 4 weeks to 3 to 5 days after initiation of therapy.[11] Uncomplicated enteric fever is treated with a single antibacterial drug.[86] However, the widespread emergence of antibiotic resistance in *Salmonella* Typhi and *Salmonella* Paratyphi A limits the arsenal of effective antibiotics in many areas. The selection of an appropriate antibiotic is dependent not only on antibiotic resistance patterns but on the severity of illness, the age of the patient, and the availability and cost of antibiotics in resource-limited settings. In addition to antibiotic therapy, supportive care, monitoring for complications, and adjunctive therapy with pulse-dose steroids for severe disease (see later) are important aspects of management of patients with enteric fever.

Chloramphenicol, Ampicillin, and Trimethoprim-Sulfamethoxazole

Chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole were the original first-line antibiotics for treating patients with enteric fever. Widespread resistance emerged in the 1980s, through dissemination of IncH1 plasmids conferring simultaneous resistance to all three agents (Fig. 100.4). Such strains remain widely disseminated and are termed MDR *Salmonella* Typhi. However, over the past several years, there has been a decline in MDR *Salmonella* Typhi concomitant with the rise in fluoroquinolone resistance.[19,38]

Because of the risk of irreversible bone marrow aplasia in up to 1 in 10,000 recipients,[119] chloramphenicol is no longer routinely used in many areas, and oral chloramphenicol is not available in the United States. However, in many resource-limited areas, oral chloramphenicol is still available, has excellent bioavailability and central nervous system penetration, and has broad-spectrum activity against many common causes of serious childhood bacterial infections, including enteric fever. Chloramphenicol reduces the mortality rate of enteric fever substantially and decreases the time to defervescence to 3 to 5 days, and was shown to be equivalent to gatifloxacin in a randomized trial,[39] but its use is associated with a high relapse rate and risk of chronic carriage.[120,121] Oral trimethoprim-sulfamethoxazole may also be used to treat individuals with susceptible strains, although delayed clearance and relapses are common. Both chloramphenicol and trimethoprim-sulfamethoxazole are bacteriostatic agents.[122] Oral amoxicillin may also be used to treat individuals with susceptible strains and may result in a roughly comparable likelihood of relapse.[123]

TABLE 100.4 Antibiotics Commonly Used in Treating Patients With Enteric Fever

	ANTIBIOTIC	ROUTE	TYPICAL PEDIATRIC DOSAGE[b]	ADULT DOSAGE	TYPICAL DURATION	COMMENTS
Original first-line agents	Chloramphenicol	PO/IV	12.5–25 mg/kg qid	2–3 g/day (divided qid)	14–21 days	These agents are effective for susceptible strains; however, multidrug resistance to all of these agents is common in many areas.
	Amoxicillin	PO	25–35 mg/kg tid	1 g tid	14 days	
	Ampicillin	IV	25–50 mg/kg q6h	2 g q6h	14 days	
	TMP/SMX	PO/IV	8–12 mg/kg/day (TMP) 40–60 mg/kg/day (SMX) (divided qid)	160 mg/800 mg qid	14 days	
Fluoroquinolones	Ofloxacin	PO/IV	15 mg/kg bid	400 mg PO bid	5–14 days	Ofloxacin and ciprofloxacin are effective for nalidixic acid–susceptible strains. Short courses of 5–7 days are acceptable in uncomplicated cases. Ten- to 14-day courses are recommended in patients requiring hospitalization or parenteral therapy. Gatifloxacin appears effective for uncomplicated cases caused by NaR strains.
	Ciprofloxacin	PO/IV	15 mg/kg bid	500 mg PO bid	5–14 days	
	Gatifloxacin	PO	10 mg/kg qd	10 mg/kg/day qd	7 days	
Third-generation cephalosporins	Ceftriaxone	IV	50–100 mg/kg qd	1–2 g IV qd	7–14 days[c]	These are an alternative to fluoroquinolones for strains with decreased fluoroquinolone susceptibility, and for empirical therapy in areas where decreased fluoroquinolone susceptibility is common.
	Cefixime	PO	10 mg/kg bid	200 mg PO bid	7–14 days	
	Aztreonam	IV	50 mg/kg q8h	2g q8h		Alternative to ceftriaxone for cephalosporin-allergic patients.
Macrolides	Azithromycin	PO	20 mg/kg qd	500–1000 mg PO qd	7 days	These are an alternative to fluoroquinolones for strains with decreased fluoroquinolone susceptibility, and for empirical therapy in areas where decreased fluoroquinolone susceptibility is common.
Eradication of carriage	Ciprofloxacin	PO	—	500–750 mg bid	28 days	If eradication is indicated for public health reasons, a trial of medical therapy is justified, even in patients with evidence of cholelithiasis. Cholecystectomy may be considered.
	Amoxicillin	PO	75–100 mg/kg/day in 3 divided doses	2 g tid	4–6 weeks	Lower efficacy than fluoroquinolones but may be considered for treatment of carriage in individuals harboring strains with fluoroquinolone resistance.

[a]See text for references.
[b]Weight-based pediatric dosage should not exceed adult maximum.
[c]Recommended that treatment continue for at least 7 days after defervescence to minimize relapse.
NaR, Nalidixic acid resistant (see text); *TMP-SMX,* trimethoprim-sulfamethoxazole.
Modified from Magill AJ, Ryan ET, Hill DR, Solomon T, eds. Hunter's Tropical Medicine and Emerging Infectious Diseases. *9th ed. Philadelphia: Saunders; 2013:568–576.*

Fluoroquinolones

In many areas of the world, fluoroquinolone antibiotics are the preferred therapy for patients with suspected enteric fever, although increasing antimicrobial resistance is occurring globally.[30,124,125] For susceptible isolates, fluoroquinolone antibiotics, such as ciprofloxacin, and the less costly, more widely available ofloxacin, result in defervescence within 3 to 4 days, which is similar to the response associated with chloramphenicol and more rapid than that for third-generation cephalosporins.[126,127] Administration of a fluoroquinolone also results in lower rates of relapse and carriage compared with those associated with use of chloramphenicol or third-generation cephalosporins.[126,127]

Because of the availability and widespread use of fluoroquinolone antibiotics in many areas of the world, fluoroquinolone resistance has become increasingly common in *Salmonella* Typhi and *Salmonella* Paratyphi A in areas where enteric fever is highly endemic.[125] In South and Southeast Asia, strains with reduced susceptibility to fluoroquinolones now predominate.[19] Fever clearance times and treatment response have worsened with this rise in fluoroquinolone nonsusceptibility.[40]

Before 2012, breakpoints for fluoroquinolone susceptibility were standard across all Enterobacteriaceae, although resistance to nalidixic acid (a nonfluorinated quinolone antibiotic) was associated with decreased clinical responsiveness to fluoroquinolone antibiotics, such as ofloxacin and ciprofloxacin.[128–131] Nalidixic acid resistance (NaR) thus became a surrogate marker for predicting clinical failure of fluoroquinolones, even for *Salmonella* Typhi and *Salmonella* Paratyphi strains that met Clinical Laboratory and Standards Institute (CLSI) criteria for fluoroquinolone susceptibility.[56] The CLSI amended its breakpoints for *Salmonella* Typhi and *Salmonella* Paratyphi A, B, and C for ciprofloxacin in 2012 and for levofloxacin and ofloxacin in 2013. For ofloxacin, strains with a minimal inhibitory concentration (MIC) of less than or equal to 0.12 μg/mL are considered susceptible. Patients with strains with an MIC of 0.12 μg/mL defervesce within an average of less than 5 days after initiation of ofloxacin therapy, and patients with strains with an MIC of 0.064 μg/mL or less defervesce in less than 4 days.[131] For ciprofloxacin, the amended breakpoint for defining susceptibility is now less than or equal to 0.06 μg/mL. A complicating factor is that many laboratories are not currently able to implement testing of these lower breakpoints for typhoidal *S. enterica* isolates. For such strains, NaR remains an appropriate marker of reduced susceptibility to fluoroquinolone antibiotics.

Although fluoroquinolones are generally well tolerated, concerns about fluoroquinolone-induced articular toxicity in immature animals have limited their use in children in some areas; however, several studies support the safety of their use in children.[132] Gatifloxacin is a dual

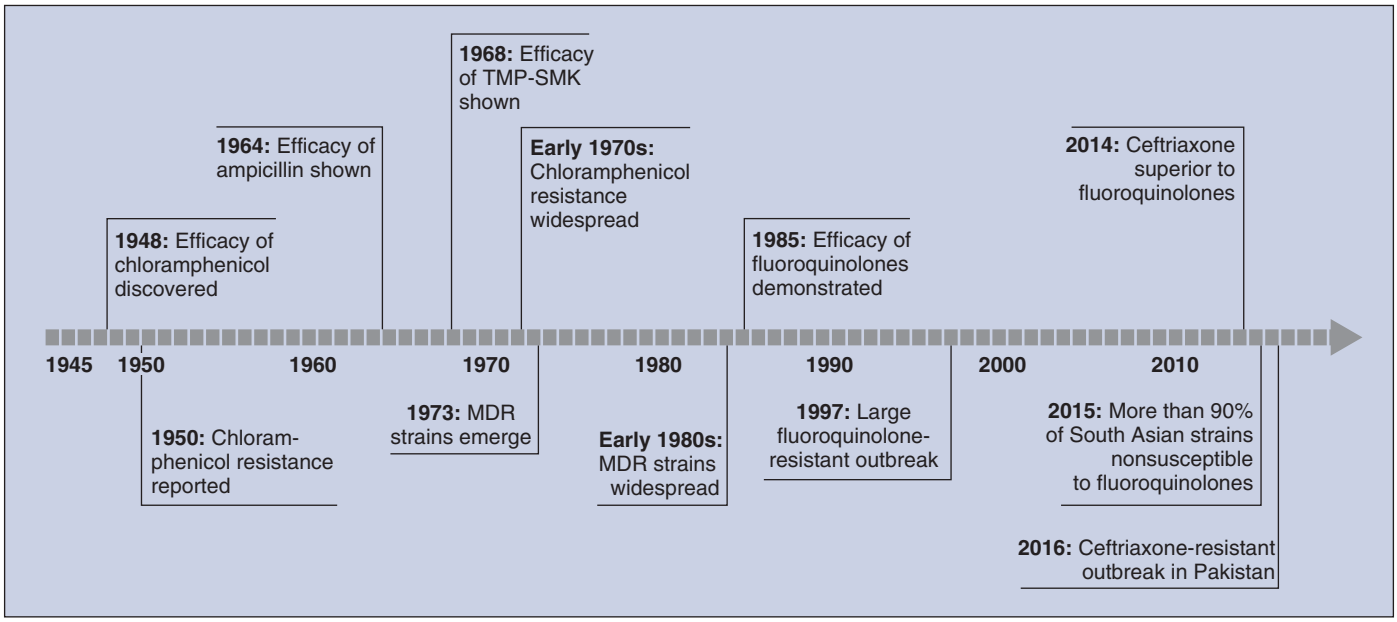

FIG. 100.4 History of antibiotic efficacy studies and the emergence of antimicrobial resistance in *salmonella* typhi. *MDR,* Multidrug resistant; *TMP-SMX,* trimethoprim-sulfamethoxazole. *(From Andrews JR, Qamar FN, Charles RC, Ryan ET. Extensively drug-resistant typhoid—are conjugate vaccines arriving just in time?* N Engl J Med. *2018:379;1493–1495.)*

mechanism-of-action fluoroquinolone that inhibits both DNA gyrase and topoisomerase IV. Gatifloxacin has been associated with increased frequency of hypoglycemia and hyperglycemia, compared with other fluoroquinolones, and for this reason it was withdrawn from the US market. However, gatifloxacin remains available in many countries where enteric fever is endemic and is reported to be clinically effective against some NaR *Salmonella* Typhi.[133] However, in Nepal, a trial comparing gatifloxacin with ceftriaxone was stopped early owing to higher treatment failure in the gatifloxacin arm as a result of fluoroquinolone resistance.[41] In documented cases of fluoroquinolone nonsusceptibility or for empirical treatment in cases acquired in regions where such strains are common, alternative agents should be used.

Cephalosporins

The emergence of *Salmonella* Typhi and *Salmonella* Paratyphi A strains with decreased susceptibility to fluoroquinolones has shifted the choice for empirical treatment of individuals with suspected severe enteric fever toward third-generation cephalosporins. However, although third-generation cephalosporins, such as ceftriaxone and cefixime, may be used, they appear inferior to fluoroquinolones for fully fluoroquinolone-susceptible strains.[127] Of note, both cefixime and short courses of parenteral ceftriaxone (≤7 days) are associated with high relapse rates during enteric fever.[134] In addition, time to defervescence is longer in patients treated with cefixime and ceftriaxone, compared with responses after administration of fluoroquinolones.[127,135] Resistance of typhoidal *S. enterica* to extended-spectrum β-lactam antibiotics has been rare and sporadically reported[42,136,137]; however, in 2017 a large outbreak of extended-spectrum β-lactam *Salmonella* Typhi was reported in Pakistan,[24] raising the concern that strains with resistance to third-generation cephalosporins may become increasingly common. Notably, the strains in this outbreak were MDR and fluoroquinolone resistant, leaving few available treatment options.

Azithromycin

Azithromycin is a highly effective drug for treating patients with enteric fever; favorable attributes include its efficacy, its established safety in children, and its excellent bioavailability and pharmacokinetics, with once-daily dosing. Azithromycin reaches high intracellular concentrations that may contribute to its high rates of cure. A 7-day course of azithromycin treatment for patients with enteric fever results in lower rates of clinical failure and substantially less relapse than third-generation cephalosporins.[138] Defervescence occurs within an average of 4 days.[129,133,138] There are only sparse reports of resistance of *S. enterica* to azithromycin to date.[43,138a] For these reasons, azithromycin is an excellent option for treating patients with enteric fever caused by MDR and fluoroquinolone-nonsusceptible *Salmonella* Typhi and *Salmonella* Paratyphi A.[138]

Other Antibiotics

Four small clinical trials have demonstrated the efficacy of aztreonam for treatment of enteric fever with comparable rates of efficacy and relapse to first-line antibiotics.[44,45] In the setting of cephalosporin-resistant *Salmonella* Typhi, carbapenems have also been successfully used to treat patients with typhoid fever, although no detailed data on efficacy and relapse rates are available.[36]

Adjunctive Therapy

Adjunctive therapy with high-dose dexamethasone has been shown to decrease mortality in cases of severe typhoid fever.[104] A double-blind trial involving 38 adult and pediatric patients with severe typhoid fever in Indonesia, conducted in 1981 and 1982, demonstrated that treatment with chloramphenicol, along with 3 mg/kg of dexamethasone administered over 30 minutes, followed by eight doses at 1 mg/kg every 6 hours, significantly reduced mortality (2 of 20 patients died in the dexamethasone arm) compared with chloramphenicol and placebo (10 of 18 patients died in the placebo arm, $P = .003$).[104] In this study, severe typhoid fever was defined as typhoid fever with shock and/or profound encephalopathy manifesting with delirium or obtundation.[104] More recent studies continue to support the concurrent use of dexamethasone and antibiotics in both children and adults with severe typhoid fever.[103,139] For this reason, administering high-dose steroids to patients who present with shock or, more often, significant mental status changes, and in whom there is strong suspicion of enteric fever, is recommended.

Supportive Care and Management of Complications

Supportive care of patients with severe enteric fever includes rehydration, nutritional management, and mobilization. Contrary to a prevailing historical opinion, it appears that fevers and malaise can be managed safely and more comfortably with a standard regimen of antipyretics.[140] One small double-blind trial showed that ibuprofen was superior to paracetamol in reducing the morbidity of fevers in enteric fever.[140]

Patients with intestinal hemorrhage should be monitored, ideally in an intensive care setting, but can generally be managed through

TABLE 100.5 Typhoid Fever Vaccines Currently Commercially Available Internationally

VACCINE	TYPE	ROUTE	DOSE AND INTERVAL	MINIMUM AGE	PROTECTION AGAINST *SALMONELLA* TYPHI	BOOSTING INTERVAL IN TRAVELERS
Ty21a	Live attenuated	Oral	Four doses (in United States) Administer one dose every other day until complete	5 yr[a]	50%–80%[b]	Every 5 yr
Vi capsule antigen	Polysaccharide	Intramuscular	1	2 yr	50%–80%	Every 2 yr
Vi tetanus toxoid conjugate vaccine[c]	Polysaccharide conjugate	Intramuscular	1	6 mo	80%[d]	Not yet defined

[a]Five years and older per World Health Organization[79]; 6 years and older per US Advisory Committee on Immunization Practices.[51]
[b]Has some efficacy against *Salmonella* Paratyphi B (see text). Additional vaccines are under development, including conjugate vaccines.
[c]Typhoid vaccines: WHO position paper, March 2018—Recommendations.[145]
[d]Preliminary data.
Modified from Magill AJ, Ryan ET, Hill DR, Solomon T, eds. Hunter's Tropical Medicine and Emerging Infectious Diseases. *9th ed. Philadelphia: Saunders; 2013:568–576.*

supportive care. Blood products, including packed red cells and plasma, may be needed in severe cases. Intestinal perforation in enteric fever is associated with a high mortality rate, but prompt surgical intervention is associated with improved survival.[82,100,141] Although the majority of perforations are single, careful inspection is required to determine if multiple perforations are present. Although simple closure may be performed in some cases, a wedge excision or segmental resection is more often necessary because the surrounding bowel is often necrotic.[142,143] Drainage of collections and peritoneal lavage should be performed before wound closure. Antibiotic regimens after perforation should be expanded to cover other intestinal microbiota, including anaerobes.

Treatment of Relapse and Chronic Carriage

Relapse usually occurs within 2 weeks of discontinuation of antibiotics, and relapsing patients typically present with milder symptoms than during the primary episode. Isolates obtained after relapse typically have the same antibiotic susceptibility as during the initial episode. The approach to treating patients with relapsed enteric fever is usually the same as for the primary episode.

Chronic carriage is defined as asymptomatic shedding of typhoidal *S. enterica* for 1 year or more. Asymptomatic shedding does not indicate a recurrence of illness or a high risk of recurrent illness. Although chronic carriage is associated with an increased risk of gallbladder carcinoma,[90] it is unknown whether the eradication of carriage reduces this risk of carcinoma, so the decision to attempt to eradicate carriage is usually based on public health considerations and is essential for individuals whose occupation involves food handling. Several antibiotics used to treat individuals with acute enteric fever reach high concentrations in bile, including fluoroquinolones. A 28-day course of ciprofloxacin has demonstrated 80% to 90% efficacy in eradication of carriage.[144] However, cholecystectomy may be necessary to eradicate carriage in individuals with cholelithiasis who continue to shed typhoidal *S. enterica* after prolonged antimicrobial therapy.[79] In an era of widespread nonsusceptibility to fluoroquinolones, there is a limited evidence base for successful treatment of carriage with antibiotics other than fluoroquinolones. Amoxicillin, administered at high doses (75–100 mg/day for 4-6 weeks), can be used, but tolerability is poor and overall eradication rates in many studies are modest.[46–48] There are even fewer data for treatment of carriers with trimethoprim-sulfamethoxazole with or without rifampin.[49,51]

Prevention

Because enteric fever is transmitted primarily through fecally contaminated water and food, the major preventive strategy to prevent enteric fever is the provision of safe water and adequate sanitation. At a municipal public infrastructure level, this requires appropriate facilities for waste disposal and sewage treatment in addition to provision of a safe water supply. In the United States, the introduction of chlorination and filtration of municipal water supplies in the early 1900s led to rapid and dramatic declines in the burden of typhoid.[52] At a household level, interventions to prevent enteric fever include hand washing with soap before preparing or eating food, avoidance of raw or uncooked foods, and local disinfection of unsafe drinking water, followed by storage in narrow-mouthed containers. Unfortunately, WHO currently estimates that 2 billion people use a drinking water source that is contaminated with feces.[53]

Historically, a heat-phenol–inactivated, whole-cell, killed typhoid vaccine was available for parenteral administration. The vaccine required multiple administrations, induced only short-term protection, and was associated with a high adverse event profile.[145] That vaccine is no longer commercially available in the United States.[146] Currently, two more effective and better-tolerated typhoid fever vaccines (Table 100.5) are commercially available in many areas: a capsular polysaccharide vaccine, licensed in the United States as Typhim Vi (Sanofi Pasteur, Swiftwater, PA), and an oral live-attenuated vaccine containing the *Salmonella* Typhi strain Ty21a, licensed in the United States as Vivotif (Valneva, Lyon, France). The polysaccharide vaccine is composed of the Vi capsular polysaccharide antigen from *Salmonella* Typhi strain Ty2, and the Vi vaccine confers 50% to 80% protection against *Salmonella* Typhi in children 2 years of age and older when administered as a single parenteral dose.[145,147–149] The Vi vaccine is not boosted by additional doses, and protection wanes rapidly after 2 years, at which point revaccination is recommended if risk is ongoing.[51] Ty21a is administered orally and also affords 50% to 80% protection against *Salmonella* Typhi.[145,150–156] Although Ty21a enteric-coated capsules are often used for travelers, a liquid formulation prepared from two sachets, one containing vaccine and a second containing a buffer, have been used for oral administration to children in resource-limited settings. The duration of immunity to the Ty21a vaccine is dependent on the number of doses received. In the United States, a four-dose every-other-day series of Ty21a is recommended and confers significant immunity for up to 7 years, although revaccination is recommended every 5 years if risk is ongoing.[51] Antibacterial drugs should not be given any time from 3 days before until 3 days after administration of a series of Ty21a. For most patients with significant primary immunodeficiencies, Ty21a should not be given; however, the Strategic Advisory Group of Experts for the WHO advises that Ty21a can be safely given to asymptomatic individuals living with human immunodeficiency virus (HIV) infection and a $CD4^+$ T-cell count greater than 200/mm^3.[145]

In 2018, WHO recommended that typhoid vaccination programs be undertaken in settings with high typhoid incidence or high burden of antimicrobial-resistant *Salmonella* Typhi.[54] However, typhoid vaccines are not currently widely used in most endemic regions and are more often used by travelers to endemic areas. For travelers receiving either the Vi or Ty21a vaccine, it is optimal that typhoid vaccination be completed at least 7 days before travel, if possible. Limitations of currently licensed vaccines include limited immunity in young children (<2 years of age) and lack of cross-protection against other causes of enteric fever, primarily *Salmonella* Paratyphi A, although Ty21a appears to provide protection against *Salmonella* Paratyphi B.[157] To improve both immunogenicity in children younger than 2 years and to induce longer-lasting protection, a number of conjugate Vi vaccines are in various stages of development. One version that conjugated Vi to *Pseudomonas aeruginosa*

recombinant exoprotein A (Vi-rEPA) achieved 90% protective efficacy against typhoid fever in a large randomized, double-blind clinical trial involving 11,000 children aged 2 to 5 years in Vietnam,[158] and has been demonstrated to be safe and immunogenic in phase II trials in infants.[159] However, this vaccine has not been licensed internationally. A Vi-tetanus toxoid conjugate vaccine (Typbar-TCV; Bharat Biotech, Hyderabad, India) was licensed in India in 2015 on the basis of immunogenicity data,[55] and was recently shown to have comparable efficacy to Typhim Vi in a human challenge model.[49] Although phase III efficacy data on this vaccine are not yet available, WHO has prequalified this vaccine and recommended its use in high-burden countries.[54] A paratyphoid A conjugate vaccine containing the O-specific polysaccharide of *Salmonella* Paratyphi A has also been reported.[160]

In non–highly endemic areas, other efforts to prevent enteric fever are aimed at limiting the role of carriers in transmission, particularly for carriers who present a significant public health risk, including food handlers and those working in child care or health care settings. In the United States, local and state health departments provide screening and work exclusion policies for individuals with enteric fever. The precise policies vary by location but may require screening of the patient and selected contacts to document clearance of bacterial shedding before discharge from public health follow-up and clearance for the performance of certain occupations.

DIFFERENTIAL DIAGNOSIS OF ENTERIC AND TYPHOIDAL FEVERS

Given the nonspecific clinical features of enteric fever and the current limitations of diagnostic testing, it is important that clinicians give consideration to the broader differential diagnosis when approaching patients with possible enteric fever. Such patients usually present with prolonged or relapsing fever, an acute febrile episode in a resource-limited area, or fever and abdominal symptoms and signs. Considerations in the differential diagnosis of enteric fever include epidemiologic risk factors, such as the relative local prevalence of invasive nontyphoidal salmonellosis, malaria, and other febrile diseases, and the type and timing of possible exposures, the duration and magnitude of fevers, the types of abdominal symptoms and signs, and laboratory features at the time of clinical presentation.

Bacteremia Associated With Nontyphoidal *Salmonella*

Although febrile gastroenteritis is the most common clinical manifestation of human nontyphoidal salmonellosis, nontyphoidal serotypes can also cause prolonged fever and bacteremia, particularly in immunocompromised patients with cell-mediated immunodeficiencies or with concomitant malaria. NTS bacteremia is a leading cause of death of young children and adults with acquired immunodeficiency syndrome (AIDS) in sub-Saharan Africa.[161] NTS can also cause abscess formation (spleen, kidneys, liver), and *Salmonella* and staphylococci are the leading causes of aortic atheroma seeding, mycotic aneurysm formation, and prolonged bacteremia.[162] Individuals with sickle cell disease and individuals with concomitant schistosomiasis are also at risk of prolonged and invasive nontyphoidal salmonellosis (see Chapter 223).[163,164]

TYPHOIDAL FEVER

A number of non-*Salmonella* infections have features that may mimic typhoid or enteric fever (Table 100.6). These illnesses have sometimes been referred to as *typhoidal fevers,* and are usually characterized as nonspecific systemic illnesses associated with fever that lasts longer than a week. Indeed, typhoid itself was named for its clinical similarity to typhus, caused by *Rickettsia* species. Typhus and typhoid are both characterized as febrile illnesses that may be prolonged and associated with encephalopathy or confusion, and if untreated, both diseases are associated with high mortality rates. In South and Southeast Asia, scrub typhus (*Orientia tsutsugamushi*) and murine typhus (*Rickettsia typhi*) are highly endemic, and are often mistaken for typhoid.[56,57] In sub-Saharan Africa, various *Rickettsia* species, including *Rickettsia africae* (the causative agent of African tick-bite fever), are common causes of nonspecific febrile illness. Leptospirosis is also a common cause of nonspecific febrile illness in many typhoid-endemic areas, and is often underdiagnosed owing to lack or underuse of diagnostics.[57] A clinical diagnosis of "typhomalarial fever," a term that appeared in the mid-19th century, highlights the overlap of the clinical syndromes of typhoid with malaria.[58] Individuals with malaria can also present with a wide range of symptoms, including prolonged or recurrent fever, headache, abdominal pain, and cough. Similarly, individuals with brucellosis may present with nonspecific fever, malaise, and abdominal pain. Fever may be prolonged, and infection during brucellosis is often characterized by its chronicity. Manifestations include osteoarticular and genitourinary involvement, granulomatous hepatitis, uveitis, and, rarely, endocarditis. Tularemia is a zoonotic infection caused by *Francisella tularensis*. Most human infections are due to exposure to small rodents or lagomorphs (rabbits), and humans usually become infected through direct contact with infected animals or animal parts, or through the bite of an arthropod as a transmission vehicle. Similar to *Salmonella, F. tularensis* is an intracellular pathogen that is able to survive and multiply within phagocytic cells, including macrophages, and infection spreads throughout the reticuloendothelial system. Without appropriate treatment, typhoidal tularemia is often fatal.

Persistent fever with nonspecific symptoms may also be seen with melioidosis, an infection caused by *Burkholderia pseudomallei*. Melioidosis is usually reported in South and East Asia and northern Australia. Melioidosis may manifest in a number of ways, ranging from a chronic febrile illness to an acute septicemia,[165] and it may include abscess formation (often in the spleen, liver, lungs, or muscles) or pneumonia. Melioidosis is associated with water and soil exposure and can affect anyone, although individuals with diabetes, immunosuppression, chronic renal disease, alcoholism, and cirrhosis are at particular risk. Clinical manifestations may occur years after initial exposure.

A typhoidal-like illness can also be seen with Q fever, caused by the intracellular bacterium *Coxiella burnetii*. Q fever is a zoonotic infection usually associated with exposure to sheep or cattle, and humans become infected through exposure to pseudospores from animal products, especially placental tissues. Individuals with Q fever may present with prolonged fever and a wide range of symptoms, including headache, pneumonia, hepatitis, and endocarditis. Rat-bite fever, caused by *Streptobacillus moniliformis,* may also cause a nonspecific febrile illness that may mimic enteric fever, especially when the route of infection is foodborne (Haverhill fever); arthritis and rash can occur.[166]

TABLE 100.6 Differential Diagnoses of Infectious Diseases to Consider in Patients With Possible Typhoid or Enteric Fever

Common Infectious Causes of Nonspecific Acute Febrile Illness in Resource-Limited Areas

Salmonella bacteremia (typhoidal and nontyphoidal serotypes)
Malaria
Dengue and other arboviral fevers
Leptospirosis
Influenza
Rickettsioses
Bartonelloses

Infectious Causes of Typhoidal Illnesses, Including Prolonged Nonlocalizing Febrile Illnesses

Persistent nontyphoidal bacteremia or abscess formation
Typhus (rickettsiosis)
Brucellosis
Typhoidal tularemia
Q fever
Bartonellosis (including trench fever)
Melioidosis
Rat-bite fever, Haverhill fever *(Streptobacillus moniliformis)*
Relapsing fever (borreliosis)
Infective endocarditis
Malaria
Babesiosis
Visceral leishmaniasis
West African trypanosomiasis
Tuberculosis
Endemic mycoses (histoplasmosis)

A number of *Bartonella* species can cause infections characterized by recurrent or prolonged fever. *Bartonella bacilliformis* is the cause of Carrión disease and is transmitted by sand flies in focal areas of Peru, Colombia, and Ecuador. During the acute illness (Oroya fever), patients present with fever and hemolytic anemia. In survivors, disease can progress to verruga peruana, a chronic illness characterized by vascular, proliferative, nodular skin lesions. In addition, *Bartonella quintana* is the cause of trench fever, a relapsing febrile illness transmitted by body lice. Trench fever usually affects individuals who are displaced or severely impoverished; onset is usually acute with high fever, headache, and retro-orbital pain, bone pain of the shins, and myalgia. Each episode of fever may last for 2 to 4 days, with relapses after 4 to 6 days. Prolonged bacteremia can be detected in 20% to 30% of patients.[167] Relapsing fever can also be caused by a number of *Borrelia* species and can be either louse-borne or tick-borne. Louse-borne relapsing fever is a human-restricted infection caused by *Borrelia recurrentis* and is associated with human body lice. Most cases of louse-borne relapsing fever are reported in Africa. Tick-borne relapsing fever has been reported in Asia, Africa, and the Americas. Relapsing fevers are characterized by relapsing and recurrent episodes of fever associated with spirochetemia. Diagnosis usually rests on detection of *Borrelia* on a thick blood smear with microscopy, although polymerase chain reaction (PCR)–based diagnosis is available. Untreated, louse-borne relapsing fever is associated with a high mortality rate. Fever may be prolonged, with many recurrences over several months.[168]

An enteric fever–like syndrome with abdominal pain may also be caused by a number of enteric pathogens, including *Yersinia enterocolitica, Yersinia pseudotuberculosis,* and *Campylobacter fetus* and *Campylobacter jejuni.* Individuals with these infections may present with a prolonged febrile illness, headache, and abdominal pain. Individuals with *Y. enterocolitica* or *Y. pseudotuberculosis* may have acute diarrhea as a major manifestation.[169,170] Most important, the prolonged enteric fever–like syndromes caused by these agents tend to occur in individuals with significant underlying disease, especially cirrhosis and iron-overloaded states.[169] Diagnosis is usually based on microbiologic culturing of blood.

Additional Causes of Prolonged or Persistent Fever

Most acute febrile illnesses resolve within 3 to 7 days. Individuals with typhoid or enteric fever may have fever that lasts for 1 to 4 weeks before resolution if left untreated. Clinicians assessing individuals with fever that lasts longer than 7 to 10 days need to consider a wide differential, both for infectious and noninfectious processes (see Chapter 56). Noninfectious causes that also need to be considered include lymphoma, malignancy, drug fever, Addison disease, and inflammatory conditions and connective tissue disorders. As described earlier, prolonged fever may also be associated with malaria, brucellosis, yersiniosis, tularemia, Q fever, melioidosis, endocarditis, tuberculosis, and endemic mycoses such as histoplasmosis. Recurrent or relapsing fever may be associated with trench fever and relapsing fever. Clinicians caring for individuals with prolonged fever in resource-limited or tropical areas or caring for individuals who have traveled to such regions need to consider these and other diagnoses. For instance, individuals with babesiosis, caused by an intraerythrocyte protozoal parasite transmitted by ticks, may also present with a nonspecific and prolonged febrile syndrome. Microscopy can confirm the diagnosis. Chronic and relapsing fever are also part of visceral leishmaniasis, a protozoal parasitic infection of the reticuloendothelial system. Individuals with visceral leishmaniasis often present with prolonged and ongoing fever, hepatosplenomegaly, pancytopenia, hypergammaglobulinemia, and absence of eosinophilia. Serologic tests and direct parasitic detection are usually used to confirm the diagnosis. Individuals with human African trypanosomiasis (sleeping sickness) can present with chronic and recurrent fever, weight loss, and lymphadenopathy before neurologic manifestations occur. Serologic tests and direct parasitic detection are usually used to confirm the diagnosis. Prolonged fever can also be associated with a number of viral syndromes, including Epstein-Barr virus (mononucleosis), characterized by prolonged fever, headache, weakness, and fatigue and may include pharyngitis, hepatosplenomegaly, and lymphadenopathy. Serologic assays are available.

Assessing an Individual With an Acute Febrile Illness in a Resource-Limited Area or After International Travel

Most individuals with typhoid or paratyphoid present with an acute febrile illness, and typhoid and paratyphoid fever are common causes of bacteremia in many resource-limited areas of the world. Most cases of typhoid fever reported in North America and Europe are related to international travel, predominantly to South Asia,[16] and affected patients usually develop fever within 1 to 3 weeks of exposure or returning from travel. Because vaccines are 50% to 80% protective and do not protect against *Salmonella* Paratyphi A, previous immunization does not preclude the possibility of enteric fever, and previous immunization can lead to cross-reactive serologic assays. Diagnosis can be confirmed by identifying *Salmonella* Typhi or *Salmonella* Paratyphi in blood cultures, but this assay is only 30% to 70% sensitive.[27] Accordingly, the clinical approach to an individual with possible typhoid fever after travel is usually based on excluding other possible entities and initiation of empirical treatment. Leading other causes of acute febrile illness in individuals at risk of typhoid include urinary, reproductive tract, and respiratory or intestinal tract infections that can be evaluated with standard approaches. Of importance, the differential diagnosis also needs to include influenza, dengue, chikungunya, Zika, leptospirosis, rickettsiosis, bartonellosis, and malaria, among others. An incubation period of less than a week before onset of fever would be most consistent with a viral cause, such as influenza or dengue. International travelers are at particular risk of influenza, and the transmission season in the Southern Hemisphere is March through September, with year-round transmission as one approaches the equator. Most individuals with influenza will have respiratory symptoms. Most individuals with dengue will present within 3 to 7 days of returning from travel to Asia or the Caribbean. Thrombocytopenia and evidence of capillary fragility, such as a positive tourniquet test, would be supportive of consideration of dengue fever (see Chapter 153). The diagnosis can be confirmed through serology or PCR. Leptospirosis is caused by exposure to animal urine, and exposure usually occurs from contaminated freshwater sources. The febrile illness can be biphasic, with conjunctival suffusion, hematuria, proteinuria, renal dysfunction, and hepatitis suggestive of the diagnosis (see Chapter 239). Rapid microscopic examination of peripheral blood should assist with establishing whether a patient may have malaria or borreliosis (see Chapter 240). A careful examination of skin for evidence of eschars or spotted rashes can assist in establishing whether the patient may have a rickettsiosis (see Chapter 186). Microbiologic culturing of blood will assist in confirming infection with a number of bacterial pathogens, including *Salmonella.*

Fever and Mesenteric Adenitis or Ileocecitis

Individuals with mesenteric adenitis or ileocecitis often present with a history of fever and abdominal pain, often localizing to the right lower quadrant. The illness needs to be differentiated from appendicitis, intraabdominal abscess formation, and diverticulitis (Table 100.7).[171] Mesenteric adenitis can be seen with *Y. enterocolitica* and *Y. pseudotuberculosis*; *Y. enterocolitica* can also cause enterocolitis and terminal ileitis. These infections are more commonly reported from Europe than the United States.[172] Fever, abdominal pain, vomiting, and diarrhea are frequent.[173] Fever can be prolonged. Ingestion of undercooked or uncooked pork and pork-related products, such as the small intestine of pigs (chitterlings), are particular risk factors.[174] Septicemia can complicate intestinal yersiniosis, especially in individuals with iron-overloaded states, immune deficiencies, cirrhosis, and alcoholism. Mesenteric adenitis can also be caused by tuberculosis, and *Mycobacterium avium* complex infection in individuals with AIDS, especially in individuals who are becoming immune reconstituted.[175] Children with HIV in Southeast Asia may present with mesenteric adenitis caused by *Talaromyces (Penicillium) marneffei.*[176] Epstein-Barr virus, parvovirus B19, and adenovirus infection may also be associated with mesenteric adenitis.[177,178]

Individuals with ileocecitis may present with fever and abdominal pain, often localizing to the right lower quadrant, potentially with signs of bowel obstruction. Noninfectious causes include Crohn disease and

ulcerative colitis. Infectious causes of ileocecitis include appendicitis, diverticular abscess formation, intestinal yersiniosis, tuberculosis, histoplasmosis, and amebiasis complicated by an inflammatory mass (an ameboma), intestinal schistosomiasis complicated by an inflammatory mass (a bilharzioma), an intestinal actinomycetoma that may include drainage fistula tracks, and intestinal angiostrongyliasis (see "Fever, Abdominal Pain, and Peripheral Eosinophilia").

Fever, Abdominal Pain, and Peripheral Eosinophilia

Individuals will sometimes present with fever, abdominal pain, and peripheral eosinophilia (>400 total eosinophils/mm^3 peripheral blood), a clinical syndrome that may be indistinguishable from enteric fever until the white blood cell count differential is available. The differential diagnosis for these individuals includes a number of entities (see Table 100.7). Fascioliasis caused by the liver fluke *Fasciola hepatica* may manifest as severe, right upper quadrant pain and high-level peripheral eosinophilia and fever. Individuals are often diagnosed incorrectly as having a pyogenic hepatic abscess and drug reaction. *F. hepatica* is transmitted to humans through ingestion of aquatic plants such as fresh watercress. During the initial phase of infection, the flukes can migrate through hepatic parenchyma, causing severe right upper quadrant pain. Imaging studies often disclose migratory hepatic lesions. After a few weeks, the worms localize to the biliary system, and the abdominal symptoms resolve. Peripheral eosinophilia can be very high during the initial stages of infection (>1000–3000 eosinophils/mm^3 of blood), when the parasites are migrating through tissue. Diagnosis is usually based on clinical recognition. Serologic assays are available.

Visceral larva migrans (VLM) may also manifest with abdominal pain, fever, eosinophilia, and hepatosplenomegaly. VLM can be caused by a number of migratory worms, most commonly the dog or cat ascarids *Toxocara canis* and *Toxocara cati,* respectively. VLM is usually an illness of very young children, 2 to 4 years of age. Diagnosis is usually based on clinical recognition. Serologic assays are available. Individuals with trichinosis can also present with fever, eosinophilia, abdominal pain, edema, and myositis. Diagnosis is usually based on clinical recognition. Serologic assays are available. Individuals with intestinal angiostrongyliasis, caused by *Angiostrongylus costaricensis,* can also present with abdominal pain and eosinophilia. Affected individuals are usually children with right lower quadrant pain, ileocecitis, or presumed appendicitis. The disease is present in areas of Latin America. A wide range of other helminthic infections can be associated with abdominal issues, intestinal irregularities, and peripheral eosinophilia, including intestinal strongyloidiasis, hookworm, trichinellosis, and schistosomiasis. Sarcocystosis and isosporiasis can also cause abdominal irregularities and peripheral eosinophilia. Individuals with gastrointestinal involvement during Churg-Strauss eosinophilic angiitis can also present with abdominal pain and eosinophilia.

TABLE 100.7 Other Causes of Fever and Intestinal Syndromes

Causes of Mesenteric Adenitis and Ileocecitis/Ileal Inflammatory Mass

Infectious

Appendicular abscess
Diverticular abscess
Yersiniosis
Salmonellosis
Mycobacterial infection
Mycoses (histoplasmosis, penicilliosis)
Amebiasis (ameboma): ileocecitis
Schistosomiasis (bilharzioma): ileocecitis
Actinomycetoma

Noninfectious

Crohn disease
Ulcerative colitis

Causes of Fever, Abdominal Pain or Intestinal Irregularities, and Peripheral Eosinophilia

Infectious

Fascioliasis *(Fasciola hepatica)*
Visceral larva migrans *(Toxocara canis, Toxocara cati)*
Angiostrongyliasis *(Angiostrongylus costaricensis)*
Schistosomiasis
Strongyloidiasis
Hookworm infection
Trichinellosis
Sarcocystosis
Isosporiasis

Noninfectious

Churg-Strauss eosinophilic angiitis
Eosinophilic enteropathy
Food allergy (e.g., gluten-sensitive enteropathy)

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The complete reference list is available online at Expert Consult.

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101 Foodborne Disease

Karen K. Wong and Patricia M. Griffin[a]

SHORT VIEW SUMMARY

Definition
- Foodborne diseases are illnesses that are acquired through ingestion of food contaminated with pathogenic microorganisms, bacterial and nonbacterial toxins, or other substances.

Epidemiology
- An estimated 48 million foodborne illnesses caused by pathogens or their toxins are acquired annually in the United States.
- Many agents that cause foodborne infection can also be acquired in other ways including ingestion of contaminated drinking or recreational water, through contact with animals or their environment, and from one person to another directly or through fomites.
- Some foodborne diseases can lead to long-term sequelae such as impaired kidney function after Shiga toxin–producing *Escherichia coli* infection, Guillain-Barré syndrome after *Campylobacter* infection, and reactive arthritis and irritable bowel syndrome after a variety of infections.
- Groups at higher risk of acquiring or experiencing more severe foodborne disease include infants, young children, pregnant women, older adults, and immunocompromised patients.
- A foodborne disease outbreak should be considered when an acute illness, especially with gastrointestinal or neurologic manifestations, affects two or more people who shared a meal or ate at the same location. However, most foodborne illnesses do not occur in the context of an outbreak.

Microbiology
- Many pathogens including bacteria, viruses, and parasites can cause foodborne disease.
- Some illnesses are caused by ingestion of chemicals (e.g., heavy metals, mushroom toxins) or preformed microbial toxins (e.g., staphylococcal toxin, botulinum toxin).

Diagnosis
- Detection of pathogens has traditionally relied on isolating bacterial pathogens in culture, visualizing parasites by microscopy, and enzyme-linked immunosorbent assays.
- Newer molecular tests present opportunities and challenges to both clinical practice and public health surveillance.
- Many intoxications must be diagnosed based on clinical suspicion alone.

Therapy
- Therapy for most foodborne diseases is supportive; replacing fluid and electrolyte losses is the most important factor in all diarrheal illnesses.
- Antimicrobial agents are used to treat parasitic infections and selected bacterial infections.
- Resistance to antimicrobial agents complicates treatment and can increase the likelihood of clinically apparent infection.

Prevention
- Individuals can reduce their risk of illness by adhering to safe food-handling practices.
- Outbreak investigation is important to identify safety gaps that may be present anywhere in the food production chain, from the farm to the table.
- Clinicians and public health practitioners can reduce risk of secondary transmission by counseling patients on prevention strategies and excluding certain patients from work, school, or daycare until transmission risk is reduced.

Foodborne diseases result from ingestion of a wide variety of foods contaminated with pathogenic microorganisms, microbial toxins, and chemicals. Many diseases transmitted commonly through food can be acquired via other routes of transmission as well. For sporadic cases (i.e., cases that are not part of recognized outbreaks), the route of transmission is generally unknown. Although most foodborne illnesses are sporadic, investigation of outbreaks is an important way to identify the types of foods and contaminants associated with foodborne illness. The major source of information for this chapter comes from foodborne disease surveillance and outbreak investigations in the United States, and the major focus is on US illnesses. During the years 2009–15, 5760 outbreaks of foodborne disease resulting in 100,939 illnesses, 5699 hospitalizations, and 145 deaths were reported to the US Centers for Disease Control and Prevention (CDC) (Table 101.1).[1] However, these figures, restricted to outbreak cases, greatly underestimate the magnitude of the problem. The actual number of foodborne illnesses in the United States is unknown but has been estimated to be approximately 48 million cases, with 128,000 hospitalizations and 3000 deaths each year (Table 101.2).[2,3] The annual cost incurred from these illnesses has been estimated to be between $51.0 billion and $77.7 billion.[4] Thus foodborne diseases are common, can be severe, and lead to considerable economic burden.

Since 1996, in several sites that now comprise approximately 15% of the US population, the CDC Foodborne Diseases Active Surveillance Network has conducted active surveillance for nine enteric pathogens transmitted commonly through food. Compared with 2013–2015, the 2016 incidence of infection with laboratory-confirmed Shiga toxin–producing *Escherichia coli* (STEC), *Yersinia*, and *Cryptosporidium* increased.[5] Interpretation of changes in incidence is complicated by changes in diagnostic tests, particularly the increased availability, use, and sensitivity of culture-independent diagnostic tests (CIDTs) and the challenges of synthesizing surveillance data from different types of laboratory tests.

The spectrum of foodborne diseases has expanded in recent decades in many ways. Noroviruses are now recognized as the most frequent cause of foodborne illness in the United States.[2] Known agents continue to be newly recognized as causes of foodborne disease such as enteroaggregative *E. coli* (EAEC)[6,7] including novel strains that produce Shiga toxin[8]; *Cronobacter* (formerly *Enterobacter*) *sakazakii*[9]; and, in South America, *Trypanosoma cruzi*, causing Chagas disease.[10] Previously uncommonly recognized food vehicles such as fresh produce and flour have become important sources.[11,12] Some pathogens have become increasingly resistant to antimicrobial drugs.[13,14]

Centralization of the food supply in the United States has increased the risk for nationwide outbreaks.[15,16] The global food trade, which is growing faster than increases in food production, forms a complex network that facilitates spread of contaminated foods throughout the world and can delay identification of the source of contamination causing outbreaks.[17]

CLINICAL MANIFESTATIONS AND PATHOGENESIS

Foodborne disease can appear as an isolated sporadic case or, less frequently, as an outbreak of illness affecting a group of people after a

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

TABLE 101.1 Mean Annual Foodborne Disease Outbreaks and Outbreak-Associated Illnesses and Hospitalizations by Etiology (Confirmed and Suspected) —Foodborne Disease Outbreak Surveillance System, United States, 2009–2015

	OUTBREAKS		OUTBREAK-ASSOCIATED ILLNESSES	
ETIOLOGY	**Total**	**%**	**Total**	**%**
Bacterial				
Salmonella	136	23	3453	30
STEC	29	5	352	3
Campylobacter	29	5	330	3
Clostridium perfringens	28	5	1119	10
Staphylococcus aureus enterotoxin	11	2	240	2
Bacillus cereus	9	2	120	1
Vibrio parahaemolyticus	7	1	40	0
Shigella	6	1	175	1
Listeria monocytogenes	5	1	55	0
Clostridium botulinum	3	1	13	0
ETEC	1	0	65	1
Staphylococcus spp.	1	0	8	0
Yersinia enterocolitica	1	0	3	0
Vibrio cholerae	0	0	2	0
Streptococcus group A	0	0	16	0
EAEC	0	0	7	0
Other *Vibrio* spp.	0	0	1	0
Vibrio vulnificus	0	0	0	0
Aeromonas hydrophila	0	0	1	0
Coxiella burnetii	0	0	1	0
Francisella novicida	0	0	0	0
Brucella spp.	0	0	1	0
Other *Clostridium* spp.	0	0	2	0
EPEC	0	0	4	0
Enterococcus faecalis	0	0	2	0
Other bacteria	5	1	67	1
Subtotal	272	46	6078	52
Chemicals and Toxins				
Scombroid toxin/histamine	14	2	43	0
Ciguatoxin	13	2	48	0
Mycotoxins	2	0	6	0
Puffer fish tetrodotoxin	0	0	1	0
Paralytic shellfish poison	0	0	2	0
Pesticides	0	0	6	0
Amnesic shellfish poison	0	0	0	0
Other chemicals	6	1	40	0
Subtotal	37	6	146	1
Parasitic				
Cryptosporidium	2	0	26	0
Trichinella	1	0	5	0
Cyclospora	1	0	62	1
Giardia	0	0	2	0
Subtotal	5	1	94	1

Continued

TABLE 101.1 Mean Annual Foodborne Disease Outbreaks and Outbreak-Associated Illnesses and Hospitalizations by Etiology (Confirmed and Suspected)[a]—Foodborne Disease Outbreak Surveillance System, United States, 2009–2015—cont'd

	OUTBREAKS		OUTBREAK-ASSOCIATED ILLNESSES	
ETIOLOGY	**Total**	**%**	**Total**	**%**
Viral				
Norovirus	267	46	5291	45
Hepatitis A	2	0	37	0
Sapovirus	1	0	19	0
Rotavirus	0	0	12	0
Astrovirus	0	0	3	0
Other	0	0	4	0
Subtotal	271	46	5366	46
Single, Multiple, and Unknown Etiologies				
Single etiology[b]	585	71	11,684	81
Multiple etiologies[c]	12	1	285	2
Unknown etiology[d]	226	27	2451	17
TOTAL	823	100	14,420	100

[a]Guidelines for reporting agencies are to consider an etiology confirmed if it meets confirmation criteria (https://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/confirming_diagnosis.html); otherwise, it is considered suspected. If more than one etiology is reported and at least two etiologies are confirmed, the outbreak is considered a confirmed multiple-etiology outbreak; otherwise, it is considered suspected. Agents that are not listed in confirmation criteria or that are not known to cause illness are sometimes reported as confirmed or suspected etiologies.
[b]The denominator for the etiology percentages is the single etiology total. The denominator for the single etiology, multiple etiologies, and unknown etiology categories is the total. Because of rounding, numbers may not add up to the single etiology total or the total.
[c]If at least two etiologies are confirmed in an outbreak, it is considered a confirmed multiple etiology outbreak; otherwise, it is considered a suspected multiple etiology outbreak.
[d]An etiologic agent was not confirmed or suspected based on clinical, laboratory, or epidemiologic information.
EAEC, Enteroaggregative *Escherichia coli;* EPEC, enteropathogenic *E. coli;* ETEC, enterotoxigenic *E. coli;* STEC, Shiga toxin–producing *E. coli.*
Modified from Dewey-Mattia D, Manikonda K, Hall AJ, et al. Surveillance for Foodborne Disease Outbreaks—United States, 2009-2015. MMWR Surveill Summ. *2018;67:1–11.*

common food exposure. A foodborne disease outbreak should be considered when an acute illness, especially one with gastrointestinal or neurologic manifestations, affects two or more people who shared a common meal. The following section divides acute foodborne diseases into a variety of syndromes based on acute signs and symptoms and typical time of onset after consumption of contaminated food. Agents most likely responsible for each syndrome are described. The incubation period in an individual illness is usually unknown, but it is often apparent in the context of the outbreak setting.

Foodborne Syndromes Caused by Microbial Agents or Their Toxins

Nausea and Vomiting Lasting Less Than 24 Hours

The major etiologic considerations are *Staphylococcus aureus* and *Bacillus cereus* (see Chapters 194 and 208). These diseases are caused by preformed enterotoxins and have a short incubation period of 1 to 8 hours. Another clue to the cause of staphylococcal and emetic *B. cereus* illnesses is that their duration is typically less than 24 hours[18,19] and often less than 12 hours.[20,21] Staphylococcal food poisoning is characterized by vomiting (87% of cases), diarrhea (89%), and abdominal cramps (72%); fever is uncommon (9%).[22] Staphylococci responsible for food poisoning produce one or more serologically distinct enterotoxins (SEA through SEV, excluding SEF), but not all cause vomiting.[18] All of these toxins are highly heat resistant and withstand ordinary cooking. They are very resistant to proteolytic enzymes and therefore pass through the stomach intact. Strains producing SEA alone account for most reported outbreaks of staphylococcal food poisoning in the United States.[20,23] In studies of rhesus monkeys, a smaller dose of SEA compared with doses of SEB, SEC, and SED was required to produce emesis.[24] The mechanisms by which enterotoxins lead to emesis may involve vagus nerve stimulation.[18]

Rarely, other enterotoxigenic coagulase-positive staphylococcal species have been implicated in outbreaks.[18] Although enterotoxigenic coagulase-negative staphylococci exist, very few reports have associated these strains with foodborne disease outbreaks.[25,26]

B. cereus strains can cause two types of food poisoning syndromes, one with an incubation period of 0.5 to 6 hours (short-incubation emetic syndrome) and a second with an incubation period of 8 to 16 hours (long-incubation diarrheal syndrome).[19] The emetic syndrome is characterized by vomiting (100% of cases), abdominal cramps (100%), and, less frequently, diarrhea (33%).[19,21] The emetic toxin is cereulide, a peptide resistant to heat and proteolysis; it is stable at pH 2 to 11. Cereulide stimulates the vagus afferent nerve by binding to the 5-hydroxytryptamine-3 receptor.[27] Rarely, fulminant liver failure may develop via impairment of fatty acid oxidation caused by the toxicity of cereulide to mitochondria.[19]

Norovirus illness is characterized by acute onset of vomiting, nonbloody diarrhea, or both, accompanied by nausea and abdominal pain. Fever occurs in about 40% of patients, is usually low grade, and lasts for less than 24 hours. Symptoms usually resolve in 2 to 3 days, but 12% of patients require medical care, and 1.5% are hospitalized for rehydration.[28,29] Noroviruses are the most common foodborne pathogens (see Chapter 176). They were estimated to cause 5.5 million foodborne illnesses per year in the United States in 2006.[2] Even more cases of acute gastroenteritis are caused by nonfoodborne transmission of noroviruses, directly from one person to another or by fomite contamination.[30] The median incubation period reported in foodborne norovirus outbreaks is 33 hours.[28] A group of related viruses in the Caliciviridae family, most notably the sapoviruses (see Chapter 176), can cause similar illness.[31]

Watery Diarrhea Without Fever Lasting 1 to 2 Days

The major etiologic considerations for this enterotoxin-mediated syndrome are *Clostridium perfringens* type A and *B. cereus.* In *C. perfringens* type A food poisoning the most common symptoms are diarrhea (91%) and abdominal cramps (73%); only 14% of patients

TABLE 101.2 Estimated Annual Number of Illnesses Caused by Pathogens That Can Be Transmitted Through Food in the United States

DISEASE OR AGENT	TOTAL NO. OF ILLNESSES	TOTAL NO. OF DOMESTICALLY ACQUIRED FOODBORNE ILLNESSES	PERCENT HOSPITALIZED	PERCENT DIED
Bacterial				
Bacillus cereus	—[a]	63,400	0.4	0
Brucella spp.	2003	839	55	0.9
Campylobacter spp.	1,322,137	845,024	17.1	0.1
Clostridium botulinum	—[a]	55	82.6	17.3
Clostridium perfringens	—[a]	965,958	0.6	<0.1
STEC O157	96,534	63,153	46.2	0.5
STEC non-O157	168,698	112,752	12.8	0.3
ETEC	—[a]	17,894	0.8	0
Other diarrheogenic *Escherichia coli*	39,871	11,982	0.8	0
Listeria monocytogenes	1662	1607	94.0	15.9
Mycobacterium bovis	208	60	55	4.7
Nontyphoidal *Salmonella*	1,229,007	1,027,561	27.2	0.5
Salmonella serotype Typhi	5752	1821	75.7	0
Shigella spp.	494,908	131,254	20.2	0.1
Staphylococcus aureus	—[a]	241,148	6.4	<0.1
Streptococcus spp.	—[a]	11,217	0.2	0
Toxigenic *Vibrio cholerae*	277	84	43.1	0
Vibrio parahaemolyticus	44,950	34,664	22.5	0.9
Vibrio vulnificus	207	96	91.3	34.8
Other *Vibrio* spp.	34,585	17,564	37.1	3.7
Yersinia enterocolitica	116,716	97,656	34.4	2.0
Parasitic				
Cryptosporidium parvum	748,123	57,616	25.0	0.3
Cyclospora cayetanensis	19,808	11,407	6.5	0
Giardia intestinalis	1,221,564	76,840	8.8	0.1
Toxoplasma gondii	173,995	86,686	2.6	0.2
Trichinella spp.	132	156	24.3	0.2
Viral				
Astrovirus	3,090,384	15,433	0.4	<0.1
Hepatitis A	35,769	1566	31.5	2.4
Norovirus	20,865,958	5,461,731	0.03	<0.1
Rotavirus	3,090,384	15,433	1.7	<0.1
Sapovirus	3,090,384	15,433	0.4	<0.1
SUBTOTAL	37,220,098	9,388,075		
Unspecified Agents[b]		38,400,000	0.2	<0.1
TOTAL		47,788,075		

[a]Recent estimates are not available.
[b]Includes known agents with insufficient data to estimate agent-specific illness, known agents not yet recognized as causing foodborne illness, substances known to be in food but of unproven pathogenicity, and unknown agents.
ETEC, Enterotoxigenic *Escherichia coli; STEC,* Shiga toxin–producing *E. coli*.
Modified from Scallan E, Griffin PM, Angulo FJ, et al. Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis. *2011;17:16–22; and Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens.* Emerg Infect Dis. *2011;17:7–15.*

experience vomiting, and only 5% report fever.[22,32] In contrast to staphylococcal food poisoning and emetic *B. cereus* disease, which are caused by preformed enterotoxins, *C. perfringens* (see Chapter 246) food poisoning is caused by toxins produced in vivo, accounting for a longer incubation period of 9 to 12 hours.[22] *C. perfringens* can produce at least 11 toxins in addition to *C. perfringens* enterotoxin (CPE). Toxinotype A strains always produce α toxin, and strains that result in gastrointestinal illness also express CPE, which is produced as the ingested vegetative cells sporulate within the intestine.[33] The toxin binds to the apical membrane of epithelial tight junctions in the small intestines, triggering formation of pores through which influx and efflux of water, ions, and other small molecules may lead to diarrhea and cytotoxicity.[34]

B. cereus strains that cause a similar long-incubation syndrome including diarrhea (96%), abdominal cramps (75%), sometimes vomiting (33%), and rarely fever[21] elaborate either separately or together two three-component enterotoxins (hemolysin BL and nonhemolytic enterotoxin). A single-protein enterotoxin (CytK) has also been described.[19,27] *B. cereus* diarrheal toxins can be detected in foods, but it has been postulated that the disease is also caused by ingested vegetative cells that produce enterotoxin within the host's intestinal tract. It is possible that both modes of infectivity coexist.

Although these illnesses last longer than staphylococcal and emetic *B. cereus* food poisoning, symptoms usually resolve within 24 to 48 hours.[19,35] However, some *B. cereus* illnesses last several days,[27,36] and in one outbreak attributed to *B. cereus*, some patients had bloody diarrhea, and three died.[37] In an outbreak of *C. perfringens* type A infections, severe necrotizing colitis developed in patients with a history of chronic, likely medication-induced, constipation.[38] A foodborne infection, fatal in about 20% of patients, is caused by *C. perfringens* type C in people with low protein intake; the disease is rare outside of Papua New Guinea, where it is sometimes called pigbel.[35,39]

Watery Diarrhea and Abdominal Cramps Lasting More Than 2 Days

The major etiologic considerations for this syndrome are enterotoxigenic strains of *E. coli* (ETEC) and *Vibrio parahaemolyticus*. Enterotoxins expressed in vivo are responsible for illness caused by ETEC[40] and by cholera toxin–producing strains of *Vibrio cholerae*.[41] Diarrhea caused by ETEC lasts a median of 6 days, often accompanied by abdominal cramping for the full duration of illness.[42] In one ETEC outbreak, uncommon symptoms included vomiting (13% of cases) and fever (19%).[42]

In the United States, many *Vibrio* spp. including *V. cholerae* cause a diarrheal syndrome; outbreaks are uncommon, but cases are reported every year.[43] The virulence factors of *V. parahaemolyticus* include both secreted toxins and effector proteins delivered directly into the cytoplasm of the host cell via type III secretion systems.[44] The median duration of diarrhea caused by *V. parahaemolyticus* is 6 days; most patients have abdominal cramping (89%), half have vomiting or fever, and 29% have bloody diarrhea.[45] Epidemic cholera, caused by cholera toxin–producing strains of *V. cholerae* O1 and O139, manifests as a profuse, watery diarrhea accompanied by muscular cramps. *V. cholerae* O141 and O75 can also produce cholera toxin and cause a similar syndrome (see Chapter 215).[46]

Diarrhea, Abdominal Cramps, and Fever

The major etiologic considerations for this syndrome are nontyphoidal *Salmonella, Shigella,* and *Vibrio* spp., especially *V. parahaemolyticus* (see Chapters 214, 215, 223, and 224).[45,47–50] *Campylobacter jejuni,* STEC, and *Yersinia enterocolitica* should also be considered and typically have a longer incubation period; also, fever is less common with STEC (see Chapters 98, 216, and 229A). Norovirus does not consistently cause fever but should be considered. These pathogens, with the exception of norovirus, can cause an inflammatory diarrhea, some by invading the intestinal epithelium and some by damaging it via secreted cytotoxins.[44,51] Bloody diarrhea and vomiting may occur in varying proportions. These illnesses usually resolve within 2 to 7 days.[47]

Nontyphoidal *Salmonella* is the most common bacterial cause of foodborne illnesses and outbreaks in the United States.[2,52] The median incubation period is 6 to 48 hours. However, *C. jejuni,* with a typical incubation period of 2 to 4 days, is the most common bacterial cause of gastroenteritis including both foodborne and nonfoodborne routes of transmission.[2]

Infrequent outbreaks of diarrhea with fever caused by *Listeria monocytogenes* (see Chapter 206) have been reported among previously healthy people.[53–55] This syndrome is characterized by frequent watery diarrhea, fever, abdominal cramps, headache, and myalgias, with a median incubation period of 20 to 31 hours.

Although febrile diarrhea is the most common presentation of *Y. enterocolitica* (see Chapter 229B) infection in young children,[56–58] in older children and adults the illness may manifest as either a diarrheal illness or without diarrhea as a pseudoappendicular syndrome; as ileocecitis, consisting of abdominal pain (resembling that of appendicitis); fever; leukocytosis; and, in some patients, nausea and vomiting.[59] Joint pain (see "Postinfection Syndromes"), beginning about 1 week after onset of diarrhea, is more common in adults.[59] Sore throat and rash can affect patients of all ages. The median duration of diarrhea is 2 weeks, but other symptoms may last longer.[60] *Campylobacter* and *Salmonella* can also cause an ileocecitis that mimics appendicitis.

Bloody Diarrhea With Minimal Fever

The distinctive syndrome of hemorrhagic colitis has been linked to STEC, most often serogroup O157 (see Chapter 224).[61] These strains produce one or both types of Shiga toxins (Shiga toxin 1 or 2). Shiga toxins, also referred to as verotoxins, are cytotoxins that damage vascular endothelial cells in target organs such as the gut and kidney.[62] Strains that produce Shiga toxin 2 are more virulent than strains that produce both Shiga toxin 1 and 2.[63] To cause disease, STEC must also possess additional virulence factors including factors that lead to adherence to the intestinal epithelium.[62] The illness is characterized by severe abdominal cramping and diarrhea, which is initially watery but may quickly become grossly bloody.[61,64] About one-third of patients report a short-lived low-grade fever that typically resolves before seeking medical attention.[61,65] Most patients fully recover within 7 days.[61] However, overall 6% (15% in children <5 years of age) of patients develop hemolytic-uremic syndrome (HUS), which is typically diagnosed about 1 week after the beginning of the diarrheal illness, when the diarrhea is resolving.[66] The fatality rate for HUS is 3% in children younger than 5 years of age and 33% in adults 60 years of age or older. For most age groups, deaths in patients without HUS are rare, but 2% of adults 60 years old or older with only hemorrhagic colitis die.[66] Non-O157 STEC are diverse in their virulence properties, causing illness ranging from uncomplicated watery diarrhea to hemorrhagic colitis and HUS. Outbreaks reported in the United States have involved serogroups O26, O103, O111, O121, O145, and O104 (serotype O104:H21, but not O104:H4).[67]

Persistent Diarrhea Lasting 2 or More Weeks

Parasites including *Cryptosporidium, Giardia,* and *Cyclospora* are the most common causes of persistent (lasting ≥14 days) foodborne diarrhea (see Chapters 279, 282, and 283). In the mid-1990s outbreaks of cyclosporiasis linked to various types of imported fresh produce were recognized in the United States.[68] The incubation period averages about 1 week (range, about 2 days to 2 or more weeks), and the most common symptom is watery diarrhea. Other common symptoms include anorexia, weight loss, abdominal cramps, nausea, and body aches. Vomiting and low-grade fever may occur. Untreated illness can last for weeks or months, with a remitting-relapsing course and prolonged fatigue.[68]

A distinctive chronic watery diarrhea, known as Brainerd diarrhea, was first described in people who had consumed raw milk.[69] After a mean incubation period of 15 days, affected individuals developed acute watery diarrhea with marked urgency and abdominal cramping. Diarrhea persisted for more than a year in 75% of patients. Several restaurant-associated outbreaks and a cruise ship–associated outbreak of a similar illness have suggested that water also transmits the agent, which has not been identified.[70–73]

Cranial Nerve Palsies and Descending Paralysis

Botulism should be strongly suspected in anyone with acute onset of symmetrical cranial nerve palsies. In some patients, illness progresses to symmetrical descending flaccid paralysis that may result in respiratory arrest (see Chapter 245). Among patients with botulism, 98% report at least one of the following symptoms: dysphagia, blurred vision, slurred speech, double vision, and change in the sound of the voice.[74] Paralysis may coincide with or be preceded by nausea in 42%, vomiting in 33%, and diarrhea in 16%.[74] Botulism is usually caused by one of four immunologically distinct, heat-labile protein neurotoxins—botulinum toxins A, B, E, and rarely F.[75] The toxins irreversibly block acetylcholine release at the neuromuscular junction. Nerve endings regenerate slowly, so recovery typically takes weeks to months[75] but is longer for some patients.[76] Foodborne botulism results from ingestion of preformed toxin. The syndromes of infant botulism and adult intestinal colonization result from ingestion of spores, with subsequent toxin production in vivo.[75,77] Clinical suspicion is important for correct diagnosis of botulism.[78]

Systemic Illness

Some foodborne diseases manifest mainly as invasive infections in immunocompromised patients. Invasive listeriosis typically affects pregnant women, fetuses, and individuals with compromised cellular immunity (see Chapter 206). In pregnant women, infection may be asymptomatic or manifest as a mild flulike illness; 20% of pregnancies in infected women end in miscarriage.[79] Neonatal listeriosis is acquired in utero or at birth and manifests as either early-onset sepsis during the first several days of life or as late-onset meningitis during the first several weeks after birth; the neonatal fatality rate is approximately 20% to 30%.[80] In elderly and immunocompromised persons, listeriosis causes meningitis, sepsis, and focal infections.[80] The median incubation period is 11 days, and 90% of cases occur within 28 days of exposure.[81]

Vibrio vulnificus can cause septicemia after ingestion of contaminated food, typically raw oysters (see Chapter 215). This severe syndrome, often accompanied by bullous skin lesions (Fig. 101.1), is seen almost exclusively in patients with impaired immunity, especially patients with chronic liver disease including cirrhosis, alcoholic liver disease, and hepatitis.[82] The association with liver disease may be related to portal hypertension, resulting in reduced hepatic phagocytic function, elevated serum iron levels that promote growth of *V. vulnificus,* or achlorhydria. The overall mortality rate is approximately 15% to 30% and varies by the timeliness of antibiotic administration.[43,49,83–86]

Occasionally, other *Vibrio* spp. including *V. parahaemolyticus* and strains of *V. cholerae* that do not produce cholera toxin cause septicemia.[44,87] Nontyphoidal *Salmonella* can cause bacteremia and focal infections, often in patients at the extremes of age or in patients with sickle cell anemia, inflammatory bowel disease, or an immunocompromising condition.[88] *Campylobacter* spp., particularly *Campylobacter fetus,* may cause systemic infection, especially in patients with immunocompromising conditions.[89]

Consumption of foods contaminated with *Toxoplasma gondii* oocysts excreted from cats or meats containing tissue cysts can cause different manifestations of toxoplasmosis depending on the host (see Chapter 278).[90] In healthy children and adults, up to 90% of infections are asymptomatic, but the remainder lead to nontender, nonsuppurative lymphadenopathy lasting weeks to months or chorioretinitis. Fever, malaise, night sweats, myalgias, sore throat, maculopapular rash, and hepatosplenomegaly may occur; other manifestations (e.g., disseminated disease, pneumonitis, hepatitis, encephalitis, myocarditis, and myositis) are rare. Both asymptomatic and symptomatic acute infections lead to latent infections that can reactivate into life-threatening central nervous system infections if a person later becomes immunocompromised. The manifestations of congenitally acquired toxoplasmosis include subclinical infection (which may reactivate during childhood or adulthood), a diverse array of abnormalities at birth (e.g., hydrocephalus, cerebral calcifications, chorioretinitis, thrombocytopenia, and anemia), and perinatal death.[90]

Several species of *Trichinella* roundworms cause trichinellosis in humans, who are only accidental hosts, when raw or undercooked pork or wild game meat contaminated with larvae is consumed (see Chapter 287). The signs and symptoms depend partly on number of larvae ingested and the person's immunity. Gastrointestinal symptoms such as nausea, diarrhea, vomiting, and abdominal cramps may develop 24 to 48 hours after ingestion, corresponding to the enteral phase of infection. This may be followed by a constellation of signs and symptoms including fever, myalgias, periorbital or facial edema, headache, or eosinophilia lasting up to several weeks to months, corresponding to the parenteral phase of infection.[91]

Other infectious agents and diseases with primary symptoms outside the gastrointestinal tract and neurologic system that can be transmitted by foods include (with a food vehicle example) group A β-hemolytic streptococci (cold egg-containing salads),[92] typhoid fever (raw produce exposed to human sewage or foods contaminated by asymptomatic human carriers),[93] brucellosis (goat's milk cheese), anthrax (meat), tuberculosis (raw milk), Q fever (raw milk), hepatitis A (shellfish or raw produce), various trematode infections (fish and aquatic invertebrates),[94] anisakiasis (fish), and cysticercosis (foods contaminated by *Taenia solium* eggs shed in feces of persons with pork tapeworms).



FIG. 101.1 Hemorrhagic bullae of the leg secondary to *Vibrio vulnificus* infection. *(From Millet CR, Halpern AV, Reboli A, et al. Bacterial diseases. In: Bolognia JL, Jorizzo JL, Schaffer JV, et al, eds.* Dermatology. *3rd ed. Philadelphia: Mosby; 2012:1187–1220.)*

Postinfection Syndromes

Although arthropathy has been reported after various enteric infections, most experts agree that the term *reactive arthritis* should be applied only to infections caused by *Salmonella, Yersinia, Campylobacter,* or *Shigella*.[95] Reactive arthritis can occur as part of the triad of aseptic inflammatory polyarthritis, urethritis, and conjunctivitis described by Reiter. Attack rates of reactive arthritis after salmonellosis vary from 1.2% in studies using more objective case definitions to 14% to 29% in studies using more subjective case definitions.[95] Although associations between HLA-B27 positivity and reactive arthritis have been identified, the association may exist only for patients with more severe joint or extraarticular involvement. In studies consisting of mostly mild cases, no associations with HLA-B27 were found.[95]

Worldwide, approximately 20% to 40% of Guillain-Barré syndrome cases have been attributed to recent *C. jejuni* infection (see Chapter 216).[96] When preceding diarrheal illness is reported, it typically occurs 1 to 3 weeks before the onset of neurologic symptoms.[97] In contrast to botulism, this syndrome is usually manifested by an ascending paralysis accompanied by sensory findings and abnormal nerve conduction velocity.

Several enteric pathogens including *Campylobacter*, STEC, *Salmonella, Shigella, Giardia,* and norovirus may lead to the development of postinfectious irritable bowel syndrome or other functional gastrointestinal disorders.[98,99]

Foodborne Syndromes Caused by Nonbacterial Toxins

A description of all nonbacterial toxins that can cause foodborne illness is beyond the scope of this chapter. Illness caused by natural substances found in staple fruits and vegetables, spices, medicinal herbs and oils, and mycotoxins (other than those in mushrooms) are not covered.[100] Likewise, food allergies[101] and illnesses caused by additives,[100] methylmercury,[102] or niacin are not covered.[103]

Nausea, Vomiting, and Abdominal Cramps Associated With Heavy Metal Ingestion

Heavy metals including copper, zinc, tin, and cadmium have caused foodborne outbreaks.[104–109] Latency periods for symptom onset most often range from 5 to 15 minutes after ingestion of contaminated beverages but can be longer with contaminated foods.[110] Nausea, vomiting, and abdominal cramps result from direct irritation of the gastric and intestinal mucosa and usually resolve within 2 to 3 hours if minor amounts are ingested. Progression to serious illness and death is possible if larger amounts are consumed.

Histamine Reaction After Eating Seafood

Histamine fish poisoning (scombroid poisoning) is characterized by symptoms resembling those of a histamine reaction. Symptoms typically begin within 1 hour of eating contaminated seafood. Flushing, rash, diarrhea, headache, perioral paresthesias, palpitations, sweating, pruritus, abdominal cramps, nausea, and vomiting are common. In severe cases, urticaria bronchospasm may also occur.[111] Histamine can accumulate in fish flesh that has a high concentration of histidine if postmortem spoilage occurs from inadequate refrigeration. Marine bacteria catalyze the decarboxylation of histidine to heat-stable histamine. Symptoms usually resolve within 24 hours. In contrast to seafood allergies, scombroid fish poisonings have a very high attack rate.[112]

Paresthesias After Eating Shellfish

Two types of shellfish poisoning manifest with paresthesias: paralytic (PSP) and neurotoxic (NSP). Mild PSP is characterized by paresthesias of the mouth, lips, face, and extremities. Larger intoxications progress rapidly to include headache, vomiting, diarrhea, dyspnea, dysphagia, muscle weakness or frank paralysis, ataxia, and respiratory insufficiency or failure.[111,113] The disease is caused by saxitoxins produced by certain dinoflagellates. Bivalve mollusks and some fish feed on these dinoflagellates; the toxins are concentrated in their flesh. Saxitoxin is heat stable and blocks the propagation of nerve and muscle action potentials by interfering with sodium channel permeability. Patients typically recover in hours to a few days.[98]

The features of NSP are similar to the features of PSP, although they are less severe. Reverse temperature perception may occur.[111,113] Brevetoxins produced by certain dinoflagellates are responsible. Brevetoxins cause an influx of sodium into nerve and muscle cells, leading to continuous activation causing paralysis and fatigue.[113] Symptoms typically resolve within 48 hours.[111]

Gastrointestinal, Neurologic, and Cardiovascular Symptoms After Eating Fish

Ciguatera fish poisoning (Table 101.3) is caused by ingestion of reef fish (e.g., barracuda, amberjack, grouper, and red snapper) and is commonly seen in tropical coastal regions.[114,115] Illness usually begins within 6 to 24 hours after eating the contaminated fish and is characterized by a broad array of neurologic, gastrointestinal, and cardiovascular symptoms including facial, perioral, and extremity paresthesias, hot and cold temperature sensation reversal, metallic taste, headache, dizziness, nausea, vomiting, bradycardia, and hypotension.[114] In severe cases, respiratory distress and death may occur. Gastrointestinal and

TABLE 101.3 Etiology of Foodborne Disease Outbreaks by Commonly Implicated Foods, Peak Season, and Geographic Predilection

ETIOLOGY	COMMONLY IMPLICATED FOODS	PEAK SEASON(S)	GEOGRAPHIC PREDILECTION
Bacterial			
Salmonella	Beef, poultry, eggs, dairy products, produce	Summer, fall	None
Staphylococcus aureus	Ham, poultry, salads, sandwiches, unpasteurized dairy products in some countries (e.g., France)	Summer	None
Campylobacter jejuni	Poultry, produce, unpasteurized (raw) milk and dairy products	Spring, summer	None
Clostridium botulinum	Home-canned vegetables, preserved fish, honey (infants)	Summer, fall	Type E common in Alaska
Clostridium perfringens	Beef, poultry, gravy	Fall, winter, spring	None
Shigella	Cold foods contaminated by food handler	Summer	None
Vibrio parahaemolyticus	Shellfish	Spring, summer, fall	Coastal states
Bacillus cereus	Fried rice, meats, vegetables		None
Yersinia enterocolitica	Pork, chitterlings	Winter	Unknown
Vibrio cholerae O1	Shellfish		Tropical, Gulf Coast, Latin America
Vibrio cholerae non-O1	Shellfish		Tropical, Gulf Coast
STEC	Ground beef, raw produce unpasteurized milk	Summer, fall	Northern United States
Viral			
Noroviruses	Salads, shellfish	Winter	None
Parasitic			
Toxoplasma gondii	Undercooked meat, raw shellfish, produce		None
Trichinella	Game meat, less commonly pork in United States		None
Cyclospora cayetanensis	Imported fresh produce	Spring, summer	None
Cryptosporidium	Unpasteurized apple cider	Summer	Northern United States
Giardia	Raw produce and a variety of other foods	Summer	Northern United States
Chemical			
Ciguatera	Barracuda, snapper, amberjack, grouper		Tropical reefs
Histamine fish poisoning (scombroid)	Tuna, mackerel, bonito, skipjack, mahi-mahi		Coastal areas
Shellfish poisoning	Shellfish		Coastal areas
Mushroom poisoning	Mushrooms	Spring, fall	Temperate
Heavy metals	Acidic beverages		None

STEC, Shiga toxin–producing *Escherichia coli.*

cardiovascular symptoms resolve in a few days, but neurologic symptoms can last for weeks or years.[111,116]

Ciguatoxins are lipid-soluble, heat-stable dinoflagellate toxins that open voltage-sensitive sodium channels in neuromuscular junctions. The dinoflagellates are consumed by small fish, which are then consumed by carnivorous fish where the toxins concentrate. Some dinoflagellates that produce ciguatoxins also produce maitotoxin. Although maitotoxin opens cell membrane calcium channels, its role in ciguatera is uncertain given its water-soluble nature.[111,116]

Rapid Paralysis After Eating Puffer Fish

Puffer fish poisoning, caused by tetrodotoxin, is rare outside of East Asia, but an outbreak in the United States resulted from fish transported in a suitcase. Rapid ascending paralysis occurs, and 14% of patients die.[111] Patients may be conscious while paralyzed.

Diarrhea and Chills Within 12 Hours After Eating Shellfish

Outbreaks of diarrhetic shellfish poisoning have been reported throughout the world including in the United States.[117,118] Diarrhea follows ingestion of filter-feeding bivalve mollusks such as mussels and scallops that contain okadaic acid produced by certain dinoflagellates. Symptoms, which can include diarrhea, chills, and vomiting, usually occur within a few hours and resolve within 3 days.[111,113] Outbreaks of azaspiracid shellfish poisoning, causing a similar syndrome, have been reported in Europe.[113]

Diarrhea, Abdominal Cramps, Memory Loss, and Disorientation After Eating Shellfish

Rare outbreaks of amnesic shellfish poisoning have been reported. Gastrointestinal symptoms, which typically occur within 24 hours of consumption, predominate in individuals younger than 40 years of age. Neurologic symptoms including headache, visual disturbances, cranial nerve palsies, anterograde amnesia, coma, and death, are more common in individuals older than 50 years of age and typically occur within 48 hours of consumption. The illness is caused by the toxin domoic acid, which is produced by certain dinoflagellates and concentrated in shellfish.[111,113]

Abdominal Cramps, Vomiting, and Diarrhea Followed by Hepatorenal Failure

Mushrooms containing cyclopeptides (amatoxins and phallotoxins) are responsible for greater than 90% of all deaths resulting from mushroom poisoning (Table 101.4). The most common implicated mushrooms are *Amanita phalloides* and *Amanita virosa*.[119] The illness is biphasic. Severe abdominal cramps, vomiting, and severe diarrhea manifest acutely and usually resolve within 12 to 24 hours. The patient then remains well for 12 to 24 hours. Two to 4 days after ingestion, hepatic and renal failure supervene. The mortality rate in adults is about 10% to 30%.[119,120] A similar syndrome occurs after ingestion of mushrooms containing gyromitrin. Although renal failure is not a feature, hemolysis, seizures, and coma may occur.[121]

Amanita smithiana mushrooms contain toxins that cause nausea, vomiting, and sometimes diarrhea 4 to 11 hours after ingestion; renal failure may develop 4 to 6 days after ingestion.[122] *Cortinarius* spp. containing orellanine cause nausea, vomiting, and diarrhea 6 to 48 hours after ingestion; renal injury may develop 2 days to 3 weeks after exposure.[121]

Miscellaneous Other Mushroom Poisoning Syndromes With Onset Within 2 Hours

Several syndromes may occur after ingestion of toxic mushrooms; identification of the syndromes is more important than knowing the associated species (see Table 101.4).[119,121,122] Species containing ibotenic acid and muscimol cause an illness that mimics alcohol intoxication and is characterized by confusion, restlessness, and visual disturbances, followed by lethargy; symptoms usually resolve within 12 hours. Muscarine-containing mushrooms cause parasympathetic hyperactivity (e.g., salivation, lacrimation, diaphoresis, blurred vision, abdominal cramps, diarrhea). Some patients experience miosis, bradycardia, and bronchospasm. Symptoms usually resolve within 24 hours. Species containing psilocybin cause hallucinations and behavior changes, which usually resolve within 8 hours. Mushrooms containing a disulfiram-like substance, coprine, cause headache, flushing, paresthesias, vomiting, and tachycardia if alcohol is consumed within 72 hours after ingestion. Species containing allenic norleucine cause gastrointestinal symptoms (e.g., anorexia, nausea, vomiting, diarrhea) within 30 minutes to 12 hours after ingestion. Progression to liver injury and acute renal failure typically occurs 4 to 6 days after ingestion. A variety of mushrooms can cause typically mild gastrointestinal irritation.[121]

TABLE 101.4 Mushroom Poisoning Syndromes, Commonly Implicated Mushrooms, and Associated Toxins

SYNDROME	COMMONLY IMPLICATED MUSHROOMS	TOXINS
Short Incubation		
Delirium, restlessness	*Amanita muscaria, Amanita pantherina*	Ibotenic acid, muscimol
Parasympathetic hyperactivity	*Inocybe* spp., *Clitocybe* spp., *Boletus* spp.	Muscarine
Hallucinations, somnolence, dysphoria	*Psilocybe* spp., *Panaeolus* spp., *Conocybe* spp.	Psilocybin
Disulfiram reaction	*Coprinus atramentarius*	Coprine
Gastroenteritis	Many	Various uncharacterized irritants
Long Incubation		
Gastroenteritis, hepatorenal failure	*Amanita phalloides, Amanita virosa,* and other *Amanita* spp.; *Galerina, Cortinarius,* and *Lepiota* spp.	Cyclopeptides (i.e., amatoxins, phallotoxins)
Gastroenteritis, muscle cramping, hepatic failure, hemolysis, seizures, coma	*Gyromitra* spp.	Gyromitrin
Gastroenteritis, acute renal failure (temporary)	*Amanita smithiana*	Allenic norleucine
Gastroenteritis, acute renal failure (often irreversible)	*Cortinarius* spp.	Orellanine

EPIDEMIOLOGY

Additional clues to the cause of a foodborne illness may be found when the illness occurs as part of an outbreak. Clinicians and public health officials can consider the type of food implicated and the outbreak setting in the context of typical epidemiologic characteristics associated with foodborne pathogen outbreaks (see Table 101.3).

Foods

Outbreaks of staphylococcal food poisoning are associated with foods of high protein content. In the United States, the foods most frequently implicated include ham, poultry, beef, potato and egg salads, pasta dishes, and sandwiches, which are thought to be contaminated during preparation by a food handler.[22] In the classic staphylococcal foodborne outbreak, a food handler's hand has a purulent skin lesion, but this is true in only a minority of outbreaks. In countries with high consumption of unpasteurized cheese such as France, dairy products are the most common vehicles. The udders of dairy animals, especially animals with mastitis, are one source.[123] In contrast, outbreaks of emetic *B. cereus* food poisoning are most often associated with starchy foods, especially rice, that have been cooked and held warm for extended periods, during which heat-resistant spores can germinate into vegetative cells that multiply and produce toxin.[19,27]

C. perfringens outbreaks usually occur after the ingestion of meat (especially beef and poultry) that has not been cooked or stored properly.[32]

Organisms have been isolated from raw meat, poultry, and fish. Outbreaks are more likely to occur when these items are held between 15°C (59°F) and 50°C (122°F) after cooking, allowing spores to germinate and vegetative cells to rapidly multiply.[33,35] *B. cereus* strains that cause the diarrheal syndrome are frequently associated with proteinaceous meat, dairy, and vegetable dishes.[27] One large *B. cereus* diarrheal outbreak was attributed to barbecued pork that was unrefrigerated for 18 hours after cooking[124]; another outbreak was traced to a meal delivery service in which food was held at and above room temperature for an extended period.[125]

E. coli O157 outbreaks were initially recognized mostly after consumption of undercooked ground beef, and this remains the most commonly recognized source in the United States. However, outbreak investigations have implicated a broad range of foods through laboratory, epidemiologic, and traceback evidence including leafy greens, apple cider, alfalfa sprouts, venison, salami, cookie dough, and flour.[12,126,127] Healthy cattle commonly carry *E. coli* O157 in their intestines and excrete it in manure, which may be used to fertilize fields; this organism is not commonly found in other food animals. Produce may become contaminated with *E. coli* O157 through environmental contamination by feces (from cattle or other animals such as feral swine or deer)[128,129] or by use of water in processing that has been contaminated with fecal matter. Outbreaks of non-O157 STEC infections have been associated with a range of food vehicles.

Nontyphoidal *Salmonella* is carried in the intestines of many animals including wild reptiles, amphibians, birds, and mammals as well as most food production animals. Consequently it is common in the environment and food chain, leading to potential contamination of many types of foods. Outbreaks have been associated with contaminated poultry, beef, fish, egg, dairy products, produce, juice, peanut butter, chocolate, cereals, and frozen processed foods. *Salmonella* serotype Enteritidis, common in poultry flocks, can internally contaminate shell eggs through an ovarian infection in the hen.[130] In the United States in the years 1998–2008, eggs and poultry were the predominant sources in outbreaks caused by *Salmonella* serotype Enteritidis[131]; these foods are also the dominant sources of sporadic infections of this serotype.[132,133]

Illnesses caused by *E. coli* O157:H7, other STEC, *Salmonella, Campylobacter, Brucella, Listeria,* and *Shigella* have been associated with consumption of raw milk. Despite these risks, raw milk is still legally sold in many states. From 1993 to 2006, 73 foodborne disease outbreaks caused by nonpasteurized milk or cheese were reported to the CDC, accounting for 1571 illnesses, 202 hospitalizations, and 2 deaths; 75% of these outbreaks occurred in the 21 states that permitted sale of nonpasteurized dairy products.[134]

Shigella outbreaks are most often associated with cool, moist foods consumed raw or that require handling after cooking such as lettuce-based salads, potato and egg salads, salsas, dips, and oysters.[135] The organism is carried by humans, not animals. Many foodborne *Shigella* outbreaks are associated with restaurants and are usually attributable to ill food workers.[135] Less than half of *Shigella* infections are estimated to be acquired through food; most are transmitted person to person directly or via fomites. The major food source for *Campylobacter* infection is poultry. Foodborne illness occurs from consumption of undercooked poultry and from consumption of other foods, especially produce, cross-contaminated from poultry in the kitchen or earlier in the farm-to-table continuum. Cattle also carry *Campylobacter,* and unpasteurized dairy products are a source.[136]

Listeriosis outbreaks have been traced to delicatessen meats, frankfurters, soft cheeses (e.g., queso fresco), and produce.[137] Delicatessen meats and frankfurters, which previously had caused large outbreaks of infections, have been infrequently implicated in outbreaks occurring since 2005, probably because of several regulatory initiatives.[138] In the United States, raw produce including raw sprouts (2008), precut celery in chicken salad (2010), whole cantaloupe (2011), and lettuce (2015–16) has been recognized as the source of listeriosis outbreaks.[138–141]

Vibrio outbreaks are rarely identified in the United States and have been caused by *V. parahaemolyticus,* toxigenic *V. cholerae,* and *Vibrio mimicus,* usually associated with the ingestion of shellfish.[45,50,87,142] Sporadic foodborne cases of *Vibrio* illness are also typically linked to ingestion of shellfish, especially oysters.[87] Toxigenic strains of *V. cholerae* have been acquired from domestically grown shellfish. Crabs transported in travelers' luggage have caused cholera.[143]

Y. enterocolitica infections are much less commonly diagnosed in the United States than in some European countries.[56,144,145] However, the use of CIDT syndrome panels is increasing detection. Outbreaks occur occasionally and have been associated with consumption of pork products and contaminated milk; produce has been implicated less frequently.[57,58,146–148] Fewer infections attributable to cross-contamination from the preparation of pork chitterlings in the household now appear.[57,144]

Although diarrhea caused by ETEC is usually thought to be acquired primarily through exposures encountered during travel outside developed countries or on cruise ships,[149,150] outbreaks caused by ETEC do occur in the United States and are most commonly associated with foods that require extensive handling to prepare and are often served cold such as seafood, fresh produce, herbs, or salads.[151–155] Foodborne ETEC infections are typically associated with a breech in hygiene and sanitation during food production, transport, or preparation.[156]

Clostridium botulinum spores can germinate into cells that can grow and produce toxins only in foods that provide an anaerobic environment, a pH of less than 4.5, low salt and sugar content, and a temperature of 4° to 121°C.[75] Foodborne botulism outbreaks have been associated with home-canned vegetables, fruits, fish, and alcohol made illegally in correctional institutions.[157–159] Additional vehicles have included baked potatoes, sautéed onions, and commercial chopped garlic in oil lacking a growth inhibitor.[160] Outbreaks caused by bottled carrot juice in 2006 and canned chili sauce in 2007 were the first related to commercial products in almost 20 years.[161,162] Honey is a recognized source of *C. botulinum* spores in infant botulism; therefore parents are advised to not feed honey to infants younger than 1 year old.[77]

In norovirus outbreaks, food is most often contaminated by a food handler; contamination can also occur directly with human fecal matter at the source of production such as shellfish caught in sewage-contaminated waters or produce irrigated with water contaminated by sewage.[28] Foods that require handling without subsequent cooking are typically implicated, including sandwiches, leafy vegetables, fruits, and shellfish.[28] In one large multistate outbreak, steamed shellfish from the Gulf Coast were implicated. These were probably contaminated by ill oystermen, who, lacking toilet facilities on their oyster boats, defecated and vomited directly into shallow oyster beds.[163]

Cyclospora infection results from ingestion of mature (infective) oocysts in contaminated food or water. Cyclosporiasis is endemic in various tropical and subtropical regions. Outbreaks of cyclosporiasis in the United States have been linked to multiple types of imported fresh produce including raspberries, basil, mesclun lettuce, and snow peas.[68,164] Detection of *Cyclospora* infections has increased markedly in recent years, at least partly due to improved detection.[165]

Foodborne toxoplasmosis is acquired through consumption of undercooked meat (especially pork, lamb, and game meat) that contains tissue cysts or uncooked foods (primarily fruits and vegetables, but also raw shellfish) contaminated with oocysts originating from cat feces.[166] Free-range organically raised meats, which are growing in popularity, may be more likely to be contaminated.[166] Trichinellosis caused by consumption of contaminated pork still occurs infrequently in the United States. However, largely because of improvements in swine husbandry in the past several decades as well as efforts to educate consumers on cooking pork thoroughly to kill any *Trichinella* larvae present, the most frequent source of trichinellosis cases and outbreaks in the United States has shifted from commercial pork to wild game meat, especially bear meat.[167]

Outbreaks of heavy metal poisoning are most often associated with acidic beverages such as tea, lemonade, fruit punch, and carbonated drinks that have been stored in corroded metallic containers for periods sufficient to leach the metallic ions from the container.[104–108,110]

Although histamine, or scombroid, fish poisoning is named after the Scombroidae family, which includes fish such as tuna, mackerel, bonito, and skipjack, many nonscombroid fish including, but not limited to, mahi-mahi, bluefish, and escolar can also cause histamine fish poisoning.[111,112,168,169] Ciguatera fish poisoning is associated with consumption of many different large reef-dwelling carnivorous fish including barracuda, red snapper, amberjack, and grouper caught between 35° north and

south of the equator.[111] Shellfish poisonings mostly occur after ingestion of bivalve mollusks, most often oysters, clams, mussels, and scallops.[113] However, cases of PSP in Florida have followed consumption of local puffer fish.[170]

The possibility that foodborne illness could be the result of an intentional contamination should also be considered. An outbreak of salmonellosis in Oregon in 1984 involved 751 people who ate or worked at 10 area restaurants. Epidemiologic investigation determined that illness was associated with eating from salad bars. A subsequent criminal investigation revealed that members of a religious commune had deliberately contaminated the salad bars.[171] In 1996 an outbreak of *Shigella dysenteriae* type 2 infections affecting 12 people was caused by consumption of deliberately contaminated muffins.[172] In 2003 ground beef was intentionally contaminated with nicotine at a supermarket.[173]

Nonfoodborne Transmission

The evaluation of a suspected foodborne outbreak may reveal other modes of transmission including consumption of water (drinking or recreational)[174,175] or contact with infected animals[176] or persons.[30]

Some pathogens incriminated in waterborne disease outbreaks are different from those most often responsible for foodborne disease. *Giardia* is a frequently recognized pathogen in outbreaks associated with drinking water including several large outbreaks traced to municipal water supplies.[177–179] Foodborne giardiasis outbreaks in the United States are rare and are usually caused by infected food handlers.[180] Giardiasis is characterized by diarrhea, nausea, abdominal pain, bloating, flatulence, and occasionally malabsorption. The incubation period is typically 1 to 2 weeks, and the duration of illness may be several weeks or occasionally longer. *Cryptosporidium* is the most common infectious cause of outbreaks caused by contaminated recreational water intended for swimming.[175] *Cryptosporidium* outbreaks caused by contaminated food and a massive outbreak caused by contaminated drinking water have occurred.[180,181] Norovirus spreads easily and can cause large outbreaks through contaminated food, water, and environments; most transmission occurs from person-to-person.[182–187] Other waterborne outbreaks have been caused by *E. coli* O157:H7,[188,189] *Shigella,*[190] hepatitis A,[191] *Salmonella* serotype Typhi,[192] nontyphoidal *Salmonella,*[193] ETEC,[194] *C. jejuni,*[136,195–197] *Toxoplasma,*[198] and *V. cholerae.*

Vulnerable Populations

Some people are more susceptible than others to acquiring a foodborne infection or to experiencing more severe illness.[199] Most host factors associated with increased risk are related to inadequate immune response. Immune-related factors include, but are not limited to, age younger than 5 years, age 65 years or older, primary immunodeficiencies, pregnancy, human immunodeficiency virus (HIV) infection, leukemia, immunosuppressive medications (e.g., chemotherapy, corticosteroids, agents used to treat autoimmune conditions), diabetes, and nutritional deficiencies.[199] Individuals with excessive iron stores, such as patients with cirrhosis and hemochromatosis, are more susceptible to infection with foodborne pathogens that grow more rapidly in the presence of iron.[199] The protection against foodborne infections conferred by gastric acid is reduced in persons who consume antacids (especially proton pump inhibitors), large volumes of liquid, or fatty foods.[199] The increasing average age and accompanying chronic illnesses in many countries means that more of the population has a heightened susceptibility to severe foodborne infections.

People with compromised immunity caused by HIV infection have higher reported rates of salmonellosis, campylobacteriosis, shigellosis, invasive listeriosis, and cyclosporiasis than persons not infected with HIV.[200,201] Most of these infections are more likely to be severe, recurrent, or persistent in such patients.[200,201] This may result from multiple aspects of host-pathogen interaction such as a lower dose needed to cause illness among these susceptible individuals owing to differences in innate, humoral, and cellular immunity. Rates of cryptosporidiosis have decreased since the advent of highly active antiretroviral therapy.

People who consume unpasteurized (raw) milk are at increased risk of acquiring foodborne infections. Immigrant and refugee populations may have certain customs that involve consumption of raw, undercooked, or fermented food.[202] Severe mushroom poisonings have occurred among immigrants who mistook poisonous mushrooms as safe mushrooms from their place of origin.[119]

Seasonality

Illnesses caused by *S. aureus, Salmonella, Shigella*, *C. jejuni, Vibrio* spp., STEC, *Giardia,* and *Cryptosporidium* are most common during the summer months. Similarly, shellfish-associated *Vibrio* infections peak during warmer months and are closely related to the temperature of the water in the oyster beds.[203] Illnesses caused by *C. perfringens* occur throughout the year but least often during the summer months.[32] Among black children in the United States, *Y. enterocolitica* infections have occurred primarily after winter holidays at which pork chitterlings are served. However, likely related to education efforts, decreased contamination of chitterlings, or both, the winter seasonality of these infections in black children diminished considerably during 2000–09. *Y. enterocolitica* infections occur throughout the year for other demographic groups.[144] The use of CIDTs has markedly increased detections of *Yersinia*.[165] Although transmitted year-round, winter is the season of peak norovirus activity, which provides additional opportunity for foodborne contamination with this pathogen.[29]

With a few exceptions, chemical food poisoning occurs throughout the year. Shellfish poisonings and ciguatera occur in association with harmful algal blooms such as a red tide. These blooms can occur at any time of the year but may be more common in late summer and fall in Florida. Mushroom poisoning is most common in the spring, late summer, and fall.

Geographic Location

The geographic setting may also provide a clue to the cause of foodborne disease. For unexplained reasons, *E. coli* O157 infections and outbreaks in the United States are more common in the northern states bordering Canada.[126,204,205] In Germany, areas of higher frequency of human *E. coli* O157 and some non-O157 STEC infections are those with higher cattle density.[206]

V. parahaemolyticus outbreaks in the United States are most frequently reported from coastal states (Gulf Coast, Atlantic, and Pacific).[45] Cases of toxigenic and nontoxigenic *V. cholerae* infection have been most often reported from the Gulf Coast of the United States.[87] Type E botulism is most common in Alaska due to consumption of fermented marine animals among Alaskan Natives.[207]

Most ciguatera fish poisoning outbreaks in the United States have been reported from the Caribbean, Florida, or Hawaii.[208] Travelers who return from these areas with the characteristic syndrome should be questioned regarding fish consumption. Transport of fish from endemic areas has caused outbreaks in nonendemic locations.[209,210] PSP and NSP outbreaks occur more frequently in coastal areas.

Globalization and centralization of the world's food supply has led to increased detection of multistate and multinational outbreaks.[15] Seemingly isolated illnesses within a geographic area may actually be part of a larger multistate or multinational outbreak.

Epidemiologic Assessment

If an outbreak of foodborne disease is suspected, public health authorities should be contacted so that it can be investigated. Investigating the outbreak is important to identify and rapidly control the source. Sometimes a common epidemiologic characteristic or meal shared by all or most patients is identified. Other outbreaks are the result of a widely distributed contaminated food, so cases may be dispersed across a broad geographic area. Ordering appropriate laboratory testing on patients is critical to identifying clusters of related cases by subtype-based surveillance.[211] In addition to identifying related strains among patients who may have shared a common exposure, subtype-based surveillance can match isolates from patients to isolates from foods or establishments.

After a food source for the infections is identified and the outbreak is halted, the investigation turns to the question of how contamination is likely to have occurred. Steps of food preparation, holding temperatures, and hygienic circumstances in the kitchen are assessed. Investigation into the sources of food ingredients may lead to assessments of the originating farm or processing plant. These assessments can result in

changes in regulatory guidance and industry practices that prevent future illnesses.

LABORATORY DIAGNOSIS

Most diarrheal illnesses do not require diagnostic testing. Guidelines exist that describe clinical presentations that should prompt stool testing in patients with diarrhea including, but not limited to, fever, bloody diarrhea, sepsis, and illness in people with certain high-risk conditions.[212] Blood cultures should be obtained from infants with sepsis or suspected enteric fever, patients with systemic manifestations of infection, patients with immunocompromising or other high-risk conditions, and patients with possible exposure to a person with suspected enteric fever.[212] Bacterial infections that are identified by culture-independent diagnostic tests and are subject to public health reporting rules should prompt culture. With culture, an isolate can be obtained and subtyped, which aids in outbreak detection; isolates can also be tested for antimicrobial susceptibility, which may change management.

Because some pathogens require specific culture media, culture conditions, or stains, diagnosis may depend on the skill of the clinician or laboratory. Some bacterial foodborne pathogens are not readily identified from cultures alone (e.g., diarrheogenic *E. coli* other than *E. coli* O157 such as non-O157 STEC and ETEC).[156,213] In addition, clinical laboratories in general do not have the capacity to test for norovirus. Therefore to identify some etiologies, human specimens must be sent to reference laboratories. Reference laboratories can identify non-O157 STEC isolates from Shiga toxin–positive specimens received from clinical laboratories,[213] identify other diarrheogenic *E. coli* (e.g., ETEC, EAEC, enteropathogenic *E. coli*, and enteroinvasive *E. coli*) by detection of virulence genes or toxins that define specific pathotypes, and identify norovirus by polymerase chain reaction (PCR).[214]

Newer commercially available molecular diagnostic assays such as assays that use multiplex PCR to simultaneously detect and distinguish between multiple enteric pathogens including several undetectable by traditional methods (e.g., ETEC, norovirus) are now widely available in the clinical setting.[215,216] These multipathogen assays can yield results within several hours. Because of the multipathogen nature of these newer molecular tests, they are likely to increase the number of illnesses for which two or more enteric pathogens are identified. Over time, this may improve understanding of pathogen interactions. However, it will become increasingly important to interpret positive results in a clinical context because some potential pathogens detected may not be causing illness.[215–217] Despite the clinical advantages of CIDTs (speed, number of detectable pathogens, and possibly lower cost), they are unable to provide information that is sometimes essential for patient care and public health purposes. Therefore bacterial culture remains necessary for antimicrobial susceptibility testing and for pathogen subtyping that aids outbreak detection and investigation. In the future, metagenomics methods may allow for subtyping and resistance testing of pathogens directly from stool specimens.

Diagnosis in Foodborne Outbreaks

In the context of a possible outbreak, determining an etiology is always important for identification of additional cases and for investigation into the possible sources of infection. To confirm the etiologic agent in outbreaks, appropriate specimens (e.g., stool or vomitus) should be tested from multiple patients. Specimen types vary by etiologic agent, and proper specimen collection and transport are important for successful diagnosis[218]; guidance is available at https://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/specimen-collection.html. In addition, as guided by epidemiologic findings, outbreak investigators may examine samples from leftover food, the food preparation environment, and food handlers. Food specimens must be sent to food microbiology laboratories that can detect bacterial or nonbacterial toxins and chemicals.

The identification, investigation, and confirmation of foodborne outbreaks of bacterial etiology have been aided greatly by standardized pulsed-field gel electrophoresis (PFGE) and whole-genome sequencing (WGS) methods in public health laboratories in the United States and Canada that are part of a subtyping network called PulseNet.[211] Bacterial isolates received from clinical laboratories that are routinely subtyped by PFGE include *E. coli* O157 and other STEC, *Salmonella, V. cholerae,* and *C. botulinum*. *Campylobacter, Shigella,* and other *Vibrio* spp. are sometimes subtyped. *L. monocytogenes* is routinely subtyped by WGS. Other pathogens may be subtyped by PFGE or WGS to confirm an outbreak. PulseNet is growing internationally, which will enable better detection of outbreaks on an international scale.

Specific Considerations About Important Pathogens

Staphylococcus aureus

Staphylococcal food poisoning is usually diagnosed clinically. Outbreaks of staphylococcal food poisoning can be confirmed by isolation of *S. aureus* of the same phage type pattern from vomitus or feces of two or more ill people or from both patient and food samples or by detection of enterotoxin or the isolation of greater than 10^5 organisms per gram in epidemiologically implicated food.[219] However, because *S. aureus* (but not the enterotoxins they produce) is heat sensitive, it is often difficult to isolate the organism from heat-treated foods. The identification of specific *S. aureus* enterotoxins (SEA through SEE) in food is accomplished most commonly by reverse passive latex agglutination or enzyme-linked immunosorbent assay platforms, which are available commercially. The tests distinguish SEA through SEE and are useful for identifying the types of enterotoxins involved in foodborne outbreaks. More recently, mass spectrometry has been used to quantify enterotoxins in foods. Although PCR detection alone of enterotoxin genes directly from contaminated foods and specimens cannot be used to confirm staphylococcal food poisoning, it can confirm isolation and direct toxin testing results.

Bacillus cereus

B. cereus food poisoning is also usually a clinical diagnosis. Outbreaks may be documented by isolating organisms from the feces of two or more ill people who shared the same meal or by isolating 10^5 or more *B. cereus* organisms per gram of incriminated food. Because nontoxigenic *B. cereus* is commonly found in foods, testing for enterotoxin genes in several isolates is advised. Molecular subtyping (e.g., by multilocus sequence typing,[220] plasmid analysis, or serotyping[221]) may be of value in confirming that isolates were derived from a common source because 14% of healthy adults have been reported to have transient gastrointestinal colonization with *B. cereus.*[222] Commercial immunoassays are available for two of the diarrheogenic enterotoxins of *B. cereus*.[19] A wild boar sperm assay has proven to be a simple and effective way to screen for cereulide-producing *B. cereus* isolates.[223] PCR assays that detect the *ces* gene (cereulide) in emetic strains have been developed.[224]

Clostridium perfringens

C. perfringens are part of the normal microbiota in many people. In addition, because less than 5% of *C. perfringens* type A strains produce CPE,[34] toxin assessment is needed to confirm a diagnosis. A reverse passive agglutination kit for detection of CPE directly from stools is commercially available. PCR can detect the *cpe* gene, but detection may not indicate expression of the protein. Therefore, detection of toxin in stool is the best indicator of infection. *C. perfringens* outbreaks can be confirmed by detection of enterotoxin or isolation of 10^6 organisms per gram of stool from two or more ill patients or by the isolation of 10^5 organisms per gram in epidemiologically implicated food.[225] Molecular subtyping can confirm that the same strains are present in both implicated foods and patients.[35] Because both heat-sensitive and heat-resistant strains of *C. perfringens* type A have been implicated in food poisoning, selective isolation procedures involving heat treatment of food and fecal specimens should not be used.

Classic Bacterial Enteropathogens

STEC (including *E. coli* O157), *Salmonella, Shigella, C. jejuni, Vibrio,* and *Y. enterocolitica* outbreaks may be detected by isolation of the organisms from the feces of ill people. However, pathogens identified as part of a routine stool culture typically include only *Salmonella, Shigella, Campylobacter,* and sometimes *E. coli* O157. To identify other bacterial pathogens in culture, the clinical laboratory needs to be notified so that special media can be used.[88] Culture-independent diagnostic

tests may also detect these organisms and are now routinely used to diagnose STEC infection. However, the tests usually do not identify species and subtypes. Culture is needed for antimicrobial susceptibility testing and for molecular subtyping (e.g., by PFGE or WGS, which are used to identify outbreaks). Infection with STEC O157 can be diagnosed by isolating sorbitol-negative *E. coli* from stools of ill patients on selective and differential media such as sorbitol-MacConkey and confirming the serogroup as O157 and then either confirming the H7 antigen, the detection of Shiga toxin, or the presence of *stx* genes that encode for the expression of the toxin. Non-O157 STEC can be identified by demonstration of Shiga toxin in stool by enzyme immunoassay or *stx* genes in stool by PCR at the clinical laboratory, with subsequent isolation of the organism by culture at a reference laboratory. In the United States, isolates of *E. coli* O157 or Shiga toxin–positive broth cultures should be forwarded to a public health laboratory for full characterization and PFGE or WGS subtyping.[213] Although immunoassays for detection of *Campylobacter* in stool are available, the accuracy of these tests varies considerably.[226] Testing of sera, especially paired serum from the acute and convalescent phases of illness, may be helpful in confirming the diagnosis of patients in outbreaks of STEC, cholera, and typhoid fever.

Botulism

Patients with suspected botulism should be treated before the diagnosis is confirmed. Tests that can help narrow the differential diagnosis include lumbar puncture (a high cerebrospinal fluid protein level is suggestive of Guillain-Barré syndrome and Miller Fisher syndrome, a variant of Guillain-Barré syndrome, which can be confused with botulism), the Tensilon test (to diagnose myasthenia gravis), and electromyography (findings of neuromuscular junction blockade, normal axonal conduction, and potentiation with rapid repetitive stimulation are suggestive of botulism).[75] Botulism may be confirmed by the demonstration of botulinum toxin in serum, gastric secretions, stool, or the incriminated food by the mouse neutralization test or by the isolation of *C. botulinum* from stool or the incriminated food; these tests can be arranged through contact with state health departments.[75] Laboratory confirmation by testing of clinical specimens can be obtained in about 70% to 75% of botulism cases.[227,228]

Protozoa

Physicians should specifically request testing for *Cyclospora* and *Cryptosporidium,* if indicated, because these pathogens are not typically detected via routine ova and parasite examination and require specialized techniques. *Cyclospora cayetanensis, Cryptosporidium,* and *Giardia* are included on the BioFire FilmArray Gastrointestinal Panel (BioFire Diagnostics, Salt Lake City, UT).[165,229] PCR assays are available for *Cyclospora*.[230] Cryptosporidiosis can be diagnosed by microscopy, PCR, or enzyme immunoassays.[231,232] Toxoplasmosis can be diagnosed by both serology and PCR.[90] Trichinellosis is diagnosed by serologic testing and, less frequently, by muscle biopsy. Additional information on the diagnosis of parasitic infections can be found at https://www.cdc.gov/dpdx/diagnosticprocedures/index.html and in Chapters 278, 279, 282, 283, and 287.

Heavy Metals

Outbreaks caused by heavy metals may be documented by demonstration of the metal in the incriminated food or stool specimens from ill patients. Marine toxin syndromes are diagnosed by clinical presentation and history of seafood consumption in the preceding 24 hours; diagnostic tests are not available in the clinical setting.[111,233] However, detection of toxin in implicated foods or certain clinical samples may be possible in coordination with the health department. Mushroom poisonings should be diagnosed by clinical suspicion and may be confirmed either by the identification of the responsible toxin in gastric contents, blood, urine, or fecal specimens or by the identification of the mushroom by a mycologist.[119]

Unknown or Rare Causes

About one-third of the reported foodborne disease outbreaks in the United States are of unknown etiology.[52] In many cases, appropriate diagnostic procedures are not conducted, or specimens are not collected in a timely manner, not transported properly, or are transported under suboptimal conditions. Many of these unknown outbreaks may be due to typical agents.[234] In others, no agent is identified despite testing, raising the possibility that etiologic agents not routinely tested for are responsible; possibilities include ETEC, EAEC, and sapovirus. The frequency of outbreaks of unknown etiology may decrease as molecular methods that can detect a wider array of pathogens become increasingly available.

Rare instances of enterococci and gram-negative rods (e.g., *Aeromonas hydrophila, Klebsiella, Enterobacter, Proteus, Citrobacter,* and *Pseudomonas*) have been reported as causes of foodborne outbreaks. However, because these organisms may be present in foods without causing illness and may be part of the normal human fecal flora, documenting their role in foodborne disease outbreaks is difficult.

THERAPY

Supportive measures are the mainstay of therapy for most foodborne illnesses. In any diarrheal illness, gastrointestinal fluid losses should be replaced. Usually any over-the-counter oral rehydration solution is sufficient; for severe dehydration, intravenous hydration may be required.[235] Early intravenous hydration may reduce the risk of oligoanuric HUS in patients with STEC O157 infection[236]; patients who develop HUS frequently require blood transfusions.[61] Antiemetics and antimotility agents offer symptomatic relief in adults, although the latter are contraindicated in patients with high fever, bloody diarrhea, or fecal leukocytes indicative of inflammatory diarrhea. Antiemetics and antimotility agents are usually not recommended for children. Supportive intensive care is essential in the management of botulism; access to mechanical ventilation greatly reduces the mortality rate.[75] The most lethal foodborne diseases are botulism, invasive listeriosis (affecting neonates, elderly adults, and immunocompromised patients), *V. vulnificus* infection (in patients with liver disease), paralytic shellfish poisoning, and long-incubation mushroom poisoning. With other pathogens, fatalities most often occur in elderly or immunocompromised patients.

Most bacterial diarrheal illnesses are self-limited and do not require antimicrobial therapy in healthy hosts who are not at the extremes of age. Empirical antimicrobial therapy may be indicated for infants; immunocompetent patients with fever, abdominal pain, bloody diarrhea, and bacillary dysentery presumptively due to *Shigella;* patients with recent international travel and fever or signs of sepsis; immunocompromised patients with severe illness and bloody diarrhea; and patients with suspected enteric fever.[212] In adults with indications for antibiotic therapy, a fluoroquinolone or azithromycin is recommended. In children, a third-generation cephalosporin may be given for infants <3 months old and for children with neurologic involvement; azithromycin is an alternative choice in children.[212] Selection of empirical antimicrobial therapy should depend on local susceptibility patterns and travel history, and therapy should be modified or discontinued when a clinically plausible organism is identified. Antibiotic treatment of nontyphoidal *Salmonella* gastroenteritis may increase the risk of long-term carriage.[237] Antibiotic treatment of STEC O157 infections may increase the risk of HUS.[238] Antibiotics are of no value in the management of staphylococcal, *C. perfringens,* and *B. cereus* food poisoning. Antimicrobial agents are lifesaving in invasive salmonellosis, invasive listeriosis, *Vibrio* septicemia, and typhoid fever. Azithromycin, doxycycline, and tetracycline shorten both the duration of choleric diarrhea and the excretion of toxigenic *V. cholerae* O1 and are first-line agents (in combination with rehydration) in patients with moderate-to-severe illness. Most diarrheal illness caused by *Campylobacter* and *Shigella* is self-limited. Early treatment of *Campylobacter* infection with fluoroquinolones, erythromycin, or azithromycin can shorten the duration of illness. However, domestically acquired fluoroquinolone-resistant *Campylobacter* infections emerged after the approval of these agents for use in poultry. Antibiotic treatment of shigellosis is recommended for patients with severe infections, patients with dysentery, or patients who are immunocompromised. Antimicrobial susceptibility testing of *Shigella* isolates is important, as resistance is common, and isolates are increasingly found that have reduced susceptibility to ciprofloxacin or azithromycin. Antimicrobial agents are also used to treat parasitic infections. More information on treating parasitic infections can be found at www.cdc.gov/parasites.

Resistant strains can complicate treatment and are associated with a greater risk of invasive infection and hospitalization.[239] Asymptomatic *Salmonella* infection may become clinically apparent when an unrelated condition is treated with an antimicrobial agent to which the *Salmonella* organism is resistant; such treatment also lowers the infectious dose.[240] The proportion of resistant isolates varies widely by serotype. *Salmonella* serotype Typhimurium resistant to multiple antibiotics emerged globally, particularly in Europe and the United States, in the 1990s.[241,242] An emerging monophasic variant of *Salmonella* serotype Typhimurium, serotype I 4,[5],12:i:-, has emerged; 59% of isolates reported to National Antimicrobial Resistance Monitoring System (NARMS) national surveillance in 2015 were resistant to ampicillin, streptomycin, sulfonamides, and tetracycline.[243]

Salmonella serotype Newport resistant to amoxicillin-clavulanate, ampicillin, ceftriaxone, chloramphenicol, streptomycin, sulfonamides, and tetracycline and with decreased susceptibility to ceftriaxone emerged in the United States in the early 2000s. It is associated with consumption of undercooked ground beef.[244] The proportion of *Salmonella* serotype Newport isolates with this resistance pattern tested in NARMS increased from none in 1996 to a peak of 25% in 2001, and it declined to 4.7% in 2015.[243] However, other resistance patterns are emerging. From 1996 through 2015 in NARMS surveillance, the percentage of *Salmonella* isolates resistant to ceftriaxone increased from 0.2% to 2.7%, and the percentage with nonsusceptibility to ciprofloxacin increased from 0.4% to 5.8%.[243] The emergence of these multidrug-resistant *Salmonella* organisms was probably related to agricultural uses of antimicrobials. This highlights the interconnected pool of pathogens between animal reservoirs and people and underlines the need for prudent use of antimicrobials in both sectors and of a One Health approach (https://www.cdc.gov/onehealth/index.html).

Medical care providers who suspect botulism should implement close observation and supportive care and immediately call their state health department's emergency 24-hour telephone number. The state health department will contact the CDC to arrange a clinical consultation by telephone and, if indicated, release of botulinum antitoxin. Management of botulism is discussed in Chapter 245.

Patients with paralytic shellfish poisoning and some patients with ciguatera may require ventilatory support, usually for only a few days. Although case reports and unblinded randomized studies have suggested that intravenous mannitol may ameliorate the acute neurologic symptoms of ciguatera, a double-blind randomized trial showed no benefit.[245] Case reports have suggested that amitriptyline and tocainide may improve persistent dysesthesias.[233] Therapy is otherwise supportive; no antitoxins are available. If not contraindicated by the presence of ileus, enemas or cathartics may be administered in an effort to remove unabsorbed toxin from the intestinal tract. Because of the severe dysesthesias associated with ciguatera, analgesics may also be required. Scombroid poisoning may be treated with a combination of H1 and H2 antihistamines. In severe cases with bronchospasm, epinephrine may be required.[246]

Therapy for short-incubation types of mushroom poisoning is primarily supportive. Patients with severe parasympathetic hyperactivity caused by muscarine poisoning may be treated with atropine.[121] Therapy for cyclopeptide poisonings includes cathartics to remove unabsorbed toxin in the minority of patents who present before the onset of severe gastrointestinal symptoms and many additional unproven measures.[119] Pyridoxine is indicated for treatment of neurologic manifestations of gyromitrin poisonings.[121]

Therapy for acute heavy metal poisoning is supportive. Emesis should be induced if it does not occur spontaneously. Antiemetics are contraindicated because retention of the toxic ions in the gut and subsequent systemic absorption may result. In severe cases of heavy metal toxicity, use of specific antidotes may be considered, but that is rarely necessary.

SURVEILLANCE

Surveillance of enteric diseases has four objectives: (1) individual case investigation for localized disease-control activities; (2) outbreak detection to protect the population and to identify gaps in control measures; (3) assessment of disease burden and trends to prioritize and assess impact of control measures; and (4) microbiologic characterization of infectious agents to improve understanding of their epidemiology, antimicrobial resistance, and virulence factors.[226] State public health laws determine reportable conditions for each state and who is responsible for reporting. State public health officials voluntarily submit data to the CDC for nationwide surveillance.

Laboratory-based surveillance, which relies on reports of test results from public health laboratories, has proven especially valuable for surveillance of foodborne diseases because of information gleaned from subtyping. For *Salmonella, Shigella,* STEC, and *Listeria,* clinical laboratories should send isolates to a reference laboratory for serotyping and molecular subtyping by PFGE or WGS. In the United States, all state public health laboratories participate in PulseNet.[211] Similarly, many US public health laboratories, as part of CaliciNet, can perform genetic sequencing of PCR products to link multiple norovirus cases to environmental sources.[247]

Although knowing the specific serotype, PFGE pattern, or WGS characteristics is rarely important in the management of a single case, this or other subtyping information is essential to the investigation of many outbreaks and is fundamental to the recognition of multistate outbreaks. This means that a diagnostic culture is of benefit not only to the patient but also to society as a whole. The use of culture-independent methods such as enzyme immunoassay or PCR that do not yield isolates that can be subtyped is increasing.[226] Until culture-independent diagnostic tests are developed that provide subtype information, it is important to reflexively culture any specimen that tests positive by a culture-independent test so that the public health functions of laboratory-based surveillance are not impaired. Culture-independent methods also do not provide information about antimicrobial susceptibility.

Efforts are underway to develop new diagnostic methods that serve both clinical and public health needs. One approach is metagenomics, the sequencing of all the genetic material in a stool sample. Although this technology has not reached a stage that is useful for routine diagnostic testing or surveillance, it was used in a retrospective study of an STEC outbreak.[248,249]

A report of illness in a single person who has attended a daycare center, family gathering, or other setting often leads to discovery of an outbreak. Outbreaks can be apparent even before the causative agent is known, and early investigation can control a source. This means that the astute clinician or microbiologist who calls the public health department epidemiologist to discuss a case plays an important role in the control of foodborne and other diseases.

PREVENTION

Monitoring of food production systems is increasingly important as the global food supply becomes more interconnected, centralized, and preprocessed for the convenience of the consumer. Prevention of foodborne disease depends on careful handling of animals, raw products, and processed foods all the way from the farm to the table and on practices that reduce or eliminate contamination in food.

Much foodborne disease can be prevented if food is selected, prepared, and stored properly. In large kitchens and in homes, careful cooking and storage are necessary to kill pathogens and to prevent their growth when food is contaminated after cooking. Bacterial pathogens grow in food at temperatures ranging from 40°F to 140°F; growth may be prevented if cold food is adequately refrigerated and hot food is held at temperatures higher than 140°F before serving. Although thorough cooking of food just before consumption eliminates the risk of many illnesses, protection against staphylococcal food poisoning is not provided because the staphylococcal enterotoxins are heat stable. Particular care in handling and cooking of raw poultry, beef, pork, shellfish, and eggs is important to prevent many foodborne diseases. Contamination in the kitchen may occur if cooked foods or ready-to-eat foods come in contact with raw foods of animal origin or with equipment such as knives and cutting boards. Poor personal hygiene by food handlers frequently contributes to norovirus, *Staphylococcus, Shigella,* and hepatitis A outbreaks.

Because they serve high-risk populations, the kitchens of hospitals and nursing homes must pay particular attention to food safety.[250] For example, routine use of pasteurized eggs instead of shell eggs for recipes

TABLE 101.5 Control and Prevention of Foodborne Diseases

General Recommendations for All People

- Thoroughly cook raw food from animal sources such as beef, pork, poultry, fish, and eggs to temperatures that eliminate most pathogens.[a]
- Wash raw fruits and vegetables before eating.
- Keep uncooked meats separate from fruits, vegetables, cooked foods, and ready-to-eat foods.
- Do not thaw meat, poultry, or fish on the counter (instead, thaw in a refrigerator, in cold water, or in a microwave oven).
- Wash hands before, during, and after preparing food and before eating food.
- Wash knives, other utensils, and cutting boards after handling uncooked foods.
- Keep refrigerators set to <40°F and freezers set to 0°F or lower, and verify with a thermometer.
- Refrigerate perishable foods within 2 hours (or within 1 hour if left out at temperatures >90°F).
- Read and follow all cooking and storage instructions on food product packaging. This is especially important for foods prepared in microwave ovens because these ovens heat foods unevenly. Even foods that appear ready to eat may require thorough cooking.
- Persons with diarrhea or vomiting possibly caused by an infectious agent should not prepare foods for others.
- Keep all animals including reptiles and amphibians away from surfaces where foods or drinks are prepared.
- Do not drink unpasteurized (raw) milk or eat foods made from unpasteurized milk. (Exception: hard cheeses made from raw milk that have been aged >60 days are generally safe to eat.)
- Do not eat home-canned foods that were not known to be adequately heat processed during canning.

Recommendations for People at High Risk Such as Pregnant Women and People With Weakened Immune Systems in Addition to Above Recommendations

Measures to Prevent a Variety of Bacterial Infections

- Do not eat uncooked sprouts.
- Do not drink prepackaged juice or juice-containing beverages that have not been processed to reduce or eliminate microbial contamination (e.g., by pasteurization).

Listeriosis Prevention Measures

- Do not eat soft cheeses such as feta, Brie, and Camembert; blue-veined cheeses; and Mexican-style cheeses such as queso blanco, queso fresco, and panela unless the package has a label that clearly states that the cheese is made from pasteurized milk. Be aware that certain soft cheeses made from pasteurized milk such as queso fresco have also caused *Listeria* infections, most likely because they were contaminated during cheese-making.
- Do not eat refrigerated pâtés or meat spreads. Canned or shelf-stable pâtés and meat spreads are safe to eat.
- Do not eat refrigerated smoked seafood unless it is contained in a cooked dish such as a casserole. Refrigerated smoked seafood such as salmon, trout, whitefish, cod, tuna, and mackerel is most often labeled as "nova-style," "lox," "kippered," "smoked," or "jerky." The fish is found in the refrigerator section or sold at delicatessen counters of grocery stores and delicatessens. Canned or shelf-stable smoked seafood is safe to eat.
- Do not eat hot dogs, luncheon meats, or delicatessen meats unless they are reheated until steaming hot.
- Avoid getting fluid from hot-dog packages on other foods, utensils, and food preparation surfaces, and wash hands after handling hot dogs, luncheon meats, and delicatessen meats.

Salmonellosis Prevention Measures

- Use pasteurized eggs.

Vibriosis, Toxoplasmosis, and Norovirus Prevention Measures

- Do not eat raw or lightly steamed oysters, clams, or other raw shellfish (especially important for patients with liver disease).

[a]Poultry: 165°F (73.9°C); ground meats: 160°F (71.1°C); intact cuts of beef, pork, ham, veal, and lamb: 145°F (62.8°C) and allow to rest for at least 3 minutes before eating; fish and shellfish: 145°F (62.8°C); egg dishes: 160°F (71.1°C).
More food safety information can be found at www.foodsafety.gov/keep/index.html.

that pool large numbers of eggs may prevent salmonellosis.[251,252] Low microbial (or neutropenic) diets are advised for some especially vulnerable patients.[199,250] Some foods pose such high risk of infection that they should be avoided by even healthy nonhospitalized persons; these include raw milk (which can transmit *Salmonella, C. jejuni,* STEC, and *Mycobacterium tuberculosis*)[134] and inadequately heat-processed home-canned foods (botulism).

Except for scombroid fish poisoning, food-handling errors resulting in chemical intoxication are different from errors leading to bacterial outbreaks. Heavy metal poisoning occurs when acidic beverages are stored in defective metallic containers. Ciguatera and shellfish poisoning occur when seafood are obtained from unsafe sources; seafood containing the toxins appear and taste normal, and cooking does not provide protection because the toxins are heat stable. Scombroid fish poisoning can be prevented through refrigeration of raw fish.[112]

The role of the clinician goes beyond that of diagnosis and treatment to prevention. This means educating patients or caregivers, especially for patients more vulnerable to foodborne disease (e.g., infants, pregnant women, older adults, and immunocompromised persons) about food safety measures. Such patients may choose to avoid high-risk foods, and everyone can benefit from following good food-handling practices (Table 101.5).

Clinicians and microbiologists have an important role in the detection of outbreaks, and rapid investigation of outbreaks can identify the source and trigger actions such as recalls or policy changes to prevent additional illnesses. Obtaining appropriate diagnostic tests for foodborne pathogens and reporting them to public health authorities are key to outbreak detection efforts. There are well-documented outbreaks of botulism,[253] salmonellosis,[254] and *E. coli* O157:H7[255] in which recognition and reporting of the initial illness could have prevented many subsequent cases.

Diagnosing and reporting of illnesses with the potential for intrafamilial spread or for spread within institutions such as daycare centers (e.g., shigellosis, *E. coli* O157:H7 infection) can prevent secondary transmission.[256] Public health authorities may exclude food handlers, recreational water staff, healthcare providers, and individuals who work in or attend schools or daycare centers from working or attending until symptoms or diagnostic tests indicate that the transmission risk is sufficiently reduced.[212] Clinicians should counsel patients with diarrhea on practicing hand hygiene and to avoid swimming, water-related activities, and sexual contact while symptomatic.

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The complete reference list is available online at Expert Consult.

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102 Tropical Sprue and Environmental Enteric Dysfunction

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SHORT VIEW SUMMARY

Definition
- Tropical sprue: Syndrome of diarrhea, malabsorption of at least two distinct nutrients, abnormal duodenal histopathology, and weight loss.
- Environmental enteric dysfunction (EED): Incompletely understood syndrome of gut mucosal inflammation, reduced intestinal absorption, and increased permeability, associated with environmental contamination, altered gut microbiota, and stunting in young children in resource-limited countries.

Epidemiology
- Sprue: Most frequent in Asia and the Caribbean islands, more frequent in adults than children; occurs in long-term travelers to endemic regions.
- EED: occurs throughout the resource-limited world.

Microbiology
- Sprue and EED: No single agent has been associated with a causal role, but small bowel overgrowth is common.

Diagnosis
- Sprue: Presence of an appropriate clinical syndrome (persistent diarrhea, malabsorption of at least two distinct nutrients, weight loss) with consistent findings on small bowel series or upper endoscopy. Response to folate and tetracycline ultimately confirms the diagnosis.
- EED: A number of potential biomarkers of EED have been investigated but there is no single biomarker that is diagnostic of EED.

Therapy
- Sprue: Removal from area of risk, treatment with folate and tetracycline.
- EED: No definitive effective treatment as yet. Ongoing trials involve nutritional supplements, water and sanitation interventions, and immunomodulators.

Prevention
- Sprue and EED: Good hygienic practices.

Tropical sprue, also called *postinfectious tropical malabsorption,* and environmental enteric dysfunction (EED) are both syndromes of enigmatic origin. Although there is substantial overlap between these two illnesses, there are also distinct features.

Tropical Sprue

Tropical sprue has been recognized since the 2nd or 3rd century AD, when Aretaeus of Cappadocia reported on "The Coeliac Affection," and was described in India in the Charaka Samhita.[1] The first mention of sprue in the modern medical literature was in 1747, when Hillary emigrated from England to Barbados and published his observations on a prolonged tropical diarrheal disease in native islanders. The English term *sprue* is an adaptation from the Dutch *sprouw,* which was originally used to refer to persistent diarrheal disease in Holland (possibly celiac disease). The term *sprue* first was used in 1880 by Manson for the persistent wasting diarrhea that occurred in tropical countries.[2] Knowledge about the cause or pathogenesis of sprue did not advance significantly until investigations were begun after recognized outbreaks during World War II. The distinction between celiac sprue and tropical sprue was not clear until the early 1970s. The distinction between sprue and EED is only just now occurring.

Environmental Enteric Dysfunction

What has been previously called tropical or environmental enteropathy[3] is currently referred to as *environmental enteric dysfunction*, indicating that there are functional deficits associated with the histopathologic changes of enteropathy, which include small intestine villous blunting or atrophy, crypt hyperplasia, and lymphocytic infiltration of the lamina propria.[4–7] Currently, this poorly understood acquired syndrome affects young children as well as adults in resource-limited areas of the world.[8–10] The functional defects include increased intestinal permeability or impaired gut barrier function, malabsorption, intestinal injury and repair, immune activation, and chronic mucosal and systemic inflammation.[4,7,9]

Although widespread efforts to decrease the global impact of childhood diarrhea—including oral rehydration, zinc supplementation, exclusive breastfeeding, vaccines to prevent enteric infections, and public health strategies to implement these interventions—have resulted in a radical reduction in the number of deaths due to childhood diarrhea,[9] there has not been a concurrent decrease in the rates of stunting in the resource-limited world. These data suggest that repeated diarrheal episodes and enteric infections themselves may not be contributing to impaired linear growth in these children, but rather that this may be the outcome of a chronic inflammatory state of the small intestine.[9]

EXPOSURES/EPIDEMIOLOGY

Tropical Sprue

Although sprue is considered a disease of tropical locales, there are distinct geographic areas of risk within the tropics. Tropical sprue has been identified most readily in Asia and the Caribbean islands, and there are isolated areas of particular risk within both hemispheres.[11,12] It is seen in northern South America, including Venezuela and Colombia, but rarely in Central America or Mexico. Currently, it remains on the Indian subcontinent, from the Himalayas to the south, but is less common than previously, and it has been recognized in Myanmar and the Philippines.[13–15] An illness consistent with sprue has been documented in patients who had undergone renal transplantation in Asia.[16] Infrequent cases of tropical sprue were documented in Africa until the 1970s; cases have been recognized in Rhodesia (now Zimbabwe) and South Africa.[17] There may be endemic foci of tropical sprue in the Middle East as well.[18]

In contrast to other endemic diarrheal illnesses in the tropical world, tropical sprue is a disease mainly of adults. Children are believed to be relatively spared, although disease has been documented in all age groups. Tropical sprue is rare in very young children; this may represent a beneficial effect of breastfeeding. In studies of family outbreaks of tropical sprue in South India, even older children developed disease at a significantly lower rate than adults.[19] The reasons underlying these observations are not known. Persistent diarrhea develops in children more commonly in parts of the world where the environment is contaminated more heavily with microbes that are potentially disease-causing.[20] Tropical sprue may be one of the causes of prolonged diarrhea and wasting in this age group as well, but studies such as small bowel intubation and cultures or biopsy have shown conflicting results, relating small bowel colonization with persistent diarrhea.

Some patterns of disease expression are of particular interest in tropical sprue. There are clear epidemics of the disease, which have

been documented best in families and villages in South India.[19] There have been descriptions of "sprue houses," in which successive tenants have developed disease. Such an epidemic pattern suggests an underlying infectious (transmissible) cause.

There have been seasonal variations in the frequency of outbreaks of tropical sprue as well. At an American military base in the Philippines,[15] an increased rate of mild tropical sprue in the setting of an increased rate of diarrheal disease was seen more often from March to July than at other times of the year for at least 4 years. In these outbreaks, sprue occurred in American military personnel and their dependents, who were eating a high-calorie, Western-style diet. This seasonal variation also lends credence to the possibility of an underlying infectious cause.

Tropical sprue occurs in expatriates living in endemic areas. Tropical enteropathy has been described in Peace Corps volunteers and has occurred sporadically in travelers.[21] Generally, tropical sprue develops in an expatriate who has lived for a prolonged time (6 months to 1 year) in an endemic area.[22] Rare cases also have been described in short-term travelers. Tropical sprue is also recognized in immigrants who leave endemic areas. Classical sprue appears to be less frequent than it was previously.

Environmental Enteric Dysfunction

Because the diagnosis of EED is less refined, the epidemiology of EED is correspondingly less well -understood. EED appears more frequently in children than does sprue, and children are at risk whenever they reside in regions where hygiene is less feasible.

EED is prevalent in low- and middle-income countries of the world with poor access to clean water, sanitation, and hygiene (WaSH) and fecal environmental contamination.[23] These include countries in sub-Saharan Africa, South Asia, and South America. Children below the age of 5 years as well as adults are affected.

CLINICAL FEATURES

Tropical Sprue

The classic clinical features of tropical sprue are nonspecific and simply reflect the symptoms of malabsorption. Changes in hygiene, increased access to medical care, and improved access to clean water supplies may all be altering the frequency and severity of malabsorption. Currently, the diagnosis of sprue often is made when the presentation is still quite subtle compared with the severe disease that was the more common presentation in the past. Malabsorptive symptoms include prolonged diarrhea, abdominal cramping, and anorexia, with or without nausea and secondary weight loss. Other associated but less common symptoms, also related to the malabsorption of specific nutrients and subsequent malnutrition or micronutrient deficiencies, may include peripheral edema, glossitis, stomatitis, and dermatitis.[24,25] Fever may occur at the onset of sprue-related diarrhea (especially in Asia). Although the presence of fever has been suggested as a means to distinguish Caribbean from Asian sprue, this distinction has not been observed consistently. Fever rarely persists for the course of disease.

Many patients can pinpoint the onset of disease. Tropical sprue rarely has an insidious onset and far more often is associated with an obvious acute episode of diarrhea that then becomes prolonged. Because the current definition of tropical sprue implies that the function of the gut was normal before the development of the disease, most typically, the diarrhea has an acute onset; however, the distinction between normal and abnormal bowel habits often may not be so clear.

Patients describe crampy abdominal pain; multiple soft or loose stools daily, often with mucus; and exacerbation of symptoms with food consumption. Patients may also complain of nausea and bloating that lead to decreased appetite and decreased oral intake. Malabsorption of specific nutrients may lead to the development of other manifestations,[26] including lactose intolerance or anemia. Anemia may convert from macrocytic to microcytic anemia. Impaired absorption of calcium, vitamin D, and magnesium also may occur, with resulting osteopenia.[27]

Environmental Enteric Dysfunction

EED can be subclinical or associated with diarrhea, enteric infections, linear growth faltering leading to stunting, and physical and cognitive impairments.[7,9] There is no single clinical feature that is specific for EED.

PATHOGENESIS

Tropical Sprue

There is a strong presumption that tropical sprue is caused by an enteric infection, perhaps in individuals predisposed by a nutritional deficiency. The facts lending support to this theory include the following: (1) often, the prolonged episode of tropical sprue is initiated by an episode of acute diarrheal disease; (2) there is an epidemic and seasonal nature to the epidemiology of the disease, as noted; and (3) the disease responds most often to treatment with antibiotics with or without nutritional supplements. Nevertheless, the precise nature of the infection(s) that lead to development of tropical sprue is less clear.

Multiple studies in Asia and the Caribbean islands have shown small bowel bacterial overgrowth in patients with tropical sprue.[21,28,29] Although some bacteria normally live in the upper small bowel of healthy persons, the organisms isolated from this region of the gut in healthy asymptomatic individuals are most often gram-positive. Streptococci, staphylococci, and lactobacilli are among the common isolates, and these are present in small numbers. In the distal small bowel, the cecum, and the colon, anaerobes and facultative gram-negative organisms predominate in normal persons. Small bowel cultures from travelers with tropical sprue show increased numbers of gram-negative rods, including *Alcaligenes, Enterobacter aerogenes,* and *Hafnia* spp. In small bowel cultures from persons with tropical sprue who were native to India, Haiti, or Puerto Rico, *Klebsiella, Escherichia coli,* and *Enterobacter cloacae* were the most common organisms. Carefully done studies in South Africa and India documented similar organisms in similar concentrations in the small bowel of asymptomatic control patients and in patients with tropical sprue, suggesting that environmental contamination may predispose to increased small bowel microbial populations.[21,28,29] In another series of patients from India, the number of organisms found in the small bowel of tropical sprue patients was the same as that found in the small bowel of healthy controls, but the types of organisms isolated varied.[28] Other organisms, especially *Enterobacter* and *Veillonella,* were isolated more frequently from the small bowel of patients with tropical sprue than from healthy controls.[30] However, the presence of particular organisms does not prove any causal effect, and all of these studies were conducted before the era of sequence-based analyses or molecular diagnostics (such as Biofire) of bacterial populations rather than relying on culture alone, which underrepresents fastidious organisms.

Gram-negative organisms isolated from the small bowel of tropical sprue patients in Haiti were found to have a secretory effect on intestinal cells, presumably by toxin production; however, these toxins have not been characterized by current methods. Other studies have further suggested that enteroaggregative *E. coli* are associated with malnutrition, with or without persistent diarrhea, and with intestinal inflammation and cytokine production.[31] In addition to its association with persistent diarrhea in children in tropical developing areas, enteroaggregative *E. coli* in patients with acquired immunodeficiency syndrome is associated with persistent diarrhea that improves with antimicrobial therapy.[32,33] Although the traditional definition of tropical sprue excludes patients with diarrhea on the basis of recognized pathogens, it is possible that improvements in diagnostic techniques, such as multiplex molecular diagnostics,[34] would permit the identification of organisms that are or have been associated with tropical sprue but previously were not able to be isolated or identified. Whether tropical sprue is distinct from EED is not clear. One possibility is that these syndromes may exist at the ends of the spectrum of a single disease, with sprue being the more advanced and symptomatic form of the more frequently asymptomatic EED.[1] Sequence-based microbial identification is another example of a significantly more sensitive technique that may be beneficial in elucidating intestinal microbiota as it contributes to the pathogenesis of tropical sprue and may clarify the relationship toEED.[1,35]

The concept that small bowel overgrowth, as it occurs spontaneously in a certain segment of the population in resource-limited countries or after an acute enteric infection, may precipitate a series of intestinal insults that proceed to full-blown tropical sprue in susceptible persons represents an attractive hypothesis for the cause of tropical sprue. The predisposition for progression from intestinal insult to tropical sprue is less easy to explain. Malnutrition, whether generalized or presenting as specific micronutrient deficiencies, may be a predisposing factor, but

is neither necessary nor sufficient, as shown by the occurrence of tropical sprue in apparently well-nourished military personnel and their dependents. Small bowel overgrowth may alter intestinal transit time and promote further overgrowth and intestinal stasis, but it cannot explain the initial colonization that induces the episode.

In vitro data suggest that small bowel colonization by *E. coli* may be increased by low levels of cytokines, as might be expected in chronic parasitic infections in resource-limited settings. As noted, certain organisms, such as enteroaggregative *E. coli,* can alter the intestinal environment by the induction of intestinal proinflammatory cytokines and intestinal inflammation.[36] There has been no genetic predisposition noted for tropical sprue as there has been for celiac sprue, and the inflammatory cytokine profile in the lymphocytes of the small bowel in patients with tropical sprue has not been described.

The processes that control the normal bacterial colonization of the small bowel are not well understood; the forces that may disrupt these normal processes to permit abnormal colonization are even less well understood. Some factors that can affect the normal small bowel colonization process include gastric acidity, which controls the entry of viable organisms into the small bowel, and intestinal mucin glycoprotein, which contains receptors for, and specifically binds, a variety of bacteria within the small bowel lumen.[37]

Bacterial binding to mucin is presumed to promote clearance of pathogenic organisms as a protection of the small bowel, but it may promote colonization by nonpathogenic organisms or promote small bowel colonization by pathogens when the mucin is damaged by malnutrition, an inflammatory process, or bacterial proteases or mucinases. Loss of the protective mucin layer in tropical sprue is suggested by evidence that the bacteria visualized are often associated tightly within the mucosa rather than being free within the intestinal lumen.[2] Damage to the protective mucin layer may also permit epithelial cell damage by other small bowel microbes and their toxins. The presence of free bile acids within the upper small bowel can alter intestinal bacterial growth rates and colonization, but bile acid concentrations have not been abnormal in patients with tropical sprue, and the bacterial organisms that have been cultured from patients with tropical sprue are not organisms that typically alter bile salt metabolism.[38]

Intestinal immunologic dysfunction has been suggested as a factor that might predispose to abnormal bacterial colonization in tropical sprue. Patients with deficiencies of secretory immunoglobulin (Ig) A are subject to more frequent and severe bouts of enteric infections. In addition to secretory IgA, lymphoid tissue is present throughout the small bowel focally in Peyer patches and diffusely as mucosal lymphocytes. When small bowel lymphocytes were characterized in patients with tropical sprue and in control patients with irritable bowel syndrome in southern India, there was no difference in the number of IgA-producing, IgG-producing, or IgM-producing lymphocytes between the two groups.[39,40] Patients with sprue had increased numbers of lymphocytes in the crypt epithelium, with a higher percentage of immunoblasts and a higher mitotic index.[41] These data can be interpreted as evidence that lymphoid activation occurs in tropical sprue, but that it is probably a secondary response to inciting microbes, rather than being the inciting process itself.

It has also been postulated that dietary fat might play a role in tropical sprue. Similar to the permissive effect of protein ingestion in the pathogenesis of pig-bel, the intake of long-chain fatty acids has been studied as a potential causative factor for tropical sprue.[42] The seasonal epidemic occurrence of tropical sprue in Puerto Rico immediately follows a traditional holiday feast of pork, which is rich in long-chain fatty acids.[43] There are several mechanisms whereby these long-chain fatty acids might contribute to the production of clinical tropical sprue. Long-chain fatty acids can alter intestinal motility and delay intestinal transit time. Plasma levels of enteroglucagon and motilin are increased significantly in patients with tropical sprue; motilin slows gastric emptying, and enteroglucagon slows intestinal transit.[44] Studies have also demonstrated abnormalities in peptide YY and neurotensin after infusion of fat in some patients with sprue, which may contribute to altered motility and bacterial overgrowth.[45] Intubation studies have shown that intestinal infusions of fat increase plasma enteroglucagon levels and decrease intestinal motor activity. Fat within the gut lumen also inhibits the mucosal sodium-potassium fluxes and the magnesium

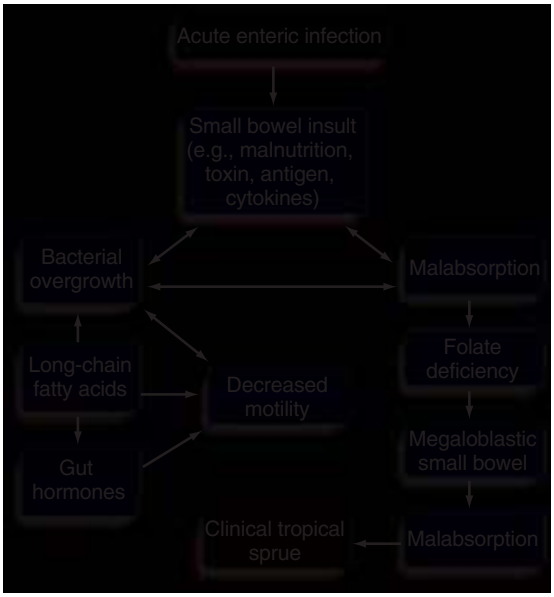

FIG. 102.1 Proposed pathogenesis of tropical sprue. The complex vicious cycle of small bowel insult that results in bacterial overgrowth and malabsorption, as well as further small bowel damage by luminal long-chain fatty acids and dysregulation of intestinal hormones, may promote disease in susceptible persons after an acute enteric infection.

adenosine triphosphatases, which can contribute to malabsorption of water and electrolytes in the intestine and raise the pH of the mucosal microenvironment.[42,46]

The elevated mucosal pH produced by intestinal fats also has been associated with increased growth of gram-negative bacteria in the lumen of the small bowel, and a switch to a high-fat diet has been associated with alterations in the intestinal microbiota.[24,47]

The elevation of mucosal pH and the presence of fatty acids within the lumen of the gut also may impair the ability of the intestine to absorb folate; folate deficiency may potentiate the intestinal dysfunction that precedes it.[48] Folate deficiency leads to a decreased number of gut epithelial cells, as assessed by DNA concentrations, and to villous atrophy. Additional structural alterations are seen in the intestine with folate deficiency, including crypt hypertrophy, villous blunting, and megaloblastic changes in the epithelial cells. These changes are nonspecific and are similar to those seen with vitamin B_{12} deficiency, celiac disease, or tropical sprue. Functionally, the folate-deficient gut is less efficient in absorbing water, electrolytes, and carbohydrates than the normal small bowel.[49] It is likely that whatever the initial insult to the gut may be in tropical sprue, the resulting folate malabsorption and deficiency contribute to the further pathogenesis of disease (Fig. 102.1).

Exocrine pancreatic insufficiency has been documented in patients with tropical sprue by the indirect pancreolauryl test.[50]

INTESTINAL ABNORMALITIES

Tropical Sprue

Although the secretory and malabsorptive syndrome seen in tropical sprue suggests preferential damage to the small bowel, functional abnormalities are seen in the large bowel as well. Functional changes in the small bowel mirror the morphologic changes seen and are most prominent in the ileum and jejunum. In tropical sprue, the jejunum is in a net secretory state, with active secretion of water, sodium, and chloride[51]; however, glucose-linked absorption remains intact, as it does for many other secretory infectious diarrheal syndromes.[52] In tropical sprue, there also is malabsorption of bile acids and vitamin B_{12} in the ileum. Bile acid malabsorption leads to fat malabsorption and malabsorption of the fat-soluble vitamins D, A, K, and E. Brush border enzymes are decreased functionally and are less efficient in digesting and absorbing disaccharides, such as lactose.[53,54] Xylose, glucose, and folate malabsorption occur, as does malabsorption of minerals such as calcium and

magnesium. Amino acid malabsorption occurs; protein metabolism is complicated further by loss of albumin in the lumen of the damaged small bowel.[55]

Most of the functional changes in tropical sprue may be related to small bowel mucosal damage consistent with the morphologic changes seen, but the hormonal regulation of the gut is also dysfunctional in this disease.[56] Postprandial insulin and gastric inhibitory peptide are reduced in tropical sprue; enteroglucagon and motilin levels are increased. In chronic tropical sprue, gastric acid secretion and secretion of intrinsic factor may also be affected. Transit time through the small bowel is slowed, as measured by breath hydrogen testing.[57–59]

In the few studies of colonic function in tropical sprue that have been done, the ability of the colon to absorb water is decreased in patients compared with controls.[60] There is speculation that the dysfunction of colonic cells may be related to damage by excess fatty acids in the gut lumen or bacterial toxins or infection. Although there is physiologic confirmation of the ability of fatty acids to disturb the absorptive function of colonocytes and small bowel enterocytes, data suggesting that colonic infections are important in the pathogenesis of tropical sprue are lacking.

HISTOPATHOLOGY

Tropical Sprue

Partial villous atrophy is the hallmark histologic change seen in the small bowel in tropical sprue, as opposed to the flattened mucosa that is characteristic of celiac sprue.[61] The villi in tropical sprue progressively shorten and thicken, forming fused leaves after about 4 months of illness.[62] These histologic changes are seen in the jejunum and the ileum, where the changes in absorption also are localized. These histologic changes are not specific for tropical sprue but may be present in severe folate deficiency or with bacterial overgrowth. In distinction, in celiac disease, blunting of villi and crypt hyperplasia are seen and lymphocytes are noted to infiltrate the crypts.

Microscopically, the mucosa is thin, with an infiltrate of chronic inflammatory cells consisting of plasma cells, histiocytes, lymphocytes, and eosinophils. As noted, these lymphocytes have been characterized, and IgA-, IgG-, and IgM-producing B lymphocytes are present in numbers equal to those of asymptomatic control patients,[40,41] suggesting that this is not a part of the pathogenesis of the illness. An increased mitotic index can be seen in the crypt cells; the nuclei of the crypt cells may also appear megaloblastic.[55,63] An increased number of goblet cells may be present, and lipoid vacuoles have been seen within the basement membrane. To date, there is no convincing evidence that tropical sprue is an immunologically mediated disease.

Environmental Enteric Dysfunction

EED is currently thought to be a consequence of continuous exposure to enteric pathogens via the oral-fecal route leading to intestinal mucosal inflammation, altered barrier integrity, and reduced absorption.[9] In addition, since the gut microbiome modulates immune and inflammatory responses, maintains intestinal barrier integrity, and determines nutritional status,[64] it is possible that dysbiosis or alterations in the composition of the microbiome may lead to dysregulated inflammatory responses, impaired barrier function, increased microbial translocation across the gut, and mucosal or systemic inflammation.[4,6] Mucosal immune responses, including T-cell activation as well as B-cell and innate immune derangements, are implicated in EED.[6] However, the underlying mechanisms are not completely understood. EED has also been reported to be associated with metabolic derangements, including carnitine deficiency, altered fatty acid oxidation, and altered bile acid metabolism.[65,66]

DIAGNOSIS

Tropical Sprue

Because the symptom complex of tropical sprue is nonspecific, the travel and exposure history of the patient are crucial in making the diagnosis. Tropical sprue should be considered in a patient who presents with chronic diarrhea, weight loss, and evidence of malabsorption. Attempts should be made to ascertain the onset of the diarrheal illness, duration of diarrheal illness, degree of weight loss, frequency and character of the stool, and any other systemic complaints, such as prolonged fever, jaundice, or itching, that might suggest alternative explanations for the diarrheal illness. Information regarding travel to, residence in, or emigration from the tropics should be requested. Although there have been sporadic case reports of mild spruelike illnesses occurring after diarrheal illnesses in temperate climates, this so-called temperate sprue is rare and a history of exposure to an endemic area should be present to even consider the diagnosis of tropical sprue.[43]

Pertinent medical history should also be obtained. There are no diagnostic physical findings for tropical sprue. Cheilosis, stomatitis, glossitis, rashes, dermatitis, koilonychia, muscle pain or weakness, peripheral neuropathy, or edema can suggest deficiencies of iron, zinc, vitamin B_{12}, folate, vitamins D and E, or protein, but these are usually secondary manifestations. Deficiencies of any of these nutrients could be present in tropical sprue because of malabsorption by the damaged small bowel.

Laboratory evaluation of a patient with suspected tropical sprue can be minimal or extensive, depending on the degree of suspicion and the urgency for diagnosis. A simple, complete blood count showing a macrocytic anemia in a high-risk patient in the appropriate clinical setting could be sufficient to proceed with other, more confirmatory diagnostic tests, such as small bowel biopsies. A more complete laboratory evaluation includes serum vitamin B_{12} and red blood cell folate levels, serum carotene concentration, or, preferably, a 72-hour fecal fat determination. Stool examination to exclude *Giardia* is useful; stool culture looking for the usual acute bacterial pathogens is less likely to be helpful in chronic diarrhea. Testing for human immunodeficiency virus may be appropriate to help define the likelihood of opportunistic intestinal infections.

Ultimately, a small bowel series with small bowel follow-through showing flattened mucosal folds, luminal dilatation, or flocculation of the barium meal can suggest tropical sprue.[67] An upper endoscopy with duodenal aspirate for parasites and biopsy can be diagnostic of tropical sprue in the appropriate clinical setting. Enhanced magnification endoscopy has been shown to be more sensitive in detecting villous atrophy than standard endoscopy and may assist in making a noninvasive diagnosis of sprue. In a study of 15 patients diagnosed with sprue by intestinal biopsy in Caracas, Venezuela, atrophy was demonstrated in 93% of patients by enhanced magnification endoscopy, whereas standard endoscopy was able to detect atrophy in only 20% of patients.[68] Documentation of abnormal transit time by small bowel follow-through or breath hydrogen testing, which also can imply bacterial overgrowth, is suggestive of but is not diagnostic of tropical sprue. The differential diagnosis that must be considered in a patient with chronic diarrhea, weight loss, and malabsorption, even in a clinical setting consistent with tropical sprue, should include giardiasis, cryptosporidiosis, coccidiosis (*Cystoisospora belli*), capillariasis, strongyloidiasis, celiac sprue (gluten enteropathy), lymphoma, intestinal tuberculosis, blind loop syndrome, pancreatic tumors, Whipple disease, and microsporidia-associated human immunodeficiency virus enteropathy. If diagnosis remains obscure, biopsy is considered to be very sensitive and will allow the syndromes to be clearly differentiated. Celiac disease, or gluten enteropathy, can be more definitively diagnosed by serologic testing for antigliadin or antiendomysial antibodies.

Environmental Enteric Dysfunction

A definitive diagnosis of histopathologic changes associated with EED requires small intestine biopsy. However, concerns about safety, cost, and ethical considerations preclude the routine use of biopsy for diagnosis. Functional changes associated with EED, including impaired gut barrier integrity or increased intestinal permeability, malabsorption, mucosal injury and regeneration, and chronic inflammation, have been proposed as biomarkers of EED.[4,6,9] However, although several studies in search of biomarkers characteristic of EED have been performed, to date there is no single biomarker that can be used to diagnose this syndrome.[7] Potential biomarkers associated with functional changes in EED are described below.

Increased Intestinal Permeability/Impaired Gut Barrier Integrity

Dual sugar absorption tests such as the lactulose:mannitol (L:M) ratio are the most widely used biomarker to assess increased intestinal

permeability or impaired gut barrier integrity.[4,6,9] These tests employ sugars that are not metabolized and are excreted intact in the urine. Large sugars such as the disaccharide lactulose do not normally cross the gut mucosal barrier unless intestinal permeability is increased, and small sugars such as the monosaccharide mannitol are absorbed in proportion to small intestine absorptive capacity. Both sugars are administered orally, and their levels are measured in the blood or urine and expressed as a ratio. In subjects with increased intestinal permeability or impaired barrier integrity, the L:M ratio is higher. Increased L:M ratios have been reported in asymptomatic children as well as those with diarrhea in Africa, Asia, and South America. Studies in the Gambia have reported an inverse relationship between L:M ratio and height-for-age.[69] However, other studies have not found similar associations.[4] Possible reasons for this variation include differences in the assay protocol, analytic and reporting methods, and criteria such as breastfeeding, fasting status, dose of sugar administered, and type and timing of sample collected.[4,9] More recent studies have not shown a correlation between L:M ratios and other biomarkers.

Fecal α_1-antitrypsin, a serum glycoprotein and protease inhibitor, is another biomarker of increased intestinal permeability and protein loss.[9] It is not normally present in the diet and is not actively secreted or absorbed. Claudins and zonulin are tight junction proteins that form barriers between intestinal epithelial cells.[7] However, none of these markers is specific for EED.

Intestinal Damage and Repair

A biomarker of small intestine mucosal damage is citrulline, a nonprotein amino acid that is minimally present in the diet, plasma levels of which reflect enterocyte mass and function.[4,9] Plasma citrulline levels depend on de novo synthesis by proximal gut epithelial cells and are reduced in EED. Intestinal fatty acid binding protein is a small intestine epithelial protein that is released into the circulation after injury to the epithelium.[7] The regenerating islet-derived proteins REG1α and REG1β have been proposed as markers of intestinal injury and repair, and therefore potentially of EED.[9] Glucagon-like peptide 2 is a trophic factor released by enteroendocrine L cells of the ileum that aids in mucosal regeneration.[7] However, none of these potential biomarkers is specific for EED. A recent study suggested that optical biopsy may be a potential minimally invasive technique for diagnosing EED.[70]

Mucosal and Systemic Inflammation

A number of markers of mucosal and systemic inflammation have been proposed as biomarkers of EED.[4,6,7,9] Mucosal inflammatory markers include fecal calprotectin, a stable calcium- and zinc-binding protein produced by neutrophils and monocytes; lactoferrin, an iron-binding glycoprotein produced by neutrophils; myeloperoxidase, a marker of neutrophil activity; and neopterin, which is produced by macrophages and dendritic cells in response to interferon-γ, produced by activated T cells. Calprotectin and lactoferrin levels are not reliable in breastfed children since these proteins are secreted in breast milk.[71] Systemic inflammatory markers include proinflammatory cytokines (interleukin [IL]-1β, IL-15, IL-17, tumor necrosis factor-α, interferon-γ, and granulocyte-macrophage colony-stimulating factor) and antiinflammatory cytokines (IL-4, IL-6, and IL-10)[9] as well as C-reactive protein and α1-acid glycoprotein, both acute-phase reactants.

Once again, none of these markers is specific for EED.

Microbial Translocation and Immune Activation

Translocation of microbes or their products across the gut leading to immune activation is believed to contribute significantly to the pathogenesis of EED.[9] Biomarkers for these processes may also serve as biomarkers of EED. These include stool neopterin, plasma lipopolysaccharide (LPS)-specific and flagellin-specific antibodies, LPS core antibody (EndoCAb), LPS binding protein, and circulating soluble CD14.[4,9] However, many of these are biomarkers of other inflammatory diseases as well, so they are not specific for EED, and assays for some of them have not been standardized.

Fecal Messenger mRNA Transcripts

Recent studies in Africa have suggested that levels of specific fecal messenger RNA transcripts for proteins involved in immune activation and inflammation may serve as biomarkers of EED using the L:M ratio as a standard.[72–74] These transcripts include those for CD53, CDX1, HLA-DRA, tumor necrosis factor-α, S100A8, MUC12, and REG1α.

THERAPY

Tropical Sprue

Treatment with folate alone improves the symptoms of tropical sprue, especially those related to anemia, but does not cure the diarrhea. Combination therapy with tetracycline and folate seems to be most effective in symptom resolution and cure of diarrhea with promotion of weight gain.[75,76] Treatment with 250 mg of tetracycline four times daily and 5 mg of folate daily for 1 month has been effective for travelers with tropical sprue, but therapy must be prolonged for 6 months or longer for residents of the tropics who have had disease of long duration. Even with prolonged therapy, relapses have been seen in this population, although these may have been caused by reexposure to an infecting organism and represent recurrent rather than relapsing disease.[77] Reports have suggested that tropical sprue in the Caribbean is more amenable to therapy than sprue in India, but these studies are difficult to compare.[78] Poorly absorbed sulfa drugs are an acceptable alternative to tetracycline in children or pregnant women.[79] A favorable symptomatic response to therapy with folate and antibiotics can provide additional evidence that tropical sprue was the cause of chronic diarrhea and malabsorption in a patient; however, even this is not specific because bacterial overgrowth in a blind loop syndrome patient would also be expected to respond. Lack of response to treatment should lead to additional diagnostic evaluation to include antibodies to gliadin or endomysium or small bowel biopsy.

Environmental Enteric Dysfunction

Several interventions to treat EED have been attempted and others are currently in development or testing. Nutritional interventions such as vitamin A, zinc, glutamine, glycine, and multiple micronutrients have yielded variable results depending on the study.[9] Treatment with the probiotic *Lactobacillus rhamnosus* GG and the antibiotic rifaximin showed no significant differences in clinical or growth outcomes.[9] L-Alanyl-L-glutamine improved weight-for-age and weight-for-height but not height-for-age.[8] Albendazole administration did not result in significant improvement in anthropometry.[8] Trials of ω-3 long-chain polyunsaturated fatty acid, a dietary essential fatty acid, resulted in improvement in mid-upper arm circumference at 9 and 12 months.[8] In a clinical trial of the effect of azithromycin on immunogenicity of oral poliovirus vaccine, biomarkers of EED were reduced although there was no effect on the immunogenicity of the vaccine, suggesting that this antibiotic may be useful in EED.[80] Several trials of WaSH interventions on nutritional status in children have been performed. Overall, these interventions had a very modest impact on stunting but not on wasting or underweight.[81] Several other trials of WaSH interventions are ongoing.[81]

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The complete reference list is available online at Expert Consult.

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103 Infectious Arthritis of Native Joints

Christopher A. Ohl

SHORT VIEW SUMMARY

DEFINITION

- Infectious arthritis is an infection of one or more joints that can be caused by bacteria, viruses, fungi, and parasites.

CLINICAL CATEGORIES

Acute Bacterial Arthritis

- Overall incidence in native joints is 2 to 12 per 100,000 per year, increased in rheumatoid arthritis (RA) and the elderly. Joint infections in persons who use intravenous (IV) drugs began to increase during the opioid abuse epidemic beginning in 2010.
- Infection is usually acquired from hematogenous dissemination, less commonly from direct joint inoculation, or from a contiguous focus. Most infections are monoarticular; 10% to 20% are polyarticular. The knee is most often involved.
- Microbiology
  - Gram-positive organisms, including *Staphylococcus aureus* and *Streptococcus* spp. predominate.
  - Gram-negative bacilli in 5% to 20% are mainly in neonates, the elderly, IV drug users, and the immunocompromised.
  - Prevalence rates of gonococcal arthritis have decreased. There are two syndromes of joint involvement: monoarticular arthritis and disseminated gonorrhea with febrile tenosynovitis and skin lesions.

Viral Arthritis

- It may occur sporadically or in community outbreaks that may be small (e.g., parvovirus B19) or explosive (e.g., chikungunya virus).
- It is caused by infection of the joint space or by immune-mediated inflammation.
- It is usually an acute symmetrical polyarticular arthritis that occurs simultaneously with other systemic symptoms of viral infection.
- Causes include parvovirus B19, chikungunya virus, rubella (which may follow immunization), hepatitis B, hepatitis C, and other viruses, including Ross River virus and o'nyong-nyong virus.
- Parvovirus B19 and chikungunya virus, in particular, may cause prolonged or chronic arthritis after acute infection.

Chronic Infectious Arthritis

- It is usually monoarticular, involving the large peripheral joints.
- It is relatively uncommon compared with acute bacterial arthritis but is increasingly seen in immunocompromised or chronically ill hosts. Etiology includes *Mycobacterium tuberculosis* and nontuberculous mycobacteria. Fungal causes include *Candida,* dimorphic fungi (e.g., *Blastomyces, Coccidioides,* and *Sporothrix*), rarely *Cryptococcus,* and molds, among others.

CLINICAL EVALUATION

- Bacterial arthritis is an emergency and should be considered in any patient with acute monoarticular arthritis and in bacteremic patients with oligoarticular joint inflammation.
- The most useful demographic risk factors are presence or absence of recent joint surgery, RA, advanced age, IV drug use, concomitant skin infection, or diabetes mellitus.
- The most helpful laboratory parameters are the synovial fluid leukocyte count and differential and examination for crystals to evaluate for gout or pseudogout.
- In patients with acute monoarticular arthritis an elevated serum procalcitonin can be predictive of bacterial joint infection.
- Diagnostic imaging should include plain film radiography. For prolonged or atypical symptoms or for deep joints, computed tomography or magnetic resonance imaging is recommended.
- Differential diagnosis includes crystalline, reactive, or traumatic arthritis, in addition to other monoarticular rheumatologic disorders.
- Identification of the infecting organism on synovial fluid Gram stain and culture remains the definitive diagnostic test. The polymerase chain reaction (PCR) assay continues to show promise.
- For gonococcal arthritis, testing should include nucleic acid amplification testing of cervical or vaginal and male urethral mucosa or urine.

THERAPY

Acute Bacterial Arthritis

- Joint drainage by repeat aspiration, arthroscopy, or arthrotomy is required.
- Empirical antibiotic selection should be based on synovial fluid Gram stain (see Table 103.5).
- IV therapy, in some instances with an oral transition, is usually prescribed for a total of 2 to 4 weeks.

Chronic Arthritis

- Therapy of *M. tuberculosis* arthritis is similar to pulmonary tuberculosis.
- Many fungal arthritides are difficult to treat and result in chronic joint disability. Treatment varies by infecting organism. Experience using the azoles (including new triazoles) and the echinocandins is growing, and in many cases these drugs are replacing amphotericin.
- Except for *Cryptococcus* and perhaps *Coccidioides* or *Sporothrix,* fungal arthritis requires surgical drainage and débridement.

SEPTIC BURSITIS

- It predominantly involves the prepatellar, olecranon, and trochanteric bursa.
- It can be caused by direct inoculation, contiguous infection, or, less commonly, from a hematogenous source.
- More than 80% of cases are due to *S. aureus.*
- Diagnosis is made from the clinical presentation and by aspirating the bursa for fluid analysis, Gram stain, and culture.
- Treatment includes antibiotics (usually 14–21 days) and daily aspiration of the bursa until sterile fluid is obtained. Surgery is rarely required. Except for severe cases, oral antistaphylococcal agents that have activity against community-acquired methicillin-resistant *S. aureus* are recommended, pending culture and sensitivity. For moderate-to-severe cases or patients with immunosuppression, intravenous (IV) antibiotics should be selected.

Infectious arthritis of single or multiple joints may be caused by any of a number of diverse microorganisms. Bacterial arthritis, also known as *suppurative, pyogenic,* or *septic arthritis,* is the most common and arguably most important joint infection and is considered a rheumatologic emergency because of its potential for rapid joint destruction with irreversible loss of function. Viral arthritis often involves multiple joints as a component of a systemic infection, and although usually self-limited, it may rarely lead to a chronic polyarticular arthritis. In contrast to the acute occurrence of bacterial and viral arthritis, joint infection caused by mycobacteria and non-*Candida* fungi usually occurs as chronic, slowly progressive monoarticular arthritis. Reactive arthritis (formerly Reiter syndrome), a sterile inflammatory spondyloarthropathy, is occasionally associated with systemic or local infection at a site remote to the joint rather than from direct infection of joint tissue. The clinical manifestations, severity, treatment, and prognosis of septic arthritis are dependent on the identity and virulence of the infecting bacterium, source of joint infection, and certain underlying host factors, such as immune status, comorbid illness, and abnormal joint architecture from disease or surgery. For patients with infectious arthritis a prompt and thorough clinical evaluation and early institution of specific treatment are essential to limit long-term sequelae.

This chapter will primarily discuss infections of native joints. For a detailed discussion of infections involving prosthetic arthroplasty see Chapter 105. Lyme arthritis caused by disseminated *Borrelia burgdorferi* (Lyme disease) infection is covered in Chapter 241.

ACUTE BACTERIAL ARTHRITIS

Epidemiology

The reported incidence of native joint septic arthritis in the general population of western Europe and the United States is between 2 and 12 cases per 100,000 per year.[1–5] Rates are increasing, particularly in the elderly, because of a larger number of at-risk patients and surgical joint procedures in recent years.[4–6] Estimates of the incidence in elderly patients and those with RA are much higher, ranging from 28 to 70 cases per 100,000 per year.[4–7] Although not well studied, socioeconomically disadvantaged populations appear to have a higher prevalence than the general population.[3,8] Bacterial arthritis is an uncommon but not rare diagnosis in the emergency department, accounting for 8% to 27% of patients presenting with one or more acutely painful joints.[9,10] Information on the epidemiology of septic arthritis is limited by the retrospective nature and varying case definitions used for many of the studies and the lack of a reliable gold standard for the diagnosis.

The published mortality rates of bacterial arthritis in adults vary between 7% and 15% but may be as high as 30% to 50% in those with significant comorbidity or multiple joint involvement.[3,11–15] Moreover, bone and joint infections that develop in a health care setting are associated with a significantly higher mortality rate, longer length of hospital stay, and greater financial cost than infections acquired in the community.[16] The morbidity of septic arthritis is considerable, with up to 50% of patients reporting permanent decreased joint function or mobility after infection, depending on the presence or absence of preexisting joint disease and identity of the infecting pathogen.[13,17,18] Despite improved antimicrobial agents and adjunct treatment measures, as well as advances in hospital care, the morbidity and mortality of septic arthritis has not changed appreciably in the past 2 to 3 decades.

Route of Infection

Bacterial arthritis is usually hematogenously acquired during overt or occult bacteremia, including that caused by endocarditis.[8,18–20] A study from Spain of cases from 1985–2011 of bacteremic osteoarticular infection noted an increase in cases, with an increased proportion of health care–related cases. Patients increasingly were found to be older, comorbidities were more common, and more cases were device related.[21] Normal, diseased, and prosthetic joints are all susceptible to hematogenous infection, although abnormal joint architecture greatly increases the risk. Thus patients with osteoarthritis, gout, and pseudogout are all at an increased risk of infectious arthritis.[5,22] The extremely vascular synovial membrane of the joint lacks a limiting basement membrane and is particularly susceptible to the hematogenous deposition of bacteria.[2,18,23] Most nosocomial cases of acute bacterial arthritis presumably occur via this route.[15,24]

Other routes of infection include direct inoculation of bacteria into the joint through surgery, trauma, animal and human bites, percutaneous puncture (such as from a nail, needle, or thorn), or from contiguous spread from adjacent infected soft tissue or bone.[2,8] A common example of the latter is septic arthritis of the small joints of the foot, complicating an adjoining diabetic foot ulcer or infection.[25] On occasion, hip joint infection may occur from contiguous spread from an intraabdominal source via the retroperitoneal space and iliac acetabulum.[26,27]

Septic arthritis is an unusual complication of arthroscopic surgical procedures of the knee, occurring in only 0.04% to 0.7% of arthroscopies and 0.14% to 2.3% of arthroscopic reconstructive procedures.[28–30] The incidence of infection after arthroscopic surgery of the shoulder may be somewhat higher, with one study reporting rates of 8.5 per 1000 rotator cuff repairs.[31] Similarly, it is also extremely rare for infection to follow a single joint aspiration or corticosteroid injection, occurring in less than 0.04% of those procedures.[5,32] Iatrogenic infections that follow joint corticosteroid injection may be caused by unusual pathogens and can occur in outbreaks or clusters related to contaminated medication, usually methylprednisolone.[33,34]

Predisposing Host Factors

Predisposing host factors for septic arthritis are listed in Table 103.1. The most important risk factor is preexisting abnormal joint architecture, particularly that caused by RA. A prospective cohort study of patients presenting with acute inflammatory arthritis found that recent joint surgery, age older than 80 years, diabetes mellitus, and RA were associated with a significant and increased incidence of septic arthritis (likelihood ratios: 6.9, 3.5, 2.7, and 2.5, respectively).[1] Prevalence was also increased for patients on hemodialysis in one study, although some of this risk was likely due to preexisting joint disease.[35] The immunosuppressive disease-modifying antirheumatic drugs (DMARDs) penicillamine, sulfasalazine, and prednisone have been identified to further increase the risk in patients with RA.[7,36] Although methotrexate therapy was found to be associated with septic arthritis in one investigation,[7] it was not so identified in a large retrospective controlled study.[36] Similarly, patients treated with anti–tumor necrosis factor (TNF) agents have increased risk for several infections, including those of the joint.[36–41] A

TABLE 103.1 Predisposing Factors in Bacterial Arthritis

Major Factors
Rheumatoid arthritis
Crystal induced arthritis (gout and pseudogout)
Advanced age
Diabetes mellitus
Chronic renal failure
Previous joint surgery
Penetrating joint injury
Intravenous drug use
Endocarditis
Immunosuppression
Organ and bone marrow transplant
Immunosuppressant therapy, including systemic corticosteroids, DMARDs, and anti-TNF agents
Minor Factors
Other joint disease
Osteoarthritis
Charcot arthropathy
Chronic systemic disease
Collagen vascular disease
Malignancy
Chronic liver disease
Sickle cell disease
Alcoholism
Hypogammaglobulinemia
Intraarticular injection (e.g., glucocorticoids)
Skin disease with or without infection
Low socioeconomic status

DMARDs, Disease-modifying antirheumatic drugs; *TNF,* tumor necrosis factor.
Data from references 1–5, 11, 20, 22, 32, 33, 41, 44, and 91.

large prospective observational study found that anti-TNF therapy doubled the risk (adjusted hazard ratio, 2.3) of septic arthritis in patients with RA. The risk was greatest in the first year of therapy and was not significantly different between the specific anti-TNF agents.[42] Moreover, opportunistic and intracellular pathogens are more likely to cause joint infections in these patients.

Regarding human immunodeficiency virus (HIV) infection, one study in Africa suggested that such patients may have a small increased risk for septic arthritis,[43] whereas other studies have found no such association.[44,45] The perceived increased risk in patients with HIV is likely related to concomitant IV drug abuse, an established risk factor for joint infection.[46,47] An important source of hematogenously derived joint sepsis is skin disease or lesions, with or without concurrent infection.[1,2] Of importance, up to 22% of patients with septic arthritis will not have an identifiable predisposing risk factor, and the negative predictive value of any one factor for joint infection is low. Thus the diagnosis must be considered in all persons presenting with inflammatory arthritis, even when risk factors are absent.[12,48,49]

Nongonococcal Arthritis

Pathophysiology

The pathophysiology of acute nongonococcal septic arthritis is complex and dependent on adherence of organisms to, and colonization of, the synovial membrane, bacterial proliferation in synovial fluid, and a resultant synovial infection with generation of a host inflammatory response.[50] Much of our understanding of these pathophysiologic mechanisms comes from studies of animal models of joint infection, particularly with *Staphylococcus aureus*.[51] After hematogenous or direct entry to the joint, bacterial adherence is facilitated by the low shear conditions of synovial fluid, and, in some cases, by joint disease or injury (traumatic or surgical). The later results in an increased amount or exposure of host-derived extracellular matrix proteins, such as fibronectin, collagen, laminin, elastin, and hyaluronic acid, which promote bacterial attachment.[23,52]

Some bacteria display a tropism for synovial infection, at least in part because of adherence characteristics of the organism. For example, *S. aureus* has surface receptors, such as fibronectin-binding protein and "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs), that recognize selected host proteins and mediate adherence to the joint extracellular matrix (see Chapter 194).[53,54] Expression of these surface receptors, as well as several other virulence factors, are influenced by genes, including those regulating quorum sensing, which promote *S. aureus* invasion and infection when host defenses are weakened.[55,56] In the case of *Kingella kingae* and *Streptococcus agalactiae*, joint tropism and synovial adherence is influenced by bacterial pili and fibrinogen-binding adhesin proteins, respectively.[52,57]

Although certain bacterial products or toxins may directly increase tissue damage in the infected joint,[23,58–61] it is the host inflammatory response to infection that is responsible for much of the joint injury.[62,63] In response to replicating bacteria and a number of bacterial products an inflammatory cell response is rapidly noted within the joint synovial membrane, which then responds with a proliferative lining-cell hyperplasia.[64] An influx of acute and chronic inflammatory cells results in the characteristic purulent inflammation of the joint and its synovial fluid. Leukocyte-derived proteases and inflammatory cytokines, including interleukin-1 (IL-1), IL-4, IL-6, IL-10, and TNF-α, either directly or indirectly result in cartilage degradation, inhibition of cartilage synthesis, and subchondral bone loss.[23,24,63,65] Intraarticular cartilage destruction may be seen in as little as 3 days.[66] In addition, the inflammatory joint infusion increases intraarticular pressure, hampering capillary blood flow to the joint, resulting in cartilage and synovial ischemia and necrosis.[18] Over time cartilage destruction leads to joint space narrowing and further erosive damage to the cartilage and underlying bone. If untreated, infection can spread from the joint to surrounding soft tissue, disrupting ligaments, tendons, and other periarticular structures and occasionally forming sinus tracts.[67,68]

Microbiology

Septic arthritis is caused by a multitude of pathogenic bacteria (Table 103.2). Except for slight decreases in the proportion of cases due to gonococci, group A streptococci, and *Streptococcus pneumoniae,* and a modest increase due to *S. agalactiae*, the distribution of bacterial organisms causing septic arthritis has changed little over time.[2–6,12,25,69–73]

TABLE 103.2 Bacteria Isolated in 2302 Compiled Cases of Bacterial Septic Arthritis in Adults

ORGANISM	NO. OF ISOLATES (% OF TOTAL)
Gram positive	
Staphylococcus aureus	1066 (46)
Staphylococci, coagulase negative	84 (4)
Streptococci	512 (22)
Streptococcus pyogenes	183 (8)
Streptococcus pneumoniae	156 (7)
Streptococcus agalactiae	69 (3)
Other streptococci	104 (5)
Gram negative	
Escherichia coli	91 (4)
Haemophilus influenzae	104 (5)
Neisseria gonorrhoeae	77 (3)
Neisseria meningitidis	28 (1)
Pseudomonas aeruginosa	36 (2)
Salmonella spp.	25 (1)
Other gram-negative rods	110 (5)
Miscellaneous (including anaerobes)	136 (6)
Polymicrobial	33 (1)

Modified from Ross JJ, Saltzman CL, Carling P, et al. Pneumococcal septic arthritis: review of 190 cases. Clin Infect Dis. *2003;36:319–327.*

Gram-Positive Bacteria

By far, the most common etiology of septic arthritis in adults is *S. aureus,* which is responsible for 37% to 65% of cases, depending on geographic location, incidence of comorbid rheumatic disease, and proportion of infections involving native joints. For patients with RA the proportion of septic arthritis caused by *S. aureus* has been reported to be higher (≈75%).[67,74] In many geographic locations the incidence of methicillin-resistant *S. aureus* (MRSA) septic arthritis has increased compared with that in the 1990s, particularly in the elderly, persons with recent orthopedic surgery, and those colonized or previously infected by MRSA.[15,16,75–79] However, some studies, particularly those from western Europe, have not noted this increase, and others have reported little change in the proportion of joint infections due to MRSA since 2000.[25,73,80] Coincident with the increased prevalence of invasive infections caused by community-acquired MRSA (CA-MRSA) since 2003, there have been several reports of native joint infection caused by this pathogen, especially in children.[16,80–86] Septic arthritis caused by CA-MRSA is associated with increased morbidity, suppurative complications, and duration of fever, antibiotics, and hospitalization, compared with that caused by methicillin-susceptible *S. aureus* (MSSA).[81,82,87] Vancomycin-intermediate, but not high-level, resistantance in *S. aureus* has rarely been reported as a cause of septic arthritis in patients with frequent exposure to health care facilities.[86,88–90] Coagulase-negative staphylococci, a common pathogen in prosthetic joint infections, are rarely a cause of native joint septic arthritis. If isolated, however, it is important to differentiate *Staphylococcus lugdunensis* from other coagulase-negative staphylococci because of its increased virulence and propensity to cause invasive infections of native tissues.[91]

Streptococcus spp. are the next most frequently isolated bacteria from adults with native joint septic arthritis.[4–6,23,25,69–73] *Streptococcus pyogenes* and other β-hemolytic streptococci from Lancefield groups C, F, and G are important pathogens within this group. *S. agalactiae* is a cause of bacterial arthritis in neonates, as well as adults with diabetes mellitus, malignancy, and genitourinary structural abnormalities, and it is prone to occur with polyarticular infection.[92–95] Population surveillance from 1995–2012 in California showed an increased incidence of septic arthritis due to this organism, with diabetes mellitus the most common underlying condition.[96] *S. pneumoniae*, traditionally thought of as a rare cause of hematogenous septic arthritis in the antibiotic era, accounted for 6% of cases in a comprehensive systematic review.[71] Polyarticular infection was frequent (36%), and many patients did not have prior or concurrent respiratory tract infection. Some studies have shown lower numbers of joint infections due to this organism, particularly in children, since the adoption

of pneumococcal conjugate vaccine.[5,6,87] Pneumococcal vaccination has, however, likely shifted the percentage of pneumococcal joint isolates from serotypes included in the vaccine to nonvaccine serotypes.

Other streptococci are a less frequent cause of septic arthritis. *Streptococcus suis*, an emerging zoonotic pathogen in China, Southeast Asia, and to a lesser extent in Europe, has been reported to cause septic arthritis in persons exposed to pigs or improperly cooked pork.[97,98] Another emerging pathogen, *Streptococcus iniae,* is associated with aquaculture fish and has occasionally been described as a cause of bacterial arthritis in fish handlers.[99] Native joint septic arthritis caused by viridans group streptococci is rare, in contrast to prosthetic joint infections, where it is a common pathogen.[100] Osteoarticular infections with the *Streptococcus bovis* group, most frequently *Streptococcus gallolyticus* subsp. *gallolyticus*, are often a manifestation of infectious endocarditis, and evaluation for endocarditis and colonic malignancy should be undertaken.[101]

Gram-Negative Bacteria

Gram-negative bacilli are cultured from approximately 5% to 20% of patients with bacterial arthritis, particularly from neonates, the elderly, IV drug users, and immunocompromised hosts.[5,6,23,73,102] The coliform bacteria are most commonly isolated from the elderly, immunocompromised, and comorbidly ill[2,25,70,72] and are increasingly found to be multidrug resistant.[103,104] *Pseudomonas aeruginosa* is an important pathogen in IV drug users and has also been described as a cause of iatrogenic septic arthritis after surgical procedures and intraarticular injections.[105,106] It has a particular affinity for fibrocartilaginous articular structures, such as the pubic symphysis and the sternoclavicular, sternochondral, and sacroiliac joints.[107–111] *Haemophilus influenzae*, once an important pathogen in young children, now rarely causes septic arthritis in populations where *H. influenzae* type b vaccine is widely used.[112] For children younger than 4 years, *K. kingae,* a resident of the normal oral flora, has replaced *H. influenzae* as the principal gram-negative cause of hematogenous bacterial arthritis.[113–117] Joint infection caused by *K. kingae* is often associated with stomatitis or upper respiratory tract infection and typically responds well to therapy with minimal articular or functional sequelae. Child-to-child transmission of this communicable bacterium occurs, and outbreaks of *K. kingae* osteoarticular infections in child care centers have been reported.[117–119]

Other Bacteria

Other nongonococcal bacteria identified in infected joints include corynebacteria, S*almonella* spp., *Neisseria meningitidis*, *Listeria monocytogenes, Burkholderia cepacia, Burkholderia pseudomallei, Legionella* spp., and *Brucella* spp. Articular infection caused by anaerobic bacteria is rarely reported.[120] An important exception, however, is *Cutibacterium acnes* (formerly *Propionibacterium acnes*), which is often isolated from infected shoulder joints after arthroscopic or open surgery.[31,121,122]

Unusual bacterial pathogens causing contiguous septic arthritis after a dog or cat bite include *Pasteurella multocida* and *Capnocytophaga* spp., and in the case of a human bite, *Eikenella corrodens* and *Fusobacterium nucleatum*.[123–126] *Streptobacillus moniliformis*, a causative agent of rat-bite fever, is occasionally isolated from the blood or synovial fluid in patients with polyarticular arthritis after a rat bite.[127,128] *Pantoea agglomerans* is the most common bacteria cultured from infected joints after direct plant thorn injury.[129] Table 103.3 lists selected clinical and epidemiologic associations with septic arthritis and their likely causative bacteria.

Cultures of synovial fluid or blood from patients diagnosed with septic arthritis yield polymicrobial flora in up to 10% of cases.[25,70] Causes of bacterial arthritis in which a pathogen is not isolated from blood or joint fluid using conventional culture techniques are *Mycoplasma hominis, Ureaplasma urealyticum, B. burgdorferi,* and *Tropheryma whipplei* (Whipple disease).

TABLE 103.3 Clinical and Epidemiologic Features Associated With Selected Bacterial Causes of Septic Arthritis

CLINICAL OR EPIDEMIOLOGIC FEATURE	ETIOLOGIC AGENT
Rheumatoid arthritis	*Staphylococcus aureus*
Intravenous drug use	*S. aureus, Pseudomonas aeruginosa*
Diabetes, malignancy	*S. aureus, Streptococcus agalactiae*
Immunocompromised hosts	*S. aureus,* streptococci, enteric gram-negative bacilli, *Listeria monocytogenes*
Neonates, children younger than 4 yr	*S. agalactiae,* gram-negative bacilli, *Kingella kingae*
Young adults, menstruating females, associated skin lesions	*Neisseria gonorrhea*
Fibrocartilaginous joints (e.g., pubic symphysis)	*S. aureus, P. aeruginosa*
Postsurgical shoulder joint	*Cutibacterium acnes*
Cat or dog bite	*Pasteurella multocida, Capnocytophaga* spp., anaerobes
Human bite	*Eikenella corrodens,* anaerobes, other oral flora (e.g., viridans streptococci)
Rat bite	*Streptobacillus moniliformis*
Postpartum women	*Mycoplasma hominis*
Ingestion of unpasteurized dairy products, residents or travelers from endemic areas	*Brucella* spp.
Residents or travelers to Southeast Asia	*Burkholderia pseudomallei* (melioidosis), *Streptococcus suis*
After plant thorn injury	*Pantoea agglomerans, Nocardia* spp.

Clinical Manifestations

Joints Involved

Nongonococcal septic arthritis is monoarticular in 80% to 90% of cases, with the knee being the predominate site of infection in up to 50% of patients.[2,5,6,20,25,130–132] Other native joints that are frequently involved in adults include the hip, shoulder, wrist, and ankle. In children hip infections predominate.[2] Infections of the peripheral joints of the hands are unusual, except in the setting of trauma, particularly animal or human bites.[125] Septic arthritis of the small joints of the foot is most often secondary to contiguous spread from skin and soft tissue ulcerations or adjacent osteomyelitis and is mostly seen in diabetic patients.[25,133] Nongonococcal polyarticular bacterial arthritis is observed in 10% to 20% of patients with septic arthritis, especially in those with RA, immunosuppression, or prolonged or intense bacteremia that is often associated with endovascular infections and intravenous drug use (IVDU).[5,6,14] These infections are usually caused by *S. aureus*. Sternoclavicular or costochondral joint infections are uncommon except in IV drug users and occasionally as a complication of occult bacteremia or subclavian vein catheterization.[110,134,135] In these cases *P. aeruginosa* is a frequent pathogen. Risk factors for septic arthritis of the pubic symphysis include female urinary incontinence surgery, participation in athletics, pelvic malignancy, and IVDU.[109]

Sacroiliac joint infection is an uncommon metastatic complication of occult or symptomatic bacteremia, usually caused by *S. aureus*.[107,136–140] IV drug abusers and patients with indwelling catheters are at risk. Patients typically present with acute onset of sacral or pelvic pain and leukocytosis. Blood cultures are often positive. Patients should be evaluated for the presence of concomitant infective endocarditis. Uncommon causes include *Salmonella* spp., including *Salmonella enterica* serovar Typhi, and brucellosis.[137,141,142] Brucellosis causes a chronic unilateral infection of the sacroiliac joint that must be distinguished from arthritis associated with inflammatory bowel disease (IBD) and ankylosing spondylitis (see Chapter 226). Injection of contaminated methylprednisolone into the sacroiliac joint led to an indolent sacroiliac joint infection caused by *Exserohilum* during a multistate outbreak of meningitis, epidural abscess, and joint infection caused by this pathogen.[143]

Clinical Presentation

Most patients with acute bacterial arthritis present with the cardinal symptoms of arthritis—pain and loss of function of one or more joints

over a 1- to 2-week period. In addition to intense pain and decreased range of motion, other symptoms of nongonococcal bacterial arthritis include swelling, redness, and increased warmth of the infected joint.[3,6,12,18,24] Joint pain is often the only focal symptom of deep or axial joint infection. In the case of sacroiliac or pubic symphysis septic arthritis, pain is intensely exacerbated by ambulation and often radiates to the back or groin, respectively, and to the hip and proximal leg in either case.[107,109,144] Although fever and malaise are commonly associated with bacterial arthritis in adults, high fever with rigors and shaking chills are typically not apparent and may be totally absent in 30% to 50% of patients.[6,18,20,24] A systematic review showed that fever was present as a physical examination finding in approximately 50% of patients, and it was found to have a sensitivity for articular infection of only 57%.[48]

Physical examination findings of infected peripheral joints include focal joint tenderness, inflammation, and effusion (Fig. 103.1). Active and passive range of motion of the joint is usually limited and results in considerable discomfort. Examination findings in patients with bacterial arthritis of the nonperipheral joints may be limited to focal tenderness over the afflicted area. Children with septic arthritis of the hip characteristically hold the hip in a flexed and externally rotated position and resist any range of motion. Weight bearing is painful. A source of infection distant from the afflicted joint, including that of the skin, may be discovered after careful clinical evaluation in up to 50% of patients.[20]

Patients who are immunocompromised, have comorbid illnesses, including RA, or are at the extremes of age often present without a fever and with significantly more subtle symptoms and signs of joint infection, frequently resulting in delayed diagnosis.[3,4,102,130,145] For patients afflicted with RA or other chronic joint disease, particularly those receiving systemic or intraarticular steroids, manifestations of a complicating joint infection may be limited to a modest increase in the severity of chronic articular inflammation and loss of joint function. In these cases it can be very difficult to differentiate joint infection from an exacerbation of their underlying disease.[11,48,146]



FIG. 103.1 (A) Bacterial arthritis of the knee showing swelling and effusion. (B) Septic third proximal interphalangeal joint demonstrating striking redness, swelling, and effusion.

Laboratory Findings

Laboratory evaluation frequently shows an elevated peripheral white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP); however, these tests are nonspecific and, if elevated, do not appreciably raise the pretest probability of septic arthritis.[24,48,147,148] Similarly, a nonelevated ESR or CRP may be found in native joint infections due to low virulence organisms.[148,149] However, in one retrospective analysis of 17 cases, a CRP exceeding 10.5 mg/dL was considered predictive of septic arthritis.[147]

Serum procalcitonin (PCT), an acute-phase reactant more sensitive and specific than ESR and CRP, may potentially be useful for the diagnosis of acute bacterial arthritis.[150,151] PCT was evaluated in a meta-analysis for the identification of osteomyelitis and septic arthritis in patients who had fever and orthopedic symptoms. For those patients with septic arthritis, using a cutoff level of 0.5 ng/mL, the diagnostic sensitivity and specificity were 65% and 88%, respectively, and the positive and negative likelihood ratios were 5.3 and 0.4, respectively.[152] Adjusting the cutoff level for positivity gives modestly different performance characteristics, and there is no clear consensus as to the optimum value.[153] Thus an elevated serum PCT might help suggest bacterial septic arthritis in a patient with an acutely inflamed joint, but, more important, a normal value is not useful for ruling it out.[150,152,154]

Arthrocentesis of an affected joint usually reveals purulent, low-viscosity synovial fluid with an elevated polymorphonuclear neutrophil count. Traditionally, a synovial fluid leukocyte count greater than 50,000 cells/mm^3 has been used to suggest a diagnosis of septic arthritis[4,18]; however, lower WBC counts are regularly encountered and do not exclude the diagnosis (Table 103.4).[48] Synovial fluid WBC counts between 25,000 cells/mm^3 and 75,000 cells/mm^3 constitute a "gray zone" for the diagnosis of bacterial arthritis, and careful clinical assessment and observation is necessary in such patients.[155] Other potential tests of synovial fluid laboratory tests have been evaluated. Although synovial fluid glucose may be depressed and protein and lactate dehydrogenase elevated, the sensitivity and specificity of these tests are low and limit their usefulness.[48] The diagnostic accuracy of an elevated synovial fluid lactic acid, PCT, or an inflammatory cytokine, such as TNF-α, has not been adequately assessed.[24,48,152,156]

Microbiologic Diagnosis

Conventional culture. Synovial fluid culture in nongonococcal infection of patients who have not previously received antibiotics will yield bacterial growth up to 80% to 90% of the time; however, Gram staining is diagnostic in only 50% of these cases.[12,72,148] False-positive Gram stains of synovial fluid can occur because of artifacts from stain, mucin, and cellular debris. There is some evidence that direct inoculation of synovial fluid into pediatric or adult blood culture media bottles or isolator culture tubes improves the recovery of pathogens,[157,158] although one investigation comparing the BACTEC (Becton Dickinson, Franklin Lakes, NJ) blood culture bottle to lysis centrifugation and conventional agar plate culture techniques found similar yields from all three methods.[159] However, using a blood culture system for synovial fluid culture may enhance the yield of fastidious organisms, such as *K. kingae* and *Brucella* spp.[160,161] Synovial fluid cultures from potentially infected shoulders should be incubated for at least 10 days, to enhance the isolation of *C. acnes*, a slow-growing bacterium that is an important cause of infection of this joint.[162]

TABLE 103.4 Diagnostic Parameters of Synovial Fluid White Blood Cell Count and Percent Polymorphonuclear Cells in Patients With Septic Arthritis

			LIKELIHOOD RATIO (95% CI)	
	SENSITIVITY (%)	SPECIFICITY (%)	Positive	Negative
>100,000 WBC/mm^3	29	99	28.0 (12.0–66.0)	0.71 (0.64–0.79)
>50,000 WBC/mm^3	62	92	7.7 (5.7–11.0)	0.42 (0.34–0.51)
>25,000 WBC/mm^3	77	73	2.9 (2.5–3.4)	0.32 (0.23–0.43)
PMN cells ≥90%	73	79	3.4 (2.8–4.2)	0.34 (0.25–0.47)

CI, Confidence interval; *PMN,* polymorphonuclear; *WBC,* white blood cell.
Modified from Margaretten ME, Kohlwes J, Moore D, et al. Does this adult patient have septic arthritis? JAMA. *2007;297:1478–1488.*

The proportion of patients with positive blood cultures in nongonococcal bacterial arthritis varies from 25% to 70%, depending on the study.[12,20] Of note, they are the sole source of the infecting organism's identity in approximately 10% of cases. Both synovial fluid and blood culture sensitivity declines in patients after antimicrobial therapy has been initiated.

False-positive synovial fluid cultures due to bacterial contamination occasionally occur and are more likely in aspirates from the wrist and shoulder. Clinical features suggesting a false-positive culture include low synovial fluid WBC counts, isolation of coagulase-negative *Staphylococcus* or *Bacillus* spp., and time to positivity of more than 48 hours.[163] *C. acnes* can be especially problematic when isolated from a native or prosthetic shoulder joint. Although this organism is a significant cause of infection of this joint, false-positive cultures also occur. Growth of the organism after 9 days of incubation is more likely to be a culture contaminant; however, careful clinical correlation is essential before discounting it as a false-positive culture.[164]

Molecular tests. Identification of bacterial DNA in synovial fluid by multiplex or broad-range polymerase chain reaction (PCR) assay shows promise for improving the etiologic diagnosis of septic arthritis, especially for infections caused by fastidious or unusual pathogens.[165–168] Identification of *K. kingae*, *Streptococcus* spp., *Mycoplasma,* and anaerobic bacteria is particularly enhanced. One technique showed a sensitivity and specificity of 95% and 97%, respectively, by using a 16S ribosomal DNA (rDNA) gene target and real-time PCR.[166] The negative predictive value in a separate study was 98%.[169] Although PCR performance is generally thought to be high, one study comparing three 16S rDNA targets reported more modest results, with sensitivities of 44% to 63% and negative predictive values of 84% to 89%.[170] Another study using multiplex PCR testing reported a negative result by PCR for *S. aureus, S. pneumoniae,* and *S. dysgalactiae* despite positive synovial fluid cultures for these organisms.[171]

In addition to the identification of fastidious organisms, other benefits of PCR include rapid results, high negative predictive values, and greater likelihood of yielding a pathogen from patients who have previously received antibiotics. Limitations of PCR include false-positive tests as a result of sample contamination, lack of antimicrobial susceptibility data, and, in the case of broad-range PCR, difficulty in confirming a specific species because of shared regions of bacterial 16S DNA. A multicenter evaluation of multiplex PCR to assess for the presence of bacterial pathogens in synovial fluid is ongoing, and early results show promise.[172] Molecular testing as a tool for the diagnosis of septic arthritis is likely on the horizon.

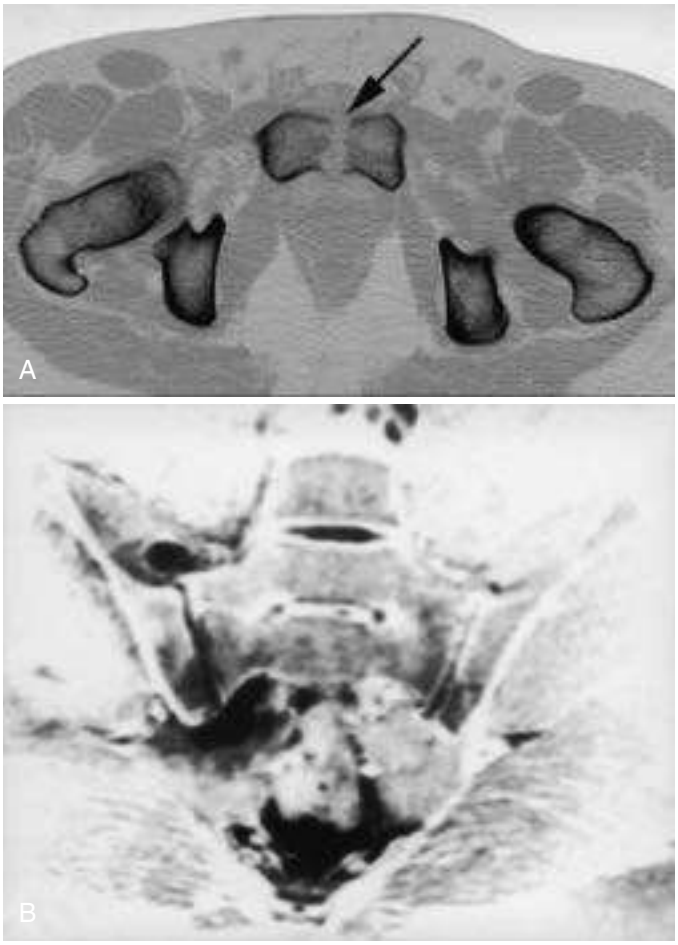

FIG. 103.2 (A) Septic arthritis of the symphysis pubis. Computed tomography of the pelvis reveals joint space widening *(arrow)* with subchondral bone resorption and disruption of the articular cortical margins. (B) Septic sacroiliac joint. Coronal T2-weighted magnetic resonance image demonstrates fluid within the right sacroiliac joint, spreading superiorly and inferiorly to form soft tissue abscesses. *(From Chew FS, Maldjian C, Leffler SG.* Musculoskeletal Imaging: A Teaching File*. Philadelphia: Lippincott Williams & Wilkins; 1999:267.)*

Radiographic Features

Imaging studies of joints and periarticular structures affected by bacterial arthritis often yield additional information helpful for establishing the diagnosis or evaluating for complications of infection.[173] Plain film radiography in early infection will typically show periarticular soft tissue swelling and fat-pad edema but normal osseous structures.[174] In advanced infection findings may include periarticular osteoporosis, joint space loss, periosteal reaction, marginal and central erosions, and destruction of subchondral bone. Plain films are useful for assessment of preexisting joint disease, metallic foreign bodies, and simultaneous osteomyelitis, or as a baseline image in monitoring for sequelae of infection.

Scintigraphy, computed tomography (CT), and magnetic resonance imaging (MRI) are highly sensitive techniques for imaging early septic arthritis. Scintigraphy, including three- or four-phase bone scanning, using technetium 99m methylene diphosphonate, are sensitive but nonspecific indicators of articular infection.[174] CT is useful for the detection of erosive bone changes, subchondral cysts, joint effusions, and periarticular soft tissue extension of infection, particularly in deep articulations, such as the hip, sacroiliac, and sternoclavicular joints and in the pubic symphysis (Fig. 103.2).[109,144,174–176]

Similar to bone scanning and CT, MRI is an extremely sensitive procedure for the evaluation of bacterial arthritis but is more specific than these other modalities.[177,178] MRI is particularly valuable for the detection of joint inflammation, effusion, and articular cartilage destruction, as well as for periarticular cellulitis, fistula, abscess, or osteomyelitis of contiguous bone. In some cases findings on MRI may discriminate between septic arthritis and underlying chronic RA.[179] However, MRI may pose difficulties distinguishing septic arthritis from sterile inflammatory arthropathies, cannot be used with certain metal joint implants, and has lower resolution for the bony cortex than CT.[23,177] Dynamic contrast-enhanced MRI, a technique that has shown usefulness in differentiating septic arthritis and transient tenosynovitis involving the hip joint, may also have use for evaluation of other joints.[180] In most US medical centers, MRI and CT have replaced scintigraphy as the preferred imaging modalities for evaluation of a potentially infected joint.

Particularly in children, sonography of the hip can provide rapid evidence of effusion and can guide prompt joint aspiration without waiting for advanced imaging.[173,181]

Gonococcal Arthritis

Epidemiology

During the 1970s and 1980s, *Neisseria gonorrhoeae* was the predominant cause of bacterial arthritis in sexually active adults and adolescents, with the majority of cases occurring in persons younger than 30 years.[182,183] Of note, the prevalence of gonococcal arthritis markedly declined as the rate of mucosal gonorrhea decreased fivefold between 1975 and 2011 to one of the lowest rates since statistics have been recorded.[184] Since 2011, however, rates of mucosal gonorrhea has rebounded, and consequently the prevalence of gonococcal arthritis has likely followed this increase.[185]

Gonococcal arthritis is one of two clinical manifestations of disseminated gonococcal infection (DGI), the other being a syndrome of tenosynovitis, dermatitis, and polyarthralgia without purulent joint infection.[182,186] There is overlap between the two conditions, and in some patients disseminated infection may progress from a bacteremic tenosynovitis-dermatitis syndrome to localized joint infection. Septic monoarticular or oligoarticular arthritis occurs in 42% to 85% of patients with disseminated gonococcal infection.[187]

DGI is usually diagnosed in persons younger than 40 years, is approximately four times more common in women than men, and complicates 0.5% to 3% of persons with mucosal gonococcal infection.[182,188] More recently the proportion of cases occurring in men is likely increasing due to the increasing percentage of mucosal infection in men with HIV and men who have sex with men (MSM).[185,189] Epidemiologic characteristics associated with DGI consist of lower socioeconomic status, nonwhite ethnicity, MSM, multiple sexual partners, and illicit drug use.[186] The incidence is markedly less in Europe than in North America, and it is considerably higher in the developing world.[182]

Pathogenesis

Gonococcal arthritis and DGI occur as a result of occult bacteremia secondary to mucosal infection of the urethra, uterine cervix, rectum, or oropharynx. Asymptomatic mucosal infection is much more likely to result in DGI than symptomatic infection and may have been sexually contracted days to months before dissemination. This, at least in part, accounts for the increased risk of disease in women for whom endocervical gonorrhea may persist without symptoms for extended periods. For these women the risk of DGI increases substantially during menstruation, pregnancy, and the direct postpartum period. One-third to one-half of all women who present with DGI will do so during these times.[187,190,191] Other host factors that increase the risk of DGI are complement deficiencies, particularly the terminal components C5 to C8,[192–195] and systemic lupus erythematosus.[196] Patients receiving eculizumab, a monoclonal antibody that inhibits terminal complement activation and thus increases risk for *Neisseria meningitidis* infection, also appear to be at higher risk for DGI.[197] Immune mechanisms, including circulating immune complexes, also likely play a part in the pathogenesis of DGI and gonococcal arthritis and may account, in part, for the low yield of cultures for *N. gonorrhoeae* in synovial fluid and skin pustules.[183]

N. gonorrhoeae possesses several virulence factors, many of them cell surface proteins, that influence pathogenesis and the ability of the organism to widely disseminate from infected mucosa. Microbial attachment to mucosal and synovial epithelium is facilitated by long pili that also play a role in inhibiting host leukocyte phagocytosis.[198] Gonococcal strains with the ability to disseminate are serum resistant and almost always express protein 1A, a principal outer membrane protein.[188,199–201] Other characteristics of strains with an increased propensity to disseminate include lack of outer membrane protein II, also called "opacity protein," and nutritional requirements for arginine, hypoxanthine, and uracil.[23,182,202] *N. gonorrhoeae* strains that cause DGI are resistant to the bactericidal effects of normal human serum, probably through changes in cell membrane lipooligosaccharide.[203]

Antimicrobial Resistance

N. gonorrhoeae displays acquired resistance to several antimicrobials, including β-lactams, tetracyclines, fluoroquinolones, and, rarely, spectinomycin.[204,205] The rapid emergence of fluoroquinolone resistance in *N. gonorrhoeae* throughout the world since 2001 has resulted in fluoroquinolones being contraindicated for empirical therapy of gonococcal infection.[204,206,207] In addition, minimal inhibitory concentrations (MICs) of the oral cephalosporin cefixime against *N. gonorrhoeae* from North America increased markedly between 2009 and 2011.[185] This, together with treatment failures using cefixime for gonococcal mucosal infections, led the Centers for Disease Control and Prevention (CDC) to no longer recommend this treatment for gonorrhea in 2010.[208,209] Amplifying the problem, MICs of azithromycin, the remaining oral drug with activity against *N. gonorrhoeae,* have also sharply increased. Between 2012 and 2017 the proportion of isolates resistant to azithromycin steadily increased from 0.3% to 4.4%.[185] In consequence, ceftriaxone the only antimicrobial with reliable activity against *N. gonorrhoeae*, is the remaining cornerstone for treatment of gonorrhea. Fortunately, in 2017 only 0.5% of isolates showed decreased susceptibility to this drug, and no isolates with elevated azithromycin MICs had elevated ceftriaxone MICs. Regrettably, precise epidemiologic data are not available on the antimicrobial susceptibility of strains of *N. gonorrhoeae* isolated from patients with DGI or gonococcal arthritis compared with strains from mucosal infections. This steady emergence of resistance of *N. gonorrhoeae* to numerous antimicrobials, particularly in the Pacific Rim and among MSM, has led to considerable concern that gonorrhea could soon attain an untreatable status.[209,210]



FIG. 103.3 Tenosynovitis of the dorsal hand accompanying monoarticular arthritis of the wrist. *Neisseria gonorrhoeae* was isolated from synovial fluid culture of the wrist.

Clinical Manifestations

Patients with DGI typically present with the classic triad of dermatitis, tenosynovitis, and migratory polyarthralgia or polyarthritis.[a] Joint symptoms may be quite severe and are often asymmetrical. Moderate fevers, chills, and general malaise are usually present. Two-thirds of patients develop tenosynovitis, usually occurring in the fingers, hands, and wrists, although the distal large and small joints of the lower limbs can also be involved (Fig. 103.3). The hip is rarely affected. Less than one-half of patients will have a true septic arthritis with a purulent joint effusion. The lesions of dermatitis are seen in two-thirds of DGI patients, are painless and nonpruritic, are few in number, and may not be noticed by the patient.[186,212] Macules, papules, and pustules on an erythematous base are most commonly seen and will often develop central necrosis (Fig. 103.4). The rash characteristically appears simultaneously with tenosynovitis and will be seen in various stages of development involving the limbs and hands (including the palms and soles), less often the torso, and rarely the face. Lesions will resolve after 4 to 5 days even if untreated, and new lesions may materialize after antimicrobial therapy is initiated.[186]

Septic gonococcal arthritis arising without tenosynovitis or skin lesions is less common than the so-called bacteremic form described previously and is clinically indistinguishable from bacterial arthritis caused by other organisms. The knees, wrists, and ankles are most commonly affected, and monoarthritis is more common than polyarthritis. Joints tend to be markedly inflamed, with large effusions. A syndrome of tenosynovitis with skin lesions may have occurred before the patient seeks medical care. Mucosal infection is usually present in patients with DGI and purulent gonococcal arthritis, although it is usually asymptomatic.

[a]References 183, 186, 187, 190, 196, 211.



FIG. 103.4 Pustular lesion overlying the fifth toe in a patient with disseminated gonococcal infection. *(Courtesy Graham International Dermatopathology Learning Center.)*

Laboratory Testing

As with nongonococcal septic arthritis, the laboratory findings of gonococcal arthritis and DGI include mild leukocytosis and elevated ESR in about 50% of patients.[186,187] Synovial fluid from joint effusions afflicted with gonococcal arthritis will frequently, but not invariably, have 50,000 to 100,000 WBCs/mm^3, predominantly neutrophils, whereas joint aspirates from patients with DGI without frank suppurative arthritis will exhibit lower cell counts.[186] *N. gonorrhoeae* is cultured from synovial fluid in approximately 50% of these cases and in 20% to 30% of patients with DGI.[182,186,187] Only 25% of Gram-stained joint aspirates from patients with suppurative gonococcal arthritis reveal intracellular and extracellular gram-negative diplococci. Significantly higher proportions of patients with DGI are found to have *N. gonorrhoeae* identified in synovial fluid by PCR assay than by conventional culture, with PCR assay sensitivity reported around 80%.[213–215] Synovial fluid PCR for *N. gonorrhoeae* is currently limited in most clinical laboratories but may be available in the near future.

For patients with DGI and gonococcal arthritis, culture yield of *N. gonorrhoeae* is much higher from mucosal sites than from synovial fluid or blood. Cultures are positive from uterine cervical swabs in 80% to 90% of women and from urethral swabs in 50% to 70% of men.[186,187,190] Culture yields from rectal and oropharyngeal swabs are lower but will sometimes give positive results. Positive blood cultures are exhibited by less than 30% of patients and are more common in those with dermatitis and tenosynovitis.[211,212,216] Gram staining and bacterial culture of skin lesions rarely yield organisms.

The use of nucleic acid amplification tests (NAATs) that are cleared by the US Food and Drug Administration (FDA) are now the recommended diagnostic testing modality for the detection of genital tract infection in women and men.[217] The performance of NAATs with respect to overall sensitivity, specificity, and ease of specimen transport is better than that of other tests available for the diagnosis of gonococcal and chlamydial infections.[218,219] Thus a mucosal NAAT should be performed for anyone with suspected gonorrhea, DGI, and gonococcal septic arthritis.[217] Mucosal NAATs should be obtained from either a cervical or self-collected vaginal swab specimen in women and from a first-catch morning urine specimen in men. Gonococcal culture is still preferred for rectal and oropharyngeal specimens and should be done in addition to a NAAT for specimens from all sites when antimicrobial susceptibility testing is indicated (e.g., suspected treatment failure).[217]

Septic Arthritis in Persons Who Inject Drugs

IVDU and addiction have been well described for more than 50 years, but since 2010 it has become a significant public health crisis, particularly opioid use disorder (OUD). In 2016 11.8 million Americans were users of prescription opiates or IV heroin.[220] Concomitant with this outbreak of OUD, nonviral IVDU infections have increased markedly in persons who inject drugs (PWID), resulting in substantial morbidity, mortality, and increased hospitalizations in this population.[221] (See Chapter 312.)

Although accurate numbers are not available, bone and joint infection in PWID is common. Estimates of the proportion of all such infections presenting in either identified or later-identified PWID increased from 3% to 12% in 2000 to 11% to 15% in 2014 and was greater in younger age groups.[221,222] Up to half of all PWID-related joint infections may be in unrecognized users; thus there are opportunities to diagnose or treat underlying drug use in patients presenting with septic arthritis, particularly those who are of younger age.[221]

Morbidity, mortality and complications of hospitalization are higher for septic arthritis in PWID than in those who do not inject drugs. One report found an adjusted odds ratio for death during hospitalization of 2.86, and repeat arthroscopic or open débridement of 1.24 and 1.68, respectively. In addition, they were more likely to leave against medical advice and have an additional 5 days of hospital stay.[222]

The bacteria responsible for joint infections in PWID are acquired either from commensal flora from the user's skin or saliva or from organisms contaminating illicit drugs, drug diluents/adulterants, or paraphernalia.[223] Occult or overt bacteremia is the most common route for joint seeding. Although commensal skin flora is the most usual origin of pathogens, contaminated water is also a frequent source. Depending on the study, *S. aureus* accounts for 50% to 90% of joint infections with methicillin-resistant isolates accounting for 25% to 40% of these.[6,223–225] *S. aureus* and MRSA are isolated more commonly in PWID than in those who do not inject drugs. Other pathogens isolated from infected joints in PWID are similar to non-IVDUs, except for higher numbers of viridians streptococci, *Bacillus* spp., *P. aeruginosa*, *Serratia marcescens*, nonfermenting gram-negative rods, nontuberculous mycobacteria, and *Candida* spp. The microbiology of joint infections in PWID has not changed appreciably over the last 3 decades, except for increases in those due to MRSA.[6,224,225]

Similar to septic arthritis in non-IVDUs, the most common joints involved in septic arthritis in PWID are the knee, hip, shoulder, and wrist.[6,223,225] Joints that are rarely infected in non-IVDUs that are more commonly septic in PWID include the sternoclavicular, sacroiliac, pubic symphysis, and cervical facet joints.[223,226] Other notable clinical features of septic arthritis in PWID include more clinical presentations associated with endocarditis, bacteremia, sepsis, and polymicrobial joint infection.

Management

Initial Approach to the Patient

Patient Assessment

The diagnosis of bacterial arthritis is considered a rheumatologic emergency and should be considered in any patient with acute monoarticular or polyarticular arthritis. Septic arthritis of the hip in a child is an extreme emergency and should be treated in the first 6 to 12 hours.[181] Distinct features of the patient's demographics, clinical history, preexisting joint disease, comorbidity, physical examination, and initial laboratory and imaging may each raise the pretest probability of septic arthritis (see discussion in "Clinical Manifestations" under "Nongonococcal Arthritis"). A method for estimating the pretest probability of an infected joint has been suggested based on clinical risk factors that are identified in the evaluation of the patient with a painful swollen joint.[48] The most useful demographic risk factors are presence or absence of recent joint surgery, RA, advanced age, concurrent skin infection, or diabetes mellitus. The most helpful laboratory parameter is the synovial fluid leukocyte count and differential (see Table 103.4). If more than one factor is present, likelihood ratios from each are multiplicative rather than additive. Evidence-based guidelines for the diagnosis and management of septic arthritis have been published and were updated without changes in 2017 by the British Society for Rheumatology Standards, Guidelines and Audit Working Group.[227]

Laboratory Assessment

As part of the clinical assessment, routine laboratory tests should be drawn for identification of comorbid conditions and as a baseline for future antimicrobial monitoring. ESR, CRP, and PCT, although not often predictive for the diagnosis of septic arthritis, may be useful for assessing response to therapy or as a prognostic marker. Cultures should

be obtained from blood, any wound contiguous with the afflicted joint, and skin lesions if present. Plain film joint radiographs should be taken, and if there is concern for soft tissue or osseous extension of infection or prolonged or atypical joint symptoms, CT and MRI should be considered.

Synovial Fluid Assessment

Arthrocentesis should be performed on all patients with inflammatory arthritis in whom joint infection is within the differential diagnosis. Skin visibly involved with cellulitis should be avoided when entering the joint. For cases of polyarticular arthritis, multiple afflicted joints should be aspirated. For axial and deep joints that are difficult to aspirate at the bedside (e.g., sacroiliac or pubic symphysis) or in the event of a "dry tap" of a peripheral joint, fluoroscopic- or CT-guided arthrocentesis is indicated. Synovial fluid examination should include cell count, leukocyte differential, and crystal examination under polarized light. Tests that need not be performed because of limited utility include viscosity, total protein, lactate dehydrogenase, glucose, lactic acid, and bacterial capsular antigens. Gram stain and aerobic and anaerobic bacterial cultures should be performed. If blood culture bottles or isolator tubes are used, they should be inoculated at the bedside. In patients who are immunocompromised, or in cases with subacute, chronic, or relapsing presentation or penetrating joint trauma, fungal and mycobacterial smear and culture are indicated.[228] All cultures should be processed promptly in the laboratory. Antimicrobial therapy should be delayed until arthrocentesis and appropriate diagnostic cultures are obtained, unless the patient shows signs of sepsis. Orthopedic consultation is often necessary for joint drainage, irrigation, and débridement, and orthopedic or rheumatologic assistance is occasionally needed for diagnostic arthrocentesis.

Other Analysis

If gonococcal arthritis is suspected, NAATs for *N. gonorrhoeae* should be obtained from genitourinary sites and/or freshly voided urine, in addition to cultures from the rectum and oropharynx. Although NAATs alone will yield a diagnosis, it is extremely important that culture also be performed in cases that have relapsed or not responded to treatment to allow for antimicrobial sensitivities of an *N. gonorrhoeae* isolate. Because of the high frequency of antimicrobial-resistant gonococcal infections, knowledge of drug susceptibility is vital for guiding therapy in these cases.

Serology is occasionally helpful for diagnosis of nonbacterial joint infections. Serologic testing for *B. burgdorferi* is indicated for patients with clinical features of Lyme arthritis in endemic areas, especially if they are at risk for tick bites (see Chapter 241). Serology, and, if available, synovial fluid or peripheral blood PCR, may also be helpful in the diagnosis of the various viral arthritides, including parvovirus B19 and chikungunya.

Differential Diagnosis

Crystalline Arthritis

The differential diagnosis of pyogenic arthritis includes several inflammatory joint diseases caused by noninfectious sources, in addition to joint infections caused by nonbacterial pathogens. Acute attacks of the crystalline joint diseases, gout, and pseudogout are perhaps the greatest mimics of bacterial arthritis. There is significant overlap in the clinical findings of these two entities because both may occur with monoarticular symptoms of warmth, swelling, pain, and erythema of the involved joint. In addition, systemic signs of inflammation, including fever and elevations of peripheral WBC counts, are often evident. For patients with a history of gouty attacks in stereotypical joints, acute joint inflammation is more likely caused by crystalline than bacterial arthritis; however, in such attacks, arthrocentesis is necessary to rule out concomitant bacterial infection.

The coexistence of crystalline and bacterial arthritis is probably not as rare as previously thought.[229–232] One retrospective study found that, of 265 joint synovial fluid aspirates that contained crystals, 1.5% of the bacterial cultures were positive.[233] Of importance, the mean synovial WBC was significantly higher in the patients who had positive cultures compared with that of the entire study population (113,000 cells/mm^3 vs. 23,200 cells/mm^3). In another study of patients with septic arthritis of the ankle, a large proportion of patients (43.5%) also had crystals found on synovial fluid analysis.[229] Because of these findings the presence of crystals in synovial fluid should not be used to rule out bacterial arthritis, and bacterial Gram stain and culture should be performed on all joint aspirates.

Other Causes of Arthritis

RA, systemic lupus erythematous, spondyloarthropathy, Still disease, rheumatic fever, and Kawasaki syndrome typically occur with subacute polyarticular arthritis associated with specific symptoms and signs of their respective diseases. Although these conditions may be confused with viral arthritides, especially parvovirus B19 or chikungunya arthritis, they are usually easily differentiated from bacterial arthritis. More important, however, is the recognition and diagnosis of bacterial arthritis in a patient with preexisting rheumatoid or other inflammatory arthritis. A sudden increase in inflammation of one or two joints in such patients should raise suspicion of complicating bacterial arthritis, even if it is not accompanied by fever or other systemic evidence of infection, and joint aspiration should be performed.

Reactive arthritis may occur spontaneously or follow a recent gastrointestinal or genitourinary infection, particularly in men.[234] The reactive arthritis may be associated with tenosynovitis and urethritis, thus mimicking disseminated gonorrhea. Patients with reactive oligoarticular arthritis, however, do not display the characteristic rash of DGI and will often have concomitant sacroiliitis, conjunctivitis, circinate balanitis, or keratoderma blennorrhagica—all conditions absent in DGI.

Hemarthrosis caused by trauma or intraarticular injury can mimic septic arthritis of the knee. In such cases a history of injury is readily apparent. For the rare case of septic arthritis after blunt, nonpenetrating trauma, an aspiration with synovial fluid analysis, and culture is required to establish the diagnosis.

Therapy

Joint Drainage

The management of acute bacterial arthritis requires prompt joint drainage, in addition to antimicrobial therapy. One approach to drainage is through daily closed-needle aspiration of affected joints until there is clinical improvement and the amount and degree of purulence of synovial fluid is minimal. Surgical modalities for drainage include arthroscopy and open arthrotomy, both of which allow visualization and irrigation of the joint, lysis of adhesions, and removal of thick purulent material and inflammatory mediators.[235–239] The choice of drainage procedure may depend on the available clinical resources and should be adapted for the individual patient and involved joint. Needle aspiration is less invasive and may be considered if the joint is easily accessible and can be adequately drained, and if the patient lacks negative prognostic factors.[235]

For bacterial infection of the knee, hip, shoulder, wrist, and ankle, drainage by arthroscopy is usually preferred and may need to be repeated for more severe infections.[236–245] For these joints an arthroscopic approach has similar or improved outcomes compared with open surgery with fewer complications and less need for a repeat drainage procedure. Risk factors for failure of a single surgical débridement include a history of inflammatory arthropathy or diabetes mellitus; involvement of the knee, shoulder; or hip; synovial fluid cell count >85,000 cells/mm^3, and *S. aureus* as the bacterial isolate.[246] Timeliness of débridement is likely an additional important prognostic indicator. One study showed that joint washout within 24 hours of diagnosis shortened hospital length of stay compared with a >24-hour delay, but pathogen type and comorbid conditions did not.[247]

Antimicrobial Therapy

Antimicrobial therapy for native joint bacterial arthritis should be initiated without delay to limit articular destruction, preferably after synovial fluid cultures have been obtained. There are no randomized controlled trials that have evaluated antimicrobial regimens for septic arthritis; thus sound evidence is lacking to guide therapeutic options. A large meta-analysis of antimicrobial therapy for bacterial arthritis did not show an advantage of any one, or combination of, therapeutic agent(s)

over another.[248] Initial antibiotic selection is dictated by synovial fluid Gram stain or likely infecting pathogens, or both, as determined by the clinical presentation (Table 103.5; also see Tables 103.2 and 103.3). Risk factors for infection by an antimicrobial resistant pathogen should be considered.

Initial antimicrobial selection. Vancomycin is indicated as empirical therapy for persons with gram-positive cocci on a synovial fluid Gram stain or as a component of therapy for those with a negative Gram stain because of the high prevalence of health care–associated MRSA and CA-MRSA joint infection. For patients with gram-negative rods on Gram stain, an antipseudomonal cephalosporin or piperacillin-tazobactam should be used unless there is a recent or concurrent infection or colonization by an extended-spectrum β-lactamase–producing pathogen, in which case a carbapenem antibiotic is preferred. Aztreonam or ciprofloxacin are less desirable alternatives due to a relatively high proportion of antimicrobial resistance to these agents in many gram-negative pathogens. Injection or infusion of antibiotics directly into the joint is not recommended. For patients with a negative Gram stain, empirical therapy is appropriate even if they have only a modest probability of septic arthritis. In these cases vancomycin plus an antipseudomonal cephalosporin or piperacillin-tazobactam should be used.

For patients with a well-documented or described history of serious allergy to penicillin, in most instances a carbapenem antibiotic can be given. For those with a remote or poorly documented or described penicillin allergy, a cephalosporin antibiotic is often tolerated. Penicillin skin testing, followed by an oral cephalosporin challenge, is useful for determining true immunoglobulin E–mediated allergy and is increasingly available for inpatients in acute care hospitals.

TABLE 103.5 Recommended Empirical Therapy for Adult Native Joint Bacterial Arthritis

GRAM STAIN	PREFERRED ANTIBIOTIC[a]	ALTERNATIVE ANTIBIOTIC
Gram-positive cocci	Vancomycin, 15–20 mg/kg (ABW) daily every 8–12 h[b]	Daptomycin, 6–8 mg/kg daily[c] *or* linezolid, 600 mg IV or PO every 12 h[c]
Gram-negative cocci[d]	Ceftriaxone, 1 g every 24 h	Cefotaxime, 1 g every 8 h[e]
Gram-negative rods[f]	Ceftazidime, 2 g every 8 h *or* Cefepime, 2 g every 8 h *or* Piperacillin-tazobactam, 4.5 g every 6 h	Carbapenem[g,h] (preferred alternative) *or* Fluoroquinolone[i] Aztreonam, 2 g every 8 hr
Gram-stain negative[f]	Vancomycin *plus* Ceftazidime *or* Cefepime *or* Piperacillin-tazobactam	Daptomycin[c] *or* linezolid[c] *plus* Carbapenem[g,h] (preferred alternative) *or* Fluoroquinolone[i] Aztreonam

[a]Unless noted otherwise, dosages are IV for persons with normal renal function.
[b]Therapeutic monitoring should target a serum area under the concentration-time curve to minimal inhibitory concentration of 400 to 600 mg-h/L.
[c]See text discussion under "Directed Therapy for MRSA Infections."
[d]Equivocal gram-negative morphology should be considered as gram-negative rods.
[e]Gram-negative cocci with epidemiology or history suggestive of gonococcal infection should be initially treated per Centers for Disease Control and Prevention Sexually Transmitted Disease Guidelines. Alternative therapies have not been suggested for patients with a history of a Stevens-Johnson syndrome or severe IgE-mediated allergy to β-lactam antibiotics. Possible empirical options for penicillin-allergic patients, pending culture sensitivities, include azithromycin, ciprofloxacin, tobramycin, gentamicin, and spectinomycin (not available in the United States).
[f]For patients with risk factors for resistant gram-negative pathogens (significant health care exposure, immunosuppression, or recent history of extended-spectrum β-lactamase gram-negative infection or colonization), drug selection should consider regional or local antibiograms.
[g]Imipenem, 500 mg every 6 h; *or* meropenem, 1 g every 8 h.
[h]Usually reserved for patients with penicillin allergy or risk factors for resistant gram-negative pathogens (significant health care exposure, immunosuppression, or history of extended-spectrum β-lactamase gram-negative infection or colonization).
[i]Ciprofloxacin, 400 mg IV every 8 h or 750 mg PO every 12 h *or* levofloxacin, 750 mg IV or PO every 24 h.
ABW, Actual body weight; *IgE,* immunoglobulin E; *IV,* intravenous; *PO,* by mouth.

Antimicrobial deescalation. Empirical treatment should be narrowed or deescalated based on the identity and antimicrobial susceptibility of bacteria identified in synovial fluid, blood, or, in some cases, ancillary cultures or test (e.g., mucosal culture or NAAT for gonococci). For cases where MSSA is isolated, therapy should be narrowed to an antistaphylococcal penicillin (e.g., nafcillin/oxacillin) or first-generation cephalosporin (e.g., cefazolin). Although ceftriaxone is an attractive option for *S. aureus* osteoarticular infections because of once-daily dosing, tolerability, and lower cost, it should be noted that it is intrinsically less active than standard antistaphylococcal agents, and clinical evidence is limited to support its use for these infections. A small retrospective cohort study that compared ceftriaxone with oxacillin for treatment of osteomyelitis (124 patients) and septic arthritis (57 patients) did demonstrate similar treatment outcomes for both groups.[249] There were fewer patients who required discontinuation of therapy because of toxicity in the ceftriaxone group. A smaller study comparing ceftriaxone with cefazolin also found similar efficacy.[250] However, until more evidence is available, the use of ceftriaxone for *S. aureus* septic arthritis remains controversial and is not recommended.

Directed therapy for MRSA infections. For MRSA joint infections, vancomycin should be continued and therapeutic serum concentration monitoring performed to achieve an area under the concentration-time curve (AUC) to MIC ratio of 400 to 600 mg-h/L.[251]

Linezolid and daptomycin are an alternative for patients with MRSA native joint infection. In prior clinical practice most clinicians have reserved their use for patients with cultures yielding vancomycin-intermediate *S. aureus;* vancomycin-resistant enterococci; or who are allergic to, intolerant of, or not clinically responding after 3 to 5 days of vancomycin. Published experience with daptomycin in bone and native joint infection is mostly limited to case reports and open-label or retrospective, observational, and post hoc studies.[252–256] However, one nested case-control study and a single prospective, randomized, controlled trial of daptomycin in osteoarticular infections demonstrated safety and similar efficacy as comparator antibiotics.[256,257] Of note, Infectious Diseases Society of America guidelines for prosthetic joint infection suggest dosing daptomycin at 6 mg/kg/day.[258] Recent publications, however, suggest that a dose of 8 mg/kg/day may be more appropriate without increased adverse events.[259,260] In recent years daptomycin usage has increased in outpatient parenteral antimicrobial therapy (OPAT) for bone and joint infections that are confirmed or suspected to be due to gram-positive pathogens. Factors likely responsible for this increased usage include convenience factors, such as once-daily dosing, lack of requirement for serum concentration monitoring and subsequent dose adjustment, and less adverse events, compared with vancomycin.[261]

Evidence for the efficacy of the oxazolidinones, linezolid or tedizolid, in bacterial arthritis includes observational studies, mostly in prosthetic joint infections and osteomyelitis, and a trial in osteoarticular infections in children.[262–268] Because linezolid is an oral agent with excellent bioavailability, it has appeal for treatment of staphylococcal joint infections. However, significant adverse drug events, including neuropathy, anemia, thrombocytopenia, and lactic acidosis, have somewhat limited its use for the prolonged therapy that is required for these infections.

There is considerable interest in using the long-acting lipoglycopeptides, dalbavancin or oritavancin, for staphylococcal (including MRSA) osteoarticular infections, including native joint septic arthritis.[269] Although expensive, these drugs can be dosed IV once per week, and for MRSA native joint septic arthritis, dalbavancin could be dosed only twice to cover the total duration of therapy.[270] Limited clinical experience suggests therapeutic efficacy for both osteomyelitis and joint infection, but further study is necessary before it can be recommended.[271,272]

Ceftaroline, a broad-spectrum cephalosporin with MRSA activity, is potentially suitable for treatment of septic arthritis, but more data is needed before it can be endorsed.[273] Like linezolid, neutropenia is associated with prolonged therapy with ceftaroline. There is very little evidence to support the use of telavancin or newer tetracycline drugs, including tigecycline, eravacycline, or omadacycline for the treatment of septic arthritis.[274]

There have been reports that the performance of vancomycin diminishes for severe infections caused by MRSA that have an isolate MIC ≥1 mg/L.[252,275,276] Further clinical studies are needed to establish

whether vancomycin in combination with rifampin or alternative drugs should be used for native joint infections caused by these organisms.

Directed therapy for other situations. For patients with a high pretest probability of septic arthritis and negative cultures who are responding to empirical therapy, the initially chosen antimicrobials should be continued to complete a full treatment course. Infections caused by animal or human bites should be treated with antimicrobials that have activity against aerobic and anaerobic oral flora, for example, ampicillin-sulbactam or amoxicillin-clavulanate, and in the penicillin-allergic patient, clindamycin plus ciprofloxacin or moxifloxacin. Gonococcal arthritis is treated initially with ceftriaxone, 1g IM or IV once every 24 hours or, alternatively, with cefotaxime or ceftizoxime, 1 g IV every 8 hours, in addition to azithromycin 1 g orally in a single dose. After improvement, oral "step-down" therapy may be substituted. The emergence of resistance to fluoroquinolones, and more recently to cefixime, limit the use of these agents for oral therapy unless culture isolates are available and sensitivity is demonstrated.[277]

Duration of therapy. The duration of therapy for septic arthritis has not been studied in controlled trials. Although therapy should be long enough to eradicate all foci of infection and thus prevent recurrence and further joint disability, the duration does not have to be as long as what is usually given for osteomyelitis. Potential variables that likely influence the duration of therapy include the particular joint involved, identity of the infecting pathogen, and host comorbidity and immune function. Traditionally, parenteral therapy for 2 to 4 weeks is prescribed, although if fluoroquinolones or linezolid (or tedizolid) are used, IV therapy can be converted to oral therapy early in the treatment course. Infections caused by *S. aureus*, including MRSA, and gram-negative bacilli generally require 4 weeks of treatment. Besides linezolid (or tedizolid), choices for oral MRSA step-down therapy after 2 to 3 weeks of parenteral therapy include trimethoprim-sulfamethoxazole (TMP-SMX), doxycycline or minocycline, or clindamycin, depending on susceptibility. Disseminated gonorrhea or gonococcal arthritis requires 1 week of treatment.

Perhaps shorter courses of parenteral antibiotics would still be effective in selected patients. A retrospective study of adult patients showed that rates of recurrent infection and sequelae of infectious arthritis did not differ among patients who received 7 days of parenteral antibiotics, 8 to 21 days, or greater than 21 days, as long as successful drainage was performed.[278] In the same study patients who had 14 days or less of total antibiotic treatment, which included initial parenteral therapy followed by oral therapy, had no difference in recurrence rates or sequelae compared with those that received longer treatment courses. Risk factors for recurrence of infection in the study included immune suppression and the lack of surgical intervention. A second retrospective study of 14 days of antimicrobial therapy for pyogenic arthritis of the fingers and wrist reported good outcomes if surgical débridement was adequate.[279] Although it is difficult to recommend a duration of antibiotic therapy based on these findings, it is reasonable to assume that a shorter duration of therapy in an immunocompetent patient who has undergone an adequate drainage procedure might be appropriate. Clinical trials to conclusively answer the question of optimal duration of therapy are needed.

Adjuvant Therapy

Current research is examining whether adjuvant therapy directed at the attenuation of microbial virulence factors or the host inflammatory response is able to reduce the sequelae of bacterial joint infection.[24,50] There is growing evidence that adjuvant early, short-course, systemic corticosteroid treatment for children with hematogenous bacterial arthritis significantly reduces the duration of symptoms, hospitalization, antibiotic therapy, and residual joint dysfunction.[280–283] Widespread application of these findings to clinical practice is pending upcoming guideline recommendations and studies in other populations of patients. Animal studies have examined TNF-α antagonists, IL-12, recombinant IL-10, and bisphosphonates as adjuvant treatments with some benefits.[24,148] Although these approaches look promising, further research is needed.

VIRAL ARTHRITIS

Arthritis or arthropathy caused by viral agents is often acute, occurs concurrently with signs and symptoms of febrile systemic illness, and resolves along with other manifestations of illness. Viruses may cause arthritis directly by infecting the synovium or indirectly through host immune-mediated responses, and much of the pathogenesis is poorly understood.[284,285]

Parvovirus B19

Perhaps the most common form of viral arthritis in developed countries is caused by human parvovirus B19. In children human parvovirus B19 infection arises with fever, rash, coryza, headache, and malaise.[286] The rash classically arises with bright red "slapped checks" and a lacy, reticular exanthem on the torso and extremities. Arthritis occurs in less than 5% of afflicted children and tends to be asymmetrical and pauciarticular, and it predominantly affects the knee.[287,288]

Adults who become infected with human parvovirus B19 do not display the classic facial erythema noted in children and present with a febrile, flulike illness with prominent polyarthralgia and joint inflammation accompanied by an absence of reticulocytosis.[284,287,289] Cases are usually seen in late winter and early spring, and regional and nosocomial outbreaks of disease have been reported.[290–293] An associated exanthem, if apparent, is a fleeting and difficult-to-appreciate rash on the torso and, less often, on the extremities, which may show some of the reticular aspects exhibited by children (Fig. 103.5). Arthritis or prominent arthralgias are more common in females than men with human parvovirus B19 infection, affecting 60% and 30% of individuals, respectively.[287,294] Characteristically, an acute and moderately severe, symmetrical polyarthritis of the proximal interphalangeal and metacarpophalangeal joints is associated with morning stiffness and somewhat resembles RA.[286,287,295] Knees, wrists, and ankles may also be involved. In approximately one-third of patients, particularly women, joint symptoms occur in episodic flares with symptom-free intervals.[286] A biphasic illness may manifest in some patients with an acute febrile illness that is separated from rash and joint symptoms by several days.[296] Diagnosis is made clinically, and a positive serologic or peripheral blood PCR test supports the diagnosis, although false-positive and -negative results occur with both tests.[284,285,297]

Although joint symptoms usually resolve within 2 weeks, persistent polyarticular arthritis may follow acute human parvovirus B19 infection in up to 20% of female patients and, in some individuals, may last up to several months, occasionally longer.[284,286,298] In such patients joint erosions and objective evidence of synovial inflammation do not develop,[286] and follow-up studies do not demonstrate long-term joint swelling or restricted range of motion consistent with true chronic arthritis.[299] Treatment of chronic human parvovirus B19 arthritis is supportive with nonsteroidal antiinflammatory drugs (NSAIDs), and, except in exceptional cases, IV immunoglobulin is not indicated.[284,300] The role of human parvovirus B19 in the pathogenesis of RA or systemic lupus erythematosus remains circumstantial and inconclusive.[285,287,301]



FIG. 103.5 Faint, lacy reticular rash of the proximal lower extremities characteristic of adult human parvovirus B19 infection.

Alphaviruses, Including Chikungunya

Several alphavirus infections are associated with arthritis and are distributed worldwide, particularly in tropical and subtropical regions.[285,302,303] These arboviruses usually cause sporadic and endemic disease; however, viral mutation or an increased prevalence of their mosquito vectors may result in periodic explosive outbreaks of fever, rash, and arthritis.[284,303,304] In 2005 an outbreak of chikungunya virus infection emerged in the islands of the Indian Ocean, afflicting more than 300,000 persons over a 2-year period before extending to South and Southeast Asia, followed by Oceania, where it was responsible for millions of additional suspected cases.[303–306] Subsequently the virus quickly spread into the New World, and cases were reported in the Caribbean in 2013, followed by South America the following year.[303] The ensuing outbreak in the Western Hemisphere peaked in early 2016 with millions of additional cases occurring over the prior 2-year period. These included several imported and a handful of locally acquired cases in the continental United States.[307] Since 2016 the virus has remained endemic throughout the tropics and subtropics worldwide.

Chikungunya virus is transmitted by the bite of the daytime-biting *Aedes* spp. mosquitoes. The presence of the mosquito vector, *Aedes albopictus*, in Europe and the Americas, together with returning travelers with extremely high levels of viremia, is a public health concern in that infection could be easily established in these previously nonendemic subtropical regions.[304,306,308,309]

Chikungunya fever is characterized by an acute biphasic febrile illness, with the initial phase manifesting headache, malaise, myalgias, symmetrical arthralgias, or polyarthritis, and in approximately 50% of cases, a skin rash (Fig. 103.6).[302–305,308,309] Joint symptoms are often quite severe (chikungunya means "that which contorts or bends up"), affecting predominantly the wrist, ankles, and small joints of the hands and feet. After 5 to 7 days of fever and a brief interlude of improved symptoms, a polyarthritis with or without tenosynovitis often recurs and may be disabling.[303,304,308,310] Some cases may resemble dengue fever or Zika virus infection, both of which are also highly endemic in regions where chikungunya fever occurs. Similar to infection caused by human parvovirus B19, a chronic form of chikungunya virus–associated arthritis has also been described.[311,312] A meta-analysis including 6532 patients with chikungunya virus arthritis showed that 43% did not fully recover after 3 months, and 21% were still symptomatic within 1 year. In many patients symptoms persisted for more than 28 months.[311] Up to one-fourth of those afflicted rated their symptoms as severe. Older age, female gender, and symmetrical joint involvement during the acute phase were associated with persistence of joint symptoms. In addition, the level of acute chikungunya viremia may also be a factor.[313] A different experience has been reported from a rheumatology clinic in the Dominican Republic, where the mean duration of symptoms was 2.5 months, and 89% of 457 patients presenting with chikungunya virus infection responded well to NSAIDs, although 72% were treated with low-dose glucocorticoids (e.g., 5–7.5 mg of prednisone daily). RA patients, all of whom had been on DMARDs, including biologic agents such as TNF-α antagonists, rituximab, or tocilizumab, did equally well.[314]



FIG. 103.6 Erythematous maculopapular rash with islands of normal skin characteristic of chikungunya virus infection. *(From Hochedez P, Jaureguiberry S, Debruyne M, et al. Chikungunya infection in travelers.* Emerg Infect Dis. *2006;12:1565–1567.)*

Diagnosis of chikungunya fever–associated arthritis is based on clinical, epidemiologic, and laboratory criteria. Tests of acute and convalescent serum with PCR and indirect immunofluorescence and enzyme-linked immunosorbent assay serology are most useful.[303–305,308,309] Other arboviruses that cause alphaviral arthritis include o'nyong-nyong, igbo-ora, Ross River, Barmah Forest, Sindbis, and Mayaro viruses.[302]

Other Causes of Viral Arthritis

Rubella is another important cause of viral arthritis in adults, particularly in the developing world, where childhood vaccination is not widespread. Joint inflammation may follow natural infection or immunization with live-attenuated vaccine.[284,315] Arthritis is thought to occur via direct infection of joint synovial tissue, although immune complexes may also play a role.[285,316,317] As with human parvovirus B19, the polyarthritis that is associated with rubella affects predominantly women and most frequently involves the small joints of the hands and, less commonly, the knees, wrists, and ankles.[285,318] Joint symptoms occur simultaneously or within a few days of the rash in 52% of adult female subjects with infection by wild-type virus and within 2 to 3 weeks in 14% of adults receiving rubella vaccine.[285,318] A placebo-controlled, randomized trial showed that 30% of seronegative, adult women receiving rubella vaccine developed acute arthropathy after vaccination, as opposed to 20% of those receiving placebo, a significant difference.[319] Arthritis after rubella vaccination or natural infection may persist for several weeks, but chronic or recurring arthritis is unusual.[315,317,320] Treatment is supportive, with NSAIDs or low-to-moderate doses of corticosteroids.[284]

In acute hepatitis B infections severe arthralgia or arthritis may be seen during the febrile prodrome before the onset of jaundice and is often seen together with urticaria.[284,321,322] A symmetrical, polyarticular arthritis typically involves the hands, wrists, knees, or ankles and resolves with the onset of jaundice. The clinical findings may resemble a RA syndrome in the acute infection period, and a positive rheumatoid factor may be seen in 40% of patients.[321] Although an association between chronic hepatitis B and RA has been hypothesized, sound evidence for this is lacking.[321,323]

Arthritis in the setting of hepatitis C virus (HCV) infection is poorly described.[284,321] Joint symptoms are noted in up to 50% of HCV-infected patients, although only 2% to 4% have frank arthritis.[284,321] It is controversial whether HCV infection is associated with a distinct inflammatory arthritis syndrome, although there is growing evidence that a rare nonerosive inflammatory arthritis mimicking RA may occur.[284,321] In chronic hepatitis B– or hepatitis C–induced essential mixed cryoglobulinemia, arthralgias, and fibromyalgia are described as occurring in approximately one-third of patients, although frank arthritis is not usually observed.[321,324,325]

Both HIV-1 and human T-cell leukemia virus 1 (HTLV-1) are retroviruses with the ability to cause arthritis or arthropathy.[284,326] Nonspecific joint pain is common in the setting of HIV infection, although true articular manifestations of HIV-1 are rare and include chronic arthralgia and oligoarticular arthritis.[284,327,328] An increased incidence of reactive and psoriatic arthritis has been observed in patients with HIV, particularly among MSM.[284,327] Some elderly patients in Japan infected with HTLV-1 have been described with a chronic joint infection termed HTLV-1–associated arthropathy, which closely resembles RA (see Chapter 168).[326,329]

Musculoskeletal complaints in survivors of Ebola virus infection is common, with 70% complaining of myalgia and arthralgia 3 weeks after hospital discharge.[330,331] Longer-term joint complications in these patients may include symmetrical polyarticular arthralgia and arthritis, enthesitis, and tendon rupture. Overt joint inflammation is usually not apparent.

CHRONIC INFECTIOUS ARTHRITIS

Chronic infectious arthritis consists of a constellation of monoarticular or, less commonly, oligoarticular joint infections that are characterized

TABLE 103.6 Infectious Causes of Chronic Monoarticular or Oligoarticular Arthritis

Bacterial
Borrelia burgdorferi
Tropheryma whipplei
Treponema pallidum
Nocardia spp.
Fungi
Candida spp.
Cryptococcus neoformans
Blastomyces dermatitidis
Coccidioides spp.
Paracoccidioides brasiliensis
Sporothrix schenckii
Aspergillus spp. and other molds, including *Rhizopus, Scedosporium,* and *Fusarium*
Mycobacteria
Mycobacterium tuberculosis
M. kansasii
M. marinum
M. avium-intracellulare complex
M. terrae
M. fortuitum, M. chelonae, M. abscessus
M. haemophilum
M. leprae
Parasites
Helminths
Filariae

by an insidious onset and indolent course, a paucity of symptoms, and progressive joint destruction that may result in considerable loss of articular function. Many of these infections arise with few symptoms and signs of joint inflammation other than subacute or chronic joint swelling, with or without effusion, and pain and stiffness during active range of motion. Although this form of infectious arthritis remains relatively uncommon compared with the incidence of acute septic arthritis, it is a significant component of the differential diagnosis of subacute or chronic joint inflammation. An increasing number of anecdotal reports indicate that it is an emerging problem in immunocompromised or chronically ill hosts. Noteworthy aspects of this entity include its ability to mimic other inflammatory joint disorders, such as RA, and its ability to arouse little clinical suspicion, resulting in considerable delays in diagnosis. Moreover, establishing a pathogen-specific diagnosis is difficult, and the response to treatment is slow and often incomplete. Inappropriate treatment—for example, systemic or intraarticular steroids for a tentative diagnosis of RA—is not uncommon and can further delay diagnosis and/or lead to more rapid or severe joint destruction. Subacute or chronic infectious arthritis is usually caused by mycobacteria or fungi, and occasionally by bacteria, such as *B. burgdorferi, T. whipplei, Treponema pallidum* (tertiary syphilis),[332] and *Nocardia* (Table 103.6).[333] Chronic arthritis caused by parasitic infection is rarely described with various helminths and filiariae, despite the large number of persons infected with these parasites each year.[334]

Fungal Arthritis

Fungal arthritis may sometimes occur in normal hosts; however, in immunocompromised or chronically ill persons, a steadily increasing frequency of infection and diversity of infecting mycotic pathogens is apparent.[335,336] Although there is some overlap between the two groups, the most common fungal pathogens isolated from infectious arthritis in healthy hosts residing in endemic regions for the dimorphic fungi are *Blastomyces dermatitidis, Coccidioides* spp., and *Sporothrix* spp., whereas in immunocompromised hosts, *Candida* spp., *Cryptococcus,* and *Aspergillus* or other molds are more often observed.[335–337] However, pulmonary and disseminated infections caused by the dimorphic fungi are increasingly diagnosed in patients with RA or IBD on TNF-α inhibitor therapy, and these drugs are likely a risk factor for chronic fungal arthritis.[338,339] Joint infection from fungal pathogens usually results from hematogenous dissemination of the organism and, except for *Candida,* has as its source a symptomatic or subclinical respiratory tract infection. On occasion, a fungus, usually a mold, is introduced into the joint via direct trauma or injury, sometimes associated with a penetrating foreign body.[336,340,341]

Candida Arthritis

Candida arthritis afflicts both native and prosthetic joints and has been increasing in frequency over the last 2 decades, parallel to the rising incidence of systemic and focal infections caused by this organism in hospitalized and chronically ill patients.[342–344] Risk factors for joint infection with this organism include loss of skin integrity, diabetes mellitus, hemodialysis, malignancy, malnutrition, premature birth, IVDU, immunosuppressive therapy (including corticosteroids), and the prolonged use of broad-spectrum antimicrobials, hyperalimentation fluid, and central IV catheters.[335,343–345] In most cases articular infection occurs through the hematogenous route, with normal joints typically afflicted in a monoarticular pattern, although polyarticular infection occurs in up to 30% of cases.[344] Infection can also occur by direct inoculation of the joint through surgery or corticosteroid injection, particularly in arthritic or surgically altered joints.[335,344,346] Patients are often heavily colonized by *Candida* at skin, gastrointestinal, or mucosal sites before joint infection. *Candida albicans* is the most commonly isolated species,[343,344] followed by *C. tropicalis*, and *C. parapsilosis*. Other less commonly cultured species include *C. glabrata, C. krusei, C. guilliermondii, C. zeylanoides, C. lambica, and C. lusitaniae.*[335,343,346–351] As in bacterial arthritis, the knee is affected in the vast majority of cases and less often the hip or other joints.[343,344] In IV drug abusers fibrocartilaginous joints, including the sternoclavicular, sacroiliac, and costochondral, may be involved. In addition, they may manifest with associated *Candida* endophthalmitis, and folliculitis of the face, scalp, and torso.[352] *Candida* arthritis may arise acutely, with complaints of marked joint inflammation and associated fever and constitutional symptoms, especially in cases accompanied by systemic candidiasis or, more insidiously, with chronic indolent joint pain and stiffness and minimal systemic manifestations.[344,346] In the latter case a long latent period is often seen between an episode of apparent or suspected occult candidemia and the onset of joint complaints. Extension of infection from the joint to adjacent bone is not uncommon.[335] Synovial fluid analysis characteristically shows polymorphonuclear leukocytosis with cell counts greater than 25,000 cells/mm^3 and a normal or low measured glucose. Gram stain of synovial fluid is not usually diagnostic, although bacterial or fungal culture yields *Candida* spp. in most cases. Blood cultures are positive in less than 30% to 50% of patients.

Cryptococcus Arthritis

Cryptococcus neoformans causes skeletal infections in less than 10% of patients with disseminated infection with this organism.[353–355] Cryptococcal arthritis is considerably less common than cryptococcal osteomyelitis, and the latter is frequently the nidus of infection for the former through local contiguous spread. The majority of patients with joint infection caused by this organism are immunocompromised because of various conditions, including acquired immunodeficiency syndrome.[355,356] The presentation of cryptococcal arthritis and associated osteomyelitis is that of a chronic joint infection, most often involving the knee.[335,355] A serum cryptococcal antigen test is often positive.[354] Diagnosis requires joint aspiration or synovial biopsy with appropriate synovial fluid or tissue fungal cultures. Of importance, with rare exceptions, osteoarticular infection represents disseminated cryptococcal disease; thus coexisting infection of the central nervous system should be ruled out.

Arthritis Due to Dimorphic Fungi

Acute infection caused by *Coccidioides immitis* or *Coccidioides posadasii* occurs in the southwestern region of the United States, northern Mexico, and a few regions of Central and South America, causing a symptomatic or subclinical primary pneumonitis. Migratory polyarthralgia and polyarticular arthritis occur in approximately one-third of these patients, manifesting as a self-limited hypersensitivity syndrome termed "desert rheumatism" or "valley-fever."[336] In about 1% of persons with coccidioidomycosis, dissemination occurs, and although any host may be afflicted, it is more likely in men, pregnant females, Blacks, Filipinos,

and immunocompromised persons, including patients with HIV infection.[357,358] Skeletal involvement occurs in approximately 30% of such cases and results in a chronic granulomatous infection of bones, joints, and periarticular tissues.[335,337,357] Joint infection may be primary or secondary to extension from contiguous osteomyelitis. Weight-bearing joints, particularly the knees and ankles, are most frequently affected, but any joint may be involved.[337,357,359] Presentation is that of subacute or chronic monoarticular or polyarticular arthritis that may progress either slowly or quite rapidly, resulting in extensive joint destruction.[357,360,361] Other manifestations of disseminated infection are not uncommon at the initial evaluation.[357] Peripheral eosinophilia may be present, and synovial fluid leukocyte counts are moderately elevated, with a lymphocytic predominance. Coccidioidal arthritis should be considered in patients presenting with chronic progressive arthritis in endemic areas. Diagnosis is suggested by an elevated titer against coccidioidal antigens on serologic testing of serum, or visualization of spherules in synovial fluid. Confirmation is by fungal culture of synovial fluid and histopathologic examination and culture of synovial or periarticular tissues.[362] In suspected cases of coccidioidal arthritis the microbiology laboratory should be alerted before sending specimens for culture because *Coccidioides* spp. are important biohazards in the clinical laboratory. Once isolated, shipping the isolate for confirmation of identity is complicated because the organism is a potential agent of bioterrorism, and its transport is strictly regulated.[363]

Similar to coccidioidomycosis, blastomycosis is primarily a pulmonary infection that may later disseminate, potentially involving the bones and joints. Skeletal infection caused by *Blastomyces dermatitidis* occurs in about one-fourth of patients with disseminated infection, and joint infection in most cases is associated with juxtaarticular osteomyelitis.[364] Monoarticular arthritis of the knee is most common, followed by the ankle, elbow, wrist, and hand.[335] Polyarticular infection is extremely rare.[365,366] An acute arthritis with abrupt onset and accompanying systemic symptoms of inflammation and a more chronic joint infection are both described.[365,367] Concomitant blastomycosis pneumonia and nodular or ulcerative skin lesions are frequently evident on examination, and periarticular draining sinus tracts are occasionally seen.[336] Synovial fluid is purulent, and organisms are readily demonstrated on cytologic examination or microscopic examination of a wet mount smear.[368] Cultures of synovial fluid or other affected locations will usually confirm the diagnosis.

Histoplasma capsulatum is an extremely rare cause of fungal arthritis in endemic areas and is usually associated with immunosuppression. A chronic monoarticular arthritis of native and prosthetic knee joints has been described.[369,370] In addition, a hypersensitivity syndrome with rash and arthralgia may accompany *Histoplasma* primary infection, which is similar to that in coccidioidomycosis.[371,372]

Sporotrichosis is a chronic infection caused by *Sporothrix* spp. that primarily affects the skin and secondarily the lymphatics after percutaneous inoculation by a contaminated foreign body that comprises plant or soil material (see Chapter 259 for new *Sporothrix* spp. names). In contrast to the other endemic mycoses, primary pulmonary infection is uncommon. Osteoarticular sporotrichosis comprises approximately 80% of extracutaneous infections and results from hematogenous dissemination rather than from direct joint inoculation. On occasion, hematogenous spread to the skin occurs at the same time. The portal of entry is usually not apparent, although a pulmonary portal is sometimes evident. At most, only 0.3% of patients with cutaneous sporotrichosis will develop arthritis.[335,373] Immunocompromised, diabetic, and alcoholic patients are at higher risk for disseminated infection and arthritis,[374–376] and the typical patient is a male involved in gardening, farming, or an outdoor occupation.[377,378] Chronic monoarticular or polyarticular sporotrichosis arthritis arises in the knees, wrists, hands, elbow, and ankle, and a tenosynovitis has also been reported.[335,378,379] Symptoms and signs are more prominent in the small joints of the wrist and ankle. Fever is uncommon, and there are usually no other focal symptoms or signs of infection, including pulmonary or cutaneous, before or during the course of arthritis.[376,378] Diagnosis is difficult because organisms are rarely found on histologic examination of joint fluid or tissue, and fungal culture of these specimens is often negative. Multiple aspirations and biopsy of affected joints may be necessary. As a result, extremely long diagnostic delays and permanent joint sequelae are common.[380]

Arthritis Due to Molds

Molds, including *Aspergillus,* are an exceedingly rare cause of septic arthritis, with the majority of cases having been reported in association with disseminated infection in immunocompromised hosts or introduction by trauma.[381,381a] One review described 31 cases of *Aspergillus* septic arthritis and noted the knee and hips to be the most frequently infected joints.[381a] Many patients had oligoarticular involvement due to hematogenous seeding. *Aspergillus fumigatus* constituted 77% of cases, followed by *A. flavus* in 13% and *A. niger* in 3%. Osteolysis and tissue extension were common. Synovial fluid WBC count varied considerably, with a median of 17,200 cells/mm^3. Prognosis was poor, with an overall survival of 65%.

Fungal arthritis caused by *Fusarium* spp., *Curvularia* spp., *Exophiala* spp., *Trichosporon asahii, Lomentospora (Scedosporium) prolificans, Scedosporium apiospermum* (*Pseudallescheria boydii*), and Mucorales have all been described and may be seen in either immunocompromised or immunocompetent patients.[382–387] A review of 43 years of reports of non-*Aspergillus* mold osteoarticular infections found antecedent trauma in children and prior surgery in adults as risk factors.[388] Hyaline molds were the most common agents reported, among which *S. apiospermum* and *L. prolificans* were most frequent. All of these pathogens usually cause severe joint infections that respond poorly to antifungal therapy.

Bone and joint infection caused by *Aspergillus* spp. and *Rhizopus* spp. have complicated arthroscopic anterior cruciate ligament reconstruction in young healthy persons.[389,390] Mold infections after intraarticular steroid injections in immunocompetent hosts have also been reported.[381,391] In the fall of 2012 a multistate outbreak of fungal infection associated with the injection of contaminated methylprednisolone acetate from a single compounding pharmacy resulted in several cases of fungal meningitis, vertebral osteomyelitis, epidural abscess, and peripheral joint infection. Altogether, there were 33 cases of peripheral joint infection caused by *Exserohilum* spp., some of which presented several months after joint injection with contaminated steroid.[34,143,392]

Therapy for Fungal Arthritis

A combined approach to therapy for fungal native joint arthritis, using medical and surgical modalities, is typically necessary for optimal results. Open or arthroscopic débridement and drainage should be performed, at which time tissue and fluid specimens can be obtained for histopathologic and microbiologic testing. However, when a diagnosis of cryptococcal, or perhaps *Sporothrix* or coccidioidal, joint infection has already been established, drainage and débridement are often not necessary.[393,394]

Historically, most of the clinical experience with therapy for fungal arthritis was with amphotericin B, with or without the addition of flucytosine. In the past 2 decades fluconazole, newer extended-spectrum triazoles (itraconazole, voriconazole, posaconazole, and isavaconazole), terbinafine, and the echinocandin-class antifungals (caspofungin, micafungin, and anidulafungin) have expanded the therapeutic options available for many fungal pathogens.[395–400] In addition, lipid preparations of amphotericin B have modestly reduced the toxicity of this drug, increasing the safety of long-term amphotericin therapy.

Treatment guidelines for native joint *Candida* arthritis are based on case reports and open-label series. Antifungal susceptibility testing should be performed on all culture isolates. Recommendations include fluconazole, 400 mg (6 mg/kg) daily for at least 6 weeks or an IV echinocandin daily for at least 2 weeks, followed by fluconazole to complete therapy.[401] Alternatively, a lipid formulation of amphotericin B, 3 to 5 mg/kg/day, can be used in place of the echinocandin, although the risk of nephrotoxicity makes this choice less appealing. Courses of therapy longer than 6 weeks are frequently necessary. Fluconazole, if used, may be given orally. Echinocandins are the preferred therapy for *C. glabrata* and *C. krusei* joint infections. Oral voriconazole can be considered for step-down therapy for septic arthritis due to *C. krusei,* as most isolates are susceptible to this drug.[401–403] The incidence of *Candida* resistance to echinocandins is quite low, whereas the proportion demonstrating azole resistance depends on the species.[402,404] Surgical

débridement, sometimes multiple procedures, is indicated in all cases of *Candida* arthritis.[401] Successful eradication of *Candida* joint infection is best confirmed by posttreatment synovial fluid analysis and culture.

For cryptococcal arthritis the choice of treatment depends on the extent of disseminated disease and the immune status of the patient. In general, initial therapy with an amphotericin B preparation plus/minus flucytosine, followed by oral fluconazole, is appropriate, but in nonimmunocompromised patients with isolated septic arthritis, treatment with fluconazole, 400 mg daily for 6 to 12 months, may be appropriate.[393]

The preferred treatment of isolated joint infection without central nervous system involvement caused by the endemic mycoses, including *Coccidioides, Blastomyces, Histoplasma,* and *Sporothrix*, is with itraconazole, 200 mg twice daily for at least 12 months, unless the patient has limb-threatening skeletal disease, in which case an induction course of an amphotericin B preparation is advised.[394,405–407] Measurement of serum itraconazole levels is suggested during therapy. Coccidioidal joint infection can also be treated with high doses of fluconazole (400–800 mg daily). With either drug, a longer duration of therapy may be necessary.[406] Available evidence suggests that, for these pathogens, therapy of arthritis with itraconazole is equivalent to that with amphotericin B. The role of voriconazole, posaconazole, and isavuconazole is unclear in the treatment of the endemic mycoses.

For bone and joint infection caused by *Aspergillus,* aggressive surgical intervention is recommended. Voriconazole does appear to be effective for therapy, and most experts recommend it preferentially to amphotericin B preparations.[381,408] Other agents with activity include posaconazole, isavuconazole, and itraconazole, but there is little experience using these agents for *Aspergillus* arthritis. Unfortunately, although still unusual in the United States, intrinsic and acquired resistance to voriconazole and other azole antifungals may be emerging in *Aspergillus* spp.[409] Treatment recommendations from the CDC for empirical coverage of contaminated steroid injection–related *Exserohilum* spp. osteoarticular fungal infections that do not involve the spine are for voriconazole alone for less severe infections and a lipid formulation of amphotericin B, in addition to voriconazole, for more severe infections.[410] The use of combination therapy with voriconazole and caspofungin in fungal arthritis caused by *Aspergillus* has been described, but the role of this remains unclear.[381]

Mycobacterial Arthritis

Tuberculosis Arthritis

Worldwide, approximately 10% to 11% of extrapulmonary tuberculosis (TB) involves the bone and joints, accounting for 1% to 3% of all TB cases.[411] The incidence is higher in the developing world and is increasing because of the escalating prevalence of HIV disease. In endemic regions of the developing world, TB arthritis is mostly a disease of children and young adults, whereas in other regions, older adults and immunocompromised hosts are predominantly afflicted. Risk factors for TB arthritis include age older than 65 years, female sex, immigration from regions with high TB endemicity, a lower socioeconomic class, incarceration, alcohol abuse, debilitating illness, IVDU, immunosuppressive drug therapy (including corticosteroids and TNF inhibitors), HIV infection, and preexisting joint disease.[411–416] Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculous arthritis have been reported.[417–419]

Clinical Features

Mycobacterium tuberculosis causes a chronic granulomatous monoarticular arthritis that is usually a result of the hematogenous dissemination associated with primary pulmonary TB.[413,415] Articular infection may remain latent for long periods before clinical presentation. It typically involves the knee, hip, and ankle but can involve any joint.[413,416,417,420] The clinical presentation is that of chronic arthritis, indistinguishable from that with other potential infectious or noninfectious causes (Fig. 103.7). Long-standing cases may develop an articular-cutaneous fistula, whereas TB arthritis of the wrist may involve the periarticular tendon sheaths arising with a carpal tunnel syndrome.[420,421] Coexisting pulmonary or other extraarticular infection may be evident, but in more than half of cases it is not.[416,417,420] Fever and other constitutional symptoms of TB are often absent. Interferon-γ release assay or tuberculin skin testing is positive in 85% to 90% of patients.[413,416,422]

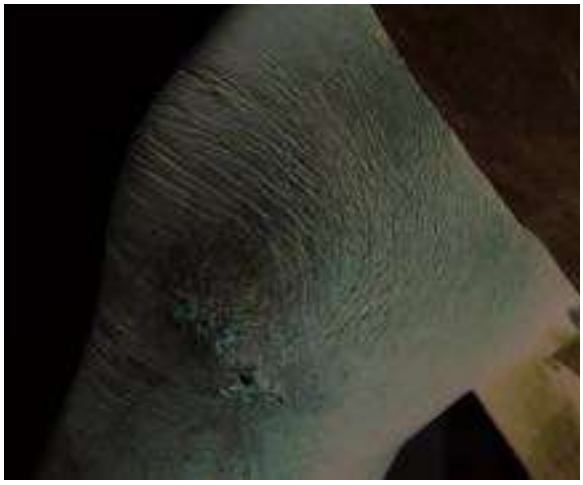

FIG. 103.7 Chronic arthritis of the knee resulting from *Mycobacterium tuberculosis,* exhibiting joint swelling, effusion, and a draining sinus fistula.

Diagnosis

To prevent unacceptable delays in diagnosis and further joint destruction, a high index of suspicion for TB arthritis must be maintained, particularly for patients who are immigrants from regions with high TB endemicity and those with other risk factors for TB. In the absence of known extraarticular TB, diagnosis requires synovial fluid analysis and, in many cases, synovial biopsy. Synovial fluid demonstrates inflammatory changes, with leukocyte counts characteristically between 10,000 cells/mm^3 and 20,000 cells/mm^3, although higher counts in the range seen with bacterial arthritis may be present.[420] Staining of synovial fluid demonstrates acid-fast bacilli in a minority of joint aspirates; however, mycobacterial culture yields *M. tuberculosis* 80% of the time. The most sensitive means of confirming the diagnosis has traditionally been synovial biopsy, in which the characteristic histopathology of caseating or noncaseating granulomas can be correlated with tissue mycobacterial staining and culture, the latter being positive in about 90% of specimens.[420] Mycobacterial susceptibility tests should be performed on all culture isolates.

The use of PCR assay of synovial fluid for the diagnosis of tuberculous arthritis has shown promise and can provide a rapid and minimally invasive diagnosis.[423–425] Sensitivity and specificity in one study was 82% and 91%, respectively, with a positive and negative predictive value of 90% and 84%, respectively. The same study found that of 98 patients with joint TB, 82.7% were positive for TB by PCR assay, 6.1% were positive by acid-fast staining, and 17.3% were positive by culture. These findings highlight the poor performance of traditional techniques when compared with PCR assaying.[423] The limitations of PCR assaying include false-positive tests caused by laboratory contamination and nonavailability in some centers, particularly the developing world. Updated guidelines suggest the off-label use of FDA-cleared NAATs for TB, including PCR, on specimens collected from sites of suspected extrapulmonary TB, including synovial fluid.[426] A positive NAAT result on synovial fluid confirms the diagnosis, but a negative result may not be used to exclude TB.

Imaging findings of TB synovitis consist initially of soft tissue swelling, but later in the disease process, synovial thickening and bone destruction may be seen. The use of MRI may help differentiate tuberculous arthritis from chronic noninfectious forms of joint inflammation, including RA.[427]

Treatment

Current recommendations from the CDC for the treatment of osteoarticular TB in adults without pulmonary TB are identical to that of extrapulmonary TB (including patients with HIV or other immunosuppressive states): isoniazid (INH), rifampin (RIF), ethambutol, and pyrazinamide for 8 weeks (ethambutol may be discontinued in isolates known to be without drug resistance), followed by INH and RIF to complete 6 months of therapy (see Chapter 249).[428] Directly observed therapy is advisable for most patients, and adjuvant corticosteroids are

generally not recommended.[413] If treated early, residual joint disease is uncommon and negligible. Data is scarce regarding treatment of osteoarticular MDR TB. Treatment of MDR and XDR TB relies on susceptibility testing and is changing sufficiently rapidly that guidelines are useful but rapidly become out of date (see Chapter 249).[419] Adjuvant surgical therapy is required only for reconstruction of unstable joints or osseous structures or drainage of periarticular abscesses. Surgical therapy may also be considered in cases where optimal therapy is not possible because of toxicity or resistance.

Nontuberculous Mycobacterial Arthritis

Nontuberculous mycobacterial arthritis, unlike that caused by *M. tuberculosis*, is usually caused by direct percutaneous inoculation of these opportunistic mycobacteria into the skin, joint, or periarticular structures. Soil and water, and rarely air, are the usual sources of these uncommon joint infections. *Mycobacterium avium-intracellulare, M. kansasii, M. marinum, M. xenopi,* and *M. terrae* are the most common causes, although virtually any atypical mycobacteria may be involved.[430–433] Skeletal and joint infection caused by the so-called rapid growers (*M. chelonae, M. fortuitum, and M. abscessus)* may follow trauma or surgery.[431,434–437] These opportunistic pathogens are usually associated with water, including tap water, and thus may invade through large or small breaks in the skin. On occasion, atypical mycobacterial joint infection is acquired through the hematogenous route, usually in immunocompromised hosts, and has been reported with *M. avium-intracellulare, M. haemophilum, M. kansasii, M. fortuitum, M. abscessus,* and *M. chelonae* (see Chapter 252).[438–441]

Chronic monoarticular arthritis and tenosynovitis caused by nontuberculous mycobacteria are well described.[430,431,442,443] *M. marinum* arthritis most often affects the small joints of the hand or wrist and their overlying tendon sheaths.[432,444] Infection is acquired through preexisting or concomitant skin trauma, often minor, during contact with fresh, brackish, or salt water, or with marine animals, such as fish and crustaceans. Diagnosis of atypical mycobacterial joint or tendon infections usually requires biopsy of infected structures for histopathologic examination and culture. If *M. marinum* is suspected, cultures should be incubated at 30°C in addition to 37°C, to facilitate growth of this organism.[442] Treatment varies depending on the identity of the isolated mycobacteria. Susceptibility testing can be performed in reference laboratories and is useful in directing therapy for some of these organisms.

Mycobacterium leprae has also rarely been associated with an inflammatory arthritis that often presents in conjunction with erythema nodosa leprosum (type 2 lepra reaction) (see Chapter 250).[334] This systemic reaction is caused by circulating immune complexes and, in its most severe form, presents with diffuse soft tissue, eye, ear, and nerve swelling with acute polyarthritis, particularly of the hands, wrists, and ankles. In addition, advanced Hansen disease may also be associated with chronic neuropathic (Charcot) arthritis of the peripheral joints.

SEPTIC BURSITIS

Septic bursitis is common, usually affecting the subcutaneous olecranon, prepatellar, or infrapatellar bursae.[445,446] It accounts for 1 to 12 cases per 10,000 hospitalizations and an incidence of 10 per 100,000 persons. Almost all cases are in males, particularly between the ages of 40 to 60 years. Bacteria are most often introduced through trauma or accidental percutaneous punctures and, very rarely, through intrabursal injection of corticosteroids or hematogenous dissemination.[445–449] Septic olecranon bursitis is four times more likely than septic prepatellar bursitis. Infection of deep bursae is rare and is usually secondary to contiguous spread from an adjacent joint infection.

More than 80% of septic bursitis is due to *S. aureus,* with the remainder due to *Streptococcus* spp. and various gram-negative bacteria, mycobacteria, and fungi.[445,447,448,450,451] Olecranon septic bursitis caused by the algal agent *Prototheca* has been reported (see Chapter 268).[445,452,453] Of note, infectious bursitis is more common during the summer months, especially that caused by *S. aureus*.



FIG. 103.8 Septic olecranon bursitis due to *Staphylococcus aureus.*

Patients with superficial bursitis present with painful swelling, redness, and increased warmth of the afflicted bursae (Fig. 103.8).[445,454] Evidence of an overlying cutaneous injury or lesion is often evident. Unlike septic arthritis, range of motion in the joint is not very restricted. However, in moderate or severe cases, pain may be extreme and the range of motion of the underlying joint reduced. Soft tissue edema and erythema may extend along the extremity and circumferentially about the joint. Associated systemic symptoms potentially include fever, chills, and malaise. Noninfectious causes of bursitis, especially that involving the olecranon bursa, can occur in conjunction with several systemic conditions, such as RA, gout, pseudogout, chondrocalcinosis, and pigmented villonodular synovitis, and must be differentiated from septic bursitis.[454] Sterile traumatic bursitis usually involves the prepatellar bursa and presents with a paucity of systemic symptoms.

The diagnosis of septic bursitis is made by aspirating the affected bursae and analyzing the fluid for WBC count and crystals. The WBC count threshold for a presumptive diagnosis of septic bursitis is greater than 1000 cells/mm^3 and is much lower than that for septic arthritis.[454] Gram staining and culture should also be performed. For chronic septic bursitis, mycobacterial and fungal smear and culture are indicated. To distinguish septic olecranon bursitis from acute arthritis of the elbow, it is helpful to evaluate whether pain is worsened by elbow extension versus flexion. Bursal pressures are increased in the latter case. Thus, for patients with septic bursitis, elbow pain is increased with joint flexion, whereas for patients with septic arthritis, synovial pressures are increased during elbow joint extension, and pain is greatest in this position.[448] Arthrocentesis should be performed if there is suspicion of joint involvement. Imaging with MRI or CT, followed by directed needle aspiration, is necessary to evaluate patients for deep bursal infection.

Treatment for septic bursitis includes antibiotics and daily aspiration of the bursae until sterile fluid is obtained.[446,454–457] In some acute cases bursectomy may be required. Antimicrobial selection should be based on bursal aspirate Gram stain as outlined in Table 103.5. Oral antimicrobials with antistaphylococcal activity are initially indicated for mild cases afflicting healthy patients with good access to medical care. Because of the high prevalence of CA-MRSA in many localities, clindamycin, TMP-SMX, and minocycline are appropriate choices because of their reliable activity against this pathogen. For more severe cases, or patients with chronic illness or immunosuppression, IV antimicrobials should be selected. In both cases definitive antibiotic therapy should be selected based on the identity and susceptibilities of cultured bacteria and continued to complete a 14- to 21-day course. The prognosis of prepatellar or olecranon septic bursitis is generally quite good, although recurrences are common and may require bursectomy when infection is quiescent.

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104 Osteomyelitis

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SHORT VIEW SUMMARY

CLASSIFICATION

- Osteomyelitis can develop as the result of contiguous spread from adjacent soft tissues and joints, hematogenous seeding, or direct inoculation of microorganisms into the bone as a result of trauma or surgery.
- The Cierny-Mader classification is based on the affected portion of the bone, the physiologic status of the host, and the local environment.
- The Lew and Waldvogel classification is based on the duration of illness (acute versus chronic), the mechanism of infection (hematogenous vs. contiguous), and the presence of vascular insufficiency.

GENERAL PRINCIPLES

- The diagnosis of osteomyelitis is suspected on clinical grounds. Confirmation usually entails a combination of radiologic, microbiologic, and pathologic tests.
- Cross-sectional imaging modalities, such as computed tomography or magnetic resonance imaging (MRI), are considered standard of care in the diagnosis of osteomyelitis.
- Repeated swab cultures of the same organism from draining wounds and sinus tracts may be of diagnostic benefit, although recovered organisms (except for *Staphylococcus aureus*) may not reflect the true pathogen.
- Surgical culture, bone biopsy, or needle aspiration is best for organism identification.
- Therapy is typically a combination of complete surgical débridement and 4 to 6 weeks of antimicrobial therapy (see Table 104.3).

OSTEOMYELITIS AFTER A CONTAMINATED OPEN FRACTURE

- Contaminated open fractures can lead to the development of osteomyelitis of the fractured bone, typically at the fracture site in 3% to 25% of cases.
- Staphylococci and aerobic gram-negative bacilli are the two most common groups of microorganisms in this setting.
- The hallmark of osteomyelitis after open fracture is nonunion of the fracture site or poor wound healing after wound closure or soft tissue coverage.
- Management of open contaminated fractures typically entails early aggressive wound irrigation and débridement, administration of parenteral and local antimicrobials, fracture fixation, and soft tissue coverage of exposed bone.

VERTEBRAL OSTEOMYELITIS AND SPONDYLODISKITIS

- The origin is hematogenous in most native cases; it may also occur postoperatively or postprocedurally.
- *S. aureus*, coagulase-negative staphylococci, and streptococci are the most common microorganisms encountered in pyogenic vertebral osteomyelitis.
- MRI of the spine is highly accurate in the diagnosis of vertebral osteomyelitis.
- Image-guided aspiration and biopsy is highly specific but lacks sensitivity.
- Most cases can be treated with a 6-week course of parenteral antimicrobial therapy without surgery.

OSTEOMYELITIS IN PATIENTS WITH DIABETES MELLITUS OR VASCULAR INSUFFICIENCY

- Patients typically have osteomyelitis contiguous with an ulcer of the lower extremity.
- The sensitivity of exposed bone or a probe-to-bone test was 60%, and the specificity was 91%.
- This category of osteomyelitis is often polymicrobial.
- Therapy typically requires a combination of broad-spectrum antimicrobial regimen and surgical débridement.

ACUTE HEMATOGENOUS OSTEOMYELITIS

- Acute hematogenous osteomyelitis of long bones occurs mainly in prepubertal children, intravenous drug abusers, and patients with indwelling central catheters.
- The most common recovered microorganism is *S. aureus*.
- Acute hematogenous osteomyelitis of long bones in prepubertal children is typically treated with a 2- to 3-week course of antimicrobial therapy.

CLASSIFICATION

Despite advances in surgical and medical management of osteomyelitis, it is still considered one of the most difficult-to-treat infectious diseases. Progressive destruction of the bone and the formation of sequestra are characteristics of this disease. Osteomyelitis can develop as the result of contiguous spread from adjacent soft tissues and joints, hematogenous seeding, or direct inoculation of microorganisms into the bone as a result of trauma or surgery.[1] When established, microorganisms produce a local inflammatory reaction that promotes bone necrosis and the formation of sequestra. *Staphylococcus aureus,* the most common microorganism recovered in osteomyelitis, preferentially causes this disease by expressing high-affinity adhesins to components of bone matrix that express fibronectin, laminin, collagen, or sialoglycoprotein. There are two major osteomyelitis classification schemes. Cierny and Mader[2] classified osteomyelitis based on the affected portion of the bone, the physiologic status of the host, and the local environment (Table 104.1). This classification lends itself to the treatment and prognosis of osteomyelitis. Stage 1 osteomyelitis typically can be treated with antimicrobial therapy alone, whereas stage 3 disease most often is managed with aggressive surgical débridement, antimicrobial therapy, and delayed orthopedic reconstruction. Lew and Waldvogel[3] classified osteomyelitis based on the duration of illness (acute vs. chronic), the mechanism of infection (hematogenous vs. contiguous), and the presence of vascular insufficiency. In contrast to the Cierny and Mader classification, the Waldvogel classification is an etiologic classification and does not implicate a specific therapeutic strategy.

Because of differences in pathophysiology, diagnosis, and management, long bone osteomyelitis, osteomyelitis resulting from open fractures, vertebral osteomyelitis, osteomyelitis in patients with diabetes mellitus and peripheral vascular insufficiency, acute hematogenous osteomyelitis, and SAPHO (*s*ynovitis, *a*cne, *p*lantar pustulosis, *h*yperostosis, and *o*steitis) syndrome are discussed separately. In addition, osteomyelitis in specific hosts (e.g., sickle cell disease), in unusual locations (e.g., clavicle), and secondary to unusual microorganisms are reviewed.

LESSONS FROM EXPERIMENTAL MODELS

Because of the lack of well-designed prospective clinical trials to guide the management of patients with osteomyelitis, recommendations about management of this disease have been primarily derived from

TABLE 104.1 Staging System of Osteomyelitis

Anatomic Type
Stage 1: Medullary osteomyelitis
Stage 2: Superficial osteomyelitis
Stage 3: Localized osteomyelitis
Stage 4: Diffuse osteomyelitis
Physiologic Class
A Host: Normal host
B Host: Systemic compromise (Bs)
Local compromise (Bi)
Systemic and local compromise (Bis)
C Host: Treatment worse than the disease

Systemic or Local Factors That Affect Immune Surveillance, Metabolism, and Local Vascularity	
SYSTEMIC (Bs)	**LOCAL (Bi)**
Malnutrition	Chronic lymphedema
Renal, hepatic failure	Major vessel compromise
Diabetes mellitus	Small vessel disease
Chronic hypoxia	Vasculitis
Immune disease	Venous stasis
Malignancy	Extensive scarring
Extremes of age	Radiation fibrosis
Immunosuppression	Neuropathy
	Tobacco abuse

From Mader JT, Shirtliff M, Calhoun JH. Staging and staging application in osteomyelitis. Clin Infect Dis. *1997;25:1303–1309.*

experimental animal models, expert opinion, and retrospective cohort studies. Experimental models have been developed mainly to study the pathogenesis and treatment of osteomyelitis and offer a more controlled approach to a heterogeneous disease.[4]

Pathogenesis in Experimental Models

In experimental models, normal bone is highly resistant to infection. Osteomyelitis in this setting can be created only after inoculation of large inocula and the creation of bone necrosis, which can result from bone trauma or surgery or can be due to the presence of foreign bodies.[5,6] When digested by osteoblasts, *S. aureus* can survive in a metabolically less active and phenotypically altered state for a prolonged period of time. This small colony variant phenotype leads to impaired antimicrobial activity, particularly with antimicrobials that interfere with cell wall synthesis, and reduced production of proinflammatory molecules.[7,8] These characteristics might explain, in part, the high relapse rate of osteomyelitis treated with a short course of antimicrobials and the long incubation period.[5,6] Muller and associates[9] studied leukocyte mobilization and phagocytosis in a guinea pig model of posttraumatic osteomyelitis. In this model, leukocyte locomotion was reduced after trauma and infection with *S. aureus* for 90 days.[9]

Antimicrobial Therapy in Experimental Models

Norden and coworkers[10] analyzed the effect of the duration of antimicrobial therapy on the rate of bone sterilization in an experimental model of chronic *S. aureus* osteomyelitis that did not include surgical débridement as part of the experimental treatment. Of cultures, 78% and 16% yielded bacterial growth after 14 and 28 days of clindamycin therapy, respectively. These data support the need for a prolonged course of antimicrobial therapy in osteomyelitis. To our knowledge, the optimal duration of antimicrobial therapy after surgical débridement in an experimental model has not been studied.

Given the importance of biofilm production and the role of rifampin in killing microorganisms present in biofilms, several animal models of staphylococcal osteomyelitis have analyzed the efficacy of rifampin, alone or in combination with β-lactams, vancomycin, macrolides, and quinolones. Combinations of rifampin with other antimicrobial agents generally were more effective in sterilizing the bone.[11,12]

The effect of fluoroquinolones on the healing process of bone fractures was assessed by Huddleston and colleagues.[13] In this study, 60 rats were divided equally into three groups, each group receiving ciprofloxacin, cefazolin, or no treatment for 3 weeks. Radiographs revealed significantly impaired healing of fractures in the ciprofloxacin-treated group compared with fractures in the cefazolin-treated group. Additional studies have shown this effect with other fluoroquinolones but not with vancomycin or gentamicin.[14,15] Quinolones should be used cautiously and when other antimicrobials are not available in cases of osteomyelitis in which fracture healing needs to occur concomitantly with antimicrobial administration. Given the inherent difficulties of conducting large prospective clinical trials in humans, the management of osteomyelitis is likely to continue to be guided by lessons learned from experimental animal models.

GENERAL PRINCIPLES

Osteomyelitis can be hematogenous or contiguous to a soft tissue infection. Acute hematogenous infectious osteomyelitis in children is discussed separately. Adults usually have vague symptoms with a subacute-to-chronic presentation. Nonspecific pain around the involved site with the absence of systemic signs and symptoms is normal. Fever, chills, local swelling, and erythema in the proximity of the involved bone are seldom seen. A draining sinus tract may be present over the involved bone. It usually evolves over several months and sometimes years.

General Principles of Diagnosis

The diagnosis of osteomyelitis is first suspected on clinical grounds. Confirmation usually entails a combination of radiologic, microbiologic, and pathologic tests. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are often elevated. The white blood cell count can be normal or elevated.

Although insensitive, a conventional radiograph is inexpensive and readily available. Abnormalities are usually seen 10 to 14 days after onset of the infection. Nuclear bone scans, although sensitive, are expensive and sometimes nonspecific. Technetium 99m (^{99m}Tc) methylene diphosphonate, gallium (Ga)-67 citrate, and indium (In) 111–labeled white blood cells commonly are used as tracers. Degenerative joint disease, bone tumors, and recent surgery can give false-positive results. These tests require more than 1 day for completion. Fluorine 18 (^{18}F) fluorodeoxyglucose positron emission tomography (PET) imaging is associated with a high sensitivity and specificity for the diagnosis of osteomyelitis, but is very expensive.[16] The high-resolution tomographic images, availability of the agents, and rapid completion are favorable traits of this imaging modality. Cross-sectional imaging modalities, such as computed tomography (CT) and magnetic resonance imaging (MRI), are considered standard of care in the diagnosis of osteomyelitis (Figs. 104.1 and 104.2). Although expensive, they are very sensitive. In certain circumstances, such as Charcot arthropathy, these imaging modalities lack specificity.[17,18] These modalities have an excellent anatomic delineation of the infectious process and an excellent resolution of the surrounding soft tissue. Given the high sensitivity of these tests, they are mostly used to rule out osteomyelitis.[18] Although the presence of hardware may limit the use of conventional MRI sequences, the use of metal artifact suppression techniques, such as multiacquisition variable-resonance image combination (MAVRIC), improves image quality, allowing use of MRI in this setting.[19] The presence of a cardiac device is not an absolute contraindication to MRI.

General Principles of Microbiologic Diagnosis

The identification of a causative microorganism is crucial. The type of organism and the associated in vitro susceptibility data help optimize medical therapy. Organism identification is best accomplished by means of surgical sampling or with needle aspiration under radiologic guidance to obtain tissues for histopathologic examination and aerobic and anaerobic bacterial culture. If a bacterial pathogen is not identified, less common causes of osteomyelitis should be considered and evaluation should be guided by the clinical context.

Swab cultures from draining wounds and sinus tracts can be of diagnostic benefit for three reasons. First, the identification of certain resistant microorganisms (e.g., methicillin-resistant *S. aureus* [MRSA],



FIG. 104.1 Magnetic resonance T2 axial image of the left tibia reveals abnormality of the middle and proximal portion of the tibia (white changes, *arrows*). During surgery, there was significant amount of purulence and osteomyelitis bone present. Cultures grew *Pseudomonas aeruginosa.*

TABLE 104.2 Microbiology of Osteomyelitis

Common (>50% of Cases)
Staphylococcus aureus
Coagulase-negative staphylococci
Occasionally Encountered (<25% of Cases)
Streptococci
Enterococci
Pseudomonas spp.
Enterobacter spp.
Proteus spp.
Escherichia coli
Serratia spp.
Anaerobes (*Finegoldia [Peptostreptococcus]* spp., *Clostridium* spp., *Bacteroides fragilis* group)
Mycobacterium tuberculosis
Rarely Encountered (<5% of Cases)
Mycobacterium avium-intracellulare complex
Rapidly growing mycobacteria
Dimorphic fungi (blastomycosis, coccidioidomycosis, sporotrichosis)
Candida spp.
Cryptococcus
Aspergillus spp.
Mycoplasma spp.
Tropheryma whipplei
Brucella spp.
Salmonella spp.
Actinomyces

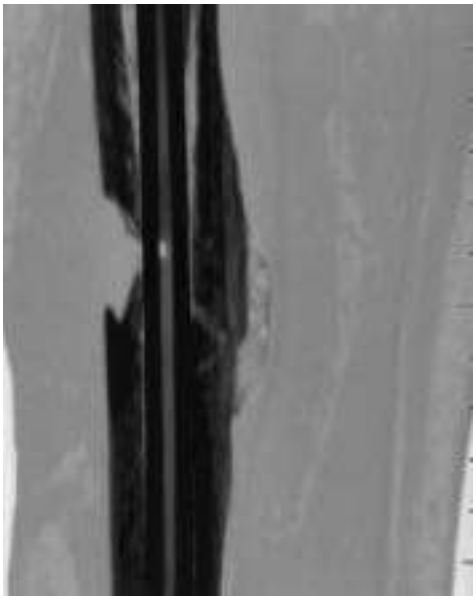

FIG. 104.2 Computed tomography reconstruction images of a nonunited femur fracture 6 months after intramedullary nailing. Surgical cultures grew *Pseudomonas aeruginosa* and *Propionibacterium acnes.*

vancomycin-resistant enterococci) indicates the need for infection-control measures. Second, the isolation of *S. aureus* from superficial cultures has a high degree of correlation with deep cultures.[20] However, the majority of sinus tract swabs obtained during *S. aureus* osteomyelitis will not grow this organism, highlighting the lack of sensitivity of this test. The recovery of other microorganisms correlates poorly with deep cultures. Third, the recovery of the same organism on two consecutive cultures, although infrequent, has been shown to highly correlate with deep surgical cultures of bones.[21]

In hematogenous long bone osteomyelitis, the infection is usually monobacterial, whereas in contiguous infection it is usually polymicrobial. *S. aureus,* coagulase-negative staphylococci, aerobic gram-negative bacteria, and *Finegoldia* (formerly *Peptostreptococcus*) spp. are the most common organisms encountered (Table 104.2). Community-acquired MRSA (CA-MRSA) has been increasingly recognized as a cause of acute long bone osteomyelitis and diskitis in children.[22] Panton-Valentine leukocidin (PVL) is a cytotoxin found in CA-MRSA strains. In children, osteomyelitis caused by PVL-positive *S. aureus* is associated with multifocal and more aggressive disease when compared with PVL-negative *S. aureus*.[23]

General Principles of Therapy

The goal of therapy for osteomyelitis is to eradicate the infection and to restore function. Most cases of osteomyelitis in adults require a combination of medical and surgical therapy for successful eradication of the infection. It has long been recognized that antimicrobial therapy alone is not curative in most cases of osteomyelitis. In 1944, with the introduction of penicillin, Key[24] wrote: "continuous drug over a long period of time will lessen the amount of discharge, but it will not cure the disease because it cannot sterilize dead bone or cavities with necrotic content and rigid walls." All antimicrobials should be withheld if possible until percutaneous aspirate or surgical deep cultures have been obtained, unless there is concomitant soft tissue infection or sepsis syndrome. Antimicrobials are usually started (Table 104.3) immediately after surgical débridement (Table 104.4).

Established Agents for Antimicrobial Therapy

β-Lactams and vancomycin are the most commonly used antimicrobials in the medical management of osteomyelitis.[25,26] Cephalosporins are commonly used in patients with osteomyelitis because of their low toxicity profile and their spectrum of activity against staphylococci and other common bacterial pathogens that cause osteomyelitis. Cefazolin has excellent activity against methicillin-sensitive staphylococci, is safe and inexpensive, and has been used extensively in medical therapy for osteomyelitis.[25] The penicillinase-resistant penicillins have excellent antistaphylococcal activity and are commonly used, but carry a higher rate of adverse effects requiring alteration in therapy during outpatient parenteral antimicrobial therapy (OPAT), compared with cefazolin.[27,28] Ceftriaxone once daily is convenient for outpatient therapy; its use in methicillin-sensitive staphylococcal osteomyelitis has been advocated by some experts.[25,29]

Vancomycin is used commonly in the treatment of osteomyelitis because of its activity against methicillin-resistant staphylococci and

TABLE 104.3 Antimicrobial Therapy for Chronic Osteomyelitis in Adults for Selected Microorganisms

MICROORGANISMS	FIRST CHOICE[a]	ALTERNATIVE CHOICE[a]
Staphylococci		
Oxacillin sensitive	Nafcillin sodium or oxacillin sodium, 1.5–2 g IV q4–6h for 4–6 wk, *or* cefazolin,[b] 1–2 g IV q8h for 4–6 wk	Vancomycin, 15 mg/kg IV q12h for 4–6 wk; some add rifampin, 600 mg PO qd, to nafcillin/oxacillin
Oxacillin resistant (MRSA)	Vancomycin, 15 mg/kg IV q12h for 4–6 wk *or* Daptomycin 6 mg/kg IV q24h[d]	Linezolid, 600 mg PO/IV q12h for 6 wk, *or* levofloxacin,[c] 500–750 mg PO/IV daily, plus rifampin, 600–900 mg/day PO for 6 wk if susceptible to both drugs
Penicillin-sensitive streptococci	Aqueous crystalline penicillin G, 20×10^6 U/24 h IV either continuously or in 6 equally divided daily doses for 4–6 wk, or ceftriaxone, 2 g IV *or* IM q24h for 4–6 wk *or* cefazolin, 1–2 g IV q8h for 4–6 wk	Vancomycin, 15 mg/kg IV q12h for 4–6 wk
Enterococci or streptococci with MIC ≥0.5 µg/mL, *or Abiotrophia* or *Granulicatella* spp.	Aqueous crystalline penicillin G, 20×10^6 U/24 h IV either continuously or in 6 equally divided daily doses for 4–6 wk, *or* ampicillin sodium, 12 g/24 h IV either continuously or in 6 equally divided daily doses; the addition of gentamicin sulfate, 1 mg/kg IV or IM q8h for 1–2 wk is *optional*	Vancomycin, 15 mg/kg IV q12h for 4–6 wk; the addition of gentamicin sulfate, 1 mg/kg IV *or* IM q8h for 1–2 wk is *optional*
Enterobacteriaceae	Ceftriaxone, 1–2 g IV q24h for 4–6 wk, *or* ertapenem 1 g IV q24h	Ciprofloxacin,[c] 500–750 mg PO q12h for 4–6 wk, *or* levofloxacin 500–750 mg PO q24h
Pseudomonas aeruginosa	Cefepime, 2 g IV q8–12h, meropenem, 1 g IV q8h *or* imipenem, 500 mg IV q6h for 4–6 wk	Ciprofloxacin,[c] 750 mg PO q12h for 4–6 wk, *or* ceftazidime, 2 g IV q8h

[a]Antimicrobial selection should be based on in vitro sensitivity data.
[b]Use 2 g for patients weighing 80 kg or more.
[c]Should be avoided, if possible, in pediatric patients and in osteomyelitis associated with fractures.
[d]Some experts recommend use of 8–10 mg/kg IV q24h.
MIC, Minimal inhibitory concentration; *MRSA,* methicillin-resistant *Staphylococcus aureus.*

TABLE 104.4 Surgical Principles in Osteomyelitis

Adequate drainage of all infected tissue
Extensive débridement of all infected tissue
Removal of all hardware
Management of dead space (flap)
Complete wound closure
Stability of infected fracture

ampicillin-resistant enterococci. In a large cohort study of 450 patients with osteomyelitis who were followed for 10 years, vancomycin was associated with a 2.5 relative risk of recurrence, compared with a penicillinase-resistant penicillin in a univariate analysis.[25] Because of the high failure rate and the increasing minimal inhibitory concentration among many staphylococcus strains, many experts now advocate the use of higher and continuous dosage of vancomycin, keeping trough levels between 15 and 20 µg/mL.[30,31] However, the data suggesting that higher trough levels improve outcomes are lacking, and rates of acute kidney injury are increased with this strategy.[32] Linezolid and tedizolid are oxazolidinone class antimicrobials with excellent activity against staphylococci, streptococci, and vancomycin-resistant enterococci. Both agents have excellent bioavailability when administered orally. Available since 2000 in the United States, linezolid has been used in patients with infections caused by vancomycin-resistant enterococci or when β-lactams or vancomycin cannot be used.[30,33] Prolonged use of linezolid has been associated with significant pancytopenia, peripheral neuropathy, optic neuritis, and lactic acidosis.[34,35] Because of its toxicity profile and experimental models showing a high failure rate, the use of linezolid in patients with osteomyelitis typically has been limited to patients with osteomyelitis caused by vancomycin-resistant enterococci or patients who are intolerant of vancomycin.[33,36] The use of linezolid therapy guided by level may ameliorate some of the risk of chronic toxicity and drug interactions when the drug is used in combination with rifampin.[37] Available since 2014, tedizolid has not been widely used for osteomyelitis. However, it may have a more attractive side-effect profile than linezolid and deserves further study.

Daptomycin, a cyclic lipopeptide antimicrobial agent, has bactericidal activity against aerobic and facultative gram-positive pathogens. The role of daptomycin in the therapy of patients with osteomyelitis is now established.[38,39] A multicenter retrospective study in Europe of 220 patients with osteomyelitis evaluated the efficacy and safety of daptomycin. Only 0.5% of patients had an elevation of creatine phosphokinase, and 75% of patients had a successful outcome.[38] However, resistance to daptomycin has occurred during prolonged therapy.[40] The role of daptomycin in osteomyelitis caused by methicillin-susceptible *S. aureus* osteomyelitis, compared with β-lactam, has not been assessed.

Oral Agents for Antimicrobial Therapy

A combination of trimethoprim-sulfamethoxazole and rifampin for 8 weeks was compared with parenteral cloxacillin for 6 weeks, followed by oral cloxacillin for 2 weeks in a randomized clinical trial of 50 patients with nonaxial oxacillin-susceptible staphylococcal osteomyelitis. No difference in the outcome was noted between the groups, emphasizing the important role of rifampin when combined with a highly bioavailable agent.[41] Pharmacokinetic interactions among drugs used in combination are increasingly recognized, particularly in those combinations using rifampin. Two small studies provide support for a pharmacologically significant decrease in clindamycin concentrations when the drug is used in combination with rifampin, but no clinical effect has yet been shown.[42,43]

First-generation quinolones, such as ciprofloxacin, have little activity against staphylococci, streptococci, and enterococci and no activity against anaerobes. Levofloxacin has good streptococcal and staphylococcal activity but variable and minimal anaerobic activity. The latest-generation quinolones, such as moxifloxacin, are broad-spectrum antimicrobials with excellent activity against gram-negative and gram-positive organisms and improved anaerobic activity. Increasing awareness of adverse effects such as tendon damage, neuropathy, and other serious complications has led to US Food and Drug Administration (FDA) to urge quinolone use only in serious infections, such as osteomyelitis. Although this class of medications remains an important option for this challenging infection, the benefits and risks must be carefully weighed on a case-by-case scenario.

New Agents for Antimicrobial Therapy

Several new agents with activity against MRSA have become available in the last several years. None of these agents have sufficient published clinical experience to replace existing agents, but they provide off-label therapeutic options in the case of clinical failure or intolerance. Ceftaroline, a fifth-generation cephalosporin that is active against MRSA via penicillin binding protein-2a binding, has been used in the setting of previous clinical failure.[44] The incidence of neutropenia during

ceftaroline use appears to be higher than with other therapy.[45] Limited retrospective reports support the use of telavancin, a lipoglycopeptide administered once daily, for osteomyelitis.[46,47] However, a significantly higher incidence of adverse events, including renal failure, was observed during phase II and III studies for other indications, suggesting that telavancin should be used only when other agents with a better safety profile cannot.[48] Dalbavancin and oritavancin are lipoglycopeptides that have the unique property of significantly prolonged half-lives, administered once weekly. Although they have been approved for skin and soft tissue infection, further study is needed before routine use can be considered for osteomyelitis.

Duration of Antimicrobial Therapy

The optimal duration of antimicrobial therapy in osteomyelitis is unknown because of the few prospective randomized clinical trials assessing the length of antimicrobial therapy in patients with osteomyelitis and the heterogeneous nature of the disease.[49] In experimental models, 4 weeks of therapy was more effective in sterilizing bone than 2 weeks of therapy. Surgical débridement was not part of these models, however, and shorter courses of therapy may be as effective when extensive surgical débridement is accomplished.[10] Because it takes 6 weeks for the débrided bone to be covered by vascularized soft tissue, and because of anecdotal experiences suggesting a higher relapse rate with a short duration of therapy, many experts advocate a total duration of 4 to 6 weeks of parenteral or highly bioavailable oral antimicrobial therapy. When the surgical débridement of all infected bone is complete or the osteomyelitic bone has been resected, some experts advocate short-duration antimicrobial therapy.[50] When stable, patients can be dismissed from the hospital, and parenteral antimicrobial therapy can be continued on an outpatient basis. Use of long-term intravenous catheters, such as peripherally inserted central catheters (PICCs), can facilitate this task.[26,51]

Hyperbaric Oxygen Therapy

Hyperbaric oxygen therapy has been used as an adjunctive measure for patients with chronic or refractory osteomyelitis.[52–54] It is hypothesized that an adequate oxygen tension is necessary for oxygen-dependent killing of organisms by the polymorphonuclear leukocytes and for fibroblast activity, leading to angiogenesis and wound healing. In addition, hyperbaric oxygen has a direct bactericidal or bacteriostatic effect on obligate anaerobic organisms. The body of evidence does suggest that there might be a favorable role in the promotion of chronic ulcer healing and in the reduction of major limb amputation.[52] Anecdotal experience in humans includes data derived from convenience retrospective cohorts of patients with recurrent and/or refractory osteomyelitis (see Chapter 50). To our knowledge, there are no adequate randomized trials assessing the efficacy of hyperbaric oxygen therapy in humans with chronic or refractory osteomyelitis, and its use in this scenario is still unproven.[53]

OSTEOMYELITIS AFTER A CONTAMINATED OPEN FRACTURE

Contaminated open fractures can lead to the development of osteomyelitis of the fractured bone, typically at the fracture site in 3% to 25% of cases, depending on the type of fracture, the level of contamination, the degree of soft tissue injury, and whether systemic and local antimicrobial therapies have been administered (see Fig. 104.2).[55–59] Patients with open fractures are usually young men in their teens or 20s. The bones of the lower extremity are typically involved, most often the tibia or fibula. Early contamination after the open fracture of bone and soft tissue eventually can lead to the development of osteomyelitis at the fracture site. Untreated, infection ultimately may lead to nonunion of the infected site, chronic osteomyelitis, or amputation.

Microbiology of Osteomyelitis After Contaminated Open Fracture

The microorganisms isolated late in this type of osteomyelitis correlate in 25% with the initial culture results at the time of débridement.[60] A variety of microorganisms have been implicated. Pathogens can include normal skin flora that contaminate the wound, organisms from contaminated soil, or nosocomial pathogens acquired because of the multiple operations that often are required to surgically repair the fracture and surrounding soft tissue envelope. Staphylococci and aerobic gram-negative bacilli are the two most common groups of microorganisms implicated in this type of osteomyelitis. Several other unusual microorganisms, such as enterococci, fungi, and atypical mycobacteria, also have been implicated.

Management of Open Contaminated Fractures to Prevent Osteomyelitis

Current management of open contaminated fractures entails early aggressive wound irrigation and débridement, administration of empirical parenteral antimicrobials, stable fracture fixation, and soft tissue coverage of exposed bone. In one study, a delay of 5 hours in surgical débridement was associated with a higher incidence of infection.[60] In contrast, a well-performed systematic review did not definitively support the need for early débridement for prevention of infection.[61] Despite the lack of clear support in the literature, we favor early débridement if possible, provided it does not have negative consequences from an operative optimization standpoint. Randomized clinical trials performed by Patzakis and colleagues[62] established the efficacy of a short duration of antimicrobial prophylaxis in the prevention of osteomyelitis in patients treated for open contaminated fractures. The use of prolonged antimicrobial therapy for prevention does not decrease the risk of infection and potentially can lead to the development of resistant microorganisms.[63,64] The addition of empirical vancomycin to cover MRSA in this setting may not be warranted.[65] Although the addition of fluoroquinolone or aminoglycoside may decrease the rate of soft tissue infection, the rate of osteomyelitis is not affected and an increase in resistant gram-negative pathogens may result.[66] Cohort studies have shown the efficacy of antibiotic-impregnated polymethyl methacrylate beads temporarily placed in and around the fracture site in the prevention of osteomyelitis after a contaminated open fracture.[67,68] The choice between external versus intramedullary fracture fixation devices in contaminated open fractures is controversial and discussed in detail elsewhere (see Chapter 105).

Signs and Symptoms of Osteomyelitis After Contaminated Open Fracture

Signs and symptoms of infection typically appear several months after an open fracture. The hallmark of osteomyelitis after open fracture is nonunion of the fracture site or poor wound healing after wound closure or soft tissue coverage. Other symptoms, such as local erythema or fever and chills, are less common. MRI or radionuclide imaging studies are typically neither sensitive nor specific for the presence of infection because of the abnormalities created by the recent previous surgery and the presence of foreign bodies.

Management of Osteomyelitis After Contaminated Open Fracture

Management of established osteomyelitis or infected nonunion after an open fracture requires débridement of the surgical site, identification of the causative microorganisms, and pathogen-directed antimicrobial therapy (see Table 104.3). If foreign bodies are retained, long-term oral antimicrobial suppression may be helpful until fracture healing occurs, at which time oral antimicrobial suppression could be discontinued. The use of quinolone and rifampin combinations has been studied in staphylococcal fracture–fixation device infection.[69,70] In sensitive staphylococcal hardware-associated osteomyelitis, a 3- to 6-month course of a quinolone and rifampin eradicated the infection in more than 80% of cases.[69,70] Given the good outcome of patients treated with other antimicrobials and concerns about the effect of quinolones on bone healing, we have not universally adopted this strategy.[13] Others have suggested use of rifampin in combination with other agents, such as linezolid or trimethoprim-sulfamethoxazole, which may avoid the toxicity concerns of quinolones while still using rifampin-based therapy.[71] If recurrence of the infection develops after discontinuation of long-term oral antimicrobial suppression and fracture bone healing, definitive therapy with removal of the foreign bodies and pathogen-directed antimicrobial therapy usually can be then undertaken without

compromising limb function. This therapeutic strategy is in contrast to prosthetic joint infection treated with débridement and retention of components, in which removal of the prosthesis typically compromises limb function.

VERTEBRAL OSTEOMYELITIS AND SPONDYLODISKITIS

Infection of the end plate and the adjacent vertebrae, variably referred to as *spondylodiskitis, disk space infection,* or *vertebral osteomyelitis,* all with or without associated epidural abscess (see Chapter 91) or psoas abscesses, is hematogenous in origin in most cases.[72–74] Potential sources of hematogenous infection are skin and soft tissue infection, genitourinary tract infection, infective endocarditis, infected intravenous sites, intravenous drug abuse, and respiratory tract infection.[73,75] Infection of the disk space and contiguous vertebra also may occur postoperatively.[75] Several studies have established the efficacy of antimicrobial prophylaxis before spinal surgery in reducing the risk of postoperative superficial or deep infection, including vertebral osteomyelitis. In one study by Schnöring and Brock,[76] 0.2% of patients receiving antimicrobial prophylaxis developed a surgical site infection, whereas 2.8% of patients developed surgical site infection when antimicrobial prophylaxis was withheld.

Clinical Signs and Symptoms of Vertebral Osteomyelitis

The clinical presentation of vertebral osteomyelitis includes localized insidious pain and tenderness in the spine area in 90% of patients. Fever is present in less than 50% of patients. Motor and sensory deficits, caused by spinal cord or nerve root compression, are present in 15% of patients.[73–76]

Microbiology of Vertebral Osteomyelitis

S. aureus, coagulase-negative staphylococci, and *Streptococcus* spp. are the most common microorganisms encountered in vertebral osteomyelitis.[73–76] *Mycobacterium tuberculosis* and *Brucella* spondylodiskitis are common in endemic regions.[77,78] Spine infections caused by gram-negative aerobic bacteria and *Candida* spp. are seen more commonly in intravenous drug abusers, immunosuppressed patients, and postoperative patients.[79,80] Immunosuppressed patients with human immunodeficiency virus infection or congenital or iatrogenic immunodeficiencies are increasingly reported with vertebral osteomyelitis caused by nontuberculous mycobacteria, with *Mycobacterium avium* complex and *Mycobacterium xenopi* dominating a review of cases over the years 1961–2014.[81]

Diagnosis of Vertebral Osteomyelitis

The diagnosis of vertebral osteomyelitis requires a high index of suspicion in at-risk patients presenting with compatible signs and symptoms. The goal of the diagnostic evaluation is to determine the extent of infection and identify the organism. Neurologic function and spinal stability always should be assessed carefully. An elevation of the ESR is present in more than 90% of cases, whereas the white blood cell count is elevated in less than 50% of patients.[73–76] Assessing for the presence of concomitant infective endocarditis should be done, especially in patients with bloodstream infection.[82,83] Plain spinal radiographs are not sensitive in the diagnosis of disk space infection. In one study, 32% of radiographs suggested diskitis.[84] MRI has proved to be an invaluable tool in detecting disk space infection and spinal cord compression (Figs. 104.3 and 104.4). Ga-67 citrate scanning seems to be a sensitive and specific method used to diagnose diskitis. In a study of 41 patients with suspected spondylodiskitis, Ga-67 scanning proved to be 100% sensitive, specific, and accurate.[85] We use Ga-67 or CT scanning when MRI cannot be performed and in cases in which MRI is inconclusive. The experience with the use of ^{18}F-fluorodeoxyglucose PET in the diagnosis of vertebral osteomyelitis is limited but appears to be promising.[86] PET/CT was found to improve specificity, positive and negative predictive values, and accuracy compared with stand-alone PET in patients with suspected osteomyelitis or implant-associated infection.[87] A prospective study comparing MRI and PET/CT found comparable sensitivity and specificity between the two modalities for diagnosing vertebral osteomyelitis.[88] However, the superior resolution of MRI allowed better detection of epidural and paravertebral infection, whereas PET/CT more readily allowed identification of areas of metastatic infection. Guidelines from the Infectious Diseases Society of America have been published to assist in the diagnosis and treatment of native vertebral osteomyelitis in adults.[89]

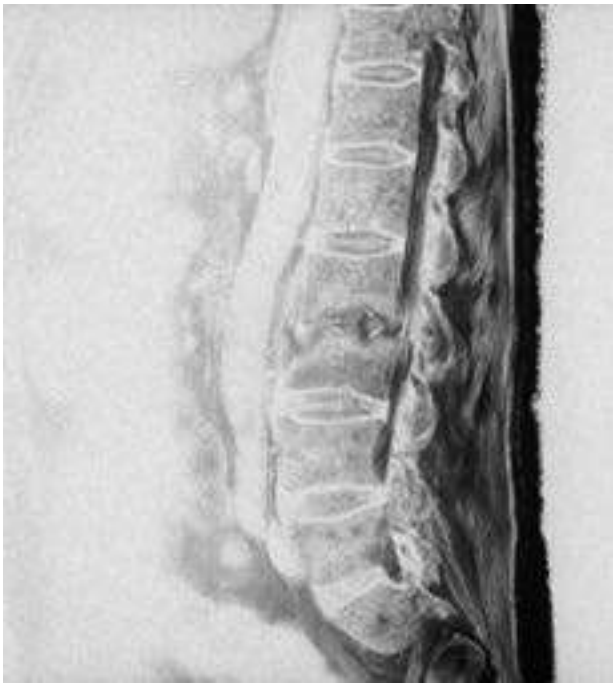

FIG. 104.3 T1-weighted magnetic resonance images show an abnormal signal in the disk between L2 and L3, with associated vertebral osteomyelitis. A fluid collection is located in the posterior part of L2 and L3, resulting in the elevation of the posterior ligament. *Staphylococcus aureus* grew from a computed tomography–guided aspirate.



FIG. 104.4 Angiogram of the lower extremity reveals an occluded bypass graft *(arrow)* in a patient with diabetes mellitus who presented with chronic gangrene and osteomyelitis of the foot.

Microbiologic Diagnosis of Vertebral Osteomyelitis

Accurate microbiologic diagnosis is critical to informing therapeutic decision making. In a significant proportion of patients with *S. aureus* diskitis, the diagnosis is often made through the detection of concomitant *S. aureus* bloodstream infection.[90] If a microbiologic diagnosis is not obtained through blood cultures, percutaneous biopsy should be performed. An analysis of the microbiology in a French prospective multicenter study of 101 patients with microbiologically confirmed vertebral osteomyelitis found that coagulase-negative staphylococci (26%), *S. aureus* (21%), *Streptococcus* spp. (13%), and Enterobacteriaceae (21%) were the most common isolates.[91] Bone biopsy can be challenging, requiring special tools and expertise. The findings of a 10-year retrospective study suggest that inflamed paravertebral soft tissue, even in the absence of abscess, may be a suitable diagnostic target.[92] Image-guided percutaneous biopsy and aspiration has a sensitivity of 38% to 60%.[93,94] If the results for the first aspirate are inconclusive, a repeat aspiration may be performed to optimize the sensitivity of the microbiologic or pathologic diagnosis. In one study, a microbiologic diagnosis was made in 80% of patients when a second biopsy was performed in those with negative cultures at first biopsy.[95] An open or a percutaneous endoscopic procedure should be reserved for patients with a nondiagnostic fluoroscopic-guided aspiration or in patients not responding to empirical antimicrobial therapy.[96]

Therapy for Vertebral Osteomyelitis

The goals of therapy should include eradicating the infection, relieving pain, preserving or restoring neurologic function, and maintaining spinal stability. Complete bed rest is not often necessary. The spine could be externally stabilized with a corset or a body brace. The treatment of vertebral osteomyelitis requires an initial 6-week course of antimicrobial therapy (see Table 104.3). A study found that a 6-week course of therapy was not inferior to a 12-week course.[97] Parenteral antimicrobial treatment may be extended in difficult cases or in cases in which undrained abscesses are being treated and do not resolve after 6 weeks. In a retrospective study from Korea, MRSA infection, undrained paravertebral or psoas abscess, and end-stage renal disease were found to be independent risk factors for recurrence. The authors suggest that in the presence of one of these risks, antibiotic treatment for longer than 8 weeks should be used to lessen the risk of recurrence.[98]

Role of Surgery for Vertebral Osteomyelitis

Surgical therapy is not necessary in most cases. Surgical débridement should be considered in patients with a large paravertebral abscess or an epidural abscesses (Fig. 104.5; also see Chapter 91), when medical management fails, or when the spine is mechanically unstable. In selected cases, percutaneous transpedicular débridement and diskectomy, performed under fluoroscopic guidance, may prevent the progression of bone destruction and deformity in the early stages of vertebral osteomyelitis and spondylodiskitis.[96] The neurologic status of the patient must be monitored closely.

Follow-Up Evaluation for Vertebral Osteomyelitis

Evaluation at the end of therapy should assess for improvement in clinical signs, symptoms, and laboratory markers of infection. Patients with successful treatment should have resolution of the systemic symptoms of infection and improvement in pain, but pain is often not resolved completely. A 25% or greater improvement in ESR and CRP at 4 to 8 weeks, compared with baseline level, is associated with successful treatment.[99,100] Follow-up MRI of patients with vertebral osteomyelitis has a limited role and may give the impression of clinical worsening, even though there otherwise is clinical improvement.[101] We advocate the use of follow-up MRI in patients with persistent elevation of inflammation markers, in patients with persistent pain, or in case of development of new neurologic signs or symptoms.[101] With effective antimicrobial therapy, spontaneous fusion between adjacent infected vertebral bodies requires 12 to 24 months.



FIG. 104.5 Signal changes within the C5 and C6 vertebral bodies and pedicles consistent with bone edema are seen, along with a small anterior epidural collection consistent with diskitis and epidural abscess.

Hardware-Associated Vertebral Infection

Early postoperative hardware-associated spine infection is treated initially with surgical débridement and retention of the hardware (see Chapter 105). Removal of spinal hardware in this situation would compromise the stability of the spine and the spinal cord. In this setting, surgical débridement is followed by a course of parenteral antimicrobial therapy (see Table 104.3).[102] Although not recommended by all experts, parenteral antimicrobial therapy at our institution is typically followed by the use of long-term oral antimicrobial suppression in this situation.[77] Long-term antimicrobial suppression should be continued until there is radiologic evidence of bone vertebral fusion. This modality is highly successful in our institution.[102] Complete bone remodeling and fusion typically are complete after 2 years. At that time, long-term oral suppression may be discontinued, with a low risk of recurrence. If there is recurrence, the spinal hardware can be removed without compromising the stability of the spine.[102] Successful therapy for late postoperative hardware-associated spine infection often involves removal of the hardware, followed by a course of antimicrobial therapy (see Table 104.3).

OSTEOMYELITIS IN PATIENTS WITH DIABETES MELLITUS OR VASCULAR INSUFFICIENCY

Osteomyelitis in patients with diabetes mellitus is typically found in the foot and less often in the hand.[103,104] The economic consequences of this type of osteomyelitis are enormous and extremity amputation is all too common. In 2014 the estimated annual cost of management of diabetic foot ulcers in the United States was $9 billion to $13 billion.[105] Fifteen percent to 25% of patients with diabetes mellitus develop foot ulcers during their lifetime. Of those, up to 24% will develop a soft tissue infection or contiguous osteomyelitis.[106] Several factors can lead to the development of a diabetic foot infection. Neuropathy, vascular insufficiency (see Fig. 104.4), and hyperglycemia can lead to a variety of consequences that ultimately lead to the development of a skin ulcer and subsequently to contiguous osteomyelitis.

Prevention of Diabetic Foot Osteomyelitis

Early recognition and management of factors that can lead to the development of foot ulcers in patients with diabetes mellitus are crucial to the delay or even prevention of osteomyelitis (see Fig. 104.6). In assessing the risk of subsequent foot ulcer in patients with diabetes mellitus, the treating physician should pay special attention to several factors (Table

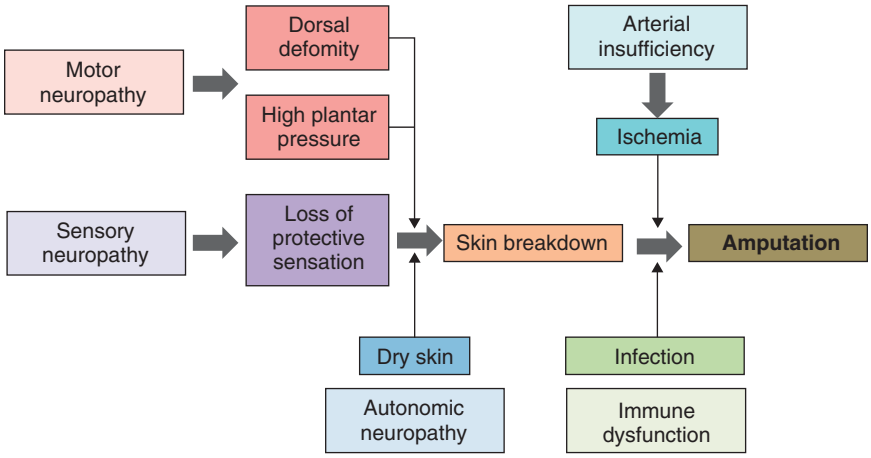

FIG. 104.6 Diagram of the multiple factors that may contribute to the development of diabetic foot infection. *(Modified from Ulbrecht JS, Cavanagh PR, Caputo GM. Foot problems in diabetes: an overview.* Clin Infect Dis. *2004;39(suppl 2):S73–S82.)*

TABLE 104.5 Factors Associated With Increased Risk of Foot Ulcers in Patients With Diabetes Mellitus

Diabetes mellitus duration over 10 years
Poor glucose control
Cardiovascular disease
Renal or retinal complications
Peripheral neuropathy
Evidence of increased local pressure (callus, erythema)
Limited joint mobility
Peripheral vascular disease
Prior history of foot ulcer
Prior history of amputation

104.5). The lower extremity macrovascular and microvascular circulation supply can be assessed through physical examination of the pedal pulses, noninvasive vascular assessments such as Doppler ultrasound, or measurement of transcutaneous oxygen pressures.[50] Patients with diabetes mellitus or vascular insufficiency should have an annual complete foot examination performed by a health care professional.[50] Strict glycemic control and smoking cessation can reduce the rate of progression of vascular diseases and neuropathy. Patients with evidence of foot irritation from local pressure should be offered well-cushioned walking shoes that redistribute the pressure in their feet.[107]

Diagnosis of Osteomyelitis in Patients With Diabetes or Vascular Insufficiency

As discussed earlier, the diagnosis of osteomyelitis in patients with diabetes or vascular insufficiency often requires multiple modalities, including a careful physical assessment of the foot and the ulcer, which can often provide clues to the presence of contiguous osteomyelitis. A chronic ulcer with a surface area of more than 2 cm^2 or a positive probe-to-bone test is associated with high positive predictive value.[108] In a systematic review of 288 pooled cases, the sensitivity of exposed bone or a probe-to-bone test was 60%, and the specificity was 91%. MRI was associated with a pooled sensitivity of 90% and a specificity of 79%.[109] Deep surgical cultures of bone and soft tissue submitted for aerobic and anaerobic cultures often revealed the presence of multiple microorganisms. This can be done by means of open débridement, needle puncture, or transcutaneous bone biopsy.[110] Although the microbiome of diabetic foot wounds may be less diverse than in intact skin,[111] the contribution of this microbiome to wound healing is not yet determined. An evaluation of the microbiome in diabetic foot osteomyelitis comparing 16S rRNA sequencing with conventional microbiologic techniques observed higher rates of anaerobes and gram-positive bacilli with the molecular assay.[112] Population-based surveillance in California from 1995 to 2012 identified an increased incidence of group B *Streptococcus (Streptococcus agalactiae)*, with diabetes present in 76.3% of patients with bone infections.[113]

Management of Osteomyelitis in Patients With Diabetes or Vascular Insufficiency

As in other forms of osteomyelitis, combined surgical and medical therapy is most often warranted (see Tables 104.3 and 104.4). Treatment failure is often due to lack of débridement, peripheral vascular disease, and failure to obtain deep cultures before antimicrobial therapy.[103] Pelvic osteomyelitis under pressure ulcers presents many diagnostic and therapeutic challenges. MRI and multiple bone biopsies for culture and histology were obtained in a study from France. Osteomyelitis was associated with the concordance of microbiology and histology, with poor correlation with MRI findings.[114]

In patients with poor arterial vascular supply, revascularization should be done if possible to provide blood flow to the débrided area or to minimize the extent of any amputation that may be required. In this subset of patients, the infected and necrotic bone is usually exposed and surrounded by poorly vascularized soft tissue. Management of dead space and adequate surgical drainage of infected bone and soft tissue often are required. When possible, antimicrobial therapy should be withheld until intraoperative deep cultures have been obtained. Antimicrobials should be administered before culture results, however, in patients with local or systemic signs of an infectious process (cellulitis, acute soft tissue infection, fever, and hemodynamic compromise). In carefully selected cases and when surgical débridement is not done, a prolonged course of antimicrobial therapy for 3 months or more has been shown to be curative and a 6-week duration of therapy may be sufficient.[50,115]

Antimicrobial Therapy for Osteomyelitis in Patients With Diabetes or Vascular Insufficiency

Because most of these infections are polymicrobial, including multiple aerobic and anaerobic microorganisms, broad-spectrum antimicrobial therapy often is required. Multiple antimicrobial regimens have been used, including piperacillin-tazobactam, ampicillin-sulbactam, ertapenem, or other β-lactams combined with metronidazole.[50] A quinolone combined with metronidazole or clindamycin also is considered an acceptable alternative and has excellent oral bioavailability. The efficacy of linezolid in the treatment of diabetic foot infection was evaluated in a large randomized clinical trial. In this study, linezolid with or without aztreonam was compared with an aminopenicillin/β-lactamase inhibitor. The linezolid arm was comparable to the aminopenicillin arm in terms of safety, clinical efficacy, and microbiologic efficacy in this particular study.[116] Surgical therapy was performed when clinically indicated. However, bone marrow suppression, peripheral

neuropathy, and optic neuritis are always a concern in prolonged linezolid therapy.

Depending on the level of surgical débridement and amputation, the duration of antimicrobial therapy varies from a few days to several weeks.[50] Some experts recommend that antimicrobial therapy be extended until the soft tissue defect and the skin have completely healed. A small (20 patients per arm) prospective, randomized trial of 6 versus 12 weeks of antibiotic therapy without surgery found no difference in the outcome of complete and persistent healing of the wound and no need for surgical therapy at the end of follow-up (12 months).[115]

ACUTE HEMATOGENOUS OSTEOMYELITIS

Hematogenous seeding of bones, albeit rare, potentially can affect any bone in the body. Acute hematogenous osteomyelitis of long bones occurs mainly in prepubertal children, elderly patients, intravenous drug abusers, and patients with indwelling central catheters. Hematogenous seeding of an intervertebral disk space can occur in adults and was discussed earlier. This section discusses mainly acute hematogenous osteomyelitis in prepubertal children.

Pathophysiology of Acute Hematogenous Osteomyelitis

Acute hematogenous osteomyelitis involves mostly the metaphyses of long bones (Cierny-Mader classification stage I). The tibia or femur is affected in most cases.[117–119] The infection can involve multiple osseous sites. Because the infection is not confined to the metaphyses, approximately half of cases of neonatal osteomyelitis also have involvement of the adjacent joint with development of septic arthritis. The predilection of infection to the metaphyseal region is explained by its peculiar anatomy.[120,121] Capillary ends of the nutrient artery make sharp loops under the growth plate. This nonanastomosing capillary system feeds into large venous sinusoids, where the blood flow becomes slow and turbulent. An obstruction of these capillaries can lead to an area of avascular necrosis. Metaphyseal capillaries lack phagocytic lining cells. Any minor trauma can lead to the development of a small hematoma, vascular obstruction, and subsequent bone necrosis. This area can be seeded from a transient bacteremia. The acute infection initially produces an inflammatory infiltration, increased bone pressure, decreased pH, and decreased oxygen tension. These factors compromise the medullary circulation and enhance the spread of infection. In infants, the infection may proceed laterally through the bone cortex or through the epiphysis and joint surfaces through capillaries that cross the growth plate.

Microbiology of Acute Hematogenous Osteomyelitis

The most common microorganisms isolated in cases of prepubertal hematogenous osteomyelitis are *S. aureus, Kingella kingae,* and *Streptococcus pyogenes.*[122] *Streptococcus pneumoniae* continues to be an important cause of hematogenous osteomyelitis, but incidence has significantly declined with immunization improvements.[123] *Haemophilus influenzae* type b, historically a common cause of long bone osteomyelitis, has become exceedingly rare since the development and widespread use of an effective vaccine in children.[124] Osteoarticular infection may be the first manifestation of disease caused by the *Streptococcus bovis* group *(Streptococcus gallolyticus).* The same caution with regard to concomitant colonic malignancy exists with osteomyelitis as with endocarditis, with 46 of 64 (71.9%) endoscopies performed in patients with osteoarticular infection showing colonic neoplasm.[125] Osteomyelitis in neonates results from hematogenous spread, especially in infants with indwelling central venous catheters. Common causative organisms in osteomyelitis of the neonate are organisms that frequently cause neonatal sepsis, such as group B *Streptococcus* spp. and *Escherichia coli. Candida* spp. and *Pseudomonas aeruginosa* are more commonly encountered in intravenous drug abusers and patients with indwelling central catheters.

In children, the diagnosis often is made in a patient with compatible radiologic and clinical findings with positive blood cultures. In adults, CT-guided aspiration or an open biopsy is often necessary to establish a definitive diagnosis.

Management of Acute Hematogenous Osteomyelitis

Most cases of acute hematogenous osteomyelitis in children can be treated with antimicrobial therapy alone.[124] Surgical débridement and intramedullary reaming are indicated if the diagnosis is in doubt, the patient has not responded clinically to antimicrobial therapy within 48 hours, infection extends into the joint, or an adequate course of antimicrobial therapy fails to cure the infection. After microbiologic specimens have been obtained, empirical antimicrobial therapy is started to cover clinically suspected organisms. When an organism is identified, the antimicrobial therapy can be changed accordingly (see Table 104.3). Switching from parenteral therapy to oral antimicrobial therapy can be done when the patient is afebrile and able to tolerate oral antimicrobials. Oral antimicrobial therapy should be given to compliant patients with close follow-up. A retrospective analysis of postdischarge antibiotics used in children and adolescents with acute osteomyelitis found comparable efficacy of oral versus intravenous antibiotics.[126] In that study of 2060 children and adolescents, 14% of the group discharged on intravenous antibiotics returned to the emergency department or required readmission secondary to complications of PICC. The typical duration of antimicrobial therapy in children is 3 weeks (see Table 104.3). In a study published in 2013, a shorter duration of antimicrobial therapy (i.e., 1 week) was compared with a 2-week duration in 53 pediatric patients with acute osteomyelitis. No difference in the outcome was observed after a median follow-up of 11.5 months.[119] Dosages of antimicrobials should be adapted to the pediatric population. Quinolones are contraindicated in pediatric patients. In adults with acute hematogenous osteomyelitis, surgical therapy often is required, followed by appropriate antimicrobial therapy based on culture and sensitivity data.

SAPHO SYNDROME

SAPHO, an acronym for *s*ynovitis, *a*cne, *p*lantar pustulosis, *h*yperostosis, and *o*steitis, is an autoinflammatory disease of unknown cause. Although incompletely understood, chronic recurrent multifocal osteomyelitis (CRMO), chronic nonbacterial osteitis, and SAPHO may all be different manifestations of the same disease. SAPHO syndrome was first described in 1972 by Giedion and coworkers.[127,128] The pathogenesis of this disease is unclear. Studies have implicated several genetic factors in the pathophysiology of this disease. Local swelling and tenderness of affected bones is often present. Systemic symptoms of fever, weight loss, and generalized malaise are rare. Osteitis typically is multifocal and affects several bones, including the chest wall bones (63%), pelvis (40%), and spine (33%).[129]

Bones of the lower limbs are affected in only 6% of cases. The mean number of active lesions per patient is five. The disease is self-limited, with spontaneous intermittent periods of exacerbation and remission. The differential diagnosis includes infectious osteomyelitis, bone malignancy, and other inflammatory arthritides. Patients require bone biopsy and cultures for diagnosis to exclude infectious osteomyelitis.

Diagnosis of SAPHO Syndrome

There are no specific laboratory tests to diagnose SAPHO syndrome. The ESR is increased in 65% of cases. Bone radiographs may show lytic erosions similar to those of infectious osteomyelitis affecting the metaphysis. With time, reactive hyperostosis can develop. Histopathology typically is nonspecific, with a combination of acute and chronic inflammatory cells. Cultures of biopsy material are typically negative for bacteria, fungi, or mycobacteria.[130] The role of *Propionibacterium acnes* in the pathophysiology of SAPHO syndrome is intriguing and may require additional investigation.[130]

Management of SAPHO Syndrome

Several therapeutic modalities, including nonsteroidal antiinflammatory drugs, pamidronate, glucocorticoids, sulfadiazine, methotrexate, tumor necrosis factor-α inhibitors, and interferon-γ (IFN-γ), have been used in case reports and small case series.[129–134] The role of continuous use of azithromycin and doxycycline was assessed in 27 patients who were treated for 16 weeks in a study by Assmann and coworkers.[130]

Patients were assessed after discontinuation of antimicrobial therapy. MRI findings, skin activity, and overall health assessment improved while patients were on antibiotics and worsened after discontinuation of antimicrobial therapy, suggesting a role for chronic use of antimicrobials.[130]

OTHER FORMS OF OSTEOMYELITIS: SPECIFIC HOSTS, UNUSUAL LOCATIONS, OR UNUSUAL ORGANISMS

Osteitis Pubis

Osteitis pubis is an infection of the symphysis pubis. It was recognized as a complication in the early era of gynecourologic surgery. Early reports postulated incorrectly that this disease was not infectious because of the nonresponse to antimicrobials and the "nonvirulent organisms" recovered during cultures. The disease is encountered after a variety of urologic and gynecologic surgical procedures, including Marshall-Marchetti-Krantz urethropexy, prolonged catheterization, inguinal hernia repair, vaginal delivery, and prostatectomy or radiotherapy for prostate cancer.[135–142] *S. aureus, Enterococcus* spp., *E. coli, Pseudomonas* spp., and *Proteus* spp. are the most commonly encountered organisms.

Sterile Osteitis Pubis

Some patients might present with a sterile form of osteitis pubis. It is believed that this form is due to an aseptic inflammation that could be triggered by surgery, by bone infarction, or in athletes. Most patients present with suprapubic pain and difficulty and pain with ambulation. In one study, the time between surgery and the diagnosis ranged from 2 to 18 months.[143] Fever and leukocytosis rarely are present.[144] An elevated ESR (>20 mm/h) is present in 67% of patients.[144] Plain radiographs may be normal early in the disease. Radiographs performed 6 months later can reveal pubic bone sclerosis, widening of the joint spaces, and rarefaction. CT and MRI are more sensitive than plain radiography and can define the soft tissue much better. Bone or indium-labeled white blood cell scanning is sensitive. Fine-needle aspiration is sometimes helpful.

The aseptic form of osteitis pubis could be managed with nonsteroidal antiinflammatory drugs and corticosteroids. Antimicrobial therapy should be administered in all other cases. In one study, surgical débridement of infected bone was required in more than 70% of cases.[144]

Osteomyelitis of the Clavicle

Osteomyelitis of the clavicle represents less than 3% of osteomyelitis cases.[117–119,145] It may be hematogenous or related to subclavian vein catheterization or neck surgery.[146] *S. aureus* is the most common organism.[114] A variety of other microorganisms have been described, including gram-negative bacteria and *M. tuberculosis*.[145,147] Given the nontraumatic nature, most cases of clavicular osteomyelitis present a therapeutic challenge. The disease may manifest with acute local pain and swelling with positive blood cultures (i.e., *S. aureus*) or may be more chronic and indolent in nature (i.e., *M. tuberculosis*). In one study, the clinical duration of the symptoms ranged from 2 weeks to 1.5 years.[148] All patients typically presented with clavicular site pain. Fever, localized swelling or a mass, and soft tissue abscesses were present in 60%, 30%, and 30% of cases, respectively.[148] Plain radiographs of the clavicle can show sclerotic or lytic changes. Acute cases secondary to *S. aureus* can be treated with parenteral antimicrobial therapy alone (see Table 104.3). Chronic cases should be treated with surgical débridement, followed by antimicrobial therapy.

Osteomyelitis in Hemodialysis Patients

Metastatic infections complicate 7% of tunneled catheter–related bloodstream infections in dialysis patients. These metastatic infections usually affect the disk space, epidural space, and joint space.[149] Because of a higher rate of *S. aureus* colonization in this patient population, most cases of hematogenous osteomyelitis are due to this microorganism. Oxacillin resistance is common among *S. aureus* isolates in these patients. Advanced diabetes mellitus and peripheral vascular disease are common among patients undergoing hemodialysis. These conditions put these patients at risk for contiguous osteomyelitis of the extremities (see "Osteomyelitis in Patients With Diabetes Mellitus or Vascular Insufficiency").

Osteomyelitis in Patients With Sickle Cell Disease

Patients with sickle cell disease are at increased risk for osteoarticular infections. Sites of bony infarction result from recurrent episodes of vasoocclusion in an expanded marrow space. These sites of necrotic bone are at risk for hematogenous seeding.[150] Acute and long bone osteomyelitis and septic arthritis are the most commonly encountered syndromes. In a large cohort study of 299 patients with homozygous sickle cell anemia in France, the prevalence of osteomyelitis was 12%.[151] *Salmonella* spp. and *S. aureus* remain the most commonly encountered microorganisms in sickle cell anemia patients with osteomyelitis.[152] Most patients are children.[152,153] Although osteomyelitis could be multifocal in this setting, long bones are commonly affected.

The differentiation between bone infarction and osteomyelitis could be challenging because their clinical and radiologic presentations are similar. A history of focalized bone pain and prolonged leukocytosis suggests osteomyelitis.[153] Sequential radionuclide bone marrow and bone scans can aid in the differentiation process.[154] CT-guided aspirate or an open biopsy with cultures sometimes is needed for a more definitive diagnosis, but the procedure can infect previously sterile, infarcted bone. Surgical and medical therapies are similar to those for osteomyelitis in patients without sickle cell disease. Empirical antimicrobial therapy should be directed against *Salmonella* and *S. aureus*.[155]

Gaucher Disease

An acute bone crisis, typically affecting the tibia in patients with Gaucher disease, the most prevalent lysosomal storage disorder, is accompanied by fever, intense local pain, and signs of inflammation. Intravascular activation of coagulation is postulated to be the cause of the ischemic insult to the bone. Bone crisis may occur in stable and treated patients with enzyme replacement therapy. Like patients with SS-hemoglobin disease (sickle cell anemia), these patients may be incorrectly considered to have acute osteomyelitis and, if biopsy is performed, may be at risk for developing osteomyelitis at the biopsy site.[156]

Osteomyelitis in Injection Drug Users

Osteoarticular infections occur more commonly in injection drug users (see Chapter 312).[157] Pathogens can reach the bones via hematogenous routes or through contiguous or direct inoculation. Multiple skeletal sites can be affected. Unusual sites of infection outside this setting are common in these patients, such as sternoclavicular, sternochondral joint, sacroiliac joint, and pubic symphysis. *S. aureus, Pseudomonas* spp., and *Candida* spp. are the most commonly encountered organisms in these patients. *M. tuberculosis* can cause vertebral osteomyelitis in these patients. *Eikenella corrodens,* a normal oral flora microorganism, can cause osteomyelitis in injection drug users who lick the needle tip or the skin before injection ("needle licker osteomyelitis").[157] Surgical and medical therapy for osteomyelitis in these patients is similar to therapy in other groups with osteomyelitis. OPAT and central venous access catheters should be used with caution in these patients, with some data suggesting an increased risk of complications.[158,159]

Skeletal Mycobacterial Infection

Extrapulmonary disease represents 20% of all tuberculosis (see Chapter 249). Infection of the musculoskeletal system represents 1% to 5% of all tuberculosis cases. Osteomyelitis caused by *M. tuberculosis* often affects the spine or a paraarticular focus. Most cases are the result of a hematogenous spread from a pulmonary source.[160,161]

The clinician should consider tuberculous osteomyelitis in patients with a past medical history of treated or untreated tuberculosis with new back pain, patients with a known positive tuberculin skin test or gamma release assay, young patients, patients coming from endemic areas with chest radiographic findings consistent with active tuberculosis

or old healed tuberculosis, patients with a household member who had tuberculosis, patients with negative bacterial cultures, or patients whose biopsy specimen of infected bone shows granulomatous inflammation. Clinical features of osteomyelitis caused by *M. tuberculosis* are pain and swelling with abscess and sinus formation. Radiographs reveal irregular cavities and areas of bone destruction with little surrounding sclerosis. Because of the presence of a sinus tract, secondary bacterial infection does occur, although infrequently.[161]

Vertebral Tuberculosis (Pott disease)

Vertebral osteomyelitis caused by *M. tuberculosis,* also called *Pott disease,* is among the most common osteoarticular manifestations of tuberculosis. In this form of vertebral osteomyelitis, in contrast to bacterial vertebral osteomyelitis, systemic symptoms are often absent. Back pain or stiffness is commonly the only symptom, and a delay in the diagnosis is often the norm. In 50% of patients with spinal tuberculosis, MRI reveals paravertebral soft tissue abscesses in addition to the bone lesion. The infection has a predilection for the anterior superior or inferior angles of the vertebral bodies, especially in the early phases of the disease.[160,162] Characteristics and challenges of the diagnosis and course of the disease are presented in a multinational report of 314 patients with a presumed diagnosis of spinal tuberculosis from high-prevalence countries (Egypt, Albania, Greece, and Turkey). Time from onset of symptoms to diagnosis was 78 days. Lumbar disease was most common (56%), followed by thoracic (49%) and thoracolumbar involvement (13%). Of 200 histopathologic examinations, 74% were consistent with tuberculosis, but a causative agent was cultured in only 41%. Mortality was 2%, but 25% developed sequelae, including structural abnormalities (kyphosis, gibbus deformity, scoliosis) and neurologic problems (paraparesis, paraplegia, and loss of sensation).[163] In contrast, in Denmark, the time from symptoms to diagnosis of tuberculosis was 19.5 days for spinal disease and 28 days for other bone and joint manifestations. Culture was diagnostic in 87%. Sequelae were present in 57% of spinal cases, with overall mortality of 25.5% among Danes compared with 1.3% among immigrants.[164]

Diagnosis of Tuberculous Osteomyelitis

Significant overlap in imaging appearances between tuberculous osteomyelitis and other forms of osteomyelitis exists. The diagnosis should rely on the presence of *M. tuberculosis* on stain or culture of a biopsy specimen. Chest radiographs show an abnormality in less than 50% of patients with musculoskeletal tuberculosis but should be obtained routinely because the existence of concomitant pulmonary tuberculosis has infection-control ramifications and may provide for an alternative area from which to obtain culture specimens. Therapy for skeletal tuberculosis is discussed in Chapter 249.

Nontuberculous Mycobacterial Osteomyelitis

Osteoarticular infections with nontuberculous mycobacteria also can occur. They are commonly seen in immunocompromised patients[165] or after contamination of a wound after trauma or surgery. *Mycobacterium marinum, Mycobacterium avium-intracellulare, Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium kansasii, and M. xenopi* all have been associated with infection.[165–170] Members of the *Mycobacterium terrae* complex (*M. arupense, M. heraklionense*, and a newly proposed species, *M. virginiense*), but not *M. terrae* sensu strictu, have been associated with tenosynovitis and osteomyelitis in the United States.[171] Disseminated osteoarticular infection with *Mycobacterium bovis* after Calmette-Guérin bacillus vaccination or intravesicular installation of Calmette-Guérin bacillus also has been reported.[172] Children and young adults with unexplained disseminated nontuberculous mycobacterial infections should be tested for the presence of inherited deficiency in the receptor for IFN-γR1 or interleukin (IL)-12β1.

Medical therapy alone is often curative, although in selected cases, surgical débridement is required. Antimicrobial agents typically used in the treatment of osteoarticular infection caused by atypical mycobacteria are the same as agents used to treat infection at other sites and are discussed in Chapters 251 and 252.

Fungal Osteomyelitis

Osteomyelitis caused by fungi is uncommon. Several observational studies and case reports have been published.[173–179] Bone lesions are most common in blastomycosis, disseminated coccidioidomycosis, and extracutaneous sporotrichosis but are seen occasionally in cryptococcosis, candidiasis, and aspergillosis. A survey of cases of spinal coccidioidomycosis collected over 50 years in the American Southwest noted that men and African Americans were disproportionately affected. A review of the management suggested that surgical therapy is indicated for mechanical instability, neurologic deficit, medically intractable pain, or progression despite antifungal therapy, which usually needs to be continued for years.[180] The typical epidemiologic risk factors and host characteristics that predispose to mycoses often provide clues as to the fungal etiology. Although most fungal osteomyelitis is hematogenous, trauma with contamination of a wound is a risk factor for fungal osteomyelitis caused by fungi, including *Scedosporium apiospermum (Pseudallescheria boydii), Lomentospora (Scedosporium) prolificans,* and *Fusarium* spp. Mold infections other than *Aspergillus* are most often associated with trauma in children and with surgery in adults.[181] Hematogenous fungal osteomyelitis usually manifests clinically as a "cold abscess" and radiologically as a well-defined osteolytic lesion with adjacent soft tissue abscess. In contrast, extracutaneous sporotrichosis causes patchy bone loss and commonly extends to contiguous joints. Surgical débridement of contiguous soft tissue should be done in patients with large collections of pus, but the role of surgery is usually limited to biopsy for diagnosis. Therapy for specific mycoses is discussed in Chapters 256 to 268.

Brodie Abscess

Brodie abscess refers to a chronic localized bone abscess. Patients with subacute cases may present with fever, pain, and periosteal elevation, whereas patients with chronic Brodie abscess are often afebrile and present with long-standing dull pain. The most common site of involvement is the distal part of the tibia. The lesion is typically single and located near the metaphysis. Of patients, 75% are younger than 25 years. Surgical débridement and culture-directed antibiotics are often curative. Cultures may be negative.

Culture-Negative Osteomyelitis

Rarely, bone culture specimens are sterile despite clinical, radiologic, and pathologic evidence of osteomyelitis. Brodie abscess and bone infarcts caused by Gaucher disease or sickle cell disease should be considered. At our institution, most of these cases are due to prior use of antimicrobial therapy. For indolent cases not responding to therapy, consideration should be given to stopping antibiotics and waiting for at least 1 month before repeating the culture.[182] Antimicrobial therapy may slow the growth of ordinarily hardy organisms.[183] When aerobic and anaerobic bacterial specimens are sterile, cultures should be obtained for fungi and mycobacteria. In selected cases, polymerase chain reaction analysis using 16S rRNA gene primers with a broad specificity for detecting bacterial DNA in bone and purulent material can be helpful.[183] If all cultures are negative, we believe that the antimicrobial regimen should be designed to cover the commonly encountered organisms that are clinically suspected, taking into account the history of prior use of antimicrobial agents.

SUMMARY

Despite important medical and surgical advances in management of patients, osteomyelitis remains extremely difficult to treat. The relapse rate can be as high as 20%. The optimal management of osteomyelitis requires a multidisciplinary team of physicians, including an orthopedic surgeon, neurosurgeon, oral surgeon, plastic surgeon, vascular surgeon, invasive radiologist, and infectious disease specialist. The usual goal of therapy is the eradication of the infection and restoration of function. Treatment of chronic osteomyelitis usually requires aggressive surgical débridement and prolonged antimicrobial therapy.

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The complete reference list is available online at Expert Consult.

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105 Orthopedic Implant–Associated Infections

Werner Zimmerli and Parham Sendi

SHORT VIEW SUMMARY

PERIPROSTHETIC JOINT INFECTION (PJI)

Diagnosis

- Determination that a prosthetic joint is infected depends on recovery of an organism from a joint aspirate or surgically obtained material from the joint, although inflammation, wound dehiscence, a draining sinus tract, joint effusion, and early loosening of the prosthesis can point toward this diagnosis.

Microbiology

- The most frequent microorganisms are *Staphylococcus aureus,* coagulase-negative staphylococci, and streptococci.
- *Cutibacterium* (formerly *Propionibacterium*) *acnes* is the most important microorganism in periprosthetic shoulder infection.
- Microorganisms establish a biofilm on the surface of the implant.

Pathogenesis

- Foreign devices are covered by host proteins (e.g., fibronectin) after implantation, favoring bacterial adherence.
- The minimal infecting dose is greater than 10,000-fold lower in the presence than in the absence of an implant.
- Granulocyte function around the implant is impaired (activation and degranulation).

Clinical Manifestations

- Acute exogenous PJI produces local signs of inflammation (wound dehiscence, secretion, erythema).
- Acute hematogenous PJI involves new-onset pain at any time after implantation, initially without local signs of infection, and new-onset articular effusion.
- In chronic PJI, pain occurs because of early loosening, local inflammation, sinus tract, and chronic articular effusion.

Treatment

- The cornerstone of successful treatment is early diagnosis.
- Cure is possible only with adequate surgery combined with appropriate antibiotic therapy.
- A treatment algorithm (see Fig. 105.1) allows one to choose the most appropriate surgical intervention: débridement with retention, one-stage exchange, two-stage exchange, removal without replacement, or suppressive therapy.
- Guidelines are available at www.idsociety.org/Organ_System/#Skeletal%20%28Bones%20&%20Joints%29.

INTERNAL FIXATION–ASSOCIATED INFECTION

Diagnosis

- Diagnosis of infection associated with implanted devices used to stabilize bone fractures, such as pins and rods, requires culture of the device, usually after surgical removal. Pain, inflammation, wound dehiscence, or loosening of the fixation device often indicates infection.

Microbiology

- Microbiology is comparable with that of PJI. Staphylococci are the most important pathogens.
- In open fractures, the wound is contaminated with environmental bacteria.

Pathogenesis

- The exogenous route plays a more important role than the hematogenous route.
- Infection may also occur via an adjacent focus (contiguous).

Clinical Manifestations

- Acute early postoperative infections cause wound healing disturbances, discharge, and erythema.
- In the case of acute symptoms that occur after an uneventful postoperative period, if systemic infection signs are dominating, pain is the most important local sign. Other local features are absent in the beginning and become apparent in the course of disease.
- Acute symptoms may also be due to reactivation of chronic posttraumatic osteomyelitis that has been silent for many years: Pain may occur without prominent systemic inflammatory signs.
- Chronic symptoms include sinus tract, pain, and implant loosening.

Treatment

- Bone fractures are less susceptible to infection if stabilized.
- In acute infections, retain implant with débridement and antimicrobial therapy until the fracture is consolidated.
- In chronic infections, the implant must be removed. When delayed infections on implants are suppressed with antimicrobials, the hardware must be removed after healing of bone fracture.

Orthopedic devices are used to stabilize bone fractures, fuse the vertebral column, correct deformities such as scoliosis, and replace damaged joints.[1,2,3] Whereas internal stabilization devices could be removed after a bone fracture is healed, prosthetic joints are kept in the body as long as their function remains full and pain free. The presence of an implant increases the risk for an infection because its local susceptibility for bacterial adherence is high.[4] Thus, even with the most meticulous infection control, implant-associated infections occur. The optimal management of orthopedic implant–associated infections requires close cooperation of experienced specialists in infectious diseases, microbiology, orthopedic and trauma surgery, and plastic surgery.

PERIPROSTHETIC JOINT INFECTION

Definition

The classic Centers for Disease Control and Prevention definition of wound infection is not applicable for implant-associated infections,[5] because superficial and deep wound infection cannot reliably be differentiated clinically.[6] Therefore, even in the case of a presumably "superficial" wound infection, revision surgery must be considered. Empirical antibiotic treatment without diagnostic workup should be avoided in the absence of life-threatening sepsis.

The definition of periprosthetic joint infection (PJI) should have a high sensitivity, in order not to delay the diagnosis of PJI. Use of the modified Infectious Diseases Society of America (IDSA) criteria increases the sensitivity of the diagnosis (Table 105.1).[3,7–11] C-reactive protein (CRP) is not a sensitive parameter in patients with PJI caused by low-virulence microorganisms.[12] The detection of microorganisms in intraoperative specimens from synovial fluid, tissue, or bone cement fragments is important. Swabs from the operative site or from a draining sinus are difficult to interpret and are not recommended. According to Bémer and colleagues,[13] four perioperative samples seeded on three culture media were sufficient for diagnosing PJI. As a criterion for PJI,

TABLE 105.1 Definition of Periprosthetic Joint Infection (at Least One Criterion Required)

- Presence of a sinus tract communicating with the prosthetic joint.
- Presence of purulence without another known etiology surrounding the prosthetic device.
- Acute inflammation consistent with infection at histopathologic examination of periprosthetic tissue (>5 granulocytes per high-power field [HPF] as average in 10 HPFs).
- Elevated leukocyte count in the synovial fluid or predominance of neutrophils, or both.[14,15]
- Growth of identical microorganism in at least two intraoperative cultures or combination of preoperative aspiration and intraoperative cultures in case of a low-virulence microorganism (e.g., coagulase-negative staphylococci, *Cutibacterium acnes*). In case of a virulent microorganism (e.g., *Staphylococcus aureus, Escherichia coli*), growth in a single specimen from synovial fluid or periprosthetic tissue, or both, and/or sonication fluid may also represent periprosthetic joint infection. However, growth in a single specimen must always be considered in the context of other criteria, and the constellation of diagnostic procedures (e.g., previous antimicrobial treatment) must be taken into account.

detection of the same microorganisms in at least two samples is required. A single positive culture of a pathogen belonging to the skin microbiome (e.g., *Cutibacterium acnes,* coagulase-negative staphylococci) does not confirm PJI. There are criteria for PJI irrespective of proof of a pathogen, as shown in Table 105.1. These include an abscess or a sinus tract that communicates with the joint or the presence of purulence in the affected joint. Other criteria for PJI include an elevated leukocyte count or the predominance of neutrophils in the synovial fluid or both,[14,15] and acute inflammation consistent with infection at histopathologic examination of periprosthetic tissue.[16,17]

Pathogenesis

Prosthetic joints are made of metal and polymers, most commonly polyethylene inlay between metal shell and metal head. Bone cement (i.e., polymethyl methacrylate [PMMA]) is frequently employed at the metal-bone interface. Biocompatible materials do not cause inflammation in the absence of infection and are therefore selected for implantation.[18]

After implantation, the device is covered by host proteins (e.g., fibronectin) favoring bacterial adherence.[19] Complement in interstitial fluid can be activated at the surface of polymeric implants, such as polyethylene particles, which results in degranulation of local neutrophils.[4] In addition, it has been shown that granulocytes interact with the nonphagocytosable surface of implants. This process is called "frustrated phagocytosis" and results in impaired granulocyte function.[4,20,21] Implants and polymer particles, which are produced in variable amounts after arthroplasty, compromise granulocyte function.[22] This interaction with wear particles, fragments from within the joint space, results in an impaired granulocyte function and favors aseptic loosening of joint prostheses.[23]

Implanted foreign material is highly susceptible to local infection. In 1957, Elek and Conen[24] showed in human volunteers that in the vicinity of suture material, the minimal abscess-forming dose is as low as 100 colony-forming units (CFUs) of *Staphylococcus aureus,* which is more than 10,000-fold lower than in the absence of foreign material. This observation was reproduced in an animal model of foreign body–associated infection using different gram-positive and gram-negative microorganisms.[20,25,26,27,28] The route of infection can be exogenous, hematogenous, or contiguous spread. The last route is rare and implicates spread from an adjacent focus of infection (e.g., osteomyelitis, deep soft tissue infection). The animal model demonstrated that microorganisms can seed on an implant from a distant site by the hematogenous route.[29] Finally, the exogenous route includes a joint inoculation of microorganisms during surgery or in the early postoperative period. Exogenous infections can also occur later via a penetrating event (e.g., trauma, injection).

Implant-associated microorganisms growing as a biofilm are protected from phagocytosis.[30] In addition, most antibiotics do not kill adherent bacteria despite penetration into the biofilm.[31,32] The lack of efficacy of many antimicrobial agents depends on the low growth rate of organisms in the biofilm.[33] As an exception, rifampin can eliminate biofilm-associated gram-positive microorganisms,[25,34,35,36] and fluoroquinolones are active against biofilms from gram-negative bacilli.[27,28,37,38]

Classification

No classification system of PJI is unanimously accepted. On the basis of the two major infection routes, PJI can be classified as either exogenous or hematogenous infection.[39] In considering the virulence of bacteria and the host response of immunocompetent patients, PJI can be classified on the basis of symptoms as acute or chronic. The traditional classification differentiates among early (those developing within <3 months after implantation), delayed (3–24 months after surgery), and late (>24 months after implantation) infection.[3,7] In clinical practice, it is more useful to classify PJI as follows:

- Acute hematogenous PJI: Infection with 3 weeks' duration of symptoms or less after an uneventful postoperative period
- Early postinterventional PJI: Infection that manifests within 1 month after an invasive procedure (surgery or arthrocentesis)
- Chronic PJI: Infection with symptoms that persist for more than 3 weeks and are beyond the early postinterventional period

This simple classification allows the differentiation between PJI, which can be potentially cured with débridement and implant retention, and the infection that requires device removal for cure.[3,7] Acute hematogenous infection is generally caused by virulent bacteria, and débridement is required in order for the device to be retained. This type of PJI can occur at any time after surgery, as long as the implant is in the body.[40–44] Early postoperative PJI manifests with poor wound healing, joint fluid accumulation, or persisting pain. In these cases, early detection with a high degree of suspicion, rapid diagnostic workup, and prompt surgical treatment are required for retention of the implant. If these (exogenous) infections are not detected early after surgery (within the first month) or if symptoms persist for more than 3 weeks, or both, chronic PJI develops, irrespective of the infection route. This form of infection requires removal of all hardware and bone cement for cure.

Risk Factors

Several risk factors have been described for the development of PJI.[6,45–55] Most data are derived from patients with total hip and knee arthroplasties. Some of the proposed risk factors have to be interpreted with caution because the corresponding studies used different statistical methods or focused on only one particular anatomic site. Risk factors for acquiring PJI can be categorized as patient characteristics, surgery-related and postoperative factors, and risk during bacteremia.[55] Goltz and colleagues[56] showed that the individual risk for PJI can be reliably predicted by using the American College of Surgeons (ACS) National Surgical Quality Improvement Program (NSQIP) Surgical Risk Calculator (http://riskcalculator.facs.org/).

Patient Characteristics

Diabetes mellitus, obesity (body mass index ≥30 [weight in kg/height in m^2]), rheumatic diseases, and immunosuppressive therapy are among the most frequently reported risk factors for developing PJI. These factors influence wound healing and predispose to many infectious diseases. Congestive heart failure, chronic pulmonary disease, peripheral vascular disease, malignancy, coagulopathy, and preoperative anemia, among other comorbidities, have been described as risk factors for PJI. Preoperative asymptomatic bacteriuria is not a risk factor.[57,58] Overall, it is rather the "net state of polymorbidity" of a patient (e.g., number of comorbidities, physical status) than a single variable that plays a unique role in infection pathogenesis. Finally, the patient's history with respect to previous tissue damage should be considered. Previous joint surgery, mainly previous arthroplasty, has been identified as a risk factor.

Surgery-Related Risk Factors

The complexity and duration of the procedure increase the risk of the joint to become inoculated with microorganisms. A prolonged operative time and multiple simultaneous joint revisions are risk factors for acquiring PJI.

Postoperative Factors

In essence, all wound complications (e.g., delayed healing, drainage or persistent dehiscence, hematoma, seroma, wet wound after initially being dry) increase the risk for infection. Also, postoperative hyperglycemia is a risk factor.[45] Finally, there are risk factors for PJI that are primarily not associated with the surgical procedure or its wound healing. These include atrial fibrillation, myocardial infarction, and a prolonged hospital stay.

Risk During Bacteremia

Urinary tract infection in the postoperative period has been identified as a risk factor for PJI. The incidence of hematogenous seeding to a joint from a remote infection is, however, low (0.1%).[59] This is different for *S. aureus*. The rate of PJI after *S. aureus* bacteremia is approximately 35%.[41–43] This high infection rate indicates that patients with previously uninfected prosthetic joints and *S. aureus* bacteremia should be carefully monitored clinically for the development of PJI. Again, rapid diagnosis of PJI may avoid an exchange of the prosthesis because these infections can be cured with débridement and implant retention.

Microbiology

As a rule, all microorganisms can cause PJI, including mycobacteria.[60,61] However, staphylococci are the most commonly isolated microorganisms. In five studies with a total of 1130 episodes of PJI, the following microorganisms were detected.[6,62,63,64,65]

- *S. aureus,* 21% to 43%
- Coagulase-negative staphylococci, 17% to 39%
- Streptococci, 7% to 12%
- Gram-negative aerobic bacilli, 5% to 12%
- Enterococci, 1% to 8%
- Anaerobic bacteria, 2% to 6%

Other microorganisms such as diphtheroids and *Candida* spp. cause PJI less frequently.

C. acnes is responsible for about 3% of periprosthetic hip and knee infection but up to 38% of periprosthetic shoulder infection.[65,66] In about 15%, polymicrobial infection occurs.[67] Infections with mixed flora are mainly observed in patients with protracted wound healing, resulting in exogenous superinfection.[68] In 4% to 15% of cases, no microorganism is detected.[6,62–65,67,69] This proportion depends on the frequency of pretreatment with antimicrobial agents, type of microorganism, and microbiologic technique.

Clinical Manifestations and Differential Diagnosis

Acute Periprosthetic Joint Infection

The description of the clinical manifestation stems mainly from hip and knee PJI. However, PJI in other joints may manifest differently.[70] Acute exogenous infection typically causes local signs of inflammation, whereas acute hematogenous infection is characterized by new-onset pain, initially without prominent local signs of infection. Acute infections are usually caused by virulent pathogens, mainly *S. aureus,* β-hemolytic streptococci, and less frequently gram-negative bacilli.[3,71]

Most early (postoperative) acute infections have an exogenous origin. Wound dehiscence, drainage, and erythema are typical signs. A systemic inflammatory response syndrome may be missing.[3,39] The differential diagnosis of postoperative acute PJI includes wound complications such as hematoma or seroma. Because the clinical differentiation of superficial and deep wound infection is not reliable,[6] each suspicious wound needs a careful orthopedic evaluation (e.g., surgical exploration of the site) in order to establish or exclude PJI.[72] Consideration of this principle increases the fraction of patients who can be cured with débridement and implant retention.

Acute-onset PJI occurring beyond the postoperative period is generally of hematogenous origin (acute hematogenous PJI). In such infections, systemic signs of inflammation are prominent. The patient typically reports profound pain at the site of the implant, although soft tissue damage is less frequent and less obviously visible than in early exogenous PJI.[3,39] Consequently, the differential diagnosis of acute PJI after an uneventful postsurgical period is narrow. It includes crystal arthropathy, which can be detected with microscopic examination of the synovial fluid and biopsy samples.[73] Venous thrombosis and arterial emboli in vessels that are in close anatomic proximity to the joint can imitate clinical signs of PJI.

All patients with acute symptoms, irrespective of the interval between prosthesis implantation and clinical manifestation, require rapid diagnostic workup because the implant can potentially be retained if symptom duration is short.[3,7,72]

Chronic Periprosthetic Joint Infection

Chronic PJI can be exogenous or hematogenous. If the joint is infected at surgery by low-virulence organisms, infection often manifests beyond the early postoperative period (≥1 month). When symptoms persist for several weeks, irrespective of infection route, the PJI becomes chronic. The key symptoms are chronic joint effusion, pain caused by local inflammation or implant loosening, and, occasionally, sinus tracts. In addition, CRP and erythrocyte sedimentation rate (ESR) do not normalize after surgery and typically fluctuate within a slightly elevated range. However, these tests are not specific. The differential diagnosis includes mechanical failure, excessive wear debris, or allergy to the implant material.[74,75] The sensitivity of CRP is limited in patients with low-virulence microorganisms. It has been shown to be normal (<8 mg/L) in 32% of patients with documented chronic PJI.[12] If early loosening is caused by mechanical failure, one-stage exchange is appropriate, whereas in patients with septic loosening the surgical strategy depends on the infecting agent.[76] Thus, it is crucial to identify PJI cases before definitive surgery. The consequence of missing a low-grade infection is an inadequate revision arthroplasty and subsequent failure. In view of the generally long-lasting course in chronic PJI, there is sufficient time for thorough diagnostic procedures.

Diagnostic Procedures

Blood Tests

Leukocyte counts are diagnostically not helpful owing to poor sensitivity and low predictive values.[77] CRP and ESR are routinely used in the diagnostic workup of PJI. Both tests have good sensitivity but poor specificity. With a threshold at greater than or equal to 30 mm/h (ESR) and at greater than or equal to 10 mg/L (CRP), the sensitivities are 91% to 97% and the specificities are 70% to 78%.[8,77,78] Thus, both tests can be used to estimate the likelihood of a PJI with negative predictive values of about 96%. Sensitivity is less for low-virulence organisms, such as *C. acnes.*[12,79,80] Procalcitonin (PCT), which is a promising biomarker in patients with respiratory tract infections, has not proved helpful in differentiating septic from aseptic loosening.[81,82] In addition, postoperative serum PCT levels are rarely elevated in patients with PJI without sepsis syndrome.[83] In contrast, interleukin-6 (IL-6) values above the cutoff value of 10 pg/L appear to have an excellent sensitivity of nearly 100% to predict the presence of PJI.[77,81] However, this test is not generally available and is costly.

Synovial Fluid Cell Counts

The threshold of leukocyte counts in synovial fluid for the diagnosis of PJI is much lower than for septic native joint arthritis. Published cutoff values show a sensitivity and specificity of about 90%. Because of differences in counting methods (automated versus manual) and interlaboratory variations in counting results, there is no uniformly accepted precise cutoff value. In hip arthroplasty, Schinsky and colleagues[15] reported optimal cutoff values of more than 4200 leukocytes per microliter, neutrophil fraction greater than 80%, or both. In patients with periprosthetic knee infection, Trampuz and colleagues[14] defined an optimal cutoff value of more than 1700 leukocytes per microliter, neutrophil fraction greater than 65%, or both. Similar results were shown by Ghanem and colleagues[84] for periprosthetic knee infection (more than 1100 leukocytes per microliter, >64% neutrophil fraction, or both). In these studies, patients with rheumatoid arthritis or joint hemorrhage, or those in the early postoperative period, were excluded. However, similar cutoff values were reported in synovial fluid of patients with and without inflammatory arthritis in periprosthetic hip infection, namely 3450 leukocytes per microliter versus 3444 leukocytes per microliter, and 78% and 75% neutrophil fraction, respectively.[85] In synovial fluid drawn shortly after surgery, the proposed cutoff values are much higher, namely,

more than 25,000 leukocytes per microliter.[86] In summary, leukocyte and differential counts are key parameters for the diagnosis of PJI, especially in patients with negative culture.

Synovial Fluid Culture

Conventional microbiologic cultures of synovial fluid have a moderate sensitivity of approximately 85% but an excellent specificity of greater than 95%.[78] However, published results often do not stratify whether the fluid was obtained by puncture, arthroscopy, or open surgery. A negative culture result does not exclude PJI. The sample sensitivity can be improved by use of polymerase chain reaction (PCR)[87] or by culturing synovial fluid in blood culture flasks.[88]

Intraoperative Samples for Culture and Histopathology

Swab cultures clearly have a lower sensitivity than cultures from periprosthetic tissue and synovial fluid, and must therefore not be used.[88] Culturing synovial fluid and periprosthetic tissue has the best accuracy, specificity, and sensitivity.[78] For the diagnosis of PJI, at least three specimens should be obtained.[7,89] The greatest accuracy is observed with four specimens processed with conventional culture and with three specimens cultured in blood culture bottles.[13,90,91]

Biopsy culture of *C. acnes* requires anaerobic conditions. Incubation time depends on multiple factors (e.g., time from sampling to laboratory, material preparation, culture media, incubation techniques), and hence may vary among institutions. Recent investigations have reported an incubation time of up to 10 days.[92,93]

Histopathologic specimens are difficult to interpret because the threshold of neutrophils per high-power field (HPF) varies among different experts, ranging from an average of 1 or more to 10 or more neutrophils when counting 10 HPFs. Many experts accept 5 or more neutrophils per HPF in ×40 magnification as a positive indicator for PJI.[17] Frozen sections can be used only in centers with experienced pathologists who are able to differentiate between mechanical failure and infection. This technique informs the surgeon in the operating room whether infection can be excluded, and therefore whether the planned one-stage exchange for early loosening can be performed.[94] In cases of chronic PJI, we recommend paraffin sections, which have been reported to be superior to frozen sections. If possible, each tissue sample should be cut into two pieces, labeled with their precise origin, and submitted one each to microbiologic and histopathologic examination. If dividing tissue sample is not possible (e.g., bone biopsies), two samples from the same location should be obtained. The comparison between culture results and histopathologic findings helps to differentiate between contamination and infection.

Sonication

Sonication of foreign-body material, such as an implant or parts of it, followed by culturing or use of molecular methods on the sonicate fluid, or both, allows detection of microorganisms in biofilms.[95] In a population with either PJI or aseptic failure, Trampuz and colleagues[96] performed a prospective trial comparing culture results from synovial fluid, periprosthetic tissue, and sonicate fluid. In this trial, the sensitivities for detecting PJI with the three different methods were 56.3%, 60.8%, and 78.5%, respectively, with specificities greater than 98%. According to a meta-analysis of 13 studies, the sensitivity of sonication culture is significantly better than that of traditional culture.[97,98] Improved sensitivity has also been reported when sonication of synovial fluid preceded PCR.[99]

Novel Diagnostic Procedures

α-Defensin Test

A quantitative and a qualitative immunoassay for α-defensin in joint fluid is commercially available (Synovasure; CD Diagnostics, Claymont, DE). α-Defensin is an antimicrobial peptide that is released by neutrophils and natural killer cells in response to inflammation. The assay has been reported to assist in the diagnosis of PJI, although its usefulness is controversial.[100,101,102] The result is not influenced by prior antibiotics, but metallosis is a potential source of false-positive results.[103,104] The necessity of sending the sample to the company for testing and the cost will be factored into the decision to use the test. In two recent studies, a lateral flow assay for rapid detection of α-defensin in synovial fluid had a limited sensitivity of 67% and 69%, respectively.[105,106] In addition, it has been shown that the sensitivity of leukocyte count is higher than that of the qualitative α-defensin test (86% vs. 54%; $P < .001$).[107] A strip test for detecting leukocyte esterase has also been used intraoperatively but has low sensitivity and gives false-positive results in bloody specimens.[108]

Molecular Diagnostics

Molecular methods have been used in synovial fluid, periprosthetic tissue specimens, and sonicate fluid from explanted prostheses or modular parts of them. Different techniques have been used, either broad-range PCR or specific multiplex PCR.[97,109,110] In patients pretreated with antibiotics, the sensitivity of broad-spectrum PCR is higher than that of culture, but still only about 70%.[87] In addition, it does not give information about the susceptibility of the microorganism with a few exceptions (e.g., *mecA* gene for methicillin resistance, *rpoB* gene for rifampin resistance). Thus it is mainly useful in combination with conventional cultures in pretreated patients. Use of specific PCR primers is potentially more sensitive for detecting defined microorganisms.[111] However, in a recent study the sensitivity of a multiplex PCR in sonication fluid (Unyvero i60 [Curetis Cie, Holzgerlingen, Germany], designed for detection of PJI) was not better than that of sonicate culture (53% vs. 58%).[112]

Imaging Procedures

Radiographs have a low sensitivity and specificity for the diagnosis of PJI. Radiolucency, osteolysis, and migration are signs of not only infection but also aseptic loosening.[113] Hence, the diagnostic role of radiography is limited. Ultrasonography is helpful in cases with joint effusion that cannot be clinically diagnosed and where guidance for joint aspiration is necessary. Computed tomography (CT) allows detection of soft tissue infection (abscesses, sinus tracts), prosthetic loosening, and bone erosion. With the use of special techniques, metallic artifacts can be minimized. However, this technique is more often used to estimate the extent of an infection than to determine whether or not an infection is present. Imaging interference occurs in the vicinity of metal implants. The indication for magnetic resonance imaging (MRI) is similar to that for CT.

With radionuclide imaging, signs of infection are visible before anatomic changes. The three-phase bone scan using a bone-seeking tracer (e.g., technetium 99m–labeled methylene diphosphonate [^{99m}Tc-MDP]) is sensitive for detecting infection but has poor specificity.[114] Bone remodeling and hence marker uptake is increased for at least 1 year after implantation.[115] Furthermore, heterotopic ossification and aseptic loosening also increase tracer uptake. The specificity can be improved to 80% to 90% with use of a more specific radiotracer such as ^{99m}Tc-ciprofloxacin (Infecton) or ^{99m}Tc–antigranulocyte monoclonal antibodies.[116,117] The use of labeled leukocytes is cumbersome and depends on the quality of the purification and labeling.[118] Unfortunately, the spatial resolution of nuclear scanning techniques is limited. Single-photon emission computed tomography plus conventional CT (SPECT-CT), which is performed with an integrated hybrid machine, offers a more precise localization of the radiotracer and should therefore be preferred. This method is mainly used with ^{99m}Tc-MDP, labeled leukocytes, or labeled antigranulocyte monoclonal antibodies. It improves the sensitivity and specificity as compared with the planar image. In 31 consecutive patients with suspected low-grade PJI, sensitivity, specificity, and accuracy improved from 66%, 60%, and 61%, respectively, to 89%, 73%, and 77%, respectively. The prevalence of PJI in this population was 29%.[119]

Positron Emission Tomography

Positron emission tomography (PET) examination plays an important role in the detection of malignant tumors. ^{18}F-fluorodeoxyglucose (FDG) accumulates in cells such as neutrophils and is intracellularly phosphorylated to a stable molecule. Therefore accumulation occurs in not only tumors but also inflammatory foci. According to a systematic review of 11 studies, including a total sample size of 635 prostheses, FDG-PET has good sensitivity (84.6%; 95% confidence interval [CI], 71%–93%) and specificity (84%; 95% CI, 68%–93%) for the detection of PJIs in

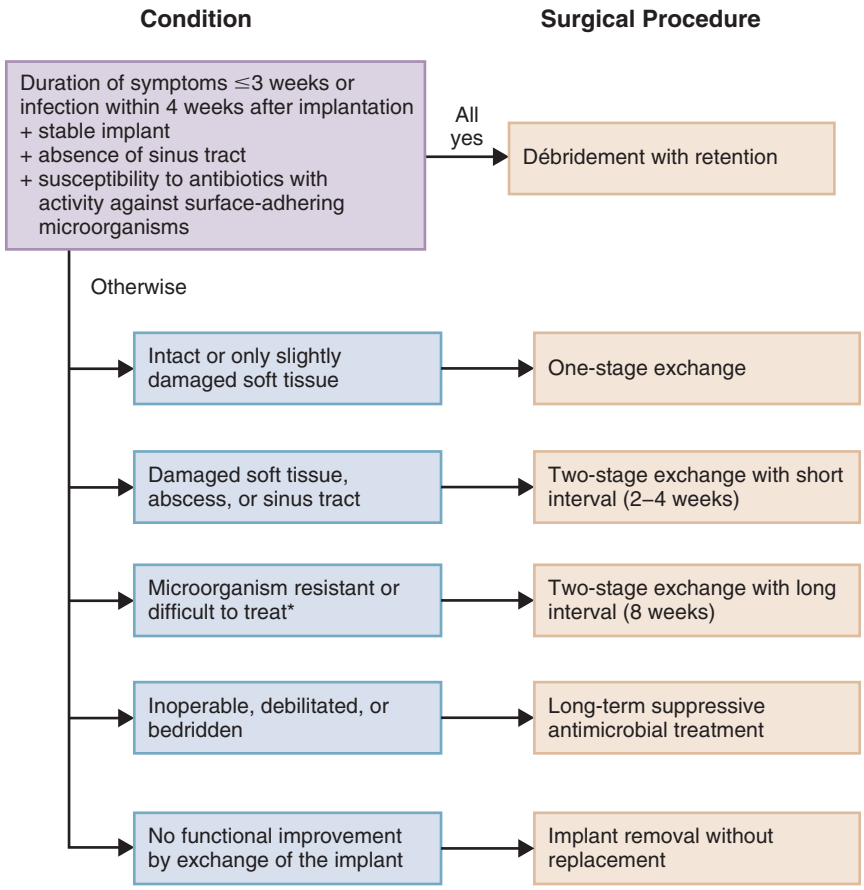

FIG. 105.1 Surgical treatment algorithm for prosthetic joint infections. *Difficult-to-treat microorganisms include microorganisms resistant to antibiotics with good oral bioavailability, rifampin-resistant staphylococci, and quinolone-resistant gram-negative bacilli and fungi. *(Modified from Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment.* Swiss Med Wkly. *2005;135:243–251.)*

hip and knee arthroplasties.[120] However, the individual studies show sensitivities between 22.2% and 100%, and specificities between 61.5% and 100%. Reasons for these inconsistent results are the nonspecific postoperative FDG uptake after total knee arthroplasty, the prolonged increased FDG uptake around the femoral neck, and the nonspecific FDG uptake around the distal part the prosthetic stem.[114,121] Thus PET cannot be recommended for routine clinical practice. In many countries costs are not covered for these novel imaging techniques.

Treatment Concepts

General Aspects

Cure at the first treatment attempt is crucial because with each treatment failure, tissue damage and functional integrity are worse. Delayed ambulation increases loss of muscle strength and increases chances of thromboembolism. Therefore, early referral to specialized centers is advised. As a rule, before starting treatment, it should be clear whether a curative or suppressive (palliative) approach will be taken. Cure is defined as a long-term pain-free functional joint with complete eradication of infection after a follow-up of 24 months following surgery. Eradication surgery requires a combination of both an appropriate surgical procedure and antimicrobial therapy. In contrast, palliative therapy is geared toward suppression of infection and thus of symptoms. In general, only minor or no surgery is performed with this strategy. Lifelong suppressive antimicrobial therapy may be necessary because the chance for cure is minimal.

The cornerstone of successful treatment is early diagnosis. The earlier the diagnosis, the less invasive is the surgical therapy. Accordingly, a high degree of suspicion is required and empirical antibiotic therapy without unequivocal diagnosis must be avoided.

Traditional standard treatment included two-stage exchange with meticulous removal of all necrotic tissue, bone cement, and the prosthesis combined with a prolonged course of antibiotics during the implant-free interval.[122] During the past 2 decades, an algorithm for the optimal surgical therapy of the different presentations was developed at our institute.[3,123] Choosing the least invasive treatment that cures infection is the most rational approach (Fig. 105.1). Because data from controlled trials comparing different surgical procedures are lacking, treatment concepts vary among different centers. Recommendations are therefore based on case series and expert opinions and published in the IDSA guidelines.[3,7,71,123] Considering the prerequisites for the successful use of each surgical procedure, all surgical interventions have a favorable outcome in more than 80% of the patients.[a]

Antimicrobial treatment without any surgical intervention is not curative but only suppressive. There are three different curative options: débridement and implant retention, one-stage exchange, and two-stage exchange. In addition, in special situations, microbiologic cure without restoration of full function can be reached with removal without replacement, arthrodesis, or amputation. There are some controversies among different specialized centers: (1) One-stage exchange is less commonly performed in the United States than it is in European centers; (2) in the case of two-stage exchange, the approach with a short interval is used in only a minority of centers; and (3) some centers continue suppressive antimicrobial therapy despite curative approach, considering the small uncertainty that the infection is not cured.[7,128] However, the benefit of stopping treatment (e.g., reducing side effects) outweighs that uncertainty. These controversial aspects will remain until controlled trials have been conducted. Fig. 105.1 shows a treatment algorithm that allows choosing the most appropriate surgical intervention based on several variables.[3,123] This algorithm has been developed for patients with hip and knee PJI.[3,62,63,129] In addition, it has also been implemented for shoulder and elbow prostheses.[66,130] In one review of shoulder

[a]References 3, 35, 36, 62, 63, 124, 125, 126, 127.

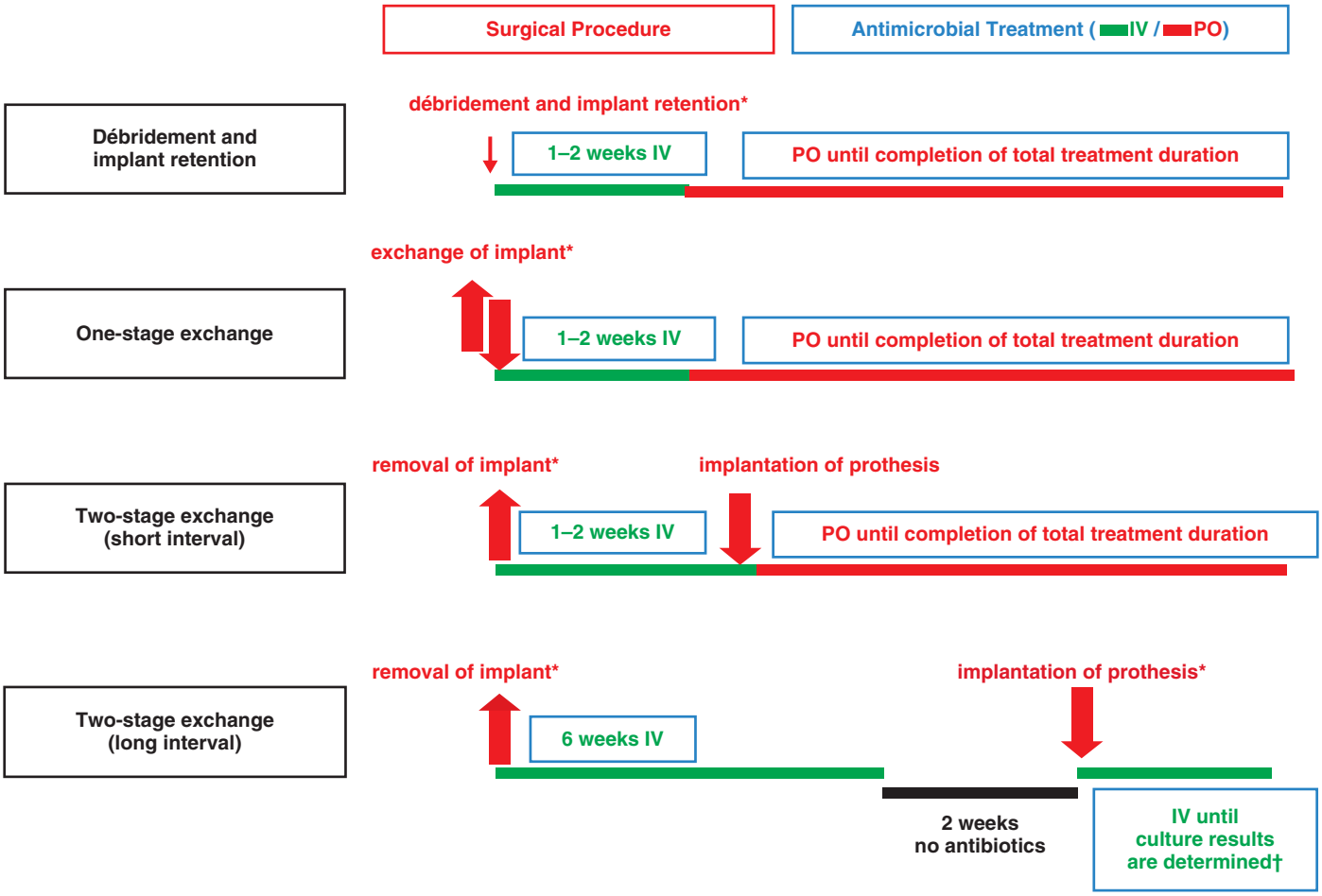

FIG. 105.2 Surgical intervention, route, and duration of antimicrobial therapy according to Zimmerli and colleagues.[3] *IV,* Intravenous route; PO, oral route; *, microbiologic sampling; †, continuation with the same antibiotic regimen. Samples are cultured for 10 to 14 days in most centers that are specialized for periprosthetic joint infection. If microbiologic results are confirmed to be negative, the antimicrobial treatment can be stopped.

periprosthetic infections, the rate of cure was 90% to 92% whether the implant was replaced in a one- or a two-stage procedure.[131] In contrast, the optimal surgical management of ankle PJI has not yet been defined.[70] In these patients, the surgical strategy is mainly dictated by the more difficult soft tissue situation. Fig. 105.2 shows the sequence of surgical interventions and the duration of intravenous and oral antimicrobial therapy.

Débridement With Retention of Implant

The success rate of this procedure is traditionally widely underestimated, because there are many published series with patients not qualifying to undergo it. However, the success rate is not lower than that of exchange surgery, provided that the following four conditions are fulfilled[b]:

- Acute infection (<3 weeks' duration of infectious symptoms or <1 month after implantation)
- Stable implant
- Pathogen susceptible to a biofilm-active antimicrobial agent (see later)
- No sinus tract and no periprosthetic abscess

The definition for duration of symptoms is based on a controlled trial.[36] Rapid and meticulous débridement of necrotic tissue is essential. In periprosthetic knee infection, the reported success rate is better after open surgery than after arthroscopic débridement (median reported success rates 86% vs. 56%).[133] In cases of open débridement, modular components should be exchanged, which results in reduction of the biofilm. The advantages of débridement with retention are the minimal invasive surgery and rapid rehabilitation.

[b]References 3, 7, 72, 124, 125, 132–135.

One-Stage Exchange

Direct exchange includes removal and reimplantation during the same surgical procedure. It can be chosen for patients with (1) a good soft tissue envelope and (2) a pathogen that is susceptible to oral antimicrobial agents with excellent bioavailability and activity on biofilms (see later). However, it is crucial to identify the causative pathogen before the surgical procedure. In general, effective antibiotic-impregnated bone cement is used.[136] However, cure rate with use of uncemented revision stems is excellent in infected total hip arthroplasties.[137]

Two-Stage Exchange

The two-stage exchange procedure starts with thorough removal of all necrotic tissue, bone cement, and all of the implant before reimplantation of a new device is performed at a second intervention.[127] After removal of all foreign material, an antibiotic-impregnated spacer, typically PMMA, is inserted in order to achieve some stability, allow some degree of mobility, prevent shrinking of the joint space, and produce a high local concentration of antibiotics. The need for local antibiotics in the spacer has never been proven in a comparative and prospective PJI trial. Interesting to note, in patients with a microorganism resistant to the antibiotic in the spacer, there is no measurable disadvantage regarding the outcome.[138] The advantage of staged exchange is the opportunity for removing all infected material before implantation of the new prosthesis. The disadvantage is the need for two surgical interventions, prolonged disability, and the interval with the biomechanically suboptimal spacer. Therefore, we favor a short interval of only 2 to 3 weeks before reimplantation, except in patients with difficult-to-treat microorganisms.[3] During the interval the bacterial load can be diminished and the soft tissue heals. This allows a treatment during one single hospitalization.

Antibiotics are not stopped before implantation, and no sampling is recommended during implantation. However, antibiotic treatment postoperatively is necessary, as in patients with débridement and retention or one-stage exchange. In patients with difficult-to-treat microorganisms (small-colony variants, rifampin-resistant staphylococci, fluoroquinolone-resistant gram-negative bacilli, fungi), early reimplantation should not be chosen.[3,139] The rationale for the long interval is the concept that difficult-to-treat microorganisms must be completely eradicated before reimplantation. These patients should be treated for 6 weeks. Because of potential adherence and persistence of these types of microorganisms on the spacer, we recommend a two-stage procedure with a spacerless interval.[139] Reimplantation should be delayed for 2 more weeks free of antibiotics in order to obtain reliable samples for microbiologic assessment. In these patients, the same antimicrobial treatment of the previous 6 weeks should be restarted after implantation of the new device. It can be definitely stopped when intraoperative culture results remain negative (see Fig. 105.2).

Antimicrobial Therapy

After microbiologic sampling, antimicrobial agents are administered by the intravenous route mainly for two reasons. First, the risk for emergence of resistance is highest during the initial phase, when the bacterial load is still high. Therefore, subinhibitory antimicrobial concentrations should be avoided. Second, enteral resorption may be compromised during the perioperative phase. Table 105.2 summarizes the pathogen-specific antimicrobial therapy, and Fig. 105.2 shows the timing of the intravenous and the oral therapy. It mainly depends on the type of surgery performed. The correct duration of treatment has never been tested in comparative studies. It is based on the reasoning that biofilm bacteria cannot be killed by host defense alone.[4] Therefore, PJI reoccurs if not all microorganisms are eliminated by débridement and antimicrobial therapy. For patients with implant retention, direct exchange, or two-stage exchange with a short interval, we propose a 3-month regimen. Some experts recommend 6 months for knee prostheses.[3,7] Several mainly observational studies, however, demonstrated

TABLE 105.2 Treatment of Implant-Associated Infections

MICROORGANISM	ANTIMICROBIAL AGENT[a]	DOSE	ROUTE
Staphylococcus spp.	Recommendation for the initial IV treatment phase (for 1–2 wk)		
Methicillin susceptible	Rifampin *plus*	450 mg q12h or 600 mg q24h[b]	PO/IV
	Nafcillin *or* oxacillin[c]	2 g q4h	IV
Methicillin resistant	Rifampin *plus*	450 mg q12h or 600 mg q24h[b]	PO/IV
	Vancomycin *or*	15 mg/kg q12h[d]	IV
	Daptomycin	8–10 mg/kg q24h[e]	IV
Staphylococcus spp.	Recommendation after completion of the initial IV treatment phase		
	Rifampin *plus*	450 mg q12h or 600 mg q24h[b]	PO
	Levofloxacin *or*	750 mg q24h to 500 mg q12h	PO
	Ciprofloxacin *or*	750 mg q12h	PO
	Teicoplanin *or*	400 mg q24h[f]	IV
	Fusidic acid *or*	500 mg q8h	PO
	Trimethoprim-sulfamethoxazole *or*	1 DS tablet q8h[g]	PO
	Minocycline[h] *or*	100 mg q12h	PO
	Linezolid *or*	600 mg q12h	PO
	Clindamycin[i]	1200–1350 mg/day divided in 3–4 doses	PO
Streptococcus spp.[j]	Penicillin G[c] *or*	18–24 million U/day divided in 6 doses	IV
	Ceftriaxone for 4 wk, *followed by*	2 g q24h	IV
	Amoxicillin *or*	1000 mg q8h	PO
	Clindamycin[i]	1200-1350 mg/day divided in 3–4 doses	PO
Enterococcus spp.[k]			
Ampicillin or amoxicillin susceptible			IV
	Ampicillin *or* amoxicillin[l] *or*	2 g q4h–q6h	IV
Ampicillin or amoxicillin resistant	Vancomycin *or*	15 mg/kg q12h[d]	IV
	Daptomycin *or*	6–10 mg/kg q24h[e]	IV
	Linezolid	600 mg q12h	IV/PO
Enterobacteriaceae	β-Lactam based on in vitro susceptibilities for 2 wk[m] *followed by*		IV
	Ciprofloxacin	750 mg q12h	PO
Enterobacter spp.[n]	Cefepime *or*	2 g q8h	IV
	Ceftazidime *or*	2 g q8h	IV
	Ertapenem *or*	1 g q24h	IV
	Meropenem	1 g q8h[o]	IV
	for 2–4 wk, *followed by*		
	Ciprofloxacin	750 mg q12h	PO
Nonfermenters[p] (e.g., *Pseudomonas aeruginosa*)	Cefepime *or*	2 g q8h	IV
	Ceftazidime *or*	2 g q8h	IV
	Meropenem	1 g q8h[p]	IV
	for 2–4 wk, *followed by*		
	Ciprofloxacin	750 mg q12h	PO
Cutibacterium spp.	Penicillin G *or*	18–24 million U/day divided in 6 doses	IV
	Clindamycin[i]	600–900 mg q8h	IV
	for 2-4 wk *followed by*		
	Amoxicillin *or*	1000 mg q8h	PO
	Clindamycin[i]	1200–1350 mg/day divided in 3–4 doses	PO
Gram-negative anaerobes (e.g., *Bacteroides* spp.)	Metronidazole	500 mg q8h	IV/PO

TABLE 105.2 Treatment of Implant-Associated Infections—cont'd

MICROORGANISM	ANTIMICROBIAL AGENT[a]	DOSE	ROUTE
Mixed infections (without methicillin-resistant staphylococci)	Ampicillin-sulbactam *or* Amoxicillin-clavulate[q] *or* Piperacillin-tazobactam *or* Imipenem *or* Meropenem for 2–4 wk, *followed by* individual regimens according to antimicrobial susceptibility	3 g q6h 2.2 g q6h 4.5 g q6h–q8h 500 mg q6h–q8h 1 g q8h[p]	IV IV IV IV IV

Antimicrobial dosage recommendations are based on normal renal and hepatic function. Antimicrobials should be chosen based on in vitro susceptibility, patient drug allergies and intolerances, and potential drug interactions or contraindications to a specific antimicrobial.
[a]For total duration of antimicrobial treatment, see text.
[b]Other dosages and intervals of administration have been reported with equivalent success rates.[145]
[c]In patients with delayed hypersensitivity, cefazolin (2 g q8h IV) can be administered. In patients with immediate hypersensitivity, penicillin should be replaced by vancomycin.
[d]Recommended doses are based on AUC_{0-24}/MIC and trough levels. Trough levels should be monitored for nephrotoxicity.[216]
[e]Recommended dose according to Infectious Diseases Society of America (IDSA) guidelines.[7] However, doses up to 10 mg/kg q24h are reported.[155]
[f]Teicoplanin: Loading dose (days 1–3 of treatment) 800 mg q24h is recommended. Not available in the United States.
[g]Double strength = trimethoprim 160 mg plus sulfamethoxazole 800 mg.
[h]Lack of data on bone penetration. Alternatively, doxycycline 100 mg q12h PO is possible.[35]
[i]Higher doses PO (e.g., up to 2400 mg/day) are possible but frequently not tolerated owing to side effects.
[j]We recommend determination of minimal inhibitory concentration for penicillin.
[k]Combination therapy with an aminoglycoside is optional because its superiority to monotherapy in PJI is unproven.[217] When using combination therapy, monitor for signs of ototoxicity and nephrotoxicity of aminoglycosides; the latter is potentiated with other nephrotoxic agents (e.g., vancomycin).
[l]In patients with hypersensitivity to penicillin, see treatment options for penicillin-resistant enterococci.
[m]In patients with hypersensitivity to β-lactam, ciprofloxacin can be administered.
[n]Cetriaxone and ceftazidime should not be administered for *Enterobacter* spp., even when tested susceptible in the laboratory. We recommend determination of MIC for cefepime. In infections due to *Enterobacter* spp., ertapenem 1 g q24h can be administered alternatively. Ertapenem is not effective against *Pseudomonas* spp. and other nonfermenters.
[o]Dosage recommended according to IDSA guidelines.[7] In Europe, 2 g q8h is suggested in case of *P. aeruginosa* infection.[35]
[p]Addition of aminoglycoside is optional.[7]
[q]Not available in the United States as IV drug.
AUC_{0-24}, Area under the time-concentration curve from time 0 to 24 hours; *DS,* double-strength; *MIC,* minimal inhibitory concentration.
Modified from Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. *2004;351:1645–1654; Sendi P, Zimmerli W. Antimicrobial treatment concepts for orthopaedic device-related infection.* Clin Microbiol Infect. *2012;18:1176–1184; and Osmon D, Berbari E, Berendt A, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America.* Clin Infect Dis. *2013;56:e1–e25.*

similar outcome results with 3-month regimens, in particular when patient selection and treatment were according to protocol.[38,125,135] The treatment course can be shortened to 6 weeks in patients treated with two-stage exchange with a long interval (see Fig. 105.2).

Initial intravenous therapy for 1 to 2 weeks is followed by long-term oral antibiotics. Oral compounds that are used in bone and joint infection should have good bioavailability and reasonable bone penetration.[35,36,38,140–144] Optimal therapy is best defined in staphylococcal implant infections. Rifampin has excellent activity on susceptible biofilm staphylococci. This has been proven in vitro, in animal models, and in several clinical studies.[25,34,36,144] There are no clinical studies proving superiority of rifampin combinations in other gram-positive or gram-negative PJIs.[35] Rifampin must always be combined with another drug for prevention of emergence of resistance. Owing to the risk of superinfection with rifampin-resistant staphylococci from the skin microbiome, we propose starting rifampin combination only in patients with dry wounds—that is, several days after definitive surgery.[145] In addition, we do not use rifampin in the short-interval concept in patients undergoing two-stage exchange. Fluoroquinolones are the first choice for the combination.[3,7,36] Because of increasing resistance, other combinations may be necessary. For each combination, possible interactions with rifampin should be considered.[146] Rifampin should be reserved for patients with implant retention, with one-stage exchange, or after early reimplantation in two-stage exchange. It has no advantage in patients with suppressive therapy and should therefore be discouraged with this type of treatment. Diligent use of rifampin lowers the risk for emergence of resistance.[147]

Because the susceptibility of methicillin-resistant staphylococci to vancomycin is decreasing, novel antistaphylococcal drugs, such as linezolid and daptomycin, may be necessary for the treatment of PJI.[148] Notably, neither is approved for bone and joint infections. Linezolid surpasses the minimal inhibitory concentration in 90% of isolates (MIC_{90}) of enterococci and staphylococci and has a good penetration in human bone.[149] Despite several observational studies in humans, linezolid efficacy in PJI cannot be unambiguously judged.[150–152] According to an observational noncomparative study, in patients with implant retention, monotherapy had a lower cure rate than combination therapy with rifampin. Notably, the best cure rate with implant retention was observed in patients with acute infections and combination therapy.[142] Serious adverse events of long-term linezolid must be considered, including peripheral neuropathy, optic neuritis, and bone marrow suppression. Because bone and joint infections generally require prolonged treatment, the use of linezolid remains controversial.

Daptomycin monotherapy has a low cure rate in animal models of implant-associated infections. However, in combination with rifampin it was highly efficacious in animal models of implant-associated infections.[153,154] Moreover, in contrast to vancomycin, daptomycin combination therapy completely prevented the emergence of rifampin resistance in *S. aureus*.[153] However, the efficacy of daptomycin for PJI cannot be conclusively judged because there are not enough clinical data. If daptomycin is considered for selected cases, the available data point toward treatment regimens with doses ranging from 8 mg/kg up to 10 mg/kg once daily and combination with rifampin.[155] During prolonged treatment, creatinine phosphokinase surveillance is necessary to rapidly detect muscle toxicity. Comedication with statins should be avoided. Also, in case of fever and dyspnea, eosinophilic pneumonia, a rare but life-threatening complication in patients with daptomycin therapy, should be considered.[156]

PJI due to gram-negative bacilli is diagnosed in 10% to 15% of the cases. The risk for these microorganisms is higher in previously treated patients.[157] Patients with gram-negative PJI who qualify for débridement and retention have a good outcome, provided that treatment with a fluoroquinolone is possible, after initial intravenous therapy (see Table 105.2).[37,38,157,158] The rationale for favoring fluoroquinolones is their activity against gram-negative biofilms.[28,37,38,159]

INTERNAL FIXATION–ASSOCIATED INFECTION

Metal hardware for internal fixation of bone fractures, spinal fusions, and corrections of scoliosis are included in this category. Although there are several similarities between the management of PJI and internal fixation–associated infections, there are also important differences. First,

eradication of infection is not always a priority, because the device could be removed after fracture healing or because bridging until definitive stabilization is possible with an external fixation device. Removal of hardware spine implants is often less optional. Second, exogenous infection after surgical treatment for open fractures plays a more important role than in PJI. Third, there is a large variety of anatomic locations where orthopedic devices are fixed, and a large variety of hardware is implanted (e.g., plates, screws, nails, rods), illustrating heterogeneous clinical constellations. Fourth, the involvement of a plastic surgeon for soft tissue coverage after infection in trauma cases plays a more important role than in PJI. The proposed treatment concepts are mainly based on observations and expert opinions. Although the interaction between microorganisms and metal work is similar in PJI and internal fixation–associated infections, classification and management concepts differ.

Definition

There are no commonly accepted criteria for the definition of internal fixation–associated infections. A consensus definition for fracture-related infections was proposed, using suggestive and confirmatory criteria.[160] A combination of clinical, laboratory, imaging, microbiologic, and histopathologic criteria is used to diagnose internal fixation–associated infections. The definition for the histopathologic criterion is not established. It should not be extrapolated from PJI (i.e., polymorphonuclear leukocyte count), because sterile bone necrosis may cause locally limited cellular inflammation. Nonetheless, histopathologic examination can be helpful in establishing the diagnosis (e.g., specific staining for mycobacteria or fungi). Significant growth of microorganisms in sonicate or conventional culture of the device, or growth of the same microorganisms in at least two biopsy samples in proximity to the device, defines infection. In addition to other criteria, a single biopsy sample with growth of a virulent pathogen (e.g., *S. aureus*) may be sufficient to fulfill infection definition. Additional signs of infection are bone loss around metal screws or an intramedullary rod (e.g., nonunion), fluid accumulation around the device, or a draining sinus.

Incidence of Infection

After internal fixation of closed fractures, the incidence of infection is <2%.[161] In patients with open fracture, the incidence of infection gradually increases with the extent of soft tissue damage. Open fractures are generally classified according to the system of Gustilo and colleagues[162] (Table 105.3). Based on this classification, it can be stated that the more severe the open fracture, the higher the incidence of infection. The classification was developed for tibial fractures, but in clinical practice the risk of infection is extrapolated to other anatomic locations. In a systematic review of the literature including 32 studies with 3060 open tibial fractures, type I fracture had an infection rate of 1.8%, and type IIIC 16.1% (see Table 105.3).[163] The use of vacuum-assisted closure may increase the risk of infection.[164]

TABLE 105.3 Open Fracture Classification According to Gustilo et al. and Corresponding Postoperative Infection Rates

FRACTURE TYPE	DESCRIPTION	INFECTION RATE
Type I	Wound is <1 cm long with a clean piercing puncture	1.8%
Type II	Laceration is >1 cm long, no extensive soft tissue damage, the puncture wound is moderately contaminated	3.3%
Type IIIA	Extensive laceration with adequate coverage of the fractured bone	5.0%
Type IIIB	Extensive loss of soft tissue with periosteal stripping and exposure of bone resulting in massive contamination	12.3%
Type IIIC	Open fracture with an arterial injury regardless of the degree of soft tissue injury	16.1%

TABLE 105.4 Classification and Characteristics of Internal Fixation Device–Associated Osteomyelitis

TIME OF ONSET OF SYMPTOMS	CHARACTERISTICS
Early infection (≤2 weeks after implantation)	*Clinical picture:* Signs of wound infection such as persistent fever, pain, erythema, swelling, wound healing disturbances *Typical microorganisms: Staphylococcus aureus,* group A streptococci, gram-negative bacilli
Delayed infection (weeks 3–10 after implantation)	*Clinical picture:* Persisting pain, low-grade fever, mechanical instability, sinus tract *Typical microorganisms:* Low-virulence microorganisms such as coagulase-negative staphylococci or mixed skin flora in case of a sinus tract
Late infection (>10 weeks after implantation)	*Clinical picture:* (1) **acute** hematogenous infection—sepsis syndrome, local pain and signs of inflammation, (2) **chronic** (delayed infection or recurrence of incorrectly treated early infection)—signs of infection after interval with bridging symptoms such as pain, wound-healing disturbances, nonunion *Typical microorganisms:* (1) *S. aureus, Escherichia coli,* (2) any microorganism, including polymicrobial infection

Pathogenesis and Classification

Similar to PJI, the two major routes of infection are exogenous and hematogenous. The relative risk for hematogenous infections is considerably lower than in patients with prosthetic joints. The exogenous route is dominant either via wound contamination due to a penetrating trauma (e.g., open fracture) or via inoculation of microorganisms into the surgical wound during or immediately after surgery (perioperative). In spinal fusions, breakdown of the skin over the hardware can also lead to exogenous infection. In addition, infection may occur via an adjacent ongoing infection, such as skin infection and soft tissue infection (contiguous). On the basis of these routes of infections, internal fixation devices can be classified into:

- Hematogenous
- Exogenous (perioperative or penetrating event)
- Contiguous

A classification according to the time interval from index surgery to infection manifestation is helpful for clinical practice, because it considers three typical features of pathogenesis: (1) infection route, (2) microorganisms, and (3) findings of clinical examination (Table 105.4).[2,165] Early and delayed infections are mainly acquired during trauma (e.g., open fracture) or implant surgery (deep surgical site infection). Early infection is generally caused by high-virulence, and delayed infection by low-virulence, microorganisms. Alternatively, delayed manifestation may be due to postponed early infection. This mainly occurs if a suspected superficial surgical site is treated with empirical antimicrobial therapy without appropriate diagnostic workup. Clinical presentation of late infections can be subclassified into acute and chronic. Acute late infection without bridging symptoms after surgery is typically caused by hematogenous seeding. Chronic late infection occurs as a result of a missed delayed infection or recurs after an inadequately treated early infection. Thus the association among infection route, microorganisms, and time point of clinical presentation cannot be reliably applied in chronic late infections. The corresponding time intervals for this classification (early, within 2 weeks; delayed, occurring in weeks 3 through 10; late, after the 10th week following surgery) have not been scientifically validated[165] but have been used in clinical practice for several decades in the context of a detailed patient history.[166] In early infections, the time of infection

can often be reliably estimated. For the decision regarding surgical management, other criteria than this classification are important (e.g., fracture stability, implant loosening, bone healing).

Microbiology

As in PJI, staphylococci are the most commonly isolated microorganisms in patients with internal fixation device–associated infections. In a study reporting microbiologic data from 777 patients with orthopedic device–associated infection or PJI, the following microorganisms were detected[167]:

- *S. aureus,* 43.5%
- Coagulase-negative staphylococci, 32.9%
- *Streptococcus* spp., 9.0%
- Gram-negative aerobic bacilli, 7.6%
- Enterococci, 3.7%
- Anaerobic bacteria, 0.1%
- Fungi, 1.2%

Regional epidemiologic data about methicillin-resistant *S. aureus* (MRSA) vary considerably among different countries.

In case of open fractures with exposed bone, a polymicrobial flora (including bacteria from the environment) contaminates the wound. Preemptive antimicrobial therapy is often administered to reduce the infection rate. A prolonged course may lead to a selection of one or more resistant microorganisms because no compound is active against all pathogens. For this reason antimicrobial agents with broad spectrum should be avoided.

Clinical Manifestations

The clinical presentation depends on the following variables:

- History of surgical procedure and preceding trauma
- Anatomic localization and surrounding soft tissue
- Time interval between inoculation (e.g., trauma, surgery) and infection manifestation
- Type of microorganism

Symptoms are either acute or chronic. Acute infections manifest typically either early after surgery or late. In early infections, poor wound healing and signs of a surgical site infection such as erythema and wound discharge are the main findings. Wound edge necrosis and hematoma are important risk factors for internal fixation–associated infections. Consequently, clinical signs of poor wound healing after internal fixation placement should rapidly raise the suspicion of infection. Acute infections are frequently caused by virulent pathogens (e.g., *S. aureus*, β-hemolytic streptococci). In acute infections that manifest late, there are two entities with slightly different clinical presentations. In the case of microbial seeding from a distant focus—which can occur any time after surgery—a recent or present history of fever and chills after an uneventful postoperative period, and elevated systemic inflammatory parameters are found. The patient has local pain, but objective signs of a local infection are initially missing. They typically appear only several days after bacterial seeding. The obvious appearance of these local findings depends on the thickness of the soft tissue covering the implant (e.g., visibility at femur versus lower tibia). Therefore it is crucial to have a high degree of suspicion in order to rapidly identify these cases. The second type of acute infection after an uneventful postoperative period is posttraumatic osteomyelitis. In this entity, previous latent infection is reactivated. This may occur even many years or decades after the device has been removed and the bone has apparently healed.[168,169] In the perspective of terminology, these infections are difficult to classify because the fracture is healed, infection pathogenesis is chronic, symptoms are acute, and reoccurrence is late. The dominating symptom is pain. Systemic inflammatory signs such as fever and chills are less frequent than in acute hematogenous cases. The keys to diagnosis are therefore the patient's history and imaging studies performed before invasive procedures. It is, however, not completely clear how and why virulent bacteria reactivate from a dormant state, causing acute symptoms, and it is not yet known what triggers this.

Infections with chronic symptoms occur either in a delayed fashion or late after internal fixation (see Table 105.4). They are due to low-virulence microorganisms (e.g., coagulase-negative staphylococci) or occur after inadequate initial treatment of any microorganism. Although initial wound healing can be observed in these infections, patients often have persisting pain or local signs of inflammation, such as intermittent discharge (sinus tract) or fluctuating erythema in the area of the scar. Impaired bone healing can be seen in imaging investigations. Again, depending on the anatomic localization, clinical signs in the surrounding soft tissue may be completely absent. CRP and ESR typically remain elevated at low levels.

Diagnostic Procedures

The benefit and the sensitivity and specificity of means to diagnose internal fixation device–associated infections mainly depend on the time interval between surgery and infection. All infections should be clinically evaluated by a multidisciplinary team of experts. In early infections, CRP, ESR, and blood leukocyte counts are not helpful to predict infection. When serial CRP measurements are performed, infection should be suspected in the case of persistent high values or significant increase after a postoperative decline. Imaging plays a minor role in patients with early infections. However, both laboratory tests and imaging may be helpful in the delayed and late period.

Microbiology and Histopathology

When infection is suspected, surgical exploration and sampling are required. Three to six biopsy specimens should be obtained in order to optimize sensitivity and specificity.[160] The main difficulty, especially in patients with poor wound healing and poor surrounding soft tissue, is the differentiation between colonizing wound flora and causative pathogens of the device-associated infection. This should be taken into account because the proportion of exogenous infection is higher than in PJI. Therefore it is crucial to explore areas with the most florid tissue inflammation and to perform biopsies in close proximity to the device. The samples should be labeled with the anatomic localization. In the case of infections with chronic low-grade symptoms manifesting at a delayed or late time point, histopathologic examination should be added in parallel. This investigation is helpful in differentiating contamination of the biopsy specimens from true culture-positive results, in particular when low-virulence bacteria of the skin flora are involved and no inflammation is seen at histopathologic examination. If anaerobes are potentially involved, special media and a rapid transport from the operating room to the microbiology laboratory should be organized in order to increase the culture sensitivity.[170] Anaerobes should be suspected in the presence of dead spaces, hematoma, and areas of tissue or bone necrosis. In the case of removal of the hardware or parts of it, cultures in enrichment broth can be performed, although there is a high risk of contamination. Sonication of removed devices may increase the sensitivity of the culture. Comparative data in patients with internal fixation devices are lacking.

Imaging

Conventional radiographs after implantation, in particular when performed in serial follow-ups, are helpful to estimate bone healing, formation of a nonunion or sequestration, implant loosening, bone loss around screws, and migration of devices. However, there are no specific signs indicating infection. Therefore, additional parameters for infection are necessary for infection diagnosis. This is also true when early device loosening is detected. Ultrasound may reveal fluid collection in the proximity of a device and help guide puncture for microbiologic sampling. However, fluid collection is mainly seen in infections with acute symptoms. Therefore, for rapid and correct diagnosis, open surgery is more appropriate than fluid sampling. CT is helpful to estimate the extent of inflamed tissue and allows the detection of bone necrosis. Similar to conventional radiographs, serial CT scans can help in estimating the success of treatment by enabling evaluation of bone healing. Nuclear medicine imaging modalities may be helpful in estimating bone viability and the presence of sequestra in posttraumatic osteomyelitis,[171] but are not routinely used for the detection of internal fixation device infections. Their sensitivity and specificities have as not been investigated in large trials, and the costs are considerable. When these modalities are used, it is critical to discuss beforehand which nuclear technique (e.g., three-phase bone scan with SPECT-CT, white blood cell scintigraphy with SPECT-CT, FDG-PET/CT) should be used to answer a specific clinical question.[171]

Treatment Concepts

General Aspects

It is essential to establish the diagnosis of implant-associated infections early. Treatment goals are fracture consolidation and prevention of chronic osteomyelitis. The longer the disease duration, the more established the biofilm formation becomes and the less likely the implant can be retained. Stable hardware can be maintained in a patient without uncontrolled sepsis who has no signs of chronic osteomyelitis. The cure rate in such patients is between 68% and 100%,[36,172,173] provided that the fracture is stable. Clinical experience and data from animal studies suggest that stable fractures are less susceptible to infection than unstable bone components.[174–176] Therefore it is important to stabilize the fracture, even in the presence of an infection. This again requires a decision to retain or exchange the fixation device when infection is diagnosed. In addition to the biofilm age, the likelihood of the fracture achieving union before implant fatigue failure must be considered. If an infected implant is retained, antimicrobial suppression is required until bone union has occurred. Whether or not the device must be removed after bone consolidation also depends on the duration of infection before treatment. In the case of early infection or acute hematogenous infection after an uneventful postoperative period, rapid diagnosis and immediate surgical and antimicrobial treatment improve the outcome. The cure rate of early staphylococcal implant-associated infections treated with a fluoroquinolone-rifampin combination was shown to be high.[36] Hence, in these cases the implant does not have to be removed after consolidation. In contrast, in patients with delayed detection of infection, the biofilm may persist on the implant. Therefore all foreign material should be removed after bone healing. Antimicrobial therapy can be stopped shortly thereafter (i.e., within 1 week).

Systemic Antimicrobial Therapy

Principles of intravenous and oral antimicrobial treatment are not different in PJI and internal fixation–associated infection (see Table 105.2). However, there are no recommendations, nor are there comparative studies defining the length of treatment. In the case of early or acute hematogenous infection after an uneventful postoperative period, we propose 3 months of antimicrobial therapy.[36] In the case of delayed presentation of infection, treatment duration depends on the surgical intervention and may be prolonged as long as the device is retained. Bone consolidation should be regularly evaluated and the device removed after fracture consolidation. Thus the treatment duration can be kept to the shortest possible time.

Local Antimicrobial Therapy

In open fractures, particularly in those with high bacterial contamination, the implantation of antibiotic-loaded beads or spacers to fill dead space is common practice.[177] Although an aminoglycoside (e.g., gentamicin, tobramycin) is the standard antibiotic in spacers, virtually any antimicrobial agent can be added to spacers and beads.[138,178] The rationale behind this procedure is to reduce the local bacterial load in poorly vascularized areas. Also, bead chains can be placed in difficult-to-reach bone areas. In open fracture, several studies have reported a reduction of infection rates when adjuvant antibiotic-loaded beads were used.[178–180] It should also be considered that there will be a selection of bacteria with emerging resistance toward the antibiotics incorporated in beads and spacers.[181] Because these materials are foreign bodies, adherence of bacteria can occur.[182] Although antibiotics are released locally, uncommonly they may have systemic unwanted effects (e.g., nephrotoxicity of gentamicin).[183] Taken together, it is judicious to rapidly reduce the dead space and local bacterial load in a contaminated open fracture. Nevertheless, antibiotic-loaded beads and spacers are foreign bodies and should be removed within a short period of time. Removal is not necessary when beads constructed from bioabsorbable material are used.[184] Their use may result in significant leakage, making the clinical interpretation of wound oozing difficult (i.e., leakage from dissolving beads vs. infection). Of note, it should be considered that a muscle flap likely contributes its part to local antimicrobial defense via excellent vascularization and penetration into the tissue. Rapid closure of the wound is essential to hinder the penetration of skin flora into the wound.

Surgical Interventions

It is important to note that patients with implant-associated infections can benefit from rapid referral to specialized centers. Before surgery, the following factors must be assessed: viability of the soft tissue and bone, bone stability, fracture healing, implant stability, duration of the infection, and magnitude of the soft tissue defect.[166] The soft tissue envelope must be well vascularized and intact to provide systemic antimicrobial agents and immune cells to the infected area. As a rule, stability of bone fragments is required for union. In patients with unstable devices, there is neither bone consolidation nor healing of infection. Therefore, such implants should be removed and replaced with either a new internal fixation device (plate or intramedullary nail) or external fixation. External fixation is preferred in cases involving difficult-to-treat microorganisms (e.g., fungi or multiresistant microorganisms). The majority of early infections can be treated with débridement and implant retention. This is also true for patients with acute symptoms after an uneventful postoperative course. Débridement includes diagnostic biopsy sampling and meticulous removal of all necrotic tissue including dead bone, abscess membranes, and granulation tissue. The first intervention is generally the most important. In case of extensive tissue necrosis or hematoma formation, more than one intervention may be necessary—although the more thorough the first intervention, the fewer additional débridements are required. In nonearly infections, the decision to retain or to remove the implant depends on multiple factors, also because biofilm formation occurs on a time-dependent continuum. In patients with a delayed infection presentation, the same procedure as for early infection may applied, although with a different antimicrobial approach (see earlier). Conceivably, shorter disease duration implies a better prognosis. In patients with a long disease duration, the implant can generally not be retained. In patients with significant skin and soft tissue defects (a priori or as a consequence of meticulous débridement), the procedure should be combined with plastic and reconstructive surgery. Uncovered soft tissue defects or delayed wound healing may result in exogenous superinfection, which is often polymicrobial. The duration of both hospitalization and antimicrobial treatment is commonly long in patients having superinfections. Important to note, the development of superinfection is not hindered, but likely enhanced, when the wound is covered with a vacuum-assisted closure device.[164]

Plate-Associated Osteomyelitis

Internal fixation with a plate may lead to a devascularized area at the interphase between plate and bone. In the case of a subcutaneous position of the plate (e.g., tibial fracture), clinical diagnosis is obvious. With rapid diagnosis, débridement with implant retention and antibiotic treatment can be performed. If the plate is in a submuscular or subfascial position (e.g., femur fracture), diagnosis is often delayed, leading to instability of the infected nonunion. After removal of plates and screws because of infection, there is usually a layer of dead cortical bone under the plate. It can be shaved off with an osteotome. The implant has to be exchanged or, alternatively, replaced by external fixation.

Intramedullary Nail–Associated Osteomyelitis

According to experimental data, medullary nailing leads to necrosis in the central part of the cortex.[185] If diagnosis is delayed, infected nonunion requires the exchange of the implant. In these cases, bioabsorbable antimicrobial-impregnated beads can be inserted into the canal.[186] A multidisciplinary team should decide for each patient whether an infected nonunion can be treated with a single-stage protocol[184] or whether a two-stage protocol including external fixation is the better option.

Pin-Tract Infection

Local inflammation surrounding fixation pins is frequent, mainly due to heat necrosis caused by drilling at excessive speed and power. Extension of infection to the bone is less frequent. Pin infection should be rapidly treated with local cleaning and oral antibiotics. According to a Cochrane review, no specific pin-site care was better than any other for reducing the risk of infection.[187] If deep bone infection occurs, pins will loosen. The reported incidence of pin-tract infection was 11%, lowest for ring (4%) and highest for hybrid fixators (20%).[188] With established pin-site bone infection, a ring sequestrum will be formed. Surgical treatment

of such infections requires removing the involved pins, excising the ring sequestrum, and administering a short course of antibiotics. If bone fragments are not yet stable, new pins should be inserted at a distant site.

PREVENTION IN ORTHOPEDIC SURGERY

Perioperative Antimicrobial Prophylaxis

Arthroplasty and Closed Fractures

Timely correct antimicrobial prophylaxis reduces postoperative infections.[189–191,192] The Surgical Infection Prevention Guideline Writers Workgroup has suggested the infusion of the first antimicrobial dose within 60 minutes before surgical incision.[193] Several studies observed even lower infection rates when antibiotic prophylaxis was administered within 30 minutes before incision.[190,194,195] However, a recently published controlled trial randomized 5580 patients (39% of them in a trauma unit) for two different prophylaxis time points.[196] The median administration times before incision were 42 (interquartile range [IQR], 30–55) versus 16 (IQR, 10–25) minutes, and the overall rate of surgical site infections was 5% in both groups. In view of surgical site infections in patients with orthopedic implants, a follow-up of only 30 days is a limitation of this trial.

Most studies investigating the prophylactic effect of antibiotic-impregnated bone cement in elective orthopedic surgery have failed to demonstrate significant differences because of the small numbers of patients.[190] However, a study including 43,149 primary and revision knee arthroplasties (Finnish Arthroplasty Register) was able to show that the lack of antibiotic-impregnated cement increases the risk for reoperation because of infection. Cases in which patients received only intravenous prophylaxis were compared with those in which patients received combined antibiotic prophylaxis (hazard ratio [HR], 1.42 [95% CI, 1.08–1.88] for primary arthroplasty; HR, 2.12 [95% CI, 1.14–3.92] for revision arthroplasty).[51]

Intravenous antimicrobial prophylaxis should be stopped after the end of surgery. Continuation does not further reduce infection rates but may increase the incidence of resistant pathogens in subsequent nosocomial infections.[197–198,199]

Compounds for antimicrobial prophylaxis are usually selected according to local epidemiology, national guidelines, and in-house practices. Most centers use first- or second-generation cephalosporins. Dual antibiotic prophylaxis (i.e., cefazolin plus vancomycin) failed to prove superiority in reducing the overall rate of surgical site infections in comparison with cefazolin monotherapy.[200] Nevertheless, if the local epidemiology points toward high MRSA or multiresistant gram-negative colonization rates, or both, the compounds used for antimicrobial prophylaxis may be adapted accordingly, based on the recommendations of local hospital infection control specialists.

In case of suspected or definite orthopedic implant–associated infections, there are additional aspects regarding the timing of antimicrobial prophylaxis. Obtaining biopsy specimens after antimicrobial agents have reached significant tissue levels decreases the sensitivity of microbiologic culture.[201] The difference in sensitivity may not be critical in chronic infections, because bacteria can be cultured from necrotic bone.[201] Preferentially, samples should be obtained just before administration of antimicrobial prophylaxis. However, when surgery is performed during ischemia (e.g., total knee arthroplasty), antibiotic prophylaxis is administered before the tourniquet is inflated, potentially influencing microbiologic results. Still, a clinical trial analyzing whether antibiotic prophylaxis during elective knee arthroplasty should be administered before tourniquet inflation or can be delayed until just before tourniquet deflation found similar low deep-tissue infection rates in both groups.[202] Thus, prophylactic antibiotics can be delayed until tourniquet deflation, thereby avoiding antibiotic carryover during sampling for microbiologic evaluation.

Open Fractures

Open fractures are at increased risk for infection (see Table 105.3). Therefore, emergency surgery with antibiotic preemptive therapy (e.g., first-generation cephalosporins for up to 24 hours) is necessary. For type I and II fractures, the same compounds used for closed fractures are indicated. In cases of massive exogenous contamination (type III open fractures), certain experts recommend antibiotics that are more active against anaerobes or gram-negatives (e.g., ampicillin-sulbactam or piperacillin-tazobactam or flucloxacillin plus an aminoglycoside).[203] However, these recommendations are based on expert opinion and observational studies, and most cited data in the literature are at least 30 years old.[204]

In type IIIB and IIIC fractures, preemptive antimicrobial therapy is recommended. Similar to the lack of data regarding the optimal compound, the optimal duration is unknown. In previous publications, a period of 10 days has been reported.[205,206] Dellinger and colleagues[189] compared in a double-blind prospective trial 1 day versus 5 days of preemptive cephalosporin treatment in patients with open extremity fractures. The rates of postoperative fracture site infections were similar. Similarly, in a retrospective case-control study, Dunkel and colleagues[207] found no difference in subsequent infection rates when a 1-day course of antibiotics was compared with prolonged treatment, even for grade III open fractures. We recommend a 1-day (or a maximum 3-day) preemptive therapy course with ampicillin-sulbactam or amoxicillin–clavulanic acid in patients with grade III open fractures. Early administration after trauma appears to be more important than the duration of preemptive therapy.[208] Closure of the wound should be strived for as soon as soft tissue conditions allow doing so in cases of open fractures. Also, rapid stabilization of the fracture is necessary. In type III fractures, initial external fixation should be preferred for a short period because of the high risk of infection with intramedullary nailing. Hence, adequate surgical procedures reduce the rate of posttraumatic infections, illustrating the importance of an interdisciplinary approach.

Prevention of Hematogenous Infection

Because implants are highly susceptible to bacterial adherence, the risk of hematogenous infection remains as long as the device is implanted.[40–43] Accordingly, means to prevent hematogenous infection are limited. Patients should be informed about the risk and reminded to rapidly contact a physician when signs of infection occur, irrespective of localization. It is important to treat obvious infections (e.g., pneumonia, skin and soft tissue infections, urinary tract infections) rapidly in order to prevent bacteremia.

A further approach is the application of hygiene measures to potential sources of bacteremia. These include *S. aureus* decolonization and dental hygiene procedures. In a double-blind, placebo-controlled, randomized study including patients undergoing elective orthopedic surgery, eradication of *S. aureus* nasal carriage with mupirocin was not effective in reducing surgical site infections. However, the rate of hematogenous *S. aureus* infections was five times lower in the mupirocin group.[209] Similarly, orodental hygiene and regular dental treatment can be recommended because these might result in reduction of risk of hematogenous PJI.[210,211] In contrast, antibiotic prophylaxis to reduce the risk of hematogenous seeding and joint contamination during dental surgery is unnecessary.[211–214] This is true for both low- and high-risk dental procedures.[210] Similarly, genitourinary procedures are not risk factors for subsequent PJI, and consequently antibiotic prophylaxis is not required.[215]

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The complete reference list is available online at Expert Consult.

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L Diseases of the Reproductive Organs and Sexually Transmitted Diseases

106 Genital Skin and Mucous Membrane Lesions

Michael H. Augenbraun

SHORT VIEW SUMMARY

Definition
- Infectious genital skin and mucous membrane lesions cover a wide spectrum of pathologic etiologies and clinical presentations, mostly manifesting as inflammatory reactions or defects in the epithelium of the genital tract in men and women.

Epidemiology
- Infectious genital skin and mucous membrane lesions are seen globally without seasonality.
- These lesions occur primarily in sexually active individuals.
- Most are due to sexually transmitted infections, but neoplastic and noninfectious inflammatory processes should be considered, especially in older individuals.
- Incubation periods may vary according to etiology: for human papillomavirus, it may potentially be weeks, months, or years after exposure; for primary syphilis, it may be weeks to months; for herpes simplex virus (HSV) outbreaks, it may be years.
- Disease may be modified in populations that are immunocompromised.
- Some conditions are currently being seen more frequently in men who have sex with men (i.e., early-stage syphilis, lymphogranuloma venereum [LGV]).

Microbiology
- Bacteria: *Treponema pallidum* (spirochete), *Haemophilus ducreyi* (gram-negative diplococcus), *Chlamydia trachomatis* L serovars (obligate intracellular pathogen), *Klebsiella granulomatis*/donovanosis (gram-negative rod)
- Viruses: HSV, molluscum contagiosum virus, human papillomavirus
- Fungi: *Candida albicans*

Diagnosis
- Clinical assessment alone may be misleading.
- Laboratory examination using microscopy and serologic testing is often helpful.
- Gram staining and light microscopy help to identify *H. ducreyi* and *Candida* spp.
- Darkfield microscopy is used to identify *Treponema pallidum*.
- Serology helps to diagnose *T. pallidum* and HSV.
- Nucleic acid amplification tests (NAATs) and less often cell culture testing are used to diagnose HSV.
- NAATs are used to identify *C. trachomatis*.

Therapy
- Therapy is best chosen based on the likely or specific etiologic agent.
- Syphilis is treated with penicillin and tetracyclines.
- HSV infection is treated with acyclovir, valacyclovir, or famciclovir.
- *C. trachomatis* causing LGV should be treated with tetracyclines.

Prevention
- Safe sex
- Partner contact tracing
- Preemptive treatment based on contact and risk

Genital lesions are of uncertain historical pedigree. Ancient Chinese medical writings dating back to 2500 BCE appear to have alluded to a "corroding ulcer" of the genitals developing a few days after coitus.[1] There are references throughout the Old Testament to genital pathology that may have been infectious in nature. Ancient Greek, Roman, and Arabic medical texts also suggest a familiarity with acute genital infections, but the descriptions are difficult to associate with any specific clinical syndrome recognized today.[2,3]

Although genital lesions originate from a wide range of infectious and noninfectious processes, they most commonly result from sexually transmitted or venereal infections (Table 106.1).[4,5,6,7] Other pathologic processes including chemical and mechanical trauma, neoplasia, and immunologic phenomena, can cause genital lesions (Table 106.2). The term *venereal,* as it relates to disease, dates to the 15th century. Its root comes from the Latin *venereus* or *venus,* meaning "from sexual love or desire." In the United States the most common causes of sexually transmitted genital lesions are herpes simplex virus (HSV) type 2, *Treponema pallidum* (syphilis), and human papillomavirus (HPV).[8]

Infectious genital lesions are unique in a number of ways compared with other infectious processes. Most are communicable and are not only a clinical concern but also a public health concern. Infectious genital lesions can harbor more than one pathogen at a time, making proper diagnosis and management a challenge.[9,10] The morphologic appearance of the lesion can differ widely from one clinical presentation to another, even when the causative organism is the same. The unpredictable nature of clinical presentation of the lesion can make a purely clinical diagnosis unreliable.[11,12,13] Inflammatory epithelial defects characteristic of these pathologies appear to enhance the transmission of other diseases, most importantly human immunodeficiency virus (HIV).[14,15–20]

This chapter focuses on the broad issues relevant to the assessment and management of genital lesions. For in-depth discussions of individual pathogens, readers are directed to the chapters that address those topics.

HISTORY OF PRESENTATION

Individuals who present with a new genital lesion and who report recent sexual activity, particularly activity with a new partner or someone with a suspected genital infection, are likely to have a sexually transmitted infection. Certain clinical circumstances suggest nonsexually transmitted pathology such as trauma, chemical irritation, or allergic hypersensitivity. A lesion that occurs proximate to sexual exposure (i.e., within hours to 1 or 2 days) may be too early to account for the incubation period of most infectious pathologies. In the case of a lesion with rapid onset, the nature of the sexual activity, a topic often left unexplored by the clinician and patient, may suggest trauma.[21,22] Traumatic genital lesions

TABLE 106.1 Infectious Causes of Genital Lesions

Sexually Transmitted Infections
Syphilis
Primary (chancre)
Secondary (condyloma latum)
Herpes simplex virus types 1 and 2
Chancroid *(Haemophilus ducreyi)*
Lymphogranuloma venereum
Granuloma inguinale (donovanosis)
Human papillomavirus
Sarcoptes scabiei
Molluscum contagiosum
Nonsexually Transmitted Infections
Folliculitis
Tuberculosis
Tularemia
Histoplasmosis
Candida (balanitis or vaginitis)
Amebiasis

TABLE 106.2 Nonvenereal Causes of Genital Lesions

Trauma
Malignancies (e.g., squamous cell carcinoma)
Behçet syndrome
Lipschütz vulvar ulcers
Peyronie disease
Fixed drug eruption
Eczema
Psoriasis
Inflammatory bowel disease
Contact dermatitis
Lichen planus
Hidradenitis suppurativa
Postinflammatory hypopigmentation
Aphthous ulcers (associated with human immunodeficiency virus)

may result from sexual assault, questions about which are also often avoided.[23,24] Recurrent lesions in someone who uses or whose partner uses latex condoms may be attributable to a latex allergy, although allergy typically results in more generalized edema and erythema of the genitals rather than a focal ulcer or papular lesion.[25] The geographic prevalence of disease may be an important factor to consider when assessing a genital lesion in a traveler.

A history of recurrent genital lesions, with or without dysesthesias preceding the development of lesions, usually suggests an infection with HSV. HSV-2 and, less commonly, HSV-1 cause a genital ulcer that is characterized by repeated symptomatic or often subclinical outbreaks separated by variable periods of quiescence during which the virus is localized to the dorsal root ganglia of adjacent sensory nerves. A variety of factors are postulated to contribute to viral shedding (which may or may not be associated with a genital ulcer) including immunosuppression, other intercurrent illnesses, sun exposure, and menses.[26,27] Data suggest that initiation of highly active antiretroviral therapy in HIV-seropositive patients may reactivate HSV-2 shedding.[28]

Host factors can be important historical determinants of etiology of a genital lesion. Patients with preexistent psoriasis or eczema or other noninfectious dermatitides may have a genital lesion related to the underlying dermatologic pathology. Nongenital autoimmune conditions may suggest an autoimmune cause for genital lesions such as lichen planus.[29] Recent history of medication use such as tetracyclines or antineoplastics in a patient presenting with a new genital lesion can prompt consideration of a fixed drug eruption. In these cases, lesions may be characterized by pigmentation or superficial ulceration.[30,31] Candidal balanitis in men has been associated with immunosuppression, contact with a partner who has vaginal candidiasis, and diabetes.[32–34] Vulvovaginal candidiasis is an extremely common problem that may manifest as focal genital lesions.[35] Although it may be more common in women with underlying immunosuppression (e.g., diabetes mellitus, HIV infection, long-term steroid use), vulvovaginal candidiasis occurs most frequently in women without such risk factors.[35,36] Autoimmune diseases such as acute reactive arthritis, Crohn disease, or Behçet syndrome may be associated with genital lesions.[37–39] The spectrum of typical and atypical genital lesions associated with HIV infection is broad and includes Kaposi sarcoma, giant condyloma acuminatum, chronic nonhealing HSV, typical HSV, syphilis, and chancroid.[40,41,42,43] This reflects both the common occurrence of coinfection with other sexually transmitted diseases and a propensity for HIV immunosuppression to modulate other conditions.

CLINICAL MANIFESTATIONS

Location

In men, lesions associated with infectious or noninfectious processes may be found on or under the prepuce, around the coronal sulcus, on the shaft of the penis, on the scrotum, on the perianal tissue, or on the inner thighs. In women, sites of involvement are equally varied. Genital lesions can appear on the mons pubis, labia, fourchette, cervix, inner thighs, perianal tissue, or anywhere in the vagina. As a result of orogenital sex, pathogens such as HSV and *T. pallidum* can also cause orolabial lesions. Anyone who engages in receptive anal intercourse is at risk for developing an infectious lesion in the perirectal area, the rectum, or the anus. Lesions of chancroid can be disseminated distant from the genitalia and the original site of infection by a process of autoinoculation.[44,45] Data suggest that as venereal chancroid rates decline, there may be an increase in nongenital yawslike cutaneous presentations in children.[46] In secondary syphilis, spirochetemia causes lesions, sometimes widely dispersed from the genitalia, that morphologically range from the classic papulosquamous rash of the palms and soles to the moist, raised lesions of condylomata lata (genitals) or mucous patches (orolabial area). *Neisseria gonorrhoeae,* a pathogen not commonly associated with genital lesions, may disseminate and cause tender, necrotic pustules, primarily on the distal extremities as part of an arthritis-dermatitis syndrome.[47] Lesions associated with scabies infestation are common in the genital region as well as intertriginous areas elsewhere including, but not limited to, the axillary folds and the interdigital spaces. Genital edema can occur after any local inflammatory process.[48]

Pain, Dysesthesias, and Systemic Symptoms

The lesions of syphilis, lymphogranuloma venereum (LGV), scabies, molluscum contagiosum, and HPV are ordinarily painless. Exceptions are noted in the medical literature.[49] Herpetic lesions are usually painful, although they may not be noticed until the clinician examines the patient and palpates or abrades the lesion. If HSV lesions are adjacent to or within the urethra, the patient can experience dysuria. Sometimes pain or other dysesthesias including pruritus may precede the development of a clinically recognizable HSV lesion, particularly during episodes of disease recurrence.[50,51] These so-called prodromal symptoms are usually milder than symptoms experienced during a primary outbreak. They may be so characteristic that patients can be reliably instructed to begin antiviral medication before lesions erupt. Chancroid ulcers are typically painful. Genital lesions from immunologically mediated noninfectious causes such as Behçet syndrome, genital extensions of Crohn disease, aphthous ulcers, and vulvitis/vestibulitis may also be exquisitely tender. Granuloma inguinale (donovanosis), a genital ulcer disease seen primarily in the tropics, is caused by the bacillus *Klebsiella granulomatis.* Although the lesions of this disease are often large and destructive, pain is absent. Most patients with exophytic genital warts are asymptomatic; a few may report pain or pruritus.

Pruritus is common with fungal infection and with ectoparasitic infestations such as scabies or lice. The pruritus associated with scabies is often described as intense and worse at night. Although pruritus may be experienced by individuals with HSV or syphilis, it is not characteristic of these conditions. Fever is occasionally seen with secondary syphilis and with primary HSV infection but usually is absent.[51,52] Headaches, fatigue, myalgias, and malaise may also accompany these infections.

Lymphadenopathy

Inguinal lymphadenopathy is a nonspecific finding characteristic of inflammatory pathology almost anywhere in the groin or either lower

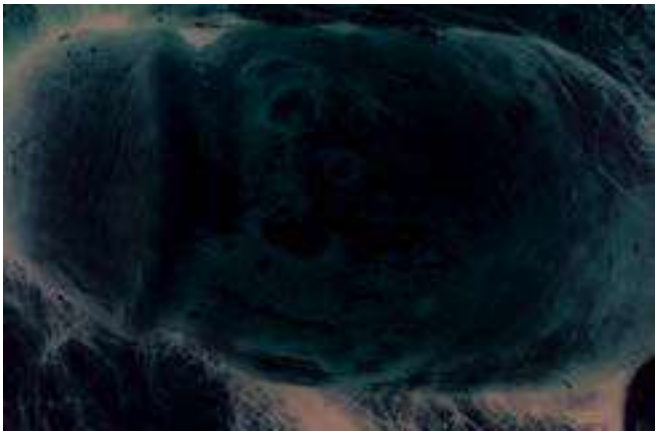

FIG. 106.1 Herpes simplex virus infection. Genital infection with herpes simplex virus.

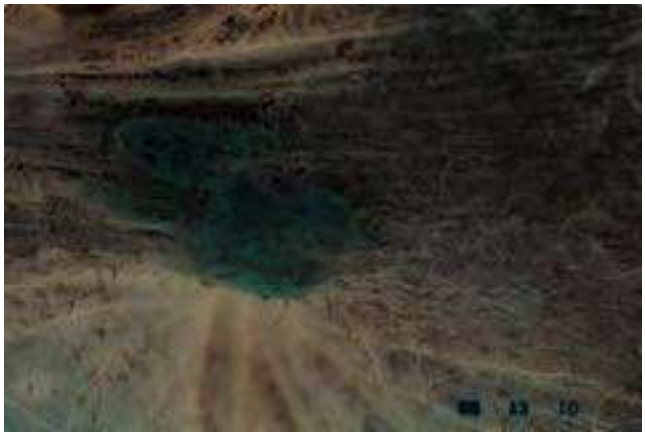

FIG. 106.2 Herpes simplex virus ulcer. An irregularly shaped herpes simplex virus ulcer.

extremity. It may also be a manifestation of systemic disease such as HIV infection, tuberculosis, or lymphoma. It often accompanies genital infection. Although the inguinal and femoral lymph nodes drain the genital region in both men and women, the inner segment of the vagina and the cervix drain into deep pelvic and perirectal lymph nodes. Anorectal lymph drainage patterns are also complex and depend on whether infection occurs above or below the dentate line (the mucocutaneous junction in the rectum). In either case, if pelvic lymph nodes are involved in inflammatory genital pathology, pelvic or rectal discomfort may be the most striking symptom.

Bilateral inguinal lymphadenopathy is typical in syphilis. Similar to the chancre of primary syphilis, it is usually painless. In secondary syphilis, as befitting a systemic process, lymphadenopathy distant from the genital area is common. Typically the inguinal, axillary, cervical, and epitrochlear nodes are involved.[52] Lymphadenopathy associated with a herpetic genital lesion is usually bilateral and, similar to the lesion, is tender. In HSV infection and in syphilis, lymphadenopathy persists for some time after resolution of the lesion.

LGV and chancroid are characterized by expansive, tender lymph nodes called *buboes*. These may be unilateral or bilateral. A central area of fluctuance often develops; if left untreated, it eventually spontaneously ruptures. Although drainage may be spontaneous, tenderness can become sufficiently severe to warrant intervention and drainage. Lymphadenitis is unusual in granuloma inguinale.

Lesion Morphology

Although the appearance of a genital lesion is not entirely specific, it can implicate an etiologic agent. HSV infections are characterized by vesicles that evolve into pustules and finally to shallow ulcers on an erythematous base (Fig. 106.1). Multiple lesions are common, and they may erupt in tightly grouped clusters. Sometimes the characteristic evolution of the lesion is not appreciated, and the patient or clinician simply observes tender ulcers that assume a wide variety of shapes and sizes because of confluence of evolving vesicles and pustules (Fig. 106.2). In a primary outbreak, lesions can reach sizes greater than 1 to 2 cm in diameter (Fig. 106.3). Immunocompromised patients such as transplant recipients, patients taking immunosuppressive drugs, and patients infected with HIV can also experience extensive herpetic ulceration of the genitals in the setting of either a primary outbreak or a recurrence.[43,53,54] At the other end of the spectrum, a substantial proportion of individuals fail to recognize HSV outbreaks because of mild or absent symptoms.[55] When instructed carefully about these types of outbreaks, most of these individuals can recognize disease.[56]

Syphilitic chancres are typically solitary, although they may rarely occur in pairs. They are round and 1 to 2 cm in diameter, with clean margins that are indurated on palpation (Fig. 106.4). The ulcer base usually lacks exudate but occasionally becomes superinfected with other bacteria.

The lesions of secondary syphilis are not chancre-like. They may start anywhere as fine macular lesions that evolve into pigmented papules,



FIG. 106.3 Primary herpes simplex infection. Severe primary herpes simplex virus infection.



FIG. 106.4 Primary syphilis. Chancre of primary syphilis.



FIG. 106.5 Secondary syphilis. Condylomata lata of secondary syphilis.

often with a fine, circumferential scale. In warm, moist areas such as the buttocks and genitals, unique lesions of secondary syphilis, known as condylomata lata, develop (Fig. 106.5). These are raised, moist nodules or plaques that are teeming with treponemes. They are highly infectious.

Chancroid lesions are similar in size to syphilitic chancres, but their edges are ragged and undermined (Fig. 106.6). The ulcer base is necrotic with a purulent exudate. Compared with the lesions of syphilis, induration of chancroid lesions tends to be less prominent, accounting for the designation of these ulcers as "soft chancres" *(ulcus molle, chancre mou)*. Despite the obvious tissue damage, adjacent inflammation is absent. Single lesions are the norm, but multiple lesions may be seen. As noted before, chancroid lesions, similar to HSV ulcers and in contrast to syphilis chancres, are tender.

The lesions of granuloma inguinale start as firm subcutaneous nodules or papules that eventually ulcerate. Typically, this ulcerative process becomes hypertrophic and bleeds easily (Fig. 106.7).[57] Local tissue destruction may be extensive. Swelling of the vulva and prepuce (causing phimosis) is common. Variants include deeply necrotic ulcers and dry, so-called cicatricial lesions that consist primarily of fibrotic tissue. Lesions occasionally are confused with squamous cell carcinoma.[58]

LGV is one of several sexually transmitted diseases caused by *Chlamydia trachomatis* and is attributable specifically to serovars L1, L2, and L3. At its earliest stage, LGV may cause a small papule or rarely a HSV-like ulcer.[59,60] This is usually asymptomatic and resolves before recognition.[61] The patient with LGV then comes to medical attention with tender inguinal lymphadenopathy as the primary process of concern. If the source of exposure is via anal intercourse, clinical manifestations may be more difficult to discern. Deep pelvic lymph nodes are commonly involved, and patients present with proctitis or proctocolitis.

Clinically visible lesions of HPV are typically caused by viral types with low oncogenic potential (i.e., types 6 and 11).[62] Most HPV infections are asymptomatic. Lesions can run the gamut from flat or relatively inconspicuous papules to verrucous, pedunculated, or large cauliflower-like masses—all referred to as *condylomata acuminata* (Fig. 106.8). Whatever the configuration, these lesions are clinically characteristic and unlikely to be confused with lesions of other etiologies. Certain HPV types (e.g., 16, 18, 31, and 33) have oncogenic potential both on moist mucosal surfaces such as ectocervix and, less commonly, on keratinized epithelium characteristic of the external genitalia. The dysplastic cytologic changes they induce are collectively referred to as squamous intraepithelial neoplasia and may be benign or malignant. Squamous cell carcinoma of the cervix, vagina, vulva, penis, or anus arises from previous HPV infection. HPV-associated carcinoma in situ on keratinized epithelium may manifest as multiple papular lesions, referred to as bowenoid papulosis (Fig. 106.9) or, when it occurs as a single plaque, as Bowen disease. Erythroplasia of Queyrat is a variant of this process that involves the glans of the penis.

The poxvirus molluscum contagiosum virus (MCV) causes benign, wartlike lesions (Fig. 106.10). Spread can be sexual or through nonsexual



FIG. 106.6 Chancroid *(Haemophilus ducreyi)*. Lesions involving the penis and the inguinal and femoral lymph nodes.



FIG. 106.7 Granuloma inguinale. Tissue smear was positive for Donovan bodies.

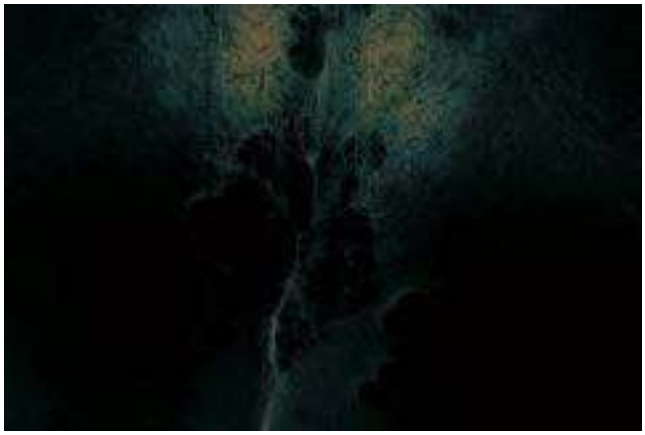

FIG. 106.8 Human papillomavirus. Genital human papillomavirus lesions.

contact in children. Lesions are small, 3 to 5 mm in diameter, multiple, and clustered in the genital or inguinal areas, perineum, or inner thighs in adults and are almost always painless. They appear pearly with an area of central umbilication, which often can be appreciated only on very close inspection. Lesions of scabies infestation range from papules to nodules with a surrounding crust (Fig. 106.11). With scratching, these lesions are often modified by excoriations or lichenification.

The use of systemic or topical antimicrobial agents before clinical evaluation can have a dramatic effect on lesion morphology in any of these various cases.

Duration

Without therapy, HSV ulcers resolve within 3 weeks in cases of primary infection. Recurrences resolve in 5 to 12 days. Except for some postinflammatory hypopigmentation, there is little scarring. Lesions that heal within days after an outbreak in the absence of therapy are probably not herpetic in nature. However, lesions proven to be herpetic and persisting for longer than 3 to 4 weeks raise the possibility of underlying immunosuppression. Syphilitic chancres and condylomata lata also resolve without therapy, usually between 3 and 12 weeks and usually without much scarring. Without therapy, the lesions of chancroid and donovanosis are slowly destructive. Scarring is typical in both of these situations. Lesions caused by HPV or MCV may persist unchanged for a prolonged time, or they may be characterized by brief periods of clinical disease alternating with resolution. Molluscum contagiosum lesions may resolve on their own after several months but may persist in immunocompromised patients.



FIG. 106.9 Bowenoid papulosis.

EPIDEMIOLOGY

Understanding of the epidemiology of genital lesions has been hampered by inconsistent access to diagnostic tools in many parts of the world. The probability of a given cause of a genital lesion varies depending on the region of the world in which the patient lives or acquires infection (Table 106.3).[63–66] Worldwide, HSV-2 is the most common cause of genital ulcer disease.[67,68]

Although previously a common cause of genital ulcer in the developing world, chancroid has become increasingly rare,[69,70] possibly related to widespread antibiotic use and incidental reduction in the reservoir or transmission, or both, of the pathogen. In the developed world, *Haemophilus ducreyi* is usually seen only in the context of focal urban outbreaks.[71,72]

In the absence of reliable serologic tests for HPV infection, it has been difficult to accurately determine the prevalence of this organism in different regions of the world. This assessment is further hampered by the asymptomatic nature of most infections. Despite these handicaps, some studies suggest that HPV may be one of the most common sexually transmitted infections in the United States.[73,74] Sometimes this infection results in the typical lesions of external genital warts known as condyloma acuminatum.[75] Although initially envisioned as a tool for preventing cervical infection and therefore avoiding the development of neoplasia, the HPV vaccine has also been associated with a decline in the prevalence of genital warts in several population-based studies.[75–78,79,80] This is not surprising, as two of the most common types of HPV, 6 and 11, cause the majority of condyloma acuminatum and are included in the currently available nonavalent vaccine. By directly immunizing vaccinees and



FIG. 106.10 Molluscum contagiosum. Lesion of molluscum contagiosum.



FIG. 106.11 Sarcoptes scabiei. Infestation with *S. scabiei.*

TABLE 106.3 Causes of Genital Ulcer Disease by Region (Percentage of Cases)

PATHOGEN	DAR ES SALAAM[4]	SOUTH AFRICA[61]	LOS ANGELES[5]	ST. LOUIS[5]	THAILAND[62]	MADAGASCAR[63]
Herpes simplex virus type 2	63	36	76	53	82	10
Haemophilus ducreyi	13	32	0	0	0	33
Treponema pallidum	2	20	0	13	0	29
>1 pathogen	10	9	0	2	2	—
Negative	21	22	24	32	16	32

where vaccine uptake has been substantial by creating significant herd immunity, the HPV vaccine may be the most effective way we currently have for controlling this common infection.

The incidence of syphilis in the United States declined so precipitously through the 1990s that the US Public Health Service embarked on an ambitious program to eliminate the disease from the country.[81] Despite these efforts, the incidence of early-stage syphilis, particularly among men who have sex with men and many of whom are also infected with HIV, has been increasing not only in the United States but also throughout the world.[82–86]

LGV occurs primarily in the tropics and subtropics.[61,87] Sporadic cases and outbreaks of LGV occur in the developed world, and the disease should be considered in travelers returning from endemic areas.[88,89] More recently, LGV outbreaks characterized by ulcerative proctitis have been noted among men who have sex with men in both Europe and North America.[90–93] Donovanosis is endemic in Papua New Guinea but has also been seen in southern Africa, parts of India, the Caribbean, and South America. It is infrequently seen in the United States.[57]

Genital Lesions in Patients With Human Immunodeficiency Virus

Although most HIV-infected patients with genital lesions manifest disease in a fashion similar to their nonimmunocompromised counterparts, it is clear that some patients, particularly patients with significant immunosuppression, experience more extensive and prolonged disease. Large, persistent, or progressive condylomata acuminata have been well described in this patient population.[94,95] In patients with HIV infection, the lesions of molluscum contagiosum can also vary in size and may number in the hundreds.[96,97] In this population, both primary and recurrent herpetic ulcers may progress to extend over large areas of the perineum.[43,98] The pain associated with such lesions can be so debilitating that it warrants hospitalization. HSV lesions in HIV-infected patients may persist well beyond the 2- to 3-week period ordinarily encountered in classic primary or recurrent disease. This finding may serve as an early sign of immunosuppression.[99–101] There has also been concern that HIV-infected patients are more likely to harbor HSV isolates that are resistant to commonly used antiviral agents.[102,103] The lesions of early-stage syphilis probably are not appreciably altered by HIV infection, although some data suggest that patients present more frequently than usual with multiple chancres. Clinical manifestations of secondary lesions may also develop before the resolution of the syphilitic chancre more commonly in HIV-infected individuals.[104,105,106] Rarely, aggressive forms of secondary syphilis have been reported in patients with HIV/acquired immunodeficiency syndrome.[107,108] Whether this truly reflects an altered presentation in this population remains unclear. Anecdotal reports of accelerated disease and failure of routine therapy have not been supported by prospective studies.[104]

Any break in the ordinarily protective barrier of the integument such as that caused by genital inflammatory lesions can serve as a conduit through which HIV is more efficiently transmitted from one sexual partner to another. Lesion exudate has been demonstrated to harbor virus.[109] There is additional concern that genital HSV-2 may increase local HIV replication, further enhancing the risk of transmission.[110] Syphilis, HSV, and chancroid infections have been associated with a greater likelihood of HIV infection in both retrospective and prospective studies.[111–114]

LABORATORY TESTING

Laboratory tests are critical to the diagnosis and proper management of genital lesions. As previously discussed, although specific pathogens can be linked to well-characterized clinical presentations, there is enough variation to ensure that clinicians relying on educated guesswork will be wrong in a significant number of cases. The following discussion is not meant to be exhaustive; for more details, reference should be made to the individual pathogens discussed elsewhere in the text.

A number of direct microscopic examinations can be performed on lesion exudate or a biopsy sample and can help make a diagnosis. Gram staining usually is not helpful in the evaluation of genital lesions. Lesion exudate is laden with nonpathogenic organisms common to genitourinary and perirectal microbiota. Under ideal circumstances, *H. ducreyi* appears as a gram-negative, slender rod or coccobacillus that aligns in a pattern referred to as "school of fish" (Fig. 106.12). Most clinicians and microbiologists are not sufficiently experienced to recognize this in the welter of other organisms typical of a lesion Gram stain, and its sensitivity and specificity are poor. *H. ducreyi* can be cultivated on special nutrient media using Mueller-Hinton–based chocolate agar, supplemented with 1% IsoVitaleX (Becton Dickinson, Franklin Lakes, NJ) and 3 μg/mL vancomycin to inhibit the growth of other organisms.[115] The rarity of this organism and the expense and limited shelf life of the media make isolation of *H. ducreyi* difficult and uncommon.

Light microscopy of syphilis chancre exudate is not useful. The spirochetes that cause syphilis do not take up standard stains well and are extremely thin. Darkfield microscopy of lesion exudate from either a chancre or condylomata lata can identify spirochetes. Incident light is angled obliquely at the stage by polarizing lenses and then reflected upward through the objective by microbes in the clinical specimen. Spirochetes appear as tightly coiled, white organisms spirally rotating against the black background of the microscopic field.[116] There are nonpathogenic treponemes, particularly in the oral cavity, which cannot be differentiated from *T. pallidum* by darkfield microscopy. In the appropriate clinical setting, a positive darkfield specimen is highly suggestive of syphilis. To perform a proper darkfield examination, ulcers must be cleaned with gauze and saline. Exudate from the lesion is then pressed against a glass slide. The specimen should not be contaminated with too much blood. A coverslip is then applied. Rapid examination of the specimen is essential because desiccation reduces the viability of organisms. Agar-based methods for the cultivation of *T. pallidum* are not routinely available. Direct fluorescent antibody testing of smears or tissue is available and, in contrast to darkfield microscopy, can differentiate between pathogenic and nonpathogenic treponemes.[117] Silver staining of biopsy material can identify spirochetes but is not commonly performed.

Serologic testing is the most commonly used method for the diagnosis of syphilitic genital lesions. The process requires two steps: a screening test that detects serum antibodies to nontreponemal antigens (e.g., rapid plasma reagin test, Venereal Disease Research Laboratory test, unheated serum reagin test), and then a confirmatory test that detects serum antibody to true treponemal antigens (e.g., fluorescent treponemal antibody absorption test, *T. pallidum* particle agglutination assay).[118] Early after the appearance of the syphilitic chancre, only the treponemal-specific test may be reactive. Repeat testing with the nontreponemal test should be considered at some time after the ulcer has formed. In rare situations, the nontreponemal test may be falsely nonreactive in secondary syphilis because of the blocking effect of excess antibody; this is known as the prozone phenomenon. In this situation, repeat testing should be performed on diluted serum specimens. Ordinarily the nontreponemal serologic test reaches its highest titer in secondary disease and declines with the onset of latency or with effective therapy. For the purpose of economy, enzyme immunoassay methodologies using

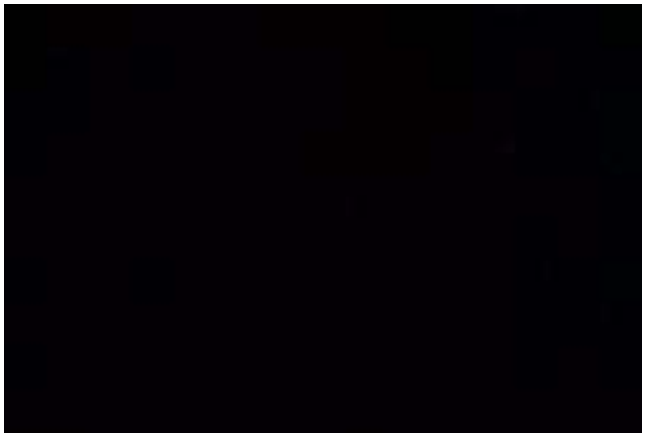

FIG. 106.12 Haemophilus ducreyi. Gram stain of *H. ducreyi.*

treponemal antigens have been introduced as screening tests. When reactive, these need to be confirmed by traditional nontreponemal specific tests. The use of this reverse algorithm has been shown to identify many more suspected cases of latent syphilis than the traditional testing algorithm.[119] The significance of a reactive enzyme immunoassay treponemal test unconfirmed by a nontreponemal test has not been clearly established.[120]

HSV-infected genital lesions can be identified by light microscopy using the Tzanck smear. In this procedure, epithelial cells are scraped from an ulcer base and stained with Wright-Giemsa stain. Multinucleated giant cells and intranuclear inclusions are characteristic of HSV infections. However, both the sensitivity and the specificity of the Tzanck smear are poor.[121] Nucleic acid amplification tests (NAATs), now recommended for HSV testing, are increasingly available and provide excellent sensitivity even in situations where culture may be negative.[122] When available, conventional cell culture also can provide a relatively rapid and accurate diagnosis, but culture has largely been replaced by NAATs. Most specimens from a genuine lesion of HSV demonstrate the pathognomonic cytopathic effect within 48 hours after collection. Fluorescein-conjugated type-specific monoclonal antibody can then be applied to these specimens for confirmation. Serologic tests for HSV antibodies are currently available and can distinguish between antibodies to viral glycoprotein G, which allows for distinction between HSV-1 and HSV-2.[123] Given the prevalent, chronic, and recurring nature of HSV genital infections, these tests are not typically used to establish the role of HSV as a cause of an acute genital lesion. Further modifications now allow these tests to be performed rapidly in the office or clinic.[124]

Klebsiella granulomatis, the cause of granuloma inguinale, or donovanosis, can be identified by staining scrapings of a lesion base with the Wright-Giemsa stain (Fig. 106.13). Surface cells alone may not harbor the organism, so biopsy is often necessary. Clusters of blue rods, with prominent polar granules and surrounded by pink capsules, are seen within infected epithelial cells and are known as Donovan bodies. Cultivation is difficult.

The diagnosis of LGV is usually based on clinical criteria. Diagnosis on the basis of a genital ulcer or lesion alone is rare. Later, after the onset of tender unilateral inguinal or femoral adenopathy, the diagnosis is most obvious. Isolation by cell culture or identification by NAATs of *C. trachomatis* from bubo drainage is diagnostic. At the present time, commercially available NAATs for chlamydia can identify the organism in rectal specimens but are not approved by the US Food and Drug Administration for this purpose. Further testing with LGV serovar-specific NAATs can be performed, but these NAATs are not widely available. Testing of serum for *C. trachomatis* antibody can be done with the use of complement fixation or microimmunofluorescence techniques but are generally not used.

The lesions of HPV also are diagnosed primarily by their clinical appearance. Resort to biopsy of lesions on keratinized tissue is infrequent. There is no cell culture system for the cultivation of HPV. The presence of virus in nonkeratinized tissue such as cervical epithelium can be assessed by cytologic methods (e.g., Papanicolaou smear) or biopsy. The cytologic change consistent with HPV is the koilocyte, with or without nuclear atypia. Cytology alone can be both insensitive and nonspecific. HPV DNA tests such as hybrid capture or polymerase chain reaction (PCR) used in conjunction with cytologic examination are accurate. Application of 3% to 5% acetic acid to suspicious mucosal lesions reveals the characteristic whitening of HPV lesions on either female or male genitalia. Serologic tests for HPV, useful in epidemiologic and vaccine studies, as of yet have no obvious role in clinical care. Their utility in aiding the diagnosis of a chronic, common problem such as HPV may be an issue.



FIG. 106.13 Klebsiella granulomatis. Biopsy of granuloma inguinale lesion showing Donovan bodies consistent with *K. granulomatis.*

Infestation with ectoparasites such as *Sarcoptes scabiei* is demonstrated by identification of the organism, eggs, or feces under direct light microscopy. This may require the unroofing of the scabies burrow bluntly with a needle or scalpel.

NAATs are an increasingly common means of diagnosing infectious diseases including *C. trachomatis* and *N. gonorrhoeae* urethritis. Efforts have been made to apply these technologies to the diagnosis of some of the more common genital lesions. Nucleic acid detection can be performed with the use of hybridization techniques, amplification techniques (i.e., PCR, transcription-mediated amplification, or some combination of both). NAATs have been developed for *H. ducreyi,*[125] *T. pallidum,*[126] and HSV.[122] These technologies have also been combined in one "multiplex" platform (M-PCR) to aid in the clinical evaluation of genital lesions.[127–133,134] The role of such tests in current clinical practice is undefined.

Routine evaluation of genital ulcer disease in the United States should at the very least include viral culture or NAATs for HSV-2 and serologic tests for syphilis. If darkfield microscopy is readily available, it should be used to identify spirochetes. Most laboratories do not have ready access to media for the cultivation of *H. ducreyi.* PCR for *H. ducreyi,* used in some research settings, is highly sensitive and specific but is not approved by the US Food and Drug Administration or widely available.

THERAPY

The goals of therapy include the elimination of the pathogen, resolution of lesions and symptoms, reduction in the risk of transmission, and, if applicable, a reduction in the frequency of recurrences.

After more than 70 years of use, penicillin remains the treatment of choice for the chancre of primary syphilis and the lesions associated with secondary disease. A single intramuscular injection of 2.4 million U of benzathine penicillin G should result in prompt conversion to darkfield-negative lesions and the onset of healing within 24 to 48 hours. Alternatives to penicillin include a course of doxycycline (100 mg orally twice daily for 14 days) or ceftriaxone (1–2 mg intramuscularly or intravenously for 10–14 days).[8] There are far fewer data and little clinical experience supporting the use of these alternative regimens.[135,136] Regardless of the therapy selected, patients treated for early-stage syphilis require serially repeated serologic tests (nontreponemal) even after lesions heal to verify an adequate response. These can be done at 6-month intervals.[21] Fourfold or two-step dilutional decreases indicate an appropriate response. There is no evidence that HIV-infected patients require additional or different therapies for early syphilis.[104] Serologic tests in these patients should be performed at intervals of 6 months or less.

HSV infections can be treated with a variety of related nucleoside analogues available both orally and intravenously. The goal of therapy may be to hasten the resolution of lesions and reduce infectivity or to suppress outbreaks in individuals who experience frequent bouts of disease. Antiviral agents inhibit DNA polymerase and require phosphorylation by viral thymidine kinase to do so. The most commonly used agent, acyclovir, was the first to be marketed. Subsequently, both valacyclovir, a prodrug of acyclovir, and famciclovir, a prodrug of another nucleoside analogue, penciclovir, were introduced for the treatment of HSV. These agents are more bioavailable than acyclovir in oral form and can be dosed less frequently. If necessary, severe HSV genital disease can be treated with intravenous acyclovir. Patients can use these agents for quick resolution of acute outbreaks, for prevention of development of lesions, or for long-term suppression of outbreaks.[137] Regular use of valacyclovir by infected patients has been shown to prevent transmission

to previously uninfected sexual partners.[138] Thymidine kinase–deficient strains of virus, although rare, are resistant to these antivirals. This phenomenon has been observed primarily, although not exclusively, in immunocompromised hosts.[102,115,139–142] Disease caused by resistant isolates can be treated with foscarnet, a DNA polymerase inhibitor unrelated to acyclovir and its congeners.[143] Foscarnet is available only for intravenous use and has been compounded for topical use. It potentially causes renal dysfunction and hypophosphatemia. Another agent that has activity against HSV is cidofovir, an acyclic nucleoside phosphonate.[144] It is also available intravenously and can be formulated for topical use. Pritelivir, a new helicase-primase inhibitor that is unaffected by thymidine kinase deficiency and is available in oral form, is currently under study (see Chapter 46).[145]

Chancroid may be cured microbiologically with a number of antimicrobial agents. Lesions are often slow to heal, and scarring may occur. Although sulfonamides and aminopenicillins were originally used in the treatment of chancroid, microbial resistance has rendered them ineffective. Erythromycin and azithromycin are currently recommended therapies. The latter can be used as a single 1-g dose orally.[8] The third-generation cephalosporin ceftriaxone, given as a single dose of 250 mg intramuscularly, also is effective, but there are concerns about diminished activity in patients with HIV coinfection.[44,146] Fluoroquinolones effectively treat chancroid but should be administered for at least 3 days and not as single-dose therapy.[147] Tender, fluctuant buboes can be aspirated for symptomatic relief.

Although symptomatic HPV infection and molluscum contagiosum may both spontaneously resolve, there is a role for treatment. Therapies include a variety of ablative techniques such as curettage, electrocautery, cryosurgery, and laser. Each of these therapies causes some tissue damage. None provides a certain cure, and recurrence is common. Topical agents include podophyllin (from the resin of the mayapple), trichloroacetic acid, and imiquimod (an immune modulator). Podophyllin should not be used on nonkeratinized epithelium or during pregnancy. Podophyllin and imiquimod also are available for use as patient-applied preparations. Topical application by patients of catechins, a green tea extract, is available and appears to be effective to some degree.[148] Intralesional interferon injections have been studied but are associated with poor response and significant side effects.[149]

Tetracycline congeners and erythromycin have long been the standard therapy for LGV. The duration of therapy is ordinarily 14 to 21 days. There are no data comparing any particular preparation or satisfactorily defining duration of therapy. Reports of successful azithromycin use for LGV have been published, but clinical trials data are lacking.[150] Donovanosis can be treated with a variety of antibiotics including doxycycline, trimethoprim-sulfamethoxazole, fluoroquinolones, and erythromycin. The course of therapy for donovanosis should be 3 weeks or until lesions are healed. Relapse may occur.

OTHER MANAGEMENT ISSUES

Because genital ulcer disease, in particular, and sexually transmitted diseases, in general, seem so closely linked to HIV transmission, it is prudent to make HIV counseling and testing services available to all patients who present with venereal genital lesions. It is also necessary for patients with genital lesions to be counseled about the communicable nature of their disease and the need in many of these instances for partner referral. Based on local public health requirements, it may be necessary to report certain infections to the health department. Sexual partners of patients with syphilis and chancroid require evaluation and therapy for those conditions. In cases of LGV and donovanosis, treatment of asymptomatic partners is of unclear benefit. Partners of individuals with HSV or HPV need counseling about exposure and the potential for disease. The utility of routine cervical Papanicolaou smears for women should be explained. Finally, methods to avoid future infections (e.g., safe sex) should be explored with the patient.

Male circumcision has been garnering attention as a potential way to reduce risk of HIV acquisition. Evidence that this strategy may also reduce risk of genital ulcer disease and other sexually transmitted diseases is conflicting.[131,151,152,153] Condom use remains an important, although not an absolute, measure to prevent infection from the common causes of diseases associated with genital lesions.

NONVENEREAL GENITAL LESIONS

Candida spp., other yeasts, and some molds sometimes cause nonvenereal genital lesions. In women, vaginal colonization with yeast is extremely common, although symptomatic disease occurs in only a fraction of colonized individuals. In a small number of patients, symptomatic disease may be recurrent. Recent use of antimicrobials is a common predisposing risk factor for candidal infections. Vulvovaginal infection is commonly characterized by a thick, whitish, pruritic vaginal discharge, but occasionally it manifests solely as vulvar and perineal disease, with inflammatory vulvitis, erythema, pruritus, superficial excoriations, and ulcerations. Distinguishing candidal lesions on the external genitalia from lesions of other common superficial ulcerative processes such as HSV lesions may at times be difficult.

Genital infection with yeast also occurs in men, a condition known as *candidal balanitis* or *balanoposthitis*. A history of a sexual partner with vulvovaginal infection may be elicited, although sexual transmission is not typical. Balanitis usually involves only the glans, which can appear taut and glossy. There may be multiple, discrete, small pustules and linear or more substantial erosions. As in women, there is often significant pruritus.[35] A proportion of individuals with candidal balanitis have diabetes mellitus.[34] Skin scrapings or vaginal fluid examined under light microscopy after the addition of potassium hydroxide reveal the pseudohyphae of *Candida* spp. Treatment with any of a variety of topical or oral azole antifungal agents is usually effective. Similar findings may be observed with Zoon balanitis, which commonly manifests as an erythematous macule on the penis in uncircumcised men. Plasmocytes predominate on histopathology. These lesions are benign, and topical azoles are generally effective.

Dermatophytes such as *Trichophyton* spp. can cause scaling and sharply demarcated lesions known as tinea corporis or tinea cruris around the genital and the anal area (Fig. 106.14). They can be asymptomatic or mildly pruritic. Infection is usually due to spread from another part of the body.

Fixed-drug eruptions, which represent systemic responses to medication, may involve the genitalia in either sex. A wide range of agents have been implicated including antibiotics such as the tetracyclines and the sulfonamides nonsteroidal antiinflammatory drugs, barbiturates,



FIG. 106.14 **Tinea corporis.**

and oral contraceptives.[30,31] Lesions occur soon after ingestion of the offending agent, with the development of erythematous or violaceous, sharply demarcated macules. They may recede with residual hyperpigmentation or evolve to form bullae and erosions. Topical medications applied to the genitals can also cause localized contact dermatitis. Other common dermatologic conditions can involve the genitals including eczema and psoriasis.[132] In eczema, pruritus leads to scratching, which then results in linear erosions. When chronic, this can cause lichenification or thickening of the skin. In psoriasis, there is epidermal hyperplasia. Lesions are red, well demarcated, and often scaling. Patients with psoriasis and who have been exposed to therapy with ultraviolet light may be at a higher risk for squamous cell carcinoma of the genitals.[133]

Aphthous ulcers of the vagina occur in Behçet syndrome and in HIV infection.[134,154] The cause of these lesions is unclear, but they are presumably the result of an immune-mediated phenomenon. These lesions are typically small, approximately 1 to 2 mm, but may be quite large in some instances. They may be superficial or deep and often are marked by surrounding erythema. The diagnosis is made by observation, a history of recurrence, and the exclusion of other causes. As with other cutaneous manifestations of Behçet syndrome, they are treated with topical or systemic steroids, colchicine, dapsone, or thalidomide. Lichen planus, another process of presumed immunologic origin, may also cause isolated genital lesions or genital lesions in association with oral lesions.[155] Lichen sclerosus is another possibly autoimmune condition that involves the external genitalia in women. Persistent low-grade inflammation eventually leads to the characteristic fibrosis of the vulva and adjoining structures with occasional extension to the perirectal tissue giving the characteristic white keyhole appearance.

Genital lesions may also arise from other processes including trauma[21,156,157] and folliculitis. Pearly penile papules are anatomic variants characterized by tightly clustered, small, uniform papules exclusively involving the coronal sulcus in a circumferential distribution. They are not rare, often appear in young adulthood, and lead anxious patients to seek clinical care, but they require no intervention. Tuberculosis can cause anogenital fistulas from adjacent, infected genital or gastrointestinal structures or, rarely, primary lesions of the genitals.[141,158,159] Genital lesions rarely result from systemic infection with other pathogens such as *Histoplasma capsulatum* and *Entamoeba histolytica*.[142,160–162] Vulvar and penile squamous cell carcinoma are rare but well-recognized clinical entities usually resulting from HPV infection.[163,164] Erythroplasia of Queryat and Bowen disease are variants of squamous cell carcinoma that occur in men.[165]

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107 Urethritis

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SHORT VIEW SUMMARY

Definition
- Urethritis is an inflammatory condition involving the urethra that is usually caused by sexually transmitted infectious pathogens.

Epidemiology
- Urethritis occurs worldwide.

Microbiology
- Common etiologic agents include *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis,* and *Mycoplasma genitalium.*

Diagnosis
- Light microscopy of urethral discharge can be helpful. The presence of polymorphonuclear leukocytes is highly suggestive of inflammation. Gram stain that reveals intracellular gram-negative diplococci suggests *N. gonorrhoeae* (or occasionally *Neisseria meningitidis*). Both can also be cultured using standard agar-based techniques. Nucleic acid amplification techniques are preferred for diagnosis of the other etiologic agents of this condition (see Table 107.1).

Therapy
- Treatment is directed toward the known or suspected pathogen. Infection with *N. gonorrhoeae* is generally treated with third-generation cephalosporins along with azithromycin, but resistance is an emerging concern. Infection with *C. trachomatis* responds to azalides and tetracyclines.

Prevention
- Condom use or abstaining from high-risk sexual activity reduces the risk for urethritis.

The symptoms of urethritis range from the trivial and often overlooked to the disabling.[1] Urethral discharge in men may be apparent at all times during the day and may be present in sufficient quantity to stain undergarments or may be scant. It may be clear, mucopurulent, or frankly purulent, and it may be white, yellow, green, or brown. Some patients report only a deviation of the first morning urine stream, whereas others report only a urethral discomfort. Occasionally, urethral discharge comes to the attention of the patient through the observation of mucus strands in the urine.

The urine stream transiently eliminates most inflammatory discharges; therefore discharge is best observed before urination. Micturition immediately preceding urethral examination may completely eliminate signs of infection.

Dysuria is common with urethritis, and men variously localize it to the meatus, the distal portion of the penis, or anywhere along the shaft. Dysuria is sometimes increased by the acidity or solute content of the urine and therefore may be most marked during the passage of concentrated first morning urine. It may be increased in the presence of irritants such as alcohol, which is an observation that sometimes leads the patient to attribute his disease to the ingestion of specific foods or fluids. Discomfort may persist between micturitions and is perceived as pain, itching, frequency, urgency, or a feeling of heaviness in the genitalia.

Discomfort experienced only during ejaculation, deep pelvic pain, or pain radiating to the back is infrequent in uncomplicated urethritis and suggests prostatitis or inflammation involving other portions of the urogenital tract such as the epididymis. Hematuria (particularly if painless) and blood in the ejaculate are uncommon in urethritis. The persistence of hematuria after cure of urethritis demands a thorough urologic evaluation.

Exclusive urethral infections are less well described in women. Dysuria is a very common symptom among women and may be due to cystitis, urethritis, or vulvitis. Urethritis is most likely due to a sexually transmitted pathogen and is often associated with concomitant cervicitis. The term *acute urethral syndrome* has been used to describe urethritis in women that is largely due to *Chlamydia trachomatis* infection; this is discussed later (see "Urethral Syndrome and Related Diseases of Women").

The epidemiology of urethritis varies with the geographic prevalence of the causative organisms. The most data are available for gonococcal and chlamydial infections. Surveillance data from the Centers for Disease Control and Prevention (CDC) reveal increases in case rates for gonococcal and chlamydial infections in both men and women throughout the United States.[2] Rates are disproportionately high for minority populations in the United States, especially among non-Hispanic blacks.[2] The highest rates are seen among men and women 20 to 24 years of age. It is unknown whether rate increases relate to enhanced screening programs, including modifications in testing procedures with addition of extragenital testing, or true increase in disease transmission.[2] Worldwide data vary considerably by locale. Gonococcal and chlamydial rates vary across Europe, although reported rates in men, especially men who have sex with men (MSM), have increased in recent years.[3]

EXAMINATION OF THE URETHRA

The male genitalia are best examined while the patient is supine. Alternatively the man can stand before the seated examiner so that the external genitalia are at approximately eye level. The entire genital area should be visualized. The underwear may reveal stains of dried discharge, suggesting that it is being produced in large amounts.

The patient is preferably examined at least 2 hours after his last micturition. If advised to restrict fluids during the day preceding the examination, the patient may be able to present for evaluation before passing the first urine of the day, which sometimes permits the recovery of very small amounts of discharge.

The entire genital area should be carefully examined because acquisition of more than one sexually transmitted infection is relatively common. Inguinal adenopathy should be palpated to evaluate for tenderness and enlargement. If lymphadenopathy is present, bilateral versus unilateral findings should be noted. The skin of the entire pubic area, scrotum, groin, and penis should be examined for lesions, and the hair should be examined for nits. The testes, epididymis, and spermatic cords should be palpated for masses or tenderness. The foreskin should be completely retracted and the glans examined. The urethral meatus should be inspected for crusting, redness, and spontaneous discharge. If no discharge is present, the urethra should be gently stripped as follows. The examiner places the gloved thumb along the ventral surface of the base

of the penis and the forefinger on the dorsum and then applies gentle pressure; the hand is moved slowly toward the meatus. This maneuver frequently expels a discharge that may be collected on a swab for examination (as described later).

If no discharge is delivered by this maneuver, the third and fourth fingers should be used to grip the penis lightly from above, just behind the glans. The thumb and forefinger can then spread open the meatus to examine for urethral redness or the presence of small amounts of discharge. Unless the patient has recently urinated or has been in a state of sexual arousal, virtually no fluid should be expressible from the urethra or observed by spreading the meatus.

If expressed material cannot be collected at the meatus, a specimen must be obtained from inside the urethra. This is best accomplished with a small urethral swab. The swab should be inserted gently at least 2 cm into the urethra, with care taken not to attempt to force the tip past any obstruction. The patient should be warned that the procedure will be uncomfortable; also, the insertion and removal of the swab should be accomplished as quickly as possible. Patients tolerate the procedure better in the supine position. If additional specimens are required for multiple examinations or cultures, separate swabs should be used, and each one should be inserted at least 1 cm deeper than the one preceding it.

Regular cotton swabs should not be used for urethral examination because their larger diameter makes insertion extremely uncomfortable and because of the possibility that the cotton or the wooden shaft may be toxic to some fastidious pathogens. Currently available nucleic acid amplification test (NAAT) kits provide swabs appropriate in size and composition to obtain a proper specimen.

The female urethra is best examined when the patient is in the lithotomy position. The entire genital area should be examined for lesions and discharge, and the vagina should be examined as described in Chapter 108. The urethral meatus may be directly visualized, and the urethra may be stripped by placing the gloved finger inside the vagina and gently moving it along the urethra. A urine specimen may be examined by light microscopy, culture, or NAATs for the presence of typical pathogens.

EXAMINATION OF THE URETHRAL SPECIMEN AND CONSIDERATION OF ETIOLOGIES

A swab that contains material from the urethra should be rolled across a clean microscope slide. Rolling rather than streaking the swab brings all its surfaces into contact with the slide and better preserves cellular morphology. The material should be air dried and fixed by gentle heating or by rinsing with methanol. Gram staining of urethral material is particularly useful in the workup of urethritis (Table 107.1), and the specimen should be examined with the use of the oil-immersion objective. Specimens obtained from within the urethra usually contain urethral epithelial cells. If recovered from near the meatus, these are typical squamous cells with a very large cytoplasmic-to-nuclear ratio; if obtained from further within the urethra, they are cuboidal epithelial cells, which are smaller and have relatively larger, less dense nuclei.

TABLE 107.1 Evaluation of Men Who Have Urethral Symptoms

DIAGNOSTIC STUDIES	COMMENTS
Gram-stained urethral smear	
Examination of first-void urine specimen for leukocytes	
Endourethral cultures or nonculture tests	
Neisseria gonorrhoeae	Culture and NAAT equally appropriate; culture can provide option for susceptibility testing
Chlamydia trachomatis	NAAT preferred
Trichomonas vaginalis	Culture or NAAT
Mycoplasma genitalium	No currently available commercial test

NAAT, Nucleic acid amplification test.

Urethral material from patients with acute urethritis contains polymorphonuclear neutrophils (PMNs) (Table 107.2). The area of the smear that contains the most PMNs should be sought. More than two PMNs per oil-immersion microscopic field is abnormal and is seen in most patients with acute symptomatic urethritis.[4] However, although the positive predictive value of urethral PMNs is quite good, the negative predictive value for infection is not. As many as 15% of men with documented urethral infection do not show significant numbers of PMNs in Gram-stained smears.[4,5] The number of PMNs in the smear is reduced by recent micturition; also, often there is considerable observer variation in the number of PMNs detected in a single specimen.[6] Therefore although purulent discharges may reveal sheets of PMNs, the minimal number of these cells that indicates disease is not known. In general the presence of even rare PMNs suggests infection, particularly in a patient who has urethral symptoms, who engages in high-risk sexual activity, or who is found to have a small amount of discharge on examination.

The distal centimeter of the urethra is colonized by normal skin or introital microbiota. The smear usually contains a variety of gram-positive and gram-negative organisms that have no particular significance. Of great diagnostic value, however, is the presence of typical gram-negative, "intracellular" diplococci (Fig. 107.1). These organisms are not randomly distributed among the cells but are seen in large numbers in a few PMNs. They are observed in more than 95% of all symptomatic patients with gonococcal urethritis and in less than 2% of all symptomatic men who cannot be shown to have gonorrhea by culture.[7] Some strains of *Neisseria gonorrhoeae* are inhibited by the concentrations of vancomycin that usually are employed in selective isolation media (i.e., Thayer-Martin, Martin-Lewis); these organisms will not be recovered by standard culture techniques.[8] Extracellular diplococci indicate gonorrhea in only 10% to 29% of all cases, and this predictive value is reduced even further in populations with a low prevalence of gonorrhea. A shortcoming of the Gram-stained smear is that coincidental infection with the agents responsible for most cases of nongonococcal urethritis (NGU) cannot be diagnosed in the presence of gonorrhea. Although a smear containing PMNs that does not reveal gram-negative intracellular diplococci suggests NGU, a smear revealing these organisms does not rule out concomitant infection with *C. trachomatis, Mycoplasma genitalium,* or *Trichomonas vaginalis.* Trichomonads are nearly impossible to identify on Gram-stained smears. Urethral material may be mixed with a small amount of saline and observed as a wet mount with the

TABLE 107.2 Objective Evidence of Urethritis

Visibly abnormal discharge (purulent, mucopurulent)
Urethral Gram stain containing ≥2 leukocytes per oil-immersion field
First-void urine specimen containing >10–15 leukocytes per high dry field

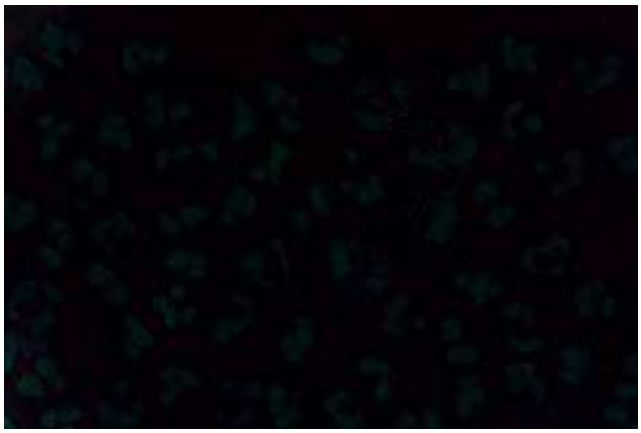

FIG. 107.1 Gram stain of urethral exudate from a man with gonorrhea. Several neutrophils contain many gram-negative cell-associated diplococci.

substage condenser racked down or the substage diaphragm partially closed. Motile trichomonads occasionally are observed but are rarely seen unless the urethral material for examination is obtained before the first voiding. Positive findings on a wet mount are diagnostic of trichomoniasis, but findings on the wet mount are often negative in infected men. Endourethral cultures and cultures of first-void urine sediment in liquid media such as modified Diamond medium are useful in the diagnosis of trichomoniasis in men.[9,10] NAATs for *T. vaginalis* infections are now available.[11] *Mycoplasma* does not have a cell wall and lacks the macromolecules in its membrane to be well-visualized by Gram stain.

After the man's urethra has been carefully examined, he may be asked to provide a divided urine specimen. The patient delivers the first 10 mL of urine into one container and a midstream urine specimen into a second container. The presence of mucus strands in the first fraction that clears in the second portion suggests urethritis. Equal aliquots of the fractions may be centrifuged and the sediments examined as wet mounts. Observation of more white blood cells in the initial fraction than in the second fraction suggests urethritis, whereas equal numbers of white blood cells in both fractions suggests cystitis or infection higher in the urinary tract. The presence of more than 10 to 15 white blood cells in 400× microscopic fields of the sediment from the initial fraction strongly suggests urethritis, but the minimum significant number of white blood cells is unknown.

The presence of white blood cells in the initial urine fraction provides no clue to the cause of urethritis. Such a finding, however, may allow an objective diagnosis of urethritis to be made in a man whose Gram-stained smear does not contain PMNs.

Some men who are infected with *N. gonorrhoeae* and many men infected with *C. trachomatis* have no symptoms. Such men often have pyuria that can be detected by examination of the first 10-mL urine sample by either microscopy[12] or leukocyte-esterase dipstick testing.[13] This approach provides a noninvasive, inexpensive method for screening men for urethral infection. Men found to have pyuria are candidates for further examination, including examination of endourethral specimens for gonococci, chlamydiae, and trichomonads.

If the urine specimen is a first morning micturition, motile trichomonads may be observed in the sediment. In one study, *T. vaginalis* organisms were recovered by culture of urethral swabs in 80% and first-void urine in 68% of infected patients. When combined, these two cultures were detected in 49 (98%) of 50 infected men.[9] As in the case of endourethral specimens, NAATs of urine are the most sensitive and specific means of identifying *T. vaginalis*.[14]

NAATs have replaced culture as the test of choice for identification of *C. trachomatis* organisms.[15] Material recovered from the urethra can be cultured on appropriate media for *N. gonorrhoeae*. Nonculture tests (i.e., NAATs) are also available for detection of *N. gonorrhoeae* organisms. These tests are considered the test of choice for diagnosis of mucosal gonococcal infections unless antibiotic susceptibility information is needed.[15] Importantly, most nonculture tests for chlamydiae are "bundled" with a nonculture test for gonococci, and some include testing for *T. vaginalis* as well.[15]

Neisseria meningitidis has been increasingly recognized as a pathogen associated with urethritis. Recent outbreaks in Ohio, Michigan, and Indiana have been in predominantly heterosexual men, and sequencing has revealed *N. meningitidis* ST11.[16,17]

Suspicion should be raised when a urethral Gram stain indicates gram-negative intracellular diplococci and a corresponding urine or urethral nonculture test is negative for the presence of *N. gonorrhoeae*. *N. meningitidis* can grow in appropriate culture media (i.e., Thayer-Martin, Martin-Lewis). *N. meningitidis* urethritis was confirmed in an outbreak investigation by culture and biochemical identification and polymerase chain reaction (PCR) testing.[16,17]

Other pathogens may also warrant consideration during urethral examination. *M. genitalium* is estimated to cause 15% to 20% of NGU cases and 30% of persistent or recurrent urethritis.[18,19] PCR assays are the most sensitive tests for this fastidious organism.[20] *Ureaplasma urealyticum* has been subdivided into two species—*Ureaplasma parvum,* which is a saprophyte, and *U. urealyticum,* which may be a cause of urethritis (see later discussion). NAATs for *U. urealyticum* are also available. Although they are present in the distal urethra, normal skin organisms (e.g., *Staphylococcus epidermidis,* α-hemolytic streptococci, propionibacteria) and vaginal organisms (e.g., *Candida albicans,* lactobacilli, *Escherichia coli, Gardnerella vaginalis*) are of no diagnostic significance.

NONINFECTIOUS URETHRITIS

The genital tract is psychologically important, and trivial symptoms are often worrisome to patients. The "worried well" make up a significant fraction of men who are seen in sexually transmitted infection clinics and in private practices. Detailed questioning as to why the patient perceives he has a genital infection may reveal guilt over masturbation or an extradyadic (e.g., extramarital) sexual contact. The urethral specimen in these cases usually reveals normal epithelial cells and no white blood cells. Some patients confuse dried remnants of semen with inflammatory discharge. Spermatozoa may be recognized on the Gram stain as gram-positive ovoids whose coloration fades gradually toward the acrosomal cap. Spermatozoa may also be recognized on the wet mount. However, the clinician must remember that symptoms and signs of true urethritis can be trivial and that microscopic examination may miss minimal inflammation, particularly if the patient has recently voided. Symptomatic patients with negative examination results should have urethral or urine specimens tested for urethral pathogens and be asked to return in several days, by which time the symptoms may have resolved or examination may provide a diagnosis. Antimicrobial treatment of symptomatic men who have neither objective evidence of urethritis nor positive cultures for urethral pathogens is inadvisable and may serve to reinforce psychosomatic contributions to their symptoms.[21] Occasionally, a patient who reports discharge actually has urinary incontinence.

Chronic irritation of the urethra can elicit a clear, mucoid discharge. Occasionally, patients who are concerned that they may have contracted a venereal disease vigorously strip the urethra looking for discharge. After several days of this trauma, a clear discharge obligingly appears that may contain a few white blood cells. A history of vigorous urethral stripping is helpful diagnostically. Patients who are receiving treatment for other forms of urethritis should be cautioned not to examine themselves too vigorously for fear that such a traumatic discharge may confuse the clinical picture. Very rarely, patients insert foreign bodies into the urethra and produce a mechanical urethritis.[22]

A heavy precipitation of crystals in the urine can suggest a discharge, and the presence of large amounts of crystalline material or calculous gravel can produce urinary discomfort. The intermittent nature of pain associated with the passage of gravel or the obvious presence of crystals on microscopic examination of the urine sediment usually confirms this diagnosis. White blood cells may be present.

Chemicals can irritate the urethra, and alcohol has long been known to produce mild dysuria. The ingestion of alcohol during treatment for gonorrhea was at one time thought to be responsible for the syndrome of postgonococcal urethritis (discussed later), although postgonococcal urethritis is now known to have an infectious etiology. Occasionally a patient develops urethral symptoms on contact with vaginal chemicals such as spermicides used by a sexual partner. The history of discomfort immediately after sexual contact may be suggestive. This condition should be diagnosed only after other possible causes have been excluded.

INFECTIOUS URETHRITIS

Gonococcal and Nongonococcal Urethritis

The classic specific etiologic agent of acute urethritis is *N. gonorrhoeae*. Urethral infection of all other causes is referred to collectively as NGU (Table 107.3). As with gonorrhea, NGU is a sexually acquired condition. NGU is more common than gonorrhea in the United States and in much of the developed world. In some developing areas, however, gonorrhea accounts for up to 80% of cases of acute urethritis.[23–25] Similar to many other sexually transmitted diseases (STDs), gonococcal urethritis and NGU have an increased incidence during the summer months, presumably because of a seasonal increase in sexual activity.



FIG. 107.2 Purulent urethral discharge from a man with gonococcal urethritis.



FIG. 107.3 Mucopurulent urethral discharge from a man with nongonococcal urethritis.

TABLE 107.3 Most Common Infectious Causes of Urethritis

Neisseria gonorrhoeae
Chlamydia trachomatis
Trichomonas vaginalis
Mycoplasma genitalium
Herpes simplex virus
Adenovirus
Ureaplasma urealyticum
Neisseria meningitidis

Compared with gonococci, the organisms that cause NGU may be relatively less prevalent among MSM than among heterosexual men with urethritis. In a study of men who had urethritis, Ciemins and colleagues[26] recovered gonococci from 45% of MSM and from 26% of men who have sex with women. Examining consecutive men attending an STD clinic, Stamm and colleagues[27] recovered gonococci from 12% of heterosexual men and 25% of MSM; in contrast, chlamydiae were recovered from 14% of heterosexual men but only 5% of MSM. Other studies demonstrate similar findings.[28–30] Studies have associated fellatio with the acquisition of gonococcal urethritis and NGU in MSM but not in heterosexual men.[31,32]

Urethritis may facilitate transmission of human immunodeficiency virus (HIV). Among patients infected with HIV, the quantity of HIV in urethral secretions is increased if the man has concomitant chlamydial or gonococcal urethritis. Treatment of urethritis reduces the urethral viral load.[33]

The clinical spectrum of gonorrhea differs from that of NGU, but there is sufficient overlap that an accurate diagnosis must be based on examination of the urethral specimen. Of men who acquire urethral gonorrhea, 75% develop symptoms within 4 days, and 80% to 90% do so within 2 weeks.[34] The incubation period for NGU is much more variable and is often longer. Incubation periods ranging from 2 to 35 days have been described, but almost 50% of men with NGU developed urethral symptoms within 4 days.[34] Therefore an incubation period of less than 1 week is not a reliable factor in the differential diagnosis. The incubation period of either infection can be prolonged by the ingestion of subcurative doses of antibiotics.

The urethral discharge is described as frankly purulent in three-fourths of patients with gonorrhea (Fig. 107.2) but in only 11% to 33% of patients with NGU.[7,35] A purulent discharge issuing from the meatus without stripping of the urethra correlates strongly with the diagnosis of gonorrhea but is also seen in 4% of patients with NGU.[7,35] Mucopurulent discharge (Fig. 107.3), consisting of thin, cloudy fluid or mucoid fluid with purulent flecks, is seen in about 50% of patients with NGU but in only 25% of patients with symptomatic gonorrhea.[7,35] The discharge is completely clear and moderately viscid in 10% to 50% of patients with NGU, principally patients who are minimally symptomatic, but in only 4% of symptomatic patients with gonorrhea.[7,35,36] A diagnosis based on the clinical characteristics of the urethral discharge is unreliable and correctly identifies the causative disorder in only 73% of all cases, even under optimal circumstances.[35] Microscopic examination always should be part of the initial evaluation.

Dysuria has been described in 53% to 75% of patients with NGU and in 73% to 88% of patients with symptomatic gonorrhea.[7] Only about 10% of patients reporting dysuria without discharge have gonorrhea; the remainder have NGU.[7] A combination of dysuria and discharge is seen in 71% of patients with gonococcal urethritis but in only 38% of patients with NGU. Therefore the combination of discharge and dysuria is associated with gonorrhea, whereas the appearance of one without the other is more frequently seen with NGU. However, the association is not sufficiently specific for differentiating these two entities. Urethral discomfort can mimic cystitis in men and women and can result in urinary frequency and urgency.

Symptoms of gonorrhea often begin abruptly, and the patient may remember the specific time of day when they were first noted. NGU usually has a less acute onset, with symptoms increasing over several days. A urethral discharge may appear days in advance of dysuria; the symptoms may wax and wane, even to the point of transiently disappearing before the patient seeks therapy. The mildness and variability of the symptoms may erroneously convince a patient with NGU that he does not have a significant disease; such patients often delay seeking medical attention.[7]

In most cases, the symptoms of infectious urethritis resolve even if the causative disorder remains untreated. Of patients with acute gonococcal urethritis, 95% who do not receive treatment are free of symptoms 6 months after contracting the disease,[37] and the symptoms of NGU gradually subside over a period of 1 to 3 months in 30% to 70% of patients.[38] How many of these asymptomatic patients remain infected and potentially infectious is unknown. Untreated gonococcal urethritis may subside to a chronic state characterized by little or no urethral discomfort and a small amount of mucoid discharge called *gleet*. This discharge contains small numbers of gonococci and PMNs.

Because of the great clinical overlap between NGU and gonococcal urethritis, a diagnosis should not be made on clinical grounds alone. Gram staining of urethral discharge material reveals typical gram-negative, intracellular diplococci in about 95% of patients with symptomatic gonococcal urethritis and is negative in about 97% of patients with symptomatic NGU.[7] Therefore in a population in which about 50% of the cases of acute urethritis are gonococcal, a positive result on Gram staining suggests gonorrhea and a negative result suggests NGU with 98% accuracy.[7] Typically shaped extracellular diplococci are found in about 15% of patients with symptomatic urethritis.[7]

The sensitivity of the culture for *N. gonorrhoeae* is less than 100%. The chances of isolating the organism are further reduced if the patient has recently taken antibiotics or if there is a delay in processing the culture. Therefore it is likely that most of the few patients with positive findings on Gram staining and negative cultures actually have gonorrhea.

In most cases of acute symptomatic urethritis, culture is unnecessary to confirm Gram stain findings diagnostic of gonorrhea. It must be remembered that results on Gram staining are negative in as many as 5% of patients who have gonorrhea, so Gram stain findings suggestive of NGU should be confirmed with a culture or nonculture test for gonococci, although therapy need not be delayed until the results are known. Results on Gram staining cannot be used to make a diagnosis of simultaneous NGU in the presence of gonorrhea. Because of the frequency with which trichomonads are missed with direct microscopic techniques, patients in whom trichomonal urethritis is suspected should be evaluated by culture of urethral and first-void urine specimens[10] or, if available, NAAT.

There is no doubt that urethritis is sexually transmitted. It occurs most frequently during the ages of peak sexual activity and in groups with a high prevalence of other STDs. It frequently occurs after sexual exposure to a new partner and is almost never seen in virgins except as a part of some systemic conditions. As the etiologic agents of urethritis have been defined, they have been isolated with high frequency from the female and male sexual partners of infected men, in whom the agents usually are carried asymptomatically.

Recognition of urethritis as an STD is important for several practical reasons. It allows definition of a population at very high risk for carrying the causative agents, namely, the sexual partners of infected patients. The prevalence of infection with these agents is sufficiently high among sexual partners to justify their treatment on epidemiologic grounds even if they are asymptomatic. Many episodes of recurrent urethritis are terminated only by the treatment of an asymptomatic sexual partner of the infected patient. Because patients with one STD are at increased risk for others, it is important to screen patients with urethritis for other STDs.

Etiology of Nongonococcal Urethritis

The organism most clearly associated with NGU, *C. trachomatis,* is discussed in detail in Chapter 180. However, this obligate intracellular parasite causes only 20% to 50% of cases of NGU.[39–41] Effective *Chlamydia* control programs have reduced the proportion of men with NGU who are infected with *C. trachomatis. C. trachomatis* is susceptible to several antimicrobial agents, including tetracyclines, sulfonamides, erythromycin, and azithromycin. Significantly, it is not reliably eradicated by cephalosporins. Chlamydiae are not recovered from at least 50% of men with NGU. The clinical features of chlamydia-negative NGU are very similar to the features of chlamydia-positive NGU.[42]

The agents responsible for chlamydia-negative NGU are incompletely identified. *U. urealyticum,* whose role in NGU is controversial, has been reclassified into two species: *U. parvum* (biovar 1) and *U. urealyticum* (biovar 2).[20] *U. parvum* may be a commensal organism, whereas *U. urealyticum* may be one of the true causative agents of chlamydia-negative NGU.[43–45] Accurate assessment of the relative contribution of ureaplasmas has been hindered by the ubiquity of the organisms that can be recovered from urethral cultures from many sexually experienced men who have no evidence of urethritis.[46] *Mycoplasma hominis* is not a cause of NGU,[47] whereas *M. genitalium* has been recovered from patients with NGU and is now a recognized cause of this condition.[48–50] Studies in which this fastidious organism was identified with the use of PCR assays identified *M. genitalium* in men with NGU more often than in symptom-free control subjects.[51–53]

Similar to *C. trachomatis, U. urealyticum* and *M. genitalium* organisms are susceptible to erythromycin, azithromycin, and, usually, tetracyclines—the agents that have been most successful in treating NGU. However, resistance may be increasing. Perhaps 2% to 7% of patients are infected with tetracycline-resistant *U. urealyticum*[54]; such patients may not be cured by tetracycline therapy. *M. genitalium* may fail to respond to therapy with commonly used therapies for NGU.[55,56]

Dysuria is described by 83% of women and 44% of men with primary herpes simplex virus (HSV) genital infection. Some men notice a clear, mucoid discharge that seems disproportionately mild relative to the degree of dysuria that they experience. HSV is recovered from the urethras of about 80% of women and 30% of men with primary infection, and HSV must be regarded as a cause of some cases of NGU. In most such instances, however, the diagnosis of HSV is obvious because of typical genital lesions. Urethral involvement is less common in recurrent disease, and dysuria is described by only 27% of women and 9% of men.[57]

T. vaginalis organisms have been identified with use of NAATs from more than 20% of men attending urban STD clinics in the United States.[58,59] Identification of *T. vaginalis* has been associated with signs and symptoms of urethritis. The syndrome is not clinically distinguishable from NGU of other causes.

Although rare urethral infection with gram-negative bacilli can be seen in men with diabetes and in men who practice insertive anal intercourse, it may occur in patients with phimosis and in patients with urethral trauma after instrumentation or indwelling catheterization.[60] Periurethral abscesses can occur in this setting. Syphilis, with an endourethral chancre, and intraurethral condylomata acuminata occasionally cause a urethral discharge. *N. meningitidis*[61] and *Moraxella catarrhalis*[62] have been isolated from patients with urethritis. One study[63] identified adenovirus more often in patients with than without urethritis. In another study, in 102 heterosexual men and MSM with urethritis, adenovirus urethritis was diagnosed by urine or urethral PCR or urethral testing. Adenovirus was the exclusive pathogen identified in 93% of the cases.[64] The most frequent symptom was dysuria (83% of patients), and the most consistent physical finding was erythema of the penile meatus (83% of patients). Urethral discharge was noted in 59% of patients, and signs of conjunctivitis were noted in 38% of patients.[64]

In summary, *C. trachomatis,* adenovirus, *M. genitalium, T. vaginalis,* and HSV have been convincingly implicated as etiologic agents in NGU. This has not yet been proven for *U. urealyticum* biovar 2.[65] A significant minority of men with NGU are not infected with any of the aforementioned organisms.

POSTGONOCOCCAL URETHRITIS

Some patients with acute gonococcal urethritis who receive a single-dose treatment with one drug experience prompt resolution followed in a few days by a recurrence of symptoms—usually a mucoid or mucopurulent discharge and sometimes mild dysuria. In other patients, the symptoms never entirely disappear and, after initial rapid improvement, stabilize at a low level. This syndrome has been referred to as *postgonococcal urethritis* (PGU)[66,67] and should be suspected in the event of findings of signs, symptoms, or laboratory evidence of urethritis 4 to 7 days after single-drug treatment for gonorrhea. PGU is a manifestation of dual urethral infection. Gonococci and the agents of NGU are extremely prevalent in sexually active populations, and they are carried simultaneously and asymptomatically by many women. Male sexual partners of these women may acquire both agents during the same sexual exposure. In the presence of gonorrhea, coincident NGU cannot be diagnosed with Gram staining. Single-dose treatment of gonorrhea with an appropriate cephalosporin such as ceftriaxone eradicates the gonococci, eliminating the symptoms of gonorrhea, but it usually spares the agents of NGU. Once the incubation period of NGU is exceeded, the patient experiences a recurrence or persistence of milder symptoms that is consistent with the latter infection.

Although PGU was originally thought to result from the consumption of alcohol or other irritants during therapy for gonorrhea, dual infection is now well established as the explanation. *C. trachomatis* has been recovered from 11% to 50% of men with gonorrhea[39]; 75% to 100% of patients with gonorrhea who are also culture positive for chlamydia will develop PGU if the gonorrhea is treated with an agent that does not eradicate chlamydia.[68] *C. trachomatis* can be recovered from almost 50% of patients with PGU, which is similar to the recovery rate in NGU. However, PGU also develops in up to 50% of the patients with gonorrhea from whom chlamydiae are not recovered. Presumably these cases are due to coinfection with gonococci and other agents of NGU such as *M. genitalium, T. vaginalis,* HSV, adenovirus, and perhaps *U. urealyticum.* As might be expected, if gonorrhea is treated with a regimen active against the agents of NGU, the incidence of PGU is lower.[68] Accordingly, current treatment schedules for gonorrhea include a second agent such as azithromycin or doxycycline. Even if not considering coinfection, currently recommended treatment for gonococcal infection includes therapy active against *C. trachomatis,* so PGU attributable to untreated *C. trachomatis* and most *M. genitalium* strains should be relatively rare.

PERSISTENT OR RECURRENT URETHRITIS

Patients with persistence or recurrence of urethral symptoms after therapy for acute gonococcal urethritis may have PGU attributable to agents of NGU, but gonococcal reinfection and frank treatment failure are also possible especially in an era when diminished susceptibility to antimicrobial agents among *N. gonorrhoeae* strains is increasing. The patient who is experiencing recurrent urethritis must be evaluated as a new patient to differentiate gonococcal from nongonococcal infection. Such patients should have objective evidence of urethritis. When taking a history of persistent or recurrent urethritis, it is important to consider reexposure due to untreated or partially treated partners as well as medication compliance. When these factors have been ruled out and the patient has received a repeat NAAT for *N. gonorrhoeae* and *C. trachomatis,* alternative pathogens such as *M. genitalium, T. vaginalis,* HSV, adenovirus, and perhaps *U. urealyticum* should be considered. If available, NAATs for *M. genitalium* and *T. vaginalis* can be performed. Empirical therapy if chosen should cover the most likely pathogens. If suspicion for reexposure is high, a patient should receive the initial regimen again. Alternatively, treatment for *M. genitalium* should be administered, and potentially treatment for *T. vaginalis* should be given for refractory urethritis.[19]

ASYMPTOMATIC URETHRAL INFECTION

Patients without specific symptoms that are referable to the urethra may be found to have signs of urethritis on physical examination. Sexually transmitted pathogens can be recovered from some patients who have neither symptoms nor signs of urethritis.

The importance of asymptomatic urethral gonococcal infection in men is well recognized.[69] Prolonged asymptomatic urethral carriage of gonococci occurs in 2% to 3% of newly infected men; however, because these men do not seek treatment and because asymptomatic urethral infection may persist for months, the prevalence of asymptomatic urethral gonococcal infections among all men harboring *N. gonorrhoeae* may be 50%.[70] This may have considerable epidemiologic significance. Random screening of asymptomatic men is unrewarding[69] except in high-risk populations. Most cases of asymptomatic urethral infection are detected after gonorrhea is diagnosed in sexual partners or if complications subsequently develop in the infected man. Up to 40% of the asymptomatic sexual partners of women with disseminated gonococcal infection or pelvic inflammatory disease are found to be infected. Asymptomatic gonorrhea may be diagnosed by examination of Gram-stained urethral material collected on a swab with a sensitivity of about 70%.[69] Therefore culture or NAAT for gonococci is the most appropriate diagnostic test.

Asymptomatic urethritis in many cases can be rapidly detected by observing PMNs in material recovered from the urethra with a swab or loop. Endourethral sampling, however, is uncomfortable and is poorly accepted by asymptomatic men. Examination of a first-void urine specimen for leukocytes or leukocyte esterase or for evidence of gonococcal or chlamydial infection with an NAAT[71,72] is a more acceptable means of examining men for asymptomatic urethral infection.[64]

Because of the frequency of asymptomatic, sexually transmitted urethral infections in men, asymptomatic sexual partners of infected individuals should always be evaluated. Because immediate diagnostic techniques are of relatively low sensitivity, such men should receive treatment at the time of their initial presentation (i.e., epidemiologic treatment).

URETHRAL SYNDROME AND RELATED DISEASES OF WOMEN

Dysuria, frequency, urgency, and nocturia are common symptoms of bacterial cystitis in women. A similar syndrome, termed *acute urethral syndrome,* occurs in women who do not have classic bacterial infection of the lower urinary tract.[73,74] The usual workup for bacterial urinary tract infection is unrewarding because less than 10^5 uropathogens are recovered from each 1 mL of urine. Some of these patients appear to have bacterial cystitis, although bacteria are recovered from the urine in smaller than usual numbers.[73,74] In other patients, the symptoms appear to be related to urethritis rather than cystitis. *Escherichia coli* sometimes causes urethritis in the absence of cystitis.[75] If ordinary bacterial pathogens associated with urinary tract infections are not isolated (even in small numbers), the condition is often caused by sexually transmitted agents.[73,74] If pyuria is absent, cultures for enteric bacteria and agents of STDs are less likely to be positive, and antimicrobial treatment is less likely to be effective; a noninfectious explanation for urethral symptoms should be sought in such patients.

N. gonorrhoeae can affect the urethra in women as it does in men and may occasionally cause the urethral syndrome.[76] Gentle stripping of the urethra as described in the earlier section "Examination of the Urethra" may deliver a purulent discharge that on Gram staining reveals typical gram-negative, cell-associated diplococci. Culture or NAAT or both will be positive for *N. gonorrhoeae.* In about three-fourths of affected women, gonococci are recovered from the endocervix as well. The syndrome responds to standard therapy for uncomplicated anogenital gonorrhea (see Chapter 212).

C. trachomatis may be recovered from the urethra in women with dysuria, frequency, and pyuria.[73,74] Urinary tract symptoms are described by about half of women in whom *C. trachomatis* is isolated from the urethra.[77] In such patients, if initial drug therapy includes antimicrobial agents that are active against chlamydiae (e.g., tetracyclines, amoxicillin, fluoroquinolones, sulfonamides, azithromycin), clinical improvement with resolution of symptoms is likely.

Dysuria is a common symptom of women with trichomoniasis. The parasite is recovered from the urethra and periurethral glands in more than 90% of women with the infection (see Chapter 280) and is associated with pyuria. Dysuria also may result from vulvar irritation such as that accompanying vaginal candidiasis, in which case the dysuria is often perceived by the patient as being external. Dysuria is unusual in patients with bacterial vaginosis.

Among sexually active women, infection with uropathogens such as gonococci, chlamydiae, and trichomonads should be ruled out before other therapies are tried.

COMPLICATIONS OF URETHRITIS

Both *N. gonorrhoeae* and *C. trachomatis* have been identified as causes of acute epididymitis among sexually active men.[78] Epididymitis could conceivably lead to infertility, although an association between infectious epididymitis and infertility in men has not been convincingly demonstrated.[79] In 20% to 30% of men with NGU, prostatic involvement can be documented; however, it is usually asymptomatic[80] and responds to standard treatments. The role of organisms that cause urethritis in the development of chronic prostatitis–chronic pelvic pain syndrome remains unclear. Krieger and Riley[81] evaluated prostatic biopsy specimens from 135 men with chronic prostatitis–chronic pelvic pain syndrome with use of PCR assays. Only 10 (8%) of the men had positive assays for pathogens such as *M. genitalium, T. vaginalis,* and *C. trachomatis.* However, 77% of subjects had evidence of 16S ribosomal DNAs, suggesting a role for previously undescribed microorganisms in chronic prostatitis–chronic pelvic pain syndrome. Urethral stricture may follow gonococcal urethritis or NGU. *N. gonorrhoeae* and *C. trachomatis* can infect the conjunctiva. Also, an oculogenital syndrome consisting of NGU and conjunctivitis may be seen in about 4% of patients with NGU[82]; it responds to standard therapy with tetracyclines and must be differentiated from reactive arthritis (see later discussion). Gonococcal and chlamydial infections in women are associated with pelvic inflammatory disease and its potential complications of tubal infertility, ectopic pregnancies, and chronic pelvic pain.

THERAPY

Specific forms of urethritis, including gonococcal, chlamydial, mycoplasmal, and trichomonal, should be treated as discussed in the appropriate chapters in Part III of this text. As a syndrome, NGU has been treated with a variety of regimens, but doxycycline and azithromycin are the current drugs of choice.[19]

Tetracyclines are usually prescribed for 7 days; there is little convincing evidence that full-dose regimens exceeding 7 days have any additional benefits.[83] Tetracycline hydrochloride is given in doses of 500 mg four times a day. The patient should be instructed to take the drug on an

empty stomach, not accompanied by milk or antacids. Alternatively, doxycycline can be administered in a dose of 100 mg orally twice daily for 7 days. This drug is highly effective, is well tolerated by patients, and can be taken with food. Twice-daily administration and fewer side effects are probably associated with better compliance. Inexpensive generic preparations are available. A doxycycline delayed-release 200-mg tablet administered daily for 7 days was as effective as twice-daily doxycycline in a randomized controlled trial for the treatment of chlamydial urethritis.[84] Doxycycline is the tetracycline of choice for treatment of NGU. Administration of doxycycline may be associated with photosensitivity reactions. Minocycline has no apparent advantages over doxycycline, and it produces dizziness in many patients.

Azithromycin is an azalide antimicrobial agent with a prolonged half-life that is active against *C. trachomatis.* A single 1-g oral dose is effective and is also more active than doxycycline against *M. genitalium.*[19]

Both doxycycline and azithromycin are highly effective and well tolerated. Generic doxycycline is inexpensive, but compliance is an issue, with the 7-day regimen not completed by patients in all instances.[85] Importantly, self-reported suboptimal adherence with a 7-day regimen of doxycycline was associated with microbiologic failure in the treatment of urethritis due to *U. urealyticum* and *C. trachomatis.*[86] Azithromycin may be more expensive, but compliance can be ensured if the drug is given under direct observation.

Erythromycin is as effective as tetracycline in chlamydial infections and is active against tetracycline-resistant ureaplasmas.[54] Erythromycin has the additional theoretical advantage of producing higher prostatic levels than those obtainable with tetracycline hydrochloride, and it may be used to re-treat patients whose symptoms are relieved by a tetracycline but who return after therapy is completed. Such patients may have a prostatic focus of infection that is not cured by tetracycline.[87] Gastrointestinal discomfort is a common and significant adverse effect of erythromycin therapy. Given the well-tolerated and effective options provided by azalide and tetracycline therapy, most clinicians no longer choose erythromycin. Recommended regimens include erythromycin base, 500 mg orally four times daily for 7 days, and erythromycin ethyl succinate, 800 mg orally four times daily for 7 days.[19] In addition to single-dose azithromycin therapy, a 5-day course of azithromycin (500 mg initially followed by 250 mg for 4 days) has been shown to be effective for both *M. genitalium* and *C. trachomatis* urethral infections.[88]

Fluoroquinolone antimicrobial agents have been evaluated in chlamydial urethritis and in syndromic NGU. Ciprofloxacin was ineffective, whereas ofloxacin (300 mg twice daily for 7 days) and levofloxacin (500 mg daily for 7 days) were effective.[19,89] In vitro studies suggest that moxifloxacin has activity against *C. trachomatis.*[90,91] Sulfonamides, including sulfisoxazole and trimethoprim-sulfamethoxazole, can also be used to treat chlamydial infections.

If all other therapies are contraindicated, an alternative treatment for chlamydial urethritis is amoxicillin. Several in vitro studies have shown amoxicillin activity against chlamydia, and clinical data suggest that it can be effective. Cure rates of *C. trachomatis* with amoxicillin treatment range from 58% to 80% in pregnant patients.[92] Therefore test of cure is necessary following amoxicillin therapy.

Even if the condition is untreated, symptoms of NGU may resolve; up to 70% of patients have complete resolution of symptoms within 6 months.[38] Resolution of symptoms does not mean that the infection is cured; asymptomatic patients may remain infected and infectious. Conversely the inflammatory response accompanying NGU may take some time to resolve even after the pathogens have been eliminated.

During treatment, the symptoms of NGU frequently resolve before the patient has completed the therapeutic regimen. Patients should be cautioned to complete the entire course of antibiotics because relapse may be more common if therapy is terminated early. To reliably differentiate a relapse from reinfection and to protect sexual partners, patients undergoing treatment for urethritis should be advised to refrain from coitus or to use condoms until both partners have completed their medication regimens and their symptoms have resolved.

Because coincident chlamydial infection is very common in men with gonorrhea and because of concerns about increasing gonococcal resistance to third-generation cephalosporins, the CDC[19] recommends that uncomplicated gonococcal urethritis should be treated with a combined regimen consisting of a single dose of a suitable cephalosporin (ceftriaxone, 250 mg intramuscularly in a single dose) followed by azithromycin (1.0 g orally in a single dose) (Table 107.4). This regimen has the advantage of providing effective single-dose therapy for gonorrhea and effective therapy for coincident, undiagnosed NGU. Disadvantages include increased cost and the potential for adverse reactions. It is also prudent to use a combination regimen to treat urethritis of undetermined origin. Fluoroquinolone-resistant strains of *N. gonorrhoeae* are widespread. Patients who have or may have gonorrhea should not be treated with fluoroquinolones unless the organism has been shown to be susceptible.[93]

TABLE 107.4 Therapy for Urethritis

DRUG AND DOSAGE	COMMENT
Ceftriaxone, 250 mg IM *plus* Azithromycin 1 g orally	For *Neisseria gonorrhoeae or Neisseria meningitidis* if documented by culture or NAAT, if suspected based on examination or history, or if gram-negative diplococci are seen on Gram stain.
Azithromycin, 1 g orally once *or* Doxycycline, 100 mg orally twice daily for 7 days	Appropriate for almost all forms of urethritis unless NAAT specifically does not demonstrate *Chlamydia trachomatis.* Azithromycin preferred for treatment of *Mycoplasma genitalium.*
Metronidazole 2 g orally once *or* Tinidazole 2 g orally once	If *Trichomonas vaginalis* has been demonstrated by light microscopy, culture, or NAAT or if these tests are not available when symptoms persist despite above-listed therapies.

IM, Intramuscularly; *NAAT,* nucleic acid amplification tests.

N. gonorrhoeae has demonstrated a consistent capacity to develop resistance and cross-resistance to previously effective antimicrobial agents. More recently a gradual but disturbing declining trend in the efficacy of cephalosporins has been noted.[94,95] Cefixime, previously recommended along with ceftriaxone for gonococcal treatment, should be used cautiously, if at all, as efficacy is no longer believed to be as reliable.[19] The CDC does not recommend cefixime as first-line therapy for gonorrhea due to reported treatment failures.[95] Suspected *N. gonorrhoeae* cephalosporin treatment failures should be reported to local public health authorities. Ongoing surveillance for emerging *N. gonorrhoeae* resistance is performed in the United States by the CDC-sponsored Gonococcal Isolate Sensitivity Project and by other public health organizations internationally. Globally there has been a gradual increase in minimal inhibitory concentrations of gonorrhea to ceftriaxone.[96–99] The World Health Organization Gonococcal Antimicrobial Surveillance Programme reported that rates of ceftriaxone resistance exceed 5% in several regions of Asia.[100]

Alternative therapeutic options are limited. Combinations with the fluoroquinolone gemifloxacin, 320 mg orally once, plus azithromycin, 2 g orally once, and gentamicin, 240 mg intramuscularly once, plus azithromycin, 2 g orally once, have been recommended as evidence-based therapies.[101] All elements in combination therapy for *N. gonorrhoeae* should be administered at the same time to achieve proper efficacy. New therapies, including solithromycin, zoliflodacin, and gepotidacin, are being evaluated at the present time and are at various stages of development.[100] In cases of *N. meningitidis* urethritis, patients were successfully treated for presumptive *N. gonorrhoeae* infection based on intracellular gram-negative diplococci. *M. genitalium* resistance to azithromycin and doxycycline has been reported worldwide. Moxifloxacin has been effective in some instances for urethritis refractory to these agents; however, fluoroquinolone resistance is now emerging.[102,103]

Patients who are receiving therapy for urethritis should be examined for other STDs and should be tested serologically for syphilis and HIV infection at the initial visit. An initial workup for trichomoniasis may be worthwhile in settings with a high prevalence of trichomoniasis in women. Direct microscopic examination of a urethral specimen for trichomonads is usually unrewarding, even if the patient can be seen before the first morning micturition. If the patient's urethritis has not

been cured by previous antibacterial therapy, or if symptoms or signs in the sexual partner suggest trichomonal infection, the patient's first-void urine sediment, an endourethral swab sample, or both can be examined with use of culture or NAAT. Empirical treatment for trichomoniasis may be warranted in such patients. Syndromic management of urethritis, in which men who have symptoms or signs of urethritis are treated with antimicrobial agents active against *N. gonorrhoeae* and *C. trachomatis* without any diagnostic studies, has been employed with success in resource-poor settings.[104]

In men whose symptoms and signs do not resolve or in whom clinical manifestations recur after appropriate treatment for urethritis, urethral Gram-stained specimens or first-void urine sediments should be examined to establish the existence of persistent urethritis. Symptoms and signs may persist in the absence of objective evidence of urethritis. Antimicrobial treatment in symptomatic men who do not have objective evidence of urethritis is of questionable value.[21]

In patients who initially receive and complete treatment for NGU but in whom urethritis is not eliminated by doxycycline or azithromycin, infection with *Trichomonas* or resistant *M. genitalium* or *U. urealyticum* should be suspected.[53,105] *M. genitalium* is the most likely etiologic agent for refractory NGU, especially if the patient was treated with doxycycline.[56,58] If not previously administered, 1 g of azithromycin can be attempted; if symptoms persist, the recommendation is to give moxifloxacin, 400 mg/day for 7 days.[19] Because these infections can be impossible to differentiate clinically, patients may be given empirical treatment with a single 2-g dose of metronidazole or tinidazole followed after 2 hours (to prevent gastrointestinal upset) by azithromycin, 1 g orally as a single dose.[19] It is important that sexual partners of patients receive the same regimen.

Some men report that their urethral symptoms disappeared while they were taking an antimicrobial agent but reappeared days to weeks after completion of therapy. Such recurrences with objective evidence of urethritis are seen in about 20% of patients with chlamydial NGU and in about 40% of patients with nonchlamydial infection. *M. genitalium* has been associated with recurrent or chronic NGU.[106]

Men with recurrent urethritis should be questioned closely about the possibility of reexposure, and attention should be given to ensuring simultaneous treatment of all sexual partners. If reexposure is likely, repeat treatment with the initial doxycycline or azithromycin regimen may be given. If the patient has not been reexposed, a recurrence of urethritis suggests the possibility that some pathogens remained in a relatively antibiotic-protected site. Prostatic involvement is common in NGU. It is possible that some men have a prostatic focus of infection. In men with repeated relapses, treatment with a 3-week course of erythromycin occasionally succeeds.[107] Patients whose relapses are not eliminated by these maneuvers should be referred for urologic evaluation to rule out anatomic abnormalities.[87] Such men probably are not infected with *Chlamydia* or *Ureaplasma*. About one-fourth of patients will be found to have a partial obstruction to urine flow, and about half of these will have urethral strictures.[87] These patients and their sexual partners do not appear to be at significant risk for infectious complications.[108] Long-term antimicrobial suppression is useful in this setting.[108]

Sexual partners of patients with sexually transmitted urethritis should receive treatment simultaneously. A woman who has been the sexual partner of a man with urethritis of undetermined origin should be given a regimen that is effective against gonococci, chlamydiae, and *M. genitalium*. A regimen combining a cephalosporin with azithromycin, as described previously, is suitable. In one study, patient-delivered partner treatment was more effective in treating partners of men who had urethritis than standard partner referral.[109] Asymptomatic male sexual partners of women known to have gonorrhea, chlamydial infection, or trichomoniasis should receive treatment even in the absence of abnormalities on direct microscopic examination.

THERAPY FOR SEXUAL PARTNERS OF MEN WITH NONGONOCOCCAL URETHRITIS

Female sexual partners of men who have urethritis are often infected with one or more of the causative microorganisms. Although infected women are often asymptomatic, the organisms are far from benign. *C. trachomatis* is a cause of acute salpingitis. Additionally, infants born to infected women may develop chlamydial conjunctivitis or pneumonia. *T. vaginalis* is an important cause of vaginitis,[14] and *M. genitalium* has been implicated as a cause of cervicitis.[51] Asymptomatic women with these infections are undoubtedly a reservoir for recurrent NGU. These considerations support the routine treatment of female sexual partners of men with NGU.

REACTIVE ARTHRITIS

Some cases of NGU appear as one element of reactive arthritis (formerly known as Reiter syndrome), which also includes arthritis, uveitis, and, often, lesions of the skin and mucous membranes.[110] The syndrome complicates 1% to 2% of cases of NGU[111] and is believed to be the most common peripheral inflammatory arthritis in young men.[112] Its pathogenesis is unclear, but it probably represents an abnormal host response to any of a number of infectious agents.[113] The syndrome is reported infrequently in women. The idiosyncratic nature of the host's response is supported by a strong correlation between the development of reactive arthritis and the presence of the human leukocyte antigen (HLA)-B27 histocompatibility antigen. This antigen has been found in 90% to 96% of patients with reactive arthritis,[114] and it has been related to uveitis and sacroiliitis.[111] Although possibly providing a clue to pathogenesis, the presence of this antigen is not diagnostic.

The inciting infection is of two types. Sexually acquired reactive arthritis (SARA) may occur after sexually transmitted urethritis.[115] Most cases in North America and Europe occur in sexually active young people.[116] Many cases occur after contact with a new partner, and some cases have been epidemiologically linked.[111] In one series, 9% of cases occurred after gonococcal urethritis, although 50% of affected patients subsequently developed PGU.[117] *C. trachomatis* is strongly implicated in the pathogenesis of SARA.[118,119] It has been recovered from the urethras of 50% of patients with SARA and from most of the men who had signs of urogenital inflammation at the time of examination.[120,121] Antibodies to *C. trachomatis* have been detected in about half of patients with SARA, and *Chlamydia*-specific cell-mediated immunity occurs in most patients with SARA.[121,122] Chlamydiae were isolated from synovial biopsy specimens in 15 of 29 patients from a number of small series and from a smaller proportion of synovial fluid specimens.[123] Chlamydial nucleic acids have been identified in the synovial membranes,[124] and chlamydial elementary bodies have been observed in joint fluid.[125] Rates of reactive arthritis have been declining in some reports despite increasing rates of *C. trachomatis* infection.[126] SARA has been reported with lymphogranuloma venereum.[127] *M. genitalium* infection has preceded SARA.[128,129] Attempts to isolate or identify mycoplasmas in affected joints have not been successful.[123]

Reactive arthritis also occurs after bacterial gastroenteritis, and it has been repeatedly described after infection with *Salmonella, Shigella, Yersinia,* or *Campylobacter.*[111,112,117] A few cases suggest that reactive arthritis may occur after antibiotic-associated colitis[130] or cryptosporidiosis.[131] Postdysenteric reactive arthritis has been reported in about 1% of patients after epidemics of gastrointestinal infection; it is considerably more common among patients who are HLA-B27 positive. Short peptides shared by HLA-B27 and enteric pathogens have raised the question of a contribution of molecular mimicry to pathogenesis.[132] Antibodies reacting with *Yersinia* proteins appear in the sera of many patients with reactive arthritis.[133]

Clinically, reactive arthritis after genital infection is indistinguishable from reactive arthritis occurring after bacterial gastroenteritis; indeed, 12% to 80% of patients with postdysenteric reactive arthritis have genital symptoms.[111,134] The age-specific and sex-specific attack rates, however, are different; 94% to 99% of cases of reactive arthritis after sexually transmitted infections occur in men. However, up to 10% of cases of postdysenteric reactive arthritis occur in women.[116] The syndrome has also been reported in sexually inexperienced children.[116]

Reactive arthritis is encountered with some frequency in HIV-infected individuals.[135] It is said to be the most common rheumatologic complication of acquired immunodeficiency syndrome. The spectrum of clinical manifestations is similar to that in other patients, but the arthritis and mucocutaneous lesions are more severe and may require more intensive therapy.

Clinical Manifestations

NGU is the initial manifestation of reactive arthritis in 80% of patients.[111,112] As with other forms of NGU, it usually occurs within 14 days after sexual exposure. Urethritis may be mild and may go unnoticed by the patient, being detectable only by physical examination performed before the first morning micturition. Gonococcal urethritis sometimes precedes reactive arthritis,[117] but coinfection with an agent of NGU is difficult to rule out. The urethral discharge may be purulent or mucopurulent, and patients may or may not report dysuria. Accompanying prostatitis, usually asymptomatic, has been described by some authors.[112,134,136] Cystitis without urethritis also has been reported and may be a manifestation, particularly in women.[134] Cervicitis has been described in women presenting with reactive arthritis.[137] The other features of reactive arthritis develop 1 to 5 weeks after the onset of urethritis.[134] Arthritis begins within 4 weeks after the onset of urethritis in 80% of patients,[111] but it precedes urethritis in about 15%.[116] The knees are most frequently involved, followed by the ankles and small joints of the feet. Sacroiliitis, either symmetrical[134] or, more frequently, asymmetrical,[116,136] develops in up to two-thirds of patients. It is more common in patients with the HLA-B27 antigen. Ankylosing spondylitis, which occurs in only about 1% of the general population, complicates a significant minority of cases of reactive arthritis,[111,138] and back pain is reported by 60% of all patients.[112] Many patients with reactive arthritis and the HLA-B27 antigen develop ankylosing spondylitis,[138] which is rare in patients without the antigen. Calcaneal spurring may be seen in one-fourth of patients with reactive arthritis[111,136] and may produce heel pain. A dactylitis resulting in sausage-shaped swelling of the digits is also characteristic.[116] Arthritis is the most persistent feature of the syndrome; it may last for months to years after other manifestations have disappeared.[116,134]

Mild bilateral conjunctivitis, iritis, keratitis, or uveitis is sometimes present but often lasts for only a few days.[134,136,139] In contrast to the conjunctivae in direct infection with *C. trachomatis,* the inflamed conjunctivae in reactive arthritis do not manifest follicular hypertrophy. Uveitis is usually anterior, acute, and unilateral.

Dermatologic manifestations occur in up to 50% of patients.[111,112] The initial lesions are waxy papules, which often display a central yellow spot; they occur most frequently on the soles and palms[134] and with decreasing frequency on the nails, scrotum, scalp, and trunk. The papules epithelialize and thicken to produce keratoderma blennorrhagicum in 10% to 25% of the patients.[112] Circinate balanitis is usually painless and occurs in 25% to 40% of all patients.[111,112,134] Circinate and ulcerative vulvitis also are described.[140] Painless erosions on the dorsum of the tongue and fauces occur most commonly with the initial episode and less frequently with recurrence. Incomplete reactive arthritis, consisting of urethritis and arthritis or arthritis alone, has been reported.[112,141]

The initial episode of reactive arthritis usually lasts 2 to 6 months, but episodes lasting for 1 year have been described.[112,134,136] Most patients feel completely well after the attack subsides, but the disease recurs in many cases, at a rate of about 15% in each 5-year period after the initial attack.[111,112,116,125] During recurrence, the genital symptoms are usually less marked and may be entirely absent.[142] More than half of patients have active disease 15 to 20 years after the initial episode,[112,138,143] the risk for residual disease being higher among patients with the HLA-B27 antigen. Almost 50% of affected individuals develop some degree of permanent disability.[138] Rare complications of reactive arthritis include pericarditis, myocarditis, first-degree atrioventricular block, and aortic insufficiency.[134,136]

Laboratory Findings

Anemia is common,[112] and the erythrocyte sedimentation rate is elevated in about 50% of patients.[116] Findings in fluid recovered at the same time from different joints may be dissimilar.[134] Synovial fluid usually contains 1000 to 200,000 white blood cells/mL, of which more than two-thirds are PMNs.[134] The glucose level in joint fluid is low in about 50% of affected joints.[134] Synovial biopsy specimens reveal nonspecific inflammatory changes.

Therapy

Treatment of reactive arthritis is controversial.[144] Because of the possibility that the inciting infection may be sexually transmitted NGU, standard antichlamydial therapy is recommended[143] and has been claimed by some authorities to reduce or eliminate urethritis.[145] Others, however, have seen no effect on the arthritis or on the overall course of the disease.[143] The relative safety of antichlamydial therapy and the frequency with which chlamydiae are isolated from patients with reactive arthritis make such treatment reasonable. Among a population in Greenland with a high prevalence of HLA-B27, treatment of patients who had urethritis or cervicitis with a tetracycline or erythromycin was associated with a lower incidence of subsequent arthritis than was treatment with penicillin or no treatment at all.[146]

Long-term antichlamydial treatment (e.g., with a tetracycline for 3 months) has been suggested, and its use was supported by the results of a double-blind, placebo-controlled trial that demonstrated an ameliorating effect on *Chlamydia*-associated, but not on enteropathogen-associated, disease.[147] The effectiveness of long-term tetracycline therapy in other arthritides[148] raises the question of whether the drug is working through an antibacterial effect on persistent, viable microorganisms or through an antiinflammatory action. That the tetracyclines are more effective in *Chlamydia*-associated disease than in other reactive arthritides suggests the former.

Administration of nonsteroidal antiinflammatory drugs (NSAIDs) is the most effective treatment.[112] Indomethacin or tolmetin is favored by some workers, and all these agents are superior to salicylates or corticosteroids. Sulfasalazine may be beneficial for patients whose symptoms do not respond to a nonsteroidal antiinflammatory drug. Cytotoxic agents such as methotrexate[149] or immunosuppressive agents such as infliximab[150] may be of value in recalcitrant cases.

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The complete reference list is available online at Expert Consult.

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108 Vulvovaginitis and Cervicitis

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SHORT VIEW SUMMARY

Definition
- Vulvovaginitis and cervicitis include infectious and noninfectious conditions involving the vulva, vagina, and cervix.

Epidemiology
- Trichomoniasis is a sexually transmitted condition.
- Bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC) are not classic sexually transmitted diseases but seldom occur in sexually inexperienced women.
- Vulvar pain syndrome and desquamative inflammatory vaginitis (DIV) are not sexually transmitted.
- Cervicitis may be sexually transmitted or idiopathic.

Microbiology
- Trichomoniasis is caused by *Trichomonas vaginalis*.
- Candidiasis is caused by *Candida albicans* and other fungal species.
- Bacterial vaginosis is associated with a complex bacterial microbiota.
- Infectious cervicitis is usually caused by Neisseria gonorrhoeae, Mycoplasma genitalium, or Chlamydia trachomatis.

Diagnosis
- Trichomoniasis and cervical infections due to *N. gonorrhoeae*, *M. genitalium*, and *C. trachomatis* can be diagnosed by identifying the causative organisms with use of culture or nonculture methods, of which nucleic acid amplification testing is the most accurate.
- BV is diagnosed using clinical criteria, including vaginal pH, odor produced when potassium hydroxide is added to vaginal fluid, and detection of "clue cells" when vaginal fluid is examined microscopically.
- VVC is diagnosed clinically and by the identification of *C. albicans* or other fungal species in cultures of vaginal fluid.
- DIV is diagnosed by the clinical appearance of the vagina and the identification of leukocytes and parabasal cells in wet preparations of vaginal fluid.

Therapy
- Trichomoniasis is treated with the oral administration of metronidazole or tinidazole.
- BV is treated with a 7-day course of oral metronidazole or with preparations for intravaginal use that contain metronidazole or clindamycin.
- VVC is treated with oral fluconazole or with preparations for vaginal use that contain nystatin, miconazole, or other antifungal agents.
- DIV is best treated with intravaginal clindamycin.
- Intravaginal administration of boric acid or corticosteroids provides symptomatic relief for DIV.

Prevention
- Judicious choice of sexual partners and regular use of condoms is preventive.

Vulvovaginal symptoms are common and frequently result in encounters of patients with the health care system, including use of folk remedies, purchase of over-the-counter (OTC) pharmaceuticals, and presentation to health care providers. In one survey[1] of female college graduates with a median age of about 30 years, almost 70% reported at least one lifetime physician-diagnosed and treated vaginal yeast infection; in addition, 29% had been treated for bacterial vaginosis (BV) and 26% for trichomoniasis. Greater than 20% of the women had been treated for a vaginal yeast infection within the prior year.

Genitourinary symptoms in women tend to lack specificity. All women have vaginal secretions, but their ability to discriminate normal from abnormal secretions is often imprecise. Similarly, vulvar discomfort is fairly common and may result from a variety of infectious and noninfectious causes. This chapter discusses vaginal discharge and vulvitis separately, with the understanding that there is considerable overlap between these syndromes and that a patient whose symptoms appear to represent one syndrome may ultimately have a quite different diagnosis.

VAGINAL SECRETIONS

The normal vaginal secretions are a physiologically important biomass. Vaginal cells contain glycogen and are continually shed into the lumen of the vagina. As the cells autolyze, glycogen depolymerizes to glucose, which serves as an energy source for bacteria known as lactobacilli. Data reported by Ravel and associates[2] demonstrated that the vaginal bacterial communities of asymptomatic North American women clustered into five groups: four were dominated by *Lactobacillus crispatus*, *Lactobacillus jensenii, Lactobacillus iners,* or *Lactobacillus gasseri,* whereas the fifth had lower proportions of lactobacilli and higher proportions of strictly anaerobic organisms. Lactobacilli metabolize glucose to lactic acid, which results in a normal vaginal pH of about 4.0.

In the studies of Ravel and associates[2] the vaginal communities of women whose microbiota was dominated by *L. jensenii* and those with higher proportions of strictly anaerobic bacteria had higher mean pH levels than the other groups. In addition, blacks and Hispanics had higher mean vaginal pH levels than whites and Asians.

The vagina is not sterile, and many species of bacteria can be isolated from vaginal specimens from healthy women, including *Prevotella, Megasphaera, Gardnerella,* and *Atopobium* spp., but lactobacilli usually dominate the vaginal microbiota. Indeed, the predominant cells in the vaginal secretions of most normal women are lactobacilli and vaginal epithelial cells (Fig. 108.1).

In addition to producing lactic acid, lactobacilli may also produce hydrogen peroxide, which is bactericidal alone and highly bactericidal in combination with physiologic amounts of myeloperoxidase and chloride.[3] Loss of the normal *Lactobacillus*-dominated vaginal microbiota increases the likelihood of exogenous infection after exposure to sexually transmitted pathogens,[4] as well as the risk for endogenous infection in association with pregnancy and gynecologic surgery.[5]

Therefore the normal vaginal secretions are a heterogeneous suspension of vaginal epithelial cells and lactobacilli in fluid that emanates from the cervix and vaginal walls (Fig. 108.2). The secretions have a pH of 3.5 to 4.6, are odorless, and do not cause itching or irritation. This acidic environment inhibits the growth of many pathogenic organisms. The normal secretions are clumpy and tend to remain in the vagina, although in some women egress of normal secretions may require use of a perineal pad. Characteristics of normal vaginal secretions are listed in Table 108.1.



FIG. 108.1 Healthy vaginal secretions. Normal vaginal secretions as seen in a suspension of vaginal secretions in 0.9% sodium chloride. Vaginal epithelial cells and lactobacilli are the primary cells seen.



FIG. 108.2 Healthy vaginal secretions on a speculum. Normal vaginal secretions on a plastic vaginal speculum. The secretions are off-white and heterogeneous.

TABLE 108.1 Characteristics of Normal Vaginal Secretions

Heterogeneous suspension of desquamated vaginal epithelial cells in water
Lactobacilli dominate the microbiota
pH of 3.5–4.6
Odorless
Do not cause itching or irritation
Usually do not soil underclothing

An appreciation of what is normal is important in dealing with patients who report an abnormal discharge. A significant minority of such patients have normal secretions. These women may have a large volume of normal secretions, relaxation of the introitus after childbirth that allows outflow of normal volumes of normal secretions, or heightened awareness of a normal amount of physiologic secretions, often as a result of other genitourinary symptoms, such as vulvar discomfort. The volume of normal secretions may increase during pregnancy, during use of hormonal contraceptive agents, and at midmenstrual cycle

Patients with a normal or physiologic vaginal discharge usually have a history of multiple self-directed or clinician-directed treatments with a wide array of topical and systemic antibacterial and antifungal agents. Recognition of the normalcy of the secretions is important so that the patient can be reassured that her secretions are normal and so that diagnostic and therapeutic attention can be focused on associated conditions, if any, that may be present.

Important characteristics of patients who present with normal vaginal secretions are listed in Table 108.2. Notable characteristics include chronicity, absence of odor, absence of vulvar discomfort (unless there is unrelated vulvitis), many unsuccessful visits to a variety of different health care providers, and many ineffective treatments.

On close questioning a number of these patients do not have sufficient external flow of vaginal secretions to stain their clothing or to require use of a perineal pad. They appreciate what they perceive to be abnormal secretions by extracting normal secretions with use of their index finger. This *finger test* is an important historical clue that the woman has normal secretions.

In addition to normal secretions, the differential diagnosis of vaginal discharge primarily includes three conditions (trichomoniasis, vulvovaginal candidiasis [VVC], and BV), an idiopathic condition known as desquamative inflammatory vaginitis (DIV), cervicitis (both infectious and noninfectious), and vulvovaginitis associated with estrogen deficiency (Table 108.3).

TABLE 108.2 Characteristics of Patients Who Report Vaginal Symptoms but Are Found to Have Normal Vaginal Secretions

Chronicity of symptoms
Many prior visits to health care practitioners
Multiple ineffective treatments with vaginal and oral antimicrobial agents
Absence of odor
Absence of itching and irritation
Minimal, if any, staining of clothing

TABLE 108.3 Differential Diagnosis of the Abnormal Vaginal Discharge

Normal (physiologic) vaginal secretions
Vaginal infections
Trichomoniasis
Vulvovaginal candidiasis
Bacterial vaginosis
Desquamative inflammatory vaginitis
Cervicitis
Infectious
Noninfectious
Estrogen deficiency

TABLE 108.4 Most Pertinent History From Patients With Vulvovaginal Symptoms

General Gynecologic History
Menstrual history
Pregnancies
Sexual preference
Contraception
Sexual relationships
Prior infections
General medical history
Allergies
Diabetes
Malignancies
Immunodeficiency
Symptoms of Vulvovaginitis
Discharge
Odor
Vulvar discomfort
Dyspareunia

APPROACH TO THE PATIENT

History

The etiologic diagnosis of vaginitis depends on a careful evaluation of the history, a physical examination, and immediate laboratory tests. Historical features are relatively nonspecific,[6] but they may direct clinical suspicion toward certain causes. If at all possible, the history should be obtained with the patient in the sitting position and before she has disrobed.

The medical history (Table 108.4) should include all of the usual gynecologic parameters, including menstrual history, pregnancies,

contraception, sexual preference, past and current sexual relationships, and prior genitourinary infections. In addition, the patient should be asked about underlying medical conditions, such as allergies, diabetes, malignancies, and immunodeficiency syndromes (primarily human immunodeficiency virus [HIV] infection), that might be associated with or influence vulvovaginal disease. Recent use of antimicrobials for any reason may result in disruption of normal vaginal microbiota and therefore cause a vaginal discharge. Patients should be asked also about any possible mechanical or chemical irritants use, such as scented panty liners, soaps, latex condoms, and so forth, that may cause subacute and chronic vaginal symptoms attributed to infections.[7]

Age

Neonates can acquire trichomonal or candidal vulvovaginitis during passage through an infected birth canal, which would argue for treating these infections in pregnant women before term. Neonatal vaginal thrush responds promptly to topical antifungal medications. Neonatal trichomoniasis can be treated with metronidazole. After the neonatal period a vaginal discharge is abnormal and should prompt a thorough examination. Vaginal candidiasis is rare in prepubescent girls. Prepubescent vaginal epithelium is thin, and the entire vagina is susceptible to infection with *Neisseria gonorrhoeae.* Gonococcal vulvovaginitis often causes profuse vaginal discharge. The diagnosis of a sexually transmitted disease (STD) in a young girl should raise the strong suspicion of child abuse, although some agents have been transmitted to children in the absence of frank sexual contact.[8] Patients in the sexually active years are more likely to have physiologic secretions or infectious vaginitis. Postmenopausal women are more likely to have atrophic vaginitis with vaginal dryness and dyspareunia.

Mode of Onset

An abrupt and identifiable time of onset of symptoms suggests infection. Vaginal discharge associated with neoplasia, estrogen depletion, or a foreign body often has a subacute onset, with symptoms progressing over a period of weeks.

Quantity of Discharge

The amount of discharge is highly variable in all conditions. Patients with VVC often have scanty discharge or no discharge at all. Atrophic discharges are commonly scanty unless infection has supervened.

External Irritation

Physiologic discharge is rarely associated with vulvar or perineal discomfort. Pruritus with a scant or absent discharge is frequently seen in candidiasis. External discomfort is an infrequent complaint in BV. Severe episodic perineal pain that sometimes prevents urination suggests herpes simplex virus (HSV) infection, which affects the labia but usually spares the vagina. Chronic discomfort (often interfering with sexual activity) should prompt consideration of a noninfectious vulvitis, such as vulvar vestibulitis.

Odor

Vaginal odor in the absence of other symptoms is the initial complaint in many cases of BV. A feculent odor may accompany anaerobic superinfection of genital lesions, or it may be noted in the presence of a foreign body or enterovaginal fistula.

Abdominal Pain

Abdominal discomfort is rare in uncomplicated vulvovaginitis, except for some cases of trichomoniasis. Women who complain of abdominal pain should be examined for evidence of urinary tract infection (UTI) and pelvic inflammatory disease.

Sexual History

Exposure to a new sexual partner increases the likelihood of STD. A history of genital symptoms in a sexual partner is helpful diagnostically. Women who have sex with women are at risk of bacterial vaginosis.[8] And although bacterial vaginosis is not considered a typical STD, women who are not sexually active do not suffer with this condition.

Other Diseases

Diabetes mellitus, acquired immunodeficiency syndrome, malignancy, and the treatments thereof, and possibly hypoparathyroidism, increase the risk for candidal vaginitis. Diseases known to impair host defenses may predispose to otherwise rare infections. Drugs used in the treatment of other diseases may predispose to vaginal infection.

Medications

Systemic or local medications may influence vaginal infection. Antibiotics that are active against the normal bacterial microbiota of the vagina predispose to candidal vaginitis.[9] Patients who are taking corticosteroids or oral contraceptives are at increased risk for development of VVC. Local medications, including vaginal douches, rarely produce a chemical vaginitis; douching immediately before examination makes diagnosis difficult.

The commencement of oral contraceptive use may be associated with increased physiologic discharge.

Physical Examination

If the patient has not had recent medical care, a general physical examination can be performed. At the least the patient's breasts should be examined if she has not had a professional breast examination during the past year. Similarly, a mammogram should be ordered if the patient's age or history dictate that one is indicated.

With the patient supine on the examining table, the pubic hair should be examined for the presence of crab lice or nits. The inguinofemoral areas should be palpated for adenopathy. Suprapubic and lower abdominal tenderness or masses can be sought by palpation.

The gynecologic examination (Table 108.5) requires adequate light. Magnification is helpful and is best provided by a colposcope. Providing the patient with a mirror allows her to participate in the examination and is useful for clarifying the location of symptoms and demonstrating important findings to the patient.

With the patient in the lithotomy position, the external genitalia should be carefully inspected. The patient should be asked to point to any areas of external itching, irritation, or other discomfort. Localization of discomfort to vulvar skin or vestibular mucosa provides useful information. The appearance of the vulvar skin and vestibular mucous membranes should be noted, with careful attention to ulcers or other discontinuity of the skin or mucosa, mucosal erythema, and visible secretions. Diffuse perineal erythema may accompany candidiasis. Diffuse reddening with small satellite lesions, usually papular or papulopustular, suggests candidiasis. The degree of perineal irritation is quite variable with all infections, but severe perivaginal irritation is uncommon with BV. Labial edema may accompany severe irritation, especially in VVC, trichomoniasis, or dermatitis.

Careful examination of all the extravaginal surfaces may reveal lesions of genital herpes, syphilis, condyloma acuminatum, molluscum contagiosum, scabies, or vulvar vestibulitis.

TABLE 108.5 Principles for the Genital Examination of the Patient With Vulvovaginal Symptoms

- Adequate illumination
- Magnification, if possible
- Give the patient a mirror
- Inspect the external genitalia
  - Lesions
  - Mucosal erythema
- Examine the vaginal mucosa
  - Erythema
  - Lesions
  - Secretions
- Examine the cervix
  - Ectropion
  - Lesions
  - Erythema
  - Endocervical secretions
- Collect vaginal and cervical specimens
- Bimanual examination

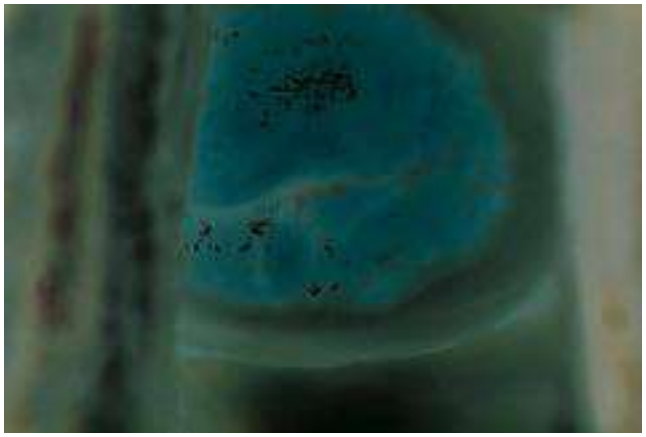

FIG. 108.3 Healthy cervix. Normal cervix with a normal ectropion.

TABLE 108.6 Specimens Obtained During the Gynecologic Examination

Vaginal secretions
pH
Whiff test
Saline wet preparation
Potassium hydroxide wet preparation
Cultures or nonculture tests
Neisseria gonorrhoeae
Chlamydia trachomatis
Candida spp.
Trichomonas vaginalis
Cervical cytologic examination (if not documented within the currently recommended time frame)

By spreading the labia with the gloved hand, one may examine the urethral meatus. The urethra may be gently stripped with a finger placed inside the vagina. Urethral discharge is not a common finding, but, if delivered, such material should be examined microscopically and cultured. The introitus and the internal surfaces of the labia minora should be examined for lesions. Vaginal discharge is sometimes observed on the labia or flowing onto the perineum. Such copious discharge is usually associated with trichomoniasis or BV but may accompany other infections.

A speculum is then inserted to expose the cervix and vaginal mucosa. A small-sized speculum is adequate for most patients and minimizes discomfort for patients who have introital lesions. A small amount of lubricant facilitates insertion without compromising the quality of the microbiologic samples to be collected. The vaginal and cervical mucosa should be inspected with attention to erythema and lesions. An ectropion, if present, should be noted (Fig. 108.3). The vaginal secretions should be described, as should any secretions emanating from the endocervical canal or from an ectropion.

Diagnostic Evaluation

Specimens obtained during the examination are listed in Table 108.6. Vaginal pH should be measured. A sample of vaginal material should be collected from the lateral vaginal wall with use of a cotton-tipped applicator. Care should be taken to avoid contamination with endocervical secretions. The collected vaginal secretions are applied to a strip of pH paper and compared with the standard chart provided by the manufacturer (Fig. 108.4). A high pH in premenopausal women indicates infections such as BV and trichomoniasis, whereas in postmenopausal women, pH is usually high due to lack of estrogen vaginal secretions, and its measurement become less useful in diagnosing infection.

A sample of vaginal secretions is then examined. The specimen may be prepared in several ways. A swab of vaginal secretions may be agitated in a tube containing about 0.5 mL of normal saline to form a suspension. Alternatively, a plastic transfer pipette may be used to introduce 2 to 3 mL of normal saline solution into the posterior fornix. The saline solution is mixed with the vaginal secretions by aspiration and reaspiration of the solution, and the resultant suspension of vaginal material is placed into a small tube.



FIG. 108.4 Testing for pH. Examination of vaginal secretions for pH with use of pH paper.

The suspension is then examined for odor (whiff test) by placing a drop on a microscope slide, adding a drop of 10% potassium hydroxide (KOH), and smelling the resultant mixture. Normal secretions have no odor. A fishy odor is indicative of BV, due to metabolism of vaginal peptides into a variety of amines that are volatile and malodorous, as well as increased vaginal transudation and squamous epithelial cell exfoliation.

A drop of the suspension of vaginal material is placed on a microscope slide, and a coverslip is added. This *wet mount* should be examined within 10 to 20 minutes of collection under high power with a bright-field microscope. Phase-contrast microscopy provides an excellent means of evaluating vaginal wet mounts. The relative numbers of epithelial cells and polymorphonuclear neutrophils (PMNs) should be noted. Because PMNs are present in physiologic endocervical discharge that collects in the vagina,[10] small numbers of PMNs may be observed in the vaginal material recovered from healthy women. A finding of more PMNs than epithelial cells in a vaginal wet preparation should raise suspicion for cervical or vaginal inflammation. Observation of relatively few PMNs does not rule out vaginal infection, however. Vaginal candidiasis can produce a discharge that contains only small numbers of PMNs. The relative absence of PMNs is characteristic of the discharge of BV.[11] In fact, the finding of many PMNs in the vaginal discharge of a patient with BV should prompt a search for simultaneous infection, such as trichomoniasis, gonorrhea, or chlamydial cervicitis. Pseudohyphae suggest vaginal candidiasis, but often only moderate or even very small numbers of yeast cells are seen in this condition. Indeed, some patients with VVC have organisms identified only by culture. The wet preparation should be scanned for motile trichomonads.

Normal squamous epithelial cells have transparent cytoplasm and small nuclei. Immature (parabasal) cells are smaller and have larger nuclei. Epithelial cells covered with tiny coccobacillary forms are called *clue cells* and are associated with BV. Clue cells are best recognized by observing the edges of epithelial cells, which may be obscured by the adherent coccobacilli. Some cells are so heavily encrusted that the nuclei are obscured. Trichomonads are best recognized by their characteristic twitching motility. The flagellae and undulating membrane may be observed by careful focusing of the microscope and adjustment of the light source. Trichomonad motility may be improved by gentle warming of the preparation. The wet mount is negative in about 38% of the women with trichomoniasis[12] (see Chapter 280), so a negative wet mount does not rule out this infection, particularly in asymptomatic women.

The bacterial microbiota can be assessed on the wet mount. Normal vaginal microbiota consists of a sparse population of bacilli. In BV the predominant organisms are tiny coccobacilli. Spermatozoa may be observed as long as 10 days after the last coitus, but motile sperm suggest sexual contact within the preceding 24 hours.[13]

Combining a drop of 10% KOH with the vaginal material on a microscope slide and applying a coverslip destroys cellular elements but leaves the bacteria and fungi unscathed, enhancing the capacity to diagnose VVC. The KOH preparation cannot be used for microscopic diagnosis of trichomoniasis or BV.

A Gram stain of vaginal material is somewhat less useful than the wet mount for differential diagnosis. Although *Candida* spp. are readily

recognized on the Gram-stain smear, trichomonads are difficult to identify. Normal vaginal microbiota consists primarily of gram-positive bacilli, which are mostly lactobacilli. In BV the normal microbiota is replaced by sheets of gram-variable coccobacilli, which often overlie the surface of epithelial cells. Women with VVC sometimes have large numbers of budding yeasts and pseudohyphae. The Gram stain is negative in many women from whom *Candida* can be cultured.[14]

Material recovered from the endocervix can be Gram stained. Normal cervical discharge usually contains moderate numbers of PMNs, and their presence is not necessarily an indication of infection.[10] The presence of large numbers of PMNs suggests cervicitis. Gram-negative, intracellular diplococci accurately diagnose gonorrhea (see Chapter 212), but extracellular diplococci are less predictive. The cervical Gram stain is positive in only about 60% of women with cervical gonorrhea. Therefore a negative Gram stain does not rule out this infection.[14]

Before the speculum is removed, specimens are obtained for examination for *N. gonorrhoeae, Chlamydia trachomatis, Mycoplasma genitalium,* and *Trichomonas vaginalis* with use of culture or nonculture methods such as nucleic acid amplification tests (NAATs). Vaginal cultures for yeast are useful to exclude fungal infection, for which the wet preparation is insensitive,[15] and to identify non–*albicans Candida* spp. Trichomonads can be sought by inoculating selective liquid media, such as the modified Diamond medium, if trichomoniasis is a diagnostic possibility.[16] Trichomonas culture is labor intensive and expensive and is not routinely performed. Sensitive and specific antigen assays are available. Finally, a cervical cytologic smear should be obtained if such an examination cannot be documented within the currently recommended time frame. Relying on the patient's history of cervical cytologic examinations is risky, because some women assume that all gynecologic examinations include a Papanicolaou (Pap) smear.

Bimanual examination for adnexal tenderness and masses should be a part of the evaluation. Adnexal tenderness is sufficiently uncommon with local vaginal infections that its presence suggests salpingitis.

TRICHOMONIASIS

Epidemiology

Trichomoniasis is one of the three most common causes of vaginal complaints, along with BV and *Candida* vulvovaginitis. Trichomoniasis prevalence is difficult to determine because it is not reported to public health authorities. In a most recent study prevalence of *T. vaginalis* was 26% among symptomatic women, 6.5% in asymptomatic patients screened, and 29% in HIV-infected women.[17]

Etiology and Pathogenesis

Trichomoniasis is caused by the protozoan *T. vaginalis.* It is a classic exogenous sexually transmitted infection (STI), such as gonorrhea and chlamydial infection. The organism is not normally present in the vagina. Transmission almost always occurs through sexual contact. After an incubation period of a few days, patients develop a purulent discharge associated with varying degrees of vulvar irritation, dysuria, and dyspareunia (Table 108.7). An abnormal odor is often present, usually signifying concomitant BV.

Diagnosis

Examination is notable for vulvar, vestibular, and vaginal erythema and a purulent vaginal discharge (Fig. 108.5). A minority of patients manifest characteristic mucosal capillary dilation, which gives the mucosa a *strawberry* appearance. Vaginal pH is almost always higher than 4.5. A positive whiff test is not unusual. The vaginal wet preparation contains an abundance of leukocytes and motile flagellated trichomonads (Fig. 108.6). In experienced hands the wet preparation is only 60% to 70% sensitive in symptomatic patients.[18] Because the organisms remain motile for 10 to 20 minutes after collection of the sample, fixation and staining is not useful.

The organism can be cultivated by inoculating a liquid medium, such as the modified Diamond medium[16] or InPouch TV (Biomed Diagnostics, White City, OR).[19] After incubation, aliquots of the liquid medium are examined daily for motile trichomonads. Culture improves the diagnostic yield, especially in asymptomatic patients. These tests are not widely available and take up to 7 days to obtain result. Before the advent of molecular testing and direct antigen assays, culture was the gold standard for detection of *Trichomonas.*

Nonculture antigen detection tests approved by the US Food and Drug Administration (FDA) include the OSOM *Trichomonas* Rapid Test (Sekisui Diagnostics, Cambridge, MA), which can be used as a point-of-care test, and the Affirm VPIII (Becton Dickinson, Sparks, MD). These tests have sensitivity that approaches that of culture.[19,20] Sensitive and specific NAATs have become the gold standard for diagnosis of *T. vaginalis,* including the APTIMA *T. vaginalis* Assay (Hologic Gen-Probe, San Diego, CA).[21,22]

A wet preparation diagnostic of trichomoniasis is highly specific because of the characteristic motility of the organisms. Diagnostic tests in which the organisms are no longer motile may lack specificity. A common clinical problem is the woman with no epidemiologic evidence for a sexually transmitted condition who has trichomonads visualized on a cervical cytologic examination.[23] In some of these cases the cytologist may have misread the smear. For such a patient the clinician should obtain confirmation of the diagnosis of trichomoniasis, by wet preparation or culture (or both), before initiating treatment and before embarking on a potentially disruptive epidemiologic investigation.

TABLE 108.7 Symptoms of Trichomoniasis

Purulent vaginal discharge
Vulvar irritation
Dysuria
Dyspareunia
Abnormal vaginal odor



FIG. 108.5 Trichomoniasis. There are purulent secretions and mucosal erythema.



FIG. 108.6 Trichomoniasis suspension. Suspension of vaginal secretions in 0.9% sodium chloride. There are leukocytes and flagellated trichomonads.

Therapy

Metronidazole and tinidazole are the only effective agents that are approved by the FDA for the treatment of trichomoniasis. A single 2-g oral dose of metronidazole or tinidazole can be prescribed. Alternatively, 500 mg of oral metronidazole can be given twice daily for 7 days. Single-dose therapy may be less effective in HIV-infected women, and a recent meta-analysis suggested the same may be true in HIV-uninfected women. A single-dose regimen, if administered under direct observation in the office or clinic, has the obvious advantage of 100% compliance. Because trichomoniasis is almost always sexually transmitted, treatment with metronidazole of all recent sexual partners, regardless of their symptoms, is an integral part of management.[24]

The aforementioned regimens containing metronidazole or tinidazole eliminate trichomonads in well over 90% of instances. Tinidazole is considerably more expensive than metronidazole. If this treatment fails (Table 108.8) the diagnosis should be reconfirmed with a wet preparation or culture. In addition, treatment of all current sexual partners should be ensured. Initial re-treatment should be with oral metronidazole, 500 mg twice daily for 7 days. If this regimen is not successful, oral metronidazole or tinidazole in a single daily dose of 2.0 g can be prescribed for 7 days.[24] If the latter regimens fail, the patient can be assumed to have clinically significant resistance, and consultation with an expert in the management of trichomoniasis should be sought. Susceptibility testing, available through the Centers for Disease Control and Prevention (CDC), should be performed.[25] Metronidazole resistance may occur in 4% to 10% of cases of vaginal trichomoniasis, and tinidazole resistance may occur in 1%.[25,26] Intravenous regimens of metronidazole have been prescribed in instances of trichomoniasis drug resistance.[27] For patients who cannot tolerate metronidazole at recommended dosages and for those who do not respond to it, tinidazole has better in vitro efficacy,[28] is better tolerated than metronidazole, and has cured most patients with metronidazole-resistant trichomoniasis.[29] Alternative regimens in nitroimidazole-resistant infections include high-dose tinidazole 2 to 3 g for 14 days, often in combination with intravaginal tinidazole.

For patients with trichomoniasis who are unresponsive to metronidazole and tinidazole, there are few therapeutic options of proven value. Nonoxynol-9 is active against *T. vaginalis* in vitro and was reported to be effective in 1 patient.[30] However, in a subsequent study, nonoxynol-9 was effective in only 3 of 17 patients.[31] Furazolidone is highly active in vitro against metronidazole-sensitive and metronidazole-resistant isolates of *T. vaginalis*[32] and was effective when given vaginally in a study conducted years ago.[33] In one study[34] vaginal acidification with boric acid was effective. In general, however, topical treatments have been disappointing,[16] presumably because of reservoirs of infection in periurethral glands and other areas that are not adequately sterilized by intravaginal medications. Specifically, topical preparations containing metronidazole (e.g., 0.75% metronidazole gel) have been ineffective in unselected patients with trichomoniasis[35] and are of no use in patients with metronidazole resistance, except as a possible adjunct to oral treatment. Repeat testing should be performed in 3 months as recommended by current CDC guidelines; the rationale of retesting is that reinfection rates are as high as 17%.[36]

T. vaginalis organisms have been shown to be estrogen dependent in vitro[37] and in vivo.[38] In one report discontinuation of estrogen replacement treatment in a postmenopausal woman was associated with resolution of vaginal trichomoniasis.[39] These data suggest that hormonal manipulation should be studied in the management of trichomoniasis that is unresponsive to metronidazole and tinidazole and for women who cannot tolerate these drugs.

TABLE 108.8 Management of Trichomoniasis Treatment Failures

Reconfirm diagnosis
Wet preparation
Culture
Confirm that all current sexual partners have been treated
Re-treat with oral metronidazole or tinidazole
Consultation with an expert
Susceptibility testing

Minor adverse reactions to metronidazole, primarily nausea and a metallic taste, are common, but most patients can tolerate the usual dosage schedules. Metronidazole allergy is unusual. In such instances desensitization has been useful.[40]

About half of women with HIV infection are also infected with *T. vaginalis*.[41] Treatment of trichomoniasis with 500 mg of metronidazole twice daily for 7 days decreases the viral load of HIV in vaginal fluid,[42] an observation concordant with the results of studies that show an association of trichomoniasis with HIV acquisition.[43]

Pregnancy

Trichomoniasis in pregnancy has been the subject of considerable interest. An association between trichomoniasis and premature rupture of the membranes was reported in 1984.[44] Cotch and colleagues,[45] using data from the Vaginal Infections and Prematurity Study, reported in 1997 on the prospective evaluation of 13,816 pregnant women. They found that trichomoniasis was independently associated with a 30% greater likelihood of preterm delivery and low birth weight, and a 40% greater likelihood of having a preterm infant of low birth weight. In a more recent study treatment of trichomoniasis in asymptomatic pregnant women with metronidazole did not prevent preterm delivery.[46] Metronidazole has traditionally been avoided during pregnancy because of largely theoretical concerns about mutagenicity and oncogenicity. However, studies and meta-analyses have not demonstrated a consistent association between metronidazole use during pregnancy and teratogenic or mutagenic effects in infants.[47–49] Therefore pregnant women who have symptomatic trichomoniasis may be treated with 2 g of metronidazole.[24] Tinidazole has not been well evaluated in pregnant women. In lactating women, breastfeeding should be stopped during treatment and for 12 to 24 hours after completion of treatment with metronidazole, and for 3 days after completion of treatment with tinidazole.[24]

VULVOVAGINAL CANDIDIASIS

VVC is the second most common cause of vaginitis after BV.

Etiology and Pathogenesis

Candida albicans and other species of *Candida* can be part of the vaginal microbiota of asymptomatic women. About 30% of unselected women are colonized. In one study of unselected women[15] two-thirds of colonized women and only 22% of uncolonized women reported symptoms, primarily vulvovaginal itching and irritation. These data suggest that colonization with *Candida* spp. usually produces symptoms, albeit mild symptoms that do not prompt the patient to seek medical attention.

C. albicans can be isolated from 80% to 90% of patients with VVC, and other yeasts account for up to 20% of cases.[50] *Candida tropicalis* is isolated from 1% to 5% and may be associated with a higher rate of recurrence after standard treatments.[51,52] *Candida* (formerly *Torulopsis*) *glabrata* accounts for about 10% of vaginal yeast isolates.[50,51,53–55] Symptomatic vaginitis caused by this organism is associated with less intense itching and dyspareunia[53] than that caused by other *Candida* spp., but the organism may be harder to eradicate with standard therapies.[51,55] The relative incidence of vaginitis caused by fungi other than *C. albicans* appears to be increasing. Non-*albicans* infections are associated with recurrent disease (accounting for 21% of recurrent vs. 12% of initial infections) and with HIV infection (22% of infections in HIV-positive women vs. 12% in HIV-negative women), especially in HIV-infected women who receive prophylaxis with imidazoles or triazoles.[51] It is believed that the widespread use of topical antifungal agents, especially in short courses, may contribute to selection for non-*albicans* yeasts, which are less susceptible to these agents than is *C. albicans*. Cases of vaginitis caused by *Saccharomyces cerevisiae* have been reported and may be associated with baking.[56,57]

Some workers have estimated that 75% of adult women will suffer at least one episode of VVC during their lifetime.[58] Inhibition of normal bacterial microbiota by antibiotics favors the growth of yeasts,[58,59] although symptomatic cases are seen after the use of antimicrobial agents that do not suppress lactobacilli.[60] VVC sometimes occurs after antimicrobial treatment of trichomoniasis or BV.

Growth of yeasts is apparently favored by high estrogen levels, although such levels also promote the growth of lactobacilli.[61–63] The

prevalence of vaginal carriage of *Candida* is higher among users of oral contraceptives than among women using other methods of birth control.[59,63] The mechanism of this estrogenic predisposition is unclear.

VVC is associated with poorly controlled diabetes mellitus, and tight glycemic control decreases the frequency of symptomatic infection.[64] However, testing for diabetes in women with recurrent VVC is not cost effective because the yield is low.[59]

It has been suggested that tight, insulating clothing predisposes to VVC by increasing vulvar warmth and moisture. In prospective studies a higher prevalence of candidal carriage and higher concentrations of organisms were found in women who wore tight rather than loose clothing.[65–67] Impairment of phagocytic cells or of cell-mediated immunity (e.g., transplantation, chemotherapy) also predisposes to VVC. Some authorities believe that women with HIV infection develop VVC more often than HIV-negative women do, especially if they have low CD4 T-cell counts.[68,69]

The contribution of sexual transmission is poorly defined. VVC increases in incidence with the onset of sexual activity,[70–72] but the incidence is also increased by the use of oral contraceptives,[59,63] the contraceptive sponge, or the intrauterine device,[67] any of which might coincide with sexual activity. Having multiple sexual partners is not associated with a higher incidence of *Candida* infection. Most women who present with VVC have no predisposing illnesses or medications.

The mechanism by which *Candida* produces disease is not well defined. Although it is postulated that differences in virulence must exist,[59] strains isolated from symptomatic women are not demonstrably different from isolates from asymptomatic carriers.[73] Filamentous forms (hyphae and pseudohyphae) are associated with active disease.[74] Pseudohyphae have been observed to penetrate vaginal epithelial cells,[75] and they are more adherent to cells than are budding yeasts (blastospores).[76] Adherence appears to be an important pathogenic feature of *Candida* spp.,[77] and sublethal concentrations of antifungal agents may ameliorate disease by reducing adherence.[78] This suggested that *Candida* organisms entered the vagina via migration from the rectum across perianal area.[79]

The severity of symptoms in VVC is not directly related to the number of yeast cells present. Indeed, very small numbers of yeasts may be present in vaginal material recovered from highly symptomatic women.[59]

Clinical Manifestations

Patients with candidal vulvovaginitis generally complain of perivaginal pruritus, often with little or no discharge (Table 108.9). Dysuria is occasionally noted and is likely to be perceived as vulvar rather than urethral. The labia may be pale or erythematous. Shallow, radial, linear ulcerations (Fig. 108.7), especially on the posterior portion of the introitus, are common. Excoriations caused by scratching are often present (Fig. 108.8). Tiny papules or papulopustules, called satellite lesions, just beyond the main area of erythema are helpful diagnostically. The vaginal walls may be erythematous. Candidal discharge is classically thick cottage cheeselike and adherent, which presents a strong clue as to the cause of the infection (Fig. 108.9). However, discharge may be thin and loose, resembling that of other vaginitides.

Diagnosis

Vaginal pH is usually normal. There is no odor when the vaginal secretions are mixed with 10% KOH. Microscopic examination of vaginal material in saline or in 10% KOH may disclose budding yeasts or mycelia (Fig. 108.10). In the symptomatic patient with a diagnostic microscopic examination, fungal cultures are not needed. Microscopic examination of vaginal secretions is incompletely sensitive and is negative in 50% of culture-confirmed VVC.[80] In accordance, cultures may be helpful to secure the diagnosis in a patient who has a compatible clinical presentation and a negative microscopic examination. It is usually expedient to treat such a patient with antifungal agents while awaiting culture results. Cultures are also useful if empirical treatment produces no response. DNA probe testing can identify the presence of yeast, although not its role in causing disease. Polymerase chain reaction (PCR) testing for yeast is not FDA cleared. Culture remains the gold standard for diagnosis.

TABLE 108.9 Symptoms of Vulvovaginal Candidiasis

Vulvar itching
Vulvar irritation
Dysuria
Dyspareunia
Abnormal vaginal discharge



FIG. 108.7 Vulvovaginal candidiasis. There is a linear ulcer of the perineal skin.



FIG. 108.8 Vulvovaginal candidiasis. There are excoriations of the skin of the labia majora due to scratching.



FIG. 108.9 Vulvovaginal candidiasis. There are adherent white patches with surrounding erythema on the cervical mucosa.



FIG. 108.10 Vulvovaginal candidiasis. Suspension of vaginal secretions in 0.9% sodium chloride. Mycelia can be seen.

TABLE 108.10 Classification of Patients With Vulvovaginal Candidiasis

Uncomplicated Infection
Sporadic or infrequent
Mild-to-moderate severity
Likely to be caused by *Candida albicans*
Patient is not immunocompromised
Complicated Infection
Recurrent infection (four or more episodes per year)
Severe infection
Caused by non-*albicans* species of *Candida*
Uncontrolled diabetes, debilitation, immunosuppression, or pregnancy

TABLE 108.11 Treatment of Vulvovaginal Candidiasis

Uncomplicated Infection
Any available topical agent, short course
Fluconazole, 150 mg as a single oral dose
Complicated Infection
Culture confirmation mandatory
Antifungal susceptibility testing may be helpful
Treatment for 10–14 days with vaginal or oral agents
Alternative topical agents
Boric acid
Consider treatment of partners
Long-term suppressive treatment for frequently recurrent disease

Therapy

The treatment of VVC is best approached by classifying the patient as having either uncomplicated or complicated infection (Tables 108.10 and 108.11).[24]

Uncomplicated VVC is a sporadic infection with *C. albicans* of mild-to-moderate severity in a woman without a history of recent candidiasis and without underlying illness, such as HIV infection or diabetes mellitus. Most patients have uncomplicated disease, and most cases respond to treatment with short courses of vaginal or oral antifungal agents. Effective agents include vaginal preparations containing nystatin, miconazole, clotrimazole, butoconazole, terconazole, and tioconazole. All except nystatin- and terconazole-containing products are available without a prescription. Self-diagnosis and self-medication with OTC antifungal agents should be limited to women who have previously had documented VVC and are experiencing a recurrence of similar symptoms; self-diagnosis in other situations is often erroneous.[81] Failure to respond to self-medication should trigger a visit to a physician. In uncomplicated disease there does not appear to be any difference in efficiency related to dosage form (vaginal tablets, suppositories, ointments, creams) or to length of treatment (1, 3, 7, or 14 days). Oral fluconazole in a single 150-mg dose is as effective as the aforementioned vaginal medications in the treatment of uncomplicated VVC[82] and has been approved by the FDA for this indication. Oral fluconazole may be less expensive than some topical preparations and is preferred over topical treatments by many women. In this dosage the side effects are few[82] and systemic toxicity is unlikely. Other oral antifungal agents, such as ketoconazole, itraconazole, and voriconazole, are effective.

Complicated VVC occurs in patients with underlying conditions that compromise the immune response, such as diabetes, HIV disease, malignancy, or treatment with immunosuppressive agents. Patients who are pregnant or who have severe disease and patients with frequent recurrences (four or more episodes during the past year) should also be considered to have complicated disease, as should women infected with species other than *C. albicans.*

Management of complicated disease should begin with a vaginal culture to confirm the diagnosis and to determine the species of any isolated yeast. More than half of patients referred because of *chronic fungal vaginitis* have negative yeast cultures and a noninfectious explanation for their symptoms.[83] It is important that these misdiagnosed patients be culled at the outset so that needless and predictably futile antifungal treatment can be avoided and attention directed to establishing an accurate diagnosis. Speciation is important for those patients who do have candidiasis because infections caused by species of *Candida* other than *C. albicans* are often more difficult to treat.[84]

Complicated VVC responds poorly to short courses of treatment with oral fluconazole[85] or topical preparations. Treatment for 7 to 14 days or longer of topical therapies or oral fluconazole every other or third day for three doses is usually required. For patients with recurrent infections, chronic suppressive treatment with an oral antifungal agent may be useful in preventing recurrences once the current infection has responded. In 2004 150 mg of fluconazole per week was evaluated.[86] Recurrences are infrequent during chronic suppressive treatment, which is continued for at least 6 months. Once suppressive treatment is discontinued, relapse occurs in about half of the patients, necessitating continued chronic suppression.

Pregnant women should be treated with topical agents for at least 7 days. Oral azole antifungal agents should not be used during pregnancy because its impact on miscarriage risk is unclear, and high doses appear to increase the risk of birth defects.

Because a significant minority of healthy women are colonized with *Candida* spp., treatment is not indicated for asymptomatic women who have positive vaginal cultures.

Resistance to antifungal agents appears to be increasing. The availability of OTC antifungal agents and the widespread use of oral agents (e.g., ketoconazole, fluconazole, itraconazole), especially in patients with HIV disease, are possible explanations. In any event, an increasing number of cases do not respond to the usual topical and oral agents. Species other than *C. albicans,* such as *Candida krusei*[87] and *C. glabrata,* are more likely to be resistant, but nonresponsive *C. albicans* isolates are also beginning to appear.[88,89]

Fungal susceptibility testing is neither widely available nor easy to interpret. The National Commission for Clinical Laboratory Standards has published guidelines.[90]

Laboratories should offer testing using these guidelines. In vitro resistance predicts treatment failure, whereas in vitro susceptibility to a given agent is more difficult to interpret.

For VVC that does not respond to the available topical and oral agents, or is caused by non–*albicans Candida* (i.e., *C. glabrata*) intravaginal boric acid has been used with success.[91] Boric acid powder (600 mg) is placed into size 0 gelatin capsules and administered vaginally for 14 days. Ninety percent cure has been achieved with intravaginal flucytosine cream or amphotericin B cream. It should be noted that boric acid capsules, amphotericin B cream, and flucytosine cream are not available commercially in the United States.

Treatment of sexual partners has no role in the treatment of sporadic uncomplicated infections, but it may be worth considering for patients who have recurrent infections. Sequential isolates from such patients tend to be identical,[92] suggesting endogenous reactivation or exogenous reinfection from the same partner. Because the responsible organism

can often be recovered from male sexual partners,[93] they cannot be ruled out as a possible source of reinfection.

An immunologic hyperreactivity reaction has been suggested as the mechanism of symptomatic and recurrent vulvovaginitis. Activation of a complex immune reaction that releases different kinds of interleukin (IL)—IL-22, IL-10, IL-17, IL-1, and IL-18—activates T cells (Th1, Th17) and has been attributed to anti-*Candida* protection. The same mechanism, if inappropriately or excessively stimulated, causes damage through stimulation of an overwhelming inflammation that impairs the anti-*Candida* defense.[94]

The advance in the knowledge of host immune responses to *C. albicans* has provided a solid background for the generation of anti-*Candida* vaccines; some are under clinical investigations, with recurrent VVC being the main indication.[95]

Use of probiotics to treat and prevent VVC is a long-standing tradition. In the absence of controlled studies the use of probiotics is not part of recommended regimens.

BACTERIAL VAGINOSIS

Many women who present to their physicians with vaginal symptoms have a specific condition, first described by Gardner and Dukes[96] in 1955, that is now referred to as BV.[97,98] Perivaginal irritation is considerably milder than in trichomoniasis or candidiasis. Dysuria and dyspareunia are correspondingly rare. Affected women are usually sexually active and often complain of vaginal odor, which frequently is described as "fishy." About 90% of patients also notice a mild-to-moderate discharge. Abdominal discomfort is occasionally present, but it is usually mild and should prompt evaluation for coincident infections such as salpingitis.

Discharge is often present at the introitus and visible on the labia minora. The labia and vulva are not erythematous or edematous. On speculum examination the vaginal walls appear uninflamed. The vagina usually contains a grayish, thin, homogeneous discharge. A pungent odor may be noted by the examiner.

The endocervix is unaffected by the process, and any cervical discharge should be physiologic. The presence of a purulent cervical discharge may result from coincident gonococcal or chlamydial infection.[99] Abnormalities on bimanual examination are unusual in uncomplicated BV and should prompt a search for other pathologic processes. There is an increased risk for endometritis and salpingitis among women with BV.[100–102]

Other vaginal infections may resemble BV; an accurate differential diagnosis depends on laboratory examination. The pH of vaginal fluid is elevated to greater than 4.6 in about 90% of women with BV.[96,103,104] A vaginal pH of 5 or higher strongly suggests BV.[105] A whiff test is positive.[103,106]

A wet mount of the vaginal fluid from patients with BV usually reveals clue cells, which are vaginal epithelial cells studded with tiny coccobacilli. These organisms are best appreciated at the edges of the cell and may be dense enough to partially obscure the nucleus. Not all cells in the specimen are clue cells, but some clue cells are seen in more than 90% of patients with BV.[107,108] A finding of increased numbers of PMNs in a patient with BV suggests the presence of a coexisting process, notably cervicitis.[99]

Culture for *Gardnerella vaginalis* can be accomplished on a variety of media (see Chapter 236), and DNA probe technology has also been used for its identification in vaginal specimens. The organism can be isolated from virtually all women with BV,[98,106] but it is also recovered from at least 50% of asymptomatic women.[98] Therefore the presence of *G. vaginalis* does not prove that a patient has BV or suggest a need for treatment. A positive culture or nonculture test for *G. vaginalis* should not be considered diagnostic of BV unless other objective evidence of BV (elevated pH, odor with 10% KOH, clue cells) is present.

Epidemiology

In the United States, according to the National Health and Examination Survey, the prevalence of BV is 29% in women age 14 to 49 years and 50% in African-American women.[109]

Bacterial vaginosis was initially described in sexually active women, and it is common in populations with a high prevalence of STDs. The precise contribution of heterosexual transmission to the overall epidemiology of the condition remains controversial. However, a recent meta-analysis[110] found a significant positive association between BV and new or multiple sexual partners and a significant negative association between BV and condom usage. The prevalence is appreciable in lesbians, among whom other STDs are relatively uncommon. In one study women who identified as lesbians had a 2.5-fold increased likelihood of BV compared with heterosexual women. Lesbian couples are mostly concordant with regard to vaginal microbiota (normal or BV), suggesting that BV is sexually transmitted in this setting.[111,112]

Pathophysiology

Microscopic examination of vaginal discharge in BV characteristically reveals a predominant microbiota of coccobacilli. On the basis of this morphology the organism most closely associated with BV was originally called *Haemophilus vaginalis*.[96] It has now been given its own genus and is called *G. vaginalis* in recognition of Gardner's initial observations (see Chapter 236). Several studies suggest a less-than-straightforward relationship between *G. vaginalis* and BV. Although Gardner regularly produced BV by inoculating fresh vaginal discharge from patients with BV into the vaginas of healthy volunteers, inoculation of a pure culture of *G. vaginalis* was far less likely to produce disease.[96] In addition, *G. vaginalis* can be isolated from about 50% of asymptomatic women.[113] Finally, the in vitro sensitivity of *G. vaginalis* to antimicrobial agents does not match the effectiveness of these agents in clinical disease. Metronidazole is highly effective therapy for BV despite the fact that *G. vaginalis* is relatively resistant to the drug in vitro.[103]

Dysbiosis

Although the pathogenesis of BV remains unclear, alternatively, it represents a dysbiosis, an imbalance of the vaginal microbiota rather than an infection. BV is characterized by a shift from the predominance of lactobacilli in the vaginal microbiota to a higher concentration of other organisms.[114]

One explanation for all of these observations is that *G. vaginalis* is not the single cause of BV. BV is actually a synergistic infection involving not only *G. vaginalis* but also other microorganisms. The total diversity of culturable vaginal organisms is dramatically increased in women with BV. Organisms usually found in the intestinal tract include *Prevotella, Megasphaera,* Coriobacteriaceae, Lachnospiraceae, *Sneathia*, *Porphyromonas, Bacteroides* spp., and *Peptostreptococcus* spp., as well as BV-associated bacteria (BVAB)-1, BVAB-2, and BVAB-3.[115,116]

Most cases of BV are also associated with motile, curved anaerobic rods that are gram negative or gram variable. These organisms have been classified into the genus *Mobiluncus*.[117] Similarly, *Atopobium vaginae* is found in high titers in the vaginal fluids of most women who have BV.[118] The precise pathogenic role of these organisms remains to be elucidated. *Mycoplasma hominis* and *Ureaplasma urealyticum* can be isolated from the vagina in many women with BV. Their role as etiologic agents of BV has not been established.

Hydrogen Peroxide Production

Hydrogen peroxide (H_2O_2)–producing lactobacilli dominate the normal vaginal microbiota[2] and appear to protect against exogenous infection. Some workers believe that an undefined change in the vaginal milieu permits the replacement of protective H_2O_2-producing lactobacilli with *G. vaginalis* and other anaerobic microorganisms as mentioned earlier.

Biofilm Formation

Evidence suggest that *G. vaginalis* is the inciting pathogen in the pathogenesis of BV. Although the other anaerobic organisms contribute to BV symptoms, *G. vaginalis* play a role in the process of biofilm development. It has the ability to adhere to host receptor sites on vaginal epithelial cells and induce the production of cytotoxic substances specific to host cells and biofilm formation.[119]

Risk Factors

Some investigators have linked douching and smoking to BV.[120–122]

Women who have BV are at increased risk for the development of infection with HSV type 2,[123] *N. gonorrhoeae,* and *C. trachomatis*.[4]

BV has been associated with an increased risk for HIV infection.[124] In one study BV was associated with HIV-1 RNA expression in the genital tract of infected women,[125] consistent with the hypothesis that BV predisposes to the acquisition of HIV-1 infection.

Diagnosis

The patient is most likely to complain of odor and of the discharge, which tends to be gray and homogeneous (Fig. 108.11). The odor is best described as "fishy" and is caused by amines such as methylamine. These amines volatilize at increased pH, which explains the propensity of the patient to notice the odor when her secretions are more alkaline (e.g., during menses, after intercourse). Vulvovaginal irritation is not usually a prominent symptom, hence the use of the term *vaginosis* rather than *vaginitis*. Table 108.12 details the symptoms usually associated with BV.

Vaginal pH is typically elevated to greater than 4.6. Odor is produced when vaginal secretions are mixed with 10% KOH. Microscopic examination of vaginal secretions suspended in 0.9% sodium chloride (NaCl) reveals few leukocytes and many small bacilli. The bacilli tend to coat vaginal epithelial cells, the so-called clue cells (Fig. 108.12), so named by Herman Gardner because they provided a *clue* to the diagnosis of this condition.[96]

Criteria for the diagnosis of BV are listed in Table 108.13. Amsel and colleagues[106] suggested that at least three of the four listed criteria (homogeneous discharge, positive whiff test, pH greater than 4.6, clue cells) should be present for the diagnosis of BV to be made. Clinicians' descriptions of the discharge tend to be poorly reproducible, and some workers prefer to require that all three of the somewhat more objective criteria be present. Criteria have been developed by Nugent and colleagues[126] for the diagnosis of BV with use of the vaginal Gram stain. This is an objective method of diagnosis that compares well[127] with the criteria of Amsel. Other tests have acceptable performance characteristics compared with Gram stain include Affirm VPIII (BD Life Sciences, Franklin Lake, NJ), a DNA hybridization probe test for high concentration of *G. vaginalis* and OSOM BVBlue test (Sekisui Diagnostics, Lexington, MA), which detects vaginal fluid sialidase activity. These tests can be used when microscopy is not available.

The proline aminopeptidase card test is not recommended due to low sensitivity. PCR assay directed at a variety of organisms associated with BV has been used in research setting. More data are needed for evaluation of its clinical utility.[116,128]

Therapy

Therapy is indicated in symptomatic women to relieve their symptoms and prevent acquisition of other STDs, such as chlamydia, gonorrhea, HIV, HSV-2, and trichomoniasis. The primary regimen for the treatment of BV is oral metronidazole, 500 mg twice a day for 7 days. A single 2.0-g dose of metronidazole, such as is used to treat trichomoniasis, is less effective and is not recommended.[129] Vaginal preparations containing 0.75% metronidazole gel[130] or 2% clindamycin cream,[131] or ovules containing 100 mg of clindamycin,[132] are effective and have few systemic side effects. They are, however, more expensive than generic oral metronidazole. Secnidazole is a nitroimidazole approved by the FDA in September 2017 for women with BV as single 2-g oral dose. Oral clindamycin also is effective, but it is not widely prescribed for this indication. Alternative regimens, such as oral tinidazole 1 g once daily for 5 days or 2 g daily for 2 days, have been shown to be effective.[133] The FDA has also cleared metronidazole administered as 750-mg extended-release tablets once daily for 7 days and a single dose of clindamycin vaginal cream; however, data on the performance of this alternative regimen are limited.[24]

Treatment failures occur fairly commonly,[134] presumably because a normal *Lactobacillus*-dominated microbiota fails to become reestablished after the anaerobes and other components of the BV microbiota have been reduced in number with use of antimicrobial agents. Recent studies have shown that persistence of *G. vaginalis* in biofilms on the vaginal wall may be associated with treatment failure.[135,136]

For patients with multiple recurrences after completion of a recommended regimen, 0.75% Metrogel twice a week for 4 to 6 months or monthly oral metronidazole 2 g with fluconazole 150 mg have been shown to reduce recurrence rates.[137,138]

For patients who have BV that is unresponsive to the currently available antimicrobial agents, intravaginal boric acid, 600 mg at bedtime, provides symptomatic relief. Once the symptoms have been controlled, the dosing interval can be increased. Some patients remain free of symptoms using boric acid capsules once or twice a week.

Probiotics have been used alone or with antibiotics for therapy and prevention of recurrence of BV. No studies support the addition of any available *Lactobacillus* or probiotic formulation as adjunctive or replacement therapy in women with BV.[139,140]

Postoperative infections occur more often in women undergoing gynecologic surgery if they have BV than if they have normal microbiota.[5] In one study[141] women who had BV and who underwent total abdominal hysterectomy were randomly assigned to receive metronidazole or no



FIG. 108.11 Bacterial vaginosis. The gray, homogeneous discharge that coats the tissues is characteristic.



FIG. 108.12 Bacterial vaginosis. Suspension of vaginal material in 0.9% sodium chloride. Many bacteria are attached to the vaginal epithelial cells (clue cells).

TABLE 108.12 Symptoms Associated With Bacterial Vaginosis

Homogeneous vaginal discharge
Fishy vaginal odor during menstruation or after intercourse
Minimal itching or irritation

TABLE 108.13 Criteria for the Diagnosis of Bacterial Vaginosis

Homogeneous vaginal discharge
Vaginal pH >4.6
Positive whiff test with 10% potassium hydroxide
Clue cells

treatment. Vaginal cuff infections occurred in none of the treated women but in 27% of the untreated women ($P < .01$). Treatment studies have demonstrated that treatment of BV before induced abortion reduces the risk for subsequent pelvic inflammatory disease.[142] Therefore it would appear prudent to screen and treat women who have BV before performing induced abortion or hysterectomy. This logic could be extended to women undergoing other invasive gynecologic procedures, although there have been no studies in those areas.

Pregnancy

Pregnant women who have symptomatic BV should be treated.[24] BV during pregnancy is associated with adverse pregnancy outcomes, including premature membrane rupture, early labor, preterm birth, and postpartum endometritis.[143–145] Treatment of pregnant women who have BV and who previously delivered a premature infant reduced the risk for preterm delivery.[146–148] In accordance, pregnant women who are at high risk for preterm delivery should be screened for BV, regardless of symptoms and, if affected, treated with oral metronidazole or oral clindamycin.[149] Topical agents do not appear to be as effective as oral agents, and the use of clindamycin cream has been associated with adverse events such as prematurity and neonatal infections.[150,151]

Treatment of pregnant women who have asymptomatic BV and who are at low risk for premature delivery is controversial.[152–154] An analysis of 21 clinical trials involving 7847 women showed that antibiotic treatment of BV during pregnancy eradicated bacterial vaginosis, but the overall risk for preterm birth was not significantly reduced.[155]

BV can be nonsymptomatic. Women without symptoms are not usually treated, although the approach to such patients may change as more is learned about the implications of having abnormal vaginal secretions. Some women with nonsymptomatic BV who receive treatment retrospectively notice improvement in vaginal discharge and odor. Although BV is not considered a classic STD, studies support the notion that sexual activity is a risk factor. Women who have never been sexually active are rarely affected.[156]

Several studies have failed to show that treatment of male sexual partners improves the outcome of women treated for BV, although these studies were not of high quality.[157] Some clinicians treat sexual partners of women who have recurrent BV or BV that is poorly responsive to treatment with metronidazole or clindamycin. Although such an approach appears to be reasonable, there is no supporting scientific evidence.

Bradshaw and colleagues[158] studied 450 women who were treated for bacterial vaginosis. Recurrent BV was associated with sexual contact with their pretreatment sexual partner and inconsistent condom use. Recurrence was halved in association with the use of estrogen-containing contraceptives.[158] These data provide further support for the importance of sexual transmission in the epidemiology of bacterial vaginosis and suggest that change in the vaginal environment produced by estrogen may facilitate restoration of normal microbiota after treatment of bacterial vaginosis.[159]

DESQUAMATIVE INFLAMMATORY VAGINITIS

Etiology and Pathogenesis

DIV is an unusual condition of unknown cause. It mimics estrogen-deficiency vaginitis and trichomoniasis but usually occurs in women of reproductive age who have normal hormonal function and no evidence of any sexually transmitted conditions.

Some investigators believe it is an inflammatory vaginitis of noninfectious etiology; others consider it as representing a defect in vaginal microbiota and have named it "aerobic vaginitis."[160,161] In a few patients DIV appears to be a local manifestation of a systemic illness, such as systemic lupus erythematosus. Patients with DIV may also have erosive lichen planus involving oral or genital mucous membranes. Some investigators[162] have suggested that DIV is always part of the lichen planus complex. The paucity of occasions when these two enigmatic conditions coexist does not support this conclusion.[163]

Diagnosis

The patient reports purulent vaginal discharge and varying degrees of vulvar irritation, dysuria, and dyspareunia (Table 108.14). There is often a history of multiple unsuccessful treatments with a variety of topical and oral antimicrobial agents. Because the disease is most often confused with trichomoniasis, frequently in patients in whom a sexually transmitted condition is highly unlikely, many patients carry the diagnosis of resistant trichomoniasis and have received several courses of metronidazole in various dosage forms and dosages.

The mucosa of the vestibule, vagina, and cervix may show diffuse (Fig. 108.13) or segmental involvement, usually in the proximal vagina. There may be superficial erosions of the mucosa, which are characteristic of this condition (Fig. 108.14).

The vaginal pH is often elevated to greater than 4.6. There is no odor when the vaginal secretions are mixed with 10% KOH. The saline

TABLE 108.14 Characteristics of Desquamative Inflammatory Vaginitis

Symptoms
Purulent discharge
Vulvar discomfort
Dyspareunia
Findings
Mucosal erythema
Purulent secretions
Parabasal cells
Treatment
Clindamycin 2% vaginal cream
Boric acid
Topical corticosteroids



FIG. 108.13 Desquamative inflammatory vaginitis. Diffuse mucosal erythema and a purulent vaginal discharge are evident.

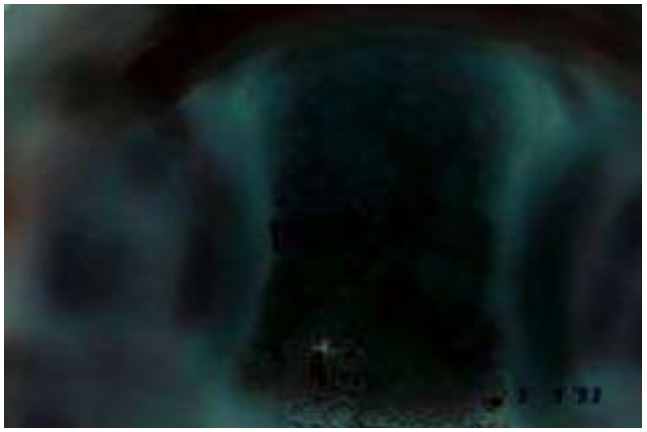

FIG. 108.14 Desquamative inflammatory vaginitis. Superficial mucosal erosions can be seen.



FIG. 108.15 Desquamative inflammatory vaginitis. The vaginal wet preparation contains many leukocytes and parabasal cells.

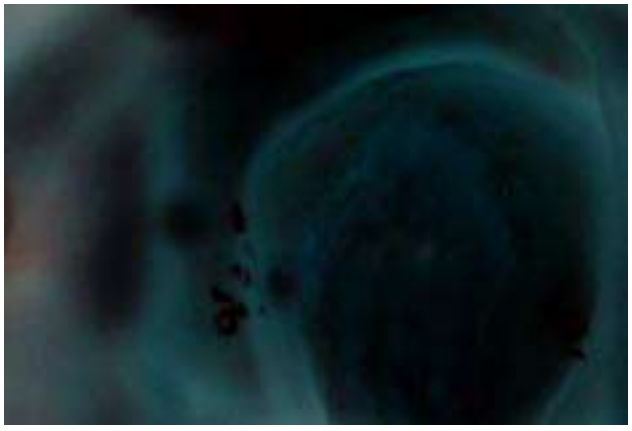

FIG. 108.16 Noninfectious purulent ectocervicitis. Purulent secretions can be seen to emanate from the ectropion.

TABLE 108.15 Cervicitis

Endocervicitis
Sexually transmitted *Neisseria gonorrhoeae, Mycoplasma genitalium, Chlamydia trachomatis* Associated urethritis, endometritis, or salpingitis may be present
Ectocervicitis
Noninfectious Inflammation in an ectropion Not associated with urethritis, endometritis, or salpingitis

wet preparation contains many leukocytes. Most of the vaginal epithelial cells are immature parabasal cells (Fig. 108.15).

Therapy

Topical corticosteroids and topical boric acid provide symptomatic relief and normalize the appearance of the mucous membranes and vaginal secretions. Relapse is predictable after these agents are discontinued. By far the most effective treatment for this condition is 2% clindamycin vaginal cream: 5 g of the cream, containing 100 mg of clindamycin, is inserted into the vagina at bedtime for 4 weeks. Most patients have a prolonged remission after this course of treatment. A few patients require a second 4-week course of treatment to induce a remission, but at least 90% of patients with DIV experience a complete remission in association with treatment with topical clindamycin.[164]

Once remission has been induced, it may be lifelong. More commonly, however, relapses occur months to years later. The relapses may also respond to re-treatment with topical clindamycin. Some patients who have very frequent relapses require continual biweekly intravaginal clindamycin or corticosteroids to remain in remission. Perimenopausal patients who are deficient in estrogen may require estrogen replacement as well as topical clindamycin to sustain a remission.

CERVICITIS

Etiology and Pathogenesis

Cervicitis is an inflammation that affects columnar epithelial cells of the endocervical glands but also can involve squamous epithelium of the ectocervix. Cervicitis may be infectious or noninfectious.[165] Infectious cervicitis is primarily caused by *N. gonorrhoeae, C. trachomatis,* or *M. genitalium.*[165]

Human papillomavirus, HSV, and *T. vaginalis* may involve the cervix but affect mainly the squamous epithelium of the ectocervix. Consideration of these important sexually transmitted organisms is beyond the scope of this chapter (see Chapters 135 and 143). Ureaplasmas and *Mycoplasma hominis* are organisms frequently found in the genital tract of women and men. Their etiologic role in disease has not been demonstrated. Noninfectious cervicitis is usually ectocervicitis, in which there is inflammation in an ectropion (Table 108.15). It is usually caused by mechanical or chemical irritation.

Symptoms and Diagnosis

The patient usually complains of a purulent vaginal discharge. The mucopurulent secretions are not irritating, so there is no vulvar discomfort or introital dyspareunia. In infectious cervicitis there may be dysuria, abnormal uterine bleeding, lower abdominal pain, or pelvic dyspareunia because gonococcal or chlamydial infection can involve the urethra, endometrium, or uterine adnexa. With noninfectious cervicitis, dysuria, abdominal pain, and deep (pelvic) dyspareunia are uncommon. There may be postcoital bleeding due to trauma to the inflamed ectropion during intercourse.

Findings on examination of the vulva and of the vaginal mucosa are usually normal. In infectious endocervicitis the purulent secretions can be seen to flow from the endocervical canal; in noninfectious ectocervicitis the purulent secretions can be seen to emanate from the ectropion (Fig. 108.16), often with clear normal secretions flowing from the endocervix. In some patients with noninfectious cervicitis the abnormal secretions are solely endocervical, presumably reflecting noninfectious endocervicitis. The other cardinal sign of cervicitis is sustained endocervical bleeding easily induced by minor trauma, such as touching the area with a swab (friability).[165] Vesicular lesions and ulcerations are present in HSV infection, whereas a strawberry cervix is characteristic of *T. vaginalis*.

In patients who have gonococcal, mycoplasmal, or chlamydial infection, urethral, uterine, or adnexal tenderness may be present, reflecting infection of these loci. The bimanual examination is usually normal in women who have noninfectious cervicitis.

Vaginal pH may be elevated. There is no odor when the secretions are mixed with 10% KOH. Wet preparations of vaginal secretions contain many leukocytes. The vaginal cells are mature. Gram-stained smears of cervical secretion confirm the presence of many leukocytes and, in gonococcal infection, may contain intracellular cocci.

In addition to microscopic examination, patients should be tested for *C. trachomatis* and *N. gonorrhoeae* by NAAT. These assays can be performed on vaginal/cervical or urine specimens. *T. vaginalis* antigen-based testing, or ideally, NAAT is also recommended.

Therapy

Studies that have attempted to correlate numbers of leukocytes in Gram-stained cervical smears with gonococcal or chlamydial infection have not produced clear-cut recommendations as to which patients should be treated for infectious cervicitis without waiting for culture results.

According to CDC guidelines, presumptive treatment with antimicrobials for *C. trachomatis* and *N. gonorrhoeae* should be provided for women with cervicitis who are at increased risk—those age younger than 25 years, those with a new sex partner, a sex partner with concurrent partners, or a sex partner who has an STI—especially if follow-up cannot be ensured.

Recommended regimens for presumptive therapy include azithromycin 1g orally in single dose or doxycycline 100 mg every 12 hours

for 7 days, and concurrent treatment for gonococcal infection (i.e., ceftriaxone 250 mg intramuscularly once plus azithromycin 1 g orally once) should be considered if the patient is at risk for gonorrhea or lives in a community where the prevalence of gonorrhea is high (>5%). For women with clinical evidence of cervicitis but at low risk for chlamydia or gonorrhea, therapy may be withheld while awaiting the results of diagnostic testing.

To minimize transmission and reinfection, women treated for cervicitis should be instructed to abstain from sexual intercourse until they and their partner(s) have been adequately treated (7 days after single-dose therapy or until completion of a 7-day regimen) and symptoms have resolved.

Patients who have positive tests for sexually transmitted bacteria should be treated with regimens recommended for the specific etiologic agent,[24] and they should be offered partner services and instructed to return 3 months after treatment for repeat testing because of high rates of reinfection. Treatment for *M. genitalium* with moxifloxacin might be considered for cases of clinically significant cervicitis that persist after azithromycin or doxycycline therapy and in which reinfection is unlikely.

Cervicitis in pregnant women can cause pregnancy and neonatal complications. Diagnosis and treatment of cervicitis in pregnant women does not differ from that in women who are not pregnant, although doxycycline should be avoided.

If tests for gonococci, *M. genitalium,* and chlamydiae are negative, antimicrobial treatment is not likely to be of benefit.

If the volume of secretions arising from an ectropion is bothersome, destruction of the endocervical mucosa of the ectropion with cryotherapy may allow the ectocervix to become reepithelialized with squamous epithelium, with a resultant diminution in the volume of ectocervical secretions.

ESTROGEN-DEFICIENCY VAGINITIS (GENITOURINARY SYNDROME)

Etiology and Pathogenesis

Estrogen-deficiency vaginitis is seen in postmenopausal women and in younger women who have become deficient in estrogen because of disease or because of treatment with pharmaceuticals that interfere with the production or the activity of estrogen.[166] This condition can also be seen during breastfeeding because of an effect of prolactin on estrogen production.[167] Without estrogen the genital mucosa thins. Glycogen is decreased, and, as a result, lactobacilli no longer dominate the vaginal microbial microbiota. Thinning of the mucosa may result in vulvar discomfort and introital dyspareunia. Symptoms include vaginal dryness, burning, irritation, dyspareunia, vulvar or vaginal bleeding, and vaginal discharge.

The thin vaginal mucosa may become infected, presumably by enteric organisms and others that are able to colonize the vagina in the absence of lactobacilli. Frequent UTIs may occur. In some instances estrogen-deficiency vaginitis overlaps with DIV.

Due to the just-described pathogenesis and involvement of the lower urinary tract, the term *genitourinary syndrome of menopause* has been linked to those symptoms.[168]

Diagnosis

The vestibular and vaginal mucosae are pale, often with patches of erythema. Vaginal secretions, if present, may be purulent. Vaginal pH is elevated. There is no odor when the secretions are mixed with 10% KOH. Microscopic examination of the secretions discloses immature (parabasal) vaginal cells with or without leukocytes. Vaginal cultures contain a variety of enteric and other bacteria.

Therapy

The primary indication for therapy is the presence of symptoms. Before initiation of therapy other causes should be excluded, such as UTI and endometrial cancer in patients with postmenopausal bleeding.

The primary defect is the absence of estrogen. Therefore definitive treatment involves estrogen replacement or cessation of antiestrogenic drugs or breastfeeding. Topical antibacterial agents containing sulfonamides or clindamycin may improve symptomatic vaginitis, and lubricating agents may relieve vaginal dryness and dyspareunia. Use of vaginal estrogen requires caution in women with high risk of estrogen-dependent tumors. In November 2016 FDA approved the use of Prasterone, another name for dehydroepiandrosterone 6.5 mg (0.5% formulation), for the treatment of genitourinary syndrome (vulvovaginal atrophy). The mechanism of action is due to aromatization of androstenedione and testosterone locally to estrol and estradiol. Ospemifene is a selective estrogen receptor modulator that acts as an estrogen agonist in the vagina. It is an oral therapy that can be used in women who cannot or prefer not to use vaginal product.

Without estrogen replacement, symptoms may recur after cessation of treatment.

VULVAR PAIN SYNDROME (FORMERLY VULVODYNIA, VESTIBULODYNIA, VULVAR VESTIBULITIS, OR FOCAL VULVITIS)

External genital discomfort is a very common symptom among women of reproductive age. Usually described as itching or burning, vulvar discomfort may or may not be associated with introital dyspareunia. There are a number of possible causes, few of which are infectious and virtually none of which are sexually transmitted. Nonetheless, the clinician should be aware of these entities to rule out infections, prescribe appropriate treatment if the diagnosis is apparent, and refer the patient to a gynecologist or dermatologist who specializes in vulvar disease if an explanation for the patient's symptoms is not readily forthcoming.

Definition and Terminology

In 2015 representatives from the International Society for the Study of Vulvovaginal Disease, the International Society for the Study of Women's Sexual Health, and the International Pelvic Pain Society developed consensus terminology and classified persistent vulvar pain into two categories[169]: (1) vulvar pain caused by specific disorder and (2) vulvodynia—vulvar pain of at least 3 months' duration, without clear identifiable cause, and that may have potential associated factors.

Vestibulitis has been eliminated from the updated terminology because the presence of inflammation (implied by "itis") has not been documented.

Vulular pain secondary to a specific disorder includes the following:
Infectious (e.g., recurrent candidiasis, herpes)
Inflammatory (e.g., lichen sclerosus, lichen planus, immunobullous disorders)
Neoplastic (e.g., Paget disease, squamous cell carcinoma)
Neurologic (e.g., postherpetic neuralgia, nerve compression, or injury, neuroma)
Trauma (e.g., female genital cutting, obstetrical)
Iatrogenic (e.g., postoperative, chemotherapy, radiation)
Hormonal deficiencies (e.g., genitourinary syndrome of menopause [vulvovaginal atrophy], lactational amenorrhea)

Vulvodynia can be as follows:
Localized or generalized or mixed
Provoked (insertional, contact), spontaneous, or mixed
Primary or secondary
Intermittent, persistent, constant, immediate, or delayed

Epidemiology

The prevalence of chronic vulvar pain (all types) ranges from 3% to 15% in self-report studies.[170] Assessing prevalence is challenging because many women with vulvar pain do not present for evaluation. In one study risk factors for the development of vulvodynia included younger age, Hispanic ethnicity, married, depression, posttraumatic stress disorder (PTSD), sleep dysfunction, and other chronic pain syndromes.[171]

Etiology and Pathogenesis

The infectious etiology of vulvar pain can be secondary to recurrent VVC, herpes, and sometimes trichomoniasis. The noninfectious etiologies of vulvodynia (localized or generalized) are not known and likely multifactorial.

Risk factors include the following:
Excessive inflammation, increase of proinflammatory products in the vulvar tissue

Neurologic proliferation and sensitization
Genetic factors via at least three potentially overlapping mechanisms: genetic polymorphisms that increase the risk of candidiasis or other infections, genetic changes that allow prolonged or exaggerated inflammatory responses, and increased susceptibility to hormonal changes associated with oral contraceptive pills
Hormonal factors: use of combined hormonal contraceptives
Psychological factors such as depression, anxiety, PTSD
Musculoskeletal and structural defect like pelvic floor muscle dysfunction, pelvic organ prolapse

Clinical Manifestations

The main complaint is significant pain upon contact with vulvar vestibule; other symptoms include irritation, raw sensation, and burning with or without dyspareunia.

Diagnosis

Sexually transmitted conditions, including genital ulcers and warts involving the labia majora and labia minora, should be obvious on physical examination. The only infection that commonly causes diffuse vulvitis is VVC, usually in association with involvement of the vestibule and vagina.

Vulvar pain syndrome (vulvodynia) is a clinical diagnosis based upon a detailed history and physical examination.

Diagnostic criteria include pain, absence of identifiable cause, duration of at least 3 months, pain with pressure point testing.[172] The latter is a cardinal sign confirmed by severe pain upon palpation of the vulva vestibule with a cotton-tipped swab or with the examiner's finger.

Laboratory evaluation is usually done to exclude other causes of the patient symptoms; vuvlvodynia does not cause laboratory abnormalities. Workup should include all other etiologies of vulvovaginitis; biopsy can be performed to exclude neoplastic and inflammatory causes such as lichen sclerosus.

Therapy

If an infectious cause is identified, it should be treated accordingly.

The treatment of vulvodynia involves a multidisciplinary approach, including psychological interventions, pelvic floor physical therapy, and vestibulectomy (for provoked vestibulodynia), and vulvar hygiene (avoiding soap or fragrance).

Treatment typically progresses from less invasive to more invasive, and several treatment options are worth pursuing.

Topical lubricants that moisturize the skin or reduce the friction during sexual contact are helpful. Medical therapy includes topical preparations such as lidocaine and estrogen cream and oral medications such as antidepressants, anticonvulsants, and injection therapy.

Submucosal injections of corticosteroids have a local antiinflammatory effect and appear to treat pain in women with vulvodynia.[173,174] Topical steroids are not recommended because of the lack of efficacy of low-dose corticosteroids and potential side effects of high-potency steroids.[175]

Of note, these treatment options are not specifically approved for vulvodynia and therefore represent off-label use.

Surgery is considered treatment of the last resort and usually not recommended. Laser therapy for vulvodynia is an area of developing research.[176]

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The complete reference list is available online at Expert Consult.

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109 Infections of the Female Pelvis

Richard H. Beigi

SHORT VIEW SUMMARY

Definition
- An acute inflammation of the epithelium and/or soft tissue of the pelvic organs

Microbiology
- Microorganisms found in the endocervix and vagina
- Bacterial vaginosis microorganisms, predominantly anaerobes
- Group B *Streptococcus* and *Escherichia coli*
- Sexually transmitted microorganisms, such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis*

Diagnosis
- Clinical diagnosis based on fever, erythema, and tenderness in the postoperative setting
- Clinical diagnosis based on risk assessment for sexually transmitted disease and an evaluation for lower genital tract inflammation in the case of pelvic inflammatory disease

Therapy for Postoperative Infections
- Broad-spectrum antibiotic therapy
- Include coverage of penicillinase-producing anaerobes
- Use antibiotic regimens effective in an anaerobic environment

Therapy for Pelvic Inflammatory Disease
- Be aware of the emerging resistance of *N. gonorrhoeae* to quinolones, azithromycin, and, to some degree, cephalosporins
- Treat concurrently for bacterial vaginosis if the diagnosis is made concurrently

Prevention
- Preoperative antibiotic prophylaxis
- Screening for sexually transmitted microorganisms

Infections of the female pelvis can be conveniently considered in three basic categories: infections related to pregnancy, infections occurring after gynecologic surgery, and pelvic inflammatory disease (PID), with its associated conditions and implications. Pelvic infections are commonly polymicrobial, with cultures revealing a mixture of both aerobic and anaerobic bacteria. These pathogenic microorganisms frequently originate from the flora of the lower genital tract. The microbial etiology of even hospital-acquired intrapartum, postpartum, and postsurgical infections reflects this because isolation of bacteria resistant to broad-spectrum antibiotic therapy is uncommon.

INTRAPARTUM, POSTPARTUM, AND POSTABORTAL INFECTIONS

Intraamniotic infection (IAI), commonly referred to as chorioamnionitis, is a clinically detectable infection of the amniotic fluid and fetal membranes during pregnancy.[1] IAI occurs in up to 10.5% of women in labor.[2] Most cases of IAI originate when vaginal microorganisms ascend into the intrauterine cavity after rupture of the membranes.[3] Approximately 2% of women with preterm labor (<37 weeks of gestation) and intact membranes have symptomatic chorioamnionitis, whereas another 20% have subclinical infection. Of women receiving ampicillin and erythromycin therapy for preterm-premature rupture of the membranes, approximately 15% will develop IAI compared with a 26% incidence for those not treated with antibiotics.[4] In full-term pregnancies, IAI is associated with dysfunctional labor. Approximately 75% of infected women require augmentation of labor with oxytocin, and approximately 35% require cesarean delivery, usually because of arrest of progress in labor.[5] A few cases, most notably those caused by *Listeria monocytogenes,* result from transplacental hematogenous spread in mothers with bacteremia.[6] The diagnosis is usually made before membrane rupture in these patients. Rare cases of IAI occurring after diagnostic amniocentesis, intrauterine transfusion, or percutaneous umbilical blood sampling have been reported. Intrauterine infection can also occur after cervical cerclage (a circumferential suture about the cervix to prevent preterm delivery in women with cervical insufficiency) in 1% to 2% of patients, and this risk may be as high as 25% if the cerclage is performed after prolapse of the membranes into the vagina. Risk factors for IAI include prolonged duration of labor or rupture of membranes, multiple vaginal examinations, young age, low socioeconomic class, nulliparity, and preexisting bacterial vaginosis.[5]

Microorganisms, such as lactobacilli and diphtheroids, are commonly found in the amniotic fluid of women in normal labor at term. Those with IAI have a select group of high-virulence microorganisms, such as group B streptococci, *Escherichia coli,* genital mycoplasmas, and pathogenic anaerobes (e.g., *Prevotella bivia*), present in significantly higher quantities, causing an inflammatory response and systemic signs of infection.[3] The first two microorganisms are found most commonly when maternal and/or neonatal bacteremia complicates intraamniotic infection.[3] Many of these microorganisms, especially anaerobic bacteria, the mycoplasmas, and *Gardnerella vaginalis*, have also been associated with bacterial vaginosis.

The clinical diagnosis of IAI is imprecise and has recently been slightly changed.[7] It is based on the presence of an isolated fever of greater than or equal to 39.0°C (102.2°F) or a lower-grade temperature (>38°C [100.4°F]) that is present on two occasions 30 minutes or more apart and the presence of one other clinical finding: maternal leukocytosis (defined as a white blood cell [WBC] count >15,000/μL), purulent cervical drainage, or fetal tachycardia. The vast majority of these gravidas will have concomitant ruptured membranes. Practically, clinicians tend to base the diagnosis on the presence of intrapartum fever plus one additional criterion anyway.[8] Maternal and/or fetal tachycardia are common with fever and add little additional information. Although the abdomen should be examined for uterine tenderness, it is often obscured by conduction anesthesia. Foul-smelling amniotic fluid is rarely appreciated. Maternal WBC counts increase with duration of labor, and cut-points suffer from a lack of precision to reliably distinguish fever from infectious and noninfectious causes. Furthermore, patients with preterm-premature rupture of the membranes before 32 weeks of gestation are candidates for antenatal steroid therapy to promote fetal lung maturity. Betamethasone leads to an increase in maternal leukocyte count and a decrease in lymphocyte count. These changes return to baseline within 3 days. Although the diagnosis is based largely on clinical findings, amniotic fluid Gram staining, WBC count (>50 cells/mm^3), and glucose concentration (<15 mg/dL) have been shown to be useful

in supporting the clinical impression, particularly in patients with intact membranes.[9] Microbial invasion of the amniotic cavity is accompanied by the presence of high amniotic fluid concentrations of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor, and a gelatinase, matrix metalloproteinase 9.[10] Measurement of these substances is costly and not readily available.

Given the imprecision of the diagnosis of IAI, antibiotic therapy should be considered in laboring gravidas with isolated fever (>39°C [102.2°F] once or >38.0°C [100.4°F] on two successive readings). Antimicrobial therapy for IAI is aimed at preventing bacteremia in the mother, as well as initiating intrapartum treatment of the fetus while awaiting delivery. Gibbs and colleagues[11] documented improved neonatal and maternal outcome when antibiotic therapy is begun intrapartum rather than immediately postpartum. Delivery of the fetus and placenta removes the sites of infection, much like draining an abscess, making this intervention the most significant part of therapy. Because group B streptococci and *E. coli* are the most common isolates from infected neonates, a combination of ampicillin plus gentamicin is a reasonable initial regimen for IAI. This regimen is sufficient to treat the mother if the delivery was vaginal, with only one additional dose of the antibiotic regimen needing to be administered postpartum. If cesarean delivery is required, up to 15% of operative patients given only ampicillin and gentamicin experience treatment failure by developing postpartum endometritis. These patients probably require continued broad-spectrum antibiotic coverage, including anaerobic coverage, and accordingly, a drug such as clindamycin and metronidazole should be added to the treatment regimen to decrease the risk for postpartum endometritis by half.[12] Other broad-spectrum regimens may be equally effective. This antibiotic regimen should be continued until the patient has been afebrile (temperature < 37.8°C [100°F]) for 24 hours.[13]

Although delivery is essential for cure, no critical diagnosis-to-delivery interval beyond which the frequency of neonatal complications escalates dramatically has been identified.[14] Accordingly, labor needs to be managed actively, but cesarean delivery should be performed only for accepted obstetric indications and not done solely for the presence of a clinical diagnosis of IAI.

Postpartum Endometritis

Postpartum infection of the uterus, the most common cause of puerperal fever, is designated endomyometritis. Cesarean delivery, particularly after labor or rupture of the membranes of any duration, is the dominant risk factor for postpartum endomyometritis (PPE).[15] The pathogenesis of this infection involves inoculation of the amniotic fluid after membrane rupture or during labor with vaginal microorganisms. The myometrium, leaves of the broad ligament, and the peritoneal cavity are then exposed to this contaminated fluid during surgery (Fig. 109.1). The reported incidence of PPE after cesarean delivery is less than 10% in patients receiving antibiotic prophylaxis. The diagnosis is uncommon after vaginal delivery. Risk factors for postcesarean endomyometritis include a prolonged duration of labor or rupture of the membranes, presence of bacterial vaginosis, number of vaginal examinations, and use of internal fetal monitoring.[16,17] Antimicrobial prophylaxis is associated with a 50% reduction in infection in all populations studied. All patients undergoing cesarean delivery, either elective or unscheduled, are candidates for antibiotic prophylaxis.[18] There is now strong evidence that antibiotic prophylaxis given for cesarean delivery before skin incision, rather than after cord clamping, decreases the incidence of postcesarean endomyometritis and total infectious morbidities without affecting neonatal outcomes.[19] In addition, a recently published randomized controlled investigation has demonstrated an additional 50% reduction in surgical site infections (SSIs) among women having a nonelective cesarean delivery during labor and/or after rupture of membranes with the addition of azithromycin to the antibiotic prophylaxis regimen.[20] Many patients who develop postcesarean endometritis despite antibiotic prophylaxis have histologic evidence of incipient infection.[21]

PPE is a polymicrobial infection caused by a wide variety of bacteria. Group B streptococci, enterococci, other aerobic streptococci, *G. vaginalis, E. coli, P. bivia, Bacteroides* spp., and peptostreptococci are the most common endometrial isolates, with group B streptococci and *G. vaginalis* the most common isolates from the blood.[22–24]

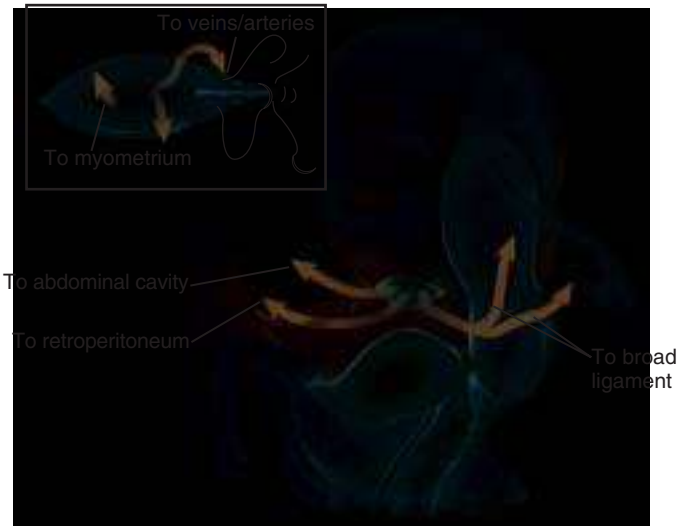

FIG. 109.1 Pathophysiology of postcesarean endomyometritis. Note the potential pathways for contaminated amniotic fluid to infect the postpartum pelvic tissues.

The isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis* from endometrium and blood suggests that these organisms can cause PPE, although good clinical responses have been obtained in patients with mycoplasmas cultured from the blood and who were treated with antibiotics not active against these organisms. These organisms have also been reported to be important pathogens associated with abdominal wound infection after cesarean delivery.[25]

Chlamydia trachomatis has been associated with a late form of PPE that occurs more than 2 days to 6 weeks after delivery in women who deliver vaginally.[26] Group A β-hemolytic streptococcal endometritis is uncommon. The source of a sporadic postpartum group A β-hemolytic streptococcal infection is typically unknown, but outbreaks of postpartum and postsurgical group A β-hemolytic streptococcal infections have been associated with colonized health care workers. Health care workers who were asymptomatic carriers of group A β-hemolytic streptococci have been identified in 15 of 21 outbreaks of postpartum and postsurgical infections reported from 1976 to 2005.[27] The infections are characterized by early onset and rapid severe progression with few localizing symptoms or physical signs. These can sometimes also be fatal if unrecognized/untreated (and even occasionally with appropriate recognition and therapy).

The diagnosis of PPE is suggested by the development of fever, usually on the first or second postpartum day. Significant fever is defined as an oral temperature of 38.5°C (101.3°F) or higher in the first 24 hours after delivery or 38°C (100.4°F) or higher for at least four consecutive hours 24 or more hours after delivery. Other consistently associated findings are lower abdominal pain, uterine tenderness, and leukocytosis. These patients may also exhibit a delay of the normally rapid postoperative return of bowel function due to an associated local peritonitis.

Patients with suspected PPE should have the uterus assessed for size, consistency, and tenderness. This requires a bimanual pelvic examination in women in whom the uterus is no longer palpable on abdominal examination.

The value of transvaginally obtained uterine cultures and the optimal method of obtaining such cultures remain matters of controversy. Although cultures obtained transvaginally are often difficult to interpret because of contaminants, they may be useful for those patients in whom initial therapy fails.[28] A test for *Chlamydia* should be performed in patients with late-onset (>7 days after delivery) mild PPE, particularly those at high risk for chlamydial infection (e.g., adolescents).

Clindamycin plus gentamicin has proved to be the most effective regimen in treating PPE, especially if PPE occurs after cesarean delivery.[29] Alternative regimens used for the treatment of PPE include one of the extended-spectrum penicillins or second-generation cephalosporins (e.g., ampicillin-sulbactam, ticarcillin-clavulanic acid, piperacillin-tazobactam,

cefotetan, cefoxitin). Failures of those regimens with poor activity against penicillin-resistant anaerobic bacteria (e.g., penicillin and gentamicin without clindamycin) are more likely. For this reason antimicrobial regimens used in the treatment of postcesarean endometritis should provide satisfactory coverage of penicillin-resistant anaerobic microorganisms (e.g., *P. bivia*). The carbapenems (imipenem-cilastatin, meropenem, ertapenem) have also proved effective in the treatment of these infections but are generally reserved for more resistant infections not usually found on an obstetric service.

Parenteral therapy should be continued until the patient's temperature has remained lower than 37.8°C (100°F) for 24 hours, the patient is pain free, and the leukocyte count is normalizing. The use of oral antibiotics after discharge has been shown to be unnecessary.[30] Women with late-onset PPE can be treated as outpatients with oral azithromycin or doxycycline therapy with or without metronidazole, depending on whether they have coexistent bacterial vaginosis. It is recommended if this is the chosen treatment route that timely clinical follow-up does occur to assure improvement and/or resolution. Doxycycline should be avoided in nursing mothers.

Early-onset PPE should respond to parenteral antimicrobial therapy within 48 hours, with the patient becoming afebrile within 96 hours. Failure to accomplish this goal frequently suggests the presence of an abdominal wound infection (or an alternative source), which occurs in 50% of these patients.[31] Because cephalosporin antibiotic prophylaxis is commonly administered to women undergoing cesarean delivery, enterococcal superinfection is another common explanation for failure to respond or relapse after treatment with regimens that are not effective against enterococci (e.g., extended-spectrum cephalosporins, clindamycin plus gentamicin). This is particularly true if the organism is isolated in pure culture or from heavy growth from an endometrial specimen. If an enterococcal superinfection is suspected, one of the following regimens should be used: (1) clindamycin or metronidazole plus ampicillin plus gentamicin, (2) ampicillin-sulbactam plus gentamicin; (3) cefoxitin or cefotetan plus ampicillin, (4) ticarcillin–clavulanic acid, or (5) piperacillin-tazobactam. Uncommonly, failure results from lack of coverage of a drug-resistant anaerobe; this can be corrected by a regimen containing either metronidazole or clindamycin. The importance of endometrial cultures for aerobes, anaerobes, and mycoplasmas will increase as antimicrobial resistance to clindamycin grows among isolates of gram-negative anaerobes.[32]

If fever persists despite apparently appropriate antimicrobial therapy, the differential diagnosis includes a wound or pelvic abscess, refractory postpartum fever, and noninfectious fever (e.g., drug fever, breast engorgement). Careful physical examination is the most important to distinguish between pelvic and nonpelvic causes of fever. Appropriate imaging studies, usually pelvic ultrasonography or computed tomography (CT), can confirm the presence of a pelvic hematoma or abscess, usually involving the space between the lower uterine segment and bladder. If present, percutaneous drainage by interventional radiology can be considered, and occasionally open surgery is required for complete management and resolution.

PPE caused by group A β-hemolytic streptococci has special epidemiologic significance.[33–35] The Centers for Disease Control and Prevention (CDC) recommends that health care worker screening be undertaken when two episodes of postpartum group A streptococcal infection are identified within a 6-month period. All health care workers present at the delivery and those who performed vaginal examinations before delivery should be screened with cultures of the nares, throat, vagina, rectum, and skin. Any health care worker who is culture positive for group A *Streptococcus* should refrain from patient care for the first 24 hours of antimicrobial therapy. If surveillance identifies additional patients or health care workers with positive cultures for group A *Streptococcus,* the isolates should be typed by sequencing the variable portion of the M-protein gene or other molecular methods to identify the strain.

Refractory Postpartum Fever of Undetermined Origin

Traditionally, the diagnosis of septic pelvic thrombophlebitis (SPT) was entertained in those patients with PPE refractory to broad-spectrum antimicrobial therapy. Once alternative explanations (e.g., wound infection, pelvic abscess, urinary tract infections, etc.) for persistent fever were ruled out, heparin therapy was used to determine whether the diagnosis was SPT or drug fever. Women with SPT responded promptly to therapeutic anticoagulation with heparin and became afebrile within 24 to 48 hours.[36] More recent analysis of this practice has made this therapeutic approach suspect.

Brown and colleagues[37] studied women who had pelvic infection and fever that persisted after 5 days despite adequate antimicrobial therapy with clindamycin, gentamicin, and ampicillin. A diagnosis of SPT was made after CT evaluation revealed uterine vein thrombosis. Women were randomly assigned to continuation of antimicrobial therapy, either alone or with the addition of heparin, until the temperature was lower than 37.5°C [99.5°F] for 48 hours. SPT (1:3000 deliveries) was diagnosed in 22% of women with prolonged infection. There was no significant difference between the responses of women with pelvic infection who were and were not given heparin therapy; in both groups, women required an average of 6 days of therapy before becoming afebrile. These results do not support the common empirical practice of heparin treatment for women with persistent postpartum infection.

Similarly, for an 8-year period (1986–94), Witlin and colleagues[38] reviewed the medical records of 31 postpartum women with the diagnosis of SPT. All of the patients demonstrated refractory febrile morbidity (mean, 5.5 ± 1.9 days before institution of heparin therapy) despite antimicrobial therapy with ampicillin, gentamicin, and clindamycin. Imaging studies (CT, ultrasonography, or both) revealed no pelvic pathologic process. The patients required an average of 4.7 ± 2.1 days (median, 5 days; range, 1–9 days) of heparin therapy before defervescence. Heparin levels were therapeutic at a mean of less than 24 hours (range, 6–24 hours). The authors suggested that currently available imaging studies cannot diagnose the entity we now define as SPT. In addition, their findings do not support the time-honored rule that SPT responds within 24 to 48 hours to therapeutic anticoagulation with heparin.

Taken together, these studies suggest that patients with refractory postpartum fever require more time to resolve their persistent fever than their more responsive counterparts and that anticoagulation does not speed this process along. Antibiotics should be discontinued in patients with persistent fever who look well, have a normal pelvic examination, and have no leukocytosis, considering the diagnosis of drug fever. More prolonged antibiotic therapy (5–6 days) with or without adjunct heparin therapy should be anticipated in those patients with persistent fever, continued pelvic organ tenderness, and persistent leukocytosis.[39]

Infections After Perineal Lacerations

Infection of the episiotomy site is an uncommon occurrence. Overall, only 0.1% of episiotomies become infected, although this rate increases to 1% to 2% for episiotomies complicated by third- or fourth-degree extensions. *Routine episiotomy is no longer recommended in obstetrics.* Modern obstetrics therefore deals with spontaneous perineal lacerations after vaginal delivery. A recent report suggests that women with third- and fourth-degree perineal tears have a lower rate of perineal wound complications if they receive antibiotic prophylaxis with cefoxitin or cefotetan at the time of repair.[40]

Shy and Eschenbach[41] classified episiotomy infections into four categories, depending on the depth of infection. The simple episiotomy infection is a local infection that is limited to the skin and superficial fascia along the episiotomy incision. In contrast to deeper infection, the associated skin changes of edema and erythema occur only adjacent to the episiotomy. The simple episiotomy infection may initially be treated with broad-spectrum antibiotics with activity against streptococci, staphylococci, Enterobacteriaceae, and anaerobes, including *Bacteroides fragilis.* If a therapeutic response is not prompt, the wound should be opened, explored, and débrided under adequate anesthesia to exclude hematoma or previously unrecognized rectovaginal communication. Ramin and colleagues[42] showed that with proper preoperative care, simple episiotomy infection is not a contraindication to early repair of dehiscence.

Infection of the two layers of the superficial fascia may occur. The more common is superficial fascia infection without necrosis, the clinical

presentation of which is neither striking nor distinctive. The skin may be erythematous and edematous, but severe systemic manifestations do not occur. If a response to broad-spectrum antibiotic therapy does not occur in 24 to 48 hours or if the clinical condition worsens during antibiotic therapy, then the episiotomy should be surgically explored.

Infection of the superficial fascia with necrosis, most commonly referred to as necrotizing fasciitis, is an uncommon yet severe infection of the subcutaneous tissues (i.e., the superficial fascia) that spreads in the fascial clefts overlying the deep fascia. The deep fascia usually, but not always, is spared; skin involvement results only secondarily, after the nutrient vessels to the skin thrombose. Because the skin is not primarily involved, the episiotomy wound may appear normal, making early recognition difficult and causing fatal delay in treatment. Despite the minimal local findings, patients may appear severely ill, with marked local pain, high fever, and prominent systemic manifestations. Most patients are diabetic. A definitive diagnosis is made at surgery, with the discovery of extensive undermining of surrounding tissues and lack of resistance in the superficial fascial plane to probing with a blunt instrument. Treatment includes broad-spectrum antibiotics (e.g., clindamycin plus ampicillin plus gentamicin) and radical débridement to include removal of all necrotic and pale tissue. It should be emphasized that treatment of this disease is primarily surgical and that less than aggressive resection of all involved tissues will lead to therapeutic failure (see Chapter 93).

In the extremely rare event of infection beneath the deep fascia, muscle may be involved, resulting in another type of episiotomy infection—myonecrosis. Myonecrosis is commonly caused by *Clostridium perfringens,* although it can occur from a neglected necrotizing fasciitis infection that invades the deep fascia and should be considered as a medical emergency. Myonecrosis of the subgluteal muscles surrounding the hip joint or the psoas muscles can also occur from bacteria introduced into this deep space by a paracervical or pudendal needle during the administration of anesthesia. These patients experience severe hip pain associated with marked limitation of motion.

Both myonecrosis and clostridial infection should be treated with surgical resection and antibiotic therapy. For clostridial infection, high-dose penicillin is the therapy of choice. Radical wide excision may be necessary. Hyperbaric oxygen therapy is at best an adjunctive measure to surgical débridement. Polyvalent gas gangrene antitoxin is probably ineffective.

Soper[43] reported an unusually severe form of clostridial myonecrosis arising from an episiotomy caused by *Clostridium sordellii.* The patient had a distinctive course characterized by the sudden onset of severe and unrelenting hypotension associated with marked, generalized tissue edema and third spacing, with increased hematocrit, marked leukemoid reaction, absence of rash or fever, and a rapidly fatal course. This *C. sordellii* toxic shock syndrome was subsequently reported in association with retention of a vaginal pack, degeneration of a cervical myoma, and postpartum endometritis.[44]

Postabortal Infections

Infection after abortion is an ascending process that occurs most commonly in the presence of retained products of conception or operative trauma. Risk factors include greater duration of pregnancy, technical difficulties, and the unsuspected presence of sexually transmitted pathogens or bacterial vaginosis.

Symptoms include fever, chills, abdominal pain, and vaginal bleeding, often with the passage of placental tissue. Postabortal infection typically has its onset within 4 days after the procedure.

Physical findings include an elevated temperature, tachycardia, tachypnea, and abdominal tenderness. In the presence of bacteremia, hypotension and frank shock may occur, and the patient may be agitated or disoriented. Pelvic examination reveals a sanguinopurulent discharge and uterine tenderness, with or without adnexal and parametrial tenderness. It is important to look for cervical or vaginal lacerations, especially with a suspected illegal abortion. Transvaginal ultrasonography can assess the intrauterine cavity for the presence of retained products of conception, suggesting the need for uterine curettage.

Septic abortion caused by *C. perfringens* has a characteristic clinical presentation and can be rapidly fatal. In severe cases massive intravascular hemolysis produces jaundice, mahogany-colored urine, and catastrophic anemia.

Laboratory evaluation of patients with more than early uncomplicated postabortal endometritis should include a complete blood cell count, urinalysis, and culture of the endometrial cavity with an endometrial suction curet; blood cultures; and CT of the abdomen and pelvis and upright chest radiographs looking for a foreign body or intrauterine gas. Simple endometritis, defined as low-grade fever associated with mild uterine tenderness after uncomplicated elective abortion, can be treated with combined parenteral and oral regimens recommended for the treatment of PID (Table 109.1). Patients with established infection, as indicated by fever higher than 38°C (100.4°F), pelvic peritonitis, or tachycardia, should be hospitalized for parenteral antibiotic therapy (Table 109.2) and prompt uterine evacuation if retained products of conception are suspected. Stubblefield and Grimes[45] reviewed the management of these cases.

Surgical removal of any residual infected tissue is essential in all but the mildest of postabortal infections. Pelvic ultrasonography can be used to confirm the presence of retained tissue. In most cases prompt

TABLE 109.1 Recommended Parenteral Regimens

Cefotetan, 2 g IV every 12 h
plus
Doxycycline, 100 mg PO or IV every 12 h
or
Cefoxitin, 2 g IV every 6 h
plus
Doxycycline, 100 mg PO or IV every 12 h
or
Clindamycin, 900 mg IV every 8 h
plus
Gentamicin loading dose, IV or IM (2 mg/kg), followed by a maintenance dose (1.5 mg/kg) every 8. Single daily dosing (3–5 mg/kg) can be substituted.

IM, Intramuscular; *IV,* intravenous; *PO,* oral.
From Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64:1–140.

TABLE 109.2 Intravenous Antibiotic Regimens for Treating Gynecologic Postoperative Infections

Localized Infection With Minimal Systemic Findings

Cefotaxime, 1 g q8h
Cefotetan, 2 g q12h
Cefoxitin, 2 g q6h
Ceftriaxone, 2 g, then 1 g q24h
Ampicillin-sulbactam, 3 g q6h
Ticarcillin-clavulanic acid, 3.1 g q4–6h
Piperacillin-tazobactam, 3.375 g q6h

Extensive Infection With Moderate-to-Severe Systemic Findings

Clindamycin, 900 mg IV q8h
plus
Gentamicin, 2 mg/kg IV, then 1.5 mg/kg q8h (or single daily dose 5 mg/kg)
with or without
Ampicillin, 2 g IV, then 1 g IV q4h
or
Ampicillin, 2 g IV, then 1 g IV q4h
plus
Gentamicin, 2 mg/kg IV, then 1.5 mg/kg q8h (or single daily dose 5 mg/kg)
plus
Metronidazole, 500 mg IV q8h
or
Imipenem-cilastatin, 500–1000 mg IV q6h
or
Meropenem, 1 g IV q8h
or
Ertapenem, 1 g IV q 24h
or
Levofloxacin, 500 mg IV q24h
plus
Metronidazole, 500 mg IV q8h

IV, Intravenous.
From Larsen JW, Hager WD, Livengood CH, et al. Guidelines for the diagnosis, treatment and prevention of postoperative infections. Infect Dis Obstet Gynecol. *2003;11:65–70.*

curettage controls the infection. If the uterus is too large to allow suction curettage, oxytocin or vaginal misoprostol administration is often successful. Concurrent laparoscopy may be needed for curettage of a uterus that was perforated at the time of abortion.

Indications for laparotomy and possible hysterectomy include failure to respond to uterine evacuation and appropriate medical therapy, perforation and infection with suspected bowel injury, pelvic and adnexal abscess, and clostridial necrotizing myonecrosis (gas gangrene). Isolation of *C. perfringens* alone does not mandate hysterectomy. Initial treatment should include high-dose penicillin (5 million units) or ampicillin-sulbactam (3 g) intravenously every 6 hours, curettage, supportive therapy, and intensive cardiovascular monitoring. Laparotomy is indicated, and hysterectomy should be considered if there is deterioration or no response.

Avoidance of unwanted pregnancies by making contraceptives available is the most important preventive measure. Screening for sexually transmitted diseases and bacterial vaginosis before performance of elective abortion is optimal but often impractical. A meta-analysis revealed a substantial protective effect of antibiotic prophylaxis in all subgroups of women undergoing induced abortion, even women in low-risk groups. Routine use of periabortal antibiotics, such as doxycycline, in the United States may prevent up to half of all cases of postabortal infections.[46,47]

Medical abortion with mifepristone (a progesterone antagonist) and misoprostol (a prostaglandin) is commonly used for elective first-trimester terminations. There have now been five reported cases of *C. sordellii* toxic shock syndrome associated with medical abortion. This syndrome is characterized by the rapid onset of profound hypotension, hypothermia, a marked leukemoid reaction, and hemoconcentration. Infection with *C. sordellii* in these cases was associated with lethal toxin production (*C. sordellii* cytotoxin L), thus explaining why the syndrome is uniformly fatal.[48]

SURGICAL SITE INFECTION AFTER GYNECOLOGIC SURGERY

The identification of SSI involves interpretation of clinical and laboratory findings, and it is crucial that surveillance programs use definitions that are consistent and standardized. The SSIs (organ or space) associated with hysterectomy are cuff cellulitis, pelvic cellulitis, and pelvic abscess.

Pathogenesis

Microbial contamination of the surgical site is a necessary precursor of SSI. The risk for SSI can be conceptualized according to the following relationship[49]:

Dose of bacterial contamination × Virulence ÷ Resistance of host patient = Risk for SSI

Quantitatively, it has been shown that if a surgical site is contaminated with more than 10^5 microorganisms per gram of tissue, the risk for SSI is markedly increased.[50] However, the dose of contaminating microorganisms required to produce infection may be much lower if foreign material is present at the site.[51]

Hysterectomy can be performed as a total vaginal hysterectomy, total abdominal hysterectomy, or laparoscopically assisted vaginal hysterectomy. The latter may also be robotically assisted. For SSIs that occur after hysterectomy performed by any method, the source of pathogens is the endogenous microbiota of the vagina. The normal vaginal microbiota consists of lactobacilli, various species of streptococci, *G. vaginalis,* strains of Enterobacteriaceae, and anaerobes. Although anaerobes predominate numerically (10:1), the concentration of lactobacilli in normal women surpasses that of the anaerobic bacteria by a factor of 100 to 1000.[52] Lactobacilli produce both hydrogen peroxide and lactic acid, which play crucial roles in protecting against the overgrowth of pathogens in the vagina. Pathogenic microorganisms, such as group B streptococci, *E. coli,* and *G. vaginalis,* and anaerobes, such as *P. bivia,* are present in increased concentrations in women without these protective microbiota or equivalent lactic acid–producing taxa (e.g., those with bacterial vaginosis).

Other factors alter the vaginal microbiota and may indirectly predispose to postoperative infection. Ohm and Galask[53,54] showed that *E. coli, Klebsiella, Proteus, Enterobacter, B. fragilis,* and enterococci are more common in 5-day postoperative vaginal cultures after abdominal or vaginal hysterectomy than in preoperative cultures from the same patients. Although several studies have documented increased enterococcal colonization after perioperative cephalosporin prophylaxis, other factors must be involved because placebo-treated groups show increased enterococcal colonization as well.

Once the vaginal epithelium is breached during excision of the cervix, microorganisms gain entry to the vaginal cuff, paravaginal tissues, and peritoneal cavity. The animal model of intraabdominal infection devised by Weinstein and colleagues[55] clarified the distinctive roles played by different bacteria in the natural history of pelvic infection. These investigators documented a biphasic response to infection consisting of an initial phase characterized by peritonitis and sepsis, in which gram-negative aerobic bacteria predominate (peritonitis stage), and a secondary phase with abscess formation, in which anaerobes predominate (abscess stage) (Fig. 109.2).

Risk Factors

Several factors, some of which are beyond the surgeon's control, influence the likelihood of the development of a postoperative infection. Febrile morbidity is more common after abdominal than after vaginal hysterectomy.[56] The incidence of postoperative infection is higher in patients of lower socioeconomic status, regardless of surgical approach. Age has inconsistently been shown to be a risk factor after hysterectomy, with premenopausal women shown to be at increased risk in some studies, especially after vaginal hysterectomy. Duration of surgery is directly correlated with postoperative infection rates.[57]

Bacterial vaginosis has been associated with an increased risk for infection after hysterectomy.[58] Available data suggest it may be prudent for patients scheduled for elective hysterectomy to undergo screening for bacterial vaginosis before the planned procedure. Those found to have bacterial vaginosis should be treated preoperatively or with metronidazole perioperatively for at least 4 days.[59]

Cuff Cellulitis

An inflammatory response at the margins of the vaginal cuff incision is a normal part of the healing process in the early posthysterectomy period. Host defense mechanisms quickly resolve this cellulitis in most patients without the need for antibiotic administration. Hemsell[60] observed that 17% of women undergoing vaginal hysterectomy and 35% of those undergoing abdominal hysterectomy had recurrent temperature elevations on the second or third postoperative day despite normal abdominal and pelvic examinations and no pain. These patients become afebrile without therapy. In a small number of cases, however, cuff cellulitis after hysterectomy requires antibiotic therapy. These women usually present within 10 days after surgery complaining of

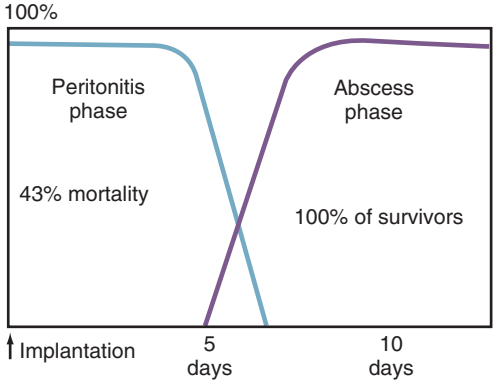

FIG. 109.2 Biphasic pathophysiology of polymicrobial pelvic infection. *(From Weinstein WM, Onderdonk AB, Bartlett JG, et al. Experimental intraabdominal abscesses in rats: development of an experimental model.* Infect Immun. *1974;10:250–1255.)*

increasing central lower abdominal and pelvic pain, increased vaginal discharge, and low-grade fever. Abdominal examination is normal or elicits only slight suprapubic tenderness to deep palpation. On bimanual examination only the vaginal surgical margin is tender, and no masses are palpable. Women with cuff cellulitis have been treated successfully as outpatients with oral antibiotics, but it is important to choose an antibiotic regimen that includes coverage for anaerobic microorganisms. Examples of such regimens include (1) amoxicillin–clavulanic acid or (2) the combination of a first-generation cephalosporin, a quinolone, or trimethoprim-sulfamethoxazole with metronidazole. Patients should monitor their temperatures at home, and clinical reevaluation should be considered if improvement in pain and temperature is not noted by 72 hours.

A well-localized collection of pus just above the vaginal cuff (vaginal cuff abscess) develops in a few patients with cuff cellulitis. In addition to fever, these women may report a sense of fullness in the lower abdomen. Bimanual pelvic examination confirms the presence of a vaginal cuff mass, and ultrasonography can confirm the presence of an abscess. Vaginal drainage facilitates cure and may be accomplished simply by dilation of the vaginal cuff in a treatment room. Some patients with larger collections may benefit from drainage under ultrasonographic or CT guidance by an interventional radiologist or an operative drainage procedure. Any purulent material recovered should be cultured for aerobic and anaerobic pathogens.[61]

Broad-spectrum parenteral antibiotics (see Table 109.2) covering both aerobic and anaerobic bacteria should be given until the patient has been afebrile for 24 to 36 hours. Many experts suggest initiating antibiotic therapy with a single broad-spectrum agent, whereas others recommend combination therapy (e.g., clindamycin plus gentamicin), even for clinically milder infections.[62]

Pelvic Abscess

The most serious late postoperative complication is a pelvic abscess. These abscesses may involve one or both residual adnexa (tubo-ovarian abscess) if the fallopian tubes and ovaries were not removed at surgery.

These infections are rare, occur almost exclusively in premenopausal women, occur despite the use of prophylactic antibiotics, and often have a latent period of many weeks between surgery and onset of symptoms.[63]

Patients may have had no apparent infection during their initial hospitalization, or, alternatively, they may have appeared to respond rapidly to the initial selection of antibiotics for presumed pelvic cellulitis, only to experience relapse after discharge. Typically, the fever curve is characterized by a high spike late in the afternoon or early evening. The leukocyte count is often in the range of 20,000/mm^3, and the erythrocyte sedimentation rate is markedly increased. Patients with postoperative adnexal abscess often have a palpable mass high in the pelvis.[64] Ultrasonography and CT both confirm the presence of a mass and help to determine whether it is loculated, related to an intraperitoneal structure, or drainable percutaneously. Because their location is removed from the vaginal cuff, pelvic/adnexal abscesses cannot be drained through the vaginal cuff unless they extend to the cul-de-sac.

Identification of a postoperative pelvic abscess does not mandate immediate drainage if it is inaccessible because antibiotic therapy alone may be successful in the treatment of this complication. The frequent isolation of a β-lactamase–producing *Prevotella* spp. from these abscesses warrants the use of clindamycin, metronidazole, or other agents effective against gram-negative anaerobes. A regimen of clindamycin plus gentamicin is frequently used. For patients whose infection fails to respond to appropriate antibiotic therapy, drainage is necessary. Ledger and colleagues[65] showed that most such antibiotic failures are caused not by antimicrobial resistance but by the unique environment of the abscess, which inhibits antibiotic effectiveness. Necrosis associated with these types of infections makes surgical exploration necessary in some cases.

If the abscess is located in the posterior cul-de-sac, drainage via colpotomy can be attempted under ultrasonographic guidance. The abscess cavity should be completely evacuated and a drain placed to prevent reaccumulation of fluid. Abscesses not located in the cul-de-sac may be drained percutaneously if they are located adjacent to the abdominal wall or are determined to be accessible by CT. A pigtail or equivalent catheter should remain in place until drainage ceases, usually 4 to 8 days.[66,67] Patients with suspected postoperative pelvic abscess who fail to respond to antibiotic therapy and cannot undergo drainage by one of the previously mentioned techniques require operative laparoscopy or laparotomy. In most cases the adnexa are involved and must be removed. Purulent material or tissue must be submitted for both aerobic and anaerobic culture.

Parenteral antibiotics should be administered until the patient has remained afebrile for 48 to 72 hours, the leukocyte count is normal, and the signs and symptoms have resolved. Most clinicians choose to treat these patients for 7 days after discharge with oral agents, such as amoxicillin-clavulanate or metronidazole. All patients should be reexamined 2 weeks after discharge to ensure that recurrence or reaccumulation of the abscess has not occurred.[68]

Osteomyelitis Pubis

Osteitis pubis has been described as a noninfectious, self-limited inflammatory condition of the symphysis pubis associated with retropubic urologic procedures.[69] It is now recognized that this condition is actually an osteomyelitis of the pubis. It is a rare infection that results from direct inoculation of the bone at the time of surgery or extension of a contiguous focus of infection. Most cases in women occur after urethral suspension or, less commonly, after radical vulvectomy or pelvic exenteration. The diagnosis is based on typical symptoms of suprapubic discomfort, difficulty with ambulation and a wide-based waddling walk, and changes on radiography or magnetic resonance imaging showing irregular bony margins and rarefaction and widening of the symphyseal joint spaces. Wound drainage, low-grade fever, moderate leukocytosis, and an increased erythrocyte sedimentation rate or alkaline phosphatase level may be present.[70]

Patients for whom clinical and radiologic findings are suggestive of this diagnosis should undergo a needle bone biopsy guided by CT.[71] Specimens should be submitted for histopathologic examination and aerobic and anaerobic culture. If pathogenic microorganisms are recovered and if the interval between onset of symptoms and diagnosis is short, a trial of antimicrobial therapy may be attempted. Patients with a poor response to this management should undergo débridement. If pathogenic microorganisms are not isolated from a needle aspirate, open surgical biopsy with débridement should be undertaken, and specimens of bone or purulent material should be cultured. Common isolates include aerobic gram-negative bacteria and staphylococcal and streptococcal species. After débridement directed antimicrobial therapy should be administered for at least 4 weeks.[72]

Sacral osteomyelitis as a complication of abdominal sacral colpopexy has been reported.[73]

PELVIC INFLAMMATORY DISEASE

PID refers to the clinical syndrome that represents a continuum of inflammation from the cervix to the endometrium, fallopian tubes, and contiguous pelvic structures: cervicitis, endometritis, salpingitis, pelvic peritonitis, and tubo-ovarian abscess.[74,75] Each year, approximately 1 million women in the United States experience an episode of symptomatic PID. Many women with PID have minimal or no symptoms.[76]

PID results from direct canalicular spread of microorganisms from the vagina or endocervix to the endometrium and fallopian tube mucosa.[77] Both *Neisseria gonorrhoeae* and *C. trachomatis* commonly cause endocervitis, and clinical symptoms of acute PID develop in 10% to 40% of women with these infections who do not receive adequate treatment.[78] In addition to *N. gonorrhoeae* and *C. trachomatis,* a wide variety of bacteria have been isolated from the upper genital tracts of women with acute, symptomatic PID, including anaerobes, gram-negative rods, streptococci, and mycoplasmas.[79,80] Many of these are the same microorganisms that are found in increased concentrations in the vagina of women with bacterial vaginosis.[81] Moreover, approximately one of every four women with presumed uncomplicated lower genital tract gonococcal or chlamydial infection is found to have histologic endometritis (subclinical PID) when evaluated by endometrial biopsy.[77] Uncommonly, respiratory pathogens, including *Haemophilus influenzae, Streptococcus pyogenes*, and *Histoplasma capsulatum* have also been isolated from the upper genital tracts of women with symptomatic PID.[82–84]

Risk Factors

Age is inversely related to the rate of PID. Sexually experienced teenagers are three times more likely to be diagnosed with PID than are women 25 to 29 years of age. A history of multiple sexual partners, an increased rate of acquisition of new partners within the previous 30 days, and frequent sexual intercourse with a single partner are all associated with an increased risk for PID.[85] Women with confirmed PID commonly have concurrent bacterial vaginosis.[86] Contraceptive choice modifies PID risk in a complex manner. Mechanical and chemical barriers decrease risk. Oral contraceptives have a variable effect, decreasing the risk for a clinical diagnosis of PID but having no effect on the rate of infertility or endometrial inflammation. Intrauterine contraceptive devices (IUDs) confer a slightly increased risk for non–sexually transmitted PID in the first month after insertion.[87] Other suggested associations with PID include douching, menses, cigarette smoking, and substance abuse.[88,89]

The improved IUDs—the levonorgestrel intrauterine system and the copper intrauterine device—are rarely associated with infection. Contamination of the endometrial cavity at insertion apparently results in a slightly increased risk for acute PID that is limited to the first 4 months of IUD use. Infections occurring after 4 months are believed to be the result of acquired sexually transmitted pathogens and not the IUD itself.[90]

A unique role for *Actinomyces* organisms in IUD-associated PID has been suggested, but this relationship remains unclear. Although as many as 4% to 8% of IUD users have *Actinomyces*-like organisms identified on Papanicolaou (Pap) smear, their presence has not been equated with pelvic actinomycosis, nor has the risk for subsequent pelvic infection been quantified.[91] In patients with cytology showing *Actinomyces* colonization, Lippes[92] showed that removal of the IUD was not necessary.

Diagnosis

The clinical diagnosis of acute PID is imprecise. Data indicate that a clinical diagnosis of symptomatic PID has a positive predictive value of 65% to 90% compared with laparoscopy. The positive predictive value of a clinical diagnosis of acute PID varies depending on epidemiologic characteristics and the clinical setting, with a higher positive predictive value among sexually active young women (particularly adolescents), among patients attending sexually transmitted diseases clinics, and in settings in which the rates of gonorrhea, chlamydia, and bacterial vaginosis are high.

Many episodes of PID go unrecognized. Although some cases are asymptomatic, others are underdiagnosed because the patient or the health care provider fails to recognize the implications of mild or nonspecific symptoms or signs (e.g., abnormal bleeding, dyspareunia, vaginal discharge). In one study chlamydial infection was noted in 29% of women experiencing persistent intermenstrual bleeding while taking oral contraceptives, suggesting the presence of endometritis.[93] Given the often subtle presentation of this disease and the significant reproductive sequelae associated with it (infertility, ectopic pregnancy, chronic pelvic pain), clinicians should maintain a low threshold for the diagnosis of PID.

Empirical treatment for PID should be considered in sexually active young women and other women at risk for sexually transmitted infections if the following minimum criteria are met and no other cause for the illness can be identified: (1) pelvic organ tenderness noted on bimanual examination with or without manipulation of the cervix and (2) microscopy showing the presence of WBCs in the vaginal secretions. Most women with PID have either mucopurulent cervical discharge or evidence of WBCs on a microscopic evaluation of the vaginal secretions (Fig. 109.3). If cervical discharge appears normal and no WBCs are found during microscopy, the diagnosis of PID is unlikely and alternative causes of pain should be investigated.[94] Additional criteria that support a diagnosis of PID include bacterial vaginosis, mucopurulent cervicitis, laboratory documentation of cervical infection with *N. gonorrhoeae* or *C. trachomatis,* oral temperature higher than 38°C (100.4°F), and elevated erythrocyte sedimentation rate or C-reactive protein level. Definitive criteria for PID include histologic evidence of endometritis on endometrial biopsy; transvaginal ultrasonography or other imaging techniques showing thickened, fluid-filled tubes with or without free pelvic fluid or tubo-ovarian complex; and laparoscopic abnormalities showing tubal purulent exudate, erythema, and edema (Fig. 109.4).

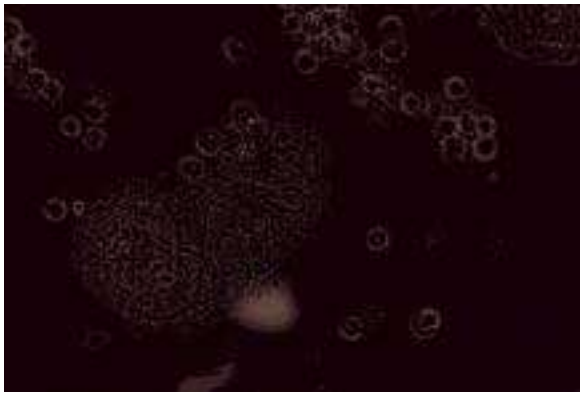

FIG. 109.3 Microscopy of a wet mount of the vaginal secretions reveals clue cells and inflammatory cells in a patient with pelvic inflammatory disease (×400).

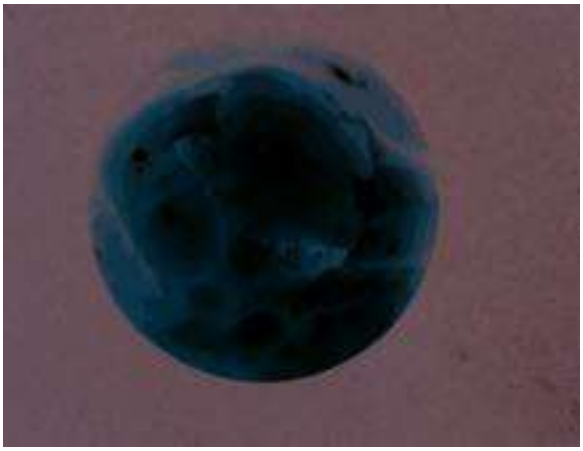

FIG. 109.4 Laparoscopic confirmation of moderate acute salpingitis showing patchy fibrin deposits on the serosal surfaces of the fallopian tubes. *(From Soper DE. Cervicitis and endometritis. In: Mandell GL, Sobel JD, eds.* Atlas of Infectious Diseases. *Vol. 9. Philadelphia: Churchill Livingstone; 1997:810.)*

Clinical diagnosis and grading of PID have poor specificity. In fact, women with PID associated with moderate-to-severe pelvic adhesions or tubal occlusion were found to have less tenderness on abdominopelvic examination and therefore to appear less ill than women with limited or no adhesions.[95] Diagnostic laparoscopy should be considered in patients for whom empirical therapy has failed and in patients with a history of recurrent PID and negative tests for chlamydia, gonorrhea, and bacterial vaginosis. Endometriosis is a common alternative diagnosis in these women.

Although rare, acute salpingitis can occur in the proximal stump of patients who have undergone surgical sterilization and in women in the first trimester of pregnancy.

Management of Acute Pelvic Inflammatory Disease

Most women with PID are treated as outpatients, reflecting the preponderance of patients with only mild-to-moderate symptoms and signs. A prospective, randomized clinical trial compared outpatient treatment with a single dose of cefoxitin intramuscularly and multidose oral doxycycline with inpatient treatment with intravenous cefoxitin and doxycycline in women with clinical symptoms and signs of mild-to-moderate PID. There were no differences in response to therapy or reproductive outcome between inpatient and outpatient regimens.[96] These data suggest that

hospitalization can be reserved for those patients with clinically severe disease (severe illness, nausea and vomiting, or high fever), those unable to follow or tolerate an outpatient oral regimen, pregnant women, and women with a clinical diagnosis of tubo-ovarian abscess.

Treatment consists of pelvic rest and antibiotics. Antibiotic regimens must provide empirical, broad-spectrum coverage of likely pathogens, including *N. gonorrhoeae, C. trachomatis,* anaerobes, gram-negative facultative bacteria, and streptococci. Several antimicrobial regimens have been effective in achieving clinical and microbiologic cure in randomized clinical trials with short-term follow-up. The need to eradicate anaerobes from women with PID has not been determined definitively. However, anaerobic bacteria associated with bacterial vaginosis have been isolated from the upper reproductive tract of women with PID, and these bacteria have been shown to cause tubal and epithelial destruction. One method of determining the appropriateness of metronidazole therapy in women with PID is to determine the presence of concurrent bacterial vaginosis. Metronidazole should be prescribed for women with the clinical diagnosis of PID and concurrent bacterial vaginosis.

The fluoroquinolones alone are no longer recommended in the treatment of PID due to the emergence of quinolone-resistant *N. gonorrhoeae*. The CDC has updated the published antibiotic treatment guidelines for acute PID (see Table 109.1, Table 109.3).[97] If parenteral cephalosporin therapy is not feasible, use of fluoroquinolones (levofloxacin, 500 mg PO once daily, or ofloxacin, 400 mg twice daily for 14 days) usually with metronidazole (500 mg PO twice daily for 14 days) may be considered if the community prevalence and individual risk for gonorrhea is low. Tests for gonorrhea must be performed before instituting therapy. If the nucleic acid amplification test result is positive for gonorrhea, a parenteral cephalosporin is recommended. If culture for gonorrhea is positive, treatment should be based on results of antimicrobial susceptibility. If the isolate is quinolone-resistant *N. gonorrhoeae* or if antimicrobial susceptibility cannot be assessed, parenteral cephalosporin is recommended, and/or consultation with an infectious disease expert is indicated in case of cephalosporin allergy. Although information regarding other outpatient regimens is limited, amoxicillin-clavulanic acid and doxycycline or azithromycin with metronidazole have demonstrated short-term clinical cure.[98]

Optimal outpatient management includes a follow-up examination performed within 72 hours after the initiation of therapy. Many patients may not return for this visit if they are symptomatically improved. Substantial clinical improvement with lysis of fever, reduction in direct or rebound abdominal tenderness, and reduction in pelvic organ tenderness with bimanual examination should be noted. If there is no response to therapy within 72 hours, patients should be reevaluated and possibly hospitalized for a potential diagnostic/therapeutic laparoscopy to confirm the diagnosis and for consideration of parenteral antibiotic therapy if they are on an oral regimen.

All male sex partners of women with acute PID should be evaluated for sexually transmitted diseases, and those who had sexual contact with the patient during the 60 days preceding the onset of symptoms in the patient should be empirically treated with regimens effective against *C. trachomatis* and *N. gonorrhoeae*. In many circumstances the male sex partner tests positive for chlamydia or gonorrhea, but the patient receiving therapy is negative, suggesting a sexually transmitted etiology and a false-negative test in the woman with PID.[99]

TABLE 109.3 Recommended Intramuscular/ Oral Regimens

Ceftriaxone, 250 mg IM in a single dose
plus
Doxycycline, 100 mg PO twice a day for 14 days
with[a] *or without*
Metronidazole, 500 mg PO twice a day for 14 days
or
Cefoxitin, 2 g IM in a single dose and Probenecid, 1 g PO administered concurrently in a single dose
plus
Doxycycline, 100 mg PO twice a day for 14 days
with or without
Metronidazole, 500 mg PO twice a day for 14 days
or
Other parenteral third-generation cephalosporin (e.g., ceftizoxime or cefotaxime)
plus
Doxycycline, 100 mg PO twice a day for 14 days
with[a] *or without*
Metronidazole, 500 mg PO twice a day for 14 days

[a]The recommended third-generation cephalosporins are limited in the coverage of anaerobes. Therefore, until it is known that extended anaerobic coverage is not important for treatment of acute PID, the addition of metronidazole to treatment regiments with third-generation cephalosporins should be considered.
IM, Intramuscular; *PID,* pelvic inflammatory disease; *PO,* oral.
From Walker CK, Wiesenfeld HC. Antibiotic therapy for acute pelvic inflammatory disease: the 2006 CDC Sexually Transmitted Diseases Treatment Guidelines. Clin Infect Dis. *2007;28(suppl 1):S29–S36.*

Management of Suspected Tubo-ovarian Abscess

Patients with suspected tubo-ovarian abscess should be hospitalized and given broad-spectrum antimicrobial drugs that include adequate coverage for gram-negative anaerobes. Failure to respond to medical therapy is suggested by a lack of defervescence within 72 hours or an increase in the size of the mass. Eighty-five percent of abscesses with a diameter of 4 to 6 cm respond to antibiotics alone, but only 40% of those 10 cm or larger respond.[100] Triple-agent therapy with ampicillin, clindamycin, and gentamicin would seem to be the regimen of choice, although other combination regimens have been used effectively.[100,101]

Surgical intervention for a tubo-ovarian abscess that does not respond to antimicrobial therapy can be performed laparoscopically, percutaneously, or transvaginally or by laparotomy. A patient with a suspected leaking or ruptured abscess should undergo immediate surgical exploration after rapid stabilization and institution of broad-spectrum antibiotics due to a nonnegligible rate of mortality associated with this clinical scenario.[102]

Sequelae

After one episode of PID a woman's risk for ectopic pregnancy increases sevenfold. Approximately 13% of women are infertile after a single episode of PID, 25% to 35% after two episodes, and 50% to 75% after three or more episodes. If a true tubo-ovarian abscess is present, only 7% to 14% of patients are able to conceive after treatment. After treatment for a tubo-ovarian complex (a less-restrictive diagnostic category than tubo-ovarian abscess), approximately two-thirds of women attempting pregnancy are unable to conceive. Other sequelae associated with PID include dyspareunia, pelvic adhesions, and chronic pelvic pain.[103] One study has suggested that screening for and treatment of cervical chlamydial infection can prevent greater than 50% of PID in a given locale.[104]

ACKNOWLEDGMENT

The author and the editors of *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases* (PPID) recognize the dedication and many contributions of Dr. David Soper to this field over many years, and his work on this chapter in the prior editions of PPID.

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110 Prostatitis, Epididymitis, and Orchitis

Catherine C. McGowan

SHORT VIEW SUMMARY

Definitions and Classification
- For the National Institutes of Health classification of prostatitis syndromes, see Table 110.2.
- Acute bacterial prostatitis is associated with lower urinary tract infection and sepsis.
- Chronic bacterial prostatitis is associated with recurrent lower urinary tract infections caused by the same bacterial clade.
- Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) occurs in absence of uropathogenic bacteria.
- Acute epididymitis and orchitis are usually due to infectious agents or local trauma.

Epidemiology
- Prostatitis represents the most common urologic diagnosis in healthy young men. Fifty percent of men may experience symptoms in their lifetimes. Prevalence is 2% to 16%.
- Nearly 90% of men evaluated for genitourinary symptoms have CP/CPPS.
- Sexually transmitted epididymitis is most common in young men, whereas infection with coliform organisms is the most common cause of epididymitis in men older than age 35. Men with bacterial epididymitis may have underlying urologic pathology or recent genitourinary tract manipulation.
- Isolated orchitis is rare.

Microbiology
- Gram-negative Enterobacteriaceae cause most episodes of bacterial prostatitis. Enterococci account for a small percentage.
- Etiology of epididymitis and orchitis reflects patient age. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* predominate in young men, and coliform or *Pseudomonas* species predominate in older men.
- Most orchitis is caused by viral infections.

Diagnosis
- Careful history, physical examination, urinalysis, and urine culture are essential.
- The lower urinary tract localization test (see Table 110.1) is the gold standard.
- Epididymitis and orchitis should be evaluated with urine or urethral nucleic acid amplification testing for *C. trachomatis* and *N. gonorrhoeae*. A urethral smear for Gram stain and a midstream urine culture are useful for establishing other etiologies.

Therapy
- Fluoroquinolones are preferred oral treatment for prostatitis. Quinolone resistance is increasingly common, especially in infections occurring after genitourinary tract instrumentation.
- Septic patients should receive empirical broad-spectrum parenteral therapy.
- Combinations of antibiotics, α-blockers, antiinflammatory agents, and pain management therapies represent the most effective treatment for CP/CPPS.
- Combination therapy with intramuscular ceftriaxone plus either azithromycin or doxycycline is recommended for *N. gonorrhoeae* infections.

ANATOMY AND PHYSIOLOGY OF THE TESTES AND MALE ACCESSORY SEX ORGANS

The testicle has two functional components: seminiferous tubules and interstitial cells. Sperm production is the primary function of the seminiferous tubules. Interstitial cells, located between the seminiferous tubules, produce sex hormones. After spermatogenesis, spermatozoa are transported from the testis into the epididymis (Fig. 110.1). Sperm move into the vas deferens, a muscular tube approximately 12 inches long that is easily palpable in the scrotum. Fructose from the seminal vesicles is the major energy source for ejaculated sperm. In addition, the seminal vesicles provide proteins that coagulate the ejaculate. Liquefaction of the semen occurs 5 to 30 minutes after ejaculation because of proteolytic enzymes from the prostate.

HOST DEFENSES OF THE MALE LOWER UROGENITAL TRACT

Organisms that ascend through the urethra cause most infections of the urogenital ducts and accessory sex organs. Mechanical factors, such as the flushing action of micturition and ejaculation, should provide some protection against infection, although the relative significance of these defenses is unclear.

A zinc-containing polypeptide termed the *prostatic antibacterial factor* is the most important antimicrobial substance secreted by the prostate.[1] Men with chronic prostatitis have significantly lower levels of zinc in their prostatic fluid than healthy men, despite normal serum zinc levels.[1,2] Other findings suggest that bacterial prostatitis and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) are associated with prostatic secretory dysfunction.[3] It is unclear whether reduced zinc concentrations precede the development of prostatic infection or result from secretory dysfunction. Prostatic secretions from patients with bacterial prostatitis contain high concentrations of immunoglobulins.[4] Several studies have shown antigen-specific antibody coating of bacteria isolated from the lower urinary tracts of patients with prostatitis.[5] The antigen-specific antibody response in prostatic secretions, predominantly secretory immunoglobulin A, is significantly greater in magnitude than the serologic response. Studies have documented that patients with prostatitis syndromes have high concentrations of cytokines and chemokines in their prostatic secretions and semen.[6,7] Although the precise mechanisms are unclear, local cytokines, chemokines, and other growth factors may contribute to the apparent increased risk of both prostate cancer and symptomatic benign prostatic hypertrophy in men with a history of prostatitis.[8–11]

The presence of leukocytes is characteristic of many inflammatory conditions of the male lower urinary tract, including prostatitis. Phagocytosis of abnormal sperm by leukocytes has been observed in infertile men with pyospermia, but the precise role and significance of prostatic fluid leukocytes is uncertain. Patients with symptoms of prostatitis have more lower urinary tract leukocytes than controls,[12] but no evidence has linked prostatitis symptoms with prostatic fluid leukocyte counts or shown that symptomatic patients with leukocytes in their prostatic secretions respond differently to treatment than symptomatic patients without inflammation.[13]

PROSTATITIS

Classification

The term *prostatitis* has been used clinically to describe various complaints referable to the lower urogenital tract and perineum in men. It has been

estimated that 50% of men experience symptoms of prostatitis at some time in their lives. Studies have suggested that the prevalence of prostatitis is 2% to 16%.[14] In otherwise healthy young men, prostatitis may be the most common urologic diagnosis. A national survey of physician visits found that there were almost 2 million visits annually for prostatitis, accounting for 8% of visits to urologists and 1% of visits to primary care physicians in the United States.[15] In the United States, the direct cost for the diagnosis and management of prostatitis was approximately $84 million in 2000, exclusive of pharmaceutical spending.[16]

The crucial clinical issue is to distinguish patients with lower urinary tract complaints associated with bacteriuria (i.e., patients who may have acute or chronic bacterial prostatitis) from the larger number of patients without bacteriuria. Further classification of patients with prostatitis depends on careful bacteriologic assessment of the lower urinary tract, which is based on sequential cultures obtained during micturition (Table 110.1). Prostatitis syndromes may be classified into four major groups: acute bacterial prostatitis, chronic bacterial prostatitis, CP/CPPS, and asymptomatic inflammatory prostatitis (Table 110.2).[17] In addition, rare patients develop granulomatous prostatitis.

Bacterial prostatitis is a common diagnosis in clinical practice, but well-documented bacterial infections of the prostate, whether acute or chronic, are uncommon. Most patients with a diagnosis of prostatitis are men with perineal, lower back, or lower abdominal pain or ejaculatory complaints. Most patients have no history of bacteriuria and little objective evidence of bacterial infection of the prostate. Patients with symptoms of prostatitis but no evidence of bacteriuria may be classified as having CP/CPPS, but as yet there are few firm data on which to base therapeutic decisions.

Acute Bacterial Prostatitis

Acute bacterial prostatitis is seldom a subtle or difficult diagnosis. Patients complain of abrupt onset of symptoms associated with lower urinary tract infection, such as urinary frequency and dysuria. Acute edema of the prostate may lead to lower urinary tract obstruction. Signs of systemic toxicity are common. Some such patients have had recent genitourinary tract procedures.[18–20] On physical examination, patients may have fever and lower abdominal or suprapubic tenderness. Findings on rectal examination are frequently impressive, with an exquisitely tender, tense prostate on palpation. Urinalysis is abnormal, with pyuria, and urine culture is positive unless the patient has received prior antimicrobial therapy. Bacteremia may occur spontaneously or may result from excessively vigorous rectal examination. Gram-negative uropathogens, especially *Escherichia coli,* are the causative agents in more than 60% of cases, although infection may be due to *Pseudomonas aeruginosa* and *Enterococcus* species.[19,20] *Escherichia coli* isolates that cause acute prostatitis in healthy young men have been shown to possess specialized urovirulence factors, especially hemolysin and cytotoxic necrotizing factor, with many strains exhibiting multiple virulence factors.[21,22] The isolation of *Staphylococcus aureus* from prostatic secretions or urine should prompt a search for a remote or endovascular source of *S. aureus* infection.

Results of antimicrobial therapy for acute bacterial prostatitis often are dramatic. Many drugs that do not penetrate into the prostate under normal conditions are effective in acute bacterial prostatitis.[23] A variety of antibiotics are appropriate for treating acute bacterial prostatitis, and recommendations for empirical therapy are based on the likelihood of the infecting organism and regional patterns of antimicrobial resistance.

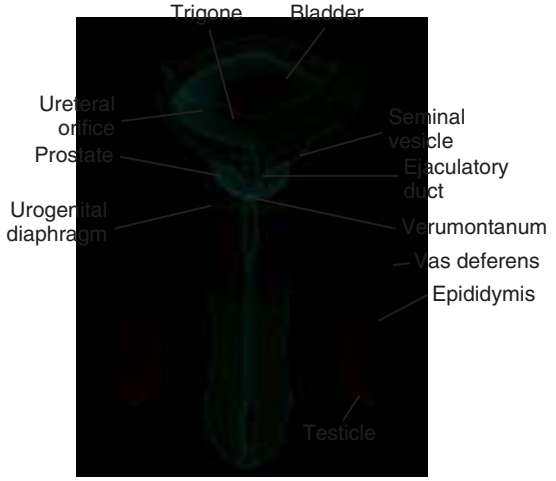

FIG. 110.1 **Anatomy of the male sex organs and lower urinary tract.**

TABLE 110.1 Lower Urinary Tract Localization Using Sequential Urine Cultures

SPECIMEN	SYMBOL	DESCRIPTION
Voided bladder 1	VB_1	Initial 5–10 mL of urinary stream
Voided bladder 2	VB_2	Midstream specimen
Expressed prostatic secretions	EPS	Secretions expressed from prostate by digital massage
Voided bladder 3	VB_3	First 5–10 mL of urinary stream immediately after prostatic massage

[a]Unequivocal diagnosis of bacterial prostatitis requires that the colony count in the VB_3 specimen greatly exceed the count in the VB_1 specimen, preferably by at least 10-fold. Many patients who have chronic bacterial prostatitis harbor only small numbers of bacteria in the prostate. In these patients, direct culture of prostatic secretions is particularly useful. Microscopic examination of the EPS is useful for identifying white blood cells and oval fat bodies (large lipid-laden macrophages characteristic of the prostatic inflammatory response).
From Stamey T. Pathogenesis and Treatment of Urinary Tract Infections. *Baltimore: Williams & Wilkins; 1980.*

TABLE 110.2 Classification of Prostatitis Syndromes on the Basis of Lower Urinary Tract Localization Studies

CONDITION	BACTERIURIA[a]	INFECTION LOCALIZED TO PROSTATE[b]	INFLAMMATORY RESPONSE[c]	ABNORMAL RECTAL EXAMINATION OF PROSTATE[d]	SYSTEMIC ILLNESS[e]
Acute bacterial prostatitis	+	+	+	+	+
Chronic bacterial prostatitis	+	+	+	–	–
Chronic prostatitis/chronic pelvic pain syndrome					–
Inflammatory subtype[f]	–	–	+	–	–
Noninflammatory subtype[g]	–	–	–	–	–
Asymptomatic inflammatory prostatitis	–	–	+	±	–

[a]Documented with an identical organism that is shown to localize to a prostatic focus when the midstream urine culture is negative.
[b]See text for diagnostic criteria.
[c]In expressed prostatic secretions, semen, postmassage urine, or prostate tissue.
[d]Abnormal findings include exquisite tenderness and swelling that may be associated with signs of lower urinary tract obstruction.
[e]Systemic findings frequently include fever and rigors and may include signs of bacteremia.
[f]Formerly termed *nonbacterial prostatitis.*
[g]Formerly termed *prostatodynia.*
From Krieger JN, Nyberg L Jr, Nickel JC. NIH consensus definition and classification of prostatitis. JAMA. *1999;282:236–237.*

In most settings, fluoroquinolones or trimethoprim-sulfamethoxazole are the preferred oral agents, due to their superior penetration into the prostate. However, quinolone resistance is increasingly common, especially in patients who develop bacterial prostatitis after genitourinary instrumentation. Nearly 90% of *E. coli* isolates were resistant to fluoroquinolones in several studies of acute prostatitis occurring after prostate biopsy.[24,25] Systemically ill patients should receive empirical parenteral therapy with a third-generation cephalosporin or a carbapenem, possibly combined with an aminoglycoside, with subsequent modification based on culture and sensitivity results. Severe sepsis after manipulation of the genitourinary tract, such as biopsy of the prostate, may be caused by *E. coli* strains that are multidrug-resistant (MDR) to common empirically used antibiotic agents.[26,27] There are limited data regarding duration of antibiotic therapy, and recommendations range from 2 to 4 weeks depending on the severity of illness. Confirmatory urine culture to ensure bacteriologic cure should be done following antibiotic completion.

Urinary retention is managed best with a suprapubic cystostomy, rather than a transurethral catheter, to avoid obstructing drainage of infected prostatic secretions into the urethra. General measures, including hydration, analgesics, and bed rest, also are indicated. The most important complications of acute bacterial prostatitis are prostatic abscess, prostatic infarction, and chronic bacterial prostatitis. Nonspecific granulomatous prostatitis may follow acute lower urinary tract infections and mimic prostate cancer both clinically and histologically.[28,29]

Chronic Bacterial Prostatitis

Chronic bacterial prostatitis is an important cause of bacterial persistence in the male lower urinary tract. Characteristically, patients experience recurrent bacterial urinary tract infections caused by the same organism.[17] Patients often are asymptomatic between episodes of bladder bacteriuria. The prostate gland is usually normal on rectal or endoscopic evaluation. Careful lower urinary tract localization studies constitute the cornerstone on which to base a diagnosis of chronic bacterial prostatitis (see Tables 110.1 and 110.2). Evaluation of pre–prostate massage and post–prostate massage samples has been proposed as an alternative to the traditional lower urinary tract localization study.[30,31] Diagnosis of chronic bacterial prostatitis based solely on symptoms, the number of leukocytes in expressed prostatic secretions, or the use of prostate biopsy specimens is inadequate.

Gram-negative rods (members of Enterobacteriaceae or pseudomonads) are the most important pathogens in chronic bacterial prostatitis. Enterococci may be the causative organisms in some cases. An etiologic role for other gram-positive cocci, such as *S. aureus,* has been suggested.[32] However, studies have demonstrated that repetitive cultures to consistently localize gram-positive bacteria in prostate-specific specimens, such as expressed prostatic secretions, are seldom reproducible in untreated patients, leading to uncertainty regarding the clinical significance of these organisms.[33]

The risk factors for the development of chronic bacterial prostatitis have not been clearly defined. Bacteria isolated from patients with chronic bacterial prostatitis, even after multiple episodes of symptomatic bacteriuria and prolonged antibiotic courses, are often antibiotic-sensitive strains. Several factors may increase the risk of failure of antibiotic therapy in some patients, including poor drug penetration into the prostatic parenchyma, bacterial biofilm formation,[34] prostatic fluid pH changes associated with infection, infected calculi that may serve as persistent foci for bacteria,[35] and incomplete medication adherence. In a retrospective review of 480 patients with acute prostatitis, factors significantly associated with progression to chronic prostatitis in 49 men (10%) were diabetes, prior manipulation, not doing cystostomy, and urethral catheterization.[36]

Depending on the results of antimicrobial sensitivity, the preferred therapy is at least 4 to 6 weeks with an oral fluoroquinolone, which results in microbiologic cure rates of 70% or more.[37,38] Treatment options for prostatitis due to emerging MDR gram-negative bacteria have been limited. Oral fosfomycin, which achieves therapeutic levels in prostatic tissue, has been reported to successfully eradicate infection with MDR *E. coli.*[39] Long-term follow-up with repeated microbiologic evaluation is needed to ensure that patients respond to treatment. Patients with chronic bacterial prostatitis who are not cured may be rendered asymptomatic by long-term suppressive treatment, based on anecdotal clinical experience. Because patients usually are asymptomatic between episodes of bacteriuria, the goal of suppressive therapy is to prevent symptomatic episodes despite the persistence of bacteria in the prostate.

Chronic Prostatitis/Chronic Pelvic Pain Syndrome

Men with CP/CPPS are the largest population with prostatitis, representing more than 90% of patients evaluated. CP/CPPS causes significant morbidity and substantially decreases patients' quality of life.[11,40] Patients may complain of various perineal and pelvic symptoms, especially pain, that may be perineal, suprapubic, infrapubic, penile, scrotal, or inguinal in location and are described as either continuous or spasmodic, and commonly as a "dull ache."[41] Other complaints include voiding difficulty, urinary symptoms, and sexual dysfunction.[42] Systemic symptoms or signs are usually absent, and physical examination generally is unremarkable.

The diagnosis of CP/CPPS is one of exclusion. Patients have no history of bacteriuria and lack objective evidence of bacterial infection of their prostatic secretions on careful lower urinary tract localization studies (see Table 110.2). Some patients with CP/CPPS have leukocytes in expressed prostatic secretions, post–prostate massage urine (voided bladder 3 [VB_3]; see Table 110.1), or semen (in the inflammatory subtype, formerly termed *nonbacterial prostatitis*), whereas others have no objective evidence of inflammation (in the noninflammatory subtype, formerly termed *prostatodynia;* see Table 110.2).[17]

The causes of CP/CPPS are uncertain and subject to ongoing multicenter investigations. Although numerous studies have found no strong evidence for a causative infectious agent,[43–45] others have reported various atypical pathogens in men with CP/CPPS.[46,47] A large prospective study of men with chronic prostatitis found that 74% had an infectious etiology implicated, including *Chlamydia trachomatis* in 37% of cases, *Trichomonas vaginalis* in 11%, *Ureaplasma urealyticum* in 5%, and classic bacterial uropathogens in 20%.[48] The methods, techniques, and control groups used in these various studies have differed considerably. Molecular studies have shown that patients with inflammatory CP/CPPS are significantly more likely to have bacterial DNA in prostatic secretions than patients without inflammation[49] or control patients with prostate cancer.[50] However, other studies have not found bacterial DNA in prostate tissue of patients with moderate to severe symptoms of CPPS.[51]

Many authorities have proposed that CP/CPPS is not an infectious disease, and that multiple extraprostatic factors may be important in the cause of this syndrome. Increased prostaglandins, autoimmunity, psychological abnormalities, neuromuscular dysfunction of the bladder neck or urogenital diaphragm, allergy to environmental agents, and nerve growth factor (a neurotrophin found to have a role in the regulation of nociceptive nerves and as a mediator and amplifier of neurogenic inflammation) all have been suggested as causative factors.[52,53]

Current therapy for symptomatic patients with CP/CPPS is unsatisfactory. The most commonly used modalities include empirical antimicrobial therapy,[54] α-blockers,[55] antiinflammatory therapy,[56] and pain management therapies.[57] A meta-analysis including 23 randomized trials of patients with CP/CPPS found that α-blockers (terazosin, tamsulosin), antibiotics (fluoroquinolones), and combinations of these therapies appeared to achieve the greatest improvement in clinical symptom scores compared with placebo.[58] However, the small statistically significant overall benefit observed may or may not be clinically significant for individual patients, and antimicrobial therapy generally is not recommended as a primary therapy, particularly in patients who have previously failed treatment with antibiotics.[59] One recent double-blind, placebo-controlled study of 60 patients found that transurethral intraprostatic injection of botulinum toxin type A decreased pain scores at 1, 3, and 6 months.[60] Experts recommend conservative therapies such as diet modification, low-impact exercise, local heat therapy, and development of personal coping skills based on their proven worth in clinical practice and with other pain syndromes.[61] More effective treatment of CP/CPPS is the focus of major research efforts. Ongoing National Institutes of Health (NIH)–funded research is focused on patient phenotyping to identify genetic, environmental, and clinical factors (e.g., associated nonurologic conditions, irritable bowel syndrome, chronic widespread pain, etc.), as well as

evidence of "central nervous system sensitization" that may be associated with the likelihood that individual patients will respond to specific therapies.[62]

Asymptomatic Inflammatory Prostatitis

The NIH consensus classification of prostatitis includes a category for patients who have a diagnosis of prostatitis determined by the presence of significant leukocytes (or bacteria or both) in prostate-specific specimens but who have no genitourinary tract symptoms.[17] These patients have prostate inflammation but have none of the usual symptoms associated with other prostatitis syndromes. It is common for patients with elevated prostate-specific antigen levels to undergo prostate biopsy for evaluation of possible prostate cancer. The most common benign pathologic diagnosis is prostatitis, based on the histologic finding of inflammatory infiltrates in the prostatic parenchyma. Some clinicians recommend a course of antimicrobial or antiinflammatory therapy in this situation.[38] These recommendations are based on the observations that acute bacterial prostatitis and exacerbations of chronic bacterial prostatitis are associated with elevations of serum prostate-specific antigen and acid phosphatase levels.[63] Whether antimicrobial therapy is beneficial for asymptomatic patients with histologic evidence of prostatitis is uncertain. The current consensus is that antimicrobial therapy is not indicated for asymptomatic patients.[64]

Histologic evidence of prostatic inflammation also may be noted in patients with no clinical history of prostatitis who have benign prostate tissue removed during surgical procedures for the treatment of bladder outflow obstruction[10] or who have radical prostatectomy for prostate cancer.[65] Inflammatory prostatitis may be diagnosed among asymptomatic men undergoing evaluation for infertility. On semen microscopic analysis, increased numbers of round cells, which could represent immature spermatozoa or white blood cells, may prompt a diagnosis of prostatitis. Other terms used in the infertility literature include *asymptomatic male genital tract infection, male accessory gland infection, prostatoseminal vesiculitis, leukocytospermia,* and *pyosemia* (*pyospermia*). Some specialists recommend antimicrobial therapy, but the proportion of these patients who have active genital tract infections is poorly defined. It would seem prudent to identify a specific genitourinary tract pathogen before recommending antimicrobial therapy for asymptomatic men who present for infertility evaluation.

Epidemiologic studies and other lines of evidence have suggested that prostatitis may be associated with an increased risk for later development of benign prostatic hypertrophy–associated events and with prostate cancer.[8,11,66,67] If these potential associations were confirmed, that would support efforts to diagnose and treat symptomatic and asymptomatic prostatitis syndromes.[65,67] However, at present, such associations are not confirmed.

Granulomatous Prostatitis

Granulomatous prostatitis is a characteristic histologic reaction of the prostate to various insults, with granulomas containing lipid-laden histiocytes, plasma cells, and scattered giant cells. In most cases, granulomatous prostatitis follows an episode of acute bacterial prostatitis.[28] There also are many specific infectious causes of granulomatous reaction by the prostate. Tuberculous prostatitis usually is secondary to tuberculosis elsewhere in the genital tract.[68] Most patients have no symptoms referable to prostatic infection. At biopsy, the granulomas may contain typical Langerhans giant cells and exhibit caseous necrosis. These infections are caused most often by *Mycobacterium tuberculosis* but also have been reported with nontuberculous mycobacteria. Iatrogenic mycobacterial prostatitis may develop in patients who receive intravesicular bacille Calmette-Guérin for treatment of transitional cell carcinoma of the bladder.[69] Prostatitis may be secondary to systemic infection with many of the deep mycoses.[70] Most cases of fungal prostatitis reported have been associated with blastomycosis, coccidioidomycosis, and cryptococcosis.[71,72] The prostate may be a focus of persistent cryptococcosis in patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). Rarely, prostatic histoplasmosis occurs in these patients.[73]

Granulomatous prostatitis is most important in the differential diagnosis for an indurated, firm, or nodular prostate. Findings on rectal examination in these patients raise the suspicion of prostatic carcinoma. Biopsy usually is necessary for diagnosis, and it is important that appropriate stains and cultures be used to detect specific causative agents.



FIG. 110.2 Computed tomography scan of a prostatic abscess lateral to the urethra *(arrow).* *(From Sobel JD, Kaye D. Urinary tract infections. In: Mandell GL, Bennett JE, Dolan R, eds.* Principles and Practice of Infectious Diseases. *6th ed. Philadelphia: Churchill Livingstone; 2005:894.)*

Prostatic Abscess

Prostatic abscess is a rare complication of prostatitis in the antibiotic era.[74] Most prostatic abscesses occur in patients with diabetes or with an immunocompromised state and in patients who have not received appropriate therapy for acute prostatitis. Foreign bodies and urinary tract obstruction are other predisposing factors. In the past, *Neisseria gonorrhoeae* was a common pathogen, but most cases now are caused by the common uropathogens. Infection generally occurs by the ascending route. On occasion, *S. aureus* is the pathogen,[32] which suggests the possibility of hematogenous infection. Blastomycosis, cryptococcosis, and nocardiosis are also well-described causes of prostatic abscess. In a prospective case series of melioidosis from an endemic region, prostatic abscess caused by *Burkholderia pseudomallei* was commonly observed.[75]

Patients are usually febrile with irritative and obstructive voiding symptoms, and they may have signs of sepsis. The clinical presentation closely resembles that of acute bacterial prostatitis. The classic abscess presentation is a fluctuant area in the prostate when palpated during rectal examination. The presentation may be subtle, however. Ultrasonography, computed tomography (Fig. 110.2), or magnetic resonance imaging of the pelvis is helpful for confirming the diagnosis or in patients with equivocal clinical findings. Treatment includes draining the abscess through a perineal or a transurethral route and appropriate antimicrobial therapy for 4 to 6 weeks.[76]

EPIDIDYMITIS

Epididymitis is an inflammatory reaction of the epididymis to various infectious agents or to local trauma. There are approximately 600,000 cases of epididymitis annually in the United States, most of which occur in men between 18 and 35 years of age.[77] Acute epididymitis has been responsible for more days lost from military service than any other disease and was responsible for 20% of urologic hospital admissions in military populations.[78]

Patients with epididymitis usually complain of painful swelling of the scrotum. The onset may be acute, over 1 or 2 days, or more gradual and often is accompanied by dysuria or irritative lower urinary tract symptoms. Many patients have a urethral discharge. Specific attention should be directed toward eliciting a past history of genitourinary tract disease or sexual exposure. Some patients may have only a nonspecific finding of fever or other signs of infection. This presentation is particularly common in hospitalized patients who have undergone recent urinary tract manipulation and who may be obtunded by medication.[79]

Tender swelling, frequently accompanied by erythema and generally unilateral, may be noted primarily in the posterior aspect of the scrotum. If the patient is examined early in the course of the disease, the swelling

may be localized to one portion of the epididymis. Later, involvement of the ipsilateral testis is common, producing an epididymo-orchitis, and it may be difficult to distinguish the testicle from the epididymis within the inflammatory mass. Scrotal examination commonly reveals the presence of a hydrocele caused by the secretion of inflammatory fluid between the layers of the tunica vaginalis. Urethral discharge may be apparent on inspection or stripping of the urethra.

There are two common types of epididymitis: nonspecific bacterial epididymitis and sexually transmitted epididymitis. Epididymitis also may occur rarely after genital trauma or with disseminated infections.

Nonspecific Bacterial Epididymitis

The most common cause of epididymitis in men older than 35 years and who are at low risk for sexually transmitted infections is infection due to coliform or *Pseudomonas* species.[80,81] In most series, gram-negative aerobic rods caused more than two-thirds of bacterial epididymitis cases. Enterococci also have been reported as pathogens.[82]

Many patients who develop bacterial epididymitis have underlying urologic pathology or have a history of recent genitourinary tract manipulation. Urologic abnormalities are especially common in children who present with epididymitis.[83] The development of epididymitis after surgery or urethral catheterization may occur weeks or, rarely, months after the manipulation. Epididymitis is particularly likely in patients who undergo urinary tract surgery or instrumentation while they are bacteriuric. Acute and chronic bacterial prostatitis is another important predisposing condition for the development of bacterial epididymitis.

Bacterial epididymitis may be an important focus of organisms causing bacteremia and local morbidity in patients with indwelling transurethral catheters. Genitourinary tract complications of acute bacterial epididymitis that occur rarely include testicular infarction, scrotal abscess, pyocele, a chronic draining scrotal sinus, chronic epididymitis, and infertility.

Tuberculous epididymitis is the most common manifestation of male genital tuberculosis, with orchitis and prostatitis seen less commonly.[84] The usual symptom is a sensation of heaviness or swelling. There is characteristic scrotal swelling with beadlike enlargement of the vas deferens. Chronic draining scrotal sinuses may be a feature. Genitourinary tuberculosis may be transmitted by direct inoculation during sexual activity and is an important cause of infertility in regions of the world where tuberculosis is endemic.[85] The systemic mycoses rarely may cause epididymitis; blastomycosis is the most common pathogen and may cause a draining sinus through the scrotal wall.[70] Brucellosis is also well described as a cause of epididymitis in patients with a history of exposure.[86] Nocardia has been described as a cause of epididymitis in immunosuppressed patients.[87] Iatrogenic cases of epididymitis and orchitis may complicate bacille Calmette-Guérin therapy for transitional cell carcinoma of the bladder.[88] Rare patients develop epididymitis as a complication of collagen vascular disorders[89,90] or as a complication of amiodarone therapy.[91]

Medical management is appropriate for most patients with bacterial epididymitis. Initial empirical treatment with agents appropriate for gram-negative rods and gram-positive cocci should be initiated pending urine culture and sensitivity results. Nonspecific measures, such as bed rest, scrotal elevation, analgesics, and local ice packs, are helpful. Surgery may be necessary for the management of complications of acute epididymal infections, such as testicular infarction, abscess, or pyocele of the scrotum.[92]

Sexually Transmitted Epididymitis

Sexually transmitted epididymitis is the most common type of epididymitis in young men. *Chlamydia trachomatis* and *N. gonorrhoeae* are the major pathogens in this population.[93] Acute epididymitis caused by sexually transmitted enteric organisms, such as *E. coli,* may also occur among men who are the insertive partner during anal intercourse.[94] Chlamydiae have been identified as the most common cause of epididymitis in younger, sexually active populations. These patients formerly were considered to have idiopathic nonspecific epididymitis.[95] One study documented infections with *C. trachomatis* in 16 (47%) of 34 cases of epididymitis in patients younger than 35 years and in only 1 of 16 cases of epididymitis in patients older than 35.[95] Patients with chlamydial epididymitis frequently do not complain of urethral discharge; however, 11 of 17 patients with epididymitis caused by chlamydiae had demonstrable discharge, usually the scant watery discharge characteristic of nonspecific urethritis. The median interval from the last sexual exposure was 10 days (range, 1 to 45 days).[95] Patients may carry chlamydiae for long periods before developing overt epididymitis.

Before the availability of penicillin, it was estimated that epididymitis occurred in 10% to 30% of men with gonococcal urethritis. In more recent studies, *N. gonorrhoeae* was identified as the cause of acute epididymitis in 16% of cases in military populations and in 21% of cases in civilians younger than 35 years.[95] Many patients with gonococcal epididymitis do not have a history of urethral discharge, and a discharge may be demonstrable in only 50% of these patients.

Underlying genitourinary tract abnormalities are uncommon in patients with sexually transmitted epididymitis. Diagnosis depends on a high index of clinical suspicion, evaluation for presence of urethritis (which may be asymptomatic), and appropriate diagnostic tests, including nucleic acid amplification testing for *C. trachomatis* and for *N. gonorrhoeae*. Specific antibiotic therapy, using drugs appropriate for chlamydial and gonococcal infections, is the most important aspect of treatment. Because of emerging antibiotic resistance identified in *N. gonorrhoeae* isolates, combination therapy with intramuscular ceftriaxone plus either azithromycin or doxycycline is currently recommended.[93] For acute epididymitis most likely caused by sexually transmitted chlamydia and gonorrhea and enteric organisms (in men who practice insertive anal sex), intramuscular ceftriaxone and oral levofloxacin or ofloxacin is recommended.

Patients should be evaluated for other sexually transmitted infections, and treatment of sexual partners is important. In general, a complete urologic workup is not indicated for patients with uncomplicated sexually transmitted epididymitis. Rare complications of sexually transmitted epididymitis include abscess formation, testicular infarction, chronic epididymitis, and infertility. Ultrasonography, particularly color-flow Doppler ultrasonography, is useful for the differential diagnosis of complicated cases of epididymitis.[96,97]

Follow-Up

Failure of the signs and symptoms of epididymitis to subside within 3 days requires reevaluation of the diagnosis and the therapy.[93] Persistent swelling and tenderness after completion of therapy suggest the need for comprehensive evaluation. Considerations in the differential diagnosis include abscess, infarction, testicular cancer, and tuberculous or fungal epididymitis. Some patients develop chronic scrotal pain that is difficult to manage and may, on occasion, merit consideration of surgical procedures.[98]

ORCHITIS

Orchitis is inflammation of the testis, but the term has also been used to describe pain localized to the testis without objective evidence of inflammation. Isolated orchitis is significantly less common than prostatitis or epididymitis. Orchitis differs from other infections of the male accessory sex glands in two important respects: bloodborne dissemination is the major route of infection, and viruses are implicated as important pathogens.

Viral Orchitis

Viral infections, particularly mumps,[99] are associated with most cases of orchitis. Although mumps rarely causes orchitis in prepubertal boys, orchitis occurs in approximately 20% of postpubertal males with mumps. Testicular pain and swelling usually begin 4 to 6 days after the onset of parotitis but may occur without parotid involvement. Orchitis is unilateral in approximately 70% of cases. Contralateral testicular swelling may occur 1 to 9 days after involvement of the first side. The clinical course is variable and ranges from mild testicular discomfort and swelling to severe testicular pain and marked swelling accompanied by nausea, vomiting, prostration, high fever, and constitutional symptoms. Epididymitis and inflammation of the spermatic cord may be noted on physical examination. Resolution of mild cases may occur in 4 to 5 days; more severe cases usually resolve in 3 to 4 weeks. In approximately half of cases, the involved testes undergo some degree of atrophy. In

older series, sterility was reported in 25% of patients with bilateral disease. More recent studies have found, however, that mumps orchitis seldom results in infertility.[100]

Coxsackie B virus produces a disease that clinically and histologically resembles mumps orchitis.[101] Infection with lymphocytic choriomeningitis virus occasionally can result in orchitis, which develops 1 to 3 weeks after the onset of the initial fever. Orchitis is usually unilateral and painful. Most cases resolve within 2 weeks.[102]

Bacterial Orchitis

With the exception of viral diseases, acute genitourinary tract infections involving only the testis are distinctly unusual. Pyogenic bacterial orchitis usually occurs because of contiguous spread from an inflammatory process in the epididymis that causes an epididymo-orchitis. Most cases of pyogenic orchitis are caused by *E. coli, Klebsiella pneumoniae, P. aeruginosa,* staphylococci, or streptococci. On occasion, acute orchitis may be caused by other organisms as a result of hematogenous seeding. This seeding seems to be a particular risk with brucellosis in endemic areas. Rare cases of syphilitic orchitis have been described.[103]

A patient with pyogenic orchitis appears acutely ill, with high fever and marked discomfort and swelling of the involved testicle. In general, the pain is described as radiating to the inguinal canal, and it frequently is accompanied by nausea and vomiting. On examination, there is usually an acute hydrocele. The testis is swollen and exquisitely tender.

The overlying scrotal skin is generally erythematous and edematous. Complications of pyogenic bacterial orchitis include testicular infarction, abscess formation, and pyocele of the scrotum. Surgery usually is required for treatment of these conditions. Orchitis can be caused by tuberculosis and blastomycosis, usually by extension from the epididymis.[84,104] Involvement of the testicle without palpable abnormality in the adjacent epididymis rarely has been observed with these agents.

UROLOGIC MANIFESTATIONS IN MEN WITH HIV INFECTION

One autopsy study of 140 patients with HIV/AIDS found that 2 of 17 cases with systemic toxoplasmosis and 4 of 65 cases of systemic cytomegalovirus infection involved the testes.[105] Other opportunistic infections involving the male genital tract include those caused by mycobacteria, cryptococcosis, *Aspergillus fumigatus* and other mycoses, *Haemophilus parainfluenzae,* and candidiasis.[106] The testes characteristically exhibit azoospermia, marked spermatogenic arrest, germ cell degeneration, peritubular fibrosis, and Leydig cell depletion. These nonspecific findings probably reflect the severe systemic disease in these patients.

Other urologic manifestations in patients with HIV infection reflect involvement of related organ systems. Bladder dysfunction may occur in patients with HIV-associated neurologic disorders. This bladder dysfunction increases the risk of urinary tract infection, which was diagnosed in 14% to 20% of HIV-positive patients in one series.[107] HIV status was an independent risk factor for bothersome lower urinary tract symptoms in a recent study of men who have sex with men, and severe urinary symptoms were more likely to be reported in HIV-positive men with a history of AIDS.[108] In another report, bacterial prostatitis was diagnosed in 17 (8%) of 209 men hospitalized for treatment of HIV-related infections.[109] The prevalence of bacterial prostatitis was observed to increase from 3% in HIV-asymptomatic patients to 14% in patients with AIDS.

SEMEN AS A VECTOR FOR HIV INFECTION

The relative contributions of behavioral and clinical risk factors to transmission of HIV are incompletely understood, but sexual activity alone provides a limited explanation. Expressing risk as a function of frequency and types of sexual contacts has not yielded straightforward patterns; this probably reflects biologic factors that determine infectiousness and the vulnerable partner's susceptibility. Stage of disease and other host factors; male circumcision; genital and plasma HIV viral load; viral characteristics, such as tropism and syncytium-inducing capability; and antiretroviral treatment have been linked to greater and lesser degrees of infectivity.[110]

Studies demonstrate that other sexually transmitted infections increase the risk of HIV transmission and acquisition[111] and suggest that their treatment may reduce the sexual transmission of HIV.[112,113] Sexually transmitted infections are believed to increase susceptibility to HIV infection by recruiting target cells and disrupting epithelial barriers. In addition, genital ulcer diseases and urethritis (gonococcal and non-gonococcal) increase the HIV viral load in semen.[114] In contrast, sexually transmitted infection treatment reduces HIV shedding.

Epidemiologic studies have suggested that direct contact with semen is the most important route for HIV sexual transmission. HIV type 1 first was isolated by cocultivation of seminal cells and donor lymphocytes.[115] Shedding of HIV in semen was associated with no significant changes in semen parameters that assessed fertility in one study of 50 semen specimens from asymptomatic or minimally symptomatic HIV-positive men.[116] In contrast, three men with AIDS all had pyospermia and grossly abnormal sperm. Abnormalities in the semen of men with AIDS likely reflect severity of disease but do not correlate with seminal shedding of HIV.

Semen is composed of cell-free seminal plasma and seminal cells (mature spermatozoa, immature sperm forms, leukocytes, and epithelial cells). Two approaches have been used to determine the likely sources of HIV within the semen. The first approach is to fractionate and analyze ejaculated semen specimens.[117] These studies have suggested that most viable (i.e., culturable) HIV in semen is cell associated, particularly with T lymphocytes and macrophages but not with motile spermatozoa. The second approach is anatomic, based on the observations that vasectomy causes dramatic reductions in seminal cells and eliminates secretions from proximal sites in the male reproductive tract. HIV RNA was assayed in seminal plasma and HIV DNA in seminal cells from 46 asymptomatic seropositive men before and after vasectomy.[118] Vasectomy produced no significant changes in levels of HIV RNA or DNA, supporting the conclusion that most seminal HIV is not associated with germinal cells but arises from distal sites in the male reproductive tract.[119]

Several lines of evidence support the idea that the male genital tract may be distinct from the systemic immune compartment.[120] Qualitative and quantitative virologic measurements from blood and genital compartments have suggested lack of association between culturability of virus in semen and viral RNA level in blood, discordant distribution of phenotypes, discordant viral RNA levels, a weak correlation between viral RNA level in semen and $CD4^+$ lymphocyte count in blood, differences in the biologic variability of viral RNA levels, and differences in the viral load response to antiretroviral therapy.[120] Phylogenetic studies have also supported the concept of compartmentalization.[121] Some, but not all,[122] studies have suggested a weak correlation between HIV RNA levels in the blood plasma and HIV RNA levels in the seminal plasma. However, neither HIV RNA blood level nor the $CD4^+$ lymphocyte count reliably predicts shedding of culturable virus or detection of viral RNA in the semen.[120] A study demonstrating that higher seminal HIV RNA levels were associated with a greater risk of heterosexual HIV transmission, independent of plasma HIV concentrations, suggests that HIV RNA in semen could be used as a marker of HIV sexual transmission risk.[123] Factors that may influence the HIV viral load in seminal fluid include systemic illness, antiretroviral therapy, urethritis, male genital tract inflammation, reactivation of genital herpesvirus infection, and seminal shedding of cytomegalovirus or other viruses. If the male reproductive tract is a distinct immunologic compartment, factors determining the infectiousness of semen may differ substantially from the factors determining HIV levels in blood, lymphatic tissue, or the central nervous system.

Whether detectable virus in the genital fluid when plasma virus is undetectable is of importance in transmission risk in serodiscordant couples is unclear based on recent clinical trials data. Three landmark studies—the HPTN 052, PARTNER, and Opposites Attract studies—have provided the strongest evidence to date that effective antiretroviral therapy prevents sexual transmission of HIV.[124–127] Across all three studies, no linked HIV transmissions were observed between mixed-HIV-status partners when the partner with HIV had virologic suppression (defined in these studies as having a plasma HIV RNA level less than either 200 or 400 copies/mL). Based on these research findings, the US Centers for Disease Control and Prevention (CDC) issued a prevention message

that people with HIV who take antiretroviral therapy as prescribed and achieve and maintain an undetectable viral load have effectively no risk of transmitting HIV through sex.[128]

SEMEN AS A VECTOR FOR ZIKA VIRUS INFECTION

Zika virus (ZIKV) is a flavivirus that has been linked to adverse birth outcomes and to the Guillain-Barré syndrome. Although ZIKV is primarily transmitted to humans by the bite of an infected *Aedes* species mosquito, it can also be transmitted through unprotected sex with an infected partner, including asymptomatic individuals. Numerous reports describe the detection of ZIKV RNA in semen, but finding ZIKV RNA in semen might not indicate the presence of infectious virus at the time of sampling, or correlate with the potential for sexual transmission of ZIKV. The largest published prospective cohort study to date included 184 symptomatic men with documented ZIKV infection from whom semen samples were obtained at 2-week intervals for 6 months following onset of illness.[129] Detectable ZIKV RNA was found in 61% of participants who submitted samples within 30 days, with the proportion decreasing to 7% or less in those who submitted samples more than 90 days after illness onset. ZIKV RNA persisted for 9 months is some men, which was consistent with previous reports. Infectious ZIKV was isolated in 3 of 78 samples with detectable RNA that were tested by culture, and all were obtained within 30 days of illness onset. Among the currently available reports of sexual transmission of ZIKV, the longest period from symptom onset in the index case to potential sexual transmission to a partner was between 32 and 41 days.[130] Current CDC guidelines for men with possible ZIKV exposure who are planning to conceive with their partner recommend waiting for at least 3 months after symptom onset (if symptomatic) or their last possible ZIKV exposure (if asymptomatic) before engaging in unprotected sex. The CDC also recommends that for couples who are not trying to conceive, men can consider using condoms or abstaining from sex for at least 3 months after symptom onset (if symptomatic) or their last possible ZIKV exposure (if asymptomatic) to minimize their risk for sexual transmission of ZIKV.[130]

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The complete reference list is available online at Expert Consult.

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M Eye Infections

111 Introduction to Eye Infections

Marlene L. Durand

Vision loss affects daily life in so many ways that most people value their sight more than any other special sense. Eye infections that result in permanent vision loss can be devastating, and many eye infections rapidly threaten sight if not promptly diagnosed and treated. Infectious disease physicians appreciate how important their help may be to ophthalmologists but may find it difficult to interpret the ophthalmologist's notes and specialized terminology. This brief review of eye anatomy and of the terminology commonly used by ophthalmologists may be helpful to the non-ophthalmologist caring for patients with eye infections.

ANATOMY

Several points about eye anatomy are worth considering.

1. From an embryologic perspective, the globe of the eye may be thought of as having three "coats" (cornea and sclera; uvea; retina) and two humors (aqueous, vitreous) (Fig. 111.1). The cornea and sclera form a protective outer coat, with the cornea as a clear window. The uvea is the highly vascularized and pigmented middle coat and is composed of the iris, ciliary body, and choroid. The retina, embryologically, is an outpouching of the brain.
2. The eye is divided into anterior and posterior *segments* by the lens (the dividing line is just behind the lens). Aqueous humor (0.25 mL) fills the anterior segment, and vitreous (4 mL) fills the posterior segment.
3. Aqueous humor is a liquid that is continuously produced and reabsorbed, with a turnover time of approximately 100 minutes. In contrast, the vitreous humor is a gel-like substance that forms in utero and is never regenerated. The rapid turnover of aqueous but persistence of vitreous is one reason why a break in the posterior lens capsule during cataract surgery greatly increases the risk of postcataract endophthalmitis. The aqueous is commonly contaminated by ocular surface flora during eye surgery, but these bacteria are usually cleared unless they reach the vitreous (see Chapter 114).
4. There is a blood-eye barrier similar in many respects to the blood-brain barrier. Many antibiotics that penetrate the blood-brain barrier also penetrate the blood-eye barrier and therefore may be good choices for treating intraocular infections.

TYPES OF EYE INFECTIONS

Patients with most types of eye infections have no systemic evidence of infection and usually feel well, aside from their eye. The exceptions are patients with endogenous endophthalmitis, some types of uveitis, and orbital infections. However, even patients with these types of eye infections may be afebrile and have a normal white blood cell count.

Conjunctivitis

Conjunctivitis is characterized by eye irritation or discomfort but not significant pain unless there is also involvement of the cornea. The latter is then termed *keratoconjunctivitis* and may lead to corneal scarring (see Chapters 112 and 113).

Keratitis

Keratitis refers to infection of the cornea (see Chapter 113). The cornea is only 0.5 mm thick in the center and has a five- to seven-cell–thick epithelium overlying the stroma and endothelium. Keratitis may occur in the epithelium only (e.g., dendritic keratitis in an initial episode of herpes simplex keratitis), the stroma or interstitium (e.g., interstitial keratitis of syphilis), or both as an infiltrate or ulceration (e.g., *Pseudomonas* keratitis). In cases of large corneal infiltrates or ulcerations, the keratitis may be appreciated even with a flashlight examination at the bedside.

Along with the overlying tear film, the cornea provides 65% to 75% of the refractive power of the eye, so keratitis usually causes decreased vision. The cornea has no blood vessels but many nerve endings, so keratitis is typically painful. Patients with decreased corneal sensation, such as from repeated attacks of herpetic keratitis, may not appreciate that they have a corneal infection until it is advanced.

Endophthalmitis

Endophthalmitis refers to bacterial or fungal infection inside the eye involving the vitreous, the aqueous, or both (see Chapter 114). Infection may be introduced exogenously (i.e., from the outside in), such as from eye surgery or eye trauma, or endogenously, from bacteremia or fungemic seeding of the eye. Of note, endophthalmitis does not serve as a *source* for bacteremia or fungemia except in rare cases of panophthalmitis (where there is both endophthalmitis and orbital cellulitis).

Uveitis

Uveitis refers to inflammation of the uvea (iris, ciliary body, choroid) or retina (see Chapter 115). Most cases are idiopathic or related to a rheumatologic condition, and the frequency of infectious etiologies varies by the type of uveitis. Uveitis is categorized by the location of maximal inflammation and thereby divided into four types: anterior (iritis, iridocyclitis); intermediate (pars planitis); posterior (retinitis, chorioretinitis, choroiditis); or panuveitis. It is important to understand the type of uveitis, because the differential diagnosis and the likelihood of an infectious etiology vary according to the anatomic category.

Periocular Infections

Periocular infections are those involving the soft tissues surrounding the globe of the eye (see Chapter 116). The term *periocular* is not precise and encompasses both *preseptal* and *orbital.* It is important to distinguish preseptal from orbital infections. Preseptal infections, also sometimes called "periorbital" infections, involve the superficial (preseptal) part of the lids and do not threaten vision. Orbital infections (orbital cellulitis, subperiosteal abscess, orbital abscess) are much more serious and are often vision threatening. The two conditions may be distinguished at physical examination; orbital infections cause one or more "orbital signs" (decreased vision, limitation in extraocular movement, proptosis), whereas preseptal infections do not. Of note, the proptosis may not be obvious at gross examination and may be detected only with use of a Hertel exophthalmometer. A difference between the eyes of 2 mm or more is significant.



FIG. 111.1 **Diagram of the eye.**

UNDERSTANDING THE OPHTHALMOLOGIST'S NOTE

The ophthalmologist's examination starts with visual acuity and intraocular pressure (IOP) measurements and then proceeds in sequence from the surface of the eye to the fundus. The ophthalmologist uses a slit lamp and indirect ophthalmoscope for a complete examination. The direct ophthalmoscope, used by most non-ophthalmologists, does not show most corneal abnormalities or the degree of intraocular inflammation or provide a full view of the fundus. Perhaps as a consequence, many serious eye infections are initially misdiagnosed as conjunctivitis by the non-ophthalmologist. Symptoms that distinguish more serious eye infections from conjunctivitis include decrease in baseline vision and eye pain. Patients with these symptoms should be referred to an ophthalmologist for an urgent examination.

Visual Acuity and Measuring Low Vision

The ophthalmologist's note usually starts with a measurement of visual acuity. This is measured both "with correction" and "without correction," meaning correction of refractive error (e.g., through use of glasses). A "pinhole" visual acuity is measured by having the patient look through a card with a pinhole in it. This mimics optimal refraction and distinguishes visual loss due to uncorrected refractive error from more serious etiologies.

Patients with low vision are particularly affected by lighting conditions, and the non-ophthalmologist should keep this in mind. Patients being examined at the bedside in a dimly lit hospital room may have better vision when examined under good lighting.

The best visual acuity the patient can achieve with each eye is always recorded. Patients with serious eye infections may have worse vision than can be measured with the Snellen eye chart; the "big E" at the top of the chart is 20/400 vision. If the patient has very low vision, it is recorded as one of the following, in descending order: "count fingers" (CF), "hand motion" (HM), or "light perception" (LP). For count fingers vision, the distance of the examiner's fingers is recorded, so "CF at 3 feet" is recorded and represents better vision than "CF at 1 foot." If the patient cannot detect a bright light shining directly in his or her eye, he or she has "no light perception" (NLP) vision, or complete blindness in that eye. Visual recovery from NLP is extremely unlikely in an infected eye unless the NLP recording is transient. Any vision better than NLP is worth saving, and an aggressive approach to eye infections is important; patients value even LP vision. In addition, there are many factors that affect vision in the setting of an acute eye infection, and some of these are reversible. The patient's vision 3 months after an acute bacterial endophthalmitis, for example, is usually much better than it is 3 days after presentation.

TABLE 111.1 Common Abbreviations

ABBREVIATION	MEANING
OD	Right eye
OS	Left eye
OU	Both eyes
V_A	Visual acuity. By convention, the right eye vision is given above the left, so the following symbol means that the right eye vision is 20/30 and left eye vision is 20/50: $V_A \begin{matrix} 20/30 \\ 20/50 \end{matrix}$
BCVA	Best corrected visual acuity (meaning with refractive error corrected, e.g., patient wearing glasses)
PH	Pinhole, used to mimic glasses in order to quickly correct for refractive error and give a measurement close to the best corrected visual acuity
CF	Count fingers vision (best vision is ability to count fingers; often given with distance of examiner's fingers from face)
CF @ 3 feet	Patient can count fingers when fingers are 3 feet away (better, e.g., than CF @ 1 foot)
HM	Hand motion (best vision is ability to detect when the examiner's hand is moving)
LP	Light perception (best vision is ability to tell when a light source, such as a flashlight, is turned on or off)
NLP	No light perception
T	Tonometry measurement of intraocular pressure; as with visual acuity, the right eye pressure is listed above the left eye pressure
IOP	Intraocular pressure—normal is 10–20 mm Hg
AC	Anterior chamber
PC	Posterior chamber (note that this is part of *anterior segment* of the eye)
IOL	Intraocular lens. This artificial lens is placed in the eye during cataract surgery to help with refraction; it is nearly always placed in the posterior chamber (the part of the anterior segment between iris and lens)
PCIOL	Posterior chamber intraocular lens (the usual location of the IOL)
K	Cornea

Intraocular Pressure

The IOP is usually measured with a tonometer, so a symbol of "T" is often listed in the ophthalmologist's note. Normal IOP is 10 to 20 mm of mercury, and both very low and very high pressures are dangerous to the eye. Very low pressure may occur in a keratitis case, for example, if the corneal infection has led to a corneal perforation and aqueous humor leak. High IOP often occurs in cases of acute endophthalmitis or infectious uveitis in which there is marked inflammation. High IOP must be treated to prevent glaucomatous damage to the eye.

Ophthalmology Examination

The ophthalmologist records the eye examination findings from front to back, starting with the lids and adnexae and ending with the funduscopic examination. Common abbreviations used are listed in Table 111.1. To illustrate the interpretation of an ophthalmology note, the following are examples of eye infection cases referred for infectious diseases consultation. Interpretation of each line of the note is listed in italics.

Case Example 1

A 65-year-old woman with multiple medical problems presented with 5 weeks of painless decrease in vision in her right eye. This had been

her better eye, with baseline visual acuity of 20/40. The onset of symptoms was 2.5 months after she had been hospitalized with pneumonia and treated with intravenous antibiotics for 10 days via a peripherally inserted central catheter.

Ophthalmologist's Examination

Exam OD (OD = right eye):

V_A sc LP; PH NI
*(**V**isual **A**cuity without correction is **L**ight **P**erception only; **P**in**h**ole examination shows **N**o **I**mprovement)*
IOP 15 *(**I**ntra**o**cular **P**ressure is 15 mm Hg)*
lids—normal
conj—mild injection *(conj = conjunctiva)*
K—mild edema *(K = cornea)*
AC—3+ cells, no hypopyon *(**A**nterior **C**hamber has 3+ white blood cells, on a scale of 0 to 4+ with 0 being normal. There is no hypopyon, meaning no layer of white blood cells in the anterior chamber. A hypopyon is illustrated in Chapter 114.)*
iris—WNL *(within normal limits)*
lens—PC IOL *(The native lens has been replaced by an artificial **i**ntra**o**cular **l**ens, placed in the **p**osterior **c**hamber, which is the usual location.)*
fundus: vitreous 4+ cells, retina hazy view—? retinal infiltrate supratemporally *(Vitreous has 4+ white blood cells, on a 0 to 4+ scale with 0 being normal. This eye therefore has marked vitritis and this produces a hazy view of the fundus, so details such as a retinal infiltrate are difficult to see. The funduscopic photograph of this case is shown in Fig. 111.2.)*

Discussion

The ophthalmologist's interpretation in this case was panuveitis. The differential diagnosis of panuveitis is discussed in Chapter 115. The patient's diagnosis was endogenous *Candida* endophthalmitis, presumably from transient candidemia 4 months earlier while she had an indwelling central venous catheter.



FIG. 111.2 Funduscopic photograph in a case of endogenous *Candida* endophthalmitis.

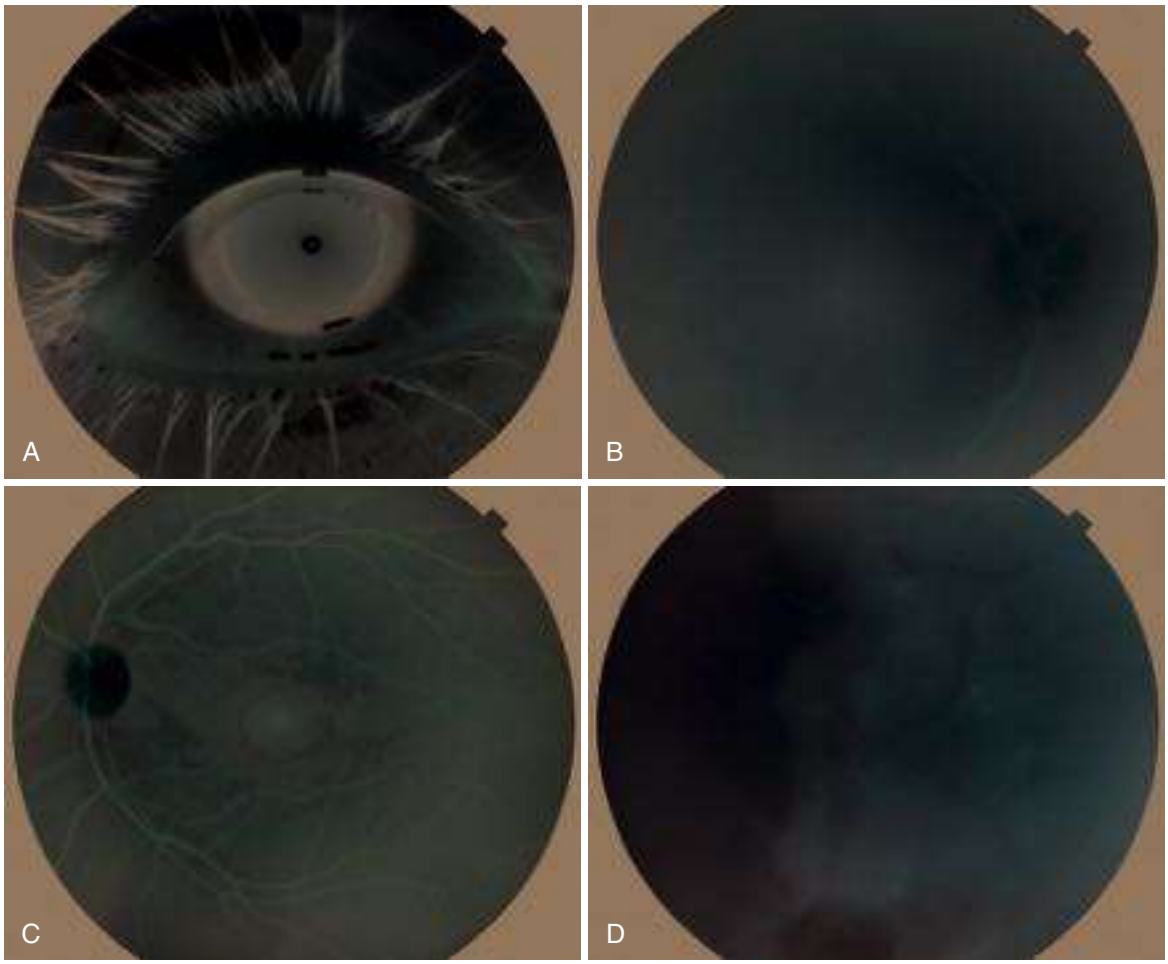

FIG. 111.3 A 19-year-old previously healthy patient with acute retinal necrosis in his right eye. The cause was herpes simplex. (A) External view. (B) Limited funduscopic view of affected eye. (C) Funduscopic view of the patient's other (normal) eye. (D) View of periphery of retina, showing the peripheral necrotizing retinitis and vasculitis (whitening and hemorrhage).

Case Example 2

A 19-year-old otherwise healthy man presented to his college infirmary with a 1-day history of right eye redness but no drainage. He had no history of prior eye problems and did not wear contact lenses. He was initially misdiagnosed with conjunctivitis and prescribed a topical antibiotic. Over the next 3 days, he developed blurring in his peripheral vision and eye pain. He was referred to an ophthalmologist.

Ophthalmologist's Examination

Exam OD *(right eye)*:
V_A—20/70
T—15 *(normal intraocular pressure)*
Lids—normal
Conjunctiva = normal
K = clear *(cornea clear)*
AC = 3+ cells *(3+ white blood cells in the anterior chamber)*
Lens = clear
Fundus: vitreous 3+ cells *(white blood cells)*, blurred disk margins, peripheral retinal whitening with vasculitis

Discussion

The external view of the right eye (Fig. 111.3A) shows injection but no discharge. The patient's eye pain and decreased vision exclude the diagnosis of conjunctivitis. The view of the fundus (Fig. 111.3B) is hazy owing to the intraocular inflammation (3+ white blood cells in aqueous and vitreous, as noted in the ophthalmologist's examination). This is in contrast with the clear view of the fundus in the patient's left (normal) eye (Fig. 111.3C). Examination of the affected eye with the indirect ophthalmoscope shows the peripheral necrotizing retinitis: areas of whitening due to retinal necrosis, and hemorrhage due to vasculitis, as noted in the ophthalmologist's note. The ophthalmologist correctly diagnosed acute retinal necrosis, a vision-threatening viral retinitis usually due to either herpes simplex or herpes zoster and discussed further in Chapter 115. This infection often occurs in otherwise healthy patients, as in this case, and is due to reactivation of latent virus.

OPHTHALMIC GLOSSARY OF INTEREST TO THE INFECTIOUS DISEASES PRACTITIONER

Aphakic, pseudophakic: An aphakic eye has no lens. A pseudophakic eye has a natural lens replaced with a prosthetic lens.

Fluorescein staining of the cornea: Useful in visualizing corneal ulcerations, such as herpetic keratitis.

Homonymous hemianopia: Loss of the left or right visual field, the same in both eyes.

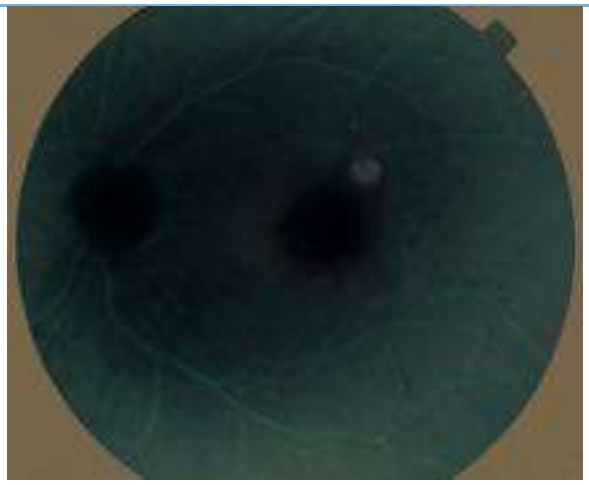

FIG. 111.4 Funduscopic photograph of a patient with reactivation ocular toxoplasmosis. New active lesion (creamy yellow) adjacent to a black "scar" from a prior episode. The new lesion threatens the macula.

LASIK (laser-assisted in situ keratomileusis): Laser eye surgery to reshape the cornea in order to correct refractive error.

OCT (optical coherence tomography): Noninvasive method to visualize the retina and subretinal tissue in cross section. Usually used for macular degeneration or diabetic retinopathy but can be used for evaluation of papilledema, subretinal fluid, and subretinal masses.

Pars plana vitrectomy: Surgical removal of a portion of the vitreous humor for diagnostic and/or therapeutic reasons (e.g., diagnosis and treatment of endophthalmitis; see Fig. 114.3).

Scotoma: An area in the visual field with altered or no vision, surrounded by normal vision.

CONCLUSION

Ophthalmologists treat most eye infections without consulting an infectious diseases specialist. A patient presenting to an ophthalmologist with decreased vision and the funduscopic findings shown in Fig. 111.4, for example, would not usually be referred to an infectious diseases physician. This case would be readily diagnosed by the retina specialist as ocular toxoplasmosis because the appearance of the fundus is classic for that disease. However, ophthalmologists may refer patients in whom a diagnosis is in question or in whom the usual treatment for a presumed diagnosis is failing. In these cases, collaboration between the ophthalmologist and the infectious diseases specialist may prove invaluable.

112 Microbial Conjunctivitis

Nalin M. Kumar, Scott D. Barnes, Deborah Pavan-Langston, and Dimitri T. Azar

SHORT VIEW SUMMARY

Definition
- Microbial conjunctivitis involves inflammation of the thin lining of the inner eyelid and front of the eyeball caused by bacteria, viruses, fungi, or parasites.

Epidemiology
- Conjunctivitis affects male and female patients of all ages.
- Viruses are the most common cause of infectious conjunctivitis, with bacteria being the second most frequent agent.
- Bacterial conjunctivitis is more prevalent in children; viral conjunctivitis is more prevalent in adults.
- Risk factors include contact lens wear, contaminated ocular medications, exposure to an infected person, vaginal versus cesarean delivery, and visits to camps and swimming pools.

Microbiology
- Nonherpetic viral conjunctivitis and bacterial conjunctivitis are the most common causes of infectious conjunctivitis.
- Common causes of bacterial conjunctivitis include *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus* spp., *Moraxella, Corynebacterium diphtheriae, Neisseria* spp., and enteric gram-negative rods.
- The most common cause of viral conjunctivitis is infection with adenovirus, but other viral causes include herpes simplex virus (HSV), enterovirus, and varicella-zoster virus.
- Neonatal conjunctivitis is commonly due to transmission from the mother during childbirth of sexually transmitted bacteria including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Diagnosis
- Symptoms of conjunctivitis include itching, increased ocular secretions, swelling of the conjunctiva or eyelids or both, pink color in the white of the eyes, and light sensitivity.
- Clinical presentation of acute bacterial conjunctivitis includes rapid onset of unilateral lid edema, adherence of the eyelids on waking, conjunctival injection, mucopurulent discharge, and involvement of the second eye within 1 to 2 days. Itching is uncommon. In contrast to viral and chlamydial conjunctivitis, there is no preauricular lymphadenopathy.
- Clinical presentation of viral conjunctivitis is acute, unilateral conjunctivitis with involvement of the second eye occurring often within 1 week. It is associated with a watery to mucous discharge and enlargement of preauricular lymph nodes.
- Routine laboratory evaluation is not usually required except in infants in the first month of life, in whom cultures and smears for bacterial, chlamydial, and herpetic causes need to be performed.
- If bacterial conjunctivitis is suspected, conjunctival scraping for identification of bacterial species by further analysis is recommended for guidance of appropriate antibiotic therapy.
- Viral conjunctivitis can often be diagnosed from signs (e.g., common cold), symptoms (e.g., watery, rather than mucous, discharge), and patient history (e.g., such as employment as a veterinarian or poultry worker). Laboratory tests are not usually necessary.

Therapy
- Use of topical antibiotics is recommended for contact lens wearers, patients with suspected cases of chlamydial and gonococcal conjunctivitis, patients with mucopurulent discharge and eye pain, and patients with preexisting ocular surface disease.
- Acute bacterial conjunctivitis is treated with a broad-spectrum topical agent such as sulfacetamide, trimethoprim-polymyxin B, or a fluoroquinolone (ciprofloxacin, levofloxacin, or moxifloxacin) usually administered as eyedrops every 3 hours when awake for 7 to 10 days. Topical azithromycin has been shown to be effective in treating bacterial conjunctivitis with a 3-day course.
- Viral conjunctivitis spontaneously resolves within days to weeks, usually without adverse sequelae to the conjunctiva, but associated keratitis may have long-term sequelae. HSV conjunctivitis in newborns should be treated with intravenous acyclovir.
- Use of topical antibiotics in viral conjunctivitis should be avoided, as they have no role and may have adverse treatment effects.
- Use of rapid antigen tests to diagnose suspected viral conjunctivitis is recommended before treatment with antibiotics.
- Adult inclusion conjunctivitis involves systemic treatment for 3 weeks with tetracycline, doxycycline, or erythromycin, with the caution to avoid tetracycline in children younger than age 8 and in pregnant or lactating women. The use of oral azithromycin has also been found to be effective.
- For hyperacute bacterial conjunctivitis, the prevalence of penicillin-resistant organisms has made ceftriaxone the treatment of choice.
- For neonatal chlamydial conjunctivitis, if erythromycin or tetracycline ointment is applied to the conjunctival surface within 1 hour after delivery, the chance of developing chlamydial conjunctivitis is reportedly almost zero. Two weeks of oral erythromycin therapy is given to a newborn with laboratory-proven chlamydial conjunctivitis; a second course may be given if adequate resolution is not achieved with the initial treatment.
- Spread of conjunctivitis is limited by following good hygiene steps including washing hands often; washing any discharge from around the eyes multiple times during the day; and using fresh washcloths, cotton balls, or tissues each time.

CONJUNCTIVITIS

Conjunctivitis affects people of all ages and is seen in all geographic locations; it is the most common inflammation of the eye and ocular adnexa.[1,2] Conjunctivitis can be divided into infectious and noninfectious causes. The most common cause of the latter is allergy. The economic impact for prevention, diagnosis, and treatment of microbial conjunctivitis is immense.[3] The various forms of conjunctivitis caused by viruses, chlamydiae, bacteria, parasites, fungi, and antigens tend to share a number of signs and symptoms, but there are some clinical differences that suggest the appropriate identification and treatment. Conjunctivitis can be either primary or secondary to systemic diseases such as chlamydia. The patient's history and the season of the year often suggest a potential diagnosis, which may then be confirmed through clinical examination and possibly laboratory evaluation. Different agents cause acute versus chronic conjunctivitis. Onset within 4 to 6 weeks before presentation is classified as acute disease. In this chapter we describe

the anatomy and physiology of the conjunctiva and the clinical presentation and laboratory testing of microbial conjunctivitis. We discuss viral conjunctivitis, bacterial conjunctivitis, and neonatal conjunctivitis, and we end with descriptions of fungal and parasitic conjunctivitis.

ANATOMY AND PHYSIOLOGY

The inner surface of the eyelid is covered by a mucous membrane called the *conjunctiva*. This membrane lining the lids (palpebral conjunctiva) is reflected on itself, forming an inferior and superior cul-de-sac, or fornix, as it then covers the surface of the globe (bulbar conjunctiva) and extends to the edge of the cornea (limbus). These fornices form a physical barrier that prevents a foreign body (e.g., contact lens) from getting into the orbital space. In addition to connecting the lids to the globe, the conjunctiva produces mucus as part of the tear film. The conjunctiva and tear film provide protection of the ocular surface from pathogens accomplished via mechanical means and via the resident immune tissue.

The conjunctiva is made up of a superficial epithelial layer overlying the substantia propria. The conjunctival epithelium possesses goblet cells, unique among stratified, nonkeratinized epithelia. These goblet cells contribute to the production of mucins in the tear film. Corneal stem cells are known to exist at the limbus and peripheral cornea. A similar search for conjunctival stem cells has produced interesting results. Conjunctival cells with stemlike activity have been identified in rabbits, and subdermal injection of clonal cultures of conjunctival epithelium in nude mice has produced cysts with goblet cells and stratified epithelium suggesting pluripotency that seems to give rise to both cell types.[4,5] The connective tissue of the substantia propria is loose and highly vascularized, properties that allow for the dramatic clinical appearances of significant edema and injection. Abundant numbers of lymphocytes, mast cells, plasma cells, and neutrophils are found throughout the connective tissue.[6] This lymphoid tissue does not form actual lymph nodes; however, its abundance, combined with the phagocytic properties of the conjunctival epithelium, demonstrates the nature of this tissue in dealing with infectious organisms.[7]

CLINICAL PRESENTATION

History and Physical Examination

Particular attention should be paid to the time course of the condition, any inciting events, prior and current medication use, and the patient's own report of the associated symptoms. The physical examination focuses on the appearance of the periorbital skin and other mucous membranes (e.g., nasal, oral); unilaterality/bilaterality; the appearance of the conjunctiva; the associated discharge; visual acuity; and any specific facial, lid, and corneal involvement. A clinical approach that describes a flow chart for diagnosis of suspected acute conjunctivitis has been described and may be useful for initial evaluation of the patient.[8]

Eye Pain

In contrast to keratitis, ocular pain is not common with most forms of conjunctivitis. There is usually some degree of irritation, most commonly described as a foreign-body sensation. Although uncommon in most forms of conjunctivitis, the presence of ulcerated lesions in the eyelid and conjunctiva in herpetic, smallpox, and vaccinia conjunctivitis may be quite painful. If the conjunctival process secondarily affects the cornea, eye pain may also become more prominent. Therefore the presence of significant eye pain should prompt a more thorough search for lid/corneal involvement or intraocular inflammation.

Itching

Almost all patients with conjunctivitis complain of variable ocular burning and itching. However, severe itching tends to be a hallmark of the allergic and toxic causes of conjunctivitis. In addition to the resident lymphoid tissue, the conjunctiva has plentiful immunoglobulin E and mast cells. Degranulation of mast cells and histamine released in response to an inciting antigen are responsible for the significant itching.

More recent studies have focused on whether itching can be regarded as a top clinical sign and symptom for conjunctivitis. Using a logistic regression model, Rietveld and colleagues[9] reported itching as one of the three indicators that provided optimal discrimination between patients with and without a positive culture. They reported that infectious conjunctivitis history and itch both made the probability of current bacterial involvement less likely. They also noted that mattering and eyelid adherence in the morning was a stronger predictor for bacterial conjunctivitis. This information has been incorporated into the practice guidelines of the Dutch College of General Practitioners.[10]

Visual Acuity

Visual acuity is usually normal or mildly decreased with conjunctivitis. Unless the cornea has become secondarily involved, vision is preserved. The ocular irritation and discharge may affect the ability to read an eye chart; a topical anesthetic agent and surface irrigation may improve the office examination of vision. A reduction in vision should prompt a search for an associated cause other than the conjunctivitis.

Conjunctival Hyperemia

The rich network of subepithelial vessels that runs throughout the conjunctiva becomes markedly dilated and congested. Against the background of the relatively avascular sclera, this hyperemia appears quite impressive. On closer examination, the hyperemia appears greater near the conjunctival periphery than in the limbal region (near the corneal border). Saccular aneurysms, petechiae, and subconjunctival or intraconjunctival hemorrhages may be present.

Periocular and Periorbital Skin

Some cases of conjunctivitis have associated skin changes. Skin lesions typical in poxviruses, immune-mediated diseases (Stevens-Johnson syndrome), and herpetic conditions are usually not difficult to recognize; however, a close examination may help identify causes that produce less obvious skin findings. Subtle vesicular changes on the lid margin may be the only sign of an otherwise unimpressive herpetic conjunctivitis. Numerous lesions of molluscum contagiosum provide an easy diagnosis, but small, isolated lesions may be missed if they are buried near the eyelid margin or obscured by the eyelashes. Ocular rosacea is quite common and usually bilateral. Caution must be used when diagnosing rosacea in unilateral cases because sebaceous gland carcinoma can manifest in such a fashion. Allergic conjunctivitis incited by antigens such as cosmetics, soaps, lotions, and some medications often has a typical periocular dermal manifestation.

Sweet syndrome (acute febrile neutrophilic dermatosis) is commonly associated with conjunctivitis.[9] The ocular manifestations of Sweet syndrome include periorbital and orbital inflammation, dacryoadenitis, conjunctivitis, episcleritis, scleritis, limbal nodules, peripheral ulcerative keratitis, iritis, glaucoma, and choroiditis. The ocular inflammation appears concurrently with skin lesions. In addition, the syndrome can manifest with optic nerve involvement.[11] Treatment of the ocular complications of Sweet syndrome includes systemic administration of corticosteroid or cyclosporine.[12]

Discharge

Ocular secretion is almost universal in conjunctivitis. Excessive tearing results from either increased lacrimation or impaired lacrimal outflow. Increased mucin production, especially relative to the aqueous component of the tear film, is a common finding. Exudate from the conjunctival surface contains varying proportions of protein and cellular debris. A serous exudate is mainly proteinaceous; a purulent exudate is more cellular (e.g., leukocytes, fibrin). The combination of proteins, fibrin, mucin, and sloughed epithelial cells can be copious depending on the cause of the inflammation. Significant matting and "sticking together" of the eyelids and eyelashes are common.

Conjunctival Edema (Chemosis) and Conjunctivochalasis

The loosely adherent subepithelial connective tissue allows the conjunctiva an impressive degree of edema. Hemodynamic changes and altered vascular integrity allow transudation through fenestrated capillaries, resulting in chemosis. This edema can be so prominent that the conjunctiva appears to be "bulging" out from between the eyelids, and it may cause exposure of the ocular surface if the eyelids cannot adequately close. Depending on the inciting agent, the chemosis may become

hemorrhagic. Acute chemosis is often self-limited, but chronic chemosis can lead to conjunctivochalasis, or laxity of the conjunctiva, with resultant redundancy sometimes draping over the lower lid margin.

Conjunctival Papillae

The palpebral conjunctiva contains connective tissue septa that provide anchorage for the tarsus (dense connective tissue providing strength to eyelids). Conjunctival inflammation may result in dilated subepithelial blood vessels that become surrounded by an infiltrate of mixed inflammatory cells (e.g., neutrophils, lymphocytes). This edema produces elevated mounds of conjunctival epithelium, with the septa restricting diffusion beyond the fibrovascular core. The mounds, or papillae, have a central red dot corresponding to the dilated capillary viewed from above. A mild papillary reaction has a velvety appearance; increasing severity or chronicity may lead to enlarged, cobblestone-like papillae. Mucus and purulent material may collect within the furrows between adjacent papillae. The conjunctiva beyond the tarsus is less likely to reveal papillae because the septal connections decrease toward the fornices. With prolonged or recurrent inflammation, the septal anchors may weaken to the point at which the papillae appear reduced because of confluence. Although papillae are fairly nonspecific, they appear more commonly in cases of bacterial and allergic conjunctivitis. They are usually much less than 1 mm in diameter, but giant papillae with diameters of 1 to 3 mm may be seen in association with contact lenses, ocular prostheses, or exposed corneal sutures.

Large cobblestone papillae on the superior tarsal conjunctiva and limbal conjunctiva are common with vernal conjunctivitis. The cobblestone papillae characteristically are easily visible with eversion of the upper lid. On slit-lamp examination, they are 1 to 8 mm in diameter, have a central core of blood vessels, and stain with fluorescein at their apices from erosion during active inflammation. Laced between and on top of the giant papillae is a ropy mucoid discharge that can form a pseudomembrane. The papillae in the upper lid may become so severe as to cause a mechanical ptosis from their weight. In vernal conjunctivitis, white dots (Horner points or Trantas dots) can occur in the corneal limbus and persist for 2 days to 1 week. Follicles are not a feature of this disease. Vernal conjunctivitis can cause a reticular subepithelial fibrosis but does not cause keratoconjunctivitis sicca and only rarely causes cicatrization of the conjunctiva.[13]

Conjunctival Follicles

Lymphoid tissue is normally present within the substantia propria of the conjunctiva except in neonates, in whom follicles are not visible. Conjunctival follicles are small, elevated clusters of lymphocytes. Small follicles can be observed in the noninflamed conjunctiva, especially in the lower fornix, and clusters of noninflamed follicles have been observed in children and adolescents in a condition called *benign lymphoid folliculosis*.[14] Follicles associated with conjunctivitis are usually of recent onset, inflamed, and enlarged. Follicles used to be distinguished from papillae. Follicles have a similar elevated appearance to conjunctival papillae; however, there is no central vascular core in conjunctival follicles. The vessels surround and peripherally encroach on the raised follicle, with the central lymphocytes and other mononuclear cells often obscuring the vessels. Except in giant papillary conjunctivitis, papillae are smaller than follicles. Follicles often have a smooth, glistening surface and are most commonly seen near the tarsus, although their presence has been described on the bulbar and limbal conjunctiva.[15] As with papillae, the presence of follicles is a nonspecific sign, but follicles are most commonly associated with viral, chlamydial, and toxic conjunctivitis.

Membranes and Pseudomembranes

Membrane formation results from intertwining of the conjunctiva with coalescence of an inflammatory coagulum, forming a yellowish white membrane overlying the palpebral conjunctiva. If the fibrinous layer is intertwined with the conjunctiva via granulation tissue, it is a true membrane and will cause bleeding when removed. Pseudomembranes have a similar appearance but are not as adherent and do not bleed when removed. Viral and bacterial causes of conjunctivitis have an increased likelihood of membrane formation, although the presence of a membrane does not rule out other causes.

Conjunctival Phlyctenules and Granulomas

A phlyctenule is a whitish, nodular collection of chronic inflammatory cells located at or near the limbus, often in the center of a hyperemic area. This represents a delayed hypersensitivity reaction generally associated with *Staphylococcus* spp. or tuberculosis. However, it can also be associated with coccidioidomycosis, candidiasis, lymphogranuloma venereum, or parasitic conjunctivitis.

Although a granulomatous nodule of chronic inflammatory cells with a fibrovascular proliferation is a classic finding in Parinaud oculoglandular conjunctivitis, it is not common with most types of conjunctivitis. A conjunctival granuloma is more indicative of an embedded foreign body or a granulomatous disease (e.g., sarcoidosis, tuberculosis), but it may be seen with chlamydial and fungal conjunctivitis.

Corneal Involvement

Because of the close proximity of the conjunctiva to the cornea, it is not surprising to see extension of the conjunctival inflammation. Corneal involvement can be as mild as punctate epithelial erosions or as severe as frank ulceration that may lead to perforation. A number of bacterial agents that can cause conjunctivitis may penetrate an intact corneal epithelium. Foreign-body sensation, pain, decreased vision, and photophobia all are signs of corneal involvement; however, many of these signs are present with the inciting conjunctivitis alone. Corneal involvement, especially with viral causes, may quickly improve with the resolution of conjunctivitis; however, some associations can lead to undesired sequelae. Continued vigilance, a high index of suspicion, and appropriate treatment of corneal involvement are necessary.

Preauricular Adenopathy

The lymphatic vessels of the eyelids drain primarily to the preauricular lymph node. The medial third of the eyelids and the conjunctiva drain to the submandibular and submental lymph nodes. Preauricular adenopathy is also a nonspecific finding, but it is often present with viral, chlamydial, herpetic, and gonococcal causes of conjunctivitis and may be absent in toxic, allergic, and nongonococcal bacterial conjunctivitis. Submandibular and submental lymphadenopathy are uncommon but are usually present in Parinaud oculoglandular conjunctivitis.

LABORATORY EVALUATION

Routine laboratory evaluation is probably not performed in most cases of conjunctivitis. Although there is agreement that all cases of suspected ophthalmia neonatorum (conjunctivitis in first month of life) should have laboratory evaluation with cultures and smears for bacterial infections, nucleic acid amplification tests (NAATs) for chlamydial infections, and polymerase chain reaction (PCR) assays for herpetic infections, there is not universal agreement regarding which other cases require laboratory evaluation and which types of evaluation are most appropriate. Because most cases of conjunctivitis are viral, it is expected that most patients will experience a self-limited course, with spontaneous resolution after only supportive therapy. The patient's history and physical examination can often predict the need for laboratory evaluation. Clear cases of viral conjunctivitis may not require conjunctival scraping for stains and cultures, but if bacterial conjunctivitis is suspected, such scrapings are recommended to guide appropriate antibiotic therapy. Indiscriminate use of antibiotics without laboratory identification of a bacterial cause may lead to the emergence of resistant organisms or may aggravate the condition as the result of a toxic or immune-mediated reaction associated with the medication. Adenoviruses are responsible for 36% of conjunctivitis cases, and one report estimated that a savings of $429 million per year could be achieved if antigen testing is done before inappropriate treatment with antibiotics.[16]

The most common procedure involves the use of a calcium alginate swab or Culturette device to collect material from the conjunctiva. Material is transferred to slides for appropriate Gram and Giemsa stains and to culture plates (e.g., blood/chocolate agar, fungal media) for further identification and sensitivity testing. Diagnosis of bacterial conjunctivitis has been reviewed by Golde and Gardiner.[17] Smears from bacterial infections reveal numerous neutrophils along with the standard epithelial

and goblet cells. Lymphocytes and monocytes are predominant in viral infections. Herpetic conjunctivitis may reveal multinucleated epithelial cells and eosinophilic, intranuclear inclusion bodies. Chlamydial conjunctivitis often reveals leukocytes, lymphocytes, and epithelial cells with basophilic, intracytoplasmic inclusion bodies; these findings are noted more frequently in children than in adults. The most common agents for bacterial conjunctivitis in children are *Haemophilus influenzae* and *Streptococcus pneumoniae,*[17] whereas the most common agents in adults are *Staphylococcus aureus* and *H. influenzae.*[18] In particular, *H. influenzae* is the causative agent in children who are younger than 6 years old, and *S. aureus* is the causative agent in children older than 6 years.[19] Identification of inclusion bodies can be difficult, and suspected cases of herpes simplex virus (HSV) should have a swab sent for PCR assay. Viral conjunctivitis may be diagnosed with the use of culture, PCR assay, or immunofluorescent techniques.

VIRAL CONJUNCTIVITIS

As a group, viral organisms are probably the most common cause of conjunctivitis. Viral conjunctivitis, often called *pink eye,* is one of the most frequent reasons for a visit to the emergency department or physician's office. Because the diagnosis can usually be made clinically, viral cultures and laboratory evaluations are not commonly performed.[9] The viral organisms typically produce an acute, unilateral conjunctivitis with involvement of the second eye occurring often within 1 week. The watery discharge and conjunctival hyperemia can be quite impressive. Preauricular adenopathy is often noted on the involved side. However, it should be noted that many cases are misdiagnosed as bacterial conjunctivitis.[2] Although many health care providers prescribe antibiotics in the mistaken notion that such discharge and hyperemia have a bacterial cause, most cases of viral conjunctivitis spontaneously resolve within days to weeks, usually without adverse sequelae. Numerous viruses can cause conjunctivitis, and many can be identified by slightly differing features of the disease course.

Adenoviral Conjunctivitis

Adenoviruses are responsible for two of the most common types of conjunctivitis (see Chapter 142). These infections are spread through respiratory fomites or by direct contact with conjunctival secretions. The incubation period varies from 5 to 10 days, with the clinical process lasting 5 to 15 days.[20] Nineteen different serotypes of adenovirus have been reported to cause conjunctivitis.[21] Once the disease course passes, immunocompetent patients are protected from a recurrent adenoviral infection caused by the same serotype.

The advent of new laboratory techniques such as real-time PCR, which can identify more pathogens than routine virus isolation, has led to more accurate diagnosis of adenoviral conjunctivitis.[22] A review of the available methods for identifying adenoviruses has suggested that a commercially available immunoassay kit (RPS Adeno Detector; Rapid Pathogen Screening Inc., Sarasota, FL) using antibodies to the hexon protein may be as sensitive and ideally suited for analysis in a clinical environment.[2,23]

Although no effective treatment for viral conjunctivitis exists, some of the symptoms may be alleviated by cold compresses, topical antihistamines, and artificial tears.[24] Current antiviral medications have not been demonstrated to be useful.[25] Topical antibiotics and the use of eye droppers can complicate the clinical presentation and increase the risk of spreading the infection.[2]

Pharyngoconjunctival Fever

Pharyngoconjunctival fever has been reported to be the most common ocular adenoviral infection.[26] The most commonly implicated serotypes in pharyngoconjunctival fever are 3, 4, and 7, but it has been associated with types 1, 5, 6, and 14 as well, having been isolated from conjunctiva, nasopharynx, and feces. The incubation period after exposure is 5 to 12 days (most commonly 8 days).[27] The triad of pharyngitis, fever, and subsequent conjunctivitis is the classic clinical presentation. The conjunctivitis is marked by a follicular reaction accompanied by a mild watery discharge, hyperemia, and chemosis (edema of the conjunctiva). The cornea may have fine, punctate erosions, and preauricular adenopathy is present in about 90% of cases. The condition usually resolves spontaneously within 2 weeks. Supportive treatment with cold compresses and artificial tears is usually sufficient.

Epidemic Keratoconjunctivitis

Epidemic keratoconjunctivitis (EKC) is associated most often with adenovirus serotypes 8 and 19, but it has also been reported with multiple serotypes including types 2 to 4, 7 to 11, 14, 16, and 29. Serotypes other than 8 and 19 may produce a similar clinical picture to the latter but do not have the tendency to widespread epidemic.[27] EKC is more severe than pharyngoconjunctival fever; it lasts 7 to 21 days, with possible corneal sequelae persisting for weeks or months.[28] The mixed papillary and follicular response in the conjunctiva is accompanied by a watery discharge, chemosis, significant hyperemia, and preauricular adenopathy.[20,29] Subconjunctival hemorrhages and conjunctival membranes are found in approximately one-third of patients with EKC, especially in the more severe cases.[30] These membranes (and pseudomembranes) are made up primarily of fibrin, leukocytes, and fibroblasts. Removal of the membrane leaves a bleeding conjunctival surface; removal of pseudomembranes is not associated with such bleeding. The presence of either type of membrane can be associated with formation of conjunctival scarring and symblepharon (adhesion of conjunctival surfaces). Corneal involvement varies from almost ubiquitous diffuse, punctate epithelial elevations to subepithelial infiltrates, seen in 20% to 50% of cases, which may persist for months or longer but usually resolve without scarring or neovascularization.[29] Treatment of EKC focuses on relieving the symptoms and minimizing the spread of this highly contagious disease. Patients are usually infectious for 10 to 14 days after onset. Cold compresses, artificial tears, and possibly decongestant eyedrops constitute the main treatment. Reduced visual acuity or disabling glare from the subepithelial infiltrates often responds to topical corticosteroids.[31] Removal of the membranes and pseudomembranes, along with administration of topical steroids, often has a significant effect on patient comfort. Meyer-Rüsenburg and colleagues[32] reviewed the prevention and treatment of EKC. They recommended the application of rigorous hygienic conditions in medical facilities to reduce viral transmission.

The structural changes occurring during the course of adenovirus EKC from the onset of the disease up to 24 weeks of follow-up have been evaluated by confocal microscopy.[33] Hyperreflective cells in the basal epithelial cell layer were found 1 week after infection. Subepithelial accumulations of dendritic cells, located mainly at the level of the Bowman layer, were also observed at 1 week. Underneath the anterior stroma, clusters of highly reflective, irregularly shaped cells were detected. At 2 weeks, follicular conjunctivitis, focal keratitis, and subepithelial infiltrates were present. Confocal microscopy revealed persistent clusters of hyperreflective basal epithelial cells intermingled with roundish cells that probably represent leukocytes. Dendritic cells had formed an intricate network and, in the anterior stroma a hyperreflective cellular plaque that corresponded to the subepithelial infiltrate was detected. At 24 weeks after onset of the symptoms, density and dimension of dendritic cell clusters were decreased, but stromal hyperreflectivity in the mid-stroma was detected.[33]

Acute Hemorrhagic Conjunctivitis

Also known as Apollo 11 disease, acute hemorrhagic conjunctivitis was initially described in Ghana during the time of the first lunar landing mission in 1969.[34] The disease is associated with coxsackievirus A24 and enterovirus 70 (see Chapter 172 for nomenclature).[35] The classic presentation is rapid onset of severe, painful papillary conjunctivitis marked by chemosis, tearing, and small subconjunctival hemorrhages. Although individual hemorrhages are noted at first, these rapidly coalesce to become confluent. The resultant hemorrhagic chemosis can reach alarming proportions. The cornea may have punctate elevations or erosions, but these rarely progress to subepithelial opacities as seen with EKC. The conjunctivitis tends to clear in 4 to 6 days, but the hemorrhages may persist. Epidemics are quite common, especially in developing countries, where up to 50% of the population may be involved. Treatment again is mainly supportive.

More recent reports have documented several outbreaks of hemorrhagic conjunctivitis with coxsackievirus A24 variant.[36–38,39,40,41] Molecular serotyping methods have been reported to be a good alternative to the

gold standard cell culture–neutralization method for diagnosis in view of its easy accessibility and expected sensitivity.

Herpes Simplex Conjunctivitis

Primary ocular involvement by HSV may manifest as an acute follicular conjunctivitis or keratoconjunctivitis with preauricular adenopathy and often with notable vesiculating periocular skin involvement. Pseudomembranes may be present in the fornices. In 1% to 5% of all HSV cases, skin lesions may be absent.[2] In the absence of frank skin vesiculation, differentiation from adenoviral infection is aided by a careful search of the lid margins for signs of herpetic blistering. Conjunctival swabs should be sent for PCR assay. In contrast to primary disease, recurrent blepharoconjunctivitis is a much more localized infection. Vesicles are localized rather than diffuse, starting as red papules, which form clear vesicles, break, and scab over to heal without scarring. Virus is present in the lesions for about 3 days, although the lesions themselves take about 1 week to heal. Conjunctivitis is usually diffuse and watery. Occasionally, rose bengal or fluorescein staining reveals a conjunctival dendritic ulcer. As opposed to the host of treatment regimens used when herpetic disease affects the cornea and other ocular components, herpetic manifestations limited to the conjunctiva require minimal supportive treatment. There is no role for antiviral agents or corticosteroids; however, an antibiotic ointment such as erythromycin may be used to prevent a bacterial superinfection. Close monitoring for corneal or adnexal involvement is necessary because this complication would necessitate a change in the treatment regimen.

Research into HSV has resulted in mechanistic insights into how this virus gains entry to ocular cells and how it spreads.[42] This includes the involvement of receptors such as nectin cell adhesion molecule 1, herpesvirus entry mediator, heparan sulfate, and paired immunoglobulin-like type 2 receptor as well as the participation of Toll-like receptors and nuclear factor kappa B.[43,44] The involvement of filopodia and endocytic pathways in the initiation and progression of the disease has also been suggested.[42] The discovery that a kinase inhibitor, BX795, inhibits HSV replication is a promising alternative to use of nucleoside analogues and may have therapeutic applications.[45]

Varicella and Varicella-Zoster Conjunctivitis

Although follicular conjunctivitis and preauricular adenopathy of varicella-zoster virus are rarely seen, approximately 4% of patients with chickenpox have conjunctival or corneal findings or both.[45] Papules may be seen on the lids, conjunctiva, and limbus. Vesicles may be found on the semilunar fold and throughout the conjunctiva. The papules form pustules, which then ulcerate as the disease progresses. Again, there is no indication for antivirals or corticosteroids in this herpetic conjunctivitis. Supportive care, with the possibility of prophylactic antibacterial ointment, is usually sufficient. However, herpes zoster ophthalmicus may lead to substantial disability if left untreated.[46,47] These complications may involve the anterior segment, optic nerve, and retina, resulting in a number of ocular pathologies including optic neuritis, glaucoma, retinal vasculitis, and uveitis.[48] (See Chapter 113.)

Variola (Smallpox) Conjunctivitis

Between 10% and 20% of individuals affected with smallpox develop severe ocular complications.[49] Additionally, approximately 7% of unvaccinated patients develop corneal lesions.[50] About 5 days after the onset of clinical disease, an exanthematous, watery conjunctivitis may develop. It frequently clears without complication. However, in some patients, pustules then appear on the bulbar conjunctiva. These painful lesions are associated with a great inflammatory reaction and purulent discharge. The lesions often extend to the cornea, leading to inflammation, scarring, and possible perforation with loss of the eye. Specific treatment is currently not established. A promising but unproven treatment is systemic and topical cidofovir; this agent has some activity against variola in vitro and against poxviruses in animal model systems.[51,52] The drug tecovirimat (ST-246; Arestvyr) may prove to be useful in treating smallpox and is being stockpiled by the US government for use in the event of a bioterrorist smallpox attack (see Chapter 132). Penicillinase-resistant antimicrobial agents should be used if the skin lesions are secondarily infected or if infection is near or involves the eyes. Daily rinsing of the eyes is important in severe cases. There are no data showing that prophylaxis or treatment of variola conjunctivitis with vaccinia immunoglobulin (VIG) has any effect, but many experts would use it to reduce the likelihood of spread.

Vaccinia Conjunctivitis

The attention to smallpox as a bioterror hazard has also brought attention to the complications of smallpox vaccine (vaccinia). Lid and conjunctival involvement is the most common form of ocular vaccinia and is similar to that seen on the arm at the site of the intentional vaccination. Initial formation of vesicles progresses to indurated pustules, which then umbilicate to open sores. The resultant scab formation may occasionally scar and leave depigmented marks in the skin. Vaccinia conjunctivitis is characterized by an acute papillary reaction and serous or mucopurulent discharge. Multifocal ulceration of the palpebral and bulbar conjunctiva occurs commonly. Conjunctival ulcers have a whitish center with surrounding injection and edema; they may be covered by a thick, yellowish gray membrane and may lead to symblepharon formation. Preauricular and submandibular adenopathy commonly accompanies vaccinia conjunctivitis.[53,54] The differential diagnoses of vaccinia lesions of the eyelid or ocular adnexa include molluscum contagiosum, keratoacanthoma, bacterial blepharitis, and HSV or varicella-zoster virus infection.[55]

Clinicians must maintain a high index of suspicion for ocular smallpox vaccine–associated adverse reactions in vaccine recipients and their close contacts. Hu and colleagues[56] reported the case of a 26-year-old woman who developed right preseptal cellulitis and blepharoconjunctivitis after contact with a vaccinated member of the military. The preseptal cellulitis resolved with antibacterial therapy, and the conjunctival infection was treated successfully with a 14-day course of topical trifluridine and a single dose of intravenous VIG.[56]

Compared with historical data on the ocular complications of smallpox vaccination, the incidence of ocular complications during the Department of Defense Smallpox Vaccination program in the United States has been low. In addition, the severity of disease seems to be less than during other vaccination periods. Perhaps these findings are the result of improved screening of vaccinees, prevaccination counseling, postvaccination wound care, and the suggested efficacy of trifluridine in the treatment of ocular vaccinia.[57]

No topical antiviral agents have been approved by the US Food and Drug Administration (FDA) for the treatment of ocular vaccinia, but topical trifluridine, cidofovir, and vidarabine have been shown to be effective in animal and uncontrolled human reports. In addition, use of antiviral ophthalmic medication and VIG has been suggested.[58] VIG has been demonstrated to be effective in treatment of lid and conjunctival lesions.[59,60] A 2002 panel convened by the US Centers for Disease Control and Prevention in Atlanta recommended the treatments shown in Table 112.1 for vaccinia conjunctivitis.[61] VIG is available from the Centers for Disease Control and Prevention.

TABLE 112.1 Treatment of Vaccinia Conjunctivitis

Mild-to-Moderate Disease (Mild Hyperemia and Edema, No Membranes or Focal Lesions)
Adults: Trifluridine (Viroptic) drops 9 times daily for 2 weeks
Children: Vidarabine 3% ointment (Vira-A) 2–5 times daily for 2 weeks
A one-time dose of VIG, 100 mg/kg IM or 6000 U/kg IV, is recommended as adjunctive therapy for moderate conjunctivitis
Severe Disease (Marked Hyperemia, Edema, Membranes, Focal Lesions, Lymphadenopathy, Fever)
A single dose of VIG, 100 mg/kg IM or 6000 U/kg IV, is recommended as adjunctive therapy; repeat in 48 hours if not improved
Adults: Trifluridine (Viroptic) drops 9 times daily for 2 weeks
Children: Vidarabine 3% ointment (Vira-A) ointment 2–5 times daily for 2 weeks
Topical antibiotic to the conjunctiva to prevent secondary bacterial infection

IM, Intramuscular; *IV,* intravenous; *VIG,* vaccinia immunoglobulin.
From Centers for Disease Control and Prevention. Smallpox: Summary of October 2002 Advisory Committee on Immunization Practices Smallpox Vaccination Recommendations. *Atlanta: Centers for Disease Control and Prevention; 2002.*

Other Viral Etiologies

Rubella, rubeola, mumps, influenza, Epstein-Barr virus, papillomavirus, molluscum contagiosum, and Newcastle disease virus all have been implicated in conjunctivitis. Rubella produces a catarrhal or follicular reaction, or both, along with the typical disease findings. Influenza viruses have also been associated with a catarrhal or follicular conjunctivitis. Rubeola (measles) produces a catarrhal or papillary reaction, often with significant discomfort and photophobia. Pale, avascular spots, similar in appearance to the oral Koplik spots, can be found in the conjunctiva.[62] Patients with mumps may develop a catarrhal conjunctivitis and punctate epithelial keratitis with severe photophobia and lacrimation but often little discomfort.[63] A follicular conjunctivitis is present in about 5% of patients with Epstein-Barr–induced mononucleosis.[64] Human papillomavirus can produce lesions on the lid margin and the conjunctiva; a catarrhal conjunctivitis may follow. Molluscum contagiosum lesions on the lid margin may cause an irritating chronic follicular conjunctivitis with punctate keratitis, superior corneal vascular pannus, and cicatricial punctal occlusion. Lesions may also occur several millimeters away from the lid margins yet still cause a follicular conjunctivitis with culture positive for virus.[65] Lesions confined only to the cornea or conjunctiva are rare but not unknown. They are usually seen in patients with immune dysfunction.[66] Newcastle disease, seen primarily in poultry workers, veterinarians, and laboratory technicians, typically produces a unilateral, follicular, and papillary conjunctivitis with hyperemia, edema, and chemosis usually in the lower fornix, mild tearing, and preauricular adenopathy.[67] Poultry workers may develop conjunctivitis from poultry infected with avian influenza viruses such as one outbreak of 78 patients with H7N7 conjunctivitis.[68]

In all of these cases, there is no specific therapy directed toward the conjunctivitis because it is almost always self-limited. Therapy directed toward the causative agent (e.g., removal of molluscum lesions) may hasten resolution of the conjunctivitis.

CHLAMYDIAL CONJUNCTIVITIS

Chlamydia trachomatis Infection

Chlamydial infections cause several important acute and chronic eye infections.[69] Studies using monoclonal antibodies to the chlamydial major outer membrane protein have identified several serotypes of *Chlamydia trachomatis.* Serotypes B, Ba, and D through K, which are often sexually transmitted, can cause a follicular conjunctivitis in an adult (inclusion conjunctivitis). The same serotypes can lead to neonatal conjunctivitis if an infected mother transmits the pathogen to the newborn during vaginal delivery. NAATs, used for genital specimens, are also useful for ocular samples.[70] Repeated infections with *C. trachomatis* serotypes A, B, Ba, and C can cause trachoma, a chronic follicular keratoconjunctivitis that remains the most common cause of preventable blindness in the world. *C. trachomatis* infection can also cause acute reactive arthritis (formerly known as Reiter syndrome),[71] a triad of urethritis, arthritis, and iridocyclitis frequently seen in sexually active young men who are positive for the HLA-B57 histocompatibility allele. In addition, several cases of Parinaud oculoglandular syndrome have been reported with lymphogranuloma venereum, a sexually transmitted disease characterized by painful inguinal lymphadenopathy and caused by *C. trachomatis* serotypes L1 through L3. Although vaccines for chlamydial infections have been developed for animals, such vaccines have not yet been generated for humans. Strategies and promising avenues for development of vaccines for chlamydial infections in humans have been summarized elsewhere.[72] Treatment with systemic antibiotics such as doxycycline and oral azithromycin of both patients and sexual partners is recommended.[24]

Chlamydia pneumoniae Infection

Chlamydia pneumoniae shares considerable homology with *C. trachomatis* and follows a similar life cycle.[72] However, it is transmitted by aerosol droplets; it can target a spectrum of cell types; and it is associated with several chronic inflammatory diseases, most notably atherosclerosis.[73] Although *C. pneumoniae* first was isolated from the conjunctiva, there are few studies on its role in ocular disorders. The organism has been detected in conjunctival swabs collected from patients with conjunctivitis,[74] but a clear association with external ocular disease is lacking. Although it has been suggested that there is an association of *C. pneumoniae* with age-related macular degeneration,[75] most evidence indicates the lack of association of *Chlamydia* infection with age-related macular degeneration.[76]

Trachoma

C. trachomatis serotypes A through C are responsible for trachoma. Active trachoma affects an estimated 84 million people; another 7.6 million have end-stage disease, of which about 1.3 million are blind.[77] The severely blinding condition is endemic in many developing countries, especially in areas of close overcrowding and poor sanitation. Trachoma is typically the result of multiple untreated infections rather than a one-time event. In some trachoma-endemic communities in the Solomon Islands, clinical signs of active trachoma are associated with nonchlamydial microbial species of several types such as *S. aureus*, Adenoviridae, coagulase-negative *Staphylococcus, H. influenzae, Moraxella catarrhalis,* and *S. pneumoniae.*[78] The initial follicular conjunctivitis begins in the upper palpebral conjunctiva and is followed by limbal follicles. Papillary hypertrophy, mucopurulent discharge, superior corneal pannus (neovascularization), and epithelial keratitis are early features of the disease. Later stages are marked by cicatrization of the conjunctiva, cornea, and eyelids.

Trachoma stands on the brink of extinction thanks to a 1998 initiative launched by the World Health Organization (WHO) entitled the WHO Alliance for the Global Elimination of Trachoma by 2020. This program advocated control of trachoma at the community level with four interrelated population health initiatives that form the *SAFE* strategy: *s*urgery for trichiasis, *a*ntibiotics for active trachoma, *f*acial cleanliness, and *e*nvironmental improvement. Evidence supports the effectiveness of this approach, and if current world efforts continue, blinding trachoma will indeed be eliminated by 2020.[79,80] In this context, the success of this approach has been demonstrated by the elimination of trachoma in Nepal.[81] As of 2017, 10 of 41 endemic countries have reported elimination of trachoma.[82] It has been suggested that the SAFE strategy be expanded to include sexual ecosystem and behavioral valuation/education, resulting in the acronym *SAFES.*[83]

The blinding complications of trachoma are the result of corneal exposure and ulceration caused by conjunctival scarring and lid deformities.[84] Two classic findings are Arlt line and Herbert pits. Arlt line is a horizontal line of conjunctival scarring found along the superior palpebral conjunctiva. Herbert pits are sharply demarcated erosions near the limbus that are filled with epithelium after the cicatrization of the limbal follicles. Once regression of the superior pannus occurs, a diffuse corneal haze may be seen. Eyelid deformities are the result of conjunctival scarring. Lids can be turned inward (entropion) or outward (ectropion), and lashes can be directed against the cornea (trichiasis), all of which contribute to an irregular ocular surface. Such irregularities can cause corneal scars, ulcers, neovascularization, and perforation. Certain interventions have been shown to be more effective at eliminating trichiasis. Full-thickness incision of the tarsal plate and rotation of the lash-bearing lid margin was found to be the best technique and preferably delivered in the community.[85]

Most trachoma programs use the WHO simplified grading system, which was specifically designed to allow rapid assessment of prevalence and severity of disease within a population. The grading system is based on the presence or absence of five clinical signs, as follows:[79]

- Trachomatous inflammation follicular: five or more follicles of larger than 0.5 mm on upper tarsal conjunctiva
- Trachomatous inflammation intense: inflammatory thickening obscuring more than half the normal deep tarsal vessels
- Trachomatous conjunctival scarring: presence of easily visible scars in the tarsal conjunctiva
- Trachomatous trichiasis: at least one eyelash rubbing on the eyeball or evidence of recent removal of in-turned eyelashes
- Corneal opacity: corneal opacity blurring part of pupil margin

Although there is no gold standard test for *C. trachomatis,* it has been determined that a clustered latent class analysis using WHO criteria and PCR assay may provide a useful method for diagnosis.[86]

Systemic tetracycline or erythromycin has been given for 3 to 4 weeks. Because the clinical response can often take several months,

topical tetracycline or erythromycin is often used twice daily for 5 days each month for 6 months.[21] This repeated topical treatment is especially useful in situations in which repeat infection is likely. Loosely based on the smallpox eradication efforts, widespread prophylactic systemic antibiotics have been tried in endemic areas in an attempt to eliminate the disease. A single dose of azithromycin was proposed as a good choice for the eradication theory.[87] The use of azithromycin for 3 to 5 days has also been proposed for treatment in patients with bacterial or trachomatous conjunctivitis.[88] With the advent of topical azithromycin, there has been support for consideration of using this eye drop as a treatment for the various ocular sequelae of trachoma.[89] Evidence has been provided that face washing alone or in combination with treatment with topical tetracycline is ineffective in treatment of active trachoma.[90]

Consensus has built on the value of mass treatment strategies for both logistic and efficacy reasons. Important unanswered questions remain about the best distribution strategies including timing of repeat mass antibiotic treatment and duration of treatment. Theoretical models suggest that biannual treatment is necessary when the baseline prevalence is greater than 50% in children. WHO currently recommends mass treatment for at least 3 years if the prevalence of trachoma in children 1 to 9 years of age is greater than 10% and then reassessment of prevalence. Evidence to guide duration of treatment is scarce; however, trachoma is unlikely to be eliminated until the SAFE strategy has been implemented for at least 3 years. A survey was published in 2012 on the clinical management, treatment options, and challenging issues facing elimination of this disease.[91]

Adult Inclusion Conjunctivitis

C. trachomatis can cause a chronic follicular conjunctivitis in adults and neonates. The adult form is usually sexually transmitted, with an estimated 1 in 300 patients with genital chlamydia developing conjunctivitis,[92] but it can occur with orogenital or hand-to-eye transmission of secretions.[93] The most common presentation comprises a unilateral red eye (although it can be bilateral), preauricular adenopathy, papillary hypertrophy, marked hyperemia, mucopurulent discharge, and a follicular reaction. Men often have a concomitant urethritis; women may have chronic cervicitis. Corneal involvement may quickly follow conjunctivitis resulting in punctate keratitis, EKC-like infiltrates, and superior limbal pannus (neovascularization). Corneal scarring and neovascularization are less common with inclusion conjunctivitis than with trachoma, and the upper and lower palpebral conjunctivae are often equally involved, as opposed to the preferentially affected upper conjunctiva in trachoma. However, severe inclusion conjunctivitis may be associated with a chronic, relapsing course leading to characteristics generally seen in trachoma.

Treatment of Adult Inclusion Conjunctivitis

Because of the prominent sexual transmission of this form of conjunctivitis, it is important to simultaneously treat all known sexual partners. Failure to do so often results in more serious sequelae associated with reinfections. Topical antibiotics are relatively ineffective, so systemic therapy is the mainstay of treatment. Tetracycline, doxycycline, or erythromycin is given for 3 weeks, with caution to avoid tetracycline in young children and in pregnant or lactating women. The use of oral azithromycin was found to be effective in the treatment of *Chlamydia* infection, although often more than one course of treatment was required.[94]

Lymphogranuloma Venereum

Certain serotypes of *C. trachomatis* (L1, L2, and L3) have been associated with systemic lymphogranuloma venereum. The associated conjunctivitis is often mild and unilateral, producing a scant, watery discharge. Although the conjunctivitis appears mild, there is impressive edema in the upper and lower eyelids. In addition to the usual preauricular lymphadenopathy, the nodes in the parotid and submaxillary region are also involved. There is a report of lymphogranuloma venereum conjunctivitis causing a keratitis leading to a corneal perforation in a patient with acquired immunodeficiency syndrome.[95] Treatment is similar to that for inclusion conjunctivitis.

BACTERIAL CONJUNCTIVITIS

There is significant disagreement on the actual incidence of bacterial conjunctivitis. Many cases of conjunctivitis are treated as if they were caused by bacterial organisms, but culture-proven bacterial conjunctivitis appears uncommon. The clinical presentation is characterized by a rapid onset of unilateral lid edema, conjunctival injection, and a mucopurulent discharge, followed by involvement of the second eye within 1 to 2 days. *Staphylococcus* spp. and *Corynebacterium* spp. are the most common organisms to colonize the lids and conjunctiva; consequently they are prominent causes of infectious conjunctivitis.[96] Although almost any bacterial organism can cause conjunctivitis given the appropriate set of conditions, the most common ones are *Staphylococcus* spp., *S. pneumoniae, Haemophilus* spp., *Moraxella, Corynebacterium diphtheriae, Neisseria* spp., and enteric gram-negative rods.[97] Wearing contact lenses has also been associated with changes in the ocular conjunctiva, making it more similar to that of the skin microbiota and that may influence the development of conjunctivitis.[98] The prevalence of microbial contamination in counterfeit and decorative contact lenses has also been reported.[99] Cases of contact lens care solution contamination by histamine-producing *Raoultella* spp. is also reported to cause keratoconjunctivitis.[100,101]

Pathogenesis

The pathogenesis of bacterial conjunctivitis usually involves a compromised epithelial surface. Although intact epithelium is an effective barrier to most organisms, *Neisseria gonorrhoeae, C. diphtheriae, Haemophilus aegyptus* (Koch-Weeks bacillus), and *Listeria monocytogenes* can penetrate such a surface through specialized attachments or toxins or both.[102] Injured epithelium or specialized attachments allow adhesion, which may result in the entry of various bacterial products and toxins. Enzymatic components such as proteases, coagulases, collagenases, and fibrinolysins combined with toxins such as those seen in *Staphylococcus* spp. and *Pseudomonas* spp. can disrupt underlying tissue, allowing further bacterial entry and possible isolation from host defense mechanisms.[103] Bacterial conjunctivitis can be clinically categorized as acute, hyperacute, or chronic on the basis of various features.

Acute (Mucopurulent) Bacterial Conjunctivitis

S. aureus, S. pneumoniae, and *H. influenzae* are the organisms that most commonly cause bacterial conjunctivitis. The acute conjunctivitis is marked by unilateral hyperemia, tearing, mucopurulent discharge, and matting of the eyelids. *S. aureus* is the most common agent in adults and children; *S. pneumoniae* and *H. influenzae* occur more frequently in children than in adults.[104] *H. influenzae* is often associated with systemic infections such as upper respiratory tract disease, and its treatment usually requires administration of systemic antibiotics. Viridans streptococci and *Streptococcus pyogenes* can produce an acute conjunctivitis, often with an associated membranous reaction. Gram-negative rods, other than *Haemophilus* spp., rarely cause acute conjunctivitis in immunocompetent patients.

Treatment of Acute Bacterial Conjunctivitis

Appropriate laboratory confirmation of bacterial conjunctivitis should be attained to guide treatment. Although many mild conjunctival infections resolve on their own, topical antibiotic treatment may speed resolution and reduce severity and morbidity.[105] Although topical antibiotics reduce the duration of the disease, comparison of outcomes between treatment and placebo groups showed little difference.[106] Treatment with a topical broad-spectrum agent such as sulfacetamide, trimethoprim-polymyxin B, or a fluoroquinolone is given for 7 to 10 days. Topical moxifloxacin has gained FDA approval to treat bacterial conjunctivitis in patients as young as 3 years of age, and topical azithromycin has been shown to be effective in treating bacterial conjunctivitis with a 3-day course.[107] Appropriate agents may be selected or altered on the basis of laboratory results. In cases of pediatric bacterial conjunctivitis (<1 year of age), erythromycin is the preferred antibiotic because of its decreased side effects.[108] However, there is greater resistance to erythromycin, and so fluoroquinolone may be a good choice because it has the lowest resistance rates regardless of the patient's age.[19]

In one study it was reported that the upper eyelid may have tarsoconjunctival crypts, and these may be a reservoir for the organisms. It was recommended that marsupialization of the crypts be performed because it obliterates the potential space and was found to be curative.[109]

Hyperacute (Purulent) Bacterial Conjunctivitis

The most frequent cause of hyperacute conjunctivitis is *N. gonorrhoeae;* a less severe form can be seen with *Neisseria meningitidis.*[110] This severe disease is most common in neonates, sexually active adolescents, and young adults. The most impressive characteristic is the copious, thick, yellowish green, purulent discharge. Marked chemosis, painful hyperemia, and eyelid edema are seen. In contrast to most cases of bacterial conjunctivitis, there is often tender preauricular adenopathy. There may be conjunctival membrane formation, and the condition may rapidly progress to corneal ulceration and perforation because *Neisseria* spp. can penetrate an intact corneal epithelium in as little as 24 hours.

Treatment of Hyperacute Bacterial Conjunctivitis

Laboratory evaluation via Gram stain and culture is important because the treatment of *Neisseria* conjunctivitis is different than that of most bacterial entities. Topical antibiotics can augment treatment, but systemic therapy is the mainstay with *Neisseria* infections. The prevalence of penicillin-resistant organisms has made ceftriaxone the treatment of choice. Gonococcal conjunctivitis without corneal involvement may be treated with one intramuscular injection of ceftriaxone. Corneal involvement usually requires hospitalization for a 3-day course of intravenous treatment. Topical antibiotic ointments and solutions have been considered, but the most important topical therapy is frequent (every 30–60 minutes) saline irrigation of the conjunctival surface and fornices to remove the inflammatory cells, proteolytic enzymes, and debris, which may be toxic to the ocular surfaces. Because up to one-third of patients with gonococcal conjunctivitis have been reported to have *Chlamydia,* concurrent treatment with tetracycline, doxycycline, or azithromycin may be indicated. A 1.5% azithromycin solution was found to be well tolerated and effective in patients with purulent bacterial conjunctivitis.[90]

Chronic Bacterial Conjunctivitis

The most common causes of chronic bacterial conjunctivitis are *Staphylococcus* spp.[111] Such infections can be difficult to eradicate because the eyelid margins and surrounding skin are heavily populated with staphylococci. Associated exotoxins are thought to be responsible for the effect on the conjunctiva, lids, and cornea. A diffuse hyperemia, minimal mucopurulent discharge, and conjunctival thickening with either a follicular or a papillary reaction are common. Eyelid involvement may manifest as redness, telangiectasia, loss of lashes, thickening, or recurrent hordeolum (stye), and ulceration at the base of the eyelashes may be seen. Maceration and ulceration of the inner and outer canthal angles may be seen in chronic blepharoconjunctivitis caused by *Moraxella* spp. Chronic staphylococcal blepharoconjunctivitis may lead to marginal corneal ulceration, most likely as a result of an immune-mediated hypersensitivity reaction. Gram-negative bacteria are more common in chronic conjunctivitis than acute conjunctivitis.[112] Organisms more often associated with the intestinal flora can be associated with chronic conjunctivitis. *Proteus mirabilis* is the most common of these, but *Klebsiella pneumoniae, Escherichia coli,* and *Serratia marcescens* have also been described.

Treatment of Chronic Bacterial Conjunctivitis

Treatment of chronic bacterial conjunctivitis demands appropriate antibiotic therapy combined with aggressive lid hygiene and possible evaluation of the lacrimal system. Laboratory evaluation may guide appropriate antibiotic treatment, often with erythromycin or bacitracin ointment. Lid hygiene involves the use of warm compresses, eyelid scrubs with nontearing shampoo, and gentle lid massage because the meibomian (sebaceous gland) orifices at the base of the eyelashes may harbor the inciting agents. The lacrimal canaliculus or sac may also serve as a bacterial reservoir requiring antibiotic irrigation and oral antibiotics. The staphylococcal hypersensitivity reaction in the cornea may require mild topical corticosteroid treatment to reduce the associated inflammation. Oral tetracycline or doxycycline may be beneficial in more severe infections.

NEONATAL CONJUNCTIVITIS

Any conjunctivitis occurring within the first 4 weeks of life is classified as neonatal conjunctivitis (ophthalmia neonatorum).[113] A mucoid discharge in the corner of the eye and persistent tearing are typical symptoms. Conjunctivitis in the newborn can be bacterial, viral, chlamydial, or toxic (reaction to chemicals). Specific identification of the cause is particularly important because there is often a potentially serious systemic infection associated with the localized ocular condition. Neonatal conjunctivitis is more commonly found if infants are delivered vaginally compared with cesarean delivery, and this may be a risk factor for this disease.[114] The cause, properties of the eye discharge, and suggested treatment are summarized in Table 112.2.

Neonatal Chemical Conjunctivitis

In 1881 Credé introduced the use of topical silver nitrate as prophylaxis against neonatal gonococcal infection.[115] The self-limited conjunctivitis, present in approximately 90% of treated newborns, usually begins a few hours after delivery and resolves in 24 to 36 hours.[116] Although quite effective against *N. gonorrhoeae,* silver nitrate has little effect on bacteria and essentially no effect on chlamydia or viruses.[117] Silver nitrate may injure epithelial cells to such a degree that they are more susceptible to the entry of other microbial agents. Silver nitrate may still be used in some countries, but hospitals in the United States have changed to

TABLE 112.2 Etiology and Treatment of Neonatal Conjunctivitis Based on Time Frame of Symptoms

TIME FRAME OF SYMPTOMS	TYPICAL CAUSE	TYPE OF EYE DISCHARGE	TREATMENT
1–24 h	Chemical		No treatment required; resolves within 2–4 days
24–48 h	*Neisseria gonorrhoeae*	Profuse, purulent	Topical irrigation with saline, IV ceftriaxone; topical atropine if corneal involvement; neonate, mother, and sexual partner should be treated for chlamydia as well
2–5 days	Gram-positive organism (e.g., *Staphylococcus aureus*)	Purulent	Bacitracin ointment for 2 weeks
5–14 days	*Chlamydia trachomatis*	Watery, followed by purulent and bloody	Erythromycin drops qid plus erythromycin suspension for 2–3 weeks or azithromycin for 3 days
6–14 days	HSV	Watery	Topical vidarabine ointment plus systemic acyclovir
5–18 days	*Pseudomonas aeruginosa*	Greenish	Gentamicin, tobramycin, or ciprofloxacin qid for 2 weeks

HSV, Herpes simplex virus; *IV,* intravenous.
Data from Neonatal conjunctivitis. https://en.wikipedia.org/wiki/Neonatal_conjunctivitis; and Makker K, Kaufman EJ. Conjunctivitis, Neonatal. StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2018.

erythromycin or tetracycline ointment. Povidone-iodine (Betadine) is markedly inexpensive and quite effective against many microbial agents; it is becoming more widely used as a prophylactic agent for newborns, especially in developing countries. The associated chemical conjunctivitis is similar in nature and course as that seen with silver nitrate. Liquid detergent capsules have also been associated with ocular conjunctivitis in children in a study from the United Kingdom.[118]

Neonatal Chlamydial Conjunctivitis

The most frequent cause of neonatal conjunctivitis in the United States is *C. trachomatis.*[119] Up to 3 million new cases of chlamydial infection occur annually,[120] with 4% to 10% of all pregnant women in the United States being diagnosed with chlamydia.[121] The infant of an untreated mother has a 30% to 40% chance of developing conjunctivitis and a 10% to 20% chance of developing pneumonia.[115] A unilateral or bilateral discharge begins 5 to 14 days after delivery. NAATs have been demonstrated to be more sensitive than other conventional methods in detecting neonatal chlamydial conjunctivitis and may be useful for the routine screening and treatment of genital *C. trachomatis* infection in pregnant women.[122–124]

Chlamydial conjunctivitis in the neonate differs from chlamydial conjunctivitis in the adult in a number of ways. No follicular response is seen in the neonate because of the inability of the immature immune system to form such a reaction. The amount of mucopurulent discharge is greater in the neonate, as is the propensity to form membranes on the palpebral conjunctiva. The infection in neonates is more responsive to topical medications. Although the typical conjunctivitis is mild and self-limited, severe cases can result in conjunctival scarring with corneal pannus and scarring. If erythromycin or tetracycline ointment is applied to the conjunctival surface within 1 hour after delivery, the chance of developing chlamydial conjunctivitis is reportedly almost zero.[125] However, topical medications cannot treat the pneumonitis and otitis media that may accompany the conjunctivitis. In a newborn with laboratory-proven chlamydia conjunctivitis, 2 weeks of oral erythromycin therapy is given; a second course may be given if adequate resolution is not achieved with the initial treatment. The mother and her sexual partners must also be treated with oral erythromycin or tetracycline (with caution in breastfeeding mothers) for 1 week.

Neonatal Gonococcal Conjunctivitis

The incidence of neonatal gonococcal conjunctivitis has decreased dramatically with effective prenatal screening and use of prophylactic antimicrobial agents in newborns. The clinical presentation begins with a hyperacute bilateral conjunctival discharge that appears within the first 24 to 48 hours after delivery. The associated purulent exudate is often so profuse that it reappears immediately after cleaning of the eye. Conjunctival membrane formation is not uncommon. *N. gonorrhoeae* can penetrate an intact epithelial surface and quickly invade the cornea, causing ulceration, perforation, and endophthalmitis if not promptly treated. Other localized gonococcal infections such as rhinitis and proctitis may be present as well as the rare but more severe disseminated infection with arthritis, meningitis, pneumonia, and septicemia, which could lead to infant death.[120] With resistance emerging against penicillin, tetracycline, and the fluoroquinolones, a single dose of intramuscular or intravenous ceftriaxone, 25 to 50 mg/kg, not to exceed 125 mg, is the preferred treatment.[126] Hospitalization and hourly saline irrigation of the conjunctival fornices are recommended; if corneal involvement cannot be ruled out because of the copious exudation, topical antibiotics are applied.[127] Povidone-iodine prophylaxis treatment has also been suggested, but its effectiveness compared with tetracycline ointment has been questioned.[128–130] Fusidic acid may prove to be a promising treatment.[131]

Nongonococcal Neonatal Bacterial Conjunctivitis

Numerous organisms can cause bacterial conjunctivitis in the newborn. Most infections are associated with gram-positive organisms such as *Staphylococcus* spp. and *Streptococcus* spp. Gram-negative organisms such as *Haemophilus* spp. and *Enterobacter* spp., *E. coli, P. mirabilis, K. pneumoniae,* and *S. marcescens* have been less commonly implicated.[132] Although *Pseudomonas aeruginosa* is a rare cause of neonatal conjunctivitis, it warrants special consideration because of its ability to rapidly cause corneal ulceration and possible perforation.[133]

Although symptoms can manifest at any time within the first month of life, nongonococcal bacterial conjunctivitis usually manifests 2 to 5 days after delivery. The clinical presentation consists of periorbital edema, chemosis, and conjunctival hyperemia and discharge. There is a higher incidence if obstruction of the nasolacrimal system is present. Conjunctival scraping for Gram stain and cultures allows for appropriate treatment—usually erythromycin ointment for gram-positive organisms and either gentamicin or tobramycin ointment for gram-negative organisms.

Neonatal Viral (Herpetic) Conjunctivitis

Herpetic conjunctivitis in the neonate is rare but can be associated with significant morbidity and mortality. HSV types 1 and 2 can be associated with conjunctivitis. In theory HSV-1 can be transmitted to the infant through oral secretions from an adult or sibling with an active cold sore, but the more common source is contact with HSV-2 during passage through an infected birth canal. Edema, conjunctival injection, and tearing usually begin within the first 2 weeks of life and may be followed by keratitis or keratouveitis. Diagnosis is commonly made by Giemsa stain but can be confirmed in 24 to 48 hours by PCR assay. If HSV is detected in neonatal conjunctivitis, the infant should be evaluated for extent of HSV infection, and intravenous acyclovir should be started immediately per American Academy of Pediatrics guidelines.[82]

PARINAUD OCULOGLANDULAR CONJUNCTIVITIS

Parinaud oculoglandular conjunctivitis refers to the association of follicular conjunctivitis and unilateral preauricular lymphadenopathy. This classification describes a type of conjunctivitis that has numerous associated causes including bacterial, viral, parasitic, mycobacterial, syphilitic, leukemic, and fungal agents. Red eye, mucopurulent discharge, and foreign-body sensation are accompanied by one or more granulomatous nodules on the palpebral conjunctiva. There is usually a visibly enlarged preauricular or submandibular lymph node on the involved side. This follicular conjunctivitis is associated with a fever and possible skin rash. *Bartonella henselae,* or cat-scratch disease, is the most common cause, but tularemia, tuberculosis, syphilis, lymphoma, mumps, Epstein-Barr virus, sporotrichosis, and sarcoidosis all have been implicated as potential causes.

Cat-scratch disease often resolves spontaneously, but 1 month of topical and systemic antibiotic therapy has been described. Because of the host of etiologic agents, an extensive workup may be warranted, with the identified cause given the appropriate systemic treatment (see Chapter 234).

PARASITIC CONJUNCTIVITIS

Leishmaniasis

A number of parasites may be associated with conjunctivitis, either by primary infection or secondarily as a response to the presence of the parasite. Blepharoconjunctivitis caused by *Leishmania* may begin as simple edema and hyperemia, with eventual progression to superficial phlyctenules in the conjunctiva and at the corneal limbus.[134] These phlyctenules may progress to abscess formation, scarring of the lids and conjunctiva, and corneal perforation. These parasites are obligate intracellular agents that are transmitted through bites of infected sand flies.

Other Parasites and Ectoparasites

The tsetse fly can infect humans with the flagellates responsible for African trypanosomiasis or sleeping sickness. Ocular effects manifest as unilateral conjunctivitis, periorbital edema, and preauricular lymphadenopathy.[135] Cryptosporidia, fly larvae, and nematodes (e.g., *Loa loa*) have also been implicated as parasitic causes of conjunctivitis.[136,137,138–140] The lid margin and lashes may be colonized by *Phthirus pubis* (lice) or *Demodex* (mites), with conjunctivitis occurring as a reaction to the organism or its waste products. Treatment for the actual conjunctivitis associated with the parasitic agents is mainly supportive;

more aggressive treatment may be necessary for the systemic parasitic condition.

FUNGAL CONJUNCTIVITIS

Although various fungal agents can be recovered from the conjunctiva, fungal conjunctivitis is rarely observed clinically. Compared with fungal keratitis, relatively few organisms have been implicated in fungal conjunctivitis. *Candida* spp., *Blastomyces* spp., and *Sporothrix schenckii* have been associated with a granulomatous conjunctivitis. These mycoses are treated with systemic antifungal agents. Conjunctival *Rhinosporidium seeberi* infection usually manifests as a fleshy, friable, red, pedunculated mass.[1] Excision of the mass with adequate margins is often curative.

Microsporidia are ubiquitous obligate intracellular fungi that are found more often in animal hosts, but the related microsporidian, *Encephalitozoon,* has been implicated as the cause of a mild conjunctivitis with punctate epithelial keratitis in immunocompromised patients.[135] *Vittaforma corneae* can cause keratoconjunctivitis in immunocompetent individuals and was responsible for a large case cluster in rugby players, probably contracted from mud splattered over their faces.[141] Symptoms of conjunctivitis may be mild and can easily be mistaken for tear film deficiencies or blepharitis; a high index of suspicion is required to make a clinical diagnosis of microsporidial conjunctivitis. Modified trichrome is a useful stain for demonstrating the organisms in smears. Oral albendazole, 400 mg twice daily, has been reported to be effective (see Chapter 43). Topical fumagillin can be obtained as Fumidil B and formulated for human use but is not approved by the FDA; fumagillin has been used to successfully treat microsporidial keratoconjunctivitis (see Chapter 270).[136] In general, medical treatment in immunocompromised patients involves long duration with frequent recurrences after discontinuation of medication.

NONINFECTIOUS VISION-THREATENING CONDITIONS ASSOCIATED WITH RED EYE

Care must be taken with any patient who presents with a red eye because there are numerous conditions that can simulate conjunctivitis including dry eye, blepharitis, endophthalmitis, cellulitis, carotid cavernous fistula, anterior segment tumors, scleritis, and subconjunctival hemorrhage.[142] As with most conditions, a thorough medical history and physical examination are helpful in the differential diagnosis. Most cases of conjunctivitis are associated with fairly painless discharge and irritation, essentially normal vision, normally reactive pupil, normal intraocular pressure, essentially clear cornea, and generally diffuse conjunctival injection. Three possible sight-threatening conditions—angle-closure glaucoma, uveitis, and corneal ulcer—often have different signs, which may enable a more proper diagnosis.

An attack of angle-closure glaucoma is associated with significant pain, often nausea, usually no discharge, generally markedly decreased vision, middilated nonreactive pupil, markedly elevated intraocular pressure, a cloudy and edematous cornea, and a more localized conjunctival injection in the limbal region. Uveitis is generally associated with mild-to-moderate pain with photophobia, essentially normal to mildly reduced vision, no discharge, a small–to–normal-sized reactive pupil, normal-to-low intraocular pressure (elevated in herpetic uveitis and Posner-Schlossman syndrome), a generally clear cornea, and a localized conjunctival injection around the limbus. Corneal ulcers are quite painful and are usually accompanied by moderately to markedly reduced vision, variable mucoid or mucopurulent discharge, normally reactive pupil, normal intraocular pressure, an opaque lesion that is easily visible in the cornea, and a generalized conjunctival injection.

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113 Microbial Keratitis

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SHORT VIEW SUMMARY

Definition
- Microbial keratitis is a vision-threatening corneal inflammatory condition caused by bacteria, viruses, fungi, or parasites.

Epidemiology
- Disease burden is higher in developing than in developed countries.
- Risk factors include contact lens wear, trauma (surgical and nonsurgical), contaminated ocular medications, altered structure of the corneal surface, and contributing systemic diseases.
- Contact lens use is the main cause for keratitis in the United States: poor lens storage and hygiene, and extended-wear use.
- Nonsurgical ocular trauma is the main cause of keratitis in developing countries.
- Trachoma is encountered more commonly in developing countries.

Microbiology
- Pathogens can be gram-negative organisms, such as *Pseudomonas aeruginosa*.
- Pathogens can be gram-positive organisms, such as *Staphylococcus* and *Streptococcus* spp.
- Viral pathogens include herpes simplex virus, varicella-zoster virus, and adenovirus.
- Outbreaks of microbial keratitis in contact lens wearers have involved *Acanthamoeba* and *Fusarium* spp.

Diagnosis
- Symptoms include severe pain and discomfort, tearing, photophobia, blepharospasm, and decreased vision.
- Clinical presentation includes conjunctival injection and discharge, decreased corneal transparency, corneal infiltrate, epithelial defect and/or stromal inflammation, corneal edema, corneal neovascularization, stromal melting, and loss of vision.
- Knowing the nature of the stromal inflammation (suppurative or nonsuppurative) and its location (focal, multifocal, or diffuse) is helpful.
- Corneal scrapings and cultures should be performed on all suspected cases of infectious keratitis.

Therapy
- Aggressive antimicrobial therapy should be initiated after scrapings and cultures of infectious keratitis.
- Fortified topical administration is the most common route of antimicrobial therapy. Subconjunctival injections, parenteral and oral routes, and antibiotic-soaked collagen shields/soft lenses are used infrequently.
- Fortified cefazolin and aminoglycoside should be used for more severe bacterial keratitis.
- Monotherapy with a fourth-generation fluoroquinolone, such as moxifloxacin, is used for bacterial keratitis.
- Antifungal therapeutic agents include topical natamycin, amphotericin B, flucytosine, fluconazole, voriconazole, and itraconazole. Oral itraconazole and voriconazole have also shown favorable outcomes when added to topical therapy. Keratoplasties are performed in recurring and unresolved keratitis.
- Novel therapies continue to develop to mitigate drug resistance problems.

Keratitis is an inflammation of the cornea produced by infectious organisms or noninfectious agents or stimuli. Microbial keratitis is a potentially vision-threatening infectious corneal inflammation event that can be caused by bacteria, viruses, fungi, or parasites.[1] Microbial keratitis is a significant public health problem. Each year in the United States, 930,000 clinic visits and 58,000 emergency department visits are due to keratitis and conditions caused by contact lens wear, which cost $175 million in direct health care expenditures and occupy over 250,000 hours of clinician time.[2] Additionally, keratitis accounted for 7.7% of medical conditions associated with hospitalization for corneal ulcers. Fungal ulcers are reported to have worse clinical outcomes than the bacterial ones because no new treatments have been introduced since topical natamycin in the 1960s.[3,4] Infectious keratitis also causes corneal opacities, which are reported to be responsible for 10% of avoidable visual impairment in some of the world's least developed countries.[5,6] Contact lens–related microbial keratitis is on the rise and may be associated with severe infections and loss of vision.[7] Specifically, the incidences of *Acanthamoeba* and fungal infections are on the rise.[8] Studies have shown that the environment also plays a role. In developing countries and tropical countries, bacterial keratitis is decreasing whereas fungal keratitis cases are also increasing.[9] In temperate countries such as the United Kingdom, bacterial keratitis accounts for 90% of the cases. Infectious keratitis requires prompt diagnosis and expedient treatment to prevent blindness or even enucleation. There are few clinical signs that distinguish microbial keratitis from corneal inflammation associated with trauma, hypersensitivity, or immune-mediated conditions. The patient's history and ocular examination, focusing on the presence or absence of an epithelial defect and/or stromal inflammation, serve as important diagnostic clues. The nature of the stromal inflammation (suppurative or nonsuppurative) and its location (focal, multifocal, or diffuse) are also helpful in making a diagnosis. Microbiologic tests are needed to establish the etiologic agent and antimicrobial susceptibility, but therapy is often begun before these results are final.

Given the rapid progression and virulent nature of many infectious agents, any corneal inflammation should be considered a threat to vision, requiring prompt evaluation and treatment. Even relatively minor corneal ulcerations may lead to significant reduction in visual acuity, should they be located on the visual axis. Corneal perforation can occur in as little as 24 hours with certain virulent organisms; subsequent endophthalmitis (inflammatory process involving the ocular cavity and adjacent structures), leading to loss of vision or even loss of the eye, is an ever-present danger in such settings.

ETIOLOGIC AGENTS AND RISK FACTORS

Microbial Agents

The conjunctival surface has been theorized to be the location of hundreds of organisms, any of which might be the origin of a given keratitis. The most commonly encountered organisms involved in microbial keratitis show tremendous geographic variance; some of the more commonly known infectious agents are shown in Table 113.1. Although the climate, vegetation, soil, and individual patient factors tend to favor specific organisms, any known organism can cause microbial keratitis, given the appropriate conditions and predisposing risk factors.

TABLE 113.1 Partial List of Causative Agents in Microbial Keratitis

Bacteria

Gram-Positive Cocci

Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus pneumoniae, Streptococcus pyogenes, viridans streptococci
Enterococcus faecalis
Peptostreptococcus spp.

Gram-Positive Bacilli

Bacillus coagulans, Bacillus cereus, Bacillus licheniformis
Brevibacillus (Bacillus) brevis, Brevibacillus (Bacillus) laterosporus
Corynebacterium diphtheriae
Clostridium perfringens, Clostridium tetani

Gram-Negative Coccobacilli

Neisseria gonorrhoeae
Moraxella lacunata, Moraxella nonliquefaciens, Moraxella catarrhalis
Acinetobacter calcoaceticus
Pasteurella multocida
Achromobacter xylosoxidans

Gram-Negative Bacilli

Pseudomonas aeruginosa, Pseudomonas stutzeri, Pseudomonas fluorescens
Burkholderia (Pseudomonas) mallei
Proteus mirabilis
Serratia marcescens
Escherichia coli
Klebsiella pneumoniae
Morganella morganii
Aeromonas hydrophila
Bartonella henselae

Mycobacteria

Mycobacterium tuberculosis, Mycobacterium chelonae, Mycobacterium gordonae, Mycobacterium mucogenicum

Actinomycetes

Nocardia spp.

Spirochetes

Treponema pallidum
Borrelia burgdorferi

Viruses

Herpes simplex virus
Varicella-zoster virus
Adenovirus
Vaccinia virus
Epstein-Barr virus
Rubeola
Enteroviruses
Coxsackievirus

Fungi

Fusarium spp.
Candida spp.
Aspergillus spp.
Acremonium spp.
Alternaria spp.
Penicillium spp.
Bipolaris spp.
Nosema spp.
Vittaforma (Nosema) corneae
Encephalitozoon spp.
Edenia gomezpompae
Exophiala phaeomuriformis

Chlamydia

Chlamydia trachomatis

Parasites

Acanthamoeba polyphaga, Acanthamoeba castellanii
Onchocerca volvulus
Leishmania brasiliensis
Trypanosoma spp.

Modified from O'Brien TP. Keratitis. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. *5th ed. New York: Churchill Livingstone; 2000:1257–1266.*

Anatomic Protection

The epithelial surface, with its tight junctions formed by desmosomes and hemidesmosomes, is remarkably resistant to the host of virulent organisms found in the surrounding ocular environment. The tear film, containing antimicrobial enzymes, combined with the mechanical action of the blinking eyelids, reduces the likelihood of microbial attachment to and survival on the corneal surface. In general, microbial agents do not cause keratitis in immunocompetent hosts or those without prior epithelial injury. However, there are exceptions in which organisms such as *Neisseria gonorrhoeae, Listeria monocytogenes, Shigella,* and *Corynebacterium* spp. may invade an intact epithelial surface.

Geographic Variation

The prevalence of various kinds of microbial keratitis varies by US regions. Fungal keratitis is present more in the southern regions, whereas bacterial keratitis is more prevalent in the northern regions. Gram-negative bacteria are most prevalent in the southern regions when compared to northern locations.[10]

In other developing countries, such as South Africa, herpetic keratitis, complicated by bacterial superinfection, is the main kind of microbial keratitis.[11]

Risk Factors

Predisposing risk factors associated with microbial keratitis usually involve disruption of the corneal epithelium, such as by contact lens wear, trauma (surgical and nonsurgical), contaminated ocular medications, and altered structure of the corneal surface. Contributing risk factors include diabetes mellitus, systemic immunodeficiency, exposure keratopathy (e.g., Graves exophthalmopathy, Bell palsy), surface alteration from or with dysfunctional tear states (e.g., Sjögren syndrome, neurotrophic cornea, chemical burn, Stevens-Johnson syndrome, medication related), anatomic abnormalities (e.g., neoplasia, cicatricial pemphigoid, traumatic lid scarring), and keratoprosthesis implantation (Boston type I keratoprosthesis).[12]

Nonsurgical ocular trauma reportedly accounted for 48% to 65% of all corneal ulcers in some developing countries,[9,13] but such trauma was responsible for only 27% of corneal ulcers reported in 1987 at one large trauma referral center in the United States.[14] Trauma has also been the leading cause of microbial keratitis in pediatric populations.[15] In a more recent survey, potential predisposing risk factors were identified in 91% of 300 cases of suspected bacterial keratitis: 50% resulting from contact lens wear, 21% from ocular surface disease, 15% from nonsurgical trauma, and 4% from corneal surgery.[16]

Contact Lenses

Contact lenses remain the most common risk factor for microbial keratitis diagnosed in the United States. The incidence of microbial keratitis ranges from 0.4 to 5.2 per 10,000 person-years for rigid gas permeable and soft contact lenses to greater than 20 per 10,000 person-years for overnight contact lens use.[17] The reported percentage of contact lens–associated corneal ulceration in the general population has increased from 0% in the 1950s and 1960s to 31% in the 1970s and more than 50% of all cases since the 1980s.[7] In a 2010 study in Northern California, Jeng and coworkers[17a] reported a substantially higher rate of corneal ulcers in contact lens wearers, compared with non–contact lens wearers. They reported the incidence of corneal ulceration in contact lens wearers as 130.4 per 100,000 person-years, compared with non–contact lens wearers, who had an incidence of ulcerative keratitis of 14.0 per 100,000 person-years.[19a]

Since the 1990s, contact lens wear has accounted for 55% to 65% of diagnosed microbial keratitis cases,[4] where *Pseudomonas aeruginosa* is reported to be the predominantly isolated microbe from the corneal and contact lens cultures as well as contact lens case cultures.[18] Recent outbreaks of microbial keratitis in contact lens wearers have involved *Acanthamoeba* and *Fusarium* spp.,[19] which historically have been involved only rarely in cases of contact lens–associated microbial keratitis. An outbreak of *Acanthamoeba keratitis* was detected in the United States in 2007, where cases had been increasing since 2004.[19a] In the United States its reported incidence is low (0.15 per million), and in the United Kingdom it is more (1.4 per million). In terms of its incidence among all keratitis cases, *Acanthamoeba* keratitis is reported to account for 1% to 1.4% of all such microbial keratitis cases in countries with less common contact lens usage compared to 4% of all such cases in countries with more common contact lens usage.[20] These outbreaks may have been associated with the improper use of certain multipurpose contact lens solutions, with two multipurpose solutions having been identified as major contributing factors to the risk of microbial keratitis. The risk of microbial keratitis is reported to also increase because of greater microbial adherence to the lens, stemming from inadequate rinsing while also failing to rub the lenses properly as part of the cleaning process.[21]

Several explanations regarding pathology of contact lens–induced microbial keratitis have been proposed. Typically, in contact lens cases, bacteria multiply because they are protected from disinfection by bacterial biofilm. Evans and Fleiszig[22] proposed that in terms of contact lens–induced *P. aeruginosa* keratitis, biochemical changes of the corneal surface, due to tear fluid and bacterial trapping against the cornea under the lens, are the likely causes of microbial keratitis. In addition, bacterial adaptation to ocular surfaces increases the risk of microbial keratitis. Blockage in efficient tear exchange between tears above and tears under contact lens areas also can lead to an imbalance in homeostasis and a decrease in antimicrobial activity of the tears trapped under the lens.[23] Shin and associates[24] have reported that the baseline eye microbiome of contact lens wearers is different from those of nonwearers.

A study in Australia was performed to establish the risk factors for moderate and severe microbial keratitis among daily contact lens wearers. Independent risk factors for moderate and severe keratitis, while adjusting for age, gender, and lens material type, included poor storage case hygiene (odds ratio [OR], 6.4), infrequent storage case replacement (OR of 5.4), solution type (multipurpose solution A; OR, 7.2), occasional overnight lens use (<1 night/wk; OR, 6.5), high socioeconomic status (OR, 4.1), and smoking (OR, 3.7).[25]

With regard to preservation and antimicrobial solutions, Lin and coworkers[26] reported excellent anti–*Staphylococcus aureus* activity from preservatives with polyaminopropylbiguanide (PAPB), chlorhexidine, and the biguanide function group, and an excellent synergistic anti–*P. aeruginosa* activity (~7 times more potent than the drugs available today) from a combination of ethylenediaminetetraacetic acid (EDTA; 5000 ppm), PAPB (5 ppm), and chlorhexidine (30 ppm). Thus the proposed multipurpose solution containing a combination of PAPB, chlorhexidine, and EDTA could help decrease the incidence of contact lens–induced microbial keratitis.

It is worth noting that fluoroquinolones alone can be ineffective against contact lens–related keratitis. Thus empirical combined regimens should be considered as a more effective option.[27]

Contact lens use in conjunction with preexisting ocular surface conditions (such as blepharitis, corneal ulcer, Stevens-Johnson syndrome) is reported to be a significant risk factor for infectious keratitis development in pediatric populations.[28] *Acanthamoeba, Staphylococcus,* and *P. aeruginosa* are the most commonly isolated microorganisms for the infectious keratitis development.

Laboratory Data and Clinical Response

Clinicians often culture the contact lens and the contact lens case in addition to the patient's cornea despite imperfect sensitivity.[29,30] False-positives are too frequent to recommend this procedure. In one study, the significance of cultures from the lens or case could be confirmed by corneal cultures in only 25% of the cases.[31] Gram-negative organisms, such as *P. aeruginosa,* which can be cultured from the lens or case, are indeed associated with contact lens–induced microbial keratitis; however, gram-positive organisms, such as *Staphylococcus* and *Streptococcus* spp., have often been shown to be responsible for a majority of these ulcers even when gram-negative organisms have been recovered from the contact lens or storage case. Contact lens use alone can produce sterile inflammatory infiltrates that resolve spontaneously with discontinued use of the lens.

CLINICAL PRESENTATION

Eye Pain

Inflammation of the cornea, one of the most richly innervated tissues in the body, generally is accompanied by severe pain and significant discomfort, which can greatly affect the physical examination. The continued movement of the eyelids over a corneal lesion further adds to the patient's discomfort during examination. Judicious use of a topical anesthetic immediately preceding the eye examination is helpful. However, long-term use of topical anesthetics has been implicated in continued epithelial defects and melting of the corneal stroma. Therefore it has become a classical teaching that topical anesthetics should not be prescribed beyond the examination process.

Conjunctival Injection and Discharge

The rapid onset of pain is often accompanied by significant conjunctival injection, tearing, photophobia, blepharospasm, and decreased vision. Discharge, so often associated with conjunctivitis, is not usually present other than in some cases of purulent bacterial keratitis. There may be some differences in the presence or absence of injection and, to some degree, tearing and discharge based on the etiologic agent (i.e., viral and parasitic keratitis may have minimal discharge; fungal keratitis may have minimal injection in the early phases).

Corneal Infiltrates

Other than the injection of limbal vessels, the noninfected regions of the cornea are generally clear. Therefore corneal inflammation appears quite different than inflammation in most other tissues. The invading organisms initiate a reaction whereby inflammatory cells from the limbal vessels and tear film migrate into the cornea, reducing its transparency. The resultant inflammatory reaction at the sites of a microbial replication are called "infiltrates." It is important to note that in patients with limited ability to mount an immune reaction, the infiltrates may be small in size, or even nonexistent, despite continued microbial growth. In patients who are able to mount a normal immune reaction, the reduction in size of the infiltrate during the course of antimicrobial therapy may be an important indicator of response to therapy.

Epithelial Defect

The infiltrate often has an overlying epithelial defect because the infectious agent generally enters the cornea through an epithelial defect that can rapidly increase in size with the microbial replication. Epithelial defects can best be visualized using a cobalt blue light after the addition of fluorescein, which will pool in areas of punctate and ulcerative defects. Infections caused by slow-growing, fastidious organisms (i.e., *Mycobacterium,* anaerobic bacteria) may have an intact epithelial surface.

Stromal Suppuration

Microbial keratitis generally produces a sharply demarcated epithelial ulceration and suppurative stromal inflammation. There is substantial crossover in the presenting characteristics of various microbial agents. Bacterial organisms generally produce a clearly defined, local inflammation; fungal elements can have a more diffuse or multifocal infiltrate. A number of slow-growing, fastidious organisms may present without an ulcer or a suppurative infiltrate.

Corneal Edema

The loss of transparency is further affected by the corneal edema that is present in almost all cases of microbial keratitis. The corneal clarity is very specifically maintained through the ability of endothelial cells to maintain a stable level of dehydration. The influx of fluid that accompanies the inflammatory cells can overwhelm the endothelium's "pumping" capacity, resulting in varying degrees of corneal edema. This

fluid may coalesce under the epithelium, forming uncomfortable bullous elevations. The discomfort may increase if the bullae rupture.

Corneal Neovascularization

Neovascularization of the cornea is a common occurrence with chronic inflammation but can present early in the course of severe keratitis. The presence of neovascularization not only affects the severity of the host inflammatory reaction but also has long-term consequences. After resolution of the inflammation, the vessels may regress slightly to the point of no longer carrying blood but still remain as empty channels ("ghost vessels"). If the neovascularization does not regress, the vessels not only provide a route for recurrent inflammatory cells but also can directly affect visual acuity and greatly decrease the potential for successful corneal transplantation.

Intraocular Inflammatory Reaction

Intraocular inflammation is not uncommon with microbial keratitis, especially with some of the more virulent gram-negative bacteria. These signs, including cell/flare, hypopyon, synechiae, and glaucoma, are less common with early fungal, viral, and parasitic etiologies, but could present during the course of any case of keratitis. Early, subtle changes may be difficult to detect and are often described as protein ("flare") and leukocytes ("cells") in the anterior chamber on slit-lamp examination. Late changes or signs of more aggressive organisms may be seen in a collection of fibrin and inflammatory white blood cells layering in the inferior portion of the anterior chamber (hypopyon). Adhesive scarring of the pupillary margin (posterior synechiae) or of the peripheral iris (peripheral anterior synechiae) may lead to an irregular pupil or elevated intraocular pressure (glaucoma), which may necessitate urgent medical or surgical treatment. This intraocular inflammation is generally considered sterile unless infection has penetrated the Descemet membrane and entered the anterior chamber.

Stromal Melting (Keratolysis)

Loss of corneal tissue (keratolysis) is a major consequence of the inflammatory reaction seen in microbial keratitis. It is most common with bacterial keratitis. Keratolysis may lead to irregular astigmatism, corneal thinning, visually significant scar formation, and, in severe cases, corneal perforation.

LABORATORY EVALUATION

The rather small area of active infection and the need to avoid unnecessary scraping dictate patient cooperation. This may be accomplished through use of topical anesthetics (although this may reduce growth of recovered organisms) in patients old enough to cooperate and may necessitate general anesthesia in children. The small amount of specimen to be obtained from the cornea requires careful advance planning and consultation with the microbiology laboratory as to how the specimen should be obtained, transported, and processed.

Several studies have shown no statistical difference in organism recovery if using sterile surgical blades, blunt platinum spatulas, or calcium alginate swabs (often dipped in trypticase soy broth). Corneal biopsies may be necessary, especially when fungal infections or late stages of *Acanthamoeba* are suspected. (Earlier stages of *Acanthamoeba* have organisms in the more superficial layers.) Shave biopsies or partial thickness 1- to 2-mm trephined specimens may be possible at the slit lamp but are better performed in a minor operating room. Lamellar keratoplasty is not usually necessary, but in cases in which a rapidly progressive, necrotizing keratitis is refractory to treatment or identification is otherwise impossible, or both, a diagnostic and therapeutic penetrating keratoplasty (host corneal removal and transplant) may be necessary.

Stains and Media Inoculation

Material from the scrapings should be transferred directly to glass slides and appropriate culture media (see Chapter 16). The slides should be clean, to avoid artifacts, and sterile, to avoid contaminating the instrument. Multiple slides are desirable to permit Gram stain; calcofluor or other fungal stain; and acid-fast stain. *Chlamydia,* herpesviruses, mycobacteria, and *Acanthamoeba* require special methods for culture.

In addition to culturing the suspected cornea, it may be helpful to culture material from the eyelids, conjunctiva, ocular medication bottles, contact lenses, storage cases, and perhaps the uninvolved eye, to establish the flora uniquely associated with the patient. If the patient had been treated before evaluation and there is uncertainty as to the diagnosis, it may be wise to consider stopping the medication for 12 to 24 hours and then proceeding with culture. Obviously, antimicrobials should not be stopped in cases of severe or rapidly progressive ulceration.

Viral Cultures

Viral keratitis is unique among microbial keratitides in that the diagnosis is often possible based on morphology and patient history. Polymerase chain reaction for herpes simplex virus and herpes zoster virus is now readily accomplished in most routine diagnostic laboratories (see Chapter 16). Viral cultures are less sensitive than polymerase chain reaction but, if used, specimens for viral culture must be placed in transport media and inoculated into cell culture the same day.

BACTERIAL KERATITIS

Etiology

Bacterial keratitis is reported to be the cause of ulcerative keratitis in 80% of the patients worldwide.[32] It is associated mostly with contact lens use in the United States.[4] In the past, several published studies have reported that bacterial pathogens are responsible for 65% to 90% of all cases of microbial keratitis.[33,34] In one large survey, a microbial organism was isolated in 49% of 5845 cases of suspected infectious keratitis; 82% were bacterial, 16% fungal, and 2% parasitic.[35] A smaller survey isolated an organism in 68% of 300 eyes suspected of having bacterial keratitis; 83% were gram-positive, 17% gram-negative, and 2% polymicrobial.[11] The majority of all bacterial keratitis is caused by five major groups: *Staphylococcus* spp.; *Streptococcus* spp. (*Streptococcus pneumoniae,* groups A to G streptococci); other gram-positive organisms (*Bacillus* and *Cutibacterium* spp.); gram-negative organisms, such as *Pseudomonas, Haemophilus, Moraxella;* and the Enterobacteriaceae (*Proteus, Serratia, Klebsiella, Enterobacter, Citrobacter*). The prominence of certain organisms responsible for bacterial keratitis has been changing over many years. *Streptococcus pneumoniae* was the most common responsible agent in the past, but other gram-positives, opportunistic commensals, *Pseudomonas,* anaerobes, and protozoa are now increasingly being reported. With the advent of refractive surgery, especially laser-assisted in situ keratomileusis (LASIK), more unusual organisms, such as *Nocardia* and *Mycobacterium* spp., are causing keratitis. The apparent changes in causal organisms could be the result of numerous factors: improved isolation techniques, less frequent culturing as highly effective broad-spectrum single agents (i.e., fluoroquinolones) have been developed, increased use of topical corticosteroids (i.e., refractive and cataract surgery), increased population of systemically immunodeficient patients, and an expansion in the use of soft contact lenses, especially extended-wear and cosmetic lenses.[36,37] A study in Toronto reported on the shifting trends in bacterial keratitis over the course of 11 years.[38] A total of 1701 consecutive corneal scrapings were taken from January 1, 2000, through December 31, 2010. A significant decrease in the percentage of gram-positive microorganisms was observed over time. The sensitivity of gram-negative isolates to tested antimicrobials was greater than 97% response for all the reported antibiotics; this was not the case for gram-positive isolates, for which resistance to the antibiotics was more common. Methicillin-resistant organisms accounted for 29.1% of all gram-positive cultures. The authors concluded that the empirical use of vancomycin in the setting of severe suspected bacterial keratitis may be justified given the shifting trends.[38]

Pathogenesis

The corneal epithelium and the Bowman membrane underneath it limit penetration of many organisms into the corneal stroma unless the barrier is breached by trauma. The few organisms that appear to invade in the absence of trauma appear to adhere and elaborate proteolytic enzymes or toxins that lyse the tissue barrier. Migration of leukocytes from the vessels in the corneal limbus into the infected cornea add to tissue destruction.[39]

Geographic Variation

Gram-positive organisms, particularly *S. aureus,* continue to be the most common agents causing bacterial keratitis in most series. The order of prevalence in one series from New York City was *S. aureus, Moraxella, Pseudomonas,* and *S. pneumoniae.*[33] A different order, *S. aureus, S. pneumoniae, Pseudomonas,* and *Moraxella,* was seen in a similar series from London.[40] *Pseudomonas* and *Streptococcus* spp. are the most common bacterial pathogens isolated in previously healthy eyes in the southern United States.[34] Bacterial ulcers are more likely to be reported from centers in developed countries, whereas fungal infections are more likely to be reported from developing countries. This is because there are more agricultural workers in the developing countries who are at greater risk of trauma.[41] The highest proportion of bacterial corneal ulcers are reported in studies from Australia, North America, the Netherlands, and Singapore. *Pseudomonas* ulcers were reported in greater proportion from a study in Bangkok, whereas the greatest proportion of staphylococcal ulcers were reported from a study in Paraguay.[41]

Gram-Positive Bacteria

The strains of staphylococci invading the cornea, usually *S. aureus,* are often resident strains from the patient's own flora.[42,43] *Staphylococcus epidermidis* is not a common infectious agent, but this organism, along with *Streptococcus* spp., can cause keratitis in immunodeficient patients and may be associated with chronic dacryocystitis.[44] In these cases of *S. epidermidis* and *Streptococcus* keratitis, ulcers may appear similar to those with staphylococcal hypersensitivity keratitis, in which corneal inflammation is thought to be a result of toxins produced by the conjunctiva-based organism or a hypersensitivity reaction to some portion of the bacterial cell wall.

Gram-positive aerobic bacilli do not often cause keratitis in immunocompetent individuals.[45] *Corynebacterium diphtheriae* is one of the organisms reported to invade intact epithelial surfaces. *Bacillus cereus* is a gram-positive rod that can cause severe ulcerative keratitis, often after an injury involving a foreign body.[46] *Nocardia* and *Mycobacterium* spp. are increasingly implicated in bacterial keratitis after refractive surgery (i.e., LASIK).

Staphylococcus hypersensitivity reactions may actually be the most common cause of keratitis. Although it is related to the bacteria, the hypersensitivity is a reaction to some exotoxin or antigen rather than direct inoculation or infection. This condition may exist in hosts who may not have any increased bacterial load other than what is considered the normal commensural load in healthy individuals. The punctate epithelial defects, marginal stromal infiltrates, phlyctenules, and peripheral ulcerations are theorized to result from either type III or type IV hypersensitivity reactions. Histologic analysis reveals the presence of plasma cells and lymphocytes in the peripheral cornea but an absence of infectious organisms. Further support for the noninfectious etiology comes from the reduction in pathology when treated with topical steroids alone.

Lichtinger and coworkers[38] reviewed the distribution, current trends, and resistance patterns of bacterial keratitis in Toronto from January 1, 2000, through December 31, 2010. They identified a decreasing trend in gram-positive isolates. The most common isolate was coagulase-negative staphylococcus.[38] In another study, Shalchi and colleagues[48] reported a high level of gram-negative keratitis and highlighted a trend of increasing gram-negative infection in cultures isolated in the United Kingdom over a 10-year period.

Gram-Negative Bacteria

Pseudomonas aeruginosa is a particularly virulent and generally most common gram-negative organism implicated in bacterial keratitis.[38] Untreated *P. aeruginosa* keratitis progresses quite rapidly from suppuration to perforation, mainly because of swift corneal destruction from the associated proteolytic enzymes.[49,50] A characteristic inflammatory ring of neutrophilic infiltrate may surround the lesion.[51,52] This "ring infiltrate" is not unique to gram-negative organisms; it can also be seen in fungal, viral, and *Acanthamoeba* keratitis. Patients in burn units and intensive care settings often have altered mental status or anatomic injuries that make ocular exposure and corneal desiccation more common. When this is combined with the frequent colonization of such units with *P. aeruginosa,* this organism can be a significant cause of keratitis. *Pseudomonas aeruginosa* was identified as the most common gram-negative bacteria isolated in cultures performed on suspected bacterial keratitis from January 1, 2000 through December 31, 2010.[38]

Less common gram-negative entities have been reported to cause keratitis. Although *Morganella morganii* keratitis may be clinically indistinguishable from *P. aeruginosa* keratitis, *Delftia* (*Pseudomonas*) *acidovorans* and *Pseudomonas stutzeri* usually have a more benign course.[53] *Serratia marcescens* has been implicated in contact lens–associated keratitis.[54] *Moraxella* keratitis may be more common in alcoholics and less common in patients with chronic ocular surface disease.[55,56]

Although *N. gonorrhoeae* is more commonly associated with conjunctivitis, it can penetrate an intact corneal epithelium and cause keratitis. The rather explosive onset and copious purulent exudates may obscure a diagnosis of keratitis. The corneal infiltration may be secondary to the effects of the conjunctival infection. *Neisseria gonorrhoeae* can produce such marked infiltration and edema that the affected conjunctiva may protrude or drape over the corneal surface, exposing the epithelium to numerous proteolytic enzymes; ulceration can quickly result. *Acinetobacter* can produce a keratitis that is clinically indistinguishable from *Neisseria* and can appear morphologically similar on Gram stain.

Mycobacteria

Mycobacterium keratitis had been decreasing in prevalence, paralleling the reduction in systemic tuberculosis. Although primary tuberculous keratitis still is uncommon, an increase in systemic immunodeficient hosts as well as incisional refractive surgery (i.e., LASIK) has been accompanied by an increase in *Mycobacterium* keratitis (*M. fortuitum, M. chelonae, M. gordonae,* and *M. avium-intracellulare*).[57,58] Infection with atypical *Mycobacterium* spp. may be indolent with mild inflammation, therefore delaying the diagnosis.[59] Several cases of mycobacterial keratitis after LASIK have been reported in the literature. The most frequently involved pathogen is *M. chelonae* (66%).[59,60] These nontuberculous species can be quite difficult to isolate and eradicate because they follow a chronic indolent course and are often resistant to conventional antitubercular medications. In the case of refractive surgery, the location under the LASIK flap causes difficulty in obtaining cultures as well as direct application of topical medications. *Mycobacterium* spp. are responsible for eventually producing keratitis in about 15% of patients with tuberculoid leprosy but in nearly 100% of those with lepromatous leprosy.[61,62]

Therapy for Bacterial Keratitis

Immediate, Aggressive Therapy

Aggressive antimicrobial therapy is the primary approach with infectious keratitis. Topical administration is the most common route, and has become the classical approach to manage bacterial keratitis. Other treatment options include antibacterial therapy using subconjunctival injections and parenteral and oral routes. Additionally, antibiotic-soaked collagen shields/soft lenses have been, or are currently being, used. The clinician must decide whether to initiate immediate antimicrobial therapy, either directed or broad spectrum, or to wait for laboratory identification of the offending agent. If bacterial keratitis is suspected, the practitioner is advised to initiate therapy immediately after obtaining corneal specimens for microbial identification. The explosive nature of bacterial keratitis suggests the need for immediate therapy, perhaps guided by results of Gram or Giemsa stains, in all suspicious cases. The rather indolent course of fungal and *Acanthamoeba* keratitis and the significant commitment to months of costly treatment might allow a delay in therapy until a causative agent is identified. If severe suppurative keratitis is seen, the Gram stain and smear will guide the selection of medication. One specific medication may be selected if only one type of bacterium (gram-positive or gram-negative) can be positively identified and the patient has not previously started an antibiotic. If two or more types of bacteria are identified, if the stain and smears are equivocal, or if the patient has been on any type of antimicrobial agent, then broad-spectrum therapy is initiated.

Hospital Admission

An important decision in treating keratitis involves hospitalization or outpatient care. Hospitalization is rarely required, but it may be necessary

for noncompliant patients or for patients with rapid necrosis or thinning. The potential for a rapid downturn and possible corneal perforation should give the clinician a low threshold for admission. The high frequency of antimicrobial administration (perhaps hourly around the clock) and close monitoring may make outpatient care impractical, especially for those living alone, living far from the hospital or clinic, or otherwise unable to comply with such demands.

Antibiotic Solutions

The route of antibiotic administration should be based on the severity of the disease but often includes hourly (or more frequent) dosing, especially in cases of severe suppurative keratitis. Although patients may prefer ointments for ease of delivery and the comfort associated with the carrier, frequent administration of topical solutions is recommended because they can penetrate ocular tissues and achieve higher concentrations better than antibiotics in ointment formulations. Commercial pharmacies do not often provide concentrated (fortified) antibiotics, but compounding and hospital-based pharmacies can easily formulate fortified antibiotics using most parenteral antibiotic preparations.

Although bacterial ulcers typically respond to treatment with available topical antibiotics, a report by the CDC on the rise of rates of antibiotic-resistant infections, such as methicillin-resistant *S. aureus,* has led to huge concern.[62a] These ocular isolates are reported to be resistant to the most commonly prescribed antibiotic class, the fluoroquinolones.[63–65] The Steroids for Corneal Ulcer Trial (SCUT) identified correlations between in vitro susceptibility and clinical outcomes, which demands that corneal culture and sensitivity testing be performed for all corneal ulcers.[66–68] Response to treatment assessment is always critical.

Another concern is poor clinical outcomes resulting from irregular astigmatism and corneal opacity even when the bacterial ulcer pathogens are susceptible to topical antibiotics. The way to have strong impact on the clinical outcomes is by identifying the factors that resolve the inflammatory response to infection that results in corneal melting and scarring.[4]

Local and Systemic Administration

Subconjunctival injection of antibiotics had been more common in the past and is one way to attain a peak concentration without compliance issues. However, the discomfort and temporary nature make this less attractive, and frequent topical administration of fortified antibiotics can achieve adequate tissue levels. Parenteral administration is uncommon and generally used only in suppurative keratitis with impending or actual perforation or bacterial extension involving the sclera.

Contact Lenses and Collagen Shields

Antibiotics have been administered through the use of collagen shields and soft contact lenses.[69] Although these methods have been used experimentally, there are no controlled clinical studies detailing efficacy and safety. New technologic advances in polymer formation and microspheric packaging of antibiotics have allowed more practical ways to actually impregnate antimicrobials into the contact lens rather than simply soaking the lens or shield in the antibiotic as is done currently. Polymer inserts have been designed to theoretically increase the duration of the drug in the tear film. A rather simple strategy to include drug delivery to the corneal tissue involves placement of temporary punctal plugs to reduce the outflow from the ocular surface.

Further, because collagenases are involved in protein degradation and keratolysis, simply stabilizing corneal melting through anticollagenases such as tetracyclines may also reduce the incidence of severe complications of infectious keratitis, such as corneal perforation.[70–72] Studies involving tetracyclines, however, have only been either in vitro or in vivo studies. High-quality randomized controlled trials in humans are needed to guide clinicians in the use of adjuvant doxycycline for the treatment of corneal ulcers.[4]

Unique Pharmacokinetics With Topical Antibiotics

Although laboratory identification is quite helpful, the standard sensitivity testing has its limitations in treating ocular infections. The minimal inhibitory concentration (MIC) determinations performed in the laboratory are based on antibiotic concentrations that are achievable through the host's serum. The concentrations achievable through direct topical administration can be many thousands of times greater than that measured in the serum after parenteral administration. This might lead to an organism being labeled "resistant" to testing with an antibiotic at achievable serum concentrations, although it may well be in the process of eradication with that same drug through the highly concentrated topical approach. This highlights the clinical response as being the best indicator of actual susceptibility, not to the exclusion of laboratory sensitivities but within the unique limitations in dealing with the direct approach in eye conditions. Direct application to the infected area may avoid dealing with numerous issues critical in systemic therapy: distribution space, first-pass clearance, absorption characteristics, toxic reaction in nontarget tissues, and impact of renal or hepatic failure.

Initial Therapy

The ideal initial antimicrobial agent should be effective (bactericidal vs. bacteriostatic) against the common or suspected corneal pathogens and have low rates of resistance, minimal toxicity to ocular tissues, comfort on administration, and rapid penetration into the ocular tissues. Traditionally, broad-spectrum therapy for suspected bacterial keratitis has been the combination of a topical cephalosporin (or vancomycin) and an aminoglycoside (tobramycin or gentamicin), all as fortified concentrations. The main disadvantages of such treatment involve the ocular irritation, difficulty of obtaining noncommercially available solutions, significant cost, and continued refrigeration of some preparations. These inconveniences have led to an increasing interest in initial single-agent therapy by using fluoroquinolones. Whether as single agents or in combination, treatment generally begins with hourly dosing and then is tapered according to the clinical response. The fortified preparations are often continued for 10 to 14 days, after which a broad-spectrum nonfortified antibiotic may be given until resolution.

Many classes of antibiotics have been used for the treatment of specific classes of organisms, often based on Gram stain and eventual identification. The advent of the broad-spectrum fluoroquinolones has altered some of these traditional classes and treatment pathways, but many still hold true. Topical cephalosporin preparations are often used in gram-positive keratitis, especially with *Staphylococcus* spp. Vancomycin is an alternative if resistance is suspected. Gentamicin and tobramycin are the most common therapeutic choices in gram-negative keratitis. Drops are readily available commercially after the clinical improvement warrants a change from the fortified preparation.

Topical Fluoroquinolones

The development of fluoroquinolones has radically altered the previously standardized treatment of bacterial keratitis. Cultures were performed on all patients and all were generally started on combination fortified antibiotics. The efficacy of the fluoroquinolones, however, has made some consider monotherapy and question the need for culturing in all cases. The agents responsible for this shift were the second- and third-generation fluoroquinolones: ciprofloxacin, ofloxacin, and levofloxacin. Fourth-generation fluoroquinolone topical antibiotics (moxifloxacin, gatifloxacin) were introduced later to reduce the risk of bacterial resistance in view of the structural modifications and dual inhibition mechanisms of these antibiotics.[73] The spectrum of coverage at the high concentrations obtained locally is quite similar for the second- and third-generation fluoroquinolones, showing activity against most gram-negative aerobes and many gram-positive organisms.[74] These fluoroquinolones have significant activity against *P. aeruginosa,* including strains that may be resistant to other antimicrobials. *Haemophilus, Neisseria,* and *Moraxella* spp. are quite susceptible to any of the fluoroquinolones. Ciprofloxacin, ofloxacin, and levofloxacin have reasonable activity topically against some *Mycobacterium* and *Chlamydia* species. The greatest gap in coverage of second- and third-generation fluoroquinolones was with *Streptococcus* spp. and anaerobic bacteria.

The fourth-generation fluoroquinolones, moxifloxacin and gatifloxacin, inhibit bacterial DNA gyrase and topoisomerase IV, which results in increased antibiotic potency against gram-positive organisms and broad-spectrum activity against gram-negative bacteria.[75] Studies of the MICs of fourth-generation fluoroquinolones showed similar or better ability to kill causative bacteria in infectious corneal ulcer than

earlier-generation fluoroquinolones.[73,76,77] Fourth-generation fluoroquinolones have higher potencies against gram-positive organisms. However, ciprofloxacin is still better than the third- and fourth-generation fluoroquinolones against gram-negative bacteria, including *P. aeruginosa*.[78] Moxifloxacin has been reported to achieve the highest conjunctival, corneal, and aqueous concentrations.[73]

Comparison of Fluoroquinolones and Fortified Antibiotics

Any new medication or alteration in standard treatment protocols is best accepted after direct comparison with the gold standard. There are several comparative studies of the fluoroquinolones and the fortified combination therapies. In a randomized, masked comparative study of 122 patients, second-generation fluoroquinolones were found to be as efficacious as fortified gentamicin and fortified cefuroxime.[79] In another multicenter, randomized study, fluoroquinolones also compared favorably with fortified tobramycin and fortified cefazolin.[80] A similarly designed multicenter study involving 324 patients showed no difference using ciprofloxacin versus fortified cefazolin and fortified tobramycin.[81] In all three studies, the patients favored the fluoroquinolone, mainly for greater ocular comfort. The only adverse effect of the fluoroquinolones was the appearance of white crystalline precipitates near the epithelial defect in 16% of patients. This precipitate was seen more frequently with ciprofloxacin than with ofloxacin, consistent with a difference in the pH of the two agents. The precipitates can impair ability to monitor the subprecipitate infiltrates. The corneal precipitates appear to have no other clinical impact, and they resolve spontaneously after the medication is stopped.

Wong and coworkers[73] reviewed three clinical trials that investigated the clinical efficacy of the fourth-generation fluoroquinolones in treating infectious keratitis. The results of the three clinical trials correlate well with the results of the in vitro studies mentioned earlier, in that fourth-generation fluoroquinolones were comparable to fortified antibiotics and were better in the treatment of infectious keratitis.[82–84] The antibiotic resistance rates of bacterial isolates were consistently lower with moxifloxacin and gatifloxacin than almost all other antibiotics.[73] A large study was conducted by Constantinou and colleagues[82] that comprised 229 patients randomized to three treatment groups (moxifloxacin 1.0%, ofloxacin 0.3%, and the combined fortified tobramycin 1.33%/cefazolin 5.0%). Constantinou and colleagues reported that none of the bacterial isolates were resistant to moxifloxacin, 2.5% were resistant to ofloxacin, 14.8% to cefazolin, 1.6% to tobramycin, and 17.5% to chloramphenicol. Clinical outcomes (cure rate, mean time to cure, clinical sign score, and rate of serious complications) were not significantly different among the three groups.[82] Parmar and associates[83] compared the effect of topical gatifloxacin 0.3% with ciprofloxacin 0.3% for the treatment of patients with bacterial keratitis and ulcer size of at least 2 mm. These authors reported that culture results revealed a significantly larger proportion of gram-positive and gram-negative bacteria to be susceptible to gatifloxacin than to ciprofloxacin: 96.2% of gram-positive cocci were susceptible to gatifloxacin versus 60.4% to ciprofloxacin; all gram-positive bacilli were susceptible to gatifloxacin, but only 75% were susceptible to ciprofloxacin; and 92.9% of gram-negative bacilli were susceptible to gatifloxacin compared with 85.7% to ciprofloxacin. Even for *P. aeruginosa,* 87.5% were susceptible to gatifloxacin, whereas only 75% were susceptible to ciprofloxacin. With patients on gatifloxacin, 95.1% showed good response and complete healing of ulcer compared with 80.9% of patients on ciprofloxacin.[83] The third study, by Shah and coworkers,[84] compared the clinical effects of moxifloxacin 0.5%, gatifloxacin 0.5%, and combined fortified tobramycin 1.3%/cefazolin 5% on bacterial keratitis. All patients had ulcer size between 2 and 8 mm. These authors reported that all bacteria isolated were susceptible to the two fourth-generation fluoroquinolones. The cure rate of the fortified antibiotics group was 90%, and that of the gatifloxacin and moxifloxacin group was 95%.[84]

In contrast to these three clinical trials, a retrospective study in the United Kingdom by Shalchi and colleagues[48] determined the scale of antibiotic resistance in microbial keratitis. They reported no trend in increasing resistance of ciprofloxacin and advised against a change to fourth-generation compounds.[48] Wong and coworkers[73] attributed this discrepancy to the different spectrum of pathogens in bacterial keratitis, with 38.9% gram-positive and 61.1% gram-negative in the UK study. Fourth-generation fluoroquinolones are known to have higher potency against gram-positive pathogens and lower potency than second-generation fluoroquinolones in the inhibition of *P. aeruginosa.* Of note is that *P. aeruginosa* only constituted 7% of all the isolates in the Constantinou study compared with 49.1% in the United Kingdom.

A Cochrane-style review of high-quality, randomized, controlled clinical trials on the management of bacterial keratitis with topical antibiotics found no significant difference in the relative risk of treatment success (defined as complete reepithelialization of the cornea) or in the time to cure, when comparing two or more topical antibiotics over at least 7 days. This review included 16 trials.[85] The aminoglycoside-cephalosporin combination reportedly caused an increase in the relative risk of minor adverse events such as chemical conjunctivitis and ocular discomfort compared to fluoroquinolones; however, it did not increase risk of major complications.[80–82,85]

In summary, fourth-generation fluoroquinolones are reasonable monotherapeutic alternatives to the combination of fortified antibiotics in the management of infectious keratitis. Further studies are needed to compare the response of *P. aeruginosa* infections to these antibiotics before concluding that fluoroquinolones are as effective as the standard combination of fortified antibiotics in the management of infectious keratitis.[73]

Proposed Therapy Guidelines

The spectrum, safety, comfort, cost, and availability of the fluoroquinolones make them a very appealing choice in treating keratitis. Although many practitioners use these medications as first-line agents, fluoroquinolones are still not recommended as empirical therapy in vision-threatening keratitis. Initial treatment, often with combination agents directed toward the likely pathogens, guided by laboratory evaluation, is still essential for such cases. General guidelines for managing keratitis are as follows; the best approach for corneal ulcers is still not definite.

1. Corneal scrapings are indicated in patients with suspected infectious keratitis when risk factors are present, when there is a large central infiltrate, or after empirical therapy has failed.
2. Culture should be done in all cases of suspected infectious keratitis in community- and hospital-based practices.
3. Fortified cefazolin and aminoglycoside should be used for more severe keratitis.
4. Monotherapy with a fourth-generation fluoroquinolone can be used for mild keratitis.

Topical Corticosteroids

The use of topical corticosteroids to decrease long-term sequelae of bacterial keratitis is controversial. An intense suppurative inflammatory reaction consisting mainly of polymorphonuclear leukocytes is induced by many bacteria responsible for keratitis. These neutrophils may destroy a significant amount of tissue through free radicals and the liberation of collagenases and gelatinases that dissolve the stroma. The rationale for corticosteroids is to prevent such tissue destruction and to improve outcomes by reducing scarring, neovascularization, and stromal melt.[86–89] Several studies have shown that concomitant use of topical steroids does not alter or reduce the bactericidal effect of antimicrobials.[90] In one study, Henry and associates[91] reported that patients using topical corticosteroids are at a higher risk of progression from keratitis to endophthalmitis; some others have reported delayed epithelial healing and worsening of infection.[92–95] A subgroup analysis of a randomized placebo-controlled study of topical steroids found that addition of corticosteroids within 2 to 3 days of initiation of topical antibiotics improved 3-month visual acuity.[96] On the other hand, a recent Cochrane review of adjuvant topical steroids for bacterial keratitis involving four randomized controlled trials found no difference in healing times or visual acuity outcomes between the topical antibiotics alone versus topical antibiotics in conjunction with topical steroids.[97–100] There is a concern that steroid use may promote a relapse of an organism that appeared to be clinically resolved but still had a low number of active bacteria. This may be the case with virulent

organisms that are difficult to eradicate and require only a low inoculum to establish an infection, as seen in some gram-negative bacteria such as *P. aeruginosa*.

Supportive Measures

Supportive measures in managing infectious keratitis are numerous. Topical cycloplegics can reduce the discomfort of ciliary spasm, reducing the associated photophobia and preventing synechiae (scarring/adhesions) of the pupil. If corneal ulceration is quite significant, a temporary soft contact lens may act as a bandage to relieve some of the discomfort while allowing repair of the stromal and epithelial surfaces without mechanical disruption from the eyelid. The contact lens itself can serve as a nidus for infection, but this should not be the case with the temporary placement of the contact lens combined with continued application of antibiotics. The change to commercial-strength antimicrobials is warranted as soon as clinically reasonable because the fortified antibiotics are epitheliotoxic and decrease epithelial healing.

Price and coworkers[101] have investigated the role of collagen cross-linking adjuvant therapy for microbial keratitis in 48 patients (confirmed bacterial etiology in 24 eyes, fungal in 7, and protozoan in 2). Despite encouraging results for superficial infiltrates, the potential benefit of this approach remains of limited value.

CHLAMYDIAL AND SYPHILITIC KERATITIS

Chlamydial Keratitis

Chlamydiae are small intracellular organisms dependent on their host cell for replication and prolonged survival.[102] Their unique multiphasic life cycle permits the pathogen to establish persistent infections, allowing the organism to escape immune clearance yet express virulence determinants that cause chronic inflammation.[103] In addition, long-lasting protective immunity does not develop after chlamydial infection, and reinfections are common.

Ocular Associations With *Chlamydia*

Chlamydiae cause a spectrum of acute and chronic ocular diseases, ranging from self-limited follicular conjunctivitis to trachoma, often with blinding sequelae.[104] Several serotypes of *Chlamydia trachomatis* can cause a follicular conjunctivitis known as adult inclusion conjunctivitis. The same serotypes can lead to neonatal conjunctivitis if an infected mother transmits the pathogen to the newborn during vaginal delivery. Repeated infections with certain serotypes can cause trachoma, a chronic follicular keratoconjunctivitis that remains the most common cause of preventable blindness in the world. In developed countries, sexually transmitted *C. trachomatis* rarely causes the sequelae of true trachoma, which is generally marked by repeat infection and chronicity.

The cicatricial phase of trachoma causes lid irregularities, leading to exposure or direct trauma from in-turned eyelids or eyelashes, which leads to corneal ulceration and opaque scarring, often long after resolution of the infective phase. The resultant effects on the cornea may predispose to bacterial superinfection, further adding to the disease morbidity.

Syphilitic Keratitis (Interstitial Keratitis)

The keratitis associated with syphilis, sometimes termed *interstitial keratitis* (IK), is not often seen in the active phase but is noted in subsequent examinations. The two major categories of IK are associated with syphilis and *Mycobacterium tuberculosis*. IK is rarely associated with acquired primary or secondary syphilis (<3% of all cases), with more than 90% caused by congenital syphilis. IK usually does not develop until the later phases of congenital syphilis, occurring in about 50% of all untreated patients. The typical presentation is during the early teen years; however, it can develop any time during the first two decades of life. Pain, photophobia, increased tearing, blepharospasm, and decreased vision are common during the acute phase. A severe iridocyclitis is present as the cornea becomes hazy, with significant reduction in vision over a few days. Neovascularization progresses centrally over the next months until the vessels coalesce in the central cornea, at which time there is a dramatic change in the disease process. After resolution of the corneal infiltrates and significant regression of the blood vessels (leaving empty channels or "ghost vessels"), some patients have a surprisingly significant return of visual acuity. Only about 10% have less than 20/200 vision; approximately 70% have between 20/20 and 20/100 vision. Lyme disease has also been implicated as a possible etiology in corneal disease, with *Borrelia burgdorferi* causing an IK similar to that seen with syphilis.[105]

Therapy for Chlamydial and Syphilitic Keratitis

Keratitis in patients with trachoma is largely due to the lid scarring or exposure resulting from the conjunctival disease and therefore requires treatment of the associated conditions to reduce the subsequent keratitis. Several generalizations can be made when treating other chlamydial eye infections. First, systemic rather than local therapy is necessary when treating adult inclusion conjunctivitis because the infected individual may harbor the pathogen in the genital tract. Similarly, newborns presenting with neonatal chlamydial conjunctivitis may harbor the organism in the lower respiratory tract and may need to be treated systemically. Individuals treated systemically do not need to be treated locally. Second, all sexual partners must be treated along with the infected patient to prevent primary infection in the partner as well as reinfection in the symptomatic individual. Third, repeat treatment may be necessary because clinical cure rates can be modest, especially for treatment of chronic chlamydial infection. Finally, antibiotics that achieve sustained tissue levels, high intracellular penetration, and low microbial resistance and require an infrequent dosing regimen are generally preferred. To this end, azithromycin is rapidly becoming the mainstay of therapy for many chlamydial infections, including *C. trachomatis*.

Therapy for Interstitial Keratitis (Syphilitic and Lyme Associated)

Syphilitic IK is an immune phenomenon that may benefit from topical corticosteroids, but antitreponemal therapy has little impact on the corneal process. However, such therapy may be necessary for other systemic manifestations of the disease and does seem to reduce the recurrence rate and likelihood of bilateral involvement. Lyme keratitis may be addressed through the use of corticosteroids, with systemic evaluation and treatment similar to that for syphilis.

VIRAL KERATITIS

Herpes Simplex Virus

Ocular herpes may be classified into three general groups: congenital and neonatal, primary, and recurrent. The global incidence of herpes simplex virus (HSV) keratitis has roughly been estimated to be 1.5 million, including 40,000 new cases of severe monocular visual impairment or blindness each year.[106] The combined incidence of epithelial and stromal keratitis in the United States has been estimated to be 18.2 per 100,000 person-years.[106] The vast majority of all ocular herpes infections are caused by HSV type 1 (HSV-1). Because infection is acquired by passage through an infected birth canal, 80% of neonatal cases are caused by HSV type 2 (HSV-2).[107,108] Multiple recurrences are far more common with genital and oral herpes than with ocular herpes. Studies have shown an 89% recurrence rate for genital and a 42% recurrence rate for oral HSV over a 1-year period. In contrast, the ocular herpes recurrence rate was 40% over a 5-year period, which is fortunate considering the visual consequences.[109,110] Epithelial ulcers ultimately respond to antiviral therapy, but stromal involvement may not readily clear, leaving a nebulous scar. In the absence of superinfection, the skin lesions heal without scarring.

Evidence has suggested that HSV-1 infection disrupts the normal equilibrium between angiogenic and antiangiogenic stimuli, leading to vascularization. Thrombospondins 1 and 2, matricellular proteins involved in wound healing, are potent antiangiogenic factors and appear to be one of the key players. Elucidating their roles in corneal scarring and vascularization may lead to improved therapies for herpes simplex keratitis.[111]

Ocular infection with HSV-1 continues to be a serious clinical problem despite the availability of effective antivirals. A 2008 review reported that despite intensive antiviral and antiinflammatory therapy, a significant percentage of patients do not respond to chemotherapy for herpetic

necrotizing stromal keratitis. Therefore the development of therapies that would reduce asymptomatic viral shedding and lower the risks of recurrent disease and transmission of the virus is crucial to decreasing the morbidity of ocular herpetic disease.[112]

Primary Herpes Simplex Virus Keratitis

Sixty percent of children are infected with HSV by age 5 years, all of whom then carry latent virus in their dorsal root ganglia.[113,114] Ocular disease resulting from primary infection or reactivation in the trigeminal ganglion may manifest in many forms, one of which is keratoconjunctivitis. In the absence of skin vesiculation, differentiation from adenoviral infection is aided by a careful search of the lid margins for signs of herpetic blistering.

Primary HSV keratitis is often atypical. Initially, there may be just a nonspecific diffuse punctate keratitis that evolves into multiple scattered microdendritic figures. There may be wandering linear serpiginous ulcers across the entire corneal surface.[109] The diffuse nature of this primary epithelial involvement is probably a function of the host's nonimmune state, which allows more widespread ulceration. Primary disease is, as a rule, confined to the epithelium in terms of clinical findings. Stromal involvement is not usually seen in this phase of the disease, presumably because the host is not immunologically programmed against the virus. Primary ocular herpes should not be confused with "first ocular occurrence." The former is a first encounter with the virus; the latter is the first eye involvement with HSV in a patient who has had a subclinical oral or nasal infection and is immune. First ocular occurrence is therefore similar to recurrent ocular herpes as described next.

Recurrent Keratitis

Patients with recurrent ocular herpes have both cellular and humoral immunity against the virus. Herpes simplex–induced eruptions of the corneal epithelium are characteristically thin, branching dendritic ulcers; wider, branching dendrogeographic ulcers; or map-shaped geographic lesions, all caused by live virus. Little inflammatory cell reaction—polymorphonuclear leukocytes but no lymphocytes—is seen in this form of ocular herpes, but many free viruses lie in intracellular and extracellular locations, particularly in the basal epithelium.[115] Signs and symptoms include tearing, irritation, photophobia, and often blurring of vision. Because the only presenting clinical finding may be a watery conjunctivitis, the patient should be asked about any trauma, previous corneal ulcers, inflammation inside the eye (iritis), nasal or oral cold sore, genital sores, recent use of topical or systemic steroid or immunosuppressive drugs, and immunologic deficiency states, malignancy, organ transplants, or chronic eczema. If corneal examination reveals dendritic or even geographic ulceration, the infectious agent is HSV until proven otherwise. In HSV keratitis, corneal sensation is reduced in about 70% of patients,[116] although it is almost never totally absent as often seen in varicella-zoster keratitis. The more marked the stromal scarring, found in more chronic cases, the more marked is the decrease in sensitivity. The corneal lesions begin as a fine, transient, punctate keratitis that coalesces to form dendritic, dendrogeographic, or geographic ulcers. In an eye that has had many recurrences or is receiving steroids without antiviral prophylaxis, the more subtle stages may be bypassed, and the cornea may simply break down in rapidly forming geographic ulceration. The actual incidence of infectious epithelial HSV keratitis in immunocompetent patients may be much higher than suspected clinically. Kodama and colleagues[116] reported that of 48 eyes with diagnoses of nonherpetic conditions, 19 were culture positive for HSV. Contrary to common opinion, recurrent epithelial infections are not always due to the patient's original HSV strain. Remeijer and associates[117] reported a study on 30 patients in whom sequential corneal HSV-1 isolates revealed that 63% were genotypically the same from recurrence to recurrence, whereas 37% were actually genetically different.

Stromal Inflammation and Intraocular Reaction

Stromal reaction is usually absent or mild and confined to the anterior layers in milder epithelial infections. On occasion, however, even mild epithelial infection may be associated with notable stromal edema and iritis. These eyes are more likely to go on to chronic recurrent immune disease and scarring, resulting in visual loss. Concurrent or future stromal disease may be minimized, as well as healing rate enhanced, by gentle débridement of the infected epithelium with a sterile cotton-tipped applicator before instituting antiviral chemotherapy.[118,119] Such débridement is thought to remove much of the immune-inciting antigen that could penetrate to deep stromal layers.

Neurotrophic Keratitis

On occasion, despite adequate antiviral therapy, epithelial ulcers do not completely heal or, having healed, break down again in an ovoid or dendritiform pattern. This is trophic, or "metaherpetic," keratopathy. Clinically, a trophic ulcer may be distinguished from an actively infected viral ulcer by the appearance of its edge. Trophic ulcers have gray, thickened borders formed by heaped-up epithelium unable to move across or adhere to the damaged ulcer base. In contrast, actively infected ulcers have discrete flat edges that may change their configuration as the ulcer erodes newly infected epithelium. Persistence of trophic ulceration over several weeks or months poses a threat to the integrity of the globe. The longer the ulcer is present, the greater is the chance of collagenolytic activity, with subsequent stromal melting (thinning) down to the Descemet membrane and perforation.

Treatment is aimed at protecting the corneal surface and damaged basement membrane because of the neurochemical and mechanical nature of the problem. Therapeutic approaches include treatment of any meibomian gland dysfunction; copious lubrication with unpreserved artificial tears, gels, or ointments, or a combination of these; lateral tarsorrhaphy; therapeutic soft contact lenses; tetracyclines; suppression of inflammation; prophylactic oral antiviral agents; autologous serum drops; and amniotic membrane transplant or, on occasion, conjunctival transplant, conjunctival flap, penetrating keratoplasty, or keratoprosthesis.[120] It has been reported that neurotrophic corneal ulcer occurred after a retrobulbar injection of chlorpromazine. The authors postulated that chlorpromazine may lead to sensory denervation to the cornea, with subsequent development of neurotrophic keratopathy, and they advise caution against this potential adverse side effect through proper patient safety, education, and postinjection management.[121]

Varicella-Zoster Virus

Contact with the varicella virus in the United States was formerly almost invariable,[122] but the use of varicella-zoster virus (VZV) vaccine as part of the recommended childhood immunizations has substantially reduced the frequency of chickenpox. Ocular involvement in chickenpox is diagnosed on the basis of an acute or recent history of chickenpox with ocular or periocular involvement with the vesicle-pustules.

VZV infection may involve small phlyctenule-like lesions that may erupt most commonly at the corneal limbus.[109,123,124] It is unclear whether these are due to live virus or to an immune phlyctenule-like reaction, or both. The cornea may develop superficial punctate keratitis: wispy, branching dendritic ulcers without terminal knobs (herpes simplex ulcers have knob-shaped endings on their branches).[125,126] Months after the acute disease, a disciform keratitis similar to that seen in HSV disease may develop.[127] This disciform reaction is steroid responsive but may recur and cause scarring similar to HSV keratitis. Less frequently reported varicella findings in the eye include dendritic keratitis and neurotrophic ulceration with corneal melting.

Herpes Zoster Ophthalmicus

Herpes zoster is a reactivation of infection with VZV. Approximately 50% to 72% of patients with periocular zoster have involvement of the ocular structures, develop chronic disease, and possibly suffer a moderate to severe degree of visual loss.[128] Of the three divisions of the fifth (trigeminal) cranial nerve, the first (ophthalmic) is by far the most frequently affected. Herpes zoster ophthalmicus (HZO) occasionally affects the maxillary division but rarely affects the mandibular division.[129] The first division of the fifth nerve, along with sympathetic branches from the ciliary ganglion, innervate the eyelids, forehead, and tip of the nose and the majority of the orbit and ocular adnexa.[130]

Clinical Manifestations of Herpes Zoster Ophthalmicus

In Liesegang's study of 94 patients with HZO, two-thirds had corneal involvement.[130] This took the form of punctate keratitis (51%), pseudodendrites (51%), anterior stromal infiltrates (41%), sclerokeratitis (1%), keratouveitis-endotheliitis (34%), peripheral ulcerative keratitis (7%), delayed mucous plaques (13%), disciform keratitis (10%), neurotrophic keratitis (25%), and exposure keratitis (11%). A delayed limbal vasculitis with or without anterior ischemic necrosis was also noted on rare occasions. Corneal disease may precede, accompany, or follow the acute disease by months to years and may recur in any of its many forms. The acute epithelial disease is considered infectious and may present as a diffuse superficial punctate keratitis or more commonly as migratory dendritic lesions that at first could be confused with herpes simplex. Piebenga and Laibson[131] also described herpes zoster dendrites that were culture negative but appeared as heaped-up, superficial plaquelike lesions, coarser than herpes simplex dendrites but lacking terminal bulbs (an important differentiating point), and staining poorly with fluorescein. These dendrites cause a foreign body sensation and are elevated, coarse, gray-white, swollen epithelial cells piled in plaques or a dendritiform shape on the corneal surface. They are both migratory and transitory and are usually associated with a neurotrophic keratitis (75%) or previous corneal inflammation (100%). Immune keratitis similar in appearance to HSV stromal disciform edema/endotheliitis may occur any time after the acute illness, most commonly first appearing after 3 to 4 months.

Corneal Anesthesia

Corneal sensation may be markedly diminished in even the mildest cases of clinically manifest herpes zoster keratitis. Sixty percent of patients have moderate-to-complete corneal anesthesia (neuroparalysis) secondary to the destructive VZV ganglionitis and to aqueous tear deficiency resulting from loss of the nasolacrimal reflex.[129,132,133] Anesthetic epithelial breakdown comprises one of the most dangerous aspects of herpes zoster keratitis. One-quarter of all HZO patients develop clinical signs of neurotrophic keratitis because of permanent corneal anesthesia. As the corneal epithelium becomes progressively unhealthier, oval epithelial defects may develop in the palpebral fissure or lower corneal area, with subsequent melting and corneal thinning. Neovascularization in these cases is a good sign and should be allowed to take place because healing often accompanies the process. Because many of these eyes are poor surgical risks, it is unlikely that a corneal graft would be successful.

Ocular Complications With Vaccinia (Smallpox) Vaccination

Recent attention to the development and use of a smallpox vaccine has brought about an interest in vaccinia keratitis; the vaccine directed against smallpox is derived from the bovine equivalent, vaccinia.[134] A study by Ruben and Lane[135] indicated that not only were the ocular complications of vaccination infrequent; they were also not notably vision threatening. Corneal involvement after autoinoculation is uncommon, with reports of approximately 1.2 cases per million primary vaccinations.[136] Other studies indicate postvaccinial keratitis in 6% to 37% of vaccinia cases with ocular involvement.[137,138] Keratitis appears more frequently in primary vaccinees than in revaccinees, which is probably a reflection of the immune status of the previously vaccinated patient. Long-term sequelae, such as madarosis (eyelash loss), punctal stenosis, and cicatricial lid changes are more common in cases with corneal manifestations (18%) than in ocular vaccinia without keratitis (2%). However, reexamination of patients with corneal vaccinia after 5 years revealed either no ocular residua or only minor corneal changes, including mild corneal scarring, ghost vessels, and subepithelial opacity with chronic conjunctivitis.[135]

Vaccinia Keratitis

Corneal manifestations of ocular vaccinia range from mild superficial punctate keratitis to interstitial or stromal keratitis, to disciform keratitis with keratic precipitates, to necrosis with perforation. As with epithelial herpes simplex or varicella-zoster keratitis, corneal epithelial vaccinia lesions stain with rose bengal early in the course of the disease and with fluorescein as an epithelial defect evolves. Direct infection of the corneal epithelium with vaccinia may present as multiple punctate lesions, as dendritiform lesions, or in a geographic pattern. All forms of vaccinia epithelial keratitis may closely resemble that seen with herpes simplex. Stromal keratitis caused by vaccinia may initially appear as scattered subepithelial opacities similar to those seen in epidemic keratoconjunctivitis. This pattern may evolve to ring infiltrates, ulceration, or stromal necrosis or scarring, which must be differentiated from *Acanthamoeba,* herpes zoster, and herpes simplex stromal keratitis.

Adenoviral Keratitis

Epidemic keratoconjunctivitis (EKC), caused by various adenovirus serotypes, is a highly contagious condition that can have explosive spread in schools, workplaces, and physicians' offices. The keratitis may have an early epithelial component, followed by a later subepithelial stage. Active viral replication within epithelial cells marks the early keratitis. The later-developing subepithelial infiltrates (SEIs) probably represent an immune response to viral antigens because microscopic analysis of SEIs reveals lymphocytes, degenerated collagen fibrils, and scarring, but no virus particles. A watery discharge, photophobia, and foreign body sensation are often accompanied by a hemorrhagic conjunctivitis with membrane formation. The keratitis progresses through a fairly orderly process of superficial epithelial keratitis, deep epithelial keratitis, and eventual SEIs.

Differential Diagnosis of Epidemic Keratoconjunctivitis

Pharyngoconjunctival fever is caused by different serotypes of adenovirus with a similar course of conjunctivitis but less commonly leads to epithelial keratitis and SEIs. Pharyngoconjunctival fever may begin in one eye but usually involves both eyes eventually. Enterovirus, molluscum contagiosum, Epstein-Barr virus, and coxsackievirus can be associated with a mild epithelial keratitis similar to the early stages of EKC. Keratitis associated with hemorrhagic conjunctivitis caused by enterovirus 70 is discussed in Chapter 112. Keratitis associated with measles (rubeola) generally is similar to that seen in EKC; however, in patients with significant malnutrition and vitamin A deficiency, measles keratitis can become a blinding disease with secondary bacterial infection and possible perforation of the globe.

Therapy for Viral Keratitis

Self-Limited Cases

Many of the cases associated with viral keratitis may not require specific antimicrobial therapy. There may be a twofold reason for this. First, a number of viral conditions are self-limited with relatively minor deleterious effects on the cornea, and second, the research and production of antiviral agents have not led to many viable treatment options, especially compared with those for bacterial keratitis. However, there are viral organisms that can cause significant visual or ocular morbidity, particularly when combined with the associated inflammatory response.

Therapy for Herpes Simplex Virus and Varicella-Zoster Virus

The management plans for both herpes simplex and herpes zoster have undergone many changes as more antiviral agents and information from basic research and clinical trials have become available. Tables 113.2 to 113.4 give the currently recommended treatments. Although topical acyclovir is just as effective as trifluridine with less ocular toxicity and is the first-line treatment for HSV keratitis in Europe, it is currently unavailable in the United States.[4,139] Acyclovir in oral form has also been shown to be effective against VZV keratitis, although the Herpetic Eye Disease Study (HEDS) I, a randomized controlled trial, found no significant improvement in the best spectacle-corrected visual acuity (BSCVA) when treating HSV stromal keratitis with oral acylovir.[4,140] On the other hand, HEDS II found that ocular HSV recurrence was 45% lower in the acyclovir group compared to the placebo group. HEDS II was a large, multicenter, randomized, placebo-controlled trial that evaluated the prolonged use of oral acyclovir for recurrent ocular HSV. The acyclovir group was reported to experience a 19% recurrence rate while the placebo group experienced a 32% recurrence rate ($P < .001$).[4,141]

TABLE 113.2 Antiviral Medications Used in Herpes Simplex Keratitis

Acute Infection

Topical or systemic medication; in the immunocompromised patient, a topical agent may be combined with a systemic agent and continued longer than the indicated period

Medication, Dosage	*Frequency*
Trifluridine (Viroptic), 1% drops	9 times daily for 7 days. May decrease dose to 5 times/day after 7 days if ulcer has healed.
Acyclovir (Zovirax), 400 mg PO[a]	tid for 14–21 days
Valacyclovir (Valtrex), 1 g PO[b]	bid for 14–21 days

Prophylaxis

Against recurrent stromal keratitis, high steroid use, and graft rejection/postoperative grafts

Medication, Dosage	*Frequency*
Acyclovir (Zovirax), 400 mg PO[a,b]	bid for 12–18 mo
Valacyclovir (Valtrex), 500 mg PO[b,c]	bid for 12–18 mo

[a]Pediatric syrup, 200 mg/tsp.
[b]Not US Food and Drug Administration–approved for this specific purpose.
[c]For use in immunocompetent hosts only.
Modified from Pavan-Langston D. Ophthalmic zoster. In: Watson C, Gershon A, eds. Herpes Zoster and Post-herpetic Neuralgia. *2nd ed. Amsterdam: Elsevier Sciences BV; 2001:119–129. Reprinted with permission from Elsevier.*

TABLE 113.3 Management of Herpes Zoster Ophthalmicus (Acute)

Antivirals

Treat for 7 days, preferably starting within 72 hours of onset of rash
- Valacyclovir (Valtrex), 1 g PO tid
- Acyclovir (Zovirax), 800 mg PO 5 times/day

Immunocompromised patients: IV acyclovir for 10 days; 10 mg/kg q8h in adults and 500 mg/m^2 q8h for children younger than 12 yr

Pain Prevention and Management

Tricyclic antidepressant (e.g., nortriptyline, desipramine), 25–75 mg PO qhs or divided dose for 3 mo (or longer, prn); start at lowest dose with antivirals or as early as possible after acute disease onset, increasing over 2–3 wk prn. Caution in patients with cardiac disease.
Nonnarcotic analgesics or short-term narcotic analgesics (e.g., oxycodone, codeine)

Dermatitis Therapy

Cool to tepid wet compresses (if tolerated) to keep dermatitis clean

Ocular Anterior Segment

Exposure keratopathy: topical antibiotic ophthalmic ointment tid
Dendritiform keratopathy: therapy for 2–3 wk (variably effective)
- 3% vidarabine ointment, *or*
- 1% trifluridine 5 times/day, *or*
- Oral antivirals (see above)

Immune keratopathy, episcleritis, scleritis, or iritis:
- Topical steroids (1%–0.125% prednisolone, 0.1% dexamethasone, or 0.2%–0.5% loteprednol), q3–4h to qid prn depending on disease severity; slow taper
- Antibiotic eyedrops or gtt/ointment prophylaxis
- Oral nonsteroidal antiinflammatory agents (e.g., ibuprofen), 400 mg PO bid–tid
- Topical antivirals unnecessary
- Cycloplegia prn for iritis: scopolamine qd

Glaucoma

Topical β-blockers (e.g., timolol or carteolol) bid
Add prn latanoprost qd, brimonidine or dorzolamide bid
No miotics (e.g., pilocarpine)
Topical steroids if glaucoma is due to inflammatory trabeculitis

Modified from Pavan-Langston D. Ophthalmic zoster. In: Watson C, Gershon A, eds. Herpes Zoster and Post-herpetic Neuralgia. *2nd ed. Amsterdam: Elsevier Sciences BV; 2001:119–129. Reprinted with permission from Elsevier.*

TABLE 113.4 Management of Herpes Zoster Ophthalmicus (Chronic or Recurrent)

Dendritiform or Immune Keratopathy, Episcleritis, Scleritis, Iritis

See Table 113.3

Tenuous, Hazy Epithelium in Anesthetic Cornea

Early lateral tarsorrhaphy and lubrication with artificial tears and tear ointments
Allow vascularization to progress to aid in healing any ulcer
Topical steroids with caution and only at low doses to minimize any inflammation

Exposure Keratopathy or Corneal Ulceration or Thinning

Lateral tarsorrhaphy
Therapeutic soft contact lens (e.g., Permalens or Kontur)
Tissue adhesive (e.g., Dermabond) for progressive thinning
Conjunctival flap, transplant, or keratoprosthesis

Glaucoma

See Table 113.3

Postherpetic Neuralgia (Drugs Below May Be Used Additively)

Tricyclic antidepressants (e.g., nortriptyline, desipramine, or other tricyclics), 25 mg titrated up to 75 mg qhs or divided dose prn. Caution if patient has cardiac disease.
Gabapentin (Neurontin), 300 mg PO bid starting dose. Efficacy may not be reached until 600 mg bid–qid. Some patients may not respond at all.
Slow-release opioids added if tricyclic antidepressants and/or gabapentin not sufficiently effective; oxycodone (OxyContin), 10–40 mg PO q12h
Capsaicin cream 1–3 times daily to skin as tolerated
Lidocaine skin patches, 12 hours on/12 hours off painful skin area
Frontal and/or nasal nerve block
Trigeminal ganglion ablation contraindicated

Modified from Pavan-Langston D. Ophthalmic zoster. In: Watson C, Gershon A, eds. Herpes Zoster and Post-herpetic Neuralgia. *2nd ed. Amsterdam: Elsevier Sciences BV; 2001:119–129. Reprinted with permission from Elsevier.*

Ganciclovir, a synthetic medication with more broad-spectrum antiviral coverage, has been reported to be effective in treating HSV-, VZV-, and cytomegalovirus-related keratitis. In addition to being just as effective as acyclovir with less ocular toxicity, ganciclovir may also be less likely to promote drug resistance.[142,143] Ganciclovir is being investigated for the treatment of VZV keratitis in a large randomized controlled trial by Northwestern University.[4]

Data in the normal population indicate that the incidence of zoster is much lower in vaccinated healthy children and adults compared with those who have suffered a natural infection.[138] The US Food and Drug Administration (FDA) approval of a live-attenuated VZV vaccine for the immunization of healthy people of all ages who have not had previous varicella, thereby reducing the incidence of varicella and its complications, was hailed as a major step in controlling this disease and is potentially essential to reducing the incidence of zoster.[144] Introduction of a more effective, adjuvanted recombinant protein VZV vaccine for adults over 50 years of age should further reduce the incidence of herpes zoster. One question that continues to be raised is, should every patient with herpes zoster receive antiviral treatment? Gnann and Whitley[145] have recommended that treatment be given to those at high risk of complications: those older than 50 years of age, immunocompromised, having moderately severe to severe pain at presentation, and having a greater degree of skin-surface involvement, and those with HZO. One study of 324 patients compared the effect of antiviral therapy on complications of HZO; the probability of an adverse outcome was 9% in untreated patients and 2% in treated ones.[146]

Sometimes, topical corticosteroids are used as adjuvant therapy to topical antivirals. HEDS I evaluated the effectiveness of corticosteroids (topical prednisolone phosphate) in treating HSV stromal keratitis. The authors reported significantly shorter time to resolution of infection in the prednisolone group, with a median of 26 days for those taking prednisolone and 72 days for those taking placebo ($P < .001$). Visual acuity at 6 months remained similar in both groups.[147]

In case of cytomegalovirus stromal keratitis, the current preferred treatment is oral valganciclovir; however, it has significant side effects, such as aplastic anemia.[148] Valacyclovir has been reported to have better ocular penetration. In addition, its treatment dosage is 1 g three times daily as opposed to acyclovir, which is 400 mg five times a day (800 mg five times a day for VZV). Less frequent dosage often aids in patient compliance.[4,149,150]

Therapy for Ocular Vaccinia

During the previous era of routine smallpox vaccination, the treatment of ocular vaccinia was based predominantly on anecdotal reports of the use of vaccinia immune globulin alone or in combination with idoxuridine or topical interferon. Many of these isolated cases were reported before the availability of more effective topical antivirals, such as trifluridine and vidarabine. In the absence of masked, controlled clinical trials, a meaningful meta-analysis of the literature is not possible. Because of this limitation, some of the current recommendations, which depart from the previous policy designed in the mid-1960s, were made by the CDC after consultation with an outside panel of corneal and external disease and infectious disease specialists.[151] The recommendations are based on principles routinely used in the treatment of other viral diseases of the ocular surface: treat active viral replication with antivirals, followed by cautious use of topical corticosteroids (when appropriate) to prevent inflammatory damage of the cornea and anterior segment. For vaccinia, there is an additional therapeutic agent, intravenous immune globulin. Because ocular vaccinia virus infections are generally self-limited, treatment should be directed toward shortening the course and limiting the severity of the disease. The evaluation and treatment of ocular complications of vaccinia virus should be performed by an ophthalmologist in a timely manner. Intravenous vaccinia immune globulin is not recommended for isolated vaccinia keratitis because of the danger of corneal opacities. ST-246 (tecovirimat [Arestvyr]) has antiviral activity against vaccinia and could prove to be useful for ocular complications of vaccinia (see Chapter 132). Although no topical antiviral is licensed by the FDA for the treatment of ocular vaccinia, topical trifluridine drops or vidarabine ointment can be used off-label for this purpose and either is likely more effective and less toxic than topical idoxuridine or cytosine arabinoside.[152,153] Topical vidarabine may be preferable for use in children because it has been available in an ointment preparation that allows less frequent dosing and is associated with less initial stinging than trifluridine. Vidarabine ointment is currently not commercially available but may be obtained through compounding pharmacists. However, if vidarabine cannot be obtained or the patient would better tolerate drops, trifluridine may be used in children just as it is in ocular herpes simplex. Topical antiviral drugs should be considered for prophylaxis of the conjunctiva and cornea if vaccinia lesions are present on the eyelid, especially if near the lid margin. The use of these drugs for prophylaxis should be balanced against the possible risk of drug toxicity and of introducing virus into the eye by frequent manipulation. Topical trifluridine may possibly have an increased risk of toxicity if used for longer than 14 days. Toxic changes to the ocular surface are almost invariably reversible with discontinuance of the drug.

Therapy for Viral Keratoconjunctivitis

The keratoconjunctivitis associated with EKC (adenoviral mediated) may not require any measures other than artificial tears and possible cycloplegics. There is no effective antiviral for EKC. However, if the condition is associated with significant pain, photophobia, and visual alteration, a course of mild topical corticosteroids, in addition to the cycloplegics, may be beneficial. There have been no definitive trials indicating whether topical steroids alter the typical duration of the disease process.

FUNGAL KERATITIS

Although many genera of molds and yeasts have been identified in fungal keratitis, it is generally much less common than either bacterial or viral keratitis.[154] Although there are great differences based on global geography, fungi have generally been responsible for less than 5% to 10% of corneal infections in most clinical series reported in the United States. Keratitis caused by molds occurs more commonly in areas with a warmer and more humid environment. These fungi are usually inoculated into the cornea by trauma involving plant or vegetable matter. Keratitis caused by *Candida albicans* is acquired from the patient's own flora.

Corticosteroid use has been implicated in altering the cornea's resistance to fungal infection, while certainly potentiating any existing fungal infection. Topical corticosteroid use for medical or surgical ocular conditions (i.e., LASIK), as well as the use of soft contact lenses as a "bandage" for postoperative or damaged corneas, may increase the likelihood of fungal keratitis.

Fungal keratitis remains a diagnostic and therapeutic challenge. Difficulties are related to establishing a clinical diagnosis, isolating the causative agent in the laboratory, and treating the keratitis effectively with topical antifungal agents. Delayed diagnosis is common, primarily because of lack of suspicion; even if the diagnosis is made accurately, management remains a challenge because of the poor corneal penetration and the limited commercial availability of antifungal agents.

Numerous fungi can cause keratitis—*Aspergillus, Curvularia, Paecilomyces, Phialophora, Blastomyces, Sporothrix, Exophiala, Scedosporium,* and *Alternaria*—but *Fusarium* spp. appear to be the most commonly isolated.[155,156] *Nocardia* and *Corynebacterium matruchotii* are rare causes of infectious keratitis but produce a corneal lesion whose appearance is nearly identical to those seen in fungal keratitis. Because the treatment would be quite different, laboratory evaluation is necessary. A number of fungi have been rarely suspected of causing keratitis, but because they are ubiquitous and easily isolated from the environment, their role in true pathogenesis is difficult to ascertain.

The incidence of fungal keratitis varies according to geographic location and ranges from 2% of keratitis cases in New York to 35% in Florida. *Fusarium* spp. are the most common cause of fungal corneal infection in the southern United States (45%–76% of fungal keratitis), whereas *Candida* and *Aspergillus* spp. are more common in northern states. In a large series of fungal keratitis from south Florida, Rosa and colleagues[156] reported that *Fusarium oxysporum* was the most common isolate (37%), followed by, in order of decreasing frequency, *Fusarium solani* (24%), *Candida, Curvularia,* and *Aspergillus* spp.[156]

Clinical Presentation

Patients with fungal keratitis generally have fewer inflammatory signs and symptoms than those with bacterial keratitis. Fungal infections may be mild and indolent, often without suppuration or an ulcerated epithelial surface in the early phases. Molds (most often *Fusarium* or *Aspergillus*) may manifest with gray-white, dry-appearing infiltrates that have a filamentous or feathery edge. The superficial lesions may even appear as strands elevating the corneal surface. Minimal stromal inflammation may be present in a focal or multifocal pattern, along with satellite lesions. Larger lesions or deep invasion may be associated with an endothelial plaque or hypopyon. As the keratitis progresses, extensive suppuration may develop, giving the appearance of a bacterial keratitis. A rapidly increasing hypopyon and anterior chamber membranes may be noted. Such rapidly progressive anterior chamber inflammation may herald fungal extension into the anterior chamber.

Fusarium Keratitis

A number of individuals have contracted *Fusarium* keratitis from contact lens wear, but this number is generally very small. In 2006, the CDC began to receive reports of an increased incidence of contact lens–associated *Fusarium* keratitis. The CDC began an investigation of the *Fusarium* keratitis outbreak, resulting in 130 confirmed cases. More than 60% of people with confirmed *Fusarium* keratitis had used a specific type of contact lens solution; the FDA has recalled that specific solution. Of the 130 confirmed cases of *Fusarium* keratitis, 37 resulted in cornea transplant surgery.[157]

Candida Keratitis

Major predisposing risk factors for keratitis caused by *Candida* spp. are prolonged epithelial ulceration, topical corticosteroid use, recent keratoplasty (corneal transplant), current corticosteroid use, and current use of a "bandage" soft contact lens (i.e., recurrent erosion, persistent

epithelial defect, etc.). *Candida* keratitis is more common in patients with a history of ocular surface disease, previous ocular herpes, exposure keratopathy, and systemic immunosuppression. In contrast to that caused by molds, *Candida* keratitis manifests as an oval epithelial ulceration with an expanding, more sharply demarcated, densely focal suppuration. In this appearance, *Candida* may mimic a gram-positive keratitis, although the inflammatory reaction is generally somewhat less than that seen with bacterial keratitis.

Therapy for Fungal Keratitis: Limited Therapy Options

The development of antifungal preparations has been similar to that for the antivirals in that there are relatively few treatment options for patients. Most agents must be specially formulated, and a number of the oral and parenteral agents cannot be processed into topical solutions because of pH or other solubility difficulties. Natamycin 5% suspension is the only topical ophthalmic antifungal commercially available in the United States. Voriconazole 1% can be prepared in the pharmacy from the intravenous formulation. In a Cochrane review of 12 randomized clinical trials of fungal keratitis, natamycin 5% suspension was superior to voriconazole 1%, although most of the superiority was found in patients with *Fusarium* keratitis. Ten trials were in India, one in Bangladesh, and one in Egypt. There were insufficient data to make conclusions about the relative efficacy of other topical drugs or combinations of topical and oral antifungals.[158] Chlorhexidine gluconate 0.05%, 0.1%, and 0.2% is available in some countries overseas and was considered to be equally effective to natamycin 5% when compared in two randomized trials in Bangladesh.[158,159]

The Mycotic Ulcer Treatment Trial I (MUTT I)[160] compared the efficacy of topical natamycin and topical voriconazole in the treatment of filamentous fungal ulcers. It found that that the group randomized to topical natamycin had an average BSCVA of 1.8 Snellen lines better at 3 months compared to the voriconazole group ($P = .006$). In particular, the *Fusarium* ulcer cases had BSCVA of 4 Snellen lines better when randomized to natamycin compared to voriconazole ($P < .001$). In fact, the Data Safety and Monitoring Committee recommended stopping the trial after enrolling 323 patients because those randomized to topical voriconazole had a statistically significant increase in the rate of corneal perforation or therapeutic penetrating keratoplasty ($P = .009$). Of note, the *Fusarium* ulcers had small scar size at 3 months when treated with natamycin. This was not the case for non-*Fusarium* ulcers. However, on day 6 of the treatment, a higher percentage of the patients were culture positive for fungus in the voriconazole group compared to the natamycin group, regardless of the type of fungus, indicating that natamycin is superior to voriconazole in the treatment of all fungi ($P < .001$). This trial has been corroborated by a second recent randomized clinical trial and the Cochrane review mentioned earlier.[158,161]

Another study reported significantly superior antifungal activity of phenylmercuric nitrate, a the commonly used ophthalmic preservative, in treating fungal keratitis in comparison to natamycin.[162] For *Fusarium* keratitis, a 0.02% solution of polyhexamethylene biguanide was found to be effective as a therapeutic option, especially in cases that were unresponsive to other common antifungal therapies.[163] Proceeding likewise, several studies[164–166] have found nanoparticles of silver more effective than natamycin against the activity of filamentous fungi in vitro.

Topical amphotericin B solution, prepared in a pharmacy, is the recommended treatment for *Candida* keratitis and may be an alternative for *Aspergillus* keratitis. Topical preparations of flucytosine, fluconazole, econazole 2%, miconazole 1%, and itraconazole have been used in clinical and experimental situations. Case reports of using oral itraconazole or oral voriconazole for mold keratitis, in addition to a topical antifungal, have suggested that the combined approach could be considered. Although topical voriconazole in comparison to topical natamycin shows lower efficacy in treating fungal keratitis, it may be effective in oral form as an adjunct to topical voriconazole.[167] The reports of successful treatment with topical voriconazole have invariably used the topical formulation in conjunction with the oral or the intravenous form.[168,169]

Oral fluconazole should be considered for *Candida* keratitis, although experience is limited. Detection of hyphae on wet mounts of corneal scrapings is difficult and mechanical débridement may be necessary because the fungi are often deep within the stroma. The greater sensitivity of calcofluor white staining of wet mounts recommends this technique for corneal scrapings. Penetration of topical agents into the corneal stroma is usually poor, and the fungal infiltration deep within the stroma is frequently unresponsive to such therapy. Cases with progressive disease unresponsive to maximal oral or topical therapy may ultimately require a penetrating keratoplasty to prevent perforation or loss of the globe because of unchecked fungal extension.

Sharma and coworkers performed a study comparing the efficacy of topical voriconazole and topical natamycin with that of intrastromal voriconazole and topical natamycin in patients with recalcitrant fungal keratitis in 2013.[170] Intrastromal injections did not offer any beneficial effect over topical therapy in this study.[170] Intracameral injection of amphotericin with or without hypopyon drainage also offers a potential adjuvant treatment for fungal keratitis.[171–175] Further investigations and evaluations of these adjuvant therapies in well-designed randomized controlled trials is important to assess their benefits.

In line with topical methods of combating fungal keratitis, Homa and colleagues[176] found that the combination of *Cinnamomum zeylanicum* essential oil (whose major component is trans-cinnamaldehyde) and natamycin was effective in inhibiting the fungal activity in *Fusarium* keratitis isolates by the reduction of its cellular metabolism and suppression of its conidia germination.

Another method used for treating keratitis is the administration of riboflavin in combination with corneal collagen cross-linking (CXL).[177–183] A significant decrease in cell morphology of both filamentous fungi and yeast that were exposed to photosensitizing riboflavin and ultraviolet light, respectively, was reported by Kashiwabuchi and coworkers.[177] Labiris and associates reported reepithelialization of corneal epithelium 5 days after CXL treatment, with no relapse (within 12-month follow-up period) in a patient who was suffering from graft ulcer 12 months after a penetrating keratoplasty procedure.[178] Shetty and coworkers[179] also reported the potency of the CXL method in resolving not only fungal but also bacterial keratitis cases that were superficial (not involving deep stromal keratitis and endothelial plaque). Although the occurrence of microbial keratitis after CXL treatment is seldom (0.0017%), moxifloxacin-resistant *S. aureus* can be the potential culprit behind such infection, which must be treated aggressively with fortified antibiotics.[184]

One important consideration to keep in mind while making the diagnosis of fungal keratitis is the morphologic similarity between *Paecilomyces* and *Penicillium* in culture. Sheybani and colleagues[185] reported the drastically different clinical outcomes of two cases of fungal keratitis from *Paecilomyces.* In one case the isolate was misidentified as *Penicillium,* resulting in less than an ideal treatment regimen, with three unsuccessful penetrating keratoplasties prior to a successful one. In the other case, the keratitis cause was correctly identified as *Paecilomyces,* resulting in a successful penetrating keratoplasty from the first attempt.

PARASITIC KERATITIS

Acanthamoeba Keratitis

Acanthamoebae are free-living protozoa ubiquitous in fresh water, well water, brackish water, and soil. These protozoa have been increasingly recognized as a worldwide cause of painful keratitis, resistant to many forms of treatment and ultimately responsible for loss of vision or even loss of the eye.[186] They are resistant to killing by freezing, desiccation, and the chlorination commonly used in municipal water supplies, swimming pools, and hot tubs. They exist as mobile trophozoites or dormant cysts. The vast majority of reported cases of amebic keratitis have been associated with contact lens use,[187,188] although corneal trauma involving contaminated water has been implicated. A significant increase in amebic keratitis was seen when saline tablets were introduced to the general public to enable preparation of homemade saline solutions. A reduction in amebic keratitis was seen after the tablets were taken off the US market; however, a recent outbreak of amebic keratitis showed that such cases still persist.

In 2006, the Illinois Department of Public Health reported an increased incidence of contact lens–associated *Acanthamoeba* keratitis,

dating from 2004. In 2007, the CDC began a retrospective review of culture-proven cases of *Acanthamoeba* keratitis in 22 centers nationwide. The review identified an association of increasing *Acanthamoeba* keratitis and the use of a contact lens solution that was different than that previously associated with the *Fusarium* keratitis outbreak. Although no specific contaminants were found in any of the solutions or the production plant, the manufacturer voluntarily removed this product from the market.[189]

To further investigate the significant increase of *Acanthamoeba* keratitis at the University of Illinois at Chicago, Joslin and coworkers[190] performed a study and reported that the contact lens solution used is independently associated with *Acanthamoeba* keratitis among soft contact lens users. However, it did not explain all cases, suggesting additional factors. In addition, a more recent study described an increase in *Acanthamoeba* keratitis after the outbreak and after the recall of the multipurpose contact lens solution. The study revealed that the yearly number of cases gradually increased from 22 in 1999 to 43 in 2003, with a marked increase beginning in 2004 (93 cases) that continued through 2007 (170 cases; $P < .0001$).[191] A statistically significant decrease in monthly cases reported, from 28 cases in June 2007 (after the recall) to 7 cases in June 2008, was followed by an increase ($P = .0004$) in reported cases thereafter; cases have remained higher than preoutbreak levels. The persistently elevated number of reported cases supports the need to understand the risk factors and environmental exposures associated with *Acanthamoeba* keratitis.[191]

Shoff and colleagues[192] performed a study to determine contact lens solution efficacy against recent clinical and tap water *Acanthamoeba* isolates and to determine whether taurine inclusion increases *Acanthamoeba* survival in contact lens solutions. They found that recent clinical and tap water *Acanthamoeba* strains, representing proven human pathogens and/or household strains, were highly tolerant of contact lens solutions. The Chicago-area tap water strain was most resilient, a concern if tap water is contributing to the *Acanthamoeba* keratitis increase. Results further differentiated resistance among genotype T4 strains, highlighting the importance of multiple strain testing.[192]

Although an ophthalmologist should be immediately consulted to treat any suspected keratitis, an excellent review of *Acanthamoeba* keratitis can be found in Chapter 273.

Clinical Signs and Symptoms With *Acanthamoeba* Keratitis

Patients with amebic keratitis have photophobia and severe pain, often out of proportion to the clinical appearance. A lengthy and worsening course shows little or no response to a variety of antimicrobial agents. Early infection is often limited to the corneal epithelium, manifesting as a diffuse epithelial keratitis, dendritic lesion, or radial keratitis. At this stage, amebic keratitis is often misdiagnosed as herpetic keratitis, and treatment is attempted with antivirals and corticosteroids. Enlarged corneal nerves have been suggested, but perineural inflammation may be a more appropriate descriptor. This radial keratitis/perineuritis is generally not seen with other types of microbial keratitis. A late finding is the "classic" ring infiltrate after progression of the gray-white superficial nonsuppurative infiltrate generally found in the central or paracentral cornea.

Onchocerciasis

The impact of parasitic keratitis is devastating in some tropical regions. Sclerosing keratitis and corneal opacification can be the result of stromal infestation by microfilariae of *Onchocerca volvulus*.[193] The host for this parasite is the black fly found in Africa and certain areas of Central and South America. The female fly deposits her eggs in vegetation and on rocks in streams and rivers. Despite the swiftly flowing cool water, the eggs persist. Onchocerciasis, or "river blindness," is one of the leading causes of blindness throughout the world.[194] The ocular lesions are seen after direct invasion of the anterior segment by the microfilariae. Early in the course of the condition, the patient will complain of tearing, photophobia, and redness. Slit-lamp examination may reveal microfilariae "swimming" in the anterior chamber or slowly moving through the cornea just under the epithelium.[195] The host tolerates the organism surprisingly well; however, a severe inflammatory reaction begins when the microfilariae die in the cornea.[196] Sclerosing keratitis is the eventual blinding complication after many long-lasting infections.

Leishmaniasis

Leishmania keratitis may appear quite similar to that of onchocerciasis.[197] *Leishmania* parasites are obligate intracellular agents transmitted through bites of infected sand flies. Human infection can take many forms, but cutaneous and mucocutaneous varieties cause edema, ulceration, and scarring of the lids or conjunctivae. Corneal involvement may begin as superficial "bumps" (phlyctenules) that progress to abscess formation and possible corneal perforation.

Microsporidia and Trypanosomes

With the increase in infections caused by the human immunodeficiency virus (HIV), microsporidial infections are being more commonly recognized (see Chapter 270). Microsporidia are ubiquitous obligate intracellular parasites now classified as fungi. Except in the immunocompromised patient, they are an unlikely cause of keratitis, generally after traumatic penetration of the stroma. *Vittaforma corneae* (formerly *Nosema corneum*) has been implicated in a few cases in Africa, India, and Japan.[198] *Nosema ocularum* and *Anncaliia* (*Nosema*) *algerae* keratitis have also been reported. The ensuing granulomatous inflammation generally leads to necrotizing thinning and perforation. HIV-positive patients may develop mild conjunctivitis with punctate epithelial keratitis caused by the related microsporidium, *Encephalitozoon cuniculi*.[199] Symptoms may be mild and can easily be mistaken for tear film deficiencies or blepharitis, or both; a high index of suspicion is required in such patients to make a clinical diagnosis of microsporidial keratitis. The tsetse fly can infect humans with the hemoflagellates responsible for African trypanosomiasis, or "sleeping sickness." Ocular effects manifest as unilateral conjunctivitis, periorbital edema, and preauricular lymphadenopathy. IK similar to that seen in syphilitic keratitis has been described.[200]

Therapy for Parasitic Keratitis

Early diagnosis of *Acanthamoeba* infection is the single most *predictive* factor in successful treatment. Most cases are initially diagnosed and treated as herpetic keratitis, further delaying proper treatment and usually allowing treatment with corticosteroids, which is proportionally correlated with a poor outcome. Early diagnosis during the epithelial stage may lead to a good visual outcome after débridement and a 3- to 4-month course of antiamebic therapy. If diagnosis is made after stromal infiltrates appear, the prognosis is more guarded because eradication is quite difficult, often requiring up to a year of antiamebic therapy. Numerous agents have been suggested in treating *Acanthamoeba,* likely indicating that the best therapy is uncertain. Diamidines (propamidine, hexamidine), biguanides (polyhexamethylene biguanide, chlorhexidine), aminoglycosides (neomycin, paromomycin), and imidazoles/triazoles (miconazole, clotrimazole, ketoconazole, itraconazole) have all been used in different combinations, depending on availability. Most of these agents are effective against the free-living trophozoite form of the organism, but none is particularly effective against the cystic stage. In vitro evaluation of topical agents against the cystic form of *Acanthamoeba* found natamycin, povidone-iodine, and acriflavine to have the best activity against this form.[201,202] No agreement exists as to the best combination, but successful outcomes have been achieved using a biguanide with or without a diamidine. Recent reports of success with topical and oral miltefosine are encouraging (see Chapter 273). Controversy exists regarding the use or benefit of corticosteroids because early use may contribute to persistence of viable cysts. Penetrating keratoplasty may be necessary, medically and therapeutically. However, even in a quiet-appearing eye, such surgery has been associated with a recurrence if performed within the first year of the diagnosis, presumably because of persistent cysts. It may be best to complete a full course of antiamebic therapy, followed by a 6-month disease-free course, before considering a penetrating keratoplasty.

The effect of methylene blue–mediated photodynamic therapy (MB-PDT) on *Acanthamoeba* was evaluated by Mito and coworkers[203] in an in vitro study. They reported that MB-PDT suppressed the respiratory activity of trophozoites in a concentration-dependent manner and that MB-PDT had a synergistic effect when used in combination with

polyhexamethylene biguanide or amphotericin B, but not with voriconazole.[203] Further studies are needed before this in vitro approach would be translated to the bedside.

Onchocerciasis was originally treated with diethylcarbamazine, but ivermectin is equally effective and less toxic.[204] Encephalitozoon conjunctivitis has responded to albendazole 400 mg PO bid (see Chapter 270), suggesting that the drug should be considered for keratitis. Experience with other agents has been discouraging. Penetrating keratoplasty has been used as a last resort.

It is important to consider the possibility of coinfection with multiple agents, especially in cases with contact lens–related keratitis.[204–207] In a study by Sharma and associates,[205] *Acanthamoeba* and *P. aeruginosa* were both found to be causing keratitis when a 20-year-old woman presented to the emergency department of their hospital with a 4-day history of progressively increasing pain, redness, photophobia, mucopurulent discharge, and decrease of vision in her right eye. Similarly, in a retrospective study, Lim and colleagues[206] identified the size of the corneal infiltrate, the prolonged course of the disease, and the decreased antibiotic sensitivity as the indicators of coinfection with multiple agents, which they term *polymicrobial keratitis.*

NOVEL THERAPEUTICS

Therapies continue to evolve for the treatment and prevention of microbial keratitis, specifically to address the serious risks of drug-resistant bacterial infections.

Duan and coworkers[208] modified emodin to synthesize haloemodin, which they used to cure *S. aureus*–induced keratitis in a rabbit model. They found that haloemodin shows antibacterial activity against gram-positive bacteria by exerting an inhibitory activity on bacterial DNA gyrase and topoisomerase I, without showing any inhibitory effect on human topoisomerases. Kolar and colleagues[209] derived a frog skin peptide called esculentin-1a(1-21)NH_2, which was tested in vitro and in vivo in a murine model of *P. aeruginosa* keratitis. Inflammatory cell recruitment was reduced by half in the treated group compared to the control mice, and no toxicity was shown in human epithelial cells up to 50 μM (MIC between 2 and 16 μM), showing promise in the development of novel topical drugs against *P. aeruginosa*. Working with the same frog skin–derived peptide and esculentin-1a and homologous esculentin-1b, Mangoni and associates[210]further found the peptide's membranolytic activity on both free-living and biofilms of *C. albicans.*

In addition, lipid-based ophthalmic drug delivery has been investigated. Carrier systems such as liposomes, lipid microsphere and lipid microtubules, solid lipid nanoparticles, and lipid suspensions and emulsions have been used to deliver drugs for the treatment of various ocular surface diseases. Ustündağ-Okur and coworkers[211] reported one such system in which they delivered oxofloxacin via nanoparticles of lipid carriers modified with chitosan oligosaccharide lactate to treat bacterial keratitis. The addition of chitosan oligosaccharide lactate prolonging the residence time of the drug.

Researchers from Australia found that N-glycan in tears bound to *P. aeruginosa* (especially the cytotoxic strain compared to the invasive strain). Binding allowed the tears to wash away the microbe.[212]

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The complete reference list is available online at Expert Consult.

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114 Endophthalmitis

Marlene L. Durand

SHORT VIEW SUMMARY

Definition

- Endophthalmitis is a bacterial or fungal infection inside the eye, involving the vitreous and/or aqueous. It is either exogenous, in which infection is introduced from the outside in, or endogenous, in which the eye is seeded from the bloodstream.

Clinical Manifestations

- Decreased vision and eye pain are usually present. Hypopyon (layer of white blood cells in aqueous humor) is commonly seen. No fever or leukocytosis is present in exogenous cases and may also be absent in endogenous cases.

Categories and Microbiology
(see Table 114.1)

- *Acute postcataract endophthalmitis.* Incidence is approximately 0.1% of cataract surgeries, with onset within 1 week postoperatively in 75%. The most common etiology is contamination of the aqueous from ocular surface flora. Gram-positive cocci cause 95% of cases, with coagulase-negative staphylococci the major pathogens (70% of all cases).
- *Chronic postcataract endophthalmitis.* Rare, this category presents as a low-grade inflammation in the aqueous postoperatively that persists for months. It may respond to topical corticosteroids initially but recurs as the drug dosage is tapered. The cause is *Cutibacterium (Propionibacterium) acnes* in most cases.
- *Postinjection endophthalmitis.* Incidence is approximately 0.1% after each intravitreal injection of an anti–vascular endothelial growth factor agent (treatment of wet macular degeneration). Injections are typically given once monthly. Major pathogens are coagulase-negative staphylococci and streptococci (25% of cases), the latter usually causing severe endophthalmitis.
- *Bleb-related endophthalmitis.* A filtering bleb is a "bleb" of conjunctiva overlying a surgically created defect in the sclera. Creation of a filtering bleb is one treatment for medically refractory glaucoma. Endophthalmitis typically occurs suddenly, months to years postoperatively; the incidence is approximately 0.5% per patient-year but may be much higher in leaking blebs. Infection is often fulminant because streptococci, enterococci, and *Haemophilus influenzae* are major pathogens.
- *Posttraumatic endophthalmitis.* Incidence is 0.9% to 10% after penetrating eye trauma ("open globe"), with the lower incidence reported using a protocol that includes 48 hours of prophylactic antibiotics. Coagulase-negative staphylococci and *Bacillus cereus* are major pathogens; *B. cereus* is most feared and causes fulminant infection.
- *Keratitis-related endophthalmitis.* Occurs when infection of the cornea extends into the aqueous. Molds are the most common etiology.
- *Endogenous bacterial endophthalmitis.* Common sources include endocarditis (*Staphylococcus aureus* and streptococci are major pathogens), intraabdominal abscess (e.g., liver abscess due to *Klebsiella pneumoniae* in East Asian nations), urinary tract infection, transient bacteremia (e.g., endoscopy, intravenous drug abuse).
- Candida *endophthalmitis.* This category is usually endogenous. Chorioretinitis, the earliest manifestation, is often asymptomatic. Chorioretinitis usually responds to systemic antifungal treatment alone, but cases with endophthalmitis (marked vitreous inflammation) also require intravitreal antifungal injection and often vitrectomy.
- *Mold endophthalmitis.* Exogenous cases occur after eye surgery, eye trauma, or as an extension of keratomycosis (fungal corneal infection), whereas endogenous cases most often occur in injection drug users or severely immunocompromised hosts. *Aspergillus* and *Fusarium* are the most common pathogens.

Diagnosis

- Exogenous cases, and some endogenous cases, require vitreous aspirate or vitrectomy for culture (aqueous may also be cultured). Endogenous cases with positive blood cultures are usually presumed to be due to the same organism.

Therapy

- Intravitreal antibiotics and often vitrectomy (surgical débridement of the vitreous) are required. Systemic antibiotics alone are not effective in treating endophthalmitis, except in cases of *Candida* chorioretinitis.

Endophthalmitis refers to bacterial or fungal infection involving the vitreous and/or aqueous humors. Most cases of endophthalmitis are caused by bacteria and present acutely. Acute endophthalmitis is a medical emergency because delayed or inadequate therapy may result in irreversible vision loss. Endophthalmitis may be either exogenous, in which organisms are introduced directly into the eye from an external source, or endogenous, in which the eye is seeded during bacteremia or fungemia. Most cases are exogenous and occur after eye surgery, intraocular injections (e.g., given for treatment of macular degeneration), eye trauma, extension of corneal infection (keratitis), or from a glaucoma filtering bleb.[1] Postcataract endophthalmitis is the most common type worldwide, but postinjection or posttraumatic endophthalmitis may be more common at some centers.[2]

In exogenous endophthalmitis, the infection is confined to the eye, and there are rarely any systemic symptoms. In endogenous endophthalmitis patients may have prominent symptoms of the underlying systemic infection (e.g., endocarditis).

CATEGORIES

It is useful to further classify endophthalmitis into several categories (Table 114.1). The presentation and typical pathogens vary by category, but treatment almost always requires the intraocular injection of antibiotics and, often, vitrectomy.

Acute Postoperative Endophthalmitis

Endophthalmitis may occur after any eye surgery, but most postoperative endophthalmitis cases occur after cataract surgery. Cataract surgery is one of the most common surgeries performed worldwide, with more than 3.5 million cataract surgeries performed annually in the United States alone. The cataract, or opacified lens, is most often broken up

TABLE 114.1 Endophthalmitis Categories and the Most Common Pathogens in Each

CATEGORY	COMMON PATHOGENS
Acute postoperative	Coagulase-negative staphylococci
Chronic postcataract	*Propionibacterium acnes*
Postinjection	Viridans streptococci, coagulase-negative staphylococci
Bleb-related	Streptococci, *Haemophilus influenzae*
Posttraumatic	*Bacillus cereus*
Keratitis related	Molds (e.g., *Fusarium*)
Endogenous	*Staphylococcus aureus,* streptococci, gram-negative bacilli
Fungal	*Candida, Aspergillus, Fusarium*

and removed by phacoemulsification, a technique using ultrasound. During cataract surgery, an incision is made through the sclera or cornea and then through the anterior lens capsule to remove the lens. An attempt is made to leave the posterior lens capsule intact. In most cases an artificial intraocular lens (IOL) is placed in the residual capsular bag. The incidence of postcataract endophthalmitis is approximately 0.1% in the United States, based on Medicare data 2010–14.[3] Other countries report rates from 0.03% to 0.2%.[4–6]

The pathogens causing postcataract endophthalmitis usually come from the patient's own eyelid or conjunctival flora, which may contaminate the aqueous during surgery.[7,8,9] The widely used "clear cornea" incision (incision through the cornea), which is sutureless and self-sealing, may increase the risk of endophthalmitis compared with the older "scleral tunnel" incision.[10,11] Clear cornea incisions may gape intermittently in the early postoperative period, leading to potential contamination.[12,13] Recent cataract surgical techniques have included femtosecond laser-assisted cataract surgery, but comparisons with standard phacoemulsification cataract surgery have not demonstrated a difference in postoperative complications.[14]

The vitreous is more susceptible to infection than the aqueous, and the risk for postcataract endophthalmitis is at least sixfold higher if the posterior lens capsule is inadvertently broken during surgery, thereby establishing a communication with the vitreous.[15,16] Silicone rather than acrylic IOLs also increase the risk for endophthalmitis.

Symptoms of endophthalmitis occur within 1 week of surgery in 75% of patients and include eye pain (74%), redness (82%), and decreased vision (94%).[16] The patient often has no symptoms for several days, and then symptoms develop rapidly within 24 hours. The pain is often not severe, and patients feel otherwise well. Signs of systemic illness are absent: There is no fever, and the white blood cell count is normal in two-thirds of patients and only mildly elevated in the rest. The physical examination is unremarkable except for the involved eye, which is usually injected and has a hypopyon (Fig. 114.1). The hypopyon is a layer of white blood cells in the anterior chamber. Slit-lamp examination reveals white blood cells in the aqueous and vitreous. Inflammation may be so severe that it obscures the funduscopic view of the retina.

In temperate climates nearly all postcataract endophthalmitis cases are due to bacteria, and 95% are due to gram-positive cocci. Coagulase-negative staphylococci are the major pathogens, causing 70% of culture-positive cases, regardless of whether a scleral tunnel or clear cornea incision was used for the surgery.[16,17] *Staphylococcus aureus* (10%), streptococci (9%), other gram-positive cocci (5%), and gram-negative bacilli (6%) account for the remaining cases. Streptococci (e.g. viridans streptococci, *Streptococcus pneumoniae,* and β-hemolytic streptococci) are associated with a particularly poor outcome. Thirty percent of patients with clinical evidence of endophthalmitis have negative or equivocal vitreous cultures.[16] In tropical countries, such as India, fungi typically cause 10% to 15% of postoperative endophthalmitis cases.[18]

Chronic Postcataract Endophthalmitis

Chronic postcataract endophthalmitis, also termed *chronic pseudophakic endophthalmitis* in reference to the IOL, is a rare, indolent infection that is almost always due to *Cutibacterium (Propionibacterium) acnes.*



FIG. 114.1 Endophthalmitis. Eye with endophthalmitis, illustrating a hypopyon. *(Courtesy Donald J. D'Amico.)*

A few cases have been caused by coagulase-negative staphylococci, diphtheroids, and fungi. Patients present with a persistent decrease in vision in the involved eye, and half also have eye pain, which is usually mild. On slit-lamp examination there are white blood cells in the anterior chamber and usually also in the anterior vitreous. A small hypopyon is present in half of patients, and in nearly all patients there is a white plaque in the residual posterior lens capsule. Patients often are misdiagnosed as having anterior uveitis and may be treated for months with topical corticosteroids, producing a waxing and waning course of intraocular inflammation. Diagnosis may be difficult because aqueous or vitreous cultures are frequently negative, even in cases in which electron microscopy of the removed artificial lens or lens capsule shows bacteria.[19] Polymerase chain reaction (PCR) testing of intraocular samples or of the explanted IOL may be helpful.[20] Biopsy of the posterior lens capsule, including the white plaque, is most likely to yield positive cultures due to *P. acnes*. Treatment of chronic *P. acnes* endophthalmitis usually requires surgical removal of the infected lens capsule and IOL, in addition to intraocular antibiotics, as relapse rates can be very high (70%) with intravitreal antibiotics alone.[21,22]

Postinjection Endophthalmitis

Neovascular, or "wet," macular degeneration accounts for approximately 10% of age-related macular degeneration (AMD) cases. In 2004 the US Food and Drug Administration approved the first anti–vascular endothelial growth factor (anti-VEGF) agent for treating wet AMD, and the annual number of intravitreal injections in the United States rapidly increased. A Medicare study found that 2.4 million intravitreal injections were given in 2012 alone.[23] Intravitreal injections of anti-VEGF agents, such as bevacizumab, ranibizumab, aflibercept, or pegaptanib, are usually repeated monthly, over many months. Each injection carries a risk of endophthalmitis, reported to be between 0.04% to 0.09%.[24,25] Intravitreal anti-VEGF agents may also be given for other indications, such as diabetic retinopathy. Symptoms of endophthalmitis usually develop within 1 week after injection and include eye pain and decreased vision. A hypopyon is found in about 80% of patients. As with postcataract endophthalmitis, gram-positive cocci cause 95% of cases, but viridans streptococci cause a much higher percentage of postinjection cases (25%) than postcataract endophthalmitis cases (9%). Patients with streptococcal endophthalmitis have a poor visual prognosis.[26]

Bleb-Related Endophthalmitis

A filtering bleb is a defect in the sclera, covered only by conjunctiva, that is surgically created to control glaucoma refractory to medical treatment. The bleb allows excess aqueous to filter out of the eye and into the systemic circulation. Because the only barrier to the aqueous humor at the site of the bleb is the thin conjunctiva, acute endophthalmitis may occur at any time, particularly if the eye becomes colonized by virulent bacteria. Bleb-related endophthalmitis usually occurs abruptly, months to years after bleb surgery. The risk of endophthalmitis in one

series was 1.3% per patient-year[27], whereas a large multicenter study from Japan found a 5-year risk of only 1%.[28] The Japanese study found that a leaking bleb increased the risk fivefold. Bleb-related endophthalmitis is typically fulminant. Patients complain of sudden onset of eye pain and decreased vision. On examination the patient has an injected eye, a hypopyon, and often a purulent bleb.[29] The major pathogens include streptococci (36%), coagulase-negative staphylococci (18%), *S. aureus* (11%), enterococci (9%), and *Haemophilus influenzae* (5%).[30]

Posttraumatic Endophthalmitis

Posttraumatic endophthalmitis develops in 0.9% to 10% of eyes that have sustained penetrating trauma.[31,32,33] Endophthalmitis is most likely to follow a lacerating injury with a metal object, whereas glass laceration injuries and blunt trauma rarely lead to endophthalmitis. Other risk factors include lens disruption, retained intraocular foreign bodies, and delay in primary closure of greater than 24 hours.[34] A computed tomography (CT) scan can be helpful in finding metallic intraocular foreign bodies, and magnetic resonance imaging (MRI) is helpful in detecting nonmetallic foreign bodies; note that a CT scan should be performed before MRI to ensure there is no metallic foreign body present at the time of MRI. *Bacillus* spp. (often *Bacillus cereus*) and coagulase-negative staphylococci are the major causes of posttraumatic endophthalmitis. Whereas eyes with endophthalmitis due to coagulase-negative staphylococci often have a good visual outcome, those with *Bacillus* endophthalmitis usually lose all useful vision.[35] Rare cases of successful therapy have been described.[36] Onset of symptoms in 12 to 24 hours after trauma, marked intraocular inflammation, and a ring corneal infiltrate are characteristic of this infection. Other causes of posttraumatic endophthalmitis include streptococci, gram-negative bacilli such as *Klebsiella* and *Pseudomonas,* and molds.[34] Unlike bacterial cases, posttraumatic endophthalmitis due to molds usually has a subacute presentation.

Keratitis-Related Endophthalmitis

An infection of the cornea, or keratitis, usually starts at the corneal surface. In severe cases of bacterial or, more often, fungal keratitis, the infection may extend through the full thickness of the cornea and into the aqueous, thereby causing endophthalmitis. The infection may continue to progress and involve the vitreous. Keratitis-related endophthalmitis is usually rare and comprises less than 1% of cases of endophthalmitis treated at most centers. Some centers, particularly from hot or tropical climates, report a higher incidence. A referral center in Iran found that 8% of all endophthalmitis cases were keratitis-related, with *Pseudomonas* being the most common virulent pathogen isolated. Coagulase-negative staphylococci were isolated more often, but these may have been ocular surface contaminants.[37] Molds cause the majority of keratitis-related endophthalmitis cases worldwide, and molds accounted for over half of such cases at a center in Florida.[38] Exogenous mold endophthalmitis is discussed as follows.

Endogenous Bacterial Endophthalmitis

Endogenous bacterial endophthalmitis is rare and results from bacteremic seeding of the eye. Only 0.04% of hospitalized patients with bacteremia have endophthalmitis.[39] Usually a significant focus of bacteremia is identified, such as endocarditis or an intraabdominal abscess, but transient bacteremia rarely may cause endophthalmitis. Endocarditis was the source in nearly 40% of cases in one United States series of 28 patients, whereas 1 patient developed endophthalmitis 2 days after upper gastrointestinal (GI) endoscopy, presumably from transient bacteremia.[40] Patients who are injection drug users also are at risk for endophthalmitis from transient bacteremia. GI or hepatic abscesses, urinary tract infections, meningitis, and infected indwelling catheters are other sources of bacteremia in endogenous endophthalmitis case series.[40,41,42] In Taiwan, Singapore, and other East Asian nations, pyogenic liver abscess due to *Klebsiella pneumoniae* is prevalent[43] and may be complicated by endogenous endophthalmitis in almost 10% of patients.[44]

The bacteria involved in endogenous endophthalmitis (e.g., *S. aureus,* streptococci, gram-negative bacilli) typically cause acute inflammation, and most patients present with eye pain and acute decrease in vision. These may be their only complaints, and the source of bacteremia may not be apparent initially. In one series half of the patients presented to an ophthalmologist.[40] In a series of 27 patients with fungal or bacterial endogenous endophthalmitis, less than 20% of patients had fever on presentation, and greater than 40% had an unremarkable general physical examination.[42] A delay in diagnosis is common, but endophthalmitis should be considered in any patient who presents with acute vitritis and hypopyon. Patients with known endocarditis should be monitored for new visual complaints and examined by an ophthalmologist promptly if these develop.

Diagnosis usually is established by culture of vitreous samples or by blood cultures in patients with endophthalmitis whose vitreous cultures fail to grow. Blood cultures are positive in approximately three-fourths of patients tested, as are vitreous cultures.[40] In North America and Europe, streptococci (*S. pneumoniae, Streptococcus anginosus* group, and group A and group B streptococci) cause 30% to 50% of cases, and *S. aureus* causes about 25% of cases; gram-negative bacilli (e.g., *Escherichia coli, Klebsiella, Serratia*) cause only one-third of cases.[40,42] In Asia gram-negative bacilli (e.g., *Klebsiella, E. coli*) cause the majority of cases, with *K. pneumoniae* accounting for 60% of cases in one study.[45] In Taiwan a syndrome of *Klebsiella* liver abscess and endophthalmitis occurring primarily in diabetic patients has been well described[46] and appears to be associated with a strain of *K. pneumoniae* exhibiting a hypermucoviscosity phenotype.[47]

Mycobacterial Endophthalmitis

Endophthalmitis caused by nontuberculous mycobacteria (NTM) is very rare, with only 19 cases seen over a 24-year period at a major eye referral hospital in Florida.[48] Nearly all cases in this series occurred after eye surgery, with cataract or glaucoma-device implant surgery accounting for two-thirds of cases; 2 cases occurred after intravitreal injections. Rare cases related to extension of keratitis have also been described.[49] Rapidly growing NTMs such as *Mycobacterium chelonae, Mycobacterium abscessus*, and *Mycobacterium fortuitum* account for the majority of cases. In most cases the endophthalmitis presents as a subacute or chronic inflammation. Treatment is difficult, and relapses are common. Hematogenously disseminated tuberculosis can involve the eye and usually presents as a uveitis, as discussed in Chapter 115.[50]

Fungal Endophthalmitis

Fungal endophthalmitis cases may be divided into two major categories by incidence and response to therapy: *Candida* endophthalmitis and mold endophthalmitis. In industrialized nations and colder climates *Candida* endophthalmitis is more common than mold endophthalmitis, whereas the reverse is true in tropical countries. *Candida* endophthalmitis is most often endogenous and usually responds well to treatment, whereas mold endophthalmitis is almost always exogenous and successful therapy is uncommon. Endophthalmitis due to *Cryptococcus* or the dimorphic fungi, *Histoplasma* and *Coccidioides,* is rare and almost always a result of disseminated disease.

Endogenous *Candida* Endophthalmitis

In the literature on endogenous *Candida* endophthalmitis the term is used variably. In some reports it is used to describe both chorioretinitis and "endophthalmitis" (i.e., infection with significant vitritis), whereas in other reports it is used only for those cases with significant vitritis (the usual meaning of endophthalmitis). A better term is ocular candidiasis, which encompasses the spectrum of intraocular infection from chorioretinitis to endophthalmitis. To avoid confusion, we will reserve "endophthalmitis" for those cases with significant vitritis, and the degree of vitritis will be described. Ophthalmologists grade the degree of vitreous inflammation on a scale of 1 to 4, with 1+ white blood cells considered mild vitritis, and 4+ cells severe vitritis. Determining the degree of vitreous inflammation is important. Ocular candidiasis with moderate-to-severe vitritis (i.e., endophthalmitis) requires intravitreal injection of amphotericin or voriconazole and usually vitrectomy, in addition to systemic therapy. In contrast, chorioretinitis with minimal or no vitritis usually resolves with systemic therapy alone. Visual outcome also depends on the initial degree of vitreous involvement.[49,51]

Candidemic seeding of the eye starts in the highly vascular choroid, so chorioretinitis is the initial manifestation seen in most prospective

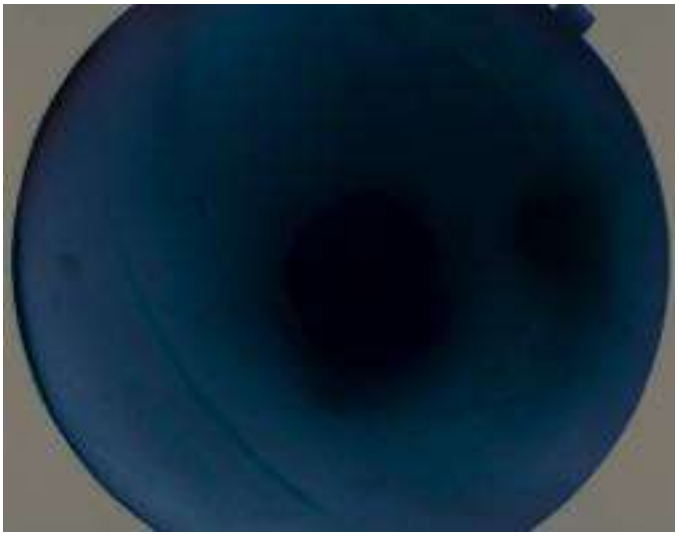

FIG. 114.2 *Candida* endophthalmitis. *Candida* endophthalmitis, with cloudy vitreous and central vitreal "fluff ball."

trials of candidemia. In chorioretinitis there are typically several white chorioretinal lesions but a clear vitreous. As infection progresses there is significant vitritis, and examination reveals a cloudy vitreous that often contains inflammatory "fluff balls" (Fig. 114.2). Chorioretinitis is much more common than endophthalmitis, and chorioretinitis often is asymptomatic. Two trials of candidemic patients found chorioretinitis in 9% to 14% but endophthalmitis in 0% to 1.6%.[52,53] Undiagnosed and untreated chorioretinitis may progress to endophthalmitis, and patients with the latter usually present with a gradual and painless decrease in vision. This presentation is common in outpatients who have had transient, usually asymptomatic, candidemia.

Risk factors for endogenous *Candida* endophthalmitis in hospitalized patients include indwelling central venous catheters, total parenteral nutrition, neutropenia, recent GI surgery or GI perforation, broad-spectrum antibiotic use, and immunosuppression, including glucocorticoid use.[54,55] The major risk factors in outpatients are indwelling central venous catheters (or recent history of such a catheter) and illicit injection drug use. Illicit intravenous drug use (IVDU) is the most common risk factor in some recent studies and accounted for 70% of cases of fungal endophthalmitis from 2001–07 in a study from Australia.[56] A recent series from a Massachusetts eye hospital found that IVDU accounted for 44% of all endogenous endophthalmitis cases diagnosed from 2006–14, and *Candida* species caused 75% of the culture-positive IVDU-related cases.[57] Rare cases of endogenous endophthalmitis in outpatients have occurred after urinary tract procedures, such as ureterolithotomy.[58]

Outpatients who present with endogenous *Candida* endophthalmitis often have a subacute presentation (i.e., 2 weeks of progressive vision loss) and may be misdiagnosed as having uveitis, especially if a history of an indwelling central venous catheter in the previous months or injection drug use is not elicited. Such patients do not usually show other signs of systemic infection, and blood cultures are usually negative because candidemia was transient and occurred days or weeks earlier. Only 6% of IVDU-related *Candida* endophthalmitis cases had positive blood cultures in one series.[57] There is often a substantial delay in diagnosis, and such a delay may lead to permanent vision loss. Although vitreous aspirate alone may yield the diagnosis in some cases, this is often nondiagnostic, and a vitrectomy for culture is usually necessary to make the diagnosis.

Exogenous *Candida* Endophthalmitis

Exogenous cases are uncommon but may develop after eye surgery, eye trauma, or keratitis.[59] Initially, only the aqueous may have inflammation, and then the infection may extend into the vitreous. Rarely, endophthalmitis may develop after corneal transplantation (postkeratoplasty endophthalmitis). The overall incidence of postkeratoplasty endophthalmitis is 0.03% in the United States, and two-thirds of these cases are due to *Candida* (one-third to bacteria).[60] Contaminated donor corneas are usually the source, and culture of the unused donor corneal rim at time of surgery may be helpful. A rim culture that grows *Candida* spp. predicts a 3% chance of developing *Candida* endophthalmitis.[61] Awareness of this increased risk may allow earlier detection and treatment of postkeratoplasty fungal endophthalmitis. Contamination of donor corneas with *Candida* may develop during storage in standard media that contain broad-spectrum antibacterial antibiotics but typically no antifungal agents.[62]

Mold Endophthalmitis

Endophthalmitis due to molds is rare in Western countries. In the United States it is most common in tropical areas, such as Florida, where 6% of 278 endophthalmitis cases treated between 1996 and 2001 were due to *Aspergillus* and other molds.[63] In tropical countries, such as India, fungal endophthalmitis is a significant problem. Molds accounted for 22% of 124 postcataract endophthalmitis cases in northern India[64] and 21% of 170 postoperative endophthalmitis cases in southern India.[65]

Mold endophthalmitis is usually exogenous, with most cases occurring after eye surgery, after penetrating eye trauma, or as an extension of fungal keratitis (keratomycosis).[59]

Postoperative mold endophthalmitis usually presents within days to weeks of eye surgery. Patients complain of eye pain and decreased vision. The surgical corneal or scleral incision may appear normal or may show evidence of wound involvement.[66] Extensive eye involvement (cornea, anterior segment, vitreous) and early presentation (37% within 1 week) characterized postcataract fungal endophthalmitis in one series from India.[67] Posttraumatic mold endophthalmitis also may present relatively quickly; in a series from India the average time to presentation after eye trauma was 7 days.[68] In endophthalmitis due to keratomycosis the diagnosis is made when there is evidence of extension of the corneal infection into the aqueous. On slit-lamp examination the fungal keratitis extends through the full thickness of the cornea, and there is significant inflammation in the aqueous. Sometimes, frondlike projections may be seen extending from the back of the cornea into the aqueous. Both corneal trauma and contact lens wear are risk factors for keratomycosis-associated endophthalmitis.

Endogenous mold endophthalmitis has been reported primarily in IV drug abusers or immunocompromised patients. The latter include organ transplant patients and patients with hematologic malignancies.[69,70] Most of these immunocompromised patients have a focus of fungal infection elsewhere, usually the lungs.[69,70]

Aspergillus is the most common cause of mold endophthalmitis, causing 50% to 90% of cases.[65,68] *Fusarium* is another common cause of mold endophthalmitis, and most cases result from *Fusarium* keratitis. Several cases occurred after a nationwide outbreak of *Fusarium* keratitis, from 2004–06, associated with a contact lens cleaning solution.[71,72] In this outbreak 6% of cases developed endophthalmitis.[71,73]

DIAGNOSIS

Endophthalmitis must be suspected clinically based on the appearance of the eye in a patient with an appropriate risk factor for endophthalmitis. The diagnosis usually is confirmed by culture of the vitreous or, less often, the aqueous. The endophthalmitis patient is afebrile and has no signs of systemic infection except in two situations: endogenous endophthalmitis, in which there is another focus of infection, and panophthalmitis, in which infection has spread from the globe to the orbit. Panophthalmitis is rare, caused by virulent pathogens (e.g., *S. pneumoniae*), and characterized by signs of endophthalmitis plus orbital cellulitis (marked edema and erythema of the eyelids, proptosis, and limitation of extraocular movements). Except for cases of endogenous endophthalmitis and panophthalmitis, blood cultures are negative, and the white blood cell count is normal or only slightly elevated.

Radiologic studies are rarely helpful in acute endophthalmitis and, except for a B scan ("brightness" scan), are not warranted before diagnostic vitreous sampling and therapy. The B-scan is an ultrasound of the globe of the eye. It can confirm the presence of vitreous inflammation or show a retinal detachment in cases in which the vitreous cannot be seen (e.g., dense cataract or extensive aqueous inflammation). The normal vitreous is echo free, whereas multiple vitreous echoes are

present when there is vitritis. The diagnosis of endophthalmitis is usually made by culturing a sample of the vitreous. The aqueous also may be cultured and is aspirated easily in the ophthalmologist's office, but aqueous cultures rarely add additional information. In one large study in which aqueous and vitreous samples were obtained, aqueous samples yielded the only positive cultures in just 4% of cases.[74] The vitreous may be sampled, either by needle aspirate or by vitrectomy (Fig. 114.3). A needle aspirate yields 0.2 to 0.3 mL of vitreous and may be performed in the ophthalmologist's office using a 27-gauge needle and syringe. A vitrectomy is performed in the operating room using a 20-gauge vitrector attached via tubing to a sterile collection bag or canister; more recently, a narrower 23-gauge vitrector has been used with success.[75] Suction is provided by a Venturi-aspiration vitrectomy machine. The vitrector simultaneously cuts and suctions the gel-like vitreous. A separate cannula placed in the vitreous provides continuous infusion of balanced salt solution to maintain eye turgor during the procedure, and this dilutes the vitreous sample. The result is a collection canister or bag containing dilute (20–100 mL) vitreous "washings." An undiluted vitreous sample called a *biopsy specimen* also may be obtained with the vitrector at the start of the case by attaching a syringe via three-way stopcock.

Gram stains of vitreous samples are positive for organisms in approximately half of bacterial endophthalmitis cases. Positive stains are highly predictive of positive cultures, but negative stains have little predictive value.[74] A unique feature of Gram stains of aqueous or vitreous samples from an inflamed eye is the pigment granule.[1] Pigment granules are most likely melanin released from the iris or retinal pigment epithelium. They appear football shaped or spherical, and the latter may be mistaken for gram-positive cocci. Their true identity is evident when the microscope focus is varied because pigment granules are highly refractile and appear copper colored with focus modulation, whereas bacteria do not (Fig. 114.4).

Vitreous washings are more likely to yield positive cultures (75%–90% of cases) than are vitreous aspirates or biopsy specimens (50%–75% of cases).[16,76] Although aspirate or biopsy samples may be cultured directly on various media (blood agar, chocolate agar, broth, and Sabouraud media), vitreous washings first should be filtered through a 0.45-μm filter. The filter paper is sterilely cut into fourths and placed onto agar plates. Before this filtration procedure, a 3-mL sample of the washings should be aspirated from the vitrectomy canister and centrifuged to produce a pellet that may be smeared on a slide for Gram stain. A negative culture does not exclude endophthalmitis, and up to one-third of clinically diagnosed endophthalmitis cases are culture negative. The rate of false-positive cultures is unknown but is reduced if positive cultures are counted as those with moderate-to-abundant growth or growth on two media. In a study of 36 patients undergoing vitrectomy for noninfectious indications, one-fifth of cultures were positive, but organisms (mostly coagulase-negative staphylococci) grew on only one medium, indicative of a low inoculum.[77]

Molecular diagnostic techniques, such as PCR assay, have been used both to diagnose unusual pathogens (e.g., *Neisseria meningitidis*,[78] *Bartonella henselae*)[79] and to improve pathogen detection.[80] Some caution must be exercised if bacteria that usually colonize the ocular surface, such as coagulase-negative staphylococci, are detected: These may represent only ocular surface contaminants rather than intraocular pathogens. False-positive rates by PCR assay of 5% to 14% have been reported in uninfected eyes.[81,82] However, this assay does appear promising. In a study from Brazil of 11 patients with postcataract endophthalmitis, PCR assay was more often positive than culture (91% vs. 75%).[83] In the control group none of 12 vitreous cultures was positive, although 2 of 50 aqueous cultures were. In a study from France of 100 postcataract cases, PCR assay was no more sensitive than culture of initial intraocular samples, before any intravitreal antibiotics were given.[84] However, PCR assay was much more sensitive (70% vs. 9%) in subsequent vitreous

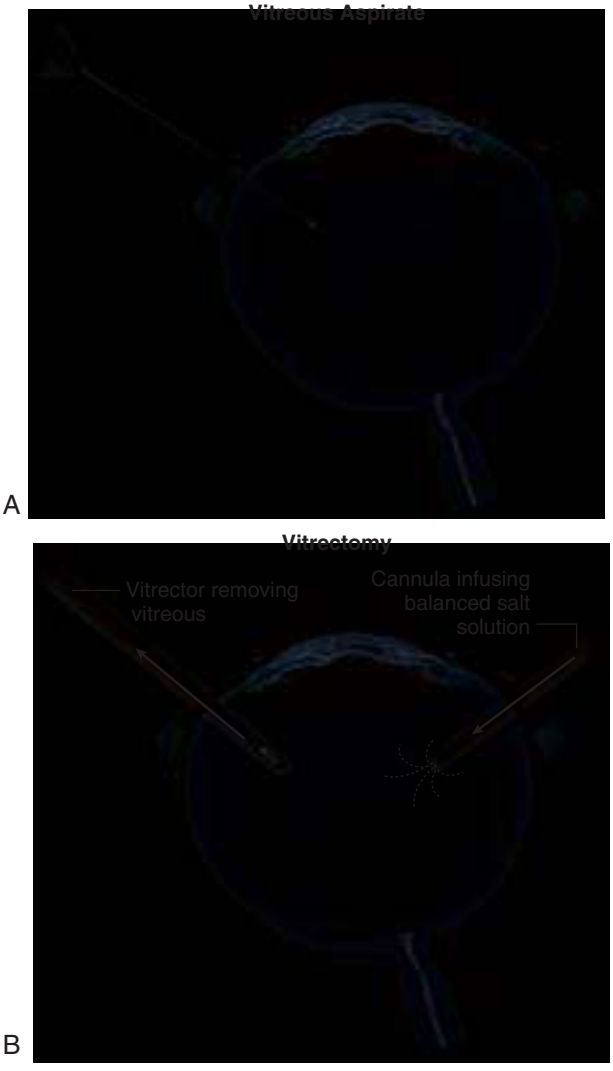

FIG. 114.3 Vitreous aspirate and vitrectomy. Diagram of vitreous aspirate (A) and vitrectomy (B).

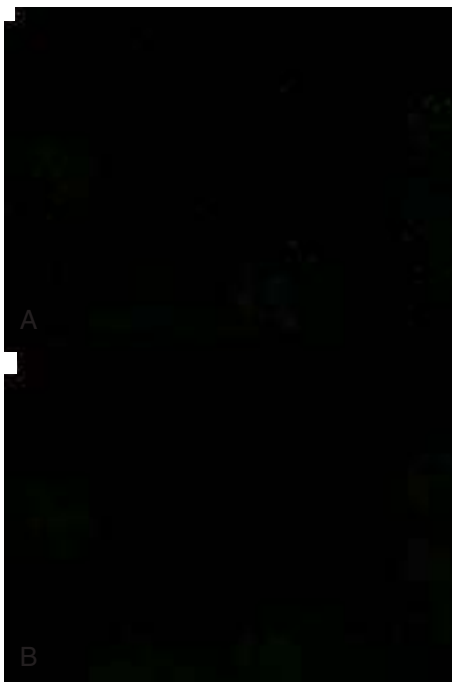

FIG. 114.4 Pigment granules, as seen on a gram stain of intraocular fluid in a case of endophthalmitis. Pigment granules appear (A) purple on Gram stain, but (B) coppery (hyperrefractile) if the fine focus knob of the microscope is rotated back and forth. *(From Durand ML. Bacterial and fungal endophthalmitis.* Clin Microbiol Rev, *2017;30:597–613.)*

cultures, reflecting either false-negative cultures due to the persistence of the intravitreal antibiotics in the vitreous sample or false-positive PCR results due to detection of dead pathogens.

THERAPY

Acute Bacterial Endophthalmitis

Acute bacterial endophthalmitis is a medical emergency because delay in giving appropriate therapy may lead to irreversible loss of vision. The most important component of treatment is direct injection of antibiotics into the vitreous by the ophthalmologist. This can be done in an office procedure room after a needle aspirate of the vitreous was sent for culture (called *tap and inject*) or at the conclusion of a vitreous débridement procedure (vitrectomy) in the operating room. The empirical intravitreal antibiotics most often used in the United States are vancomycin, 1 mg, plus ceftazidime, 2.25 mg. Each agent is diluted in 0.1 mL of sterile water or normal saline. Amikacin, 0.4 mg, may be used in place of ceftazidime for patients with significant cephalosporin allergies or for treatment of ceftazidime-resistant organisms, but in general, intravitreal aminoglycosides are avoided due to the risk (although very rare) of macular infarction.[85] Macular infarction from intravitreal aminoglycosides causes a blind eye, and there is no known treatment.[86]

Intravitreal antibiotics persist in the vitreous only 24 to 48 hours.[87] In severe cases of endophthalmitis one antibiotic injection may be insufficient to cure the infection. This is especially true in patients who did not undergo a vitrectomy as their initial procedure but only an office tap and inject. If such patients fail to improve by 24 to 48 hours, a vitrectomy with repeat antibiotics (either vancomycin or ceftazidime) is usually indicated. Vitrectomy rather than a tap is in fact the initial procedure of choice in patients presenting with fulminant infection because a vitrectomy débrides the abscess-like infected vitreous rapidly, leading to quicker clearing of infection and improved visual outcome.[16,88]

Systemic antibiotics alone are not effective for treating any type of endophthalmitis but are used for treating the underlying systemic infection in endogenous endophthalmitis cases. Whether systemic antibiotics that achieve good vitreous levels and target the most common pathogens could be beneficial as adjunctive therapy in treating exogenous bacterial endophthalmitis is unknown. Theoretically, such systemic antibiotics could effectively extend the half-life of intravitreal antibiotics. The only large randomized trial undertaken to evaluate this was the Endophthalmitis Vitrectomy Study (EVS), which evaluated patients with postcataract endophthalmitis.[16] The EVS concluded that the addition of systemic antibiotics to intravitreal antibiotics was not beneficial, but the systemic antibiotics used in the study (IV amikacin plus ceftazidime) had poor efficacy against staphylococci, the etiology of 80% of cases. In addition, amikacin penetrates the blood-eye barrier poorly.[83,89] The value of adjunctive intravitreal corticosteroids (dexamethasone, 0.4 mg) is unknown because no large randomized controlled trials (RCTs) have been conducted. A 2017 Cochrane review of the literature identified only three randomized trials, none listed in a clinical trial registry, and these had a combined total of only 95 participants.[90] The review concluded that the effectiveness of adjunctive corticosteroid therapy could not be determined based on available literature. In acute bacterial endophthalmitis of any type (postcataract, endogenous, bleb-related), removal of any synthetic IOL that is present is usually not necessary. In chronic pseudophakic endophthalmitis, exchange or removal of the IOL is necessary in the majority of cases. In mold endophthalmitis the IOL should be removed.

Chronic Postcataract Endophthalmitis

Chronic postcataract endophthalmitis due to *P. acnes* requires at least vitrectomy and intravitreal vancomycin for treatment, but 50% of cases recur with this therapy alone.[91] The addition of posterior capsulectomy to this regimen reduced the recurrence rate in one study,[91] but not in another.[92] Exchanging the IOL for a new one markedly improved the success rate. The combination of total capsulectomy, IOL exchange or removal, vitrectomy, and intravitreal antibiotics cured all infections in both studies, including refractory infections that had failed earlier therapies. Systemic antibiotic therapy is not indicated for this condition.

Fungal Endophthalmitis

Endogenous *Candida* Endophthalmitis

All patients with ocular candidiasis require systemic antifungal therapy. Patients with *Candida* chorioretinitis but no endophthalmitis (i.e., minimal or no vitritis) usually respond to systemic antifungal therapy alone. In patients with chorioretinitis in which there is a macula-threatening lesion, intravitreal antibiotics are recommended in addition, to provide immediate high intraocular antifungal levels. The intravitreal antibiotics used are amphotericin, 5 to 10 μg in 0.1 mL sterile water, or voriconazole, 100 μg in 0.1 mL sterile water. Voriconazole may be used for sensitive strains.

Treatment of endogenous *Candida* endophthalmitis (i.e., significant vitritis) includes immediate intravitreal antifungal therapy in addition to systemic antifungal therapy in all patients and a vitrectomy in nearly all cases. Vitrectomy is performed with local (retrobulbar) anesthesia rather than general anesthesia, but vitrectomy still may not be possible in very ill inpatients with candidemia. However, vitrectomy appears to be beneficial in patients with significant vitritis. A study of 12 patients with *Candida* endophthalmitis (i.e., significant vitritis) reported a good outcome in the 7 patients who underwent early vitrectomy, but blindness or scotoma in 4 of 5 patients in whom vitrectomy was delayed more than 1 week or not performed.[93]

Systemic therapy is indicated for both *Candida* chorioretinitis and endophthalmitis. Systemic fluconazole and voriconazole achieve excellent vitreous levels (70% and 40%, respectively, of plasma levels), and these agents are favored over amphotericin due to better intraocular penetration and lower systemic toxicity.[94] For endophthalmitis due to fluconazole-susceptible *Candida* strains, fluconazole is the treatment of choice and is prescribed as a loading dose of 800 mg (12 mg/kg), followed by 400 to 800 mg (6–12 mg/kg) daily. The dose is reduced for patients with renal dysfunction. Although some fluconazole-resistant *Candida* species are also voriconazole resistant, voriconazole should be used for voriconazole-susceptible strains, or in patients intolerant of fluconazole but tolerant of voriconazole. Voriconazole is given as a loading dose of 400 mg (6 mg/kg) IV twice daily for 2 doses, followed by 200 to 300 mg (4 mg/kg) IV or orally twice daily. Voriconazole has more drug-drug interactions and is more expensive than fluconazole, and serum voriconazole levels should be monitored beginning approximately 1 week after starting therapy. In cases of endophthalmitis caused by *Candida* strains that are resistant to fluconazole and voriconazole, the 2016 Infectious Diseases Society of America guidelines recommend IV liposomal amphotericin (3–5 mg/kg, IV daily), with or without flucytosine, 25 mg/kg four times daily.[95,96,97]

Echinocandins successfully treat most cases of candidemia, including candidemia due to fluconazole-resistant strains. However, they are not recommended for treating *Candida* endophthalmitis due to poor penetration into the vitreous. It is possible, but not proven, that echinocandins may be adequate to treat *Candida* chorioretinitis. A study of eight eyes in seven patients who were receiving IV micafungin at the time of vitrectomy or enucleation demonstrated very poor micafungin levels in the vitreous (average 0.5% of plasma levels), but a good level in the choroid in the one eye in which this was measured (5.8 μg/mL, or 34% of plasma level).[98] There have been case reports of failure in endophthalmitis (significant vitritis) but success in chorioretinitis.[99,100] In addition, a large randomized prospective trial of echinocandins (micafungin vs. caspofungin) for treating invasive candidiasis included 11 patients with *Candida* chorioretinitis, and 7 (64%) had successful outcomes.[101] The study does not mention whether adjunctive therapies were used (vitrectomy, intravitreal antifungal agents).

Exogenous *Candida* Endophthalmitis

Many cases of exogenous *Candida* endophthalmitis primarily involve the aqueous humor, and these may be treated with either intracameral amphotericin or voriconazole (for susceptible strains). The usual dose is 5 μg of amphotericin or 50 μg of voriconazole. Voriconazole is preferred over amphotericin for sensitive species because intracameral amphotericin can cause a transient increase in eye pain, hypopyon, or intraaqueous inflammation.[102,103] Intracameral voriconazole has a half-life of only 22 minutes in rabbits,[104] so a similarly short half-life is presumed in humans.

Topical voriconazole 1% solution applied to the cornea every 2 hours in one study produces high levels in the aqueous after 24 hours,[105] so topical voriconazole may be given at regular intervals to prolong the intracameral level of the drug after intracameral injection. Antibiotics injected into the aqueous do not usually reach the vitreous, so a separate intravitreal injection of either amphotericin (5–10 μg) or voriconazole (100 μg) should be given in most cases. Even in cases in which there is no apparent vitritis, intravitreal injection of antifungal agent should be considered (in addition to intracameral antifungal) because occult infection may already be present. In cases in which there is marked vitritis, vitrectomy should be performed in addition to intravitreal amphotericin or voriconazole. Systemic fluconazole (for susceptible strains) or voriconazole (for fluconazole-resistant strains, such as *Candida krusei* or some strains of *Candida glabrata*) is recommended in all cases of exogenous *Candida* endophthalmitis, in addition to intravitreal antifungal therapy.

In nearly all cases of postcataract *Candida* endophthalmitis, the IOL should be removed. In cases that occur after corneal transplantation, the infected cornea is often replaced with a new donor cornea at the onset of endophthalmitis.

Mold Endophthalmitis

Mold endophthalmitis should be treated with a combination of vitrectomy, intracameral and/or intravitreal antifungal therapy, removal of any foreign material such as an IOL, and systemic antifungal therapy.

In cases of exogenous endophthalmitis primarily involving the aqueous, such as occurs with progression of keratomycosis, intracameral amphotericin, 5 μg, or voriconazole, 50 μg, should be given in addition to systemic voriconazole (for susceptible fungi). Any foreign material, such as an IOL, synthetic glaucoma filtering valve, or foreign body introduced by trauma, must be removed. For cases resulting from progression of fungal keratitis, corneal transplantation is almost always required when endophthalmitis is diagnosed, to rapidly debulk the infection. An intravitreal injection of amphotericin or voriconazole is often given in exogenous cases even if there is no apparent vitritis because of concern for occult infection. Patients with exogenous mold endophthalmitis involving the vitreous should receive a vitrectomy plus intravitreal amphotericin, 5 to 10 μg, or voriconazole, 100 μg (for susceptible fungi),[106,107–108] plus systemic voriconazole (for susceptible fungi). Intravitreal injections of either amphotericin or voriconazole may be repeated at least 48 hours after the first injection if there is no improvement and may be repeated more than once if necessary.

Systemic voriconazole is recommended for all cases of exogenous mold endophthalmitis due to susceptible molds, such as *Aspergillus* and *Fusarium*.[109,110] These molds are the most common causes of exogenous and endogenous mold endophthalmitis, and systemic voriconazole achieves good intraocular levels. IV voriconazole is preferred for initial treatment to ensure adequate levels before switching to oral therapy. For cases of exogenous endophthalmitis involving the vitreous and due to voriconazole-resistant molds, adjunctive liposomal amphotericin may be given in addition to vitrectomy and intravitreal amphotericin injections (and removal of IOL, etc.). However, the potential risk of toxicity versus benefit of adjunctive systemic liposomal amphotericin therapy must be weighed for a particular case, and this evaluation must take into account the response to initial vitrectomy and intravitreal amphotericin. Systemic therapy with posaconazole has been used with success in a few case reports.[111,112] Fluconazole is not recommended due to poor activity against molds. Use of isavuconazole for treating mold endophthalmitis has not been reported.

Treatment for endogenous mold endophthalmitis due to transient fungemia (e.g., after IVDU) includes vitrectomy, intravitreal amphotericin or voriconazole, and systemic voriconazole (for susceptible molds). For endogenous cases in patients ill with fungemia, the systemic antifungal chosen should be the one optimal for the systemic infection. Such patients are often too ill for vitrectomy but can tolerate intravitreal injections of antifungal agents.

VISUAL OUTCOME

The final visual outcome after acute bacterial endophthalmitis usually cannot be determined for several months because sequelae of intraocular inflammation resolve slowly. Patients who lose all vision (no light perception) almost never regain it, however. Some predictions can be made based on presenting visual acuity and the causative organism, factors that are related. In the EVS, patients who presented with the worst vision—light perception only—were much more likely to have infection from virulent bacteria (streptococci, *S. aureus,* gram-negative bacilli) and a poor visual outcome. Factors that correlated with virulent bacteria in the EVS included presentation with swollen eyelids, afferent pupillary defect, corneal infiltrate, larger hypopyon (≥1.5 mm), or loss of a red reflex.[113] Patients did best if they presented with a mild decrease in vision or had vitreous cultures that were negative or grew coagulase-negative staphylococci. In the EVS, outcome closely correlated with the pathogen recovered.[114] Greater than 80% of eyes with coagulase-negative staphylococci had at least 20/100 vision at follow-up 9 to 12 months later, and only 4% had severe visual loss (<5/200). Results in culture-negative endophthalmitis cases were similar. In contrast, 20/100 or better vision was achieved in only 56% of gram-negative cases, 50% of *S. aureus* cases, and 30% of streptococcal cases. Similar large outcome studies are not available for other types of endophthalmitis, but in general, outcomes reflect the virulence of the predominant organisms. Some of the worst outcomes are seen in posttraumatic endophthalmitis due to *B. cereus,* in which salvage of the eye is rare.[115] The generally poor visual outcome in bleb-related endophthalmitis reflects the fact that most cases are due to streptococci and *H. influenzae.* Endogenous bacterial endophthalmitis also usually has a poor outcome, although early diagnosis and prompt treatment with vitrectomy and intravitreal antibiotics has saved useful vision even in eyes with virulent organisms.[40]

Visual outcome after chronic *P. acnes* endophthalmitis may depend on the chronicity of the infection. In a review of 36 patients diagnosed with this infection over 22 years, the visual outcome was 20/40 or better in half of the patients but worse than 20/400 in one-fifth of patients.[91]

The visual prognosis for endogenous *Candida* endophthalmitis is difficult to determine because many reviews combine cases of chorioretinitis with endophthalmitis cases.[42] Patients with chorioretinitis alone usually have excellent visual outcomes. *Candida* endophthalmitis also may have a good outcome if treated with vitrectomy, intravitreal antifungal therapy, and systemic therapy. The prognosis in mold endophthalmitis in the pre-voriconazole era was poor, with rare salvage of useful vision. However, several recent cases treated in the voriconazole era with the aggressive management described earlier have had excellent visual outcomes (20/20–20/60).[116,117]

PREVENTING ENDOPHTHALMITIS

Prophylaxis for Eye Surgery

The value of any type of prophylaxis for postoperative endophthalmitis is unknown.[118,119,120] Standard prophylaxis involves only the use of topical povidone-iodine on the conjunctiva and eyelids immediately before surgery. A multicenter European study, European Society of Cataract and Refractive Surgeons (ESCRS), conducted from 2003–06 found that use of prophylactic intracameral (into the anterior chamber) cefuroxime, 1 mg/0.1 mL, at the end of cataract surgery was associated with a significantly lower rate of endophthalmitis than non-use, 0.06% versus 0.3%.[121] As a consequence, many centers worldwide now use this prophylaxis. Adoption of intracameral cefuroxime prophylaxis has not been universal, however, because of criticism that the ESCRS control group's endophthalmitis rate was surprisingly high (0.3%, whereas typical rates are 0.1%). Although adoption of intracameral cefuroxime prophylaxis has reduced endophthalmitis rates in some centers, other sites have not seen a benefit.[122] The ESCRS study was the only RCT to evaluate intracameral antibiotic prophylaxis. Ophthalmologists have used other intracameral antibiotics for prophylaxis, such as intracameral moxifloxacin or vancomycin, without benefit of RCTs.[123] However, since 2014, a rare but potentially blinding adverse reaction to prophylactic intraocular vancomycin has been described. This reaction is labeled "hemorrhagic occlusive retinal vasculitis" and is of unknown etiology, although clearly associated with recent intraocular vancomycin injection.[124,125,126] Symptoms include a delayed onset of painless vision loss (1–21 days), mild aqueous and vitreous inflammation, retinal hemorrhages in areas of ischemia, and involvement of retinal venules.[126] Visual

outcomes have been very poor, with 67% of eyes left with 20/200 or worse vision and 20% left with no light perception.[126] Intraocular vancomycin should not be given for prophylaxis.

The value of giving postoperative topical antibiotics is also unknown, and no RCT has assessed this. However, ophthalmologists commonly prescribe postoperative topical antibiotics, partly due to the concern about potential contamination postoperatively through a clear cornea incision. In Sweden, where prophylactic intracameral cefuroxime is used but topical postoperative antibiotics are not, endophthalmitis rates are low.[118]

Prophylaxis for Intravitreal Injections

Intravitreal injections are performed as an office procedure, and gowns, gloves, and masks are rarely used. However, the high incidence of postinjection endophthalmitis caused by viridans streptococci has led to the realization that the ocular surface may be contaminated by oral flora from either the patient or the ophthalmologist during the procedure. As a consequence, use of masks or observing silence in the room during the injection is recommended.[118,120] Topical antibiotics should not be used for prophylaxis after intravitreal anti-VEGF injections because these have been associated with an increased risk of postinjection endophthalmitis.[127]

Prophylaxis for Eye Trauma

Prompt repair of penetrating eye (open globe) injuries is important in preventing posttraumatic endophthalmitis. A brief course of prophylactic antibiotics at the time of presentation with an open globe appears to be beneficial, based on the results of several series.[31,33,128,129] Two centers have achieved very low rates of posttraumatic endophthalmitis (0.9%) using a prophylactic regimen of IV vancomycin plus either ceftazidime (one center) or cefepime (the other center).[33,129] Prophylactic antibiotics are started on presentation and continued for 48 hours.

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115 Infectious Causes of Uveitis

Marlene L. Durand

SHORT VIEW SUMMARY

Definition and Categories

- *Uveitis* means inflammation of the uvea (iris, ciliary body, choroid) or retina.
- Uveitis is divided into categories by the site of greatest inflammation:
  - Anterior (iritis, iridocyclitis)
  - Intermediate (pars planitis)
  - Posterior (choroiditis, retinitis, chorioretinitis)
  - Panuveitis
- Noninfectious causes of uveitis (idiopathic, autoimmune) are more common than infectious causes, but the frequency of each varies depending on category.
- Approximately 10% of anterior uveitis cases have an infectious etiology.
- Approximately 50% of posterior uveitis cases have an infectious etiology.

Epidemiology and Etiology

- The most likely infectious etiologies vary by uveitis category and by location in the world (see Table 115.1).
- Infectious causes of anterior uveitis include herpes simplex virus (HSV) (90% of infectious etiologies), varicella-zoster virus (VZV), syphilis, tuberculosis (TB), and Lyme disease.
- Infectious causes of intermediate uveitis are rare and include Lyme disease.
- Infectious causes of posterior uveitis include ocular toxoplasmosis, acute retinal necrosis (HSV, VZV, sometimes cytomegalovirus [CMV]), CMV retinitis (severely immunocompromised patients), ocular *Toxocara,* syphilis, cat-scratch disease, and *Candida* endophthalmitis. West Nile virus may cause uveitis, most commonly a multifocal chorioretinitis
- Infectious causes of panuveitis include syphilis, TB, and *Candida* endophthalmitis.
- Some bacterial etiologies of uveitis are seen primarily in tropical regions or developing countries; these include leptospirosis (panuveitis without retinal or choroidal lesions), leprosy (anterior uveitis), and *Brucella* (chronic relapsing uveitis). Viral infections caused by Chikungunya virus, Ebola virus, and Zika virus may also produce various types of uveitis.

Diagnosis

- Diagnosis varies by etiology; see text. Ocular syphilis is presumed in cases of uveitis with positive specific treponemal serology. Polymerase chain reaction of aqueous or vitreous for HSV, VZV, and CMV may be helpful in some chronic anterior uveitis cases (aqueous) and in some cases of acute retinal necrosis (vitreous higher yield than aqueous, but vitreous sampling carries more risk).

Therapy

- Therapy varies by etiology; see text. Acute retinal necrosis is a medical emergency and requires antiviral treatment directed against HSV and VZV; consider treatment that is also effective against CMV in immunocompromised patients. Ocular toxoplasmosis is discussed in Chapter 278. Ocular TB and ocular syphilis should be treated the same way as TB meningitis and neurosyphilis, respectively.

Uveitis means inflammation of the uvea. The uvea is the pigmented, vascular middle layer of the eye embryologically, sandwiched between the cornea-sclera outer protective layer and the retina. The word *uvea* comes from the Latin word *uva,* meaning *grape,* a translation by Roman anatomists (e.g., Galen) of the Greek term, used because of the grapelike appearance of this highly vascular layer when the white sclera was stripped away. The uvea is composed of the iris, ciliary body, and choroid (see Chapter 111). The *iris* regulates the amount of light that reaches the retina, the *ciliary body* produces aqueous humor and supports the lens, and the *choroid* helps to nourish the retina.

Retinitis is included as a type of uveitis even though the retina is not part of the uvea because the retina is often involved when there is underlying choroidal inflammation. Uveitis is classified by the ocular structures involved. Several anatomic classification schemes exist, but all divide uveitis into anterior, intermediate, posterior, and panuveitis categories (Table 115.1). The site of greatest inflammation determines the category. The International Uveitis Study Group classification is commonly used. In anterior uveitis, inflammation involves the iris (iritis), anterior ciliary body (cyclitis), or both (iridocyclitis). Anterior uveitis is characterized by white blood cells (WBCs) in the aqueous humor. There are often keratic precipitates (cells on the corneal endothelial surface) and iris lesions. Intermediate uveitis refers to inflammation involving the anterior vitreous, ciliary body, and adjacent portion of the retina (called *peripheral* retina). Posterior uveitis refers to inflammation involving the choroid (choroiditis), the retina (retinitis), both the choroid and the retina (chorioretinitis), or the retinal vessels (retinal vasculitis). There may be inflammation of the vitreous and/or optic nerve. Panuveitis involves all three parts of the uvea. Uveitis may also extend to involve the cornea (keratouveitis) or sclera (sclerouveitis).

Other terms commonly used in uveitis appear in Fig. 111.1 in Chapter 111. The eye is divided into anterior and posterior segments by the lens. The iris divides the anterior segment further into anterior and posterior chambers. Aqueous humor fills the anterior segment and is produced and resorbed constantly, with a turnover time of 100 minutes. The posterior segment, a term not to be confused with posterior chamber, is filled with the gel-like vitreous. The vitreous is produced in utero and never regenerated, although it may be surgically removed (vitrectomy) and replaced with clear fluids such as saline.

In addition to anatomic location, uveitis is classified as granulomatous or nongranulomatous. *Granulomatous* does not mean there are granulomas on pathology, but rather describes a type of inflammation in which the WBCs are condensed into clumps rather than uniformly dispersed. In granulomatous anterior uveitis, *granulomatous* or *mutton fat* keratic precipitates form on the endothelial surface of the cornea. These are greasy in appearance and more yellow than nongranulomatous *(granular)* keratic precipitates (Fig. 115.1). There may also be clusters of WBCs in the iris, called *Busacca nodules* if in the iris stroma and *Koeppe nodules* if at the pupillary margin. Topical corticosteroid treatment may convert a granulomatous anterior uveitis into a nongranulomatous anterior uveitis. In granulomatous uveitis that involves the posterior segment of the eye, there may be WBCs in large clusters *(snowballs)* in the vitreous, in exudates adjacent to retinal vessels *(candle wax drippings)*, or as granulomas within the choroid (e.g., multifocal choroiditis). Granulomatous uveitis is typical of infections such as tuberculosis (TB), syphilis, and toxoplasmosis, although all may have a nongranulomatous presentation. Two types of autoimmune uveitis, sarcoidosis and Vogt-Koyanagi-Harada syndrome, usually produce a granulomatous uveitis. Nongranulomatous inflammation is more characteristic of autoimmune



FIG. 115.1 Granulomatous keratic precipitates. Granulomatous keratic precipitates, also called *mutton fat* keratic precipitates. The white precipitates represent white blood cells that have condensed on the endothelial surface of the cornea in anterior uveitis. They do not represent granulomas. Granular keratic precipitates are similar, but the condensations are much smaller, so the white "dots" appear finer. *(Courtesy Audrey C. Melanson.)*

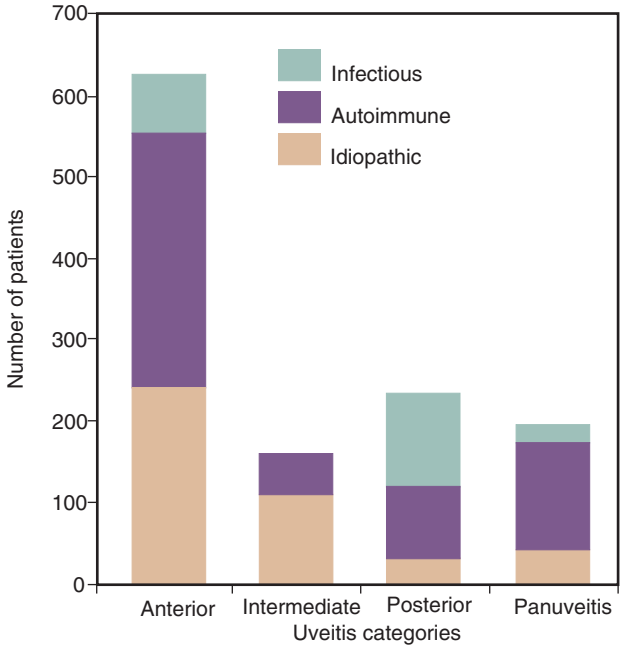

FIG. 115.2 Etiologies of uveitis. Bar graph of the causes of uveitis by anatomic category (anterior, intermediate, posterior, panuveitis), one bar for each category. Each bar is divided into three segments, a different color for each etiology: infectious, autoimmune, and idiopathic. *(Height of each bar and each segment based on data by Rodriguez A, Calonge M, Pedoza-Seres M, et al. Referral patterns of uveitis in a tertiary eye care center.* Arch Ophthalmol. *1996;114:593–599.)*

TABLE 115.1 Classification of Uveitis and Major Infectious Etiologies in Each Category

CATEGORY	OCULAR FINDINGS	MAJOR INFECTIOUS ETIOLOGIES (%)[A]
Anterior (iritis, cyclitis, iridocyclitis)	WBCs in aqueous, keratic precipitates, iris nodules, synechiae	Herpes simplex (10%); syphilis (<1%); TB (<1%); Lyme disease (<1%); leprosy (<1%)
Intermediate	WBCs or *snowballs* in vitreous, pars plana *snow bank*	Lyme disease (<1%)
Posterior (choroiditis, chorioretinitis, retinitis)	Lesions in choroid, retina, or both; vitritis in some	*Toxoplasma* (25%); CMV (12%)[b]; ARN (6%); *Toxocara* (3%); syphilis (2%); *Candida* (<1%)
Panuveitis	WBCs in aqueous and vitreous	Syphilis (6%); TB (2%); *Candida* (2%)

[a]Percentage of uveitis cases in each category (not of total uveitis cases), based on 1237 cases of uveitis seen at Massachusetts Eye & Ear Infirmary, Boston, 1982–92. (Rodriguez A, Calonge M, Pedoza-Seres M, et al. Referral patterns of uveitis in a tertiary eye care center. *Arch Ophthalmol.* 1996;114:593–599.)
[b]Series was before use of highly active antiretroviral therapy, and rate for CMV retinitis would be lower now.
ARN, Acute retinal necrosis; *CMV,* cytomegalovirus; *TB,* tuberculosis; *WBCs,* white blood cells.

uveitis such as Behçet disease, reactive arthritis, and ankylosing spondylitis.

EPIDEMIOLOGY

Uveitis has a prevalence of 70 to 115 cases/100,000 population in the United States and may account for up to 10% of blindness worldwide.[1,2] Uveitis can occur at any age but is less common in children than in adults. Surveys from around the world report a mean age at presentation of 35 to 45 years old.[3] Uveitis is more common in women than men in most studies. Anterior uveitis is the most common type of uveitis, accounting for up to 90% of uveitis cases in community-based ophthalmology practices.[4–6] Major referral eye centers report a relatively high incidence of posterior and panuveitis cases, likely due to the vision-threatening nature of these conditions. A large study from a referral center in India reported that posterior uveitis (11%) and panuveitis (22%) accounted for one-third of cases, whereas intermediate (10%) and anterior (57%) uveitis accounted for the remaining cases.[3]

Uveitis may be caused by autoimmune conditions, infections, or, rarely, trauma, but up to 50% of cases are idiopathic. Some cases of intraocular inflammation masquerade as uveitis (masquerade syndromes) but have other causes such as malignancy (e.g., ocular–central nervous system [CNS] lymphoma).

Infections cause approximately 20% to 50% of uveitis cases, with the higher frequency seen in tropical countries. The most common infectious etiology varies by country and region. In Iran, infections caused 20% of uveitis cases with herpetic infections and TB as the most common infectious etiologies.[7] In a South African study, infections accounted for 47% of uveitis cases, with syphilis (16%) being the most common infectious etiology,[8] whereas a study from Vietnam reported an infectious etiology in 27% of uveitis cases, with the most common being TB (9%).[9]

The likelihood of an infectious etiology is greater in some categories of uveitis, such as posterior uveitis, than others. This is illustrated in Fig. 115.2, and the frequency of infections is listed by category in Table 115.1. Understanding the types of infectious etiologies by category is helpful in considering possible etiologies for a patient with uveitis. In anterior uveitis cases in the United States, most cases are idiopathic (40%) or associated with a rheumatologic condition (45%) such as seronegative arthropathies, human leukocyte antigen (HLA)-B27–associated disease, reactive arthritis, and juvenile rheumatoid arthritis. The most common infectious cause is herpes simplex, which accounts for almost 10% of anterior uveitis cases, whereas syphilis, TB, and Lyme disease each cause less than 1%. Intermediate uveitis most often has an unknown etiology (69%) in the United States or is due to sarcoidosis (22%) or multiple sclerosis (8%); infections are extremely rare.[5] However, in India, intermediate uveitis has an infectious etiology in 11% of cases including leptospirosis (7%) and TB (4%).[3] Posterior uveitis has an infectious etiology in more than 40% of cases, with ocular toxoplasmosis the most common cause, accounting for 20% to 40% of posterior uveitis cases. Other infectious causes include cytomegalovirus (CMV) retinitis, acute retinal necrosis (ARN), *Toxocara,* syphilis, and *Candida* in the United States. Major noninfectious diagnoses in a US study were lupus erythematosus, sarcoidosis, and birdshot retinochoroidopathy, an eye disease of unknown etiology (8% each).[5] In panuveitis, infections cause 10% of cases and include syphilis, TB, and *Candida.*[5] The remaining

90% of cases are caused by inflammatory conditions such as sarcoidosis, Behçet disease, systemic lupus erythematosus, multifocal choroiditis and panuveitis, and Vogt-Koyanagi-Harada syndrome or are idiopathic.

CLINICAL MANIFESTATIONS

Because infectious uveitis usually represents a chronic process, patients often present with no systemic complaints. Patients with anterior uveitis typically present with eye pain and decreased vision. The eye is often injected, especially near the limbus *(ciliary flush)*, and slit-lamp examination shows cells in the anterior chamber. There may be keratic precipitates, iris nodules, or synechiae (adhesions) between the iris and either the cornea or the lens. The vitreous has few, if any, cells, and the retina is normal. Patients with intermediate uveitis present with floaters or blurred vision but typically no pain or photophobia. The aqueous is quiet, but vitreous cells are characteristic and are often clumped into so-called snowballs. There may be a white exudate or *snow bank* over the pars plana. In posterior uveitis, patients often have painless loss of vision as their primary symptom. There are usually few cells in the anterior chamber, but the funduscopic examination shows lesions in the retina or choroid or both. There may be retinal vasculitis or periphlebitis (venous sheathing). There may be many cells in the vitreous, typical of toxoplasmosis, or no vitritis, typical of CMV retinitis. Panuveitis is characterized by inflammation in both anterior and posterior segments of the eye.

Different pathogens produce different clinical manifestations when they infect the eye. The following is an alphabetical list of various infectious etiologies of uveitis and the most common clinical manifestations of each. Some of these infectious causes of uveitis are common worldwide (e.g., anterior uveitis due to herpes simplex, or posterior uveitis due to ocular toxoplasmosis), while others are rare (e.g., brucellosis, Whipple disease). Still others are rare except in outbreak situations (e.g., Ebola virus).

Acute Retinal Necrosis

Acute retinal necrosis (ARN) is a rapidly progressive necrotizing retinitis that mainly affects immunocompetent patients. The etiology of nearly all cases is herpes simplex virus (HSV) or varicella-zoster virus (VSV).[10] In immunocompromised patients, CMV may also be the etiology. The American Uveitis Society has established the following four features as diagnostic criteria for ARN: (1) focal well-demarcated areas of retinal necrosis located in the peripheral retina, (2) rapid circumferential progression of necrosis, (3) occlusive vasculopathy, and (4) prominent inflammation (WBCs) in the vitreous and aqueous.[10] A study of 18 patients with ARN (negative for human immunodeficiency virus [HIV]) who underwent vitreous biopsy found polymerase chain reaction (PCR) evidence of VZV in 12 specimens and HSV in 4 specimens; 2 specimens were negative by PCR.[11] CMV is a rare cause of ARN but must be considered in immunocompromised patients. Epstein-Barr virus (EBV) has been found by vitreous PCR in some cases of ARN, but only in cases in which vitreous PCR was also positive for VZV.[11] Some patients with ARN due to HSV have a history of congenital herpes or herpes encephalitis months to years earlier, but these cases are uncommon.[12,13] ARN typically begins with a unilateral anterior uveitis. Patients may have mild eye pain or photophobia followed by decreased vision in the affected eye. Funduscopic examination with an indirect ophthalmoscope shows one or more foci of retinal necrosis in the peripheral retina, an area not usually seen with a direct ophthalmoscope (Fig. 115.3). The areas of retinitis have sharply demarcated borders and typically spread circumferentially and posteriorly. Vascular sheathing develops along with a dense vitritis. Necrosis of areas of the retina leads to retinal detachment in many patients. Retinal detachment occurs weeks to months after onset of ARN. One study found that more than 50% of patients developed a retinal detachment, and this occurred 3 weeks to 5 months after onset of ARN.[11] Treatment with laser to encircle areas of retinal necrosis may help prevent retinal detachment. Treatment with intravenous acyclovir halts progression of the retinitis in ARN in most cases. ARN may involve the other eye several months after the first eye even despite therapy. In an early study, ARN developed in the other eye in 70% of patients who had not received antiviral treatment and in 13% of treated patients.[14] In cases that progress despite intravenous acyclovir, intravitreal foscarnet and other systemic antiviral agents may be essential to halt the rapid progression of this blinding infection (see "Therapy").



FIG. 115.3 Acute retinal necrosis. Funduscopic photograph of acute retinal necrosis showing loss of retinal features in the peripheral retina due to retinal necrosis.

Brucellosis

Brucellosis is one of the most widespread zoonoses worldwide, with endemic regions in the Middle East, Africa, Central Asia, India, China, and Central and South America. Cases may be seen in nonendemic countries in travelers or immigrants from endemic regions. Uveitis may develop as part of the illness. A prospective study from Turkey of 147 patients with brucellosis found uveitis in 5% (anterior in 6 patients and posterior in 1 patient).[15] The authors noted that osteoarticular complications of brucellosis such as spondylitis were more common in patients with ocular involvement. Ocular involvement may occur during acute or chronic brucellosis. A report from Iran described a case of bilateral acute optic disk swelling with retinal hyperemia occurring 1 week after the febrile illness.[16] Chronic relapsing uveitis may be a sign in chronic, untreated brucellosis. In a 2008 study of 1551 patients with brucellosis seen in Peru over a 26-year period, 0.7% of patients with acute brucellosis and 8% of patients with chronic brucellosis had ocular findings.[17] Uveitis accounted for more than 80% of cases, with posterior uveitis accounting for half of the cases. The diagnosis of brucellosis may be missed for years. Tabbara and Al-Kassimi[18] described a Saudi Arabian patient with a 9-year history of relapsing uveitis with episodes responding to corticosteroids. The patient also had a history of recurrent fevers, but brucellosis was not diagnosed until she developed a paravertebral abscess due to *Brucella melitensis*. The uveitis responded to antibiotic therapy.

Cat-Scratch Disease

Bartonella henselae causes Parinaud oculoglandular syndrome in 2% to 5% of patients with cat-scratch disease (CSD). This syndrome consists of a necrotic granuloma with ulceration of the conjunctival epithelium and regional lymphadenopathy (preauricular, submandibular, cervical). The other major ocular manifestations of CSD are neuroretinitis and optic neuritis, although other posterior segment manifestations (e.g., vitritis, focal retinitis, choroiditis, intraretinal white spots, retinal vasculitis) have been described.[19] Most cases of presumed ocular CSD rely on positive serologic testing for *B. henselae* for diagnosis, so it is possible that some of these cases have another etiology but a positive CSD serology from prior and unrelated cat exposure. Rare cases have had positive PCR testing of intraocular fluids. *Bartonella grahamii* has been identified by PCR amplification and sequence analysis in the intraocular fluid of an HIV-seronegative patient with bilateral neuroretinitis and behavioral changes; *Bartonella elizabethae* has been

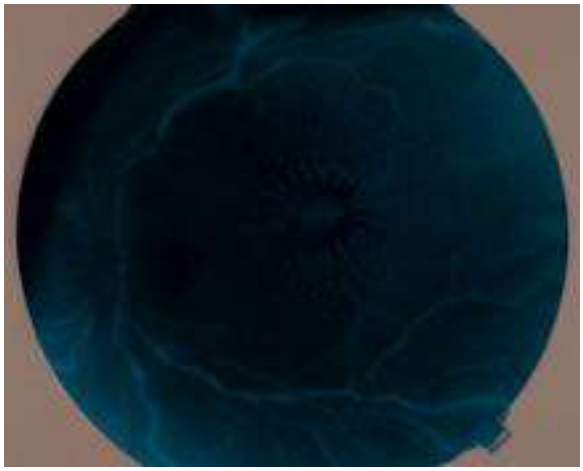

FIG. 115.4 Cat-scratch disease neuroretinitis. Funduscopic photograph of cat-scratch disease neuroretinitis, showing macular star in a 9-year-old patient who developed unilateral visual loss after acquiring a kitten. *(Courtesy Dr. Simmons Lessell.)*

implicated as the pathogen in another patient with neuroretinitis by serologic antibody studies exclusively.[20,21]

Neuroretinitis is a clinical diagnosis with characteristic eye findings of optic disk edema and a macular star, a striking sunburst pattern around the macula (Fig. 115.4). Patients present with a fairly sudden loss of vision. The macular star resolves in 2 to 3 months, and vision usually spontaneously recovers in almost all patients. Approximately two-thirds of patients with neuroretinitis are seropositive for *B. henselae*.[22] Optic neuropathy is another common manifestation of ocular CSD. In 53 patients ranging in age from 8 to 65 years with CSD-related optic neuropathy, two-thirds had flu-like symptoms at the time of presentation, including fever and headache in approximately one-fourth.[19] Bilateral involvement was present in 17%, and half also had neuroretinitis. Significant visual complications occurred in 9%, primarily due to retinal vascular occlusion. Antibiotics were given in 74%, and there was no correlation with visual outcome. Antibiotics such as doxycycline, quinolones, or macrolides, with or without rifampin, have been given to treat ocular CSD, but the benefit is uncertain.[23] The benefit of corticosteroids is also unclear. In a retrospective study of patients treated over a 20-year period in Israel, 12 of 24 eyes (50%) treated with antibiotics alone had significant visual acuity improvement versus 14 of 16 eyes (88%) treated with a combination of antibiotics plus corticosteroids, but the association with improved visual acuity and combination therapy remained significant only if doxycycline-based regimens were included.[23] The study could not answer whether antibiotics alone or corticosteroids alone were beneficial, when compared with no treatment as numbers were too small, but 100% of eyes ($n = 3$) treated with corticosteroids alone and 60% of eyes ($n = 5$) with no treatment also had significant improvement in visual acuity.

Chikungunya Virus

Chikungunya virus is transmitted by mosquitoes and causes an acute febrile illness after an incubation period of 3 to 7 days (can range from 2 to 12 days). Chikungunya virus has been reported from more than 60 countries worldwide. Major outbreaks have occurred in India; countries in the Indian and Pacific Oceans; other parts of Asia; Africa; and, since 2013, the Caribbean and South America. Cases in the United States are primarily seen in travelers who have recently returned from an endemic area; 156 cases were reported to the US Centers for Disease Control and Prevention in 2017, all in returned travelers.[24] The illness is characterized by the acute onset of high fever and severe joint pains; joint symptoms are usually bilateral and symmetrical. Uveitis typically occurs several weeks after the acute illness, although onset may be during the illness or months later. The most common eye findings are anterior uveitis (iridocyclitis), retinitis, and optic neuritis.[25,26] The retinitis or retinochoroiditis is characterized by areas of whitening in the posterior pole with surrounding retinal and macular edema; there may be an associated occlusive vasculitis.[27] Most patients recover good visual acuity.

Cytomegalovirus Anterior Uveitis

CMV has been implicated in some cases of chronic or recurrent anterior uveitis.[28] Similar to HSV and VZV, these cases have been unilateral and chronic, and some have had sectoral iris atrophy. The patients are immunocompetent and have no CMV retinitis or other signs of CMV disease. Similar to HSV and VZV, serology shows evidence of past rather than acute infection. CMV cases typically have diffuse keratic precipitates and marked intraocular pressure elevation at the time of the uveitis flares.[28] Diagnosis has been made by PCR of the aqueous. In some cases, there is response to oral valganciclovir hydrochloride. However, optimal duration of treatment is unknown. In some cases, recurrence of anterior uveitis has occurred while on therapy as well as soon after stopping the medication.[28,29] It is unclear whether local CMV reactivation is the primary cause of the uveitis or whether it reflects a secondary reactivation in response to another cause of the inflammation.[29]

Cytomegalovirus Retinitis

Although CMV may cause acute retinal necrosis in an immunocompromised host, it more typically causes a characteristic CMV retinitis. CMV retinitis affected more than 30% of patients with acquired immunodeficiency syndrome (AIDS) before highly active antiretroviral therapy (HAART), but now it is rare in countries where HAART is widely available. However, CMV was still the most common cause of ocular lesions in HIV-positive patients seen at a tertiary eye center in India from 1993 to 2010.[30] CMV retinitis is seen primarily in HIV-positive patients who are HAART-naïve or who have failed HAART; most have $CD4^+$ T-cell counts less than 50 cells/mm^3 (average 15 cells/mm^3 in one study).[31] CMV retinitis may also occur in other severely immunocompromised patients, such as organ transplant recipients. Patients with CMV retinitis usually present with painless loss of vision. Eye findings typically include white retinal infiltrates; retinal vasculitis, which may have a frosted branch angiitis pattern; and multiple retinal hemorrhages. An important clinical feature is the absence of significant vitreous inflammation. As a consequence, the view of the retina is usually clear. This is in contrast to ocular toxoplasmosis, in which vitritis is common. In one study, patients with HIV who developed CMV retinitis while on HAART had less severe disease, but more often bilateral eye involvement, than patients who were HAART-naïve.[30]

Dengue

Dengue is a flavivirus infection transmitted by mosquito bites, primarily *Aedes aegypti.* The four serotypes of dengue are widely distributed in tropical areas worldwide. Although conjunctival hemorrhage has long been recognized in dengue fever, uveitis is now increasingly being recognized and was reported in 137 of 1719 patients (7.9%) in one tertiary referral hospital.[32] Decreased vision is the most common symptom. Anterior, intermediate, and posterior uveitis may occur, with symptoms sometimes beginning months after the initial illness.[33] Macular edema or hemorrhage is the most common retinal manifestation. Involvement is bilateral in more than 70% of cases.[25] Optic neuritis also may occur. Iritis manifests as eye pain, redness, and photophobia. Prognosis for vision is good with anterior uveitis but less so in posterior segment disease.

Ebola Virus

Ebola virus disease (EVD) was first described in 1976 in central Africa, but the largest outbreak in history occurred in West Africa in 2014–16. The first case occurred December 2013, and the epidemic peaked in the fall of 2014. Greater than 28,600 cases and 11,300 deaths were reported—99.9% from Guinea, Sierra Leone, and Liberia[34]—and many of the 17,000 survivors experienced late sequelae during convalescence including uveitis. Onset of uveitis in EBV may occur weeks after recovery from the acute illness, and virus may persist in intraocular fluids for months. In 2015 a case of panuveitis developing 14 weeks after EVD onset (8 weeks after discharge from the hospital) was described, and aqueous fluid samples tested positive for Ebola virus by culture and reverse-transcription PCR.[35] The patient recovered vision after treatment

with corticosteroids and the oral antiviral favipiravir, but other EVD survivors lost vision from the uveitis. The incidence of uveitis and other ocular findings following EVD has been reported to range from 13% to 34%. A study of 96 EVD survivors in Liberia found that 22% developed EVD-associated uveitis, and 3% developed optic neuritis; 39% of eyes with uveitis had less than 20/400 vision.[36] Posterior uveitis was the most common type in this study (62% of uveitis cases). A prospective study of 341 EVD survivors in Guinea found that 14% developed uveitis, 2% developed episcleritis, and 1% developed interstitial keratitis; uveitis was most often unilateral (78%) and anterior (48%).[37] Relapses of uveitis in this study occurred up to 13 months after clearance of Ebola virus in the blood. The length of time that Ebola virus can persist in intraocular fluids has infection control implications for patients who require eye surgery. However, a study of 50 EBV survivors in Sierra Leone who required cataract surgery had no Ebola virus detected by reverse-transcription PCR in aqueous, vitreous, or conjunctival samples at a median of 19 months following EVD diagnosis.[38]

Herpetic Anterior Uveitis

Anterior uveitis due to HSV or VZV is often presumed in a patient who presents with anterior uveitis and has a history of an earlier episode of herpetic keratitis. Of the 10% of anterior uveitis cases that are due to infection, most are caused by HSV, primarily HSV type 1. The anterior uveitis represents reactivation of latent HSV infection, rather than new HSV infection, in nearly all cases. The patient is otherwise well at the time of anterior uveitis, without systemic illness, active cold sores, or other evidence of reactivation HSV infection aside from the uveitis. Most patients with HSV-related anterior uveitis have either a history of HSV keratitis or active corneal infection at the time of uveitis. HSV keratitis is common, and approximately 40% of patients with ocular herpetic disease have recurrent episodes of anterior uveitis.[39] Iritis in an eye with previous herpetic keratitis should be considered herpetic until proven otherwise.[40] Herpetic anterior uveitis is nearly always unilateral. Patients experience eye pain, redness, and photophobia. The cornea may appear cloudy, and slit-lamp examination may reveal interstitial keratitis typical of active recurrent HSV keratitis or corneal scars from prior episodes. Anterior chamber inflammation may be mild to severe, and there may be a hypopyon or keratic precipitates or both; keratic precipitates may be small, large, or stellate. Anterior uveitis due to HSV may occur in a patient with no history or findings suggesting herpetic keratitis, but this is thought to be uncommon.[41] In the absence of clinical evidence or history of HSV keratitis, some clues that may suggest herpetic anterior uveitis include unilateral disease, decreased corneal sensation, posterior synechiae, acute increase in intraocular pressure (from inflammation of the trabecular meshwork), and iris atrophy (patchy, sectoral, or diffuse).[42] VZV reactivation may cause a similar anterior uveitis. Sectoral iris atrophy is particularly suggestive of HSV or VZV as the etiology.[43] PCR studies of the aqueous in patients with no history of keratitis but recurrent episodes of anterior uveitis with sectoral iris atrophy found HSV in 83% and VZV in 13% of patients.[44]

Leprosy

More than half of patients affected by leprosy in a study from Nepal had ocular complications.[45] This was also the case in a study from India of 150 patients with leprosy, in which visual impairment was seen in 12.6% and blindness due to leprosy was seen in 3.3%.[46] Common eye findings included corneal scarring from corneal hypoesthesia and trichiasis and anterior uveitis (iridocyclitis). Most cases of ocular disease in this study and others have occurred in patients with multibacillary (lepromatous) disease of several years' duration. The uveitis in leprosy and its complications (glaucoma, cataract) are the primary cause of blindness from this disease. Uveitis is nearly always an iridocyclitis that is bilateral, chronic, and relapsing. In contrast to ocular TB, choroidal involvement is rarely seen.[47,48]

Leptospirosis

Leptospirosis is a common cause of uveitis in tropical countries and generally occurs during the immune phase of this biphasic illness. Uveitis may occur 2 days to 4 years after the initial systemic illness but usually occurs 3 to 6 months later.[49] An outbreak of uveitis involving 73 patients in southern India occurred in 1994, several months after heavy rains and flooding.[50] Uveitis was bilateral in half of the patients, and 96% had panuveitis. Nearly all patients had had a febrile illness consistent with leptospirosis 1 to 10 months earlier. Leptospiral DNA was detected by PCR in aqueous samples of 80% of patients.[51] Eye findings are typically either a nongranulomatous hypopyon uveitis or a panuveitis with marked vitritis and retinal vasculitis.[49] The retinal vasculitis affects primarily retinal veins. There may be optic disk papillitis and neuroretinitis with a macular star. Retinal and choroidal lesions are absent in leptospiral panuveitis, which helps to distinguish it from sarcoidosis, toxoplasmosis, and ARN. An analysis of 107 leptospiral uveitis cases from southern India found that several different *Leptospira interrogans* serovars were involved, but the most common was serovar Australis.[52] Some patients had infection with more than one serovar. Visual outcome did not seem to be influenced by the number or type of infecting serovars.

Lyme Disease

Uveitis rarely occurs in Lyme disease, but when it does the findings are protean. As with syphilis, ocular Lyme disease has been associated with anterior uveitis, intermediate uveitis, optic neuritis, neuroretinitis, retinal vasculitis, choroiditis, and panuveitis.[53] Uveitis is usually seen in the late stage of Lyme disease.[54] It may accompany Lyme arthritis. In a European prospective study of 84 patients with Lyme arthritis, 4% had ocular inflammation (keratitis, anterior uveitis, intermediate uveitis).[55] Intermediate uveitis, which rarely has an infectious etiology, may be the most common type of uveitis due to Lyme disease in Europe.[56] Establishing a causal relationship with Lyme disease in uveitis is complicated by the limitations of Lyme serology testing. In a retrospective study of 440 patients with optic neuritis in a hyperendemic area on Long Island, New York, Lyme disease was causal in only 1 of 28 patients with positive enzyme-linked immunosorbent assay (ELISA) tests for Lyme disease.[57] Of the remaining patients, 24 patients had a negative Lyme disease test by Western blot, and 3 had syphilis.

Presumed Ocular Histoplasmosis Syndrome

Presumed ocular histoplasmosis syndrome (POHS) is a syndrome characterized by multiple scars in the choroid *(histo spots)*, neovascularization, peripapillary atrophy, and clear vitreous and aqueous. POHS affects young and middle-aged adults and is a major cause of choroidal neovascularization in patients younger than 50 years of age. POHS causes permanent loss of vision in at least 2000 people in the United States annually and is associated with the HLA-DR15 (a subtype of HLA-DR2) and HLA-DQ6 haplotypes.[58] The etiology is unknown. Rare cases of fungal endophthalmitis have been seen in patients with disseminated histoplasmosis. A case report described acute bilateral chorioretinitis in a 16-year-old immunocompetent boy in Tennessee who developed acute disseminated histoplasmosis. Chorioretinitis did not respond to antifungal therapy alone but resolved after the eventual addition of corticosteroids. By 3 months after presentation, all chorioretinal lesions had resolved and no histo spots developed.[59] However, the association of *Histoplasma capsulatum* with POHS is presumptive and based primarily on epidemiologic grounds. In the United States, POHS is most prevalent in the Midwest, the region endemic for histoplasmosis. A study published in 1972 found that 4.4% of people living in an endemic area who tested positive by a histoplasmin skin test had ocular histo spots.[60] Other support for *H. capsulatum* as the etiology is lacking, and POHS has been found in areas where *H. capsulatum* is absent such as the Netherlands.[61]

Progressive Outer Retinal Necrosis

Progressive outer retinal necrosis (PORN) is a very rapidly progressive viral retinitis that involves the deeper ("outer") layers of the retina and occurs mainly in HIV-infected patients with low $CD4^+$ T-cell counts. It is rare in the United States and other countries in which HAART is widely available. Patients with HIV and PORN typically have $CD4^+$ counts less than 100 cells/mm^3 (average 20 cells/mm^3), but rare cases have been described in HIV-positive patients with $CD4^+$ T-cell counts

greater than 100 cells/mm^3.[62] A few cases of PORN have been described in other immunosuppressed patients such as organ transplant recipients.[63] Nearly all cases of PORN are due to VZV, although CMV and HSV have been described. Patients present with vision loss that may be unilateral or bilateral, and examination shows multiple peripheral lesions in the deep (outer) layers of the retina initially. These lesions rapidly coalesce to involve the full thickness of the retina. PORN resembles ARN but is distinguished from it clinically by the following three features: (1) involvement of the outer retina, (2) absence of any significant inflammation in the vitreous or aqueous humor, and (3) absence of involvement of the retinal vasculature. Optic neuropathy may precede PORN in rare cases.[64] The retinitis in PORN usually progresses rapidly despite systemic antiviral therapy, often resulting in blindness within days of presentation. A few successes have been achieved by a combination of systemic and intravitreal therapy (see "Therapy").

Syphilis

The incidence of ocular syphilis appears to be increasing, coincident with the rising incidence of syphilis.[65–67] Ocular syphilis may be the presenting feature of syphilis, especially in older adults.[68] Syphilis may involve the cornea as an interstitial keratitis or the sclera as a nodular scleritis.[69] Uveitis, however, is the most common manifestation of ocular syphilis and is often granulomatous. Syphilis may produce anterior uveitis, intermediate uveitis, posterior uveitis, or panuveitis. Syphilitic anterior uveitis is granulomatous in two-thirds of patients[70] and bilateral in half of patients. Interstitial keratitis, iris nodules, dilated iris vessels, and iris atrophy may be seen. The most common form of posterior uveitis is multifocal chorioretinitis, but other manifestations include focal chorioretinitis, pseudoretinitis pigmentosa, retinal necrosis, neuroretinitis, and optic neuritis. A pale optic nerve head from prior syphilitic optic neuritis may mimic glaucomatous optic atrophy. Chorioretinitis was the type of uveitis seen in 15 of 20 patients with syphilitic posterior uveitis in one review.[71] A specific type of focal chorioretinitis, acute posterior placoid chorioretinitis, has been described in syphilis for more than 20 years and is characterized by large, often solitary yellow lesions that are typically in the macula.[72] Retinal vasculitis may occur in ocular syphilis, and branch retinal vein occlusions have been described.[73]

Uveitis may occur in either congenital or acquired syphilis. Typical findings in congenital disease include interstitial keratitis and so-called salt-and-pepper fundi. Interstitial keratitis does not usually occur until the patient is a teenager or young adult. It may be accompanied by an anterior uveitis. The patient may have no other stigmata of congenital syphilis. Glaucoma may result from the inflammation. In acquired syphilis, onset of uveitis may occur in secondary or tertiary syphilis. The most common ocular finding in secondary syphilis is iritis, which accounts for more than 70% of eye findings.[73] Symptoms are often acute in onset. In contrast, when ocular syphilis develops in tertiary disease, patients often have slowly progressive decrease in vision as their only symptom. The eye findings are protean and include all of the previously listed findings. In contrast to patients with secondary disease, patients with tertiary disease are often middle-aged or older. They often have no knowledge of prior exposure to syphilis, which likely occurred decades earlier. The diagnosis may be missed if only the rapid plasma reagin (RPR) or VDRL test is checked because these tests are often negative in tertiary syphilis. In a series of 50 patients with a reactive fluorescent treponemal antibody absorption (FTA-ABS) test and eye findings consistent with active or inactive ocular syphilis (e.g., chorioretinitis, optic atrophy, iritis, interstitial keratitis), the average age was 59, and the VDRL test was reactive in only 24%.[74]

All patients with presumed ocular syphilis should have a lumbar puncture to exclude concomitant neurosyphilis, which may be present in 40% of patients.[74] Normal cerebrospinal fluid does not exclude ocular syphilis because ocular syphilis is frequently present without evidence of neurosyphilis. Patients who test positive for HIV have a higher rate of concurrent ocular syphilis and neurosyphilis.[73,75] All patients with ocular syphilis should be tested for HIV. In a study of 24 patients treated for ocular syphilis between 1998 and 2006, 11 patients were found to be HIV positive, and this was a new diagnosis in 7 patients.[76] HIV-positive patients are more likely than HIV-negative patients to have acute, bilateral uveitis with more extensive eye involvement (vitreous, retina, and optic nerve involvement simultaneously).[73,77]

It is likely that nearly all cases of uveitis and positive specific syphilis serologies represent infection with *Treponema pallidum* subsp. *pallidum*. It is possible, however, that some patients from areas endemic for yaws or bejel have positive serologies owing to exposure to these nonvenereal *T. pallidum* spp. in childhood but have uveitis from another etiology. Commercial laboratories often employ ELISA kits that use one or more cloned antigens or a lysate of the Nichols strain of *T. pallidum*. These kits vary in specificity and sensitivity, with false-positive results being common in low-risk populations. Additionally, some authors maintain that uveitis may be a late manifestation of yaws or bejel.[78–80] The eye findings they describe are similar to findings seen in ocular syphilis, and the diseases cannot be distinguished from syphilis serologically. The possible distinction would be important to the patient because of social implications, although it would not change therapy.

False-positive test results (e.g., low-titer RPR, rarely FTA-ABS) may also occur in patients with uveitis, especially as many have underlying rheumatologic conditions that increase the risk of a false-positive test. These are discussed later (see "Diagnosis").

Ocular Toxocariasis

Ocular toxocariasis usually affects children. It can be asymptomatic or cause unilateral decrease in vision. Although either dog *(Toxocara canis)* or cat *(Toxocara cati)* roundworms may cause ocular toxocariasis, *T. canis* is the more common etiology (see Chapter 290). A survey of ophthalmologists performed by the Centers for Disease Control and Prevention found that only 64% of patients with newly diagnosed ocular toxocariasis owned pets.[81] *Toxocara* infection occurs most commonly through ingestion of contaminated soil, and children exposed to playgrounds or sandboxes contaminated with dog or cat feces are at increased risk.[81]

There are three types of ocular manifestations: (1) peripheral chorioretinal granuloma (50% of cases), (2) posterior pole chorioretinal granuloma (25%), and (3) diffuse panuveitis (25%).[82,83] In nearly all cases, only one eye is involved. Diagnosis may be difficult, especially in panuveitis cases. Because the infection is confined to the eye, serology is often negative, and there is usually no peripheral eosinophilia or other signs of systemic parasitic infection. In addition, patients without ocular toxocariasis may have positive serologic tests due to incidental or past exposure to *Toxocara*. Data from the Third National Health and Nutrition Survey of a representative sample of the US population age 6 or older found that 13.9% were positive for *Toxocara* antibodies.[84] The diagnosis is often clinical, based on the appearance of the eye findings, particularly in an older child. A vitreous aspirate for evaluation of local *Toxocara* immunoglobulin G (IgG) compared with serum levels (Goldmann-Witmer coefficient) may be helpful in diagnosis.[85] However, vitreous aspiration is contraindicated in children in whom retinoblastoma is in the differential diagnosis.

In rare cases, ocular toxocariasis may be in the differential diagnosis of sporadic retinoblastoma. The distinction may be difficult, particularly in children older than 5 years of age in whom retinoblastoma is rare, but ocular toxocariasis is more common. All such cases should be evaluated by an experienced pediatric oncologic ophthalmologist because much of the distinction relies on the clinical examination. There are some clues in distinguishing retinoblastoma from ocular toxocariasis. The average age of unilateral cases of retinoblastoma is 2 years, and only 8% of retinoblastoma cases occur in children older than 5 years.[86] In contrast, ocular toxocariasis is typically seen in older children, with most cases occurring in children 4 to 8 years of age in some studies and even older in other studies. A retrospective study of ocular toxocariasis cases seen over 20 years in a uveitis center found that the average age was 16.5 years.[83] Another differentiating feature is that intraocular calcifications as seen on orbital ultrasound or computed tomography (CT) are characteristic of retinoblastoma but are not seen in ocular toxocariasis. Subtle findings on eye examination also help distinguish the entities. The presence of posterior synechiae and cataract argue against retinoblastoma and for chronic uveitis such as that caused by *Toxocara* or chronic endophthalmitis.[85] Both ocular

toxocariasis and retinoblastoma may have vitreous cells; however, these are WBCs in *Toxocara* infection. Shields and colleagues[86] at Wills Eye Hospital in Philadelphia described the vitreous cells in retinoblastoma as appearing different from WBCs—"soft and round, often appearing much larger and smoother in configuration than inflammatory cells." Diagnostic vitrectomy or aspirate must be avoided in cases of suspected retinoblastoma. Any instrumentation of the eye may spread the retinoblastoma to the orbit, and because of this risk, a child who undergoes vitreous aspirate for *Toxocara* diagnostic testing is committed to prophylactic chemotherapy if retinoblastoma is found instead. Orbital extension of retinoblastoma is the most significant factor predictive of eventual metastases and is associated with a very high mortality rate.[86]

Toxoplasmosis

Ocular toxoplasmosis is the most common infectious cause of posterior uveitis in the United States. Toxoplasmosis is a worldwide infection that may cause a majority of posterior uveitis cases in highly endemic areas such as Brazil and France. The characteristic funduscopic findings in ocular toxoplasmosis include a creamy yellow chorioretinal lesion adjacent to an old scar and marked vitreous inflammation. Ocular toxoplasmosis is discussed in detail in Chapter 290.

Tuberculosis

Ocular complications of systemic TB are rare. Ocular TB was diagnosed in only 1.4% of 10,524 patients seen in the eye clinic of a Boston sanatorium between 1940 and 1966.[87] A 1996 report of 1005 patients in India with active TB also found evidence of ocular disease in only 1.4%.[88] Ocular disease may be more common in HIV-positive patients with TB. A study of 100 patients hospitalized for TB in Madrid found presumed ocular TB in 24% of 45 HIV-positive patients compared with 13% in 55 HIV-negative patients.[89] Ocular TB was seen in 3.8% of HIV-positive patients with ocular lesions seen in a tertiary eye center in India; all had pulmonary TB as well. Patients presented with conjunctival involvement, subretinal abscess, choroidal tubercles, and panophthalmitis.

Ocular TB may manifest without evidence of systemic disease, so the diagnosis is often presumptive. Most patients have no history of TB, and half have normal chest radiographs.[90] Eye pathology is rarely available before vision is lost and the eye is enucleated. Biopsies of the uvea risk vision and are rarely performed. In a review of 40 cases with histologic evidence of intraocular TB reported between 1869 and 1991, only 1 case was proven by biopsy, and the rest were proven after loss of the eye.[91] Cultures of the aqueous or vitreous with rare exceptions are negative.[92] Diagnosis by PCR of aqueous or vitreous samples has been reported,[93,94] but the sensitivity and specificity of this test are unknown. Most reported PCR-positive cases have no culture confirmation, and many have no evidence of systemic disease.[92,93] A positive purified protein derivative (PPD) skin test or interferon-γ release assay blood test alone is of little help in determining if a patient with uveitis has ocular TB. In patients from TB-endemic countries, the incidence of latent TB is high (as is the incidence of active TB). It has been estimated that a patient with uveitis and a positive PPD test has only a 1% probability of having ocular TB.[95] Factors that make the diagnosis more likely include a history of TB or evidence of active disease, and the presence of eye findings typical of ocular TB such as multifocal choroiditis or granulomatous anterior uveitis.

Based on studies of patients with systemic TB and presumed ocular involvement, the spectrum of ocular findings has been described. TB can involve any part of the eye, but the most common finding is focal or multifocal choroiditis. Active or healed choroiditis was found in 78%[88] and 94%[89] of patients with systemic disease and presumed ocular TB in two reports. In earlier studies, choroidal involvement was seen in 30% to 40% of similar patients.[87] Choroidal *tubercles* are usually one-quarter to several disk diameters in size and are seen most often in the posterior pole rather than the periphery of the fundus. The number of lesions ranges from 1 to 60; however, most patients have fewer than 5.[91] One or both eyes may be involved. Lesions are yellow, white, or gray. In active disease, overlying vitreous inflammation may be present. Inactive choroidal lesions appear as scars. The appearance of choroiditis is not pathognomonic for TB, and similar lesions may be seen in sarcoidosis, syphilis, and, rarely, metastatic disease. The choroidal tubercles may coalesce and produce a type of uveitis called serpiginous-like choroiditis.[96] Chronic anterior uveitis, usually granulomatous (i.e., with mutton-fat keratic precipitates or iris nodules or both), is the next most common manifestation of ocular TB.[95] There may be an associated vitritis or choroiditis. A third common manifestation is retinal vasculitis or periphlebitis, often called Eales disease.[91] In 1880, Henry Eales, a British ophthalmologist, described recurrent retinal and vitreous hemorrhages in young men that he thought were immunologically mediated. The mechanism of Eales disease is still controversial, with some proponents arguing for direct infection of the retinal vessels and others for immune reaction to tuberculoprotein.[91] However, PCR studies of removed epiretinal membranes from eyes with Eales disease have demonstrated *Mycobacterium tuberculosis* genome in many cases, although cultures were negative.[97] Eales disease is most common in patients from India, Pakistan, and Afghanistan.[91] In India, 1 in 135 patients referred to a specialty eye center have Eales disease.[98] Typical features include vascular sheathing of retinal veins (periphlebitis), vitreous inflammation, and peripheral retinal capillary occlusion. Capillary occlusion leads to neovascularization and subsequent retinal hemorrhages (Fig. 115.5). The term *Eales disease* is sometimes used to refer to idiopathic occlusive retinal vasculitis rather than specifically to TB-related eye findings consistent with this. Whether most cases are associated with TB or respond to anti-TB therapy is unclear. A review of 500 patients with Eales disease seen in India in 1985–95 found that 95% were men, mean age was 30 years, 81% had bilateral eye involvement, and 97% had a positive Mantoux tuberculin skin test (of 360 patients tested).[99] The authors noted the high baseline prevalence of latent TB in India during the study period (70%–90%) and that only 15 patients of those evaluated by a pulmonary physician had evidence of active (nonocular) TB. Other manifestations of ocular TB include scleritis, interstitial keratitis, and optic neuritis.

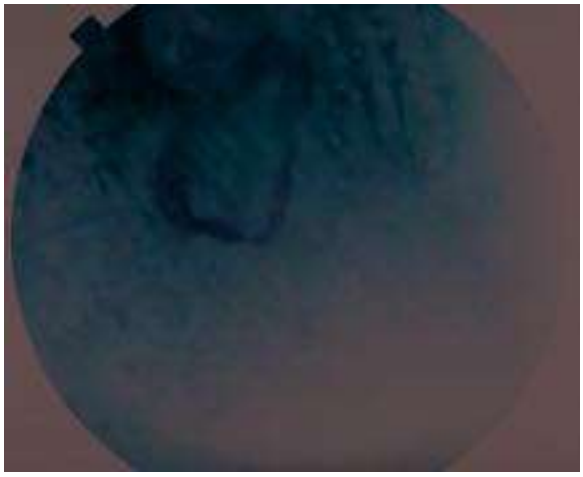

FIG. 115.5 Ocular tuberculosis. Retinal photograph showing signs of Eales disease in a patient from India: retinal neovascularization surrounded by a large yellow ring of fibrovascular proliferation in the vitreous. *(From Raizman MB, Haas JJ. Case 4: 1998. A 32-year-old man with vitreous hemorrhage and mediastinal lymphadenopathy. Case records of the Massachusetts General Hospital.* N Engl J Med. *1998;338:313–319.)*

West Nile Virus

The incidence of ocular involvement in West Nile virus (WNV) infection is unknown, but it may be common in patients with severe disease. The most common eye finding is multifocal chorioretinitis, and this is bilateral in nearly all cases. In an outbreak in Tunisia in 2003 involving 29 patients hospitalized with severe WNV, 80% had multifocal chorioretinitis, although 65% had no eye symptoms.[100] Retinal vasculitis was also common. Onset of lesions occurred 3 to 30 days after onset of acute illness. The ocular disease was self-limited, and chorioretinal lesions evolved into scars that resembled laser scars. A report of seven patients with WNV in the United States also found that bilateral multifocal

chorioretinitis was the most common finding, occurring in 86% of eyes.[101] Other findings included retinal hemorrhages, chorioretinal streaks, vitritis, retinal vasculitis, and optic atrophy. A majority of patients in both studies were diabetic. Outcomes were good in most cases, although the few patients with optic atrophy or occlusive retinal vasculitis had permanent loss of vision.

WNV may also produce retinal findings in some birds. A study of 13 hawks with naturally acquired WNV infection found that all had similar funduscopic findings, with exudative chorioretinal lesions or scars in a linear or geographic pattern.[102]

Whipple Disease

Whipple disease is rare, and ocular findings are even more so. Bilateral granulomatous panuveitis in one patient has been described. This patient had iris and retinal nodules as well as vitritis and a chronic course over 4 years before diagnosis.[103] A case of Whipple disease in a patient with a long-standing renal transplant included findings of bilateral vitritis and chorioretinitis.[104] A review of 11 patients with ocular Whipple disease found that chronic uveitis was the most common manifestation, affecting 9 patients, whereas 1 patient had isolated bilateral disk swelling and 1 had Parinaud syndrome.[105]

Zika Virus

Zika is a flavivirus infection transmitted by mosquito bites, largely *A. aegypti*, and was originally seen in Africa and Asia. Zika was first described in the Western Hemisphere in 2015, when an epidemic began in Brazil, rapidly spreading throughout the country. Zika is currently a risk in multiple parts of the world including Central and South America, Mexico, the Caribbean, and parts of Asia and Africa (see Chapter 153). Affected adults may experience a nonpurulent conjunctivitis or anterior uveitis,[106] whereas infants infected in utero may have a broad spectrum of retinal and optic disk findings.[25] Retinal findings are very rare in adults. An eye center in Florida reported bilateral chorioretinitis (diffuse, confluent, placoid, and multifocal lesions) in an immunocompromised woman from Puerto Rico who was undergoing chemotherapy for lymphoma.[106] The chorioretinal lesions spontaneously resolved over the following 5 months.

ENDOPHTHALMITIS MIMICKING UVEITIS

Endophthalmitis usually manifests acutely, but two types that usually have a subacute or chronic presentation may mimic, and be initially misdiagnosed as, uveitis. These are discussed next and detailed in Chapter 114.

Chronic Postcataract Endophthalmitis

Patients with a history of several months of unilateral anterior chamber inflammation following cataract surgery in the same eye should be suspected of having chronic pseudophakic endophthalmitis rather than anterior uveitis. Nearly all such cases are due to *Propionibacterium acnes*. Clues to the diagnosis include the history of onset after cataract surgery and the finding on eye examination of white plaques in the posterior lens capsule. Aqueous or vitreous cultures are usually negative. Chronic postoperative endophthalmitis may also be due to fungi, as discussed next.

Chronic Endophthalmitis Due to Fungi

By convention, intraocular inflammation due to fungi is considered endophthalmitis rather than uveitis. Chronic endophthalmitis due to fungi may occur postoperatively or following seeding of the eye during occult fungemia (endogenous fungal endophthalmitis) and may be initially mistaken for a noninfectious uveitis. Examination of the eye may reveal findings that suggest the diagnosis of fungal endophthalmitis, however. These findings include a "clumped" appearance of inflammation in the aqueous humor in post-cataract surgery cases, for example, or fluffy white lesions in the vitreous or on the retina in endogenous endophthalmitis cases. In cases due to occult fungemia, the patient's medical history also may provide clues that suggest a fungal etiology, such as risk factors for candidemia (e.g., intravenous drug abuse or a recent indwelling central venous catheter) or risk factors for fungemia due to molds (e.g., immunocompromise from organ transplantation or chemotherapy, or recent intravenous drug abuse). Fungal endophthalmitis is discussed further in Chapter 114.

DIAGNOSIS OF INFECTIOUS UVEITIS

Patients with infectious uveitis often present a diagnostic dilemma to ophthalmologists and infectious diseases physicians. The diagnosis is nearly always made clinically, primarily on the basis of the eye examination by the ophthalmologist. Ophthalmologists do not need to refer cases with typical eye findings (e.g., classic *Toxoplasma* chorioretinitis) to infectious diseases physicians, so the infectious disease specialist sees only cases that have atypical features or are rapidly progressive. The diagnosis often cannot be proven by studies on the aqueous or vitreous, and these are the parts of the eye that can be sampled relatively safely, although vitreous sampling has some risk.

Approach to the Patient With Uveitis

An infectious diseases physician consulting on a case of uveitis should (1) obtain a copy of the ophthalmologist's notes for review (see Chapter 111 for help with interpretation of these notes); (2) determine what anatomic category of uveitis is involved—that is, anterior, intermediate, posterior, or panuveitis; and (3) ask the ophthalmologist what his or her differential diagnosis is based on the findings of the eye examination alone. The anatomic category limits the likely infectious and noninfectious etiologies, as does the pace of the infection. The anatomic category is determined by the ophthalmologist based on the site of greatest inflammation.

Pathology of vitreous samples is important in establishing a diagnosis of ocular lymphoma, and intraocular cultures are essential in cases of "uveitis" due to chronic *Candida* or *P. acnes* endophthalmitis. Molecular diagnostic techniques such as PCR have been helpful in viral retinitis, but the sensitivity appears to be low in other types of uveitis. The technique for sampling aqueous or vitreous is discussed in Chapter 111.

PCR has proven most useful in herpetic uveitis cases, such as herpetic anterior uveitis and ARN. The aqueous or vitreous may be tested for HSV, VZV, or CMV. Yamamoto and coworkers[107] found that PCR tests of aqueous samples from 7 patients with recurrent iridocyclitis of suspected viral etiology were positive for either HSV (6 samples) or VZV (1 sample), whereas samples from 17 control eyes were negative. A retrospective study from Florida of 53 patients with anterior uveitis found PCR of the aqueous humor to be positive for HSV in 8% (4 of 49), VZV in 3% (1 of 35), and CMV in 2% (1 of 47).[108] In a study of 28 patients with ARN, PCR was positive in 27 (96%) for HSV, VZV, or CMV.[109] Another study using PCR of aqueous in ARN or PORN diagnosed these viruses in 86% of cases.[110] A study of 433 PCR tests of 133 patients with possible infectious chorioretinitis, analyzed for HSV, VZV, CMV, EBV, and toxoplasmosis, identified 81% of the 95 patients with a final clinical diagnosis of infectious uveitis.[111]

The sensitivity and specificity of aqueous or vitreous PCR for most other types of uveitis are not yet known, although sensitivity appears to be low enough that a particular etiology should not be excluded from the differential diagnosis based on a negative PCR result. The gold standard is usually clinical diagnosis or response to therapy or both because most agents of uveitis cannot be cultured. In a study from France that included 55 patients with clinically diagnosed ocular toxoplasmosis who also underwent vitreous sampling, the sensitivity of PCR was only 27%, although specificity was 100%.[112] Similarly, in a series of 9 patients with ocular Whipple disease who underwent vitreous sampling, all had periodic acid–Schiff–positive macrophages, but the sensitivity of vitreous PCR was only 60% (3 of 5 tested).[107] For ocular TB, both false-positive and false-negative PCR results have been described. Gupta and colleagues[94] reported that 10 of 17 patients (60%) with presumed ocular TB had positive aqueous PCR assays for *M. tuberculosis,* with the clinical diagnosis based on a positive PPD test or abnormal chest radiograph or both plus the absence of another uveitis diagnosis. However, PCR was also positive in 23% of the control group of patients with uveitis who had a negative PPD test and normal chest radiograph. False-negative PCR tests may also occur in some PCR assays because aqueous and vitreous specimens may contain inhibitors.[113]

Positive serologic tests such as IgG antibodies to *Toxoplasma,* HSV, VZV, or CMV also may be of little help given the high prevalence of

such antibodies in the general population. However, negative serologies to these pathogens may be helpful and may effectively exclude these diagnoses in the appropriate clinical setting. A specific treponemal test for syphilis (e.g., *T. pallidum* particle agglutination or FTA-ABS test) should be ordered on all patients with uveitis. If this test is positive and eye findings are consistent with ocular syphilis, the patient should be treated for ocular syphilis with high-dose intravenous penicillin. It is possible that the positive specific test for syphilis represents an incidental finding and that the uveitis has another etiology, but patients require treatment for the possibility of syphilitic uveitis because it is nearly impossible to exclude. Syphilis can produce almost any ocular manifestation.[114] Such patients should also have a lumbar puncture, but a normal cerebrospinal fluid profile will not exclude the diagnosis of ocular syphilis. The *T. pallidum* particle agglutination assay (TPPA) is preferred because the FTA-ABS test may be falsely positive in some patients, especially patients with rheumatologic conditions.[115] RPR also should be checked, although false-positive tests are common in autoimmune conditions, and all positive RPR tests must be confirmed with a specific treponemal test. A negative RPR test does not exclude ocular syphilis because it may be negative in tertiary syphilis; these RPR-negative, presumed ocular syphilis cases typically have an indolent presentation. Fulminant cases of syphilitic uveitis, characterized by marked intraocular inflammation, are nearly always due to secondary syphilis, and in these cases, the RPR titer is usually high. Treatment for presumed syphilis should be started immediately in these fulminant cases and not delayed until confirmatory specific treponemal test results return.

For both medical and social reasons, it is important to determine that a positive syphilis serologic test is not falsely positive. Although it is well known that nontreponemal tests such as RPR may be falsely positive in a variety of patient populations, the FTA-ABS test may be falsely positive as well, particularly in patients with rheumatologic conditions. A prospective cohort study of 50 patients with rheumatologic conditions and at least one serologic test positive for syphilis found that the specificity of the FTA-ABS test was only 68% in this population.[115] In addition, FTA-ABS may be falsely positive in patients with Lyme disease. ELISA tests for syphilis have also been plagued with false-positive results as well as variability among laboratories. Commercial laboratories often use ELISA kits that employ one or more cloned antigens or a lysate of the Nichols strain of *T. pallidum*. These kits vary a great deal in specificity and sensitivity. Any patient with uveitis and a positive FTA-ABS test should have confirmation with a *T. pallidum* particle agglutination assay (TPPA) or enzyme immunoassay. Because results from these confirmatory tests may take more than a week, therapy for possible ocular syphilis should be started as soon as the diagnosis is suspected if delay in therapy could compromise vision.

Radiologic studies of the eye or orbit are sometimes helpful in uveitis, but primarily for evaluation of noninfectious etiologies. Magnetic resonance imaging of the brain and orbit may be helpful in suspected cases of ocular-CNS lymphoma if brain lesions are found, although eye disease may precede CNS lesions by months. Fluorescein angiography of the eye may show retinal vascular patterns consistent with certain diseases such as viral-induced vasculitis in CMV. Indocyanine green angiography of the eye was found to be useful in detecting and following subclinical choroidal lesions in eight patients with presumed ocular TB.[116] Chest CT may be helpful in distinguishing sarcoidosis from TB in patients with granulomatous uveitis because these diseases often produce similar eye findings. In a study of 50 patients with uveitis suspected to have ocular sarcoidosis, CT was positive for sarcoidosis in 20%, although none of the patients had pulmonary symptoms.[117]

THERAPY

Acute anterior uveitis due to HSV is treated with topical corticosteroids and oral acyclovir or valacyclovir. Long-term prophylactic oral acyclovir (400 mg twice daily) seems to be beneficial in preventing recurrences of herpetic stromal keratitis[118] and anterior uveitis.[119]

ARN due to HSV or VZV is usually treated with high-dose intravenous acyclovir (10 mg/kg every 8 hours in patients with normal renal function) for 1 to 2 weeks followed by valacyclovir or famciclovir for 6 weeks to several months.[11,120] The optimal duration is unknown. For severe cases, intravenous acyclovir for 1 to 2 weeks followed by valacyclovir, 1 to 2 g every 8 hours, for several weeks is often given and may be followed by several months of oral acyclovir, 400 mg twice daily. Intravitreal injections of foscarnet are also typically given in severe cases. More recently, oral valacyclovir usually given at 2 g every 8 hours (twice the standard dose) has been favored by many ophthalmologists in initial treatment of ARN. A retrospective review of 62 patients with ARN (90% unilateral) treated between 1992 and 2016 at a specialty hospital in London with either intravenous acyclovir (33 patients) or oral valganciclovir (29 patients) found no difference in visual outcome, although only one-third of patients had a good visual outcome.[119] In immunocompromised patients presenting with ARN, CMV could be an etiology, and treatment with intravenous ganciclovir should be given instead of acyclovir until or unless CMV can be excluded (e.g., seronegative patients). Retinitis can progress rapidly (within 24 hours) in ARN, and the goal of initial therapy in ARN is to halt progression of retinitis and prevent involvement of the other eye. The infectious diseases physician should work with the retina specialist on a daily basis to determine if retinitis is progressing despite intravenous acyclovir. If retinitis is worsening despite therapy, an empirical switch to intravenous foscarnet should be considered as well as intravitreal injections of foscarnet. Cases of ARN in immunocompetent hosts that progressed despite intravenous acyclovir have responded to combination therapy with intravitreal ganciclovir or foscarnet injections plus systemic ganciclovir, foscarnet, or cidofovir.[121–123] Repeated intravitreal foscarnet injections may be required. Intravitreal foscarnet may reduce the rate of retinal detachment, a common complication of ARN, particularly VZV ARN.[124] VZV ARN tends to be more severe and progress more rapidly than HSV ARN.[124] For any type of ARN, because retinitis may occur in the second eye several months after onset of ARN in the first eye, oral antiviral therapy (e.g., acyclovir, valacyclovir, famciclovir) is often continued for several months following initial intravenous therapy. For immunosuppressed patients, suppressive antiviral therapy should probably be given for the duration of immunosuppression, which may be indefinite in many cases.

PORN has a dismal outcome despite therapy in most cases. In countries where resources are limited, treatment may be especially difficult. A center in South Africa used outpatient treatment with repeated intravitreal injections of ganciclovir over weeks to months (median, 12 injections) in 50 eyes of HIV-positive patients with PORN, and only 13% of eyes lost all vision (no light perception) compared with 60% in earlier published studies.[125] The best outcomes have been reported in case reports and small series using intravitreal and systemic antiviral therapies. The importance of starting antiretroviral therapy in patients not already receiving this has also been emphasized. There have been successes with repeated intravitreal injections with foscarnet and ganciclovir, in addition to prolonged combination intravenous therapy with these agents and the initiation of antiretroviral therapy in HIV-positive patients. In one patient with bilateral PORN, vision was lost in one eye, but an aggressive treatment led to visual recovery in the other eye.[126] This treatment included intravenous foscarnet and ganciclovir for 7 months and concurrent nearly twice-weekly intravitreal injections with foscarnet, 1.2 mg/0.05 mL, and ganciclovir, 2 mg/0.05 mL (total 58 injections over 6 months). Antiretroviral therapy was also initiated, and anti-VZV therapy was stopped when $CD4^+$ T-cell count increased to 100 cells/mm^3. The patient maintained 20/20 vision in the treated eye 2 years later.

Ocular syphilis should be treated the same as neurosyphilis, with 10 to 14 days of intravenous penicillin (3 to 4 million U every 4 hours in patients with normal renal function). A short course of systemic corticosteroids (e.g., oral prednisone, 60–80 mg daily) may be required during the initial few days of penicillin therapy to decrease intraocular inflammation rapidly in patients presenting with marked intraocular inflammation. Many experts follow intravenous penicillin with injections of 2.4 million U of intramuscular benzathine penicillin once weekly for 3 weeks. All patients with presumed ocular syphilis should be tested for HIV. All patients should have a lumbar puncture to exclude concurrent neurosyphilis before starting intravenous penicillin, if possible. However, in acute cases with vision-threatening uveitis, therapy should not be delayed if a lumbar puncture cannot be performed in a timely way. The reason for performing a lumbar puncture is to determine if there is

concurrent neurosyphilis (i.e., with CNS involvement) because such patients will require a follow-up lumbar puncture at 6 months to document resolution of infection.

Ocular TB should be treated with the same medications and duration of therapy as TB meningitis, although ethambutol is avoided if possible because of potential ocular toxicity. A short course of systemic corticosteroids may be necessary initially if there is sight-threatening inflammation.

The treatment of CMV retinitis is discussed in Chapter 137, the treatment of ocular toxoplasmosis is discussed in Chapter 278, and the treatment of ocular toxocariasis is discussed in Chapter 290. Treatment of Lyme uveitis should be the same as for neuroborreliosis, preferably with intravenous ceftriaxone. Chronic endophthalmitis due to *P. acnes* or *Candida* is discussed in Chapter 114.

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116 Periocular Infections

Marlene L. Durand

SHORT VIEW SUMMARY

Definition
- Periocular infections include infections of the eyelids, lacrimal system, and orbit.
- Eyelid infections include hordeolum, stye, blepharitis, and superinfected chalazion.
- Lacrimal system infections include dacryoadenitis (infection of the lacrimal gland), canaliculitis (infection of the canaliculi that collect tears in the medial canthus and drain into the lacrimal sac), and dacryocystitis (infection of the lacrimal sac).
- Preseptal cellulitis involves the soft tissues in front of the orbital septum. The orbital septum comprises fibrous tissue that extends from the orbital rim into the tarsal plate of the lids.
- Orbital infections include orbital cellulitis, subperiosteal abscess, and orbital abscess. These may lead to cavernous sinus thrombophlebitis.

Microbiology
- Lid infections: *Staphylococcus aureus,* streptococci
- Dacryoadenitis: In acute cases, *S. aureus,* streptococci, rarely *Haemophilus influenzae,* Epstein-Barr virus, and herpes zoster. Most chronic cases are noninfectious, but *Mycobacterium tuberculosis* is an infectious cause.
- Canaliculitis: *Actinomyces israelii,* also staphylococci, streptococci in primary infections; in patients with punctal plugs, unusual organisms (e.g., *Pseudomonas, Mycobacterium chelonae*) may be the pathogen.
- Dacryocystitis: *S. aureus,* streptococci; also gram-negative bacilli, anaerobes
- Preseptal cellulitis: *S. aureus* or streptococci in localized infections. Preseptal cellulitis rarely may result from bacteremia due to *H. influenzae,* group A streptococci, or *Streptococcus pneumoniae.*
- Orbital infections: *S. aureus,* including methicillin-resistant *S. aureus, Streptococcus anginosus (milleri),* other streptococci (including *S. pneumoniae), H. influenzae,* other gram-negative bacilli, anaerobes. Mixed infections are common.

Diagnosis
- Clinical examination in lid infections and lacrimal system infections.
- Preseptal cellulitis must be distinguished from orbital infections (orbital cellulitis, subperiosteal and orbital abscess). Orbital infections almost always have one or more of the following "orbital" findings: proptosis, which may not be grossly apparent but can be measured as 2 mm or more difference in the Hertel exophthalmometer measurements; ophthalmoplegia; and decreased vision. Preseptal cellulitis has none of these features, only lid edema and erythema. Computed tomography (CT) scan should be performed on any patient with orbital findings. CT should be considered in children who present with what appears to be severe preseptal cellulitis because they may have subperiosteal abscess (see text).

Therapy
- Varies by diagnosis; see text.
- Orbital infections are much more serious than preseptal cellulitis, and all orbital infections must be treated with intravenous antibiotics. Subperiosteal abscesses usually require surgical drainage, and orbital abscesses almost always do. Drainage of an adjacent infected sinus may be indicated.

Periocular infections include infections of the eyelids, lacrimal system, and orbital soft tissues that surround the globe of the eye. These infections may affect vision if not recognized and treated appropriately.

EYELID INFECTIONS

Anatomy

Each eyelid contains a fibrous tarsal plate that gives the lid its firmness (Fig. 116.1). Within each tarsal plate are 20 to 25 meibomian (or tarsal) glands (Fig. 116.2). These glands may be seen as faint yellow lines on the inner surface of the everted lid, extending perpendicular to the lid margin. Meibomian glands are sebaceous glands that secrete sebum, an oily substance. Sebum prevents the tear film from evaporating too quickly from the ocular surface. At the lid margin, adjacent to the eyelash follicles, are smaller sebaceous glands called *glands of Zeis.*

Hordeolum

A hordeolum is an acute infection of a sebaceous gland of the lid, usually caused by *Staphylococcus aureus.* An internal hordeolum is an infection of a meibomian gland, and patients present with lid swelling, erythema, and tenderness. Internal hordeola may point toward either the skin or the conjunctival surface. An external hordeolum (stye) is an infection of a gland of Zeis, and patients present with a painful pustule that points to the lid margin. Internal and external hordeola usually respond to frequent warm compresses. Topical bacitracin or erythromycin ointment may be used at night. Incision and drainage of the lesion is rarely required. There have been no randomized studies of the various medical treatments commonly prescribed for acute internal hordeola (e.g., warm compresses, antibiotics, lid scrubs), so the effectiveness of various treatments is unknown.[1] Methicillin-resistant *Staphylococcus aureus* (MRSA) may play a role in some cases.[2]

Chalazion

A chalazion is a sterile granulomatous reaction to inspissated sebum within an obstructed meibomian gland. It may result from an internal hordeolum or arise de novo.[3] Patients present with a nontender nodule within the lid that points to the conjunctival surface. Chalazia may become large and press on the ocular surface, distorting vision. Most chalazia resolve within 1 month, but recurrences are common in patients with chronic blepharitis. A randomized prospective trial treating patients with primary chalazia that had persisted for at least 1 month, despite conservative measures, found that intralesional triamcinolone injection was as effective as incision and curettage.[4] A three-way randomized trial comparing 4 to 6 weeks of hot compresses alone versus hot compresses plus either topical tobramycin or topical tobramycin-dexamethasone found that complete resolution occurred in only 18% of patients, and there was no significant difference between the groups.[5] Lesions with a lower pretreatment duration were more likely to resolve completely. Acute superinfection of a chalazion may occur, which should be treated with antibiotics (Fig. 116.3). Persistent or recurrent chalazia should be biopsied to exclude sebaceous cell carcinoma of the lid.

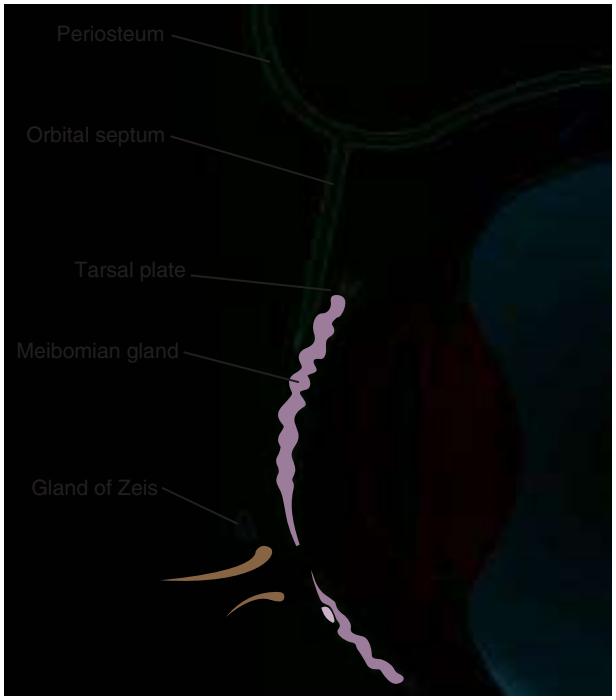

FIG. 116.1 Diagram of the anterior portion of the eye and orbit, illustrating the orbital septum and tarsal plate of the eyelid. Infections anterior to the orbital septum are described as preseptal, whereas infections posterior to the septum are considered orbital. Meibomian glands lie within the tarsal plate.

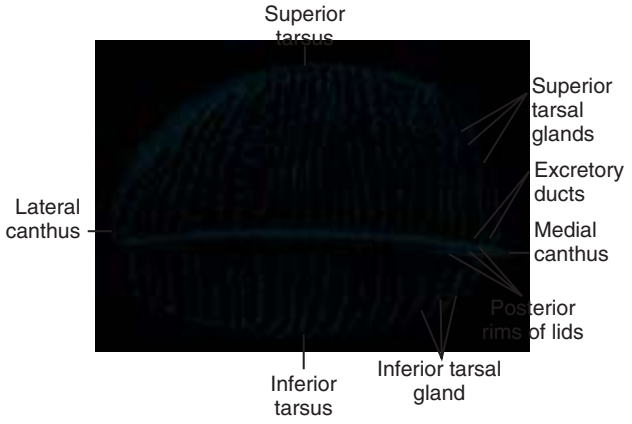

FIG. 116.2 The meibomian glands. *(From Warwick RE.* Wolff's Anatomy of the Eye and Orbit. *Philadelphia: Saunders; 1976.)*



FIG. 116.3 Superinfected chalazion.

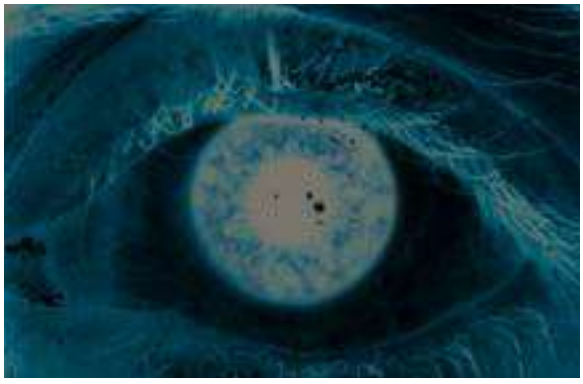

FIG. 116.4 Photograph of eyelids with marginal blepharitis.

Blepharitis

Blepharitis (Fig. 116.4) is an inflammation of the eyelids, usually involving the lid margins. It is one of the most common conditions seen by ophthalmologists. Blepharitis is usually chronic and may lead to ocular surface disease, such as chronic conjunctivitis, functional tear deficiency, and corneal inflammation or infection (keratitis). The American Academy of Ophthalmology classifies blepharitis as either anterior or posterior, with anterior blepharitis involving the lid skin, base of eyelashes, and eyelash follicles, whereas posterior blepharitis involves the meibomian glands.[6] Anterior blepharitis is further categorized as either "staphylococcal" or seborrheic. There is significant overlap in symptoms between categories. Patients with scaling, crusting, erythema of lid margins are considered to have staphylococcal blepharitis, although evidence for a role of staphylococci is minimal, and supporting studies are older than 30 years.[6] Patients with seborrheic blepharitis have greasy scaling of the eyelids and usually also of eyebrows and scalp. Patients with meibomian gland dysfunction typically have thickened lid margins with pouting or plugged meibomian orifices, and the meibomian secretions expressed by gentle massage of the lids ranges from turbid fluid to thick, cheesy material.[6] Rosacea and seborrheic dermatitis are often present in patients with meibomian gland dysfunction. Treatment of chronic blepharitis is usually with twice-daily gentle eyelid scrubs using either diluted baby shampoo or a dedicated commercial eyelid cleanser; a recent study found the latter to be superior.[7] A topical antibiotic ointment (e.g., bacitracin) applied to the lid margins may be helpful in cases with acute inflammation. Oral tetracycline may be helpful if there is associated rosacea. Cultures of eyelid margins are not routinely performed but may be useful in some cases because, rarely, cases may be due to unexpected bacterial or fungal pathogens.[8] Phthiriasis palpebrarum is infestation of the eyelashes by crab lice. Patients have pruritus of the lid margins and blepharoconjunctivitis and are often initially misdiagnosed as having an allergic or atopic condition. The lice, which burrow into the lid margin, may be difficult to see.[9] Herpes simplex blepharitis has been described in adults and children, may be recurrent, and is occasionally bilateral.[10,11] Blastomycosis is a rare cause of a granulomatous blepharitis (Fig. 116.5). *Demodex* mites are common ectoparasites of the skin and may infest the lid margins; infestation is associated with cylindrical dandruff around the lashes. *Demodex folliculorum* can be found in the lash follicle and *Demodex brevis* in sebaceous and meibomian glands.[12] The possible association between *Demodex* and chronic blepharitis was first postulated 50 years ago.[13] Studies of topical treatments have found that lid scrubs with tea tree oil (from the leaf of the tree *Melaleuca alternifolia*) are effective in eradicating *Demodex,*[14] and such scrubs have effectively treated some cases of chronic blepharitis.[12] A case of chronic blepharitis that mimicked sebaceous gland carcinoma

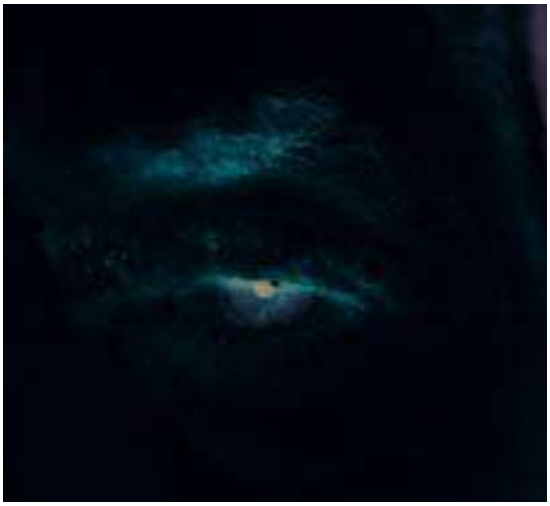

FIG. 116.5 Blastomycosis involving the eyelid in an otherwise healthy man with a normal chest radiograph. *(Courtesy Dr. John Bennett.)*

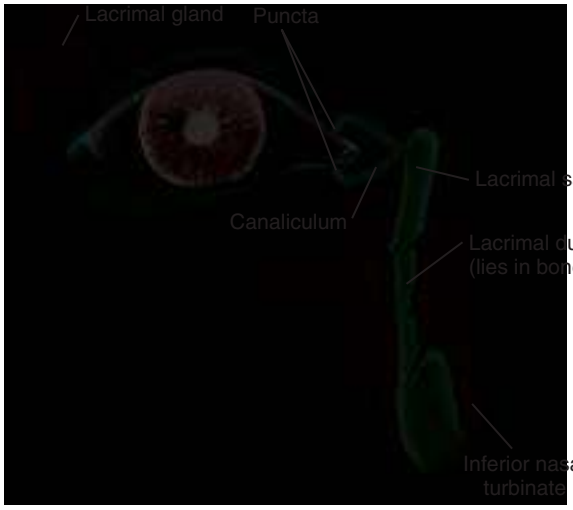

FIG. 116.6 The lacrimal system.

was found to be due to *Demodex* on lid biopsy and responded to tea tree oil lid scrubs.[15]

INFECTIONS OF THE LACRIMAL SYSTEM

Anatomy

The lacrimal gland is located beneath the upper outer orbital rim (Fig. 116.6). It produces tears that flow across the eye and then drain through the puncta, canaliculi, lacrimal sac, and lacrimal duct into the nasal cavity. The only parts of the lacrimal system that are visible on examination are the puncta and sometimes the lacrimal gland. The size of the lacrimal gland varies, but a portion may be visible in some patients when the upper lid is everted and the patient looks down and in. The gland then appears as a pink mass under the conjunctiva, just under the lateral part of the upper orbital rim.

Dacryoadenitis

Dacryoadenitis is an inflammation of the lacrimal gland. Infections are rare and may be acute or chronic. Patients with acute dacryoadenitis present with a tender area of erythema and swelling in the lateral part of the upper lid. Acute dacryoadenitis may lead to preseptal or orbital cellulitis or may suppurate into an abscess[16]; *S. aureus* and streptococci are common pathogens. A series of 11 cases reported *S. aureus* in more than half (6 cases), followed by *Streptococcus pneumoniae* (2), mixed skin flora (2), and *Haemophilus influenzae* (1).[17] There are case reports of acute suppurative dacryoadenitis due to *Pseudomonas,* brucellosis, and cysticercosis.[18–20] Epstein-Barr virus may cause acute nonsuppurative dacryoadenitis in mononucleosis, which may be unilateral or bilateral.[21,22] Herpes zoster has been the cause of acute dacryoadenitis that preceded (by 2 days) the onset of a vesicular rash in the first division of the trigeminal nerve.[23,24] A study found that dacryoadenitis was present in one-third of patients with *Acanthamoeba* keratitis, although direct infection of the lacrimal gland was not found.[25] Chronic infectious dacryoadenitis is rare, but most reports describe *Mycobacterium tuberculosis* as the cause.[26,27] Most cases of chronic dacryoadenitis are inflammatory rather than infectious, however. Sjögren disease and sarcoidosis are the most common associated diseases, although cases of Crohn's disease and granulomatosis with polyangiitis presenting as chronic dacryoadenitis have been described.[28,29] Granulomatous inflammation of bilateral lacrimal glands was seen in one patient receiving interferon-α and ribavirin for hepatitis C; evaluation for sarcoidosis was negative.[30] Thyroid orbitopathy may rarely present with lacrimal gland enlargement.[31] Tumors are an important cause of lacrimal gland enlargement, and dacryoadenitis has been a presenting sign in rare cases of lymphoma and leukemia.[32]

Canaliculitis

Canaliculitis may occur spontaneously or develop after placement of a silicone or temperature-sensitive acrylic polymer punctal plug in the canaliculi. These plugs are used to treat dry eyes, and canaliculitis results in approximately 8% of patients with long-term follow-up.[33] Symptoms of canaliculitis include tearing and irritation in the medial portion of the affected eyelid, and examination reveals a swollen, "pouting" punctum and erythema of the adjacent nasal conjunctiva. There may be a unilateral discharge and conjunctivitis. The lower canaliculus is affected more often than the upper. A yellow-green exudate and yellowish concretions may be expressed from the involved punctum in many cases. The concretions, called *sulfur granules,* are formed by *Actinomyces israelii,* the organism in the majority of cases.[34] A literature review of 188 reported cases found that the major organisms were *Actinomyces* (30%), streptococci (12%), and staphylococci (10%).[35] The most common pathogen may differ depending on region and whether the canaliculitis is primary or secondary to punctal plugs. In a series from India of 74 patients with primary canaliculitis, staphylococci were the major organisms (39%),[36] whereas in a series from Taiwan of 76 patients with canaliculitis, streptococci (28%) were most common in primary canaliculitis and *Pseudomonas* (46%) in plug-related canaliculitis.[37] Various other organisms have been reported, including *Mycobacterium chelonae* (in several plug-related infections),[38] *Eikenella corrodens,* and *Streptococcus anginosus (milleri)* group,[39] *Arcanobacterium haemolyticum,*[40] *Citrobacter freundii,*[41] *Aggregatibacter aphrophylus*,[42] and *Nocardia asteroides*. Treatment of canaliculitis requires removal of canalicular material and concretions and removal of a punctal plug if present. After this, topical antibiotics are usually given.[37] On occasion, surgical exploration is required.

Dacryocystitis

Dacryocystitis, or inflammation of the lacrimal sac, is the most common infection of the lacrimal system. It arises because of obstruction of the lacrimal duct, pooling of tears in the lacrimal sac, and subsequent infection. Obstruction may be congenital or may result from trauma, tumors, infection, or inflammation of the duct. Acute dacryocystitis symptoms include pain, swelling, and erythema near the nasal corner of the eye (Fig. 116.7). There is usually epiphora (excessive tearing) and a purulent discharge. Infants often have lacrimal duct obstruction with epiphora, but acute dacryocystitis rarely complicates the obstruction. The most common causes of acute dacryocystitis are *S. aureus* and streptococci, but gram-negative bacilli also play a role. One series of 137 patients found that *Pseudomonas* (12%) was the second most common pathogen after *S. aureus* (30%).[43] Treatment requires antibiotic therapy (e.g., ampicillin-sulbactam) and usually incision and drainage of a lacrimal sac abscess. In one study, incision and drainage was an outpatient procedure requiring only local anesthesia in approximately 80% of cases.[44] A repeat drainage procedure was required within 1 month in 8%. Chronic or recurrent dacryocystitis usually requires a surgical procedure, dacryocystorhinostomy (DCR). Cultures taken when purulence was found at the time of DCR surgery in one series were



FIG. 116.7 Acute dacryocystitis. *(From Durand ML. Periocular infections. In: Schlossberg D, ed.* Clinical Infectious Disease. *2nd ed. Cambridge, UK: Cambridge University Press; 2015:117.)*

polymicrobial in 40%, and the most frequent pathogens were *S. aureus* (20%), streptococci (12%), and gram-negative bacilli (56%), with *H. influenzae* (20%) the most common gram-negative organism.[45] Another study also found that *H. influenzae* was the predominant gram-negative bacillus recovered.[46] Anaerobes were found in 19% in that study. Fungi are very rare causes of dacryocystitis, but a case of mucormycosis involving the lacrimal sac has been described.[47] *Rhinosporidium seeberi,* an aquatic protistan parasite seen especially in tropical climates, such as southern India, may cause chronic dacryocystitis. A report from India described 50 patients with ocular rhinosporidiosis over a 2.5-year period, including 26% with lacrimal sac involvement.[48] Bloody discharge from the puncta was a feature of lacrimal sac infection, and at surgery, a pink, vascularized growth was found in the lacrimal sac.

Patients with an episode of acute dacryocystitis and who do not ultimately undergo a DCR procedure may have further episodes of acute dacryocystitis. One study found this risk was 25%.[44]

PRESEPTAL CELLULITIS AND ORBITAL INFECTIONS

Anatomy

The orbital septum is a thin, fibrous membrane that serves as a barrier between the superficial lids and the orbit. The septum arises from the orbital periosteum at the orbital rim and extends to the tarsal plates of the eyelids (see Fig. 116.1). Infections anterior to the septum are preseptal, and infections posterior to the septum are orbital. *Preseptal cellulitis* involves only the lids and not the orbit, whereas *orbital cellulitis* involves the soft tissues (fat, muscle) contained within the bony orbit (Fig. 116.8). The bony orbit is shaped like a cone placed horizontally, apex tilted medially. It is surrounded by the paranasal sinuses for much of its circumference: the frontal sinus superiorly, the maxillary sinus inferiorly, and the ethmoid medially. The floor of the frontal sinus is also the roof of the orbit. The medial orbital wall, the paper-thin lamina papyracea, is also the lateral wall of the ethmoid sinus. It contains multiple foramina for nerves and blood vessels, and some people also have natural defects called *Zuckerkandl dehiscences.* For these anatomic reasons, ethmoid sinusitis is the most common cause of sinus-related orbital infection. Periosteum (periorbita) lines the orbit, and infection from the ethmoid sinus may cross the lamina papyracea and collect beneath the medial periorbita as a *subperiosteal abscess*. An *orbital abscess* may result from sinusitis without antecedent subperiosteal abscess or orbital cellulitis. The venous drainage of the middle third of the face and paranasal sinuses is primarily through the orbital veins, which drain inferiorly to the pterygoid plexus and posteriorly to the cavernous sinus. As a consequence, *cavernous sinus thrombophlebitis* may occur as a complication of a sinus or orbital infection (see Chapter 91).

Epidemiology

Preseptal cellulitis is much more common than orbital cellulitis. In a review of 315 pediatric patients (age 18 years or younger) with either infection treated in two adjacent Boston hospitals between 1980 and 1998, 94% were preseptal cellulitis cases.[48a] Both conditions occur most often in young children: 75% of patients in the Boston study were younger than 5 years. Children with orbital cellulitis tend to be slightly older than children with preseptal cellulitis. The average age of orbital cellulitis patients in the Boston study was 5 years versus 3 years for preseptal cellulitis patients. Frequently, the term *orbital cellulitis* is used in the literature imprecisely to include cases of subperiosteal and orbital abscess in addition to cellulitis. The distinction is important clinically because abscesses usually require surgical drainage. Orbital cellulitis is the most common orbital infection in some pediatric series and subperiosteal abscess in others, whereas orbital abscess is rare (6% of cases in one series).[49] Subperiosteal abscess comprised 72% of cases in a series of 101 children with orbital infections.[50]

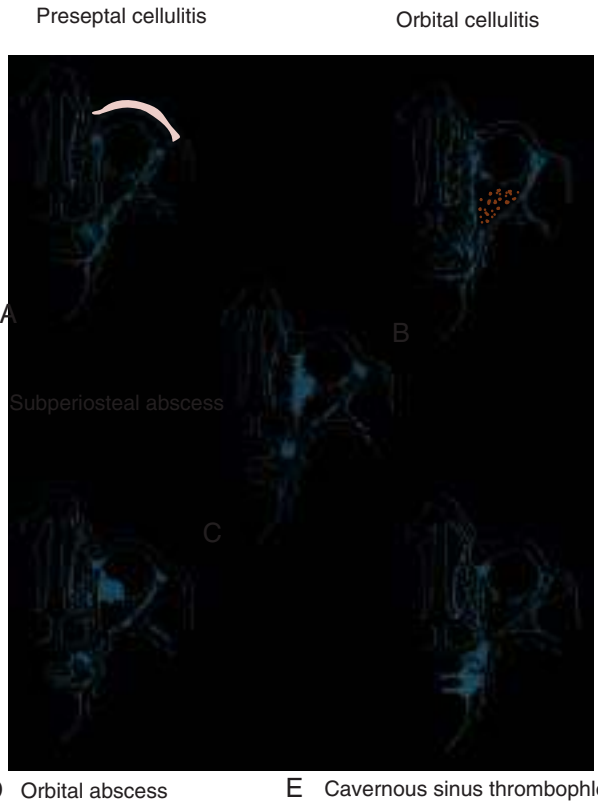

FIG. 116.8 Five diagrams illustrating preseptal cellulitis (A), orbital cellulitis (B), subperiosteal abscess (C), orbital abscess (D), and cavernous sinus thrombophlebitis (E). *(Modified from Chandler JR, Langenbrunner DJ, Stevens FR. The pathogenesis of orbital complications in acute sinusitis.* Laryngoscope. *1970;80:1414–1428.)*

Etiology and Bacteriology

Sinusitis causes up to 90% of all preseptal and orbital infections. Other causes of preseptal cellulitis include eyelid skin infections and (rarely) bacteremia. Other causes of orbital infections include dental infections, orbital trauma or surgery, and lacrimal system infections.

Preseptal cellulitis is usually caused by sinusitis. Cultures of the sinuses in these cases usually reveal the usual acute sinusitis pathogens, including *S. pneumoniae* and *H. influenzae*, although *S. aureus* may also play a role. Two other causes of preseptal cellulitis include bacterial superinfection of a rash or break in the eyelid skin and bacteremic seeding. The first may follow trauma, an insect bite, or herpetic lid lesions (herpes simplex or zoster). The pathogens are usually *S. aureus* or group A streptococci. Preseptal cellulitis that is part of a facial cellulitis or erysipelas is included in this category. Rare cases of group A streptococcal preseptal cellulitis have been complicated by either streptococcal toxic shock syndrome or eyelid necrosis.[51,52] *Pseudomonas aeruginosa* also caused lid necrosis as a complication of blepharitis and preseptal cellulitis in one case.[53] Other unusual causes of preseptal cellulitis include ringworm,[54] atypical mycobacteria,[55] and anthrax.[56] The second cause,

bacteremic seeding of the lids, occurs in infants and young children (usually younger than 3 years). This syndrome has become much less common since the introduction of *H. influenzae* type b (Hib) vaccine in 1990. Before the Hib vaccine, preseptal cellulitis was associated with bacteremia in 10% to 33% of cases, with 80% to 100% of these cases due to *H. influenzae*.[57–59] Studies in the Hib vaccine era have found that bacteremia is a very rare cause of preseptal cellulitis; a study of 35 such patients found none had positive blood cultures.[50] Streptococci, especially *S. pneumoniae* and group A streptococci, are the main causes of bacteremia now,[60] although nontypeable *H. influenzae* is still an occasional pathogen.[61]

Subperiosteal abscess is caused by ethmoid sinusitis in nearly all cases. Abscess cultures show *S. pneumoniae, S. anginosus*, group A streptococci, *H. influenzae,* and *S. aureus* as the major pathogens. A study from Toronto reported that, of 18 cases of subperiosteal abscess with positive cultures from surgical drainage, *S. anginosus* (44%) and *H. influenzae* (22%) were the major pathogens.[50] A study of children with sinogenic orbital cellulitis or subperiosteal abscess evaluated the changing bacteriology since the widespread use of the 7-valent pneumococcal conjugate vaccine (PCV7) and found a marked decrease in infections due to *S. pneumoniae* or viridans streptococci but an increase in *S. aureus* infections, including MRSA.[62] Brook and Frazier[63] found that subperiosteal abscesses in adults were also polymicrobial, with a similar mixture of aerobes and anaerobes. These cultures agreed with maxillary sinus puncture cultures obtained from the same patients. A study of 94 children hospitalized in Toronto between 2004 and 2009 with orbital cellulitis (45% of cases), subperiosteal abscess (47%), or orbital abscess (8%) found that *S. anginosus (milleri)* was the most common pathogen (44% of culture-positive cases), followed by *S. aureus,* group A streptococci, *S. pneumoniae*, and *H. influenzae*.[64] Anaerobes, including *Fusobacterium* and *Eikenella,* were isolated in 14% of culture-positive cases. A study of 53 patients (two-thirds adults) with sinogenic orbital or subperiosteal abscess found that the major pathogens were streptococci (37%), *S. aureus* (28%), gram-negative bacilli (17%), and anaerobes (19%).[65] MRSA accounted for 6.5% of cases; one-third of cultures grew more than one pathogen.

Orbital cellulitis and orbital abscess are usually caused by sinusitis, but rare cases occur after penetrating trauma, orbital surgery, canalicular surgery, peribulbar anesthesia for eye surgery, endophthalmitis, dental abscess, dacryocystitis, or dacryoadenitis.[66–70] These nonsinusitis etiologies may be more common in adults than children. In a study from Australia, 91% of children with orbital cellulitis or abscess had sinusitis, whereas only half of adults did.[71] The remaining adults had dacryocystitis, trauma, endophthalmitis, and secondarily infected nasal tumor as etiologies. There was no case of posterior extension of preseptal cellulitis in either children or adults in this study, and such cases are very rare.

An unusual cause of preseptal or orbital cellulitis is pneumococcal bacteremia. Cellulitis of the head and neck region from bacteremic pneumococcal infection is well described in patients with lupus erythematosus or hematologic disorders.[72,73] Patients with lupus who develop this may just have started corticosteroid therapy. Pneumococcal orbital cellulitis has also been described in a previously healthy adult without bacteremia.[74] *Pseudomonas* preseptal or orbital cellulitis may occur in neutropenic cancer patients secondary to *Pseudomonas* bacteremia.[75,76] Community-acquired MRSA is an important cause of orbital cellulitis in neonates and is associated with bacteremia.[77,78]

Clinical Manifestations

Preseptal and Orbital Cellulitis

Preseptal cellulitis must be distinguished from orbital cellulitis, a much more dangerous infection. The term *periorbital cellulitis,* sometimes used for preseptal cellulitis, should be avoided because it does not make this distinction clear. In preseptal and orbital cellulitis the lids are red and swollen. The lids may be swollen shut, but it is essential to examine the eye to evaluate visual acuity and extraocular movement. In preseptal cellulitis, vision is normal, there is no afferent pupillary defect, extraocular movements are full and painless, and there is no proptosis. In contrast, patients with orbital cellulitis have some degree of ophthalmoplegia or proptosis, or both. There is often deep eye pain and pain with eye movement. Proptosis may not be grossly apparent and should be measured (e.g., with the Hertel exophthalmometer); a difference of 2 mm or more is significant. Vision may be decreased, and an early warning sign may be an afferent pupillary defect. Fever and leukocytosis, usually present in children with preseptal or orbital cellulitis, may be absent in adults. Fever was present in 70% of pediatric cases but only 30% of adult cases in one series.[71]

Orbital and Subperiosteal Abscesses

Patients with an orbital or subperiosteal abscess usually present with marked lid swelling and erythema, eye pain, proptosis, marked ophthalmoplegia, and often vision loss. Most have fever. Because the abscess is medial or superomedial in nearly all cases, the eye is typically fixed looking "down and out" (Fig. 116.9).

Orbital Apex, Superior Orbital Fissure, and Cavernous Sinus Syndromes

Orbital apex syndrome is characterized by marked ophthalmoplegia and vision loss. The cranial nerves of the orbital apex are involved, which include the optic nerve and cranial nerves III, IV, VI, and the first division of V. There is often an afferent pupillary defect due to involvement of the optic nerve and hypoesthesia of the forehead due to involvement of the first division of cranial nerve V. Etiologies include vascular (e.g., carotid cavernous fistula), inflammatory (e.g., giant cell arteritis, Wegener's disease), neoplastic (e.g., lymphoma, head and neck cancers, neural tumors), and infectious.[79] In infectious orbital apex syndrome, unlike orbital cellulitis, marked vision loss and ophthalmoplegia may occur with minimal or no lid swelling or erythema. Overt signs of orbital inflammation may worsen subsequently. This syndrome is usually caused by infection in the adjacent posterior ethmoid or sphenoid sinuses, and most cases are due to invasive mold infections. Orbital apex syndrome is a well-known presentation of mucormycosis.[80] *Aspergillus* may cause an orbital apex syndrome in immunocompromised or normal hosts and may have a subacute presentation.[81,82] *Pseudallescheria boydii* has been described as an etiology in rare cases of invasive fungal infection involving the orbital apex.[83] Rare cases of orbital apex syndrome are due to bacteria.[84] Visual loss is usually irreversible.

If the infection is localized immediately anterior to the orbital apex, a "superior orbital fissure syndrome" may occur. This syndrome has the same cranial neuropathies as orbital apex syndrome except there is no involvement of the optic nerve. If the infection is posterior to the orbital apex, a "cavernous sinus syndrome" may occur. This has the same cranial neuropathies as orbital apex syndrome except with the added involvement of the second division of cranial nerve V and sometimes the oculosympathetic fibers. In addition, because the cavernous sinus is a venous plexus that extends to the opposite side, bilateral cranial neuropathies are typical. The superior orbital fissure, orbital apex, and cavernous sinus are contiguous, and the etiologies are similar.[79] Infections rarely respect the precise anatomic locations these syndromes imply, and infections may be in the cavernous sinus, for example, without having all the features of the cavernous sinus syndrome. Infectious etiologies for all of these syndromes include fungi, bacteria such as *S. aureus,* streptococci including *S. anginosus (milleri),* gram-negative bacilli, syphilis, and herpes zoster. Herpes zoster ophthalmicus (HZO) rarely may be complicated by complete unilateral ophthalmoplegia or



FIG. 116.9 **Patient with orbital abscess (eye looks "down and out").**

orbital apex syndrome. In a review of 20 cases, HZO preceded ophthalmoplegia in 75% and occurred concurrently in 20%.[85]

Cavernous Sinus Thrombophlebitis

Septic cavernous sinus thrombophlebitis is rare and should be suspected in any patient with orbital cellulitis who develops contralateral signs of orbital inflammation (lid swelling, proptosis, ophthalmoplegia) (see also Chapter 91). Spread to the opposite eye occurs through the cavernous sinus and usually occurs within 24 to 48 hours of the initial unilateral orbital findings.[86] Patients may also present with bilateral findings, including lid edema, chemosis, proptosis, ptosis, and ophthalmoplegia, or they may present with signs of bilateral neuropathies of some or all of cranial nerves III, IV, and VI but without the lid edema and erythema that typifies orbital cellulitis. The latter is especially true in cases of cavernous sinus thrombophlebitis that arise from skin infections of the middle third of the face or in dental infections, rather than primary orbital infections. In cavernous sinus thrombophlebitis there may be decreased sensation over the forehead and sometimes cheek due to involvement of the first or second division of cranial nerve V. Trigeminal nerve involvement may be seen in a quarter of patients with cavernous sinus thrombophlebitis but is not a feature of usual bacterial orbital cellulitis.[87] Early cases may present with unilateral findings of orbital cellulitis and cavernous sinus syndrome but with persistent headache and lethargy. This was illustrated in a case report of a child with cavernous sinus thrombophlebitis, sphenoid sinusitis, and *S. anginosus (milleri)* bacteremia in whom the clues to more serious infection were unrelenting headache, fever, and lethargy.[88] Visual loss may occur from venous congestion and ischemia. Patients are usually febrile and may be lethargic or obtunded. There is often sphenoid and posterior ethmoid sinusitis. *S. aureus* is the major pathogen, and cases of MRSA have also been described.[89] Other pathogens include streptococci, especially *S. anginosus (milleri)* group; anaerobes; and gram-negative bacilli.[90–92] Two cases with a subacute presentation involved *Actinomyces* in one and *Aggregatibacter actinomycetemcomitans* in the other.[93,94] In both cases, patients initially were misdiagnosed as having Tolosa-Hunt syndrome, an idiopathic, steroid-responsive inflammatory process involving the cavernous sinus.

Laboratory and Radiologic Studies

Laboratory studies (white blood cell count, blood cultures) should be obtained in all patients with preseptal or orbital cellulitis. Leukocytosis with a left shift is present in most patients. Blood cultures are rarely positive in older children and adults but may be positive in up to 8% of young children, as noted earlier.

The most helpful study in evaluating a patient with orbital infection is computed tomography (CT). A CT scan should be performed in any patient with orbital signs (ophthalmoplegia, proptosis, decreased vision, or a combination of these) because it is essential to identify an abscess that may require urgent drainage. Repeat scans should also be obtained in any patient with presumed uncomplicated orbital cellulitis who fails to improve or worsens on intravenous (IV) antibiotics alone. A CT scan may not be necessary in many cases of preseptal cellulitis because the diagnosis may be made clinically. However, several reports highlight the fact that in children orbital signs may be absent, yet they may have an orbital or subperiosteal abscess. Some authors advocate CT for all children with preseptal cellulitis, however, and report occasional cases of subperiosteal abscess that presented similar to preseptal cellulitis, with no proptosis, visual decrease, or ophthalmoplegia.[95] A retrospective study that included 111 children (median age, 7) with orbital or subperiosteal abscess on CT found that proptosis, ophthalmoplegia, and pain with eye movement were risk factors for abscess, but half lacked these findings.[96] This study found that marked lid inflammation with edema extending beyond the lid margins, high white blood count (neutrophil count > 10,000), and previous antibiotic therapy were also risk factors for abscess.

If performed, CT in preseptal cellulitis shows lid edema but no proptosis or inflammation ("streaking") of the orbital fat. Findings in orbital cellulitis usually include proptosis, streaking of the intraconal fat, and edema of the medial rectus muscle. In subperiosteal or orbital abscess there is a low-density mass effect with or without enhancement.[97] An air-fluid level within the mass is even more specific for abscess.[98] Lateral displacement of the medial rectus and displacement of the periosteum away from the lamina papyracea are findings that suggest subperiosteal abscess (Fig. 116.10). CT results alone lead to misdiagnoses, however, and cannot always be relied on to determine the need for surgery. In one study, CT missed the diagnosis for 2 of 10 subperiosteal abscesses and 1 of 5 orbital abscesses.[97] Another review of 159 patients with orbital complications of sinusitis described 4 patients who developed blindness from orbital abscess.[99] The abscess was not diagnosed by CT in any of these 4 patients before surgery.

If orbital apex syndrome is suspected, magnetic resonance imaging (MRI) or high-resolution CT with slice thickness of 3 mm or less, or both, should be obtained.[100] If cavernous sinus thrombosis is suspected, MRI with venography (MRV) should be performed. Findings in cavernous sinus thrombosis include flattening or bowing of the lateral wall of the cavernous sinus (best viewed on coronal images) and filling defects within the contrast-enhancing cavernous sinus.[86] Dilation of the superior ophthalmic vein due to venous obstruction is an indirect sign of cavernous sinus thrombosis. Contrast-enhanced thin-section CT also has a very high sensitivity for detecting cavernous sinus thrombophlebitis, and CT is the usual initial study performed in patients who present with orbital infections. Radiologists correctly diagnosed seven of eight cases of cavernous sinus thrombophlebitis in one retrospective study in which the images were viewed without any clinical information.[101] The differential diagnosis of bacterial orbital cellulitis includes orbital pseudotumor, tumor, and invasive fungal disease (invasive sinus aspergillosis and mucormycosis). Orbital pseudotumor is an idiopathic disease, more common in adults than children, that often manifests with painful ophthalmoplegia. It may appear with inflammatory proptosis, mimicking orbital cellulitis.[102] Patients with tumors of the orbit may present with acute inflammation mimicking orbital cellulitis. This has been described in primary ophthalmic rhabdomyosarcoma and retinoblastoma.[103,104] Rhinocerebral mucormycosis, which frequently manifests as an orbital cellulitis, is discussed in Chapter 258. Mucormycosis should be considered in any patient who presents with orbital cellulitis and who has risk factors for mucormycosis (e.g., poorly controlled diabetes mellitus, hematologic malignancies, immunosuppression, deferoxamine therapy). In contrast with typical bacterial orbital cellulitis, patients with rhinocerebral mucormycosis may have minimal lid erythema, more pain in the forehead or temple than in the eye, and early onset of decreased sensation in the first and second divisions of cranial nerve V. Invasive sinus aspergillosis usually invades from the sphenoid sinus and may manifest as a subacute orbital apex syndrome.

Therapy

Preseptal cellulitis due to sinusitis should be treated with antibiotics active against *S. aureus, S. pneumoniae,* and *H. influenzae*. Blood cultures should be obtained in very young children and in any patient with a fever or other signs of systemic illness. Initial antibiotics should be

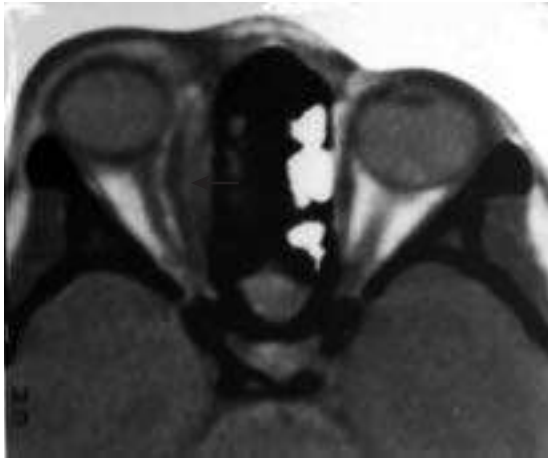

FIG. 116.10 Computed tomography scan of subperiosteal abscess (*arrow* points to abscess).

given intravenously in these cases. In older children and adults with mild preseptal cellulitis due to sinusitis, initial antibiotics may be oral (e.g., amoxicillin-clavulanate). In cases of preseptal cellulitis due to a skin lesion (e.g., superinfected abrasion or insect bite), oral antibiotics may be given if the infection is mild and there is no sign of systemic illness. Antibiotics that have activity against MRSA, in addition to streptococci and methicillin-susceptible *S. aureus*, should be chosen (e.g., trimethoprim-sulfamethoxazole plus amoxicillin). In countries (e.g., the Netherlands) or communities that have a very low incidence of MRSA, inclusion of an anti-MRSA antibiotic may not be necessary.

All patients with orbital infections (cellulitis, subperiosteal or orbital abscess) should be promptly treated with IV antibiotics and monitored closely for signs of visual compromise. Blood cultures should be drawn in all cases and sinus cultures obtained in patients with sinusitis. Nasal screening cultures for MRSA colonization may be helpful. Initial antibiotics should be broad spectrum with coverage against *S. aureus,* including MRSA; streptococci, including *S. pneumoniae*; *H. influenzae* and other gram-negative bacilli; and anaerobes. A regimen such as IV vancomycin, metronidazole, and ceftriaxone would provide this coverage and also provide adequate antibiotic levels in the central nervous system (CNS) in cases in which posterior spread of infection is a concern. In patients who are highly penicillin-allergic or intolerant of third-generation cephalosporins, levofloxacin or ciprofloxacin could be substituted for ceftriaxone. If there is no concern for CNS spread, IV vancomycin plus ampicillin-sulbactam (or piperacillin-tazobactam) would be a reasonable initial regimen. If *Pseudomonas* is a consideration (e.g., patients who are immunocompromised, have known sinus colonization with *Pseudomonas,* or who have received multiple antibiotics in recent months), then an antipseudomonal agent should be included. Antibiotics should be tailored based on culture results (e.g., blood, sinus, abscess drainage). Older children and adults with subperiosteal abscess usually require prompt surgical drainage in addition to broad-spectrum IV antibiotics, but young children with small, medial subperiosteal abscesses may respond to IV antibiotics alone. A study of 68 children with subperiosteal abscesses reported that two-thirds responded to medical therapy alone, whereas one-third required surgery.[105] The children who required surgery were older (8.3 vs. 6.2 years) and had larger abscesses on CT scan (>10 mm). Children with subperiosteal abscesses who are initially managed medically must be followed very closely, and any worsening should prompt surgical drainage of the abscess. Patients with orbital abscesses nearly always require immediate surgical drainage, in addition to broad-spectrum empirical therapy. Broad-spectrum combination antibiotic therapy (e.g., vancomycin, metronidazole, and a third- or fourth-generation cephalosporin) should also be used as initial therapy for acute bacterial cavernous sinus thrombosis secondary to orbital cellulitis. Because this infection carries a high risk of intracranial complications (e.g., brain abscess, subdural empyema), any regimen should include antibiotics that cross the blood-brain barrier. Surgical drainage of the primary focus of infection (e.g., sinusitis or dental abscess) should be performed, and patients should be monitored closely for any intracranial extension that may require surgical drainage.[106] The use of anticoagulation has been controversial[107] and is discussed further in Chapter 91.

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The complete reference list is available online at Expert Consult.

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117 Viral Hepatitis

Jules L. Dienstag

SHORT VIEW SUMMARY

ACUTE HEPATITIS

- Acute clinical illnesses caused by the five hepatitis viruses (hepatitis A virus [HAV], hepatitis B virus [HBV], hepatitis C virus [HCV], hepatitis D virus [HDV], and hepatitis E virus [HEV]) are similar.
- Illness ranges from asymptomatic to fulminant.
- Asymptomatic infections are 10 to 30 times more common than symptomatic ones.

CHRONIC HEPATITIS

- Chronic hepatitis affects more than 400 million people worldwide.
- HBV, HCV, and HDV cause chronic hepatitis.
- HEV causes protracted and chronic hepatitis only in immunosuppressed patients.

INDIVIDUAL HEPATITIS VIRUSES (SEE TABLE 117.1)

Hepatitis A Virus

- HAV is an RNA virus that is a member of the *Hepatovirus* genus.
- HAV is transmitted via the fecal-oral route.
- It typically causes an acute, self-limited illness, more often symptomatic in adults than in children.
- HAV is more severe in patients with preexisting chronic hepatitis B or C.
- Relapsing and cholestatic hepatitis may occur.
- Diagnosis is by serology (immunoglobulin M [IgM] anti-HAV) (see Table 117.2).
- Treatment beyond supportive care is usually not necessary.
- For prevention, highly effective HAV vaccines are available; these have markedly reduced incidence where used.
- Passive immunization with immune globulin intramuscularly is effective in postexposure prophylaxis.

Hepatitis B Virus

Virology and Epidemiology

- HBV is a double-stranded DNA virus in the *Orthohepadnavirus* genus and the Hepadnaviridae family; 10 genotypes have been identified.
- Worldwide, 250 million people are infected chronically with HBV.
- One million deaths annually result from complications of chronic infection—cirrhosis, hepatocellular carcinoma (HCC).
- Prevalence in Asia is estimated at greater than 10%, resulting primarily from perinatal transmission, after which the likelihood of acquiring chronic infection is 90%.
- In adults, transmission of hepatitis B occurs primarily as a result of high-risk behaviors (injection drug use, sexual activity) or after occupational exposure but resolves in more than 95% of otherwise healthy persons.

Diagnosis

- Acute hepatitis B is a symptomatic infection typified by right upper quadrant pain, nausea, malaise, jaundice, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and IgM antibody to hepatitis B core antigen.
- Different categories of chronic infection are diagnosed on the basis of serologic testing and HBV DNA level (see Table 117.5).
- Liver biopsy is not required for diagnosis but may inform treatment decisions in chronic infection.

Treatment

- Indications are as follows (see Table 117.7):
  - Hepatitis B e antigen (HBeAg)–positive or HBeAg-negative patients with ALT greater than two times the upper limit of normal or moderate-to-severe hepatitis on liver biopsy and HBV DNA >20,000 IU/mL
  - HBeAg-negative patients with low-level HBV DNA but evidence of fibrosis or moderate-to-severe hepatitis on liver biopsy
  - Cirrhosis associated with chronic HBV irrespective of HBV DNA level
  - Inactive carrier (hepatitis B surface antigen [HBsAg] positive) preemptively when starting chemotherapy or other immunosuppressive therapy (including anti–tumor necrosis factor)
- Therapy is as follows (see Table 117.8):
  - Tenofovir disoproxil fumarate (TDF) as administered at 300 mg daily, or entecavir 0.5 mg daily (1-mg dose approved for prior lamivudine resistance but not a viable alternative to adding tenofovir); in patients with reduced creatinine clearance (estimated glomerular filtration rate [eGFR] <60 mL/min) or evidence of proximal tubular injury or osteoporosis, and according to some authorities all patients >60 years old, tenofovir alafenamide 25 mg daily is used instead of TDF.
  - Administration of 48 weeks of pegylated interferon (PEG IFN) in noncirrhotic HBeAg-positive patients is also an option.
  - Consider stopping oral therapy in HBeAg-positive patients 6 to 12 months after HBeAg seroconversion (HBeAg negative and anti-HBe positive); there is a ≥40% chance of HBeAg seroconversion after 5 years of therapy.
  - Indefinite treatment is used in patients with chronic HBeAg-negative hepatitis B; consider stopping therapy for HBsAg seroconversion.

Prevention

- Universal vaccination of infants begins at birth (three or more intramuscular doses).
- Prenatal maternal HBsAg screening is performed. If mother is HBsAg positive, hepatitis B immune globulin 0.5 mL is given at birth along with vaccination.
- Tenofovir 300 mg is administered starting in the third trimester in women with HBV DNA >2× 10^5 IU/mL.

Hepatitis D Virus

- HDV is a percutaneously transmitted, circular RNA virus (genus: *Deltavirus*) that requires HBV (or other hepadnaviruses) to replicate and persist.
- Acute coinfection can occur and follows the clinical course of acute HBV infection.
- HDV superinfection results in chronic infection in patients with prior chronic hepatitis B and has clinical features similar to those of chronic hepatitis B infection, although with increased severity and risk of progression to cirrhosis.
- The diagnosis is made by means of serologic testing for antibodies to HDV or by polymerase chain reaction (PCR) for HDV RNA.
- Oral antivirals for hepatitis B are not active against HDV, but prolonged treatment with PEG IFN may benefit a proportion of patients.

SHORT VIEW SUMMARY—cont'd

Hepatitis C Virus

Virology and Epidemiology

- HCV is a single-stranded RNA virus (genus: *Hepacivirus*) with six main genotypes (1–6); it exists as quasispecies because of its high mutation rate.
- HCV requires host lipid membrane assembly and secretion apparatus for replication.
- Worldwide, 71 million patients have chronic HCV infection, with more than 3 million residing in the United States.
- Transmission occurs predominantly through injection drug use, but other modes include blood transfusion before the availability of screening or nonsterile practices in which blood or body fluids are exchanged. (Note: The risk of hepatitis C is not increased in people with tattoos or in health workers.)
- Chronic HCV infection develops in at least 85% of patients with acute infection and, in the absence of treatment, results in cirrhosis in approximately 20% of patients after 20 years of infection.
- The annual incidence of HCC in persons with HCV-associated cirrhosis is 1% to 4%.
- Hepatitis C is the leading indication for liver transplantation in the United States.

Diagnosis

- Symptomatic hepatitis and jaundice develop in fewer than 10% to 20% of patients with acute hepatitis C, which often portends viral clearance.
- Chronic hepatitis C tends to be asymptomatic, although patients often complain of fatigue.
- Patients with HCV-associated cirrhosis may present clinically with complications of end-stage liver disease such as variceal bleeding, hepatic encephalopathy, or ascites.
- The detection of antibody to HCV is the initial diagnostic test, but PCR testing for HCV RNA distinguishes ongoing chronic infection (commonly) from prior exposure with subsequent clearance (rare).

Treatment

- The goal of therapy in chronic hepatitis C is to achieve a sustained virologic response (SVR), which is defined as undetectable HCV RNA 24 weeks after completion of therapy (12 weeks has been adopted as the SVR end point in contemporary clinical trials).
- The availability of direct-acting antivirals (DAAs), which are highly potent, well tolerated, and can be administered orally, has resulted in dramatic advances in treatment of hepatitis C. The September 2017 guidelines for treatment of hepatitis C from the American Association for the Study of Liver Diseases and the Infectious Diseases Society of America are presented as standard of care as follows:
  - Standard of care first-line agents for DAA-naïve patients, September 2017, are summarized in Table 117.9.
    - Genotype 1
      - Sofosbuvir-ledipasvir for 12 weeks (no cirrhosis or compensated cirrhosis; if no cirrhosis, not black, and HCV RNA $<6\times 10^6$ IU/mL, treatment duration 8 weeks)
      - Glecaprevir-pibrentasvir for 8 weeks (no cirrhosis) or 12 weeks (compensated cirrhosis)
      - Grazoprevir-elbasvir for 12 weeks (not recommended for genotype 1a if baseline elbasvir NS5A resistance present)
      - Sofosbuvir-velpatasvir for 12 weeks
    - Genotype 2
      - Glecaprevir-pibrentasvir for 8 weeks (no cirrhosis) or 12 weeks (compensated cirrhosis)
      - Sofosbuvir-velpatasvir for 12 weeks
    - Genotype 3
      - Glecaprevir-pibrentasvir for 8 weeks (no cirrhosis) or 12 weeks (compensated cirrhosis) (not recommended for PEG IFN–ribavirin–experienced patients)
      - Sofosbuvir-velpatasvir for 12 weeks (not recommended for PEG IFN–ribavirin–experienced patients; for baseline Y93H resistance-associated substitutions, add ribavirin or consider sofosbuvir-velpatasvir-voxilaprevir)
      - Additional regimens in compensated cirrhosis
        - Grazoprevr-elbasvir plus sofosbuvir for 12 weeks (not recommended for PEG IFN–ribavirin-naïve patients)
        - Sofosbuvir-velaprevir-voxilaprevir for 12 weeks (not recommended for PEG IFN–ribavirin-naïve patients)
    - Genotype 4
      - Glecaprevir-pibrentasvir for 8 weeks (no cirrhosis) or 12 weeks (compensated cirrhosis)
      - Sofosbuvir-velpatasvir for 12 weeks
      - Sofosbuvir-ledipasvir for 12 weeks (not recommended for PEG IFN–ribavirin–experienced patients with compensated cirrhosis)
      - Grazoprevr-elbasvir for 12 weeks (for PEG IFN–ribavirin–experienced, only for relapsers)
    - Genotype 5 or 6
      - Glecaprevir-pibrentasvir for 8 weeks (no cirrhosis) or 12 weeks (compensated cirrhosis)
      - Sofosbuvir-ledipasvir for 12 weeks
      - Sofosbuvir-velpatasvir for 12 weeks

Hepatitis E

- HEV is an RNA virus (genus: *Hepevirus*).
- It spreads through fecally contaminated water in endemic areas.
- HEV causes acute, self-limited hepatitis, similar to HAV, in normal hosts.
- Case-fatality rates are 0.9% to 2.8% in men and 20% in pregnant women, particularly in the third trimester.
- In immunosuppressed patients, particularly solid-organ transplant recipients, HEV can cause protracted infection, chronic hepatitis, and cirrhosis.
- Diagnosis is by serologic tests (IgM antibody to HEV) or PCR in blood or stool.
- With regard to treatment, acute hepatitis E is usually self-limited; chronic hepatitis E responds to ribavirin (preliminary data suggest responsiveness to sofosbuvir) therapy.
- Highly effective vaccines consisting of recombinant capsid proteins have been developed for use in endemic areas. One (Hecolin) is licensed in China.

Viral hepatitis is an infection that predominantly affects the liver but may also have systemic clinical manifestations. It is estimated that as many as 400 million persons have chronic viral hepatitis,[1] which, therefore, is the most common cause of chronic liver disease. Although the exact burden of acute viral hepatitis is not known, at least 1.4 million cases of hepatitis A occur worldwide annually. Thus, viral hepatitis is a major public health problem. The vast majority of cases of hepatitis are caused by one of five hepatotropic viruses: hepatitis A virus (HAV); hepatitis B virus (HBV); hepatitis C virus (HCV); hepatitis D (delta) virus (HDV); and hepatitis E virus (HEV). HBV, HCV, and HDV also cause chronic hepatitis, whereas HAV does not. HEV causes acute hepatitis in normal hosts but can cause protracted and chronic hepatitis in immunosuppressed patients (Table 117.1). Approximately 15% to 17% of cases of hepatitis have features similar to hepatitis caused by known viruses but remain unexplained and are therefore areas of search for occult viral agents. Putative viral agents for such cases, primarily in transfusion-associated hepatitis, have been described (hepatitis G, hepatitis GB, transfusion-transmitted virus, SEN virus),[2–4] but these do not appear to be human pathogens. Liver involvement may also be seen on occasion as part of systemic infections with herpes simplex virus, Epstein-Barr virus (EBV), varicella-zoster virus, enteroviruses, adenoviruses, yellow fever virus, mumps, rubella, and rubeola, but these viruses do not cause infections that primarily affect the liver.

The acute clinical illnesses caused by the five hepatitis viruses (A–E) range from asymptomatic to fulminant and fatal. The chronic infections caused by HBV, HCV, and HDV range from subclinical persistent infections to rapidly progressive chronic liver disease with cirrhosis and increased risk for hepatocellular carcinoma (HCC). HEV can also cause protracted and chronic infections in immunosuppressed patients (see later).

TABLE 117.1 Hepatitis Viruses and Characteristics of Infection

CHARACTERISTIC	A	B	C	D	E
Virus family	Picornaviridae	Hepadnaviridae	Flaviviridae	Unassigned	Hepeviridae
Genus	*Hepatovirus*	*Orthohepadnavirus*	*Hepacivirus*	*Deltavirus*	*Hepevirus*
Nucleic acid	RNA	DNA	RNA	RNA	RNA
Incubation period (days)	15–48 (mean 30)	30–180 (mean 60–90)	15–160 (mean 50)	30–180 (mean 60–90)	14–60 (mean 40)
Mode of transmission					
Fecal-oral	Yes	No	No	No	Yes
Sexual	Possible	Yes	Rare	Yes	No
Blood	Rare	Yes	Yes	Yes	No[a]
Chronic infection	No	Yes	Yes	Yes	Yes[b]
Cirrhosis and hepatocellular carcinoma	No	Yes	Yes	With hepatitis B	No[c]

[a]Can be bloodborne in endemic areas.
[b]Acute hepatitis in normal hosts, protracted and chronic infection only in immunosuppressed patients.
[c]Cirrhosis can occur after chronic infection, which is confined to immunosuppressed patients.

Discussion of viral hepatitis in this chapter is divided into the following two sections: "Acute Viral Hepatitis" and "Chronic Viral Hepatitis."

ACUTE VIRAL HEPATITIS

Clinical Manifestations

The clinical manifestations of acute viral hepatitis are similar among the five hepatitis viruses, and no clinical features distinguish unequivocally one from the others, although certain epidemiologic patterns of transmission may suggest a particular virus.

Asymptomatic infections with the hepatitis viruses are approximately 10 to 30 times more common than symptomatic ones. These are most often recognized by rises in liver biochemical test results along with serologic markers of viral infections in patients being evaluated for nonspecific constitutional complaints (see later). The incubation periods of hepatitis vary with the individual virus (see later), and prodromal symptoms are common but quite varied in the individual patient. Approximately 25% of patients report the onset of symptoms as a "flulike" illness. Symptoms include malaise, fatigue, myalgia, arthralgia, and headache. Anorexia, nausea, and vomiting may occur, sometimes associated with alterations in olfaction and taste. Pharyngitis, coryza, and cough may also be present. Low-grade fever is common and more frequent in hepatitis A and E, rather than in hepatitis B or C, except when hepatitis B is associated with a serum sickness–like syndrome, in which a fever of 39.5°C to 40°C may be present. Dark urine and clay-colored stools may appear, usually 1 to 5 days before the onset of icterus, although a substantial portion of patients never become jaundiced. With the onset of jaundice, the constitutional symptoms generally abate but may continue during the entire icteric period. Right upper quadrant tenderness and discomfort are present as the liver enlarges, and in some patients a cholestatic clinical picture may develop, suggesting extrahepatic biliary obstruction. Splenomegaly and cervical lymphadenopathy are seen in 10% to 20% of patients.

As patients recover, constitutional symptoms resolve, but usually some liver enlargement persists, as do elevations of liver biochemical tests (see later). The duration of the posticteric phase is variable and ranges from 2 to 12 weeks. It is usually more prolonged in hepatitis B and C. Complete resolution of clinical and laboratory abnormalities is to be expected 1 to 2 months after the onset of jaundice in acute hepatitis A and 3 to 4 months after the onset of jaundice in 75% of cases of uncomplicated acute hepatitis B or C. In hepatitis E, acute cases are similar to those in hepatitis A, but protracted infection may occur in hepatitis E in immunosuppressed patients, and severe and fatal infection can occur in pregnant women (see later).

Laboratory Features

Serum aspartate aminotransferase (AST) and the alanine aminotransferase (ALT) activities increase to variable levels during the prodromal phase of acute hepatitis and precede elevations in bilirubin. The magnitude of AST and ALT elevations do not correlate closely with the extent of liver damage. Peak levels vary from 400 to 4000 international units (IU)/L, and maximums are usually reached when the patient becomes icteric. Jaundice, when present, reaches levels of 5 to 20 mg/dL and is usually equally divided between conjugated and unconjugated fractions. Neutropenia and lymphopenia may be present initially, followed by lymphocytosis with atypical lymphocytes. Prothrombin times should be followed because a prolonged value may indicate a severe hepatic synthetic defect, severe hepatocellular necrosis, and a poor prognosis. A diffuse but modest elevation of the serum globulin fraction is often present, and antibodies to smooth muscle and to other cell constituents can be seen.

Prognosis of Acute Hepatitis

The prognosis of patients with hepatitis A is excellent; nearly all previously healthy patients recover fully and without sequelae. Similarly, 95% to 99% of otherwise healthy, immunocompetent patients with acute hepatitis B recover completely. Hepatitis C is even less severe than hepatitis B during the acute phase, and fatalities are rare, although the precise case-fatality rate is unknown; chronic hepatitis C follows acute infection in approximately 85% of cases. Patients with simultaneous acute hepatitis B and acute hepatitis D do not necessarily have a more severe disease than those with acute hepatitis B alone, and the duration of hepatitis B infection will determine the chronicity of hepatitis. In contrast, hepatitis D superinfection of established chronic hepatitis B infection often leads to clinical deterioration (see later). Waterborne acute infection with HEV has a mortality rate of 0.6% to 2.8%[5] in men and up to 20%[6,7] in pregnant women. HEV can also cause chronic and severe hepatitis in immunosuppressed patients.[8]

Certain clinical and laboratory findings may be associated with more complicated and protracted courses of acute viral hepatitis. These include advanced age and serious underlying medical conditions. Presenting clinical features such as ascites, peripheral edema, and hepatic encephalopathy indicate a poorer prognosis. A prolonged prothrombin time, low serum albumin, hypoglycemia, and very high serum bilirubin indicate more severe hepatocellular damage. Patients with those clinical and laboratory findings should be hospitalized promptly.

Fulminant Hepatitis

Fulminant hepatitis is defined as severe liver failure developing within 8 weeks of the onset of symptoms and is the most feared complication of acute hepatitis. It is seen primarily with hepatitis B and D but may occur rarely in hepatitis A infection in older patients and in patients who also have chronic hepatitis B or C infection.[9] Hepatitis B infection of patients with underlying chronic hepatitis C may also lead to fulminant hepatic failure. Fulminant hepatitis is hardly ever seen in patients with hepatitis C alone. As noted earlier, hepatitis E may lead to acute liver failure uncommonly in men and in up to 20% of pregnant women.[5–7]

Considerable geographic variations exist in the causes of acute liver failure. Hepatitis E is the leading cause in India, and hepatitis B is the leading cause in France and Japan. Patients with acute fulminant

hepatitis usually present with hepatic encephalopathy, which may evolve into a deep coma. Liver size is reduced, bilirubin rises, and prothrombin time is markedly prolonged, even as aminotransferase levels drop. Ascites and peripheral edema are present, consistent with hepatic failure. Cerebral edema is common, and brainstem compression may occur. Also seen are gastrointestinal bleeding, sepsis, and respiratory failure. Cardiovascular collapse and renal failure are terminal events. The mortality rate is high (>80% in patients with deep coma), but those who survive have a complete clinical and laboratory recovery. Liver transplantation, if it can be performed in time, may be lifesaving in such patients. Drug-induced (acetaminophen) toxicity is the leading cause of hepatic failure in the United Kingdom and the United States.[10] Viral hepatitis was identified in only 12% of cases of fulminant hepatitis in the United States; HBV and HAV accounted for 7% and 4% of cases, respectively.[11,12]

Complications of Acute Hepatitis

Uncommonly, hepatitis A can be associated with relapsing hepatitis, occurring weeks to months after apparent recovery from hepatitis A.[13] Also uncommonly, a clinical picture of protracted cholestatic hepatitis may be present in patients with hepatitis A and persist for up to a year. Even with these complications, however, hepatitis A remains self-limited and does not progress to chronic liver disease.

A variety of immune-mediated complications may be associated with hepatitis B and C. During the prodromal phase of hepatitis B, a serum sickness–like syndrome may develop, characterized by arthritis or arthralgias, rash, angioedema, and, rarely, hematuria and proteinuria. Essential mixed cryoglobulinemia (EMC) and associated lymphoproliferative disorders can complicate chronic (not acute) hepatitis C, and hepatitis C may be associated with abnormalities in lipoprotein and glucose metabolism. The risk of chronic infection after acute hepatitis B in otherwise healthy adults is appropriately 1%, whereas the corresponding risk of chronicity after acute hepatitis C is 85% to 90%. Both chronic hepatitis B and C are associated with an increased risk of HCC. Chronic hepatitis B and C are discussed in the subsequent section of this chapter and in Chapters 145 and 154.

Agents of Viral Hepatitis

The characteristics of individual viruses that cause hepatitis are reviewed in Table 117.1. Additional details regarding epidemiology, virology, and pathogenesis are presented in chapters devoted to the individual pathogens.

Hepatitis A Virus (Also See Chapter 174)

HAV is a nonenveloped positive-strand virus that is 27 to 28 nm in diameter and is a member of the *Hepatovirus* genus in the Picornaviridae family. Human HAV strains are divided into four genotypes but appear to be of only one serotype. HAV is relatively stable under a variety of environmental conditions but can be inactivated by commonly used disinfecting chemicals, including hypochlorite and bleach (see Chapter 174). Person-to-person transmission by the fecal-oral route is the primary means of HAV transmission throughout the world.[14] Before the introduction of HAV vaccine, children were the major source of person-to-person transmission. Although foodborne and waterborne transmission are increasingly uncommon in the developed world, sporadic foodborne outbreaks continue to be reported, often linked to food products imported from developing countries. In 2017, a surge of new acute hepatitis A infections occurred via person-to-person spread, concentrated among homeless persons, persons using injection drugs, and their close personal contacts.

HAV replicates in the liver and is excreted in high concentrations in the stool and bile. The incubation period of illness is 15 to 45 days (mean of 30 days), and infectivity of virus in stool is present from 21 days before to 8 days after onset of jaundice. The highest concentration of virus in stool is in the 2-week period before jaundice develops (Fig. 117.1). Viremia begins during the prodrome and extends through the period of increased serum aminotransferase levels. Shedding of virus in stool may occur for a longer period in children than in adults. Although chronic shedding of HAV does not take place, virus shedding may recur during relapsing acute illness.

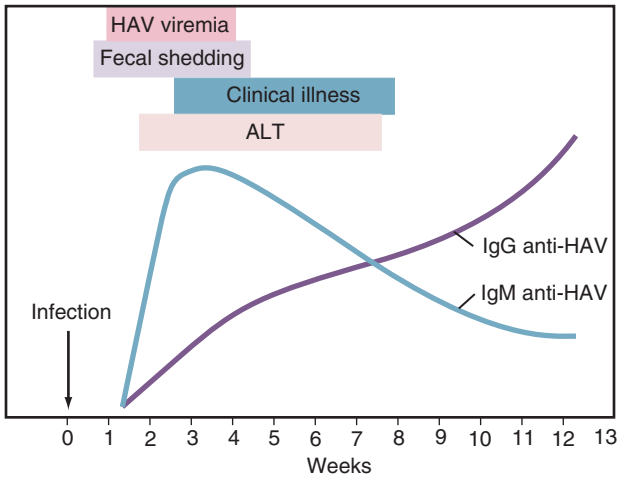

FIG. 117.1 Clinical, virologic, and serologic course of acute hepatitis A virus *(HAV)* infection. *ALT,* Alanine aminotransferase; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M.

Hepatitis A is typically an acute self-limited disease, but its clinical expression varies with age. The vast majority of infections are silent in children younger than 5 years, and rates of symptomatic infection and jaundice increase in older children and adults. Hepatitis A often begins with a mild prodrome; after 1 to 7 days dark urine and jaundice may appear. Two-thirds of patients recover by 2 months, 85% by 3 months, and nearly all by 6 months. Recovery is full and without sequelae, and chronic infection does not occur. Relapsing disease may occur after a typical initial course in 3% to 20% of patients. Occasionally, cholestatic hepatitis may complicate an acute course. HAV is a rare cause of fulminant hepatitis in the developed world but can cause severe hepatitis in individuals with preexisting hepatitis B or C and the elderly. During outbreaks of hepatitis A in homeless populations, mortality rates of approximately 3% to 4% have been reported.

Hepatitis B Virus (Also See Chapter 145)

HBV infects more than 250 million people worldwide.[15] It is the leading cause of chronic hepatitis, cirrhosis, and HCC. The sequelae of chronic infection account for more than 1 million deaths annually.

A partially double-stranded DNA virus in the Hepadnaviridae family, HBV replicates through reverse transcription from an RNA intermediate. The complete viral particle is 42 nm in diameter, outnumbered by abundant 22-nm spherical and filamentous subviral particles. HBV is divided into 10 genotypes (A–J) and multiple subgenotypes. The primary hosts for HBV are humans, but the envelope protein, hepatitis B surface antigen (HBsAg), has been detected in nonhuman primates. The primary cell for HBV replication is the hepatocyte, although a robust in vitro culture system has not been established. HBV does not appear to be directly cytopathic to the hepatocyte, and liver injury appears to be mediated by virus-specific cellular and humoral immune responses (see Chapter 145).

HBV is transmitted by cutaneous and mucosal exposure to infectious blood or bodily fluids, such as semen, saliva, cervical secretions, and tears, and can survive up to 7 days on environmental surfaces. The typical mode of transmission of HBV varies in part with the prevalence of infection. Perinatal transmission is the predominant mode in high-prevalence areas, whereas horizontal transmission, particularly in early childhood, accounts for most cases in intermediate-prevalence areas. Unprotected sexual intercourse and intravenous drug use are the major routes of spread in low-prevalence areas.

The sequence of virologic markers of acute, self-limited HBV infection is depicted in Fig. 117.2. The incubation period of hepatitis B is 30 to 180 days (mean, 60–90 days). The first marker is HBsAg within 1 to 12 weeks, usually in 8 to 12 weeks. HBsAg is ordinarily detected 2 to 6 weeks before ALT or AST elevations and clinical symptoms and remains detectable during the entire course of HBV infection. In typical acute

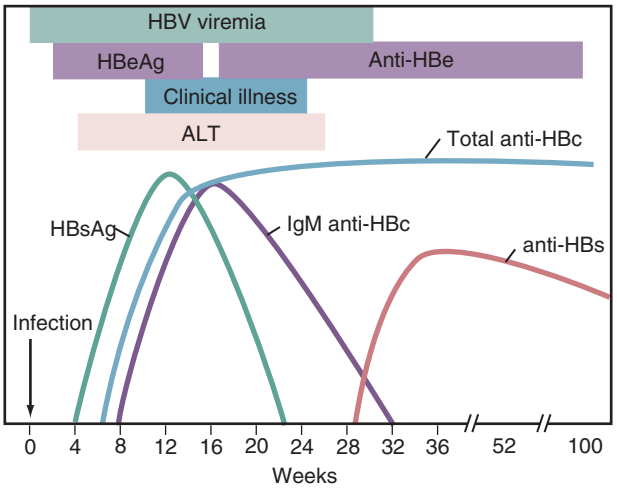

FIG. 117.2 Clinical, virologic, and serologic course of acute hepatitis B virus *(HBV)* infection. *ALT,* Alanine aminotransferase; *HBc,* hepatitis B core; *HBe,* hepatitis B envelope; *HBeAg,* hepatitis B e antigen; *HBs,* hepatitis B surface; *HBsAg,* hepatitis B surface antigen; IgM, immunoglobulin M.

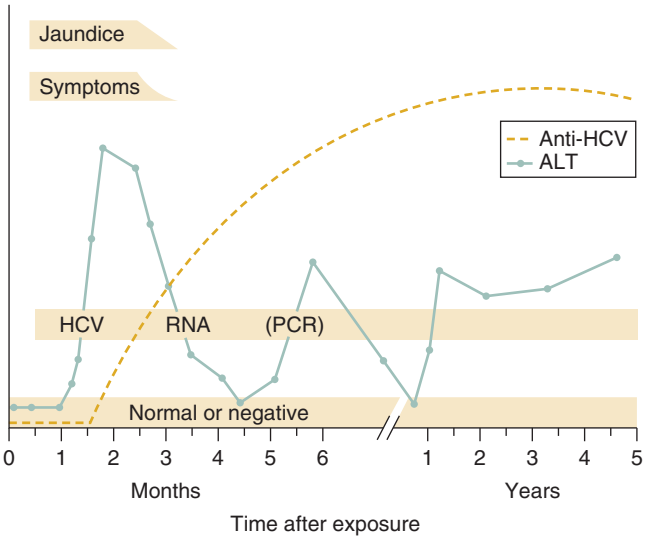

FIG. 117.3 Typical clinical, virologic, and serologic course of chronic hepatitis C virus *(HCV).* ALT, Alanine aminotransferase; PCR, polymerase chain reaction; RNA, ribonucleic acid. *(From Hoofnagle JH. Chronic hepatitis. In: Goldman L, Ausiello D, eds.* Cecil Medicine. *23rd ed. Philadelphia: Saunders; 2008.)*

cases, HBsAg becomes undetectable by 1 to 2 months after jaundice and rarely persists beyond 6 months. After HBsAg clears, anti-HBs becomes detectable and persists indefinitely. Anti-HBc can be present weeks to months before detection of anti-HBs. Hepatitis B e antigen (HBeAg), which appears concurrently with or shortly after the appearance of HBsAg, is associated with high levels of HBV replication and tends to be replaced by antibody to HBeAg (anti-HBe) beyond approximately 3 months.

Hepatitis C Virus (Also See Chapter 154)

HCV is a positive-strand RNA virus in the genus *Hepacivirus* of the family Flaviviridae. HCV is roughly spherical and is 55 nm in diameter. There are at least six major genotypes or clades, and a minor seventh genotype has been identified. The genotypes are highly diverse, but the extent of serotypic variation among genotypes is not well understood. The high level of virion turnover coupled with the absence of proofreading by the NS5B polymerase results in generation of multiple variants ("quasispecies") in an infected person. The use of autonomously replicating viral replicons and the establishment of a complete cell culture system have enabled detailed studies of HCV replication and identification of specific viral functions as targets for antiviral chemotherapy (see "Chronic Viral Hepatitis" later).

After infection, HCV RNA can be detected within days of exposure. The incubation period of hepatitis C is from 15 to 160 days (mean, 50 days). Viremia peaks 8 to 12 weeks after infection and then plateaus or drops to lower levels and persists in 85% to 90% of individuals (Fig. 117.3); the risk of chronicity after acute HCV infection has been linked to non-C *IL28B* haplotypes.[16] The major consequence of persistent HCV infection is hepatic fibrosis, which may progress to life-threatening cirrhosis and increased risk of HCC. This generally occurs more than 20 years after infection but can develop more rapidly in persons older than 50 years or those with immunosuppression or human immunodeficiency virus (HIV) infection (see Chapter 154).[17]

HCV RNA can be detected in blood, saliva, tears, seminal fluid, ascitic fluid, and cerebrospinal fluid; however, HCV infection is most often transmitted through percutaneous exposure to blood. In economically developed countries, most new HCV infections are related to intravenous drug use. HCV may also be transmitted between sexual partners and perinatally, although these modes of transmission are inefficient and much less frequent than in HBV infections. In developed nations, antibody to HCV is prevalent in 1% to 2% of the population. The World Health Organization (WHO) estimated that more than 185 million persons worldwide were infected with HCV; however, modeling of global HCV RNA prevalence data in 2015 suggested that the current prevalence of viremic HCV infection is 1% of the global population, corresponding to 71 million infected persons.[18]

Hepatitis D Virus (Also See Chapter 146)

HDV is a 32-nm circular, single-stranded RNA virus (genus: *Deltavirus*) that requires the presence of HBV or another hepadnavirus for replication. Therefore, HDV either can infect the host at the same time as HBV or can superinfect a host that is already infected with HBV. HDV has a viral envelope derived from HBV and has a similar tropism for hepatocytes. An in vitro culture system, however, has not been established for its growth, and thus the mechanism for injury of hepatocytes with HDV remains unclear. HDV prevalence generally mirrors prevalence of HBV worldwide; eight genotypes have been identified, but genotype 1 is predominant. It is primarily transmitted via parenteral routes, although sexual and intrafamilial transmission can also occur.

Simultaneous infection (coinfection) of HBV and HDV generally manifests as an acute self-limited hepatitis with full recovery, and chronic infection is seen in 2% of patients, driven primarily by chronic HBV infection. More severe acute infection can be seen, and fulminant hepatitis can occur. In contrast, HDV superinfection of a person with chronic HBV infection usually results in a severe infection, followed by chronic hepatitis D infection in 90% of patients. Fulminant hepatitis may occur and is 10 times more common in superinfection with HDV than in coinfection with HDV and HBV. In patients with chronic HDV infection, HDV is the dominant virus because it suppresses HBV replication. Once chronic HDV is established, the course of hepatitis is accelerated, and cirrhosis occurs in 60% to 80% of patients. If HDV is present, the risk of HCC is higher than with HBV infection alone.

Hepatitis E Virus (Also See Chapter 178)

HEV is a single-stranded, nonenveloped RNA virus that is 27 to 34 nm in diameter. It is the single member of the genus *Hepevirus* in the family Hepeviridae. HEV has four genotypes in humans (and a fifth genotype not detected in humans) and up to 24 subtypes. HEV is primarily enterically transmitted. Genotypes 1 and 2 are spread by means of fecally contaminated water in endemic areas and may be spread through blood transfusion, particularly in endemic areas. Person-to-person transmission is uncommon but has been documented in households. Highest rates of seroprevalence are in Asia, Africa, the Middle East, and Central America. Sporadic cases occur in Western countries, and, surprisingly, a relatively high seroprevalence (21%) has been found in the United States, apparently related to infection with attenuated genotypes 3 and 4, which are of limited pathogenicity and which have been linked to animal-reservoir (predominantly swine) exposure.

The incubation period of illness is 14 to 60 days (mean, 40 days), and clinical illness appears to be similar to acute hepatitis from other

hepatotropic viruses, although perhaps more severe than that seen with HAV. Fulminant hepatitis can occur, with case-fatality rates of 0.9% to 2.8% in men and up to 20% in pregnant women, particularly in the third trimester.[5–7] The reason for the markedly increased mortality in pregnant women is unclear but may be related to increased virus replication observed in pregnancy.[19] HEV can be detected in stool for 1 week before the onset of illness and for up to 2 weeks afterward (Fig. 117.4). Hepatitis E viremia is generally short in duration, but on occasion it can last up to 4 months.

HEV is not a cause of chronic hepatitis, except in immunosuppressed patients. These include solid-organ recipients, HIV-infected patients, and patients receiving chemotherapy for hematologic malignancies. In such patients, chronic HEV infection and progressive clinical liver disease have been reported to develop.[8,20–22]

Diagnosis

Serologic tests are available to enable making the diagnosis of acute hepatitis A, B, C, D, and E. An algorithm for a diagnostic approach to a patient with acute hepatitis begins with four serologic tests for the most common causes of viral hepatitis in the United States: HBsAg, immunoglobulin M (IgM) antibody to HAV (anti-HAV), IgM anti-HBc, and anti-HCV. Table 117.2 shows the interpretation of the results of those tests. Detection of HBeAg may be useful as a marker for viral replication and for relative infectivity late in the course of illness. Sensitive and specific tests are available for HBV DNA (see Chapter 145) and HCV RNA (see Chapter 154), and these are particularly useful in assessing the effect of antiviral therapy (see "Chronic Viral Hepatitis" later). If hepatitis E is under consideration, a serologic test for IgM antibody to HEV (IgM anti-HEV) or polymerase chain reaction (PCR) assay for HEV RNA in blood or stool can be sought from the Centers for Disease Control and Prevention.

Liver biopsies are not indicated in patients with acute, self-limited hepatitis. In protracted cases or in patients with cholestatic disease, liver biopsy may be helpful in establishing the diagnosis or differentiating the patient's presentation from that associated with other causes of liver injury. Typical findings in acute viral hepatitis include lobular disarray, apoptosis of hepatocytes, mononuclear cell infiltrates in portal and periportal areas, and cholestasis. Liver biopsies are seldom indicated in cases of fulminant hepatitis, and percutaneous biopsies are often contraindicated because of coagulation defects or thrombocytopenia.

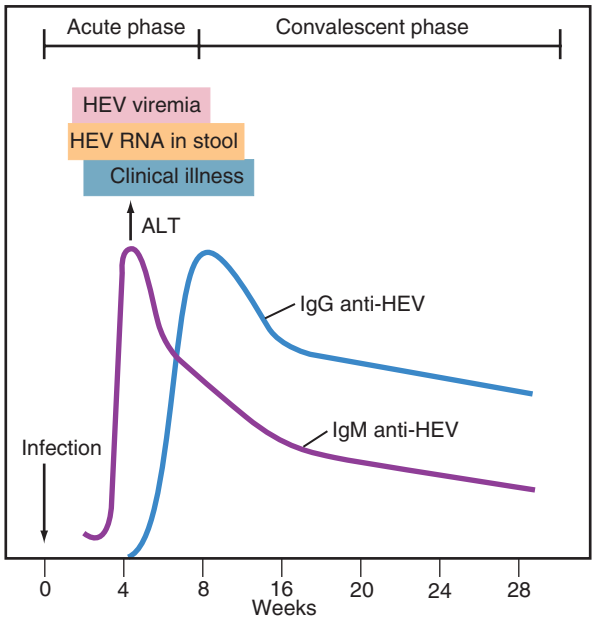

FIG. 117.4 Time course of clinical, virologic, and serologic events during acute hepatitis E virus *(HEV)* infection. *ALT,* Alanine aminotransferase; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *RNA,* ribonucleic acid.

Differential Diagnosis

A number of clinical entities can be considered in the differential diagnosis of acute hepatitis. These include drug-induced liver injury, ischemic hepatitis, autoimmune hepatitis, Budd-Chiari syndrome, Wilson disease, and syndromes related to pregnancy, such as acute fatty liver of pregnancy and the syndrome of hemolysis, elevated liver enzymes, and low platelets (HELLP).

Other viruses may cause acute hepatitis on occasion. EBV is a relatively common cause, and 90% of patients with heterophile-positive mononucleosis have mild AST and ALT elevations, at two to five times normal (see Chapter 138). Cytomegalovirus (CMV) infection may also be associated with mild elevations of AST and ALT in otherwise healthy individuals but can be associated with more severe and systemic disease in immunosuppressed patients (see Chapter 137). Herpes simplex virus may cause a generalized infection that is associated with hepatic abnormalities, most commonly in neonates and immunocompromised children and adults.

Yellow fever may be associated with acute hepatitis and can be severe. Serum AST is usually higher than ALT, probably because of skeletal or myocardial injuries or both. In severe cases, aminotransferase levels may be greater than 2000 IU/L. Serum bilirubin is most often in the range of 5 to 10 mg/dL. Yellow fever should be suspected in a recent traveler to enzootic areas who did not receive adequate immunization. Acute hepatitis may also be seen in patients with leptospirosis[23] and Q fever.[24]

Treatment

Most cases of acute viral hepatitis do not require specific treatment, other than generally supportive and symptomatic measures. Other than for clinically severe cases, hospitalization is not required. Focused or

TABLE 117.2 Simplified Diagnostic Approach in Patients Presenting With Acute Hepatitis

	SEROLOGIC TESTS OF PATIENT'S SERUM			
DIAGNOSTIC INTERPRETATION	**HBsAg**	**IgM Anti-HAV**	**IgM Anti-HBc**	**Anti-HCV**
Acute hepatitis B	+	–	+	–
Chronic hepatitis B	+	–	–	–
Acute hepatitis A superimposed on chronic hepatitis B	+	+	–	–
Acute hepatitis A and B	+	+	+	–
Acute hepatitis A	–	+	–	–
Acute hepatitis A and B (HBsAg below detection threshold)	–	+	+	–
Acute hepatitis B (HBsAg below detection threshold)	–	–	+	–
Acute hepatitis C	–	–	–	+

HAV, Hepatitis A virus; *HBc,* hepatitis B core; *HBsAg,* hepatitis B surface antigen; *HCV,* hepatitis C virus; IgM, immunoglobulin M.
Modified from Longo D, Fauci A, Kasper D, et al. Harrison's Principles of Internal Medicine. *18th ed. New York: McGraw Hill; 2011.*

prolonged bed rest is also not necessary for full recovery, although patients may feel more comfortable with some restriction of physical activity. A high-caloric diet is desirable, but because many patients may experience nausea later in the day, the major caloric intake may be best tolerated early in the day. Intravenous feeding may be necessary if the patient cannot tolerate oral feeding. Glucocorticoid therapy has no proven value in acute viral hepatitis.

In acute hepatitis B, the prognosis is excellent and specific antiviral therapy is not necessary. In rare cases of severe acute hepatitis B, however, treatment with a nucleoside analogue at oral doses used for chronic hepatitis B is recommended by many consultants (see "Chronic Hepatitis B" later), even though its efficacy in acute hepatitis B has not been established in clinical trials.

Because progression to chronic infection is the rule after acute hepatitis C, treatment of acute cases of hepatitis C is recommended and is supported by a meta-analysis of data from clinical trials.[25] Still, because >20% (and up to 50% in some experiences) of acute hepatitis C cases resolve spontaneously, current recommendations are to observe for 6 months after onset of acute hepatitis C and to reserve treatment for those who do not recover spontaneously. Treatment consists of 8 to 12 weeks of therapy with one of the four current-generation, all-oral, highly effective direct-acting antiviral (DAA) agents used for chronic hepatitis C (see later discussion of treatment of chronic hepatitis C).[26] In the setting of an acute needlestick injury derived from a patient with known or suspected hepatitis C, the individual who was stuck should be monitored with HCV RNA and AST and ALT tests, and should receive treatment for acute hepatitis C infection as noted earlier.

As noted earlier, most acute cases of hepatitis E are also self-limited and do not require specific therapy; however, uncommonly, acute cases may be severe, and ribavirin (RBV) has been used effectively in individual cases with apparently good clinical responses and reduction of HEV RNA levels in blood.[27,28] Supporting the use of RBV in severe acute hepatitis E are observations of the effectiveness of RBV therapy in achieving a sustained virologic response (SVR) in organ-allograft recipients with chronic hepatitis E.[29] Hepatitis E can be a severe and fatal disease in pregnant women, and therapy is necessary in such cases; however, RBV is contraindicated in pregnancy because of its teratogenicity. Studies of its potential use in this setting are worthy of consideration.[30] Anecdotal effectiveness of sofosbuvir (SOF) in severe, persistent hepatitis E has been reported but not confirmed in cinical trials.

Prevention

Hepatitis A

Both passive and active immunoprophylaxis are available against hepatitis A (see Chapter 316). Immune globulin (IG) preparations used to contain sufficient anti-HAV concentrations to provide protection; however, as reported in 2017, the reduction in levels of anti-HAV in plasma donors has been reflected by reduced potencies of commercial IGs used for hepatitis A prophylaxis.[31] Therefore the US Public Health Service has updated IG doses for postexposure or preexposure prophylaxis. Instead of 0.02 mL/kg,[32] the recommended IG dose has been increased to 0.1 mL/kg (7 mL in a 70-kg adult) for preexposure prophylaxis in persons planning up to a month of travel to an endemic area and for postexposure prophylaxis (household, sexual, institutional). For travelers to endemic areas who plan up to 2 months of travel or longer, the IG dose is 0.2 mL/kg (to be repeated every 2 months for travel lasting longer than 2 months).[33] Administration is recommended as early as possible after exposure and is effective as late as 2 weeks after exposure. Prophylaxis is not necessary for those who have already received hepatitis A vaccine or for those known to have serum anti-HAV. For postexposure prophylaxis against HAV for individuals 12 months to 40 years of age, a single dose of hepatitis A vaccine is as effective[34] and is preferred to IG when practical for both preexposure and postexposure prophylaxis. For those younger than 12 months or older than 40, immunocompromised persons, and those with chronic liver disease, IG is recommended at the new doses described earlier.

Highly effective inactivated hepatitis A vaccines are available and have been part of the childhood immunization schedule since 2006 in the United States. Based on persistent levels of anti-HAV after vaccination, protection has been predicted to last at least 25 to 30 years.[35] The availability of this vaccine has resulted in a dramatic reduction of hepatitis A in all areas where vaccine use has been extensive (see Chapter 174). Patients with chronic hepatitis B or C have an increased risk of severe hepatitis when infected with HAV and should therefore also receive hepatitis A vaccine.

Hepatitis B

Vaccines against hepatitis B are now part of childhood immunization schedules in the United States and in most developed countries; the first dose should be administered within the first 24 hours of life. Hepatitis B vaccine is now recommended for individuals with frequent exposures to HBV infections, such as health care workers exposed to blood and household and sexual contacts of persons with chronic HBV infection. Included as recommended candidates for the vaccine are injection drug users, residents and staff of institutions for developmentally disabled persons, adults with chronic liver disease, adults with end-stage renal disease, adults with HIV infection, and diabetics younger than age 60.[36] HBV vaccines are recombinant preparations (Recombivax HB [Merck, Rahway, NJ] and Engerix-B [GlaxoSmithKline, Brentford, London]), which are available individually or as part of combinations with HAV vaccine (Twinrix [GlaxoSmithKline]) or with DPT (diphtheria, pertussis, tetanus) and inactivated polio vaccine for children (Pediarix [GlaxoSmithKline]) (see Chapter 316). A new recombinant hepatitis B vaccine with a novel adjuvant (CpG 1018, a CpG-containing oligonucleotide sequence that activates Toll-like 9 receptors), Heplisav-B [Dynavax, Berkeley, CA], was approved in 2017 for adults aged 18 or older; two intramuscular injections given a month apart were shown to induce protective antibody in a higher proportion than three intramuscular injections (at time 0, 1 month, and 6 months) with Engerix-B, especially among vaccine recipients with type 2 diabetes.

For postexposure prophylaxis after exposure to HBV, a combination of hepatitis B immune globulin (HBIG) (0.06 mL/kg) for rapid presence of antibody and hepatitis B vaccine (full course) for long-lasting immunity is recommended for those who have not been vaccinated. This is recommended for percutaneous inoculation or transmucosal exposure to HBV-containing bodily fluids and for sexual contact with an individual with hepatitis B (see Chapter 316). All pregnant women should be tested for circulating HBsAg, and HBIG (0.5 mL) should be given to infants as soon as possible after birth but within 12 hours of delivery in conjunction with a dose of hepatitis B vaccine. Additional doses of vaccine are indicated at 1 month and 6 months of age.

Hepatitis C

IG is not effective in prevention of hepatitis C. Development of vaccines to hepatitis C is impeded by the extensive viral heterogeneity of HCV. For persons with multiple sexual partners or sexually transmitted diseases, the likelihood of transmission of HCV is increased, and use of condoms is recommended. Persons with hepatitis C should avoid sharing razors, toothbrushes, and nail clippers with sexual partners and family members.

Hepatitis D

No product is available specifically to prevent transmission of HDV. The major approach to prevention of hepatitis D is prevention of hepatitis B through vaccination with HBV vaccines. Persons with chronic HBV infection should avoid percutaneous exposure and intimate contact with persons who have HDV infection.

Hepatitis E

IG, even produced from populations where hepatitis E is highly endemic, does not appear to provide protection against infection with HEV.[37]

A recombinant hepatitis E capsid protein vaccine (Hecolin [Xiamen Innovax Biotech, Xiamen, China]) was highly protective against infection with HEV (100% efficacy) in a large-scale trial and was licensed in China in 2011.[38] Another recombinant HEV capsid protein vaccine developed by GlaxoSmithKline in collaboration with the US Army has also been shown to be highly effective (95.5% protection against HEV infection after three doses)[39] but has not been approved by the US Food and Drug Administration (FDA) (see Chapter 178 for further details). Vaccines against HEV would be directed at areas of high prevalence for infection, as noted earlier.

CHRONIC VIRAL HEPATITIS

The term *chronic viral hepatitis* is used to describe protracted hepatocellular necrosis and inflammation, often with fibrosis, that lasts longer than 6 months and is caused by HBV, HCV, HBV-associated HDV, or HEV. Nonviral causes of chronic hepatitis that may be confused clinically with chronic viral hepatitis include autoimmune hepatitis; metabolic-genetic disorders (Wilson disease, hereditary hemochromatosis, α_1-antitrypsin deficiency); alcoholic liver disease; nonalcoholic fatty liver disease; drug-induced liver disease; and granulomatous disorders. The chronic viral hepatitides, however, are readily distinguished from these other forms of chronic hepatitis with serologic or virologic testing. Classified histologically by the degree of hepatocellular necrosis and inflammation (grade) and fibrosis (stage; Table 117.3),[40–42] chronic viral hepatitis may be mild (even sometimes without liver injury at all, representing an inactive carrier state), moderate, or severe. With the potential to culminate in cirrhosis, portal hypertension, and even HCC, chronic viral hepatitis ranks as the fifth most common cause of human deaths worldwide and the tenth most common cause of deaths in the United States. In the United States, chronic viral hepatitis has been estimated to result in annual economic losses that exceed $1 billion. Left to follow its natural history, chronic viral hepatitis could account for an even more substantial health and economic burden in coming generations; however, a vaccine to prevent hepatitis B and highly effective antiviral therapies for both hepatitis B and C have already been shown and will continue to reduce the morbidity and mortality of this human scourge.

Neither of the enteric RNA hepatitis viruses, hepatitis A and hepatitis E, causes chronic hepatitis; however, acute hepatitis A can be followed by a protracted, cholestatic syndrome that may last many months.[43] Hepatitis E can cause a protracted and chronic hepatitis in immunosuppressed patients (see later). Similarly, rare instances have been reported in which classic autoimmune hepatitis was preceded and presumably triggered by an episode of acute viral hepatitis A, B, or C.[44–46] Both CMV and EBV have been implicated as agents of acute hepatitis, primarily in patients with immunocompromise, but in immunocompetent patients with systemic acute CMV or EBV infection (e.g., infectious mononucleosis), transient elevations of serum aminotransferase and alkaline phosphatase activities are common. Neither agent is a likely contributor to chronic hepatitis.

In all forms of chronic viral hepatitis, liver injury does not result directly from a cytopathic effect of the virus but instead from an ongoing host-mediated cytolytic T-cell response to virus-infected hepatocytes that is ineffective in achieving adequate clearance of all virus-infected cells.

TABLE 117.3 Contemporary Histologic Classification of Chronic Hepatitis According to the Modified Histologic Activity Index (HAI) of Ishak and Colleagues and the METAVIR Scale

	HAI	METAVIR
Necroinflammatory Activity (Grade)		
Periportal necrosis, bridging necrosis	0–4	0–3, 0–1
Intralobular necrosis, confluent	0–6	
Intralobular necrosis, focal	0–4	0–2
Portal inflammation	0–4	0–3
TOTAL	0–18	A0–A3[a]
Fibrosis (Stage)		
None	0	F0
Portal (some)	1	F1
Portal (most)	2	F1
Bridging (few)	3	F2
Bridging (many)	4	F3
Incomplete cirrhosis	5	F4
Cirrhosis	6	F4
TOTAL	6	4

[a]Necroinflammatory activity: A0, none; A1, mild; A2, moderate; A3, severe.

Chronic Hepatitis B

In persons in whom HBV infection fails to clear during acute infection, chronic infection is characterized serologically by persistence in serum of the HBV envelope protein, HBsAg. Among cases of chronic hepatitis B, several important distinctions, based on levels of HBV replication, epidemiologic considerations, and viral variants, merit attention (Table 117.4).

Distinctions Based on Relative Levels of Hepatitis B Virus Replication

Among patients with chronic hepatitis B, a distinction can be drawn between those with highly replicative HBV infection (>10^5–10^6 IU/mL) and those with levels of replication below this threshold. In the relatively replicative phase of chronic hepatitis B, levels of HBV DNA are high (detectable in the past with insensitive hybridization assays or, currently, at levels that exceed 10^5 to 10^6 IU/mL with sensitive amplification assays such as PCR and transcription-mediated amplification [TMA]); transmissibility of infection to contacts is favored; HBeAg, another serologic marker of HBV replication, is detectable; the expression of HBV nucleocapsid antigens (primarily hepatitis B core antigen [HBcAg]) in hepatocytes is demonstrable; and clinical markers of liver injury are present. Such patients tend to have moderate-to-severe chronic hepatitis B.[47]

At an annual incidence of approximately 10% to 15%, persons with highly replicative chronic hepatitis B lose markers of high replication and undergo spontaneous conversion to a relatively low replicative state characterized by limited infectivity and liver injury and loss of HBeAg and of detectable hepatocyte expression of HBcAg.[48–51] Such patients tend to have clinically mild chronic hepatitis or are even inactive carriers of the virus. Unlike hepatitis C, in which any level of virus replication can be associated with liver injury, in chronic HBV infection a threshold exists, on the order of approximately 10^3 to 10^4 IU/mL, below which liver injury is negligible or absent.[52–54] The distinction in level of HBV replication between these two relative phases of chronic HBV infection is reflected by a dramatic difference in infectivity. Babies born to mothers with high-level HBV replication (HBeAg-reactive, HBV DNA ≥10^6 IU/mL) have a 90% chance of chronic HBV infection, compared with babies born to mothers with low-level HBV replication, whose likelihood of chronic HBV infection is 10% or less.[55] Similarly, a person who sustains a needlestick injury from a needle contaminated by blood from someone with highly replicative chronic HBV infection has a greater than 30% chance of infection, but the likelihood of infection is only 0.1% if the needlestick derives from a person with low-level HBV replication.[56] Similar distinctions occur between the two groups in sexual transmission. Inactive hepatitis B carriers, who have low-level viremia, absent or negligible histologic activity, normal liver biochemical test results, and minimal infectivity, can undergo HBsAg–to–anti-HBs

TABLE 117.4 Distinctions Among Patients With Chronic Hepatitis B

Distinction Based on Levels of HBV Replication
HBV DNA ≥10^5–10^6 IU/mL vs. ≤10^3–10^4 IU/mL Wild-type HBeAg reactive vs. HBeAg nonreactive
Distinctions Based on Epidemiologic Considerations
High-prevalence areas: perinatal acquisition, host tolerance, clinically inapparent acute infection, chronicity >90%, high risk of cirrhosis and HCC Low-prevalence areas: adolescent or adult sexual or percutaneous acquisition, host intolerance, clinically apparent acute hepatitis, chronicity <1%, low risk of cirrhosis and HCC
Distinction Based on Viral Variants
Wild-type, HBeAg-reactive vs. precore or core-promoter (HBeAg-negative) variants

HBeAg, Hepatitis B e antigen; *HBV,* hepatitis B virus; *HCC,* hepatocellular carcinoma; *IU,* international units.

seroconversion (i.e., the serologic hallmark of recovery from hepatitis B) but at a frequency of only 1% to 2% per year.[57,58] In the setting of immunologic compromise (e.g., cytotoxic chemotherapy, immunosuppression for organ transplantation, anti–tumor necrosis factor-α [TNF-α] therapy for inflammatory bowel disease or rheumatologic disorders, HIV infection), inactive carriers and even persons who have recovered from hepatitis B can have reactivation, potentially severe, of hepatitis B.[59–61] Among the complications of chronic hepatitis B is HCC, postulated to be associated with incorporation of HBV DNA (but not at a recognized uniform site) into the host genome.[62–64] Ironically, conversion from a state of free episomal HBV DNA to one in which HBV DNA is integrated into the host genome tends to occur, in nature, as HBV infection converts from highly replicative to minimally replicative. In all likelihood, integration of HBV DNA into host DNA does not rely on this change in replicative status; instead, both integration and reduced replication tend to occur after prolonged HBV infection. Areas of high HCC and HBV prevalence overlap geographically[63]; the relative risk of HCC among persons with HBsAg is 100-fold higher than in persons without this marker of HBV infection.[62] Observations have shown that among persons with HBsAg, the risk of HCC resides primarily among those with markers of high-level HBV replication.[65,66] Similarly, severity and progression of chronic hepatitis B are more pronounced in patients with high levels of HBV replication.[49,67,68] Thus the level of HBV replication has a profound influence over the clinical expression of chronic hepatitis B.

Distinctions Based on Epidemiologic Considerations

Chronic hepatitis B follows acute HBV infection in more than 90% of neonates born to mothers with chronic hepatitis B but in only approximately 1% of immunocompetent adults with clinically apparent acute hepatitis B.[69–71] In Asia and sub-Saharan Africa, the prevalence of HBV infection is high, exceeding 10% of the population, and in many Western countries, such as the United States, prevalence rates of less than 1% are the rule. These differences in frequency translate into marked distinctions in clinical expression of HBV infection.

In high-prevalence areas, women of childbearing age have the high likelihood of infection characteristic of the population at large; consequently, the risk of perinatal transmission of HBV infection from infected mother to her baby is high and represents the most common mode of HBV transmission in the population. Acquired in the perinatal period, HBV infection is unlikely to be accompanied by clinically apparent acute hepatitis but almost universally results in chronic infection, a reflection of what appears to be tolerance of the virus by the host immune system,[69] even though some immunologic responses to HBV have been demonstrated in newborns. Although most such infected persons can have mild asymptomatic chronic hepatitis B during childhood and early to middle adulthood, when high-level HBV replication tends to be accompanied by no more than minimal necroinflammatory liver injury, the lifetime risk of succumbing to chronic hepatitis B in such high-HBV-prevalence populations can reach 40%, the result of liver injury linked to high HBV replication in the middle decades and beyond, culminating in cirrhosis and HCC.[62]

In contrast, in low-prevalence areas (e.g., United States, western Europe), the limited frequency of HBV infection in the general population does not support a large cohort of infected women of childbearing age; therefore, the frequency of perinatal transmission is low. Instead, most HBV infections in such Western countries occur during adolescence and early adulthood, enhanced by behaviors (e.g., sexual activity, injection drug use, occupational exposure to blood and contaminated instruments) that foster the spread of bloodborne agents. Such infections result typically in a robust host-immune response to the presence of HBV on hepatocyte membranes, recognized clinically as acute hepatitis, and the cytolytic T-cell response is usually sufficient to eradicate HBV infection. Chronicity of hepatitis B under these circumstances is rare (≤1%),[70,72] and even among those with chronic hepatitis B in such low-prevalence HBV populations, progression to cirrhosis, and especially to HCC, is much less likely. Therefore, hepatitis B tends to be a chronic progressive disorder associated with a risk of HCC in high-prevalence populations but an acute self-limited illness with a low risk of HCC in low-prevalence populations.

What appears to drive the distinction between hepatitis B acquired in infancy and hepatitis B acquired in adulthood is the level of immunologic tolerance to HBV—high in the former group and low in the latter. In fact, most persons with chronic hepatitis B do not recall having had an acute hepatitis-like illness (i.e., an episode in which the host immune system mounted a vigorous attack on HBV-infected liver cells). In this vein, the clinical expression of HBV infection in the host with immunocompromise resembles the pattern that occurs in high-prevalence early-life acquisition areas (high likelihood of chronic infection and its consequences) rather than the pattern in low-prevalence adulthood-acquired infection. Some authorities distinguish between an immune-tolerant phase of chronic HBV infection during the early decades of life (high-level HBV replication with near-normal biochemical markers of inflammation, ALT activity, and liver injury) and an immune-clearance phase in later decades (with levels of liver injury more appropriate to the level of HBV replication). This categorization, of course, does not apply to patients who acquire HBV infection during adulthood and who are immunologically intolerant of the virus from the outset of infection. Even among those infected at birth (e.g., as is the rule in high-HBV-prevalence Asian countries), liver inflammatory activity and fibrosis occur even in early decades,[73] and many infected persons have protracted periods of inactive liver injury during the so-called immune-clearance period. Therefore, a more accurate distinction after perinatally acquired infection is between relatively high levels of immunologic tolerance (or a dissociation between HBV replication and necroinflammatory liver injury) during the early decades of life and substantially lower levels of tolerance (and a link between HBV replication and necroinflammatory liver injury) during later decades. Ultimately, over the lifetime of such patients, except during the early decades, the degree of progression of liver disease does correlate with the level of HBV replication.[65–68]

At least 10 genotypes of HBV have been identified, varying in geographic distribution, but understanding of the impact of genotype on the severity and consequences of chronic hepatitis B is evolving.[74] For example, genotype C is more likely than genotype B to result in progressive chronic hepatitis B and HCC and to be associated with later HBeAg seroconversion.[75–77] Generally, HBV genotype considerations tend to be clinically irrelevant in most settings.

Distinctions Based on Viral Variants

The distinction between high-replication HBeAg-reactive and low-replication HBeAg-negative chronic hepatitis B has been complicated by the recognition of a variant of chronic hepatitis B, HBeAg-negative chronic hepatitis B. In this subset of patients with chronic hepatitis B, HBeAg is undetectable, but other markers of enhanced HBV replication (HBV DNA, intrahepatocytic HBcAg), elevation of serum aminotransferase activity, and histologic evidence of chronic liver injury are present. With mutations in the precore region of the HBV genome (precore mutations or core-promoter mutations [see Chapter 145]), these patients have HBV infection in which the gene for the soluble nucleocapsid HBeAg protein cannot be transcribed or translated.[78–81]

In general, these mutations are acquired later in the natural history of chronic hepatitis B among persons infected early in life (i.e., they follow infection with wild-type HBV infection).[82] The exception to this observation is patients with precore-mutant fulminant hepatitis B in whom HBeAg-negative infection is transmitted perinatally or horizontally from persons with precore-mutant hepatitis B.[83,84] Still, all patients with these mutations have circulating serum anti-HBe instead of HBeAg. The most common precore mutation represents a G-to-A mutation at nucleotide 1896, which results in a stop codon in the precore gene, where initiation of HBeAg transcription occurs. In HBV genotype A, the nucleotide at position 1858 is C, adjacent, complementary, and strongly bound to nucleotide G at position 1896 in the loop structure of this part of the viral genome. Because the C_{1858}-G_{1896} bond is so strong, mutations have to occur at both of these sites for a precore mutation to occur, which is rare; therefore, precore mutations are seldom seen in patients with HBV genotype A. In contrast, in genotypes other than A, the nucleotide at position 1858 is T, adjacent, complementary, and unstably bound to nucleotide 1896 in the HBV genome loop structure. Because the bond between T_{1858} and G_{1896} is so unstable, G_{1896}A mutations can occur readily, without the need for a complementary mutation at position 1858. Therefore,

precore mutations occur primarily among persons with HBV genotypes other than A.[85] Consequently, precore mutations and HBeAg-negative chronic hepatitis tended to be relatively uncommon in parts of the world (e.g., the United States) in which genotype A predominates but are common in other parts of the world (e.g., Mediterranean countries, Europe, Asia) in which other genotypes are prevalent.[85,86] Although wild-type chronic hepatitis B predominates in the United States, almost all contemporary cases of chronic hepatitis B in Europe and the Mediterranean are HBeAg negative; in the United States, HBeAg-negative hepatitis B, once rare, now accounts for up to 40% of cases, fueled by migration from these other countries.[74,82]

Levels of HBV DNA tend to be lower in patients with HBeAg-negative chronic hepatitis B, but episodic flares in necroinflammatory activity are common, as are severe and progressive chronic hepatitis and its consequences, cirrhosis and HCC.[58] Important distinctions in response to antiviral therapy between wild-type HBeAg-reactive and mutant HBeAg-negative chronic hepatitis B are discussed subsequently.

Pathophysiology and Natural History

Like the other human hepatitis viruses, HBV is not cytopathic but instead engenders an endogenous cytolytic T-cell response to virus-infected hepatocytes.[87] Although nucleocapsid HBV antigens appear to be the target for cytolytic T cells, the complex cellular and humoral host factors, and the interaction among these factors, that determine the severity, duration, and outcome of hepatitis B are poorly understood. As noted, some authorities have postulated that periods of relative inactivity followed by periods of accelerated liver injury represent an immune-tolerance phase and an immune-intolerance or immune-clearance phase, respectively, in the natural history of chronic hepatitis B.[54] Such a model, however, is too simplistic and fails to account for demonstrated immune responses or for low-level or episodic liver injury that occurs during periods postulated to represent a tolerance phase, for periods of quiescence that can occur later in the natural history of chronic hepatitis B, for reactivations that can occur at any time during the course of chronic hepatitis B, or for waxing and waning necroinflammatory activity that occurs so commonly in HBeAg-negative chronic hepatitis B. Currently, the consensus view is that viral proteins are presented by antigen-presenting cells to $CD4^+$ (Th1) lymphocytes and to $CD8^+$ (Th2) lymphocytes. In acute, self-limited HBV infection, the $CD4^+$/Th1 response dominates, supporting cytolytic T-cell destruction of viral antigen-expressing hepatocytes. In chronic hepatitis B, Th2 responses dominate, and the weaker cytolytic T-cell response to HBV-infected hepatocytes is insufficient to clear virus-infected cells but sufficient to maintain inefficient but persistent hepatocyte injury. Evidence in experimental chimpanzee infection, however, suggests that this adaptive cellular immune response plays a secondary or "mop-up" role after the innate immune response, which clears 90% of the virus before, and without any, liver injury.[88–91] Evidence has been marshaled as well to support the presence of T-cell exhaustion in chronic hepatitis B. The pathogenesis and natural history of chronic hepatitis B are covered in more detail in Chapter 145.

Clinical Manifestations

In chronic hepatitis B, symptoms run the gamut from absent to severe, debilitating, and life-threatening.[67] Among inactive hepatitis B carriers and persons with mild-to-moderate chronic hepatitis B, symptoms are usually absent, although some with mild-to-moderate chronic hepatitis B report fatigue and, less commonly, right upper quadrant discomfort or "fullness." More severe and advanced cases can be associated with fatigue and jaundice, but persons with compensated cirrhosis may have no symptoms at all. Decompensated cirrhosis may be accompanied by fatigue, jaundice, loss of muscle mass (weight loss), ascites, edema, bruising (coagulopathy), gastrointestinal bleeding (gastroesophageal varices or portal hypertensive gastropathy), and hepatic encephalopathy. As is the case for acute hepatitis B and its associated serum-sickness-like prodromal syndrome, chronic hepatitis B may be complicated by immune-complex manifestations, including cutaneous vasculitis, arthritis, glomerulonephritis, and generalized vasculitis.[92]

Viral and laboratory markers of HBV infection are summarized in Table 117.5. The biochemical hallmark of chronic hepatitis B is elevation of serum aminotransferase activity, with normal to near-normal alkaline phosphatase activity. In severe, progressive, and decompensated chronic hepatitis B, bilirubin levels can increase (hepatic excretory dysfunction); albumin can fall, and prothrombin time can become prolonged (hepatic synthetic defect); and hypersplenism can occur (primarily thrombocytopenia and leukopenia). Autoantibodies are usually absent but may be present at low levels.

Histologic features of chronic viral hepatitis vary from absence of necrosis and inflammation to architectural distortion and fibrosis characteristic of cirrhosis. In general, histologic injury (grade) and fibrosis (stage) are categorized as mild, moderate, or severe. The level of necroinflammatory activity or injury is based on periportal necrosis, portal inflammation, and intralobular necrosis. Mild activity is confined to portal tracts, and necrosis and inflammation spilling beyond the limiting plate of periportal hepatocytes (interface hepatitis) signify a more severe injury with increased potential for progression. The degree of fibrosis is based on the localization and extent of scar tissue; fibrosis confined to portal areas is mild, and fibrosis that reaches beyond the portal tract (septal or bridging fibrosis), especially that which links portal tracts to other portal tracts or to central veins, connotes a more advanced and progressive process (see Table 117.3).[1,93]

Treatment

The goal of antiviral therapy in chronic hepatitis B is prevention of clinical and histologic progression, which, in turn, can be achieved with eradication or, more likely, suppression of HBV replication to below the threshold for liver injury (10^3–10^4 IU/mL).[53] In patients with HBeAg-reactive chronic hepatitis B, HBeAg to anti-HBe seroconversion tends to be associated (in up to approximately 80% of cases) with a sustained reduction in HBV replication that persists even after therapy is discontinued. Therefore, in HBeAg-reactive chronic hepatitis B, HBeAg seroconversion is a potential end point of therapy, after a consolidation period. For noncirrhotic HBeAg-positive patients, therapy should be continued for an additional 6 months after HBeAg seroconversion. Moreover, Lee and colleagues[94] found a higher rate of durable HBeAg seroconversion after extending treatment with lamivudine to 12 months, and most authorities would recommend a full 12-month consolidation period after HBeAg seroconversion in patients who had acquired HBV infection in infancy (as is common among Asian patients) and even potentially longer (or indefinitely) in cirrhotic patients.[94–97] In patients

TABLE 117.5 Viral and Laboratory Markers of Hepatitis B Virus Infection

DIAGNOSIS	HBsAg	Anti-HBs	IgM Anti-HBc	IgG Anti-HBc	HBeAg	Anti-HBe	ALT
Acute hepatitis	+	–	+	–	+	–	Elevated
Recovered (immune)	–	+	–	+	–	+	Normal
HBeAg-positive chronic hepatitis B	+	–	–	+	+	–	Elevated
HBeAg-negative chronic hepatitis B	+	–	–	+	–	+	Elevated (may be intermittent)
Vaccinated (immune)	–	+	–	–	–	–	Normal
Inactive carrier	+	–	–	+	–	+	Normal

ALT, Alanine aminotransferase; *HBc,* hepatitis B core (antigen); *HBe, HBeAg,* hepatitis B e antigen; *HBs, HBsAg,* hepatitis B surface antigen; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M.

with HBeAg-negative chronic hepatitis B, however, HBeAg seroconversion is not an option, and a sustained response (i.e., maintained even after cessation of treatment) is a less realistic goal; long-term suppressive therapy is usually necessary.[82] Chronic HBV infection is associated with the presence in hepatocytes of stable covalently closed circular HBV DNA (cccDNA). Although proponents of one type of therapy over another invoke relative efficacy of a drug in reducing cccDNA, no therapies have been shown to eradicate this stable form of HBV DNA, whereas all therapies that reduce HBV replication have also been shown to reduce cccDNA. Similarly, persons with spontaneous HBeAg seroconversion have a concomitant reduction in serum and liver HBV DNA and in hepatic cccDNA. Successful antiviral therapy with any agent is likely to have a beneficial impact on the long-term natural history of chronic hepatitis B.[98–100] Similarly, oral agents have been shown to retard the clinical progression of HBV-associated compensated cirrhosis and advanced fibrosis,[101,102] and an approximately 30% reduction in the number of patients with end-stage chronic hepatitis B listed per year for liver transplantation coincided with the introduction and wide use of oral antivirals between the years of 2000 and 2006.[103]

According to recommendations of the American Association for the Study of Liver Diseases (AASLD),[104] candidates for antiviral therapy have HBV DNA levels of more than 2×10^3 or $\times 10^4$ IU/mL (standardized IU/mL have replaced copies/mL [1 IU = 5.6 copies/mL] in contemporary usage), usually with elevated ALT levels and with histologic evidence of liver injury. Patients with HBeAg-reactive (with HBV DNA levels $>2 \times 10^4$ IU/mL) and HBeAg-negative (with HBV DNA levels $>2 \times 10^3$ IU/mL) chronic hepatitis B and with ALT two or more times the upper limit of normal (ULN) are candidates for therapy, according to the AASLD and, with slight modifications, guidelines from other societies.[105–107] Patients who meet HBV DNA criteria but have ALT less than two times the ULN constitute an indeterminate group in whom a liver biopsy or a noninvasive measure of liver fibrosis (e.g., liver elasticity) may be useful to guide treatment decisions (when moderate-to-severe inflammation, necrosis, fibrosis, or a combination of these are present). In the European Association for the Study of the Liver (EASL) guidelines, the threshold for treatment in both HBeAg-positive and HBeAg-negative chronic hepatitis B is HBV DNA $>2 \times 10^3$ IU/mL and an ALT level greater than the ULN. For patients who do not meet HBV DNA and ALT thresholds, included in factors to be considered that favor therapy are age above 40, family history of HCC, previous treatment history, or extrahepatic immune-complex manifestations. All patients with compensated cirrhosis with any detectable level of HBV DNA, regardless of ALT level, should be treated. Patients with decompensated chronic hepatitis B are not candidates for interferon (IFN) therapy, which is contraindicated because of ineffectiveness and intolerability, but are candidates for therapy with any level of HBV DNA or ALT and have been shown to derive therapeutic benefit from nucleoside or nucleotide analogues. Antiviral therapy is not indicated in inactive hepatitis B carriers (with persistently normal ALT, undetectable or very low HBV DNA [$<2 \times 10^3$ IU/mL], and normal to near-normal histologic findings).

Eight antiviral drugs have been approved in the United States by the FDA for treatment of chronic hepatitis B: injectable recombinant IFN-α,[108] pegylated (PEG) IFN-α,[109,110] and six oral agents—the nucleoside analogues lamivudine,[111–114] entecavir,[115,116] and telbivudine,[117,118] and the nucleotide analogues adefovir dipivoxil,[119,120] tenofovir disoproxil fumarate (TDF),[121] and tenofovir alafenamide[122,123] (also see Chapter 47). The relative antiviral potencies of the approved drugs are listed in Table 117.6. Of the eight, PEG IFN-α, entecavir, and the two tenofovir formulations are used as first-line agents.

TABLE 117.6 Relative Potencies of Approved Antiviral Drugs for Chronic Hepatitis B

	LOG_{10} REDUCTION OF HBV DNA IN HBeAg-REACTIVE DISEASE
Interferon-α	Not reported
Adefovir	3.5
Pegylated interferon	4.5
Lamivudine	5.5
Tenofovir	6.2
Telbivudine	6.5
Entecavir	6.9

HBeAg, hepatitis B e antigen; *HBV,* hepatitis B virus; *LOG,* logarithm.

Interferon-Based Therapy for Chronic Hepatitis B

Although reports that describe antiviral activity of IFNs in patients with chronic hepatitis B date back to the mid-1970s,[124] not until the late 1980s was the convincing efficacy of IFN-α in chronic hepatitis B shown in controlled clinical trials.[108,125] IFNs induce host antiviral gene expression and signal-transduction pathways and enhance cytolytic T-cell activity targeted at HBV-infected hepatocytes. A meta-analysis of 24 randomized controlled trials that involved 1299 patients showed that those with standard IFN-α treatment (5–10 million units subcutaneously for 16–24 weeks[126]) had a 25% advantage in normalization of ALT activity, a 25% advantage in HBeAg loss, a 23% advantage in clearance of HBV DNA as measured with hybridization assay (detection threshold of approximately $\geq 10^5$–10^6 virions/mL), and a 6% advantage in HBsAg loss.[127] Relapse is infrequent after a successful HBeAg response, and among patients with IFN-associated HBeAg responses, posttreatment HBsAg seroconversions occur in up to 80% over the decade after therapy in Western patients,[98,100,127–129] but not in Asian patients.[130,131] In addition, the natural history of chronic hepatitis B has been shown to be improved after successful IFN therapy; during the decade after successful IFN therapy, both long-term survival and freedom from hepatic decompensation have been reported,[99] as has a reduction in the frequency of HCC.[130,132] The first approved IFNs for hepatitis B were recombinant IFN-α preparations administered three times a week or every other day; however, long-acting once-weekly PEG IFN has supplanted standard IFN-α. A polyethylene glycol moiety is covalently linked to IFN through a reaction called pegylation, which renders the drug more stable and less immunogenic. Two forms are available: PEG IFN-α2a and PEG IFN-α2b. Although they differ in size (40 kD vs. 12 kD) and dosage regimen (180 μg vs. 1.5 μg/kg/wk), their efficacy appears similar; however, PEG IFN-α2a is the only PEG IFN approved for hepatitis B. IFN is administered via subcutaneous injection, and resistance does not emerge during therapy, as it does for some of the oral agents (see subsequent discussion).

Large-scale trials of 48 weeks of treatment with PEG IFN-α2a 180-μg injections weekly versus lamivudine 100 mg by mouth daily versus combination PEG IFN–lamivudine were conducted in HBeAg-positive and HBeAg-negative populations. In HBeAg-positive patients, at the end of the 48-week treatment period, HBV DNA was suppressed by 4.5 $\log_{10}$, 5.8 $\log_{10}$, and 7.2 $\log_{10}$ in the three groups, respectively. Although HBeAg seroconversions occurred during therapy in comparable proportions of all three groups (27%, 20%, and 24%, respectively), 24 weeks after cessation of therapy, HBeAg seroconversions were most common, 32%, in the PEG IFN monotherapy arm, compared with 27% in the combination arm and 19% in the lamivudine monotherapy arm.[109]

In HBeAg-negative patients, during 48 weeks of treatment, HBV DNA was suppressed by 4.1 $\log_{10}$, 4.2 $\log_{10}$, and 5.0 $\log_{10}$ in the PEG IFN monotherapy, lamivudine monotherapy, and combination therapy groups, respectively. Yet 24 weeks after completion of therapy, HBV DNA was suppressed to undetectable ($<10^2$ copies/mL [studies were done before adoption of international units per milliliter]) in only 19%, 20%, and 7% of the three groups, respectively.[110] The degree of HBV DNA suppression in the PEG IFN arms was durable 6 months later, at the end of 2 years (17%), at the end of 3 years (13%–18%), and at 5 years (12%)[133,134]; however, this is the only trial to demonstrate such short-term and long-term benefits of PEG IFN for HBeAg-negative chronic hepatitis B. In a randomized trial of 48 weeks of PEG IFN plus RBV versus PEG IFN in 138 patients with HBeAg-negative chronic hepatitis B, the addition of RBV conferred no benefit, but the more important outcome was the limited efficacy of PEG IFN–based therapy. Only 7.5% of the study population achieved suppression of HBV DNA

to less than 400 copies/mL at week 24 after treatment, and HBsAg loss did not occur in any trial participant.[135] In a study of patients with HBV genotype D, HBeAg-negative patients treated with PEG IFN for a median of 23 months,[136] *IL28B* genotype "CC" was associated with a higher rate of HBsAg loss (29%) than non-CC genotypes (13%); the beneficial impact of CC over non-CC genotypes has also been reported for HBeAg-reactive chronic hepatitis B.[137] Another predictor of PEG IFN responsiveness in chronic hepatitis B is the quantitative HBsAg level—a decline within the first 12 to 24 weeks and a reduction to less than 20,000 IU/mL at week 24. If these milestones are not achieved, therapy will be ineffective and can be stopped.[138,139]

These observations notwithstanding, in general, the value of PEG IFN in HBeAg-negative patients remains controversial because the benefits of therapy may not outweigh its side effects and intolerability.

Toxicities of IFN include systemic flulike symptoms; malaise; fatigue; marrow suppression (cytopenias); emotional liability (manifesting primarily as irritability, depression, or anxiety); hair loss or thinning; weight loss; and autoimmune events, most commonly thyroiditis. Side effects are usually manageable but limit therapy in those with marked hypersplenism and severe psychiatric disorders.

During IFN-based therapy, ALT flares that are typically well tolerated can occur, except in patients with advanced cirrhosis, in whom such flares can precipitate hepatic decompensation. Patients treated with IFN need frequent monitoring of blood counts and thyroid-stimulating hormone and close medical supervision. Because of its better efficacy and preferable once-weekly administration, PEG IFN has replaced standard IFN as first-line therapy. In practice, PEG IFN is discontinued after 1 year of therapy, whereas the oral agents are continued until treatment end points are met; the extension of therapy with oral agents can yield ultimate serologic response rates comparable with or exceeding those of PEG IFN (see subsequent discussion).

Direct Oral Antiviral Agents

Lamivudine. Although lamivudine has been superseded by newer, more effective oral agents, many important lessons gleaned from experiences with lamivudine apply to antiviral therapy with other nucleoside analogues. Lamivudine, the (−)enantiomer of β-L-2′,3′-dideoxy-3′-thiacytidine and an analogue of deoxycytidine, is a reverse transcriptase inhibitor that results in HBV and HIV RNA chain termination (see Chapter 128). The first nucleoside analogue, approved in 1998, for the treatment of chronic hepatitis B, lamivudine, 100 mg daily administered orally, reduces HBV DNA replication by a median of up to 5.5 logs.[115,117,140] In HBeAg-reactive chronic hepatitis B, 1 year of lamivudine treatment, whether in treatment-naïve or IFN-experienced patients, is associated with HBeAg loss in just under one-third, HBeAg seroconversion in 16% to 21%, histologic improvement in 50% to 60%, and a return to normal of aminotransferase activity in 40% to 75%.[111–117] Comparable results have been achieved in children.[141] Suppression of HBV DNA to levels undetectable with hybridization assays (with detectability thresholds of 10^5–10^6 virions/mL) is achieved in almost all patients[111–114] and to levels undetectable with PCR amplification assays (thresholds of 10^2–10^3 virions/mL) in 30% to 44%.[115,117] Unlike IFN, lamivudine is effective in patients with high-level HBV DNA, but like IFN, lamivudine is more likely to result in HBeAg loss and seroconversion among patients with high baseline aminotransferase levels.[112,142,143] In fact, for the subgroup of patients with ALT levels five or more times the ULN, whether Caucasian or Asian, lamivudine therapy achieves HBeAg seroconversion in approximately 55%. In contrast, for patients with normal aminotransferase activity, common in Asian populations, HBeAg responses are rarely achieved. HBeAg seroconversions are more likely if HBV DNA suppression is profound, confined to patients with HBV DNA suppressed to less than 10^4 virions/mL.[53] If HBeAg responses occur and therapy is stopped after 1 year, the response is durable in more than 80% of patients and (at least in Western cohorts, but not in Asians) may be followed by HBsAg seroconversion (>20% after 2–3 years).[95,96,144,145] Lamivudine responses are more durable among patients with more protracted treatment duration after HBeAg response[97,146,147] and among those with HBV genotype B than with C.[147] Lamivudine-associated HBV DNA suppression was found to be substantially more profound in patients with HBV subtype *ayw* (genotype D) than in those with subtype *adw* (genotype A).[148] If HBeAg remains detectable during therapy, cessation of therapy results in a return to baseline virologic and biochemical levels; long-term therapy, then, is indicated to maintain clinical benefit, and HBeAg seroconversions have been shown to increase over time with duration of therapy, approaching 50% after 5 years.[144,145,149]

During a year of lamivudine therapy, progression of fibrosis is retarded,[150] and with more protracted therapy, histologic benefit continues. Moreover, in patients with compensated cirrhosis treated for 3 years cumulatively, regression of cirrhosis has been documented histologically to occur in two-thirds of patients.[151,152]

In HBeAg-negative chronic hepatitis B, an HBeAg serologic response is not a goal; instead, responsiveness is measured with biochemical, virologic, and histologic end points. In this subgroup of patients treated for a year, biochemical responses are achieved in approximately 60% to 80% and histologic responses in two-thirds.[116,117,153] Undetectable HBV DNA as measured with PCR can be achieved in approximately 60% to 70% of patients, and HBV DNA can be suppressed by up to 4.7 $\log_{10}$ copies/mL.[116,117] When therapy is discontinued, a return to baseline is almost invariable; therefore, in order to maintain benefit in HBeAg-negative chronic hepatitis B, long-term therapy is necessary.[154,155]

During lamivudine treatment, flares in ALT activity accompany HBV DNA suppression in approximately 40% of patients, and in the absence of a sustained response (i.e., HBeAg response), posttreatment ALT flares occur in approximately 20% to 30% of patients.[112,156] Flares in biochemical activity may be no more common in patients with lamivudine treatment than in matched placebo recipients, but posttreatment flares are more common in treated patients.[145] Usually, posttreatment flares are mild and asymptomatic, but severe, even life-threatening, posttreatment flares have been reported, primarily in patients with cirrhosis with marginal hepatic compensation before treatment.[157] Therefore, whenever lamivudine is discontinued, patients should be monitored closely for several months.

Although patients with decompensated cirrhosis are not candidates for IFN therapy, they can respond to lamivudine, which can stabilize, retard, or even reverse clinical and laboratory markers of hepatic failure.[158–160] In one report of 23 patients referred to a liver transplantation center after the availability of lamivudine, the survival rate at 4 years was 60%. In contrast, among 23 patients referred to the same center in the period immediately before the availability of lamivudine, all patients for whom donor livers were not available died within just over a year.[159] In addition, a placebo-controlled trial of lamivudine in patients with compensated cirrhosis (or advanced hepatic fibrosis) showed a significantly higher frequency of disease progression (≥2-point elevation in Child-Turcotte-Pugh compensation score, life-threatening complications of cirrhosis, emergence of HCC) in the placebo group compared with the lamivudine group, which provided proof of principle that nucleoside-analogue maintenance therapy can prevent hepatic decompensation in patients with cirrhosis.[101] The observation that lamivudine treatment can retard the clinical progressions of cirrhosis adds to the evidence that drug treatment can improve the natural history of chronic hepatitis B, as has been shown in Western patients treated with IFN for chronic hepatitis B.[99]

Besides ALT flares that accompany treatment-related suppression of HBV DNA (similar to those seen during IFN therapy and to those that occur during spontaneous HBeAg seroconversion) and that accompany cessation of treatment (as viral replication resumes), no adverse effects of therapy were identified in registration trials of lamivudine compared with placebo, nor have adverse events been linked to lamivudine since its approval.[156] Unfortunately, when used as monotherapy, lamivudine is associated with viral resistance.[161] Mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) region of the HBV polymerase emerge in approximately a quarter of patients treated with lamivudine for a year.[53,111–114,153,161–163] The most common of these are methionine-to-valine or methionine-to-isoleucine mutations in polymerase domain C at amino acid 204 (M204V, M204I), often accompanied by upstream leucine-to-methionine mutations (L180M) in domain B.[164–166] With each successive year of lamivudine therapy, the frequency of YMDD mutations increases, reaching 70% at 5 years.[167] Initial observations suggested that YMDD-mutant hepatitis B was less "replicatively competent" in vitro,[162,168] which corresponded with the

biochemical, virologic, and histologic benefit (compared with pretreatment baseline) maintained after transient ALT and HBV DNA elevations accompanying the emergence of the mutations.[161] Although HBeAg seroconversions continue to occur after the emergence of YMDD mutations, the frequency of HBeAg responses is lower in YMDD-mutant chronic hepatitis B, and ultimately, clinical benefit is lost in some patients.[144,151,161,167,169] This clinical observation may be explained by the emergence of compensatory mutations in other polymerase domains.[165] In patients with immunologic compromise, such as those with HIV infection or those receiving immunosuppressive therapy after liver transplantation, the emergence of YMDD variants occurs earlier after the initiation of lamivudine therapy and is more likely to precipitate severe acute hepatitis-like flares and hepatic decompensation.[170] Severe flares can occur, as reported most frequently in Asian patients,[171] and are more common in patients with marginally compensated cirrhosis.[172] Moreover, in persons with HBV-HIV coinfection, treatment with lamivudine monotherapy results in universal rapid HIV resistance[173]; therefore, testing for HIV infection should be done before the initiation of lamivudine therapy for hepatitis B. When HBV-HIV coinfection is identified, lamivudine monotherapy is contraindicated; instead, combination drug therapy should be instituted, and if lamivudine is used, the 300-mg HIV dose should be given.

When lamivudine was introduced, no other nucleoside analogues were available (the value of lamivudine-IFN combination therapy was never established in YMDD-variant HBV infection). Because patients tended to do well clinically, certainly compared with baseline pretreatment status, for at least 1 or 2 years after the emergence of YMDD-mutant hepatitis B[144,151,161]; because cessation of lamivudine after YMDD mutations resulted in reversion to wild-type HBV and its attendant higher ALT and HBV DNA levels[163]; and because other antivirals were not available, the general approach was to continue lamivudine after the emergence of YMDD variants unless clinical benefit was lost. Now that other antivirals are available to which YMDD variants are responsive,[164,166,174,175] breakthrough resistance to lamivudine is managed with the addition of another antiviral drug (addition of adefovir or replacement by tenofovir; see later discussion).

The optimal duration of lamivudine or any oral therapy has not been determined. In HBeAg-reactive chronic hepatitis B, treatment may be stopped 6 months after HBeAg seroconversion, and the durability of the response is anticipated to be greater than 80%.[95,96] Data suggest that Asian patients should be treated even longer before stopping therapy.[97] For patients who have not achieved an HBeAg response, and for patients with HBeAg-negative chronic hepatitis B, lamivudine or any oral treatment may have to be extended indefinitely.

As the first oral antiviral introduced for hepatitis B, lamivudine has been studied extensively and has an excellent safety record. On the other hand, its poor resistance profile and the subsequent availability of newer, more potent, more effective, and less resistance-prone agents (see subsequent discussions) have reduced dramatically the current use of lamivudine and rendered it almost obsolete; it is no longer recommended as first-line therapy.

Adefovir dipivoxil. Adefovir dipivoxil, approved by the FDA in 2002, is the oral prodrug of adefovir, an acyclic phosphonate nucleotide analogue of adenosine monophosphate with antiviral activity against HBV and other hepadnaviruses, retroviruses such as HIV, and herpesviruses. Although doses of 30 mg or more daily are associated with nephrotoxicity, at a daily oral dose of 10 mg, adefovir dipivoxil suppresses HBV replication by medians of approximately 3.5 $\log_{10}$ in HBeAg-reactive hepatitis B and 3.9 $\log_{10}$ in HBeAg-negative hepatitis B, in both treatment-naïve and IFN-failed patients.[119,120] In a phase III clinical trial among HBeAg-reactive patients, adefovir treatment, 10 mg daily for 48 weeks, resulted in histologic improvement in 53%, return of ALT to normal in 48%, undetectable HBV DNA with PCR assay (<400 virions/mL) in 21%, loss of HBeAg in 24%, and HBeAg seroconversion in 12%.[120] A 30-mg group included in this study but abandoned because of nephrotoxicity achieved even better results: histologic response in 59%, biochemical response in 55%, undetectable HBV DNA with PCR assay in 29% (median 4.76 $\log_{10}$ reduction in copies/mL of HBV DNA), HBeAg loss in 27%, and HBeAg seroconversion in 14%.[120] As is the case for IFN and lamivudine, adefovir is more likely to result in HBeAg seroconversion in patients with elevated baseline ALT levels; for both the 10-mg and 30-mg doses of adefovir, the frequency of HBeAg seroconversion increased to 21% for the subset of patients with baseline ALT levels five or more times the ULN.[120] HBeAg seroconversion during adefovir therapy results in a durable response that allows treatment to be discontinued; in the absence of an HBeAg response, indefinite continuation of therapy is necessary.

In a phase III clinical trial among HBeAg-negative patients, adefovir treatment, 10 mg daily for 48 weeks, resulted in histologic improvement in 64%, return to normal of ALT in 72%, and undetectable HBV DNA with PCR assay (<400 virions/mL) in 51%.[119] Reactivation is the rule when treatment is discontinued, as observed after short-term IFN or lamivudine therapy. The histologic efficacy of adefovir is similar in patients with both mild and advanced fibrosis. In addition, adefovir reduces $\log_{10}$ HBV DNA levels comparably across all HBV genotypes.[176] Whether cross-genotype activity represents an advantage of adefovir over lamivudine is debatable; HBV genotyping was not available when lamivudine registration trials involving approximately 1000 patients were done, and the number of patients with lamivudine treatment in reports of variable responses across genotypes is too small to be conclusive.[147,148] In addition, HBeAg responses to adefovir did differ, ranging from 7% to 20%, among HBV genotypes A to D, but the numbers of HBeAg responses were too small for adequate statistical comparisons.[176] Like lamivudine, adefovir appears to retard progression of fibrosis in patients treated for a year.[119,120] Although reports have appeared in the literature to suggest that adefovir reduces cccDNA in treated patients, in fact, cccDNA is reduced (but not to undetectable levels) in patients who undergo HBeAg seroconversion spontaneously and in those who achieve seroconversion when treated with IFN, lamivudine, or adefovir.[177]

Adefovir, a flexible acyclic nucleotide that is similar to its natural substrate, is less likely to be sterically hindered by mutated amino acids in the HBV polymerase YMDD binding site pocket.[164] During courses of adefovir therapy that lasted 48 to 60 weeks in patients with immunocompetence, immunocompromise (e.g., persons with HIV coinfection or liver allograft recipients), HBeAg reactivity, and HBeAg negativity, no viral resistance was encountered.[178] Moreover, lamivudine-resistant, YMDD-mutant HBV responds to adefovir.[174,175] In clinical trials of adefovir for lamivudine-resistant YMDD-mutant hepatitis B, adefovir monotherapy (i.e., a switch from lamivudine monotherapy to adefovir monotherapy) reduced HBV DNA by a median of 4 $\log_{10}$ copies/mL and was just as effective as adefovir-lamivudine combination therapy (i.e., the addition of adefovir to lamivudine).[174,175] On the basis of this observation, some authorities had recommended switching to adefovir monotherapy in patients who become lamivudine resistant; however, when this is done, especially if any delay is introduced between the cessation of lamivudine and the addition of adefovir, ALT flares are encountered in approximately one-third of patients.[175] In addition, in vitro studies indicate that adefovir is actually more effective (half maximal inhibitory concentration [IC_{50}] is lower and resistance is reduced) in lamivudine-resistant than in wild-type HBV.[165,166] Finally, adefovir resistance (see subsequent discussion) occurs more frequently in patients who *switch* from lamivudine to adefovir compared with those in whom adefovir is *added* to lamivudine.[179,180] These observations argue for maintenance of lamivudine when adefovir is added because of lamivudine resistance. Although no resistance occurs during 48 or 60 weeks of adefovir therapy,[178] adefovir resistance does begin to emerge during the second year of treatment and can reach 30% at 5 years.[181] These mutations occur in polymerase domain D, usually an asparagine-to-threonine mutation at residue 236 (N236T),[182] but also in domain B, an alanine-to-valine mutation at residue 181 (A181V). These adefovir-associated mutations respond to lamivudine.

Safety of adefovir at a dose of 10 mg daily is similar to that of placebo.[119,120] Elevations of ALT occur during and after cessation of therapy, just as observed with lamivudine treatment. At doses of 30 mg or more a day of adefovir, however, nephrotoxicity is encountered.[120] A distal renal tubular acidosis occurs, with falling phosphorus and rising creatinine levels. At a dose of 10 mg, adefovir has a high therapeutic index, and creatinine elevations of 0.5 mg/100 mL or more were not observed in the registration trials of adefovir.[119,120] At doses of 30 mg or more, nephrotoxicity is not encountered before 6 to 8 months of

therapy, and when it occurs, it is reversible. Creatinine monitoring is recommended when adefovir is used, and reductions in dose frequency are mandated for patients with reduced creatinine clearance (CrCl; for CrCl 20 to 49 mL/min, every other day; for CrCl 10 to 19 mL/min, every third day; for patients undergoing hemodialysis, once a week after dialysis).

Although adefovir filled an important niche when it was introduced, providing effective therapy for lamivudine resistance, the drug has several limitations. Adefovir remains the least potent of all available oral agents in suppressing HBV DNA and in achieving HBeAg seroconversion, and it suppresses HBV DNA relatively slowly in most patients and hardly at all (by <2 $\log_{10}$) in a sizable proportion of treated patients.[183] Therefore, adefovir has been supplanted by its superior successor tenofovir (see subsequent discussion) and is no longer considered first-line therapy.

Entecavir. Entecavir (approved in 2005) is a cyclopentyl guanine nucleoside analogue that interferes with priming of the HBV polymerase, with reverse transcription of the minus strand of DNA and with DNA-dependent synthesis of the positive strand of HBV DNA. At a daily dose of 0.5 mg given for 48 weeks, entecavir was shown to be superior to lamivudine in preliminary and registration trials.[115,116] In patients with HBeAg reactivity, entecavir results in HBeAg seroconversion in 21%, reduces HBV DNA by a median of 6.9 $\log_{10}$ (5.0 $\log_{10}$ in patients with HBeAg negativity), suppresses HBV DNA to undetectable levels (<10^2 $\log_{10}$ copies/mL) with PCR assay in 67% (90% in HBeAg-negative patients), returns aminotransferase levels to normal in 68% (78% in HBeAg-negative patients), and improves histologic findings in 72% (70% in HBeAg-negative patients). Continuation of entecavir after the first year has been shown to result in increased HBeAg seroconversion rates (up to 39% at year 3) and HBsAg loss (5% at 2 years).[184,185] Data extending the duration of entecavir therapy for up to 5 years with a concomitant increase in dose from 0.5 mg to 1.0 mg daily in patients who did not seroconvert after 1 year are now available.[186] An additional 23% (33 of 141) achieved HBeAg seroconversion with treatment, although only 1.4% (2 of 145) lost HBsAg. A retrospective analysis of the 94 Asian patients enrolled in this extension study found that 95% of patients maintained an HBV DNA level of less than 300 copies/mL, and 76% had normalized levels of ALT at 5 years.[187] Important to note, long-term therapy was well tolerated, and resistance occurred in only 1 patient.

Although entecavir is cross-resistant with lamivudine, the blood levels achieved with daily doses of 0.5 or 1 mg exceed the increased IC_{50} for YMDD-variant hepatitis B in most patients.[165] At an increased dose of 1 mg daily, entecavir has also been proven effective in, and approved for use in, lamivudine-resistant chronic hepatitis B.[188] In previously treatment-naïve patients, entecavir resistance has not been encountered during the first year of therapy in phase II or phase III clinical trials; with its high barrier to resistance, entecavir has been observed to maintain its favorable resistance profile in cohorts followed for up to 5 years (1.2%).[184,189,190] On the other hand, resistance does occur at an unacceptable rate (from 7% at 1 year up to 43% at 4 years) among treatment-experienced patients with preexisting lamivudine resistance. In this setting, entecavir resistance requires the emergence of both YMDD-mutant HBV and a second mutation in domain B (T184A or I169T), C (S202G/I), or D (M250V).[191] Therefore, although approved for this indication, entecavir has not been embraced for the treatment of lamivudine-resistant HBV infection.

Although entecavir was found in preclinical toxicity testing to be associated with hepatic, pulmonary, and brain tumors in rodents, similar toxicity has not been detected in other animal species or in humans.[192,193] On the contrary, entecavir therapy has been associated with a reduction in HCC and in all-cause mortality.[194–196] Entecavir has a safety profile indistinguishable in clinical trials from that of lamivudine, but post-treatment ALT flares are much less frequent,[115,116] and the durability of HBeAg responses is comparable with that of the other oral agents. Initially believed to have no antiviral activity against HIV, entecavir does have low-level anti-HIV activity and cannot be used as monotherapy in patients with HBV-HIV coinfection who need treatment for HBV infection.[197] The entecavir dose should be reduced for patients with CrCl <50 mL/min.

Because of its high potency; high efficacy in achieving biochemical, serologic, virologic, and histologic endpoints; high barrier to resistance; beneficial impact on the natural history of chronic hepatitis B; and excellent short-term and long-term tolerability and safety profile, entecavir is recommended as first-line antiviral treatment for chronic hepatitis B.

Telbivudine. Telbivudine (approved in 2006) inhibits HBV polymerase, interfering with plus-strand synthesis of HBV DNA; in large-scale registration human trials, at an oral dose of 600 mg/day, it achieved a median 6.4 $\log_{10}$ (HBeAg-positive patients) and 5.2 $\log_{10}$ (HBeAg-negative patients) reduction in HBV DNA at 52 weeks.[117] In HBeAg-positive and HBeAg-negative patients, telbivudine suppressed HBV DNA to undetectable (<10^2 $\log_{10}$ copies/mL) in 60% and 88%, reduced ALT to normal in 77% and 74%, and improved histologic findings in 65% and 67%, respectively. HBeAg seroconversion occurred in 22% at the end of 1 year, with an increase to 30% at the end of year 2.[198] As an L-nucleoside, telbivudine is cross-resistant with lamivudine. Although telbivudine resistance at 1 year was less common (in 5% of HBeAg-positive and 2% of HBeAg-negative patients) than lamivudine resistance, by year 2[117] telbivudine resistance had increased to 22% and 9%, respectively.[198] The combination of telbivudine plus lamivudine is not additive and achieves no greater inhibition of HBV DNA than telbivudine alone.[118] In fact, none of the currently available nucleoside or nucleotide analogues provide more profound HBV suppression in combination with any others than the more potent one used alone.

Telbivudine has the same excellent tolerability profile as lamivudine (except for creatine kinase elevations), and durability of HBeAg responses after cessation of therapy is similar to that of the other antivirals (approximately 80%). Despite its high potency, telbivudine has not been widely embraced as therapy for hepatitis B because of its poor resistance profile and its inferiority to other available drugs; it is no longer recommended as a first-line agent for hepatitis B, and its production and distribution were discontinued after December 2016.

Tenofovir. Two formulations of tenofovir are available: TDF, approved for hepatitis B in 2008, and TAF, approved in 2016.

Tenofovir disoproxil fumarate (TDF), another acyclic nucleotide, approved as an inhibitor of HIV reverse transcriptase, has similar activity in vitro against wild-type and lamivudine-resistant hepatitis B. In trials among patients with immunocompetence and immunocompromise (e.g., with HIV-HBV coinfection and after liver allograft) and treatment-naïve or lamivudine-resistant patients, TDF, 300 mg daily, reduced HBV replication by more than 6 logs; achieved HBV DNA suppression much more rapidly and consistently, with negligible nephrotoxicity and resistance; and was superior to adefovir.[116,120,199,200] In a registration trial,[121] TDF (compared with adefovir; data shown for 48 weeks, except as noted) reduced HBV DNA by 6.2 $\log_{10}$ in HBeAg-reactive patients and by 4.6 $\log_{10}$ in HBeAg-negative patients and to levels undetectable with PCR in 76% and 93%; resulted in normal ALT in 68% and 76%; and improved histologic findings in 74% and 72%, respectively. HBeAg seroconversion was achieved in 21% at the end of 1 year, 27% at the end of 2 years, 34% at the end of 3 years, and 40% at the end of 5 years; HBsAg loss was recorded in 3% at 1 year, 6% at 2 years, and 8% at 3 and 5 years,[121,201,202] and no resistance was encountered through year 3[202] and year 5[90] and beyond to year 8. Among 348 patients who completed 5 years of therapy with TDF and who underwent liver biopsies at baseline and at the end of treatment, 87% had histologic improvement, 51% had a regression in fibrosis score, and 74% (71 of 96) no longer had cirrhosis (Ishak fibrosis score 5 or 6) as defined as a greater than or equal to 1-unit decrease in score.[102]

The safety profile of TDF continues to be reassuring in more than 5 years of patient experience on therapy. The rates of nephrotoxicity and renal tubular dysfunction are approximately 1%. In a 5-year, treatment-extension study, only 9 of 585 (2%) patients experienced a serious adverse event attributed to TDF. These side effects included an increase in ALT or AST, increase in lactate dehydrogenase (LDH), and three reports of a reduction in bone mineral density.[102] For patients with CrCl <50 mL/min, the TDF dose should be reduced.

TDF is effective and recommended for patients with resistance to lamivudine (common when this drug was in popular use) or entecavir (rare). In the past, in patients with lamivudine resistance, adefovir was added to lamivudine (to avoid ALT flares and adefovir resistance), but tenofovir preparations (TDF or TAF; see later) are so potent and have

such a high barrier to resistance that the current recommendation is to switch from lamivudine or entecavir and rely on tenofovir monotherapy. In clinical trials, such monotherapy is as effective as combination therapy, without the emergence of resistance during 3 to 5 years of observation.[203–205]

On the basis of potent suppression of HBV DNA, efficacy data, compelling evidence of histologic improvement, a high barrier to resistance, an excellent safety and tolerability profile, and a reduction in the long-term consequences of chronic hepatitis B (e.g., HCC),[196] TDF is recommended as first-line therapy for chronic hepatitis B; moreover, classified as pregnancy category B, tenofovir should be considered for women with chronic hepatitis B during their childbearing years and during pregnancy, when therapy is indicated.

Tenofovir alafenamide (TAF) is a prodrug of tenofovir that must be activated to tenofovir in hepatocytes, allowing for higher targeted delivery of the drug to the liver and a 90% reduction in systemic exposure, reducing significantly the risk of proximal renal tubular injury and loss of bone density (phosphate wasting as a result of proximal tubular injury). At an oral dose of 25 mg daily, TAF is as effective and well tolerated as 300 mg daily of TDF with a comparably high barrier to resistance. Two randomized, double-blind, phase III, noninferiority trials of TAF versus TDF were conducted, one in HBeAg-reactive and one in HBeAg-negative patients.[122,123] Primary end points for TAF versus TDF at week 48 were as follows, respectively. In *HBeAg-reactive* patients, HBV DNA was below 29 IU/mL in 64% and 67%; ALT was normal (in central laboratory) in 72% and 67%; HBeAg loss or seroconversion occurred in 14% and 10% and in 12% and 8%. HBsAg loss was negligible, occurring in 4 of 576 (1%) and 1 of 288 (0.3%). Mean reductions in bone density were lower for TAF (e.g., hip −0.10%) than for TDF (hip −1.72%), mean increases in serum creatinine were smaller for TAF (0.01 mg/dL) versus TDF (0.03 mg/dL), and median reductions in CrCl (estimated glomerular filtration rates) were lower for TAF (−0.6 mL/min) than for TDF (−5.4 mL/min). In *HBeAg-negative* patients, HBV DNA was below 29 IU/mL in 94% and 93%; ALT was normal (in central laboratory) in 83% and 75%. No study subjects lost HBsAg. Mean reductions in bone density were lower for TAF (e.g., hip −0.29%) versus TDF (hip −2.16%). Mean increases in serum creatinine were comparably small for TAF (0.01 mg/dL) and TDF (0.02 mg/dL), but median reductions in CrCl (estimated glomerular filtration rates) were lower for TAF (−1.8 mL/min) than for TDF (−4.8 mL/min). These findings were similar at week 96, the more substantial ALT reduction with TAF was sustained over 96 weeks, and no tenofovir resistance occurred in either group through 96 weeks. Between weeks 96 and 120, when initial TDF recipients were switched to TAF, earlier differences between TDF and TAF in ALT normalization, renal function, and bone density all resolved.

TAF is now one of the first-line antiviral drugs for chronic hepatitis B recommended for patients with impaired renal function (CrCl <50 mL/min), those with reduced bone density, anyone at risk for renal injury, and patients older than age 60, who are more prone to the potential nephrotoxicity of TDF.[106] The dose of TAF should be reduced for patients with CrCl below 15 mL/min.

Other Agents for Chronic Hepatitis B

Approved as therapy for HIV infection, but not approved officially for HBV infection, the cytosine analogue emtricitabine is similar in structure, activity, and resistance to lamivudine (see Chapter 128).[206] Although it has no advantage over lamivudine, emtricitabine is available (for HIV/AIDS) in a combination tablet with tenofovir, which some clinicians use when combination therapy is advisable (e.g., for patients with already established L-nucleoside resistance).

Clevudine, a pyrimidine nucleoside analogue that was approved for use in Korea, was being evaluated in clinical trials versus adefovir as comparator. Although clevudine has been purported to eradicate cccDNA, this clinical end point was not documented, and although HBV DNA is much slower to rebound after clevudine therapy, clinical trials showed no advantage of clevudine in potency or resistance profile over available agents.[207] In 2009, development of this drug was halted because of the emergence of severe myopathy after prolonged use.

Although the current armamentarium of antiviral drugs is effective in suppressing HBV replication profoundly and in achieving short-term and long-term improvements in clinical end points, most patients require therapy of indefinite duration; therefore efforts are underway to evaluate inhibitors that interfere with other components of the HBV life cycle and that enhance host immune responsiveness to HBV. Among the viral targeting strategies are inhibitors of viral entry, HBV core assembly, cccDNA, and HBV secretion; novel technologic approaches such as RNA interference and gene editing with CRISPR/Cas9 are being investigated. Immunomodulatory approaches being applied include reconstitution of innate and adaptive immune responsiveness, Toll-like receptor agonists, T-cell vaccines, checkpoint inhibitors, IFN gene agonists, and retinoic acid inducible gene-I (RIG-I) activation. These studies are in their early stages, and, to date, none has been successful in "curing" HBV infection—that is, in achieving HBsAg loss and seroconversion, the pattern of recovery from infection, and in eliminating the need for continued antiviral therapy.[208]

Optimal Use of Available Agents for Hepatitis B

The trend in drug development has been in the direction of agents with progressively more profound inhibition of HBV replication. Although the proportion of HBeAg-reactive patients who achieve HBeAg seroconversion after 1 year of therapy (approximately 20%) is no greater for the more potent oral agents than for the less potent,[115,117,121] patients treated with any drug who achieve the most robust inhibition of HBV DNA are the most likely to achieve HBeAg responses and to avoid drug resistance.[117,209] Thus, the more potent the antiviral in inhibiting HBV replication, the better the outcome is likely to be (see Table 117.6). Potentially, combination therapy with high-potency, low-resistance agents could achieve even more limited resistance and treatment synergies, such as improved antiviral kinetics.[210,211] To date, however, advantages of combination therapy have not been shown in clinical trials.[109,118] For example, in a trial of combination tenofovir and entecavir therapy, after 2 years the benefit of dual antiviral therapy compared with entecavir monotherapy was modest and restricted to a small subset of patients with HBeAg and very high baseline HBV DNA ($\geq 10^8$ IU/mL), in whom HBV DNA was reduced to less than 50 IU/mL in 79% versus 62% of patients, respectively; however, even in this subset, the difference in rates of HBeAg loss and HBeAg seroconversion were not statistically significant between the groups.[190] Therefore, currently, combination therapy is restricted to patients with established drug resistance,[174,175,180] and, as aforementioned, for the contemporary high-potency, low-resistance agents, monotherapy appears to be just as effective as combination therapy.[203–205]

Recommendations for Therapy

Recommendations or guidelines for the treatment of chronic hepatitis B have been issued by the AASLD,[105] the EASL,[106] the Asian Pacific Association for the Study of the Liver,[107] and several other official bodies (Table 117.7). PEG IFN, entecavir, or tenofovir is the preferred first-line therapy for chronic hepatitis B; however, the availability of entecavir and tenofovir in resource-limited settings across the world continues to be an issue.[212] Although an approved first-line treatment with an excellent frequency of 1-year HBeAg seroconversion, PEG IFN may not be as competitive as the first-line oral agents entecavir and tenofovir, the efficacy, simplicity of use, tolerability profile, and high barrier to resistance of which are appealing to physicians and patients. As discussed later, any advantage in achieving HBeAg seroconversion after a 1-year course of PEG IFN injection therapy is overcome by extending oral-agent therapy beyond a year, and HBeAg seroconversion frequencies increase with each year of additional oral-agent therapy, associated rarely, if at all, with the emergence of resistance variants. Moreover, therapy with entecavir or tenofovir has been shown to be associated with histologic evidence of regression of fibrosis and cirrhosis after 3 to 5 years of therapy.[102,213] Patients with hepatitis B genotype A have particularly favorable rates of HBeAg seroconversion and even HBsAg loss with PEG IFN compared with patients with non-A genotypes,[214] and some authorities favor PEG IFN in this subgroup of patients; however, patients with genotype A are also highly responsive to oral-agent therapy. Additional details for the antiviral drugs for hepatitis B are presented in Chapter 47.

A consensus from the AASLD recommendations[105] is that HBeAg-negative patients with normal ALT activity and HBV DNA levels below

TABLE 117.7 Recommendations and Guidelines of the American Association for the Study of Liver Diseases for the Treatment of Chronic Hepatitis B

HBeAg	HBV DNA (IU/mL)	ALT	TREATMENT	FIRST-LINE AGENTS
Chronic Hepatitis B Without Cirrhosis				
+	>2 × 10^4	>2 × ULN	Recommended	PEG IFN, ETV, TDF, TAF
+	>2 × 10^4	≤2 × ULN	Not recommended; consider assessment of fibrosis[a]; consider for age >40, family history of HCC, extrahepatic manifestations	
–	>2 × 10^3	>2 × ULN	Recommended	PEG IFN, ETV, TDF, TAF
–	>2 × 10^3	<2 × ULN	Not recommended; consider assessment of fibrosis[a]; consider for age >40, family history of HCC, extrahepatic manifestations	
–	≤2 × 10^3	Normal	Not recommended[b]	
Cirrhosis				
±	Detectable (Regardless of HBV DNA level)	Normal or ↑	Recommended	Compensated: treat with ETV or TDF, TAF for detectable HBV DNA (even <2 × 10^3 IU/mL, regardless of ALT level
±	Regardless of HBV DNA level	Normal or ↑	Recommended	Decompensated[c]: treat indefinitely with ETV or TDF, TAF
±	Undetectable (PCR[d])	Normal or ↑	Not recommended	[c]

Table is updated to reflect supplanting of the less effective drugs by the best available antiviral drugs.[104] Guidelines issued by the European Association for the Study of the Liver are similar, with the following exceptions: HBV DNA threshold of >2 × 10^3 IU/mL for HBeAg positive or HBeAg negative; ALT threshold greater than upper limit of normal.[218]

[a]If liver biopsy reveals advanced necroinflammatory activity or fibrosis, or if noninvasive assessment of fibrosis reveals advanced fibrosis, consider treatment.

[b]Inactive carrier.

[c]If decompensated, coordinate care with a liver transplantation center.

[d]<10^3 IU/mL.

ALT, Alanine aminotransferase; *ETV,* entecavir; *HBeAg,* hepatitis B e antigen; *HBV,* hepatitis B virus; *HCC,* hepatocellular carcinoma; *IU,* international units; *PCR,* polymerase chain reaction; *PEG IFN,* pegylated interferon; *TAF,* tenofovir alafenamide; *TDF,* tenofovir disoproxil fumarate; *ULN,* upper limit of normal.

the threshold associated with liver injury (≤2 × 10^3 IU/mL; i.e., inactive hepatitis B carriers) are not candidates for antiviral therapy. On the other hand, patients with HBV DNA levels greater than 2 × 10^4 IU/mL (for HBeAg-reactive patients) or greater than 2 × 10^3 IU/mL (for HBeAg-negative patients), and ALT levels greater than two times the ULN, are considered candidates for therapy. Recommendations issued by the EASL are similar, but the HBV DNA threshold for treatment is 2 × 103 IU/mL in both HBeAg-positive and HBeAg-negative patients, and the ALT threshold is above the ULN.[106] Such patients have been shown to benefit from antiviral therapy and are assumed to have at least moderate necroinflammatory activity and fibrosis at liver biopsy; therefore, liver biopsy is not necessarily needed. For HBeAg-reactive patients with HBV DNA greater than 2 × 10^4 IU/mL, but normal or minimally elevated ALT levels less than two times the ULN, common among those who acquire HBV infection at birth, sustained responses are not likely, and treatment is not recommended (except for patients aged ≥40, a family history of HCC, previous treatment history, or extrahepatic immune-complex manifestations). For patients who meet HBV DNA criteria for treatment but with normal to near-normal ALT (less than two times the ULN per AASLD; less than the ULN per EASL), a liver biopsy or a noninvasive assessment of liver fibrosis is recommended to assess the extent of liver injury and fibrosis, which, in turn, is helpful in the decision of whether or not to treat.[105,106] Some authorities suggest that therapy should be instituted earlier in life among those with high HBV replication but low ALT.[215,216] These thresholds and criteria, however, do not meet the most rigorous evidence-based standard, and adopting them has never been shown to result in clinical benefit.[105,217]

Treatment of patients with compensated cirrhosis has been shown to delay clinical progression.[101] All patients with cirrhosis and HBV DNA >2 × 10^3 IU/mL, independent of ALT level, should be treated according to the aforementioned AASLD guidelines; treatment is also indicated for compensated cirrhotic patients with HBV DNA <2 × 10^3 IU/mL. AASLD guidelines are essentially the same as EASL guidelines for patients with compensated cirrhosis, which include as candidates for therapy patients with any detectable level of HBV DNA, regardless of ALT level. For decompensated cirrhosis in the presence of any detectable HBV DNA, with any level of ALT, urgent therapy may be lifesaving, but care should be coordinated with a liver transplantation center. Patients with cirrhosis should be treated with oral agents, not PEG IFN.

Choice of Antiviral Agents

On the basis of demonstrated superiority, PEG IFN has supplanted standard IFN, entecavir has supplanted lamivudine, and tenofovir has supplanted adefovir; although better than lamivudine, telbivudine has a poor resistance profile, which limits its appeal. Therefore, for most patients with chronic hepatitis B who are candidates for therapy, either PEG IFN, entecavir, or tenofovir is a potential first-line treatment (see Tables 117.7 and 117.8).[104,218,219] Several authorities recommend IFN-based therapy as the first-line approach, focusing on its apparent immunomodulatory activity, the brief duration of treatment, the higher frequency of HBeAg seroconversion after a year of therapy, the potential for HBsAg seroconversion, and the absence of resistance.[109,110,219–223] In fact, ALT elevations attributed to the immunologic effects of IFN are seen just as commonly during nucleoside or nucleotide analogue therapy,[112,120] and restoration of endogenous cytolytic T-cell activity or function occurs during nucleoside or nucleotide analogue therapy, as the level of HBV DNA is reduced.[224–226] Some favor PEG IFN for the subgroups most likely to respond (i.e., those with high ALT, low HBV DNA levels, genotypes A and B, and early age)[222,223]; however, these are also the populations most likely to respond to nucleoside or nucleotide analogues. Moreover, HBsAg seroconversions were no more common in patients with IFN treatment than with lamivudine treatment in a head-to-head trial (although these HBsAg results were not included in the published report),[113] and the frequency of HBsAg loss during PEG IFN therapy can be accomplished with the newer oral agents after 1 to 2 years or longer. Similarly, rates of HBeAg seroconversion with oral agents extended beyond 1 year are comparable with those achieved with a year of PEG IFN[a] but without the need for injections, side effects, or intensive clinical and laboratory monitoring. Although PEG IFN requires

[a]References 131, 132, 168, 171, 172, 180, 204.

TABLE 117.8 Approved Antiviral Drugs for Chronic Hepatitis B

	IFN	PEG IFN	LAM	ADV	ETV	TBV	TDF	TAF
Dose	5 MU/day[a]	180 µg/wk	100 mg/day	10 mg/day	0.5 mg/day	600 mg/day	300 mg/day	25 mg/day
Route	Subcutaneous	Subcutaneous	Oral	Oral	Oral	Oral	Oral	Oral
Duration in trials	4 mo	48 wk	48–52 wk	48 wk	48 wk	52 wk	48 wk	48 wk
Tolerability	Poor	Poor	Excellent	Excellent[b]	Excellent	Excellent	Excellent[b]	Excellent
HBeAg seroconversion	18%–20%	27%[c]	16%–21%	12%	21%	22%	21%	10%
Log_{10} HBV DNA reduction								
HBeAg positive	?[d]	4.5	5.5	3.5	6.9	6.5	6.2	Not reported, likely same as TDF
HBeAg negative	?[d]	4.1	4.7	3.9	5.0	5.2	4.6	Not reported, likely same as TDF
HBV DNA PCR negative								
HBeAg positive	?	25%	44%	21%	67%	60%	76%	64%
HBeAg negative	?	63%	73%	64%	90%	88%	93%	94%
ALT normalization								
HBeAg positive	?[e]	39%	75%	61%	68%	77%	68%	72%
HBeAg negative	?[e]	38%	79%	77%	78%	74%	76%	83%
HBsAg loss during treatment	up to 8%	3%–4%	≤1%	0%	2%	<1%	3%	≤1%
Viral resistance	None	None	15%–30%	None	None	2%–5%	0%	0%
>1 yr	None	None	5 yr: 70%	5 yr: 29%	≤1%	2 yr: 9%–22%	0%	0%
Cost (US $)	5600[a]	18,500	2500	6600	8700	6000 (no longer available)	13,000	12,000

Data shown are maximum reported values for up to 1 year (48–52 weeks) of treatment in registration trials. These comparisons are head-to-head only for PEG IFN versus LAM, ETV versus LAM, TBV versus LMV, and TDF versus ADV.
[a]5 MU daily to 10 MU three times per week for 16 to 24 weeks; the cost is that of a complete course, not of a year of therapy.
[b]Creatinine monitoring recommended.
[c]At 32% 24 weeks after therapy.
[d]When these studies were conducted, HBV DNA was measured with insensitive hybridization assays.
[e]ALT is generally normal in those with adequate serologic/virologic response; reports rarely include ALT levels at the end of treatment. In HBeAg-reactive patients, a meta-analysis showed a 23% advantage in ALT normalization (after therapy) in treated versus untreated patients.[670]
ADV, Adefovir; *ALT,* alanine aminotransferase; *ETV,* entecavir; *HBeAg,* hepatitis B e antigen; *HBsAg,* hepatitis B virus surface antigen; *HBV,* hepatitis B virus; *IFN,* interferon; *LAM,* lamivudine; *MU,* million units; *PCR,* polymerase chain reaction; *PEG IFN,* pegylated interferon; *TAF,* tenofovir alafenamide; *TBV,* telbivudine; *TDF,* tenofovir disoproxil fumarate.

therapy of a brief finite duration and is not associated clinically with resistance, the need for subcutaneous injections and often intolerable systemic side effects reduce its appeal. In addition, resistance to the newest oral agents, entecavir and tenofovir, is absent to negligible even after several years of therapy.[184] Furthermore, oral agents are effective in a substantial proportion of patients with failure to respond to IFN,[114] including patients with immunocompromise[170,227,228] and patients with hepatic decompensation.[158–160]

For patients with lamivudine resistance, the *addition* of adefovir while continuing lamivudine has been recommended,[173,175,179,180,211] but tenofovir is a better choice. When resistance to lamivudine emerged, adefovir as a second drug that is not cross-resistant was added; however, because of its higher potency and barrier to resistance, tenofovir monotherapy ("switch" rather than "add") suffices. Although entecavir at double the regular dose is approved for treatment of lamivudine-resistant hepatitis B,[188] the frequency of emergent entecavir resistance in this population is unacceptably high.[184] Because the stakes are so high when resistance emerges in patients with decompensated cirrhosis and in organ allograft recipients, for these populations, initial therapy with two agents may be advisable,[104,218,229] although not proven to be superior to entecavir or tenofovir alone. Similarly, because of the risks of treatment failure in patients with cirrhosis, stopping antiviral therapy after HBeAg seroconversion may not be the best strategy.[104,218]

Special Patient Populations

Pregnancy

Experience with lamivudine and tenofovir in pregnant women with HIV infection suggests that both are safe during pregnancy. For pregnant women who meet routine criteria for antiviral treatment, guidelines are the same as for nonpregnant women. Although antiviral treatment during late pregnancy (weeks 28–32 of gestation) reduces levels of maternal HBV DNA, studies have not shown that such antiviral therapy reduces the frequency of maternal-fetal transmission of HBV infection below levels achieved with administration of HBIG and hepatitis B vaccine to newborns. The exception is in mothers with high-level viremia,[104,218,230] now defined as $>2 \times 10^5$ IU/mL. In HBeAg-positive pregnant women with HBV DNA $>2 \times 10^5$ IU/mL, treating mothers during the third trimester (gestational weeks 30–32) until postpartum week 28 with TDF was shown to reduce the rate of mother-to-child transmission to 0% from 7% (per-protocol analysis; to 5% from 18%, intention-to-treat analysis) in an untreated control group.[231] On the basis of preclinical teratogenicity studies, entecavir is classified as pregnancy category C, whereas tenofovir is classified as category B[218]; because of this theoretical advantage, the vast experience with tenofovir in HIV-infected pregnant women, and the demonstrated benefit of TDF in late trimester pregnancy, tenofovir is preferable to entecavir for treatment of pregnant women.[105]

Liver Transplantation

Antiviral therapy with any of the available antiviral agents in patients with decompensated chronic hepatitis B may delay the need for liver transplantation; however, once liver transplantation is undertaken, HBV reinfection of the new liver occurs universally unless preemptive antiviral measures are instituted.[232] Before the availability of such antiviral therapy, most patients undergoing liver transplantation could manage well clinically for several years, despite HBV reinfection; however, in an unpredictable proportion, severe allograft-compromising and life-threatening HBV-associated liver injury supervened. The most aggressive form of HBV reactivation, fibrosing cholestatic hepatitis, represents overwhelming expression of HBV in the hepatocyte.[233] Characterized histologically by fibrosis and cholestasis out of proportion to necroinflammatory activity, this dreaded form of hepatitis B is more reminiscent

of direct virus-induced cytopathic injury than indirect cell-mediated cytotoxicity characteristic of virus-associated liver injury in the immunocompetent host. The likelihood of recurrent hepatitis B (i.e., hepatitis associated with HBV infection) correlates with the level of HBV replication before transplantation; however, even patients with low-level HBV replication before liver transplantation can have recurrent hepatitis B because immunosuppression, especially with corticosteroids, enhances HBV replication.[234,235] In fact, even recipients of donor livers from anti-HBs–positive or anti-HBc–positive donors have had hepatitis B reactivation after liver transplantation.[236] Patients who undergo liver replacement for fulminant hepatitis B may be an exception to the rule of universal reinfection; in such cases, rapid and overwhelming elimination of HBV-infected hepatocytes depletes the substrate for new HBV replication.

All patients on the liver transplantation waiting list with HBV-related indications should receive antiviral therapy. Currently, centers that perform liver transplantation follow a protocol of preemptive prophylaxis with a combination of entecavir or tenofovir and intravenously administered, high-dose HBIG beginning during the anhepatic phase of the procedure and continuing daily for the first week, followed by periodic readministration indefinitely thereafter.[237,238] Rarely, however, HBV envelope (HBsAg) escape mutations emerge during HBIG therapy after liver transplantation.[239–241] The addition of a nucleoside or nucleotide analogue to this regimen has dramatically reduced the risk of recurrent hepatitis B after transplantation.[170,242] Moreover, availability of these potent nucleoside or nucleotide analogue antiviral agents—IFN is not effective—has permitted exploration of strategies to change the route of administration (intramuscular vs. intravenous) and to reduce the dose, frequency, and duration of HBIG treatment.[232,243,244] Although the success of posttransplantation lamivudine therapy was hampered by the emergence of resistance,[170] the addition of adefovir, either as rescue therapy or as primary therapy, overcame the problem of posttransplantation lamivudine resistance and its often dire clinical consequences. Therefore, currently, the outcome of liver transplantation for hepatitis B is comparable with that of the procedure for nonviral types of end-stage liver disease.[232,242] Now that newer, better oral agents are available, they have replaced lamivudine and adefovir, and in selected patients may even replace HBIG therapy.[245] Entecavir monotherapy without HBIG was shown in a report from a single center to be highly effective. At 8 years after transplantation, 92% of treated patients were HBsAg negative, and 100% had undetectable HBV DNA; the 9-year survival was 85%, and no deaths resulted from recurrent HBV infection.[246] In patients at high risk for posttransplantation hepatitis B, a combination of HBIG and an oral agent is recommended.[106]

HIV Coinfection (Also See Chapter 124)

Patients with HIV-HBV coinfection tend to have high levels of HBV DNA and more advanced histologic disease but with relatively limited elevations of aminotransferase activity.[247] Moreover, occasionally, immunologic reconstitution associated with successful antiretroviral therapy (ART) results in severe exacerbations of hepatitis B in patients with coinfection.[248] IFN is not effective in this subset of patients with immunocompromise.[54] Lamivudine, when administered as part of the ART drug cocktail used to treat patients with HIV infection, is given at a dose of 150 mg twice daily, three times the HBV dose, and is complicated frequently by lamivudine resistance,[249] which limits the value of lamivudine. When, in the past, adefovir was a first-line treatment for hepatitis B, it lowered HBV DNA levels by 4 to 5 $\log_{10}$ copies/mL and maintained this effect without resistance even after more than 2 years of treatment.[228,250] Eclipsing adefovir, tenofovir, which is effective against both HIV and HBV and now a popular component of ART, is the first-line agent to be used in this population.[250] Currently, combination therapy that includes the nucleotide analogue TDF and an L-nucleoside (e.g., with the combination tablet that includes tenofovir and emtricitabine) is recommended (monotherapy with anti-HIV agents is not advisable), and ideally, both HIV and HBV infections should be treated simultaneously.[104,218,219,251–253] If an HBV-HIV coinfected patient is a candidate for therapy for hepatitis B but not HIV, single agents that have activity against HIV (lamivudine, tenofovir, entecavir) are contraindicated because of the risk of HIV resistance to monotherapy. Currently, treating both HBV and HIV infections is the preferable strategy; all patients with HIV-HBV coinfection should receive tenofovir-based ART (containing TDF or TAF), independent of CD4 count.[106]

Immune-Complex Disease

Immune-complex glomerulonephritis and generalized vasculitis (polyarteritis nodosa) can occur in patients with chronic hepatitis B.[92] Because these extrahepatic manifestations occur in settings of viral antigen excess, reduction of hepatitis B replication can reverse the process. Early trials of IFN showed improvements in nephrotic syndrome and in clinical findings in generalized vasculitis,[254] but oral nucleoside or nucleotide analogues, especially now entecavir and tenofovir, have replaced IFN in the management of these cases. Patients with immune-complex extrahepatic manifestations should receive tenofovir or entecavir (PEG IFN, which can exacerbate immune-complex–mediated extrahepatic manifestation, is not recommended) regardless of whether or not they meet HBV DNA and ALT treatment thresholds in treatment guidelines.

Oncology Patients Who Need Cytotoxic Chemotherapy

When cytotoxic chemotherapy is administered to HBV-infected patients with malignant disease, levels of HBV replication are enhanced and cell-mediated immune injury of HBV-infected hepatocytes is suppressed. When cycles of chemotherapy are completed, however, restoration of cytolytic T-cell function occurs at a time of residual high-level HBV antigen expression on hepatocyte membranes, initiating a burst of hepatocytolysis and severe, often fatal, reactivation of hepatitis B.[30,32] Preemptive antiviral therapy with nucleoside or nucleotide analogues, initiated before chemotherapy (preemptive therapy), has been shown to prevent such reactivations of hepatitis B.[255] The risks of HBV reactivation and the advantages of preemptive antiviral therapy are similar for patients undergoing immunosuppressive or immunomodulatory (e.g., anti-TNF and other anticytokine) therapy. Current data do not support preemptive antiviral treatment for anti-HBs–reactive persons requiring immunomodulatory therapy; however, the risk is sufficiently high for antiviral therapy in anti-HBc–reactive patients treated with the most potent immunomodulators (e.g., rituximab).[59,60,582] The optimal duration of antiviral therapy after discontinuation of immunosuppressive chemotherapy remains to be defined.[105,106,256]

Chronic Hepatitis D

HDV is a defective agent that requires HBV (or hepadnaviruses of other species) to replicate and that depends absolutely on the persistence of HBV to establish chronic infection (see Chapter 146).[257–260] When acute coinfection with both agents occurs simultaneously, the duration of infection is determined by the duration of HBV infection; therefore, HDV does not increase the frequency of chronicity of acute HBV infection. When HDV superinfection occurs in a person already infected chronically with HBV, chronic hepatitis D follows almost invariably (with rare exceptions), maintained by chronic HBV infection. Clinically, hepatitis D superinfection may be recognized as a flare in aminotransferase activity in a person with chronic hepatitis B, and in general, chronic hepatitis B is converted to more severe chronic hepatitis after HDV superinfection; for example, severe, even fatal, chronic hepatitis can follow in an inactive hepatitis B carrier superinfected with HDV.

Clinical features of chronic hepatitis D are similar to those of chronic hepatitis B, except for the increased severity and more rapid progression to cirrhosis and end-stage liver disease; in addition, both early mortality and the risk of HCC are increased in patients with hepatitis D.[258] When hepatitis D was described originally, the more severe cases attracted clinical attention, and hepatitis D was believed to be invariably severe; however, mild hepatitis, even quiescent liver disease (inactive carriage), has been recognized, and the disease can become indolent after an early period of severe hepatitis lasting several years.[261] In addition, three genotypes have been identified, with distinct geographic distribution, and genotype 2 has been associated with milder disease.[258] Although focal outbreaks of hepatitis D continue to emerge throughout the world, in Mediterranean countries, where hepatitis D was first recognized, the frequency of hepatitis D declined dramatically during the 1990s, attributed

to changes in migration patterns, improvements in socioeconomic conditions, adoption of HIV-avoidance behavior among injection drug users, and hepatitis B vaccination programs (immunity to hepatitis B protects against infection with hepatitis D).[262] Still, HDV superinfection of persons with chronic hepatitis B continues to be observed, even in low-prevalence countries, among active injection drug users.[263]

Although chronic hepatitis D tends to be more severe than chronic hepatitis B, and to require concomitant chronic hepatitis B, these generalizations do not prevail in one clinical situation. After liver transplantation for chronic hepatitis D, hepatitis D reinfection can occur without high levels of HBV replication and without liver injury. Even in the absence of maneuvers to prevent the reestablishment of HBV infection (HBIG plus antiviral agents), the outcome of hepatitis D after liver transplantation is comparable with that after nonviral liver disease.[237,264,265] On the other hand, if after liver transplantation for hepatitis D, high-level HBV infection emerges, severe hepatitis D may follow. Although hepatitis D, like other viral hepatitides, is likely to injure hepatocytes indirectly via cytolytic T-cell activation, and although autoimmune markers (anti-LKM3) are common in hepatitis D,[266] the mechanism of HDV-associated liver disease has not been defined definitively.

Serologically, the diagnosis of hepatitis D is established by the presence of circulating antibody to HDV (anti-HDV). Unlike anti-HBc in HBV infection, the distinction between IgM and immunoglobulin G (IgG) anti-HDV is not useful clinically. Although not widely used clinically, testing is available in a limited number of specialized and research laboratories to detect HDV RNA with PCR.[267] Curiously, patients with chronic hepatitis D may harbor liver-kidney microsomal antibodies type 3 (anti-LKM3) that recognize uridine diphosphate glucuronosyltransferase and that are distinct from similar LKM antibodies observed in type 2 autoimmune hepatitis and chronic hepatitis C (anti-LKM1) and certain types of drug-induced hepatitis.[266]

Treatment

None of the currently available nucleoside or nucleotide analogues active against hepatitis B has been shown to be effective in patients with hepatitis D.[258] IFN-α at doses and durations recommended for hepatitis B are ineffective for chronic hepatitis D; however, IFN therapy extended to 12 months and at a dose of 9 million units three times a week can result in clinical improvement (return to normal of aminotransferase activity and an improvement in liver histologic findings) in more than two-thirds of treated patients, despite the fact that therapy has limited impact on HDV replication and that clearance of HDV RNA is unusual.[268] Although aminotransferase activity remained normal for 6 months after therapy in half of patients treated this way, ultimately, clinical reactivation followed after cessation of therapy in many patients. On the other hand, biochemical remission was maintained in a proportion of treated patients, even 14 years after completion of therapy, and in some, clearance of HDV RNA and regression of fibrosis were documented.[258,269] An anecdotal report of a sustained biochemical, virologic, and histologic response after 12 years of IFN therapy (5 million units daily) in one patient[270] raises the possibility that protracted treatment may be the best approach for patients with chronic hepatitis D. Therefore, some investigators have advocated chronic therapy until eradication of HDV RNA is achieved. PEG IFN has also been shown to have efficacy in chronic hepatitis D[271]; 48 weeks of PEG IFN resulted in sustained clearance of HDV RNA (as of 24 weeks after therapy) in a quarter of patients.[272] Ultimately, novel antiviral approaches for the treatment of chronic hepatitis D (clevudine, now abandoned, was shown to be active in woodchucks with HDV infection[273]) are necessary.[274]

As noted previously, liver transplantation for end-stage chronic hepatitis D has an excellent prognosis; still, the same protocol of HBIG plus a nucleoside or nucleotide analogue used after transplantation for chronic hepatitis B is recommended for patients with chronic hepatitis D to minimize the likelihood of recurrent hepatitis B and its deleterious impact on posttransplantation HDV infection.

Chronic Hepatitis C

In adults with clinically apparent acute hepatitis B, the likelihood of chronic infection is less than 1%.[70,72] In marked contrast, acute hepatitis C is followed by chronic HCV infection in at least 85% of cases.[5,275] Contributing to this high frequency of chronic HCV infection are its high replication rate (10^{11}–10^{12} virions per day with a virion half-life of 2 to 3 hours)[276] without polymerase error proofreading, molecular heterogeneity driven by a high mutation rate (1 mutation per synthesized genome), and, consequently, the virus's ability to change sufficiently and rapidly enough in the face of the evolutionary pressure of host immunity to circumvent neutralizing antibodies.[277] In addition to viral genotypes 1 to 6, the nucleotide sequences of which can differ by as much as 30% to 50%, HCV exists as multiple genetically distinct quasispecies, differing in nucleotide sequence by up to 5% (see Chapter 154).[278,279] Believed to have diverged phylogenetically from related viral agents more than 10,000 years ago and to have evolved in humans for this extended period, HCV has established itself firmly among humans, infecting as many as 175 to 185 million worldwide according to previous estimates; however, when reassessed in 2015, modeling of global prevalence of hepatitis C viremia was more consistent with a prevalence of 71 million worldwide. In developed nations, antibody to HCV is prevalent in 1% to 2% of the population.[18] In the United States alone, as many as 1.6% to 1.8% of the population have been infected (approximately 4 million in the 1990s; by 2010, 1% were estimated to be viremic, corresponding to approximately 3 million people, a reduction proposed to have been associated with deaths in the infected population), and the Public Health Service has estimated an annual death toll of 8000 to 10,000.[5,280–282]

The impact of hepatitis C is difficult to minimize. Chronic hepatitis C contributes to approximately 25% to 40% of all chronic liver disease[283] and accounts for up to 40% of all patients undergoing liver transplantation.[284–286] As of 2007, annual mortality associated with hepatitis C surpassed mortality associated with HIV/AIDS in the United States,[287] and, as of 2012, mortality associated with hepatitis C surpassed mortality of all 60 other nationally reportable infectious diseases.[288] Economic estimates of the annual hepatitis C–related direct medical care costs incurred in the United States exceed $1 billion.[285] These statistics may seem surprising in light of the documented decline of acute hepatitis C cases reported over the past several decades. Initially, hepatitis C was recognized in recipients of transfused blood and blood products, but the frequency among transfused persons declined from more than 30% in the 1960s, to more than 10% in the 1970s (exclusion of "commercial" blood donors),[289] to approximately 5% in the 1980s (adoption of "surrogate" screening tests that identified blood donors with an increased risk of bloodborne viral infections),[290–292] to less than 4% in the early 1990s (adoption of HCV-specific screening tests of donor blood).[293–295] During the 1990s, with progressively increasing sensitivity of donor screening tests for anti-HCV, the frequency of transfusion-associated hepatitis C fell to almost undetectable, effectively imperceptible levels, estimated now to be 1 in 103,000 units transfused.[296] This remarkable reduction in transfusion-associated hepatitis C, however, hardly made a dent in the overall frequency of reported cases, most of which occurred in groups other than blood recipients. In the 1990s, however, the overall frequency of reported cases of acute hepatitis C fell by more than 80% (to approximately 35,000), mirroring a similar decline among injection drug users, who adopted behavioral changes designed to minimize acquisition of HIV infection.[297]

Despite the fact that the annual incidence of new HCV infections declined substantially during the last decade of the 20th century, the burden of long-established chronic infections continues to grow and is expected to triple or quadruple over the next generation.[284,285,298] The relatively recent decline in new cases has had limited impact on the large reservoir of chronic infections established, in most instances, 4 to 5 decades ago.[281] Of the 3 to 4 million people estimated on the basis of serologic surveys to have chronic hepatitis C in the United States, less than one-third to one-half have come to clinical attention, mostly as a result of serendipitous discovery (e.g., when donating blood or during routine laboratory testing for other reasons).[299,300] In serologic surveys of the US population, three-quarters of persons found to harbor HCV infection were in the baby boomer age cohort (born from 1945 through 1965); whereas the prevalence in other birth cohorts was only 0.8%, in baby boomers, the prevalence was 3.2%, and 73% of HCV-related mortality occurs in this cohort. Therefore the US Public Health Service

has recommended birth-cohort screening in all persons born between 1945 and 1965.[301–303] This recommendation acknowledges the fact that in many cases an obvious epidemiologic source is not apparent and that most of the pool of currently infected persons was exposed during the 1960s and 1970s in adolescence and early adulthood; now that they are in their 50s to 70s many have moderate-to-advanced liver disease. Implementation of this one-time age-cohort screening policy has been predicted to identify approximately 800,000 cases of hepatitis C; to prevent 200,000 cases of cirrhosis, 47,000 cases of HCC, and 120,000 HCV-related deaths; and to be cost-effective.[303] In North America, although the several-decades-old pool in baby boomers has been the predominant source of HCV-infected persons, a new epidemic of acute hepatitis C has emerged during the first two decades of the 21st century among a new generation of young injection drug users (who have not adopted the precautions that led to a reduction in reported cases in the 1990s); furthermore, the spike in the number of new cases continues to be amplified by the opioid epidemic plaguing adolescents and young adults.[304–307]

Pathophysiology and Natural History

Long refractory to in vitro cultivation, HCV can now be replicated in cell culture, which has facilitated understanding of the viral life cycle.[308,309] One of the envelope proteins of HCV, E2, contains a binding site for a surface protein on hepatocytes and lymphocytes, CD81, which may represent a cellular receptor for the virus.[310] HCV also binds to the tight-junction protein claudin 1 and to other liver-surface proteins. Because HCV and lipids rely on the same low-density lipoprotein (LDL) assembly and secretion pathway, HCV appears to be able to "hijack" the LDL assembly and secretion pathway, which allows the virus to "masquerade," invisible to the adaptive immune response, as a lipoprotein; this ability to evade the immune system may contribute to the success of HCV as a chronic passenger in its human host.[311] Like the other hepatitis viruses, HCV is not cytopathic; instead, the presence of virus-infected hepatocytes initiates a cascade of host cellular immunologic events that culminate in clearance of HCV-infected hepatocytes. Early insight has been gleaned from studies of cell-mediated immunity to HCV polypeptides, and the host, genetic, and viral factors that distinguish between the small minority who recover and the large majority who proceed to harbor chronic infection after acute HCV infection are now beginning to be understood. From studies of cell-mediated immunity, a general consensus has emerged that attributes recovery to a more robust, broadly targeted $CD4^+$ and $CD8^+$ T-cell response to HCV proteins, and chronic infection or disease to an absent or minimal and narrowly directed HCV-specific T-cell response.[5,312–315] For spontaneous resolution of hepatitis C, $CD4^+$ T cells, specifically, have been found to be essential for viral clearance.[316] In addition, intrahepatic $CD4^+$ and $CD8^+$ responses against a wide spectrum of HCV epitopes have been shown to correlate with viral clearance and recovery from HCV infection, as has the presence of intrahepatic HCV-specific IFN-γ–producing T cells.[317] Moreover, genomic analysis of the progression of HCV infection in chimpanzees has revealed transcriptional changes in the IFN-α response that correlate with the duration of infection; and genomic signals for antigen-processing and presentation and for the adaptive immune response are expressed in association with viral clearance.[318] Also contributing to chronic HCV infection are mutations in $CD8^+$ T cell–targeted viral epitopes that allow HCV to escape immune-mediated clearance, and upregulation of inhibitory receptors on exhausted (functionally impaired) T cells.[319] For HCV infection to be established chronically, as noted previously, the host immune response to HCV is usually too limited in impact to contain the infection; in addition, this feeble immunologic response allows superinfection with a different genotype or viral isolate (absence of heterologous immunity) or even, under experimental conditions in chimpanzees, reinfection with the same viral isolate (absence of homologous immunity).[320–324] Accordingly, prospects for an effective hepatitis C vaccine to prevent infection are challenging.[325] On the other hand, neutralizing antibodies have been identified against broadly conserved HCV epitopes[326]; potentially, this advance in understanding the humoral immune response to HCV could be exploited to pursue passive immunoprophylaxis with globulin preparations or active immunization with vaccines.

Host factors that have been suggested to correlate with a higher frequency of chronicity after acute infection include male gender; older age (adults more than children); ethnicity (blacks more than whites); clinically mild, inapparent, or anicteric acute infection; certain extended human leukocyte antigen (HLA) haplotypes; and immunologic compromise.[327] From genome-wide association studies (GWASs) among patients treated with IFN-based antiviral therapy emerged the recognition that *IL28B*-related haplotypes coding for IFN lambda 3 (IFN-λ3) correlate strongly with response to antiviral therapy; the favorable CC genotype was associated with sustained virologic responsiveness (approximately 80%), whereas the unfavorable genotype TT reflected refractoriness to responsiveness (approximately 30%).[328] The same genotypes have also been shown to be associated with relative likelihood of HCV clearance after acute infection: CC is associated with a greater than 50% chance of resolution, TT with an approximately 25% chance of resolution, and heterozygous CT with an approximately 30% chance of resolution.[16] Resolution after acute hepatitis C is even more strongly linked to the combination of the *IL28B* CC genotype and an HLA class II allele near *DQB1*03:01*.[329] Further functional analysis of the link between resolution of acute HCV infection and *IL28B* has revealed a frameshift variant upstream of *IL28B* encoding IFN-λ3 on chromosome 19q13.13 that creates a novel IFN gene, *IFN-λ4,* associated with impaired HCV clearance.[330] Further GWAS results have permitted mapping of the mixed histocompatibility complex variant most closely associated with spontaneous HCV clearance to a 50-kilobase region on chromosome 19, rs8099917, approximately 7000 base pairs upstream of *IL28B*.[331]

The natural history of hepatitis C is variable.[327–330,332–339] As noted previously, up to 85% to 90% of acutely infected persons acquire chronic infection. Progression to cirrhosis among those with chronic hepatitis C has been observed even in patients with asymptomatic and otherwise clinically, biochemically, and histologically mild disease. An analysis of 57 adequately sized and documented published studies showed that, among adults (mean age of infection, 42 years) with transfusion-acquired chronic hepatitis C, progression to cirrhosis occurred in approximately 25% over the course of 20 years.[336] A similarly high 20-year, 22% rate of progression to cirrhosis, probably magnified by selection bias, was recorded in cohorts of patients (mean age of infection, 29 years) referred to liver clinics; however, recorded 20-year rates of progression were lower in community-acquired hepatitis C (mean age of infection, 26 years) at 7% and in blood donors (mean age, 22 years) at 4%.[336] In a systematic review of 111 studies (of 33,121 patients) of prognosis of chronic hepatitis C, Thein and colleagues[340] found that rates of progression from histologic fibrosis stage to stage varied (nonlinear progression), in that the overall progression to cirrhosis after 20 years was 16%, but that the 20-year rate of progression to cirrhosis varied according to study type: 18% for cross-sectional retrospective studies (attempt to time the duration of infection retrospectively, based on history of potential exposure, in patients presenting for clinical care), 7% for retrospective-prospective studies (i.e., prospective follow-up studies of subjects identified retrospectively after outbreaks of apparently acute hepatitis C), 18% for studies in clinical settings, and 7% for studies in nonclinical settings (e.g., blood donation center).

Histologic features are perhaps the best predictors of disease progression.[341,342] Among a cohort of Japanese patients who underwent serial liver biopsies over a 20-year period, progression to cirrhosis was limited in those with histologically mild necroinflammatory activity and fibrosis.[343] In contrast, those with moderate and severe hepatitis or fibrosis had progression to cirrhosis almost invariably over 20 to 10 years, respectively.[343] Therefore, baseline liver biopsy is helpful not only to define the degree of liver injury that transpired in the decades before clinical presentation but also to predict the pace of histologic progression over the ensuing 1 to 2 decades.[344,345] In a US cohort of 123 untreated patients who underwent paired biopsies a median of almost 4 years apart, histologic progression of fibrosis occurred in 39%, histologic stability in 37%, and improvement in 24%.[346] Although some investigators have devised models to assess linear progression of fibrosis in chronic hepatitis C,[341] others have emphasized that the rate of progression is variable, not linear.[340,346,347] In this US cohort, the best predictors of histologic progression of fibrosis were advanced age, grade of histologic necroinflammatory activity (especially periportal necrosis), and level

of aminotransferase activity.[346] Although a number of anecdotal reports have documented the presence of severe hepatitis and even, rarely, cirrhosis in patients with normal aminotransferase activity,[348] such severe histologic changes could have derived from an earlier period of biochemical activity, and in large groups of patients with normal aminotransferase levels, histologic studies have shown that the overwhelming majority of patients, with few exceptions, have mild histologic features.[300,349,350] In addition, in several longitudinal studies of patients with chronic hepatitis C and persistently normal aminotransferase activity, the absence of histologic progression over 5 years or more has been documented.[346,351,352] Therefore the rationale exists for monitoring patients with persistently normal aminotransferase activity without therapeutic intervention.[353] Still, because as many as a quarter of patients with normal aminotransferase activity followed for several years ultimately have aminotransferase elevations,[351] even patients with normal aminotransferase levels should be monitored regularly. In addition, patients with normal aminotransferase activity respond as well as those with elevated aminotransferase activity to antiviral therapy and are not excluded as treatment candidates (see subsequent discussion).

Variability in progression of chronic hepatitis C remains a confounding clinical feature of the disease. Yet to be explained are extreme differences in outcome between cohorts of young women who acquired hepatitis C from contaminated anti-D Rh immunoglobulin at the time of childbirth in 1977–79 (17- to 20-year progression to cirrhosis of only 0% to 2%[332,335]; 35-year progression to cirrhosis, in 2013, 19%[354]) and cohorts of recipients of HCV-contaminated intravenous globulin (<11-year progression to cirrhosis in 30%).[334] Similarly, the limited progression of transfusion-related chronic hepatitis C in young children compared with that in adults has been described but not explained.[355] For patients who acquired hepatitis C after transfusion in the 1970s, long-duration follow-up studies lasting 20 years (the Transfusion Transmitted Viruses Study) showed progression to cirrhosis in approximately 20% but no increase in overall mortality (and only a slight, almost negligible, increase in liver-related mortality) compared with a matched, transfused control group in whom hepatitis did not develop.[327,338] Therefore, for someone whose perspective is molded by experiences in a blood bank—where asymptomatic healthy persons with hepatitis C are identified during blood-donor screening and where disease progression in transfusion-related hepatitis C is slow and clinically inapparent—hepatitis C appears to be a slowly progressive and mild disease. In contrast, in a tertiary-care hospital with a liver-transplantation program, referrals that funnel in primarily the sickest patients along the spectrum of disease activity[333] bias hepatologists and transplant surgeons to the view that hepatitis C is an invariably progressive and fatal disease. A more balanced reality exists between these two extremes. In most patients, chronic hepatitis C is a slowly progressive liver disease that may have few clinical consequences over the first 2 decades; progression to cirrhosis occurs in 2% to 4% of young children and young women and in less than 10% of community-based young adult (<40 years) patient cohorts but in about 20% to 25% of referral center–based cohorts of older adults.[327,336] What is not known but is likely is whether, given a sufficiently long time (e.g., 3–5 decades or more), hepatitis C is progressive in most patients.

Neither level of HCV RNA, HCV genotype, nor HCV quasispecies diversity correlates with the degree and rapidity of progression of chronic hepatitis C, but more advanced liver disease is found in patients with higher liver iron levels, almost certainly an indirect reflection of duration of infection.[327] Several factors have been shown to accelerate or to be associated with accelerated progression of fibrosis in chronic hepatitis C, including advanced age at the time of infection; male gender; excessive alcohol use; coinfection with HBV or, as is common in hemophiliacs with multiple transfusions and in injection drug users, HIV; persistently elevated aminotransferase activity[346]; certain extended HLA haplotypes[356]; and concomitant other type of liver disease (e.g., hemochromatosis, steatohepatitis).[300,327,357] Fibrosis associated with steatosis has been recognized to be more common in patients with HCV genotype 3,[358] but hepatic steatosis associated with obesity and insulin resistance may also be associated with a higher frequency of cirrhosis in patients with chronic hepatitis C.[359,360] The intimate relation between HCV replication and the LDL assembly and secretion pathway contributes to the observed association between hepatic steatosis and progression of fibrosis.[311] Blacks, in whom acute hepatitis C is more likely to be followed by chronic hepatitis C, are actually less likely to have progression to cirrhosis than whites,[361] although in one report, the estimated risk of fibrosis progression in blacks was statistically indistinguishable from (albeit 10% lower than) that in whites.[362] Severity of chronic hepatitis C is thought to be increased in patients with acute hepatitis A, based primarily on a report of fulminant hepatitis in a group of patients with chronic hepatitis C superinfected with HAV.[363] This report, however, documented a unique experience not replicated by other observers.

Among patients with compensated cirrhosis resulting from chronic hepatitis C, long-term studies of the natural history of the disease have shown a very good prognosis, a 10-year survival rate of 80%.[364] Other investigators, however, have reported more rapidly progressive decompensation and mortality.[365] Once evidence for hepatic decompensation appears, the survival rate falls dramatically to 50%.[364] In most experiences, in patients with cirrhosis, decompensation occurs at an incidence rate of 4% to 5% per year, a mortality rate of 2% to 6% per year, and an HCC rate of 1% to 4% (up to 7% in some reports) per year.[364,366,367] In a study cohort of 1050 patients selected because of failed previous IFN-based therapy and advanced fibrosis (Ishak stage 3 or 4) or cirrhosis (Ishak stage 5 or 6; see Table 117.3), the rate of clinical decompensation was 18% over 3.5 years,[368] and the 5-year incidence of HCC was 5%, higher in the cirrhotic group at 7% than in the advanced fibrosis group at 4.1%.[369] In this cohort, the 5-year risk of HCC could be stratified into low (0.4% observed), medium (4.8% observed), and high (17.8% observed) risk based on a model in which risk increased with age, black race, alkaline phosphatase, presence of esophageal varices, smoking history, and lower platelet count.[369]

HCC, however, rarely occurs until HCV infection has been established for approximately 20, but more often 30, years, and almost all such patients are already cirrhotic or at least have advanced fibrosis.[369,370] HCV does not integrate into the human host genome, as does HBV. In vitro studies suggest that HCV core protein and nonstructural protein NS3 can transform mammalian cells in culture,[371] and HCV core protein has been shown to induce HCC in transgenic mice,[372] but the mechanism of hepatocarcinogenesis in chronic hepatitis C is unknown. Like other chronic liver diseases associated with cirrhosis and HCC (e.g., hemochromatosis, autoimmune liver disease, α_1-antitrypsin deficiency, nonalcoholic steatohepatitis, alcoholic cirrhosis), chronic hepatitis C may lead to liver cancer by promoting endlessly repetitive cycles of hepatocyte regeneration and repair, resulting ultimately in the emergence of malignant clones. Among patients with cirrhosis and hepatitis C, excessive alcohol intake, hepatic iron overload, and concomitant HBV infection appear to increase the risk of HCC. Currently, chronic hepatitis C accounts for approximately one-third of all cases of HCC in the United States, where the incidence and mortality of all causes of HCC have doubled over the past 25 years and of HCV-associated HCC tripled during the 1990s.[373,374] In Japan, where currently HCV infection accounts for 90% of all cases of HCC, the frequency of HCV-associated HCC tripled over 4 decades.[275] As the US cohort of those who acquired HCV infection in young adulthood matures to middle-aged adults, now predominantly otherwise healthy and in the prime of their careers, predictions made in the years 2000–03 were that, discounting the potential impact of antiviral therapy on the natural history of chronic hepatitis C, over the ensuing decades of the 21st century the frequency of cirrhosis would increase by more than 500%, of HCC by more than 250%, of liver-related deaths by more than 200%, and of both hepatic decompensation and liver transplantation by more than 60%.[285,298,375]

Clinical Manifestations

The most typical patient with recently discovered hepatitis C is asymptomatic, but fatigue is one of the more common clinical features in symptomatic persons. Overall, clinical features of chronic hepatitis C, and of decompensated cirrhosis associated with hepatitis C, are similar to those of chronic hepatitis B. Laboratory test abnormalities in chronic hepatitis B and C are similar; the principal abnormality is an elevation of serum aminotransferase activity, usually ALT exceeding AST, until cirrhosis supervenes, when, in general, AST exceeds ALT.[376] More characteristic of chronic hepatitis C than other forms of chronic liver

disease are episodic fluctuations in aminotransferase activity, postulated to result from bursts of hepatic necroinflammatory activity accompanying the emergence of new quasispecies that overcome host immunologic containment of HCV.[322,323] With progressive chronic hepatitis C and evolving fibrosis, hypersplenism occurs and platelet and white blood cell counts fall. In patients with compensated cirrhosis associated with hepatitis C, laboratory indicators of hepatic synthetic function—prothrombin time and serum albumin—remain normal, but these markers become abnormal in decompensated cirrhosis. Impaired hepatic excretory function tends to be maintained before the emergence of severe, end-stage cirrhosis, when bilirubin increases.

Chronic hepatitis C can be accompanied by circulating autoantibodies, including nuclear antibodies, especially liver-kidney microsomal antibody (anti-LKM1), similar to that seen in autoimmune hepatitis type 2.[46] Assays for circulating immune complexes (such as those that detect aggregated immunoglobulins or cold-precipitable globulins [cryoprecipitates]) can be detected in up to half of all patients with chronic hepatitis C,[377] but only a small fraction of patients have immune-complex disorders (see subsequent discussion).

Histologic features of chronic hepatitis C are similar to those observed in chronic hepatitis B (see previous discussion).[378,379] Fibrosis stage 3 in the 6-point Ishak scale (or F2 in the F0–F4 METAVIR staging system; see Table 117.3) represents septal fibrosis (i.e., scar tissue extending beyond the portal tract and bridging portal to portal or portal to central zones of the liver lobule).[40,42] Fibrosis stages higher than 3 (>F2) are usually progressive and are accepted as histologic criteria for timely treatment.[343]

Disease manifestations unrelated to the liver have been described in patients with chronic hepatitis C, and HCV RNA has been detected in extrahepatic sites, such as lymphocytes and the spleen.[380,381] Among the extrahepatic diseases linked with HCV infection are autoimmune disorders such as Sjögren (sicca) syndrome and immune-complex disorders such as EMC (with a spectrum from mild leukocytoclastic cutaneous vasculitis [palpable purpura, arthralgias] to membranoproliferative glomerulonephritis).[5,382] In patients with immune-complex disease, HCV RNA and anti-HCV have been found to be concentrated in circulating immune complexes.[383] EMC is associated not only with immune-complex diseases but also with lymphoproliferative disorders, such as monoclonal gammopathy of unknown source and B-cell non-Hodgkin lymphoma, also reported to be more prevalent in patients with hepatitis C (with or without EMC).[377,384–386] Lichen planus and porphyria cutanea tarda have also been linked with chronic hepatitis C; whether hepatitis C can cause neurologic disorders and impaired cognition is controversial. Hepatitis C has also been linked with insulin resistance and hepatic steatosis (metabolic syndrome), and type 2 diabetes mellitus.[360,387,388]

Hepatitis C Virus–HIV Coinfection (Also See Chapter 124)

Because of the common, bloodborne routes of acquisition of these two viral agents, approximately one-third of patients with HIV infection (three-quarters in the subset with injection drug use) are coinfected with HCV.[389] As the introduction of ART reduced dramatically the frequency of life-threatening opportunistic infections and malignant diseases and improved survival, hepatitis C emerged as an important cause of morbidity and mortality in persons with HIV. The level of viremia is amplified, the rate of hepatic fibrosis is higher, the course of chronic hepatitis C is accelerated, and the frequency of liver failure is more pronounced in patients with HIV-HCV coinfection.[390–392] Since the introduction of ART for HIV infection, mortality from end-stage liver disease caused by HCV infection in this patient population has increased fivefold.[393] Occasionally, an acute hepatitis-like biochemical flare follows the initiation of ART therapy in patients with HIV-HCV coinfection; ART-related immune recovery resulting in cytolytic T-cell injury of HCV-infected hepatocytes has been invoked to explain this observation.[394]

Treatment

When recombinant IFN-α was first approved and introduced in the early 1980s, the duration of therapy (at a dose of 3 million units administered subcutaneously three times a week) was 6 months. The therapeutic end point in early clinical trials was a return to normal of ALT activity (end-treatment response), which occurred in approximately half of all treated patients during therapy but that was sustained 6 months after therapy in fewer than a quarter.[395,396] When assays were introduced to detect the presence of HCV RNA with reverse-transcription PCR, application of these assays showed that SVRs (defined during most of the IFN-based–therapy era as undetectable HCV RNA for at least 24 weeks after the cessation of therapy), a more stringent end point than biochemical outcome, occurred in fewer than 10% of patients treated for 6 months.[396] Higher doses, more frequent administration, and different preparations (e.g., α2b, α2a, consensus, leukocyte) of IFN failed to increase the frequency of SVRs, as did induction therapy with higher doses and more frequent administration during the early months of therapy.[353,396–402] Doubling the duration of therapy to 12 months, however, doubled the frequency of SVR to approximately 20%.[396,398,399] The efficacy of therapy was doubled again to approximately 40% when the oral guanine nucleoside RBV was added to IFN treatment.[403,404] RBV has a minimal impact on HCV replication and is ineffective when used alone[405,406]; however, this nucleoside analogue could potentially result in immunologic modulation (shift from Th2 response to Th1 response), inhibition of host inosine monophosphate dehydrogenase (IMPDH) activity, depletion of intracellular guanosine triphosphate pools,[407] induction of viral mutational catastrophe,[408] enhancement of the effect of IFN through an increase in IFN-stimulated gene expression (which is suppressed by HCV NS5A), and a direct, albeit subtle, antiviral effect.[409–412] Although the mechanism of RBV activity in HCV infection is not known, the addition of RBV to IFN, with dose based on patient weight, reduces the frequency of virologic relapse at the end of therapy, increasing substantially the efficacy (SVR) of therapy.[402–404] Moreover, achievement of SVR after the completion of therapy has been shown to translate into a durable response characterized by maintenance of virologic, biochemical, clinical, survival, and histologic benefit (including regression of cirrhosis), equivalent to a "cure" in almost all cases.[413–416] As discussed later in this chapter, the IFN era has been surpassed (IFN-based therapy is no longer recommended for hepatitis C), and the current generation of orally administered, highly effective, well-tolerated, high-barrier-to-resistance DAAs achieves cure in almost all treated patients, across all genotypes, and across all patient populations, including those that had been less responsive to IFN-based therapy—a remarkable success story!

Pegylated Interferon and Ribavirin: Standard of Care 2001 to 2011—Treatment Principles Established

The efficacy of antiviral therapy was improved again by the introduction of PEG IFNs, long-acting IFNs bound to polyethylene glycol, α2b in 2001 and α2a in 2002. In patients with chronic hepatitis C, PEG IFN and RBV are no longer the standard of care, but important principles of antiviral therapy were established during the PEG IFN decade. Moreover, even after the introduction of first-generation protease inhibitors (PIs) for genotype 1 in 2011, PEG IFN–RBV remained the standard of care for genotypes 2 and 3, and, for genotype 1, PEG IFN–RBV remained the backbone of therapy to which direct antiviral agents were added when PIs and then polymerase inhibitors were introduced. Therefore, an appreciation of the role of PEG IFN and RBV remains relevant in the era of early-generation direct antivirals.

Limiting degradation of circulating IFNs, pegylation prolongs the half-life of IFNs substantially, permitting once, instead of thrice, weekly injections; PEG IFN monotherapy doubles the frequency of SVR relative to monotherapy with its non-PEG IFN counterpart.[417–420] Moreover, the addition of daily oral RBV to PEG IFN increases the frequency of SVR to approximately 55%.[421–423] Two PEG IFNs were approved: (1) the smaller, linear, 12-kDa, partially renally excreted PEG IFN-α2b,[418,421] and (2) the larger, branched, 40-kDa, non–renally excreted PEG IFN-α2a.[417,419,420,422,423] The two are comparable in efficacy when administered with RBV,[422,423] although the 40-kDa molecule has a longer half-life and, because of its large size, a more restricted (8-L) volume of distribution, allowing a common dose (180 μg) to be used over a wide range of patient weights. The smaller, 12-kDa molecule has a much larger (20-L) volume of distribution and is administered on the basis of weight (1.5 μg/

kg). At standard doses, neither PEG IFN–RBV combination has a higher efficacy than non-PEG IFN–RBV combination therapy for patients who weigh more than 85 kg.

The side effects of PEG IFN and RBV are similar to those of standard IFN (primarily flulike symptoms, marrow suppression, irritability or depression, and thyroiditis [the most common of potential autoimmune reactions]) plus those of RBV (hemolytic anemia [mean, 2–3 g/dL reduction in hemoglobin], nasal or chest congestion, pruritus, drug rashes, and gout); however, neutropenia is more profound, resulting in dose reductions more frequently, in patients treated with PEG (18%–20%) than with non-PEG (5%–8%) IFNs.[423] Because of the risk of anemia in patients with ischemic cardiovascular or cerebrovascular disease, RBV should be avoided in such patients and in patients with marked anemia. In addition, because it is teratogenic in animals, RBV should not be used in pregnant women or their sexual partners, and contraceptive use should be practiced by women of childbearing age and their sexual partners during and for several months after RBV therapy. Excreted by the kidneys, RBV is contraindicated in persons with renal insufficiency. The side effects of PEG IFN plus RBV are not inconsequential and require substantial support from those supervising therapy. In the registration trials of combination PEG IFN–RBV therapy, side effects or laboratory abnormalities led to dose reductions in 36% to 45% and drug discontinuation in 10% to 14%.[421,422] Although neutropenia is common, infections are not,[424] even when absolute neutrophil counts fall below 500/mm^3; therefore, white blood cell growth factors are almost never required. For patients with RBV-associated anemia, options included dose reduction or the addition of erythropoietin injections, which were shown to minimize RBV dose reductions and to improve symptoms of anemia (quality of life) but not to increase the frequency of SVR.[425–427]

For patients with genotypes 2 and 3, PEG IFN and RBV dose reductions had no impact on SVR rates.[428,429] In contrast, patients with genotype 1 did have reductions in SVR when they were not fully adherent to planned doses and durations of therapy.[428–430] An analysis to determine which of the two drugs was more important to maintain at full or near-full doses was undertaken in 936 patients with genotype 1 in whom treatment with standard IFN had failed and who were then retreated with PEG IFN and RBV.[430] Maintenance of PEG IFN to more than 60% of the prescribed dose during the first 20 weeks of therapy was found to be crucial to achieving SVR; however, reducing the RBV dose during the first 20 weeks had no negative impact on SVR if the PEG IFN dose was not reduced and RBV was not discontinued before completion.[431] These observations in re-treated prior nonresponders—that maintaining the PEG IFN dose during therapy is more important than maintaining the RBV dose—appeared to apply also to treatment-naïve patients being treated for the first time, as long as the cumulative RBV dose did not fall below 60% of anticipated therapy.[432] Although completing the full duration of planned RBV therapy was usually necessary, patients with suppression of HCV RNA to undetectable levels within 2 weeks of initiation of therapy were an exception.[433] The observation that reduction of RBV doses had little impact on SVR does raised doubt about the need for or value of maintenance of RBV doses with erythropoietin.[427]

In registration trials of PEG IFN-α2a and PEG IFN-α2b with RBV, RBV doses, definitions of response and of adverse effects (e.g., differences in objectivity of recording depression), and baseline characteristics (e.g., average weight, percent male, percent with bridging fibrosis or cirrhosis) of the study populations were not entirely equivalent,[421–423] and comparisons between the trials of the two PEG IFNs should be interpreted cautiously. In these registration trials, both PEG IFNs with RBV were tested versus a comparator arm of standard IFN-α2b with RBV. In these combination therapy trials, PEG IFN-α2b plus RBV was comparable in tolerability with standard IFN-α2b plus RBV, and PEG IFN-α2a plus RBV was somewhat better tolerated.[421,422] Nevertheless, in these trials, among patients with genotype 1, SVR was achieved in 42% of those in the trial of PEG IFN-α2b (albeit with a suboptimal RBV dose)[421] and in 46% to 51% of those in the trials of PEG IFN-α2a.[422,423] Further suggesting that PEG IFN-α2a is more effective against genotype 1 is the observation that among patients with genotype 1 and a high level of HCV RNA (>2 × 10^6 copies/mL), PEG IFN-α2a, but not PEG IFN-α2b, plus RBV achieved SVR more frequently than non-PEG IFN-α2b plus RBV.

In the only head-to-head trial of PEG IFN-α2b and PEG IFN-α2a (the IDEAL trial, supported by the manufacturer of PEG IFN-α2b) for 48 weeks in 3070 patients with HCV genotype 1, two doses of PEG IFN-α2b (standard 1.5 μg/kg and lower 1 μg/kg, with weight-based RBV doses ranging from 800 to 1400 mg and with RBV dose reductions for side effects at 200-mg decrements) were compared with PEG IFN-α2a (at the standard 180-μg dose, with weight-based RBV doses limited to the standard 1000 and 1200 mg and with RBV dose reductions by half, per package insert).[434] Because of potentially higher RBV doses and smaller RBV dose reductions for anemia in the PEG IFN-α2b groups, this trial might have been predicted to favor PEG IFN-α2b; however, patients randomized to the PEG IFN-α2a arm actually received higher median weight-based RBV doses (13.4 mg/kg) than those in the two PEG IFN-α2b arms (12.4–12.6 mg/kg). Whether the odds were weighted to favor one type of PEG IFN over the other, however, SVRs occurred in comparable proportions of all three groups: 39.8% and 38% in the two PEG IFN-α2b groups, 1.5 and 1.0 μg/kg, respectively, and 40.9% in the PEG IFN-α2a group. Adverse events, drug discontinuations for adverse events, serious psychiatric side effects, hematologic adverse effects, erythropoietin use, and cumulative RBV doses administered were indistinguishable across the three groups.[435] Although the two PEG IFNs were found to be of equivalent efficacy in this head-to-head trial, several meta-analyses have supported higher efficacy for PEG IFN-α2a than for PEG IFN-α2b.[436–438]

In patients with chronic hepatitis C, aminotransferase levels fall during IFN therapy, without the transient ALT flare characteristic of IFN treatment for chronic hepatitis B; however, in a small proportion of patients, ALT levels remain elevated during successful PEG IFN therapy, even after suppression of HCV RNA, returning to normal only after completion of therapy. A two-phase kinetic reduction in HCV RNA follows IFN-based therapy, with the first, steep-sloped phase lasting 2 to 3 days and representing inhibition of virus replication or release and with the second, less steep-sloped phase lasting weeks to months and believed to represent turnover of infected hepatocytes.[275,439] Studies of standard IFN-RBV combination therapy and of PEG IFN–RBV therapy showed convincingly that a 6-month course of therapy sufficed for patients with HCV genotypes 2 and 3, and a full year was necessary for optimal benefit in patients with genotype 1.[403,404,423] The duration of therapy and RBV dose were not assessed equally in registration trials of the two PEG IFNs with RBV; however, regardless of which PEG IFN was chosen, all treatment guidelines included a recommendation for treatment of patients who had the more refractory genotype 1 with a full year of combination therapy and daily doses of RBV—for PEG IFN-α2a, 1000 mg (for patients weighing <75 kg) or 1200 mg (for patients weighing 75 kg or more); for PEG IFN-α2b, a range of weight-based daily RBV doses from 800 mg for weight <65 kg, 1000 mg for weight 65 to 85 kg, 1200 mg for weight 85 to 105 kg, and 1400 mg for weight >105 kg. For patients with the more favorable genotypes 2 and 3, 6 months of therapy and a daily RBV dose of only 800 mg sufficed.[345,440–444] The likelihood of SVR was approximately 80% in patients with genotypes 2 and 3[421–423]; although comparable results could be achieved in patients with genotypes 2 and 3 when treated with non-PEG IFN plus RBV,[421,422] improved convenience and tolerability favored PEG IFN in these patients.

Hepatitis C genotype was an important predictor of PEG IFN–RBV response (2 and 3 more responsive than 1 and 4), as noted previously. On the basis of a GWAS of patients in the IDEAL trial, investigators found that the IFN-λ3-associated *IL28B* genotype of the host was a strong determinant of treatment responsiveness. As noted earlier, approximately 80% of patients with the favorable CC genotype achieved SVR, whereas only approximately 30% of patients with the unfavorable TT genotype achieved SVR.[328] The same genotypes have also been shown to be associated with relative likelihood of HCV clearance after acute infection, with CC favoring clearance and TT favoring chronicity.[16]

Other variables that correlated with reduced responsiveness to PEG IFN–RBV therapy included high-level HCV RNA; advanced histologic grade (necrosis and inflammation) and stage (fibrosis); higher HCV quasispecies diversity; higher hepatic iron levels; age >40 years; obesity; insulin resistance; hepatic steatosis; male gender; black ethnicity[361,435,445–447]; Latino ethnicity[448]; limited adherence (<80% of prescribed IFN, <80%

of prescribed RBV, <80% of prescribed duration of therapy)[429]; and immunologic compromise.[401,421,422,449–454] *IL28B* genotype contributes to the lower responsiveness of black patients, who are more likely to harbor non-CC genotypes, but race-associated differences in virus-specific CD4-positive T-cell responses at baseline (lower in blacks) and upregulated expression on cytolytic T cells and on natural killer cells of "programmed cell death-1" receptors (associated with functional CD8-positive T-cell impairment and exhaustion) also contributed to the reduced antiviral responsiveness in black patients.[455,456] Patients who did not achieve a ≥2-$\log_{10}$ reduction in HCV RNA during the first 12 weeks of therapy had a ≤3% likelihood of achieving an SVR. That is, absence of an early virologic response (EVR) was a strong predictor of nonresponsiveness; in contrast, among patients who achieved an EVR, approximately two-thirds had an ultimate SVR.[422,428] Therefore the absence of an EVR at 12 weeks was adopted widely as an important milestone, after which treatment was discontinued.[345,428]

Rapid virologic responses (RVRs) were defined as reductions of HCV RNA to undetectable levels within 4 weeks of initiation of antiviral therapy. Among patients with genotype 1 who achieved an RVR, 24 weeks of PEG IFN–RBV therapy was just as effective as 48 weeks of therapy, especially in those with low-level HCV RNA at baseline (<800,000 IU/mL), who could achieve SVR rates of approximately 90%.[457–460] Abbreviation of therapy to 24 weeks for RVR was shown to be effective also for patients with genotype 4.[461] Early analyses suggested that for patients with genotypes 2 and 3, the duration of PEG IFN–RBV therapy could be truncated from 24 to 12 or 16 weeks.[462,463] In the largest trial to test this strategy,[464] 24 weeks of therapy was superior to 16 weeks and, as shown in another trial,[465] even with daily RBV doses as high as 1400 mg. Although a full 24 weeks appeared to be the best regimen in patients with genotypes 2 and 3, patients with low-level viremia and an RVR were candidates for shortening of therapy, especially if tolerability of therapy was poor.

Tailored pegylated interferon and ribavirin therapy. As noted previously, the duration of therapy could be shortened for patients with genotype 1 (less securely shown for genotypes 2 and 3) who achieved rapid reductions in HCV RNA (RVRs), especially those with a low baseline HCV RNA. This observation supported the trend to improve outcomes by tailoring antiviral therapy based on patient characteristics (e.g., weight), viral factors (e.g., genotype and baseline level of HCV RNA), and initial drug response (e.g., presence or absence of RVR). Although shortening of therapy was helpful in patients with RVR, patients with genotype 1 who did not achieve RVR (slow responders) and who took longer to suppress HCV RNA to undetectable levels (including those with a ≥2 $\log_{10}$ reduction but with HCV RNA still detectable at 12 weeks; i.e., with an EVR) were shown to benefit from the extension of therapy to 72 weeks.[459,460,466–468] Therefore, the longer the time necessary to suppress HCV RNA to undetectable levels, the longer therapy was to be continued thereafter.[458,459]

For patients with genotype 3, but not 2, treated for 24 weeks with PEG IFN-α2a and RBV, a reduced daily RBV dose of 400 mg was found to be just as effective in yielding SVR as the full 800-mg dose.[469] In general, however, in most clinical trials, patients with genotype 3 did not respond quite as well as those with genotype 2,[459,463,465] and consideration tended to be given to lowering the threshold for extending therapy to 48 weeks in patients with genotype 3. In addition, many authorities preferred to treat patients with cirrhosis who had genotypes 2 and 3 for a full 48 weeks.

For patients with genotype 1 who are relatively refractory to treatment because of a high baseline HCV RNA level (>800,000 IU/mL) and because they are overweight (>85 kg), increasing the weekly PEG IFN-α2a dose to 270 μg and the daily RBV dose to a fixed 1600 mg was shown in a prospective randomized controlled trial of 188 patients to improve the rate of SVR (47%) compared with standard PEG IFN-α2a and RBV doses (SVR, 28%) or with an increase over the standard regimen in the dose of one drug but not the other (32% for increased RBV only; 36% for increased PEG IFN only). As might be anticipated, tolerability was lower in the group receiving higher doses of both drugs.[470] For patients treated with PEG IFN-α2b and RBV, a large (>5000 participants), community-based trial of weight-based RBV therapy (the WIN-R trial) showed that for patients with genotype 1 (but not 2 and 3), increasing the range of daily RBV doses from 800 to 1400 mg (including the 1400-mg dose for patients weighing >105 kg) was more effective, especially in overweight patients, than the standard 1000- to 1200-mg range without increased intolerability.[471] Similarly, another trial of 150 patients with genotype 1 treated with PEG IFN-α2b and RBV showed the benefit of increasing starting RBV doses from 13.3 mg/kg (800–1400 mg daily; SVR up to 29%) to 15.2 mg/kg (1000–1600 mg daily; SVR up to 49%), an SVR benefit that was not enhanced by the use of erythropoietin.[427] Finally, the IDEAL trial[435] showed that a higher, 1400-mg, daily RBV dose combined with PEG IFN-α2b achieved excellent rates of SVR, among the highest in the trial (42%–45%), in patients weighing >105 kg. Moreover, reduced-dose PEG IFN-α2b (1 μg/kg) was just as effective as full-dose PEG IFN-α2b (1.5 μg/kg)[435]; this reduced dose was considered potentially for patients in whom side effects of full-dose PEG IFN-α2b were intolerable. In short, tailoring PEG IFN or RBV doses and treatment duration to individual patients was found to improve the outcome of PEG IFN–RBV therapy. In contrast to the value of tailoring IFN-based therapy, the new generation of pangenotypic DAAs is so effective that the need for tailoring therapy has been largely surpassed (with the minor exception of patients undergoing initial therapy when resistance-associated substitutions [RASs] are present at baseline for one of the recommended regimens, but mostly for retreatment of patients when RASs emerge during failed initial therapy; see later).

Clinical benefits of interferon-based therapy. Successful treatment with IFN-based regimens has been associated with improved survival and with a reduction in complications of chronic hepatitis C in most analyses,[414,415,472–474] although not all.[402] Similarly, antiviral therapy has been calculated to be cost-effective,[475,476] even in patients with mild chronic hepatitis C.[477] In addition, therapy can retard the progression of, and even reverse, fibrosis and cirrhosis.[413,414,416,478,479] Many reports have appeared in the literature purporting to show that IFN treatment of patients with cirrhosis and hepatitis C, even in the absence of an SVR, can reduce the frequency of HCC.[480–484] These predominantly retrospective studies, however, were potentially flawed by a lead-time bias; treated patients may have had less advanced disease than untreated patients in these trials. Therefore, less advanced disease, not IFN treatment, may have accounted for the reduced frequency of this late neoplastic complication of chronic hepatitis C. Confidence in the conclusions of these studies is also minimized by other subject selection biases; incomparability of subjects, treatment, and monitoring among trials; inadequately addressed confounding variables (such as alcohol use); and lower publication frequency of studies with negative results.[485] In fact, in other retrospective and prospective studies, IFN treatment had no beneficial impact on the risk of HCC in patients with cirrhosis caused by chronic hepatitis C.[364,486,487] In addition, in a large, lengthy, prospective, randomized controlled trial (the HALT-C Trial) of maintenance PEG IFN therapy for 3.5 years in 1050 patients with chronic hepatitis C, advanced fibrosis, and prior failure to respond to IFN-based therapy (i.e., in the absence of an SVR), PEG IFN therapy had no impact on the incidence of HCC, even out to 5 years,[368,369] although a beneficial impact on late-emerging HCC after 5 years was suggested in patients with baseline cirrhosis (Ishak fibrosis score 5 or 6) but not advanced fibrosis (Ishak fibrosis score 3 or 4).[488] On the other hand, among patients of any stage of fibrosis who achieved an SVR following IFN-based therapy, the risk of HCC was reduced.[489] Similar benefits follow SVRs achieved after DAA therapy (see later).

First-Generation NS3-4A Protease Inhibitors for Chronic Hepatitis C: Boceprevir and Telaprevir—Standard of Care for Genotype 1, 2011–13

The first DAAs—telaprevir (TVR) and boceprevir (BOC)—gained FDA approval in May 2011 specifically for the treatment of genotype-1 chronic hepatitis C. BOC and TVR are linear ketoamide compounds that cause reversible covalent inhibition at the protease catalytic site. The NS3-4a viral protease is required for cleavage of the HCV polyprotein into mature proteins. Neither of these PIs could be used as monotherapy because of the rapid emergence of resistant variants; however, when these PIs were added to PEG IFN–RBV, the percentage of patients with genotype 1 who achieved SVR improved dramatically, both for treatment-naïve patients and treatment-experienced patients (i.e., who in whom

prior IFN-based treatment had failed). In the pivotal registration trials of TVR (ADVANCE)[490] and BOC (SPRINT 2)[491] in treatment-naïve patients, study patients were randomized to standard-of-care PEG IFN–RBV therapy versus triple therapy with PEG IFN–RBV plus a PI. In the ADVANCE trial, patients received PEG IFN-α2a–RBV–TVR for 8 or 12 weeks followed by PEG IFN–RBV for up to another 40 or 36 weeks, respectively. This trial included response-guided therapy (RGT); patients who achieved an undetectable HCV RNA level at weeks 4 and 12 (an extended rapid virologic response [eRVR]) could truncate PEG IFN–RBV therapy to a total of 24 weeks, whereas patients who did not achieve an eRVR but cleared virus by week 24 continued PEG IFN–RBV therapy for 40 to 36 more weeks. In the 12-week triple-drug therapy arm, 75% of patients achieved an SVR, compared with 44% of patients receiving PEG IFN–RBV therapy alone, and, even in the 8-week triple-therapy arm, SVRs occurred in 69%. (Of note, since the trials of the first of the DAAs, an SVR has been defined as undetectable HCV RNA through posttreatment week 12 (SVR_{12}), which was shown to be the equivalent of the former 24-week milestone.) On the basis of RGT rules, 58% of TVR-treated study patients met eligibility criteria to discontinue therapy at 24 weeks and had SVR rates of 83% to 89%, respectively, for the 8-week triple-therapy group and for the 12-week triple-therapy group; the likelihood of an SVR in patients who did not meet shortened-therapy criteria was lower—50% and 54%, respectively—but still better than in patients treated for 48 weeks in the PEG IFN–RBV control group who did not achieve an eRVR (39%). A follow-up study in 540 treatment-naïve participants (ILLUMINATE) confirmed the value of RGT with TVR; among the 65% of study patients who achieved an eRVR, 92% went on to an SVR.[492]

In the SPRINT 2 trial of BOC in treatment-naïve subjects, patients were randomized to 48 weeks of PEG IFN–RBV versus PEG IFN–RBV–BOC triple therapy; this trial included 4 weeks of lead-in therapy with PEG IFN–RBV for all subjects and both an RGT arm (BOC–PEG IFN–RBV for 24 weeks if HCV RNA was undetectable at weeks 8 and 24 or a full 48 weeks of triple therapy if RGT criteria were not met) and a fixed-duration arm (PEG IFN–RBV–BOC for 44 weeks following the 4-week PEG IFN–RBV lead-in). Rates of SVR rates were 63% in the RGT arm (44% meeting criteria for shortened, 28-week, therapy), 66% in the full 48-week-therapy arm, and 38% in the PEG IFN–RBV control arm. The rate of SVR was 67% to 69% in nonblack patients, but only 42% to 53% in blacks. Among subjects in the RGT arm who did not meet criteria for shortened therapy, SVRs were no more common than in the PEG IFN–RBV control arm.

REALIZE (TVR)[493] and RESPOND-2 (BOC)[494] were the registration trials in treatment-experienced patients with genotype-1 chronic hepatitis C who had not achieved SVRs during prior courses of IFN-based therapy: relapsers who had an end-of-treatment response (undetectable HCV RNA) but whose HCV RNA reemerged after therapy; partial responders who had a greater than or equal to 2-$\log_{10}$ IU/mL reduction in HCV RNA but never cleared viremia during previous IFN-based therapy; and null responders, in whom levels of HCV RNA fell by less than 2 $\log_{10}$ during previous therapy. The registration trial of TVR in prior nonresponders, REALIZE, involved all three categories of nonresponders, did not include RGT, but did have a lead-in arm. Subjects in the PEG IFN–RBV–TVR treatment arms (12 weeks of triple therapy with or without a 4-week lead-in phase followed by PEG IFN–RBV for the remainder of 48 weeks) had indistinguishable SVRs of 66% and 64%, respectively, compared with the control group, which received PEG IFN–RBV for 48 weeks and had a 17% rate of SVR. The likelihood of an SVR was inversely proportional to the relative level of treatment refractoriness during previous IFN-based therapy—31% in prior null responders, 57% in prior partial responders, and 86% in prior relapsers.

The registration trial of BOC in prior nonresponders, RESPOND-2, involved former relapsers and partial responders but not null responders and included both a 4-week PEG IFN–RBV lead-in period and RGT. Patients were randomized to 4 weeks of lead-in PEG IFN–RBV therapy followed by either triple-drug RGT (PEG IFN–RBV–BOC through week 36 for an eRVR [defined as undetectable HCV RNA at week 8 and at all subsequent time points through week 36] but for a full 44 weeks of triple-drug therapy if eRVR was not achieved) or fixed-duration triple-drug therapy (triple-drug therapy for 44 weeks after lead-in) or to a 48-week PEG IFN–RBV control group. An SVR was achieved in up to 52% of prior partial responders and in up to 75% in prior relapsers (differences between the RGT and fixed-duration triple-drug arms were small and related to unexplained slightly lower on-treatment responses in the RGT group that occurred during the initial 36 treatment weeks, when regimens were identical). In a subsequent open-label trial among 42 prior null responders, an SVR was achieved in 38% after 48 weeks of therapy (44 weeks of PEG IFN–RBV–BOC following 4 weeks of PEG IFN–RBV lead-in therapy).

Essential conclusions from trials of both PIs in treatment-experienced patients are that prior relapsers attained an SVR at rates exceeding those of treatment-naïve patients (69% for BOC and 86% for TVR); prior partial responders had intermediate SVR rates of 52% (48-week BOC arm) to 57% (48-week TVR arm); and prior null responders had the lowest SVR rates, in the 30% range. Pretreatment bridging fibrosis or cirrhosis at biopsy had no effect on SVR rates in prior relapsers but was associated with a poorer outcome in prior partial and, especially, in prior null responders. For example, among TVR-treated null responders, SVRs occurred in 41% of TVR-treated subjects with minimal fibrosis, in 39% with bridging fibrosis, but in only 14% with cirrhosis (for comparison, 10% of PEG IFN–RBV–treated prior null responders with cirrhosis had SVRs). For both PIs, responsiveness correlates with *IL28B* genotype (CC better than non-CC), ethnicity (white better than black), fibrosis stage (low stage better than high stage), HCV genotype subtype (1b more responsive than 1a), and, when available, level of HCV RNA reduction during lead-in PEG IFN–RBV therapy (≥1 $\log_{10}$ reduction better than <1 $\log_{10}$ reduction). Unlike the case for PEG IFN–RBV therapy, for PI-based triple-drug therapy, age, baseline HCV RNA level, and insulin resistance have little or no influence on treatment responsiveness.

Recommendations based on randomized controlled trials and on data extrapolated by FDA analysis from the body of data generated in treatment-naïve and treatment-experienced patients[495,496] were issued in 2011 for TVR and BOC, as follows. For treatment-naïve patients with chronic hepatitis C, genotype 1, treatment consisted of TVR or BOC along with PEG IFN–RBV. For TVR, 750 mg three times a day with a fatty diet, along with weekly PEG IFN and twice-daily, weight-based RBV, was administered for 12 weeks, followed by an additional 12 weeks of PEG IFN–RBV for patients with an eRVR (HCV RNA undetectable, but ≤1000 IU/mL, at weeks 4 and 12) and for an additional 36 weeks for patients without an eRVR (but with HCV RNA undetectable at week 24). For BOC, after 4 weeks of PEG IFN–RBV lead-in therapy, patients received BOC 800 mg three times daily with food along with weekly PEG IFN and twice-daily, weight-based RBV for 24 weeks (total duration of therapy, 28 weeks), if HCV RNA was undetectable at weeks 8 and 24. If HCV RNA was detectable at week 8, less than 100 IU/mL at week 12, but undetectable at week 24, triple-drug PEG IFN–RBV–BOC was continued for another 12 weeks (through week 36), followed by an additional 12 weeks of PEG IFN–RBV (total duration of therapy 48 weeks). Patients with cirrhosis treated with either TVR or BOC were treated for a full 48 weeks, without response-guided shortening of therapy.

For treatment-experienced patients who had relapsed after prior IFN-based therapy, the treatment regimen was the same as that for treatment-naïve patients. Data in relapsers with cirrhosis were too limited for a definitive recommendation about the duration of therapy.

For treatment-experienced patients who were prior partial responders and noncirrhotic, RGT with BOC (4 weeks of lead-in therapy with PEG IFN–RBV, followed by BOC along with PEG IFN and RBV through week 36 if HCV RNA was undetectable at weeks 8 and 24, but, if HCV RNA is detectable at week 8 but undetectable at week 24, followed by an additional 12 weeks of PEG IFN–RBV, through week 48) or full 48-week TVR-based therapy (12 weeks of triple-drug therapy followed by 36 weeks of PEG IFN–RBV therapy) was recommended. In BOC-treated patients, if HCV RNA did not fall by at least 1 $\log_{10}$ IU/mL during lead-in with PEG IFN–RBV, a full 44 weeks of triple-drug therapy was considered.

If a decision was made to treat null responders, a full 48 weeks of therapy was recommended (for TVR, 12 weeks of triple therapy followed by 36 weeks of PEG IFN–RBV; for BOC, 4 weeks of lead-in PEG IFN–RBV, followed by 44 weeks of triple-drug treatment, ending at week 48).

For these regimens, failure to achieve HCV RNA reductions at critical treatment milestones was shown to be invariably predictive of nonresponse, justifying absolute stopping rules for futility—for TVR, if HCV RNA was greater than 1000 IU/mL at weeks 4 or 12, or if HCV RNA remained detectable at week 24; for BOC, if HCV RNA was greater than or equal to 100 IU/mL at week 12, or if HCV RNA remained detectable at week 24. If PEG IFN–RBV was to be discontinued for any reason, the PI also had to be discontinued in order to protect the patient from being treated with PI monotherapy, which would engender the emergence of resistance.

Triple therapy did not avert the toxicities of PEG IFN–RBV, and both TVR and BOC could worsen the anemia induced by PEG IFN–RBV, mandating close laboratory monitoring for anemia and requiring red blood cell transfusions in some cases, especially in prior null responders with cirrhosis. Rectal burning occurred in approximately one-third of TVR-treated patients, and dysgeusia (altered sense of taste) was common to both PIs (approximately one-third of BOC-treated patients and approximately 10% of TVR-treated patients). Moreover, in TVR trials, 6% of patients withdrew because of the development of a severe, confluent, maculopapular, pruritic rash of the trunk and extremities, which in some cases necessitated systemic corticosteroid therapy; careful monitoring was necessary to identify rashes when they were early, mild, and easily reversible. Both drugs were administered every 8 hours with food, which, for TVR, had to be a 20-g fat meal. Lastly, both drugs are metabolized via the P-450 (CYP3A4) system, accounting for multiple drug-drug interactions, including with many medications (e.g., statins) taken in the middle-aged population in which hepatitis C is most prevalent. Careful review of coadministered medications was paramount before initiation of treatment. The rigors of triple-drug PI therapy—multiplied intolerability, complexity of RGT and stopping rules, thrice-daily dosing and high pill burden, multiple drug-drug interactions, and so on—and the imminent availability of next-generation and all-oral direct antiviral therapy (see later) tempered initial enthusiasm for this generation of direct antivirals. Although first-generation PI-based triple-drug therapy was the recommended standard of care from 2011 to 2013 for patients with genotype-1 chronic hepatitis C, in practice, the treating community became progressively more conservative as time elapsed, in anticipation of simpler, more effective DAA regimens to come. For patients with mild disease and low-stage fibrosis, treatment was postponed, whereas initiation of first-generation therapy was more selective, limited to specific populations of patients who warranted immediate therapy, such as prior relapsers who have bridging fibrosis or cirrhosis on biopsy or other subgroups in whom mitigating the risk of progressive liver disease and its complications commanded a high priority.

The most frequently described mutation in NS3/4A was R155K, which occurred at the catalytic site where BOC and TVR bind. This mutation is found more commonly in genotype 1a–infected patients because one nucleotide change creates the amino-acid substitution in 1a, whereas two nucleotide changes are required for the same substitution in genotype 1b. This common variant may explain the consistently higher SVR rates observed in genotype 1b patients on triple therapy. Although most nonresponders to PI-based triple-drug therapy acquired resistance variants, these variants were not archived and were supplanted by wild-type HCV within 2 years of stopping therapy in almost all patients.

Once IFN-free regimens consisting of NS5A inhibitors and improved PIs were introduced in 2016–17 (see later), TVR and BOC were discontinued; they are no longer recommended as treatment for hepatitis C. PEG IFN–based treatments have now been supplanted by combinations of orally administered, small-molecule HCV inhibitors with few side effects, simple dosing, treatment courses as brief as 8 to 12 weeks, pangenotypic efficacy, and negligible resistance.

Second-Generation NS3-4A Protease Inhibitor and First-Generation Nucleoside Polymerase Inhibitor for Chronic Hepatitis C: Simeprevir and Sofosbuvir—Standard of Care, 2014–15

Simeprevir (SMV), a second-generation PI for HCV genotype 1, was approved in November 2013; the pangenotypic nucleoside polymerase inhibitor SOF was approved in December 2013.

SMV, with antiviral activity against HCV genotype 1 (1b > 1a), had improved pharmacokinetic properties compared with those of first-generation PIs and, therefore could be taken once a day. Like first-generation PIs, SMV had to be taken with PEG IFN–RBV and with food. Approximately a third of patients with HCV genotype 1a harbor an NS3 polymorphism, Q80K, which renders them refractory to the antiviral activity of SMV; if SMV therapy was being considered, Q80K testing was advisable, and, if results were positive, discouraged the use of SMV.

In two phase III clinical trials among treatment-naïve participants with chronic hepatitis C (QUEST-1 and QUEST-2), SMV 150 mg daily plus PEG IFN–RBV for 12 weeks, followed by another 12 weeks of PEG IFN–RBV, yielded an SVR in 80% (compared with 50% of control subjects treated with PEG IFN–RBV). In participants with genotype 1a and a Q80K variant, SMV triple-drug therapy was no more effective than PEG IFN–RBV, and the efficacy of SMV-based triple-drug therapy was reduced to 58% in participants with cirrhosis. Phase III trials included RGT (if HCV RNA was <25 IU/mL at week 4 and undetectable at week 12, the treatment course could end at week 24; if these milestones were not met [but stopping rules not violated], treatment was extended to 48 weeks); however, in the 8% of study participants who did not meet RGT milestones for shortened therapy, only 25% experienced an SVR. Therefore, RGT was not recommended for SMV. The efficacy of SMV-based triple-drug therapy was similar in prior relapsers to IFN-based therapy, achieving (in the phase III PROMISE trial) an SVR in 79% (compared with 37% in a PEG IFN–RBV–treated control group). Phase III trials did not include treatment-experienced nonresponders, but, based on phase II trials, SMV-based triple-drug therapy was approved for partial responders and null responders, for all of whom a full 48 weeks of therapy (12 weeks of SMV with PEG IFN–RBV, followed by 36 weeks of PEG IFN–RBV) was recommended; in these phase II trials, an SVR was achieved in 85% of prior relapsers, 70% of prior partial responders, and 45% of prior null responders. These treatment approaches applied to patients with any stage of fibrosis, including those with cirrhosis. If an HCV RNA suppression milestone of ≤25 IU/mL was not met by week 4, further treatment was futile, and therapy was to be stopped; if HCV RNA was not suppressed to ≤25 IU/mL at week 12 or 24 (by which time SMV had been completed), PEG IFN–RBV was to be stopped for futility. In black patients, SMV therapy was approximately 10% less effective in achieving an SVR than in white patients. SMV is metabolized in the liver by cytochrome P-450 3A (CYP3A); therefore, concomitant administration of SMV along with CYP3A inducers or inhibitors could amplify or reduce SMV exposure, and it was important that prescribers consult prescribing information and/or the website www.hep-druginteractions.org before prescribing SMV. In addition to the side effects of PEG IFN–RBV, SMV-based therapy was associated with photosensitivity, rash, and mild hyperbilirubinemia (Janssen Therapeutics, Titusville, NJ, November 2013).[497–501] Fourth-generation PIs and second-generation NS5A inhibitors became standard of care in 2017 (see later) and reduced the appeal of SMV; it is no longer recommended as first-line treatment for hepatitis C (www.hcvguidelines.org).

SOF was the first non-PI DAA to be approved. SOF is a uridine nucleoside polymerase inhibitor with one of the best profiles among the new oral hepatitis C antiviral agents. It is very potent, has a high barrier to resistance and pangenotypic activity, is very well tolerated with few adverse events, requires only once-daily oral administration, and appears to be relatively free from major drug-drug interactions.[502,503] In clinical trials, SOF has been studied in all genotypes (1–6); in treatment-naïve patients and prior null-responders to PEG IFN–based therapy; in prior TVR and BOC nonresponders, with PEG IFN–RBV or in IFN-free regimens; in combination with RBV or with NS5A inhibitors; and for treatment periods as brief as 8 to 12 weeks to as long as 24 weeks.[504–506]

In a phase III, IFN-free trial of SOF plus RBV—in patients with genotypes 2 and 3 who were IFN intolerant, ineligible, or unwilling (55% *IL28B* non-CC; 16% cirrhotic)—78% achieved SVR (93% genotype 2, 61% genotype 3) versus 0% in placebo recipients.[506] Similarly, among prior PEG IFN–RBV nonresponders with genotypes 2 and 3 treated with open-label SOF-RBV (IFN free) for 12 or 16 weeks, 50% (86% genotype 2, 30% genotype 3) and 73% (94% genotype 2, 62% genotype

3), respectively, experienced SVRs.[506] In phase III trials, among patients with genotypes 1 and 4 to 6, open-label SOF plus PEG IFN–RBV for 12 weeks yielded SVRs of 90% (compared with 60% in historical control subjects); 89% in genotype 1; and 97% in genotypes 4 to 6 (NEUTRINO trial).[505] In treatment-naïve participants with genotypes 2 and 3, SVRs occurred in 67% of patients treated with either SOF and RBV for 12 weeks or PEG IFN–RBV for 24 weeks; again, SVR was more frequent in SOF-RBV recipients with genotype 2 (97%) than those with genotype 3 (56%) (FISSION trial).[505] IFN-free combinations of SOF plus NS5A inhibitors yielded near-100% SVRs in both treatment-naïve participants and prior null responders with genotypes 1, 2, and 3 after treatment durations as brief as 12 weeks (ledipasvir [LDV]) and 24 weeks (daclatasvir) (see later). A combination fixed-dose pill containing 400 mg of SOF plus 90 mg of LDV to be used with or without RBV for 8 weeks (treatment-naïve, noncirrhotic patients) to 12 weeks (treatment-experienced, cirrhotic patients) (LONESTAR, ION-1, ION-2, ION-3 trials) was approved in October 2014 (see later).[507–510] The combination of SOF and SMV was also found to be very effective in patients with genotype 1 and was a recommended first-line regimen until superseded by later-generation DAAs (see later).[511]

SOF may cause significant bradycardia when administered with amiodarone, particularly if a β-blocker is also being administered. Therefore, administration of amiodarone along with SOF-containing regimens should be avoided. SOF was approved for combination therapy of chronic hepatitis C with genotypes 1, 2, 3, and 4, including in patients with HCC awaiting liver transplantation and those with HCV and HIV-1 coinfection, in whom efficacy is comparable to that in patients with HCV monoinfection. SOF can be used in patients with mild-to-moderate renal impairment but is not recommended for patients with severe renal failure (CrCl <30 mL/min). Therapeutic acid suppression (e.g., proton pump inhibitors [PPIs]) raise gastric pH, which reduces the solubility of SOF; therefore, if a PPI is to be used, both the PPI (equivalent to ≤20 mg of omeprazole) and SOF should be taken together under fasted conditions. The dosage of SOF is 400 mg orally every day, and combination regimens are listed in Table 117.9, which reflect the September 2017 recommendations of the AASLD and the Infectious Diseases Society of America (IDSA), which have collaborated to update consensus treatment recommendations (www.hcvguidelines.org). Those specific recommendations are further discussed later.

First-Generation NS5A Inhibitors, Second-Generation Nucleoside Polymerase Inhibitors, and Third-Generation NS3-4A Protease Inhibitors—Standard of Care, 2015 to September 2017

The AASLD and IDSA have been formulating and updating recommendations for treatment of HCV-infected patients (www.hcvguidelines.org). The recommendations issued in January 2015 and February 2016 were

TABLE 117.9 AASLD and IDSA Recommendations for Treatment of Hepatitis C: September 2017

FIRST-LINE TREATMENT RECOMMENDATIONS IN DAA-NAÏVE PATIENTS*		
GENOTYPE	**NO CIRRHOSIS**	**COMPENSATED CIRRHOSIS**
1	GLE-PIB × 8 wk SOF-LDV × 8-12 wk (8 wk for noncirrhotic, nonblack patients with HCV RNA <6 × 10^6 IU/mL without HIV infection or PEG IFN–RBV experience) SOF-VEL × 12 wk GRZ-ELB × 12 wk	GLE-PIB × 12 wk GRZ-ELB × 12 wk SOF-LDV × 12 wk SOF-VEL × 12 wk
2	GLE-PIB × 8 wk SOF-VEL × 12 wk	GLE-PIB × 12 wk SOF-VEL × 12 wk
3	GLE-PIB × 8 wk SOF-VEL × 12 wk	GLE-PIB × 12 wk SOF-VEL × 12 wk GRZ-ELB + SOF × 12 wk SOF-VEL-VOX × 12 wk
4	GLE-PIB × 8 wk GRZ-ELB × 12 wk SOF-VEL × 12 wk SOF-LDV × 12 wk	GLE-PIB × 12 wk GRZ-ELB × 12 wk SOF-LDV × 12 wk SOF-VEL × 12 wk
5 or 6	GLE-PIB × 8 wk SOF-LDV × 12 wk SOF-VEL × 12 w	GLE-PIB × 12 wk SOF-LDV × 12 wk SOF-VEL × 12 w

GENOTYPE	**FIRST-LINE TREATMENT RECOMMENDATIONS IN DAA-EXPERIENCED PATIENTS (DURATION 12 WK FOR ALL CHOICES)***		
Experience with	PI + PEG IFN–RBV	SOF-containing DAA, no NS5A	NS5A
1	GLE-PIB SOF-LDV SOF-VEL	GLE-PIB SOF-VEL SOF-VEL-VOX	SOF-VEL-VOX
2	Not relevant	GLE-PIB SOF-VEL	No recommendation
3	SOF-VEL-VOX	SOF-VEL-VOX	SOF-VEL-VOX
4	SOF-VEL-VOX	SOF-VEL-VOX	SOF-VEL-VOX
5 or 6	SOF-VEL-VOX	SOF-VEL-VOX	SOF-VEL-VOX

AASLD, American Association for the Study of Liver Diseases; *DAA,* direct-acting antiviral; *ELB,* elbasvir 50 mg[a,b]; *GLE,* glecaprevir 300 mg[b,c]; *GRZ,* grazoprevir 100 mg[b,c]; *HCV,* hepatitis C virus; *IDSA,* Infectious Diseases Society of America; *LDV,* ledipasvir 90 mg; *PEG IFN,* pegylated interferon; *PI,* protease inhibitor; *PIB,* pibrentasvir 120 mg[b]; *RBV,* ribavirin; *SOF,* sofosbuvir 400 mg[d]; *VEL,* velpatasvir 100 mg; *VOX,* voxilapervir 100 mg[c]. (Note: drugs with endings "-buvir" are polymerase inhibitors, "-asvir" are NS5A inhibitors, and "-previr" are protease inhibitors.)

*Order of listing does not convey preference for one regimen over another. See www.hcvguidelines.org for caveats/minor exceptions, alternative treatments, treatment in special populations, situations that merit baseline resistance testing, and information on drug-drug interactions.

[a]Baseline high-fold NS5A resistance-associated substitutions for ELB should not be present in patients with genotype 1a.

[b]DAA regimens GLE/PIB and GRZ/ELB are recommended for patients with stage 4–5 kidney disease (creatinine clearance <30 mil/min).

[c]US Food and Drug Administration warning letter issued in October 2015 that hepatic decompensation and failure have been reported with protease inhibitor-containing DAAs, which are not recommended for patient with decompensated cirrhosis (Child-Pugh class B and C, see text).

[d]SOF-containing DAA combinations are not recommended for stage 4–5 chronic kidney disease (creatinine clearance <30 mil/min).

based on the approval of the fixed combination of LDV and SOF (Harvoni; Gilead Sciences, Foster City, CA); the fixed combination of paritaprevir, ritonavir, and ombitasvir, plus dasabuvir (Viekira Pak; AbbVie, North Chicago, IL) plus or minus RBV; the fixed combination of grazoprevir and elbasvir (Zepatier; Merck, Whitehouse Station, NJ); the NS5A inhibitor declatasvir (Daklinza; Bristol-Myers Squibb, Princeton, NJ); and the fixed combination of paritaprevir, ritonavir, and ombitasvir (Technivie; AbbVie). These and subsequently approved treatments are IFN-free regimens that rely on DAAs, such as next-generation NS3/4 PIs (names ending in *-previr*), nucleoside and nonnucleoside NS5B polymerase inhibitors (names ending in *-buvir*), and inhibitors of NS5A (a membrane-associated phosphoprotein that is part of the HCV RNA replication complex; names ending in *-asvir*).[503,512] (These drugs are discussed further in Chapter 47.) As a class, first-generation PIs had good efficacy in treatment-naïve patients and prior relapsers but only fair efficacy in prior nonresponders, a low barrier to resistance, a narrow genotype specificity, challenging tolerability, and multiple drug-drug interactions.[503]

Second-generation PIs—SMV, as discussed earlier, and ritonavir-boosted paritaprevir[513]—have improved pharmacokinetics (once-daily instead of thrice-daily administration), reduced regimen complexity, efficacy across genotypes, a higher barrier to resistance, improved tolerability, and fewer drug-drug interactions. SMV when combined with PEG IFN–RBV (as reviewed earlier) or with SOF and ritonavir-boosted paritaprevir in the absence of PEG IFN, with one or more other direct oral antivirals (polymerase inhibitors, NS5A inhibitors, RBV, or a combination of these), has been shown to achieve SVR rates greater than 90% in both treatment-naïve patients and prior null responders with genotype-1 HCV infection, including those with the more refractory *IL28B* non-CC host genotypes and viral genotype 1a.

The NS5A inhibitors suppress HCV replication rapidly and profoundly but tend to have less-than-ideal resistance profiles[503]; however, some in this class have been reported to have excellent resistance profiles and broad genotype specificity. Drug class combinations that have been approved are described here.

Ledipasvir-sofosbuvir (Harvoni). LDV-SOF was approved in October 2014 for treatment of hepatitis C, genotypes 1a, 1b, 4, and 6 (see Table 117.9). LDV, 90 mg, an NS5A inhibitor, is combined with SOF, 400 mg, an NS5B inhibitor, reviewed earlier. Based on the results of phase III trials in treatment-naïve noncirrhotic patients (ION-3),[509] treatment-naïve cirrhotic and noncirrhotic patients (ION-1),[508] and treatment-experienced cirrhotic and noncirrhotic patients (ION-2)[510] with genotype-1 chronic hepatitis C, the duration of therapy is 12 weeks for treatment-naïve patients and for treatment-experienced patients without cirrhosis. For treatment-naïve nonblack patients without cirrhosis who have HCV RNA levels of <6 million IU/mL, 8 weeks of therapy has been shown to be sufficient. For treatment-experienced patients with cirrhosis, 24 weeks of therapy is required. Clinical trials in both treatment-naïve and treatment-experienced patients have demonstrated SVR rates of 91% to 100%.[507–510] Moreover, studies with and without RBV have indicated that addition of RBV does not improve efficacy of LDV-SOF. Moreover, this combination therapy has been shown to be effective[514] and is now indicated for patients with decompensated cirrhosis; however, it has not been shown to be effective in patients with advanced renal failure. The combination of LDV-SOF is given once daily and is generally well tolerated. The most common side effects are fatigue, insomnia, headache, and nausea and are generally mild in intensity. As noted earlier, SOF may cause severe bradycardia when administered with amiodarone, particularly if a β-blocker is also being administered. Therefore, administration of amiodarone along with SOF-containing regimens should be avoided. As with other SOF-containing regimens, LDV-SOF is not recommended for patients with severe renal impairment (CrCl <30 mL/min).

Drugs that induce P-gp, such as St. John's wort and rifampin, may reduce the concentration of both LDV and SOF. Gastric acid inhibitors can also reduce LDV concentrations and should be used at low doses if possible. Ideally, PPIs and LDV-SOF should not be used together; however, if PPI treatment is necessary, LDV-SOF should be taken with food 4 hours prior to 20 mg of omeprazole. LDV and SOF are further discussed in Chapter 47.

Ritonavir-booster paritaprevir, ombitasvir, and dasabuvir (Viekira Pak). Paritaprevir is an NS3/4A PI boosted by ritonavir, ombitasvir is an NS5A inhibitor, and dasabuvir is a nonnucleoside NS5B inhibitor. Initially, the regimen was formulated as ritonavir-boosted paritaprevir and ombitasvir in a single tablet (taken once daily) plus dasabuvir in a separate tablet taken twice daily. Later, an extended-release version (Viekira XR) was approved, consisting of three individual tablets (ritonavir-boosted paritaprevir, ombitasvir, and long-acting dasabuvir) taken once daily (with or without twice daily RBV). The combination of ritonavir-boosted paritaprevir, ombitasvir, dasabuvir, and RBV (i.e., up to five drugs) was shown to be an effective all-oral, IFN-free regimen and was approved in December 2014 (the extended-release version approved in July 2016) for treatment of genotypes 1a and 1b (see Table 117.9). The combination without dasabuvir (Technivie) was approved for genotype 4 in July 2015. Clinical trials have demonstrated that this regimen is highly effective in treatment-naïve patients with and without cirrhosis and in those in whom therapy with PEG IFN and RBV has failed.[515–519] High rates of SVRs have been noted, ranging from 93% to 100%, with the highest efficacy seen in genotype-1b infection. In 473 noncirrhotic, treatment-naïve patients, the combination of ritonavir (100 mg)-boosted paritaprevir (150 mg once daily), ombitasvir (25 mg once daily), dasabuvir (250 mg twice daily), and weight-based RBV for 12 weeks achieved an SVR_{12} in 96.2% (SAPPHIRE-I).[515] For genotype 1a, efficacy was approximately 7% lower without RBV (90%) than with RBV (97%), whereas for genotype 1b, SVRs were achieved in 99% with or without RBV (PEARL-III and PEARL-IV).[516] In 297 noncirrhotic, treatment-experienced participants, this five-drug combination was associated with an SVR_{12} in 96.3%: 95.3% in relapsers, 100% in partial responders, and 95.2% in null responders (SAPPHIRE-II).[517] In a group of 380 treatment-naïve and treatment-experienced participants with well-compensated cirrhosis, the same five-drug treatment regimen for 12 weeks or 24 weeks yielded an SVR_{12} in 91.8% and 95.9%, respectively. In the treatment-naïve subgroup, 12- and 24-week SVR_{12} rates were 94.2% and 94.6%, respectively. In the treatment-experienced subgroup, 12- and 24-week SVR_{12} rates were 96.6% and 100%, respectively, in relapsers; 94.4% and 100%, respectively, in partial responders; and 86.7% and 95.2%, respectively, in null responders (TURQUOISE-II).[518] The rate of SVR_{12} after treatment with this drug combination was indistinguishable between study participants with genotypes 1a and 1b.[515,517,518] Null-responder cirrhotic patients with genotype 1a treated for 12 weeks responded less well (SVR_{12} 80%) than those treated for 24 weeks (SVR_{12} 92.9%).[518]

For genotype 1a, the combination is given with weight-based RBV (in two divided doses taken twice daily) for 12 weeks (no cirrhosis) or 24 weeks (compensated cirrhosis). For genotype 1b, this drug combination is given for 12 weeks, without RBV (no cirrhosis) or with RBV (compensated cirrhosis). For genotype 4, the regimen is given for 12 weeks with weight-based RBV but without dasabuvir. As noted earlier and reviewed later, this three-component fixed-dose regimen is available as Technivie.

In October 2015, the FDA issued a warning letter, which is added to prescribing information for both Viekira Pak and Technivie, noting that hepatic decompensation and failure have been reported with these medications. Patients who receive these drugs should be monitored closely for hepatic decompensation, and administration of medications should be stopped if hepatic decompensation is noted. Currently, all PI-containing DAAs for hepatitis C, although recommended in compensated cirrhosis (Child-Pugh class A), are contraindicated in patients with decompensated cirrhosis (Child-Pugh classes B and C).

The regimen has been generally well tolerated, with a variety of common, usually mild side effects. These include fatigue, asthenia, insomnia, headache, and pruritus. Hyperbilirubinemia (primarily unconjugated) and elevations in ALT were seen, which resolved during treatment or immediately thereafter. Components of the regimen are substrates and inhibitors of important metabolic enzymes, and drug interactions can occur, such as with rifampin, St. John's wort, anticonvulsants, salmeterol, and ethinyl estradiol–containing products. These drugs are discussed further in Chapter 47.

Now that more effective, simpler regimens are available, paritaprevir-ombitasvir-dasabuvir combinations are no longer recommended as first-line therapy (www.hcvguidelines.org).

Elbasvir-Grazoprevir (Zepatier). Elbasvir-grazoprevir is an oral fixed-dose combination that consists of an NS5A replication complex inhibitor, elbasvir (50 mg), and an NS3/4A PI, grazoprevir (100 mg). The elbasvir-grazoprevir combination was approved in January 2016 for treatment of HCV genotypes 1a, 1b, and 4 (see Table 117.9). Approval was based on the results of clinical trials in patients with HCV genotypes 1, 4, and 6, both in treatment-naïve and treatment-experienced patients, without cirrhosis or with compensated cirrhosis. A regimen of one tablet taken daily for 12 weeks resulted in high rates of SVR_{12} in treatment-naïve patients (95%–100%).[520] An SVR_{12} of 92% to 97% was found in treatment-experienced patients who received elbasvir-grazoprevir for 12 to 16 weeks with or without concomitant RBV.[521] Patients coinfected with HIV and HCV also had high SVR_{12} responses (92%).[522]

Resistance to elbasvir-grazoprevir in HCV 1a infections is conferred by baseline polymorphisms to HCV NS5A at positions M28, Q30, L31, or Y93. These result in apparent decreased efficacy of the 12-week regimen, and if present, extension of therapy to 16 weeks and addition of RBV are recommended.[523,524]

Elbasvir-grazoprevir is generally well tolerated. In pooled data from phase II and III clinical trials, the most common adverse events were fatigue (11%), headache (10%), and nausea (5%), similar to rates in placebo recipients.[523–525] ALT elevations greater than five times the ULN occurred in 1% of participants, usually at or after 8 weeks of therapy, and mostly resolved at or after completion of therapy. Anemia was observed when RBV was included in therapy.

For patients with compensated cirrhosis (Child-Pugh class A), dose adjustment is not necessary, but elbasvir-grazoprevir, like any PI-containing DAA combination, should not be used in patients with moderate or severe hepatic impairment[523,524] (Child-Pugh class B or C). Dose reduction is not necessary in patients with renal insufficiency, including those on hemodialysis; unlike SOF-containing regimens, which have not been shown in clinical trials to be effective in patients with renal failure (CrCl <30 mL/min), elbasvir-grazoprevir is effective in this population subset.

Potential drug-drug interactions are important considerations in the use of elbasvir-grazoprevir.[524,525] Both are substrates of cytochrome P3A (CYP3A) and should not be used with strong CYP3A inducers such as efavirenz and rifampin. CYP3A inhibitors, such as ketoconazole, and inhibitors of organic anion transporting polypeptide 1B1 (OATP1B1), such as cyclosporine, may raise levels of elbasvir-grazoprevir and should not be used concomitantly.

Daclatasvir (Daklinza). Daclatasvir is an oral medication that is an inhibitor of HCV phosphoprotein 5A (NS5A) and may also inhibit phosphorylation of NS4A. It was approved by the FDA in July 2015 for treatment of patients with HCV genotype 3 and in February 2016 for patients with genotypes 1a and 1b in combination with SOF, with and without RBV. Before, declatasvir was superseded by more effective pangenotypic drug combinations, the AASLD/IDSA guidelines also included a recommendation for its use in treatment of genotype-2 infections. The recommended dose of daclatasvir is one 60-mg tablet per day, along with one tablet of SOF (400 mg) per day.

Clinical trials of daclatasvir plus SOF have shown high rates of SVR_{12} in genotypes 1, 2, and 3 infections. In a study of 334 patients, an SVR_{12} was 98% for genotype-1 treatment-naïve or treatment-experienced patients, 92% for genotype 2–infected patients, and 89% for genotype 3–infected patients.[526] In patients with cirrhosis and Child-Pugh class A (compensated) and Child-Pugh class B or C (decompensated), treatment with daclatasvir plus SOF and RBV resulted in SVR_{12} of 94% to 56%.[527] Patients with HIV-HCV coinfection also responded well to daclatasvir and SOF (SVR_{12} of 97% to 98%).[528] Dose adjustments are not necessary for renal or hepatic impairment. Resistance to daclatasvir plus SOF in genotype-1a infection was associated with polymorphisms of amino acids at NS5A positions M28, Q30, L31, and Y93.[529,530]

Daclatasvir was generally well tolerated in reported studies. When administered along with SOF, the most commonly reported adverse events were fatigue (14%), headache (14%), nausea (8%), and diarrhea (5%). Because SOF can cause serious bradycardia when administered with amiodarone, particularly if a β-blocker is also being administered, administration of amiodarone along with the combination of daclatasvir and SOF should be avoided.

Daclatasvir is a substrate of CYP3A, and drug levels will be reduced when this agent is administered with strong inducers of CYP3As. Administration with strong CYP3A inducers such as rifampin, phenytoin, or St. John's wort should be avoided. If a moderate inducer of CYP3A, such as efavirenz, needs to be given, the daclatasvir dose may be increased to 90 mg/day. If a strong inhibitor of CYP3A is given, the dose of daclatasvir may be reduced to 30 mg/day.

Daclatasvir is an inhibitor of P-glycoprotein transporter (P-gp), OATP1B1 and OATP1B3, and breast cancer resistance protein (BCP). Daclatasvir may increase the levels of drugs that are substrates of these transporters, such as digoxin, rosuvastatin, and dabigatran. Because of the availability of more effective, pangenotypic drug combinations, declatasvir is no longer recommended as first-line therapy for hepatitis C (www.hcvguidelines.org).

Paritaprevir-ritonavir-ombitasvir (Technivie). Technivie is an oral fixed-dose combination that consists of three of the four components in Viekira Pak (paritaprevir-ritonavir-ombitasvir [discussed previously]) but does not include the fourth component, dasabuvir, which does not have activity against genotype 4. The regimen consists of two tablets taken daily, each containing paritaprevir (75 mg; two-tablet total dose 150 mg), ritonavir (50 mg; total dose 100 mg), and ombitasvir (12.5 mg; total dose 25 mg) along with meals. Technivie plus RBV received FDA approval in July 2015 for treatment of HCV genotype 4 in patients without cirrhosis but was recommended in the February 2016 AASLD/IDSA guidelines for patients with genotype 4 who have compensated cirrhosis. In October 2015, the FDA issued a warning letter that is added to prescribing information for both Technivie[531,532] and Viekira Pak, stating that hepatic decompensation and failure have been reported with these medications. Patients who receive these medications should be monitored closely for hepatic decompensation, and, if noted, administration of Technivie and Viekira Pak should be stopped.

The clinical trial on which approval of Technivie was based consisted of 135 participants with HCV genotype-4 infections without cirrhosis who were treated with Technivie with or without RBV for 12 weeks. SVR_{12} was achieved in 91% (40 of 44) of treatment-naïve patients treated without RBV, in 100% (42 of 42) of treatment-naïve patients treated with RBV, and in 100% (49 of 49) of treatment-experienced patients treated with the RBV-containing regimen.[533,534] Overall, the regimen was generally well tolerated, and the most common side effects consisted of fatigue, weakness, insomnia, and pruritus, most commonly in the RBV-containing regimen. Further discussion of adverse effects and drug-drug interactions of paritaprevir-ritonavir-ombitasvir can be found in the Viekira Pak section presented earlier.

Second-Generation NS5A Inhibitors and Fourth-Generation NS3-4A Protease Inhibitors—Standard of Care, September 2017

The AASLD/IDSA hepatitis C treatment guidelines (www.hcvguidelines.org) were updated in September 2017, as summarized in Table 117.9, to reflect approval of new drug combinations SOF-velpatasvir (VEL), SOF-VEL-voxilaprevir (VOX), and glecaprevir (GLE)-pibrentasvir (PIB). From among the other previously approved DAAs described earlier, only LDV-SOF and grazoprevir-elbasvir (see earlier) were retained as first-line agents.

Sofosbuvir-velpatasvir (Epclusa). With the introduction of the aforementioned combination DAAs, near-complete efficacy was achieved for genotypes 1, 2, 4, 5, and 6, but genotype 3 remained the most refractory and challenging, especially for patients with cirrhosis in whom PEG IFN–RBV therapy had failed. This last "frontier" was addressed with the development of a pangenotypic DAA regimen containing a single-pill, daily combination of the nucleoside polymerase inhibitor SOF, 400 mg, and the second-generation, low picomolar EC_{50} (in vitro drug concentration that achieves 50% of virus replication), high-barrier-to-resistance NS5A inhibitor VEL, 100 mg. SOF-VEL was approved in June 2016 for genotypes 1 to 6, and the approval was extended in August 2017 for patients with HIV infection. Efficacy of this pangenotypic combination was demonstrated in a series of trials, ASTRAL-1 to ASTRAL-4.[535–537]

In 626 patients (including treatment-naïve patients, treatment-experienced patients, noncirrhotics, and compensated cirrhotics) with

genotypes 1, 2, 4, 5, and 6, treatment with SOF-VEL achieved SVR_{12} in 97% to 100% (ASTRAL-1). In ASTRAL-1, among patients receiving SOF-VEL, treatment was well tolerated; adverse events occurred in indistinguishable percentages of SOF-VEL recipients (78%) and 116 placebo recipients (77%).[535] In 134 patients with genotypes 2 (ASTRAL-2) and 277 patients with genotype 3 (ASTRAL-3), of whom 26% to 29%, respectively, had failed prior antiviral treatment, and of whom 29% to 30%, respectively, had compensated cirrhosis, SOF-VEL for 12 weeks achieved SVR_{12} rates of 99% and 95%, respectively, superior to outcomes in control groups receiving the previous standard of care, SOF with weight-based RBV for 12 weeks in genotype 2 (n = 132, SVR_{12} 94%) and 24 weeks in genotype 3 (n = 275, SVR_{12} 80%).[536] Moreover, SOF-VEL was highly effective and superior to SOF-RBV in the most refractory patient subsets, those with genotype 3 who had cirrhosis and/or had failed prior IFN-based therapy. Among treatment-naïve participants, the rate of SVR_{12} for SOF-VEL versus SOF-RBV was 98% versus 90% in noncirrhotics and 93% versus 74% in compensated cirrhotics. The differences were even more pronounced in treatment-experienced participants: 91% versus 71% in noncirrhotics and 89% versus 58% in compensated cirrhotics.

In 267 patients with Child-Pugh class B decompensated cirrhosis (78% genotype 1, 4% genotype 2, 15% genotype 3, 3% genotype 4, <1% genotype 6; 55% treatment-experienced [44% with PEG IFN–RBV, 11% with a PI regimen], 75%–83% with ascites), SOF-VEL achieved SVR_{12} in 83% treated for 12 weeks and in 86% treated for 24 weeks; a third group treated with SOF-VEL-RBV for 12 weeks had a slightly higher SVR_{12} of 94%. Efficacy was comparable across genotypes, except for patients with genotype 3, in whom the SVR_{12} rate for the 12-week and 24-week SOF-VEL regimens were both only 50%, but 85% in the 12-week SOF-VEL-RBV regimen.[537] Baseline NS5A RASs did not affect responsiveness, except in cirrhotic patients with baseline Y93H, who had a slightly reduced SVR_{12} of 84% and in whom the addition of RBV has been recommended. Based on these data, SOF-VEL for 12 weeks is recommended as initial treatment for all genotypes, for noncirrhotics and compensated cirrhotics, and for retreatment in subgroups of treatment-experienced patients with genotype 1 and 2. For retreatment of patients with genotypes 3 to 6, the more effective triple combination of SOF-VEL-VOX (see later) is recommended (see Table 117.9). Like other SOF-containing DAA combinations, SOF-VEL is not recommended in patients with CrCl <30 mL/min or in patients taking amiodarone, especially with β-blockers. For all DAA treatment, before one initiates therapy, checking for drug-drug interactions is advisable (www.hcvguidelines.org).

Sofosbuvir-velpatasvir-voxilaprevir (Vosevi). First-line DAA regimens for chronic hepatitis C are so effective that the "cure" rate approaches 100%; however, a small subset of patients do not achieve SVR_{12}—up to 6% for genotype 1 and up to 12% for genotype 3. One of the latest-generation combination DAAs, the polymerase inhibitor SOF (400 mg), the NS5A inhibitor VEL (100 mg), and the high-potency, high-barrier-to-resistance pangenotypic PI VOX (100 mg), addresses this efficacy gap. In a randomized, double-blind, placebo-controlled, phase III trial involving 263 patients with genotype 1 in whom a course of an NS5A-containing DAA treatment had failed (114 patients with other genotypes were also included but were all assigned to the triple-combination group), 96% of SOF-VEL-VOX–treated participants (12-week course of daily treatment) versus 0% of placebo recipients achieved SVR_{12} (POLARIS-1).[538] The SVR_{12} was 91% for genotype 4, 95% for genotype 3, 97% for genotype 1 (1a and 1b indistinguishable), and 100% for the small numbers with genotypes 5 and 6. Noncirrhotics had higher SVR_{12} than compensated cirrhotics (99% vs. 93%), but the presence of baseline NS5A RASs did not influence efficacy (SVR_{12} in 98% without and in 97% with baseline RASs). In a complementary randomized, double-blind, positive-controlled trial among prior nonresponders to DAA regimens that did not contain an NS5A inhibitor (POLARIS-4), patients with genotypes 1 to 3 were randomized to receive 12 weeks of SOF-VEL-VOX (n = 163) versus SOF-VEL (n = 151); 19 additional patients with genotype 4 were treated with SOF-VEL-VOX without randomization, for a total SOF-VEL-VOX cohort of 182. SVR_{12} occurred in 98% of the triple combination group versus in 90% of the double-combination group.[538] SVR_{12} rates were higher for SOF-VEL-VOX than for SOF-VEL for all genotypes except for 1b (and, in genotype 4, none received SOF-VEL): genotype 1a, 98% versus 89%; genotype 1b, 96% versus 95%; genotype 2, 100% versus 97%; genotype 3, 94% versus 85%; genotype 4 100%. SOF-VEL-VOX responsiveness was indistinguishable between noncirrhotics (98%) and compensated cirrhotics (96%); as described earlier for SOF-VEL, responsiveness to SOF-VEL in this trial was reduced in compensated cirrhotics (86%) compared with noncirrhotics (94%). The presence of baseline NS5A or NS3 RASs did not influence efficacy (SVR_{12} in 99% without and in 100% with baseline RASs) in SOF-VEL-VOX recipients; similarly, in the SOF-VEL arm of POLARIS-4, SVR_{12} rates were the same in those with and without baseline NS5A or NS3 RASs (90% vs. 89%), consistent with the roughly 10% inferiority of SOF-VEL to SOF-VEL-VOX. Between POLARIS-1 and POLARIS-4, the number of baseline RASs (of any kind, NS3 and/or NS5A) had no influence on SVR_{12}—97% with one RAS, 98% with two, and 100% with three or more than four. In these trials, side effects in the SOF-VOL-VOX group were mild and uncommon; the four most common (in 12%–27%) were headache, fatigue, nausea, and diarrhea; the SOF-VEL-VOX side-effect profile was similar to that in placebo recipients and SOF-VEL recipients—for these four adverse events, 8% to 20% and 5% to 28%, respectively.

The potential for an 8-week course of SOF-VEL-VOX was pursued in treatment-naïve patients. Two SOF-VEL-VOX phase III trials in which an 8-week versus a 12-week course were compared showed that 8 weeks was inferior, yielding SVR_{12} rates of 95% versus 98% for all genotypes and for both compensated cirrhotics and cirrhotics (with the exclusion of cirrhotic patients with genotype 3), primarily because of the lower 8-week-course response in genotype 1a (92%) (POLARIS-2). An 8- versus 12-week regimen of SOF-VEL-VOX was evaluated separately in patients with genotype 3 and cirrhosis; 96% in both duration groups achieved SVR_{12}. (POLARIS-3).[539]

Based on these trials, the triple combination of SOF-VEL-VOX is recommended for patients with genotype 1 in whom a prior non-NS5A, SOF-containing regimen or an NS5A-containing regimen has failed, and for patients with genotypes 3 to 6 in whom any DAA regimen has failed. For cirrhotics with genotype 3 in whom a prior NS5A regimen had failed, SOF-VEL-VOX efficacy was slightly lower, leading to the recommendation that RBV be added. This triple-drug DAA combination is not recommended for treatment-naïve patients, except for patients with genotype 3 and cirrhosis (see Table 117.9). Like all other SOF-containing DAA combinations, SOF-VEL-VOX is not recommended along with concomitant amiodarone and in patients with creatinine clearance <30 mL/min; like all other PI-containing DAA combinations, SOF-VEL-VOX is not recommended in patients with decompensated cirrhosis. SOF-VEL-VOX can be taken with 20 mg of omeprazole (but the safety of other PPIs has not been evaluated). For all DAA treatment, before one initiates therapy, checking for drug-drug interactions is advisable (www.hcvguidelines.org).

Glecaprevir-pibrentasvir (Mavyret). The fixed-dose combination of two pangenotypic, high-potency, high-barrier-to-resistance DAAs, the PI GLE (300 mg) and the NS5A inhibitor PIB (120 mg) (total doses of GLE and PIB achieved with three fixed-combination pills taken once a day with food), has proven to be highly effective across all genotypes; in treatment-naïve noncirrhotic patients, an 8-week course suffices. In phase II and III trials of >2300 treatment-naïve and treatment-experienced noncirrhotic patients, this DAA achieved SVR_{12} in 99.0% to 99.7% of patients with genotypes 1, 2, and 4 to 6 and 95% with genotype 3; across all genotypes in noncirrhotic patients, 8 weeks of therapy was equivalent in efficacy to 12 weeks (phase III trials ENDURANCE-1 to ENDURANCE-4 and phase II trials SURVEYOR-1 and SURVEYOR-2).[540–543] In patients with compensated cirrhosis, both treatment-naïve and treatment-experienced (IFN-RBV–based or SOF-RBV ± PEG IFN), 12 weeks of GLE-PIB achieved SVR_{12} in 99% across genotypes 1, 2, and 4 to 6 (phase III EXPEDITION-1) and in up to 98% of patients with treatment-naïve genotype 3 (phase II SURVEYOR-2). In DAA-treatment–experienced patients (approximately a third each with PI experience only, NS5A inhibitor only, and both PI and NS5A experience) with genotypes 1 and 4, with or without compensated cirrhosis, GLE-PIB achieved SVR_{12} just as frequently after 12 weeks of treatment (89%) as after 16 weeks of treatment (91%) (phase II MAGELLAN-1). In this trial, both the number of previous DAA treatments and the number of baseline RASs

were associated with degradation of GLE-PIB treatment. For example, in the cohort treated for 12 weeks, the GLE-PIB–associated SVR_{12} fell from 100% for prior PI only, to 88% for prior NS5A inhibitor only, to 79% for prior PI and NS5A inhibitor; SVR_{12} rates were 100% for patients with no baseline RASs and baseline RASs limited to NS3 (PI) only but 83% for patients with NS5A baseline RASs[544]

Like all DAAs that include a PI, GLE-PIB should not be used in decompensated cirrhotics, but the combination is effective and recommended for patients with severe renal impairment. In a phase III trial among 104 patients with stage 4 or 5 renal disease (CrCl <30 mL/min), with or without cirrhosis, whether treatment naïve or treatment experienced, the SVR_{12} rate across all genotypes was 98%.[545] GLE-PIB should be taken with food. Drug-drug interactions should be considered prior to therapy (www.hcvguidelines.org).

Considerations in Direct-Acting Antiviral Therapy of Chronic Hepatitis C

The role of resistance testing. Because first-line DAA treatments are so highly effective, including in patients with baseline RASs, baseline testing for RASs is not recommended routinely. The exceptions are (1) elbasvir-grazoprevir—patients with genotype 1a (not genotype 1b) should be tested for elbasvir resistance, which, if present, justifies extending therapy to 16 weeks and adding RBV or selecting an alternative regimen; and (2) VEL-SOF—in treatment-naïve cirrhotic patients with genotype 3 who are to be treated with VEL-SOF, resistance testing is recommended, and, if a Y93H RAS is detected, RBV should be added.

For patients in whom an initial DAA course (or courses) has failed, pretreatment resistance testing is not recommended when treating with GLE-PIB or SOF-VEL-VOX, because these combinations are so effective against all RASs. For prior nonresponders to DAA regimens who are to be treated with elbasvir-grazoprevir, LDV-SOF, or VEL-SOF, however, resistance testing can help guide therapy. As was the case for treatment-naïve patients to be treated with elbasvir-grazoprevir, treatment-experienced patients with genotype 1a (not genotype 1b) should be tested for elbasvir resistance, which, if present, justifies extending therapy to 16 weeks and adding RBV or selecting an alternative regimen. For patients with genotype 1a (not 1b) who are to be treated with LDV-SOF, resistance testing should be considered, and if resistance is present, LDV-SOF treatment should be extended to 24 weeks for cirrhotics, and, in both noncirrhotic and cirrhotic patients, RBV should be added, or a different DAA regimen should be selected. For treatment-experienced patients with genotype 3 who are to be treated with VEL-SOF, resistance testing is recommended, and, if a Y93H RAS is detected, RBV should be added. A detailed primer on resistance testing, including a summary of the most common RASs for each DAA combination, appears in the AASLD/IDSA hepatitis C treatment guidelines (www.hcvguidelines.org).

Reactivation of hepatitis B. Cases of hepatitis B reactivation in DAA-treated patients with HBV-HCV coinfection began to appear in the medical literature in 2015[546,547]; by 2017, 29 such patient reports had been compiled, 28 from the FDA Adverse Event Reporting System and one case from the literature, over a 3-year period (2013–2016).[548] In a meta-analysis of 770 patients (from 36 studies) with chronic hepatitis C who were HBsAg reactive, HBV reactivation (a virologic outcome defined as an increase in HBV DNA by >2–4 $\log_{10}$ or to $>2 \times 10^3$ IU/mL) occurred in comparable proportions of patients managed with IFN-based therapy (14.5%) and those managed with DAA therapy (12.2%). The differences between the two groups were that for patients receiving IFN-based treatment, HBV reactivation occurred later, usually at the end of therapy or in the posttreatment period, whereas for DAA-treated patients HBV reactivation occurred earlier, within the first 4 to 12 weeks, always during therapy. In addition, HBV reactivation associated with "hepatitis," defined as an ALT elevation greater than 2 to 3 times the lowest level achieved during therapy or more than 10 times the ULN, and severe hepatitis cases (with liver failure or jaundice or necessitating liver transplantation) occurred in the DAA group (12.2%) but not in the IFN-based group (0%).[549]

In October 2016, for all DAAs, the FDA issued a black box warning about the risk of HBV reactivation (including severe cases of fulminant hepatitis, liver failure, and death) in HBV-HCV coinfected patients. All patients with chronic hepatitis C who are to be treated with DAAs should be screened for HBV infection. Therefore, before beginning DAA therapy for hepatitis C, all patients should be tested for HBV infection; preemptive hepatitis B antiviral therapy should be instituted in HBsAg-reactive patients, and close monitoring for HBV reactivation should be done in anti-HBc–reactive or anti-HBs–reactive/anti-HBc–reactive patients (www.hcvguidelines.org).

Does DAA cure increase the risk of HCC? The risk of HCC in patients with chronic hepatitis C and advanced fibrosis/cirrhosis is 1% to 4% per year,[369,550] especially after ≥3 decades of HCV infection. During the IFN-treatment era, the rate of HCC after treatment-associated SVR was shown to be reduced.[415,473,551] For example, over a 10-year posttreatment observation, HCC occurred in 5.1% of patients with SVR but in 21.8% of patients without SVR—that is, achievement of SVR was associated with a greater than fourfold reduction in HCC.[473] What these studies drove home was the fact that, among patients with established advanced fibrosis, cure of HCV infection reduces but does not eliminate the risk of HCC entirely. This important observation (and similar observations in the reduction but not elimination of liver failure and liver-related death) suggested that, ideally, patients with chronic hepatitis C should be treated before advanced fibrosis is established, allowing virologic cure to be accompanied by prevention of late-disease clinical complications.

The risk of HCC after IFN-associated SVR has been well documented.[552–554] In 2016, reports from Spain and Italy appeared to suggest that the risk of HCC recurrence after treatment and initial HCC occurrence were higher than expected in DAA-treated patients with SVR.[555,556] In contrast, and consistent with the data cited earlier about the reduction in HCC after SVR, a series of subsequent reports, including meta-analyses of multiple experiences—almost 14,000 patients involved in 41 studies, and data from Australia, North America (e.g., the Veterans Administration database), France, Italy, and the United Kingdom)—demonstrated that the risk of HCC after DAA-induced SVR was reduced compared with that in untreated patients and comparable to that after IFN-induced SVR.[557–563] A consensus emerged from these studies to indicate that the higher risk of HCC in DAA-treated patients reflected a cohort bias, in that DAA treatment was applied to an older, sicker population, more heavily weighted to advanced fibrosis or cirrhosis and including decompensated liver disease. In effect, the reason for the increased risk of HCC after DAA treatment was not the DAA treatment but more advanced liver disease in the DAA-treated patients. Thus, successful DAA treatment and cure (SVR) reduces the risk of HCC but does not eliminate it, and the fact that more patients with advanced liver disease are being treated with DAA means that, as was the case after IFN-induced SVR, continued HCC surveillance is necessary after SVR in patients with advanced fibrosis, a treatment recommendation that has been adopted widely (www.hcvguidelines.org).[564,565]

Candidates for Therapy

Evidence-based, authoritative treatment recommendations are issued and updated by a joint panel of the AASLD and IDSA[566] and the EASL.[567] The AASLD/IDSA guidelines are updated online whenever new DAA treatments are introduced (www.hcvguidelines.org). Patients with chronic hepatitis C, with detectable HCV RNA, with or without elevated aminotransferase activity, and with all grades of hepatitis and stages of fibrosis are candidates for antiviral therapy. The goal of therapy is to achieve SVR, which has been shown to reduce all-cause mortality and the health consequences of hepatitis C, including decompensated (end-stage) liver disease and HCC. The only exception are patients with a brief life expectancy, which is unlikely to be extended by eradication of HCV infection. Treating patients with advanced fibrosis (METAVIR stage F3) and cirrhosis (METAVIR stage F4) merits a high priority; however, treatment early in the natural history of chronic hepatitis C is the only way to prevent all the dire consequences of HCV-associated liver disease. In patients with histologically milder hepatitis C, progression was shown to be slow[345]; however, patients with histologically mild hepatitis respond as well as or better than patients with more severe hepatitis. Because histologic grade (absence vs. presence of cirrhosis) influences the choice and duration of DAA therapy (see Table 117.9), because fibrosis stage is predictive of future progression of chronic hepatitis C, and because the presence of cirrhosis determines the need for recommended surveillance screening for HCC and esophageal varices,

a baseline assessment of disease stage is recommended as a prelude to antiviral therapy.[344,345,440,441,443] Given how progressively simple and effective antiviral therapy has become with DAAs, and given advances in noninvasive measures of fibrosis (e.g., liver elasticity), a baseline liver biopsy before therapy is no longer necessary for most patients with chronic hepatitis C[568,569]; however, all patients should undergo an assessment of fibrosis stage. Whether antiviral therapy with IFN-based therapy prolongs survival in treatment-naïve persons and in prior nonresponders with cirrhosis was the subject of debate in the past,[b] but many definitive studies have shown that achieving SVR in cirrhotics, either with IFN-based therapy or DAA therapy, improves survival.[473,570–572] Therapy with DAAs has also been shown to reduce both liver and nonliver complications (cardiovascular events, bacterial infections), portal hypertension, and hepatic fibrosis.[571,573–575]

In patients with decompensated cirrhosis, PEG IFN–based antiviral therapy regimens, ineffective and poorly tolerated, are not recommended,[353] several reports to the contrary notwithstanding.[576] Instead, current DAA regimens containing combinations of nucleoside inhibitors (e.g., SOF) and NS5A inhibitors (e.g., LDV, VEL, daclatasvir) are treatment options, usually with RBV, that result in high rates of SVR_{12} (albeit not quite as effective as these DAAs in patients with compensated liver disease) and improvements in hepatic compensation (e.g., increased albumin, reduced Model for End-Stage Liver Disease [MELD] score, and reduced need for liver transplantation).[514,537,577,578] On the other hand, DAA combinations that include a PI (grazoprevir, VOX, ombitasvir) are not recommended in decompensated cirrhosis, because of either hepatic complications (including liver failure and death) or absence of data.

For patients who did not respond to the best available IFN-based antiviral regimen, potentially, maintenance therapy with PEG IFN could have retarded histologic and clinical progression. Supporting this hypothesis was the demonstration of a histologic benefit in three-quarters of patients with IFN treatment, including a sizable proportion without virologic response.[396] In addition, in prior nonvirologic responders to IFN monotherapy, a preliminary controlled trial of additional treatment versus no treatment for 2 years showed histologic benefit in the treated group.[579] Definitive clinical trials undertaken to determine whether maintenance therapy with PEG IFN can prevent histologic progression in virologic nonresponders, however, showed no benefit, and such maintenance therapy is not recommended.[368,580–582] Fortunately, now that more potent oral DAAs are available, the proportion of patients who remain nonresponders is growing vanishingly small.

As described earlier for the first-line DAA combinations, prior nonresponders to IFN-based therapy and prior nonresponders to DAA therapy have high SVR_{12} rates when re-treated with DAAs. A summary of current recommendations appears in Table 117.9 and www.hcvguidelines.org.

In the pivotal registration clinical trials of antiviral therapy for chronic hepatitis C, restrictive entry and exclusion criteria confined the treated population to a narrow subset without comorbid medical conditions, active alcoholism or other substance use, uncontrolled neuropsychiatric disorders, and so on.[583] Patients with these other conditions can be treated, however, if the other medical conditions can be managed effectively.[345,440] In most clinical trials, persons with advanced age were not included, and for many elderly patients with chronic hepatitis C, longevity is not affected by the disease or its treatment. In elderly persons, however, DAA therapy is as effective as in younger patients.[584–586]

In children, progression of chronic hepatitis C tends to be slow, and the disease follows a relatively benign course in most cases.[355] Unfortunately, progression to cirrhosis has been reported in a small proportion of those with childhood-acquired hepatitis C. In clinical trials of antiviral therapy in this population, efficacy is similar to that achieved in adult populations.[345,587] Data on safety and efficacy of the new DAA agents in children younger than age 18 are limited, but adolescents aged 12 to 17 have been treated effectively (SVR_{12} ≥87%) with LDV-SOF (genotype 1) and SOF-FRBV (genotypes 2 and 3).[588,589] Data on other DAAs and in children aged 3 to 11 are anticipated (www.hcvguidelines.org).

When they were first released, DAAs were very expensive; the most costly was $94,500 for a 12-week course of LDV-SOF. Fortunately, these exorbitant costs have come down, and the initial cost of the most recent of the new DAA regimens (GLE-PIB) is $26,400. Despite the high costs of DAAs, they have been shown to be cost-effective (e.g., for the most expensive, LDV-SOF).[590] Based on the known prevalence of chronic hepatitis C in the United States, the known rate of progression to cirrhosis and its complications, and the efficacy of DAA therapy, modeling has predicted that the hepatitis C–associated disease burden (liver-related death, HCC, decompensated cirrhosis, and liver transplantation) will fall by 50% to 70% between 2015 and 2030.[591]

Challenge to Benefits of Direct-Acting Antiviral Therapy

In a 2017 Cochrane review of the benefits and potential harms of DAA therapy, the authors challenged the validity of SVR_{12} as a meaningful end point, impugned the large body of clinical trials as being subject to bias in favor of benefit and against harms, and concluded that data were insufficient to demonstrate that such therapy reduced HCV-associated morbidity (including cirrhosis, decompensated liver disease, and HCC) and mortality.[592] This conclusion was rejected vociferously in responses by the AASLD and EASL,[593,594] among other organizations. In fact, the Cochrane reviewers included only studies that had placebo or untreated control groups, which eliminated many contemporary DAA-treatment trials for which an untreated control group would have been unethical. In addition, the rejection by the Cochrane reviewers of SVR as a meaningful end point had already been considered carefully and accepted by the FDA and regulatory agencies of other countries, which recognized SVR as a meaningful treatment end point for hepatitis C. For a disease such as hepatitis C that progresses slowly and insidiously in most patients, the long-term effects of DAA treatment on late clinical outcomes (progression to cirrhosis, hepatic decompensation, HCC, and death) take decades to measure. Moreover, the relation between immediate posttreatment SVR and improvements in these late outcomes already had been demonstrated definitively for IFN-based therapy; DAA treatment had already been shown to lower fibrosis stage, to improve MELD scores (and result in "delisting" patients on the liver transplantation waiting list) and reduce portal hypertension in patients with decompensated disease, to eliminate the deleterious impact of HCV reinfection after organ transplantation, to reverse extrahepatic manifestations of hepatitis C (e.g., renal disease, vasculitis, B-cell lymphoproliferative disorders including lymphoma), and to improved health-related quality of life. As stated bluntly in the EASL rebuttal, "The inability of the authors of the Cochrane review to determine a clinical benefit of DAA-based treatment of hepatitis C unfortunately reflects their flawed methodological approach and their ignorance of the natural history of HCV infection and associated systemic diseases."[594]

Similar imbroglios followed (1) the 2012 US Preventive Services Task Force (USPSTF) conclusion that data supporting screening for hepatitis C were inadequate,[595,596] followed subsequently by a retraction, and (2) the conclusion of a National Institutes of Health (NIH) Consensus Development Conference on management of hepatitis B in 2008 that data were insufficient to recommend antiviral therapy for hepatitis B[597] (for very similar reasons to the Cochrane reviewers' denial of clinical benefit of DAA therapy in hepatitis C). The notion that eliminating HCV does not translate to improving clinical outcomes of chronic hepatitis C is patently absurd, unsupportable, and well refuted by a robust body of clinical trial data and peer-reviewed literature. Not surprisingly, a strong consensus has emerged rejecting the validity and clinical relevance of the conclusions of the Cochrane review and bemoaning the deleterious effect this misguided analysis might have on efforts that are underway and supported by public health leadership (e.g., US Public Health Service, WHO, National Academy of Medicine) to reduce the global impact of hepatitis C (and B) on morbidity and mortality.

Special Patient Populations

Liver Transplantation

End-stage chronic hepatitis C is the most frequent indication for liver transplantation.[286,598] Recurrent HCV infection is reestablished invariably in the new liver and may be associated with an increase in the frequency of acute rejection episodes,[599,600] but clinical progression during the early years after transplantation may be limited, and overall early survival

[b]References 364, 366, 368, 414, 415, 487.

unaffected. In contrast, even during the first 5 years after liver transplantation, histologic progression appears to be accelerated; more than half of patients have moderate-to-severe hepatitis and approximately 10% have advanced fibrosis or cirrhosis,[601,602] and eventually, survival is impaired.[603,604] A small proportion of patients with chronic hepatitis C have early reactivation of hepatitis, and in those with early and difficult-to-manage rejection, added immunosuppression enhances HCV replication and may amplify HCV-associated liver injury.[600] Furthermore, management is confounded by difficulty in distinguishing between rejection and viral liver injury on the basis of often overlapping clinical and histologic features. The most aggressive form of recurrent hepatitis C after liver transplantation is fibrosing cholestatic hepatitis, a rapidly progressive form of liver injury, described previously in patients with hepatitis B undergoing liver transplantation, and characterized by progressive fibrosis, cholestasis, and severe jaundice out of proportion to necroinflammatory activity.[605] PEG IFN–RBV–based antiviral therapy for hepatitis C, whether begun preemptively immediately after transplantation or introduced after the emergence of recurrent HCV-associated liver injury, was disappointingly ineffective after liver transplantation, sometimes suppressing HCV replication but resulting only rarely in an SVR.[451,606] What is more, PEG IFN and RBV are poorly tolerated after liver transplantation, necessitating dose reductions.[606] Because immunosuppression enhances HCV replication,[607] attempts are made to minimize the use of immunosuppressive drugs after liver transplantation for chronic hepatitis C.[608] With all its limitations, however, PEG IFN–RBV–based antiviral therapy did appear to slow disease progression after liver transplantation, as reported in a study of 81 patients monitored histologically and hemodynamically.[609]

Trials of DAAs have shown that these regimens are effective in achieving SVR in patients with HCV infection after liver transplantation. Before transplantation, use of SOF with RBV in patients with hepatitis C and decompensated cirrhosis who were on the waiting list and who were treated for up to 48 weeks showed that more than 90% could experience complete suppression of HCV RNA before transplantation; if HCV RNA had been undetectable for at least 30 days before transplantation, recurrent HCV infection in the allograft could be prevented in almost all.[610] Moreover, approximately a third to a fifth of patients with decompensated cirrhosis on the liver transplantation waiting list improve sufficiently to be "delisted" for transplantation.[611] After transplantation, DAA treatments shown to be effective include SOF-RBV, which achieved a 70% SVR_{12} in patients with compensated liver disease but only 59% in patients with decompensated liver disease after transplantation.[612,613] The largest body of data involve LDV-SOF, which is nearly completely effective with or without RBV after liver transplantation in patients with compensated liver disease, again less so in patients with decompensated cirrhosis (Child-Pugh class B and C).[514,614–616]

Other DAA regimens that have been observed to be effective after liver transplantation include daclatasvir-SOF and SMV-SOF; although data are limited, GLE-PIB and SOF-VEL-VOX are likely to find a place in treatment of HCV infection after liver transplantation (www.hcvguidelines.org). In all patients treated after liver transplantation, consideration of drug-drug interactions is important, especially with calcineurin inhibitors. Based on available data, the AASLD/IDSA guidelines include the following as first-line regimens after liver transplantation: GLE-PIB, LDV-SOF, daclatasvir-SOF, and VEL-SOF (see www.hcvguidelines.org for details and for alternative-choice DAA regimens).

The timing of DAA treatment before liver transplantation in patients who are potential transplantation recipient candidates remains unsettled. If patients are treated before transplantation and are cured of hepatitis C, they may be denied the availability of donor organs from HCV-infected donors, potentially lowering their MELD scores, increasing their waiting time, and reducing their chances for obtaining a timely allograft—a situation labeled as MELD "purgatory" or "limbo."[617] Although policies vary across liver transplantation centers, the trend is to treat before transplantation patients with low-to-intermediate MELD scores (e.g., <20) who are sufficiently stable and not in imminent need of organ replacement, but to postpone DAA treatment until after transplantation (likely to be nearly completely effective) in patients with high MELD scores and advanced stages of liver decompensation (e.g., ≥20, in whom the efficacy of DAA treatment is reduced.[618,619]

HIV Coinfection (Also See Chapter 124)

Patients with HIV-HCV coinfection could respond to antiviral therapy with standard or PEG IFN plus RBV, but the likelihood of an SVR was approximately one-half to two-thirds that expected in immunocompetent patients with HCV infections alone,[345,620–625] PEG IFN–RBV treatment had to be administered for a full 48 weeks for all genotypes, and the target daily dose of RBV had to be reduced often to 600 to 800 mg,[622,623,625] although full doses had been recommended.[254,626] PEG IFN–RBV antiviral therapy of patients with HIV-HCV coinfection was shown to result in reduced antiretroviral drug hepatoxicity[627,628] and did not appear to have a deleterious effect on progression of HIV disease, but patients with coinfection tolerated PEG IFN and RBV less well than patients with HCV infection alone. Moreover, because RBV is an IMPDH inhibitor, its use had the capability to potentiate the activity and toxicity of the purine analogue didanosine; therefore, these two drugs could not be used together.[629] Similarly, zidovudine may exacerbate RBV-associated anemia, and stavudine-associated lipoatrophy may be enhanced by RBV.[626] Whether abacavir reduces PEG IFN–RBV efficacy is controversial.[630] Application of first-generation PI-based therapy (TVR–PEG IFN–RBV) in HIV-HCV coinfected patients was more effective (71% SVR_{24}) than PEG IFN–RBV therapy (41% SVR_{24}). No significant drug-drug interactions were apparent between TVR and ART, no HIV breakthroughs occurred in patients being treated with ART during PI-based therapy for hepatitis C, and safety and tolerability were similar to those in HCV monoinfected patients.[631]

The old standard-of-care PEG IFN–RBV and first-generation PIs have been supplanted in HIV coinfected patients by all-oral DAA regimens. High response rates in HIV-HCV coinfected patients are indistinguishable from those in HCV-monoinfected patients,[632–635] and treatment recommendations are the same for both groups. The importance of drug-drug interactions between DAAs for HCV infection and ART for HIV infection necessitates close attention, however (see www.hcvguidelines.org).

Immune-Complex Disease

The response to IFN-based antiviral therapy was variable and often disappointing in patients with cutaneous vasculitis or glomerulonephritis resulting from HCV-associated EMC. Although some patients responded during IFN-based therapy, SVR was unlikely, and many such patients needed indefinite maintenance of antiviral therapy.[353,636–639] Experience with current-generation DAAs has been encouraging. In patients with cryoglobulinemic vasculitis, the DAA combinations SOF-daclatasvir, SOF-RBV, SOF-SMV, LDV-SOF, and others have achieved SVR_{12} in 82% to 100% and clinical remission of vasculitis in 71% to 90%.[640–644] In refractory HCV-associated immune-complex syndromes, options include plasmapheresis, cytotoxic therapy, and B-cell blockade with monoclonal anti-CD20 (rituximab).[440,645]

B-Cell Lymphoma

In patients with B-cell, non-Hodgkin lymphoma associated with chronic HCV infection, antiviral therapy has been reported to achieve disease remission, initially with IFN-based therapy[646–648] and more recently with DAA therapy.[649,650]

Other Special Populations

The efficacy of DAA therapy is high in patients with renal failure. Although SOF-containing combinations are effective and can be used in patients with low-stage chronic kidney disease, SOF-based DAAs have not been shown to be effective in patients with CrCl <30 mL/min (stage 4 or 5 chronic kidney disease). In contrast, elbasvir-grazoprevir and GLE-PIB have been shown to be effective and are recommended in patients with stage 4 or 5 chronic kidney disease (see www.hcvguidelines.org). Treatment with DAAs is also effective in persons who inject drugs, but such patients benefit from concomitant addiction treatment, needle-exchange programs, and opioid agonist therapy delivered by multispecialty experts in addiction medicine; after cure in this population, reinfection rates of approximately 25% have been observed, but treating patients who inject drugs comes with an added benefit of reducing the spread of HCV infection in the population at

large. Reinfection with HCV has been noted as well in HIV-infected men who have sex with men, who also respond well to DAA therapy (see www.hcvguidelines.org).

Chronic Hepatitis E

Initially, HEV had been considered to cause acute hepatitis exclusively, albeit on occasion severe, particularly in pregnant women,[30,651] but HEV has been recognized as a cause of protracted and chronic hepatitis in immunocompromised patients. These have primarily involved solid organ transplant recipients, including liver, kidney, and kidney-pancreas transplants.[652] Chronic hepatitis E has also been reported in HIV-infected patients,[653] in a patient with lymphoma taking rituximab,[654] and in patients with hematologic disorders on chemotherapy.[655,656] In the largest series of reported patients with organ transplants, HEV infection led to chronic hepatitis in two-thirds of patients and cirrhosis in 10%.[657]

Chronic infection is associated with HEV genotype 3. The clinical manifestations of chronic HEV are similar to those of other chronic viral hepatitides.[30] Infected organ-transplant patients have relatively few symptoms, and most do not present with jaundice. Liver test results are usually only modestly abnormal (e.g., ALT levels are approximately 300 IU/L). Liver histopathologic findings in patients with chronic HEV infection are similar to those seen in chronic hepatitis C. Sequential liver biopsies may reveal rapid progression to liver fibrosis.[658]

In addition to liver involvement, extrahepatic complications are also seen. These include neurologic manifestations, such as Guillain-Barré syndrome, Bell palsy, peripheral neuropathy, muscle wasting, acute transverse myelitis, and acute meningoencephalitis.[659,660] Chronic glomerulonephritis and cryoglobulinemia can also be seen. HEV-associated neurologic and kidney manifestations may completely or partially resolve if HEV is cleared.

Treatment

Chronic HEV infection will clear, and associated chronic hepatitis will resolve in approximately one-third of organ transplant patients whose immunosuppression is diminished, particularly if drugs that target T-cell function are reduced.[657] For the remaining 70% whose hepatitis E cannot be resolved by reduction of immunosuppression, specific antiviral therapy has been used. PEG IFN and RBV, either individually or in combination, have been used in a small case series with encouraging results.[661–664] A retrospective case series was reported of 59 solid organ transplant patients with chronic hepatitis E who were treated with RBV monotherapy. The median time between diagnosis of HEV infection and beginning therapy was 9 months (range, 1–82 months). The median dose of RBV was 600 mg/day (range, 29–1200 mg) with a median treatment of 3 months (range, 1–18 months). At the end of therapy, HEV clearance was observed in 95% of patients. A relapse of HEV replication occurred in 10 patients when RBV was stopped. An SVR was observed in 78% (46 of 59) of patients and in 4 relapsers who were re-treated for longer periods of time. Anemia was the main side effect, which necessitated reductions in RBV dose, erythropoietin use, and blood transfusions.[29]

On the basis of this retrospective case series, the authors recommend therapy of chronic hepatitis E with RBV for 3 months and additional courses for those in whom relapse occurs. Determination of optimal dose and duration of therapy will require prospective, controlled studies. Resistance to RBV has been observed in patients with hepatitis E in whom RBV treatment failed.[665]

The hepatitis C antiviral agent SOF has activity against HEV in vitro, and anecdotal reports suggest that SOF at a daily dose of 400 mg reduces, but does not eliminate, HEV RNA in patients with HEV infection after immunosuppression. Further study of higher SOF doses has been suggested.[666–669]

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The complete reference list is available online at Expert Consult.

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O Acquired Immunodeficiency Syndrome

118 Global Perspectives on Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

Peter Piot

SIZE OF THE PROBLEM

By the end of 2016, the estimated number of people infected with human immunodeficiency virus (HIV) worldwide was 36.7 million (range, 30.8 million–42.9 million), from a cumulative total of approximately 76.1 million persons infected since the beginning of the epidemic. Table 118.1 shows global estimates of the HIV epidemic.[1–3] On a global scale, the HIV epidemic is slowing down, although with unacceptably high levels of new HIV infections and acquired immunodeficiency syndrome (AIDS) deaths. In every day of 2016 alone, about 5000 persons became infected with HIV, and 1.0 million persons died of AIDS-related illnesses in 2016, mostly because of inadequate access to prevention and treatment of the infection.[4] In a growing number of communities, HIV infection has become endemic, with a fairly constant or slowly declining level of new infections over a long period of time.

Fig. 118.1 shows a world map with HIV prevalence rates by country. Eastern and southern Africa continues to bear a disproportionate share of the global burden of HIV, with around half of all people living with HIV infection and around 42% of AIDS deaths in 2016 occurring in that subregion. Altogether, sub-Saharan Africa is home to more than 70% of all people infected with HIV.[5] Most new HIV infections occur in sub-Saharan Africa, Asia, and Eastern Europe and Central Asia.[6] Young people aged 15 to 24 are thought to account for around 40% of all new HIV infections among adults.[7]

Women account for just over half (51%) of all people infected with HIV worldwide; in most affected regions, such as Africa and the Caribbean, nearly 60% of people who have HIV infection are women. HIV infection rates in young women (15–24 years) are twice as high as those in young men, with substantial increases in sub-Saharan Africa, where HIV infections among young women aged 15 to 24 made up 66% of new infections among young people.[6] From 2001 to 2016, the proportion of women infected with HIV increased in many regions (Fig. 118.2).

In 2016, around 2 million adolescents aged 10 to 19 years were living with HIV, nearly 85% of whom lived in sub-Saharan Africa. An estimated 260,000 adolescents were newly infected with HIV globally in 2016. In sub-Saharan Africa, three in four new HIV infections among 15- to 19-year-olds were among girls, and HIV-related illnesses remain the second leading cause of death for young women aged 15 to 24 years in Africa.[8] In 2016, 37% of global new HIV infections in adults were among young people aged 15 to 24.[9] At current HIV incidence rates, the significant increase in the youth population in many countries with high HIV burden will lead to a growing proportion of the young adult population living with HIV, infected perinatally or sexually. Programs need to increase their meaningful engagement with young people, because treatment adherence among adolescents is generally lower and treatment failure rates are comparatively higher than in other age groups.[10]

Trends suggest that HIV prevention efforts are having an impact in several of the most affected countries. East and West African countries, Côte d'Ivoire, several Caribbean countries, most Southeast Asian countries, and southern India have all seen downward trends in national HIV prevalence percentages. There were more than 1,200,000 fewer new HIV infections globally in 2016 than at the peak of the epidemic in the late 1990s.[11] Global goals to expand access to treatment to 30 million people by 2030 are on track, but the goal to reduce new global HIV infections to 500,000 or fewer by 2020 is off target. The steepest declines in HIV incidence have been seen in eastern and southern Africa, the region of the world most affected by the epidemic, with a 29% decline in infections between 2010 and 2016. In contrast, over the same period, new HIV infections rose by 60% in Eastern Europe and Central Asia.[10]

To monitor country-level progress, the Joint United Nations Programme on HIV and AIDS (UNAIDS) developed the 90-90-90 indicators, aiming for a world by 2020 in which 90% of people know their HIV status, 90% of all people diagnosed with HIV are on treatment, and 90% of all people receiving treatment will be virally suppressed. At the global level in 2016, 70% of people living with HIV know their status (range, 51%–84%), 77% of those diagnosed with HIV are on treatment (range, 57%–89%), and 82% of people on treatment are virally suppressed (range, 60%–89%). When the gaps across this "treatment cascade" are combined, this means that 44% of all people living with HIV were virally suppressed in 2016, short of the 73% required for achievement of the 90-90-90 targets. However, certain countries—both high-income areas such as Sweden and the United Kingdom, and low- and middle-income countries (LMICs) such as Cambodia and Botswana—are on track to achieve the targets by 2020.[10] In addition, there are major discrepancies among and within countries in terms of achieving these targets.

Surveillance for HIV infection worldwide is probably the most complete and consistent among global health issues since the mid-1990s. The selection of surveillance populations depends on the type of epidemics and the local context. In epidemics with at least 1% prevalence rates in the adult population and in which transmission is mostly heterosexual, the recommended surveillance population is pregnant women who attend antenatal clinics. In epidemics in which HIV has not spread into the general population, the primary focus of the surveillance system is among groups at higher risk for infection with HIV, such as sex workers, men who have sex with men (MSM), and injection drug users. In addition, more than 50 countries, mostly in Africa where heterosexual transmission of HIV is predominant, have conducted national population-based household surveys with HIV and syphilis testing.[12] These surveys give a more precise picture of HIV prevalence rates in men and women in rural areas, populations previously not well covered by figures from

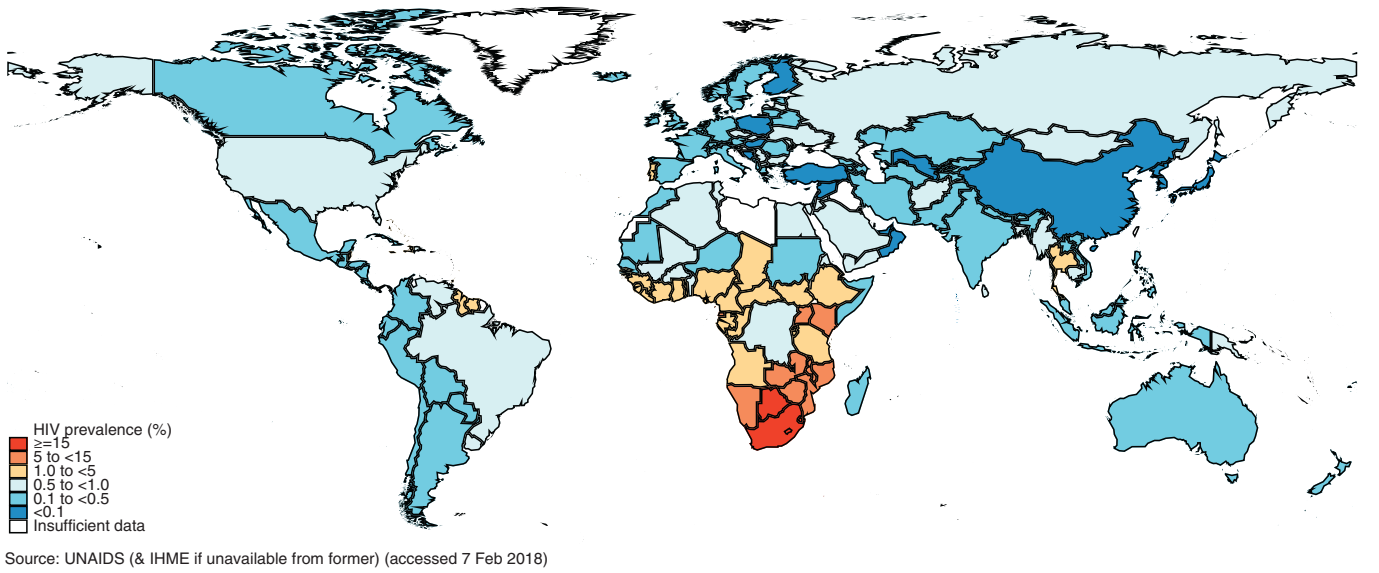

FIG. 118.1 Human immunodeficiency virus prevalence by country, 2016. *(Data from UNAIDS. http://aidsinfo.unaids.org; and Institute for Health Metrics and Evaluation. Global Burden of Disease Study 2015 [GBD 2015]. HIV incidence, prevalence, and mortality 1980–2015. http://ghdx.healthdata.org/record/global-burden-disease-study-2015-gbd-2015-hiv-incidence-prevalence-and-mortality-1980-2015. Accessed February 7, 2018.)*

TABLE 118.1 Global Summary of the Human Immunodeficiency Virus (HIV) Epidemic as of December 2016

Number of People With HIV in 2016	
Total	36.7 million
Adults	34.5 million
Women	17.8 million
Children <15 yr	2.1 million
People Newly Infected With HIV in 2016	
Total	1.8 million
Adults	1.7 million
Children <15 yr	160,000
AIDS Deaths in 2016	
Total	1.0 million
Adults	890,000
Children <15 yr	120,000

AIDS, Acquired immunodeficiency syndrome.
Data from UNAIDS.[3]

antenatal clinic attendees, but may also underestimate the extent of HIV infection among mobile populations and some of the most affected or vulnerable populations. Therefore several surveillance approaches are needed to achieve the most accurate estimates of HIV infection.[13]

Western Europe

The epidemiology of HIV infection in North America is extensively discussed in Chapter 119. Except for this section, this chapter deals mainly with the situation in the developing world. Western Europe had an estimated 651,380 persons living with HIV in 2015.[14] The number of annual reported new diagnoses of HIV infection almost tripled between 1999 and 2005 to reach nearly 20,000.[15] In 2011, the figure rose again to 30,000.[16] In 2016, the highest proportion of HIV transmission in European Union/European Economic Area (EU/EEA) countries was reported to be from sex between men (40%), followed by heterosexual transmission (32%); in 23% of the cases, the transmission mode remained unknown.[17] HIV infection diagnoses among MSM in the EU/EEA region increased from 8493 cases in 2007 and peaked at 10,079 cases in 2014, and are now declining slightly (9274 in 2016), whereas the number of cases among injection drug users decreased from 1450 cases in 2007 to 860 cases in 2016, although increases were observed in 2011 owing to outbreaks in Greece, Lithuania, and Romania.[18] More than half of the heterosexually transmitted HIV cases originated in migrants from countries with high HIV prevalence rates, although there has been a decline in foreign-born cases of HIV owing to sharp decreases among migrants originating from countries with generalized HIV epidemics (from 5873 in 2007 to 3388 in 2016). The highest rates of new HIV diagnoses in 2016 in the EU/EEA were reported in Latvia (18.5 per 100,000 population; 365 cases), Estonia (17.4; 229 cases), and Malta (14.5; 63 cases). The lowest rates were reported in Slovakia (1.6; 87 cases) and Hungary (2.3; 228 cases).[17]

The annual number of newly diagnosed HIV infections has declined from a peak of nearly 8000 to 5164 in 2016.[19] The decline in new infections may be linked to reduced HIV transmission among MSM; and among heterosexual men and women, the decline is potentially linked to a fall in migration from high HIV-prevalence countries. In Central Europe (as defined by the World Health Organization [WHO]), 5772 new HIV cases were diagnosed in 2016, with 1269 cases diagnosed in Poland and 625 in Romania.[17]

Eastern Europe and Central Asia

New HIV infections in Eastern Europe and Central Asia rose by 60% between 2010 and 2016, with 190,000 new infections in 2016, a rise more pronounced than in any other world region. Treatment coverage is only 28% (22%–32%) in the region; between 2010 and 2016, AIDS–related deaths had increased by 27%.[2] The most HIV infections in this region were from the two countries with the largest populations: the Russian Federation, with 1.16 million cumulative reported diagnoses in mid-2017,[20] and Ukraine, with around 247,000 cumulative reported diagnoses.[17] In the Russian Federation, the annual number of new infections dramatically increased from less than 40,000 in 2006 to more than 60,000 in 2011[16] and to 103,438 in 2016.[10] Ukraine has an adult HIV prevalence rate of 0.9% as of 2016.[3]

Of the new HIV cases reported in Eastern Europe and Central Asia, 42% were among people who inject drugs.[10] In Russia, the European Centre for Disease Prevention and Control (ECDC) estimates that for cases in which the transmission route is known, 49% of the diagnoses were among people who use drugs and 49% were ascribed to heterosexual transmission.[17] Large numbers of young and unemployed men inject and share drugs. Syringe exchange programs have recently increased,

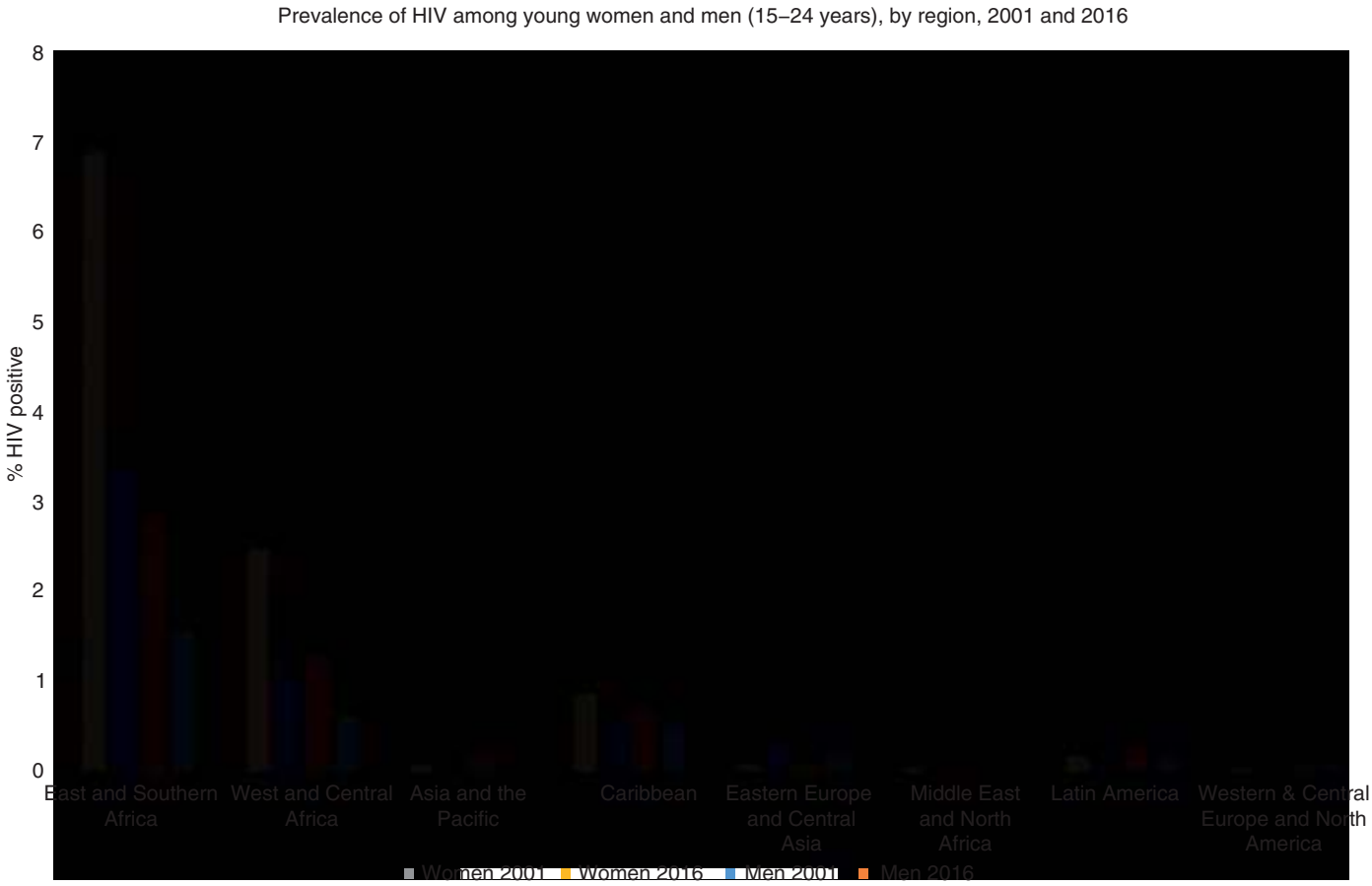

FIG. 118.2 Prevalence of human immunodeficiency virus *(HIV)* among young women and men (15–24 years old), by region, 2001 and 2016. *(Data from UNAIDS.* http://aidsinfo.unaids.org. *Accessed February 6, 2018.)*

but coverage is too limited to make an impact on the HIV epidemic curve; and in Russia, substitution therapy with methadone is still against the law. As Russia's epidemic matures, AIDS mortality rates will contribute significantly to the demographic decline of the country.

Elsewhere, the epidemics are comparatively small. In Eastern Europe between 2007 and 2016, the overall increase in HIV cases was driven by an increase in sexual transmission of HIV, which doubled for heterosexual transmission and rose ninefold for transmission through sex between men. Transmission through injection drug use decreased by 39% during this period.

In addition to that of HIV infection, major epidemics of tuberculosis and syphilis have occurred in Central Asia against a background of political and economic instability, increased mobility, drug use, and degradation of public health infrastructure.

Sub-Saharan Africa

AIDS more heavily affects sub-Saharan Africa than any other region of the world. In 2015, 29.4 million people in the region were estimated in the Global Burden of Disease (GBD) Study to be infected with HIV.[14]

Africa and the Caribbean are the only regions in which cases in women exceed cases in men.[21] Epidemics in sub-Saharan Africa vary significantly in scale. National adult HIV prevalence rates range from around a very low prevalence of 0.5% in some countries of West Africa (i.e., Senegal, Niger, Mauritania), to around 15% to 25% in southern Africa, to around 27% in Swaziland.[3] GBD estimates suggest that around 1.8 million people in sub-Saharan Africa became infected with HIV in 2015,[14] which was a decline from 2.2 million new infections in 2001.[16] Although AIDS was previously (in 2005) the single largest cause of mortality in the region, AIDS is now the second major cause of mortality, behind diarrhea, lower respiratory infections, and other common infectious diseases.[22] An estimated 10.9 million children have been orphaned by AIDS in this region.[8]

National adult HIV prevalence rates in 2016 exceeded 12% in eight countries,[3] all in southern Africa. Sub-Saharan Africa bears a disproportionate share of the global burden of HIV, with 85% of the world's HIV-positive pregnant women living in that region in 2014.[23] Of the children living with HIV in 2014, 91% lived in sub-Saharan Africa, although new infections in children in eastern and southern Africa decreased by 56% from 170,000 in 2010 to 77,000 in 2016; in West and Central Africa in the same period, new infections among children decreased by 33% from 90,000 in 2010 to 60,000 in 2016.[10] In South Africa, Tanzania, Uganda, Mozambique, Ethiopia, Namibia, and Swaziland, the number of children newly infected with HIV decreased by over 60% from 2009 to 2014.[24]

HIV prevalence and incidence in a growing number of African countries have declined significantly. The number of people acquiring HIV was reduced substantially in sub-Saharan Africa between 2001 and 2016. For example, the rate of new HIV infections dropped by 90% in both Senegal and Côte d'Ivoire and by 87% in Niger. In South Africa and Swaziland, with the largest number of HIV infections and the highest rate of infection respectively, reductions amounted to 55% in both countries. In West and Central Africa, Burundi's rates dropped 85%, and those in the Democratic Republic of the Congo (DR Congo), 84%. Other countries with declines included Guinea-Bissau, Cameroon, and Mali, with reductions of more than 60%. However, even when there has been a 46% reduction overall in the number of people acquiring HIV in sub-Saharan Africa, the region represented 64% of all new HIV infections in 2016.[3] A modeling study conducted in urban Kenya, Zimbabwe, and urban Haiti suggested that changes in patterns of HIV prevalence are consistent with behavior change.[25]

In Uganda, urban antenatal HIV seroprevalence rates peaked around 1992 (15%–30%), followed by a steady decline by 2002, most markedly among women aged 15 to 24 years.[26] This decline was associated with an increase in median age of sexual debut and a decline in multiple

partnerships among men, although no significant changes were found in extramarital sex among men. Between 1995 and 2000, condom use at last sex with a nonregular partner increased from 35% to 59% in men and 20% to 39% in women, respectively. In 2005, the national HIV prevalence rate was estimated to be 6.7%, with large regional variations. In particular, in the conflict-affected region of northern Uganda with 1.7 million internally displaced persons, the HIV prevalence rate was estimated to be close to 10%, which is very high for a rural population.[27] The national HIV prevalence rate fell slightly to 6.0% in 2016, and new infections were reduced by 47% from 2001 to 52,000 in 2016.[3,28]

There is significant heterogeneity in progress in reducing HIV incidence across eastern and southern Africa. For example, Swaziland is close to achieving the 90-90-90 targets (of 73% of all people living with HIV being virally suppressed), with 68% of all people living with HIV being virally suppressed. In 5 years from 2011 to 2016, incidence among adults fell by nearly half (44%).[29] This situation contrasts with Botswana, where 78% of people living with HIV are virally suppressed, but in this case treatment scale-up has not been associated with a meaningful decline in new HIV infections.[30] Young women are still disproportionally infected by HIV as compared with young men. A national household survey undertaken in South Africa in 2012 showed sustained high levels of HIV infection among young females. For example, among 15- to 24-year-olds, female prevalence (11.4%) was 3.9 times higher than that of males (2.9%).[31] Young women with older partners were also at increased risk for HIV infection. Among men and women, increasing partner numbers and inconsistent condom use were significantly associated with HIV infection. In a survey in 2005, young people who reported participation in at least one program for behavioral change were less likely to be infected with HIV.[32]

An understanding of sexual networks is crucial to understanding the rapid heterosexual spread of HIV, particularly in southern Africa. Why is southern Africa HIV hyperendemic? A multiplicity of risk factors, such as the lack of male circumcision, male sexual violence, concurrent and multiple partnerships, and migratory patterns, operates to create a "perfect storm"; no single factor in itself can explain such HIV levels, because all of them individually exist elsewhere. The practice of multiple sexual partnerships has been shown to be the most important risk factor in the transmission of HIV,[33] but the characteristics of each sexual partnership (e.g., whether it is transactional or whether payment is involved, older age of the male partner,[34] earlier age of the female partner, existence of concurrent partnerships) have been associated with HIV transmission.

The comparative impact on the spread of HIV of concurrent multiple relationships, defined as sexual relationships that overlap in time as opposed to serial multiple relationships, is less clear. Early modeling work concluded that, for the same number of partners per person, sexual partner networks that include concurrent partnerships lead to larger HIV epidemics than do networks without concurrent partnerships, especially at the beginning of the HIV epidemic curve.[35] However, empirical evidence so far has not confirmed such mathematical modeling. A study in five cities in sub-Saharan Africa and one in KwaZulu-Natal found no association between concurrency and HIV prevalence level.[36–38]

In the countries in southern Africa in which HIV prevalence rates are extremely high, the probability that one's partner is infected with HIV is around 1 in 3 to 1 in 6. This probability results in much higher levels of heterosexual exposure to HIV than occur anywhere else in the world and makes any new partnership, whether concurrent or not, a critical risk factor. Multiple and concurrent partnerships are often linked to mobility (including labor-related mobility), in that people may have different partners at different residences; also, those who stay behind may themselves have other partners, and living near major transport roads or locations with migrant labor generates "hot spots" for HIV transmission as demonstrated in KwaZulu-Natal.[39,40]

The disease burden of HIV infection in sub-Saharan Africa is high and will probably grow considerably higher. In 2016 alone, despite major progress in antiretroviral therapy (ART) coverage, around 730,000 sub-Saharan Africans died of AIDS, which constituted 73% of the global total.[2] The HIV adult prevalence rate in West and Central Africa is 2%. Guinea-Bissau has the highest HIV prevalence in West Africa, at 3.1%. In Central Africa, the estimated prevalence rates in all countries are 4% or lower, with the exception of Equatorial Guinea at 6.2%. HIV-2 is primarily found in West Africa but has also been confirmed in other African countries. The highest prevalence of HIV-2 infection is found in Guinea-Bissau, with prevalence rates as high as 8.0% in 1987 to 1988 and a decline to 2.5% in 2011. In contrast to the increasing spread of HIV-1, the prevalence rate of HIV-2 has remained rather stable in West Africa and has declined in Guinea-Bissau and in the Gambia,[41] probably the result of the higher transmissibility of HIV-1 compared with that of HIV-2. A mathematical modeling study predicts HIV-2 infection to fall below 0.1% in rural Guinea-Bissau by 2050.[42]

Middle East and North Africa

The number of HIV infections in Middle Eastern and North African countries is very low.[2] The number of people newly infected in the Middle East and North Africa continued to decline from its peak at 21,000 in 2005 to 18,000 in 2016. So far, the spread of the virus appears to be limited to those who pay for sex, MSM, and injection drug users in large cities. Most of the AIDS cases have been reported from Djibouti, Somalia, and Sudan, with only Djibouti having an estimated HIV prevalence rate higher than 1% (1.3%). Morocco, Egypt, Iran, Lebanon, Yemen, and Tunisia are at 0.1% HIV seroprevalence levels, or below, among the general population.[3] Iran has made needles and syringes available in pharmacies in an effort to cut the spread of HIV infection among an estimated 200,000 drug injectors, with half of them sharing injection equipment.[16] Libya's epidemic has been growing, with almost 90% of the 6000 to 10,000 HIV infections in Tripoli attributed to injection drug use. Transmission through contaminated injection equipment has also been reported in Bahrain and Oman. Because of the illegality of homosexuality in this region, concern exists that HIV may spread undetected among MSM.[43]

Asia and the Pacific

Overall, in Asia and the Pacific, an estimated 5.1 million people had HIV in 2016, including 270,000 people who were newly infected during the previous year. Approximately 170,000 died of AIDS-related illnesses in 2016.[2] The national HIV prevalence rate is highest in Southeast Asia (0.3%), with wide variations in epidemic trends among different countries.[44] Approximately 2.1 million people in India were estimated to have HIV in 2016, with a national adult HIV prevalence rate of around 0.3%.[3] The epidemic is extremely heterogeneous; for example, in Mumbai and Pune and in Karnataka, Andhra Pradesh, and Tamil Nadu states, HIV prevalence rates of 2% to 4% have been reported in pregnant women, in contrast to Calcutta and Delhi, in which prevalence rates have remained at less than 1%. A report suggests that the prevalence rate in pregnant women in Mumbai had fallen to 0.6% by 2012.[45] Injection drug use is the predominant mode of transmission in the northeastern states near Myanmar, where prevalence rates among drug users exceed 70%. In Manipur state, many of the women with positive HIV test results are sex partners of male injection drug users. HIV prevalence levels among injection drug users usually far exceed levels among sex workers. HIV among injection drug users is now also appearing in other parts of India, such as Punjab.[46,47] Over 90% of HIV-positive injection drug users in Manipur and its neighboring state of Mizoram are reported to be coinfected with the hepatitis C virus (HCV) which presents a challenge to ART treatment because coinfected patients were found to be less tolerant to several ARTs.[48]

In China, HIV infection was first noted in Yunnan province, which borders the Golden Triangle where Myanmar, Thailand, and Laos meet. The prevalence rate of HIV infection in injection drug users there climbed rapidly to 70% or more in the early 1990s, and HIV infection first emerged in sex workers in this region. Prevalence of HIV among injection drug users declined from 12.3% in 2008 to around 6% in 2014.[49] Around 115,000 persons in China were infected with HIV in 2015 according to data from China's Centre for Disease Control and Prevention,[50] with sexual transmission now the primary mode of HIV transmission. Although overall HIV prevalence remains low, with 0.037% of the population living with HIV, the provinces of Yunnan, Sichuan, and Guangxi each have over 50,000 people living with HIV; among MSM, HIV prevalence is 7.7%.[49] In cities and among young MSM, incidence may be particularly high; a 2014 study suggested that

HIV incidence among young MSM was 18.9 per 100 person-years in Guiyang, 10.6 per 100 person-years in Beijing, and 5.6 per 100 person-years in Shanghai.[51] Since 2005, China has established more than 1500 methadone clinics and needle exchange sites.[49] Examples in Sichuan show that these programs can reduce the reuse of nonsterile needles by almost half.

China's HIV prevalence rate remains low (estimated at <0.1% of the total population), but the epidemic continues to grow in several parts of the country, and among groups such as young MSM and individuals older than 60, with figures from China's Centre for Disease Control and Prevention suggesting that 15% of new infections in 2015 were among this latter age group.[50] The AIDS epidemic remains a potential challenge, particularly with regard to improving coverage of prevention of mother-to-child transmission and reducing AIDS-related deaths, which stood at 21,000 in 2014.[49] Several factors in China fuel it, including rapid economic growth and higher disposable income for men, commercial sex, injection drug use, high mobility of workers, and stigma and discrimination experienced by MSM and people living with HIV. China has dramatically improved its AIDS response since an initial phase of denial. This commitment has been translated into laws and policies, growing HIV prevention efforts, and increased access to treatment. China increased the number of people receiving HIV treatment by nearly 50% in 2011 alone,[16] and in 2014 59.0% of all adults and children living with HIV were receiving ART and over 90% of people on treatment were virally suppressed.[49]

The most heavily affected countries of Southeast Asia are Cambodia, Thailand, and Myanmar, with injection drug use and commercial sex driving HIV transmission. In Thailand, the epidemic has spread in overlapping waves through injection drug users and sex workers, their clients, and the female partners of clients, including female spouses. The prevalence rate among sex workers rose from 3% in 1989 to 30% in 1996, and the rate among sexually transmitted disease (STD) clinic attendees rose from nearly zero to 9% over the same period, before declining in the late 1990s. Convincing evidence shows a fall in risky behaviors after extensive programs to promote condom use in brothels and to discourage men from visiting them. The number of new annual HIV infections in Thailand continues to decline, from 21,000 in 2005 to 6400 in 2016, although the decline in HIV prevalence has been slowing in recent years as more people receive ART. The HIV prevalence rate among injection drug users has declined over the past 20 years but remains high, with an HIV prevalence of 19.02% among people who inject drugs in 2016.[52] Similarly, infection rates remain high among key populations such as transgender people and MSM, with HIV prevalence of 9.15% among this latter group. Programs for injection drug users and MSM are still inadequate. The percentage of sex workers reached with HIV prevention programs amounted to 57%, whereas the percentage of sex workers reporting the use of a condom with their most recent client was reported at 95% in 2011.[21] Estimates from UNAIDS suggest that 91% of people living with HIV knew their status in 2016, with 69% treatment coverage for adults living with HIV.[52]

The epidemic in Myanmar also shows signs of a decline, with a decrease in new infections from 24,000 in 2005 to 11,000 in 2016. The male-to-female ratio has changed significantly over time from almost 8 to 1 in 1994 to a steady increase in the proportion of women infected by 2008. However, the elevated prevalence rate of HIV among key populations at higher risk is of concern, with HIV prevalence of 26.3% among people who inject drugs in Myanmar, 6.4% among MSM, and 4.7% among prisoners.[52] The epidemic in Cambodia has shown a steep drop in HIV, with a prevalence of 0.6% in 2016 and fewer than 1000 new infections. The epidemic in Vietnam declined from 24,000 new infections in 2005 to 11,000 new infections in 2016. Commercial sex and injection drug use remain the most common risk factors for HIV infection in Vietnam. However, the HIV prevalence rates among pregnant women increased from 0.03% in 1994 to 0.5% in 2011. In 2007, the number of men living with HIV infection was 3.0 times higher than the number of women, with this ratio gradually decreasing to 2.6 by 2012. Although frequently ignored because of prevailing social attitudes, MSM are an emerging risk group, with 8.2% of MSM in Vietnam living with HIV in 2016. However, the epidemic is now present across the general population, with the growth in HIV infections among clients of sex workers and women at low risk.

An HIV prevalence rate of 0.9% has been documented in Papua New Guinea, fueled mainly through heterosexual transmission. Risky behavior is widespread, with research showing high rates of unsafe sex with multiple partners, sexual violence, and the frequent failure to use condoms.[53]

Injection drug use currently is the major mode of HIV transmission in several countries in Asia (Fig. 118.3). In China, with the largest estimated population of injection drug users (2.3 million–2.9 million) and an HIV prevalence rate of 6.4%[54] in users, HIV infection rates have been rising. In Vietnam, HIV prevalence among injection drug users was 30% in 2011, and it stabilized through to 2012. However, in certain areas HIV prevalence remains at high levels in well-established epidemics (Quang Ninh, 56%; Ho Chi Minh City, 55%; Can Tho, 45%), and prevalence is increasing in newer epidemics in northwestern provinces of Vietnam. In Malaysia, among injection drug users there is an HIV prevalence rate of over 26%. The first HIV outbreak in China occurred among injection drug users in Yunnan province in the late 1980s, with the epidemic rapidly expanding to its neighboring provinces. Despite large investments in prevention services for drug users, less than 25% of injection drug users in Yunnan obtain their own injecting equipment and have rates of sharing injecting equipment below 50%.[40,55]

New HIV epidemics in MSM are now firmly established across Asia. These data represent an alarming trend because male-male sexual activity in the region is diverse, often completely hidden, and beyond the reach of current HIV prevention efforts. Similarly, recent studies show increasing HIV prevalence rates among MSM in Bangkok, which rose from 17% in 2003 to 28% in 2007. The increasing sex industry in parts of Asia is of concern, and sex workers and male clients of sex workers are likely to be the most important groups to target for prevention programs.[39]

Latin America and the Caribbean

With an estimated 115,000 people newly infected with HIV in 2015, the HIV prevalence rate in Latin America and the Caribbean remains generally stable, but the epidemic is growing. The total number of people estimated to be infected with the virus is 2.1 million; annual new infections increased by 3% in Latin America between 2010 and 2015, and regional declines in new infections have been greater for children then adults. There are significant disparities among countries, with more than 20% decline in new infections in countries such as El Salvador, Colombia, Nicaragua, and Uruguay since 2010; in contrast, there were significant increases in new infections between 2010 and 2016 in Chile (34%), Guatemala (23%), Costa Rica (16%), Honduras (11%), and Panama (9%). In the Caribbean, new infections have remained relatively static; in Cuba, HIV infections more than doubled between 2010 and 2016, whereas in the same period new infections declined by nearly a quarter in Haiti and Trinidad and Tobago.[10] HIV transmission continues to occur among populations at higher risk for exposure, including sex workers, their clients, injection drug users, and particularly MSM.[16,56] The epidemic in Latin America was similar initially to that in North America and Europe, with most cases occurring among MSM and injection drug users. Systematic surveillance in the region is limited, and the picture is complex, with male-to-male transmission predominating in major cities. Pooled prevalence estimates suggest an HIV prevalence of 14.9% for MSM in Central and South America, and 25.4% for MSM in the Caribbean.[20] Reliable data on HIV rates among injecting drug users are limited, but there are indications of a decline in HIV infections among this population; for example, in Argentina, HIV prevalence among people who inject drugs declined from 7.6% in 2005 to 0.4% in 2013.[57] In Latin America, HIV prevalence decreased by nearly 20% among young people in 2011, with higher decreases among young men (33%), the group in which the majority of HIV infections occur in Latin America. In 2012, it was estimated that 43% of adolescents living with HIV (aged 10–19) in Latin America and the Caribbean were female, the lowest proportion of any region.[58] A large proportion of MSM often also have sex with women, which leads to an increased number of HIV infections among women. Bisexual behavior of male partners is an important source of HIV infection for women in several Latin American

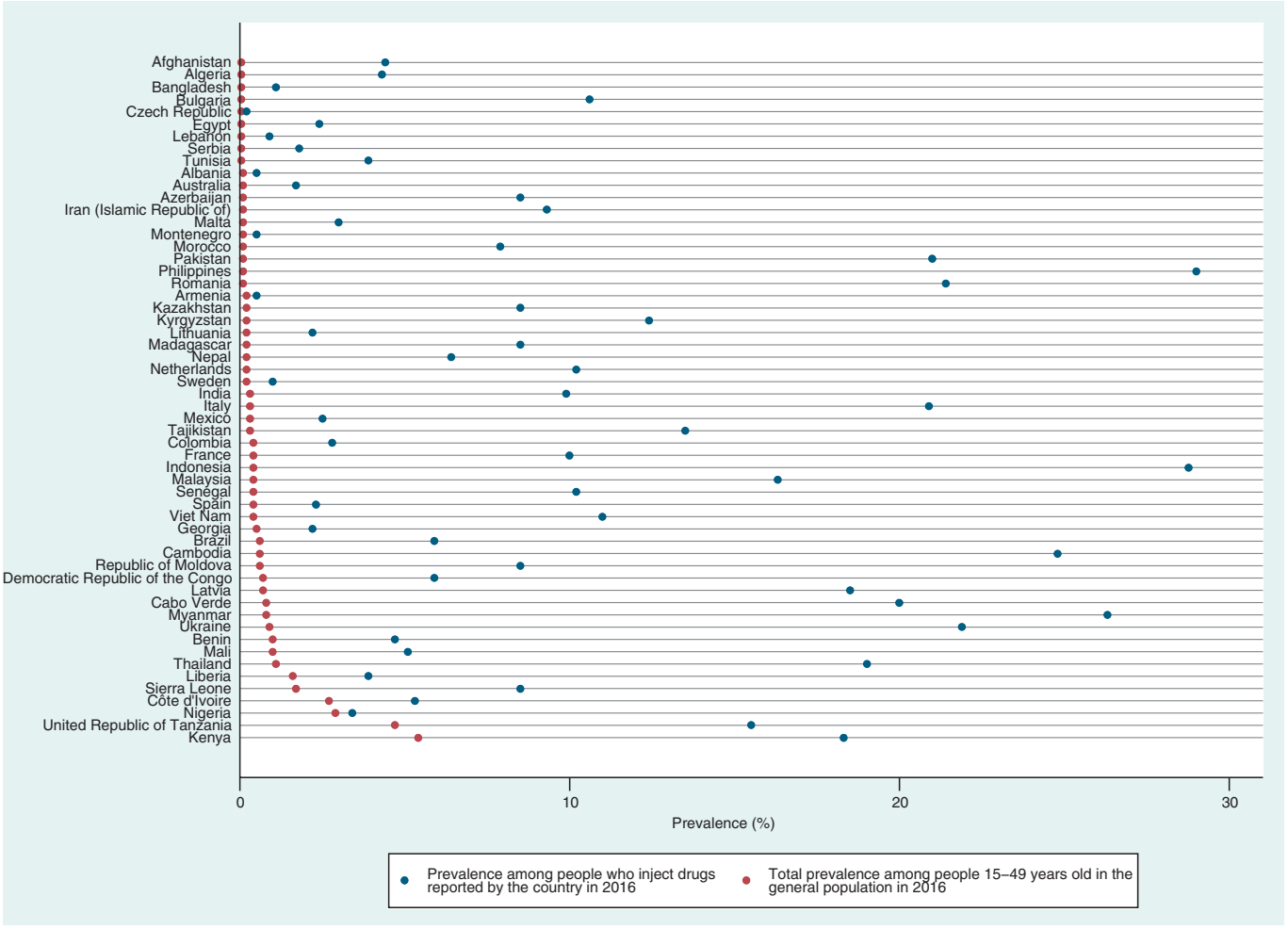

FIG. 118.3 Human immunodeficiency virus trends among injection drug users in selected countries, 2016. *(Data from UNAIDS.* http://aidsinfo.unaids.org. *Accessed February 6, 2018.)*

countries. HIV rates in pregnant women are generally low (around 0.5% or less), although estimates of prevalence rates are higher in countries of the Caribbean such as Haiti, where prevalence as high as 2.5% among pregnant women has been reported.[59]

Over one-third of all people with HIV in Latin America and the Caribbean region reside in Brazil, where in 2016 an estimated 830,000 people were living with HIV, which corresponds to 0.6% of the adult population. Although initially concentrated primarily among MSM, the epidemic subsequently spread to injection drug users and eventually into the general population, with increasing numbers of women becoming infected. A large proportion of infections among women may be attributed to the behavior of male sexual partners. However, unprotected sex between men remains an important factor. In 2014, the HIV prevalence rate among MSM was 10.5%.[60] Transgender women across the region, and particularly in Brazil, are at high risk of HIV infection and violence. In a 2017 respondent-driven sampling study, almost one-third of transgender women were estimated to be living with HIV; among this sample, new HIV diagnoses were associated with black race, and history of sex work and cocaine use.[61] HIV prevalence among injection drug users in Brazil has declined in some cities, as a result of harm-reduction programs and changes from injected to inhaled drugs, and mortality rates among drug users have also declined.[62] A 2015 study using data from eight Brazilian cities found that an estimated 9.9% of people who inject drugs were living with HIV.[63]

Brazil remains one of the world's first success stories in its political determination to secure antiretroviral drugs while also sustaining resources for prevention and care. More than 60% of the 830,000 living with HIV in need of treatment are receiving antiretroviral drug therapy in Brazil.[52]

Of the Central American countries, Honduras has been especially broadly affected by heterosexual transmission. However, overall, HIV infections are highest among female sex workers and their clients (there is a prevalence of 5.3% among sex workers overall in Honduras), among MSM (prevalence of 11.7%), and among transgender people (prevalence of 11.9%). Since 2010, new HIV infections have declined by 29%; however, in the same period, AIDS-related deaths have increased by 11%.[64]

In the Caribbean, the adult HIV prevalence rate is estimated to be 1.3%.[3] HIV prevalence is highest in the Dominican Republic and Haiti, which together account for around 70% of the 310,000 people infected with HIV in the Caribbean.[16,56] The HIV prevalence rates show clear signs of decline in these two countries, but AIDS remains one of the leading causes of death among adults. Declining HIV rates are also documented in Barbados and in Trinidad and Tobago. Although new infections are increasing in Cuba (from 1600 annual new infections in 2010 to 3300 new infections in 2016), 70% of people living with HIV have access to treatment, and Cuba was the first country validated by WHO to have eliminated mother-to-child transmission of HIV and syphilis. HIV infection in the Caribbean is a true mosaic of different epidemiologic patterns determined by the local interaction of various risk determinants for HIV transmission.

In 2016, HIV prevalence rates among MSM were 31.6 % in Trinidad and Tobago, 4.9% in Guyana, and 18.2% in Haiti.[21] Drug use and sex for drugs (mainly cocaine and crack) are major risk factors in the Bahamas, Bermuda, Puerto Rico, and Trinidad. Finally, heterosexual transmission is predominant in Haiti and in the neighboring Dominican Republic.[16,56] Among sex workers, an HIV prevalence rate of 8.4% has been found in Haiti, with 2.8% in Jamaica and 6.1% in Guyana.[52]

DYNAMICS OF THE SPREAD OF HUMAN IMMUNODEFICIENCY VIRUS

Basically, no difference exists among the various modes of transmission of HIV in the developing world and industrialized countries. Selected aspects of HIV transmission in the LMICs and other determinants of HIV spread are reviewed briefly in this section.

Modes of Transmission

Worldwide, HIV infection is predominantly sexually transmitted. Unprotected heterosexual intercourse accounts for most cases of HIV infection in sub-Saharan Africa and some countries in the Caribbean and Asia, but the situation is somewhat different in the rest of the world.

The low average efficiency of penile-vaginal intercourse for transmission of HIV in the absence of amplifying factors has now been well documented, especially for transmission from women to men.[65] A sustained and fulminant heterosexual epidemic can be explained only by a common occurrence of factors that amplify heterosexual transmission, probably in addition to high-risk sexual behavior patterns.[66]

Factors that enhance the efficiency of heterosexual transmission of HIV include higher viremia or more advanced immunodeficiency in the infecting partner, acute primary infection, receptive anal intercourse, sex during menses, lack of male circumcision, and the presence of other STDs. Viral load of HIV-1 has been shown to be the primary determinant of transmission risk in HIV-discordant couples,[67,68] and suppressing viral load through ART reduced HIV transmission in discordant couples by 96%.[69] Indeed, 5-year follow-up of the landmark treatment-as-prevention trial HIV Prevention Trials Network (HPTN) 052 observed no genetically linked infections in discordant heterosexual couples when HIV-1 infection was stably suppressed by ART in the index participant.[70] This is the foundation for the current strategy of ART as prevention.

Other factors that may increase the risk for heterosexual transmission but are less well documented include the use of various desiccating vaginal agents, traumatic sexual intercourse, hormonal contraception, and cervical ectopy.[71,72]

Data from three randomized controlled trials undertaken in South Africa, Kenya, and Uganda show that male circumcision reduces the risk for heterosexually acquired HIV infection in men by approximately 60%.[73–75] This evidence strongly supports the findings of numerous observational studies.[76] At this stage, what, if any, protective effect male circumcision may have on a woman's risk for acquiring HIV during sexual intercourse is unclear. A study in Uganda and Zimbabwe found that it neither increased nor decreased a woman's risk for infection.[77] A more recent mathematical modeling analysis conducted in Zimbabwe and Kenya suggested a 46% reduction in the rate of male-to-female HIV transmission, suggesting that women also benefit greatly from male circumcision.

Conventional STDs have attracted much attention as a risk factor because many are treatable with relatively inexpensive antibiotics and antiviral agents.[78] Evidence from prospective studies showed that genital ulcers (e.g., chancroid, syphilis, genital herpes) particularly enhance the sexual transmission of HIV by a factor of 3 to 8.[79,80] Early diagnosis and treatment of STDs reduced the spread of HIV infection in two randomized trials in Tanzania[81] and Uganda,[82] where a 38% to 42% decline in the rate of newly acquired HIV infections was observed in the interventions communities. However, later studies found no effect.[83] Perhaps STD treatment as a form of HIV prevention is more effective early in an epidemic. Since the first description of AIDS in the early 1980s, severe mucocutaneous herpes simplex virus (HSV) infections have been associated with the immunodeficiency syndrome. HSV type 2 (HSV-2) is also the most common cause of genital ulcer disease, with prevalence levels above 80% in Africa.[84] HSV-2 infection may facilitate HIV acquisition and potentially increase HIV genital shedding and infectiousness. A meta-analysis of prospective observational cohort studies showed that male and female patients with seropositive results for HSV-2 had a threefold higher risk for acquiring HIV compared with individuals with HSV-2–seronegative results.[85] More recently, in two cohort studies in Uganda and Zimbabwe, hazard ratios for HIV acquisition were 2.8 and 4.4 for prevalent HSV-2 infection, respectively, and 4.6 and 8.6 for incident HSV-2 infection, respectively.[86]

Several trials have been conducted to measure the effect of acyclovir suppressive therapy in reduction of HIV acquisition with HIV seronegativity, reduction of HIV transmission from persons with dual HIV and HSV-2 infections, and reduction of HIV disease progression. Two controlled trials reported no efficacy of HSV-2 suppressive therapy on reduction of HIV acquisition,[87,88] undermining the extensive evidence from epidemiologic studies.[89]

The relative risks of gonorrhea, chlamydial infection, and bacterial vaginosis for HIV acquisition in women are smaller than those of genital ulcers. However, because these STDs are far more common in most populations than are genital ulcers, their contribution to the heterosexual spread of HIV may also be greater. Several studies have found that genital shedding of HIV is greatly enhanced in the presence of urethral, cervical, or vaginal inflammation.[90,91] For example a recent cohort study in Uganda showed an association between recurrent bacterial vaginosis and risk of HIV; bacterial vaginosis may trigger an innate immune response that upregulates cytokines associated with HIV acquisition.[92] Overall, the case for STD control to reduce HIV transmission has been considerably weakened; and with the appearance of antiretroviral-based prevention interventions, STD treatment may no longer be a core HIV prevention strategy.

Mother-to-child transmission during pregnancy, delivery, or breast-feeding is a major mode of spread of HIV. The prevention of HIV transmission from mother to child is a success story in many countries, with a few such as Cuba, Thailand, Belarus, and Armenia having declared elimination of such transmission.[93,94] In Botswana, Mozambique, Namibia, South Africa, Swaziland and Uganda—designated "priority countries" in the Global Plan to eliminate new HIV infections in children—90% or more of pregnant women living with HIV were accessing treatment in 2015. During the five years of the Global Plan, four Africa countries reduced new infections by 80% or more: Uganda (86%), Burundi (84%), South Africa (84%) and Swaziland (80%).[95] In 2016, globally, an estimated 160,000 children became infected with HIV; this is a 47% reduction since 2010, when about 300,000 children were newly infected with HIV most of whom live in sub-Saharan Africa.[2] Mother-to-child transmission of HIV can occur during pregnancy, at the time of delivery, or postnatally via breast-feeding. Breast-feeding by mothers who are HIV positive (in the absence of ART for either mother or infant) can account for up to one-third of HIV infections among infants in sub-Saharan Africa. HIV transmission risk through breast-feeding is enhanced during primary maternal HIV infection acquired after the infant has been put to the breast.[96] The additional risk for postnatal transmission attributable to breast-feeding is estimated at about 14% (range, 5%–20%). Duration of breast-feeding, maternal plasma and breast milk viral load, cracked nipples, mastitis, breast abscess, and infant thrush increase the risk for transmission of HIV. Exclusive breast-feeding is associated with a reduced risk for HIV transmission as compared with mixed feeding.[97]

The situation has greatly improved in numerous countries, from 10% of pregnant women with HIV having access to medicines that prevent HIV transmission to their children in 2004 to more than 30% at the end of 2007, to 61% in 2011, and to 77% in 2015.[98]

Injection drug use is estimated to account for about 30% of new infections outside Africa.[99] HIV has spread considerably among injection drug users in parts of Asia, Eastern Europe, and Central Asia (where an estimated 51% of new infections are among injection drug users), the Middle East and North Africa (where an estimated 28% of new infections are among injection drug users), Latin America, and the Caribbean; this spread has sometimes occurred where injection has replaced smoking and inhaling as a more cost-effective way of drug administration.

Estimates from 2015 suggest that around 15.6 million people inject drugs worldwide.[100] The largest numbers of injectors are found in China, the United States, and Russia, where estimates of HIV prevalence rates among injectors were 12.4%, 8.7%, and 30.4%, respectively.[100] Worldwide, 2.8 million people who inject drugs are estimated to be HIV positive.[101] Injection drug–associated HIV epidemics are characterized by a high degree of regional and local heterogeneity. Explosive epidemics have occurred in both developing and developed countries, with documented HIV incidence rates reaching as high as 20% to 30% per year. Several interventions have been developed to reduce the spread of HIV among

injection drug users, including programs that promote sterile syringe acquisition, drug use treatment, and community outreach—jointly referred to as "harm reduction." Substitution treatment, and methadone maintenance in particular, has been associated with reduced injection frequency and declines in needle sharing, sexual risk behaviors, and HIV seroconversion. In cities in which cocaine and methamphetamine are the main drugs of use, little is available in terms of drug abuse treatment.[102]

A systematic review on coverage of HIV prevention, treatment, and care services in injection drug user populations concluded that coverage is still very low worldwide. The study concluded that although the number of countries with HIV prevention services for injection drug users is growing, the level of coverage remains poor. The study identified 82 countries implementing needle and syringe programs and 70 countries providing opioid substitution therapy, an increase of 5 and 8 countries, respectively, since the last audit had been published in 2008.[103] A systematic review drawing on 106 studies suggested that where drug use is criminalized, this has a negative impact on HIV prevention and treatment.[99]

Transfusion with HIV-contaminated blood continues to be a source of HIV infection in some parts of the developing world as a result of the recruitment of voluntary and unpaid donors and the absence of implementation of national policies for transfusion, proper screening of collected blood, and a strategy for its rational use.[104] Clinical indications for appropriate blood transfusions are often not met outside of large cities in the low-income countries.

Nosocomial transmission of HIV through injection with nonsterile syringes and needles occurs, but its relative contribution to the spread of HIV in the developing world is not well documented, although it is probably no more than 5%, with women and children at greater risk because of frequent anemia. The mean probability of HIV infection from puncture with a contaminated needle was estimated to be 0.23% (range, 0.00%–2.38%).[105] The potential for HIV transmission with unsterilized needles in medical settings is probably weak, but localized outbreaks in Romania and Libya among infants and young children through intravenous contamination have shown that it can occur when basic hospital hygiene is not applied. In 2001, it became widely known that in a number of Chinese provinces, but mostly in Henan in Central China, paid blood donation for plasmapheresis did not respect basic practices of infection control. This resulted in HIV infections, with estimates ranging from less than 100,000 to several hundreds of thousands. Those criminal practices seem to have ceased, but it is unclear whether those responsible for them were ever punished.

Reducing the Spread of Human Immunodeficiency Virus

Historically, the HIV epidemic is still in an early phase in most parts of the world, with continuing geographic spread and changing epidemiologic patterns. It is the complex mix and interaction of direct (behavioral) and indirect risk and vulnerability factors that determine how and when HIV spreads in the population.[106,107]

Safer Sexual Behavior

Sexual behavior is the most important determinant of HIV spread in most parts of the world; about 85% of HIV transmission is sexual, whether via penile-vaginal or anal sex.[56] Such behavior is heterogeneous among and within populations. Sexual behavior surveys around the world have documented this heterogeneity in terms of sexual orientation, marriage patterns, age of sexual debut, number of partners, and rates of casual, transactional, and commercial sex.[108–111] In addition, these studies found that men have generally more casual partners than women; that their female partners are often much younger, with age differences of 10 years or more; that in many societies, women before or within marriage also disclose several partners; and that in some societies, higher socioeconomic status and urban settings are associated with a higher number of partners. However, according to mathematical models, sexual mixing patterns are equally important in determining the spread of HIV, particularly in early stages of the epidemic.[112,113] The "core group" concept postulates that a relatively small proportion of the population contributes to maintenance of the epidemic; this pattern was first described in the 1970s with respect to the epidemiology of gonorrhea in the United States.[114] However, for a chronic viral infection such as HIV, this concept may be more relevant during the emerging phase of the epidemic than for epidemics with very high levels of HIV infection that already occur among persons at a very young age and with relatively low-risk behavior, such as in southern Africa.

The behavior of one's partner is as relevant for the risk for HIV infection as one's own behavior. In parts of sub-Saharan Africa, in discordant couples, as many couples have women with HIV as have men with HIV. However, outside of Africa, in most settings, up to 80% of women with HIV in long-term stable relationships have been infected by their partner. Data suggest that an increasing proportion of women with HIV infection in Latin America and most of Asia have their husbands as their only sexual contact.[16]

The rate of condom use in high-risk behaviors plays a major role in the control of HIV transmission. Despite much early skepticism and moral concerns, condoms are increasingly being used in many parts of the developing world, particularly in high-risk populations and where social marketing programs are dynamic.[115–117] In these groups, both increases in condom use and high rates of consistent condom use have been documented and have resulted in fewer HIV infections. Numerous studies have also shown moderate-to-high rates of condom use in the general population of many developing countries (Fig. 118.4).[117] For example, data collected by the Brazilian Ministry of Health in 2006 showed that 51% of men and 32% of women reported having used a condom during their last sexual encounter with a casual partner.[118] In Zimbabwe, where a decline in HIV prevalence has been documented, more than 70% of men reported using a condom the last time they had high-risk sex.[119] However optimistic the trends in condom use are in the world, the rise in condom use generally applies only to a suboptimal fraction of all sexual encounters. Condom use is much lower in rural areas, and condom promotion has had little impact in the context of steady sexual relationships.[120] Across the world, promotional efforts to encourage consistent condom use are poor, particularly within regular partnerships, in which condom use can be a very sensitive issue.[56,121] Globally, in 2005, estimates were that a condom was used in less than 10% of sex acts involving a nonregular partner. In most high-prevalence (>2%) countries between 2000 and 2016, male condom use increased significantly; for example, condom use at last high-risk sex (with a nonmarital, noncohabiting partner) was highest in Botswana (94%, 2013), Zimbabwe (85%, 2015), Namibia (80%, 2013), and Malawi (76%, 2015). However among 41 countries with data available for young people (aged 15–24 years) for the period 2011 to 2016, condom use at last high-risk sex in the previous year was less than 50% among young women in 31 countries and among young men in 18 countries.[10]

Country data available on the percentage of adults aged 15 to 49 who had more than one sexual partner in the past 12 months who reported use of a condom during last intercourse vary greatly worldwide. Females aged 15 to 49 reported condom use during their last intercourse at a rate of 12% in India, 35% in DR Congo, 48% in Ukraine, 55% in Swaziland, and 66% in Namibia, illustrating the variation worldwide.[21]

The use of condoms has played a role in the significant reduction of HIV incidence rate in MSM in the high-income countries; among intensively counseled discordant couples[122,123]; in large populations of men and women in Thailand, Uganda, and Senegal; and among sex workers in Kenya, DR Congo, India, and Bolivia. These best practices show that effective HIV prevention is possible, particularly when HIV prevention is comprehensive and sustained.

Male Circumcision

Studies in Kenya, South Africa, and Uganda indicated that male circumcision reduced the risk of female-to-male sexual HIV transmission by roughly 60%.[73–75,124] Since then, medically performed voluntary male circumcision is part of combination prevention programs in populations in which male circumcision is not the rule. By 2014, 3.2 million voluntary male medical circumcisions were taking place, in 14 priority countries in eastern and southern Africa; this declined to an estimated 2.6 million in 2016, presenting a challenge to global targets to reach 25 million voluntary circumcisions between 2016 and 2020.[10]

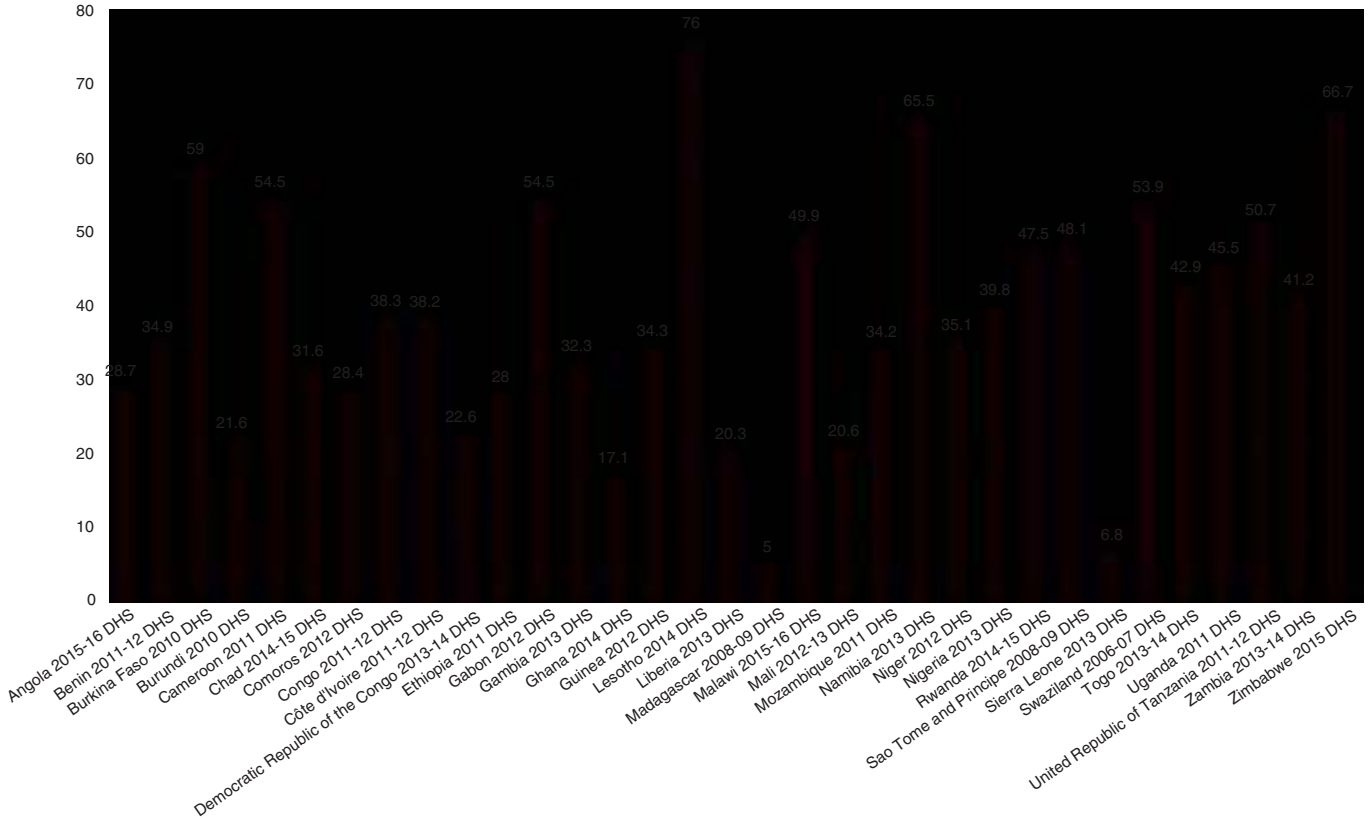

FIG. 118.4 Percentage of condom use at last high-risk sex among female adults (15–49 years old) in Africa, 2016. "High-risk" sex is defined as sex with a nonmarital, noncohabiting partner. *(Data from UNAIDS. http://aidsinfo.unaids.org. Accessed February 6, 2018.)*

However, circumcision of boys at birth is still not widely recommended or practiced in sub-Saharan Africa and would be the longer-term and less costly solution. In sub-Saharan Africa, rates of circumcision vary throughout the region, with important implications for HIV prevention efforts. In southern Africa, where one in three adults is infected in some countries, the rate of circumcision among men is lower than 30% in countries such as Botswana, Malawi, Namibia, Rwanda, Swaziland, Uganda, Zambia, and Zimbabwe.[10] Recent data from Uganda show that 5 years after the male circumcision intervention trial was performed, high effectiveness among the men who were circumcised still remained, with a 73% protective effect against HIV infection.[125]

Preexposure Prophylaxis

Recent advances in pharmaceutical-based HIV prevention have opened new and important approaches to combination prevention. Whereas a trial of a microbicide gel with tenofovir in South Africa found a 39% protection among women,[126] another large trial did not find effectiveness of a tenofovir gel.[127] More recently, the ASPIRE trial, which tested a vaginal ring containing dapivirine, showed that HIV incidence was 37% lower in the dapivirine group versus the placebo group. Researchers found that use of the ring was associated with protection against HIV in women older than 21, but not in women younger than 21, whose adherence was lower.[128] More research with various products is being conducted, but, as with oral preexposure prophylaxis (PrEP), adherence to the use of the microbicide is a key factor for effectiveness.

PrEP with an antiretroviral drug that is used before sex among HIV-negative individuals has been a major addition to the prevention armamentarium. Randomized controlled trials testing precoital use of different preexposure prophylactic products resulted in significant reductions in infection for various groups, such as women (39% reduction), MSM (44% reduction), uninfected partners in HIV serodiscordant partnerships (~70%), and young heterosexuals.[129] However, two other studies produced conflicting results, finding no efficacy. The main reason for the difference in these results seems related to low adherence to prophylaxis. In 2012, the US Food and Drug Administration approved one of the PrEP products (i.e., Truvada gel). Since then, trials of oral on-demand PrEP among MSM in the United Kingdom (the PROUD study) and France (ANRS IPERGAY) have shown significant reductions in new HIV infections relative to placebo.[130,131] Research is growing into long-acting injectable forms of PrEP, such as injectable cabotegravir, which could be a useful alternative for those who struggle to adhere to daily oral PrEP. Although PrEP may have a crucial role to play in prevention and is highly acceptable to many high-risk groups, access needs to be expanded together with support to promote adherence. National regulatory approval for PrEP has been granted in high-income countries such as the United States, France, Australia, Canada, Belgium, Norway, Israel, Portugal, Scotland, and Luxembourg. Among LMICs, approval for PrEP as been granted in Brazil, South Africa, Kenya, Zimbabwe, Namibia, and Thailand. PrEP coverage in LMICs is still thought to be low; the number of people who started on PrEP between 2012 and early 2017 has been estimated at nearly 250,000, of whom the vast majority (220,000) lived in the United States. Thus, scaling up PrEP rollout and increasing accessibility remain challenges.[10]

Treatment as Prevention

Five-year follow up of the landmark treatment-as-prevention trial HPTN 052 observed no genetically linked infections in discordant heterosexual couples when HIV-1 infection was stably suppressed with ART in the index participant.[70] However, evidence for the efficacy of treatment as prevention is still somewhat uncertain at the population level. The ANRS Treatment as Prevention trial, a cluster RCT in KwaZulu-Natal, found no reduction in HIV incidence in the 11 communities where HIV-positive participants received ART regardless of CD4 count, compared with the control arm, in which ART was offered in line with national guidelines. This may have been due to difficulties linking people to testing and treatment services, certain participants' choice not to access treatment, and the complex geography of sexual networks that extend beyond individual communities.[132] Early findings from the PopART universal test-and-treat intervention in Zambia show that after just 1 year, 87% of women and 78% of men living with HIV knew their status, although

linkage to care and ART initiation remains a challenge to reaching 90-90-90 targets.[133] Encouragingly, evidence from the Opposites Attract study in Australia, Thailand, and Brazil showed no linked HIV transmissions between discordant homosexual male couples, across 16,889 acts of condomless anal intercourse, when the HIV-positive partner had an undetectable viral load.[134]

Combination Prevention

There is wide agreement that a combination prevention strategy is the best approach to prevent new infections.[135] Biomedical interventions need to be introduced together with behavioral and structural approaches for the first to be successful. There is also a need to test in community trials what the optimum program design and content of a combination prevention strategy might be. Issues to consider are what key populations should be targeted and the need to provide a better understanding of the existent cultural barriers to the provision of such services.

The range of available prevention tools may continue to expand in coming years. For example, a safe and even moderately efficacious HIV vaccine could prove transformative for HIV prevention. A phase IIb proof-of-concept study, Imbokodo, is underway in South Africa, aiming to enroll 2600 HIV-negative women in sub-Saharan Africa and evaluate the safety and efficacy of a vaccine. The regimen is based on "mosaic" immunogens, which can stimulate immune responses against a wide variety of global HIV subtypes, and is drawing on results from early-stage clinical trials such as APPROACH, which evaluated seven different prime-boost vaccines.[136] In tandem, the HVTN 702 study, a phase IIb/III trial, is evaluating a new regimen in South Africa based on the regimen used in the RV144 trial in Thailand, the only candidate vaccine ever found to show some protection against HIV.[137]

Advances in bioinformatics may allow for improved phylodynamic analysis, to better understand patterns of HIV transmission and resistance in specific social networks and thus help target prevention efforts. For example, analysis of sequence data in the United States in 2017 demonstrated recent and disproportionately rapid transmission among young Hispanic/Latino MSM, allowing public health resources and campaigns to be tailored to this high-risk group.[138]

Demography and Social Context

Investigators took a long time to recognize that the determinants of the HIV epidemic involve more than multiple sexual and drug-using partners. There is increasing awareness that personal behavior is critically influenced and conditioned by the sociodemographic, cultural, and legal contexts within a society.[106,107,139,140] Some factors have an indirect effect; for example, the inconsistencies in harm-reduction policies and practices are obstacles to the prevention of the transmission of HIV through injection drug use.[102]

In developing countries, with demographic growth rates of more than 3% in some African countries and age pyramids that reflect the slow decline in mortality rates in the age group of those younger than 5 years, adolescents and the more sexually active age groups account for a high proportion of the population. This demographic by itself often results in higher incidence rates of STDs such as HIV infection.[141,142] In sub-Saharan Africa, the population will increase from 0.8 billion to over 2 billion by 2050, with around 605 million young people between 10 and 24 expected to live in sub-Saharan Africa by 2050.[143]

In 2008, the world reached an invisible but momentous milestone: for the first time in history, more than half the human population, 3.3 billion people, were living in urban areas.[144] Furthermore, the total urban population in sub-Saharan Africa is expected to increase from 298 million in 2010 to about 1.1 billion in 2050.[145] Urban population growth is driven partly by rural-to-urban migration of young adults seeking jobs and other livelihood opportunities. Estimates from UN-Habitat show that about 70% of all urban residents in sub-Saharan Africa live in slums.[144] The experiences of the urban poor are characterized by reliance on a cash economy, overcrowding, poor environmental sanitation, lack of security, lack of social and health services, and high levels of mobility and riskier sexual practices. These experiences have often led to the growth of marginalized conditions that have been associated with higher rates of STDs.[146] In many sub-Saharan African countries, the HIV prevalence rate is significantly higher in urban than in rural areas and is also higher in slums than in nonslum urban areas. Moreover, women who live in slums are particularly at risk, with higher HIV prevalence rates than those of both men and rural women.[56]

With globalization of the economy and increased inequalities, rural-to-urban movement migrations occur all over the developing world and have played a major role in the spread of HIV in some parts of the world, such as in Central and southern Africa[147] and also in the Mekong, Nepal, and China.[148] The Greater Mekong subregion includes a vast number of migrants and mobile people.[149] Border-crossing points are meeting places for many transport workers, workers without families, traders, tourists, border police, and military personnel; and the use of alcohol and drugs and commercial or casual sex are common.

With the insight that social, political, and economic contexts often contribute to health risk and HIV vulnerability, some HIV prevention programs have begun to include structural interventions. Structural interventions have been used extensively in public health policy for a broad range of issues, including injury prevention, smoking cessation, obesity control, and motor vehicle safety. These interventions address the determinants of health and seek to change the health environment of individuals, communities, and organizations. In HIV prevention, examples of such interventions include income-generating activities for women infected with HIV, conditional cash transfers, empowerment of women, enactment of laws to prevent HIV infection discrimination in the workplace, sex education programs in schools, antialcohol campaigns, and decriminalization of homosexuality Hard evidence that "structural interventions"[150] can be effective is still limited but was demonstrated for cash transfer in adolescent schoolgirls in Malawi and for a program to reduce violence against women in South Africa.[151,152] The effects of structural interventions on prevention outcomes may be complex and indirect; for example, a conditional cash transfer program was found not to reduce HIV incidence among young women in South Africa.[153] However, conditional cash transfers reduced these young women's risk of intimate partner violence by 34%, by reducing women's engagement in sexual partnerships and delaying sexual debut. These behaviors can in the longer term protect against HIV acquisition.[154]

The simplistic assumptions that "poverty causes AIDS" or that "AIDS is a disease of poverty" have been overturned. Higher-income groups and people who are better educated usually live in urban settings and may have higher-risk sex than those who live in rural areas. However, the HIV prevalence rate is not necessarily higher because more educated persons use condoms more often and have different sexual networks.[12] Economic inequality or disparities in wealth have been shown to be an important factor underlying HIV vulnerability. Poverty clearly makes it more difficult for individuals and communities to cope with the consequences of HIV infection and AIDS.[150]

At the microlevel, the evidence is more mixed. In some contexts, disposable income drives people into transactional sex.[149] Especially among women and girls, lack of income can impede access to HIV and other health information and services and can increase food insecurity.[155,156] Food insecurity in southern Africa was associated with increased transactional sex or multiple sexual partners. Among women who attended antenatal clinics in Soweto, South Africa, 21% said they had exchanged sex for goods or money; these women were considerably more likely to be HIV positive compared with the other women in the study.[157]

Gender

Gender inequalities are now widely recognized to drive HIV epidemics. In diverse regions, a greater percentage of women are HIV positive or face disproportionate risk for infection. In 2017, more than half (17.8 million) of the 34.5 million adults (15 years of age or older) living with HIV infection are women[6]; this figure is higher than the estimated 15.4 million in 2007 and the 13.8 million in 2001. In sub-Saharan Africa, 56% of adults infected with HIV in 2017 were women. The Caribbean region has experienced an increase in the number of HIV-infected women, with women representing 35% of newly infected adults, and women are more affected than men are by HIV.[6] Young women (aged 15–24) in the Caribbean may be especially vulnerable to HIV infection; for example in Haiti, women aged 20 to 24 are three times more likely

to be HIV positive than men of the same age.[158] Survey data show that 9% to 24% of women aged 15 to 24 reported having had sex with a man at least 10 years older than themselves in the previous 12 months. These age-disparate partnerships, especially when compounded by other risk factors such as concurrent sexual partners and infrequent condom use, can increase HIV risk.[159] The gender discrepancy in HIV infections is especially glaring among young people in sub-Saharan Africa, where women aged 15 to 24 years accounted for 23% of new HIV infections. Boys and young men in the same age group accounted for 11% of new infections in sub-Saharan Africa. In certain southern African localities, young women account for as much as 91% of infections among 15- to 19-year-olds[160] (Table 118.2).[161,162] This vulnerability does not match sexual risk behaviors; indeed, demographic and health surveys in 14 countries have shown that the proportion of young men aged 15 to 19 years who reported having had more than one sexual partner in the previous 12 months varied from 12% to 46%, with a median of 21%. For young women, the results were much lower, varying from 2% to 15%, with a median of 4%.[12] Studies suggest that in settings where young women have limited access to education, employment, and economic resources, age-disparate sexual relationships often involve potential economic benefits.[163] Wide age disparities between young women and older men play an important role in the high HIV infection levels found among young women because of power imbalances and economic dependence in such relationships.[164] In a Botswana study among 8000 men, the odds of unprotected sex rose by 28% for every year's increase in age difference between men and their female partners. Furthermore, phylogenetic data from recent research in KwaZulu-Natal, South Africa, shows that age-disparate sex (sex between women younger than 25 and men on average 8.7 years older) drives the HIV transmission cycle in this high-prevalence region.[34]

Condom use was shown to be least likely in relationships with the widest age disparities.[165] In rural Zimbabwe, for example, the HIV prevalence rate was approximately 16% among teenage girls whose last partner was younger than 5 years older than themselves, but among girls with partners 10 or more years older, the prevalence rate was twice as high.[166] Similarly, among South African women aged 15 to 24 years, the HIV prevalence rate was found to be 30% in those with partners at least 5 years older, compared with 19% in those with partners younger than 5 years older.[167]

Numerous studies in many parts of the world have linked sexual violence and intimate partner violence with heightened HIV infection risks for women.[168] Lifetime prevalence rate estimates of forced sex by an intimate partner varied from 4% in Serbia and Montenegro to 46% in Bangladesh and Ethiopia provinces.[169] Exposure to intimate partner violence can increase women's risk for HIV infection through forced sex with an infected partner, through limited or compromised negotiation of safer sex practices,[170] and through increased sexual risk-taking behaviors. Studies in Kenya and Tanzania show that women who have been subjected to violence by their partners are up to three times more likely to acquire HIV than women who have not experienced violence.[171,172]

Economic and social empowerment can help reduce some forms of violence against women. In South Africa, for example, a microfinance project targeting rural women was combined with training on HIV, gender norms, and domestic violence. Within 2 years, women's risks for intimate partner violence were reduced by more than half.[173] In South Africa, Stepping Stones is an HIV prevention program that aims to improve sexual health through building stronger, more gender-equitable relationships with better communication and less violence between partners.[174] A randomized controlled trial of the program found, in addition to a reduction in HIV infection, that the men in the program disclosed lower rates of perpetrating severe intimate partner violence at 12 and 24 months after intervention.[175]

Stigma and Discrimination

The spread of HIV is disproportionately high among many groups that experience discrimination and a lack of human rights protection. Since the beginning of the pandemic, reports of harassment and violence against sex workers, AIDS widows, bisexuals, transgender people, MSM, street children, and drug users have been widespread, occurring on all continents. In 2012, UNAIDS reported that 61% of countries now have

TABLE 118.2 Percentage of People Identified as Living With HIV and Incident Tuberculosis Who Received Treatment for Both HIV and Tuberculosis, 2016

<25%	25%–50%	51%–75%	76%–100%
	Argentina		
	Bhutan		
	Bolivia (Plurinational State of)		
	Bosnia and Herzegovina		
	Botswana		
	Brazil		
	Burkina Faso		
	Cabo Verde		
	Cameroon		
	Central African Republic		
	China		
	Colombia		
	Democratic Republic of the Congo	Azerbaijan	
	Dominican Republic	Bahamas	
	Ethiopia	Belarus	
	Gambia	Benin	
	Guinea	Burundi	
	India	Côte d'Ivoire	
	Iran (Islamic Republic of)	Cambodia	
	Jamaica	Djibouti	
	Kenya	Ecuador	
	Lao People's Democratic Republic	Egypt	
	Latvia	El Salvador	
	Lebanon	Eritrea	
	Lesotho	Georgia	
Afghanistan	Malaysia	Guatemala	
Bahrain	Mali	Guyana	
Bangladesh	Mauritius	Haiti	
Barbados	Mongolia	Honduras	
Bulgaria	Morocco	Kyrgyzstan	
Chad	Mozambique	Malawi	
Comoros	Myanmar	Malta	
Congo (Brazzaville)	Netherlands	Mexico	Albania
Equatorial Guinea	Niger	Nicaragua	Armenia
Gabon	Peru	Norway	Belize
Ghana	Serbia	Oman	Brunei Darussalam
Guinea-Bissau	Sierra Leone	Paraguay	Costa Rica
Indonesia	South Africa	Republic of Moldova	Cuba
Iraq	South Sudan	Romania	Dominica
Ireland	Sir Lanka	Russian Federation	Estonia
Kiribati	Suriname	Rwanda	Israel
Kuwait	Syrian Arab Republic	Saudi Arabia	Jordan
Liberia	Thailand	Senegal	Kazakhstan
Madagascar	Timor-Leste	Swaziland	Namibia
Nepal	Uganda	Tajikistan	Panama
Nigeria	Ukraine	Togo	Saint Lucia
Pakistan	United Republic of Tanzania	Trinidad and Tobago	Sao Tome and Principe
Papua New Guinea	Uruguay	Turkey	Slovenia
Philippines	Venezuela (Bolivarian Republic of)	Viet Nam	Tunisia
Somalia	Zambia	Zimbabwe	United Arab Emirates

Data from UNAIDS. http://aidsinfo.unaids.org. Accessed February 6, 2018.

some form of antidiscrimination law in place to protect people infected with HIV, although in many instances such laws are not enforced.[21] For example, in 2016, 93 countries reported the existence of accountability mechanisms to challenge discrimination in health care settings, but in almost one-third (30 of 93) of these countries, civil society and non-governmental partners reported that the mechanisms were not functioning.[10]

Factors that contribute to AIDS-related stigma include misconceptions about modes of transmission, the fatality of AIDS, widespread taboos about the sexual and drug use behaviors that are risk factors for infection, homophobia, and blaming of individuals for infection.

Stigma within health care systems is also common and can result in the transfer of HIV-positive patients to facilities with inferior care or in the disregard for patients' rights and confidentiality. However, research in rural Haiti suggests that the introduction of high-quality HIV care can lead to a reduction in stigma, with the result of increased uptake of HIV testing. Rather than stigma, logistic and economic barriers determine who will access such services.[176] A study in Botswana found that stigmatizing attitudes had lessened 3 years after the national program for universal access to ART was introduced. ART access was a factor in reducing stigma but did not eliminate stigma altogether and did not lessen the fear of stigma among people infected with HIV.[177]

Stigmatizing processes also play an important role in HIV risk, transmission, and the quality of life for people with AIDS. In China, a study with mobile populations found an association between increased levels of stigmatizing attitudes toward people infected with HIV and a reported unwillingness to engage in harm-reduction activities.[178] Comparative studies from Uganda and India show that individuals are more reluctant to seek HIV testing or care for fear of ostracism, humiliation, and unemployment and rejection from family members, colleagues, and the community as a whole.[179]

On a global scale, stigma has discouraged policy makers, political leaders, and also civil society leaders from prioritizing or even acknowledging AIDS as an urgent national issue.[180]

Interventions that have in part succeeded in reducing stigma related to HIV infection have integrated skill building (e.g., for caregivers and health care workers) with health information and communication activities, including media campaigns with opinion leaders and role models. Some stigma reduction interventions appear to work, at least on a small scale and in the short term, but many gaps remain, especially in relation to scale and duration of impact.[181]

IMPACT OF PREVENTION AND CARE PROGRAMS

The most important lesson from the past decades is that the spread of HIV can be reversed or prevented on a large scale, including in some of the poorest countries of the world. For example, the latest data suggest that between 2010 and 2016, the annual number of new HIV infections (all ages) declined by 16% to 1.8 million.[10] The debate over the relative priority of HIV prevention versus treatment and care should be viewed with skepticism.[182] The most successful responses to the epidemic combine prevention and care strategies to prevent future infections and improve the quality of life for both infected and uninfected individuals. One particular example is the case of Kenya, where the government's National AIDS Control Council has developed a strategic framework, spanning 2014–15 to 2018–19, to address structural drivers of the epidemic and provide comprehensive access to HIV prevention, treatment, and care services. It supports tailor-made prevention programs in each county, devised according to the local epidemiology and state of the AIDS response. The strategy has the flexibility to include innovation. Thus in 2017, the government launched self-test kits as part of its "Be Self Sure" campaign, and rollout of PrEP through public health facilities for groups at risk, including young people, serodiscordant couples, sex workers, and people who use injection drugs.[183]

Despite repeated claims of a single or simple intervention to stop the transmission of HIV, all real-world evidence points to the need for a combination of approaches to HIV prevention. Only when communities and decision makers come to terms with the complexity of HIV prevention and responses will programs succeed.[106] Whereas the precise mix of elements of "combination prevention" needs to be tailored to the local epidemiology and resources, in general it includes condom use, behavioral interventions, medical male circumcision, and expanded use of ART for infected persons and PrEP for uninfected persons at risk for infection. In addition, for maximal impact, resources should be directed to areas where transmission risk in a country is highest.

The effectiveness of national responses to the AIDS pandemic ultimately will determine how extensively HIV will spread. This effectiveness requires, in the first place, political and financial commitment to HIV prevention, which is still insufficient. Many countries need two to three times more financial resources than presently available to control the AIDS epidemic.

In 2016, investment in HIV programs in LMICs reached $19.1 billion, up from $16.8 billion in 2011, $10 billion in 2007, $8.3 billion in 2005, and $250 million in 1996, when UNAIDS was founded. Extraordinary efforts resulted in over 20.9 million people receiving ART as of June 2017.[2] International funding for the AIDS response in LMICs peaked at almost $10 billion (constant 2016 US dollars) in 2013, before declining to around $8.1 billion in 2016. Domestic spending became the majority of HIV expenditures in LMICs for the first time in 2011; whereas domestic spending in LMICs rose by an average of 11% each year from 2006 to 2016, the rate of that increase slowed to 5% in 2015 to 2016.

Access to antiretroviral drugs that prevent mother-to-child transmission of HIV has also greatly improved. Worldwide, 76% of pregnant women living with HIV had access to antiretroviral medicines to prevent transmission of HIV to their infants in 2016.[2] There is substantial interregional variation; in sub-Saharan Africa, antiretroviral coverage for pregnant women in 2016 was 77%, in Latin America it was 74%, but coverage was only 40% in the Middle East and North Africa.[184] However, the data demonstrate a substantial scale-up, given that antiretroviral coverage for pregnant women was only 45% in LMICs in 2008,[185] 33% of women in 2007, and 14% in 2005. Cuba, Thailand, Belarus, and Armenia have been certified as having eliminated vertical transmission through the expansion of access to services for prevention of mother-to-child transmission.[93,94] In Botswana, South Africa, Namibia, and Swaziland, the percentage of ART coverage for prevention of mother-to-child transmission is 95%; in the Central African Republic, it is 81%, in Ethiopia, 69%; and in Ghana, 56%. A few countries in sub-Saharan Africa still have to make progress, with less than 50% coverage (i.e., Nigeria, Republic of Congo, Guinea, and Somalia, among others).[21] The increased availability of point-of-care rapid tests for diagnosis of HIV infection has the potential to expand the already crucial role of voluntary counseling and testing in prevention and care. Health care centers with adequate resources can provide immediate prevention education or referral to treatment and care services, thus drastically reducing loss to follow-up among patients.

There is still a gap in expanding access to HIV testing services so that people can know their status; in 2016 an estimated 30% of people living with HIV infection did not know their HIV status, with particular gaps among young people and men.[10] Increasingly, couples testing and provider-initiated approaches in clinical settings are being promoted (i.e., health care providers routinely initiate an offer of HIV testing in a context in which the provision of, or referral to, effective prevention and treatment services is assured).[186] Self-testing (also known as home testing) holds significant promise. It may help reach people living with HIV who do not use facility-based testing and is a potential means of addressing testing disparities among groups tested less frequently, such as men and people from key populations.[187] Self-testing may be particularly acceptable to MSM, although common concerns about self-testing among MSM include the lack of counseling, possible user error, and accuracy.[188] Research carried out through the HIV Self-Testing Africa (STAR) project in Malawi suggests that self-testing is highly accepted by young people, if costs are kept low and their autonomy and confidentiality are respected.[189] A study in Zambia found that peer educators providing self-testing kits to female sex workers could improve testing uptake and rapid linkage to care.[190] WHO issued guidance for World AIDS Day 2016 to help encourage scale-up of self-testing rollout as an approach to be offered in addition to conventional testing.

Assisted partner notification, tracing sexual or drug-injecting partners of people diagnosed with HIV, may be another promising approach to improving testing uptake and rapidly linking HIV-positive individuals

to care. A recent meta-analysis of three randomized trials demonstrated that assisted partner notification services resulted in a 1.5-fold increase in adoption of HIV testing services among partners compared with passive referral, with few instances of violence or harm recorded.[191] This supports the rationale for scaling up the implementation of assisted partner notification services, together with HIV self-testing services, in line with 2016 WHO guidelines.[192]

Although there has been major progress in access to ART, there are several barriers to the provision of ART, resulting in a declining cascade of treatment access, adherence, and reduction in viral load. The percentage of people living with HIV who are virally suppressed was as low as 30% in the United States,[193] 17% in Lithuania, 7% in Venezuela, and 19% in Cameroon.[52] Most often reported reasons are related to inconsistent pattern of diagnosis, linkage to care, differences in levels of CD4 cell count for initiation, and retention in care.[194]

Considerably increased resources have been brought into countries for AIDS programs by major global health initiatives, particularly the Global Fund to Fight AIDS, Tuberculosis and Malaria and the President's Emergency Plan for AIDS Relief (PEPFAR). As a result, services to people with HIV have rapidly expanded. In many countries, infrastructure and laboratories have been strengthened; in some, primary health care services have been improved. The negative effect of AIDS on the health workforce has been lessened by the provision of ART to HIV-infected health care workers, by training, and, to an extent, by task shifting.[195] Of ongoing concern is the long-term sustainability of efforts in terms of the future burden of HIV and availability of the necessary funding.[196] In 2016, $19.1 billion of funding was available to support HIV efforts in LMICs, which will need to rise by one-third to $26.2 billion by 2020 to meet global targets to reduce the burden of HIV/AIDS.[197] The impact of reduced resources for AIDS is still unclear, but it should negatively affect some optimistic scenarios predicting the end of AIDS and an HIV-free generation. What is now needed is a long-term strategy to reduce the epidemic to low endemic levels—something that is realistic with the existing prevention and treatment tools.[198]

IMPACT OF THE AIDS EPIDEMIC

AIDS has been shown to affect not only individual patients and their relatives but also, in the worst affected countries, communities at large, with a long-term impact on households, the health sector, demography, and economic and social systems. With an estimated 19.4 million HIV infections and with an adult HIV prevalence rate of 7%, at least 10 times higher than in most other parts of the world, the magnitude of the impact in southern and eastern Africa on the population and economies is bound to be of a different scale.[158] In addition, within Africa, huge and widening differences in the spread of HIV are seen, which implies that the consequences of the epidemic differ substantially. The HIV prevalence rate among pregnant women in southern Africa is as much as five times higher than in western Africa and three times higher than in eastern Africa.[56]

Perhaps the most visible aspect of the burden of AIDS used to be the large number of men and women with AIDS in the hospitals of many African countries, where they may make up at least 50% of all adult patients in the absence of access to ART. However, as a result of the widespread access to ART, there are fewer and fewer patients with HIV in the hospitals of the continent, even if AIDS remains the second major cause of death in Africa.[22] Among people with HIV, tuberculosis is the most common and deadly opportunistic infection; it accounts for 40% of all adult deaths, although the cause of death in patients infected with HIV in the developing world is not well studied.[199] Recent data suggest that 57% of tuberculosis cases among people living with HIV were not diagnosed or treated, leading to 390,000 deaths from tuberculosis among people living with HIV in 2015.[200]

The impact of HIV infection on mortality, burden of disease, and the health system in major parts of Africa was enormous before the current wider access to ART. In a large study of a representative sample of patients who died during hospitalization in Abidjan, Côte d'Ivoire, tuberculosis, bacteremia with gram-negative rods, and cerebral toxoplasmosis caused 53% of the deaths.[201] Tuberculosis was found in half of the cadavers with an AIDS-defining pathologic process, compared with only 4% for *Pneumocystis jiroveci* pneumonia. In 2001, in the city of Pointe-Noire, Republic of the Congo, where by law all bodies should be registered at the morgue before they can be legally buried, a clinical examination of all bodies registered at the morgue was performed. Overall, 1309 adult deaths were investigated and the bodies tested for HIV. Forty-five percent of the deaths were from AIDS. The AIDS mortality rate among adults was 6.3 per 1000 for women and 4.9 per 1000 for men. In the most productive age group, 25 to 44 years, mortality was tripled by AIDS.[202]

In rural South Africa, since 1992, deaths have been rigorously monitored with a validated verbal autopsy instrument to establish probable cause. The dynamics of the mortality transition were investigated with comparison of the periods 2002 to 2005 and 1992 to 1994. Since 1994, all-cause mortality increased substantially because of a sixfold rise in deaths from infectious disease that affected most age and gender groups and a modest increase in deaths from noncommunicable diseases. The change in female risk for death from HIV infection and tuberculosis was almost double the change in male risk. The burden of disorders requiring chronic care increased disproportionately compared with those requiring acute care. The implications for primary health care systems are substantial, with integrated chronic care based on scaled-up delivery of ART needed to address this expanding burden.[203]

In countries of southern and eastern Africa, the direct effects of AIDS on the health sector have been dramatic, with greatly increased service needs associated with caring for people with HIV. Before the availability of ART, in Zambia, Malawi, and South Africa, for example, the availability of health workers was affected by morbidity and mortality, absenteeism due to personal or family illness, and attrition due to employment changes or family illness.[204] A study in four South African provinces found that an estimated 16% of health workers employed in the public and private health facilities had HIV in 2002.[205] In addition, in some African countries, 35% to 45% of physician posts and up to 15% of nurse posts are vacant.[206]

A growing number of persons with HIV infection and the 19.5 million people on chronic ART, 95% of whom live in LMICs,[207] put additional pressure on health services that are already under great strain. Absorbing this growing burden is a major challenge for health care systems in the worst affected countries.[208] A report in Tanzania concluded that the services of half the current health workforce are necessary to deliver ART to everyone in need.[209] The impact of AIDS on health services also applies to countries with much lower HIV prevalence rates. For example, in Vietnam in 2007, AIDS absorbed nearly 5% of all public health spending. Vietnam, with an adult HIV prevalence rate of 0.4%, has 250,000 people infected with HIV; a similar number of people are living with HIV in Swaziland, where adult prevalence is more than 27% but the population is about 1.3 million.[52]

The long-term demographic impact of AIDS is becoming clearer. The estimates from the GBD Study of 2017 confirmed that HIV/AIDS remains one of the leading causes of disease burden and death, although it is now the second highest cause of death in sub-Saharan Africa thanks to ART, and no longer the highest.[22,210] Since 2001, annual incidence of HIV infection has fallen in 38 countries, most of them in sub-Saharan Africa.[210] Research by Walker and Ghys[211] estimated that 10% of child mortality in sub-Saharan Africa as a whole was the result of HIV infection, but this proportion has decreased substantially as a result of successful prevention programs for mother-to-child transmission.[212]

In addition, the number of orphans—children and adolescents (0–17 years) with parents who died of AIDS—reached about 13.4 million worldwide as of 2015, down from a peak of 15 million in 2009.[8] In countries with a generalized epidemic, a significant proportion of orphaned children are orphaned because of AIDS; for example, 74% of orphaned children in Zimbabwe are orphaned owing to AIDS, and 63% in South Africa.[213] Orphans are less likely than other children to have basic material needs met and are more likely to be underweight.[56,214]

GLOBAL RESPONSES TO AIDS

Nearly 4 decades of response to AIDS are finally beginning to deliver results. In 2017, on a global basis, fewer people were infected with HIV, primarily because of scaling up of HIV prevention efforts, and fewer died of AIDS, partly because of increased access to treatment. Globally,

there were almost 1 million fewer deaths in 2016 than at the peak of AIDS-related deaths in 2005 (a fall of 48%, from 1.9 million AIDS-related deaths in 2005 to 1 million in 2016).[2] The decline in AIDS-related deaths has been particularly significant in eastern and southern Africa, with a 62% decrease from 1.1 million deaths in 2004 to 420,000 in 2016, attributable to the rollout of ART in the region.[10] This progress has been achieved through political leadership at the levels of both government and civil society, programs on the ground, and a spectacular increase in financing for the national response.

In June 2001, the United Nations General Assembly held a Special Session on HIV/AIDS at which the Declaration of Commitment on HIV/AIDS was adopted; this declaration has since served as a blueprint for action against the worsening pandemic, and has been updated as part of the Sustainable Development Goals, with a commitment to end AIDS as a public health problem by 2030 (reference on SDG Declaration).[214a] AIDS is inextricably linked to the other Sustainable Development Goals; education, gender equality, and poverty eradication are all vital in fighting it.[106]

After the General Assembly Special Session, the Global Fund to Fight AIDS, Tuberculosis and Malaria was established as a public-private financing mechanism among governments, civil society, and the private sector to raise and dispense resources worldwide to combat these three diseases. By May 2017 a total of $33.8 billion had been disbursed, over 50% of which was spent on HIV/AIDS activities.[215] In 2003, President George W. Bush launched PEPFAR, which is the largest funder of AIDS efforts in the developing world and the major financier of the Global Fund. From the financial years of 2004 to 2017, PEPFAR disbursed $72.7 billion to combat HIV/AIDS and tuberculosis.[216] Combined, these mechanisms have saved millions of lives, and are undoubtedly major achievements in international development (Fig. 118.5). Globally, $19.1 billion was available from all sources for the HIV/AIDS response in LMICs in 2016, although the estimated annual need by 2020 is $26.2 billion. International donor funding decreased in 2016 to $8.1 billion (from a peak of nearly $10 billion in 2013). Furthermore, although domestic spending in LMICs rose by an average of 11% each year from 2006 to 2016, the rate of that increase slowed to 5% between 2015 and 2016.[10]

Despite the progress accomplished in access in recent years, and although the number of new HIV infections has fallen in many countries and regions, the AIDS epidemic is not over in any part of the world. Current efforts to slow the AIDS pandemic are still inadequate (Fig. 118.6).

More progress has been accomplished in recent years on HIV treatment than on prevention. Globally, 76% of pregnant women living with HIV had access to antiretroviral therapies to prevent mother-to-child transmission in 2016, from 9% in 2004.[2] Antiretroviral drugs have succeeded in delaying the onset of illness, reducing mortality from AIDS, improving the quality of life for people infected with HIV, and probably limiting the spread of HIV. In rural Malawi, for example, deaths from AIDS were averted by the rapid scale-up of free ART, which led to a decline in adult mortality.[217] After years of debate, the dramatic drops in the prices of antiretroviral drugs because of generic manufacturing and differential pricing, combined with specific financing mechanisms, have made ART a reality in low-income nations (Fig. 118.7), but in 2017 approximately 15.8 million people who need ART still do not have access to it.

The nearly 2 million new HIV infections that continue to occur each year worldwide stresses the need for scaling up of prevention programs while broadening access to antiretroviral treatment.[123]

The future of the response to HIV requires continuous advocacy to remind policy makers that the AIDS pandemic is one of the defining issues of our time and that AIDS remains one of the most severe epidemics in modern history.[218,219] Rising rates of infections in certain regions such as Eastern Europe and Central Asia, and continuing high rates especially among young people and key populations, should serve as a wake-up call from complacency. There is an emerging gap between



FIG. 118.5 UNAIDS estimates for resources available for human immunodeficiency virus in low- and middle-income countries, 2000 to 2015. *(Data from Kates J, Wexler A, Lief E. Financing the Response to HIV in Low- and Middle-Income Countries: International Assistance from Donor Governments in 2015; July 15, 2016. https://www.kff.org/global-health-policy/report/financing-the-response-to-hiv-in-low-and-middle-income-countries-international-assistance-from-donor-governments-in-2015/. Accessed June 2016.)*

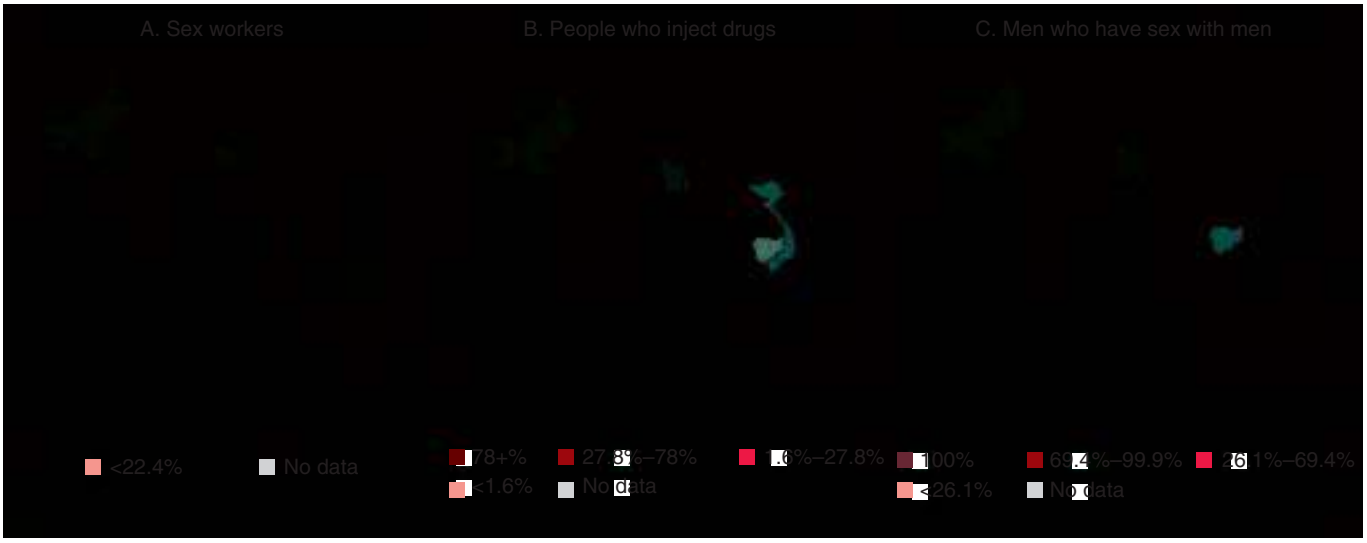

FIG. 118.6 Percentage of most at-risk populations in selected Asian countries reached by human immunodeficiency virus prevention programs, 2016. (A) Sex workers. (B) People who inject drugs. (C) Men who have sex with men. *(Data from UNAIDS. http://aidsinfo.unaids.org. Accessed February 6, 2018. Most recent data as of 2016.)*

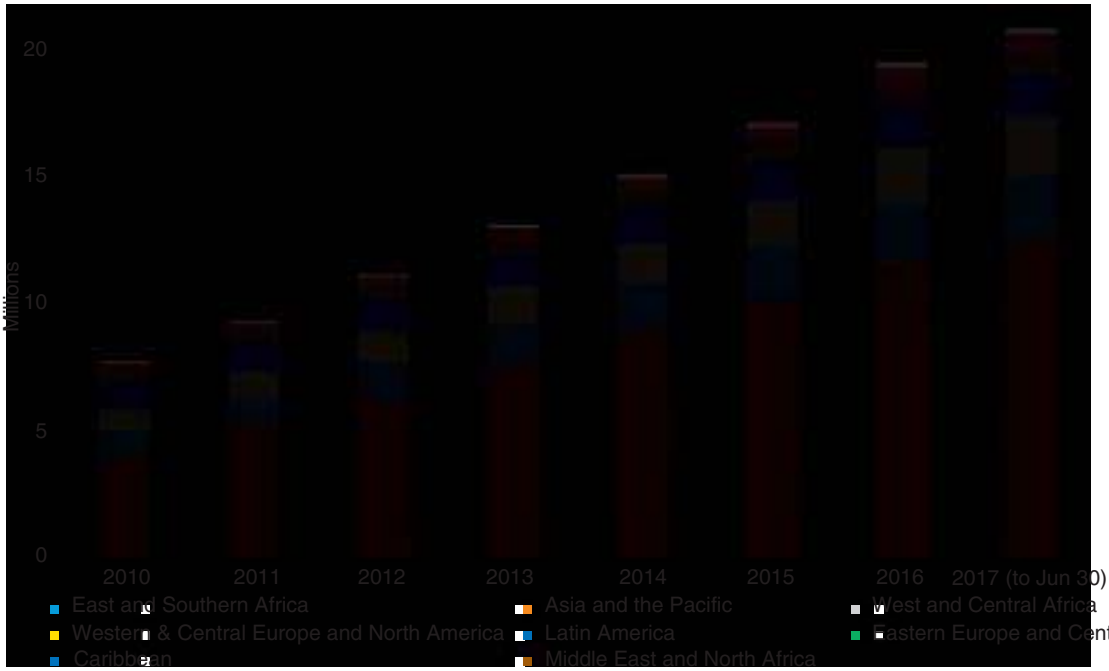

FIG. 118.7 Trends in the number of people identified as living with human immunodeficiency virus who are receiving antiretroviral therapy by region, 2010 through June 30, 2017. *(Data from UNAIDS. http://aidsinfo.unaids.org. Accessed February 26, 2018.)*

available resources and needs, and arguably the momentum to integrate human rights and social justice into the HIV/AIDS response is stalling in the context of a "shrinking civil society space."[220] Stigma, criminalization, and discrimination remain key nonmedical barriers that continue to drive new infections and hinder prompt testing and linkage to treatment.[221] Long-term strong leadership is needed to keep HIV prevention and treatment at the forefront of social policy and action, with scientific evidence and human rights as the basis for the response. This requires a broad-based coalition of people from civil society, business, faith-based organizations, and community groups and activists, and a stronger connection with other global health issues. Research and development for new prevention tools, including PrEP, and vaccines that can be made available and affordable across the world, need to be strengthened.

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119 Epidemiology and Prevention of AIDS and HIV Infection, Including Preexposure Prophylaxis and HIV Vaccine Development

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Acquired immunodeficiency syndrome (AIDS) is the most severe manifestation of a clinical spectrum of illness caused by infection with human immunodeficiency virus (HIV). The syndrome is defined by the development of serious opportunistic infections, neoplasms, or other life-threatening manifestations resulting from progressive HIV-induced immunosuppression. AIDS was first recognized in mid-1981, when unusual clusters of *Pneumocystis jirovecii* pneumonia and Kaposi sarcoma were reported in young, previously healthy homosexual men in New York City, Los Angeles, and San Francisco.[1,2] The subsequent documentation of cases among persons with hemophilia, blood transfusion recipients, and persons who inject drugs (PWIDs) and their sex partners suggested that a transmissible agent was the primary cause of the immunologic defects characteristic of AIDS. In 1983, 2 years after the first reports of AIDS, a cytopathic retrovirus was isolated from persons with AIDS and associated conditions such as chronic lymphadenopathy.[3,4] By 1985 serologic tests to detect evidence of infection with HIV had been developed and licensed.

Blood obtained in 1959 from an adult Bantu man living in what is now the Democratic Republic of Congo represents the oldest known case of HIV-1 infection in the world.[5] HIV sequences from that sample and others from the same region in Africa suggest that humans acquired a common ancestor of the HIV-1 M group by cross-species transmission under natural circumstances in approximately 1933 (1919–45).[6] HIV infection has become pandemic, affecting every region of the world, and is a major cause of morbidity and mortality, particularly among young adults. HIV is spread primarily through heterosexual contact, with women now accounting for more than half of new HIV infections in adults.[7] Transmission through transfusion of blood and blood products has been virtually eliminated in countries that have systematically instituted HIV antibody screening of donated blood and plasma and heat treatment of clotting factors. In developed countries sharp declines in the incidence and mortality of AIDS have been noted after the use of active antiretroviral therapy (ART) became widespread in 1996.[8] As a result, the number of persons living with HIV infection continues to rise, and evidence suggests that new infections in the United States remain stable, except among men who have sex with men (MSM) and, in particular, black young MSM, in whom rates have increased.[9] In the United States the HIV epidemic increasingly affects women, minorities, persons living in the Southeast, and the poor.[10,11] The control and prevention of HIV infection, whether on a global or an individual scale, must be grounded in an understanding of the changing epidemiology of HIV.

HIV AND AIDS SURVEILLANCE IN THE UNITED STATES

The Centers for Disease Control and Prevention (CDC) collects, analyzes, and disseminates surveillance data on HIV infection and AIDS. All 50 states, the District of Columbia, and all US territories require the reporting of AIDS cases to local health authorities by name, and they in turn use a uniform surveillance case definition and case report form to report cases to the CDC.[12]

The initial AIDS surveillance case definition, which was established soon after the first reports of unexplained illnesses associated with cellular immunodeficiency in homosexual or bisexual men, formally listed the opportunistic infections and neoplasms indicative of underlying immunosuppression.[13] In the absence of previously described causes of immunosuppression, a diagnosis of one of these conditions was defined as AIDS. The definition did not include the less severe manifestations of HIV infection and was designed to be highly specific and provide a standard means to monitor trends of severe immunodeficiency caused by what was then an unknown agent.

The AIDS surveillance case definition was modified in 1985, 1987, 1993, 2008, and again in 2014.[14–17] Each revision to the AIDS surveillance case in the United States has been done in response to diagnostic and therapeutic advances and to improve standardization and compatibility of surveillance data regarding persons at all stages of HIV disease.

In 1993 the AIDS surveillance definition was expanded to include evidence of severe immunosuppression (<200 $CD4^+$ T lymphocytes/μL or a $CD4^+$ T-lymphocyte percentage of total lymphocytes of <14) and a series of clinical conditions that, in the presence of HIV infection, were considered AIDS defining (Table 119.1).

Evaluation studies have shown that AIDS surveillance has provided complete and timely information on diagnosed cases of AIDS in the United States. A national multicenter study published in 1992 used computerized medical records in six areas to determine that 92% of persons with AIDS-defining conditions were reported to local health departments.[18] Of these previously reported cases, 67% were reported to local health departments within 2 months of the date of diagnosis. Studies of state and local death certificate information and national vital statistics found that the completeness of reporting persons with AIDS ranged from 80% to 96% and that 70% to 90% of HIV-related deaths were reported to AIDS surveillance groups.[19–21] Therefore the reporting of AIDS in the United States has been among the most complete of all reportable diseases and conditions.[22,23] However, advances in HIV treatment have slowed the progression of HIV disease for infected persons and contributed to a decline in AIDS incidence; as a result, many persons infected with HIV may never develop AIDS. Therefore data from the AIDS reporting system, although still important, have become less informative about current trends in HIV transmission. This led public health authorities, in 1999, to recommend that all states and territories include surveillance for HIV infection and not just for AIDS.[24] In addition, a revised surveillance case definition for HIV infection that incorporated the reporting criteria for HIV infection and AIDS into a single case definition was recommended by the Council of State and Territorial Epidemiologists at its 1998 annual meeting and published by the CDC in 1999.[25]

TABLE 119.1 AIDS-Defining Conditions

Bacterial infections, multiple or recurrent[a]
Candidiasis of bronchi, trachea, or lungs
Candidiasis, esophageal
Cervical cancer, invasive[b]
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1-month duration)
Cytomegalovirus disease (other than liver, spleen, or nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV related
Herpes simplex, chronic ulcer(s) (>1-month duration); bronchitis, pneumonitis, or esophagitis
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1-month duration)
Kaposi sarcoma
Lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia[a]
Lymphoma, Burkitt (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
Mycobacterium avium-intracellulare complex or *Mycobacterium kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis, any site (pulmonary[b] or extrapulmonary)
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jirovecii pneumonia
Pneumonia, recurrent[b]
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome of HIV infection

[a]Children younger than 13 years.
[b]Added in the 1993 expansion of the AIDS surveillance case definition for adolescents and adults.

TABLE 119.2 Surveillance Case Definition for HIV Infection in Persons Age ≥6 Years: United States, 2014

STAGE	LABORATORY EVIDENCE[a]	CLINICAL EVIDENCE
Stage 0	Negative or indeterminate HIV test within 180 days of the first confirmed positive test *or* Detection of HIV antigen or nucleic acids before an antibody test is positive	None required (acute retroviral syndrome is possible)
Stage 1	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count ≥500 cells/μL *or* $CD4^+$ T-lymphocyte percentage ≥26	None required (but no AIDS-defining condition)
Stage 2	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of 200–499 cells/μL *or* $CD4^+$ T-lymphocyte percentage of 14–25	None required (but no AIDS-defining condition)
Stage 3 (AIDS)	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of <200 cells/μL *or* $CD4^+$ T-lymphocyte percentage of <14[b]	*or* documentation of an AIDS-defining condition (with laboratory confirmation of HIV infection)[b]
Stage unknown[c]	Laboratory confirmation of HIV infection *and* no information on $CD4^+$ T-lymphocyte count or percentage	*and* no information on presence of AIDS-defining conditions

[a]The $CD4^+$ T-lymphocyte percentage is the percentage of total lymphocytes. If the $CD4^+$ T-lymphocyte count and percentage do not correspond to the same HIV infection stage, select the more severe stage.
[b]Documentation of an AIDS-defining condition supersedes a $CD4^+$ T-lymphocyte count of ≥200 cells/μL and a $CD4^+$ T-lymphocyte percentage of total lymphocytes of ≥14. Definitive diagnostic methods for these conditions are available in Appendix C of the 1993 revised HIV classification system and the expanded AIDS case definition. (Centers for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep*.1992;41[RR-17]:1–19)
[c]Although cases with no information on the $CD4^+$ T-lymphocyte count or percentage or on the presence of AIDS-defining conditions can be classified as stage unknown, every effort should be made to report $CD4^+$ T-lymphocyte counts or percentages and the presence of AIDS-defining conditions at the time of diagnosis. Additional $CD4^+$ T-lymphocyte counts or percentages and any identified AIDS-defining conditions can be reported as recommended. (Council of State and Territorial Epidemiologists. Laboratory Reporting of Clinical Test Results Indicative of HIV Infection: New Standards for a New Era of Surveillance and Prevention [Position Statement 04-ID-07]. 2004. http://www.cste.org/ps/2004pdf/04-ID-07-final.pdf.)
From Schneider E, Whitmore S, Glynn KM, et al; Centers for Disease Control and Prevention. Revised surveillance case definitions for HIV infection among adults, adolescents, and children age <18 months and for HIV infection and AIDS among children aged 18 months to <13 years–United States, 2008. MMWR Recomm Rep. *2008;57(RR-10):1–12.*

In the 2008 revision of the surveillance case definition for adults and adolescents, the CDC required laboratory-confirmed evidence of HIV infection to meet the surveillance case definition. Diagnostic confirmation of an AIDS-defining condition alone without laboratory-confirmed evidence of HIV infection is no longer sufficient to classify an adult or adolescent as HIV infected for surveillance purposes. The incorporation of laboratory confirmation of HIV infection into the surveillance case definition was also consistent with the 2007 recommendations of the World Health Organization (WHO).[26]

The 2014 revision consolidated the case definition to apply to persons of all ages, revised the laboratory and diagnostic criteria to eliminate the confirmatory Western immunoblot from the testing algorithm, and accounted for the detection and discrimination of HIV-1 and HIV-2 infections (also see Chapter 120).[27] This revised algorithm reduces the rate of false-positive tests and the number of tests needed to diagnose HIV infection. For laboratory confirmation of HIV infection, the revised surveillance case definition accepts a positive combined HIV-1/2 antigen/antibody test, followed by positive nucleic acid testing. With the laboratory confirmation of HIV infection and the $CD4^+$ T-lymphocyte count, cases are classified into one of five HIV infection stages: 0, 1, 2, 3, or unknown (Table 119.2). Stage 0 is established by either a negative or indeterminate result that is followed by a positive result on subsequent testing within 180 days or the detection of HIV p24 antigen or nucleic acids before an antibody test is positive. If the criteria for stage 0 are met, the stage is defined as 0 regardless of $CD4^+$ T-lymphocyte cell counts, percentages, or the presence of opportunistic illness diagnoses. In stage 1 the person has no AIDS-defining conditions and either a $CD4^+$ T-lymphocyte cell count of ≥500 cells/μL or a percentage of ≥26. In stage 2 there are no AIDS-defining conditions, and the $CD4^+$ T-lymphocyte cell count is between 200 and 499 cells/μL or a percentage between 14 and 25. Stage 3 corresponds to AIDS; the patient has a $CD4^+$ T-lymphocyte cell count of less than 200 cells/μL (or a percentage <14) or documentation of an AIDS-defining condition. Finally, when the $CD4^+$ T-lymphocyte cell count is not available or there is no information of an AIDS-defining condition, the patient is classified as HIV infected, stage unknown. One must stress that the revised case definition is intended for public health surveillance and not for clinical diagnosis.

HIV INFECTION AND AIDS IN ADULTS

Incidence and Prevalence of AIDS and HIV in the United States (Also See Chapter 118 for Global Data)

The CDC estimates that 1,122,990 persons age ≥13 years were living with diagnosed or undiagnosed HIV infection in the United States at the end of 2015, for an overall HIV prevalence of 418.7 per 100,000 population.[28] As more people came to know their HIV status, the percentage of persons living with undiagnosed HIV decreased from 16.9% in 2010 to 14.5% in 2015 and thus, at year-end 2016, 991,447 persons were living in the United States with diagnosed HIV infection for a prevalence of 306.6 per 100,000 population.[29] The majority of those

living with HIV were nonwhite (65%), and 67% were MSM. The HIV prevalence rates for blacks (1435.4/100,000) and Hispanics (587.6/100,000) were, respectively, 7.3 and 2.9 times the rate for whites (196.7/100,000).[29]

In 2008 the CDC published the first national HIV incidence estimates, using novel laboratory assays that differentiate recent versus long-standing HIV infection.[30] This analysis showed that an estimated 56,300 new HIV infections (95% confidence interval [CI], 48,200 to 64,500) occurred in 2006, for an overall incidence rate of 22.8 cases per 100,000 population.[11] The revised number of new infections was substantially higher than the previous estimate of 40,000 annual new infections in the United States.[31] Since then, HIV incidence has declined from 41,800 in 2010 to 38,500 in 2015, with the rate decreasing from 16.3 in 2010 to 14.4 in 2014.[28] Seventy-three percent of new infections occurred among men, 42.1% among blacks, and 65.1% among MSM. The highest rates are for blacks/African Americans at 49.5 per 100,000 population. From 2010–15 there was an increase in new infections among persons age 25 to 34 years with no significant change in persons ≥55 years and a decrease in all other age groups. HIV diagnoses are not evenly distributed across states and regions. Southern states now account for more than half (52%) of all HIV diagnosis. The rate of HIV diagnosis among adults and adolescents in 2017 in the United States and six dependent areas was 11.8 per 100,000 population and varied from a high of 46.3 in the District of Columbia to 0.0 in American Samoa and the Republic of Palau (Fig. 119.1).

Serologic Monitoring of the HIV Epidemic

In 2003 the CDC implemented the National HIV Behavioral Surveillance System (NHBS) to estimate and monitor HIV behaviors, prevalence, and trends among persons at risk of HIV infection. This survey is conducted in rotating annual cycles, with each one of three risk groups (MSM, PWIDs, and high-risk heterosexuals) surveyed each year. In general, the patterns of HIV transmission observed in these studies in the United States are similar to those observed through AIDS case surveillance—higher rates of HIV infection are found among men than women, among blacks and Hispanics than whites, and among persons 20 to 45 years of age than those in other age groups. In addition, the National Health and Nutrition Examination Survey (NHANES), a program started in the 1960s to assess the health and nutritional status of noninstitutionalized adults and children in the United States, has been used to estimate HIV prevalence. NHANES data suggests that the prevalence of HIV infection among adults age 18 to 59 years residing in households in the United States was 0.39% for the period 2007–12.[32]

Homosexual and bisexual men remain a major population with an increased prevalence of HIV infection. In the 2014 NHBS survey among MSM, the overall HIV prevalence was 22%, but it was 36% among black MSM, 17% among Hispanic MSM, and 15% among white MSM.[9]

In contrast to the epidemic among homosexual and bisexual men, the epidemic among PWIDs has been more concentrated geographically within the United States. Among PWIDs the initial HIV seroprevalence studies demonstrated very high rates of HIV infection in the Northeast and along the Atlantic Coast and low rates on the West Coast and in cities in other areas.[33,34] Surveys conducted in drug treatment centers in 1997 showed a median HIV seroprevalence rate of 14.8% (range, 0%–37.7%) among PWIDs entering drug treatment programs.[35] Similar findings have been noted in subsequent studies; HIV seroprevalence among PWIDs admitted to drug treatment centers in Baltimore (25%) and Newark (24%) was high, whereas it was low in San Francisco (7%), Seattle (5%), Denver (2%), and Detroit (1%).[36,37] However, among most large metropolitan areas, the HIV prevalence rates among PWIDs

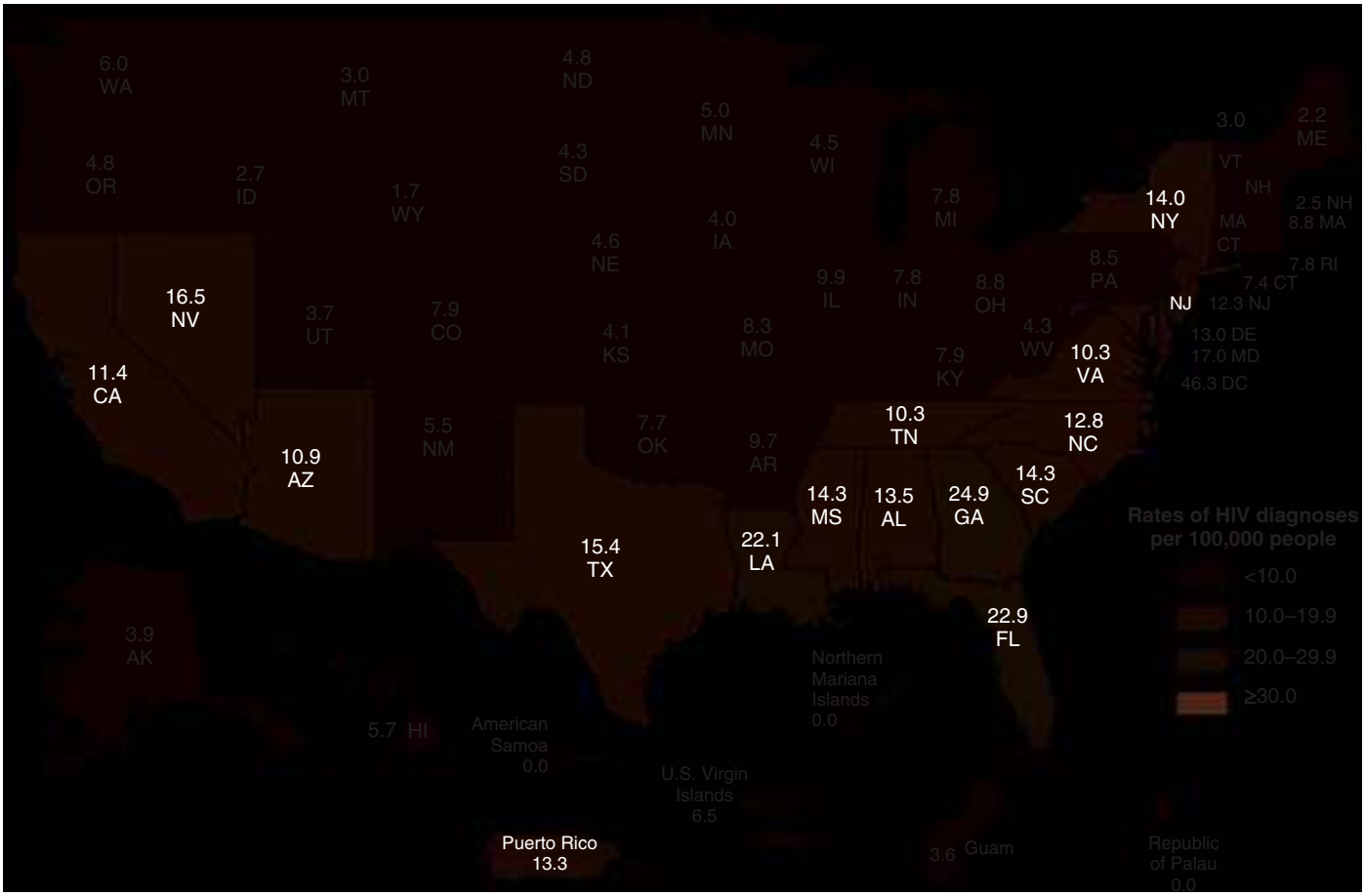

FIG. 119.1 Rates of HIV diagnoses in the United States, 2017. *(From the Centers for Disease Control and Prevention.* HIV in the United States by Region. https://www.cdc.gov/hiv/statistics/overview/geographicdistribution.html. *Accessed January 23, 2019.)*

continues to be less than 10%, and approximately 40% have a prevalence of less than 5%.[38,39] The number of new HIV infections attributed to injection drug use (IDU) in the United States had decreased in recent years, but as a result of the opioid epidemic, outbreaks of HIV infection among PWIDs are now occurring in the United States, with the CDC recently identifying 220 counties in 26 states as potentially vulnerable to HIV outbreaks among PWIDs.[40]

HIV infection acquired through heterosexual contact is the primary route of infection among women, particularly those who are members of minority groups.[41,42] Despite this, the number of reported cases of HIV/AIDS attributable to high-risk heterosexual contact has declined for both men and women. Despite this decline, more than 7000 women were diagnosed with HIV in 2016, and women represent 19% of new HIV diagnoses in the United States.[29]

The data from a cohort study conducted among drug users in Baltimore, a high-incidence and high-prevalence city, suggest that the incidence of HIV has doubled among drug-using men who also engage in homosexual sex and among women who have had a recent sexually transmitted infection, suggesting that sexual behavior is a major determinant of risk even among drug users.[43]

Surveys conducted in clinical settings indicate that the HIV infection rate is higher than it is in a more representative sample of the general population.[44–47] For this reason, since 2006 the CDC has recommended opt-out HIV screening as part of routine clinical care in all health care settings.[48] In many areas tuberculosis (TB) patients have high rates of HIV infection, and HIV screening should be provided to all those diagnosed with TB.[47] Among patients with syphilis the overall HIV seroprevalence was 15.7%; among men the seroprevalence was 27.5% and among women 12.4%.[49] However, among MSM and those with syphilis the seroprevalence ranged from 64.3% to 90%.

HIV prevalence rates among adolescents attending adolescent medicine clinics in three metropolitan areas (Baltimore, Houston, and New York City) that collected data each year from 1993–97 showed an overall HIV prevalence rate of 0.4% (range, 0.2%–0.5%).[50] The rates were the same for male and female patients (0.4%) and were approximately the same among patients 13 to 19 years of age (0.4%) and those 20 to 24 years of age (0.5%). However, the rates were higher among black patients (0.6%) than among Hispanic and white patients (0.1%).

Studies of HIV seroprevalence in entrants to correctional facilities have indicated a wide range of rates, with the highest in areas with a moderate-to-high incidence of AIDS.[51] From 1991–92 the median HIV seroprevalence was 2.9% (range, 0%–15%) in 35 correctional facilities in 17 metropolitan areas.[52] The rates ranged from 1% to 12.5% for men and 0% to 24% for women, which is a reflection of the high rates of drug use in these persons. Among entrants to the New York State prison system between 1987 and 1997, 12% of men and 18% of women were infected with HIV.[53] Between 1992 and 1998 data from nearly 500,000 HIV tests performed in correctional facilities in the United States demonstrated an overall HIV seroprevalence of 3.4%, 56% of which were among persons newly identified as HIV infected.[54] More recent data suggests that nationally the HIV prevalence among incarcerated persons has decreased, and it is now 1.5%. However, in state prisons in Florida, Maryland, and New York the prevalence exceeds 3%.[55]

As previously stated, although surveillance for HIV infection has traditionally focused on the incidence of AIDS and the prevalence of HIV, new diagnostic technologies that allow the estimation of incident HIV infection have become available, including the use of the sensitive–less sensitive enzyme immunoassay (EIA) test (or the serologic testing algorithm for recent HIV seroconversion [STAHRS]) and the measurement of HIV RNA by polymerase chain reaction (PCR) in pooled blood specimens that test negative for HIV (see Chapter 120).[30,56] Having an HIV surveillance system that focuses on incident rather than prevalent infections undoubtedly allows better monitoring of the HIV epidemic.[57] AIDSVu (http://aidsvu.org) is an interactive online mapping tool that allows users to visualize and explore HIV surveillance data at the state, county, and ZIP code levels.

Exposure Categories

Since the first cases of AIDS were reported in 1981 in the United States, cases in MSM have consistently represented the largest number of AIDS cases reported. The cases of AIDS among recipients of blood or blood components and among persons with hemophilia increased dramatically during the mid-1980s, but since 1987 the numbers have declined; after the implementation of HIV antibody testing in March 1985, no more than 5 cases of transfusion-associated HIV infection per year were reported to the CDC during the subsequent years, compared with 714 cases in 1984.[58] Since the initiation of HIV antibody screening of donated blood and plasma, heat treatment of clotting factors, P24 antigen testing, and, since 1999, nucleic acid amplification testing (NAAT) of donor samples, the window period for potential HIV transmission by blood transfusion has decreased to 12 days, and the estimated risk for HIV transmission by blood products is now 1 in 1.4 to 1 in 1.8 million units.[59]

Heterosexual contact cases consist of persons who report exposure to a person with or at increased risk for HIV infection (e.g., a person who uses injects drugs [PWID]) or those born in countries where heterosexual transmission is the major route of HIV infection (e.g., areas of sub-Saharan Africa and some Caribbean countries). In 1992, for the first time the number of cases diagnosed among women infected through heterosexual contact exceeded the number infected through IDU and represented 68% of cases among women reported in 2002.[28] Blacks and Hispanics account for greater than 75% of all persons reported with AIDS attributed to heterosexual contact.[60] For AIDS cases reported in 2006, 17% of men and 73% of women reported having acquired HIV through high-risk heterosexual contact. In the past persons with sex partners of unknown HIV infection or risk status were classified in the undetermined category, but since September 2000 the procedures for investigating cases reported without risk have changed from ascertaining risk for all reported cases to estimating risk distributions from statistical models and population-based samples. Selected follow-up and investigation of heterosexual contact cases have identified other sources of exposure to HIV infection for some persons, especially men.[61,62] Nonetheless, it is unlikely that such misclassification bias has significantly influenced national trends.[63]

Of the 30,870 cases of HIV infection among adults and adolescent males reported in 2017, 4% occurred in MSM who are also PWIDs. Except for this group, persons with more than one reported mode of exposure to HIV are counted only once in a hierarchy of exposure categories.[29]

The cases in persons with no reported exposure to HIV through any of the recognized routes are classified as "no risk reported or identified." This category includes cases that are being followed up by local health department officials; cases in persons whose exposure history is incomplete because they died, declined to be interviewed, or were lost to follow-up; and cases in persons who were interviewed or for whom other follow-up information was available and no mode of exposure was identified. Previous studies indicate that when follow-up information is obtained, an established exposure mode can be identified for greater than 90% of these persons, and they are subsequently reclassified into the appropriate exposure category.[64] Although the surveillance and investigation of cases with an undetermined risk can assist in detecting unusual modes of transmission (e.g., transplantation), such instances remain rare.[65]

AIDS Trends

The 1993 change in the AIDS case definition[16] dramatically altered trends in the incidence of AIDS because of a large increase in reported AIDS cases. In 1993, the first year that the new case definition was used, 103,500 AIDS cases were reported to the CDC among persons 13 years or older, compared with 49,016 cases in 1992. In 1994, 1995, and 1996 the number of AIDS cases reported annually decreased, in contrast to the artificial peak in the number of cases reported in 1993. Since the implementation of the revised case definition, more than half of all cases have been reported based on a $CD4^+$ T-lymphocyte count of less than 200 cells/μL. However, in 1995 and 1996 the occurrence of AIDS-defining opportunistic illnesses and death among persons with AIDS decreased 7% and 25%, respectively, for the first time in the epidemic.[16,66] Declines in the incidence of AIDS continued from 1998–99, but these declines began to level off, and there was essentially no change from 1999–2001. From 2002–06 the estimated number of newly diagnosed

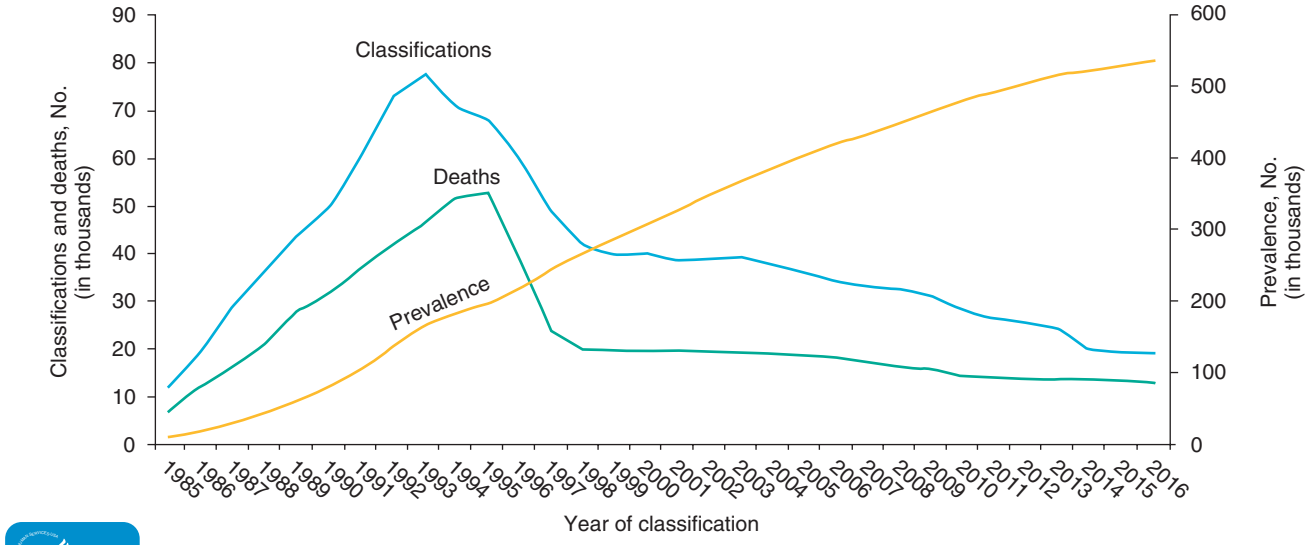

Note. Deaths of persons with HIV infection, stage 3 (AIDS) may be due to any cause.

FIG. 119.2 Stage 3 (AIDS) classifications, deaths, and persons living with diagnosed HIV infection ever classified as stage 3 (AIDS) 1985–2016: United States and six dependent areas. *(From the Centers for Disease Control and Prevention; National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention Division of HIV/AIDS Prevention.* Trends in HIV Infection Stage 3 (AIDS). https://npin.cdc.gov/publication/trends-hiv-infection-stage-3-aids. *Accessed January 23, 2019.)*

AIDS cases remained stable, with 37,852 cases reported in 2006, for a rate of 12.3 per 100,000 population in the United States. From 2007–10 the estimated number of annual AIDS diagnoses continued to remain stable, but the rate continued to decline, for an estimated rate of AIDS diagnoses in 2010 of 10.8 per 100,000 population.[67] After the introduction of highly active ART, the number of deaths and new AIDS cases began to decline. Between 1995 and 1998 AIDS deaths declined by 63% (from 51,760–18,823) and continued to decline from 16,947 in 2002 to 14,061 in 2006, a 17% decrease; but since then, rates have stabilized, with approximately 15,000 deaths per year. In 2016 there were 15,428 deaths, for a rate of 4.8 per 100,000[29] (Fig. 119.2). Surveillance now monitors HIV infection and classifies as stage 3 what used to be called AIDS. In 2017 the rate of HIV infections classified as stage 3 (AIDS) was 5.4 per 100,000.[29]

Demographic Characteristics of HIV Infection

Men accounted for 81% of HIV infections diagnosed in 2017, and HIV rates were much higher for men (23.1/100,000) than women (5.2/100,000).[29] From 2012–17 the HIV infection rate was highest for persons age 25 to 29 years (32.9), followed by persons age 20 to 24 years (28.7). The only age group for which the rate of HIV infection increased during that timeframe is that between the ages of 25 and 29 years. Since 1991 the percentage of AIDS cases reported among persons 50 years or older has also steadily increased, and in 2017 they represented 17% of all HIV diagnosis.[29] The CDC estimated that 6.4% of incident HIV infections occurred among persons 55 years or older between 2010 and 2016.[28]

In 2017 26.2% of the persons diagnosed with HIV infection were white, 43.5% were black non-Hispanic (39% of reported cases among men and 60% among women), and 24.7% were Hispanic (Table 119.3). In 2017 the rate of HIV diagnoses was 41.10 per 100,000 for blacks and 16.1 per 100,000 for Hispanics, compared with 5.1 per 100,000 for whites.[29] Rates among American Indian/Native Alaskans have slightly increased since 2012; however, only 212 cases were reported in 2017 in these populations.[29]

Hispanics with AIDS represent different countries of origin and cultures.[68] The rate of AIDS is the highest for Hispanics in the Northeast and Puerto Rico. IDU is the predominant exposure mode in Puerto Rican–born men and women with AIDS who are residing in either Puerto Rico or the US mainland. The AIDS rate among Hispanics is the lowest in the West. Hispanics with AIDS in this area are primarily from Mexico or Central and South America and are less likely to report a history of IDU.

TABLE 119.3 Characteristics of Persons Reported With Acquired Immunodeficiency Syndrome and Rates by Year of Report: United States, 1997 and 2010

	1997		2010	
	NO. (*n* = 60,634)	RATE[a]	NO. (*n* = 33,015)	RATE[a]
Sex[b]				
Male	47,312 (78%)	44.0	24,749 (71%)	20.0
Female	13,322 (22%)	11.5	8242 (29%)	6.4
Race/Ethnicity				
White non-Hispanic	20,197	10.4	8875	4.4
Black	27,075	83.7	16,118	43.0
Hispanic	12,466	37.7	6636	13.7
Asian/Pacific Islander	448	4.5	44	9.7
American Indian/ Native Alaskan	206	10.4	170	7.2

[a]Per 100,000 population, extrapolating from the official 1980 census count and 2009 postcensus estimates. http://222.census.gov/ipc/www/idb/.
[b]Rates by sex are only for adult/adolescent cases of acquired immunodeficiency syndrome.
From Centers for Disease Control and Prevention. HIV/AIDS surveillance report. MMWR Morb Mortal Wkly Rep. *1997;9:1–43; and Centers for Disease Control and Prevention. HIV/AIDS surveillance report.* MMWR Morb Mortal Wkly Rep. *2006;18:1–55.*

Geographic Distribution of HIV Infection

The first cases of AIDS reported in the United States were clustered among homosexual and bisexual men and PWIDs in the major metropolitan areas of the East and West Coasts.[69] Since then, AIDS cases

have been reported from all 50 states, the District of Columbia, Puerto Rico, the Virgin Islands, and Guam. However, HIV and AIDS cases are distributed unevenly across the United States.[28] In 2017 HIV rates were highest in the South (16.12/100,000 population), followed by the Northeast (10.6/100,000 population), and lowest in the Midwest (7.4/100,000 population)

The geographic distribution of HIV/AIDS has changed over time. The southern region of the United States accounted for 52% of HIV diagnoses in 2017.[29] Between 1999 and 2003 there was a 13.8% increase in the incidence of AIDS in the southern region of the United States, more than any other region of the country.[70] In most of the United States the majority of people diagnosed with HIV live in metropolitan areas, but in the South 23% of new HIV diagnoses are among people living in suburban and rural areas. In 2017 the six cities with the highest rates of HIV diagnoses per 100,000 population were all in the South: Miami-Fort Lauderdale-West Palm Beach, FL (35.8); Orlando-Kissimmee-Sanford, FL (28.6); Atlanta-Sandy Springs-Roswell, GA (27.3); New Orleans–Metairie, LA (27.0) and Baltimore, MD (26.8); and Baton Rouge, LA (26.9).[29]

Clinical Manifestations of HIV Infection

Spectrum and Progression of HIV Infection

The spectrum of HIV infection ranges from an asymptomatic state to severe immunodeficiency and associated serious secondary infections, neoplasms, and other conditions.[71] Initial or primary infection with HIV can be followed by an acute mononucleosis-like illness. The features of this acute illness associated with seroconversion include fever, lymphadenopathy, sweats, myalgia, arthralgia, rash, malaise, lethargy, sore throat, anorexia, nausea, vomiting, diarrhea, headache, photophobia, and mucocutaneous ulcers.[72,73] Less common manifestations have also been reported, including a variety of neurologic conditions (e.g., aseptic meningitis, myelopathy, radiculopathy, peripheral neuropathy, and Guillain-Barré syndrome),[74] *Candida* esophagitis,[75] and mucocutaneous ulceration (see Chapter 122).

Estimates of the frequency of symptoms among those with primary HIV infection range from 40% to 90%. The signs and symptoms of acute HIV infection are usually manifested days to weeks after exposure.[76–78] In a comprehensive review of primary HIV infection, the interval between exposure and symptomatic illness was reported to range on average from 2 to 4 weeks, with the duration of illness lasting from 1 to 2 weeks.[75] However, the diagnosis of acute HIV infection is frequently missed as the symptoms associated with acute HIV-1 infection are nonspecific. In a group of 23 persons at risk of HIV infection, 87% were symptomatic, and 95% of them sought medical care, yet few had the correct diagnosis made at the first clinic visit.[72] In a prospective study, fever, myalgia, rash, night sweats, and arthralgia occurred more frequently among patients with primary HIV infection, but no targeted symptoms allowed for screening for primary infection.[73] Acute HIV infection should therefore be included in the correct setting in the differential diagnosis of any unexplained febrile illness (see Chapter 122).[76]

The diagnosis of acute HIV infection cannot be made with the commonly available serologic tests (third-generation immunoassays) because they detect antibodies and become positive approximately 3 weeks after the initial infection.[79] Studies of homosexual men,[80,81] persons with hemophilia,[80,82] and intravenous (IV) drug users[83] have consistently demonstrated the development of detectable HIV antibodies within 3 to 12 weeks after infection. Epidemiologic studies and case reports, as well as modeling techniques,[80] suggest that seroconversion beyond 6 months is very uncommon. The laboratory diagnosis of primary HIV infection should be made by requesting a plasma HIV RNA assay, which has a sensitivity of 100% and specificity of 97.4%.[73] The test for the presence of detectable p24 antigen is less sensitive (88.7%) but more specific (100%) than the plasma HIV RNA assay for the diagnosis of primary infection,[73] and the detection of p24 antigen is now used routinely in the screening of blood donors.[84] Therefore the presence of viral p24 antigen or high-titer HIV RNA in a patient with a negative test for HIV-1 antibodies establishes the diagnosis of acute HIV infection (see Chapter 120). False-positive HIV RNA tests have been described, but they are not reproducible and have values less than 3000 copies/mL.[85] With the availability of fourth-generation HIV tests that detect both p24 antigen and antibodies (immunoglobulins G and M [IgG and IgM]), HIV infection can be identified sooner than with third-generation immunoassays. In a study that did NAAT on HIV-negative specimens after third- and fourth-generation immunoassays, NAAT increased HIV detection rate by 2.2% after a third-generation immunoassay and by only 0.7% after fourth-generation immunoassay.[86]

After primary infection with HIV, the risk for disease progression increases with the duration of infection. Most cohort studies that have evaluated the natural history of HIV infection have been conducted in the United States and Europe and show that AIDS develops in less than 5% of HIV-infected adults within 2 years of infection; without therapy, AIDS develops in approximately 20% to 25% within 6 years of infection and in 50% within 10 years.[87–90] Approximately 5% to 8% of HIV-infected individuals remain clinically asymptomatic, with normal $CD4^+$ T-lymphocyte counts for more than 8 years after infection. These individuals are called "long-term nonprogressors."[91–93]

Differences in the rate of progression may be due to the route of infection, the size of the viral inoculum, the pathogenicity of the infecting viral strain, or the immunologic status of the host. For example, in one analysis of nearly 700 HIV-infected transfusion recipients, the estimated risk for the development of AIDS was 33% for persons within 5 years of infection and 49% within 7 years.[94] Among recipients infected for similar periods, AIDS developed more rapidly in those who received blood from donors who progressed to AIDS soon after donation (50%) than in those who received blood from other HIV-infected donors (26%). In the same study the recipients in whom AIDS developed had received significantly more units of blood at the time of infection than HIV-infected recipients without AIDS did, which raises the possibility that the underlying clinical status leading to multiple transfusions, particularly the degree of immunosuppression, or exposure to other viral cofactors may also have affected disease progression.

AIDS-Indicator Diseases

Three clinical conditions accounted for greater than 75% of all initial AIDS-indicator conditions reported in 1992: *P. jirovecii* pneumonia (42%), HIV wasting syndrome (20%), and candidiasis of the esophagus (15%). The prevalence of several AIDS-indicator diseases was higher in MSM than in heterosexual men or women. In general, the reported frequency of AIDS-indicator diseases is similar for men and women with similar modes of exposure to HIV. However, among PWIDs, esophageal candidiasis, cytomegalovirus (CMV) disease and retinitis, and herpes simplex virus (HSV) disease have been reported more frequently in women than men.[95]

Because most AIDS-indicator infections result from the endogenous reactivation of previously acquired pathogens, the frequency of reported opportunistic infections probably reflects, in part, the geography-specific prevalence of endemic infections. For example, toxoplasmosis and cryptococcosis are more likely to develop in African and Haitian patients.[96] Similarly, the risk of extrapulmonary TB among foreign-born persons with AIDS in the United States is the highest in those from Haiti, the Philippines, Central America, and Africa.[97] Among US-born persons, those at increased risk for extrapulmonary TB include residents of the South and Northeast, blacks and Hispanics, and PWIDs.[98]

Expansion of the surveillance case definition for AIDS in the United States in 1993 to include immunologic criteria (a $CD4^+$ T-lymphocyte cell count of <200/μL) caused a substantial distortion of the trend in the incidence of AIDS-defining diseases. Therefore the CDC developed a procedure for estimating the incidence of AIDS-defining opportunistic infections among persons reported solely on the basis of immunologic criteria; this procedure allowed trends in the incidence of opportunistic infections in persons with AIDS to be estimated as though the case definition had not changed.[98] Changes in the incidence and prevalence trends of AIDS-defining diseases between 1991 and 1996 demonstrated that, for homosexual/bisexual men, significant decreasing trends occurred for 11 opportunistic infections, including *Mycobacterium avium-intracellulare* disease, *P. jirovecii* pneumonia, CMV retinitis, Kaposi sarcoma, esophageal candidiasis, CMV disease, extrapulmonary cryptococcosis, toxoplasmic encephalitis, TB, HSV infection, and disseminated histoplasmosis. In

contrast, for PWIDs decreasing trends were seen for only five opportunistic infections (*P. jirovecii* pneumonia, esophageal candidiasis, TB, chronic HSV infection, and chronic cryptosporidiosis), and an increase occurred for recurrent pneumonia.[99] The decreasing trend for some AIDS-defining opportunistic infections, such as *P. jirovecii* pneumonia, was probably related to the increasing use of ART and prophylaxis against *P. jirovecii* pneumonia. For example, among adults and adolescents with a single AIDS-defining disease reported through 1996, the proportion with *P. jirovecii* pneumonia decreased from 50% in 1988 to 39% in 1996. Although declines were seen in everyone with AIDS during this period, they were the most striking for homosexual/bisexual men and persons with hemophilia or a coagulation disorder. The differences in trends for homosexual/bisexual men and PWIDs may reflect differences in socioeconomic status, access to medical care, or adherence to preventive medications. The increasing use of effective ART, which began in 1995/1996, had caused a dramatic decrease in the incidence of AIDS-defining opportunistic illnesses by the end of 1996,[8] and the incidence of AIDS could no longer be reliably estimated. The decline in AIDS-defining opportunistic illnesses that began in 1996 has continued. With the increasing use of effective ART, AIDS-defining illnesses are occurring mainly among persons with newly diagnosed HIV infection at the time of AIDS, those known to be infected but who do not seek or receive care, and those for whom treatment has failed.[100–103] Among patients who begin ART the unusual presentation of opportunistic infections can occur as a result of immune reconstitution.[104]

In 1993 three new AIDS-defining illnesses were added to the surveillance case definition: recurrent bacterial pneumonia, invasive cervical carcinoma, and pulmonary TB.[16] Several studies have shown that persons with HIV-related immunosuppression are at increased risk of bacterial pneumonia,[105] which can result in significant morbidity and mortality.[19,20] One study among PWIDs found that the annual incidence rate of bacterial pneumonia was five times higher in those who were infected with HIV than in those who were seronegative PWIDs.[105] *Streptococcus pneumoniae* is the most commonly isolated bacterial pathogen and has been reported to precede the onset of other AIDS-defining conditions in 57% to 81% of persons with HIV infection.[106] In a population-based survey in San Francisco, the rate of pneumococcal bacteremia (89% of the HIV-infected patients with bacteremia had pneumonia as a major clinical syndrome) among persons with AIDS was nearly 100 times higher than rates reported before the HIV epidemic.[107] The risk of pneumonia in HIV-infected patients is inversely related to their $CD4^+$ T-lymphocyte counts (see Chapter 123).

Precursor lesions to invasive cervical cancer, such as cervical dysplasia, neoplasia, and genital papillomavirus infection, are more commonly diagnosed in HIV-infected women than in other women (also see Chapter 126).[108] In a study comparing HIV-seropositive with HIV-seronegative women with normal baseline cervical cytology, women positive for oncogenic human papillomavirus had a higher 2-year incidence of squamous intraepithelial lesions than HIV-negative women, regardless of $CD4^+$ count. Among women infected with nononcogenic human papillomavirus types, the incidence of squamous intraepithelial lesions at 2 years was significantly higher among HIV-infected women with a $CD4^+$ count of less than 200 cells/μL than among those with higher $CD4^+$ counts and HIV-negative women.[109] The risk of cervical cancer among HIV-infected patients has not changed with the advent of potent ART.[110]

HIV Infection and Tuberculosis

After several decades of declining incidence, the number of new cases of TB in the United States began to increase in 1986.[111] Many factors contributed to the resurgence of TB, but the HIV/AIDS epidemic was in part a major cause of these excess cases of TB.[112] Now once again, the overall case rates of TB in the United States are declining, with 4.6 new cases per 100,000 population (a total of 13,767 cases) reported in 2006[113] and an estimated 4.0% prevalence of latent TB infection in the general population. The percentage of TB cases with known HIV infection also decreased from 15.0% in 1993 to 6% in 2006[114]; however, the full spectrum of the overlap between HIV and TB is not known, and more than 100,000 persons in the United States are thought to be coinfected with HIV and *Mycobacterium tuberculosis*.[111,112] Comparisons of AIDS and TB registries conducted by the 50 states and Puerto Rico revealed that 14% of persons with TB in 1993 and 1994 (27% among those 25–44 years of age) also appeared in the AIDS registry.[115] The overlap in the demographic and geographic characteristics of the two diseases is evident by surveys of HIV seroprevalence in TB clinics and by the fact that 80% of those with TB and AIDS were found in New York, California, Florida, Georgia, New Jersey, Illinois, and Texas. Both TB and HIV infection disproportionately affect racial and ethnic minorities and the urban poor.[111] In one analysis black and Hispanic adults who died from AIDS were nearly three times more likely than whites to also have TB.[116]

HIV infection is a strong risk factor for the development of active TB in persons with latent *M. tuberculosis* infection. In a prospective study of PWIDs with documented positive tuberculin skin tests, the observed incidence of active TB was 7.9 per 100 person-years for 49 HIV-infected persons versus no cases among 62 HIV-seronegative persons. The risk of active TB in HIV-seropositive persons in this study—14% over 2 years—contrasts strikingly with the estimated 10% lifetime risk in HIV-negative persons with latent TB infection.[117] In addition, HIV-infected persons are at increased risk for the development of active, symptomatic TB after their initial exposure and subsequent infection with *M. tuberculosis*.[118] Outbreaks of TB among HIV-infected individuals in correctional facilities, AIDS clinics, and hospital wards suggest that the development of active TB after exposure is greatly increased among HIV-infected persons. Finally, molecular epidemiology studies conducted in San Francisco and New York suggest that one-third of the cases in San Francisco and 40% in New York are due to recent transmission rather than reactivation.[119,120]

Drug-resistant TB is more common in persons with HIV infection than in those with TB but without HIV infection.[115] In a multivariate analysis conducted by the CDC between 1993 and 1996, being infected with HIV was a risk factor for isoniazid and rifampin monoresistance and multidrug-resistant TB.[121] In a study conducted in New York City the proportion of isolates resistant to one or more anti-TB drugs increased from 10% from 1982–84 to 23% in 1991.[122] Among the isolates of *M. tuberculosis* with primary resistance to isoniazid or rifampin, 75% came from patients known to be infected with HIV.[122] The increased risk of drug-resistant TB among HIV-infected persons may reflect a higher proportion of the disease resulting from recently acquired drug-resistant strains[119,120] and other factors, such as decreased absorption of oral antimycobacterial drugs among HIV-infected persons.[123] Outbreaks of multidrug-resistant TB (e.g., resistant to both isoniazid and rifampin and/or other drugs) have been characterized by (1) a high prevalence of HIV infection among the outbreak cases (range, 20%–100%), (2) a high mortality rate among persons infected with resistant strains (range, 72%–89%), (3) a short median interval between diagnosis and death (range, 4–16 weeks), and (4) nosocomial transmission to health care workers.[115,124–126] The emergence of extensively drug-resistant TB (resistant to both isoniazid and rifampin and to any fluoroquinolone drug and at least one of three second-line injectable drugs [amikacin, kanamycin, or capreomycin]) among HIV-infected persons in South Africa raises concerns about the possibility of epidemics of virtually untreatable TB.[127]

Mortality of Persons With HIV Infections and AIDS

The estimated number of deaths among persons with HIV/AIDS increased steadily through 1995, when approximately 50,000 persons were estimated to have died of AIDS. In 1996 the estimated number of deaths from AIDS decreased to 37,525 and has continued to drop every year since then to 17,347 in 2000, 14,061 in 2006, and 12,287 in 2016 (see Fig. 119.2).[29] Since 2012 the number of deaths reported among persons with stage 3 (AIDS) HIV infection has remained stable, with approximately 13,000 deaths per year and a rate of 4 per 100,000 population.[29] The dramatic declines in AIDS mortality seen after 1996 were largely due to the availability of combination ART[26,128,129] and have been more rapid among whites and homosexual and bisexual men but have occurred in all populations.[10] The percentage decrease in mortality has been the smallest among black women and in the South.

In 1993 HIV infection became the most common cause of death among persons age 25 to 44 years.[130] In 1994 72% of HIV-related deaths occurred in persons age 25 to 44 years, in whom it was the leading cause of death, accounting for 19% of the deaths in this age group. After 1996, when AIDS mortality began to decline, and while HIV infection is no longer a leading cause of death for Americans overall, it remains a leading cause of death among black men age 25 to 44 years.

To put into perspective the impact that AIDS-related mortality has had, the years of potential life lost before the age of 65 years because of AIDS have been calculated.[131] From 1989–90 the years of potential life lost before age 65 years that could be attributed to AIDS and HIV infection increased by 13%. In 1993 AIDS had become the fifth leading cause of years of potential life lost before age 65 years, and in 1994 it became the fourth. That year it was estimated that approximately 49,500 persons died of AIDS.[130] At a population level the improvement in survival for AIDS patients as a result of the introduction of highly active ART in 1996 can be seen in a study conducted in New York City, where the overall cumulative survival at 24 months increased from 43% among patients diagnosed with AIDS from 1990–95 to 76% among those diagnosed from 1996–98.[128] It has been estimated that at least 3 million years of life have been saved in the United States as a direct result of the treatment of HIV infection.[132]

HIV/AIDS IN CHILDREN

Through the end of 2017, a total of 9573 cases of AIDS in children younger than 13 years had been reported in the United States.[29] The number of AIDS diagnoses reported in children younger than 13 years is now quite small in the United States, with 33 cases reported in 2017. HIV diagnosis is also uncommon, with 99 cases and a rate of 0.2 per 100,000 population reported in 2017.[29] The spectrum of AIDS-defining opportunistic infections and malignancies in children overlaps considerably with those that are included in the surveillance case definition for adults and adolescents; however, some differences can be noted (see Chapter 127).[133] Three important exceptions for children younger than 13 years are the inclusion of lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia, recurrent bacterial infections, and the exclusion of a threshold $CD4^+$ T-lymphocyte count (e.g., <200 $CD4^+$ T lymphocytes/μL). Longitudinal evaluations of lymphocyte subsets in both HIV-infected and HIV-uninfected children suggest some measure of prognostic value.[134,135] However, because normal newborns and young infants have prominent lymphocytosis,[136] moderate declines in $CD4^+$ T lymphocytes by adult standards can represent significant impairment in young children.[134,137]

Pediatric cases are ordered into a hierarchy of mutually exclusive exposure categories, and most children reported with AIDS acquire HIV infection perinatally from their mothers.[138] Among the 287 children of age less than 13 years reported through the end of 2016, 86% had acquired HIV infection perinatally. Because most children acquire HIV infection from their mothers, the racial, ethnic, and geographic distributions of children with AIDS parallel those of women with AIDS. Sixty percent of the pediatric HIV infections and 69% of AIDS cases reported in 2017 were among black non-Hispanics.

From 1984–92 the estimated number of children with perinatally acquired AIDS diagnosed each year increased and peaked at 905 in 1992.[138] In 1994, as a result of a clinical trial that demonstrated the efficacy of zidovudine in reducing perinatal HIV transmission by two-thirds,[139] the Public Health Service issued recommendations for the use of zidovudine and counseling and HIV testing of pregnant women in the United States.[140,141] With the implementation of these recommendations, the number of children with perinatally acquired HIV infection has decreased dramatically in the United States, with an estimated 21,956 cases of perinatally acquired HIV presented between 1994 and 2010 (Fig. 119.3).

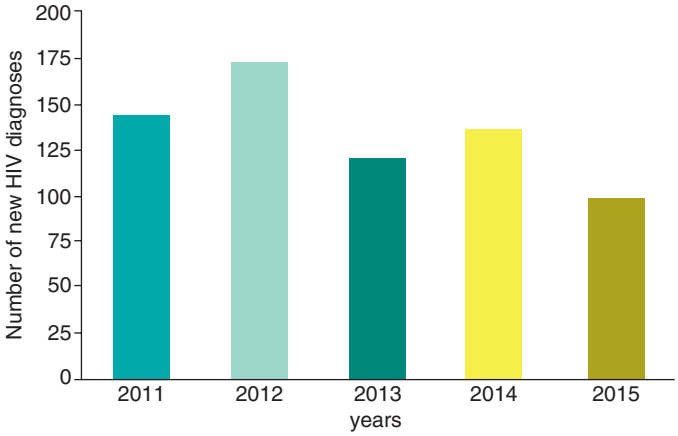

FIG. 119.3 Diagnoses of perinatal HIV infections in the United States, 2011–15. *(From the Centers for Disease Control and Prevention. HIV Among Pregnant Women, Infants, and Children. https://www.cdc.gov/hiv/group/gender/pregnantwomen/index.html. Accessed January 23, 2019.)*

MODES OF TRANSMISSION

More than 2 decades after the initial studies were conducted to determine the ways in which HIV is transmitted, surveillance and epidemiologic data throughout the world continue to support only three primary modes of transmission: sexual contact; exposure to blood, largely through IDU and occasionally through transfusion; and perinatal transmission from infected mothers to their infants.

Sexual Transmission

Sexual contact is the predominant mode of HIV transmission throughout the world. However, the geographic distribution of cases attributable to homosexual and heterosexual transmission varies markedly.[7] Whereas heterosexual transmission is the major mode of spread of HIV infection in Africa and the Caribbean, in North America and Europe male-to-male sexual transmission continues to account for the majority of cases among men, and heterosexual transmission accounts for a smaller number of new infections except among women.[142]

The likelihood of acquiring or transmitting HIV infection through a single sexual contact is directly related to certain correlates of exposure, such as the infectiousness of the source partner, the susceptibility of the exposed individual, the number of partners, and the prevalence of HIV infection in the population. Sexual transmission of HIV is relatively inefficient, but behavioral and biologic factors influence the likelihood of HIV transmission in a given sexual encounter. For example, anal sex has been consistently found to be a greater risk than vaginal sex, which in turn is a higher risk than oral sex,[143] and the coexistence of a sexually transmitted infection (most notably the presence of genital ulcerative disease) greatly increases the infectiousness as well as the susceptibility of the individual.[144–146] Carefully performed longitudinal cohort studies conducted in Africa have helped us understand the risk of HIV transmission per coital act. In a study conducted in Uganda the probability of HIV transmission per coital act was the highest from subjects with early infection (8.2 cases/1000 coital acts), it declined to 0.7 to 1.5 per 1000 coital acts with established infection, and it increased again to 2.8 cases per 1000 coital acts in advanced infection.[147] Index partner HIV viral load was the strongest predictor of transmission at each stage of infection. Taking into consideration that patients with acute infection have extremely high viral loads, the risk for HIV transmission from patients with acute infection has been estimated to be as high as 1 case per 50 coital acts.[148] The estimated per-act HIV transmission risk (expressed as per 10,000 exposures) has been estimated to be 138 for receptive anal intercourse, 11 for insertive anal intercourse, 8 for receptive penile-vaginal intercourse, and 4 for insertive penile-vaginal intercourse.[149] In a prospective cohort study of 2189 high-risk homosexual and bisexual men conducted in San Francisco, Denver, and Chicago with 2633 person-years of follow-up, 60 seroconversions were observed. The estimated per-contact risk of acquiring HIV from unprotected receptive anal intercourse was 0.82% when the partner was known to be HIV infected and 0.27% when partners of unknown serostatus were included.[150] In a systematic review and meta-analysis of the literature on HIV-1 infectiousness through anal intercourse, the per-act risk was 1.4%, and the per-partner risk was 40.4% for unprotected receptive

anal intercourse, with no significant difference between per-act risks for heterosexuals and MSM.[151] The per-partner risk for unprotected insertive anal intercourse and combined unprotected receptive and insertive anal intercourse risks were 21.7% and 39.9%, respectively. These data support that unprotected anal intercourse is a high-risk practice for HIV transmission, but that there is probably substantial variation in infectiousness.

Infectiousness of the Source Partner

The variability in the infectiousness of the source partner has been suggested by observations that some persons appear to be highly efficient transmitters of HIV through sexual contact. Such efficiency is manifested by the infection of a high proportion of an individual's sexual partners,[152] often after only a single contact.[153,154]

It has been thought for some time that HIV transmission appears to be more efficient late in the course of HIV infection.[147,155–159] More recently, primary HIV infection has been recognized as a period associated with increased infectiousness likely related to the much higher viral load in blood and genital secretions of persons with acute infection.[148,160,161] From a public health point of view, this association is extremely important because increased infectiousness would precede recognition of infection by the individual.[162,163] In general, the higher the plasma viral load, the more likely HIV transmission will occur, given similar exposure. In a study conducted in Africa there were no cases of seroconversion among heterosexual discordant partners when the infected partner had serum HIV RNA of less than 1500 copies/mL and the per-person risk of transmitting HIV-1 increased by a factor of 2.5 each time the viral load in the HIV-infected person increased by a factor of 10.[164] In 2008 the Swiss National AIDS Commission published a statement stating that individuals living with HIV who achieved and maintained an undetectable viral load for at least 6 months and did not have other sexually transmitted infections did not transmit HIV through sexual contact.[165] This became known as "The Swiss Statement" and was quite controversial when first published. Since then, results of several studies have provided strong evidence that suppression of HIV viral replication with antiretrovirals (ARVs) dramatically reduces the infectiousness of the HIV-1–infected individual and the subsequent risk of transmission through sexual contact. In a randomized controlled trial conducted among serodiscordant couples, treatment with ART led to a 96% reduction in the probability of HIV transmission and, more important, on long-term follow up of study participants, there were no linked transmissions when the viral load of the infected partner was suppressed.[166,167] Outside of the clinical trial setting the PARTNER study demonstrated zero HIV transmissions among serodiscordant heterosexual and MSM couples when the HIV-infected partner had a suppressed viral load on ART.[168] These and other studies have now confirmed that there is a negligible risk (likely zero risk) of sexual transmission of HIV when the HIV-infected sex partner has durably suppressed viral replication on ART.[169] Taken together, these findings have resulted in the "Undetectable equals Untransmittable (U = U) concept which was accepted by the CDC as a public health principle in 2017.[170]

Similarly, the risk for perinatal transmission of HIV increases as the viral load in the mother increases, and no cases in one study occurred when the maternal viral load in plasma was less than 1000 copies/mL, 16.6% when the viral load was between 1001 and 10,000 copies/mL, 21.3% when the viral load was between 10,001 and 50,000 copies/mL, 30.9% when the viral load was between 50,001 and 100,000 copies/mL, and 40.6% when the viral load was greater than 100,000 copies/mL.[171]

The probability of saliva from HIV-infected persons transmitting HIV through oral-oral or oral-genital sexual contact is low but real.[172] HIV is found in very low concentrations in the saliva of infected persons,[173,174] and since 1987, 21 case reports of 42 potential instances of oral transmission of HIV have appeared.[172] Such transmission is difficult to prove or rule out because most persons with HIV and a history of oral sexual contact report genital contact as well.

Epidemiologic and laboratory data indicate that genital ulcer disease in the source partner is an important factor facilitating HIV transmission. Genital ulcers probably increase the infectiousness of both male and female source partners.[175,176] In a prospective study of more than 400 men who acquired a sexually transmitted disease (STD), including HIV infection, from a group of prostitutes in Nairobi, Kenya, seroconversion to HIV was independently associated with the concurrent acquisition of genital ulcer disease.[177] In a study in Malawi, patients with genital ulcer disease were associated with lower $CD4^+$ T-lymphocyte cell counts and higher plasma HIV RNA than HIV-infected patients without genital ulcer disease.[178] Furthermore, a similar study in Uganda suggested that genital ulcers, caused mainly by HSV-2, are associated with higher viral loads in both incident and prevalent HIV-infected patients.[179] Genital ulcer disease may have augmented the women's infectiousness by increasing viral shedding in the female genital tract through a local inflammatory response mediated by the recruitment and activation of HIV-infected macrophages and lymphocytes to and on the disrupted mucosal surface.[177,180] The recovery of HIV from genital ulcers in HIV-infected women supports this hypothesis.[181] These data suggest that the treatment of genital ulceration and HSV-2 suppressive therapy may be associated with a reduction in HIV viremia, thus potentially reducing HIV transmission and disease progression. However, controlled studies of antiviral therapy for genital HSV infections did not show a reduced risk in the acquisition or transmission of HIV infection.[182–184] In the United States MSM account for the large majority of primary and secondary syphilis cases among males, and having syphilis significantly increases the risk of being diagnosed with HIV in the future.[185]

Nonulcerative STDs may also enhance the sexual transmission of HIV. Among men, urethritis and gonorrhea are associated with the increased detection of HIV in semen,[186,187] and appropriate antibiotic treatment diminishes the amount of HIV present in genital secretions.[188]

Susceptibility of the Recipient Partner

As previously mentioned, the presence of genital and anorectal ulceration or mucosal disruption secondary to infection has emerged as one of the most consistent and biologically plausible factors affecting the transmission and acquisition of HIV infection through sexual contact. Although ulcers that disrupt the genital epithelium can serve simply as a portal of entry for HIV, they may have a more complex role in HIV transmission. Genital ulcers cause an inflammatory response that in turn may increase the number of stimulated T lymphocytes at the surface of the ulceration and thus increase the number of susceptible cells.[178,180]

A number of epidemiologic studies have demonstrated that a history of genital ulcer disease in the recipient partner is a risk factor for acquiring HIV in both heterosexual and homosexual men[180,189] and women.[176,189] Although this association may not be surprising, two seroepidemiologic studies of homosexual men have convincingly demonstrated that genital ulcer disease is not simply a marker for increased sexual activity.[180,190] Instead, infection with HIV was independently associated with serologic evidence of prior syphilis or infection with HSV-2, the two most common causes of genital ulcers among homosexual men in the United States. In another study among female prostitutes in Nairobi, 60% of seroconverting women experienced one or more episodes of genital ulcers before seroconverting.[176]

Nonulcerative STDs, such as gonorrhea and chlamydial infection, also facilitate the acquisition of HIV infection by causing mucosal disruption of the genital tract.[176] Other nonulcerative STDs (e.g., urethritis, cervicitis, balanitis, bacterial vaginosis, and genital warts) also increase the risk of acquiring HIV infection.[191–193] Observational studies over many years strongly suggested that circumcised men were unlikely to acquire HIV. Three randomized trials have demonstrated that circumcision can decrease the acquisition of HIV infection rates by 50% to 60% in circumcised men who largely engaged in heterosexual intercourse.[194–196] However, this benefit has not been confirmed for MSM.[197,198] The higher frequency of balanitis in uncircumcised men has been postulated as a partial explanation for the increased risk of HIV observed among uncircumcised men in developing countries.[177,199,200]

Noninfectious causes of ulceration of the genital tract may also pose a risk for the sexual transmission of HIV. For example, the frequent use of the nonoxynol-9 contraceptive sponge by female prostitutes in Nairobi was associated with increased rates of genital ulcers, vulvitis, and HIV seroconversion in one study.[201] The increased risk for HIV acquisition may be attributable to chemical irritation from the spermicide

or to mechanical trauma, both of which can result in inflammation and ulceration.[202] The importance of the treatment of sexually transmitted infections in the HIV epidemic was made evident by a study in Tanzania showing that prompt management of sexually transmitted infections decreased the incidence of HIV infection by 42%.[203] Certain traumatic sexual practices that result in rectal mucosal disruption and lesions have been associated with HIV infection. Early epidemiologic studies of homosexual men found that receptive anal intercourse, "fisting," and douching increased the risk of HIV transmission.[204–206] Subsequent studies have both supported these findings and extended the association of receptive anal intercourse to the heterosexual transmission of HIV.[157,162,207]

Although cases of AIDS have been reported among lesbian and bisexual women, most were infected through IDU or sex with HIV-infected men.[197–199] Female-to-female transmission of HIV appears to be very rare.[200] However, cases of female-to-female transmission of HIV have been reported.[201–203]

No consistent association between oral contraceptive use by women and acquisition of HIV infection has been found. In one study women who reported taking oral contraceptives had a reduced risk of HIV infection.[158] However, in a longitudinal cohort study of Nairobi prostitutes and a cross-sectional study of sex partners at an STD clinic in Nairobi, oral contraceptive use was an independent risk factor for HIV seroconversion and prevalent infection, respectively.[176,204] In contrast, a study of prostitutes in Zaire[189] and one of pregnant women in a rural US community with a high prevalence of HIV infection[79] found no association between HIV infection and the use of oral contraceptives. The presence of cervical ectopy has been associated with an increased risk of HIV seropositivity among long-term female partners of HIV-positive men in Nairobi.[204] Because oral contraceptives are associated with higher rates of ectopy, the relationship, if any, between oral contraceptives and HIV requires further study.[205] There are growing concerns that hormonal contraceptives, and specifically progestogen-only injectables, such as depot medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-EN), may increase the risk of HIV acquisition when used by HIV uninfected women or, when used by women living with HIV, may increase the risk of female to male HIV transmission.[206,208] As a result of these data WHO issued, in 2017, an updated guidance statement for the use of hormonal contraception by women at risk of HIV. In this new document WHO has changed the recommendation for both methods from "use without restriction" to "benefits outweigh theoretical or proven risks."[209] Although anal and vaginal intercourse are the two sex practices associated with the greatest risk of transmission, other sexual activities involving exposure to semen or blood also carry a potential risk of transmission. For example, seroconversion for the HIV antibody has been documented after receptive oral intercourse with ejaculation.[172]

Finally, a mutation in the chemokine receptor gene that may render the rare homozygous host relatively resistant to HIV infection has been identified (see Chapter 121). When this mutation is present in the heterozygous state, it does not prevent infection.[210] The presence of this mutation varies according to race, with 11% and 1.7% homozygosity among whites and blacks, respectively.[160]

Transmission Through Injection Drug Use

Among PWIDs HIV is transmitted by parenteral exposure to HIV-infected blood through sharing of contaminated needles and other injection equipment. Specific factors that have been associated with HIV infection among PWIDs include the duration of IDU, frequency of needle sharing, number of needle-sharing partners, number of injections, median number of injections in "shooting galleries," and prevalence of HIV infection in the area of residence.[34,211–213] The rate of HIV infection among PWIDs varies widely among different geographic areas. In the United States the rate of HIV infection has been the highest in the Northeast.[35,36]

Most studies have found higher rates of HIV infection associated with cocaine and heroin injection than with heroin injection alone, probably because of the greater frequency of cocaine injections.[214–216] Another possible explanation is the greater likelihood of the exchange of sex for drugs among cocaine users.[217] Among PWIDs poor socioeconomic conditions, homelessness, and minority race and ethnicity are associated with an increased frequency of risk behavior and higher rates of HIV infection.[218–221] Among PWIDs enrolled in a study in Baltimore from 1988–89, the 703 HIV-infected users were more likely to have a history of syphilis (16.8%) than the 2218 uninfected PWIDs (11.3%).[222] The higher rates of STD among HIV-infected PWIDs than uninfected users suggests that some infections are transmitted through unsafe sexual practices rather than IDU itself.[43,222,223]

Many PWIDs have changed their drug use behavior to reduce the risk of HIV infection.[224,225] Substance use treatment, street outreach programs, syringe services programs, AIDS educational efforts, and HIV counseling and testing programs have all been shown to be effective in reducing, although not eliminating, the risk of HIV transmission in these populations.[226] However, worldwide coverage of HIV prevention, treatment, and care services for PWIDs is very low, and thus interventions that are known to be effective have not been implemented.[227] ARV drugs given to HIV-negative PWIDs have been shown to decrease HIV-1 incidence by 49%.[228]

Transmission by Blood and Other Tissues

The recipients of unscreened blood or blood products from HIV-infected donors are at high risk for HIV infection. HIV has been transmitted through receipt of whole blood, blood cellular components, plasma, and clotting factors.[229,230] The likelihood of a person becoming infected with HIV after receiving a single-donor blood product documented to be HIV positive approaches 100%.[231,232] Other blood or plasma products, such as hepatitis B immune globulin, immune serum globulin, Rh(D) immune globulin, and hepatitis B vaccine, are prepared by using one of several fractionation processes that inactivate HIV; the use of these products has not been associated with transmission.[233,234]

Before serologic testing for HIV was begun in 1985, 0.04% of 1,200,000 donations in the United States were estimated to be HIV positive.[235] During this time an estimated 29,000 blood or blood product recipients were exposed to HIV; because many died of underlying conditions, 12,000 of these persons were estimated to have survived long enough for AIDS to develop.[236] Confidential unit exclusion and direct donor deferral as well as the institution of HIV antibody screening in 1985, followed by additional tests for antibodies to HIV-2 and p24 antigen in 1996, markedly decreased the risk of HIV infection through blood or blood products. With the implementation of p24 antigen testing, the risk of HIV transmission through the transfusion of screened blood was estimated to be 1 in 200,000 to 1 in 2,000,000 per unit transfused in the United States.[237,238] Further reduction in the risk of transfusion-transmitted HIV in the United States occurred after the US Food and Drug Administration (FDA) approved the implementation of NAAT of blood units in 2002, which reduces the window period to approximately 12 days.[239] In the clinical trials that led to the approval of NAAT by the FDA, a total of 7 HIV-1–positive and 88 hepatitis C virus (HCV)-positive donations that would otherwise not have been diagnosed were detected in more than 20 million donations tested. As a result of additional testing, estimated risk for HIV transmission by blood products is now 1 in 1.4 to 1 in 1.8 million units.[59] The risk of HIV infection for patients with hemophilia who received concentrated clotting factors composed of blood components from potentially thousands of donors was substantial before 1985,[240,241] but now it is exceedingly low. HIV transmission by transplantation of the liver, heart, kidney, pancreas, bone, and possibly skin has been reported.[65,242,243] Relatively avascular tissues, such as corneas and processed tissues, have not been associated with transmission.[65,244,245] AIDS developed in several transplant recipients after receiving a variety of organs from a single HIV-negative cadaver donor. The donor was subsequently found to be HIV infected by culture and PCR.[65]

Perinatal Transmission

Vertical transmission of HIV from an infected woman to her infant can occur during gestation (in utero), at the time of delivery (intrapartum), or postpartum through breastfeeding. Significant progress has been made in elucidating risk factors that influence transmission during these three periods, detecting infection in the newborn earlier and more reliably, and preventing perinatal transmission with the use of ARV drugs (see Chapter 128).

The occurrence of intrauterine infection is supported by the detection of HIV both in fetal tissue as early as 8 weeks[246] and in placental tissue infected in vivo and in vitro.[247] In addition, the 30% to 50% of infected infants who test positive by PCR or HIV culture at birth[248–250] also suggests intrauterine transmission of HIV. The proportion of infants who become infected in each trimester of pregnancy is unknown, but transmission to the infant early in pregnancy would presumably allow viral replication to reach a level sufficient for detection by culture or PCR.[251] HIV-infected infants who test negative by PCR or HIV culture at birth may have become infected late in pregnancy or during the intrapartum period. Accumulating information suggests that a sizable proportion of vertical transmission may occur during the intrapartum period.[252,253] Both vaginal and cesarean deliveries present frequent and varied opportunities for the infant to be exposed to infected maternal blood and cervicovaginal fluids. Although many studies have found statistically similar rates of transmission for vaginal and cesarean section delivery,[254–257] there are data from meta-analysis and randomized clinical trials suggesting that elective cesarean section reduces the risk of perinatal HIV transmission even when accounting for the use of ARV drugs.[258] As a result, the percentage of deliveries performed via elective cesarean section in HIV-infected pregnant women increased from 20% from 1994–98 to 44% from 1998–2000.[259] The isolation of HIV from breast milk,[260] as well as reports of breastfeeding mothers who infected their infants after they had acquired HIV infection through postpartum blood transfusions, provided initial evidence for postnatal HIV transmission.[261] Subsequent evaluation has focused on estimating the added, or attributable, risk of perinatal transmission conveyed by breastfeeding. Several prospective cohort studies that compared breastfed and bottle-fed infants have detected higher rates of HIV infection in breastfed children.[253–255,262,263] The attributable risk of transmission through breastfeeding ranges from 14% to 29% and was determined on the basis of data from developing countries where ART has not been used.[264] Mothers who themselves acquired HIV infection in the postpartum period were more efficient transmitters, presumably because of the increased viral burden associated with primary HIV infection.[265,266] For these reasons it has been recommended in the United States since 1985 that HIV-infected women abstain from breastfeeding their infants. Epidemiologic studies[263] and mathematical models[267] have evaluated the competing risks of acquiring HIV infection by breastfeeding and the increased morbidity and mortality associated with alternatives to breastfeeding in developing countries. Both approaches have found that for children in many developing countries, the benefits from breastfeeding outweigh the risk of HIV transmission through breastfeeding. However, the risk of not breastfeeding varies greatly between and within developing countries, so the situation needs to be considered on an individual basis.

Prospective studies of infants born to women with HIV infection before the use of ARV drugs have found rates of transmission ranging from 13% to 40%,[254–256,268] with the highest rates of perinatally acquired HIV infection, which approach 40%, reported from Africa.[268] The disparity in these rates most likely reflects differences in the severity of the maternal disease stage, nutritional status, rates of breastfeeding, study design, completeness and length of follow-up, and use of different diagnostic criteria.[137] The risk of perinatal transmission appears to vary by the disease stage of the mother. Mothers at both extremes of the clinical spectrum of HIV infection, with either acute primary infection[199,252] or advanced symptomatic disease,[265,268] have been reported to be more likely to transmit HIV to their infants than asymptomatic seropositive women. The most important risk factor to determine the likelihood that an infant will acquire HIV perinatally is the HIV viral load of the mother.[269,270] However, mother-to-child transmission has been observed across the entire range of plasma HIV RNA levels, including in women with very low or undetectable levels of maternal HIV-1 RNA on ART.[271]

In addition to maternal risk factors, obstetric factors that disrupt the maternal-fetal barrier can increase perinatal transmission. In one study among Zairian women the presence of histologic chorioamnionitis and funisitis was associated with an overall twofold increase in the risk of transmission.[272] Preterm delivery, prolonged rupture of membranes (>4 hours), the use of illicit drugs during pregnancy, a low antenatal $CD4^+$ count, and low birth weight are also associated with increased risk for perinatal HIV transmission.[273,274]

Infants born to HIV-infected mothers have passively acquired the maternal antibody to HIV, which persists for 12 to 18 months. For infants 0 to 6 months of age, PCR and viral culture offer the greatest sensitivity and specificity for detecting HIV infection (see Chapters 120 and 127).[275] Nonetheless, these tests can detect only one-half or less of perinatally infected infants, which is a reflection of very low viral burden, sequestration of the virus in other tissues, or recent transmission to the infant either late in the third trimester or at the time of delivery.[276] Other options for diagnosing HIV infection in infants include HIV-specific IgA assays and an in vitro antibody production assay such as the ELISPOT (enzyme-linked immunosorbent spot).[251,277] However, the overall sensitivity and specificity of these tests are less than those of PCR and viral culture, especially for infants younger than 3 months.

Transmission of HIV in Health Care Settings

Percutaneous, mucous membrane, and cutaneous exposure to blood-contaminated body fluids can occur frequently in the health care setting.[278–280] Such exposure has resulted in occupationally acquired HIV infection in health care workers (see Chapter 298).[281–285] Data from several prospective surveillance projects among health care workers indicate that the average risk of seroconversion after a needlestick injury with HIV-infected blood is approximately 0.3%.[281–283] Percutaneous injury, usually inflicted by a hollow-bore needle, is the most common mechanism of occupational HIV transmission. The transmission of HIV has also been reported after mucous membrane and cutaneous exposure to blood, and in those instances the risk is estimated to be 0.09%.[286] As of December 2013 the CDC had received reports of 58 confirmed and 150 possible cases of HIV seroconversion temporally associated with occupational exposure to HIV among health care personnel in the United States.[287] HIV seroconversion temporally associated with an occupational exposure was not documented. In a retrospective study conducted by the CDC it was found that the risk of transmission of HIV to health care workers increased when the device causing the injury was visibly contaminated with blood, the device had been used for insertion into a vein or artery, the device caused a deep injury, or the source patient died within 2 months after the exposure.[288]

Because health care workers are more likely than patients to have contact with blood in the health care setting, the risk of HIV transmission from patient to health care worker clearly exceeds that of health care worker to patient.[289,290] Transmission of HIV from a health care worker to patients has been documented in two instances: in a dental practice in Florida[291,292] and from an orthopedic surgeon in France.[293] In the cluster of cases in Florida the precise events that resulted in the dentist transmitting HIV to 6 of approximately 1100 patients tested for HIV in this practice remain unknown. However, the 6 patients had no confirmed exposure to HIV other than receiving treatment from the dentist, and each was infected with a viral strain that was very similar to that of the dentist but dissimilar from other HIV-infected persons in the local area.[291,292] The very small risk of a health care worker transmitting HIV to a patient probably depends on several factors, including the type of procedure; the technique, skill, and medical condition of the health care worker; and the titer of circulating virus.[289,294,295] Aside from these two instances, the investigation of 22,759 patients of 53 other HIV-infected health care workers has not identified other episodes of transmission of HIV from a health care worker to a patient.[296]

Two patients undergoing nuclear medicine procedures have been reported to have been infected through inadvertent IV injections of blood or other material from HIV-infected patients.[297] In addition, transmission of HIV through percutaneous or mucocutaneous exposure to blood or other body substances has occurred in homes in which health care has been provided.[298,299] Transmission of HIV from patient to patient through improper sterilization or reuse of contaminated needles and syringes has been reported in Romania and the former Soviet Union.[300,301] Similarly, a report from Australia suggested that a breach in infection control precautions caused HIV to be transmitted from one patient to four others during minor surgical procedures

performed on the same day by an HIV-negative surgeon.[302] A more detailed review of transmission in the health care setting can be found in Chapter 298.

Other Modes of Transmission

Although HIV has been isolated from a variety of body fluids,[174,260,303–306] only blood, semen, other genital secretions, and breast milk have been implicated as sources of infection. HIV infection is acquired through exposure to blood, principally through IDU and receipt of contaminated blood, blood products, organs, and tissues. Exposure of nonintact skin to blood after a motor vehicle accident and a sports injury has been reported to result in HIV infection, but these occurrences are rare.[307,308]

Vaginal or anal intercourse is the predominant way in which persons are exposed to HIV-infected semen and cervicovaginal fluids. However, transmission of HIV through intravaginal insemination with unprocessed donor semen[309,310] and intrauterine insemination with processed semen[311] has been reported. Although data regarding the magnitude of the risks are conflicting,[309–311] there is no evidence that any procedure can reliably eliminate HIV from semen.[311]

Laboratory and epidemiologic studies indicate that the infectiousness of saliva from HIV-infected persons through human bites or occupational contact is extremely low. Furthermore, definitive attribution of HIV transmission to contact with saliva is difficult because saliva is often comingled with blood in these settings. The low risk of saliva-mediated HIV transmission is probably attributable to the very low concentrations of HIV in the saliva of infected persons[173,174] as well as the presence of HIV inhibitory activity in saliva.[312] One case report of two siblings infected with HIV suggested a bite as the route of transmission for the previously uninfected child.[313] However, because the bite did not break the skin or result in bleeding, the precise mode of transmission remains uncertain. Multiple epidemiologic studies, including occupational and household contact studies, have found no evidence of transmission via a human bite.[314] Similarly, studies of health care workers monitored prospectively after percutaneous, mucous membrane (e.g., during the administration of cardiopulmonary resuscitation), or nonintact skin exposure to saliva from HIV-infected patients have not detected any instances of HIV antibody seroconversion.[282,315]

To examine the risk of HIV transmission through casual contact, studies have evaluated more than 1000 nonsexual household contacts of both adults and children with HIV infection.[314,316–318] In these households transmission of HIV was found only among sex partners, children born to infected mothers, and persons who themselves had risk factors for HIV infection. However, eight case reports have described household transmission of HIV unassociated with sexual contact, IDU, or breastfeeding. Five of the eight reports were associated with documented or probable blood contact.[299,319–321] Two reports involved nursing care of terminally ill persons with AIDS in which blood exposure might have occurred but was not documented; in both reports skin contact with other secretions and excretions occurred.[299,322] In the last report a bite was suggested but not documented to have resulted in transmission.[313]

Laboratory and epidemiologic studies have produced no evidence of the replication of HIV within insects, in vitro mechanical transmission of HIV, or transmission through biting or bloodsucking insects.[323–325] The potential role of insect-mediated HIV infection was evaluated in a study of residents in a southern Florida community with a high rate of HIV infection.[326] HIV seropositivity was not associated with either epidemiologic or laboratory evidence of exposure to mosquitoes, as measured by the presence of antibodies to five arboviruses. Additional studies in Africa failed to establish an association between the presence of malaria antibodies and HIV.[327]

HIV INFECTION AND AIDS OUTSIDE THE UNITED STATES

HIV infection is pandemic, affecting almost all countries (see Chapter 118). Through December of 2017, United Nations Programme on HIV/AIDS (UNAIDS) and WHO estimate that approximately 36.9 (31.1–43.9) million persons are living with HIV worldwide, including 1.8 (1.3–2.4) million children younger than 15 years. UNAIDS/WHO estimate that in 2017, 21.7 (19.1–22.6) million people living with HIV were on ART, 940,000 (670–1.3 million) died from an AIDS-related illness, and 1.8 (1.4–2.4) million persons were newly infected with HIV, of which 70% occurred in sub-Saharan Africa, the most severely affected region of the world. Greater than 95% of all HIV-infected persons live in low- and middle-income countries (see Chapter 118). The modes of transmission of HIV are similar throughout the world, but the relative frequency varies considerably among countries and regions. In western Europe, North America, and Australia, as well as some parts of South America and the Caribbean, homosexual and bisexual men and PWIDs remain the predominantly affected groups.[328] In northern Europe most AIDS cases have occurred among homosexual and bisexual men, whereas in southern Europe, greater than 60% of persons with AIDS are PWIDs.[329] Although new HIV infections are declining globally, the only region of the world where new HIV infections are increasing is eastern Europe, with two countries, the Russian Federation and Ukraine, contributing 75% of all cases in the WHO European Region and 92% of cases in the East.[329–332]

In sub-Saharan Africa and some areas of the Caribbean, heterosexual contact is the most common mode of transmission.[328,333–339] IDU is now an important route of transmission of HIV in many developing countries and countries in transition, including those of the former Soviet Union.[331] The receipt of contaminated blood products remains a source of HIV transmission in many developing countries where HIV testing of blood and blood products is not regularly available, and medical injections with contaminated medical devices continue to result in some HIV infections.[327,328,332]

A more extensive discussion of AIDS in the developing world is presented in Chapter 118.

HIV-2

HIV-2 is a second human immunodeficiency virus that can result in severe immunodeficiency in some individuals but generally does so less frequently than in HIV-1–infected individuals. Compared with HIV-1 infection, asymptomatic infection is more common in HIV-2–infected individuals, virus loads in blood are lower, and transmission to partners or neonates is less frequent.[342–344]

HIV-2 likely emerged after cross-species transmission of simian immunodeficiency virus (SIV) from sooty mangabeys to humans. Infection with HIV-2 was first reported in western Africa in 1986.[345] Although cases of HIV-2 infection have since been reported in other parts of Africa, several European countries, Canada, the United States, Brazil, and India,[346] the virus continues to be found mostly among heterosexual persons in western Africa, but an increasing number of cases have been recognized in Europe, India, and the United States.[347–349] Data from surveillance and serologic surveys indicate that the prevalence of HIV-2 infection in the United States is extremely low.[337,338] In 1992 the FDA recommended that all blood donations be screened with serologic assays for HIV-1 and HIV-2. The serologic testing of more than 24 million blood donations found no HIV-2–infected persons.[338] Similarly, surveys conducted among persons presumably at increased risk of infection with retroviruses through sexual contact and IDU have found very low rates of HIV-2 infection.[346,349] In one survey, performed from 1988–1990, of 31,533 persons at high risk for HIV infection in the United States, 10% were found to be infected with HIV-1, but only 2 persons (0.006%) were seropositive for HIV-2.[350]

Accumulating information suggests that the modes of transmission for HIV-1 and HIV-2 are similar. Worldwide, HIV-2 infections have been diagnosed predominantly in men and women infected through heterosexual contact and to a lesser extent in homosexual men, PWIDs, transfusion recipients, and persons with hemophilia.[346,351] The lower level of shedding of HIV in genital secretions may help explain the different transmission rates between HIV-1 and HIV-2.[352] Although the perinatal transmission of HIV-2 has been reported, numerous studies suggest that HIV-2 is transmitted less efficiently than HIV-1 from mother to child.[351–356] The natural histories of HIV-1 and HIV-2 infection appear similar in that both are characterized by a broad spectrum of disease. However, the incubation period from the time of initial infection to the eventual development of AIDS may be longer for HIV-2.[351,357–359]

HIV-1 and HIV-2 are genetically and immunologically distinct. However, nucleotide sequence analysis indicates that HIV-1 and HIV-2 share a similar genomic organization, suggesting a common evolutionary origin.[360] Overall, the nucleotide sequence homology for HIV-1 and HIV-2 is approximately 40%; the *gag* and *pol* genes for the two viruses are approximately 60% homologous.[360] These genetic similarities can result in frequent serologic cross-reactions between HIV-1 and HIV-2. Current FDA-approved HIV diagnostic tests do not differentiate between HIV-1 and HIV-2 infection, but because the Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad, Hercules, CA) allows differentiation between HIV-1 and HIV-2 infection, the CDC has proposed a new HIV testing algorithm that first performs a fourth-generation test, followed by an HIV-1/2 differentiation to determine if the patient has antibodies to HIV-1 or HIV-2 (see Chapter 120).[361] HIV-2 is intrinsically resistant to the non-nucleoside reverse-transcriptase inhibitors and enfuvirtide.[362] Although dual infection with both HIV-1 and HIV-2 has been reported, they are uncommon outside endemic areas. In one study from France, among 3700 HIV-infected patients, only 17 were identified as dually infected.[363] Blood donations in the United States are currently tested for evidence of both HIV-1 and HIV-2 antibodies.

PREVENTION OF HIV INFECTION

The prevention of HIV infection must be based on strategies that interrupt sexual, bloodborne, and perinatal transmission of the virus. Such strategies must be grounded in an understanding of the epidemiology of HIV infection and knowledge of the science of human behavior. These science-based strategies are the foundation for the design, implementation, and evaluation of prevention efforts. A number of behavioral interventions have been tested and applied to reduce HIV-associated risk behaviors across a variety of populations. In general, these programs are theory driven and emphasize the development of the cognitive, social, and technical skills associated with safer sex and drug use practices.[364] CDC has compiled a compendium of evidence-based HIV behavioral interventions (EBIs).[365] These interventions have been rigorously evaluated and have demonstrated evidence of efficacy. The current EBIs include 74 HIV risk-reduction behavioral interventions for which there is best evidence for 44 and good evidence for 30.[366] However, it should be noted that these interventions have been proven to reduce HIV-related risky behaviors but not HIV incidence in randomized controlled trials. As a number of biomedical interventions, in particular ARV drugs, have been proven to be effective for HIV prevention, the concept of "combination prevention" has emerged. Combination prevention is defined as rights-based, evidence-informed, and community-owned programs that use a mix of biomedical, behavioral, and structural interventions prioritized to meet the HIV prevention needs of particular individuals and communities and to have the greatest sustained impact on reducing new infections.[367]

Prevention of Sexual Transmission

Strategies for the prevention of the sexual transmission of HIV have focused up to very recently on promoting sexual abstinence, reducing unsafe sexual behavior (unprotected anal intercourse or decreasing the number of partners), encouraging proper condom use, and treating sexually transmitted infections.[368] The consistent use of latex condoms has been shown to be effective for the prevention of HIV transmission at the level of both the individual and the population.[369–371] Multiple epidemiologic studies of heterosexual couples in which one partner is HIV positive and the other HIV negative indicate that the correct and consistent use of latex condoms can significantly reduce the transmission of HIV and other STDs.[372–375] Natural skin condoms do not prevent the transmission of HIV and should not be used. Similarly, petroleum-based lubricants increase the likelihood of rupture of latex condoms and should be avoided.

The effectiveness of condoms to prevent the heterosexual transmission of HIV has been estimated to be 87%, but it may be as low as 60% or as high as 96%.[376,377] Many factors can affect the effectiveness of condoms in reducing the risk of HIV transmission, such as condom breakage, leakage, and slippage. Although condom breakage can occur, it appears to be uncommon, particularly in developed countries, where studies have found breakage rates of 2% or less for vaginal or anal intercourse.[370,372] Similarly, low rates of slippage (i.e., <1%) have been reported.[377] The effectiveness of condoms during anal intercourse is probably lower because condom breakage and slippage may be considerably higher than they are during vaginal intercourse.[378]

Intravaginal pouches ("female condoms") also require correct and consistent use.[379,380] The female condom has been shown to be as effective as the male condom for the prevention of sexually transmitted infections,[380–382] and its contraceptive failure rate has ranged from 11% to 26%, depending on the consistency and correctness of usage.[381] However, the contraceptive failure rate of a condom may not accurately reflect its effectiveness in reducing heterosexual HIV transmission.[370,372]

Currently available spermicides do not protect against the transmission of HIV and other sexually transmitted infections, and nonoxynol-9 might increase the risk for HIV sexual transmission. Three randomized, controlled trials of the use of nonoxynol-9 by commercial sex workers in Africa failed to demonstrate any protection against HIV infection,[383,384] with one showing an increased risk of HIV transmission with the use of a vaginal sponge containing a high dose of nonoxynol-9.[385] Nonoxynol-9–containing contraceptives also failed to protect against infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in two randomized trials, one among prostitutes in Africa and one among US women recruited from an STD clinic.[386,387] The irritative effects of spermicides on the vaginal epithelium may in fact facilitate rather than reduce HIV transmission.[388] Despite these data, contraceptives containing nonoxynol-9 continue to be commonly used in family planning clinics.[389] Several other available topical microbicides that have been studied in clinical trials thus far have also failed to demonstrate efficacy.[390,391] A placebo-controlled study of a polynaphthalene sulphonate gel (PRO2000), completed in 2009 in Malawi, South Africa, Zambia, Zimbabwe, and the United States, reported that HIV infections were reduced by 30% in women who used PRO2000 vaginal gel; however, this difference did not reach statistical significance.[392] The use of a microbicide gel containing the ARV tenofovir has demonstrated efficacy in reducing HIV incidence. In a study conducted in South Africa (CAPRISA 004) this product demonstrated an overall reduction in HIV acquisition of 39% and 54% among women with high gel adherence.[393] This data was not confirmed by the VOICE (MTN-003) trial, where a 1% tenofovir vaginal gel was no better than placebo in preventing HIV acquisition.[394] The quest to develop an effective vaginal microbicide is critically important as female-controlled methods to prevent HIV and STDs that can be safely and effectively used by women are urgently needed.

Despite the demonstrated benefits of condom use, multiple studies have found relatively low rates of consistent condom use among sexually active homosexual men and heterosexual men and women. The factors that influence condom use are complex.[395] Concern about decreased sexual pleasure or a partner's lack of cooperation, inadequate communication skills, the temporal effects of drugs or alcohol, the use of other methods for contraception, and cultural influences have been correlated with low rates of condom use.[395–398]

As previously stated, three randomized trials of male circumcision have demonstrated that circumcision provides a protected benefit of between 50% and 60% against the acquisition of HIV infection among men who engage predominantly in heterosexual intercourse.[194–196] Although these results are clearly encouraging and studies have proven the protective effect of male circumcision, there still remain many challenges in the implementation of this intervention to obtain the full impact of circumcision on HIV incidence among those populations the most heavily affected by HIV.[399]

Partner notification is another mechanism to assist in the prevention of sexual transmission of HIV. Even though many HIV-infected individuals cooperate in notifying at least some of their sex partners, others do not.[400,401] Although the effectiveness of contact tracing and partner notification has been hotly debated,[402] it is probably an effective prevention strategy,[403,404] particularly when it is targeted to acute HIV infection.[405] The efficacy of partner notification by the health department appears to be substantially more effective than notification by the infected person or the patient's physician.[401,406,407]

The control of sexually transmitted infections is also an important intervention to reduce the sexual transmission of HIV. Data from a study in Tanzania in which a syndrome approach was used in the

treatment of symptomatic sexually transmitted infections led to a 42% decrease in HIV incidence.[408] In contrast, in the Rakai district of Uganda a community-based trial of mass treatment for sexually transmitted infections failed to show a difference between the treatment and control arms.[409] The reasons for the lack of consistency in the results of these two trials are multiple and not yet fully explained. Nevertheless, despite lack of proven efficacy in the prevention of HIV infection, the diagnosis and treatment of sexually transmitted infections remains important for the individual as well as for public health.

Preexposure Prophylaxis

ARVs have shown to be highly effective for the prevention of HIV when administered prophylactically to HIV-uninfected but at-risk individuals (preexposure prophylaxis [PrEP]) and when administered after sexual exposure (postexposure prophylaxis).[410] In the iPrEx (Initiativa Profilaxis Pre-Exposición [Preexposure Prophylaxis Initiative]) randomized trial, daily administration of coformulated tenofovir plus emtricitabine as PrEP among MSM decreased the risk of HIV infection by 44%.[411] However, among participants with detectable levels of tenofovir, there was a 92% reduction in risk of HIV acquisition. Subsequent studies using also tenofovir coformulated with emtricitabine have shown as much as an 85% decrease in HIV transmission when adherence is four or more tablets per week.[412] Studies among heterosexual couples have found high level of protection when adherence was also high[413] and no reduction in HIV acquisition when adherence was low.[394,414] The Partners PrEP trial was a randomized placebo-control study of oral daily tenofovir or tenofovir coformulated with emtricitabine for HIV prevention among HIV-uninfected serodiscordant heterosexual couples in Uganda and Kenya. Among participants of both sexes, the efficacy of tenofovir alone was 67% and 75% for tenofovir-emtricitabine. Efficacy estimates were not statistically different between men and women. Among participants assigned to tenofovir-emtricitabine who had detectable drug levels, there was a 90% reduction in HIV acquisition.[413] In contrast, in the FDF2, the FEM-PrEP, and the VOICE studies, low levels of adherence did not reduce the risk of HIV acquisition among participants.[394,414,415] Taken together, these studies suggest that daily oral PrEP with tenofovir-emtricitabine is also effective among heterosexual couples but that adherence needs to be high (Table 119.4). Of importance, in all studies the drugs have been extremely safe and well tolerated. Based on these findings, in 2013 the FDA approved the use of coformulated tenofovir plus emtricitabine for preexposure prophylaxis to prevent sexual acquisition of HIV.[415]

ARVs can also be given after exposure to prevent HIV acquisition (postexposure prophylaxis). In 2005 the CDC published guidelines for ARV postexposure prophylaxis after sexual, IDU, and other nonoccupational exposures to HIV. In these guidelines it is recommended that persons seeking care 72 hours or sooner after nonoccupational exposure to blood, genital secretions, or other potentially infected body fluids of a person known to have HIV infection be offered a 28-day course of ART.

As previously stated, reducing the viral load through the use of ART dramatically reduces the risk for transmission through sexual contact.[166] In a randomized controlled trial ART given to HIV-infected persons, beginning at a CD4 count of 350 to 550 cells/mm^3, reduced sexual transmission of HIV-1 by 96%. This is by far the most effective intervention to date for the prevention of HIV infection. The realization that treatment is prevention has led to a variety of test-and-treat strategies.[416]

Prevention of Transmission by Injection Drug Use

The prevention and treatment of IDU are critical for reducing HIV transmission among PWIDs,[417] and several studies have documented that significantly lower rates of drug use and related risk behavior are practiced by PWIDs who are in treatment.[418,419] In addition, substance abuse treatment can serve as an entry point for medical care. However, the impact of substance use treatment on HIV infections has been observed only for those users who remain in treatment for at least 1 year.[420] For this reason, brief detoxification programs are not considered effective strategies for HIV prevention unless they are followed by a longer course of treatment. Furthermore, an estimated 80% of active drug users in the United States are not in treatment because of choice or the unavailability of treatment.[421] An integrated approach to service delivery for persons who use drugs that incorporates science-based prevention strategies is critical for the prevention of HIV and other infectious diseases among substance abusers.[422] The removal of restrictions on the purchase of needles and syringes,[423] needle and syringe exchange programs,[424] the proper use of bleach for the disinfection of drug injection equipment,[425] and medication-assisted treatment, such as methadone maintenance programs,[426] are all effective interventions for the prevention of HIV infection among PWIDs. Community outreach-based interventions that include education about HIV transmission and prevention and the distribution of condoms and bleach kits have also demonstrated significant changes in the frequency of drug use and drug-related behaviors.[427] Particularly useful have been risk reduction interventions delivered through social networks by peer educators.[428] Improvement in selected drug use behavior has been reported, including a decrease in the sharing of drug-injection equipment and an increase in the use of bleach for cleaning equipment,[427–431] although the duration of such behavior has not been studied extensively. The overwhelming majority of studies have found that needle exchange programs lead to reduction in the sharing of syringes among program participants,[432,433] do not result in increased drug use among participants or in the recruitment of first-time users, and reduce the transmission of HIV among PWIDs.[434–437] A December 2011 US Congressional ban on the use of federal funds for any program that distributes sterile needles or syringes for hypodermic injection of illegal drugs was lifted in early 2016, largely in response to a severe needle-associated HIV and hepatitis C outbreak in Indiana. PrEP has also proven to be effective among PWIDs. In the Bangkok Tenofovir Study the use of oral tenofovir reduced the acquisition of HIV by 48.9%, and in participants who had detectable tenofovir levels, there was a 73.5% reduction in risk of HIV acquisition. As a result, the CDC published guidelines in 2013 recommending daily oral PrEP with tenofovir-emtricitabine (or tenofovir alone) for PWIDs at

TABLE 119.4 Selected Studies of Preexposure Prophylaxis Using ARV to Prevent HIV Infection

STUDY	ARV	POPULATION	*N*	SITES	EFFICACY ON HIV INCIDENCE
CAPRISA 004[393]	TDF gel	Women	889	South Africa	39% reduction
iPrEx[411]	TDF/FTC	MSM	2499	South America, Africa, South East Asia	44% reduction
Partners PrEP[414]	TDF TDF/FTC	Heterosexual serodiscordant couples	4758	Africa	67% TDF 75% TDF/FTC
TDF2[415]	TDF/FTC	Heterosexual men and women	1200	Botswana	62% efficacy
FEM-PrEP[413]	TDF/FTC	Women	1950	Kenya and South Africa	Stopped for futility
MTN-003 VOICE[394]	TDF tablet and gel 1% TDF/FTC	Women	5029	Uganda, Zimbabwe, South Africa	TDF tablet and gel stopped for futility. TDF/FTC also not effective

ARV, Antiretrovirals; *FTC,* antiretroviral drug emtricitabine; *MSM,* men who have sex with men; *TDF,* antiretroviral drug tenofovir.

risk of HIV infection.[307] In summary, there is clear evidence that a combination of prevention approaches could eliminate HIV transmission among PWIDs. Unfortunately, the implementation of many of these interventions has been limited by public or political opposition.[438]

Prevention of Transmission Through Blood and Other Tissues

The first report of transfusion-associated AIDS was in 1982. In 1983 blood banks initiated the voluntary self-exclusion of donors with risks for HIV infection. In 1985 the first serologic assays for the HIV antibody became available, and the use of these HIV serologic tests to screen blood donations dramatically decreased the risk of transfusion-associated HIV transmission.[236,237] The serologic identification of repeat donors with HIV infection (including the detection of p24 antigen and NAAT), screening of blood for hepatitis C and human T-lymphotropic virus types I and II, and reduction in the number of transfusions performed have also lowered the risk of transmission.[237–239,439] The risk of HIV infection in the United States through the transfusion of blood or blood products is currently extremely low and is estimated to be 1 in 1.4 to 1 in 1.8 million units.[59] Organ and tissue donors should be evaluated and serologically screened in a manner similar to that for blood donors.[440,441] In addition, donations of semen and bone from a living donor may be quarantined until subsequent testing has definitively ruled out the possibility of delayed seroconversion in the donor. The prompt administration of ARV drugs for postexposure prophylaxis after transfusion of contaminated blood prevented HIV infection in one patient.[442]

Prevention of Transmission in the Health Care Setting

The effective prevention of HIV transmission in the health care setting requires a multifaceted approach to reduce the frequency of occupational blood exposure among health care workers. Such a strategy includes engineering controls that do not rely on worker compliance (e.g., self-sheathing needles), safe work practices and techniques, personal protective equipment, and training.[443,444] In particular, a reduction in percutaneous injuries will require the development of puncture-resistant gloves, the redesign of needles and other sharp instruments, or both. In 1987 the CDC recommended that the principle of "universal precautions" be incorporated in programs for infection control.[445] Under universal precautions, blood and certain other body fluids from *all* patients are considered to be potentially infective. Universal precautions include the appropriate use of hand washing and protective barriers, care in the use and disposal of needles and other sharp instruments, and appropriate disinfection and sterilization of reusable equipment. For occupational exposure to HIV among health care workers, the CDC recommended the use of zidovudine for postexposure prophylaxis in 1990.[446] This recommendation was further supported by a case-control study involving health care workers from the United States, France, Italy, and the United Kingdom that showed that the risk of HIV seroconversion after occupational exposure decreased by approximately 81% with the use of zidovudine.[288] Subsequent recommendations have incorporated the newer ARV drugs as well as risk stratification for the type of exposure in the management of occupational exposure to HIV.

Prevention of Perinatal Infection

The primary prevention of perinatally acquired HIV infection must center on routine, voluntary counseling and HIV antibody testing and ART of pregnant women living with HIV (see Chapter 126).[447,448] Because a substantial proportion of women may not initially acknowledge high-risk behavior or know the infection status of their partners, routine HIV testing and counseling must be considered a standard of care when managing pregnant women.[449] In 1994 the results of a randomized, double-blind clinical trial (Pediatric AIDS Clinical Trials Group Protocol 076) found that zidovudine therapy administered to HIV-infected women during pregnancy, at the time of labor, and postpartum to their infants was associated with a 67.5% reduction in the risk of perinatal HIV transmission.[139] Based on these results, a Public Health Service task force issued recommendations for the use of zidovudine for the reduction of perinatal HIV-1 transmission.[140] Several subsequent studies have confirmed the benefits of zidovudine for the prevention of perinatal HIV-1 transmission, even when the drug is given for a much shorter period than it was in the original Protocol 076 trial.[450,451] Advances in understanding the pathogenesis of HIV infection and the availability of laboratory tests to monitor the disease (such as HIV RNA) and better ARV drugs have led to an update in 2008 in the recommendations for the use of ARV drugs in pregnant women.[452] The implementation of these recommendations, which include universal prenatal HIV counseling and testing, ARV prophylaxis, scheduled cesarean delivery, and avoidance of breastfeeding, has resulted in significant public health benefits. The current risk of perinatal HIV transmission in the United States is less than 2%,[453] and the number of children in whom AIDS attributed to perinatal HIV transmission was diagnosed has decreased from a peak of 954 in 1992 to 14 in 2010,[67] and the elimination of perinatal HIV transmission in the United States is a feasible goal. Unfortunately, in most of the world perinatal HIV transmission continues to be an important way in which children acquire HIV infection globally, and further efforts are necessary to reduce mother-to-child HIV transmission.

The prevention of postnatal transmission of HIV infection through breastfeeding must take into account the likelihood of competing risks for morbidity and mortality associated with feeding alternatives in developing countries. In 1985, after the first case report implicating HIV transmission from breast milk and the isolation of HIV from breast milk, the CDC recommended that HIV-seropositive women not breastfeed their infants.[454] This recommendation was intended for mothers in the United States, where alternative, safe, and nutritious substitute feeding methods are readily available.[261] In 1992 WHO and the United Nations International Children's Emergency Fund developed a consensus statement on HIV transmission related to breastfeeding, which stated that "in settings where the primary causes of infant deaths are infectious diseases and malnutrition, breast-feeding should remain the standard advice to pregnant women, including those who are HIV infected."[455] By 1996 UNAIDS had published a revised statement that supported breastfeeding in all populations for whom other safe options are not available, irrespective of HIV infection rates, but recommended counseling for women about the risks of HIV transmission through breastfeeding.[456]

When children born to HIV-infected women can be ensured uninterrupted access to nutritionally adequate breast milk substitutes that are safely prepared and fed to them, they are at less risk of illness and death if they are not breastfed. However, when these conditions are not fulfilled, in particular in an environment where infectious diseases and malnutrition are the primary causes of death during infancy, artificial feeding substantially increases a child's risk of illness and death. In those settings it is preferable for women to provide exclusive breastfeeding to their infants rather than mixed feeding (breast milk plus food supplements) because the risk of HIV transmission is substantially lower when the infant receives only breastfeeding.[457] The importance of providing safe alternatives to breastfeeding among HIV-infected women has been highlighted by several randomized trials on the prevention of perinatal transmission in breastfeeding populations. For example, in the PETRA (PErinatal TRAnsmission) trial, the benefits of combination ART diminished considerably as a result of breastfeeding.[458] In the South African Intrapartum Nevirapine Trial (SAINT), breastfeeding was the most significant risk factor for transmission from mother to child when ART was administered to prevent intrapartum and early postpartum HIV transmission.[459] Recent trials providing ART to the mother during breastfeeding have proven to be an effective approach to reducing HIV infection through breast milk.[460]

COUNSELING AND HIV ANTIGEN/ANTIBODY TESTING

Early recognition of HIV infection through HIV antibody testing has been one of the primary objectives of HIV prevention efforts. However, with the realization that treatment of HIV infection has prevention benefits, HIV testing has become an even more important preventive intervention.[461,462] The major benefits of HIV testing programs are (1) linkage to care and prevention services of HIV-seropositive persons, (2) counseling to promote the behavior change necessary to reduce

HIV transmission, and (3) referral to PrEP programs of high-risk individuals found to be HIV uninfected when these programs are available.

Physicians have an essential role in this public health effort. As the principal providers of primary health care, they are the most frequently named by the general public as the desired source for HIV testing.[463] The number of persons who have been tested for HIV has increased significantly in recent years, and it is now estimated that 46% of adults and adolescents in the United States report having ever been tested for HIV infection.[464] However, many who are tested for HIV do not return for their results. The CDC estimates that 31% of those who tested HIV positive in 2000 at publicly funded testing sites of the CDC failed to return for their results.[461] The approval by the FDA of rapid HIV tests, such as the OraQuick HIV rapid test (OraSure Technologies, Bethlehem, PA) and the subsequent CLIA (Clinical Laboratory Improvement Amendment) waiver of this test, offers the possibility for more persons to be tested for HIV and receive their results on the same day, eliminating the need for a second visit.[465] Although some concerns have been expressed about the reliability of rapid tests, several studies have demonstrated that the sensitivity and specificity of the commercially available rapid tests are comparable to those of standard EIA testing (see Chapter 120).[466] Rapid tests may have poor positive-predictive value in low-prevalence settings.[467]

In an effort to make HIV testing more accessible and available in clinical settings, the CDC issued counseling and referral guidelines in 2006.[447] These guidelines recommend that an "opt-out" approach be implemented for HIV testing in health care settings, in which all patients between the ages of 13 to 64 are notified that they will be tested for HIV unless they decline. Prevention counseling is no longer a part of routine HIV testing but is still recommended for persons known to engage in behaviors that increase their risk of acquiring HIV infection. The guidelines also recommend annual testing for persons considered to be at high risk. Although this approach has been shown to be cost-effective in several studies,[468,469] it has still not gained wide acceptance as a screening strategy.[470] In 2013 the US Preventive Services Task Force updated their 2005 recommendations on HIV screening and recommended that clinicians screen adolescents and adults age 15 to 65 years and that younger adolescents and older adults who are at increased risk should also be screened. Both these recommendations received a grade A recommendation.[471]

Couples voluntary counseling and testing (CVCT) has been used as an HIV prevention intervention for heterosexual couples in Africa for more than 20 years.[472] The critical difference between the CVCT model and the conventional model of individual HIV testing is that the couple receives all counseling and testing at the level of the dyad and allows the implementation of prevention and care interventions based on their joint HIV status. CVCT has recently been adopted for male-male couples in the United States.[473]

Health care workers who perform invasive procedures that are considered prone to exposure (e.g., procedures that include digital palpation of a needle tip in a body cavity or the simultaneous presence of the health care worker's fingers and a needle or other sharp object in a poorly visualized or highly confined anatomic site) should know their HIV antibody status. The mandatory testing of health care workers for the HIV antibody is not recommended.[474]

The use of short-term case management to link newly diagnosed HIV infected persons into care has been shown to be effective and is a recommended intervention to decrease the possibility that HIV-infected persons may fail to enter care.[475,476] HIV-infected persons should also be instructed to notify sex or needle-sharing partners and refer them for HIV counseling and testing. If HIV-infected persons are reluctant to directly inform their partners, physicians may offer to inform the partners or seek the assistance of the local health department. Confidentiality is very important, to protect these individuals and not discourage them from seeking HIV testing.

Persons found to be HIV seropositive should be referred for medical evaluation, including immunologic ($CD4^+$ T-lymphocyte cell counts) and virologic (quantitation of HIV RNA) monitoring, screening for other STDs, prophylaxis against certain opportunistic illnesses, vaccinations, ART, and other preventive and therapeutic services.[477]

VACCINES FOR HIV TYPE 1 INFECTION

The development of a safe and effective HIV-1 vaccine is one of the highest biomedical research priorities. Effective vaccination offers the best hope for the ultimate control of the HIV-1 epidemic,[478] but no vaccine is near clinical use despite more than 35 years of intensive research in this field. HIV-1 vaccine development presents unprecedented scientific challenges.[479,480] Among the most important roadblocks is our current lack of understanding of the immune correlates of protection against HIV-1 and our inability to induce broadly reactive neutralizing antibody (NAb) responses by vaccination. In addition, enormous genetic variation exists among HIV-1 strains worldwide. Nevertheless, new vaccine candidates aimed at eliciting virus-specific cellular and humoral immune responses have been able to afford partial protection in certain nonhuman primate models, and several novel passive and active immunization strategies are currently being tested for clinical efficacy. In this chapter we review recent progress in the clinical development of candidate HIV-1 vaccines.

Animal Models

The lack of an accepted small animal model for HIV-1 infection has slowed vaccine development. Nonhuman primates have therefore been required for the preclinical evaluation of candidate HIV-1 vaccines. Chimpanzees can be infected with HIV-1, but most viruses are nonpathogenic in this species.[481,482] The most extensively studied nonhuman primate model involves SIV infection of rhesus macaques (*Macaca mulatta*).[483] SIV and HIV-1 are similar in genetic structure and organization, and certain pathogenic strains of SIV cause an AIDS-like disease in macaques, including opportunistic infections.[484,485] As a result, SIV infection of rhesus macaques has emerged as a popular model for testing HIV-1 vaccines. A related model, involving chimeric simian-human immunodeficiency virus (SHIV) infection of macaques, has also been used.

The importance of humoral immune responses is illustrated by studies in rhesus macaques in which passive infusions of HIV-1–specific neutralizing monoclonal antibodies afforded complete protection against infection with pathogenic SHIV isolates.[486,487] Although vaccine-elicited antibody responses have been shown to provide a degree of protection against homologous and heterologous viral challenges in certain settings,[488–492] it has proven extraordinarily difficult to generate broadly reactive NAbs by vaccination using recombinant envelope proteins.

Vector-based strategies have also been explored in rhesus macaques. Poxviruses have been explored extensively as candidate HIV-1 vaccine vectors but have proven only weakly immunogenic.[493–498] Replication-incompetent, recombinant adenovirus serotype 5 (rAd5) vectors have been shown to elicit robust cellular immune responses in nonhuman primates[499,500] but have shown no protection against SIVmac251 challenges.[9,10,118] Studies with Ad26/Env vaccines have shown partial protection against both SIVmac251 and SHIV-SF162P3 challenges in rhesus macaques, with functional non-NAbs as the correlate of protection in these models.[488,501] Another promising vaccine study showed that CMV vectors afforded early and profound virologic control in approximately half of the vaccinated animals after SIV challenge.[502]

Human Trials

More than 50 candidate HIV-1 vaccines have been studied in more than 30,000 HIV-1–uninfected healthy volunteers since 1987. The study of candidate HIV-1 vaccines in humans poses several unique problems above and beyond those encountered in studies of other experimental vaccines. Volunteers need to be fully informed of the potential hazards of immunization and of the limitations of our knowledge regarding HIV-1 transmission, pathogenesis, and immune control. Volunteers must be made aware that they may become seropositive for HIV-1 by conventional screening assays,[503–507] and the recent adoption of an "opt-out" policy for routine HIV-1 testing increases the potential for false-positive results to occur.[508,509] Volunteers must also be counseled not to abandon behaviors to reduce risk for acquisition of HIV-1 infection because of a presumption that the vaccine under study will prove effective. A detailed description of the procedures used in the conduct of phase I studies of HIV-1 candidate vaccines in humans, including measures taken to address the preceding issues, has been published.[510]

The purpose of phase I studies is to examine the immunogenicity and safety of vaccine candidates in humans. A wide variety of different types of vaccine candidates have been evaluated in humans. For most phase I and phase II studies (Table 119.5), individuals at low risk for acquisition of HIV-1 infection are enrolled to avoid the potentially confounding effects of intercurrent HIV-1 infections. In contrast, phase IIb and III studies are carried out in participants at higher risk for acquisition of HIV-1 infection (Table 119.6).[511–518]

Purified envelope protein,[513–515,519,520] synthetic peptides,[521–530] live vector–based,[531–559] and DNA[560–564] vaccines have been explored in clinical trials. In addition, various adjuvant strategies have been studied, including alum, MF59, and plasmid cytokines, such as granulocyte-macrophage colony-stimulating factor, IL-2, IL-12, and IL-15. Various delivery modalities have also been assessed for DNA vaccines, including intramuscular injection, bioinjector (compressed air injection system), and electroporation.[510,565–573]

Phase II Clinical Studies

Six phase II studies of candidate HIV-1 vaccines by the AIDS Vaccine Evaluation Group (AVEG) and its successor, the HIV Vaccine Trials

TABLE 119.5 Phase II Studies of Candidate HIV-1 Vaccines in HIV-1–Seronegative Volunteers

VACCINE	COMPOSITION	HIV-1 STRAINS	SPONSOR	REFERENCES
rgp120	Protein	SF-2 + MN	Chiron/Biocine, Aventis Pasteur	574
vCP205, rgp120	Canarypox prime, protein boost	MN/LAI (vCP205), SF-2 (rgp120)	Aventis Pasteur, Chiron/Biocine	575
vCP1452, rgp120	Canarypox prime, protein boost	MN/LAI (vCP1452), MN + GNE8 (rgp120)	Aventis Pasteur, VaxGen	76
VRC-HIVDNA-016-00-VP, VRC-HIVADV014-00-VP	DNA prime, rAd5 boost	Clade B HXB2 *gag,* NL4-3 *pol,* NY5/BRU *nef*; clade A 92rw020, clade B HXB2/BaL, clade C 97ZA012 *env*	NIH VRC	77
pGA2/JS7, MVA/HIV62	DNA prime, rMVA boost	BH10, IIIB, HXB-2/ADA *gag,* protease, reverse transcriptase, *env, tat, vpu, rev*	Geovax	578
Ad26.Mos.HIV, MVA.Mos, and clade C Env gp140	rA26 prime, rAd26, rMVA, ± protein boost	Synthetic bivalent mosaic inserts (*env, gag,* and *pol*)	Janssen, NIH, MHRP	579

MHRP, Military HIV Research Program; *NIH VRC,* National Institutes of Health Vaccine Research Center; *rAD5,* recombinant adenovirus serotype 5; *rgp120,* recombinant glycoprotein 120; *rMVA,* modified vaccinia Ankara (vaccine); *vCP205,* canarypox vector 205.

TABLE 119.6 Completed Efficacy Studies (Phase IIb, III) of Candidate HIV-1 Vaccines in HIV-1–Seronegative Volunteers

VACCINE	START	SAMPLE SIZE	LOCATION	TARGET POPULATION	HIV INFECTION RATE IN VACCINE GROUP (%)	HIV INFECTION RATE IN PLACEBO GROUP (%)	VACCINE APPROACH; HIV-1 STRAINS	VACCINE DEVELOPER	REFERENCES
AIDSVAX B/B	1998	5403	North America, Netherlands	MSM	6.7	7.0	rgp120: MN, GNE-8	VaxGen	513
AIDSVAX B/E	1999	2546	Thailand	IVDU	8.4	8.3	rgp120: MN, A244	VaxGen	516
Step Study (HVTN 502)	2004	3000	North America, Caribbean, Australia	MSM; sexual exposure	4.6[a]	3.1	Ad5 *gag/pol/nef*: clade B *gag*-CAM-1, *pol*-IIIB, *nef*-JR-FL	Merck	511
Phambili Study (HVTN 503)	2006	3000[b]	South Africa	Sexual exposure	Stopped early 4.54[c]	Stopped early 3.70	Ad5 *gag/pol/nef*: clade B *gag*-CAM-1, *pol*-IIIB, *nef*-JR-FL	Merck	517
ALVAC-HIV (vCP1521) AIDSVAX B/E (RV144)	2003	16,402	Thailand	Sexual exposure	0.192[d]	0.279	Canarypox and rgp120	Sanofi Pasteur, VaxGen	601
DNA/Ad5 (HVTN 505)	2009	2504	United States	MSM	2.8%[e]	2.3%	DNA prime, Ad5 boost. Clade B HXB2 *gag,* NL4-3 *pol,* NY5/BRU *nef*; clade A 92rw020, clade B HXB2/BaL, clade C 97ZA012 *env*	NIH VRC	518

Ad5, Adenovirus serotype 5; *HVTN,* HIV Vaccine Trials Network; *IVDU,* intravenous drug user; *MSM,* men who have sex with men; *rgp120,* recombinant glycoprotein 120; *VAX,* vaccine; *vCP,* canarypox vector.

[a]Yearly HIV-1 incidence in MSM population (n = 1836) as of October 17, 2007.

[b]Target enrollment was 3000. Actual enrollment was 801 before study termination on September 19, 2007 and unblinded in October 2007. At the time the study was stopped, 56 (7%) of subjects had received all three planned injections.

[c]Yearly HIV-1 incidence per 100 person-years, through August 31, 2009.

[d]Yearly HIV-1 incidence per 100 person-years.

[e]Stopped early April 22, 2013 by the Data Safety Monitoring Board because futility criteria were met. Study was also unblinded.

Network (HVTN), have been performed. The first study (AVEG 201) evaluated two recombinant glycoprotein 120 (rgp120) subunit vaccines in 296 HIV-1–seronegative subjects in six demographic groups: four with higher-risk behavior and two with lower-risk behavior.[574] After three immunizations, greater than 87% of subjects developed NAbs to the homologous isolate, which persisted for at least 2 years in 57% of subjects who were tested. However, the study showed somewhat lower antibody responses to one of the vaccines (HIV-1_{SF-2} gp120) in the IV drug user group and, to a lesser extent, in heterosexual partners of HIV-1–infected individuals. The reasons for this have not been explained, but this study illustrates the importance of assessing immunogenicity in groups other than low-risk subjects.

The second phase II study was a collaboration between AVEG and HIVNET (AVEG 202–HIVNET 014).[575] This study examined a prime-boost regimen consisting of the canarypox vector vCP205 with an rgp120 (SF-2) boost in 435 subjects, of whom 60 were lower risk and 375 were higher risk. The vaccines were generally well tolerated in these groups. Greater than 90% of subjects who received the prime-boost regimen developed NAbs against the homologous T-cell line adapted (TCLA) virus, and one-third of those who received regimens containing vCP205 developed HIV-1–specific $CD8^+$ T-lymphocyte responses. There appeared to be no significant difference in immune responses between the higher- and lower-risk groups in this study.

The HVTN completed a phase II study of vCP1452, which expressed gp120, the transmembrane portion of gp41, *gag*, a portion of *pol*, and several $CD8^+$ T-lymphocyte epitopes from *nef* and *pol* (HVTN 203). The vector was administered at a dose of $10^{7.26}$ mean tissue culture infectious dose ($TCID_{50}$) at a regimen of 0, 1, 3, and 6 months to 330 subjects. The vaccine was well tolerated, but the overall immunogenicity was disappointing. Vaccinees developed HIV-1–specific $CD8^+$ T-lymphocyte responses at a cumulative frequency of less than 30% at any point throughout the study and less than 15% at any single time point.[227] Why vCP1452 was less immunogenic in this phase II study than other canarypox constructs in phase I studies is not clear. Raising the dose of vCP1452 to 10^8 significantly increased reactogenicity but did not augment immunogenicity.[576]

The second phase II trial conducted by the HVTN assessed the safety and immunogenicity of a DNA prime/rAd5 boost regimen developed by the National Institutes of Health Vaccine Research Center (NIH VRC; HVTN 204). This vaccine contained six DNA plasmids (*gag, pol*, and *nef* from clade B and *env* from clades A, B, and C) and four rAd5 vectors (*gag-pol* from clade B and *env* from clades A, B, and C). This placebo-controlled study enrolled 480 subjects irrespective of baseline Ad5 titer in the Americas (United States, Brazil, Jamaica, and Haiti) and South Africa and administered 4 mg of DNA at 0, 1, and 2 months, followed by an rAd5 boost (1×10^{10} viral particles) at month 6. The vaccine regimen was well tolerated and moderately immunogenic with cellular immune responses directed at global potential T-cell epitopes, detected to *env* and *gag* in 54% and 55% of subjects, respectively. This regimen also induced modest binding antibody responses in subjects to the envelope glycoprotein Con S (95%), clade A (84%), clade B (95%), and clade C (93%) gp140 oligomers, but limited NAb responses to Tier 1 viruses.[577] This regimen was advanced into a phase IIb efficacy study (HVTN 505) in Ad5-seronegative, circumcised MSM and who are at high risk for acquiring HIV-1 (which is described later).

A third phase II trial has been conducted by the HVTN, which assessed the safety and immunogenicity of an modified vaccinia Ankara (MVA) alone and a DNA prime/MVA boost regimen (HVTN 205). These vaccines, pGA2/JS7 DNA (expressing *gag, pol, env, tat, rev,* and *vpu* genes) and MVA/HIV62 (expressing *gag, pol*, and *env* genes), were studied in 300 vaccinia-naïve individuals, of whom 75 received placebo. The vaccines were given in two different regimens—3 mg of DNA given at 0 and 2 months, followed by MVA given at 4 and 6 months (DDMM regimen) versus three doses of the MVA given at 0, 2, and 6 months (MMM regimen). Both of these regimens were found to be generally safe and well tolerated. However, differences in the immune responses elicited were observed, with the DDMM regimen eliciting higher rates of T-cell responses and the MMM regimen inducing higher rates of antibody responses.[578] Further studies of these products are being planned.

A fourth phase II trial assessed the safety and immunogenicity of a bivalent mosaic HIV-1 construct including Env/Gag/Pol antigens delivered by a recombinant adenovirus serotype 26 (Ad26) vector as a prime and boosted with Ad26 or MVA vectors expressing homologous mosaic Env/Gag/Pol antigens with or without a clade C envelope gp140 protein.[579] The mosaic inserts are bioinformatically derived complimentary antigens designed to cover the majority of globally circulating HIV-1 strains.[580–582] In this multiarm study the Ad26 prime/Ad26+gp140 regimen was found to be the most immunogenic (with antibody and T-cell responses identified in 80% to 100% of participants) and has been selected for further assessment (see later).

Phase IIb and III Efficacy Studies

Determination of the efficacy of a candidate HIV-1 vaccine requires the conduct of large-scale, rigorously controlled clinical trials. Such trials present formidable scientific and logistic challenges and require extensive resources. As a result, the criteria to determine that a candidate HIV-1 vaccine has sufficient promise to proceed into an efficacy trial remain a matter of substantial controversy. Nevertheless, it is also appreciated that properly conducted efficacy trials may provide important information regarding correlates of protection that may not be obtained otherwise, even if the candidate vaccine is only minimally effective. This information could then be "fed back" to investigators for appropriate modification or revision of vaccine development efforts.

The first phase III trial of a candidate HIV-1 vaccine involved the VaxGen AIDSVAX B/B vaccine.[513] This vaccine consisted of rgp120 proteins from two clade B isolates (MN and GNE-8) in alum and was administered at months 0, 1, 6, 12, 18, 24, and 30 in a multicenter study. The experimental design was a double-blinded, randomly allocated, placebo-controlled study (vaccine-to-placebo ratio of 2:1 in 5403 subjects, of whom 5109 were MSM and 308 were women at heterosexual risk for HIV-1 infection. At the end of the 3-year period of observation, the rate of the major study end point (HIV-1 infection) was virtually the same in the vaccine group (6.7%) and the placebo group (7%) (see Table 119.6).

A second phase III study was conducted in Thailand with a similar rgp120 vaccine (AIDSVAX B/E) derived from a clade B isolate (MN) and a primary clade E isolate (CM244).[515] The experimental design was similar to that described previously, except that the vaccine-to-placebo ratio was 1:1, and the study population consisted of 2546 PWIDs. The 3-year cumulative rate of infection was 8.3% in the placebo group and 8.4% in vaccine recipients. Taken together, these studies indicate that the rgp120 approach offered no evidence for protection against acquiring HIV-1 infection.

A phase III trial (RV144) of a prime-boost vaccine regimen consisting of the canarypox vector vCP1521 expressing HIV-1 *gag* and *pol* (subtype B) and CRF01_AE (subtype E) gp120 boosted by AIDSVAX B/E has been conducted in Thailand. The vaccine regimen involved administration of the canarypox ALVAC vector at months 0, 1, 3, and 6 and the rgp120 protein at months 3 and 6. Each subject was followed for 3 years to assess for both acquisition of HIV-1 infection and HIV-1 disease progression. This study enrolled a community-based population of 16,402 (1:1 randomization) subjects not at especially high risk for HIV-1 acquisition (see Table 119.6). The modified intention-to-treat analysis (excluding HIV-1 infections present at baseline) showed a modest vaccine efficacy of 31.2% ($P = .04$) but no effect on viral load setpoint in those subjects who became HIV-1 infected.[516] This observation of modest vaccine efficacy triggered an exhaustive search for potential correlates of efficacy, which identified IgG responses to the variable regions (particularly V2) of the HIV-1 envelope as potentially protective immune responses (see Table 119.6).[583–585]

In 2016 a phase IIb/3 study was initiated in South Africa (HVTN 702) to assess the RV144 regimen in another context to verify the RV144 finding noted earlier. The vaccines were slightly modified from the RV144 regimen to resemble the circulating strains in South Africa. In HVTN 702, ALVAC-HIV (vCP2438) is given as a prime (months 0 and 1) and boosted with ALVAC-HIV and an MF59 adjuvanted bivalent subtype C gp120 protein (months 3, 6, and 12; NCT#02968849). This study is fully enrolled ($n = 5400$ women) and is in active follow-up (Table 119.7).

TABLE 119.7 Efficacy Studies of Candidate HIV-1 Vaccines or bNAbs in HIV-1–Seronegative Volunteers Currently Underway

VACCINE/bNAb	NAME	START	SAMPLE SIZE	LOCATION	TARGET POPULATION	NCT#
ALVAC (vCP2438), bivalent clade C gp120/MF59	HVTN 702	2016	5400	South Africa	Men/women	2968849
VRC01[a]	HVTN 703/ HPTN 081	2016	1900	sub-Saharan Africa	Women	2568215
VRC01[a]	HVTN 704/ HPTN 085	2016	2700	Americas	MSM, transgender	2716675
Tetravalent Ad26.Mosaic, clade C gp140/alum	HVTN 705	2018	2600	sub-Saharan Africa	Women	3060629

[a]bNAb.
Ad26, Adenovirus serotype 26; *bNAbs,* broadly neutralizing antibodies; *gp120,* glycoprotein 120; *HPTN,* HIV Prevention Trials Network; *HVTN,* HIV Vaccine Trials Network; *MSM,* men who have sex with men; *NCT,* ClinicalTrials.gov identifier number; *vCP,* canarypox vector.

Two phase IIb efficacy studies of the Merck rAd5-*gag/pol/nef* vaccine given at months 0, 1, and 6 have also been conducted. These studies aimed to determine whether the HIV-1–specific cellular immune responses elicited by this vaccine[586] would reduce either HIV-1 acquisition or viral load setpoint after infection. One study was conducted in the Americas, the Caribbean, and Australia (HVTN 502 or "Step") and enrolled 3000 participants at high risk for HIV-1 infection. A parallel study was begun in South Africa (HVTN 503 or "Phambili"). The Step Study was terminated early at the first Data Safety Monitoring Board meeting in September 2007 because of a determination of futility in finding significant protection from infection between the vaccine and placebo groups. The rates of infection in the vaccine and placebo groups were not significantly different (see Table 119.6), and HIV-1 viral loads were not reduced in vaccinees compared with placebo subjects, despite the induction of HIV-1–specific cellular immune responses in most vaccinees.[511,512,587] In addition, the data suggested that vaccinees who had preexisting Ad5 immunity and who were uncircumcised may have had increased risk for HIV-1 acquisition, especially during the early period after vaccination.[588] Subsequent analyses, using an HIV-1 sieve approach, suggested that the vaccine may have elicited immune responses that exerted immunologic pressure on the infecting HIV-1 virus in vaccine recipients who became HIV-1 infected.[589,590] No increased HIV-1 acquisition was observed in vaccinees who did not have preexisting Ad5 immunity and who were circumcised. A second study of this vaccine regimen was carried out in Phambili. It was halted and unblinded shortly after it was initiated due to the results from the Step trial—with 801 of the planned 3000 participants enrolled. An evaluation of HIV-1 acquisition of these limited data found no evidence for vaccine benefit in preventing HIV-1 acquisition or on HIV-1 viral load setpoint (see Table 119.6).[591] Although the biologic basis for the potentially increased rate of HIV-1 acquisition in the Step Study remains unclear, these data suggest potential advantages of using vaccine vectors that are not subject to substantial preexisting vector immunity.

The fourth concept to be tested in an efficacy trial is HVTN 505, a phase IIb study of a DNA prime followed by rAd5 boost HIV-1 vaccine regimen (DNA/rAd5) developed by the VRC at the NIH. This vaccine included envelope antigens from HIV-1 clades A, B, and C. This study enrolled 2504 volunteers in the United States who were MSM or transgender volunteers at high risk for acquiring HIV-1. At the first planned interim Data Safety Monitoring Board review for efficacy in April 2013, the study was stopped for lack of efficacy because futility criteria had been met for both coprimary end points: HIV-1 acquisition and reduction in HIV-1 viral load setpoint in those who became HIV-1 infected.[592] At present, no further development of rAd5-based HIV-1 vaccine candidates is planned. It is unclear whether the failure of these rAd5 vector-based vaccines represents the failure of these specific vaccine products or the failure of the T-cell–based vaccine concept in general.

A fifth concept (phase IIb, HVTN 705) is undergoing field testing based on the bivalent mosaic antigen concept delivered by an Ad26 vector and boosted with an alum adjuvanted clade C gp140 protein (NCT#03060629). This study is recruiting 2600 women in sub-Saharan Africa at risk for HIV-1 infection. The primary end point is prevention of HIV-1 infection. This study is ongoing.

Broadly Neutralizing Antibodies Against HIV-1

The development of single-cell antibody cloning techniques has led to the recognition and eventual generation of monoclonal broadly neutralizing antibodies (bNAbs) against HIV-1 from patients infected with HIV-1. This has led to consideration of their use in immunoprophylaxis and immunotherapy of HIV infection.[593] Passive transfer of bNAbs to macaques has been shown to prevent infection of macaques with SHIV[594] and also to suppression of SHIV in infected animals.[595] Properties of bNAbs, such as extensive somatic hypermutation, make it extraordinarily difficult to elicit them by conventional immunization approaches.

bNAbs have been administered to uninfected[596] as well as HIV-1 infected volunteers[597,598] in phase 1 trials. The most extensively studied of the bNAbs, VRC01, is undergoing phase 2b studies to determine its efficacy in prevention of acquisition of HIV-1 infection. Both studies were initiated in 2016 in persons at increased risk for the acquisition of HIV-1 infection. The first study is in the Americas in men and transgender persons who have sex with men (n = 2700; HVTN 704/HPTN085, NCT#02716675).[599] The companion study is evaluating the prophylactic efficacy in women (n = 1900) in sub-Saharan Africa (HVTN703/HPTN081, NCT#02568215).[599]
Both of these studies are fully enrolled and in active follow-up. Multiple bNAbs with improved breadth and prolonged half-life have been developed and are in phase I testing[600] (NCT#03387150).

Early Phase Clinical Trials

A number of novel HIV-1 vaccine concepts are currently being pursued in early phase clinical trials. Studies are planned to evaluate novel vectors, such as CMV vectors, and novel Env immunogens that aim to induce bNAb responses. Although such immunogens do not yet exist, early phase clinical trials are in progress to evaluate native-like Env trimers and to determine whether engineered immunogens can trigger germline variable antibody genes that are believed to be required for neutralization breadth. In addition, studies will determine whether a series of immunogens can recapitulate B-cell development pathways that have been defined for broadly neutralizing monoclonal antibodies. Meanwhile, a series of early phase clinical trials are underway to evaluate whether cocktails of broadly neutralizing monoclonal antibodies can be used for passive protection.

Summary

Multiple HIV-1 vaccine approaches have been evaluated, and several large clinical efficacy trials are currently underway to evaluate novel HIV-1 vaccine strategies that should inform the field over the next several years. Future priorities of the field involve the development of vaccines that elicit broadly reactive NAbs and antibody cocktails for passive protection.

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The complete reference list is available online at Expert Consult.

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120 Diagnosis of Human Immunodeficiency Virus Infection

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SHORT VIEW SUMMARY

Definition

- Human immunodeficiency virus (HIV) detection is the cornerstone of the medical and public health response to the HIV epidemic. HIV detection is accurate and sensitive, and precise assays have been designed for three general purposes: individual diagnosis, epidemiologic surveillance, and donor screening for blood and tissue products.

Epidemiology

- By the end of 2017 more than 36 million individuals were living with HIV infection throughout the world. New initiatives to eliminate HIV worldwide have adopted a strategy of "90-90-90": 90% of HIV-infected individuals know their diagnosis, 90% of those diagnosed undergo combination antiretroviral therapy (cART), and 90% of those undergoing cART have HIV RNA levels below the limit of detection in commercial assays. The first "90" represents a key landmark and depends on accurate diagnosis. In the Unites States the prevalence of HIV infection is approximately 1.1 to 1.2 million individuals and approximately 85% of individuals know their status. The second "90" also depends on testing; new testing modalities permit diagnosis of HIV in a single visit, which will facilitate engagement in care. Eradication approaches will rely heavily on preexposure prophylaxis (PrEP) regimens, and as a result, many more individuals will require routine testing during PrEP. Testing with robust performance characteristics will be essential to evaluate individuals, potentially in the many millions, undergoing long-term PrEP.

Microbiology

- Worldwide, HIV is highly genetically diverse, and HIV testing modalities are designed to detect all HIV variants. Within an individual the genetic diversity of HIV early in infection is limited but increases with duration of infection, reflecting both the intrinsic mutation rate of HIV and strong selection pressures, including immune responses. At present, useful tools to detect HIV infection include detection of viral components (HIV RNA and p24 antigen) and humoral responses (commonly detected by enzyme-linked immunosorbent assay in various formats or by Western blotting and immunofluorescence-based assays, which are less frequently used).

Diagnosis

- Diagnosis proceeds from history, physical examination, and laboratory studies. Laboratory analysis is a two-step sequential process using a highly sensitive screening test, followed by a highly specific supplemental assay.
- A variety of formats are available, including rapid and home testing, that are approved by the US Food and Drug Administration (see Table 120.1). In many cases it is possible to perform both the screening and the supplemental assays within a single visit.
- The Centers for Disease Control and Prevention recommends all individuals age 18 to 65 years should be tested for HIV infection in an opt-out fashion. Testing has been adopted by the US Preventative Services Task Force, and the cost of opt-out testing is substantially underwritten through current insurance and the Affordable Care Act.
- No test is perfect, and familiarity with assay limitations is essential to ensure accurate identification of HIV infection.

Therapy

- HIV diagnosis is essential to introduce specific ART for treatment of infection.
- Screening assays for HIV infection are essential but not sufficient to initiate ART, and supplemental testing is essential to initiate cART.
- Individuals who have been exposed to HIV can undergo postexposure prophylaxis. Testing after completion of the prophylaxis regimen is essential to determine whether infection has occurred. Continued evaluation and referral for PrEP may be indicated.
- Individuals who are at risk for HIV infection but are not infected should be evaluated for PrEP. Regimens for PrEP are distinct from regimens for therapy for infected individuals, and it is essential to evaluate individuals carefully for HIV infection in such circumstances.
- Specific guidelines are available for testing, treatment, and prophylaxis.

Prevention

- PrEP represents a strategy to treat high-risk individuals with ART to prevent HIV infection. Careful evaluation is needed to rule out HIV infection before initiating PrEP. Expansion of HIV diagnostic approaches beyond clinic settings facilitates early identification of HIV-infected individuals. As individuals with early infection are highly infectious, expansion of testing can improve prevention efforts.

Human immunodeficiency virus (HIV) infection results in a progressive immunodeficiency that has resulted in approximately 35 million deaths worldwide (https://www.who.int/gho/hiv/en). By the end of 2017, more than 36 million individuals were living with HIV infection throughout the world[1] (http://www.unaids.org/en/resources/fact-sheet). Accurate, sensitive, and precise assays have been designed for three general purposes: patient diagnosis and clinical management, epidemiologic surveillance, and donor screening for blood and tissue products. As such, HIV diagnosis has been a fundamental cornerstone to establishing the extent of epidemic spread worldwide (Fig. 120.1)

As the public health response to the HIV epidemic has evolved, several key events have focused even greater attention on HIV diagnostics. The development of affordable combination antiretroviral therapy (cART) combined with the infrastructure for reliable delivery has enabled international public health efforts to propose successive expansion of therapy and, most recently, an ambitious "90-90-90" initiative targets advancing elimination of HIV transmission (90% of HIV-infected individuals know their diagnosis, 90% of those diagnosed undergo cART, and 90% of those undergoing cART have HIV RNA levels below the limit of detection in commercial assays) to eliminate HIV spread. The first critical "90" objective in this initiative is evaluation for HIV infection and has had variable success worldwide[2] but is the basis for subsequent steps in overall elimination of infection. As described, HIV diagnosis has come to rest on critical events surrounding diagnosis: the five Cs (https://www.who.int/hiv/topics/vct/about/en/): "*c*onsent, *c*onfidentiality, *c*ounseling, *c*orrect test results, and *c*onnection/linkage to prevention care and treatment"; diagnosis is therefore critical to eliminating HIV, and ensuring these five goals has become a more compelling imperative.[3] In addition, recent studies demonstrating the benefit to initiating therapy early in the course of HIV infection, as well as expansion of the numbers

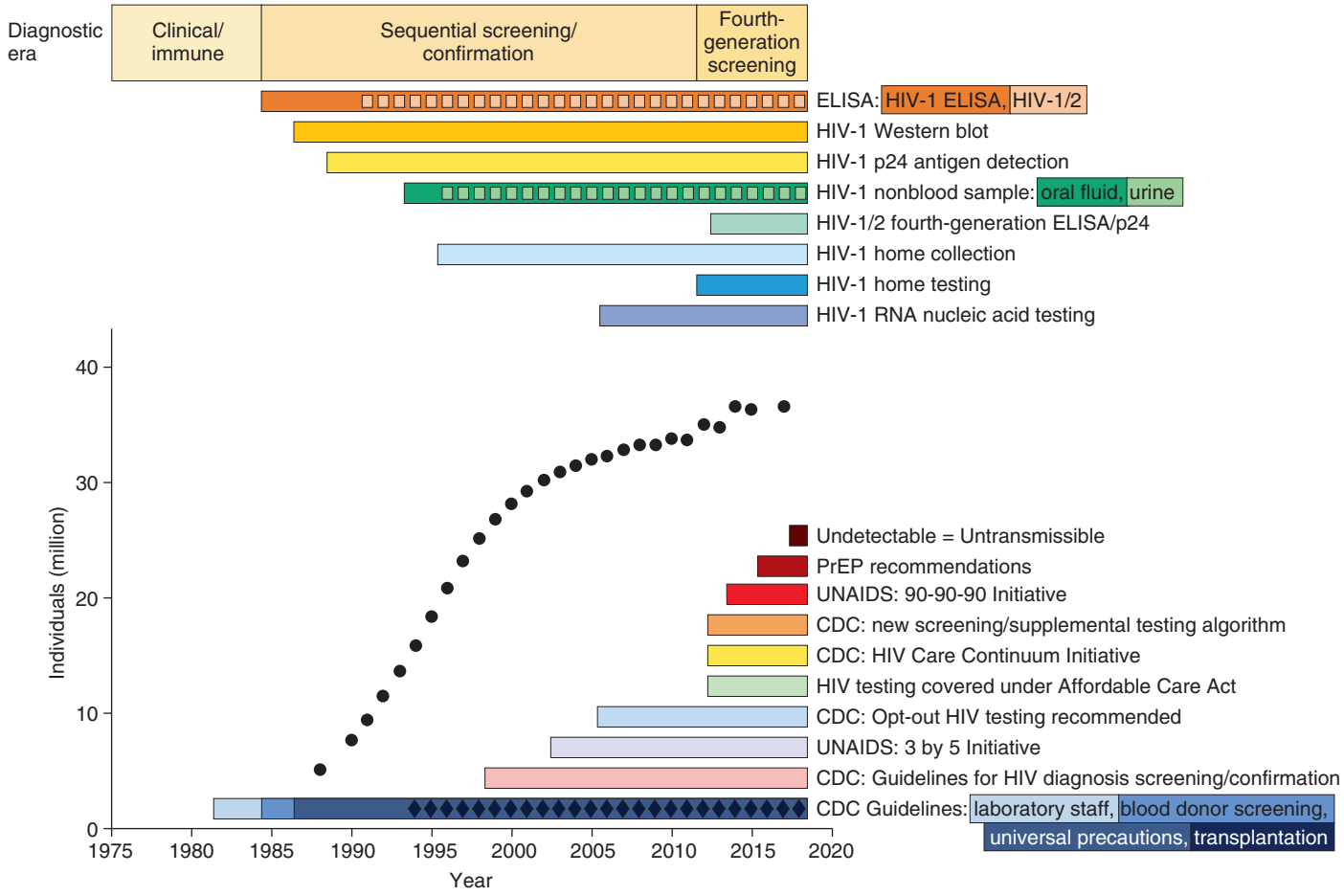

FIG. 120.1 HIV/AIDS diagnosis: three eras of diagnostic approaches. Diagnosis of HIV infection includes clinical and laboratory testing and requires appropriate implementation. Diagnostic approaches have evolved since the first cases of AIDS were recognized in the early 1980s. These eras can be divided chronologically (*x*-axis) and by the phase of the epidemic worldwide (*y*-axis). Above the epidemic curve are depicted laboratory modalities, and below the curve are implementation landmarks and public health initiatives. Initially, AIDS was diagnosed as a clinical syndrome in the setting of specific opportunistic infections or neoplastic diseases; laboratory methods were limited frequently and consisted of studies of immune responses, enumeration of CD4 and CD8 cells, and determining CD4/CD8 ratios in peripheral blood. The development of sequential serologic testing with a variety of techniques to detect HIV antigen and anti-HIV antibody represented the first laboratory techniques to detect HIV and took place as an expanding epidemic was recognized. This period was marked by successive testing modalities that extended the ability of testing to be initiated beyond clinical facilities. In parallel, public health agencies developed guidelines, initiatives, and infrastructure that did not substantially alter the initial trajectory of spread but did contribute to limiting the total disease burden worldwide. A third era of diagnosis emerged with the development of testing modalities that can provide both screening and supplementary diagnostic information in a single visit; advancements coincided with a point where spread remained stable and as wide-ranging changes in recommendations for tests, treatments, and PrEP, and targeted goals ("90-90-90") were implemented. These initiatives, supported by expanded diagnostics, are likely to tip the balance in favor of efforts to eliminate HIV. *CDC,* Centers for Disease Control and Prevention; *ELISA,* enzyme-linked immunosorbent assay; *PrEP,* preexposure prophylaxis; *UNAIDS,* United Nations Programme on HIV/AIDS.

of individuals for whom testing is recommended, has placed an additional burden on optimizing performance characteristics to detect HIV early after infection occurs and to do so in large populations with low incidence of HIV infection. The second "90" relies on the reliable and responsible linkage to care after a positive diagnosis. Diagnostic methodologies have become quite flexible; such capability permits greater opportunity for seamless linkage to care. Adapting the diagnostic approach and setting for diverse risk groups has been a critical development and remains an important responsibility in the public health response to HIV infection. The recent National HIV/AIDS Strategy for the United States[4] reflects the broad shift in emphasis in expanding HIV diagnostics beyond traditional clinical settings.

Advances in diagnostics have been critical to the development of a feasible and scalable response to the epidemic and have often driven the medical and public health response itself. At the same time, limitations of the diagnostics and their appropriate administration can lead to equivocal results at a critical time for potentially infected individuals, Health care professionals represent gatekeepers of diagnosis; no test (or testor) is perfect, and thorough understanding of HIV diagnostic capabilities is an essential responsibility for health care workers at all levels. HIV infection is a life-altering diagnosis with social, medical, and potentially legal consequences. Misdiagnosis of HIV infection has profound consequences for patients and their contacts; liability issues have been justifiably extreme.[5,6]

Diagnosis of HIV infection is not simply the result of interpretation of a laboratory test, but proceeds as for the evaluation of any other illness, from careful history and physical examination to indicated laboratory studies. The present chapter will survey the methods, strategies, and circumstances for diagnosis of HIV infection.

BACKGROUND/PERSPECTIVE

The current state of the art HIV diagnosis is the product of continuous advances in laboratory techniques coordinated with progressive expansion in implementation. HIV detection remains a two-step sequential process consisting of a highly sensitive *screening* test, followed by a highly specific *supplemental* or *confirmatory* assay. In the United States and many developed countries, diagnostics can be conveniently divided into three periods (see Fig. 120.1): (1) An early period (1981–85), before the

identification of the virus, in which diagnosis relied on clinical evaluation with minimal laboratory input; this early approach, although highly specific, was insensitive and identified patients only after a long "window period." (2) A prolonged period (1985–2013), during which serologic diagnostics were used in a strategy of sequential testing and then during which assays for screening and confirmation accurately identified most HIV-infected individuals with successively improved sensitivity and specificity and a progressively shortened window period; however, diagnosis required multiple visits. (3) A recent period (2013 to the present), in which screening includes detection of HIV antibody and antigen in fourth-generation assays and supplemental assays discriminate HIV-1 and HIV-2 infections (see Fig. 120.1). Of importance, this fourth-generation–based approach can be accomplished in many cases within a single visit. These recent developments have coincided with an expansion of use of diagnostics for evaluation of individuals undergoing preexposure prophylaxis (PrEP) and postexposure prophylaxis (PEP) for HIV infection. As the knowledge base expanded, improvements in HIV diagnostic testing improved and drove expansion of testing services and locations. As shown in Fig. 120.1, the combination of improvements in testing modalities, expansion of settings for testing, and increased public health initiatives to engage at-risk populations has strong potential to tip the balance of the epidemic versus prevention struggle in favor of HIV eradication.

Laboratory Advances

In early studies (1981–85) the diagnosis of acquired immunodeficiency syndrome (AIDS) was made by identification of characteristic infections or neoplasms in appropriate risk groups. Before development of molecular techniques for HIV detection, the use of peripheral CD4 cell counts and percentage CD4 cells and, more accurately, use of CD4/CD8 cell ratios was used as a marker of immunodeficiency and used in screening.[7] The recovery of a transmissible agent, in cultures of susceptible cells with reproducible cytopathology associated with virus-like particles containing reverse-transcriptase enzyme activity characteristic of the retrovirus family[8–13] (Fig. 120.2), paved the way for future diagnostics. When virus isolation was reliably positive, this test could be considered a gold standard of infection; virus isolation was, however, cumbersome, time consuming, and was not sufficiently sensitive for routine screening. The discovery that serum from patients with AIDS contained antibodies that recognized viral gene products[8,9,14,15] and that reactivity was lifelong in the majority of infected individuals permitted large-scale investigations of appropriate serologic detection assays.

Several standard laboratory procedures used to detect HIV gene products, including radioimmunoprecipitation, Western blotting (WB), enzyme-linked immunosorbent assays (ELISAs), and immunofluorescence were adapted to form the basis for the earliest HIV detection systems.[16] The first ELISA HIV test kits received US Food and Drug Administration (FDA) approval for use in blood donor screening in the United States in March 1985[17] and ushered in a new era of diagnosis, beginning with HIV blood donor screening. All donations reactive in a single test were discarded, and units with repeat reactivity were considered positive for viral antibodies. Within approximately 3 months, more than 1 million units of donated blood were screened in the United States, with 0.25% reported repeatedly reactive.[17] Two principal technical issues of the first generation of HIV ELISA assays were a high rate of false-positive results arising from a variety of clinical conditions and laboratory artifacts[18] and a small but ominous number of false-negative results resulting from the inability to detect the presence of HIV antibodies early in infection (so-called window period) before full seroconversion.[19,20]

To address false-positive results, highly specific tests, including WB, immunofluorescence, and radioimmunoprecipitation were incorporated as confirmatory assays on all repeat ELISA-reactive samples.[21–23] Although all confirmatory assays are relatively labor intensive, WB procedures proved most useful, efficient, and specific; the WB technique was soon established as a widely used gold standard test for confirmation and was FDA approved for use in the United States in 1987.

Technical advancements in ELISA assays also improved detection of sensitivity early HIV infection during the window period. The development of infectious molecular clones of HIV[24–26] permitted detailed virus characterization of components of the AIDS virus (see Fig. 120.2), facilitating understanding of replication and importantly provided access to cloned HIV genes that could be used to further develop specific diagnostics. A second-generation of ELISA assays decreased false-positive

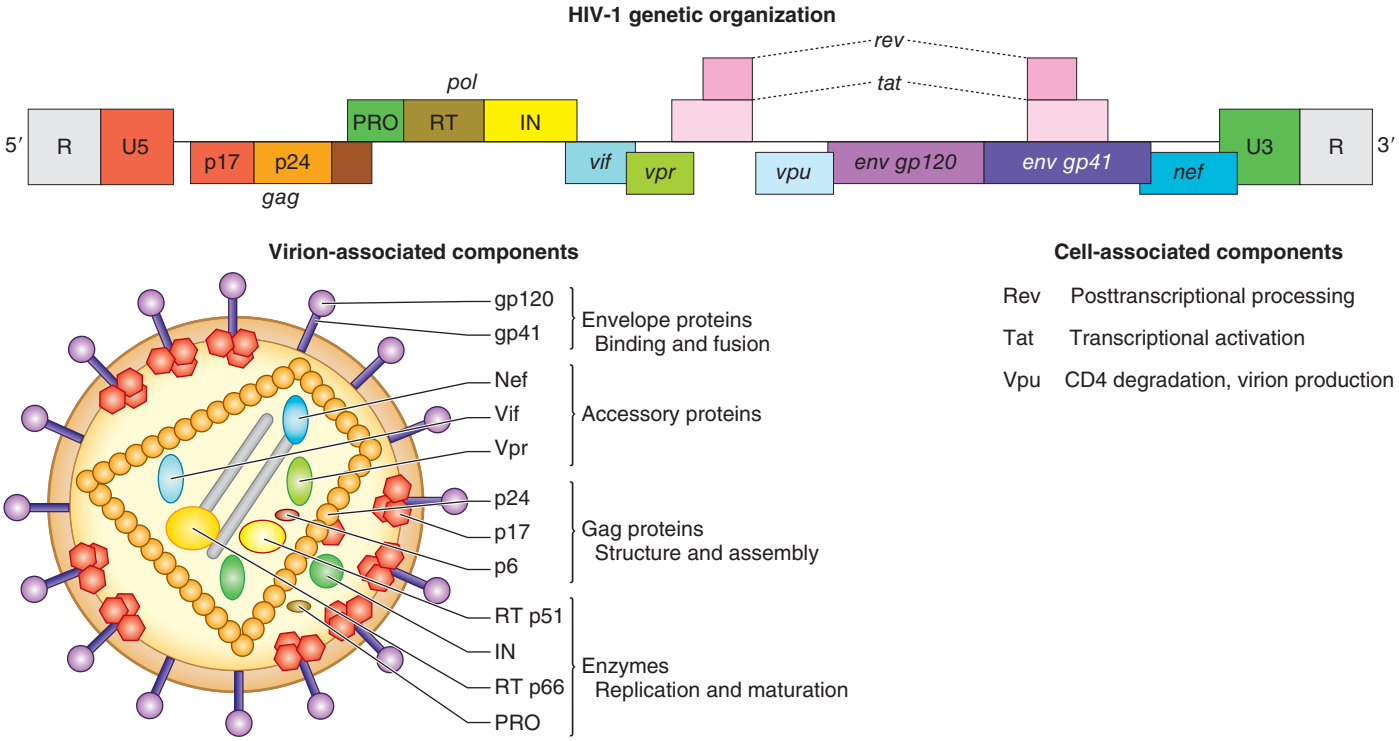

FIG. 120.2 Organization of HIV-1. HIV-1 is an enveloped retrovirus with a positive-stranded genome (two copies/virion) that contains genes for proteins with structural enzymatic and regulatory functions, as indicated.

rates by using recombinant antigens and synthetic peptides instead of infected cell lysates, resulting in greater sensitivity.[27–31] Introduction of an assay to detect the presence of viral antigen (p24) in 1989 to detect HIV was especially useful during early virus infection and improved detection of recent HIV infection[32–36]; p24 antigen detection kits were FDA approved in 1989.

In 1986 a second human immunodeficiency virus, HIV-2, was identified in West African natives by studying individuals with an AIDS-like immunodeficiency who had nonreactive or unusual HIV-1 serologic results,[37,38] and shortly thereafter, cases of HIV-2 were detected in the United States, Canada, and Europe[39–44]; changes in ELISA assays to include HIV-2 antigens ensued, and combination ELISA kits were introduced in 1991 (see Fig. 120.1), which were subsequently adapted into a number of distinct formats to produce flexible, rapid tests that would be useful in resource-limited settings[45] and for use with alternative body fluids, including urine and saliva (see Fig. 120.1).[46–50]

ELISA assays to detect HIV antibody have remained a cornerstone for HIV screening. Confirmatory assays, immunofluorescence and more commonly WB, delivered excellent specificity; WB approaches provided additional information regarding early HIV infection. Progressive emergence of HIV RNA, p24 antigen, and serologic reactivity forms the basis for staging early HIV infection, standardized as six successive "Fiebig stages" (Fig. 120.3). Emergence of serologic reactivity with WB to the HIV integrase protein, p31, is typically delayed after infection occurs, and p31 reactivity distinguishes Fiebig V HIV stage (p31 negative) from Fiebig VI stage (p31 positive). Confirmatory testing with WB techniques had disadvantages as well, including a requirement for specialized equipment and an inability to provide a rapid simple version of the assay, requiring additional visits to arrive at a diagnosis. In addition, a substantial number of blots with indeterminate results required additional evaluation and limited the utility and cost-effectiveness of this modality; in response, alternative approaches using dual ELISA assays were developed within developing countries and World Health Organization (WHO). Ultimately, new strategies were developed that bypassed WB entirely.

Inability to detect HIV during early infection (window period) remains a critical limitation in HIV testing. Methods to detect HIV p24 antigen decreased the window period and became required for blood donor screening in 1996. Improvements in ELISA approaches using "sandwich" antibody techniques resulted in greater specificity in screening and greater sensitivity in detecting antibody during the window period and ushered in a new third generation of HIV ELISA assays.[51,52] Nevertheless, the HIV window period remained unacceptably long, and new approaches to detect HIV nucleic acid, denoted nucleic acid testing (NAT), were developed for diagnosis. NAT is more sensitive than third-generation ELISA assays in detecting window period HIV-1.[53] NAT (for HIV-1 only) was approved for use in screening plasma donors in 2001 and for use in individual blood donors in 2002, and qualitative HIV-1 RNA detection for diagnosis was approved in 2006, which reduced window period, on average, 6 days earlier than p24 assays in seroconversion panels.[54]

In independent efforts to increase detection of window period infections, fourth-generation assays have been developed that use a combination of third-generation ELISA with sensitive p24 antigen detection.[52,55–58] The first antibody-antigen (Ab-Ag) diagnostic assay was FDA approved in 2010 for detection of both HIV-1 and HIV-2; although not as sensitive as NAT, these fourth-generation tests represented marked improvements in detecting early infection, with a median detection time 7 days earlier than that for third-generation ELISAs. Fourth-generation tests that were positive did not distinguish whether the test was positive because of the p24 reactivity or the anti-HIV reactivity. Early infection was therefore inferred, with fourth-generation assays positive and standard ELISAs negative. Newer assays, denoted as fifth-generation assays by some, do distinguish between p24 antigen positivity and serologic reactivity, permitting a more precise diagnosis (see Fig. 120.3). Moreover, these new assays could be performed in a rapid format, permitting diagnosis of HIV infection in a single visit. Together these assays, detecting HIV antigen and antibody and differentiating HIV-1 and HIV-2, have ushered in the current HIV serology + p24 antigen era of HIV diagnosis and serve as supplementary assays in a revised, simplified, and streamlined diagnostic algorithm.

HIV testing was developed to detect HIV-subtype B, the most common subtype in the United States and Europe. The majority of infections worldwide are, however, non–subtype B, and early generation assays had variable sensitivity to detect these non-B subtypes; inclusion of additional antigens and peptides has improved sensitivity such that the current versions of ELISA, WB, and NAT assays will detect B and non-B subtypes with equivalent sensitivity and specificity.

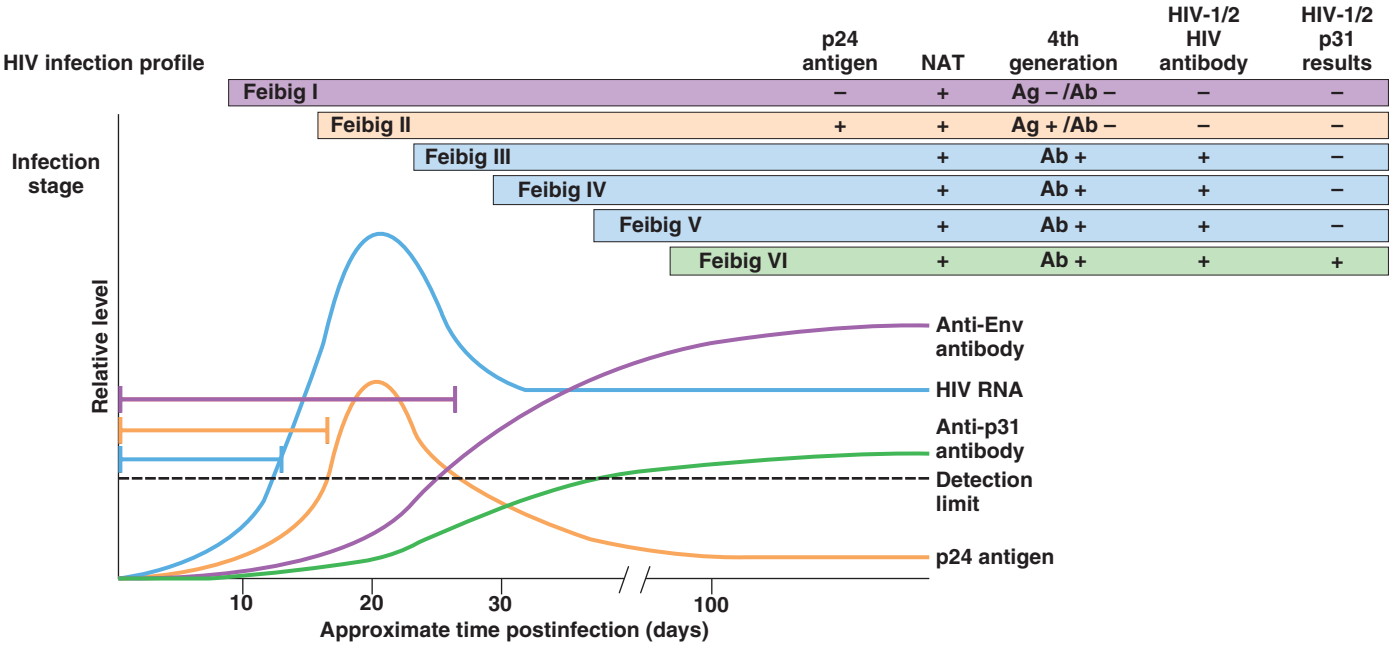

FIG. 120.3 HIV infection profile. After HIV infection, intense viral replication results in high levels of HIV RNA and HIV p24 protein in plasma. Subsequently, increases in anti-HIV antibody are detected, and a variable window period exists between infection and detection by different testing modalities. HIV RNA levels are detected earliest, followed by HIV p24 antigen and antibody production. Antibody to the HIV p31 protein emerges last. Using a combination of testing modalities for detection of HIV RNA, p24 antigen, and anti HIV antibody, individuals with early HIV infection can be categorized into a series of infection stages (Fiebig stages) as indicated. The earliest stage (Fiebig I), with only HIV RNA detectable, and the latest stage (Fiebig VI), in which antibody to HIV proteins, including p31, is present. As indicated, all testing modalities will identify later Fiebig stages, but only HIV nucleic acid testing (NAT) will detect Fiebig I; fourth-generation assays can detect Fiebig II and later.

As testing procedures for blood donation expanded, variations in laboratories carrying out these evaluations were expected, and test kit evaluation procedures were established with the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) to evaluate and limit variability[59–61] and worldwide under WHO auspices.[62] MPEP is a voluntary quality assessment program providing well-curated sample sets for testing. Proficiency in HIV testing is of paramount importance, and early surveys of laboratories revealed significant variation and issues with reproducibility.[63] Reporting results of HIV testing was initially haphazard and potentially confusing,[64] leading to uncertainty. Performance rapidly improved, high-quality control measures were documented,[65,66] and guidelines for screening donor organs before transplantation were established by the FDA, with the first interim rule in 1993 requiring donor screening and record keeping. MPEP has been a highly successful CDC initiative and was extended to evaluate proficiency in rapid HIV tests.

Implementation of HIV Diagnostics

Implementation of HIV diagnostic testing has evolved over the last 30 years, although certain goals have remained: Under the majority of circumstances, testing is voluntary and confidential. Identification of so-called "key populations" in the United States, currently including gay, bisexual, and other men who have sex with men (MSM) of all races and ethnicities; black women and men; people who inject drugs; Latino men and women; youth age 13 to 24 years; people in the southern United States; and transgender women.[4] In concert with identifying these key populations, HIV diagnostics were progressively expanded (see Fig. 120.1); in the United States the CDC, FDA, and other governmental authorities provided a series of successive recommendations to broaden testing, which were driven to some degree by the improved performance characteristics and the development of formats that made such expansions feasible, scalable, and reliable. In parallel, programs disseminating information regarding HIV and public service initiatives raised awareness of the need for increasing testing. The first National HIV Testing Day was designated on June 27, 1995, and an important subsequent approach has been administered through HIV.gov to designate a day to focus on specific at-risk communities; beginning in 1999 (National Black HIV/AIDS Awareness Day, February 7) and continuing through 2016 with National Transgender HIV Testing Day (April 18), these events encourage testing using progressively broad approaches, including social media, to engage specific key populations. Similar initiatives have been developed elsewhere and under the auspices of United Nations Programme on HIV/AIDS (UNAIDS) and WHO. Combining these programs with mobile health initiatives and flexible testing modalities has great potential to reach populations who have not routinely engaged health care.[67] As programs for preexposure and PEP expand, rigorous HIV diagnostics will be necessary, and familiarity with the limitations of testing modalities will be essential.

Among the challenges HIV testing encountered early in the epidemic was expanding diagnostics beyond blood donation facilities and implementing a public health measure that was confidential, voluntary, and effective. In the United States and elsewhere, so-called "alternative sites" were established shortly after approval of screening ELISA assays; these alternative sites were independent of blood centers and were permitted to perform voluntary and confidential HIV testing with pretest and posttest counseling procedures.[68] The logistics of screening at alternative sites were often controversial,[69] but mandatory testing of patient populations was rejected. During the screening confirmation period, screening using ELISA assays and confirmation with WB or immunofluorescence was done under defined laboratory conditions only, as simple and rapid testing for HIV were not available. With guidelines for screening and confirmatory studies provided to laboratory staff, blood donor screening was soon followed by development of universal precautions and guidelines for evaluating tissue for transplantation (see Fig. 120.1).

In the United States the introduction of rapid HIV-1 testing procedures, combined with new guidelines in 2001 to recommend screening of all pregnant women and simplification of the consent process, was followed by additional recommendations in 2003 to make testing routine in health care settings and for use of rapid testing during labor and delivery, thus expanding HIV testing beyond traditional health care facilities and permitting support of point-of-care real-time HIV diagnosis. Benchmark results of these advances have been analyzed using a number of statistical approaches. The National Center for Health Statistics surveyed more than 188,000 noninstitutionalized individuals age 18 to 64 years and incorporated questions regarding HIV testing. After an initial increase in individuals tested over the years 1987–95, the total proportion of the population who had ever undergone testing and the rate at which individuals were tested within a year has been calculated (see Fig. 120.2).[70] The total proportion of the population in developed countries who have undergone HIV testing is relatively high (e.g., 40% in the United States [see Fig. 120.2][71] and 50% in France[72]). Of note, the percentage of individuals reporting having been tested in the previous 12 months (10.4%, see Fig. 120.2) has remained unchanged since 2001, despite the promotion of rapid testing modalities and expansion of testing resources, although the proportion of people with HIV aware of their status is about 80% in the United States.[73]

In an effort to reach the substantial proportion of undiagnosed individuals, the CDC revised recommendations in 2006[71] to make HIV testing a part of routine screening for all individuals presenting for care, incorporating an "opt-out" strategy, where patients implicitly agree to testing upon presenting for care; testing is done unless the patient specifically declines. Opt-out testing for HIV infection represents a landmark shift in emphasis for HIV diagnosis; under this recommendation, patients are informed that HIV testing will be performed unless individuals opt out of testing. In this scenario counseling is available and assent is inferred. Although not universally implemented, opt-out testing represents an important change in approach to testing and has been increasingly used.

In an effort to address substantial stigma associated with HIV testing, a number of non–clinic-based and home-based opportunities for HIV testing have been developed. Development of rapid and simple assays for HIV led to true home testing; home testing permits privacy but not readily accessible counseling or interpretation, and availability of health care professionals to provide counseling will be essential.

Opt-out testing includes availability of counseling and the opportunity to refuse testing and is entirely distinct from mandatory testing, which requires testing be performed regardless of consent or degree of counseling. In general, mandatory testing has been repeatedly rejected, based in large measure on concerns for individual dignity.[74,75] Mandatory testing has, however, been supported under states' authority to protect the community interest against epidemic disease (as initially proposed and implemented for smallpox).[76] Testing of inmates in federal corrections facilities was mandated beginning in 1998, and a number of states have mandatory testing in state prisons.[77–79] In certain circumstances, especially sexual offenses, HIV testing has been court ordered; test results are made available to victims and in some cases to prison administrators. HIV testing of infants is mandatory in several states. Testing as a part of military recruitment is required in the United States and in at least 26 other countries.[75] Mandatory testing is required by a number of governments for immigrants entering as a worker, student, temporary or permanent resident; testing performed in US laboratories may or may not be acceptable for this purpose (a list of such countries and whether US testing is accepted is available at www.WHO.int). In the face of involuntary HIV testing, governing bodies, such as WHO, have categorically rejected mandatory HIV testing,[75] and the issue remains debated in the United States[78]; testing practices vary by country and can be influenced by political and unforeseen events.[80] The recent influx of asylum seekers in various countries has prompted some jurisdictions to implement mandatory testing of refugees. The rate of HIV positivity in these groups has been reported, and generally reflects rates from countries from which individuals are fleeing.[81]

Reporting HIV Infection

Initially, reporting of HIV diagnoses for epidemiologic purposes was deemed unnecessary, as the HIV-1 epidemic was tracked using AIDS case definition and mortality statistics. Early identification of key populations focused surveillance efforts on groups such as MSM, intravenous drug users, and commercial sex workers. With the advent of reliable testing modalities and effective ART, resulting in the dramatic

decline in death and in AIDS diagnoses, the presence of HIV infection represents the most useful marker of prevalence. In the United States reporting takes place on the state and federal level.[82] Names reporting and partner notification followed initial recommendations, raising privacy concerns, and partner notification is required in New York State as of 1998[83] and has been considered in others. Names reporting is standard practice in the United States; names of individuals newly diagnosed with HIV infection are reported to state health departments, where data is maintained in confidential databases. Personal identifying information is removed from the dataset when the data are reported to the CDC. Early in the epidemic, WHO endorsed worldwide unlinked anonymous surveys.[84]

Individual states vary in their approach to HIV testing, and specific laws regarding HIV testing, counseling, and reporting have been compiled (https://www.cdc.gov/hiv/policies/law/states/index.html). Even fully implemented, universal testing represents a passive surveillance approach and does not provide sufficient detection for individuals who do not engage health care, and active programs are likely to be required to identify all infected individuals, or even achieve "90-90-90" goals. Pilot programs of active screening are relatively labor intensive but have demonstrated success[85] in identifying HIV infection. Newer surveillance models using mobile, ehealth, and home testing options to enhance surveillance have been reviewed.[86] WHO standards for enhanced testing involves endorsement of home testing and testing through voluntary assisted-partner notification (2017; http://www.who.int/hiv/topics/vct/hts-new-opportunities/en/index2.html).

TERMINOLOGY AND PERFORMANCE CHARACTERISTICS

Laboratory approaches for detecting the presence of HIV infection have been developed, typically with a particular application intended, such as blood safety, patient diagnosis, or epidemiologic surveillance[87]; current assays for patient diagnosis (Table 120.1) have utility for established HIV infection, and some assays have utility for diagnosis of early HIV infection. A number of specific terms have been applied in describing HIV testing, procedures, and results (see Table 120.1). In the United States the FDA has regulatory oversight for testing, and not all modalities have FDA-approved versions. Serologic tests are broadly divided into screening assays and supplemental (formerly popularly described as "confirmatory") tests (see Table 120.1); some assays, such as the qualitative HIV RNA detection assay introduced in 2006, are approved as either a screening or a supplementary assay. No test, however, can be used as both a screening and a supplemental assay for an individual, and all individuals continue to require at least two assays for diagnosis. Serologic screening assays are available in a number of ELISA or p24 antigen assay formats for initial testing. Results of screening tests are considered reactive or nonreactive. In the United States supplemental assays, such as GEENIUS (Bio-Rad, Hercules, CA) or previously WB or immunofluorescence assays, have been used. Home sample collection of dried blood spots includes both a screening and supplementary phase, whereas home testing for HIV testing is for HIV screening only.

No test is perfect, and assay imperfections are quantitated using a number of characteristics: sensitivity, specificity, false-positive rate, and false-negative rate. For assays of HIV infection, both false-negative rate and false-positive rate have profound implications. Sensitivity (Table 120.2) relates to how many infections are missed by testing; inadequate sensitivity has great impact both in blood surveillance and individual diagnosis. In both circumstances inability to detect infection potentially exposes others to infection and misses critical opportunities for counseling and therapy. Specificity (see Table 120.2) describes the proportion of uninfected individuals who test negative for HIV. Decreased specificity results in false-positive findings, prompting profound patient distress and extensive additional evaluation. Decreased specificity compromises HIV testing from a patient diagnosis (fear of positive testing) and public health (inadequate description of the epidemic, unnecessary use of funds) standpoint. In general, the optimization of any test ultimately pits sensitivity at odds with specificity; the greater the sensitivity of a test, the more likely the possibility that false-positives may occur, whereas increasing specificity results in increased numbers of false-negatives (Fig. 120.4). To maximize both specificity and sensitivity for detection of HIV infection, a single test is inadequate. Instead, a sequential strategy has been designed. Testing is initiated with a highly sensitive ELISA screening (>99.5% sensitive); in this phase, false positives may be significant (1%–10%). The screening phase is followed, however, by a highly specific confirmatory test (>99.5% specific), and the initial false positives are excluded. As a result, HIV testing has among the highest level of sensitivity and specificity for any medical diagnostic procedure.

When deciding whether to use HIV testing in individual circumstances, it is essential to know estimates of what the probability is that HIV infection is present if the testing is positive, and what the probability is that HIV is not present if the HIV test is negative. In this regard, knowledge of the positive predictive value (the proportion of all positive results that are actually true positives) and negative predictive value (the proportion of all negative results that are true negatives) of HIV testing is quite useful (see Table 120.2). Even at high specificity and sensitivity, positive predictive values and negative predictive values are dependent upon the disease prevalence (see Fig. 120.3). As a result, no single test for HIV is useful or efficient in low-prevalence populations. The combination of a repeatedly reactive screening assay followed by a highly specific confirmatory assay yields an effective approach for diagnostic purposes, even in low-prevalence populations.

Test characteristics are defined in licensing trials using ideal conditions under which performance characteristics are defined (test efficacy), whereas performance may vary under conditions of actual field use (test effectiveness). Initial trials of kits may use relatively small numbers of samples, and with such small sample sizes, no false positives or negatives may be obtained. In such circumstances, test sensitivity/specificity may be reported as "100%." Reporting of such absolutes of any test may be somewhat misleading because it is more useful to report such findings taking into account the sample size and values, such as "<1 false positive/sample size." This may be more informative, and performance characteristics become even more important as modalities are used for evaluation of individuals undergoing PrEP or PEP.

Numerous screening and supplementary assays have been developed using a variety of patient materials to determine the presence of HIV infection (see Table 120.1); all kit names and manufacturers of the FDA-approved assays are available at www.fda.gov. In addition, WHO has evaluated specificity, sensitivity, negative predictive value, positive predictive value, and cost of all test kits the organization supports for distribution throughout the world. UNAIDS (Report 16, Geneva 2009, http://www.who.int/diagnostics_laboratory/evaluations/hiv/en/).

SPECIFIC LABORATORY METHODS FOR DETECTION OF HIV INFECTION

Immune Responses to HIV

Infection with HIV results in virus replication in a variety of cell types expressing surface markers such as CD4 and a coreceptor such as CXCR4 or CCR5. The genetic diversity of HIV-1 during this early infection period is limited, and it is likely that the majority of individuals are infected with a single variant, with slow, predictable accumulation in genetic diversity reflecting both the intrinsic mutation rate of HIV-1 and strong purifying selection, likely including immune responses.[88,89] Viral replication results in humoral and cellular responses, leading to viral protein processing and display of viral antigen fragments on infected cell surfaces, prompting an immune reaction (see Fig. 120.3). Cellular reactivity represents the earliest detectable immunity to HIV,[90–92] but such responses have not yet been exploited for use in early diagnosis of HIV infection.

Humoral responses represent a reliable method to determine the presence of true HIV infection. Antibody production after HIV infection follows a period of intense virus replication, as demonstrated by relatively high viral RNA levels and p24 antigen production preceding development of antibodies (schematic in Fig. 120.3). The precise kinetics of antibody production to individual HIV proteins is incompletely understood, and significant variability has been detected[93]; in general, however, immune responses to HIV-1 appear similar to other infections, with immunoglobulin M (IgM) followed by appearance of IgG production.[94–98]

The HIV window period (see Fig. 120.3) is the time after infection has occurred but before evidence of HIV infection is detectable. The HIV diagnostic window represents a vulnerable period, particularly

TABLE 120.1 HIV Testing Platforms and Modalities to Detect HIV RNA, p24, and Antibody

TRADENAME	INFECTIOUS AGENT	FORMAT	SPECIMEN	FDA-APPROVED HIV-1/2 SCREENING	HIV-1/2 DIFFERENTIATION OR SUPPLEMENTAL ASSAY	HIV-1 ONLY	HIV-1 ANTIGEN	HIV-1/2 AB	USEFUL FOR ACUTE INFECTION DETECTION	RAPID POINT-OF-CARE TESTING
HIV-1 Nucleic Acid Testing										
APTIMA HIV-1 RNA Qualitative Assay[440]	HIV-1	TMA	Plasma/serum	Yes	Supplemental	Yes			Yes	
HIV-1/2 ELISA/HIV-1 p24										
Elecsys HIV Combi PT[441]	HIV-1, HIV-2	Immunochemiluminescence	Plasma/serum	Yes		Yes	Yes	Yes	Yes	
VITROS HIV-1/HIV-2 Reagent Pack and Calibrator[442]	HIV-1, HIV-2	Immunometric	Serum/ plasma	Yes		Yes	Yes	Yes	Yes	
BioPlex 2200 HIV Ag-Ab Assay[443]	HIV-1 and HIV-2	Multiplex flow immunoassay	Serum, plasma	Yes		Yes	Yes	Yes	Yes	
ADVIA Centaur HIV Ag-Ab Combo (CHIV) Assay[444]	HIV-1, HIV-2	Microparticle chemiluminometric immunoassay	Serum	Yes		Yes	Yes	Yes	Yes	
ARCHITECT HIV Ag-Ab Combo[445]	HIV-1, HIV-2	Chemiluminescent EIA	Plasma/serum	Yes		Yes	Yes	Yes	Yes	
GS HIV Ag-Ab Combo EIA[446]	HIV-1, HIV-2	EIA	Plasma/serum	Yes		Yes	Yes	Yes	Yes	
Alere Determine[447]	HIV-1, HIV-2	Immunoassay	Plasma/serum	Yes		Yes	Yes	Yes	Yes	Yes
Geenius HIV 1/2 Supplemental Assay[448]	HIV-1, HIV-2	HIV detection test	Blood		Supplemental and differentiation		Yes	Yes	Yes	
HIV-1/2 ELISA										
SURE CHECK HIV 1/2 ASSAY[449]	HIV-1, HIV-2	Rapid immunoassay	Fingerstick and venous whole blood, serum, plasma					Yes	Less sensitive	Yes
HIV 1/2 STAT-PAK Assay[450]	HIV-1, HIV-2	Rapid immunoassay	Fingerstick and venous whole blood, serum, plasma	Yes				Yes	Less sensitive	Yes
OraQuick ADVANCE Rapid HIV-1/2 Antibody Test[451]	HIV-1, HIV-2	Rapid immunoassay	Oral fluid, plasma, whole blood (venipuncture and fingerstick)	Yes				Yes	Less sensitive	Yes
Chembio DPP HIV 1/2 Assay[452]	HIV-1, HIV-2	Rapid immunochromatographic assay	Oral fluid, serum, plasma, whole blood	Yes				Yes	Less sensitive	Yes
INSTI HIV-1/HIV-2 Antibody Test[453]	HIV-1 and HIV-2	Rapid immunoassay	Plasma/whole blood (venipuncture and fingerstick)	Yes				Yes	Less sensitive	Yes
Reveal Rapid HIV-1 Antibody Test[454]	HIV-1	Rapid immunoassay	Serum/plasma	Yes		Yes			Less sensitive	Yes
Uni-Gold Recombigen HIV-1/2[455]	HIV-1, HIV-2	Rapid EIA	Whole blood (venipuncture, fingerstick)	Yes				Yes Yes	Less sensitive	Yes
Genetic Systems HIV-1/HIV-2 Plus O EIA[456]	HIV-1, HIV-2	EIA	Serum/plasma/cadaveric serum	Yes				Yes	Less sensitive	

Continued

TABLE 120.1 HIV Testing Platforms and Modalities to Detect HIV RNA, p24, and Antibody—cont'd

TRADENAME	INFECTIOUS AGENT	FORMAT	SPECIMEN	FDA-APPROVED HIV-1/2 SCREENING	HIV-1/2 DIFFERENTIATION OR SUPPLEMENTAL ASSAY	HIV-1 ONLY	HIV-1 ANTIGEN	HIV-1/2 AB	USEFUL FOR ACUTE INFECTION DETECTION	RAPID POINT-OF-CARE TESTING
Uni-GoldRecombigen HIV-1/2[455]	HIV-1, HIV-2	Rapid EIA	Serum, plasma	Yes				Yes	Less sensitive	
Avioq HIV-1 Microelisa System[457]	HIV-1	EIA	Serum, plasma, dried blood spot, oral fluid	Yes		Yes			Less sensitive	
ABBOTT PRISM HIV O Plus Assay[458]	HIV-1, HIV-2	ChLIA	Plasma/serum/cadaveric serum	Yes				Yes	Less sensitive	
VITROS HIV-1/HIV-2 Reagent Pack and Calibrator[442]	HIV-1, HIV-2	Immunometric	Plasma/serum	Yes				Yes	Less sensitive	
ADVIA Centaur HIV 1/O/2 Rapid Test[459]	HIV-1, HIV-2	Microparticle chemiluminometric immunoassay	Plasma/serum	Yes				Yes	Less sensitive	
Home Access/Home Detection										
OraQuick In-Home HIV Test[460]	HIV-1, HIV-2	Immunoassay	Oral fluid	Yes					Not sensitive	
Home Access HIV-1 Test System[461]	HIV-1	Dried blood spot collection device	Dried blood spot		Supplemental	Yes			Less sensitive	
HIV-1 Western Blot[a]										
Cambridge Biotech HIV-1 Western Blot Kit[462]	HIV-1	WB	Serum/plasma		Yes	Yes			Not as sensitive as ELISA	
GS HIV-1 Western Blot[462]	HIV-1	WB	Serum/plasma		Yes	Yes			Not as sensitive as ELISA	
OraSure HIV-1 Western Blot Kit[463]	HIV-1	WB	Oral fluid		Yes	Yes			Not as sensitive as ELISA	
GS HIV-1 Western Blot[462]	HIV-1	WB	Dried blood spot		Yes	Yes			Not as sensitive as ELISA	

[a]No longer in general use.
Ag-Ab, Antigen-antibody; *ChLIA,* chemiluminescent immunoassay; *EIA,* enzyme immunoassay; *ELISA,* enzyme-linked immunosorbent assay; *HIV,* human immunodeficiency virus; *TMA,* transcription-mediated amplification; *WB,* Western blot.

TABLE 120.2 Test Performance Definitions and Derivations

TEST PERFORMANCE CHARACTERISTICS	DEFINITION	FORMULA
True HIV positive	Number of individuals who are actually HIV infected	A
True HIV negative	Number of individuals who are actually HIV uninfected	B
False HIV positive	Number of positive HIV test results/total samples without HIV infection	C
False HIV negative	Number of negative test results/total samples with HIV infection	D
Total number of individuals with HIV infection	HIV-infected population	A + D
Total number of individuals without HIV infection	HIV-free population	B + C
Total number of positive tests	—	A + C
Total number of negative tests	—	B + D
Sensitivity	Number of positive test results/total samples with HIV infection	A/A + D
Specificity	Number of negative test results/total samples without HIV infection	B/B + C
Positive predictive value	Proportion of those with HIV-positive assay who are actually HIV infected	A/A + C
Negative predictive value	Proportion of those with HIV-negative assay who are actually HIV uninfected	B/B + D
Test efficacy	Test performance under ideal conditions	
Test effectiveness	Test performance under practical conditions	
Number of persons with HIV infection who test positive	—	A
Number of persons without HIV infection who test negative	—	B
Number of HIV-positive test results and not infected	—	C
Number of HIV-negative test results with the disease	—	D
Total number of persons	—	A + B

HIV, Human immunodeficiency virus.

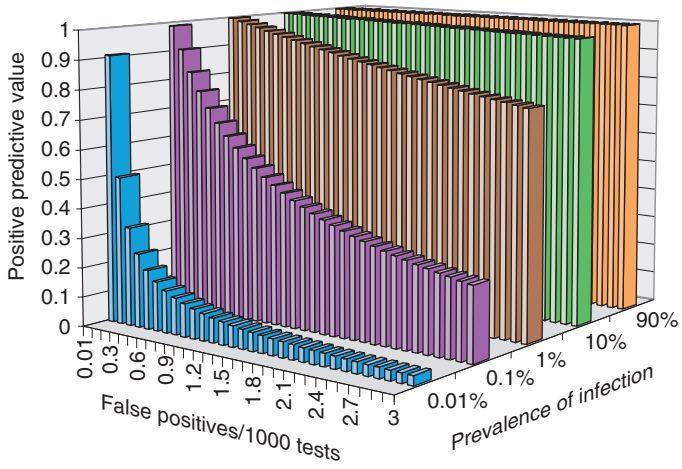

FIG. 120.4 Positive predictive value of HIV supplemental assays is a function of disease prevalence. At very low prevalence of infection in the population (0.01%), the positive predictive value of testing declines sharply with any false-positive testing. As the prevalence of infection in the overall population increases, the positive predictive value improves regardless of a few false-positive results. The HIV screening step eliminates the vast majority of HIV-uninfected individuals; those who screen reactive and are referred for supplemental testing therefore constitute a population with a high prevalence of HIV infection. As such the overall positive predictive value remains high despite relatively few false-positive supplemental results.

from a blood safety standpoint, and several major efforts have been launched to minimize its duration. Initial studies suggested the HIV window period was on the order of 2.1 months,[99] but progressive improvements in ELISA methodology reduced window periods to less than 4 weeks. As shown in Fig. 120.3, HIV p24 antigen or HIV RNA is often detectable before antibody responses, and use of sensitive HIV antigen and nucleic acid have reduced the window period to the current minimum. Limited studies with other HIV antigens have demonstrated early detectable reverse transcriptase after infection.[100] It is important to note, however, that studies of viral and immune kinetics to estimate the duration of the HIV window period use established seroconversion panels consisting of serial samples obtained from patients with known exposure history.[101] The levels of HIV-1 viremia vary in patients, and the rate of change of HIV RNA levels or production of antibodies during acute HIV infection is unpredictable; thus generalizations regarding the timing of seroconversion in such panels (or in newly infected patients) remain imprecise. Instead, analyses describe only average changes in the window period relative to an established comparator method. In general the average window period with third-generation antibody tests is 22 days. Standard antigen testing in fourth-generation assays decreases the window period to approximately 16 days, and NAT further reduces this period an additional 4 to 6 days.[54,102]

Once established, HIV seroreactivity is typically lifelong, although several exceptions have been reported.

1. Late in the course of HIV-1 infection, in the presence of profound immunodeficiency, antibody levels may decline, potentially confusing serodiagnosis. Levels of HIV expression remain relatively high, and detection of HIV RNA is likely to be positive.
2. In rare cases HIV infection may proceed in the absence of detectable serologic markers.[103–113] Typically, such patients have been characterized by rapid disease progression.
3. Early introduction of ART may delay the development of full antibody responses to HIV infection.[114] In addition, introduction of ART may permit humoral immunity so that immunosilent infection may be detected.[115,116] Therapy may decrease the relative levels of anti-HIV antibodies and may affect detection by certain diagnostic platforms.[117,118]

In these unusual circumstances it is typically straightforward to reconstruct the clinical course and establish HIV diagnosis; careful history and dual antigen-antibody (Ag-Ab) and especially NAT assays are useful for clarification.

HIV Screening Assays

Serologic Techniques to Detect Anti-HIV Antibody

Standard ELISA Assays

HIV-1 ELISA assays were the earliest approved serologic tests for HIV infection and remain the most sensitive commercial assays for infection. HIV-1/2 ELISA assays have evolved over a number of generations to

maximize sensitivity and specificity. All assays capture HIV antibody using immobilized HIV antigens. In their earliest iterations, whole-cell lysates of infected cells were used to coat ELISA wells. Incubation with patient sera permitted anti-HIV antibody to bind to the immobilized antigens and the bound antibody was identified using enzyme-conjugated antihuman IgG. The amount of measured enzyme activity is proportional to the amount of bound antibody. Readout for ELISA assays is quantitative (e.g., optical density or fluorescence measurements), and reactivity is defined as enzyme activity exceeding an established threshold. Techniques to produce recombinant HIV-1 antigens[119–121] and chemically synthesized HIV peptides eliminated the use of cell lysates, thereby increasing specificity and sensitivity. Third-generation assays used a sandwich technique with enzyme-coupled HIV antigens and took advantage of the bivalent or multivalent nature of antibodies to improve specificity. Only antibodies bound to ELISA wells that also bind HIV antigens generate a signal; nonspecifically bound antibodies would be less likely to bind HIV antigens.[122] Sandwich technology also expands subtypes of antibodies detected. In direct ELISA assays the conjugate is directed against a specific antibody subtype (e.g., IgG), whereas sandwich technology permits detection of any antibody class, including IgM. Sandwich ELISA thus increased ability to detect HIV antibodies early in HIV infection,[123] and has been adapted in new formats, including immunochromatography, and has been expanded to detect HIV p24 antigen in addition to detecting anti-HIV antibodies (see later); the combination of advances in immunochromatography and sandwich technology to detect p24 antigen forms the basis for the most sensitive HIV screening assays (e.g., Alere Determine; Abbott, Abbott Park, IL; see Table 120.1). Screening tests have also been adapted for epidemiologic use in detecting incident HIV infection.[124]

Efforts to improve HIV detection during HIV-1 seroconversion period have led to combining the standard third-generation ELISA assay with a p24 antigen detection assay with increased sensitivity; microparticles and sandwich strategies are incorporated into microparticle technologies. Monoclonal antibodies to HIV p24 are immobilized onto microparticles, and along with HIV-1 antigens and HIV-2 peptides, the patient sample to be analyzed is added to the well, and any antibody and/or antigen is captured to form a complex. After a wash step, enzyme-labeled anti-p24 antibody and HIV-1 and HIV-2 antigens are added to form antibody-antigen-antibody or antigen-antibody-antigen conjugate complexes; finally, a substrate is added to provide a detectable signal proportional to the amount of bound enzyme.

Particle Agglutination Assays

HIV antigen antibody reactions have been used to develop relatively rapid, simple assays that do not require colorimetric readout. Such assays, denoted particle agglutination (PA), are based on the ability of sera containing HIV antibodies to cross link small particles containing HIV antigens on the surface (Fig. 120.5). PA has advantages of sensitivity and relatively high inherent specificity because the presence of bivalent or multivalent reactions is necessary for agglutination to occur. PA assays are relatively easy to perform and require little equipment and as such have important advantages for resource-poor areas.[125–130] Assays based on particle agglutination have been FDA approved[45]; PA are subject to reader interpretation, are time sensitive, and have no permanent records of agglutination reactions.[45,131] Nevertheless, in large field trials and hospital-based studies, PA platforms are quite robust, performed well for HIV screening,[132–134] and have reported utility with dried blood spots.[135] Moreover, advances in particle research with miniaturization and use of paramagnetic particles has been useful in development of new detection methodologies, including new fourth-generation tests.

Alternative ELISA Formats: Simple/Rapid Tests

Despite excellent performance characteristics, there are significant drawbacks of current HIV screening assays, including delays from the time of sample collection to reporting and the complexity of assay procedure. In the United States, publicly funded programs perform approximately 2.1 million HIV tests; in 2000, 30% of patients with positive results and 39% with negative results did not return to obtain test findings. The need for simple HIV detection assays for point-of-care sites and resource-poor circumstances has spurred development of a class of more user friendly kits known as "rapid" and "simple/rapid." Simple and rapid tests are manufactured in a variety of formats, and WHO and the CDC differ in details of definition (storage requirements, time required to obtain results). In general, these assays use lateral or capillary flow of sample along a solid support to permit interaction with an embedded antigen; controls are included to identify nonspecific reactivity. Simple tests react with antigen in variable storage requirements at ambient temperature using whole blood as substrate and require little or no equipment. Rapid tests are completed in less than 15 minutes (WHO) or in 30 minutes or less (CDC). Simple/rapid tests require confirmation but have been used in circumstances of emergency detection (e.g., pregnant women at time of delivery).

Sensitivity of rapid testing methods is sufficiently high with non-B HIV subtypes and with group O viruses,[136,137] and several kits include detection of HIV-2. In seroconversion panels, however, rapid testing may be less sensitive than conventional assays; Makuwa and coworkers[136] found rapid tests were positive 2 to 8 days after a conventional third-generation ELISA using a seroconversion panel including subtypes A, B, and C. Similarly, WHO surveys noted that in some panels, p24 antigenemia was detectable before simple/rapid assay–positive results. Brauer and colleagues[138] recently evaluated the only currently available fourth-generation rapid assay, finding a detrimental low sensitivity for p24 (10%) in seroconversion panels, implying that most of acute infections would be missed with this assay.

Nevertheless, strategies have been evaluated to use sequential independent rapid testing assays exclusively for HIV screening and confirmation.[139,140] Dual ELISA formats may yield higher false-positive results.[140]

Use of rapid HIV testing has resulted in increases in frequency of patient populations receiving test results, including pregnant women,[141] individuals attending sexually transmitted disease (STD) and outpatient clinics,[142] and patients presenting in emergency departments.[143] Modeling studies using the performance characteristics of the Single Use Diagnostic System (Abbott) rapid test kit suggested significant increases in numbers of individuals who would have learned their serostatus.[144] Rapid HIV testing permits early counseling and discussion of risk reduction and therapy but is challenged by the consequences of immediately discussing unexpected test results. For example, in a randomized study of rapid versus conventional testing in pregnant women, more women in the rapid testing arm were likely to obtain their serostatus results but were *less* likely to return for ART compared with women who received their results in a traditional follow-up appointment.[145]

Development of rapid and simple assays and worldwide experience with ELISA formats as supplemental assays led to new studies of the potential for alternative strategies. In domestic US settings this was rigorously analyzed[146] in the context of FDA sensitivity/specificity requirements, and it was concluded that some dual formats excluding WB may provide appropriate performance characteristics and may have fewer indeterminate or discordant results. These initial studies fueled new initiatives to develop supplemental assays that aid in the diagnosis of HIV infection. In parallel, advances in immunochromatographic applications for serologic detection offered new and useful modalities to detect HIV.

Saliva as Source of Patient Material for ELISA Assay

The difficulty, expense, and risk involved in obtaining blood for HIV detection has led to investigation of alternative sources for diagnosis. Several investigators noted the presence of anti-HIV antibodies in saliva, which contains little, if any, infectious virus[47,48,147] and can be obtained safely without the risk of needlestick injury.[148,149] Oral fluid consists of saliva, bacteria, mucus, debris, and a crevicular transudate containing significant and detectable levels of anti-HIV antibodies in infected individuals.[49,150] The presence of anti-HIV antibody appears to be transudative in origin and generally, although not exclusively, is the result of local synthesis.[151,152] Both IgA[48] and IgG classes are present, but anti–HIV-1 IgG is present in greater abundance; even so, anti–HIV-1 antibodies in saliva are nearly 1000 times lower in concentration than

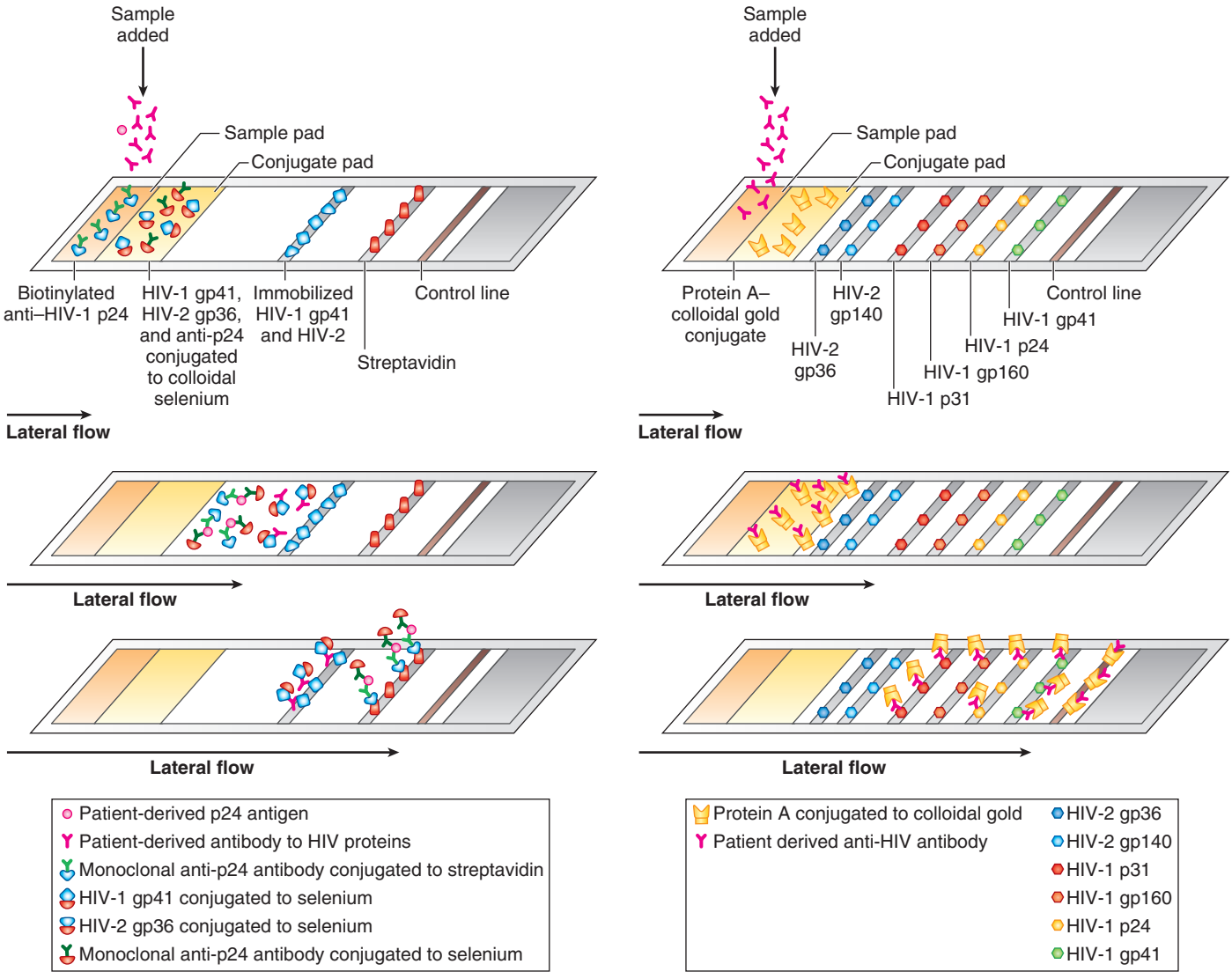

FIG. 120.5 Current ELISA strategies for HIV detection. (A) Fourth-generation detection of HIV antigen and antibody by immunochromatography. Sample is applied to the sample pad impregnated with monoclonal anti-p24 antibody conjugated to biotin. If HIV p24 is present in the sample, it binds to anti–p24-biotin conjugate; sample is carried through the conjugate pad containing HIV-1 gp41 and HIV-2 gp36, each conjugated to selenium, as well as monoclonal anti-p24 conjugated to selenium. If p24 antigen–anti-p24–biotin is present, it is bound by anti-p24 conjugated to selenium. The p24-antibody sandwich continues to travel along the strip by lateral flow until the complex reaches a line of immobilized streptavidin, which captures the complex via binding to avidin; as these complexes accumulate they generate a pink-red precipitate. In parallel, patient-derived anti–HIV-1 gp41 or HIV-2 anti-gp36 binds gp41-selenium or gp36-selenium, respectively, and those conjugates travel by lateral flow along the strip until they are captured by a line of immobilized gp41 or gp36, creating a pink-red precipitate as they accumulate. These sandwiched antibody or antigen precipitates are then visualized by the operator. A final line, which will react with any antibody serves as a positive control. (B) Immunochromatographic supplemental assay to detect HIV. Sample containing antibodies to HIV-1 (p24, p31, gp160, gp41) or HIV-2 (gp140, gp36) is applied and travels by lateral flow. The sample first encounters protein A conjugated to colloidal gold; protein A will bind antibody molecules regardless of antigen specificity, and HIV antibody–protein A conjugate continues to travel along the strip until it encounters corresponding immobilized proteins, where the complex accumulates and gradually creates a pinkish-red color, which is visualized by the operator. A final line binding all antibodies represents a positive control that the sample was applied and lateral flow took place. *Ag-Ab,* Antigen-antibody; *ELISA,* enzyme-linked immunosorbent assay.

in serum.[153] Nevertheless, IgG levels remain sufficiently high for measurement by both ELISA and WB techniques.[46,154,155]

Oral fluid detection of HIV-1 has excellent performance characteristics, comparable to that detected using serum.[46,154,155] Recently, O'Connell and coworkers[118] reported several false-negative results using oral fluid testing in patients with HIV infection who have undergone ART early in the course of HIV infection. It is possible that partial resolution of polyclonal gammopathy reduced transudative IgG levels, thereby reducing sensitivity. Obviously, patients on ART will be identified as HIV infected, but there are circumstances where such patients may be unwilling to disclose their status. Such false-negative results may have an impact on new HIV testing development, as new assays will likely be tested in positive, control, HIV-infected patients who may be undergoing therapy.[118] Instances of false-positive oral HIV testing have been reported.[156] The etiology of such occurrences remains uncertain, although recent data suggest an increasing false-positive rate as test kits reach expiration dates.[157]

Although little, if any, infectious HIV is present in saliva, HIV DNA was detected in crevicular fluid samples from patients with gingivitis or periodontitis, even in the absence of local bleeding.[158,159] The source

of HIV nucleic acid in these circumstances is unclear, although oropharyngeal shedding may contribute.[160]

Saliva IgA has been investigated as source material to diagnose HIV infection in newborns. Early studies in newborns and infants suggested that determining anti-HIV IgA might represent a useful assay to specifically identify HIV infected infants[161]; more recently, several studies have suggested lower sensitivity and greater nonspecific reactivity for saliva IgA detection.[162,163]

Although use of nonbloody oral fluid reduces possibilities for exposure to HIV, there remain concerns for potential exposure to other infectious agents, particularly tuberculosis.[164]

Urine as Source of Patient Material for ELISA

Urine as a source of patient material for HIV detection offers relative ease of collection and high sensitivity.[165] As levels of HIV IgG in urine are relatively low (estimated in the range of 1 mg/L), sensitive techniques are required for detection; ELISA assays were developed and were approved in 1996, followed by WB assays in 1998. There are no FDA-approved simple/rapid tests for urine approved as of 2009. False-positive results have been identified with urine,[166] although performance characteristics are generally excellent. One potential advantage of urine is that a single sample can be used to investigate the presence of chlamydia and gonorrhea in addition to HIV-1.[167]

SEROLOGIC TECHNIQUES TO SCREEN FOR HIV ANTIGENS

Acute HIV-1 infection is characterized by relatively high levels of viremia compared with the levels of various anti-HIV antibodies[168] (see Fig. 120.4). As a result, there is a short but significant period in early infection during which p24 antigen is present in the absence of specific anti-p24 antibody. ELISA techniques have been developed to specifically detect p24 using polyclonal patient-derived IgG preparations or monoclonal antibodies to coat ELISA wells. Wells are incubated with dilutions of patient sera, washed, and bound p24 is detected using a second anti-HIV antibody conjugated to a colorimetric detection system. HIV antigen capture assays detect as little as 5 to 20 pg of p24. Assuming all p24 in blood is from intact virions and that there are approximately 3000 p24 molecules per virion, the limit of p24 detection is in the range of approximately 40,000 to 200,000 virion particles.

Clinical application of p24 antigens revealed that newly infected patients had transient p24 detectable levels,[33,35] and that p24 antigenemia in established disease suggested a poor prognosis.[34] As antibody to p24 develops, complexes of p24 with endogenous antibody interfere with laboratory detection, and p24 sensitivity is reduced.[36] Methods incorporating pH adjustment or heat treatment to dissociate p24 Ag-Ab complexes have resulted in increased p24 detection sensitivity, and immune complex dissociation results in more frequent detection of antigenemia late in disease.[169,170] The combination of heat denaturation and signal amplification using biotinylated tyramide has extended the lower limit of signal detection to 0.5 pg p24 (≈4000 virion particles) has been reported,[171–173] suggesting this assay may be particularly attractive in resource-limited areas without the capability to perform quantitative HIV-1 RNA levels. Gag p24 protein is a relatively well-conserved protein among HIV-1 variants, and commercially available kits have performed consistently well even with diverse HIV-1 subtypes, but new p24 panels have been developed to represent the wide diversity of subtypes for sensitive detection.[174]

FOURTH-GENERATION HIV ANTIGEN-ANTIBODY ASSAYS

Improvements in sensitivity in p24 detection, combined with improvements in sandwich antibody testing, which could both be adapted to immunochromatographic formats, permitted development of rapid fourth-generation testing modalities, which have become the standard for initial evaluation of HIV-infected individuals. Fourth-generation testing (see Fig. 120.5A) involves application of the test sample into a lateral flow immunochromatographic device, in which patient sera or plasma is applied to a sample pad, where it first encounters anti-p24 antibody conjugated to biotin p24 Ag-Ab conjugates, and HIV antibodies travel by lateral flow to a conjugate pad containing HIV detection reagents conjugated to selenium, including a second anti-p24 antibody and peptides from HIV-1 (gp41) and from HIV-2 (gp36). Ag-Ab complexes continue to migrate by lateral flow and are captured by gp41 peptide, gp36 peptide, and streptavidin immobilized in lines perpendicular to the lateral flow. Note that lines are visualized only by accumulation of selenium conjugate; as a result, only "sandwiched" Ag-Ab conjugates will yield a visual result. This strategy takes advantage of the increases in sensitivity and specificity afforded by the sandwich approach; the lateral flow adaptation permits simultaneous detection of both antigen and antibody (see Fig. 120.5A).

In a second immunochromatographic application, supplementary assays were developed to detect and discriminate antibodies to HIV-1 and HIV-2 (see Fig. 120.5B) and detect antibodies to specific viral antigens. These rapid assays now provide supplemental assays to detect HIV infection in individuals with reactive fourth-generation assays and to discriminate between HIV-1 and HIV-2 (see Fig. 120.5B). In addition, these lateral flow devices were built after long experience with WB and included detection of HIV-1 p31 so that these new rapid assays could also provide information regarding early HIV infection (p31 negative corresponds to Fiebig V infection). The success of these newer assays has enabled whole-scale replacement of the WB or other prior confirmatory assays and permitted completion of HIV diagnostics in a single visit in many cases, facilitating linkage to care.

FALSE-POSITIVE AND FALSE-NEGATIVE RESULTS IN SCREENING TESTS FOR HIV ANTIBODY

HIV screening assays are designed for highest available sensitivity and specificity; sensitivity is a more paramount requirement for screening because the consequences of a false-negative screening test, which permits HIV infection to proceed undiagnosed, are more profound than a false positive, which will be further evaluated with a confirmatory test. Currently, the false-positive rate in a low-risk population remains low (0.06%–0.12% in the blood donor population),[175] suggesting practical test effectiveness is consistent with performance efficacy. Despite high specificity and sensitivity of HIV screening assays, false-negative and false-positive results may occur. Technical difficulties in assay execution may represent a cause for either false-negative reaction or false-positive results and may represent the most common cause of a false-positive or -negative test result.

False-negative results from ELISA methodology are rare but may arise from the lack of antibodies of sufficient avidity to generate a colorimetric signal above the assay background threshold. Early HIV infection, as described earlier, represents a common cause of low-level antibody production; rare immunosilent HIV infection, without development of antibody response despite HIV replication, and other underlying conditions, such as neoplasms/chemotherapy and common variable immunodeficiency (CVI), represent potential causes of false-negative HIV screening assays; patients with CVI have had detectable seroconversions.[176] Unusual circumstances, such as extensive transfusion, may sufficiently dilute endogenous antibody in infected individuals to yield false-negative HIV results[177]; robust antibody reactivity is not universally present.[178] In the past, technical difficulties, especially in early second-generation assays containing limited numbers of synthetic antigens, represented significant sources of false-negative results[179–181]; currently, such artifacts are highly unlikely sources of false-negative results.

False-positive screening results are more common than false-negative results and may arise from a number of reasons, generally classified as technical artifacts, chronic medical conditions, multiparity, and unusual circumstances. Reports of false-positive ELISA assays are frequent and often occur in the setting of testing patients with low pretest probability for HIV infection; as a result, it is not always certain whether the false-positive result was associated with a particular medical condition or was the result of the false-positive rate of the test itself.

Several medical conditions may contribute to falsely reactive HIV ELISA tests, including chronic alcohol use, rheumatic disease, congenital bleeding disorders, syphilis, and neurocysticercosis. In certain rheumatic diseases, polyclonal antibody production has been a persistent source

of false-positive HIV ELISA results. Specific reactivity for gp41 has been identified in some cases, and p24 reactivity is responsible for false positives in Sjögren syndrome; reactivity is generally of low avidity and has been eliminated by thiocyanate washing.[182] Recent vaccination with hepatitis B[183] or rabies[184,185] has been reported to result in false-positive HIV screening results, although the frequency of these events is likely to be quite low.[186] In 1992 influenza vaccination was reported to be associated with increased false-positive HIV testing; an extensive analysis traced increases in false positives to certain ELISA kit lots, and frequent false reactivity after routine influenza vaccination has not been reported. Multiparous women also have a higher rate of false-positive screening tests for HIV, although the etiology remains uncertain. Fourth-generation assays also have false-positive results; recently, the presence of heterophile antibodies has been reported to result in false-positive assay results.[187]

Acute infections may yield false-positive HIV screening assays; recent infection with dengue, malaria, and hepatitis B has been reported to yield false reactivity. False-positive results have been reported in patients with leprosy using early ELISA assays,[188,189] with antibodies to lipoarabinomannan of *Mycobacterium leprae* implicated as potential cross-reacting antibody,[190] although a study using double-sandwich ELISA did not reproduce the high rate of false-positive results in this population.[191] As primary HIV infection may present as an acute viral syndrome with fever, rash, and numerous constitutional symptoms, thoughtful interpretation of any screening assay in patients with such symptoms is warranted. Recent survey of patients with documented acute mononucleosis did not identify a positive HIV screening assay result.[192] However, several patients with primary HIV infection have been reported with false-positive serologic tests for Epstein-Barr virus infection noted during evaluation.

The presence of HIV infection may affect serologic detection of other infections: False-positive results for syphilis, Ebola, Marburg, and Lassa fever have been reported in patients with HIV infection; HIV infection has been reported to result in occasional false-negative results in human T-cell lymphotropic virus (HTLV)-1/2 assays.[193]

With time and widespread application it is possible that new sources of false-positive results may occur. In needlestick circumstances, samples for HIV testing from inpatients may be obtained from indwelling catheters; samples drawn in such a fashion may become contaminated with vehicles for drugs administered through the catheter. One diluent, propylene glycol, has been reported to give a false-positive result with HIV ELISA assays.[194]

NONSEROLOGIC TECHNIQUES TO SCREEN FOR HIV

Hybridization and Amplification Assays to Detect HIV Nucleic Acids

Use of nucleic acid testing (NAT) represents an adjunct to, but not a replacement for, serologic methods for HIV detection. Various formats can detect HIV-1 RNA with great sensitivity, and new real-time polymerase chain reaction (PCR) methods have been developed capable of detecting a single copy of HIV-1 RNA.[195] Clinical methods for HIV nucleic acid detection for use in monitoring HIV infection have included nucleic acid sequence-based amplification (NASBA), branched DNA (bDNA), and PCR amplification; NASBA formats have detected as low as 22 to 31 copies/mL.[196,197] The first of these methods to be FDA approved for donor screening or diagnosis is a "TMA/HPA" system (Fig. 120.6) consisting of a transcription-mediated amplification (TMA) and hybridization protection assay (HPA), named Aptima, developed by Gen-Probe (San Diego, CA).[54] This modality has been approved *both* for screening and confirmation of infection and only detects HIV-1.

The principle of the TMA/HPA assay is to generate large numbers of HIV RNA copies from HIV-1 for detection by specific hybridization to chemiluminescent probes (see Fig. 120.6). Plasma samples are extracted, and HIV-1 RNA is reverse transcribed to complementary DNA (cDNA) using exogenous murine leukemia virus reverse transcriptase. The primer for the cDNA reaction contains the promoter sequence for the T7 bacteriophage RNA polymerase, followed by sequences complementary to HIV-1. The resulting cDNA product contains T7 promoter sequences linked to HIV-1 sequences, which are used as a template for added T7 RNA polymerase, a high-efficiency enzyme that rapidly transcribes

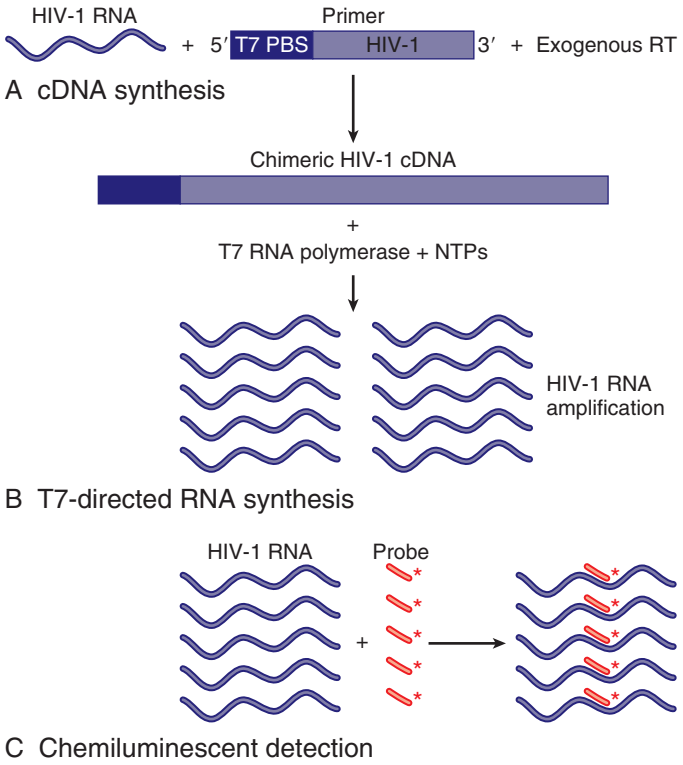

FIG. 120.6 Detection of HIV nucleic acid. Detection of HIV nucleic acid by transcription mediated amplification/hybridization protection assay. (A) Complementary deoxyribonucleic acid *(cDNA)* synthesis. (B) T7-directed ribonucleic acid *(RNA)* synthesis. (C) chemiluminescent detection. *NTPs,* Nucleotide triphosphates; *PBS,* phosphate-buffered saline; *RT,* reverse transcriptase.

multiple copies from the chimeric T7-HIV cDNA. The RNA copies are visualized by addition of chemiluminescent probes, which hybridize to HIV-1 sequences; excess probe is quenched; and the luminescence from the RNA-probe hybrid is quantitated (see Fig. 120.6). The assay is qualitative only and does not yield specific copy number; sensitivity of the assay is in the range of 13 copies/mL plasma.[54] TMA/HPA detected a limited panel of non-B subtypes of HIV-1 equally well. The TMA/HPA assay was sufficiently sensitive to detect HIV RNA 12 days earlier than standard third-generation ELISA and 6 days earlier than p24 assays. Thus one primary application of TMA/HPA is in diagnosis of early HIV-1 infection; the Aptima system was approved for screening and confirmation of HIV-1 in 2006 and should supplant use of bDNA or PCR assays for diagnosis.

Similarly, TMA/HPA technology was developed for blood donor screening. In the United States trials for blood donor screening by NAT screened more than 20 million donations and found seven HIV-infected samples that scored negative for HIV antibody.[102] NAT systems detected a number of HIV-1 infections that would have been missed by previously licensed test methods, confirming the increased sensitivity of these systems.[198] Similarly, trials in Europe, South Africa, and Japan[199–202] and in case reports[203–207] have documented, either in real time or retrospectively, identification of patients in seroconversion windows, using NAT, who had scored negative using p24 antigen detection. By contrast, there are also reports of undetected HIV-1 in the setting of relatively low HIV-1 RNA levels, especially in minipools of 16 or 24 plasma samples,[102] Retrospective analysis of a blood transmission case (before NAT) revealed a relatively low viral RNA level (estimated 40 copies/mL)[208]; the benefit of NAT over traditional testing has been estimated to reduce the window period by 2 to 6 days. Using NAT technology, the risk of HIV infection through transfusion was estimated as 1 per 1,576,000[209]; four cases of p24 antigen negative blood component were identified in more than 19,000,000 screened units.[210] In consequence, the risk of HIV-1

transmission via blood components is reduced, but not completely eliminated, by incorporating NAT into screening procedures; the residual risk of HIV infection in United States is estimated at 1 per 2,135,000.[210] Similar or lower risks have been estimated from other reporting countries,[211,212] and although there are concerns regarding subtype sensitivity and sample preparation, many blood centers throughout the world have incorporated NAT as a component of HIV screening. The relative cost-effectiveness of NAT remains a concern.[199,201,213] NAT is approved in the United States for blood-donor screening using plasma, and assays must be able to detect 100 copies 95% of the time; several assays exceed this limit.[197] Standard subtype B virus preparations have been established as quantitative controls.[214] Testing can be performed on single samples or minipools of plasma and may be combined with testing for hepatitis C and B.

TMA/HPA requires trained personnel and is more expensive than traditional HIV testing or routine HIV RNA quantitation assays, and routine assays have been used for diagnosis in resource-limited settings. NAT has, however, reported both false-positive and false-negative results in plasma.[215,216] A value of 10,000 copies/mL has been proposed as a cutoff for the HIV-1 RNA level above which is suggestive of HIV infection in the nondonor population. NAT has not been extensively used for cadaveric specimens; RNA is chemically labile, and delays in sample processing may present particular challenges in detection by this method. NAT is effective in detecting HIV in serum or dried blood spots,[217] so plasma (nonheparinized) is a more ideal starting material, especially for low copy-number detection.

In unusual circumstances HIV-infected individuals may have TMA/HPA-negative results. As described in Chapter 121, the spectrum of HIV infection includes long-term nonprogressors (LTNPs) with persistently high CD4 cell numbers and relatively low HIV-1 RNA levels.[218] Although LTNPs are HIV-1 ELISA reactive and WB positive, screening with current NATs could not be expected to reliably identify such individuals as HIV infected. As reported by Migueles and coworkers,[219] the HIV-1 RNA levels in LTNPs with naturally suppressed viremia are approximately two copies/mL plasma, below the limit of reported detection for TMA/HPA.[219] At present, NAT systems for patient analysis only detect HIV-1; currently, there are no FDA-approved HIV-2 NAT kits. In the United States, NAT blood bank screening procedures do not evaluate the presence of HIV-2 infections, although assays for HIV-2 RNA have been developed to monitor known infections and have been useful in characterizing samples identified from blood donors.[220]

NAT performance characteristics have been established largely with subtype B virus. HIV-1 RNA detection assays used to monitor HIV infection have been adapted for use with alternative HIV subtypes. A collaborative study of 28 laboratories from 16 countries evaluated an HIV-1 RNA Genotype Reference Panel for use with NAT[221] that contained six pure subtypes plus two recombinants (CRF01_AE and URF A/G) from group M and two isolates from group N and O, respectively. Despite some interassay differences, subtypes A to D and AE were detected consistently, but some assays had difficulty with the detection and quantification with subtypes F and G and the representative of group N; most assays failed to detect group O. More recent NAT assays have been designed to overcome the impact of HIV diversity on HIV detection, but some peculiar strains can still affect NAT efficiency. In a case report Foglieni and coworkers[222] described a repeat blood donor seroconverting to anti-HIV antibodies but showing undetectable HIV RNA at diagnosis and 2- to 3-log lower viral load during follow-up when compared with other NATs targeting different regions; genome sequencing revealed a B/F recombinant with mutations affecting primers and probe annealing. Genetic variation in HIV subtypes have prompted WHO to standardize strategies for testing for HIV and other NATs.[223]

HIV SUPPLEMENTAL AND CONFIRMATORY ASSAYS

HIV-1/2 ELISA for Confirmation of HIV Infection

In the United States and many western countries the requirements for optimum sensitivity and specificity in HIV testing have generally been supplied by sequential ELISA-WB formats. ELISA assays have been used to provide confirmation of HIV infection, especially in developing countries with limited resources to support WB or immunofluorescence assays. As described later, WHO has established several strategies incorporating dual ELISA formats with distinct design formats for diagnosis algorithms. In 2013 the FDA expanded the approval for the HIV MultiSpot ELISA screening test to include confirmation as part of a new CDC-proposed algorithm. MultiSpot uses sample concentrated onto a solid-phase membrane to overcome relatively slow, diffusion-mediated interactions between immobilized ligands and free analytes in solution.[224] The initial HIV-1/2 discriminatory assay, MultiSpot, consisted of immobilized ligands, including recombinant gp41, an immunodominant gp41 peptide, and a gp36 immunodominant peptide representative of HIV-2 isolates, as well as a procedural control consisting of goat antihuman IgG. Patient samples are added to a cartridge, which is then concentrated onto the membrane, and antibodies reacting with immobilized ligands are detected with goat antihuman antisera. In a series of studies the sequential use of a third-generation HIV ELISA, followed by MultiSpot, was equivalent to the standard ELISA-WB algorithm.[225–228] The MultiSpot approach also detected HIV antibodies in individuals with decreases in anti-gp41 antibodies as the result of ART. A newer discriminatory assay (see Fig. 120.5B) distinguishes HIV-1 and HIV-2 in an straightforward immunochromatographic approach with equivalent performance characteristics that have supplanted the prior MultiSpot assay.

WESTERN BLOTTING

HIV WB represents a specific method to detect the presence of serologic reactivity to individual viral antigens. WB has a specificity of greater than 99% and was the practical gold standard confirmatory test for HIV infection in the United States for many years (see Fig. 120.1). New supplementary assays have replaced WB as a confirmatory or supplemental assay; detailed description of this technique can be found in Chapter 122 of the eighth edition of *Principles and Practice of Infectious Diseases*.

IMMUNOFLUORESCENCE

Indirect immunofluorescence assay (IFA) is a standard virologic technique to identify the presence of antibodies by their specific ability to react with viral antigens expressed in infected cells; bound antibodies are visualized by incubation with fluorescently labeled antihuman antibody.[229,230] A requirement for antibodies to demonstrate reactivity with characteristic staining patterns provides additional specificity to the interpretation. Indirect immune fluorescence had been used extensively as a confirmatory assay in HIV diagnosis,[230,231] and continues to be used in laboratories with extensive experience with the assay. The presence of HIV antibodies is demonstrated by intense cytoplasmic immunofluorescence in cells observed in multiple fields as determined by an experienced examiner. Negative results consist of no significant difference in background fluorescence staining in positive and negative cells; results that are neither positive or negative (denoted "other" or "indeterminate") may be obtained as well when intense fluorescence is present in uninfected cells and infected cells. Conditions interfering with immunofluorescence include severe lipemia, hyperbilirubinemia, paraproteinemia, and autoimmune diseases. IFA for HIV-2 has been described,[232] but no FDA-approved kits for this purpose are available in the United States.

Performance characteristics for IFA remain excellent and in good agreement with other supplemental/confirmatory tests such as WB results.[233] In a MPEP survey no false positives were detected, and 14.3% of 215 results were indeterminate. Commercial IFA assays are relatively rapid, straightforward, and inexpensive[234] but require special equipment and expertise; nevertheless they may be particularly suited for programs with limited resources.[235] In the United States IFA has largely been replaced by fourth-generation testing. The Home Access system, approved in 1996, however, continues to use IFA as confirmatory testing.[236]

Radioimmunoprecipitation assay (RIPA) is a standard technique to identify the presence of antibodies by their ability to react with

radiolabeled antigens.[237–239] Antigens may be labeled by iodination or metabolic labeling, in which HIV-infected cells are grown in the presence of radioactive amino acids, resulting in the incorporation of label into HIV proteins. Lysates of radiolabeled cells are incubated with dilutions of patient serum; complexes of radiolabeled antigen-HIV antibody are harvested using reagents such as Sepharose-bound protein A. Radioimmunoprecipitations are highly specific and were used as early confirmatory assays.[238] In some circumstances, RIPA may be more sensitive than WB reactivity. Huisman and coworkers[237] detected early Gag p24 reactivity before ELISA reactivity, and Saah and coworkers[240] noted the utility of radioimmunoprecipitation in resolving indeterminate WB early in infection. RIPA is still likely less sensitive than p24 assay or NAT, as described in case reports of analyses of serial specimens from seroconversions.[241] Techniques to distinguish HIV-1 and HIV-2 have been reported.[242] Radioimmunoprecipitation assays are relatively labor intensive, have specific equipment and laboratory requirements, and do not have commercial versions approved by the FDA.

VIROLOGIC TECHNIQUES TO DETECT HIV INFECTION

Isolation of HIV by in vitro cultivation techniques remains a useful method for establishing HIV infection. Although the levels of HIV in plasma may be relatively high, the proportion of circulating viremia that is capable of transmitting infection is relatively low; thus use of serum or plasma for HIV cultivation is relatively insensitive. Use of peripheral blood mononuclear cells (PBMCs) from HIV-infected individuals as a source of HIV has a much higher rate of positive isolation, especially if PBMCs are CD8 depleted. PBMCs may be cocultivated with uninfected donor PBMCs, activated with phytohemagglutinin, and cultivated with interleukin-2 to increase detection. To increase detection of HIV with CCR5 specificity, monocyte-derived macrophages may be used for cocultivation.

DIAGNOSTIC ALGORITHMS AND PRACTICAL APPLICATION OF HIV DETECTION ASSAYS

Several algorithms for HIV detection have been devised, depending upon the purpose of the detection. In the United States the diagnostic algorithm underwent major revision as new fourth-generation assays were approved for confirmation of HIV infection; in that setting the CDC recommended new diagnostic approaches, which are now universally accepted in the United States (Fig. 120.7). In the new algorithm fourth-generation testing represents the first stage in testing; samples reactive by either Ag or Ab reactivity are subjected to HIV-1/2 differentiation assay as a supplemental assay (see Fig. 120.7). In the cases of discordant results (differentiation assay is negative), early HIV infection may be suspected, and HIV-1 NAT is recommended (see Fig. 120.7). In any case of uncertainty, repeat testing is typically quite useful, as the repeat samples will typically be from a later time point in the HIV infection profile, permitting a more mature serologic response to develop.

HIV-1/2 Screening Phase

Screening for HIV begins during the patient interview. As described earlier, HIV testing is imperfect, with a significant false-positive rate in low-prevalence populations. For patient diagnostic purposes, efficient application of HIV testing should be implemented after some risk assessment and with serious consideration of scientific and ethical consequences.[243]

In the United States the CDC revised guidelines describe populations recommended for HIV testing.[71] New guidelines recommend routine screening for HIV infection in all persons 13 to 64 years of age presenting in all health care settings, unless HIV prevalence is <0.1%. Testing is recommended for any patient presenting with an STD, viral hepatitis, or tuberculosis. Repeat screening is indicated for persons with elevated risk for HIV infection, for persons engaging in sex with a new partner, and in persons with occupational HIV infection.

All screening ELISA tests with reactive results are automatically repeated in duplicate; repeatedly reactive samples are referred for confirmation. In cases of discordant results an independent blood sample should be obtained, and a screening HIV-1/2 ELISA should be repeated; use of an independent approved HIV-1/2 ELISA kit using a different detection strategy may be considered. Nonreactive results identify patients who are at elevated risk but do not have detectable infection. Such individuals represent a critical population that requires continued care and counseling.

Acute HIV Infection

Individuals in whom acute HIV infection is strongly suggested represent a potentially difficult but important group to diagnose correctly and quickly, especially if early ART is under consideration. In general, a fourth-generation Ab-Ag ELISA and appropriate HIV-1 RNA detection provide essential information in a timely fashion. In contrast, other

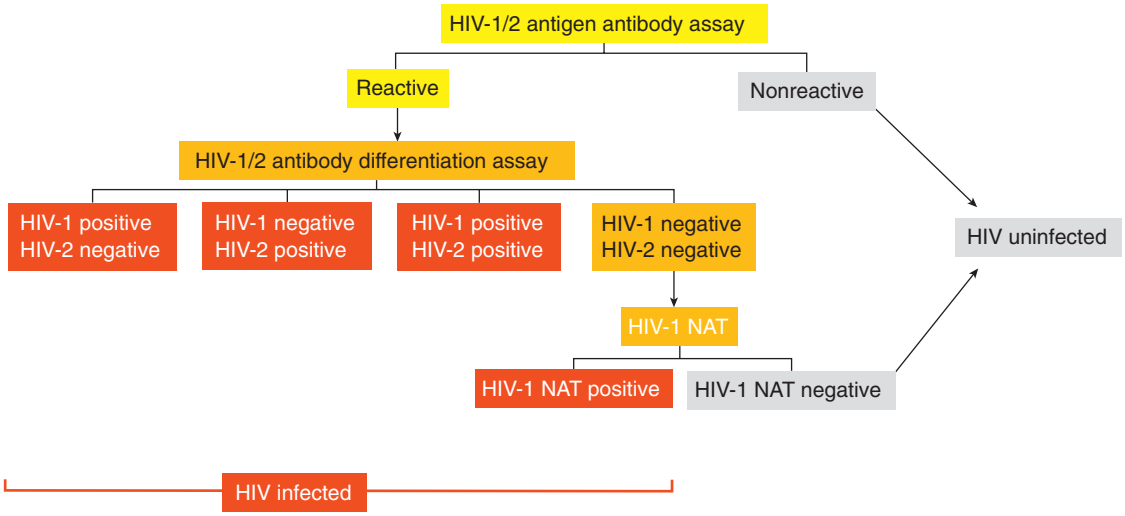

FIG. 120.7 HIV testing algorithm. Initial testing is performed with HIV-1/2 antigen antibody assay (see Table 120.1; HIV-1/2/HIV-1 p24 platform; assay depicted in Fig. 120.5A). Samples that are reactive for either antigen or antibody are referred for supplemental testing with HIV-1/2 antibody differentiation assay (see Table 120.1 for assays, strategy depicted in Fig. 120.5B). Where HIV antibody is present, the differentiation assay confirms the presence of HIV antibody and distinguishes HIV-1 from HIV-2; if antibody is not detected, the sample may be from an early HIV infection, and the sample is referred for HIV-1 NAT. If the NAT is negative, then the patient is not infected, and the initial HIV-1/2 antigen-antibody assay was a false-positive result. *NAT,* Nucleic acid testing.

rapid testing modalities, especially oral, urine, and home testing modalities, have decreased sensitivity during the acute infection period.

Repeated reactivity with HIV-1/2 ELISA, positive qualitative NAT assay, or positive p24 antigen represents a positive screening test for HIV infection; the presence of positive NAT or p24 is strongly suggestive of early HIV infection. TMA/HPA qualitative NAT is approved as a screening *and* a supplemental assay (but not both in a single individual). Thus, in the acute HIV infection before seroreactivity by ELISA-WB, a positive p24 and positive NAT represents HIV infection. In circumstances of discordant results a repeat specimen should be obtained. Even where p24 and NAT are positive, repeated serologic testing is advisable, especially if early ART is considered. Serologic reactivity evolves in a relatively reproducible fashion. Fiebig and colleagues[243a] proposed an early HIV infection staging system based on NAT, p24, and serologic reactivity patterns (Fig. 120.3): Fiebig I–positive HIV RNA, negative p24 antigen, negative third-generation enzyme immunoassay (EIA); Fiebig II–positive HIV RNA, positive p24 antigen, negative third-generation EIA; Fiebig III–positive HIV RNA, positive p24 antigen, positive third-generation EIA, negative WB; and Fiebig IV–positive HIV RNA, positive or negative p24 antigen, positive third-generation EIA, and indeterminate WB. All subjects had to have a nonreactive EIA by non–IgM-sensitive EIA. The corresponding mean cumulative durations from onset of HIV infection, according to Fiebig and coworkers, are 5 (Fiebig I), 10.3 (Fiebig II), 13.5 (Fiebig III), and 19.1 (Fiebig IV) days.

HIV-1/2 Supplemental Analysis

Samples repeatedly reactive in screening assays are referred for supplemental testing (see Fig. 120.7). These supplemental/confirmatory tests approved by FDA include HIV differentiation assays (e.g., GEENIUS), WB, IFA, and qualitative NAT. In current practice ELISA differentiation assays are now most commonly used, replacing long-standing use of WB assays. New assays are flexible and can be performed in a rapid fashion, permitting more effective linkage to care.

Test Counseling

Pretest and posttest counseling sessions represent critical opportunities for health care professionals to impact HIV transmission and are an integral part of the HIV testing process. Patient counseling is a paramount concern in patient diagnosis, and use of specially trained counselors should be considered. In general, typical pretest counseling includes a discussion of the importance and limitations of the assays; a discussion of behaviors to reduce risk of contracting HIV; implications of positive results, including effects on employment; and insurance. Consent for HIV testing, including implied opt-out testing, should be explicitly documented as part of clinical evaluation, and in some circumstances this may require written signed consent from patients. Posttest counseling involves a discussion of the results and their accuracy, the implications of negative and positive results, and consideration of behaviors that may prevent transmission or acquisition. When testing is performed outside the health care circumstances, opportunities for pretest and posttest counseling remain essential.

Notification strategies are traditionally carried out in a face-to-face manner, to permit broad discussion and to address patient concerns. Telephone counseling was found to be surprisingly effective in home collection strategies,[244] and alternatives to face-to-face discussions may be advantageous in certain circumstances. Tsu[245] compared direct (face-to-face) and telephone notification (patients called to obtain results) in a randomized trial of 351 at-risk and homeless youth; a significantly higher proportion of individuals in the telephone notification group received testing results and posttest counseling compared with those who returned to receive information face to face. Counseling has always been a component of home collection services and has been incorporated into new home testing, including web-based information, 24-hour telephone contacts, and referral services.

Alternative Strategies for Screening/Confirmation HIV Detection

Although the sequential screening and supplementary assays is a sensitive and specific approach for HIV detection and has been useful in United States and developed countries, it is relatively expensive, labor intensive, and requires special equipment; alternative strategies are essential for detection of HIV antibodies in resource-poor circumstances. Initially, dual ELISA strategies, using assays with different principles or antigens, have demonstrated >99% sensitivity and specificity in field tests using HIV subtypes appropriate for resource poor areas.[246–254] Poor-quality testing remains an infrequent but preventable error; improving both test quality and implementation of test algorithms will be essential in widespread expansion of initiatives and procedures for HIV testing.[255]

WHO and UNAIDS[137,256,257] initially developed three alternative strategies for detection of HIV infection: designed for surveillance of blood supply (strategy I), surveillance and diagnosis (strategy II), and diagnosis (strategy III). Subsequently, diagnosis recommendations have been clarified,[258] and current recommendations include ELISA strategies: two sequential reactive ELISA results for HIV diagnosis in high-prevalence areas (>5% HIV infection) and three sequential ELISA reactive results in low-prevalence (≤5% HIV infection) areas.

SPECIFIC CIRCUMSTANCES AND SPECIAL POPULATIONS

HIV Diagnosis in Individuals Undergoing Preexposure Prophylaxis

Several studies have directly addressed the use of ART either as PrEP or as early treatment to prevent HIV transmission and have shown these approaches to have great promise as tools for HIV prevention[259] (see Chapter 119). Testing that gives accurate HIV infection status is important so that only uninfected individuals are given PrEP. Detection of acute infection is important as demonstrated by results from the PrEP study. In this study two individuals with undetected acute HIV infection were randomized to the PrEP arm of the study and developed drug resistance, highlighting the importance of accurate diagnoses when implementing PrEP; guidance for the use of PrEP[260] recommends HIV testing before PrEP. Since the development of HIV testing, more than 40 million individuals have been identified with HIV infection. The success of PrEP and PEP requires that many more individuals will need to be followed and sequentially tested for HIV infection, placing an enormous burden on testing modalities in correctly detecting HIV in low-prevalence and early infection populations. The approach to evaluation of all PrEP and PEP circumstances should have three components: at the initial visit, a careful history establishing the time line of all potential exposure events; second, relevant physical examination and laboratory tests should be performed to determine whether HIV infection is already present at the time of the visit, which may predate the presenting history; and finally, the timeline of ongoing visits to track the effect of PrEP or PEP interventions must be established, which will include additional testing. Clinicians should strongly consider that individuals presenting for either PrEP or PEP may already have early or acute HIV infection, and HIV screening modalities should be chosen accordingly. Similarly, repeat testing is essential during the course of PrEP and PEP and after discontinuation to exclude a window period infection that was suppressed and not detectable by routine testing during PEP.

Widespread implementation of PrEP strategies will have a profound effect on HIV spread, but successful programs require rigorous diagnostic accuracy. PrEP consists of a two–antiretroviral drug combination and is inadequate as therapy for established HIV infection. Incident infections during PrEP are commonly due to nonadherence with PrEP regimens, less frequently to initiation of PrEP during the occult window period infection,[261] and rarely due to true PrEP regimen failure.[262] Laboratory assays for HIV infection should use assays with the highest sensitivity. Fourth-generation Ag-Ab combination assays are likely to become the standard for routine evaluation; oral screening assays are likely to be inadequate because of decreased sensitivity.[263–266] Ongoing evaluation should be individualized. Some individuals undergoing PrEP may use self-testing or other testing modalities that are less sensitive to detect early HIV infection; an accurate history and precise understanding of the performance characteristics of testing is essential for comprehensive patient evaluation; and there may be circumstances requiring more frequent monitoring and NAT evaluation.

Detection of HIV-2 and HIV-1/2 Dual Infections

HIV-2 is a retrovirus with a structure and replication program similar to HIV-1 but is distinct from HIV-1 in origin, epidemiology, spread, disease progression, genetic organization, and nucleic acid sequence[37,38,267,268] (see Chapter 169). HIV-2 infections originally reported in West Africa are now present in Europe, especially Portugal, and in South Asia, including India.[269] The CDC reports that African countries with at least 1% HIV-2 prevalence include Angola, Cape Verde, Côte d'Ivoire, Gambia, Guinea-Bissau, Mali, Mauritania, Mozambique, Nigeria, and Sierra Leone. In addition, Benin, Burkina-Faso, Ghana, Guinea, Liberia, Niger, São Tomé, Senegal, and Togo are reported to have significant numbers of HIV-2 infections.[270] New initiatives have been implemented to track HIV-2 epidemiology.[271] Infection with HIV-2 does not prevent infection with HIV-1,[272] sequential HIV-2 to HIV-1 infections have been documented,[273] and dual HIV-1 plus HIV-2 infections are reported in West Africa and in India.[269,274,275] The prevalence of HIV-2 remains low and may be decreasing in some areas of West Africa.[276] In the United States HIV-2 remains infrequent but not rare.[277] The CDC reported 242 cases in 1987–2010[278]; Torian and colleagues[279] documented 62 HIV-2 infections in New York City between 2000 and 2008,[279] and Hollenbeck and coworkers[280] identified 12 patients in Providence, Rhode Island. The current prevalence in the United States is not known precisely. Individuals from countries with substantial populations of HIV-2 in West Africa and elsewhere emigrate to the United States, and the understanding of geographic distribution is useful in patient evaluation; as summarized by Gandhi and coworkers,[281] the HIV-2 epidemic has undergone complex dynamics and may be declining in West Africa, especially in younger individuals.

The ability to detect HIV-2 infection, discriminate HIV-2 from HIV-1, and identify HIV-1 plus HIV-2 dual infections has always been important from an epidemiologic and blood safety standpoint, but specific diagnosis has become essential in the era of effective ART. Specifically, HIV-2 reverse transcriptase is not sensitive to nonnucleoside reverse-transcriptase inhibitor (NNRTI) antiretrovirals, such as nevirapine, efavirenz, etravirine, or delavirdine. This is due to a Y188L polymorphism naturally occurring in HIV-2. As a result, patients with HIV-2 infection (or HIV-1/2 coinfection) should not be treated with NNRTI-based regimens, and misdiagnosis of HIV-1/2 coinfection as HIV-1 monoinfection may rapidly lead to complex drug resistance.

Initially, 60% to 90% of sera from HIV-2 infected individuals reacted with HIV-1 ELISA kits[282–284]; in the United States specific screening assays for HIV-2 were not initially developed because of the relatively few HIV-2 infections. Instead, combination screening assays[285] were developed to improve the sensitivity of HIV-1 assays, often incorporating synthetic peptides, including those from immunodominant domains of HIV glycoproteins.[286–292] Use of cross-absorption tests,[293] competitive ELISA,[294,295] and PCR assays were reported to distinguish infections.[296]

The development of an HIV-1/2 ELISA platform (e.g., GEENIUS [Bio-Rad]) that distinguishes whether reactivity is derived from HIV-1 or HIV-2 represents a particularly useful advance. The combination of rapid screening assay and point-of-care supplemental confirmatory assay offered, for the first time, an FDA-approved method for diagnosing HIV-2 infection in the United States.

Methodology for HIV-2 RNA levels has not been standardized to the same degree as HIV-1, and no FDA-approved tests are available for diagnosis; HIV-2 RNA assays are available for monitoring established disease.[268] IFAs have been used to distinguish HIV-1 and HIV-2 infection,[232] but currently there are no FDA-approved HIV-2 IFA test kits. HIV-2 cultivation from infected individuals has been reported,[297] but the frequency of infectious virus recovery was lower than that detected with HIV-1.[297]

As newly diagnosed cases of HIV are frequently referred for early initiation of ART, identifying the presence of HIV-2 either as a single infection or as HIV-1/2 dual infection is an absolute imperative. Gottleib and coworkers[268] have highlighted that achieving 90-90-90 targets for HIV-2 has lagged behind HIV-1, with a number of potential explanations. Torian and her colleagues[279] have documented a prolonged (>1 year) delay in documenting the presence of HIV-2 after a first diagnosis with HIV infection. In light of consequences for the emergence of drug resistance if NNRTI-containing regimens are initially used, careful assessment for HIV-infection must be considered in all individuals with HIV infection originating from, or ties to, West Africa. The CDC recommends HIV-2 testing for sex partners or needle-sharing partners of HIV-2–infected persons or individuals from countries with significant HIV-2 populations, individuals who have received transfusions or nonsterile needle exposures in countries with significant HIV-2 populations, and children of women known to be HIV-2 infected. Thoughtful consideration for HIV-2 must also be considered in individuals from West Africa who have been previously identified with HIV-1 and in whom HIV-2 has not been specifically evaluated; Torian and coworkers[279] noted that greater than 60% of the HIV-2 diagnoses had an initial diagnosis of HIV-1 only; the absence of testing does not mean the absence of infection.

Little is known regarding the HIV-2 window period; recently Cazals and colleagues[298] described HIV-2 seroconversion in detail in a single patient with seroconversion dynamics similar to that reported for HIV-1. An individual with symptoms consistent with HIV seroconversion had ELISA seroreactivity and p24 antigen reactivity within 21 days of symptom onset with an HIV Ag-Ab combination[298]; HIV-2 WB was indeterminate, but additional WB 50 days after symptom onset was positive. Performance characteristics of HIV testing assays in acute or recent HIV-2 infection have not been otherwise analyzed; third-generation HIV1/2 ELISA assays were less sensitive in detecting early HIV-2 infection.[299] There are few available seroconversion samples for HIV-2; use of experimentally infected primates with simian immunodeficiency virus (SIV; a close relative of HIV-2) has been suggested, but this method relies on the efficiency of detecting cross-species antibody, and the kinetics of an immune response may not be identical to HIV-2 infection in humans.

Detecting Non-B HIV-1 Types and Subtypes Other Than Subtype B

HIV-1 is composed of four principal groups: a large group, M (main), representing the majority of HIV-1 pandemic viruses; O (outlier); N (not M and not O[300,301]), and the recently isolated group P. The latter strain was identified in 2009 in a Cameroonian woman, and it is closely related to gorilla SIV (SIVgor), suggesting a different cross-species transmission compared with SIV in chimpanzees (SIVcpz).[302]

Group M viruses have been classified by phylogenetic analysis into a series of nine subtypes (A–J, except for E), five sub-subtypes, a growing number of circulating recombinant forms (circulating recombinant forms [CRFs], 58 so far), and an immeasurable number of unique recombinant forms (URFs). Non-B viruses account for almost 90% of HIV-1 infections globally and, due to travels and migratory waves, showed an increasing prevalence in previously subtype B–restricted areas (i.e., Europe and North America). In the past such variants were relatively difficult to detect with same sensitivity as subtype B, although more recent versions of ELISA assays have been optimized to include detection of non-B subtypes.[303,304] Group O viruses are more distantly related to the group M, have been identified at relatively low frequency in localized areas of central Africa,[305] and may have emerged after zoonotic events from chimpanzee or gorilla reservoirs.[306,307] Other groups are quite rare; group N ($N = 13$) and P ($N = 2$) viruses detected in West Africa are likely zoonotic events from nonhuman primates. Of note, all types and groups are detected by HIV ELISA assays, even groups N and P.[308]

The performance characteristics for many HIV-1 kits have been optimized using HIV-1 subtype B. Incorporation of HIV peptides with broad cross-reactivity into third-generation ELISAs has enabled detection of non-B subtypes, including more distantly related type O.[309–312] A peptide-based ELISA assay has been reported using V3 peptides to accurately discriminate among HIV and SIV viruses,[313]including group O, which detected group M and was used to identify the existence of group N isolates.[300] HIV-1 M subtypes, group O, and group N viruses are detected by ELISA, WB, and fourth-generation assays techniques. Qualitative TMA/HPA testing has also performed well in detecting non-B subtypes; all A, C, D, E, F, G, N, and O subtypes were detected, although as described later ("HIV Testing for Blood Donor Screening"), some viruses have not been detected in blood product surveillance. As

discussed before, PCR-based assays for viral load measurements also have difficulty detecting and reliably quantifying HIV-1 RNA when testing diverse genetic variants of HIV-1, especially group O and N. Different assays may yield discordant viral load results. No extensive data are available regarding detection of CRFs.[54]

Detecting HIV-1 Dual Infections

Two strains of HIV can infrequently infect the same person before host immunity develops, known as coinfection; otherwise, if the second strain is transmitted after seroconversion, this condition is named superinfection. One of the strongest markers of superinfection is the high frequency, at least 20% of the HIV pandemic, of recombinant viral strains worldwide. Clinical implications of HIV dual infection are acquisition of drug-resistant strains during effective therapy, thus leading to virologic failure and onward transmission of drug resistance if recombination occurs, and the impact on disease progression. Cornelissen and coworkers[314] showed that dual infection is associated with faster CD4 decline in patients with recent HIV infection. Detecting superinfection is difficult and hinges on detecting genetic differences between viral strains within the same individual: Frequent sampling; specific diagnostic assays; and sensitive sequencing methods, such as single-genome sequencing or next-generation sequencing, are fundamental. A new multiregion hybridization PCR assay in three regions of the HIV-1 genome (*gag p17, vpu,* and *nef*) was developed to detect the most common subtypes and CRFs in West Central Africa,[315] providing sufficient sensitivity and specificity so that it could be used as a screening tool in areas where multiple subtypes cocirculate with high prevalence, including areas of Europe.[316] Dual infections are difficult to detect and therefore difficult to quantify; studies show wide incidence variations, from no detected cases to rates close to the rate of incident HIV in the background population. This is due to wide variation of laboratory methods, sample timing and frequency, HIV-1 subtype, ongoing risk behavior, and ART use.

HIV Testing During Pregnancy or at Delivery

Diagnosis of HIV in pregnant women is a public health priority; the advent of effective therapy to reduce the risk of transmission has made such arguments more compelling. Diagnosis in pregnancy may be complicated by increased false-positive rates of ELISA screening assays with increased parity. In addition, women may present at delivery without previous evaluation, and rapid tests may be the only available modality to evaluate HIV infection expeditiously. In a study of 858 women diagnosed in labor, Constantine and coworkers[317,318] have suggested that a single-screening ELISA at delivery is sensitive, although the use of two rapid ELISA assays have performance characteristics as for the standard ELISA-WB strategy. Current guidelines for standard prenatal care include information regarding HIV testing; routine opt-out testing, except in jurisdictions where testing requires written informed consent; and testing early in pregnancy and in the third trimester in individuals at high risk for HIV infection. Some experts recommend repeat testing for all pregnant women in the third trimester. For women who present at delivery with unknown serostatus, 24-hour availability of rapid testing along with initiation of ART based on a single positive rapid result is recommended.[319]

HIV in Perinatal Diagnosis

Serodiagnosis of HIV infection in the perinatal period is complicated by the prolonged persistence of maternal antibody for approximately 18 months after delivery. Methods for diagnosing infection include the serial Aptima HIV-1 qualitative assay (plasma) and DNA PCR testing of peripheral blood mononuclear cells (whole blood or dried blood spots[320,321]). The sensitivity and specificity of DNA and RNA testing methods have been reviewed[322]; detection of non-B subtypes by qualitative RNA testing (Aptima) has had excellent reported results, and DNA PCR may be suboptimal with false-negative results documented.[323] The recent FDA approval of qualitative RNA testing (Aptima) for use in diagnosis (including pediatric diagnosis) may make this test the preferred modality. Alternative techniques to measure the presence of early HIV infection include direct HIV cultivation using techniques standardized by the AIDS Clinical Trials Group to evaluate HIV infection; a recent summary reports use of sequential sampling and analysis using DNA/RNA or p24 testing. Two positive samples obtained after ≥1 month and after ≥4 months indicates the presence of HIV infection. Negative HIV infection is based on two DNA or RNA or p24 determinations at ≥1 month and ≥4 months of age, or two negative serologic tests obtained separately at ≥6 months of age; repeating serologic evaluation at 18 months in individuals with prior negative results is common.[322] HIV-1 NAT early after birth must be interpreted with care because infection may take place at parturition, and HIV-1 RNA levels in plasma may be relatively low at delivery even in true infection. Early introduction of ART after delivery may also confound diagnosis; HIV RNA and DNA levels may be suppressed as a result of therapy, and complete antibody seroconversion may not take place in the setting of early ART. In such circumstances perhaps the only method to identify the presence of infection is close monitoring and serial testing after completion of a defined course of ART.

Maternal-fetal transmission of HIV-2 is much less common than HIV-1 (mother-to-child transmission rate of 4% vs. 15%–45%, respectively) but may occur especially if women are infected during pregnancy. Diagnosis of HIV-2 infection is similar to HIV-1, although Faye and coworkers[324] have reported that assays may be less sensitive than those for HIV-1. A full discussion of diagnosis of HIV infection in newborn can be found in the report by Read.[322]

HIV Testing for Blood Donor Screening

Laboratory methods for HIV detection were first implemented in populations as screening tools for blood products; more than 14,000 recipients of blood products as a risk factor for HIV infection have developed AIDS. Cases have decreased markedly; in the United States 40 adults and adolescents have been infected or developed AIDS after receiving blood screened as negative for HIV antibody,[325] but none since 2008.[326] Elsewhere, rare infections have occurred since the advent of NAT testing[327–330]; in some cases, transmissions are due to genetic differences of transmitted virus not detected by NAT, highlighting the need for vigilance in understanding genetic variability of HIV within countries. The residual risk for HIV infection in the United States is currently <1 : 1,000,000 units transfused.[331]

Blood surveillance procedures are designed to provide optimum safety of blood products; the screening phase begins with an interview and questionnaire, and donors may be screened out based on history; donors not screened out sign consent for donation and for HIV-1/2 testing with HIV-1/2 ELISA and NAT. Any sample reactive on either assay is discarded, and the sample is repeated in both screening assays; if the repeat screen is negative, a second screening assay is performed. If the second screening assay is negative, the sample is considered negative and the donor is not deferred. If the second screening assay is positive, the sample is considered repeatedly reactive and sent for confirmatory WB; positive WBs are repeated with a separate sample; and the results of ELISA and WB are reported to the patient with appropriate posttest counseling and referral. An individual with donor status of positive screening and with a negative confirmatory test remains under consideration. WHO recommendations for screening for blood safety involves a single screening assay for HIV infection; if reactive in a single test, the donation is discarded and the test repeated. The risk from blood donation in developed countries is relatively low[332]; recent analysis demonstrated the successful expansion of NAT screening over the 1998–2008 period, both in terms of numbers of countries screening and in terms of advances in technology and automation.[333] The majority of NAT screening is now conducted with multiplexed HIV/hepatitis C virus (HCV)/hepatitis B virus assays, based on either transcription-mediated amplification (TIGRIS [Gen-Probe]/ULTRIO [Grifols, Los Angeles, CA] systems) or multiplex polymerase chain reaction (MPX), performed on highly automated instrument platforms that ensure reliable results with excellent sensitivity and specificity.[334] Serologic screening must, however, be maintained, even with the most sensitive NAT testing performed on individual donations; rare low-viremic HIV-infected subjects (i.e., elite controllers) may indeed be seropositive but NAT

negative, given their unique control over viral replication. Overall, in the United States the estimated risk of HIV from transfusion is <1 per 10^6 units transfused.

Worldwide, blood safety in general varies considerably; in an estimated 39 countries, WHO reported that blood donations are not routinely tested for HIV, and 47% of donated units in low-income countries are tested in the absence of quality assurance (http://www.who.int/bloodsafety/global_database/GDBS_Summary_Report_2011.pdf). Dedicated efforts to improve safety have been implemented; in 2003 the President's Emergency Plan for AIDS Relief (PEPFAR) began supporting programs in 14 targeted countries in sub-Saharan Africa and the Carribean,[156] with improvements in blood donation infrastructure, increases in numbers of units donated, and decreases in HIV-reactive units. Challenges persist: a UNAIDS survey recently reported that >99% of all donations are appropriately screened for HIV in high-income and upper-middle-income countries; 76.2% to 83.2% of donations are appropriately screened for HIV in lower-middle-income and low-income countries.[335]

NAT has demonstrated a substantial impact worldwide. More than 270 million units have been screened by HIV NAT since its inception, and 244 (0.9/1 million) were NAT positive/serology negative. Although residual risk calculations may be overestimates,[336,337] the residual risk for HIV infection in the United States remains <1:1,000,000 units transfused.[331] Rare infections have occurred since the advent of NAT testing[327–330]; not all contaminated units result in infection.[338] In some cases transmissions are due to genetic differences of transmitted virus not detected by NAT, highlighting the need for vigilance in understanding genetic variability of HIV within countries.[337] Worldwide the demonstrated success of NAT has not yet been realized in all countries. The WHO Global Database on Blood Safety survey[339] reported that in 2013 176 of 180 reporting countries screened blood donations for HIV; 70 countries used antibody testing only, 106 reported use of antigen + antibody testing, and a total of 44 countries used NAT.

HIV Testing in Tissue Procurement for Transplantation

HIV transmission from organs transplanted from infected individuals occurred before the availability of sensitive and specific testing. In adults and adolescents there have been at least 50 cases of HIV transmission through transplantation or artificial insemination, sometimes involving multiple recipients. Strikingly, a 38-year-old male donor who tested negative for HIV and HCV antibodies at the time of donation transmitted HIV and HCV to four recipients.[340] A transmission event happened in 2009 from a living donor that resulted negative for HIV antibodies 79 days before organ recovery; a retrospective investigation revealed a reactive NAT on a stored sample obtained 11 days before transplant.[341] Both in the United States and internationally, donor screening before organ recovery has been based on ELISA testing since its introduction in 1985; after the implementation of NAT assays, greater than 50% of organ procurement organizations recently adopted this additional test to overcome the risk associated with the antibody window period. Such further screening raised the issue of losing more life-saving organs due to false-positive results: HIV transmission is an extremely rare event in the United States, and there is no nationwide policy for HIV screening for living donors. The CDC recommendations address (1) repeated donor testing with a combination of an HIV serologic test and HIV NAT as close to the time of organ donation as feasible logistically, but no longer than 7 days preceding organ donation; (2) all living donors should be advised of their obligation to avoid behaviors that would place them at risk for acquiring HIV infection before transplant surgery; and (3) for living donors with a history of high-risk behaviors (e.g., high-risk sexual activity or injection drug use) identified during evaluation, individualized counseling and a detailed discussion of specific strategies to avoid high-risk behaviors should be provided.

HIV Testing in Vaccine, Prevention, Postexposure, and Gene Therapy Studies

One recent issue concern in laboratory evaluation of HIV-1 infection is evaluation of patients enrolled in vaccine trials using one or more HIV antigens for immunization. If the vaccines themselves are effective immunogens, it is possible that patients will artifacually "seroconvert" for HIV in the absence of true infection. For example, individuals immunized with a single gene product (e.g., gp160) may develop specific gp160 reactivity.[342] If such individuals already have a nonspecific reactivity for p24, then the combination of reactivity to gp160 and to p24 will result in positive HIV screening and supplemental assays. Current and planned candidate vaccines include multiple HIV components, and effective immune responses may affect positive routine testing. Vaccine-induced seropositivity has important social implications, and vaccine recruitment trials clearly and plainly address possible outcomes.[343] Alternatively, patients in vaccine trials may become truly infected, and authentic infection requires identification. Careful history and physical examination are essential; application of NAT in such circumstances may yield useful results in establishing the presence of infection,[344] and individuals receiving HIV DNA vaccines have not yielded positive HIV NATs. CD4 counts and percentage of CD4 cells in peripheral blood are also useful because serial samples are likely available for comparison in vaccine studies.

HIV Testing in Gene Therapy Studies Using Lentiviral Vectors

The recent advent of lentiviral vectors for gene therapy raises possibilities that portions of the vector may be expressed and potentially result in false-positive HIV testing. The backbone for a number of gene therapy vectors is a lentivirus (typically HIV), which can infect nondividing cells, thereby expanding their range of cell types in which therapeutic genes can be introduced. The genomes of these vectors are highly deleted, but RNA produced by the vectors can contain HIV and can therefore be detected by HIV RNA assays. These vectors do not encode HIV *gag* or *env* genes, and serologic assays for HIV antigens or p24 assays have been nonreactive. As a consequence, such individuals may have HIV testing results similar to recent HIV infection (HIV RNA positive/antibody negative). An early analysis of large series of individuals who have received autologous cells transduced with lentiviral vectors did not document reactive serologies or detectable retroviral nucleic acid in plasma.[345] More recently, however, patients undergoing gene therapy have had detectable HIV RNA in plasma using conventional quantitative HIV RNA assays.[346–348] In all of these cases detailed analysis revealed that authentic HIV infection was not present. It is imperative that analyses of such cases proceed in a rigorous, methodical, and thoughtful fashion. Thorough understanding of the vectors and viral RNA assay are essential. Analysis of sequential samples is necessary; sequencing the nucleic acid present in plasma, if present in sufficient quantities, will provide useful information, and patient-derived sequences identical to vector-derived sequences will identify the source of the positive HIV RNA result as the vector.

In certain gene therapy settings, defective retroviral vectors have been used to complement patient gene deficiencies; cells from which these defective viruses are propagated include other "helper" viruses and may contribute to development of malignancy in certain situations.[349] Neither the nucleic acids nor gene products from the vectors or the helper cell lines are remotely related to HIV and are not sources of cross-reaction.

HIV Infection but Viral RNA Less Than 50 Copies/mL: HIV Elite Controllers

A small fraction of individuals infected with HIV do not progress to CD4 lymphopenia and do not have HIV viral RNA levels >50 copies/mL plasma in commercial assays; more sensitive single-copy assays do detect the presence of HIV in these so-called "elite controller" patients, and ongoing low-level HIV replication is detectable using sufficiently sensitive assays.[219,350,351] Despite the low level of plasma viremia and substantial CD4 cell numbers, all such patients are ELISA reactive and fully WB positive. As a result, patients who are newly diagnosed with HIV and have reactive HIV-1/2 ELISA tests and are WB positive but have HIV RNA levels <50 copies/mL are likely to be elite controllers; the presence of a fully positive WB makes early infection much less likely. All such patients should be repeatedly evaluated to document

the presence of HIV infection and absence of viremia; such patients are actively studied in tertiary care centers,[352–355] and referral for additional evaluation is often useful.

HIV Testing in the Setting of Acute Retroviral Syndrome or Other Acute Illness

Primary HIV infection may present as an acute viral syndrome with fever, lymphadenopathy, and rash (see Chapter 122). Diagnosis near the time of infection has obvious social, medical, and public health benefits, but not all screening strategies are equally effective in detecting primary HIV infection. TMA/HTA assays (Aptima[356]) are currently the most sensitive qualitative FDA-approved assays for acute HIV-1 infection. FDA-approved rapid/simple assays may not be as sensitive as standard third-generation ELISA assays. New fourth-generation ELISAs, as described earlier, have excellent performance characteristics and can detect antigens approximately 5 to 7 days after the appearance of nucleic acid. Of importance, the data published to date indicate the assays available in the United States are capable of detecting acute HIV infection in greater than 80% of individuals who are NAT positive but nonreactive or indeterminate in antibody-only assays. The window period for HIV infection may vary from patient to patient, and thus the value of a negative screening test must be weighed carefully but not exclusively. As outlined earlier, other acute infections may yield false reactivity in HIV screening assays. Similarly, primary HIV infection may present with relatively nonspecific symptoms and may give false-positive results for acute EBV infection. Thus review of such cases should be directed and comprehensive.

HIV Testing in Occupational Exposure

HIV-1 infection may be transmitted as an occupational exposure (see Chapter 298) in the hospital setting as a result of needlestick injury, transmission during surgery, and so forth; in outpatient dental circumstances; or other instances where contact favoring transmission may occur. It is essential that the serostatus of the donor patient be established as expeditiously as possible. Although testing in these circumstances is time sensitive, it still requires the same elements of counseling, consent, and confidentiality as standard HIV-1 testing. In the United States, in the opinion of the American Medical Association Council of Ethical and Judicial Affairs, involuntary HIV testing is acceptable in the event a health care professional underwent exposure but the patient refuses to be tested.[357] By contrast, WHO has unequivocally rejected mandatory HIV testing,[75] except in screening of blood or blood-manufactured materials or in screening of donors before procedures involving transfer of bodily fluids, for instance, organ donation, artificial insemination, and transplantation.

In occupational exposures it is important to test the exposed worker at the time the event took place to establish baseline serostatus. Counseling and HIV testing should be performed on the exposed worker immediately, to rule out the possibility of preexisting but undiagnosed infection. Rapid ELISA testing may be used, especially if the donor is not thought to be acutely HIV-1 infected. In the event of a nonreactive rapid ELISA, it would be prudent to obtain routine ELISA and TMA/HPA tests to address the unlikely possibility that the rapid assay was insensitive to an early HIV-1 infection. Judicious use of RNA assays may also be used. It is essential to carefully scrutinize testing of donors in this circumstance because hospitalized or acutely ill patients may have a variety of conditions that may yield a falsely reactive HIV-1/2 ELISA. In the event the serostatus cannot be established, initiating ART is an appropriate option (see Chapter 298).

HIV Testing in Idiopathic CD4 Lymphopenia

Idiopathic CD4 lymphopenia (ICL) is an immunodeficiency characterized by repeated CD4 cell numbers <300 cells/μL (<20%), without a coexisting condition thought to be a cause of immunodeficiency, and the patient is HIV negative.[358] ICL is obviously completely distinct from HIV infection, but patients with persistent CD4 lymphopenia and HIV nonreactive ELISA may be referred for additional evaluation. Careful history and repeated HIV testing with judicious use of appropriate NAT will clarify these cases quickly so that they may be referred for appropriate and targeted care.

Factitious HIV Infection and AIDS Phobia

Cases of factitious HIV infection have been reported in the context of psychiatric dysfunction.[359–362] The advent of multiple testing modalities, including home collection systems, may complicate patient presentation in factitious HIV. Careful history, application of routine algorithms, repeat standard assays, use of appropriate counseling or psychiatric consult services, and common sense may resolve most of these delicate cases.

Similarly, a number of individuals have reported substantial fear of HIV infection because of experiencing flulike symptoms. These cases are typically isolated occurrences but may occur in groups.[363] In general these cases are typically resolved by routine evaluation and sequential testing; in the setting of coexisting obsessive-compulsive, anxiety, or paranoia disorders, such patients obviously require primary therapy for underlying illness as well, and infectious disease consultants play a key role in providing information, counseling, and dispelling myths while performing appropriate testing.

HIV Testing to Estimate Duration of Infection and Incidence

The HIV epidemic has been tracked by prevalence using rates of clinical disease (AIDS) and more recently by HIV seroprevalence. Estimates of incidence rates of HIV infection have been relatively difficult to obtain but are an important characteristic of epidemics, especially in area where infection rates may be changing rapidly. Application of serologic and nucleic acid assays has been useful in estimating duration of infection,[364] with a general goal of identifying individuals within 24 to 48 weeks of infection.

Serologic assays have been developed taking advantage of two general characteristics of early infection: (1) decreased avidity of early IgG and IgM antibodies relative to avidity of antibodies in established infection and (2) delayed emergence of antibodies to p31 until after several months of infection. Assays based on either observation have been developed. Initial studies used "sensitive/less sensitive" (S/LS) ELISA assays and quantitative immunocapture assays for estimates of HIV-1 incidence rates.[124,365,366] An S/LS (or "detuned") ELISA approach uses a dual ELISA assay strategy that consists of a standard sensitive third-generation ELISA to detect all HIV infections and a detuned ELISA that does not detect early infections because of the decreased antibody quantity and avidity early in infection. Samples that are HIV reactive using the standard assay and nonreactive on the detuned assay are classified as recent infections. S/LS assays are generally applicable in epidemiologic studies of HIV-1 to characterize high-risk populations, incidence trends, and peak age incidence of infection.[367–369] Subtype performance has not been extensively studied, and the assay is somewhat labor intensive.

A quantitative immunocapture assay (Aware BED; Calypte Biomedical, Portland, OR) represents a second-generation incidence assay designed to address some limitations of S/LS approach.[370] Quantitative immunocapture assay measures the relative levels of HIV gp41-specific IgG as a fraction of the total IgG. The gp41 peptide is a synthetic peptide consisting of an 18–amino-acid immunodominant region of gp41 from subtypes B, E, and D, each ligated to a spacer, aminobutyric acid, and then conjugated in parallel as a branched-chain peptide.[370] Field testing with >600 samples revealed a linear relationship between standardized detection of the BED peptide and duration of infection. The BED assay has been used in the United States[124,371] and elsewhere[372–375] to detect recent infections and estimate incidence rates.

In addition, an avidity index assay can be performed to discriminate between recent seroconversions and established infections; this assay is based on the comparison of test signal (measured as sample/cutoff ratio) between two replicates of fourth-generation assays in which the second replicate is added with guanidine hydrochloride, a denaturation agent that elutes low-avidity and -affinity antibodies after Ag-Ab bonds have formed. This application has been effective in discriminating recent from chronic HIV infections in field testing of subtype B and non-B HIV.[376]

Anti-p31 antibodies are detected in newer discriminating supplemental assays; the absence of anti-p31, indicating relatively early HIV

infection (Fiebig stage V, HIV infection likely <100 days). Not all assays detect antibody to p31 with equal sensitivity; Tuaillon and coworkers[377] reported the GEENIUS assay to be less sensitive in detecting anti-p31 compared with New Lav Blot I WB (Bio-Rad) or INNO-LIA HIV I/II Score Dot Blot (Fujirebio, Malvern, PA) assays; as such GEENIUS may overestimate early infection compared with other assays. Overall, as described by Ananworanich and coworkers,[378] the development of fourth-generation and NAT assays has permitted a reevaluation of standard staging of early HIV infection and a new (fourth-generation) staging proposal that correlates with T-, B-, and natural killer–cell concentrations and cell-associated HIV DNA levels. As Robb and coworkers[379] have reported, early HIV infection is heterogeneous, with wide variation in HIV RNA levels. New approaches developed to discriminate HIV reactivity[380,381] may lead to improved diagnostics and greater understanding of this critical period.

Nucleic acid assays have also been used to estimate duration of HIV infection. Kouyos and coworkers[382] from the SWISS HIV Cohort evaluated whether HIV viral evolution and sequence diversity could correlate with time from infection. During acute/early HIV infection, viral quasispecies are highly homogeneous, whereas they become more and more diverse within the following years. Sequence data obtained from routine genotyping using Sanger sequencing for clinical purposes (i.e., evaluation of drug resistance in protease and reverse transcriptase) show that the number of ambiguous nucleotides increased with the age of infection, and a fraction of ambiguous nucleotides greater than 0.5% provides strong evidence against a recent infection event, 1 year before sampling.[382] Andersson and coworkers[383] and Grossman and coworkers[384] demonstrated that this approach is useful in non-B subtypes. Additional approaches have been developed using other genome regions such as *gag*,[385] new next-generation sequencing assays instead of Sanger sequencing,[386–388] and additional measures of genetic variation (e.g., generalized or Shannon entropy[385,387]). High-resolution amplicon melting temperature,[389] clustering algorithms,[390] Bayesian-derived time to recent common ancestor,[391] and Hamming distance[392] have also been used. Assays are useful with plasma but at present not with dried blood spots.[393,394]

Recency assays, including both serologic and nucleic acid–based testing, have been limited by elevated "false recency" rates, including misclassifying advanced HIV infection as recent. A combined biomarker approach has demonstrated success superior to single assays alone.[395] Laeyendecker and colleagues[394] demonstrated the combination of LAg (limiting antigen) Avidity and Bio-Rad Avidity assays, combined with elevated viral RNA levels, were superior to single-assay approaches. These approaches, resulting in recent infection testing algorithms (RITAs), which integrate early infection serologic assays with viral RNA levels, provide critical information regarding trends in the course of the HIV epidemic. These approaches have clear usefulness in epidemiologic assays for HIV infection; they also serve useful benefit in patient management, especially in partner notification and contact tracing. Surveys of HIV specialists in England and Northern Ireland, where RITAs have been available for years, revealed that physicians were well acquainted with assays and were confident in communicating essential information, including contact tracing without undue patient anxiety.[396]

Home Collection and Self-Testing for HIV

The development of simple/rapid tests present new opportunities and the possibility of HIV diagnosis outside traditional testing facilities. Two kinds of HIV testing are available outside the health care facilities: home collection devices and home testing. Home collection devices are available in the United States and are FDA approved (Home Access; Home Access Health, Hoffman Estates, IL). Patients purchasing a home collection kit must contact the distributor using a toll-free telephone number, register a unique identifier contained in the kit, provide demographic data, and receive an educational pretest counseling session. Packaged material contains consent documents, and consent is implied when the individual registers the test by telephone. Patients prepare the dried blood spot sample, which is shipped for laboratory testing. After a short (3–7 days) processing time, patients contact the toll-free number for results, which are available through an interactive voice response system or directly from a counselor. Testing includes both screening with HIV-1 ELISA (window period of 3 months) and confirmatory testing (immunofluorescence) for reactive screening results.[236] All indeterminate and positive results are delivered by trained counselors. Home collection is considered a safe, confidential, and FDA-approved service and has been available in the United States since 1996.

In July 2012 FDA approved the OraQuick InHome HIV Test (OraSure Technologies, Bethlehem, PA) (see Table 120.1), intended as an over-the-counter test for consumer use as an aid in the diagnosis of HIV-1 and HIV-2. Such a test is not to be used by individuals younger than 17 years and is not intended to be used with specimens other than oral fluid. The kit provides a test result in 20 minutes, and results are for screening only; additional confirmatory testing is essential. Of critical concern, the window period for in-home testing (3 months) is relatively long, and nonreactive results obtained shortly after potential transmission events may provide a false sense of security in the setting of a potentially infectious period.

Self-testing expands the population that tests for HIV[397] and can increase frequency of testing; it is unclear whether lack of additional counseling may miss opportunities to address risk behavior.[398] Home testing can be quite reliable, and extensive review of performance characteristics[399] in general revealed only minor difficulties in self-testing related to sample collection and overall directions. Implementation is not uniformly successful in all circumstances, however; in a population of commercial sex workers in Uganda, Oldenberg and colleagues[400] measured a 15% to 23% incorrect interpretation of test images of strong positive, strong negative, and inconclusive home test results, and as high as a 61% incorrect interpretation of weak positive results. Study participants were peer-trained with the home testing kits and had limited health education and concomitant drug use, which represented possible correlates for increased frequency of incorrect interpretation, although such limitations do approximate real-world conditions. In addition, self-testing has been used successfully, can alleviate stigma, and can reach a patient population that does not typically engage the health care system or HIV testing.[397] Self-testing may be attractive for use as a "point-of-sex" procedure.[401–403] As the window period for home testing remains considerable, widespread replacement of clinic-based testing with self-testing could actually result in increased spread in high-prevalence circumstances[404]; health care and public health–efforts workers caring for at-risk individuals who use self-testing should incorporate detailed and ongoing education discussions and posttest counseling. In a randomized study comparing standard care or provision of self-test kits with or without additional active follow-up, Wray and coworkers[405] found individuals receiving self-test kits and follow-up telephone contacts with counselors had increased rates of repeat testing, risk reduction counseling, and PrEP referrals.

Self-testing has gained demonstrated acceptance in a number of studies worldwide[406–409] and is recommended as an additional approach for HIV testing by WHO.[410] In 2015 a large initiative, HIV Self-Testing Africa (STAR), was implemented in Africa; by 2018 STAR distributed over 2 million self-testing kits in Eswatini, Lesotho, Malawi, South Africa, Zambia, and Zimbabwe, and new clinical trials (https://clinicaltrials.gov/ct2/show/NCT02718274) are investigating the role of additional engagement to initiate ART. STAR will also evaluate acceptance and economic benefits of self-testing.[411,412] Current challenges include issues of implementation scale-up,[413,414] case reporting, and partner notification requirements.[410] New studies are in process to investigate HIV self-testing in a number of at-risk populations (https://clinicaltrials.gov/ct2/results?cond=HIV+self+testing&term=&cntry=&state=&city=&dist=). These new studies will evaluate the utility of self-testing in various settings, ranging from deployment of self-testing kits in villages in Africa and China, to evaluating health approaches to expand home testing in individuals not typically engaged in health care in the United States. These and similar studies are likely to yield important new insights regarding self-testing and linkage to care that may widely expand the numbers of individuals undergoing testing. As a consequence, health care professionals engaging individuals newly identified with HIV infection should be well acquainted with the performance characteristics and limitations of these assays.

Other Human Retroviral Infections and New Retroviral Zoonoses

HIV-1 and HIV-2 epidemics arose from two independent zoonotic infections from distinct primate species, with zoonotic events unlikely. There are more than 40 SIV variants in nonhuman primates, with only a few having established infection with human-to-human transmission. Several key viral variants may have contributed to enhancing transmission.[415] The precise circumstances of these cross-species events remain uncertain, and practices and behaviors continue so that new zoonotic retroviral infection remains possible. Evaluation of patients with immunodeficiency will undoubtedly involve testing for known retroviruses, and the possibility of novel infections should be considered. Primate hunter/butcherers exposed to animals in which infection with SIV is endemic may show evidence of exposure using sensitive laboratory-based assays to detect cell-mediated immunity to SIV[416] but are consistently negative in HIV-1/2 ELISAs. There are, however, numerous distinct retroviruses in primate species, and the sensitivity of currently approved assays for the detection of more distantly related viruses is uncertain but is likely to be low. Yang and coworkers[417] have reported the design of new peptide ELISA assays using Env gp120 V3 sequences from several HIV-1 M, N, and O isolates and from diverse SIV isolates. Such assays, which have demonstrated robust performance in field detection of HIV and SIV, may have additional utility in screening potentially contaminated bushmeat and thereby prevent future zoonotic events.[418]

New zoonotic infections have been detected in persons from Central Africa with extensive contact with primate blood and body fluids. Using sequential strategy of HTLV serology, followed by amplification using primers with sequences shared by primate T-cell leukemia viruses, two primate retroviruses were identified in humans.[419] Primate T-cell viruses are genetically distinct from HIV-1 and HIV-2 and should not yield reactive HIV-1/2 ELISA or NAT results.

Spumaretroviruses are members of the Spumaretrovirinae, a subfamily of Retroviridae and are distinct from the Orthoretrovirinae subfamily to which HIV belongs. Spumaviruses are present in a number of mammal families (e.g., bovine, equine, feline), including numerous nonhuman primate species and Old and New World monkeys, chimpanzees, gorillas, baboons, and oranguatans.[420] Most macaques are seroreactive by 3 years of age. Spumaviruses of cows, cats, and horses do not infect humans, but simian spumaviruses are readily transmissible to humans. Typically, primate veterinary handlers, bushmeat hunters, and others with occupational or other direct primate contact[421,422] may be infected. Although foamy virus infection in vitro results in dramatic cytopathic effect, and simian foamy virus (SFV) may augment SIV pathogenicity,[421] foamy viruses have not been associated with disease in humans.[421] In the Democratic Republic of Congo, Switzer and coworkers[423] measured a seroprevalence rate of 0.5% among individuals in rural villages with routine and close contact with nonhuman primates, and higher rates (2.2%–11.1% PCR positive) have been reported by Engel and coworkers[424] in Bangladesh and by Muniz and coworkers[425] in Brazil, where 18% of primate handlers were seropositive for SFV, but additional study is essential to document human-to-human transmission. SFV is not closely related to HIV or SIV and should not be responsible for reactive HIV-1 serology or NAT.

Cross-species transmission of retroviruses from other species has been considered as a possible consequence of xenotransplantation, as candidate species for tissue donation all harbor endogenous retroviruses, especially porcine endogenous retrovirus (PERV) in pig species.[426,427] Endogenous retroviruses present in animal genomes may not be expressed in the host species but may be inducible upon culture in vitro and transmitted to human cells.[428] Receptors for PERV are expressed in a variety of tissues,[429] and PERV is resistant to most current antiretrovirals.[430] Thus one concern is that endogenous retrovirus production may not be detected at time of harvest but may be induced after transplantation. Concerns for potential retroviral expression with development of zoonotic disease from other porcine and other tissue has prompted debate and recommendations on testing of xenotransplanted tissue for the presence of retroviral sequences and the ability to induce retroviruses in vitro from candidate tissues.[431,432] No evidence, however, of PERV transmission to humans has been demonstrated,[433] and PERV genomes undergo inactivation due to extensive APOBEC (a cellular cytidine deaminase)–mediated hypermutation.[434] Similarly, studies in nonhuman primates have not detected PERV in experimental xenotransplants,[435] although transmission to severe combined immunodeficiency disease (SCID) or nucleotide oligomerization domain (NOD)/SCID mice has been reported in pancreatic islet transplants.[435,436] PERV antigens are serologically distinct from HIV.[437]

REGULATORY ISSUES

Regulation of HIV Testing Modalities

HIV infection and AIDS are conditions with active constituencies among the public and relatively high-profile media attention. Although the scientific debate over the cause of AIDS has justifiably been put to rest,[438] and HIV testing has been largely accepted, material in the lay press continues to circulate that has erroneously criticized or misinterpreted HIV testing sensitivity and accuracy (e.g., www.aliveandwell.org, www.duesberg.com, virusmyth.net, http://ed-sherbeyn.com). Physicians, scientists, and the public require accurate information regarding the materials and procedures used for HIV testing (UNAIDS.org/special). Numerous individual kits are available for HIV testing; in the United States the CDC surveys laboratories performing HIV testing through routine performance evaluations (MPEP), and FDA reviews quality control and licensure of these products. A list of FDA-approved tests is maintained at www.fda.gov/cber/products/testkits.htm. Worldwide, WHO has supported distribution of kits to resource-poor countries since 1998 and maintains a list of kits meeting WHO performance specifications (www.WHO.int); WHO recommends that any kit manufactured for distribution should be approved by regulatory agencies in the home country of manufacture.

Regulation of HIV Reporting

HIV prevalence and incidence data represent critical strategic information and the basis for public health response to the epidemic. Reliable and responsible reporting of HIV infection is essential to achieve accurate surveillance and maintain public trust. Integrating reporting into public health programs planning eradication of HIV infection and clinical programs that link individuals to care will maximize the use of these data. The availability of home testing and anonymous testing modalities expands the populations that may access HIV testing, especially among key populations, but will provide, at best, haphazard reporting. An exhaustive analysis[439] reached the critical conclusion that although passive epidemiologic approaches have been useful, active data collection from key populations is essential for comprehensive understanding of the HIV epidemic, to develop useful datasets for use in monitoring programs addressing HIV-infected individuals and to evaluate their effectiveness.[439] Achieving "90-90-90" objectives requires use of an expanded HIV diagnostic database. Development and use of unique identifiers for patients, especially in key populations, will be useful in linking individuals across clinical and epidemiologic platforms and may address limitations of current data collection strategies.

CONCLUSION

HIV diagnosis is performed for a variety of purposes: epidemiologic surveillance, individual testing, and protecting supply of blood products and tissues for transplantation. Establishing the diagnosis of HIV infection in individuals is a process that optimally requires a coordinated effort among patients, health professionals, and the laboratories. Of critical importance, the use of HIV testing modalities in PrEP and PEP circumstances places a substantial burden on the performance characteristics of testing modalities to detect the presence of HIV in low-prevalence populations and in early HIV infection. Not all testing modalities are equally useful for these demanding circumstances. The contribution of infectious disease specialist in diagnosis will be invaluable and should proceed from medical history and physical examination to laboratory diagnostic testing.

ACKNOWLEDGMENTS

We thank John Coffin, Henry Masur, H. Clifford Lane, John Mellors, Richard T. Davey, Michael A. Polis, Olivia Fankuchen, Joseph Kovacs, Malcolm A. Martin, Klaus Strebel, Francois Clavel, Indira Hewlett, Susan Leitman, Julia A. Metcalf, and Tina Levin for insightful discussions, and are grateful to NIH Infectious Diseases fellows for useful conversations.

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The complete reference list is available online at Expert Consult.

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121 The Immunology of Human Immunodeficiency Virus Infection

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The interactions between the human immunodeficiency virus (HIV) and the human immune system are extraordinarily complex, as evidenced by the highly variable rates of disease progression observed in HIV-infected individuals. Indeed, even individuals who were infected from a common source may experience widely divergent clinical outcomes.[1,2] HIV subverts the immune system by infecting $CD4^+$ T cells that normally orchestrate immune responses and by activating the immune system and inducing a replication-permissive microenvironment that the virus uses to its own advantage. The discoveries that in addition to the CD4 molecule, the primary receptor for HIV, certain chemokine receptors function as essential HIV coreceptors for HIV entry into target cells, whereas other receptors facilitate entry or propagation have expanded the scope of host factors that play a role in the pathogenesis of HIV-induced disease.

The lack of recognizable and consistent correlates of protective immunity in HIV infection continues to hamper vaccine development and immunotherapeutic approaches to HIV disease. Robust HIV-specific immune responses, both humoral and cell mediated, are mounted by most infected individuals. Nonetheless, the vast majority of untreated HIV-infected individuals experience inexorable disease progression, resulting in profound immunodeficiency, despite the presence of these robust antiviral immune responses. In this regard, qualitative in addition to quantitative aspects of virus-specific immune responses clearly are important in the containment of viral replication.

The progress that has been made to date in understanding the pathogenesis of HIV infection is unparalleled. The availability of effective combination antiretroviral therapy (ART) has had extraordinary clinical benefits for patients, has diminished the transmissibility of virus from an infected to an uninfected individual, and has also provided important insights into the immunologic and virologic factors associated with the control of HIV infection and disease progression. Studies on cohorts of well-defined HIV-infected individuals, including long-term nonprogressors/elite controllers (LTNP/ECs) and those initiating ART early after infection, have also provided important insights into HIV immunopathogenesis. However, fundamental questions remain regarding the nature and scope of the precise pathogenic mechanisms of HIV disease. It is clear that HIV induces dysfunction of almost every element of the immune system and that the pathogenesis of HIV disease is multifactorial.[3–5] In this regard, a great deal is known about how HIV hijacks the immune system by targeting lymphoid tissues and perturbing the immunoregulatory balance; however, any attempt to manipulate these enormously complex and intertwined immunologic parameters for the purpose of inhibiting HIV replication without untoward effects is a daunting task. Nonetheless, progress in the understanding of how viral replication can be restricted with immunotherapeutic interventions is providing new treatment and potentially curative opportunities.[6–11]

HIV ENTRY AND DISSEMINATION

HIV Receptors and Entry Into Cells

CD4 was identified as the major cellular receptor for HIV fusion and entry in 1984.[12,13] Additional factors for viral entry were suspected, yet remained elusive for many years until the recognition of HIV coreceptors. In late 1995 and early 1996, a series of papers was published that altered our understanding of how HIV enters a target cell. The first report, by Cocchi and coworkers,[14] identified the CC chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES (*r*egulated on *a*ctivation, *n*ormal *T*-cell *e*xpressed and *s*ecreted), also known as CCL3, CCL4, and CCL5, respectively, as major components of $CD8^+$ T-cell–derived HIV suppressor factors. They observed that these chemokines, in combination, could inhibit the infection of activated $CD4^+$ T cells by certain strains of HIV-1, HIV-2, and simian immunodeficiency virus (SIV). Subsequently, a chemokine receptor, CXCR4, together with CD4, was required for entry of X4 viruses into target cells but was not used by R5 strains.[15]

In a separate line of research, Paxton and coworkers studied a population of individuals who had been exposed repeatedly to HIV-infected partners but who remained uninfected.[16] They identified two individuals whose $CD4^+$ T cells were refractory to infection with R5 strains of HIV, but were easily infectable with X4 strains. In addition, cells from these individuals produced high levels of MIP-1α, MIP-1β, and RANTES, the same chemokines previously identified as suppressors of HIV infection. Shortly thereafter, the chemokine receptor CCR5 was identified and shown in a series of papers to be a coreceptor for cellular entry of R5 strains (Fig. 121.1).[17–21] Subsequently, a mutant allele of the *CCR5* gene was described that contained a 32-bp deletion resulting in a truncated nonfunctional coreceptor for HIV entry.[22,23] Because CCR5-utilizing HIV is almost universally responsible for primary infection, individuals homozygous for the *CCR5* mutation are almost completely protected from HIV-1 infection.[24] Heterozygosity for the *CCR5* mutation results in reduced expression of CCR5 on the cell surface. Although heterozygosity for *CCR5* does not appear to afford protection against HIV-1 infection, it may result in slowed progression of disease in HIV-infected individuals.[24] Conversely, several polymorphisms in the *CCR5* gene and its ligands have been associated with more rapid disease progression.[25] Since the report of a single case of long-term control of HIV-1 in the absence of ART by genetic targeting of CCR5,[26] deletion of the *CCR5* gene through either ablation followed by stem cell transplantation from a *CCR5*-deficient donor or gene therapy to block or delete *CCR5* has been proposed as one of several potential strategies for eradicating HIV from infected individuals.[9] Nonetheless, the reproducibility and practicality of such an approach currently remains in doubt, especially for the tens of millions individuals currently living with HIV in resource-poor countries.

Over the past two decades, many other receptors have been suggested to play a role in HIV entry into target cells. There is a general consensus that CD4 and a chemokine coreceptor, usually either CCR5 or CXCR4, are essential for viral entry into target cells, whereas other receptors may serve merely to facilitate infection or transmission of HIV. In this regard, several pattern recognition receptors (PRRs), including dendritic cell (DC)–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) and related calcium-dependent lectins are regarded as important attachment receptors for HIV and may play an important role in early events of transmission (see next section).[27,28] Attachment factors can also enhance cell-to-cell spread via the formation of virologic synapses (see Fig. 121.1). The integrin $\alpha_4\beta_7$ has been shown to bind the HIV envelope glycoprotein gp120 and serve as an attachment receptor for HIV.[29] Most recently, treatment with an antibody that targets $\alpha_4\beta_7$ has been shown to prevent and restrict SIV replication in a nonhuman primate (NHP) model.[30] The interaction between $\alpha_4\beta_7$ and gp120 may be especially important for replication in the gut-associated lymphoid

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

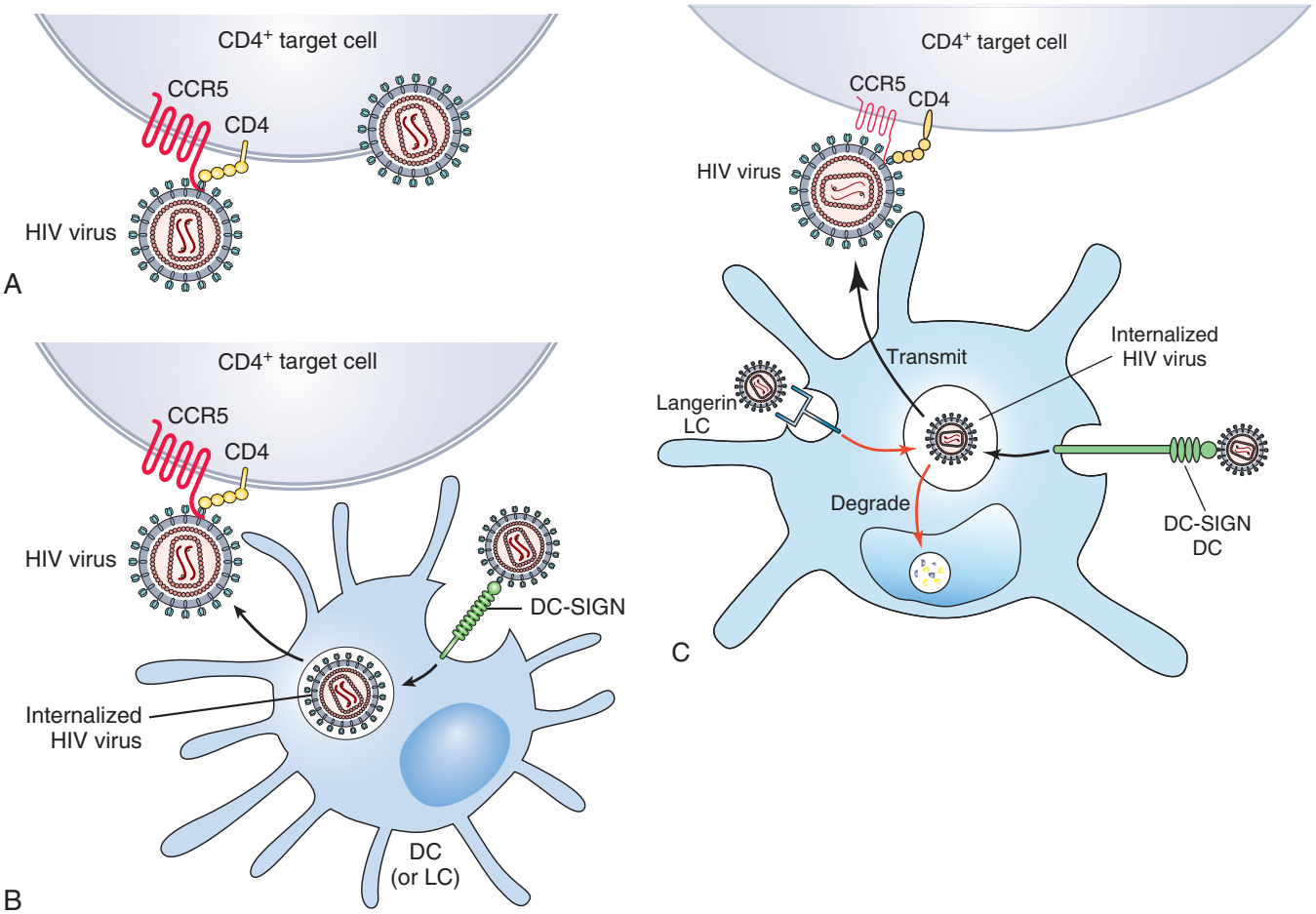

FIG. 121.1 Model of virus-cell infection and cell-to-cell transmission of human immunodeficiency virus *(HIV).* (A) CCR5-utilizing strains of HIV (R5) infect $CD4^+$ target cells by binding to both CD4 and the chemokine receptor CCR5, followed by fusion and entry into the cell. (B) Cell-to-cell spreading of virus can occur by transinfection, whereby a DC or LC captures virus through a C-type lectin receptor such as DC-SIGN or langerin, a process thought to occur both at the site of transmission and in lymphoid tissues. After capture, the DC internalizes the virus into a cellular vesicle and transfers it to a target $CD4^+$ T cell, whereas the LC degrades the virus in a TRIM5α/langerin-dependent process. However, HIV degradation in the LC may be reversed by inflammation and coinfections. (C) Passage of virus from one infected cell to another uninfected target cell also occurs, and both processes can be enhanced by interactions between various ligands and host attachment receptors; for example, the binding of HIV envelope gp120 to the integrin $\alpha_4\beta_7$ leads to binding of LFA-1 to ICAM-1 through a cascade of activation steps. These interactions coalesce to form virologic synapses that can enhance virus spreading by physical proximity and possibly by excluding neutralizing antibodies. *DC,* Dendritic cell; *DC-SIGN,* dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin; *LC,* Langerhans cell.

tissues (GALT), where this integrin is preferentially expressed on resident $CD4^+$ T cells.[28] In addition, the binding of gp120 to $\alpha_4\beta_7$ leads to activation of another integrin, lymphocyte function–associated antigen 1 (LFA-1), which also enhances the replication efficiency of HIV. This receptor may also play a role in the facilitation of HIV transmission across genital mucosal surfaces.[28]

Dissemination of HIV Infection

It remains unclear which cell type is actually the first to become infected after exposure to HIV. The most frequent route of exposure occurs through sexual transmission (Fig. 121.2), with less frequent routes including blood and mother-to-child transmission.[31] However, regardless of the route of entry, rapid migration to regional lymph nodes is followed by dissemination via the bloodstream to various lymphoid tissues (see Fig. 121.2). Massive depletion of memory $CD4^+$ T cells in the GALT has been shown to occur very early after SIV and HIV infections,[32–35] although there is some debate as to whether the depletion is a result of direct infection, bystander effects, or both.[36]

In early studies of macaques exposed to SIV intravaginally, myeloid dendritic cells (mDCs) in the vaginal mucosa were identified as the first cells to contain SIV DNA at approximately 2 days after exposure.[37] Improvements in in situ hybridization and imaging techniques have provided better insight into the early events of virus transmission, particularly in NHP models, although there still remains debate regarding which cells are infected first. The answer may depend on the type of mucosal transmission, whether vaginal, rectal, or penile, with important variables that include target cell availability and attributes of the mucosal epithelial barrier.[38,39] Nonetheless, DCs, especially the specialized DCs called Langerhans cells (LCs), in addition to macrophages, interstitial DCs, and resting $CD4^+$ T cells are all potentially among the first cells to capture and/or replicate HIV.[28,40] Various studies of heterosexual transmission in NHPs have also shown that both cell-free and cell-associated virus in semen, including virus associated with macrophages and to a lesser extent with $CD4^+$ T cells, can penetrate the vaginal epithelium and rapidly disseminate to lymphoid tissues.[41] Given the difficulties of studying early events of HIV infection in humans, various organ cultures, including intact human cervical tissue explants, have been used as models of HIV transmission. Exposure of cervical explants to HIV has demonstrated that intraepithelial $CD4^+$ T cells and LCs rapidly bind virus.[42,43] HIV target cells, including macrophages and $CD4^+$ T cells, and stromal DCs and LCs are also present in the male genital tract and are especially abundant in the foreskin.[31] The very strong protective effect of circumcision on HIV transmission is suggestive of a role for cells of the foreskin in susceptibility to HIV infection.[44] These areas of research have also advanced considerably over the past several years, at least in part driven by a growing interest in preventing

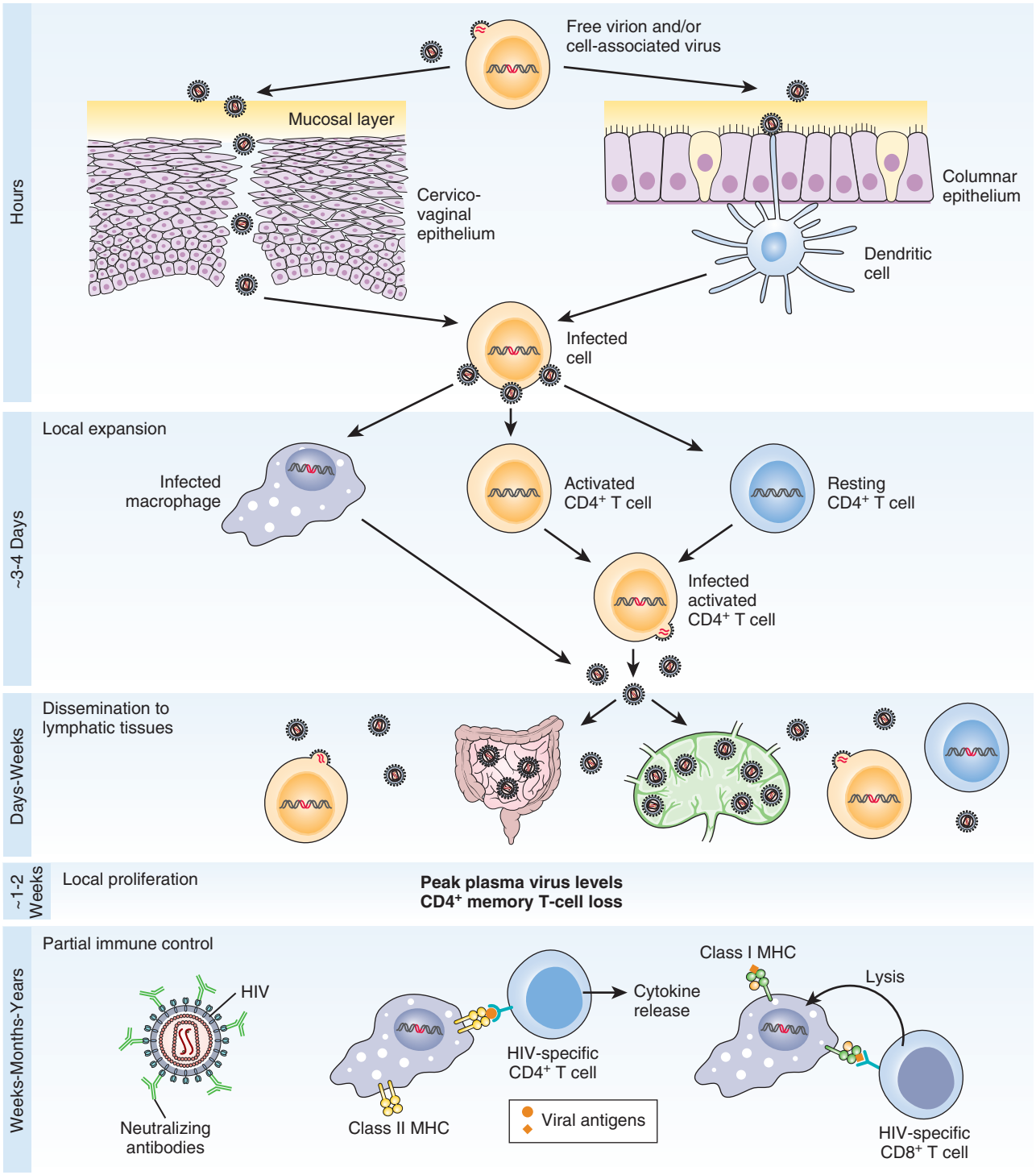

FIG. 121.2 Initial infection and dissemination of human immunodeficiency virus *(HIV)* infection. The sequence of events after vaginal or rectal transmission includes the following: (1) crossing the mucosal barrier; (2) interaction of virus with host cells, such as dendritic cells, that transport HIV to the paracortical regions of draining lymphoid tissues, leading to infection of $CD4^+$ T cells and systemic dissemination of HIV infection in various lymphoid tissues; macrophages can also be infected in this manner, although they are not felt to efficiently produce virus for wide dissemination; and (3) induction of HIV-specific immune responses, beginning with $CD8^+$ T cells and following with antibodies that provide incomplete control of viremia. *MHC,* Major histocompatibility complex. *(Modified from Haase AT. Perils at mucosal front lines for HIV and SIV and their hosts.* Nat Rev Immunol. *2005;5:783–792; with permission.)*

transmission with topical microbicides and various inhibitors that can directly target HIV, including inhibitors of CCR5 binding to HIV or neutralizing antibodies.[45] There has also been considerable interest in identifying the earliest events of HIV transmission, given the evidence that the majority of mucosal infections are established by a single or a few viruses; furthermore, factors such as microbreaches, hormones, and inflammatory responses can affect transmission.[31,39] A better understanding of these events is thought to be essential for advancing HIV vaccine strategies and other strategies aimed at reducing the rates of HIV transmission.[31]

DCs and LCs are capable of retaining infectious virus on their surface or within endosomal compartments for extended periods of time, thus

allowing HIV to remain shielded before encountering a target cell for productive replication. It is somewhat unclear how retention of infectious virus is mediated, although several lectin and nonlectin receptors expressed on DCs and LCs have been proposed.[27] Given that some of the proposed HIV capture molecules such as DC-SIGN are expressed on DCs but not on LCs, it is likely that this process can be achieved by means of a variety of different mechanisms. For example, whereas degradation is the predominant outcome when HIV is endocytosed into LCs by the C-type lectin langerin (see Fig. 121.1) and the host restriction factor TRIM5α (tripartite motif-containing protein 5α) is recruited,[46] this antiviral effect may be abrogated by coinfections and inflammatory responses.[27] Collectively, the preponderance of evidence indicates that LC–T-cell or DC–T-cell conjugates enhance HIV replication via what has been termed a *virologic synapse* (see Fig. 121.1). Furthermore, whereas LCs are thought to be largely sessile, DCs are uniquely poised to carry HIV from tissues in which the initial rounds of viral replication occur to the regional lymph nodes, where $CD4^+$ T cells become infected after contact with DCs. This leads to subsequent rounds of virus replication and spread in the absence of HIV-specific immune responses. Thus lymphoid tissue plays a key role in the initiation and dissemination of HIV infection.

HIV-SPECIFIC IMMUNE RESPONSES

Although the immune response to HIV has been extensively studied, a number of fundamental issues remain unresolved. Among the most important of these is that it remains unclear how HIV avoids immunologic control in most infected individuals. It is striking that unlike the situation with most other viral infections, the human host does not consistently mount an immune response that is able ultimately to eliminate the virus from the body or even suppress it. NHP models have been useful for the study of retrovirus-specific immunity, immunotherapies, and vaccines. Through passive transfer studies or studies involving depletion of $CD8^+$ T cells in experimental animals, insight into the various facets of virus-specific immune responses have been extended from correlative studies in humans. It is known that passive transfer of virus-specific antibodies can protect against retroviral infection, and cellular immune responses can control viral replication in NHP models; in addition, there are indications for similar outcomes in humans. Furthermore, insight into the breadth and magnitude of HIV-specific immune responses in HIV-infected individuals is beginning to inform new strategies for the development of effective prophylactic or therapeutic vaccines.

Humoral Immune Responses

In most individuals who acquire HIV, the virus rapidly establishes a persistent infection and evades the antibody response of the host. Although antibody responses capable of binding multiple HIV encoded proteins can be detected weeks to months after infection, the response to the surface glycoprotein (Env) is the most important with regard to effector functions against virions or infected cells.[47] The constant fragments (Fc) of immunoglobulin G (IgG) antibodies bound to Env expressed on infected cells can in turn bind to receptors on effector cells (FcγRs). These cells can mediate antibody-dependent cellular phagocytosis (ADCP) in the case of monocytes, macrophages, or DCs or antibody-dependent cellular cytotoxicity (ADCC) in the case of perforin-bearing cells such as natural killer (NK) cells or monocytes.[48] Other antibody effector functions include those that directly lyse virions such as the binding of IgG constant regions (Fc) to C1q, to initiate the classical complement cascade. By far the most extensively studied antiviral function, possibly because of its dominant role in protection against infection, is neutralization through blockade of viral entry to target cells by binding to Env. Any single antibody may have one or several of these effector functions, such as HIV-specific neutralizing antibodies that also mediate ADCC.

There are several features of the Env protein that pose challenges for mounting broad and potent antibody responses to diverse HIV isolates. The Env subunits gp120 and gp41 noncovalently associate and trimerize into the functional viral spike that is very heavily glycosylated. These glycosylated surfaces are poorly immunogenic and provide the virus with what has been termed a *glycan shield*.[49] Some conserved epitopes such as those at the apex of the trimer or the CD4 binding site can be partially occluded by glycosylation, which would limit antibody access. Perhaps the greatest challenge to developing broad and potent recognition of HIV is the level of genetic variation. The sequence diversity of Env is high within geographically defined populations and even within a single infected patient. Mutations that occur during the process of reverse transcription and a prolonged duration of infection generate highly diverse viral sequences that coexist in the plasma.[50,51] The level of diversity of HIV is considerably higher than that of most human RNA viruses and is likely a major contributor to the paucity of cross-neutralizing antibodies in the majority of HIV-infected individuals. Even when compared with influenza, which is also an RNA virus that undergoes high rates of mutation, the HIV variability in one individual is often greater than that of the influenza virus circulating in the community for an entire season.[52] HIV viral sequences may vary between 10% to 16% in the plasma of a given chronically infected individual.[51]

Despite these challenges the prevalence of sera with broad neutralizing activity is not rare in chronically infected people. Depending on the neutralization criteria that are used in defining breadth of sera, antibodies with broad neutralizing potential have been shown to arise in approximately 20% to 50% of HIV-infected individuals.[53–56] Sera from these patients are active against difficult-to-neutralize primary isolates and across highly diverse clades of viruses to which the patient has not been exposed. However, such antibodies may take 1 to 2 years to develop in infected individuals.[57,58] Although antibodies that bind Env can be detected in the plasma within weeks of HIV infection,[59] these early antibodies are largely nonneutralizing and do not protect against superinfection.[60,61] Relatively high levels of antibodies that have neutralizing potential appear several weeks later, although their breadth is limited and their ability to eliminate the circulating virus is inadequate[49,62] (see Fig. 121.2). This is at least partly the result of the selective pressure exerted by the antibody response that drives HIV genetic variants to arise rapidly. It is thought that these diverse circulating envelope sequences may represent a constantly evolving target that contributes to the ability of HIV to evade the humoral immune response. As Env changes, the humoral immune response coevolves with regard to shifting specificities, mode of recognition, and affinity maturation (Fig. 121.3). The end result in some patients is the development of antibodies capable of neutralizing highly diverse isolates by targeting accessible conserved epitopes.

Over the past decade, study of the sera and cells from such patients has resulted in extraordinary advances in our understanding of the nature of a broadly neutralizing antibody (bNAb) response. These advances have been largely driven by the formation of well-defined cohorts of patients with broad neutralizing sera[63–68], Env-specific peripheral blood B-cell isolation through microculture or fluorescent probe sorting, and isolation of Ig genes and reexpression as monoclonal antibodies.[69,70] These developments led to an explosion in the number of HIV-specific monoclonal antibodies isolated, resulting in a deconvolution of the specificities responsible for the activity of broadly neutralizing sera, and an understanding of the structure, function, and evolution of these antibodies. The targets of these bNAbs fall into five major groups: the CD4-binding site, a second variable loop (V2) glycan at the trimer apex, a site at the base of V3 centered on the Asn332-glycan on gp120, the membrane-proximal external region on gp41, and epitopes that bridge the interface between gp120 and gp41 (Fig. 121.4).[47] Many of these antibodies are atypical compared with monoclonal antibodies for other viruses. They may have very high levels of somatic hypermutation; often have insertions and deletions, long heavy-chain complementarity–determining 3 regions, or restricted germline use; or may be polyreactive. In some cases patients who ultimately developed broadly neutralizing sera had participated in longitudinal study protocols involving frequent sampling of serum and peripheral blood mononuclear cells (PBMCs) since acute infection.[71–73] Use of these samples combined with next-generation sequencing has permitted detailed studies of how bNAbs arise in some individuals. At present the challenge for the field is how to best use this information to develop immunotherapies or passive or active prophylaxis.

Experiments using passive administration of bNAbs in animals have provided an important proof of concept that they have potent



FIG. 121.3 Coevolution of human immunodeficiency virus (HIV) and the antibody response. In response to HIV infection, the antibodies expressed by naïve B cells that bind the HIV envelope undergo somatic hypermutation and increase their affinity. This selects for viral variants that are not recognized. In response, new antibody specificities may then be expanded or existing specificities may further mutate. This process continues over 1 to 2 years, ultimately resulting in the generation of broadly neutralizing antibodies in some patients.

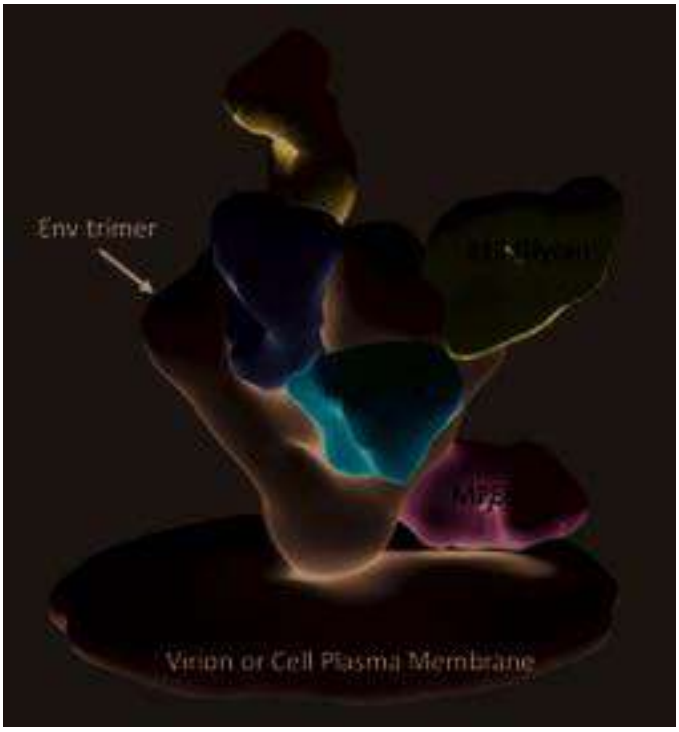

FIG. 121.4 Conserved sites of vulnerability on human immunodeficiency virus envelope glycoprotein. The figure depicts the binding of examples of broadly neutralizing antibodies, here labeled with their respective binding sites. *bs,* Binding site; *MPER,* membrane proximal external region. *(Image courtesy A. Ward; with permission.)*

immunotherapeutic effects. BNAbs can dramatically lower viremia in chimeric simian-human immunodeficiency virus (SHIV)–infected rhesus macaques or HIV-infected humanized mice. Although mutational escape is commonly observed during monotherapy, it is less often observed during administration of antibody combinations. In addition, passive antibody administration to macaques during acute or chronic infection with some SHIVs has resulted in sustained suppression of viremia after the antibody is cleared, suggesting that passive antibodies have the potential to modulate the T-cell response.[74,75] The potential role of neutralizing antibodies as immunotherapies, alone or in combinations with antiretrovirals, should become clearer after completion of several clinical trials using these strategies that are underway in humans.

There is also a large body of data that suggest that antibodies can likely provide complete protection from HIV infection. Numerous bNAbs have been shown to provide sterilizing immunity against mucosal challenges in SHIV-rhesus macaque challenge models.[47] The levels required to protect these animals are well within the range of titers detected in many HIV-infected patients, suggesting that they are likely achievable in uninfected vaccinees. Although stimulating bNAbs through vaccination is a desirable goal, generating such a response has proven extremely challenging. Thus far, most vaccines in humans or experimental animals have generated antibody responses capable of neutralizing only very easy to neutralize viruses or responses specific to only the immunizing strain. As noted earlier, these antibodies take months to years to develop and are in many ways atypical. However, it is not completely clear whether prolonged development and high levels of somatic hypermutation are requirements for generation of these antibodies or simply reflect chronic replication and mutation in an immunocompromised host. Nonetheless, immunization strategies that might recapitulate the pathways to the generation of bNabs are currently the subject of intense interest. These strategies include immunogens designed to better engage naïve B cells, partial Env deglycosylation, or immunogens

derived from the study of Env and bNAb ontogeny to be used in progressive vaccination with sequential immunogens, to initiate and then broaden the antibody response. Although the induction of a bNAb response is an extremely difficult goal to achieve, weakly or nonneutralizing antibodies can provide some, albeit more modest, level of protection, as reviewed by Excler and colleagues.[76] In addition, considerable optimism has been generated by a vaccine trial in humans demonstrating 31% overall efficacy in preventing infection in participants, primarily those at heterosexual risk of HIV infection in Thailand.[77] Participants were vaccinated with a combination of an attenuated poxvirus encoding HIV gene products, followed by an HIV envelope protein boost. Although the precise mechanism of action remains incompletely understood, the vaccine did not induce neutralizing antibodies against circulating strains, and protection from infection may have operated through an antibody-mediated cellular cytotoxicity mechanism. The role of such responses may become more apparent after the completion of several protocols that build on this strategy. In addition to these studies, efforts are underway to use antibodies in passive prophylaxis.[78] These approaches include vectored prophylaxis, by means of delivery of DNA or mRNA encoding bNAbs, or passive antibody therapy by means of intravenous or intramuscular injection. In some cases bNAbs have been engineered to include multiple specificities in a single antibody or to dramatically extend half-life.[79–81] A large multinational prophylaxis trial of passive administration of the bNAb VRC01 is currently in progress. It appears likely that the aforementioned passive prophylactic or immunotherapeutic clinical trials will provide considerable insights regarding potential selection of resistant isolates, and the levels of antibodies necessary for control of, or protection against, HIV infection.

Cellular Immune Responses

Cytotoxic T Lymphocytes

Major histocompatibility complex (MHC) class I–restricted, HIV-specific, $CD8^+$ T-lymphocyte responses are found in the peripheral blood within the first few months of HIV infection and are detected during the chronic phases of infection in most HIV-infected individuals.[82] During the chronic phase of infection, $CD8^+$ T cells specific for each of the known HIV-1 gene products are found in the peripheral blood with bulk cytotoxicity assays, limiting dilution assays for cytolysis, and interferon-γ (IFN-γ) secretion.[83] Several lines of evidence suggest that HIV-specific $CD8^+$ T cells play an important role in restriction of virus replication. First, the temporal association of the peak of the virus-specific cytotoxic response with the decline of viremia during acute HIV infection of humans or SIV infection of macaques is thought to represent the effect of virus-specific $CD8^+$ T cells in restricting retroviral replication in humans.[82,84,85] Second, indirect evidence of an important role of $CD8^+$ T cells in restriction of HIV replication in humans comes from strong associations between restriction of virus replication and certain MHC class I alleles and functional links with epitopes presented by these alleles.[86–90] Third, $CD8^+$ T-cell depletion by exogenous monoclonal antibodies has been shown to abrogate restriction of virus replication in SHIV-infected and SIV-infected monkeys.[91–94] Last, the HIV-specific $CD8^+$ T cells of patients that control HIV replication for many years have been shown to be functionally superior to those without immunologic control of virus replication (see "Long-Term Nonprogressors/Elite Controllers").[83] Based on the aforementioned evidence, it is now generally accepted that $CD8^+$ T cells are an important component of immune system–mediated restriction of HIV replication. However, although HIV-specific $CD8^+$ T cells likely exert some level of control over HIV replication, this restriction is generally incomplete, because viral RNA levels reach 10^3 to 10^6 copies/mL plasma in the vast majority of HIV-infected individuals during the chronic phase of infection in the absence of ART.

Several viral factors unique to HIV likely contribute to the ability of HIV to evade the cellular immune response. Mutations produced during the reverse transcription process, combined with high levels of replication over a prolonged period, result in a highly diverse population of viruses that circulate in a given patient. It is thought that this extraordinary level of viral diversity results from the development of mutant viruses that escape immune recognition. Selection of "escape" mutations has been documented in HIV-infected humans and SIV-infected macaques. Longitudinal studies of viral sequences and $CD8^+$ T-cell responses to known motifs have shown the appearance of mutations that no longer bind to the MHC class I molecule.[95] However, it should be noted that the interplay of host and viral factors is extraordinarily complex, given the large number of viral epitopes targeted by the immune response, the timing of gene expression during the viral replication cycle, and the complement of MHC class I alleles. The host $CD8^+$ T-cell response is limited by the ability of the MHC class I alleles to bind to various viral epitopes, and viral replication is limited by the degree to which an escape mutation impairs viral replicative capacity, or "fitness." In addition, the $CD8^+$ T-cell response to each of the proteins within the autologous virus is very high in frequency and very broad.[96,97] Escape mutations may be found within a single epitope, but it is likely that other conserved epitopes within the same gene remain as targets.[96] Strong associations between escape mutations, or viral fitness, and virus load have not been found.[98,99] Finally, putative escape mutations are also found in viral sequences in the plasma of LTNP/ECs at the same frequency as MHC-matched progressors.[96,97] Taken together, these data suggest that although escape mutations clearly can occur, they do not appear to be the critical driving force in the loss of immunologic control in the vast majority of HIV-infected patients.

In addition to the high levels of diversity generated during HIV infection, other viral factors may contribute to the poor ability of the immune system to control HIV replication. HIV Nef, Tat, and Vpu are each capable of downmodulating surface expression of MHC class I molecules necessary for the recognition of infected cells, as reviewed by Wonderlich and coworkers.[100] The effect of Nef is to diminish cell surface expression of human leukocyte antigen-A (HLA-A) and HLA-B molecules, leaving HLA-C and HLA-E unaffected.[101] It has been proposed that these effects may permit infected cells to avoid lysis by HLA-A– or HLA-B–restricted T cells, which dominate the cellular immune response, yet also avoid lysis by NK cells, which are inhibited by the presence of HLA-C or HLA-E. However, there is not uniform agreement that this is a predominant mechanism by which HIV avoids immunologic control. LTNP/ECs (see later), who can maintain immunologic control for many years, typically have intact MHC and HIV *nef* sequences.[89,102] Several investigators have demonstrated the ability of autologous HIV-infected cells to stimulate cytokine secretion or cytolysis by class I–restricted HIV-specific $CD8^+$ T cells. In addition, the high frequencies of HIV-specific $CD8^+$ T cells found in the peripheral blood of most HIV-infected patients rapidly fall to very low levels after initiation of ART.[103] This indicates that in the absence of therapy, HIV-infected cells stimulate and maintain high frequencies of HIV-specific $CD8^+$ T cells. Although infected cells are recognized, viral replication is not fully suppressed. At present, the relative role of MHC downregulation in the loss of restriction of HIV replication remains unclear.

Another parameter of the $CD8^+$ T-cell response that may provide enhanced restriction of virus replication is the breadth or number of HIV peptides to which a patient may respond. However, when the total $CD8^+$ T-cell response was measured during acute or chronic infection, no relationship was found between the breadth of the response and levels of plasma HIV viremia.[97,104,105] Several reports have described a narrowly focused $CD8^+$ T-cell response in LTNP/ECs or in individuals treated with ART during acute infection and who have restricted virus replication.[89,106,107] Because HIV-specific $CD8^+$ T cells of most chronically infected patients persist at high frequencies and respond to a broad array of HIV peptides, it does not appear likely that inadequate breadth of the response is a major contributor to poor restriction of virus replication during chronic infection.

Several lines of evidence have suggested that the inability of $CD8^+$ T cells to control HIV may lie not in the quantity of these cells but rather in the qualitative properties of the response. Some data have suggested that disrupted HIV-specific $CD8^+$ T-cell function in progressors is associated with increased expression of programmed death (PD)–1 molecules[108] or decreased CD127 (interleukin [IL]-7Rα chain)[109,110] expression, but other studies have noted that these parameters are restored during ART, suggesting that they may be a consequence rather than a cause of the loss of control of viral replication.[111,112] The HIV-specific $CD8^+$ T cells of LTNP/ECs maintain greater frequencies of "polyfunctional" cells, named for their ability to degranulate and produce several

cytokines, including IL-2.[113] HIV-infected LTNP/ECs have also been differentiated by increased proliferative capacity of HIV-specific $CD8^+$ T cells linked to enhanced expression of perforin.[114] These functions have been found to be necessary for the killing of autologous HIV-infected $CD4^+$ T cells.[115] It is thought that the relative absence of these functions in progressors may represent a mechanism whereby HIV avoids immunologic control.[115,116]

Recent studies in the SIV/SHIV Rhesus macaque infection model have provided further evidence that the $CD8^+$ T-cell response may be used to suppress, or even cure lentiviral infection.[94,117] In a series of studies, rhesus macaques were immunized with a rhesus cytomegalovirus (CMV) recombinant vaccine encoding HIV gene products that are targets of the cellular immune response. The vaccine caused a chronic CMV infection that induced a persistent SIV-specific $CD8^+$ T-cell response. In those animals that were infected, a profound level of suppression of the pathogenic SIV challenge virus was observed. In 50% of infected animals, the pathogenic challenge virus was reduced below detection threshold levels and thought to have been cleared.[117] Interesting to note, the data suggest that nonclassical MHC class II– and MHC class I-E–restricted $CD8^+$ T-cell responses broadly targeting an average of 35 epitopes mediate this control.[118,119] In a more recent study, macaques treated with potent and broadly neutralizing anti–HIV-1 antibodies during acute SHIV infection unexpectedly facilitated emergence of $CD8^+$ T-cell immunity able to durably suppress virus replication.[74] Thus, in addition to an envelope-specific humoral immune response to an HIV vaccine, the $CD8^+$ T-cell response may provide an important second line of defense against breakthrough infections that might lead to long-term control of the virus. Although highly speculative, based on the results in the rhesus macaque model, it is theoretically possible that the $CD8^+$ T-cell response to vaccination might result in virus clearance in some instances.

Soluble $CD8^+$ T-Cell–Secreted Factors

In addition to cytolysis, other mechanisms of $CD8^+$ T-cell–mediated antiviral activities have been described. $CD8^+$ T cells of HIV-infected patients secrete soluble factors that can inhibit viral replication in the absence of cell killing. This noncytolytic antiviral activity was initially discovered in vitro after use of $CD8^+$ T cells from HIV-infected patients.[120] Years later, the suppressive activity against R5 viruses was shown to be mediated in large part by the CC chemokines MIP-1α, MIP-1β, and RANTES.[14] These molecules are natural ligands for CCR5, a coreceptor for R5 strains of HIV-1, and inhibit viral replication primarily by blocking virus entry. In addition to the CC chemokines, poorly characterized soluble factor(s) act after viral entry to suppress HIV transcription in infected cells. These factor(s) have been termed *$CD8^+$ T-cell antiviral factors* (CAF).[121] Although not required for this suppressive activity, maximal suppression of HIV replication is observed under conditions wherein cell contact is maintained and cells are HLA matched.[122,123] This suppressive activity has been shown to be greater in PBMCs of HIV-infected patients than in uninfected control subjects. A novel anti-HIV chemokine produced by activated $CD8^+$ T cells, XCL1/lymphotactin, has been shown to inhibit a broad spectrum of HIV-1 isolates, irrespective of their coreceptor-usage phenotype.[124] At present, it remains incompletely understood whether suppressive factors are secreted in an antigen-specific manner.

$CD4^+$ T-Cell Responses

It has been demonstrated in several animal models of viral pathogenesis that virus-specific $CD4^+$ T cells are critical for the induction or maintenance of an effective $CD8^+$ T-cell response that mediates restriction of virus replication. Many viral infections of humans or experimental animals typically result in induction of $CD4^+$ T-cell responses that can be demonstrated by proliferation to virus antigens in vitro long after elimination or control of infection, which is a reflection of the persistence of these virus-specific memory $CD4^+$ T cells. Unlike most other infections of humans, HIV infection is characterized by the absence or extremely low level of HIV-specific $CD4^+$ T-cell proliferative responses in the vast majority of untreated patients. Of note, $CD4^+$ T cells from untreated HIV-infected individuals also have defective proliferative responses to non-HIV antigens.[125] In this regard, strong HIV-specific $CD4^+$ T-cell responses are found in relatively rare LTNP/ECs who restrict HIV replication in the absence of ART.[126–128] In addition, proliferative responses to HIV antigens have been found in patients who were treated early during acute infection and who then had restricted HIV replication when ART was withdrawn.[128] Because HIV infects $CD4^+$ T cells, it was believed that the early loss of HIV-specific proliferative responses may be the result of infection and deletion of HIV-specific $CD4^+$ T cells in the lymphoid tissues on encountering the virus.[129] However, several lines of evidence indicate that HIV-specific $CD4^+$ T cells persist in patients with progressive disease. Many reports have documented the persistence of HIV-specific $CD4^+$ T cells in most patients by intracellular cytokine staining after stimulation with HIV antigens.[130–132] HIV-specific $CD4^+$ T cells also persist at the expected frequency and with the expected phenotype and functions in progressors when compared with responses to other viral antigens.[133] IL-2 production and proliferation of HIV-specific $CD4^+$ T cells was abrogated during an interruption of antiviral therapy, but restored in patients receiving ART, suggesting that diminished proliferation of these cells may be partly a result of the presence of high levels of HIV antigen. This effect is similar to that observed during viremia with other human viruses. Thus there is now general agreement that HIV-specific $CD4^+$ T cells persist at the expected frequencies during the chronic phase of infection in patients with progressive disease, although there is some controversy over whether disruptions of function persist. In addition, the role of depletion or disruption of the functions of HIV-specific $CD4^+$ T cells during acute infection or end-stage disease remains to be defined.

Host Genetic Factors

Among the most reproducible and powerful host factors to affect HIV disease is the impact of HLA alleles on viral load and disease progression. Since its initial description, the association between HLA-B*57/58 and B*27 with slower disease progression and B*35 with more rapid disease progression has been repeatedly observed, including in four genome-wide association studies.[95] Associations between these "protective alleles" and reduced viral load is not simply due to their enrichment in LTNP/ECs, but has been observed even in progressor cohorts.[134] Having a protective allele is neither necessary nor sufficient for immunologic control of HIV, given that approximately 20% of LTNP/ECs lack such alleles and they are found in progressors at the same frequency as in the general population.[107] In addition, an association has been observed between reduced viral load and a micropolymorphism in HLA-C,[135,136] and an association with reduced viral load has been made in patients who carry a subset of B alleles and a killer inhibitory receptor.[137] Unlike other protective alleles, this combination is believed to function through the action of NK cells expressing the 3DL1 inhibitory receptor. Although the mechanisms of each of these HLA effects remain incompletely understood, important clues continue to come from in vitro studies of $CD8^+$ T-cell and NK-cell function.

As noted earlier (see "HIV Entry and Dissemination"), the chemokine receptor genotype has an impact on rates of progression of disease. For example, individuals who carry one copy of the mutant CCR5-Δ32 allele have an increased chance of experiencing a slow rate of disease progression compared with individuals who are homozygous for wild-type alleles.[138] In addition, some CCR2 alleles have been associated with slower rates of disease progression. Despite the association between *CCR5* genotype and slower progression of HIV disease, this factor does not appear to be a dominant influence in the determination of the state of long-term nonprogression; neither CCR5-Δ32 nor CCR2 alleles have been found to be enriched in LTNP/ECs who control HIV replication.[89,102]

Long-Term Nonprogressors/Elite Controllers

Important clues regarding mechanisms underlying a successful immune response to HIV-1 have come from the study of rare examples of naturally occurring immunologic control of HIV replication. Although rates of disease progression vary widely among HIV-infected individuals, the median time between infection and development of acquired immunodeficiency syndrome (AIDS) is approximately 10 years. It has become clear that a small percentage of untreated HIV-infected individuals show no evidence of disease progression, despite prolonged infection.[139,140]

In addition to LTNP/ECs, other individuals who remain HIV seronegative, despite multiple exposures to HIV, have been identified, suggesting that elements of protection exist but may not be readily detected with standard measures of the immune response. This group is likely heterogeneous with regard to the mechanisms of resistance to HIV infection, as reviewed by Lederman and coworkers.[141] Studies of LTNP/ECs and highly exposed seronegative patients have advanced our understanding of the pathogenesis of HIV disease and have increased optimism that some forms of immunologic protection from infection or control of viral replication may be harnessed in prophylactic or therapeutic vaccines.

Definitions of LTNP/ECs have varied.[142] One early definition that was commonly used included documented HIV infection for more than 7 years, $CD4^+$ T-cell count higher than 500 cells/μL without significant decline over time, no symptoms of HIV disease, and no history of ART. Because definitions of nonprogressors were created empirically, it is not surprising that these individuals constitute a heterogeneous group. Many who were originally defined by these clinical criteria have now gone on to develop progressive disease. However, there remains a small subgroup of untreated nonprogressors who have now been infected for 20 years and maintain normal $CD4^+$ T-cell counts and plasma viral RNA levels less than 50 copies/mL of plasma. Mechanisms that may determine a course for LTNP/ECs during HIV infection include host genetic factors, effective immunologic control of virus replication, and/or infection with an attenuated strain of HIV (Table 121.1). Although patients with normal $CD4^+$ T-cell counts and low levels of plasma virus are a heterogeneous group, a small subset of patients with truly nonprogressive HIV infection and control of virus replication in the absence of ART are likely to hold important clues to the basis of an effective immune response to HIV.

Host Immune Response Factors

A considerable amount of evidence now suggests that HIV-specific $CD8^+$ T cells play a major role in the maintenance of low viral load in most LTNP/ECs. First, the HLA-B*57 allele is found in approximately 65% of patients in several different cohorts.[142] In addition, the HIV-specific $CD8^+$ T-cell response in these patients is highly focused on B57-restricted peptides, suggesting that the B57 molecule likely plays a direct role in restriction of virus replication in these individuals.[87,89] There are reports documenting viruses sufficiently pathogenic to cause progression to be transmitted to a second patient, who then becomes an LTNP/EC. In this case, both individuals carried the B*5703 allele.[143] Thus the recipient was able to mediate immunologic control despite B*57-associated escape mutations in the virus. In addition, there are cases of virus strains that cause progression in one partner being transmitted by superinfection to a nonprogressor, and that virus being controlled.[144,145] In addition, "elite controller" rhesus macaques have been described that are enriched for the Mamu B*08 and B*17 alleles.[146,147]

TABLE 121.1 Possible Mechanisms of Long-Term Nonprogression/Elite Control With HIV Infection

Host Genetic Factors
HLA type
Heterozygosity for 32-bp deletion in chemokine receptor CCR5
Mannose-binding lectin alleles
Tumor necrosis factor c2 microsatellite alleles
Gc vitamin D–binding factor alleles
Host Immune Response Factors
Effective CTL responses: "polyfunctionality," proliferation, perforin production, cytotoxicity
Secretion of CD8 antiviral factor
Secretion of chemokines that block HIV entry coreceptors CCR5 (e.g., MIP-1α, MIP-1β, and RANTES) and CXCR4 (e.g., SDF-1)
Maintenance of functional lymphoid tissue architecture
Virologic Factors
Infection with attenuated strains of HIV

CTL, Cytotoxic T lymphocyte; *HLA,* human leukocyte antigen; *MIP,* macrophage inflammatory protein; *RANTES,* regulated on activation, normal T-cell expressed and secreted; *SDF,* stromal cell-derived factor.

In some cases, epitopes restricted by these alleles have motifs similar to those that bind HLA-B27 or HLA-B57 in humans. Furthermore, transient $CD8^+$ T-cell depletion of SIV-infected elite controller macaques resulted in a dramatic increase in viral replication.[91] Taken together, these data argue strongly that immunologic control in most LTNP/ECs is not a consequence of infection with attenuated viruses but rather is mediated by HIV-specific $CD8^+$ T cells.

The most recent data regarding immunologic control in LTNP/ECs suggest that the superior ability of these patients to control HIV compared with progressors is mediated not by the quantity of HIV-specific $CD8^+$ T cells but rather by their qualitative function.[83] A number of studies using quantitative techniques that examined the response to a broad array of HIV gene products have not found higher frequencies or breadth of HIV-specific $CD8^+$ T cells in LTNP/ECs compared with progressors. The HIV-specific $CD8^+$ T cells of LTNP/ECs maintain greater frequencies of polyfunctional cells, named for their ability to degranulate and produce several cytokines, including IL-2.[112,113] However, these cells make up an extremely small subset of the total HIV-specific $CD8^+$ T-cell response, and many LTNP/ECs demonstrate few or no such cells.

By far, the property that most clearly distinguishes the HIV-specific immune response of LTNP/ECs from other patients is the ability to suppress HIV replication and/or kill HIV-infected cells in vitro. For some time, it had been known that HIV-specific $CD8^+$ T cells of LTNP/ECs maintain greater proliferative capacity and ability to upregulate perforin on stimulation compared with progressors.[114] More recently, the $CD8^+$ T cells of LTNP/ECs were shown to have a greater capacity to restrict HIV replication in vitro.[116,148,149] In addition, increases in perforin and granzyme B production were shown to result in a dramatically increased ability of $CD8^+$ T cells of LTNP/ECs to kill autologous HIV-infected $CD4^+$ T cells compared with progressors.[112,115] Although causality must ultimately be demonstrated, the abilities to proliferate, produce perforin, and kill HIV-infected $CD4^+$ T cells appear to be qualities within the HIV-specific $CD8^+$ T-cell response that are clearly associated with immunologic control in LTNP/ECs.

The relationship between humoral immune responses to HIV and disease progression remains incompletely understood. LTNP/ECs, as a group, do not have high levels of neutralizing antibodies.[63,139,140,150] In many cases, they may have low levels of binding or neutralizing antibodies believed in part to be due to lower levels of antigen driving the humoral immune response. In studies that examined clinical parameters that might be associated with bNAbs, the only correlation found was a positive correlation with viral load.[63,64] Some studies have demonstrated that sera that can neutralize a broad panel of primary isolates can be found in patients that have relatively high $CD4^+$ T-cell counts, but are viremic.[63–68] Although such patients tend to have slow rates of disease progression, they are a heterogeneous group. In these patients, antibodies may not be causing slower disease progression, but rather the association may be a consequence of the need for both an intact immune system and the presence of viral antigen to have such a response. At present, it does not appear likely in most cases that maintenance of neutralizing antibodies plays a major role in determining the state of nonprogression.

Virologic Factors

Infection with attenuated strains of HIV may account for nonprogression in a small subset of individuals. The most extensively characterized association of attenuated viral strains and nonprogression is that of an Australian cohort of nonprogressors who were infected by transfusion from a single nonprogressor donor.[151] Viruses from these individuals contained deletions in the *nef* gene and in the U3 region of the long terminal repeat (LTR). Other anecdotal reports have implicated other defective HIV genes with nonprogression, although such cases appear to be the exception rather than the rule.[152] Many of the patients described in the original Australian cohort ultimately developed progressive disease.

RESERVOIRS OF HIV INFECTION

There is unequivocal evidence from multiple lines of investigation that HIV persists in almost all infected individuals receiving effective ART. The most powerful demonstration of the inability of ART to eradicate HIV infection comes from in vivo studies of individuals who began ART during the chronic stage of HIV infection, achieved and maintained

suppression of plasma HIV RNA for several years, and subsequently interrupted therapy. Interruption of ART resulted in a rapid rebound of plasma viremia in 95% of patients.[153] More recent studies have largely confirmed these findings, although there is evidence that a proportion of individuals who initiated ART early control their plasma viremia for variable periods of time after discontinuation of therapy.[11] Although the reasons for this difference are unclear, a delineation of factors involved, including origin of rebounding plasma HIV, effect of time to establish long-lived reservoirs, and role of the immune system, are currently being investigated. A number of studies have suggested that replication-competent virus persists in the $CD4^+$ T-cell compartment in infected individuals receiving effective ART and that early establishment of HIV reservoirs in peripheral lymphoid tissues, including the GALT, are the main impediments to eradicating HIV.[154]

It was demonstrated by using highly sensitive assays with a limit of detection of a single copy of HIV RNA per milliliter of plasma that persistent low levels of plasma viremia from an unknown origin remain in the vast majority of individuals with undetectable plasma HIV when standard assays are used.[155,156] In addition to important HIV reservoir sites in the GALT and peripheral lymphoid tissues, putative reservoir sites include the reproductive tract, bone marrow, and central nervous system (CNS).[156–158] In addition to the high HIV burden in $CD4^+$ T cells, other cells that can potentially contribute to the viral reservoir include DCs and macrophages, in addition to microglial cells, specialized macrophages that reside in the CNS.[157,158] Genetic variability has been demonstrated in HIV isolated simultaneously from the plasma and several reservoir sites, including the reproductive tract and the CNS, indicating that there may be compartmentalization of HIV in different sites.[158] Such compartmentalization may provide a sanctuary for HIV that may be relatively impenetrable by antiretroviral drugs; however, it is unclear whether these sanctuary sites contribute significantly to the persistence of HIV in the presence of ART.

Latent Reservoirs of HIV

The pool of latently infected cells in the resting $CD4^+$ T-cell compartment has been one of the most extensively studied persistent reservoirs to date and is considered to be a major impediment to HIV eradication.[159–161] HIV may enter resting $CD4^+$ T cells, at which point a varying degree of reverse transcription of the HIV genome may occur in these cells (Fig. 121.5).[162,163] This period of preintegration latency may last hours to days; in the absence of an activation signal, unintegrated proviral DNA loses its capacity to initiate a productive infection. If these cells become activated, however, reverse transcription proceeds to completion, followed by nuclear translocation and integration of proviral DNA into cellular DNA.[162,163] It has been clearly demonstrated that a pool of resting $CD4^+$ T cells that carry replication-competent HIV persists in essentially all infected patients who were receiving ART and in whom plasma viremia was suppressed below levels of detectability.[159–161] In addition, this HIV reservoir is established during the earliest stages of HIV infection. The initiation of ART as early as 10 days after infection with

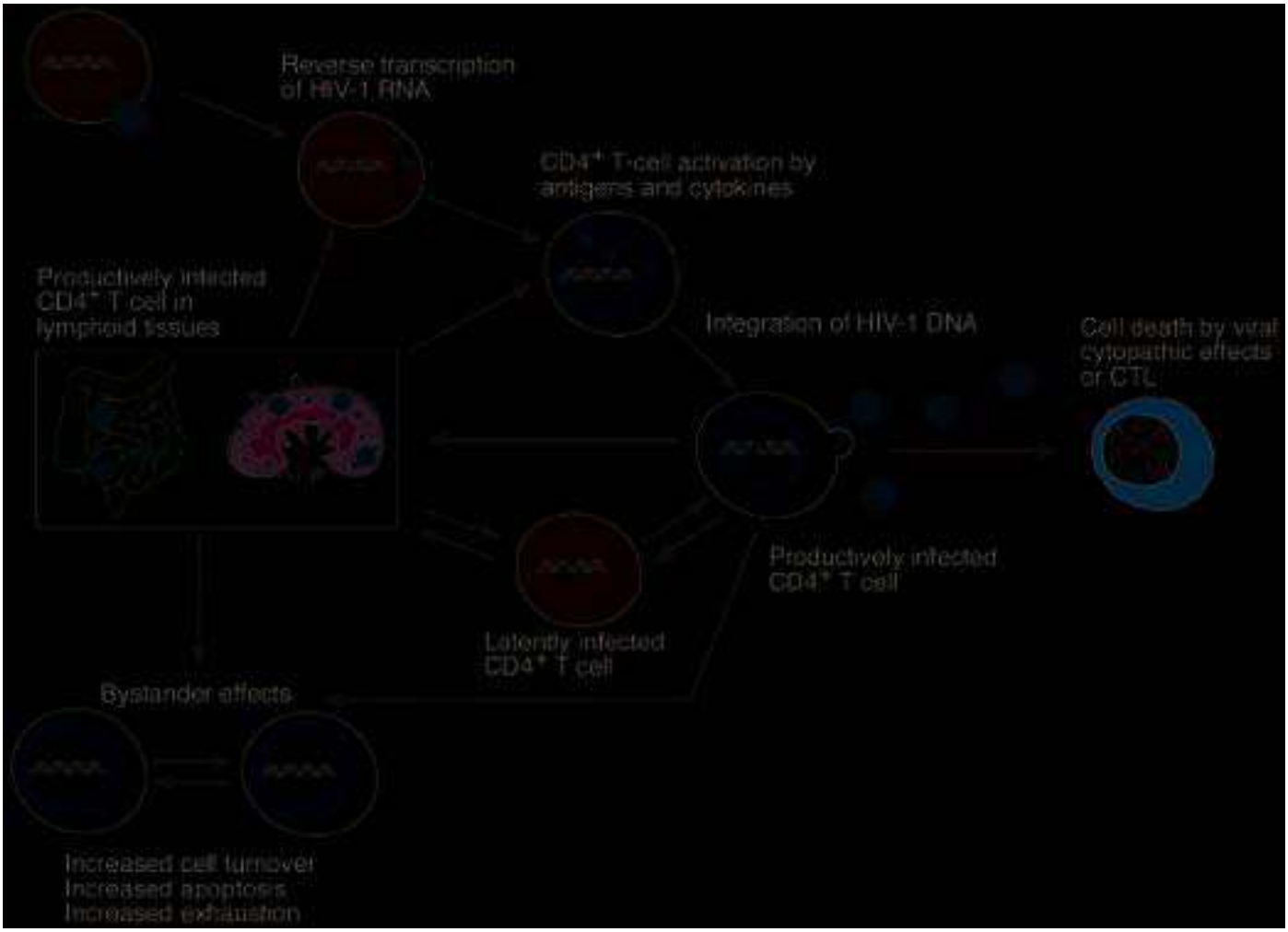

FIG. 121.5 Direct and indirect effects of human immunodeficiency virus *(HIV)* infection. HIV preferentially replicates in activated $CD4^+$ T cells, although virus entry can occur before activation. Productive HIV replication proceeds as follows: entry into cell, reverse transcription of HIV RNA, integration into host genome, and formation of new virions. Consequences of productive HIV infection in $CD4^+$ T cells include cell death by cytolysis and other mechanisms, spreading of virus to new targets, and establishment of long-lived latently infected reservoirs. The availability of high numbers of HIV target cells in lymphoid tissues leads to high levels of viral replication in most untreated individuals, and in turn leads to bystander effects that reflect chronic immune activation. *CTL,* Cytotoxic T lymphocyte.

HIV does not prevent the establishment of the resting CD4+ T-cell reservoir of HIV.[164] Furthermore, several studies have shown that despite effective ART, HIV persists in the resting CD4+ T-cell reservoir for years,[154] including in the recently characterized stem cell memory T cells, which have been shown to be long-lived, quiescent, and relatively insensitive to HIV cytopathic effects.[165] It has been estimated that it would take decades of continuous ART, using the currently available drugs, to eradicate HIV, and these estimates assume 100% effectiveness of ART and that the CD4+ T-cell latent reservoir is the only viral reservoir present in infected individuals. These sobering data and the unlikelihood of certain of the assumptions have led many to conclude that the latent in addition to other viral reservoirs present major impediments to eradication of HIV.

Lymphoid Tissues

Secondary lymphoid tissues, especially those of lymph nodes and GALT, represent major sites of HIV replication early, after, and throughout all stages of infection. In the absence of ART, HIV infection is characterized by a dichotomy in viral load between peripheral blood and lymphoid tissue, in which the frequency of infected cells in lymphoid tissues far exceeds that in peripheral blood.[154] Early findings on tissue sections excised from axillary lymph nodes have demonstrated that up to 25% of CD4+ T cells present in the germinal centers (GCs) harbor HIV DNA.[166] More recent analyses performed on tissues isolated from GALT have demonstrated massive depletion of CD4+ T cells in the lamina propria within several weeks after infection.[34,35] Depletion of CD4+ T cells in lymphoid tissues (and in peripheral blood owing to a direct link with lymphoid tissues) has also been shown to persist throughout all stages of HIV infection in untreated individuals.[156,158] Collectively, these data emphasize the role of secondary lymphoid tissues as critical reservoirs for HIV in vivo.

The significant role of secondary lymphoid tissues in ongoing HIV replication during all stages of disease in the absence of ART suggests that these compartments may also play a significant role in the maintenance of viral reservoirs in the presence of ART. Initiation of ART leads to a rapid decrease in lymph node viral burden, and within 24 weeks the levels of HIV RNA in the GCs decrease significantly.[167] However, even with prolonged ART, it is almost universally possible to detect HIV RNA within B-cell follicles of lymph nodes, where follicular dendritic cells (FDCs) have been proposed as long-lived repositories for intact HIV virions. Similar to DCs, FDCs have been shown to retain infectious HIV within endosomal compartments that can recycle to the surface and transmit virus to target CD4+ T cells.[168] The GALT also harbor HIV that is not completely cleared with ART; levels of HIV DNA in CD4+ T cells remain severalfold higher in GALT compared with the peripheral blood in those whose levels of plasma HIV RNA had remained below the limit of detection for several years. There are also indications that macrophages in mucosal, adipose, and pulmonary tissues may be a long-lived source of infectious HIV, although most of these observations have been made in animal models and need further evaluation in humans.[158,169] Finally, there is also evidence that virus in myeloid cells is acquired by phagocytosis of infected CD4+ T cells.[170]

Strategies for HIV Eradication

Over the past several years, there has been a flurry of discussion regarding the possibility that HIV can be eradicated from HIV-infected individuals.[b] Such a pursuit has accelerated with evidence that one such individual, commonly referred to as the "Berlin patient," has been cured of HIV.[26] His case is unique and unlikely to be clinically applicable, given that the approach taken was to treat an underlying leukemia with chemotherapy and to perform a stem cell transplantation from a donor who was homozygous for the HIV resistance gene *CCR5-Δ32*. The post-transplantation therapy also included chronic immunosuppressive therapy for the prevention of transplant rejection. Despite numerous attempts since 2009, similar attempts to eradicate HIV involving bone marrow or stem cell transplantation or gene therapy have failed to replicate the outcome of the Berlin patient.[9,11] Alternative cure strategies are also being pursued, including the use of agents that could reverse HIV latency and/or use modalities involving gene therapy or molecules engineered for virus-specific killing.[9] Finally, there are also discussions of using immune-based interventions as part of a combination of approaches to control HIV replication in infected individuals through the induction of an immune response that could prevent viral rebound in the absence of ART. In this case, the more appropriate term would be sustained ART-free virologic remission, whereby the virus, although still present in the body, would be kept in check by the individual's own immune system.[9,154]

[b]References 9, 11, 155, 156, 169, 171, 172.

IMMUNE DYSFUNCTION CAUSED BY HIV INFECTION

A wide array of immune system deficits is associated with HIV infection. Abnormalities in the function of all limbs of the immune system, including T and B lymphocytes, antigen-presenting cells, and NK cells, have been described. Immunodeficiency may become so profound in the late stages of disease that HIV-specific antibody and cytolytic T-lymphocyte responses diminish in the face of high levels of ongoing viral replication.

Lymphoid Tissues

The dysfunctions induced by HIV can be best appreciated in the context of the microenvironment in which the virus replicates. Most CD4+ T cells, which comprise the main targets for HIV replication, reside in secondary lymphoid tissues, such as lymph nodes and GALT, in which immune responses are generated. Early studies conducted before the availability of effective ART demonstrated that the clinically asymptomatic phase of HIV infection is characterized by lymphoid hyperplasia, whereas advanced HIV infection is marked by a striking disruption and atrophy of lymphoid tissue architecture.[173] Follicular involution, hypervascularity, and fibrosis are some of the histopathologic changes evident in lymph nodes from patients with advanced HIV disease. FDCs are eliminated during disease progression and the resulting disturbances in follicular structure have important implications regarding the pathogenesis of HIV-related immunodeficiency. The ability to mount immune responses against new antigens and the ability to maintain memory responses are dependent on the maintenance of an intact follicular architecture, which is severely impaired when the FDC network is destroyed.[168] Fibroblastic reticular cells (FRCs), another constituent of lymph nodes that provide the structural backbone and framework for the generation and maintenance of immune responses, are also progressively affected by the lack of containment of HIV replication.[168]

In studies on the effect of HIV infection on lymph node architecture, the inflammation caused by persistent viral replication contributes to lymphatic tissue damage and limits the potential for immunologic reconstitution after ART. Deposition of collagen occurs mainly in the paracortex region of lymph nodes, which is populated mainly by T cells, and as such is thought to be associated with the loss of total CD4+ T cells in general, and naïve CD4+ T cells in particular.[174] Early initiation of ART may help limit the HIV-related damage and enable normalization of CD4+ T-cell populations. A distinct subset of lymphoid tissue memory CD4+ T cells, called T follicular helper (Tfh) cells, are present in GCs and play a critical role in their formation and maintenance.[175] Tfh cells provide and receive survival and differentiation signals by interacting with cognate B cells during the GC reaction. In lymph nodes of chronically infected HIV-viremic individuals, there is an accumulation of Tfh cells in GCs, which has been correlated with hypergammaglobulinemia and frequencies of GC B cells.[176] However, it is unclear whether these Tfh cells can provide adequate help to GC B cells, given that antibody responses to HIV and other pathogens are impaired in HIV-infected individuals and that Tfh cells are major targets for active HIV replication.[177]

In GALT, massive depletion of CD4+ memory T cells, especially Th17+ CD4+ T cells, appears to occur within weeks after infection. Although possible explanations for the profound level of destruction at mucosal surfaces were first reported within the controlled setting of SIV infection,[32,33] findings of extensive depletion of CD4+ T cells in GALT during the acute phase of HIV infection had been reported before the SIV studies.[34] Later studies established that Th17 CD4+ T cells were preferentially lost in the GALT of HIV-infected individuals

and after pathogenic SIV infection.[178] The consequences of massive depletion of CD4+ T cells in GALT on the course of HIV disease remain somewhat speculative and mainly based on animal models; however, it has been proposed that the depletion compounds immune activation, a hallmark of HIV disease (see Fig. 121.5), through the disruption of mucosal integrity and leakage of inflammatory products.[39] Despite these uncertainties, and as with studies on the effect of early ART on lymph node architecture, early treatment appears to help restore the intestinal CD4+ T-cell pool.[179]

CD4+ T Cells

CD4+ T-cell dysfunction, both quantitative and qualitative, is a major hallmark of HIV disease. The opportunistic infections observed with advancing disease are primarily caused by defects in CD4+ T-cell number and function that result directly or indirectly from HIV infection, although debate remains regarding the pathogenic mechanisms of CD4+ T-cell depletion. Direct effects of HIV on CD4+ T-cell function include direct infection of these cells, with resultant cell death that leads to loss of absolute cell numbers. Indirect effects of HIV infection result in decreased CD4+ T-cell production and differentiation, altered activation and migration, dysregulation of effector functions, and other defects in T-cell helper function.[180] Furthermore, there is evidence that differences in phenotypic and functional attributes of HIV-specific and opportunistic pathogen–specific CD4+ T cells contribute to HIV disease progression and susceptibility to opportunistic infections.[181] For example, CD4+ T-cell responses against tuberculosis (TB) are lost earlier than those directed against CMV in HIV-infected individuals, in part because TB-specific CD4+ T cells tend to be more susceptible to HIV than their CMV-specific counterparts.[182] These differences are also thought to help explain at least in part the progressive yet somewhat selective susceptibility of HIV-infected individuals to opportunistic pathogens.[183]

T cells from HIV-infected individuals manifest various phenotypic and functional abnormalities that begin early after infection and worsen with disease progression.[183] Functionally, CD4+ T cells are assessed according to their capacity to expand and differentiate into subsets that produce characteristic cytokines and chemokines (Th1, Th2, and Th17), and/or characteristic function, as with Tfh cells that promote B-cell affinity maturation and T-regulatory cells (Tregs) that regulate effector responses. Defects in early HIV infection include depletion in the GALT of Th17 cells, which mainly secrete IL-17, IL-22, and IL-21 and are responsible for host responses against extracellular bacteria and fungi at mucosal sites.[39] Given the importance of Th17 responses in protection of fungal infections, loss of Th17 cells may explain the early and common manifestations of mucosal candidiasis in HIV infection.[184] Defects associated with chronic HIV disease and ongoing viral replication include decreases in response to and production of IL-2, particularly with IL-2 as part of a polyfunctional immune response; this latter defect is most clearly associated with poor outcome.[185] Although single IFN-γ–producing CD4+ T cells are not affected until late in disease, these cells have poor proliferative capacity compared with the polyfunctional populations and predominate throughout the chronic phase in most untreated individuals.

Functional attributes of a given T-cell population can generally be linked to a distinct phenotypic profile, although there is more plasticity among helper T cells than previously thought.[185,186] A number of surface markers are typically used to define various stages of CD4+ T-cell differentiation, from naïve to central, effector, and terminally differentiated memory populations. Effector memory CD4+ T cells, which are primed for immediate effector response, including secretion of IFN-γ and cytotoxicity, are relatively short-lived and have a low capacity to self-renew.[187] Central memory CD4+ T cells, which are better preserved in HIV-infected individuals who spontaneously control viral replication than in those who do not, have a high expansion capacity and are relatively long-lived as a result of autocrine production of IL-2.[188] In addition, HIV disease progression has been associated with skewing toward more differentiated and senescent CD4+ T cells,[189] including loss of CD4+ T cells expressing CD28 (i.e., the major costimulatory receptor of the B7-CD28 family, which is needed for normal activation of T cells and expressed on central but not effector memory T cells).[187] Furthermore, there is an increase in the frequency of CD4+ T cells expressing high levels of inhibitory members of the B7-CD28 family, namely PD-1, TIGIT, and CTLA-4, in HIV-infected individuals with persistent viremia.[7,108,110,190] This phenomenon has been termed T-cell exhaustion, a dysfunctional manifestation of HIV persistence that includes impairment of cytokine production and proliferative potential. Such defects can be reversed ex vivo by blocking the immunoregulatory molecules, referred to as checkpoint inhibitors. Inhibition of immunologic checkpoints has been increasingly used to treat various cancers by removing the blocks to immune control of the neoplasm. However, the reversibility of exhaustion in chronic viral infections in humans remains unknown.[8]

A variety of additional mechanisms probably also contribute to the observed functional defects in T cells. Preferential infection by HIV of CD4+ memory T cells,[191] and in particular HIV-specific CD4+ T cells,[192] and those of the Th17 and Tfh lineages,[187] helps explain loss of an effective response against the virus itself and other pathogens. In this regard, disruptions and reductions in the CD4+ T-cell receptor (TCR) repertoire is observed with progressive HIV disease, and particularly in association with aging; these disruptions are not restricted to a particular subset of CD4+ T cells but are thought to help explain the impaired response to pathogens in individuals with advancing HIV disease and age.[5,193] Likely mechanisms involved in these disruptions, which are more frequently encountered as people with HIV age and live longer, include thymic involution and shrinkage of the naïve T-cell pool, in addition to the loss of T-cell populations with self-renewal potential and the accumulation of those with exhausted and senescent properties.[194] Although early initiation of ART appears to stabilize and even improve the TCR repertoire, other promising modalities, such as cytokine-based therapies with IL-7, have shown potential; however, more studies are needed to address repertoire restoration.[179]

Mechanisms of CD4+ T-Cell Depletion

Even though CD4+ T cells were shown more than 30 years ago to be the cells most deleteriously affected in HIV-infected individuals, there remains considerable debate regarding the relative contribution of various mechanisms for the depletion of CD4+ T cells during the course of HIV infection. As with many other areas of HIV immunopathogenesis, evaluations of patients who control viremia without treatment for prolonged periods of time, those who begin effective ART during acute or chronic phases of infection, and those who interrupt treatment have provided fundamental insights into the understanding of the potential contributions of increased destruction, decreased production, and redistribution as mechanisms for CD4+ T-cell depletion in HIV-infected individuals.

Increased Destruction

Direct infection. The observations that CD4+ T cells are the principal targets of HIV infection in vivo[12,13] and that HIV infection of CD4+ T cells in vitro causes cytopathogenicity have led to a reasonable assumption that direct infection of CD4+ T cells in vivo results in their depletion (see Fig. 121.5). However, quantitative studies of the frequency of HIV-infected cells in vivo suggest that single-cell killing by direct infection with HIV may not be the predominant mechanism of CD4+ T-cell depletion. In this regard, the proportion of productively infected peripheral blood CD4+ T cells in HIV-infected individuals rarely exceeds 1 in 100, even in advanced disease.[195] Viral burden and levels of virus expression are far greater in lymphoid tissues, including GALT and lymph nodes, than in peripheral blood.[34,35,166,173] Furthermore, qualitative analyses showing a higher susceptibility of certain CD4+ T-cell subsets, and in particular those that are HIV specific,[192] has led to a reevaluation of the mechanisms of HIV pathogenesis. Tfh cells in lymph nodes,[177] and Th17 cells in mucosal tissues,[39] have been shown to be particularly more susceptible to HIV infection than other CD4+ T cells. Although these distinctions may not be enough to account solely for the progressive, albeit slow, loss of CD4+ T cells that occurs over the course of HIV disease, their critical role in immune responses and homeostasis certainly helps explain the incapacity of the immune system to contain HIV.

Death by apoptosis and pyroptosis. Apoptosis is the morphologic description of a form of programmed cell death critical to physiologic homeostasis in almost every organ system; pyroptosis is similar in form but is associated with inflammatory responses to microbes.[196,197] Apoptosis

is mediated by a series of effectors (e.g., caspases) and regulators and proceeds by one of two major pathways: the extracellular pathway, triggered by the binding of a ligand to a death receptor; and the intracellular pathway, triggered by mitochondrial events after stress signals from within the cell.[197] Mechanisms of apoptosis associated with HIV infection include cytolysis of infected cells through activation of granzymes, death of infected cells by virus-induced cytopathogenicity, death of bystander cells from proapoptotic viral proteins released from infected cells, and death resulting from HIV-induced immune activation and proinflammatory cytokines such as TNF-α.[198] Pyroptosis is induced after abortive HIV infection of $CD4^+$ T cells,[199] whereby the presence of HIV after cell-to-cell spread is sensed, inducing the formation of an inflammasome that ultimately triggers cell death via caspase-1 and release of the proinflammatory cytokine IL-1β.[198]

It has been suggested that aberrant intracellular signals transduced by HIV proteins, including gp120 and Tat, might prime $CD4^+$ T cells for apoptosis. However, whereas this mode of depletion of $CD4^+$ T cells by induction of apoptosis in HIV-infected cells has been shown in numerous in vitro models, the vast majority of cell death that occurs in vivo during the chronic phase of infection results from activation-induced and inflammation-induced bystander mechanisms associated with apoptosis and pyroptosis.[197,198] The massive depletion of $CD4^+$ T cells in GALT that occurs during acute SIV and HIV infection is likely to involve multiple mechanisms.[200] Fas-mediated apoptosis is also the primary mechanism of apoptosis resulting from HIV-induced activation of $CD4^+$ T cells, in addition to $CD8^+$ T cells, B cells, and NK cells (see Fig. 121.5).[201,202] Also, both Fas- and TNF-related apoptosis-inducing ligand (TRAIL)-mediated pathways of apoptosis have been linked to the induction of IFN-induced genes, a major mechanism of HIV-mediated immune dysregulation during the chronic phase of infection in viremic individuals that has been shown to affect all major lymphocyte populations.[196,202,203] In addition to activation-induced cell death mediated by ligation of death receptors, there is also intrinsic apoptosis induced by activation that results in decreased expression of prosurvival members of the Bcl-2 family including Bcl-2 and Bcl-X_L. Both these prosurvival proteins are downregulated in activated $CD4^+$ and $CD8^+$ T cells isolated from HIV-infected individuals.[201] Studies on the immunotherapeutic potential of cytokines such as IL-7 and IL-15 have suggested that part of their mechanism of action is to increase survival of T cells by increasing intrinsic levels of Bcl-2 and other prosurvival members of this family.[204] However, the increased survival may also promote HIV persistence.[205]

Lymphocyte turnover. Mathematical models of lymphocyte turnover derived through analysis of immediate changes in circulating $CD4^+$ T-cell counts in individuals, after the initiation of ART, have led to estimates that approximately 2×10^9 $CD4^+$ T cells are destroyed and replenished daily.[206] These seminal findings initially led to the suggestion that increased turnover, which is defined by increased proliferation and cell death, of $CD4^+$ T cells results from homeostatic compensation caused by HIV-mediated killing of $CD4^+$ T cells. However, later studies using a variety of techniques to measure lymphocyte proliferation, including Ki-67 antigen, 5-bromo-2′-deoxyuridine (BrdU), and $^2H_2$-glucose to evaluate the effects of HIV on cell turnover have revealed a more complicated view. Several investigators have demonstrated that the increase in cell turnover during ongoing viral replication not only affects $CD4^+$ T cells but also is observed in cells that are not direct targets of HIV and SIV, namely $CD8^+$ T cells, B cells, and NK cells.[207–209] These observations led to the conclusion that increased cell turnover is mainly caused by HIV-induced immune activation and increased homeostatic proliferation in response to lymphopenia. Consistent with this view is the observation that $CD4^+$ T-cell counts are maintained in natural SIV infections in which chronically high levels of virus replication occur in the absence of immune activation.[210] A similar analysis has been made for HIV-2, wherein $CD4^+$ T-cell numbers are maintained and disease progression is slow compared with HIV-1, although levels of virus replication are also lower in HIV-2 infection.[180] One lingering question with regard to the view that increased cell turnover is driven by increased immune activation is how to explain why $CD4^+$ T cells are gradually depleted while $CD8^+$ T cells are maintained until late-stage disease. Although there is no clear answer to this conundrum, several possibilities have been proposed, including a greater susceptibility of $CD4^+$ than $CD8^+$ T cells to the detrimental effects of immune activation and/or a lower regenerative capacity for $CD4^+$ compared with $CD8^+$ T cells. There are also indications that differences in homeostatic regulation between $CD4^+$ and $CD8^+$ T-cell compartments may help explain why the CD4:CD8 ratio is skewed in HIV infection and does not always recover with ART.[211–213]

Decreased Production

Decreased production of $CD4^+$ T cells could occur as a result of reduced thymic output. Proposed mechanisms for reduced thymic output in HIV infection include disruption of the thymic microenvironment, direct HIV infection and depletion of $CD4^+$ thymocytes, reduction in thymocyte proliferation, and destruction of thymocytes by apoptosis.[194] Douek and coworkers first demonstrated that recent thymic emigrants in HIV-infected individuals are significantly decreased as a consequence of HIV infection.[214] The percentage of circulating and lymph node naïve $CD4^+$ T cells carrying signal-joint T-cell receptor excision circle (TREC) gene products, a marker of recent thymic emigration, was significantly reduced in HIV-infected individuals compared with age-matched control subjects. Initiation of effective ART resulted in a significant increase in signal-joint TRECs in $CD4^+CD45RO^-CD27^+$ naïve T cells in the periphery; the latter finding suggested that the thymus remains functional in these individuals, who were past adolescence, and may contribute to immune reconstitution. Advances in approaches for measuring the various forms of TREC and new cell-surface markers for tracking thymic emigrants, including CD31, have been used to confirm decreased thymic output in HIV disease.[215] These findings are also supported by observations of increased thymic output after initiation of ART in HIV-infected individuals, especially in individuals who initiate therapy early after infection.[5] An enhanced capacity for immune reconstitution when ART is initiated early after HIV infection may help explain the more rapid recovery of $CD4^+$ T cells in these individuals.[216,217]

The disruption of normal hematopoiesis may contribute to the depletion of $CD4^+$ T cells during HIV infection. Although there is limited evidence for direct infection of $CD34^+$ progenitor cells and a role for the bone marrow in HIV persistence, abnormalities in bone marrow architecture and stromal auxiliary cells are observed in HIV-infected individuals. Such defects may thus compromise the bone marrow's ability to serve as the primary source of lymphocyte precursors, a process that is worsened by the effects of aging.[193]

Redistribution. Although the role of redistribution in the loss of $CD4^+$ T cells is unclear and difficult to ascertain, data from both HIV and SIV infections indicate that there is significant trafficking of $CD4^+$ T cells from the peripheral blood to lymphoid tissue in acute and chronic infection. The trafficking of lymphocytes is mediated, in part, through the expression of homing receptors, which guide cells from the peripheral blood into lymphoid tissues via extravasation that involves cross-linking of their ligands on endothelial venule cells. In this regard, several investigators have suggested that redistribution of $CD4^+$ T cells back from lymphoid tissue to the peripheral blood contributes significantly to the increase in $CD4^+$ T cells after the initiation of ART.[218]

$CD8^+$ T Cells

Dysregulation of $CD8^+$ T-cell numbers and function is evident throughout the course of HIV disease. After acute primary infection, $CD8^+$ T-cell counts usually rebound to supranormal levels and may remain elevated for prolonged periods. Increases in $CD8^+$ T cells during all but the late stages of disease may partly reflect the effects of chronic immune activation that affect both $CD4^+$ and $CD8^+$ T cells, albeit with distinct responses in terms of rates of proliferation, death, and regeneration. Although many explanations for the inverse ratio of CD4 to CD8 remain speculative, there is general agreement that the inversion is not simply a reflection of $CD4^+$ T cells being direct targets for HIV.[211–213] As with $CD4^+$ T cells, $CD8^+$ T cells of HIV-viremic individuals manifest an increased cell turnover and decreased levels of TREC. However, there are some indications that compared with $CD4^+$ T cells, $CD8^+$ T cells are less dependent on thymic function and an intact lymphoid tissue architecture for homeostasis and have a lower deficit to overcome after the acute phase of massive cell depletion in GALT.[174,213] In addition, changes in

lymphoid tissue architecture that occur with progressive HIV disease, including increased fibrosis and loss of FRC networks, are thought to cause an increased relative proportion of $CD8^+$ T cells in the circulation, thus accounting at least in part for the low CD4:CD8 ratio in the blood.[219]

Furthermore, whereas both activation and death rates are increased for $CD4^+$ T cells, there is likely a net increase in activation for $CD8^+$ T cells that may be explained by differences in how $CD4^+$ and $CD8^+$ T cells become activated in HIV-infected individuals.[220] In this regard, the degree of T-cell activation, as measured by the expression of the activation marker CD38 on $CD8^+$ but not on $CD4^+$ T cells, remains one of the strongest predictors of time to HIV disease progression. Even in patients receiving ART, the level of activation on $CD8^+$ T cells is inversely correlated to gains in $CD4^+$ T-cell counts.[221]

During HIV disease progression, $CD8^+$ T cells also undergo alterations that reflect a skewing within the $CD8^+$ T-cell compartment. Increased frequencies of $CD8^+$ T cells with reduced expression of CD28 and increased expression of CD57 are observed in HIV disease, possibly reflecting expansion of immunosenescent cells that normally occurs in the elderly.[193] Data from numerous studies have suggested that impaired HIV-specific $CD8^+$ T-cell activity in HIV-viremic individuals is at least in part associated with a skewed effector memory $CD8^+$ T-cell population that displays low polyfunctionality, proliferative capacity, and effector function.[220,222] Furthermore, HIV-induced T-cell exhaustion, which includes upregulation of the inhibitory receptors, including PD-1, LAG-3, TIGIT, and Tim-3, among others, has been described both for HIV-specific $CD8^+$ T cells and in the general $CD8^+$ T-cell population.[223] Several transcriptional factors that control T-cell effector function and differentiation, including BATF,[224] T-bet, and Eomes, are also modulated in exhausted $CD8^+$ T cells and may control the extent of dysregulation,[223] even after successful reduction of viremia by ART.[225] Other $CD8^+$ T-cell functions may be impaired during HIV disease progression, such as loss of noncytolytic, non–MHC-restricted $CD8^+$ T-cell–derived HIV suppression.[226]

B Cells

Dysregulation of B cells and an intrinsic loss of function are likely responsible, in part, for the increase in certain bacterial infections seen in advanced HIV disease in adults, and for the morbidity and mortality associated with bacterial infections in HIV-infected children who cannot mount an adequate humoral response to common bacterial pathogens.[183] The number of circulating B cells is decreased in HIV-infected individuals; this likely reflects a combination of effects associated with HIV viremia, including the expansion of apoptosis-prone subsets and redistribution of cells into lymphoid tissues.[227] One of the hallmarks of chronic HIV viremia is hypergammaglobulinemia,[228] a consequence of generalized immune activation and the many inflammatory cytokines that favor terminal differentiation of B cells.[229] The increase in immunoglobulins occurs for all classes and subclasses of antibody, although IgG1 is the most affected, and the increase is largely polyclonal.[230] The association between hypergammaglobulinemia and ongoing HIV replication was first demonstrated in longitudinal studies in which initiation of ART led to the normalization of serum levels of both immunoglobulins and the corresponding antibody-secreting cells in the blood (plasmablasts or plasma cells).[231] In lymphoid tissues, as mentioned earlier (see "Immune Dysfunction Caused by HIV Infection"), more recent studies have shown strong correlations among HIV-induced hyperplasia, hypergammaglobulinemia, and frequencies of Tfh and GC B cells, the latter accounting for up to 50% of cells in biopsy specimens.[232] Chronic HIV plasma viremia has also been associated with the appearance of various subpopulations of B cells in the peripheral blood that express reduced levels of CD21, some of which are responsible for the increased secretion of immunoglobulins and the decreased response to B-cell stimuli, an abnormality that was first demonstrated in 1983. [228] Several of these phenotypic and functional aberrations can be reversed by reduction of HIV plasma viremia by ART, suggesting that control of viremia may contribute to the restoration of the humoral arm of the immune system, especially when ART is initiated during primary infection.[230]

Several defects in B cells from HIV-infected individuals have been attributed to the expansion of subsets that are rare in the peripheral blood of healthy individuals.[230] In addition to the plasmablasts, described earlier, that are associated with hypergammaglobulinemia, there is also an expansion of immature transitional B cells and tissue-like memory B cells bearing features of exhaustion. The expansion of immature transitional B cells in the peripheral blood of HIV-infected individuals is associated with increased serum levels of IL-7 and $CD4^+$ T-cell lymphopenia, with the strongest effects observed in advanced HIV disease and in individuals treated with exogenous IL-7.[233] In contrast, the expansion of tissue-like memory B cells, defined by reduced expression of CD21 and CD27, the marker of classic B-cell memory, in the peripheral blood of HIV-infected individuals is associated with chronic HIV viremia.[234] Tissue-like memory B cells show signs of HIV-associated exhaustion, manifesting with the following: increased expression of multiple inhibitory receptors and altered expression of homing receptors, similar to exhausted virus-specific T cells; decreased replication history and somatic hypermutation required for an effective antibody response; and decreased proliferative responses. Furthermore, the HIV-specific B-cell response is enriched within this exhausted B-cell compartment, indicating that B-cell exhaustion may contribute to the poor antibody response against HIV observed in HIV disease. Finally, correlates of B-cell dysfunction observed in HIV-infected viremic individuals also included an increased frequency of B-cell neoplasms, which is likely a reflection of transformation arising from increased HIV-induced B-cell turnover in the face of impaired T-cell immunoregulation.[235]

Classic memory B cells are defined by the expression of CD27 on B cells that are resting and long-lived. Of note, the decrease in the frequency of resting memory B cells in HIV-infected individuals occurs early after infection and is not reversed by ART, although there are some indications that early initiation of ART helps normalize the B-cell compartment.[236] These observations likely explain, at least in part, the reduced memory B-cell responses that have been reported against various T-cell–dependent and T-cell–independent immunogens and against HIV itself. In this regard, several recently developed methods for probing, expanding, and isolating HIV-specific B cells have become available and are providing new insights into the antibody response against HIV. As discussed earlier (see "Humoral Immune Responses") potent and bNAbs arise after several years of infection in a relatively small percentage of untreated individuals for reasons that remain unclear but likely involve sustained antigenic stimulation from persistent HIV replication. It is hoped that a better understanding of the HIV-specific antibody and B-cell responses in HIV-infected individuals will lead to better strategies for an effective antibody-based vaccine.

Natural Killer Cells

NK cells provide innate immune defense against virus-infected cells, certain tumor cells, and allogeneic cells. Activation of NK cells is governed by a balance of signals received from the triggering of cytotoxic activating and inhibitory receptors, the latter of which recognize MHC class I molecules and prevent cytolysis.[237] NK cells can be divided into three subsets based on the expression of CD56; most NK cells in the peripheral blood of healthy individuals are composed of $CD56^{dim}$ NK cells, compared with a minority that are $CD56^{bright}$. This latter subset tends to have a more regulatory function, secreting high levels of cytokines such as IFN-γ and TNF-α, whereas the former subset has a more cytotoxic function. The third subset, $CD56^{neg}$ NK cells, are almost absent in healthy individuals, yet become overexpressed in HIV-infected individuals, especially those who experience chronic viremia.[238] A reduction in the ability of NK cells to lyse target cells is observed throughout the course of HIV disease, and this defect increases with disease progression. Loss of function is associated with the increased frequency of $CD56^{neg}$ NK cells. These functional defects are associated with increased frequencies of $CD56^{neg}$ NK cells and are accompanied by alterations in expression of receptors that regulate NK-cell activity, namely the upregulation of certain inhibitory receptors and the downregulation of several activating receptors.[239]

There is little evidence that HIV productively infects NK cells in vivo; however, there is evidence that many HIV proteins, including envelope and accessory proteins, perturb NK cells through evasion properties that reduce the ability of NK cells to kill infected target cells.[239] NK cells are also the primary mediators of the ADCC response, an antibody constant (Fc) region-mediated effector function that may be responsible for some of the nonneutralizing antibody-mediated protective effects

observed in the RV144 HIV vaccine trial.[240] In HIV-infected individuals, there are indications that ADCC is involved in LTNP/EC control, although there is also evidence of HIV-mediated evasion.[239] Several studies, reviewed by Carrington and coworkers,[241] have also found an association between genotype of certain NK cell–related HLA molecules and control of HIV viremia and disease progression, suggesting a role for NK cells in the control of HIV disease. In this regard, mutations in HIV accessory genes of chronically infected individuals likely have been shown to arise in response to pressure from killer-cell immunoglobulin-like receptors (KIR), providing additional evidence that HIV evolves to evade NK cell–mediated responses against the virus.[242]

Monocytes and Macrophages

Cells of the monocyte-macrophage lineage play key roles in the immunopathogenesis of HIV disease. These cells are likely involved in facilitating the transmission and establishment of HIV infection, as described earlier (see "Dissemination of HIV Infection"), although their role in maintaining the viral reservoir in ART-treated individuals is less certain.[243] Dysfunction of these cells also occurs in HIV-infected individuals, especially with regard to impaired host defense against intracellular pathogens and their contribution to the immune-activating effects associated with ongoing HIV replication (see "Role of Immune Activation in the Pathogenesis of HIV Infection").[244]

Monocytic cells express CD4 and numerous HIV coreceptors on their surface, including CCR5 and CXCR4, and can theoretically serve as targets of HIV infection for both R5- and X4-tropic strains. However, unlike infection of $CD4^+$ T cells, HIV coreceptor usage is more restricted to CCR5 in the monocyte-macrophage lineage.[243] Differences in receptor-binding affinities and mechanisms of cell-cell spread and host factors likely explain the more restricted replication of HIV in this lineage despite the expression of appropriate HIV receptors.[243,244] Whereas circulating monocytes are rarely found to be infected unless differentiated into macrophages ex vivo, the presence of HIV in vivo can be detected in tissue macrophages, including resident microglial cells in the brain, and pulmonary alveolar macrophages.[243,244] Lymphoid tissue macrophages were identified as prolific producers of HIV in the setting of advanced HIV disease two decades ago, [245] and more recently as a source of potent pro-inflammatory factors.[246]

In pre-ART years, HIV-induced CNS disease was common and involved infection and activation of microglia and perivascular macrophages in the CNS. The process resulted in neurologic impairment, including encephalopathy, neuropathy, astrocytosis, and cerebral vasculitis. The inflammatory processes linked to HIV-associated dementia have been associated with several factors, including chemokines and cytokines that are produced by activated macrophages.[243,244] Increased levels of lipopolysaccharide (LPS), a highly inflammatory bacterial product that preferentially binds to macrophages through interactions with CD14 and Toll-like receptors, are observed in the serum of HIV-infected individuals. The binding of LPS to monocytes may promote disruption of the blood-brain barrier, further enhancing the migration of activated monocytes into the CNS and thus contributing to HIV-associated dementia.[247] Even in individuals whose HIV viremia is controlled by ART, there is evidence for residual neurocognitive disorders that are likely to be associated with residual macrophage activation.[244]

Dendritic Cells

DCs are among the first cells to encounter HIV after mucosal exposure and are probably responsible for transporting the virus to lymphoid organs, thus facilitating infection of $CD4^+$ T cells and viral dissemination (see "Dissemination of HIV Infection"). Whereas DCs express several different chemokine receptors that can be used as HIV coreceptors for entry,[248] there is limited evidence that DCs become productively infected in vivo. Instead, DCs are more likely to be involved in capturing HIV, forming conjugates with $CD4^+$ T cells by way of long flexible filopodia, and contributing to the formation of infectious viral synapses.[249] This sequence of events is thought to be highly efficient at promoting the transmission of HIV virions from DCs to $CD4^+$ T cells. Several C-type lectin receptors, including DC-SIGN and langerin, which are differentially expressed on subsets of DCs, play an important role in the capture of HIV virions by DCs.[27]

Early studies provided conflicting data on the dysfunction of DCs during HIV infection. Interpretation of these studies was complicated by the use of different DC purification techniques, existence of multiple poorly defined DC subsets, and differential culture conditions used by investigators. The delineation of DCs into subsets that reflect origin, location, and function—namely mDCs and plasmacytoid dendritic cells (pDCs)—has helped clarify their dysregulation in HIV infection. The mDCs are further classified by tissue location and circulation, and although their importance in antigen presentation is well established, their sensing properties and how HIV evades them have been more recently described.[196] Viral infections are sensed by cells of the innate immune system through PRRs such as TLRs. Type I IFNs, including IFN-α, produced primarily by pDCs, are one of the first sensing mechanisms induced by viral infections, including HIV. In this regard, recent findings suggest that type I IFNs can block SIV infection, but that in chronic infection, they have a detrimental effect (see "Cytokines and HIV Disease: Dysregulation of Cytokine Production").[250] HIV infection can also lead to a decrease in the number of circulating pDCs through mechanisms that are not well delineated but may involve direct infection with HIV or direct interactions with the HIV envelope, redistribution to lymphoid tissues, and apoptosis.[40,251] Several mechanisms of immune dysfunction in HIV disease have also been attributed to pDCs, including secretion of factors that induce Treg cells, which then suppress the antiviral response, and induction of apoptosis-mediating ligands on pDCs.[252] Finally, there are also indications that the increase in Tregs and reciprocal depletion of Th17 cells in the gut of HIV-infected individuals is in part due to inflammatory factors secreted by chronically activated mDCs and pDCs.[253]

ROLE OF IMMUNE ACTIVATION IN THE PATHOGENESIS OF HIV INFECTION

The end result of HIV infection is profound immunodeficiency; however, paradoxically, HIV infection is associated with hyperactivation of the immune system throughout most of the course of disease.[228] HIV subverts the immune system by inducing immune activation, using this milieu toward its own replicative advantage, and causing widespread damage to the immune system. Whereas it is well accepted that the replicative cycle of HIV infection is most efficiently achieved in activated cells,[162,163] most of the damage caused by HIV is not the result of direct infection of $CD4^+$ T cells but, rather, the widespread bystander activation, dysregulation, and cell death of $CD4^+$ T cells and other cells of the immune system.[213] In this regard, numerous studies have demonstrated that the level of $CD8^+$ T-cell activation, as measured through expression of CD38 and HLA-DR, is a better correlate of HIV disease progression than HIV viremia.[4] More recently, alterations in expression and secretion of factors or biomarkers, especially those associated with the myeloid lineage such as soluble CD14 and D-dimer, among others,[244] have also been proposed as indicators of immune activation induced by both active viral replication and by the residual effects of viremia in individuals receiving effective ART.[179] The persistence of immune activation in individuals receiving ART is thought to explain, at least in part, their increased risk of comorbidities, such as cardiovascular and liver diseases, type 2 diabetes, osteoporosis, and malignancies.[254,255]

With regard to T cells, and to a lesser extent B cells and NK cells, the evidence for HIV-induced immune activation is severalfold. There is a high frequency of cells expressing markers of activation and cell cycling that is accompanied by homeostatic dysregulation and cell death during ongoing HIV replication.[187,213] Many of these manifestations are attenuated or reversed by effective ART. One of the most widely accepted indirect pieces of evidence that immune activation plays a major role in HIV immunopathogenesis is the nonpathogenic outcome of SIV infection in its natural host. In the case of naturally SIV-infected sooty mangabeys, high levels of SIV viremia occur in the absence of generalized immune activation and progressive $CD4^+$ T-cell depletion.[210] In further contrast to HIV infection, which almost invariably leads to AIDS if left untreated, SIV infection in natural hosts almost never leads to disease progression. Over the years, the differences between pathogenic HIV infection in humans and nonpathogenic SIV infection in nonhuman primates, such as sooty mangabeys, have been extensively investigated.

The most consistent feature of these comparisons remains the lack of generalized immune activation in natural SIV infection, with a muting of the innate immune response, including systemic inflammation and a strong type I IFN response after the acute phase, being the most important difference observed between naturally controlled SIV and uncontrolled HIV infections.[169,210]

The underlying causes of HIV-induced immune activation, increased cell turnover, and eventual depletion of $CD4^+$ T cells remain a matter of debate. There is a growing body of evidence, mainly from NHP models,[169] that the early innate immune proinflammatory response that occurs at mucosal sites leads to recruitment and activation of adaptive immune cells, including HIV target cells, which then allow the virus to disseminate to secondary lymphoid tissues for rapid amplification before an effective adaptive immune response. A number of studies have shown that when this initial phase of viral replication is blunted,[30,74] immunologic and/or virologic control ensues and disease progression can be averted. In the absence of early intervention, the early massive depletion of $CD4^+$ T cells in mucosal and other lymphoid tissues leads to a chronic state of increased proliferation, activation, and cell death. The eventual systemic depletion of $CD4^+$ T cells that characterizes AIDS is also explained by a pronounced loss of naïve and central memory $CD4^+$ T cells, both of which are strictly regulated and may be more prone to regenerative failure than other lymphocytes.[187]

There are important consequences of generalized immune activation that likely contribute to the inefficiency of the HIV-specific immune response and to the eventual impairment of the regenerative potential of the entire immune system. The increase in immune activation and inflammation that arises from persistent HIV replication is accompanied by an expansion of exhausted T cells and B cells and by increased levels of immunoregulatory factors such as IL-10.[256] Exhaustion is defined by decreased proliferative and effector function, alterations in homing receptor expression patterns consistent with migration to sites of inflammation, and increased expression of inhibitory receptors,[190] such as PD-1, 2B4, LAG-3, CD160, and Tim-3 on $CD8^+$ T cells, and PD-1 and CTLA-4 on $CD4^+$ T cells.[108,110] A reversal of HIV-specific exhausted T cells has been demonstrated with PD-1 and CTLA-4 antagonists, many of which are used as checkpoint blockers in treatment of certain cancers.[8]

The effect of generalized immune activation on the immune system also includes gradual destruction of lymph node architecture and changes in the composition of lymphoid tissues that impede immune function. The changes in lymph node architecture that occur with HIV infection and persistent viremia include progressive damage to follicular structures, from hyperplasia to lysis, atrophy, and involution; each stage has increasing deleterious effects, both on the generation of effective immune responses and on T-cell homeostasis.[168] Collagen deposition and fibrosis have also been shown to contribute to defects in normal T-cell homeostasis, and similar effects have been proposed for B cells.[168,218]

CYTOKINES AND HIV DISEASE: DYSREGULATION OF CYTOKINE PRODUCTION

One important facet of chronic immune activation induced by HIV infection is the increased production of proinflammatory cytokines and chemokines. Early after infection, high levels of TNF-α, IP-10, IFN-γ, and IL-6 are found in serum. Several cytokines have been shown to upregulate HIV expression in infected cells.[257] High in vivo levels of cytokines, which are unique to acute HIV at least in potency and breadth,[258] are commonly referred to as a cytokine storm.[254] Levels of other proinflammatory and immunoregulatory cytokines increase over the course of HIV disease, including IL-1β, IL-10, and transforming growth factor-β (TGF-β), the last of which is particularly evident in lymphoid tissues and is thought to contribute to the deposition of collagen and ensuing damage to the stromal FRC network on which lymphocytes migrate.[168,218] The production of proinflammatory cytokines and chemokines is thought to originate, at least in part, from innate immune cells such as inflammatory monocytes and neutrophils as a result of increased expression of PRRs such as Toll-like receptors.[5] In this regard, the triggering of Toll-like receptors, as part of the innate immune responses against HIV or because of mucosal leaching of microbial products such as LPS, is thought to lead to secretion of proinflammatory cytokines by macrophages and DCs.[254]

The role of pDCs in shaping the cytokine milieu in HIV-infected individuals is considered potentially very important, although difficult to ascertain because of the low frequency of pDCs in the circulation. Although numbers of pDCs may be decreased in the peripheral blood of HIV-infected individuals, their induction and secretion of copious amounts of IFN-α is most likely to occur as part of the host innate immune response to HIV. In this regard, the induction of type I IFNs as part of the early response to HIV can be highly beneficial, as shown in a recent NHP model.[250] However, it is the chronic effect of these pathways that is thought to be detrimental, and numerous studies have shown that ongoing HIV replication is associated with the induction of numerous IFN-induced genes in all major lymphocyte populations.[196] Among these IFN-induced genes are ones coding for death receptors and ligands that have been associated with increased susceptibility to apoptosis of lymphocytes isolated from HIV-viremic individuals. These include Fas and FasL and the ligand TRAIL and accompanying death receptors DR4 and DR5. These latter receptors have also been associated with T-cell exhaustion,[259] as has TNF most recently, which is one of the cytokines strongly induced by HIV and now being proposed as a critical link between inflammation and T-cell dysregulation in chronic viral infections.[260]

Another disruption in cytokine networks observed in HIV disease is a progressive loss of the ability to produce immunoregulatory cytokines, such as IL-2, that reflects changes in $CD4^+$ and $CD8^+$ T-cell effector function (see "Cellular Immune Responses"). Whereas functional impairment was once attributed to loss of Th1 cytokines (secretion of IL-2 and IFN-γ) and possible dominance of Th2-like responses (i.e., secretion of IL-4, IL-5, and IL-10) during progression of HIV disease, many of these paradigms have been complicated by plasticity between subsets and the identification of other $CD4^+$ T-cell subsets. These include Th17 cells, which reside primarily in skin and mucosal tissues, produce IL-17 and IL-22, and play important roles in antibacterial and antifungal immunity.[261] In mucosal tissue dysregulation associated with HIV-induced immune activation, the secretion of indoleamine 2,3-dioxygenase, an immunomodulatory enzyme, by DCs leads to suppression of Th17 cells while inducing the development of Tregs.[262] Although the role of Tregs in HIV disease remains controversial, there does appear to be a consensus that the balance of Tregs and Th17 is disrupted in HIV-infected individuals as a result of persistent immune activation and that these changes are, in part, driven by increases in Treg suppressive cytokines, such as TGF-β and IL-10.[261]

Role of Cytokines as Therapeutic Agents in HIV Infection

The use of cytokine-based therapies aimed at immune reconstitution and immune modulation in HIV disease has expanded over the past decade, particularly with the knowledge that ART alone is unable to restore $CD4^+$ T-cell numbers and function completely. However, each approach has faced challenges, and the prospects of cytokine-based therapeutic interventions remain unclear.

Most cytokine-based therapies have been aimed at T-cell deficiencies in the common gamma chain (γc) family of cytokines. Among this family, IL-2 initially received the most attention because it was first considered as a therapeutic agent in 1983.[263] Several years of trials and detailed analyses have determined that intermittent administration of IL-2, although associated with a sustained expansion of naïve and memory regulatory $CD4^+$ T cells and diminution in levels of activation and apoptosis, does not appear to provide sustained clinical benefits.[264] Two additional members of the γc family of cytokines—IL-7 and IL-15—are being considered as therapeutic agents to improve T-cell function in HIV-infected individuals.

IL-7 is primarily produced by stromal and epithelial cells in the thymus, lymph nodes, and intestinal epithelium and binds to IL-7Rα (CD127) complexed with common γc.[211] IL-7 is considered a major regulator of thymocyte development and of the survival and homeostasis of naïve and memory T cells. In HIV-infected individuals, high levels of IL-7 are detected in serum, especially in advanced $CD4^+$ T-cell lymphopenia.[265] These increased levels of IL-7 are thought to result from lower clearance due to decreased presence of cells expressing

CD127.[211] Nonetheless, several clinical studies in humans have also demonstrated that administration of IL-7 leads to improvement of T-cell homeostasis, repertoire, and function without inducing increased Tregs as had been shown to occur with IL-2 administration.[8] There are also indications that IL-7 is less toxic than IL-2 and may also improve reconstitution of lymphoid tissues. However, concerns remain, including that HIV may cause $CD4^+$ T cells to be refractory to IL-7 signals and that IL-7 may lead to increased HIV replication.[266] IL-15, also a member of the γc family, is produced by antigen-presenting cells, including monocyte-macrophages and DCs, and binds to the IL-2Rβ complexed with common γc. In both HIV-infected and HIV-negative individuals, IL-15 induces many of the same responses as IL-2. However, in contrast with IL-2 and IL-7, IL-15 may be most effective at driving the expansion and tissue emigration of $CD4^+$ and $CD8^+$ effector memory T cells. However, these findings were described in an SIV model, and it remains to be seen how IL-15 will modulate T cells in HIV-infected individuals. There are also concerns that IL-15 may be difficult to implement as part of a clinical regimen of immune reconstitution, given that it can accelerate disease progression when administered during the acute phase of infection, and also accelerate lymphoid tissue damage and fibrosis.[211] Finally, IL-21, also a member of the γc family, is produced primarily by Tfh and Th17 cells and has been proposed to enhance HIV-specific cell-mediated and humoral responses based on findings in animal models and LTNP/ECs.[204]

CONCLUSIONS

HIV is the quintessential opportunist, as illustrated by its ability to subvert activation of the immune system to its own replicative advantage. The virus can disarm multiple components of the host immune response by direct and indirect mechanisms. Further understanding of the interactions between the virus and host that lead to dysfunction and depletion of multiple components of the immune system should aid in the development of preventive and therapeutic strategies. Whereas effective ART currently remains the best approach for delaying HIV disease progression, several therapeutic interventions are being considered, including those intended to boost the immune system, such as vaccines, or to reverse HIV-induced cellular exhaustion, such as checkpoint inhibitors. Other immune-based therapies, including bNAb-based interventions with and without ART, are being considered. There are also indications that therapeutics aimed at dampening HIV-induced immune activation may be beneficial, especially early during the course of infection. Finally, agents aimed at purging HIV from latent and other viral reservoirs are also being evaluated, although such modalities aimed at eradicating HIV from infected individuals have been unsuccessful thus far. However, regardless of the approach, there is growing agreement that virologic remission involving some degree of induced immunologic control may be a more attainable goal than achieving complete eradication.

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The complete reference list is available online at Expert Consult.

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122 General Clinical Manifestations of Human Immunodeficiency Virus Infection (Including Acute Retroviral Syndrome and Oral, Cutaneous, Renal, Ocular, Metabolic, and Cardiac Diseases)

Timothy R. Sterling and Richard E. Chaisson

SHORT VIEW SUMMARY

ACUTE RETROVIRAL SYNDROME
- This syndrome is the initial manifestation of human immunodeficiency virus (HIV) infection.
- One-half to two-thirds of patients present with an acute mononucleosis-like illness (see Table 122.1), often with a truncal exanthem.
- Symptoms generally resolve within 10 to 15 days.

PERSISTENT GENERALIZED LYMPHADENOPATHY
- This disorder occurs in 50% to 70% of people with HIV infection and usually affects the cervical, submandibular, occipital, and axillary regions.
- In patients treated with antiretroviral therapy (ART), previously involuted nodes may enlarge.

METABOLIC AND ENDOCRINE ABNORMALITIES
- Hypogonadism, adrenal insufficiency, constitutional wasting, lipid abnormalities, insulin resistance, lipodystrophy, and osteoporosis have been reported.

ORAL DISEASE
- Oral candidiasis, oral hairy leukoplakia, gingivitis, and periodontitis may occur.

CUTANEOUS DISEASE
- Dermatologic consequences include cutaneous infections (herpes simplex virus, varicella-zoster virus, molluscum contagiosum, scabies, bacillary angiomatosis, and Kaposi sarcoma).

RENAL DISEASE
- Multiple causes of renal disease may affect patients with HIV infection as well as a specific HIV-related nephropathy, which includes proteinuria, mildly elevated serum creatinine concentration reflecting glomerular injury, mesangial proliferation, and tubular degeneration.

CARDIAC DISEASE
- Accelerated atherosclerosis with myocardial infarction is seen in patients with HIV infection who have been treated with ART and appears to be associated with elevations in proinflammatory cytokines and prothrombotic markers.
- Myocarditis and pericarditis are also found.

IMMUNE RECONSTITUTION SYNDROMES
- Immune reconstitution syndromes are seen after ART and can involve *Mycobacterium tuberculosis, Mycobacterium avium* complex, *Cryptococcus neoformans,* cytomegalovirus, hepatitis B, hepatitis C, lymphoma, and other opportunistic diseases.

Human immunodeficiency virus (HIV) infection results in a wide range of clinical consequences from asymptomatic infection despite active viral replication to severe immunodeficiency with life-threatening opportunistic disease. In people infected with HIV, ongoing viral replication produces a steady decline in and eventual ablation of cell-mediated immunity as well as marked immune activation and inflammation, all of which give rise to diverse manifestations of opportunistic disease. Acquired immunodeficiency syndrome (AIDS), in which the infected host can no longer control opportunistic organisms or malignancies that rarely cause illness in immunocompetent individuals, is the most advanced stage of this illness. Major advances in treatment with antiretroviral drugs have dramatically altered the clinical course of HIV infection and converted what was once a highly fatal disease into a manageable chronic condition. For individuals with access to antiretroviral therapy (ART), median life expectancy following infection has increased from 10 to 12 years to 25 to 50 years, depending on age at infection.[1] Despite these impressive improvements in survival, treated chronic HIV infection is associated with immune activation and chronic inflammation that can result in damage to a number of organ systems. Current research is focused on finding a cure (or functional cure) for HIV infection and treating its inflammatory complications to prevent cardiovascular, renal, endocrinologic, and neurologic consequences.

HISTORY

Disease caused by HIV was first described in late 1980 and early 1981 with outbreaks of Kaposi sarcoma and opportunistic infections due to *Pneumocystis jirovecii,* herpes simplex, and cytomegalovirus (CMV) in young men who have sex with men (MSM).[2–7] In 1982 the US Centers for Disease Control and Prevention (CDC) developed a case definition based on clinical, immunologic, and epidemiologic features for what was termed the *acquired immunodeficiency syndrome.*[8] Soon after the initial case reports of AIDS, additional cases were reported in heterosexual injection drug users, immigrants from Haiti,[8–12] people with hemophilia, recipients of blood transfusions, and Africans.[13,14] As the groups of people at risk for AIDS expanded, clinicians noted an increasing spectrum of clinical manifestations of AIDS-associated immunodeficiency such as generalized lymphadenopathy, idiopathic thrombocytopenia, oral candidiasis, and a constitutional wasting syndrome.[15–18] The now archaic term *AIDS-related complex* was coined to describe the signs and symptoms of immunodeficiency recognized with increasing frequency in people at risk for AIDS.[19,20] After HIV was first described in 1983–84,[21–23] serologic tests to identify people infected with HIV were developed that allowed large serologic surveys of at-risk populations to estimate the number of individuals infected with the virus and to delineate the spectrum of HIV-associated diseases.

Over almost 40 years, case definitions and classifications of HIV disease have evolved to reflect a growing understanding of pathogenesis, natural history, and therapeutics. Many of the common manifestations from the earliest days of the epidemic are rarely seen in high-income countries and are becoming less frequent in low-income and middle-income countries.

NATURAL HISTORY OF HIV INFECTION

The clinical spectrum of untreated HIV infection includes primary infection (acute retroviral syndrome) (Table 122.1), asymptomatic infection, early symptomatic infection, and advanced immunodeficiency with opportunistic complications and, ultimately, death. Fig. 122.1 shows a schematic diagram of the key immunologic, viral, and clinical features of HIV infection in untreated individuals. Untreated HIV infection is characterized by active viral replication that results in progressive immunodeficiency and serious clinical consequences. Although many patients may be unaware of their infection, HIV is usually not virologically latent, and chronic viremia, inflammation, and progressive immunodeficiency result in significant impairment and death. Viral load or viremia is monitored by measurement of HIV-1 RNA in plasma, and immunologic status is reflected by the absolute number of $CD4^+$ lymphocytes or the proportion of lymphocytes that express $CD4^+$. Primary HIV infection is characterized by a high concentration of HIV-1 RNA in plasma and suppression of the $CD4^+$ cell count. Plasma viremia declines precipitously with antibody seroconversion and the development of an anti-HIV immune response, usually reaching a steady-state level or setpoint within 6 to 12 months.[24,25] In most untreated asymptomatic patients, the $CD4^+$ count declines gradually over several years. The slope of decline is a function of the plasma viral load. Plasma viremia increases, accompanied by a more rapid decline in $CD4^+$ count, before the onset of symptomatic disease. As the viral load increases and the $CD4^+$ count falls, the risk for opportunistic infections, malignancies, wasting, neurologic complications, and death increases substantially.

There is considerable variation in the progression of HIV disease, with some individuals progressing from infection to AIDS in less than 5 years[26] and so-called long-term nonprogressors remaining asymptomatic without treatment or evidence of immunologic decline for many years.[26,27] Long-term nonprogressors appear to fall into at least two categories. Most have detectable viremia but maintain $CD4^+$ counts that provide adequate protection from the development of opportunistic disease. These individuals generally have gradual loss of $CD4^+$ lymphocytes, however, and eventually progress to advanced immunodeficiency. A much smaller group of individuals (<1%) are so-called elite controllers, who have undetectable HIV viral loads and maintain normal $CD4^+$ counts.[28] This group is of considerable interest because of their ability to remain aviremic. However, studies have shown that such individuals have increased immune activation, have ongoing viral replication in $CD4^+$ cells, and experience higher rates of hospitalization for cardiovascular conditions and other non–AIDS-related illnesses than individuals treated with ART.[29–31] Nonetheless, understanding the mechanisms whereby these patients control HIV is of potential value for the development of HIV vaccines and functional cures for HIV.

Before the availability of effective ART, the rate of progression from initial HIV infection to AIDS among MSM in San Francisco was 9.8 years.[32] Other studies estimated the period from infection to AIDS to be 7 years for transfusion recipients and 10 years for hemophiliacs and injection drug users.[33] In the absence of treatment, survival is short after diagnosis of clinically defined AIDS. Studies of survival of the first patients with AIDS in San Francisco and New York found a median survival of 9 to 12 months, with most patients dead within 2 years.[34,35] Patients with a diagnosis of an opportunistic infection had the most rapid mortality, whereas survival was significantly longer in patients with an initial diagnosis of Kaposi sarcoma. Subsequent studies revealed that survival after diagnosis of AIDS was directly related to the $CD4^+$ count at diagnosis. In most studies before the availability of effective ART, median survival after diagnosis of AIDS was estimated to be between 12 and 18 months.[36] The mean survival time after a $CD4^+$ count of 200 cells/mm^3 was 38 to 40 months.[37,38]

A number of laboratory tests have been correlated with progressive immunodeficiency, the development of AIDS, and mortality. Taken together, however, the $CD4^+$ lymphocyte count and plasma viral load are the best prognostic markers for subsequent disease course in an HIV-infected individual. The $CD4^+$ count, a specific test for cellular immunocompetence, is a sensitive predictor of the development of symptomatic HIV infection and AIDS in the near term because it reflects current immunologic capacity.[39–43] Conversely, the plasma viral load (HIV-1 RNA) is an extremely useful predictor of disease course over a more extended period and is strongly associated with the rate of subsequent $CD4^+$ count decline.[44–52] A more rapid decline in $CD4^+$ count, faster clinical progression, and decreased survival all are associated with a higher baseline viral load. In a study of HIV-infected MSM (homosexual or bisexual men) enrolled in the Multicenter AIDS Cohort Study, the risk for progression to AIDS and death was highly correlated with plasma viral load at study entry, independent of $CD4^+$ count.[47,48] Baseline plasma viral load was a stronger predictor of progression and mortality than $CD4^+$ count. In addition, the average annual decline in the $CD4^+$ count of HIV-infected men varied according to their initial viral load, decreasing by 36 $CD4^+$ cells per year in men with baseline

TABLE 122.1 Symptoms and Signs of Acute Retroviral Syndrome in 209 Patients

SYMPTOM OR SIGN	NO. WITH FINDING	FREQUENCY (%)
Fever	200	96
Adenopathy	154	74
Pharyngitis	146	70
Rash	146	70
Myalgia or arthralgia	112	54
Thrombocytopenia	94	45
Leukopenia	80	38
Diarrhea	67	32
Headache	66	32
Nausea, vomiting	56	27
Elevated aminotransferase levels[a]	38	21
Hepatosplenomegaly	30	14
Thrush	24	12
Neuropathy	13	6
Encephalopathy	12	6

[a]Based on 178 subjects.
Modified from Niu MT, Stein DS, Schnittman SM. Primary human immunodeficiency virus type 1 infection: review of pathogenesis and early treatment intervention in human and animal retrovirus infections. J Infect Dis. *1993;168:1490–1501.*

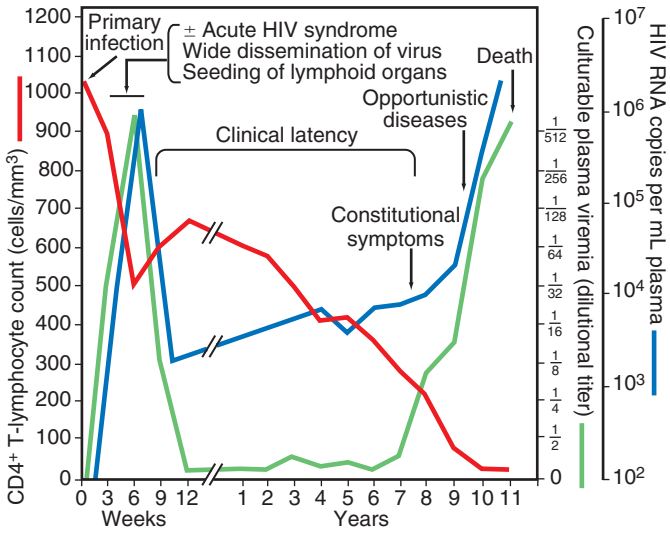

FIG. 122.1 Natural history of HIV infection in the absence of therapy in a hypothetical patient. *(From Fauci AS, Pantaleo G, Stanley S, et al. Immunopathogenic mechanisms of HIV infection.* Ann Intern Med. *1996;124:654–663.)*

TABLE 122.2 Probability of Developing AIDS in 1604 Men in the Multicenter AIDS Cohort Study

BASELINE VIRAL LOAD[C]	BASELINE CD4⁺ COUNT[D]	NO. STUDIED	NO. WITH AIDS	% AIDS AT 3 YEARS	% AIDS AT 6 YEARS	% AIDS AT 9 YEARS
<500	>750	66	3	0	1.7	3.6
	<750	56	13	3.7	9.6	22.3
501–3000	Any	257	90	2.0	16.6	35.4
3001–10,000	>750	93	39	3.2	14.2	59.7
	<750	300	179	8.1	37.7	62.4
10,001–30,000	>750	64	42	9.5	36.7	62.4
	351–750	259	194	16.1	54.9	76.3
	≤350	73	63	40.1	72.9	86.2
>30,000	>500	141	105	32.6	66.8	76.3
	351–500	121	111	47.9	77.7	94.4
	201–350	104	92	64.4	89.3	92.9
	<200	70	67	85.5	97.9	100

[a]1987 Centers for Disease Control and Prevention case definition.
[b]Based on baseline HIV branched-chain DNA viral load and CD4⁺ cell count.
[c]HIV RNA copies/mL of plasma by branched-chain DNA. Viral load determined by reverse-transcriptase polymerase chain reaction approximately twofold greater.
[d]CD4⁺ cells/mm³.
Data from Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4⁺ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med. *1997;126:946–954.*

HIV-1 RNA less than 500 copies/mL and by 77 CD4⁺ cells per year in men with baseline HIV-1 RNA higher than 30,000 copies/mL.[47] Using the viral load and CD4⁺ count together, however, provides the best prognostic estimate of subsequent clinical course (Table 122.2 and Fig. 122.2). Put in the context of HIV pathogenesis, the viral load measures the replicative rate of the infection and its destructive potential for the cellular immune system, and the CD4⁺ count gauges the extent of immune compromise and the present risk for opportunistic disease. Rather than initiate ART on all HIV-infected individuals as early as possible, guidelines for initiating ART have historically been based on CD4⁺ counts and plasma viral load due to ART toxicity with prolonged use. In addition, opportunistic infection prophylaxis has been based on the current CD4⁺ count, which determines the short-term risks for developing specific infections (e.g., *P. jirovecii* pneumonia or disseminated *Mycobacterium avium* complex infection) and death. Frequent monitoring of CD4⁺ counts was previously recommended for patients not on ART to determine when treatment was indicated and for patients on ART to assess their response to therapy. However, as studies have demonstrated the long-term value of treating all HIV-infected individuals regardless of CD4⁺ count, this is now recommended.[53] Use of plasma viral load to monitor treatment response is now preferred, with monitoring of CD4⁺ counts recommended at the start of treatment and periodically for individuals with initially low counts to determine when to discontinue opportunistic infection prophylaxis. No CD4⁺ cell monitoring is considered necessary for individuals with initial counts >500 cells/mm³.

The rate of progression of HIV infection in population-based studies varies depending on age, with older individuals generally having a more rapidly progressive course.[54–58] Patients who experience more severe symptoms during acute retroviral syndrome tend to have higher viral loads after seroconversion and progress more rapidly than patients who seroconvert without symptoms.[59] Women have approximately half-$\log_{10}$ lower HIV-1 RNA than men after seroconversion, but this difference diminishes with time from seroconversion.[60–62] Although HIV-1 RNA is an important predictor of subsequent disease progression in both women and men,[47,63,64] there is no sex difference in HIV disease progression, particularly when women and men have equal access to care.[65–67] There do not appear to be racial differences in HIV-1 RNA levels[68] or the natural history of HIV disease progression.[65,69] Geographic differences in the risk of infections that can occur at higher CD4⁺ counts such as tuberculosis, salmonellosis, and some bacterial pneumonias can result in shorter survival times for individuals in low-income and middle-income countries.

Other laboratory studies that predict the development of AIDS in populations of HIV-infected people include total lymphocyte count less than 1000 cells/mm³, total white blood cell count less than 4000 cells/mm³, and hematocrit less than 40 mL/dL.. Although these tests are readily available and inexpensive, they do not discriminate short-term risks for individuals as well as CD4⁺ counts and are generally not used for clinical management. Other markers of HIV disease progression that have been validated in clinical studies but are not widely used include the HIV p24 antigen, serum β_2-microglobulin, neopterin, acid-labile interferon-α, anti-p24 antibody, and soluble CD8. These so-called surrogate markers are measures of virus presence or host immune responses to HIV. These measures do not provide prognostic information independent of the viral load and have therefore been supplanted by quantitative plasma HIV-1 RNA monitoring in developed countries. Low-cost alternatives to flow cytometric quantification of CD4⁺ lymphocytes for application in resource-poor settings are now

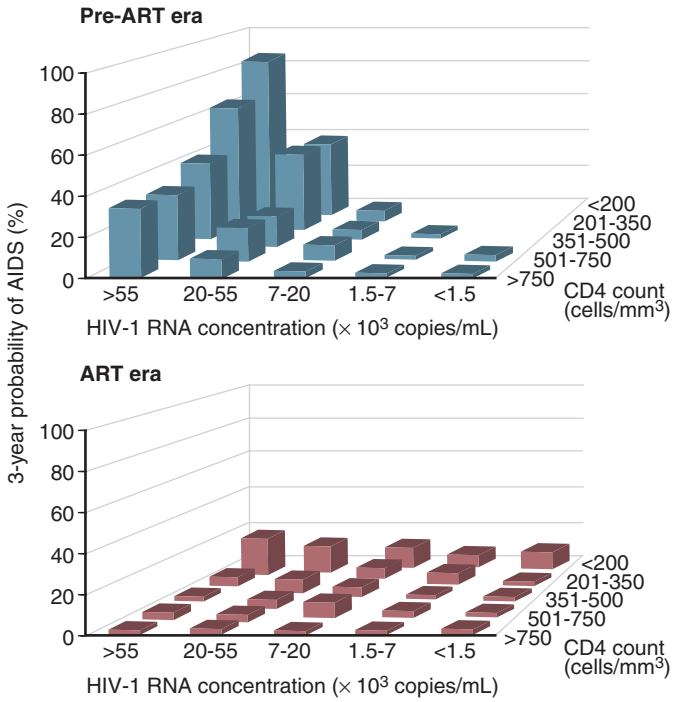

FIG. 122.2 Prognosis according to CD4⁺ cell count and viral load in the pre–antiretroviral therapy (ART) and ART eras. These data were from 12,574 adult patients starting ART with a combination of at least three drugs. Kaplan-Meier estimates of the probability of AIDS at 3 years are shown. *(From Egger M, May M, Chene G, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies.* Lancet. *2002;360:119–129.)*

available and include manual assays that use enzyme-linked immunosorbent assay or bead-based formats.[70]

CLASSIFICATION OF HIV INFECTION

In the early years of the HIV pandemic, surveillance case definitions for AIDS were necessary for tracking the growth of the epidemic; as awareness of the clinical spectrum of HIV infection grew, the number of conditions diagnostic of AIDS increased. In addition, staging systems for HIV were developed by the CDC, World Health Organization (WHO), Walter Reed Army Institute of Research, and others, incorporating clinical and laboratory features into discrete categories with differing prognoses. Distinguishing between HIV infection and AIDS was historically useful for epidemiologic purposes, but the difference is arbitrary and is less meaningful from a clinical perspective in an era of potent ART. At the present time, two classification systems are in wide use. The 2014 CDC Revised Surveillance Case Definition for HIV infection includes four stages based on laboratory testing, $CD4^+$ lymphocyte count and percentage, and clinical evidence including AIDS-defining conditions (Table 122.3).[71]

The CDC classification system for HIV infection recognizes the prognostic significance of the $CD4^+$ count in individuals with HIV infection, but there is considerable variation in risk for opportunistic complications and prognosis in individuals with $CD4^+$ counts <200 cells/mm^3. Individuals with $CD4^+$ counts <50 cells/mm^3, for example, are generally considered to have advanced HIV disease and are at much higher risk for the development of opportunistic infections such as CMV disease or disseminated *Mycobacterium avium* complex infection and death. The CDC classification system was initially developed at a time when the inevitable course of HIV infection was progression toward advanced immunodeficiency and death and when drug therapy was of limited and transient efficacy in stemming the course of the disease. According to the CDC classification system, HIV-infected individuals are classified on the basis of the most advanced stage that they have reached.[71] In the present era, patients treated with ART usually experience marked improvement in cellular immune function and may have a normal or near-normal life expectancy with dramatically lower risk for developing opportunistic disease than they had before receiving treatment.[72] There is no current mechanism for reclassifying patients on the basis of immunologic and clinical improvement resulting from ART, a situation that understandably curtails use of the CDC system.

The WHO classification system (Tables 122.4, 122.5, and 122.6) is used primarily in resource-limited countries. However, there are limitations that make it difficult for this staging system to be uniformly implemented. Many of the classifications require the diagnosis of opportunistic infections that cannot be readily confirmed in most resource-limited settings; clinical criteria for establishing presumptive or definitive diagnoses may be useful. Estimates of weight loss and other constitutional symptoms are also difficult in such settings. The CDC and WHO staging systems are compared in Table 122.7.

CLINICAL MANIFESTATIONS

HIV infection causes disease manifestations of three principal types: (1) an acute viral illness seen in the initial weeks of infection and associated with a high viral load and an intense host immune response; (2) immunologically mediated processes related to host responses to chronic viral infection in the absence of ART (e.g., lymphadenopathy, thrombocytopenia, HIV-related dementia) or persistent inflammation

TABLE 122.3 HIV Infection Staging Systems

STAGE	LABORATORY EVIDENCE[A]	CLINICAL EVIDENCE
Stage 0	Negative or indeterminate HIV test result within 180 days before first confirmed positive HIV test result of any type *or* Sequence of tests that demonstrate the presence of HIV-specific viral markers such as p24 antigen or nucleic acid (RNA or DNA) 0–180 days before or after antibody test that had a negative or indeterminate result	None required (but no AIDS-defining condition)
Stage 1	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of >500 cells/mm^3 *or* $CD4^+$ T-lymphocyte percentage of ≥29	None required (but no AIDS-defining condition)
Stage 2	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of 200–499 cells/mm^3 *or* $CD4^+$ T-lymphocyte percentage of 14–28	None required (but no AIDS-defining condition)
Stage 3 (AIDS)	Laboratory confirmation of HIV infection *and* $CD4^+$ T-lymphocyte count of <200 cells/mm^3 *or* $CD4^+$ T-lymphocyte percentage of <14[b]	*Or* documentation of AIDS-defining condition (with laboratory confirmation of HIV infection)[b]
Stage unknown[c]	Laboratory confirmation of HIV infection *and* no information on $CD4^+$ T-lymphocyte count or percentage	*And* no information on presence of AIDS-defining conditions

[a]The $CD4^+$ T-lymphocyte percentage is the percentage of total lymphocytes. If the $CD4^+$ T-lymphocyte count and percentage do not correspond to the same HIV infection stage, select the more severe stage.
[b]Documentation of an AIDS-defining condition supersedes a $CD4^+$ T-lymphocyte count of ≥200 cell/μL and a $CD4^+$ T-lymphocyte percentage of total lymphocytes of ≥14.
[c]Although cases with no information on $CD4^+$ T-lymphocyte count or percentage or on the presence of AIDS-defining conditions can be classified as stage unknown, every effort should be made to report $CD4^+$ T-lymphocyte counts or percentages and the presence of AIDS-defining conditions at the time of diagnosis. Additional $CD4^+$ T-lymphocyte counts or percentages and any identified AIDS-defining conditions can be reported as recommended.
Data from Selik RM, Mokotoff ED, Branson B, et al; Centers for Disease Control and Prevention. Revised surveillance case definitions for HIV infection—United States, 2014. MMWR Recomm Rep. *2014;63(RR03):1–10.*

TABLE 122.4 World Health Organization Clinical Staging of Established HIV Infection

HIV-ASSOCIATED SYMPTOMS	WHO CLINICAL STAGE
Asymptomatic	1
Mild symptoms	2
Advanced symptoms	3
Severe symptoms	4

WHO, World Health Organization.

TABLE 122.5 World Health Organization Immunologic Classification for Established HIV Infection

	AGE-RELATED CD4 VALUES			
HIV-ASSOCIATED IMMUNODEFICIENCY	<11 Months (% $CD4^+$)	12–35 Months (% $CD4^+$)	36–59 Months (% $CD4^+$)	>5 Years (Absolute No./mm^3 or % $CD4^+$)
None or not significant	>35	>30	>25	>500
Mild	30–35	25–30	20–25	350–500
Advanced	25–29	20–24	15–19	200–349
Severe	<25	<20	<15	<200 or <15%

TABLE 122.6 World Health Organization Clinical Staging of HIV/AIDS for Adults and Adolescents With Confirmed HIV Infection

Clinical Stage 1

Asymptomatic
Persistent generalized lymphadenopathy

Clinical Stage 2

Moderate unexplained weight loss (<10% of presumed or measured body weight)[a]
Recurrent respiratory tract infections (e.g., sinusitis, tonsillitis, otitis media, pharyngitis)
Herpes zoster
Angular cheilitis
Recurrent oral ulceration
Papular pruritic eruptions
Seborrheic dermatitis
Fungal nail infections

Clinical Stage 3

Unexplained[a] severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhea for longer than 1 month
Unexplained persistent fever (>37.6°C [99.7°F]), intermittent or constant, for longer than 1 month)
Persistent oral candidiasis
Oral hairy leukoplakia
Pulmonary tuberculosis (current)
Severe bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, or bacteremia)
Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis
Unexplained anemia (<8 g/dL), neutropenia (<0.5 × 10^9/L), or chronic thrombocytopenia (<50 × 10^9/L)

Clinical Stage 4[b]

HIV wasting syndrome
Pneumocystis jirovecii
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital, or anorectal, for longer than 1 month, or visceral at any site)
Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)
Extrapulmonary tuberculosis
Kaposi sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extrapulmonary cryptococcosis including meningitis
Disseminated nontuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy

Chronic cryptosporidiosis (with diarrhea)
Chronic isosporiasis
Disseminated mycosis (coccidioidomycosis or histoplasmosis)
Recurrent nontyphoidal *Salmonella* bacteremia
Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumors
Invasive cervical carcinoma
Atypical disseminated leishmaniasis
Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

[a]Unexplained refers to when the condition is not explained by other causes.
[b]Some additional specific conditions can also be included in regional classifications (such as reactivation of American trypanosomiasis [meningoencephalitis and/or myocarditis]) in the World Health Organization region of the Americas and disseminated penicilliosis in Asia.

even following ART initiation (e.g., cardiovascular disease, dyslipidemia, hypercoagulability, other metabolic disorders); and (3) opportunistic diseases resulting from impaired host responses as the cellular immune system is damaged or ablated. The major clinical syndromes most frequently seen in HIV-infected individuals fall into the last category. Potent ART has added two new categories of clinical manifestations that may be commonly encountered in patients with HIV infection: (1) immune reconstitution syndromes with exacerbations of previously silent or adequately treated infections, especially mycobacterial infections,[73,74] and (2) a syndrome of lipodystrophy with fat loss and redistribution, elevated serum triglyceride and cholesterol levels, and insulin resistance seen in patients receiving ART, especially with protease inhibitors.[75,76] The clinical features of immune reconstitution syndromes are discussed later in this chapter, and the manifestations of drug toxicity related to the treatment of HIV are discussed in Chapter 128.

Although the clinical manifestations of HIV infection do not vary according to HIV subtype, the incidence of specific opportunistic infections is profoundly influenced by geography and the prevalence of infectious diseases in particular regions. HIV-1 infection increases susceptibility to tuberculosis, and the incidence of tuberculosis in HIV-infected individuals is extremely high in sub-Saharan Africa, where tuberculosis is endemic. In this setting, tuberculosis is the most common opportunistic infection in people with HIV infection and the leading cause of death. Approximately 22% of all deaths from tuberculosis worldwide are associated with HIV disease, mostly in sub-Saharan Africa.[77] Malaria is also endemic in many developing countries and occurs with increased frequency and severity in HIV-infected individuals, particularly during pregnancy.[78] Opportunistic infections such as *P. jirovecii* pneumonia, *M. avium* complex disease, CMV retinitis, non-Hodgkin lymphoma, and HIV encephalopathy, which are relatively common in developed countries, are uncommon in developing countries such as those in West Africa.[79] In regions where it is endemic (e.g., the Mediterranean, Central America, South America, Africa, and Asia), leishmaniasis occurs with increased frequency among HIV-infected individuals. Similarly, *Trypanosoma cruzi* (South America), histoplasmosis (Ohio and Mississippi river valleys), and *Talaromyces marneffei* (Thailand, China, Hong Kong) occur with increased frequency in certain regions.

The incidence of specific opportunistic diseases has been determined for several large cohorts of HIV-infected patients. In a cohort of more than 1200 patients with $CD4^+$ counts less than 300 cells/mm^3 before the availability of ART, the most common opportunistic infection was *Candida* esophagitis (13.3 cases per 100 person-years). *P. jirovecii* pneumonia, disseminated *M. avium* complex, CMV disease, and AIDS dementia complex occurred at rates of 5 to 9 cases per 100 person-years. The relatively lower incidence of *P. jirovecii* pneumonia reflects the use of specific prophylaxis with trimethoprim-sulfamethoxazole or aerosolized pentamidine, which dramatically lowers the risk for this infection even in the absence of ART.[80,81] Less common were toxoplasmosis, cryptococcal meningitis, herpes zoster, the wasting syndrome, and Kaposi sarcoma (2–4 cases per 100 person-years). The least common complications were non-Hodgkin lymphoma, tuberculosis, progressive multifocal leukoencephalopathy, and cryptosporidiosis (1–2 cases per 100 person-years). Similar results have been found in other cohorts of patients in developed countries.[82] Considerably less information is available on the natural history of HIV infection in developing countries, but the spectrum of disease in patients presenting with HIV-related illnesses is different, with tuberculosis, cryptococcosis, bacterial sepsis and pneumonia, herpes zoster, and gastroenteritis predominating.[83]

Early clinical findings may also predict disease progression in HIV-infected individuals who have not developed opportunistic disease. Oral candidiasis and oral hairy leukoplakia are markers of immunosuppression and herald the development of AIDS in many patients.[43,84,85] Generalized lymphadenopathy is a common clinical finding in early HIV infection but does not predict progression to AIDS.[86] The occurrence of an opportunistic disease increases the risk for death independently of the $CD4^+$ cell count[87,88] including death due to non–AIDS-related causes.[89] This may be caused not only by morbidity related to the complication itself but also by an increase in immune activation and inflammatory responses leading to upregulation of HIV replication, with acceleration of HIV disease progression. A number of studies have demonstrated increases in HIV viral load in patients with acute opportunistic infections.[90–93] Although viral load generally decreases after the acute illness, it generally does not return to premorbid levels.

Effect of Antiretroviral Therapy on Natural History of HIV Infection

Even before the era of effective combination ART, it was clear that nonsuppressive ART and prophylaxis against *P. jirovecii* pneumonia had substantially altered the natural history of AIDS, prolonging the median survival of treated patients with AIDS to 2 to 3 years.[94–98] ART and prophylaxis against *P. jirovecii* pneumonia and *M. avium* complex prolonged the time from HIV infection to AIDS, decreased the incidence of opportunistic complications, and improved overall survival.[99–106]

TABLE 122.7 Comparison of World Health Organization and Centers for Disease Control and Prevention Staging Systems

WHO STAGE[B]	WHO T-LYMPHOCYTE COUNT AND PERCENTAGE[C]	CDC STAGE[D]	CDC T-LYMPHOCYTE COUNT AND PERCENTAGE
Stage 1 (HIV infection)	$CD4^+$ T-lymphocyte count of ≥500 cells/mm^3	Stage 1 (HIV infection)	$CD4^+$ T-lymphocyte count of ≥500 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of ≥29
Stage 2 (HIV infection)	$CD4^+$ T-lymphocyte count of 350–499 cells/mm^3	Stage 2 (HIV infection)	$CD4^+$ T-lymphocyte count of 200–499 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of 14–28
Stage 3 (advanced HIV disease)	$CD4^+$ T-lymphocyte count of 200–349 cells/mm^3	Stage 2 (HIV infection)	$CD4^+$ T-lymphocyte count of 200–499 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of 14–28
Stage 4 (AIDS)	$CD4^+$ T-lymphocyte count of <200 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of <15	Stage 3 (AIDS)	$CD4^+$ T-lymphocyte count of <200 cells/mm^3 or $CD4^+$ T-lymphocyte percentage of <14

[a]For reporting purposes only.
[b]Among adults and children ≥5 years of age.
[c]Percentage applicable for stage 4 only.
[d]Among adults and adolescents (≥13 years of age). CDC also includes a fourth stage, stage unknown; laboratory confirmation of HIV infection but no information on $CD4^+$ T-lymphocyte count or percentage and no information on AIDS-defining conditions.
CDC, Centers for Disease Control and Prevention; *WHO,* World Health Organization.

Changes in plasma HIV-1 RNA and $CD4^+$ lymphocyte counts resulting from ART have been shown to be strong predictors of clinical regression of HIV disease and restoration of cellular immunity.[107–109]

The use of combination ART and the introduction of protease inhibitors in the mid-1990s led to dramatic changes in the natural history of treated HIV disease (Figs. 122.3, 122.4, and 122.5).[110–115] In the United States as a whole, deaths attributed to AIDS have decreased by 85% since peaking at approximately 50,000 in 1995 to approximately 6700 in 2014.[111,112,116] Studies among cohorts of HIV-infected individuals in multiple settings demonstrate the beneficial effect of ART on clinical disease progression and death (Fig. 122.6).[117] Global improvements in survival of people with HIV infection have now been documented as a result of the expansion of access to ART to almost 20 million people worldwide through efforts of initiatives such as the US President's Emergency Program for AIDS Relief and the Global Fund for AIDS, TB and Malaria as well as efforts by national governments to scale up treatment of HIV infection. According to UNAIDS, deaths from AIDS decreased by almost half between 2005 and 2016, from 1.9 million to 1.0 million.[118–120] As discussed later on, as deaths due to AIDS have dramatically declined, morbidity and mortality in people with HIV infection is now more frequently caused by chronic diseases not traditionally classified as related to HIV infection. In the HIV Outpatient Study cohort, for example, declines in AIDS-related mortality between 1997 and 2004 were accompanied by a relative increase in deaths due to non–AIDS-related causes such as liver disease, non–AIDS-related cancers, and cardiovascular disease.[114] The Antiretroviral Therapy Cohort Collaboration reported that among more than 1800 deaths in 13 HIV clinical cohorts that occurred between 1996 and 2006, about half were due to AIDS, and the remainder were due to non–AIDS-related cancers, cardiovascular disease, trauma, liver disease, and other causes; the rate of AIDS-related deaths fell steadily with increased time on ART.[121] In the United States in 2014, almost half of deaths in people with HIV were attributed to non–AIDS-related causes. Among non–AIDS-related causes of death in Europe, non–AIDS-defining cancers were the leading cause.[122]

Effective therapy has not only decreased the incidence of new opportunistic infections but has also led to resolution of preexisting conditions.[123] In some cases the immune restoration resulting from ART can alter the clinical presentation of specific opportunistic infections, as in the case of focal mycobacterial lymphadenitis or CMV vitritis.[124] It may also unmask opportunistic infections that were not evident before ART initiation such as tuberculosis.[125,126] It is becoming increasingly clear that the immunologic changes resulting from ART represent at least a partial immune reconstitution, although the recovery of antigen-specific immunity appears to lag behind $CD4^+$ cell count increases.[127–130] The clinical manifestations of immune reconstitution syndromes are discussed at the end of this chapter. The incidence of new opportunistic infections in patients who have had satisfactory virologic and immunologic responses to ART is extremely low, even when primary prophylaxis has been discontinued.[131,132] Moreover, reactivation of previously diagnosed opportunistic infections such as *M. avium* complex infections and CMV retinitis appears to be uncommon in patients with immune recovery who discontinue maintenance therapy.[133,134]

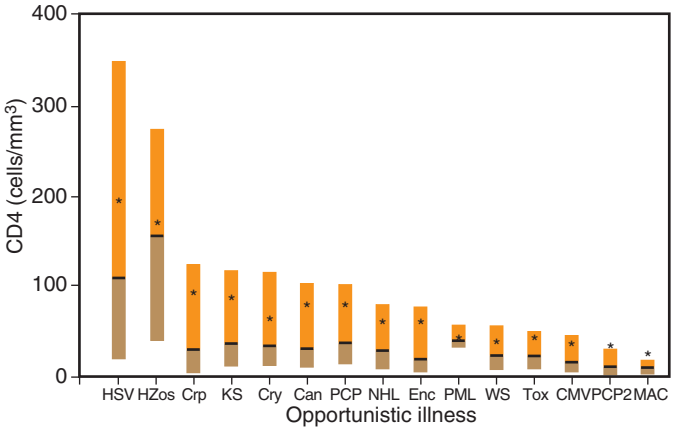

FIG. 122.3 Range of $CD4^+$ lymphocyte counts at the time of diagnosis of opportunistic diseases in HIV-infected patients. Boxes represent the 25th to 75th percentiles, bars represent medians, and asterisks represent means. *Can, Candida* esophagitis; *CMV,* cytomegalovirus; *Crp,* cryptosporidiosis; *Cry,* cryptococcosis; *Enc,* HIV encephalopathy; *HSV,* herpes simplex virus; *HZos,* herpes zoster; *KS,* Kaposi sarcoma; *MAC, Mycobacterium avium* complex; *NHL,* non-Hodgkin's lymphoma; *PCP,* first episodes of *Pneumocystis jirovecii* pneumonia; *PCP2,* recurrent *P. jirovecii* pneumonia; *PML,* progressive multifocal leukoencephalopathy; *Tox,* toxoplasmosis; *WS,* wasting syndrome. *(From Moore RD, Chaisson RE. Natural history of opportunistic disease in an HIV-infected urban cohort.* Ann Intern Med. *1996;124:633–642.)*

Over the past 4 decades, the natural history of HIV infection and our understanding of it have undergone considerable change. The clinical course of HIV disease in patients receiving ART is likely to evolve further in the coming years, with additional manifestations and complications of long-standing infection and use of antiretroviral drugs becoming apparent as larger numbers of patients are treated for longer periods of time.

Acute Retroviral Syndrome

The initial manifestation of HIV infection in one-half to three-quarters of recently infected individuals is a mononucleosis-like illness referred to as acute retroviral syndrome. Whereas the first reports noted a high prevalence of symptoms in acutely infected men,[135,136] other studies have found a wide range of symptomatic acute HIV infections.[137–139] In one study of 378 subjects with acute retroviral syndrome, injection drug users had or reported symptoms less frequently than persons who

acquired HIV through sexual transmission.[140] Most health care workers with occupationally acquired HIV had acute retroviral syndrome after exposure.[141,142] A recent intensive study of blood donors in Thailand reported that 78% of 430 adults diagnosed before HIV antibody seroconversion had symptoms of acute retroviral syndrome.

The clinical features of acute retroviral syndrome are nonspecific and variable.[143–148] The onset of the illness ranges from 1 to 6 weeks after exposure to the virus but peaks at 3 weeks. Table 122.1 lists the signs and symptoms of acute retroviral syndrome reported in 209 cases.[146] Fever, sweats, malaise, myalgias, anorexia, nausea, diarrhea, and a nonexudative pharyngitis are prominent symptoms.[146–153] Many patients report headaches, photophobia, and meningismus. Two-thirds of patients may have a truncal exanthem that may be maculopapular, roseola-like, or urticarial. Findings of skin biopsies are nonspecific, with perivascular

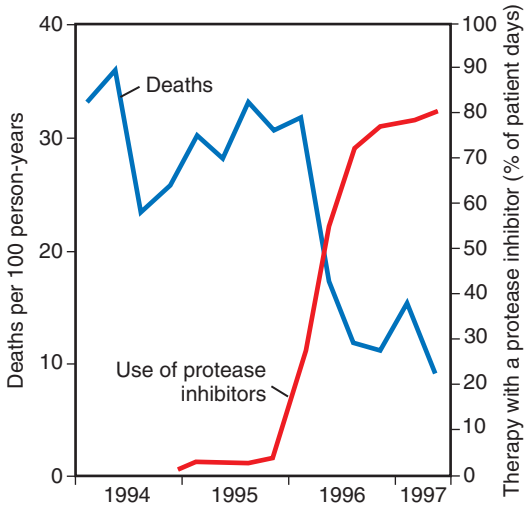

FIG. 122.4 Incidence of death and use of protease inhibitors in HIV-infected patients and CD4⁺ count lower than 100 cells/mm³ in the HIV Outpatient Study. *(From Palella FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection.* N Engl J Med. *1998;338:853–860. © 1998 Massachusetts Medical Society.)*

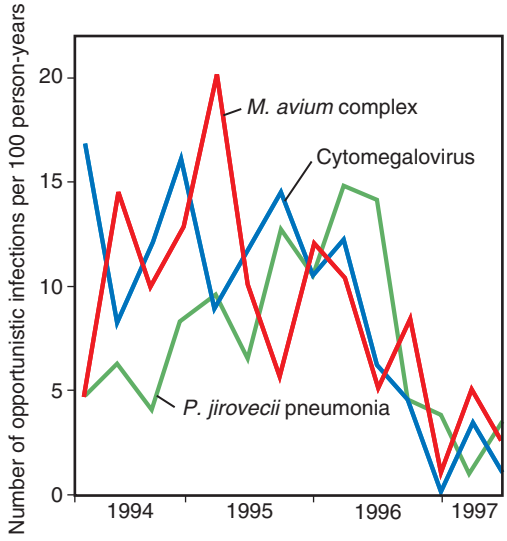

FIG. 122.5 Incidence of selected opportunistic infections in HIV-infected patients and CD4⁺ count lower than 100 cells/mm³ in the HIV Outpatient Study in the era before and after the introduction of protease inhibitors. *(From Palella FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection.* N Engl J Med. *1998;338:853-860. © 1998 Massachusetts Medical Society.)*

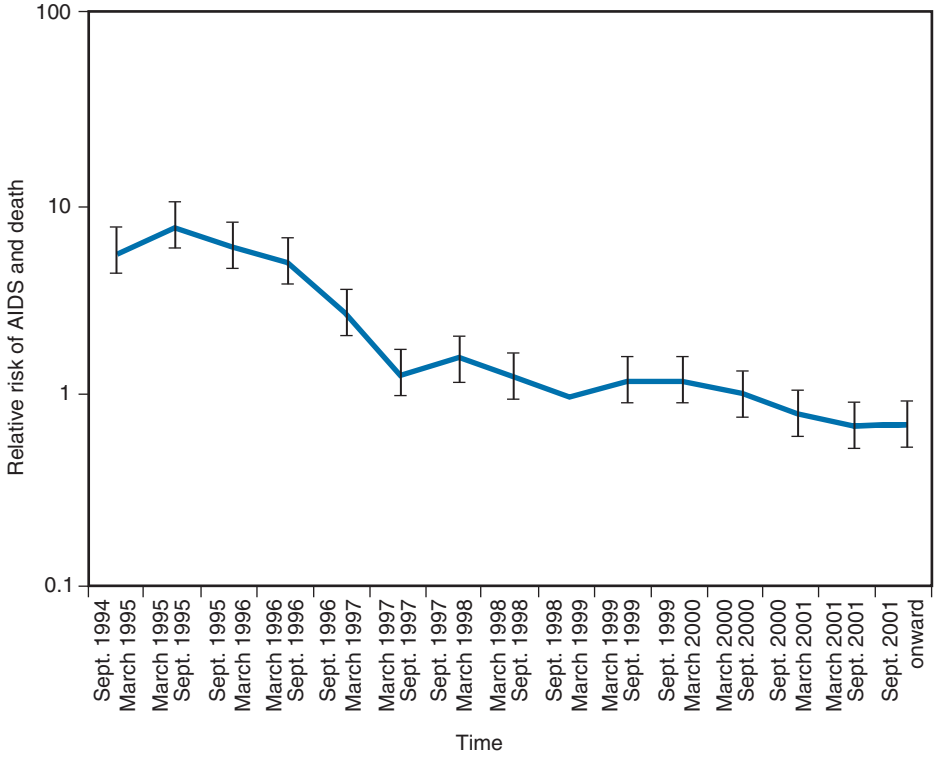

FIG. 122.6 Relative risk of AIDS or death since the introduction of antiretroviral therapy (ART) adjusted for CD4⁺ count at recruitment, age, previous ART treatment, and AIDS status. Data are from 9803 HIV-infected patients seen at 70 treatment centers in Europe, Israel, and Argentina. Vertical bars represent 95% confidence intervals. *(From Mocroft A, Ledergerber B, Katlama C, et al. Decline in the AIDS and death rates in the EuroSIDA study: an observational study.* Lancet. *2003;362:22–29.)*



FIG. 122.7 Aphthous ulcer. *(Courtesy Dr. Stephen Raffanti.)*

lymphocytic infiltrates and dermal mononuclear cell infiltrates.[154] In addition to aseptic meningitis, neurologic symptoms occur in a minority of patients and may include encephalitis, peripheral neuropathy, and an acute ascending polyneuropathy (Guillain-Barré syndrome).[155] Physical examination frequently reveals cervical, occipital, or axillary lymphadenopathy; rash; and, less commonly, hepatosplenomegaly. Oral aphthous ulcerations (Fig. 122.7) have been reported in several cases; these may involve the esophagus. Oral and esophageal candidiasis during the seroconversion illness has been reported. Symptoms generally resolve in 10 to 15 days. A wide range of acute opportunistic infections have been reported in patients with acute retroviral syndrome including *P. jirovecii* pneumonia, cryptococcal meningitis, and *Candida* esophagitis. Their occurrence is probably due to the depression of the $CD4^+$ cell count that generally accompanies acute HIV infection.

Laboratory evaluation of patients with acute retroviral syndrome shows a reduced total lymphocyte count, elevated sedimentation rate, negative heterophil antibody test, and elevated aminotransferase and alkaline phosphatase levels.[146] When lymphocyte phenotyping is performed, a characteristic pattern is observed.[156] Initially the total lymphocyte count including both $CD4^+$ and $CD8^+$ T lymphocytes decreases with a normal $CD4^+/CD8^+$ ratio. Within several weeks the $CD4^+$ and $CD8^+$ cell populations both begin to increase. The rise in $CD8^+$ cell numbers is relatively greater than the rise in $CD4^+$ cells, and the $CD4^+/CD8^+$ ratio is inverted. In the weeks that follow the $CD8^+$ cell population increases markedly because of HIV-specific $CD8^+$ T lymphocytes. The $CD4^+/CD8^+$ ratio usually remains inverted as the acute illness resolves, primarily because of excess numbers of $CD8^+$ cells. In patients with neurologic symptoms the cerebrospinal fluid may show a lymphocytic pleocytosis with normal levels of protein and glucose.[157]

Tests for detecting acute HIV infection include plasma HIV RNA, which becomes positive about 5 days after infection, and HIV p24 antigen, which may be detected after 10 days; antibody reactivity on enzyme immunoassay testing is not found until 14 to 21 days.[158] Fiebig and colleagues[158] characterized the stages of acute HIV infection by the timing of the appearance of viral markers and immune responses in plasma donors who were seroconverting. In Fiebig stage 1 (3–8 days), HIV-1 RNA is detectable, but p24 antigen and anti-HIV antibodies cannot be detected. Stage 2 (4–8 days) is marked by the appearance of p24 antigen in blood and cerebrospinal fluid. Stage 3 is marked by detection of anti-HIV antibodies by sensitive enzyme immunoassay, stage 4 is marked by the first appearance of bands on a Western blot, and stage 5 is marked by conversion of the Western blot to positive on multiple bands.[157,158] The cumulative time of Western blot seroconversion ranges from 15 to 28 days. Use of a fourth-generation, ultrasensitive enzyme immunoassay shortens the time to detection of antibodies.[159] Therefore plasma HIV RNA is the most sensitive marker for acute HIV infection and is the preferred test when evaluating patients suspected to have acute retroviral syndrome.[160] Typical RNA levels range from 10^5 to more than 10^6 copies/mL of plasma, and the titers decline as the $CD8^+$ cytotoxic T-cell and antibody responses subsequently increase. Low-level ($<10^4$) false-positive HIV RNA tests may occur, but high-level viremia is diagnostic of acute infection in the absence of anti-HIV antibodies.

The differential diagnosis of acute retroviral syndrome includes a number of other illnesses—infectious mononucleosis and other viral infections such as influenza, viral hepatitis, measles, rubella, primary herpes simplex virus (HSV) infection, and CMV and secondary syphilis. Evaluation of patients presenting with an illness consistent with acute retroviral infection should include a careful history to elicit risks for HIV infection; laboratory tests to rule out mononucleosis, CMV, and syphilis; HIV antibody and plasma RNA tests; and complete blood cell counts and differential. There is potential benefit in treating acute HIV with ART because there is evidence that this may lower the viral setpoint, lead to enhanced $CD4^+$ and $CD8^+$ HIV-specific responses, decrease the severity of acute disease,[161,162] and decrease levels of immune activation and inflammation.[163] Early treatment does not appear to prevent establishment of reservoirs of latently infected resting $CD4^+$ cells, although it may decrease the size of the reservoirs in adults.[164] In infants, very early treatment may result in remission of HIV viremia following cessation of treatment for several years, as illustrated by the "Mississippi Child."[165] However, the remission of viremia is not permanent.[166] Current guidelines recommend ART for all HIV-infected individuals; thus initiating treatment in early HIV infection (the day of HIV diagnosis if resources and support are available) is appropriate (see Chapter 128).[167–169]

Persistent Generalized Lymphadenopathy

Infection with HIV is associated with a high prevalence of persistent generalized lymphadenopathy (PGL), often beginning with acute retroviral syndrome. In the early 1980s, PGL was recognized as a prodromal state to the development of AIDS in MSM who were otherwise healthy.[15,17] The pathogenesis of PGL is related to the rapid infection of $CD4^+$ cells in lymph nodes by HIV after initial infection. Although enlarged peripheral lymph nodes may be the most evident sign of recent HIV infection, intestinal lymph nodes and Peyer patches in the gut are an even more important target of early HIV infection with infection of large proportions of $CD4^+$ T cells and dendritic cells resulting in obliteration of normal nodal architecture and loss of function. Disruption of the intestinal mucosa can then lead to bacterial translocation and systemic immune activation, a process that may persist throughout the course of infection.[170,171]

The syndrome of PGL is defined as the presence of two or more extrainguinal sites of lymphadenopathy for a minimum of 3 to 6 months for which no other explanation can be found. Biopsy specimens of lymph nodes from such patients usually reveal a follicular hyperplasia without specific pathogens. PGL develops in 50% to 70% of HIV-infected individuals. The most frequently involved node groups are the posterior and anterior cervical, submandibular, occipital, and axillary chains; epitrochlear and femoral nodes may also be enlarged. Physical examination usually reveals symmetrical, mobile, rubbery lymph nodes ranging from 0.5 to 2 cm. Localized (i.e., asymmetrical) adenopathy and rapid nodal enlargement are not characteristic and suggest another infectious or malignant process. Mediastinal and hilar adenopathy is not characteristic of the syndrome; however, abdominal computed tomography often reveals enlarged mesenteric and retroperitoneal adenopathy in patients with HIV infection. The natural history of HIV infection in patients with PGL does not differ significantly from that of HIV infection in patients without PGL.[86,172] Involution of enlarged lymph nodes, with degeneration of follicular germinal centers and loss of hyperplasia, often accompanies progression of HIV infection to advanced disease.

In patients treated with ART, previously involuted lymph nodes may again enlarge as HIV-specific and other T cells are replenished. In addition, focal lymphadenitis with constitutional symptoms may occur in patients with previously silent infections 1 to 2 months after starting ART. These reversal or unmasking reactions, or immune reconstitution syndromes, are reminiscent of reversal reactions seen in multibacillary forms of leprosy, heralding a return of pathogen-specific T-cell responses.

The differential diagnosis of PGL includes HIV infection and a wide variety of other processes associated with generalized lymphadenopathy such as sarcoid, secondary syphilis, and Hodgkin disease. In patients with

HIV infection, lymphadenopathy may also be caused by mycobacterial infections, Kaposi sarcoma, and lymphoma.[173] An uncommon cause of lymphadenopathy in patients with HIV infection is multicentric Castleman disease.[174,175] Castleman disease is an angioproliferative, hyperplastic process of lymph nodes and other lymphoid tissues showing characteristic histologic findings, with either hyaline vascular or plasma cell variants. In patients with HIV in particular, multicentric Castleman disease is the most common presentation, with involvement of lymph nodes, liver, spleen, and other organs. Although the pathogenesis of Castleman disease is not fully understood, infection with Kaposi sarcoma–associated herpesvirus (human herpesvirus type 8) is believed to underlie a large proportion of cases. Nearly all patients who have HIV infection and Castleman disease are also infected with human herpesvirus type 8.[176,177] In contrast to PGL, multicentric Castleman disease is associated with constitutional symptoms and multiple organ involvement in most patients with HIV infection. The diagnosis is established on histopathology. Treatment with the anti-CD20 monoclonal antibody rituximab, alone or in combination with chemotherapy such as liposomal doxorubicin, has led to an overall survival of >90% at 5 years.[178,179]

Constitutional Disease and Wasting

HIV infection is often completely asymptomatic; however, some patients present with nonspecific constitutional symptoms in the months or years after primary infection but before opportunistic disease is diagnosed. Patients commonly report being easily fatigued and the need to reduce their normal activities. Debilitating fatigue is uncommon in the early years of infection. Low-grade fevers (temperature <38°C [100.4°F]), occasional night sweats, and intermittent diarrhea are also reported. Severe wasting, with loss of more than 10% of body weight, is generally a finding of advanced HIV disease. The exact incidence of constitutional symptoms, fatigue, and weight loss is not known, and the cause is varied and often multifactorial. The differential diagnosis of these findings includes intercurrent minor illnesses, endocrinologic abnormalities, anemia, and psychological or psychiatric disorders.

In African patients with HIV infection, a wasting illness termed *slim disease* has been described.[180] These patients have debilitating fatigue, fevers, sweats, protracted diarrhea, and severe weight loss. Opportunistic or conventional pathogens are not found, but the patients waste away and die of severe malnutrition and terminal secondary infections. This illness has been encountered in developed countries as well but far less commonly than in Africa—a pattern that suggests underdiagnosis of opportunistic diseases in Africa. Several studies of African patients with enteropathic slim disease found that most had enteric pathogens or microsporidia when a thorough evaluation was performed.[181,182] In Abidjan, Côte d'Ivoire, 37% of patients who died with a diagnosis of slim disease were found at autopsy to have disseminated tuberculosis,[183] and the presence of tuberculosis at autopsy was strongly associated with the degree of wasting.[184] The definition of wasting syndrome in the United States is the presence of unexplained constitutional disease for longer than 1 month with a temperature higher than 38.3°C (100.9°F), diarrhea, and loss of more than 10% of baseline body weight. A thorough evaluation to identify specific pathogens that would explain the symptoms and be amenable to treatment is essential before wasting syndrome is diagnosed, and usually a specific cause can be implicated.

In patients with more advanced HIV disease with high viral loads and severe depletion of $CD4^+$ cells, constitutional disease (fatigue, weight loss, malaise, fever) usually heralds the onset of opportunistic infections or malignancies. In one study of HIV-infected outpatients with fever, a specific cause could be identified for 83%.[185] Common causes of fever in these patients included *P. jirovecii* pneumonia, *M. avium* complex bacteremia, catheter-related bacteremia, bacterial pneumonia, sinusitis, lymphoma, and drug reactions. Fever lasting longer than 2 weeks was more often associated with AIDS-defining illnesses.

Anxiety disorders and depression are common in patients with HIV infection,[186–188] and studies have suggested an increased prevalence of affective disorders among HIV-infected individuals. Injection drug users, in particular, have a high prevalence of affective disorders that may result in somatic complaints. Moreover, the physical effects of opiates and withdrawal from stimulants such as cocaine and amphetamines cause fatigue and other constitutional symptoms.

Metabolic and Endocrine Abnormalities

A number of metabolic and endocrinologic disturbances have been identified in patients with HIV infection.[189–191] The pathogenesis of metabolic and endocrine dysfunction in people with HIV is thought to be multifactorial and may result from activation of proinflammatory cytokines and procoagulant mediators by HIV infection, T-cell activation resulting directly and indirectly from HIV, bacterial translocation due to intestinal mucosal disruption, and activation of other infectious agents such as CMV by HIV-induced immunodeficiency.[192] The spectrum of complications appearing in ART-naïve patients differs from that seen in treated patients with viral suppression, and in the latter group, aging and lifestyle may contribute to some manifestations.

Hypogonadism, particularly depression of testosterone or dihydrotestosterone levels, has been reported in men and women with HIV infection and weight loss or wasting.[193,194] Elevated levels of myostatin-immunoreactive protein, a muscle catabolic agent, have been found in men with HIV and wasting.[195] In most clinical studies, however, wasting has been found in association with decreased caloric intake, elevated catabolism caused by opportunistic infections, or chronic diarrhea.[196–199] In advanced HIV, severe wasting, whatever the cause, is strongly associated with mortality risk.[200] Weight loss has remained an important predictor of mortality even in the era of ART.[201]

The prevalence of osteoporosis is more than three times greater in HIV-positive individuals than HIV-negative control subjects.[202] HIV-positive individuals are also at increased risk of bone fracture.[203]

In contrast to the wasting illness seen in patients with advanced untreated HIV infection, patients receiving ART may have lipid abnormalities including hypertriglyceridemia and high low-density lipoprotein cholesterol and low high-density lipoprotein cholesterol levels. They may also have lipodystrophy, obesity, and insulin resistance.[76,204] These abnormalities are seen more commonly with protease inhibitor–based therapy, but they also occur with nonnucleoside reverse-transcriptase inhibitor therapy. Significant weight gain and diabetes mellitus have been reported in patients switching from efavirenz to integrase strand transfer inhibitors.[205,206] The lipid and glucose abnormalities may contribute to an increased risk for cardiovascular disease including myocardial infarction.[207] Recent abacavir use has been associated with an increased risk of myocardial infarction.[208] In the Multicenter AIDS Cohort Study, HIV-infected men on ART with suppressed viral loads were overall less likely to have good metabolic health,—a composite of normal blood pressure, serum triglycerides, serum cholesterol, fasting blood glucose, and waist circumference—than HIV-seronegative men. However, these differences were largely seen in nonobese men, although the prevalence of obesity was slightly lower in HIV-infected men.[209]

Oral Disease

Abnormalities of the oral cavity occur throughout the course of HIV infection. Primary HIV infection has been associated with severe aphthous stomatitis and with oropharyngeal and esophageal candidiasis. As the infection progresses and immunologic impairment proceeds, numerous oral complications arise. In the late stages of disease, oral manifestations are highly prevalent and frequently severe.[210–212] A number of studies have demonstrated that the occurrence of oral lesions such as candidiasis and hairy leukoplakia is associated with an increased risk for progression to AIDS.[213–215]

Oral Candidiasis

Candida infections of the hard and soft palates, buccal mucosa, tongue, pharynx, and hypopharynx are observed frequently. *Candida albicans* is the species most commonly identified, but *Candida tropicalis, Candida glabrata,* and *Candida krusei* infections also occur. Contrary to systemic *Candida* infections, which appear to result from defects in phagocyte function and number, mucosal *Candida* infections result from impaired cellular immunity. The incidence of candidiasis increases with progressive cellular immunodeficiency, particularly as $CD4^+$ counts fall below 200 to 300 cells/mm^3.[216]

The most common form of candidiasis is thrush (pseudomembranous candidiasis). Characteristic cottage cheese plaques that can be removed with a tongue blade are seen on the soft palate, tonsils, and buccal mucosa (Fig. 122.8). Less often, thrush involves the lateral and posterior



FIG. 122.8 Oral candidiasis (thrush). *(Courtesy Dr. Stephen Raffanti.)*



FIG. 122.9 Angular cheilitis. *(Courtesy Dr. Stephen Raffanti.)*

aspects of the tongue, the hard palate, and the hypopharynx. *Candida* infection can produce flat erythematous plaques distributed in the same way as the pseudomembranous form of the disease but without the characteristic white exudate. This atrophic form of candidiasis is underdiagnosed because many clinicians are unfamiliar with its appearance. Atrophic candidiasis of the tongue also occurs. Less frequently, *Candida* can cause a nonscrapeable white plaque similar to that seen in hairy leukoplakia (see next section). In contrast to the corrugated lesions and hairlike projections seen in oral hairy leukoplakia, candidal lesions are smooth. This hypertrophic form of disease may involve the lateral border of the tongue, palate, and buccal mucosa. *Candida* infection of the lateral lip (angular cheilitis) is another common complication. Angular cheilitis can cause pain, fissures, erythema, and difficulty opening the mouth (Fig. 122.9). Physical examination, wet mount preparation, and response to antifungal therapy establish the diagnosis.

The diagnosis of candidiasis is frequently made on the basis of physical examination alone. A potassium hydroxide preparation of scraped material from a plaque is diagnostic and can be performed easily in most clinical settings. Cultures for *Candida* are rarely necessary unless antimicrobial resistance is suspected in patients with thrush refractory to azole therapy. A biopsy specimen of oral lesions can be used to distinguish various forms of leukoplakia. A therapeutic trial of antifungal agents can also help establish a diagnosis.

Oral Hairy Leukoplakia

Oral hairy leukoplakia is a raised white lesion of the oral mucosa that is usually seen on the lateral margin of the tongue.[217] The frequency of occurrence of oral hairy leukoplakia increases as the $CD4^+$ count decreases.[216] Oral hairy leukoplakia appears to be caused by the replication of Epstein-Barr virus in the epithelium of keratinized cells on the surface of the tongue and buccal mucosa.[218] Other herpesviruses have also been isolated from cultures of biopsy specimens of lesions; however, their role in the pathogenesis of oral hairy leukoplakia is unclear. The diagnosis is established by visual inspection, failure to scrape off the lesion with a tongue blade, failure of the lesion to respond to antifungal therapy, and biopsy material or scrapings in which Epstein-Barr virus can be identified. Oral hairy leukoplakia is usually asymptomatic, although large lesions may impair taste, hinder eating, and cause discomfort. There is usually clinical improvement with ART.

Gingivitis and Periodontitis

Severe gingivitis (linear gingival erythema) and periodontitis (necrotizing ulcerative periodontitis) have been observed in patients with HIV disease.[219] The onset of symptoms is often insidious but may be abrupt. Pain is often severe; patients may note foul breath, bleeding gums, and loosening of teeth. Physical examination may reveal a bright red marginal line on the gingiva, necrosis and ulceration of interdental papillae, gingival erosion, exfoliation of enamel, and loose teeth. The cause of gingivitis and periodontitis is unclear. Cigarette smoking may be an important cofactor in the pathogenesis of periodontitis. Mixed cultures of aerobic and anaerobic flora have been obtained from gingival biopsy samples. More severe, ulcerating gingivitis can be caused by infections with gram-negative bacilli, particularly *Klebsiella pneumoniae* and *Enterobacter cloacae*. Infections tend to be chronic, but débridement, irrigation, and topical antiseptic agents or metronidazole therapy may control some cases.

Oral Ulcers

A number of ulcerative lesions may occur in the oral cavity of patients with HIV infection. HSV types 1 and 2 may cause primary or recurrent oral ulcers. These lesions generally appear as small smooth ulcers on an erythematous base on the lips, buccal mucosa, hard palate, or gums. The ulcers may be single or multiple and are often painful. Episodes may last for several weeks; acyclovir may be beneficial. CMV may rarely cause solitary large ulcers in patients with disseminated CMV infection. Aphthous stomatitis is manifested by single or multiple painful ulcers, often with exudate or necrosis, which may appear on the buccal and labial mucosa and lateral margin of the tongue (see Fig. 122.7). These ulcers do not occur more commonly than in HIV-seronegative individuals, but episodes are more severe and prolonged.[220] The ulcers may be treated with topical corticosteroids or thalidomide if persistent.[221–223] The cause of oral ulcers is best determined by biopsy and viral culture, although minor lesions may be observed without specific therapy in many cases. Several drugs including zalcitabine, zidovudine, and dapsone have been reported to cause oral and gastrointestinal ulcers.

Other Oral Lesions

The purple-red lesions of Kaposi sarcoma may occur at any site in the mouth, but the palate is most common. Lesions may become large and nodular. Non-Hodgkin lymphoma may arise in the mouth as a swelling or ulcers; biopsy is required for diagnosis. Oral warts caused by human papillomavirus infection may be seen, but they are not malignant precursors. Ketoconazole and zidovudine can cause brown oral pigmentation. Salivary glands such as the parotid gland may be enlarged by infiltration with $CD8^+$ lymphocytes or benign lymphoepithelial cysts. These cysts often respond to ART.[224]

Musculoskeletal Complications

Polymyositis complicates HIV infection in a small number of patients and can occur at any stage of HIV infection.[225] Clinical features include myalgias, weakness of the proximal muscles, muscle tenderness, wasting, and fatigue.[226] Creatine kinase and other muscle enzyme concentrations are usually elevated, although they do not correlate with disease severity; electrophysiologic studies are consistent with a myopathy.[227,228] Nucleoside reverse-transcriptase inhibitors have been associated with a polymyositis-like clinical picture in a small proportion of patients who receive these agents. Initially described with zidovudine, the mechanism of this

myopathy is inhibition of mitochondrial DNA polymerase, which is distinguished on electron microscopy by ragged myofibrils.[229] Other nucleoside reverse-transcriptase inhibitors also can cause mitochondrial toxicity resulting in neuropathies, liver impairment, and lactic acidosis.[230] The more recently developed agents in this class such as tenofovir, emtricitabine, and lamivudine are less likely to cause mitochondrial damage.

Pyomyositis has been reported in patients with advanced HIV. Skin flora, particularly *Staphylococcus aureus,* are usually recovered from wound cultures, and preexisting skin diseases such as prurigo nodularis are a risk factor.[231,232]

Although rheumatologic findings in patients with HIV disease are not unusual, the extent to which HIV infection is associated with these disorders is not always clear. Defining a specific arthropathy caused by HIV is difficult because many patients with HIV infection are already at increased risk for inflammatory joint disease. For example, injection drug users may develop septic arthritis caused by pyogenic bacteria, particularly *S. aureus.* MSM may have an increased risk for gonococcal arthritis or postinfectious reactive arthritis (formerly Reiter syndrome) associated with genital or gastrointestinal tract infections. Immune complex deposition related to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection may also be associated with arthritis in patients with HIV infection. Although some animal retroviruses are clearly associated with arthropathies, the situation with HIV remains unclear.

Osteopenia and avascular necrosis of bone occur at a higher frequency in HIV-infected individuals than in HIV-seronegative people in the general population,[233–235] and the incidence of avascular necrosis appears to be increasing.[236] Risk factors for avascular necrosis include corticosteroid therapy, lipid-lowering agents, and testosterone.[235] Although avascular necrosis has been described in individuals receiving ART,[237] such therapy does not appear to affect the risk for avascular necrosis.[238] Bone mineral density decreases modestly shortly after ART initiation but does not appear to decrease with time on ART, and in some studies it has increased.[233]

Cutaneous Manifestations

Dermatologic consequences of HIV infection include primary cutaneous opportunistic infections and malignancies, which may also disseminate to the viscera, and systemic opportunistic diseases with skin involvement.[239,240]

Viral Infections of the Skin and Mucous Membranes

A wide range of viruses involve the skin in immunosuppressed patients with HIV infection. The exanthem of acute HIV infection is an erythematous morbilliform eruption of the trunk and upper arms that occurs 2 to 4 weeks after infection and is usually associated with fever, headache, arthralgias, night sweats, pharyngitis, or thrush.[136,241,242] The rash resolves within 5 to 7 days. HSV (see Chapter 135) frequently causes morbidity in patients with advanced HIV disease.[243] Serology shows previous infection with HSV-2 in more than 90% of MSM with HIV infection; it is less prevalent in other groups. Although HSV-2 recurs frequently even in nonimmunosuppressed hosts, it recurs more frequently and for prolonged periods in patients with HIV infection. HSV-2, a common pathogen of the sacral root dermatomes, often causes outbreaks in the buttocks, perineum, scrotum (or vulva), and shaft and glans of the penis. Characteristic lesions of HSV appear first as painful erythematous papules; later, they vesiculate and ulcerate, and pustules may form. Chronic ulcers may become granulated, verrucous, or bloody (Fig. 122.10). Herpes simplex proctitis is associated with severe rectal pain, fever, tenesmus, and obstipation. External lesions may be absent, and the diagnosis is established by anoscopic or sigmoidoscopic examination and cultures. Giant perirectal ulcers and lesions at other sites that yield thymidine kinase–resistant strains of HSV-2 have occurred in patients who were previously treated with acyclovir. HSV infections are diagnosed by the typical appearance and distribution of the lesions, viral culture, or polymerase chain reaction assay. Tzanck preparations may show giant cells, which suggest HSV infection but generally have low sensitivity. Some physicians base diagnosis on how patients respond to an empirical trial of acyclovir. Orolabial HSV infections in HIV-infected patients may be caused by HSV-1 or HSV-2. Although primary infections may occur after patients acquire HIV, recurrences are the more common manifestation of HSV infection. Often a prodrome of tingling and pain precedes the appearance of painful vesicles and ulcers. Lesions may be found on the lips, buccal mucosa, gingiva, soft palate, uvula, and tongue. HSV disease may recur chronically in patients with advanced immunosuppression.

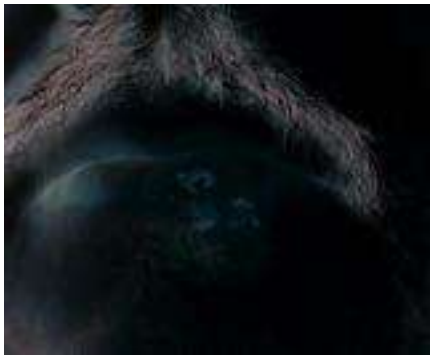

FIG. 122.10 Herpes simplex cheilitis. *(Courtesy Dr. Stephen Raffanti.)*



FIG. 122.11 Herpes zoster in dermatomal distribution. *(Courtesy Dr. Stephen Raffanti.)*

Varicella-zoster virus (shingles) is often reactivated in patients with HIV infection (see Chapter 136),[244] typically when the $CD4^+$ count is 200 to 500 cells/mm^3. There have been reports of herpes zoster after initiation of ART,[245] suggesting a role of the host immune response in this clinical manifestation. Herpes zoster may occur early in the course of HIV infection, but the incidence in late HIV disease is 5% to 10% annually.[238,246–249] Dermatomal outbreaks are most common, and a substantial proportion of patients may have several dermatomes involved (Fig. 122.11). Recurrent episodes at the same or different sites, chronic (nonremitting) zoster, and dissemination are often seen.[250] Shingles is often characterized by radicular pain and itching several days before erythematous papules appear, and vesiculation occurs within several days. Lesions are often extremely pruritic, and excoriation with secondary bacterial infection commonly occurs. Over a period of 4 to 7 days, lesions form bullae and crusts and begin to heal, although some patients have chronic herpes zoster. Cranial and thoracic dermatomes, followed by lumbar and sacral roots, are most often involved. Outbreaks along the ophthalmic branch of the trigeminal nerve may result in corneal involvement and lead to scarring and opacification that impair vision. A substantial proportion of patients may experience postherpetic scarring and pain. In patients with HIV infection who acquire primary varicella (chickenpox), the acute infection may progress to a chronic form in weeks to months.

Despite the frequency of disseminated CMV disease in late-stage AIDS, cutaneous manifestations are unusual. However, vesicles, bullae,



FIG. 122.12 Molluscum contagiosum. *(Courtesy Dr. Stephen Raffanti.)*



FIG. 122.13 Kaposi sarcoma. *(Courtesy Dr. Stephen Raffanti.)*

and hyperpigmented indurated plaques all have been described. Infections with human papillomavirus, the causative agent of condylomata acuminata, are more prevalent in HIV-infected people than in the general population. In addition to genital lesions (see Chapter 143), warts are often seen in periungual locations, on the feet (plantar warts), and in bearded areas of the face.

Molluscum contagiosum, a cutaneous poxvirus infection, is seen more often in HIV-infected people than in other populations (see Chapter 133). Most patients have $CD4^+$ counts less than 200 cells/mm^3. The agent is transmitted by sexual or other close contact; reactivation of remote infection may cause outbreaks in immunosuppressed hosts. Molluscum lesions are small firm papules with a pearly white umbilicated surface distributed on the face, trunk, or genital areas. The lesions are usually painless and can be differentiated from herpetic lesions by the absence of erythema, smaller size, and resolution of lesions without ulcerating or crusting (Fig. 122.12). Biopsy may be necessary to exclude more serious causes of cutaneous lesions such as cryptococcosis, pyogenic granuloma, and basal cell carcinoma. Liquid nitrogen is used effectively to treat this condition. Lesions may also resolve after initiation of ART, with a resultant increase in $CD4^+$ count.[251]

Bacillary Angiomatosis

Bacillary angiomatosis is associated with cutaneous and visceral involvement that produces lesions characterized by vascular proliferation, hemorrhage, and necrosis.[252–256] The disease was first described in 1983 in a patient with AIDS with subcutaneous nodules with vascular proliferation and evidence of bacterial involvement by electron microscopy.[255] Subsequently the cause of bacillary angiomatosis was attributed to the organisms *Bartonella henselae* and *Bartonella quintana* (see Chapter 234). *B. henselae* infection has been associated with cat and flea exposure and *B. quintana* infection with low socioeconomic status, homelessness, and exposure to lice.[257] *Bartonella* organisms have been cultured from skin lesions, blood, liver, bone, and other sites.[252–254,256]

Patients with bacillary angiomatosis usually present with one or several cutaneous lesions, although disseminated disease is common. The typical skin lesions are purple-red nodules or plaques that can ulcerate and crust. Lesions may be mistaken for cutaneous Kaposi sarcoma, skin tags, or basal cell carcinoma. Visceral disease may include hepatitis (bacillary peliosis), splenic or osseous lesions, bacillemia, pneumonitis or, less often, involvement of other organs.[258] Bacillary peliosis is a characteristic illness in which patients present with fever, right upper quadrant pain, hepatomegaly, and elevation of liver enzyme levels, particularly alkaline phosphatase.[259,260] Imaging studies of the liver may reveal echogenic defects; histologically, lesions have a cystic appearance, with vascular proliferation, hemorrhage, and necrosis.

The diagnosis of bacillary angiomatosis is best made by biopsy of involved sites. Hematoxylin and eosin stains of biopsy specimens from skin lesions show proliferation of small blood vessels in the dermis or cutis, enlarged endothelial cells with abundant cytoplasm, and necrotic and granulomatous changes. Warthin-Starry stains show perivascular accumulations of bacilli; these findings may be confirmed by electron microscopy, although this is not usually necessary. The diagnosis can also be established by culture of the organism in several special media or by detection of *Bartonella* DNA by polymerase chain reaction assay.[261] Serologic assays for anti-*Bartonella* antibodies are available through the Special Pathogens Branch of the CDC. The natural history of the infection in patients with HIV is for relapses to occur in the absence of prolonged therapy with erythromycin or doxycycline. Fluoroquinolones, other macrolides, and trimethoprim-sulfamethoxazole also have activity against *Bartonella*.



FIG. 122.14 Seborrheic dermatitis. *(Courtesy Dr. Stephen Raffanti.)*

Kaposi Sarcoma

Kaposi sarcoma is a vascular neoplastic disorder that in the United States is seen predominantly in HIV-infected MSM. Human herpesvirus type 8, which is transmitted sexually, has been implicated in the pathogenesis of Kaposi sarcoma (see Chapter 140).[226–265] Although Kaposi sarcoma also affects visceral organs, the characteristic findings are cutaneous red-purple nodules or plaques (Fig. 122.13). Sites commonly involved include the legs, feet, mucous membranes, hard palate, nose, trunk, and scalp.[266] Lesions of Kaposi sarcoma are often difficult to distinguish from lesions of bacillary angiomatosis; biopsy is required for diagnosis.

Other Cutaneous Manifestations

Various other skin disorders have been described in patients with HIV infection. Seborrheic dermatitis, an inflammatory condition of sebaceous glands that may be associated with dermatophytic superinfection, is an early complication (Fig. 122.14). Erythema and scaling of midline areas of the forehead, face, and groin are typical findings. Psoriasis occurs in 5% of HIV-infected individuals, with scaly reddish plaques, onycholysis, nail pitting, and subungual hyperkeratosis. Associated psoriatic arthritis

occurs more frequently than in HIV-seronegative individuals with psoriasis. Tinea infections of the scalp, trunk, inguinal and perineal areas, extremities, and feet are also common. Onychomycoses, or fungal infections of the fingernails and toenails, are common, although usually asymptomatic, causing only cosmetic changes. Bacterial folliculitis may be localized or disseminated in patients with HIV infection, and relapses frequently occur. *S. aureus* is the most common causative pathogen. Community-acquired methicillin-resistant *S. aureus* skin infections occur with increasing frequency in HIV-infected patients; risk factors include high-risk sex, drug-using behavior, and environmental exposure, but not immune status.[267] Eosinophilic folliculitis (Fig. 122.15) is an inflammatory condition associated with raised pruritic nodules with a pustular head on an erythematous base; it is similar to bacterial folliculitis. Biopsy specimens of these lesions reveal intense infiltration of eosinophils and absence of polymorphonuclear cells and organisms. Xerosis and ichthyosis are also common in patients with advanced HIV disease and may be refractory to therapy with emollients and antiinflammatory agents; antihistamines may provide symptomatic relief. Prurigo nodularis appears as nodules and papules caused by chronic rubbing and scratching. This is precipitated by one of the many causes of pruritus in HIV-infected patients, such as xerosis, eosinophilic folliculitis, and atopic dermatitis.

Disseminated cryptococcosis and histoplasmosis may cause mucocutaneous papules, nodules, pustules, or ulcers (Figs. 122.16 and 122.17). Biopsy and culture establish the diagnosis. Molluscum contagiosum can also cause small papules; the causative agent is molluscum contagiosum virus, a poxvirus. In Thailand and southern China, *T. marneffei* is a common opportunistic fungal infectious agent in patients with AIDS.[268] Patients can present with umbilicated papules, subcutaneous nodules, or morbilliform eruptions. Diagnosis is established by identifying the ellipsoid organism with central septation and characteristic red pigment production when grown in culture. Nontuberculous mycobacteria such as *M. avium* complex and *M. haemophilum* may cause cutaneous papules, pustules, abscesses, lymphadenitis, or ulcerations. Culture is required for diagnosis.



FIG. 122.15 Eosinophilic dermatitis. *(Courtesy Dr. Stephen Raffanti.)*



FIG. 122.16 Cryptococcosis. *(Courtesy Dr. Stephen Raffanti.)*

Scabies

Sarcoptes scabiei var. *humanus* is the mite responsible for scabies, a common ectoparasitic infestation, in HIV-infected individuals. Scaly pruritic papules or hyperkeratotic plaques may occur on the palms, soles, trunk, or extremities. Characteristic burrows between the fingers and on the wrists are not always seen. Norwegian (crusted) scabies (Fig. 122.18) is a severe and highly contagious manifestation of this disease, seen particularly with advanced immunosuppression.[269] Permethrin 5% cream and ivermectin are effective therapies.[270]

Renal Disease

A number of renal abnormalities have been described in patients with HIV infection since early in the epidemic, and the spectrum of conditions recognized has expanded over time, as has our understanding of the pathogenesis of these disorders.[247] Similar to metabolic disease, kidney dysfunction may result from direct effects of HIV itself, immune-mediated responses to HIV infection, opportunistic infections, and chronic inflammation in individuals successfully treated with ART. In addition, drug toxicity from antiretroviral agents and drugs used to treat opportunistic diseases and other complications is a major cause of renal problems in patients with HIV infection. Metabolic disorders such as



FIG. 122.17 Histoplasmosis. *(Courtesy Dr. Stephen Raffanti.)*



FIG. 122.18 Norwegian scabies (in a patient with wasting syndrome). *(Courtesy Dr. Stephen Raffanti.)*

diabetes and cardiovascular disease increase the risk of developing kidney damage as well, and risk factors for cardiovascular disease and chronic kidney disease are multiplicative.[271] Other conditions that mediate renal pathologic processes including injection drug use and HBV and HBC infection may also affect the kidneys.

Pentamidine, foscarnet, and the aminoglycosides can cause acute tubular necrosis, and indinavir, sulfadiazine, and intravenous acyclovir can cause intratubular obstruction by crystal formation. The widely used nucleoside reverse transcriptase inhibitor tenofovir disoproxil fumarate (TDF) accumulates in renal tubular cells via active transport by organic anions and can cause tubular dysfunction including proteinuria, diabetes insipidus, acute tubular necrosis, and, unusually, Fanconi syndrome. Patients with a normal glomerular filtration rate at the onset of treatment with TDF experience modest declines in glomerular filtration rate over time but rarely progress to chronic kidney disease. Individuals with underlying kidney impairment or low body weight may have a marked loss of renal function after initiating TDF, and the drug is not recommended for patients with chronic kidney disease. A newer formulation of a tenofovir prodrug, tenofovir alafenamide (TAF), does not undergo organic anion transport and does not accumulate in renal tubular cells, although it does achieve higher intracellular concentrations in peripheral blood mononuclear cells. TAF had less nephrotoxicity in clinical trials and resulted in improvement in renal dysfunction in patients switched from TDF-containing regimens. TAF is now recommended to replace TDF in US and other treatment guidelines.[162] Thrombotic microangiopathy and thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome have been reported in HIV-infected individuals and are associated with high HIV-1 viral loads and advanced immunosuppression. Hypertension may be a prominent feature, and the condition may respond to ART.[272] Immune complex–mediated glomerular diseases such as those associated with immunoglobulin A (IgA) nephropathy[273] and HCV infection[274] have also been reported.

The predisposition of patients of African descent to develop renal disease has been linked to polymorphisms in the *APOL1* apolipoprotein gene, which is hypothesized to have provided evolutionary protection against trypanosomal infections in Africa.[275,276] Although a variety of renal pathologic processes occur in patients of all races, and chronic renal insufficiency due to a variety of causes is not uncommon, black patients develop end-stage renal disease (ESRD) considerably more often than white patients, a consequence of the *APOL1* polymorphisms.[277] One study noted that more than 90% of patients with biopsy-proven HIV-associated nephropathy (HIVAN) had *APOL1* risk alleles. The pathogenesis of *APOL1*-associated nephropathies is not well understood but may involve changes in ion flux, mitochondrial dysfunction, and increases in inflammation.[278]

Manifestations of HIVAN include proteinuria, mildly elevated serum creatinine levels, and normal-sized echogenic kidneys on renal ultrasonography. Biopsy specimens show focal and segmental glomerulosclerosis on histopathology with glomerular injury, mesangial proliferation, tubular degeneration with microcyst formation, and capillary collapse.[279] Patients usually have detectable HIV RNA in the plasma.[279,280]

Renal dysfunction in patients with HIV disease is usually diagnosed incidentally when patients present with opportunistic infections and have a $CD4^+$ count less than 200 cells/mm^3.[281] Asymptomatic proteinuria, up to 5 g/day, is often the initial finding, and the serum creatinine level is often normal or only mildly elevated. The albumin concentration is almost always low, as is true for most patients with AIDS with opportunistic infections, and the blood pressure is usually normal. Renal biopsy most often shows focal and segmental glomerulosclerosis (collapsing glomerulosclerosis) with severe tubulointerstitial disease and proliferative microcyst formation. Immunofluorescence studies often reveal deposits of IgM and C3, and electron microscopy shows tubuloreticular inclusion bodies,[281] underscoring the association of this genetic association, but the presence of HIVAN in the small number of individuals without risk alleles indicates that other factors are also involved.[282,283]

The clinical course of HIVAN progresses quickly, usually because many other opportunistic processes occur simultaneously.[284] The optimal management for HIVAN includes rapid initiation of ART,[285,286] short courses (8–12 weeks) of corticosteroids,[287] and use of angiotensin-converting enzyme inhibitors for patients who can tolerate them.[288] Immunosuppressive therapy with cyclosporine has been tried but is probably not superior to corticosteroid therapy.[289]

A recent large observational cohort study demonstrated that the risk of ESRD remains high among HIV-infected individuals but is declining with improvements in viral suppression.[290] ESRD remains more frequent in black patients with HIV infection. Kidney transplantation to HIV-positive recipients appears to be feasible from HIV-negative as well as HIV-positive donors as long as viral suppression is maintained.[291,292]

Ocular Complications

Ocular diseases are extremely common manifestations of HIV disease with a wide variety of causes ranging from a benign HIV retinopathy to sight-threatening viral opportunistic infections.[293–295] As HIV disease progresses, the risk for ocular complications increases appreciably.

Before widespread initiation of ART, HIV retinopathy occurred in approximately one-third of all patients with HIV, with the prevalence higher in patients with low $CD4^+$ counts. The most frequent finding is a cotton-wool spot, a small pale lesion that is thought to represent transient focal retinal ischemia. HIV retinopathy may also produce microaneurysms and retinal hemorrhages. The condition is generally benign, although some patients have developed visual defects attributed to this condition.

The most common and serious ocular complication of HIV disease is retinitis, most often caused by CMV (see Chapter 137). CMV is ubiquitous in patients with HIV infection and causes serious morbidity in patients with AIDS. CMV is transmitted by the same routes as HIV, and almost all patients with sexually acquired HIV infection are also infected with CMV. Similar to other herpesviruses, CMV may infect cells latently and be reactivated when host defenses are impaired. Asymptomatic CMV viruria and viremia may be found in one-third to one-half of patients with advanced HIV disease. The risk for CMV retinitis is determined largely by the $CD4^+$ count and by the CMV DNA level in the peripheral blood. For patients with $CD4^+$ counts less than 200 cells/mm^3, the annual risk is 4% to 12%. Further risk stratification is aided by CMV DNA measurements, with aviremic patients having less than 1% risk per year, even at low $CD4^+$ counts.[296]

CMV has a unique predilection for the retina, with 90% of end-organ disease in patients with HIV infection being retinitis.[297] Other involved sites include the colon, esophagus, stomach, adrenals, pancreas, brain, and lungs. The onset of CMV retinitis may be insidious or rapid. Patients complain of painless, progressive visual loss, blurring, and floaters. CMV retinitis usually arises unilaterally, although it may subsequently progress to the contralateral retina. Funduscopic examination of the involved eye typically reveals coalescing white exudates in a vascular pattern, with surrounding hemorrhage and edema. Lesions are often peripheral initially, involve the fovea later, and result in visual loss. Retinal detachment may occur as a late complication.

The advent of ART has resulted in astonishing declines in the incidence of CMV retinitis. Several population-based studies have reported 60% to 90% reductions in the incidence of this disease. Improvement in cell-mediated immunity with ART results in suppression of CMV DNA in plasma, with a subsequent decrease in the risk for disease. It appears that patients with treated CMV retinitis who receive ART may safely discontinue anti-CMV therapy if their $CD4^+$ count rises to more than 100 cells/mm^3 for at least 3 to 6 months.[298,299] For patients with $CD4^+$ counts less than 50 cells/mm^3, education regarding retinitis symptoms and regular ophthalmologic examinations are recommended. Patients complaining of ocular symptoms should undergo a thorough ophthalmologic examination. Retinal findings may include cotton-wool spots or lesions of infectious retinitis. Less common retinal infections include toxoplasmosis, pneumocystosis, varicella-zoster virus, and ocular syphilis, which is usually a diffuse intraocular process. Cotton-wool spots are prevalent in patients with AIDS but do not appear to predict the development of other retinal disease. The cotton-wool spots are distributed in a vascular pattern similar to that of CMV but do not have the irregular pattern of full retinal exudates and hemorrhages characteristic of CMV retinitis. An ophthalmologist or other highly trained observer should examine any patient with signs or symptoms of retinitis promptly because delay in therapy can result in irreversible visual loss. Cultures of the blood and urine yield CMV in 60% and

80% of cases, respectively, although the diagnosis rarely rests entirely on these results.

Patients who have had CMV retinitis frequently experience acute retinal detachment. Erosion of the retinal border at the site of a necrotic lesion allows the retina to be lifted off underlying tissues. Patients complain of sudden loss of vision ("like a curtain falling" in front of the affected eye). Surgical reattachment is often partially successful in restoring vision, although progressive visual loss may ensue. Treatment options include oral valganciclovir, intravenous ganciclovir, intravenous foscarnet, and intravenous cidofovir in combination with ART (see Chapter 137).[298]

Varicella-zoster retinitis is a severe necrotizing retinitis (progressive outer retinal necrosis) that may occur in patients with advanced HIV disease and low $CD4^+$ cell counts, although some cases occur at earlier stages of HIV disease.[300] Patients most often note rapid visual loss. Funduscopic findings include peripheral necrosis, occlusive vasculopathy, optic neuritis, and vitreal and scleral inflammation, and the syndrome is termed *acute retinal necrosis.* Varicella-zoster retinitis usually occurs in the absence of zoster at other sites, but a history of varicella-zoster virus disease is common, and the virus may be isolated from tissue samples in some patients.[301] Blindness ensues despite therapy in most patients, although responses to intravenous ganciclovir and foscarnet plus intravitreal injections of ganciclovir or foscarnet or both have been reported.[298] Optimization of ART is also recommended.

Ocular toxoplasmosis occurs in patients with advanced immunodeficiency, and many, but not all, have cerebral toxoplasmosis. *Toxoplasma* retinitis is characterized by discrete, rounded, pale exudates. Lesions are usually discrete foci of retinal inflammation without hemorrhage or vasculopathy. Vitreal inflammation is common. The diagnosis is made by observation by an experienced ophthalmologist.

P. jirovecii may cause a choroiditis that mimics CMV retinitis.[302] The lesions are typically posterior, are yellow-orange, and do not cause vitreal inflammation. Choroidal pneumocystosis occurs most often in patients with previous *P. jirovecii* pneumonia, particularly in patients taking aerosolized pentamidine for prophylaxis.

Cardiac Manifestations

Early clinical observations suggested that HIV infection spared the heart, as memorialized in the activist/playwright Larry Kramer's award-winning play *The Normal Heart.* Subsequent experience showed that cardiac involvement in HIV and AIDS is not unusual.[303,304] Cardiac abnormalities in patients with HIV infection may include opportunistic infections or diseases of the myocardium (e.g., *T. gondii, Trypanosoma cruzi*) or pericardium (e.g., mycobacteria, Kaposi sarcoma), left ventricular dysfunction and dilated cardiomyopathy, cardiac autonomic abnormalities, and vascular heart disease such as pulmonary hypertension. Infectious endocarditis may also occur in patients with HIV infection, especially injection drug users, but there is little evidence to suggest that the risk is increased after accounting for behaviors. Marantic endocarditis is a manifestation of late-stage HIV disease and is sometimes noted at autopsy.

In patients with long-standing but treated HIV infection, accelerated atherosclerosis with myocardial infarction is increasingly common.[207] Population-based studies have demonstrated that rates of myocardial infarction and cardiac death are increased in HIV-infected veterans compared with control subjects, and HIV-infected men in the Multicenter AIDS Cohort Study have an increased prevalence of coronary calcification and total (but not calcified) coronary artery plaque than HIV-negative men.[305,306] The risk for coronary disease is associated with age, duration of HIV infection, nadir $CD4^+$ lymphocyte count, and duration of ART, suggesting in part that chronic immune activation plays a mechanistic role in the development of cardiac disease. This is consistent with the results of the Strategies for Management of Antiretroviral Therapy (SMART) study,[307] in which patients randomly assigned to interrupted ART had increased rates of cardiac death, which was associated with elevations in proinflammatory cytokines and prothrombotic markers.

Myocardial disease in HIV-infected individuals is surprisingly common, particularly in late-stage disease. Infectious myocarditis has been reported with a number of opportunistic infections, notably *T. gondii.* In an autopsy-based study in France, for example, 12% of patients with AIDS who died were found to have cardiac toxoplasmosis.[308] Most patients with cardiac toxoplasmosis also had cerebral involvement, although several patients had isolated toxoplasmic myocarditis. Myocarditis may be seen in acute *T. gondii* infection, and coincident toxoplasmic pneumonitis may present a clinical picture of diffuse pulmonary infiltrates and cardiac insufficiency. Other opportunistic agents causing myocardial involvement include mycobacteria and fungi.

A more common and underdiagnosed disorder in HIV-infected individuals is cardiomyopathy with left ventricular dysfunction, which may result in congestive heart failure.[309] Several large cohort studies in the pre-ART era found that 8% to 12% of patients with HIV infection had echocardiographic evidence of left ventricular dysfunction, and the incidence of dilated cardiomyopathy with severe congestive heart failure (New York Heart Association class III or IV) was 15% to 18% per year.[310,311] Clinical evaluation of patients may reveal only complaints of fatigue and exertional dyspnea, and physical examination may show tachycardia without rales or overt signs of congestive heart failure. Echocardiography shows global hypokinesis and enlargement of all four chambers, with a modestly to severely reduced left ventricular ejection fraction and increased end-diastolic volume index. Chest radiography is frequently unhelpful. The cause of HIV-related cardiomyopathies is multifactorial, with conflicting data on the causative role of cardiotropic viruses, vitamin deficiencies, and cardiotoxic drugs. However, there is compelling evidence that HIV itself is involved in the pathogenesis of cardiomyopathy in a large proportion of patients.[310,311] HIV has been identified in myocardial tissue by in situ hybridization in several studies and found to be positive in one-third to two-thirds of patients with myocarditis. In addition, an inflammatory cellular infiltrate composed of major histocompatibility complex class I–expressing $CD8^+$ cells is found in a large proportion of patients, suggesting an autoimmune mechanism in this process. Other viruses also implicated in dilated cardiomyopathy in patients with HIV infection include Epstein-Barr virus, coxsackieviruses, and CMV. Supportive treatment with digoxin, diuretics, and afterload reduction is usually of symptomatic benefit, and some patients with inflammatory myocarditis respond to corticosteroid therapy. Nonetheless, the prognosis for patients with this finding is poor,[312] although combination ART might change this.

Pericardial effusions have been reported in varying proportions of patients in a number of studies, many of which have had selection bias as a limitation. In patients with more advanced illness in hospital-based settings, pericardial effusions can be common. For example, a Portuguese study found that 41% of 181 HIV-infected patients had pericardial effusions.[313] Most effusions in this setting are asymptomatic or arise with signs and symptoms of opportunistic disease at other sites. The causes of pericardial disease in patients with HIV infection are diverse, but opportunistic infections and malignancies are the agents most commonly implicated. Mycobacterial infections, especially tuberculosis, are frequently associated with pericardial involvement in areas in which coinfection with HIV and *M. tuberculosis* is common, and nontuberculous mycobacteria may also invade the pericardial space. Other infections seen in the pericardium include bacterial infections, fungal infections (e.g., *Cryptococcus*), and viral cardiopulmonary infections. Kaposi sarcoma and non-Hodgkin lymphoma may also cause pericardial effusion. Cardiac tamponade or cardiac dysfunction resulting from effusions is unusual in most series, and in most cases pericardiocentesis is not necessary as a therapeutic maneuver. One study, however, reported that 40% of patients with an HIV-related pericardial effusion had signs of tamponade.[314]

Hematologic Manifestations

HIV infection can affect all three hematologic cell lines—leukocytes, red blood cells, and platelets. Although neutropenia and anemia are seen primarily in advanced disease, thrombocytopenia can occur in early-stage disease (e.g., HIV-related thrombocytopenia) or late-stage disease (thrombotic thrombocytopenic purpura). Neutropenia may be caused by HIV infection itself or may be an adverse effect of therapy. In one study, neutropenia was an independent risk factor for bacterial infection after controlling for $CD4^+$ count.[315] In another study of 71 patients with an absolute neutrophil count less than 1000 cells/mm^3 for a median of 13 days, only 6 (8%) developed culture-proven infection.[316]

The risk for infection increased with decreasing $CD4^+$ counts, but most episodes were mild and self-limited. Anemia is common in advanced HIV infection and is an independent risk factor for mortality.[317,318] HIV-related thrombocytopenia is a result of both immune-mediated platelet destruction (as is seen in idiopathic thrombocytopenic purpura) and impaired platelet production.[319] Patients are usually asymptomatic, even with profound thrombocytopenia. The thrombocytopenia usually responds to ART; this has been best studied with zidovudine but has also been shown with ART.[320,321] Thrombotic thrombocytopenic purpura in an HIV-infected person was first described by Jokela and colleagues in 1987.[322] The classic diagnostic pentad includes thrombocytopenia, microangiopathic hemolytic anemia, renal failure, fever, and neurologic abnormalities, although not all patients have all these findings. In addition, given the high frequency of many of these findings in patients with advanced AIDS, such a diagnosis may not always be considered. The incidence of thrombotic thrombocytopenic purpura has appeared to be decreasing in the ART era.[323]

Non–AIDS-Related Cancers

Malignancies termed *AIDS-related* were those originally identified occurring as epidemics in populations in which they were usually rare (e.g., Kaposi sarcoma and non-Hodgkin lymphoma in young MSM). These conditions were included in the CDC early case definition of AIDS to ensure sensitivity of the surveillance process. Cervical cancer was added to the case definition subsequently when cases appeared out of proportion in HIV-infected women. In recent years, however, a number of cancers have been detected in populations with HIV infection in excess of what would be expected based on rates in HIV-seronegative people in the general population. Both AIDS-related and non–AIDS-related cancers remain an important cause of illness and death in people with HIV infection; in a large North American cohort between 1995 and 2009, as use of ART dramatically increased and life expectancy improved, cancers accounted for 10% of AIDS deaths.[324–326] Increases in non–AIDS-related cancers have been notable in particular for liver cancer, lung cancer, head and neck tumors, and anal cancer. The use of ART has substantially reduced the incidence of AIDS-related cancers but appears to have little effect on rates of non–AIDS-related malignancies. As the prevalence of HIV infection increases due to declining mortality and as the infected population ages, more non–AIDS-related cancers can be anticipated.

Immune Reconstitution Syndromes

ART is associated with dramatic reductions in HIV-1 RNA and increases in $CD4^+$ lymphocyte counts. In addition to decreasing HIV-related mortality and the incidence of opportunistic infections significantly (as discussed earlier), the improvement in immune function can be associated with paradoxical worsening of underlying opportunistic infections.[327,328] The clinical presentation of immune reconstitution inflammatory syndrome (IRIS) varies according to the pathogen (see later discussion). Not every patient who has immunologic improvement with ART experiences IRIS; the risk factors for the development of these syndromes are not well understood.

Tuberculosis

Paradoxical worsening of tuberculosis was initially described in HIV-seronegative patients with tuberculosis before the HIV era, but it appears to be more common in HIV-infected patients.[74] Clinical manifestations include fever, adenopathy (e.g., cervical, thoracic, intraabdominal), and worsening pulmonary infiltrates. Central nervous system tuberculomas, pleural effusions, and psoas abscesses have also been described.[73,74,329,330] Diagnoses such as lymphoma, adverse drug reaction, and tuberculosis treatment failure related to nonadherence, malabsorption, or drug resistance must be excluded before the diagnosis of tuberculosis IRIS can be established. Treatment with ART has been associated with an increased risk for IRIS in some, but not all, studies; the incidence rate ranges from 7% to 43%.[73,74,329–332] Patients who experience IRIS with ART usually do so within 2 to 3 weeks of starting therapy. Clinical management includes continuation of antituberculosis therapy and ART and the use of nonsteroidal antiinflammatory drugs for symptomatic relief. For severe manifestations such as compromise of the airways or venous return to the heart, corticosteroids (e.g., prednisone 1.5 mg/kg/day for 2 weeks followed by 0.75 mg/kg/day for 2 weeks[332a]) may be given. Unmasking of previously unrecognized tuberculosis after ART initiation has also been reported, although this may represent progression of untreated tuberculosis that is coincidental to the start of HIV therapy.[125,126]

Mycobacterium avium Complex Disease

Focal or diffuse lymphadenitis developing within 2 to 3 months of initiation of ART has been described in several patients with low baseline $CD4^+$ counts.[124,333] Mycobacteremia is generally not present, but cultures of lymph nodes usually grow *M. avium*. There have also been reports of focal osteomyelitis developing in patients who discontinue primary or secondary *M. avium* prophylaxis after sustained increases in $CD4^+$ counts during ART.[334,335] Treatment is the same as for *M. avium* infection in the absence of immune reconstitution.

Cytomegalovirus Disease

CMV retinitis in the setting of immune reconstitution (immune recovery uveitis) has been described in patients with and without a history of CMV retinitis before initiation of ART.[336–338] Development of CMV disease at extraocular sites (e.g., bloodstream, colon, pancreas) in patients without prior CMV disease has also been described.[339] Among patients with a history of CMV retinitis who subsequently initiate and respond to ART, 18% to 63% develop immune recovery uveitis.[340,341] In one study the median time of ART until development of uveitis was 43 weeks.[340] The optimal management is unclear. Oral corticosteroids with or without concomitant anti-CMV therapy are often used.

Infection With Varicella-Zoster Virus

There have been several reports of the development of herpes zoster in patients receiving ART. In two series, herpes zoster developed in 7% to 8% of patients receiving combination ART and occurred within 17 weeks of initiation of therapy.[342,343] In one report, herpes zoster was associated with an increase in $CD8^+$ lymphocytes.[342] The clinical manifestations are usually not severe and respond to acyclovir or famciclovir.

Viral Hepatitis

Patients infected with HCV can develop acute hepatitis or cirrhosis during ART.[344,345] It is difficult to discern whether elevations in hepatic aminotransferase levels are caused by toxicity of ART or immune reconstitution: there is no reliable clinical or laboratory parameter that distinguishes between these two entities. In a study of 60 patients coinfected with HIV and HCV who completed at least 16 weeks of ART, immune recovery was associated with a persistent increase in HCV RNA, particularly in patients with baseline $CD4^+$ count less than 350 cells/mm^3.[346,347] Other studies have found that hepatotoxicity in coinfected patients who respond to ART is associated with increased HCV-specific immune responses and T-cell activation.[348] This suggests that at least in some cases hepatotoxicity is associated with immune reconstitution. There have also been reports of acute hepatitis developing during ART in patients with prior HBV infection in whom increased HBV RNA levels are detected.[349] Patients with chronic HBV infection are at increased risk for hepatotoxicity from ART, and withdrawal of antiretroviral drugs that have activity against HBV such as tenofovir or lamivudine may result in worsening of hepatic function and increased aminotransferase levels, which may be caused by immune mechanisms or loss of viral suppression.[350] Management of patients with chronic hepatitis is discussed in Chapter 117.

Other Diseases

Paradoxical worsening in the setting of immune reconstitution has also been described after initiation of ART with infection with *Cryptococcus neoformans,*[351,352] *P. jirovecii* pneumonia,[353,354] progressive multifocal leukoencephalopathy,[355] and Kaposi sarcoma.[356] It has also been seen with Hodgkin and non-Hodgkin lymphoma.[357–359] Of particular concern is IRIS that involves the central nervous system because of the high associated morbidity and mortality. This has been seen with infection with *C. neoformans* and progressive multifocal leukoencephalopathy in addition to tuberculosis.

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123 Pulmonary Manifestations of Human Immunodeficiency Virus Infection

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SHORT VIEW SUMMARY

EPIDEMIOLOGY

- Human immunodeficiency virus (HIV) infection increases the risk of infectious and noninfectious respiratory conditions.
- Most complications occur in those not receiving antiretroviral therapy.
- Effective antiretroviral therapy has reduced, but not eliminated, the excess risk of pulmonary infections such as *Pneumocystis jirovecii* pneumonia (PCP) and to a lesser extent bacterial pneumonia.
- Noninfectious conditions, such as chronic obstructive pulmonary disease and lung cancer, are assuming greater importance as HIV-infected individuals live longer.

APPROACH TO THE PATIENT

- The differential diagnosis is influenced by the stage of immunosuppression, antiretroviral therapy, prophylaxis for opportunistic infections, and the local epidemiology of conditions such as tuberculosis and endemic fungal infections.
- Lower CD4 cell counts increase the risk of all pulmonary infections, including those that also occur at higher CD4 cell counts.
- Findings on chest radiography can help guide the differential diagnosis (see Table 123.2).

PNEUMOCYSTIS JIROVECII PNEUMONIA

- Most cases of PCP occur at CD4 cell counts of fewer than 200 cells/mm^3 in patients not receiving antiretroviral therapy.
- Patients with PCP typically report cough and fever of at least several weeks' duration.
- Radiographic findings include diffuse interstitial infiltrates most commonly, but cavities, pneumothoraces, pleural effusions, and normal radiographs can occur.
- Diagnosis relies on identification of the organism in respiratory secretions, often through induced sputum or bronchoalveolar lavage, which is more sensitive.
- Serum (1→3)-β-d-glucan is also highly sensitive for the diagnosis of PCP.

BACTERIAL PNEUMONIA

- *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most commonly isolated pathogens.
- With worsening immunosuppression, pneumonias due to *Staphylococcus aureus, Pseudomonas aeruginosa* (especially in the setting of neutropenia), *Nocardia* spp., and *Rhodococcus equi* can occur.

MYCOBACTERIAL PNEUMONIA

- The features of *Mycobacterium tuberculosis* pulmonary infection vary based on the degree of immunosuppression.
- HIV-infected patients with respiratory symptoms who are from tuberculosis-endemic areas or have other risk factors for tuberculosis, who have a positive test for latent tuberculosis without a history of prophylactic therapy, or who have a personal history of tuberculosis without documentation of appropriate treatment should be placed in negative air pressure rooms until the diagnosis of pulmonary tuberculosis is excluded.
- Sputum nucleic acid amplification tests can hasten diagnosis, particularly in smear-negative cases.

FUNGAL PNEUMONIA

- Fungal pneumonia tends to occur with CD4 cell counts <100 cells/mm^3, often as a component of disseminated fungal infection (cryptococcosis, histoplasmosis, coccidioidomycosis, blastomycosis).
- Histoplasmosis and coccidioidomycosis are important causes of pneumonia in those who have resided in or traveled to endemic areas; *Blastomyces* rarely causes pulmonary infection in patients with HIV.
- Pulmonary aspergillosis usually occurs in severely immunocompromised individuals with neutropenia or corticosteroid use.

VIRAL PNEUMONIA

- During the influenza season, influenza is the most common diagnosis in HIV-infected patients with fever and respiratory symptoms, although adenovirus, respiratory syncytial virus, and parainfluenza virus can cause a similar presentation.

NONINFECTIOUS RESPIRATORY DISORDERS

- Pulmonary Kaposi sarcoma usually occurs with cutaneous or mucosal disease.
- Pulmonary lymphoma causes nodules, masses, or pleural effusions and is usually diagnosed via video-assisted thoracoscopic surgery (VATS).
- Lymphocytic interstitial pneumonitis, a rare cause of interstitial infiltrates, is typically diagnosed on biopsy. It primarily occurs in children but may develop in adults, particularly those with higher CD4 counts than are seen with many opportunistic infections.

Respiratory complications of human immunodeficiency virus (HIV) infection remain a significant source of morbidity and mortality, even after the introduction of combination antiretroviral therapy in 1996.[1] Although the incidence rate of these complications has since declined, they are frequently the sentinel event that brings a person with unknown HIV status to medical attention. They also commonly occur in patients with poor adherence to their HIV treatment.

EPIDEMIOLOGY

The Pulmonary Complications of HIV Infection Study followed 1100 patients with HIV from 1988 to 1994.[2] The study also included 167 control subjects who were negative for HIV. The study preceded the availability of protease inhibitors and the widespread use of combination antiretroviral therapy, but it provided the most accurate data regarding the incidence of specific pulmonary infections among individuals with HIV. Importantly, the study included the era after which prophylaxis against *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) pneumonia (PCP) became the standard of care. During the 5 years of follow-up, acute bronchitis was the most common lower airway infection; it occurred twice as often in those with HIV infection as in controls. By far, the two most common acquired immunodeficiency syndrome (AIDS)–defining complications were bacterial pneumonia and PCP. These occurred with approximately equal frequency, and follow-up showed higher rates of pneumonia with declining CD4 cell counts. Other specific causes of opportunistic infections occurred relatively infrequently and included infection with cytomegalovirus (CMV), *Aspergillus*, cryptococci, and herpes simplex virus.

The use of combination antiretroviral therapy has dramatically diminished the incidence of all of these complications, with some evidence of a greater decline in PCP than in bacterial pneumonia.[1,3] For the former, this decline occurred independently of PCP prophylaxis.[4] However, even among individuals with normal or near-normal CD4 cell counts who are undergoing antiretroviral therapy, the rate of bacterial pneumonia is significantly higher among those infected with HIV than

in uninfected control subjects.[5,6] Long-term administration of anti-*Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX) in particular and *Mycobacterium avium* complex prophylaxis with macrolide antibiotics likely reduces the incidence of bacterial pneumonia.[7–10] Treatment with TMP-SMX, however, has been shown to increase the rate of colonization with resistant bacteria, especially pneumococci.[11]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of a person with HIV infection with respiratory symptoms is exceedingly broad and includes infectious, neoplastic, and cardiovascular etiologies (Table 123.1). However, as noted previously, certain conditions (in particular, PCP and bacterial pneumonia) occur far more commonly than others; hence, the diagnostic evaluation and initial empirical therapy should be focused toward these diagnoses.

Certain historic, clinical, and radiographic clues help determine the likelihood of specific opportunistic infections and are discussed in the following sections.

TABLE 123.1 Causes of Pulmonary Disease Associated With HIV Infection

Most Common
Bacterial
No organism identified, but responsive to antibacterial therapy
Streptococcus pneumoniae
Haemophilus influenzae
Mycobacterial
Mycobacterium tuberculosis[b]
Fungal
Pneumocystis jirovecii
Less Common but Clinically Important in Some Settings
Bacterial
Pseudomonas aeruginosa
Staphylococcus aureus
Enterobacteriaceae
Legionella spp.
Nocardia spp.
Rhodococcus equi
Mycobacterial
Mycobacterium kansasii
Mycobacterium avium complex
Fungal
Cryptococcus neoformans
Histoplasma capsulatum
Coccidioides spp.
Aspergillus spp.
Blastomyces dermatitidis
Talaromyces marneffei
Viral
Influenza
Cytomegalovirus
Herpes simplex virus
Adenovirus
Respiratory syncytial virus
Parainfluenza virus
Parasitic
Toxoplasma gondii
Strongyloides stercoralis
Microsporidia spp.
Cryptosporidium parvum
Noninfectious
Chronic obstructive pulmonary disease
Lung cancer
Kaposi sarcoma
Non-Hodgkin lymphoma
Primary pulmonary hypertension
Congestive heart failure
Lymphocytic (or lymphoid) interstitial pneumonitis
Abacavir hypersensitivity

[a]Causes are listed within categories in approximate order of frequency.
[b]Frequency of *M. tuberculosis* infection is highly dependent on local rates of tuberculosis and the patient's exposure history.

Clinical or Laboratory Stage of Immunosuppression

The majority of cases of PCP occur in individuals with CD4 cell counts of fewer than 200 cells/mm.[3,12–14] If the CD4 cell count is unavailable, helpful clinical correlates are the presence of oral candidiasis, which is known to correlate with advanced HIV-related immunosuppression and the risk of PCP, and other clinical markers of advanced HIV disease, such as weight loss. Progressive immunosuppression increases the risk of all pulmonary infectious processes, including those that may occur with relatively preserved CD4 cell counts, such as bacterial pneumonia and tuberculosis (TB).

Tempo of the Illness

Individuals with HIV with bacterial pneumonia typically present in much the same way as those without HIV infection: with a relatively acute illness (measured in days) characterized by fever and chills and sometimes accompanied by pleuritic pain and sputum production.[15] PCP, in contrast, typically manifests as a subacute to chronic illness of several weeks' duration, with the most prominent symptoms being fever and shortness of breath. Other helpful clues to PCP include chest tightness (specifically a sense of difficulty in taking a full breath) and a report that a typical day's activities (climbing stairs, conversing on the telephone) are now associated with dyspnea.

Receipt and Type of *Pneumocystis* Prophylaxis

Patients receiving TMP-SMX for *Pneumocystis* prophylaxis rarely have breakthroughs of PCP if they are compliant with therapy. In contrast, second-line prophylaxis, such as atovaquone, dapsone, and aerosolized pentamidine, is associated with small but significant rates of treatment failure.[7,16–18] As noted previously, TMP-SMX reduces the risk of bacterial infections, including pneumonia. However, given the high rates of pneumococcal infection among patients with HIV, as well as the concurrently high rate of pneumococcal resistance to TMP-SMX and macrolides, receipt of these drugs for prophylaxis is partially protective at most.

Receipt of Antiretroviral Therapy

In addition to its obvious beneficial effect on HIV RNA levels and CD4 cell counts, patients who receive continuous antiretroviral therapy have a lower rate of pneumonia than a comparable group undergoing intermittent therapy. This was demonstrated in a randomized, controlled trial comparing immediate to deferred antiretroviral therapy in patients with CD4 cell counts greater than 500 cells/mm.[19] The favorable effect of HIV treatment on reducing pneumonia incidence may be in part the result of a reduction in pulmonary viral replication and improvement in lung CD4 cell function and number.[20]

History of Opportunistic Processes

Some patients with HIV appear to have prominent B-cell dysfunction and thus are at a much greater risk for the development of encapsulated bacterial infections, in particular those from *Streptococcus pneumoniae* and *Haemophilus influenzae*. Such individuals frequently have a history of multiple visits or hospitalizations for bacterial pneumonia,[10] as well as episodes of otitis media, bronchitis, and other bacterial respiratory infections. In addition, persons with a history of *Pneumocystis* infection are at greater risk for relapse, in particular if they are not receiving antiretroviral therapy or PCP prophylaxis with TMP-SMX.[7]

Injection Drug Use and Smoking

Persons with HIV who are active users of intravenous drugs are more likely to have invasive bacterial infections, including pneumonia, compared with individuals with HIV acquired from other routes.[6] As with other populations, smoking increases the risk of pneumonia

regardless of whether the patient is receiving antiretroviral therapy or not.[19] Chronic obstructive pulmonary disease (COPD) is an independent risk factor for community-acquired pneumonia, pulmonary TB, and PCP in persons living with HIV; HIV infection is an independent risk factor for acute exacerbations of COPD.[21–23]

Local Epidemiology or Past Residence

The incidence of TB as a complication of HIV infection varies markedly depending on local rates of TB and the patient's exposure history. In the United States, a majority of TB cases occur in people who have emigrated from TB-endemic areas; many of these areas (such as sub-Saharan Africa and parts of the Caribbean) also have high background rates of HIV infection. In addition, homelessness and incarceration both increase the risk of TB exposure and subsequent disease. A person's past residence or travel history may elicit potential exposure not only to TB but also to diseases caused by endemic fungi, such as histoplasmosis, coccidioidomycosis, and blastomycosis.

Characteristic Radiographic Findings

Although no radiographic finding is pathognomonic for a specific microbiologic diagnosis, certain patterns suggest various diagnoses (Table 123.2). Patients with bacterial pneumonia are more likely to have a focal infiltrate on chest radiography, whereas those with PCP typically show diffuse interstitial infiltrates.[15,24] Importantly, many exceptions to these generalizations exist, including the presence of a normal chest radiograph in up to 10% of those with PCP or TB and the occasional occurrence of diffuse interstitial infiltrates in persons with bacterial pneumonia, in particular that caused by *H. influenzae*.[25–27] In fact, given the wide range of radiographic findings that potentially result from PCP (including cavities, pneumatoceles, pleural effusions, nodules, and pneumothoraces), no radiographic appearance can entirely rule out the diagnosis of PCP in an HIV-infected host with advanced immunosuppression.[28]

TABLE 123.2 Radiographic Appearance of Pulmonary Diseases in HIV Infection

Diffuse Interstitial Infiltrates
Pneumocystis jirovecii
Mycobacterium tuberculosis, especially with advanced HIV disease
Histoplasma capsulatum
Coccidioides spp.
Cryptococcus neoformans
Toxoplasma gondii
Cytomegalovirus
Influenza
Lymphocytic interstitial pneumonitis
Abacavir hypersensitivity
Focal Consolidation
Pyogenic bacterial pneumonia from *Streptococcus pneumoniae, Haemophilus influenzae*
M. tuberculosis
Legionella spp.
Rhodococcus equi
Hilar Adenopathy
M. tuberculosis
H. capsulatum
Coccidioides spp.
Non-Hodgkin or Hodgkin lymphoma
Mycobacterium avium complex
Cavitary Disease
Pyogenic bacterial pneumonia from *Pseudomonas aeruginosa, Staphylococcus aureus,* Enterobacteriaceae
M. tuberculosis
C. neoformans
R. equi
Aspergillus spp.
Nocardia spp.
M. avium complex
P. jirovecii
Nodules or Masses
M. tuberculosis
C. neoformans
Aspergillus spp.
H. capsulatum
Nocardia spp.
Non-Hodgkin lymphoma
Kaposi sarcoma
Lung cancer
Normal Radiograph
P. jirovecii
M. tuberculosis

TRIAGE OF PATIENTS: INFECTION CONTROL ISSUES

Appropriate triage of patients with HIV-related respiratory infections is a frequently encountered dilemma because of the higher rates of TB in this context and the often atypical presentation of the disease. Because no clinical or radiographic presentation can entirely exclude TB, practitioners must have a high degree of suspicion and a low threshold to admit such patients to negative airflow rooms until TB has been ruled out or an alternative diagnosis has been established.[29]

Patients with HIV and respiratory disease are admitted to negative airflow rooms if any of the following conditions are present:

1. Any clinical presentation highly consistent with TB (cavitary lung disease, subacute course, weight loss)
2. Prior residence in a highly TB-endemic area
3. Other risk factors for TB exposure, such as contact with an active case, homelessness, or incarceration
4. History of positive tuberculin skin test or interferon-γ release assay results without preventive therapy
5. History of TB without documentation that appropriate treatment has been completed

The use of these criteria minimizes, but does not eliminate, the chance that a person with HIV is a source of a nosocomial exposure to TB.

DIAGNOSTIC TESTS

Diagnostic tests should begin with noninvasive studies, usually while empirical therapy for the most likely diagnoses (bacterial pneumonia and, if suspected, PCP) is started. These tests include a complete blood count with differential, a lactate dehydrogenase (LDH) test, two sets of blood cultures, a sputum Gram stain and culture, a serum cryptococcal antigen test, and a chest radiograph. If TB is being considered, a sputum acid-fast smear and culture should also be obtained and processed at a laboratory that is experienced in TB diagnostics and can provide results of acid-fast staining promptly (in 24–48 hours). Nucleic acid amplification tests can also aid rapid diagnosis with sputum (see Chapter 16) and hasten the time to discontinue respiratory precautions in patients with possible TB. The purpose of the blood cultures is to facilitate diagnosis of bacterial pneumonia, most notably from *S. pneumoniae,* with which bacteremia in patients with HIV occurs in up to 60% of cases.[30] Although not universally available, urinary pneumococcal antigen testing may provide a more rapid diagnosis of pneumococcal pneumonia than that provided by cultures. The reported sensitivity and specificity of this test are 81% and 98%, respectively.[31] Given the low specificity of sputum Gram stain and the low sensitivity of sputum culture, a positive blood culture or urinary antigen assay is often the only method for a specific etiologic diagnosis in a patient with bacterial pneumonia.

As noted in Chapter 269, LDH levels are generally elevated in patients with PCP, especially when the disease is severe enough to necessitate hospitalization. A normal level suggests an alternative diagnosis, and, conversely, the level of elevation correlates with the severity of the disease. Because many conditions cause an increase in LDH, the test has a low specificity for PCP but is a helpful diagnostic clue pending more definitive tests.[30] In many centers, an induced sputum sample (with hypertonic saline solution to induce cough) is the initial test of choice for diagnosis of PCP and sometimes TB. The sensitivity of this test for PCP varies widely in published reports, with a summary of several analyses citing an overall sensitivity of 55%.[32] Factors that may account for the varying sensitivity include patient cooperation with the test, persistence of the respiratory therapist in obtaining the

sample, and experience of the diagnostic laboratory in identifying the organism. Centers that use immunofluorescence staining for diagnosis likely have a higher sensitivity than those that use alternative staining methods (Giemsa, toluidine blue).[32,33]

If no definitive diagnosis has been identified, the decision to proceed with other diagnostic tests, both noninvasive and invasive, depends on whether the patient has responded to initial therapy. In clinical practice, a prompt clinical response to empirical antibiotic therapy directed at the most common pathogens in HIV-related pneumonia (*S. pneumoniae* and *H. influenzae*) serves as indirect evidence that alternative processes have not been overlooked.[30] For patients without clinical improvement and for a still unknown diagnosis, expedited referral for fiberoptic bronchoscopy with bronchoalveolar lavage (BAL), with or without a transbronchial biopsy, is recommended. Because BAL alone is highly sensitive for the diagnosis of PCP and because biopsy carries a risk of pneumothorax and pulmonary hemorrhage, many bronchoscopists prefer to perform BAL alone initially, reserving biopsy for a repeat procedure if still indicated. The main indication to proceed with a biopsy at the initial procedure is a high likelihood of an alternative diagnosis to PCP; this situation arises when a patient is taking TMP-SMX for PCP prophylaxis and has a relatively high CD4 cell count or an atypical-appearing chest radiograph, or if a diagnosis requiring biopsy for identification is highly suspected. Examples of such conditions include CMV or *Aspergillus* infection, lymphocytic interstitial pneumonitis, and malignant diseases other than Kaposi sarcoma (KS). Additional diagnostic studies depend on the degree of patient immunosuppression, epidemiologic risk factors, clinical course, and radiographic appearance. For example, a patient from a histoplasmosis-endemic area with chest imaging that shows hilar adenopathy along with diffuse infiltrates should undergo testing for histoplasmosis urinary antigen. Alternatively, someone with a history of neutropenia and receipt of corticosteroids who presents with pleuritic pain and pulmonary nodules may ultimately need video-assisted thoracoscopic surgery (VATS) for diagnosis of pulmonary aspergillosis. The various diagnostic strategies for patients with HIV-related pulmonary disease are summarized in Table 123.3.

TABLE 123.3 Diagnostic Tests Used in Patients With HIV Infection and Pulmonary Disease

TEST	COMMENT
Indicated in All Patients	
Complete blood count with differential	If neutropenia is present, empiric therapy covering *Pseudomonas aeruginosa* is indicated
Lactate dehydrogenase	Elevated in most patients with PCP; nonspecific
Blood cultures	Helpful for diagnosis of bacterial pneumonia, in particular from *Streptococcus pneumoniae*
Expectorated sputum	Often not available; Gram stain and culture have low sensitivity and specificity, especially after antibiotics have been started
Induced sputum for PCP stain, acid-fast bacilli stain, and culture	Initial test of choice in most centers for diagnosis of *Pneumocystis jirovecii,* although sensitivity varies widely
Indicated for Selected Patients Not Responding to Initial Empirical Therapy	
Fiberoptic bronchoscopy with BAL ± transbronchial biopsy	BAL highly sensitive for diagnosis of PCP; Kaposi sarcoma often diagnosed with visualization of characteristic purple endotracheal or endobronchial plaques, with no biopsy because of risk of bleeding Biopsy often necessary to establish alternative diagnoses such as cytomegalovirus, *Aspergillus,* or lymphocytic interstitial pneumonitis.
Video-assisted thoracoscopic biopsy	Useful in diagnosis of peripheral nodules, masses not reachable with bronchoscopic biopsy
Serum cryptococcal antigen	Nearly 100% sensitive for diagnosis of disseminated disease in patients with HIV infection; if positive, cerebrospinal fluid examination is mandatory to exclude meningitis
Serum (1→3)-β-D-glucan	Component of many fungal cell walls, including *P. jirovecii*; elevated in many patients with HIV-related PCP, including some with negative induced sputum examinations
Urinary histoplasmosis antigen	Indicated in patients residing in or from histoplasmosis-endemic areas, in particular if imaging shows diffuse or reticulonodular infiltrates and hilar adenopathy
High-resolution computed tomography scan	May help identify abnormalities not evident on chest radiography; normal study makes PCP highly unlikely
Gallium scan	Negative study rules out PCP; however, positive results are nonspecific, and other tests (high-resolution computed tomography scan, sputum induction) provide more useful information with greater rapidity

BAL, Bronchoalveolar lavage; *HIV,* human immunodeficiency virus; *PCP, Pneumocystis jirovecii* pneumonia.

SPECIFIC PATHOGENS

Pneumocystis Jirovecii Pneumonia

Early in the HIV epidemic, the principal risk factor for development of HIV-related PCP was determined to be a reduced CD4 cell count. Data from the Multicenter AIDS Cohort Study showed that the risk of PCP was markedly increased in patients with CD4 cell counts of fewer than 200 cells/mm^3; thrush and fever were other independent predictors.[12] In the postprophylaxis era, patients with PCP usually have advanced immunosuppression and CD4 counts below 100 cells/mm^3.[1] In one series of patients receiving care at an urban hospital, the median CD4 cell count for a first-time case of PCP was 36 cells/mm^3; for a recurrent case, it was 10 cells/mm^3.[34]

Care providers need to be aware that PCP remains a frequent sentinel opportunistic infection for patients who are not aware they are HIV positive and that this is also a risk factor for severe disease. In one retrospective series from Baltimore, 77% of patients with diagnosed PCP were not receiving prophylaxis and 33% were not previously known to have HIV infection. Patients not undergoing preventive therapy accounted for 100% of the deaths, 85% of the hospital days, and 100% of the cases admitted to the intensive care unit.[35] In large part because of this phenomenon and the advanced immunosuppression associated with the disease, short-term mortality from PCP remains significant despite the declining incidence of PCP that began in the early 1990s and has continued after the introduction of potent therapy in 1996.[36,37]

Risk factors for a poor clinical outcome in PCP include hypoxemia, extensive bilateral pulmonary involvement, other concurrent pulmonary infections, recurrent rather than primary disease, elevated LDH levels, and an alveolar-arterial gradient of greater than 30 mm Hg.[38–40] Because of the tendency for PCP to worsen after initiation of therapy (postulated as the result of an enhanced inflammatory response to dying organisms), adjunctive corticosteroids are indicated for partial pressure of oxygen less than 70 mm Hg or an alveolar-arterial gradient of greater than 35 mm Hg, because this intervention has been shown to reduce the risk of respiratory failure and death.[41,42] A Cochrane review concluded that the evidence supported the use of adjunctive corticosteroids to treat HIV-infected patients with PCP and substantial hypoxemia, although the number of appropriately conducted trials was small.[43] Although concern exists that use of adjunctive corticosteroids could worsen concomitant opportunistic infections (in particular, TB and CMV infections), these complications of steroid therapy in this context have been rare.[44] It is not essential to rule out concurrent infections before the use of steroids for PCP, although one should remain vigilant for such processes in a patient at risk.

Since the introduction of combination antiretroviral therapy, studies have identified additional risk factors for poor outcome, including older age, use of treatments other than TMP-SMX, concurrent culture of CMV from BAL fluid, and a CD4 cell count less than 50 cells/mm^3.[38,45] One retrospective study suggested that when controlling for other factors, receipt of combination antiretroviral therapy at the time of treatment for PCP is associated with improved survival.[46] Supporting this finding were the results of a controlled clinical trial that compared early versus delayed antiretroviral therapy in the setting of acute opportunistic infections, the bulk of which were PCP; the results showed that early

therapy was associated with a significantly reduced risk of HIV disease progression or death.[47]

Because culture of the organism in clinical laboratories is not practical, definitive diagnosis relies on visualization of cysts in respiratory secretions. As discussed previously, induced sputum is often the first diagnostic procedure, followed by BAL obtained with bronchoscopy, which has a sensitivity of 95% to 100%. Identification of cysts on appropriate stains is diagnostic of PCP because an asymptomatic carrier state does not appear to exist in HIV-infected individuals; however, persistence of organisms through and even after therapy is common and does not reflect failure of treatment.[48] A newer diagnostic technique is polymerase chain reaction (PCR) of respiratory secretions; however, this technique has been studied primarily in HIV-negative patients.[49] Serum (1→3)-β-D-glucan (β-glucan) is a component of the fungal wall of many fungi, including *P. jirovecii.* Measurement of serum β-glucan is a reliable serologic marker for PCP, particularly when respiratory symptoms are present.[50–53] In addition, the test may support the diagnosis of PCP when the clinical presentation is suggestive but sputum results are negative.[54]

Bacterial Pneumonia

Although impairment of cell-mediated immunity is the most notable HIV-related immune deficit, abnormalities in both humoral immunity and, in the late stages of disease, neutrophil function, occur.[55–57] As a result, bacterial infections, and particularly bacterial respiratory infections, frequently complicate HIV disease. In a study done after the availability of effective HIV therapy, the rate of bacterial pneumonia among patients with HIV was still over 10-fold higher than that in HIV-negative control subjects.[58] Because the encapsulated bacteria (in particular, *S. pneumoniae*) are more intrinsically virulent than the opportunistic infections of advanced HIV disease, these infections may occur at any stage of HIV disease and may be the sentinel opportunistic process in a person otherwise unaware of HIV infection. Among those with HIV infection, risk factors for the development of bacterial pneumonia are progressive immunodeficiency (as measured by the absolute CD4 cell count), cigarette smoking, and injection drug use.[59] Neither the use of TMP-SMX for PCP prophylaxis nor a history of immunization against *S. pneumoniae* is sufficiently protective against bacterial pneumonia to warrant a change in empirical therapy in consideration of this diagnosis.[60] Mortality from bacterial pneumonia is higher in patients with HIV who have a CD4 cell count of less than 100 cells/mm^3, radiographic progression of disease on therapy, and shock.[61] As with other opportunistic infections, use of antiretroviral therapy decreases the risk of bacterial pneumonia, but not to the rate of individuals who are HIV negative, even when the CD4 cell count normalizes.[3,6] Most patients with HIV-related community-acquired bacterial pneumonia respond promptly to empirical therapy. One study suggested that the prognosis is similar to that in patients without HIV infection, despite increased susceptibility to the disease.[62]

The most commonly identified pathogen in HIV-related bacterial pneumonia is *S. pneumoniae,* generally followed by *H. influenzae.*[63,64] Although both typically cause an acute illness and focal consolidation on chest radiography, *H. influenzae* may rarely cause a more subacute illness with diffuse interstitial infiltrates, suggestive of PCP. Gram-negative bacilli and *Staphylococcus aureus* (including methicillin-resistant strains) assume increasing importance as immunosuppression worsens, presumably from both neutrophil dysfunction and selective pressure of other antimicrobials. *Pseudomonas aeruginosa* infection has been associated with neutropenia, prior treatment with cephalosporins, and CD4 cell counts of less than 50 cells/mm^3. Another important feature of pseudomonal respiratory infections in patients with HIV is their predilection for relapse; chronic colonization typically ensues, with relapse rates after therapy between 25% and 86%.[65–67] Although pathogens of atypical pneumonia have been identified in HIV-infected hosts, in most published series they occur relatively infrequently, if at all.[63] *Nocardia* spp. are an important cause of pulmonary disease in immunocompromised hosts, including those with HIV infection.[68] The relative rarity of nocardiosis compared with other opportunistic processes may be due to the use of TMP-SMX for PCP prophylaxis, although TMP-SMX does not completely prevent *Nocardia* infection. The disease is usually subacute to chronic and limited to the lung, where cavitation may occur; it therefore may appear similar to TB.[69] Dissemination with positive blood cultures is also possible, especially in patients with advanced immunosuppression.



FIG. 123.1 Chest computed tomography in patient with human immunodeficiency virus with pulmonary and disseminated infection from *Rhodococcus equi,* showing a consolidation with cavitation in the lingula. Sputum and blood cultures were both positive for the organism, which was visualized on both Gram and acid-fast staining of respiratory secretions.

Rhodococcus equi can cause both localized pulmonary and disseminated disease in HIV-infected hosts, who appear to be particularly susceptible to this pathogen. A gram-positive coccobacillus, *R. equi* can be mistaken for routine oral flora on sputum smears; a clinical clue is that the organism also is weakly acid fast. The typical presentation is one of a chronic pneumonia (with cough, sputum production, and sometimes hemoptysis) with radiographs that show cavitary disease and often pleural effusions (Fig. 123.1); however, diverse radiographic findings have been reported.[70] Bacteremia commonly accompanies *Rhodococcus* pneumonia and may be the most reliable way to make the diagnosis.[71] Because treatment necessitates prolonged therapy with multiple agents, persistence or recurrence of symptomatic infection is common unless the immune status can be improved with antiretroviral therapy.[70]

Mycobacterial Pneumonia

HIV infection markedly increases the risk of the development of TB, as either progression of primary disease or reactivation. As noted previously, the likelihood of a pulmonary process being the result of TB is strongly influenced by the exposure history, which is related to the patient's current or past residence and the presence of other known risk factors, such as homelessness or incarceration. A majority of TB cases in the United States occur in foreign-born individuals, and rates are particularly high in immigrants from highly TB-endemic regions.[72]

The clinical presentation of TB in patients with HIV depends on the degree of immunosuppression.[25,29,73,74] Those with higher CD4 cell counts (>400 cells/mm^3) present similarly to those without HIV, with upper lobe cavitary disease and a low risk of extrapulmonary dissemination; the presence of constitutional symptoms is variable. At more advanced stages of immunosuppression, patients are more likely to have disseminated disease accompanied by prominent constitutional symptoms and unusual pulmonary manifestations such as mediastinal-hilar adenopathy, focal lower lobe or diffuse interstitial infiltrates, and pleural effusions. In these cases, sputum acid-fast smears are less likely to be positive than in those with cavitary disease, and diagnosis may ultimately be made with alternative methods such as isolator blood cultures (positive in one-fourth to one-half of patients) or tissue biopsy of an involved site (lymph node, bone marrow, or liver). Nonetheless, even with negative acid-fast bacillus smears on respiratory secretions, sputum or BAL cultures may ultimately be positive even with relatively subtle parenchymal abnormalities. Sputum nucleic acid amplification tests, some of which simultaneously assess for rifampin resistance, can hasten diagnosis, particularly for smear-negative cases.[75]

With respect to atypical mycobacteria, the respiratory tract may act as a portal of entry for *M. avium* complex, but it is rarely a primary cause of respiratory symptoms.[76,77] As a result, recovery of the organism from respiratory secretions may be a marker of colonization and a harbinger of disseminated disease but is not diagnostic of pulmonary infection and does not necessarily warrant treatment. Among the non-TB mycobacteria, *Mycobacterium kansasii* is the most likely to cause pulmonary disease in persons with HIV. It occurs uncommonly, mostly during advanced HIV disease, and has been associated with a subacute to chronic course that includes constitutional symptoms and cough; radiographic abnormalities include cavities, nodules, or infiltrates.[78–80] Isolation of the organism from sputum or BAL specimens generally warrants therapy because asymptomatic colonization is unusual.

Fungal Pneumonia

A variety of fungi other than *P. jirovecii* may cause pneumonia in patients with HIV. The most common are *Cryptococcus neoformans;* the endemic fungi *Coccidioides* species, *Histoplasma capsulatum,* and *Blastomyces dermatitidis;* and the mold *Aspergillus fumigatus.* These organisms tend to cause pneumonia only in the setting of severe immunosuppression (CD4 cell count <100 cells/mm^3). Although the lungs act as a portal of entry and may be the initial site of symptoms, hematogenous dissemination commonly occurs in the setting of advanced HIV.

Cryptococcal pneumonia typically has few symptoms; most patients present with meningitis and then are found to have a pulmonary infiltrate. However, some may have fever and cough; other findings may include dyspnea, chest pain, and hemoptysis.[81–83] Nonpulmonary symptoms depend on whether dissemination has occurred, most commonly to the central nervous system, where basilar meningitis can lead to headache, cranial neuropathies, and, in more severe cases, depressed consciousness. On occasion, physical examination reveals white papular lesions on the skin that mimic molluscum contagiosum. A wide range of radiographic abnormalities may occur, including diffuse interstitial infiltrates, focal consolidation, cavitary disease, nodules, adenopathy, and pleural effusions, with more extensive disease seen in those with lower CD4 cell counts. BAL cultures are usually positive, as is the serum cryptococcal antigen; antibody testing has no significant role. Even in the absence of central nervous system symptoms, a cerebrospinal fluid examination is mandatory in patients with AIDS who have cryptococcal pneumonia and a positive serum cryptococcal antigen because meningitis may be chronic or subacute and produce minimal symptoms. Not surprisingly, patients with HIV with higher CD4 cell counts and lower serum cryptococcal antigen titers are more likely to have localized pulmonary disease.[81]

Pulmonary histoplasmosis, coccidioidomycosis, and blastomycosis are generally limited to the geographic regions where these fungi are endemic, although, rarely, these infections may reactivate many years after travel or residence in one of these areas.[84] *Histoplasma capsulatum* is found worldwide, most notably in the Mississippi, Ohio, and St. Lawrence River valleys; the Caribbean; southern Mexico; and Central America. In patients with AIDS, pulmonary histoplasmosis is most commonly seen as a disseminated disease in an individual with a CD4 cell count of less than 100 cells/mm^3, with fever, wasting, adenopathy, diarrhea, and mucosal lesions accompanying the pulmonary process.[85,86] Cough and dyspnea are the most common pulmonary symptoms; typical radiographic findings are diffuse interstitial or reticulonodular infiltrates. The presence of hilar or mediastinal adenopathy may help distinguish histoplasmosis from *P. jirovecii,* but the clinical presentations in susceptible hosts overlap significantly. The diagnostic test of choice for histoplasmosis is detection of polysaccharide antigen in urine or blood; in disseminated histoplasmosis, these tests have reported sensitivities of 92% and 100%, respectively.[87] The antigen test does not distinguish between histoplasmosis and blastomycosis, but the latter rarely occurs in HIV-positive patients. These tests have supplanted antibody and skin testing. Blood cultures that use the lysis-centrifugation method and BAL fluid cultures are also often positive for histoplasmosis in patients with HIV. Because the organism grows slowly, results of histoplasmosis antigen testing are often available before the cultures turn positive.

Coccidioides spp. are endemic to the southwestern United States, northern Mexico, and parts of South and Central America. In endemic areas, they may cause pulmonary disease in individuals with HIV, usually affecting those with severe immunosuppression (CD4 cell counts <200 cells/mm^3).[88,89] As with histoplasmosis, pulmonary findings are often accompanied by clinical evidence of dissemination, including involvement of the skin, lymph nodes, bone, and meninges. Chest radiographic abnormalities are diverse and may include alveolar infiltrates, nodules, adenopathy, cavities, and pleural effusions. Isolation of the organism in respiratory secretions, usually through bronchoscopy, is generally necessary for the diagnosis. A positive isolator blood culture confirms the presence of disseminated disease. Presumptive diagnosis of coccidioidomycosis can also be made via urinary antigen testing, which has a sensitivity of 71% in patients with severe forms of the disease.[90] Although 80% of patients with HIV with pulmonary coccidioidomycosis have positive serology results, these titers have more of a role in monitoring response to therapy than in establishing the diagnosis. Skin testing with coccidioidal antigen has no useful role diagnostically and is no longer commercially available.

Of the endemic fungi, *B. dermatitidis* is the least likely to act as an opportunistic pathogen in patients with HIV.[91] The organism is found in the midwestern and south central United States. In a case series of blastomycosis in 15 patients with HIV, 12 had evidence of pulmonary involvement and 5 also had disseminated disease at the time of diagnosis. The predominant signs at presentation were fever, weight loss, and cough.[92]

Pulmonary aspergillosis in patients with HIV infection occurs almost exclusively in those with advanced HIV-related immunosuppression (CD4 cell count <50/mm^3). Frequently, other risk factors for aspergillosis are present as well, such as receipt of corticosteroids or neutropenia.[93–96] The specific organism is usually *A. fumigatus* or *Aspergillus flavus.* Respiratory tract disease is the most common manifestation; despite the predilection for the organism to invade blood vessel walls and disseminate, focal central nervous system disease from *Aspergillus* in patients with AIDS appears to occur more commonly from contiguous spread from the sinuses, orbits, and ears.[97]

Two types of *Aspergillus* respiratory disease have been described: invasive pseudomembranous tracheitis and invasive pneumonitis. In the former, fever, cough, dyspnea, and wheezing are common signs; the diagnosis is established when endoscopic examination reveals an exudative pseudomembrane adherent to the tracheal wall. In invasive pneumonitis, fever and cough may be accompanied by pleuritic pain and hemoptysis. Radiographic abnormalities in both of these forms overlap and can show diffuse infiltrates, cavities, and focal wedge-shaped abnormalities that reflect pulmonary infarction. A definitive diagnosis requires identification of fungal organisms consistent with *Aspergillus* on a biopsy specimen in a patient with the appropriate clinical syndrome. Not uncommonly, a presumptive diagnosis of pulmonary disease is made when *Aspergillus* spp. are cultured from respiratory secretions in a patient with fever, cough, infiltrates, and severe immunosuppression.

Viral Pneumonia

Although patients with HIV infection are at risk for viral pneumonitis, these conditions are rarely diagnosed in clinical practice, though the introduction of multiplex PCR diagnostic assays may increase the likelihood of diagnosis, at least of common respiratory viral pathogens. Cytomegalovirus presents a clinical quandary in patients with advanced HIV disease. Isolation of CMV from BAL specimens is relatively common, yet most patients with positive BAL cultures have an alternative diagnosis (especially PCP or bacterial pneumonia) and may have improvement without specific therapy directed at CMV.[98–100] Nonetheless, in a patient with advanced HIV disease (CD4 cell count <50 cells/mm^3), interstitial infiltrates on chest radiography, and no alternative diagnosis established, CMV may be the sole responsible pathogen. This diagnosis is confirmed when histopathology (samples are usually obtained via transbronchial biopsy or VATS) shows intracellular inclusions typical of CMV; cultures are confirmatory rather than diagnostic, and noninvasive blood testing (antibody or PCR) cannot provide definitive evidence of CMV pulmonary disease. Ganciclovir or valganciclovir therapy is indicated for cases confirmed with histopathology or with sufficient evidence to suggest that CMV may be acting as a copathogen when treatment of another diagnosis is associated with a suboptimal response.

During influenza season, patients with HIV may present with typical symptoms of influenza, consisting of an acute febrile illness associated with cough, myalgias, sore throat, and rigors. One prospective study found that during the winter, even when extensive testing for other respiratory viruses was done, influenza was the most common specific diagnosis among outpatients with HIV who presented with fever and respiratory symptoms.[101] Overall disease severity was mild. However, pulmonary complications from influenza and death can occur in HIV-infected individuals.[102] Although influenza vaccine is indicated for all individuals with HIV, not all patients receive the vaccine, and they may mount a suboptimal response to the vaccine with progressive immunodeficiency.[103] Chest radiographs are often negative, which suggests that, as with patients without HIV, primary influenza pneumonia is unusual[104,105]; major complications ensue with bacterial superinfection. The diagnosis of influenza is established either on clinical grounds during high influenza prevalence or with use of a reverse-transcriptase PCR influenza diagnostic test on respiratory secretions.

As with other populations with immunosuppression, pneumonitis in patients with HIV has been reported as a result of herpes simplex virus, adenovirus, respiratory syncytial virus, and parainfluenza virus. These causes of pneumonia are rare in patients with HIV but should be considered when clinicians cannot identify an alternative diagnosis. For herpes simplex virus, viral isolation in respiratory secretions usually indicates evidence of reactivation in the upper oropharynx rather than primary pneumonitis, but pulmonary or tracheal infection may occur, especially in the context of endotracheal or nasogastric intubation.

Parasitic Pneumonia

Among the various parasitic infections that occur in patients with HIV, none primarily affects the respiratory tract. However, rarely, they can lead to pulmonary disease as part of generalized dissemination or from focal lung involvement. Pulmonary toxoplasmosis is a rare form of severe pneumonia that occurs predominantly in patients with markedly depressed immune function (CD4 cell count <50 cells/mm^3).[106]

The clinical presentation of pulmonary toxoplasmosis is similar to that of PCP but, unlike PCP, it may be accompanied by a sepsis-like syndrome with hypotension. Radiographic abnormalities are diverse and consist most commonly of interstitial infiltrates but also potentially of nodules, effusions, or a mass lesion; as with PCP, an elevated LDH is a commonly reported laboratory finding.[107] Diagnosis is established with identification of *Toxoplasma* tachyzoites on Giemsa stain of BAL fluid or tissue obtained on biopsy; alternative techniques such as PCR or tissue culture are available in commercial laboratories and research settings.

Despite its association with defects in cell-mediated immunity, disseminated infection to the lung of *Strongyloides stercoralis* is surprisingly rare in individuals with HIV, even in areas highly endemic for both HIV and *Strongyloides*. Indeed, many patients with HIV with *Strongyloides* superinfection syndrome have other classic risk factors, including receipt of corticosteroids, severe wasting, or human T-cell lymphotropic virus type 1 coinfection. Clinicians should consider this diagnosis in a patient with HIV, especially with the previously mentioned risk factors, who presents with pneumonitis, gram-negative sepsis, and sometimes meningitis.[108,109] Identification of *Strongyloides* larvae on a centrifuged BAL specimen is diagnostic; eosinophilia is variably present, and its absence does not exclude the diagnosis.

Although cryptosporidiosis and microsporidiosis predominantly involve the gastrointestinal tract, both can, rarely, colonize the lung and lead to pulmonary disease.[110,111] Diagnosis is made through direct visualization of the organism on respiratory secretions or histopathology, with appropriate stains: modified acid-fast stain for cryptosporidiosis and the modified trichrome stain for microsporidiosis. Because antimicrobial treatment for these conditions is suboptimal, improvement of immune function with antiretroviral therapy is the preferred treatment.

Neoplastic and Other Noninfectious Pulmonary Complications of HIV Infection

The two neoplasms of the lung that are considered AIDS-defining conditions are pulmonary KS and non-Hodgkin lymphoma. Patients with pulmonary KS usually have evidence of cutaneous or mucosal disease; in one series, only 16% had disease limited to the lungs.[112,113] The disease may be asymptomatic even with extensive abnormalities on chest radiograph (Fig. 123.2); these abnormalities are typically nodular infiltrates with or without pulmonary effusions, with chest computed tomography showing a peribronchovascular distribution of infiltrates. Diagnosis is generally made through direct visualization of characteristic purplish plaques on bronchoscopy; because these lesions are highly vascular and quite typical, biopsy is often avoided due to the risk of hemorrhage. Treatment with chemotherapy is indicated for symptomatic pulmonary disease. However, as with cutaneous KS, antiretroviral therapy can induce significant improvement in pulmonary KS and sustained remissions; in some cases, antiretroviral therapy can even obviate the need for chemotherapy.[114,115] Conversely, HIV treatment has also rarely been associated with inflammatory worsening of KS, as a form of the immune reconstitution inflammatory syndrome.[116] Such cases should be managed with continued antiretroviral therapy combined with chemotherapy to control flares of the disease.



FIG. 123.2 Chest computed tomography in patient with human immunodeficiency virus with biopsy-confirmed pulmonary Kaposi sarcoma. Despite the extensive bilateral lung nodules seen on this study, the patient's only respiratory symptom was a mild cough. Nearly complete clearance was achieved with chemotherapy and combination antiretroviral therapy.

The radiographic findings in pulmonary lymphoma are diverse and may consist of nodules, masses, and pleural effusions.[117] Although the diagnosis can sometimes be established through BAL with cytology, transbronchial biopsy, or thoracentesis, these procedures have a low yield, and VATS is often necessary.[118] Nonspecific findings that suggest lymphoma are elevations in LDH and a positive positron emission tomography scan corresponding to the area of abnormality on chest imaging. Pleural effusion lymphocytes contain human herpesvirus 8 proteins in both KS and primary effusion lymphoma, but the cytology in the latter is distinctive.

In many centers, the most common pulmonary neoplasm encountered in patients with HIV is lung cancer.[119] Risk factors for the development of lung cancer are similar to those in non–HIV-infected populations, with smokers having the greatest risk.[120] HIV-infected patients who smoke now lose more years of life from smoking than from HIV.[121] Unlike the AIDS-defining cancers, KS and lymphoma, the incidence of lung cancer in HIV patients is likely to increase because of the prolonged survival associated with antiretroviral therapy. As with other non–AIDS-related malignant diseases, the risk appears to be greatest for those with lower nadir CD4 cell counts.[122]

Although lymphocytic interstitial pneumonitis is seen more commonly in children with HIV, this complication may, rarely, develop in adults as well.[123] The presentation is similar to a related condition, nonspecific interstitial pneumonitis, with cough, shortness of breath, and constitutional symptoms. Diffuse reticulonodular or interstitial infiltrates are seen on chest imaging, which makes differentiation from PCP difficult. However, compared with patients in whom HIV-related opportunistic infections develop, those with lymphocytic interstitial pneumonitis usually have a relatively preserved or even normal CD4 cell count.

Because diagnosis is made through histopathology and exclusion of infectious etiologies, transbronchial or VATS biopsy is indicated. Several case reports have shown that antiretroviral therapy may lead to substantial improvement in lymphocytic interstitial pneumonitis.[124,125] For unresponsive cases, corticosteroids may be effective.[123]

HIV infection is a risk factor for the development of pulmonary hypertension.[126,127] It occurs at all stages of HIV disease, with variable CD4 cell counts and HIV RNA levels at the time of presentation. In some series, women represent a disproportionate number of the cases.[128] The predominant symptom is exertional dyspnea; cough, fatigue, and chest pain may also be present. The key to the diagnosis is recognition that the symptoms are not related to a primary pulmonary infectious process; physical examination signs consistent with right ventricular hypertrophy or failure are helpful but often not present initially. An echocardiogram shows evidence of right atrial hypertrophy and elevated pulmonary pressures; this is then confirmed with right heart catheterization. Management of pulmonary hypertension is the same as for HIV-negative hosts, with prostaglandin agonists (epoprostenol), endothelin receptor antagonists (bosentan, ambrisentan), diuretics, and anticoagulation therapy; sildenafil may also have a role, but clinicians should be cautious about drug interactions with some antiretroviral agents. Studies have not consistently shown a beneficial response to antiretroviral therapy.[129,130]

Patients with HIV are at greater risk for the development of COPD, particularly emphysema, than are HIV-negative subjects.[131] Some observational studies suggest that antiretroviral therapy may decrease the likelihood of developing this condition.[5,132] Cigarette smoking is highly prevalent among patients with HIV but is an area of prevention that is often overlooked by providers.

Abacavir is a nucleoside reverse transcriptase inhibitor that causes a systemic reaction in approximately 5% of individuals, usually within 1 to 6 weeks of starting the drug[133] (see Chapter 128). As part of this multisystem reaction, cough and dyspnea may occur, along with fever, rash, fatigue, malaise, gastrointestinal symptoms, and arthralgias.[134] Clinicians who evaluate patients who have recently been started on abacavir must be aware that this nucleoside reverse transcriptase inhibitor can cause a systemic hypersensitivity reaction because continued abacavir treatment in the face of this reaction can be fatal. Symptoms generally resolve slowly after cessation of the drug. Abacavir should never be resumed in such circumstances because rechallenge can lead to an immediate and life-threatening recurrence of the hypersensitivity reaction. Because abacavir hypersensitivity can mimic other processes, in particular influenza, helpful clinical clues that make recognizing hypersensitivity more likely are worsening of symptoms after each dose, gastrointestinal symptoms, and rash.[135] A strong association is seen between the presence of the human leukocyte antigen (HLA)-B*5701 allele and the risk of development of abacavir hypersensitivity[136]; therefore patients must be tested for HLA-B*5701 before initiation of abacavir, and the drug is contraindicated in those who are positive. Such a strategy markedly reduces the risk of severe hypersensitivity.

TREATMENT

Treatment of opportunistic infections of the lung and other sites is discussed in Chapter 129.

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The complete reference list is available online at Expert Consult.

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124 Gastrointestinal, Hepatobiliary, and Pancreatic Manifestations of Human Immunodeficiency Virus Infection

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SHORT VIEW SUMMARY

Definition
- Gastrointestinal and hepatobiliary diseases are a common cause of morbidity in persons with human immunodeficiency virus (HIV) infection and often the result of both host factors and exposures.

Disorders of the Esophagus
- Disorders of the esophagus typically manifest as dysphagia or odynophagia and affect up to one-third of patients with acquired immunodeficiency syndrome (AIDS).
- Infectious causes include *Candida,* cytomegalovirus (CMV), herpes simplex virus, varicella-zoster virus, mycobacteria, *Histoplasma,* and *Pneumocystis jirovecii.*
- Noninfectious causes include reflux esophagitis and pill esophagitis.
- Malignant causes include esophageal carcinoma, lymphoma, and Kaposi sarcoma.
- Presumptive diagnoses can often be made with a careful history and physical examination.
- Upper endoscopy with biopsy of lesions is highly sensitive in many cases.

Disorders of the Stomach
- Gastric disorders can result from opportunistic infections but more commonly are not related to HIV-induced immunosuppression.
- *Helicobacter pylori,* CMV, and Kaposi sarcoma are common causes of gastric disorders.
- Upper endoscopy and gastric biopsies are often needed for definitive diagnosis.

Disorders of the Liver
- Because of shared modes of transmission, hepatitis caused by acute or chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) is common.
- Anti-HCV and anti-HBV treatment with oral antivirals is recommended for all persons with HIV and viral hepatitis coinfection (see Chapters 117, 145, and 154).
- Drug-induced liver injury has been observed with some antiretroviral agents and other drugs used in persons with HIV infection.

Disorders of the Biliary Tree, Gallbladder, and Pancreas
- Acalculous cholecystitis and cholangitis are found primarily in advanced AIDS and may involve the pancreas.
- CMV, *Cryptosporidium, Cystoisospora,* and microsporidia are most commonly implicated in cases of cholangitis.
- AIDS-related acalculous cholecystitis and cholangitis are rare in settings where antiretroviral therapy (ART) is widely used, and non–AIDS-related gallbladder and biliary diseases may be more common in such settings.
- Endoscopic retrograde cholangiopancreatography can be used for both diagnosis and treatment.
- Didanosine and systemic pentamidine can cause pancreatitis.
- Pancreatic infections with mycobacteria, *Cryptococcus, Toxoplasma gondii, P. jirovecii,* and CMV have been described, often in disseminated disease.

Disorders of the Small and Large Intestine
- Causes of enterocolitis include bacterial, protozoal, and viral pathogens (see Table 124.3).
- *Clostridioides difficile* (formerly *Clostridium difficile*) is the most common cause of diarrhea in persons with HIV infection.
- CMV, *Cryptosporidium,* and *Mycobacterium avium-intracellulare* complex infections are more common in the setting of severe immunodeficiency.
- HIV-associated enteropathy causes culture-negative diarrhea and may improve with ART.
- Anal cancer and high-grade anal dysplasia due to human papillomavirus are common in persons with HIV infection; screening is recommended for persons at risk.

Diseases of the gastrointestinal system frequently complicate human immunodeficiency virus (HIV) infection and mark its progression to acquired immunodeficiency syndrome (AIDS). Many HIV-related gastrointestinal diseases, such as *Candida* esophagitis, biliary cryptosporidiosis, and cytomegalovirus (CMV) colitis, represent opportunistic infections that are the result of advanced immunosuppression, whereas other gastrointestinal processes such as hepatitis B virus (HBV) or hepatitis C virus (HCV) infection may occur at any stage of HIV disease. The likelihood and nature of gastrointestinal manifestations of HIV depend on both host and environmental factors; infectious complications are typically a product of both exposure to potential pathogens and immunocompetence. With the advent of potent antiretroviral therapy (ART), the incidence and spectrum of HIV-related gastrointestinal manifestations have changed dramatically.[1] However, in the setting of severe immunodeficiency the broad range of diagnostic considerations mandates the systematic evaluation of gastrointestinal signs and symptoms. Conversely, persons with effectively treated HIV infection are highly unlikely to experience gastrointestinal manifestations of opportunistic pathogens.

DISORDERS OF THE ESOPHAGUS

Esophageal diseases occur commonly, affecting up to one-third of patients with AIDS and typically produce symptoms of dysphagia and odynophagia, which may be the result of esophageal ulceration caused by infectious pathogens or noninfectious processes.[2] Infectious esophagitis is most often due to infection with *Candida albicans* but may also be caused by viruses such as herpes simplex virus (HSV), CMV, and varicella-zoster virus (VZV), and less commonly by other infectious agents including primary HIV infection. Noninfectious processes such as reflux esophagitis, pill esophagitis (doxycycline), and malignancies (esophageal carcinoma, lymphoma, and Kaposi sarcoma) may be clinically indistinguishable from infectious esophagitis. Furthermore the risk of esophageal (and gastric) carcinoma may be higher in patients with AIDS compared with the general population.[3] Esophageal ulcerations are often caused by CMV (≈45%), idiopathic ulcers (≈40%), and HSV (≈5%), and may involve symptoms of localized pain.[4] In the era of effective ART, the clinical spectrum of esophageal disease has changed substantially; the occurrence of infectious and idiopathic esophageal ulceration has decreased significantly, whereas noninfectious esophageal diseases, such as gastroesophageal reflux disease and dysmotility disorders, may account for an increasing proportion of esophageal symptoms.

Regardless of etiology, most esophageal processes are associated with dysphagia or odynophagia, and persistent or intermittent retrosternal pain, nausea, anorexia, and weight loss may occur. The onset of symptoms may be acute but more typically follows an indolent course. Nonesophageal manifestations of disease may be found in patients with odynophagia;

for example, oral candidiasis frequently accompanies *Candida* esophagitis, and active CMV infection may be found in other anatomic sites such as the retina or colon.[5] However, among patients with advanced immunodeficiency, the broad range of disease processes and the possibility of multiple etiologies mandate a methodical approach to evaluation and management of esophageal symptoms.

Patients with high $CD4^+$ cell counts (>350 cells/mm^3) who present with typical symptoms of gastroesophageal reflux disease may be empirically treated with medications to reduce gastric acid secretion. However, because the absorption of some antiretroviral drugs (e.g., atazanavir) may be reduced in the setting of acid suppression, clinicians should be cognizant of concurrent medications. Patients with more advanced HIV disease, with or without the presence of oral candidiasis, should be empirically treated for esophageal candidiasis with antifungal therapy (e.g., fluconazole, 100–200 mg/day).[6,7] Presumptive oral antifungal therapy is highly effective and is usually preferable to initial diagnostic upper endoscopy.[8] If esophageal symptoms resolve with oral antifungals, the diagnosis of candidiasis can be established empirically. However, failure of symptoms to respond to empirical antifungal therapy within 14 to 21 days indicates a need for further evaluation. Most patients with advanced HIV disease (up to 77%) who do not respond to antifungal therapy have esophageal ulceration rather than evidence of persistent esophageal candidiasis.[9] Upper endoscopy with biopsy is a highly sensitive procedure for establishing a specific diagnosis and is the preferred approach to evaluation of patients with odynophagia or dysphagia who do not respond to empirical antifungal therapy. Upper gastrointestinal contrast radiography may reveal characteristic abnormalities, but these findings are relatively insensitive and nonspecific, whereas upper endoscopy may yield a treatable pathologic diagnosis in most patients.

The endoscopic appearance of esophageal candidiasis resembles cheesy friable plaques that may involve the entire esophagus, whereas viral esophagitis is usually associated with diffuse erythematous ulceration of the mucosa. CMV esophagitis frequently causes numerous large shallow ulcerations, whereas HSV esophagitis is typically seen as superficial confluent ulcers in the distal esophagus and may be associated with concurrent nonesophageal lesions. Idiopathic or aphthous ulcers may have an endoscopic appearance similar to that of CMV ulcers and represent a diagnosis of exclusion. Noninfectious processes such as drug-induced ulcers and malignant diseases may affect the esophagus, with the most common tumors being Kaposi sarcoma, primary lymphoma, and adenocarcinoma. Persons with HIV infection may also have conventional esophageal pathology such as reflux esophagitis.

For lesions visualized with endoscopy, a biopsy should be performed and tissue sections prepared for histopathologic stains to identify viral inclusion bodies (CMV), multinucleated giant cells (HSV), and direct evidence of HSV or CMV or invasive fungi. Immunohistochemical stains for CMV and HSV are helpful. In some settings, polymerase chain reaction (PCR) assay has been used to detect organisms from nondiagnostic ulcer biopsy specimens; however, contamination with oral secretions limits its clinical utility.[10] Cultures for fungi and viruses may be helpful, although positive results for *C. albicans* may not indicate esophagitis. Antifungal drug sensitivity testing may be useful if azole-resistant candidiasis is suspected.[11] The yield of endoscopy with biopsy and culture is high, and more than one pathologic process may be found. However, multiple biopsies (more than three) may be needed to exclude the diagnosis of viral esophagitis. Lesions that do not respond to appropriate therapy should be reevaluated with endoscopy with biopsy and culture to confirm the diagnosis and, if indicated, with drug sensitivity testing.

DISORDERS OF THE STOMACH

Gastric disorders in persons with HIV infection may be the result of opportunistic infections but are often unrelated to immunodeficiency, even among patients with advanced HIV disease. Patients with gastric disease may present with protean symptoms such as nausea, vomiting, early satiety, and anorexia; abdominal pain and hematemesis may also be present. Some disease processes, such as CMV gastritis and gastrointestinal Kaposi sarcoma, may be associated with extragastric involvement.

Gastritis and gastroduodenal ulcers may be found in persons living with HIV (PLWH) with upper gastrointestinal symptoms and may be the result of *Helicobacter pylori* infection. In some studies, the prevalence of *H. pylori* has been lower in PLWH compared with HIV-negative individuals.[12] In sub-Saharan Africa, *H. pylori* was detected in 88% of HIV-negative individuals and 51% of PLWH, among whom the prevalence decreased in persons with lower $CD4^+$ cell counts.[13] *H. pylori* infection may be detected with serology, urea breath testing, histologic evaluations, and stool *H. pylori* antigen testing, which has emerged as the preferred test for diagnosis and treatment efficacy.[14] PLWH may also have altered gastric function including decreased secretion of gastric acid and intrinsic factor. Hypochlorhydria may impair the absorption of some medications such as ketoconazole, itraconazole, and atazanavir, and may also permit bacterial overgrowth.[15]

CMV may cause gastric inflammation or ulceration alone or in association with esophageal ulceration. The appearance of gastric CMV may be diverse and includes thickened edematous gastric folds, erosive gastritis, and superficial or deep ulcerations. The radiographic features may be nonspecific and may even be masslike, suggestive of malignant disease. Other gastric infections have been reported including infection with *Cryptosporidium, Mycobacterium avium-intracellulare* complex, histoplasmosis, leishmaniasis, and syphilis. Malignant lesions that involve the stomach may also be associated with upper gastrointestinal symptoms. Gastrointestinal Kaposi sarcoma, associated with human herpesvirus type 8 infection, complicates cutaneous disease in 50% of patients and most commonly involves the stomach.[16] Gastric Kaposi sarcoma lesions may be asymptomatic but can also cause nausea, abdominal pain, and, rarely, severe hemorrhage. AIDS-related lymphomas may also involve the gastrointestinal tract; these lesions tend to be multifocal but may rarely be gastric mucosa–associated lymphoid tissue lymphomas associated with *H. pylori.*[17] Although the incidence of AIDS-related lymphoma has decreased in the era of ART, the risk of gastric carcinoma is elevated (up to 44% higher) in PLWH compared with the general population.[3] In light of these diverse diagnostic considerations, patients with persistent upper gastrointestinal symptoms, particularly patients with low $CD4^+$ cell counts, should be evaluated for opportunistic infection and malignancy disease. The definitive diagnosis of upper gastrointestinal pathology often requires endoscopic evaluation with biopsy and cultures. Kaposi sarcoma lesions typically appear as a violet-blue submucosal mass without ulceration or bleeding but may appear as linitis plastica. Biopsies of these lesions may fail to confirm Kaposi sarcoma in up to two-thirds of cases. However, when cutaneous Kaposi sarcoma has been histologically confirmed, the endoscopic appearance of the lesion may be sufficient to establish the diagnosis. Biopsies of gastroduodenal ulcerations should be performed and specimens sent for viral culture and histologic staining for evidence of viral inclusions, fungi, and *H. pylori* infection. Multiple biopsies may be needed to exclude viral pathogens, and additional stains and cultures may be helpful in diagnosing specific infections, such as acid-fast stains and culture for *M. avium-intracellulare* complex.

DISORDERS OF THE BILIARY TREE AND GALLBLADDER

Diseases of the gallbladder and biliary tree that affect PLWH include common, non–AIDS-associated conditions such as cholelithiasis and AIDS-associated conditions such as acalculous cholecystitis and cholangiopathy. Patients typically present with postprandial pain, fever, right upper quadrant tenderness, and an elevated serum alkaline phosphatase level. Ultrasound or computed tomography may reveal evidence of acute cholecystitis or cholangitis related to cholelithiasis; in patients with AIDS, these studies may suggest acalculous cholecystitis or AIDS-associated cholangitis.[18] During acute or chronic acalculous cholecystitis, the gallbladder generally appears thickened and edematous, with obliteration of the gallbladder lumen without evidence of gallstones. Nonvisualization of the gallbladder with radionuclide hepatobiliary scintigraphy is also suggestive. If a cholecystectomy is performed, operative specimens should be sent for microbiologic and histopathologic evaluation because before the advent of potent ART, opportunistic pathogens were identified in more than 50% of cases.[19] CMV, *Cryptosporidium,* and microsporidia are the pathogens most commonly associated with acalculous cholecystitis; however, multiple organisms may be recovered, and often no etiologic agent is identified after extensive microbiologic evaluation. Although the exclusion of opportunistic

infection is critical in the evaluation of patients with HIV with signs and symptoms of acute cholecystitis, the incidence of AIDS cholangiopathy is very low in settings where ART is widely used and should be primarily considered in patients with a $CD4^+$ cell count less than 50 cells/mm^3. Although rare, the HIV-1 protease inhibitor atazanavir may lead to the development of biliary stones containing significant concentrations of the actual drug; thus patients treated with atazanavir who present with symptomatic cholelithiasis warrant consideration of a change to another antiretroviral agent.[20]

If noninvasive radiographic imaging indicates intrahepatic or extrahepatic biliary ductal dilation, endoscopic retrograde cholangiopancreatography may be necessary for diagnosis and management of AIDS-associated cholangiopathy. Four common patterns of cholangiographic abnormalities are revealed with endoscopic retrograde cholangiopancreatography: stenosis of the papilla of Vater with dilated extrahepatic biliary tract, sclerosing cholangitis, a combination of sclerosing cholangitis and papillary stenosis, and choledochal long stenosis or strictures. Endoscopic collection of bile from the common bile duct and duodenal or papillary biopsy may identify an opportunistic pathogen or malignancy. Similar to acalculous cholecystitis, cholangitis is associated with opportunistic infections by CMV, *Cryptosporidium,* or microsporidia in more than half of cases. For example, among 82 patients with HIV in whom cryptosporidiosis developed during a waterborne outbreak, 24 (29%) had evidence of biliary involvement that was associated with $CD4^+$ cell counts less than 50 cells/mm^3.[4,21] If papillary stenosis is present, endoscopic sphincterotomy may produce relief of symptoms and biochemical resolution of cholestasis, although in some case series the clinical effectiveness of this procedure has been disappointing.[22]

DISORDERS OF THE LIVER

Hepatic abnormalities are common in PLWH and are often associated with elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase levels. Liver disease may result from acute or chronic viral hepatitis, HIV-related opportunistic infections, or noninfectious processes such as drug-induced liver injury (DILI), alcohol, nonalcoholic fatty liver disease, or malignancy (Table 124.1). Because of shared routes of transmission, chronic infection with HBV or HCV or both frequently complicates HIV disease,[23] and liver disease caused by these pathogens in an important cause of morbidity and mortality in the era of effective ART.[24–26]

Compared with only 5% of adults without HIV infection, approximately 20% of PLWH who acquire acute HBV infection after childhood will develop chronic HBV infection, characterized by hepatitis B surface antigenemia (HBsAg) and active viral replication.[27] In addition, spontaneous HBV reactivation has been reported in anti–hepatitis B surface antibody-positive patients with severe immunosuppression.[28] Although chronic HBV infection does not appear to adversely impact the natural history of HIV disease, patients with HIV and HBV coinfection are at increased risk of liver-related morbidity and mortality compared with patients with HBV infection alone.[29] For example, among men with and without HIV infection followed longitudinally in the Multicenter AIDS Cohort Study (MACS), Thio and colleagues[29] reported that men with HIV and HBV coinfection were approximately 19 times more likely to die of liver disease compared with men infected with HBV alone. These and other data support the recommendation that all PLWH should be screened for evidence of resolved or active HBV infection.[30] Testing for HBsAg, hepatitis B surface antibody, and anti–hepatitis B core antibody (anti-HBc total) is recommended before the initiation of ART because some reverse transcriptase inhibitors used to treat HIV drugs are also active against HBV. Individuals found to be susceptible to HBV infection should undergo vaccination. Patients with chronic HBV (defined as persistent HBsAg >6 months) should undergo further evaluation to determine HBV replication status (e.g., hepatitis B e antigen and serum HBV DNA) and liver disease activity (e.g., serum ALT level or other serum markers of liver damage, liver biopsy, or transient elastography).

Patients with HIV and chronic HBV infection characterized by active HBV replication (e.g., detectable HBV DNA) are at increased risk for the development of cirrhosis, end-stage liver disease (ESLD), and hepatocellular carcinoma (HCC), and should be monitored for the development of cancer with liver ultrasound every 6 to 12 months.[31] Treatment for both HBV and HIV is recommended for all coinfected patients, regardless of serum ALT or HBV DNA level.[32] The goal of HBV therapy in coinfected patients is the prevention of liver disease, which may be achieved through sustained suppression of HBV replication and in some cases seroclearance of hepatitis virus e antigen or hepatitis virus s antigen or both. Four antiretroviral drugs approved by the US Food and Drug Administration for treatment of HIV (lamivudine, emtricitabine, tenofovir disoproxil fumarate, tenofovir alafenamide) also suppress HBV replication by inhibition of HBV DNA polymerase.[31] Similarly, entecavir, a guanosine analogue approved for treatment of chronic HBV infection, also inhibits HIV reverse transcriptase and, when used in the absence of HIV suppression with antiretrovirals, can lead to emergence of HIV-resistance mutations (M184V) that confer decreased susceptibility to lamivudine and emtricitabine.[33] To prevent the development of resistance, these dually active drugs must be used only as part of an effective antiretroviral combination therapy regimen. Oral adefovir and telbivudine are also approved for treatment of chronic HBV infection but are not recommended as first-line therapies. Peginterferon-alfa administered by weekly subcutaneous injection may also be effective in treatment of chronic HBV; however, few data exist regarding the effectiveness and tolerability in patients with HIV and HBV coinfection.[34] Treatment guidelines recommend that all HIV-infected patients with chronic HBV infection be treated for both infections with combination ART that includes tenofovir plus emtricitabine or lamivudine. Treatment of one viral infection but not the other is not recommended (see Chapter 145).

HCV coinfection is also common. Globally, HCV prevalence in PLWH is approximately 6.2%, which is about sixfold greater than the prevalence in HIV-negative populations. Among PLWH, HCV prevalence varies according to HIV risk group: 82.4% of persons who inject drugs, 6.4% of men who have sex with men (MSM), and 4.0% of heterosexually

TABLE 124.1 Selected Causes of Hepatic Disease in Persons With HIV

Viruses
Hepatitis A
Hepatitis B
Hepatitis C
Hepatitis D
Hepatitis E
Epstein-Barr
Cytomegalovirus
Herpes simplex
Adenovirus
Varicella-zoster
Fungi
Histoplasma capsulatum
Cryptococcus neoformans
Coccidioides immitis
Candida albicans
Pneumocystis jirovecii
Talaromyces marneffei
Protozoa
Toxoplasma gondii
Cryptosporidium parvum
Microsporidia
Schistosoma
Bacteria
Mycobacteria
Mycobacterium avium-intracellulare complex
Mycobacterium tuberculosis
Bartonella henselae (peliosis hepatis)
Malignant Disease
Kaposi sarcoma (HHV-8)
Non-Hodgkin lymphoma
Hepatocellular carcinoma

HHV-8, Human herpesvirus 8; *HIV,* human immunodeficiency virus.

exposed persons.[35] Heterosexual HCV transmission is uncommon but may be more likely in persons with partners who have HIV and HCV coinfection. Likewise, HCV transmission between HIV-infected MSM is relatively common, particularly among men who engage in unprotected anal receptive intercourse in the setting of noninjection drug use, and HCV sexual transmission networks have been observed.[36] Acute HCV infection may be clinically silent and detected by asymptomatic elevations in serum ALT levels. Although some PLWH may spontaneously clear acute HCV, most will progress to chronic HCV infection, which is often characterized by asymptomatic elevations in serum ALT and AST levels, but leads to progressive hepatic fibrosis that may lead to cirrhosis that may be followed by hepatic decompensation or HCC or death.[37] Patients with ESLD are profoundly symptomatic with manifestations of portal hypertension including ascites, esophageal varices, and splenomegaly; decreased hepatic synthetic function including hypoalbuminemia, thrombocytopenia, and coagulopathy; and hepatic encephalopathy. Liver transplantation is an option for management of PLWH who present with ESLD or HCC.[38] Extrahepatic manifestations of HCV infection such as membranous glomerulonephritis, porphyria cutanea tarda, and cryoglobulinemia with or without vasculitis may also occur.

On one hand, HIV coinfection adversely affects the natural history of HCV disease, with enhanced HCV replication and accelerated hepatic fibrosis progression, presumably because of HIV-related immunosuppression. On the other hand, conflicting reports exist regarding the impact of HCV infection on the natural history of HIV disease, and for most persons, HCV does not adversely affect HIV disease or its treatment with ART.[39] In the era of effective ART, HCV infection is an important cause of morbidity and mortality in PLWH.[4] As such, current management guidelines recommend that all HIV-infected patients be screened for HCV infection with an assay to detect anti-HCV. Negative anti-HCV results may be observed in PLWH who have advanced immunodeficiency (e.g., $CD4^+$ cell count <100 cells/mm^3) or in PLWH with early, acute HCV infection; in such cases, blood HCV RNA should be assessed when HCV infection is suspected in persons with negative anti-HCV results (e.g., elevated ALT levels). Patients with positive HCV antibody results should have further HCV RNA testing to confirm active HCV replication. Patients should be counseled to prevent liver damage and HCV transmission, evaluated for the presence of chronic liver disease, and offered curative HCV treatment.[2] Because alcohol ingestion accelerates the progression of liver disease, patients should be advised to abstain from alcohol. Assessments of disease severity should include a history and physical examination for signs and symptoms of chronic liver disease and measurement of blood albumin, creatinine, sodium, prothrombin time, total and direct bilirubin, and platelet count. The magnitude of HCV RNA in the blood provides only limited information regarding disease severity. Noninvasive radiographic imaging may reveal hepatic parenchymal abnormalities; mass lesions; or evidence of ascites, splenomegaly, or varices. However, imaging studies cannot reliably exclude the presence of significant liver fibrosis. Specific liver disease staging is recommended for all coinfected patients by using either noninvasive serum markers (e.g., AST-to-platelet ratio index, fibrosis-4 index), liver stiffness measurement by elastography, or liver biopsy.[40,41] In addition to hepatic fibrosis staging, histologic evaluation may be useful to exclude other causes of hepatic disease such as nonalcoholic liver fatty liver disease or DILI.

All PLWH with active HCV infection should be considered for HCV treatment with direct-acting antivirals. In clinical trials and real-world studies, approximately 95% of PLWH have achieved sustained virologic response (HCV cure) following treatment with oral HCV direct-acting antiviral regimens including the agents listed in Table 124.2. The observed sustained virologic response rates in PLWH are similar to rates observed in HIV-negative individuals; accordingly the American Association for the Study of Liver Diseases and Infectious Diseases Society of America guidance states that "HIV/HCV-coinfected persons should be treated and re-treated the same as persons without HIV infection, after recognizing and managing interactions with antiretroviral medications."[42,43] For example, because of inhibition or induction of cytochrome P-450 3A/4, the HCV nonstructural 3/4A protease inhibitors (grazoprevir, glecaprevir, and voxilaprevir) may be combined only with selected antiretroviral agents. Details of recommended HCV treatments and drug interactions can be found at www.hcvguidelines.org and www.hep-druginteractions.org, respectively (see Chapters 117 and 154).

Other infectious processes may also involve the liver. Viral pathogens such as CMV, Epstein-Barr virus, HSV, and adenovirus can cause hepatocellular damage, usually in the setting of disseminated disease. Other infectious pathogens such as mycobacterial and fungal agents may lead to granulomatous inflammation, characterized by elevated alkaline phosphatase levels and nonspecific hepatic granuloma. *M. avium-intracellulare* complex is the most common opportunistic pathogen that affects the liver and is associated with manifestations of systemic disease including fever, abdominal pain, and wasting.[44] Extrapulmonary *Mycobacterium tuberculosis* infection may be associated with hepatic involvement in 5% to 10% of HIV-related cases. Disseminated fungal infections with organisms including *Cryptococcus neoformans, Histoplasma capsulatum, Coccidioides immitis, Penicillium marneffei,* and *C. albicans* may produce a granulomatous response in liver.

Pneumocystis jirovecii hepatic infection may occur rarely, particularly during aerosolized pentamidine prophylactic therapy. *Bartonella henselae* can cause peliosis hepatis, a vascular proliferative liver infection, in the absence of cutaneous lesions. Peliosis hepatis occurs in patients with AIDS, who may present with fever, weight loss, and hepatosplenomegaly with an elevated serum alkaline phosphatase level. The diagnosis of bartonellosis can be made with Warthin-Starry silver staining of biopsy specimens, culture of the blood or tissue plated on heart infusion agar with 5% rabbit blood, and PCR assay.[45] Blood culture for acid-fast bacilli is usually sufficient to establish the diagnosis of disseminated mycobacterial infection. *C. neoformans* and *H. capsulatum* may be rapidly detected with evidence of antigenemia. The evaluation of other potential causes may require liver biopsy, which can yield a treatable diagnosis in approximately 60% of patients with HIV with unexplained fever and liver abnormalities. Hepatic tissue should be sent for microbiologic evaluation and culture and for routine and specific histologic staining

TABLE 124.2 Clinical Trials of Direct-Acting Antiviral Therapies in Patients With Hepatitis C Virus/HIV Coinfection

TRIAL	TOTAL NO. HIV/HCV COINFECTION PATIENTS	HCV REGIMEN	TREATMENT DURATION (weeks)	SVR (%)
ION-4	335	Sofosbuvir/ledipasvir	12	96
ERADICATE	50	Sofosbuvir/ledipasvir	12	98
C-EDGE	218	Grazoprevir/elbasvir	12	96
ALLY-2	203	Sofosbuvir + daclatasvir	8–12	76–97
TURQUOISE-1	63	Paritaprevir/ritonavir/ombitasvir + dasabuvir ± ribavirin	12–24	92
C-WORTHY	59	Grazoprevir/elbasvir ± ribavirin	12	92
ASTRAL-5	106	Sofosbuvir/velpatasvir	12	95
EXPEDITION-2	153	Glecaprevir/pibrentasvir	8–12	98

HCV, Hepatitis C virus; *HIV,* human immunodeficiency virus; *SVR,* sustained virologic response.

for acid-fast bacilli and fungal pathogens. Hepatic mass lesions are commonly the result of malignant disease, although infectious processes may resemble mass lesions radiographically. Before potent ART, the most common hepatic malignant disease was Kaposi sarcoma in the setting of cutaneous disease. Abdominal imaging with contrast-enhanced computed tomography may reveal enhanced lesions located in the capsular, hilar, and portal areas, with invasion into the liver parenchyma. The definitive diagnosis of hepatic Kaposi sarcoma requires biopsy with histologic examination; however, the risk of hemorrhage after a biopsy may be increased because of the vascular nature of the Kaposi sarcoma lesion. Non-Hodgkin lymphomas may involve the liver, usually in association with lymph node or visceral involvement or both, although primary hepatic lymphoma has been reported. Patients may present with weight loss, fever, night sweats, and abdominal pain (termed *B symptoms*); jaundice may occur with intrahepatic or extrahepatic bile duct obstruction. Radiographic imaging typically reveals solitary or multiple hepatic mass lesions and involvement of abdominal lymph nodes. Biopsy of the hepatic lesion or involved lymph nodes is needed to confirm the diagnosis of lymphoma. With effective ART, the incidence of Kaposi sarcoma and lymphoma involving the liver has declined, but HCC related to HBV or HCV has been increasingly recognized in patients with HIV, particularly patients with cirrhosis; such patients may have evidence of advanced liver disease, elevated serum α-fetoprotein level, and radiographic evidence of a mass lesion.[46]

Among noninfectious liver diseases, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis are common in PLWH. In a meta-analysis of 10 studies, the prevalence of NAFLD in PLWH evaluated by liver imaging was 35%, and the prevalence of nonalcoholic steatohepatitis in PLWH evaluated by liver biopsy was 42%.[47] Risk factors for NAFLD in PLWH are similar to risk factors reported for HIV-negative persons including high body mass index, high waist circumference, type 2 diabetes, hypertriglyceridemia, and hypertension. In addition, older nucleoside reverse transcriptase inhibitors including stavudine and didanosine were associated with hepatic steatosis; in more recent analyses, the association of HIV disease and its treatment with NAFLD is less clear.[48–50] PLWH with evidence of hepatic steatosis and abnormal liver enzymes require further evaluation for severity of liver damage including assessment of degree of fibrosis with liver biopsy or transient elastography with controlled attenuation parameters. NAFLD treatment is usually directed at control of metabolic disease including weight loss and diabetes. Although specific NAFLD treatments are under investigation, these have not been approved by the US Food and Drug Administration or investigated in PLWH.

DILI is a common cause of liver abnormalities in PLWH, and infection with HIV or HCV or both may enhance the toxicity of some medications such as antituberculosis drugs.[51] Patients typically present with elevated serum ALT and AST levels, although some drugs such as macrolide antibiotics (e.g., azithromycin) and trimethoprim-sulfamethoxazole induce a cholestatic or mixed liver injury pattern. DILI is often asymptomatic and detected through routine monitoring of serum liver enzymes. HIV-1 protease inhibitors, particularly tipranavir/ritonavir, and nonnucleoside reverse-transcriptase inhibitors, particularly nevirapine, have been associated with severe DILI after the initiation of therapy, which may be more common in the setting of chronic HBV or HCV infection.[51–55] In addition to elevations of serum ALT and AST levels, nucleoside analogue reverse-transcriptase inhibitors such as zidovudine and stavudine have been associated with mitochondrial toxicity, which leads in some cases to fatal hepatomegaly with severe steatosis and lactic acidosis.[56–58] Antiretroviral medications, particularly didanosine, have also been associated with noncirrhotic portal hypertension and nodular regenerative hyperplasia; however, the incidence of this condition is extremely low.[59–61]

DISORDERS OF THE PANCREAS

Diseases that involve the pancreas may be caused by processes unrelated to HIV infection such as alcohol, cholelithiasis, and hyperlipidemia or may be caused by HIV-related opportunistic infections or medication toxicity.[62] AIDS cholangiopathy caused by CMV, *Cryptosporidium,* or microsporidia may involve the juxtaampullary portion of the pancreatic duct. Mycobacterial infections of the pancreas have been described including pancreatic abscess related to *M. tuberculosis* and disseminated *M. avium-intracellulare* complex. Fungal pathogens such as *C. neoformans* and *Candida* spp., *Toxoplasma gondii, P. jirovecii,* and protozoal pathogens may involve the pancreas, typically in the setting of disseminated disease. Pancreatic CMV inclusions are frequently observed in autopsy specimens from patients with disseminated CMV disease, although clinical pancreatitis is infrequently recognized before death.[63] Infection with mumps may also cause pancreatitis.

Drug-induced pancreatic inflammation and dysfunction may also occur in patients with HIV disease. Didanosine may frequently cause asymptomatic hyperamylasemia; clinical pancreatitis has been observed in 1.2% to 6.7% of didanosine recipients, and fulminant pancreatic toxicity has been reported.[64] Systemic pentamidine therapy for *P. jirovecii* pneumonia is toxic to pancreatic beta-islet cells and can cause pancreatitis and symptomatic hyperglycemia or hypoglycemia; in contrast, prophylaxis with aerosolized pentamidine rarely leads to pancreatic dysfunction.[65] The risk of pancreatitis in patients receiving intravenous pentamidine is dose related, with most cases occurring after 2 weeks of therapy. Overall the incidence rate of acute pancreatitis has been low (1.27 cases per 1000 person-years) in some settings.[66]

DISORDERS OF THE SMALL AND LARGE INTESTINE

Symptomatic disease of the small intestine and colon remains common among patients with AIDS and may be caused by a diverse range of infectious agents; however, among PLWH treated with ART, the incidence of acute enterocolitis has declined substantially. Small bowel diseases generally produce bloating, nausea, cramping, and profuse diarrhea and may be associated with malabsorption and weight loss. In contrast, colitis may produce lower abdominal discomfort and cramping, urgency, and tenesmus and typically causes frequent small-volume diarrhea. Medication-related diarrhea may be observed at any stage of HIV disease, and gastrointestinal adverse effects may lead to discontinuation of antiretroviral medications.[67] Furthermore, with prolonged life expectancy the finding of colorectal malignant diseases such as adenocarcinoma is expected to increase, and recommendations for routine screening colonoscopy should be followed.[68] Inflammatory bowel diseases (e.g., ulcerative colitis, Crohn disease) have been reported in patients with HIV with diarrhea.

The differential diagnosis for enterocolitis in a severely immunocompromised patient is extensive and includes bacterial, protozoal, and viral pathogens (Table 124.3). Bacteria that are more common in patients with HIV infection include *Salmonella* spp., *Shigella, Campylobacter jejuni, Escherichia coli* (enterotoxigenic, enteroadherent, and enteroaggregative), and *Listeria monocytogenes.* Furthermore, among MSM, shigellosis is predominantly a sexually transmitted pathogen, particularly among men with HIV.[69] Nontyphoidal salmonellosis is associated with bacteremia in one-half of infections and may be recurrent in PLWH.[70] Less commonly, bacteremia may occur with *Shigella* and *Campylobacter* infection. *C. difficile* infection may also be more common among patients with HIV disease, particularly among hospitalized patients, patients who have recently undergone antibiotic therapy, and patients with advanced AIDS.[71] Small bowel overgrowth can occur and has been associated with hypochlorhydria and wasting. Other, less common bacterial causes of enterocolitis include *Aeromonas, Plesiomonas, Yersinia,* and *Vibrio* spp. Mycobacterial infections of the small bowel are usually associated with late-stage HIV disease and disseminated *M. avium-intracellulare* complex, although enteritis caused by *M. tuberculosis* has been reported. Parasites and fungi that infect the small and large bowel include spore-forming protozoa, *Cryptosporidium,* microsporidia such as *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, *Cystoisospora, Cyclospora, Entamoeba histolytica,* and *Giardia lamblia.* Amebic infection and giardiasis may occur at any stage of HIV disease and are commonly associated with conventional risk factors, such as sexual practices and travel-related exposures. Disease caused by *Cryptosporidium* is more common and severe in patients with HIV with advanced immunosuppression and leads to persistent infection (60%), biliary disease (29%), and even fulminant disease (8%).[72] Similarly, microsporidiosis causes diarrhea in patients with advanced HIV disease ($CD4^+$ cell count <50 cells/mm^3) and may be associated with cholangiopathy. Less commonly,

TABLE 124.3 Causes of Lower Gastrointestinal Tract Disease in Persons With HIV

Causes of Enterocolitis
Bacteria
Campylobacter jejuni and other spp.
Salmonella spp.
Shigella flexneri
Aeromonas hydrophila
Plesiomonas shigelloides
Yersinia enterocolitica
Vibrio spp.
Mycobacterium avium-intracellulare complex
Mycobacterium tuberculosis
Escherichia coli (enterotoxigenic, enteroadherent)
Bacterial overgrowth
Clostridioides difficile (formerly *Clostridium difficile*) (toxin)
Parasites
Cryptosporidium parvum
Microsporidia *(Enterocytozoon bieneusi, Septata intestinalis)*
Cystoisospora belli
Entamoeba histolytica
Giardia lamblia
Cyclospora cayetanensis
Viruses
Cytomegalovirus
Adenovirus
Calicivirus
Astrovirus
Picobirnavirus
HIV
Fungi
Histoplasma capsulatum
Causes of Proctitis
Bacteria
Chlamydia trachomatis
Neisseria gonorrhoeae
Treponema pallidum
Viruses
Herpes simplex
Cytomegalovirus

HIV, Human immunodeficiency virus.

diarrhea caused by *Cystoisospora* and *Cyclospora* has been reported in patients with advanced immunosuppression.[1]

CMV is the most significant viral cause of enterocolitis and leads to fever, abdominal pain and tenderness, bloody diarrhea, and, rarely, intestinal perforation or toxic megacolon. As with its appearance in the esophagus, CMV enterocolitis occurs with severe immunodeficiency and produces mucosal inflammation with superficial ulceration.[73] Proctitis in patients with HIV disease typically reflects sexually transmitted infections such as HSV infection, *Chlamydia trachomatis* infection, syphilis, and gonorrhea, particularly among MSM. CMV proctitis is less common but may occur in the setting of extensive CMV colitis. The incidence of human papillomavirus–associated anal dysplasia and cancer is high in PWLH, particularly MSM; anal cancer screening is recommended for individuals at risk.[74,75]

Patients with HIV with diarrhea or other symptoms of enterocolitis should be carefully evaluated for identification of treatable conditions. History should focus on the nature and duration of symptoms; concomitant medications; travel; and other exposures such as food, water, or sexual contact. The standard diagnostic evaluation should include stool leukocyte examination for identification of inflammatory causes of diarrhea such as bacterial pathogens and CMV and for guiding empirical therapy; other infectious agents such as protozoa and *M. avium-intracellulare* complex are typically not associated with the finding of fecal leukocytes. Stool should be cultured for enteric bacterial pathogens, assayed for *C. difficile* toxin, and examined for ova and parasites. Stool specimens should be examined on at least three occasions with modified acid-fast stain to identify *Cryptosporidium, Cyclospora,* and *Cystoisospora.* Special trichrome staining may be useful for the identification of microsporidia. PCR multiplex panels for gastrointestinal pathogens may be useful.

If noninvasive stool studies are not diagnostic and symptoms persist, endoscopic evaluation with biopsy may prove helpful, particularly in patients with chronic diarrhea and severe immunodeficiency ($CD4^+$ cells count <100 cells/mm^3).[76] Patients with signs and symptoms suggestive of large bowel involvement should undergo colonoscopy and biopsy. The sensitivity of endoscopic biopsy for the diagnosis of CMV disease is high, and colonoscopy may be used to identify disease limited to the right side of the colon, which may be missed during flexible sigmoidoscopy. Upper endoscopy with duodenal biopsy is useful for patients with symptoms of small bowel disease or patients with persistent symptoms and negative evaluation of the lower gastrointestinal tract. In addition to hematoxylin and eosin staining, duodenal histologic specimens should be stained with fungal stains and modified acid-fast stain for *Cryptosporidium* and should undergo electron microscopy for microsporidia. PCR assay of biopsy specimens may also prove valuable in the diagnosis of some pathogens such as microsporidia. Small bowel aspirates are generally not useful in the evaluation of unexplained diarrhea.

Approximately 20% to 50% of patients with chronic diarrhea have a negative gastrointestinal tract evaluation. Patients without an identifiable cause of diarrhea may have HIV-associated enteropathy, the pathology of which is not fully understood but likely involves depletion of gut-associated $CD4^+$ cells.[77,78] Histologic evaluation of small bowel biopsy specimens may reveal a decrease in villous surface area and crypt cell proliferation in the absence of inflammation. For many pathogens, few specific therapies are available, but potent ART may effectively control diarrhea in patients with HIV enteropathy and in patients with microsporidiosis and cryptosporidiosis.

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125 Neurologic Diseases Caused by Human Immunodeficiency Virus Type 1 and Opportunistic Infections

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SHORT VIEW SUMMARY

Definition
- Human immunodeficiency virus (HIV) and associated infections can adversely affect any aspect of the central and peripheral nervous system.

Epidemiology
- Ten percent of HIV-positive patients initially present with neurologic disease.
- Thirty percent to 50% of HIV-positive patients have neurologic complications.
- Eighty percent of HIV-positive patients have nervous system involvement at autopsy.

Cognitive Manifestations
- HIV-associated neurocognitive disorder (HAND) occurs in 15% of patients with acquired immunodeficiency syndrome (AIDS) and can be the first manifestation of disease in 3% to 10% of patients.
- Detailed neurocognitive testing may be required to diagnose HAND.

Central Nervous System (CNS) Mass Lesions
- Toxoplasmosis is the most frequent cerebral mass lesion. Incidence depends on the seropositivity of the population. Empirical treatment with pyrimethamine and sulfadiazine is useful when clinical and radiologic findings are consistent with the diagnosis.
- Primary CNS lymphoma is the most common HIV-associated brain malignancy and is associated with Epstein-Barr virus infection.
- Progressive multifocal leukoencephalopathy is caused by JC polyomavirus, which induces lytic infection of oligodendrocytes, causing multifocal CNS demyelination.

Spinal Cord
- Vacuolar myelopathy is present at autopsy in 17% to 46% of patients with AIDS.
- Presenting symptoms are gait disturbance, weakness, sensory changes, and urinary dysfunction.
- Diagnosis is one of exclusion.
- Etiology is unclear.

Peripheral Nervous System
- HIV-associated distal sensory polyneuropathy is the most common peripheral neuropathy seen in HIV. Symmetrical paresthesia, numbness, and painful dysesthesia of the lower extremities can occur.
- Nucleoside neuropathy, mononeuritis multiplex, demyelinating neuropathies, and diffuse infiltrative lymphocytosis syndrome–associated neuropathy are other types of neuropathies seen in HIV patients.
- Clinical presentation, electrophysiologic studies and nerve biopsy help differentiate these entities.

Neurologic manifestations are frequent in human immunodeficiency virus type 1 (HIV-1) infection. In the era before combined antiretroviral therapy (ART) or in settings where antiretroviral drugs are not available, neurologic disease constituted the initial presentation in 10% of patients, and neurologic complications developed in 30% to 50% during the course of the disease.[1,2] Autopsies have shown involvement of the nervous system in up to 80% of cases.[3,4] With the advent of ART, the overall incidence of the most frequent human immunodeficiency virus (HIV)–associated neurologic diseases has decreased. The most consistent impact has been a decreased incidence of acquired immunodeficiency syndrome (AIDS)–associated dementia, but decreases in HIV-associated polyneuropathy and central nervous system (CNS) opportunistic infections have also been observed.[5,6,7,8,9]

Diagnosis of neurologic complications in patients with HIV-1 infection poses a particular challenge for clinicians. Indeed, HIV-infected individuals are often severely debilitated and present with multiple constitutional symptoms related to systemic infections or tumors that might overshadow or mimic a primary neurologic condition. In addition, HIV-infected individuals are usually treated with a combination of prophylactic drugs and a rapidly growing number of antiretroviral medications. Drug interactions and neurologic side effects of these medications are common, which adds another level of complexity for care providers. However, certain rules can be applied to facilitate the understanding of these challenging cases:

1. The spectrum of neurologic manifestations in HIV-1–infected individuals depends on their degree of immunosuppression, reflected by $CD4^+$ T-lymphocyte counts, and the speed of disease progression, as estimated by measurement of plasma HIV-1 viral load.
2. Multiple pathologies may coexist in the context of immunosuppression. The peripheral nervous system and CNS are frequently affected concomitantly in HIV-1–infected individuals, and opportunistic infections of the brain may be superimposed on primary HIV-1–associated neurologic disorders.
3. Antiretroviral medications and prophylactic drugs taken by HIV-infected individuals often cause neurologic side effects, which must be included in the differential diagnosis of these patients.
4. Immune recovery associated with ART may be associated with an inflammatory reaction within the CNS.

PRINCIPAL NEUROLOGIC MANIFESTATIONS OF HIV TYPE 1 INFECTION

Meningeal Syndrome

Patient Otherwise Asymptomatic, $CD4^+$ T-Lymphocyte Counts >200 Cells/μL: Aseptic Meningitis

Clinical presentation. Headache, stiff neck, and fever, associated with nausea and vomiting, can be the first manifestations of HIV-1 infection. It is a self-limited illness that subsides spontaneously after several weeks. In some cases, transient cranial neuropathies may develop, affecting mostly the fifth, seventh, and eighth cranial nerves.[10]

In addition, patients may present with symptoms of encephalopathy, and postmortem examination of individuals who died of unrelated causes in this early stage showed mild meningeal inflammation, focal cerebral white matter myelin damage, and perivascular inflammatory infiltrates and gliosis.[11,12]

Laboratory investigations. Cerebrospinal fluid (CSF) analysis shows a moderate lymphocytic pleocytosis (10–100 cells/μL), which is typical of viral meningitis.[13] This aseptic meningitis can occur as soon as 1 week after the primary infection, when HIV-1 conventional serology

is still negative. HIV-1 RNA, however, should be detectable in blood and CSF, and the HIV-1 p24 antigen might be detected in the blood. Repeat serology testing after 6 weeks usually helps to clarify this situation.

Treatment. Interestingly, early onset of aseptic meningitis has not been associated with late neurologic manifestations in HIV-1 infection, and patients may remain asymptomatic for many years before developing other symptoms of HIV-1 infection. If not properly diagnosed at the time of their acute illness, these patients may therefore unwittingly infect a number of sexual partners. It is therefore crucial to include HIV-1 in the differential diagnosis of aseptic meningitis. This condition might recur at any time throughout the course of the disease, although CSF pleocytosis becomes less common with advanced immunosuppression. Treatment is symptomatic. The decision to start on antiretroviral medications should be based on current guidelines that generally recommend treatment of initial HIV-1 infection (see Chapter 119).

Patient at Any Stage of HIV-1 Infection and CD4$^+$ T-Lymphocyte Counts at Any Level: Syphilitic Meningitis

Clinical presentation. In the United States, up to 6% of HIV-infected individuals have a history of syphilis. *Treponema pallidum* infection can also occur at any time during HIV-1 infection and mimics neurologic complications of AIDS. Indeed, both conditions may cause acute or chronic meningitis, myelopathy, cranial or peripheral neuropathies, cerebrovascular disease, and dementia. Therefore it is paramount to distinguish neurosyphilis from latent disease with positive serology and normal physical examination in HIV-infected patients because it has a direct impact on treatment. Neurosyphilis can occur as early as 1 year or as late as 30 years after initial infection. In this chapter, we discuss only syphilitic meningitis; other neurologic complications of this disease are covered in Chapter 237.

Laboratory investigations. Elevation of protein concentration and leukocyte counts can be found in the CSF of approximately 15% of patients with primary syphilis and up to 40% of patients with secondary syphilis. Some of these patients eventually experience spontaneous cure of this earlier CNS infection, but the persistence of asymptomatic CSF abnormalities for more than 5 years in the untreated patient is highly predictive of the development of clinical neurosyphilis.

Persistent mononuclear pleocytosis and elevated protein concentration can be found in the CSF of HIV-1–infected patients with neurosyphilis,[14] as well as an elevated immunoglobulin G synthesis rate and oligoclonal bands. This is of no help in establishing the diagnosis of neurosyphilis in the context of HIV-1 infection because these findings occur as well in asymptomatic HIV-1 seropositive patients, especially when they have detectable HIV-1 RNA in their CSF. Using either the criterion of elevated CSF protein of greater than 50 mg/dL or white blood cell counts greater than 10 cells/μL in HIV-positive patients with reactive rapid plasma reagin may lead to overdiagnosis of neurosyphilis in the absence of clinical symptoms. Follow-up lumbar puncture after 12 months showed persistence of CSF abnormalities in 62% of cases.[15] A positive CSF Venereal Disease Research Laboratory (VDRL) test result establishes the diagnosis of neurosyphilis if the spinal tap is not bloody. However, this test may be negative in HIV-1 infection.[16,17] A reactive CSF fluorescent treponemal antibody absorbed test increases the likelihood of *T. pallidum* infection but is less specific because it can result from treated neurosyphilis or from contamination of the CSF with small amounts of blood containing antibody at the time of the lumbar puncture.

Acute symptomatic syphilitic meningitis is usually the earliest clinical manifestation of neurosyphilis, which occurs within the first year of infection and can be associated with cranial nerve palsies, including isolated eighth nerve palsy, and signs and symptoms of hydrocephalus. CSF abnormalities are similar to those of asymptomatic neurosyphilis, except that the CSF VDRL test result is nearly always positive (also see Chapter 237).

Treatment. HIV-1–infected individuals with a positive serum rapid plasma reagin test result, unexplained CSF pleocytosis, and elevated protein concentration, as well as symptoms consistent with neurosyphilis, should be treated with intravenous penicillin even in the absence of a positive VDRL test result in the CSF. Treatment consists of intravenous penicillin G (3–4 million units IV every 4 hours for 10–14 days). In case of allergy to penicillin, ceftriaxone (2 g IM or IV once daily for 10–14 days) is another option. A careful follow-up of these patients is necessary, and a repeat spinal tap 1 month after onset of treatment should show a normalization of the CSF cellularity and protein concentration. Normalization of serum rapid plasma reagin predicted normalization of CSF and clinical abnormalities 13 months after treatment in more than 90% of a cohort composed mostly of HIV-infected men. This was more frequent in those receiving ART than in untreated patients.[18] Some patients have a transient increase in CSF HIV-1 viral load to more than 100,000 copies/mL associated with neurosyphilis, which subsides after antibiotic treatment. Neurosyphilis may amplify intrathecal HIV replication, possibly through immune activation that persists even after syphilis treatment.[19] As is the case with other opportunistic infections of the brain, this viral burden is likely carried by activated circulating lymphocytes and monocytes coming to combat the CNS infection and should not be interpreted as a manifestation of HIV-1 encephalitis.

Patient With AIDS, CD4$^+$ T-Lymphocyte Counts of <200 Cells/μL: Cryptococcal Meningitis

Clinical presentation. This is the most common opportunistic meningitis in AIDS, which affected 10% of patients before the ART era[20] but has decreased in incidence since then (also see Chapter 262).[21] Cryptococcal meningitis differs from aseptic meningitis in that meningismus is present in fewer than 40% of cases, and patients may present with only fever and headache, which become progressively more debilitating. Confusion, blindness, or altered state of consciousness occurs in severe cases.

Laboratory investigations. The detection of *Cryptococcus neoformans* antigen titers by enzyme immunoassay, latex agglutination, or lateral flow assay in the CSF provides a rapid diagnosis, which is confirmed subsequently by CSF culture. Because false-positive results occur with all the antigen tests, confirmation by culture is essential to establish the diagnosis of cryptococcal meningitis. Other CSF findings include markedly elevated opening pressure, mononuclear pleocytosis, elevated protein and decreased glucose concentration in 50% of the cases, and direct detection of the organism by India ink staining in 80% of the cases. Serum cryptococcal antigen is usually detected in cryptococcal meningitis in AIDS patients, and blood and urine cultures may also be positive.

Brain imaging is usually negative. The most common finding on magnetic resonance imaging (MRI) is dilated Virchow-Robin spaces, but findings can also include pseudocysts, cryptococcomas, and hydrocephalus.[22] Poor prognosis factors include altered mental status, absence of CSF pleocytosis, CSF antigen titer greater than 1:1024, a positive blood culture, and hyponatremia.

Treatment. Treatment should begin with amphotericin B (0.7–1.0 mg/kg) with flucytosine (100 mg/kg PO in four divided doses per day) until the patient is clearly responding and for not less than 2 weeks, which is associated with significantly increased rates of yeast clearance from CSF and decreased mortality compared with amphotericin B alone. Combination of fluconazole with amphotericin B showed no survival benefit.[23] Use of fluconazole alone as initial therapy is associated with much slower culture conversion and is not recommended.[24] Consolidation therapy then consists of fluconazole (400 mg PO daily) administered for 8 weeks or until CSF cultures are sterile. Lipid formulations of amphotericin B may be used for patients with renal insufficiency, and itraconazole may be substituted for fluconazole if patients can tolerate fluconazole but is clearly inferior to the latter. Lifelong maintenance therapy using fluconazole (200 mg/day) has been previously recommended to prevent relapses.[25] Updated recommendations state that maintenance therapy can be discontinued in HIV-negative patients if they have received 6–12 months of maintenance therapy. In HIV-positive patients, maintenance therapy can be discontinued if CD4$^+$ counts reach greater than 100 cells/μL, and there is a sustained undetectable or very low HIV RNA level for 3 or more months.[26]

The outcome is generally favorable within 2 weeks, but early mortality still reaches 6%.[27] In a large multicountry study of HIV-associated

cryptococcal meningitis predominantly in resource-limited settings, 2-week mortality was 17% and 1-year mortality was 41%.[28] In Botswana, the in-hospital mortality was significantly lower among those patients who were on ART at the time of diagnosis of cryptococcal meningitis compared with untreated patients (8% vs. 21%).[29] Complications such as increased intracranial pressure greater than 200 mm H_2O occur in nearly all patients. Such a complication should be recognized and treated aggressively with mechanical drainage, including repeat lumbar punctures, temporary external lumbar drainage, or intraventricular shunts.[30,31] Corticosteroids, acetazolamide, and mannitol have not been shown to be effective. Neurologic deterioration after commencing ART occurs in 26% of patients and is caused by cryptococcosis-associated immune reconstitution inflammatory syndrome (IRIS).[32] It is more frequent if ART is given 7 days after compared with 28 days after onset of antifungal therapy.[33] ART should be deferred until 5 weeks after diagnosis and treatment of cryptococcal meningitis because ART commenced within 1 to 2 weeks is associated with higher mortality.[26,34] Persistent CSF cryptococcal growth at ART initiation and poor $CD4^+$ T-cell increases on ART are strong predictors of cryptococcosis-associated IRIS.[32] Management of cryptococcosis-associated IRIS includes continuation of ART, antifungal therapy, and a course of corticosteroids.[35]

Differential Diagnosis of Meningitis

Other CNS infections in AIDS include tuberculosis, histoplasmosis, aspergillosis, coccidioidomycosis, and infections with *Acanthamoeba, Candida albicans, Trypanosoma cruzii,* herpes simplex and herpes zoster viruses, and *Nocardia asteroides.* Geographic differences account for variations in local prevalence. Estimates indicate that more than 1 million people worldwide have tuberculosis and HIV coinfection, with the highest number of individuals in sub-Saharan Africa. CNS tuberculosis can present in numerous ways, including as a tuberculoma, an abscess, a spinal cord lesion, and most commonly as tuberculous meningitis (TBM). TBM is a highly treatable condition in resources-limited settings (RLS) because its presentation is commonly subacute and antituberculosis treatment is widely available. However, TBM diagnosis remains a challenge throughout the world. Acid-fast bacilli staining on CSF has a sensitivity of 10% to 20% that varies greatly on the basis of technical expertise.[36] CSF culture has a sensitivity of approximately 50% and can take as long as 6 weeks to become positive.[37] The Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA) is a molecular diagnostic test that showed 95% sensitivity for the diagnosis of TBM on CSF in a small study in Uganda. As a result, the World Health Organization has endorsed it as the test of choice for the diagnosis of TBM, when available.[38] In RLS, treatment is often initiated empirically with minimal objective testing for guidance except for hypoglycorrhachia and high CSF total protein, for which biochemical tests are available.

Bacterial meningitis is more commonly a problem for HIV-infected patients in RLS compared with resource-rich settings.[39,40] HIV-infected patients are at risk for pneumococcal infection,[41] and pneumococcal meningitis usually arises from spread of an untreated primary infection. These cases are more likely in RLS where patients present at later stages of disease and have limited access to health services.

COGNITIVE AND MOTOR SYNDROMES

HIV Type 1–Associated Neurocognitive Disorder

Clinical presentation. This common CNS complication of HIV-1 infection occurs in 15% of patients with AIDS and can be the first manifestation of the disease in 3% to 10%.[42] In the past, this condition has also been named AIDS dementia complex, HIV-1 encephalopathy, or HIV-associated major cognitive disorder. A less severe entity named HIV-1–associated mild neurocognitive disorder occurs in an additional 20% to 25%, and asymptomatic neurocognitive impairment (ANI) is used to categorize individuals with subclinical impairment.[43,44] Risk factors in uncontrolled HIV infection include an AIDS-defining illness, increased age and survival duration, lower nadir of $CD4^+$ T-cell counts, and higher baseline HIV viral loads.[45] Risk factors for neurocognitive decline in well-controlled HIV-infected individuals include glomerular filtration rate 50 mL/min or less, HIV infection for 15 years or longer, education level 12 years or less, and cerebrospinal fluid protein greater than 45 mg/dL.[46]

The clinical characteristics of this disorder can be subdivided into three main categories: cognitive, behavioral, and motor (Table 125.1).

Initial symptoms are usually subtle and include difficulty with memory, slowness of thought, and trouble concentrating. Complex mental activities become more time consuming and difficult to perform. A loss of interest in social and professional activities soon follows, and such apathy and social withdrawal may be mistaken for depression. Although cognitive and behavioral symptoms are prominent in most patients, some mainly present with motor dysfunction, which includes decreased coordination, altered handwriting, loss of balance, and gait instability.

The mental status examination reveals minor psychomotor slowing, inattention, decreased short-term memory, inability to perform simple calculations, and frontal release signs. Ocular movement testing shows saccadic pursuit. Other frequent findings are brisk reflexes, mild postural tremor, slowing of rapid alternating movements, and gait instability when performing half-turns. If untreated, dementia becomes more global, profoundly impairing orientation, memory, and cognition. Confusional or psychotic episodes have been reported, but seizures are a rare occurrence. Despite the extent of the cerebral involvement, there is usually no aphasia, apraxia, or other signs of discrete cortical dysfunction, except in terminal stages. Therefore this syndrome has been classified as a frontal-subcortical dementia. A rapid bedside evaluation can be performed with the Montreal Cognitive Assessment (MoCA) or the Ascertain Dementia 8-Item Informant Questionnaire (AD8).[47,48] However, these screening tests are not as sensitive as a detailed neuropsychological evaluation, which should include tests of attention, memory, and psychomotor speed such as digit span and verbal fluency tests and the Trail Making Test, the Grooved Pegboard Test, the Symbol Digit Modalities Test, and the Rey Auditory Verbal Learning Test.[49] The International HIV Dementia Scale can be used to identify individuals at risk of HIV-associated dementia despite language barriers.[50,51]

Unfortunately, cognitive dysfunction persists despite ART. A cross-sectional study of 1555 HIV-infected adults in the United States showed neuropsychological impairment in 52%. Prevalence estimates for specific HIV-associated neurocognitive disorder (HAND) diagnoses were 33% for ANI, 12% for mild neurocognitive disorder, and 2% for HIV-associated dementia.[52] In recent longitudinal studies that followed patients with HAND for 3 to 4 years, neurocognitive status remained stable in approximately 61% to 77% of patients, declined in 13% to 23%, and improved in 10% to 16%.[53,54] Patients with ANI developed symptomatic neurocognitive decline earlier than those with normal cognitive function.[55]

Laboratory investigations. Numerous groups have detected early neurologic dysfunction in HIV-1–infected asymptomatic individuals.[56,57,58] Subtle electrophysiologic abnormalities can be found in early HIV-1 infection (on electroencephalography, evoked potentials, and nerve conduction studies), but they do not seem to have a predictive value for the later onset of AIDS dementia, which occurs generally when the $CD4^+$ T-lymphocyte counts are less than 200 cells/μL. CSF analysis shows mild lymphocytic pleocytosis in 25% of patients and elevated protein in 55%,[59] which can also be found in nondemented patients. Increased CSF levels of neurofilament light protein and neopterin have been reported in HAND, but their use in patients' management is limited because these markers are also elevated during opportunistic infections

TABLE 125.1 Clinical Triad in Human Immunodeficiency Virus Type 1–Associated Neurocognitive Disorder

COGNITIVE	BEHAVIORAL	MOTOR
Forgetfulness	Apathy	Gait instability
Mental slowing	Social withdrawal	Poor coordination
Decreased concentration	Lack of spontaneity	Leg weakness

of the CNS and because these assays are not readily available in the clinical setting.[60,61]

Measurement of the HIV-1 CSF viral load has been evaluated as a surrogate marker in HIV-1–infected individuals with cognitive dysfunction. A wide overlap was found between CSF HIV-1 viral load values of demented and nondemented individuals, and concomitant opportunistic infections of the CNS must be ruled out because they also contribute to transient elevation of HIV-1 viral burden in the CSF.[62] These findings suggest that there may be two phases of HIV-1 infection in the CSF. First, a transitory infection of the CNS may occur early in the disease via trafficking lymphocytes. These viral strains have a lymphotropic phenotype (using CXCR4 coreceptor) and may respond to therapy in parallel to plasma virus load. Second, a more autonomous infection of CNS monocytes/macrophages may take place at a later stage, with macrophage tropic strains (using CCR5 coreceptor).[63] Such patients may need higher drug penetration in the CNS. Whether measurement of the HIV-1 CSF viral load provides an accurate representation of the viral replication occurring in the CNS is unclear. However, subsets of patients with higher CSF than plasma viral loads, known as CSF escape, have been recognized, in whom intrathecal viral replication correlates with neurologic deficits. Because HIV-1–associated dementia complex is a diagnosis of exclusion, CSF examination is indicated for bacterial, fungal, and acid-fast bacteria cultures; cryptococcal antigen; VDRL test; and cytology. In addition, performing CSF polymerase chain reaction (PCR) for JC virus (JCV), the agent of progressive multifocal leukoencephalopathy (PML), is indicated because this condition is often difficult to distinguish from HIV-1 encephalopathy on brain imaging studies (see "Progressive Multifocal Leukoencephalopathy" later; also see Chapter 144).

Imaging studies. Computed tomography (CT) and MRI may show greater subcortical atrophy than cortical atrophy, which is not proportional to the degree of dementia. The progression of brain atrophy is worse in older individuals with HIV than normal controls despite viral suppression.[64] MRI may also demonstrate multiple hyperintense signals in T2-weighted images, which are nonenhancing, poorly demarcated, and localized bilaterally in the subcortical white matter (Fig. 125.1). MRI is superior to CT for distinguishing these abnormalities from confounding illnesses such as PML. Unlike lesions of PML, there is no associated hypointensity in T1-weighted images. The severity of global brain atrophy and signal changes in basal ganglia correlates with cognitive impairment.[65]

Brain biopsy and histologic analysis. There is no indication to perform a brain biopsy in patients with HAND, unless imaging studies suggest the presence of another process. Postmortem examination reveals encephalitis with multinucleated giant cells and microglial nodules, as well as astrocytosis and perivascular mononuclear cell infiltrates. HIV-1 has been found in microglial nodules, perivascular macrophages, and multinucleated giant cells. The latter are the result of the fusion of infected macrophages. The virus can also infect astrocytes.[66] HIV-1 has also been found in endothelial cells,[67] and abnormalities of the cerebral microcirculation are characterized by increased cellularity and pleomorphism of endothelial cells, as well as prominent perivascular aggregates of HIV-infected macrophages. These findings are sometimes related to microinfarcts and are postulated to give rise to increased vascular permeability.[68] Indeed, early enhancement has been detected in the basal ganglia of patients with HAND, suggesting a disruption of the blood-brain barrier (BBB) in these regions.[69] HIV-1 does not infect glial cells in adults, whereas a limited expression of viral regulatory gene products has been demonstrated in glial cells from children with AIDS.[70] White matter pallor involving primarily the centrum semiovale is a hallmark of HIV-1 encephalopathy in adults, which may be caused by seepage of macromolecules and edema fluid in the brain parenchyma through the permeable BBB and may cause injury to the myelinated fibers. Finally, HIV-1 does not infect neurons. Therefore quantitative neuronal loss, decrease in cell size, or dendritic injury found in the cortex of demented patients[71] may only be secondary to infection of other cells and associated immune activation.[72]

Treatment. Although the pathophysiology of HAND is not entirely elucidated, HIV-1 remains the main triggering factor, and it is therefore reasonable to prevent viral replication in the CNS. However, antiretroviral drugs have variable penetration through the BBB, and evaluation of CNS concentration and pharmacokinetics of these medications is incomplete. On the basis of available pharmacologic data, antiretrovirals can be classified according to their penetration through the BBB. A CNS penetration effectiveness (CPE) score[73] is given to each medication going from 1 (low) up to 4 (high) (Table 125.2). The CPE score of an ART regimen is calculated by summing the score of each individual antiretroviral. Drugs with high CNS penetration include zidovudine, nevirapine, and ritonavir-boosted indinavir.

Since 1996, the availability of protease inhibitors (PIs) and the use of three or more drugs in ART regimens have made a major impact on the incidence of neurologic disease in HIV-1–infected patients.[74,75,76] This is somewhat surprising; PIs in general have a very low CSF-to-plasma ratio because they are highly protein bound. Nevertheless, the incidence of HAND, which was estimated at 6.49 per 1000 person-years pre-1997, decreased to 0.66 by 2003–2006, an approximately 10-fold decrease.[74,77] Although ART is now the treatment of choice for

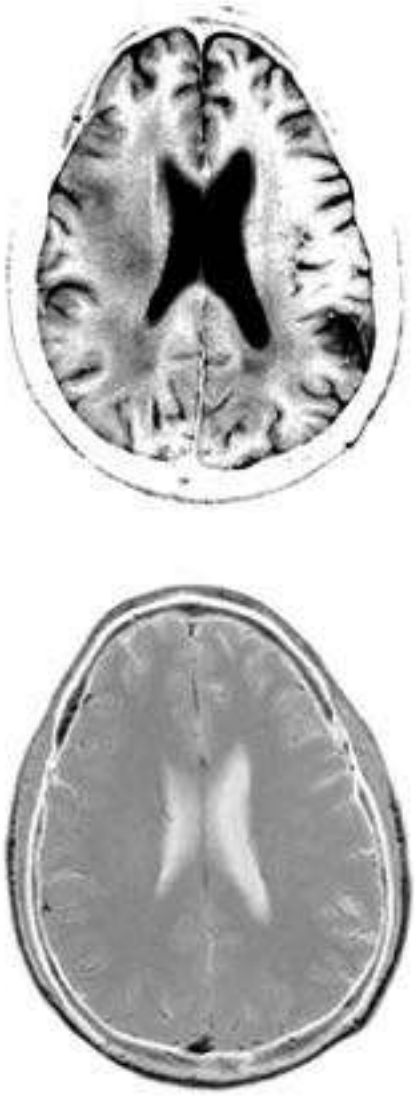

FIG. 125.1 Brain magnetic resonance images of a 42-year-old man with human immunodeficiency virus–associated neurocognitive disorder. The T2-weighted image (A) shows bilateral, ill-defined hyperintense signal in the periventricular white matter and centrum semiovale, which does not enhance with gadolinium injection on the T1-weighted image (B). The ventricles are slightly enlarged, consistent with subcortical atrophy.

TABLE 125.2 Central Nervous System Penetration Effectiveness Score for Antiretroviral Drugs

	4	3	2	1
Nucleoside reverse-transcriptase inhibitors	Zidovudine	Abacavir Emtricitabine	Didanosine Lamivudine Stavudine	Tenofovir
Nonnucleoside reverse-transcriptase inhibitors	Nevirapine	Delavirdine Efavirenz	Etravirine	
Protease inhibitors	Indinavir-r	Darunavir-r Fosamprenavir-r Indinavir Lopinavir-r	Atazanavir-r Atazanavir Fosamprenavir	Nelfinavir Ritonavir Saquinavir-r Saquinavir Tipranavir
Fusion/entry inhibitors		Maraviroc		Enfuvirtide
Integrase inhibitors		Raltegravir		

[a]Numerical score of 4 is highest and 1 is lowest by method of Letendre et al.[73]
-r, Ritonavir-boosted.

advanced HIV-1 disease, the optimal drug combination for HAND has not been established. Whether regimens containing several drugs with good penetration through the BBB were advantageous has been investigated. In ART-experienced patients with $CD4^+$ T-cell counts of less than 200 cells/μL, those who received multiple CNS penetrating agents had a greater reduction of CSF HIV-1 RNA.[78,79,80] These results have been confirmed in recent studies, showing that higher CPE scores of long-term ART are associated with better viral suppression in CSF[72,73,74] and decreased risk of cognitive impairment.[81,82,83,84] However, a recent randomized trial of ART with high CNS penetration did not show any benefit in HAND; this could possibly be due to the short duration of the study and unbalanced randomization.[85] One study also showed that regimens with higher CPE scores may be associated with increased risk of HIV dementia.[86] Other studies have shown that discontinuation of efavirenz was associated with cognitive improvement in scheduled treatment interruption regimens[87] and in otherwise asymptomatic HIV-infected individuals,[88] suggesting that some antiretrovirals might have neurotoxic properties, which was also demonstrated in vitro.[89] The possible neurotoxic effects of efavirenz were also highlighted by a study that found worse neurocognitive function in patients on long-term efavirenz compared with those taking lopinovir-ritonavir.[90]

The hypothesis that discordant mutations are present in blood and CSF has been confirmed by the analysis of HIV-1 genotypes in paired samples. Mutations of the reverse-transcriptase and protease genes of CSF strains, but not in the corresponding blood isolates, were detected in 10 of 23 subjects (43%). Interestingly, mutations conferring resistance to low or high CSF-penetrating agents occurred at similar frequencies.[91] Reverse-transcriptase mutation patterns differed in 14 of 21 (67%) paired samples from plasma and CSF in one study, and HIV RNA responses and reverse-transcriptase genotypes were discordant between CSF and plasma in some subjects.[92,93] A recent study suggests a role of M184V/I mutations in influencing CNS reservoirs of HIV and CSF escape.[94] In a study of HIV-infected children starting ART, 8 of 11 had identical resistance patterns in both CSF and plasma at baseline, whereas at week 48 of treatment, only 1 of 9 children had similar genotypes, suggesting discordant viral evolution in these two compartments.[95] CSF viral escape with progressive neurologic dysfunction despite well-controlled plasma viral load and good immunologic response on ART is now well recognized.[94] In general, these patients had a higher CSF viral load compared with plasma. Clinical characteristics of patients differed based on plasma HIV RNA levels preceding the CSF escape, with low-level viremia or suppressed patients being more symptomatic.[94] These patients have CSF pleocytosis and elevated protein concentration, MRI abnormalities, and unique CSF resistance mutations compared with plasma. They have clinical and radiologic improvement after optimization of ART.[96] Failure to recognize the occurrence of different genotypic resistance patterns in plasma and CSF may lead to uncontrolled viral replication in the CNS and worsening neurologic symptoms despite evidence of viral clearance in the periphery. These resistant isolates may, in turn, spill back into the circulation and contribute to the progression of HIV-1 disease. Therefore genotypic analysis of CSF HIV-1 strains is indicated in patients with cognitive dysfunction who have good virologic response to ART in the plasma but persistence of an elevated HIV-1 CSF viral load.

In addition to antiretroviral treatment, other experimental compounds have been tested for the treatment of HAND, but without evidence of clear benefit so far. These include a calcium channel blocker (nimodipine), antioxidants (vitamin E, thioctic acid, CPI-1189), a tumor necrosis factor-α antagonist (pentoxifylline, CPI-1189), a noncompetitive *N*-methyl-D-aspartate inhibitor (memantine), peptide T, rivastigmine, selegiline, minocycline, and a combination of paroxetine and fluconazole.[97,98] Use of computerized cognitive training as in other neurodegenerative disorders is being tried for HIV patients as well.[99]

CENTRAL NERVOUS SYSTEM MASS LESIONS

In patients with AIDS presenting with change in mental status or abnormal neurologic examination results, brain lesions are frequently revealed on CT or MRI. These can be quite extensive and may represent life-threatening emergencies.

Toxoplasma Encephalitis

At the beginning of the AIDS epidemic, *Toxoplasma* encephalitis (TE) was the most common cerebral mass lesion in patients with AIDS. TE is caused by a reactivation of latent infection by the protozoan *Toxoplasma gondii* as a result of progressive loss of cellular immunity. The incidence of TE depends on the seropositivity for *T. gondii* in a specific population. Seroprevalence can vary widely between countries and even within a country depending on factors such as country of birth, climate, and socioeconomic status. The seroprevalence of toxoplasmosis in the United States for individuals ages 12 to 49 years decreased from 14.1% in the 1980s to 6.7% in 2009–2010. The reason for this decrease in seroprevalence is unclear but may be due to improved soil/cat feces–related hygiene as well as food storage.[100] The incidence of TE has decreased thanks to widespread prophylaxis for *Pneumocystis jirovecii* pneumonia using trimethoprim-sulfamethoxazole, which also prevents CNS toxoplasmosis.[101] Since the era of ART, there has been a further trend for a decreased incidence of this condition,[21] and it accounted for only 28% of focal brain lesions occurring in patients with AIDS in 1998[102] and for 26% of patients with neurologic disorders in 2004.[103] Nevertheless, TE continues to be a severe health problem. Patients who develop TE nowadays either did not receive or had not taken ART adequately.[104]

Clinical presentation. Almost 90% of patients with TE have $CD4^+$ T-lymphocyte counts less than 200 cells/μL, and 75% have $CD4^+$ counts less than 100 cells/μL at the time of clinical presentation. The most common symptoms include headache, confusion, fever, and lethargy. Cases may also occur with a strokelike presentation.[105] Seizures develop in up to 30% of patients.[106] Seventy percent of patients have focal signs

on the neurologic examination, such as hemiparesis, cranial nerve palsies, ataxia, and sensory deficits. The clinical presentation is usually subacute, ranging from a few days to a month (see Chapter 278).

Laboratory investigations. As is the case in the general population, serum anti-*Toxoplasma* immunoglobulin G antibodies can be detected in patients with TE, whereas immunoglobulin M antibodies are less commonly found, supporting the notion that most cases represent a reactivation of latent infection. Measurement of antibody titers is not helpful to establish the diagnosis. On enzyme-linked immunosorbent assay, only 7% of patients known to be seropositive for *T. gondii* had lost their antibodies at the time of presentation.[107] Therefore, a negative serology orients toward another entity, whereas a positive serology is not diagnostic (see Chapter 278).

A slight increase in CSF protein concentration and a moderate mononucleated pleocytosis (<60 cells/μL) are common but nonspecific, and may be due to the underlying HIV-1 infection. A slight decrease in the CSF glucose has been reported but is not a constant finding. The PCR technique has been less useful for detection of *T. gondii* DNA in the CSF compared with other pathogens, with a sensitivity of only 44% to 65% and a specificity of 100%.[108] However, this low sensitivity may be improved by the use of stage-specific PCR primers.[109] CSF analysis is therefore more useful to rule out other infectious processes than to confirm the diagnosis of TE.

Imaging studies. Neuroimaging with head CT or MRI demonstrates CNS lesions in almost all cases, with the exception of the rare diffuse encephalitic form of toxoplasmosis. Lesions are multiple in two-thirds of the cases, and 90% of them display ring enhancement after administration of contrast material. MRI is more sensitive than CT in detecting multiple lesions. These are generally localized at the corticomedullary junction, in the white matter, or in the basal ganglia; are surrounded by edema; and induce mass effect on surrounding structures (Fig. 125.2). Unfortunately, the neuroradiologic characteristics of TE are not pathognomonic and may be observed in other conditions such as primary CNS lymphoma (PCNSL) (see "Primary Central Nervous System Lymphoma" later).

Brain biopsy. Because of the good response to therapy, histologic examination is not required for the diagnosis of TE, and an empirical therapeutic trial is recommended when the clinical and radiologic

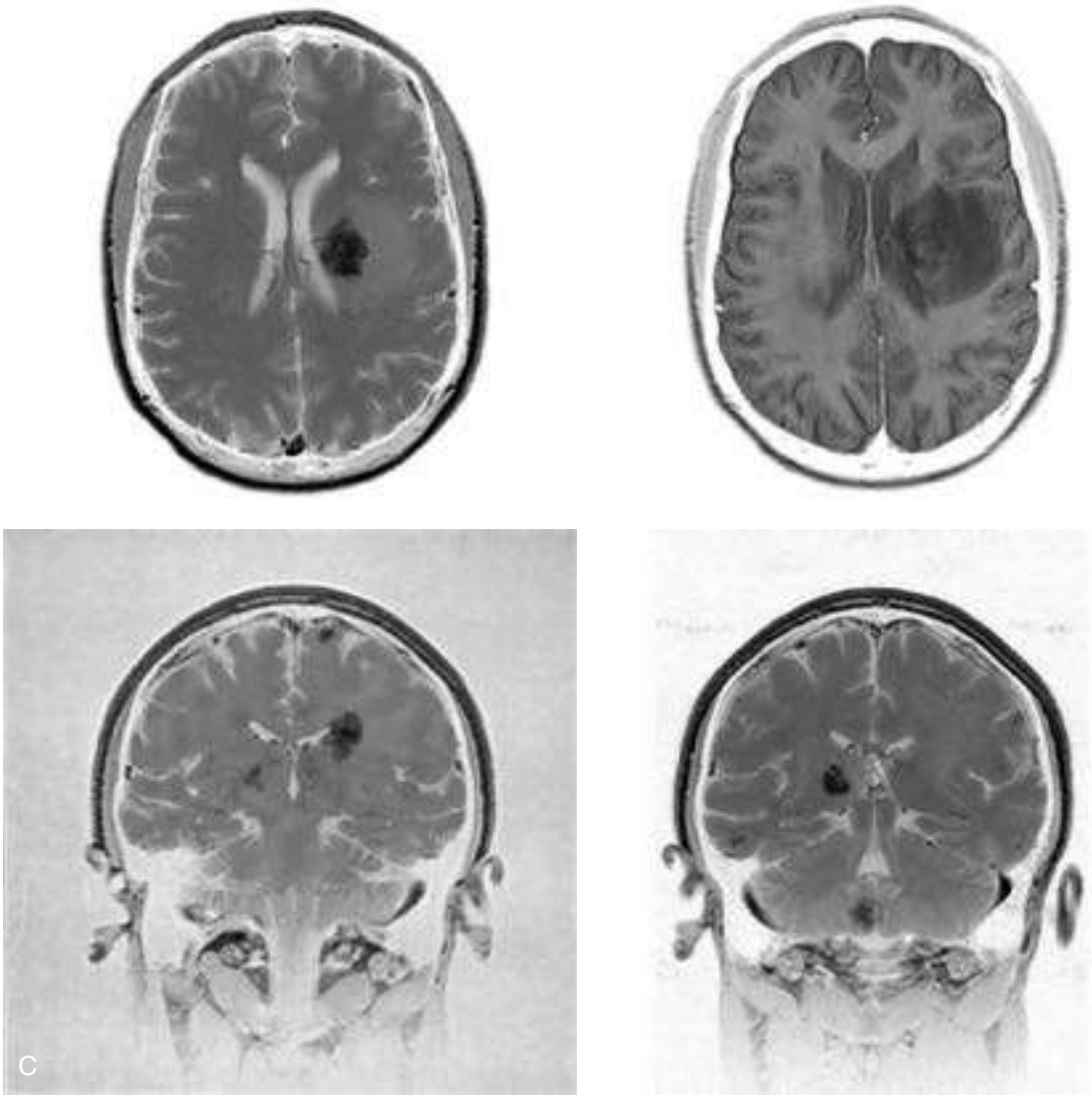

FIG. 125.2 Brain magnetic resonance images of a 38-year-old man with acquired immunodeficiency syndrome and *Toxoplasma* encephalitis. The T1-weighted image after gadolinium injection shows a large enhancing lesion in the left frontal lobe (A), surrounded by edema, as demonstrated on the T2-weighted image (B). Additional smaller enhancing lesions can be seen on coronal cuts in the right thalamus and at the left convexity (C) and in the right cerebellum and right temporal lobe (D).

findings are consistent with this diagnosis. Histologic examination shows mainly necrotic abscesses with blood vessel thrombosis and necrosis. Cysts containing bradyzoites, the dormant form of *T. gondii,* coexist with numerous active tachyzoites.

Treatment. Treatment consists of a combination of pyrimethamine and sulfadiazine, which cause a synergistic and sequential block on the folic acid metabolism necessary for the development of the parasite. Standard acute therapy consists of pyrimethamine (200 mg PO the first day of treatment, followed by 50–75 mg/day PO), sulfadiazine (1000–1500 mg PO in four divided doses), and folinic acid (10–25 mg/day PO for 6 weeks).

Clindamycin (600 mg IV or 450 mg PO four times daily) is an adequate alternative to sulfadiazine in the previous regimen for patients allergic to sulfonamides. Side effects, which consist of cytopenia, rashes, diarrhea, and increased liver enzymes, have been reported in 40% to 70% of patients receiving pyrimethamine and sulfadiazine and in 36% of those receiving pyrimethamine and clindamycin. These can cause early discontinuation of therapy. Atovaquone (1500 mg PO two times daily) can also be combined with pyrimethamine and folinic acid. Although corticosteroids are frequently prescribed to diminish cerebral edema, their use has been shown to be neither beneficial nor harmful in TE.[110] Because high doses of steroids also decrease the size of CNS lymphoma lesions, they should be administered only in cases with impending cerebral herniation during the initial medical treatment of presumed TE so as not to cloud the diagnosis. Neurologic improvement is clinically apparent in more than half the cases by day 3 of therapy and in most cases by day 7. Nevertheless, the death rate at 1 year varies from 10% to 60%.[103,111] Persistent neurologic sequelae remained in 37% of survivors.[112] A failure to improve or a worsening of the symptoms should prompt repeat imaging studies by days 10 to 14 to determine the need for a brain biopsy. TE-associated IRIS presenting simultaneously with initiation of ART has been reported in six patients, including five who were supposed to take effective prophylaxis. Brain biopsy showed intense angiocentric infiltrate consisting predominantly of $CD8^+$ T lymphocytes in two of them.[113]

Secondary prophylaxis. *Toxoplasma gondii* is sensitive to treatment only when in tachyzoite form. Because dormant cystic forms may rupture and reinitiate the infectious process at any time, maintenance therapy is necessary to prevent a relapse, which is likely to occur after a delay of 6 to 8 weeks of interruption of treatment. Standard maintenance therapy consists of pyrimethamine (25–50 mg/day) with folinic acid (10–25 mg/day) and either sulfadiazine (2–4 g/day in four divided doses) or clindamycin (450 mg PO four times daily). Patients with sustained $CD4^+$ T-cell counts higher than 200 cells/μL for more than 3 months can discontinue their secondary prophylaxis.[114] Relapse occurred in only 1 of 22 (5%) patients.[115]

If the diagnosis is made in a timely fashion and the patient does not become intolerant to the treatment, TE is currently an opportunistic infection with a relatively high therapeutic success rate, and death is usually caused by other complications of AIDS.

Primary Central Nervous System Lymphoma

This condition, which affected 2% of patients with AIDS at the beginning of the epidemic, has seen its incidence decrease considerably in the ART era.[21] In 1998, it accounted for only 12% of AIDS-related focal brain lesions.[102] Its radiographic appearance makes it the principal differential diagnosis of TE.

Clinical presentation. The onset of symptoms is generally subacute, lasting weeks to months. Confusion, lethargy, and memory loss are the most frequent symptoms. As the disease progresses, hemiparesis, aphasia, seizures, and cranial nerve palsies occur.[116] Fever, headaches, and constitutional symptoms are generally absent, which helps distinguish PCNSL from TE. At the time of diagnosis, the average $CD4^+$ T-lymphocyte counts are usually very low (≈50 cells/μL).

Laboratory investigations. A mild mononuclear pleocytosis (<30 cells/μL) and an increase in the protein concentration in the CSF are common findings in patients with PCNSL but are nonspecific and may be due to the underlying HIV-1 infection. High protein levels (≤590 mg/dL) have been reported in patients with extensive lymphomatous infiltration of both cerebral hemispheres. Hypoglycorrhachia is a rare finding.

It is important to perform cytologic analysis of the CSF because the presence of atypical or malignant lymphomatous cells can establish the diagnosis. Flow cytometry immunophenotyping has at least 25% higher sensitivity than conventional cytomorphologic methods for the detection of malignant cells.[117] Systemic extracerebral lymphomas, which have an increased incidence in AIDS patients, can also cause lymphomatous meningitis but do not generally spread to the brain itself. Similarly, PCNSL does not metastasize systemically. The Epstein-Barr virus (EBV) genome can be detected in tumor cells of nearly all PCNSLs, but only in some systemic lymphomas of AIDS patients, and rarely in primary brain lymphoma tissue from patients without immunodeficiency.[118] When testing is performed in a research setting, detection of EBV DNA by PCR in the CSF has a reported sensitivity of 80% to 90% and a specificity of 87% to 98% for the diagnosis of PCNSL. Measurement of the EBV CSF viral load by quantified PCR in the CSF may improve the sensitivity of this test[119] and may be clinically useful in monitoring responses to treatment.[120] However, other groups have found EBV DNA in the CSF of patients who did not have PCNSL, which raises doubts regarding the true predictive value of this test when performed in the clinical setting.[121,122]

Imaging studies. Head CT or MRI usually shows findings consistent with a CNS tumor. Solitary mass lesions are as frequent as multiple lesions.[116] Most lesions display some degree of enhancement, which is usually nodular or patchy. Ring enhancement, identical to that commonly seen in TE, can occur and correlate with central tumor necrosis. Subependymal enhancement seems more specific of CNS lymphoma, but this is a rare feature. Lesions are frequently located in the corpus callosum, the periventricular white matter, or the cortex. Involvement of the posterior fossa occurs only in 10% of cases.[123] Lesions can be surrounded by edema, which may induce variable mass effect on neighboring structures (Fig. 125.3).[124] Magnetic resonance imaging is more sensitive than CT in revealing multiple lesions, which can be useful if a biopsy is being considered. Thallium-201 single-photon emission CT shows an accumulation of isotope in the tumor due to increased metabolic activity.[125] In one series comparing enhancing brain lesions caused by toxoplasmosis and lymphoma, the thallium index of each lesion, measured as the ratio of the mean uptake in the lesion to that of the corresponding contralateral side, was increased and a significant predictor of lymphoma only in lesions 2 cm or larger, yielding 100% sensitivity and 89% specificity.[126] However, the usefulness of this method in differentiating CNS lymphoma from opportunistic infections may depend heavily on the resolution of the images, and appropriate technology may not be available in every center. Fluorodeoxyglucose–positron emission tomography scan has also shown utility in differentiating PCNSL from nonmalignant disease in a small series.[127]

Brain biopsy. If the CSF cytology fails to reveal lymphomatous cells and if EBV PCR is negative in the CSF, an image-guided stereotactic brain biopsy using CT or MRI is the only way to ascertain the diagnosis. The macroscopic appearance of these tumors is generally that of a multifocal, diffusely infiltrating, and expanding nonhemorrhagic mass, without well-demarcated borders, simulating the appearance of an infiltrating glioma. However, well-circumscribed, largely necrotic masses can also resemble abscesses. Microscopic analysis reveals a variety of patterns, including large cells, small noncleaved cells, and immunoblastic or mixed cellular components. These are generally of B-lymphocyte lineage. EBV is present in tumor cells, suggesting that this virus may have a role in tumorigenesis,[128] as is the case in Burkitt lymphoma and in nasopharyngeal carcinoma.

Treatment. The response to steroids seen in lymphomas of non-AIDS patients is not always seen in AIDS patients. In patients with altered mental status, debilitating focal symptoms, or impending herniation, dexamethasone (10 mg IV or PO, followed by 4 mg IV or PO every 6 hours) can provide temporary improvement. Current treatment guidelines recommend the use of high-dose methotrexate in combination with ART. A study of 51 patients treated with this regimen demonstrated a median overall survival of 5.7 years, with 5- and 10-year rates of 48% and 41%, respectively.[129] In the past, treatment of CNS lymphoma in AIDS consisted of whole-brain radiation therapy with a total of 3000

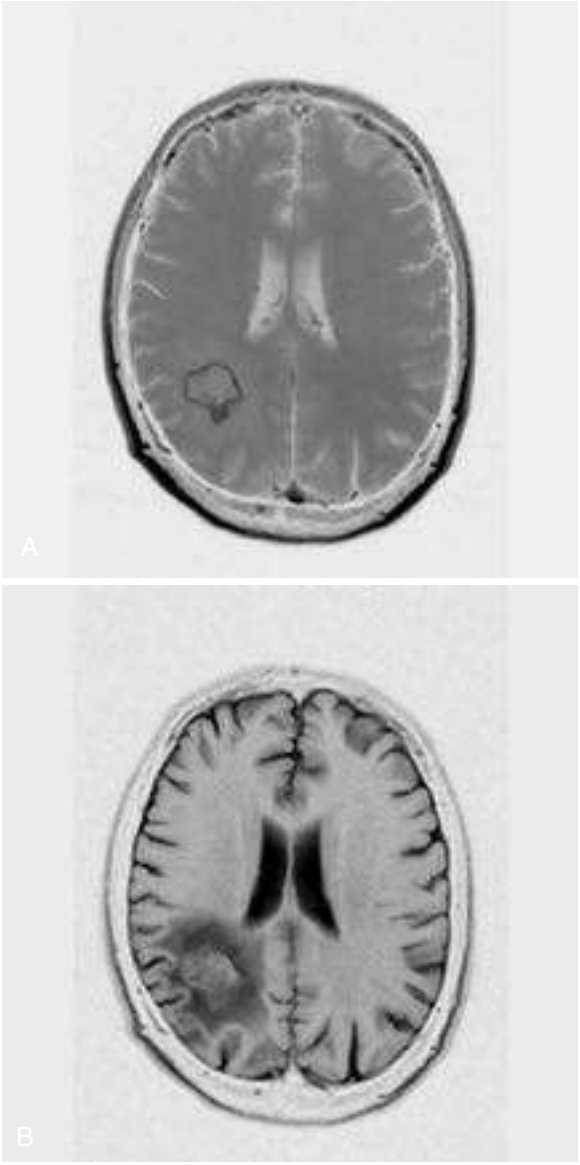

FIG. 125.3 Brain magnetic resonance images of a 50-year-old man with primary central nervous system lymphoma. (A) A 2-cm ring-enhancing lesion is present in the right parietal lobe, surrounded by edema. (B) The T2-weighted image shows low signal intensity centrally, which is more consistent with cellular proliferation than with an infectious process.

cGy over a 3-week period. Steroids were added to decrease peritumoral edema and mass effect.[123] The palliative response rate before the ART era was 53%.[130] In a series of 25 patients in the United States seen between 1995 and 2001, the median survival was 87 days. However, patients who received ART after diagnosis had a longer survival; 6 of 7 patients receiving ART were alive compared with 0 of 18 untreated patients at a median follow-up time of 1.8 years. Furthermore, the median survival was only 29 days for 11 patients who received neither radiation therapy nor ART.[131] The estimated 3-year overall survival rate of HIV-associated PCNSL after whole-brain radiation therapy was 64%. Results were influenced by the performance status at presentation, with patients with good performance status having a 100% 3-year overall survival versus 38% in those with poor performance status.[132] However, combination treatment including radiation therapy and chemotherapy is difficult to tolerate because of high toxicity. Additionally, whole-brain radiation therapy is associated with worsened cognitive function and white matter disease.[133] Immune recovery induced by an effective ART leads to dramatic improvement in survival of patients with AIDS-associated PCNSL.[134]

Progressive Multifocal Leukoencephalopathy

Before the AIDS era, PML was a rare disease affecting mainly patients with chronic lymphocytic leukemia, those with non-Hodgkin lymphoma, or organ transplant recipients.[135] At the beginning of the AIDS epidemic, up to 5% of patients developed PML.[136] Despite the availability of ART, up to 28% of focal brain lesions in AIDS patients were attributed to PML in 1998,[102] which equals the number of TE cases. The incidence of PML in ART-treated patients is 0.6 to 1.3/1000 person-years.[137,138] PML is caused by the polyomavirus JCV. This double-stranded DNA virus infects 90% of the normal adult population worldwide and remains quiescent in the kidneys without causing any disease. In the setting of immunosuppression, JCV is reactivated and induces a lytic infection of oligodendrocytes, causing multifocal demyelination of the CNS (see Chapter 144).[139]

Clinical presentation. Classic PML usually develops when $CD4^+$ T-lymphocyte counts decrease to fewer than 200 cells/μL.[136] The most common presenting symptoms are limb weakness (hemiparesis or monoparesis), altered mental status, gait ataxia, and visual symptoms, including hemianopsia, diplopia, and third nerve palsy. Approximately 80% of patients have focal neurologic findings.[140] However, PML lesions can occur anywhere in the CNS white matter, particularly at the subcortical level, although the optic nerves and the spinal cord are rarely involved.[141] Because subcortical lesions prevent the transmission of information to and from the cortical areas, presentation implying cerebral cortical dysfunction, such as aphasia, apraxia, memory loss, and visual agnosia, does not rule out this diagnosis. In fact, seizures, which are usually considered to be of cortical origin, occur in up to 44% of PML patients.[142] Some symptoms can also be attributed to HAND, which is often superimposed on other CNS pathologies in the end stages of AIDS.

Laboratory investigations. Laboratory analyses are necessary to establish the diagnosis of PML.[143] Conventional CSF analysis is normal or shows a moderate increase in protein concentration and a mild mononucleated pleocytosis (<25 cells/μL), which is nonspecific in the context of HIV-1 infection. Before the ART era, detection of JCV DNA in the CSF by PCR had a sensitivity of 74% to 92% and a specificity of 92% to 96% for the diagnosis of PML.[144,145] In patients receiving ART, the sensitivity of this test has decreased to 58%, probably because of decreased JCV replication in the context of a recovering immune system.[146] Nevertheless, measurement of JC viral load in the CSF appears to be of value as a correlate of PML disease activity and survival.[145,147,148]

Imaging studies. The hallmark of PML is patchy or confluent areas of low attenuation on CT or hyperintensity on fluid-attenuated inversion recovery or T2-weighted and hypointensity on T1-weighted MRI (Fig. 125.4). MRI is twice as sensitive as CT in distinguishing multiple lesions. These generally do not enhance after administration of contrast material, and they are not surrounded by edema, and, hence, substantial mass effect on surrounding structures is absent. However, 8% of lesions can show faint, peripheral, and irregular enhancement. Lesions are usually bilateral, asymmetrical, well demarcated, and localized preferentially in the periventricular areas and the subcortical white matter.[149] Involvement of the deep gray matter structures, including the basal ganglia and thalamus, can nevertheless be found in up to 17% of cases. In addition, a hyperintense cortical signal can be seen adjacent to PML lesions in the underlying white matter,[150] and is associated with a risk of seizures. Normal findings on CT or MRI do not rule out PML because microscopic lesions might be smaller than the resolution power of these tests. One such case showed multiple small foci of demyelination disseminated among the cortical U fibers at autopsy.[151]

HIV-1 encephalopathy can be easily mistaken for PML on brain imaging studies. As in PML, white matter lesions without mass effect or contrast enhancement are the radiologic hallmarks of this disease. However, HIV-1 encephalopathy lesions tend to be symmetrical and less clearly demarcated than the lesions of PML and are not associated with focal neurologic deficits. Proton magnetic resonance spectroscopy and magnetization transfer studies have shown promising potential in differentiating PML lesions from HIV-1 encephalopathy.[152] The typical proton magnetic resonance spectroscopy pattern in PML lesions includes a decreased *N*-acetylaspartate–to-creatine ratio, consistent with axonal

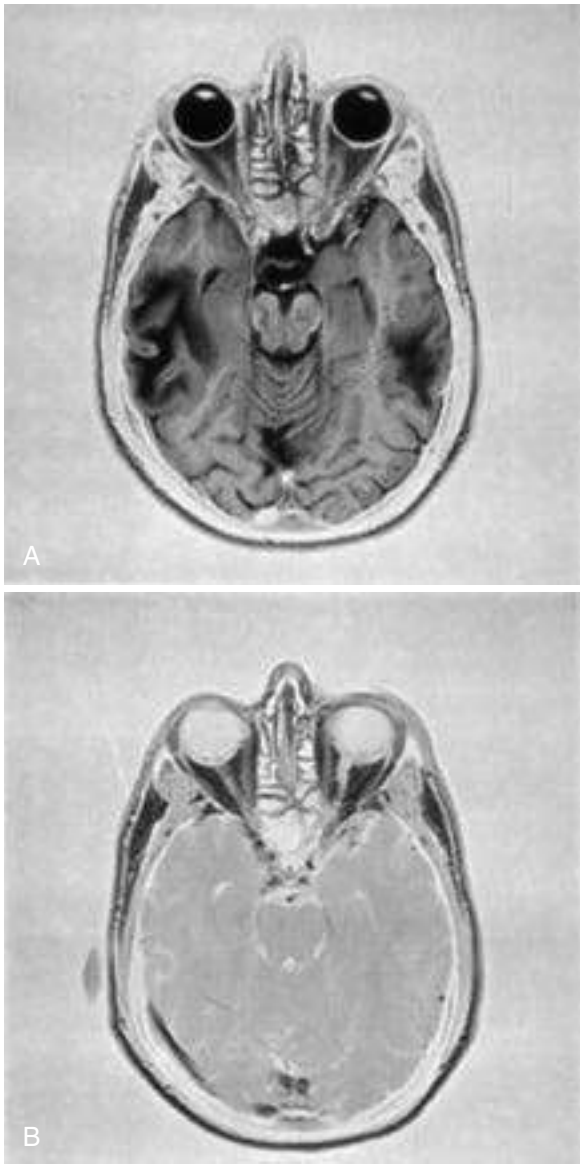

FIG. 125.4 Brain magnetic resonance images of a 43-year-old man with progressive multifocal leukoencephalopathy. (A) Prominent areas of high signal intensity are present in the subcortical white matter of both temporoparietal lobes on T2-weighted images. (B) The affected areas are hypointense on T1-weighted images, do not enhance with gadolinium, and are not associated with mass effect or edema.

compromise; increased choline/creatine ratio, indicating cell membrane breakdown and turnover; and an occasional increase in lipid/lactate and *myo*-inositol levels.[153,154,155] In one study, patients who had the longest survival had the highest *myo*-inositol level, consistent with glial activity and inflammation in PML lesions.[153] Metabolic alterations consistent with inflammation detected on proton magnetic resonance spectroscopy in PML lesions are associated with a cellular immune response against JCV. This inflammatory reaction, occurring early after disease onset, appears to be instrumental in the containment of PML.[156]

Brain biopsy. When the JC viral load is too low to be detected by PCR in the CSF, a brain biopsy may be necessary to establish the diagnosis of PML.[157] Such cases have become more frequent in the era of ART.[102] Histologic examination shows areas of demyelination in subcortical white matter or at the gray-white junction, as well as large, hyperchromatic oligodendrocytic nuclei that stain positively for JCV by immunohistochemistry and contain large amounts of JC virions detectable by electron microscopy. Other histologic features of PML include large, bizarre astrocytes with lobulated nuclei and lipid-laden macrophages engaged in removing myelin breakdown products. Demyelinating lesions may be entirely circumscribed within the cortical gray matter.[139]

Treatment. There is no specific treatment for PML. Numerous therapeutic attempts in HIV-infected patients have included cytosine arabinoside,[158] interferon-α,[159] topotecan,[160] cidofovir,[161,162] mirtazapine, and mefloquine,[163] but none showed any benefit compared with antiretroviral treatment alone. Serotonin receptors are used by JCV to enter astroglial cells in vitro.[164] Therefore serotonin receptor blockers such as mirtazapine, which downregulate receptors on the cell surface, have been used empirically with variable success.[165]

It is important to realize that there is a large diversity in the natural evolution of PML. In patients with AIDS who are profoundly immunosuppressed, the course of the disease used to be rapidly progressive, leading to death within 2 to 4 months from the time of symptom presentation. However, approximately 10% of patients had a protracted course and survived more than a year. Since the ART era, median survival has increased to 10.5 months,[166] and 40% to 50% of the patients survive PML, although some have devastating neurologic sequelae.[167] Predictive factors for longer survival include $CD4^+$ T-lymphocyte counts greater than 300 cells/μL at disease onset, as well as absence of hyperperfusion in PML lesions as measured by arterial spin labeling.[168] Study of the cellular immune response against JCV showed that detection of JCV-specific cytotoxic T lymphocytes in the peripheral blood mononuclear cells of HIV-infected patients with PML was associated with long-term survival, whereas those with undetectable cellular immune responses against the virus had a progressive neurologic disease and a fatal outcome.[169,170,171,172] Strategies aimed at boosting this immune response may prove useful for preventing uncontrolled replication of JCV and the spread of PML.[173] For the time being, optimization of ART and avoidance of immunosuppression remain the best available therapeutic option for patients with PML.

Inflammatory Progressive Multifocal Leukoencephalopathy

Inflammatory forms of PML occur frequently in the setting of IRIS in patients taking ART. This appears to be related to infiltration of cytotoxic $CD8^+$ T-cells that engage JCV-infected oligodendrocytes.[174] PML-associated IRIS is characterized by contrast-enhancing lesions and even swelling on neuroimaging studies or inflammatory infiltrates on a brain biopsy specimen,[175] or both, and their outcome is usually favorable,[176] but fatal cases have been reported.[177] PML-associated IRIS often causes a paradoxical worsening of neurologic symptoms and can be distinguished from the natural evolution of PML by magnetic resonance spectroscopy.[178] Clinicians should not disregard the diagnosis of PML in the presence of contrast-enhancing brain lesions and should use caution before treating these immunosuppressed individuals with steroids.

Cytomegalovirus Encephalitis

Cytomegalovirus (CMV) may cause necrotizing focal encephalitis and ventriculoencephalitis.[179] It occurs in patients with $CD4^+$ counts of fewer than 50 cells/μL and is often concomitant with CMV infection of other organs, including retinitis, adrenalitis, and pneumonitis (see Chapter 137). The incidence of CMV disease, including CMV encephalitis (CMVE), has decreased considerably since the availability of ART.[180]

Clinical presentation. Patients with CMVE show features similar to those with AIDS dementia but tend to have a more acute onset and more prominent confusion/disorientation or apathy/withdrawal. Hyponatremia and cranial nerve involvement, which are usually not present in AIDS dementia, are also helpful for establishing the diagnosis.[181]

Laboratory investigations. The conventional CSF examination is generally nonspecific because it is either normal or shows a slight protein increase and mononucleated pleocytosis. CMV culture often remains negative. However, the detection of CMV DNA by PCR in the CSF is both sensitive and specific.[182]

Imaging studies. Focal necrotizing lesions associated with periventricular and meningeal enhancement or hydrocephalus may be seen on brain imaging studies.[183] Although MRI has better resolution than CT, it often lacks sensitivity for diagnosing CMVE.[184]

Brain biopsy. Microglial nodule encephalitis with CMV inclusions can be readily diagnosed by histologic examination. Interestingly, similar findings can be found at autopsy in 6% to 40% of patients with AIDS and dementia.[179] However, autopsy findings of CMV infection of the brain do not always correlate with the presence of cognitive dysfunction.

Treatment. The management of CMVE is difficult.[185] In one autopsy-confirmed series, half of the patients were taking maintenance doses of ganciclovir for the treatment of CMV retinitis. CMVE developed in others during first full-dose inductions with ganciclovir or foscarnet for retinitis.[183] The possibility of viral resistance to antivirals should be considered if treatment failure occurs.

The treatment for CMVE is induction therapy with ganciclovir at 5 mg/kg/day IV every 12 hours and foscarnet 60 mg/kg every 8 hours or 90 mg/kg IV every 12 hours for 14 to 21 days. Maintenance therapy can include a low dose combination of ganciclovir and foscarnet, foscarnet monotherapy, ganciclovir monotherapy, and valganciclovir monotherapy. The duration may be indefinite or until CD4 count is greater than 100 cells/mm^3. If ganciclovir resistance is suspected, foscarnet should be used, with foscarnet induction at 90 mg/kg IV every 12 hours for 14 to 21 days, followed by maintenance 90 to 120 mg/kg/day indefinitely. Blood levels of oral valganciclovir at a dose of 900 mg are equivalent to those of intravenous ganciclovir at a dose of 5 mg/kg. The prognosis is usually poor, with median survival not exceeding 5 weeks. Cidofovir may be used in patients in whom ganciclovir or foscarnet has failed or who have become intolerant to these drugs. The cidofovir dose is 5 mg/kg/wk for 2 weeks, followed by 5 mg/kg every 2 weeks. This drug is nephrotoxic and should be given with intravenous hydration and high doses of probenecid before and after cidofovir injection (see Chapter 137).

Miscellaneous Mass Lesions and Rationale for Brain Biopsy

Brain biopsy used to be considered the gold standard for the diagnosis of CNS mass lesions in AIDS. The biopsy is the most specific, but not always the most sensitive, test. Indeed, sensitivities of 64% to 96% have been reported in AIDS patients. In addition, this procedure is not without significant risks in this patient population. Brain biopsy has a mortality rate of 0% to 3%, a major morbidity rate of 0.5% to 9%, and a minor morbidity rate of 2% to 4%.[186,187,188,189]

Brain biopsy is often impractical because of the location of the lesion. In addition, several disease processes can coincide in patients with multiple lesions, and multiple biopsies are rarely performed. Finally, change of therapy indicated by the result of the biopsy is not always possible in patients with advanced AIDS, and overall survival was improved by only a couple of months before the era of ART.[187,189]

The availability of molecular diagnosis by PCR in the CSF has considerably changed the management of these patients. A decision analysis was performed on 136 consecutive HIV-1 patients presenting with mass lesions between 1991 and 1995.[188] After 3 weeks of empirical therapy for TE, patients with progressive disease underwent a brain biopsy. CSF PCR amplification for *T. gondii,* EBV, and JCV DNA was performed in 66 patients. The presence or absence of mass effect surrounding the brain lesions, knowledge of the patient's serologic status for *T. gondii,* and a prophylactic regimen with trimethoprim-sulfamethoxazole, as well as PCR results, were used in this analysis. The probability of TE was 0.87 in *Toxoplasma*-seropositive patients with mass effect who were not receiving trimethoprim-sulfamethoxazole, but only 0.59 for those receiving prophylaxis. For *Toxoplasma*-seropositive patients receiving trimethoprim-sulfamethoxazole, the probability of PCNSL was 0.36. Conversely, in *Toxoplasma*-seronegative patients with mass effect, the likelihood of PCNSL was 0.74, which increased to 0.96 if the EBV PCR result was positive in the CSF. Among focal brain lesions without mass effect, the probability of PML was 0.81, which increased to 0.99 if JCV DNA was detected in the CSF.

Therefore brain biopsy is not necessary to confirm a diagnosis of PML, and JCV PCR in the CSF is now being used as a diagnostic test and for inclusion of patients in treatment studies.[190] Bypassing the need for tissue diagnosis is more controversial in patients with suspicion of PCNSL who have a positive result on EBV PCR in the CSF. Because treatment consists of whole-brain radiation, the risk of a false-positive PCR result has to be balanced with the risks of brain biopsy and by the fact that this technique does not always yield a definitive diagnosis.

An algorithm for the management of HIV-1–infected patients with CNS mass lesions is shown in Fig. 125.5.

SPINAL SYNDROME

Myelopathy is a frequent finding at autopsy in patients with AIDS and is probably underrecognized clinically. It can be primarily HIV-1–associated or caused by other opportunistic infections or tumors.

Vacuolar Myelopathy

Vacuolar myelopathy (VM) is present at autopsy in 17% to 46% of patients with AIDS.[191,192,193] This disorder occurs with advanced

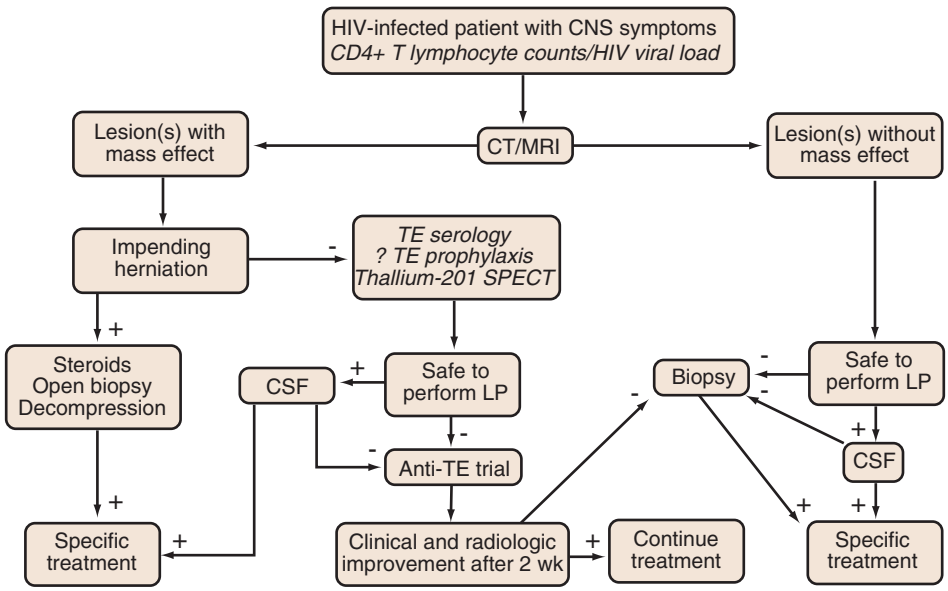

FIG. 125.5 Management of the human immunodeficiency virus (HIV) type 1–infected patient with central nervous system (CNS) mass lesions. The elements in italics represent data that contribute to the decision-making process (see text for details). *CSF,* Cerebrospinal fluid; *CT,* computed tomography; *LP,* lumbar puncture; *MRI,* magnetic resonance imaging; *SPECT,* single-photon emission computed tomography; *TE, Toxoplasma* encephalitis.

immunosuppression, and the symptoms are often overlooked or attributed to debilitation.

Clinical presentation. Symptoms are often overshadowed by coexisting central or peripheral nervous system impairment, such as AIDS dementia, which occurs late in the course of the disease.[191] Usually, patients report progressive, painless gait disturbance; weakness and sensory disturbances in the legs; impotence in men; and urinary frequency and urgency. The evolution is usually progressive and leads to severe paralysis of the legs and loss of sphincter control. Neurologic signs include spastic paraparesis, hyperreflexia, extensor plantar responses, and mild sensory impairment, with vibratory and position sense being disproportionately affected. There is usually no associated sensory level.

Laboratory investigations. CSF analysis either is normal or demonstrates a slight increase in protein and lymphocytosis frequently seen in HIV-1 infection. Opposite to what is observed in AIDS dementia, the CSF HIV-1 viral load is not increased in patients with VM.[194] However, CSF examination is a useful test to rule out other treatable infections (see "Histologic Studies" later).

Imaging studies. Findings on MRI of the spinal cord are usually normal in patients with VM. However, this examination is useful to rule out an extradural or intradural mass lesion or an epidural abscess.[195]

Histologic studies. Because an anatomic diagnosis is not possible, VM remains a clinical diagnosis of exclusion. At autopsy, discrete or coalescent 10- to 100-µm vacuoles containing cellular debris or macrophages and, rarely, axonal swelling can be seen in the white matter of the spinal cord, involving principally the posterior or lateral columns, or both. These lesions are usually symmetrical and more frequent at the middle to lower thoracic levels. Vacuoles appear to be the result of focal swelling within the myelin sheath. Ultrastructural studies indicate both axonal and myelin injury,[196] although axonal destruction is seen only in areas of intense vacuolation. The etiology of VM is still unknown. Consistent with the absence of an increased HIV-1 CSF viral load, immunohistochemistry studies did not demonstrate an association between HIV-1 antigens and VM.[192,197] Therefore the role of HIV-1 may only be indirect. Because histologic findings are similar to those of subacute combined degeneration of the spinal cord and because patients with VM usually have normal serum levels of vitamin B_{12} and folic acid, it was suggested that abnormal metabolism of the vitamin B_{12}–dependent transmethylation pathway may be important in the pathogenesis of VM. Indeed, *S*-adenosylmethionine and methionine were decreased in the CSF and serum of patients with VM, respectively.[198] The underlying cause of this metabolic disorder is unclear. Macrophage activation might generate substrates that are metabolized by methylation and therefore trigger a local deficit of methyl group donors in the spinal cord, leading to myelin vacuolization.

Treatment. An open-label pilot clinical trial suggested that patients with VM may benefit from supplements of L-methionine, but a double-blind placebo controlled trial demonstrated no benefit.[199,199a] However, there are also reports indicating a role for ART in the treatment of myelopathy in an antiretroviral-naïve HIV-infected patient[200] and for the combination of lopinavir-ritonavir in an ART-experienced patient.[201] Because there is no way to confirm the diagnosis of VM outside of a postmortem examination, it is possible that the patients who responded to various ART formulations were instead affected by HIV-1 myelitis,[202] which has the same histologic features as HIV-1 encephalitis, and therefore may benefit from antiretroviral treatment.

Differential Diagnosis of a Noncompressive Myelopathy

Other etiologies of noncompressive myelopathy in patients with advanced HIV-1 infection include other viral infections such as CMV, varicella-zoster virus, and herpes simplex virus types 1 and 2, as well as human T-cell lymphotropic virus type 1. Human T-cell lymphotropic virus type 1 is also transmitted sexually or through transfusion of cellular blood products and is the agent of a chronic spastic paraparesis called human T-cell lymphotropic virus type 1–associated myelopathy (see Chapter 168).[203] Syphilitic meningomyelitis and rare fungal or parasitic infections are diagnosed by appropriate serologies and cultures.[204]

PERIPHERAL NERVOUS SYSTEM SYNDROMES

Peripheral neuropathies have become the most common neurologic complication of HIV infection. Neuropathies include several entities that are thought to be directly associated with HIV and occur at different stages of immunosuppression. Other forms are caused by opportunistic pathogens, such as CMV. Finally, the peripheral nervous system can also be affected by antiretroviral treatment toxicities.

HIV-Associated Neuropathies

Inflammatory Demyelinating Polyneuropathies

These entities can occur at the time of seroconversion but are generally diagnosed in seropositive patients who are otherwise asymptomatic and not yet profoundly immunosuppressed, although some cases have also been reported in late stages of the disease.[205] Acute inflammatory demyelinating polyneuropathy (AIDP) has clinical features similar to those of Guillain-Barré syndrome. Sensory symptoms such as paresthesias may precede an acute, progressive weakness of distal and proximal muscles of two or more limbs, associated with areflexia. Respiratory muscles may be involved, and patients sometimes require assisted ventilation.[206] Sensory signs are usually mild, even in the setting of severe weakness. The maximal weakness is usually reached within the first 4 weeks. Patients with a more protracted course are affected by the chronic form of inflammatory demyelinating polyneuropathy (CIDP), which may be monophasic or relapsing.[207,208] In regions with a high prevalence of HIV, CIDP is commonly associated with HIV infection. At a single hospital in Johannesburg, South Africa, 43% of all CIDP cases over a 2-year period occurred in HIV-infected individuals.[209] A severe case of AIDP was observed in a patient with advanced AIDS 4 weeks after starting on ART, concomitant with immune reconstitution.[210] AIDP should therefore be included in the growing number of ART-induced IRISs in HIV-infected individuals.

Laboratory investigations. The CSF analysis often differs from that of HIV-1–seronegative patients with Guillain-Barré syndrome by the presence of a mononuclear pleocytosis of 20 to 50 cells/µL. The CSF protein concentration is usually elevated to levels as high as 250 mg/dL, and a polyclonal gammaglobulinemia can be detected.

Electrophysiologic studies. Demyelination is demonstrated by decreased motor nerve conduction velocities or prolonged distal latencies and minimum F-wave latencies in two or more nerves. Conduction block is often prominent.

Nerve biopsy. Sural nerve biopsy generally demonstrates the presence of a perivascular and endoneural mononuclear cell infiltrate with macrophage-mediated segmental demyelination. In severe cases, wallerian-like degeneration of axons can be seen. Similar to Guillain-Barré syndrome occurring in immunocompetent individuals, the etiology of AIDP in HIV-infected patients is thought to be autoimmune. Anti–peripheral nerve myelin antibodies have been found in HIV-1–positive patients with AIDP, as well as increased levels of soluble CD8 and neopterin in the CSF, indicating an abnormal immune activation. CMV was found at autopsy in the peripheral nerves of one patient with AIDS who presented with AIDP.[211]

Treatment. Spontaneous recovery is usually the outcome of AIDP, although HIV-1–infected patients tend to have a more severe course with a slower recovery than immunocompetent individuals with Guillain-Barré syndrome. Plasmapheresis is indicated if the illness is sufficiently severe to warrant treatment. Patients with CIDP benefit from treatment with prednisone or plasmapheresis.[212,213] However, prednisone should be used with great caution in patients with immunosuppression. Other treatments such as intravenous immunoglobulin have also been used successfully in HIV-1–positive patients with inflammatory demyelinating polyneuropathy, sometimes in combination with plasmapheresis.[214] In cases of AIDP occurring in late stages of the disease, a trial of ganciclovir may be warranted if other treatments are not efficient.[211]

Distal Sensory Polyneuropathy

Distal sensory polyneuropathy (DSPN) is the most common cause of peripheral neuropathy in HIV-1 infection, which is symptomatic in 38% of patients and asymptomatic in an additional 20%.[214,215] Moreover,

histologic abnormalities can be detected at autopsy in most patients dying of AIDS.[216] Distal sensory polyneuropathy occurs despite ART; 87% of affected individuals have an HIV-1 virus load less than 400 cps/mL, and 70% have $CD4^+$ T-cell counts greater than 350/μL.[217] DSPN is associated with older age, low CD4 nadir, diabetes, and past nucleoside analogue reverse-transcriptase inhibitor (NRTI) exposure. In the United States, HIV-positive African-American women had a higher incidence of DSPN (41.3%) compared with whites (34.8%) and Hispanics (24.7%).[218] Older age, taller height, PI use, and female sex have been identified as risk factors for development of DSPN.[219] Depression, prior opioid use, and certain iron regulatory gene polymorphisms have been identified as additional risk factors for distal neuropathic pain.[220,221]

Clinical presentation. DSPN is characterized by the progressive onset of symmetrical paresthesia, numbness, and painful dysesthesia of the lower extremities. The pain is often described as an aching or burning sensation and is worse on the soles of the feet. Some patients have a lower pain threshold (hyperalgesia) or pain induced by nonnoxious stimuli (allodynia), such as the contact with bed covers at night. Symptoms may remain stable or progress over months and ascend in a length-dependent fashion up the legs. Fingertips may become affected when symptoms reach the knees. Although wearing shoes and walking may exacerbate the pain for some patients, others report maximal discomfort when they are barefoot in bed. Therefore DSPN may have a major negative impact on patients' ability to ambulate and the quality of their sleep. Perception of noxious stimuli, temperature, and vibrations is usually more affected than light touch and proprioception. DSPN may even have several forms because some patients only have decreased sensation for noxious stimuli and temperature, indicating dysfunction of small unmyelinated sensory fibers; some only have decreased vibration sense and proprioception, consistent with dysfunction of large myelinated fibers; and others have decreased sensation for all modalities. Hyporeflexia of the lower extremities is a common finding, and gait ataxia with a positive Romberg sign is present in severe cases. Weakness is rarely found on the examination or is confined to the intrinsic foot muscles. Autonomic dysfunction is increasingly recognized and can be present in up to 90% of patients with severe DSPN and in 30% of patients with mild to no DSPN.[222]

Laboratory investigations. CSF analysis shows only nonspecific findings with mild elevation of protein concentration and mononucleated pleocytosis, which is common in HIV-1 infection.

Electrophysiologic studies. Nerve conduction studies show low-amplitude or absent sural nerve action potentials. Sensory and motor nerve conduction velocities are normal or only mildly reduced. Electromyographic studies demonstrate acute denervation and chronic reinnervation in distal leg muscles. These findings are consistent with an axonal distal symmetrical, predominantly sensory, polyneuropathy.

Nerve and skin biopsy. Sural nerve biopsy confirms the diagnosis of axonal degeneration of myelinated and unmyelinated axons. Punch skin biopsies show evidence of decreased epidermal nerve fiber density in the distal leg. In patients with advanced HIV infection, epidermal nerve fiber density correlated with the clinical and electrophysiologic severity of DSPN.[223] A modest dorsal root ganglion (DRG) neuronal loss has also been demonstrated in DSPN,[224] as well as selective degeneration of the axons and myelin sheaths in the cervical and upper thoracic levels of the gracile tracts. This process therefore represents the degeneration of the centrally directed extension of the sensory neurons.[225]

Inflammatory infiltrates around peripheral nerve fibers and in DRGs consist of activated macrophages, with local release of cytokines such as tumor necrosis factor-α, interferon-γ, and interleukin-6.[216] As is the case in VM and, to some extent, in HIV encephalitis, productive HIV-1 replication in the peripheral nerves and DRGs is sparse and limited to monocyte/macrophages. Therefore the presence of activated macrophages secreting inflammatory cytokines, rather than the virus itself, seems to account for most of the peripheral nerve damage.[226] How activated macrophages penetrate DRGs and peripheral nerve fibers is unclear, and it has been hypothesized that the blood-nerve barrier, like the BBB, may be affected in HIV-infected patients.[216] Finally, experimental models in animals suggest that it is still possible that HIV, or HIV-1 proteins, may have a direct toxic effect on the neuropathic pain of DSPN.[227]

Because a similar type of polyneuropathy is common in the HIV-1–seronegative population, other etiologies such as vitamin B_{12} deficiency, neurotoxic medications, alcoholism, and diabetes mellitus should be ruled out.

Treatment. There is no available therapy that leads to the regeneration of nerve fibers and DRGs and reversal of symptoms. Suppression of viral replication with ART is unfortunately not associated with clinical improvement in DSPN. Therefore treatment of neuropathic pain in DSPN is purely symptomatic and is aimed primarily at attenuating the painful dysesthesia and improving the quality of life of these patients. Treatment should not be administered if numbness and decreased sensation for noxious stimuli and temperature are the only symptoms because they do not improve.

Nucleoside Neuropathy

Clinical presentation. Dose-dependent neurotoxicity of the NRTIs didanosine and stavudine (d4T) has been detected in approximately 30% of patients taking these medications.[228] The risk is increased in patients on regimens containing a combination of didanosine and d4T.[229] The clinical presentation and electrophysiologic study results were indistinguishable from those of DSPN, and the diagnosis can be established only by a temporal association between initiation of a dideoxynucleoside NRTI and the onset of the symptoms, which can occur as early as 1 week. Discontinuation of the offending medication may lead to symptomatic improvement over several weeks in two-thirds of patients, although it is often preceded by a transient worsening of symptoms known as "coasting."[230]

Laboratory investigations. The pathogenetic mechanism of nucleoside neuropathy appears to be most likely related to nucleoside-induced mitochondrial dysfunction. Indeed, NRTIs inhibit mitochondrial polymerase in vitro, resulting in tissue-specific injury. Zidovudine toxicity affects mainly skeletal muscle (see "Muscular Complications From Therapies in HIV" later), whereas didanosine and d4T cause pancreatitis in addition to neuropathy. Nucleoside neuropathy occurs more frequently in individuals with preexisting DSPN, and administration of NRTIs to healthy animals does not result in neuropathy.[216] These data suggest that NRTIs may only exacerbate an inflammatory process triggered by activated macrophages in peripheral nerve fibers or DRGs of HIV-infected individuals. Clinicians should therefore use caution before removing didanosine or d4T from successful ART regimens, unless there is a clear temporal association between the administration of these medications and the onset of the symptoms or an elevated serum lactate level.[231] A list of neurotoxic medications that have been associated with DSPN in HIV-infected patients is shown in Table 125.3.

Treatment. First-line therapies include nonsteroidal antiinflammatory drugs and acetaminophen, as well as topical application of capsaicin[232,233] or 5% lidocaine gel.[234] Anticonvulsant medications are the second line of treatment. Medications such as carbamazepine and phenytoin that are commonly used in other forms of painful neuropathies are contraindicated because both are metabolized by the liver, which may cause unwanted interactions in patients taking PIs. Gabapentin is metabolized by the kidneys and is usually well tolerated by HIV-infected individuals.[235] Treatment starts using 300 mg PO at bedtime and is increased to 300 mg PO three times daily over 1 week. The extent of symptomatic relief is variable, and some patients may need doses as high as 1200 mg three times daily for a significant reduction in their discomfort. Fatigue and sleepiness are infrequent but may be limiting in some patients. Pregabalin, which is similar to gabapentin, is used as well, although studies have failed to show any benefit over placebo.[236] Another anticonvulsant, lamotrigine, showed substantial pain reduction in a subgroup of patients receiving neurotoxic NRTIs but no difference compared with placebo in patients with DSPN who were not receiving nucleosides.[237]

Amitriptyline, which is commonly used for the treatment of diabetic neuropathy, is not superior to placebo in HIV-infected patients with DSPN.[238] However, adjunction of tricyclics with lower anticholinergic side effects, such as nortriptyline, may benefit patients who remain symptomatic on adequate doses of anticonvulsants. In refractory cases, a combination of anticonvulsant, tricyclic, nonsteroidal antiinflammatory, and topical medications may be necessary to achieve significant relief.

TABLE 125.3 Neurotoxic Medications Used in Human Immunodeficiency Virus-1 Infection

NAME	CLINICAL PRESENTATION
Nucleoside Reverse-Transcriptase Inhibitors	
Zidovudine	Myopathy
Didanosine	DSPN
Stavudine	DSPN
Nonnucleoside Reverse-Transcriptase Inhibitors	
Efavirenz	Vivid dreams
Antiviral Agent	
Foscarnet	Seizures
Antibacterial Agents	
Isoniazid	DSPN
Dapsone	DSMP
Metronidazole[a]	DSPN
Antineoplastic Agents	
Vincristine	DSMP, CN
Cisplatin	DSPN

[a]High doses only.
CN, Cranial neuropathy; *DSMP,* distal sensory motor polyneuropathy; *DSPN,* distal sensory polyneuropathy.

Narcotic analgesics should be used as the last resort because of their addictive potential in the context of a chronic pain syndrome. Tramadol shares properties with opioid analgesics but is less likely to cause dependence and lead to abuse.[239] Long-acting opioid agonists such as fentanyl patches should be preferred to short-acting agents. Smoked cannabis may be as efficient as oral drugs for treatment of neuropathic pain.[240,241] Numerous experimental drugs and therapies have been disappointing in the treatment of DSPN, including mexiletine, memantine, prosaptide, peptide T, recombinant human nerve growth factor, plasmapheresis, and acupuncture (for reviews, see Simpson[242] and Gonzalez-Duarte and colleagues[243]). Depletion of acetylcarnitine, a substrate in the production of energy during β-oxidation of free fatty acids, was implicated in the pathogenesis of DSPN,[244] but this was not confirmed in a larger sample of patients.[245]

Mononeuritis Multiplex

Patients with previously asymptomatic HIV-1 infection and $CD4^+$ T-lymphocyte counts greater than 200 cells/μL, as well as patients with AIDS and profound immunosuppression, can be affected by mononeuritis multiplex.

Clinical presentation. Patients present with acute onset of sensory or motor deficit limited to one or more peripheral nerves. Involvement of a facial or laryngeal nerve has also been reported. The asymmetrical nature of this disorder and the prominent weakness differentiate it from other HIV-associated neuropathies. The course can be self-limited in early HIV-1 infection[246] or more severe in patients with advanced disease.

Laboratory investigations. CSF analysis is nonspecific and shows only a mild elevation of protein concentration and a mononuclear pleocytosis. Cryoglobulinemia was reported in one case.[247]

Electrophysiologic studies. Nerve conduction studies reveal a reduction of the amplitude of sensory nerve action potentials and compound muscle action potentials, as well as a mild reduction in nerve conduction velocities in the distribution of single nerves. The electromyographic examination is also consistent with focal or asymmetrical multifocal axonal degeneration, although considerable overlap may exist with DSPN and inflammatory demyelinating polyneuropathy.

Nerve biopsy. Similar to its clinical presentation, the nerve biopsy specimen of patients with mononeuritis multiplex shows a spectrum of pathologies rather than a single pattern. Axonal degeneration and perivascular inflammatory infiltrates are found in patients with early HIV-1 infection and limited clinical involvement. Patients with AIDS and CMV infection usually have mixed axonal and demyelinating lesions with inflammatory infiltrates also containing polymorphonuclear cells, as well as, sometimes, characteristic cytomegalic inclusion bodies.[248] The most aggressive form consists of a necrotizing arteritis with necrosis of endoneurial or epineurial vessels and might be caused by circulating immune complexes.

Treatment. Mild forms of mononeuritis multiplex developing in patients who are otherwise asymptomatic might improve without specific treatment. Others might benefit from therapies such as plasmapheresis[247] or intravenous immunoglobulin.[249] Corticosteroids and cyclophosphamide should be reserved for aggressive cases of mononeuritis multiplex with vasculitis proved by nerve biopsy. In late HIV-1 infection, especially in patients with concurrent systemic CMV infection, empirical therapy with ganciclovir for CMV should be considered.[250]

Progressive Polyradiculopathy

Because it occurs late in the course of HIV-1 infection, in patients with low $CD4^+$ T-lymphocyte counts and concurrent systemic illnesses, progressive polyradiculopathy is often underrecognized.

Clinical presentation. Initially patients report lower extremity and sacral paresthesia and, sometimes, radicular pain in the cauda equina distribution. These symptoms are followed by a rapidly progressive areflexive paraparesis and ascending sensory loss, often accompanied by urine retention. The upper extremities are relatively spared. A thoracic sensory level, if present, indicates concomitant medullary involvement, but other features indicating upper motor neuron damage, such as spasticity and hyperreflexia, are usually absent. A prominent infection with CMV, mainly retinitis, esophagitis, or colitis, is conspicuously present in a majority of cases.

Laboratory investigations. In contrast to other peripheral nervous system diseases associated with HIV-1 infection, the CSF analysis is useful in establishing the diagnosis. A marked polymorphonuclear cell pleocytosis, elevated protein concentration, and hypoglycorrhachia are the hallmarks of this syndrome.[251] CSF cultures demonstrate the presence of CMV in 60% of the cases.[252] Cultures may take as long as 2 weeks to grow, and CSF samples must be kept on ice immediately after the lumbar puncture. As is the case with CMVE,[182] CMV DNA may be detectable in the CSF by PCR. Because of the frequent concomitant systemic infection with CMV, blood culture may also be positive for this virus. Urine culture is nonspecific because many AIDS patients shed CMV in the urine asymptomatically. Cytologic studies can reveal cytomegalic cells with intranuclear and intracytoplasmic CMV inclusions.

Electrophysiologic studies. Electromyographic examination is useful to differentiate this syndrome from AIDP. Severe and widespread proximal axonal damage in lumbar nerve root distribution is correlated by fibrillation potentials, complex repetitive discharges, and motor unit recruitment patterns in lower extremity muscles. Motor nerve conduction velocities are minimally altered, but affected muscles display prolonged or absent F waves. These findings are consistent with extensive denervation of the lower extremity muscles, which is characteristic in this syndrome.

Nerve biopsy. Because of the radicular localization of the lesions, a nerve biopsy is not helpful for diagnosis. Autopsy studies reveal a severe inflammation associated with necrosis of the ventral and dorsal nerve roots. Cytomegalic inclusions can be detected within the nucleus and cytoplasm of Schwann, ependymal, and endothelial cells, which are also positive for CMV by in situ hybridization studies. Similar findings have been reported in cranial nerves at the site of exit from the brainstem.

Despite the strong association of polyradiculopathy and CMV in patients with AIDS, other possibilities include neurosyphilis and lymphomatous meningitis, which should be ruled out by the CSF analysis.

Treatment. The treatment of CMV-associated progressive polyradiculopathy is similar to that of CMVE (see earlier). It consists of intravenous ganciclovir or foscarnet, or both.[253] Newer anti-CMV medication such as valganciclovir and cidofovir have not been evaluated for progressive polyradiculopathy. Phenotypic and genotypic characterization of viral

isolates should be considered in case of resistance to treatment.[254] The clinical response is variable but usually best if therapy is started within days after the onset of symptoms.

Because the results of PCR for CMV DNA in the CSF can take 1 to 2 weeks to obtain, empirical treatment can be justified in this entity, especially if the workup reveals a polymorphonuclear pleocytosis in the CSF or widespread systemic infection with this virus. The rationale is to prevent irreversible necrosis of the nerve roots. In patients who are already paraplegic several weeks after the onset of their symptoms, treatment can achieve stabilization, but no real improvement should be expected. Neutropenia is the most common dose-limiting toxicity of ganciclovir and may preclude concomitant use of other myelotoxic drugs such as zidovudine. Concomitant treatment with granulocyte colony-stimulating factor may become necessary in that setting.

Diffuse Infiltrative Lymphocytosis Syndrome–Associated Neuropathy

A small subset of HIV-infected patients develops persistent CD8 hyperlymphocytosis and a Sjögren syndrome–like illness associated with multivisceral CD8 T-cell infiltration, known as *diffuse infiltrative lymphocytosis syndrome*. These individuals usually have parotid enlargement and generally have higher $CD4^+$ T-lymphocyte counts, fewer opportunistic infections, and longer survival times than do other HIV-infected patients.[255] Some of these patients may develop a primary B-cell lymphoma.

Clinical presentation. Some of these patients present with an acute or subacute sensorimotor distal symmetrical neuropathy, which is always painful.[256]

Electrophysiologic studies. Electromyographic and nerve conduction study results are consistent with axonal neuropathy.

Nerve biopsy. A nerve biopsy specimen shows marked angiocentric $CD8^+$ infiltrates without mural necrosis and abundant expression of HIV-1 p24 protein in macrophages. The lymphocytic infiltrate is polyclonal in most patients. The HIV-1 proviral load in peripheral nerve is much higher than in other types of HIV-associated neuropathy.[257]

Treatment. Zidovudine and steroid therapy were associated with improvement in a small group of patients.[256] The prevalence of diffuse infiltrative lymphocytosis syndrome has significantly decreased in the ART era, suggesting that this condition is an antigen-driven response against HIV and should be treated with anti-HIV therapy.[258]

Amyotrophic Lateral Sclerosis–Like Syndrome

During the past 20 years, at least 19 cases of amyotrophic lateral sclerosis (ALS) or ALS-like syndromes have been reported in HIV-infected individuals.[259,260,261] These cases differed from classic ALS because they occurred in younger patients, were unusually rapidly progressive, and improved after the institution of ART. Case studies at autopsy exhibited pathology outside the motor neuron pool. The etiology of this syndrome is unclear because HIV-1 does not infect neurons. It has also been suggested that ALS is caused by another, yet unidentified, retrovirus, and the retroviral enzyme reverse transcriptase has been found in 50% to 56% of sera from HIV-1–negative ALS patients, but not in CSF, at levels comparable to those of HIV reverse transcriptase, compared with 7% to 19% of controls.[262,263] However, this causality is not proven, and there is no indication for administering antiretroviral treatment to HIV-1–negative patients with ALS. ALS and HIV-1 infection can also be coincidental,[264] and these data indicate that HIV-1 should be included in the differential diagnosis of ALS.[265]

MUSCULOSKELETAL SYNDROMES

Muscular disorders have been described in HIV-1–infected individuals and can occur at any stage of the disease.

HIV-Associated Myopathies

HIV-associated polymyositis occurs in 0.22% of HIV-infected patients.[266] Patients report mainly lower extremity weakness, characterized by difficulty in rising from a chair or climbing stairs, as well as fatigue. Myalgias are present in as many as half of the cases, and the neurologic examination reveals proximal symmetrical lower extremity weakness.[267] Cutaneous rash or involvement of the extraocular and facial muscles is absent. There is a mild elevation of creatine phosphokinase in the serum (median, ≅500 IU/L), although creatine phosphokinase can be as high as 7500 IU/L in some cases.[266] This may be an incidental finding that orients toward a diagnosis in cases in which muscle strength is still intact. The creatine phosphokinase level correlates with the degree of myonecrosis seen on a muscle biopsy specimen, but not with the weakness. Electromyographic testing may reveal polyphasic small motor unit potentials, fibrillations, and increased insertional irritability, but it can also be normal in 30% of the cases.[266] Muscle biopsy is the most definitive test and shows endomysial lymphocytic infiltrates without rimmed vacuoles or chronic myopathic features similar to those seen in idiopathic polymyositis.[268] Infiltrating cells are predominantly $CD8^+$ T cells and macrophages.[269] Interestingly, almost one-half of patients with HIV-1 polymyositis also had diffuse infiltrative lymphocytosis syndrome. Because both conditions are extremely rare, their association is not likely to be coincidental.[266] Similar to idiopathic polymyositis, patients with HIV-1 polymyositis have had a favorable response with corticosteroids (prednisone 1 mg/kg/day) for an average of 9 months. However, the risk of long-term immunosuppressive therapy should be carefully considered in this population of patients. Other immunologically based therapies such as azathioprine, methotrexate, or intravenous immunoglobulin have also been successful.[266]

Inclusion body myositis seen in HIV-infected patients is similar to sporadic inclusion body myositis except that it has younger age of onset and can sometimes respond to immunosuppressive therapies. A recent study showed significant overlap between HIV-associated polymyositis and inclusion body myositis, with all patients eventually developing finger and wrist flexor weakness, rimmed vacuoles on biopsy, or anti-NT5C1A autoantibodies—features most consistent with inclusion body myositis.[270]

Rare cases of nemaline rod myopathy as well as muscle involvement with diffuse infiltrative lymphocytosis syndrome have also been reported in HIV-infected individuals.[255] All these syndromes must be differentiated from HIV-1 wasting syndrome and may easily be overlooked in a population of debilitated patients who often present with generalized weakness. In addition, it may occur simultaneously with CNS or peripheral nervous system complications of HIV-1 infection.

Muscular Complications From Therapies in HIV

Myopathy occurs in 17% of patients treated with zidovudine for periods longer than 270 days.[271] The clinical presentation is indistinguishable from HIV-associated myositis.[267] In zidovudine myopathy, biopsy results reveal numerous ragged red fibers and abnormal mitochondria.[272] Zidovudine-induced mitochondrial toxicity is mediated through the inhibition of the enzyme γ-DNA polymerase, which is responsible for the replication of mitochondrial DNA. This induces an energy shortage within the muscle, which results in overt myopathy over time.

In zidovudine-induced myopathy, treatment consists of zidovudine withdrawal. Objective improvement in muscle strength is expected to occur in most patients after 8 weeks.[273]

More worrisome is the prospect of statin-associated rhabdomyolysis.[274] Indeed, increased cholesterol and triglyceride values are frequent in HIV-infected patients on ART, requiring therapy with statins with the aim of preventing cardiovascular complications. Most statins are metabolized by the liver cytochrome P-450 isoenzyme 3A4. Similarly, PIs are both substrates for and inhibitors of cytochrome P-450 isoenzyme 3A4 and substrates for P-glycoprotein, a bidirectional drug transporter that is also inhibited by statins to varying degrees. Pharmacokinetic studies have shown that PIs may greatly increase statin concentrations. Statin-associated rhabdomyolysis occurs within weeks from onset of treatment and may be fatal.[275]

HIV-associated lipodystrophy syndrome is increasingly recognized in patients treated with ART. Increased lipid content in muscles has been seen in imaging studies as well as muscle biopsies of such patients.[276] HIV-infected patients on ART often complain of arthralgias, and cases of frozen shoulder, tendonitis, and temporomandibular joint dysfunction have been described within a year of starting on indinavir. A survey using an anonymous questionnaire answered by 292 HIV-infected

TABLE 125.4 Neuromuscular Syndromes in Human Immunodeficiency Virus Type 1 (HIV-1) Infection

DIAGNOSIS	DISEASE STAGE	CLINICAL FEATURES	DIAGNOSTIC STUDIES	TREATMENT
AIDP	Early > late	Weakness more than sensory loss	CSF: ↑ WBCs	Early: IVIG, steroids, plasmapheresis
CIDP			↑↑ Protein NCSs: demyelination	Late: consider ganciclovir/foscarnet
MM	Early or late	Multiple painful mononeuropathies	NCSs: multifocal axonal neuropathy Biopsy: inflammation/vasculitis CMV	Early: none Late: steroids/cyclophosphamide Ganciclovir/foscarnet
Nucleoside	Any stage	Distal sensory loss	NCSs: distal axonopathy	Nucleoside withdrawal
Neuropathy		Neuropathic pain	Increased serum lactate	
DSPN	Late	Distal sensory loss Neuropathic pain	NCSs: distal axonopathy	NSAIDs, capsaicin AED, tricyclics
PP	Late	Progressive flaccid paraparesis, urinary dysfunction, LS pain	CSF: increased WBCs (PMNs), CMV PCR+	Ganciclovir/foscarnet Cidofovir
DILS	Late	Sjögren syndrome, distal motor and sensory loss, pain	NCSs: axonal neuropathy Biopsy: $CD8^+$ T cells, HIV-1	Zidovudine/ART Steroids
Zidovudine	Any stage	Proximal weakness	EMG: ± irritative	Zidovudine withdrawal
Myopathy		Myalgias	Biopsy: ragged red fibers	
Polymyositis	Any stage	Proximal weakness Myalgias	EMG: ± irritative Biopsy: inflammatory infiltrates	Steroids, IVIG Immunosuppressants
ALS-like	Late	Weakness, dysphagia	EMG: neurogenic	ART

AED, Antiepileptic drug; *AIDP,* acute inflammatory demyelinating polyneuropathy; *ALS,* amyotrophic lateral sclerosis; *ART,* antiretroviral therapy; *CIDP,* chronic inflammatory demyelinating polyneuropathy; *CMV,* cytomegalovirus; *CSF,* cerebrospinal fluid; *DILS,* diffuse infiltrative lymphocytosis syndrome; *DSPN,* distal sensory polyneuropathy; *EMG,* electromyography; *IVIG,* intravenous immunoglobulin; *LS,* lumbosacral; *MM,* mononeuritis multiplex; *NCS,* nerve conduction studies; *NSAID,* nonsteroidal anti-inflammatory drug; *PCR,* polymerase chain reaction; *PMNs,* polymorphonuclear leukocytes; *PP,* progressive polyradiculopathy; *WBCs,* white blood cells.

patients in Europe indicated that arthralgias were more frequent in patients taking PIs, especially indinavir (40.6%) or ritonavir plus saquinavir (41.9%), compared with nonnucleoside reverse-transcriptase inhibitors (NNRTIs) (26.5%) or NRTIs (25.3%).[277] Indinavir causes urolithiasis by crystallizing in the urinary tract, and indinavir crystals have been found in the joint fluid of patients with frozen shoulder. Temporary interruption of the PI or replacement with an NNRTI should be considered on the basis of the patient's discomfort, ART history, and HIV-1 genotype studies.

Opportunistic Infections and Tumor Infiltrations

Opportunistic agents causing pyomyositis include *Mycobacterium tuberculosis, Mycobacterium avium intracellulare,* CMV, and *T. gondii.* Muscle involvement can occur with Kaposi sarcoma and non-Hodgkin lymphoma.[255]

SEIZURES

HIV-positive patients have an increased risk to develop seizure activity due to HIV-associated brain lesions and metabolic disturbances. The incidence of seizures depends on the degree of immunosuppression.

CNS mass lesions and meningitis are the major causes of seizures, but no identifiable cause other than HIV-1 infection can be detected in up to 25% of cases.[278] Unless a reversible cause can be readily identified and treated, anticonvulsant therapy should be initiated after the first seizure.

Antiepileptic drugs (AEDs) and antiretrovirals can adversely affect each other when coadministered. The adverse effects can include breakthrough seizures, virologic failure, or drug toxicity. One of the recommendations is that it may be important to avoid cytochrome P-450 enzyme-inducing AEDs in people on antiretroviral regimens that include PIs or NNRTIs because pharmacokinetic interactions may result in virologic failure.[279] This particularly applies to the older generation AEDs—primidone, phenytoin, phenobarbital, and carbamazepine.

In addition, protein binding must be considered. Most AEDs and PIs are highly protein bound, as are other medications taken by HIV-infected patients, such as trimethoprim-sulfamethoxazole. This may result in competition for available protein binding sites. Valproic acid and phenytoin commonly displace other drugs from albumin, which may result in increased free drug levels, side effects, and toxicity. Conversely, PIs may also displace AEDs such as carbamazepine from protein binding sites and result in toxicity. There have been no studies of the outcome of seizure management in HIV-infected patients on ART. Until these data are available, clinicians should favor the use of AEDs that have no effect on the cytochrome P-450 system and have limited protein binding, such as levetiracetam, lacosamide, gabapentin, and pregabalin.[280] The choice of the AED or combination thereof depends on the type of seizure presented by the patient. In the case of status epilepticus or emergencies in which intravenous treatment is indicated, traditional AEDs such as phenytoin or phenobarbital should be used temporarily in the acute phase and should then be replaced by medications that have lower interaction potential with PIs once seizure control has been achieved.

The principal neuromuscular syndromes found in HIV-1 infection are listed in Table 125.4. A summary of the principal neurologic complications of HIV infection is provided in Fig. 125.6.

TEMPORAL TRENDS AND AGING IN NEUROLOGIC MANIFESTATIONS OF HIV INFECTION

The spectrum of CNS complications of HIV-1 diseases is constantly evolving, and both quantitative and qualitative changes have been noted in recent years.[76,281] Patients are living longer with ART, but they may become resistant to antiretrovirals. The clinical presentation of known diseases such as PML may be altered by ART-induced immune reconstitution,[176] and new entities, such as severe HIV-associated leukoencephalopathy, have been described in ART-experienced individuals.[281,282] Expanded tropism of JCV to cerebellar neurons has been demonstrated, which may have implications in the pathogenesis of cerebellar atrophy occurring in HIV-infected individuals.[283] In addition, PIs are associated with a marked increase in serum cholesterol and triglycerides. Therefore long-term HIV-1–infected individuals are at increased risk of developing cerebrovascular events.[283,284] Stroke usually occurs in younger patients infected with HIV-1 compared with HIV-negative individuals, and, in some cases, it may be caused by a vasculopathy.[285]

The number of people living with HIV who are 55 years of age and older is expected to more than double between 2013 and 2045.[286] As this population becomes older, age-related entities such as Alzheimer dementia are expected to overlap with HIV-associated neurologic

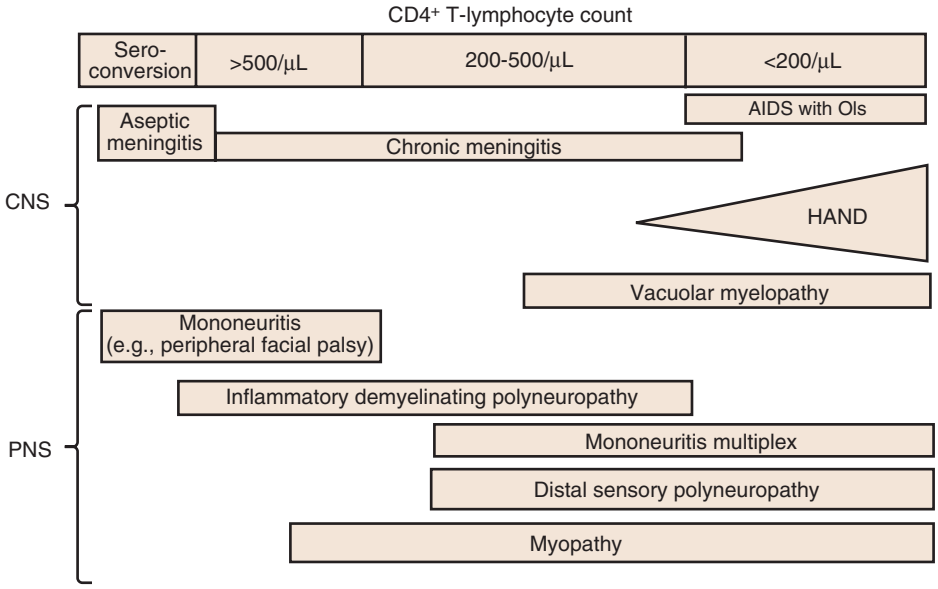

FIG. 125.6 Occurrence of the principal neurologic complications of human immunodeficiency virus (HIV) type 1 infection according to the degree of immunosuppression as measured by CD4⁺ T-lymphocyte counts (see text for details). *AIDS,* Acquired immunodeficiency virus; *CNS,* central nervous system; *HAND,* HIV-associated neurocognitive disorder; *OIs,* opportunistic infections; *PNS,* peripheral nervous system.

conditions and add another layer of complexity to management of these patients.[287,288,289] Not surprisingly, older age and diabetes were associated with increased frequency of dementia in HIV-infected patients.[290,291] In addition, cerebral small vessel ischemic vascular disease may cause white matter lesions and cortical atrophy, thus compounding the effect of HIV on the CNS of older patients.[292] Because of these trends and the fact that new clinical entities may potentially warrant different treatments, a brain biopsy should be considered in cases in which molecular diagnosis cannot be obtained, and postmortem analysis should be sought for all patients who did not respond to treatment.

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126 Human Immunodeficiency Virus Infection in Women

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Human immunodeficiency virus (HIV) infection has had a profound impact on the health of women worldwide. By the end of 2016, 52% of the more than 36.7 million people living with HIV infection were women.[1] HIV infection and resultant acquired immunodeficiency syndrome (AIDS) are the leading causes of death among women in their reproductive years (ages 15–49).[2] Sub-Saharan Africa remains the most severely affected region, with nearly 1 in 20 adults (4.9%) living with HIV infection (more than half of whom are women) and accounting for 69% of all people living with HIV infection worldwide.[2] In the United States, 7% of all cases of AIDS reported as of 1985 occurred in women; this proportion increased to 26% of all AIDS cases newly diagnosed in 2001; by 2015, 19% of all new HIV diagnoses were among women.[3]

With the advent of increasing access to potent HIV antiretroviral therapy (ART) worldwide, new HIV infection rates have decreased overall and by 50% or more in many countries including in sub-Saharan Africa.[2] However, there were profound differences in infection rates among young men and women in 2016; new infections among women ages 15 to 24 years were 44% higher than rates among men in the same age group.[2] Although all people infected with HIV continue to face stigma, discrimination, and injustice, factors such as gender inequity, differential access to health service, and sexual violence are particularly prominent hardships among women.[2] This chapter discusses the epidemiology of HIV infection in women, transmission of HIV to women, prevention of heterosexual HIV transmission, pregnancy and risk for perinatal transmission, and clinical manifestations and management issues for women infected with HIV.

EPIDEMIOLOGY

Epidemiology in the United States

Risk Factors for and Changing Patterns of Transmission

When AIDS was first recognized in 1981,[4] it was considered to be a disease of men who have sex with men (MSM) and of injection drug users. However, with the rapid increase in the number of women infected with HIV in the United States and globally, heterosexual transmission of HIV has emerged as the most common risk factor for HIV infection worldwide. By 2001, women accounted for 26% of new AIDS diagnoses (11,164 of 43,158 cases), 18% of the 816,149 cumulative AIDS cases, and 32% of newly reported HIV diagnoses (11,394 of 35,575 cases) in the United States. Since then, annual HIV diagnoses have declined 20% among women from 2010 to 2014; in 2015 women made up 19% (7402) of the 39,513 new HIV diagnoses.[5] Overall, per the Centers for Disease Control and Prevention (CDC), women represented 20% (248,270) of the 1,216,917 cumulative AIDS diagnoses in the United States from the beginning of the epidemic through the end of 2015.[3]

Heterosexual contact has become the predominant mode of exposure for women in the United States, accounting for 86% of new HIV infections among women in the United States in 2015.[5] Cases associated with injection drug use among women have declined since 1992, with 13% of cases now attributed to injection drug use. However, there are racial and ethnic disparities in types of transmission risk among women throughout the United States, with overall higher rates of transmission due to injection drug use in 2015 among white women (32%).[3] Most new HIV infections in the United States continue to occur through male-to-male sexual contact (70% in 2016), but increases in HIV incidence among young African-American and Hispanic gay and bisexual men may be helping to drive the ongoing epidemic in women.[5] For instance, in a large study of African-American men who have sex with men and women in three cities (Chicago, Philadelphia, Los Angeles), 39% of the sample (584 men) were HIV-positive.[6] Of men who were HIV-positive, 46% reported sex without condoms with HIV-negative or unknown male partners and 45% with HIV-negative or unknown female partners.

Bisexual activity in men who do not identify as gay or disclose their bisexual activities has been labeled as "on the down low" activity.[7] Another study among men who have sex with men and women (MSMW) found differences in sexual behaviors compared with men who have sex with men only (MSMO).[8] This study was conducted among MSM who participated in the National HIV Behavioral Surveillance study and included 2042 (11.9%) participants who identified as MSMW. Compared with MSMO, MSMW had higher numbers of sexual partners, higher numbers of casual partners, higher rates of sex without condoms, and higher rates of sexually transmitted infections (STIs) and were more likely to engage in transactional sex for either money or drugs. Therefore tailored interventions to reduce HIV risk behavior among MSMW may have implications for reducing HIV transmission rates among women.[3]

Female-to-female sexual transmission of HIV remains exceedingly rare.[9] In populations of women with HIV infection who report having sexual contact only with women, most have had a secondary risk factor such as injection drug use.[9] In a study published in 1995 of 498 lesbian and bisexual women frequenting public venues in San Francisco and Berkeley, California, 6 (1.2%) were HIV infected.[10] This population showed high rates of high-risk behaviors (10% reported injection drug use, and 40% reported unprotected sex with men, some of whom were bisexual men and male injection drug users), and no evidence of sexual transmission between women was found. These data and other more recent studies suggest that the frequency of female-to-female HIV transmission remains very low.[9]

The epidemic of drug use continues to influence HIV risk among women in the United States, despite the fact that injection drug use plays a less prominent role in the spread of infection now than in the past. In 1996, after exclusion of the 24% of women whose initial mode of transmission was not reported, 45% of women with AIDS reported injection drug use or heterosexual contact with an injection drug user (18%).[11] By 2001, after excluding the 42% of women without a reported risk behavior, only one-third of women with newly diagnosed AIDS reported injection drug use, and 14% reported heterosexual contact with an injection drug user.[12] By 2015, the percentage of HIV infections attributed to injection drug use was highest among white women (32%), but the overall rates of transmission among women due to injection drugs are now at an all-time low at 13%.[13]

Needle exchange programs have long been known to decrease spread of HIV among intravenous drug users and are a powerful tool to combat new infections in the context of the recently declared opioid epidemic in the United States.[13] In 2015, in Austin, Indiana, a rural town of about 4000 residents with an historically very low prevalence of HIV infection, almost 200 individuals were found to be newly infected with HIV as a result of injection of oxymorphone.[14] A public health emergency was declared, and following national media exposure and a CDC-led investigation,[15] the state expanded free HIV and hepatitis C virus testing and partner services, established local HIV treatment services, and provided immediate access to health insurance. Through an executive

governmental order, an emergency syringe-exchange program, the first in Indiana, was started; within 6 months, 277 persons who injected drugs had enrolled, and more than 97,000 sterile syringes were distributed and returned. Currently, persons who inject drugs can access sterile needles and syringes through syringe services programs[16] and through pharmacies without a prescription. Although less common, access to sterile needles and syringes may also be possible through a prescription written by a physician and through other health care services. Syringe services programs are an effective component of a comprehensive integrated approach to HIV prevention among intravenous drug users, are becoming more prevalent across the United States, and may serve as an additional tool to combat HIV incidence among women.

Finally, HIV transmission through intravaginal insemination of donor semen has been reported.[17] Use of assisted reproductive technologies, which include sperm washing and intracytoplasmic sperm injection, reduces horizontal transmission from an HIV-infected man to an HIV-negative woman to almost zero.[18–21] Although these techniques are promising, they require specialized laboratories, are expensive, and are typically not covered by insurance and therefore are not accessible to most couples (see "Strategies to Prevent Heterosexual Transmission" and "Fertility Issues"). With the advancement in powerful biomedical prevention strategies such as "treatment as prevention" and preexposure prophylaxis (PrEP), assisted reproduction with sperm washing may no longer be necessary within serostatus-discordant couples, as the risk of transmission from a partner living with HIV with virologic suppression is extremely low.[22]

Racial and Ethnic Disparities in HIV Incidence

HIV disproportionately affects women of color: among all women with an HIV diagnosis in the United States in 2015, 61% (4524) were African American, 19% (1431) were white, and 15% (1131) were Hispanic/Latina.[5] To this day, the HIV diagnosis rate for black women remains 16 times as high as that of white women and almost 5 times that of Hispanic women.[3] Although the number of HIV diagnoses among African-American women decreased 42% from 2005 to 2014, the rate is still disproportionally higher than rates in women of other races and ethnicities.[23] A number of factors seem to increase risk among African-American women for acquisition of HIV, including higher rates among African-American men, higher prevalence of STIs in the African-American population, stigma, poverty, structural racism, less access to health care, and higher rates of incarceration, all of which can disrupt social networks and decrease the number of available partners for women.[3] Of note, the highest rates of new HIV diagnoses among women were among black women living in the northeastern (52.4/100,000) and southern (36.1/100,000) states.[24]

Age Patterns in Women Living With HIV in the United States

HIV incidence is more common in women of childbearing age; in 2015, 66% of new HIV infections among women were diagnosed in girls and women between 15 and 44 years of age. However, an increasing percentage of new infections among women (currently 34%) occur in women 45 years old or older. Estimates indicate that by 2030, more than 70% of people living with HIV in the United States will be 50 years old or older.[25,26] Older women may not recognize themselves as being at risk for HIV infection,[27] may experience more stigma within their communities than younger women,[28–30] and are more likely to present to HIV care later in the course of infection. Older women living with HIV have particular psychosocial and medical needs,[31] including frailty,[32] worsening neurocognitive function,[33] issues with sexuality,[34,35] bone loss,[36] and menopause.[37] Therefore, designated care paradigms for women aging with HIV, tailored to address both the medical and the psychosocial gaps in this unique population, are indicated.

HIV Care Continuum Among Women

Within the United States, more than 1.1 million people are living with HIV infection, and 12% of women are still unaware of their infection, a rate that has been decreasing over time.[23] Overall in the United States, the typical HIV care continuum outcomes are still inadequate: Among women living with HIV, 88% have had HIV diagnosed; 64% are linked to care; 50% are retained in care; and 48% have achieved the goal of ART, specifically virologic suppression.[38] The gravity of the HIV epidemic among women in the United States is often not appreciated by individuals at risk and by the broader community.[39] A cohort study of high-risk women in the northeastern and southeastern states found an annual HIV incidence of 0.32%; older age, substance use, and knowing that a partner had HIV were associated with higher HIV prevalence.[40] With the increasing use of routine rapid HIV testing of adults in the United States and in the era of universal ART (starting ART as soon as HIV is diagnosed, regardless of CD4 T-cell count), more people, including women, should be identified earlier and started promptly on ART. The goals of the National HIV/AIDS Strategy, a comprehensive plan to address the HIV epidemic established by the United States in 2010 and now updated to 2020, are to reduce new infections, increase access to care and improve health outcomes, reduce HIV-related health disparities, and achieve a more coordinated national response[41]; these goals are especially important to address among women.

Worldwide Epidemiology

Disproportionate Impact on Young Women

Updated estimates from the Joint United Nations Programme on HIV/AIDS (UNAIDS) (2017 update)[2] continue to report more HIV infections worldwide than at any point in history, with 36.7 million currently infected. Since the start of the epidemic, there have been 76.1 million infections overall and 35 million people have died of AIDS-associated illnesses.[1] Of the 36.7 million people living with HIV as of the end of December 2016, 34.5 million are adults, and more than 50% (17.8 million) of those are women. HIV infection has been reported from all over the world, but more than 95% of all infections are in resource-limited settings, and two-thirds of people living with HIV reside in sub-Saharan Africa.[2] Global efforts have been mounted to address the epidemic, and there has been significant progress, with new HIV infections declining by an estimated 11% among adults from 2010 to 2016 and by 47% among children.[2] Despite this progress, approximately 1.8 million people were newly infected with HIV in 2016, which equates to approximately 5000 new infections per day. Of those 5000 new infections a day, 64% were in sub-Saharan Africa, and 4500 were in adults; of those, 22% were in young women (ages 15–24).

Despite this progress, the rate of decline for new HIV infections has varied significantly by region, by age group, and, importantly, by sex. Young women continue to be a highly affected group when it comes to new infections in high-prevalence settings compared with men in the same age group. For instance, in 2016, new infections in young women 15 to 24 years of age were 44% higher than in men in the same age group.[2] Young women in this age group represented 26% of all new infections in eastern and southern Africa in 2016, although this demographic represents only 10% of the population.[2] These discrepant rates reflect the biologic and social vulnerability of young women to HIV infection,[42] young women partnering with older men,[43] and inaccurate perceptions of young women of their own risk,[44] all of which require targeted interventions and an intense focus.

As of World AIDS Day, December 1, 2017, UNAIDS provided an update in a landmark document called "Right to Health"[45] that 20.9 million people (57%) of the 36.7 million people infected worldwide were on ART. These rates were generally not discrepant by sex; indeed, in many countries, women had higher rates of ART access than men, likely due to accessing ART through antenatal settings.[2] Annual AIDS-related deaths have declined by 48% from 2005 to 2016 (from 1.9 million to 1.0 million) largely due to global scale-up of ART.[2]

Update on Perinatal Transmission Rates

Rates of perinatal transmission from mother to child during pregnancy or breastfeeding have decreased as access to prevention of maternal-to-child transmission (PMTCT) services has increased. New infections in children declined by 47% from 2010 to 2016, with 300,000 new infections in 2010 compared with 160,000 new infections reported as of December 2016.[2] This decrease corresponds to an increase in antiretroviral (ARV) coverage for pregnant women from 47% in 2010 to 76% by 2016 (Fig. 126.1).[2]

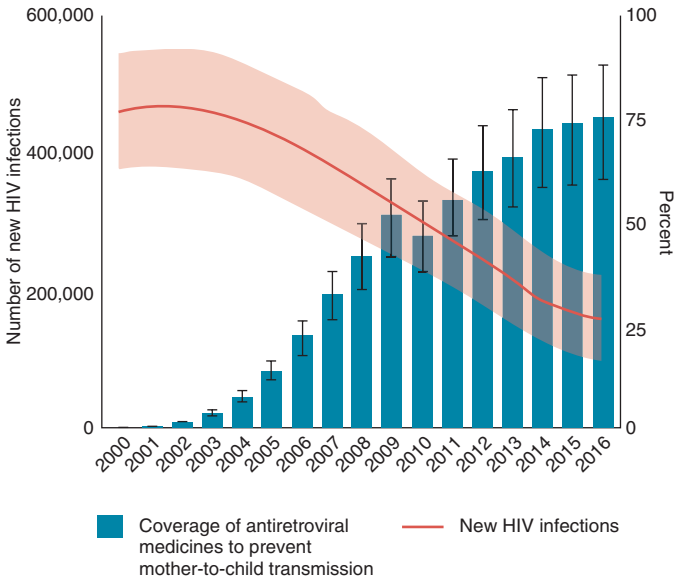

FIG. 126.1 New human immunodeficiency virus (HIV) infections among children (age 0–14 years) and coverage of antiretroviral regimens to prevent mother-to-child transmission globally 2000–2016. *(From UNAIDS Data 2017. http://www.unaids.org/sites/default/files/media_asset/20170720_Data_book_2017_en.pdf. Accessed December 15, 2017.)*

Risk Factors for HIV Transmission to Women Worldwide

Worldwide, HIV infection is spread primarily through heterosexual contact, although injection drug use and its subsequent contribution to HIV transmission varies geographically. Because female drug users have sexual partners who are at high risk for HIV infection and because injection drug use can lead to exchange of sex for drugs or money, there may be underrecognition and underreporting of cases spread through dual mechanisms. Several more recent studies suggest that unprotected anal sex between men may be a more important factor in the epidemics in sub-Saharan Africa than is commonly believed. These men may then transmit HIV infection to women if they have sex with both men and women.[46] Gay men and other MSM accounted for 12% of new infections globally in 2015, whereas sex workers and people who inject drugs accounted for 5% overall.[2]

Regional Variation in HIV Incidence and the Impact on Women

As summarized earlier, sub-Saharan Africa remains the hardest hit in terms of the HIV epidemic. Nearly two-thirds of people living with HIV reside in sub-Saharan Africa, and 64% of new infections in 2016 occurred in this region.[2] Despite this continued prevalence and incidence, the largest reductions in HIV incidence worldwide were observed in eastern and southern Africa with approximately 40,000 fewer new adult HIV infections reported in the region in 2015 than in 2010. More gradual declines in incidence have been observed in the Asia and Pacific region and in western and central Africa.[2] Moreover, rates of new adult HIV infections were relatively static in Latin America and the Caribbean, western and central Europe, North America, the Middle East, and North Africa, whereas the annual numbers of new HIV infections in eastern Europe and central Asia have increased significantly over the past several year (57% increase from 2015 to 2016).[2] Because the implications for subpopulations such as women are unclear in regions where data are not accurately recorded, efforts are underway to collect and analyze national data with greater accuracy and precision.

AIDS-Related Mortality and Its Impact on Women and Families

The high mortality rate for HIV/AIDS continues to have a major impact on families. As of 2016, approximately 17 million children and adolescents worldwide had been orphaned by loss of one or both parents to AIDS.[2] More than 90% of these children live in sub-Saharan Africa. Saving the lives of parents through access to ART in countries with limited health care resources and helping to alleviate poverty and improve education are central to the global response to the orphan crisis.[47] The United States Agency for International Development (USAID) and the U.S. President's Emergency Plan for AIDS Relief Orphans and Vulnerable Children Program[23] have provided funding and technical support to protect, care for, and support children affected by HIV. These agencies invest in programs that enable children to stay in school; help extended family members serve as primary caregivers; and provide legal and child protection to improve access to essential services and prevent and respond to child abuse, exploitation, and family separation. A variety of interventions have also focused on keeping orphaned adolescents safe from HIV acquisition during the vulnerable period of young adulthood.[48]

In 2014, UNAIDS launched a campaign to end the AIDS epidemic with the 90-90-90 targets: By 2020, 90% of people living with HIV should know their status, 90% of people living with HIV who know their status should be on treatment, and 90% of people on treatment should achieve virologic suppression.[49] In 2015, the United Nations General Assembly adopted their new Sustainable Development Goals (SDGs), which included a target to end the AIDS epidemic by 2030.[50] With the science showing that starting ART as early as possible both keeps people living with HIV in better health[51,52] and prevents HIV transmission,[53] many countries have adopted the recent World Health Organization (WHO) recommendations of treating everyone living with HIV with ART regardless of $CD4^+$ counts or viral load.[54] According to the *Ending AIDS: Progress Towards the 90-90-90 Targets* report, by 2016, 70% of all people living with HIV globally knew their HIV status, 77% of whom were accessing ART (20.9 million people or more than half of all people living with HIV), and more than 82% of people on treatment were virally suppressed.[49] These target achievements did not vary significantly between men and women worldwide.

TRANSMISSION AND ACQUISITION IN WOMEN AND PREVENTIVE STRATEGIES

Heterosexual Transmission

Efficiency of Transmission

Globally, heterosexual transmission accounts for the spread of HIV infection in approximately 90% of people living with AIDS, and the fastest growing subset of the epidemic is in women due to heterosexual transmission.[2] However, mechanisms of heterosexual transmission remain poorly understood.[55] HIV has been isolated from semen of HIV-infected men and from cervicovaginal secretions of HIV-infected women. However, HIV is not transmitted consistently by sexual contact. Although some individuals become infected after a single exposure to HIV-infected semen, others remain uninfected despite hundreds of exposures.[56,57] This lack of transmission may be due to the amount of virus in the semen or cervicovaginal fluid, the host immune response, the relative virulence of HIV isolates, or some combination thereof.

HIV-infected women can transmit HIV to their uninfected male sexual partners, but heterosexual transmission seems to be more efficient from men to women than from women to men.[58,59] The HIV risk by exposure type in the absence of ART was estimated in a systematic review, which found that the risk of HIV infection for receptive penile-vaginal intercourse was 1 in 1250 compared with 1 in 2500 for insertive penile-vaginal intercourse.[60] In comparison, the risk of receptive anal intercourse is much higher with a risk of 1 HIV infection per 72 exposure acts.[60] As noted earlier, female-to-female HIV transmission has been reported but is exceedingly rare.[9,61] The explanation for this difference in ease of HIV spread from men to their female partners relates to the larger volume of semen compared with cervicovaginal secretions transferred in the coital act and to the higher concentration of HIV on average in seminal fluid. Other factors include increased surface area and thinner epithelium in the female genital tract, rendering it more susceptible to infection. Finally, changes in the protective immune response driven by hormonal fluctuation over the menstrual cycle in women may increase the risk of HIV acquisition.

Factors Associated With Transmission

Factors that have been associated with a greater likelihood of transmitting HIV between heterosexual partners include high HIV viral loads or more advanced immunodeficiency in the infecting partner, the presence of any STI including ulcerative and nonulcerative disease in either partner, sexual activity during menses, receptive anal sex, unprotected vaginal sex, traumatic sex, and increased number of sexual contacts (Table 126.1).[62–64] Baeten and associates[65] demonstrated that genital HIV-1 RNA levels independently predicted HIV-1 transmission risk even after adjusting for plasma HIV-1 RNA concentrations, suggesting that genital HIV viral load could be used as an independent marker of HIV-1 sexual transmission risk.

Male circumcision was shown in randomized trials in Africa to decrease female-to-male HIV transmission by 60%, making male circumcision a highly effective strategy for HIV prevention.[66,67] Because male circumcision provides only partial protection, however, higher risk behaviors could nullify the effect of circumcision. Additionally, circumcision among HIV-infected men may not directly decrease acquisition of HIV among women in serostatus-discordant couples.[66] Roll-out of voluntary circumcision is an active HIV prevention strategy recommended by WHO.[68] Implementation of male circumcision is challenging and has experienced variable success rates around the world because it requires surgical training, aseptic techniques, acceptability, availability, and cultural and religious considerations.[69,70]

The dynamic nature of the female genital tract, cyclical reproductive hormones, and hormonally mediated immune defenses create a "window of vulnerability" to HIV and other infectious agents when the humoral, cell-mediated, and innate immune defenses are suppressed during the ovulatory phase of the menstrual cycle.[71] Cervical mucus and cervicovaginal mucus have been shown to impede the diffusion of HIV, helping to elucidate why conditions such as bacterial vaginosis that can disrupt this protective mucus have been associated with increased HIV acquisition risk in women.[72]

Impact of Hormonal Contraception on HIV Transmission and Acquisition

Hormonal contraceptives, specifically the progestin-only injectable depot medroxyprogesterone acetate (DMPA), have been associated with increased risk for HIV-1 transmission and acquisition in some studies, mostly retrospective studies or studies designed to assess different outcomes.[73] Heffron and coworkers[74] conducted secondary data analyses from the Partners in Prevention HSV/HIV Transmission Study among 3790 heterosexual HIV-discordant couples enrolled in two longitudinal studies in seven African countries. Incidence of HIV infection among female hormonal contraception nonusers was 3.8/100 person-years compared with 6.9/100 person-years among users of injectable forms of contraception ($P = .04$) and 5.9/100 person-years in oral contraceptive users ($P = .33$).[74] Among male partners, the incidence of HIV infection was 1.5/100 person-years when the female partners were HIV-positive and using hormonal contraception compared with 2.6/100 person-years in male partners of female partners using injectable drugs ($P < .05$) and 2.5/100 person-years in male partners of female partners using oral contraceptives ($P = .31$).[74]

Several biologically plausible mechanisms have been postulated to explain how various hormonal contraceptive methods could increase risk of HIV acquisition in women, including thinning or disruption of epithelial barriers, alterations in HIV target cells, or changes in secreted immune defense molecules.[75–78] Polis and colleagues[73] performed a systematic review of studies that assessed the impact of hormonal contraceptives on the risk of HIV acquisition and 31 relevant studies for their analysis: 24 specific to oral contraceptives, 24 specific to progestin-only injectables, and 3 including progestin-containing implantable contraceptives. There was no evidence to suggest that oral contraceptive pills or contraceptive implants increased the risk of HIV acquisition; however, this systematic review indicated that DMPA increased the risk of HIV acquisition by a hazard ratio of 1.4.[73] Due to the observational nature of these studies, there were several confounding factors that could not be controlled for, including misreporting of condom use or difference in sexual behavior. Clinicians and patients need to weigh the potential risk for hormonal contraception on HIV transmission or acquisition risk with known risks for unwanted pregnancy, with its known morbidity and mortality,[79] and the efficacy of implantable or injectable forms of contraception.

Given the substantial public health implications of limiting contraception options for women at risk of acquiring HIV and the previously mentioned limitations with the observational nature of the data, WHO convened a meeting of stakeholders in December 2016 and in 2017 published their most recent guidance statement on hormonal contraceptive methods for women at high risk of HIV and living with HIV.[80] They recommended that the medical eligibility criteria (MEC) for combination hormonal contraceptives, progestogen-only pills, and levonorgestrel and etonogestrel implants remain at 1 (no concern) among women at high risk of HIV infection. However, the MEC recommendation for injectables (DMPA and norethisterone enanthate) was changed to 2, indicating some concern that their use may lead to an increased risk of HIV acquisition.[80] The CDC generally adopted the same guidance as WHO for inclusion in the US MEC guidance for the use of contraceptives, also changing the MEC category for DMPA from 1 (no concern) to 2 (some concern), based on the theoretical risks that DMPA may increase the risk of HIV acquisition in women already at risk.[81]

TABLE 126.1 Risk Factors Associated With Sexual Transmission of HIV

Sexually transmitted infections
Ulcerative/nonulcerative diseases
Genital tract inflammation
HIV disease
Higher viral loads
Lower $CD4^+$ levels
Acute HIV infection
Lack of effective antiretroviral therapy
Lack of heterozygosity or homozygosity for inactivating 32-base pair deletion in chemokine receptor gene (*CCR5*)
Anatomic factors
Lack of circumcision
Cervical ectopy
Leukocytospermia
?Hormonal contraception
Sexual practices
Receptive anal intercourse
Sexual activity during menses
Bleeding during intercourse (disruption of vaginal mucosa through trauma)
Lack of barrier protection
HIV viral features
Syncytium formation
Certain viral clades

HIV, Human immunodeficiency virus.

Sexually Transmitted Infections

STIs, particularly infections associated with genital ulcers, increase the efficiency of HIV transmission and the susceptibility to HIV infection.[82] Genital ulcerative diseases, which include syphilis, chancroid, and genital herpes, are thought to enhance the access of HIV to mucosal tissues, lymphatic drainage, and systemic lymphocytes.[82,83] Epidemiologic studies suggest that nonulcerative STIs also increase susceptibility to HIV infection; female sex workers in Zaire had an increased risk for HIV seroconversion even with nonulcerative STIs,[84] and seroconversion was more likely in HIV serostatus–discordant sex partners if the previously HIV-negative sex partner had ulcerative and nonulcerative STIs.[85] Nonulcerative STIs, such as gonorrhea, chlamydial infection, and trichomoniasis, seem to increase the number of lymphocytes, monocytes, and Langerhans cells in the endocervix in susceptible seronegative women, providing more potential targets for HIV infection.[86–88] Similarly, nonulcerative STIs may increase the number of cells harboring HIV in the genital tracts of HIV-positive transmitters.[86,87]

Bacterial vaginosis has been associated with higher concentrations of HIV RNA in the genital tract of HIV-infected women,[89] increased risk for HIV transmission to male partners,[90] and a 60% increased risk for HIV acquisition in women.[91] Cohen and coworkers[90] studied 2236 HIV-seropositive women and their HIV-uninfected male partners from

the African Partners in Prevention HSV/HIV Transmission Study to see if bacterial vaginosis was associated with increased HIV transmission. Incidence of HIV in men whose HIV-infected female partners had bacterial vaginosis was 2.91 versus 0.76 per 100 person-years in men whose female partners had normal flora (hazard ratio [HR], 3.62; 95% confidence interval [CI], 1.74–7.52). After controlling for sociodemographic factors, sexual behavior, male circumcision, STIs, pregnancy, and plasma HIV-1 RNA levels in female partners, bacterial vaginosis was associated with a greater than threefold increased risk for female-to-male HIV-1 transmission (adjusted HR, 3.17; 95% CI, 1.37–7.33).[90] However, there were several limitations affecting the generalizability of these results, including that all the participants were involved in a clinical trial and underwent couples HIV counseling and testing, and index participants had baseline $CD4^+$ counts greater than or equal to 250 cells/mm^3 and were seropositive for herpes simplex virus type 2 (HSV-2). Mitchell and associates[89] studied 53 HIV-infected women in the United States and Kenya prospectively and found that HIV shedding was greater in women coinfected with certain bacterial vaginosis–associated species only if on ART. Among these women, vaginal *Lactobacillus* spp. were associated with a lower risk of genital HIV shedding, whereas certain bacterial vaginosis–associated species increased that risk.[89] Bacterial vaginosis commonly recurs despite appropriate antibiotic therapy, and strategies such as partner treatment, disruption of the biofilm with boric acid, and improvement of *Lactobacillus* colonization have been largely unsuccessful in recurrent cases.[92]

HIV Infection Within the Genital Tract

Much research has been dedicated to isolation, identification, and quantification of the amount of HIV present within cervicovaginal secretions. The presence of HIV in the female genital tract is necessary for heterosexual transmission of HIV and for perinatal transmission of HIV during labor and delivery.[93] HIV-1 has been isolated from cervical and cervicovaginal secretions obtained using cervicovaginal lavage, vaginal aspirates, and vaginal or cervical swabs or wicks.[94] Although viral loads in genital secretions tend to be lower after initiation of effective ARV medications, detectable HIV in genital secretions has been reported in men and women with undetectable plasma HIV levels.[95–97] Patients with a low plasma virus burden still may potentially transmit HIV to a sexual partner or perinatally to an infant through pregnancy or breastfeeding, although the risk is low.[98,99]

HIV has been detected in menstrual blood, and having intercourse during menses increases the risk for infecting the male partner.[100] Menstruation introduces approximately 80 mL of blood into the genital tract over a 3- to 5-day period. This menstrual blood is likely to contain HIV-infected cells and free virus, reflecting the HIV viral load of peripheral blood. Menstrual blood also changes the microbiota in the vagina and increases the pH in the vagina to the neutral range, which improves the viability of HIV shed in menses and HIV in semen deposited in the vagina during intercourse.[101] Higher levels of HIV in the genital tract may be seen with conditions that increase the vaginal pH such as blood in the vagina, bacterial vaginosis, lack of *Lactobacillus,* menopause, intercourse, and possibly the use of some forms of birth control.[74,102,103] The hormonal changes of the menstrual cycle also play a role, specifically in that genital viral loads are lower in the periovulatory phase and highest during menses, suggesting that local factors may affect the genital viral load compartment independent of plasma viral load.[104]

Strategies to Prevent Heterosexual Transmission

Barrier Methods

Barrier contraceptives constitute an effective means of preventing HIV transmission when used consistently. One study found no heterosexual transmission events among 124 HIV serostatus-discordant couples who used condoms consistently and a seroconversion rate of 4.8 per 100 person-years among 121 serostatus-discordant couples who used condoms inconsistently.[85] Diaphragms are not an effective barrier form of protection against HIV acquisition.[105] Given inconsistent use of condoms as a barrier form of protection against both HIV and pregnancy, most experts recommend dual forms of contraception in the context of HIV risk.[106]

Treatment of Sexually Transmitted Infections

Treatment of STIs alone is unlikely to significantly decrease transmission of HIV and susceptibility to HIV infection. In a Tanzanian clinical trial conducted in subjects in rural communities, universal STI screening and treatment of symptomatic cases of STI resulted in a 40% decrease in HIV incidence compared with a village in which no STI screening or treatment was available.[107] However, a randomized, placebo-controlled trial in HIV-negative, HSV-2–seropositive women in Africa and MSM in Peru and the United States demonstrated that acyclovir 400 mg twice daily suppressed HSV-2 genital ulcers but was not effective in reducing HIV-1 acquisition in HSV-2–seropositive women and MSM.[108] Other studies of strategies to treat STIs in individuals at risk of HIV infection or individuals with HIV have been similarly ineffective, leading to a focus on more effective strategies of combating HIV infection as outlined further on.

Microbicides

A woman-controlled method of reducing the risk of HIV acquisition would help combat the biologic and social vulnerability to HIV in women. One such approach involves a gel, cream, or ring applied locally in the vagina, cervix, or anus to deliver product that can reduce the risk of HIV. However, until more recently, the history of microbicide research has been discouraging.[109,110] Although nonoxynol-9, a commonly used spermicidal agent, has anti-HIV activity in vitro, there have been safety concerns after reports of vaginal ulceration and inflammation during clinical studies.[111,112] A placebo-controlled trial of nonoxynol-9 in sex workers in four countries did not show a protective effect of nonoxynol-9 on HIV-1 transmission and actually showed an association with an increased risk of HIV acquisition when the product was used more frequently.[112] Nonoxynol-9 may cause toxic effects enhancing HIV-1 infection and should not be used as a potential HIV prevention method.

Assessment of other non–ARV-based microbicides, such as carrageenan (Carraguard) vaginal gel, over many studies and years have been largely unsuccessful.[113] Therefore, current microbicides in trials are focused on directly acting antiviral agents.[110] Vaginal tenofovir gel (used either pericoitally or daily) showed promise in early studies[114] but was not shown to be protective in larger studies, likely owing to poor adherence.[115,116] Barriers to adherence included messiness associated with a vaginal gel, lack of discreteness, and bulky packaging. Two trials tested the efficacy of a vaginal ring containing a nonnucleoside reverse transcriptase inhibitor (NNRTI), dapivirine, in reducing HIV acquisition in young African women. The dapivirine vaginal ring provided modest protection against HIV infection in both trials.[117,118] Issues with adherence to the ring were also seen in these studies, however, with no efficacy seen among younger women (ages 18–21 years) likely due to low adherence. When participants used the ring most of the time, HIV protection approached 70%.[117] Future studies of the microbicide ring should focus on extended use of the vaginal ring and safety in adolescents and pregnant women. Finally, the Microbicide Trials Network has started phase I trials of a vaginal ring that will provide contraception as well as HIV protection. This ring combines dapivirine with levonorgestrel, a contraceptive hormone, both of which are slowly released over the course of 3 months. It is hoped that this ring is the first among many combination rings that will be tested to protect against unwanted pregnancy and HIV risk.[119]

Treatment as Prevention

The introduction of potent combination ART in 1996 and the public health approach to HIV treatment in health care resource–limited settings in 2002 have changed the course of the HIV/AIDS epidemic.[120] ART to reduce the infectiousness of individuals with HIV[98,121,122] and oral PrEP for uninfected individuals to prevent HIV acquisition[123] are the most promising approaches for decreasing the spread of the virus. ART given to HIV-infected partners with the purpose of achieving and maintaining full virologic suppression has been shown to prevent linked HIV transmission in a randomized controlled study from the HIV Prevention Trials Network (HPTN 052) among serostatus-discordant couples.[122] In this study, early treatment of the HIV-infected members

of the couples resulted in a 96% reduction in transmission to non–HIV-infected partners.[122]

These results were confirmed in two observational studies of serostatus-discordant couples (PARTNERS study and Opposites Attract) in which the partner living with HIV had already achieved viral suppression on ART, and there was no use of PrEP by the HIV-negative partner or condoms by the couple. After approximately 75,000 combined sex acts, there were no HIV transmissions within partnerships in both cohorts.[121,124] A meta-analysis concluded that there is minimal risk for sexual transmission to women in heterosexual serostatus-discordant partnerships when the HIV-positive male partner has full viral suppression, with caveats regarding lack of information on sexual intercourse type, STIs, and condom use.[125] Indeed, a growing movement, Undetectable = Untransmissible, implies that there is essentially no risk of sexual transmission from a person with HIV who is consistently virologically suppressed on ART.[54] Therefore ART should be a key component for all combination prevention strategies, especially in the context of ongoing efforts to improve worldwide access to ART.

Preexposure Prophylaxis in Women

Another strategy to decrease the sexual transmission of HIV involves the use of ART for primary prevention (i.e., PrEP) among individuals at risk for HIV (Table 126.2).[126] Although randomized clinical trials of PrEP have demonstrated the efficacy of daily oral tenofovir disoproxil fumarate (TDF)–containing regimens for reduction of HIV acquisition, this efficacy has been highly variable across trials. There was a relative reduction in HIV acquisition of 44% in MSM and transgender women (Preexposure Prophylaxis Initiative [iPrEx] trial),[127] 67% to 75% in heterosexual HIV serostatus-discordant couples in which the HIV-negative partner knew the HIV-positive status of his or her partner (Partners PrEP),[128] 62% in heterosexual men and women (Botswana TDF2),[129] and 49% in individuals who inject drugs (Bangkok Tenofovir Study [BTS]).[130] However, two placebo-controlled studies of daily oral TDF-based PrEP conducted specifically among young sexually active African women at risk for HIV infection (FEM-PrEP and VOICE) failed to demonstrate efficacy of daily PrEP in reducing HIV acquisition.[115,131]

FEM-PrEP and VOICE together enrolled greater than 5000 women, but the results in these trials were not consistent with subgroup analyses among women in trials that enrolled heterosexual couples (Partners PrEP, TDF2, BTS) where efficacy was convincingly demonstrated. In Partners PrEP, which included 1785 Kenyan and Ugandan women with an HIV-infected partner (52% of the overall study population), efficacy among women was 66% with TDF/emtricitabine (FTC) and 71% with TDF, and efficacy remained between 64% and 84% even among subgroups of women at the highest risk.[128,132] In the TDF2 study conducted among heterosexual men and women in Botswana, efficacy of daily oral TDF/FTC among the 557 women in the trial (45.7% of the overall study population) was 49%, although statistical power was reduced by the limited number of end points in each subgroup.[129] In BTS, which included 489 women who used injection drugs in Thailand (20% of the overall study population), efficacy of daily oral TDF in women was 79% in reducing HIV acquisition.[130]

Overall, high adherence was noted in trials that reported high PrEP efficacy, and low adherence was noted in trials without efficacy. Of critical importance to the interpretation of PrEP trials was the incorporation of pharmacologic measures of adherence, where drug levels were measured in a biomatrix such as plasma.[133] HIV acquisition across studies, including studies that enrolled women, was associated with lower PrEP adherence as adjudicated by pharmacologic or objective measures. For instance, in both FEM-PrEP and VOICE, self-reported

TABLE 126.2 Clinical Trials of Oral Preexposure Prophylaxis in Which Women Were Enrolled

STUDY	STUDY POPULATION	LOCATION	INTERVENTION	OUTCOME	COMMENTS
TDF2[129]	1219 sexually active adults; 55% male, 45% female; 94% unmarried; approximately 90% age 21–29 years	Botswana	Daily oral TDF/FTC vs. placebo	63% protection (95% CI, 22%–83%; $P = .01$)	>30% did not complete study; greater TDF blood concentrations in subjects who remained HIV uninfected compared with subjects who acquired HIV; cannot draw definitive conclusions for women and men separately
Partners PrEP Study[403]	4758 heterosexual serostatus-discordant couples; 38% negative female partner, 68% negative male partner; median age 33 years	Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, Zambia	Daily oral TDF, TDF/FTC, or placebo	67% protection with TDF alone; 75% protection with TDF/FTC; TDF vs. FTC/TDF not statistically significant in primary analysis	TFV detected in blood samples from 82% of subset of HIV-uninfected subjects vs. 31% of subjects who acquired HIV; detection of TFV in blood associated with 86%–90% protection
FEM-PrEP[131]	2120 heterosexual women at high risk for infection age 18–35 years	Kenya, South Africa, Tanzania	Daily oral TDF/FTC vs. placebo	Trial discontinued in April 2011 due to lack of efficacy for HIV prevention	TFV consistently detected in blood samples from <30% of subset of HIV-uninfected subjects
VOICE (MTN-003)[115]	5029 heterosexual women age 18–45 years in high-prevalence areas	South Africa, Zimbabwe, Uganda	Daily oral TDF, FTC/TDF, or placebo or daily vaginal TDF gel or placebo gel	TDF and TDF gel discontinued early due to lack of efficacy for HIV protection. No study drug significantly reduced risk for HIV acquisition: HIV incidence was 5.7 per 100 person-years (range, 0.8–9.9 per 100 person-years). Effectiveness was –48.8% for TDF; –4.2% for TDF/FTC; and 14.7% for TDF gel	Adherence to study drugs was low[404]: TFV was detected in 30% of oral TDF arm, 29% in oral TDF/FTC arm, and 25% in TDF gel arm
HPTN 052[98]	1763 heterosexual serostatus-discordant couples; 50% negative female partner, 50% negative male partner; 94% married; 61% age 26–40 years	Botswana, Kenya, Malawi, South Africa, Zimbabwe, Brazil, India, Thailand	Immediate or delayed oral antiretroviral therapy in partner with HIV	96% protection. No linked infections seen when HIV infection stably suppressed by ART over 5-year follow-up	Suppression of viremia on therapy ensured by routine monitoring

ART, Antiretroviral therapy; *CI,* confidence interval; *FTC,* emtricitabine; *HIV,* human immunodeficiency virus; *TDF,* tenofovir disoproxil fumarate; *TFV,* tenofovir.
Modified from Kashuba ADM, Patterson KB, Dumond JB, et al. Pre-exposure prophylaxis for HIV prevention: how to predict success. Lancet. *2012;379:2409–2410.*

adherence was greater than 90%, but adherence rates to the active drug were less than 40% among women in FEM-PrEP and approximately 30% among women in VOICE when based on plasma tenofovir levels.[115,131] Furthermore, more than half of the women in VOICE had no tenofovir detected in plasma during any study visit, suggesting that most women in this trial were not taking the provided PrEP drug at all.[115] In Partners PrEP, by contrast, 82% of participants who did not acquire HIV had detectable plasma tenofovir levels; detectable tenofovir levels were associated with 86% to 90% risk reduction in HIV acquisition.[128] Similarly, in TDF2, 80% of participants who did not acquire HIV had detectable plasma TDF levels, and participants who did not acquire HIV were more likely than participants who did to have detectable study drug.[129] In BTS, 66% of participants overall had detectable plasma tenofovir levels; detectable levels were associated with a 70% risk reduction in HIV acquisition.[130]

Although adherence is critical, there may be biologic differences among men and women that explain some of the discrepant findings in the pivotal PrEP clinical trials. For instance, PrEP efficacy in FEM-PrEP and VOICE differed from that in iPrEx (conducted among men and transgender women), despite similar proportions of participants with detectable study drug. Poor adherence may have greater consequences for women than men in the context of PrEP due to sex differences in tissue levels of drug. Specifically, concentrations of TDF are up to 100-fold higher in rectal tissue than vaginal tissue after TDF administration,[134–136] suggesting that higher levels of adherence (six or seven doses per week) to daily TDF/FTC–based PrEP may be necessary for protection from vaginal exposure.[137] Concentration thresholds for tenofovir or its metabolites in various biomatrices that correlate with protection from HIV infection have been estimated for MSM based on pharmacokinetics modeling in conjunction with incidence data from PrEP studies. For instance, a regimen of four doses a week of TDF/FTC seems to provide 96% protection against HIV for MSM.[138,139] Similar data to allow for analogous modeling studies in women are urgently needed. Finally, although bacterial vaginosis may impact concentrations of 1% tenofovir gel in the vaginal tract, pathogenic species of bacterial vaginosis do not affect tenofovir concentrations in the cervicovaginal tract from oral TDF/FTC–based PrEP,[140] allaying concerns that disruptions in vaginal flora may impact the efficacy of PrEP in women.[141]

REPRODUCTIVE CARE OF WOMEN LIVING WITH HIV

Women living with HIV have long been stigmatized against childbearing,[142] and such stigma is still lingers today.[143] On the one hand, health care providers of women living with HIV should discuss reproductive desires at every patient visit to ensure that they are supporting those desires. On the other hand, because more than half of all pregnancies among women with HIV in the United States are unintended and contraception is underused,[22] it is important that comprehensive family planning and preconception care be integrated into routine health visits. Providers should initiate and document a nonjudgmental conversation with all women of reproductive age concerning their reproductive desires because women may be reluctant to initiate this discussion.[144,145] All women should be offered effective and appropriate contraceptive methods to reduce the likelihood of unintended pregnancy, including hormonal contraception and intrauterine devices, and offered emergency contraception as appropriate.[146] Providers should be aware of potential interactions between ARV medications and hormonal contraceptives that could lower contraceptive efficacy (Table 126.3). For women who desire pregnancy, providers should discuss options for safer conception, as discussed next.

Conception for Women Living With or at Risk of HIV

Reproductive Desires

With the improved clinical outlook of individuals with HIV, women living with HIV are increasingly interested in having children. In a national survey of people with HIV under care in the United States in 1998, 28% to 29% desired children in the future; among respondents desiring children, 69% of women and 59% of men expected to have one child or more in the future.[147] In a survey of women living with HIV under care in an American university HIV clinic in 2004, most of the 118 women (91%) had borne children, and one-third wanted to have children in the future, suggesting that women with HIV have reproductive patterns similar to HIV-negative women.[148] In a cross-sectional survey conducted by telephone in 2006 to 2007, 61% of the 700 women with HIV on ART believed they could safely have children, although 59% of them thought that society strongly urged them not to have children.[143] More recent studies have suggested that among women receiving HIV care, fertility intent may be higher than these prior estimates.[149–151]

Fertility Issues

Factors associated with fertility desires and intentions in women living with HIV are listed in Table 126.4. Reduced fertility among women with HIV has been observed in both developed and developing countries. Lower fertility rates may be due to reproductive choices, previous infection with other STIs, an effect of HIV infection or induced immunosuppression (i.e., anovulation, effect on sperm, higher rates of spontaneous abortion), and decreased sexual activity. The decrease in the conception rate in three African cities due to the HIV epidemic was estimated to be 16% to 26%.[152] In one study of infertile women with HIV and their partners, the women often presented with unexplained tubal occlusions and the men had abnormal semen parameters.[153] Anovulation is another relatively frequent cause of infertility and is associated with low $CD4^+$ counts, high HIV RNA levels, and a history of substance use.[154] Among concordant couples, the male infertility factor may also be important. Men with advanced HIV infection are more likely to have low testosterone levels and abnormal semen analyses compared with HIV-negative control subjects.[154] A significant positive correlation between sperm count and $CD4^+$ count has been observed.[155] However, as ART becomes increasingly available, these differences in fertility among HIV-positive and HIV-negative women and men are narrowing, indicating ART might improve fertility among women living with HIV.[156,157]

If fertility treatment is required, a limited amount of data have shown that in vitro fertilization (IVF) outcomes in couples with HIV, including clinical pregnancy and live birth rates, are comparable to couples without HIV and with no cases of horizontal or vertical transmission.[158,159] HIV infection does not appear to affect ovarian response among women with HIV who are otherwise healthy,[160] and there is no evidence that HIV infection influences ovarian aging.[161] Most fertility centers are open to assisting HIV-infected couples or individuals with conception.[162] The guidelines from the American Society for Reproductive Medicine published in 2015[163] state that "there is no ethical reason to withhold fertility services at clinics with the necessary resources to provide care to HIV-infected individuals and couples who are willing to use recommended risk-reducing therapies. Clinics without sufficient resources to offer care should assist in referral to providers equipped to manage such patients."[164]

Safe Conception

Serostatus-discordant couples attempting conception have several options for strategies to prevent sexual transmission of HIV. Couples should be counseled on HIV transmission risks and prevention strategies and come to an agreement using shared decision making. Most importantly, the infected partner should receive ART and achieve optimal virologic suppression before attempting conception. If the man is living with HIV and the woman is not, donor sperm insemination can also be offered. Other options for preventing HIV transmission but attempting conception include (1) timed intercourse around the time of ovulation to minimize HIV exposure (sexual intercourse without a condom limited to the 2–3 days before and the day of ovulation [peak fertility])[146]; (2) the use of PrEP with TDF/FTC for the uninfected member of the couple; (3) intrauterine or intravaginal insemination if the woman is living with HIV and the man is not; and (4) sperm washing if the man is living with HIV, ideally once he has achieved nondetectable plasma HIV RNA levels.[165] Sperm washing is based on the premise that HIV-infective material exists primarily in the seminal fluid and not within sperm cells. The sperm have no receptors for HIV, in contrast to the plasma and nonsperm cells, which may contain virus. The technique

TABLE 126.3 Drug Interactions Between Antiretroviral Agents and Hormonal Contraceptives

ARV DRUG	EFFECT ON CONTRACEPTIVE DRUG LEVELS AND CONTRACEPTIVE EFFECTS ON ART AND HIV	CLINICAL STUDIES	DOSING RECOMMENDATION/CLINICAL COMMENT FOR CONTRACEPTION OPTIONS
EFV	COC • No effect on EE concentrations • ↓ active metabolites of norgestimate LN AUC ↓ 83%; norelgestromin AUC ↓ 64% • Etonogestrel (in COC) C24 ↓ 61% DMPA • No effect on DMPA levels Etonogestrel implant • Etonogestrel AUC ↓ 63%–82% LN Implant • LN AUC ↓ 47% • LN (emergency contraception) AUC ↓ 58% Changes in ARV levels and/or effects on HIV COC • No effect on EFV concentrations • EFV C12 ↓ 22%; was under therapeutic threshold in 3/16 subjects DMPA • No effect on HIV disease progression • No effect on EFV concentrations LN implant • No effect on HIV disease progression	COC • Pregnancy rates no difference • Pregnancy rate higher (13%) in women using COCs and EFV vs. COCs alone • Progesterone >3 (surrogate for ovulation) in 3/16 • No ovulations DMPA • No increase in pregnancy • Low progesterone Etonogestrel implant • Pregnancy rate higher with EFV vs. no ART but still lower than other hormonal methods • Presumptive ovulation in 5% LN implant • 12% pregnancy rate • 15% pregnancy rate • Pregnancy rate higher with EFV vs. no ART but still lower than other hormonal methods	Consider alternative method (or a reliable method of barrier contraception) in addition to COC/POP/implants No additional contraception needed with DMPA[a]
ETR	EE: AUC ↑ 22% NE: No significant effect	COC • No ovulations	No additional contraceptive protection is needed
NVP	EE: AUC ↓ 29% and no change in 2 different studies NE: AUC ↓ 18% Etonogestrel (in COC) C24 decreased 22% DMPA: No significant change LN implant • LN AUC ↑ 35% Changes in ARV levels and/or effects on HIV COC • NVP no significant effect DMPA • No effect on HIV disease progression LN implant • No effect on HIV disease progression	COC • No increase in pregnancy rate • No ovulation DMPA • No increase in pregnancy rate • No ovulations Etonogestrel implant • No increase in pregnancy rate LN implant • No increase in pregnancy rate	No additional contraceptive protection is needed
RPV	EE: AUC ↑ 14% NE: No significant change Changes in ARV levels and/or effects on HIV COC • No change in RPV levels vs. historical controls	COC • No change in progesterone	No additional contraceptive protection is needed
ATV/r	EE: AUC ↓ 19% Norgestimate: AUC ↑ 85% POP • NE AUC ↑ 50%	N/A	No additional contraceptive protection is needed
DRV/r	EE: AUC ↓ 44% NE: AUC ↓ 14%	N/A	Consider an alternative method (or a reliable method of barrier contraception) in addition to COC/POP/implants No additional contraception needed with DMPA[a]
LPV/r	EE AUC ↓ 55% NE AUC ↓ 17% Patch • EE AUC ↓ 45% • Norelgestromin AUC ↑ 83% DMPA • AUC ↑ 46% Etonogestrel implant • Etonogestrel AUC ↑ 52% Changes in ARV levels and/or effects on HIV Patch • LPV/r level ↓ 19% DMPA • No effect on HIV disease progression • LPV/r no change	COC • Increased pregnancy rate, but CIs overlap Patch • No ovulations DMPA • No pregnancies, no ovulations • Increased pregnancy rate, but CIs overlap Etonogestrel implant • No increase in pregnancy rate LN implant • No increase in pregnancy rate	No additional contraceptive protection is needed
SQV/r	↓ EE Changes in ARV levels and/or effects on HIV COC • SQV/r no change	N/A	Consider alternative method (or reliable method of barrier contraception) in addition to COC/POP/implants No additional contraception needed with DMPA[a]
ATV	COC • EE AUC ↑ 48% • NE AUC ↑ 110%	N/A	Prescribe oral contraceptive that contains no more than 30 μg of EE, or recommend alternative contraceptive method No additional contraception needed with DMPA/POP/implants

TABLE 126.3 Drug Interactions Between Antiretroviral Agents and Hormonal Contraceptives—cont'd

ARV DRUG	EFFECT ON CONTRACEPTIVE DRUG LEVELS AND CONTRACEPTIVE EFFECTS ON ART AND HIV	CLINICAL STUDIES	DOSING RECOMMENDATION/CLINICAL COMMENT FOR CONTRACEPTION OPTIONS
ATV/c	Drospirenone AUC ↑ 2.3-fold EE AUC ↓ 22%	N/A	**Contraindicated** with drospirenone-containing hormonal contraceptive due to potential for hyperkalemia Consider alternative or additional contraceptive method
DRV/c	Drospirenone AUC ↑ 1.6-fold EE AUC ↓ 30%	N/A	In combination with drospirenone-containing COCs, clinical monitoring is recommended due to potential for hyperkalemia Consider alternative or additional contraceptive method
IDV	COC • EE AUC ↑ 22% • NE AUC ↑ 26%	COCs • No pregnancies among women taking IDV and COCs	No additional contraceptive protection is needed
NFV	COC • EE AUC ↓ 47% • NE AUC ↓ 18% DMPA • No change NFV • AUC ↓ 18%	COCs • 1 small study suggested that women using COCs and NFV may have had higher pregnancy rates than women using COCs alone DMPA • No pregnancies, no ovulations • $CD4^+$ count/HIV RNA: no change	Can consider alternative method (or reliable method of barrier contraception) in addition to this method No additional contraception needed with DMPA[a]
MVC	COC • No significant effect on EE or LN	N/A	No additional contraceptive protection is needed
RAL	COC • EE no change • Norgestimate AUC ↑ 14%	N/A	No additional contraceptive protection is needed
DTG	COC • No significant effect on norgestimate or EE • DTG AUC no change	N/A	No additional contraceptive protection is needed
EVG/c	EVG/COBI/FTC/TDF COC • Norgestimate AUC ↑ 126% • EE AUC ↓ 25%	N/A	No additional contraceptive protection is needed

[a]Because the hormonal levels achieved with DMPA are substantially higher than are required for contraception, any small reduction in hormonal level due to ARVs is unlikely to reduce contraceptive effectiveness.

ART, Antiretroviral therapy; *ARV,* antiretroviral; *ATV,* atazanavir; *ATV/c,* atazanavir/cobicistat; *ATV/r,* atazanavir/ritonavir; *AUC,* area under the curve; *CHC,* combination hormonal contraceptives; *CI,* confidence interval; *COBI,* cobicistat; *COC/P/R,* combined oral contraceptives/patch/ring; *DMPA,* depot medroxyprogesterone acetate; *DRV/c,* darunavir/cobicistat; *DRV/r,* darunavir/ritonavir; *DTG,* dolutegravir; *EE,* ethinyl estradiol; *EFV,* efavirenz; *ETR,* etravirine; *EVG,* elvitegravir; *EVG/c,* elvitegravir/cobicistat; *HIV,* human immunodeficiency virus; *IDV,* indinavir; *LN,* levonorgestrel; *LPV/r,* lopinavir/ritonavir; *MVC,* maraviroc; *N/A,* no data available; *NE,* norethindrone; *NFV,* nelfinavir; *NVP,* nevirapine; *PI,* protease inhibitor; *PI/r,* ritonavir boosted-protease inhibitor; *POP,* progesterone-only oral contraceptive pills; *RAL,* raltegravir; *RPV,* rilpivirine; *RTV,* ritonavir; *SQV/r,* saquinavir/ritonavir; *TDF,* tenofovir disoproxil fumarate.

Data from Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in adults and adolescents living with HIV. Department of Health and Human Services. http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf; and Department of Health and Human Services. Recommendations for the use of antiretroviral drugs in pregnant women with HIV infection and interventions to reduce perinatal HIV transmission in the United States. Updated November 2017. http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf.

TABLE 126.4 Factors Associated With Fertility Desires and Intentions in Women Living With HIV Infection

POSITIVE INFLUENCES	NEGATIVE INFLUENCES
Younger age	Personal health concerns
No children	Having had one or more children
Antiretroviral therapy	Concerns about infecting partner
Interventions to prevent mother-to-child transmission	Concerns about infecting child
Partner's/family members' wish for children	Negative or judgmental attitudes of health care workers, family, and community
HIV-related stigma	HIV-related stigma
New male partner	Fear of disclosure

Modified from Hoyt MJ, Storm DS, Aaron E, et al. Preconception and contraceptive care for women living with HIV. Infect Dis Obstet Gynecol. *2012;2012:604183.*

uses density-gradient fractionation to separate sperm from seminal plasma and nonsperm cells.[166] However, with the increasing availability of potent ART regimens and higher rates of HIV viral suppression on effective ART, the use of sperm washing, given its expense and the need for specialized expertise, is rapidly waning. Finally, the risk of HIV superinfection or infection with a resistant virus is negligible when both partners are on ART and have fully suppressed plasma viral loads.[146,167]

PrEP with oral TDF/FTC for uninfected partners is an additional strategy to decrease the risk for HIV sexual transmission among couples trying to conceive. In a prospective study of 53 serostatus-discordant couples (man with HIV) opting to use only timed intercourse with or without PrEP, the pregnancy rate after natural conception was 26% for the first attempt and increased to 75% after 12 attempts.[168] No cases of HIV transmission occurred. To participate in this study, the male partner had to have undetectable HIV RNA in the plasma (<50 copies/mL), no reports of STIs, and no unprotected sex with other partners. Luteinizing hormone in the urine was tested to determine the optimal time of conception, and oral PrEP with TDF alone was given at the luteinizing hormone level peak and 24 hours later. Fertility evaluations were suggested after six unsuccessful attempts, and advanced age in the female partner was found to be a predictor for infertility.[168]

Contraception for Women With or at Risk of HIV

Protection against STIs is a critical aspect to consider when determining the optimal contraceptive plan for women living with or at risk of HIV. The most effective methods to prevent forward transmission of both STIs and HIV in women living with HIV are barrier methods. Although individuals with HIV have relied on barrier methods for the prevention of STIs, including HIV, WHO categorizes male and female condoms as only somewhat effective contraceptives.[169] Male condoms also require partner cooperation, so dual methods are recommended for optimal birth control among women with or at risk of HIV.

Impact of Hormonal Contraceptives on HIV Progression

At the present time, available highly effective reversible birth control options include hormonal methods such as the pill, vaginal ring, transdermal patch, implant, and levonorgesteral intrauterine device (IUD) and the nonhormonal copper IUD. Technologies for a dual purpose microbicide (dapivirine) and contraceptive (levonorgesteral) vaginal ring are in development as summarized in "Strategies to Prevent Heterosexual Transmission." In terms of the question of HIV disease progression in women with HIV using contraceptives, a systematic review identified 13 high-quality studies that evaluated the risk of HIV disease progression and hormonal contraception use.[170] Only 1 of the 13 studies demonstrated an increased risk of disease progression with concomitant use of hormonal contraceptives. A secondary analysis from a randomized trial of 595 women showed an increased risk of declining $CD4^+$ count among 302 women randomly assigned to hormonal contraceptives (DMPA or combined oral contraceptive [COC] pills) compared with 293 women randomly assigned to the copper IUD (HR, 1.56; 95% CI, 1.08–2.26 for DMPA; HR, 1.69; 95% CI, 1.09–2.64 for COC).[171] However, loss to follow-up and changing contraceptive method were common; therefore the intent-to-treat analysis failed to show an association between hormonal contraception and HIV disease progression.[171] Conversely, a more recent and larger study in a prospective cohort of 2269 women with HIV in sub-Saharan Africa found that the use of injectable contraceptives (DMPA and norethisterone enanthate), but not COCs, was associated with a lower likelihood of disease progression, defined as a composite measure of ART initiation, $CD4^+$ count decreasing to less than 200 cells/mm^3, or atraumatic death (adjusted HR, 0.74; $P = .04$ for injectable contraceptive users; adjusted HR, 0.83; $P = .5$ for COC users).[172] Taken together, these data do not indicate a serious concern for the hastening of HIV disease progression with hormonal contraceptive use.

Drug-Drug Interactions Between ART and Hormonal Contraceptives

Another potential concern raised with hormonal contraceptives and HIV is the potential for drug-drug interaction between the hormonal contraceptive and ARVs, resulting in a decrease in systemic ART exposure or exogenous hormone concentrations. A few pharmacokinetics studies have found that exogenous hormone exposure influenced ART exposure. One study found statistically lower efavirenz (EFV) concentrations when given with an oral contraceptive pill containing ethinyl estradiol/desogestrel.[173] Another study of the contraceptive transdermal patch (ethinyl estradiol/norelgestromin) plus lopinavir/ritonavir–based ART identified significantly lower ritonavir exposures.[174] Also, one study observed slightly higher nevirapine (NVP) and lower nelfinavir exposure when these ARVs were combined with DMPA.[175] Despite these statistically significant changes, the changes in exposures were not lower than 80% of previously observed concentrations, and therefore the changes are unlikely to be clinically significant. In addition, most studies of drug interactions between more modern ARVs currently in use and hormonal contraceptives do not show a significant lowering in exposure to the former with the latter.[176] Based on these data, the impact of hormonal contraceptives on ART exposure, if any, is small and unlikely to be clinically significant. Overall, the available data indicate that hormonal contraceptives do not impact the rate of disease progression for women living with HIV, either by primary impact on HIV disease or by secondary impact on ART pharmacokinetics.

However, clinicians need to be aware of the potential for pharmacologic interactions between most protease inhibitors and NNRTIs with both the ethinyl estradiol and the progestin components of hormonal contraceptives, as shown in Table 126.3.[22,176] Both ethinyl estradiol and progestins are metabolized by the cytochrome P-450 (CYP) enzyme system. Generally, protease inhibitors are CYP inhibitors, and NNRTIs are CYP inducers, possibly leading to increasing hormone exposure and reduced hormone exposure, respectively. A reduced progestin exposure could impact contraceptive efficacy, as the contraceptive effect is primarily due to the progestin. An increase in progestin exposure is generally well tolerated. Reduced ethinyl estradiol exposure could lead to an increased amount of breakthrough bleeding and thus might lead to reduced adherence to the contraceptive but would not primarily impact contraceptive efficacy. Increased ethinyl estradiol exposure could lead to increased side effects such as breast tenderness, headache, and nausea, which could similarly negatively impact contraceptive adherence. More concerning, increased estrogen exposure is related to estrogen-induced hepatic production of clotting factors and subsequent thrombosis-related complications such as venous thromboembolism, myocardial infarction, and cerebrovascular accident. Moreover, increases in the circulating levels of angiotensinogen with increased estrogen exposure could lead to increases in blood pressure. These effects are largely theoretical, and widespread tolerability concerns due to the effects of ART on increasing hormonal contraceptive levels have not been observed.

In terms of ART and the effectiveness of contraceptives, to date only EFV-based ART has been reported to lead to clinically significant drug-drug interactions that decrease the effectiveness of hormonal contraceptives (Table 126.3).[22,176] This concern is most relevant to levonorgestrel-based and etonogestrel-based implants, but not DMPA. Two studies have shown that DMPA in conjunction with selected protease inhibitor–based and NNRTI-based regimens remains efficacious in suppressing ovulation with clinically insignificant changes in medroxyprogesterone acetate levels and with no significant changes in ARV levels.[175,177,178] However, two pharmacokinetics studies evaluated levonorgestrel and etonogestrel subdermal implants in Ugandan women living with HIV on EFV-based ART, on NVP-based ART, and on no ART.[179,180] In both studies, the researchers found a significant drop in the geometric mean for both levonorgestrel and etonogestrel concentration levels over the first 6 months of having the implant placed when women were on EFV-based ART. In the first study, three pregnancies occurred within the first year of having the levonorgestrel implant placed (3/20 = 15%) in women on EFV-based ART, with no pregnancies or significant drops in levonorgestrel levels in the NVP-based or no-ART arms.[179] Another study followed a large cohort of 24,560 women contributing 37,635 years of follow-up with 3337 incident pregnancies in East Africa.[181] In women using implants, adjusted pregnancy incidence was three times higher for women on EFV-based ART than for women on NVP-based ART (1.1 per 100 person-years [95% CI, 0.72–1.5] for NVP-based ART users; 3.3 per 100 person-years [95% CI, 1.8–4.8] for EFV-based ART users [adjusted incidence rate ratio, 3.0; 95% CI, 1.3–4.6]). In women on DMPA, the adjusted pregnancy incidence was not significantly different for women on either EFV-based ART or NVP-based ART. However, overall, women on implants had lower contraceptive failure rates than women receiving all other contraceptive methods except for IUDs; the overall conclusion was that effective contraceptive options including implants should be made available for women worldwide.[181]

In terms of the interaction between EFV and emergency contraception, oral levonorgestrel is the hormone in Plan B, the most prevalent over-the-counter emergency contraception option for women. Levonorgestrel levels appeared to drop after administration of EFV and Plan B.[182] Although the minimal effective concentration of levonorgestrel for emergency contraception is unknown, the pharmacokinetics information suggests that higher doses (greater than the standard 1.5 mg) might be needed in women taking EFV to prevent pregnancy. A study in the AIDS Clinical Trials Group (ACTG) is underway to study this question.

Other non–EFV-based ARVs do not have significant drug-drug interactions with hormonal contraceptives. Maraviroc is a substrate of

CYP3A enzymes, but it is neither an inducer nor an inhibitor of the CYP3A system, and no significant interactions with hormonal agents have been demonstrated.[176] Likewise, no clinically significant interactions between hormonal contraceptives and unboosted integrase inhibitors including raltegravir or dolutegravir have been noted. However, hormonal therapies with at least 30 µg ethinyl estradiol are recommended when taking elvitegravir and the booster cobicistat (COBI), a CYP3A4 inhibitor, because of the COBI effect of the combination on ethinyl estradiol levels.[176] One study investigated interactions between the transdermal contraceptive patch (ethinyl estradiol/norelgestromin) and lopinavir/ritonavir.[174] The 45% decrease in the area under the curve of ethinyl estradiol from this study with the patch was comparable to results from interaction studies with oral hormonal contraceptive therapies.[174] To date, pharmacokinetics studies between the contraceptive intravaginal ring and ARV agents have not been published.

The IUD, both copper and levonorgestrel, is widely used internationally and increasingly being used in the United States. Both of these methods are safe and effective in women with HIV and are not associated with HIV transmission.[183–185] The levonorgestrel-releasing intrauterine system has been shown to be safe, effective, and well tolerated and to reduce menstrual bleeding in HIV-infected women.[186,187] There are no known pharmacokinetics interactions between the levonorgestrel-releasing IUD and ART.[176] IUDs do not influence genital tract HIV RNA shedding, and their use among HIV-infected women is not associated with increased risk for transmission of the virus to sexual partners.[185,187,188] WHO MEC for contraceptive use has a level 3 recommendation (use of IUD is not usually recommended unless other more appropriate methods are not available or not acceptable) for women with WHO stage 3 or 4 clinical disease, but for all other women infected with HIV, including women with mild HIV disease (WHO stage 1 or 3), IUD use falls into WHO level 2 recommendation (the method can generally be used because the benefits of the method outweigh the risks).[169]

HIV AND PREGNANCY

Guidelines on Use of Antiretroviral Agents in Pregnant HIV-Infected Women

Since mid-1997, the US Department of Health and Human Services (DHHS) Panel on Antiretroviral Guidelines for Adults and Adolescents has issued recommendations for the use of ARV agents in adults living with HIV. These recommendations are updated on a regular basis, with the most recent information available on the AIDS*info* website, a web-based service of DHHS (http://AIDSinfo.nih.gov).[189]

The DHHS Perinatal Guidelines panel revises its recommendations for the use of ARV drugs in pregnant women living with HIV on an ongoing basis, and the recommendations are also fully accessible online.[22] Although there are no controlled clinical trials demonstrating the safety or efficacy of most ARV agents during pregnancy, both panels recommend that ART in pregnant women be the same as for nonpregnant women, unless clear fetal or maternal contraindications exist. Combination ARV regimens both to benefit the health of the mother and to prevent perinatal HIV transmission should be provided to all pregnant women living with HIV, regardless of $CD4^+$ count and HIV RNA copy number. The greatest risk factor for perinatal transmission is maternal HIV viral load, which is why reducing HIV viral loads to undetectable via effective ART benefit both the pregnant mother and the unborn fetus.[146]

The DHHS Perinatal Guidelines state that pregnancy is not a reason to defer standard suppressive ART, but the guidelines outline unique considerations for ART use including potential need to alter dosing as a result of physiologic changes associated with pregnancy, potential for adverse short-term or long-term effects on the fetus and neonate, and efficacy in reducing the risk for perinatal transmission.[146] The current pharmacokinetics and toxicity data in human pregnancy and recommendations for use in pregnancy for approved ARV agents are presented in Table 126.5.[22]

Perinatal Transmission: Timing, Risk Factors, and Strategies to Eliminate

Before the use of ARV medications, estimates of the frequency of perinatal transmission ranged from 13% in Europe to more than 60% in Africa, with frequencies of 14% to 33% reported in the United States.[190] As the number of women who became infected with HIV during their childbearing years increased, so would the number of children infected perinatally unless access to ART could be ensured to prevent maternal-to-child transmission. Effective ARV medications for PMTCT can reduce perinatal HIV transmission to less than 1% in the absence of breastfeeding and to less than 5% by 6 months of age in breastfeeding infants.[191,192] WHO has called for the "virtual elimination" of pediatric HIV infection, and access to ARV agents for PMTCT continues to increase; in 2011, only 57% of pregnant women living with HIV in low-income and middle-income countries were receiving ARVs, but by 2016, 76% of the estimated 1.4 million pregnant women living with HIV globally received ART.[193] A growing number of countries including Armenia, Belarus, Cuba, and Thailand have been formally validated for having eliminated mother-to-child transmission of HIV.[193] Fig. 126.1 in "Update on Perinatal Transmission Rates" under "Worldwide Epidemiology" summarizes the increase of ART access among pregnant women worldwide as tabulated by UNAIDS.[2]

There has been much progress along the road to ART for all people living with HIV, including all pregnant women living with HIV. In 2013, WHO revised guidelines for PMTCT to recommend lifelong three-drug ART for all women regardless of $CD4^+$ count or clinical stage of disease (Option B+).[192] These guidelines override any former recommendations of using ART only if the $CD4^+$ count was a certain value, using ART for women with high $CD4^+$ counts during pregnancy only, or stopping ART after breastfeeding had ceased. Once-daily EFV with two nucleosides such as TDF plus lamivudine (3TC) (or FTC) remains the recommended first-line regimen for pregnant women globally. In the past, EFV had been avoided during pregnancy due to concerns primarily originating from teratogenicity studies in animals. However, data from the Antiretroviral Pregnancy Registry (APR) showed that the rate of birth defects reported in association with EFV is similar to that of other ARVs.[194,195] In the latest iteration of the guidelines, dolutegravir has been listed as an alternative first-line regimen for people living with HIV worldwide, and studies to examine the safety of dolutegravir in pregnancy and for prevention of perinatal transmission are underway.

Outdated recommendations for PMTCT have been studied in cost-effectiveness analyses and examined in relationship to mortality benefit.[191] Although the recommendations by WHO to provide Option B+ (lifelong ART for all pregnant women regardless of $CD4^+$ count) were provided in 2013,[192] the overall treatment guidelines issued by WHO for all adults living with HIV infection changed in 2015.[54] These guidelines stipulate that all people living with HIV—regardless of $CD4^+$ count, sex, pregnancy status, hepatitis B status, etc.—should be on ART. The overwhelming individual-level and public health benefits of putting all people living with HIV on ART led to this change in guidelines, and the concept of varying strategies to prevent perinatal transmission is now fading into the past with this new dictum.

Barriers to ART provision for pregnant women in settings with limited health care resources still exist. These barriers include distance between antenatal care sites where HIV diagnosis is made and ART sites where treatment is started; transportation costs; and human resource constraints that lead to long waiting times, frequent ART stock shortages, and scheduling difficulties. The CDC reported on the implementation of Option B+ in the third quarter of 2011 in Malawi, when the Malawi Ministry of Health began a program in which all pregnant and breastfeeding women with HIV were eligible for lifelong ART regardless of $CD4^+$ status.[196] In Malawi, the number of pregnant and breastfeeding women started on ART per quarter increased by 748%, from 1257 in the second quarter of 2011 before program implementation to 10,663 in the third quarter of 2012, which was 1 year after implementation of Option B+ policy.[196] More than 77% of women in Malawi started on ART while pregnant or breastfeeding continued on ART 1 year later, a finding similar to that of the national ART program. The removal of the barrier of requiring $CD4^+$ counts, decentralization of ART into all antenatal care sites, and training of nearly all nurses and clinical officers on the new integrated PMTCT/ART guidelines greatly facilitated the increase in the number of pregnant and breastfeeding women started on ART in Malawi. Option B+ as well as the parent policy of having all people living with HIV worldwide on ART has been an important innovation

TABLE 126.5 Antiretroviral Drug Use in Pregnant Women Living With HIV: Pharmacokinetics and Toxicity Data in Human Pregnancy and Recommendations for Use in Pregnancy

ARV DRUG	PK IN PREGNANCY	CONCERNS IN PREGNANCY	RATIONALE FOR RECOMMENDED USE IN PREGNANCY
NRTIs/NtRTIs		Potential maternal and infant mitochondrial toxicity	Used as part of combination regimens; use of single or dual NRTIs alone is not recommended for treatment of HIV infections
Abacavir (ABC)	PK not significantly altered; no change in dose indicated High placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects); hypersensitivity reactions occur in approximately 5%–8% or more of nonpregnant persons; rate in pregnancy is unknown; previous screening for HLA-B5701 should be done and documented as negative before starting ABC	One of several preferred NRTIs for dual nucleoside backbone of combination regimens in pregnancy ABC *should not be used* in patients who test positive for HLA-B*5701 because of risk of hypersensitivity reaction Abacavir/3TC is preferred NRTI combination for use in pregnancy Patients should be educated regarding symptoms of hypersensitivity reaction
Emtricitabine (FTC)	PK study shows slightly lower levels in third trimester compared with postpartum; no clear need to increase dose High placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects) If HBV coinfection, possible HBV flare if drug is stopped	One of several preferred NRTIs for dual nucleoside backbone of combination regimens in pregnancy Tenofovir/FTC is preferred NRTI combination for use in pregnancy
Lamivudine (3TC)	PK not significantly altered; no change in dose indicated High placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects); well-tolerated, short-term safety demonstrated for mother and infant If HBV coinfection, possible HBV flare if drug is stopped	One of several preferred NRTIs for dual nucleoside backbone of combination regimens in pregnancy ABC/3TC and tenofovir/FTC (or with 3TC) are preferred NRTI combinations for use in pregnancy
Tenofovir alafenamide (TAF)	No data are available on placental transfer of TAF	Insufficient data to assess for teratogenicity in humans; no evidence of teratogenicity in rats Renal function should be monitored because of potential for renal toxicity	
Tenofovir disoproxil fumarate (TDF)	Limited studies in human pregnancy; data indicate AUC lower in third trimester than postpartum, but trough levels adequate High placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects); clinical studies in monkeys show decreased fetal growth and reduction in fetal bone porosity within 2 months of starting maternal therapy Human studies demonstrate no consistent link to low birth weight, but data are conflicting about potential effects on growth outcomes later in infancy If HBV coinfection, possible HBV flare if drug is stopped	One of several preferred NRTIs for dual nucleoside backbone of combination regimens in pregnancy TDF is preferred NRTI in combination with 3TC or FTC in women with chronic HBV infection Because of potential for renal toxicity, renal function should be monitored Tenofovir/FTC (or with 3TC) is preferred NRTI combination for use in pregnancy
Zidovudine (ZDV)	PK not significantly altered; no change in dose indicated High placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects); well-tolerated, short-term safety demonstrated for mother and infant	Alternative NRTI for dual nucleoside backbone of combination regimens (ZDV/3TC) in pregnancy; should not be included in regimen if significant toxicity or d4T use
NNRTIs		Hypersensitivity reactions, including hepatic toxicity, and rash more common in women; unclear whether increased in pregnancy	NNRTIs are recommended for use in combinations with two NRTI drugs
Efavirenz (EFV)	AUC decreased during third trimester compared with postpartum, but nearly all exceeded target exposure levels, and no change in dose is indicated Moderate placental transfer to fetus	Significant malformations (anencephaly, anophthalmia, cleft palate) were observed in 3 (15%) of 20 cynomolgus monkeys receiving EFV during first trimester at dose comparable to human therapeutic exposure FDA advises women to avoid becoming pregnant while taking EFV and to avoid using EFV in first trimester, as fetal harm may occur EFV should be continued in pregnant women receiving virologically suppressive EFV-based regimen, as ARV drug changes during pregnancy may be associated with loss of viral control and increased risk of perinatal transmission	EFV plus a preferred two-NRTI backbone is alternative initial regimen in pregnancy Concern because of birth defects seen in primate study, but data not borne in human studies and extensive experience in pregnancy Preferred regimen in women who require coadministration of drugs with significant interactions with PIs or prefer convenience of coformulated, single-tablet, once-daily regimen Screening for antenatal and postpartum depression is recommended
Etravirine (ETR)	Limited PK studies in human pregnancy; in four pregnant women, drug levels and AUC similar to nonpregnant women, suggesting no dose modification needed Variable placenta transfer, usually in moderate to high categories, 0.19–4.25 (data from 19 mother-infant pairs).	Limited experience in human pregnancy; no evidence of teratogenicity in rats and rabbits	Safety and PK in pregnancy data are insufficient to recommend use during pregnancy Not recommended in ART-naïve populations

TABLE 126.5 Antiretroviral Drug Use in Pregnant Women Living With HIV: Pharmacokinetics and Toxicity Data in Human Pregnancy and Recommendations for Use in Pregnancy[a]—cont'd

ARV DRUG	PK IN PREGNANCY	CONCERNS IN PREGNANCY	RATIONALE FOR RECOMMENDED USE IN PREGNANCY
Nevirapine (NVP)	PK not significantly altered; no change in dose indicated High placental transfer to fetus	No evidence of human teratogenicity Increased risk for a symptomatic hypersensitivity reaction, often with rash, and potentially fatal liver toxicity among women with $CD4^+$ counts >250 cells/mm^3 when first initiating therapy; unclear whether pregnancy increases risk	NVP is not recommended because of greater potential for adverse events, complex lead-in dosing, and low barrier to resistance NVP should be used with caution when initiating ART in women with $CD4^+$ counts >250 cells/mm^3 Use NVP and ABC together with caution; both can cause hypersensitivity reactions within first few weeks after initiation
Rilpivirine (RPV)	Moderate to high placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects)	RPV plus a preferred two-NRTI backbone (RPV/TDF/FTC) is an alternative initial regimen but is not recommended with pretreatment HIV RNA >100,000 copies/mL or $CD4^+$ count <200 cells/mm^3 Do not use with proton-pump inhibitors
PIs		Hyperglycemia, new-onset or exacerbation of diabetes mellitus, and diabetic ketoacidosis reported with PI use; unclear whether pregnancy increases risk Data conflicting regarding risk for preterm delivery in women receiving PIs	PIs are recommended for use in combination with two NRTI drugs
Atazanavir (ATV)/ritonavir (RTV) ATV/cobicistat (COBI)	Two of three intensive PK studies of ATV with RTV boosting during pregnancy suggest that standard dosing results in decreased plasma concentrations compared with nonpregnant adults; ATV concentrations further reduced by approximately 25% with concomitant TDF use Low placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects) Must be given with low-dose RTV-booster regimen during pregnancy Theoretical concern regarding increased indirect bilirubin levels causing significant exacerbation in physiologic hyperbilirubinemia in neonates has not been observed in clinical trials to date	One of preferred PIs for use in combination regimens in pregnancy; must be given with low-dose RTV once daily Package insert recommends increased ATV dosing for ARV-experienced pregnant women in second and third trimester also receiving either TDF or H2 receptor antagonist or ARV-naïve pregnant women receiving EFV ATV should not be used in patients receiving both TDF and H2 receptor antagonist or in ARV-experienced patients also taking EFV
Darunavir (DRV)/RTV	PK study demonstrated plasma concentrations were decreased by 17%–35% with once-daily and twice-daily dosing in third trimester Low placental transfer to fetus	No evidence of teratogenicity in mice, rats, or rabbits. No evidence of human teratogenicity	One of preferred PIs for use in combination regimens in pregnancy when used with low-dose RTV twice daily in pregnancy Better tolerated than LPV/RTV
Lopinavir (LPV)/RTV	AUC decreased in second and third trimesters with standard dosing; PK studies suggest increasing to LPV 600 mg/150 mg bid or LPV 500 mg plus RTV 125 mg bid in second and third trimesters Low placental transfer to fetus More nausea than preferred agents	No evidence of human teratogenicity; abundant experience and established PK in pregnancy	One of alternative PI-boosted regimens for use in combination regimens in pregnancy PK studies suggest need for increased dosing in second and third trimesters If standard dosing is used, monitor virologic response and LPV drug levels, if available Once-daily LPV/RTV is not recommended in pregnancy
RTV (as a single PI)	Phase I/II study in pregnancy demonstrates lower levels during pregnancy compared with postpartum Minimal placental transfer to fetus	No evidence of human teratogenicity (can rule out twofold increase in overall birth defects) Limited experience at full dose in human pregnancy; should be used as low-dose RTV boosting with other PIs	RTV as single PI is not recommended because of inferior efficacy and increased toxicity
INSTIs			
Dolutegravir	High placental transfer to fetus	No evidence of teratogenicity in mice, rats, or rabbits Preliminary data suggest no increased risk of teratogenicity in humans Safety and PK in pregnancy data are limited but increasing experience in pregnancy	One of alternative INSTI-based regimens for use in combination regimens in pregnancy Available as fixed-dose combination with ABC, requiring HLA-B5701 testing, administered once daily In nonpregnant adults, associated with lower rates of INSTI resistance than RAL and therefore suggested for women with acute infection in pregnancy
Elvitegravir (EVG/c (available in fixed-drug combination TDF/FTC/EVG/COBI (brand name: Stribild[b]) and EVG/COBI/TAF/FTC brand name Genvoya[c])	Evidence of high placental transfer of EVG and low transfer of COBI	No evidence of teratogenicity in rats and rabbits with all four components of medications If HBV coinfection present, possible HBV flare if drug stopped postpartum	EVG/c **is not recommended** for initial use in pregnancy For women who become pregnant while taking EVG/c, consider switching to more effective, recommended regimen If EVG/c regimen is continued, viral load should be monitored frequently, and TDM (if available) may be useful

Continued

TABLE 126.5 Antiretroviral Drug Use in Pregnant Women Living With HIV: Pharmacokinetics and Toxicity Data in Human Pregnancy and Recommendations for Use in Pregnancy[a]—cont'd

ARV DRUG	PK IN PREGNANCY	CONCERNS IN PREGNANCY	RATIONALE FOR RECOMMENDED USE IN PREGNANCY
Raltegravir (RAL)	High placental transfer to fetus	No evidence of human teratogenicity (can rule out 1.5-fold increase in overall birth defects)	One of preferred INSTI-based regimens for use in combination regimens in pregnancy Case report of markedly elevated liver transaminases with use in late pregnancy Severe, potentially life-threatening, and fatal skin and hypersensitivity reactions have been reported in nonpregnant adults Has been associated with rapid viral load reduction Useful when drug interactions with PI regimens are a concern Twice-daily dosing required

[a]Approved ARV medications that are toxic in pregnancy or less potent than other available ARV medications and therefore not recommended for use in pregnancy have not been included in this table, including stavudine, didanosine, enfuvirtide, fosamprenavir, indinavir, nelfinavir, saquinavir, tipranavir, and maravoric.
[b]Stribild package insert: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/203100s030lbl.pdf.
[c]Genvoya package insert: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/207561s002lbl.pdf.
3TC, lamivudine; *ABC,* abacavir; *ART,* antiretroviral therapy; *ARV,* antiretroviral; *ATV,* atazanavir; *AUC,* area under the curve; *COBI,* cobicistat; *d4T,* stavudine; *DRV,* darunavir; *EFV,* efavirenz; *ETR,* etravirine; *EVG/c,* elvitegravir/cobicistat; *FDA,* US Food and Drug Administration; *FTC,* emtricitabine; *HBV,* hepatitis B virus; *HIV,* human immunodeficiency virus; *INSTI,* integrase strand transfer inhibitor; *LPV,* lopinavir; *NNRTI,* nonnucleoside reverse-transcriptase inhibitor; *NRTI,* nucleoside reverse-transcriptase inhibitor; *NtRTI,* nucleotide reverse-transcriptase inhibitor; *NVP,* nevirapine; *PI,* protease inhibitor; *PK,* pharmacokinetics; *RAL,* raltegravir; *RPV,* rilpivirine; *RTV,* ritonavir; *TAF,* tenofovir alafenamide; *TDF,* tenofovir disoproxil fumarate; *TDM,* therapeutic drug monitoring; *ZDV,* zidovudine.
Modified from US Public Health Service Task Force. Department of Health and Human Services. Recommendations for the use of antiretroviral drugs in pregnant women with HIV infection and interventions to reduce perinatal HIV transmission in the United States. Updated November 14, 2017. http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf.

that has the potential to accelerate the goal of eliminating mother-to-child transmission of HIV worldwide.

Timing of Perinatal Transmission

HIV can be transmitted from a woman living with HIV to her child during intrauterine gestation, at delivery, or in the postpartum period through breastfeeding. HIV-1 has been isolated from fetal blood samples taken before elective terminations[197] and from fetal tissues at 8 weeks of gestation. In half of the infants who eventually are proven to be HIV-infected, HIV-1 can be identified by culture, polymerase chain reaction, or p24 antigen detection at or shortly after birth, which suggests that they were infected in utero before delivery.[198] HIV-infected infants who test negative for HIV by polymerase chain reaction or culture at birth may have been infected late in pregnancy or during birth. The differences in onset and progression of HIV infection in infants may reflect timing of infection, where infants infected earlier in pregnancy typically have more rapid progression to AIDS than infants infected at birth, who present with asymptomatic HIV infection and have prolonged survival.[199] Perinatal transmission is thought to occur near or during delivery in most cases.[192,200] Twin studies in the pre-ART era were used to further understanding of the timing of perinatal HIV transmission. Goedert and coworkers[201] studied 22 pairs of HIV seropositivity–discordant twins, and in 18 of the 22 pairs, the presenting twin was the infected sibling. Data from the International Registry of HIV-Exposed Twins suggest that the risk for infection in the twin who is born first is twice that in the second-born twin (26% and 13%, respectively).[201] The greater infection rates of first-born twins may be linked to increased contact with maternal secretions during birth.

Risk Factors for Perinatal Transmission

Perinatal transmission is a multifactorial process, influenced by viral, immune, and clinical factors in the mother and the infant (Table 126.6). Maternal plasma viral load is the strongest predictor of the risk for transmitting HIV perinatally.[202] In the pre-ART era, women with more advanced HIV disease and lower $CD4^+$ and higher $CD8^+$ T-cell counts were at increased risk to transmit HIV perinatally.[203,204] In a nested case-control study within a prospectively followed cohort of HIV-infected women, Thea and colleagues[205] found that high maternal viral load increased the likelihood of perinatal transmission in women without AIDS. Primary HIV infection in pregnancy or during breastfeeding associated with acute high-titer HIV viremia also is associated with increased rates of transmission. No threshold has been observed below which transmission does not occur; women with undetectable plasma HIV RNA have transmitted HIV perinatally. In a collaboration of studies, Ioannidis and coworkers[206] identified 44 cases of perinatal HIV-1 transmission among 1202 women with HIV-1 RNA viral loads less than 1000 copies/mL at delivery or at the measurement closest to delivery. The transmission rate was approximately 1% for women on ART, which was significantly lower than the 9.8% rate for untreated mothers. Multiple RNA measurements were obtained during pregnancy or at the time of delivery in 12 cases, of which 10 had a mean viral load greater than 500 copies/mL.[206] However, these are rare events, and perinatal HIV-1 transmission may be almost eliminated with combination ARV prophylaxis accompanied by complete and persistent suppression of maternal viremia.[192,206]

Use of ARV regimens for perinatal prophylaxis that are not completely suppressive (e.g., zidovudine [ZDV] or NVP monotherapy) may result in the development of resistance mutations and possibly increase the risk for perinatal transmission or progression in infected infants. Perinatal transmission of HIV with mutations associated with ZDV resistance has been described.[207] The current Recommendations for Use of Antiretroviral Drugs in Pregnant Women With HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States advise that all pregnant HIV-infected women should receive a combination ARV drug regimen to prevent perinatal transmission regardless of plasma HIV RNA copy number or $CD4^+$ cell count, and women with detectable HIV RNA should have ARV drug resistance studies performed before starting or modifying ART.[22]

Many reports have correlated perinatal transmission with numerous factors, including virologic, immunologic, sociodemographic, and lifestyle factors, which are listed in Table 126.6. Other factors are less well proven such as the role of HIV genotype. HIV-1 can be classified into at least nine different genotypes (clades) based on differences in the envelope region of the viral genome.[190] The distribution of subtypes differs around the world: Subtype B predominates in the United States and Europe; subtypes A, C, and D predominate in Africa; and subtypes B and E predominate in Thailand.[190] The role of subtypes in perinatal transmission is as yet undefined, but some epidemiologic studies have suggested that subtype E may be transmitted sexually more efficiently than subtype B, perhaps accounting for at least some of the differences in transmission rates seen between countries.[208]

In women with HIV who are not virally suppressed, various obstetric factors are believed to be associated with increased perinatal HIV transmission including chorioamnionitis; placenta previa; preterm delivery; and invasive interventions such as scalp monitoring, chorionic villus sampling, amniocentesis, cord blood sampling, and placental biopsy.[209,210]

TABLE 126.6 Potential Factors Influencing Perinatal Transmission of HIV Especially in the Setting of HIV Viremia

Maternal Factors
Lack of effective antiretroviral therapy
Advanced HIV disease as measured by
Clinical staging
Low $CD4^+$ lymphocyte count
Higher viral loads
p24 antigenemia
Primary HIV infection
Viral phenotype: syncytium inducing
Viral genotype: virulent mutant strain of HIV
Coinfection with other sexually transmitted diseases
First-born twins
Obstetric events
Vaginal delivery
Invasive procedures or fetal monitoring during labor
Prolonged premature rupture of membranes (>4 hours)
Older maternal age
Cigarette smoking and illicit drug use during pregnancy
Breastfeeding
Unprotected sexual intercourse with multiple partners
Fetal or Placental Factors
Chorioamnionitis
Prematurity
Low birth weight
Labor or Birth Canal Factors
Cervicovaginal viral load
Local HIV-specific immune response
Maternal-fetal transfusion of blood
Immune Factors
Humoral
Neutralizing antibody
Antibody-dependent cellular cytotoxicity
gp120 V3 loop antibody
MHC concordance and homozygosity
Maternal HLA-A*2301
Other
Cell Mediated
Cytotoxic T lymphocytes
$CD8^+$ suppression
Mucosal immunity
Other

HIV, Human immunodeficiency virus; *MHC,* major histocompatibility complex.
Modified from Sprecher S, Soumenkoff G, Puissant F, et al. Vertical transmission in a 15-week fetus. Lancet. *1986;2:228, © by The Lancet Ltd., 1986; and Bryson YJ. Perinatal HIV-1 transmission: recent advances and therapeutic interventions.* AIDS. *1996;3:533–542.*

In women on suppressive ART, data suggest that invasive procedures such as artificial rupture of membranes, amniocentesis, and episiotomy should be considered, if clinically indicated, as they would be for women without HIV. Promotion of safer sexual practices including condom use and limiting sexual partners and abstaining from drug, tobacco, and alcohol use also may reduce the risk for perinatal transmission.[192]

Use of Antiretroviral Drugs to Decrease Perinatal Transmission

The goals of ART in pregnancy are to optimize maternal health, to provide maximal suppression of the viral load, to prevent perinatal HIV transmission, to prevent horizontal HIV transmission to sexual partners, and to avoid potential maternal or fetal toxicity. This section reviews data on the use of ARV medications in pregnancy and the current guidelines for ART to decrease perinatal HIV transmission. In the subsequent section, the use of ARV medications in pregnancy and the optimal management of pregnant HIV-infected women are addressed.

Combination drug regimens are considered the standard of care for both the treatment of HIV infection and the prevention of perinatal HIV transmission.[146] One of the major achievements in HIV research was the demonstration by the Pediatric AIDS Clinical Trials Group Protocol 076 Study (PACTG 076) in February 1994 that administration of ZDV to the pregnant woman and her infant could reduce the risk for perinatal transmission by 67.5%.[211,212] In PACTG 076, women with $CD4^+$ counts greater than 200 cells/mm^3 were randomly assigned to a ZDV treatment group or to a control group in which placebo was given. The women in the treatment group received 100 mg of ZDV five times daily starting between 14 and 34 weeks of gestation and continued throughout pregnancy, followed by intrapartum ZDV (given as an intravenous loading dose of 2 mg/kg over 1 hour during labor, then a continuous intravenous infusion of 1 mg/kg/h until delivery), with oral administration of ZDV to the infant (syrup 2 mg/kg every 6 hours) for 6 weeks. The rate of perinatal transmission was decreased to 8.3% in the 205 women who received ZDV from a rate of 25.5% in the 204 women who received no ART ($P = .00006$).[211] In August 1994, the US Public Health Service Task Force issued recommendations for the use of ZDV for reduction of perinatal HIV-1 transmission,[213] followed in July 1995 by recommendations for universal prenatal HIV-1 counseling and HIV-1 testing with informed consent for all pregnant women in the United States.[214] Subsequent epidemiologic studies in the United States, France, and other countries with higher level health care resources showed successful use of the PACTG 076 ZDV regimen in the real world coupled with increased antenatal HIV counseling, and testing rapidly demonstrated dramatic reductions in perinatal transmission rates.[146,215,216]

Subsequent clinical trials and observational studies demonstrated that combination ARV prophylaxis (initially dual and then triple combination therapy) given to the mother antenatally was associated with further declines in transmission to less than 2%.[217–219] It is currently estimated that less than 100 infected infants are currently born each year in the United States.[5] However, although new perinatal HIV infections are becoming rare in countries with good access to health care resources, infections continue to occur, and the birth of an infected infant is a sentinel event representing missed opportunities and barriers to prevention of this infection.[146] Important obstacles to eradication of perinatal transmission of HIV in the United States include the continued incidence of HIV infection among women of childbearing age; delayed or lack of prenatal care, particularly in women with substance use or mental health issues; acute (primary) infection in pregnant women and in women who are breastfeeding; poor adherence to prescribed ARV regimens; and lack of full implementation of routine, universal prenatal HIV counseling and testing.[146]

Potential Mechanisms of Antiretroviral Drugs to Reduce Perinatal Transmission

There are several mechanisms through which ARV drugs can reduce perinatal transmission. One important mechanism is by decreasing maternal viral load in the blood and genital secretions via antenatal drug administration, particularly in women with high viral loads. However, ARV drugs have been shown to reduce the risk for transmission even among women with HIV RNA levels of less than 1000 copies/mL.[220] Additionally, the level of HIV RNA at delivery and receipt of antenatal ART are each independently associated with the risk for transmission, suggesting that ARV prophylaxis does not work solely through reduction in viral load.[212,218]

An additional mechanism of protection is preexposure infant prophylaxis provided by administration of ARV drugs that cross the placenta from the mother during labor, resulting in adequate systemic drug levels in the infant at a time of intensive exposure to maternal genital tract virus during passage through the birth canal.[146] Infant PrEP is provided through administration of drug to the infant after birth; this would protect against cell-free or cell-associated virus that might have obtained access to the fetal/infant systemic circulation through maternal-fetal transfusion during uterine contractions occurring in labor or through systemic dissemination of virus swallowed by the infant during passage through the birth canal.[146]

It is likely that efficacy of ARVs in reducing perinatal transmission is multifactorial, and each of these mechanisms is contributory. The efficacy of ARV regimens administered during labor or to the neonate or both in reducing perinatal transmission demonstrates the importance

of the preexposure and postexposure components of prophylaxis in decreasing perinatal transmission.[146,221–223]

Combination ARV regimens are far superior to single-drug regimens in decreasing HIV viral load and perinatal transmission. A longer three-part regimen (e.g., starting at or before 28 weeks of gestation) given antenatally, intrapartum, and postpartum was superior to a shorter two-part antepartum/intrapartum (e.g., starting at 36 weeks of gestation) or intrapartum/postpartum regimen in preventing perinatal transmission; hence current guidelines recommend that all pregnant women with HIV should be treated with combination suppressive ART as soon as possible, and that ART be continued for life.[146] Longer duration of ART in pregnancy has been associated with reduced transmission rates compared with shorter treatment duration.[146,224]

Research on the optimal management of infants born to women with HIV has informed the current Pediatric HIV Guidelines.[225] Data from PACTG 1043 showed that 1746 infants born to women living with HIV who received no ARV prophylaxis during pregnancy were randomly assigned to three infant prophylaxis regimens: the standard 6-week ZDV regimen; 6 weeks of ZDV plus three doses of NVP given during the first week of life (at birth, 48 hours later, and 96 hours after the second dose); and 6 weeks of ZDV plus 2 weeks of 3TC/nelfinavir.[146,217,226] The risk for intrapartum transmission was significantly lower with the two-drug and three-drug arms (2.2% and 2.5%, respectively) compared with ZDV alone (4.9%) ($P = .046$ for each arm compared with ZDV alone).[146] Although transmission rates with the two combination regimens were similar, neutropenia and anemia have been associated with the three-drug regimens, including 3TC in infants.[146,226] In the United States, data from PACTG 316 demonstrated that the addition of a two-dose oral NVP regimen (200 mg intrapartum/2 mg/kg dose to neonates) to the standard antepartum combination ARV regimens used for prophylaxis or treatment in pregnant women is not recommended because it does not appear to provide additional efficacy in reducing transmission and may be associated with the development of NVP resistance.[146,217]

Impact of Pregnancy on HIV Infection

Although both pregnancy and HIV infection are immunosuppressive conditions, large studies in the United States and Europe failed to show that pregnancy accelerates HIV replication or disease progression, even in the pre-ART era.[227–229] Pregnancy does not seem to result in acceleration of HIV disease in most HIV-infected women; however, women with more advanced disease who are not virally suppressed tend to experience disease progression over a shorter period than that typical of women with less advanced disease, suggesting that the pregnancy may enhance progression of HIV disease in such cases.[230]

Impact of HIV Infection on Pregnancy Outcomes

Women taking ART may be at increased risk for adverse pregnancy outcomes including preterm birth or delivery (delivery before 37 weeks of gestation), low-birth-weight infants (<2500 g), and small-for-gestational-age infants (birth weight <10th percentile expected for gestational age).[194] Most studies that showed an association between HIV and adverse pregnancy outcomes involved women not on ARV or starting ART. A large meta-analysis of 11,224 women in 14 European and American studies did not demonstrate, for example, an increased rate of preterm delivery among women using ART during pregnancy.[231] The PROMISE trial compared ZDV alone with lopinavir/ritonavir–based ART combined with a dual nucleoside reverse-transcriptase inhibitor (NRTI) backbone of either ZDV/3TC or TDF/FTC.[232] Compared with women receiving ZDV alone, higher rates of extremely preterm delivery were reported in women receiving ZDV/3TC/lopinavir/ritonavir ($P < .001$), but not in women receiving TDF/FTC/lopinavir/ritonavir ($P = .77$). In contrast, extremely preterm delivery rates were higher among women receiving TDF/FTC/lopinavir/ritonavir than women receiving ZDV/3TC/lopinavir/ritonavir ($P = .04$).[233] These rates of very preterm delivery were not significantly different compared with women receiving ZDV alone ($P = .10$). The potential mechanism of action by which protease inhibitors may increase a woman's risk of preterm delivery is unknown.

Of the 15 studies that have addressed effects of ART on birth weight, 5 demonstrated an association between any ART use and low birth weight.[22] When comparing the initiation of monotherapy in pregnancy versus ART initiated before pregnancy and continued during pregnancy, ART was associated with small gestational weight for age (HR, 1.34; 95% CI, 1.05–1.7).[233] Zash and colleagues[234] reported on an observational birth outcomes surveillance study in Botswana that compared all live births and stillbirths (>24 weeks) with exposure to three-drug ART from conception. Among 11,932 HIV-exposed infants, 5780 (48.4%) were exposed to ART from conception. Adverse birth outcomes were more common among HIV-exposed infants than infants not exposed to HIV (39.6% vs. 28.9%; adjusted relative risk [RR], 1.40; 95% CI, 1.36–1.44). The risk for any adverse birth outcome was lower among infants exposed from conception to TDF, FTC, and EFV compared with other ART regimens including NVP-based and lopinavir/ritonavir–based ART.[234] As ART becomes more widely available for pregnant women in settings with limited health care resources, it will be critical to monitor carefully pregnancy outcomes, including congenital anomalies, preterm birth, stillbirth, and infant mortality, to assess risks and benefits of the different regimens used to treat women living with HIV and maximize HIV-free survival in infants.[22]

Antepartum Care of Women Living With HIV

Medical care of the pregnant woman living with HIV requires coordination and communication among prenatal care providers, primary and HIV specialty care providers, obstetric providers, and other needed service providers including mental health and drug abuse treatment services and public assistance programs.[146] An initial evaluation of a pregnant woman living with HIV should include an assessment of HIV disease status and recommendations regarding initiating, continuing, or modifying ART (see Chapter 128). In addition to the usual antenatal assessments, this assessment should include evaluating past and current $CD4^+$ count, current plasma HIV RNA copy number, past and current ART, and previous ARV drug use in pregnancy; assessing the need for prophylaxis against opportunistic infections such as *Pneumocystis jirovecii* pneumonia or *Mycobacterium avium* complex; and reviewing results of previous and current HIV ARV drug resistance studies, prior HLA B5701 assays, and assessing supportive care needs.[22] The history should identify any factors known to be associated with enhanced perinatal transmission such as a history of STIs, drug and alcohol use, tobacco use, and high-risk sexual activity including lack of condom use, as altering these practices may decrease perinatal transmission risk. A complete physical examination including a pelvic examination can reveal concurrent conditions that may warrant therapy. Plasma HIV RNA levels of pregnant women with HIV should be monitored at the initial visit, 2 to 4 weeks after initiating or changing ARV medications, monthly until RNA levels are undetectable, and then at least every 3 months during pregnancy.[146] HIV RNA levels also should be assessed at approximately 34 to 36 weeks of gestation to inform decisions about mode of delivery and about optimal treatment of the newborn (see Chapter 127). $CD4^+$ count should be monitored at the initial antenatal visit and every 3 to 6 months during pregnancy. HIV drug resistance studies should be performed before starting ARV regimens in all ARV-naïve women whose HIV RNA levels are above the threshold for resistance testing (i.e.>500 to 1000 copies/mL) or who have suboptimal virologic response to ART started during pregnancy; however, therapy should not be delayed while waiting for resistance testing results. ART may need to be modified based on resistance assay result. Ongoing routine obstetric care and decisions about initiating, continuing, or adjusting ART should be the same as for nonpregnant women living with HIV.

DHHS guidelines recommend advising women living with HIV in the United States and other regions with good health care resources where safe alternative infant feedings exist to refrain from breastfeeding to avoid postnatal transmission of HIV to their infants through breast milk.[235] Women living with HIV should also avoid premastication of food for their infants, a potential risk factor for transmission.[236,237]

Decisions regarding the use and choice of an ARV regimen should be individualized based on the woman's health, previous treatment, and known or suspected drug resistance. The known benefits and known

and unknown risks of such therapy during pregnancy should be considered and discussed (see Table 126.5). Risks of these drugs during pregnancy should be placed in perspective by also discussing benefits of ART for the health of the infected woman and for reducing the risk for HIV transmission to her infant. The importance of adherence to ART should be emphasized. Depending on individual circumstances, provision of support services, mental health services, and drug abuse treatment may be essential to ensure adherence of the infected woman to ART regimens and ultimate success of ART for her own health and for preventing perinatal transmission.[22]

Although the risk for teratogenicity is greatest during the first 10 weeks of gestation, data to date do not support major teratogenic effects of any of the ARV drugs.[146] In most cases, women who present for obstetric care on fully suppressive ARV regimens should continue their current regimens. All pregnant women should initiate ART as soon as possible, including in the first trimester. The clinical, immunologic, and virologic status of the mother must be weighed against the potential effect on the fetus. Discontinuing ARV medications may result in a rebound of viral load, however, which may have an impact on HIV transmission, disease progression, and subsequent ability to obtain rapid virologic control. Although the rates of HIV infection are low in women with undetectable or low HIV RNA levels, there is no threshold below which the lack of transmission can be ensured.[146] Dual combination therapy (i.e., ZDV/3TC) without the addition of a third drug (i.e., protease inhibitor, NNRTI, or integrase strand inhibitor) is not recommended because of the potential for inadequate viral suppression and rapid development of resistance, most commonly the M184V mutation.[146,238]

HIV-Infected Pregnant Women Who Have Never Received Antiretroviral Drugs

All pregnant women living with HIV should receive potent combination ART, regardless of $CD4^+$ count or plasma HIV RNA copy number, that generally consists of two NRTIs plus an integrase strand inhibitor, NNRTI, or boosted protease inhibitor, with consideration regarding continuing the ARV regimen after delivery being the same as for nonpregnant individuals (Table 126.7).[189] Studies of ZDV in prevention of perinatal transmission suggest that an important mechanism of infant PrEP is transplacental drug passage. Thus when selecting an ARV regimen for pregnant women, the two preferred dual NRTI agent backbones with high placental transfer include abacavir (ABC), 3TC, FTC, and TDF.[146] Although ZDV/3TC was a preferred NRTI combination for ARV-naïve pregnant women in the past, based on efficacy studies in preventing perinatal transmission and extensive experience with safe use in pregnancy, this combination is now an alternative regimen due to known side effects, and ABC/3TC (fixed-dose combination), TDF/FTC (fixed-dose combination), and TDF/3TC are now considered preferred NRTIs (see Table 126.7). TDF/FTC and ABC/3TC are the preferred two NRTI components for pregnant adults with HIV based on increased experience with use in pregnancy, availability as a single combination tablet with once-daily dosing, enhanced activity against hepatitis B, and less frequent toxicity compared with ZDV/3TC. As TDF has potential renal toxicity, TDF-based dual NRTI combinations should be used with caution in patients with renal toxicity. Although tenofovir alafenamide (TAF), a prodrug of tenofovir, has shown improvements in renal function and bone density, there are inadequate studies

TABLE 126.7 Initial Combination Regimens for Antiretroviral-Naïve Pregnant Women

	DRUGS	COMMENTS
Preferred Regimens		
Two-NRTI backbones	ABC/3TC	Available as fixed-dose combination; can be administered once daily; should not be used if HLA-B*5701–positive because of risk of hypersensitivity reaction or with ATV/r or with EFV if pretreatment HIV RNA >100,000 copies/mL
	TDF/FTC or TDF/3TC	Fixed-dose combination available and can be given once daily; TDF has potential renal toxicity and should be used with caution in patients with renal insufficiency
PI regimens	ATV/r + preferred two-NRTI backbone	Once-daily administration; extensive experience in pregnancy; maternal hyperbilirubinemia; no clinically significant neonatal hyperbilirubinemia or kernicterus reported, but neonatal bilirubin monitoring recommended; cannot be administered with proton-pump inhibitors; specific timing recommended for dosing with H2 blockers
	DRV/r + preferred two-NRTI backbone	Better tolerated than LPV/r; increasing use in pregnancy; given twice daily in pregnancy
INSTI regimen	RAL + preferred two-NRTI backbone	Increasing experience in pregnancy; rapid viral load reduction; twice-daily administration; avoids drug interactions with PIs
Alternative Regimens		
Alternative NRTI backbone	ZDV/3TC	Fixed-dose combination available; must be given twice daily; potential for hematologic toxicity
Alternative PI regimen	LPV/r + preferred two-NRTI backbone	Abundant experience in pregnancy; more nausea than preferred agents; twice-daily administration; dose increase recommended in third trimester
Alternative NNRTI regimen	EFV + preferred two-NRTI backbone	Concern because of birth defects in primate study; risk in humans is unclear; convenient single tablet; once-daily regimen; avoids drug interactions with PIs; screening for antenatal and postpartum depression is recommended
Alternative NNRTI regimen	RPV/TDF/FTC (or RPV + preferred two-NRTI backbone)	RPV not recommended with pretreatment HIV RNA >100,000 copies/mL or $CD4^+$ count <200 cells/mm^3; do not use with proton-pump inhibitor; available in co-formulated single pill; once-daily regimen
Insufficient Data in Pregnancy to Recommend Routine Use in ART-Naïve Women		
	BIC, COBI, EVG/COBI/TDF/FTC fixed-drug combination, EVG/COBI/TAF/FTC fixed-drug combination, FPV, MVC	
Not Recommended for Use in ART-Naïve Pregnant Women		
	d4T, ddI, FPV/r, IDV/r, NFV, RTV, SQV/r, ETR, NVP, T20, TPV/r, ABC/3TC/ZDV regimen by itself	

[a]Drugs are listed alphabetically. See Table 126.5 for additional information regarding individual drugs.
3TC, Lamivudine; *ABC,* abacavir; *ART,* antiretroviral therapy; *ARV,* antiretroviral; *ATV/r,* atazanavir/ritonavir; *BIC,* bictegravir, *COBI,* cobicistat; *d4T,* stavudine; *ddI,* didanosine; *DRV/r,* darunavir/ritonavir; *DTG,* dolutegravir; *EFV,* efavirenz; *ETR,* etravirine; *EVG,* elvitegravir; *FPV/r,* fosamprenavir/ritonavir; *FTC,* emtricitabine; *HIV,* human immunodeficiency virus; *IDV/r,* indinavir/ritonavir; *INSTI,* integrase strand transfer inhibitor; *LPV/r,* lopinavir/ritonavir; *MVC,* maraviroc; *NFV,* nelfinavir; *NRTI,* nucleoside reverse-transcriptase inhibitor; *NNRTI,* nonnucleoside reverse transcriptase inhibitor; *NVP,* nevirapine; *PI,* protease inhibitor; *RAL,* raltegravir; *RPV,* rilpivirine; *RTV,* ritonavir; *SQV/r,* saquinavir/ritonavir; *T20,* enfuvirtide; *TAF,* tenofovir alafenamide; *TDF,* tenofovir disoproxil fumarate; *TPV,* tipranavir; *T20,* enfuvirtide; *ZDV,* zidovudine.
Modified from US Public Health Service Task Force. Department of Health and Human Services. Recommendations for the use of antiretroviral drugs in pregnant women with HIV infection and interventions to reduce perinatal HIV transmission in the United States. Updated November 14, 2017. http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf.

of its use in pregnancy, and thus it cannot be recommended for use in pregnancy at this time.[146]

The important study HPTN 052, a multinational, randomized trial evaluating treatment of HIV-infected individuals within serostatus-discordant couples, demonstrated that immediate initiation of ART among HIV-infected participants with $CD4^+$ counts between 350 cell/mm^3 and 500 cell/mm^3 reduced both HIV-related events or death and sexual transmission of HIV to their HIV-negative partners by more than 96%.[122] Of the 28 transmissions that were virologically linked to the infected partner, only 1 occurred in the immediate-therapy arm compared with participants who started ART only after their $CD4^+$ counts decreased to less than 250 cells/mm^3 (deferred study arm) (HR, 0.04; 95% CI, 0.01–0.27; $P < .001$).[98] All pregnant women should be initiated or maintained on a combination ART regimen during pregnancy to prevent perinatal transmission and to continue on suppressive ART lifelong, given current data on the benefits and risks to the woman and its prevention of sexual transmission to her sexual partners.[146]

HIV-Infected Pregnant Women Who Are Currently Receiving Antiretroviral Therapy

Women who have been receiving ART for HIV infection should generally continue treatment during pregnancy. Discontinuation of therapy could lead to an increase in viral load, a subsequent decline in immune status, disease progression, and adverse consequences for both the fetus and the woman including increased risk for transmission. EFV, an alternative NNRTI for nonpregnant adults, is now also an alternative NNRTI for pregnant women with HIV who have started it before pregnancy. There have been concerns regarding use of EFV in the first trimester and potential for neural tube defects based on nonhuman primate data and retrospective case reports. However, an updated meta-analysis including data on 2026 women with first-trimester EFV exposure from 21 prospective studies did not find an increased relative risk of overall birth defects in infants born to women receiving EFV-based versus non–EFV-based regimens (RR, 0.78; 95% CI, 0.56–1.08).[195] Because unnecessary ARV drug changes during pregnancy may be associated with loss of viral control and increased risk for perinatal transmission, EFV-based regimens may be continued in pregnant women, provided that the ARV regimen is well tolerated and results in virologic suppression. In women who present on drugs not recommended for initial use because of concerns about viral breakthrough (i.e., fixed-dose combinations of EVG/COBI/TDF/FTC or EVG/COBI/TAF/FTC), providers should consider switching to more effective recommended regimens. If an EVG/COBI regimen is continued, viral load should be monitored frequently, and therapeutic drug monitoring (if available) may be useful.[146]

Stopping Antiretroviral Therapy During Pregnancy

Discontinuation of ART during pregnancy may be indicated in some situations including serious treatment-related toxicity, pregnancy-induced hyperemesis, acute illnesses or planned surgeries that preclude oral intake, lack of available medication, and patient request.[146] Women who are having an elective cesarean section can take oral medications before the scheduled surgery and restart drugs after surgery; given that most ARV medications are now given once or twice daily, the women either would not miss any doses or at most receive the postpartum dose a few hours late.[146]

If discontinuation of ART is indicated, all ARV drugs should be stopped simultaneously and reintroduced simultaneously as soon as possible. However, drugs with long half-lives such as NVP and EFV may be detected for 21 days or longer after discontinuation.[239–241] As other drugs with shorter half-lives are cleared, only the NNRTI may persist, resulting in functional monotherapy that can increase the risk for selection of NNRTI-resistant mutations. To prevent this functional monotherapy, some experts recommend either stopping the NNRTI first and continuing the other ARV drugs for a period of time[240] (at least 7 days is recommended) or switching from an NNRTI-based regimen to a protease inhibitor–based regimen or integrase inhibitor–based regimen.[146] The current recommendation is for lifelong ART, so most experts would recommend switching to another better-tolerated regimen with the goal of obtaining and then maintaining viral suppression.

HIV-Infected Pregnant Women Who Have Previously Received Antiretroviral Therapy but Are Not Currently Receiving Medication

There is concern that some women with previous time-limited use of ARV drugs during previous pregnancies may have developed genotypic resistance to one or more components of the initial ARV regimen, potentially limiting the efficacy of standard regimens (i.e., regimens containing the dual NRTI backbone of ZDV/3TC or ABC/3TC). Given the lack of substantive data, it is reasonable to make preliminary decisions about ARV regimens based on results of previous resistance testing. In general, ART should be initiated before receiving results of current ARV resistance studies because longer ART has been associated with reduced transmission rates compared with shorter treatment periods.[224,242] Careful monitoring of virologic response to the chosen ARV regimen is important, and adjustments to therapy should be guided by repeat resistance testing in consultation with a clinician experienced in HIV treatment.

Monitoring of the Woman and Fetus During Pregnancy

Plasma HIV RNA levels of pregnant women with HIV should be monitored at the initial visit, 2 to 4 weeks after initiating or changing ARV drug regimens, monthly until RNA levels are undetectable, and at least every 3 months during pregnancy. HIV RNA levels (viral load) should also be assessed at 34 to 36 weeks of gestation to inform decisions about mode of delivery and about optimal treatment of the newborn (see "Risk Factors for Perinatal Transmission").[146] The recommended monitoring of viral load in pregnancy is more frequent than in nonpregnant women because of the need to lower viral load as rapidly as possible to decrease transmission risk.

$CD4^+$ counts should be monitored at the initial antenatal visit and every 3 to 6 months in pregnant patients on ART. Monitoring of $CD4^+$ count can be performed every 6 months in patients on combination ART with consistently suppressed viral load who have $CD4^+$ counts well above the threshold for opportunistic infection risk.[146]

ARV drug resistance testing should be performed on women who have persistently detectable plasma HIV RNA levels despite receiving ARV drugs. Because NRTI drugs may be associated with development of lactic acidosis, pregnant women receiving NRTI drugs should have hepatic enzymes and electrolytes monitored more frequently, and any new symptoms of potential hepatotoxicity should be evaluated thoroughly.

Because of physiologic changes such as hemodilution during pregnancy, the CD4 T-cell percentage may be more stable than the absolute $CD4^+$ count during pregnancy.[243] Monitoring for complications of ARV drugs during pregnancy should be based on what is known about side effects of the drugs that the woman is receiving (see Table 126.5). An ultrasound scan in the first trimester is recommended for confirmation of gestational age and to guide potential timing of scheduled cesarean delivery, if needed, as scheduled cesarean deliveries for prevention of HIV transmission should be performed at 38 weeks of gestation in the setting of maternal HIV viremia.[146] Most experts would recommend assessment of fetal anatomy in women who have received combination ART, given the limited data on the effect of combination therapy on the fetus. If the patient is not seen until later in gestation, second-trimester ultrasound can be performed to both scan anatomy and determine gestational age.[146]

Although data are still limited, the risk for transmission does not appear to be increased with amniocentesis or other invasive diagnostic procedures in women receiving effective ART resulting in viral suppression.[244,245] This is in contrast to the pre-ART era, when amniocentesis and chorionic villus sampling were associated with a 2- to 4-fold increased risk for perinatal transmission (see "Risk Factors for Perinatal Transmission," earlier). Some experts consider chorionic villus sampling and cordocentesis too risky to offer to women living with HIV and recommend limiting invasive procedures to amniocentesis, but existing data on transmission risk associated with these procedures are limited.[246]

Women with HIV receiving ART during pregnancy should receive glucose screening with a standard 1-hour, 50-g glucose loading test at 24 to 28 weeks of gestation.[146] Some experts recommend performing earlier glucose screening in women with ongoing protease inhibitor–based therapy initiated before pregnancy, similar to recommendations for women with a high-risk factor for glucose intolerance such as maternal obesity, advanced maternal age, and family history of diabetes mellitus.[146]

Teratogenicity

The potential harm to the fetus from maternal ingestion of a specific drug depends not only on the drug itself but also on the dose ingested; the gestational age of the fetus at exposure; the duration of exposure; the interaction with other agents to which the fetus is exposed; and, to an unknown extent, the genetic makeup of the mother and the fetus.[146] Based on the preponderance of studies indicating no difference in rates of birth defects for first-trimester compared with later ARV drug exposures, women can be counseled that ART during pregnancy generally does not increase the risk of birth defects.

EFV use in pregnancy has received increased scrutiny because of studies on small nonhuman primates. Significant malformations were observed in 3 of 20 infant cynomolgus monkeys receiving EFV from gestational days 20 to 150 at a dose resulting in plasma concentrations comparable to systemic human exposure at therapeutic dosage.[247] The malformations included anencephaly and unilateral anophthalmia in one monkey, microphthalmia in one monkey, and cleft palate in the third monkey. In humans, sufficient numbers of first-trimester exposures to EFV have been monitored in the APR to detect at least a twofold increase in the risk of overall birth defects, without any such increase detected. The current Perinatal Guidelines do not include the restriction of use before 8 weeks of gestation, consistent with both the British HIV Association and WHO guidelines for use of ARV drugs in pregnancy (which note that EFV can be used throughout pregnancy).[146]

The major toxicity of NRTIs is related to effects on mitochondrial DNA synthesis; these drugs have varying affinity for mitochondrial DNA polymerase gamma, resulting in depletion and dysfunction of mitochondrial DNA.[146] Clinical disorders linked to mitochondrial toxicity may be subtle (mild peripheral neuropathy or myopathy) or fulminant (hepatic steatosis, lactic acidosis, and liver failure). The relative potency of NRTIs in inhibiting mitochondrial DNA polymerase gamma in vitro is highest for zalcitabine, followed by didanosine, stavudine, ZDV, 3TC, ABC, and TDF (TAF).[147] Toxicity related to mitochondrial dysfunction has been reported to occur in infected patients receiving long-term treatment with NRTIs and generally has resolved with discontinuation of the drugs; a possible genetic susceptibility to these toxicities has been suggested.[248]

A comprehensive review of use of TDF in pregnant women for treatment of HIV or hepatitis B (or for PrEP) found no evidence of an increased risk of pregnancy loss, stillbirth, preterm birth, small gestational weight for age, or infant mortality compared with similar women receiving placebo or alternate ARV drug regimens.[249] In the APR, sufficient numbers of first-trimester exposures have been monitored to detect at least a 2-fold increase in risk of overall birth defects for darunavir, didanosine, EFV, indinavir, raltegravir, rilpivirine, and stavudine; no such increases have been detected to date. For ABC, atazanavir, FTC, 3TC, lopinavir, nelfinavir, NVP, ritonavir, TDF, and ZDV, sufficient numbers of first-trimester exposures have been monitored to detect at least a 1.5-fold increase in risk of overall birth defects and a 2-fold increase in risk of birth defects in the more common classes, cardiovascular and genitourinary systems; no such increases have been detected to date.[146] Human data on teratogenicity of all ARV agents approved by the US Food and Drug Administration (FDA) can be found in Appendix B: Supplement: Safety and Toxicity of Individual Antiretroviral Agents in Pregnancy of the Perinatal Guidelines[146] and are briefly summarized in Table 126.5.

Intrapartum Antiretroviral Therapy

Women should continue taking antepartum combination ART on schedule as much as possible during labor and before scheduled cesarean delivery.[146] Intravenous ZDV administration is recommended for pregnant women with HIV RNA greater than 1000 copies/mL or unknown HIV RNA levels near delivery, regardless of antepartum regimen or mode of delivery. Many experts choose to continue to give intravenous ZDV to all HIV-positive women during labor and to neonates regardless of the antepartum ARV regimen, even though intrapartum prophylaxis has not been associated with a lower risk for transmission in women with HIV RNA less than 400 copies/mL at delivery.[146] There are inadequate data to determine whether administration of intravenous ZDV to women with HIV RNA between 50 and 999 copies/mL provides any additional protection against perinatal transmission, but some experts recommend administering intravenous ZDV to these women because the transmission risk is slightly higher when HIV RNA is in the range of 50 to 999 copies/mL compared to <50 copies/mL.[146]

For women who have received antepartum ARV drugs but have suboptimal viral suppression near delivery (i.e., HIV RNA >1000 copies/mL), scheduled cesarean delivery at 38 weeks of gestation is recommended to minimize perinatal transmission of HIV, and intravenous ZDV administration should begin 3 hours before the scheduled operative delivery.[146] If ZDV was not used in the antenatal ART regimen because of known or suspected ZDV resistance, intrapartum ZDV is still recommended in women with HIV RNA >1000 copies/mL near delivery except in women with documented histories of hypersensitivity. The addition of intrapartum/neonatal single-dose NVP is not recommended.[146]

Women with unknown HIV status who present in labor should have rapid HIV antibody testing performed and intravenous ZDV initiated if the test is positive (without waiting for results of the confirmatory test) and infant ZDV therapy initiated.[146] Factors that may increase risk of infection include diagnosis of a sexually transmitted disease, illicit drug use, exchange of sex for money or drugs, multiple sexual partners during pregnancy, a sexual partner at risk of or with known HIV infection, signs and symptoms of acute HIV infection, or living in a region with an elevated incidence of HIV in women of childbearing age.[146] If the expedited antigen/antibody results are positive, an HIV-1/HIV-2 antibody differentiation test and an HIV-1 RNA assay should be done as soon as possible; maternal (intravenous ZDV) and infant (combination ARV prophylaxis) ARV drugs should be initiated pending results of the differentiation test. If the follow-up HIV antibody testing is negative, results of the HIV RNA test should be reviewed to rule out acute infection as a cause of the initial positive test before ART is stopped. The choice of intrapartum/postpartum ART regimen for women without antepartum ART should include intravenous ZDV immediately to prevent perinatal transmission of HIV. Although intrapartum/neonatal ARV medications will not prevent perinatal transmission that occurred before labor, most transmission occurs near to or during labor and delivery.[146]

PrEP for the fetus can be provided by giving mothers a drug that rapidly crosses the placenta, producing fetal systemic ARV drug levels during intensive exposure to HIV in maternal genital secretions and in blood during birth. Most experts recommend ARV agents that cross the placenta well; ZDV and other NRTIs, NNRTIs, and the integrase strand inhibitor raltegravir cross the placenta well, whereas protease inhibitors do not (see Table 126.5). If HIV antibody is positive, adding ARV agents to the neonatal portion of the intrapartum/neonatal ZDV regimen can further reduce perinatal transmission of HIV for mothers who have received no antepartum ARV drugs (see "Care of Newborn").[146]

If HIV antibody is negative, infant ARVs can be stopped.[146] In the past, HIV was considered a relative contraindication to operative vaginal delivery with forceps or vacuum. More recent data from greater than 3000 deliveries between 2008 and 2016 suggest that operative delivery, if indicated based on best obstetric practices, is a safe option in women who are virally suppressed.[146,245]

Artificial rupture of membranes performed in the setting of ART and virologic suppression is not associated with increased risk of perinatal transmission and can be performed for standard obstetric indications.[146] Women experiencing postpartum hemorrhage due to uterine atony are often managed with oral or parenteral methylergonovine (Methergine) or other ergot alkaloid as a first-line agent. However, methylergonovine should not be coadministered with drugs that are potent CYP3A4 enzyme inhibitors such as protease inhibitors and COBI. The concomitant use of ergotamines and these CYP3A4-inhibiting drugs has been associated with exaggerated vasoconstrictive responses. If alternative treatments for postpartum hemorrhages are not available, methylergonovine should be used in as low a dose and for as short a time as possible.[146]

Practitioners who provide health care for HIV-infected pregnant women and their neonates should report prospectively all cases of prenatal exposure to any ARV medication (either alone or in combination) to the APR (Research Park, 1011 Ashes Drive, Wilmington, NC 28405; telephone: 1-800-258-4263; fax: 1-800-800-1052; www.APRegistry.com).[194] Treatment and prophylaxis of opportunistic infections during pregnancy should follow guidelines similar to those for nonpregnant women.[250] Pregnant women who develop active opportunistic infections including tuberculosis should receive a drug regimen developed by obstetric and infectious diseases specialists. As with ARV medications, the potential benefits of prophylactic agents must be weighed against their potential risks. Pneumococcal, hepatitis B, and inactivated influenza vaccines may be given if indicated during pregnancy. Live vaccines such as for rubella, measles, mumps, and varicella are contraindicated in patients with severe immunosuppression ($CD4^+$ count <200 cells/mm^3) and during pregnancy, labor, and the early postpartum period.

Care of Newborn

Currently all newborns perinatally exposed to HIV should receive postpartum ARV drugs to reduce the risk of perinatal transmission of HIV.[146] The selection of a newborn ARV regimen should be determined based on maternal and infant factors that influence risk of HIV transmission. The uses of ARV regimens in newborns include the following:

- **ARV prophylaxis:** The administration of one or more ARV drugs to a newborn without confirmed HIV infection to reduce the risk of HIV acquisition.
- **Empirical HIV therapy:** The administration of a three-drug combination ARV regimen to newborns at highest risk of HIV acquisition. Empirical HIV therapy is intended to be preliminary treatment for a newborn who is later confirmed to have HIV but also serves as prophylaxis against HIV acquisition for newborns who are exposed to HIV in utero, during the birthing process, or during breastfeeding and who do not acquire HIV.
- **HIV therapy:** The administration of a three-drug combination ARV regimen at treatment dosages (ART) to newborns with confirmed HIV infection (see Tables 127.2 and 127.3).[146]

For newborns whose mothers have received ART during pregnancy with sustained viral suppression near delivery and for whom there are no concerns related to maternal adherence, a 4-week ZDV prophylaxis regimen can be used. Newborns at higher risk of HIV acquisition should receive a combination ARV regimen, ARV prophylaxis, or empiric HIV therapy based on the clinician's assessment of risk.[146] Newborns of women with unknown HIV status who test positive on expedited HIV testing performed during labor or shortly after birth should be initiated on an ARV regimen based on the clinician's assessment of risk. If supplemental testing is negative, the ARV regimen can be discontinued. For newborns with confirmed HIV, ART should be initiated as soon as possible. In the United States, the use of ARV drugs other than ZDV, 3TC, and NVP cannot be recommended for any indication in premature newborns (<37 weeks of gestational age) because of lack of dosing and safety data.[146] Providers with questions about ARV management of perinatal HIV exposure should consult the Clinician Consultation Center at the University of California, San Francisco for perinatal HIV consultation at 1-888-448-8765, which provides free clinical consultation on all aspects of perinatal HIV including newborn care.

Postpartum Follow-Up of HIV-Infected Women

ART should be continued in all HIV-infected women after delivery to reduce the risk of disease progression and to prevent HIV sexual transmission. Plans for modifying ART after delivery should be made in consultation with the woman and her HIV care provider, ideally before delivery, taking into consideration the preferred regimens for nonpregnant adults.[146] The immediate postpartum period poses unique challenges for ART adherence,[251–254] and new or continued supportive services including mental health and substance abuse treatment should be ensured before hospital discharge. A number of studies have suggested that postpartum depression is common among women with HIV.[255] The US Preventive Services Task Force recommends screening all women for postpartum depression using a validated tool; this is especially important for women living with HIV who appear to be at increased risk for postpartum depression and for poorer ART adherence during the postpartum period. Women should be counseled that postpartum physical and psychological changes and the stresses and demands of caring for an infant may make adherence more difficult, and additional support may be needed during this period.[146]

Women with a positive rapid HIV antigen/antibody test result during labor require comprehensive follow-up. This comprises confirmation of HIV infection, full health assessment including evaluation for associated medical conditions, counseling related to newly diagnosed HIV infection, and assessment of the need for opportunistic infection prophylaxis.[146]

Breastfeeding is not recommended for women living with HIV in the United States, where safe, affordable, and feasible alternatives are available and culturally acceptable. However, clinicians should be aware that women may face social, familial, and personal pressures from cultures where breastfeeding is important, as women may fear that formula feeding would reveal their HIV status.[146] Therefore it is important to address these possible barriers to formula feeding during the antenatal period.

Contraceptive counseling is a critical aspect of postpartum care. Although condoms are universally recommended for prevention of STI and HIV transmission, the unintended pregnancy rate with condom use alone is high. The postpartum period provides an opportunity to review and optimize women's health care including cervical cancer screening; routine immunizations; future reproduction options and need for contraception; mental health and substance abuse treatment as indicated; and assessment for signs of postpartum depression and alcohol and drug use, which may interfere with adherence.[146,235,251] For a detailed discussion of postnatal care of neonates born to women living with HIV, see Chapter 127.

Guidelines for prevention of perinatal HIV and information on the management of HIV during pregnancy are changing rapidly. The reader is encouraged to obtain the most current information about HIV and women from up-to-date sources on the Internet. One of the best sources on HIV/AIDS is AIDS*info* (see "Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States"), which offers current information on research, clinical trials, and treatment to patients and health care providers.[22] There are links to the most current guidelines for management of women during pregnancy and for information on the safety and toxicity of ARV agents in pregnancy.[146] The US government also has published *A Guide to the Clinical Care of Women with HIV/AIDS, 2013 Edition* (https://hab.hrsa.gov/sites/default/files/hab/clinical-quality-management/womenwithaids.pdf). Because optimal HIV care must be individualized and incorporate rapidly changing knowledge, consultation with an HIV/AIDS expert is strongly recommended.

CLINICAL MANIFESTATIONS OF HIV INFECTION IN WOMEN

In the beginning of the HIV epidemic, initial data on clinical manifestations of HIV infection were derived from predominantly male cohorts. Subsequent data on the clinical manifestations of HIV infection in women suggested few sex differences in nongynecologic opportunistic processes and HIV disease progression. Women can present with gynecologic disease, however, that is influenced by their HIV-induced immunosuppression, as summarized in this section.

Opportunistic Processes and Nongynecologic Malignancies

Although Kaposi sarcoma is infrequent among women, HIV infection dramatically increases the risk for this malignancy. In the multicenter Women's HIV Interagency Study (WIHS), the largest cohort study of HIV infection in HIV-infected and at-risk women in the United States, the standardized incidence ratio for this malignancy was 275.8.[256] Kaposi sarcoma has a different presentation in women than in men. Women with this malignancy may have a highly aggressive disease course and an increased incidence of noncutaneous disease, lymphedema, lymph

node disease, and visceral Kaposi sarcoma.[257] Radiographic findings can include nodular opacities, peribronchovascular opacities, thickened interlobular septa, pleural effusions, and lymphadenopathy.[258] Women also may have gynecologic involvement. There is at least one case report of Kaposi sarcoma manifesting as a vulvar mass[259] and two cases diagnosed by cervical biopsy.[260]

The incidence of non–AIDS-defining cancers in patients living with HIV has increased more recently. In a cohort of Italian patients from 1985 to 2011, the incidence rates before and after the introduction of ART were examined, and standardized incidence ratios (SIRs) were used to compare the cancer risk of subjects with HIV with the age-matched and sex-matched general population.[261] In women, the risks were higher than expected for cancer of the vulva (SIR, 69.2), Hodgkin lymphoma (SIR, 7.5), anal cancer (SIR, 41.2), and lung cancer (SIR, 4.8). However, in a more recent review in the United States, the incident lung cancer rates among WIHS participants found these rates did not differ by HIV status or by time period (before ART vs. after ART).[262] Women with HIV and lung cancer are typically young smokers (mean age, 40 years) with stage IV adenocarcinoma who have a poor prognosis. All WIHS women with lung cancer were noted to be smokers, and the risk increased with cumulative tobacco exposure.[262]

Multiple reviews of breast cancer have not found an excess incidence among women with HIV compared with women without HIV. Patients with HIV infection and breast cancer tend to present with more advanced stage and aggressive disease.[263] A summary of case reports of women in the pre-ART era found histopathology results uniformly describing poorly differentiated or undifferentiated tumors. These reviews substantiate the observation that women with HIV and breast cancer generally, but not always, present at a younger age and have increased bilateral disease, unusual histology, and a poorer outcome compared with women without HIV.[263] Chemotherapy-induced myelosuppression can be problematic, and patients often require hematopoietic growth factor and dose reduction during treatment of their breast cancer.[264]

Population studies of women with HIV have shown lower than expected cases of breast cancer in selected African and Western countries.[265] One explanation is competing mortality. Pantanowitz and Dezube[265] offer additional hypotheses including the possibility that the immunodeficiency state may be protective and women with HIV could have fewer risk factors compared with the general female population. HIV infection may actually be protective for breast cancer at the molecular level. Neoplastic breast cells express *CXCR4,* but not *CCR5*. In vitro, binding of HIV to the *CXCR4* receptor induces apoptosis of neoplastic breast cells.[266] In a nested case-control study among WIHS and HIV Epidemiology Research Study (HERS) participants living with HIV, a significantly higher proportion of the controls had *CXCR4*-tropic HIV compared with cases with breast cancer (28% vs. 9%).[266] Breast cancer risk was decreased with *CXCR4* tropism and menopause, after adjustment for $CD4^+$ count, HIV RNA level, and ARV drug exposure.[266] Women living with HIV should follow guidelines established for the general female population for mammographic screening.

Gynecologic Infections and Disease

Human Papillomavirus Infections

Cervical Infection

Human papillomavirus (HPV) infection, particularly with HPV-16 and HPV-18, is the major risk factor for development of cervical cancer,[267] most anal cancers,[268] and some vulvar and vaginal cancers.[269] Additionally, HPV-6 and HPV-11 can cause condylomata acuminata, or genital warts.[270] Women living with HIV are two to three times more likely than women without HIV to have detectable levels of HPV DNA in cervicovaginal specimens due to increasing rates of acquisition and decreased clearance.[271,272] Women with higher HIV viral load and lower $CD4^+$ counts are at elevated risk of HPV acquisition; lower $CD4^+$ counts were also associated with decreased HPV clearance.[271] Therefore women with HIV are more likely to have HPV-related disease including vulvar, cervical, and anal dysplasia and cancer and condylomata acuminata.[273–277] In the United States, women living with HIV have significantly higher rates of cervical cancer than women in the general population,[278] and there is a direct relationship between $CD4^+$ counts and cervical cancer risk.[279] Bacterial vaginosis and *Trichomonas vaginalis* infections, which are less likely to be cleared in women with HIV, increase the risk for acquisition or reactivation of HPV infection, which suggests that the local cervicovaginal milieu may play a role in the susceptibility to HPV infection.[280]

Women with HIV are more likely to progress from HPV infection to HPV-related disease such as low-grade[281–283] or high-grade[284–287] squamous intracellular lesions, likely related to HIV-associated immunosuppression.[273] Two studies found that persistence of HPV and presence of low-grade squamous intraepithelial lesions (SILs) were stronger predictors of developing high-grade SILs than HIV status.[285,288] Crack cocaine has been associated with oncogenic HPV infection, SILs, and a decrease in the clearance of SILs over time.[289] Vitamin A may play a protective role. In one study, women living with HIV with low serum retinol levels (<1.05 mol/L) were more likely to have cervical SILs (multivariate odd ratio, 1.75; $P = .02$) even after adjustment for HPV status, nutritional status, and HIV disease stage.[290] In the era of ART,[271] data regarding the impact of ART on the incidence of HPV infection are conflicting, with some data indicating lowering of HPV incidence[291,292] (all and high-risk HPV types) by ART, and other data failing to show an impact of ART on HPV incidence.[293,294]

Women living with HIV should be screened for cervical cancer and precancers similarly to women without HIV with a few notable exceptions.[295] Given the higher prevalence of HPV-related cervical disease in women with HIV, screening for cervical cancer with a Pap test should begin 1 year after the onset of sexual activity, but no later than 21 years of age.[250] Pap tests should continue annually until there are three consecutive negative results, and then tests can occur at 3-year intervals until 30 years of age, when co-testing with an HPV test should begin at 3-year intervals (provided that three normal Pap tests were obtained previously).[250] Screening for cervical cancer should continue indefinitely for women living with HIV. Young women 21 to 24 years of age who have an atypical squamous cells of undetermined significance result should have a reflex HPV test; if positive, they should be triaged to colposcopy rather than repeat Pap test. Young women with HIV have a high rate of progression of abnormal cytology,[288] and 30% of adolescents who were perinatally infected had abnormal Pap tests at the time of their first test.[296] Generally, women living with HIV with cervical intraepithelial neoplasia should be managed similarly to women without HIV according to the American Society for Colposcopy and Cervical Pathology Guidelines.[297] Low-grade or high-grade cervical lesions are less likely to regress in the setting of HIV infection; therefore it may be preferable to proceed with management for young women if adherence to care is a concern.[250]

Extracervical Infection

Approximately 95% of all vulvar intraepithelial neoplasia (VIN), the precursor to vulvar cancer, is associated with HPV infection.[298] Similar to cervical HPV infection, vulvar HPV infection and associated VIN are more prevalent in women with HIV infection.[277,299,300] Women with HIV develop VIN at a younger age, and the lesions are more often multifocal.[275] Additionally, women with HIV are at increased risk of recurrent VIN and progression to invasive vulvar carcinoma.[275] The impact of ART on the natural history of VIN is not known due to the small number of studies all with small sample size.[300,301] There is no screening procedure for vulvar cancer; however, close examination of the vulva and vagina is particularly important for women with HIV. Biopsy should be performed promptly for any lesions identified as suspicious for VIN or vulvar cancer.[250]

Genital warts are most commonly caused by HPV-6 or HPV-11. Women with HIV may have larger or more numerous genital warts, which may be more difficult to treat.[300,302] Many genital warts regress without treatment, but refractory lesions require biopsy to rule out intraepithelial neoplasia. When treatment is requested due to patient discomfort or request or extensive lesions, topical treatments such as patient-applied imiquimod or provider-applied trichloroacetic acid are usually attempted first. Surgical treatments are used for dense lesions that are less likely to respond to topical therapy.[250]

Anal cancer has been associated with HPV-16 infection; however, for individuals living with HIV, the fraction of anal cancer attributable to HPV-16 is smaller.[303] The prevalence of high-grade anal intraepithelial

neoplasia and anal cancer is higher in women with HIV compared with women without HIV.[304,305] Some studies have evaluated the cost-effectiveness of screening for anal intraepithelial neoplasia and anal cancer in women with HIV using anal cytology.[306–308] At the present time, there are no national recommendations for routine screening of anal cancer for women with HIV. However, anal cancer screening with cytologic screening or high-resolution anoscopy is recommended by some experts in the care of women with HIV.[250,307] Anal cytology should be collected only if there is availability of high-resolution anoscopy,[250] as any abnormal cytology should be evaluated with high-resolution anoscopy.

Prevention of Human Papillomavirus Infection

All three HPV vaccines have been shown to prevent HPV infection and thus HPV-related cancers. Studies of two of these vaccines (bivalent and quadrivalent vaccines) have shown the HPV vaccination is safe and effective in girls and women living with HIV ranging from 13 to 26 years of age; however, the immune response is lower among women with HIV compared with women without HIV, especially if $CD4^+$ counts were <200 cells/mm^3.[309,310] HPV vaccination is recommended for girls and women with HIV 9 through 26 years old, ideally before the onset of sexual activity.[250] Consistent male condom use has also been shown to decrease risk of HPV infection by 70% in women.[311] Additionally, male circumcision has been shown to decrease the rate of HPV infection of the penis in randomized controlled trials,[312,313] and observational studies have shown an association between male circumcision and decreased cervical cancer risk.[314,315] However, evidence is currently insufficient to recommend adult circumcision for decreasing penile HPV risk and prevention of cervical cancer.[250]

Vaginal Infections

Bacterial Vaginosis

Bacterial vaginosis has been the most frequent vaginal infection in some US cohorts of women living with HIV, occurring in 35% to 47% of women.[316,317] Factors associated with bacterial vaginosis among US women with HIV include alcohol use, smoking, douching, and multiple sex partners.[316,318] HIV infection and induced immunosuppression may modify the course of bacterial vaginosis. Bacterial vaginosis is more persistent in women with HIV compared with women without HIV, particularly women with lower $CD4^+$ counts.[319] Although women with HIV may have more severe or persistent bacterial vaginosis infections,[319] there are no unique treatment recommendations. Bacterial vaginosis has been associated with various adverse outcomes such as preterm birth, transmission and acquisition of STIs including HIV,[90,320] and increased genital HIV shedding[89] possibly due to the increased local inflammatory response.[321] Bacterial vaginosis–associated organisms that have been significantly associated with genital HIV shedding include BVAB3, *Leptotrichia,* and *Sneathia*.[89] Treatment can normalize vaginal flora back to the protective lactic acid–producing bacteria and should be considered as a prevention strategy for STI and HIV acquisition and prevention.[89]

Vulvovaginal Candidiasis

The prevalence and cumulative incidence of candida vaginal colonization[322] as well as vulvovaginal candidiasis (VVC)[323] are greater in women living with HIV than in women without HIV. Lower $CD4^+$ count and higher viral load have been associated with VVC in women with HIV.[323] The symptoms of VVC are similar to symptoms experienced by women without HIV.[323] Therapy for VVC in women with HIV should not differ from therapy in women without HIV, but prolonged (i.e., 7–14 days) courses of conventional antimycotic treatments may be necessary for women with severe VVC, very low $CD4^+$ counts, or additional immunocompromising conditions such as diabetes or receipt of corticosteroid treatment.

Trichomonal Vaginitis

Trichomonal vaginitis is one of the most common STIs in women with and without HIV, affecting an estimated 3.7 million persons in the United States.[324] Proportions of women with HIV with a diagnosis of vaginal infection caused by *Trichomonas* have ranged from 6% to 27% in various US cohorts.[325] Reinfection is common, occurring in 36% of women with HIV, and is associated with a history of another STI (RR, 1.52; 95% CI, 1.08–2.14).[325] Becoming pregnant may be protective (RR, 0.59; 95% CI, 0.39–0.87).[325] A large clinical trial of women living with HIV determined a 7-day course of metronidazole is more effective than a single dose for treatment of *Trichomonas*.[326] A concurrent bacterial vaginosis infection was associated with failure of single-dose metronidazole treatment.[327]

Pelvic Inflammatory Disease

Several studies performed in the United States and Africa compared the presentation and course of pelvic inflammatory disease (PID) in women with and without HIV. Four studies[328–331] found that women with HIV had a lower admission white blood cell count, and five studies[328–330,332,333] noted that women with HIV were more likely to have tubo-ovarian abscesses or require surgical intervention. One study from the pre-ART era found that women with HIV were more likely to have persistent fevers refractory to antibiotic therapy,[328] and another study found higher temperatures and mean clinical severity scores.[332] Barbosa and colleagues[331] noted that women with HIV were more likely to remain febrile 48 hours after initiation of antimicrobial therapy. By day 5 or 6 of therapy, however, there was no statistically significant difference in the presence of fever.

The microbiology of PID in these studies is similar to that described in women without HIV but with PID, although the frequency of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* may be lower among women with HIV.[310,334] One study showed *Mycoplasma* spp. and *Streptococcus* spp. were more likely to be isolated in endometrial biopsy specimens of women with HIV ($P < .05$).[330] Another study of Kenyan women with PID found that the prevalence of bacterial vaginosis was significantly higher in women with HIV with low $CD4^+$ counts,[334] and in a South African study it was noted that *T. vaginalis* infection of the lower genital tract was significantly associated with a clinical PID diagnosis in women with HIV ($P = .002$).[335] Taken together, these studies suggest HIV infection might increase the risk of more severe PID; however, there is no indication that the response to therapy is different for women with HIV compared with women without HIV. Women with HIV should receive the conventional recommended antibiotic regimens.[336]

Genital Ulcer Disease

The most common causes of genital ulcer disease among women with HIV are HSV and syphilis. Women with lower $CD4^+$ counts are more likely to have active and severe genital ulcer disease due to HSV.[336] Because HIV seems to modify the course of HSV, the CDC has specific recommendations regarding the treatment of episodic infections or suppression for people living with HIV. In general, doses of acyclovir, famciclovir, and valacyclovir are higher, and duration of treatment is longer.[336] Persistent large ulcerations resistant to HSV treatments may herald either acyclovir resistance or the presence of idiopathic aphthous ulcerations, described subsequently. ART reduces the severity and frequency of symptomatic genital herpes, but subclinical genital HSV shedding still occurs.[337,338]

In women with advanced HIV disease, idiopathic vulvar or vaginal ulcers are rare manifestations[339–341]; the ulcers can be intractable, progress to fistula formation, and cause severe bleeding. In a national retrospective review of 29 women with idiopathic genital ulcers (defined by either negative HSV and syphilis test results or a nondiagnostic ulcer biopsy specimen), the median $CD4^+$ count was only 50 cells/mm^3, and 68% had a previous AIDS-defining event.[341] In 37% of patients, oral ulcers coexisted; in 19%, genital ulcers progressed to fistula formation. Although the numbers were small, there was often a good response to either corticosteroid treatment (topical, intralesional, or systemic) or initiation of ART in ARV-naïve women.[341] Thalidomide also was used successfully in the pre-ART era.[342]

CMV rarely can cause disease in the lower female genital tract. Women present with labial, vulvar, and cervical ulcerations, and typically they are severely immunocompromised. They often have coexistent cytomegaloviral retinal or gastrointestinal tract disease. In addition to painful genital ulcers, some women have had fevers and significant cervical bleeding. Women with this manifestation generally responded to intravenous ganciclovir (see Chapter 137).[343–345]

SEX DIFFERENCES IN HIV INFECTION AND TREATMENT RESPONSES

In the beginning of the HIV epidemic, initial data on the natural history of HIV infection were derived from predominantly male cohorts. As data from women became available, however, a few differences in the natural history of HIV infection by sex began to emerge. Beyond natural history, we also examine sex differences in virology, immunology, HIV reservoir, pharmacokinetics, treatment adherence, and treatment outcomes and toxicities in this section.

Sex Differences in Clinical Progression

Studies from early in the history of the HIV epidemic reported faster rates of progression to clinical outcomes and AIDS in women than men.[346,347] However, these differences, on further adjustment, seemed to be due to lower access to effective ARVs or late entry into care in women. Furthermore, higher rates of psychosocial factors among women in the United States, such as substance use, mental illness, stigma, and poverty, seemed to contribute to disparate care outcomes, rather than inherent biologic differences by sex.[348–350] Studies conducted after more wide-scale dissemination of ART worldwide showed similar clinical presentations and rates of progression to AIDS and death for women and men.[351,352] In the most recent era, a meta-analysis examining 31 studies with a total sample size of 86,233 men and 117,719 women in low-income and middle-income countries revealed that men living with HIV have a significantly greater hazard rate of all-cause mortality compared with women.[353] This effect seemed secondary to later diagnoses in men (with women being diagnosed earlier and entering care, for example, through the antenatal care setting). Other factors included reduced engagement and retention in care, later initiation of ART, and lower adherence rates to ART in men compared with women.[353]

Virologic and Immunologic Differences Between Men and Women Off ART

There are distinct biologic differences between women and men in the natural history of and immunity to HIV-1 infection.[354] HIV-1 RNA levels are approximately 0.5 $\log_{10}$ lower (range, 2-fold to 6-fold) in women than men early on in HIV infection[355] for approximately 5 to 7 years following seroconversion.[356] As $CD4^+$ counts decline and HIV-1 progresses off ART, this sex difference in HIV viral loads abates and then disappears. Despite having lower viral loads earlier in HIV disease than men, women do not have a slower rate of progression to AIDS. Indeed, for a given viral load early on in infection, women may actually progress more quickly to AIDS than men, possibly secondary to immunologic differences by sex.

Women have higher absolute $CD4^+$ counts than men (approximately100 cells/mm^3) early on in infection.[357,358] However, women actually have a more rapid depletion of $CD4^+$ counts during the natural history of HIV disease. A possible mechanism is that interleukin-7 levels tend to be higher (by 40%) in HIV-1–infected women compared with men, and there is an inverse correlation between circulating interleukin-7 levels and $CD4^+$ counts.[358] Other mechanisms underlying sex-based differences in the immune response are incompletely understood but have precedence in disparate rates of autoimmune diseases by sex,[354] differential responses to vaccination, and sex-specific responses to other infections. For instance, there are sex-specific differences in varicella-zoster virus reactivation rates,[359,360] fulminant hepatitis E infection,[361,362] and rates of spontaneous hepatitis C clearance.[363] Differential responses to vaccines by sex may further be mediated by hormonally mediated differences in the induction of Toll-like receptor response genes.[364]

Female-predominant sex hormones (i.e., progesterone, estrogen) have direct effects on immune responses. Estrogen downregulates tumor necrosis factor-α expression,[365,366] and progesterone-induced blocking factor influences the cytolytic function of lymphocytes including natural killer cells.[367] Women in the third trimester of pregnancy, when estrogen and progestin levels are very high, demonstrate transiently reduced peripheral blood CD4 T-cell counts. Finally, human lymphocytes express a glucocorticoid receptor with a distinct progesterone-binding domain, with progesterone exerting a dose-dependent inhibitory effect on *CCR5* expression in activated T cells[368] leading to lower *CCR5* density in women.[369] *CCR5* coreceptor expression on CD4 T cells has been linked to lower viral setpoints,[370] which could contribute to the virologic differences between men and women seen early on in infection.

Sex Differences in the HIV Reservoir and Possible Impact on Cure Strategies

Other sex-specific factors might influence the biology of the HIV reservoir. HIV persistence results from HIV seeding reservoir sites such as lymph nodes, thymus, central nervous system, and genital tract. Research efforts have focused on decreasing or even eradicating the reservoir in an attempt to elicit HIV remission or cure.[371] Although none of the current strategies being pursued have had success to date, the research on cure is ongoing and active and is employing a variety of strategies to explore the reduction of the HIV reservoir. Sex differences in HIV persistence or the reservoir may lead to the need for tailoring strategies attempting HIV cure by sex.

There are limited studies examining sex differences in HIV persistence when men and women are on ART. However, one analysis compared levels of the HIV-1 reservoir in a well-matched cohort of men and premenopausal women on ART with documented and long-standing virologic suppression.[372] Levels of HIV-1 DNA were not significantly different between the groups, but cell-associated HIV-1 RNA and low-level viremia by a highly sensitive assay were lower in women than in men. Consistent with this finding, markers of T-cell activation were also significantly reduced in women compared with men.[372] Other studies have suggested a higher incidence of posttreatment control in women[373] and prolonged time to viral rebound after ART is discontinued.[374]

One major strategy being pursued on the pathway to cure is to reverse HIV latency in the reservoirs. However, current methods known to aid in latency reversal are insufficient and have not led to eradication. In a genome-wide short hairpin RNA screen, one group identified the estrogen receptor ESR-1 as a cellular factor critical to reversing HIV-1 latency in a cell model.[375] If estrogen is directly repressive of HIV-1 transcription in latency models, estrogen blockade may help potentiate provirus latency reversal with known agents (e.g., histone deacetylase inhibitors) in women. This observation is the basis of an ongoing study in the Adult ACTG of a combination of tamoxifen and vorinostat (a histone deacetylase inhibitor) and their effects on viral reactivation among postmenopausal HIV-infected women on ART. Further work is needed to study the impact of female-specific hormones and their cycling in the context of reproductive-aged women on the reservoir, the impact of decreased concentrations of female hormones on the reservoir in postmenopausal women, and how to harness these findings into strategies directed toward cure.

Sex Differences in Responses to Antiretroviral Therapy and Pharmacokinetics

Treatment Outcomes

In general, women have equal rates of achieving virologic suppression on effective ART compared with men. In fact, women are more likely to be virologically suppressed than men in many regions of the world[49,376] where women are linked into care earlier (due to accessing care through antenatal settings) than men. In general, the choice of ARV regimen does not change according to the sex of the individual. DHHS Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living With HIV provides the latest recommendations for the treatment of HIV and is updated frequently.[189]

In contrast, a number of studies have shown that women may have more adverse effects on ART than men, an observation that was more common with the older ARV agents. For example, women are more likely to experience rash and hepatic toxicity on NVP (an NNRTI) than men.[377] These toxicities on NVP occur more frequently when patients are started on the agent at higher $CD4^+$ counts: >250 cells/mm^3 for women and >400 cells/mm^3 for men.[189] The older thymidine analogue NRTIs stavudine and didanosine were associated with higher rates of lactic acidosis among women than men.[377] Finally, there are higher rates of gastrointestinal side effects, perturbations in lipids, and likely lipodystrophy (body changes that involve central fat accumulation and

peripheral atrophy) in women than men on protease inhibitor–based therapies.[377] The higher rate of adverse effects in women compared with men on ART can have consequences including a higher rate of discontinuation due to tolerability issues.[378]

Pharmacokinetics

Differences in adverse effects among women and men are most likely due to sex differences in the pharmacokinetics of ARVs.[379,380] In 1999, the National Institutes of Health published a six-volume report entitled the *Agenda for Research on Women's Health for the 21st Century,*[381] which, among other dicta, claimed that because "gaps in knowledge remain regarding the behavior of drugs in women … [and] over-reliance on traditional male-oriented medical practices may ill serve women … gender-related pharmacokinetic and pharmacodynamic differences" must necessarily be assessed. The FDA mandated in 1998 that applications for new drugs must include data that are sex-stratified, providing information on the safety, tolerability, and effectiveness of the drug by sex. However, a 2001 investigation by the US General Accounting Office revealed that more than one-third of the FDA-approved drugs since the 1998 mandate were approved without provision of any sex-specific information.[382] In HIV medicine, even if women are enrolled in clinical trials investigating new drugs, the data on pharmacokinetics, side-effect profiles, and efficacy are not always provided, or the numbers of women in the trial are often too low to stratify analyses by sex.

Despite this, a few pharmacokinetics comparisons of various ARVs between men and women have shown that women in general tend to have higher exposure to various ARVs at the same dose than men.[380,383] There are a number of postulated mechanisms for differential ARV concentrations by sex, as each of the components of the pharmacokinetics cascade (drug absorption, distribution, metabolism, and distribution) can be influenced by either female-predominant hormones or differences in organ/body size by sex. For example, in terms of absorption, women have slower emptying times in the stomach than men, which may lead to increased rates of drug absorption. Moreover, the emptying time may vary throughout the menstrual cycle depending on progesterone levels, which vary by phase of the cycle. In terms of distribution, ARVs are distributed by plasma binding proteins (e.g., albumin and α_1-acid glycoprotein). Estrogen decreases α_1-acid glycoprotein concentrations, which may lead to greater concentrations of free drug in the plasma. In terms of metabolism, female hormones have differential effects on CYP enzymes, the main metabolizing enzymes at the level of the liver for a variety of ARVs. In terms of elimination, women have smaller organs and may clear drugs less quickly as a result. Overall, the sex differences in pharmacokinetics parameters for ARVs tend to lead to higher drug concentrations in women compared with men, which may explain the higher rates of toxicities on ARVs among women.[380]

Sex Differences in Comorbidities

Given the increasing uptake of ART across the world and the subsequent reductions in HIV-related morbidity and mortality, there is a burgeoning focus on noncommunicable or comorbid complications of chronic HIV infection. Men and women are at increased risk for comorbid diseases at relatively earlier ages compared with the general population. Such comorbidities include cardiovascular disease, cerebrovascular disease, osteopenia and osteoporosis, various cancers, and geriatric syndromes such as falls and frailty,[384–392] all of which may have sex differences in incidence and prevalence in the general population. Beyond sex differences in hormones playing a differential role in the incidence of comorbidities among women, higher rates of immune activation and inflammation[354,393] and higher rates of smoking[394] have been postulated to play a role in comorbidity prevalence and severity among women living with HIV in particular.

Men are still at more risk of cardiovascular and cerebrovascular disease than women, even with concomitant HIV infection. Recent evidence has shown higher rates of ischemic stroke in women with HIV compared with women without HIV.[395] Women with HIV are also more at risk for osteopenia and osteoporosis[396] than men living with HIV, as in the general population. In terms of cancers, breast cancer does not seem to have a higher incidence in women with HIV than women in the general population, and indeed it is postulated that *CXCR4* positivity on breast cancer cells (and their subsequent destruction by HIV) may lead to lower rates of breast cancer in women living with HIV than in women without HIV.[266] However, all people living with HIV are at a higher risk than their non–HIV-infected counterparts for Epstein-Barr virus–associated lymphomas, human herpesvirus 8–associated cancers such as Kaposi sarcoma and Castleman syndrome, HPV-associated cancers including cervical cancer and anal cancer, and some non–AIDS-related cancers such as colon cancer and lung cancer.[388]

Finally, it is important to note that, although not related to immunosuppression from HIV, some concomitant mental health conditions are more prevalent in women in the United States living with HIV than men.[397] Poverty, stigma, and structural racism contribute to these differences,[350] especially as women with HIV in the United States are more likely to be racial or ethnic minorities than men (see "Epidemiology in United States"). Finally, the high rates of intimate partner violence among women with HIV in the United States puts this group more at risk for nonadherence to ART, nonsuppressed viral loads, and death.[398]

Sex Differences in Aging

Women with HIV may enter menopause earlier than their non–HIV-infected counterparts and have a higher rate of vasomotor symptoms.[399] In women with HIV, lower $CD4^+$ count, injection drug use, and low physical activity all have been associated with an earlier onset of menopause than in women without HIV.[400] Women with HIV may also have a greater severity of vasomotor symptoms with menopause than their non–HIV-infected counterparts, necessitating close attention to symptoms and hormone replacement therapy when needed.[400]

INCLUSION OF WOMEN IN CLINICAL RESEARCH

Women and minority populations have not been represented in clinical trials of HIV medications and interventions to the extent that reflects their burden of disease.[401] Many treatment strategies for women are extrapolated from studies performed almost exclusively in men. Despite the importance of the HIV cure research agenda, women account for only 8.3% of subjects in studies related to cure, which does not correspond to the prevalence of infection in women either domestically or globally.[402] Given the sex-based differences in immune responses and virologic parameters detailed in this chapter, it is imperative to enroll adequate numbers of women in cure trials and to continue to unravel basic mechanisms related to the effects of sex hormones on HIV persistence and the reservoir to guide eradication strategies that may be tailored to women.

Pharmaceutical companies have increasingly focused on women-specific studies only after an ARV agent is approved, leading to use of a medication in women without knowledge of sex-specific differences in tolerability or efficacy. Strategies to include greater numbers of women in HIV-specific studies could include revisiting the strict enrollment criteria around pregnancy, reviewing overly stringent contraceptive requirements, having after-hours study visits, and providing transportation and child care.[399] Given the importance of sex differences in HIV medicine and the burden of the epidemic among women, an increasing focus on enrolling adequate numbers of women in clinical trials and performing sex-stratified analyses to help differentiate tailored strategies for women is essential.

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127 Pediatric Human Immunodeficiency Virus Infection

Geoffrey A. Weinberg and George K. Siberry[a]

SHORT VIEW SUMMARY

EPIDEMIOLOGY

- A remarkable decline in US pediatric human immunodeficiency virus (HIV) infection has occurred because most currently infected children are surviving into young adulthood, and there has been tremendous success in preventing mother-to-child transmission (MTCT). Such success may be achieved globally wherever effective HIV screening and therapy for pregnant women are accomplished.
- Influx of HIV-infected immigrants, refugees, and adoptees, in addition to residual domestic MTCT, will continue to contribute to a small but definite presence of pediatric HIV infection in the United States.
- An alarming increase in adolescent acquisition of HIV infection is occurring among young men who have sex with men, especially African-American and Hispanic men.

ELIMINATION OF NEW PEDIATRIC HIV INFECTION IN THE UNITED STATES

- Prevention of pediatric HIV infection critically depends on prevention of HIV MTCT, which in turn depends on primary prevention of HIV infection in women, universal HIV testing of pregnant women to identify those who are HIV infected, and appropriate antiretroviral therapy in HIV-infected women and their infants.
- Each episode of perinatal HIV infection in the United States should be viewed as a sentinel public health event, indicating a woman whose HIV infection was undiagnosed before or during pregnancy or a woman with a known diagnosis who did not receive appropriate interventions during prenatal care to prevent MTCT.
- Prevention of adolescent HIV infection involves similar strategies to those used for adults (safer sex, avoidance of intravenous or injection drug use) but with special attention to the adolescent's unique developmental and biopsychosocial challenges. Additional challenges may be posed by interrupted health insurance, homelessness, and stigma among young men who have sex with men.

THERAPY FOR AGING HIV-INFECTED YOUTH

- Disclosure of the diagnosis of perinatal HIV infection to the aging child requires discussion and planning and is a process that may take place over months to years based on several factors, including the developmental level of the child, and the parent's or caretaker's readiness to disclose. Disclosure is best accomplished by early adolescence to more fully engage youth in their own care, preferably before the age of sexual debut.
- Adherence with medical care may suffer during adolescence because of issues revolving around psychosocial developmental, confidentiality, peer pressure, and socioeconomic factors. This contributes to the substantial presence of antiretroviral resistance of HIV strains in adolescents with perinatal HIV infection.
- Transition from the pediatric care model to the adult care model is a multifaceted process that requires time and planning to keep the aging adolescent successfully engaged in care.
- Complications of long-standing perinatal HIV infection and its therapy among maturing youth include impaired growth and bone mineral accrual, possibly increased cardiovascular risk factors, increased incidence of behavioral and psychiatric disorders, and increased risk for cervical dysplasia and preterm delivery among young women.

Although the pathogenesis of human immunodeficiency virus (HIV) infection and the general virologic and immunologic principles underlying the use of combination antiretroviral therapy (cART) are similar for HIV-infected children and adults, unique considerations apply to infants, children, and adolescents.[1] These include (1) the acquisition of infection through perinatal exposure for the vast majority of children (i.e., a primary infection acquired in the context of an immature immune system); (2) in utero exposure to antiretroviral medications, HIV infection, and altered inflammatory milieu; (3) differences in immunologic markers, viral load, and diagnostic strategies in young infants; (4) changes in pharmacokinetic parameters with age; (5) considerations related to the formulation and palatability of medications, and fewer medication options adequately tested and available for use by children[2,3]; (6) issues of adherence in infants and young children dependent on others for medication administration and in adolescents facing adherence challenges from developmental issues, including peer pressure; and (7) challenges in the disclosure of HIV diagnosis to children and adolescents. The salient pediatric-specific issues in the diagnosis, clinical manifestations, and treatment of pediatric HIV infection are addressed in a previous edition of this textbook and elsewhere.[3,4–13,14–16,17,18] Table 127.1 lists frequently updated Internet-based sources of information.

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

The focus of this chapter is on current successes and the remaining challenges in domestic pediatric and adolescent HIV infection.

CURRENT EPIDEMIOLOGY OF DOMESTIC PEDIATRIC AND ADOLESCENT HIV INFECTION

The epidemic of acquired immunodeficiency syndrome (AIDS) was first recognized in adults in the United States in 1981, although sporadic cases in the United States and Europe had occurred since at least the mid-1970s.[19–23] In retrospect, it is known that pediatric cases occurred almost as early as adult cases.[24–26] During the peak of the US pediatric HIV epidemic, approximately 10% of people living with HIV infection in the United States were children (<13 years of age) and adolescents (13–19 years of age); currently in the United States, children and adolescents together account for less than 1% of people living with HIV infection.[27] This remarkable 90% decline in the prevalence of pediatric HIV infection is the result of striking advances in both the prevention of mother-to-child transmission (MTCT) of HIV, reducing the incidence of new infection, and the increased survival of infected children aging into young adulthood.[27,28]

Since reporting began in 1981, approximately 9600 cases of AIDS in children younger than 13 years have been reported by the Centers for Disease Control and Prevention, with most occurring in children of color (59% black, 21% Hispanic, 16% white).[27,29,30] The greatest

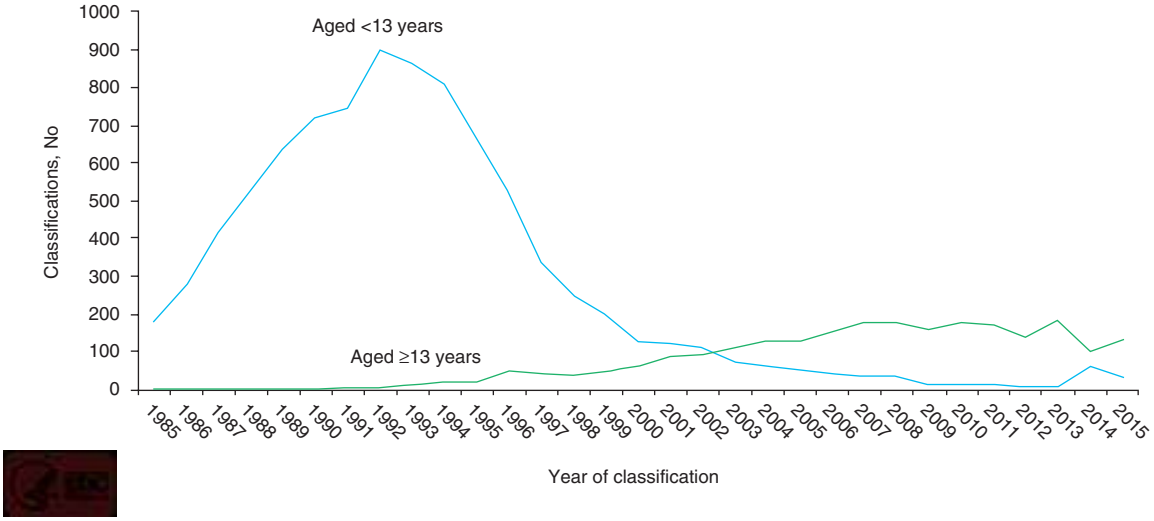

FIG. 127.1 Acquired immunodeficiency syndrome (AIDS) cases in the United States and six dependent areas acquired perinatally, by year of, and age at, diagnosis, 1985–2015. *(From Centers for Disease Control and Prevention. Pediatric HIV surveillance slide set.* https://www.cdc.gov/hiv/library/slideSets/index.html. *Accessed June 1, 2018.)*

TABLE 127.1 Useful Websites for Information on Pediatric HIV/AIDS

WEBSITE	SPONSOR	COMMENTS
https://aidsinfo.nih.gov	US National Institutes of Health	Continuously updated clinical practice guidelines for management of HIV infection and HIV–related opportunistic infections; information on clinical trials and other research efforts
https://www.hivguidelines.org/	New York State Department of Health AIDS Institute	Updated practice guidelines and chapters on management issues
https://www.cdc.gov/hiv	Centers for Disease Control and Prevention (CDC)	Compendium of information, websites, telephone and fax numbers; Spanish language links
https://www.niaid.nih.gov/ and https://www.oar.nih.gov/	National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) NIH Office of AIDS Research (OAR)	Summary of NIAID and NIH-wide activities and information on HIV/AIDS topics
www.nccc.ucsf.edu	Clinician Consultation Center, University of California, San Francisco	Telephone consultation and Internet-based information on perinatal HIV issues including antepartum and intrapartum management of women with HIV infection, and postpartum management of HIV-exposed infants (perinatal HIV hotline: 888-448-8765); also, management of children and youth with HIV infection (telephone consultation: 800-933-3413)
http://www.who.int/hiv/en	World Health Organization (WHO)	Information, practice guidelines, and other HIV resources for international providers
http://www.unaids.org/	Consortium of United Nations Agencies including WHO and United Nations Children's Fund (UNICEF)	Information and statistics relevant to international providers
https://aidsetc.org/	Health Research Services Administration (HRSA)	Educational and consultation resources
https://www.accessdata.fda.gov/scripts/cder/daf/gov/scripts/cder/daf/	US Food and Drug Administration (FDA)	Access latest information about FDA-approved drugs

All sites accessed December 14, 2018.
AIDS, Acquired immunodeficiency virus; *FDA,* US Food and Drug Administration; *HIV,* human immunodeficiency virus.

number of pediatric AIDS cases (cumulative through 2016, recognizing that case numbers of HIV infection, not just AIDS, are likely much greater) have been reported in New York (2430), Florida (1569), New Jersey (810), California (710), Texas (411), Pennsylvania (374), and Puerto Rico (404). The mode of infection for more than 95% of the cumulatively reported cases of pediatric AIDS is MTCT. Less than 1% of cases have been reported to have no identifiable risk factor; early in the epidemic, some cases were the result of transfusion of tainted blood components. Rarely, cases have been attributed to sexual abuse or assault and only very rarely to household, school, child care, and hospital or clinic exposure. Breastfeeding is an important mode of HIV transmission in lower resource settings, but in the United States, where nutritionally complete infant formula and clean water are readily available, HIV-infected women are counseled to avoid breastfeeding.[5,17,31,32] Premastication or prewarming of infant food by an HIV-infected caregiver is a possible source of pediatric HIV infection and also is contraindicated.[33,34]

In the 5 years after antenatal, intrapartum, and neonatal zidovudine MTCT prophylaxis was implemented as the standard of care in the United States for pregnant women with HIV infection, the number of pediatric AIDS cases reported in the United States decreased by 86% among infants younger than 1 year and by 78% in children 1 to 5 years of age.[28,35–40] These changes reflected a clear success in decreasing perinatal transmission and in better antiretroviral therapy (ART) and prophylactic medications to prevent progression of pediatric HIV infection to AIDS. In 1991 the annual number of perinatally infected infants in the United States peaked at 1650; by 2013, only 69 perinatally infected children were born, an affirmation that the combination of clinical investigation, public health policy implementation, and continued antiretroviral drug development has sharply curtailed the rate of perinatally acquired pediatric HIV/AIDS in the United States (Fig. 127.1).[28–29,30] The total number of HIV-exposed births (i.e., pregnancies among HIV-infected women) is likely decreasing but is still substantial, such that continued efforts to prevent MTCT will be required.[1,29,30]

Similar remarkable decreases in MTCT of HIV have been reported from the United Kingdom, Ireland, France, and wherever prenatal testing and identification of HIV-infected pregnant women and provision of cART to pregnant women and their infants is achieved.[41–43]

Pediatric HIV infection in the United States is now considered as a condition that could be eliminated—a nearly unimaginable concept just 2 decades ago. Globally, however, the 2.1 million children younger than 15 years living with HIV accounted for approximately 6% of all 36.7 million people living with HIV; 160,000 new pediatric infections represented 9% of all incident infections, and 120,000 child deaths accounted for 12% of all AIDS-related deaths.[44] New infections in children across the world have declined by 47% since 2010, as the proportion of HIV-infected pregnant women with access to cART has increased from 47% to 76%. The influx of HIV-infected immigrants, refugees, and international adoptees, and uncommon instances of MTCT, will continue to contribute to a small but definite presence of HIV-infected children in the United States for a number of years to come.

In addition, some areas of the United States continue to experience MTCT of HIV from missed opportunities in case identification of, or care provision for, infected pregnant women.[45] Public health efforts must be strengthened to truly eliminate domestic pediatric HIV infection.

In contrast to the tremendous success in decreasing new perinatal HIV infections through aggressive MTCT prevention programs, the incidence of HIV infection in US youth remains stable. The lack of substantial progress in reducing HIV infection in youth represents a confluence of the majority of perinatally infected children now surviving into adolescence and the continued acquisition of HIV infection during adolescence through sexual contact (especially among young men who have sex with men) and, to a lesser extent, intravenous drug use.[46] In 2016, 21% of the newly reported infections with HIV were in adolescents and young adults ages 13 to 24 years.[46] Among these youth with new HIV diagnoses, most (55%) were black or Latino (23%), and more than 80% were young men who have sex with men.

REMAINING CHALLENGES IN DOMESTIC PEDIATRIC HIV INFECTION

Elimination of New Pediatric HIV Infection

The reader is referred to Chapter 126 for a more detailed discussion of ART of pregnant women living with HIV; essential points are reiterated here, along with newer information on management of the HIV-exposed infant.

Maternal Determinants of Mother-to-Child Transmission

The MTCT rate is estimated to range from 13% to 39% in infants born to HIV-infected women who did not receive ART during pregnancy or intrapartum.[5,17,35] In the United States, the transmission rate decreased from 25% to 8% in 1995, after incorporation of an antenatal, intrapartum, and neonatal regimen of zidovudine (Pediatric AIDS Clinical Trials Group [PACTG] 076 study) into standard-of-care guidelines, and currently the rate of MTCT in the United States is less than 2%.[5,27,29,30] Correlates of MTCT include late initiation of, or absence of, cART during pregnancy, maternal HIV viral load greater than 1000 copies/mL, low $CD4^+$ T-cell count, prolonged rupture of membranes, preterm delivery, concomitant genital ulcerative disease, and seroconversion during pregnancy.[6,47–51] Cesarean section is recommended for the prevention of transmission if viral load suppression (<1000 copies/mL) has not been achieved by delivery.[5,6,52] It is clear that vertical transmission is most significantly decreased by the use of ART in pregnant women and their neonates.[1,5] Furthermore, MTCT is significantly lower in women who start ART before conception, compared with those starting ART during pregnancy.[5,53] When analyzed independently of plasma viral load, the MTCT rate of pregnant women followed in the French Perinatal Cohort who initiated ART before conception was 0.2% (6/3505), compared with 1.1% (50/4570) who initiated therapy during the pregnancy.[53] Remarkably, none of the subset of 2651 pregnant women who received ART from before conception and maintained less than 50 copies/mL plasma viral load at delivery transmitted HIV infection to their infants (0/2651; MTCT rate, 0%; 95% confidence interval, 0%–0.1%).[53]

In the absence of ART, the majority of MTCT occurs during labor and delivery (50%), with an additional 25% occurring antenatally and 25% postnatally through breastfeeding among women not receiving ART.[1,5] Acute HIV infection late in pregnancy or during breastfeeding greatly increases the rate of HIV MTCT.[5] Preventive therapies and other measures have differential effects depending on the stage of pregnancy in which they are applied and whether infants are breastfed. Appropriately designed antenatal ART optimizes maternal health and prevents antenatal and intrapartum transmission.[1,5,31] Interventions initiated at delivery have no effect on those infants already infected in utero but can decrease the rate of intrapartum transmission.[5,54,55] Even neonates born to HIV-infected women who did not receive antenatal and intrapartum therapy may benefit from ART if it is begun in the first 12 hours after birth.[37,39,56] When HIV infection is newly diagnosed in a pregnant woman or child, it is essential to recommend HIV testing to the mother's other children regardless of age.[5,17]

Postpartum Mother-to-Child Transmission

The rate of infection among infants of HIV-infected women who breastfeed is notably greater than that among infants of women who do not breastfeed, even if antiretroviral agents were administered during pregnancy and intrapartum.[5,17,31,32] HIV has been detected in both the cellular and the cell-free fractions of human breast milk, and high maternal plasma viral load is likely a risk factor for transmission through breastfeeding.[57–59] In high-resource settings such as the United States, all HIV-infected women should completely avoid breastfeeding to prevent HIV transmission to their infants.[5,17] Although there remains a medical contraindication to breastfeeding in order to maximize prevention of HIV transmission, there is an increasing recognition that some women in the United States and similar industrialized nations face social, cultural, and other reasons that may lead them to breastfeed despite such counseling, making it important for providers to enable honest conversations about infant feeding intentions and to provide women who insist on breastfeeding additional information to minimize risk.[60–62] When women living with HIV infection continue cART after delivery, postpartum MTCT rates decline even with breastfeeding.[5,6,31] Thus in lower-resource settings such as sub-Saharan Africa, the further decrease in HIV transmission through avoidance of breastfeeding needs to be balanced against the substantially increased risk for respiratory and diarrheal disease morbidity and mortality in infants who are not breastfed.[5,6,31] The World Health Organization has suggested that each country should decide if it should adopt the strategy of counseling mothers to breastfeed while receiving antiretroviral interventions, or of counseling to avoid all breastfeeding, when considering how to provide infants the greatest chance of HIV-free survival.[31]

Elimination of Mother-to-Child Transmission

Prevention of pediatric HIV infection critically depends on primary prevention of HIV infection in women (including those who are pregnant), universal HIV testing of pregnant women to identify those who are HIV infected, and, finally, appropriate ART in HIV-infected women and their infants.[1,5] Currently, each episode of perinatal HIV infection in the United States should be viewed as a sentinel public health event, indicating a woman whose HIV infection was either undiagnosed before or during pregnancy, or a woman with a known diagnosis who did not receive adequate prenatal care including appropriate interventions to prevent MTCT.[1,30,45] One or more missed opportunities for prevention (defined as lack of prenatal care; lack of early maternal HIV testing; lack of prenatal, intrapartum, or infant antiretroviral prescriptions; failure to perform scheduled cesarean delivery for eligible women; and breastfeeding by HIV-infected women) were present in 74% of perinatally HIV-infected infants (and 52% of uninfected but perinatally HIV-exposed infants) among 8000 births in 15 US jurisdictions during 2005 to 2008. Although substantial challenges remain in ensuring universal prevention, identification, and appropriate therapy of HIV infection among girls and women of childbearing age, near elimination of US MTCT

is achievable with current initiatives such as universal HIV opt-out testing of adolescents, adults, and pregnant women; early treatment of HIV-infected men and women to reduce transmission to heterosexual partners; and solidification of standard ART for infected pregnant women during antenatal and intrapartum periods, along with infant prophylaxis.[1,5,6,17] Residual MTCT from acute HIV infection acquired late in pregnancy or during breastfeeding of previously uninfected women or from transmission of antiretroviral-resistant HIV will likely make achieving zero cases of perinatal HIV transmission impossible, but reductions of another order of magnitude from the current 100 to 200 infected births per year is feasible.

MANAGEMENT OF THE HIV-EXPOSED INFANT

General Measures

Decisions surrounding mode of delivery—avoidance of the routine use of fetal scalp electrodes for fetal monitoring and, if possible, avoiding the use of forceps or vacuum extraction—are made at delivery.[5,6] In the United States and other resource-rich areas where safe and affordable infant formula is available, breastfeeding is contraindicated except in extraordinary circumstances (see earlier).[5,31,60–62] Standard (universal) precautions are indicated in the delivery room and nursery; once the infant's condition has been stabilized, bathing, administration of vitamin K, and ophthalmia neonatorum prophylaxis are performed, as is routine with non–HIV-exposed infants.

Antiretroviral Agents

All newborns exposed to HIV should receive antiretroviral drugs in the neonatal period to reduce perinatal transmission of HIV; selection of the appropriate type of antiretroviral regimen is guided by the level of transmission risk as judged by consideration of factors such as maternal use of cART and maternal viral load at delivery, maternal health, mode of delivery of the infant, and gestational age at delivery. Current thinking is that HIV MTCT risk falls along a spectrum, and that consequently different antiretrovirals are indicated for different scenarios of infant perinatal HIV exposure.[5,6] The risk of MTCT is greater for infants born to:

- Mothers who received neither antepartum nor intrapartum antiretroviral drugs
- Mothers who received only intrapartum antiretroviral drugs, or who initiated antiretroviral drugs in only the late second or the third trimester of pregnancy
- Mothers who received antepartum and intrapartum antiretroviral drugs, but who have detectable viral load near delivery (usually defined as confirmed HIV RNA level above the lower limits of detection of an ultrasensitive assay, although some experts would use a threshold of >400–1000 copies/mL)
- Mothers with acute or primary HIV infection during pregnancy or breastfeeding
- Mothers with unknown HIV status who test positive at delivery (or whose newborns have a positive HIV antibody test result)

An infant born to a mother living with HIV infection who received cART throughout pregnancy, had sustained viral load suppression (below detectable RNA level) near delivery, and who had no concerns related to ART adherence is considered to be at low risk of perinatal HIV acquisition.

Historically, the use of antiretroviral drugs in the newborn period was referred to as *antiretroviral prophylaxis*; recently, the different scenarios of risk noted earlier have generated the terminology of neonatal prophylaxis, empirical treatment, and treatment of infants for HIV infection.[5,6] In this context, *neonatal antiretroviral prophylaxis* refers to the administration of antiretroviral drugs to a newborn without confirmed HIV infection to reduce the risk of HIV acquisition. Antiretroviral prophylaxis may include the routine administration of a *single agent*, usually zidovudine, or *combinations* of two (zidovudine and nevirapine) antiretroviral drugs (or even three antiretrovirals, such as zidovudine, lamivudine, and either nevirapine or raltegravir). *Empirical HIV therapy* refers to the administration of a three-drug combination antiretroviral regimen to newborns at highest risk of HIV acquisition, intended primarily to serve as early treatment for a newborn who is likely later confirmed to have acquired HIV; it also serves as antiretroviral prophylaxis against HIV acquisition for those newborns who are exposed to HIV in utero, during the birthing process, or during breastfeeding and who do not acquire HIV. Finally, the term *HIV therapy* is used to denote administration of a three-drug combination antiretroviral regimen to newborns with HIV infection confirmed through early birth detection (see later section on infant diagnosis). It is noteworthy that, with the important exception of nevirapine, the neonatal antiretroviral dosing for prophylaxis, empirical therapy, and therapy is the same as that for treatment with all antiretroviral drugs currently recommended for newborns. For those infants with documented HIV infection, therapy will be given lifelong per current practice guidelines.[5,6,18] As newer agents or newer data become available for use in newborns, it is possible that other additional differences in dosing will emerge.

The precise interval during which newborn antiretroviral prophylaxis or empirical HIV therapy can be initiated and still be of benefit is undefined; however, most studies support providing prophylaxis as early as possible after delivery, preferably within the first 6 to 12 hours of life.[5,6]

Table 127.2 provides an overview of neonatal antiretroviral management according to risk of perinatal HIV in the newborn. Data supporting these recommendations are discussed in the US Department of Health and Human Services (DHHS) Pediatric Antiretroviral Guidelines.[5] Single- and multiple-agent antiretroviral prophylaxis recommendations have been derived from both randomized controlled clinical trials (e.g., the PACTG 076 and National Institute of Child Health and Human Development–HIV Prevention Trials Network [NICHD-HPTN] 040/PACTG 1043 trials) and observational studies.[5] Recommendations for empirical therapy have been formulated mainly by consensus, after the realization that infected infants given cART very early in infancy can suppress HIV replication and even lessen their viral reservoir, although the infection is not cured.[5] Empirical therapy recommendations are consistent with use of postexposure prophylaxis and are being studied in current clinical trials.[5]

Table 127.3 summarizes the dosing recommendations for antiretroviral agents in newborns. Additional information about dose selection for newborns, including premature infants (<37 weeks' gestational age), can be found in the guidelines[5] and by contacting the National Perinatal HIV Hotline (888-448-8765), a federally funded service providing free clinical consultation for difficult cases to providers caring for pregnant women living with HIV and their newborns.

Diagnosis of HIV Infection in the HIV-Exposed Infant

Proper use of diagnostic tests for pediatric HIV infection is heavily dependent on the age of the child (also see Chapter 120).[4,5,17,63] Beyond the age of 18 months, standard serologic testing evaluations with a fourth-generation antibody-antigen immunoassay that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen, followed by (if reactive) a supplemental antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies, may be performed, as for an adult.[5,17,63]

In contrast, infants born to HIV-seropositive women pose a special diagnostic challenge because they are seropositive at birth, whether or not they are infected, owing to transplacental passage of maternal antibody to HIV. Because maternal antibody is detectable in the infant for as long as 18 months after birth, tests that rely on detection of HIV antibody are not useful for diagnosis in children younger than 18 months.[4,5,17] Rather, tests that detect the presence of the virus itself must be performed. Nucleic acid tests (NATs), including DNA polymerase chain reaction (PCR) and RNA assays such as reverse-transcriptase PCR or transcriptase-mediated amplification assays are the most widely available of these tests and are equally preferred for the diagnosis of HIV infection in infants.[5,17,63] Recommended testing of infants born to HIV-infected women includes two or three HIV NATs, performed at ages 14 to 21 days, 1 to 2 months, and 4 to 6 months (Fig. 127.2).[5,17] Some experts perform an additional NAT in the first 48 hours of life to identify infected infants early, especially in infants at higher risk for in utero infection (e.g., no antenatal antiretroviral treatment or high level of maternal viremia in late pregnancy). Many such high-risk infants will also be those receiving enhanced antiretroviral prophylaxis regimens (i.e., multiagent antiretroviral prophylaxis or empirical HIV treatment),

TABLE 127.2 Newborn Antiretroviral Management According to the Risk of HIV Acquisition

CATEGORY OF RISK GROUP	DESCRIPTION OF RISK GROUP	NEONATAL ARV MANAGEMENT *(CURRENT TERMINOLOGY)*[b]
Low risk of perinatal HIV transmission	Mothers received standard cART during pregnancy with sustained viral suppression near delivery and no concerns related to ARV drug adherence	*(Neonatal single agent ARV prophylaxis)* 4 weeks of ZDV
Higher risk of perinatal HIV transmission	Mothers who received neither antepartum nor intrapartum ARV drugs Mothers who received only intrapartum ARV drugs, or who initiated ARV drugs in only the late second or third trimester of pregnancy Mothers who received antepartum and intrapartum ARV drugs, but who have detectable viral load near delivery Mothers with acute or primary HIV infection during pregnancy or breastfeeding	*(Neonatal combination agent ARV prophylaxis)* 6 weeks of ZDV PLUS three doses of NVP, given within 48 h of birth, 48 h after first dose, and 96 h after second dose (NVP given at prophylaxis dose) OR *(Empirical HIV therapy)* 6 weeks of three-drug combination—either ZDV + 3TC + NVP (NVP given at prophylactic dose) or ZDV + 3TC + RAL
Presumed newborn HIV exposure	Mothers with unknown HIV status who test positive at delivery or postpartum, or whose newborns have a positive HIV antibody test	*(Empirical HIV therapy)* (some experts would choose to use *Neonatal combination agent ARV prophylaxis* in this scenario)
Newborn with confirmed HIV infection	Newborn with confirmed positive HIV NAT result	*(HIV therapy)* cART with ZDV + 3TC + NVP (treatment doses) or ZDV + 3TC + RAL

[a]Modified from Panel on Antiretroviral Therapy and Medical Management of Children Living with HIV. US Department of Health and Human Services guidelines for the use of antiretroviral agents in pediatric HIV infection, December 14, 2018. https://aidsinfo.nih.gov/. Accessed December 14, 2018.
[b]For explanation of current terminology, see text. All ARVs are to be given to infant as soon as possible after delivery, preferably within the first 6 to 12 hours of life. Dosing information given in Table 127.3.
ARV, Antiretroviral; *cART,* combination antiretroviral therapy; *HIV,* human immunodeficiency virus; *NAT,* nucleic acid test; *NVP,* nevirapine; *RAL,* raltegravir; *3TC,* lamivudine; *ZDV,* zidovudine.

TABLE 127.3 Newborn Antiretroviral Dosing Recommendations

DRUG	ORAL DOSING
ZDV (zidovudine): Prophylaxis and treatment dosage *Note:* ZDV is the only agent available as an intravenous formulation for newborns unable to tolerate oral agents. The intravenous dose is 75% of the oral dose, administered at the same intervals.	*≥35 weeks' gestation at birth* Birth to age 4–6 weeks: • 4 mg/kg/dose twice daily *≥30 to <35 weeks' gestation at birth* Birth to age 2 weeks: • 2 mg/kg/dose twice daily *Age 2 weeks to 4–6 weeks:* • 3 mg/kg/dose twice daily *<30 weeks' gestation at birth* Birth to age 4 weeks: • 2 mg/kg/dose twice daily Age 4–6 weeks: • 3 mg/kg/dose twice daily
3TC (lamivudine): Prophylaxis and treatment dosage	*≥32 weeks' gestation at birth* Birth to age 4 weeks: • 2 mg/kg/dose twice daily Age 2 weeks to 4–6 weeks: • 4 mg/kg/dose twice daily
NVP (nevirapine): Prophylaxis dosage	*Birth weight 1.5–2 kg* • 8 mg/dose once daily (actual dose, NOT mg/kg) *Birth weight >2 kg* • 12 mg/dose once daily (actual dose, NOT mg/kg)
NVP: Treatment dosage	*≥37 weeks' gestation at birth* Birth to age 6 weeks: • 6 mg/kg/dose twice daily *34–37 weeks' gestation at birth* Birth to age 1 week: • 4 mg/kg/dose twice daily Age 1–6 weeks: • 6 mg/kg/dose twice daily
RAL (raltegravir): Prophylaxis and treatment dosage	*≥37 weeks' gestation and ≥2 kg at birth* Birth to age 1 week: • 1.5 mg/kg/dose once daily Age 1–4 weeks: • 3 mg/kg/dose twice daily Age 4–6 weeks: • 6 mg/kg/dose twice daily

Modified from Panel on Antiretroviral Therapy and Medical Management of Children Living with HIV. US Department of Health and Human Services guidelines for the use of antiretroviral agents in pediatric HIV infection, December 14, 2018. https://aidsinfo.nih.gov/. Accessed December 14, 2018.

leading some experts to recommend that an additional NAT be performed 2 to 4 weeks after cessation of antiretroviral administration (i.e., at 8–10 weeks of age), because of the theoretical possibility that early multiagent antiretroviral use may decrease the diagnostic sensitivity of NAT assays (see Fig. 127.2).[5] Any positive NAT result constitutes presumptive evidence of HIV infection; a second diagnostic test should be performed immediately, using either the same assay on a different blood sample or one of the other assays (i.e., RNA assay if DNA PCR assay was used, or DNA PCR assay if RNA assay was used) on the original sample to confirm the diagnosis. Two or more negative NAT results (and the absence of any other positive NAT result), both performed at 2 or more weeks of age and at least one performed at 1 or more month of age, are sufficient to *reasonably* exclude HIV infection in the nonbreastfed infant. Two or more negative NAT results (and the absence of any other positive NAT result), one performed at 4 or more weeks of age and one performed at 4 or more months of age, are sufficient to *definitively* exclude HIV infection in the nonbreastfed infant.[5,17] Some experts continue to perform a serologic test (enzyme immunoassay) at 15 to 18 months to confirm the absence of HIV infection (seroreversion), even in those children with all negative NAT results in infancy.

ADOLESCENT HIV INFECTION

Prevention of Infection

The routes of transmission of HIV in adolescents are similar to those in adults (mostly sexual contact and, to a smaller extent, intravenous drug use), such that prevention goals are similar to those used in adult medicine—serologic testing, counseling on safer sex practices, pregnancy planning, evaluation for use of preexposure prophylaxis (PrEP)[5,17] (see Chapter 119), and avoidance of intravenous drug use. However, the adolescent's ongoing psychosocial development, independence in seeking and following health care recommendations, and frequent difficulty in modifying current behavior for future benefits may complicate prevention efforts and necessitate a different focus from that used for adults.[5,13,14–16,17–18] In addition, special focus on the increasing demographic of young men who have sex with men, especially those of ethnic and racial minorities, may be required to successfully decrease new adolescent HIV infections.[46]

Issues in the Care of Aging HIV-Infected Youth

Disclosure of the HIV Diagnosis

Disclosing to a child that he or she has had HIV infection since birth is simultaneously one of the most difficult and important aspects of

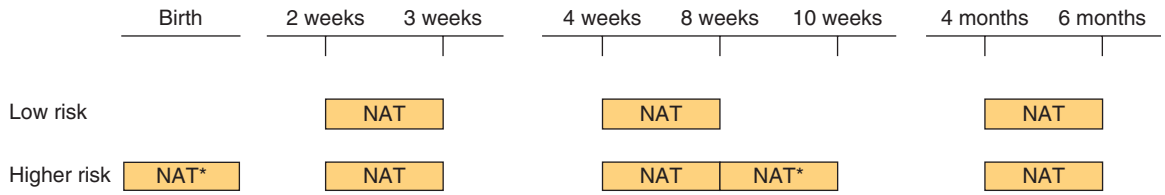

FIG. 127.2 Recommended virologic testing schedule for infants exposed to HIV by perinatal transmission risk. Low Risk: Infants born to mothers who received standard antiretroviral therapy *(ART)* during pregnancy with sustained viral suppression (usually defined as confirmed HIV RNA level below the lower limits of detection of an ultrasensitive assay) and no concerns related to maternal adherence. Higher Risk: Infants born to mothers living with HIV who did not receive prenatal care, did not receive antepartum or intrapartum antiretrovirals *(ARV),* received intrapartum ARV drugs only, mothers who initiated ART late in pregnancy (late second or third trimester), were diagnosed with acute HIV infection during pregnancy, who had detectable HIV viral loads close to the time of delivery, including those who received combination ARV drugs and did not have sustained viral suppression. *For higher-risk infants, additional virologic diagnostic testing should be considered at birth and 2 to 4 weeks after cessation of ARV prophylaxis (i.e., at 8–10 weeks of life). *NAT,* nucleic acid test. *(Modified from Panel on Antiretroviral Therapy and Medical Management of Children Living with HIV. US Department of Health and Human Services guidelines for the use of antiretroviral agents in pediatric HIV infection, December 14, 2018.* http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf. *Accessed December 14, 2018.)*

caring for such children. The process for disclosure should be discussed and planned with the parents (or caretakers) and generally represents a process that takes place over many visits and several years, based on multiple factors, including the child's developmental level and questions about reasons for medications and medical visits, the child's health status, the approaching age of potential sexual debut, and the parent's or caretaker's readiness for disclosure to the child.[5,17,64–68] Parents' concern about revealing their own HIV status when disclosing information about HIV to their infected children can contribute to reluctance and delays in disclosure to children.[65] Observational studies show that most children learn of their diagnosis at 8 to 10 years of age.[64,67,68] However, delayed disclosure is unfortunately common, even occurring after sexual debut in some cases.[66] It is important and developmentally appropriate to disclose perinatal HIV infection no later than early adolescence to more fully engage youth in their own care goals, enable them to discuss their HIV status with sexual partners, and allow them to draw on social support to improve their health outcomes.[66,67] Research gaps and remaining challenges in this important area of research and care have been reviewed.[68]

Care Refusal and Low Adherence Among HIV-Infected Youth

The advent of effective cART has transformed perinatal HIV infection from a progressive, fatal infection into a chronic disease.[5,14–16,17,18] In addition to the concern for the development of cardiovascular and other long-term complications, survivors of perinatal HIV infection must also confront the usual challenges of adolescence and adulthood faced by peers without HIV infection.

Despite frequent contact with the pediatric medical care system, HIV-infected adolescents are often inexperienced with adult health care provision systems, may fear or deny their HIV infection, and may distrust any information given to them by the medical establishment.[5] Adolescents may also have complex biopsychosocial issues such as low self-esteem, chaotic and unstructured lifestyles, peer pressures not to be singled out with illness, and a lack of family support, all of which may reduce adherence with prescribed therapy.[5,17] The concrete thought processes of adolescents might make it difficult for them to understand why medications are necessary during periods of asymptomatic infection, especially if they have experienced any adverse effects of medication. Adolescents with perinatal HIV infection have often developed substantial antiretroviral resistance after serial, nonsuppressive regimens through childhood and continue to struggle with medication adherence.[69] Finally, in adolescents with behaviorally acquired HIV infection, ART is less likely to be initiated even in the era of universal treatment recommendations, and adolescents in adult care sites are more likely to discontinue ART than those receiving care at pediatric and adolescent clinics.[70,71]

Treatment regimens for adolescents must balance the goal of prescribing a maximally potent antiretroviral regimen with a realistic assessment of existing and potential support systems for the adolescent to adhere to such a regimen.[5,18] Rather than focusing simply on choosing "the right antiretroviral regimen" and avoidance of opportunistic illnesses, care plans for HIV-infected youth may require a strong focus on school success and career choices, reproductive health and plans for marriage and families, and transition to adult medical care.

Transition of HIV-Infected Youth From the Pediatric Care Model to the Adult Care Model

Transition to the adult medical care model takes time and advance planning because the pediatric and adult chronic-care models are different.[5,17,72–75,76] Transition is "a multifaceted, active process that attends to the medical, psychosocial, and educational or vocational needs of adolescents as they move from the child-focused to the adult-focused health-care system."[73] Care models for children and adolescents with perinatally acquired HIV tend to be family centered, consisting of a multidisciplinary team of physicians, nurses, social workers, and mental health professionals. Many adolescents have had long-standing relationships with pediatric clinic staff and are often reluctant or anxious about adjusting to a new provider. In the pediatric setting, they have depended on substantial psychosocial support and are accustomed to a family-oriented setting that is not as easily externally identified as a clinic for treating people with HIV infection as are many adult care settings. Most adolescents are unfamiliar with the typical adult care model, which tends to be more individual centered, manages higher patient volumes, and has stricter consequences for lateness and missed appointments. In addition, the transition to adult care is often complicated by loss of insurance coverage that was relatively stable before adulthood.[74,75] Opportunities for the pediatric care provider and adult medical care provider to communicate early about timing of and expectations for transition of adolescents can lead to a smoother process. Pediatric care providers should have a formal plan to transition adolescents to adult care that considers factors such as adherence and HIV clinical status, presence of cognitive impairment or mental illness, level of family or other social support, financial and housing stability, and history of incarceration. Several groups have proposed specific models and approaches for transition programs.[74,76]

ADULT COMPLICATIONS OF PERINATAL HIV INFECTION

In many respects, the long-term complications of perinatal HIV infection and its treatment will be similar to those for HIV infection acquired in adulthood. However, there may be additional effects of exposures to HIV itself or to ART during critical periods of growth and development that lead to unique or more severe adverse outcomes as perinatally infected youth age through adulthood.

Many perinatally infected adolescents have had impaired growth during long periods of poorly controlled HIV infection in earlier childhood before the advent of combination antiretroviral regimens. As a result, their final adult stature is likely to be permanently shorter than their genetic potential, despite recovery of body mass.[77–80]

Similarly, perinatal HIV infection is associated with higher risk for poor bone mineral accrual during the first 20 years of life, when bone

mass normally is accrued to produce the early adulthood peak; the resulting compromise of peak bone mass may put these young adults at higher risk for fractures and osteoporosis at an early age.[81]

It is established that cardiovascular risk factors in childhood predict cardiovascular disease in adulthood and that atherosclerosis has a clinically silent onset early in life.[82–84] It has also been reported that HIV infection in adults appears to be an independent risk factor for cardiovascular disease, even with effective ART.[85] Taking these observations together, there is substantial concern that the prolonged exposure to dyslipidemia, chronic inflammation, and other cardiovascular risk factors related to HIV and its treatment throughout childhood may create particularly early onset and high risk for cardiovascular disease as perinatally HIV-infected youth reach adulthood.

Although active cART has drastically lowered the rate of pediatric HIV encephalopathy, the rates of cognitive deficits, attention deficits, and psychiatric disorders appear to remain higher than expected among perinatally HIV-infected youth.[15] Determining an appropriate comparison group confounds these analyses, but there are data supporting significantly higher rates of behavioral, educational, and pharmacologic interventions among perinatally infected youth.[86] In addition, perinatally infected youth 6 to 17 years of age have notable cognitive, academic, and social functioning deficits despite being in relatively good HIV virologic and immunologic control.[87]

Female adolescents with HIV infection have a high rate of cervical dysplasia (approaching 60%) and should undergo cervical Papanicolaou testing at HIV diagnosis, in 6 months, and then (if results are normal) yearly, rather than waiting until 21 years of age.[16] Contraceptive counseling regarding estrogen-containing oral contraceptives is complicated by the use of some concurrent antiretroviral agents. More than 100 pregnancies among US adolescent and young adult women with perinatally acquired HIV infection now have been reported.[66,88] Common but not consistent features among these reported pregnancies include greater rates of preterm birth, cervical dysplasia, and sexually transmitted infections, although not increased MTCT of HIV.[66,88]

The actual risks for adverse long-term growth, bone, cardiovascular and other health outcomes are not completely known, because the first wave of perinatally infected youth has just reached early adulthood. Thus, it will be important for adult medical care providers to be able to identify which of their adult patients acquired HIV infection perinatally, to monitor them appropriately, and to continue to research long-term complications of perinatally acquired HIV infection.

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128 Antiretroviral Therapy for Human Immunodeficiency Virus Infection

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SHORT VIEW SUMMARY

DEFINITION
- Treatment of human immunodeficiency virus (HIV) infection uses a combination of at least three drugs to arrest virus replication and disease progression.

THERAPY CHOICES
- Antiretroviral therapy targets and inhibits HIV-specific enzymes.
  - Nucleoside and nucleotide reverse-transcriptase inhibitors (see Table 128.1)
  - Nonnucleoside reverse-transcriptase inhibitors (see Table 128.2)
  - Protease inhibitors (see Table 128.3)
  - Integrase inhibitors (see Table 128.4)
- Maraviroc and ibalizumab are the only drugs to target a host protein.
  - Entry inhibitors (see Table 128.4)

THERAPY STRATEGIES
- Preferred regimens will generally use two nucleoside reverse-transcriptase inhibitors (one of which is lamivudine or emtricitabine) in combination with an integrase strand transfer inhibitor.

THERAPY GOALS
- The goal of antiretroviral therapy, in all patients, is durable suppression of viral load to levels undetectable in commercial assays.

In June of 1981, the description of five cases of acquired immunodeficiency in the United States began our modern understanding of the human immunodeficiency virus (HIV) epidemic.[1] While HIV was first recognized clinically in the 1980s, recent work has shown that the virus may have been introduced into the United States through New York City around 1970.[2] Although HIV prevention and vaccine development efforts have continued since that time, the rapid advances made in the development and implementation of combination antiretroviral therapy (ART) have transformed care of the patient with HIV. In the developed world, substantial reductions in HIV-associated morbidity and mortality have changed an HIV diagnosis from a potential death sentence into a manageable chronic illness.[3] The life expectancy of adults with well-controlled HIV infection is beginning to approximate the life expectancy of uninfected persons.[4] These successes in developed nations should not obscure the magnitude of the ongoing worldwide HIV epidemic, because dedicated efforts to export these therapeutic advances to developing and impoverished nations have often met financial, organizational, and, in some cases, political, obstacles. Since the US Food and Drug Administration approval of zidovudine in 1987, antiretroviral agents have been developed that target many of the critical steps in the HIV replication cycle: entry, reverse transcription, integration, and proteolytic processing (Fig. 128.1). The preponderance of clinical evidence now demonstrates the superiority of integrase inhibitor–containing regimens as first-line therapy for HIV infection. While there are still roles for protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) in certain clinical situations, they are now less likely to be used as part of an initial treatment regimen. The clinical use of combination HIV ART requires attentive consideration of the pharmacology of, the clinical experience with, and the emergence of resistance to antiretroviral agents.

ANTIRETROVIRAL AGENTS

Nucleoside and Nucleotide Reverse-Transcriptase Inhibitors

Nucleoside and nucleotide reverse-transcriptase inhibitors (NRTIs) block the RNA-dependent DNA polymerase—reverse transcriptase (RT)—from synthesizing viral complementary DNA (cDNA) from HIV RNA. Drugs from this class were the first antiretrovirals to enter clinical use but, because of drug-associated toxicities, these early agents have largely been replaced in clinical practice by newer drugs with improved therapeutic, toxicity, and dosing profiles. All drugs in this class are analogues of native nucleotides, and almost all of them share the common motif of lack of a 3′-hydroxyl (OH) group on their ribose ring, which prevents the addition of nucleotides to the elongating proviral DNA strand; this effectively terminates proviral DNA synthesis (Fig. 128.2 and Table 128.1). The structural exception is tenofovir, which causes chain termination because of the lack of an intact ribose moiety.

Drugs in this class must be phosphorylated by intracellular kinases into their active triphosphate form before they can effectively inhibit RT, and all of these agents can, to a much lesser extent, inhibit the activity of normal cellular DNA polymerases—most notably the mitochondrial DNA (mtDNA) polymerase-γ (pol-γ). This NRTI-associated inhibition of mitochondrial function and replication accounts for certain drug-specific adverse effects, for instance, hyperlactatemia, lactic acidosis, hepatic steatosis, peripheral neuropathy, myopathy, and lipoatrophy. The dideoxynucleoside RT inhibitors exhibit the tightest binding to and the most inefficient exonucleolytic removal ("proofreading") from DNA pol-γ; this leads to the greatest degree of mtDNA synthesis inhibition via chain termination.[5–7] The rank order of NRTIs associated with mitochondrial dysfunction is dideoxyinosine > stavudine > zidovudine ≫ lamivudine-emtricitabine = abacavir = tenofovir. Lamivudine, emtricitabine, abacavir, and tenofovir are the NRTIs least likely to be associated with adverse drug effects resulting from mitochondrial dysfunction.

Lamivudine-Emtricitabine

Lamivudine [(−)2′,3′-dideoxy-3′-thiacytidine; 3TC] is a cytosine analogue with activity against HIV and hepatitis B virus (HBV). Emtricitabine (2′,3′-dideoxy-5-fluoro-3′-thiacytidine; FTC) is chemically related to lamivudine. Given their similar activities and toxicities, these drugs are discussed together. Of importance, however, these drugs should not be used together clinically. Lamivudine and emtricitabine can be taken with or without food and require dose reductions in patients with renal insufficiency. These drugs have minimal DNA pol-γ inhibitory effects.

Lamivudine or emtricitabine forms part of the backbone of nearly all currently recommended antiretroviral regimens. Emtricitabine is available in several fixed-dose combinations with other antiretroviral drugs, including tenofovir, efavirenz, rilpivirine, and elvitegravir. Lamivudine is available coformulated with abacavir, with zidovudine, or with both abacavir and zidovudine. Comparisons of lamivudine and emtricitabine

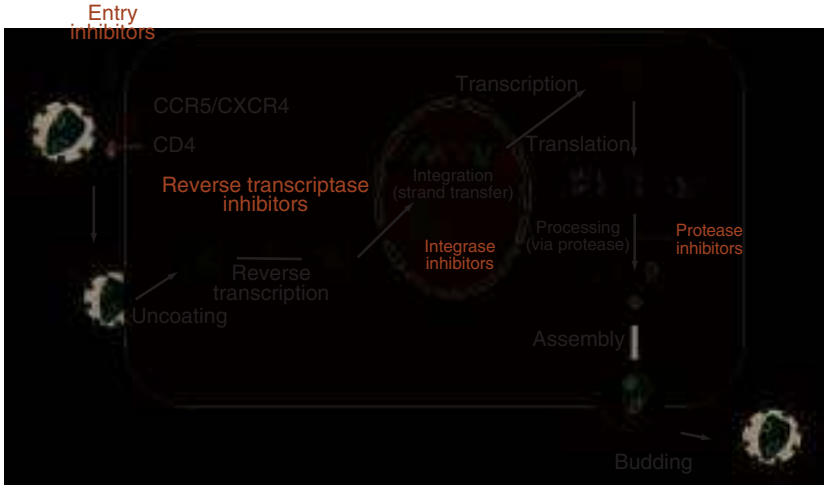

FIG. 128.1 The human immunodeficiency virus replication cycle. Combination antiretroviral therapy disrupts multiple viral processes to suppress replication. Fusion inhibitors can block the virus-coreceptor interaction and late stages of virus–cellular membrane fusion. Reverse transcription in the cytoplasm is inhibited by structural analogues of native nucleotides/nucleosides. Newly formed viral complementary DNA is incorporated into preintegration complexes and actively transported into the nucleus. Viral integrase catalyzes both the cytoplasmic processing of viral DNA and chromosomal integration (known as strand transfer); integrase inhibitors block strand transfer. Once viral DNA is transcribed and translated, homodimeric viral proteases cleave and process polypeptides into mature virions; protease inhibitors render these virions noninfectious. Virions bud from the infected cellular surface and are released, continuing the cycle. *CCR5,* C-C motif of chemokine receptor 5; *CXCR4,* C-X-C chemokine receptor 4.

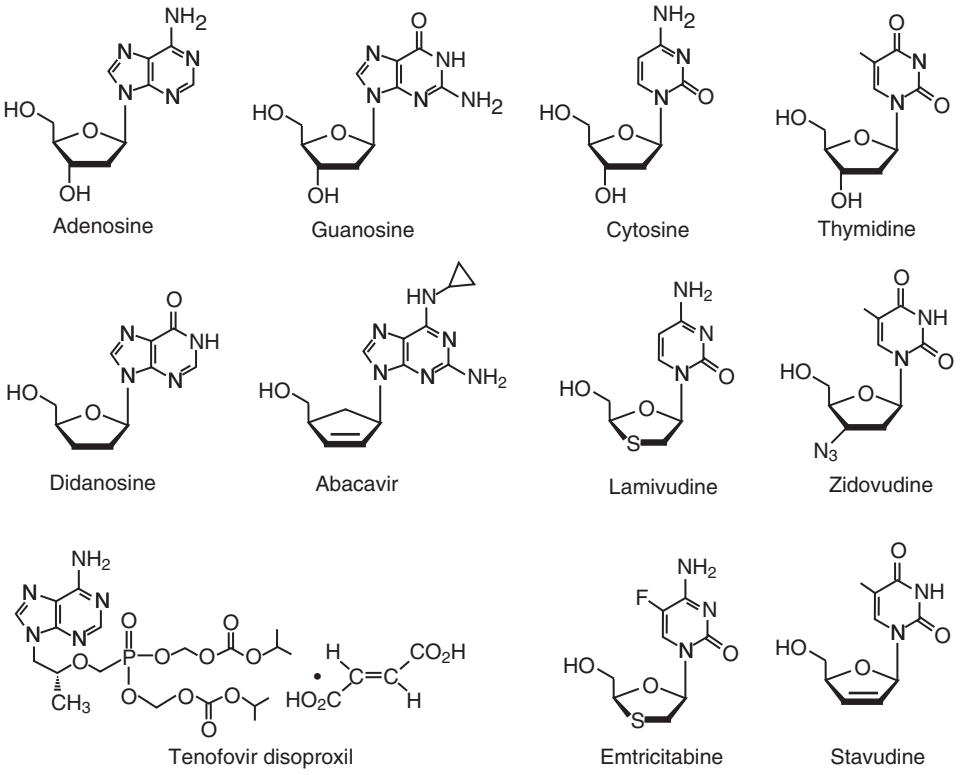

FIG. 128.2 Nucleoside and nucleotide analogue reverse-transcriptase inhibitors. Shown on top are the naturally occurring nucleosides (adenosine, guanosine, cytosine, and thymidine); below each of them are analogues used in antiretroviral therapy.

suggest the two drugs are clinically equivalent.[8] In general, regimens with an emtricitabine–tenofovir disoproxil fumarate (TDF) NRTI backbone have outperformed regimens with a zidovudine-lamivudine backbone.[9]

Lamivudine and emtricitabine are each well tolerated. Headache, nausea, fatigue, and neutropenia have been reported. Care should be taken when discontinuing lamivudine or emtricitabine in patients coinfected with HBV because HBV rebound and worsening hepatitis can occur.

High-level resistance to lamivudine and emtricitabine, up to 1000-fold, is conferred by a point mutation at position 184 in the *RT* gene (M184V). This mutation comes with a fitness cost to HIV that results in a 0.3 to 0.6 $\log_{10}$ reduction in plasma HIV RNA levels.[10,11] This fitness cost to HIV is often exploited clinically by continuing lamivudine or emtricitabine therapy in patients harboring virus with the M184V mutation. M184V can also hypersensitize HIV to inhibition by zidovudine, restore sensitivity to zidovudine in the presence of thymidine analogue mutations (TAMs),[12] and delay or prevent the emergence of TAMs.[10]

TABLE 128.1 Approved Nucleoside and Nucleotide Reverse-Transcriptase Inhibitors

AGENT	TRADE NAME	ORAL BIOAVAILABILITY (%)	SERUM HALF-LIFE (hr)	INTRACELLULAR HALF-LIFE OF TRIPHOSPHATE (hr)	ELIMINATION	ADULT DOSE[a]	DOSAGE FORMS
Zidovudine	Retrovir	64	1.1	3–4	Hepatic glucuronidation Renal excretion	300 mg twice daily During labor and delivery: 2-mg/kg IV loading dose, followed by 1 mg/kg/h until umbilical cord is clamped	300-mg tablet 100-mg capsule 50-mg/5-mL syrup 10-mg/mL solution for IV infusion
Didanosine	Videx Videx EC	40 fasted	1.5	25–40	Cellular metabolism	≥60 kg: 400 mg once daily 25 kg < weight < 60 kg: 250 mg once daily	125-, 200-, 250-, and 400-mg capsules 2- and 4-g powder packets (make 20-mg/mL solution)
Stavudine	Zerit	86	1.1	3	Renal excretion	≥60 kg: 40 mg twice daily <60 kg: 30 mg twice daily	15-, 20-, 30-, and 40-mg capsules 1-mg/mL oral solution
Lamivudine	Epivir	86	2.5	12–18	Renal excretion	300 mg once daily or 150 mg twice daily	100-, 150- and 300-mg tablets 5- and 10-mg/mL solutions
Abacavir	Ziagen	83	1.5	3.3	Hepatic glucuronidation and carboxylation	600 mg once daily or 300 mg twice daily	300-mg tablet 20-mg/mL solution
Tenofovir disoproxil fumarate (TDF)	Viread	39 with meal	12–14	>11[b]	Renal excretion	300 mg once daily	150-, 200-, 250-, and 300-mg tablets 40-mg/g oral powder
Emtricitabine	Emtriva	93	8–10	>24	Renal excretion	200 mg once daily Solution: 240 mg once daily	200-mg capsule 10-mg/mL solution
Zidovudine + lamivudine	Combivir[c]					One tablet twice daily	Zidovudine 300-mg/lamivudine 150-mg tablet
Abacavir + lamivudine	Epzicom[c] Kivexa[d]					One tablet once daily	Abacavir 600-mg/lamivudine 300-mg tablet
TDF + emtricitabine	Truvada[c]					One tablet once daily	TDF 300-mg/emtricitabine 200-mg, TDF 250-mg/emtricitabine 167-mg, TDF 200-mg/emtricitabine 133-mg, TDF 150-mg/emtricitabine 100-mg tablets
Zidovudine + lamivudine + abacavir	Trizivir[c]					One tablet twice daily	Zidovudine 300-mg/lamivudine 150-mg/abacavir 300-mg tablet
TDF + emtricitabine + efavirenz	Atripla[c]					One tablet once daily on an empty stomach	TDF 300-mg/emtricitabine 200-mg/efavirenz 600-mg tablet
TDF + emtricitabine + rilpivirine	Complera Eviplera[c,d]					One tablet once daily with a meal	TDF 300-mg/emtricitabine 200-mg/rilpivirine 25-mg tablet
TDF + emtricitabine + elvitegravir + cobicistat	Stribild[c]					One tablet once daily with food	TDF 300-mg/emtricitabine 200-mg/elvitegravir 150-mg/cobicistat 150-mg tablet
Tenofovir alafenamide (TAF) + emtricitabine + elvitegravir + cobicistat	Genvoya					One tablet once daily with food	TAF 10-mg/emtricitabine 200-mg/elvitegravir 150-mg/cobicistat 150-mg tablet
TAF + emtricitabine + rilpivirine	Odefsey					One tablet once daily with a meal	TAF 25-mg/emtricitabine 200-mg/rilpivirine 25-mg tablet
TAF + emtricitabine	Descovy					One tablet once daily	TAF 25-mg/emtricitabine 200-mg tablet
Abacavir + lamivudine + dolutegravir	Triumeq[c]					One tablet once daily	Abacavir 600-mg/lamivudine 300-mg/dolutegravir 50-mg tablet
Bictegravir + emtricitabine + TAF	Biktarvy					One tablet once daily	Bictegravir 50-mg/emtricitabine 200-mg/TAF 25-mg tablet

[a]For pediatric dose, see Chapter 127.
[b]Diphosphate form in activated cells.
[c]Pharmacokinetic properties are similar to those of the component drugs used separately.
[d]Trade name as marketed in Europe.

Abacavir

Abacavir—(1*S, cis*)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl)]-2-cyclopentene-1- methanol (ABC)—is a synthetic guanosine analogue administered orally as either 300 mg twice daily or 600 mg once daily. Mild hepatic dysfunction necessitates a decrease in dose to 200 mg twice daily; the use of abacavir in patients with moderate-to-severe hepatic dysfunction is contraindicated. The use of abacavir with ribavirin or ganciclovir can increase the likelihood of mitochondrial, hepatic, and pancreatic toxicities. Abacavir is not a substrate for or inhibitor of the cytochrome P-450 (CYP) enzyme complex, can be taken with or without food, and is metabolized primarily by glucuronidation. Abacavir is available as fixed-dose combinations with lamivudine, and with lamivudine and zidovudine.

Until recently, abacavir-lamivudine was typically classified as an alternative nucleoside backbone combination for antiretroviral-naïve patients. However, recent guidelines now include certain abacavir-lamivudine–containing combinations as recommended initial regimens.[13–15] Results from the AIDS Clinical Trials Group (ACTG) 5202 trial that compared TDF-emtricitabine to abacavir-lamivudine, in combination with either efavirenz or ritonavir-boosted atazanavir (atazanavir/r), demonstrated reduced virologic responses in participants with initial viral loads greater than 100,000 copies/mL who were randomized to receive abacavir-lamivudine.[16] In participants with baseline viral loads less than 100,000 copies/mL, similar times to virologic failure for the NRTI backbones of abacavir-lamivudine or TDF-emtricitabine were observed when used in combination with efavirenz or atazanavir/r.[17] Abacavir use was associated with a shorter time-to-regimen modification when combined with boosted atazanavir or efavirenz and a shorter time-to-safety event when combined with efavirenz. A similarly designed smaller study also comparing abacavir-lamivudine with TDF-emtricitabine, combined with ritonavir-boosted lopinavir (lopinavir/r), showed no differences in responses among individuals with high initial viral loads.[18] A 48-week study that included prescreening for the human leukocyte antigen (HLA)-B*5701 allele demonstrated superior virologic efficacy of TDF-emtricitabine over abacavir-lamivudine when either was combined with efavirenz.[19] In another trial in treatment-naïve subjects, the addition of abacavir to a three-drug regimen of zidovudine, lamivudine, and efavirenz did not improve treatment results.[20]

Several studies have evaluated switching a patient's NRTI backbone regimen to include abacavir. Substitution of either abacavir or TDF for stavudine or zidovudine in patients with lipoatrophy led to significant increases in limb fat.[21] Two studies that investigated the effects of a switch to TDF-emtricitabine or abacavir-lamivudine from the subject's existing NRTI backbone demonstrated no statistically significant differences in treatment failure rates, increased lipids or cardiovascular events with abacavir, or decreased bone mineral density (without increased fracture rate) associated with tenofovir-containing therapy.[22,23]

Abacavir is a weak inhibitor of DNA pol-γ and exhibits few mitochondrial-associated toxicities. The most significant acute adverse effect of abacavir use is a potentially fatal hypersensitivity syndrome. The presence of fever, abdominal pain, and rash, usually within 2 weeks of starting abacavir, generally requires discontinuation of the drug. This syndrome has been linked to the presence of the HLA-B*5701 allele, found in 5% to 8% of white populations with HIV. Screening for the HLA-B*5701 allele led to reductions in both clinically diagnosed and immunologically confirmed hypersensitivity reactions, and screening is now recommended before considering abacavir therapy.[24] Patients who are HLA-B*5701 positive should not receive abacavir. Data from a large cohort study raised concerns about increased rates of myocardial infarction in patients taking abacavir.[25] An association between abacavir and cardiovascular event risk has subsequently been corroborated, weakly corroborated, or refuted in a series of studies.[26–35] No consensus on abacavir-associated cardiovascular risk has yet been reached. Caution should therefore be exercised when considering abacavir therapy in patients at high risk for cardiovascular disease.

Reduced phenotypic susceptibility and resistance to abacavir can be conferred by the *RT* gene mutations K65R, L74V, Y115F, and M184V.[36] L74V reduced abacavir susceptibility twofold, K65R by roughly threefold, and Y115F by threefold, whereas the M184V mutation in isolation did not reduce abacavir activity unless paired with more than two TAMs.[37–40] The combination of M184V with either L74V or K65R resulted in a fivefold to eightfold decrease in abacavir susceptibility. As is the case for other NRTIs, high-level abacavir resistance accompanies the Q151M complex and T69 insertion mutations.

Tenofovir

Tenofovir is a nucleotide adenosine 5′-monophosphate derivative that, because of poor oral bioavailability, is commercially available in two prodrug forms: tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide fumarate (TAF). Tenofovir has activity against both HIV and HBV. TDF is administered orally at a dose of 300 mg once daily without regard to food and is also available as fixed-dose combinations with emtricitabine, as well as with emtricitabine and either efavirenz, rilpivirine, or elvitegravir-cobicistat. TAF is available in fixed-dose combinations with emtricitabine as well as emtricitabine with either rilpivirine, elvitegravir-cobicistat, or bictegravir. Dosing for TDF should be decreased in patients with renal insufficiency; TAF is not recommended for patients with creatinine clearance less than 30 mL/min. No dose adjustment is needed for hepatic impairment, although TAF has not been studied in patients with severe liver disease. After ingestion, TDF or TAF is hydrolyzed to tenofovir and then phosphorylated and incorporated into HIV DNA, causing chain termination. TAF administration results in higher intracellular concentrations and lower plasma concentrations of tenofovir, when compared to TDF.[40a] Tenofovir has low affinity for cellular DNA polymerases, including the mtDNA pol-γ. The majority of tenofovir is excreted unchanged in the urine via glomerular filtration, although the drug is also actively secreted across the renal tubule. Tenofovir is not a substrate for or inducer of the CYP enzymes but can reduce atazanavir concentrations; ritonavir and atazanavir increase tenofovir exposure. Tenofovir and didanosine should not be coadministered.

Tenofovir prodrugs are now included in many preferred first-line antiretroviral regimens, and can also be used in treatment-experienced patients whose virus lacks the K65R mutation. TDF or TAF, in combination with either lamivudine or emtricitabine, is often used in patients coinfected with HIV and HBV. In general, regimens that include a nucleoside backbone of TDF or TAF and either emtricitabine or lamivudine, along with either a boosted PI, an NNRTI, or an integrase inhibitor, are likely to achieve durable HIV responses.[9,41–44] There are exceptions to this rule. Some patients receiving tenofovir prodrugs in combination with emtricitabine (or lamivudine) and nevirapine have experienced early virologic failure,[45,46] and certain triple nucleoside combinations that include TDF (e.g., TDF-lamivudine-abacavir and TDF-lamivudine-didanosine) have fared less well than some comparator regimens.[47] The triple-NRTI combination of TDF-zidovudine-lamivudine has antiviral activity but has never been compared directly with more standard regimens.[48]

There has been some concern about the cumulative nephrotoxic potential of TDF, given its structural similarity to the nephrotoxic nucleosides adefovir and cidofovir. In an observational cohort, the use of TDF was associated with a greater decline in renal function than was seen with other NRTIs.[49] Decreased glomerular filtration rates (GFRs) in patients receiving TDF were modest within 6 months of starting therapy (–14 mL/min/1.73 m^3) and appeared to stabilize after 18 months (GFR decline of –19 mL/min/1.73 m^3); these findings were not associated with an increased rate of TDF discontinuation.[50] TDF, in combination with boosted PIs, was associated with greater declines in renal function compared with TDF in combination with NNRTIs, although it is not always clear that observed decreases in GFR in patients on TDF are clinically significant.[51] More recent large studies have confirmed an association between TDF and renal dysfunction; 50% of patients after 10 years of TDF therapy had estimated GFRs below 90 mL/min/1.73 m^3.[52,53] Monitoring of renal function in patients receiving TDF (serum creatinine, urinalysis, and urine electrolytes) is warranted. TDF is also associated with decreases in bone mineral density,[54–57] although the precise clinical implications of these findings remain to be fully elucidated. Bone loss associated with a TDF-containing regimen can be mitigated by vitamin D and calcium supplementation.[58] TAF has noninferior virologic efficacy and appears to have less renal and bone toxicity compared to TDF, defined as lower serum creatinine increases,

changes in proteinuria, and percent change in bone mineral density at the hip and spine.[59] Switching from TDF to TAF was associated with maintenance of virologic suppression and improved bone mineral density.[60] However, fasting lipid levels may increase with TAF when compared to TDF. In patients switching from coformulated TDF and emtricitabine to coformulated TAF and emtricitabine, median total, low-density lipoprotein, and triglyceride levels increased by approximately 10 mg/dL; total cholesterol–to–high-density lipoprotein ratios did not change.[60a]. Over 96 weeks, increases over baseline levels in total (29 mg/dL), high-density lipoprotein (6 mg/dL), and low-density lipoprotein (17 mg/dL) cholesterols, along with triglycerides (19 mg/dL), were significantly greater in participants who received the fixed-dose combination of TAF, emtricitabine, elvitegravir, and cobicistat when compared to participants who received the fixed-dose combination of TDF, emtricitabine, elvitegravir, and cobicistat.[60b]

Resistance to tenofovir prodrugs is selected by the K65R mutation and confers a twofold reduction in tenofovir activity. The TAM1 pathway (41L, 210W, 215Y/F) reduces tenofovir susceptibility fourfold, whereas the TAM2 pathway has less of an effect on tenofovir activity.[61–63] Insertion mutations at position 69 confer high-level resistance to the NRTIs, including TDF, whereas tenofovir retains significant activity in the presence of the Q151M complex.[64–66]

Integrase Strand Transfer Inhibitors

After viral entry into a target cell and subsequent uncoating, HIV RNA is reverse transcribed in the cytoplasm. The viral cDNA is then processed and transported to the nucleus, where integration into the cellular genome occurs as a result of strand transfer. HIV integrase catalyzes both the cDNA processing and strand transfer, and integrase strand transfer inhibitors (INSTIs; see Fig. 128.3 and Table 128.2) block the strand transfer reaction, thereby inhibiting HIV types 1 and 2 (HIV-1 and HIV-2) replication.[67] The safety profiles and superior efficacy of INSTI-containing regimens is reflected in their recommended first-line use worldwide.

Raltegravir

Raltegravir was the first approved antiretroviral drug that inhibits the catalytic activity of HIV integrase. Raltegravir is administered orally, 400 mg twice daily with or without food, or as two 600-mg tablets orally once daily. No dose adjustment is recommended for patients with mild-to-moderate hepatic dysfunction or renal insufficiency. Raltegravir is not known to induce or inhibit the CYP enzyme complex, and is primarily metabolized by glucuronidation. Caution is suggested when raltegravir and inducers of glucuronidation enzymes (rifampin, rifabutin, phenytoin, or phenobarbital) are used concurrently. When coadministered with rifampin, the raltegravir dose should be doubled to 800 mg twice daily. Antacids that contain aluminum or magnesium reduce raltegravir absorption; coadministration is not recommended. Calcium carbonate–containing antacids may be coadministered with raltegravir dosed 400 mg twice daily but not with 1200 mg once daily.

Raltegravir may be used in treatment-experienced patients with HIV and is considered a recommended treatment option by several guideline panels, when coadministered with a tenofovir prodrug (TDF or TAF) and emtricitabine.[13–15] The combined results of two similar phase III trials demonstrated a greater suppression to plasma viral loads less than 50 copies/mL through 48 weeks of treatment in chronically infected, treatment-experienced subjects receiving raltegravir compared with placebo, in both groups combined with optimized background therapy.[68,69] In a 48-week study in treatment-naïve subjects, a raltegravir-based regimen resulted in noninferior virologic outcomes to efavirenz-based combination therapy, with fewer central nervous system (CNS) side effects; these benefits persisted over 5 years of raltegravir-based therapy.[70,71]

A raltegravir once-daily regimen, administered as two 600-mg tablets, may be used in treatment-naïve patients with HIV or in virologically suppressed people with HIV who had been receiving a twice-daily raltegravir-containing regimen. In a phase III study of raltegravir 1200 mg once daily versus raltegravir 400 mg twice daily, both in combination with coformulated emtricitabine and TDF, in 797 treatment-naïve people with HIV, noninferior rates of virologic suppression at 48 weeks were observed in participants who received raltegravir 1200 mg once daily.[71a] Mean changes from baseline $CD4^+$ T-cell counts and the proportion of drug-related adverse events were similar between the two groups. The proportion of participants with HIV-1 RNA levels less than 40 copies/mL at 48 weeks was 89% and 88% in participants who received raltegravir 1200 mg once daily or 400 mg twice daily, respectively. Among participants with baseline HIV-1 RNA levels greater than 100,000 copies/mL, rates of suppression with once- or twice-daily raltegravir were similar (87% vs. 84%, respectively). Raltegravir resistance occurred in four participants (1%) randomized

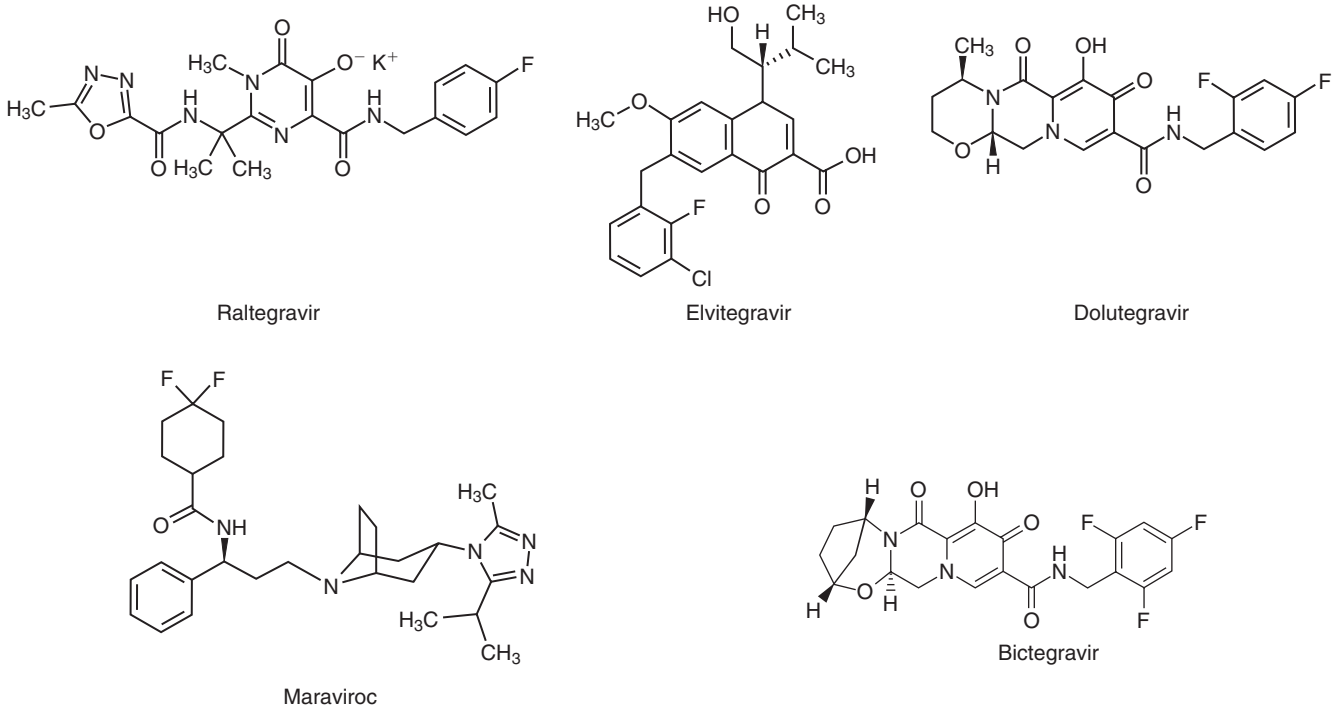

FIG. 128.3 **Entry inhibitor and integrase strand transfer inhibitors.**

TABLE 128.2 Approved Entry Inhibitors and Integrase Inhibitors

AGENT	TRADE NAME	BIOAVAILABILITY (%)	SERUM HALF-LIFE (hr)	ELIMINATION	ADULT DOSE[a]	AVAILABILITY
Enfuvirtide	Fuzeon	84	3.8	Catabolism to constituent amino acids	90 mg subcutaneously twice daily	90-mg single-use vials
Maraviroc	Selzentry Celsentri[b]	33	14–18	Hepatic metabolism via CYP3A	300 mg twice daily	25-, 75-, 150- and 300-mg tablets 20-mg/mL solution
Ibalizumab	Trogarzo	100	64	Intracellular catabolism	2000 mg IV loading dose, then 800 mg IV every 2 wk	150-mg/mL solution
Raltegravir	Isentress	Not established	9	UGT1A1-mediated glucuronidation	Treatment-naïve: 400 mg twice daily or 1200 mg once daily Treatment-experienced: 400 mg twice daily	400- and 600-mg tablets Chewable 25- and 100-mg tablets 100-mg packet
Dolutegravir	Tivicay	Not established	14	UGT1A1-mediated glucuronidation Hepatic metabolism via CYP3A	Treatment- or INSTI-naïve: 50 mg once daily INSTI-experienced or when coadministered with UGT1A/CYP3A inducers: 50 mg twice daily	10-, 25-, and 50-mg tablets

[a]For pediatric dose, see Chapter 127. Elvitegravir is not available as a single drug.
[b]Trade name as marketed in Europe.
CYP3A, Cytochrome P-450 isoenzyme 3A; *INSTI,* integrase strand transfer inhibitor; *UGT1A1,* UDP glucuronosyltransferase 1A1.

to once-daily raltegravir and no participants who received twice-daily raltegravir; concomitant emtricitabine resistance was seen in all four participants with raltegravir resistance mutations. Similar virologic suppression rates were maintained after 96 weeks of follow-up (81% vs. 80%, respectively). The results of this study differ from a prior study of raltegravir dosed at 800 mg once daily that demonstrated inferior virologic efficacy when compared to twice-daily dosing.[71b]

Two raltegravir switching strategies have been explored with disparate results. In a study of treated, virologically suppressed patients on a lopinavir/r regimen, participants were randomized either to continued PI therapy or to a raltegravir regimen.[72] A switch to raltegravir was associated with improved lipid profiles but at a cost of greater virologic failure rates. Raltegravir switching was virologically inferior to maintaining a lopinavir/r regimen. In a second study that enrolled fewer patients with prior virologic failure, a raltegravir switch met noninferiority criteria compared with continuing a boosted PI-based regimen.[73]

Adverse effects that occur more often in raltegravir-treated than placebo subjects have included fatigue, nasopharyngitis, rash, herpes zoster infection, elevations of alanine aminotransferase and aspartate aminotransferase, and increased triglycerides. Increases in creatine kinase, myalgia, and proximal myopathy have been associated with raltegravir use.[74,75] Neuropsychiatric side effects have been reported with raltegravir (e.g., depression and insomnia) but the precise relationship between drug and effect requires further study. HIV resistance to raltegravir is conferred by amino acid substitutions that occur in proximity to the integrase catalytic residues.[67,68] Raltegravir is considered to have a low genetic barrier to resistance; single mutations (E92Q, Q148H, N155H) confer 5-fold to 10-fold decreases in drug sensitivity and contribute to virologic failure.[76]

Elvitegravir

Elvitegravir is an INSTI that is available as the fixed-dose combination of 150 mg elvitegravir, 300 mg TDF, 200 mg emtricitabine, and 150 mg cobicistat or as elvitegravir, cobicistat, emtricitabine, and 10 mg TAF. Elvitegravir absorption is increased with food; fixed-dose combinations should be taken once daily with a meal. Cobicistat is a CYP3A inhibitor with no antiviral activity that is used to enhance exposure to elvitegravir, a CYP3A4 substrate, and is analogous to ritonavir boosting of PIs. Pharmacokinetic boosting extends the half-life of elvitegravir to 9 hours. The fixed-dose combination of elvitegravir that contains TDF should not be used in patients with a creatinine clearance less than 70 mL/min and must be discontinued when the creatinine clearance falls below 50 mL/min. The fixed-dose combination of elvitegravir with TAF should not be used in patients with creatinine clearances below 30 mL/min. Cobicistat inhibits tubular secretion of creatinine and results in a 0.1- to 0.2-mg/dL increase in serum creatinine; GFRs are not affected. No dose adjustments are required in patients with mild-to-moderate hepatic insufficiency. Elvitegravir coformulated with TAF is not recommended in patients with severe hepatic impairment. The fixed-dose combinations of elvitegravir can alter the concentrations of drugs that are metabolized by the CYP2D6 and CYP3A isoenzymes.

The fixed-dose elvitegravir-containing products are currently recommended by the US Department of Health and Human Services (HHS) Antiretroviral Guidelines Panel for initial therapy in certain clinical situations for people with HIV.[14] Two phase III studies demonstrated the noninferiority of coformulated elvitegravir, cobicistat, emtricitabine, and TDF when compared with either coformulated efavirenz, emtricitabine, and TDF or the combination of atazanavir/r and coformulated emtricitabine and TDF after 48 weeks of therapy.[77,78] Elvitegravir coformulated with TDF remained noninferior to the atazanavir/r-containing regimen after 96 weeks.[79] Overall rates of antiretroviral drug resistance were similar between the coformulated elvitegravir-containing and coformulated efavirenz-containing regimens; drug resistance was not observed in the atazanavir/r-containing regimen. Of 13 participants whose virus developed resistance on coformulated elvitegravir, 3 demonstrated triple-class resistance and 9 had mutations to both elvitegravir and emtricitabine (M184I/V). Elvitegravir selects for resistance at positions E92, G140, Q148, and N155 of integrase and should not be used in subjects with prior resistance to raltegravir.

The fixed-dose combination of elvitegravir, cobicistat, emtricitabine, and TAF has been evaluated as initial therapy in two studies in people with HIV and creatinine clearances greater than 50 mL/min.[59] Patients randomized to either TAF or TDF coformulated with elvitegravir, cobicistat, and emtricitabine demonstrated noninferior rates of virologic suppression. At 48 weeks, 92% of participants who received coformulated elvitegravir and TAF and 90% of participants who received coformulated elvitegravir and TDF had plasma HIV-1 RNA levels less than 50 copies/mL. Virologic failure with resistance developed in similar proportions (0.8%–0.6%) in the two arms. Eight of 12 patients with resistance developed primary INSTI mutations, all developed M184V, and 1 selected for K65R. The most common side effects were nausea, diarrhea, and headache. Noninferiority was again seen at week 96, but by week 144, elvitegravir coformulated with TAF demonstrated superior virologic efficacy to elvitegravir coformulated with TDF (84% vs. 80%, with plasma HIV-1 RNA levels <50 copies/mL).[60b,79a]

The most common adverse events associated with coformulated elvitegravir are nausea and diarrhea. Neuropsychiatric adverse events have been reported in people receiving INSTIs, including elvitegravir.[79b]

Clinically relevant drug-drug interactions are possible with the elvitegravir-containing fixed-dose combinations and are mainly due to the CYP3A and CYP2D6 modulation by cobicistat. Antacids reduce cobicistat levels, whereas histamine type 2 (H2) receptor antagonists and proton pump inhibitors do not; the administration of elvitegravir-containing regimens and antacids should be separated by 2 hours. Coformulated elvitegravir is contraindicated in patients taking lovastatin, simvastatin, and rifampin.

Dolutegravir

Dolutegravir is an INSTI that has a higher genetic barrier to resistance than raltegravir or elvitegravir, does not require pharmacologic boosting, and can be taken with or without food.[80] In antiretroviral-naïve patients, dolutegravir has been administered orally as 50 mg once daily; a 50 mg twice-daily dose has been studied in treatment-experienced patients with genotypic resistance to raltegravir.[81,82] Dolutegravir has a half-life of 14 hours, is primarily metabolized by glucuronidation, and does not inhibit or induce CYP isoenzymes.[83] Dolutegravir can inhibit tubular secretion of creatinine, resulting in increased serum creatinine concentrations without changes in glomerular filtration.[84] Polyvalent cations can reduce dolutegravir absorption; dolutegravir-containing regimens should be taken 2 hours before or at least 6 hours after antacids or cation-containing laxatives. Dolutegravir is available in 10-mg, 25-mg, and 50-mg tablets and in a fixed-dose once-daily combination of dolutegravir, abacavir, and lamivudine, and has been coformulated with rilpivirine. Dolutegravir in combination with abacavir-lamivudine or a tenofovir prodrug (TDF or TAF) with emtricitabine is recommended as an initial regimen for antiretroviral-naïve patients.[14,15]

A dose-ranging phase IIb study of dolutegravir 50 mg with the fixed-dose combination of abacavir 600 mg and lamivudine 300 mg demonstrated superiority to a fixed-dose combination of efavirenz, emtricitabine, and TDF.[85] This difference in virologic response was driven primarily by discontinuation of the efavirenz-containing regimen at a higher rate than is typically seen in clinical practice. A phase III study in antiretroviral-naïve participants demonstrated virologic noninferiority of a once-daily dolutegravir regimen compared with twice-daily raltegravir in combination with a two-NRTI backbone.[81] Dolutegravir was superior to ritonavir-boosted darunavir (darunavir/r) when combined with either abacavir and lamivudine or TDF and emtricitabine,[86] and, when combined with abacavir and lamivudine, demonstrated superiority to an efavirenz-containing regimen.[87] Once-daily dolutegravir was associated with statistically significant increases, albeit small, in serum creatinine (0.1 mg/dL) over 48 weeks. Nausea, headache, nasopharyngitis, and diarrhea were observed in similar proportions of participants receiving dolutegravir or raltegravir. A phase III study of treatment-naïve participants with HIV demonstrated the superior efficacy, safety profile, and time to viral suppression of a dolutegravir plus abacavir-lamivudine regimen through 48 weeks when compared with the fixed-dose combination of efavirenz, TDF, and lamivudine[87]; the dolutegravir-containing regimen remained superior at week 144.[87a] In treatment-naïve women with HIV, the fixed-dose combination of dolutegravir, abacavir, and lamivudine demonstrated superior virologic efficacy when compared to a regimen of TDF, emtricitabine, and atazanavir/r.[87b]

The most common adverse reactions to dolutegravir are headache and insomnia. Dolutegravir has been associated in case report series with neuropsychiatric side effects, although the precise mechanism of these effects and their relationship to dolutegravir require further investigation.[79b,87c–87g]

Dolutegravir use in the first trimester of pregnancy may be associated with a risk of neural tube defects.[87h–87j] While a definitive association has not been established and additional clarifying data are anticipated, three HHS Antiretroviral Guidelines Panels have jointly issued interim recommendations on the use of dolutegravir in persons with HIV who are pregnant or of child-bearing potential.[87j] A negative pregnancy test is recommended prior to initiating dolutegravir. Those persons currently receiving dolutegravir should be counseled on the potential risks of neural tube defects within the first 12 weeks after conception. For those on dolutegravir who may be in this window of risk, switching to a non–dolutegravir-containing ART regimen is recommended. Persons who are pregnant and are 8 weeks or more from their last menstrual period may initiate or continue dolutegravir-based regimens. This warning is based on neural tube defects detected in 4 infants born to 426 women in Botswana; data from additional births in 500 women who used dolutegravir since conception are expected in the near future.[87j]

Most major integrase inhibitor resistance mutations decrease dolutegravir susceptibility, including E92Q, G140S/A, and Q148H/R/K. The exception is Q155H; Q155H-containing virus retains in vitro dolutegravir sensitivity.[88] In a study of 24 patients with raltegravir-resistant virus, 75% of participants who received 24 weeks of dolutegravir 50 mg twice daily, with an optimized background regimen, achieved plasma HIV-1 RNA levels less than 50 copies/mL.[82] The presence of more than two INSTI resistance mutations at baseline was associated with virologic failure. Given the limited patient enrollment and duration of follow-up in this study, the use of dolutegravir in patients with INSTI resistance mutations should be restricted to research studies or to those patients without additional antiretroviral treatment options, until additional outcomes data are reported.

Bictegravir

Bictegravir is an INSTI that is available as the fixed-dose combination of 50 mg bictegravir, 200 mg emtricitabine, and 25 mg TAF. The fixed-dose combination does not require boosting and can be taken without regard to food. Bictegravir has a half-life of 18 hours, is highly protein bound, and is metabolized by glucuronidation and the CYP3A4 isoenzyme. CYP3A4 is, however, neither induced nor inhibited. Bictegravir inhibits the tubular secretion of creatinine and results in an approximately 0.1-mg/dL increase in serum creatinine after 48 weeks of use; GFRs are not affected. The fixed-dose combination that contains bictegravir should not be used in patients with a creatinine clearance less than 30 mL/min or in patients with severe hepatic impairment. Bictegravir should not be used in combination with rifampin, rifamycins, or dofetilide. Bictegravir in combination with emtricitabine and TAF is recommended as an initial regimen by the HHS Antiretroviral Guidelines Panel.[14]

Two phase III studies demonstrated the noninferiority of the fixed-dose combination of bictegravir, emtricitabine, and TAF when compared to two dolutegravir-containing regimens. In a study that compared coformulated bictegravir, emtricitabine, and TAF to the fixed-dose combination of dolutegravir, abacavir, and emtricitabine for initial therapy in 629 adults with HIV, noninferior rates of virologic suppression to less than 50 HIV-1 RNA copies/mL were observed at 48 weeks.[88a] Fewer than 10% of enrolled participants were women. The proportions of participants with HIV-1 RNA levels less than 50 copies/mL after 48 weeks of therapy were 92.4% versus 93% in an intention-to-treat analysis and 99.3% versus 98.6% in the per-protocol (missing-as-excluded) analysis. HIV-1 resistance to either fixed-dose combination was not observed. Greater rates of nausea and drug-related nausea were observed with the fixed-dose combination of dolutegravir, abacavir, and emtricitabine, a finding attributed to previously observed greater rates of nausea with abacavir, relative to TDF or TAF. Serum creatinine increases of 0.1 mg/mL at week 48 were seen with both antiretroviral regimens.

In a study that compared the fixed-dose combination of bictegravir, emtricitabine, and TAF to dolutegravir and coformulated emtricitabine and TAF for initial therapy in 645 adults with HIV, noninferior rates of virologic suppression to less than 50 HIV-1 RNA copies/mL were observed after 48 weeks of therapy.[88b] The study population was 12% female. The proportion of participants with HIV-1 RNA levels less than 50 copies/mL at 48 weeks were 89% versus 93% in an intention-to-treat analysis and 99% versus 99.7% in the bictegravir and dolutegravir arms, respectively, in a per-protocol analysis. While the incidence and severity of adverse events were similar in the two treatment arms, study drug-related adverse events were significantly less common with the bictegravir fixed-dose combination. No participant developed treatment-emergent HIV-1 resistance with either antiretroviral regimen. Serum creatinine increases of 0.1 mg/mL at week 48 were seen with both antiretroviral regimens.

The bictegravir-containing fixed-dose combination may also be considered for use in virologically suppressed people with HIV who switch regimens. Two unpublished studies evaluated the safety and efficacy of switching to a bictegravir-containing fixed-dose combination

from regimens composed of either dolutegravir, abacavir, and lamivudine or coformulated abacavir and lamivudine or coformulated emtricitabine and tenofovir in combination with atazanavir or darunavir. A total of 1140 participants were studied. At 48 weeks, similar rates of continued virologic suppression were observed between participants who switched to the fixed-dose combination of bictegravir, emtricitabine, and TAF and those who maintained their prescribed antiretroviral regimen.[88c,88d]

Nonnucleoside Reverse-Transcriptase Inhibitors

NNRTIs bind RT but, in contrast to NRTIs, do so in a pocket far from the active site (Fig. 128.4 and Table 128.3). NNRTIs are noncompetitive inhibitors that induce conformational changes in RT, thereby reducing its activity. Available NNRTIs have long half-lives (25–55 h), do not require phosphorylation, and are HIV-1 specific; they have no activity against HIV-2. The NNRTI binding pocket on RT is nonessential to enzymatic function and can tolerate mutations without significant loss of RT activity. NNRTIs are hepatically metabolized and are substrates for the CYP enzymes. The potential for clinically relevant drug-drug interactions is, therefore, higher in NNRTIs than NRTIs.

The barrier to HIV-1 resistance is low for NNRTIs. Single-point mutations in RT can inactivate all members of this class, with the exception of etravirine. Given this low resistance barrier, NNRTIs are often used early in therapy when the probability of HIV resistance to these agents is lowest and the combined protective effect of three fully active drugs is maximized. NNRTI resistance mutations do not affect in vitro viral fitness, but it is not clear whether this observation has clinical relevance.[89] However, drug discontinuation in patients harboring NNRTI resistance mutations often leads to the loss of circulating viruses with mutations over time, suggesting at least some in vivo fitness disadvantage to those viruses.

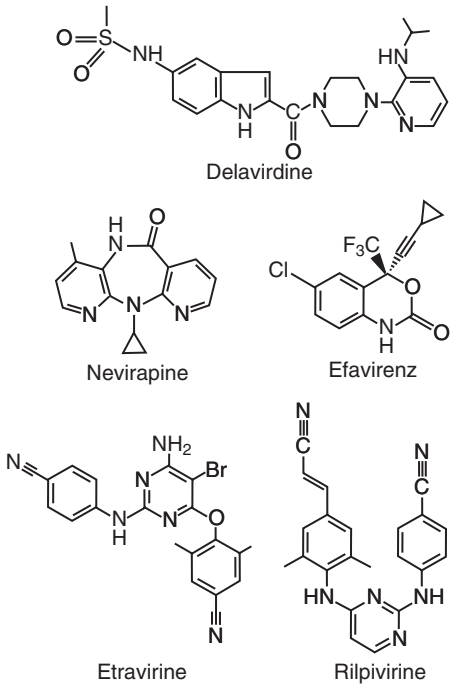

FIG. 128.4 **Non-nucleoside reverse-transcriptase inhibitors.**

Efavirenz

Efavirenz is an NNRTI that is administered orally as a 600-mg tablet once daily, usually in the evening. Efavirenz is also available as a coformulation with TDF and emtricitabine. Efavirenz should be taken on an empty stomach; a high-fat meal increases bioavailability and toxicity. No dose adjustment is needed in renal failure. Efavirenz is a major substrate of CYP2B6 and 3A4; a moderate inhibitor of CYP2C9, 2C19, and 3A4; and an inducer of CYP2B6 and 3A4. Concomitant use with other drugs acting at CYP3A4 carries a high likelihood of drug-drug interactions. All PIs are substrates of CYP3A4, and drug-specific dose adjustments are necessary.[14] There is no significant interaction between efavirenz and fluconazole, but an efavirenz dose adjustment is needed when coadministered with voriconazole.

Efavirenz is now an alternative agent for use in initial antiretroviral regimens according to some guidelines panels.[20,54] Superior virologic outcomes have been seen when efavirenz-based therapy was compared with some PI-based (nelfinavir, indinavir, and lopinavir-ritonavir) or triple NRTI-based regimens.[47,90–93] Comparable virologic outcomes were noted in a comparison to an atazanavir-, an elvitegravir-, or a raltegravir-containing regimen.[70,77–79,94] However, an efavirenz-containing regimen was inferior to a dolutegravir-containing regimen in one comparative trial.[87] Concerns exist regarding certain CNS-related toxicities of efavirenz and a possible association with suicidality.[95]

As with other NNRTIs, efavirenz can cause rash and hepatotoxicity, although less commonly than nevirapine. The two major toxicities of efavirenz are CNS adverse effects and teratogenicity. Efavirenz may cause QTc prolongation, and alternative antiretrovirals should be considered in patients at higher risk for torsades de pointes. Efavirenz use has been associated with neural tube defects in human embryos following first-trimester exposure, and CNS birth defects in nonhuman primates have been observed.[96] Alternative regimens should be considered in sexually active persons not using effective contraception. Efavirenz can be initiated in persons who present for antenatal care after the first trimester, or continued in persons who present for care during the first

TABLE 128.3 Approved Non-Nucleoside Reverse-Transcriptase Inhibitors

AGENT	TRADE NAME	ORAL BIOAVAILABILITY (%)	SERUM HALF-LIFE (hr)	ELIMINATION	ADULT DOSE[a]	DOSAGE FORMS
Nevirapine	Viramune	>90	45 after first dose 25–30 after 2 wk	Hepatic metabolism via CYP3A4 and 2B6	200 mg once daily for 14 d, then, if no rash develops, 200 mg twice daily or 400 mg XR once daily	100-, 200-, 400-mg tablets 50-mg/5-mL solution
Delavirdine	Rescriptor	85	5.8	Hepatic metabolism via CYP3A4	400 mg three times daily	100- and 200-mg tablets
Efavirenz	Sustiva Stocrin[b]	40–45	40–55	Hepatic metabolism via CYP2B6 and 3A4	600 mg once daily on an empty stomach	50- and 200-mg capsules 600-mg tablet
Etravirine	Intelence	Unknown	41 ± 20	Hepatic metabolism via CYP3A4, 2C9, and 2C19	200 mg twice daily after meals	25-, 100-, and 200-mg tablets
Rilpivirine	Edurant	Unknown	50	Hepatic metabolism via CYP3A	25 mg once daily with a meal	25-mg tablet

[a]For pediatric dose, see Chapter 127.
[b]Trade name as marketed in Europe.
CYP, Cytochrome P-450; *XR,* extended-release.

trimester. A definitive association between efavirenz use and birth defects has not been observed in one meta-analysis.[96a]

Up to 50% of patients starting efavirenz can experience some CNS adverse effects, although these usually decrease over the first several weeks of therapy and can be partially ameliorated by evening dosing. Adverse CNS effects include vivid dreams, insomnia, dizziness, and difficulty concentrating. Hallucinations, depression, suicidal ideation, and psychosis have been reported less commonly. Efavirenz is often avoided in persons with preexisting psychiatric conditions. A higher rate of suicidality was observed among efavirenz-treated patients in ACTG studies, an association not subsequently seen in analyses of three large observational cohorts or a retrospective cohort study.[95,96b–96d]

Efavirenz resistance is most commonly conferred by the K103N substitution in RT, but all NNRTI mutations at positions 100, 106, 181, 188, 190, and 225 abrogate efavirenz activity.[97] Y181C is uncommonly selected during efavirenz therapy. The standard commercial genotypic assays cannot reliably detect resistance mutations present at less than 20% of the viral population.[98] Consequently, some importance should also be placed on ascertaining past NNRTI treatment history as a surrogate guide to the possible presence of key resistance mutations.

Significant interactions can occur with antituberculosis therapies, particularly rifampin, although a large study demonstrated comparable virologic outcomes in patients receiving concomitant efavirenz-based therapy and antituberculosis treatment.[99] Like nevirapine, efavirenz decreases plasma methadone levels and can precipitate acute withdrawal.[100]

Rilpivirine

Rilpivirine is an NNRTI that is administered orally as a 25-mg tablet once daily with food. It is also available as a fixed-dose combination with dolutegravir or with emtricitabine and TDF or TAF. Fixed-dose combinations that contain rilpivirine have received increased interest recently primarily due to their smaller pill size, an advantage for some patients. No dosage adjustment is recommended for patients with renal dysfunction, including patients who require hemodialysis or peritoneal dialysis, or patients with mild-to-moderate hepatic insufficiency. The use of rilpivirine in patients with severe hepatic impairment has been insufficiently studied. Rilpivirine has a half-life of 50 hours and is metabolized by CYP3A4-mediated hepatic oxidation; no inhibition or induction of CYP isoenzymes has been reported. Rilpivirine absorption is sensitive to gastric pH; proton pump inhibitors should not be coadministered, and H2 receptor antagonists and antacids require spacing of doses. Rifamycins and efavirenz decrease rilpivirine concentrations, whereas PIs and azole antifungals increase plasma rilpivirine levels. Supratherapeutic rilpivirine plasma levels may prolong the cardiac QTc interval.

Two similar randomized phase III trials demonstrated noninferior efficacy of rilpivirine compared with efavirenz over 48 to 96 weeks of therapy when combined with TDF and emtricitabine.[101,102] However, a pooled analysis demonstrated lower response rates and higher rates of virologic failure in patients who received rilpivirine and had either baseline plasma viral loads greater than 100,000 copies/mL or adherence less than or equal to 95%, relative to patients taking efavirenz.[103] The incidence of serious adverse events was similar for rilpivirine and efavirenz, although rilpivirine had lower rates of discontinuation because of adverse events and fewer grade 2 to 4 laboratory abnormalities. Rates of virologic failure-associated drug resistance after 96 weeks were higher with rilpivirine and were more likely to be accompanied by NRTI resistance-associated mutations, most commonly at position 184 of RT (M184I or V). The 2018 HHS Antiretroviral Guidelines Panel lists the fixed-dose combination of rilpivirine, tenofovir (TDF or TAF), and emtricitabine as one of the recommended initial regimens in certain clinical situations for use in ART-naïve patients with $CD4^+$ T-cell counts greater than 200 cells/mm^3 and plasma viral loads less than 100,000 copies/mL.[14]

The most common rilpivirine-associated NNRTI resistance mutation is E138K, a mutation that confers resistance to all NNRTIs, including etravirine. The K101E, Y181C, and V189I NNRTI resistance mutations can also be selected by rilpivirine therapy; K103N has not been reported. Adverse effects associated with rilpivirine use include depressive disorders, headache, insomnia, and rash. Rash is significantly less likely to occur with rilpivirine than efavirenz.[103]

Protease Inhibitors

HIV relies on its aspartyl protease to cleave Gag and Gag-Pol polyproteins into their essential structural and enzymatic (RT, integrase) components. Many human monomeric aspartyl proteases (e.g., renin, pepsin) exist, but it is the homodimeric structure of HIV-1 and HIV-2 protease that selectively binds to, and is inhibited by, PIs (Fig. 128.5 and Table 128.4). Although viral particles can still be formed in the presence of PIs, they are rendered noninfectious. As a class, PIs are hepatically metabolized via the CYP isoenzyme CYP3A4. Ritonavir is a potent inhibitor of CYP3A4 metabolism; this property has been exploited to beneficially increase, or "boost," plasma drug levels of other PIs by coadministering subtherapeutic doses of ritonavir. CYP3A4 inhibition can lead, however, to drug-drug interactions with other medication classes that include immunosuppressants, antiarrhythmics, antimycobacterials, other antiretroviral drugs, hydroxymethylglutaryl coenzyme A reductase inhibitors (statins), methadone, and oral contraceptives. Atorvastatin, lovastatin, rosuvastatin, and simvastatin either are contraindicated with PI-based antiretroviral therapies or have maximum dosing limits.

PIs can be effective components of initial, second-line, and salvage antiretroviral regimens, although dyslipidemias can be a problem with some agents, and may develop within weeks to months of starting PI-based therapy.[104–107] PIs have also been implicated in the development of insulin resistance.[108–111] Although certain PIs may increase the risk of insulin resistance and diabetes, this is unlikely to be a class-wide effect and, in certain cases, may have more to do with a regimen's NRTI backbone.[112–118] Recent large observational cohort studies have found an association between PI use and an increased risk of cardiovascular events.[26,31] Atazanavir was a PI exception, and male patients with HIV who take atazanavir may have lower rates of cardiovascular events when compared to men receiving other antiretroviral regimens.[118a,119] Some data suggest a possibly increased risk of preterm delivery in pregnant persons receiving PI-based ART.[120]

PIs often have a higher genetic barrier to resistance than either NNRTIs or integrase inhibitors and typically require multiple mutations to substantially lose antiviral activity, although exceptions exist (e.g., saquinavir, nelfinavir). With most PIs, a major (or primary) mutation limits antiviral effectiveness and is followed by one or more minor (or secondary) mutations that by themselves do not dramatically affect phenotype but can improve viral fitness.[97] Ritonavir-boosted PIs select resistance mutations based on the main PI and not the low-dose ritonavir. The genotypic mutation patterns associated with PIs can be particularly complex and challenging to interpret; phenotypic resistance testing can often help resolve these clinical ambiguities.[121] Limited data exist on PI resistance mutations selected during boosted PI therapy in treatment-naïve patients, primarily because these patients usually fail with resistance to the NRTI backbone and not to the boosted PI.[97]

Boosted PI monotherapy has been studied as a strategy to limit drug toxicities and regimen cost. Unfortunately, boosted PI monotherapy leads to inferior rates of viral suppression and the emergence of drug resistance in treatment-naïve patients.[122] Boosted PI monotherapy has also been studied as maintenance, or consolidation, therapy to maintain viral suppression; results have been less effective than combination therapy.[123,124] Simultaneous double ritonavir-boosted PI therapy has also not demonstrated benefit over the more traditional single-boosted PI approach.[125,126]

Boosted Atazanavir

Atazanavir can be administered either as a 400-mg capsule once daily in treatment-naïve patients, or preferably as a 300-mg capsule combined with either 100-mg ritonavir or 150-mg cobicistat, both once daily, for treatment-naïve and treatment-experienced patients. Both boosted and unboosted atazanavir should be taken with food. No dose adjustment is needed for renal dysfunction in treatment-naïve patients, but a recommendation exists not to use atazanavir in treatment-experienced dialysis patients. Moderate hepatic impairment necessitates a dose reduction of atazanavir to 300 mg once daily, and the drug should not be used in severe (Child-Pugh class C) hepatic insufficiency. Atazanavir is metabolized by CYP3A4 and can also affect CYP2C8 and UDP glucuronosyltransferase 1A1 substrates. Methadone steady-state levels appear unaffected by atazanavir.[127] The absorption of atazanavir requires a low

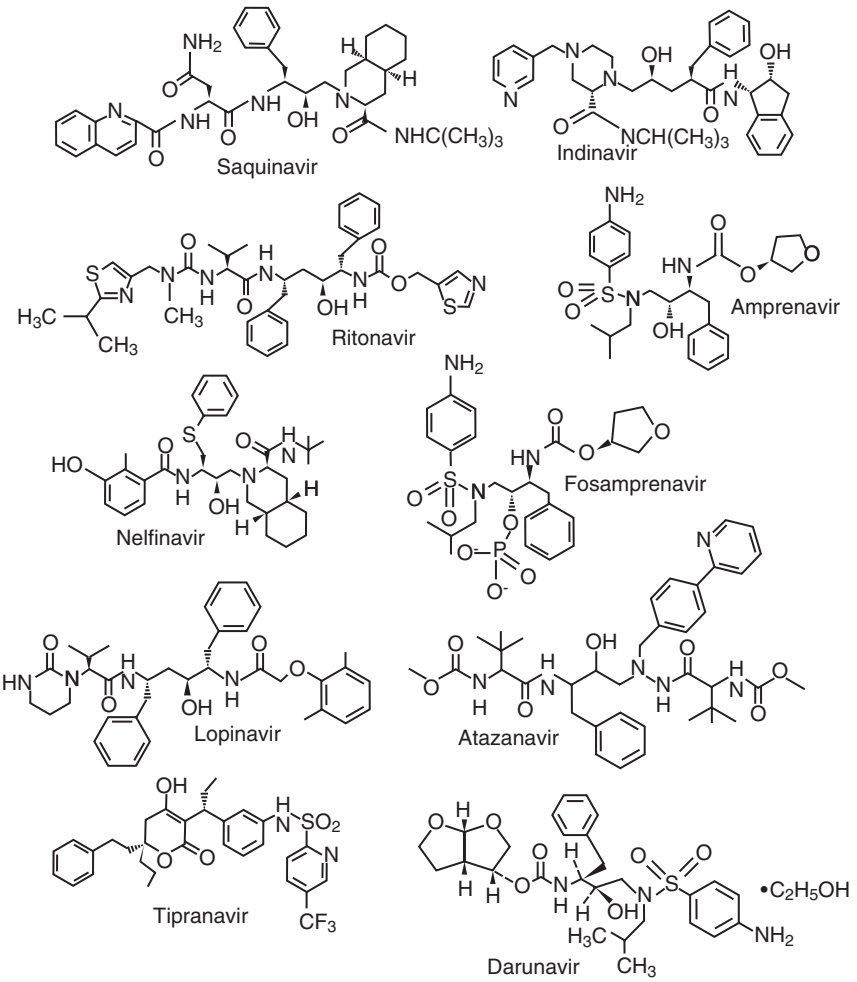

FIG. 128.5 **Protease inhibitors.**

gastric pH; interactions with antacids and H2 antagonists are possible. Atazanavir and proton pump inhibitors should not be coadministered in treatment-experienced patients.[14] Nevirapine should not be coadministered with either boosted or unboosted atazanavir.

The recommendation for ritonavir boosting of atazanavir in treatment-naïve patients is derived from two studies, one that directly compared boosted and unboosted atazanavir over 96 weeks and a second that compared atazanavir/r with lopinavir/r over 48 weeks.[41,128] Similar virologic response rates were seen with unboosted and boosted atazanavir, although there were more treatment failures and atazanavir resistance in participants receiving an unboosted atazanavir-containing regimen. Atazanavir/r demonstrated noninferiority to lopinavir/r over 48 weeks of therapy. There were fewer cases of dyslipidemia and diarrhea in participants receiving atazanavir/r, although this trial used an older lopinavir/r formulation that is no longer available. Atazanavir/r had similar antiviral efficacy compared with efavirenz when used with the NRTI backbones of either abacavir and lamivudine or TDF and emtricitabine.[129] Using a combined end point of antiviral efficacy and tolerability, atazanavir/r was inferior to either darunavir/r or raltegravir when used in combination with TDF and emtricitabine.[130] Atazanavir/r demonstrated similar virologic efficacy when compared to the fixed-dose combination of elvitegravir, cobicistat, TDF, and emtricitabine over 144 weeks.[130a] The CYP3A inhibitor cobicistat was found to have similar efficacy and tolerability compared to ritonavir when administered with atazanavir-TDF-emtricitabine.[131] Atazanavir/r has recently been evaluated more specifically in women. In a study of 575 women that compared atazanavir/r to cobicistat-boosted elvitegravir, both with TDF-emtricitabine, virologic suppression rates to viral loads less than 50 copies/mL were superior with the elvitegravir-containing fixed-dose combination after 48 weeks (87% vs. 81%, respectively).[131a] A 495-women study of a fixed-dose combination of dolutegravir, abacavir, and lamivudine versus atazanavir/r plus TDF and emtricitabine demonstrated significantly greater virologic suppression to HIV-1 RNA levels less than 50 copies/mL at 48 weeks for the dolutegravir-containing regimen when compared to the atazanavir-containing regimen (82% vs. 71%, respectively).[87b] The study was designed as a noninferiority study with a margin of −12%; the mean difference of superior virologic suppression with the dolutegravir-containing fixed-dose combination was 10.5%.

Adverse effects associated with atazanavir include nausea, hyperbilirubinemia with jaundice and scleral icterus, rash, cholelithiasis, and nephrolithiasis.[132–135] Atazanavir has less effect on serum cholesterol and triglycerides than do other PIs and was not associated with an increased risk of cardiovascular disease events.[119] Ritonavir boosting of atazanavir comes at a cost of more hyperbilirubinemia and a greater adverse effect on serum lipid profiles. The primary atazanavir resistance mutation is the I50L substitution; I84V also significantly limits drug activity. Minor mutations occur at positions 10, 16, 20, 24, 32, 33, 34, 36, 46, 48, 53, 54, 60, 62, 64, 71, 73, 82, 85, 90, and 93. In general, the presence of three or more of these mutations predicts a loss of virologic response to atazanavir.[136] For the practicing clinician, the simplest approach may be to order a resistance phenotype that will integrate all of the aforementioned genotypic information in a more concise and straightforward manner.

Boosted Darunavir

Darunavir is a PI with activity against some PI-resistant viruses. Its efficacy was first validated when boosted with ritonavir in treatment-experienced patients. On the basis of additional trials, darunavir/r is considered as a first-line PI in patients initiating ART. No dose adjustments are needed for patients with mild or moderate renal insufficiency

TABLE 128.4 Approved Protease Inhibitors

AGENT	TRADE NAME	ORAL BIOAVAILABILITY (%)	SERUM HALF-LIFE (hr)	ELIMINATION	ADULT DOSE[a]	DOSAGE FORMS
Saquinavir	Invirase	4	1–2	Hepatic and intestinal metabolism via CYP3A4	1000 mg twice daily with ritonavir 100 mg twice daily	200-mg capsule 500-mg tablet
Ritonavir	Norvir	70	3–5	Hepatic metabolism via CYP3A4 and 2D6	Not recommended as single agent	100-mg capsule and tablet 80-mg/mL solution
Indinavir	Crixivan	60–65	1.8	Hepatic metabolism via CYP3A4	800 mg every 8 h Drink ≥1.5 L of water daily	200- and 400-mg capsules
Nelfinavir	Viracept	70–80	3.5–5	Hepatic metabolism via CYP2C19, CYP3A4, and CYP2D6	1250 mg twice daily or 750 mg three times daily, with meals	250- and 625-mg tablets
Fosamprenavir	Lexiva Telzir[b]	—	7–11	Hepatic metabolism via CYP3A4 Biliary excretion	Treatment-naïve: 1400 mg once daily with ritonavir 100- or 200- mg once daily, or 700 mg twice daily with ritonavir 100 mg twice daily PI-experienced: 700 mg twice daily with ritonavir 100 mg twice daily	700-mg tablet 50-mg/mL suspension
Lopinavir + ritonavir	Kaletra	—	5–6	Hepatic metabolism via CYP3A4	Two tablets twice daily	Lopinavir 100-mg/ritonavir 25-mg and lopinavir 200-mg/ritonavir 50-mg tablets Lopinavir/ritonavir 80-mg/20-mg per mL solution
Atazanavir	Reyataz	—	7	Hepatic metabolism via CYP3A4	Treatment-naïve: 300 mg once daily with ritonavir 100 mg once daily, or cobicistat 150 mg once daily, with food. Treatment-experienced or with TDF: 300 mg once daily with ritonavir 100 mg once daily or cobicistat 150 mg once daily, with food	100-, 150-, 200-, and 300-mg capsules 50-mg packet
Tipranavir	Aptivus	—	5–6	Hepatic metabolism via CYP3A4	500 mg twice daily with ritonavir 200 mg twice daily	250-mg capsule 100-mg/mL solution
Darunavir	Prezista	—	15 (with ritonavir)	Hepatic metabolism via CYP3A4	Treatment-naïve: 800 mg once daily with ritonavir 100 mg once daily, or cobicistat 150 mg once daily, with food. Treatment-experienced: 800 mg once daily with ritonavir 100 mg once daily, or cobicistat 150 mg once daily, with food; or 600 mg twice daily with ritonavir 100 mg twice daily, with food	75-, 150-, 600-, and 800-mg tablets 100-mg/mL suspension

[a]For pediatric dose, see Chapter 127. Consult product monograph for appropriate dose when low-dose ritonavir is used for pharmacokinetic enhancement.
[b]Trade name as marketed in Europe.
CYP, Cytochrome P-450; *PI,* protease inhibitor; *TDF,* tenofovir disoproxil fumarate.

or with mild or moderate hepatic impairment. No data are available on dosing in severe renal failure; the use of darunavir in patients with severe hepatic impairment is not recommended.

In treatment-experienced patients, darunavir is administered with meals as a 600-mg tablet twice daily with ritonavir (100 mg) or as an 800-mg tablet once daily with 100 mg ritonavir for patients with no genotypic darunavir resistance mutations.[137] In treatment-naïve patients, darunavir/r has been studied at an 800-mg/100-mg once-daily dose. The CYP3A inhibitor cobicistat was found to boost darunavir levels in a manner similar to ritonavir,[138] and a fixed-dose combination tablet of darunavir and cobicistat was approved by the FDA in 2015. Darunavir contains a sulfa moiety and should be used with caution in patients with sulfonamide allergies. Darunavir can be used in conjunction with atazanavir, efavirenz and etravirine. It is not recommended for use, however, with lopinavir/r, saquinavir, lovastatin, or simvastatin.

Two randomized phase IIb studies (POWER-1 and POWER-2) in treatment-experienced patients with, on average, three primary PI resistance mutations and an 80-fold or greater baseline change in lopinavir susceptibility demonstrated greater viral load reductions and CD4 lymphocyte increases after 24 weeks of therapy in patients receiving darunavir/r compared with an investigator-selected PI, both with optimized background therapy.[139] At 48 weeks, a greater proportion of darunavir/r-treated than comparator PI-treated subjects had plasma HIV-1 RNA levels less than 50 copies/mL (45% vs. 10%, respectively). In a separate trial comparing darunavir/r to lopinavir/r in lopinavir-naïve, treatment-experienced patients, darunavir/r was noninferior to lopinavir/r in achieving plasma HIV-1 RNA less than 400 copies/mL at 48 weeks.[140] In treatment-naïve patients, darunavir/r was noninferior to lopinavir/r after 48 weeks of treatment in achieving HIV-1 RNA levels less than 50 copies/mL.[141] The dose of darunavir/r studied in treatment-naïve subjects was 800 mg/100 mg once daily, rather than the 600 mg/100 mg twice-daily dose previously studied in treatment-experienced subjects. Adverse effects seen with darunavir include diarrhea, nausea, headache, and nasopharyngitis; 0.5% of subjects enrolled in clinical trials of darunavir/r developed acute hepatitis. Postmarketing cases of liver injury and death have been reported. Coformulated darunavir and cobicistat was evaluated in combination with TAF plus emtricitabine or TDF plus emtricitabine and demonstrated similar, albeit low by modern standards, rates of virologic suppression at 48 weeks (77% vs. 84%, respectively).[141a]

Genotypic data from the screening samples of the POWER studies suggest that several darunavir-associated resistance mutations exist. The I50V mutation, one that is also selected by amprenavir, confers the greatest resistance, with a more than fourfold change increase. However,

a single darunavir-associated resistance mutation, even I50V, does not confer resistance; two or more mutations are required. Mutations V11I, I54L, G73S, and L89V confer the least resistance, with each contributing a less than twofold increase in resistance. Mutations V32I, L33F, I47V, I54M, L76V, and I84V lead to an intermediate twofold to fourfold increase. Treatment-experienced patients should preferably not start darunavir (or tipranavir) without first obtaining a virus-resistance phenotype. This is because the limited clinical experience with these PIs may initially compromise the accuracy of the genotypic algorithms used to predict drug sensitivity.

Ritonavir

Ritonavir was originally marketed and used as a stand-alone PI dosed at 600 mg orally twice daily. Gastrointestinal side effects, particularly diarrhea and nausea, were common and limited the drug's tolerability. Dyslipidemias, vomiting, altered taste, and paresthesias were also reported. Today, ritonavir is usually used at lower doses (100–200 mg twice daily) to inhibit CYP3A4 and improve the bioavailability and increase the half-life of other PIs. These low ritonavir doses decrease, but do not entirely eliminate, its gastrointestinal side effects. Ritonavir inhibits the metabolism of all available PIs but is not used in conjunction with nelfinavir. Ritonavir boosting improves PI efficacy and decreases the occurrence of PI resistance after virologic failure, albeit at a cost of an increased incidence of clinically significant drug-drug interactions.

Drugs Used Less Commonly or of Historical Interest

As ART has evolved and new agents have been introduced, medications and regimens of prior historical importance have now largely been replaced by antiretrovirals with improved efficacy and safety profiles. A compendium of drugs of historical interest follows, together with other drugs more recently approved but of limited utility.

Nucleoside and Nucleotide Reverse-Transcriptase Inhibitors (See Fig. 128.2)

Zidovudine

Zidovudine (ZDV, AZT) is a thymidine analogue that is administered orally as a 300-mg pill twice daily. More commonly, however, zidovudine is coformulated with lamivudine and administered twice daily. For patients with renal impairment necessitating peritoneal dialysis, hemodialysis, or continuous venovenous hemofiltration, zidovudine dosing should be adjusted to 100 mg every 8 hours. Zidovudine can be taken with or without food and has an oral bioavailability of 64%, resulting in part because of first-pass glucuronidation in the liver. Zidovudine is neither a substrate for, nor an inducer of, the CYP enzyme complex. The short plasma half-life and concentrations of zidovudine do not accurately reflect the more robust intracellular concentrations of the zidovudine phosphorylated forms. Zidovudine should never be coadministered with stavudine (d4T) because of antagonism, demonstrated both in vitro and in vivo.[142,143]

As the first antiretroviral agent approved in the United States (in 1987), a wealth of clinical outcomes data has been generated with zidovudine as either monotherapy or dual therapy.[144–148] The use of zidovudine in effective three-drug regimens has been systematically evaluated since 1996.[149] A study that compared the efficacy of six combination regimens demonstrated the superiority of zidovudine in combination with lamivudine and efavirenz and established this zidovudine-containing regimen as the standard for initial ART at the time.[90] Since then, however, tenofovir-containing regimens have largely replaced zidovudine-containing regimens as the preferred first-line regimens because of superior virologic outcomes, reduced drug resistance, and less lipoatrophy.[9,150] If the preferred tenofovir-containing regimens cannot or should not be used, current guideline recommendations also generally do not list zidovudine as an alternative nucleoside backbone choice.[13,14] Zidovudine, used as monotherapy or in combination, has also been widely used to prevent mother-to-child transmission of HIV; rates of transmission in the United States and many other countries are now less than 2%, often using zidovudine-containing regimens.[151–153]

The most common zidovudine adverse effects are headache and malaise. Other common side effects include anorexia, nausea, and vomiting. These symptoms often improve during the first month of therapy. Zidovudine can also cause dose-limiting toxicities of anemia and granulocytopenia. Of greatest long-term concern is the interference of zidovudine with the normal function of mtDNA pol-γ. Lactic acidosis, hepatic steatosis, peripheral neuropathy, lipodystrophic/lipoatrophic changes, and myopathy all appear to be related to the mitochondrial toxicities associated with zidovudine. Because of fewer toxicities and superior clinical outcomes observed with newer NRTIs, zidovudine is no longer frequently used as a first-line antiretroviral agent in developed countries. The combination of zidovudine and lamivudine remains first-line therapy, however, for pregnant persons with HIV.

Two resistance pathways have been described in patients failing zidovudine (or stavudine) therapy, most often when used as monotherapy or dual therapy in the pre–highly active ART era. TAMs accumulate in the *RT* gene either at positions 41, 210, and 215 (TAM1 pathway) or at positions 67, 70, and 219 (TAM2 pathway). Although the factors responsible for the emergence of one pathway over the other remain unclear, the TAM1 pathway confers higher-level zidovudine resistance and greater cross-resistance to other NRTIs than does the TAM2 pathway. Mutations from both pathways can be seen in patients, and TAMs can continue to accumulate over time, especially in those patients who continue zidovudine in the presence of either a partially suppressive or overtly failing regimen.

Stavudine

Stavudine (d4T) is a thymidine analogue that has similar antiretroviral activity as zidovudine but has significant toxicities that limit its clinical use. These include peripheral neuropathy, hyperlactatemia, lactic acidosis, hepatic steatosis, lipoatrophy, and pancreatitis. Stavudine is antagonistic to zidovudine, and also should not be coadministered with didanosine because of concerns for possibly fatal lactic acidosis that appears to have a female predominance.[154] Stavudine offers no advantages in virologic outcome over TDF and should not generally be used as first-line ART.[54] Nevertheless, because of coformulation with other agents and cost concerns, it may still be used as part of initial regimens in some parts of the developing world.

Didanosine

Didanosine (ddI) is functionally an adenosine analogue that is administered in a delayed-release enteric-coated form. Dosing is weight-based and should be reduced commensurate with creatinine clearance. There is no specific recommendation for dosing in the setting of hepatic impairment, although the potential for toxicities in this situation should be closely monitored. Didanosine absorption is decreased in the presence of food, and the drug should be taken 30 minutes before or 2 hours after a meal. As with other dideoxynucleotides, didanosine may be associated with mitochondrial-associated toxicities. Most commonly, didanosine can cause peripheral neuropathy and pancreatitis and should not be coadministered with drugs having similar toxicity profiles. Didanosine use has been associated with a reversible 1.5-fold increased relative risk of myocardial infarction, even after adjustment for baseline cardiovascular risk.[25] However, these data were not confirmed in a second study.[155] Didanosine resistance during the monotherapy and dual-therapy era was most often conferred by the L74V mutation in the *RT* gene. In the era of combination therapy, this mutation is less commonly seen. Resistance to didanosine can also be conferred by the 69 insertion complex and the Q151M complex.[61] Didanosine can also select for the signature tenofovir mutation K65R in vitro, although the significance of this in vivo is unknown.[156] Largely because of associated toxicities and inferior virologic efficacy, current guidelines no longer recommend didanosine-based regimens for initial therapy in antiretroviral-naïve patients.[13–15]

Nonnucleoside Reverse Transcriptase Inhibitors (See Fig. 128.4)

Nevirapine

Nevirapine is administered orally with or without food at 200 mg once daily for the first 2 weeks, then 200 mg twice daily thereafter. Nevirapine

induces it own metabolism via CYP3A4, and the dose increase 2 weeks into therapy is necessary to maintain adequate plasma drug levels. No dose adjustment is needed for renal impairment short of dialysis, and the use of nevirapine in the setting of hepatic dysfunction is not recommended. Nevirapine is a minor substrate for CYP2B6 and 2D6 and a weak inhibitor of CYP1A2, 2D6, and 3A4. It is a major substrate and inducer of CYP3A4 and a strong inducer of CYP2B6. As such, multiple clinically relevant drug-drug interactions are possible. Antibiotics with antimycobacterial activity, such as rifampin, rifabutin, and clarithromycin, should not be coadministered with nevirapine. Inferior virologic outcomes were observed in patients with HIV receiving nevirapine-based ART while on treatment for tuberculosis.[99] Nevirapine decreases serum concentrations of some PIs and decreases plasma concentrations of oral contraceptives and methadone.[157] A careful review of drug-drug interactions on a patient-by-patient basis is warranted before starting nevirapine. Nevirapine crosses the placenta and is known to enter breast milk.[158–160]

Nevirapine can be an effective component of a three-drug antiretroviral regimen.[161,162] Nevirapine has lost favor relative to other NNRTIs, particularly efavirenz, for initial ART, largely because of inferior clinical effectiveness and problematic toxicities that include rash, Stevens-Johnson syndrome, and hepatic necrosis.[163,164] A study of more than 23,000 participants demonstrated an increased risk of death and acquired immunodeficiency syndrome (AIDS)–defining illness in subjects starting a nevirapine-based regimen, relative to efavirenz.[165] After 12 months of combination therapy, participants on nevirapine had smaller CD4 cell count increases and markedly higher rates of virologic failure compared with efavirenz regimens, although disagreement on some of these conclusions exists.[166]

An elevated risk of liver damage contraindicates the use of nevirapine in two subsets of patients: women with CD4 counts greater than 250 cells/mm^3 and men with CD4 counts greater than 400 cells/mm^3.[167,168] Nevirapine use should be avoided in pregnant persons with CD4 counts greater than 250 cells/mm^3.[169–172] Single-dose nevirapine, however, is associated with alarming rates of NNRTI resistance in both mothers and infants, and combination therapies are now generally recommended in these situations.[173–177] In women previously exposed to single-dose nevirapine, combination therapy with lopinavir/r is superior to nevirapine-based therapy.[178]

Resistance to nevirapine is conferred by any of the following mutations in RT: K103N, V106A/M, Y181C, Y188L, and G190A/S. All of these NNRTI mutations reduce nevirapine sensitivity by 50-fold or greater.[97,179] The existence of any of these mutations in a patient's HIV genotype precludes the effective use of nevirapine.

Etravirine

Etravirine is an NNRTI that retains some activity in the presence of certain NNRTI resistance mutations. The drug acts as an allosteric inhibitor of HIV-1 RT.

Etravirine is administered orally as two 100-mg pills (200 mg) twice daily after a meal. Ingestion without food decreases etravirine exposure by 50%. No dose adjustment is required for patients with renal insufficiency or mild-to-moderate hepatic insufficiency. As with other NNRTIs, etravirine has a relatively long half-life (41 ± 20 hours). As a substrate and inducer of CYP3A4 and an inhibitor of other CYP isozymes, etravirine has important drug-drug interactions. The drug cannot be coadministered with tipranavir, fosamprenavir, or atazanavir. Etravirine should not be used with another NNRTI or any unboosted PI and should not be given with rifampin, clarithromycin, erythromycin, or anticonvulsants such as phenytoin or carbamazepine. Etravirine has not been associated with fetal risk in animal studies, and there are no studies to define the risk of etravirine use in pregnant persons.

Two similar randomized, double-blind, placebo-controlled trials of etravirine and darunavir versus darunavir, both with optimized background therapy, demonstrated greater virologic suppression to less than 50 copies/mL after 24 weeks in subjects receiving etravirine and darunavir.[180,181] Subjects in these trials had active or historical genotypic evidence of NNRTI resistance-associated mutations. A smaller randomized, phase IIb placebo-controlled trial of etravirine versus placebo, both with optimized background therapy, however, did not demonstrate a significant reduction in viral loads in etravirine-treated subjects after 48 weeks; in this study, subjects receiving etravirine had a median of one NNRTI resistance mutation at study entry.[182] In PI-naïve subjects with baseline NRTI and NNRTI resistance mutations, an etravirine-containing regimen was inferior to a regimen containing any PI, boosted or unboosted.[183] An etravirine regimen dosed once daily was associated with fewer adverse neuropsychiatric side effects than efavirenz over 48 weeks and similar viral efficacy, although the study was not powered to demonstrate virologic noninferiority.[184]

Resistance to etravirine is associated with up to 17 different mutations in RT.[185] The most important resistance mutations appear to be Y181C and G190A, and these require the presence of other mutations to limit etravirine activity; K103N does not confer etravirine resistance. The most common adverse effects seen with etravirine are rash and nausea. The rash, described as erythema or a papular eruption, usually begins in the second week of therapy and lasts for a median of 10 days.[182] As with other NNRTIs, severe dermatologic reactions have been reported.

Protease Inhibitors (See Fig. 128.5)

Saquinavir

Saquinavir is administered as two 500-mg tablets (1000 mg) along with a ritonavir 100-mg tablet, both twice daily. The use of unboosted saquinavir is not recommended. Saquinavir bioavailability is improved with high-calorie, high-fat meals and should be taken within 2 hours of a full meal. The inhibition of CYP3A4 by saquinavir and ritonavir leads to drug-drug interactions. For example, amiodarone, midazolam, lovastatin, simvastatin, and St. John's wort should not be coadministered. Concurrent use of rifampin is contraindicated because of hepatic toxicity, and levels of oral contraceptives are decreased. A complete review of a patient's medications is warranted before the use of ritonavir-boosted saquinavir (saquinavir/r) therapy.

Early clinical experience with saquinavir was compromised by the poor bioavailability of a hard capsule formulation.[149] A soft gel capsule formulation improved bioavailability and clinical outcomes but has since been replaced by the current 500-mg tablet.[186,187] Twice-daily saquinavir/r was compared with lopinavir/r, both in combination with emtricitabine-TDF, and demonstrated similar virologic and immunologic outcomes.[188] From the perspective of patient adherence, lopinavir/r retains a lower pill burden, can be taken without food, and can be stored above 34°C. A once-daily saquinavir/r dosing schedule has also been tested but demonstrated low drug trough concentrations; this dosing schedule is not currently recommended.[189]

Adverse effects of saquinavir/r are most commonly nausea, vomiting, diarrhea, and abdominal discomfort. Saquinavir/r can prolong the QT and PR electrocardiographic intervals and lead to serious arrhythmias. The major saquinavir resistance mutation is L90M within protease, although G48V can also be selected. Minor mutations can occur at positions 10, 24, 54, 62, 71, 73, 77, 82, and 84.

Indinavir

Indinavir is not recommended for initial ART, boosted or unboosted, because of pill burden and the risk of nephrolithiasis.[14] Unboosted indinavir is administered as two 400-mg capsules every 8 hours. A boosted regimen combines two 400-mg indinavir capsules with 1 to 2 100-mg ritonavir capsule(s), twice daily. Indinavir must be taken with water, either 1 hour before or 2 hours after a meal. Meal requirements do not apply to ritonavir-boosted indinavir (indinavir/r). Indinavir has low solubility at physiologic pH and can crystallize in the kidney and urine.[190,191] Patients should increase their water intake to decrease the risk of nephrolithiasis associated with indinavir/r. CYP3A4 inhibition by indinavir can lead to multiple drug-drug interactions; a thorough review of a patient's medications is warranted before indinavir therapy. The main adverse effects seen with indinavir are nephrolithiasis, unconjugated hyperbilirubinemia without jaundice, abdominal pain, nausea, and dry skin and lips. Indinavir has been used in combination regimens with some success but has been largely replaced in recent years by less toxic and more tolerable PI regimens.[192,193] The major indinavir resistance mutations selected during therapy are M46L, V82A, and I84V. Minor mutations can occur at positions 10, 20, 24, 32, 36, 54, 71, 73, 76, 77, and 90.

Nelfinavir

Nelfinavir is not generally recommended for initial antiviral therapy because of inferior viral efficacy relative to other PIs, notably lopinavir/r, and the NNRTI efavirenz. Nelfinavir is administered as two 625-mg tablets (1250 mg total) twice daily with meals. It is primarily metabolized by CYP2C19 and CYP3A4 and is not boosted with ritonavir. Increasing doses of ritonavir did not necessarily increase the observed area under the curve of nelfinavir.[194] The potential for drug-drug interactions is significant, as for other PIs, and a thorough review of a patient's medications should be undertaken before nelfinavir therapy. Nelfinavir-induced viral suppression, when used in combination with two NRTIs, compares unfavorably with that induced by efavirenz, nevirapine, lopinavir/r, and ritonavir-boosted fosamprenavir (fosamprenavir/r), in antiretroviral-naïve patients.[90,195–198] Nelfinavir should not be used in PI-experienced patients.[199] Adverse effects with nelfinavir include loose stools or diarrhea, hypercholesterolemia, and hypertriglyceridemia. The major nelfinavir resistance mutation is D30N, and L90M is selected less commonly. Minor mutations can occur at amino acid positions 10, 36, 46, 71, 77, 82, 84, and 88 within protease.

Fosamprenavir

Fosamprenavir, the phosphorylated prodrug of amprenavir, has improved oral bioavailability and efficacy compared with the no longer available amprenavir. Pill burden has also been improved but is still greater than with some other PIs. Once ingested, fosamprenavir is converted to amprenavir in the gut. Fosamprenavir is administered as a 700-mg tablet with a 100-mg ritonavir tablet, both twice daily; a once-daily regimen of fosamprenavir 1400 mg with 100 to 200 mg ritonavir has also been used. The twice-daily regimen is preferred; once-daily fosamprenavir/r should not be used in PI-experienced patients and its use in treatment-naïve patients cannot be recommended until efficacy is demonstrated in a sufficiently large randomized clinical trial. Fosamprenavir is metabolized by CYP3A4 and is excreted in feces. The potential for drug-drug interactions is significant. No dose adjustment is required for renal insufficiency but progressive dose reductions are needed in patients with worsening hepatic dysfunction.

Early clinical experience with amprenavir demonstrates viral efficacy in combination regimens.[200] Patients receiving twice-daily fosamprenavir/r demonstrated similar viral load reductions and CD4 cell count increases when compared with those seen in nelfinavir-treated patients.[198] Less virologic failure was seen in fosamprenavir/r-treated patients than in those treated with nelfinavir. A regimen containing twice-daily fosamprenavir/r demonstrated noninferiority to a lopinavir/r-containing regimen in the proportion of patients achieving plasma HIV RNA levels less than 50 copies/mL after 48 weeks of therapy.[116] Similar increases in plasma lipid levels were seen with either therapy.

Adverse effects of fosamprenavir/r therapy include diarrhea, hypertriglyceridemia, and rash. Fosamprenavir contains a sulfonamide moiety that may explain the increased incidence of dermatologic side effects relative to other PIs. Patients with a known sulfonamide allergy should be monitored when starting fosamprenavir/r therapy. The major fosamprenavir resistance mutation is I50V, and I84V is selected less commonly.[97] The valine substitution at position 50 selected by fosamprenavir is different than the leucine substitution selected at the same position by atazanavir (I50L); these mutations do not confer cross-resistance. Minor mutations can occur at amino acid positions 10, 32, 46, 47, 54, 73, 76, 82, and 90.

Lopinavir/Ritonavir

Lopinavir is available only as a coformulation with ritonavir. First-pass metabolism limited lopinavir/r plasma concentrations, whereas ritonavir boosting dramatically improved drug trough concentrations.[201] The most recent formulation of lopinavir-r is a heat-stable tablet that is administered as two lopinavir/r 200-mg/50-mg tablets twice daily. This tablet can be taken without regard to food, although lopinavir/r solution should still be taken with meals. Lopinavir/r can also be given once a day, although not to pregnant persons. Decreased clearance is not expected with renal insufficiency, and some increase in lopinavir/r exposure occurs with worsening hepatic dysfunction. Significant CYP3A4 inhibition occurs, and a patient's medications should be thoroughly reviewed before lopinavir/r therapy.

Lopinavir/r has demonstrated efficacy in combination regimens in treatment-naïve and treatment-experienced patients, and it has been frequently used as the comparator PI in noninferiority trials of new or newly formulated PIs.[196,202] Lopinavir/r is superior to nelfinavir, whereas atazanavir/r, darunavir/r, fosamprenavir/r, and saquinavir/r have demonstrated noninferior virologic outcomes when compared with lopinavir/r in treatment-naïve subjects.[41,116,141,196,203,204] Virologic suppression on a lopinavir/r and two-NRTI regimen can be maintained for at least 7 years.[205] A comparison of lopinavir/r or efavirenz, either with two NRTIs, demonstrated superior virologic efficacy of an efavirenz-containing regimen but greater CD4 cell count gains with the lopinavir/r-containing regimen.[91] Less drug resistance associated with virologic failure was seen in subjects randomized to lopinavir/r.

Adverse effects seen with lopinavir/r are primarily gastrointestinal: diarrhea or loose stools, nausea, and, less commonly, vomiting. Hypercholesterolemia and hypertriglyceridemia are reported in patients taking lopinavir/r. The major lopinavir/r resistance mutation is V82A, although V32I and I47A can also be selected. Lopinavir/r resistance requires the accumulation of multiple mutations, often six or more, before the drug loses clinical effectiveness.[206,207] This is unlikely to occur in treatment-naïve patients receiving lopinavir/r as part of recommended combination therapy. Minor mutations have been documented at positions 10, 20, 24, 33, 46, 50 (I50V), 53, 54, 63, 71, 73, 76, 84, and 90. When mutations V32I and I47A are found together with mutations at position 46, they are associated with high-level lopinavir/r resistance.[208,209]

Tipranavir

Tipranavir is a nonpeptidic PI approved for use in treatment-experienced patients with resistance to multiple other PIs. The drug is administered at 500 mg twice daily with ritonavir 200 mg twice daily, and should be taken with a high-fat meal. No dose adjustments are required for patients with renal or mild hepatic impairment; tipranavir is contraindicated in patients with moderate-to-severe liver dysfunction. Tipranavir capsules are thermally labile and must be kept below 77°F. Complex drug-drug interactions occur as a result of the net effect of tipranavir and ritonavir inducing P-glycoprotein and inhibiting CYP3A4. As a consequence, tipranavir cannot be coadministered with rifampin, several antiarrhythmics, lopinavir, saquinavir, and amprenavir. Numerous other tipranavir interactions exist, including increases in rifabutin levels and significant reductions in the levels of coadministered methadone and oral contraceptives. Tipranavir has been shown to induce adverse fetal effects in animal studies; no studies assessing risk in pregnant persons have been performed.

The efficacy of tipranavir in treatment-experienced subjects with PI-resistant virus was demonstrated in two randomized trials, RESIST-1 and RESIST-2.[210] Subjects enrolled in these trials were required to have at least one primary PI resistance mutation but could not have more than three at codons 33, 82, 84, or 90. After 48 weeks, more subjects randomized to tipranavir-ritonavir plus optimized background therapy achieved viral loads of less than 50 copies/mL than did subjects receiving a ritonavir-boosted comparator PI (23% vs. 10%, respectively).[210] Tipranavir-ritonavir has also shown sustained virologic responses in pediatric and adolescent patients.[211]

Tipranavir has been associated with severe hepatotoxicity and intracranial hemorrhage. Thirteen of 6840 patients receiving tipranavir in clinical trials developed intracranial hemorrhages, and for this reason, patients at risk for bleeding from trauma, surgery, or other medical conditions (e.g., hemophilia), or who are taking other drugs that increase the risk for bleeding, should not receive tipranavir. The most common adverse effects seen with tipranavir are nausea and diarrhea, in part because of the increased dosage of ritonavir required for boosting. Tipranavir contains a sulfonamide moiety; the proportion of patients with a sulfonamide allergy that react unfavorably to tipranavir is unknown.

Entry Inhibitors

HIV entry shares common mechanisms with several other enveloped viruses.[212] The HIV-1 surface glycoproteins gp120 and gp41 mediate



FIG. 128.6 Human immunodeficiency virus entry. Entry *(from left to right)* requires CD4, envelope glycoproteins assembled as a heterotrimer, and a coreceptor, in this case CCR5. Binding of CD4 to glycoprotein gp120 exposes a flexible third hypervariable (V3) loop. The V3 loop interacts with both the N-terminus and the second extracellular loop (ECL2) of CCR5, leading to the release of gp41 by a currently unknown mechanism. Gp41 inserts into the target cell membrane, and rearranges and brings the HR1 and HR2 domains together to form a six-helix bundle. Viral and cellular membranes then approximate, leading to fusion. *CCR5,* C-C motif of chemokine receptor 5; *HR1,* heptad repeat 1; *HR2,* heptad repeat 2. *(Reprinted with permission from Louis B. Henderson, PhD, and the Physicians' Research Network, Inc. See Tsibris A. Update on CCR5 inhibitors: scientific rationale, clinical evidence, and anticipated uses.* https://www.prn.org/images/pdfs/86_tsibris_athe.pdf. *Accessed December 2007.)*

viral binding to and membrane fusion with host target cells (Fig. 128.6 and see Table 128.2). The *ENV* gene product is first translated as gp160 and then cleaved intracellularly into gp120 and gp41. On the viral membrane, there is a noncovalent association of one molecule of gp120 with one molecule of gp41.[213] Three of these units aggregate on the membrane surface to form the gp120/gp41 heterotrimer.[214–216] The association of gp120 with gp41 in the trimer traps gp41 in a conformationally metastable state, the energy from which can later be exploited to accelerate the rate of fusion.[217]

Binding of gp120 to its primary receptor on the cell surface, CD4, is the first step leading to membrane fusion. After CD4 binding, sequential binding of coreceptor occurs, although some interactions of CD4 with gp120 lead to inappropriate shedding of gp120 and inactivation of the fusion reaction.[218] If gp120 is not shed, fusion requires the binding of either the C-C motif of chemokine receptor 5 (CCR5) or C-X-C chemokine receptor 4 (CXCR4) to proceed.[219–223] The interaction of gp120 with CD4 forms the coreceptor binding site.[224] Coreceptor recognition is defined by several structural elements of gp120 that include the V1-V2 hypervariable region, the bridging sheet (an antiparallel, four-stranded β sheet that connects the inner and outer domains of gp120), and most importantly, the V3 hypervariable loop.[224–227] According to current models of HIV-1 entry, sequential binding of gp120 to CD4 and the coreceptor leads to the release of gp41 from its metastable conformation. The hydrophobic N-terminal fusion domain of gp41, now free, inserts into the target membrane.[217,228,229] Two trimeric coiled-coil structures in gp41, termed heptad repeat 1 (HR1) and heptad repeat 2 (HR2), rearrange to form a six-helix bundle that leads to the approximation of the two membranes and eventual fusion.[212] Enfuvirtide is a peptide mimetic of gp41 HR2 that interferes with the HR1-HR2 association, whereas maraviroc binds to CCR5, changes its conformation, and prevents gp120-CCR5 binding (see Table 128.2).[230]

Enfuvirtide

Enfuvirtide is a 36-amino-acid synthetic peptide that is administered as a 90-mg subcutaneous injection twice daily; the location of the injection does not affect bioavailability. No dose adjustment is required for hepatic or renal impairment. No clinically significant drug interactions with enfuvirtide have been reported. Studies in animals demonstrated no teratogenic effects of enfuvirtide, and there are no formal studies in pregnant persons to assess fetal risk from enfuvirtide use.

Two similar randomized, open-label trials in treatment-experienced subjects with advanced HIV disease (median CD4 count <100 cells/μL) demonstrated greater viral load reductions when enfuvirtide was added to an optimized background regimen.[231,232] Enfuvirtide use in enfuvirtide-naïve subjects also adds virologic benefit when used in combination with darunavir, tipranavir, maraviroc, and raltegravir, underscoring the added benefit to patients when at least two active drugs are included in salvage regimens.[68,139,210,233] Enfuvirtide is not currently recommended for treatment-naïve patients.

The need for twice-daily injections, along with the adverse effects that accompany those injections, have limited the clinical use of enfuvirtide. Injection site reactions that include pain, erythema, nodules, cysts, induration, and pruritis are seen in 98% of patients injecting enfuvirtide. Nausea, diarrhea, fatigue, and insomnia have also been reported. Resistance to enfuvirtide is conferred by amino acid substitutions in the HR1 region of gp41, most commonly at positions 36, 38, 40, and 43.[97]

Maraviroc

Maraviroc is a CCR5 antagonist (see Fig. 128.3 and Table 128.2). HIV entry requires viral gp120 to sequentially bind CD4 and a surface coreceptor, either CCR5 or CXCR4. Maraviroc is an allosteric inhibitor of the gp120-CCR5 interaction that blocks HIV attachment to target cells and thereby reduces viral replication.

Maraviroc is typically administered as 300 mg twice daily; the drug has not yet been adequately studied in patients with renal or hepatic dysfunction. Maraviroc is a substrate for CYP3A. This necessitates a dose reduction to 150 mg twice daily during coadministration with CYP3A inhibitors such as PIs (with the exception of ritonavir-boosted tipranavir), ketoconazole, or clarithromycin, and the dose increases to 600 mg twice daily during coadministration with CYP3A inducers, such as efavirenz, nevirapine, and some anticonvulsants. All patients should be screened for viral coreceptor usage before starting maraviroc, and this drug should not be used in patients with evidence of circulating CXCR4-using virus. Phenotypic and genotypic coreceptor usage assays are commercially available, although a phenotypic assay was used to determine coreceptor usage in the major maraviroc clinical trials. A phenotypic tropism assay is therefore generally preferred in routine clinical practice.

Maraviroc may be used in treatment-experienced and treatment-naïve patients in the United States. Two similar clinical trials in treatment-experienced subjects with CCR5-using viruses compared maraviroc versus placebo, both with optimized background therapy.[233,234] After 48 weeks, a greater proportion of maraviroc-treated subjects achieved plasma viral loads less than 50 copies/mL and had greater increases in $CD4^+$ T-cell counts than did placebo recipients. For subjects taking at least three active drugs, similar proportions in the placebo and maraviroc dosing arms achieved undetectable HIV RNA levels. Maraviroc confers no virologic benefit in subjects with dual/mixed virus.[235] In a 48-week study in treatment-naïve subjects, maraviroc was compared with efavirenz, either in combination with zidovudine-lamivudine; the results for HIV RNA levels less than 50 copies/mL did not meet criteria set to demonstrate noninferiority for maraviroc in this study.[236] Adverse effects

seen with maraviroc include cough, fever, upper respiratory infections, rash, musculoskeletal symptoms, abdominal pain, and dizziness. A modest increase in liver-related adverse events was seen in subjects receiving maraviroc, most commonly elevated transaminases and increased bilirubin.

High-level maraviroc resistance can occur by the selection of amino acid substitutions in the third hypervariable (V3) loop of gp120. The intrinsic sequence variability of this region, however, has precluded the identification of canonical genotypic resistance mutations. In clinical trials, genotypic maraviroc resistance has been uncommon compared with escape via selection of minority CXCR4-using viral populations that circulate below the detection limit of coreceptor usage assays. Therefore, changes in coreceptor usage, rather than emergence of true resistance, are the dominant mechanism for virologic escape from maraviroc. In clinical trials to date, discontinuation of maraviroc generally resulted in a loss of detectable CXCR4-using viruses and a reappearance of CCR5-using viral populations.

Ibalizumab

Ibalizumab is a humanized monoclonal antibody that binds CD4 and inhibits postattachment steps in HIV-1 entry. Ibalizumab is not believed to interfere with normal CD4 function and does not prevent HIV-1 gp120 binding to CD4. Ibalizumab is administered intravenously as a single loading dose of 2 g, followed by an 800-mg intravenous infusion every 2 weeks thereafter. The clinical experience with ibalizumab is extremely limited when compared to other antiretroviral medications. The primary and only safety evaluation of ibalizumab comes from a 40-participant study conducted over 25 weeks.[236a] Participants were heavily treatment-experienced, harbored multidrug-resistant HIV-1, and were failing a salvage antiretroviral regimen. Baseline viral loads and CD4 counts were 4.5 $\log_{10}$ copies/mL and 73 cells/mm^3, respectively. In combination with an optimized background regimen that contained at least one drug to which a participant's virus was susceptible, 43% of participants achieved viral load levels less than 50 copies/mL after 25 weeks. Less virus response was observed at lower CD4 counts and higher baseline viral loads. The most common side effects seen in this small study were diarrhea, dizziness, nausea, and rash; creatinine elevations occurred in 10% of participants. Another formulation of ibalizumab was administered intramuscularly, was well tolerated, and reduced viral loads by up to 1.2 logs.[237] The use of ibalizumab should be restricted to patients with multidrug-resistant HIV-1 who are failing a salvage treatment regimen.

Another monoclonal antibody, PRO 140, which binds to CCR5, has been administered subcutaneously to patients with CCR5-topic HIV-1 infection, and was reported to reduce mean viral load activity by 1.65 $\log_{10}$.[238]

INITIATING ANTIRETROVIRAL THERAPY

Recommendations regarding when to initiate ART for HIV infection have varied widely over the years. Overwhelming data now support universal treatment for people with HIV, regardless of CD4 counts. For all patients, regardless of duration of infection or prior treatment experience, the goal of therapy is the reduction of plasma viral load to less than 50 copies/mL. Guidelines from expert panels are published and periodically updated; comprehensive online versions of these recommendations are available.[13–15]

When to Begin Therapy?

The decision to begin ART for any patient must balance the burden and toxicity of the drug regimen against the benefits of decreased HIV-related morbidities and increased life expectancy (Table 128.5). Although clinicians are most comfortable considering the deleterious consequences of HIV infection in discrete quanta of CD4 counts (e.g., <50, <200, >350 cells/mm^3), there is a continuum, without clear demarcation, in the risk of progression to AIDS and death across the range of declining CD4 counts, from 650 cells/mm^3 or more to less than 50 cells/mm^3.[239–242] This includes causes of death not usually attributed to HIV infection itself.[243–249] As the risks associated with ART have decreased because of more potent drug combinations, lower pill burdens that improve adherence, and newer medications with fewer short-term and long-term toxicities, the risk-benefit ratio of initiating ART earlier in the course of disease has shifted toward beginning therapy at higher CD4 cell counts.[250]

TABLE 128.5 When to Initiate Antiretroviral Therapy in Adolescents and Adults

Recommend Initiation of Therapy
All patients with HIV, regardless of CD4 count
Conditions Favoring Early Initiation of Therapy
Acute or recent HIV infection, *or* AIDS-defining conditions, including HIV-associated dementia and AIDS-associated malignancies, *or* Pregnancy, *or* HIV-associated nephropathy, *or* Lower CD4 counts (<200 cells/mm^3), *or* Acute opportunistic infections, *or* HIV RNA >100,000 copies/mL, *or* CD4 decline >100 cells/mm^3/yr, *or* Hepatitis B coinfection, *or* Hepatitis C coinfection

AIDS, Acquired immunodeficiency disease; *HIV,* human immunodeficiency disease; *RNA,* ribonucleic acid.
Modified in part from Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Washington, DC: US Department of Health and Human Services. https://aidsinfo.nih.gov/contentfiles/lvguidelines/glchunk/glchunk_11.pdf. Accessed August 4, 2015; and Thompson MA, Aberg JA, Hoy JF, et al. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society–USA panel. JAMA. *2012;308:387–402.*

Most treatment guidelines now recommend ART for all patients with HIV, regardless of CD4 count.[14] The level of evidence to support therapy initiation guidelines is strongest for CD4 counts less than 200 cells/mm^3. Patients who initiate therapy with CD4 counts between 200 and 350 cells/mm^3 have reduced rates of death, opportunistic infections, and serious non-AIDS events and an increase in rates of maximal virologic suppression and CD4 cell count rises.[241,251–261] Between 350 and 500 cells/mm^3, cohort studies and randomized controlled trials have demonstrated a decreased risk of HIV-related disease progression, relative to patients who initiated therapy at CD4 counts less than 350 cells/mm^3.[262–266]

A randomized controlled trial of 4685 participants (the Strategic Timing of AntiRetroviral Treatment [START] study) recently demonstrated that early initiation of ART in patients with a CD4 count greater than 500 cells/mm^3 was associated with reduced disease progression and mortality when compared with delayed therapy.[267] In this study, individuals were randomized to start ART immediately or to defer until the CD4 count decreased to 350/mm^3 or AIDS developed. The primary end point (development of a serious AIDS or non-AIDS event) occurred in 42 participants in the immediate group versus 96 participants in the deferred group (hazard ratio, 0.43; 95% confidence interval, 0.30–0.62; $P < .001$). The majority of primary end points in both groups occurred when the CD4 count was greater than 500/mm^3. A second study, the Temprano trial, enrolled 2056 individuals in Côte d'Ivoire with CD4 cell counts greater than 500/μL and demonstrated that ART started at CD4 cell counts greater than 500/μL reduced the risk of serious infection and death by 44% when compared with ART initiation using World Health Organization guidelines at the time.[268] Together these two clinical end point trials provide convincing evidence that ART should be started as soon as possible in nearly all patients with HIV once the diagnosis is made.

If ART should begin as soon as possible, how soon is too soon? Immediate ART initiation following HIV diagnosis has recently been studied in both resource-limited and resource-rich settings. An unblinded randomized controlled trial of single-visit ART initiation in Johannesburg, South Africa, enrolled 377 participants and demonstrated greater rates of virologic suppression, defined as HIV-1 RNA levels less than 400 copies/mL within 10 months of study enrollment, in participants randomized to "rapid" ART when compared to standard ART initiation (64% vs. 51%,

respectively).[268a] Approximately 70% of patients in the rapid ART arm initiated same-day therapy, with 96% of rapid arm participants starting within 1 month. A 703-participant study of same-day ART initiation in Haiti demonstrated greater proportions of those participants randomized to same-day ART retained in care and virologically suppressed at 12 months, when compared to participants who initiated ART within 3 weeks of diagnosis.[268b] In San Francisco, California, participants enrolled in a program that offered ART initiation on the same day of diagnosis had faster times to virologic suppression when compared to participants offered standard-of-care ART initiation, and median time from care to ART was reduced by 96% (27 days vs. 1 day).[268c,268d] A rapid-entry program of economically and socially disenfranchised people with HIV in Atlanta, Georgia, reduced the time to ART initiation and the time to virus suppression.[268e] The accumulating data suggest that ART initiation on the same day as HIV diagnosis is feasible and demonstrates benefits at the individual- and population-based levels.

Treatment of HIV infection across CD4 strata may play an important role in limiting HIV transmission, a strategy known as "treatment as prevention." An observational study of nearly 3400 heterosexual patients with HIV and their uninfected partners (i.e., serodiscordant couples) demonstrated a 92% reduction in HIV transmission rates when the partner with HIV received ART.[269] In the HPTN 052 study, serodiscordant heterosexual couples in which the partner with HIV had a median CD4 count of approximately 450 cells/mm^3 were randomized to immediate or delayed ART and followed prospectively.[270] A 96% reduction in HIV transmission was observed in participants who received early ART. Early ART participants had increased CD4 counts and decreased rates of extrapulmonary tuberculosis. These data were the first from a randomized controlled trial to definitively demonstrate both a personal benefit to patients with HIV from early ART initiation and a public health benefit from a reduction in the rates of HIV transmission.

Although treatment guidelines play a useful role in helping physicians decide when to start therapy, patient willingness and readiness to start lifelong therapy are critical, and the role of meticulous adherence in ART success is undeniable.[271–273] The deferral of therapy until adherence can be maximized is preferable to suboptimal or incomplete therapy.

What to Begin With?

Several patient and virus factors need to be considered when choosing an initial regimen (Table 128.6). These include existing comorbidities (e.g., cardiovascular, renal, or psychiatric disease), potential adverse drug effects and interactions with other medications the patient may be receiving, pill burden, pregnancy or pregnancy potential, convenience, resistance testing results, access, cost, and potential patient adherence. Determining the drug susceptibility of a patient's HIV isolate is also an important step in constructing a combination antiretroviral regimen. Based on the results of genotypic testing, a regimen can be constructed that maximizes the probability of virologic suppression while minimizing adverse effects, toxicities, and pill burden for the patient. Fixed-dose combinations have become a mainstay of initial therapeutic regimens and have simplified the available choices. Most regimens in the United States will consist of a dual-NRTI backbone in combination with an INSTI.

TABLE 128.6 What Antiretroviral Regimens to Start

RECOMMENDED REGIMENS
Dolutegravir-abacavir-lamivudine[b,c]
Dolutegravir + tenofovir (TDF or TAF)-emtricitabine[b,c]
Raltegravir + tenofovir (TDF or TAF)-emtricitabine
Bictegravir-TAF-emtricitabine

[a]Recommendations from the 2018 U.S. Department of Health and Human Services (HHS) Antiretroviral Guidelines Panel.[14]
[b]Only for patients who are HLA-B*5701 negative.
[c]Dolutegravir may be associated with a risk of neural tube defects.[87h–87j] See reference 14 for HHS guidance regarding use.
TAF, Tenofovir alafenamide fumarate; *TDF,* tenofovir disoproxil fumarate.

Dual-NRTI Backbone

Three two-NRTI fixed-dose combinations are commercially available: tenofovir prodrug (TDF or TAF) plus emtricitabine, abacavir plus lamivudine, and zidovudine plus lamivudine. When considering TAF versus TDF, in general TAF has less bone and kidney toxicity, whereas TDF is associated with lower lipid levels. The combination of zidovudine and lamivudine, long the most widely used fixed-dose NRTI combination, has been replaced by tenofovir prodrug (TDF or TAF) and emtricitabine in the developed world as the most commonly used dual-NRTI combination.

Two studies have directly compared abacavir-lamivudine and TDF-emtricitabine, either in combination with lopinavir/r (the HEAT study) or in conjunction with a comparison of efavirenz and atazanavir/r (ACTG 5202). In the HEAT study, a similar proportion of patients in either treatment arm had achieved viral loads of less than 50 copies/mL after 48 weeks, regardless of initial screening viral loads.[18] In the larger ACTG 5202 trial, however, abacavir-lamivudine-treated subjects with initial viral loads greater than 100,000 copies/mL had an elevated hazard ratio of virologic failure compared with subjects receiving TDF-emtricitabine, forcing an independent data safety monitoring board to recommend that this arm of the study be discontinued.[16] Abacavir-lamivudine should therefore be used with caution in patients with viral loads greater than 100,000 copies/mL, pending further data in this population. Similar viral efficacy was observed in a study of abacavir-lamivudine in combination with dolutegravir, regardless of pretreatment plasma HIV-1 RNA levels.[87] Concerns have also been raised about a possibly increased cardiovascular risk associated with concomitant abacavir use, although more data are needed.[25,155] Until such information is available, caution is advised regarding abacavir use in patients with preexisting cardiac risk factors.[14]

Regimens containing a single NRTI in combination with an NNRTI and a PI, or NRTI-sparing regimens have been studied, generally with inferior results. Subjects randomized to receive an NRTI-NNRTI-PI combination discontinued treatment more frequently because of side effects than subjects receiving either a dual-NRTI–NNRTI or dual-NRTI–PI combination regimen.[274] An NRTI-sparing regimen of efavirenz and lopinavir/r was more likely to be associated with drug resistance, had increased frequencies of lipid elevations, and showed greater rates of virologic failure in patients with viral loads greater than 100,000 copies/mL when compared with a dual-NRTI–efavirenz or dual-NRTI–lopinavir/r combination regimen.[91] An NRTI-sparing regimen of raltegravir plus darunavir/r resulted in high rates of virologic failure.[275] Based on these findings, combination therapy without a dual nucleoside backbone is not generally recommended for initial ART, although trials of NRTI-sparing regimens continue.

Initial INSTI-, NNRTI-, or PI-Based Therapy

NNRTI-, PI-, and INSTI-based therapies, in combination with a dual-NRTI backbone, all provide effective suppression of HIV-1 replication and reconstitution of CD4 cell counts. The use of NNRTIs in initial therapy has been largely eclipsed by the successful development of more effective PI- and INSTI-based combination therapies. In general, integrase inhibitor–based regimens have demonstrated either noninferiority (raltegravir and elvitegravir) or superiority (dolutegravir) to some NNRTI- or PI-based regimens when tolerability and virologic and immunologic outcomes were compared. While the choice of whether to use an NNRTI, a boosted PI, or an INSTI in combination with two NRTIs as initial therapy needs to be individualized, INSTI options are preferred.[13–15]

Initial Integrase Inhibitor–Based Therapy

Treatment with integrase inhibitor–based therapy represents all of the regimens currently recommended by the HHS Antiretroviral Guidelines Panel.[14] Dolutegravir is dosed once daily while raltegravir is administered once or twice daily; bictegravir is coformulated into one once-daily pill. Although raltegravir and elvitegravir have not been directly compared as initial therapy in a clinical trial, raltegravir may be considered over fixed-dose coformulated elvitegravir in settings in which fewer drug-drug interactions, no food requirement, and no association with proximal renal tubulopathy or inhibition of creatinine secretion are relevant. One

study has demonstrated the noninferiority of dolutegravir when compared with raltegravir.[81]

Initial PI-Based Regimens

Several clinical trials have evaluated which PIs to use initially. Darunavir/r is preferred in certain clinical scenarios unless patients are intolerant to ritonavir-associated side effects. Atazanavir/r, darunavir/r, fosamprenavir/r, and saquinavir/r have all demonstrated noninferiority to lopinavir/r.[116,141,203,276] Atazanavir/r has fewer gastrointestinal side effects and may induce fewer changes in lipid profiles than lopinavir/r but is associated with more hyperbilirubinemia than other regimens, a toxicity that increases treatment discontinuation when compared with darunavir/r.[129,276] Nelfinavir and indinavir no longer have important roles in initial therapy.[196,198]

Alternative NNRTI-Based Regimens

Depending on comorbidities, in certain clinical situations alternative (e.g., efavirenz-based) regimens may be preferable to recommended regimens, recognizing the low genetic barrier to resistance of this class. Efavirenz is preferred over nevirapine for therapy in most treatment-naïve patients because of less toxicity and possibly greater efficacy. In pregnant persons and persons of child-bearing age who might become pregnant, alternatives to efavirenz should be considered, and nevirapine may be preferable. Similarly, in individuals with preexisting psychiatric disturbances, some prefer nevirapine or rilpivirine over efavirenz because of efavirenz-associated neuropsychiatric side effects. Rilpivirine may be considered in patients with pretreatment HIV-1 RNA level loads less than 100,000 copies/mL and CD4 counts greater than 200 cells/mm^3.

Triple NRTI and Four-Drug Therapy

Three-drug NRTI therapy has been compared with other regimens in clinical trials and is not generally recommended.[92,277] A comparison of TDF versus efavirenz, both combined with abacavir-lamivudine, resulted in a high rate of virologic nonresponse in treatment-naïve TDF-treated subjects, together with high rates of the M184V (98% of subjects tested) and K65R (54%) resistance mutations.[47]

No advantages have been demonstrated for four-drug versus three-drug initial regimens. In early trials, the addition of a PI to a three-drug regimen of two NRTIs plus an NNRTI provided no additional benefit.[90] The addition of a fourth drug to two-class, three-drug therapy confers no additional benefit. In a randomized, double-blind trial, standard three-drug therapy (zidovudine-lamivudine-efavirenz) had similar efficacy to four-drug therapy (zidovudine-lamivudine-efavirenz-abacavir), even in subjects with initial viral loads greater than 100,000 copies/mL.[20] In subjects with virologic suppression on a zidovudine-lamivudine-abacavir regimen, the addition of either TDF or efavirenz resulted in similar rates of virologic suppression maintenance; treatment failures were also similar.[278]

Two-Drug Therapy

While combinations of three active drugs remain the mainstay of ART, researchers have begun to explore the role of initial two-drug therapy in investigational regimens. An 805-participant study in France of treatment-naïve participants randomized to raltegravir twice daily or coformulated TDF and emtricitabine once daily, both with darunavir/r, demonstrated noninferior rates of virologic and clinical failure with raltegravir plus darunavir/r over 96 weeks, when compared to the more standard three-drug regimen of TDF, emtricitabine, and darunavir/r.[278a] There were caveats, however. Participants with baseline CD4 counts below 200 cells/mm^3 had more failures in the two-drug arm and there was a trend toward more failure in the two-drug arm in patients with viral loads greater than 100,000 copies/mL, a finding noted in two other studies that evaluated ritonavir-boosted darunavir plus raltegravir.[275,278b] A two-drug combination of lopinavir/r and lamivudine had noninferior rates of virologic suppression when compared to lopinavir/r and two NRTIs at 48 weeks.[278c] While this regimen is impractical due to pill burden, the side effect profile of lopinavir/r, and the need for twice-daily dosing, it does highlight the general point that some two-drug regimens may hold promise as initial ART. Studies are currently ongoing to evaluate additional two-drug combinations, specifically dolutegravir plus lamivudine and darunavir/r plus lamivudine.

Interrupting Therapy

Temporary discontinuations of antiretroviral treatment, whether referred to as structured treatment interruptions or intermittent therapy, have been studied in patients with HIV infection as a strategy to minimize drug toxicities and cost, decrease treatment fatigue, improve quality of life, stimulate HIV-specific immune responses, or minimize the emergence of drug-resistance viruses. In many of these respects, interruptions of therapy have been unsuccessful. The body of available evidence suggests a lack of benefit to structured treatment interruptions, and a large study demonstrated potential harm with this approach.[240,279] Several other studies have also supported the proposition that discontinuing therapy has detrimental effects on outcome.[280–282] Taken together, the available evidence on the use of treatment interruptions in HIV infection suggests a lack of benefit and the potential for harm with treatment interruption. Treatment interruptions are, thus, not a recommended strategy, and their use should generally be limited to the research setting as part of a clinical trial.

LABORATORY TESTING DURING ANTIRETROVIRAL THERAPY

CD4-based criteria are most widely used to determine when ART should be initiated. Resistance testing, HLA-B57 typing, and plasma viral RNA load monitoring are also important laboratory tests that assist the clinician in designing the most effective and patient-specific antiretroviral regimen. When CCR5 antagonist therapy is being considered, coreceptor tropism testing is essential. Routine monitoring of hepatic and renal function, along with serum lipids, fasting glucose, and hematologic parameters, should be undertaken when appropriate (Table 128.7).

CD4 T-Cell Counts

CD4 T-cell counts should be determined when an HIV diagnosis is made and monitored at intervals thereafter. However, the value of frequent (every 3–4 months) CD4 count monitoring in HIV clinical management has been called into question.[283] Patients with plasma viral loads less than 200 copies/mL and CD4 counts greater than 300 cells/mm^3 had a greater than 97% chance of maintaining durable CD4 counts greater than 200 cells/mm^3 for 4 years. Once combination ART is started, the CD4 count may reasonably be expected to increase between 50 to 150 cells/mm^3 in the first year and 50 to 100 cells/mm^3 in the second year.[284,285] In patients initiating therapy when CD4 counts fall below 200 cells/mm^3, only a minority reconstitute their CD4 counts to greater than 500 cells/mm^3 after 4 years.[284] CD4 counts can often reach levels considered normal in patients who initiate therapy with CD4 counts greater than 350 cells/mm^3.[286] For patients with consistently suppressed viral loads who have been on ART for 2 years or more, the HHS guidelines recommend yearly testing for those with CD4 counts of 300 to 500 cells/mm^3 and optional testing for those with CD4 counts greater than 500 cells/mm^3.[14]

HLA-B*5701 Screening

Prospective screening for the HLA-B*5701 allele reduces substantially, but does not eliminate, the risk of an abacavir hypersensitivity reaction.[24,287] All patients being considered for an abacavir-containing regimen should first undergo HLA-B*5701 testing. Patients who test positive for this allele should not receive abacavir therapy. Abacavir skin patch testing is not recommended.

Plasma Viral Load Monitoring

Viral load monitoring is necessary to assess the response to ART and the durability of virologic suppression. The goal of all ART, whether in treatment-naïve or treatment-experienced patients, living in developed or developing countries, is suppression of plasma HIV RNA to undetectable levels (<50 copies/mL). Viral load should be measured before starting therapy, 2 to 8 weeks later, and then at 4- to 8-week intervals until HIV RNA is no longer detectable. At least a 1 $\log_{10}$ reduction in viral load should be expected at 4 weeks, with a decline in plasma HIV RNA to less than 50 copies/mL by 16 to 24 weeks of therapy. More

TABLE 128.7 Laboratory Monitoring Before and During Antiretroviral Therapy

	ENTRY INTO CARE	STARTING[a] OR MODIFYING ART	2–8 WEEKS POST-ART	EVERY 3–6 MONTHS	EVERY 6 MONTHS	EVERY 12 MONTHS	TREATMENT FAILURE
CD4 T-cell count	✔	✔		✔[b]		✔[c] (after 2 yr)	✔
HIV viral load	✔	✔	✔[d]	✔[e]	✔[e]		✔
Resistance testing	✔	✔[f]					✔
HLA-B*5701 testing		✔[g]					
Tropism testing		✔[h]					✔[h]
Hepatitis B serology (HBsAb, HBsAg, HBcAb)[i,j,k]	✔	✔				✔	
Hepatitis C screening (HCV Ab or RNA)[l]	✔					✔[m]	
Basic chemistries[n]	✔	✔	✔	✔			
ALT, AST, total bilirubin	✔	✔	✔	✔			
CBC with differential	✔	✔			✔		
Fasting lipid profile[o]	✔	✔				✔	
Fasting glucose or hemoglobin A_{1c}	✔	✔		✔[p]		✔[p]	
Urinalysis[q]	✔	✔			✔[r]	✔	
Pregnancy test		✔[s]					

[a]If ART initiation occurs soon after the HIV diagnosis and entry into care, then repeat baseline testing is not needed.
[b]During the first 2 years of ART or if viremia develops while the patient is on ART or the CD4 count is <300 cells/mm^3.
[c]After 2 years on ART with consistently suppressed viral load, check every 12 months for CD4 count 300–500 cells/mm^3; for CD4 count >500 cells/mm^3, CD4 monitoring is optional.
[d]If HIV RNA is detectable at 2–8 weeks, repeat testing every 4–8 weeks until viral load is <200 copies/mL.
[e]For patients on ART, viral load is typically measured every 3–4 months. This interval may be extended to every 6 months for adherent patients with stable CD4 counts and consistently suppressed viral loads.
[f]Resistance testing should include genotypic drug resistance testing for mutations in the reverse transcriptase and protease genes. If there is a clinical concern for transmitted INSTI resistance, integrase resistance mutation testing may be included.
[g]If considering abacavir.
[h]If considering a CCR5 antagonist or for failure of a regimen that includes a CCR5 antagonist.
[i]ART regimens for patients with HIV and HBV infection should include TAF or TDF plus either lamivudine or emtricitabine.
[j]If HBsAb, HBsAg, and HBcAb are negative, administer HBV vaccine series.
[k]An isolated positive HBcAb is most consistent with resolved HBV infection and loss of HBsAb. Consider HBV viral load testing for confirmation.
[l]HCV antibody testing may be inadequate to screen for HCV infection acquired in the past 6 months or HCV infection of any duration in patients with CD4 counts <100 cells/mm^3.
[m]Repeat screening for at-risk patients: injection drug users, MSM, jailed persons, and people with percutaneous/parenteral exposure to blood in unregulated settings.
[n]Serum sodium, potassium, chlorine, bicarbonate, BUN, creatinine, fasting glucose, and creatinine-based estimated glomerular filtration rate. Measure serum phosphorus in patients with chronic kidney disease on a TAF- or TDF-containing regimen.
[o]If normal at baseline test, check yearly. If borderline or abnormal, check every 6 months.
[p]If normal, check every 12 months. If abnormal at last measurement, check every 3–6 months.
[q]Urine glucose and protein should be checked before starting a TAF- or TDF-containing regimen
[r]If on a TAF- or TDF- containing regimen, check every 6 months. Otherwise, check urinalysis yearly.
[s]In woman with child-bearing potential.
ALT, Alanine aminotransferase; *ART,* antiretroviral therapy; *AST,* aspartate aminotransferase; *BUN,* blood urea nitrogen; *CBC,* complete blood count; *HBcAb,* hepatitis B core antibody; *HBsAb,* hepatitis B surface antibody; *HBsAg,* hepatitis B surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HCV Ab,* hepatitis C virus antibody; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen; *INSTI,* integrase strand transfer inhibitor; *MSM,* men who have sex with men; *TAF,* tenofovir alafenamide fumarate; *TDF,* tenofovir disoproxil fumarate.
Modified from US Department of Health and Human Services, Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. Accessed April 13, 2018.

rapid virus decay kinetics may be observed with INSTI-based therapy, although the clinical significance of this is unknown.[87] Viral blips, or transient viral load increases to between 50 and 1000 copies/mL in a patient with previously suppressed plasma HIV RNA, are occasionally seen but do not appear to be associated with eventual virologic failure and do not necessitate a change in therapy.[288,289] In some cases, blips may be the results of temporary lapses in patient adherence to antiretroviral regimens.[290] True virologic failure is defined as a persistently detectable viral load in a patient with previously suppressed HIV RNA or the inability to achieve an undetectable viral load after 24 to 48 weeks of therapy. Although multiple causes of virologic failure are possible, a detectable viral load in either scenario should prompt HIV drug resistance testing. The term *low-level viremia* specifically refers to confirmed detectable plasma HIV-1 RNA levels less than 200 copies/mL.

The initiation of effective ART in developing countries can result in initial treatment outcomes that are similar to those seen in resource-rich nations.[291] The lack of available viral load monitoring in many resource-poor countries, however, can complicate efforts to achieve and sustain durable virologic suppression on ART.[292,293] One potential consequence of using CD4- or clinically based criteria to guide treatment decisions is the emergence of HIV-1 drug resistance in patients who may unknowingly continue a partially suppressive antiretroviral regimen. High rates of HIV resistance have been observed in some developing countries that institute ART programs without frequent viral load monitoring.[294–297] The accumulation of resistance carries the risk of compromising both first- and second-line therapeutic regimens, and efforts to bring cost-effective viral load monitoring to patients with HIV worldwide should be encouraged.

HIV Drug Resistance Testing

Virologic failure that results from resistance to antiretroviral agents is a major cause of treatment failure.[298–301] The clinical significance of antiretroviral drug resistance was demonstrated not long after the

introduction of the nucleoside analogues.[302,303] Subsequent studies have shown an increased risk of virologic failure when patients are treated with drugs to which the virus shows genotypic or phenotypic resistance.[304,305] Resistance testing for HIV protease and RT mutations is cost-effective under several clinical circumstances and is currently recommended at the time of HIV diagnosis, before initiating therapy in pregnant persons, and whenever a treatment regimen fails with HIV RNA levels greater than 1000 copies/mL.[120,306–311] The resistance test should, if possible, be performed while the patient is still on the failing regimen. The role of testing for integrase inhibitor resistance in patients with newly diagnosed HIV is less clear. A recent modeling study suggested that, with the currently low base case prevalence of transmitted integrase inhibitor resistant virus (around 0.1%), integrase resistance testing of patients newly diagnosed with HIV would result in worse clinical outcomes and would not be cost-effective.[311a]

Genotypic resistance assays sequence HIV genes from patient isolates and report the presence of mutations that confer resistance; these assays are generally preferred for antiretroviral-naïve patients. Phenotypic resistance assays rely on cultured patient HIV isolates and report fold changes in sensitivities in the presence or absence of drugs. Phenotypic tests are usually more expensive than genotypic tests but can be useful in the interpretation of more complex resistance patterns. Virtual phenotypic resistance assays make use of a library of known matched genotypes and empirically tested phenotypes to predict a patient's phenotype based on known genotype results. All resistance assays have limitations when determining resistance to recently approved antiretrovirals. Clinical experience is often helpful to evaluate the number and type of genotypic mutations and the magnitude of phenotypic fold changes in drug susceptibility that are clinically meaningful and correspond to a loss of in vivo drug activity. Technical issues can sometimes prevent successful resistance testing when plasma HIV RNA levels are less than 500 to 1000 copies/mL.

Determining HIV Coreceptor Usage

The coreceptor tropism assay used most widely in clinical trials is a phenotypic assay that requires the amplification of *env* sequences from plasma HIV-1 RNA.[312] A commercial genotypic assay is available and can be used to determine coreceptor usage but has not been validated with clinical outcomes, making it more challenging for the practicing clinician to interpret. Currently, the HHS Antiretroviral Guidelines Panel recommends phenotypic testing as the preferred assay for measuring tropism.[14] As with resistance testing, viral loads greater than 1000 copies/mL are needed to most accurately perform coreceptor usage testing. Test results supplied to the clinician will indicate whether a virus is R5, X4, or dual/mixed. The term *dual/mixed* refers to the fact that the current coreceptor usage assay cannot distinguish between the presence of one virus that uses either receptor for viral entry (dual-tropic) or mixed viral populations in the same patient sample—populations that use either CCR5 or CXCR4. For clinical purposes, this distinction is not as important as knowing whether the sample contains any X4 virus. Although the initially approved phenotypic assay only detected X4 viruses if they constituted 5% to 10% of the "quasipecies" present, assays with improved sensitivity for detection of CXCR4, using or dual/mixed virus, are now available.[313] Changes in coreceptor usage, rather than emergence of true resistance, represent the dominant mechanism of virologic escape from CCR5 antagonists.[233]

CHANGING THERAPY

Clinical assessment, together with measurement of HIV RNA levels and CD4 cell counts, should be used to assess the need to change therapy. If the patient experiences drug toxicity or intolerance or is unable to adhere to therapy, a change in regimens may be required. In these situations, it may be appropriate simply to replace the offending drug by another that is better tolerated and exhibits similar potency.

Clinical situations that should prompt consideration for changing therapy include a poor early virologic response to therapy, failure to suppress plasma HIV-1 RNA to undetectable levels by 4 to 6 months after initiation, repeated detection of virus in plasma after initial suppression to undetectable levels, a persistent and significant decline in the CD4 T-cell count, and clinical deterioration.

The selection of a new regimen in patients with virologic, immunologic, and clinical failure should involve consideration of the history of previous antiretroviral drug exposure, current drug resistance patterns, other drugs with the potential for drug interactions, and individual comorbid conditions. At least two, and preferably three, fully active drugs should be included in the new regimen, ideally using agents from at least one new class. With the multiple drugs available, the goal of the new regimen should always be to durably suppress plasma HIV RNA levels to below detectable limits of the most sensitive available assay.

SPECIAL CIRCUMSTANCES

Preexposure Prophylaxis

ART has been studied as possible HIV prevention for at-risk, HIV-uninfected populations. The effectiveness of a 1% TDF-containing vaginal gel was studied in a randomized controlled trial of South African women, where its use before and after potential HIV exposures was associated with a 39% reduction in HIV incidence.[314] Greater risk reduction was observed in women with high levels of gel adherence. A study of 2499 HIV-uninfected men and transgender women who have sex with men demonstrated a 44% risk reduction of HIV infection over 1 year of follow-up in participants randomized to receive oral TDF-emtricitabine daily, compared with placebo.[315] This risk reduction was strongly tied to adherence and varied from 73% in participants with 90% or greater adherence to 32% when adherence was less than 50%. A large study of serodiscordant heterosexual Kenyan and Ugandan couples found that daily oral TDF use reduced the incidence of HIV infection by 62% and that daily TDF-emtricitabine reduced HIV incidence by 73%.[316] Similar findings were reported in a 1200-person Botswanan study where daily oral TDF-emtricitabine reduced HIV incidence by 63%.[317] In general, preexposure prophylaxis reduces the risk of HIV infection and on-demand preexposure prophylaxis reduces HIV infection risk specifically in men who have sex with men.[318,319]

Not all studies have demonstrated benefits to preexposure prophylaxis. A study of daily oral TDF-emtricitabine in African women did not demonstrate reduced HIV incidence rates and was associated with increased rates of side effects.[320] A separate study of African women showed no HIV incidence reduction with the use of oral TDF or daily TDF vaginal gel; adherence to study drugs was low.[321] The Centers for Disease Control and Prevention has issued interim guidelines on the use of HIV chemoprophylaxis.[322,323] These guidelines emphasize that patients considered for preexposure prophylaxis should be at an ongoing high (for men having sex with men) or very high (for heterosexuals) risk for HIV acquisition. Subjects receiving preexposure prophylaxis should be treated with the fixed-dose combination TDF-emtricitabine once daily. Regular HIV antibody testing, counseling, sexually transmitted infection screening, and serum creatinine measurements should be performed. There are currently insufficient data to recommend TAF-containing regimens for preexposure prophylaxis.

Postexposure Prophylaxis

Exposures to HIV can occur both inside and outside of the health care setting. For health care personnel, the risk of HIV exposure results from percutaneous or mucous membrane exposure to HIV-infected body fluids, most commonly blood. Nonoccupational exposure can occur with any exchange of body fluids, most commonly sexual activity (voluntary or forced) or intravenous drug use. Saliva that does not contain blood is associated with a negligible risk of HIV transmission.[324] Few data exist in humans regarding optimal postexposure prophylaxis (PEP), so in many cases, treatment decisions will be based on guidelines from national advisory panels, anecdotal clinical experiences, and patient preferences.

Occupational HIV Exposures

Prospective studies have suggested a roughly 0.3% and 0.09% risk of HIV transmission from percutaneous and mucous membrane exposure, respectively.[325,326] The risk of HIV transmission increases with the severity of the exposure; a deep puncture from a hollow-bore needle containing HIV-infected blood with a high viral load confers greater risk than a superficial cut from a suture needle in a patient with an undetectable

viral load. Transmission can occur in both settings, however, and prompt evaluation by occupational health services is imperative. If the source person is known to be HIV positive, PEP should be considered.[326] For blood or fluid sources with unknowable HIV status (e.g., from a disposed needle in a sharps container), PEP is generally not recommended. The absolute and relative risk reductions in HIV transmission from the use of PEP are unclear, but PEP should begin as soon as possible after exposure. Animal studies suggest the optimal duration of PEP may be approximately 4 weeks, although the tolerability of regimens can limit patient adherence.[327–333] Because of toxicities and tolerability, older guidelines had recommended a tiered approach for health care workers needing PEP, wherein more severe exposures received combination three-drug therapy and less severe exposures receive two-drug therapy. However, with the improved dosing schedules and tolerability of newer medications, all PEP regimens should now contain at least three drugs regardless of the severity of the exposure.[326] Any PEP regimen should attempt to maximize adherence (e.g., low pill burdens, no food restrictions), limit toxicities, and account for any drug resistance in the source HIV. The delay associated with HLA-B*5701 typing before abacavir use should generally preclude its inclusion in PEP regimens. Consultation with persons with expertise in HIV ART is advised.

Nonoccupational HIV Exposures

Nonoccupational postexposure prophylaxis (nPEP) is recommended for patients presenting within 72 hours of exposure to known HIV-infected body fluids. Under these circumstances, nPEP should consist of combination three-drug ART for 28 days.[334] HIV seroconversion on nPEP, when used for voluntary sexual and intravenous drug use exposures, has been reported to occur at a rate of 1% in one nonrandomized study; the availability of nPEP does not appear to increase risk behavior.[335–337] The use of prophylaxis in sexual assault victims is recommended, but the acceptance of and adherence to nPEP in this group may be low.[338] A decision to offer nPEP when the source patient is unavailable, and thus has an unknown HIV status, should be made on an individual basis. HIV postexposure prophylaxis is not generally recommended in the setting of bombings or other mass-casualty events.[339]

CONCLUSIONS AND FUTURE DIRECTIONS

Remarkable progress has been made since the advent of ART for HIV infection in the mid-1980s, turning a once almost uniformly fatal disease into a generally treatable infection. Newer regimens offer greater convenience and less toxicity than ones previously used, and emerging data suggest that ART should be initiated earlier during the natural history of HIV infection than was previously recommended.

Many challenges remain for patients on therapy, including adherence to complex regimens, emergence of drug-resistant virus variants, and the development of complications of therapy. Close monitoring of patients on therapy remains essential, using available laboratory tests, such as CD4 cell counts and viral load measurements, to evaluate success or failure. Progress is also being made in the rollout of ART in the developing world, although major challenges remain because of the costs and infrastructure needs required for sustainable programs. As the rollout and optimization of antiretroviral treatment programs continue in the developing world, viral load testing and resistance monitoring will be increasingly important to minimize the morbidity associated with suboptimal treatment regimens.

The successes of ART have permitted a consideration of the possibility of HIV cure. The experience of the "Berlin patient," a man with HIV presumably cured after allogeneic stem cell transplantation from a CCR5 Δ32-homozygous donor, provided the proof-of-concept that the HIV reservoir could be eradicated.[340] The best research approach to pursue HIV cure on a broader scale remains unsettled. Research efforts currently focus on eradication approaches to evaluate the roles of latency reversal agents, broadly neutralizing antibodies, and compounds with novel mechanisms of action, with the hope of ushering in a new era of potent and selective eradication therapeutics.

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129 Management of Opportunistic Infections Associated With Human Immunodeficiency Virus Infection

Henry Masur

SHORT VIEW SUMMARY

Definition
- Acquired immunodeficiency syndrome (AIDS) is an immunologic disease caused by human immunodeficiency virus (HIV), a retrovirus that is characterized by declining immunity and increased susceptibility to characteristic opportunistic infections and cancers.
- AIDS-related opportunistic infections are defined as infections that occur with increased frequency or severity in patients with HIV infection.

Epidemiology
- The incidence of HIV-related opportunistic infections in an individual patient depends on the patient's degree of immunosuppression and environmental and person-to-person exposures.
- Occurrence of specific infections may be due to primary infection (initial acquisition of the pathogen), reinfection, or reactivation of latent infection (secondary infection).

Microbiology
- The organisms that cause HIV-related opportunistic infections include bacteria, fungi, viruses, and protozoa.
- The constellation of infections that occur in AIDS is characteristic of this syndrome: *Pneumocystis* pneumonia, *Toxoplasma* encephalitis, cytomegalovirus retinitis, pneumococcal pneumonia, disseminated *Mycobacterium avium* complex, cryptosporidiosis, cryptococcal meningitis, and tuberculosis. The occurrence of these infections individually or in a cluster should prompt consideration of underlying HIV infection in any patient without another clear predisposing condition.

Diagnosis
- Given the broad range of pathogens that can cause infectious syndromes in patients with HIV infection and the potential toxicities of therapeutic agents, specific microbiologic diagnoses should be established in preference to empirical treatments when possible.

Therapy
- Specific agents can successfully treat most, but not all, HIV-related opportunistic infections (see Table 129.2). Prognosis depends on the severity of the acute illness, the presence of comorbidities, and availability of effective and well-tolerated therapies. For a few HIV-related opportunistic infections such as cryptosporidiosis and JC virus encephalitis, there is no effective specific therapy; effective treatment relies on improving immune response by initiating effective antiretroviral therapy (ART).

Prevention
- ART is the most effective preventive strategy for reconstituting immunity and reducing (but not eliminating) the risk of opportunistic infections.
- Primary prophylaxis regimens for *Pneumocystis jirovecii* and *Toxoplasma gondii* are effective for patients not on ART (admittedly a rare group who would take prophylaxis but not ART) with $CD4^+$ T-lymphocyte counts less than 200 cells/mm^3 or for patients starting ART with $CD4^+$ T-lymphocyte counts less than 100 cells/mm^3.
- Long-term suppressive therapy is important for preventing relapse and recurrence of certain infections while awaiting ART-induced immune reconstitution.
- Immunization is an important strategy for preventing viral and bacterial diseases in patients with HIV infection.

The quality and duration of survival for patients with human immunodeficiency virus (HIV) infection has improved remarkably since acquired immunodeficiency syndrome (AIDS) was first recognized in the early 1980s.[1–5] Much of the early improvement in prognosis before the availability of potent antiretroviral therapy (ART) was the result of anti-*Pneumocystis* prophylaxis and effective management of individual acute opportunistic infections.[6–9] Since 1995 the expanding number of well-tolerated and highly effective antiretroviral drugs has permitted the development of multidrug regimens that have durably improved $CD4^+$ T-cell counts and reduced HIV viral load, thus reducing the incidence of opportunistic infections and neoplasms and prolonging survival. For patients who are linked to care and who manifest durable responses to long-term ART, survival of patients with HIV infection is currently almost equal to that of individuals without HIV infection.[4,5]

HIV-related opportunistic infections and neoplasms continue to occur in the United States and abroad. In the United States and globally, many HIV-infected persons are not receiving ART and thus continue to develop the complications of untreated HIV infection. In the United States despite efforts to provide everyone with access to ART, substantial numbers of patients are unaware of their HIV infection or have not been able to access ART.[10–13] In Washington, D.C., for example, it was estimated that in 2016, 10% to 15% of HIV-infected persons had not been tested for HIV and were thus unaware that they were infected. Among individuals who were tested, 34% had a $CD4^+$ count <200 cells/mm^3 at the time they were first tested, and some presented with an acute opportunistic infection as the initial manifestation of HIV infection. Among persons who were aware of their HIV infection, 26% were not retained in care, and 37% did not achieve durable viral suppression.[14]

Thus in the United States a substantial number of HIV-infected individuals are susceptible to opportunistic infections and neoplasms. Such HIV-related complications are seen regularly in health care facilities throughout the United States including urban and rural areas in all geographic regions.

HIV-related opportunistic infections do not behave clinically exactly like opportunistic infections seen in other immunosuppressed populations. For example, cytomegalovirus (CMV) manifests in HIV-infected persons primarily as retinitis or colitis (and almost never as pneumonitis), whereas in stem cell transplant recipients CMV manifests as pneumonitis or colitis. Toxoplasmosis manifests mainly as encephalitis in patients with HIV infection, but in patients with cancer or organ transplants it is more likely to manifest as visceral or disseminated disease. *Pneumocystis* pneumonia is far more likely to be an acute illness in organ transplant recipients than in HIV-infected persons, who often develop indolent disease (which ultimately becomes life threatening or fatal if untreated). Trimethoprim-sulfamethoxazole (TMP-SMZ) is associated with much more frequent allergic responses in patients with HIV infection compared

with patients with cancer or patients who have received stem cell or solid-organ transplants.

The management of HIV-related opportunistic infections continues to evolve as new diagnostic tests and new therapies are developed. The current guidelines for management of HIV-related opportunistic infections from the National Institutes of Health, Centers for Disease Control and Prevention, and Infectious Diseases Society of America are updated multiple times per year.[8]

As patients with HIV infection live longer, new clinical syndromes are emerging. The additional years of survival permit hepatitis C virus (HCV), hepatitis B virus (HBV), and human papillomavirus (HPV), for example, to have more impact. More patients with HIV and either HCV or HBV are living long enough to develop cirrhosis, decompensated liver disease, and hepatocellular carcinoma.[15–21] More patients with HPV are living long enough to develop HPV-related cancers of the cervix, rectum, and oropharynx.

In the current era, the causes of death for patients with HIV have changed.[17–19] Patients have an enhanced inflammatory state despite durable viral suppression.[22–24] This advanced inflammatory state appears to accelerate cardiovascular, hepatic, and renal disease and appears to have a role in enhancing the development of cancers. Liver disease has become a major cause of death in patients with HIV in the United States.[17–19] Malignant neoplasms other than Kaposi sarcoma and lymphoma, most of which are associated with chronic viral infection, are also more frequent causes of death than in the past.[25–28] Patients may also be affected by concurrent health risks involving opioids, alcohol, tobacco, and obesity, which also affect their long-term survival.

Efforts to recognize HIV infection early and to initiate ART before immunosuppression is substantial are the most important approaches to prevention of HIV/AIDS–related opportunistic infections. Similarly, early recognition of opportunistic infections and prompt initiation of appropriate treatment will minimize the impact of opportunistic infections when they do occur. Health care resources used to manage opportunistic infections are unequivocally well spent if applied with a strategy that emphasizes prevention and that provides aggressive recognition of and therapy for acute syndromes.[29,30]

IMMUNOLOGIC MONITORING

Patients with HIV infection differ from most other populations of immunosuppressed patients in that there is a highly sensitive and highly specific laboratory test to determine susceptibility to individual opportunistic pathogens. The $CD4^+$ T-cell count is a valuable marker to determine when patients are at increased risk for development of a specific opportunistic infection.[31–35] For example, for patients with HIV infection, *P. jirovecii* pneumonia (PCP) occurs rarely in patients who have $CD4^+$ T-cell counts greater than 200 to 250 cells/mm^3,[8,31,32] and disseminated *Mycobacterium avium-intracellulare* complex (MAC) occurs rarely in patients with $CD4^+$ counts greater than 50 cells/mm^3.[8,36,37] The $CD4^+$ T-cell count is helpful for focusing a diagnostic evaluation. For instance, if a patient with a $CD4^+$ count of 700 cells/mm^3 develops cough, fever, and diffuse interstitial pulmonary infiltrates, the likelihood that this syndrome is caused by PCP is extremely low (but not zero). Therefore sputum examination or bronchoalveolar lavage for *Pneumocystis* is not initially indicated for such a patient, and most attention when processing respiratory secretions and choosing empirical therapy should be directed at common bacterial and viral pathogens. In contrast, if the $CD4^+$ count were 25 cells/mm^3 in the same patient, the search for *Pneumocystis* in sputum or bronchoalveolar lavage would be an important focus because PCP is so common in this patient population. If a patient with HIV infection develops chronic fever and weight loss without focal findings, disseminated MAC becomes a more important consideration if the $CD4^+$ T-cell count is less than 50 cells/mm^3 than if the $CD4^+$ count is more than 300 cells/mm^3, in which case tuberculosis (TB), endemic mycoses, and HIV-related malignant neoplasms (especially lymphoma) would be more appropriate considerations.

Although current $CD4^+$ T-cell counts provide a useful estimate of susceptibility to infections, they are not perfect predictive tools. For example, although more than 90% of cases of PCP occur in patients with $CD4^+$ T-cell counts less than 200 cells/mm^3, some cases occur in patients with $CD4^+$ counts in the range of 200 to 300 cells/mm^3, and a few occur in patients with $CD4^+$ counts greater than 300 cells/mm^3.[31–34] Moreover, if the most recent $CD4^+$ count and viral load were obtained many months before the patient's presentation or if the patient's adherence to ART is uncertain, it will be difficult to judge the patient's current immune status.

A possible concern has been that $CD4^+$ T-cell counts in patients receiving ART may not accurately reflect the clinical susceptibility to opportunistic infections. Evaluation of several large databases demonstrated that ART does not alter the relationship between $CD4^+$ T-cell counts and the occurrence of opportunistic infections in any substantial manner, regardless of how low the nadir $CD4^+$ count was before initiation of ART.[38]

When ART is initiated, it is important to recognize that some syndromes that may appear to be infectious are immunologic or inflammatory reactions induced by the initiation of ART—that is, immune reconstitution inflammatory syndrome (IRIS). Most of these syndromes that occur soon after ART initiation, especially in patients with low $CD4^+$ T-cell counts, appear to be reactions to latent organisms or residual antigen, rather than active infections due to replicating organisms.

$CD4^+$ T-cell counts are not the only laboratory predictors of opportunistic infection. HIV viral load in the peripheral blood is an independent predictor. For patients with detectable HIV viremia, with each log increase in titer, the likelihood of occurrence of an opportunistic infection increases. For patients with HIV viral loads that are below the level of assay detection (e.g., <50 copies/μL), the risk of opportunistic infection is reduced substantially at any $CD4^+$ T-cell count compared with patients with measurable viremia.[39–43]

Pathogen-specific assays are also useful for predicting or recognizing the occurrence of opportunistic infections. Assays for TB (purified protein derivative skin test or interferon-γ release assay), *Cryptococcus* (serum cryptococcal antigen), *Toxoplasma* (serum antibody), HBV (hepatitis B surface antigen [HBsAg], hepatitis B surface [HBs] antibody, hepatitis B core antibody [HBc]), and HCV (HCV serology and polymerase chain reaction [PCR]) are examples of tests that help inform prevention and management strategies.[8,44–48]

Although prospective monitoring for risk of opportunistic infection focuses on laboratory-based measures, clinical findings can be useful predictors of opportunistic infection susceptibility as well. For example, the development of otherwise unexplained oropharyngeal candidiasis or oral hairy leukoplakia, wasting, and any type of pneumonia can each be indicators of current susceptibility to PCP or other opportunistic infections.[8]

SPECTRUM OF OPPORTUNISTIC PATHOGENS

Many of the opportunistic diseases that characterize HIV-induced immunosuppression occur in patients with HIV infection much more frequently than in almost any other patient group. For example, without prophylaxis or effective ART, PCP ultimately develops in at least 80% of HIV-infected patients in North America.[8,33] The annual attack rate for patients with $CD4^+$ T-cell counts less than 100 cells/mm^3 is about twice that for patients with severe combined immunodeficiency syndrome and more than 10 times the rate for patients with organ transplantation, solid tumors, or most hematologic malignant neoplasms.[49] Disseminated MAC was rarely recognized in humans before the advent of HIV infection, yet it occurred in 30% to 50% of patients with advanced HIV infection in North America before ART and specific chemoprophylaxis became standard of care.[8,36,37]

Cerebral toxoplasmosis, chronic cryptosporidiosis, chronic microsporidiosis, and Kaposi sarcoma are examples of other processes that occur only rarely in individuals other than patients with HIV infection. Their presence should strongly suggest that HIV testing be performed unless there is another obvious cause of severe immunosuppression.

Environmental exposure is an important determinant of the infectious complications of HIV infection. Thus management of HIV-infected patients benefits from careful assessment of geographic and occupational exposures. HIV-infected patients likely have enhanced susceptibility to certain pathogens if they work in certain occupations such as child care (cryptosporidia, *Streptococcus pneumoniae,* and enteric bacterial pathogens), correctional facilities or homeless shelters (TB), and veterinary

facilities (cryptosporidia). Geography is also important (*Histoplasma* in the Midwest, *Coccidioides* in the Southwest, *Trypanosoma cruzi* in Brazil, TB in South Africa and South East Asia). Some pathogens are ubiquitous, such as *Streptococcus pneumoniae, Candida,* herpes simplex virus type 1 (HSV-1), and CMV. Other pathogens are not so universal and occur only because of specific exposure to an infected individual or an environmental source. These exposures may be respiratory (e.g., TB, endemic mycoses, and *Pneumocystis*), enteric (e.g., *Salmonella, Cryptosporidium,* and *Microsporida*), vector-borne (e.g., *Leishmania, Bartonella,* trypanosomes), contact mediated (e.g., methicillin-resistant *Staphylococcus aureus* [MRSA]), or sexual (e.g., HSV-2, human herpesvirus 8 [HHV-8], *Treponema pallidum*).[8,50–59]

Traditionally, most HIV-associated opportunistic infections were thought to be caused by reactivation of latent infection, but this conclusion was based primarily on speculation rather than data. Some episodes of opportunistic infection in adults clearly represent primary infection rather than reactivation. Moreover, for some patients, second episodes of disease such as TB and PCP have been caused by strains that are clearly different from the strain causing the initial episode, suggesting that acquisition of a new strain rather than reactivation of the initial disease-causing strain can produce acute infection.[60–66]

INFECTIONS DUE TO PATHOGENS THAT ARE NOT OPPORTUNISTIC

Patients with HIV infection develop nonopportunistic as well as opportunistic infections. HIV-infected patients are just as likely to acquire common, community-acquired, or hospital-acquired pathogens as non–HIV-infected patients. Therefore if a patient with HIV infection develops a respiratory syndrome, diagnostic considerations should include common causes of disease in the community including influenza virus, *Mycoplasma pneumoniae,* and *Legionella* infections. Diarrhea is more likely caused by nonopportunistic viruses, microbial toxins, and bacteria that are circulating in the community than by cryptosporidia or microsporidia.[67–70]

Behavioral factors can influence the relative frequency of causative pathogens. *S. aureus* (especially MRSA) is more common among men who have sex with men[50,51] and persons who inject drugs compared with the general population. Enteric pathogens are overrepresented among men who have sex with men compared with the general population. Thus in considering the differential diagnosis of various syndromes, common community-acquired pathogens and pathogens associated with certain lifestyles or exposures should not be overlooked.

MANAGEMENT OF ANTIRETROVIRAL THERAPY FOR PATIENTS WITH ACUTE OPPORTUNISTIC INFECTION

If a patient who has not been on ART develops an acute opportunistic infection, providers must decide when to initiate ART, as all patients with HIV infection should be treated expeditiously with ART. The immune augmentation that ART produces may help facilitate recovery from an acute opportunistic infection, but definitely will ultimately be beneficial in preventing HIV-related opportunistic complications and prolonging survival.[71] However, complications are associated with initiation of ART, which must factor into the decision about when to initiate ART.

For ART-naïve patients, the initiation of ART may produce a clinically apparent, enhanced inflammatory response (IRIS) to the presenting clinical syndrome that can cause considerable morbidity.[8,72–74] For instance, in terms of the site of clinical disease that is being treated, initiating ART after PCP therapy is started can exacerbate pulmonary dysfunction when the enhanced immune and inflammatory responses interact with remaining *Pneumocystis* organisms or antigen in the lung. Similarly a patient with cryptococcal or tuberculous meningitis may develop dangerously increased intracranial pressure (ICP) when ART is initiated and the inflammatory response to meningeal organisms or antigen is enhanced.[72–78]

ART-induced IRIS may also unmask a subclinically infected site that had latent organisms or residual antigen due to an infection the patient was known to have or due to a pathogen that was not known to be present. For example, for a patient being treated for pulmonary TB, ART-associated IRIS may manifest as meningitis, pericarditis, or lymphadenitis, as the IRIS involves sites that did not initially appear clinically to be infected. For an HIV-infected patient not known to have an opportunistic infection, for example, when ART is started, IRIS may manifest as retinitis due to CMV that was not clinically apparent before ART or lymphadenitis due to MAC that was not known to be present. Some of these IRIS presentations may be mild and self-limiting. Others can be life threatening, can be difficult to distinguish from new opportunistic infections, and may warrant therapy with corticosteroids or immunosuppressive biologic agents.

Delivering ART to a patient who is seriously or critically ill presents special pharmacologic challenges. Patients may be unable to absorb oral drugs because they are intubated, they have severe nausea, or they have poor gastrointestinal absorption due to cryptosporidiosis or microsporidiosis. In such cases, clinicians must decide whether to try to optimize pharmacokinetics to reach targeted serum levels, given uncertainty about absorption, or to withhold therapy until ingestion and absorption are more predictable.

The current recommendation for patients not already receiving ART is that ART should be initiated within the first 2 weeks of treatment of the opportunistic infection.[8,71] This recommendation is based on studies that showed waiting longer was associated with the development of additional opportunistic infections and decreased survival. Exactly when in that window period ART should be started depends on clinical judgment related to the severity of the patient's infectious syndrome, the projected ability to absorb oral drugs, and potential drug interactions. For patients with severe disease and complicated management, longer intervals to initiation of ART may be appropriate.

There are notable exceptions to the general recommendation to start ART within 2 weeks of diagnosing an opportunistic infection. For opportunistic infections that have no effective specific therapy such as cryptosporidiosis and JC encephalitis, initiating ART is the only intervention likely to provide benefit, and thus ART should be started as soon as feasible.[79–83] For cryptococcal or tuberculous meningitis, ART should not be initiated until 2 to 10 weeks of therapy for the opportunistic infection have been completed, especially if patients have increased ICP or low cerebrospinal fluid (CSF) white blood count at time of diagnosis.[8] Clinically apparent pericardial disease warrants similar considerations.

For patients already receiving ART when an opportunistic infection is diagnosed, ART should generally be continued if possible. However, as discussed earlier, drug absorption issues or potential drug interaction complexities may be sufficiently compelling that temporary ART interruption may be the best management strategy.

DRUG INTERACTIONS

Some drugs used for treatment or prevention of opportunistic infections can interact with each other, with drugs used to treat concurrent conditions such as seizures or depression or anxiety, or with antiretroviral agents.[8] Such interactions can alter the efficacy and toxicity of prescribed drugs. This most often occurs with drugs that share the same hepatic cytochrome metabolic pathway. Protease inhibitors and nonnucleoside reverse transcriptase inhibitors (NNRTIs) and boosting agents (e.g., cobicistat and ritonavir) as well as rifampin, rifabutin, azoles, atovaquone-proguanil, and quinolones need special attention because they all are metabolized by hepatic enzymes. For example, darunavir, ritonavir, and lopinavir-ritonavir can each increase the rifabutin area under the concentration-time curve (AUC) substantially. Ritonavir decreases the AUC of voriconazole. Coadministration of voriconazole and efavirenz leads to decreased voriconazole AUC and increased efavirenz AUC.[8] Useful tables are available in the US Department of Health and Human Services Guidelines for Antiretroviral Therapy in Adolescents and Adults as well as websites, package inserts, and published investigations of specific drug-drug interactions.[8]

PREVENTION OF OPPORTUNISTIC INFECTIONS

Antiretroviral Therapy

The optimal approach to prevention of opportunistic infections is to initiate effective ART. As noted earlier, ART can probably never restore

immune function to normal, and some pathogens such as *Mycobacterium tuberculosis,* herpes zoster, and *Streptococcus pneumoniae* will still cause more frequent disease in HIV-infected patients than in non–HIV-infected patients, even with comparable $CD4^+$ T-cell counts. However, the lower the $CD4^+$ T-cell count, the higher the incidence is of each of these opportunistic infections.

Ideally all patients with HIV infection start ART within a few days of establishing their HIV diagnosis. Chemoprophylaxis without ART was shown to be effective and extends survival modestly. However, very few patients who do not take ART would take chemoprophylaxis for PCP or toxoplasmosis. Thus chemoprophylaxis without ART is rarely clinically relevant in this era.

Patients who start ART with $CD4^+$ T-cell counts less than 200 cells/mm^3 should take PCP prophylaxis and *Toxoplasma* prophylaxis (patients who are anti-*Toxoplasma* immunoglobulin G [IgG] positive) until their $CD4^+$ counts exceed 200 cells/mm^3 and 100 cells/mm^3, respectively, although admittedly their risk of these opportunistic infections is substantially decreased once HIV viral loads fall below the limit of assay detection, regardless of $CD4^+$ count. Chemoprophylaxis against MAC for patients with $CD4^+$ counts below 50 cells/mm^3 was previously recommended but is no longer recommended due to the rarity of MAC infection in the United States since effective ART regimens were introduced.[8]

Ensuring currency of immunizations for HIV-related pathogens is also important. Being up-to-date for pneumococcal and *Haemophilus* vaccines, zoster vaccine (if age appropriate), hepatitis B vaccine, and influenza vaccine are important strategies.[8]

Empirical Versus Specific Therapy

An issue that frequently arises when patients have acute infectious processes is whether to use immediate empirical antimicrobial regimens or to withhold therapy until a specific diagnosis is established by invasive techniques. Such decisions require clinical judgment. Evidence of substantial immunosuppression or clinically severe disease would logically indicate the need to start empirical therapy quickly rather than waiting for diagnostic test results. In addition, there may be situations in which the diagnosis is so predictable (e.g., retinitis with hemorrhage and exudates in a patient with a $CD4^+$ T-cell count <50 cells/mm^3; a cerebral mass lesion in a patient who is *Toxoplasma* IgG positive and has a $CD4^+$ count <50 cells/mm^3; or a patient with dysphagia, oral candidiasis, and a $CD4^+$ count <50 cells/mm^3) that expensive or invasive diagnostic tests are not mandatory if the patient responds as expected to empirical therapy.

Many AIDS-related opportunistic infections relapse within weeks or months after acute therapy is stopped if effective ART cannot be initiated and maintained.[8] Experience during the first decade of AIDS clearly documented that PCP, toxoplasmosis, cryptococcosis, disseminated MAC infection, and CMV retinitis would relapse if lifelong suppressive pathogen-specific therapy was not maintained. For a few opportunistic infections such as mucosal candidiasis, herpes simplex infections, and herpes zoster infections, such secondary prophylaxis has not been indicated because the disease is not life threatening; recurrences could be easily treated; and pill burden, cost, drug toxicity, and potential drug interactions appear to outweigh the small potential benefit.

Patients who respond to ART, as manifested by sustained increases in $CD4^+$ T-cell counts, are very unlikely to experience opportunistic infection relapse (with the exception of coccidioidomycosis) and thus do not need to receive prolonged long-term maintenance therapy beyond what is recommended in current guidelines.[84–88] There are sufficient data to recommend that for patients who have $CD4^+$ T-cell counts above the thresholds suggested in well-established, evidence-based guidelines,[8] primary prophylaxis (preventing a first episode of disease) or secondary prophylaxis (preventing relapse or recurrence) can be discontinued. Prophylaxis should be restarted if the $CD4^+$ T-cell count subsequently falls below the threshold indicated in the guidelines.[8]

Tables 129.1, 129.2, and 129.3 summarize current recommendations from the National Institutes of Health, Centers for Disease Control and Prevention, and Infectious Diseases Society of America for prophylaxis to prevent first episodes (Table 129.1); to treat acute opportunistic infections (Table 129.2); and to start, discontinue, and restart prophylaxis (Table 129.3).

MANAGEMENT OF SPECIFIC OPPORTUNISTIC INFECTIONS

Pneumocystis jirovecii Pneumonia

PCP was the clinical manifestation that originally suggested to clinicians that a new syndrome, AIDS, was occurring in patients who appeared to be previously healthy.[89,90] Until those cases, PCP had been infrequently recognized in the United States in any population because immunosuppressive regimens were not as intense as current approaches and diagnostic tools were less developed.

PCP continues to be a commonly recognized complication of HIV infection in North America and Western Europe. As more patients are receiving ART and as the $CD4^+$ T-cell count at ART initiation rises, PCP is not as common as it was in the pre-ART era. However, there are many HIV-infected patients who have not been prescribed ART or in whom the virus is not durably suppressed, and thus PCP continues to occur. PCP occurs worldwide, although in some areas of the world it is rarely documented (see Chapter 269).

PCP can be almost completely prevented by either ART-induced immune reconstitution or specific chemoprophylaxis. As noted previously, many patients do not recognize that they have HIV infection until they develop PCP. Some patients are aware of their HIV infection but do

Text continued on p. 1774

TABLE 129.1 Prophylaxis to Prevent First Episode of Opportunistic Disease

OPPORTUNISTIC INFECTIONS	INDICATION	PREFERRED	ALTERNATIVE
Pneumocystis pneumonia (PCP)	$CD4^+$ count <200 cells/mm^3, *or* $CD4^+$ <14%, *or* $CD4^+$ count >200 but <250 cells/mm^3 if monitoring $CD4^+$ cell count every 3 mo is not possible *Note:* Patients who are receiving pyrimethamine-sulfadiazine for treatment or suppression of toxoplasmosis do not require additional PCP prophylaxis	TMP-SMZ 1 DS PO daily, *or* TMP-SMZ 1 SS daily	TMP-SMZ 1 DS PO 3 times weekly *or* Dapsone 100 mg PO daily or 50 mg PO bid, *or* Dapsone 50 mg PO daily + (pyrimethamine 50 mg + leucovorin 25 mg) PO weekly, *or* (Dapsone 200 mg + pyrimethamine 75 mg + leucovorin 25 mg) PO weekly, *or* Aerosolized pentamidine 300 mg via Respirgard II nebulizer every mo, *or* Atovaquone 1500 mg PO daily, *or* (Atovaquone 1500 mg + pyrimethamine 25 mg + leucovorin 10 mg) PO daily
Toxoplasma gondii encephalitis	*Toxoplasma* IgG-positive patients with $CD4^+$ count <100 cells/mm^3 *Note:* All regimens recommended for primary prophylaxis against toxoplasmosis are also effective as PCP prophylaxis	TMP-SMZ 1 DS PO daily	TMP-SMZ 1 DS PO 3 times weekly, *or* TMP-SMZ 1 SS PO daily, *or* Dapsone 50 mg PO daily + (pyrimethamine 50 mg + leucovorin 25 mg) PO weekly, *or* (Dapsone 200 mg + pyrimethamine 75 mg + leucovorin 25 mg) PO weekly, *or* Atovaquone 1500 mg PO daily, *or* (Atovaquone 1500 mg + pyrimethamine 25 mg + leucovorin 10 mg) PO daily

TABLE 129.1 Prophylaxis to Prevent First Episode of Opportunistic Disease—cont'd

OPPORTUNISTIC INFECTIONS	INDICATION	PREFERRED	ALTERNATIVE
Mycobacterium tuberculosis infection (i.e., treatment of LTBI)	(+) screening test for LTBI, with no evidence of active TB, and no prior treatment for active TB or LTBI, *or* Close contact with a person with infectious TB, with no evidence of active TB, regardless of screening test results	(INH 300 mg + pyridoxine 25–50 mg) PO daily × 9 mo, *or* INH 900 mg PO biw (by DOT) + pyridoxine 25–50 mg PO daily × 9 mo	Rifampin 600 mg PO daily × 4 mo, *or* Rifabutin (dose adjusted based on concomitant ART) × 4 mo, *or* (Rifapentine [see dose below] PO + INH 900 mg PO + pyridoxine 50 mg PO) once weekly × 12 wk *Rifapentine dose:* 32.1–49.9 kg: 750 mg *or* 50 kg: 900 mg Rifapentine recommended only for patients receiving raltegravir- or efavirenz-based ART regimen For patients exposed to drug-resistant TB, select anti-TB drugs after consultation with experts or public health authorities
Streptococcus pneumoniae infection	For individuals who have not received any pneumococcal vaccine, regardless of $CD4^+$ count, followed by: If $CD4^+$ count ≥200 cells/mm^3 If $CD4^+$ count <200 cells/mm^3 For individuals who have previously received PPV23 Revaccination: If age 19–64 yr and ≥5 yr since first PPV23 dose If age ≥65 yr and ≥5 yr since previous PPV23 dose	PCV13 0.5 mL IM × 1 PPV23 0.5 mL IM at least 8 wk after PCV13 vaccine PPV23 can be offered at least 8 wk after receiving PCV13 or can wait until $CD4^+$ count is increased to ≥200 cells/mm^3 One dose of PCV13 should be given at least 1 yr after last receipt of PPV23 PPV23 0.5 mL IM or SQ × 1 PPV23 0.5 mL IM or SQ × 1	PPV23 0.5 mL IM × 1
Influenza A and B virus infection	All HIV-infected patients	Inactivated influenza vaccine annually (per recommendation for the season) Live-attenuated influenza vaccine is *contraindicated* in HIV-infected patients	
Syphilis	For individuals exposed to a sex partner with a diagnosis of primary, secondary, or early latent syphilis within past 90 days, *or* For individuals exposed to a sex partner >90 days before syphilis diagnosis in the partner, if serologic test results are not available immediately and the opportunity for follow-up is uncertain	Benzathine penicillin G 2.4 million U IM for 1 dose	For penicillin-allergic patients: Doxycycline 100 mg PO bid for 14 days, *or* Ceftriaxone 1 g IM or IV daily for 8–10 days, *or* Azithromycin 2 g PO for 1 dose: *not recommended* for MSM or pregnant women
Histoplasma capsulatum infection	$CD4^+$ count ≤150 cells/mm^3 and at high risk because of occupational exposure or living in a community with hyperendemic rate of histoplasmosis (>10 cases/100 patient-yr)	Itraconazole 200 mg PO daily	
Coccidioidomycosis	New positive IgM or IgG serologic test in patients who live in disease-endemic area and with $CD4^+$ count <250 cells/mm^3	Fluconazole 400 mg PO daily	
Varicella virus	Preexposure prevention: Patients with $CD4^+$ counts ≥200 cells/mm^3 who have not been vaccinated, have no history of varicella or herpes zoster, or are seronegative for VZV *Note:* Routine VZV serologic testing in HIV-infected adults and adolescents is not recommended Postexposure prevention: Close contact with a person with chickenpox or herpes zoster and is susceptible (i.e., no history of vaccination or of either condition or known to be VZV seronegative)	Preexposure prevention: Primary varicella vaccination (Varivax), 2 doses (0.5 mL SQ each) administered 3 mo apart If vaccination results in disease because of vaccine virus, treatment with acyclovir is recommended Postexposure prevention: Varicella-zoster immune globulin (VariZIG) 125 IU per 10 kg (maximum 625 IU) IM, administered as soon as possible and within 10 days after exposure *Note:* VariZIG is exclusively distributed by FFF Enterprises (Temecula, CA) Individuals receiving monthly high-dose IVIG (>400 mg/kg) are likely to be protected if last dose of IVIG was administered <3 wk before exposure	Preexposure prevention: VZV-susceptible household contacts of susceptible HIV-infected patients should be vaccinated to prevent potential transmission of VZV to their HIV-infected contacts Alternative postexposure prevention: Acyclovir 800 mg PO 5 times per day for 5–7 days, *or* Valacyclovir 1 g PO TID for 5–7 days These alternatives have not been studied in patients with HIV If antiviral therapy is used, varicella vaccines should not be given until at least 72 h after last dose of the antiviral drug
Hepatitis A virus (HAV) infection	HAV-susceptible patients with chronic liver disease or who are injection drug users or MSM	Hepatitis A vaccine 1 mL IM × 2 doses at 0 and 6–12 mo IgG antibody response should be assessed 1 mo after vaccination; nonresponders should be revaccinated when $CD4^+$ count >200 cells/mm^3	For patients susceptible to both HAV and hepatitis B virus (HBV) infection: Combined HAV and HBV vaccine (Twinrix), 1 mL IM as a 3-dose (0, 1, and 6 mo) or 4-dose series (0, 7, and 21–30 days and 12 mo)

Continued

TABLE 129.1 Prophylaxis to Prevent First Episode of Opportunistic Disease—cont'd

OPPORTUNISTIC INFECTIONS	INDICATION	PREFERRED	ALTERNATIVE
Hepatitis B virus (HBV) infection	Patients without chronic HBV or without immunity to HBV (i.e., with anti-HBs <10 IU/mL) Patients with isolated anti-HBc: vaccinate with 1 standard dose of HBV vaccine and check anti-HBs 1–2 mo after; if >100 IU, no further vaccination needed; if titer is <100 IU, vaccinate with full series Early vaccination is recommended before $CD4^+$ count falls below 350 cells/mm^3 In patients with low $CD4^+$ cell counts, vaccination should not be deferred until $CD4^+$ count reaches >350 cells/mm^3 because some patients with $CD4^+$ counts <200 cells/mm^3 do respond to vaccination	HBV vaccine IM (Engerix-B 20 µg/mL or Recombivax HB 10 µg/mL), 0, 1, and 6 mo, *or* HBV vaccine IM (Engerix-B 40 µg/mL or Recombivax HB 20 µg/mL), 0, 1, 2, and 6 mo Combined HAV and HBV vaccine (Twinrix), 1 mL IM as 3-dose (0, 1, and 6 mo) or 4-dose series (0, 7, and 21–30 days and 12 mo) or Heplisav 2-dose series (0, 1 mo) 0.5 mL IM Anti-HBs should be obtained 1–2 mo after completion of vaccine series Patients with anti-HBs <10 IU/mL are considered nonresponders and should be revaccinated with another 3-dose series	Some experts recommend vaccinating with 4 doses of double dose of either HBV vaccine
Herpes zoster	Age 50 yr or older	2-dose series of recombinant zoster vaccine (RZV) 0.5 mL IM 2–6 mo apart	
Human papilloma virus (HPV)		Recombinant 9-valent vaccine 0.5 mL IM (0, 1, and 6 mo)	

ART, Antiretroviral therapy; *bid,* twice daily; *DOT,* directly observed treatment; *DS,* double-strength; *HBs,* hepatitis B surface antibody; *HIV,* human immunodeficiency virus; *Ig,* immunoglobulin; *IM,* intramuscular; *INH,* isoniazid; *IVIG,* intravenous immune globulin; *LTBI,* latent tuberculosis infection; *MSM,* men who have sex with men; *PCV13,* 13-valent pneumococcal conjugate vaccine; *PO,* per os (oral); *PPV23,* 23-valent pneumococcal polysaccharide vaccine; *SQ,* subcutaneous; *SS,* single-strength; *TB,* tuberculosis; *TMP-SMZ,* trimethoprim-sulfamethoxazole; *VZV,* varicella-zoster virus.
From Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. http://aidsinfo.nih.gov/guidelines. Accessed March 1, 2019.

TABLE 129.2 Treatment of AIDS-Associated Opportunistic Infections

OPPORTUNISTIC INFECTION	PREFERRED THERAPY	ALTERNATIVE THERAPY	OTHER COMMENTS
Pneumocystis pneumonia (PCP)	Patients who develop PCP despite TMP-SMZ prophylaxis can usually be treated with standard doses of TMP-SMZ Duration of PCP treatment: 21 days <u>For moderate-to-severe PCP:</u> TMP-SMZ: (TMP 15–20 mg and SMZ 75–100 mg)/kg/day IV given q6h or q8h; may switch to PO after clinical improvement <u>For mild-to-moderate PCP:</u> TMP-SMZ: (TMP 15–20 mg and SMZ 75–100 mg)/kg/day, given PO in 3 divided doses, *or* TMP-SMZ: 160 mg/800 mg or DS 2 tablets PO tid <u>Secondary prophylaxis, after completion of PCP treatment:</u> TMP-SMZ DS: 1 tablet PO daily, *or* TMP-SMZ (80 mg/400 mg or SS): 1 tablet PO daily	<u>For moderate-to-severe PCP:</u> Pentamidine 4 mg/kg IV daily infused over ≥60 min; can reduce dose to 3 mg/kg IV daily because of toxicities, *or* Primaquine 30 mg (base) PO daily + (clindamycin 600 mg q6h IV or 900 mg IV q8h) or (clindamycin 450 mg PO q6h or 600 mg PO q8h) <u>For mild-to-moderate PCP:</u> Dapsone 100 mg PO daily + TMP 5 mg/kg PO tid, *or* Primaquine 30 mg (base) PO daily + (clindamycin 450 mg PO q6h or 600 mg PO q8h), *or* Atovaquone 750 mg PO bid with food <u>Secondary prophylaxis, after completion of PCP treatment:</u> TMP-SMZ DS: 1 tablet PO 3 times weekly, *or* Dapsone 100 mg PO daily, *or* Dapsone 50 mg PO daily + (pyrimethamine 50 mg + leucovorin 25 mg) PO weekly, *or* (Dapsone 200 mg + pyrimethamine 75 mg + leucovorin 25 mg) PO weekly, *or* Aerosolized pentamidine 300 mg monthly via Respirgard II nebulizer, *or* Atovaquone 1500 mg PO daily, *or* (Atovaquone 1500 mg + pyrimethamine 25 mg + leucovorin 10 mg) PO daily	

TABLE 129.2 Treatment of AIDS-Associated Opportunistic Infections—cont'd

OPPORTUNISTIC INFECTION	PREFERRED THERAPY	ALTERNATIVE THERAPY	OTHER COMMENTS
Toxoplasma gondii encephalitis	Treatment of acute infection: Pyrimethamine 200 mg PO 1 time, followed by weight-based therapy: If <60 kg, pyrimethamine 50 mg PO once daily + sulfadiazine 1000 mg PO q6h + leucovorin 10–25 mg PO once daily, *or* If ≥60 kg, pyrimethamine 75 mg PO once daily + sulfadiazine 1500 mg PO q6h + leucovorin 10–25 mg PO once daily Leucovorin dose can be increased to 50 mg daily or bid Duration for acute therapy: At least 6 wk; longer duration if clinical or radiologic disease is extensive or response is incomplete at 6 wk After completion of acute therapy, all patients should be initiated on long-term maintenance therapy	Treatment of acute infection: Pyrimethamine (leucovorin)[a] + clindamycin 600 mg IV or PO q6h, *or* TMP-SMZ (TMP 5 mg/kg and SMZ 25 mg/kg) IV or PO bid, *or* Atovaquone 1500 mg PO bid with food + pyrimethamine (leucovorin)[a], *or* Atovaquone 1500 mg PO bid with food + sulfadiazine 1000–1500 mg PO q6h (weight-based dosing, as in preferred therapy), *or* Atovaquone 1500 mg PO bid with food	Refer to http://www.daraprimdirect.com for information regarding how to access pyrimethamine If pyrimethamine is unavailable or there is a delay in obtaining it, TMP-SMZ should be used in place of pyrimethamine-sulfadiazine For patients with a history of sulfa allergy, sulfa desensitization should be attempted using one of several published strategies Atovaquone should be administered until therapeutic doses of TMP-SMZ are achieved Adjunctive corticosteroids (e.g., dexamethasone) should be administered only when clinically indicated to treat mass effect associated with focal lesions or associated edema; discontinue as soon as clinically feasible Anticonvulsants should be administered to patients with a history of seizures and continued through acute treatment but should not be used as seizure prophylaxis If clindamycin is used in place of sulfadiazine, additional therapy must be added to prevent PCP
	Long-term maintenance therapy: Pyrimethamine 25–50 mg PO daily + sulfadiazine 2000–4000 mg PO daily (in 2–4 divided doses) + leucovorin 10–25 mg PO daily	Long-term maintenance therapy: Clindamycin 600 mg PO q8h + (pyrimethamine 25–50 mg + leucovorin 10–25 mg) PO daily, *or* TMP-SMZ DS 1 tablet bid, *or* TMP-SMZ DS 1 tablet once daily, *or* Atovaquone 750–1500 mg PO bid + pyrimethamine 25 mg + leucovorin 10 mg PO daily, *or* Atovaquone 750–1500 mg PO bid + sulfadiazine 2000–4000 mg PO daily (in 2–4 divided doses), *or* Atovaquone 750–1500 mg PO bid with food	
Mycobacterium tuberculosis disease	After collecting specimen for culture and molecular diagnostic tests, empirical TB treatment should be started in individuals with clinical and radiographic presentation suggestive of TB *Initial phase (2 mo, given daily, 5–7 times per week by DOT):* INH + [RIF or RFB] + PZA + EMB *Continuation phase:* INH + (RIF or RFB) daily (5–7 times per week) *Total duration of therapy (for drug-susceptible TB):* Pulmonary, drug-susceptible TB: 6 mo Pulmonary TB and culture-positive after 2 mo of TB treatment: 9 mo Extrapulmonary TB with CNS infection: 9–12 mo Extrapulmonary TB with bone or joint involvement: 6–9 mo Extrapulmonary TB in other sites: 6 mo Total duration of therapy should be based on number of doses received, not on calendar time	Treatment for drug-resistant TB *Resistant to INH:* (RIF or RFB) + EMB + PZA + (moxifloxacin or levofloxacin) for 2 mo; followed by (RIF or RFB) + EMB + (moxifloxacin or levofloxacin) for 7 mo *Resistant to rifamycins ± other drugs:* Regimen and duration of treatment should be individualized based on resistance pattern and clinical and microbiologic responses and in close consultation with experienced specialists	Adjunctive corticosteroids improve survival for TB meningitis and pericarditis. See text for drug, dose, and duration recommendations All rifamycins may have significant pharmacokinetic interactions with antiretroviral drugs Therapeutic drug monitoring should be considered in patients receiving rifamycin and interacting ART Paradoxical IRIS that is not severe can be treated with NSAIDs without a change in TB or HIV therapy For severe IRIS reaction, consider prednisone and taper over 4 wk based on clinical symptoms For example: *If receiving RIF:* prednisone 1.5 mg/kg/day for 2 wk, then 0.75 mg/kg/day for 2 wk *If receiving RFB:* prednisone 1.0 mg/kg/day for 2 wk, then 0.5 mg/kg/day for 2 wk A more gradual tapering schedule over a few mo may be necessary for some patients
Disseminated *Mycobacterium avium-intracellulare* complex (MAC) disease	At least 2 drugs as initial therapy with: Clarithromycin 500 mg PO bid + ethambutol 15 mg/kg PO daily, *or* (Azithromycin 500–600 mg + ethambutol 15 mg/kg) PO daily if drug interaction or intolerance precludes use of clarithromycin *Duration:* At least 12 mo of therapy, then can discontinue if no signs and symptoms of MAC disease and sustained (>6 mo) $CD4^+$ count >100 cells/mm^3 in response to ART	Addition of a third or fourth drug should be considered for patients with advanced immunosuppression ($CD4^+$ counts <50 cells/mm^3) or high mycobacterial loads (>2 log CFU/mL of blood) or in the absence of effective ART Third or fourth drug options may include: RFB 300 mg PO daily (dosage adjustment may be necessary based on drug interactions) Amikacin 10–15 mg/kg IV daily or streptomycin 1 g IV or IM daily, *or* Moxifloxacin 400 mg PO daily or levofloxacin 500 mg PO daily	Testing of susceptibility to clarithromycin and azithromycin is recommended NSAIDs can be used for patients who experience moderate-to-severe symptoms attributed to IRIS If IRIS symptoms persist, short-term (4–8 wk) systemic corticosteroids (equivalent to 20–40 mg prednisone) can be used

Continued

TABLE 129.2 Treatment of AIDS-Associated Opportunistic Infections—cont'd

OPPORTUNISTIC INFECTION	PREFERRED THERAPY	ALTERNATIVE THERAPY	OTHER COMMENTS
Bacterial respiratory diseases *(with focus on pneumonia)*	Empirical antibiotic therapy should be initiated promptly for patients presenting with clinical and radiographic evidence consistent with bacterial pneumonia. The recommendations listed are suggested empirical therapy. The regimen should be modified as needed once microbiologic results are available. Empirical outpatient therapy: PO β-lactam + PO macrolide (azithromycin or clarithromycin) *Preferred β-lactams:* high-dose amoxicillin or amoxicillin/clavulanate *Alternative β-lactams:* cefpodoxime or cefuroxime, *or* *For penicillin-allergic patients:* Levofloxacin 750 mg PO once daily or moxifloxacin 400 mg PO once daily *Duration:* 7–10 days (minimum of 5 days). Patients should be afebrile for 48–72 hours and clinically stable before stopping antibiotics Empirical therapy for non-ICU hospitalized patients: IV β-lactam + macrolide (azithromycin or clarithromycin) *Preferred β-lactams:* ceftriaxone, cefotaxime, or ampicillin-sulbactam *For penicillin-allergic patients:* Levofloxacin 750 mg IV once daily or moxifloxacin 400 mg IV once daily Empirical therapy for ICU patients: IV β-lactam + IV azithromycin, *or* IV β-lactam + (levofloxacin 750 mg IV once daily or moxifloxacin 400 mg IV once daily) *Preferred β-lactams:* ceftriaxone, cefotaxime, or ampicillin-sulbactam Empirical therapy for patients at risk of *Pseudomonas* pneumonia: IV antipneumococcal, antipseudomonal β-lactam + (ciprofloxacin 400 mg IV q8–12h or levofloxacin 750 mg IV once daily) *Preferred β-lactams:* piperacillin-tazobactam, cefepime, imipenem, or meropenem Empirical therapy for patients at risk for methicillin-resistant *Staphylococcus aureus* pneumonia: Add vancomycin IV or linezolid (IV or PO) to baseline regimen Addition of clindamycin to vancomycin (but not to linezolid) can be considered for severe necrotizing pneumonia to minimize bacterial toxin production	Empirical outpatient therapy: PO β-lactam + PO doxycycline *Preferred β-lactams*: high-dose amoxicillin or amoxicillin/clavulanate *Alternative β-lactams*: cefpodoxime or cefuroxime Empirical therapy for non-ICU hospitalized patients: IV β-lactam + doxycycline Empirical therapy for ICU patients: *For penicillin-allergic patients*: Aztreonam IV + (levofloxacin 750 mg IV once daily or moxifloxacin 400 mg IV once daily) Empirical therapy for patients at risk of *Pseudomonas* pneumonia: IV antipneumococcal, antipseudomonal β-lactam + aminoglycoside + azithromycin, *or* Above β-lactam + aminoglycoside + (levofloxacin 750 mg IV once daily or moxifloxacin 400 mg IV once daily), *or* *For penicillin-allergic patients:* Replace β-lactam with aztreonam	Fluoroquinolones should be used with caution in patients in whom TB is suspected but is not being treated Empirical therapy with a macrolide alone is not routinely recommended because of increasing pneumococcal resistance Patients receiving a macrolide for MAC prophylaxis should not receive macrolide monotherapy for empirical treatment of bacterial pneumonia For patients started on IV antibiotic therapy, switching to PO therapy should be considered when they are clinically improved and able to tolerate oral medications Chemoprophylaxis can be considered for patients with frequent recurrences of serious bacterial pneumonia Clinicians should be cautious about using antibiotics to prevent recurrences because of the potential for developing drug resistance and drug toxicities
Salmonellosis	All HIV-infected patients with salmonellosis should receive antimicrobial treatment due to an increase of bacteremia (by 20- to 100-fold) and mortality (by up to 7-fold) compared with HIV-negative individuals. Ciprofloxacin 500–750 mg PO (or 400 mg IV) q12h, if susceptible Duration of therapy: *For gastroenteritis without bacteremia*: If CD4$^+$ count ≥200 cells/mm^3: 7–14 days If CD4$^+$ count <200 cells/mm^3: 2–6 wk *For gastroenteritis with bacteremia*: If CD4$^+$ count ≥200/mm^3: 14 days or longer duration if bacteremia persists or if infection is complicated (e.g., if metastatic foci of infection are present) If CD4$^+$ count <200 cells/mm^3: 2–6 wk Secondary prophylaxis should be considered for: Patients with recurrent *Salmonella* gastroenteritis ± bacteremia, *or* Patients with CD4$^+$ <200 cells/mm^3 with severe diarrhea	Levofloxacin 750 mg (PO or IV) q24h, *or* Moxifloxacin 400 mg (PO or IV) q24h, *or* TMP-SMZ 160 mg/800 mg (PO or IV) q12h, *or* Ceftriaxone 1 g IV q24h, *or* Cefotaxime 1 g IV q8h	PO or IV rehydration if indicated Antimotility agents should be avoided Role of long-term secondary prophylaxis in patients with recurrent *Salmonella* bacteremia is not well established; must weigh benefit against risks of long-term antibiotic exposure Effective ART may reduce frequency, severity, and recurrence of *Salmonella* infections

TABLE 129.2 Treatment of AIDS-Associated Opportunistic Infections—cont'd

OPPORTUNISTIC INFECTION	PREFERRED THERAPY	ALTERNATIVE THERAPY	OTHER COMMENTS
Mucocutaneous candidiasis	For oropharyngeal candidiasis; initial episodes (for 7–14 days): *Oral therapy:* Fluconazole 100 mg PO daily	For oropharyngeal candidiasis; initial episodes (for 7–14 days): *Oral therapy:* Itraconazole oral solution 200 mg PO daily, *or* Posaconazole oral suspension 400 mg PO bid for 1 day, then 400 mg daily *Topical therapy:* Clotrimazole troches, 10 mg PO 5 times daily, *or* Miconazole mucoadhesive buccal 50-mg tablet—apply to mucosal surface over canine fossa once daily (do not swallow, chew, or crush), *or* Nystatin suspension 4–6 mL qid or 1–2 flavored pastilles 4–5 times daily	Long-term or prolonged use of azoles may promote development of resistance Higher relapse rate for esophageal candidiasis seen with echinocandins than with fluconazole use Suppressive therapy usually not recommended unless patients have frequent or severe recurrences If decision is to use suppressive therapy: *Oropharyngeal candidiasis:* Fluconazole 100 mg PO daily or thrice weekly, *or* Itraconazole oral solution 200 mg PO daily *Esophageal candidiasis:* Fluconazole 100–200 mg PO daily, *or* Posaconazole 400 mg PO bid *Vulvovaginal candidiasis:* Fluconazole 150 mg PO once weekly Isavuconazole not approved for treatment of esophageal candidiasis
	For esophageal candidiasis (for 14–21 days): Fluconazole 100 mg (up to 400 mg) PO or IV daily, *or* Itraconazole oral solution 200 mg PO daily	For esophageal candidiasis (for 14–21 days): Voriconazole 200 mg PO or IV bid, *or* Isavuconazole 200 mg PO as loading dose, followed by 50 mg PO daily, *or* Isavuconazole 400 mg PO as loading dose, followed by 100 mg PO daily, *or* Isavuconazole 400 mg PO once weekly, *or* Anidulafungin 100 mg IV 1 time, then 50 mg IV daily, *or* Caspofungin 50 mg IV daily, *or* Micafungin 150 mg IV daily, *or* Amphotericin B deoxycholate 0.6 mg/kg IV daily, *or* Lipid formulation of amphotericin B 3–4 mg/kg IV daily	
	For uncomplicated vulvovaginal candidiasis: Oral fluconazole 150 mg PO for 1 dose, *or* Topical azoles (clotrimazole, butoconazole, miconazole, tioconazole, or terconazole) for 3–7 days For severe or recurrent vulvovaginal candidiasis: Fluconazole 100–200 mg PO daily for ≥7 days, *or* Topical antifungal ≥7 days	For uncomplicated vulvovaginal candidiasis: Itraconazole oral solution 200 mg PO daily for 3–7 days	
Cryptococcosis	Cryptococcal meningitis: *Induction therapy (for at least 2 wk, followed by consolidation therapy):* Liposomal amphotericin B 3–4 mg/kg IV daily + flucytosine 25 mg/kg PO qid *Note:* Flucytosine dose should be adjusted in patients with renal dysfunction	Cryptococcal meningitis: *Induction therapy (for at least 2 wk, followed by consolidation therapy):* Amphotericin B deoxycholate 0.7 mg/kg IV daily + flucytosine 25 mg/kg PO qid, *or* Amphotericin B lipid complex 5 mg/kg IV daily + flucytosine 25 mg/kg PO qid, *or* Liposomal amphotericin B 3–4 mg/kg IV daily + fluconazole 800 mg PO or IV daily, *or* Amphotericin B deoxycholate 0.7 mg/kg IV daily + fluconazole 800 mg PO or IV daily, *or* Fluconazole 400–800 mg PO or IV daily + flucytosine 25 mg/kg PO qid, *or* Fluconazole 1200 mg PO or IV daily	Addition of flucytosine to amphotericin B has been associated with more rapid sterilization of CSF and decreased risk for subsequent relapse Patients receiving flucytosine should have either blood levels monitored (peak level 2 h after dose should be 30–80 μg/mL) or close monitoring of blood counts for development of cytopenia Flucytosine dose should be adjusted in patients with renal insufficiency Opening pressure should always be measured when LP is performed; repeated LPs or CSF shunts are essential to effectively manage increased ICP Corticosteroids and mannitol are ineffective in reducing ICP and are *not* recommended Corticosteroids should not be routinely used during induction therapy except for management of IRIS
	Consolidation therapy (for at least 8 wk, followed by maintenance therapy): Fluconazole 400 mg PO (or IV) daily *Maintenance therapy:* Fluconazole 200 mg PO daily for at least 12 mo	*Consolidation therapy (for at least 8 wk, followed by maintenance therapy):* Itraconazole 200 mg PO bid for 8 wk—less effective than fluconazole *Consolidation therapy (for at least 8 wk, followed by maintenance therapy):* Itraconazole 200 mg PO bid for 8 wk—less effective than fluconazole	
	For non-CNS, extrapulmonary cryptococcosis and diffuse pulmonary disease: Treatment same as for cryptococcal meningitis Non-CNS cryptococcosis with mild-to-moderate symptoms and focal pulmonary infiltrates: Fluconazole, 400 mg PO daily for 12 mo		

Continued

TABLE 129.2 Treatment of AIDS-Associated Opportunistic Infections—cont'd

OPPORTUNISTIC INFECTION	PREFERRED THERAPY	ALTERNATIVE THERAPY	OTHER COMMENTS
Histoplasmosis	Moderately severe to severe disseminated disease: *Induction therapy (for at least 2 wk or until clinically improved):* Liposomal amphotericin B 3 mg/kg IV daily *Maintenance therapy* Itraconazole 200 mg PO tid for 3 days, then 200 mg PO bid Less severe disseminated disease: *Induction and maintenance therapy:* Itraconazole 200 mg PO tid for 3 days, then 200 mg PO bid *Duration of therapy:* At least 12 mo Meningitis: *Induction therapy (4–6 wk):* Liposomal amphotericin B 5 mg/kg/day *Maintenance therapy:* Itraconazole 200 mg PO bid to tid for ≥1 yr and until resolution of abnormal CSF findings Long-term suppression therapy: *For patients with severe disseminated or CNS infection after completion of at least 12 mo of therapy and patients who relapse despite appropriate therapy:* Itraconazole 200 mg PO daily	Moderately severe to severe disseminated disease: *Induction therapy (for at least 2 wk or until clinically improved):* Amphotericin B lipid complex 3 mg/kg IV daily, *or* Amphotericin B cholesteryl sulfate complex 3 mg/kg IV daily (not sold in US) Alternatives to itraconazole for maintenance therapy or treatment of less severe disease: Voriconazole 400 mg PO bid for 1 day, then 200 mg bid, *or* Posaconazole 400 mg PO bid Fluconazole 800 mg PO daily Meningitis: No alternative therapy recommendation Long-term suppression therapy: Fluconazole 400 mg PO daily	Itraconazole, posaconazole, and voriconazole may have significant interactions with certain ARV agents. These interactions are complex and can be bidirectional Therapeutic drug monitoring and dosage adjustment may be necessary to ensure triazole antifungal and ARV efficacy and reduce concentration-related toxicities Random serum concentration of itraconazole + hydroitraconazole should be >1 μg/mL Clinical experience with voriconazole or posaconazole in treatment of histoplasmosis is limited Acute pulmonary histoplasmosis in HIV-infected patients with $CD4^+$ counts >300 cells/mm^3 should be managed as nonimmunocompromised host
Coccidioidomycosis	Clinically mild infections (e.g., focal pneumonia): Fluconazole 400 mg[b] PO daily, *or* Itraconazole 200 mg[b] PO bid Bone or joint infections: Itraconazole 200 mg[b] PO bid Severe, nonmeningeal infection (diffuse pulmonary infection or severely ill patients with extrathoracic, disseminated disease): Lipid formulation amphotericin B 3–5 mg/kg IV daily, *or* Amphotericin B deoxycholate 0.7–1.0 mg/kg IV daily *Duration of therapy:* continue until clinical improvement, then switch to a triazole Meningeal infections: Fluconazole 400–800 mg[b] IV or PO daily	Mild infections (focal pneumonia): *For patients who failed to respond to fluconazole or itraconazole:* Posaconazole 300 mg delayed-release tablet[b] PO bid × 1 day, then once daily, *or* Posaconazole 400 mg PO suspension[b] PO bid, *or* Voriconazole 200 mg[b] PO bid Bone or joint infection: Fluconazole 400 mg[b] PO daily Severe, nonmeningeal infection (diffuse pulmonary infection or severely ill patients with extrathoracic, disseminated disease): Some specialists add a triazole (fluconazole[b] or itraconazole[b]) 400 mg per day to amphotericin B therapy and continue triazole once amphotericin B is stopped Meningeal infections: Itraconazole 200 mg[b] PO tid for 3 days, then 200 mg PO bid, *or* Voriconazole 200–400 mg[b] PO bid, *or* Posaconazole 300 mg delayed-release tablet[b] PO bid × 1 day, then once daily, *or* Posaconazole 400 mg oral suspension[b] PO bid, *or* Intrathecal amphotericin B deoxycholate when triazole antifungals are ineffective	Relapse can occur in 25%–33% of HIV-negative patients with diffuse pulmonary or disseminated diseases. Therapy should be given for at least 12 mo and usually much longer; discontinuation depends on clinical and serologic response and should be done in consultation with experts Therapy should be lifelong in patients with meningeal infections because relapse occurs in 80% of HIV-infected patients after discontinuation of triazole therapy Therapeutic drug monitoring and dosage adjustment may be necessary to ensure triazole antifungal and antiretroviral efficacy and reduce concentration-related toxicities Intrathecal amphotericin B should be given only in consultation with a specialist and administered by an individual with experience with the technique
Cytomegalovirus (CMV) disease	CMV retinitis: *Induction therapy (followed by long-term maintenance therapy)* *For immediate sight-threatening lesions (within 1500 μm of the fovea):* Intravitreal injections of ganciclovir (2 mg) or foscarnet (2.4 mg) for 1–4 doses over 7–10 days to achieve high intraocular concentration faster, *plus* Valganciclovir 900 mg PO bid for 14–21 days, then 900 mg once daily *For peripheral lesions:* Valganciclovir 900 mg PO bid for 14–21 days, then 900 mg once daily	CMV retinitis: *For immediate sight-threatening lesions (within 1500 μm of the fovea):* Intravitreal therapy as listed in Preferred Therapy, plus one of the following: *Alternative systemic induction therapy (followed by long-term maintenance therapy):* Ganciclovir 5 mg/kg IV q12h for 14–21 days, *or* Foscarnet 90 mg/kg IV q12h or 60 mg/kg q8h for 14–21 days, *or* Cidofovir 5 mg/kg/wk IV for 2 wk; saline hydration before and after therapy and probenecid 2 g PO 3 hours before dose, followed by 1 g PO 2 hours and 8 hours after dose (total 4 g). *Note:* This regimen should be avoided in patients with sulfa allergy because of cross-hypersensitivity with probenecid	Choice of therapy for CMV retinitis should be individualized, based on location and severity of lesions, level of immunosuppression, and other factors (e.g., concomitant medications and ability to adhere to treatment) Given the evident benefits of systemic therapy in preventing contralateral eye involvement, reducing CMV visceral disease, and improving survival, whenever feasible, treatment should include systemic therapy The ganciclovir ocular implant, which is effective for treatment of CMV retinitis, is no longer available. For sight-threatening retinitis, intravitreal injections of ganciclovir or foscarnet can be given to achieve higher ocular concentration faster Routine (i.e., every 3 mo) ophthalmologic follow-up is recommended after stopping long-term maintenance therapy for early detection of relapse or IRU, and then periodically after sustained immune reconstitution

TABLE 129.2 Treatment of AIDS-Associated Opportunistic Infections—cont'd

OPPORTUNISTIC INFECTION	PREFERRED THERAPY	ALTERNATIVE THERAPY	OTHER COMMENTS
	Long-term maintenance: Valganciclovir 900 mg PO daily for 3–6 mo until ART-induced immune recovery	Long-term maintenance (for 3–6 mo until ART-induced immune recovery: Ganciclovir 5 mg/kg IV 5–7 times weekly, *or* Foscarnet 90–120 mg/kg IV once daily, *or* Cidofovir 5 mg/kg IV every other week with saline hydration and probenecid as above	IRU may develop in the setting of immune reconstitution Treatment of IRU: Periocular corticosteroid or short courses of systemic steroid Initial therapy in patients with CMV retinitis, esophagitis, colitis, and pneumonitis should include initiation or optimization of ART
	CMV esophagitis or colitis: Ganciclovir 5 mg/kg IV q12h; may switch to valganciclovir 900 mg PO q12h once patient can tolerate oral therapy *Duration:* 21–42 days or until symptoms have resolved Maintenance therapy is usually not necessary but should be considered after relapses	CMV esophagitis or colitis: Foscarnet 90 mg/kg IV q12h or 60 mg/kg q8h for patients with treatment-limiting toxicities to ganciclovir or with ganciclovir resistance, *or* Valganciclovir 900 mg PO q12h in milder disease and if able to tolerate PO therapy, *or* *Duration:* 21–42 days or until symptoms have resolved For mild disease, if ART can be initiated without delay, consider withholding CMV therapy	
	Well-documented, histologically confirmed CMV pneumonia: Experience treating CMV pneumonitis in patients with HIV infection is limited. Use of IV ganciclovir or IV foscarnet is reasonable (doses same as for CMV retinitis) Optimal duration of therapy and role of oral valganciclovir have not been established. CMV neurologic disease: *Note:* Treatment should be initiated promptly Ganciclovir 5 mg/kg IV q12h + (foscarnet 90 mg/kg IV q12h or 60 mg/kg IV q8h) to stabilize disease and maximize response; continue until symptomatic improvement and resolution of neurologic symptoms Optimal duration of therapy and role of oral valganciclovir have not been established Optimize ART to achieve viral suppression and immune reconstitution		
Herpes simplex virus (HSV) disease	Orolabial lesions (for 5–10 days): Valacyclovir 1 g PO bid, *or* Famciclovir 500 mg PO bid, *or* Acyclovir 400 mg PO tid Initial or recurrent genital HSV (for 5–14 days): Valacyclovir 1 g PO bid, *or* Famciclovir 500 mg PO bid, *or* Acyclovir 400 mg PO tid Severe mucocutaneous HSV: Initial therapy acyclovir 5 mg/kg IV q8h After lesions begin to regress, change to PO therapy as above; continue until lesions are completely healed Long-term suppressive therapy *For patients with severe recurrences of genital herpes or patients who want to minimize frequency of recurrences:* Valacyclovir 500 mg PO bid Famciclovir 500 mg PO bid Acyclovir 400 mg PO bid Continue indefinitely regardless of $CD4^+$ cell count.	For acyclovir-resistant HSV: *Preferred therapy:* Foscarnet 80–120 mg/kg/day IV in 2–3 divided doses until clinical response *Alternative therapy:* IV cidofovir (dose as in CMV retinitis), *or* Topical trifluridine, *or* Topical cidofovir, *or* Topical imiquimod Duration of therapy: 21–28 days or longer	Patients with HSV infections can be treated with episodic therapy when symptomatic lesions occur or with daily suppressive therapy to prevent recurrences Topical formulations of trifluridine and cidofovir are not commercially available Extemporaneous compounding of topical products can be prepared using trifluridine ophthalmic solution and IV formulation of cidofovir

Continued

TABLE 129.2 Treatment of AIDS-Associated Opportunistic Infections—cont'd

OPPORTUNISTIC INFECTION	PREFERRED THERAPY	ALTERNATIVE THERAPY	OTHER COMMENTS
Varicella-zoster virus (VZV) disease	Primary varicella infection (chickenpox): *Uncomplicated cases (for 5–7 days):* Valacyclovir 1 g PO tid, *or* Famciclovir 500 mg PO tid *Severe or complicated cases:* Acyclovir 10–15 mg/kg IV q8h for 7–10 days May switch to oral valacyclovir, famciclovir, or acyclovir after defervescence if no evidence of visceral involvement Herpes zoster (shingles): *Acute localized dermatomal:* For 7–10 days; consider longer duration if lesions are slow to resolve Valacyclovir 1 g PO tid, *or* Famciclovir 500 mg tid *Extensive cutaneous lesion or visceral involvement:* Acyclovir 10–15 mg/kg IV q8h until clinical improvement is evident May switch to PO therapy (valacyclovir, famciclovir, or acyclovir) after clinical improvement (i.e., when no new vesicle formation or improvement of signs and symptoms of visceral VZV), to complete 10- to 14-day course Progressive outer retinal necrosis: (Ganciclovir 5 mg/kg ± foscarnet 90 mg/kg) IV q12h + (ganciclovir 2 mg/0.05 mL ± foscarnet 1.2 mg/0.05 mL) intravitreal injection biw Initiate or optimize ART Acute retinal necrosis (ARN): (Acyclovir 10–15 mg/kg IV q8h) + (ganciclovir 2 mg/0.05 mL intravitreal injection biw × 1–2 doses) for 10–14 days, followed by valacyclovir 1 g PO tid for 6 wk	Primary varicella infection (chickenpox): *Uncomplicated cases (for 5–7 days):* Acyclovir 800 mg PO 5 times/day Herpes zoster (shingles): *Acute localized dermatomal:* For 7–10 days; consider longer duration if lesions are slow to resolve Acyclovir 800 mg PO 5 times/day	In managing VZV retinitis, consultation with an ophthalmologist experienced in management of VZV retinitis is strongly recommended Duration of therapy for VZV retinitis is not well defined and should be determined based on clinical, virologic, immunologic, and ophthalmologic responses Optimization of ART is recommended for serious and difficult-to-treat VZV infections (e.g., retinitis, encephalitis)
Progressive multifocal leukoencephalopathy (PML) (JC virus infections)	There is no specific antiviral therapy for JC virus infection. The main treatment approach is to reverse immunosuppression caused by HIV Initiate ART immediately in ART-naïve patients Optimize ART in patients who develop PML in phase of HIV viremia on ART	None	Corticosteroids may be used for PML-IRIS characterized by contrast enhancement, edema, or mass effect and with clinical deterioration

[a]Pyrimethamine and leucovorin doses are the same as for preferred therapy.
[b]Fluconazole, itraconazole, posaconazole, and voriconazole may have significant interactions with other medications including ARV drugs. These interactions are complex and can be bidirectional.
AIDS, Acquired immunodeficiency syndrome; *ART,* antiretroviral therapy; *ARV,* antiretroviral; *bid,* twice daily; *biw,* biweekly; *CFU,* colony-forming unit; *CNS,* central nervous system; *CSF,* cerebrospinal fluid; *CYP3A4,* cytochrome P-450 3A4; *DOT,* directly observed therapy; *DS,* double-strength; *EMB,* ethambutol; *G6PD,* glucose-6-phosphate dehydrogenase; *HIV,* human immunodeficiency virus; *ICP,* intracranial pressure; *ICU,* intensive care unit; *IM,* intramuscular; *INH,* isoniazid; *IRIS,* immune reconstitution inflammatory syndrome; *IRU,* immune recovery uveitis; *IV,* intravenous; *LP,* lumbar puncture; *mo,* month(s); *NSAIDs,* nonsteroidal antiinflammatory drugs; *PI,* protease inhibitor; *PO,* per os (oral); PZA, pyrazinamide; *q,* every; *RFB,* rifabutin; *RIF,* rifampin; *SS,* single-strength; *TB,* tuberculosis; *tid,* thrice daily; *TMP-SMZ,* trimethoprim-sulfamethoxazole; *TVR,* telaprevir; *wk,* week(s); *yr,* year(s); *ZDV,* zidovudine.
From Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. http://aidsinfo.nih.gov/guidelines. Accessed March 1, 2019.

TABLE 129.3 Indications for Discontinuing and Restarting Opportunistic Infection Prophylaxis in HIV-Infected Adults and Adolescents

OPPORTUNISTIC INFECTION	INDICATION FOR DISCONTINUING PRIMARY PROPHYLAXIS	INDICATION FOR RESTARTING PRIMARY PROPHYLAXIS	INDICATION FOR DISCONTINUING SECONDARY PROPHYLAXIS/LONG-TERM MAINTENANCE THERAPY	INDICATION FOR RESTARTING SECONDARY PROPHYLAXIS/LONG-TERM MAINTENANCE
Pneumocystis pneumonia (PCP)	$CD4^+$ count increased from <200 to >200 cells/mm^3 for >3 mo in response to ART Can consider when $CD4^+$ count 100–200 cells/mm^3 if HIV RNA remains below limits of detection for at least 3–6 mo	$CD4^+$ count <100 cells/mm^3 $CD4^+$ count 100–200 cells/mm^3 and with HIV RNA above detection limit of assay	$CD4^+$ count increased from <200 cells/mm^3 to >200 cells/mm^3 for >3 mo in response to ART Can consider when $CD4^+$ count 100–200 cells/mm^3 if HIV RNAs remain below limits of detection for at least 3–6 mo If PCP occurs at $CD4^+$ count >200 cells/mm^3 while not on ART, discontinuation of prophylaxis can be considered once HIV RNA levels are suppressed to below limits of detection for at least 3–6 mo *Note:* If PCP occurs at $CD4^+$ count >200 cells/mm^3 while on ART, continue PCP prophylaxis for life, regardless of how high $CD4^+$ cell count rises as a consequence of ART	$CD4^+$ count <100 cells/mm^3 $CD4^+$ count 100–200 cells/mm^3 and with HIV RNA above detection limit of assay

TABLE 129.3 Indications for Discontinuing and Restarting Opportunistic Infection Prophylaxis in HIV-Infected Adults and Adolescents—cont'd

OPPORTUNISTIC INFECTION	INDICATION FOR DISCONTINUING PRIMARY PROPHYLAXIS	INDICATION FOR RESTARTING PRIMARY PROPHYLAXIS	INDICATION FOR DISCONTINUING SECONDARY PROPHYLAXIS/LONG-TERM MAINTENANCE THERAPY	INDICATION FOR RESTARTING SECONDARY PROPHYLAXIS/LONG-TERM MAINTENANCE
Toxoplasma gondii encephalitis (TE)	$CD4^+$ count increased to >200 cells/mm^3 for >3 mo in response to ART Can consider when $CD4^+$ count 100–200 cells/mm^3 if HIV RNA remains below limits of detection for at least 3–6 mo	$CD4^+$ count <100 cells/mm^3 $CD4^+$ count 100–200 cells/mm^3 and with HIV RNA above detection limit of assay	Successfully completed initial therapy, receiving maintenance therapy and free of signs and symptoms of TE, and $CD4^+$ count >200 cells/mm^3 for >6 mo in response to ART	$CD4^+$ count <200 cells/mm^3
Microsporidiosis	NA	NA	No signs and symptoms of nonocular or ocular microsporidiosis and $CD4^+$ count >200 cells/mm^3 for >6 mo in response to ART	No recommendation
Salmonellosis	NA	NA	Resolution of *Salmonella* infection and after response to ART with sustained viral suppression and $CD4^+$ counts >200 cells/mm^3	No recommendation
Cryptococcal meningitis	NA	NA	<u>If following criteria are fulfilled:</u> Completed initial (induction and consolidation) therapy, and Received at least 1 yr of maintenance therapy, and Remain free of symptoms of cryptococcal infection, and $CD4^+$ count ≥100 cells/mm^3 for >3 mo, and with suppressed plasma HIV RNA in response to ART	$CD4^+$ count <100 cells/mm^3
Histoplasma capsulatum infection	$CD4^+$ count >150 cells/mm^3 for 6 mo while on ART	For patients at high risk of acquiring histoplasmosis, restart at $CD4^+$ count <150 cells/mm^3	<u>If following criteria are fulfilled:</u> Received itraconazole for >1 yr, and Negative fungal blood cultures, and $CD4^+$ count ≥150 cells/mm^3 for ≥6 mo in response to ART, and Serum *Histoplasma* antigen <2 ng/mL	$CD4^+$ count <150 cells/mm^3
Coccidioidomycosis	$CD4^+$ count ≥250 cells/mm^3 and with viral suppression while on ART	Restart at $CD4^+$ count <250 cells/mm^3	<u>Only for patients with focal coccidioidal pneumonia:</u> Clinically responded to ≥6 mo antifungal therapy, with $CD4^+$ count ≥250 cells/mm^3, and with viral suppression while on ART Should continue monitoring for recurrence with serial chest radiographs and coccidioidal serology every 6–12 mo <u>For patients with diffuse pulmonary or disseminated nonmeningeal diseases:</u> Therapy is at least 12 mo and usually much longer; discontinuation depends on clinical and serologic response and should be done in consultation with experts <u>For meningeal diseases</u>: Suppressive therapy should be continued indefinitely even with increase in $CD4^+$ count on ART	No recommendation
Cytomegalovirus (CMV) retinitis	NA	NA	CMV treatment for at least 3–6 mo and with $CD4^+$ count >100 cells/mm^3 for >3–6 mo in response to ART Therapy should be discontinued only after consultation with an ophthalmologist, taking into account anatomic location of lesions, vision in contralateral eye, and feasibility of regular ophthalmologic monitoring Routine (i.e., every 3 mo) ophthalmologic follow-up is recommended after stopping therapy for early detection of relapse or immune restoration uveitis and then periodically after sustained immune reconstitution	$CD4^+$ count <100 cells/mm^3

ART, Antiretroviral therapy; *HIV,* human immunodeficiency virus; *NA,* not applicable.
From Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. http://aidsinfo.nih.gov/guidelines. Accessed March 1, 2019.

not adhere to prescribed chemoprophylaxis or ART. Therefore PCP continues to be a substantial cause of morbidity and mortality despite the availability of approaches that can markedly reduce its incidence.

Pneumocystis causes disease almost exclusively in the lungs. Extrapulmonary disease occurs but is uncommon. Patients characteristically have chest tightness or exercise intolerance as very early symptoms, before routine chest radiographs demonstrate infiltrates or before arterial blood gases reveal hypoxemia. If therapy is to have the greatest chance to succeed, patients and clinicians must be trained to initiate diagnostic evaluation at this stage, before pulmonary dysfunction is severe.[89,91]

Even with very mild manifestations of disease, organisms can be detected readily from sputum or bronchoalveolar lavage,[92–95] allowing initiation of therapy on an outpatient basis at a stage when prognosis is excellent. These secretions can also be analyzed for other pathogens, thus leading to diagnoses other than PCP. In many cases, PCP can be distinguished on a clinical basis with a high (but not complete) degree of certainty from bacterial pneumonia or viral pneumonia by the duration of symptoms, characteristics of the sputum, and radiologic manifestations. PCP can be especially difficult to reliably distinguish from certain other infectious and noninfectious processes that can manifest subacutely as symmetrical interstitial infiltrates including TB, histoplasmosis, and nonspecific interstitial pneumonitis.[96–99] Therefore, it is important to establish a specific diagnosis to ascertain that the correct pathogen is being treated and to avoid the toxicities, cost, and inconvenience of unnecessary drugs.

Establishing a specific diagnosis also has epidemiologic implications in terms of ascertaining the isolation precautions and contact tracing that are needed (e.g., such considerations are very different for TB as opposed to PCP). However, given the cost of a diagnostic evaluation, in some settings it may be necessary to treat cases of presumptive PCP empirically. If patients do not improve, the yield of a diagnostic evaluation of *Pneumocystis* infection should not be diminished substantially within the first several days or even 2 weeks after initiation of therapy, which is quite different from the experience with PCP in other patient populations. Empirical diagnoses by definition preclude the possibility of testing for active TB, eliminating the potential for early identification of TB and reduction of transmission. The availability of induced sputum examination provides a very sensitive, relatively low-cost method for diagnosis of PCP.[95] The visualization of *Pneumocystis* by colorimetric or immunofluorescent stain in sputum, bronchoalveolar lavage, or tissue is definitive for diagnosis of PCP.

Nucleic acid amplification systems for PCP that use oral washes, gargles, sputum, or bronchoalveolar lavage have not yet been standardized and validated for clinical use.[100,101] More and more laboratories are using nucleic acid amplification, however, so the clinician must be cognizant of how to interpret the results.

A negative nucleic acid amplification test on a bronchoalveolar lavage is very strong evidence that the cause of the pulmonary dysfunction is something other than *Pneumocystis,* assuming the laboratory has validated its assay. A negative result on an induced sputum is less useful. A positive result in a bronchoalveolar lavage or sputum is consistent with either colonization of the airways or acute disease. In theory the copy number of sequences in the specimen is helpful: a high copy number is suggestive of active disease, and a low copy number is more suggestive of colonization. However, given the variability in specimen acquisition, such suggestions are far from definitive. In contrast, detection of organisms by direct visualization is definitive evidence that the pneumonia is due to *Pneumocystis,* presumably because direct visualization is less sensitive and thus a positive result indicates a substantial number of organisms, indicating disease rather than colonization.

β_1-Glucan detection in serum or bronchoalveolar lavage is not sufficiently sensitive or specific to be diagnostically useful.[102–106] Some experts use this test as supportive of a diagnosis of PCP, but it is clearly not definitive. Some experts also follow results serially to document decreasing organism burden, which is logical but not proven to be useful.

The likelihood that a patient with AIDS will survive an episode of PCP depends on the severity of pulmonary dysfunction at the time of initiation of therapy, the patient's ability to tolerate available regimens, the presence of comorbidities, and the severity of the patient's immunologic dysfunction. An alveolar-arterial gradient greater than 30 mm Hg, a severely abnormal chest radiograph, a large number of organisms detected on lavage or biopsy, and comorbid conditions are strongly correlated with a poor prognosis.[89] Any drug therapy is more likely to be successful if it is started at a time when pulmonary dysfunction is mild and if other severe opportunistic infections or neoplasms are absent. Second, third, and subsequent episodes of PCP do not necessarily carry a worse prognosis than the first episode.[107,108] More relevant to prognosis is the severity of the acute disease, the presence of comorbidities, and the patient's current $CD4^+$ T-lymphocyte count, and HIV viral load.

TMP-SMZ is the treatment of choice for acute PCP because of its convenience of administration, high degree of efficacy, and manageability of associated toxicities (see Table 129.2).[8,109,110] No agent has been shown to have a higher efficacy for PCP than TMP-SMZ. There is no clear reason to prefer intravenous over oral TMP-SMZ in patients with mild disease who adhere to the treatment and who are without potential gastrointestinal dysfunction. Patients usually improve clinically within 4 to 8 days in terms of fever, respiratory rate, arterial-alveolar gradient, and dyspnea, although there may be an initial worsening during the first 8 to 72 hours of therapy if adjunctive corticosteroid therapy is not given.[87,111–114]

Survival for mild episodes treated with TMP-SMZ has improved steadily during the past 3 decades.[109–114] For patients with an initial room-air partial pressure of oxygen (Po_2) greater than 70 mm Hg, survival has improved from 85%–90% to 95%–99% in optimal circumstances.[109–114] Patients with significant concomitant disorders do not have such good results. This improvement in patient outcome for patients with moderate-to-severe disease presumably reflects better supportive treatment in critical care units and the better understanding that TMP-SMZ can be continued despite non–life-threatening toxicities. In addition, better alternative agents have been available since the early 1990s for patients who truly have treatment failure with TMP-SMZ or who are unable to tolerate the drug.[8,115–117]

In vitro resistance of *Pneumocystis* to TMP-SMZ was not described until the 1990s. Some human isolates contain mutations in the dihydropteroate synthase enzyme, the target for sulfonamides. When these same mutations occur in other organisms (e.g., *S. pneumoniae* or *Plasmodium* spp.), they produce microbiologic and clinical resistance. Further information is needed to determine whether these mutations in fact confer sulfonamide resistance to *Pneumocystis* that is clinically significant and whether the frequency of such resistance will become sufficient to warrant new therapeutic and prophylactic strategies.[118–122] The recent cloning of the human species of *Pneumocystis* will likely provide insights into resistance mechanisms and new targets for therapy.[123]

Common adverse reactions to TMP-SMZ include rash, nausea, vomiting, granulocytopenia, transaminase elevations, nephritis, and hyperkalemia.[87,124] These reactions do not invariably require discontinuation of TMP-SMZ therapy. The rashes, which commonly occur between days 8 and 12 of therapy, may be limited in extent and associated with a degree of pruritus that the patient can tolerate for 21 days. They are more frequent in patients with higher $CD4^+$ T-cell counts. Life-threatening desquamating processes (e.g., Stevens-Johnson syndrome) are rare in HIV-infected patients, although a few fatal cases have been described. Severe febrile, hypotensive episodes that resemble septic shock in terms of hemodynamics have also been reported. Granulocytopenia is most often a dose-related phenomenon that may resolve partially or completely if the dose of TMP-SMZ is reduced by 25%. Granulocytopenia responds to leucovorin administration only rarely. A report that leucovorin administration can diminish the likelihood of therapeutic response to TMP-SMZ was intriguing but not conclusive and has not been replicated.[125] Nausea and vomiting can be troublesome complications of TMP-SMZ therapy. Severe nausea may result from very high sulfonamide levels and may resolve if the dose is reduced such that sulfonamide levels 2 hours after dosing are 100 to 150 μg/mL.[126] Transaminase levels may fluctuate to three to five times normal until therapy is stopped; they usually return promptly to baseline values if they were caused by TMP-SMZ rather than another drug or a coexisting process such as hepatitis A virus, HBV, or HCV.

Overall, adverse reactions have required discontinuation of TMP-SMZ therapy in about 25% of cases.[8,89,124] Although it has not been proved

by a prospective study, adverse reactions can probably be reduced without sacrificing efficacy by lowering the recommended dose of TMP-SMZ from TMP 20 mg/kg/day (with SMZ 100 mg/kg/day) to TMP 15 mg/kg/day (with SMZ 75 mg/kg/day).[109] Gradual dose escalation of TMP-SMZ can reduce the impact of adverse effects, but this approach is appropriate in selected patients when trying to institute prophylaxis, not when instituting therapy in patients with acute illness.[127,128]

Parenteral pentamidine is effective therapy for PCP.[8,109] The intravenous regimen is inconvenient to administer, however, and the adverse reactions associated with it can be life threatening. Renal dysfunction, hypoglycemia, hyperglycemia, granulocytopenia, and hypotension are reported in 10% to 50% of patients.[8,109,129–132] If pentamidine is administered over a period of at least 60 minutes in 100 to 150 mL of dextrose in water, clinically important hypotension is unusual.[131] The renal dysfunction associated with pentamidine can be severe. If the serum creatinine level rises by more than 1 to 2 mg/dL, strong consideration should be given to withholding therapy for a few days or changing to an alternative agent. Pentamidine prolongs the QT interval, and this has led to torsades de pointes in a few patients with fatal outcomes, especially if patients had a preexisting QT prolongation or were receiving other drugs that prolong the QT interval. Hypoglycemia can be a life-threatening complication of pentamidine therapy; it can occur at any juncture during therapy or for many weeks after therapy has been completed.[132] Hypoglycemia occurs more frequently in patients who have pentamidine-induced renal dysfunction. The unpredictability of the hypoglycemia adds an element of risk to inpatient or outpatient use of this drug. Life-threatening hypoglycemia is sufficiently uncommon, however, that this effective agent is still recommended for patients with severe disease who cannot tolerate TMP-SMZ. Lowering the dose of parenteral pentamidine from 4 to 3 mg/kg/day has been advocated to reduce toxicity; whether this also reduces efficacy is unknown. Aerosol pentamidine is not sufficiently effective for use to treat PCP.[133]

Dapsone is rarely used in this era for therapy or prophylaxis of PCP, but this drug does have clinical activity against *Pneumocystis*.[109,134–136] Dapsone (100 mg orally every day) plus TMP (5 mg/kg orally every 8 hours or 300 mg orally every 8 hours) appears to be as effective as TMP-SMZ but less toxic.[8,111,134–136] This regimen is not frequently used because it is less convenient to administer than TMP-SMZ (there is no tablet that combines dapsone and TMP). Dapsone-based regimens have no major advantages in terms of efficacy or safety. Rashes are common among HIV-infected patients treated with dapsone, but a 21-day course of therapy can usually be completed without interruption. Methemoglobinemia can occur and can be life threatening. Oral dapsone alone has some efficacy when 100 mg is administered every day for 21 days, but there is probably not enough activity to warrant use of this agent as single-drug therapy. Higher doses are not well tolerated. Dapsone should not be administered to patients who have had immediate hypersensitivity reactions to sulfonamides.[134]

Atovaquone is a hydroxynaphthoquinone that affects mitochondrial electron transport in microorganisms and therefore has a mechanism of action distinct from that of TMP-SMZ or pentamidine.[115,137] Atovaquone is available as an oral suspension but is not available in a parenteral form. For patients with mild or moderate PCP, it has a high degree of efficacy and is extremely well tolerated. A large, prospective, double-blind study demonstrated that atovaquone, although very effective and much better tolerated than TMP-SMZ, is associated with more treatment failures.[115] Because atovaquone, in contrast to TMP-SMZ, has no antibacterial activity, it is possible that patients in the atovaquone-treated groups were more likely to experience a deterioration of their condition owing to unrecognized, concurrent bacterial processes. Atovaquone is better tolerated than intravenous pentamidine.[137] The absorption of atovaquone suspension can be erratic, especially in patients who cannot consume fatty foods with this medication. Atovaquone takes several days to reach steady-state levels. It should not be given to patients with significant gastrointestinal dysfunction and should not be initiated as primary therapy in patients with severe disease. Mutations that could confer atovaquone resistance have been identified in human isolates, but their clinical relevance remains to be determined.[138] The primary toxicities associated with atovaquone are nausea, abdominal pain, low-level transaminase elevations, and rash. Atovaquone is a reasonable treatment option for patients with mild or moderate PCP who cannot tolerate TMP-SMZ and who are good candidates for oral therapy.

Clindamycin plus primaquine is also effective therapy for PCP.[8,110,117] Despite the fact that primaquine can be given only orally, this regimen has been used successfully in patients with mild, moderate, and severe disease. Clindamycin plus primaquine is associated with toxicity including rash, serum aminotransferase elevation, diarrhea, and hemolysis. It is a reasonable regimen for patients who are unable to tolerate other regimens. Clindamycin-primaquine is also a reasonable option for patients with definitively established PCP who are unequivocally failing therapy with TMP-SMZ.

Regardless of which specific agent is chosen as the initial therapy for PCP, adjunctive corticosteroid therapy is indicated for any patient whose initial room-air Po_2 is less than 70 mm Hg.[8,111–115] Three prospective trials demonstrated that the frequencies of ventilatory failure and mortality can be reduced substantially by the prompt use of corticosteroids.[111–115] Physiologically, adjunctive corticosteroids appear to prevent much of the decline in oxygenation that characteristically occurs during the first 3 days of treatment.[114] This decline may be caused by the inflammatory response elicited by dying organisms. Adjunctive corticosteroids may also provide benefit for patients with an initial room-air Po_2 greater than 70 mm Hg. Physiologic evidence indicates improved lung function in such patients, but so few patients with mild PCP develop respiratory failure or die that it is difficult to substantiate a survival benefit. The safety of the 21-day regimen is well substantiated. Reactivation of TB, CMV, or Kaposi sarcoma is unusual. It is unclear whether such short courses of corticosteroids could predispose patients to osteonecrosis.[139,140] The frequency of TMP-SMZ–related rash is not diminished by corticosteroids.[141]

If an HIV-infected patient with PCP fails to improve while receiving conventional therapy, there are no controlled data to indicate which modifications in therapy are optimal.[8,142–146] The mean time to improvement for HIV-infected patients treated with conventional therapy is 4 to 8 days,[87,109] so therapeutic failure probably should not be diagnosed until patients have received 4 to 8 days of therapy. Clinicians often feel compelled to alter therapy earlier, however, especially if the patient's condition is deteriorating rapidly as opposed to failing to improve. If a patient has not improved after 5 to 10 days of therapy, repeat diagnostic procedures should be considered to determine whether another treatable (infectious or noninfectious) condition is present. Bronchoalveolar lavage is the procedure of choice. *Pneumocystis* is often present in lavage or tissue for at least 3 to 4 weeks after initiation of therapy, even in patients who respond promptly, so its presence after 7 to 10 days of therapy does not necessarily imply that therapy is ineffective. A decision regarding treatment effectiveness should be based on clinical and laboratory parameters such as oxygenation, ventilation, and fever. The presence of extensive intraalveolar exudate or extensive fibrosis after 7 to 10 days of therapy is a more ominous sign. Open lung biopsy is rarely necessary to establish a diagnosis of PCP, but it can occasionally be useful for identifying other processes. Kaposi sarcoma of the lung is usually apparent on bronchoscopy because of endobronchial lesions that are obvious to the bronchoscopist.[147] However, if such lesions are not seen on bronchoscopy, Kaposi sarcoma of the lung is one treatable process that is difficult or impossible to diagnose reliably from cytology or from transbronchial biopsy specimens. Nodular lesions on chest computed tomography, extensive intrabronchial lesions, and the presence of a bloody pleural effusion may be helpful clues that Kaposi sarcoma is the cause of pulmonary dysfunction. CMV and lymphoma are other processes that may be identified by cytology or by some form of biopsy more readily than by sputum assessment.

If *Pneumocystis* infection is the only identifiable cause of the pulmonary dysfunction after 7 to 10 days of therapy and no improvement has been observed, there are several therapeutic interventions to consider: (1) switch from TMP-SMZ to parenteral pentamidine or clindamycin-primaquine; (2) add corticosteroids to conventional therapy if they have not already been added; (3) use two specific therapies concurrently (e.g., TMP-SMZ plus pentamidine); and (4) add ART. Each of these approaches has been associated with a successful outcome in some cases.[142–144] A controlled trial is needed to determine the best approach, but such trials are difficult to perform because of the large size, complexity,

and cost of a valid study. Whether patients with AIDS with PCP should be supported aggressively with intensive care, mechanical ventilation, or other interventions depends on issues specific to each individual patient, as would be the case for any patient who is critically ill. The most reasonable approach would be to individualize each management plan in terms of the days of therapy that have been completed, the therapeutic alternatives that are available, and the concomitant processes that are present. The patient's wishes and the availability of resources need to be taken into account. Published data indicate that some patients with AIDS with PCP can survive intubation and mechanical ventilation and lead independent lives for years after hospital discharge. The best candidates for intensive care and mechanical ventilation are patients who had a good functional status before PCP, patients who have presented with no other serious opportunistic processes, patients who have received fewer than 7 days of specific therapy, and patients who have clearly articulated a desire for aggressive support.

As mentioned previously, for patients who were not receiving ART when PCP was diagnosed, ART is generally not initiated immediately, but is started within 2 weeks of the PCP episode.[71] An important study demonstrated that patients who started ART within 2 weeks had better survival than patients who started after 2 weeks, presumably because additional HIV-associated complications were prevented by the earlier introduction of therapy.[71] There are multiple reasons not to initiate ART immediately after the diagnosis of PCP: inability to take oral drugs, drug interactions with agents used during the acute disease, adverse effects of ART being confused with adverse effects of other recently added drugs, and IRIS against *Pneumocystis* or another pathogen are among the issues that influence this decision.[8] However, in challenging cases early initiation of ART may be a desirable strategy.

For the rare patients receiving ART at the time that PCP is diagnosed (an unusual occurrence most often related either to very recent initiation of ART or nonadherence to ART), ART is usually continued if the patient can tolerate it. Such decisions are complex, related to the patient's ability to reliably absorb oral ART during an acute illness as well as to potential drug interactions.

Prevention of PCP is a major priority in the management of HIV infection.[8] Prevention of PCP is logical because episodes are frequent (at least 80%–90% of HIV-infected patients in North America develop an episode at some point if they have received neither anti-*Pneumocystis* prophylaxis nor ART), morbidity and mortality due to PCP can be substantial, low-cost drugs are available that are effective, and the period of high susceptibility can be defined.[8,49] Prospective and retrospective studies have shown that most primary episodes of PCP occur in patients with $CD4^+$ T-cell counts less than 200 cells/mm^3.[8,33,49] Other documented predictors of the occurrence of PCP, independent of the $CD4^+$ T-cell count, are otherwise unexplained oropharyngeal candidiasis (e.g., no concurrent corticosteroids or antibacterials), high HIV viral load, wasting syndrome, previous AIDS-defining event, and prior pneumonia of any type.[8] These parameters should be added to the list of factors encouraging primary prophylaxis.

When ART is initiated and is effective, the HIV viral load declines rapidly, and the patient's risk of PCP decreases quickly regardless of the measured $CD4^+$ T-cell count. As the $CD4^+$ T-cell increases with a viral load below the level of assay detection, management strategies have been proposed suggesting that PCP prophylaxis is not necessary. However, if the patient's $CD4^+$ T-cell count was very low (e.g., less than 100 cells/mm^3) when ART was started, the $CD4^+$ count may not rise above 200 cells/mm^3 for many months. In such cases, PCP may be rare, yet PCP does occur, and thus most clinicians would continue PCP prophylaxis until the $CD4^+$ T-cell count is above 200 cells/mm^3.[148,149]

Before the era of ART, secondary prophylaxis (prevention of second or subsequent episodes of PCP) was indicated for everyone who had a documented PCP episode because the 1-year recurrence rate is about 65% for patients who receive no prophylaxis.[8,49] As mentioned earlier, there is considerable evidence suggesting that discontinuation of primary or secondary prophylaxis is appropriate for patients who respond to ART and who manifest $CD4^+$ T-cell counts that are persistently approximately 200 cells/mm^3 (see Table 129.3).[150]

TMP-SMZ is the preferred prophylactic regimen for any HIV-infected patient who can tolerate it (see Tables 129.1 and 129.2).[8,150–157] If administered at a dose of 160 mg TMP plus 800 mg SMZ (i.e., one double-strength tablet once daily), episodes of PCP are extremely uncommon among patients who adhere to the regimen. Trials have demonstrated that TMP-SMZ is much more effective for either primary or secondary prophylaxis than aerosolized pentamidine or dapsone-containing regimens.[151–156] TMP-SMZ also has the benefit, based on retrospective analyses, of reducing the frequency of toxoplasmosis,[157] and it probably has a beneficial effect on reducing the frequency of pneumococcal, staphylococcal (methicillin-sensitive *S. aureus* and MRSA), and *Haemophilus* spp. infections.[150,151]

There is not convincing evidence that clindamycin-primaquine or intravenous pentamidine should be used for PCP prophylaxis for patients with HIV infection. However, there are some small studies suggesting that intravenous pentamidine is effective in pediatric hematopoietic stem cell recipients.[158,159]

Comparative trials have confirmed that TMP-SMZ is not nearly as well tolerated as aerosolized pentamidine. Due to side effects of rash, pruritus, fever, granulocytopenia, thrombocytopenia, anemia, hepatitis, nephritis, nausea, or vomiting, 20% to 40% of patients cannot tolerate TMP-SMZ. Reducing the dose of TMP-SMZ by 50% (i.e., a single-strength tablet daily) or reducing the frequency to two or three times weekly lowers the toxicity.[152,156] In a study comparing TMP-SMZ at a dose of one double-strength tablet daily with TMP-SMZ at a dose of one double-strength tablet three times weekly, there was no difference in efficacy when the data were evaluated based on an intent-to-treat analysis.[156] However, there were more failures on the intermittent regimen when occurrences of PCP were analyzed based on the regimen patients were actually taking at the time that PCP was diagnosed. This finding has suggested to some investigators that the intermittent regimen is less effective.

Because TMP-SMZ is the preferred regimen, strategies to increase patient tolerance of this regimen are important. In two controlled studies, gradual dose escalation of TMP-SMZ at the time at which prophylaxis is restarted was shown to increase tolerability.[127,128]

There are several alternatives for patients who cannot tolerate TMP-SMZ. Dapsone is rarely used anymore. However, there are trials that show that daily dapsone or weekly dapsone-pyrimethamine has an efficacy comparable to that of aerosolized pentamidine (i.e., not as effective as daily TMP-SMZ when used as prophylaxis for PCP). Dapsone-containing regimens, especially dapsone-pyrimethamine regimens, are effective as prophylaxis against toxoplasmosis.[135,136,153] Similar to TMP-SMZ, dapsone or dapsone-pyrimethamine is poorly tolerated by a substantial number of patients; fever, rash, pruritus, and hemolysis occur. About 20% of patients who cannot tolerate TMP-SMZ also cannot tolerate dapsone-containing regimens.[134] Aerosolized pentamidine, although not as effective as TMP-SMZ, has a definite ability to reduce the frequency of PCP when used for primary or secondary prophylaxis. The manner in which aerosolized pentamidine is delivered to the patient is a major determinant of efficacy and safety.[160] Because different nebulizers deliver different spectra and different densities of particle sizes, they deliver different amounts of drug to the lung. Only the Respirgard II jet nebulizer and the Fisons ultrasonic nebulizer have been studied in large, well-controlled trials with clinical end points. Aerosolized pentamidine is well tolerated by most patients. Coughing and wheezing can be ameliorated or prevented by nebulized albuterol. A bitter taste is often reported. Cases of pancreatitis and renal dysfunction have been attributed to aerosolized pentamidine, but it is not always certain that aerosolized pentamidine was the cause.

A major concern related to the use of aerosolized pentamidine is environmental contamination with drug and respiratory aerosols, which is created when patients cough or become disconnected from the nebulizer. Health care workers and patients may inhale enough pentamidine to develop detectable urine levels of the drug.[161] The clinical importance of this is unknown. More important, if the patient has pulmonary TB, the environmental contamination produced by dispersed respiratory particles has considerable potential to spread TB.[162] Patients need to be carefully screened for pulmonary TB before aerosolized pentamidine prophylaxis is initiated.

Atovaquone has been assessed as prophylaxis in trials comparing it with either dapsone alone or aerosolized pentamidine.[163,164] In both

situations, atovaquone was equally effective as the alternative, and it was better tolerated than dapsone. As indicated earlier, optimal atovaquone absorption is dependent on ingestion of high-fat meals concurrent with drug administration.

If patients cannot tolerate TMP-SMZ, aerosolized pentamidine, or atovaquone, there are several poorly studied options. The best option for patients intolerant of the normally used therapies is to try to maximize the patient's ability to tolerate TMP-SMZ by using a dose-escalation strategy or an intermittent (three times weekly) regimen. Aerosolized pentamidine can be administered by employing doses greater than the approved regimen (300 mg twice monthly or 600 mg once monthly, rather than the approved dose of 300 mg monthly),[165] although experience documenting the superiority of twice-monthly pentamidine is limited. Results with clindamycin-primaquine have been disappointing. Whether the need for alternative regimens will grow substantially depends on the clinical relevance of sulfonamide resistance.

Before effective ART was available, there were analyses of why patients experienced breakthrough PCP while receiving prophylactic therapy. These analyses demonstrated that breakthroughs usually occurred in patients who were not receiving TMP-SMZ (i.e., they were receiving other regimens for prophylaxis), patients who were not adherent to prophylaxis, or patients who had very low $CD4^+$ T-cell counts with high viral loads.[166,167] These factors are undoubtedly relevant, but the major focus for PCP prevention in this era should be institution of effective ART.

Toxoplasma gondii

Toxoplasma gondii (see Chapter 278) causes disease in patients with HIV infection primarily by reactivation of latent disease rather than by primary infection.[168–173] Patients almost always have IgG antibodies against *Toxoplasma* (although insensitive enzyme-linked immunosorbent assays may fail to detect such antibodies), have fairly advanced immunosuppression ($CD4^+$ T-cell counts <50 cells/mm^3), and have not been receiving TMP-SMZ prophylaxis. Because the seroprevalence of toxoplasmosis is much higher in some areas such as Western Europe (50%–80%) and South America than in the United States (11%) (i.e., there is a higher incidence of latent infection), those areas have much higher frequencies of AIDS-associated toxoplasmosis because the latent disease has the potential to reactivate when patients are severely immunosuppressed.[174,175]

In patients with HIV infection, toxoplasmosis manifests most often as focal cerebral disease with fever, headache, confusion, motor defects, and seizures. Retinochoroiditis, pneumonitis, disseminated disease, diffuse and nonfocal cerebral disease, and a sepsis-like syndrome all have been reported, but these are not as frequent as focal lesions of the central nervous system (CNS).

If an HIV-infected patient with a $CD4^+$ T-cell count less than 100 cells/mm^3 presents with a space-occupying cerebral lesion that involves gray matter, the differential diagnosis in North America should focus on two entities: toxoplasmosis and lymphoma. Fungal, mycobacterial, viral, and nonlymphoma neoplastic processes also manifest as space-occupying lesions, but these entities are causative processes seen less commonly in the United States and Western Europe than *Toxoplasma* and lymphoma. Progressive multifocal leukoencephalopathy (PML) should manifest differently because it affects primarily white matter. The incidence of malignant neoplasms that are not included as AIDS-defining conditions is increasing; thus clinicians must also be alert to the possibility that CNS masses represent metastatic tumor.[27,28,176,177] Also, in the era of ART, CNS lymphoma appears to be increasing in frequency compared with CNS toxoplasmosis.

Clinical or imaging characteristics help to distinguish lymphoma from toxoplasmosis but are not definitive.[178–183] Magnetic resonance imaging is more sensitive than computed tomography for radiologic diagnosis of *Toxoplasma* encephalitis. Positron emission tomography or single-photon emission computed tomography may also be helpful in distinguishing *Toxoplasma* encephalitis from primary CNS lymphoma.[179,178] However, no imaging technique is completely specific.

If safe and feasible, a lumbar puncture should be performed for *T. gondii* PCR assay.[180–186] A positive CSF PCR assay for Epstein-Barr virus (EBV) is moderately specific for primary CNS lymphoma.[187] Some positive EBV PCR results have been documented in patients with proven cerebral toxoplasmosis—that is, the CSF is EBV PCR positive, but the lesion is caused by *Toxoplasma*. For toxoplasmosis, detection of the *Toxoplasma* organism by CSF PCR should be virtually diagnostic that *Toxoplasma* is the causative agent; however, the test is not standardized across laboratories. Published results have shown that some laboratories can achieve high specificity but low sensitivity. Sensitivity may be low after specific therapy has been started.

Although most cases of *Toxoplasma* encephalitis are clinically diagnosed by response to empirical therapy or by PCR assay of the CSF, an unequivocal diagnosis of *Toxoplasma* encephalitis requires a brain biopsy, which is most commonly performed by a stereotactic CT-guided needle biopsy. Hematoxylin and eosin stains can be used for detection of *T. gondii,* but sensitivity is significantly increased if immunoperoxidase or immunofluorescent staining is used. The detection of *Toxoplasma* in tissue samples by PCR is not a standardized approach and can be difficult to interpret because *Toxoplasma* can be present in brain tissue as a latent organism unrelated to the process that is causing the space-occupying lesion.

If an HIV-infected patient with a circulating $CD4^+$ T-cell count less than 50 cells/mm^3 and positive serum anti-*Toxoplasma* antibody presents with a CNS mass lesion involving gray matter, most clinicians would treat the patient empirically for toxoplasmosis.[188–192] A definitive diagnostic study (i.e., brain biopsy) has morbidity associated with it, and the diagnostic yield may be only 50% if toxoplasmosis is the cause depending on the size of the biopsy specimen and skill of the pathologist. The cysts and tachyzoites of *T. gondii* can be difficult to recognize in fragments of necrotic brain tissue, and even several small needle biopsy samples may miss the area that has abundant organisms. When patients are treated with either sulfadiazine-pyrimethamine or clindamycin-pyrimethamine, unequivocal improvement clinically and radiologically should occur within 10 to 21 days. If such improvement does not occur, a biopsy should be performed to establish whether the cause is an infectious or a neoplastic process other than toxoplasmosis.[188–192] Pyrimethamine and sulfadiazine are becoming more difficult to find in some regions. TMP-SMZ has not been as extensively evaluated in published literature as pyrimethamine and sulfadiazine, but several small trials or observational cohorts suggest that TMP-SMZ has comparable efficacy and safety to sulfadiazine plus pyrimethamine.[191–194] TMP-SMZ has the advantage of being available as an oral or an intravenous preparation.

Corticosteroids to reduce inflammation may be necessary in patients with signs of increased ICP. As there are no well-defined parameters to determine when corticosteroid therapy is indicated, clinical judgment must be used. The administration of corticosteroids can make early evaluation of the clinical and radiologic response to specific therapy difficult because the observed improvement may be solely the result of corticosteroid therapy and unrelated to the anti-*Toxoplasma* regimen employed. Doses of corticosteroids should be tapered as soon as feasible.

Although some clinicians institute antiseizure drugs prophylactically, most experts would initiate such therapy only if a seizure occurs.

For patients who have a clinical and radiologic response, anti-*Toxoplasma* therapy should be continued lifelong, in the absence of an ART-induced increase in the $CD4^+$ T-cell count, because relapses occur in the same sites manifesting initially if therapy is discontinued, even after 8 to 12 months of treatment. If the $CD4^+$ T-cell count rises substantially (e.g., to levels >200 cells/mm^3) due to ART and the patient has received at least 6 months of anti-*Toxoplasma* therapy, the anti-*Toxoplasma* therapy can be safely discontinued, provided that the lesion has largely resolved on cerebral imaging and the patient is neurologically stable (see Table 129.3).[8,195,196]

Treatment failures are unusual for patients with toxoplasmosis who are able to tolerate both pyrimethamine and sulfadiazine or TMP-SMZ. Radiologically proven failures in patients who are adhering to their drug regimen should raise the possibility that toxoplasmosis is not the correct or the only diagnosis.

Adverse reactions to sulfadiazine (leukopenia, rash, elevated levels of aminotransferases, nausea, nephritis) and to pyrimethamine (leukopenia, thrombocytopenia) are common. TMP-induced or pyrimethamine-induced leukopenia often does not respond to leucovorin therapy,

although a short course of leucovorin (10–20 mg orally or intravenously every 6 hours) should be administered. For patients unable to tolerate sulfadiazine, clindamycin plus pyrimethamine is also effective (see Table 129.2).[188,190]

Immune reconstitution syndromes associated with toxoplasmosis have only rarely been reported.[197,198] The potential for such syndromes to occur should be considered before initiating ART, especially in patients who have elevated ICP.

TMP-SMZ offers considerable protection as primary prophylaxis for *Toxoplasma*-seropositive patients with $CD4^+$ T-cell counts less than 100 cells/mm^3.[8,157] Dapsone-pyrimethamine and atovaquone also have substantial efficacy. However, prevention should focus primarily on improving immune function by instituting ART. As with other opportunistic infections, susceptibility to toxoplasmosis is reduced substantially as soon as the viral load is suppressed.

Herpes Simplex Virus and Varicella-Zoster Virus

In the United States, 60% of the general adult population is seropositive for HSV-1, and 17% is seropositive for HSV-2. For HIV-positive individuals, 95% are seropositive for HSV-1 or HSV-2.[199–201] HSV (see Chapter 135) is a frequent cause of oral, genital, and perirectal ulcerations in patients with HIV infection who have low $CD4^+$ T-cell counts and who are not receiving effective ART. Ulcers in such patients can be several centimeters in diameter.

The response of acyclovir-sensitive HSV lesions is usually prompt and occurs within 3 to 10 days.[202,203] Therapy should continue until the lesions are crusted over or epithelialized. Relapses occur with high frequency. If relapses occur quickly or often, long-term suppressive therapy may be necessary. Acyclovir-resistant isolates occur primarily in patients who have low $CD4^+$ T-cell counts and who have received long-term suppressive therapy. Foscarnet and cidofovir are active against herpes simplex and have been used successfully against acyclovir-resistant strains.[204–207] Cases of foscarnet-resistant and cidofovir-resistant HSV have also been reported. Favorable experience has been reported with topical preparations of trifluridine, foscarnet, imiquimod, or cidofovir.[208,209] If lesions recur, the causative virus may be a drug-susceptible strain even if the prior episode was caused by a drug-resistant strain.

Chemoprophylaxis is not routinely recommended to prevent occurrences of HSV-1 or HSV-2 disease. If recurrences are severe or frequent, such suppression could be considered using valacyclovir or famciclovir.

Esophagitis due to HSV occurs but is not as common as *Candida* esophagitis. Disseminated herpes simplex viral infections and focal visceral manifestations, including encephalitis, are unusual in HIV-infected patients, even in patients with very low $CD4^+$ T-cell counts.

The incidence of herpes zoster is 15-fold higher in HIV-infected individuals than in age-matched control subjects.[210,211] ART does not diminish the incidence of herpes zoster substantially.[210,211] In fact, zoster occurs with increased frequency in the initial 6 months after starting ART, which may represent a form of either "unmasking" or IRIS. Dermatomal herpes zoster lesions are usually similar in extent and distribution to lesions seen in immunocompetent patients (see Chapter 136). Dissemination of varicella-zoster virus (VZV) is an unusual event.[212] If dissemination does occur, visceral disease is unusual, and cutaneous manifestations can be quite variable in presentation. A high index of suspicion should be maintained that hyperkeratotic, verrucous, or ecthymatous lesions in patients with low $CD4^+$ T-cell counts could be caused by VZV infection.

Neurologic disease due to VZV is well described in association with HIV infection. Manifestations include CNS vasculitis, multifocal leukoencephalopathy, ventriculitis, myelitis, cranial nerve palsies, aseptic meningitis, progressive outer retinal necrosis, and acute retinal necrosis.

For dermatomal disease, acyclovir, valacyclovir, or famciclovir therapy hastens the crusting of lesions and aids in preventing postherpetic neuralgia and preventing recurrences in HIV-infected patients (see Table 129.2).[8] Therapy is indicated within the first week of rash onset or if lesions have not fully crusted. Treatment should probably continue until all lesions are scabbed or crusted, even if this requires more than 7 to 10 days. Patients with zoster ophthalmicus and neurologic complications would be logical candidates for high-dose intravenous acyclovir, probably with adjunctive corticosteroids. Many clinicians are reluctant to use corticosteroids to treat dermatomal disease in patients with HIV infection despite evidence regarding a beneficial effect in patients without HIV infection.[213,214] Acyclovir-resistant isolates rarely occur,[215] and some of these cases have been treated successfully with parenteral foscarnet or cidofovir.

About 5% of patients with HIV infection do not have detectable antibodies to VZV, although routine serologic testing is not recommended. Seronegative patients may benefit from ensuring that all household contacts have been immunized, thus avoiding exposure of the patient to a family member with varicella. If seronegative patients are exposed to this virus, primary varicella may result and can be severe.[216] The live-attenuated varicella vaccine can be considered for HIV-infected adults with $CD4^+$ T-cell counts greater than 200 cells/mm^3.[8] Seronegative HIV-infected patients who are exposed to varicella probably benefit from VariZIG (administered immediately after exposure, but within 10 days of exposure) or acyclovir (initiate 7–10 days after exposure) (see Chapter 136).

Two vaccines now are available for non–HIV-infected individuals to prevent herpes zoster and postherpetic neuralgia in people ≥50 years of age: RZV (a recombinant glycoprotein E vaccine) and ZVL (a live-attenuated vaccine). The live-attenuated vaccine to prevent herpes zoster (Zostavax) can be considered for HIV-infected patients with $CD4^+$ T-cell counts greater than 200 cells/mm^3.[8,208,217] For preventing zoster in HIV-infected individuals, there are not enough data currently available to make recommendations regarding the inactivated recombinant vaccine.[218,219]

Cytomegalovirus

CMV retinitis was one of the earliest manifestations that clinicians recognized as characteristic of AIDS. During the first decade of the AIDS epidemic, CMV retinitis was a devastating and common occurrence in patients with this new syndrome. In that era, CMV retinitis caused severe visual impairment because no specific therapy was available for CMV, and no durably effective therapy was available for HIV.

CMV infection, as assessed by serology, is almost universal among HIV-infected patients who have acquired HIV infection through homosexual contact (see Chapter 137). In contrast, only about 75% of HIV-infected patients who are heterosexual are seropositive for CMV.[220,221]

Historically, before either specific anti-CMV prophylaxis or ART was available, 21% to 44% of patients developed CMV-associated disease at some point during their illness.[7,222–224] HIV-infected patients with circulating $CD4^+$ T-cell counts less than 50 cells/mm^3 are often viremic and viruric with CMV, which does not imply that they have disease due to CMV.[225,226] The likelihood of development of CMV-associated disease is related to both the degree of immunosuppression and the quantity of circulating CMV. The latter can be assessed by a variety of quantitative systems that detect antigen or nucleic acid in circulating blood, although this is not done routinely. A strategy to intervene in high-risk patients (i.e., patients with low $CD4^+$ T-cell counts and detectable CMV above some defined threshold) may be plausible because oral agents are available, but such an approach does not seem necessary in the era of effective ART.

Retinitis is the most commonly recognized disease caused by CMV.[7,221–224,227–229] Most cases occur at $CD4^+$ T-cell counts less than 50 cells/mm^3. CMV retinitis has the potential to involve and rapidly damage the macula and optic disk, to cause retinal detachments, and to result in irreversible visual impairment and ultimately in blindness. Disease is usually recognized unilaterally at first but may present or become bilateral.[229–231]

The diagnosis of CMV retinitis is usually made clinically. Obtaining retinal or vitreous material for examination is risky (detached retina or secondary infection can result). The appearance of CMV retinitis is characteristic to an experienced ophthalmologist, and CMV causes most cases of retinitis that occur in HIV-infected patients whose HIV viremia is not durably suppressed and who have $CD4^+$ T-cell counts less than 100/mm^3.

If effective treatment is not initiated, bilateral disease occurs in most cases, and blindness can occur.[231,232] Therefore therapy is urgent when

disease is recognized unless the lesions are small and peripheral in patients not receiving ART, in which case anti-CMV therapy could possibly be withheld while ART is initiated if the patient is closely monitored.

A variety of therapeutic approaches have been used employing intravenous ganciclovir, intravenous foscarnet, intravenous cidofovir, oral valganciclovir, local injections of antiviral drugs, and sustained-release ganciclovir implants (see Table 129.2).[8,233–241] Many strategies have relied on sustained-release ganciclovir implants, but these are no longer marketed. Unless these implants again become available, alternative therapeutic strategies are necessary, although published studies of such alternative approaches are sparse.

For extensive retinitis or vision-threatening retinitis, many ophthalmologists use a strategy of immediate intraocular ganciclovir injections followed promptly by intravenous ganciclovir, followed by oral valganciclovir. For small peripheral lesions, oral valganciclovir alone is a reasonable option, especially if ART is being initiated. These patients must be followed closely by an ophthalmologist.

Intravenous ganciclovir and intravenous foscarnet are equally effective in terms of inducing remission of retinitis. Intravenous cidofovir is also effective.[8,237–239] Ganciclovir is usually the intravenous drug of choice because of its more favorable toxicity profile compared with foscarnet or cidofovir. New lesions or progressive disease may be identified during the first 7 days of therapy: These do not necessarily imply a poor response. Considerable improvement in inflammation, edema, and hemorrhage will be recognized in responders before the end of 21 days of therapy. Serial PCR monitoring of CMV viremia may be a plausible method to monitor therapy but is not usually recommended, and it has not been shown to be useful (see Chapter 137).

Maintenance regimens using intravenous ganciclovir or intravenous foscarnet were more commonly administered in an earlier era before effective ART, but without ART these regimens only prolonged the interval until relapse by several weeks. The mean time to progression for patients receiving ganciclovir or foscarnet maintenance regimens without effective ART is 50 to 59 days.[242]

Oral valganciclovir is an attractive alternative to intravenous therapy for maintenance regimens and for initial regimens in some patients. Oral valganciclovir has pharmacokinetics similar, but not identical, to intravenous ganciclovir. Its oral bioavailability is predictable for patients with normal gastrointestinal function, but the oral route is not preferred as the sole initial therapy for patients with severe or vision-threatening disease. Since the advent of oral valganciclovir, oral ganciclovir is no longer available. Other drugs including letermovir are being developed that could have a role in prophylaxis in the near future.[243]

The major toxicity of ganciclovir and valganciclovir is bone marrow suppression with neutropenia and thrombocytopenia. Confusion, nausea, vomiting, aminotransferase elevation, and inhibition of spermatogenesis also occur. Granulocyte colony-stimulating factor may be useful to permit continued ganciclovir or valganciclovir therapy. Foscarnet is nephrotoxic and can cause nausea, vomiting, anorexia, seizures, hypocalcemia, and hypomagnesemia. Foscarnet generally must be infused over 60 minutes, after salt loading with a 60-minute infusion of normal saline, and therefore requires more infusion time than ganciclovir therapy. Cidofovir is not used often but has the advantage that it can be given once every 2 weeks after two consecutive weekly doses, obviating the need for permanent intravenous access. Cidofovir is nephrotoxic and should not be given to patients with serum creatinine >1.5 mg/dL, and each dose should be administered with probenecid after hydration with 1 L of normal saline. The long half-life can be a disadvantage if toxicity occurs.

Esophagitis, colitis, pneumonitis, and encephalitis are life-threatening syndromes caused by CMV that have been documented to respond to therapy.[244–249] A specific diagnosis for these syndromes should be established by histology or cytology because they are indistinguishable from syndromes caused by other pathogens on the basis of clinical criteria alone. Culture of CMV or CMV PCR from tissue, secretions, or excretions is insufficiently specific for CMV-caused disease to be used as a basis for therapy except in the case of neurologic disease. Detection of CMV by culture or, more often, by PCR in CSF is highly suggestive that CMV is the cause of a compatible neurologic syndrome.[250]

For patients with esophagitis, colitis, or rectal ulcers, improvement in clinical symptoms is usually noted during the first week of therapy with valganciclovir, ganciclovir, or foscarnet. Improved performance status and increased weight are often noted, especially if therapy results in reduced dysphagia or reduced diarrhea.

There is considerably less knowledge about ganciclovir therapy for CMV pneumonia because there are so few well-documented cases. No consensus exists regarding the specific criteria for establishing this diagnosis short of lung biopsy, and many patients with no response to ganciclovir therapy had severe and advanced lung damage before ganciclovir treatment was started. Ganciclovir therapy for bone marrow or stem cell transplant recipients is usually given concurrently with immune serum globulin or hyperimmune globulin. However, even though there are no convincing data that immune or hyperimmune globulin enhances efficacy in transplant populations, this strategy is often employed for patients with HIV infection and CMV disease.

Recurrence of CMV retinitis after effective therapy is extremely unusual if the $CD4^+$ T-cell count rises to more than 50 to 100 cells/mm^3 after institution of ART.[8] It is reasonable to stop maintenance therapy if the $CD4^+$ T-cell count has been greater than 50 to 100 cells/mm^3, the lesion is inactive, and regular ophthalmologic follow-up is possible.[8] Clinicians need to distinguish between the recurrence of CMV retinitis and immune reconstitution vitritis because management of immune reconstitution syndromes is quite different from management of CMV retinitis.[251–254]

Drug-resistant CMV isolates are not as common in the era of ART as they were previously.[255–261] Isolates that are resistant to ganciclovir may be susceptible to foscarnet depending on the mutation that occurred.

Because CMV frequently causes specific organ damage in patients with HIV infection and may contribute to the febrile wasting syndromes associated with untreated HIV disease, specific prevention of CMV disease was a logical goal in the era when ART was not highly effective. Strategies based on intervention with intravenous or oral regimens when CMV is detected by a nucleic acid or antigen assay of serum or peripheral white blood cells are logical, but benefit has not been demonstrated over and above what can be achieved with ART. Patients who will not take ART are unlikely to take an anti-CMV agent.

Patients could consider methods to reduce infection or reinfection by practicing safe sex, avoiding infected needles, and receiving only blood products that have been determined to be free of CMV or have been filtered. Child care providers in child care facilities are also at increased risk due to contact with infected secretions. However, ART is the clearly effective intervention.

When oral ganciclovir was the only oral drug available, it was used to prevent primary CMV disease; studies suggested that this drug was effective in the era before effective ART.[262,263] Even then, few clinicians used oral ganciclovir as primary prophylaxis because of the cost, toxicity, and inconvenience of this drug. Valganciclovir has replaced oral ganciclovir as a marketed product because it is more potent. Oral valganciclovir has not been studied extensively for primary prophylaxis. However, there is little role for specific CMV prophylaxis in this era when ART is so effective.

When ART is initiated, CMV IRIS can occur either at a site of CMV disease that had been recognized and treated or at a site that was not clinically recognized to be infected with CMV.[251–254] IRIS associated with CMV is most commonly recognized in the retina and typically occurs in patients with $CD4^+$ counts less than 50 cells/mm^3 at the time ART was started. IRIS may manifest in patients who start ART after CMV was diagnosed, or CMV IRIS may manifest in patients starting ART who never had prior retinal disease, even if screened by careful ophthalmologic examination before ART. Immune reactivation syndromes typically have more inflammation in the anterior or posterior chambers than CMV disease and typically occur in the first 4 to 12 weeks after ART is started. These lesions can be difficult to distinguish from active CMV retinitis. Therapy has not been studied in large clinical trials but usually includes periocular corticosteroids or short courses of systemic corticosteroids. Some clinicians use oral valganciclovir in conjunction with corticosteroid therapy to treat presumed CMV IRIS on the presumption that replicating virus rather than residual antigen alone is present.

Epstein-Barr Virus and Human Herpesvirus 6 and 7

EBV (see Chapter 138) has been implicated in the pathogenesis of oral hairy leukoplakia and primary CNS lymphoma and may have a role in the pathogenesis of some cases of nonspecific pneumonitis, lymphadenopathy, systemic lymphoma, fever, or wasting. There is currently insufficient evidence to warrant specific testing for EBV except for diagnostic CSF testing when CNS lymphoma is being considered. Detection of EBV by qualitative CSF PCR assay in a patient with a CNS mass lesion is suggestive of primary CNS lymphoma but is not unequivocally specific.[187]

Human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) (see Chapter 139) can be isolated from some patients with HIV infection. It is unclear how often these viruses cause clinically important syndromes such as fever, rash, pneumonitis, or cerebritis in this patient population. Encephalitis due to HHV-6 is the best described disease entity in this population. If there is a strong suspicion that HHV-6 could be the cause of a clinical syndrome, ganciclovir or foscarnet therapy (or both) is the preferred choice for therapy based on in vitro studies. Cidofovir also has activity against HHV-6 and HHV-7.

JC Virus Encephalitis (Progressive Multifocal Leukoencephalopathy)

JC virus encephalitis (PML) is a unifocal or multifocal demyelinating process caused by JC virus (see Chapter 144). This disorder produces characteristic white matter lesions and manifests subacutely as cognitive impairment, focal motor deficits, and seizures.[264] Clinically, PML must be distinguished from AIDS dementia complex, CMV encephalitis, and cerebral infarction. Imaging is helpful in identifying these disorders. Detection of JC virus in the CSF by nucleic acid amplification is helpful diagnostically, but some patients with positive JC virus in the CSF have neurologic disease due to some other process.[265–267] Detection of JC virus or antibody outside the CNS is not helpful for clinical management.

Examples of dramatic clinical and radiologic improvement after institution of ART have been reported, although some patients worsen after institution of ART owing to an immune reconstitution syndrome.[268–272] No specific antiviral therapy has been effective.

Candida Species

Mucosal candidiasis is a hallmark of HIV infection. One of the earliest clinical observations in the era before there was a serologic test for HIV infection was that the presence of oral candidiasis was almost diagnostic of AIDS in patients who did not have some other obvious risk factor such as high-dose corticosteroid therapy, pregnancy, recent antibacterial therapy, or diabetes mellitus. Vaginal candidiasis was not so specifically suggestive of HIV infection because there are many reasons for women to develop vaginal candidiasis other than HIV/AIDS (i.e., pregnancy, concurrent antibacterial therapy, corticosteroid therapy).

Stomatitis, esophagitis, vaginitis, and proctitis caused by *Candida albicans* infection are common and often respond to topical therapy (nystatin or clotrimazole), oral therapy (itraconazole, fluconazole, voriconazole, posaconazole), or intravenous therapy (fluconazole, voriconazole, posaconazole, isavuconazole, caspofungin, micafungin, anidulafungin, or amphotericin B). Oral fluconazole is convenient and relatively inexpensive and therefore is preferred unless there is a strong suspicion that the pathogen is resistant to fluconazole. Fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole all inhibit specific hepatic enzymes of the cytochrome P-450 class, resulting in elevated levels of drugs metabolized by those enzymes such as protease inhibitors and NNRTIs.[8]

There is usually no urgent reason to institute antifungal therapy for any of these candidal mucosal disorders other than patient discomfort. Esophagitis in this patient population is rarely associated with bleeding, perforation, fungemia, or disseminated fungal disease, in contrast to the experience with neutropenic patients after antineoplastic chemotherapy.

Stomatitis, esophagitis, and proctitis often recur after specific therapy is discontinued if $CD4^+$ T-cell counts remain low. Chemoprophylaxis is not routinely recommended. However, fluconazole administration may have to be continued for long durations if recurrences are frequent or severe and the $CD4^+$ T-cell counts remain low (see Table 129.2). Patients with no response to oral azole therapy after 1 to 2 weeks usually have $CD4^+$ T-cell counts less than 50 cells/mm^3 or extensive prior exposure to fluconazole that has led to fluconazole resistance (or both). *Candida* isolates that are clinically and microbiologically resistant to fluconazole have been described in patients on long-term azole therapy.[272,273] Resistance to echinocandins has also been described.[274]

If there is no response to a 7-day course of oral fluconazole (200 mg or more every day), higher doses of fluconazole are rarely effective. Other options include itraconazole-cyclodextrin solution, voriconazole, posaconazole, isovuconazole, an echinocandin, or a short course of intravenous liposomal amphotericin B.

Disseminated candidiasis is not characteristic of AIDS. If candidemia occurs, it is usually associated with an intravenous catheter or intravenous substance abuse. Treatment is similar to that in other patient populations, with particular attention directed at removing infected intravenous lines.

Cryptococcus neoformans, Histoplasma capsulatum, and *Coccidioides immitis/Coccidioides posadii*

Meningitis is the most frequent manifestation of cryptococcosis in HIV-infected patients.[275–285] Patients with cryptococcal meningitis usually present with fever, headache, neck stiffness, or photophobia. Most, but not all, have $CD4^+$ T-cell counts less than 50 cells/mm^3. Extraneurologic manifestations such as pneumonia or skin lesions with or without apparent neurologic disease also occur.

In patients with cryptococcal meningitis, CSF typically demonstrates elevated protein levels and elevated numbers of mononuclear cells and decreased glucose concentration. In some patients, one or all of these CSF parameters may be normal. The cryptococcal antigen tests in serum and CSF are almost always positive, and 75% of patients have an elevated opening pressure when a lumbar puncture is performed. Baseline factors predicting a poor therapeutic response in patients with meningitis include altered mental status (e.g., confusion, lethargy, obtundation), CSF antigen titer >1:32, decreased CSF leukocyte count (<20 cells/mm^3), age <35 years, positive blood cultures for *Cryptococcus,* hyponatremia, and positive CNS culture for *Cryptococcus.*

As with other opportunistic infections, early recognition and institution of therapy are important for maximizing the likelihood of a favorable response. Diagnosis is readily established by culture or cryptococcal antigen testing of blood or CSF. Long-term outcomes improve if effective ART is initiated.[286]

The best-studied therapy for cryptococcal meningitis is amphotericin B administered intravenously plus flucytosine given orally until clinical improvement has occurred and at least for 2 weeks; this is followed by fluconazole, 400 mg orally every day for 8 weeks, followed by fluconazole, 200 mg orally every day for life unless $CD4^+$ T-cell counts rise above 100 to 200/mm^3 (see Table 129.2).[8,277–285] Both amphotericin B deoxycholate and liposomal amphotericin have been used (see Table 129.2). Liposomal amphotericin B, in a dose of 3 to 4 mg/kg/daily, is recommended as the preferred amphotericin B formulation for primary induction therapy, based on clinical experience and reduced renal toxicity compared with amphotericin B deoxycholate. Flucytosine, when combined with amphotericin B, accelerates fungal clearance from the CSF and reduces the rate of relapse.[279,281,287] Flucytosine is associated with potential bone marrow suppression and hepatotoxicity. Patients receiving flucytosine should have either blood levels monitored (peak level 2 hours after dose should be between 25 and 100 μg/mL) or close monitoring of complete blood cell counts to identify developing cytopenias.

Fluconazole therapy is used as initial therapy to treat cryptococcal meningitis with a high degree of success in many parts of the world outside the United States, but fluconazole even at doses of 2000 mg/day is not as effective as amphotericin B.[8,281–283] There is no specific reason to use voriconazole, posaconazole, isavuconazole, or itraconazole to treat cryptococcal disease.[284] *Cryptococcus* rarely becomes clinically resistant to amphotericin B or fluconazole. Susceptibility testing is available only as a research procedure.

The clinical status of patients with cryptococcal disease may deteriorate because of the consequences of increased ICP that result from cryptococcal disease independent of any effects of ART or IRIS.[288,289] Clinical signs of increased ICP need to be sought, especially at presentation and during acute induction therapy. The baseline opening pressure

should be measured at the time of the initial lumbar puncture in patients with focal neurologic findings or altered mental status. An imaging study of the CNS may be useful before the initial lumbar puncture and subsequently if the clinical status deteriorates. If the opening pressure is high (>25 cm H_2O), consideration should be given to reducing the pressure by repeated lumbar punctures or by insertion of a CSF drain or shunt. One approach used by experienced clinicians is to remove the volume of CSF that reduces opening pressure by 50% as often as needed by assessing clinical parameters and to resort to a shunt if symptoms of increased pressure continue to recur after many lumbar punctures. Corticosteroids, mannitol, and acetazolamide are not recommended.[8,290]

Monitoring titers of cryptococcal polysaccharide antigen in serum or CSF is of no value in determining response to therapy and is not recommended.[8,283] If new symptoms or clinical findings subsequently develop, a repeat lumbar puncture, with measurement of opening lumbar pressure and CSF culture, should be performed. Many experts would repeat the CSF culture after 2 weeks. Because a positive culture after 2 weeks of therapy predicts relapse, these experts would consider a positive culture at this time point to be an indication to extend the duration of induction therapy.

After at least 2 weeks of successful induction therapy (i.e., substantial clinical improvement and perhaps a negative CSF culture after repeat lumbar puncture), therapy with amphotericin B and flucytosine can be discontinued, and consolidation therapy can be initiated with fluconazole 400 mg daily. This therapy should continue for at least 8 weeks. Subsequently, fluconazole should be reduced to 200 mg daily and continued as long-term maintenance therapy to complete at least 1 year of azole therapy.

Maintenance fluconazole therapy can be stopped for patients who are asymptomatic, have completed 6 months of therapy, and have had a sustained increase in $CD4^+$ T-cell counts with ART to more than 100 to 200 cells/mm^3 for at least 6 months (see Table 129.3).[8] Some clinicians would repeat the lumbar puncture before stopping therapy to assess the antigen titer and the presence of viable organisms, but there is no evidence that this is necessary.

About 30% of patients with cryptococcal meningitis develop IRIS when ART is initiated.[8,291–300] IRIS can be a life-threatening complication of starting ART for patients with cryptococcal meningitis, with substantial headache, fever, photophobia, lymphadenopathy, pneumonitis or progressive obtundation, herniation, and death. The manifestations of IRIS are difficult to distinguish from treatment failure. Initiating ART at 5 weeks after diagnosis, as compared with 1 to 2 weeks after diagnosis, was shown to increase survival, although the incidence of IRIS was not significantly different between the two groups.[293]

A randomized study examined adjunctive therapy with dexamethasone in patients with cryptococcal meningitis in Asia and Africa and found that it did not reduce mortality and was associated with more adverse effects and disabilities.[297] Based on limited data in North America and on expert opinion, the optimal ART strategy is to delay initiation of ART at least until after completion of antifungal induction therapy (the first 2 weeks) and possibly until the total induction/consolidation phase (10 weeks) has been completed. Delay in ART may be particularly important for patients with increased ICP. Thus the timing of ART administration should be considered between 2 and 10 weeks after the start of antifungal therapy; the precise starting and subsequent dates of therapy should be monitored carefully for complications caused by IRIS such as elevated ICP.[8]

For nonmeningeal cryptococcosis, IRIS is much less common. ART can probably be initiated within 2 to 4 weeks of starting anticryptococcal therapy. Non-CNS, extrapulmonary cryptococcosis and diffuse pulmonary disease should be treated similarly to CNS disease. HIV-infected patients with apparent nonmeningeal disease should always have lumbar puncture to determine if subclinical meningeal disease is present. For mild-to-moderate symptoms and focal pulmonary infiltrates, treatment with fluconazole (400 mg daily for 12 months) combined with effective ART is recommended. Treatment is identical for patients with an isolated positive serum cryptococcal antigen test.[300]

Histoplasmosis is a well-recognized complication of HIV infection, often occurring as the AIDS-defining disease in patients with $CD4^+$ T-cell counts less than 150 cells/mm^3, especially in certain geographic areas such as the Mississippi and Ohio River valleys of the United States, Puerto Rico, and much of Latin America.[301] Patients with low $CD4^+$ T-cell counts, particularly patients with $CD4^+$ counts less than 150 cells/mm^3, are likely to present with extrapulmonary manifestations such as fever, meningitis, abdominal pain, diarrhea, or shock. Patients with higher $CD4^+$ counts (i.e., >300 cells/mm^3) may present with disease confined to the lungs. Diagnosis is characteristically established by direct microscopy or culture (bronchoalveolar lavage, bone marrow, or blood) or by antigen detection (urine, blood, or bronchoalveolar lavage). Although a sensitive and specific assay is available only from specialized laboratories, the sensitivity of the test most commonly used is high in urine (95%), serum (85%), bronchoalveolar lavage (70%), and CSF (50%).[301–305] *Histoplasma* meningitis can be especially difficult to document and must sometimes be treated presumptively in patients with disseminated disease.

Acute therapy for moderate or severe nonmeningeal disease should consist of intravenous liposomal amphotericin B for at least 14 days for most patients.[8,306–310] Meningeal disease requires a longer course of amphotericin B (i.e., at least 4–6 weeks). For patients with mild, nonmeningeal disease, itraconazole is also effective (see Table 129.2). Serum levels of itraconazole should be monitored. Serum and urine *Histoplasma capsulatum* polysaccharide antigens are useful for monitoring both the initial and the long-term phases of treatment. These tests predict treatment failure or relapse.[8,310] As with most HIV-associated opportunistic infections, patients with acute disease need lifelong maintenance therapy with itraconazole to prevent relapses unless they receive effective ART.[311]

Coccidioidomycosis can cause especially severe disease in patients with HIV infection, particularly patients with $CD4^+$ cell counts less than 250 cells/mm^3 and who have traveled to or resided in the southwestern United States and parts of Central or South America (see Chapter 265). Patients can develop focal or diffuse pneumonia, meningitis, lymphadenitis, hepatitis, or skin disease, or they can be asymptomatic with a positive serology.[312] The diagnosis is difficult to establish: direct microscopy; culture; and assays for IgG, IgM, complement-fixing antibody, antigen, and nucleic acid have been used.[313]

For patients with diffuse pulmonary infiltrates due to *Coccidioides* and $CD4^+$ T-cell counts less than 50 cells/mm^3, therapy should consist of the amphotericin B deoxycholate or the liposomal form of amphotericin B.[8] If meningeal involvement is present, therapy should consist of fluconazole or, alternatively, itraconazole; some refractory cases may require systemic and intrathecal amphotericin B twice weekly; some of these patients will require a CSF shunt. Focal coccidioidal pneumonia or other mild disease in patients with $CD4^+$ T-cell counts more than 50 cells/mm^3 and relatively mild disease can probably be treated adequately with fluconazole alone, 400 mg orally every day, or itraconazole.

Primary prophylaxis for *Coccidioides* is recommended by many experts based on a new positive IgG or IgM serology plus a $CD4^+$ T-cell count less than 250 cells/mm^3.[8] These experts would test seronegative individuals yearly if the patients reside in and travel to endemic areas.

After acute disseminated or meningeal disease due to *Coccidioides*, fluconazole or itraconazole maintenance therapy should probably be continued lifelong regardless of $CD4^+$ T-cell count, which differs from recommendations for all other HIV-related opportunistic infections; that is, for other infections, maintenance therapy can be stopped when reconstitution goals are met.[8]

Bacterial Pneumonia

Patients with HIV infection have a higher incidence of respiratory infections than patients without HIV infection (see Chapter 123).[314–319] The frequency of these infections is inversely related to the $CD4^+$ T-cell count. These respiratory infections include upper tract (sinusitis, otitis, and bronchitis) and lower tract (pneumonia, empyema) disease. *S. pneumoniae* is the most commonly identified bacterial cause. Infection due to encapsulated *Haemophilus influenzae* is not common among adults in North America. *Pseudomonas* spp. and *S. aureus* pneumonia have been reported to be frequent causative agents for respiratory disease.

Diagnosis and therapy do not differ from the approaches established for patients without HIV infection. Patients with HIV infection and

low CD4+ T-cell counts develop more severe pneumococcal disease than non–HIV-infected patients, as characterized by bacteremia and focal complications such as effusions and empyemas. Patients with higher CD4+ T-cell counts appear to have disease that is comparable in severity to patients without HIV infection.[320]

The frequency of bacterial infections is reduced by prophylactic or therapeutic regimens prescribed for other indications such as TMP-SMZ (e.g., for PCP) and azithromycin or clarithromycin (e.g., for MAC). Granulocyte colony-stimulating factor can reduce the frequency of bacterial infections in severely neutropenic (non–HIV-infected) patients with cancer, and it has been used for neutropenic patients with HIV infection. Because the frequency of pneumococcal disease can be reduced by the administration of pneumococcal vaccine to patients with CD4+ T-cell counts greater than 200 cells/mm^3, immunization with both the conjugate and the polysaccharide vaccines should be given following published guidelines (see Chapter 316).[8] Immunization should probably be repeated if the initial vaccine was administered when the CD4+ count was less than 200 cells/mm^3 and later increased to more than 200 cells/mm^3 due to ART. HIV-infected patients also should receive annual influenza immunization with the inactivated vaccine. Reducing patient exposure by immunizing family members for pneumococcus, hemophilus, and influenza is also indicated.

Mycobacterium Species Infection

Mycobacterium tuberculosis

TB is the leading cause of morbidity and mortality among people living with HIV worldwide, with 1.2 million new HIV-infected persons reported with TB and 390,000 deaths in 2015.[321] However, TB is uncommon among US-born patients who have not had exposure to known cases and who have not spent time in high-risk environments such as correctional facilities, shelters, drug treatment centers, or TB endemic areas outside the United States.[322]

Rates of TB in the United States are declining, with 2.9 new cases of TB disease per 100,000 population (9287 cases) reported in 2016, a decline of 2.7% from 2015. The incidence of HIV-related TB has declined more rapidly than the rate of active TB in the general population in part due to the widespread use of ART.

TB is a special risk in patients with HIV infection because the conversion rate from latent disease to active disease is 35 to 162 per 1000 person-years of observation for patients with HIV, as opposed to 12.9 per 1000 person-years for the general population.[8,323] TB can occur at any CD4+ T-cell count, although the risk increases with progressive immunodeficiency. ART results in a prompt and marked decrease in the incidence of TB, and this effect has been documented in settings with low and high case rates. Even when patients develop sustained viral suppression and a CD4+ T-cell count in "normal" ranges, however, HIV-infected patients remain at higher risk for developing active TB than the general population.

All persons with HIV infection should be tested for TB with either a PPD or an interferon-γ release assay (IGRA). A patient should not be assessed with both tests because of uncertainty related to interpretation of discordant results. The test should be repeated if it was negative and the CD4+ T-cell count increases above 200 cells/mm^3 from a level below that. If the patient is exposed to a known case of TB or has a high risk for exposure, the screening test should be repeated.

Latent tuberculous infection is defined by a positive IGRA or the presence of a positive tuberculin skin test (≥5 mm of induration at 48–72 hours in HIV-infected persons with no clinical or radiographic evidence of TB disease). IGRAs have higher specificity (92%–97% vs. 56%–95%), better correlation with surrogate measures of exposure to *M. tuberculosis,* and less cross-reactivity with bacille Calmette-Guérin vaccination and nontuberculous mycobacteria. Three IGRAs are approved by the US Food and Drug Administration and available in the United States. Progressive immunodeficiency reduces the sensitivity of the tuberculin skin test and IGRA.[324–326]

All previously untreated patients with a positive test for latent TB and all patients with close recent contact with active TB should be treated.[8] Treatment of latent TB should be with isoniazid or one of the recommended alternatives once the presence of active disease has been ruled out (see Table 129.1).

The manifestations of active TB among patients with HIV/AIDS depend on host immune status. For patients who have CD4+ T-cell counts greater than 350 cells/mm^3, manifestations of pulmonary disease are not substantially different from manifestations in the general population. For patients with lower CD4+ counts, lower lobe pulmonary disease, miliary disease, cavitation, effusions, adenopathy, and extrapulmonary disease are more common than in patients with higher CD4+ counts or non–HIV-infected patients.[327]

The initial evaluation of a patient suspected to have any form of HIV-related TB should always include a chest radiograph and perhaps a computed tomography scan even in the absence of pulmonary symptoms or signs. For patients with HIV infection, however, chest radiography is not a sensitive screen for pulmonary TB. Patients with low CD4+ T-cell counts can have TB culture-positive sputum despite normal chest radiographs. Sputum smear and culture are often useful in symptomatic patients being evaluated for possible TB disease who have a normal chest radiograph and patients who appear to have only extrapulmonary disease.[328]

As with non–HIV-infected patients, TB can be diagnosed by smear, culture, or nucleic acid probe of a respiratory sample or some other tissue or fluid.[8,328–330] The use of a nucleic acid amplification test is recommended on at least one specimen from all patients being evaluated for suspected pulmonary TB because this test has excellent sensitivity and specificity and can distinguish TB from nontuberculous mycobacteria.[330] Because this test is rapid, it can also guide decisions about isolation precautions. HIV infection does not alter the sensitivity of sputum culture, but patients with advanced immunodeficiency often have negative sputum smears.[8,329–332]

Because standard mycobacterial cultures for TB may take weeks to months to grow, rapid diagnosis is preferred in patients with HIV infection given the risk of rapid clinical progression and the risks of transmission to other highly susceptible individuals. Nucleic acid amplification tests provide rapid diagnosis of TB and some may also provide resistance testing for rifampin and isoniazid. These tests are useful in patients with positive and negative smears and can distinguish *M. tuberculosis* from other mycobacteria. These tests are more sensitive than AFB smear, being positive in 50% to 80% of smear-negative, culture-positive specimens with sensitivity approaching 90% if three specimens are assessed.[329,332] Nucleic acid amplification tests can also be used on extrapulmonary specimens with the caveat that the sensitivity is often lower than in sputum specimens.

Treatment of suspected TB for HIV-infected individuals is the same as for non–HIV-infected persons.[333–335] Regimens should include an initial four-drug combination of isoniazid, rifampin, ethambutol, and pyrazinamide. If rapid drug susceptibility testing indicates resistance to rifampin, an initial multidrug-resistant TB regimen (usually including a fluoroquinolone and either an aminoglycoside or capreomycin) should be used and later adjusted once complete susceptibility results are available. All HIV-infected patients should ideally receive directly observed therapy. Drug-susceptible TB should be treated with a 2-month intensive phase of the four drugs. Ethambutol can be discontinued when susceptibility to isoniazid and rifampin has been confirmed. Isoniazid and a rifamycin are used in the continuation phase of therapy, generally recommended as an additional 4 months of treatment for uncomplicated TB. Daily regimens (5–7 days per week) are preferred over intermittent regimens (two or three times per week), which perform well in non–HIV-infected persons. For HIV-infected patients, twice-weekly or thrice-weekly dosing during the intensive phase has been associated with an increased risk of treatment failure or relapse that includes acquired drug resistance to the rifamycin class.

For HIV-infected patients, regimens that include once-weekly or twice-weekly dosing during the continuation phase of therapy have also been associated with increased risks of treatment failure or relapse with acquired rifamycin resistance. Therefore daily (5–7 days per week) dosing is also recommended during the continuation phase of therapy.

The outcomes of 6-month regimens (2 months of isoniazid, rifampin, ethambutol, and pyrazinamide followed by 4 months of isoniazid and rifampin) given as directly observed therapy to patients with HIV coinfection have been comparable to outcomes in non–HIV-infected populations. Some experts prefer 9- to 12-month regimens, but there

is no compelling evidence that such longer regimens produce better results in settings where ART is available.[333–335]

Courses longer than 6 months (e.g., 9 months, 12 months, 18 months, or longer) are indicated in certain patients with high burdens of disease, slow therapeutic response, or involvement of the meninges or bone. A 6- to 8-week course of corticosteroids is indicated for patients with severe pericardial or meningeal disease.[336,337] For ART-naïve patients, ART should be started within 2 weeks after TB treatment initiation when the $CD4^+$ T-cell count is <50 cells/mm^3 and within 8 weeks of starting anti-TB treatment in patients with higher $CD4^+$ T-cell counts.[8,338–342]

IRIS is frequently associated with initiation of TB therapy in patients with HIV infection.[338–342] IRIS may manifest as clinical exacerbation at sites previously known to be involved by active disease or at sites that had been clinically silent until enhanced immunity caused clinical manifestations in response to viable or nonviable organisms. This is particularly relevant to patients with TB meningitis and pericarditis. In patients with TB meningitis and low $CD4^+$ T-cell counts, early ART may exacerbate meningeal inflammation leading to mild, moderate, or severe disease, and thus the timing of ART initiation must be based on clinical judgment.

When TB occurs in patients already on ART, treatment for TB must be started immediately. ART should be modified to reduce the risk for drug interactions and to maintain virologic suppression. Drug interactions between antituberculous drugs (especially rifamycins) and antiretroviral drugs (especially protease inhibitors and NNRTIs) need to be carefully considered and appropriate adjustments made.[8]

Patients with pulmonary TB should have monthly sputum smears and cultures to document culture conversion on therapy, defined as two consecutive negative cultures. Patients with susceptible TB should convert sputum cultures to negative by 2 months of TB therapy, although patients with advanced disease (i.e., cavitary TB) may take longer to convert sputum cultures to negative. Patients who have not had sputum culture conversion at 4 months should have sputum sent for resistance testing.

Relapse of TB is uncommon if patients have strains susceptible to all major antituberculous drugs and if they complete the recommended course of therapy. Some apparent relapses are reinfections with new, different strains of TB.[60–62]

Mycobacterium avium Complex

MAC disease was a characteristic complication of AIDS in North America for the first 25 years of the HIV epidemic. MAC has been much less common since the widespread use of ART.[1,2,8,343] MAC most often manifests as a systemic process characterized by fever, weight loss, elevated serum alkaline phosphatase levels, and substantial anemia.[343–345] Wasting, diarrhea, or lymphadenopathy may be seen. Diagnosis is most readily established by blood culture or by biopsy of affected tissue. Culture of organisms from respiratory secretions, stool, or urine does not unequivocally establish the presence of invasive disease or the need for therapy. Patients colonized with MAC do not necessarily develop disease due to MAC, although they are more likely to develop such disease than noncolonized persons with HIV infection. Therefore a positive culture of sputum, stool, or urine is not necessarily an indication for therapy.

Initial treatment regimens usually include either clarithromycin or azithromycin plus ethambutol (see Table 129.2). Testing of isolates for clarithromycin or azithromycin resistance is recommended for all clinically significant isolates, although the occurrence of such primary resistance is unusual.[8] Because there is more experience with clarithromycin than with azithromycin, the former drug is usually preferred.[8] If clarithromycin or azithromycin is used as monotherapy, relapse occurs in most patients in less than 1 year. Therefore clarithromycin plus ethambutol is the recommended regimen unless there is reason to suspect that the isolate is macrolide-resistant.[8,344,345]

Additional drugs such as rifabutin, amikacin, ciprofloxacin, moxifloxacin, or linezolid may be added if the disease is severe or extensive, if the isolate is drug-resistant, or if the patient is extremely immunosuppressed. Some experts advocate a three-drug regimen of clarithromycin, ethambutol, and rifabutin or a quinolone or an aminoglycoside.

Most patients with drug-susceptible isolates demonstrate defervescence and lower quantities of circulating MAC within 2 to 4 weeks after initiation of therapy, unless the disease is extensive, drug adherence is poor, or absorption or pharmacokinetic issues result in suboptimal serum tissue concentrations of drug. If patients do not respond within the first 1 to 2 months of therapy, MAC susceptibility testing should be repeated, and serum drug-level monitoring should be considered.

If MAC bacteremia recurs after a period of clinical and mycobacterial improvement, organisms have often become resistant to the drugs used in the treatment regimen. It is logical to choose a new regimen based on susceptibility results, although, as noted, such testing has not been validated as clinically useful for most drugs. A multidrug regimen is typically recommended, although there are no adequately powered trials to provide evidence-based guidance. Whether the macrolide should be continued despite clinical or in vitro resistance is controversial.

A repeat blood culture during therapy is necessary only if patients have not responded clinically after 4 to 8 weeks. Therapy should be continued for a minimum of 12 months based on trials done before the era of effective ART. Therapy can be discontinued after 12 months in patients who are asymptomatic and have had an ART-induced increase in $CD4^+$ T-cell counts to greater than 100 cells/mm^3 for at least 6 months.[8]

For patients with disseminated MAC who are ART-naïve, many clinicians would not initiate ART for the first 2 to 4 weeks of anti-MAC therapy to reduce the likelihood of IRIS and simplify assessment of management strategy if adverse events occur.[8] Most MAC disease seen in the United States currently is not due to disseminated disease with bacteremia, but is rather due to IRIS syndromes after ART initiation. MAC is probably the most common cause of lymphadenopathy following ART initiation. The enlargement of lymph nodes leads to local symptoms and physical findings. Biopsy of affected nodes or organs is usually necessary to be certain that the pathology is not due to active fungal disease, TB, or a malignant neoplasm such as lymphoma. Patients with IRIS due to MAC are not usually bacteremic, although biopsy of the affected node or organ usually demonstrates MAC by histology or culture or both. There is no consensus about whether such patients need to be treated for MAC rather than continuing ART alone, but most clinicians do treat MAC for several months with one of the above-described regimens.

Chemoprophylaxis for MAC is no longer recommended because all patients should be on ART promptly, which has greatly reduced the incidence of MAC. Chemoprophylaxis for MAC disease in previous years had been a logical management strategy in the United States because this disease was frequent, severe, and difficult to treat in the long term and because there are parameters for identifying patients who are at highest risk.[36,37,346,347] Patients at risk include patients with a $CD4^+$ T-cell count less than 50 cells/mm^3 and patients with a respiratory or gastrointestinal tract that is colonized with MAC. Clarithromycin, azithromycin, and rifabutin are effective chemoprophylactic agents in terms of reducing the incidence of disease and reducing mortality.

Enteric Pathogens: *Salmonella, Shigella,* and *Campylobacter* Species and *Clostridiodes difficile* (formerly *Clostridium difficile*)

Rates of bacterial enteric infections are at least 10-fold higher among HIV-infected adults not treated with ART compared with the general population.[69,348–350] These rates decline when patients are administered ART. The risk of bacterial diarrhea varies inversely with the $CD4^+$ T-cell count and is especially high among patients with $CD4^+$ counts <200 cells/mm^3. As with non–HIV-infected persons, the most common isolates are *Salmonella, Shigella,* and *Campylobacter*. *Clostridiodes difficile* (formerly *Clostridium difficile*) infection is also common among HIV-infected patients; a low $CD4^+$ T-cell count is an independent risk factor for *C. difficile* disease.

The most likely source for most enteric infections in HIV-infected patients is ingestion of contaminated food or water. However, sexual activity is well documented to have the potential for direct or indirect fecal-oral exposure, thus increasing the risk of infections, especially with *Shigella* and *Campylobacter*.[348–350]

For *Salmonella, Shigella,* and *Campylobacter,* patients with HIV infection present with acute, self-limiting gastroenteritis, diarrhea, or bacteremia with or without extraintestinal involvement. Initial therapy

is similar to therapy for non–HIV-infected persons. Patients with HIV infection, especially patients with low $CD4^+$ T-cell counts, may have more persistent diarrhea, more severe diarrhea with bloody stools, and more bacteremia. Whereas 7- to 14-day courses of therapy may be sufficient for patients with mild-to-moderate disease, other patients may require longer courses of therapy. Although there are no well-defined guidelines, 2- to 6-week courses of therapy are used by many experts.

Relapses after therapy are common with *Salmonella* spp. and probably other enteric bacterial infections as well.[348–350] For such patients, prolonged long-term antibacterial suppressive therapy may be appropriate until the $CD4^+$ T-cell count increases to greater than 200 cells/mm^3.

Patients with HIV infection are predisposed to enteric infections with non-*jejuni,* non-*coli Campylobacter* spp. such as *Campylobacter fetus* as well as *Helicobacter* spp. such as *Helicobacter cinaedi* and *Helicobacter fennelliae.* These organisms require special techniques to be identified and are more likely to be reported from blood rather than from stool.

HIV-infected patients with gastrointestinal infections require no different approaches to diagnosis or initial therapy than non–HIV-infected persons. Given their enhanced susceptibility to enteric pathogens, HIV-infected patients should be particularly careful about exposures to high-risk foods (e.g., raw eggs, undercooked seafood or poultry) or high-risk pets (e.g., stray animals or dogs or cats younger than 6 months) or to pathogen transmission involved with exposure to infants and children (e.g., daycare).[8]

Cryptosporidium, Cystoisospora, Cyclospora, and *Microsporida* Species

Cryptosporidium, Cystoisospora, Cyclospora, and *Microsporida* spp. all cause chronic diarrheal syndromes in HIV-infected patients with low $CD4^+$ T-cell counts.[351–354] Each can cause biliary and pancreatic disease by infecting the biliary-pancreatic ducts as well. Some *Microsporida* spp. (see Chapter 282) can also cause systemic disease, and certain species can produce keratitis. These pathogens can be identified in stool by a variety of tests depending on the specific pathogen including direct microscopic techniques, antigen tests, nucleic acid amplication tests, and tissue biopsy. For corneal disease, microsporidia can be identified by corneal scrapings.

The results of therapy for cryptosporidiosis (see Chapter 282) have been disappointing. No pharmacologic or immunologic therapy directed specifically against *Cryptosporidium* has been shown to be consistently effective in patients with HIV infection when used without ART. Some clinicians use paromomycin therapy, but clinical trials have not shown benefit.[355] Nitazoxanide may be effective in some patients.[356] For cryptosporidiosis and microsporidiosis, the use of antimotility drugs, combined with enhancing immune function with ART, is currently the best option.[8,357]

Prevention of cryptosporidiosis should focus on environmental control because no drugs are known to be effective for chemoprophylaxis.[8] In some areas where outbreaks have been linked to the local drinking water supply or where contamination of water is likely, water for drinking should be boiled for 1 minute or filtered through a device capable of removing particles as small as 1 mm in diameter. Individuals at risk should also avoid contact with farm animals, infected humans, or pets that are at high risk (e.g., dogs and cats younger than 6 months, stray animals, animals with diarrhea).[8]

Cystoisospora and *Cyclospora* infections (see Chapter 283) can be treated effectively with TMP-SMZ.[358] Patients usually respond symptomatically within 7 to 10 days, but the relapse rate is high unless TMP-SMZ is continued lifelong for patients whose $CD4^+$ T-cell counts remain low. If patients respond to ART with sustained increases in $CD4^+$ counts and sustained suppression of HIV viral loads, it is logical to presume that long-term maintenance therapy for isosporiasis or cyclosporiasis can be discontinued. Ciprofloxacin is also active against *Cyclospora.*[358]

Albendazole has activity in vitro and in vivo against some microsporidia. Albendazole can have a beneficial effect on diarrhea caused by *Encephalitozoon intestinalis,* disseminated disease caused by *Encephalitozoon hellem* or *Encephalitozoon cuniculi,* and disease caused by *Nosema* spp. or *Trachipleistophora* spp.[359,360] Albendazole has not been as effective in treating the major cause of microsporidial diarrhea, which is *Encephalitozoon bieneusi.* Some patients with *E. bieneusi* diarrhea experience transient symptomatic improvement after albendazole therapy, but there is little evidence for microbiologic improvement or improvement in absorption, and relapse often occurs promptly after drug therapy is discontinued.

Microsporidial keratitis has been treated successfully with prolonged topical administration of fumagillin.[361] Patients with microsporidial keratitis should be assessed for systemic microsporidiosis, and systemic therapy with albendazole should be considered. Because little is known about the transmission of microsporidia to humans, no specific recommendations for prevention can be made. It is logical to presume that prevention of food and water contamination by animals would decrease transmission of this organism.

Treponema pallidum

Syphilis is often recognized in HIV-infected patients either because of characteristic lesions of primary or secondary disease or because a screening serology result for *Treponema pallidum* is positive. There is currently a resurgence of syphilis in HIV-infected patients in many areas. The clinical manifestations of syphilis as well as its diagnosis and treatment do not differ dramatically in HIV-infected patients and non–HIV-infected patients (see Chapter 237).[362–365] Reports of ocular syphilis among patients with HIV infection have led to speculation about overrepresentation of this syndrome among HIV-infected patients.[366,367]

Syphilis can have diverse manifestations of primary, secondary, and tertiary disease. *T. pallidum* should enter into the differential diagnosis of dermatologic, ocular, and neurologic syndromes.

Neurosyphilis in patients with HIV infection presents special challenges. Neurosyphilis can occur at any stage of syphilis. Manifestations of neurosyphilis in patients with HIV infection are similar to manifestations in patients who do not have HIV infection. However, clinical manifestations of neurosyphilis, such as concomitant uveitis or meningitis, may be more common in patients with HIV infection.[368–370]

No single laboratory test can be used to establish a definitive diagnosis. The diagnosis of neurosyphilis depends on a combination of CSF tests (CSF cell count or protein and CSF Venereal Disease Research Laboratory) in the setting of reactive serologic test results and neurologic signs and symptoms. CSF abnormalities are common in patients with early-stage syphilis and are of unknown significance in the absence of neurologic signs or symptoms.

Patients with HIV infection with neurosyphilis (or ocular or otic syphilis) should receive intravenous aqueous crystalline penicillin G or procaine penicillin for 10 to 14 days.[8] Following therapy for syphilis, retreatment should be considered for patients with early-stage syphilis who have persistent or recurring clinical signs or symptoms of disease or a sustained fourfold increase in serum nontreponemal titers after an initial fourfold decrease following treatment. True treatment failure is probably an unusual event for any stage of syphilis.

Bartonella Species

Bartonella henselae, the cause of cat-scratch disease, and *Bartonella quintana,* the cause of trench fever (see Chapter 234), cause a spectrum of almost unique clinical syndromes in patients with HIV infection.[371–373] *B. henselae,* associated with cat exposure and also transmitted by fleas, causes disease primarily in patients with $CD4^+$ T-cell counts less than 50 cells/mm^3. Manifestations may be acute or very indolent and include cutaneous and subcutaneous angiomatous papules, which can be confused with Kaposi sarcoma, and angiomatous masses in the liver (peliosis hepatis), lymph nodes, lungs, and CNS, which can manifest as mass lesions. Bartonellosis can also manifest as fever alone.

B. quintana, transmitted by body lice, causes fever, cutaneous lesions, bone lesions, and endocarditis. *B. quintana* is epidemiologically very different from *B. henselae* because it is associated with lice and homelessness. This organism rarely, if ever, causes peliosis hepatis.

The diagnosis of bartonellosis is usually established by Warthin-Starry stain of tissue, by culture of blood or tissue using enriched agar, or by PCR assay of serum.[8] Antibody titers can be useful if patients seroconvert, although antibody synthesis may be deficient in patients with low $CD4^+$ T-cell counts, and elevated titers may not be seen for many weeks or months after the acute event.

Most experience with therapy has been with either erythromycin or doxycycline, although azithromycin is frequently substituted for erythromycin. At least 2 g daily of erythromycin base should be given for at least 12 weeks for patients with cutaneous disease.[8] Doxycycline with or without rifampin is preferred for CNS disease and severe manifestations and should be given at a dose of 100 mg every 12 hours. Clinical response may be seen during the first week. Cutaneous lesions may require 1 to 2 months to resolve; hepatic lesions may require 2 to 3 months. Hepatic and osseous lesions should be treated initially with intravenous erythromycin. Rifampin may be useful as an adjunct to the primary drug. A Jarisch-Herxheimer reaction can be seen in response to the first few drug doses. Treatment failures and relapses occur.

Prevention of bartonellosis should focus on reducing exposure to the vectors—the body louse (for *B. quintana*) and cats (especially young cats and stray cats) and cat fleas (for *B. henselae*).

Kaposi Sarcoma and Human Herpesvirus 8

Seroprevalence of HHV-8 is 1% to 5% in the general population but higher in certain geographic areas and among men who have sex with men (13%–35%).[374–377] Most patients with chronic HHV-8 infection are asymptomatic. HHV-8 is associated with Kaposi sarcoma as well as less common neoplastic processes including primary effusion cell lymphoma, Kaposi sarcoma herpesvirus inflammatory cytokine syndrome, and multicentric Castleman disease (see Chapter 140).

Seropositive patients with HHV-8 viremia have a markedly increased likelihood of developing Kaposi sarcoma.[376,377] Serologic testing for antibody to HHV-8 is not routinely performed. A PCR assay to quantitate circulating HHV-8 in peripheral blood is useful primarily for diagnosis and management of patients with multicentric Castleman disease.

Kaposi sarcoma and primary effusion cell lymphoma occur most commonly in patients with $CD4^+$ T-cell counts less than 200 cells/mm^3, whereas multicentric Castleman disease can occur at any $CD4^+$ T-lymphocyte count. Since the introduction of ART, Kaposi sarcoma has become less frequent in the United States and Western Europe.[27,28]

HHV-8 is inhibited by ganciclovir, foscarnet, and cidofovir in vitro. Ganciclovir may have a role in treating multicentric Castleman disease but has no role in treating other HHV-8–associated malignancies.[378–381]

The presence of Kaposi sarcoma does not necessarily mandate institution of specific therapy, especially if lesions are few and inconspicuous. Lesions may regress in patients who respond to ART. Local measures can be useful, including excision, irradiation, and intralesional injection with chemotherapy. Kaposi sarcoma can cause life-threatening disease by obstructing a vital structure such as the larynx, bronchus, biliary tract, or bowel. Kaposi sarcoma can occasionally infiltrate a vital organ such as the lung and cause fatal hypoxemia. In these life-threatening situations, either radiation therapy or cytotoxic chemotherapy is necessary to produce a rapid and substantial response.

The optimal mode of therapy depends on the location and extent of Kaposi sarcoma. A variety of chemotherapeutic regimens have been used to treat extensive or life-threatening disease with some success. Liposomal doxorubicin and paclitaxel demonstrate similar response rates and progression-free survival, although liposomal doxorubicin exhibits less high-grade toxicity relative to paclitaxel. Therefore paclitaxel is generally preferred as first-line therapy. For multicentric Castleman disease and for Kaposi sarcoma herpesvirus inflammatory cytokine syndrome, there is no standardized therapy. There may be a role for antibody therapy against interleukin-6.[382,383]

Viral Hepatitis

Liver disease is an increasingly important cause of morbidity and mortality in patients with HIV infection.[17,19,384–387] HCV and HBV are commonly recognized causes of liver disease in patients with HIV infection, but alcohol; antiretroviral drugs; and a host of other infectious, neoplastic, and toxic processes can adversely affect the liver (see Chapter 117). All HIV-infected patients should be screened for hepatitis A, B, and C.[8]

The incidence of HCV among HIV-infected patients depends on the specific population being evaluated; because this virus is more efficiently spread by blood exposure than sex, intravenous substance users and recipients of unscreened blood products are more likely to be infected than men who have sex with men. Cirrhosis develops within a median of 20 years of HCV acquisition among HIV-infected persons, although the range of onset is highly variable. The natural history of HCV is clearly accelerated among patients with HIV coinfection. Cirrhosis is more likely to occur in older patients, male patients, alcohol users (>20 to 50 g/day), and patients with decreased $CD4^+$ T-cell counts.[388–392]

All patients with HIV infection should be screened for HCV using immunoassays to detect antibody. If the antibody test is negative and there is high suspicion for HCV infection, testing for HCV RNA should be performed. All coinfected patients should be treated for both HIV and HCV. The goal of therapy of the individual patient is prevention of fibrosis, cirrhosis, hepatocellular carcinoma, and death. Updated guidance for the management of HCV in monoinfected (HCV) and coinfected (HIV/HCV) patients, sponsored by the Infectious Diseases Society of America and the American Association for the Study of Liver Diseases, is available at http://www.hcvguidelines.org.[393] This guidance is updated online because new drug approvals and new studies are appearing rapidly in the current era. Recommended regimens rely on directly acting agents alone. There is no role for interferon therapy and only limited roles for ribavirin. Drug interactions between ART and directly acting agents against HCV must be considered when initiating HCV therapy. The regimens for treating HCV in HIV-infected patients are no different from the regimens in non–HIV-infected patients. The response rates are also similar and are not affected by $CD4^+$ T-cell count. Recommendations for treatment are discussed in detail in Chapters 117, 145, and 154.

HBV is a major cause of HIV-associated liver disease worldwide.[387,394–396] In the United States, however, it is much less common than HCV among HIV-infected patients, occurring in 5 to 10 of HIV-infected patients nationally. HBV is transmitted neonatally as well as by blood and by sex.

Initial testing for patients with HIV infection should include serologic testing for HBsAg, HBc antibody (anti-HBc total), and HBs antibody (anti-HBs). If the patient has acute infection, HBsAg can be detected 4 weeks (range, 1–9 weeks) after exposure, and anti-HBc IgM is usually detectable when the patient is initially symptomatic.

Chronic HBV infection is defined as HBsAg detected on two occasions at least 6 months apart. Patients with chronic HBV infection should be tested for HBV e antigen (HBeAg), antibody to HBeAg (anti-HBe), and HBV DNA. Active disease, which can be HBeAg negative or positive, can be distinguished from inactive disease by the presence of serum HBV DNA and persistent or fluctuating alanine aminotransferase elevation. Patients with past infection that has cleared are HBsAg negative with positive anti-HBs and anti-HBc (see Chapter 145).

Some patients test positive for anti-HBc alone (i.e., negative anti HBs and serum HBV DNA negative). This may represent individuals with resolved infection who were anti-HBs and anti-HBc antibody positive, but whose anti-HBs titers have fallen below the level of assay detection. Alternatively this may signify active infection in the liver. This may also represent a false-positive result. There is no convenient method to detect which of these alternatives is applicable to an individual patient.

Before ART is initiated, all persons who test positive for HBsAg should be tested for HBV DNA. After ART is initiated, the test should be repeated every 3 to 6 months to ensure effective HBV suppression during ART, or patients should be treated with an ART regimen that contains two drugs with anti-HBV activity (e.g., tenofovir and lamivudine or tenofovir and emtricitabine).

Because HBV reactivation has been observed in patients with HBV infection during HCV treatment, patients with HCV/HIV coinfection and active HBV infection (i.e., positive HBsAg test or positive circulating bDNA and perhaps with HBc antibody positivity alone) should receive ART that includes two agents with anti-HBV activity before initiating HCV therapy. HBV reactivation should be considered in patients with current HBV infection who develop elevated liver enzymes during or immediately after HCV therapy.

The treatment of HBV is discussed in Chapters 117 and 145. For patients who are coinfected with HIV and HBV, any ART regimen should include two agents that are active against HBV with two goals: to prevent exacerbation of HBV hepatitis due to initiation of ART (IRIS) and to prevent progression of HBV liver disease.

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130 Chronic Fatigue Syndrome (Systemic Exertion Intolerance Disease)

N. Cary Engleberg

SHORT VIEW SUMMARY

Definition
- As defined in a 1994 Centers for Disease Control and Prevention/National Institutes of Health consensus conference, chronic fatigue syndrome (CFS) is a disorder involving 6 or more months of unexplained, profound fatigue with *at least four* of eight associated symptoms (see Table 130.2).
- A new name, "systemic exertion intolerance disease," was proposed by the Institute of Medicine in 2015, along with somewhat modified diagnostic criteria (see Table 130.3).
- Other names used for this disorder include "postinfectious fatigue," "chronic mononucleosis," "myalgic encephalomyelitis" (United Kingdom/Canada), and "chronic fatigue and immune dysfunction syndrome" (United States).

Epidemiology
- Prevalence is estimated at 0.2% to 1% of the population, depending on the definitions used.
- CFS occurs in women three to seven times more frequently than in men.
- It occurs in all socioeconomic strata and among all races.

Etiology
- CFS may be a sequela of various infectious diseases (e.g., herpesvirus infections, influenza, Q fever, brucellosis, Lyme disease, giardiasis) but is not known to be directly related to any ongoing chronic infection (see Table 130.1).
- Subtle disturbances of immune function are not consistent and not known to be causative of symptoms.
- Hypothalamus-pituitary-adrenal axis or autonomic disturbances may occur, but correcting these subtle anomalies does not improve symptoms of CFS.
- Prior or concurrent history of psychiatric disorders is common.

Diagnosis
- Diagnosis is clinical and usually based on the 1994 consensus definition (see Table 130.2).
- Modified diagnostic criteria have also been proposed by the Institute of Medicine in 2015 (see Table 130.3).
- There is no valid laboratory test to rule in or to rule out CFS.

Therapy
- Pharmacologic therapies are directed at relieving symptoms of pain, sleep disruption, and depression.
- No antimicrobial and immune therapies have been proven to be helpful.
- Nonpharmacologic therapies (cognitive-behavioral therapy and graded exercise therapy) have been repeatedly shown to be helpful in most patients.

Prevention
- There are no known preventive measures.

Chronic fatigue syndrome (CFS) refers to an illness that consists of profound, prolonged fatigue associated with other somatic or neuropsychological symptoms. The diagnosis is based on the patient's subjective report of a compatible symptom cluster in the absence of any medical or psychiatric condition that might account for the complaints. Attempts to ascribe CFS to a single, coherent cause have been fruitless. The available evidence favors the notion that the syndromal definition identifies a heterogeneous population of patients in whom fatigue, pain, cognitive complaints, and viral-like symptoms, such as low-grade fever, sore throat, and tender lymph nodes, are the final common consequences of a variety of different causes. The current, prevailing etiologic hypothesis is that the disorder is a multifactorial condition in which genetic and environmental factors (including infection) interact to produce a disturbed capacity to manage and to control stress, fatigue, and pain.

Other names for this disorder include *chronic fatigue and immune dysfunction syndrome* (or CFIDS) in the United States and *myalgic encephalomyelitis* (or ME) in Great Britain and Canada. Most authorities in the United States preferred the designation *chronic fatigue syndrome* because it is defined by the clinical features of the illness without implication of a specific etiology or characteristic pathology. ME has a much more complex and varied set of diagnostic criteria.[1] However, in recent years combined designations (CFS/ME or ME/CFS) have been used frequently in the literature to accommodate all points of view. A new name, *systemic exertion intolerance disease* (or SEID), has been proposed by the Institute of Medicine (IOM).[2] This new name attempts to capture the central characteristics as well as its severity and complexity with a much simplified diagnostic rubric. *Postviral fatigue* and *postinfectious fatigue* are less strictly defined designations for chronic idiopathic fatigue when the condition is perceived to be induced by an infectious disease and persists after resolution of the infection.

HISTORY

Although popular interest in CFS has been a relatively recent phenomenon, a historical perspective suggests that the illness is not new.[3] For several centuries an illness resembling CFS has been described repeatedly in the medical literature by different names. The illness has been attributed variously to neurologic, cardiovascular, endocrine, and infectious causes. The proximate association of infections, especially influenza, with chronic fatigue (i.e., neurasthenia) was appreciated in the late 19th century.[4] In the 1950s Spink[5] found that nearly 20% of patients with serologic evidence of brucellosis developed lingering symptoms of fatigue, weakness, myalgic pain, mental confusion, and depression in the absence of evidence for continued, active infection, whether or not they had received treatment. He hypothesized that the development of "chronic brucellosis" involved an infection and a psychological predisposition. Later studies by Imboden and coworkers[6] confirmed this impression by showing that patients with chronic brucellosis scored unfavorably on the Minnesota Multiphasic Personality Inventory (MMPI) relative to patients who had recovered from acute brucellosis. To test the hypothesis that a psychological propensity precedes the chronic fatigue illness, these authors conducted a retrospective cohort analysis of military personnel and dependents in Maryland after an outbreak of Asian influenza during the winter of

1957–58.[7] All subjects had completed the MMPI in August 1957, just before the epidemic. Prolonged convalescence from influenza was correlated with preexisting, unfavorable scores on certain subscales of this test. The typical MMPI profile associated with prolonged postinfluenzal symptoms was nearly identical to that observed in patients with chronic brucellosis.

In 1985 two large series of patients with prolonged fatigue and other symptoms were reported to have elevated antibody titers against Epstein-Barr virus (EBV) compared with healthy control subjects.[8,9] In the same year a large outbreak of chronic fatigue with associated symptoms and serologic tests suggesting chronic EBV infection occurred in the area of Lake Tahoe, Nevada.[10] It was proposed that idiopathic chronic fatigue might be due to "chronic mononucleosis." This hypothesis was appealing because it had long been observed that persistent fatigue may follow documented acute mononucleosis in a small proportion of cases.[11–14] However, several subsequent investigations failed to confirm a role for active EBV replication in the persistence of this clinical syndrome.[15–19] Numerous other infectious agents have been proposed as the cause of CFS, including *Candida albicans, Borrelia burgdorferi,* enteroviruses, cytomegalovirus, human herpesvirus 6, spumavirus, retroviruses, *Bornavirus,* and xenotropic murine retrovirus (XMRV) (Table 130.1). The evidence that active infection with any of these agents causes a significant proportion of chronic fatigue cases is either inconclusive or refuted by subsequent investigations.

To stimulate productive research on this clinical problem, the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) sponsored a series of research conferences between 1985 and 1994. One goal of these conferences was to prepare a workable consensus definition of CFS that could be applied uniformly by investigators studying the epidemiology, clinical features, etiology, and treatment of the disorder (Table 130.2). In 2013 the US Department of Health and Human Services, in coordination with a number of other governmental agencies, contracted with the IOM to conduct a study to identify the evidence for various diagnostic criteria for CFS, develop evidence-based clinical criteria, and recommend whether new terminology for CFS should be developed. The IOM's report, published in 2015, proposed somewhat modified diagnostic criteria (Table 130.3) and also proposed that CFS be renamed *systemic exertional intolerance disease.*[2] Before this report there were already a total of eight case criteria defining chronic fatigue illness.[20] The IOM report offers a new diagnostic rubric to unify the field and facilitate timely diagnosis. Better understanding by physicians and the public and less encumbrance of research by multiple case definitions should follow.

EPIDEMIOLOGY

Fatigue is one of the most common complaints encountered in general medical practice. In most patients the complaint is eventually attributed to a diagnosable medical condition or is short lived. According to the most recent consensus conference definition, severe fatigue that remains unexplained after baseline physical and laboratory examinations and persists for more than 6 months is designated as *idiopathic chronic fatigue*. Patients with idiopathic chronic fatigue who also complain of four or more of the associated symptoms listed in Table 130.2 may be considered to have CFS.[21]

Because the case definition requires a medical evaluation, it can be applied only to study the prevalence of CFS in populations who seek medical attention. In a general medical practice in Boston, idiopathic chronic fatigue was reported by 8.5% of patients, but only 0.3% could be diagnosed with CFS.[22] The CDC ascertained the national prevalence of CFS by conducting case finding through a network of physicians in four cities. The prevalence, age, and sex distribution were remarkably similar in the four cities. The prevalence ranged from 3 to 11 per 100,000 population. Patients were predominantly women (7:1) and clustered in the 30- to 50-year age group.[23] Similar estimates of prevalence were reported from studies in Australia and the United Kingdom.[24,25] The characteristic predominance of upper-middle-class white women in their 30s and 40s resulted in the pejorative term "yuppie flu." These and other observations are biased, however, by reliance on clinic-based case ascertainment. When a random telephone survey was conducted in the San Francisco area, a different epidemiologic pattern emerged.[26] The

TABLE 130.1 Proposed Infectious Causes of Chronic Fatigue Syndrome

PROPOSED ETIOLOGIC AGENT	REFERENCES: Suggestive Studies	REFERENCES: Negative Studies
Epstein-Barr virus	8, 9, 187	15, 17, 18, 48, 188, 189
Cytomegalovirus	190, 191	15, 48, 188, 189
Human herpesvirus 6	49, 50, 192, 193	15, 48, 194, 195, 189
Human herpesvirus 7	183, 196	50, 189, 192, 195
Human herpesvirus 8	—	50, 189, 197
Enteroviruses	198–200	15, 48, 201–204
Parvovirus	43, 205	206, 207, 189
GB virus-C		208
Human spumavirus	209	—
Bornavirus	210, 211	212
Human retrovirus	213	214–216
Xenotropic murine retrovirus (XMRV)	49, 50	54, 56–58 59 (retraction of 49) 60 (retraction of 50)
Borrelia burgdorferi	217	218, 219
Brucella spp.	220	5
Mycoplasma spp.	221, 222	223
Candida albicans	224, 225	226

TABLE 130.2 CDC/NIH Consensus Conference Definition of Chronic Fatigue Syndrome

Clinically evaluated, unexplained chronic fatigue for >6-mo duration, which is not lifelong or the result of ongoing exertion and is not alleviated substantially by rest. Fatigue is associated with a significant reduction in occupational, educational, social, or personal activities *plus* ≥4 of the following concfurrent symptoms:

- Impaired memory or concentration
- Sore throat
- Tender cervical or axillary lymph nodes
- Muscle pain
- Multijoint pain
- New headaches
- Unrefreshing sleep
- Postexertional malaise

CDC, Centers for Disease Control and Prevention; *NIH,* National Institutes of Health.

Modified from Fukuda K, Straus SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med. *1994;121:953–959.*

TABLE 130.3 IOM Diagnostic criteria for Systemic Exertion Intolerance Disease

Diagnosis requires that the patient have the following three symptoms:

1. A substantial reduction or impairment in the ability to engage in preillness levels of occupational, educational, social, or personal activities; that persists for more than 6 months and is accompanied by fatigue, which is often profound; is of new or definite onset (not lifelong); is not the result of ongoing excessive exertion, and is not substantially alleviated by rest *and*
2. Postexertional malaise[a] *and*
3. Unrefreshing sleep[a]

At least one of the two following manifestations is also required:

1. Cognitive impairment[a] *or*
2. Orthostatic intolerance

[a]Frequency and severity of symptoms should be assessed. The diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome should be questioned if patients do not have these symptoms at least half of the time with moderate, substantial, or severe intensity.

IOM, Institute of Medicine.

From Institute of Medicine. Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Redefining an Illness. *Washington, DC: National Academies Press; 2015.*

overall prevalence of subjects reporting a CFS-like illness was 0.2% of the population, and the female predominance was less dramatic (2.9:1). The age distribution was the same as that seen in clinic-based studies, but the distribution of cases by income showed higher rates in persons with family incomes less than $40,000, suggesting that the perception of "yuppie flu" is an artifact of health care use by the affected populations. The rates of CFS-like illness were higher in African Americans and Hispanics than in whites and Asian Americans, and there was no preponderance of cases in any individual occupational group. Similar findings have been reported in other community-based studies.[27,28] These studies suggest that there is a high frequency of chronic fatigue in all communities; however, review of individual medical records reveals a medical or psychiatric condition to account for a majority of the cases. In addition, the prevalence of idiopathic chronic fatigue (usually about 1%–6%) is much greater than the number that meets the CDC consensus criteria for CFS (0.1%–0.3%).

Investigators in the United Kingdom used a population-based birth cohort database to estimate the occurrence of "chronic disabling fatigue" (CDF) in adolescents, defined by fatigue of ≥6 months duration, but not necessarily meeting CFS criteria. At the age of the 13, the point prevalence was 1.3%,[29] and there was an association with a family adversity score. A subsequent analysis drawn from the same database found a point prevalence of 1.9% at age 16.[30] After excluding subjects with severe depression, the prevalence of CDF dropped to 0.6%. Of interest, female gender comparable to that seen in adults appeared among 16-year-olds but not 13-year-olds. A longitudinal assessment of CDF in this population showed an increasing prevalence between ages 13 and 18 years; however, 75% recover within 2 to 3 years.[31]

Outbreaks of idiopathic illness consistent with CFS have been reported occasionally since the 1940s.[32] In many of these outbreaks the involvement of an infectious agent is unlikely because certain subgroups of the population at risk were affected disproportionately. Large hospital outbreaks in Los Angeles and London affected the professional staff but not the hospitalized patients or nonprofessional staff.[33,34] An acute outbreak of neuromyasthenia in New Zealand resulted in CFS in many of the affected individuals. A 10-year follow-up of these cases indicated that most patients had recovered partially or completely.[35]

ETIOLOGY AND PATHOGENESIS

Attempts to elucidate the pathophysiology of CFS have been hampered by several methodologic problems. Foremost among these is the problem of selecting a homogeneous group of subjects for study from among patients identified by the working definition. In a symptom cluster analysis of patients meeting the CDC criteria, at least two distinct subgroups emerged: one having numerous syndromal and nonsyndromal symptoms with high severity scores, and a second, larger group with limited symptoms and only moderate severity.[36] A recent genetic and clinical symptom analysis suggests the existence of seven discrete subgroups.[37]

It has long been appreciated that some patients have an acute onset associated with an infectious disease or other definable stressor, whereas others describe an insidious and progressive onset. Most patients have past or current psychiatric disorders,[38–40] whereas some have no past or present psychiatric symptoms. There is no reason to assume a priori that patients with these diverse clinical circumstances have the same disorders simply because they meet the CDC criteria at the time of presentation.

Role of Infection

Infectious causes of CFS have been proposed (see Table 130.1), but there is no reproducible evidence that any single agent is responsible for any significant proportion of these illnesses. Postinfectious fatigue cases indistinguishable from CFS may immediately follow a diagnosed infection (e.g., EBV infection, influenza, brucellosis, giardiasis), including some that are highly localized geographically (e.g., Lyme disease, Q fever, Ross River virus infection).[7,41–44] In a prospective study Hickie and coworkers[14] reported that postinfectious fatigue occurs at a rate of approximately 12% whether the instigating infection is EBV, Q fever, or Ross River virus. Most of these patients met criteria for CFS as well. Others have reported that postinfectious and idiopathic cases have indistinguishable clinical and psychosocial features.[45] Prolonged convalescence from infectious mononucleosis is a well-recognized phenomenon.[11–13] A more recent prospective study of adolescents with infectious mononucleosis showed that at 6 and 24 weeks of follow-up, 13% and 4%, respectively, had prolonged fatigue that met CDC criteria.[46] The use of steroids during the acute phase of mononucleosis was not associated with prolonged postinfectious fatigue in this study.

The occurrence of clinically indistinguishable "postinfectious" fatigue states after several unrelated infections supports the concept that CFS is a nonspecific sequela to a variety of illnesses rather than a disease with a single, specific infectious cause. Controversy persists about whether chronic fatigue can be triggered by any infectious or traumatic event or whether only a particular type of infection or trauma is necessary. However, patients seen in general practice for common infections do not have an increased frequency of prolonged fatigue relative to patients seen for other medical problems.[47]

Although there was never any direct virologic evidence favoring chronic, persistent EBV infection as a cause of CFS, a significant body of negative research has accumulated to reject this hypothesis. These studies showed no significant differences in serologic titers,[15,48] shedding of virus in saliva,[19] blood lymphocyte-transforming activity,[19,18] or EBV-specific cytotoxic lymphocytes[18] between patients and healthy control subjects. In addition, a large treatment study compared intravenous and oral acyclovir with placebo in CFS patients and failed to show any benefit.[17] Similarly, negative microbiologic data have been collected for other specific infectious agents (see Table 130.1). The possibility that CFS patients may reactivate latent viruses more frequently than healthy individuals has been proposed,[49,50] but it is not clear how or whether viral reactivation affects the ongoing symptom complex.[51] For example, Chia and Chia[52] have recently observed that enterovirus RNA and capsid protein VP1 are found four times more frequently in the stomachs of CFS patients than in healthy control subjects. However, like many similar virologic observations, it is not clear whether persistent enterovirus is a cause or a consequence of the syndrome.

A high-profile controversy concerning an infectious etiology for CFS occurred after a 2009 publication implicating XMRV in 67% of 101 patients but only 3.7% of 218 healthy control subjects.[53] A separate research group reported a similar disparity in the frequency of murine leukemia virus–related sequences among CFS patients and healthy blood donors.[54] Initially, criticism of these reports from established researchers focused on poorly described selection of cases and control subjects, potential sources of bias, and uncertainty about the generalizability of the findings.[55–57] However, the most serious challenge came from DNA sequence analysis showing that viruses in an established murine cell line are ancestral to viruses detected in the human samples, suggesting that the human samples were contaminated with mouse DNA.[58] Several subsequent studies failed to show any association of XMRV with CFS,[59–62] and the two studies that initially found an association were eventually retracted (see Table 130.1).[54,63]

Role of the Autonomic Nervous System

In 1995 researchers at Johns Hopkins University reported that chronic fatigue patients had abnormal responses to a 45-minute tilt-table test protocol.[64] Virtually all of the patients had syncope or reproduction of fatigue symptoms, whereas only about one-third of healthy control subjects had an abnormal response. These investigators proposed that the persistent fatigue may be attributable to neurally mediated hypotension. Subsequent studies have provided mounting evidence that younger patients with CFS have increased sympathetic activity at rest with exaggerated cardiovascular response to orthostatic stress. Resting catecholamine levels are increased, and thermoregulatory responses suggest increased sympathetic activation at rest.[65,66] This may be manifest as positional orthostatic tachycardia syndrome without hypotension.[67] However, Katz and coworkers[68] failed to find any difference in the frequency of orthostatic intolerance among adolescents 6 months after infectious mononucleosis, whether they met criteria for CFS or had completely recovered.

Attempts to treat CFS as neurally mediated hypotension have not been successful. The investigators who demonstrated abnormal tilt-table

testing in young patients with CFS used combinations of fludrocortisone, atenolol, and sertraline with increased salt intake to treat a cohort of patients. Initially, they reported a favorable durable response in 47% of 19 patients.[64] However, two subsequent controlled trials failed to show any benefit using this approach to therapy.[69,70]

Role of the Immune System

Some of the proposed inciting and perpetuating factors involve disruptions in various biologic systems (e.g., the immune system, skeletal muscle, heart, CNS). Although subtle alterations in some of these systems have been identified in patients, similar changes are observed in individuals without symptoms. Differences in various measures of immune function (e.g., cytokine levels, in vitro lymphocyte function, flow cytometry) have been observed by comparing CFS patients and healthy persons; however, many of the studies are inconsistent with one another or have not been reproduced. One prospective study of postinfectious fatigue failed to find any significant differences in the levels of eight cytokines comparing patients and control subjects.[71] In another study alterations in cytokine profiles were only found in patients with a disease duration of less than 3 years.[72]

The most consistent immune alterations have included an increase in T lymphocytes expressing activation markers,[73] for instance, in the number of lymphocytes bearing the CD45RA differentiation marker.[74] These findings suggest mild activation of the cellular immune system, but the relevance of these alterations is unclear, given the inconsistent immunologic differences among monozygotic twin pairs that are discordant for CFS.[75] It is also not clear that a change in lymphocyte subsets occurs in conjunction with clinical improvement.[76]

Another immunologic finding more common in CFS patients than in healthy control subjects is a reduction in natural killer cell function and in the number of lymphocytes bearing the CD16 marker.[77,78] This finding raised the concern that malignancies might occur at an increased rate in CFS. Analysis of cancer registries after the large 1985 Lake Tahoe outbreak did not support this notion.[79] Subsequently, Chang and coworkers[80] queried the Surveillance, Epidemiology, and End Results (SEER)-Medicare registry that includes 1.2 million cancer cases and compared them with 100,000 control subjects. The frequency of a CFS diagnosis more than 1 year before selection was 0.5% in both the cancer and control groups; however, an extensive subgroup analysis showed a small increase in the odds of CFS in patients with non-Hodgkin lymphoma. Two studies of all-cause mortality showed no increased rates for CFS patients.[81,82]

Abnormalities of B lymphocytes have been present less consistently among CFS patients. However, a Norwegian research group reported a transient beneficial effect of rituximab therapy in a majority of 15 treated CFS patients compared with 15 placebo-treated control subjects.[83] This observation has been followed up with an open-label, one-armed (uncontrolled) phase II study suggesting clinical response during the period of B-cell depletion.[84]

Dysregulation of RNase L has been studied as a common feature of CFS. Normally, the presence of double-stranded (ds)RNA (i.e., from viruses) activates expression of the enzyme 2′,5′-oligoadenylate synthetase. The 2′,5′-adenylate oligonucleotides generated from this activity bind to and activate RNase L, which degrades viral and some host RNAs. CFS patients are purported to have increased levels of 2′,5′-adenylate oligonucleotides and RNase L activity.[85,86] In addition, a low-molecular-weight RNase L molecule has been detected more frequently in CFS patients than in healthy control subjects (88% vs. 32%).[87,88] Activation of this pathway in CFS could be regarded as evidence of a response to viral infection or, alternatively, as evidence of disturbed immune regulation that results in unnecessary degradation of host RNA.

A synthetic dsRNA rintatolimod (Ampligen; Hemispherx Biopharma, New Brunswick, NJ) activates 2′,5′-oligoadenylate synthetase via Toll-like receptor 3 (TLR-3) and generates RNase L. This drug, given intravenously, has been reported to benefit CFS patients in two randomized, placebo-controlled trials.[89,90] However, the US Food and Drug Administration has repeatedly rejected a new drug application for Ampligen based on concerns about study design and efficacy. In any case, the failure of the drug to make CFS worse argues against exaggerated RNase L levels and RNA degradation as a cause of fatigue.

Role of the Central Nervous System

Because idiopathic chronic fatigue usually includes neuropsychological symptoms and subtle alterations of hormones regulated at the hypothalamic level, the hypothesis that the central nervous system (CNS) is the principal site of the pathophysiology has gained support in recent years. Accumulating data from CNS imaging studies support this notion, but the significance of these findings still is unclear.[49,91–95] Investigators have observed regions of reduced cerebral blood flow in CFS patients relative to healthy control subjects[96,95]; however, these findings were not confirmed in a study of single-photon emission computed tomography scanning in monozygotic twins discordant for CFS.[97]

Urinary free cortisol levels have been shown to be lower in patients with CFS than in age-matched and sex-matched healthy control subjects, although the means of both groups are within the defined normal range.[98,99] Exaggerated adrenal responsiveness to corticotropin infusions in these patients suggests that a subtle defect in hypothalamic-pituitary-adrenal (HPA)-axis activity exists.[100] The disturbance also results in a flattening of the diurnal pattern of cortisol secretion and enhanced sensitivity of the axis to negative feedback.[101,102] Nater and coworkers[103] reported an attenuation of morning salivary cortisol in medication-free CFS patients compared with well-matched control subjects but noted that the effect was limited to women. Reduced HPA-axis activity and hyperresponsiveness have also been observed in fibromyalgia and in posttraumatic stress disorder, whereas enhanced activity of the axis is typical of major depressive disorder.[104] In contrast, gonadotropin levels are not affected by either CFS or fibromyalgia.[105] These findings suggest the presence of a common pathophysiology in CFS and other stress-related disorders that is centered in the CNS.

The finding of depressed HPA-axis activity as a feature of chronic fatigue illnesses motivated a study at the NIH in which patients received either replacement hydrocortisone or placebo for 3 months. This study and a subsequent trial suggest that there was minor improvement in the hydrocortisone group during treatment.[106] There was also profound and sustained suppression of the HPA axis and loss of bone density, which prompted investigators to recommend against the use of steroids for treatment.[106,107] Another attempt to boost the HPA axis pharmacologically was made in a randomized controlled trial (RCT) of the drug galantamine (a central stimulant of HPA-axis activity). The study showed no substantial benefit.[108] Therefore, although HPA-axis disturbances may be present in CFS, attempts to correct these disturbances do not relieve symptoms. This suggests that any fundamental disturbance occurs at a more complex level in the brain.

The notion that the pathophysiology of CFS might involve enhanced sympathetic activity was addressed by conducting a randomized, double-blind, placebo-controlled trial of low-dose clonidine in 120 adolescent CFS patients.[109] Compared with placebo, clonidine resulted in lower levels of plasma norepinephrine and reduced C-reactive protein levels. However, the clonidine-treated patient also had fewer steps per day, suggesting that pharmacologic reduction of sympathetic outflow did not increase physical activity.

As in the earlier studies of brucellosis and influenza by Imboden and coworkers,[7] contemporary investigators have found that when patients present with a "viral illnesses" in general practice, psychiatric morbidity, belief in vulnerability to viruses, and attributional style at initial presentation are more important predictors of fatigue 6 months later than are "viral" symptoms during the initial infection.[110] Several studies have reported that a prior history of depression is frequently present in chronic fatigue states and may represent an important predisposing condition.[110–113] Perhaps the most convincing observations come from the National Child Development Study in the United Kingdom, a study that prospectively collected health and psychosocial data on huge cohorts of individuals from birth to adulthood. Cohorts born in 1946, 1958, and 1970 were analyzed in separate studies that came to similar conclusions. In the 1946 cohort 3035 participants were assessed for CFS by self-report and a structured interview at age 53 years. The study found that those with psychopathology assessed by standard interview instruments at ages 15 and 36 years were 2.65 times more likely to report CFS later in life. Moreover, the likelihood of CFS was greatest in those with the most severe psychiatric disorders.[114] This study was confirmed using the 1958 cohort of 11,419 participants.[115]

Those with psychopathology when assessed at age 33 years were 2.8 times more likely to report CFS at age 42 years. In addition, this analysis showed that psychopathology and sedentary behavior during childhood and extremes of physical activity in adulthood are not associated with CFS later in life. The association between CFS and psychopathology was highlighted by a study that found an increased suicide rate in individuals with a diagnosis of CFS.[82]

Role of the Genome

To investigate a possible genetic predisposition to chronic fatigue illnesses, family history and twin studies have been conducted. In one study CFS patients reported higher rates of fatigue illness in family members than medical control patients.[116] Similarly, twin studies in Australia and Great Britain suggest that disabling fatigue of greater than 1-month duration occurs more frequently in monozygotic than in dizygotic twins.[117,118] Applying the CDC criteria for CFS, a study in the United States showed a concordance of 38% in monozygotic twins versus 11% in dizygotic twins.[119] However, a microarray study of the transcriptomes of discordant, monozygotic twins failed to identify any biologically active marker that can distinguish the twins with CFS from those with no fatigue.[120]

With the capacity to perform genome-wide analysis, researchers now have the opportunity to search for either disease-specific alleles or to find genes that are dysregulated in CFS patients. To identify disease-specific alleles, Smith and coworkers[121] looked for polymorphisms in genes involved in the serotonergic system and found three CFS-associated alleles of the *HTR2A* gene, which encodes a G protein–coupled serotonin receptor. Polymorphisms in this gene have previously been associated with a variety of psychiatric disorders. Similarly, Rajeevan and coworkers[122] found single nucleotide polymorphisms (SNPs) in the glucocorticoid receptor *(NR3C1)* gene that occurs with relatively high frequency in CFS compared with control subjects. In a comprehensive genomic screening study Goertzel and coworkers[123] defined a panel of 28 SNPs that predicts the presence of CFS with 76% accuracy The three most relevant genes in the panel were *NR3C1, TPH2* (encoding neuronal tryptophan hydroxylase, involved in serotonin synthesis), and *COMT* (encoding catecholamine-*O*-methyl transferase). Identification of these genes suggests genetic predispositions involving the HPA axis, serotonergic system, and autonomic nervous system, respectively. These findings are consistent with functional studies that show disturbances in the targeted physiologic systems, even though the studies have identified different CFS-associated alleles.

Reconciling the differences in these genetic studies may be difficult if CFS proves to be a collection of disorders with different manifestations and predispositions. Nevertheless, research on related disorders provides strong evidence for genetic predisposition in similar functional disorders. A recent report by Binder and coworkers[124] showed that certain SNPs affecting the expression of FK506 binding protein 5 predict the development of posttraumatic stress disorder (PTSD) after adult trauma among inner-city African Americans who experienced abuse as children. Thus the combination of early psychological trauma and a genetic variant that influences the responsiveness of the glucocorticoid receptor results in hyperresponsiveness of the HPA axis in adulthood. Similarly, certain genotypes of the *MAOA* (monoamine oxidase) gene have been found to predict adult consequences in mistreated children.[125] Whether these specific genes will prove to play a role in some CFS variants is yet to be determined; however, a pathophysiologic model of genetic predisposition plus environmental challenge as the substrate for the disorder is an approach to understanding these disorders that is gaining traction.[126]

If this simple hypothesis is correct, then it should be possible to observe differences in gene expression in CFS patients versus control subjects. In accordance, a transcriptional analysis of 30,000 genes in 7 patients with postinfectious fatigue and 8 matched control subjects at four time points after documented primary EBV infection identified a panel of 35 genes with expression that correlated temporally with the clinical course.[127] Similarly, Kerr and coworkers[128] compared the transcriptomes of 25 CFS patients with those of 50 unrelated, healthy control subjects. They found 88 genes with differential expression in CFS patients. Subsequently, they identified 21 SNPs among these genes that distinguish subjects with CFS from normal and depressed subjects.[129] Unfortunately, the panels of dysregulated genes identified by these two laboratories are mutually exclusive, underscoring the heterogeneity of the syndrome, its definition, and the inherent problem of patient and control selection in CFS research.

CLINICAL MANIFESTATIONS

CFS occasionally develops in the aftermath of an identifiable infectious disease, such as infectious mononucleosis or influenza. More often, however, an infectious triggering event is not confirmed. Flulike symptoms may persist or develop de novo after the acute onset. These symptoms include sore throat, low-grade fever, tender adenopathy, generalized myalgia, migratory arthralgia, and headache. In contrast, objective physical findings corresponding to these subjective complaints, such as pharyngitis, a temperature greater than 100.5°F, and palpable adenopathy, are rare after the resolution of the initial illness. The presence of significant objective muscle weakness or frank arthritis should suggest an alternative diagnosis.

Persistent, disabling fatigue is the cardinal symptom of CFS. Exhausting fatigue that lasts hours to days may follow even modest exertion. Patients often report that they have a limited allotment of energy each day and cannot function when it is depleted. In consequence, Wessely and Powell[130] undertook a prospective study using standardized interview instruments to compare the perception of fatigue among patients with "postviral fatigue" and control groups with neuromuscular disorders or major depression. They determined that the features of physical fatigue were similar in all three groups, but complaints of mental fatigue (e.g., poor concentration, poor memory, sleepiness, decreased muscle strength), physical fatigue after mental activity, and fatigue at rest were more common in the CFS and depression groups. Mental fatigue was uncommonly reported by patients with neuromuscular disorders unless they also had a psychiatric diagnosis, and the only feature that distinguished the CFS patients from the depression patients was the tendency of the former to attribute their fatigue to a physical rather than affective cause.

Patients may describe difficulty with concentration and memory, although actual deficits are not consistently demonstrable using neuropsychological testing. Either insomnia or excessive sleep may be reported. A thorough sleep history helps to determine whether formal polysomnography is indicated to rule out a primary sleep disorder. Sleep disorders are common among patients who present with fatigue, even among patients who meet symptomatic criteria for CFS.[131–133] Any or all of the symptoms may occur persistently or occur with striking seasonality. Some cases of CFS have seasonal variation comparable with that seen with seasonal affective disorder.[134]

Most patients who meet symptom criteria for CFS also have a past or current history of a psychiatric disorder (e.g., depression or anxiety disorder).[39–40] The treating physician must determine whether a preexisting psychiatric disorder accounts for all of the patient's complaints. If so, CFS should not be diagnosed. In contrast, altered mood states that occur in the context of the CFS are often transient and reactive to the physical disability and discomfort.

It is also important for the clinician to realize that the definition of CFS intersects with several other functional somatic disorders, such as fibromyalgia (FM), irritable bowel syndrome (IBS), multiple chemical sensitivity syndrome, temporomandibular joint dysfunction, and interstitial cystitis, to name a few. Several of these disorders may occur in the same patient, and their concurrence may reflect our inability to adequately understand or define them. A recent population-based survey suggests that CFS-like illness is associated with lifetime prevalences of major depression, chronic widespread pain (i.e., FM), and IBS of 57%, 41%, and 16%, respectively.[135] Moreover, an individual with any one of these three diagnoses is 14 times more likely to have a CFS-like illness than one who has none of these three comorbid conditions. A unified view of etiology and management of these disorders is presented in a review by Henningsen and coworkers.[136]

LABORATORY FINDINGS

No laboratory tests are diagnostic for CFS. Laboratory evaluation requires testing only for the purpose of ruling out unrecognized medical

conditions that account for the symptoms. The minimal evaluation consists of a complete blood count, serum chemistry profile, urinalysis, and thyroid function testing.[21] Additional tests may be indicated when the history or quality of the patient's symptoms suggests specific alternative diseases (e.g., chronic infection, collagen vascular disease, neurologic disorder, neoplasm). A low-titer antinuclear antibody test is found in 15% to 54% of patients with CFS.[137,138] Antibodies against DNA and extractable nuclear antigens are typically absent. Instead, antibodies directed against insoluble nuclear matrix proteins are responsible for the nuclear fluorescence.[138,139] The significance of these autoantibodies is unknown.

When groups of patients with CFS are compared with control groups, significant differences have been found in many laboratory or radiologic results (e.g., in hormone levels, CNS radiologic tests, measures of cellular immunity). These findings may provide some insight into the pathophysiology of chronic fatigue states, but they have no diagnostic value in individual patients. In all of these examples there is significant overlap between the fatigued and normal groups, and the tests cannot be used as a reliable marker for the disorder in an individual. For example, although study groups of patients may have disturbances in the HPA axis, neither blood nor urine cortisol levels are sufficiently discriminatory to be of value in diagnosis of individual patients. Levels should be obtained only when clinical findings indicate a need to rule out frank adrenal insufficiency.

Orthostatic intolerance is included among the diagnostic criteria for CFS proposed by the IOM (see Table 130.3). This criterion refers to symptoms (e.g., fainting, lightheadedness, spatial disorientation, feeling dizzy or lightheaded when standing) and does not require the performance of a tilt-table test.[2] Exercise intolerance is not easily quantified. Snell and coworkers[140] observed that clinical cardiopulmonary testing did not distinguish 51 subjects with CFS from 10 sedentary but not disabled control subjects (all women) at an initial test. However, if retested 24 hours later, CFS subjects performed less well, particularly with respect to the workload at ventilatory threshold, whereas the control subjects reproduced their initial test results. Using this 2-day testing protocol, the authors correctly classified 49 of the 51 CFS cases and 9 of the 10 control subjects.

Changes in the perfusion of certain areas of the brain are suggested by studies using single-photon emission computed tomography,[91,92,95] Similarly, small areas of localized increased signal intensity, bilateral reduced white matter volume, and anisotropy of the right anterior arcuate fasciculus have been reported more frequently in brain magnetic resonance imaging studies of CFS patients than in those of healthy control subjects,[49,93] although different research groups have reported different anatomic findings. The causes of these findings are unknown, and their relationship to symptoms is not clear. Because the findings are not specific for CFS, CNS imaging should be used only when it is medically indicated to rule out a structural abnormality.

Because immunologic tests (e.g., flow cytometry, cytokine levels, in vitro lymphocyte function) have no diagnostic or prognostic significance, they are of value only in the context of a research study and should not be ordered as part of the routine evaluation for idiopathic chronic fatigue. Laboratory evaluation of the 2′,5′-oligoadenylate synthetase pathway[86] is not sufficiently specific to be helpful in diagnosis.

MANAGEMENT

General Principles

The case definitions for CFS, ME, and SEID all describe an idiopathic disorder diagnosed on the basis of clinical features, not on causation (see Tables 130.2 and 130.3). Consequently, specific medical or psychiatric therapy is rational only when an alternative or coexisting diagnosis is present. Antimicrobial therapy is not indicated unless there is clear evidence of a specific, active infection producing disease manifestations. Uncontrolled trials of successful antiviral therapy have been reported, but failed RCTs of antimicrobial therapy have been cited as evidence that conventional infectious agents are not a perpetuating factor in CFS and do not require treatment.

In my opinion, conservative therapy for idiopathic fatigue syndromes is appropriately nonspecific and should be focused on remediation of symptoms and physical rehabilitation instead of specific, presumed causes.[141] Because CFS is a heterogeneous disorder, the outcomes of treatment studies are influenced heavily by the method of selecting patients. Also, noncontrolled treatment observations are of minimal value in these disorders because most blinded controlled trials have shown a robust placebo effect.

The treatment of individual patients is empirical. It is useful to objectify the symptoms as much as possible so that the response to any intervention can be assessed independently by both the physician and the patient. Medications should be evaluated in an additive or sequential manner so that there can be no confusion about their efficacy or adverse effects. With the large range of symptoms among these patients, physicians are faced with a variety of treatments from which to choose, and patients may inquire about unconventional or alternative therapies. In the absence of evidence for efficacy, empirical treatment choices should be guided by a concern for safety and cost.

Pharmacologic Therapy

Medications may be useful for the treatment of symptoms, including nonnarcotic pain relievers for myalgia, arthralgia, or headache; nonaddictive sleep aids for sleep disruption; and psychoactive agents for depression or anxiety. Various vitamins and "nutritional supplements" have no proven, consistent benefit and may be costly.[142–146] Costly "immune-enhancing" therapies, such as intravenous immune globulin, immunostimulants, and cytokines, have failed to provide benefit in controlled studies.[147–152] A recent study examined the effect of anakinra, an antagonist of the interleukin (IL)-1α and IL-1β receptor, administered for 4 weeks in a placebo-controlled trial.[153] Patients in the study were 50 women, aged 15 to 59 years with severe fatigue attributed to CFS, who were followed for 20 weeks. No clinically significant effect on fatigue severity was found.

Carefully controlled treatment trials do not show a consistent benefit from any single pharmacologic agent. It has been useful to extrapolate from more conclusive studies of fibromyalgia patients because this idiopathic condition has significant overlap with CFS. Although the diagnosis of fibromyalgia is based on a subjective report of pain and musculoskeletal tenderness, symptoms of profound fatigue, sleep disruption, and cognitive or emotional difficulties are common accompaniments. In addition, fibromyalgia and CFS share certain biologic features (e.g., autonomic and neuroendocrine findings). Amitriptyline and serotonin-norepinephrine reuptake inhibitors (SNRIs) are moderately beneficial in fibromyalgia,[154] and common experience suggests that chronic fatigue patients with pain and sleep disorder may respond in a similar fashion.[155,156] A meta-analysis of 18 RCTs that evaluated four agents in fibromyalgia patients—milnacipran, duloxetine, pregabalin, and sodium oxybate—showed them all to have equivalent efficacy in achieving a 50% reduction of pain, with a combined mean of 31.6% of treated patients achieving this goal in all studies.[157] This mean was significantly superior to the placebo response rate, but a remarkable proportion of patients taking placebo also achieved this degree of pain reduction (18.6%). One large placebo-controlled trial of fluoxetine therapy for CFS failed to show benefit; however, this study was criticized because the treated subjects had long-standing, disabling symptoms[158] A 3-year follow-up study comparing patients who received antidepressant therapy with those who did not suggests a significant, sustained benefit of treatment; however, this analysis did not clarify the duration of therapy.[159] SNRIs, selective serotonin reuptake inhibitors, and pregabalin are available, safe, and relatively inexpensive; however, patients treated with these agents often drop out because of adverse effects or a significant nocebo (negative placebo) effect.[157] The most recent NIH workshop on therapies of CFS20 concluded that evidence was available, albeit of only fair quality, that rintatolimod, a TLR-3 agonist, may have improved some measures of exercise performance and functions of daily living.[90,160]

Nonpharmacologic Therapy

Discussions about the "reality" of CFS are decidedly unhelpful and often insulting to patients. Similarly, framing the illness in a manner that is inconsistent with the patient's perceptions (e.g., as "depression" in the absence of formal psychiatric criteria) is likely to evoke resistance and nonadherence. The best approach is the one that is most consistent

with current understanding of CFS: to recognize and validate the patient's symptoms as part of an idiopathic syndrome. In doing so, it is essential to educate the patient about the unexplained nature of the fatiguing illness and to correct any misinformation that the patient may have about its cause or treatment. This approach allows the physician to enlist the patient's support in pursuing the rational management agenda.

All patients should be counseled regarding exercise and sleep hygiene. Many patients have significant disruptions in their sleep patterns that should be corrected gradually. Daytime napping should be limited or avoided altogether because this behavior may further disrupt nighttime sleep quality. Melatonin is a popular over-the-counter sleep remedy used by many patients; however, the rationale for this therapy is unclear, given that CFS patients have normal levels and timing of endogenous melatonin secretion.[161]

Although some patients report relief of symptoms with dietary alterations, there is little reliable experimental evidence to inform changes in diet. A recent RCT showed that a low-sugar, low-yeast diet, recommended by some, was no more efficacious in relieving fatigue or improving quality of life than simple, healthy eating.[162] Highly restrictive diets that may impair general nutrition should be discouraged.

Among psychological therapies, cognitive-behavioral therapy (CBT) is considered the most effective modality in CFS patients. This approach involves a restructuring of the patient's beliefs concerning the causes of the illness and an objective assessment of the symptoms and disabilities. A 2008 meta-analysis reported successful outcome in about half of the patients entering therapy and a mean drop-out rate of 16%. The authors concluded that this modality was moderately effective.[163] The development and use of an online CBT program for Dutch adolescents likely improves the cost-effectiveness of this method as well. A randomized, open-label trial in the Netherlands showed that an Internet-based CBT program resulted in eightfold likelihood of recovery than randomization to usual care in a tertiary treatment center during a 6-month treatment period.[164] A long-term follow-up study (mean follow-up, 2.7 years) showed that the eventual recovery rate was similar in both CBT and "usual care" subjects, but the CBT-assigned subjects achieved a faster and sustained recovery after completing the online program.[165]

Individual counseling provided in general medical practice may serve the same functions and seems to be as effective as formal CBT.[166,167] However, classic insight-oriented psychotherapy rarely is indicated but may be helpful in patients who experience significant, ongoing emotional stress at home or work.

Exercise has been traditionally advocated in the treatment of fibromyalgia. Several studies have confirmed the widely held view that light aerobic exercise is also beneficial in CFS.[168–170] A systematic review of all treatment options for CFS in 2001 suggested that CBT and a program of graded exercise therapy (GET) were the only therapeutic options with credible benefit.[171] By 2011 a large randomized parallel-group trial (the *P*acing, *g*raded *A*ctivity, and *C*ognitive-behavior therapy: a randomized *E*valuation [PACE] study) was published. In this study CBT and GET both resulted in less fatigue and superior physical functioning when compared with treatment by a medical specialist or "adaptive pacing," a program in which patients control their own activity level in accordance with their perception of energy and tolerance.[172] CBT and GET also resulted in lower health care costs per quality-adjusted life year than the other nonpharmacologic therapies mentioned earlier.[173] The beneficial effects of these interventions in the PACE study were then subjected to mediation analysis that showed the most important mediator to be fear avoidance of exercise.[174] The authors concluded that abating the patient's fear of exercise should be a critical goal of CBT and GET.

Since then a 2015 NIH review of treatment similarly concluded that counseling therapies and GET were the only treatments to demonstrate benefit with at least a moderate level of evidence.[20] An updated Cochrane review of current evidence for GET reached similar conclusions.[175]

Readers of the literature and physicians who encounter patients with this syndrome should be aware that there is an active and sometimes vitriolic controversy between advocates of psychophysically oriented interventions and those who favor a standard medical (sometimes virologic) model of the disorder. The alternative hypothesis views exercise and even CBT as potentially harmful. The flavor of this controversy is apparent from the reviews of Hooper and Prins and the comments that followed[176–179] and in some of the comments after the publication of the PACE trial and the meta-analysis published by the NIH Pathways to Prevention Workshop in 2015.[180–185] Despite this opposition, there is no evidence-based benefit of bed rest as a treatment for CFS, and many physicians will likely see the potential for continuous inactivity to reinforce illness behavior and to facilitate complicating myofascial pain syndromes. In addition, there is objective evidence in adolescents and young adults with CFS of reduced range of motion of large joints and the spine compared with healthy control subjects matched for age and joint hypermobility.[186]

SUMMARY

Prolonged idiopathic fatigue states may arise spontaneously or during the convalescence from an infectious disease. When associated with other symptoms, diagnostic criteria for CFS may be met, although the distinction between syndromal and nonsyndromal chronic fatigue may be artificial. No laboratory tests are available to confirm or exclude the diagnosis of CFS. Various infections have been proposed as occult causes of the syndrome, but none has been implicated in any significant proportion of the patients who meet the clinical definition. In contrast, most experimental evidence points to a reversible derangement of certain CNS functions in persons with genetic predisposition. Treatment modalities such as exercise, CBT, and medications that act in the CNS seem to provide the most benefit.

As noted in the 2015 IOM report,[2] significant questions remain with respect to the etiology, pathophysiology, and treatment of CFS, which require rigorous and systematic research for improved understanding.

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A Viral Diseases

131 Biology of Viruses and Viral Diseases

James D. Chappell and Terence S. Dermody

HISTORY

Viruses have substantially influenced human health, interactions with the ecosphere, and societal history and structures. Because of their sheer numbers and ubiquity, these organisms are the single most important cause of infectious disease morbidity and mortality worldwide. The first viruses were identified as the 19th century ended. New virus identification has since continued with growing momentum. Emerging or reemerging viral diseases include bat-origin respiratory coronaviruses[1] and reoviruses[2–5]; swine-, avian-, and bat-origin influenza viruses[6,7,8]; globally spreading alphaviruses and flaviviruses that threaten the health of adults, children, and the unborn[9–11]; and highly lethal and transmissible filoviruses, such as Ebola virus.[12]

Increasing recognition of the environmental virus reservoir has been paralleled by a growing appreciation for an internal community of human viruses, collectively described as the *virome*. The human virome encompasses acute and persistent viral infections, chromosomally integrated endogenous viral elements, and bacteriophages.[13] Virome composition and dynamics carry prodigious implications for human health through direct effects on cells and tissues, interactions with innate and adaptive arms of the immune system, and influence on the bacterial microbiota. The human virome is a priority research front that will help explain virus-host interactions on the fulcrum between health and disease.

Insights into viral biology and disease have expanded in reciprocal relationship with advancements across the continuum of scientific knowledge, including the fields of microscopy, protein and nucleic acid chemistry, cell biology, immunology, and clinical medicine. In recent years, x-ray crystallography and high-resolution cryoelectron microscopy have allowed visualization of virus structures at an atomic level of resolution. Functional domains of many viral structural and enzymatic proteins have been defined, which is fostering development of new strategies to diagnose viral illnesses and design effective antiviral therapies. Viral genome detection, now represented by a multitude of routinely used target- and signal-amplification methods, has proven superior to conventional serologic assays and culture techniques for the diagnosis of many viral diseases such as those caused by enteroviruses, hepatitis viruses, herpesviruses, human immunodeficiency virus (HIV), and various respiratory and enteric viral pathogens, to name a few. Furthermore, multiplexed molecular assays ("syndromic panels") are becoming standard approaches for the simultaneous detection and differentiation of diverse viral etiologies that manifest as overlapping clinical presentations, such as acute respiratory illness and gastroenteritis. Rapid developments in nucleotide sequencing technology are permitting the application of these tools to highly sensitive and discriminatory virus detection in clinical specimens.

One of the most exciting advances in contemporary virology is the means to repurpose viruses for improving human health through systematic viral genome modification with predictable outcomes. Technologies now exist whereby specific mutations and foreign genetic material can be efficiently engineered into the genomes of most human viruses. Such approaches have been exploited in the rational design of vaccines and development of viral vectors for use in gene transduction and cancer treatment. Furthermore, these powerful techniques regularly drive research into viral pathogenesis, host responses to infection, and viral and host determinants of contagion.

Knowledge accumulation about viruses and viral diseases has been immense since the revolutionary discovery in 1882 of tobacco mosaic virus, a form of infectious agent previously unknown to the world. However, ongoing breakthrough discoveries in virology repeatedly reveal our finite comprehension of these fascinating entities, understandings of which are inseparably entwined with scientific exploration of their complex hosts.

VIRUS STRUCTURE AND CLASSIFICATION

The first classification of viruses as a group distinct from other microorganisms was based on the capacity to pass through filters of a small pore size (filterable agents). Initial subclassifications were based primarily on pathologic properties such as specific organ tropism (e.g., hepatitis viruses) or common epidemiologic features such as transmission by arthropod vectors (e.g., arboviruses). Current classification systems are based on the following: (1) the type and structure of the viral nucleic acid and the strategy used in its replication; (2) the type of symmetry of the virus capsid (helical vs. icosahedral); and (3) the presence or absence of a lipid envelope (Table 131.1).

Virus particles—virions—can be functionally defined as a delivery system that surrounds a payload. The delivery system consists of structural components used by the virus to survive in the environment and bind

TABLE 131.1 Classification of Viruses

FAMILY	EXAMPLE	TYPE OF NUCLEIC ACID	GENOME SIZE (kb OR kb PAIR)	ENVELOPE	CAPSID SYMMETRY
RNA-Containing Viruses					
Picornaviridae	Poliovirus	SS (+) RNA	7–9	No	I
Astroviridae	Astrovirus	SS (+) RNA	6–8	No	I
Caliciviridae	Norwalk virus	SS (+) RNA	7–8	No	I
Togaviridae	Rubella virus	SS (+) RNA	10–12	Yes	I
Flaviviridae	Yellow fever virus	SS (+) RNA	9–13	Yes	S
Coronaviridae	Coronavirus	SS (+) RNA	26–32	Yes	H
Rhabdoviridae	Rabies virus	SS (–) RNA	11–15	Yes	H
Paramyxoviridae	Measles virus	SS (–) RNA	13–18	Yes	H
Filoviridae	Ebola virus	SS (–) RNA	19	Yes	H
Arenaviridae	Lymphocytic choriomeningitis virus	2 SS (ambisense) RNA segments	11	Yes	S
Bunyaviridae	California encephalitis virus	3 SS (ambisense) RNA segments	11–19	Yes	H
Orthomyxoviridae	Influenza virus	6–8 SS (–) RNA segments	10–15	Yes	H
Reoviridae	Rotavirus	10–12 DS RNA segments[a]	19–32	No	I
Retroviridae	Human immunodeficiency virus	2 identical SS (+) RNA segments	7–13	Yes	S
DNA-Containing Viruses					
Hepadnaviridae	Hepatitis B virus	Circular DS DNA with SS portions	3–4	Yes	I
Parvoviridae	Human parvovirus B19	SS (+) or (–) DNA	4–6	No	I
Polyomaviridae	JC virus	Circular DS DNA	5	No	I
Papillomaviridae	Human papillomavirus	Circular DS DNA	7–8	No	I
Adenoviridae	Adenovirus	Linear DS DNA	26–48	No	I
Herpesviridae	Herpes simplex virus	Linear DS DNA	125–240	Yes	I
Poxviridae	Vaccinia virus	Linear DS DNA	130–375	Yes	Complex

[a]Reovirus and orbivirus, 10 segments; rotavirus, 11 segments; Colorado tick fever virus, 12 segments.
(+), Message sense; (–), complement of message sense; *DS,* double-stranded; *H,* helical; *I,* icosahedral; *S,* spherical; *SS,* single-stranded.
Data from Condit RC. Principles of virology. In: Knipe DM, Howley PM, eds. Fields Virology. *6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013:21–51.*

to host cells. The payload contains the viral genome and often includes enzymes required for the initial steps in viral replication. In almost all cases, the delivery system must be removed from the virion to allow viral replication to commence.

In addition to mediating attachment to host cells, the delivery system also plays a crucial role in determining the mode of transmission between hosts. Viruses containing lipid envelopes are sensitive to desiccation in the environment and, for the most part, are transmitted by the respiratory, parenteral, and sexual routes. Nonenveloped viruses are stable to harsh environmental conditions and are often transmitted by the fecal-oral route.

Viral genomes exist in a variety of forms and sizes and consist of DNA or RNA (see Table 131.1). Animal virus genomes range in size from 3 kb, encoding only three or four proteins in small viruses such as the hepadnaviruses, to more than 300 kb, encoding several hundred proteins in large viruses such as the poxviruses. Viral genomes are single- or double-stranded and circular or linear. RNA genomes are composed of a single molecule of nucleic acid or multiple discrete segments, which can vary in number from as few as two in the arenaviruses up to 12 in some members of the Reoviridae. Viral nucleic acid is packaged in a protein coat, or capsid, that consists of multiple protein subunits. The combination of the viral nucleic acid and the surrounding protein capsid is often referred to as the *nucleocapsid* (Fig. 131.1).

Structural details of many viruses have now been defined at an atomic level of resolution (Fig. 131.2). General features of virus structure can be gained from examination of electron micrographs of negatively stained virions and thin-section electron micrographs of virus-infected tissues and cultured cells. These techniques allow rapid identification of viral size, shape, symmetry, and surface features; presence or absence of an envelope; and intracellular site of viral assembly. Cryoelectron microscopy and computer image processing techniques are used to determine the three-dimensional structures of spherical viruses at a level of resolution far superior to that of negatively stained electron micrographs. A major advantage of cryoelectron microscopy is that it allows studies of viruses to be performed under conditions that do not alter native virion structure. Moreover, advances in cryoelectron microscopy have extended the achievable resolution of particle-associated proteins to atomic levels, sufficient to recognize characteristic features of secondary structural elements.[14] Image reconstructions of cryoelectron micrographs, sometimes in combination with x-ray crystallography, also can be used to investigate structural aspects of various virus functions, including receptor binding[15–17] and interaction with antibodies.[18,19] Identification of key motifs, such as receptor-binding sites or immunodominant domains, provides the framework for understanding the structural basis of virus-cell interactions. Electron tomography with image reconstruction has been applied to architectural studies of viruses and intracellular foci of virus replication, rendering exquisite three-dimensional representations of particle organization and revealing the structure and subcellular origins of virus assembly centers.[20,21]

A number of general principles have emerged from studies of virus structure. In almost all cases, the capsid is composed of a repeating series of structurally similar subunits, each of which in turn is composed of only a few different proteins. The parsimonious use of structural proteins in a repetitive motif minimizes the amount of genetic information required to encode the viral capsid and leads to structural arrangements with symmetrical features. All but the most complex viruses exhibit either helical or icosahedral symmetry (see Table 131.1). Viruses with helical symmetry contain repeating protein subunits bound at regular intervals along a spiral formed by the viral nucleic acid. Interesting

to note, all known animal viruses that show this type of symmetry have RNA genomes. Viruses with icosahedral symmetry display twofold, threefold, and fivefold axes of rotational symmetry, and viral nucleic acid is intimately associated with specific capsid proteins in an ordered packing arrangement.

The use of repeating subunits with symmetrical protein-protein interactions facilitates the assembly of the viral capsid. In most cases, viral assembly appears to be a spontaneous process that occurs under the appropriate physiologic conditions and often can be reproduced when recombinant viral proteins are expressed in the absence of viral replication.[22,23] For many viruses, assembly of the capsid proceeds through a series of intermediates, each of which nucleates the addition of subsequent components in the assembly sequence.

One of the least understood aspects of viral assembly is the process that ensures that the viral nucleic acid is correctly packaged into the capsid. In the case of viruses with helical symmetry, there may be an initiation site on the nucleic acid to which the initial capsid protein subunit binds, triggering the addition of subsequent subunits. The genomes of most DNA-containing viruses are inserted into preassembled capsid intermediates (procapsids) through adenosine triphosphate–driven mechanisms.[24] In preparations of many icosahedral viruses, empty capsids (i.e., capsids lacking nucleic acid) are frequently observed, indicating that assembly may proceed to completion without a requirement for the viral genome.

In some viruses, the nucleocapsid is surrounded by a lipid envelope acquired as the virus particle buds from the host cell cytoplasmic, nuclear, or endoplasmic reticular membrane (see Fig. 131.1). Inserted into this lipid bilayer are virus-encoded proteins (e.g., the hemagglutinin [HA] and neuraminidase proteins of influenza virus and gp41 and gp120 of HIV), which are exposed on the surface of the virus particle. These viral proteins usually contain a glycosylated hydrophilic external portion and internal hydrophobic domains that span the lipid membrane and anchor the protein into the viral envelope. In some cases, another viral protein, often termed a *matrix protein,* associates with the internal (cytoplasmic) surface of the lipid envelope, where it can interact with the cytoplasmic domains of the envelope glycoproteins. Matrix proteins may function in stabilizing the interaction between viral glycoproteins and the lipid envelope, directing the viral genome to intracellular sites of viral assembly or viral budding. Matrix proteins also can influence a diverse set of cellular functions to create a permissive replication environment, such as inhibition of host cell transcription[25,26] and evasion of the cellular innate antiviral response.[27]

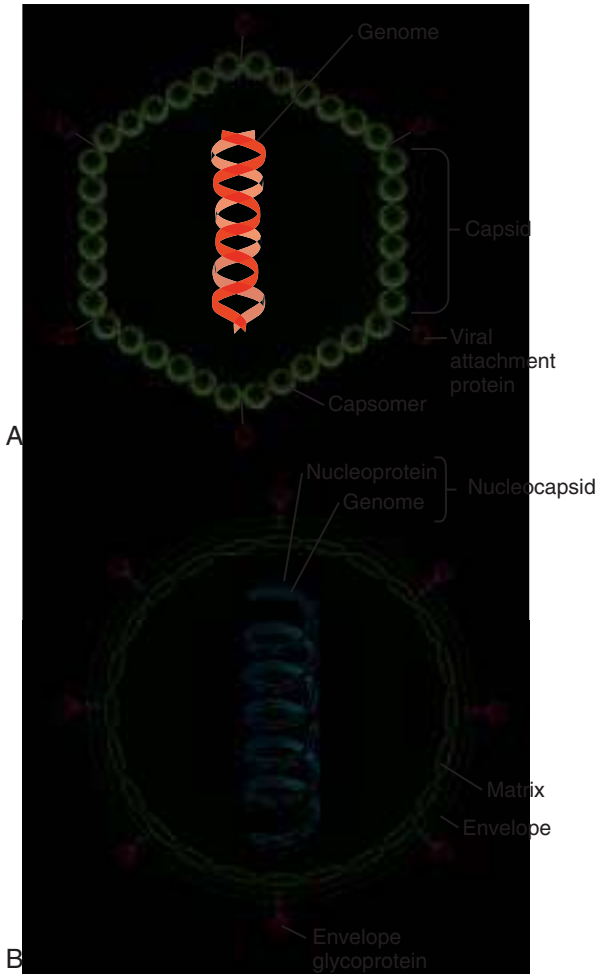

FIG. 131.1 Schematic diagrams illustrating the structure of a nonenveloped icosahedral virus (A) and an enveloped helical virus (B). Nucleocapsid: combination of a viral nucleic acid and surrounding protein capsid.

VIRUS-CELL INTERACTIONS

Viruses require an intact cell to replicate and can direct the synthesis of hundreds to thousands of progeny viruses during a single cycle of infection. In contrast to other microorganisms, viruses do not replicate by binary fission. Instead, the infecting particle must disassemble in order to multiply itself.

Attachment

The interaction between a virus and its host cell begins with attachment of the virus particle to specific receptors on the cell surface. Viral proteins that mediate the attachment function (viral attachment proteins) include the following: single-capsid components that extend from the virion surface, such as the attachment proteins of adenovirus,[28] reovirus,[29] and

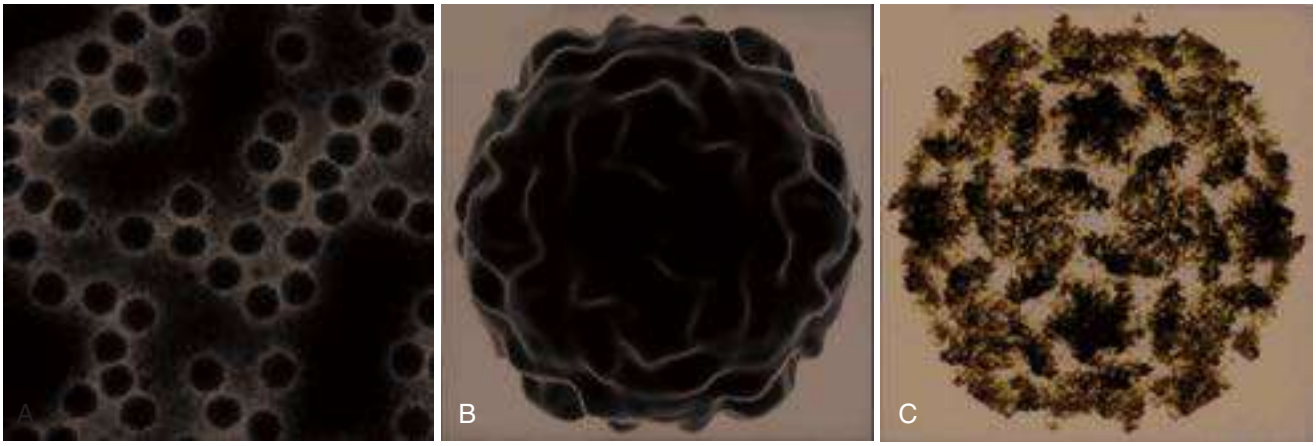

FIG. 131.2 Structural studies of poliovirus. (A) Negative-stained electron micrograph. (B) Three-dimensional image reconstruction of cryoelectron micrographs. (C) Structure determined by x-ray crystallography. *(Courtesy Dr. James Hogle, Harvard University.)*

rotavirus[30,31]; surface glycoproteins of enveloped viruses, such as influenza virus[32,33] (Fig. 131.3) and HIV[34,35]; viral capsid proteins that form binding pockets that engage cellular receptors, such as the canyon formed by the capsid proteins of poliovirus[36] and rhinovirus[37]; and viral capsid proteins that contain extended loops capable of binding receptors, such as foot-and-mouth disease virus.[38] Studies of the attachment of several diverse virus groups, including adenoviruses, coronaviruses, herpesviruses, lentiviruses, and reoviruses, indicate that multiple interactions between virus and cell occur during the attachment step. These observations indicate that a particular sequence of binding events between virus and cell optimizes specificity and contributes significant stability to the association.[39]

One of the most dynamic areas of virology concerns the identification of virus receptors on host cells. This interest stems in part from the critical importance of the attachment step as a determinant of host species tropism and target cell selection by many viruses. Numerous virus receptors have been identified (Table 131.2), and three important principles have emerged from studies of these receptors. First, viruses have adapted to use cell surface molecules designed to facilitate a variety of normal cellular functions. Virus receptors may be highly specialized proteins with limited tissue distribution, such as complement receptors, growth factor receptors, or neurotransmitter receptors, or more ubiquitous components of cellular membranes, such as integrins and other intercellular adhesion molecules, glycosaminoglycans, or sialic acid–containing oligosaccharides. Second, many viruses use more than a single receptor to mediate multistep attachment and internalization. For example, adenovirus binds coxsackievirus-adenovirus receptor (CAR)[40] and the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$[41]; herpes simplex virus (HSV) binds heparan sulfate[42–44] and herpesvirus entry mediator (HVEM/HveA),[45] nectin 1 (PRR1/HveC),[46] or nectin 2 (PRR2/HveB)[47]; HIV binds CD4[48,49] and chemokine receptors CXCR4[50,51] and CCR5[52–54]; and reovirus binds sialylated glycans,[55,56] junctional adhesion molecule A (JAM-A),[57,58] and NgR1.[59] Third, in many cases, receptor expression is not the sole

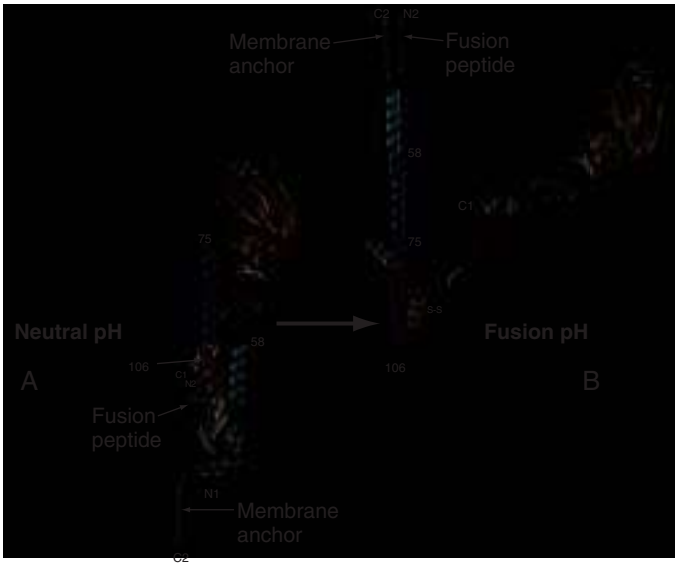

FIG. 131.3 The folded structure of the influenza virus hemagglutinin (HA) and its rearrangement when exposed to low pH. (A) The HA monomer. HA1 is blue, and HA2 is multicolored. The receptor-binding pocket resides in the virion-distal portion of HA1. The viral membrane would be at the bottom of this figure. (B) Conformational change in HA induced by exposure to low pH. Note the dramatic structural rearrangement in HA2, in which amino-acid residues 40 to 105 become a continuous alpha helix. *Dashed lines* indicate regions of undetermined structure. This model of HA in its fusion conformation is a composite of the HA1 domain structure and the low-pH HA2 structure. *(Modified from Russell RJ, Kerry PS, Stevens DJ, et al. Structure of influenza hemagglutinin in complex with an inhibitor of membrane fusion.* Proc Natl Acad Sci U S A. *2008;105: 17736–17741.)*

TABLE 131.2 Receptors and Entry Mediators Used by Selected Human Viruses

VIRUS	RECEPTOR
Adeno-associated virus	AAVR[291]
Adenovirus	Coxsackievirus and adenovirus receptor (CAR)[40,286] CD46[287,288] Desmoglein 2[289] Integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$[41] Sialic acid–containing oligosaccharides[290]
Coronavirus	9-*O*-acetylated sialic acid–containing oligosaccharides (HCoV-OC43[292] and HCoV-HKU-1[293]) Aminopeptidase N (HCoV-229E)[294,295] Angiotensin-converting enzyme 2 (SARS-CoV[296] and NL63[297]) Dipeptidyl peptidase 4 (MERS-CoV)[298] Carcinoembryonic antigen–related cell adhesion molecule 5 (MERS-CoV)[299] α2,3-linked sialic acids (MERS-CoV)[300]
Coxsackievirus	Integrin $\alpha_v\beta_3$[301] Decay-accelerating factor (CD55)[302,303] Coxsackievirus and adenovirus receptor (CAR)[40] Intercellular adhesion molecule 1 (ICAM-1)[304] GRP78/BiP[305] Heparan sulfate[306] Sialic acid[307]
Cytomegalovirus	Heparan sulfate[308,309] Integrins $\alpha_2\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_3$[310] Platelet-derived growth factor-α receptor[311]
Ebola virus	T-cell immunoglobulin and mucin domain 1 (TIM-1)[315] Niemann-Pick C1 cholesterol transporter[316,317]
Echovirus	Integrin $\alpha_2\beta_1$[312] Decay accelerating factor (CD55)[313,314]
Enterovirus 68	ICAM-5 (telencephalin)[318]
Enterovirus 71	P-selectin glycoprotein ligand-1 (PSGL-1)[319] Scavenger receptor B2 (SR-B2)[320]
Epstein-Barr virus	Complement receptor 2 (CD21)[321,322] MHC class II protein[75]

TABLE 131.2 Receptors and Entry Mediators Used by Selected Human Viruses—cont'd

VIRUS	RECEPTOR
Hantaviruses	β_3 Integrins[323]
Henipaviruses	Ephrin-B2[324,325] Ephrin-B3[326]
Hepatitis A virus	Mucin-like protein TIM-1[327]
Hepatitis C virus	CD81[328,329] Scavenger receptor B1 (SRB1)[330,331] Claudin[332] Occludin[333]
Herpes simplex virus	Heparan sulfate[42–44] Herpesvirus entry mediator (HVEM/HveA)[45] Nectin 1 (PRR1/HveC)[46] Nectin 2 (PRR2/HveB)[47]
Human herpesvirus 6A	CD46[334]
Human herpesvirus 6B	CD134[335]
Human immunodeficiency virus	CD4[48,49] Chemokine receptor CXCR4[50,51] Chemokine receptor CCR5[52–54]
Human metapneumovirus	Integrin $\alpha_v\beta_1$[336]
Human T-cell leukemia virus	Glucose transporter GLUT-1[337] Neuropilin-1[338]
Influenza virus	Sialic acid–containing oligosaccharides[33,339]
Kaposi sarcoma herpesvirus	Heparan sulfate[340] Integrin $\alpha_3\beta_1$[341] Ephrin-A2[342]
Measles virus	CD46 (vaccine strains)[343,344] Signaling lymphocyte-activation molecule (SLAM)[345] Nectin-4[346,347]
Mumps virus	Sialic acid[348]
New World hemorrhagic fever arenaviruses (e.g., Junin virus)	Transferrin receptor 1[349]
Norovirus	Histo-blood group antigens[350,351] Gangliosides[352]
Old World hemorrhagic fever arenaviruses (e.g., Lassa fever virus)	α-Dystroglycan[353] Lysosome-associated membrane protein-1[354]
Parvovirus B19	Erythrocyte P antigen (globoside)[355]
Poliovirus	Poliovirus receptor (PVR, CD155)[191]
Polyomavirus (BK)	Gangliosides GD1b and GT1b[356,357]
Polyomavirus (JC)	Serotonin receptor 5HT2A[358] LSTc pentasaccharide[72]
Polyomavirus (Merkel cell)	Heparan sulfate[359] Sialic acid[360,361]
Rabies virus	Neural cell adhesion molecule (CD56)[362] Nerve growth factor receptor (P75NTR)[363]
Reovirus	Sialic acid–containing oligosaccharides[55,56] Junctional adhesion molecule-A (JAM-A)[57] Nogo receptor-1 (NgR1)[364] β_1 integrins[365]
Rhinovirus (type A, major group, and type B)	Intercellular adhesion molecule 1 (ICAM-1)[366–368]
Rhinovirus (type A, minor group)	Low-density lipoprotein receptor[369]
Rhinovirus (type C)	Cadherin-related family member 3[370]
Rotavirus	Sialic acid–containing oligosaccharides[371,372] Histo-blood group antigens[373] Integrins $\alpha_2\beta_1$, $\alpha_4\beta_1$, $\alpha_v\beta_3$, $\alpha_x\beta_2$[374,375]
Rubella virus	Myelin oligodendrocyte glycoprotein (MOG)[376]
Sindbis virus	Natural resistance–associated macrophage protein (NRAMP)[377]

determinant of viral tropism for particular cells and tissues in the host. Therefore, although receptor binding is the first step in the interaction between virus and cell, subsequent events in the viral replication cycle also must be supported for productive viral infection to occur.

Several viruses bind receptors expressed at regions of cell-cell contact.[60] JAM-A, which serves as a receptor for reovirus[57] and feline calicivirus,[61] and CAR, which serves as a receptor for some coxsackieviruses and adenoviruses,[40] are expressed at tight junctions[62,63] and adherens junctions.[64,65] Junctional regions are sites of enhanced membrane recycling, endocytic uptake, and intracellular signaling.[66] Therefore it is possible that viruses have selected junction-associated proteins as receptors to usurp the physiologic functions of these molecules. In this regard, interactions of coxsackievirus with decay-accelerating factor elicit a tyrosine kinase–based signaling cascade that mediates subsequent interactions of the virus with CAR in tight junctions.[67] Structures of viral proteins or whole viral particles in complex with sialic acid have been determined for some viruses, including the influenza virus HA[33,68] (see Fig. 131.3), polyomavirus,[69–72] foot-and-mouth disease virus,[73] reovirus attachment protein σ1,[55,56] and the VP8 domain of rotavirus capsid protein VP4.[31] Sialic acid binding in each of these cases occurs in a shallow groove at the surface of the viral protein. However, the architectures of the binding sites differ. Structures of complexes of viral proteins or viral particles and cell surface protein receptors have also been determined. These include adenovirus fiber knob and CAR,[74] Epstein-Barr virus (EBV) gp42 and major histocompatibility complex (MHC) class II protein,[75] HSV glycoprotein D and HVEM/HveA,[76] HIV gp120 and CD4,[35] measles virus HA and CD46[77] and SLAM (signaling lymphocyte-activation molecule),[78] reovirus σ1 and JAM-A,[58] and rhinovirus and ICAM-1 (intercellular adhesion molecule 1).[79] In several of these cases, the viral attachment proteins engage precisely the same domains used by their cognate receptors to bind natural ligands.

Penetration and Disassembly

Once attachment has occurred, the virus must penetrate the cell membrane, and the capsid must undergo a series of disassembly steps (uncoating) that prepare the virus for the next phases in viral replication. Enveloped viruses including some paramyxoviruses and retroviruses enter cells by fusion of the viral envelope with the cell membrane (Fig. 131.4). Attachment of these viruses to the cell surface induces changes in viral envelope proteins required for membrane fusion. For example, the binding of CD4 and certain chemokine receptors by HIV envelope glycoprotein gp120 induces a series of conformational changes in gp120 that lead to the exposure of transmembrane protein gp41.[80,81] Fusion of viral and cellular membranes proceeds through subsequent interactions of the hydrophobic gp41 fusion peptide with the cell membrane.[82,83,84,85]

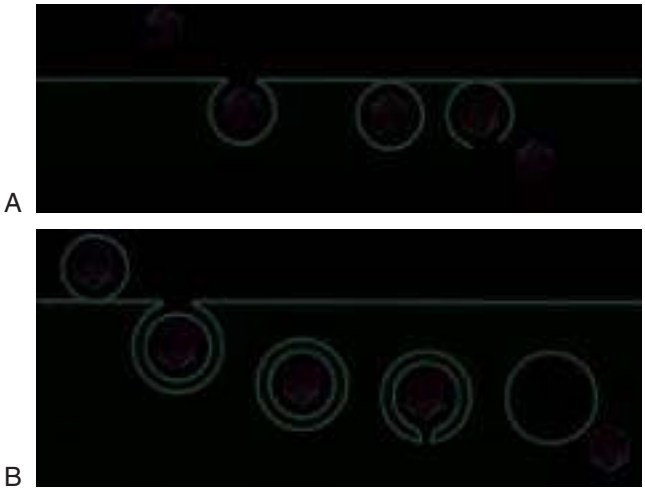

FIG. 131.4 Mechanisms of viral entry into cells. Nonenveloped (A) and enveloped (B) virus internalization by receptor-mediated endocytosis.

Other viruses enter cells by some form of receptor-mediated endocytic uptake (see Fig. 131.4). For several viruses, virus-receptor complexes induce formation of clathrin-coated pits that invaginate from the cell membrane to form coated vesicles.[86] These vesicles are rapidly uncoated and fuse with early endosomes, which sort internalized proteins for recycling to the cell surface or other cellular compartments, such as late endosomes or lysosomes. For other viruses, virus-receptor complexes are taken into cells by caveolae in lipid rafts.[86] Enveloped viruses such as dengue virus,[87] influenza virus,[88] and Semliki Forest virus[89] exploit the acidic environment of the endocytic compartment to induce conformational changes in surface glycoproteins required for membrane fusion. High-resolution structures of the influenza virus HA at acidic pH illustrate a dramatic conformational alteration leading to the fusion-active state (see Fig. 131.3).[88]

Endocytic uptake and acidification are also required for entry of some nonenveloped viruses such as adenovirus,[90,91] parvovirus,[92] and reovirus.[93,94] In these cases, acidic pH may facilitate disassembly of the viral capsid to enable subsequent penetration of endosomal membranes. In addition to acidic pH, endocytic cathepsin proteases are required for disassembly of several viruses, including Ebola virus,[95] Hendra virus,[96] reovirus,[97] and Middle East respiratory syndrome[98] and severe acute respiratory syndrome (SARS)[99] coronaviruses.

In contrast to enveloped viruses, nonenveloped viruses cross cell membranes using mechanisms that do not involve membrane fusion. This group of viruses includes several human pathogens, with adenoviruses, picornaviruses, and rotaviruses serving as prominent examples. Despite differences in genome and capsid composition, each of these viruses must penetrate cell membranes to deliver the genetic payload to the interior of the cell. Capsid rearrangements triggered by receptor binding,[100,101] acidic pH,[90,91] or proteolysis[102,103] serve essential functions in membrane penetration by some nonenveloped viruses. Although a precise understanding of the biochemical mechanisms that underlie viral membrane penetration is incomplete, small capsid proteins of several nonenveloped viruses, such as adenovirus,[104] poliovirus,[105] and reovirus,[106] are required for membrane penetration, perhaps by forming pores in host cell membranes.

Genome Replication

Once a virus has entered a target cell, it must replicate its genome and synthesize its proteins. Replication strategies used by single-stranded, RNA-containing viruses depend on whether the genome can be used as messenger (m)RNA. Translation-competent genomes, which include those of the coronaviruses, flaviviruses, picornaviruses, and togaviruses, are termed *plus* (+) *sense* and are translated by cellular ribosomes immediately following entry of the genome into the cytoplasm. For most viruses containing (+) sense RNA genomes, translation results in synthesis of a large polyprotein that is cleaved into several smaller proteins through the action of viral and sometimes host proteases. One of these proteins is an RNA-dependent RNA polymerase (RdRp), which replicates the viral RNA. Genome replication of (+) sense RNA-containing viruses requires synthesis of a minus (−) sense RNA intermediate, which serves as template for production of (+) sense genomic RNA.

A different strategy is used by viruses containing (−) sense RNA genomes. The genomes of these viruses, which include the filoviruses, orthomyxoviruses, paramyxoviruses, and rhabdoviruses, cannot serve directly as mRNA. Therefore viral particles must contain a copackaged RdRp to transcribe (+) sense mRNAs using the (−) sense genomic RNA as template. Genome replication of (−) sense RNA-containing viruses requires synthesis of a (+) sense RNA intermediate, which serves as a template for production of (−) sense genomic RNA. Mechanisms that determine whether (+) sense RNAs are used as templates for translation or genome replication are not well understood.

RNA-containing viruses belonging to the family Reoviridae have segmented, double-stranded (ds) RNA genomes. The innermost protein shell of these viruses (termed a *single-shelled particle* or *core*) contains an RdRp that catalyzes the synthesis of (+) sense mRNA using as a template the (−) sense strand of each dsRNA segment. The mRNAs of these viruses are capped at their 5′-termini by virus-encoded enzymes and then extruded into the cytoplasm through channels in the single-shelled particle.[107] The (+) sense mRNAs also serve as a template for

replication of dsRNA gene segments. Viral genome replication is thus completely conservative; neither strand of parental dsRNA is present in newly formed genomic segments.

The retroviruses are RNA-containing viruses that replicate using a DNA intermediate. The viral genomic RNA is (+) sense and single stranded; however, it does not serve as mRNA following viral entry. Instead, the retrovirus RNA genome is a template for synthesis of a double-stranded DNA copy, termed the *provirus.* Synthesis of the provirus is mediated by a virus-encoded RNA-dependent DNA polymerase or *reverse transcriptase,* so named because of the reversal of genetic information from RNA to DNA. The provirus translocates to the nucleus and integrates into host DNA. Expression of this integrated DNA is regulated for the most part by cellular transcriptional machinery. However, the human retroviruses HIV and human T-cell leukemia virus (HTLV) encode proteins that augment transcription of viral genes. Intracellular signaling pathways are capable of activating retroviral gene expression and function, inducing high levels of viral replication in response to certain stimuli.[108] Transcription of the provirus yields mRNAs that encode viral proteins and genome-length RNAs that are packaged into progeny virions. Such a replication strategy results in persistent infection in the host because the viral genome is maintained in the host cell genome and replicated with each cell division.

With the exception of the poxviruses, viruses containing DNA genomes replicate in the nucleus and for the most part use cellular enzymes for transcription and replication of their genomes. Transcription of most DNA-containing viruses is tightly regulated and results in the synthesis of early and late mRNA transcripts. The early transcripts encode regulatory proteins and proteins required for DNA replication, whereas the late transcripts encode mainly structural proteins. Several DNA-containing viruses, such as adenovirus and human papillomavirus (HPV), induce cells to express host proteins required for viral DNA replication by stimulating cell-cycle progression. For example, the HPV E7 protein binds the retinoblastoma gene product pRB and liberates transcription factor E2F, which induces the cell cycle.[109,110] To prevent programmed cell death in response to E7-mediated unscheduled cell-cycle progression, the HPV E6 protein mediates the ubiquitylation and degradation of tumor suppressor protein p53.[111–113]

Some DNA-containing viruses, such as the herpesviruses, can establish latent infections in the host. Unlike the retroviruses, genomes of the herpesviruses do not integrate into host chromosomes but instead exist as plasmid-like episomes. Mechanisms that govern establishment of latency and subsequent reactivation of replication are not well understood. However, microRNAs encoded by cytomegalovirus (CMV) and perhaps other herpesviruses may promote persistence by targeting viral and cellular mRNAs that control viral gene expression and replication and innate immune responses to viral infection.[114,115]

A fascinating aspect of virus-cell interactions is the replication microenvironments established in infected cells. Viral replication is a sophisticated interplay of transcription, translation, nucleic acid amplification, and particle assembly. Furthermore, infection must proceed under sensitive pathogen surveillance systems trained on virus-associated molecular patterns (e.g., unmethylated CpG dinucleotides in viral DNA genomes and exposed 5′ triphosphates of many viral RNAs) and replicative intermediates (e.g., dsRNA generated during RNA virus replication) that may impose impassable blocks to infection.[116] Partitioning of the viral replication machinery from the surrounding intracellular milieu satisfies a spatial requirement to concentrate viral proteins and nucleic acid for efficient genome amplification and encapsidation while simultaneously shielding viral products from cellular sensors that provoke antiviral innate immune responses. Hence, as a general rule, viral replication is a localized process, occurring within morphologically discrete cytoplasmic or nuclear structures variously termed *viral inclusions* (or *inclusion bodies*), *virosomes, viral factories,* or *viroplasms.* These entities are novel, metabolically active organelles formed by contributions from both virus and cell. Many highly recognizable features of viral cytopathic effect observed using light microscopy, such as dense nuclear inclusions or refractile cytoplasmic densities, represent locally concentrated regions of viral nucleic acid and protein.

Membrane-associated replicase complexes appropriated by (+) sense RNA viruses are perhaps the most conspicuous examples of compartmentalized viral replication. In cells infected by these viruses, intracellular membranes originating from the endoplasmic reticulum (ER; e.g., picornaviruses[117,118]), ER-Golgi intermediate compartment and *trans*-Golgi network (e.g., flaviviruses[119]), endolysosomal vesicles (e.g., alphaviruses[120]), and autophagic vacuoles (e.g., poliovirus[121]) are reduplicated and reorganized by viral proteins into platforms that anchor viral replication complexes consisting of the RdRp and other RNA-modifying enzymes required for RNA synthesis. Viruses with dsRNA genomes also form replication organelles composed of host-derived membranes,[122] the nature of which is less clear. The assembly pathway of rotavirus, a dsRNA virus, involves budding of immature particles into the ER, where a lipid envelope is transiently acquired and subsequently replaced by the outermost protein shell.[123]

The tight relationship of RNA virus replication to cellular membranes is less predictable for DNA viruses. For example, in distinction to the supporting role of autophagy in the replication of some RNA viruses, autophagosomes (stress-induced, double-membraned vesicles that remove noxious cytoplasmic materials to lysosomes for degradation) defend against infection by HSV-1, which encodes a protein that inhibits induction of autophagy and accentuates viral virulence.[124,125] The replication and assembly complexes of many DNA viruses, including adenoviruses, herpesviruses, papillomaviruses, polyomaviruses, and parvoviruses, are associated with promyelocytic leukemia (PML) nuclear bodies,[126,127] which have been ascribed functions in diverse nuclear processes encompassing gene regulation, tumor suppression, apoptosis, and removal of aggregated or foreign proteins.[128] It appears that DNA viruses exploit PML bodies in a variety of ways, which include consolidation and disposal of misfolded viral proteins, sequestration of host cell stress-response factors that block infection, and segregation of interfering cellular DNA repair proteins from sites of viral replication.[129]

The life cycles of all viruses that replicate in eukaryotic cells are physically and functionally intertwined with the cytoskeleton. Many viruses with nuclear replication programs, such as adenovirus, HSV, and influenza virus, are transported by motor proteins along microtubules toward the nucleus, resulting ultimately in release of the viral genome into the nucleoplasm through nuclear pores.[130] The microtubule network is also conscripted as an egress pathway by a number of enveloped viruses (e.g., HIV, HSV, and vaccinia virus) for conveyance of immature particles to sites of virion budding.[131] Furthermore, microtubules and actin filaments may serve as anchorage points for nucleoprotein complexes that coordinate genome expression or amplification with cytoplasmic replication programs, exemplified by parainfluenza virus (PIV),[132] reovirus,[133] and vaccinia virus.[134] Because the cytoskeleton is a decentralized organelle linking cellular structural elements to the metabolic and transport machineries, it is not surprising that viruses capitalize on this highly integrative system, which provides a stable platform for replication and enables purposeful movement of virions or subviral components within cells to facilitate the requisite insulation of viral assembly from disassembly.

Release

Exit of progeny virions from an infected cell classically has been understood to occur via budding by enveloped viruses (directly from the cell membrane as in the case of HIV[135] or into a secretory pathway as exemplified by hepatitis C virus [HCV][136]) or cell lysis by nonenveloped viruses. In addition, outgoing particles have been considered to be typically disconnected infectious units, independent of one another. These concepts of virus release likely are oversimplified because emerging evidence supports an alternative mode of viral egress involving extracellular vesicles.[137] A variety of nonenveloped and enveloped viruses appear to migrate through the extracellular milieu as micropopulations within cell-derived vesicles originating from multivesicular bodies or autophagosomes. This transit strategy confers many possible advantages to virus survival—for example, invisibility to immune surveillance systems and genetic robustness allowing for rapid adaptation to different replication environments. The group paradigm of a viral infectious unit carries numerous implications for theoretical and experimental virology, particularly in the areas of viral evolution, quasispecies dynamics, clonality, population diversity within an infectious focus, and cell attachment and entry mechanisms.

Cell Killing

Viral infection can compromise numerous cellular processes, such as nucleic acid and protein synthesis, maintenance of cytoskeletal architecture, and preservation of membrane integrity.[138] Many viruses are also capable of inducing genetically programmed mechanisms of cell death including apoptosis and pyroptosis.[139,140,141] Apoptotic cell death is characterized by cell shrinkage, membrane blebbing, condensation of nuclear chromatin, and activation of an endogenous endonuclease, which cleaves cellular DNA into oligonucleosome-length DNA fragments.[142] These changes occur according to predetermined developmental programs or in response to certain environmental stimuli. In some cases, apoptosis may serve as an antiviral defense mechanism to limit viral replication by destruction of virus-infected cells or reduction of potentially harmful inflammatory responses elicited by viral infection.[143] In other cases, apoptosis may result from viral induction of cellular factors required for efficient viral replication.[139,140] In general, RNA-containing viruses, including influenza virus, measles virus, poliovirus, reovirus, and Sindbis virus, induce apoptosis of host cells, whereas DNA-containing viruses, including adenovirus, CMV, EBV, HPV, and the poxviruses, encode proteins that block apoptosis. For some viruses, the duration of the viral infectious cycle may determine whether apoptosis is induced or inhibited. Viruses capable of completing an infectious cycle before induction of apoptosis would not require a means to inhibit this cellular response to viral infection. Interesting to note, several viruses that cause encephalitis are capable of inducing apoptosis of infected neurons (Fig. 131.5).[144–146]

Pyroptosis also has been recognized as a form of programmed cell death in response to intracellular pathogens.[147] This system is stimulated by cellular sensors *(inflammasomes)* of microbial components, triggering activation of caspases and subsequent cleavage of gasdermin D and precursor forms of the proinflammatory mediators interleukin (IL)-1β and IL-18 into biologically active products. Membrane pore formation mediated by a gasdermin D–derived polypeptide leads to release of mature IL-1β and IL-18 and cytolemmal rupture. The precise roles of pyroptosis in viral replication and pathogenesis await results of ongoing studies. CD4 T-cell decline during HIV infection has been linked to pyroptosis.[148]

ANTIVIRAL DRUGS

(Also see Chapters 44 to 48.)

Knowledge of viral replication strategies has provided insights into critical steps in the viral life cycle that can serve as potential targets for antiviral therapy. For example, drugs can be designed to interfere with virus binding to target cells or prevent penetration and disassembly once receptor engagement has occurred. Steps involved in replication of the viral genome are also obvious targets for antiviral therapy. A number of antiviral agents inhibit viral polymerases, including those active against herpesviruses (e.g., acyclovir), HIV (e.g., zidovudine), hepatitis B virus (HBV) (e.g., entecavir), and HCV (e.g., sofosbuvir). Drugs that inhibit viral proteases are used to treat HCV[149] and HIV[150] infection. These drugs block the proteolytic processing of viral precursor polyproteins and serve as potent inhibitors of replication. Other viral enzymes also serve as targets for antiviral therapy. The influenza virus neuraminidase is required for the release of progeny influenza virus particles from infected cells. Oseltamivir, peramivir, and zanamivir bind the neuraminidase catalytic site and efficiently inhibit the enzyme.[151] These drugs have been used in the prophylaxis and treatment of influenza virus infection.[152]

Better understanding of viral replication strategies and mechanisms of virus-induced cell killing is paving the way for the rational design of new antiviral therapeutics. One of the most exciting approaches to the development of antiviral agents is the use of high-resolution x-ray crystallography and molecular modeling to optimize interactions between these inhibitory molecules and their target viral proteins. Such structure-based drug design has led to the development of synthetic peptides (e.g., enfuvirtide) that inhibit HIV entry by blocking gp41-mediated membrane fusion.[153] Other vulnerable steps in HIV replication are targets of drugs approved for patient treatment, including entry inhibitors that interfere with gp120 binding to CCR5[154] and agents that prevent proviral integration into cellular DNA through inhibition of viral integrase activity[155] (see Chapter 128). Viral replication complexes have been successfully targeted in the development of highly effective regimens for treatment of HCV infection. Inhibitors of the HCV NS5A protein disrupt its essential function in the formation of membranous structures that scaffold viral RNA replication and virion assembly. In a combination antiviral approach analogous to the treatment of HIV infection, NS5A inhibitors are coadministered with drugs that specifically target the HCV RdRp and NS3-4A protease, which creates a high genetic barrier to resistance through interference with multiple steps in the viral replication program. Notably, combination therapy is rapidly becoming a matter of first principles in the research and development of new antiviral drugs and durable therapeutic strategies (see Chapter 47).

Despite promising advances in rational antiviral drug design, current therapeutic approaches to some viral infections rely heavily on compounds with less specific mechanisms of action. One such agent, interferon-α (IFN-α), efficiently inhibits a broad spectrum of viruses and is secreted by diverse cell types as part of the host innate immune response. Recombinant IFN-α is used to treat certain patients with HBV infections (see Chapter 145). Ribavirin, a synthetic guanosine analogue, inhibits the replication of many RNA- and DNA-containing viruses through complex mechanisms involving inhibition of viral RNA synthesis and disturbances in intracellular pools of guanosine triphosphate.[156,157] This drug is used to treat some HCV infections and is sometimes administered in aerosolized form to treat respiratory syncytial virus (RSV) lower respiratory tract infection in hospitalized children and in severely ill and immunocompromised persons. Ribavirin therapy reduces the mortality associated with certain viral hemorrhagic fevers, such as that caused by Lassa virus.[158] Broader-spectrum therapies exemplified by IFN-α and ribavirin are considered for use against emerging pathogens and other susceptible viruses for which biochemical and structural information is insufficient to design high-potency agent-specific drugs.

VIRUS-HOST INTERACTION

One of the most formidable challenges in virology is to apply knowledge gained from studies of virus-cell interactions in tissue culture systems to an understanding of how viruses interact with host organisms to cause disease. Virus-host interactions are often described in terms of pathogenesis and virulence. *Pathogenesis* is the process whereby a virus



FIG. 131.5 Reovirus-induced apoptosis in the murine central nervous system. Consecutive sections of the hippocampus prepared from a newborn mouse 10 days after intracranial inoculation with reovirus. Cells were stained with (A) hematoxylin and eosin, (B) reovirus antiserum to detect viral antigen, and (C) antiserum to detect the activated form of apoptosis protease caspase-3. Cells that stain positive for reovirus antigen or activated caspase-3 contain a dark precipitate in the cytoplasm, including neuronal processes. Scale bars, 100 μm. *(Modified from Danthi P, Coffey CM, Parker JS, et al. Independent regulation of reovirus membrane penetration and apoptosis by the μ1 Φ domain.* PLoS Pathog. *2008;4:e1000248.)*

interacts with its host in a discrete series of stages to produce disease. *Virulence* is the capacity of a virus to produce disease in a susceptible host. Virulence is often measured in terms of the quantity of virus required to cause illness or death in a predefined fraction of hosts infected with the virus. Virulence is dependent on viral and host factors and must be measured using carefully defined conditions (e.g., virus strain, dose, and route of inoculation; host species, age, and immune status). In many cases, it has been possible to identify roles played by individual viral and host proteins at specific stages in viral pathogenesis and to define the importance of these proteins in viral virulence. Recently, the concept of microbial "pathogenic potential" has been introduced as a metric of its capacity to cause disease.[159] Pathogenic potential attempts to relate disease expression in the form of symptoms or death to organism dose and communicability.

Entry

The first step in the process of virus-host interaction is the exposure of a susceptible host to viable virus under conditions that promote infection (Fig. 131.6). Infectious virus may be present in respiratory droplets or aerosols, in contaminated food or water, or in a body fluid or tissue (e.g., blood, saliva, urine, semen, or a transplanted organ) to which the susceptible host is exposed. In some cases, the virus is inoculated directly into the host through the bite of an animal vector or through the use of a contaminated needle. Infection also can be transmitted from mother to infant through virus that has infected the placenta or birth canal or by virus in breast milk. In some cases, acute viral infections result from the reactivation of endogenous latent virus (e.g., reactivation of HSV giving rise to herpes labialis) rather than de novo exposure to exogenous virus.

Exposure of respiratory mucosa to virus by direct inoculation or inhalation is an important route of viral entry into the host. A simple cough can generate up to 10,000 small, potentially infectious aerosol particles, and a sneeze can produce nearly 2 million. The distribution of these particles depends on a variety of environmental factors, the most important of which are temperature, humidity, and air currents. In addition to these factors, particle size is an important determinant of particle distribution. In general, smaller particles remain airborne longer than larger ones. Particle size also contributes to particle fate after inhalation. Larger particles (>6 μm) are generally trapped in the nasal turbinates, whereas smaller particles may ultimately travel to the alveolar spaces of the lower respiratory tract.

Fecal-oral transmission represents an additional important route of viral entry into the host. Food, water, or hands contaminated by infected fecal material can facilitate the entry of a virus via the mouth into the gastrointestinal tract, the environment of which requires viruses that infect by this route to have certain physical properties. Viruses capable of enteric transmission must be stable to gastric acidity and resistant to bile salts. Because conditions in the stomach and intestine are destructive to the lipids contained in viral envelopes, most viruses that spread by the fecal-oral route are nonenveloped. Interesting to note, many viruses that enter the host via the gastrointestinal tract require proteolysis of certain capsid components to infect intestinal cells productively. Treatment of mice with inhibitors of intestinal proteases blocks infection by reovirus[160] and rotavirus,[161] which demonstrates the critical importance of proteolysis in the initiation of enteric infection by these viruses. The host microbiota is essential for infection by some viruses.[162–164]

To produce systemic disease, a virus must cross the mucosal barrier that separates the luminal compartments of the respiratory, gastrointestinal, and genitourinary tracts from the host's parenchymal tissues. Studies with reovirus illustrate one strategy used by viruses to cross mucosal surfaces to invade the host after entry into the gastrointestinal tract.[165,166] After oral inoculation of mice, reovirus adheres to the surface of intestinal microfold cells (M cells) that overlie collections of intestinal lymphoid tissue (Peyer's patches). In electron micrographs, reovirus virions can be followed sequentially as they are transported within vesicles from the luminal to the subluminal surface of M cells. Virions subsequently appear within Peyer's patches and then spread to regional lymph nodes and extraintestinal lymphoid organs such as the spleen. A similar pathway of spread is used by poliovirus[167] and HIV,[168] suggesting that M cells represent an important portal for viral invasion of the host after entry into the gastrointestinal tract.

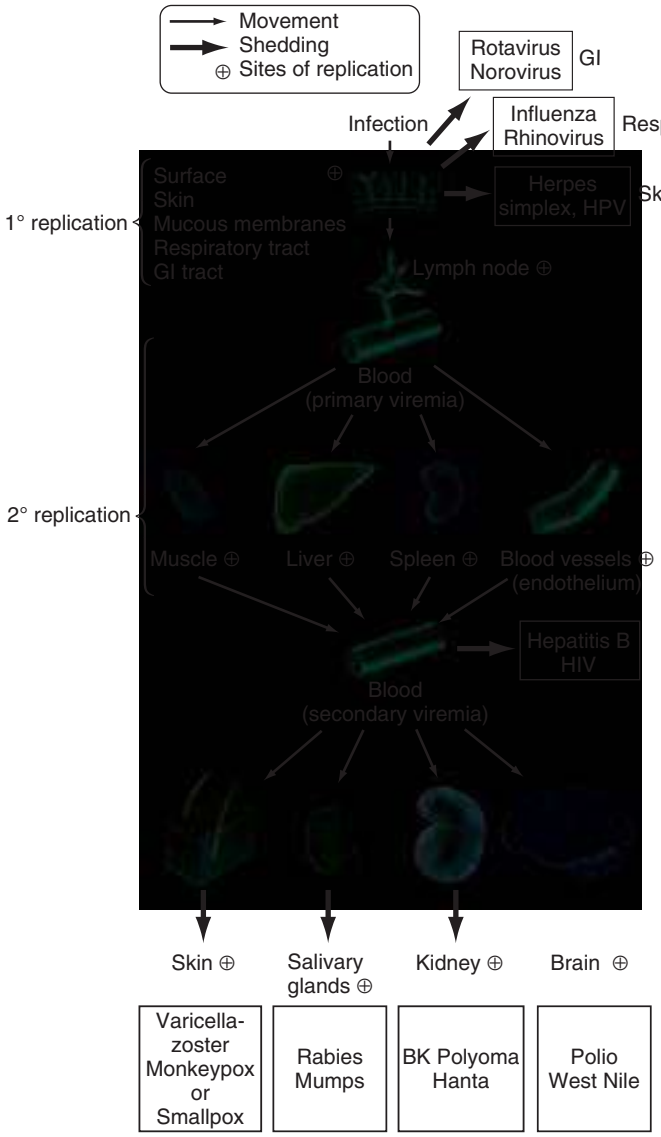

FIG. 131.6 Entry and spread of viruses in human hosts. Some major steps in viral spread and invasion of target organs are shown, represented by example viruses. Neural spread is not illustrated. *GI,* Gastrointestinal; *Resp.,* respiratory; *HIV,* human immunodeficiency virus; *HPV,* human papillomavirus. *(Modified from Nathanson N, Tyler KL. Entry, dissemination, shedding, and transmission of viruses. In: Nathanson N, ed.* Viral Pathogenesis. *Philadelphia: Lippincott-Raven; 1997:13–33.)*

Spread

Once a virus has entered the host, it can replicate locally or spread from the site of entry to distant organs to produce systemic disease (see Fig. 131.6). Classic examples of localized infections in which viral entry and replication occur at the same anatomic site include respiratory infections caused by influenza virus, rhinovirus, and RSV; enteric infections produced by norovirus and rotavirus; and dermatologic infections caused by HSV (cold sores and genital ulcers) and HPV (warts). Other viruses spread to distant sites in the host after primary replication at sites of entry. For example, poliovirus spreads from the gastrointestinal tract to the central nervous system (CNS) to produce meningitis, encephalitis, or poliomyelitis. Measles virus and varicella-zoster virus (VZV) enter the host through the respiratory tract and then spread to lymph nodes, skin, and viscera. Pathobiologic definitions of viruses based on spread potential have begun to blur amid accumulating evidence that model agents of localized infection may disseminate to distant sites. For example,

rotavirus, an important cause of acute gastroenteritis in children, replicates vigorously in villous tip epithelial cells of the small intestine but also frequently invades the bloodstream, the clinical significance of which is unclear.[169] Influenza virus is another case in point; viral RNA in blood is detected at a substantial frequency in hematopoietic cell transplant recipients and correlates with more severe disease and increased mortality.[170] Reports of rhinovirus viremia have begun to reshape thinking about the pathogenesis and disease spectrum of rhinovirus infections, which probably account for more episodes of serious respiratory illness than traditionally appreciated.[171,172]

Release of some viruses occurs preferentially from the apical or basolateral surface of polarized cells, such as epithelial cells. In the case of enveloped viruses, polarized release is frequently determined by preferential sorting of envelope glycoproteins to sites of viral budding. Specific amino-acid sequences in these viral proteins direct their transport to a particular aspect of the cell surface.[173,174] Polarized release of virus at apical surfaces may facilitate local spread of infection, whereas release at basolateral surfaces may facilitate systemic invasion by providing virus access to subepithelial lymphoid, neural, or vascular tissues.

Many viruses use the bloodstream to spread in the host from sites of primary replication to distant target tissues (see Fig. 131.6). In some cases, viruses may enter the bloodstream directly, such as during a blood transfusion or via an arthropod bite. More commonly, viruses enter the bloodstream after replication at some primary site. Important sites of primary replication preceding hematogenous spread of viruses include Peyer's patches and mesenteric lymph nodes for enteric viruses, bronchoalveolar cells for respiratory viruses, and subcutaneous tissue and skeletal muscle for alphaviruses and flaviviruses. In the case of reovirus, infection of endothelial cells leads to hematogenous dissemination in the host.[175,176]

Pioneering studies by Fenner with mousepox (ectromelia) virus suggested that an initial low-titer viremia (primary viremia) serves to seed virus to a variety of intermediate organs, where a period of further replication leads to a high-titer viremia (secondary viremia) that disseminates virus to the ultimate target organs (Fig. 131.7).[177] It is often difficult to discriminate primary and secondary viremias in naturally occurring viral infections. However, replication of many viruses in reticuloendothelial organs (e.g., liver, spleen, lymph nodes, and bone marrow), muscle, fat, and even vascular endothelial cells can play an important role in maintaining viremia.[176]

Viruses that reach the bloodstream may travel free in plasma (e.g., enteroviruses and togaviruses) or in association with specific blood cells.[178] A number of viruses are spread hematogenously by macrophages (e.g., CMV, HIV, and measles virus) or lymphocytes (e.g., CMV, EBV, HIV, HTLV, and measles virus). Although many viruses have the capacity to agglutinate erythrocytes in vitro (a process called hemagglutination), only in exceptional cases (e.g., Colorado tick fever virus) are erythrocytes used to transport virus in the bloodstream.

The maintenance of viremia depends on an interplay among factors that promote virus production and those that favor viral clearance. A number of variables that affect the efficiency of virus removal from plasma have been identified. In general, the larger the viral particle, the more efficiently it is cleared. Viruses that induce high titers of neutralizing antibodies are more efficiently cleared than those that do not induce humoral immune responses. Finally, phagocytosis of virus by cells in the host reticuloendothelial system can contribute to viral clearance.

A major pathway used by viruses to spread from sites of primary replication to the nervous system is through nerves. Numerous diverse viruses, including Borna disease virus, HSV, poliovirus, rabies virus, reovirus, and Venezuelan equine encephalitis (VEE) virus, are capable of neural spread. Several of these viruses accumulate at the neuromuscular junction after primary replication in skeletal muscle.[179,180] HSV appears to enter nerve cells via receptors that are located primarily at synaptic endings rather than on the nerve cell body.[181] Spread to the CNS by HSV,[182] rabies virus,[179,180] and reovirus[183,184] can be interrupted by scission of the appropriate nerves or by chemical agents that inhibit axonal transport. Neural spread of some of these viruses occurs through the microtubule-based system of fast axonal transport.[185]

Viruses are not limited to a single route of spread. VZV, for example, enters the host by the respiratory route and then spreads from respiratory epithelium to the reticuloendothelial system and skin via the bloodstream. Infection of the skin produces the characteristic exanthem of chickenpox. The virus subsequently enters distal termini of sensory neurons and travels to dorsal root ganglia, where it establishes latent infection. Reactivation of VZV from latency results in transport of the virus in sensory nerves to skin, where it gives rise to vesicular lesions in a dermatomal distribution characteristic of zoster or *shingles*.

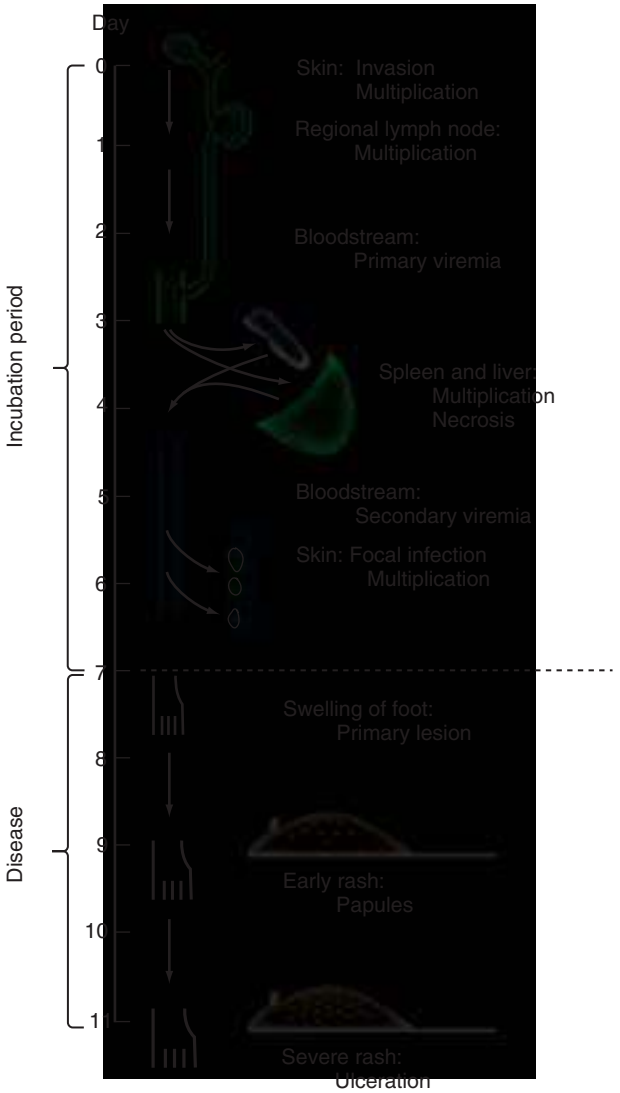

FIG. 131.7 Pathogenesis of mousepox virus infection. Successive waves of viremia are shown to seed the spleen and liver and then the skin. *(From Fenner F. Mousepox [infectious ectromelia of mice]: a review.* J Immunol. *1949;63:341–373.)*

Poliovirus also is capable of spreading by hematogenous and neural routes. Poliovirus is generally thought to spread from the gastrointestinal tract to the CNS via the bloodstream, although it has been suggested that the virus may spread via autonomic nerves in the intestine to the brainstem and spinal cord.[186,187] This hypothesis has been supported by experiments using transgenic mice expressing the human poliovirus receptor.[188] When these mice are inoculated with poliovirus intramuscularly in the hind limb, virus does not reach the CNS if the sciatic nerve ipsilateral to the site of inoculation is transected.[189] Once poliovirus reaches the CNS, axonal transport is the major route of viral dissemination. Similar mechanisms of spread may be used by other enteroviruses.

Tropism

The capability of a virus to infect a distinct group of cells in the host is referred to as *tropism*. For many viruses, tropism is determined by

the availability of virus receptors on the surface of a host cell. This concept was first appreciated in studies of poliovirus when it was recognized that the capacity of the virus to infect specific tissues paralleled its capacity to bind homogenates of the susceptible tissues in vitro.[190] The importance of receptor expression as a determinant of poliovirus tropism was conclusively demonstrated by showing that cells not susceptible for poliovirus replication could be made susceptible by recombinant expression of the poliovirus receptor.[191] In addition to the availability of virus receptors, tropism also can be determined by postattachment steps in viral replication, such as the regulation of viral gene expression. For example, some viruses contain genetic elements, termed *enhancers,* which act to stimulate transcription of viral genes.[192,193] Some enhancers are active in virtually all types of cells, whereas others show exquisite tissue specificity. The promoter-enhancer region of John Cunningham (JC) polyomavirus is active in cultured human glial cells but not in HeLa cervical epithelial cells.[194] Cell-specific expression of the JC virus genome correlates well with the capacity of this virus in immunocompromised persons to produce progressive multifocal leukoencephalopathy, a disease in which JC virus infection is limited to oligodendroglia in the CNS.

Specific steps in virus-host interaction, such as the route of entry and pathway of spread, also can strongly influence viral tropism. For example, encephalitis viruses such as VEE virus are transmitted to humans by insect bites. These viruses undergo local primary replication and then spread to the CNS by hematogenous and neural routes.[195] After oral inoculation, VEE virus is incapable of primary replication and spread to the CNS, illustrating that tropism can be influenced by the site of entry into the host. Influenza virus buds exclusively from the apical surface of respiratory epithelial cells,[196] which may limit its capacity to spread within the host and infect cells at distant sites.

A wide variety of host factors influence viral tropism. These include age, nutritional status, immune responsiveness, and certain genetic polymorphisms that affect susceptibility to viral infection. Age-related susceptibility to infection is observed for many viruses, including reovirus,[197,198] rotavirus,[199,200] and RSV.[201–203] The increased susceptibility in young children to these viruses may in part be due to immaturity of the immune response but also may be related to intrinsic age-specific factors that enhance host susceptibility to infection. Nutritional status is a critical determinant of the tropism and virulence of many viruses. For example, persons with vitamin A deficiency have enhanced susceptibility to measles virus infection.[204,205] Similarly, the outcome of most viral infections is strongly linked to the immune competence of the host.

The genetic basis of host susceptibility to viral infections is complex. Studies with inbred strains of mice indicate that genetic variation can alter susceptibility to viral disease through a variety of mechanisms.[206] These can involve differences in immune responses, variability in the ability to produce antiviral mediators such as IFN, and differential expression of functional virus receptors. Polymorphisms in the expression of chemokine receptor CCR5, which serves as a coreceptor for HIV,[52–54] are associated with alterations in susceptibility to HIV infection.[207,208] Furthermore, a single amino-acid polymorphism in the human rhinovirus C receptor, CDHR3, influences its abundance at the cell surface and the subsequent severity of viral infection.[209]

Added to effects of host genetic diversity, the intrinsic nonviral (i.e., principally bacterial) microbiota has come to attention as an important variable in host susceptibility to viral infection. Local microbial flora may exert a positive or negative influence on viral infectivity through mechanisms that may involve direct interactions between viruses and bacterial products or intermediary functions of the host immune system.[210] Notable examples of viral infections modulated by commensal microbes include mouse mammary tumor virus,[163] norovirus,[164] poliovirus,[162,214] and reovirus.[162]

Persistent Infections

Many viruses are capable of establishing persistent infections, of which two types are recognized: chronic and latent. *Chronic viral infections* are characterized by continuous production of virus for prolonged periods of time. Congenital infections with rubella virus and CMV and persistent infections with HBV and HCV are examples of chronic viral infections. *Latent viral infections* are characterized by maintenance of the viral genome in host cells in the absence of viral replication. Herpesviruses and retroviruses can establish latent infections. The distinction between chronic and latent infections is not readily apparent for some viruses, such as HIV, which can establish both chronic and latent infections in the host.[216–218] Viruses capable of establishing persistent infections must have a means of evading the host immune response and a mechanism of attenuating their virulence. For example, HCV[219] and HIV[220–222] are capable of extensive antigenic variation resulting in escape from neutralizing antibody responses by the host.

Several viruses encode proteins that directly attenuate the host immune response; for example, the adenovirus E3/19K protein[223] and CMV US11 gene product[224] block cell surface expression of MHC class I proteins, resulting in diminished presentation of viral antigens to cytotoxic T lymphocytes (CTLs). The poxviruses encode a variety of immunomodulatory molecules including CrmA, which blocks T-cell–mediated apoptosis of virus-infected cells.[225] In some cases (e.g., the CNS), preferential sites for persistent viral infections are not readily accessible by the immune system,[226] which may favor establishment of persistence. The central position and potent effects of innate immune responses in the control of viral infections, mediated primarily by the type 1 IFN system, has driven myriad survival adaptations and countermeasures among virtually all known human viruses. Specialized proteins or nucleic acids may function in viral antagonism of innate immunity, or viruses may adopt a passive strategy of sequestering provocative signals from cellular sensors of microbial invasion.[227,228]

Viruses and Cancer

Several viruses produce disease by promoting malignant transformation of host cells. Work by Peyton Rous with an avian retrovirus was the first to demonstrate that viral infections can cause cancer.[229] Rous sarcoma virus encodes an oncogene, v-*src,* which is a homologue of a cellular proto-oncogene, c-*src.*[230,231] Cells infected with Rous sarcoma virus become transformed.[232–236] Several viruses are associated with malignancies in humans. EBV is associated with many neoplasms, including Burkitt lymphoma, Hodgkin disease, large B-cell lymphoma, leiomyosarcoma, nasopharyngeal carcinoma, and multicentric Castleman disease. HBV and HCV are associated with hepatocellular carcinoma. HPV is associated with cervical cancer and a variety of anogenital and esophageal neoplasms. Kaposi sarcoma–associated herpesvirus is associated with Kaposi sarcoma and primary effusion lymphoma in persons with HIV infection.

Often, linkage of a virus to a particular neoplasm can be attributed to transforming properties of the virus itself. For example, EBV encodes several latency-associated proteins that are responsible for immortalization of B cells; these proteins likely play crucial roles in the pathogenesis of EBV-associated malignancies.[237] Similarly, HPV encodes the E6 and E7 proteins that block apoptosis[111–113] and induce cell cycle progression,[109,110] respectively. It is hypothesized that unregulated expression of these proteins induced by the aberrant integration of the HPV genome into host DNA is responsible for malignant transformation.[238] The tumorigenicity of polyomaviruses is mediated by a family of viral proteins known as tumor (T) antigens. Reminiscent of the HPV E6 and E7 proteins, T antigens induce cell cycling and block the ensuing cellular apoptotic response to unscheduled cell division.[239] The normally episomal polyomavirus genome becomes integrated into cellular DNA during neoplastic transformation of nonpermissive cells unable to support the entire viral replication program, which would otherwise culminate in cell death. Discovery of a human polyomavirus clonally integrated into cells of an aggressive form of skin cancer, Merkel cell carcinoma,[240] substantiates the long-standing suspicion that polyomaviruses can promote neoplasia in humans.

In other cases, mechanisms of malignancy triggered by viral infection are less clear. HCV is an RNA-containing virus that lacks reverse transcriptase and a means of viral genome integration. However, chronic infection with HCV is strongly associated with hepatocellular cancer.[241] It is possible that increased cell turnover and inflammatory mediators elicited by chronic HCV infection increase the risk of genetic damage,

which results in malignant transformation. Some HCV proteins also may contribute to neoplasia. For example, the HCV core protein can protect cells against apoptosis induced by a variety of stimuli, including tumor necrosis factor-α (TNF-α).[242]

VIRAL VIRULENCE DETERMINANTS

Viral surface proteins involved in attachment and entry influence the virulence of diverse groups of viruses. For example, polymorphisms in the attachment proteins of influenza virus,[243,244] polyomavirus,[245] reovirus,[246] rotavirus,[247] and VEE virus[248] are strongly linked to virulence and can be accurately termed *virulence determinants*. Viral attachment proteins can serve this function by altering the affinity of virus-receptor interactions or modulating the kinetics of viral disassembly. Important to note, sequences in viral genomes that do not encode protein also can influence viral virulence. Mutations that contribute to the attenuated virulence of the Sabin strains of poliovirus are located in the 5′ nontranslated region of the viral genome.[249] These mutations attenuate poliovirus virulence by altering the efficiency of viral protein synthesis. In another variation on the theme of virulence regulation by nontranslated RNA, a long noncoding RNA, known as polyadenylated nuclear (PAN) RNA, produced by Kaposi sarcoma–associated herpesvirus downregulates the host antiviral response and is required for induction of lytic replication from latency.[250]

A number of viruses encode proteins that enhance virulence by modulation of host immune responses. Illustrative examples include the influenza A NS1 protein, which interferes with activation of cellular innate immune responses to viral infection,[251] and translation products of the adenovirus E3 transcriptional unit, which serve to prevent cytotoxic T-cell recognition of virus-infected cells and block immunologically activated signaling pathways that lead to death of infected cells.[223,252] In many cases, these proteins are dispensable for viral replication in cultured cells. In this way, immunomodulatory viral virulence determinants are analogous to classical bacterial virulence factors such as various types of secreted toxins.

HOST RESPONSES TO INFECTION

The immune response to viral infection involves complex interactions among leukocytes, nonhematopoietic cells, signaling proteins, soluble proinflammatory mediators, antigen-presenting molecules, and antibodies. These cells and molecules collaborate in a highly regulated fashion to limit viral replication and dissemination through recognition of broadly conserved molecular signatures, followed by virus-specific adaptive responses that further control infection and establish antigen-selective immunologic memory. The innate antiviral response is a local, transient, antigen-independent, perimeter defense strategically focused at the site of virus incursion into an organ or tissue. Mediated by ancient families of membrane-associated and cytosolic molecules known as *pattern recognition receptors* (PRRs), the innate immune system detects pathogen-associated molecular patterns (PAMPs), which are fundamental structural components of microbial products including nucleic acids, carbohydrates, and lipids.[253] Viral PAMPs in the form of single-stranded (ss)RNA, dsRNA, RNA 5′ triphosphate, and DNA evoke the innate immune response through two groups of PRRs: the transmembrane Toll-like receptors (TLRs) and the cytosolic and nuclear nucleic acid sensors. The latter include retinoic acid inducible gene-I (RIG-I)-like receptors and DNA sensors such as cyclic GMP-AMP synthase.[254] Nucleic acid binding by PRRs activates signaling pathways leading to the production and extracellular release of IFN-α, IFN-β, and proinflammatory cytokines such as IL-1β and IL-18. IFN-α and IFN-β engage the cell surface IFN-α/β receptor and thereby mediate expression of hundreds of gene products that corporately suppress viral replication and establish an intracellular antiviral state in neighboring uninfected cells. Well-described IFN-inducible gene products include the latent enzymes dsRNA-dependent protein kinase (PKR) and 2′,5′-oligoadenylate synthetase (OAS), both of which are activated by dsRNA.[255] PKR inhibits the initiation of protein synthesis through phosphorylation of translation initiation factor eIF2α. The 2′,5′-oligoandenylates generated by OAS bind and activate endoribonuclease RNase L, which degrades viral mRNA. In addition to mediating an intracellular antiviral state, IFN-α/β also stimulates the antigen-independent destruction of virus-infected cells by a specialized population of lymphocytes known as natural killer (NK) cells.[256] Important to note, IFNs bridge innate and adaptive antiviral immune responses through multiple modes of action, which include enhancing viral antigen presentation by class I MHC proteins,[257] promoting the proliferation of MHC class I–restricted $CD8^+$ CTLs,[258] and facilitating the functional maturation of dendritic cells.[259] Proinflammatory mediators IL-1β and IL-18 pleiotropically stimulate and amplify the innate immune response through induction of other inflammatory mediators, immune cell activation, and migration of inflammatory cells into sites of infection.[260] These molecules perform essential functions in host antiviral defense.[261]

The adaptive immune response confers systemic and enduring pathogen-selective immunity through expansion and functional differentiation of viral antigen–specific T and B lymphocytes. Having both regulatory and effector roles, T lymphocytes are centrally positioned in the scheme of adaptive immunity. The primary cell type involved in the resolution of acute viral infection is the $CD8^+$ CTL, which induces lethal proapoptotic signaling in virus-infected cells on recognition of endogenously produced viral protein fragments presented by cell surface MHC class I molecules. Less frequently, $CD4^+$ T cells, which recognize MHC class II–associated viral oligopeptides processed from exogenously acquired proteins, also demonstrate cytotoxicity against viral antigen-presenting cells.[262] The usual function of $CD4^+$ T lymphocytes is to orchestrate and balance cell-mediated (CTL) and humoral (B lymphocyte) responses to infection. Several classes of helper T (Th) $CD4^+$ cell subsets have been defined based on characteristic patterns of cytokine secretion, gene-expression profiles, and effector activities.[263,264,265,266,267] Th1, Th2, Th9, Th17, Th22, T-regulatory (Treg), and T-follicular helper (Tfh) cells comprise distinguishable mature populations derived from undifferentiated $CD4^+$ precursor lymphocytes. Th1 and Th2 lymphocytes are usually associated with the development of cell-mediated and humoral responses, respectively, to viral infection. Understanding continues to evolve about the exact roles of other Th subsets in viral disease and antiviral immunity. For certain persistent viral infections, such as those caused by HIV and HSV, Treg cells might exacerbate disease through suppression of CTLs or, paradoxically, might ameliorate illness by attenuating immune-mediated cell and tissue injury.[265] Tfh cells promote differentiation of antigen-specific memory B lymphocytes and plasma cells within germinal centers.[266] Therefore Tfh cells likely occupy a central place in the humoral response to viral infection and vaccination. Although Tfh cell functions are not unique to antiviral responses, chronic viral infections including HBV and HIV appear to stimulate proliferation of these cells.[268,269]

The primacy of cell-mediated immune responses in combating viral infections is revealed by the extreme vulnerability of individuals to chronic and life-threatening viral diseases when cellular immunity is dysfunctional. Those with acquired immunodeficiency syndrome (AIDS) exemplify the catastrophic consequences of collapsing cell-mediated immunity; progressive multifocal leukoencephalopathy caused by JC polyomavirus, along with severe mucocutaneous and disseminated CMV, HSV, and VZV infections, are frequent complications of diminishing numbers of $CD4^+$ T cells. Similarly, iatrogenic cellular immunodeficiency associated with hematopoietic stem cell and solid-organ transplantation or antineoplastic treatment regimens predisposes to severe, potentially fatal infections with herpesviruses and respiratory viral pathogens such as adenovirus, coronavirus, influenza virus, PIV, and RSV,[270,271] all of which normally produce self-limited illness in immunocompetent hosts. Prevention and management of serious viral respiratory infections are significant challenges in myelosuppression units because of the communicability of respiratory viruses and paucity of effective drugs to combat these ubiquitous agents. Individuals with significantly impaired cell-mediated immunity also are at increased risk for enhanced viral replication and systemic disease after immunization with live-attenuated viral vaccines (e.g., measles, mumps, and rubella [MMR] and VZV vaccines). Hence, live viral vaccines are generally contraindicated for immunocompromised persons (see Chapter 316). TNF-α inhibitor therapy, increasingly used to manage a variety of inflammatory and rheumatologic diseases, enhances the risk of HBV reactivation, with potentially life-threatening consequences.[272]

Preventive and interventional HBV treatment strategies are necessary to circumvent complications of uncontrolled viral replication in these patients.

In contrast to cell-mediated immune mechanisms, humoral responses are usually not a determinative factor in the resolution of primary viral infections. One notable exception is a syndrome of chronic enteroviral infections in the setting of agammaglobulinemia.[273] However, for most human viral pathogens, the presence of antibody is associated with protection against initial infection in vaccinees or reinfection in hosts with a history of natural infection.[274] Longitudinal studies indicate that levels of protective serum antibodies (induced by natural infection or immunization) to common viruses, including EBV and measles, mumps, and rubella viruses, are remarkably stable, with calculated antibody half-lives ranging from several decades to thousands of years.[275] The protective role of antibodies on secondary exposure is frequently explained as interruption of viremic spread when a hematogenous phase is involved, such as occurs with measles, mumps, and rubella viruses, poliovirus, VZV, and most arboviruses. Nevertheless, most human viruses, excluding arthropod-transmitted agents, enter their hosts by penetration of a mucosal barrier, frequently undergoing primary replication in mucosal epithelium or adjacent lymphoid tissues. Neutralizing immunoglobulin A (IgA) exuded onto mucosal epithelial surfaces may protect against primary infection at this portal of viral entry. A classic example is gut mucosal immunity induced by orally administered Sabin poliovirus vaccine containing live-attenuated virus. Secretory IgA against poliovirus blocks infection at the site of primary replication and consequently interrupts the chain of viral transmission, although fully virulent revertant viruses arise at regular frequency in vaccine recipients, who may develop disease and also transmit revertant strains to nonimmune individuals.[276] Clinical and experimental studies of immunity to HIV have led to the recognition that resident immune responses at exposed mucosal surfaces are likely critical components of host resistance to primary HIV infection, and achievement of potent mucosal immunity has emerged as an important consideration for the design of candidate HIV vaccines.[277] Despite the appearance of serum neutralizing antibodies to HIV several weeks after infection, viral eradication is thwarted by selection of neutralization-resistant variant strains from a mutant pool, which is perpetually replenished because of extreme plasticity within neutralization determinants on the viral envelope glycoproteins.[278] Identification of epitopes bound by broadly neutralizing antiviral antibodies has provided potential new targets for structure-based vaccine design[279]; such a strategy has yielded a promising RSV vaccine candidate.[280]

Protection against viral infection by serum immunoglobulins is often correlated with antibody-mediated neutralization of viral infectivity in cultured cells. Antibodies interrupt the viral life cycle at early steps, which may include cross-linking virion particles into noninfectious aggregates, steric hindrance of receptor engagement, and interference with viral disassembly.[281] It is presumed that virus neutralization in cell culture by human serum is reflective of antibody activity in the intact host, but the mechanistic basis of infection blockade and disease prevention by antibodies in vivo is difficult to define precisely. For example, exclusively in vivo functions of the humoral antiviral response include Fc-mediated virion phagocytosis[282,283] and antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC responses require effectors from both the innate and adaptive systems, NK cells and antibodies, respectively.[284] The basis of ADCC is FcγRIIIA (CD16) receptor-dependent recognition by NK cells of virus-specific IgG bound to antigens expressed on the surface of infected cells, leading to release of perforin and granzymes from NK cells that eventuate in target cell apoptosis. Neutrophils, lymphocytes, and macrophages also possess Fc receptors and may participate in ADCC. New vaccine strategies are being developed to elicit these responses.

Although exuberant innate and adaptive antiviral immune responses are essential to restoration and preservation of normal host physiology in the face of viral infection, viruses paradoxically can undermine delicate systems of immune homeostasis. One such example is celiac disease, a complex T-cell-mediated intestinal disorder with an autoimmune component characterized by an inflammatory immune response to dietary gluten that occurs exclusively in individuals expressing human leukocyte antigen (HLA) DQ2 or DQ8. Approximately 30% to 45% of the US population carries a DQ2 or DQ8 allele, yet <1% of the population develops the disease. This observation suggests that additional environmental factors, such as viral infections, contribute to disease induction in genetically susceptible individuals. Infection of mice with some (but not all) strains of reovirus abrogates tolerance to fed antigen. Interferon regulatory factor 1, a transcription factor important in the antiviral immune response, is a key mediator of virus-induced loss of tolerance to fed antigen and celiac disease development. Moreover, humans with celiac disease have higher titers of antireovirus antibodies relative to unaffected controls, suggesting that reovirus may be an environmental trigger for the disease.[285]

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132 Orthopoxviruses Vaccinia (Smallpox Vaccine), Variola (Smallpox), Monkeypox, and Cowpox

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SHORT VIEW SUMMARY

Definition
- Orthopoxvirus infections can cause a spectrum of febrile rash illnesses in humans, ranging from fairly benign, localized skin infections to severe systemic infections.
- Vaccinia (smallpox vaccine), variola (smallpox), monkeypox, cowpox, and several newly discovered orthopoxvirus species are known to cause human disease.

Epidemiology
- Most orthopoxvirus infections are zoonotic, with humans serving as accidental hosts.
- Vaccinia virus continues to be used as a vaccine as well as a subject and tool for biomedical research, and it causes sporadic disease in vaccinees, contacts of vaccinees, laboratory workers, and farmworkers with occupational exposure to cattle.
- Variola, the causative agent of smallpox, caused significant morbidity and mortality worldwide before its eradication in 1980.
- Monkeypox virus causes intermittent human infections, primarily in Central Africa and West Africa, although isolated outbreaks have been identified in the United States and Sudan.
- Human cowpox virus infection was historically associated with occupational exposure to cattle; however, more recently, pet rats, pet cats, and zoo and circus elephants have been implicated.

Microbiology
- Orthopoxviruses are a group of large, complex, double-stranded DNA viruses that replicate in the cytoplasm of the host cell.

Diagnosis
- Diagnostic laboratory testing for orthopoxvirus infections can include polymerase chain reaction, viral culture, and electron microscopy of rash lesion material as well as serologic testing of serum.

Therapy
- Vaccinia immune globulin intravenous is licensed for treatment of certain complications of vaccinia vaccine administration and is available through the US Centers for Disease Control and Prevention.
- Tecovirimat is an antiviral compound licensed for treatment of human smallpox disease and is available through the Centers for Disease Control and Prevention.
- The development and evaluation of vaccines and therapeutics for orthopoxvirus infections is an active area of research.

Prevention
- Orthopoxviruses induce cross-reactive antibodies that protect against infection from other orthopoxvirus species.
- Vaccination with smallpox vaccine (i.e., vaccinia virus) can be used to protect individuals at high risk of orthopoxvirus disease.
- Other control measures focus on educational outreach to decrease the risk of exposure to likely zoonotic vectors.

BACKGROUND

The genus *Orthopoxvirus* belongs to the family of Poxviridae, a group of large, complex, double-stranded DNA viruses that replicate in the cytoplasm of the host cell and are defined by their genomic, structural, and antigenic similarities.[1,2] Humans can be infected by members of multiple poxvirus genera, but they are usually accidental hosts. Most human infections are zoonotic, and animal exposure and geographic location give clues to the etiologic agent. The orthopoxvirus variola, however, is a selective human pathogen. Variola is the causative agent of smallpox, which in 1980 was declared by the World Health Organization (WHO) to be eradicated worldwide. Other orthopoxviruses known to infect humans are cowpox, vaccinia, and monkeypox. New orthopoxviruses that cause localized human disease have been identified in the past 5 years (AK2015, NY_v014; discussed later). Species of poxvirus genera that infect humans other than within the *Orthopoxvirus* genus are discussed in Chapter 133. Comprehensive book chapter reviews and monographs on poxvirus virology are available.[3–5]

The ability of sera raised against one orthopoxvirus species to cross-neutralize another species is one of the fundamental reasons for cross-protection provided with vaccination. Vaccinia virus is the orthopoxvirus species now characterized as the constituent of smallpox vaccine. Other orthopoxviruses used as smallpox vaccines include horsepox virus and cowpox virus.[6] Because of the continued characterization of orthopoxviruses as causes of human illness and in part because of the concern that variola could be used as an agent of bioterrorism or as a biological weapon, there is continued interest and research on orthopoxviruses and poxviruses. The demonstration that orthopoxviruses can be created *de novo* using synthetic biology techniques has added a new dimension to these concerns.[7] Research on vaccinia is targeting fundamental viral properties and use of the virus as a (vaccine) vector as well as potential use as an oncolytic virus.[8,9] The emergence in 2003 of human clinical illness caused by monkeypox in the United States and ongoing disease in Central Africa, renewed reports of human monkeypox in West Africa in 2017, continued recurrence of human vaccinia infections in Latin American countries, and cowpox infections in Eurasia have also increased the need for knowledge of, and treatments for, these viral pathogens. Variola virus and the Congo Basin clade of monkeypox virus are considered select agents by the US Department of Health and Human Services and the US Department of Agriculture; these considerations impose additional restrictions on use in research laboratories and on reporting on discovery of these agents in clinical specimens.[10]

MORPHOLOGY AND CHEMICAL STRUCTURE

The poxviruses described in this chapter and Chapter 133 belong to the family Poxviridae, subfamily Chordopoxvirinae.[1] The eight genera of vertebrate poxviruses are *Orthopoxvirus, Parapoxvirus, Avipoxvirus,*

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Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus, and *Yatapoxvirus.* Only species of *Orthopoxvirus, Parapoxvirus, Molluscipoxvirus,* and *Yatapoxvirus* are known to infect humans. The last three are discussed in Chapter 133. Orthopoxvirus virions are large and brick shaped (as are the virions of yatapoxvirus and molluscipoxvirus). Poxvirus virions range in length from 220 to 450 nm and in width and depth from 140 to 260 nm.[11,12] The electron microscopic appearance of virions varies with sample preparation.[3] On cryoelectron microscopy of unstained, unfixed vitrified specimens, vaccinia and other orthopoxviruses appear as smooth, rounded rectangles; a uniform core is surrounded by a 30-nm membrane. In conventional thin sections, the core appears dumbbell shaped and is surrounded by a complex series of membranes. On negative stain, most virions have short surface tubules 10 nm in diameter and are referred to as M (mulberry) forms; a few virions, slightly larger and electron dense, appear to have a thick (20- to 25-nm) membrane or capsule (C form) (Fig. 132.1). Poxvirus particles contain about half of the approximately 200 potential virus genome-encoded proteins; virions are composed of structural proteins and enzymes including a virtually complete RNA polymerase system for primary transcription of viral genes.[3] The genome, which is within a nucleoprotein complex (nucleosome) inside the core, consists of a single linear molecule of double-stranded DNA that is composed, depending on the strain, of approximately 130 to 375 kb pairs of DNA and is covalently closed at each end; the ends are hairpin-like telomeres. Complete genome DNA sequences have been reported for several different species of the orthopoxviruses described in this chapter; GenBank entries are compiled at a dedicated website (https://www.viprbrc.org/brc/home.spg?decorator=pox).

During virus replication,[3,13,14] virion morphogenesis begins in the cytoplasm in areas known as cytoplasmic viral factories. Thin-section electron microscopic observations of cells early after infection show crescent-shaped membrane structures that progress to ovoid or circular structures, termed *immature virions,* which enclose a dense nucleoprotein complex. Primary transcription precedes the production of the crescents (cup shaped in three dimensions) and the immature virions. The bilayer surface membrane of the immature virion differentiates, with one layer becoming the outer membrane and the other becoming the core membrane, forming an intracellular mature virion (MV). A small portion of MVs may be further processed to acquire a bilayer envelope of Golgi intermediate compartment membrane that contains specific viral proteins. The intracellular enveloped virion (IEV) then moves along cellular microtubules to the cell surface, where actin polymerizes behind the IEV. IEVs exit the cell through distinctive microvilli by fusing the outermost lipoprotein layer with the plasma membrane, releasing the MV within the inner lipoprotein layer. The released particle is the extracellular enveloped virion (EEV); enveloped virions (EVs) that stay attached to the outer surface of the cell are cell-associated enveloped virions (CEVs). MVs and EVs are mature infectious particles, each with distinct surface antigenic properties. Enveloped and nonenveloped forms are believed to have different attachment sites on the cell[3]; however, the common result is fusion of the MV membrane with the plasma membrane, followed by entry and uncoating of the particle, release of viral contents into the cell, and initiation of virus-controlled transcription of early-, intermediate-, and late-class proteins.[3] Reviews of fusion and entry processes are available.[5,15,16] Secretion or expression of viral proteins modulates the host immune response or intracellular signaling to permit

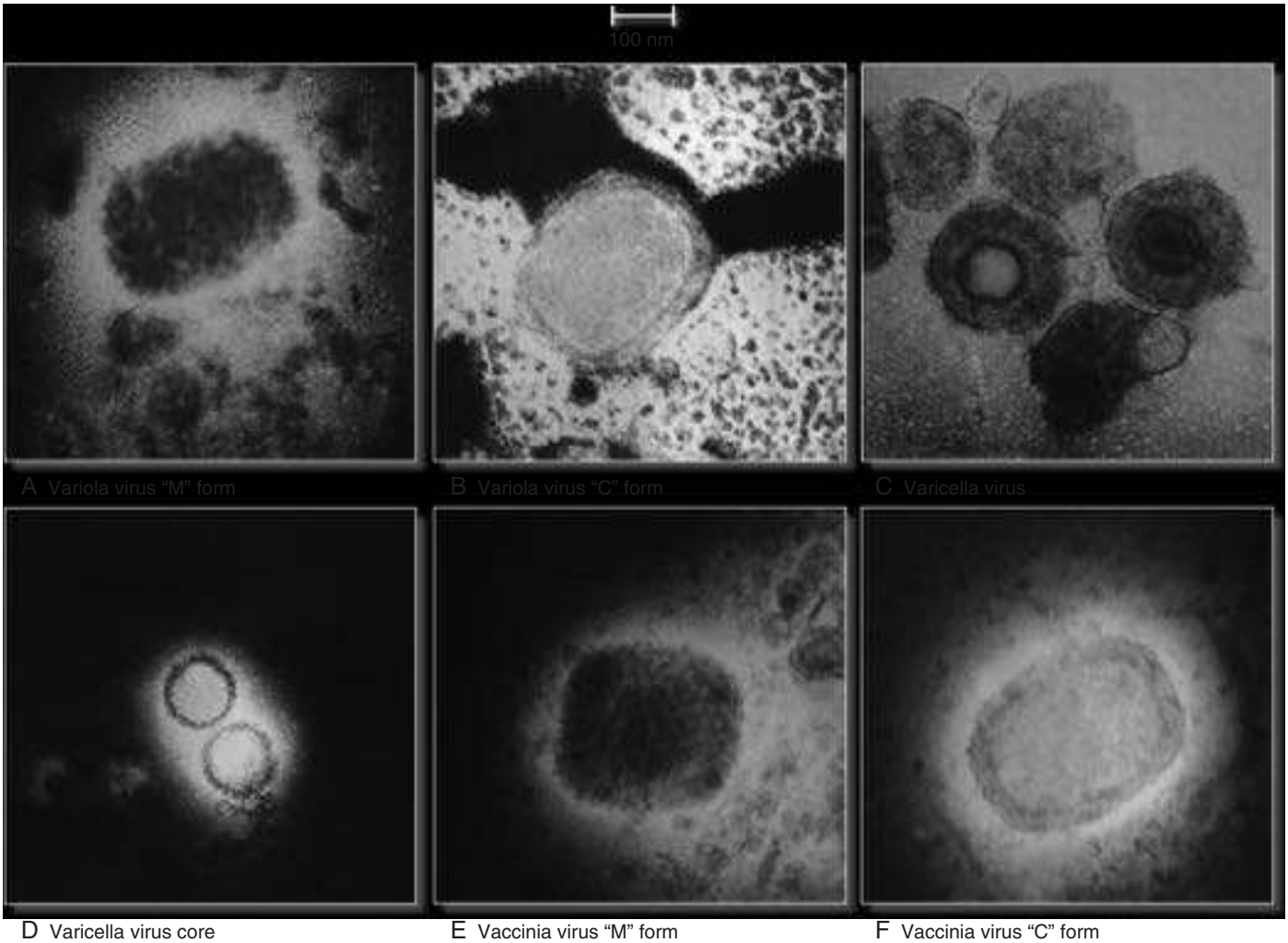

FIG. 132.1 (A–F) Electron micrographs of variola, varicella, and vaccinia virions. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library.* http://phil.cdc.gov/phil/default.asp. *Image ID 5 2426. Electron micrograph taken in 1975 by Dr. James Nakano, CDC.)*

the subsequent assembly, morphogenesis, release, and infectivity of progeny virions.

The dissemination of naturally released virions (EVs) is an important aspect of pathogenesis, and genes have been identified (e.g., *B5R*) that code for proteins necessary for the proper development of EEVs and expression of virulence. Host cell factors are also involved in EEV dissemination.[13,14,17]

PATHOGENESIS

Poxvirus infections evolve either as a localized, fairly benign skin infection or as a systemic infection. Systemic infection results in viral dissemination, with the formation of generalized skin lesions, and usually involves some degree of morbidity and mortality. The pathogenesis of human systemic orthopoxvirus infections is largely extrapolated and modeled from studies of the pathogenesis of the related orthopoxvirus infections in animals: mousepox (ectromelia) in mice, rabbitpox in rabbits, and monkeypox in nonhuman primates and prairie dogs.[18–23] In addition, extensive literature exists on animal models used to examine the role of virally encoded proteins predicted to be involved in viral pathogenesis or used to examine the effect of therapeutic methods or both.[24–27] The general paradigm for systemic orthopoxvirus pathogenesis is that the virus enters the host through a respiratory route, a mucosal surface, or a break in the skin and replicates locally. The virus then spreads through local lymphatics, causing a primary viremia, and subsequently spreads to the reticuloendothelial system. Replication in these organs results in the secondary viremia, usually associated with fever. The virus then ultimately seeds skin, causing a characteristic pock rash.

The host immune response probably involves all arms of the immune system. Complement, interferon, natural killer cells, and inflammatory cells are implicated in the early innate response; poxvirus-specific antibodies (including neutralizing antibodies) are subsequent components of the humoral response, and poxvirus-specific cytotoxic lymphocytes are involved in cellular clearance of infection.[28–35] Within their large genomes, poxviruses encode a number of proteins predicted to modulate the host's immune response, which affect both viral survival and disease pathogenesis. Various poxvirus-encoded proteins are predicted to bind and interfere with the function of host cytokines, chemokines, interferon, and complement. Other poxvirus-encoded proteins may interfere with apoptosis. Articles on immunomodulatory properties of poxviruses review specific properties of these virally encoded gene products.[5,36–38]

VACCINIA (SMALLPOX VACCINE)

Vaccinia is an orthopoxvirus and is the most studied of all poxviruses. It continues to be useful as a tool in medical research and is the focus of renewed interest as a vector for recombinant vaccines.[8] As such, it can pose an infection risk to laboratory personnel, and multiple exposures and infections have been documented in laboratory workers.[39] At some point, vaccinia replaced cowpox and horsepox[67] as the smallpox "vaccine." Genotypic analysis suggests that vaccine strains of vaccinia virus are most closely related to cowpox viruses from continental Europe and horsepox.[40] A single strain of the virus, derived from the New York City Board of Health (NYCBOH) strain, is used for smallpox vaccine in the United States. Other strains have been used outside the United States.

Smallpox vaccine is administered with a special bifurcated needle designed to hold a small standardized inoculum of a live virus suspension between its prongs. The skin over the deltoid or triceps is pierced multiple times with the needle with enough vigor to allow a trace of blood to appear after several seconds. Within 2 to 5 days of inoculation, a papule forms at the vaccination site. This papule evolves into a vesicle and then a pustule, reaching its maximum size (about 1 cm in diameter) by 8 to 10 days after vaccination, after which it dries to a scab, which usually separates by day 14 to 21.[41,42] An areola may encircle the site, and umbilication may occur as the lesion evolves. Low-grade fever is sometimes observed in children but rarely in adults. Regional lymphadenopathy may also occur. A scar at the inoculation site often provides lifelong evidence of successful vaccination, although the presence of a scar may not guarantee a history of successful smallpox vaccination because it may have resulted from bacterial superinfection or vaccination with the Calmette-Guérin bacillus.

Immunity Resulting From Vaccination

Vaccinia immunization is cross-protective against other orthopoxvirus infections including variola. Preexposure vaccine efficacy is estimated to be 100% for 1 to 3 years after vaccination,[43] and reports from the smallpox eradication efforts noted that "smallpox rarely occurs during the 4 or 5 years after successful vaccination in infancy."[44] Complete protection against smallpox after vaccination is not lifelong, although data suggest that substantial protection may persist for 15 to 20 years.[44–46] Experiences with monkeypox in the United States in 2003 suggest that immunity is not completely protective against systemic orthopoxvirus disease acquisition for more than 20 years after vaccination.[47,48] Protection against death from the disease may persist even longer than protection against disease.[49,50]

Likely because of the relatively long asymptomatic incubation period of systemic orthopoxvirus diseases (10–14 days), vaccinia is also effective as a postexposure prophylaxis when given to contacts of patients with smallpox. Vaccination should be performed as soon as possible after exposure; interpretation of data from the eradication program suggests that vaccination may not be as effective if given more than 3 days after the exposure.[51–54] Effectiveness was greater for individuals vaccinated previously.

Despite more recent advances, the correlates of immunity against smallpox are poorly understood. Humoral responses including neutralizing antibody correlate with protection in both animal[55] and human[56–58] studies. Cell-mediated and T-cell responses are also believed to be critical for successful vaccination.[59] T-cell responses have been documented up to 35 years after vaccination.[60–62] Neutralizing antibody responses are also long-lived.[61,63,64]

Complications Resulting From Vaccination

Of all vaccines used today, the smallpox vaccine has one of the highest rates of adverse events.[41,65–72] Major complications include progressive vaccinia, eczema vaccinatum, generalized vaccinia, accidental infection, postvaccinial encephalitis, and myopericarditis.

Progressive vaccinia, previously termed *vaccinia necrosum* or *vaccinia gangrenosum,* is a rare, often fatal vaccine complication in persons with severe deficiencies of cellular immunity.[65] In 1 year (1968) in the United States, five cases among 6 million primary vaccinations and six cases among 8.6 million revaccinations were found.[70] Four of these 11 patients died. Progressive vaccinia is characterized by progressive, often painless, growth and spread of the vaccine virus beyond the inoculation site, often leading to necrosis, sometimes with metastases to other body sites.[73] This diagnosis should be considered if the vaccination site lesion continues to progress and expand without apparent healing more than 15 days after vaccination.[74] Initially, limited or no inflammation is present at the site, and histopathologic examination shows an absence of inflammatory cells.[75] Management of progressive vaccinia has historically included aggressive therapy with vaccinia immune globulin (VIG), methisazone, débridement, and whole-blood transfusions from previously vaccinated individuals. The last-mentioned procedure, designed to bolster cell-mediated immunity, often resulted in a graft-versus-host reaction.[73] Methisazone is now regarded as ineffective therapy. Supportive measures and attention to prevention of secondary bacterial infections are beneficial, and newer therapeutic agents such as tecovirimat (TPOXX or ST-246) (discussed further in Chapter 48), cidofovir, and brincidofovir (CMX-001), may also have a role, although their efficacy is unproven (discussed further in "Therapy" and in Chapters 46 and 48).[41,66] VIG is available from the Centers for Disease Control and Prevention (CDC) as an intravenous formulation. In 2009, a severe case of progressive vaccinia developed in a member of the US Armed Forces after vaccination against smallpox and a subsequent diagnosis of acute myelogenous leukemia (subtype M0) and initiation of chemotherapy. The patient recovered after intensive treatment including multiple doses of vaccinia immune globulin intravenous (VIGIV), oral and topical tecovirimat, brincidofovir, and imiquimod.[67,68]

Eczema vaccinatum can occur in people with a history of atopic dermatitis (eczema) regardless of disease severity or activity. This complication is the clinical result of local spread or dissemination from the primary vaccination site in such persons or the result of inadvertent

contact of another's unscabbed vaccination site with a susceptible atopic individual's skin.[76,77] The clinical presentation is a localized or generalized papular, vesicular, or pustular rash anywhere on the body or localized to previous eczematous lesions. Systemic illness with fever, malaise, and lymphadenopathy may occur. In the 1968 national survey, 66 cases (no deaths) among 14.5 million vaccinations (4.6 cases per 1 million) and 60 cases (1 death) among several million contacts were found. Treatment of eczema vaccinatum includes administration of VIGIV (6000 U/kg, with repeat doses or higher doses or both depending on severity of symptoms and response to treatment),[43] hemodynamic support with fluid replacement and electrolyte monitoring, and skin care.[41] In one study, early VIG administration reduced the mortality rate from 30% to 40% to 7%.[78] In 2007 a severe case of eczema vaccinatum was successfully treated with a combination of multiple doses of VIGIV, a single dose of cidofovir, a multiple-day course of oral tecovirimat, intensive skin care management, and skin grafting.[79]

Generalized vaccinia is a nonspecific term that is used to describe a vesicular rash that develops after vaccination. True generalized vaccinia is believed to represent the end product of viremic spread of virus. However, documentation of virus in suspected generalized vaccinia vesicular rash lesions has been extremely rare, leading to the proposal of a new dermatologic manifestation termed *postvaccinial nonviral pustulosis* to explain these findings.[80,81] No predisposing factors have been identified. Treatment is generally not necessary because the generalized rash is self-limited. The lesions evolve and resolve more quickly than the primary vaccination site, presumably because of a developing immune response to the virus. This complication is estimated to occur in approximately 242 of every 1 million primary vaccinations.[41] In general, this complication of vaccination does not necessitate the administration of VIG unless it is severe and the patient is systemically ill or the patient has an underlying immunocompromising condition. Treatment with nonsteroidal antiinflammatory drugs or oral antipruritics may provide symptomatic relief.[41]

Postvaccination encephalomyelitis (PVEM) is a rare but serious complication that usually occurs only in primary vaccinees. The frequency of its occurrence differs widely from country to country and with the strain of vaccinia virus used in the vaccine. In the survey conducted in the United States in 1968, the frequency was 2.9 to 12.3 per 1 million first-time vaccinees.[41] The incidence of PVEM was lower with the NYCBOH vaccinia virus strain than with the strain used in other countries.[82] No predisposing factors are known, although host factors are believed to be important; the pathophysiology is not well understood. Cases have variably displayed clinical and diagnostic features suggestive of a postimmunization demyelinating encephalomyelitis/acute demyelinating encephalomyelitis or direct viral invasion of the nervous system.[83–85] This postvaccination reaction typically occurs 11 to 15 days after vaccination. Symptoms of PVEM include fever, headache, vomiting, confusion, delirium, disorientation, restlessness, drowsiness or lethargy, seizures, and coma. The cerebrospinal fluid can have an elevated pressure but generally has a normal cell count and chemistry profile.[86–88]

Children younger than 2 years can also develop a rare postvaccination encephalopathy (PVE) similar to PVEM. Acute onset of PVE occurs earlier in the postvaccination period (6–10 days after vaccination), involves the same symptoms as PVEM, and may include hemiplegia and aphasia.[4,41]

The diagnosis of PVE or PVEM is one of exclusion because no specific tests are available to confirm the diagnosis of this complication, and many other infectious and toxic etiologies can result in a similar clinical picture. More recent cases have shown the presence of orthopoxvirus-reactive antibodies in cerebrospinal fluid.[89,90] The role of antiviral medications is unclear; VIG, although effective prophylactically, has not shown clear benefit after symptom onset. A few cases have responded to some combination of intravenous immune globulin, steroids, and VIG.[89] A review of the pathogenesis of acute demyelinating encephalomyelitis and suggested therapies is available (also see Chapter 89).[91]

Accidental infection occurs when virus from the vaccination site is transferred to another site or to another person through intimate skin contact. It usually occurs in individuals receiving primary vaccination rather than a repeat vaccination. Accidental self-inoculation, which most commonly occurs on the face, mouth, lips, or genitalia, is usually not serious and requires no specific treatment. Inoculation of the conjunctiva, cornea, or eyelid is more serious and can be sight threatening if not evaluated and treated appropriately. In the years 1963–68, ocular vaccinia was observed in 348 people (259 vaccinees and 66 contacts). Of these, 22 had evidence of corneal involvement, and 11 had permanent defects.[92] No controlled trials of therapeutics exist; current topical optic antivirals (trifluorothymidine, vidarabine)[93] have in vitro activity against vaccinia, and their off-label use for this purpose has been recommended by some ophthalmologists (see Chapter 113).[41]

Cardiac adverse events are rare and had not been reported before 2003 in any person vaccinated with the NYCBOH strain. Myocarditis had been reported after vaccination with the vaccine strains used in Europe and Australia,[94,95] and in the US military population, myopericarditis was documented in 18 of 230,734 primary vaccinees immunized with the NYCBOH strain in 2002–03.[96] Clinical studies suggest that myopericarditis may occur in 1 in 175 adults who receive primary vaccination against smallpox.[97] However, few documented cases of dilated cardiomyopathy exist, and most cases appear to be mild and self-limited.[98,99] Arrhythmias and myocardial ischemia have also been described, but the association with vaccination is not as clear.[100–104]

Vaccination programs implemented in 2002–03 designed to help civilian public health preparedness and military preparedness for the possible use of smallpox as a weapon of bioterrorism documented lower adverse event rates than previously seen, in part because of stringent criteria and education programs to screen persons at risk for complications.[100,105,106] Nonetheless, instances of generalized rash, which may arise 10 to 14 days after vaccination, continued to be reported. Diagnosing true generalized vaccinia, which represents virus presumably spread hematogenously, on a clinical basis alone is often difficult, as it can be confused with a form of erythema multiforme or erythematous urticaria eruptions that may be immunologically mediated. Laboratory identification of virus within the disseminated rash may differentiate these conditions. Studies of recent vaccination efforts have also identified focal and generalized folliculitis associated with vaccination.[107]

The recommendations of the Advisory Committee on Immunization Practices on vaccinia vaccination are available at https://www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/smallpox.html. Additional clinical guidance for the use of smallpox vaccines in a smallpox emergency have been published.[108] The Advisory Committee on Immunization Practices recommends vaccination as a safeguard for laboratory and health care workers who are at high risk for orthopoxvirus infection.[109] In the United States the CDC Drug Service provides the vaccine after CDC approval of a formal request for this purpose by the administering physician. Vaccinia immunoglobulin is available to treat possible postvaccination complications, which can be severe.[110] In the United States the use of a cell culture–grown clonal derivative of the NYCBOH/Dryvax (Wyeth Pharmaceuticals, Collegeville, PA) smallpox vaccine has replaced use of Dryvax. Studies and development of this new vaccine, ACAM2000, have been previously reviewed.[97,111]

Vaccinia Virus as a Zoonosis

Vaccinia virus infections are not generally regarded as naturally occurring, although vaccinee-to-cattle and cattle-to-human transmissions occurred on farms during the smallpox eradication campaign. Sporadic outbreaks of infection caused by the vaccinia virus subspecies buffalopox virus, which involve transmission between milking buffalo, cattle, and people, have been reported mainly in India but also in Egypt, Bangladesh, Pakistan, and Indonesia. Vaccinia-like lesions have been observed on the animals' teats and the milkers' hands. Biologic data and limited DNA analyses of isolates from an outbreak in India in 1985 suggest that buffalopox virus may be derived from vaccinia virus strains transmitted from humans to livestock during the smallpox vaccination era.[112–114] A vaccinia virus possibly related to the vaccine strain used during smallpox eradication in Brazil was found in cattle and farm worker handlers in rural Rio de Janeiro.[115,116] Subsequent reports and surveillance efforts indicate that these vaccinia virus infections are ongoing and that at least two clades or subgroups of vaccinia are circulating in Brazil, likely representing low-level endemic disease.[117–119] Vaccinia virus infections of cattle and farm workers have also been identified in



FIG. 132.2 Smallpox lesions on skin of the trunk. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library. http://phil.cdc.gov/phil/default.asp. Image ID 5 284. Photograph taken in 1973 by James Hicks, CDC.)*



FIG. 132.3 Smallpox lesions on palms. This child was infected with the smallpox virus and on day 8 of the rash shows the typical lesions on his palms. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library. http://phil.cdc.gov/phil/default.asp. Image ID 5 3303. Photograph taken in 1972 by Dr. Paul B. Dean, CDC.)*

Colombia.[120] The ecologic factors that support the persistence of vaccinia in this region of the world remain unknown and are a topic of study.[121,122] In addition, at least two instances of human infection with a vaccinia-vector recombinant rabies virus vaccine in a bait dispersed to control rabies in wildlife have been reported; the bait was retrieved by a pet dog in both cases.[123,124]

VARIOLA (SMALLPOX)

In 1980 the WHO General Assembly declared that smallpox had been eradicated; destruction of the remaining virus stocks, initially scheduled for 1999, has been delayed in an effort to permit research for improved preparedness in the event smallpox recurs as the result of the malevolent use of variola virus. The virus has a strict human host range and no animal reservoir. Variola major strains produced a disease with a severe prodrome, fever, and prostration. The virus was most often transmitted between humans via large-droplet respiratory particles inhaled by susceptible persons who had prolonged close face-to-face contact with an infectious person; it was spread less commonly via aerosol or direct contact with the rash lesion or sloughed crust material from the scab.[82,125] A toxemia or other form of systemic shock led to case-fatality rates of up to 30%. Secondary attack rates among unvaccinated contacts within households ranged from 30% to 80%. Variola minor strains (alastrim, amass, or kaffir viruses) produced less severe infection and case-fatality rates of less than 1%, although secondary attack rates among unvaccinated contacts within households also ranged from 30% to 80%. The last naturally occurring smallpox case was in Somalia in October 1977, although a fatal laboratory-associated infection with variola major virus occurred at the University of Birmingham, England, in August 1978.[82]

Naturally acquired variola virus infection caused a systemic febrile rash illness. For ordinary smallpox, the most common clinical presentation, after an asymptomatic incubation period of 10 to 14 days (range, 7–17 days), was fever, with the temperature quickly rising to about 103°F, sometimes with dermal petechiae. Associated constitutional symptoms included backache, headache, vomiting, and prostration. Within 1 or 2 days after incubation, a systemic rash appeared that was characteristically centrifugally distributed (i.e., lesions were present in greater numbers on the oral mucosa, face, and extremities than on the trunk). Lesions were commonly manifested on the palms and soles (Figs. 132.2 and 132.3). Initially, the rash lesions appeared macular, then papular, enlarging and progressing to a vesicle by days 4 to 5 and a pustule by day 7; lesions were encrusted and scabby by day 14 and sloughed off (Fig. 132.4). Skin lesions were deep-seated and in the same stage of development in any one area of the body. Milder and more severe forms of the rash were also documented. Less severe manifestations (modified smallpox or variola sine eruptione) occurred in some vaccinated individuals, whereas hemorrhagic or flat-pox types of smallpox



FIG. 132.4 Smallpox lesions at day 17 of rash on a 5-year-old convalescing Indonesian child. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library. http://phil.cdc.gov/phil/default.asp. Image ID 5 2041. Photograph taken in 1963 by J.D. Millar, CDC.)*

are thought to have developed as a result of impaired immune response of patients.

Variola major smallpox was differentiated into the following four main clinical types:

1. Ordinary smallpox (90% of cases) produced viremia, fever, prostration, and rash; mortality rates were generally proportionate to the extent of rash. With the WHO classification, mortality rates ranged from less than 10% for "ordinary discrete" smallpox to 50% to 75% for the rarer "ordinary confluent" presentation.
2. (Vaccine)-modified smallpox (5% of cases) produced a mild prodrome with few skin lesions in previously vaccinated people and a mortality rate well under 10%.
3. Flat smallpox (5% of cases) produced slowly developing focal lesions with generalized infection and an approximate 50% fatality rate.

4. Hemorrhagic smallpox (<1% of cases) induced bleeding into the skin and the mucous membranes and was invariably fatal within a week of onset.

A discrete type of the ordinary form, with a typical febrile prodrome and rash, resulted from alastrim variola minor infection.[82] The WHO established a classification system for smallpox case types based on disease presentation and rash burden. The hemorrhagic and flat types have already been briefly described. The ordinary type was subdivided into three categories on the basis of the extent of rash on the face and the body. In the ordinary confluent category, no area of skin was visible between vesiculopustular rash lesions on the trunk or the face. Patches of normal skin were visible between rash lesions on the trunk in ordinary semiconfluent disease and on the face in ordinary discrete disease. (Vaccine)-modified disease arose with sparse numbers of lesions. Infection conferred lifelong immunity.[82,126]

Before its eradication, smallpox as a clinical entity was relatively easy to recognize, but other exanthematous illnesses were mistaken for this disease.[125–127] For example, the rash of severe chickenpox, caused by varicella-zoster virus, was often misdiagnosed as that of smallpox. However, chickenpox produces a centripetally distributed rash and rarely appears on the palms and soles. In addition, in the case of chickenpox, prodromal fever and systemic manifestations are mild, if they occur at all; the lesions are superficial in nature; and lesions in different developmental stages may be present in the same area of the body. Other diseases confused with vesicular-stage smallpox included monkeypox, generalized vaccinia, disseminated herpes zoster, disseminated herpes simplex virus infection, drug reactions (eruptions), erythema multiforme, enteroviral infections, scabies, insect bites, impetigo, and molluscum contagiosum. Diseases confused with hemorrhagic smallpox included acute leukemia, meningococcemia, and idiopathic thrombocytopenic purpura. The CDC, in collaboration with numerous professional organizations, has developed an algorithm for evaluation of patients with smallpox. The algorithm assists in differential diagnoses of the vesiculopustular stage of rash. The algorithm and additional laboratory testing information are available at https://www.cdc.gov/smallpox/clinicians/diagnosis-evaluation.html. The differential diagnosis of febrile vesicular pustular rash illnesses is presented in Table 132.1.

TABLE 132.1 Differential Diagnosis of Febrile Vesicular Pustular Rash Illnesses That May Be Confused With Smallpox

DISEASE	CLUES
Varicella	Most common in children younger than 10 years; children do not usually have a viral prodrome
Disseminated herpes zoster	Immunocompromised or elderly persons; rash looks like varicella, usually begins or erupts in dermatomal pattern
Impetigo *(Streptococcus pyogenes, Staphylococcus aureus)*	Honey-colored crusted plaques with bullae are classic but may begin as vesicles
Drug eruptions	Exposure to medications
Erythema multiforme minor	Target or bull's-eye lesions; often follows systemic viral infections such as herpes simplex virus; may include palms and soles
Erythema multiforme (including Stevens-Johnson syndrome)	Involves conjunctivae and mucous membranes
Enteroviral infections (especially hand-foot-and-mouth disease)	Seasonal—summer and fall
Disseminated herpes simplex virus	Similar to varicella
Scabies and insect bites	Pruritus; patient not febrile
Molluscum contagiosum	May disseminate in immunosuppressed individuals
Generalized vaccinia	History of vaccination with smallpox vaccine or contact with vaccinated individual
Monkeypox	Travel to endemic area; animal exposure

Modified from Centers for Disease Control and Prevention. Evaluating patients for smallpox: acute generalized vesicular or pustular rash illness protocol. https://www.cdc.gov/smallpox/clinicians/algorithm-protocol.html.

MONKEYPOX

Monkeypox virus was so named because it was first detected in captive Asiatic monkeys; however, the virus has been found naturally only in Africa (although it emerged in the United States as a result of global commerce), and evidence points to rodents as important reservoir hosts. Reviews of human monkeypox infection are available.[128–130]

Monkeypox was first recognized by Von Magnus in Copenhagen in 1958 as an exanthem of primates in captivity. The disease was later seen in other captive animals including primates in zoos and animal import centers. Particular attention was focused on it in 1970 when smallpox surveillance activities in Africa revealed cases of human monkeypox, clinically indistinguishable from smallpox, particularly in Zaire (now the Democratic Republic of the Congo [DRC]). Serosurveys and virologic investigations in the 1980s in the DRC by the WHO indicated that monkeys are sporadically infected, as are humans; three-fourths of cases, mainly in children younger than 15 years, resulted from animal contact; vaccinia vaccination has about 85% protective efficacy; monkeypox virus probably has a broad host range including squirrels (*Funisciurus* spp. and *Heliosciurus* spp.); and human monkeypox has a secondary attack rate of 9% among unvaccinated contacts within households (i.e., it is much less transmissible than smallpox). Since 1970 the disease has been seen in the DRC, Liberia, Ivory Coast, Sierra Leone, Nigeria, Benin, Cameroon, Gabon, Central African Republic, and South Sudan; most cases have been in the DRC, which in 1980 had a population of about 30 million (338 cases were discovered prospectively during WHO-intensified monkeypox surveillance in Zaire in 1981–86). Human monkeypox has continued to be reported from the DRC, mainly in children younger than 15 years. On the basis of reported monkeypox onset dates in a largely retrospective study complicated by a concurrent outbreak of chickenpox, about 250 serosubstantiated cases of monkeypox occurred among 0.5 million people in 78 villages, from February 1996 to October 1997. About three-fourths of the cases appeared to result from human-to-human transmission; however, the secondary attack rate of 8% among unvaccinated contacts within households appeared to be about the same as in the 1981–86 surveillance.[128,131]

Sporadic outbreaks continue to occur and cause concern,[128,132] but the most detailed clinical, epidemiologic, and ecologic information about virology laboratory–confirmed disease in Africa was obtained before 1988. Initial animal surveys in Zaire detected monkeypox-specific antibodies in 85 of 347 squirrels (25%) sampled but from none of 233 terrestrial rodents. Monkeypox-specific antibody has been detected in very few monkeys, which, similar to humans, are probably only occasional hosts.[133] Subsequent work[131] in the DRC found evidence of orthopoxvirus seroreactivity in some small terrestrial mammals tested including Gambian rats *(Cricetomys emini)* and elephant shrews *(Petrodromus tetradactylus)*. Studies in the 1980s that used direct virus sampling of trapped animals revealed virus in only one *Funisciurus* species.

In 2003 monkeypox infection of humans was identified in the United States as a result of exposure to ill prairie dogs, probably infected after exposure to infected West African small mammals imported as exotic pets.[134] From that work, a Gambian rat *(Cricetomys gambianus)*, rope squirrel (*Funisciurus* sp.), and dormouse (*Graphiurus* sp.) from the affected African shipment of exotic species originating in Ghana and implicated in the US monkeypox outbreak were found to be infected with monkeypox with viral isolation and nucleic acid detection (polymerase chain reaction).[134]

Pathogenesis

The pathogenesis of human monkeypox is surmised to be similar to that of smallpox: an acute febrile exanthem with an incubation period of about 12 days. During the incubation period, the virus is distributed initially to internal organs and then to the skin.[82,129] The main differences are a greater degree of lymphadenopathy and a lower capacity for human

case-to-case spread. The major concerns are the source of infection and the mode of transmission.

Clinical Manifestations

In general, the clinical features of disease as seen in Central Africa are those of a classic or modified case of smallpox. The most obvious difference is the pronounced lymphadenopathy, which involves the submandibular, cervical, and sublingual regions.

Most cases occur in unvaccinated children. In the DRC in 1981–86, 291 cases (86%) occurred in children younger than 10 years, and only 12 of them (4%) had vaccination scars. The illness lasts 2 to 4 weeks. Of 292 unvaccinated patients, 22 (7.5%) had a mild illness with fewer than 25 skin lesions and were not incapacitated; 55 (19%) had 25 to 99 lesions, were incapable of most physical activity, and needed nursing; and 218 (75%) had more than 100 lesions, were totally incapacitated, and needed intensive nursing. Complications occurred in about 40% of patients; the most common were bacterial skin infections (16%), respiratory (12%) and gastrointestinal (5%) disorders, and keratitis (3.8%). The overall mortality rate was approximately 10%; however, all the deaths occurred in unvaccinated children, in whom the group mortality rate is about 15%.[129] In the United States, disease in the 2003 outbreak appears to have been milder, and only 3 of the 37 laboratory-confirmed cases had complications or serious disease: keratitis, encephalopathy (with diffuse cortical, thalamic, and brainstem edema; meningeal enhancement; and left thalamic and right parietal signal abnormality on magnetic resonance imaging),[134] and upper respiratory tract lymphadenitis with dysphagia and airway compromise.[135] No deaths were observed. Although routes of exposure and host factors may have been significantly different in the African and US outbreaks, evidence of differences between DRC/Congo Basin and US/West African viral strains/clades is also evident.[136–138] Additional animal and human studies are beginning to define some of the viral genetic differences that may contribute to the differences in pathogenicity and apparent interhuman transmissibility of the two viral clades.[139,140] The skin lesions of monkeypox are illustrated in Fig. 132.5.

Diagnosis

Until 2003, human monkeypox had not been detected outside Africa. Clinical diagnosis may present a problem because fewer physicians now have experience with smallpox, which human monkeypox closely resembles. In vaccinated individuals, the rash may be more pleomorphic and not in a uniform stage of development.[129] Access to a virology laboratory should permit detection of the virus with electron microscopy and molecular methods, and this detection provides a diagnosis. In some circumstances, distinguishing between monkeypox and tanapox may be important. In the past, differentiation between monkeypox and variola was essential and could be done with examination of the pock appearance (hemorrhagic or not) and presence of pocks produced on chorioallantoic membrane at 39°C, a temperature that inhibits smallpox. Currently an array of nucleic acid diagnostic techniques permits the speciation of these viruses.[141–146]

The close serologic relationships among orthopoxviruses make detection of monkeypox-specific antigens difficult, but methods are available and being refined that may be of value, particularly for epidemiologic studies of monkeypox virus in its natural reservoirs.[11,133] An immunoglobulin M capture assay was shown to be sensitive and specific in identification of monkeypox cases 4 to 56 days after rash onset; this assay may have similar use in the identification of human smallpox cases.[147]



FIG. 132.5 Monkeypox skin lesions.

Epidemiology and Control

Management of individual cases has been supportive, with case-to-case spread reduced with isolation and, if available, the use of smallpox vaccine for contacts. Potential use of newer therapeutics is discussed at the end of this chapter. Human cases in Africa occur in villages in the rain forests, where a variety of animals are captured for food. Infection of children may be explained by their playing or working with carcasses. The results of the comprehensive surveys carried out in the 1980s indicated that infected individuals were principally unvaccinated children and that case-to-case spread was unusual. Control measures are based on interposing a buffer zone of cleared land between the arboreal reservoir and cultivated land, the development of animal husbandry as a source of meat, and education in the handling of wildlife, with emphasis on any trapping done by previously vaccinated individuals; continued vaccination was not thought necessary.[129,133]

Only occasional human cases were reported from Central Africa after cessation of routine surveillance activities following smallpox eradication, but a resurgence occurred in 1996–97 that has not yet been fully explained. Increased political unrest leads to population displacement and breakdown of routine control measures, and the levels of vaccine-induced immunity decline with time. A potentially serious finding that requires clarification is the observation that human case-to-case transmission appears to have occurred more frequently in 1996–97 than earlier.[132] A study of an outbreak in the Republic of the Congo also showed that the virus was transmitted quite efficiently between successive generations of human transmission associated with a hospital compound; six generations of uninterrupted interhuman transmission were identified and supported with laboratory studies.[148] Human monkeypox continues to be reported in the Congo Basin, and mildly symptomatic monkeypox or other orthopoxvirus infections may coexist in the area.[149–151] Congo Basin clade monkeypox was reported in Cameroon, Central Africa Republic, and Republic of Congo in 2017.[152] A sizable outbreak of monkeypox was reported in Nigeria in 2017 with multiple states affected. As of September 2018, more than 100 confirmed cases of monkeypox have been reported from Nigeria.[153] Comparison of the genomes of smallpox virus and monkeypox virus strains isolated up to 1986 suggested that they have evolved discretely,[154] and the results of complete genome analysis[155] confirm this observation. Laboratory workers studying monkeypox virus should be vaccinated with vaccinia and handle the virus in certified biosafety cabinets. Biosafety Level 2 (BSL-2) containment, according to the *Biosafety in Microbiological and Biomedical Laboratories* definition, should be the minimum containment used. BSL-3 laboratory practices provide additional biosafety protection. As mentioned previously, additional select agent requirements apply to use of the Congo Basin clade of this virus.

COWPOX

Cowpox sometimes occurs as a rare occupational infection of humans and can be acquired by contact with infected cows; more often, other animals (e.g., infected rats, pet cats, and zoo and circus elephants) have been sources of the disease. More recently, two cases of laboratory-acquired human infection with cowpox virus were documented.[156,157] Phylogenies show that cowpox virus is a diverse species and is geographically restricted; it has been isolated from humans and various animals in Europe and adjoining regions of Asia.[158–160] A serosurvey of wild animals in Great Britain found orthopoxvirus antibodies in a portion of bank and field voles and wood mice that were collected, which is consistent with small rodents being reservoir hosts for cowpox virus.[161]

Clinical Manifestations

Most information is available from a detailed analysis of 54 human cases investigated in 1969–93.[159] Lesions are generally restricted to the hands and face, and most patients (72%) have only one lesion. Multiple lesions may be caused by multiple primary inoculations, by autoinoculation, and occasionally by lymphatic or viremic spread. Lesions in humans occur mainly on the fingers, with reddening and swelling; autoinoculation of other parts of the body may occur, and systemic severe infections have been reported, often in individuals with immunosuppressive conditions.[162] Skin lesions are initially similar to lesions of a primary vaccinia virus vaccination; the site becomes papular, and in 4 to 5 days a vesicle develops. The lesion passes through macular, papular, vesicular, and pustular stages before forming a hard black crust. The lesion is usually painful, and erythema and edema are common at the late vesicular and pustular stages. Usually, lymphadenitis, fever, and general malaise occur, often referred to as influenza-like. These features are usually severe in children; 16 of 54 patients (30%) were hospitalized. Most patients take 6 to 8 weeks to recover; for some patients, recovery takes more than 12 weeks. Scarring is usually permanent.

NEWLY DESCRIBED ORTHOPOXVIRUSES

Human infection with a novel orthopoxvirus was detected in the country of Georgia.[163] Two men developed poxlike lesions on their hands after tending to ill cows. Both eventually recovered fully with some scarring. Although cowpox was suspected, DNA sequencing revealed a novel virus that, although related to known Old World orthopoxviruses, had significant phylogenetic branching divergences. Epidemiologic investigations suggested that cows were likely to be incidental hosts of this novel virus, whereas the natural reservoir may be small mammals such as shrews and rodents.[163] Other orthopoxviruses or closely related viruses have been characterized as the cause of human infections. These infections include the localized rash lesions in a resident of Alaska (AK2015)[164] and the infection of a renal transplant patient on an immunosuppressive regimen with a progressively spreading rash illness (NY-v014).[164,165]

ORTHOPOXVIRUS LABORATORY DIAGNOSTICS

Electron microscopy of vesicle fluid or extracts of crusts is particularly valuable because it distinguishes between parapoxvirus, herpesvirus, and other presumptive orthopoxvirus infections. Of 24 cases of cowpox in which adequate material was available, electron microscopy was successful in 23. Similar sensitivity was achieved in the diagnosis of smallpox.[12] Many protein-based diagnostic approaches have also been used[5,11] and further developed.[166] Nucleic acid–based diagnostics[142–144] are now commonly used to diagnose orthopoxvirus infections and to speciate them. Within currently defined species, not all strains of cowpox are identical, and genomic analysis may show differences of epidemiologic value. As well, although the phylogenetic differences are not as great within the monkeypox virus and vaccinia virus species, diagnostic techniques have been developed to differentiate monkeypox virus clades and vaccinia strains.

Virus may be isolated on the chorioallantoic membrane, where the production of characteristic hemorrhagic pocks is diagnostic. A cytopathic effect occurs in many cell lines (Vero, MRC-5, RK13), and detection of A-type inclusions is usually diagnostic for human cowpox virus infections, as it would be if found in biopsy material.

THERAPY

An active area of research involves the development and evaluation of therapeutics for orthopoxvirus infections. VIGIV is prepared from serum from individuals who have received smallpox vaccination. It has been licensed by the US Food and Drug Administration and is available through the CDC (https://www.cdc.gov/smallpox/clinicians/vaccine-medical-management6.html). Although controlled clinical trials of the efficacy of VIGIV have not been carried out, it has been used in the treatment of eczema vaccinatum, progressive vaccinia, and generalized vaccinia.[41] It can be considered for use in ocular complications of vaccinia except for vaccinia keratitis, in which it may be harmful (see Chapter 113). No clear benefit has been shown for vaccinia immune globulin alone in treatment of smallpox, but it may be useful in the treatment of other orthopoxvirus infections (see later). Comprehensive reviews of the history and latest developments in poxvirus antivirals and therapeutics have been published.[5,93,167] In addition, such compounds are being tested on experimental models of variola-infected nonhuman primates[168] and on other animal models of orthopoxvirus infections.[25,26,169] The earliest drug compounds shown to have activity against orthopoxviruses including vaccinia were thiosemicarbazone derivatives. Initial case studies and case series suggested that they were effective in prophylaxis of smallpox and in treatment of progressive vaccinia and eczema vaccinatum.[170,171] However, subsequent double-blind controlled trials of smallpox prophylaxis revealed no benefit.[172]

A number of different classes of therapeutics have been tested for potential systemic or topical use against orthopoxviruses. These include compounds predicted to interfere with specific viral enzymes and cellular targets.[167] In vitro studies have usually focused on compounds currently licensed or under phase I or II study, although some novel compounds have also been studied. Promising candidates have been studied in small animal models (mouse, rabbit, and others) and in nonhuman primates. Because monkeypox and smallpox both cause human illness, with mortality rates that range from 10% to 40% in nonvaccinated individuals, models have been designed to evaluate drug efficacy in systemic lethal disease created by intranasal, aerosol, or (historically) intracerebral virus challenge. Intravenous challenges classically have been designed to evaluate the effect of drug on rash development or illness progression, although higher challenge doses of virus have been used for a lethal model.[173] The primary animal models currently used involve a challenge of aerosolized or intranasal virus, which results in pulmonary disease and lethality. Models of localized rash lesions have involved scarification of animal skin. Treatment models of keratitis involve scarification of corneal tissue. Work evaluating the potential use of antivirals for treatment of systemic complications of vaccination (progressive vaccinia and eczema vaccinatum) has used immunodeficient mouse populations.

The most studied compounds are inhibitors of DNA polymerase. Some nucleoside analogue compounds with activity against herpesviruses, most notably acyclovir and its derivatives, do not have activity against poxviruses. Other compounds with antiherpesvirus activity do show in vitro and in vivo activity against poxviruses, specifically 5-iodo-2′-deoxyuridine, adenine arabinoside, and trifluorothymidine.[174–177] Because of their systemic toxicity, these compounds have also been used topically for treatment of orthopoxvirus (and herpesvirus) ocular infections. Of the three compounds, trifluorothymidine appears to be most widely available. Many phosphonate-nucleoside analogues (e.g., cidofovir) have antiorthopoxvirus activity. In vitro, cidofovir has been shown to be active against the orthopoxviruses cowpox, vaccinia, monkeypox, and variola.[26,178,179] In vivo studies with cidofovir have shown it to successfully protect challenged animals when given prophylactically or early in the evolution of disease, often before the onset of overt symptoms. Cidofovir has known renal toxicity and is administered with hydration and probenecid. It has a long intracellular half-life but is not orally bioavailable; the alkoxyalkyl ester analogue of cidofovir, 1-*O*-hexadecyloxypropyl cidofovir (brincidofovir), is orally bioavailable and has been reported to have a protective effect in mice challenged with aerosolized cowpox, mice challenged with a lethal inoculum of mousepox virus, and rabbits challenged with a lethal inoculum of rabbitpox virus.[179–181] Brincidofovir has also been shown to be active against variola virus in tissue culture.[182] Other nucleoside analogues are also under study.[183,184]

Tecovirimat (ST-246 or TPOXX) is a novel orally bioavailable compound with antiorthopoxvirus activity, characterized to inhibit orthopoxvirus release. Genetic characterization of viral mutants resistant to tecovirimat indicate the viral target is the orthopoxvirus homologue of the vaccinia F13 protein, which is needed for wrapping of the virus before its release as an enveloped viral particle.[185] The drug has been shown to be active against multiple orthopoxvirus species, including monkeypox and variola virus.[186,187] Tecovirimat, used prophylactically before the onset of symptoms or therapeutically after the onset of symptoms, has been shown in a variety of small animal

models to prevent disease or to significantly mitigate illness severity and mortality.[188–190] As mentioned earlier in the section on vaccinia virus, it has been used, in combination with other therapeutic methods, in the successful treatment of severe cases of eczema vaccinatum and progressive vaccinia. Animal studies have shown the drug has synergistic benefit when combined with brincidofovir.[191] The drug was approved by the US Food and Drug Administration in July 2018 for the treatment of human smallpox disease. (Tecovirmat is discussed further in Chapter 48.)

Other antivirals tested against orthopoxviruses have predicted cellular targets. Ribavirin, an inosine monophosphate dehydrogenase inhibitor, shows in vitro activity against a number of orthopoxviruses[192] and has shown antiorthopoxvirus activity in animal models of vaccinia-induced keratitis[193] and mouse tail pock lesions.[194] Case reports of the use of ribavirin and VIG in the treatment of progressive vaccinia are available.[195] Initial therapy with ribavirin alone was ineffective at stemming new lesions; however, with the addition of VIG, new lesion development was stopped.

Additional studies of compounds targeting cellular kinases are of interest as another potential antiorthopoxvirus therapeutic strategy. Various cellular kinases (Abl, Src, and others) have been shown to be involved in the egress of virus, and blocking their function provides a different "antiviral" therapeutic mechanism of action.[196,197] Studies of an ErbB kinase inhibitor (CI-1033) showed benefit in treatment of vaccinia-infected mice; its benefit was augmented with the use of a monoclonal antibody that neutralizes MV viral particles. In vitro, CI-1033 appeared to inhibit the local release of virus from an infected cell, so as to impede the ability of the virus to subsequently infect other surrounding (uninfected) cells.[198]

Comprehensive summaries of antiorthopoxvirus therapeutic development are available,[5,167,17,199–202] and this is an area of active research. For example, a study evaluating a 19th century therapy for smallpox involving a botanical preparation of the carnivorous plant *Sarracenia purpurea* demonstrated antiorthopoxvirus activity against vaccinia, monkeypox, and variola viruses.[203]

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133 Other Poxviruses That Infect Humans: Parapoxviruses (Including Orf Virus), Molluscum Contagiosum, and Yatapoxviruses

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SHORT VIEW SUMMARY

Definition
- Parapoxviruses, molluscum contagiosum, and yatapoxviruses are among the nonorthopoxvirus infections of humans.

Epidemiology
- Parapoxvirus infections most commonly occur in individuals with occupational exposures to infected sheep, cattle, or goats.
- Molluscum contagiosum infection occurs worldwide and is spread through mild skin trauma, fomites, and sexual transmission.
- Yatapoxvirus infections are rarely reported; infections are acquired through exposure to infected animals and potentially arthropod vectors and are usually geographically restricted to Central Africa and East Africa.

Microbiology
- Poxviruses are a diverse group of large, complex double-stranded DNA viruses that replicate in the cytoplasm of the host cell.

Diagnosis
- Parapoxvirus, molluscum contagiosum, and yatapoxvirus infections are often diagnosed clinically based on their characteristic clinical features in combination with appropriate exposure and travel histories.
- Diagnostic laboratory testing for poxviruses may include polymerase chain reaction assay, electron microscopy, viral culture, and serology.

Therapy
- Parapoxvirus infections are usually self-limited; treatment with topical and intralesional cidofovir as well as imiquimod has been anecdotally reported.
- Multiple modalities for treatment of molluscum contagiosum have been described including cryotherapy, mechanical curettage, and chemical treatments with podophyllin-podofilox, cantharidin, iodine, and tretinoin.

Prevention
- Transmission of parapoxviruses, molluscum contagiosum, and yatapoxviruses can be prevented by avoiding exposures to animal vectors, properly covering lesions, and observing good hand hygiene practices.

PARAPOXVIRUSES

Parapoxviruses, which are found worldwide, are common pathogens of sheep, goats, and cattle. Human infection, characterized by localized epithelial lesions, is an occupational hazard for people who handle infected animals. Parapoxvirus infection in sheep and goats is usually referred to as *sore mouth, scabby mouth, contagious pustular dermatitis/ecthyma,* or *orf,* and the corresponding human infection is referred to as *orf.* Taxonomically the relevant parapoxvirus species is referred to as *Orf virus*; synonyms are contagious pustular dermatitis virus and contagious ecthyma virus. Parapoxvirus infection of dairy cattle is usually referred to as *paravaccinia, pseudocowpox,* or *ring sores,* and the human equivalent is referred to as *paravaccinia, pseudocowpox,* or *milker's nodes.* The virus species is referred to as *pseudocowpox virus*. Parapoxvirus infection of beef cattle is referred to as *stomatitis papulosa.* The parapoxvirus species associated with beef cattle is referred to as *bovine papular stomatitis virus*. Other zoonotic infections caused by tentative members of the *Parapoxvirus* genus and shown to infect humans are derived from camel exposure (contagious ecthyma or Ausdyk disease) or, less often, from seals (sealpox) and deer.[1,2,3] The parapoxvirus of red deer in New Zealand is another parapoxvirus species. Lesions in animals are found on the skin, in the oropharyngeal mucosa, and on external surfaces. Other parapoxviruses documented to cause ungulate, pinniped, or other nonhuman animal infections may also cause human illness. Detailed reviews of members of this genus of Poxviridae are available.[4–7]

Morphology and Composition of the Agent

The parapoxviruses have a unique appearance among poxviruses, as revealed with negative-stain transmission electron microscopy. Most poxvirus virions are brick shaped, but parapoxvirus particles are oblong, rounded, or ovoid. In addition, members of the *Parapoxvirus* genus have a characteristic M form that can be observed with negative-stain electron microscopy: one long spicule wraps the particle, giving a crisscross effect.[8] The stability of the virus in scabs is correlated with possible transmission of the virus through fomites.

The parapoxvirus genome consists of a linear double-stranded DNA of about 135 kilobase pairs with covalently closed terminal hairpins; the genome is relatively high in G+C content and is smaller than other poxvirus genomes. Complete genome sequences of Orf virus and bovine papular stomatitis virus have been reported.[9] Several proteins have been described to have potential roles in viral pathogenesis[10] including a chemokine-binding protein,[11] an interleukin-10 homologue,[12] a vascular endothelial growth factor homologue,[13] an interferon resistance gene,[14] and a cytokine-binding protein.[15]

Pathogenesis and Immune Response

Infection, which occurs via cuts and scratches, usually remains localized in the epithelium or oral mucosa. Human lesions of orf are produced by hypertrophy and proliferation of epidermal cells, which is often marked and perhaps related to the endothelial growth factor homologue encoded by the virus and to leukocyte infiltration. Histologic examination of human lesions shows many small multilocular vesicles within the dermis; true macrovesicles rarely occur.[16,17] Generalized symptoms of lymphadenopathy, malaise, and disseminated lesions are uncommon, and the immune response is not protective against disease recurrence on a lifelong basis.[17,18] Second attacks occur in 8% to 12% of individuals.[17,19]

Clinical Manifestations

Detailed descriptions of human disease progression are available,[16–18] as are illustrations (Fig. 133.1).[20,21] In brief, infection manifests as localized lesions at the site of inoculation by a diseased animal. The portal of entry is usually a break in the skin. Six stages of clinical disease are

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FIG. 133.1 Human orf virus infection. Nodule caused by orf virus after contact with a lamb being sacrificed for a holiday—Massachusetts 2010. *(From Centers for Disease Control and Prevention. Human orf virus infection from household exposures—United States, 2009-2011.* MMWR Morb Mortal Wkly Rep. *2012;61:245–248.)*

described. After a brief incubation period of 3 to 5 days, lesions begin as (pruritic) erythematous macules and then raise to form papules, often with a target appearance (days 7–14). Lesions become nodular or vesicular, and orf lesions often ulcerate after 14 to 21 days; this ulceration has been referred to as the *acute stage.* Complete healing can take 4 to 6 weeks and is characterized by a regenerative papilloma and regressive stages where normal epithelium is seen once again.[17] Very large granulomatous lesions occur, more frequently in individuals who are immunocompromised, and these may need surgical removal; other, less invasive therapeutic methods may now be feasible and are discussed later in this chapter.[22] Milker's node lesions may have a more nodular appearance, without ulceration. Parapoxvirus infections reported in handlers of reindeer and musk-oxen in Norway are more granulomatous and persist for months.[23] Erythema multiforme and Stevens-Johnson syndrome have been associated with parapoxvirus infections.

Diagnosis

Polymerase chain reaction diagnostic tests generic for parapoxvirus[24,25] and orf[25,26] have been reported; however, the infection is usually clinically diagnosed on the basis of exposure history and the presence of a characteristic lesion. Negative-stain transmission microscopy of lesion material examined by a skilled observer can be diagnostic if the characteristic structure is observed. Virus isolation in tissue culture usually requires primary ovine or bovine cells and may be difficult to attain.[27] The development of immunologic sera specific for parapoxviruses is another source for diagnostic reagents.[28]

Epidemiology

Infection with parapoxvirus is an occupational hazard of farm workers, abattoir workers, veterinarians, students, and others with frequent exposure to sheep, cattle, or goats. Human orf infection is most common in the spring, a time when the bottle-feeding of lambs may predispose humans to exposure risks, and in the fall, when slaughtering and shearing occur.[17] Of 191 cases of orf or milker's nodule with a known source surveyed from 1978–95, 84% had an ovine source, and 16% were transmitted by cattle. An additional 32 cases occurred in abattoir workers.[29] Human orf infections have also been associated with exposures during household meat processing or animal slaughter, particularly in association with religious holidays.[30,31] Another study evaluating a cluster of cases in children in the Midwest United States found that facial contact with infected animals often led to disease acquisition in children.[25] Most workers at risk are infected at some point in their career, and reinfection is not uncommon. Infected individuals should take care not to further infect themselves with autoinoculation or to spread infection to contacts, including animals. The vaccine used to control orf in sheep is fully virulent and has caused human infection.

Therapy

In most cases, the disease is self-limited. Anecdotal reports of the use of 3% cidofovir topical cream[32,33] have described apparent beneficial effects; however, no controlled trials are available. Topical treatment with the Toll-like receptor/interferon modulating compound imiquimod has also shown benefit in a number of cases,[34,35] including the case of a patient with a giant orf lesion that failed to respond to topical and intralesional cidofovir.[36]

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum, a disease that causes a benign self-limited skin "tumor" or papular eruption, occurs worldwide and is regarded as a specific human infection.[37] Although no evidence exists of disease transmission between humans and other animals, lesions that resemble molluscum and contain pox virions have been detected in species other than humans (e.g., horses and chimpanzees).

Description of the Agent

Four subtypes, characterized by restriction endonuclease digests, have been described.[38] Disease presentation by all subtypes appears to be similar. The genome of molluscum contagiosum virus subtype I has been sequenced.[39] This genome encodes several novel gene products involved in its pathogenesis and in evasion of the immune system including an interleukin-18–binding protein[40] and apoptosis inhibitors,[41] among others.

Pathogenesis and Pathology

Molluscum contagiosum lesions have long been known to have a distinctive pathology. In 1841 the first description of characteristic molluscum bodies—Henderson-Paterson bodies—was provided by Henderson and Paterson. Onset of infection occurs when the virus begins replication in the lower layers of the epidermis[42] and then extends upward. The incubation period is quite variable and can be lengthy (2–7 weeks; as long as 6 months has been suggested). The epidermis hypertrophies and extends down into the underlying dermal strata. Characteristic inclusions (Henderson-Paterson bodies, or molluscum bodies) are formed in the prickle cell layer and gradually enlarge as cells age and migrate to the surface. These cells are replaced by hyperplasia of the basal cell layer. The structure of the basement membrane remains intact; the hypertrophied epidermal cells, with their cytoplasm occupied by a large acidophilic granular mass (the molluscum body), project above the skin to appear as a tumor.[43] Little to no inflammatory infiltrate is seen until late in disease, just before natural resolution of the lesion occurs.[44]

Clinical Manifestations

Infection occurs after breakage of the skin. The characteristic lesion begins as a small papule and, when mature, is a discrete, 2- to 5-mm-diameter, smooth, dome-shaped, pearly or flesh-colored nodule that is often umbilicated (Fig. 133.2). A cheesy off-white, sometimes yellowish, material is easily expressed from lesions. Usually, 1 to 20 lesions occur, but occasionally, hundreds may be seen. Because of multiple simultaneous infections, or mechanical spread, these lesions may become confluent along the line of a scratch, and satellite lesions are occasionally seen.

In children, lesions occur mainly on the trunk and proximal extremities. In adults, they tend to occur on the trunk, pubic area, and thighs, but in all cases, infection may be transmitted to other parts with autoinoculation.[44] In patients infected with human immunodeficiency virus (HIV), molluscum infections occur along the beard line in male patients; with facial involvement, ocular involvement such as lesions on the bulbar conjunctiva has been found.[45] Individual lesions last for about 2 months, but the disease usually persists for 6 to 9 months.[46] Severe and prolonged infection tends to occur in individuals with impaired cell-mediated immunity, including patients with HIV infection.[37,47]

Diagnosis

The clinical appearance of lesions is generally sufficiently characteristic to permit clinical diagnosis. Brick-shaped virions can usually be seen in large numbers if the cheesy material expressed from the lesion is examined with transmission negative-stain electron microscopy. The virus has

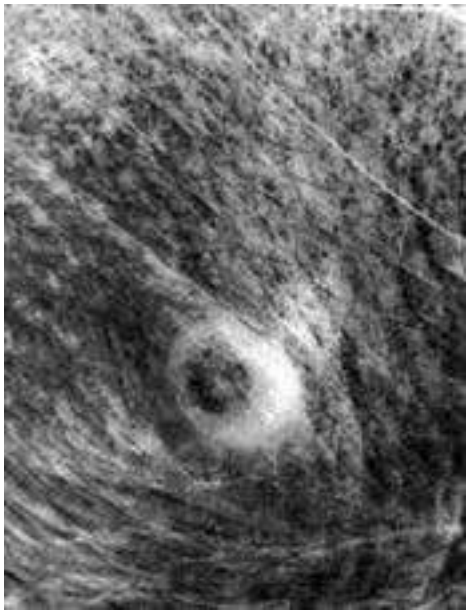

FIG. 133.2 Molluscum contagiosum. *(From Wood MJ. Skin and soft tissue infection. In: Farrar WE, Wood MJ, Innes JA, et al, eds.* Infectious Diseases Text and Color Atlas. *Hong Kong: Gower Medical Publishing; 1992.)*

not been cultured in standard tissue culture systems. The characteristic histopathology of these lesions is diagnostic; polymerase chain reaction methods have been described.[48,49] Similar-appearing umbilicated lesions have occasionally been seen in patients with acquired immunodeficiency syndrome (AIDS) with disseminated cryptococcosis.

Epidemiology and Control

The virus occurs worldwide, and reports of increasing disease have paralleled reports of AIDS. Traditional modes of transmission are associated with mild skin trauma and in some cases fomites (shared towels). Among adults the disease is often sexually transmitted, and genital lesions are common.[37,50] Atopic dermatitis and pubic hair removal, which may result in minor skin trauma or deficits in the epidermis, have emerged as possible risk factors for infection.[51,52,53]

Molluscum contagiosum presents a significant concern for individuals whose children are in daycare or school situations, where concerns about potential transmission to other children may exist. Covering of lesions and hand hygiene after contact with lesions should prevent transmission in these situations.

Therapy

Infection is benign and recovery is usually spontaneous, but treatment may be sought for cosmetic reasons, particularly for facial or multiple lesions. Various treatments have been tried[37] including cryotherapy,[54] mechanical curettage,[54,55] and chemical treatments (podophyllin/podofilox, cantharidin, iodine, and tretinoin).[55–57] Irritation is an adverse effect of many of the chemical treatments. Topical application of an antiviral 3% cidofovir cream or suspension[58,59] has been reported to be beneficial, as has potentially immune-modulating cimetidine.[60] Topical imiquimod therapy has been reported to be beneficial,[61] but two unpublished, large, randomized clinical trials failed to show efficacy.[62] The absence of well-controlled trials makes assessment of the efficacy of various therapeutic regimens difficult for the clinician. A retrospective medical chart review and telephone survey failed to show a beneficial effect of any therapy compared with no therapy on time to clearance or rate of recurrence of lesions.[63] For patients with AIDS and molluscum, the use of highly active antiretroviral therapy, with improved $CD4^+$ cell counts, appears to be efficacious.

YATAPOXVIRUSES

The genus *Yatapoxvirus* includes tanapox virus and Yaba monkey tumor virus.

Tanapox

Human infection with tanapox virus, which was first recognized in the Tana River basin area of Kenya in 1957, was best characterized during post–smallpox eradication surveillance efforts. An account of 264 laboratory-confirmed cases from Zaire (now the Democratic Republic of Congo), with color illustrations, is available,[64] as is information on the virus itself.[65] The genome of the virus has been sequenced.[66] Yaba-like disease virus of monkeys is a variant of the species of virus that causes tanapox in humans.[7,67] Recent anecdotal reports of human disease outside Africa have been published and illustrate the need to consider poxvirus causes of illness in travelers returning from and emigrants from areas where the virus is endemic.[68–70]

Pathophysiology and Clinical Manifestations

Tanapox infection begins with a short febrile illness of 2 to 4 days with temperatures of 38°C to 39°C (100.4°F to 102.2°F) that is sometimes accompanied by headache, backache, or prostration. The eruption of a lesion is often heralded by pruritus at the site. The lesion appears as a hyperpigmented macule, often with central elevation. The macule then evolves to a papule, with palpable induration. Fever and systemic symptoms wane as the lesion manifests. The papule then becomes more "pocklike" but contains no fluid; umbilication or the formation of a pseudocrust has been reported at this stage. Typically the papule evolves into a firm, deep-seated, elevated nodule. At the end of the first week, the lesion is surrounded by erythema and by indurated skin. Regional adenopathy is common at this stage. After this stage, lesions either ulcerate or become larger nodules, up to 2 cm in diameter. In the African series, maximum size was usually reached within 2 weeks, and then the local inflammatory response began to wane and the lesion began to granulate. Resolution of lesions occurred within 6 weeks.[64]

Most cases (78% in one series[57]) involve a solitary nodule; however, as many as 10 lesions on one individual have been described. The most common location for lesions (72%) is the lower extremities, and the least common locations are the face and parts of the body that are normally covered by clothing.[64] Infection appears to confer lifelong immunity.

Diagnosis

For diagnosis of tanapox, the limited geographic distribution should be considered, as should travel history. Unique clinical features that allow the differentiation of tanapox from other orthopoxvirus infections include the nodular nature of the rash lesion, local adenopathy, paucity of lesions, benign disease course, and protracted course of rash resolution. As well, the solid nodular/ulcerated lesions are larger and develop more slowly than the lesions of monkeypox, but they are smaller and develop more rapidly than tropical ulcers.

Tanapox virus can be detected with electron microscopy, and the virions usually appear enveloped.[71] This finding does not exclude the possibility of infection with other morphologically similar brick-shaped poxviruses; nucleic acid testing[68,69] on-lesion extract could be used for that purpose. Tanapox virus grows in a number of cell lines (e.g., owl monkey kidney, Vero, MRC-5, BSC-1), but not on cell adhesion molecules.

Epidemiology and Control

Tanapox virus is restricted to Africa, principally to Kenya and the Democratic Republic of the Congo, and likely has a simian reservoir.[67] Cases of direct primate-to-human transmission, via a break in skin, have been described in animal handlers, although such cases appear to be extremely rare.[72,73] Several factors have led to speculation that an insect or arthropod intermediary may be involved in transmission of tanapox virus to humans. Persons confirmed to have tanapox infection have denied contact with nonhuman primates but have reported arthropod and culicine mosquito bites before infection. Also, in patients in whom multiple lesions developed, no evidence was found that the virus had been spread mechanically.[64] Furthermore, the seasonal variation of human tanapox infections follows the activity of local arthropod populations. No human-to-human transmission has been reported. With the exception of vaccination, measures for the prevention of monkeypox are applicable to tanapox.

Yaba Monkey Tumor Virus

Yaba monkey tumor virus is a distinct species of *Yatapoxvirus*. It was originally isolated as the cause of a cutaneous tumor on a rhesus monkey *(Macaca mulatta)*. In Asiatic monkeys, the virus causes benign histiocytomas that resolve in 1 to 2 months.[74] Serosurveys have suggested that African green monkeys are the natural host of Yaba monkey tumor virus.[75]

Clinical Manifestations

Accidental needlestick infections of human animal handlers and deliberate infections of human volunteers show that humans have localized skin lesions that develop at the site of inoculation.[76] Human infections have not been recently reported.

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The complete reference list is available online at Expert Consult.

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134 Introduction to Herpesviridae

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Although there are hundreds of herpesviruses that infect nearly all animals, eight herpesviruses naturally infect humans (Table 134.1). A ninth herpesvirus, herpes B virus, which naturally infects macaques, can cause fatal encephalitis in humans. This chapter is an overview of herpesviruses that infect humans. Individual herpesviruses are discussed in subsequent chapters (see Chapters 135–141).

CLASSIFICATION

Members of the Herpesviridae are distinguished from other virus families by their structure and genome. Herpesviruses contain double-stranded DNA surrounded by an icosahedral nucleocapsid, which is wrapped inside a tegument consisting of several viral and cellular proteins and then surrounded by an envelope studded with viral glycoproteins (Fig. 134.1).[1] The envelope is derived from host cell membranes. Herpesviruses range from 120 to 260 nm in diameter. Virions contain a characteristic set of viral proteins as well as some host cell proteins.

Human herpesviruses are subdivided into three subfamilies (see Table 134.1). The Alphaherpesvirinae include herpes simplex virus (HSV) types 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), and herpes B virus. These viruses are latent in neurons of sensory ganglia, and infection of cultured cells leads to rapid destruction of the cells. They usually cause mucocutaneous infections in healthy individuals. The Betaherpesvirinae include cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and human herpesvirus 7 (HHV-7). These viruses have a more limited host range, replicate slowly in cell culture, and establish latency in mononuclear cells. The Gammaherpesvirinae include Epstein-Barr virus (EBV) and Kaposi sarcoma–associated herpesvirus (KSHV, also known as HHV-8). These viruses establish latency in lymphoid cells and cause lytic infection in epithelial cells or fibroblasts. The Betaherpesvirinae and Gammaherpesvirinae can cause lymphoproliferation with mononucleosis.

EBV and HHV-6 are present as one of two types, referred to as types A and B. EBV types can differ depending on the geographic location of the infected individual. Although HSV-1 and HSV-2 share 50% or more sequence identity, different types of EBV and HHV-6 are much more closely related.

GENOME STRUCTURE AND PROTEINS

The human herpesviruses (HHVs) consist of 125,000 to 229,000 bp of double-stranded DNA (see Table 134.1), and their overall guanosine and cytosine content varies from 36% (for HHV-7) to 70% (for HSV-2). The genomes consist of long unique regions and short repeated regions. These repeats, as well as single nucleotide polymorphisms, are often useful for molecular epidemiologic studies because restriction endonuclease digestion results in different-sized fragments that can help to indicate whether different persons are infected with different strains of virus. Thus, after transplantation, the sizes of the repeated regions can help to indicate whether the viruses originate from the donor or the recipient.[2] EBV has terminal repeats at the ends of its genome. The number of terminal repeats is fixed in cells that were infected with the same clone of virus but varies in number if the cells were infected with different viral clones.[3] The viral genome is linear inside the virion but circularizes in infected cells. Portions of many herpesvirus genes overlap in the genome; most herpesvirus genes are not spliced.

HHVs encode 71 to several hundred genes.[4] Herpesviruses encode a core set of approximately 40 proteins that are conserved among all the herpesvirus species and include proteins involved in nucleic acid synthesis (e.g., viral DNA polymerase), nucleic acid metabolism (e.g., ribonucleotide reductase), protein modification (e.g., protein kinases), and virion structure (major capsid protein; glycoproteins B, H, and L). In addition, members of the three subfamilies of the Herpesviridae each contain a conserved set of genes unique to each subfamily, such as genes encoding proteins important for virus entry. Herpesviruses have a lytic phase of replication that results in cell death and a latent phase of replication in which no or a very limited set of viral proteins are made.

VIRUS REPLICATION

Herpesviruses use at least two principal methods to enter cells.[5] Virus can enter cells by endocytosis and subsequent fusion of the virion envelope to the endocytic membrane, which allows entry of the nucleocapsid into the cytoplasm. Alternatively, the virion envelope can fuse to the cell membrane on the cell surface to deliver the nucleocapsid directly to the cytoplasm. Viral glycoproteins bind to receptors on the cell membrane, which allows entry into the cell. Herpesviruses often have more than one cell surface receptor (see Table 134.1). The viral nucleocapsid is transported from the cytoplasm to the nucleus, where the linear viral DNA circularizes, and thus DNA replication can begin. Replication proceeds in an orderly pattern of viral gene expression. The immediate-early viral genes are initially expressed; they encode proteins that regulate viral gene expression. This is followed by the early viral genes, many of which encode enzymes important for viral DNA replication or protein phosphorylation. Last, the late viral genes are made, many of which encode structural proteins, including the viral glycoproteins and nucleocapsid proteins. Viral genes are transcribed in the nucleus, and the proteins are synthesized in the cytoplasm. Herpesvirus nucleocapsids are assembled in the nucleus and undergo envelopment at the inner nuclear membrane, deenvelopment at the outer nuclear membrane, and reenvelopment at the cytoplasmic membrane, and then exit the cell. Lytic replication of herpesviruses inhibits host cell RNA and protein synthesis.

VIRUS LATENCY AND REACTIVATION

The mechanisms by which herpesviruses establish and maintain latency are not well understood. Some viruses, such as HSV, express one family of latency-associated transcripts that do not encode proteins but may be important to prevent apoptosis.[6] CMV expresses multiple viral RNAs.[7] Other viruses express proteins during latency. EBV expresses the EBV

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

TABLE 134.1 Biologic Features of Herpesviruses That Infect Humans

VIRUS	SUBFAMILY[a]	GENOME SIZE (kbp)	RECEPTOR(S)	SITES OF LATENCY
Human Virus				
HSV-1 (HHV-1)	Alpha	152	Nectin-1, nectin-2 TNFRSF14, 3-OS-HS	Sensory and cranial nerve ganglia
HSV-2 (HHV-2)	Alpha	152	Nectin-1, nectin-2 TNFRSF14	Sensory and cranial nerve ganglia
Varicella-zoster virus (HHV-3)	Alpha	125		Sensory and cranial nerve ganglia
Cytomegalovirus (HHV-5)	Beta	229	PDGFR α, EGFR Integrin $\alpha_2\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_3$	Monocytes, macrophages, $CD34^+$ cells
HHV-6	Beta	165	CD46, CD134	$CD34^+$ cells, monocytes, macrophages
HHV-7	Beta	145		CD4 cells
Epstein-Barr virus (HHV-4)	Gamma	172	CD21, CD35, EphA2, MHC class II Integrins $\alpha_v\beta_6$, $\alpha_v\beta_8$	Memory B cells
Kaposi sarcoma–associated herpesvirus (HHV-8)	Gamma	165	Integrin $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_1$, DC-SIGN, EphA2	B cells
Simian Virus				
Herpes B virus (Macacine herpesvirus 1)	Alpha	150	Nectin-1	Sensory and cranial nerve ganglia

[a]Alpha, Beta, and Gamma refer to subfamilies Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae, respectively.
DC-SIGN, Dendritic cell–specific ICAM-3–grabbing nonintegrin; *EGFR,* epidermal growth factor receptor; *EphA2,* ephrin receptor tyrosine kinase A2; *HHV,* human herpesvirus; *HSV,* herpes simplex virus; *MHC,* major histocompatibility complex; *PDGFR,* platelet-derived growth factor receptor; *3-OS-HS,* 3-*O* sulfated heparan sulfate; *TNFRSFI4,* tumor necrosis factor receptor superfamily, member 14.
Modified from Straus SE. Introduction to herpesviridae. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. *6th ed. Philadelphia: Churchill Livingstone; 2005:1756–1762.*

nuclear antigen 1 protein, which allows viral episomes to partition to dividing latently infected B cells.[8] EBV and KSHV encode a number of latency proteins expressed in different types of virus-associated malignancies. Herpesvirus genomes exist in a circular, episomal form during latency in specific cell types. HHV-6 is an exception; up to 1% of healthy persons have HHV-6 integrated in their chromosomes.[9]

Several mechanisms have been proposed for maintaining latency in herpesviruses. These include expression of viral microRNAs during latency that inhibit expression of immediate-early viral genes[10,11] and methylation of histone proteins associated with lytic genes, which results in compaction of chromatin and silencing of the lytic genes.[12,13] In addition, certain viral and cellular proteins, which are normally in the nucleus of cells undergoing herpesvirus replication, may be sequestered to the cytoplasm during latency so that the viral proteins cannot activate gene expression.[14]

Latent viral genomes can reactivate to produce infectious virus. Reactivation can be stimulated in some virus-infected cells by radiation, trauma to nerves (in the case of the Alphaherpesvirinae), hyperthermia,[15] or hypoxia.[16] Reactivation is more common in immunosuppressed or immunocompromised hosts that have impaired T-cell immunity. For example, in the absence of antiviral prophylaxis, 50% or more of seropositive transplant recipients reactivate HSV, EBV, HHV-6, and HHV-7. Stress can also lead to reactivation; CMV reactivation occurs in 33% of seropositive immunocompetent patients in intensive care units.[17] Reactivation allows the virus to be transmitted to other individuals, thereby perpetuating virus infection over time to other generations.

PATHOGENESIS

Many herpesviruses, such as HSV, EBV, CMV, HHV-6, and HHV-7, are shed from the oral mucosa without symptoms despite the presence of neutralizing antibody and virus-specific T cells.[18] It is during asymptomatic shedding, rather than during symptomatic disease, that most viruses are transmitted from person to person. In contrast, VZV is only transmitted when patients have varicella or zoster.

Symptomatic disease due to some herpesviruses is associated with lytic virus replication, resulting in skin lesions due to HSV or VZV or visceral lesions due to HSV, VZV, or CMV. Other diseases, such as erythema multiforme associated with HSV or hemolytic anemia associated with CMV or EBV, are due to the immune response to the virus. Most symptoms from infectious mononucleosis associated with EBV are due to the proliferation of T cells that respond to the infection rather than to lytic destruction of virus-infected B cells.

Most human encounters with herpesviruses are asymptomatic or induce very mild symptoms. Many persons infected with HSV-1 or HSV-2 are asymptomatic, and young children and infants infected with CMV and EBV are usually asymptomatic. In contrast, most persons infected with VZV present with chickenpox and most with HHV-6 have fever. Infections are rarely fatal except in highly immunocompromised persons. It is in the best interest of the virus for the host to survive so that the infection can be transmitted to others. Whereas some herpesviruses (e.g., HSV, VZV, CMV, HHV-6) infect a wide range of cells in the body, others (e.g., EBV, HHV-7, KSHV) have a more narrow host range. Most herpesviruses spread in the blood. VZV, EBV, HHV-7, and KHSV disseminate in the bloodstream by lymphocytes, and CMV and HHV-6 by monocytes; HSV viremia has been reported in some persons with primary genital infection and in severely immunocompromised persons.

EPIDEMIOLOGY

Nearly all adults are infected with HSV-1, VZV, EBV, HHV-6, and HHV-7 (Table 134.2). Fifteen percent to 25% of adults in the United States are infected with HSV-2, 40% to 70% are infected with CMV, and less than 3% are infected with KSHV. Rates of HSV-1, HSV-2, CMV, EBV, and KSHV are higher in developing countries than in the United States.

Herpesviruses are usually spread by direct contact because the enveloped viruses do not survive long in the environment; VZV is the exception and is spread by airborne transmission. HSV, EBV, CMV, and HHV-6 are spread by infected saliva; HHV-7 and KSHV are also likely spread by saliva. Sexual contact results in spread of HSV, CMV, KSHV, and perhaps EBV. Intrauterine infection with HSV, VZV, and CMV can occur; most infants infected with HSV acquire the infection at the time of delivery. CMV, EBV, and KSHV have been transmitted by organ transplantation, and CMV and EBV have been spread by blood transfusion.

CLINICAL SYNDROMES

Several herpesviruses cause similar clinical syndromes, including vesicular skin lesions, retinitis, hepatitis, encephalitis, and mononucleosis;

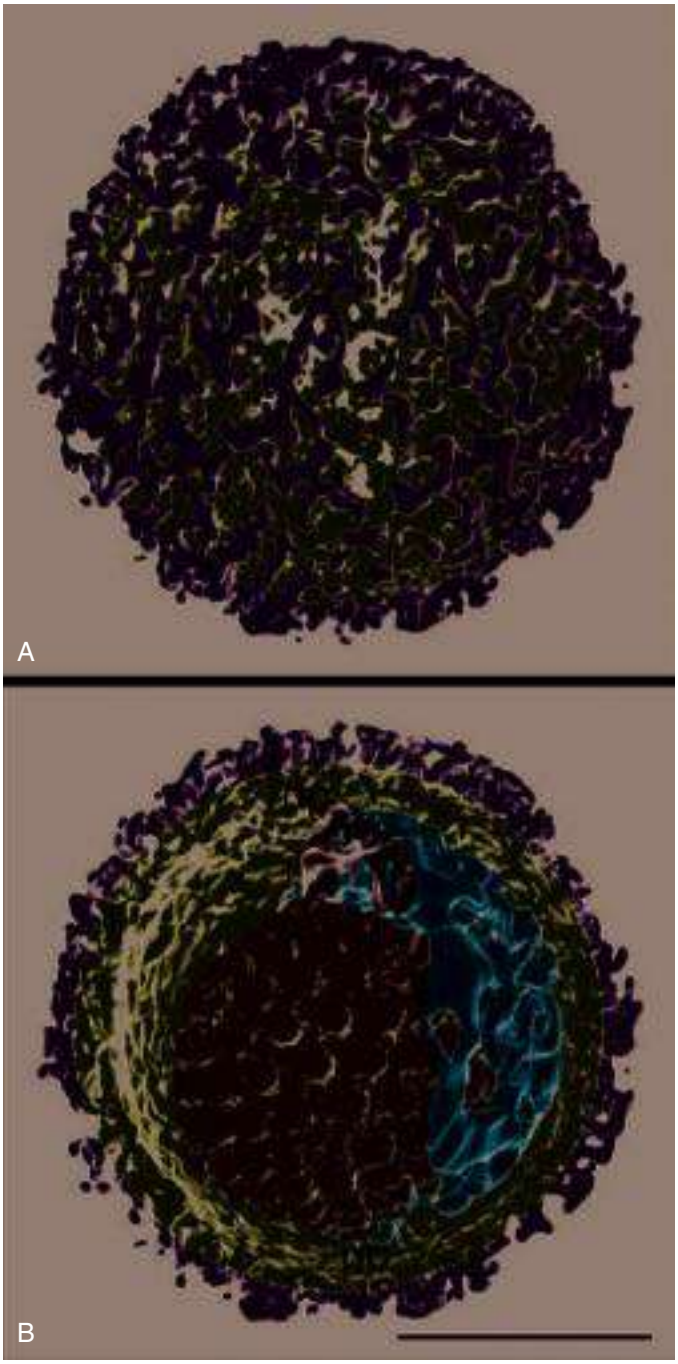

FIG. 134.1 Structure of a herpes simplex virus virion based on cryoelectron tomography. (A) The outer surface of the virion shows viral glycoproteins *(yellow)* embedded in the viral membrane *(blue).* (B) The interior of the virion shows the viral nucleocapsid *(light blue)* surrounded by the protein tegument *(orange),* which is inside the viral envelope *(blue and yellow). dp,* Distal pole; *pp,* proximal pole. Scale bar = 100 nm. *(From Grünewald K, Desai P, Winkler DC, et al. Three-dimensional structure of herpes simplex virus from cryo-electron tomography.* Science. *2003;302:1396–1398, with permission.)*

virus-specific diagnostic tests are needed to distinguish the virus that causes a particular syndrome (Table 134.3; see also Table 134.2).

Herpesviruses cause more severe disease in persons with impaired cellular, but not with impaired humoral, immunity (see Table 134.2). Patients with impaired T-cell immunity, such as organ transplant recipients receiving immunosuppression therapy, hematopoietic cell transplant recipients, patients with acquired immunodeficiency syndrome (AIDS), or patients with congenital T-cell or natural killer (NK) cell deficiencies, have more severe herpesvirus infections.[19,20] Patients infected with human immunodeficiency virus (HIV) and low CD4 cell counts often have severe herpesvirus infections. Persistent erosive mucocutaneous oral or anogenital HSV infections may occur, and resistance to acyclovir is not uncommon. VZV can cause verrucous skin lesions or small vessel central nervous system vasculitis or radiculopathy in patients with AIDS. CMV can cause encephalitis, retinitis, colitis, or radiculopathy; however, pneumonitis is rare in patients with AIDS. EBV is associated with central nervous system lymphomas or non-Hodgkin lymphoma, whereas KSHV is associated with Kaposi sarcoma, primary effusion lymphoma, and Castleman disease in patients with AIDS. Interestingly, HHV-6 and HHV-7 rarely cause disease in patients with AIDS. Infection with HSV-2 increases the rate of transmission and infection with HIV.[21]

IMMUNITY

Humoral immunity contributes to protection from primary infection with herpesviruses. Antibody acquired transplacentally protects neonates from HSV, VZV, and CMV; infection of neonates can cause severe disease when mothers develop primary infection and lack virus-specific antibody near the time of birth. VZV and CMV infection during fetal development is associated with fetal growth restriction and birth defects. Antibody to VZV (varicella-zoster immune globulin) or CMV (CMV hyperimmune globulin) reduces the severity of primary disease in immunocompromised persons. Antibodies have numerous activities than can inhibit infection; these including neutralization of infection, antibody-dependent cellular cytotoxicity, complement-mediated cellular cytotoxicity, and antibody-dependent cellular phagocytosis. Most herpesviruses spread in the body from cell to cell rather than as free virions; therefore the effect of antibody is limited once infection is well established.

The observation that patients with hypogammaglobulinemia are not at risk for developing severe herpesvirus infections, whereas those with impaired cellular immunity can have life-threatening disease, indicates the important role of cellular immunity in controlling the severity of herpesvirus disease. Numerous virus epitopes are recognized by $CD4^+$ and $CD8^+$ T cells, and few appear to be dominant in HSV and CMV infection.[22,23] $CD4^+$ T cells produce cytokines that are critical for controlling virus infection, especially interferon-γ and tumor necrosis factor-α. $CD8^+$ T cells can kill virus-infected cells, and in some cells can induce cleavage of viral proteins to prevent virus replication without killing the cells. Cellular immunity is critical for controlling the severity of acute infection and reducing virus reactivation.

Patients with mutations in genes in the innate immunity pathway important for interferon signaling, such as *TLR3, UNC93B1, IRF3, TRAF3, TICAM1,* and *TBK1,* have an increased likelihood to develop HSV encephalitis.[24] Mutations in genes important for T-cell function, including *SH2D1A*, *BIRC4, ITK, CD27, CD70*, and *MAGT1,*[25,26] can result in severe EBV disease, and mutations in *STIM1* can result in severe KSHV disease.[27] Mutations in other genes important for cellular immunity, including *PIK3CD, PIK3R1, GATA2*, *IKBKG*, *IFN-γR1, IL2Rγ, FCGR3A,* and *DOCK8,* can result in severe infections with multiple herpesviruses.[20] Patients with NK cell defects are especially prone to severe herpesvirus infections.[28] Polymorphisms in *HLA* genes are associated with symptomatic HSV[29] or EBV infection,[30] whereas polymorphisms in *TLR2*[31] or *IL10*[32] are associated with increased severity of HSV or EBV infection, respectively.

Herpesviruses encode numerous genes that inhibit cellular immune mechanisms.[33–35] EBV and KSHV encode homologues of the antiapoptotic protein BCL2, whereas HSV, CMV, and HHV-6 encode other proteins that inhibit apoptosis. Many herpesviruses encode proteins that inhibit recognition of infected cells by $CD4^+$ and $CD8^+$ T and NK cells. HSV, EBV, and CMV encode proteins that inhibit the TAP protein that is required for processing major histocompatibility complex (MHC) class I molecules, and KSHV encodes proteins that enhance endocytosis of MHC class I molecules from the cell surface. HSV, EBV, and CMV also encode proteins that inhibit MHC class II, and CMV and KSHV encode proteins that inhibit NK cells. HSV, VZV, EBV, CMV, and KSHV encode proteins that inhibit interferon. HSV encodes a glycoprotein (gE) that inhibits the activity of antibody, whereas HSV and KSHV encode proteins that inhibit complement. EBV and CMV encode interleukin (IL)-10 homologues, and KSHV encodes an IL-6 homologue. HHV-6 and HHV-8

TABLE 134.2 Features of Herpesvirus Infections and Seroepidemiology

VIRUS	PRIMARY INFECTION IN HEALTHY PERSONS	INFECTION IN IMMUNOCOMPROMISED PERSONS	SEROPREVALENCE (%)		
				HEALTHY ADULTS	
			HEALTHY CHILDREN	United States	Developing World
Herpes simplex virus 1	Gingivostomatitis Keratoconjunctivitis Cutaneous herpes Genital herpes	Gingivostomatitis Keratoconjunctivitis Cutaneous herpes Visceral infections	20–40	50–70	50–90
Herpes simplex virus 2	Genital herpes Cutaneous herpes Gingivostomatitis Aseptic meningitis Neonatal herpes	Genital herpes Cutaneous herpes Disseminated infection	0–5	15–25	20–60
Varicella-zoster virus	Varicella	Disseminated infection	50–75	85–95	50–80
Cytomegalovirus	Mononucleosis Hepatitis Congenital cytomegalic inclusion disease	Hepatitis Retinitis Other visceral infections	10–30	40–70	40–80
Epstein-Barr virus	Mononucleosis Hepatitis Encephalitis	Polyclonal and monoclonal lymphoproliferative syndromes Oral hairy leukoplakia	10–30	80–95	90–100
Human herpesvirus 6	Exanthem subitum Infantile fever and seizures Encephalitis	Fever and rash Encephalitis Bone marrow suppression	80–100	60–100	60–100
Human herpesvirus 7	Exanthem subitum Childhood fever and seizures Encephalitis	Encephalitis	40–80	60–100	40–100
Kaposi sarcoma–associated herpesvirus	Febrile exanthem Mononucleosis?	Kaposi sarcoma Castleman disease Primary effusion lymphoma	<3	<3	10–60
Herpes B virus	Mucocutaneous lesions Encephalitis	Not reported	0	0	0

Modified from Straus SE. Introduction to herpesviridae. In: Mandell GL, Bennett EJ, Dolin R, eds. Principles and Practice of Infectious Diseases. *6th ed. Philadelphia: Churchill Livingstone; 2005:1756–1762.*

TABLE 134.3 Clinical Syndromes Associated With Human Herpesviruses

SYNDROME	HSV-1	HSV-2	VZV	CMV	EBV	HHV-6	HHV-7	KSHV	HERPES B VIRUS
Gingivostomatitis	+	+	–	–	–	–	–	–	–
Genital lesions	+	+	–	–	–	–	–	–	–
Keratoconjunctivitis	+	+	+	–	–	–	–	–	–
Cutaneous lesions	+	+	+	–	–	–	–	+	+
Neonatal infection	+	+	+	+	–	–	–	–	–
Retinitis	+	+	+	+	–	–	–	–	–
Esophagitis	+	+	+	+	–	–	–	–	–
Pneumonitis	+	+	+	+	+	+	–	–	–
Hepatitis	+	+	+	+	+	+	–	–	–
Meningitis	–	+	+	–	–	+	–	–	–
Encephalitis	+	+	+	+	+	+	+	–	+
Myelitis	+	+	+	+	+	+/–	–	–	+
Mononucleosis	–	–	–	+	+	+	–	+?	–
Hemolytic anemia	–	–	+	+	+	–	–	–	–
Leukopenia	–	–	+	+	+	+	–	–	–
Thrombocytopenia	–	–	+	+	+	+	–	–	–

CMV, Cytomegalovirus; *EBV,* Epstein-Barr virus; *HHV,* human herpesvirus; *HSV,* herpes simplex virus; *KSHV,* Kaposi sarcoma–associated herpesvirus; *VZV,* varicella-zoster virus. *Modified from Straus SE. Introduction to herpesviridae. In: Mandell GL, Bennett JE, Dolin R, eds.* Principles and Practice of Infectious Diseases. *6th ed. Philadelphia: Churchill Livingstone; 2005:1756–1762.*

encode chemokines, and CMV, EBV, HHV-6, HHV-7, and KSHV encode chemokine receptor homologues.[36] Herpesviruses also express microRNAs that contribute to immune evasion.[37,38] Many herpesviruses activate the inflammasome, a complex of proteins that in turn activate IL-1β and IL-18, which have antiviral activities; these same viruses often encode proteins that inhibit the inflammasome.[39,40] Together these immune evasion proteins allow the virus to avoid destruction by the host immune system.

ONCOGENESIS

Two human herpesviruses, EBV and KSHV, are oncogenic in humans, whereas the other human herpesviruses are not associated with cancer.[41] Oncogenic viruses must be maintained in cells and be transmitted to progeny cells. Oncogenic viruses express a limited set of viral proteins during latency to avoid detection by the immune system and to provide functions needed to immortalize the cell. EBV and KSHV encode proteins that activate cellular gene expression and B-cell signaling pathways. EBV LMP-1 functions as an oncogene and mimics the activity of CD40 to activate nuclear factor kappa B and signal transducers and activators of transcription (STATs), whereas KSHV encodes several proteins (e.g., K1, K12, ORF74) with transforming activity. EBV is associated with B-cell malignancies including Hodgkin disease, non-Hodgkin lymphoma, and Burkitt lymphoma, as well as epithelial cell cancers such as gastric carcinoma and nasopharyngeal carcinoma. Similarly, KSHV is associated with Kaposi sarcoma (an endothelial cell cancer) and two B-cell malignancies—primary effusion lymphoma and Castleman disease.

DIAGNOSIS

Most primary herpesvirus infections, such as herpes gingivitis, genital herpes, varicella, and roseola, are diagnosed by their clinical symptoms. Serology is useful for confirming acute or previous infection. Type-specific serologies are available for HSV-1 and HSV-2, either of which can cause oral or genital disease.

Polymerase chain reaction (PCR) assay is the standard test for detection of HSV, EBV, CMV, or HHV-6 encephalitis; culture of virus from the cerebrospinal fluid is much less likely to be positive.[42] Intrathecal synthesis of antibody to VZV is more sensitive for diagnosis of VZV encephalitis than is PCR assay.[43] PCR assay of cerebrospinal fluid is also useful for the diagnosis of HSV meningitis and can be an important clue for EBV central nervous system lymphoma in highly immunocompromised patients. Quantification of CMV and EBV DNA in the blood is useful for monitoring the risk for disease due to these viruses and for response to therapy in transplant recipients. Culture of herpesviruses is much less sensitive than the PCR assay but is useful for analysis of drug-resistant virus. Because asymptomatic shedding is common for most herpesviruses, a positive culture does not necessarily indicate that the virus is causing disease. Direct fluorescent antibody testing is useful for detection of VZV in skin lesions.

Lytic replication of herpesviruses causes characteristic intranuclear inclusions in tissues. CMV and HHV-6 cause both intranuclear and cytoplasmic inclusions, whereas HSV and VZV result in only intranuclear inclusions (Fig. 134.2). Immunohistochemistry with monoclonal antibodies can be used to identify specific viruses in tissues.

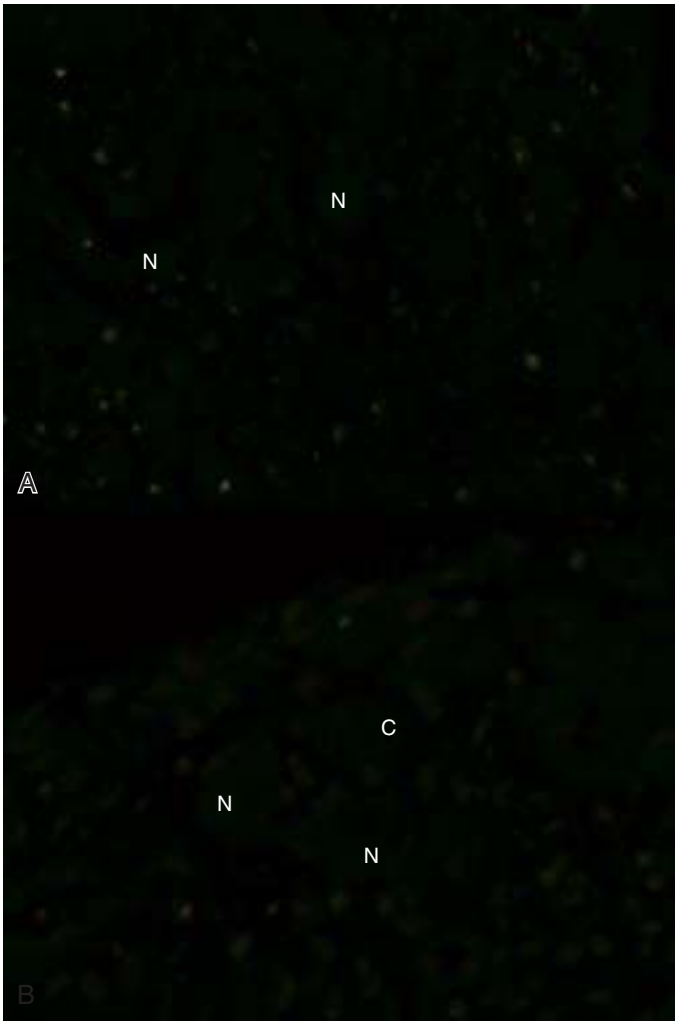

FIG. 134.2 (A) Intranuclear inclusions in cells infected with herpes simplex virus. (B) Intranuclear and cytoplasmic inclusions in cells infected with cytomegalovirus. *C,* Cytoplasmic; *N,* intranuclear. *(Courtesy David Kleiner, Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD.)*

THERAPY

Nearly all US Food and Drug Administration–approved oral or intravenous antiviral agents for herpesvirus infections act at the same step of virus replication; they inhibit the viral DNA polymerase.[44] Some antiviral agents are phosphorylated by the HSV or VZV thymidine kinase (acyclovir, penciclovir) or CMV UL97 protein kinase (ganciclovir), whereas other antiviral drugs do not require phosphorylation by viral proteins (foscarnet, cidofovir). In general, foscarnet and cidofovir are more toxic to cells because they are active in both infected and uninfected cells. Valacyclovir, valganciclovir, and famciclovir are prodrugs that are converted to their active form (i.e., acyclovir, ganciclovir, penciclovir, respectively) by cellular enzymes. Brincidovir, which is under development, has activity against most, if not all, of the herpesviruses and is a lipid conjugated form of cidofovir without significant renal toxicity.[45] In 2017, letermovir, a viral terminase inhibitor important for viral DNA cleavage and packaging,[46] was approved for prophylaxis of CMV in hematopoietic cell transplant recipients. Other drugs under development that act at steps other than the viral DNA polymerase include maribavir for CMV,[47] which inhibits the viral protein kinase UL97; pritelivir, a helicase-primase inhibitor for HSV [48]; and amenamevir, a helicase-primase inhibitor for HSV and VZV[49] (see Chapter 46).

Resistance of HSV, VZV, or CMV to antiviral drugs usually only occurs in highly immunocompromised persons and is usually due to mutations in the HSV or VZV thymidine kinase or CMV protein kinase; foscarnet or, less often, cidofovir is used to treat drug-resistant HSV, VZV, or CMV. Resistance to the latter two agents can occur due to mutations in the viral DNA polymerase. Antiviral therapy has not been proven to be clinically effective for HHV-6, HHV-7, and KSHV. Antiviral therapy is not beneficial for reducing symptoms of EBV disease, with the exception of oral hairy leukoplakia. Corticosteroids are occasionally used to treat severe manifestations of EBV, which are due to the T-cell proliferative response to the virus rather than to lytic replication in B cells.

Currently available antiviral agents inhibit lytic replication, not latent replication, and therefore do not affect the latent reservoir of viral DNA. One approach that has been suggested to reduce the latent reservoir of herpesvirus DNA is to activate lytic replication in latently infected cells (e.g., using a histone deacetylase inhibitor) and then treat with an inhibitor of lytic replication (acyclovir or ganciclovir). This approach has been used in vitro[50] and in a small number of patients with

EBV-positive lymphomas.[51] Another approach is to infuse human leukocyte antigen–matched cytotoxic T cells to destroy latently EBV-, CMV-, or HHV-6–infected cells.[52]

PREVENTION

Varicella-zoster immune globulin is used to decrease the severity of disease in immunocompromised persons exposed to chickenpox or shingles, and CMV immune globulin is used to decrease disease in organ transplant recipients at high risk for CMV disease. Hyperimmune globulin was not shown to prevent congenital CMV in one study[53]; additional trials are ongoing. Acyclovir reduces recurrences of HSV and severity of varicella in uninfected immunocompromised persons exposed to VZV; ganciclovir or foscarnet reduces the risk for CMV disease in transplant recipients who often have virus reactivation. While CMV can reactivate in patients with acute disease in the intensive care unit, a study of ganciclovir to reduce CMV reactivation disease in CMV-seropositive adults with critical illnesses did not support its use for prophylaxis.[54]

Currently licensed vaccines are only available for VZV to prevent varicella and zoster.[55] The live-attenuated vaccines differ only in the titer of the vaccine virus used; the live shingles vaccine is approximately 14 times the titer of the varicella vaccine. Both vaccines induce humoral and cellular immunity. The varicella vaccine reduces the risk and severity of varicella, but does not prevent infection. In 2017, a glycoprotein subunit vaccine was licensed to prevent zoster, and is particularly effective in the elderly.[56] Vaccine-induced antibody is the correlate of protection against varicella, while VZV-specific CD4 T cells correlate with protection from zoster; the correlate of protection against other herpesviruses in not well understood. Live-attenuated vaccines are not likely options for EBV and KSHV, which contain oncogenes and other genes that induce lymphocyte proliferation. Candidate vaccines for herpesviruses that are farthest along in clinical trials are subunit vaccines. Although a glycoprotein D vaccine for genital herpes failed to show efficacy for protection against genital herpes infection in women,[57] additional vaccines are under development.[58] A glycoprotein B vaccine for CMV reduced infection in healthy women[59,60] and reduced virus reactivation in a subset of organ transplant recipients in phase II trials.[61] A CMV DNA vaccine reduced virus reactivation in hematopoietic stem cell transplant recipients[62] but was unsuccessful in a phase 3 trial. Additional CMV vaccines are in clinical trials and under development.[63] A glycoprotein gp350 vaccine reduced the rate of EBV infectious mononucleosis, but did not prevent asymptomatic infection, in healthy young adults.[64]

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135 Herpes Simplex Virus

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SHORT VIEW SUMMARY

Definition
- Herpes simplex virus (HSV) is a human alphaherpesvirus of two types, HSV-1 and HSV-2.
- Both types cause common infections in otherwise healthy children and adults, with varied clinical manifestations that are generally more severe in patients with immunosuppression.

Epidemiology
- HSV-1 and HSV-2 have a worldwide distribution and have no known animal reservoirs.
- HSV-1 is acquired more frequently and earlier than HSV-2. Approximately 3.7 billion adults have antibodies to HSV-1 by their 40s.
- HSV-2 seroprevalence correlates with onset of sexual activity and is consistently higher in women than men.
- Global incidence of HSV-2 has been estimated at 19.2 million new cases per year.
- HSV-2 increases risk of HIV-1 acquisition and transmission.

Microbiology
- HSV is an enveloped virus that is 160 nm in diameter and has a linear, double-stranded DNA genome.
- HSV-1 and HSV-2 have approximately 50% homology, and homologous sequences are distributed throughout the genome map.
- HSV infection occurs through attachment to cells via ubiquitous receptors, reflecting a wide tissue range of infections in the host including sensory neurons, which allow lifelong latency.
- Viral replication occurs through nuclear and cytoplasmic phases including attachment, fusion, DNA replication, capsid formation, and egress.
- Latency occurs within neural ganglia and is associated with transcription of only a limited number of virus-encoded RNAs.

Clinical Manifestations
- HSV has been isolated from nearly all visceral and mucosal sites. Clinical manifestations depend on the anatomic site, antigenic type (1 or 2) of the virus, and age and immune status of the host.
- Initial (primary) infections are usually more severe than recurrent ones, but reactivation of latent infection can result in frequent clinical manifestations.
- Orofacial infection (gingivostomatitis and pharyngitis) is the most frequent initial clinical manifestation of HSV-1 infection. Recurrent lesions on the vermilion border of the lip (herpes labialis) are the most frequent manifestation of latent infection.
- Clinical aspects of primary genital infections by HSV-1 or HSV-2 are similar, but recurrences are more frequent with HSV-2. Complications include aseptic meningitis, transverse myelitis, and sacral radiculopathy. Extragenital lesions may occur during the course of primary genital infection.
- HSV can cause vision-threatening eye infections such as keratitis and retinitis.
- Herpes simplex encephalitis is the most commonly identified cause of acute, sporadic viral encephalitis in the United States. Magnetic resonance imaging is the neuroimaging technique of choice, and lumbar puncture is diagnostic.
- Esophagitis, pulmonary infections, hepatitis, and overwhelming disseminated disease may be caused by HSV, most commonly in immunocompromised patients. Severe skin infections can be seen in patients with eczema.
- Neonatal infections may occur through contact with HSV-infected secretions. Infants younger than 6 weeks have the highest frequency of visual and central nervous system involvement.

Diagnosis
- Clinical criteria are used for consideration of the diagnosis of HSV infection, but laboratory tests are needed to confirm the diagnosis.
- Diagnosis can be made by detection of HSV DNA by polymerase chain reaction assay in lesion scrapings, fluids, or tissue. Viral isolation in tissue culture is three to four times less sensitive than DNA molecular techniques.
- Serology is diagnostic of established infection. Western blot most accurately discriminates HSV-1 and HSV-2 infections.

Therapy
- Acyclovir, valacyclovir, or famciclovir is used to treat mucocutaneous and visceral infections (see Table 135.3).
- Primary or recurrent genital infections may be treated to limit duration of symptoms. Individuals with frequent recurrences may be treated with suppressive therapy.
- Herpes simplex encephalitis and meningitis are treated with high-dose acyclovir (10 mg/kg intravenously every 8 hours for 14–21 days).
- Acyclovir-resistant HSV strains can be treated with foscarnet or cidofovir.

Prevention
- There is currently no licensed vaccine to prevent either HSV-1 or HSV-2.

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) produce a wide variety of illnesses including mucocutaneous infections, infections of the central nervous system (CNS), and occasional infections of visceral organs; some of these conditions may be life threatening. HSV-2 is biologically and epidemiologically linked to increased acquisition and transmission of human immunodeficiency virus type 1 (HIV-1) infection. Effective chemotherapy for HSV infection is available but does not appear to alter the effects of recent or prior HSV-2 infection on HIV acquisition or transmission.

The word "herpes" (from the Greek, "to creep") has been used in medicine since antiquity. Cold sores (herpes febrilis) were described by the Roman physician Herodotus in AD 100.[1] Genital herpes was first described by John Astruc, physician to the king of France in 1736: the first English translation of his treatise on venereal disease appeared in 1754.[2,3] Transmission of infection in orolabial lesions was documented in the late 19th century. Experimental transfer of disease to rabbits was accomplished in the early 20th century, and HSV was grown in vitro in 1925.[4,5] In the 1960s, Nahmias and Dowdle[6] reported two antigenic types of HSV with different sites of viral recovery.

DESCRIPTION OF VIRUS

Microbiology Virus Structure

The eight known human herpesviruses (HHVs) are divided by genomic and biologic behavior into three groups: alphaherpesviruses

(HSV-1, HSV-2, and varicella-zoster virus [VZV]), betaherpesviruses (cytomegalovirus [CMV], HHV-6, and HHV-7), and gammaherpesviruses (Epstein-Barr virus and Kaposi sarcoma–associated herpesvirus, or HHV-8). Herpesviruses share morphologic traits including an internal core containing double-stranded DNA, an icosahedral capsid with 162 capsomers, an amorphous material surrounding the capsid called a tegument, a lipid envelope with surface viral glycoproteins, and an overall diameter of about 160 nm.[7] Despite these similarities, the biologic and epidemiologic features of each of the herpesviruses are different. Although HSV-1 and HSV-2 are the two most closely related herpesviruses, the two agents are serologically and genetically distinct.[6]

The genome of HSV is a linear, double-stranded DNA molecule (molecular weight approximately 1×10^8 kDa) that encodes about 90 transcriptional units, 84 of which appear to encode proteins. Sequences from both terminal ends of the genome repeat in an inverted fashion, dividing the genome into two unique components.[8] The overall sequence homology between HSV-1 and HSV-2 is about 50%,[9] with homologous sequences distributed over the entire genome map. Most polypeptides specified by one viral type are antigenically related to polypeptides of the other viral type. Many type-specific regions unique to HSV-1 and HSV-2 proteins do exist, however, and some appear to be important in evading host immunity. Interspecies recombinants of HSV-1 and HSV-2 are widely circulated despite divergence of these viruses 6 to 8 million years ago.[10,11]

HSV is genomically stable, and restriction endonuclease or sequence analysis of viral DNA can distinguish between the two subtypes and among strains of each subtype.[12,13] Isolates from epidemiologically related sources, such as sexual partners, mother-infant pairs, or victims of a common-source outbreak, are often nearly identical.[14–17] The variability of clinical strains of HSV-1 and HSV-2 is such that HSV isolates obtained from two individuals can be differentiated,[18] and superinfection within a single person can be identified using nucleotide sequencing.[19] Globally, nucleotide and protein identity among strains is high, with a maximum nucleotide divergence of 0.4% and approximately 90% protein identity.[20,21]

The degree of sequence diversity across the globe is only partially characterized,[11,20,22,23] but whole-genome sequencing has been extended more recently to a variety of clinical isolates.[24] Although hot spots of sequence variability are increasingly being identified in specific genes,[21] the role of this variability in HSV pathogenesis is not understood at the present time.

Virus Replication

Viral replication has nuclear and cytoplasmic phases. The initial steps of replication include attachment and fusion between the viral envelope and cell membrane to liberate the nucleocapsid into the cytoplasm of the cell. The initial attachment to the cell membrane involves the interactions of viral glycoproteins C and B with cellular heparan sulfate.[25,26] Subsequently, viral glycoprotein D binds to cellular coreceptors that belong to the tumor necrosis factor receptor family of proteins, the immunoglobulin superfamily (nectin family), or both.[27] The ubiquity of these receptors underscores the wide host range of herpesviruses, and their presence on sensory neurons implicates them in development of neuronal infection and latency.[28,29]

After attachment, the deenveloped tegument capsid structure is transported to nuclear pores where viral DNA is released into the nucleus. The virion envelope fuses with the host cell membrane and releases several functional proteins. The virion host shutoff protein shuts off synthesis (by increasing cellular RNA degradation), whereas VP16 turns on transcription of immediate early (IE) genes of HSV replication,[30] some of which are important determinants of neurovirulence in animal models. Others are required for synthesis of a subsequent polypeptide group, the β or early polypeptides. Many β-proteins are regulatory proteins and enzymes required for DNA replication including the targets (such as viral DNA polymerase and DNA helicase) of most antiviral drugs in current use or development.[31] Transcription of the viral genome, replication of viral DNA, and assembly of capsids take place in the nucleus.[32] The late (γ) class of HSV genes, which are structural and assist with viral egress, requires viral DNA replication for expression. DNA replication takes place in a "rolling circle" pattern similar to a roll of toilet paper. Specific viral genes "clip" the end of the viral DNA into the procapsid.

After nucleocapsids are assembled in the nucleus, envelopment occurs as the nucleocapsids bud through the inner nuclear membrane into the perinuclear space. In some cells, viral replication in the nucleus forms type A basophilic Feulgen-positive bodies that contain viral DNA and eosinophilic inclusion bodies that are devoid of viral nucleic acid or protein and represent a "scar" of infection. Virions are then transported through the endoplasmic reticulum and Golgi apparatus to the cell surface. The entire viral production cycle takes 4 to 6 hours, and an infected cell survives for 16 to 20 hours. HSV is cytopathic to cells that harbor the full cycle of HSV replication.[33]

Molecular Features of Latency

Some neuronal cells survive HSV infection and continue normal cellular functions but maintain viral genomes in a repressed state termed *latency*.[34,35] Latency is associated with transcription of only a limited number of virus-encoded RNAs.[36,37] The molecular mechanisms by which latency is established, maintained, and altered are incompletely understood. Subsequent activation of the viral genome may occur, resulting in the normal pattern of regulated viral gene expression, replication, and release of HSV, although without apparent damage to the infected neuron. The release of virions from the latently infected neuron to the peripheral endings (neurites) follows a complex process of anterograde transport through neuronal axons.[38,39] Subsequent viral entry into epithelial cells can result in viral replication; this entire process is termed *reactivation*.[40,41]

Although infectious virus typically cannot be cultured from sensory or autonomic nervous system ganglia dissected from cadavers, maintenance and growth of the neural cells in tissue culture results in production of infectious virions (explantation) and in subsequent permissive infection of susceptible cells (cocultivation).[42,43] HSV replication was first detected in neurons during reactivation in vitro, which suggested that the neuron harbors latent virus in vivo.[36] Viral DNA and RNA have since been found in neural tissue in the absence of infectious virus.[37] Individual neurons infected with multiple strains of virus have been documented in severely immunosuppressed patients[44] and more recently in immunocompetent individuals,[45] suggesting that the ganglia can be reseeded with HSV throughout chronic infection or reinfection, or both, through exogenous contact with new strains due to incomplete immunity.

Three noncoding RNA latency-associated transcripts (LATs) are the only transcripts in abundance in the nuclei of latently infected neurons.[36,46,47] In both trigeminal and dorsal root ganglia, LATs appear to maintain, rather than establish, latency.[48] LAT transcript abundance and low genome copy number correlate with subnuclear positioning of HSV genomes around the centromere.[49] Chromatization of HSV DNA appears to play a vital role in silencing expression of lytic replication genes.[50,51] Although certain viral transcripts are known to be necessary for reactivation from latency, the molecular mechanisms of HSV latency are not fully understood, and strategies to interrupt or maintain latency in neurons are in developmental stages.[52] Latency-competent deletion mutants of the LAT genomic region have reduced reactivation efficiency.[46,53] In addition, substitution of HSV-1 LAT for HSV-2 LAT induces an HSV-1 reactivation pattern.[48]

HSV-1 LATs promote the survival of acutely infected neurons perhaps by inhibiting apoptotic pathways.[54,55] LAT-derived microRNA is highly expressed during latency and appears to silence expression of the key neurovirulence factor infected cell protein 34.5 (ICP34.5)[56] and prevent expression of ICP0, an IE protein vital to HSV reactivation, through antisense binding of messenger RNA (mRNA). However, exit from latency appears to correlate with derepression of all, rather than just a small cluster of, HSV replication genes.[57] Evidence suggests that latency is not bimodally defined from lytic infection: specifically, there is evidence of cell-to-cell spread within ganglia following primary infection and expression of lytic genes during latency.[58,59] Thus within a single ganglion, HSV-infected neurons may vary significantly across a spectrum varying from deep latency to full lytic reactivation.

Dynamics of Herpes Simplex Virus Latency and Reactivation

Although latency is the predominant viral state on a per-neuron basis, the high frequency of oral and genital tract reactivation for HSV-1 and HSV-2 suggests that the virus is rarely quiescent within the entire biomass of ganglionic tissue.[60,61] Experimental data suggest that HSV-2 is frequently and nearly continually shed into genital mucosa. Mathematical models of HSV-2 reactivation predict that viral release from the ganglia occurs in a nearly continuous drip,[60] whereas only a single HSV particle from neurons is necessary to spark viral replication in genital epithelial cells.[60] Studies employing microdissection plus real-time polymerase chain reaction (PCR) of individual neurons from cadaveric trigeminal ganglia explants revealed that many more neurons (2%–10%) harbor HSV than would be predicted by in situ hybridization studies for LAT.[62,63] Moreover, viral copy number is highly variable among neurons, with extremely high levels in certain neurons.[62,63] HSV DNA copy numbers are similar in LAT-positive and LAT-negative neurons, adding uncertainty to the role that LATs play in preventing reactivation. In mice, IE, early, and late transcripts are abundant within latently infected ganglia, again suggesting a highly dynamic state among HSV-infected neurons, with some exhibiting deep latency and others showing evidence of lytic reactivation.[64]

EPIDEMIOLOGY

HSVs have a worldwide distribution and are found in the most remote human populations. There are no known animal vectors for HSV-1 or HSV-2, and although experimental animals are easily infected, humans appear to be the only natural reservoir. HSV-1 resulted from ancient codivergence with *Homo sapiens* from chimps, whereas HSV-2 was transmitted across species to a precursor of modern humans roughly 1.4 to 3 million years ago.[11,65] Multiple HSV-1 gene fragments, many of ancient origin, have been detected in whole HSV-2 genomes, suggesting frequent coinfection and genetic recombination.[66]

Herpes infection is the predominant cause of genital ulcers throughout the world. This is due to an overall decrease in *Treponema pallidum* and chancroid infections in most populations,[67] increase in PCR usage for HSV detection, and increase in HSV-2 reactivation frequency among individuals coinfected with HSV and HIV, rather than an increase in worldwide HSV prevalence.

Herpes Simplex Virus Type 1

Infection with HSV-1 is acquired more frequently and earlier than infection with HSV-2. More than 90% of adults have antibodies to HSV-1 by their 40s. Prevalence of antibody to HSV-1 increases with age and demonstrates an inverse correlation with socioeconomic status. In post–World War II era Western populations, 80% to 100% of middle-aged adults of lower socioeconomic status had antibodies to HSV-1 compared with only 30% to 50% of adults in higher socioeconomic groups.[68–70] Serosurveys continue to show a decline in the age-specific prevalence rates for HSV-1 infection in both the United States and globally, although socioeconomic class distinctions remain.[71] However, in much of Asia and Africa, HSV-1 infection is nearly universal and is acquired early in childhood (Fig. 135.1). Although primary HSV-1 and HSV-2 are clinically indistinguishable, in the United States, genital HSV-1 is now more common in whites, whereas primary genital HSV-2 remains more common in blacks.[72] HSV-1 now accounts for approximately 140 million cases of genital herpes.[70] This increased frequency of sexually acquired HSV-1 infections as well as an increased proportion of neonatal HSV cases due to HSV-1 may be accounted for by the decrease in HSV-1 acquisition in childhood.[73–75]

Herpes Simplex Virus Type 2

Antibodies to HSV-2 start to appear during puberty and correlate with initiation of sexual activity.[71,76] Widespread use of serologic testing has provided a detailed characterization of a worldwide HSV-2 pandemic over the past 2 decades (Fig. 135.2).[71,77,78] Global HSV-2 prevalence is estimated at 11.3% (417 million people) among individuals 15 to 49 years of age, with 19.2 million new infections in 2012.[79] Surveys generally indicate very high levels of infection in Africa, although risk varies within each country according to sex and region.[80] Seroprevalence is

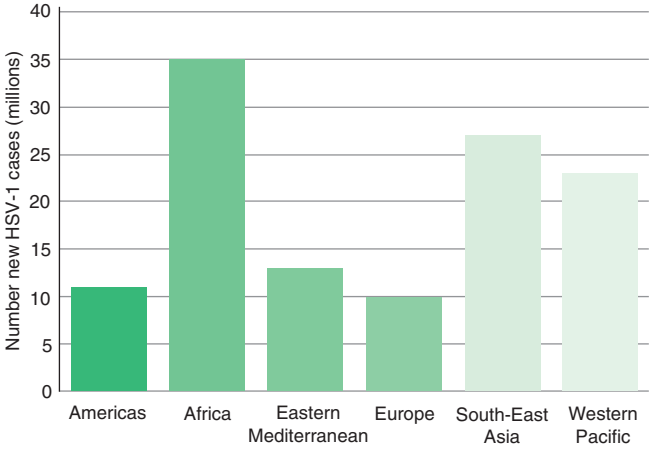

FIG. 135.1 Global seroincidence of herpes simplex virus type 1 *(HSV-1)* infection. *(From Looker KJ, Magaret AS, May MT, et al. Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012.* PLoS One. *2015;10:e0140765.)*

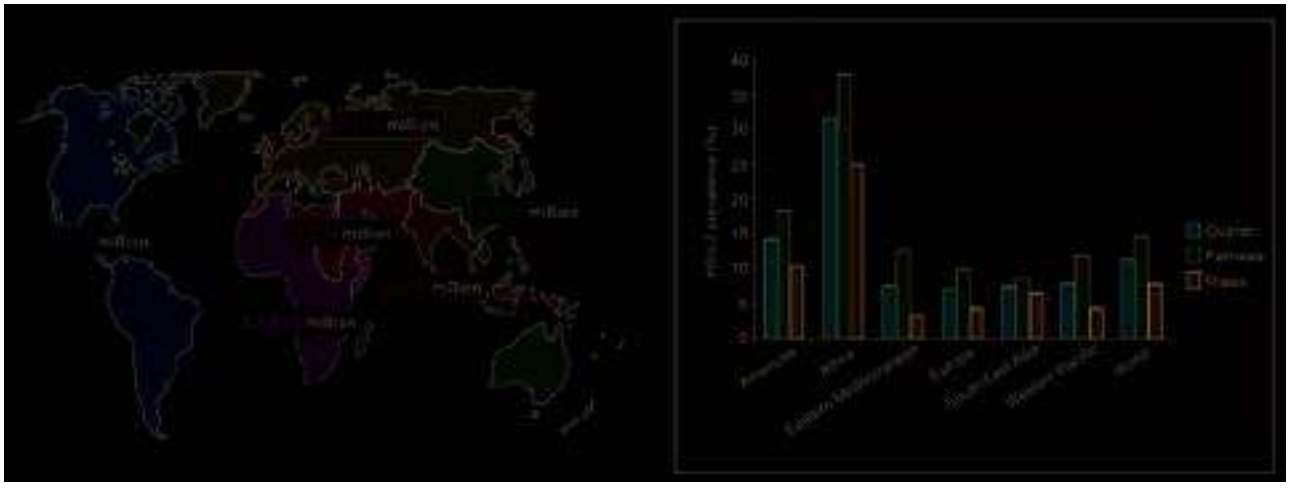

FIG. 135.2 Global herpes simplex virus type 2 *(HSV-2)* prevalence by geographic region. *(From Looker KJ, Magaret AS, Turner KM, et al. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012.* PLoS One. *2015;10:e114989.)*

lower in Europe, Australia, Latin America, and Asia, although it remains highly dependent on the risk of the group being evaluated. In the United States, nationwide surveys showed an increase in HSV-2 seroprevalence from 16.4% to 21.7% of adults between 1979 and 1991, with a decrease to 16% from 2005 to 2010.[71,77,81] The cumulative lifetime incidence of HSV-2 is 25% in white women, 20% in white men, 80% in black women, and 60% in black men. The higher rates of HSV-2 among African Americans may reflect patterns of sexual networking rather than differences in high-risk individual behavior.[82]

Although global incidence decreases with age (Fig. 135.3), local incidence of HSV-2 infection is often difficult to estimate owing to the common nature of asymptomatic seroconversion, attenuation of symptoms due to prior HSV-1 infection, location of lesions in nonvisible locations (e.g., perianal), and differential access to health care and diagnostics. For instance, among HSV-seronegative women in the control arm of an HSV-2 vaccine trial, only half of seroconversions were clinically symptomatic.[83] Nevertheless, data are accumulating and vary between populations based on risk characteristics and geography. Vaccine and condom prevention studies conducted in serodiscordant American couples document HSV-2 seroincidence levels between 6.7 and 8.6 infections per 100 person-years for women and 1.5 to 3.7 per 100 person-years for men.[84–86] Seroincidence in several cohorts of high-risk urban youth was 11.7 cases per 100 person-years.[87] In African populations characterized by high preexisting HSV-2 seroprevalence, seroincidence was 1.8 to 12.9 per 100 person-years, with higher acquisition rates among persons infected with HIV-1 and seronegative women in a monogamous relationship with a seropositive man.[88–90] Among adolescent cohorts in sub-Saharan Africa, incidence exceeding 20 per 100 person-years has been reported,[91,92] although far lower rates have been noted in certain regions.[93]

Herpes Simplex Virus Type 2 Risk Factors

HSV-2 prevalence in a population is defined by geographic region, sex, sexual habits, and study population. Prevalence of HSV-2 in women is consistently higher than in men (see Figs. 135.2 and 135.3).[68,69,75,78,79] HSV-2 infection exceeds 80% among HIV-infected individuals, individuals recruited from sexually transmitted disease clinics, and men who have sex with men (MSM).[94,95] HSV-2 antibody levels are closely related to the lifetime number of sexual partners, age of sexual debut, and history of other sexually transmitted diseases.[96,97] HSV-2 infection is nearly universal among female sex workers with many exposures, suggesting a genetic phenotype of complete protection is rare.[98]

Cofactors for genital HSV-2 acquisition risk at the level of an individual are well defined from prospective trials. Despite equivalent shedding rates,[99] women are consistently at higher risk for HSV-2 acquisition than men. Possible explanations include greater mucosal surface area, higher likelihood of asymptomatic ulcers (which may facilitate transmission) in men, or high-risk placement within sexual networks. It is uncertain whether past HSV-1 infection reduces HSV-2 infection risk. However, people with prior HSV-1 infection are three times as likely to acquire HSV-2 subclinically.[83] Overall, one-third of source partners within serodiscordant couples deny a history of genital lesions.[100]

In contrast to bacterial sexually transmitted diseases, HSV-2 is commonly transmitted within long-term couples rather than casual sexual relationships. Longitudinal studies of such couples showed transmission rates ranging from 3% to 12% per year.[86,101] Although incidence of transmission within serodiscordant couples is relatively low in clinical trials, the median time to transmission within discordant couples in natural history cohorts is 3 months,[102] with a median number of only 40 sex acts before transmission, or an approximate 3.5% per-coital risk.[86]

Knowledge of a long-term partner's HSV-2–positive status decreases transmission incidence by 50%, highlighting the importance of formal diagnosis and disclosure of infection.[86,102] Consistent condom use decreases HSV acquisition among women, and chemoprophylaxis of the source partner decreases transmission by roughly 50%.[86,101] Circumcision decreases both HIV-1 and HSV-2 acquisition risk in men[103–105] but does not appear to impact transmission rates of HSV-2 to female partners.[106]

TRANSMISSION

Transmission of HSV most frequently occurs through close contact with a person who is repeatedly shedding virus at a peripheral site, at a mucosal surface, or in genital or oral secretions.[107–109] In 1921, Lipschutz[5] inoculated material from genital herpetic lesions into the skin of humans, eliciting clinical infection within 48 to 72 hours in six persons and within 24 days in one case. It is now understood that infection occurs by inoculation of virus onto susceptible mucosal surfaces (e.g., oropharynx, cervix, conjunctivae) or through small cracks in the skin. Because HSV is readily inactivated at room temperature and by

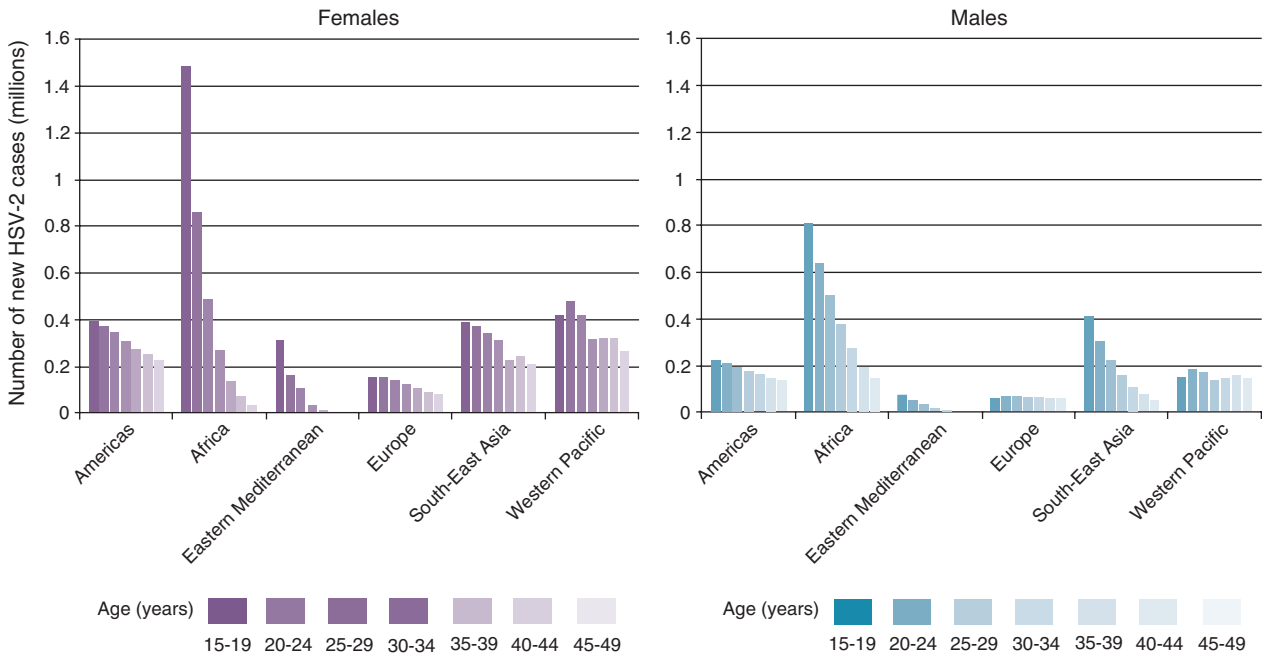

FIG. 135.3 Estimated herpes simplex virus type 2 *(HSV-2)* incidence by sex, age, and geographic region. *(From Looker KJ, Magaret AS, Turner KM, et al. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012.* PLoS One. *2015;10:e114989.)*

drying, aerosol and fomitic spread are unusual. Transmission of HSV-1 from orogenital contact is increasingly recognized, perhaps because of reduction in age-specific prevalence of HSV-1 at the time of sexual debut.[110] Spread of HSV-1 infection from oral secretions to other skin areas is a hazard of certain occupations (e.g., dentists, respiratory care unit personnel), and laboratory-acquired and nosocomial outbreaks in hospital or nursery personnel have been reported.[111] Herpes gladiatorum outbreaks among wrestlers are well recognized.[112] Transmission of HSV can occur in infants born to mothers excreting HSV at delivery.[113] Anal and perianal infections with HSV-1 or HSV-2 are common among sexually active populations of MSM.[114] Most cases occur within 5 days of contact, highlighting the short incubation period of primary infection.

Precise virologic determinants of transmission likelihood are poorly understood. For HIV infection, a clear relationship between genital and plasma HIV viral load and per-coital risk for HIV transmission is established.[115,116] However, because genital HSV-2 levels fluctuate extremely rapidly over hours both on and off therapy,[117,118] the precise degree to which source partner viral load during sex impacts likelihood of transmission is unknown.[119] Nevertheless, modeling suggests that a certain viral load threshold influences sexual transmission.[120]

Subclinical or asymptomatic shedding of HSV in oral and genital secretions is a hallmark of infection,[99,107,121] both in immune competent and immunocompromised persons,[122] and transmission occurs more commonly during asymptomatic shedding.[100,123] Prolonged asymptomatic shedding episodes lasting several days are well described, even among immunocompetent patients taking daily antiviral therapy.[99,118] Frequency of symptomatic recurrences poorly predicted likelihood of transmission in a prospective trial, again highlighting the high transmission risk for asymptomatic shedding.[124]

Frequency of detectable shedding is markedly heterogeneous among individuals seropositive for HSV-2, likely influencing the variability in reported per-coital transmission rates.[121] DNA polymerase inhibitors that decrease frequency of asymptomatic shedding as well as peak HSV-2 titers during recurrence also decrease transmission within serodiscordant couples.[101]

PATHOGENESIS

Primary Infection

Exposure to HSV at mucosal surfaces or abraded skin permits entry of the virus and initiation of its replication in cells of the epidermis and dermis.[125] Initial HSV infection is often subclinical, without apparent lesions. In animal models and human subjects, both clinical acquisition and subclinical acquisition are associated with sufficient viral replication to permit infection of either sensory or autonomic nerve endings, with the subsequent potential for lifelong reactivation.[37,61,125,126] After traversing the neuroepithelial gap and entering into the neuronal cell, the virus or nucleocapsid is transported intraaxonally to the nerve cell bodies in ganglia.[127] For HSV-1 infection, trigeminal ganglia are most commonly infected, although extension to the inferior and superior cervical ganglia also occurs.[34,35] With genital infection, sacral nerve root ganglia (S2 to S5) are most commonly affected.[40] Autonomic ganglia also appear to be infected, albeit clinical manifestations of such infections are relatively uncommon.[128] In humans, the interval from inoculation of virus in peripheral tissue to spread to the ganglia is unknown.

High-level viral replication occurs in ganglia and contiguous neural tissue during primary infection, although lytic transcripts also are detectable during and between reactivations in mouse and guinea pig models.[58,125,126,129] After initial inoculation of the neural ganglion, virus spreads to other mucosal skin surfaces by centrifugal migration of infectious virions through peripheral sensory nerves. This mode of spread explains the characteristic development of new lesions distant from the initial crop of vesicles in patients with primary genital or orolabial HSV infection, the large surface area over which these vesicles may be visualized, and the recovery of virus from neural tissue distant from neurons innervating the inoculation site.[130] Contiguous spread of virus also likely occurs via autoinoculation.[117] Viremia is present during approximately 25% of primary HSV-2 infections, and its presence may affect the natural history of HSV-2 disease in terms of site, severity, and frequency of reactivation.[131]

During primary HSV-2 infection, a local resident T-cell population has yet to be established in mucosa or ganglia. Therefore innate immune cells and antigen presenting cells represent early immune responders in biopsies of active lesions and are likely to be critical in eliminating infected cells during first exposure to the virus.[132]

Ganglionic Cell–Mediated Immunity

After resolution of primary disease, viral DNA is present in 2% to 11% of ganglion cells in the anatomic region of initial infection.[62] Therefore many neurons may contribute to reactivation, the frequency and severity of which may be influenced by host T-cell responses at the ganglion level as well as the previously described molecular features of latency. Although it is unknown whether reactivating stimuli transiently suppress these immune cells or independently upregulate transcription of lytic genes, or both, it is well documented that $CD8^+$ T cells juxtapose to HSV-1 latently infected neurons in human trigeminal ganglia.[133] These activated cells are reactive to a broad array of early and late HSV antigens.[134] In murine models, resident $CD8^+$ lymphocytes and lymphocyte-derived cytokines appear to be important in preventing infectious virions from being transported down the length of the axon for release in the basal layer of the epidermis[135–137] and can block reactivation both with interferon-γ release[138] and granzyme B degradation of IE protein ICP4.[139] In addition, in animal models, the latent viral load in the entire biomass of ganglia correlates positively with number of neurons infected and rate of reactivation but inversely with number of $CD8^+$ T cells present.[140,141] Glutamine, which promotes proliferation of activated T cells, decreased ganglionic HSV-2 reactivation in a murine system.[142]

Mucosal Cell–Mediated Immunity

Studies involving genital mucosal responses to HSV-2 infection indicate that viral-host interactions in the mucosa dictate clinical expression of disease. Histologic examinations reveal that herpetic lesions consist of a thin-walled vesicle or ulceration in the basal region; multinucleated infected cells that may include intranuclear inclusion bodies; necrosis; and an acute inflammatory infection including neutrophils, natural killer (NK) cells, B cells, and T cells.[132] A distinct subset of HSV-2–specific $CD8^+$ T cells that persist in genital skin conduct immune surveillance directed at containing virus at the point of release into the epithelium and are first responders capable of controlling HSV-2 and aborting clinical lesions.[143,144] Once virus reaches the dermal-epidermal junction, there are two possible outcomes: subclinical shedding or recurrence defined clinically by a skin blister and ulceration (Fig. 135.4).

Host effector cell density at the precise site of viral release into the genital/oral mucosa appears to be the main influence in dictating clinical and subclinical expression of disease.[145] These cells were shown to lack chemokine-receptor expression required for lymphocyte egress, express gene signatures of T-cell activation and antiviral activity, and produce cytolytic granules during virologic quiescent time periods.[143,146] $CD8^+$ memory T-cell trafficking into genital mucosa is temporally associated with clearance of virus from genital lesions,[147,148] and $CD8^+$ T-cell density at the specific site of infection is likely a key predictor of the extent of viral spread and potential.[149] Low levels of plasma HSV-specific $CD8^+$ cytotoxic T-lymphocyte precursors, rather than low $CD4^+$ lymphocyte counts, predict frequent and severe HSV-2 recurrences in untreated as well as treated HIV-1–infected patients.[150,151] HSV-2–specific $CD8^+$ and $CD4^+$ T cells appear to persist for many years in genital skin previously involved in an HSV-2 reactivation.[143,149,152] Heterogeneous spatial density of $CD8^+$ T-cell levels across the mucosa is a likely explanation for persistent breakthrough shedding.[143,149,153]

Innate Host Factors Impacting Pathogenesis and Disease Severity

Herpesviruses are evolutionarily ancient, infect a broad range of hosts, and have developed complex mechanisms for immune system evasion. Both HSV-1 and HSV-2 encode proteins that are directed at subverting innate and acquired responses.[154,155] ICP47 inhibits the transporter activity protein–mediated transport of viral peptides to the endoplasmic reticulum/Golgi apparatus; this prevents HSV peptide presentation by major histocompatibility class I molecules needed for $CD8^+$ cytotoxic

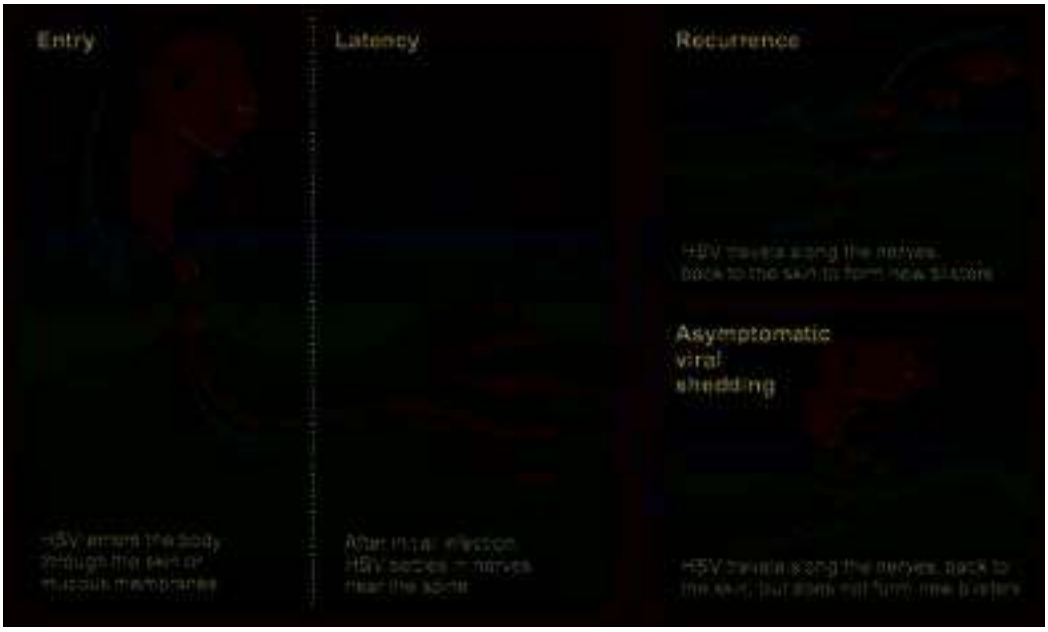

FIG. 135.4 **Schemata of herpes simplex virus *(HSV)* recurrence and asymptomatic viral shedding.**

T-cell responses to HSV.[155,156] The virion host shutoff protein rapidly degrades host and viral mRNA on host cell infection, which suppresses the interferon α/β antiviral innate response.[157] HSV glycoprotein J inhibits cytotoxic T-lymphocyte–induced apoptosis of infected cells and results in increased viral replication.[158,159] The antiviral function of several interferon-stimulated gene products is counteracted by HSV proteins, including ICP34.5, ICP0, and US11.[160,161]

Defects in innate immunity are associated with severe HSV-1 and HSV-2 infections. Fatal severe HSV-1 infections occur in individuals with innate immunity defects in NK cells and plasmacytoid dendritic cells.[162,163] Specific deficiencies in effective innate function involving the nuclear factor kappa B regulator,[164] Toll-like receptor (TLR)-3,[165] and TLR signaling protein UNC-93B[166] all are associated with familial and childhood HSV encephalitis. These mutations are rarely associated with severe cases of other infections.[167]

The innate immune response impacts the overall course of genital herpes as well. Defects in interferon receptors in mice promote enhanced infection.[168] TLR-2 polymorphisms are associated with increased HSV-2 shedding and recurrence rate,[169] and a randomized clinical trial found that a topically applied TLR-7 and TLR-8 agonist resulted in decreases in shedding.[170] Similarly, variation at the apolipoprotein E allele is associated with increased shedding.[171] Most host genetic determinants of frequency and severity of reactivation remain unidentified.

Cell-Mediated Host Factors Impacting Pathogenesis and Disease Severity

The role of the innate immune response is often not clearly differentiated from acquired immunity. Interferons that are influenced by innate cells (plasmacytoid dendritic cells, NK-T cells, and NK cells) may alter the balance of the Th1/Th2 response to HSV-2 and trigger similar transcription pathways in innate and acquired immune cells. Dendritic cells infiltrate genital lesions along with $CD4^+$ lymphocytes, and differences in antigen presentation may be factors in viral clearance.[172–175]

Animal models suggest that multiple cell populations including NK cells, macrophages, various T lymphocytes, and lymphokines generated by these cells play a role in host defense against HSV infection.[176,177] Experimental ablation of lymphocytes indicated that T cells play a major role in viral containment and prevention of lethal disseminated disease, although antibodies also help reduce viral titer in neural tissue.[178] In animals, passive transfer of primed lymphocytes confers protection against rechallenge.[176] Maximal protection usually requires the activation of multiple T-cell subpopulations including cytotoxic T cells and T cells responsible for delayed hypersensitivity.[179,180] In humans, serious systemic manifestations of disease can occur in patients with T-cell immunodeficiency resulting from HIV, organ transplant, chemotherapy, or certain inherited disorders.[181–183]

There is an evolving appreciation of the complexity of the cellular immune response to HSV-2. The impressive natural breadth and structure of the HSV-directed T-cell repertoire in regard to HSV target antigens is increasingly defined.[184,185] The compartmentalization of the acquired response is also better understood: responses in the neural ganglia, CNS, and mucosal sites all may play different roles.[137,144] Biopsy specimens of human herpetic lesions show that the predominant infiltrating cell is initially the $CD4^+$ lymphocyte,[132,186] displaying activation markers such as interleukin-2 receptor, dopamine receptor, and intercellular adhesion molecule-1 and secreting large amounts of γ-interferons.[187] In animal models of primary HSV infection, $CD4^+$ T cells release specific cytokines (CXCL-9 and CXCL-10), which are critical for subsequent $CD8^+$ T-cell function and infiltration.[188] This dense infiltrate of HSV-specific $CD8^+$ T cells as well as the number of circulating HSV-specific $CD4^+$ T cells is associated with clearance of HSV-2 from genital lesions, and these cells are disproportionately represented in the mucosa compared with the serum.[150,189] Persistence of ganglionic $CD8^+$ T cells in murine models is associated with experimental containment of reactivation.[140] However, plasma levels and the functionality of these cells or dendritic cells have, to date, not correlated with lesion or shedding rate, again pointing to the prominence of mucosal viral-host interactions in influencing disease expression.[190]

Some aspects of HSV disease may be related to cell-mediated immunopathologic events. In experimental animals, stromal keratitis associated with HSV-1 infection is precipitated by HSV-specific T cells. Molecular cross-reactivity between the HSV proteins and cellular proteins did not appear to play a role in this phenomenon in one study.[191] Thus the underlying mechanism of this immunopathologic syndrome has not been identified. The role of immunopathogenesis in oral and genital lesions is also unknown.

Humoral Host Factors Impacting Pathogenesis and Disease Severity

Agammaglobulinemic patients appear to handle HSV infection normally. However, neonatal HSV is more likely to occur in vaginally delivered infants when the mother has primary rather than recurrent infection, because placental antibodies are generated after primary infection and increase in avidity over time.[192–194] These data suggest cellular immunity is required for viral clearance, whereas antibodies may be required for

reducing acquisition. These factors must be considered in the design of immunotherapeutics versus prophylactic vaccines.

During natural infection, antibodies are broadly generated against tegument, capsid, and other nonessential viral glycoproteins, although the importance of antibody response magnitude, breadth, tissue compartment specificity, and subclass is not defined for HSV infection.[178] Surface viral glycoproteins necessary for attachment also participate as antigens that are recognized by antibodies mediating neutralization and immune-mediated cytolysis (antibody-dependent cell-mediated cytotoxicity).[195] Monoclonal antibodies specific for each of the known viral glycoproteins confer experimental protection against subsequent neurologic disease or ganglionic latency.[196] In an ex vivo model of human reactivation, adequate neutralizing antibody concentrations prevented the successful transmission of HSV from dorsal root ganglion neurons to keratinocytes, despite successful anterograde transport of fully assembled particles down the length of the sensory neuron.[197] Effector functionality of antibodies appears to be a key component of protection in animal studies.[198–200] Although several vaccines have shown promise in animal vaccine studies, evaluation of these vaccines in humans has been slow.[84,85] Interestingly, immunization with a glycoprotein deletion strain (ΔgD-2) conferred protection against lethal challenge in an animal model.[27]

SPECTRUM OF DISEASES CAUSED BY HERPES SIMPLEX VIRUS

HSV has been isolated from nearly all visceral and mucocutaneous sites, but clinical manifestations and infection course depend on the anatomic site involved, antigenic type of the virus, and age and immune status of the host. Presentations vary from subclinical mucosal shedding to overwhelming sepsis or encephalitis. First episodes of HSV disease, especially primary infections, are frequently accompanied by systemic signs and symptoms, involve both mucosal and extramucosal sites, have a higher complication rate, and have a longer duration of symptoms and viral shedding from lesions.[201,202] Conversely, asymptomatic primary infection is also common. Both viral subtypes can cause genital and orofacial infections, and infections caused by the two subtypes are clinically indistinguishable. However, the frequency of reactivation is influenced by anatomic site and virus type.[203]

OROFACIAL HERPES SIMPLEX VIRUS INFECTION

Gingivostomatitis and pharyngitis are the most frequent clinical manifestations of first-episode HSV-1 infection,[204,205] usually result from primary infection, and are most commonly seen in children and young adults.[206,207] Clinical signs and symptoms, which include malaise, myalgias, inability to eat, irritability, and cervical adenopathy, last 3 to 14 days. Lesions may involve the hard and soft palate, gingiva, tongue, lip, and face (Fig. 135.5). HSV-1 and HSV-2 infection of the pharynx usually results in exudative or ulcerative lesions of the posterior pharynx, tonsillar pillars, or both. Lesions of the tongue, buccal mucosa, or gingiva occur later in the course in one-third of cases. Fever lasts 2 to 7 days. It can be difficult to clinically differentiate HSV pharyngitis from bacterial pharyngitis, *Mycoplasma pneumoniae* infections, and pharyngeal ulcerations of noninfectious causes (e.g., Stevens-Johnson syndrome). Recurrent herpes labialis is the most frequent clinical manifestation of reactivation. No substantial evidence suggests that reactivation of orolabial HSV infection is associated with symptomatic recurrent pharyngitis. Release of HSV from the trigeminal ganglia may be associated with asymptomatic salivary virus excretion, intraoral mucosal ulcerations, or herpetic ulcerations on the vermilion border of the lip or external facial skin. About 50% to 70% of seropositive patients undergoing trigeminal nerve root decompression and 10% to 15% of seropositive patients undergoing dental extraction develop orolabial HSV disease a median of 3 days after these procedures.[208,209]

In immunosuppressed patients, infection may extend into mucosal and deep cutaneous layers. Friability, necrosis, bleeding, severe pain, and inability to eat or drink may result.[210] The lesions of HSV mucositis are similar to oral lesions caused by cytotoxic drug therapy, trauma, or fungal or bacterial infection.[211] Persistent and disabling ulcerative HSV infections are common in patients with acquired immunodeficiency syndrome (AIDS).[212] HSV and *Candida* infections often occur concurrently. Systemic acyclovir therapy reduces the frequency of clinical reactivation, speeds the rate of healing, and relieves the pain of mucosal HSV infections in immunosuppressed patients.[210,211] Patients with atopic eczema or burns may acquire severe orofacial HSV infections (eczema herpeticum), which may rapidly involve extensive areas of skin with occasional systemic dissemination.[213] Extensive eczema herpeticum typically resolves promptly with delivery of intravenous acyclovir.[214]



FIG. 135.5 Primary herpes simplex virus gingivostomatitis in a child extending to involve the cheek, chin, and periocular skin.

Erythema multiforme is also associated with HSV infections, and evidence suggests that HSV infection is the precipitating event in about 75% of cases of cutaneous erythema multiforme.[215] HSV antigen is often present in circulatory immune complexes and skin lesion biopsy samples from these patients.[216] Patients with severe HSV-associated erythema multiforme are candidates for chronic suppressive oral antiviral therapy.[217]

Complications of Oral Herpes Simplex Virus Type 1

HSV-1 and VZV are implicated as common causes of Bell palsy (facial paralysis of the mandibular portion of the facial nerve).[218] HSV DNA was found in ganglionic fluid in a high percentage of patients undergoing decompressive surgery for Bell palsy, suggesting recent reactivation as the cause of disease.[219] Although one trial showed faster and more frequent resolution of facial paralyses with the prompt use of antiviral therapy directed at HSV-1 or VZV, more recent data on antiviral therapy for Bell palsy suggest no benefit, perhaps related to the delayed timing of initiation of therapy. Corticosteroids are a mainstay of therapy and correlate with improved short-term and long-term resolution of symptoms (a common regimen is prednisone, 60–80 mg/day for 7 days).[220–222] There is no consensus on use of antiviral agents plus corticosteroids versus corticosteroids alone for the treatment of Bell palsy.

GENITAL HERPES SIMPLEX VIRUS INFECTION

First-episode primary genital herpes is associated with prolonged duration of symptoms, lesions (10–20 days), and viral shedding. This is particularly true for primary infection (i.e., HSV-1 and HSV-2 antibody negative), which is the case for about half of first-episode cases.[201] About 25% of patients with a first clinical episode of symptomatic genital herpes already have HSV-2 directed antibodies, suggesting asymptomatic acquisition occurred in the past.[223] First episodes of genital herpes caused by HSV-2 in patients who had prior HSV-1 infection are associated with less frequent systemic symptoms and faster healing than primary genital herpes, although rates of recurrence are the same.[201–203] The clinical courses of first-episode genital herpes caused by HSV-1 and HSV-2 are similar,[72,224] but frequency of subsequent recurrences in the first year after acquisition differ considerably: 55% and 90%, respectively.[203,224] Genital HSV-1 acquisition is rare after HSV-2 infection.[225] Prior oral HSV-1 infection appears to protect against genital HSV-1 infection, although the degree of this protection is unknown.

In 70% of women and 40% of men, first-episode genital herpes is accompanied by fever, headache, malaise, and myalgias. Pain, itching, dysuria, vaginal and urethral discharge, and tender inguinal lymphadenopathy are the predominant local symptoms and persist for several days after systemic symptoms. Local symptoms often peak between days 7 and 11 of detectable shedding, whereas inguinal tenderness can persist for several weeks. Widely spaced bilateral lesions of the external genitalia are characteristic on examination (Figs. 135.6 and 135.7). Lesions may be present in varying stages including vesicles, pustules, painful erythematous ulcers, crusting (on dry surfaces), or reepithelialization (mucosal surfaces). Multiple small ulcers often coalesce into one larger ulcer. If untreated, formation of new ulcers between days 4 and 10 of infection is common. The mean time from the onset of a primary genital HSV lesion to complete healing is 19.5 days for women and 16.5 days for men.[201,202]

Local Complications of Genital Herpes Simplex Virus Infection

The complications of genital herpes are related to local extension, spread to extragenital sites, or both.[113,226] Complications occur more frequently in women than in men.[201] A clear mucoid discharge and dysuria are present in first episodes of HSV infection in 83% of women and 44% of men. The severity of dysuria is often out of proportion to the urethral discharge seen on examination and the mild inflammation detected on urinalysis. HSV-2 cervicitis, when symptomatic, is notable for purulent or bloody vaginal discharge and can be difficult to clinically differentiate from *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection. When present on speculum examination, cervical ulceration or necrosis is specific for HSV-2 infection. HSV-2 is present on the cervix and urethra in more than 80% of women with first-episode infections and 20% of women with recurrent lesions (Fig. 135.8).[227,228] Traditional colposcopy and Pap test lack sensitivity for cervical HSV infection.[229] Moreover, HSV can be isolated from the urethra and urine of men and women without external genital lesions and from the urethra of 5% of women with dysuria-frequency syndrome.[228–230]

Occasionally, HSV genital tract disease is manifested by endometritis and salpingitis in women and prostatitis in men.[231,232] Both HSV-1 and HSV-2 can cause symptomatic or asymptomatic rectal and perianal infections. HSV proctitis is usually associated with rectal intercourse.[233,234] Symptoms include anorectal pain, anorectal discharge, tenesmus, and constipation. Sigmoidoscopy reveals ulcerative lesions of the distal 10 cm of the rectal mucosa. Rectal biopsy specimens show mucosal ulceration, necrosis, polymorphonuclear and lymphocytic infiltration of the lamina propria, and, occasionally, multinucleated intranuclear inclusion–bearing cells. External perianal lesions are present in only about one-half of cases.[233] Antiviral therapy speeds healing.[233–235] Perianal herpetic lesions are also found in immunosuppressed patients receiving cytotoxic therapy. Extensive perianal herpetic lesions, HSV proctitis, or both are common among HIV-infected patients.[212] Subclinical perianal shedding of HSV is detected both in heterosexual men and in women who report no rectal intercourse.[235,236] This phenomenon is due to the establishment of latency in the sacral dermatome from prior genital tract infection, with subsequent reactivation in epithelial cells in the perianal region.



FIG. 135.6 **Primary genital herpes simplex virus type 2 infection of the vulva.**



FIG. 135.7 **Chancroidal herpes simplex virus lesion on penis.** *(From Corey L. Herpes simplex virus infections. In: Mandell GL, series ed; Rein MF, ed.* Atlas of Infectious Diseases. Vol. V. Sexually Transmitted Diseases. *Philadelphia: Churchill Livingstone; 1996. Courtesy H.H. Handsfield, MD.)*

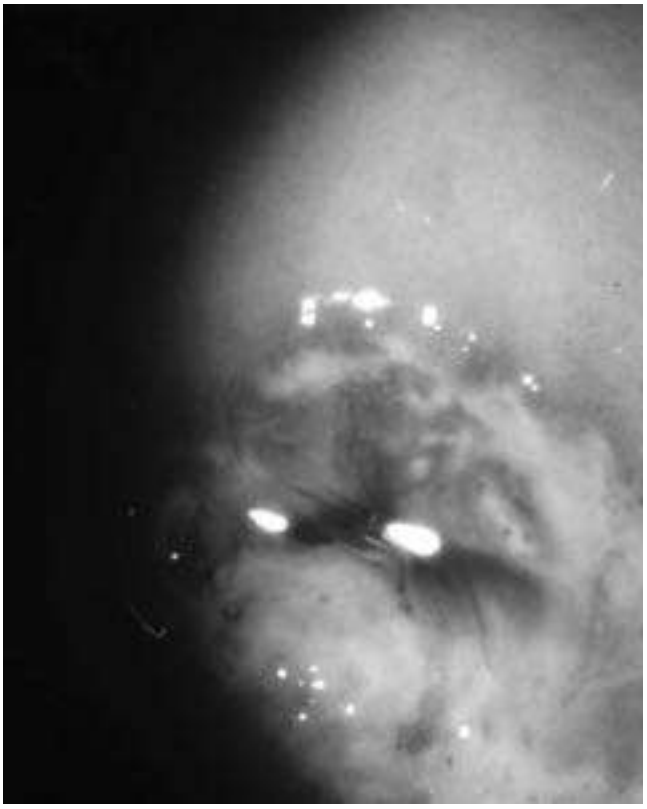

FIG. 135.8 Herpes simplex virus cervicitis. *(From Corey L. Herpes simplex virus infections. In: Mandell GL, series ed; Rein MF, ed.* Atlas of Infectious Diseases. Vol. V. Sexually Transmitted Diseases. *Philadelphia: Churchill Livingstone; 1996.)*

Aseptic Meningitis, Transverse Myelitis, and Sacral Radiculopathy

Both HSV-1 and HSV-2 have been isolated from cerebrospinal fluid (CSF), although overt viral meningitis is much more common with HSV-2.[237,238] In one series, 36% of women and 13% of men with primary genital HSV-2 infection had stiff neck, headache, and photophobia on two consecutive examinations.[201] Among these patients, hospitalization was necessary for 6.4% of women and 1.6% of men for aseptic meningitis. There was also a high frequency of CSF pleocytosis in patients without overt clinical evidence of meningeal irritation in a study of primary genital herpes in the early 1900s, suggesting that meningeal involvement may be quite common with primary genital herpes.[239]

Fever, headache, vomiting, photophobia, and nuchal rigidity are the predominant symptoms of HSV aseptic meningitis. Meningeal symptoms usually start 3 to 12 days after the onset of genital lesions. Symptoms generally reach a maximum 2 to 4 days into the illness and gradually recede without sequelae over 2 to 3 days. The CSF is usually clear, and opening pressures may be somewhat elevated. White blood cell counts in the CSF usually range from 10 to 1000 cells/mm.[3] The pleocytosis is predominantly lymphocytic in adults, although early in the course of disease and in neonates a predominantly polymorphonuclear response may be seen. The CSF glucose level is usually more than 50% of the blood glucose level, although hypoglycorrhachia has been reported.[240] The CSF protein is usually slightly elevated. In cases of aseptic meningitis, HSV may be isolated from the CSF, although HSV DNA detection by PCR assay is a considerably more sensitive diagnostic test and is now the mainstay for diagnosis.[238] The differential diagnosis of aseptic meningitis is broad, but the presence of both neurologic involvement and genital ulcerations narrows the possibilities to include sacral herpes zoster, Behçet syndrome, collagen vascular disease, inflammatory bowel disease, and porphyria. Although uncommon, aseptic meningitis may be the sole presenting sign of new HSV-2 acquisition. Use of systemic antiviral chemotherapy early in the course of primary genital herpes decreases subsequent development of aseptic meningitis. Controlled trials of intravenous acyclovir for established HSV meningitis have not been conducted. However, intravenous acyclovir, 10 mg/kg every 8 hours for 7 to 10 days, is recommended for hospitalized symptomatic patients.

Benign recurrent lymphocytic meningitis, or Mollaret meningitis, is characterized by recurrent episodes of meningitis lasting 3 to 7 days and resolving without neurologic sequelae.[241] Although the differential diagnosis for chronic meningitis is broad, HSV-2 is responsible for most recurrent cases.[242] In one small series of patients with meningitis during first-episode genital HSV-2 infection, 27% had possible symptomatic evidence of recurrent meningitis, and 10% had documented recurrent disease verified by HSV DNA in the CSF.[243] Prophylactic doses of acyclovir are often encouraged for patients with frequent recurrent meningitis.

Autonomic nervous system dysfunction can occur in association with genital HSV infection.[244,245] Manifestations include hyperesthesia or anesthesia of the perineum, lower back, or sacral regions; urinary retention; and constipation. This complication occurs more frequently in women than men but is sometimes present in men with HSV proctitis. Physical examination reveals a large bladder, decreased sacral sensation, and poor rectal and perineal sphincter tone. Impotence and absent bulbocavernous reflexes have been noted in men. CSF pleocytosis may be present in some patients. Electromyography usually reveals slowed nerve conduction velocities and fibrillation potentials in the affected area, and urinary cystometric examination shows a large atonic bladder. Most cases resolve within 4 to 8 weeks.

Transverse myelitis has also been reported in association with primary genital HSV infection.[246] Decreased deep tendon reflexes and muscle strength in the lower extremities as well as the previously described autonomic nervous system signs and symptoms are present. Residual neurologic dysfunction may occur. Whether autonomic nervous system and spinal cord dysfunction results from viral invasion of the CNS or an unusual immunologic response to infection is unknown.

Extragenital Lesions

Extragenital lesions commonly develop during the course of primary genital herpes and are more common in women than in men. Extragenital lesions are most frequently located in the buttock, groin, or thigh area, although the finger and eye can also be involved. Among patients with primary HSV-2, 9% develop extragenital lesions, most commonly on the buttocks. Among patients with primary genital HSV-1, 25% acquire extragenital lesions, most commonly in or around the mouth.[225] The distribution of lesions on the extremities or areas near the genital lesions and their typical occurrence 2 weeks into the course of disease suggest that most extragenital lesions develop by autoinoculation of virus or by viral reactivation in another part of the affected dermatome rather than viremic spread.[201,225] However, the common demonstration of plasma viremia during the course of primary HSV infection suggests that viremic spread may also be a factor.[131,247] Both HSV-1 and HSV-2 have been shown to be rare causes of pelvic inflammatory disease. Although this may represent dual infection with other sexually transmitted pathogens such as *N. gonorrhoeae* and *C. trachomatis,* extension of HSV infection into the uterine cavity with laparoscopic evidence of HSV-positive vesicular lesions on the fallopian tube has been reported.[231]

Disseminated Infection

Bloodborne dissemination as manifested by multiple vesicles over widespread areas of the thorax and extremities occurs rarely in individuals with primary mucocutaneous herpes.[248,249] Cutaneous dissemination usually occurs early in the disease and is often associated with aseptic meningitis, hepatitis, or pneumonitis. Other rare complications of primary genital HSV-2 infection include monarticular arthritis,[250] thrombocytopenia,[251] adrenal necrosis,[252] and myoglobinuria.[253] Pregnancy may predispose to severe visceral dissemination of primary genital HSV disease.[254,255] Reactivation of genital HSV in immunosuppressed patients, especially patients with impaired cellular immune responses, can be associated with interstitial pneumonia, hepatitis, and meningitis, similar to the manifestations of disseminated infection of the neonate.[256,257]

Disseminated visceral infections in immunosuppressed and pregnant patients are associated with high mortality and should be treated immediately with systemic antiviral chemotherapy.

Superinfection

Bacterial superinfection of genital herpes in immunocompetent patients is a rare complication. Pelvic cellulitis appears as an advancing erythema and swelling of the perineal area, and patients should receive systemic antimicrobial therapy. Fungal vaginitis is frequently encountered toward the end of genital herpes–associated symptoms and often leads to recurrence of pruritus and increased discharge; concurrent yeast infection occurs more frequently in women with genital herpes.[201] Bacterial vaginosis also appears to be more common in individuals seropositive for HSV-2.[258,259]

RECURRENT MUCOCUTANEOUS HERPES SIMPLEX VIRUS INFECTIONS

In contrast to first episodes of genital infection, the symptoms, signs, and anatomic sites of infection of recurrent genital or orolabial herpes are usually localized to a defined mucocutaneous site.[260,261] Local symptoms such as pain and itching are mild to moderate compared with first episodes of infection, and the duration of the episode is shorter. Lesions are usually confined to one side, and the involved area is on average one-tenth that of primary infection.[201,262] Recurrent orolabial HSV infection tends to be of shorter duration than genital HSV infection. Orolabial lesions usually pass through clinical stages of infection more rapidly, and the median time from onset of tingling to healing averages 5 days.[204]

Among immunocompetent individuals who acquire HSV-1 orally and genitally, HSV-1 reactivates more frequently in the oral region than in the genital region. Similarly, for HSV-2, reactivation in the genital region is 8 to 10 times more frequent than oral reactivation of HSV-2.[224,263] In experimental animal systems, both sacral and trigeminal ganglia contain latent virus, but reactivation differs according to the anatomic site of infection.[264] In a murine model, inserting the latency-associated transcripts of HSV-2 into an HSV-1 virus increased reactivation in sacral nerve root ganglia, indicating that viral factors influence site of reactivation.[47]

Both oral and genital HSV reactivations are frequently associated with prodromal symptoms, which occur in the absence of lesions in 20% of episodes.[260] Prodromal symptoms vary from a mild tingling sensation, occurring 0.5 to 48 hours before eruption, to shooting pains in the buttocks, legs, or hips 1 to 5 days before eruption. In many patients, the prodromal symptoms are the most bothersome part of the episode. HSV is present on mucosal surfaces more frequently during the prodrome, suggesting that viral reactivation is associated with these symptoms.[236,262] The severity and mean duration of pain are greater in women (5.9 days) compared with men (3.9 days), as is the likelihood of dysuria (27% vs. 9%), although dysuria is normally external, and isolation of HSV from the urethra is considerably less common than during the first HSV-2 episode. The mean duration of shedding (approximately 4 days in both sexes) is shorter than during primary infection but highly variable both between and within individuals over time.[265]

The diverse clinical spectrum of recurrent HSV infection is increasingly recognized. First, subclinical reactivation of virus on mucosal surfaces is common.[236] In addition, studies of both orolabial and genital ulcerative lesions have found a surprisingly high frequency of HSV isolated from "atypical" clinical syndromes (approximately 33%) including linear fissures or serpiginous ulcers without an erythematous base (see Fig. 135.7).[266] Even among experienced clinicians, false-positive and false-negative clinical diagnoses of genital herpes are common.[83,267,268] Therefore in the absence of a known HSV diagnosis, we recommend that all ulcerative lesions on the oral and genital mucosa be sampled for HSV.[269] A definitive etiologic diagnosis is best established by demonstration of viral nucleic acid or isolation of virus from the affected area. The detection of HSV-2 does not rule out coinfection with *Treponema pallidum* or *Haemophilus ducreyi,* which should be considered in the appropriate clinical and epidemiologic context.[268,270]

Importantly, although 80% of seroprevalent individuals deny genital lesions, the vast majority are not truly asymptomatic but have genital lesions that they do not recognize as herpetic. In several studies, seropositive women and men who were previously unaware of their diagnosis were educated regarding signs and symptoms of genital herpes. During follow-up periods of just a few months, 48% to 62% of these subjects developed classic recurrent lesions.[108,261,271] In one study, localized genital symptoms developed in an additional 25%.[108] The rate of subclinical shedding was the same among men and women with recognized and unrecognized genital HSV infection, although frequency and duration of recurrences were greater in subjects with previously recognized disease.

FREQUENCY OF REACTIVATION AND RECURRENCE

The major morbidity of genital HSV-2 infection is a result of the high frequency of reactivation.[272] Of patients who present with symptomatic first-episode genital HSV-2 infection, 90% experience clinical reactivation of infection, and 98% experience subclinical HSV-2 shedding in genital mucosa.[273–276] The median clinical recurrence rate is between four and five episodes per year, although there is great heterogeneity among patients: 20% of patients report more than 10 clinical recurrences during the year after primary infection.[203] Studies of recurrence rates of genital HSV-2 lesions show a steady but gradual decrease in recurrence rates over time. In one study, annual recurrences of genital herpes decreased from an average of 5 to 2 per year over a 5- to 8-year period. The decrease most commonly occurred 3 to 5 years after acquisition. However, there was again great variability, with 20% of patients reporting increased recurrences over time.[277] Both recurrence and asymptomatic shedding persist at high levels in most patients 10 years after initial infection.[278]

HSV-1 infection recurs much less frequently in the genital tract than HSV-2. Less than 5% of people with primary HSV-1 infection of the genital tract will have more than four lifetime recurrences.[224] Recurrence frequency is slightly higher for men and for people with prolonged duration of primary infection (>35 days).[203]

Subclinical Herpes Simplex Virus Type 2 Shedding

Subclinical or asymptomatic viral shedding is a critical concept for understanding the epidemiologic and transmission features of genital and orolabial HSV infections.[100,123,279,280] Previous studies indicated that two-thirds of mucosal HSV-1 or HSV-2 shedding episodes are subclinical.[236,273] Protocols with frequent swabbing for HSV-2 in the anogenital tract detect a high number of previously missed shedding episodes that last less than 6 hours, indicating that subclinical HSV episodes constitute more than three-fourths of the virologic reactivation in an individual (Fig. 135.9).[121,281] Shedding episodes of HSV may last from less than 2 hours to several days and, similar to clinical recurrences, occur most frequently after acquisition and then gradually decline to stable levels over a 1- to 2-year period. In women, the anatomic sites of asymptomatic shedding are often spatially dispersed across numerous genital sites[282,283] and include the cervix, vulva, anus, and urethra.[236] Anal reactivation is seen without a history of anal interactions, as the anal region has overlapping innervation with the vulvar area. In men, shedding occurs from the penile skin, urethra, anus, and occasionally semen.[276] Among MSM, perianal shedding is more common, whereas the converse is true for men who acquire HSV via heterosexual contact, suggesting that the site of initial inoculation and latency influence the subsequent pattern of reactivation. Similar patterns of subclinical reactivation occur in the oropharynx where frequent shedding correlates with frequent recurrence.

Subclinical reactivation can be detected by either culture or PCR. HSV DNA detection by PCR assay is three to four times more sensitive than viral isolation.[269] Studies of antiviral therapy have shown that long-term daily therapy reduces viral DNA detection by 70% to 95% (from 28% to 8% of days), indicating that HSV DNA as detected by PCR assay on mucosal surfaces represents the replicating form of the virus and hence is infectious.[273,284] Most sexual and maternal-fetal transmissions occur during episodes of subclinical shedding. Similarly, subclinical shedding can cause primary genital HSV-1 infections through orogenital sexual activity. Therefore counseling of patients with genital



FIG. 135.9 Anatomic distribution of genital shedding in two women who had 22 genital samples collected over a 30-day period. The quantity of virus shed in each region is shown according to the heat map. (A) Widespread genital shedding in a woman with asymptomatic shedding. (B) Diffuse shedding in a woman with a symptomatic genital lesion on days 1 to 5, days 11 and 12, and days 15 to 19. The site of the lesion is highlighted in black. *(From Johnston C, Corey L. Current concepts for genital herpes simplex virus infection: diagnostics and pathogenesis of genital tract shedding.* Clin Microbiol Rev. *2016;29:149–161.)*

herpes needs to emphasize the potential for infectivity regardless of symptoms and provide appropriate strategies to decrease the risk to patients' sexual partners.

HERPETIC WHITLOW

Herpetic whitlow (HSV infection of the finger) may occur as a complication of primary oral or genital herpes by inoculation of virus through a break in the epidermal surface or by direct introduction of virus into the hand through occupational or some other type of exposure.[285] Before the increased use of gloves in health care settings, HSV-1 was most commonly isolated from herpetic infections of the hand. With the increasing prevalence of HSV-2, herpetic whitlow due to HSV-2 is encountered more frequently, and one survey found HSV-2 as the predominant causative agent.[225] Clinical signs and symptoms of herpetic whitlow include the abrupt onset of edema, erythema, and localized tenderness of the infected finger. Vesicular or pustular lesions of the fingertip can be difficult to distinguish from lesions of pyogenic bacterial infection. Fever, lymphadenitis, and epitrochlear and axillary lymphadenopathy are common. The infection may recur. Prompt diagnosis (to avoid unnecessary and potentially exacerbating surgical therapy or transmission) is essential. Antiviral chemotherapy to speed the healing process is recommended.

HERPES GLADIATORUM

HSV may infect almost any area of skin. Mucocutaneous HSV infections of the thorax, ears, face, and hands occur in outbreaks among wrestlers. Transmission of these infections is facilitated by trauma to the skin sustained during matches. Prompt diagnosis and therapy are required to contain the spread of this infection and reduce microepidemics among wrestling teams.[286]

EYE INFECTIONS

HSV infection of the eye is the most frequent cause of corneal blindness in the United States.[287,288] HSV keratitis commonly results from reactivation of latent, orally acquired, trigeminal ganglia–derived HSV-1. It arises with an acute onset of pain, blurring of vision, chemosis, conjunctivitis, and characteristic dendritic lesions of the cornea (Fig. 135.10). Use of topical glucocorticoids may exacerbate symptoms and lead to involvement of deep structures of the eye.[289] Débridement, topical antiviral treatment, interferon therapy, or a combination of these methods hastens healing. Primary disease is often self-limited, but recurrences are common, and the deeper structures of the eye may sustain irreversible scarring due to immunopathologic injury. Both eyes are involved in approximately 5% of cases. Asymptomatic shedding appears to occur, although different studies report highly variable frequencies of viral detection.[290,291]

HSV-1 blepharitis and conjunctivitis are distinguishable from other self-limited viral conjunctivitis cases by the presence of vesicles on the eyelid margin. Chorioretinitis occurs in neonates or in patients with HIV infection who have disseminated infection.[292] HSV and VZV can also cause acute necrotizing retinitis, a devastating condition that leads to painless vision loss and affects both eyes about 25% of the time. This

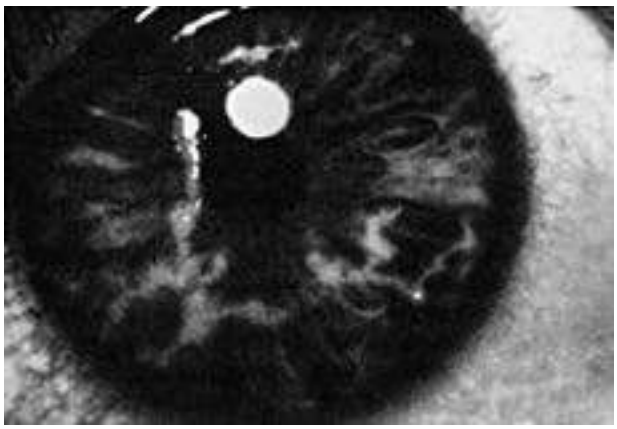

FIG. 135.10 Herpes simplex virus type 1 dendritic keratitis. *(From Pavan-Langston D, ed.* Ocular Viral Disease. Vol. 15. *Boston: Little, Brown; 1975:19–36.)*

entity can occur in immunocompetent individuals, pregnant women, and HIV-1–infected patients. Retinal necrosis is rapid, and prompt systemic antiviral chemotherapy, systemic corticosteroids to reduce inflammation, vitreal biopsy with PCR assay, and laser retinopexy are indicated. Residual blindness is common.[288,293,294]

HERPES SIMPLEX VIRUS ENCEPHALITIS

HSV is the most commonly identified cause of acute, sporadic viral encephalitis in the United States, accounting for 10% to 20% of all cases.[295] The estimated incidence is approximately 2.3 cases per 1 million persons per year. In contrast to enteroviral infections, cases are distributed throughout the year, and the age distribution appears to be biphasic, with peaks at 5 to 30 years of age and more than 50 years of age.[296] HSV-1 causes more than 95% of cases.[297,298]

The pathogenesis of HSV encephalitis varies. In children and young adults, primary HSV infection may result in encephalitis; presumably, exogenously acquired virus enters the CNS by neurotropic spread from the periphery through the olfactory bulb. Most adults with HSV encephalitis have clinical or serologic evidence of mucocutaneous HSV-1 infection before the onset of symptoms.[298] However, in about 25% of patients examined, the HSV-1 strains from the oropharynx and brain tissue of the same patient differ; thus some cases may result from reinfection with another strain of HSV-1 that reaches the CNS.[299] Reactivation of latent HSV-1 infection in trigeminal or autonomic nerve roots may be associated with extension of virus into the CNS through nerves innervating the middle cranial fossa. HSV DNA has been demonstrated by DNA hybridization in brain tissue obtained at autopsy from healthy adults.[298] Therefore reactivation of long-standing latent CNS infection is an additional potential mechanism for the development of HSV encephalitis.

The clinical hallmark of HSV encephalitis is acute onset of fever and focal neurologic (especially temporal lobe) symptoms. Differentiation of HSV encephalitis from other viral encephalitides, focal infections, and noninfectious processes is difficult.[300] The most sensitive noninvasive method for early diagnosis of HSV encephalitis is demonstration of HSV DNA in CSF by PCR, although uncommonly PCR testing may become positive only a few days after onset. Although titers of CSF and serum antibodies to HSV increase in most cases of HSV encephalitis, they rarely do so earlier than 10 days into the illness and as such are generally not helpful in establishing an early clinical diagnosis.[301–303]

Magnetic resonance imaging is the neuroimaging technique of choice for detection of abnormalities associated with HSV encephalitis, and gadolinium-enhanced lesions are often seen in the temporal lobe. Brain biopsy was performed extensively in the past to make the diagnosis of HSV encephalitis and has a low complication rate; demonstration of HSV antigen, HSV DNA, or HSV replication in brain tissue obtained by biopsy is highly sensitive. Brain biopsy is infrequently used now, but it provides the best opportunity to identify alternative, potentially treatable causes of encephalitis and may be considered when the clinical presentation is atypical or the diagnosis remains unclear.[300]

Most authorities recommend empirical use of intravenous acyclovir to patients with presumed HSV encephalitis until the diagnosis is confirmed or an alternative diagnosis is made. All suspected or confirmed cases should be treated with intravenous acyclovir at a dose of 30 mg/kg/day in three divided doses for 14 to 21 days.[304] Cases of clinical recurrence of encephalitis after therapy stopped have been reported that required more treatment. For this reason, some authorities prefer to treat initially for 21 days, and many continue therapy until HSV DNA has been eliminated from the CSF. Even with therapy, neurologic sequelae are frequent, especially in patients older than 35 years of age.

VISCERAL AND PULMONARY HERPES SIMPLEX VIRUS INFECTIONS

HSV infection of visceral organs usually results from viremia, and multiple-organ involvement is common. Occasionally, the clinical manifestations of HSV infection involve only the esophagus, lung, or liver. HSV esophagitis may result from direct extension of oropharyngeal HSV infection into the esophagus or by reactivation and spread of HSV to the esophageal mucosa through the vagus nerve.[305,306] It is a well-known complication in patients with AIDS and should be differentiated from *Candida* and CMV infections and aphthous ulcers by histologic examination. The predominant symptoms of HSV esophagitis are odynophagia, dysphagia, substernal pain, and weight loss. The distal esophagus is most commonly involved with multiple oval ulcerations on an erythematous base, with or without a patchy white pseudomembrane. With extensive disease, diffuse friability may involve the entire esophagus. Neither endoscopic nor barium examination differentiates HSV esophagitis from esophageal ulcerations related to *Candida,* thermal injury, radiation, or corrosives. Endoscopically obtained secretions for cytologic examination and culture provide the most useful diagnostic material. Systemic antiviral chemotherapy usually reduces symptoms and heals ulcerations.

HSV pneumonitis is uncommon except in severely immunosuppressed patients and may result from extension of herpetic tracheobronchitis into lung parenchyma.[307–309] Because oral shedding of HSV can lead to contamination, a positive HSV culture from a respiratory specimen should be interpreted with caution in an unlikely host or in the absence of radiologic evidence of disease. However, demonstration of virus from lower respiratory tract specimens should be evaluated promptly to determine whether there is evidence of tracheobronchitis or true parenchymal lung disease. HSV-1 pneumonia usually manifests as a focal necrotizing pneumonitis. Hematogenous dissemination of virus from sites of oral or genital mucocutaneous disease may also occur and produce bilateral interstitial pneumonitis. Bacterial, fungal, and parasitic copathogens are commonly present, and the mortality rate from untreated HSV pneumonia in immunosuppressed patients is high (>80%).[307,308] HSV has also been observed in association with acute respiratory disease syndrome.[310,311] Most authorities believe the presence of HSV in tracheal aspirates in such settings is due to reactivation of HSV in the tracheal region and localized tracheitis in patients with long-standing intubation. Such patients should be evaluated for the potential extension of HSV infection into lung parenchyma. Controlled trials evaluating the role antiviral agents for HSV play in the morbidity and mortality of acute respiratory disease syndrome have not been conducted.

HSV is an uncommon cause of hepatitis in immunocompetent patients. HSV infection of the liver is associated with fever, abrupt elevations of bilirubin and serum aminotransferase levels, and leukopenia (<4000 white blood cells/μL). Disseminated intravascular coagulation may also develop.[252]

GENITAL HERPES SIMPLEX VIRUS INFECTION IN HIV-INFECTED PATIENTS

Persistent HSV infection is a common clinical presentation of HIV infection.[212] HSV reactivation, especially perianal shedding in men and

subclinical vulvar shedding in women, is more frequent in HIV-positive patients than HIV-negative control subjects. As with the HIV-negative population, mucosal HSV-2 reactivation rates vary considerably among patients.[235,312] For example, HSV DNA was detected on 30% to 80% of days in two cohorts of HIV-positive patients.[312,313] Low $CD4^+$ counts and high HIV viral loads are associated with an increased frequency of HSV-2 shedding,[151,235,314,315] although not HSV-1 oral shedding.[316] Highly active antiretroviral therapy appears to reduce the frequency of genital lesions substantially but only modestly reduces the frequency of subclinical shedding.[151] HSV shedding and lesion rates appear to increase transiently after the initiation of antiretroviral therapy, perhaps relating to yet unidentified host or viral factors associated with immune reconstitution or immune reconstitution inflammatory syndrome.[317,318]

Increased Risk of HIV-1 Acquisition in Herpes Simplex Virus Type 2–Infected Patients

Perhaps the most impactful epidemiologic complication of HSV-2 infection is its role in increasing sexually acquired HIV-1 in women, heterosexual men, and MSM. Among MSM, antibodies to HSV-2 are detectable in most HIV-positive men[319] and in one-fourth of HIV-1–negative men.[95] Within discordant HIV-1 heterosexual partnerships both in the developed and the developing world, HSV-2 infection is common among potential transmitters (70% and 95%, respectively) and individuals at risk for HIV-1 infection.[320]

Case-control and cohort studies have shown that prior HSV-2 infection is associated with an increased risk for acquisition of HIV,[321–325] and mathematical models suggest that in many areas of the world it appears to be a substantial driver of micro-outbreaks of HIV-1.[326] Per-contact probability of HIV-1 acquisition is affected equally by HSV-2 serostatus and HIV-1 plasma viral load in the source partner (Table 135.1). Meta-analyses performed on all available prospectively conducted studies spanning four continents estimated consistently elevated risks for HIV-1 acquisition in persons with HSV-2 infection across sex and sexual preference.[320,327] One study indicated that approximately one-fourth of infections in a high-prevalence city in sub-Saharan Africa were directly attributable to HSV-2 and that HSV-2 facilitated spread of HIV into low-risk populations.[326] A separate review estimated a population attributable risk of 20% in populations with moderate HSV-2 prevalence such as the United States and 45% in populations with very high HSV-2 seroprevalence.[328] However, two well-designed clinical trials showed no effect of standard-dose acyclovir HSV-2 treatment on the acquisition of HIV-1 among HSV-2–positive persons,[329,330] despite reduced frequency of genital lesions and HSV-2 shedding. This lack of effect of antiviral therapy may relate to the minimal effect acyclovir has on subclinical shedding and subclinical inflammation in the genital tract.[152] More recent modeling suggests that equivalent route of transmission may drive some apparent HSV-2 attributable risk due to HIV-1.[331]

Besides its effect on acquisition among HIV-1–infected patients, HSV-2 also influences both mucosal and plasma HIV replication. HIV virions can be detected in genital herpes lesions, and higher HIV-1 titers are found in genital secretions during episodes of subclinical HSV-2 reactivation.[332,333] The regulatory proteins ICP0 and ICP4 can upregulate the rate of HIV replication in vitro,[334–336] and coinfection of epithelial cells by HSV-2 and HIV results in a higher copy number of HSV virions.[335] Additionally, transfection of HSV-2 genes into $CD4^+$ T cells enhances HIV-1 replication. HSV imprints a lasting focal inflammatory response of HIV-susceptible cells that is unaffected by antiviral therapy. Herpetic lesions are associated with an influx of activated $CD4^+$ lymphocytes,[152,186] which persist in the region after the lesion heals.[152] Detailed in situ studies indicate that an inflammatory focus of chemokine (C-C motif) receptor 5–expressing $CD4^+$ T cells as well as dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin–expressing dendritic cells remains after clinical healing.[152] These dendritic cell–$CD4^+$ T-cell interactions markedly facilitate both the passage and the replication capacity of $CD4^+$ T cells in vivo and have been shown to be an important focus of early HIV infection in nonhuman primates.[142] Antiviral therapy with acyclovir does not alter these findings,[152] which may explain why anti-HSV-2 therapy does not reduce HIV acquisition or transmission. HSV-2 shedding rate and genital inflammation appear to be higher in African relative to American heterosexual cohorts,[337,338] which may add to the enhanced risk of HIV-1 acquisition in this population.

TABLE 135.1 Per-Contact Probability of HIV-1 Acquisition Stratified by Plasma HIV-1 RNA in HIV-1 Seropositive Partner and HSV Serostatus in the Susceptible Partner

HIV-1 PLASMA RNA IN SOURCE PARTNER (copies/mL)	PER-CONTACT PROBABILITY IN HIV-1–SUSCEPTIBLE PARTNER	
	HSV-2 Positive	HSV-2 Negative
<1700	0.0001	0.00004
1700–12,499	0.0023	0.0005
12,500–38,499	0.0018	0.0002
>38,499	0.0036	0.0007

HIV, Human immunodeficiency virus; *HSV,* herpes simplex virus.
Modified from Quinn T, Wawer M, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N Engl J Med. *2000;342:921–929.*

Impact of Herpes Simplex Virus Type 2 Infection on HIV Transmission Probability From Coinfected Patients

Plasma HIV viral load is an important determinant of sexual transmission,[339] and both plasma and genital HIV-1 viral load are approximately 30% higher in HSV-2–infected patients during and for several weeks after HSV-2 recurrence (clinical and subclinical). These findings add plausibility to the observations that genital ulcer diseases increase the probability of HIV transmission[313,323,340,341] and that HSV-2 may enhance maternal-fetal HIV transmission.[342,343] Frequency of HSV-2 shedding correlates with HIV plasma viral load: twice-daily valacyclovir lowers HIV-1 plasma viral load by 0.2 to 0.5 $\log_{10}$ and decreases the frequency and mean quantity of HIV-1 shed in the genital tract.[341,344] Acyclovir also reduces HIV-1 viral load by 0.25 $\log_{10}$ and increases time to AIDS, which may be useful in select patients without access to HIV-targeting antiretroviral therapy. The relative decrease in HIV viral load correlates with the dose of acyclovir or valacyclovir used, independent of drug effect on genital HSV shedding, which suggests a direct antiviral effect on HIV.[345–348] Acyclovir did not lower HIV-1 transmission rate within serodiscordant couples, however, in a randomized clinical trial.[348]

The mechanism by which acyclovir reduces HIV-1 viral load is unresolved. It is possible that HSV-2 DNA polymerase inhibitors decrease HIV-1 viral load indirectly by decreasing the overall level of T-cell activation relating to HSV-2 recrudescence. However, acyclovir also acts directly on HIV-1 reverse transcriptase and may even predispose to development of clinically important HIV-1 reverse transcriptase mutations.[349] Acyclovir requires HSV thymidine kinase for its initial phosphorylation step; therefore in vivo, one would expect that its anti-HIV activity would require coinfection of a significant number of cells with both HIV-1 and HSV-2. A direct antiviral effect on HIV is suggested by the correlation of HIV viral load with valacyclovir dose, independent of drug effect on genital HSV shedding.[345] Yet the reductions in HIV plasma RNA appear only after 6 to 12 weeks and are not seen with episodic use of these agents. Although acyclovir can reduce viral load, its effects are not universal, and hence the impact of acyclovir on limiting the pace of HIV progression to AIDS does not warrant its routine clinical use in all patients coinfected with HIV and HSV-2.[346,347]

HERPES SIMPLEX VIRUS INFECTIONS IN IMMUNOCOMPROMISED HOSTS NOT INFECTED WITH HIV

Organ transplant recipients, patients undergoing cancer chemotherapy, and patients compromised by malnutrition or disorders of skin integrity such as burns or eczema are at risk for the development of severe HSV infections,[350–352] usually due to reactivation of endogenous virus. Primary

infection is relatively rare. HSV can rarely be transmitted via a solid-organ graft, which can be lethal, although this has not been observed in the antiviral era.[353]

The probability and severity of HSV disease is in part determined by the intensity of immunologic suppression. For instance, in the era before the routine use of acyclovir during chemotherapy, the HSV disease rate was considerably higher following hematopoietic stem cell transplantation (HCT) (70%–80%) relative to solid-organ transplantation (SOT) (40%–50%).[354–362] Most HSV infections occurred within the first 30 days after SOT or HCT when T-cell immunosuppression is most profound.[182,363] Higher disease rates are also observed following T-cell depletion with OKT3 or antithymocyte globulin following SOT.[364] HSV-1–seropositive patients who received a bone marrow transplant from an HSV-seronegative donor are at higher risk for recurrence and are more likely to develop acyclovir-resistant strains of HSV than patients who received marrow from HSV-1–positive donors.[365] Haploidentical and cord transplants as well as prolonged corticosteroid use for graft-versus-host disease also portend a higher risk of HSV recurrence.[366–368]

Most kidney, liver, and bone marrow transplant recipients excrete HSV-1 in saliva during the first 2 to 3 weeks after grafting.[369] Although these reactivations are often initially asymptomatic, extensive mucocutaneous ulcerations may also occur (Fig. 135.11) and, if persistent, extend to the esophagus or lung. Genital HSV-2 in immunocompromised patients may manifest as typical self-limited lesions or persistent chronic ulcerations and may occasionally mimic hemorrhoids or human papillomavirus disease.[370] Besides having the potential to cause extensive and disfiguring mucocutaneous infections, in rare cases HSV may disseminate to visceral organs such as adrenal glands, liver, bone marrow, and gastrointestinal tract in such patients. Blood PCR is usually the diagnostic test of choice and is important to distinguish infection from bacterial sepsis, CMV hepatitis, or other causes of fulminant liver failure.[371–373]

Because of the high frequency of disease in untreated patients, the potential for severe and even fatal manifestations, and the difficulty in distinguishing HSV from chemotherapy-related mucositis, routine prophylaxis against HSV during the initial period after HCT, initiation of cytoreductive chemotherapy, or SOT is the standard of care to shorten the course or prevent mucocutaneous HSV infection. Prophylactic acyclovir dramatically reduced the 2-year incidence of HSV disease following HCT from 30% to 0.2%[374] and reduced bacteremia with oral pathogens in patients after chemotherapy in a single trial.[369]



FIG. 135.11 Severe mucocutaneous herpes simplex virus type 1 infection in a bone marrow transplant patient. *(From Corey L. Herpes simplex virus infections. In: Mandell GL, series ed; Rein MF, ed.* Atlas of Infectious Diseases. Vol. V. Sexually Transmitted Diseases. *Philadelphia: Churchill Livingstone; 1996.)*

Antiviral resistance is an important clinical issue in the immunocompromised host. Thymidine kinase mutations that confer drug resistance appear to develop sequentially in immunocompromised hosts who are not controlling infections mucosally.[375] Resistance develops more frequently in HCT patients receiving short-term prophylaxis or repeated treatment for recurrent disease than in patients on long-term high-dose prophylaxis.[374,376–380] Antivirals directed at other sites of viral replication and more recently immunotherapeutic compounds[381–383] are being developed as alternative treatments to acyclovir-resistant strains.

NEONATAL HERPES

Infants acquire infection through contact with HSV-infected secretions, usually at the time of delivery.[192,292,384] Of neonatal herpes, 90% is perinatally acquired, 5% to 8% is congenital, and the remaining minority is acquired postnatally.[292] HSV-2, which causes 70% of neonatal HSV infections, nearly always occurs during delivery. Although congenitally infected infants have been reported, these infants almost invariably are born to mothers who had primary HSV-1 or HSV-2 infection during pregnancy.[385,386] Features of congenital HSV-2 include microcephaly, hydrocephalus, and chorioretinitis. Neonatal HSV-1 infections may also be acquired through postnatal contact with health care workers or immediate family members who have symptomatic or asymptomatic orolabial HSV-1 infection.

Neonates (infants <6 weeks old) have the highest frequency of visceral or CNS infection or both of any HSV-infected population. If not treated, neonatal herpes undergoes dissemination or develops into CNS infection in more than 70% of cases. Without therapy, the overall rate of death from neonatal herpes is 65%; less than 20% of neonates with CNS infection develop normally.[292,387] CNS morbidity is less severe with HSV-1 than with HSV-2 infection.[388] Although skin lesions are the most commonly recognized features of disease, many infants do not acquire visible lesions until well into the course of disease.[389] Cutaneous involvement alone is not associated with mortality, albeit subclinical CNS infection may be present.

Intravenous acyclovir has markedly improved the outcome of infants with neonatal HSV infection. Early initiation of therapy is an important factor in influencing outcome, especially with CNS infection, and prolonged therapy with oral suppression is now recommended. High-dose (60 mg/kg/day) intravenous acyclovir divided in three daily doses for 21 days (14 days for cutaneous involvement only) reduces mortality and morbidity, but long-term disabilities are still common, especially in infants with HSV-2 infection involving the CNS.[390] Continuation of therapy with daily oral acyclovir for 6 months improves neurodevelopmental outcomes in infants who survive acute infection.[390]

Prevention Measures for Neonatal Herpes

Infants born by cesarean section to women before the rupture of membranes or by vaginal delivery to women with no evidence of recent HSV infection are at minimal risk for the development of HSV infection, and most hospitals do not recommend segregating the infant from the rest of the infants in the neonatal nursery. If a more cautious approach is desired, the infant can be put into an Isolette incubator to make hospital personnel aware of the necessity to use wound and skin precautions and proper hand-washing techniques.

Infants born to women with active lesions should be placed in isolation. Viral cultures, liver function studies, and CSF examinations should be obtained, and the infant should be closely observed for the first month of life. Any symptoms of neonatal disease (e.g., poor feeding, fever, hypothermia, skin lesions, lethargy, or seizures) should be investigated expeditiously for evidence of neonatal HSV infection, and empirical therapy should be administered before the availability of diagnostic test results.

Management of contact between infant and mother should be handled on an individual basis. In women who acquire primary genital herpes late in pregnancy, the high incidence of extragenital lesions suggests that separation of mother and infant is warranted until therapy has produced a clinical and virologic response. Because recurrent maternal genital herpes is rarely associated with dissemination of disease or the development of extragenital lesions in exposed extremities, protection

of the infant from exposure to infected genital secretions is adequate. When handling the infant in the hospital, the mother should wear a gown and observe proper hand-washing techniques. Orolabial herpes presents a greater risk of postnatal acquisition of HSV infection to the neonate than genital herpes.[14] Thus nursery personnel and other adults with external lesions caused by HSV should not engage in intimate contact with a neonate.

HERPES SIMPLEX VIRUS INFECTION IN PREGNANCY

Incidence data for neonatal HSV infection are similar to data for neonatal HIV infection before the advent of routine antiretroviral use in pregnancy and are higher than incidence data for congenital syphilis, toxoplasmosis, and congenital rubella during endemic years.[113] The prevalence of genital HSV infection during pregnancy as well as the incidence of neonatal HSV infection is influenced by socioeconomic status, age, and past sexual activity.[391] In the United States, 22% of all pregnant women and 55% of non-Hispanic black pregnant women are HSV-2 seropositive.[392] However, the highest risk for transmitting HSV in the perinatal period occurs during the acquisition of HSV near the time of labor.[113,393] Table 135.2 depicts the frequency of neonatal infection in relation to maternal serologic states. One of the major unanswered questions of HSV in pregnancy is the low frequency of reported neonatal HSV-2 infections among African infants born to HSV-2–seropositive women in sub-Saharan Africa, despite high HSV-2 seroincidence at child-rearing age and evidence of subclinical shedding near term.[394–396]

Clinical Course of Genital Herpes in Pregnancy

The clinical manifestations of recurrent genital herpes including frequency of subclinical versus clinical infection, duration of lesions, pain, and constitutional symptoms tend to be similar in pregnant and nonpregnant women. Recurrences increase in frequency over the course of pregnancy,[397,398] and women who enter pregnancy with HSV-2 seropositivity experience normal neonatal outcomes including birth weight and gestational age.[399] First-episode infections in pregnancy have more severe consequences for mother and infant.[385,393,400,401] Maternal visceral dissemination during the third trimester as well as prematurity or fetal growth restriction occasionally occur. The acquisition of primary HSV-1 or HSV-2 disease in pregnancy carries the risk for potential transplacental transmission of virus to the neonate and can result in spontaneous abortion, although this is relatively uncommon.[402] We recommend antiviral treatment of newly acquired genital HSV during pregnancy with 7 to 10 days of acyclovir, 400 mg three times a day, or valacyclovir, 500 to 1000 mg twice daily, to reduce the likelihood of disseminated infection in the mother. The effect of this intervention on reducing congenital or perinatal transmission to the infant is unknown.

TABLE 135.2 Transmission Rates of Neonatal HSV by Maternal HSV Serologic Status Among Women Who Delivered at the University of Washington and Madigan Army Hospitals

MATERNAL HSV SEROSTATUS	NO./TOTAL (%) OF INFANTS WITH NEONATAL HSV	RATE PER 100,000 LIVE BIRTHS[a]
HSV seronegative	6/11,115 (0.054)	54 (19.8–118)
HSV-1 seropositive only	6/23,480 (0.026)	26 (9.3–56)
All HSV-2 seropositive	3/13,795 (0.022)	22 (4.4–64)
HSV-2 only	2/5761 (0.035)	35 (4.2–126)
HSV-1 and HSV-2	1/8034 (0.012)	12 (0.3–7.0)

[a]95% confidence level.
HSV, Herpes simplex virus.
Modified from Brown ZA, Ashley RL, Selka S, et al. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. JAMA. *2003;289:203–209.*

Surveillance Strategies for Neonatal Transmission of Herpes Simplex Virus

Criteria for laboratory screening and surveillance as well as delivery procedures for women with recurrent genital HSV infections are questions frequently encountered by physicians caring for pregnant women.[403] The high HSV-2 prevalence rate in pregnancy and low incidence of neonatal disease (1 in 6000–20,000 live births) indicate that only a low proportion of infants are at risk for acquiring HSV (see Table 135.2).[74] Therefore cesarean section is not warranted for all women with recurrent genital disease.[404] Because intrapartum transmission of infection accounts for most cases, only women who shed HSV at delivery need to be considered for abdominal delivery.[192,404] Several studies showed no correlation between recurrence of viral shedding before delivery and the presence of viral shedding at term.[384,404,405] Hence weekly virologic monitoring and amniocentesis are not recommended.

The frequency of transmission from mother to infant is markedly higher in women who acquire HSV near term (30%–50%) compared with women who reactivate HSV-2 at delivery (<1%).[192] Although maternal HSV-2 antibody is protective, HSV-1 antibody offers little or no protection against neonatal HSV-2 infection.[406] Primary HSV-1 genital infection leads to a particularly high risk for transmission and accounts for an increasing proportion of neonatal HSV infections.[192,194] Moreover, during reactivation, HSV-1 appears more transmissible to the neonate than HSV-2.[406] Only 2% of women who are HSV-2 seropositive and undergo swabbing of cervical secretions at delivery have detectable virus, and only 1% of infants exposed in this manner develop infection, presumably because of the protective effects of maternally transferred antibodies and perhaps lower viral titers during reactivation.[192,393] Despite the low frequency of HSV transmission in this setting, 30% to 50% of infants with neonatal HSV infection are born to mothers with established genital herpes.[407]

HSV shedding at time of delivery (detected by isolation via cervicovaginal swab) is the greatest risk factor for intrapartum HSV transmission (relative risk, 346); however, culture-negative, PCR-positive cases of intrapartum transmission are well described. New acquisition of HSV (odds ratio [OR], 49), isolation of HSV-1 versus HSV-2 (OR, 35), cervical versus vulvar HSV detection (OR, 15), use of fetal scalp electrodes (OR, 3.5), and young maternal age confer further risk for transmission, whereas abdominal delivery is protective (OR, 0.14).[192] Physical examination poorly predicts the absence of shedding,[408] and PCR far exceeds culture in terms of sensitivity and speed. Therefore PCR detection at the onset of labor should be considered to aid clinical decision making for HSV-2–seropositive women. Because cesarean section appears to be an effective means of reducing maternal-fetal transmission, patients with recurrent genital herpes should be encouraged to come to the hospital early at the time of delivery for careful examination of the external genitalia and cervix plus swab for viral isolation. Women who have no evidence of lesions should have a vaginal delivery. The presence of active lesions on the cervix or external genitalia is an indication for abdominal delivery.[407]

This policy results in the exposure of some infants to episodes of asymptomatic cervical or vulvar shedding, or both.[408] The identification of HSV-exposed infants provides important information to the attending pediatrician. If first-episode exposure has occurred (e.g., if HSV serologic tests show that the mother is seronegative or if the mother is HSV-1 seropositive and the isolate at delivery is HSV-2), many authorities would initiate antiviral therapy for the infant with intravenous acyclovir.[409,410] At a minimum, viral cultures and PCR should be performed on samples obtained from the throat, nasopharynx, eyes, and rectum of these infants immediately and at 5- to 10-day intervals. Lethargy, skin lesions, or fever should be evaluated promptly. All infants from whom HSV is isolated 24 hours after delivery should be treated with intravenous acyclovir at recommended treatment doses.

The duration of ruptured membranes in women with clinically apparent lesions does not have a well-defined relationship to the transmission of HSV to the infant.[271] Delivery of infants by cesarean section, even in women with intact membranes, occasionally results in neonatal herpes. Prolonged contact with infected secretions may increase the risk for acquisition. Many authorities recommend that if

membranes are ruptured for more than 4 to 6 hours, cesarean section should no longer be considered protective against HSV transmission. However, transmission can occur from exposure to external genital lesions alone. Thus in women with recurrent genital herpes who have active external genital lesions during labor, we still recommend abdominal delivery.

Prevention of Herpes Simplex Virus Acquisition in Pregnancy by Use of Antiviral Agents

Controversy exists regarding the use of antiviral therapy during pregnancy. Antiviral therapy given after 36 weeks of gestation reduces, but does not totally eliminate, HSV-2 recurrence at delivery.[410,411] Systematic reviews suggest that the frequency of cesarean delivery can also be reduced.[412,413] Although mathematical models suggest such an approach may be cost-effective in reducing cesarean deliveries, there are no data supporting the ability of this approach to reduce neonatal infection. Because the risk for neonatal transmission is low among HSV-2–seropositive women, the routine use of antiviral chemotherapy among HSV-2–positive pregnant women would result in many women treated with few cases of neonatal HSV prevented even if the effects on reducing maternal-fetal transmission are high. As such, we do not believe that routine use of antiviral therapy at the end of gestation should be recommended until better information on efficacy and potential neonatal safety are assessed. As described earlier, a more logical approach that requires further study would be to screen low-risk seropositive mothers at delivery with rapid PCR and follow infants with HSV-2 exposure intensively after birth.

Targeting HSV-2–seronegative women could be an effective means to decrease mother-to-infant transmission, as 2% of women in one survey seroconverted during pregnancy, and one-third of these seroconversions occurred during the third trimester. Potential approaches range from counseling for routine abstinence in all women after 34 weeks of gestation to the use of routine serologic screening to identify HSV-susceptible women. Such women then would be counseled about the importance of engaging in only protected coitus during the latter parts of pregnancy. Of particular interest are the 20% of women who are in a serodiscordant relationship and are at high risk for primary infection (3.5% for HSV-1, 20% for HSV-2).[414] A third possible type of screening program could be to identify and treat the HSV-2–positive sex partners of seronegative pregnant women with antiviral therapy and observe them serially for seroconversion, although the expense would be substantial.[415] Clinical and demographic information do not differentiate women at high versus low risk for transmitting HSV to their infants.[416] Pregnancy may also enhance HSV acquisition, making standard prophylactic measures such as condoms potentially less effective.[417] Because nearly 30% of neonatal HSV is due to HSV-1, attention to reducing HSV-1 acquisition through orogenital sex is also important.

DIAGNOSIS OF ACTIVE HERPES SIMPLEX VIRUS INFECTIONS

Clinical criteria are critical for considering the diagnosis of HSV infections. Nevertheless, given the gravity of diagnosing a lifelong viral sexually transmitted infection, we believe that appropriate management of the patient should always include laboratory confirmation of the infection. A clinical diagnosis can often be inferred when characteristic multiple vesicular lesions appear on an erythematous base. In this situation, given the prolonged morbidity of first-episode genital HSV infection and the ability of antiviral agents to mitigate systemic symptoms and prevent development of new crops of ulcers, it is appropriate to initiate oral antiviral treatment while awaiting laboratory confirmation.

Herpetic lesions may also resemble skin ulcerations of other causes.[96,267] Mucosal HSV infection can appear as urethritis or pharyngitis without cutaneous lesions. When there is clinical uncertainty, laboratory diagnosis is essential to guide therapy.[83]

HSV infection is best confirmed by demonstration of HSV DNA in lesion scrapings or by isolation of virus in tissue culture.[267,269] The sensitivity of viral isolation is higher in vesicular lesions than ulcerative lesions, during first rather than recurrent episodes of disease, and in samples from immunosuppressed rather than immunocompetent patients. HSV DNA detection is the preferred diagnostic method, if available, because it is three to four times more sensitive than viral isolation, is less affected by variation in specimen transport, and is cost-effective as compared with culture.[269] Laboratory confirmation permits subtyping of the virus, which may help predict frequency of reactivation after first-episode oral or genital HSV infection, site of CNS infection, and likelihood of drug resistance.[418]

HSV causes a discernible cytopathic effect in a variety of cell culture systems, generally within 48 to 96 hours after inoculation. Spin-amplified culture with subsequent staining for HSV antigen shortens the time needed to identify HSV to less than 24 hours. Staining of scrapings from the base of the lesions with Wright, Giemsa (Tzanck preparation), or Papanicolaou stain demonstrates characteristic giant cells or intranuclear inclusions of HSV infection. These cytologic techniques are useful as quick office procedures to confirm the diagnosis, but they do not differentiate between HSV and VZV infections, they are relatively insensitive, and correct identification of giant cells requires experience.

DIAGNOSIS OF ESTABLISHED HERPES SIMPLEX VIRUS INFECTIONS

Development of type-specific serologic assays has markedly improved our understanding of HSV-1 and HSV-2 seroprevalence,[419–421] and these assays are useful clinical management tools. Most commercially available assays measure antibodies to purified HSV-1–specific or HSV-2–specific proteins such as glycoproteins gG1 and gG2, which are antigenically distinct between the two subtypes and thus allow for detection of HSV-2 in the presence of HSV-1 antibodies and vice versa. Seroconversion to gG1 and gG2 may take weeks to months to occur, and hence detection of these antibodies generally means long-standing infection.[419,422–424] The Western blot assay, which measures additional HSV-1–specific and HSV-2–specific antibodies, is the most accurate available serologic test and has a sensitivity and specificity of greater than 98% for distinguishing HSV-1–specific and HSV-2–specific antibodies.[425,426] Assays that use whole viral extracts or antigens are inaccurate and should not be used for any stage of clinical diagnosis or epidemiologic studies.[427,428] These assays are still sold commercially, however, and used in some laboratories.

Acute-phase and convalescent-phase serum can be useful in demonstrating seroconversion during primary HSV-1 or HSV-2 infection. Only 5% of patients with recurrent orogenital HSV infections have a fourfold or greater rise in HSV antibody titer in the interval between collection of two samples. Immunoglobulin M antibodies are not useful for defining recent or past acquisition.[429]

OVERVIEW OF THERAPIES FOR HERPES SIMPLEX VIRUS INFECTIONS

The advent of antiviral drugs for HSV-1 and HSV-2 infections has made management of these infections a part of standard clinical practice (see Chapter 46). For mucocutaneous and visceral HSV infections, acyclovir and its related compounds valacyclovir and famciclovir are the mainstays of therapy. Several antiviral agents are available for topical use in HSV eye infections, including idoxuridine, trifluorothymidine, topical vidarabine, and cidofovir. However, there is no role for topical therapy in mucocutaneous disease.[226] For HSV encephalitis and neonatal herpes, intravenous acyclovir should be used. Acyclovir-resistant virus can be encountered in immunocompromised hosts and is treated with foscarnet, cidofovir, or possible topical agents such as imiquimod. Although the possibility of acyclovir resistance is typically not of clinical importance in immunocompetent patients, a possible exception may be HSV keratitis, where prophylaxis may predict development of resistance.[430] Allergies to acyclovir are rare, and desensitization is theoretically possible, although uncommonly employed.

Acyclovir was the first antiviral agent clearly demonstrated to be effective against HSV infections. It is an acyclic nucleoside analogue that is a substrate for HSV-specific thymidine kinase and is selectively phosphorylated by HSV-infected cells to acyclovir monophosphate.[431,432] Cellular enzymes then phosphorylate acyclovir monophosphate to

acyclovir triphosphate, a competitive inhibitor of viral DNA polymerase. Acyclovir triphosphate is incorporated into the growing DNA chain of the virus and causes chain termination. Acyclovir has potent in vitro activity against HSV-1, HSV-2, and VZV,[433] with partial activity against CMV.

Numerous trials of acyclovir in mucocutaneous HSV infections of the immunocompetent and immunosuppressed host have been conducted.[434–439] Famciclovir, the oral formulation of penciclovir, is also clinically effective in the treatment of a variety of HSV-1 and HSV-2 infections.[440–443] Valacyclovir, a valyl ester of acyclovir, allows greater bioavailability of the active compound than acyclovir,[444–446] making valacyclovir useful for once-daily suppressive therapy and for short-course, 1- or 2-day treatment of orogenital HSV-1 infection.[447,448] Ganciclovir has activity against both HSV-1 and HSV-2, but because it is more toxic than acyclovir, valacyclovir, and famciclovir, it is generally not recommended for treatment of HSV infections.[449] Anecdotal clinical observations suggest it may also be less effective. Table 135.3 outlines treatment options for the use of these compounds. Despite the demonstrated effectiveness of the guanosine analogues, most infected people remain untreated.

TABLE 135.3 Antiviral Chemotherapy for HSV Infection

	Dosage/Regimen	Comment
Mucocutaneous HSV Infections		
Infections in Immunosuppressed Patients		
Acute symptomatic first or recurrent episodes	Oral acyclovir, 400 mg qid, famciclovir, 500 mg PO tid, or valacyclovir, 1 mg PO bid, for 7–10 days is effective. In severe cases, IV acyclovir, 5 mg/kg q8h, is given.	Treatment duration may be 7–14 days.
Suppression of reactivation disease	IV acyclovir, 5 mg/kg q8h, valacyclovir, 500 mg PO bid, or oral acyclovir, 400–800 mg 2–3 times per day, prevents recurrences during the immediate 30-day posttransplantation period.	Suppression of clinical HSV-2 is routine for patients undergoing stem cell and organ transplant. Valacyclovir, 2 g 4 times daily, is also effective in preventing CMV infection. Valacyclovir, 4 g 4 times daily, has been associated with TTP after extended use in HIV-positive patients. In HIV-infected patients, oral famciclovir, 500 mg bid, is effective in reducing clinical and subclinical reactivations of HSV-1 and HSV-2. If using acyclovir in HIV-infected patients, we generally start with the lower dose of 400 mg twice daily and increase to 800 mg twice daily if breakthrough recurrences occur. *Note:* Once-daily dosing of valacyclovir, 500 mg to 1 g, should be avoided in HIV-infected patients owing to concerns regarding lower efficacy.
Symptomatic recurrent genital herpes in HIV-1–infected patients	Oral acyclovir, 400 mg tid × 5–10 days Valacyclovir, 1000 mg bid × 5–10 days Famciclovir, 500 mg PO bid × 5–10 days	
Infections in Immunocompetent Patients		
Genital Herpes		
First episodes	Oral acyclovir, 400 mg tid (V) or 200 mg 5 times per day (I) × 7–10 days Oral valacyclovir, 1000 mg bid × 7–10 days (I) Famciclovir, 250 mg tid × 7–10 days (I) IV acyclovir, 5 mg/kg q8h for 5 days, is given for severe disease or neurologic complications such as aseptic meningitis.	Treatment can be extended if healing is incomplete after 10 days of therapy.
Symptomatic recurrent genital herpes	**Oral acyclovir,** 400 mg tid × 5 days (V), **800 mg PO tid × 2 days** or bid × 5 days (II) **Valacyclovir, 500 mg bid × 3 days** (I) or 1 g daily × 5 days (I) **Famciclovir,** 125 mg bid for 5 days (I), **1 g bid for 1 day** (I), or **500 mg once then 250 mg PO bid × 3 doses** (I)	All these therapies are effective in shortening lesion duration. Short-course options (1, 2, or 3 days of therapy) should be considered based on increased convenience, likelihood of adherence, and reduced cost and are **listed in bold.** Given the brief period of viral replication and rapid evolution of lesions, patients should be given drugs for self-administration when prodromal symptoms occur.
Suppression of recurrent genital herpes	Oral acyclovir, 400 mg bid (I) Valacyclovir, 500 mg daily (I) or 1000 mg daily (I) or 250–500 mg bid (I) prevents symptomatic reactivation. Patients with frequent reactivation (<9 episodes/yr) can take valacyclovir 500 mg daily; patients with >9 episodes/yr should take valacyclovir 1000 mg/daily or 500 mg bid. Famciclovir, 250 mg bid (I)	Consider in patients with frequent (>6 episodes) or severe recurrences, in immunocompromised patients, or as an adjunct to prevent transmission.
Orolabial HSV Infections		
First episode	Oral acyclovir, 15 mg/kg (up to 200 mg) 5 times per day (II) or 400 mg tid (V) × 7 days Famciclovir, 500 mg bid (V) Valacyclovir, 1000 mg bid (V) × 7 days	
Recurrent episodes	Oral acyclovir, 400 mg 5 times per day × 5 days (II) **Valacyclovir, 2000 mg bid × 1 day** (I) **Famciclovir, 1500 mg once** (I)	Self-initiated therapy with topical 1% penciclovir cream q2h during waking hours (I); topical acyclovir cream, 5% 5 times per day × 4 days (I). Short-course options should be considered based on increased convenience and likelihood of adherence and are **listed in bold.** Given the brief period of viral replication and rapid evolution of lesions, patients should be given drugs for self-administration when prodromal symptoms occur.
Suppression of reactivation of orolabial HSV	Oral acyclovir, 400 mg bid (II), or valacyclovir, 500 mg or 1000 mg daily (II), or famciclovir, 500 mg bid (V)	Consider in patients with frequent (>6 episodes) or severe recurrences, in immunocompromised patients, or as an adjunct to prevent transmission.

TABLE 135.3 Antiviral Chemotherapy for HSV Infection—cont'd

	Dosage/Regimen	Comment
Herpetic Whitlow		
	Oral acyclovir, 200 mg 5 times daily for 7–10 days	
HSV Proctitis		
	Oral acyclovir, 400 mg 5 times per day, is useful in shortening the course of infection.	In immunosuppressed patients or in patients with severe infection, IV acyclovir, 5 mg/kg q8h, may be useful.
Herpetic Eye Infections		
		In acute keratitis, topical trifluorothymidine, vidarabine, idoxuridine, acyclovir, penciclovir, and interferon all are beneficial. Débridement may be required; topical corticosteroids may worsen disease (see Chapter 113).
CNS HSV Infections		
HSV encephalitis	IV acyclovir, 10 mg/kg q8h (30 mg/kg/day) for 14–21 days	
HSV aseptic meningitis	IV acyclovir, 30 mg/kg/day for 7–10 days	No studies of systemic antiviral chemotherapy exist.
Autonomic radiculopathy		No studies are available.
Neonatal HSV Infections		
	Acyclovir, 60 mg/kg/day divided into 3 doses × 21 days	Monitoring for relapse should be undertaken; most authorities recommend continued suppression with oral acyclovir suspension for 3–4 mo.
Visceral HSV Infections		
HSV esophagitis	IV acyclovir, 15 mg/kg/day	In some patients with milder forms of immunosuppression, oral therapy with valacyclovir or famciclovir is effective.
HSV pneumonitis		No controlled studies exist. IV acyclovir, 10 mg/kg q8h, should be considered.
Disseminated HSV Infections		
		No controlled studies exist. IV acyclovir, 10 mg/kg q8h, nevertheless should be given. No definite evidence indicates that therapy decreases risk of death.
Erythema Multiforme–Associated HSV		
		Anecdotal observations suggest that oral acyclovir, 400 mg bid or tid, or valacyclovir, 500 mg bid, suppresses erythema multiforme.
Surgical Prophylaxis		
		Several surgical procedures such as laser skin resurfacing, trigeminal nerve root decompression, and lumbar disk surgery have been associated with HSV reactivation. IV acyclovir, 3 mg/kg, and oral acyclovir, 800 bid, valacyclovir, 500 bid, or famciclovir, 250 bid, is effective in reducing reactivation. Therapy should be initiated 48 h before surgery and continued for 3–7 days.
Infections With Acyclovir-Resistant HSV		
	IV foscarnet, 40 mg/kg q8h, should be given until lesions heal. IV cidofovir, 5 mg/kg once weekly, may also be effective.	Imiquimod is a topical alternative, as is topical cidofovir gel 1%, which is not commercially available and must be compounded at a pharmacy. These topical preparations should be applied to lesions once daily for 5 consecutive days.

[a]Note: I, II, III, IV, and V in parentheses represent level of evidence.
CMV, Cytomegalovirus; *CNS,* central nervous system; *HIV,* human immunodeficiency virus; *HSV,* herpes simplex virus; *IV,* intravenous; *PO,* per os (orally); *TTP,* thrombotic thrombocytopenic purpura.
Modified from Cernik C, Gallina K, Brodell RT. The treatment of herpes simplex infections: an evidence-based review. Arch Intern Med. *2008;168:1137–1144; and Spruance S, Aoki FY, Tyring S, et al. Short-course therapy for recurrent genital herpes and herpes labialis: entering an era of greater convenience, better treatment adherence, and reduced cost.* J Fam Pract. *2007;56:30–36.*

Treatment of Primary Genital Herpes Simplex Virus Infection

Primary infection often manifests as prolonged severe genital ulcerations and sometimes nervous system involvement. Even patients with first-episode herpes who have mild clinical manifestations initially can develop severe or prolonged symptoms. Therefore all patients with first episodes of genital herpes should receive antiviral therapy for 7 to 10 days with one of several regimens (see Table 135.3), ideally within 72 hours of onset. Treatment extension beyond this time point is indicated if healing is slow. Adjunctive treatment with sitz baths is helpful for dysuria in female patients. If urinary retention develops, intermittent or indwelling bladder catheterization may be necessary until symptoms resolve.

Suppressive Therapy for Persistent Genital Herpes Simplex Virus Infection

Patients with recurrent genital HSV infection can be managed with no therapy if recurrences are mild and infrequent; episodic therapy can be used for recurrences; daily suppressive therapy is an option for patients with frequent or severe recurrences. The choice of episodic versus long-term suppressive therapy should also be individualized based on patient preference. Factors that may favor daily suppressive therapy are high frequency of recurrences (more than six per year), good patient adherence to antiviral therapy, lack of documented toxicity, affordability, patient wish to decrease risk for transmission to a partner, and patient anxiety over uncontrolled recurrences.

Suppressive therapy decreases recurrences and asymptomatic shedding by 70% to 80% and increases both median time to recurrence and quality of life. Many patients receiving such therapy report having experienced no symptomatic outbreaks. Patients on suppressive therapy report higher quality-of-life indices than patients on episodic treatment.[450] Safety and efficacy have been documented among patients receiving daily therapy with acyclovir for 6 years and with valacyclovir or famciclovir for 1 year.[451]

Although acyclovir and its derivatives are clinically effective, they do not completely suppress asymptomatic shedding, even when given at maximal doses three times per day.[451] Patients on daily therapy still experience short episodes of asymptomatic shedding. The viruses obtained from such episodes are drug sensitive. These breakthrough episodes appear to occur due to rapid kinetics of viral expansion during drug trough levels.[118,452]

Episodic Therapy for Persistent Genital Herpes Simplex Virus Infection

Shorter courses of therapy are increasingly being used for treatment of recurrent episodes of mucocutaneous HSV-1 or HSV-2 infection in immunocompetent patients. One-day courses of famciclovir and valacyclovir are clinically effective, more convenient, and generally less costly than longer courses of therapy.[439,441,453–456] Optimal outcomes are seen with early initiation of therapy (6–24 hours postinfection). This is easiest to achieve if the patient is prescribed medication between recurrences to allow initiation of self-treatment at home at the first sign of prodrome.

Therapy for Severe Herpes Simplex Virus Infection

Intravenous acyclovir (30 mg/kg/day, given as a 10-mg/kg infusion over 1 hour at 8-hour intervals) is effective in reducing the morbidity and mortality associated with HSV encephalitis.[304] Again, early initiation of therapy is a critical factor in outcome. The major side effect of intravenous acyclovir is transient renal insufficiency, usually caused by crystallization of the compound in the renal parenchyma. This adverse reaction can be avoided if the medication is given slowly over 1 hour and the patient is well hydrated. Because CSF levels of acyclovir average only 30% to 50% of plasma levels, the dosage of acyclovir used for treatment of CNS infection (30 mg/kg/day) is double that used for the treatment of mucocutaneous or visceral disease (15 mg/kg/day). For disseminated neonatal HSV, high-dose intravenous therapy is recommended (60 mg/kg/day in three divided doses) for 21 days.[457]

Prophylaxis of Herpes Simplex Virus in Immunocompromised Hosts and Development of Drug Resistance

Patients undergoing stem cell transplantation or solid-organ transplantation are at high risk of herpesvirus reactivation. In most patients, intravenous acyclovir or oral valacyclovir is highly effective in preventing HSV reactivations; high doses of valacyclovir also reduce CMV reactivations.[449] Although acyclovir-resistant strains of HSV are well described in these populations,[458–460] stem cell transplant recipients receiving daily suppressive antiviral therapy are less likely to develop acyclovir-resistant HSV compared with those who received episodic therapy with outbreaks.[374]

Patients with late-stage HIV infection are also at risk of developing severe, persistent mucosal infections. Acyclovir prophylaxis is indicated for patients with severe or frequent recurrences. Although resistant HSV-1 strains have been documented in local mucosal replicating sites in immunocompetent hosts receiving therapy and may be archived in the latent pool,[45] archived drug resistance does not typically have an effect on treatment outcome for HSV-1 and HSV-2 in immunocompetent hosts.[461]

Treatment of Drug-Resistant Herpes Simplex Virus

Most acyclovir-resistant strains of HSV have a deficiency in thymidine kinase, the enzyme that phosphorylates acyclovir.[462,463] Thus cross-resistance to famciclovir is usually found (see Chapter 46). Occasionally an isolate with altered thymidine kinase specificity arises and is sensitive to famciclovir but not to acyclovir. In some patients infected with thymidine kinase–deficient HSV, higher doses of acyclovir are associated with clearing of lesions.[464] In others, clinical disease progresses. Isolation of HSV from persisting lesions despite adequate dosages and blood levels of acyclovir should raise the suspicion of resistance.

Therapy with foscarnet, which does not require phosphorylation by viral thymidine kinase, should be initiated for resistant HSV infections and is usually successful, although side effects from foscarnet including renal insufficiency, electrolyte wasting, nausea, paresthesias, and seizures are common.[465,466] Because of its toxicity and cost, intravenous foscarnet is reserved for patients with extensive mucocutaneous infections and laboratory-confirmed resistance. Of note, foscarnet can independently lead to genital ulceration.

Cidofovir is a nucleotide analogue and exists as a phosphonate or monophosphate form that also has anti-HSV activity. Most thymidine kinase–deficient strains of HSV are sensitive to cidofovir, which can be used topically or given intravenously on a weekly basis. Cidofovir ointment speeds healing of acyclovir-resistant lesions,[467,468] but the drug itself also can cause mucocutaneous ulcerations. The intravenous form very commonly causes renal failure, a Fanconi-like syndrome with proteinuria and bicarbonate wasting, neutropenia, and rash and gastrointestinal distress due to concurrent probenecid dosing.

Trifluridine ointment is useful for ophthalmic HSV infection and is anecdotally useful for resistant mucosal HSV infection in patients with AIDS.[469] Resiquimod, a topical TLR7 and TLR8 agonist, decreased shedding and recurrence rate of anogenital HSV-2, but further commercial development of this agent has ceased.[170] We and others have had success treating refractory ulcers due to drug-resistant HSV with imiquimod,[470] although this intervention has no effect on long-term recurrence rate.[471]

Novel Antiviral Agents for Herpes Simplex Virus Type 1 and Type 2 Infections

Amenamevir[472] and pritelivir[473] belong to a new class of antiviral compounds that inhibit the helicase/primase complex. Phase II studies have shown safety and a marked reduction in both HSV-2 shedding and days with genital lesions in immunocompetent patients.[31,474] These drugs were active against acyclovir-resistant viruses,[475–477] and thus inhibitors of these highly conserved complexes have potential as therapy for resistant HSV infections in immunosuppressed patients. Amenamevir and pritelivir are being studied for HSV patients with multiple orofacial and genital lesions.[478] Additional studies of these inhibitors for advancement to licensure are under consideration (see Chapter 46 for further discussion).[479]

COUNSELING AND PREVENTION

Many patients with first-episode genital HSV infection experience substantial morbidity and as a result miss work or school. Yet a significant proportion, particularly men, do not seek health care advice.[480] Many patients report feelings of depression and fear of rejection and discovery. These negative feelings tend to subside over time, although not completely.[481] Psychological distress can be both a cause and a consequence of genital lesions.[482] Therefore during acute illness, it is best to ensure symptomatic palliation by recommending antiviral therapy, antiinflammatory pain medicines, sitz baths, and local drying of the lesions. The patient should usually be taught about the chronicity of infection and natural history of recurrence and asymptomatic reactivation on later visits. Women's concerns regarding issues relating to pregnancy and childbirth should be addressed as well. The misconception that HSV causes cancer should be dispelled.

Some patients in primary care, pregnant women, or patients at sexually transmitted disease clinics who are asymptomatic will have their infection diagnosed from serologic study rather than symptoms. This is also likely to be a source of distress for patients, and the above-described counseling strategies should be used. Particular emphasis should be placed on recognition of subtle genital ulcers. Multiple

resources including websites (http://www.ashasexualhealth.org/) and printed materials are available to assist patients, their partners, and clinicians who become involved in counseling.

Finally, strategies for prevention of HSV-2 transmission should be emphasized to patients, particularly patients within serodiscordant relationships. Partially effective strategies include full disclosure to the susceptible partner, condom use, abstinence during symptomatic lesions or prodromes, and antiviral therapy.[101,102,483] Valacyclovir (500 mg daily) reduces transmission of genital herpes by 50% by suppression of episodes of subclinical shedding and is a primary, albeit imperfect, prevention strategy within serodiscordant partnerships.[101] The possibility of asymptomatic transmission should also be addressed.

Herpes Simplex Virus Vaccines

To date, no licensed vaccine is available for either HSV-1 or HSV-2. Based on the large public health burden of HSV and the continued spread of the HSV-2 epidemic worldwide, there is a need for such a vaccine.[484] Recommendations for increased research and development of a next generation of herpes simplex vaccines have been formulated by the US National Institute of Allergy and Infectious Diseases.[485] A review of the status of HSV vaccine research and development has been published on behalf of the World Health Organization Product Development for Vaccines Advisory Committee.[486] Most HSV vaccine studies have tested the monomeric surface glycoprotein gD2 and/or EB1. Consistent results have not been seen, and development of these vaccines has largely been discontinued.[487–489]

A deeper knowledge of factors possibly associated with protection against HSV is needed. It is hoped that the development of other successful herpesvirus vaccines such as those for CMV and VZV will spur further research on HSV-2. Advances in understanding T-cell immune responses to HSV have fostered the development of immunotherapeutic vaccines with the goal of limiting HSV-2 shedding, recurrences, and transmission among seropositive individuals.[490–496] Practically speaking, clinical trials of therapeutic vaccines are faster, require much smaller sample sizes, and potentially allow a rapid and less expensive pathway to licensure. In a recent phase II trial, a vaccine consisting of HSV-2 glycoprotein D2, a truncated ICP4, and Matrix-M2 adjuvant demonstrated a 50% reduction in shedding rate at 6 months.[497] A variety of additional approaches are entering human clinical trials.

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The complete reference list is available online at Expert Consult.

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136 Chickenpox and Herpes Zoster (Varicella-Zoster Virus)

Richard J. Whitley

SHORT VIEW SUMMARY

Definition
- Varicella-zoster virus (VZV) is an alphaherpesvirus that causes chickenpox and herpes zoster.

Epidemiology
- Chickenpox is the primary infection occurring in childhood.
- Chickenpox is usually a benign infection but can cause life-threatening disease in an immunocompromised host.
- Chickenpox is more likely to occur in late winter and early spring.
- Herpes zoster is the consequence of reactivation of latent virus, occurring mainly in elderly and immunocompromised individuals.
- Herpes zoster causes significant pain in many individuals, particularly elderly individuals.
- There is no seasonal predilection for occurrence of herpes zoster.

Microbiology
- VZV is a double-stranded DNA virus. Following primary infection, latency is established in sensory ganglia.

Diagnosis
- Diagnosis of chickenpox and herpes zoster is usually clinical.
- Chickenpox is characterized by a maculopapular, vesicular, and papular rash in all stages of evolution.
- Herpes zoster is usually a unilateral vesicular rash. Dissemination can occur in immunocompromised patients.
- A Tzanck smear of lesion scrapings may demonstrate intranuclear inclusions; however, its sensitivity as a diagnostic test is low.
- Polymerase chain reaction (PCR) can be applied to lesion scraping to detect VZV DNA and is the diagnostic procedure of choice.
- Viral culture can be used to make a diagnosis, but it is less sensitive than PCR.

Therapy
- Three drugs are licensed for treatment of VZV infections (see Table 136.1).
- Chickenpox in children 2 to 16 years of age can be treated with acyclovir at a dosage of 20 mg/kg four times per day for 5 days. For older patients, the dosage of acyclovir is 800 mg five times a day.
- The prodrugs valacyclovir and famciclovir are used by some experts to treat chickenpox.
- Herpes zoster can be treated with acyclovir at a dosage of 800 mg five times daily for 7 to 10 days, with valacyclovir at a dosage of 1 g three times daily for 7 to 10 days, or famciclovir at a dosage of 500 mg three times daily for 7 to 10 days.
- Herpes zoster is likely to require pain control with analgesics and medications such as pregabalin.

Prevention
- High-titer varicella-zoster immune globulin (VariZIG) can be administered to high-risk patients to attempt to prevent infection.
- A VZV vaccine is available to prevent chickenpox (Varivax). It is a two-dose series with the first administered at 12 to 15 months of age and the second administered between 4 and 6 years. This two-dose series has dramatically decreased the incidence of chickenpox and its associated complications.
- Two vaccines are approved to prevent herpes zoster in older individuals. A high-titer live VZV vaccine (Zostavax) and an inactive adjuvanted VZV vaccine (Shingrix) are available for adults older than 50 years of age. Each vaccine can reduce the incidence of herpes zoster. In 2018, the Advisory Committee on Immunization Practices recommendations stated that Shingrix is preferred to Zostavax and that Shingrix can be administered to adults who have previously received Zostavax.

Varicella-zoster virus (VZV) causes two distinct clinical diseases. Varicella, more commonly called chickenpox, is the primary infection and results from exposure of a person susceptible to the virus to someone who is actively infected. Chickenpox is ubiquitous and extremely contagious, but for the most part it is a benign illness characterized by a generalized exanthematous rash. It occurs seasonally and in epidemics. Recurrence of infection results in the localized entity known as *herpes zoster,* often referred to as *shingles,* a common infection among elderly and immunocompromised individuals. Live attenuated vaccines for the prevention of chickenpox (Varivax) and herpes zoster (Zostavax) are licensed in the United States. An inactivated adjuvanted vaccine (Shingrix) is licensed for prevention of herpes zoster in older adults in the United States. Vaccination is recommended for use in healthy children and in susceptible adults to prevent chickenpox (see Chapter 316). Both herpes zoster vaccines are recommended for adults older than 50 years of age to decrease the incidence of shingles, although Shingrix is preferred in the Advisory Committee on Immunization Practices (ACIP) recommendations (see later).

Historically, the incidence of chickenpox was that of the annual birth rate, but incidence has been tremendously reduced with widespread vaccination. Surveillance for varicella in three counties in California, Texas, and Pennsylvania from 1995 to 2000 showed reductions in cases of varicella by 71% to 84% in 1999 and 2000,[1] with only 500 cases occurring in 2004.[2] By 2010, varicella incidence declined by 98% in the two remaining sites (Antelope Valley, California, and West Philadelphia, Pennsylvania) (Fig. 136.1).[3,4] Since the implementation of a two-dose vaccine series, the incidence of chickenpox has decreased to approximately 5 cases in 100,000 in states with adequate reporting.[2] Approximately 1 million cases of herpes zoster, which result in more than 2 million physician visits annually, occur yearly in the United States. This approximation likely is an underestimation of disease occurrence. Many individuals with herpes zoster require long-term follow-up medical care for postherpetic neuralgia (PHN).

HISTORICAL OVERVIEW

Shingles has been recognized since ancient times as a unique clinical entity because of the dermatomal vesicular rash; however, chickenpox was often confused with smallpox.[5] In 1875 Steiner successfully transmitted VZV by inoculation of the vesicular fluid from a person with chickenpox to human "volunteers."[6] The infectious nature of VZV was further defined by von Bokay,[7,8] who observed chickenpox in people who had close contact with other people with herpes zoster. He correctly described the mean incubation period of approximately 2 weeks for the development of chickenpox in susceptible patients and the average range in days. In 1925, Kundratitz[9] showed that the inoculation of vesicular fluid from patients with herpes zoster into

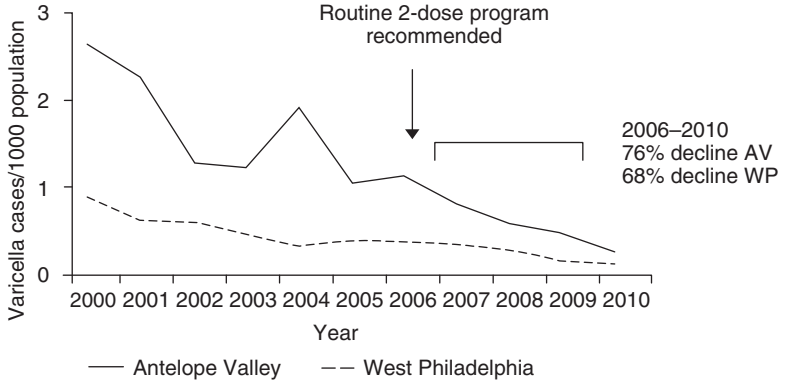

FIG. 136.1 Impact of a two-dose varicella vaccination program in three sentinel counties. *(From Bialek SR, Perella D, Zhang J, et al. Impact of a routine two-dose varicella vaccination program on varicella epidemiology.* Pediatrics. *2013;132:e1134-1140.)*

susceptible individuals resulted in chickenpox. Similar observations were reported by Brunsgaard[10] and others,[11] and in 1943 Garland[12] suggested that herpes zoster was the consequence of the reactivation of latent VZV.

Since early in the 20th century, similarities in the histopathologic features of skin lesions and in epidemiologic and immunologic studies indicated that varicella and herpes zoster were caused by the same agent.[13,14] Tyzzer[15] described the histopathologic features of skin lesions resulting from VZV infections and noted the appearance of intranuclear inclusions and multinucleated giant cells. These descriptions came from histologic studies performed on serial skin biopsy specimens that were obtained during the first week of illness. The histopathologic descriptions were amplified by Lipschitz in 1921[16] for herpes zoster.

Isolation of VZV in 1958 permitted a definition of the biology of this virus.[14] Viral isolates from patients with either chickenpox or herpes zoster demonstrated similar changes in tissue culture, specifically the appearance of eosinophilic intranuclear inclusions and multinucleated giant cells. These findings are virtually identical to findings present on clinically available biopsy material. Taken together, these data provided a universal acceptance that both diseases were caused by VZV. By 1958, Weller and colleagues[14,17-19] established that there were neither biologic nor immunologic differences between the viral agents isolated from patients with these two clinical entities. Later studies identified VZV by rigorous biochemical methods.[20] Viral DNA from a patient with chickenpox who subsequently developed herpes zoster was examined by restriction endonuclease analysis, and the molecular identity of these two viruses was verified.[21]

PATHOGEN AND ITS REPLICATION

VZV is a member of the Herpesviridae family and shares structural characteristics with other members of the family. The virus has icosapentahedral symmetry and contains centrally located double-stranded DNA with a surrounding envelope. The size of the virus is approximately 150 to 200 nm, and it has a lipid-containing envelope with glycoprotein spikes.[22,23] The naked capsid has a diameter of approximately 90 to 95 nm.[24-26] The DNA contains 125,000 base pairs, or approximately 80 megadaltons, and encodes about 75 proteins. The organization of the viral genome is similar to that of other herpesviruses. There are unique long (105-kb) and unique short (5.2-kb) regions of the viral genome. Each unique sequence contains terminal repeat sequences. With replication, the unique short region can invert on itself and result in two isomeric forms.[22,27,28] Notably, a large portion of the VZV genome is colinear with herpes simplex type 1 (HSV-1). For example, within the unique long region of VZV, 56 of the 62 genes have HSV-1 homologues. Similarly, in the unique short region all four genes have homologues.

Eight VZV glycoproteins have been identified: gB, gC, gE, gH, gI, gK, gL, and gM/N. These glycoproteins have been the subject of intense investigative interest because they represent the primary markers for both humoral and cell-mediated immune responses.[22]

Only enveloped virions are infectious, likely accounting for the lability of VZV. Furthermore, the envelope is sensitive to detergent, ether, and air-drying. VZV is highly cell associated and spreads from cell to cell by direct contact. Virus can be isolated in a variety of continuous and discontinuous cell culture systems of human and simian origin. Approximately 8 to 10 hours after infection, virus-specific immunofluorescence can be detected in the cells immediately adjacent to the initial focus of infection. This parallels the microscopic observation of the radial spread of the cytopathologic process.[29,30] Electron microscopic studies demonstrate the appearance of immature viral particles within 12 hours of the onset of infection. As with HSV, the naked capsids acquire their envelope at the nuclear membrane, being released into the perinuclear space where large vacuoles are formed.[24,31] Infectious virus is then spread to adjacent cells after fusion of plasma membranes.

EPIDEMIOLOGY OF VARICELLA-ZOSTER VIRUS INFECTIONS

Chickenpox

Humans are the only known reservoir for VZV. Chickenpox follows exposure of the susceptible or seronegative person to VZV and represents the primary form of infection. Although it is assumed that the virus is spread by the respiratory route and replicates in the nasopharynx or upper respiratory tract, retrieval of virus from individuals incubating VZV has been uncommon. However, the application of polymerase chain reaction (PCR) techniques to nasopharyngeal secretions of exposed and susceptible individuals has detected VZV DNA and supports this hypothesis.[32] Chickenpox was a common infection of childhood and affects both sexes equally and people of all races. To a certain extent, the virus is endemic in the population at large; however, it becomes epidemic among susceptible individuals during seasonal periods of late winter and early spring.[33] Intimate contact appears to be the key determinant for transmission.

With the implementation of childhood vaccination programs in some countries, the epidemiology of chickenpox is changing dramatically with a decreasing incidence. However, many countries still do not employ universal immunization; as a consequence, the epidemiology of infection remains as known historically.

Overall, chickenpox is a disease of childhood because 90% of cases occur in children younger than 13 years. Typically the virus is introduced into the susceptible school-aged or preschool child. In a study by Wells and Holla,[34] 61 of 67 susceptible children in kindergarten through the fourth grade contracted chickenpox. Approximately 10% of individuals older than 15 years are considered susceptible to VZV infection. The incubation period of chickenpox (i.e., the time interval between exposure of a susceptible person and the time the vesicular rash develops in an index case) is generally regarded to be 14 to 15 days, but disease can appear within a range of 10 to 20 days.[35,36] Secondary attack rates among susceptible siblings within a household are between 70% and 90%.[37] Patients are infectious for approximately 48 hours before the period of

vesicle formation and generally for 4 to 5 days thereafter until all vesicles are crusted.

Although chickenpox exists worldwide among children, it occurs more frequently in adults who reside in tropical regions than in adults who reside in other geographic areas. Stokes[38] noted a higher incidence of chickenpox among soldiers serving abroad during World War II, in whom the incidence was 1.41 to 2.27 per 1000 persons annually. These rates contrast with rates in the United States, which were approximately half those reported among the soldiers.

Herpes Zoster

The epidemiology of herpes zoster is different. VZV characteristically becomes latent after primary infection within the dorsal root ganglia. Reactivation leads to herpes zoster, a sporadic disease. Histopathologic examination of the nerve root after infection with VZV demonstrates characteristics indicative of VZV infection. In people who die after recent herpes zoster infection, an examination of the dorsal root ganglia reveals satellitosis, lymphocytic infiltration in the nerve root, and degeneration of the ganglia cells.[39,40] Intranuclear inclusions can be found within the ganglia cells. Although it is possible to demonstrate the presence of VZV by electron microscopy, it has not been possible to isolate virus in cultures as has been done after HSV infection, usually from explants of dorsal root ganglia. Nevertheless, the application of PCR to extracted ganglionic DNA demonstrates latent viral DNA in neurons of sensory ganglia, including dorsal root, cranial, and enteric ganglia.[41] The biologic mechanism by which VZV establishes latency remains unknown.

Herpes zoster is a disease that occurs at all ages. It affects approximately 1 million annually or 20% or more of the population throughout the life span, although mainly elderly adults.[42,43] Herpes zoster occurs in individuals who are seropositive for VZV or, more specifically, in individuals who have had chickenpox. Reactivation appears to depend on a balance between virus and host factors. Most patients who develop herpes zoster have no history of exposure to other people with VZV infection at the time of the appearance of lesions. The highest incidence of disease varies between 5 and 10 cases per 1000 for people older than 60 years.[17] Approximately 4% of patients experience a second episode of herpes zoster; however, recurrences of dermatomal lesions are usually caused by HSV. This finding was verified in the Shingles Prevention Study on vaccination.[44] In a 7-year study by McGregor,[45] the annualized rate of herpes zoster was 4.8 cases per 1000 patients, and three-fourths of the patients were older than 45 years. Immunocompromised individuals have a higher incidence of both chickenpox and shingles.[46-49] Herpes zoster occurs within the first 2 years of life in children born to women who have had chickenpox during pregnancy. These cases probably reflect in utero chickenpox with reactivation early in life.

PATHOGENESIS

Chickenpox occurs in susceptible individuals who are exposed to virus after close personal contact. Histopathologic findings of human VZV infections, whether chickenpox or herpes zoster, are virtually identical. The vesicles involve the corium, or dermis. As viral replication progresses, the epithelial cells undergo degenerative changes characterized by ballooning, with the subsequent appearance of multinucleated giant cells and prominent eosinophilic intranuclear inclusions. Under unusual circumstances, necrosis and hemorrhage may appear in the upper portion of the dermis. As the vesicle evolves, the fluid becomes cloudy as a consequence of the appearance of polymorphonuclear leukocytes, degenerated cells, and fibrin. Ultimately, either the vesicles rupture and release infectious fluid or the fluid gradually becomes reabsorbed.

As noted earlier, transmission is likely by the respiratory route, followed by localized replication at an undefined site, with lymphatic spread and seeding of the reticuloendothelial system and, ultimately, viremia. The occurrence of viremia in patients with chickenpox is supported by the detection of virus in peripheral blood mononuclear cells and by the diffuse and scattered nature of the skin lesions, and it can be verified in selected cases by the recovery of virus from the blood.[50] The mechanism of VZV reactivation that results in herpes zoster is unknown.

CLINICAL MANIFESTATIONS

Chickenpox

Chickenpox is generally a benign, self-limited disease in immunocompetent children, the incidence of which has markedly decreased with more widespread use of the varicella vaccine. There are fewer than 14 deaths per year in the United States in immunocompetent children.[51] For a healthy unimmunized child, chickenpox-associated mortality is fewer than 2 per 100,000 cases, although this is higher in infants.[22] This risk increases by more than 15-fold for adults. The presenting manifestations of chickenpox are a rash, low-grade fever, and malaise. In a few patients, a prodrome of the reported symptoms may occur 1 to 2 days before the onset of the exanthem. For the most part, chickenpox in an immunocompetent child is associated with lassitude and a temperature of 100°F to 103°F of 3 to 5 days' duration. Subsequent constitutional symptoms include malaise, pruritus, anorexia, and listlessness; these symptoms gradually resolve as the illness abates. The skin manifestations, which are the hallmark of infection, consist of maculopapules, vesicles, and scabs in varying stages of evolution. The lesions initially contain clear vesicular fluid, but over a short period of time they pustulate and scab. Most lesions are small, having an erythematous base with a diameter of 5 mm to as large as 12 to 13 mm. The lesions can be round or oval; central umbilication occurs as healing progresses. The lesions have often been referred to as "dew drop–like" during the early stages of formation. If they do not rupture within a few hours, the contents rapidly become purulent in appearance. Lesions appear on the trunk and face and rapidly spread centrifugally to involve other areas of the body. Successive crops of lesions generally appear over a period of 2 to 4 days. Thus early in the disease, the hallmark of the infection is the appearance of lesions at all stages, as noted previously. The lesions can also be found on the mucosa of the oropharynx and the vagina; however, these sites are less commonly involved. The crusts completely fall off within 1 to 2 weeks after the onset of infection and leave a slightly depressed area of skin.

Immunocompromised children, particularly children with leukemia, have more numerous lesions, often with a hemorrhagic base. Healing takes nearly three times longer in this population.[46] These children are at greater risk for visceral complications, which occur in 30% to 50% of cases and can be fatal in 15% of cases in the absence of therapy. A notable complication of chickenpox is secondary bacterial infection of the lesions, often in association with gram-positive organisms. Streptococcal toxic shock is a rare but potentially lethal complication of varicella. Infection in the neutropenic host can be systemic, beyond cutaneous involvement.

The most frequent noncutaneous site of involvement after chickenpox is the central nervous system (CNS); the neurologic abnormalities are manifested as acute cerebellar ataxia or encephalitis.[35,52-54] Cerebellar ataxia has been estimated to occur in 1 in 4000 cases among children younger than 15 years. Cerebellar ataxia can appear as late as 21 days after the onset of rash. It is more common, however, for acute cerebellar ataxia to manifest within 1 week of the onset of the exanthem. An extensive review by Underwood[54] of 120 cases demonstrated that ataxia, vomiting, altered speech, fever, vertigo, and tremor all were common on physical examination. Cerebrospinal fluid (CSF) from these patients often demonstrates lymphocytosis and elevated levels of protein. This is usually a benign complication in children and resolves within 2 to 4 weeks. PCR techniques can detect VZV DNA in the CSF.[55]

A more serious CNS complication is encephalitis, which can be life threatening in adults. Encephalitis is reported to occur in 0.1% to 0.2% of individuals with chickenpox.[53] Underwood's review[54] reported this illness to be characterized by depression in the level of consciousness with progressive headaches, vomiting, altered thought patterns, fever, and frequent seizures. The duration of disease in these patients is at least 2 weeks. Some patients experience progressive neurologic deterioration that leads to death. Mortality in patients who develop encephalitis has been estimated to range from 5% to 20%, and neurologic sequelae can occur in 15% of survivors.

A serious and life-threatening complication is the appearance of varicella pneumonitis, a complication that occurs more commonly in adults and in immunocompromised individuals.[33,52] Among adults, it is estimated to occur in 1 in 400 cases of infection; not infrequently, in

the absence of clinical symptoms, it appears 3 to 5 days into the course of illness and is associated with tachypnea, cough, dyspnea, and fever. Pregnant women during the second and third trimesters of gestation are particularly vulnerable to pneumonitis with primary infection. Chest radiographs usually reveal nodular or interstitial pneumonitis. Varicella pneumonitis can be life threatening when it occurs in pregnant women during the second or third trimester.

In a prospective study of male military personnel, radiographic abnormalities were detected in nearly 16% of enlisted men who developed varicella, although only one-fourth of these men had evidence of cough.[56] Only 10% of the men with radiographic abnormalities developed evidence of tachypnea, indicating that asymptomatic pneumonitis may exist more commonly than was initially predicted. Other manifestations of noncutaneous and nonneurologic involvement include the appearance of myocarditis, nephritis, bleeding diatheses, and hepatitis. Furthermore, isolated abdominal presentations of chickenpox have been reported.[32,41]

Perinatal varicella is associated with a high death rate when maternal disease develops 5 days before delivery or up to 48 hours postpartum.[57,58] In large part, this is the consequence of the newborn failing to receive protective transplacental antibodies as well as the immaturity of the neonatal immune system. Under such circumstances, mortality has been reported to be 30%. Affected children have progressive disease involving visceral organs, especially the lung. The outcome in these children was summarized by Brunell.[59] Congenital varicella, although uncommon, is characterized by skin scarring, hypoplastic extremities, eye abnormalities, and evidence of CNS impairment.[60]

Varicella has been associated epidemiologically with the development of Reye syndrome and coadministration of aspirin. Therefore the administration of aspirin is contraindicated in individuals with varicella. Cutaneous complications include the development of secondary skin infections, especially infections caused by *Staphylococcus aureus.*

Chickenpox in the Immunocompromised Patient

Chickenpox in an immunocompromised child or adult is a cause of significant morbidity and mortality. As noted previously, the duration of healing of cutaneous lesions can be extended by a minimum of threefold. However, a more important problem is the progressive involvement of visceral organs. Data from a variety of immunocompromised patient populations ranging from patients with lymphoproliferative malignancies and solid tumors to bone marrow transplant recipients indicate a broad spectrum of disease. In the absence of therapy, approximately one-third of children develop progressive disease with involvement of multiple organs, including the lungs, liver, and CNS.[46] Most of these children develop pneumonitis within the first week after the onset of infection, as do 20% of all children who acquire chickenpox. Mortality in this patient population is approximately 15% to 18%.[46,61,62] Patients with lymphoproliferative malignancies who require continuous chemotherapy appear to be at greatest risk for visceral involvement.

In individuals undergoing hematopoietic stem cell transplantation, the incidence of VZV infections over the first year has been estimated to be 30% by 1 year after transplantation. Of these infections, 80% occurred within the first 9 months after transplantation, and 45% of patients had cutaneous or visceral dissemination (see Chapter 307). Overall, 23 deaths occurred in one prospective series.[48] Risk factors identified for the acquisition of VZV infection included age between 10 and 29 years, diagnosis other than chronic myelogenous leukemia, posttransplant use of antithymocyte globulin, allogeneic transplant, and acute or chronic graft-versus-host disease. Graft-versus-host disease increases the probability of visceral dissemination significantly. Patients receiving HSV prophylaxis with acyclovir 800 mg orally twice daily or valacyclovir 500 mg orally twice daily or receiving treatment for cytomegalovirus reactivation with ganciclovir derive a degree of protection against reactivation of VZV.

Herpes Zoster

Herpes zoster, or shingles, is characterized by a unilateral vesicular eruption with a dermatomal distribution. Thoracic and lumbar dermatomes are most commonly involved (Fig. 136.2). Herpes zoster may involve the eyelids when the first or second branch of the fifth cranial nerve is affected, but herpes zoster ophthalmicus is a sight-threatening condition. Although lesions on the tip of the nose are said to presage corneal lesions, absence of such skin lesions does not guarantee corneal sparing. Keratitis may be followed by severe iridocyclitis, secondary glaucoma, or neuroparalytic keratitis. Ophthalmologic consultation should be requested for any patient with suspected herpes zoster ophthalmicus. Generally the onset of disease is heralded by pain within the dermatome that precedes the lesions by 48 to 72 hours. Early in the disease course, erythematous, maculopapular lesions appear and rapidly evolve into a vesicular rash. Vesicles may coalesce to form bullous lesions. In the normal host, these lesions continue to form over 3 to 5 days, with the total duration of disease being 10 to 15 days. However, it may take 1 month before the skin returns to normal. Rarely, individuals will develop an entity known as *zoster sine herpete,* which refers to dermatomal pain in the absence of lesions and is confirmed by serologic boosting of antibody responses.[22]



FIG. 136.2 **Herpes zoster involving the lumbar dermatome.**

Unusual cutaneous manifestations of herpes zoster in addition to herpes zoster ophthalmicus include involvement of the maxillary or mandibular branch of the trigeminal nerve, which results in intraoral involvement with lesions on the palate, tonsillar fossa, floor of the mouth, and tongue. When the geniculate ganglion is involved, Ramsay Hunt syndrome may occur, with pain and vesicles in the external auditory meatus, loss of taste on the anterior two-thirds of the tongue, and ipsilateral facial palsy.

No known factors are responsible for the precipitation of episodes of herpes zoster. If herpes zoster occurs in children, the course is generally benign and not associated with progressive pain or discomfort. In adults, systemic manifestations are mainly associated with pain, as noted in the following paragraphs.

The most significant clinical manifestations of herpes zoster are the associated acute neuritis and, later on, PHN. Modeling of pain attributed to herpes zoster defines three phases of disease: acute, subacute, and chronic.[63] Historically the latter two make up PHN. As identified later, the impact of therapy on each phase can be defined. PHN, although uncommon in young people, may occur in 25% to 50% of patients older than 50 years.[64-66] Up to 50% of adults older than 50 years have debilitating pain that persists for more than 1 month. PHN may cause constant pain in the involved dermatome or consist of intermittent stabbing pain. Pain may be worse at night or on exposure to temperature changes; at its worst, neuralgia can be incapacitating.[67]

Extracutaneous sites of involvement include the CNS, as manifested by meningoencephalitis or encephalitis. The clinical manifestations are similar to manifestations of other viral infections of the brain. However, a rare manifestation of CNS involvement by herpes zoster is granulomatous cerebral angiitis, which usually follows zoster ophthalmicus. Involvement of the CNS with cutaneous herpes zoster is probably more common than recognized clinically. Frequently, patients who undergo CSF examination for other reasons during episodes of shingles are found to have evidence of pleocytosis without significantly elevated protein levels. These patients are without signs of meningeal irritation and infrequently complain of headaches. A notable neurologic complication is the late appearance of cerebral angiitis after herpes zoster ophthalmicus.

Angiitis typically manifests as an ischemic stroke and can occur several months after herpes zoster of the trigeminal ganglia. This problem has been noted in several patients and defined as being progressive with a high mortality rate. Other nervous system manifestations of chickenpox include meningitis, transverse myelitis, and Reye syndrome.

Classically, VZV infection involves sensory ganglia; however, motor paralysis can occur as a consequence of the involvement of the anterior horn cells, in a manner similar to that encountered with polio. Patients with involvement of the anterior horn cells are particularly likely to have excruciating pain. Other neuromuscular disorders associated with herpes zoster include Guillain-Barré syndrome, transverse myelitis,[68] and myositis.[69] Herpes zoster in an immunocompromised patient is more severe than in a healthy person. Lesion formation continues for up to 2 weeks, and scabbing may not occur until 3 to 4 weeks into the disease course.[70] Patients with lymphoproliferative malignancies are at risk for cutaneous dissemination and visceral involvement, including varicella pneumonitis, hepatitis, and meningoencephalitis. Although some immunocompromised patients with dermatomal zoster who remain febrile and develop progressive lesions outside the dermatome may become severely ill, disseminated herpes zoster is rarely fatal even in immunocompromised patients.

Herpes zoster has been recognized as a frequent infection in individuals with human immunodeficiency virus (HIV) infection, occurring in 8% to 11% of patients. Although the occurrence of cutaneous dissemination is infrequent, complications such as VZV retinitis, acute retinal necrosis, and chronic progressive encephalitis have been reported.[71] The use of anti–tumor necrosis factor-α monoclonal antibodies has been associated with an increased incidence of herpes zoster.[72]

Chronic herpes zoster may also occur in immunocompromised patients, particularly patients with HIV infection. Patients have experienced new lesion formation with an absence of healing of the existing lesions. These syndromes can be particularly debilitating and have been associated with the isolation of VZV isolates resistant to acyclovir.

DIAGNOSIS

The diagnosis of both chickenpox and shingles is usually made by history and physical examination. In the first part of the 21st century, the differential diagnosis of varicella and herpes zoster is less confusing than it was 20 to 30 years ago. Smallpox or disseminated vaccinia was confused with varicella because of the similar appearance of the cutaneous lesions, and it could again pose a problem in the era of bioterrorism. With the worldwide eradication of smallpox, these disease entities only serve to confound the diagnosis if used by a bioterrorist or as a complication of vaccination. The characteristic skin rash of chickenpox with lesions in all stages of development provides the basis for the clinical diagnosis of infection. The presence of pruritus, pain, and low-grade fever also helps establish the diagnosis of chickenpox. The localization and distribution of a vesicular rash make the diagnosis of herpes zoster highly likely; however, other viral exanthemas can occasionally be confused with this disease.

Impetigo and varicella can also be confused clinically. Impetigo is usually caused by group A β-hemolytic streptococci, often follows an abrasion of the skin or inoculation of bacteria at the site of the skin break, and can be associated with the formation of small vesicles in the surrounding area. Systemic signs of disease may be present if progressive cellulitis or secondary bacteremia develops. Unroofing lesions and performing a Gram stain of the scraping of the base of the lesion should reveal gram-positive cocci in chains, suggestive of streptococci, or gram-positive cocci in clusters, suggestive of staphylococci, another cause of vesicular skin lesions, or both organisms. Treatment for these latter infections is distinctly different from treatment for chickenpox and requires administration of an appropriate antibiotic.

In a smaller number of cases, disseminated vesicular lesions can be caused by HSV. In these cases, disseminated HSV infection is usually a consequence of an underlying skin disease such as atopic dermatitis or eczema. An unequivocal diagnosis can be made only by isolation of the virus in tissue culture.

Disseminated enteroviral infections, particularly those caused by group A coxsackieviruses, have been reported to cause widespread distal vesicular lesions. These rashes are more commonly morbilliform in nature, with a hemorrhagic component rather than a vesicular or vesiculopustular appearance. Generally, these infections occur during the enterovirus season in late summer and early fall and are associated with lesions of the oropharynx, palms, and soles. This latter finding is helpful in distinguishing enteroviral disease from chickenpox.

Unilateral vesicular lesions in the dermatomal pattern should immediately lead the clinician to suspect a diagnosis of shingles. HSV and coxsackievirus infections can also cause dermatomal vesicular lesions. In such situations, diagnostic viral cultures remain the best method of establishing the cause of infection. Confirmation of the diagnosis is possible through the isolation of VZV in susceptible tissue culture cell lines or by the demonstration of either seroconversion or serologic rises using standard antibody assays of acute and convalescent serum specimens. A Tzanck smear, performed by scraping the base of the lesion, can demonstrate multinucleated giant cells; however, the sensitivity of this test is no better than 60% and does not distinguish HSV from VZV. Commercially available reagents are useful for direct fluorescent antibody staining of smears obtained from scraping vesicular lesions. With atypical skin lesions, such smears have adequate sensitivity and specificity to guide early management decisions. In some laboratories, PCR has become the diagnostic technique of choice because of sensitivity, specificity, and specimen stability. However, its expense and lack of uniform performance standards have limited routine diagnostic use. Useful antibody assays include immune adherence hemagglutination assay, fluorescence antibody to membrane antigen assay, and enzyme-linked immunosorbent assay.[73] The application of PCR to the CSF can be used to detect VZV DNA and therefore infections of the CNS. A positive PCR in the CSF may be seen in dermatomal zoster, and it is most useful when typical skin lesions of herpes zoster may not be present. PCR may detect evidence of VZV DNA is a nasal swab in individuals with herpes zoster, even before the appearance of rash.

THERAPY

The medical management of chickenpox and shingles in the normal host is directed toward reduction of complications. For chickenpox, hygiene is important, including bathing, astringent soaks, and closely cropped fingernails to avoid a source for secondary bacterial infection associated with scratching of the pruritic skin lesions. Pruritus can be decreased with topical dressing or the administration of antipruritic drugs. Soaks with aluminum acetate, or Burow solution, in the management of herpes zoster can be both soothing and cleansing.[74] Acetaminophen should be used to reduce fever in patients with chickenpox because of the association between aspirin and Reye syndrome.

Acyclovir is approved in the United States for the treatment of both chickenpox and herpes zoster in the normal host (Table 136.1). Oral acyclovir therapy in healthy children, adolescents, and adults shortens the duration of lesion formation by about 1 day, reduces the total number of new lesions by approximately 25%, and diminishes constitutional symptoms in one-third of patients.[22,23,74-77] The American Academy of Pediatrics recommends therapy for adolescents and adults as well as high-risk groups of patients (e.g., premature infants, children with bronchopulmonary dysplasia) within 24 hours of onset of disease. In children 2 to 18 years old, the oral dosage is 20 mg/kg four times daily for 5 days (maximum 800 mg daily). Adolescents and adults can receive up to 800 mg five times a day. Oral therapy of herpes zoster in the normal host accelerates cutaneous healing and reduces acute neuritis.

Acyclovir has been evaluated in controlled studies for all herpesvirus infections. Acyclovir is a guanine derivative that has a high degree of selectivity for the inhibition of VZV replication because of its selected phosphorylation and activation by the virus-coded thymidine kinase and its subsequent selective inhibition of the viral DNA polymerase. It is estimated that the concentration of acyclovir required to inhibit VZV replication in vitro is 2.1 to 6.3 μM, which is easily achieved after intravenous administration of acyclovir.[78] However, such concentrations are not easily achieved even after administration of high-dose oral acyclovir as summarized.[79] The recommended dosage for acyclovir is 10 mg/kg administered intravenously every 8 hours or, as suggested by some, 500 mg/m^2 intravenously every 8 hours, especially in children

TABLE 136.1 Antiviral Therapy of Varicella-Zoster Infection

INFECTION	DRUG	ROUTE	DOSAGE
Chickenpox (immunocompetent host)	Acyclovir	Oral	20 mg/kg (maximum 800 mg) 4–5 times daily × 5 days
Chickenpox (immunocompromised host)	Acyclovir	IV	10 mg/kg q8h × 7 days
Herpes zoster (immunocompetent host)	Valacyclovir Famciclovir Acyclovir	Oral Oral Oral	1 g tid × 7 days 500 mg q8h × 7 days 800 mg 5 times daily × 7–10 days
Herpes zoster (immunocompromised host)	Acyclovir Valacyclovir Famciclovir	IV Oral Oral Oral	10 mg/kg q8h × 7 days 800 mg 5 times daily × 7 days 1 g tid × 7 days[a] 500 md tid × 10 days[a]

[a]Not approved for this indication by the US Food and Drug Administration.
IV, Intravenous.
Modified from Baden L, Dolin R. Antiviral chemotherapy. In: Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J, eds. Harrison's Principles of Internal Medicine. *19th ed. New York: McGraw-Hill Education; 2015:1174.*

and in patients at risk for complications; otherwise oral treatment is the preferred management strategy.

The prodrugs of acyclovir and penciclovir—valacyclovir and famciclovir, respectively—have been licensed for therapy of herpes zoster.[80,81] The use of valacyclovir results in enhanced oral bioavailability, approximately 60%, compared with poor oral absorption of acyclovir. Oral bioavailability of famciclovir is approximately 80%. Both drugs appear superior to acyclovir for acceleration of cutaneous healing and are at least equally, if not more, efficacious for resolution of pain. Valacyclovir is administered to adults at a dosage of 1 g three times daily for 7 to 10 days.[80] The dosage of famciclovir is 500 mg three times daily for 7 to 10 days[80] Both medications are well tolerated. These medications primarily affect the acute and subacute phases of disease, as noted earlier. All three licensed therapeutics in North America are generic.

Resistance to acyclovir has been encountered in immunosuppressed patients including patients with hematologic-oncologic conditions and HIV-infected patients.[82-86] Foscarnet has been used to treat herpes zoster with resistant VZV. Dosage recommendations are 60 mg/kg every 8 hours adjusted for renal function for at least 10 days or until lesions are healed. Cidofovir has been used to treat acyclovir-resistant VZV, although experience in its use is limited.[82]

The concomitant administration of corticosteroids and an antiviral remains controversial. In one study, such regimens failed to affect PHN, although resolution of acute neuritis was accelerated.[87] This study was not placebo controlled. A placebo-controlled trial, using a 2×2 factorial design, demonstrated significant improvement in quality of life.[88] Patients older than 50 years who received acyclovir (800 mg five times daily for 3 weeks) and tapering doses of prednisone (60 mg daily for 7 days, 30 mg daily for 7 days, and 15 mg daily for 7 days) experienced resolution of acute neuritis, were able to sleep uninterrupted, and returned to their usual activity levels more promptly than did control subjects and had lower analgesic requirements. Complications were not encountered; however, patients at risk for complications of high-dose steroid therapy were excluded.

Management of varicella pneumonitis and other complications requires excellent supportive nursing care in addition to evaluation on an individual basis of the potential need for antiviral therapy. The management of acute neuritis and PHN can be particularly problematic. The judicious use of analgesics ranging from nonnarcotic to narcotic derivatives is required, and drugs such as amitriptyline hydrochloride, fluphenazine hydrochloride, lidocaine patches, gabapentin, and pregabalin may be used.[89-91] Furthermore, intrathecal administration of narcotics has been reported to be of value.[92]

PREVENTION

In the normal host, prophylaxis of chickenpox is achieved via vaccination. The potential for transmission of VZV in the hospital to immunosuppressed patients, particularly children, is a serious problem. Patients who require hospitalization because of varicella are a source of nosocomial infection. Because approximately 10% of adults are seronegative, the risks in the hospital environment can be high. Individuals most likely to become infected are nurses and other medical personnel providing care to infected patients. Airflow can be a means of transmission of infection from one area to another in the hospital environment.

In immunocompromised individuals who have not been previously exposed to chickenpox, the administration of varicella-zoster immune globulin (VariZIG[93]) (see Chapter 316) has been shown to be useful for both prevention and amelioration of symptomatic chickenpox.[94-97] VariZIG should be administered to an immunodeficient patient younger than 15 years who has a negative or unknown history of chickenpox, who has not been vaccinated against VZV, and who has had contact in the household with a playmate or in a shared hospital room for more than 1 hour. Administration of VariZIG may prolong the incubation period of illness.

Recent guidelines also recommend administration of VariZIG to a pregnant woman who is known to be seronegative and who has had a significant exposure. VariZIG should also be administered to a newborn infant whose mother had onset of chickenpox fewer than 5 days before delivery or up to 48 hours postpartum. For preterm hospitalized infants, guidelines for use have been recommended by the American Academy of Pediatrics Red Book Committee.[98] The use of VariZIG for susceptible immunocompetent individuals older than 15 years must be evaluated on an individual basis.

A vaccine was licensed for the prevention of chickenpox in immunocompetent individuals in 1995.[99-105] Studies performed indicated protection after vaccination, but disease occasionally occurred, especially in small outbreaks, and led to an ACIP recommendation of two doses of vaccine for children as implemented in 2006 (see Chapter 316). The Oka strain of VZV was developed by Takahashi and colleagues in Japan and studied as a vaccine extensively in both healthy and leukemic children. In immunocompromised children, serologic evidence of host response after vaccination has been achieved in 89% to 100% of vaccinated individuals. However, vaccine-induced rash is not uncommon and occurs in variable percentages of patients from approximately 6% to 47%. The factor most predictive of the appearance of rash is the degree of immunosuppression. Specifically, for children with acute lymphoblastic leukemia, the likelihood of rash can be 40% to 50%. The subsequent occurrence of natural varicella after community exposure was decreased in larger control studies and averaged 8% to 16%. Vaccination did not appear to increase the likelihood of subsequent herpes zoster during the period of follow-up.

The ACIP recommends routine childhood vaccination with two doses of vaccine.[106] In clinical trials, the development of antibody responses was higher than in the immunocompetent host and was between 94% and 100%. Vaccine-induced rash was far less common in these individuals and occurred at a frequency of 0.5% to approximately 19% overall, with the rate for subsequent appearance of varicella after community exposure averaging between 1% and 5%. The impact of this vaccine is now being appreciated.

The Oka vaccine was also evaluated in adults to prevent shingles, but a significantly higher titer of live attenuated virus was required to elicit significant and durable increases in cell-mediated immune (CMI) responses. Thus Zostavax was developed specifically for protection against herpes zoster. The zoster vaccine contains, on average, 19,400 plaque-forming units (PFU) per dose,[107] whereas the chickenpox vaccines contain either approximately 9800 PFU per dose (quadrivalent measles, mumps, rubella, and varicella vaccine)[108] or 1350 PFU per dose (monovalent varicella vaccine).[93] A zoster vaccine, to be effective, should boost an older person's CMI responses and therefore mimic immunologic benefit of exposure of VZV-immune adults to chickenpox.[109]

Several dose-ranging studies of vaccine defined a boost in waning CMI responses in older individuals.[110-112] The Shingles Prevention Study evaluated the high-titer, live attenuated zoster vaccine[44] in nearly 40,000 subjects older than 60 years of age with a mean follow-up of 3 years. Benefit was defined in three specific areas. First, the incidence of herpes zoster was 50% lower in the vaccine group than in placebo recipients (5.4 cases per 1000 person-years vs. 11.1 cases per 1000 person-years, $P < .001$). For patients who developed herpes zoster during the study, vaccine virus was not detected by PCR. Second, the incidence of PHN was 67% lower among vaccine recipients (0.5 cases per 1000 person-years vs. 1.4 cases per 1000 person-years, $P < .001$). In addition, the median duration of pain among subjects in whom herpes zoster developed was shorter in the vaccine group, albeit of marginal clinical value (21 days vs. 24 days, $P = .003$). Third, vaccination significantly decreased the burden of illness overall for patients who developed zoster (as assessed by an area under the curve evaluation, $P = .008$). When patients were analyzed according to two age groups, younger elderly (60–69 years old) and older elderly (≥70 years old), differences in vaccine efficacy were noted. Vaccination was more effective in preventing herpes zoster among the younger elderly group versus the older elderly group. However, it prevented PHN and burden of illness to a similar extent in both groups. The risk of subsequent development of herpes zoster does not appear to be increased in vaccine recipients.[113]

In 2006, the US Food and Drug Administration approved the zoster vaccine for prevention of herpes zoster in adults 60 years of age or older[44,107] and subsequently for adults older than 50 years in 2011.[114] However, the Centers for Disease Control and Prevention and ACIP recommend the zoster vaccine only for individuals 60 years of age or older because of concerns for possible waning of protection during the later years, when the risks of herpes zoster are greatest.[115] A recent study described the waning of efficacy of the zoster vaccine; efficacy in regard to the incidence of zoster persisted for 8 years, whereas efficacy in regard to the burden of illness related to zoster lasted for 10 years.[115] Use of a second, "booster" dose of vaccine to address the problem of waning immunity is under consideration.[115] This vaccine is not indicated for the treatment of PHN or herpes zoster.

In January 2018 the ACIP approved an adjuvanted inactivated subunit vaccine (Shingrix) to prevent herpes zoster.[116] The approval was based on the results of two randomized, placebo-controlled clinical trials in which two doses of Shingrix were administered to more than 30,000 participants 50 years of age or older[117] and 70 years of age or older.[118] Efficacy was 96.6% to 97.4% in participants 50 to 69 years of age and 91.3% in participants ≥70 years of age. Prevention of postherpetic neuralgia was 88.5% to 91.2%. The two-dose regimen of Shingrix resulted in frequent mild-to-moderate local and systemic reactions, although serious adverse reactions were no more frequent than in patients receiving placebo, and administration of Shingrix to previous recipients of Zostavax was shown to be well tolerated and immunogenic.[119] The ACIP recommended that Shingrix be approved for prevention of herpes zoster in individuals ≥ 50 years of age and that it was preferable to Zostavax.[116] It also recommended that Shingrix can be given to previous recipients of Zostavax. Additional follow-up studies of Shingrix are planned, including administration to immunocompromised individuals.[117]

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The complete reference list is available online at Expert Consult.

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137 Cytomegalovirus

William J. Britt

SHORT VIEW SUMMARY

Definition

- Human cytomegalovirus (HCMV) is a widespread cause of infection in normal and immunocompromised hosts.
- HCMV is a major cause of multiorgan dysfunction in immunocompromised patients, including allograft recipients and individuals with untreated or poorly controlled HIV/AIDS.
- HCMV is the most common intrauterine viral infection and can result in neurodevelopmental sequelae in infants infected in utero.
- HCMV has also been associated with a number of other diseases in normal hosts, including immune senescence.
- HCMV rarely causes symptoms in normal individuals, and almost all acute infections are clinically silent.

Epidemiology

- HCMV infection is ubiquitous, with over 50% of the US population infected by adulthood and over 100% infected in many regions of world. HCMV infection results in lifelong persistence with intermittent shedding and thus circulates continuously.
- Community spread of HCMV occurs through contact with oral and genital secretions. Infants commonly acquire HCMV through breastfeeding. HCMV is a sexually transmitted infection.
- In allograft recipients, HCMV infections can be acquired from transplanted allografts or from reactivation of virus in previously infected allograft recipients. Increasing risk of disease in this population is associated with increasing suppression of T-lymphocyte responses.
- Reinfection is common in immunocompromised persons and has also been demonstrated in immunocompetent individuals following exposure to new strains of HCMV.

Microbiology

- HCMV is a member of the Betaherpesvirinae subfamily of the Herpesviridae family of herpesviruses. It is a large, enveloped, double-stranded DNA virus (235,000 base pairs) encoding over 150 proteins.
- Serologic assignment of strains of HCMV is not available, but serologically distinct strains have been identified. Next-generation sequencing has demonstrated multiple viral genotypes (quasispecies) in infected individuals.

Diagnosis

- In immunocompromised hosts, HCMV can cause protean manifestations; thus accurate diagnosis requires laboratory testing.
- Although HCMV can be recovered by tissue culture, serology and nucleic acid amplification testing are mainstays of diagnostics for HCMV.

Therapy

- Several antiviral agents are available for prophylaxis and treatment of HCMV infections in immunocompromised hosts, but they have significant toxicities, and prolonged use can result in the development of resistance and treatment failure (see also Chapter 46).
- In some transplantation centers, HCMV hyperimmune globulins are employed together with antiviral chemotherapy for high-risk patients.

Prevention

- Attention to infection control measures, such as hand hygiene and contact precautions, has been shown to reduce the spread of HCMV.
- Humanized and human monoclonal antibodies with potent in vitro virus neutralizing activity are in clinical trials for treatment and prevention of severe HCMV infections.
- Development of vaccines for prevention of HCMV infection and the modulation of HCMV disease represents a high priority of both government agencies and pharmaceutical companies.

Human cytomegalovirus (HCMV) was isolated independently in the laboratories of Drs. Thomas Weller, Margaret Smith, and Wallace Rowe in the 1950s and was designated as cytomegalovirus (CMV) by Weller.[1–3] Interestingly, Weller isolated HCMV from an infant with findings that were consistent with congenital CMV infection and later demonstrated that sera from infants with clinical findings of cytomegalic inclusion disease were reactive with the virus.[4,5] This observation was of considerable interest because cytomegalic inclusion disease of the newborn, a syndrome that included hepatosplenomegaly, petechial rashes, intracranial calcifications, microcephaly, and histopathologic findings of large, inclusion-bearing cells in tissues from affected infants, had long been suspected to be caused by an infectious agent.[6–8] In contrast to the association of HCMV and cytomegalic inclusion disease of the newborn, the clinical importance of infections with HCMV in adults was limited to its association with heterophil-negative mononucleosis, until it was linked to a number of disease syndromes in solid-organ transplant (SOT) recipients and shown to be an important cause of pneumonitis and death in hematopoietic transplant recipients.[9–14] Subsequently, HCMV was shown to be one of the most important opportunistic infections in patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), contributing to both morbidity and mortality in these patients.[15–20] Although highly active antiretroviral therapy has dramatically changed the natural history of HIV infection, HCMV still represents a clinically important pathogen in a subset of these patients.[21,22] Over the last 2 decades, the spectrum of disease associated with HCMV in transplant recipients has broadened considerably, particularly in its role in chronic rejection and loss of the transplanted graft. In addition, HCMV infections in nonimmunocompromised individuals have been linked to a number of diseases associated with chronic inflammation, including coronary artery disease, exacerbations of symptoms in patients with inflammatory bowel disease, immune senescence in the aged population, multiple sclerosis, and human cancers.[23–39] In several instances, the contribution of HCMV infection to specific disease remains only an association; however, it is important to consider that the impact of HCMV infections on the health of a population could be significant because over 60% of adults in the United States are infected with HCMV, and in many regions of the world over 95% of the population is infected.[40,41] Thus as our understanding of the biology of this large DNA virus increases, it is highly likely that its role in the expression of a number of human diseases will be further defined and, perhaps, provide new avenues for improved therapeutics for management of these diseases.

MICROBIOLOGY

Virus Structure

HCMV, or human herpesvirus (HHV)-5, is the prototypic member of the Betaherpesvirinae subfamily of the family of Herpesviridae. Other members of the Betaherpesvirinae subfamily that infect humans include HHV-6 and HHV-7 and the large number of species-specific CMVs that infect other mammals. HCMV is the largest and most complex herpesvirus, with a 235,000 double-stranded DNA genome that encodes approximately 165 open reading frames.[42,43] The HCMV virion is approximately 220 nm in diameter, and virion proteins are distributed in three regions: the capsid, tegument, and envelope.[44,45] Tegumented subviral particles are enveloped in the cytoplasm of infected cells by budding into viral glycoprotein-enriched membranous vesicles prior to egress from the cell (Fig. 137.1). The icosahedral capsid is approximately 130 nm in diameter and although only about 20% larger than the capsid of herpes simplex virus (HSV), the HCMV capsid must package a genome that is over 50% larger than that of HSV.[44,46] Thus HCMV is likely near its capacity to package viral genomes, and acquisition of new genetic material during virion genome replication is likely selected against secondary to constraints imposed by the size of the HCMV genome. Conversely, deletion or truncation of viral genes that are not essential for virus replication could potentially increase the efficiency of genome packaging and virus replication, resulting in the persistence of viral mutants lacking genes not essential for virus replication, a phenomenon observed during in vitro passage of HCMV. The mature HCMV virion contains over 100 virus-encoded proteins as well as host proteins acquired during the complex nuclear and cytoplasmic assembly pathway of the particle.[47,48] The capsid contains four well-studied proteins that range in size from 150 to 8.5 kDa and are homologous in terms of size and function with capsid components of other herpesviruses.[45] In addition to virion tegument proteins and envelope glycoproteins that share homologous structural and functional features with their counterparts in other herpesviruses, HCMV also encodes a significant number of nonstructural proteins that can be detected within the infected cell but not in the virion. Nonstructural viral proteins provide a large number of functions required for efficient replication of the viral genome and assembly of progeny virions. In addition, virus encoded nonstructural proteins provide essential functions required for the regulation of cellular responses to HCMV infection, including significant alterations in cellular intermediary and lipid metabolism, inhibition of apoptosis and necroptosis, immune evasion of innate and adaptive immune responses, and maintenance of genome persistence during latency.[45]

Replication

HCMV replication follows a temporally regulated pattern of viral gene expression that is characteristic of the replication of herpesviruses. This includes immediate-early genes, expressed shortly after infection, that have been shown to regulate intrinsic host cell responses to infection as well initiating the transcription of viral genes essential for genome replication and virus assembly. Early viral genes represent the second class of viral genes expressed during productive infection. Protein products of these viral genes function primarily in viral DNA replication and packaging but also regulate host cell functions to optimize virus replication. Importantly, HCMV DNA replication results in an exponential increase in the number of viral genomes and ultimately, progeny virions. Thus functions critical for genome replication, such as those provided by viral genes encoding the DNA polymerase, terminase complex, and phosphotransferase, represent targets of currently licensed antiviral agents. Lastly, late viral genes are those encoding proteins such as components of the outer tegument layer and envelope, which are required for assembly of the infectious virion.

Both replication of HCMV DNA and the morphogenesis of the virion capsid take place in the nucleus. Following structural maturation of the capsid, newly synthesized concatemeric viral DNA is cleaved by a virus-encoded terminase that results in packaging of unit-length, linear genomic DNA.[49–51] Subsequently, viral DNA-containing capsids acquire an inner layer of tegument proteins during their egress from the nucleus, including essential interactions between proteins and capsid protein, that stabilize the interaction between the capsid and the inner tegument layer of the virion.[52] As the immature particle is transported along the cellular cytoskeleton, additional tegument proteins are applied to the maturing particle until the tegumented particle is enveloped in a modified intracellular membranous compartment distal to the *trans*-Golgi network that has been designated as the viral assembly compartment.[53,54] Formation of this intracellular compartment requires extensive reorganization of the cellular secretory and endocytic systems, and its formation is essential for efficient virion envelopment and infectious virion production.[53–56] The envelope of HCMV is structurally complex and is formed by lipid-containing host cell membranes that contain well over 20 different virus-encoded proteins. Several virion envelope proteins have been shown to be targets of virus-neutralizing antibodies and in experimental animal models have been shown to induce protective antiviral responses (see Fig. 137.1). These findings, together with correlations between antibody responses in humans and control of HCMV infections, have led to the selection of these components of the virion as potential targets for vaccine induced immunity. There is no evidence that host cell proteins are excluded from or selectively incorporated into the virion envelope during HCMV envelopment; thus host cell proteins can also be found in the virion envelope. Although specific functions for virion-associated host cell proteins in the infectious cycle of HCMV have been suggested based on in vitro findings, definitive evidence of an essential role of virion host cell proteins in HCMV replication is limited.[57,58]

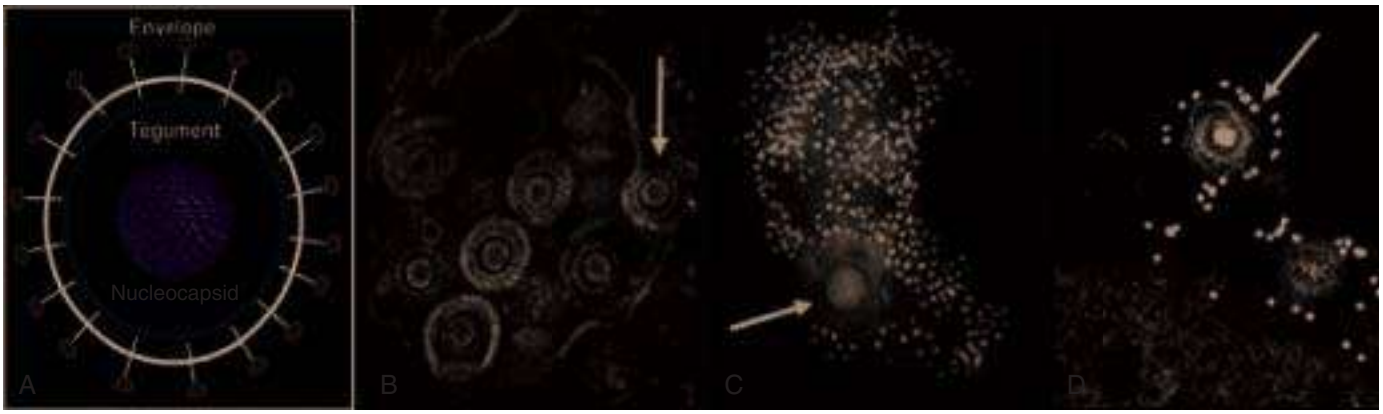

FIG. 137.1 Morphology of HCMV particles. (A) Cartoon of HCMV virion with nucleocapsid shown in yellow, the surrounding tegument layer of proteins indicated by the red oval, and outermost region, the envelope, studded with virion glycoproteins. (B) Electron micrograph showing intracytoplasmic vacuole with tegumented particle budding into vacuole *(arrow)* that also contains enveloped particles. (C) Single virion treated with detergent to remove envelope and tegument proteins detected with antitegument antibodies and electron-dense round nanogold particles (magnification ×70,000). The nucleocapsid is apparent in the lower left *(arrow)*. (D) Arrow indicates envelope of extracellular virions decorated with antienvelope antibodies and nanogold particles (magnification ×70,000).

Following envelopment, the virus is released from the cell either through a cellular exocytic pathway or after lysis of the cell. Alternatively, virus may spread cell-to-cell by less well-defined mechanisms. Regardless of the mode of spread, infectivity of progeny virions is absolutely dependent on assembly of a complete virion envelope, specifically the incorporation of virion envelope proteins, including glycoproteins B, H, L, O, M, and N, that have been shown to be required for early steps of virus entry into permissive cells. Mature, infectious progeny can be detected approximately 48 hours after infection, making the eclipse period (interval from infection to progeny virus production) of HCMV over twice as long as that of HSV and nearly eight times as long as influenza virus.

Persistence in the Host

In addition to shared strategies for replication and regulation of host cell responses, once infected, herpesviruses persist for the lifetime of the host. Although mechanisms of HCMV persistence are less well understood than those of alphaherpesviruses or gammaherpesviruses, HCMV persistence at the level of the infected host and not within an individual cell likely includes both a low-level chronic productive infection together with a state of true latency, in which only a limited number of viral genes are expressed and genome replication does not occur. This latter paradigm of restricted expression of HCMV genes during latency has recently been challenged utilizing more contemporary methodologies to define viral gene transcription during latency. These studies have suggested that a large number of viral genes are transcribed during latency in $CD34^+$ cells.[59,60] HCMV persistence has been demonstrated in epithelial cells from a number of organs, including the salivary gland, breasts, intestines, and prostate; however, it is unclear if HCMV DNA detected in these tissues represents a source of infectious virus that could be reactivated from latency, although studies in small animal models have demonstrated reactivation from latently infected salivary glands.[61] In contrast, HCMV latency has been extensively studied in $CD34^+$ myeloid mononuclear cells, a cell type that can be mobilized from the bone marrow and trafficked as $CD14^+$ cells to different tissues. Reactivation of HCMV from latency in $CD34^+$ myeloid mononuclear cells has been shown following differentiation of latently infected myeloid cells into tissue macrophages, thus providing a mechanism for infectious virus dissemination from a reservoir of latently infected cells.[62,63]

Viral functions interacting with the host cell that allow establishment and regulation of latency are incompletely defined and include modifications of cellular chromatin that restrict transcription from a number of viral promoters.[64] In addition, major changes in host cell metabolism and expression of immunomodulatory cytokines have been described.[65,66] Although a number of in vitro and in vivo models have provided new insight into the establishment and maintenance of HCMV latency, this aspect of the biology of HCMV remains incompletely defined and is an active area of study. Finally, whether a chronic low-level productive infection can be differentiated from latency with asynchronous but frequent reactivations in an infected host is likely of limited importance to the clinical significance of persistent HCMV infection in the normal host. Yet some level of productive infection appears to be maintained in the infected host, because studies have shown stability over decades of antiviral antibody responses against both nonstructural and structural virion proteins, and HCMV-specific $CD8^+$ T-lymphocyte responses from persistently infected individuals are directed at a wide variety of both nonstructural and structural virion proteins, including proteins expressed late in the replicative cycle.[67] These data argue that during HCMV persistence, the adaptive immune response of an infected host is presented with a large number of virus-encoded proteins similar to the immune system of individuals undergoing acute HCMV infection.

Host Interaction and Responses

The highly restricted species specificity of CMVs has been associated with large numbers of orthologous viral genes that regulate host cell responses to infection. These genes are often very similar in their functions and in some cases exist in blocks of genes that are colinear in the genomes of different CMVs. It is believed that these viral genes have coevolved with the host to enable CMVs to successfully persist but with the cost of strict species specificity, thus preventing maintenance of CMVs in intermediate hosts. CMVs utilize multiple strategies to ensure persistence in human populations. CMVs are efficiently transmitted from infected mothers to their infants in the perinatal period and as a sexually transmitted infection. In both cases, the ensuing infection is routinely associated with asymptomatic infection with prolonged virus shedding, thus providing an efficient mechanism for maintenance of this virus in a population. In addition, immunity to CMV is incompletely protective and reinfection with genetically diverse strains of HCMV has been well described in normal individuals.[68–72]

A large number of viral functions are thought to optimize HCMV infection and persistence in the host cell. These include the inhibition of cell death pathways (apoptosis, necroptosis, and pyroptosis) that are activated by virus infection, control of the cell cycle to optimize viral DNA replication, inhibition of cellular stress responses such as the unfolded protein response in the endoplasmic reticulum, modification of host cell metabolism, inhibition of cell death during HCMV replication, and perhaps most widely studied, evasion of host innate and adaptive immune responses[73–88] (Table 137.1). A discussion of the mechanisms by which HCMV can modify these host responses is beyond the scope of this chapter; however, the extraordinarily complex relationship between HCMV and the host immune response warrants a brief description because the most robust disease phenotypes associated with HCMV infection are seen in the immunocompromised host.

Following HCMV infection, expression of both immediate and early genes modifies innate responses. These range from blocking cellular intrinsic resistance mechanisms, such as silencing initial viral gene transcription by the host death domain–associated protein (Daxx) of the promyelocytic leukemia nuclear body; to inhibition of both the nuclear interferon-inducible protein 16 and cytosolic (cyclic GMP-AMP synthase), DNA sensor pathways limiting type I interferon gene expression; and finally limiting nuclear factor-κB–driven inflammatory responses.[89–96] A number of viral effector functions are responsible for inhibition of these early host cell responses, including virus-encoded microRNAs and proteins within the tegument of incoming virions, and viral proteins expressed as products of immediate-early genes.[89,94,97–100] A more well-described group of viral gene products target the antiviral activities of cellular innate responses such as natural killer (NK) cells and activated macrophages. Reported immune evasion mechanisms that inhibit NK cell activity include limiting cell surface expression of NK cell–activating ligands, expression of viral proteins that mimic major histocompatibility complex (MHC) molecules, and expression of inhibitory NK ligands such as human leukocyte antigen (HLA)-E.[101–110] Direct evidence supporting an important role of HCMV immune evasion functions that target NK cell activity in HCMV infections comes from studies in animal models, including recent studies in rhesus macaques that have clearly demonstrated that in the absence of virus-encoded NK cell immune evasion functions, CMV infection was dramatically attenuated.[111] Finally, $CD4^+$ and $CD8^+$ T-lymphocyte recognition of HCMV-infected cells has been shown to be significantly inhibited by

TABLE 137.1 HCMV Modifications of Host Cell Responses to Facilitate Replication and Persistence

HOST CELL FUNCTION	HCMV MODIFICATION	REFERENCES
Cell cycle regulation	Inhibition of cell cycle progression	73–77
Glucose and lipid metabolism	Increased utilization of glucose and altered lipid metabolism	78, 80–84
Stress response	Inhibition of cellular stress response	79, 82, 85
Cell death pathways	Inhibition of apoptosis and necroptosis	86–88
Host immune responses to viral infection	Inhibition of intrinsic, innate, and adaptive immune responses	90–96, 103, 105, 107, 110, 111, 114

mechanisms that include degradation of both class I and II MHC molecules and interference with peptide loading of class II MHC molecules.[105,112–114] The in vivo importance of viral-encoded immune evasion functions that limit recognition of HCMV-infected cells by effectors of the adaptive immune response has been modeled in rhesus macaques. In these experiments, mutant rhesus CMVs that failed to degrade class I MHC molecules secondary to deletion of orthologues of HCMV immune evasion genes were shown to be highly attenuated in macaques.[115]

Pathogenesis of HCMV Infection

Although the findings of end-organ disease in multiple organ systems following HCMV dissemination in immunocompromised individuals has suggested that HCMV disease can readily be explained by lytic virus replication and cytopathology, the magnitude of clinical disease observed in some HCMV-infected individuals often is inconsistent with the level of virus replication. Such observations have suggested that mechanisms other than viral cytopathology could contribute to some clinical manifestations of HCMV infection, and that the severity of disease cannot be directly attributed to the level of virus replication in an individual patient. Thus components of the host innate and adaptive immune responses likely contribute to the clinical expression of HCMV infection in some patients. However, it is important to note that an accurate quantification of the contribution of virus replication and that of host responses to disease following CMV infection has only been accomplished in animal models of HCMV infections.[116–118] Thus the distinction between the effects of virus replication and host responses to the clinical expression of HCMV infection should perhaps only be a starting point for the discussion of mechanisms of HCMV disease in different patient populations. A categorization to simplify the discussion of the plethora of clinical manifestations associated with HCMV infection disease would be (1) clinical manifestations associated with high levels of virus replication in severely immunocompromised patients in which tissue damage can be attributed to viral infection, and (2) a more diverse set of disease phenotypes in patients with residual immunity in which host responses to HCMV could represent a significant component of clinically apparent disease. Admittedly, these distinctions are an arbitrary attempt to describe different mechanisms of disease, and the spectrum of clinical disease that is observed in most patients with HCMV infection likely reflects variable combinations of both host responses and direct effects of virus replication, including virus persistence driving ongoing host innate and adaptive immune responses.

Pathogenesis of HCMV in the Setting of Immunosuppression and Virus Dissemination

The clinical spectrum of disease in immunocompromised patients with uncontrolled HCMV replication was well appreciated by clinicians caring for allograft recipients prior to the advent of effective antiviral therapy.[10–14] End-organ disease (including hepatitis), bone marrow dysfunction, colitis, pneumonitis, and, less commonly, central nervous system (CNS) disease (including encephalitis) were reported in both solid-organ and hematopoietic allograft recipients. Concomitant with the recognition of HCMV as a cause of disease in allograft recipients, clinicians linked the severity of disease to the degree of immunosuppression. Disseminated HCMV infection with a similar spectrum of end-organ disease was also common in HIV/AIDS patients in the era prior to highly active antiretroviral therapies.[17,19,20] In contrast to the infrequent occurrence in allograft recipients, retinitis was one of the most recognizable clinical expressions of disseminated HCMV infection in patients with HIV/AIDS.[119–122] In patients with widely disseminated infection, replicating HCMV could be recovered from peripheral blood, often from buffy coat leukocytes, and histopathologic findings consistent with HCMV infection, including large cells with characteristic nuclear inclusions, so-called owl's eye inclusions secondary to the clear halo surrounding the inclusion (Fig. 137.2). Interestingly, in more recent studies in which the level of HCMV viremia could be more accurately quantified with polymerase chain reaction (PCR), higher viral loads were associated with the presence of owl's eye inclusions in tissue sections from HCMV-infected patients, suggesting a direct correlation between detection of histopathologic findings of HCMV infection and the level of virus replication.[123]

Early findings in both solid-organ allograft and hematopoietic transplant recipients demonstrated that control of virus replication and dissemination was dependent on the timely development of HCMV-specific T-lymphocyte responses and were subsequently confirmed in a number of studies in transplant recipients.[124–133] Correlations were demonstrated between T-lymphocyte responses and the risk for invasive HCMV disease and, importantly, direct evidence of the importance of HCMV-specific T-lymphocyte responses was initially provided by the transfer of ex vivo expanded HCMV-specific $CD8^+$ T lymphocytes into patients at risk for HCMV infection and disease.[134] Similarly, the risk for invasive HCMV infections in HIV/AIDS patients before the widespread use of highly active antiretroviral agents was directly related to the loss of HCMV-specific T-lymphocyte responses.[135–137] In these patients, the risk of HCMV disease fell dramatically with the introduction of effective HIV antiretroviral therapy, and the decrease in invasive HCMV disease was directly correlated with the reconstitution of adaptive immune responses.[135]

From observations in both transplant recipients and patients with HIV/AIDS, the control of HCMV replication requires the presence of effective HCMV-specific T-lymphocyte responses, whether HCMV infection is classified as a primary infection, such as that which could occur after transplantation with a graft from an infected donor (Donor+/Recipient−), or following recrudescence of infection in an individual who was previously immunocompetent.[128,129,138,139] When effective antiretroviral agents became available, HCMV replication could be inhibited, dissemination limited, and virus replication significantly inhibited in end organs. Thus significant benefit from antiviral therapy was observed in

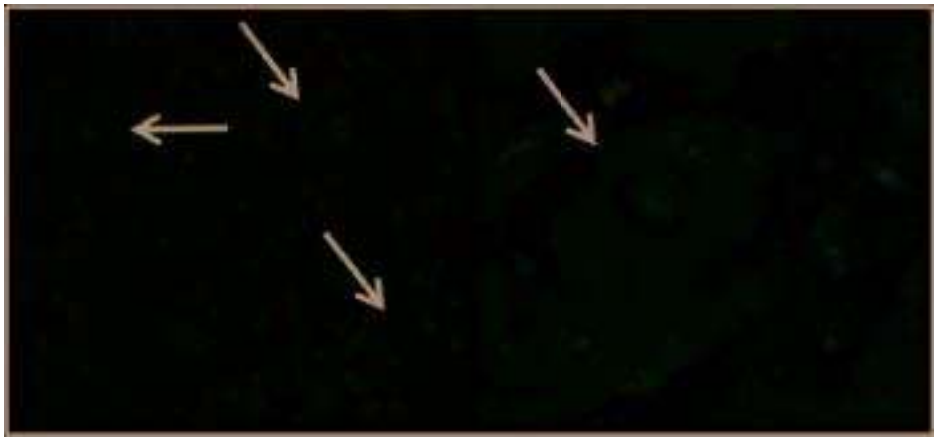

FIG. 137.2 Characteristic cytomegalic cells with owl's eye inclusions *(arrows).* Note size of HCMV-infected cells and nuclei as compared to surrounding uninfected cells. (Hematoxylin and eosin staining; magnification ×20 *[left]*, ×40 oil immersion *[right]*.)

these patients, particularly early in the posttransplantation period when suppression of adaptive immune responses is often most intense.[140] However, as has been demonstrated both in clinical studies of antiviral treatment, prophylaxis, or both, and by transfer of HCMV-specific $CD8^+$ T cells to suppress HCMV replication in transplant recipients, failure to adequately reconstitute HCMV-specific adaptive immunity can result in HCMV disease late in the posttransplantation period.

Pathogenesis of Diseases That Lack a Direct Relationship to Levels of Virus Replication

In early studies, solid-organ allograft recipients with HCMV infections and high levels of virus replication often succumbed to multiorgan disease, whereas a leading cause of death in hematopoietic transplant recipients was HCMV pneumonia/pneumonitis.[141] In contrast to findings in other organ systems in these patients, patients with fatal HCMV pneumonia often exhibited only focal areas of HCMV infection and once clinical disease became evident, treatment with antivirals often provided limited benefit. Similarly, in immunocompromised patients with HCMV colitis, severe symptomatology can be observed that is discordant with widely separated and focal areas of colonic involvement, findings that suggest that disease in these patients likely includes components of the host response. Findings from studies in solid-organ allograft recipients have also suggested that lytic virus replication leading to tissue damage was an unlikely explanation for the spectrum of disease in these patients.[142–145] Furthermore, the majority of HIV/AIDS patients with clinically apparent HCMV encephalitis were found to have focal encephalitis with areas of microglial nodules and not a necrotizing encephalitis that is more characteristic of uncontrolled virus replication.[142] These clinical observations suggest that diseases associated with low levels of HCMV replication are not the result of direct viral damage but are indirect and presumably secondary to host responses to HCMV infection. Furthermore, HCMV infection has been proposed to contribute to chronic rejection of renal and heart allografts in numerous clinical studies and in experimental animal models of allograft rejection.[146–148,149–152,153–158] Although precise mechanisms that could account for the contribution of HCMV infection to allograft rejection remain to be completely defined, findings from patient specimens and from experimental animal models strongly argue that the contribution of ongoing HCMV-induced inflammation in the graft together with incompletely controlled lytic HCMV infection could be responsible for disease in these patients.

HCMV infection in nonimmunocompromised hosts has long been associated with a number of chronic diseases, including atherosclerotic heart disease and more recently aging and immune senescence.[23,24,27,30–32,159–172] A discussion of specific mechanisms associated with HCMV infection that contribute to chronic disease is beyond the scope of this chapter, but common to many of the proposed mechanisms is the capacity of HCMV to induce chronic inflammation in the persistently infected host. This latter biologic feature of HCMV infection in normal individuals is perhaps best illustrated by finding that up to 10% to 15% of the total peripheral blood $CD8^+$ T-lymphocyte responses of normal individuals infected with HCMV, and even a greater percentage in aged individuals, can be directed at HCMV.[67,170] As described previously, the suite of immune evasion functions encoded by HCMV presumably contribute to its capacity to efficiently express a limited set of viral genes and in some cases, replicate in sites of inflammation during persistence. Interestingly, host inflammatory responses appear to contribute to HCMV gene expression.[173–182] Thus lifelong persistence of HCMV in normal individuals leads to a symbiosis between HCMV and the host that results in some level of chronic activation of innate and adaptive immunity.

Routes of Transmission and Sources of HCMV Infection

Infection with HCMV is common in most populations in the world and in most areas of the world, and is acquired during early childhood. In the United States, over 50% of the population is infected with HCMV, with the prevalence of infection dependent on both race and socioeconomic status.[40] The rates of HCMV infection in the United States increase steadily throughout adult life, with increased rates of infection noted during adolescence.[41,183–185] In southern Asia, Africa, and many countries in South America, the HCMV seroprevalence is nearly 100% in adults and similarly high in young children and adolescents.[40,186–195] In adults, community acquisition of HCMV follows exposure to saliva, urine, and genital secretions, including semen, from an infected individual. Intimate contact, including sexual activity, is an important route of HCMV transmission, because higher rates of HCMV infection have been described in populations with high rates of sexually transmitted infections.[15,196–201] HCMV is considered a sexually transmitted infection in adults.

A second important risk factor for HCMV infection is exposure to young children. Infants and children can acquire HCMV through intrauterine infection, during parturition, through exposure to breast milk containing HCMV, and through exposure to other children infected with HCMV. Intrauterine infection resulting in congenital HCMV infection occurs in approximately 1 in 200 births in the United States and perhaps as often as 1 in 100 live births in some regions of the world.[195,202–205] Furthermore, HCMV is readily transmitted by breast milk, and between 50% and 70% of breastfed infants of HCMV seropositive women will become infected, depending on the duration of breastfeeding.[206–210] Thus breast milk transmission likely contributes to the high HCMV seroprevalence in children reported in Asia, South America, and Africa. Regardless, if infected with HCMV early in life as the result of an intrauterine infection, during delivery, or following ingestion of HCMV-infected breast milk, infants routinely shed significant amounts of infectious virus in saliva and urine for prolonged periods of time.[208] Thus infected infants can serve as reservoirs for spread of this virus under conditions that favor close contact with an infected child, such as those provided by parents or other caregivers, family, individuals living in crowded conditions with an infected infant, or other young children and caregivers in group care facilities.[211–216] Furthermore, the spread of HCMV among young children within families and within group care settings is more frequent when children are under 2 years of age.[217,218]

HCMV can be acquired in hospitalized patients through exposure to blood products and following transplantation of an organ from a HCMV-infected donor. Nosocomial transmission was infrequent even prior to the introduction of universal precautions in health care facilities.[219] Transmission of HCMV by blood products containing HCMV has been recognized for decades, and an extensive literature describes various attempts to limit HCMV transmission by this route. Perhaps the most universally accepted approach is to utilize only blood products from HCMV seronegative donors. However, the seroprevalence of HCMV is over 50% in most donor populations, and thus such an approach would greatly compromise the supply and availability of blood products. In high-risk recipient populations such as extremely premature infants and individuals undergoing allogeneic hematopoietic stem cell transplantation (HSCT), utilization of leukocyte-reduced blood from HCMV-seronegative donors has been advocated,[220,221] whereas other investigators have argued that the use of unselected leukocyte-reduced blood products is sufficient.[222,223–227] A recent survey of transfusion practices indicated that the standard of practice in over 90% of transfusion services included the use of leukocyte-reduced blood products, but the use of leukocyte-depleted products from HCMV seronegative donors for high-risk populations was not as consistent.[228] This variability in the donor selection based on HCMV seroreactivity was reflected in the conclusions in a publication from the American Association of Blood Banks and by a recent review of this topic.[229,230] In contrast to the contentious debate about the transmissibility of HCMV in blood products, HCMV transmission by an allograft from an infected donor is established. Transmission has occurred following transplantation of solid organs, as well as from hematopoietic cells.

CLINICAL MANIFESTATIONS

Infection in the Immunocompetent Host (Infectious Mononucleosis)

HCMV is commonly acquired throughout life, and infection is subclinical in almost all infected individuals. HCMV was first well described as a cause of an infectious mononucleosis syndrome, and in early studies, HCMV was estimated to account for between approximately 10% and 20% of cases of heterophil-negative mononucleosis in adults.[9,231–235] The

clinical expression of HCMV mononucleosis has been reported to include fever, malaise, and myalgias that can be persist for weeks. Pharyngitis appears to be less frequent and less severe than that seen in infectious mononucleosis caused by Epstein-Barr virus. Laboratory abnormalities can include hemolytic anemia, thrombocytopenia, reactive lymphocytosis, and elevated aminotransferases. Similar findings have been reported in children acutely infected with HCMV, although the incidence of symptomatic HCMV in normal children is unknown but likely to be exceedingly low.[236] Even in individuals with asymptomatic infections, virus can be shed from saliva, urine, and vaginal secretions for weeks to months.[237] Treatment is symptomatic, and clinical and laboratory abnormalities are self-limited.

Transfusion-Acquired Infection

In addition to mononucleosis syndromes, HCMV has been demonstrated to be a cause of clinical disease in transfusion-acquired infections in normal hosts. Transfusion-associated HCMV infections in surgical patients were well described in older literature, but have been dramatically reduced by the use of leukocyte-reduced blood products (see "Routes of Transmission and Sources of HCMV Infection"). There has been more recent interest in the contribution of HCMV infection to the morbidity and possibly mortality in severely ill patients with septic shock and patients with thermal burns.[238–246] In this patient population, HCMV reactivates from an existing latent infection, and replicating virus can be detected over a broad time interval in these patients, ranging from within the first week of hospitalization to several weeks.[238,239,242] Although most case series do not describe increased mortality secondary to replicating HCMV in patients with severe sepsis or burns, it appears that HCMV replication in these patients is associated with an increased length of stay in an intensive care unit.[238–240,242] A randomized trial using ganciclovir treatment to control viremia and virus shedding in patients with severe sepsis failed to demonstrate that treatment was associated with altered levels of the proinflammatory cytokine interleukin-6 in the sera from these patients or length of intensive care unit stay when compared to control patients.[247] Thus HCMV replication in these patients may be only an indirect marker of systemic inflammation and does not directly impact the clinical course of these patients. Alternatively, this initial trial could have selected noninformative end points for analysis, and additional trials will be required to definitively address the role of HCMV in the morbidity of patients with severe sepsis or burns.

Association With Inflammatory Bowel Disease

A consistent association between HCMV and exacerbation of symptoms of inflammatory bowel disease (IBD) has been described in numerous case reports and in several larger patient series, particularly in patients with steroid-refractory ulcerative colitis.[248–255] It is estimated that about 25% of patients with IBD who present with severe colitis will have active HCMV infections, a rate that increases to over 30% in patients with symptoms that failed to respond to steroid treatment.[29,248,256,257] Although definitive evidence that HCMV infection can lead to the development of IBD has not been reported, clinical observations have strongly argued that the presence of HCMV in the intestinal mucosa contributes to the severity of flares of disease in these patients. The diagnostic criteria for HCMV in patients with IBD and severe colitis have included the detection of HCMV DNA in blood by PCR and, more definitively, the detection of HCMV in intestinal biopsy specimens by immunohistochemistry, PCR, or both.[28,258] The severity of colitis in these patients, including intestinal inflammation, has been shown to be related to the number of HCMV inclusion-bearing cells in biopsy specimens and the quantity of HCMV DNA in biopsy specimens.[259,260] Consistent with the observed relationship between virus replication and disease severity, treatment with antiviral agents has been shown to hasten resolution of clinical symptoms and to reduce the need for surgical interventions in IBD patients with flares of colitis.[248,251,259,261–263] Finally, well-defined mechanisms that account for the role of HCMV infection in the development of severe colitis in patients with IBD remain to be defined; however, recent studies have suggested that HCMV infection may alter the phenotype of intestinal macrophages, resulting in the release of proinflammatory mediators and increased intestinal inflammation.[264,265]

HCMV Infections in the Immunocompromised Host

HCMV Infections in Solid-Organ Transplant Recipients (See Also Chapter 308)

In the mid-1960s, Rifkind and colleagues described histopathologic findings consistent with HCMV infection in tissue specimens from patients undergoing renal transplantation and isolated infectious HCMV from these patients.[10,11,266,267] These investigators also linked immunosuppression with clinical disease associated with this recently described virus. Shortly thereafter, other investigators confirmed these reports.[268] HCMV has subsequently become one of the most recognized causes of disease in allograft recipients. An illustration of the importance of HCMV infection in the posttransplantation period is illustrated by an early report of an *epidemic* of HCMV in a transplant recipient population that was thought to have originated in a hemodialysis unit utilized by allograft recipients prior to transplantation.[269] HCMV infections continue to represented one the most frequent posttransplantation infection in both SOT and HSCT recipients, occurring in approximately 50% of allograft recipients. Prior to the widespread use of antiviral prophylaxis, HCMV infection and disease ranged from about 50% in renal, liver, and heart transplant recipients to over 70% in lung allograft recipients.[270–272] Although substantial progress in treatment with antiviral drugs coupled with sensitive and rapid diagnostics have resulted in decreased in morbidity and mortality associated with HCMV infections in allograft recipients, HCMV remains a common infection in the posttransplantation period, and clinically apparent infections can develop in as many as 20% of high-risk recipients.[273–278]

With the dramatic reduction in blood product transfusion transmission of HCMV, the major sources of HCMV infection in SOT recipients are virus present in the transplanted organ (donor CMV-positive [D+]) or reactivation of persistent infection (recipient CMV-positive [R+]) following immunosuppression of allograft recipients.[279,280] Transplantation of an organ from an HCMV-seropositive donor into an HCMV-seronegative recipient (D+/R–) represents a high risk for both virus transmission and the severity of the ensuing infection, whereas transplantation of an organ from a seropositive donor into a seropositive recipient (D+/R+) or an organ from a seronegative donor into a seropositive recipient (D–/R+) represent an intermediate risk (Table 137.2). Transplantation of an organ from a seronegative donor into a seronegative recipient (D–/R–) is classified as low risk. These risk stratifications were derived from observations of patients infected during the first 6 months

TABLE 137.2 Identified Clinical Risks for Significant HCMV Infections in Allograft Recipients

ALLOGRAFT RECIPIENT	RISK CATEGORY	REFERENCES
Solid-Organ Transplant		
Donor+/Recipient–	High	279, 280
Donor+/Recipient+ or Donor–/Recipient+	Intermediate	273, 280
Donor–/Recipient–	Low	
T-cell depletion (antithymocyte globulin; anti-CD52; anti-CD25)	Can modify risk stratification	281, 286, 289, 291
Hematopoietic Stem Cell Transplant		
Donor+/Recipient+ or Donor–/Recipient+	High	387–389
Donor+/Recipient–	Intermediate	387, 389
Donor–/Recipient–	Low	
Degree of HLA mismatches between donor and recipient; umbilical cord stem cell transplant into + recipient; T-cell depleting antibodies; graft-versus-host disease requiring steroid therapy	Can modify risk stratification	390, 391, 393

HLA, Human leukocyte antigen.

following transplantation, when immunosuppression was most intense, and during a period of time when antiviral therapies were not available. It was argued that HCMV infection following D+/R− transplantation was equivalent to primary infection in a recipient with a compromised immune system and that clinical symptoms developed more frequently in seronegative recipients than seropositive recipients, secondary to residual adaptive immunity to HCMV in seropositive recipients. While this remains a paradigm in SOT recipients, an analysis of a large series of SOT recipients demonstrated that while about 20% of D+/R− transplant recipients developed symptomatic HCMV infections, 8.1% of D+/R+ and 9% of D−/R+ recipients also developed symptomatic HCMV infections.[273] Thus when the total number of patients in all risk categories were considered, the number of CMV-positive recipients with symptomatic infection was equivalent to the number of patients in the high risk D+/R− group with symptomatic infection.[273] Lastly, different T-lymphocyte–depleting and nondepleting antibodies that are commonly used during induction of immunosuppression and for treatment of allograft rejection have been reported to differentially increase the risk for HCMV infection in the posttransplantation period (see Table 137.2). Three antibody preparations, a polyclonal antithymocyte globulin and two monoclonal antibodies, anti-CD25 (basiliximab) and anti-CD52 (alemtuzumab), have been reported to confer different risks for HCMV infection and disease in the posttransplantation period.[281–290] A consensus on the relative risk for increased HCMV infections and disease conferred by each antibody is difficult to define from available literature, although a recent review has suggested that use of rabbit anti-thymocyte globulin is associated with a higher rate of HCMV infection in recipients as compared to the monoclonal anti-CD52 or anti-CD25 antibodies.[291]

The importance of the level of suppression of T-lymphocyte responses as a risk for HCMV infection and disease in SOT recipients in the posttransplantation period in contemporary studies have confirmed observations made more than 3 decades ago that demonstrated the relationship between HCMV T-lymphocyte responses and disease in the posttransplantation period.[292] Furthermore, the finding that both seronegative and seropositive SOT recipients could develop invasive HCMV infections, when viewed together with more contemporary data, has led to the paradigm that in an individual allograft recipient, the level of HCMV-specific T-cell immunity in the posttransplantation period is the major determinant in the control of HCMV infection and disease. More recently, standardized assays that can accurately quantify HCMV-specific T-lymphocyte responses have convincingly shown that the level of T-lymphocyte responses in the posttransplantation period can define the risk of HCMV infection in allograft recipients.[129,130,293–301] Thus exposure to HCMV in the posttransplantation period, whether from a persistent infection or from a transplanted organ, presents a risk for infection and disease that can be modified by the level of HCMV-specific T-lymphocyte responsiveness.

The widespread use of antiviral prophylaxis and, in some centers, rigorous monitoring for HCMV replication followed by aggressive preemptive therapy has resulted in HCMV infections that become apparent late (defined as >120 days, or after discontinuing antiviral prophylaxis) following transplantation. Although combined into a single category as late infections, biologically these infections are significantly different in terms of their incidence, risk factors, and clinical expression. It has been suggested that late posttransplantation infections be further subdivided into postprophylaxis HCMV infection and disease that occurs within the first 6 months following discontinuation of antiviral prophylaxis, and truly late-onset infection and disease that occurs over 6 months after prophylaxis is discontinued.[277] Regardless of the terminology employed to describe different populations of SOT recipients with HCMV infections, it appears that HCMV infection in these two groups differs fundamentally in its mechanisms of disease, and therefore in risk factors for infection and clinical expression of HCMV infection in each group. As an example, the conventional paradigm suggesting increased clinical risk for HCMV infection in D+/R− SOT recipients has been reported as a risk factor for infection and disease shortly following discontinuation of prophylaxis, whereas this category of SOT recipients was not suggested to be at higher risk for late-onset disease.[274,277,302–307] Importantly, both categories of late infections have been attributed to the effective suppression of HCMV replication by antiviral therapy, which in turn is thought to result in delayed development of adaptive immunity, specifically T-lymphocyte responses to HCMV, whereas some investigators have suggested that ganciclovir could inhibit T-lymphocyte proliferation directly and result in a more global deficit in T-lymphocyte responses.[127,308–311] It is of interest to note that the role of antiviral prophylaxis in the development of late-onset disease is unclear.[307,312] Lastly, the role of T-lymphocyte–depleting antibodies in the induction of immunosuppression is also thought to be a clinically significant risk for the development of late HCMV infections and disease.

The clinical manifestations of HCMV infection in the posttransplantation period can be protean, and symptomatic HCMV infections are not infrequent, occurring in perhaps as high as 20% of high-risk allograft recipients even with antiviral prophylaxis. Together with the presence of comorbid conditions and other infections in these patients, the diagnosis of infection based solely on clinical presentation is unreliable, with the possible exception of HCMV retinitis, which can present with characteristic ophthalmologic findings. The most commonly described clinical manifestation of HCMV in the SOT recipient has been referred to as the "CMV syndrome."[313] The clinical criteria for the diagnosis of CMV syndrome have varied among transplantation centers, which has made the comparison of results from clinical trials in different centers difficult to interpret. Recently in an attempt to establish common criteria, Ljungman and colleagues suggested criteria for CMV syndrome that include detection of HCMV in the blood together with at least two of the following criteria: (1) fever >38°C for >2 days, (2) new or increased fatigue/malaise, (3) leukopenia or neutropenia, (4) >5% reactive lymphocytes, (5) platelet counts <100,000 cells/μL or <20% of platelet counts if starting platelet count is <115,000 cells/μL, and (6) elevation of hepatic aminotransferases (Table 137.3).[313]

In contrast to the CMV syndrome in SOT recipients, end-organ disease has been well described in both SOT and HSCT HCMV-infected allograft recipients. Clinical manifestations of HCMV infection that have been described in these patients include pneumonia, gastrointestinal disease, hepatitis, retinitis, nephritis/cystitis, pancreatitis, myocarditis, and CNS disease, with gastrointestinal tract infection and disease being the most common clinical manifestation of HCMV infection (see Table 137.3). In each case, with the exception of retinitis, a definitive diagnosis requires demonstration of HCMV in tissue specimens from the affected organ and, if possible, quantitation of the amount of virus. The level of HCMV in the blood can serve to alert to the possibility of end-organ disease, but diagnosis of organ involvement requires detection of HCMV in the organ system. In cases of suspected HCMV pneumonia, quantitation of virus in bronchoalveolar lavage (BAL) fluid has been suggested to be of considerable value in the evaluation of SOT (and HSCT) recipients with pneumonia, and may lead to withholding of treatment from patients who may only be shedding virus and who do not have invasive infection. Considerable effort to establish levels of HCMV DNA in BAL specimens that are predictive of invasive HCMV disease is ongoing, and, although variable, values of HCMV DNA that are predictive of pneumonia in patients have been reported for lung transplant recipients, other SOT recipients, and HSCT recipients.[314–317] However, as illustrated in one case report, caution has been suggested in the

TABLE 137.3 Clinical Expression of HCMV Infections in Solid-Organ Transplant Recipients

CLINICAL MANIFESTATION	CLINICAL AND LABORATORY FINDINGS
CMV syndrome	Fever New or increased fatigue/malaise Leukopenia or neutropenia Reactive lymphocytes Thrombocytopenia Elevation of hepatic aminotransferases
End-organ disease	Gastrointestinal disease (colitis, hepatitis, esophagitis) Pneumonitis (CMV+ BAL) CNS (retinitis, encephalitis)
Allograft dysfunction	Allograft rejection (chronic)

BAL, Bronchoalveolar lavage; *CMV,* cytomegalovirus; *CNS,* central nervous system.

interpretation of a low or absent HCMV DNA signal in a BAL specimen when other clinical and laboratory findings are consistent with the diagnosis of HCMV pneumonia.[318]

Of note, these clinical manifestations of HCMV disease in transplant recipients reflect end-organ disease associated with high levels of virus replication and usually present clinically in the early posttransplantation period or within months after discontinuing antiviral prophylaxis. In contrast, the clinical manifestations of end-organ disease and CMV syndrome can be more variable in late-onset disease, and in SOT recipients, late-onset HCMV disease may not be associated with significant viremia and evidence of disseminated infection.[307,319,320] Furthermore, routine care of SOT recipients late after transplantation may be provided by community physicians who may not have high index of suspicion for atypical presentations of HCMV infection, and thus patients may be diagnosed late in the course of the infection.[277] In SOT recipients, late-onset HCMV infection has been correlated with allograft loss and increased all-cause mortality in some studies, whereas these associations have not been reported in the analyses of other SOT populations.[302,321–323,324–326]

Finally, HCMV infections in the posttransplantation period have long been associated with an increased incidence of fungal and bacterial infections in allograft recipients.[327] Whether the increased risk for nonviral infections in these patients represents an indirect effect of HCMV on the capacity of the host to mount protective immune responses, or merely represents a role of HCMV as sentinel of status of immunity in these patients, continues to be debated.[148]

HCMV Infection and Allograft Survival and Function in Solid-Organ Transplantation

An important but yet unanswered question in the biology of HCMV infection in allograft transplantation is the role infection with this virus plays in the function and survival of the allograft. As was noted previously, an extensive literature has argued that HCMV infection can impact graft function acutely as well as altering long-term graft function and survival.[157,276,328–340] The association between HCMV infection and graft dysfunction, graft loss, or both has been most extensively described in cardiac and renal allograft recipients.[149,276,331,341–347] Cardiac allograft vasculopathy (CAV) is a well-described condition associated with rejection of cardiac allografts, displaying characteristic histopathologic finding in the coronary arteries of the allograft that include intimal fibromuscular thickening with concentric narrowing of the vessel lumen that is often associated with inflammation in all layers of the coronary arteries.[348–350] Development of CAV is associated with graft dysfunction and loss, and often requires cardiac retransplantation. Although the mechanisms that induce CAV have not been defined, a pathway associated with endothelial damage followed by migration and proliferation of smooth muscle cells and ultimately fibrosis and thickening of the intima has been suggested.[351,352] Damage to the endothelium is thought to involve a combination of innate and adaptive immune effector functions, including antibodies that could mediate graft rejection, although the precise definition and composition of these effectors remains to be precisely determined.[353–361] Thus HCMV infection in the donor or recipient has been associated with the development of CAV, but a unifying mechanism that can account for the role of this virus infection and the development of CAV remains to be defined. Because of the limitations of invasive studies in humans, the role of HCMV infection in the development CAV cannot be easily studied with sufficient precision to define a mechanism(s) of disease, particularly in a complex population such as transplant recipients with underlying disease and exposure to immunosuppressive agents. Perhaps even more challenging is the task of dissecting the role of HCMV in CAV development in the face of an ongoing alloreaction that may include both T-lymphocyte and antibody-mediated rejection of the cardiac allograft. Studies utilizing antiviral drugs and analysis of HCMV-specific T-lymphocyte responses have suggested that control of HCMV replication can limit CAV in cardiac allograft recipients, thus providing some insight into potential mechanisms of disease in these patients that can be explored in relevant models.[149,342,362–365] Fortunately, small animal models of cardiac allograft transplantation have been developed in which CMV infection results in decreased graft survival with histopathologic changes that resemble findings reported in human cardiac allografts with evidence of rejection.[155,352,366–368] Ideally, these models will allow direct hypothesis testing of mechanisms that contribute to CAV that cannot be carried out in a clinical population.

Although the control of episodes of acute rejection of a renal allograft has improved significantly, chronic renal allograft rejection resulting in the loss of a functioning graft continues to represent a major clinical issue in renal allotransplantation. Results from decades-old studies have linked HCMV infection with renal allograft loss, although as with CAV in cardiac allografts, the mechanism(s) through which HCMV contributes to allograft loss remains undefined. Chronic rejection in renal transplant recipients is histologically characterized by tubulointerstitial nephritis and tubular atrophy leading to the loss of proximal tubules.[369] HCMV infection has been proposed to contribute to both the development and progression of chronic renal allograft rejection. Importantly, the findings of chronic renal allograft rejection have also been modeled in animal systems, thus allowing for more controlled studies of the role of CMV in this process.[158,370–378] However, it should also be noted that even though HCMV infection in renal allograft recipients has been repeatedly associated with an increased incidence of graft dysfunction in both the early and late posttransplantation period, whether HCMV infection has a direct role in this chronic inflammatory process remains somewhat contentious.[152,156,157,329,379–382] Inflammation remains linked to graft rejection, and it has been proposed that viral gene expression contributes to the inflammatory responses in renal allografts undergoing chronic rejection. Interestingly, studies in humans have demonstrated HCMV-encoded proteins in >90% of failing renal allografts.[164,383] Thus the presence of HCMV in the allograft could influence not only the intensity, but the nature of chronic inflammation associated with this alloreaction, as has been demonstrated in small animal models of HCMV-associated graft dysfunction.[154] Lastly, CMV infection of allografts in small animal models has been shown to induce increased angiogenesis as well as transcription programs that resemble that of wound healing.[150,180,384] Thus several lines of evidence point to HCMV as a proximal cause of graft dysfunction and tubulointerstitial nephritis and tubular atrophy in renal allografts. Hopefully, informative animal model systems that have been developed to investigate the role of HCMV infection in allograft rejection will help define mechanisms associated with this process.[158,372,376–378]

HCMV Infections in Hematopoietic Stem Cell Transplant Recipients (See Also Chapter 307)

The importance of HCMV infection in the outcomes of HSCT recipients has long been recognized.[14] HCMV infection in the early posttransplantation period has been associated with a decrease in survival, including decreased survival unrelated to relapse of the underlying malignancy following transplantation for treatment of leukemias and lymphomas.[338,339,385,386] With very intensive monitoring, HCMV reactivation occurs in about 40% to 50% of all recipients of HSCTs, depending on the risk factor for HCMV reactivation or acquisition of HCMV. In contrast to SOT recipients, among whom the high-risk group for HCMV infection and disease is defined as seronegative recipients of a donor-seropositive organ (D+/R−), this combination has been defined as an intermediate-risk group in HSCT recipients (see Table 137.2).[387,388] The remarkable difference between HSCT and SOT allograft recipients was illustrated by comparison of the incidence of viremia in D+/R− HSCT recipients (3.7%) and D+/R− SOT recipients (78%) when results in a single center were analyzed.[388] A potential explanation for the difference between HSCT and SOT recipients is that in HSCT D+/R− transplant recipients, the efficiency of HCMV transmission appears to be low and has been correlated with the total number of nucleated cells in the allograft.[389] Thus the HCMV serostatus of the recipient is a major determinant of the risk of HCMV reactivation and disease in HSCT recipients. Additional characteristics of the donor and recipients have been described that identify HSCTs with an increased risk for HCMV infection and disease. These include (1) a related donor with one HLA-A, -B, or -DR mismatch or an unrelated donor with one HLA-A, -B, -C, or -DR mismatch; (2) use of T-lymphocyte–depleting strategies, including antithymocyte globulin or alemtuzumab; (3) umbilical cord stem cell HSCT in seropositive recipients; (4) HLA-haploidentical HSCT;

and (5) graft-versus-host disease requiring steroid therapy (see Table 137.2).[390–394]

Clinical expression of HCMV infections in HSCT recipients can range from asymptomatic virus shedding to end-organ disease and death, particularly in patients with HCMV pneumonia. Thus the differentiation between patients with HCMV reactivation and asymptomatic shedding versus those patients who will progress to end-organ disease cannot be dependent on clinical assessments and can only be accomplished utilizing algorithms that incorporate frequent virologic monitoring that includes quantitative measures of virus replication. Overall, in HSCT recipients, HCMV shedding/infection occurs in over 50% of patients at risk for HCMV reactivation in the early posttransplantation period, with a median onset between 40 and 60 days posttransplantation.[339,386–389] End-organ disease in these patients included a spectrum of manifestations similar to that observed in SOT recipients, with gastrointestinal disease being most frequently described. In contrast to SOT recipients, HCMV pneumonia remains a significant source of morbidity and mortality in HSCT recipients. HCMV pneumonia has been reported to account for about 30% of total cases of end-organ disease in HSCT recipients; however, as compared to disease in the gastrointestinal tract, HCMV pneumonia is accompanied by higher rates of morbidity and mortality, with a fatal outcome reported in nearly 50% of some patient populations.[140,395,396] Although this case fatality rate remains unacceptably high, it must be contrasted with mortality rates from HCMV pneumonia of nearly 100% prior to the introduction of virologic monitoring and preemptive antiviral therapy.[141,397,398]

With improved control of HCMV infections in the early posttransplantation period provided by effective antiviral therapies, late-onset HCMV infection has become increasingly important and accounts for significant morbidity and mortality in HSCT recipients. Although HCMV infections late after transplantation are relatively infrequent (on the order of 5%), these infections are associated with decreased overall survival in this population, and mortality rates of nearly 50% have been described in some centers.[140,387,399–401] Clinical manifestations of late-onset disease can include end-organ dysfunction that has been catalogued for HCMV infections early after transplantation and similarly appears to be related to deficits in adaptive immunity, specifically HCMV-specific T-lymphocyte responses, as has been reported early after HSCT.[402] Attempts to monitor reconstitution of HCMV-specific T-lymphocyte responses utilizing class I MHC tetramer assays and interferon responses following ex vivo stimulation have been reported.[139,301,403–408] Although these reports suggested potential utility of this strategy in monitoring reconstitution of HCMV-specific T-lymphocyte responses, there has been only limited introduction of these approaches into posttransplantation monitoring of HSCT recipients. Finally, there are reports that late-onset fungal infections are associated with HCMV reactivation in the early posttransplantation period.[409]

Congenital HCMV Infection

HCMV is the most common cause of congenital (present at birth) viral infection. The prevalence of this intrauterine infection ranges from 6 in 1000 in the United States to over 10 in 1000 in South America, Asia, and Africa.[195,202–205] Although a comprehensive discussion of the natural history of this important perinatal infection is beyond the scope of this chapter, a brief description of characteristics of maternal HCMV infections during pregnancy and the outcomes of pregnancy can serve to highlight significant aspects of this perinatal infection.

Similar to some other perinatal infections such as rubella and toxoplasmosis, HCMV can cross the placenta during pregnancy and establish an intrauterine infection. Natural history studies in urban populations in the Southeastern United States defined many of the salient features of congenital HCMV infections. In these populations, maternal HCMV infections were categorized as primary infections during pregnancy (*de novo* appearance of HCMV-specific immunoglobulin G [IgG] during pregnancy) or recurrent infections in women with preconceptional HCMV-specific IgG antibodies (nonprimary) that have been argued to result from reactivation of HCMV in persistently infected women. Although almost all maternal HCMV infections during pregnancy are subclinical, extensive natural history studies that prospectively monitored women during pregnancy determined that intrauterine transmission occurs in about 30% of women undergoing primary HCMV infection during pregnancy.[204,410,411] This rate of transmission has been confirmed in several maternal populations, although underlying deficits in maternal immunity, such as those associated with HIV/AIDS infections, can be associated with increased rates of intrauterine HCMV transmission.[412–414] The rate of intrauterine transmission associated with nonprimary infection is unknown but commonly has been suggested to be on the order of 1% to 2%, although this number represents an extrapolation of data from natural history studies and not from prospective studies.[415] From these early studies, it was proposed that the most severely affected infants with congenital HCMV infection were born to women with primary infection during pregnancy.[416] In addition, the intrauterine transmission rate of HCMV was proposed to be about 20- to 30-fold more frequent in women with primary infection during pregnancy as compared to women with nonprimary infections. Thus the paradigm that preconceptional maternal immunity to HCMV can limit intrauterine transmission and also modify the severity of the intrauterine infection was established, although a number of more contemporary observations have challenged this long-held but unverified concept.[415,417]

The severity of the intrauterine infection, and more importantly the degree of CNS damage, associated with congenital HCMV infection have been correlated with neurodevelopmental status of the fetus such that infections in the late first trimester or early second trimester are more commonly associated with more severe infection and CNS damage. However, this observation has also been described in congenital infection following maternal rubella or toxoplasma infections.[418–420] From existing natural history studies, about 10% to 15% of congenitally infected infants will present in the newborn period with some evidence of congenital infection and be classified as having a symptomatic infection. This identification of infants with symptomatic congenital HCMV infection is of clinical importance because it allows stratification of congenitally infected infants in terms of their risk for long-term neurodevelopment sequelae. Some 30% will have long-term neurologic sequelae as a result of infection during neurodevelopment.[410,421] Infants with evidence of congenital HCMV infections (symptomatic infection) can exhibit characteristic findings initially described in infants with cytomegalic inclusion disease. These include micrencephaly, jaundice, hepatosplenomegaly, petechial rashes, evidence of neurologic disease (e.g., seizures or abnormal tone and posture), chorioretinitis, and, rarely, evidence of extramedullary hematopoiesis.[422,423] More recently, investigators have also included evidence of intrauterine growth restriction as a clinically apparent manifestation of symptomatic congenital HCMV infection.

Laboratory abnormalities can include elevated liver transaminases and elevated bilirubin, anemia, and thrombocytopenia.[410,421] Cerebrospinal fluid (CSF) pleocytosis has been described in infants with symptomatic congenital HCMV infections and more commonly, CNS involvement has been diagnosed by neuroimaging. Case series have described intracranial calcifications and altered brain development such as cerebellar hypoplasia, and deficits in cortical migration, including pachygyria and even lissencephaly.[424–430] It should be noted that such severely affected infants represent only about 30% of the cases of symptomatic congenital HCMV infections and less than 5% of all cases of infants with congenital HCMV infection.[421] Prior to the availability of antiviral therapies, the mortality rate in the newborn period was approximately 10% in infants with severe symptomatic congenital HCMV infections.[431] Well over 90% of infants with congenital HCMV infections will not exhibit clinical findings in the newborn that can be attributed to this infection, and thus can only be identified if a newborn screening program is in place that can detect HCMV shedding in the newborn period. Long-term neurodevelopmental sequelae are a hallmark of congenital HCMV infections. Sequelae resulting from damage to other end-organ systems, such as the liver, are rarely observed.

Hearing loss is the most frequently identified sequela.[432–439] It has been estimated that hearing loss associated with congenital HCMV infections may account for approximately 25% of all cases of hearing loss in children.[440] An important characteristic of hearing loss associated with congenital HCMV infection is that only about 50% of infected infants will have hearing loss in the newborn period, and therefore only a fraction of infected infants at risk for hearing loss will be identified during newborn auditory screening programs. The remaining 50% can

develop hearing loss during childhood, and existing hearing loss may progress during this time.[441] Neurodevelopmental delays have been well documented in infants with symptomatic congenital HCMV infections, particularly those with CNS structural abnormalities, but several studies have failed to demonstrate neurodevelopmental delays in infants with asymptomatic infections.[442–445]

In dramatic contrast to the protection from congenital infection that is afforded by preconceptional immunity to rubella and toxoplasmosis, maternal immunity to HCMV that is present prior to pregnancy does not prevent transmission of the virus to the fetus or symptomatic infection in the newborn. This observation was made over 3 decades ago and has been confirmed in a number of different studies and maternal populations.[203,416,446–455] As was previously noted, there remains an ongoing debate about the level of protection provided by preexisting maternal immunity both in terms of protection from transmission and prevention of severe symptomatic infections.[415,417] However, several unique observations in the natural history of congenital HCMV infections suggest that it will be difficult to quantify the protection provided by maternal immunity, because this effect could be unique to a maternal population. As an example, the incidence of congenital HCMV infection increases with increasing maternal seroprevalence, such that maternal populations with the highest seroprevalence have higher rates of congenital HCMV infection in their offspring.[456] It is of considerable interest that in contrast to other perinatal virus infections, such as rubella, the rate of congenital HCMV infection does not reach a plateau when plotted against the maternal HCMV seroprevalence. This finding, when modeled, suggests that the incidence of congenital HCMV infections that result from primary maternal infections will never approach the number of congenital HCMV infections that follow nonprimary infections.[457] Furthermore, it is well documented that symptomatic congenital HCMV infections can follow nonprimary infections, further demonstrating that not only is maternal immunity incompletely protective in terms of intrauterine transmission, but it may also not prevent the development of symptomatic congenital infections.[68,70,203,435,458,459] This potential shift in the paradigm that maternal immunity is protective and can prevent significant intrauterine infections and long-term sequelae has implications that HCMV vaccines might be able to prevent the sequelae of congenital HCMV infection. Lastly, the source of nonprimary maternal infections has long been believed to be the reactivation of a persistent infection or a recurrence. However, with the availability of newer technologies coupled with findings from basic studies of HCMV, investigators have been able to demonstrate that immunocompetent adults with immunity to HCMV, including pregnant women, can be reinfected with new strains of HCMV that differ both antigenically and genetically from strains of virus in the persistently infected host.[68–72,460] Thus the current understanding of the relationship between maternal immunity and the outcome of maternal infections with HCMV during pregnancy, whether congenital HCMV infection follows primary or nonprimary maternal infections, continues to be incomplete.

DIAGNOSIS OF HCMV INFECTIONS

Although HCMV-specific serology and, in some centers, isolation of HCMV remain important components in the diagnosis of HCMV infection, viral antigen detection and nucleic acid amplification technologies (NAATs) have replaced these older methodologies and now represent the mainstay of diagnostic approaches for detection of HCMV in clinical specimens (Table 137.4). A major hurdle to the optimization of care of allograft transplant recipients and other immunocompromised patients has been the standardization of both NAATs and antigen detection between transplant centers. When the variability in quantitation of diagnostic assays is combined with the inherent variability of patient populations and individual center-specific protocols for management of transplant recipients, the translation of findings from different centers into coherent management strategies has been difficult. More recently, several steps have been taken to remedy this issue, including standardization of results from NAATs and reporting quantified results from antigen detection assays. These steps have provided a foundation for integration of results from different transplant centers, and thus provide a larger number of evaluable patients for comparative analysis of outcome data, as discussed in the following sections.

TABLE 137.4 Diagnostic Approaches for Detection of HCMV Infections

ASSAY	APPLICATIONS	PERFORMANCE CHARACTERISTICS
Nucleic Acid Detection		
In situ hybridization	Detection of HCMV nucleic acids in tissue specimens	Sensitive detection of HCMV in tissues specimens Technically demanding
Nucleic acid amplification technologies	Quantitative determination of HCMV nucleic acids in blood, body fluids, and tissue specimens	Standardized assays Can quantify HCMV genomes in tissues and body fluids Rapid turnaround Can be used to guide therapy and detect development of antiviral resistance
Antigen Detection		
Immunological detection of immediate-early antigen	Rapid detection of HCMV in assays of virus infectivity	Increased sensitivity of detection of infectious HCMV in clinical specimens Requires tissue culture facilities
Immunohistochemistry	Detection of HCMV in tissue specimens	Increased sensitivity of detection of HCMV in tissue specimens Only semiquantitative
Antigenemia	Quantitative determination of HCMV in blood	Can be used in preemptive treatment protocols and for monitoring treatment Requires technical expertise Limited value in patients with leukopenia
Virus isolation	Determination of virus shedding or presence of virus in tissue	Sensitivity dependent on quality of specimen and viral load in specimen Requires tissue culture facilities Prolonged period of culture can be necessary (up to 28 days)
Serology		
Virus-specific immunoglobulin G (IgG)	Determination if infected with HCMV; detection of seroconversion	Standardized assays High positive and negative predictive values in immunocompetent hosts
Virus-specific immunoglobulin M (IgM)	Detection of recent HCMV infection	Standardized assays HCMV-specific IgM can persist for weeks to months, limiting value in assignment of timing of acquisition of infection
Virus-specific IgG avidity	Estimation of interval from IgG seroconversion	Useful to estimate timing of infection in immunocompetent hosts Value uncertain in allograft recipients
Histopathology	Detection of HCMV in tissues	Characteristic findings Insensitive in comparison to immunohistochemistry and nucleic acid amplification technologies

Quantitative Nucleic Acid Amplification Technologies

Quantitative NAATs have become the methodology of choice for the detection and quantitation of HCMV in specimens from both normal and immunocompromised individuals. In general, NAATs for HCMV detection are primarily quantitative PCR (qPCR) amplification of DNA using different primer sets. NAATs-based assays that are based on the amplification of HCMV RNA have also been utilized in monitoring both SOT and HSCT recipients.[461–464] The detection of HCMV RNA was proposed to be a more relevant measure of HCMV replication than assays employing qPCR amplification of blood because several studies demonstrated that HCMV DNA can be amplified from leukocytes obtained from normal, immunocompetent individuals with past HCMV infection secondary to the presence of latently infected myeloid cells. This finding raised the possibility that results from PCR amplification of HCMV DNA in whole blood would fail to quantify the level of virus replication.[465,466] Although this concern is well founded, practical experience with qPCR-based assays of whole blood or plasma have been successfully employed to quantify virus replication, primarily through serial measurements. Thus qPCR amplification of HCMV DNA has largely replaced assays of HCMV RNA and qualitative PCR assays for the detection of HCMV in transplant recipients.

Multiple formats for qPCR quantification of HCMV DNA have been developed, and multiple protocols for DNA extraction and preparation of PCR analysis have been reported. As could have been anticipated from such laboratory-specific approaches, the major hurdle for implementation and interpretation of screening and monitoring strategies utilizing qPCR has been the lack of standardization of sample preparation and PCR assays, and perhaps most importantly, the standardization of the quantity of HCMV DNA detected in each assay format.[467–469] Harmonization of sample preparation and of qPCR assays represents a significant challenge, since the preparation of tissue specimens for PCR analysis in many laboratories is generic and not specific to a single infectious agent. Similarly, the experience of the laboratory with different PCR formats will dictate the preference of the laboratory for a particular qPCR assay. However, a very simple solution was provided by supplying reference standards for HCMV DNA that allowed calibration of each assay system and thus enabled comparison of results from different laboratories.[470,471] Utilizing these calibration standards, interlaboratory variability could be controlled.[469,472] Lastly, even with HCMV DNA calibration standards, the variability of results among different formats, particularly different commercial systems, can be observed in the extremes of the performance characteristics of each assay.[469] Thus until PCR protocols are standardized to produce similar performance in characteristics, such as the limit of detection of input HCMV DNA, seamless integration of qPCR findings from different transplant centers will remain problematic.

A second issue surrounding a universally accepted NAATs-based approach for detection and monitoring HCMV infections in immunocompromised hosts is the selection of a blood component for assay. There is little debate about the value of other fluids, such as BAL fluid, CSF, urine, or tissue, but the choice between using whole blood or plasma for the detection of HCMV by qPCR remains unsettled. Multiple laboratories have reported that assays using whole blood are more sensitive than those using plasma, although there is good concordance between the results from whole blood and plasma.[473–477] Lastly, simplicity of sample preparation favors the use of whole blood, since an accurate amount of sample can be processed prior to an additional centrifugation step required to prepare plasma.

Antigen Detection

The generation and characterization of HCMV-specific monoclonal antibodies led quickly to the development of assays for detection of HCMV antigens. These early assays became the standard approach for rapid detection of HCMV in SOT and HSCT recipients, until the widespread introduction of NAATs. Many of these assays continue to be used in diagnostic laboratories and in the management of HCMV infections in allograft recipients, particularly in situations that require the identification of HCMV in body fluids and tissue specimens (see Table 137.4). One of the initial assays utilized monoclonal antibodies to identify the protein product of a viral immediate-early gene (*IE-1*) that is expressed within hours after infectious virus is seeded onto permissive human primary cell monolayers. This assay has been further modified by a low-speed centrifugation–enhanced inoculation to increase the efficiency of infection and therefore the sensitivity of the assay. Specimens of urine, saliva, BAL fluid, genital secretions, and, rarely, minced tissue have been assayed using this approach. The assay is relatively rapid, requiring about 12 to 16 hours from receipt of specimen to reporting of results; has minimal interobserver error in its interpretation; and compares favorably in sensitivity to tissue culture isolation of HCMV. Older studies have suggested that this assay may more be sensitive than standard tissue culture methods for detection of HCMV, although similar comparative studies were not performed by other laboratories.[478] However, this assay is labor intensive and less amenable to high-throughput screening unless adapted to microtiter plate formats, requires tissue culture facilities, and requires relatively expensive instrumentation depending on the method for detection of the HCMV monoclonal antibody. Additional applications for HCMV antigen detection include use of HCMV-specific antibodies in immunohistochemistry protocols to detect HCMV in tissue sections, both frozen and paraffin-embedded tissue (Fig. 137.3). These assays can complement NAATs approaches to demonstrate tissue-invasive HCMV infection and importantly can provide direct evidence of end-organ infection and disease. In contrast to NAATs-based assays for detection of HCMV in tissue, antigen detection assays are extremely labor intensive and require technical expertise to optimize the assay for each tissue and fixation condition.

In the early 1990s, the antigenemia assay was introduced as an approach for monitoring HCMV in the blood of immunocompromised patients. This assay was based on the detection of the HCMV tegument protein pp65 (ppUL83) in circulating leukocytes, primarily polymorphonuclear leukocytes (PMNs).[479,480] Detection of HCMV infection in patients is based on the detection of this viral protein in samples of peripheral blood that have been enriched for PMNs and spotted onto glass slides. The slides are processed using an anti-HCMV pp65 antibody followed by detection of the pp65 protein within PMNs. The assay is technically straightforward, and has a rapid turnaround time as compared to culture or even IE-1 detection in cell monolayers inoculated with HCMV, and the results can be quantitatively expressed as the number of HCMV-positive cells per 50,000 to 200,000 PMNs. Quantitation of the results from this assay has been and continues to be used in some transplant centers to guide institution of preemptive antiviral therapy,

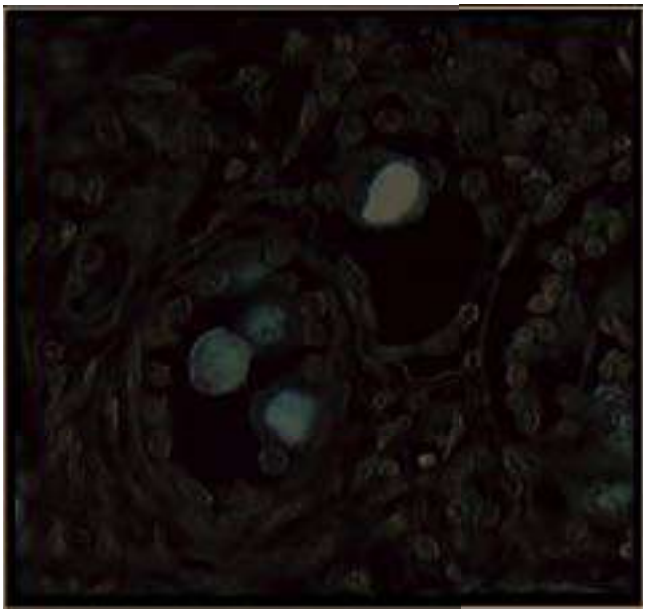

FIG. 137.3 Immunohistochemical detection of HCMV infected cells. Viral nuclear antigen (immediate-early gene *IE-1*) present in renal epithelium is indicated by brown-stained nuclei (magnification ×33).

to detect HCMV infection after discontinuation of prophylaxis, and to monitor the response to antiviral therapy. The clinical utility of this assay compared favorably to early-generation NAATs-based assays in monitoring HCMV infections in SOT and HSCT recipients, perhaps because results from the antigenemia assay offered a high positive predictive value of clinically significant levels of HCMV replication in these populations.[481–485] However, the assay also suffered several drawbacks, such as the requirement to have sufficient numbers of circulating leukocytes for valid test results, inherent difficulties in standardizing results across different centers secondary to limited dynamic range of the assay, operator dependence, and inability to process large numbers of samples concurrently; also, in some centers the antigenemia assay had only a moderate negative predictive value.[484,486–489] Because of these limitations, most large-volume transplant centers have transitioned to newer-generation quantitative NAATs-based assays for monitoring HCMV infections in both SOT and HSCT recipients. However, it must be noted that comparative studies within single institutions have provided evidence for the concordance of findings from the antigenemia assay and NAATs.[395,490]

Virus Culture

Isolation of replicating HCMV from tissue specimens or blood requires significant time and effort as well as facilities for maintaining primary human cells (see Table 137.4). As a result, this approach to the diagnosis of HCMV is utilized primarily in centers with dedicated diagnostic virology laboratories. HCMV grows slowly, and primary isolates are highly cell associated and require prolonged culture. A culture must be held for 4 weeks before it is considered negative. Identification of infected cells utilizes recognition of characteristic cytopathology induced by HCMV and is usually verified by either antigen detection within cells from the infected culture or NAATs-based assays of the culture. Recovery of infectious virus from tissue specimens can also be challenging, secondary to microbial contamination of the specimen and toxicity of tissue homogenates. In most cases, tissue specimens are subjected to antigen detection technologies such as immunohistochemistry or NAATs.

Serology

Serologic demonstration of previous and current HCMV infection is of value, particularly in identifying previously infected individuals as part of pretransplantation screening of donors and recipients (see Table 137.4). Several formats for detection of HCMV IgG antibodies are available commercially. Although serial samples can demonstrate either *de novo* IgG seroconversion or increasing antibody titers in acutely infected individuals, in most cases laboratories must rely on avidity determinations to estimate the chronicity of an HCMV infection. These assays utilize avidity maturation of HCMV-specific IgG antibodies to estimate the duration of an infection. Low-avidity antibodies that are bound to an immobilized antigen can be readily released by exposure to a low concentration of urea or to a chaotrope such as guanidine. These antibodies have been shown to develop early in infection, whereas high-avidity antibodies that require higher concentrations of the chaotrope to be released from the antigen are found later in infection following maturation of the antibody response. In most individuals, this follows a relatively predictable time course that can then be extrapolated to estimate the chronicity of the infection. These assays are widely used in serologic studies of pregnant women who also have HCMV-specific immunoglobulin M antibodies as an approach to estimate the chronicity of HCMV infections during pregnancy.[491–495] The detection of higher-avidity HCMV-specific IgG antibodies following allograft transplantation has been described, but its value in management of HCMV infections in either SOT or HSCT recipients has not been defined.[496]

Diagnosis of Congenital Infections

The diagnosis of congenital HCMV infections contrasts with the diagnosis of HCMV infections in immunocompromised patients, primarily because it is qualitative. Although quantitation of the level of infection (viral load) has been correlated with severity of infection and long-term outcome, the wide variance in outcome as a function of viral load has limited the clinical value of quantitative results from NAATs in the management of congenitally infected infants.[410,497–500] Diagnosis of congenital HCMV requires the demonstration of HCMV or HCMV DNA in urine, saliva, or blood from an infant within the first 2 weeks of life by isolation of replicating virus, detection of viral antigens, or NAATs. Detection of HCMV DNA or HCMV after 4 weeks of life cannot be unequivocally defined as congenital (present at birth) HCMV infection. Newborn infants can excrete HCMV following exposure to genital tract secretions containing HCMV during vaginal delivery and from ingestion of breast milk from a seropositive mother. Contemporary diagnostic approaches include qPCR analysis of saliva and urine, because large amounts of HCMV can be found in these fluids in congenitally infected infants.[202,501–504] Finally, fetal infection can be diagnosed by characteristic findings of imaging studies, including ultrasound and magnetic resonance imaging, and by the detection of HCMV in amniotic fluid, usually by NAATs.[505,506] Importantly, false-negative results have been reported in amniotic fluid when testing takes place before 17 weeks of gestation, or too early (<7 weeks) following maternal seroconversion.[505–509] Finally, in contrast to the limited clinical value of quantitation of viral load in congenitally infected infants, investigators have suggested that quantitation of the viral load in amniotic fluid can be correlated with the severity of the fetal infection, and thus with the potential long-term damage associated with this intrauterine infection.[510–512]

THERAPY OF HCMV INFECTIONS (see also Chapter 46)

Antiviral drugs with activity against HCMV have been available for nearly 3 decades, yet the treatment of clinically significant infections caused by this virus remains challenging. This is secondary to the modest efficacy of available drugs and to their toxicity, which limits their use in specific patient populations and often requires dose modifications that further limit their activity. In addition, clinically significant resistance to single agents can develop during treatment. Currently licensed agents include (1) ganciclovir (GCV), a deoxyguanosine nucleoside analogue that can inhibit the viral DNA polymerase and also function as a chain terminator; (2) foscarnet, a pyrophosphate analogue that directly inhibits the viral DNA polymerase; (3) cidofovir, a cytidine deoxynucleoside analogue that also inhibits the viral DNA polymerase; and (4) letermovir, the newest antiviral for HCMV, an agent that inhibits function of UL51 and UL56 of the HCMV terminase complex (UL51, UL56, and UL89). Additional agents include maribavir, an inhibitor of UL97, and brincidofovir, an analogue of cidofovir that has less renal toxicity; both are currently in late clinical development.

GCV and valganciclovir, its L-valyl prodrug with improved oral bioavailability, are currently the first-line agents for treatment and prophylaxis of HCMV infections. GCV requires phosphorylation by the HCMV-encoded kinase UL97 as an initial modification, followed by additional phosphorylations by host kinases to produce the triphosphate form of GCV that functions as an inhibitor of the viral DNA polymerase and DNA polymerization.[513–515] The toxicity of GCV is almost exclusively bone marrow suppression, resulting in reversible neutropenia and less often thrombocytopenia. Because it is not metabolized to any extent and is excreted almost unchanged into the urine, dosing can depend on renal function. Foscarnet is an inhibitor of many herpesviruses and is often used when myelosuppression limits GCV use or GCV resistance develops during treatment, or both. This agent has been shown to provide clinical benefit in immunocompromised patients, including patients with HIV/AIDS and HCMV end-organ disease, and in HSCT recipients requiring preemptive therapy for HCMV infections.[397,516,517] It is excreted almost unchanged into the urine, and its major toxicity is reversible renal impairment.[517] Cidofovir has been shown to be a potent inhibitor of several different viral polymerases other than HCMV, including poxviruses, polyomaviruses, and adenoviruses. Its clinical use is limited by significant nephrotoxicity that can result in irreversible acute kidney injury.[518–522] The most recently licensed agent, letermovir, has recently been shown to be useful in the prophylaxis of high-risk HSCT recipients, since myelosuppression has not been associated with its use.[394] Its use in SOT recipients remains to be demonstrated, but it appears to have limited significant toxicity at least in these initial trials, suggesting that it may have more widespread indications.

Maribavir has been shown to directly inhibit the HCMV viral kinase UL97, and it has been shown to inhibit critical steps in virion

morphogenesis and, potentially, in the function of the viral polymerase.[523–526] Extensive preclinical studies led many investigators to suggest that this agent would be active in treatment of HCMV infections. This enthusiasm was further supported by the demonstration of its activity in a limited trial in HSCT recipients.[527] However, a pivotal clinical trial failed to show that maribavir improved outcome of HSCT recipients over placebo.[528] Importantly, there remains some controversy over the end points that were utilized to estimate the clinical benefit provided by maribavir in this later trial and in a subsequent trial that suggested some benefit.[529,530] Lastly, brincidofovir is an oral analogue of cidofovir that has been shown to have significantly less nephrotoxicity than the parent drug, although case reports of brincidofovir-associated acute kidney injury have been reported.[531,532,533] In a small trial, this agent was shown to be active in suppression of HCMV infection in HSCT recipients; however, treatment with this agent was associated with diarrhea that in some cases was dose limiting.[532]

Prophylaxis and Preemption

The treatment of HCMV infections with antiviral agents has been most well studied in allograft recipients, although there is an extensive literature on the use of antivirals to treat HCMV infection in HIV/AIDS patients prior to the extensive use of combination antiretroviral therapies. Two approaches have been described in transplant recipients: preemptive therapy and prophylaxis. Prophylaxis protocols often incorporate a preemptive strategy after discontinuation of prophylaxis. The selection of one of these approaches over the other is often dictated by the preference and experience of the transplant center, the type of transplantation, and the risk for HCMV infection and disease in the allograft recipient. Preemptive treatment of HCMV infection was first deployed in HSCT recipients and utilized serial quantitative measures of HCMV in various body fluids as early indicators of HCMV infection. Results from virologic monitoring were combined with predetermined criteria for significant levels of HCMV, and if indicated, antiviral therapies were initiated until resolution of the HCMV infection, or significant reduction in the quantity of virus. This approach evolved from early studies that demonstrated that monitoring HSCT recipients for HCMV shedding in the posttransplantation period, followed by GCV treatment and prophylaxis until day 100 posttransplantation, significantly reduced the incidence of HCMV pneumonia and improved overall outcome.[534,535] The initial preemptive therapy approaches were followed by protocols incorporating the presence of antigenemia as an indication for initiating GCV treatment.[536]

Of interest is that the results from these very early studies could not definitively demonstrate a clear advantage of antiviral prophylaxis or preemptive therapy in patient outcome, since each approach had unique advantages as well as limitations. Even today, there continues to be discussion as to which is the most effective approach. In SOT recipients, reports of studies in renal, liver, and heart allograft recipients argue that universal prophylaxis results in more favorable outcomes, whereas other studies have suggested that outcomes in patients managed by preemptive strategies are similar to those in patients given prophylaxis.[537–539,540–545] In contrast to SOT recipients, prophylaxis with GCV in HSCT recipients is associated with delayed engraftment, and some 20% to 30% of patients who received GCV prophylaxis in the first 100 days posttransplantation developed neutropenia.[546] An increased risk of bacterial and fungal infections is observed in patients with prolonged cytopenias secondary to delayed engraftment and neutropenia.[536,547] Thus universal prophylaxis in HSCT recipients utilizing a myelosuppressive agent such as GCV cannot be viewed similarly to that in SOT recipients, in whom reversible neutropenia can be managed by dose adjustments. Perhaps the availability of an effective antiviral such as letermovir that can be used in prophylactic protocols in HSCT recipients will lead to hybrid prophylaxis–preemptive treatment strategies in these patients.

Two major disadvantages of universal prophylaxis strategies that continue to invite discussion are (1) development of late-onset disease secondary to suppression of antigen stimulation during reconstitution of T-lymphocyte responses, and (2) the selection of antiviral drug–resistant viruses. The longstanding controversy surrounding the impact of early suppression or treatment of HCMV infections and the risk of late-onset disease has not been resolved. Similarly, concerns regarding the development and selection of antiviral drug–resistant viruses by widespread use of antiviral drugs as prophylaxis for HCMV infections in allograft recipients are valid. There are numerous reports of the development of clinically significant resistance in HCMV in patients treated with antivirals. Reports from studies in SOT recipients have described rates of GCV resistance ranging from approximately 5% to 30% in patients treated with GCV in the posttransplantation period.[548–551,552] Several risk factors were noted for development of GCV resistance. These include higher viral loads; high-risk (D+/R–) SOT recipients, including those receiving multivisceral allografts; and interestingly, suboptimal dosing of GCV prophylaxis.[548,550–552] A study from a reference laboratory reported that of over 500 submitted specimens, some 30% had mutations in UL97 or UL54 that have been associated with antiviral resistance.[553] Antiviral resistance has also been well described in HSCT recipients, although perhaps not as frequently as in SOT recipients, because of the less frequent use of GCV prophylaxis in these patients.[554,555,556,557]

Viral Resistance

Identification of antiviral drug–resistant viruses traditionally employed the demonstration of a resistant phenotype, that is, reduction in the yield of infectious virus in an in vitro assay in which varying concentrations of antiviral drug are added to the culture. The results are generally reported as the effective concentration of a drug at which a 50% decrease in virus production is observed. Such assays are of limited value for clinical use because (1) adaptation of a potential virus swarm to in vitro propagation may occur, (2) weeks of virus propagation are required to perform the assay, (3) there may be an inability to capture resistance patterns of minor populations, and (4) there are difficulties in standardization of assays between laboratories. These assays have been replaced with genotyping assays, which can rapidly identify mutations in the targets of antiviral drugs that confer resistance. These assays rely on PCR amplification of HCMV genes targeted by antivirals such as viral kinase UL97 (confers GCV resistance), viral DNA polymerase UL54 (confers resistance to GCV, foscarnet, cidofovir, and brincidofovir), and the terminase complex (UL51, UL56, UL89), and on Sanger sequencing of the products to identify mutations that have been associated with drug resistance.[558,559,560] Next-generation sequencing of these products can be very informative and can identify minor populations of variant sequences associated with drug resistance, although the clinical relevance of minor virus populations remains incompletely defined.[561–563] Clinical observations have also demonstrated the importance of antiviral resistance testing of relevant patient specimens, since compartmentalization of antiviral resistance has been shown for specimens from one compartment (aqueous fluid of eye or CSF) but not another (blood).[564,565]

Therapy of Congenital Infections

Treatment of congenital HCMV infections with currently licensed antiviral agents cannot be accomplished in utero, a time point in the infection that would likely provide the greatest benefit because of the assumed toxicity of these agents on the developing fetus. However, there are scattered case reports describing administration of GCV to pregnant women as a treatment for fetal HCMV infection.[566] Thus the current understanding of the potential role of these agents in modifying the natural history of congenital HCMV infections has been derived from studies of exclusively GCV and its oral analogue, valganciclovir, in the treatment of newborns with clinical stigmata of congenital HCMV infections. In two large trials, one utilizing parentally administered GCV and the second utilizing oral valganciclovir, treatment of newborn infants with symptomatic congenital HCMV infections demonstrated that both approaches could suppress virus replication and limit end-organ dysfunction, and were generally well tolerated except for dose-limiting myelosuppression, which required dose reductions.[567,568] Outcomes from these initial trials were encouraging, since the trials demonstrated a reduction in the number of infants with hearing loss compared to historical controls.[567] In the second study, infants were treated with oral valganciclovir for a 6-month period, and although the primary end point of differences in hearing loss at 6 months was not met, overall there were benefits from GCV treatment during limited follow-up.[568] Together, results from the first and the second trial suggested an improvement

in long-term hearing outcomes of treated infants with symptomatic congenital HCMV infections, particularly in the maintenance of functional hearing.[568] Other trials using GCV treatment of infants with symptomatic congenital HCMV infections with different durations of therapy but all beginning therapy in the newborn period also demonstrated a benefit of treatment.[569,570] A current trial is underway that will utilize the second strategy of treatment with oral valganciclovir of infants with asymptomatic congenital HCMV infections. A number of case reports and small series have described the outcome of GCV treatment of infants with congenital HCMV infections, including infants with hearing losses associated with congenital HCMV infections. Data from these reports are encouraging, but without well-controlled studies with adequate follow-up it will be difficult to determine the value of GCV treatment in infants with asymptomatic congenital HCMV infections, since only 15% could potentially benefit from antiviral therapy, and the remaining 85% will have a low risk of exhibiting neurodevelopmental sequelae from this intrauterine infection. Lastly, about 20% of infants will develop neutropenia that will require dose modifications during treatment, and the long-term consequences of treatment with this nucleoside analogue that can be incorporated in host DNA must be weighed against presumed benefits of treatment.

Passive Immunotherapies for the Prevention and Treatment of HCMV Infections

The development of immunoprophylaxis and treatment of HCMV infection in immunocompromised patients, including fetuses infected in utero, has been an active area of research for over 2 decades. Early studies of HCMV demonstrated the capacity of anti-HCMV antibodies to neutralize infectious virus in in vitro assays, and small animal models of HCMV infections also demonstrated the in vivo protective activity of virus-neutralizing antibodies. With the development of technologies to isolate human immunoglobulins from donor plasma and to formulate preparations for intravenous infusion, there was great interest in these pooled blood products for the prophylaxis and treatment of infectious diseases. CMV hyperimmune globulins (CMV-IGs) were prepared from donor plasma screened to contain high titers of anti-HCMV antibodies and not from immunized donors. In addition, donor pools were identified using antigen-binding assays that had been described to correlate with in vivo protection but were not selected based on activities in virus neutralization assays. However, this donor selection strategy increased the relative HCMV-specific antibody-binding activity as well as the virus-neutralizing activity in these products. It is interesting that comparison of two commercially available products (Cytotect CP and Cytogam) revealed little difference in functional activity between the products.[571] Initial studies using high-titer anti-HCMV immunoglobulins provided evidence of the value to this approach in SOT recipients, as initially shown in renal and liver allograft recipients.[572–574] More recently, the therapeutic value of CMV-IG when combined with GCV has been shown in thoracic SOT and multivisceral SOT recipients.[575,576,577–580] In contrast to the utility of CMV-IG in both prophylaxis and treatment of SOT recipients, trials in allogeneic HSCT failed to provide equivocal evidence of benefit from intravenous immune globulins.[581] In agreement with older studies, more recent meta-analyses of studies of the value of CMV-IG in HSCT have suggested that this approach has little benefit for outcome following HSCT.[582,583]

Studies in small animal models of congenital HCMV infections provided evidence that serum from immune animals could prevent or ameliorate intrauterine infection with HCMV.[584,585] Thus it was proposed that HCMV antibodies transferred to pregnant women undergoing a primary HCMV infection could potentially modify the maternal infection and, following transplacental passage, achieve a therapeutic effect in the infected fetus. Nigro and colleagues reported that treatment of pregnant women undergoing a primary HCMV infection resulted in both a decreased transmission rate and improved outcomes.[586] This study was not controlled and included significant biases in the selection of women who would be treated. A subsequent study that in was controlled, used the same CMV-IG product, and avoided the biases present in the first study failed to demonstrate any reduction in mother-to-fetus transmission in women undergoing primary infection.[587] Although there were no significant differences in the outcomes of infected newborns born to treated and untreated women, there were more adverse outcomes in control, noninfected women treated with CMV-IG.[587] A current large, multicenter study sponsored by the National Institutes of Health will hopefully define the role of CMV-IG treatment in women undergoing primary HCMV infection during pregnancy.

In addition to CMV-IG, several human or humanized monoclonal antibodies have been studied in immunocompromised individuals. These are antibodies against the major virion envelope glycoproteins, including the pentamer complex (UL75, UL115, UL128, UL129, UL131) and glycoproteins gB and gH. Results from early studies in immunocompromised hosts were not encouraging, at least for one of the products.[588,589] More recently, very potent virus-neutralizing monoclonal antibodies directed at the pentamer complex and individual components of this viral envelope complex (gH, gL, UL128) and gB have been characterized, and at least a combination of two of these have been studied in early-phase clinical trials, which showed some beneficial effects on reduction of frequency of CMV-associated disease.[590]

Vaccines for the Prevention and Treatment of HCMV Infections

The search for prophylactic and therapeutic vaccines for HCMV infections has been ongoing for over 4 decades.[591] The first candidate vaccine that provided some evidence that vaccination against HCMV could modify the course of HCMV infections in SOT recipients was reported by Plotkin and colleagues.[592] This vaccine (Towne) was derived from a clinical isolate extensively passaged in tissue culture and described as attenuated, although at the time there was little understanding of determinants of viral virulence. The Towne vaccine protected vaccinated volunteers from low-dose wild-type, low-passage virus (clinical isolate) challenge, but when the challenge dose was increased only minimally, protection induced by the vaccine waned.[593] This vaccine has subsequently been tested in HCMV-seronegative women and failed to prevent virus acquisition in vaccinated women.[594] Several reasons have been proposed for the limited activity of this vaccine, but most argue that is overly attenuated and replicates poorly in vaccinated individuals. However, the development of replication-attenuated HCMV vaccines remains an important strategy in the quest for an efficacious vaccine.[595–598] More recently, a replication-defective HCMV that expresses a complete set of viral genes associated with in vivo pathogenicity is in advanced preclinical development.

In contrast to limited studies of replication-competent or replication-defective HCMV vaccine candidates, or both, subunit (viral protein) and DNA-based candidate vaccines have been evaluated in clinical trials. The most widely studied was an adjuvanted viral envelope glycoprotein (gB) vaccine. An initial evaluation of this candidate vaccine in seronegative women suggested transient efficacy in prevention of infection.[599] A follow-up study in normal adolescent females failed to provide evidence of prevention of infection as compared to placebo.[600] At best, the results from these studies demonstrated immunogenicity of the vaccine formulation, but limited evidence of protective immunity. Several explanations have been proposed for the limited protective activity of this candidate vaccine, including the use of a nonnative viral protein as an immunogen, the potentially transient nature of the protective responses, and the lack of a meaningful surrogate marker of protection in these populations that could be correlated with responses to this vaccine. The same formulation has also been studied in SOT recipients with perhaps more encouraging results.[601] In SOT (renal and liver) recipients, pretransplantation vaccination with the adjuvanted HCMV gB vaccine resulted in increased antibody titers to gB in both HCMV-seronegative and HCMV-seropositive recipients, and led to a decreased duration of HCMV viremia and use of GCV as compared to recipients of the placebo.[601] Further development of this candidate vaccine has not progressed, however. A second candidate vaccine based on DNA immunization has been tested in clinical trials. This candidate vaccine includes plasmid DNA encoding two immunogenic HCMV proteins, gB and pp65, and has been shown to be immunogenic in normal individuals and in HSCT recipients. Results from early-phase trials suggested some protective activity in HSCT.[602–604] Other candidate vaccines currently under study

include modified vaccinia Ankara virus–vectored HCMV glycoprotein-based and peptide-based vaccines.[605–607]

Vaccines that can modify the natural history of congenital HCMV infection and reduce morbidity and mortality, including long-term graft loss associated with HCMV infections, must be viewed as a priority for both government and industry support. Significant hurdles in our understanding of the biology of this virus remain, and it can be argued that the empiricism of previously successful approaches for vaccine development may not be applicable to HCMV. Perhaps this is most clearly reflected in the variability of definitions of protective efficacy that have been suggested in published studies. Hopefully, clinically meaningful end points of protection can be established for the evaluation of vaccines in each target population for future clinical trials, such as those recently described to aid in antiviral drug development and evaluation in transplantation patients.[313]

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138 Epstein-Barr Virus (Infectious Mononucleosis, Epstein-Barr Virus–Associated Malignant Diseases, and Other Diseases)

Eric C. Johannsen and Kenneth M. Kaye

SHORT VIEW SUMMARY

Definition
- Infectious mononucleosis is a clinical syndrome characterized by pharyngitis, fever, lymphadenopathy, and the presence of atypical lymphocytes on a peripheral blood smear. Primary Epstein-Barr virus (EBV) infection is the most common cause of this syndrome.

Virology and Epidemiology
- EBV is a herpesvirus that establishes lifelong latent infection in B lymphocytes.
- Replication occurs in oral epithelium, and infectious EBV is frequently present in the saliva of asymptomatic seropositive individuals.
- EBV is transmitted predominantly through exposure to infected saliva, frequently as a result of kissing.
- Seroprevalence approaches 95% in adults, and EBV is distributed throughout the world.
- In childhood primary EBV infection is usually asymptomatic or a nonspecific illness.
- Frequency of presentation as infectious mononucleosis increases with age to about 50% of primary infections by adolescence.
- EBV is tightly linked with several malignancies, including endemic Burkitt lymphoma, nasopharyngeal carcinoma, and lymphoproliferative disease (LPD).

Microbiology
- EBV is a gamma-1 herpesvirus, genus *Lymphocryptovirus*.
- EBV is a double-stranded DNA virus that is enveloped.
- EBV is also known as human herpesvirus 4.

Clinical Manifestations
- Infectious mononucleosis is generally a self-limited, spontaneously remitting syndrome.
- Complications may occur, including splenic rupture, neurologic manifestations such as encephalitis, autoimmune hemolytic anemia, and mild hepatocellular enzyme elevations.

Diagnosis
- The appearance of nonspecific, heterophile antibodies (immunoglobulin M [IgM] reacting with sheep or horse red blood cells) can distinguish primary EBV infection from other causes of infectious mononucleosis.
- The presence of IgM viral capsid antigen antibodies is closely correlated with acute EBV infection. Heterophile antibodies in a person with clinical infectious mononucleosis is sufficient to establish the diagnosis.
- EBV serology may be helpful in atypical cases and in children, who are frequently heterophile negative.
- Primary human immunodeficiency virus infection is the most important differential diagnostic consideration.
- Serial measurement of EBV viral loads may be useful in the detection of EBV-associated malignancies in immunosuppressed individuals, especially for LPD.

Therapy
- Treatment of mononucleosis is primarily supportive.
- Corticosteroids may be helpful in managing mononucleosis complications such as airway impingement from tonsillar enlargement.
- Antiviral therapy is of no proven benefit in infectious mononucleosis.
- EBV-associated LPD may be treated with decrease of immune suppression, rituximab, or adoptive immunotherapy, depending on the cause (see later).

Prevention
- There is currently no EBV vaccine, but this is an area of active investigation.

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus. Infection with EBV is common, worldwide in distribution, and largely subclinical in early childhood. EBV has been established as the causative agent of heterophile-positive infectious mononucleosis, which occurs most frequently in late adolescence or early adulthood. In addition, EBV is causally associated with the development of malignant diseases, including Burkitt lymphoma, lymphoproliferative disease (LPD), Hodgkin lymphoma, primary central nervous system (CNS) lymphomas in acquired immunodeficiency syndrome (AIDS), and nasopharyngeal carcinoma (NPC), based on seroepidemiologic data and the detection of EBV genomes in these tumors. Some epidemiologic studies describe an association between EBV and autoimmune diseases, particularly multiple sclerosis; however, a causal relationship is not established.

HISTORY

Historical accounts of infectious mononucleosis often attribute the initial description of the disease to Filatov or Pfeiffer, who nearly simultaneously at the end of the 19th century described an illness characterized by malaise, fever, hepatosplenomegaly, lymphadenopathy, and abdominal discomfort.[1,2] This illness came to be known as Drusenfieber (glandular fever); however, without specific techniques with which to establish the diagnosis, the concept of Drusenfieber as a clinical entity fell into disrepute. Between 1910 and 1920 a number of observers reported cases of apparent spontaneous remission of leukemia, with a clinical course that is consistent with resolution of infectious mononucleosis.[3,4] The establishment of infectious mononucleosis as a clinical entity is credited to Sprunt and Evans,[5] who in 1920 described six cases of fever, lymphadenopathy, and prostration that occurred in previously healthy young adults. The authors pointed out the mononuclear lymphocytosis that developed in each of the patients and contrasted the pathologic appearance of these lymphocytes with the uniform lymphocyte morphology observed in children with other infections. Two years later Downey and McKinlay[6] described additional cases of infectious mononucleosis and provided a more detailed morphologic description of the atypical lymphocyte. The recognition of atypical lymphocytosis as a hematologic marker for the disease led to more accurate descriptions of the clinical manifestations of this illness.

A major advance occurred in 1932, when Paul and Bunnell,[7] investigating immunologic mechanisms in serum sickness, unexpectedly encountered high titers of spontaneously occurring sheep red blood cell (RBC) agglutinins (heterophile antibodies) in the sera of patients with infectious mononucleosis.

During the 1940s and 1950s substantial efforts were made to detect a causative agent for infectious mononucleosis. Attempts to culture etiologically related bacteria and viruses from patients with infectious mononucleosis proved unsuccessful. The disease could not be transmitted to animals. Interpretation of experimental attempts to transmit the disease to humans was hindered by the failure to appreciate the widespread occurrence of asymptomatic infection in preadolescents and the absence of a serologic marker of immunity.[8–10]

The identification of EBV followed the description by Burkitt[11] in 1958 of an unusual lymphoma with a predilection for the head and neck. The geographic distribution of this tumor paralleled that of certain mosquito-borne diseases in Africa, and a search for an etiologically related arbovirus was undertaken. Epstein and associates[12] in 1964 described the presence of particles that resembled herpesviruses in tissue cultures of biopsy specimens from patients with Burkitt lymphoma. An indirect immunofluorescent assay for detecting anti-EBV antibodies was developed by Werner and Gertrude Henle,[13] and high titers were detected in patients with Burkitt lymphoma. Additional studies revealed that 90% of American adults had demonstrable EBV antibodies as well.[13] The development of infectious mononucleosis in a technician in the Henles' laboratory, from whom sequentially obtained sera were analyzed for EBV antibody, suggested that acute EBV infection may be associated with this illness.[14] Large-scale epidemiologic studies[15–18] showed that heterophile-positive infectious mononucleosis occurred in patients without preexisting EBV antibody, and conversely, heterophile-positive infectious mononucleosis was always accompanied by acquisition of EBV antibodies. These epidemiologic studies indicated that subclinical EBV infection also occurred. With specific antibody tests for EBV, it became apparent that 10% to 20% of the cases of mononucleosis, of which most were heterophile negative, were caused by other agents, the most frequent of which was cytomegalovirus (CMV). This chapter deals primarily with EBV-induced infectious mononucleosis and EBV-associated malignancies.

DESCRIPTION OF EPSTEIN-BARR VIRUS

Physical Properties

EBV, or human herpesvirus 4, is a gamma-1 herpesvirus. Like the other members of the Herpesviridae family, EBV has a double-stranded DNA genome encased in an icosahedral protein nucleocapsid surrounded by a lipid envelope embedded with viral glycoproteins. Herpesviruses also have an amorphous protein layer, the tegument, which lies between the capsid and envelope. The B95-8 laboratory strain of EBV, the first herpesvirus genome sequenced, was found to have a 12-kilobase (kb) deletion and the wild-type EBV genome, which is approximately 172 kb in size and encodes about 90 proteins and 25 microRNAs (miRNAs).[19,20]

Life Cycle

Primary infection with EBV results from exposure to the oral secretions of seropositive individuals through kissing, sharing of food, or other intimate contact. It is generally accepted that EBV infection spreads to B lymphocytes after initial productive (lytic) infection of oral epithelial cells. Direct evidence of epithelial infection in immunocompetent hosts has been difficult to obtain. Tonsillar biopsies from patients with primary EBV infection did not reveal any infected epithelial cells, but infected lymphocytes were readily seen.[21,22] EBV undoubtedly has clinically significant tropism for epithelial cells, as is seen in NPC and oral hairy leukoplakia. It remains possible that significant infection of oral epithelial cells occurs in nontonsillar sites or that an initial round of lytic replication precedes spread to the B-cell compartment and the onset of symptoms.[23,24] Indeed, there is evidence that many herpesviruses transiently infect epithelial cells on multiple occasions before they establish lifelong infection within the appropriate latency cell type.[25] Studies using banked samples have demonstrated that during EBV's 30- to 50-day incubation period, the virus is detectible in low levels in the blood. Symptoms of infectious mononucleosis coincide with a rapid rise in serum EBV viral loads and increased detection in saliva.[26] Infected B lymphocytes incite an intense cytotoxic T-cell response, and these T cells constitute the atypical lymphocytosis characteristic of primary EBV infection.[27,28] In healthy individuals most infected B lymphocytes are cleared through immune surveillance, but between 1 and 50 B cells per million remain quiescently infected and serve as the reservoir for lifelong infection of the individual.[29,30] Thus EBV shares the properties of lifelong latency and persistence with other members of the herpesvirus family. In contrast to that of alphaherpesviruses (herpes simplex virus [HSV] and varicella-zoster virus [VSV]), shedding of infectious EBV particles into the saliva from periodic reactivation of latently infected cells is entirely asymptomatic. This shedding occurs in otherwise healthy persons but is more frequent in immunosuppressed hosts (Table 138.1).

TABLE 138.1 Frequency of EBV Shedding

POPULATION DESCRIPTION	OROPHARYNGEAL SHEDDING RATE (RANGE)	REFERENCE
EBV-seronegative individuals	0	89
Seropositive healthy adults	12%–25%	85–87, 89–91
Solid-tumor patients	27%	86, 87
HIV-1–infected individuals	50%	88
Renal transplant recipients	56%–70%	85, 87
Infectious mononucleosis patients	50%–100%	89–91, 348
Critically ill leukemia or lymphoma patients	74%–92%	86, 87

EBV, Epstein-Barr virus; *HIV,* human immunodeficiency virus.

The host range of the virus is limited. In vitro cultivation of the virus has been described primarily in B lymphocytes and also in nasopharyngeal epithelial cells of humans and certain nonhuman primates.[31] EBV binds to its receptor, the cluster of differentiation 21 (CD21) molecule, through an interaction with its major envelope glycoprotein, gp350. CD21 or complement receptor 2 transduces signals important for B-lymphocyte proliferation and can also be expressed by follicular dendritic cells and nasopharyngeal epithelial cells.[32–38] Another EBV glycoprotein, gp42, binds class II major histocompatibility complex (MHC) molecules, which serve as coreceptors for infection of B cells.[39–43] Gp42 also promotes B-cell infection by forming a heterotrimeric complex with the gH/gL EBV glycoproteins, masking a motif on gH that is important for infection of epithelial cells.[44] EBV virions released from infected B lymphocytes contain lower levels of gp42 and infect epithelial cells more efficiently than virions derived from epithelial cells. This reciprocal tropism is proposed to enhance EBV shuttling between B lymphocytes and the oral epithelium.[45]

Latent Infection and Growth Transformation

Epstein-Barr Virus Drives B Cells to Proliferate

After infection with EBV, B lymphocytes enter the cell cycle and proliferate continuously in a process termed *transformation* or *immortalization;* these cells can be propagated in vitro indefinitely.[46] This ability of EBV to convert peripheral blood B cells into immortalized lymphoblastoid cell lines is widely used in genomic studies as a means of preserving DNA samples from volunteer donors for future use.[47] In vivo EBV-driven B-cell proliferation is observed during infectious mononucleosis, in which it probably serves to expand rapidly the pool of infected B lymphocytes. These B lymphocytes are usually rapidly cleared from the circulation.[48–51] However, in the absence of an intact immune response, EBV infection can result in life-threatening LPD.[52,53] The growth-transforming properties of EBV can act in concert with genetic and environmental cofactors to cause malignant diseases in immunocompetent hosts as well.[54,55]

Epstein-Barr Virus Genome Is Circularized in Latent Infection and Expresses a Subset of Viral Genes

EBV infection of B lymphocytes is characterized by a state of viral latency in which the genome circularizes in the nucleus and is replicated

TABLE 138.2 Patterns of EBV Latent Gene Expression

				EBV-ASSOCIATED MALIGNANT DISEASES				
		Acute Infection	Healthy Carrier	Latency III	Latency II		Latency I	
EBV GENE PRODUCT	FUNCTION	*IM*	*PBB*	*LPD*	*HL*	*NPC*	*GC*	*BL*
EBNA1	EBV genome maintenance	+	?	+	+	+	+	+
EBNA2	Activates expression of EBV/host genes	+	—	+	—	—	—	—
EBNA3s[a]	Represses p16/INK4A tumor suppressor expression	+	—	+	—	—	—	—
EBNALP	Coactivates with EBNA2	+	—	+	—	—	—	—
LMP1	Mimics CD40 signaling	+	—	+	+	+	—	—
LMP2	Mimics BCR signaling	+	+	+	+	+	±	—
miRNAs	Block expression of host RNAs	+	?	+	+	+	+	+
EBERs	Noncoding, highly expressed RNAs	+	+	+	+	+	+	+

[a]Includes EBNA3A, EBNA3B, and EBNA3C.
BCR, B-cell receptor; *BL,* Burkitt lymphoma; *EBERs,* EBV-encoded RNAs; *EBNA,* EBV nuclear antigen, *EBV,* Epstein-Barr virus; *GC,* gastric cancer; *HL,* Hodgkin lymphoma; *IM,* infectious mononucleosis; *LMP,* latent infection membrane protein; *LPD,* lymphoproliferative disease; *miRNAs,* micro–RNAs; *NPC*, nasopharyngeal carcinoma; *PBB,* peripheral blood B cell; *PCNSL,* primary central nervous system lymphoma.
Note: Because EBV-positive gastric cancers sometimes express LMP2 and, rarely, LMP1, these tumors have been classified as latency I or II by different experts.

as an episome in concert with host chromosomes by cell enzymes. The infection is latent in the sense that viral particles are not being produced, but it is anything but quiescent. Limited viral gene expression persists, and these genes exert effects on the infected cell. In vitro latent infection of B lymphocytes with EBV is characterized by the expression of latent infection membrane proteins 1 and 2 (LMP1 and LMP2), six EBV nuclear antigens (EBNAs), and two small, nuclear, noncoding RNAs (EBV-encoded RNAs [EBERs]) that are transcribed by RNA polymerase III (Table 138.2).[56] Additional EBV transcripts have been detected in latent infection and are termed *complementary strand transcripts* or *Bam*H1 A rightward transcripts. Translation of these transcripts into proteins has not been shown, but they appear to serve as precursors for two of three clusters of EBV-encoded miRNAs whose role in EBV biology remains unclear.[57,58] Recombinant reverse genetic analysis has determined that only LMP1, EBNA1, EBNA2, EBNA3A, EBNA3C, and EBNALP are critical for B-cell growth transformation.[56] The mechanisms by which these EBV gene products promote B-lymphocyte growth have been the subject of intense investigation.

Functions of Epstein-Barr Virus Genes Expressed in Latent Infection

After the virus gains entry to susceptible B lymphocytes, EBNA2 and EBNALP are the first proteins expressed. EBNA2 is an acidic transactivator that acts as the major switch to turn on latent virus gene expression and several B-cell gene products (including c-MYC, c-FGR, CD21, and CD23). It has no intrinsic sequence-specific DNA binding capacity but rather is targeted to promoters by binding to a host DNA binding protein RBP-Jκ (also called CBF1 or CSL), a downstream component of the Notch signaling pathway.[59,60] By an incompletely understood mechanism, EBNALP cooperates with EBNA2 to activate expression of the remaining nuclear proteins and LMP1 and LMP2.[61] *LMP1* is the major EBV-encoded oncogene, and its expression in transgenic mice results in B-cell lymphomas.[62,63] It constitutively activates signaling pathways that mimic the growth and survival signals given to B cells by $CD4^+$ T lymphocytes through the CD40 surface glycoprotein. LMP1 sends this signal through its cytoplasmic tail, which binds a set of second-messenger proteins similar but not identical to those used by CD40.[64,65] Unlike CD40, LMP1 does not require the presence of ligand to form patches in the cell membrane but self-associates constitutively, approximating its cytoplasmic tails to activate signaling.[66] This results in the activation of nuclear factor κB (NF-κB), c-JUN, upregulation of adhesion molecules (intercellular adhesion molecule 1, lymphocyte function-associated antigen 1 [LFA-1], and LFA-3), cytokine production, B-cell proliferation; and induction of an antiapoptotic state.[48,55] A second EBV latent membrane protein, LMP2, mimics another signal necessary for B-cell survival.[67] By interacting with signaling molecules of the B-cell receptor (BCR), LMP2 mimics BCR engagement by constitutive patching in the membrane in a manner analogous to LMP1. LMP2 probably also interferes with normal signaling through the BCR by antigenic stimulation to inhibit activation of lytic viral replication (discussed subsequently). Of interest, LMP2 is not necessary for EBV-mediated outgrowth of B cells in vitro but is probably a critical component of the viral strategy in vivo. The nuclear protein EBNA1 acts to promote the replication of the viral genome by the host machinery when the virus is in the latent, episomal state and to ensure proper segregation of the EBV genome to both daughter cells. The EBNA3 proteins also interact with the recombination signal binding protein (RBP)-Jκ DNA binding protein and promote B-lymphocyte growth and survival by silencing expression of the $p16^{INK4}$ and $p14^{ARF}$ tumor suppressor gene products.[68,69] The function of the highly expressed, noncoding EBERs is incompletely understood.

Patterns of Epstein-Barr Virus Gene Expression Vary in Different Malignancies

EBV-associated malignant diseases are exclusively associated with latent infection and latent gene expression. Three general patterns of expression of EBV-encoded proteins have been observed in association with latency (see Table 138.2).[48,49] Expression of all latent genes is seen in LPD in immunosuppressed hosts, in primary CNS lymphoma of patients with AIDS, and during primary EBV infection (infectious mononucleosis), and this program of gene expression is often referred to as *latency III.*[54] EBV-associated NPCs, Hodgkin lymphoma, and T-cell lymphomas exhibit a more restricted pattern of EBV gene expression (latency II) that includes LMP1, LMP2, EBNA1, and the EBERs and EBNA1.[70–72] In Burkitt lymphoma (latency I) only EBERs and EBNA1 are expressed.[54] The more restricted patterns of latent gene expression in some tumors are probably in part the result of the intense immune response against viral proteins.

Lytic Infection

Latent infection can be activated to lytic infection by stimulation of host B cells by certain chemicals, calcium ionophores, or antibodies to surface immunoglobulin.[73] The physiologic signals that reactivate EBV lytic replication are unknown, but signaling through the B-cell receptor after antigenic stimulation is a possible scenario. After this inciting event, two EBV-encoded transcriptional activators are expressed: *BZLF1* and *BRLF1*. Expression of these immediate early genes leads to a cascade of events that culminate in the production of early EBV gene (early antigen [EA]) products responsible for viral replication (e.g., thymidine

kinase, DNA polymerase) and late (structural) genes of the virus, including viral capsid antigens (VCAs).[74] Lytic infection produces EBV virions and can cause host cell death.

EPIDEMIOLOGY

Serum Antibody Prevalence

Antibodies to EBV have been found in all population groups studied, and most studies have shown no predilection for either gender. Antibodies are acquired earlier in life in developing countries than in industrialized countries, but by adulthood 90% to 95% of most populations have demonstrable EBV antibodies.[75,76] In the United States and in Great Britain EBV seroconversion occurs before the age of 5 years in about 50% of the population.[76–78] A second wave of seroconversion occurs midway through the second decade of life. EBV seroconversion may occur at a younger average age in the southern United States than in other areas of that country.[79] Lower socioeconomic groups have a higher EBV antibody prevalence than more affluent age-matched control groups. In the United States EBV antibody prevalence among individuals aged 6 to 19 years decreased from 72% in 2003–04 to 65% in 2009–10. The change was mainly due to decrease in EBV antibodies among non–Hispanic white individuals.[80]

Two strains of EBV have been defined on the basis of viral gene sequences expressed during latency and their ability to transform B lymphocytes.[73] The strains (type 1 [A] or 2 [B]) are not distinguishable serologically, making estimates of their distribution challenging. Available epidemiologic data show a high rate of type 1 EBV infection worldwide, whereas the prevalence of type 2 EBV infection (or coinfection) may be less than 10% in the developed world.[81] Type 2 EBV infection is more prevalent in sub-Saharan Africa and Papua New Guinea and observed more frequently in immunocompromised people.[82]

Incidence of Infection

Clinically apparent infectious mononucleosis is more common in populations in which primary EBV exposure is delayed until after the first decade of life. The disease is diagnosed most frequently among adolescents of higher socioeconomic groups in industrialized countries.[83] The incidence of infectious mononucleosis in a large epidemiologic study in the United States was 45.2 cases per 100,000 per year and was highest in the 15- to 24-year-old age group.[84] The incidence was the same for women as for men, but the peak age-specific incidence occurred 2 years earlier in women. No clear seasonal incidence has been noted.

Methods of Spread

The virus persists in the B-cell compartment for the life of the infected host and can be cultured from throat washings from 10% to 20% of healthy adults, from 50% of kidney transplant recipients, and from greater proportions of those critically ill with leukemia or lymphoma (see Table 138.1).[85–87] Approximately 50% of men with human immunodeficiency virus type-1 (HIV-1) infection who have sex with men shed EBV in oropharyngeal secretions.[88] Low titers of EBV are present in throat washings of persons with infectious mononucleosis.[89–91] Susceptible roommates of students with infectious mononucleosis or with inapparent EBV infection have EBV seroconversion no more frequently than the general susceptible college population.[17,79] Only 6% of those with infectious mononucleosis cite previous contact with another case of infectious mononucleosis.[84] EBV DNA or protein, or both, have also been identified in parotid duct and uterine cervical epithelia, although the implications of this distribution are unclear with respect to viral transmission.[92,93]

EBV, like other herpesviruses, is relatively labile in the laboratory, and the virus has not been recovered from environmental sources, including fomites. These data suggest that EBV is a widespread agent that is not particularly contagious and that most cases of infectious mononucleosis are probably contracted by intimate contact between susceptible individuals and asymptomatic shedders of EBV. Among young adults, spread of the virus may be facilitated by the transfer of saliva with kissing.[94,95] EBV infection has been linked to sexual intercourse; however, other studies have found that kissing with coitus conferred no additional risk of EBV infection compared with kissing without coitus.[96,97] Serologic evidence suggests that the virus may also be spread among susceptible individuals within families.[98,99] In malaria-endemic regions, EBV infection is usually acquired within the first 6 months of life, and this has been linked to high EBV loads in breast milk.[100] Although EBV has been detected in breast milk from other parts of the world,[101] transmission by this route appears to be very inefficient outside of sub-Saharan Africa. EBV has also been spread via blood transfusion and after open heart surgery as the postpump perfusion syndrome.[102] Most postpump perfusion infectious mononucleosis is, however, heterophile negative and attributable to CMV.

Although several apparent epidemics of infectious mononucleosis have been described, these reports have not been substantiated with EBV serologic data and have lacked rigorous epidemiologic, clinical, or laboratory support. Some of these have resulted from errors in the performance of Monospot tests.[103] On the basis of the previously discussed information, true epidemics of infectious mononucleosis are unlikely to occur.

Public Health Impact

College and military populations experience the highest morbidity from infectious mononucleosis, although cases occur in other groups as well. Infectious mononucleosis accounted for 5% of all hospitalizations of University of Wisconsin students, with an incidence rate of 450 admissions per 100,000 students per year. Other American universities have reported similar incidence rates.[104,105] Approximately 12% of susceptible college students undergo EBV seroconversion yearly.[17,18] Many of these infections are subclinical (see subsequent discussion).[17,79] Although primary EBV infection may be clinically apparent in only about 10% of military cases, infectious mononucleosis ranked fourth as the cause of days lost because of illness in Army personnel.[106,107] Detailed information about the impact of infectious mononucleosis on the general population is not available because infectious mononucleosis is not a reportable disease in most states. However, morbidity from infectious mononucleosis likely is generally underestimated because a specific diagnosis may not be made and the nonspecific illness can be attributed to a variety of other causes.

PATHOGENESIS

Host Immune Response

Immune Response Controls Epstein-Barr Virus Infection and Is the Cause of Mononucleosis Symptoms

EBV presents a formidable challenge to the immune system. At the height of acute infection up to 20% of peripheral blood B lymphocytes may express EBNA, and 0.005% to 0.5% of circulating mononuclear cells are capable of forming continuous cell lines if cultured in vitro.[108,109] The immune response to EBV-infected transformed lymphocytes is complex and involves both humoral and cell-mediated immune mechanisms.[27,110] An intact immune response is critical to prevention of the unchecked proliferation of these cells as seen in LPD but is also responsible for most of the symptoms of infectious mononucleosis. The increase in prevalence in symptomatic acute EBV infection with age of seroconversion is probably the result of differences in the immune responses of different age groups.

Cellular Response to Epstein-Barr Virus

The cellular immune response to EBV is complex and well integrated and includes $CD8^+$ and $CD4^+$ cytotoxic T-lymphocytes (CTLs) and natural killer (NK) cells.[28,111–114] The massive atypical lymphocytosis of infectious mononucleosis is composed primarily of antigen-stimulated $CD8^+$ cytotoxic T cells. In one study 40% of circulating $CD8^+$ T cells were reactive against a single EBV epitope.[115] These lymphocytes probably produce most of the signs and symptoms of infectious mononucleosis through the abundant production of cytokines, including tumor necrosis factor, interleukin-1 (IL-1), and IL-6.[116] During acute infection $CD8^+$ T cells specific for lytic antigens predominate, but with convalescence, a shift occurs toward cells that recognize latent proteins, particularly the EBNA3 proteins.[27,115,117] T cells reactive against EBV latent proteins are sufficiently numerous that unselected mononuclear cells from EBV-immune adults suppress the outgrowth of autologous EBV-infected B lymphocytes in vitro.[114] An expansion of EBV-specific $CD4^+$ lymphocytes has also been

described in infectious mononucleosis but is small in magnitude, and its significance in containing acute EBV infection is unclear.[118,119]

Humoral Response to Epstein-Barr Virus

The humoral immune response to EBV has been extensively studied, primarily as a means to diagnose EBV infection (see "Laboratory Diagnosis" section for detailed discussion). In general, specific antibodies directed against EBV lytic antigens (VCA and EA) are demonstrable in most patients with infectious mononucleosis. By contrast, antibody responses to the latency-associated EBV nuclear antigens (EBNA1, EBNA2, EBNA3s, and EBNALP) do not develop until convalescence.[27] The significance of any of these antibody responses to containment of EBV infection is not established; however, antibodies to EBV surface glycoproteins have been shown to prevent experimental EBV infection.[120–122]

Heterophile Antibodies Generated Early During Primary Epstein-Barr Virus Infection Have No Known Role in Pathogenesis

For unclear reasons, primary EBV infection is associated with the synthesis of large amounts of antibodies reactive against antigens found on sheep, horse, and beef red cells. These so-called heterophile antibodies are a heterogeneous group of predominantly immunoglobulin M (IgM) antibodies that do not react with specific EBV proteins.[123] Detection of these antibodies in sera of patients with mononucleosis syndromes predicts acute EBV infection with high specificity and adequate sensitivity and is discussed in the section "Laboratory Diagnosis." No good correlation is found between the heterophile titer and the severity of the illness, and no clearly defined role exists for heterophile antibodies in the pathogenesis of EBV disease or in immune clearance of the virus.

Epstein-Barr Virus Immune Evasion

EBV has evolved multiple strategies to elude this aggressive immune response. The EBV BCRF1 protein shares 70% homology with the cytokine IL-10. This EBV protein is functional and is thought to mimic IL-10 inhibition of interferon-γ (IFN-γ) synthesis by mononuclear cells in the peripheral blood. Thus BCRF1 expression during lytic infection is expected to promote a shift toward Th2 differentiated $CD4^+$ effectors that can provide B-cell help but do not promote the $CD8^+$ responses needed to kill EBV-infected cells.[124,125] Another EBV protein, *Bam*H1 rightward frame 1 (BARF1), can function as a soluble receptor for colony-stimulating factor 1 and may interfere with the ability of this cytokine to enhance expression of interferon-α (IFN-α) from monocytes.[126] EBV also encodes a Bcl2 homologue that is expressed during lytic replication and may act to prevent apoptosis of the host cell.[127] Finally, the virus has evolved a strategy for ensuring its persistence in the memory B-cell compartment. After acute infection resolves, most latent proteins are no longer expressed to circumvent the strong immune pressure exerted against EBV. However, in any cycling cell, EBV must express EBNA1 to ensure that its genome is replicated. To prevent targeting of this key protein, EBNA1 contains a sequence of expanded glycine-alanine repeats—not necessary for its function in genome maintenance—capable of inhibiting proteasomal processing of the protein.[128] Without this processing, EBNA1 peptides cannot be presented on class I MHC molecules, and cells that express EBNA1 may elude immune surveillance.

Histopathologic Findings

Because biopsies are rarely obtained in patients with uncomplicated infectious mononucleosis, most data come from pathologic examination of tissues obtained from fatal cases or from cases with atypical features in which biopsy specimens were obtained for diagnostic evaluation. During the acute phase of the illness lymph nodes throughout the body are moderately enlarged. Individual nodes reveal increased numbers of enlarged, moderately active lymphoid follicles. Germinal centers are also enlarged, with cores that contain blast cells, histiocytes, and lymphocytes. Although the reticulin framework remains intact, invasion by the hyperplastic pulp makes its borders less distinct.[129] In studies of spleens obtained at autopsy or at surgery after rupture, the organ is usually two to three times its normal weight.[130] The splenic capsule and trabeculae are edematous, thinned, and invaded by lymphoid cells. Most of the increased splenic size is the result of hyperplasia of the red pulp. Throughout the red pulp, pleomorphic blast cells are evident. The spleen is often congested with focal, particularly subcapsular, hemorrhages. The white pulp is relatively normal. Tonsillar biopsy specimens obtained during the course of mononucleosis reveal intense proliferation with numerous mitoses.[131] Bone marrow aspirate and biopsy specimens are often strikingly normal when compared with the florid changes noted in peripheral blood. Biopsy specimens are usually normocellular to mildly hypercellular. Small granulomas may be present, but these are not specific for mononucleosis and have no prognostic significance.[132,133]

Changes in hepatic histologic features are usually mild. Hepatocytes show minimal swelling and vacuolization. Pleomorphic lymphocytic and monocytic portal infiltration is usually evident. Bile ducts may be minimally swollen, but frank biliary stasis is rare.[134,135] A number of histopathologic changes have been reported in the nervous system in fatal cases of infectious mononucleosis.[131,136,137] These changes include neuronal degeneration, perivascular cuffing, perivascular hemorrhage, and astrocytic hyperplasia. Little mononuclear infiltration may be present despite demonstrable degenerative changes in the neurons of the cortex, basal ganglia, cerebellum, or spinal cord.

CLINICAL MANIFESTATIONS

Infectious Mononucleosis (Primary Infection)

Spectrum of Illness

EBV induces a broad spectrum of illness in humans. Classic or typical infectious mononucleosis is an acute illness characterized clinically by sore throat, fever, and lymphadenopathy; serologically by the transient appearance of heterophile antibodies; and hematologically by a mononuclear leukocytosis that consists, in part, of atypical lymphocytes (Table 138.3). An individual case may have most but not necessarily all the aforementioned characteristics. Specific serologic tests for EBV infection indicate that infection results in a spectrum of clinical manifestations. Attempts to exclude cases that fail to meet the classic criteria for infectious mononucleosis result in artificial and often misleading distinctions.

The age of the patient has a profound influence on the clinical expression of EBV infection. In children primary EBV infection is often asymptomatic. Young children may be more likely to exhibit rashes, neutropenia, or pneumonia than individuals with primary EBV infection at an older age.[138] Clinically apparent infections in very young children are heterophile negative in about one-half of the cases.[139] The proportions of clinically apparent disease and of heterophile-positive cases increase with age. By 4 years of age 80% of children with primary EBV infection are heterophile antibody positive.[140] During the course of the illness 90% of the adolescents with clinically apparent infectious mononucleosis should be heterophile positive.

In patients of college age the ratio of clinically apparent to inapparent EBV infection ranges from 1:3 to 9:1.[17,79,96] In two prospective series a much higher rate (89%) of symptomatic infection was observed when students were evaluated at 8-week intervals compared with 25% when students were evaluated 3 years after enrollment.[96,97] In military recruits this ratio has been as low as 1:10.[107] Because of previously existing immunity, the disease is less common in older patients. When it does occur, however, clinical and serologic manifestations are similar to those found in adolescents.[141] In general, EBV infection is inapparent or is a self-limited illness that lasts 2 or 3 weeks. In rare cases the disease can be devastating and can be accompanied by severe prostration, major complications, and even death,[142] as discussed subsequently.

Symptoms and Signs

Most cases of infectious mononucleosis consist of the clinical triad of sore throat, fever, and lymphadenopathy (Table 138.4). Epidemiologic studies suggest that the incubation period of acute infectious mononucleosis is 30 to 50 days. Viral shedding in oral secretions has been observed for up to 36 days before onset of symptoms,[90,96,143] although a recent study found that oral virus could be detected only 1 week before symptoms.[26] The onset may be abrupt, but often several days of prodromal symptoms can be elicited, including chills, sweats, feverish sensations, anorexia, and malaise. Retro-orbital headaches, myalgias, and feelings of abdominal fullness are other common prodromal

TABLE 138.3 Manifestations of Epstein-Barr Virus–Induced Infectious Mononucleosis

Clinical
Fever
Sore throat
Lymphadenopathy
Hematologic
>50% mononuclear cells
>10% atypical lymphocytes
Serologic
Transient appearance of heterophile antibodies
Permanent emergence of antibodies to Epstein-Barr virus

TABLE 138.4 Signs and Symptoms of Primary EBV and Infectious Mononucleosis

	INFECTIOUS MONONUCLEOSIS[a]		PRIMARY EBV[b]
Symptom	**Percentage**	**Range (%)**	**Percentage (%)**
Sore throat	82	70–88	83
Malaise	57	43–76	59
Headache	51	37–55	42
Anorexia	21	10–27	39
Myalgias	20	12–22	36
Chills	16	9–18	
Nausea	12	2–17	
Abdominal discomfort	9	2–14	8
Cough	5	5	
Vomiting	5	5	
Arthralgias	2	2	
Sign	**Percentage**	**Range (%)**	**Percentage (%)**
Lymphadenopathy	94	93–100	68
Pharyngitis	84	69–91	
Fever	76	63–100	38
Splenomegaly	52	50–63	
Upper eyelid edema	50	<20–50	
Hepatomegaly	12	6–14	
Palatal enanthem	11	5–13	
Jaundice	9	4–10	
Rash	20	0–23	

[a]Data for Infectious Mononucleosis from references 104, 144, 145, 147, 487.
[b]Primary EBV infection refers to new EBV infection and includes asymptomatic cases, those cases with symptoms but not meeting criteria for infectious mononucleosis, and those meeting criteria (77%) for infectious mononucleosis. From prospective study of primary EBV infection by Balfour.[96]
EBV, Epstein-Barr virus.

symptoms. The most frequent symptom is sore throat, which may be the most severe the patient has experienced.[144,145] Other patients seek medical attention because of prolonged fever or malaise and less frequently because of incidentally encountered lymphadenopathy. Rarely, the first manifestation of illness is one of the complications of infectious mononucleosis described subsequently.

The signs of infectious mononucleosis are summarized in Table 138.4. Fever is present in greater than 90% of patients with infectious mononucleosis. The fever usually peaks in the afternoon with temperatures of 38° to 39° C, although a temperature as high as 40° C is not uncommon. In most cases fever resolves over a 10- to 14-day period. Bilateral upper eyelid edema (the Hoagland sign) occurs only during the first few days of illness and has been reported in up to 50% of cases in some series[145,146] but less frequently in others.[147] Tonsillar enlargement is usually present, occasionally with tonsils meeting at the midline. The pharynx is erythematous, with an exudate in about one-third of cases. Palatal petechiae may be seen in 25% to 60% of cases but are not diagnostic of infectious mononucleosis. The petechiae are usually multiple, are 1 to 2 mm in diameter, occur in crops that last 3 to 4 days, and are usually seen at the junction of the hard and soft palate.[148] However, palatal petechiae are not unique to infectious mononucleosis and can also be observed in rubella or accompanying group A streptococcal pharyngitis. Cervical adenopathy, usually symmetrical, is present in 80% to 90% of patients. Posterior adenopathy is most common, but submandibular and anterior adenopathies are quite frequent as well, and axillary and inguinal adenopathies also occur. Individual nodes are freely movable, are not spontaneously painful, and are only mildly tender to palpation. The results of examination of the lungs and heart are usually normal. Abdominal examination may detect hepatomegaly in 10% to 15% of cases, although mild tenderness to fist percussion over the liver is present somewhat more frequently.[144,147] Jaundice is present in approximately 5% of cases.[145] Splenomegaly is present in about one-half of cases if sought carefully over the course of the illness. The splenomegaly is usually maximal at the beginning of the second week of illness and regresses over the next 7 to 10 days. The results of neurologic examination are generally normal, although occasional complications may occur (see subsequent discussion).

Complications

Most patients with infectious mononucleosis recover uneventfully. Complications that occasionally occur have been extensively reported in the literature. Even these complications have generally resolved fully, although rare fatalities have been reported.

Dermatologic Complications

Rash may accompany infectious mononucleosis and may be macular, petechial, scarlatiniform, urticarial, or erythema multiforme–like. Historically, rash was felt to be rare, occurring in about 5% of patients, whereas the administration of antibiotics, particularly ampicillin was observed to result in a pruritic, maculopapular eruption in 90% to 100% of patients (Fig. 138.1); the rash could appear either during or after cessation of the antibiotic.[149,150] The ampicillin-related rash does not necessarily predict future intolerance to ampicillin.[151,152] More recently, reports have indicated a higher rate of rash of 20% in infectious mononucleosis in the absence of antibiotics, with perhaps little or no further increase in rash incidence after the administration of antibiotics.[153,154] The reasons underlying these differences in rash incidence are unclear.

Acute, painful genital ulcers, typically involving the labia minora (Lipschutz ulcers) can occur in up to 30% of prepubertal or adolescent females during infectious mononucleosis.[146] These lesions are often greater than 1 cm diameter with characteristic purple edges and a necrotic base. They are not sexually transmitted but due to their appearance and location can prompt evaluations of sexual abuse, which can be distressful for patients and families. These lesions may be misdiagnosed as HSV or Behçet syndrome. Lipschutz ulcers remit spontaneously within 6 weeks without scarring. Treatment is supportive.

Primary EBV infection is the most common cause of Gianotti-Crosti syndrome (papular acrodermatitis of childhood).[146] Lesions, which can be asymptomatic or slightly pruritic, are papular and located in a symmetrical distribution on the cheeks, buttocks, and extensor surfaces of extremities and usually resolve within several weeks. Gianotti-Crosti syndrome typically occurs in children age 6 years or younger but can occur in adolescents. Due to the younger age of these patients, many of the typical symptoms of mononucleosis may be absent.

Hematologic Complications

Autoimmune hemolytic anemia occurs in 0.5% to 3% of the patients with infectious mononucleosis.[155,156] Cold agglutinins, almost always of the IgM class, are present in 70% to 80% of cases.[157] Anti-I specificity has been reported in 20% to 70% of cases.[158,159] Most, but not all, cases



FIG. 138.1 Patient with infectious mononucleosis and ampicillin-induced rash. Maculopapular rash extends over the trunk and extremities. Rash frequently has a violaceous hue and is often accompanied by pruritus. *(Courtesy Dr. Stephen Gellis.)*

of autoimmune hemolytic anemia in infectious mononucleosis are mediated by antibodies of this specificity.[160–163] The hemolysis usually becomes clinically apparent during the second or third week of illness and subsides over a 1- to 2-month period.[164] Corticosteroids may hasten recovery in some cases. Hemophagocytic syndrome, a rare complication of EBV infection, is discussed in a subsequent section.

Mild thrombocytopenia is common in infectious mononucleosis. Platelet counts less than 140,000/mm^3 were noted in 50% of patients with uncomplicated infectious mononucleosis in one series.[165] Profound thrombocytopenia with bleeding occurs rarely,[166] but platelet counts less than 1000/mm^3 and deaths from intracerebral bleeding have been reported.[167,168] The mechanism for the thrombocytopenia is not known. The presence of normal or increased numbers of megakaryocytes in the marrow, coupled with reports of antiplatelet antibodies, suggests that peripheral destruction of platelets may occur, possibly on an autoimmune basis.[158,162,169] Corticosteroids have been reported to be beneficial for the thrombocytopenia in some, but not all, cases.[166–168,170] For refractory cases splenectomy may be indicated.[169]

Neutropenia is seen rather frequently in uncomplicated infectious mononucleosis. The neutropenia is usually mild and self-limiting, although deaths associated with bacterial sepsis or pneumonia, or both, have been reported.[171–178] Anaerobic sepsis without associated granulocytopenia, presumably of pharyngeal origin, has also been reported.[179]

Splenic Rupture

Splenic rupture is a rare but dramatic complication of infectious mononucleosis. Lymphocytic infiltration of the capsule, trabeculae, and vascular walls, coupled with rapid splenic enlargement, predisposes the organ to rupture. The incidence of rupture is highest in the second or third week of illness but may be the first sign of infectious mononucleosis. Abdominal pain is uncommon in infectious mononucleosis,[180] and splenic rupture must be strongly considered whenever abdominal pain occurs. The onset of this pain may be insidious or abrupt. Pathologic examination of some ruptured spleens has revealed subcapsular hematomas that suggest that rupture may be preceded by intermittent subcapsular bleeding. The pain, usually in the left upper quadrant, may radiate to the left scapular area. Left upper quadrant tenderness to palpation, with or without rebound tenderness, is usually present along with peritoneal signs or shifting dullness. In rare cases splenic rupture is unaccompanied by pain and is manifested as shock. Laboratory findings include a falling hematocrit and in some cases an elevated left hemidiaphragm. The abdominal catastrophe may reverse the usual differential count of infectious mononucleosis and evoke a neutrophilia. Confirmatory findings should not be awaited if splenic rupture is suspected. Prompt splenectomy is the treatment of choice, although nonoperative observation and splenorrhaphy have a role in the management of selected patients with subcapsular splenic hematoma.[181,182] Because a history of trauma may be elicited in about one-half the cases of splenic rupture,[183] elimination of contact sports, attention to constipation, and caution in splenic palpation are prudent measures for at least the first month after diagnosis (see "Therapy" section).

TABLE 138.5 Neurologic Complications of Infectious Mononucleosis

Encephalitis[185–189]
Meningitis[185]
Guillain-Barré syndrome[189]
Optic neuritis[191]
Retrobulbar neuritis[192]
Cranial nerve palsies[189]
Mononeuritis multiplex[193]
Brachial plexus neuropathy[194]
Seizures[185,189]
Subacute sclerosing panencephalitis[195]
Transverse myelitis[196]
Psychosis[197]
Demyelination[198]
Hemiplegia[199]

Neurologic Manifestations

Neurologic complications, which occur in less than 1% of the cases, can dominate the clinical presentation (Table 138.5).[184–199] On occasion, these neurologic signs can be the first or only manifestation of infectious mononucleosis. In many cases the heterophile antibody determination is negative, atypical lymphocytes may be low in number or delayed in appearance, and the diagnosis must be made by changes in EBV-specific antibodies.[184,185,190] The encephalitis seen with infectious mononucleosis may be acute in onset and rapidly progressive and severe but is usually associated with complete recovery. The encephalitis is commonly manifested as a cerebellitis but may also be global.[186–188] The clinical presentation may also resemble that of aseptic meningitis. In both encephalitis and meningitis changes in the spinal fluid are mild. The opening pressure is normal or slightly elevated. A predominantly mononuclear pleocytosis may be present, with most cell counts much less than 200/mm.^{3} Atypical lymphocytes have been seen in the cerebrospinal fluid (CSF) in a number of cases. The protein level is usually normal to mildly elevated, and the glucose concentration is usually normal. Low titers of EBV VCA can be found in the CSF.[189] Cases of Guillain-Barré syndrome, Bell palsy, and transverse myelitis have been reported in primary EBV infection.[190] Although neurologic complications are the most frequent cause of death in infectious mononucleosis, the benign outcome of most of these episodes should be emphasized.[200] Eighty-five percent of the patients with neurologic complications recover completely.[184]

Hepatic Manifestations

Hepatic manifestations consist largely of self-limited elevations of hepatocellular enzyme levels, which are present in 80% to 90% of the cases of infectious mononucleosis.[201] Fulminant hepatitis is rarely seen in primary EBV infection and suggests an underlying immunodeficiency. In such cases hepatitis appears to result from infiltration of the liver by EBV-infected lymphocytes and reactive cells rather than EBV infection of hepatocytes.[202,203]

Renal Manifestations

Abnormal urinary sediment is common in acute infectious mononucleosis.[204,205] Microscopic hematuria and proteinuria are the most frequently

noted abnormalities.[206] Overt renal dysfunction is, however, extremely rare, although sporadic cases of acute renal failure in association with infectious mononucleosis have been reported.[207] The renal manifestations of infectious mononucleosis have been hypothesized as usually attributable to interstitial nephritis from renal infiltration by activated T lymphocytes.[207] Renal dysfunction in association with EBV-associated rhabdomyolysis has also been reported, although not all cases of rhabdomyolysis are accompanied by renal dysfunction.[208]

Cardiac Manifestations

Clinically significant cardiac disease is uncommon. Electrocardiographic abnormalities, usually confined to ST-T wave abnormalities, were reported in 6% of the cases in one series.[209] Pericarditis and fatal myocarditis have also been observed.[210,211]

Pulmonary Manifestations

Pulmonary manifestations of infectious mononucleosis are rare.[212–215] Early studies reported the presence of interstitial infiltrates in 3% to 5% of the cases. However, systematic examination for other causes of nonbacterial pneumonias (e.g., *Mycoplasma*) was not carried out in these studies, and whether these infiltrates were related to EBV infection is not clear. Severe pneumonia has, however, been reported, and in at least one instance EBERs, which indicate EBV infection of cells, were found in pulmonary tissue.[216,217] The attribution of pulmonary lesions to EBV infection should be made only after other pathogens have been carefully excluded.

Death

Death from infectious mononucleosis is rare.[200,218] Death may occur as a result of overwhelming EBV infection or from complications of the disease. Neurologic complications of the illness, splenic rupture, and upper airway obstruction are the most frequent causes of death from infectious mononucleosis in previously healthy persons. Deaths from complications associated with granulocytopenia, thrombocytopenia, hepatic failure, and myocarditis have also been reported.[a]

Clinical Course

Most cases of infectious mononucleosis resolve spontaneously over a 2- to 3-week period. The sore throat is usually maximal for 3 to 5 days and then gradually resolves over the course of a week to 10 days. Patients remain febrile for 10 to 14 days, but in the last 5 to 7 days the fever is usually low grade and associated with little morbidity. The prostration associated with infectious mononucleosis is generally more gradual in its resolution. As the illness resolves, patients often have days of relative well-being that alternate with recrudescence of symptoms.

Genetic Disorders Associated With Severe Epstein-Barr Virus Disease

EBV is the only virus to induce B-cell proliferation, which is primarily controlled by NK and T cells. Certain genetic disorders disrupt NK- or T-cell function, resulting in uncontrolled EBV infection. Some of these disorders affect only EBV disease, whereas others affect EBV and other infections.[221] Immunodeficiencies specific for EBV can be either X-linked or autosomal. Genetic testing should be considered in patients with severe EBV disease in the absence of known risk factors; for instance, EBV LPD in the absence of known immune suppression.

X-linked Genetic Predispositions to Severe Epstein-Barr Virus Disease

An X-linked syndrome has been described in which boys, without other evidence of immunodeficiency, develop overwhelming primary EBV infection with demonstrable virus in lymph nodes, spleen, thymus, and other organs.[222,223] This syndrome has been designated *X-linked lymphoproliferative disease 1* (XLP1) and is sometimes referred to as Purtilo syndrome or Duncan disease. Affected boys develop a large proliferation of polyclonal B and T cells in response to primary EBV infection that frequently results in fulminant hepatitis and hemophagocytic syndrome (discussed subsequently). Patients who survive primary EBV infection frequently develop progressive agammaglobulinemia, or they may develop lymphoma within several years after initial infection.[224–229] This disorder was linked to mutations in the signaling lymphocyte activation molecule (SLAM)–associated protein (SAP) gene in 1998.[230] SAP is an important mediator of signal transduction of SLAM and SLAM-related receptors found on T and NK cells.[231,232] Elegant studies of female XLP carriers has demonstrated that the critical defect responsible for EBV susceptibility arises from the inability of $CD8^+$ T cells to recognize antigens presented on B lymphocytes.[233] Thus the specificity of SAP mutation for EBV results from the propensity of this virus to infect and transform B cells.

Mutations in the X-linked inhibitor of apoptosis *(XIAP)* gene results in XLP2; this syndrome is a distinct clinical entity with a high incidence of hemophagocytosis and minimal risk of LPD.[234]

The X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia (XMEN) syndrome is caused by mutations in the magnesium transporter 1 (MAGT1), and can result in B-cell lymphoma.[235] Preliminary data in two patients with XMEN suggests that magnesium supplementation reduces the numbers of EBV-infected cells in blood and therefore may be of clinical benefit in this disease.[236]

Autosomal Genetic Predispositions to Severe Epstein-Barr Virus Disease

Several rare autosomal-recessive genetic disorders have been linked with severe EBV infection.[221] Mutations in the genes responsible for these disorders include *ITK* (IL-2–inducible T-cell kinase), *CD27*, *CORO1A* (coronin actin-binding protein 1A), and *LRBA* (lipopolysaccharide [LPS]-responsive beige-like anchor protein). These disorders can lead to EBV-associated lymphoproliferative syndrome. *CD27* or *ITK* mutations can also lead to *hemophagocytic lymphohistiocytosis* (HLH) (see later).

Genetic Disorders That Predispose to Severe Epstein-Barr Virus Disease and Other Infections

A number of autosomal genetic disorders can result in severe EBV disease and a predisposition to other infections, including those caused by bacteria or other viruses.[221] These include mutations in *PIK3CD* (phosphoinositide-3-kinase [PI3K] catalytic subunit 110delta), *CTPS1* (cytidine triphosphate [CTP] synthase 1), *STK4* (serine threonine kinase 4), *GATA2* (GATA binding protein 2), *MCM4* (minichromosome maintenance complex component 4), *FCGR3A* (Fc gamma receptor 3A [CD16a]), *CARD11* (caspase recruitment domain-containing protein 11), or *ATM* (ataxia telangiectasia mutated). Severe combined immunodeficiency (SCID) can be due to mutations in multiple genes. Mutation of *WAS* (Wiskott-Aldrich syndrome protein) can lead to severe EBV disease or other infections and is X-linked, rather than autosomal.

Chronic Active Epstein-Barr Virus Infection

Persistent EBV infection has been suggested as a frequent cause of fatigue and malaise in young and middle-aged adults.[237–240] This speculation has arisen from reports of a syndrome characterized by fatigue, sore throat, mild cognitive dysfunction, and myalgias initially noted in association with an apparent increase in antibody titers to the EBV EA complex (see "Laboratory Diagnosis" section).[237,238] These reports have included primarily young adults, usually with a female preponderance, who report a nonspecific symptom complex more reminiscent of the prodrome of infectious mononucleosis than of the syndrome itself (often known as *chronic mononucleosis syndrome* or *chronic fatigue syndrome*). These patients have been noted either sporadically[237,238,241] or in epidemic clusters.[240] The initial suggestion that the syndrome is attributable to EBV has become untenable on the basis of serologic and epidemiologic observations.[241,242] Investigation of the syndrome has been hampered by the vagueness of the symptoms and the absence of objective laboratory diagnostic criteria. A consensus case definition that focuses on fatigue rather than on EBV as the central feature of the syndrome has emerged.[243,244] The chronic fatigue syndrome is discussed in more detail in Chapter 130.

[a]References 163, 176, 200, 211, 219, 220.

In contrast to the nonspecific syndrome just noted, patients in whom EBV appears to play a direct role in ongoing objective organ system dysfunction have been identified.[245–249] These cases have been termed *chronic active EBV* (CAEBV) infection and are extremely rare in the United States. CAEBV is more frequent in Asia and South America, where, in striking contrast to other EBV LPDs, it has been associated with EBV infection of NK or T cells.[250–252] To distinguish CAEBV from other nonspecific syndromes, diagnostic criteria have been proposed.[253–255] First, patients have severe illness that lasts more than 6 months, began as primary EBV infection, and is associated with markedly elevated titers to EBV lytic antigens (VCA immunoglobulin G [IgG] ≥ 1:640 or EA IgG ≥ 1:160) or EBV DNA level in the blood (>300 copies/μg DNA). Second, histologic evidence of major organ involvement, such as interstitial pneumonia, hemophagocytosis, uveitis, lymphadenitis, or persistent hepatitis, is present. Third, affected tissues should contain elevated amounts of EBV DNA, RNA, or proteins by in situ hybridization or immunohistochemical staining. The prognosis for these patients is poor, with most dying of progressive pancytopenia and hypogammaglobulinemia or NK-/T-cell nasal lymphoma within a few years, although survival for more than 10 years after diagnosis has been observed.[248] Presentation before age 8 years, without thrombocytopenia, or with an NK-cell phenotype is associated with an improved prognosis.[256] Antiviral therapy with acyclovir or ganciclovir is of no proven benefit, but case reports of adoptive immunotherapy and bone marrow transplantation for patients with CAEBV have been found.[247,257–261] The pathogenesis of CAEBV is not well understood but is probably the result of an immune defect that permits the proliferation of EBV-infected T or NK cells.[262] Certain genetic defects have been linked to CAEBV and hemophagocytic lymphohistocytosis (described later). The limited number of cases of CAEBV diagnosed in the United States often includes disease associated with EBV infection of B cells.[261] Whether B-cell CAEBV represents a distinct clinicopathologic entity is an intriguing but academic question because the prognosis is similarly poor and treatment options are no different than NK- or T-cell CAEBV.

Epstein-Barr Virus–Associated Hemophagocytic Lymphohistiocytosis

The hemophagocytic syndrome is characterized by excessive lymphocyte and macrophage (histiocyte) activation and infiltration of bone marrow, lymph nodes, spleen, and liver, with prominent phagocytosis of erythrocytes and nucleated cells.[263] Although the hemophagocytic syndrome can occur as a consequence of XLP syndrome or CAEBV, it can present as a distinct clinical entity in the absence of these diseases and has been called HLH. Children are primarily affected, usually before the age of 3 years, with high fevers, pancytopenia, liver dysfunction, and coagulopathy.[263,264] HLH usually develops as the sequela of a viral infection, most commonly primary EBV infection. Most, if not all, cases of HLH are associated with a monoclonal proliferation of T cells that are usually $CD8^+$.[264–266] In EBV-associated HLH most infiltrating T lymphocytes are monoclonally infected with EBV.[267–269] These unregulated proliferating T cells are thought to account for the markedly elevated levels of tumor necrosis factor (TNF)-α, IFN-γ, macrophage-specific colony-stimulating factor, IL-6, IL-10, IL-18, and soluble IL-2 receptor that typify HLH and drive macrophage activation.[270,271] The disease group familial hemophagocytic lymphohistiocytosis (FHL) comprises rare diseases that result from mutations in genes responsible for the maturation or release of CTL- or NK-cell cytotoxic granules, or for target cell entry of these granule proteins. These genes exhibit autosomal-recessive inheritance and are due to mutations in *PRF1* (perforin), *UNC13D* (MUNC13-4 protein), *STX11* (syntaxin 11), or *STXBP2* (MUNC18-2). These genes are responsible for FHL2, FHL3, FHL4, and FLH5, respectively.[221] FHL2, 3, and 5 are also associated with CAEBV. Mutations in *ITK* or *CD27* (described earlier) can also lead to HLH. Untreated, the prognosis of EBV-associated HLH is poor. However, treatment with the etoposide, dexamethasone, and cyclosporine-based HLH-94 protocol has been associated with survival rates of approximately 75%.[272] A newer protocol, HLH-2004, has been published, which confirmed the efficacy of etoposide and dexamethasone, but the benefit of cyclosporine has been questioned.[273,274]

Oral Hairy Leukoplakia

As previously stated, reactivation of lytic EBV replication with viral shedding in the saliva is usually entirely asymptomatic. An important exception to this rule is *oral hairy leukoplakia* (OHL), which arises as a corrugated or "hairy" white lesion usually on the lateral surface of the tongue but sometimes elsewhere. This nonmalignant lesion is seen in AIDS and other states of immunosuppression and is caused by unchecked lytic replication of EBV.[275,276] The diagnosis of OHL is based on the typical appearance of the lesions in the appropriate clinical setting. The differential diagnosis includes oral candidiasis, which, unlike OHL, can be removed with gentle scraping of the tongue. Alternatively, thrush may be diagnosed with a potassium hydroxide wet mount or should respond to an empirical trial of antifungal therapy. Biopsy for histology and in situ hybridization or immunofluorescence staining for EBV is rarely necessary but confirms the diagnosis. Polymerase chain reaction (PCR) detection of EBV in "oral scrapes" is neither sensitive nor specific for OHL.[277]

Epstein-Barr Virus–Associated Malignant Diseases

EBV is an extremely well-adapted parasite that establishes lifelong latent infection without lasting adverse effects in about 95% of the human population. However, in immunosuppressed hosts, the growth-transforming properties of EBV can result in malignant disease. EBV in conjunction with environmental or genetic factors, or both, can rarely result in malignant disease in immunocompetent hosts (Table 138.6).

TABLE 138.6 Prevalent EBV-Associated Malignant Diseases

MALIGNANT DISEASE	EBV ASSOCIATION	POPULATION AT RISK	COFACTORS
Lymphoproliferative disease	~90%	Transplantation patients	Immunosuppression
Hodgkin lymphoma	~50%, depending on histologic subtype	Children (developing countries) Young adults (western countries)	Unknown
Diffuse large B-cell lymphoma	~10%	?Elderly	Unknown
Nasopharyngeal carcinoma	100% undifferentiated	Southern Chinese, Inuit	Genetic predisposition and dietary factors
Gastric cancer	~4%[a] 20% 30%[a] 100% squamous	Unknown	Unknown
Burkitt lymphoma	>95% endemic ~20% sporadic ~40% HIV associated	African children Independent of $CD4^+$ count	*c-myc* translocations (all) Malaria (endemic only)

AIDS, Acquired immunodeficiency syndrome; *CNS,* central nervous system; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus.
Modified from Kieff E, Rickinson AB. Epstein-Barr virus and its replication. In: Knipe D, Howley P, Griffin D, et al, eds. Fields Virology. *5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:2603–2654.*

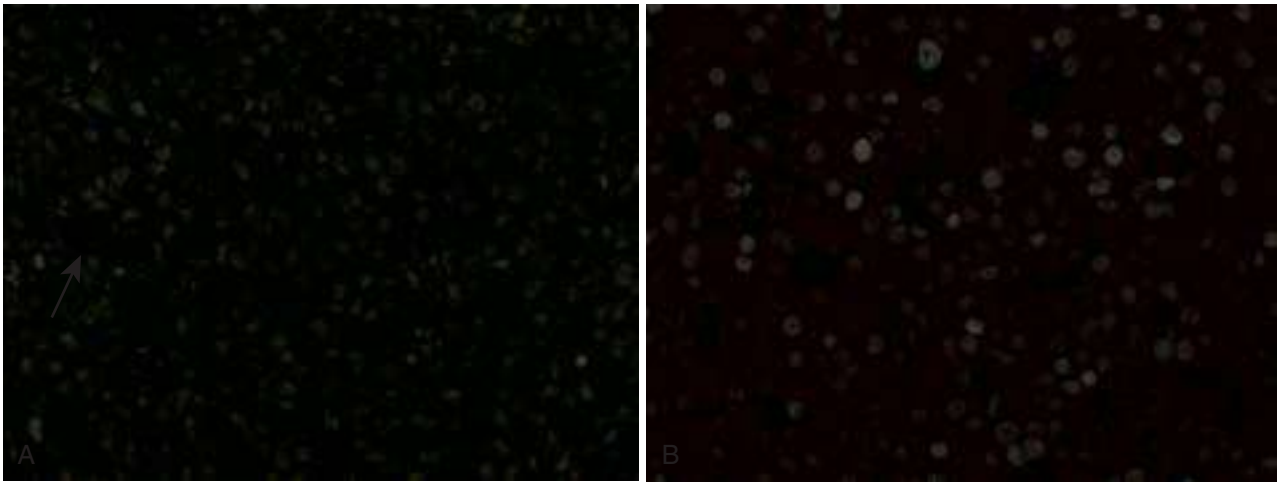

FIG. 138.2 Posttransplant lymphoproliferative disease involving the colon. (A) Tumor is composed of large, atypical lymphoid cells (hematoxylin and eosin). Scattered macrophages *(arrow)* are seen, producing "starry-sky" appearance. (B) In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA *(brown)* shows variably intense nuclear staining in most tumor cells, indicating EBV infection. (Original magnification, ×400.) *(Courtesy Dr. Jeffery Kutok.)*

Lymphoproliferative Disease

In the absence of effective immune surveillance, uncontrolled proliferation of EBV-infected B lymphocytes may occur. This disorder is referred to as *LPD* and represents the in vivo equivalent of the immortalized B-cell lines seen with EBV infection in vitro. Proliferating B cells in LPD express all EBV latent proteins (latency III), including the EBNA3 proteins that are normally strong targets for $CD8^+$ cytotoxic T cells (see Table 138.2).[27,56] Patients with LPD typically present with symptoms similar to those of infectious mononucleosis or with fever and lymphomatous infiltration of lymph nodes, spleen, liver, bone marrow, kidney, lung, CNS, or intestine (Fig. 138.2). The frequency of this disease in solid-organ and bone marrow transplant recipients has led to the designation *post-transplant lymphoproliferative disease* (PTLD), but it can be seen in any patient receiving high-dose immune suppression or in those with inherited disorders that affect T-cell immunity. Patients with more severe cellular immune impairment, such as those receiving T-cell–depleted bone marrow transplants, cord transplants, haploid identical stem cell transplants, or antithymocyte globulin, are at increased risk for PTLD, as are those with primary EBV infection after transplantation.[52,53] Notably, the timing of risk for PTLD differs in stem cell versus solid-organ transplant recipients because of differences in immune suppression. In stem cell transplants the overall risk is 1%, and this risk is greatest within the first 5 months after transplantation when the immune suppression is severe and before immune reconstitution.[278] In solid-organ transplantation the risk is more prolonged because of the need for long-term immunosuppression. PTLD is most common in multivisceral transplantation (up to 33%); least common in renal, liver, and heart transplantation (≈1%–2.5%); and intermediate in lung (≈6%) or intestinal (≈10%) transplantation.[279–281] Up to half of PTLDs occur in the setting of primary EBV infection. These may arise early after transplantation and often occur in children, who are more likely to be EBV seronegative.[282] The cells of origin for PTLD may be either host derived or donor derived. Most often, PTLD occurs in host-derived cells in solid-organ transplantation and in donor-derived cells in allogeneic stem cell transplantation.[283–286]

Burkitt Lymphoma

Burkitt lymphoma is a high-grade lymphoma with characteristic small, noncleaved B cells and is endemic in equatorial Africa. Endemic Burkitt lymphoma is geographically associated with *Plasmodium falciparum* malaria and usually arises as a tumor of the jaw. Although the fact that greater than 90% of Burkitt lymphomas are EBV associated has been long appreciated, the role of the virus in its pathogenesis is unclear because most of the EBV transforming genes are not expressed. In fact, viral gene expression is restricted to *EBNA1* and the EBERs (latency I; see Table 138.2).[49,54,55] It is unlikely that EBV is merely a passenger because terminal repeat analysis of EBV genomes has confirmed that the viral infection occurred before expansion of the tumor.[287] Also, persons in endemic regions with elevated titers to EBV lytic antigens are at high risk for Burkitt lymphoma.[288] Children from an endemic region with high risk of Burkitt lymphoma were shown to be infected with EBV earlier in life and had elevated viral loads during infancy compared with those from a nonendemic area.[289] In addition to EBV association, virtually all Burkitt lymphomas contain a chromosomal translocation that involves the *c-myc* oncogene and an immunoglobulin heavy- or light-chain locus. The unregulated expression of this potent oncogene probably supplants the need for expression of many of the EBV-transforming genes that otherwise would serve as targets for immune surveillance. In addition to the endemic form of the disease, sporadic Burkitt-like lymphomas are seen that typically arise as abdominal masses. These lymphomas also contain *c-myc* translocations but are less consistently associated with EBV (only about 25% of cases).[290] Persons with HIV are at increased risk for Burkitt-like lymphoma, independent of degree of immunodeficiency.[291]

Hodgkin Lymphoma

Hodgkin lymphoma is an unusual malignant disease in that the malignant Hodgkin and Reed-Sternberg (HRS) cells constitute as little as 1% of the tumor. The balance of the tumor mass is composed of an infiltrate of reactive mononuclear and stromal cells. An infectious etiology for Hodgkin lymphoma was proposed as early as 1966 on the basis of the epidemiology of the disease, but definitive evidence was slow to evolve because of technical difficulties presented by the scarcity of the HRS cells.[292–294] Subsequently, EBV DNA and protein expression were shown in HRS cells from some forms of Hodgkin lymphoma.[295,296] Furthermore, symptomatic EBV mononucleosis increases the risk (relative risk, 4.0) for EBV-positive Hodgkin disease as was shown in a Scandinavian study; Hodgkin disease occurred a median of 4 years after the mononucleosis syndrome.[297] The strongest associations are with the mixed cellularity (Fig. 138.3) and lymphocyte-depleted histologic subtypes.[70] No association with the lymphocyte-predominant subtype could be proved, and this is now considered a distinct, non–EBV-associated entity. There is, however, general agreement that in EBV-associated Hodgkin lymphoma, the malignant HRS cells represent postgerminal center B cells that express a latency II EBV gene pattern (*LMP1, LMP2, EBNA1,* and EBERs; see Table 138.2). EBV genomes, when present in HRS cells, are monoclonal with terminal repeat analysis, which suggests that EBV infection preceded the development of the malignant disease.[296] Many HRS contain "crippling mutations" in their immunoglobulin genes or fail to express surface immunoglobulins and thus lack a critical anti-apoptotic signal normally transmitted by the B-cell receptor (BCR).

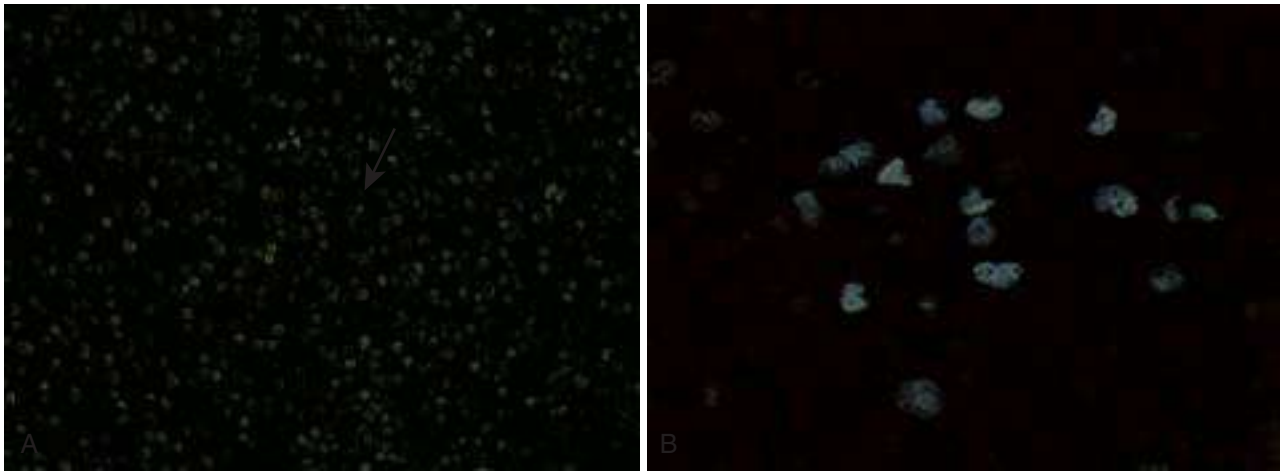

FIG. 138.3 Mixed cellularity classic Hodgkin lymphoma. (A) Lymph node architecture is effaced by infiltrate composed of small lymphocytes, epithelioid histiocytes, plasma cells, eosinophils, and Hodgkin and Reed-Sternberg (HRS) cells (*arrow;* hematoxylin and eosin). (B) In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA *(brown)* shows EBV infection in malignant HRS cells. (Original magnification, ×400.) *(Courtesy Dr. Jeffery Kutok.)*

Expression of EBV *LMP2A* can serve as a surrogate BCR signal and may allow the survival of cells otherwise destined to undergo apoptosis from failure to express functional immunoglobulin.[298] Activation of NF-κB signaling is also typical of HRS cells, which suggests activation of this pathway by LMP1.[299] In some EBV-negative HRS cells, inhibitor of kappa Bα *(IκBα)* gene mutations have been reported that could serve as an alternative means of constitutively activating the NF-κB pathway.[300] Speculation is tempting that EBV gene expression can serve as one step in the malignant transformation of HRS cells that is circumvented by other mutational events in EBV-negative forms of the disease.

Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common type of high-grade non-Hodgkin lymphoma, accounting for about 40% of cases worldwide.[301] These lymphomas are diffuse in the sense that they lack the normal architecture of more differentiated lymphomas (e.g., follicular). Lymphomas exhibiting DLBCL morphology are quite heterogeneous and are thought to represent multiple distinct diseases. An important advance was the use of gene expression profiling to subdivide DLBCL into germinal center B-cell–like (GCB) and activated B-cell–like (ABC) subtypes, with the latter carrying a worse prognosis.[302] About 10% of DLBCL are EBV positive, most exhibiting the ABC phenotype. EBV-positive DLBCLs were originally reported to be most prevalent in elderly patients and attributed to waning cellular immunity with advancing age.[303] In the setting of HIV infection, the frequency of EBV positivity in DLBCL increases to about 60%.[290] However, EBV-positive DLBCL are increasingly appreciated to occur in younger immunocompetent persons and have most recently been reclassified as EBV+ DLBCL-NOS.[301,304] The NOS (not otherwise specified) is to ensure that tumors that can already be classified as well defined, rare EBV-associated lymphomas (see Table 138.7) are not included in this category. One rare lymphoma meriting special mention is primary CNS lymphoma. This subtype of DLBCL bears a striking resemblance to PTLD and is highly associated with EBV, with rates approaching 90%. As with PTLD, it occurs in the setting of profound immunosuppression; those with the lowest $CD4^+$ counts for the longest time are at greatest risk. The prognosis is poor, and excluding more treatable conditions such as CNS toxoplasmosis is essential.

Nasopharyngeal Carcinoma

NPC is a rare disease in most western countries, but its prevalence rate approaches 50 per 100,000 in southern China and among the Inuit in Alaska.[305] An association between EBV and NPC was first suggested with the observation that patients with this malignant disease had elevated IgG and immunoglobulin A (IgA) titers to EBV lytic antigens (VCA and EA).[306] The undifferentiated form (Fig. 138.4) is EBV associated in nearly 100% of cases, whereas squamous NPCs are inconsistently EBV associated, particularly outside endemic regions. The undifferentiated form bears some resemblance to Hodgkin lymphoma in that the tumor consists of EBV-positive cells (of epithelial origin in this case) that express a latency II gene pattern infiltrated with reactive, nonmalignant lymphoid cells.[71,307,308] Terminal repeat assays have confirmed that these epithelial cells contain monoclonal EBV genomes, placing EBV infection early in the genesis of the malignant disease as seen in EBV-associated B-cell neoplasia.[309] In addition to EBV, evidence indicates that genetic and environmental factors may have roles in tumor development.[310–312] Recent reviews of epidemiologic data indicate that the incidence of NPC is gradually declining and that mortality has fallen substantially. Potential explanations for this improvement include expanded population

TABLE 138.7 Rare EBV-Associated Cancers or Proliferative Disorders

DISEASE	% EBV POSITIVE	REFERENCE
EBV Infection of B Cells		
Lymphomatoid granulomatosis	100	488
Primary effusion lymphoma	75–90	489
Pyothorax-associated lymphoma	70	490
Plasmablastic lymphoma	75–90	491
EBV-positive mucocutaneous ulcer	100	492
Angioimmunoblastic T-cell lymphoma	100[a]	493
Primary CNS lymphoma	10–90[b]	494
EBV Infection of T Cells		
Nasal NK-/T-cell lymphoma	100	495
Chronic active EBV	100	496
Hemophagocytic lymphohistiocytosis	75	497
Nonhepatosplenic gamma-delta T-cell lymphoma	50	498
Hydroa vacciniforme–like lymphoma	100	324
EBV Infection of Nonlymphoid Cells		
Leiomyosarcomas (associated with immunosuppression)	100	499

[a]This T-cell lymphoma is characterized by infiltration of EBV-positive B cells.
[b]~10% in HIV-negative patients, ~90% in HIV-positive patients.
CNS, Central nervous system; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus.

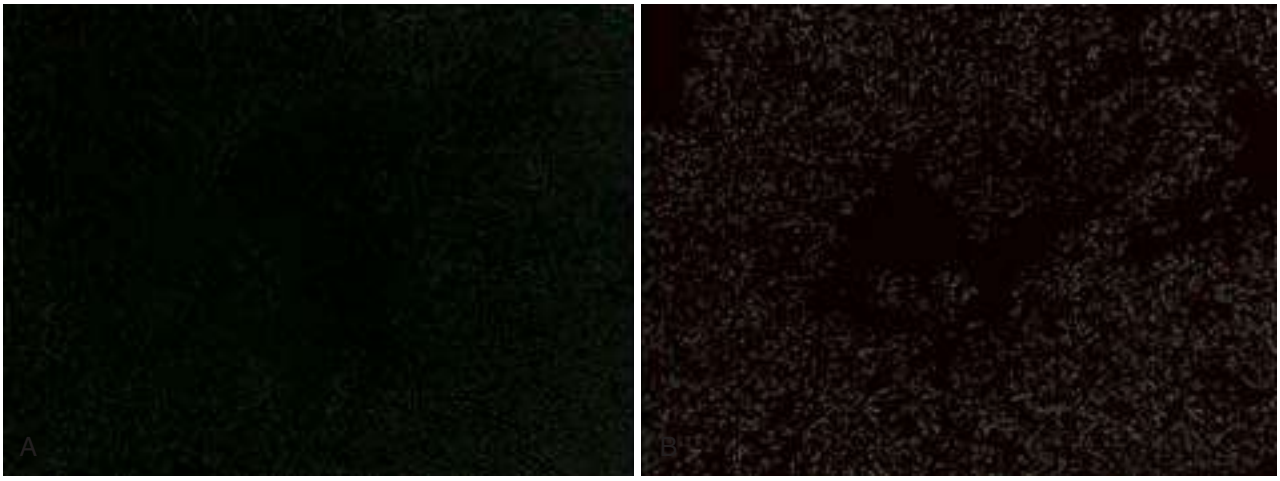

FIG. 138.4 Nasopharyngeal carcinoma (NPC). (A) Nests of metastatic undifferentiated NPC in a fibrous stroma in lymph node (hematoxylin and eosin). Metastases often lack infiltrating lymphocytes. (B) In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA *(brown)* shows EBV infection in most cells in the same area of tissue. (Magnification, ×100.) *(Courtesy Dr. Miguel Rivera.)*

screening and advances in radiotherapy and treatment with systemic agents.[313]

Gastric Carcinoma

Since the first report of an EBV-positive gastric carcinoma in 1990, numerous studies have confirmed that approximately 10% of gastric carcinomas worldwide are EBV associated.[314,315] Lymphoepithelioid gastric cancers in which malignant epithelial cells are surrounded by lymphoid stroma are EBV positive in up to 80% of cases, as are a small fraction of gastric cancers with typical morphology.[316] The proportion of EBV-positive tumors also varies inversely with gastric cancer incidence, ranging from approximately 17% in the United States and Germany to 4% in China.[317] EBV genomes are monoclonal by terminal repeat analysis consistent with EBV infection occurring as an early event in tumorigenesis.[316] EBV latent gene expression in gastric carcinomas consists of *EBNA1,* EBERs, EBV miRNAs, and variable *LMP2A* expression, and rarely LMP1, consistent with either a latency I or II pattern.[318] The role(s) played by these EBV gene products in gastric cancer tumorigenesis remains to be defined.

Other Malignant Diseases

Nasal NK-/T-cell lymphomas (Table 138.7) are angiocentric lymphomas that typically present as a midline facial destructive disease (lethal midline granuloma) but can also arise at other extranodal sites.[319] They are highly associated with EBV, and, like NPC and Hodgkin lymphoma (HL), malignant cells typically express a latency II gene pattern.[320] As discussed previously, persons with CAEBV are at high risk of development of this subtype of peripheral T-cell lymphoma.[321,322] A similar angiocentric malignant disease, *lymphomatoid granulomatosis* (LG), is now known to be a distinct clinical entity caused by EBV-infected proliferating B lymphocytes with an exuberant reactive T-cell infiltrate. Patients with LG typically present with pulmonary lesions and synchronous brain, skin, kidney, or liver lesions that can be easily mistaken for disseminated fungal infections.[323] Another rare EBV-associated cutaneous NK-/T-cell LPD is hydroa vacciniforme–like lymphoma, seen primarily in children in Central and South America and Asia.[324] An EBV association has also been reported in some breast cancers, hepatocellular cancers, and smooth muscle tumors, but the contribution of EBV to the pathogenesis of these malignant diseases remains to be established.[325,326]

In addition to the Burkitt-like lymphomas and DFCL, persons with HIV (or other immunosuppression) are at increased risk for an unusual EBV-associated lymphoma, *primary effusion lymphoma* (PEL).[327,328] These human herpesvirus 8 (Kaposi sarcoma–associated herpesvirus)–related lymphomas are often coinfected with EBV. PELs derived their name from a tendency to arise within potential body cavities, such as the pleural, pericardial, or peritoneal spaces, and frequently follow an aggressive clinical course. *Pyothorax-associated lymphoma* is sometimes confused with PEL but differs in that it is strictly EBV (not Kaposi sarcoma–associated herpesvirus)-associated, forms an identifiable mass lesion, arises in patients with long-standing pleural-based inflammation, and is seen in patients without HIV.[329] In pediatric patients with AIDS, EBV has been reported in leiomyosarcomas in HIV-infected individuals and also in transplant recipients.[330] Another study reported EBV in leiomyosarcomas in both HIV-infected and also transplant recipients.[331]

Multiple Sclerosis and Other Autoimmune Diseases

Viruses have long been suspected as environmental triggers for autoimmune diseases in genetically predisposed individuals. EBV has been a candidate on the basis of seroepidemiologic studies that link it to systemic lupus erythematosus, rheumatoid arthritis, and *multiple sclerosis* (MS).[332–335] Although definitive proof is lacking, patients with MS are more likely to be EBV seropositive than age-matched control subjects.[334] This difference is most notable in pediatric patients in whom the rate of EBV seropositivity of control subjects is much lower.[336] Patients with MS have higher titers to EBV antigens, whereas elevated titers are not observed in other viruses, including CMV, VZV, and HSV.[336–340] Furthermore, prospective studies have shown that antibody titers, particularly to EBNA1, are elevated more than 10 years before the onset of MS symptoms.[341–343] In addition, a history of symptomatic infectious mononucleosis is associated with a twofold increased risk of development of MS relative to asymptomatic primary EBV infection.[344] Finally, Cepok and associates[345] determined that the two most frequent MS-specific oligoclonal IgG bands in the CSF of MS patients recognized peptides present in the EBV proteins EBNA1 and BRRF2. Thus a body of data supports an association of EBV infection with MS. Whether these observed differences in immune responses to EBV cause MS or are merely an epiphenomenon that results from the same immunologic dysregulation that causes MS remains to be seen.

LABORATORY DIAGNOSIS

Infectious Mononucleosis

Hematologic Findings

The central hematologic manifestation of the illness is a circulating lymphocytosis. At presentation, a relative and absolute mononuclear lymphocytosis is found in about 70% of the cases. The lymphocytosis peaks during the second or third week of illness, and monocytes and lymphocytes account for 60% to 70% of the total white cell counts of 12,000 to 18,000/mm.^{3} However, higher white cell counts are not uncommon, and occasional patients manifest 30,000 to 50,000 leukocytes/mm.^{3} Atypical lymphocytes are the hematologic hallmark of infectious mononucleosis and account for about 30% of the differential count at

their zenith.[145,147] The wide range in the atypical lymphocytosis is well recognized, and some cases show none or only a few atypical lymphocytes, whereas 90% or greater of the circulating lymphocytes may be atypical in other cases. These atypical lymphocytes are composed largely of reactive $CD8^+$ cytotoxic T cells, and their degree of elevation correlates with symptom severity.[96] Atypical lymphocytes are not pathognomonic for infectious mononucleosis and can be observed with CMV infection, primary HIV infection, viral hepatitis, toxoplasmosis, rubella, mumps, and roseola and in drug reactions (Table 138.8).[346,347] The atypical lymphocyte is generally larger than the mature lymphocyte encountered in peripheral blood. The cytoplasm is often vacuolated and basophilic, and its edges have a rolled-up appearance with a tendency to flow around adjacent RBCs on a peripheral smear. Nuclei are often lobulated and are eccentrically placed. Although the cells may appear quite immature, the heterogeneity of morphologic and tinctorial characteristics of such cells helps to distinguish atypical lymphocytes from the more uniform lymphoblasts of acute lymphocytic leukemia.[6,346]

A relative and absolute neutropenia is evident in 60% to 90% of the cases, and neutrophils that remain in circulation exhibit a mild left shift.[172,173] In most cases the neutropenia is mild, with total granulocyte counts of 2000 to 3000/mm,[3] although profound granulocytopenia has also been reported.[171,174–178,225,348] The neutropenia is usually self-limited, and counts rise gradually toward normal by a month after presentation.[172]

Thrombocytopenia is also common, and 50% of the patients in one series manifested platelet counts of less than 140,000/mm^3.[165] Although cases of profound thrombocytopenia with bleeding have been reported,[166–170] these cases are rare and contrast markedly with the generally benign course of the common mild thrombocytopenia.

TABLE 138.8 Differential Diagnosis of Atypical Lymphocytosis

Epstein-Barr virus primary infection (infectious mononucleosis)
Cytomegalovirus primary infection (heterophile-negative mononucleosis)[a]
Human herpesvirus 6 primary infection (roseola)
Primary human immunodeficiency virus infection
Toxoplasmosis
Acute viral hepatitis
Rubella, mumps
Drug reactions (e.g., phenytoin, sulfa)

[a]Cytomegalovirus is the most common cause of heterophile-negative mononucleosis.

Heterophile Antibodies

Heterophile antibodies are low-affinity IgM antibodies with broad specificity for predominantly carbohydrate antigens that can react with molecules found on the surface of a number of nonhuman erythrocytes (hence heterophile = other loving). Originally described by Paul and Bunnell[7] as sheep erythrocyte agglutinins, they play no role in EBV immunity but may be a consequence of polyclonal B-cell infection by the virus. Various technical improvements, such as preadsorption of serum against other antigens or use of purified beef erythrocyte extracts as the detection reagent, have increased the specificity of this test to around 95% to 99% for primary EBV infection. Commercial spot kits such as the Monospot test are available and generally equivalent to traditional heterophile antibody assays based on erythrocyte agglutination.[349] Occasional false-positive heterophile tests have been reported in patients with lymphoma or hepatitis, but the rarity of this event makes confirmation of a positive Monospot test result with EBV-specific serology unnecessary.[350–352] Three cases of false-positive Monospot tests in the setting of primary HIV infection have been reported.[353] One study of 132 patients with positive Monospot test results found no instances of primary HIV infection.[354] However, the exact rate of false-positive heterophile results among patients with primary HIV infection is not known. Heterophile antibodies are, however, relatively insensitive for diagnosing primary EBV infection, with sensitivities ranging from 70% to 90% for adults and adolescents and less than 50% in children.[349] When absent at the onset of illness, heterophile antibodies may appear later in the course. Thus, in the appropriate clinical setting, a positive heterophile test is sufficient to confirm the diagnosis of infectious mononucleosis, but a negative test does not exclude it.

Epstein-Barr Virus–Specific Antibodies

In addition to the transient heterophile antibodies, infection with EBV results in the development of virus-specific antibodies. Antibodies are formed to structural proteins or VCAs, nonstructural proteins expressed early in the lytic cycle or EAs, and nuclear proteins expressed during latent infections or EBNAs. A determination of EBV-specific antibodies is rarely necessary for the diagnosis of infectious mononucleosis because 90% of the cases are heterophile positive, and few false-positive results are obtained if the test is properly performed (see previous discussion). For heterophile-negative cases and for diagnosis in atypical cases, a determination of EBV antibodies may help to establish a cause (Table 138.9).[355]

TABLE 138.9 Antibodies to Epstein-Barr Virus

ANTIBODY SPECIFICITY	TIME OF APPEARANCE IN INFECTIOUS MONONUCLEOSIS	PERCENTAGE OF EBV-INDUCED MONONUCLEOSIS CASES WITH ANTIBODY	PERSISTENCE	COMMENTS
Viral Capsid Antigens				
IgM VCA	At clinical presentation	100	4–8 wk	Highly sensitive and specific; major diagnostic utility
IgG VCA	At clinical presentation	100	Lifelong	High titer at presentation and lifelong persistence make IgG VCA more useful as epidemiologic tool than as diagnostic tool in individual cases
Early Antigens				
Anti–EA-D	Peaks at 3–4 wk after onset	70	3–6 mo	Correlated with severe disease; also seen in nasopharyngeal carcinoma
Anti–EA-R	2 wk to several mo after onset	Low	2 mo to >3 yr	Occasionally seen with unusually severe or protracted illness; also seen in African Burkitt lymphoma
Latent Antigen				
EBV nuclear antigen	3–4 wk after onset	100	Lifelong	Late appearance helpful in diagnosis of heterophile-negative cases

EA, Early antigen; *EBV,* Epstein-Barr virus; *IgG,* immunoglobulin G; *IgM* immunoglobulin M; *VCA,* viral capsid antigen.

Viral Capsid Antigen Antibodies

Antibodies to VCA as measured with immunofluorescence arise early in the course of the illness and are seen at presentation in most cases. IgG antibodies to VCA are usually present at titers of 80 or greater on the first visit to a physician. Because these initially detected levels are close to peak VCA titers, a fourfold rise in titer is seen in only 10% to 20% of the cases. After recovery, detectable titers of VCA IgG antibody are maintained for life. Thus IgG VCA antibody titers may be of little help in the diagnosis of infectious mononucleosis. Conversely, IgM antibodies to VCA are sensitive and specific for infectious mononucleosis. IgM antibody titers are present in about 75% of patients at the onset of illness, and 95% will eventually develop them.[356] Titers fall rapidly thereafter, and in only 10% of the cases are titers greater than 5 retained by 4 months after diagnosis.[357,358] IgM VCA antibodies are not seen in the general population; thus their presence is virtually diagnostic of acute EBV infection.

Early Antigen Antibodies

Serum antibodies to EAs are also seen with indirect immunofluorescence, and two distinct patterns of fluorescence emerge.[355,358] Certain sera stain both nuclei and cytoplasm diffusely (anti–EA-D), whereas the staining of other sera is restricted (anti–EA-R) to cytoplasmic aggregates. Anti–EA-D antibody is found in about 70% of patients with acute infectious mononucleosis (see Table 138.9). Anti–EA-D titers arise later in the course of illness than those to VCA and disappear after recovery. Anti–EA-D antibodies may be found in the sera of patients with advanced NPC but are absent from the general population. The appearance of anti–EA-D antibodies in a patient with IgG VCA antibodies suggests recent EBV infection. Unfortunately, only 70% of EBV-induced cases manifest anti–EA-D antibodies. The presence and titer of anti–EA-D antibodies correlate with the duration and severity of clinical illness.[358] Anti–EA-R antibodies are only occasionally seen in infectious mononucleosis (see Table 138.9). They are present more often in protracted or atypical cases, arise after the anti–EA-D antibodies peak, and remain detectable for up to 2 years.[359] Anti–EA-R antibodies are also present in higher titers in patients with African Burkitt lymphoma and occasionally in healthy persons who also have high VCA titers.[360] Currently, commercial laboratories typically do not differentiate anti–EA-R and anti–EA-D.

Epstein-Barr Nuclear Antigen Antibodies

Antibodies to EBNAs appear late in the course of all cases of infectious mononucleosis and persist for life.[361] The appearance of EBNA antibodies in a patient who was previously VCA positive and EBNA negative is strong evidence of recent EBV infection. These antibodies may be reactive against any of the six nuclear proteins expressed during latent infection. Neutralizing antibodies to EBV also appear late in the course of infectious mononucleosis and reach maximal levels 6 to 7 weeks after the onset of illness.[362] Neutralizing antibodies persist at stable titers (mean, 40) for life. The appearance or a rise in titer of neutralizing antibodies to EBV also indicates recent EBV infection. Neutralizing antibodies are, however, difficult to measure, and tests for them are not routinely available.

Culture of Epstein-Barr Virus

EBV may be cultured from oropharyngeal washings or from circulating lymphocytes of 80% to 90% of patients with infectious mononucleosis.[90,91,94,108,363] Cultivation of the virus is, however, not routinely available in most diagnostic virology laboratories. This, coupled with the ubiquity of virus shedding in both healthy persons and in those with unrelated illnesses, renders cultivation of the virus of little clinical use (see Table 138.1). Rapid diagnostic techniques based on DNA hybridization or monoclonal antibody techniques have also been developed but for similar reasons are not helpful in the diagnosis of mononucleosis.[364–366] Of interest, up to 50% of memory B cells are infected with EBV during infectious mononucleosis, compared with 1 in 10^4 to 1 in 10^6 memory B cells that contain virus in healthy individuals.[367,368]

Epstein-Barr Virus Viral Load

EBV DNA can be detected in lymphocytes and plasma early in the course of infectious mononucleosis. Detection of viral DNA in plasma is otherwise infrequent in healthy individuals.[369] Low levels of EBV DNA can be detected in blood up to 3 weeks before onset of infectious mononucleosis symptoms in some individuals, and levels increase rapidly close to the onset of illness.[26] EBV viral load in blood is initially high in mononucleosis but then rapidly declines.[370]

The detection of EBV DNA from blood is being increasingly used for a number of EBV-associated diseases. In its most modern form DNA is quantitated with real-time PCR to determine a specific copy number. Although the use of EBV loads holds much promise, the technique is currently hampered by a lack of standardization across different centers. For instance, different sample types are used to assay viral loads and can include whole blood, plasma, or peripheral blood mononuclear cells. EBV is a cell-associated virus and therefore typically is found in the peripheral blood mononuclear cell component; but in certain disease states, notably NPC, it can be found in high levels in plasma, likely because of cell death and release of episomal DNA into the circulation. Further, extensive variability in quantitation can be found as a result of a lack of standardized DNA extraction techniques and gene amplification targets.[371,372] Taken together, these factors have resulted in a lack of validated data from multicenter trials. Thus individual centers often have their own protocols for monitoring viral load and decision points for intervention or preemptive strategies, such as for prevention of LPD (see discussion of PTLD later). A significant advance has been the establishment of WHO International Standard for EBV nucleic acid amplification techniques.[373] This has improved but not entirely eliminated the interlaboratory variation with these assays. At present, quantitative tracking of individual patients using a single reference laboratory remains the most accurate assessment of trends.[371,372]

Other Laboratory Abnormalities

Liver function test results are abnormal in almost all cases of infectious mononucleosis.[201,374,375] Levels of the hepatocellular enzymes aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase are most commonly elevated, and one of the three is abnormal in about 90% of the cases. Elevations are usually mild, with individual values in the range of two to three times the upper limit of normal. Elevation to greater than 10 times the upper limit of normal necessitates a search for another diagnosis.[201] The alkaline phosphatase level is elevated in about 60% of the cases.[374,375] Mild elevation of the bilirubin level is noted in approximately 45% of cases, although frank jaundice occurs in only about 5%. Elevations are maximal in the second week of illness and decline gradually over a 3- to 4-week period.

Cryoproteins are present in modest amounts in 90% to 95% of patients.[159,376] The cryoproteins are generally mixed cryoglobulins of IgG and IgM classes. When the cryoglobulins are dissociated, antibody of anti-i or anti-I, or both, specificities is usually seen.[376,377]

Posttransplant Lymphoproliferative Disease

The use of EBV loads has been intensively studied in PTLD. Although standard protocols are not available and controlled trials have not been performed, a number of studies report that the EBV viral load can be predictive of PTLD, especially in the setting of T-cell–depleted stem cell transplants or in umbilical cord blood transplantation, because the incidence of PTLD is higher for these patients.[369,378–380] The higher incidence is due to the importance of T-cell immunity in controlling EBV infection. Umbilical cord blood is EBV naïve, and because most transplant recipients are EBV positive, these patients are at particularly high risk for PTLD. Many transplantation centers therefore now monitor EBV loads and view elevated values as evidence for increased risk of PTLD in stem cell transplantation. However, the approach to monitoring EBV loads is not standardized and varies among institutions. One international leukemia working group recommended weekly monitoring for at least 3 months in posttransplantation in high-risk hematologic stem cell transplantation recipients.[381] Some centers now routinely treat

high EBV loads with rituximab, an anti-CD20 antibody that kills B cells and rapidly lowers EBV load, or with other approaches such as adoptive immunotherapy (discussed subsequently).[382,383] Rituximab lowered the rate of PTLD compared with historical control subjects in one study.[384]

The use of EBV viral loads is less clear after PTLD in solid-organ transplantation, and large studies are necessary to define protocols. After solid-organ transplantation, EBV viral loads tend to remain consistently high, without a clear risk for development of PTLD.[385–388] A rapid increase in viral loads may be of concern for development of PTLD. EBV load monitoring is particularly important after transplantation in individuals who are EBV seronegative, especially if the donor is EBV positive because these patients are at higher risk for PTLD after primary infection.[282] Similar to the situation with hematologic stem cell transplantation, there is no standardized approach to monitoring EBV loads. For instance, one renal transplant group recommends monitoring EBV loads monthly for the first 3 to 6 months after transplantation, followed by every 3 months for the first year, whereas another group states there are insufficient data to recommend routine monitoring and that testing should be individualized. Those with increasing EBV loads are often managed with reduced immunosuppression and sometimes with rituximab.[389,390]

Nasopharyngeal Carcinoma

NPC is difficult to diagnose in its early stages; therefore patients typically present with advanced disease. The most common initial presenting symptom is a neck mass (Fig. 138.5). Diagnosis requires endoscopy to visualize the nasopharynx and histologic examination of biopsy tissue.[391] Radiologic studies are helpful in revealing the extent of disease (Fig. 138.6). Patients with NPC have elevated levels of serum IgA directed against EBV VCA and EA.[392–394] The elevation of the IgA antibodies may occur several years before the onset of NPC. In light of this finding, a program of screening individuals for elevated EBV IgA VCA and EA titers has been instituted in southern China, where NPC is one of the leading malignant diseases. Individuals with elevated IgA titers are then observed closely for the development of disease. This screening program enhanced the diagnosis of NPC in earlier as opposed to more advanced stages of disease.

Detection of EBV DNA in nasopharyngeal brush biopsies has also been proposed in one study as a possible screening mechanism in high-risk populations. This study detected EBV DNA in 19 of 21 brush biopsies from patients with recently diagnosed NPC but only in 1.3% of control subjects.[395]

Cell-free EBV DNA in plasma is commonly detected in patients with NPC.[391] Cell-free EBV DNA is postulated to be released into the circulation on tumor cell death. In two studies by the same authors[396,397] of patients with NPC, quantitative analysis of the concentration of DNA in plasma was useful in monitoring patients for recurrence of disease. Further, patients with NPC presenting with higher plasma level viral loads have poorer outcomes and higher rates of early recurrence or metastasis after radiotherapy.[398,399] In a study from China the presence of persistent EBV DNA in plasma over a 4-week period was a useful screening tool to identify asymptomatic individuals with NPC. NPC was present in 11% of those with persistently positive EBV DNA in plasma. The carcinoma was identified at an earlier stage of disease and resulted in better patient outcomes compared with historical control subjects. However, only 1% of those with EBV DNA in plasma in a US study had NPC, indicating the positive predictive value for this approach is much lower in nonendemic regions.[400,401]



FIG. 138.5 Patient with nasopharyngeal carcinoma and neck mass *(arrow).*

Central Nervous System Lymphoma in Acquired Immunodeficiency Syndrome

PCR detection of EBV DNA in CSF has been useful in the diagnosis of CNS lymphoma in patients with HIV.[402–405] Nearly all primary CNS lymphomas in HIV disease are EBV associated, as discussed previously. Whereas patients with HIV without CNS lymphoma rarely have detectable EBV DNA in CSF, EBV DNA is frequently detected when CNS lymphoma is present. Therefore CSF PCR for EBV used in conjunction with radiologic studies may reduce the need for brain biopsy in certain instances. Quantification of EBV DNA in CSF may also be useful for monitoring the effects of CNS lymphoma therapy.[406]

DIFFERENTIAL DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS

In most cases the diagnosis of infectious mononucleosis is straightforward. The clinical manifestations of sore throat, fever, lymphadenopathy, and malaise coupled with atypical lymphocytosis and a positive heterophile test result establish the diagnosis of EBV-induced infectious mononucleosis.[407] Difficulties arise, however, when the clinical manifestations are less striking, particularly when the heterophile test results are negative.

Heterophile-Negative Infectious Mononucleosis Caused by Epstein-Barr Virus

Heterophile-negative infectious mononucleosis may be caused by several different agents, including EBV. As previously noted, the heterophile test is highly specific for primary EBV infection but not sensitive, especially in the pediatric age group.[139,140] On these occasions the diagnosis rests on the demonstration of appropriate changes in specific EBV serologic tests (see Table 138.9). Because the preclinical phase of EBV infection is over 30 days, most patients will have developed EBV antibodies at the time of clinical presentation. Detection of EBV DNA in blood can also be used to confirm the diagnosis in the appropriate clinical scenario but is rarely necessary in practice.

Cytomegalovirus as a Cause of Heterophile-Negative Infectious Mononucleosis

The most frequent cause of heterophile-negative infectious mononucleosis in most populations is CMV.[408] Although differentiation of individual cases of EBV-induced versus CMV-induced infectious mononucleosis may be difficult, certain features are more common in CMV infections. CMV more frequently follows transfusion and is more often manifested as a typhoid-like syndrome without sore throat and lymphadenopathy. Splenomegaly may be slightly more prominent with CMV-induced disease, whereas the atypical lymphocytosis is usually less intense in CMV-induced infectious mononucleosis. In age-matched control subjects the results of liver function tests are less elevated when the agent is

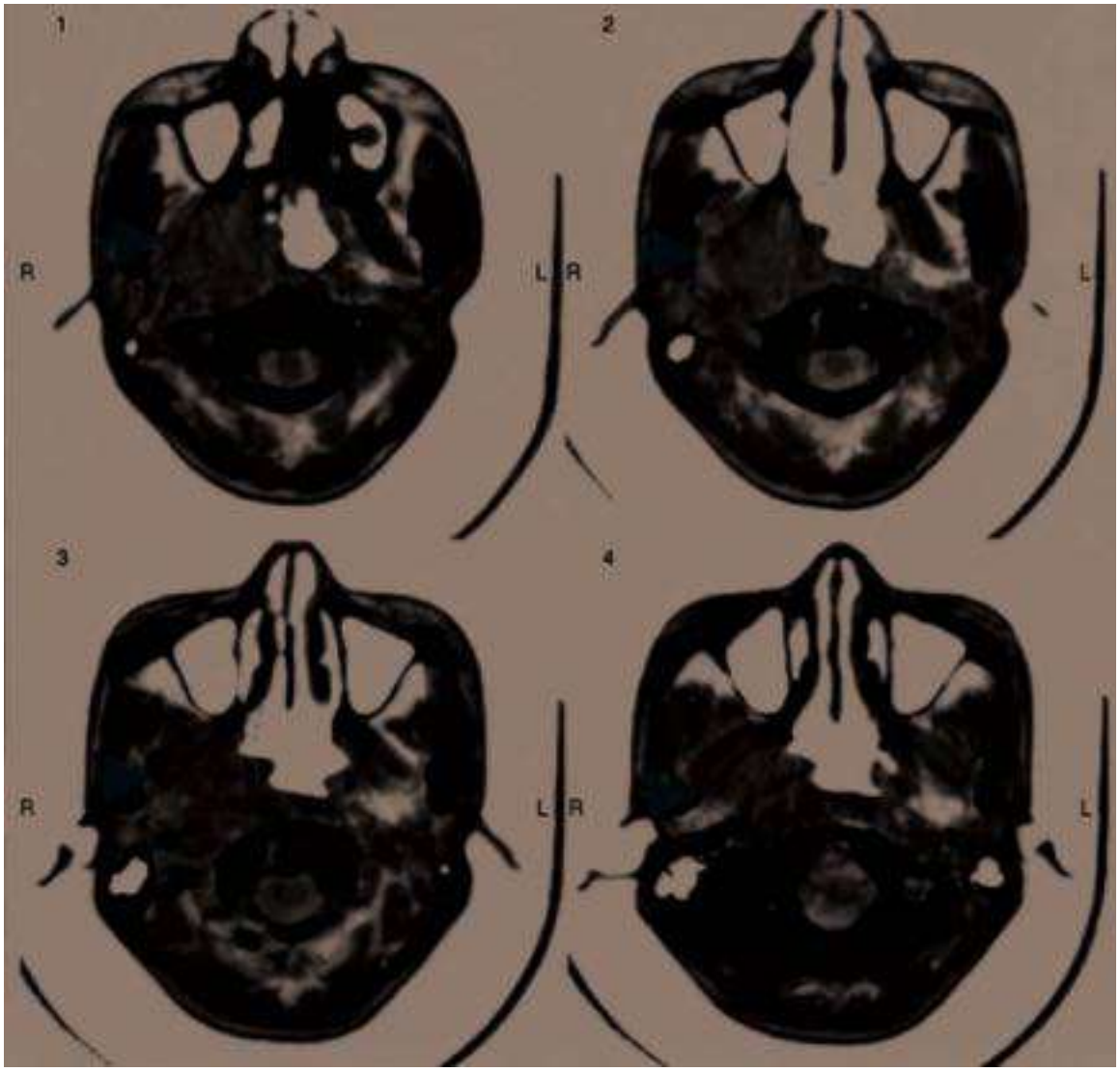

FIG. 138.6 Computed tomographic scan images of 25-year-old man with nasopharyngeal carcinoma *(arrows).* Tumor involves right parapharyngeal space. *(Courtesy Dr. Yi Zeng.)*

CMV. The illness may be attributed to CMV with serologic evidence of acute CMV infection and no evidence of acute EBV infection.

Primary Human Immunodeficiency Virus Infection

Patients with primary HIV infection may also present with fever, lymphadenopathy, and pharyngitis.[409–411] Such patients may also have a maculopapular rash and signs of aseptic meningitis. Patients with primary HIV infection are typically heterophile negative; however, rare cases of heterophile-positive primary HIV infection have been reported.[353] Thus serum or plasma should be sent for HIV RNA (viral load) as part of the evaluation of heterophile-negative infectious mononucleosis and may even be appropriate in heterophile-positive patients at high risk (see Chapter 120). Patients with primary HIV infection typically have negative or indeterminate HIV serology but can usually be diagnosed by combined antibody/antigen (fourth generation) tests.

Group A Streptococcal Pharyngitis

A streptococcal sore throat may also mimic infectious mononucleosis clinically. Adenopathy is generally submandibular and anterior cervical, and splenomegaly is absent in streptococcal sore throat. Culture of group A β-hemolytic streptococci from the throat is supportive but not conclusive evidence for this diagnosis because colonization with the organism can be common in this population of patients. If infectious mononucleosis is suspected in a patient with group A streptococci cultured from the pharynx, a positive heterophile, or positive EBV serologies can be used to confirm the diagnosis.

Hepatitis A, B, or C

Viral hepatitis may result in fever, lymphadenopathy, malaise, and an atypical lymphocytosis. In general, the atypical lymphocytosis is of lesser magnitude and accounts for less than 10% of the leukocytes. In viral hepatitis hepatocellular enzyme levels are usually markedly elevated at the initial visit, whereas in infectious mononucleosis the results of liver function tests are only mildly elevated initially and rise gradually over a 1- to 2-week period. In addition, specific serologic tests are currently available for the detection of infection with hepatitis A, B, and C viruses.

Toxoplasmosis and Other Infections

Acute toxoplasmosis may also give rise to an infectious mononucleosis-like illness. Usually the degree of the lymphocytosis is mild, and a diagnosis can be made with serologic tests for *Toxoplasma.* Rubella may also occasionally be manifested by fever, lymphadenopathy, and a mild atypical lymphocytosis, but the appearance of the exanthem and the clinical course of the illness are generally not confused with those of infectious mononucleosis. A serologic diagnosis of recent rubella infection can be obtained if the diagnosis remains in doubt. Anaplasmosis can

give rise to fever and an atypical lymphocytosis, and atypical lymphocytes can account for greater than 10% of the white blood cell differential.[412] These patients will typically lack the pharyngitis and lymphadenopathy that is present in EBV infection. In addition, *Anaplasma* infection is limited to regions where its tick vector is endemic. Diagnosis of *Anaplasma* infection can be made by PCR detection of *Anaplasma* nucleic acid in blood. Infectious lymphocytosis of childhood is a disease of uncertain cause that is characterized by fever, lymphadenopathy, occasionally diarrhea, and a lymphocytosis that consists almost exclusively of small mature lymphocytes. The disease is most common in the pediatric age group, may occur in epidemics, and is not associated with EBV infection.[413]

THERAPY

Infectious Mononucleosis

Supportive

Treatment of infectious mononucleosis is largely supportive because greater than 95% of the patients recover uneventfully without specific therapy. The level of activity is generally tailored to what the individual patient can tolerate comfortably. To avoid trauma to the spleen, contact sports or heavy lifting should be avoided during the first month of illness and until any splenomegaly has resolved. Ultrasound scan examination can be used to monitor spleen size. If constipation is present, it should be treated with a gentle laxative. Acetaminophen or nonsteroidal antiinflammatory agents can be helpful in relieving the sore throat and in suppressing the fever. Sore throat may be further alleviated with gargling with warm salt water.

Antiviral Agents

Acyclovir, ganciclovir, and foscarnet inhibit EBV replication in vitro.[414–416] However, these agents target the viral DNA polymerase, which is expressed only during lytic infection. Because EBV infection is predominantly latent, it is not surprising that these agents are ineffective in treatment of infectious mononucleosis. Further, the clinical symptoms and signs of infectious mononucleosis are largely the result of the vigorous immune response directed against EBV. A meta-analysis of five randomized controlled trials showed no significant benefit of acyclovir in the treatment of infectious mononucleosis. These trials included patients with mild, moderate, and severe mononucleosis. As expected, viral shedding from the oropharynx, where lytic replication commonly occurs, was reduced, but inhibition of shedding was lost 3 weeks after withdrawal of the antiviral agent.[417–421]

Corticosteroids

Corticosteroids should not generally be used in uncomplicated infectious mononucleosis. A double-blind, placebo-controlled trial showed that the combination of acyclovir and prednisolone did not reduce the duration of symptoms or result in an earlier return to work.[420] Other studies with corticosteroids have indicated that corticosteroids decrease the period of febrility and hasten the resolution of tonsillopharyngeal symptoms but do not reproducibly affect lymphadenopathy or liver and spleen involvement.[422–426] One particular reason to avoid corticosteroids in uncomplicated disease is that they have rarely been linked with complications such as encephalitis and myocarditis.[48,426] In addition, there is a theoretical risk that corticosteroids may inhibit the host immune response, resulting in a larger reservoir of latently infected cells that could potentially put patients at risk for EBV-associated malignant disease.

Corticosteroids may be helpful in cases of complicated infectious mononucleosis.[426–428] Tonsillar enlargement that causes airway compromise may respond rapidly to corticosteroids, eliminating the need for tracheostomy. Corticosteroids may also be helpful in autoimmune hemolytic anemia, severe thrombocytopenia, and aplastic anemia. Some investigators also advocate the use of corticosteroids for CNS involvement, myocarditis, or pericarditis. In selected cases of severe or prolonged prostration, corticosteroids may be of benefit. If corticosteroids are administered in these situations, treatment should be initiated in doses equivalent to 60 to 80 mg of prednisone per day given in a split daily regimen. The response is usually rapid, and the dosage can be tapered over a 1- to 2-week period.

Lymphoproliferative Disease

The overall mortality rate remains high, at about 50%, for LPD. Outcome appears to be enhanced with early diagnosis and treatment.[429] Multiple methods are used in the treatment of LPD, but large trials are still necessary to determine the best approaches.[52,53,282,430] The approach to therapy differs for monoclonal versus polyclonal LPD and depending on whether LPD arises in hematopoietic stem cell or solid-organ transplantation. The mainstay of LPD therapy in solid-organ transplantation is reduction of immune suppression. This strategy is logical because LPD most likely results from ineffective immune surveillance of EBV-infected B cells. Reduction of immune suppression leads to regression of tumors in up to 50% of cases. However, this approach is usually ineffective in stem cell transplantation because these patients receive high-dose chemotherapy and radiation to ablate the immune system and are dependent on engraftment of donor immune cells.[429] Reduction of immunosuppression can increase the risk of graft rejection. Surgical resection and radiotherapy are often used in localized LPD and can be combined with reduction of immune suppression in solid-organ transplant recipients with good results.

Rituximab, a monoclonal antibody directed against the CD20 antigen, found on most B cells, has become a mainstay of LPD treatment and can be given as a single agent or in combination with cytotoxic chemotherapy.[52,53,430] Binding of this antibody to B cells produces cell death through complement fixation or antibody-dependent cell-mediated cytotoxicity. Response rates range from approximately 70% to 100% with rituximab in different studies, and these differences may be a result of the timeliness of diagnosis.[429] At some centers rituximab is administered preemptively to patients without overt LPD when rising EBV DNA levels are observed in the blood.[431] Rituximab is occasionally given prophylatically in other settings but must be used judiciously as it does result in further immunosuppression due to profound B-cell depletion for up to 8 months.

Antiviral therapy is not of proven efficacy and not recommended for LPD.[432,433] Most LPD cells are latently infected by EBV and therefore do not express the EBV DNA polymerase. Experimental approaches aiming to induce lytic infection, and therefore induce expression of the EBV DNA polymerase, in LPD cells with treatment with arginine butyrate followed by ganciclovir have been reported.[434] By contrast, administration of acyclovir or ganciclovir prophylactically to high-risk pediatric solid-organ transplant recipients (donor EBV positive, recipient EBV negative) has been observed to reduce the risk of subsequent LPD development.[435,436]

Adoptive immunotherapy is another approach to LPD treatment. It is generally reserved for relapsed or refractory cases due to expense and limited availability of this therapy. It is particularly useful in allogeneic stem cell recipients, where LPD usually arises from donor cells and the donor is often available to harvest CTLs. This strategy is based on reconstitution of a cellular immune response against EBV to treat the infected tumor cells. Allogeneic stem cell recipients with LPD have been treated with unselected, donor mononuclear cells.[437,438] This approach results in response rates of up to 90% but also results in a high rate of graft-versus-host disease (GVHD) because of the presence of the infused alloreactive T cells. To avoid GVHD another approach has been to infuse selected, donor-derived, EBV-specific CTLs.[439–441] Marking of transferred T cells has shown that they persist up to 9 years.[442] This approach has also been used with success as prophylaxis for LPD in hematopoietic stem cell transplant recipients. In one study none of the 101 patients who received EBV-specific T-cell infusions as prophylaxis developed PTLD, and 11 of 13 patients with PTLD infused with EBV-specific T cells achieved complete remission.[443]

Adoptive immunotherapy can also be used in solid-organ transplant recipients. The organ recipient's CTLs can be expanded in vitro and then infused back into the patient.[444] PTLD during the first year after transplantation tends to exhibit type III latency, whereas PTLD that occurs in later years tends to have type I or II latency. Types I and II latency have programs of expression of fewer EBV latent genes, so these tumors are less immunogenic for CTL infusion and also tend to be more aggressive.[443] The infused CTLs do not expand as robustly as

in hematopoietic stem cell transplantation and only exhibit transient persistence, perhaps at least partially because of continued immunosuppression.[429] A limitation to this method occurs in primary EBV infection, in which rapid-onset LPD may not allow time to generate EBV-specific CTLs. Newer approaches to enhance the speed of CTL preparation from the typical 8 to 12 weeks with traditional expansion methods include direct selection of T cells using specific viral peptides in the context of class I human leukocyte antigen (HLA) molecules (tetramers) and isolation of IFN-γ–secreting T cells in response to EBV antigens.[443] Rapid expansion of virus-specific T cells has also been performed using antigen-presenting dendritic cells that have been engineered to express specific viral antigens with isolation of specific CTL cells in 10 days.[445,446] Another approach in solid-organ transplants has been to use closely matched allogeneic CTLs. A bank of about 100 CTLs from healthy donors has been used on a best-possible HLA match basis to treat LPD.[447] In a phase II multicenter trial, 33 patients with LPD who had failure with conventional therapy were treated with these allogeneic CTLs.[448] A response rate of 52% was seen at 6 months, and 14 patients achieved complete remission. Long-term follow-up at 4 to 9 years after the last CTL infusion showed that 12 of the 14 patients remained in complete remission, whereas two died—one of recurrent PTLD and the other from an unrelated infection.[449]

Measurement of the EBV DNA load in the blood may be helpful for prediction of response to treatment of LPD. In one study a decrease in the EBV DNA load within 72 hours correlated with response to therapy in seven responders, and all nonresponders had an increased EBV load at 72 hours.[450] Of note, however, after treatment with rituximab, peripheral blood mononuclear cell EBV DNA levels can fall even in the setting of tumor progression.[451]

Epstein-Barr Virus Targeted Therapy in Associated Malignant Diseases

A comprehensive discussion of multimodality therapy for all EBV-associated malignant diseases is beyond the scope of this chapter. The success of EBV immunotherapy in LPD has prompted its investigation in other EBV-associated malignant diseases. These malignant diseases, however, present challenges for immunotherapy that LPD does not. First, EBV gene expression is more limited in other tumors and, unlike LPD, they do not express the immunodominant EBNA3 proteins. Second, because other EBV-associated malignant diseases arise in the setting of an apparently intact immune response, it is not obvious that immunotherapy should work. Several small clinical trials of CTL infusions have shown they are generally well tolerated and have some efficacy for EBV-positive Hodgkin lymphoma and NPC.[452–460] The development of immune checkpoint inhibitors targeting the inhibitory programmed death protein 1 (PD-1) receptor found on lymphocytes, or its ligands PD-L1 and PD-L2, is an important new avenue for cancer immunotherapy.[461] Checkpoint inhibitor therapy appears to be particularly effective in tumors expressing "neoantigens" due to high somatic DNA mutation rates.[462] EBV-associated cancers upregulate the PD-L1 ligand and by definition express foreign antigens, making them attractive candidates for checkpoint inhibitor therapy.[463,464] Preliminary animal model studies have demonstrated checkpoint inhibitors can inhibit growth of EBV-associated lymphomas.[465] In summary, limited data suggest that EBV immunotherapy in patients with advanced EBV-positive Hodgkin lymphoma or NPC is well tolerated and may be beneficial. Checkpoint inhibitors, targeting the PD-1 signaling pathway, may assume an increasing role in future therapy of EBV-associated cancers. By contrast, antiviral drugs have no established role in the treatment of EBV-malignant diseases.

Oral Hairy Leukoplakia

Oral hairy leukoplakia differs from most EBV-related diseases in that the EBV infection is predominantly lytic rather than latent. In this setting of active lytic infection, agents such as acyclovir, ganciclovir, and foscarnet are effective in therapy.[466–469] Topical therapy, such as use of podophyllum resin, has also been shown to have efficacy against OHL.[470,471] In the setting of HIV-related OHL, oral lesions usually regress with the institution of effective antiretroviral therapy.

PREVENTION

Public Health Measures

Because the spread of virus requires intimate contact, isolation of patients with infectious mononucleosis is not necessary. Elevated viremia is seen for several months after recovery, so consideration should be given to postponement of blood donation by patients with infectious mononucleosis for at least 6 months after the onset of illness.

Vaccine

EBV vaccine development has been an elusive goal for many years, and substantial research activity is being devoted to this area, as recently reviewed.[472] Because EBV infection does not cause severe disease in most instances, a vaccine must be particularly safe.[50] However, there are about 125,000 cases of infectious mononucleosis in the United States each year and approximately 200,000 new cases of EBV-associated malignancy across the world.[473] Development of a vaccine that could reduce or eliminate this burden of disease remains a significant goal. The only herpesvirus vaccine currently licensed by the US Food and Drug Administration is a live-attenuated varicella-zoster vaccine. Because of EBV's associations with malignant diseases, acceptance of a live-attenuated vaccine is highly unlikely.

The goals of an EBV vaccine are not yet clearly defined[282] and likely will be determined based on the levels of protection provided by different vaccine formulations. Complete protection from infection, at first glance, appears to be the primary goal, but its attainment may be limited by the biology of the virus (see subsequent discussion). Another potential goal is prevention of symptomatic infection of infectious mononucleosis, without necessarily prevention of lifelong latent viral infection. In such a case determination is critical of whether the vaccine also provides any protection against complications of EBV, such as EBV-related malignancy or fulminant primary infection in XLP. Notably, symptomatic infectious mononucleosis is associated with a 3.4-fold increased risk of EBV-related Hodgkin lymphoma, so a vaccine that prevents symptomatic infection without necessarily preventing infection could be of significant benefit.[474] In addition, because elevated EBV viral loads in blood can predict development of EBV-associated malignancy in transplant recipients, and because EBV viral loads are elevated at the onset of NPC, an EBV vaccine that induces better immune control of infection and prevents increased viral loads may potentially prevent EBV malignancies.[473] To address these issues a meeting was held at the US National Institutes of Health in 2011. The group concluded that the goals of future EBV vaccine research should be the prevention of infectious mononucleosis and EBV-associated malignancies. Another recommendation was to identify surrogate markers that predict EBV-associated malignancies, to be able to more rapidly assess the effectiveness of vaccines before the advent of malignancy.[474]

Two major approaches have been taken to EBV vaccine development. The most common approach taken with EBV vaccines is to induce EBV neutralizing antibody directed against the viral glycoprotein gp350, the most abundant glycoprotein on the virus, and which binds to the EBV cellular CD21 receptor.[475] Immunization with gp350 protects against EBV-induced lymphomas in an animal model.[476] Of interest, despite initial expectations, cell-mediated immunity appears to play an important role in the gp350 vaccination prevention of lymphoma in this model.[477] A small trial in China with recombinant vaccinia virus expressing gp350 protected six of nine children from EBV infection at 16 months compared with none of 10 control subjects.[478] However, the use of live vaccinia-based vaccines is unlikely to become widespread.[282] Of importance, phase I and II studies of a purified gp350 vaccine have recently shown protection from symptoms of infectious mononucleosis but did not prevent asymptomatic infection with EBV, consistent with the potential goal of reducing cases of symptomatic mononucleosis. Of note, one initially EBV-seropositive participant experienced an oligoarthritis reaction, which may have been related to the vaccine.[479–481] A recent discovery found that presenting the CD21 binding component of gp350 in a symmetrical array on self-assembling nanoparticles induced potent neutralizing antibodies in mice and nonhuman primates and improved protection in a mouse model.[482] It is possible that such an approach may enhance the effectiveness of gp350 vaccines.

The second approach has been to develop a vaccine with known EBV class I–restricted MHC CTL epitopes.[483–485] Although such a vaccine would not necessarily be designed to prevent primary infection, it is expected to ameliorate the symptoms of mononucleosis.[483] Another important potential use of such a vaccine would be to boost the CTL response to avoid development of, or possibly treat, EBV-associated malignant diseases. A significant number of EBV epitopes recognized by CTLs have now been identified. A phase I trial has been completed in Australia with a single EBNA3 EBV epitope. In this trial two placebo recipients became EBV infected, and one had symptomatic mononucleosis; four vaccine recipients acquired EBV infection but none were symptomatic.[486] To generate a broad-based CTL response, a vaccine containing multiple EBV epitopes is necessary. In addition, because CTLs from individuals with different HLA alleles recognize different EBV epitopes, inclusion of relevant epitopes in a vaccine is important. Therefore current efforts have fused multiple peptide epitopes together for use in vaccines.[50,483]

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139 Human Herpesvirus Types 6 and 7 (Exanthem Subitum)

Jeffrey I. Cohen[a]

SHORT VIEW SUMMARY

Definition
- Human herpesviruses 6 and 7 (HHV-6 and HHV-7) cause exanthem subitum or febrile seizures in young children and reactivate frequently in highly immunocompromised hosts. They can also cause encephalitis in immunocompromised hosts.

Epidemiology
- Most adults are seropositive for HHV-6 and HHV-7.
- The average age of infection with HHV-6 is about 1 year, and for HHV-7 is 2 years.
- About 50% of hematopoietic transplant recipients and 20% to 33% of organ transplant recipients have HHV-6 and HHV-7 DNA in the blood.
- HHV-6 DNA is integrated in the chromosomes of 1% to 2% of persons and is transmitted in the germline DNA.

Microbiology
- HHV-6 and HHV-7, like cytomegalovirus, are betaherpesviruses.

Diagnosis
- Exanthem subitum is usually diagnosed clinically, but seroconversion to HHV-6 or HHV-7 antibody positivity can be used.
- Diagnosis of HHV-6 or HHV-7 disease in immunocompromised persons is difficult owing to the high frequency of asymptomatic reactivation and the finding that up to 2% of persons have HHV-6 DNA integrated in their chromosomes.
- HHV-6 limbic encephalitis is diagnosed based on clinical signs and symptoms and HHV-6 DNA in the cerebrospinal fluid.
- Detection of HHV-6 protein or RNA in tissues is more specific than HHV-6 DNA for diagnosing virus-associated disease in immunocompromised persons.

Therapy
- No therapy has been shown to be effective for treatment of HHV-6 or HHV-7, but both viruses are sensitive to ganciclovir, foscarnet, and cidofovir in vitro.
- Ganciclovir, foscarnet, or both have been used to treat immunocompromised persons with HHV-6 or HHV-7 disease, especially with limbic encephalitis.

Human herpesviruses 6 and 7 (HHV-6 and HHV-7) are both members of the Betaherpesvirinae subfamily. Both viruses infect T cells, are present ubiquitously, and can cause exanthema subitum (or roseola infantum). In addition, both viruses frequently reactivate in highly immunocompromised patients but rarely cause serious disease in these patients.

HUMAN HERPESVIRUS TYPE 6

History

HHV-6 was discovered by Salahuddin and colleagues[1] in 1986 in patients with lymphoproliferative disorders and human immunodeficiency virus (HIV). Subsequently, two variants of HHV-6 were described: HHV-6A and HHV-6B.[2] HHV-6B was shown to be an etiologic agent of exanthema subitum,[3] whereas HHV-6A has rarely been associated with disease.[4]

Description of the Virus

HHV-6 is a member of the *Roseolovirus* genus of betaherpesviruses and shares a number of features with cytomegalovirus (CMV), including numerous homologous viral proteins and similar genomic structures.[5] HHV-6A and HHV-6B, which share 90% nucleotide sequence identity, are sufficiently different in their sequences and in their cell tropism that they could be classified as separate species of herpesviruses. The receptor for HHV-6A is CD46 and for HHV-6B is CD134, both of which interact with a complex consisting of viral glycoproteins gH, gL, gQ1, and gQ2. The HHV-6 genome contains about 165 kilobase pairs of DNA.

Epidemiology

More than 95% of adults are seropositive for HHV-6. Maternal antibody to HHV-6 declines during the first 5 months of life. About 40% to 50% of children are infected by 1 year of age, and 77% to 82% are infected by 2 years of age (Fig. 139.1).[6,7] The peak of infection occurs at 9 to 21 months. Approximately 90% of infections in children are symptomatic; in one study, 40% of infants with HHV-6 were seen by a physician.[6] There is no seasonal peak for primary HHV-6 infection.[6] HHV-6 is usually transmitted horizontally, presumably by infected saliva from close contacts with children. Outbreaks of HHV-6 infection have been reported at daycare centers.

HHV-6 infects peripheral blood mononuclear cells (PBMCs) and cells in the liver, salivary glands, endothelial cells, and central nervous system (CNS). HHV-6 DNA was detected in PBMCs from 22% and in cervical swabs from 7.5% of pregnant women.[8] HHV-6 was also detected in about 1% of cord blood samples and in fetal blood, indicating a potential for fetal disease. Congenital infection with HHV-6 is most often due to chromosomally integrated HHV-6 (ciHHV-6) passed in the germline DNA. In one study, 86% of congenital infections were from ciHHV-6, and 14% were due to transplacental infection.[9] These infants have 10^5 or 10^6 copies of HHV-6 DNA per microgram of cellular DNA. HHV-6 DNA persists in the blood intermittently after primary infection in most children and can reactivate in healthy children without apparent illness.[10] HHV-6 has been transmitted by organ transplantation[11] and has been transmitted to hematopoietic stem cell transplant (HSCT) recipients by ciHHV-6 from donor cells.[12]

HHV-6 frequently reactivates in immunocompromised patients. About 50% of HSCT and about 33% of solid-organ transplant recipients reactivate HHV-6 as defined by detection of viral DNA in the peripheral blood.[13,14] More than 95% of HHV-6 reactivations in HSCT recipients are due to HHV-6B. Reactivation of the virus usually occurs within the first month after transplant, and reactivation is increased with reduced cellular immunity, particularly in patients receiving anti-CD3 antibody or corticosteroids, and in those who have undergone allogeneic or cord blood transplants.[14,15] Half of adults who receive cord blood transplants have reactivation lasting a median of 4 years after transplant.[16] Reactivation has also been reported in 54% of critically ill patients who are not otherwise immunocompromised[17] and frequently occurs in patients with drug reaction with eosinophilia and systemic symptoms (DRESS).

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

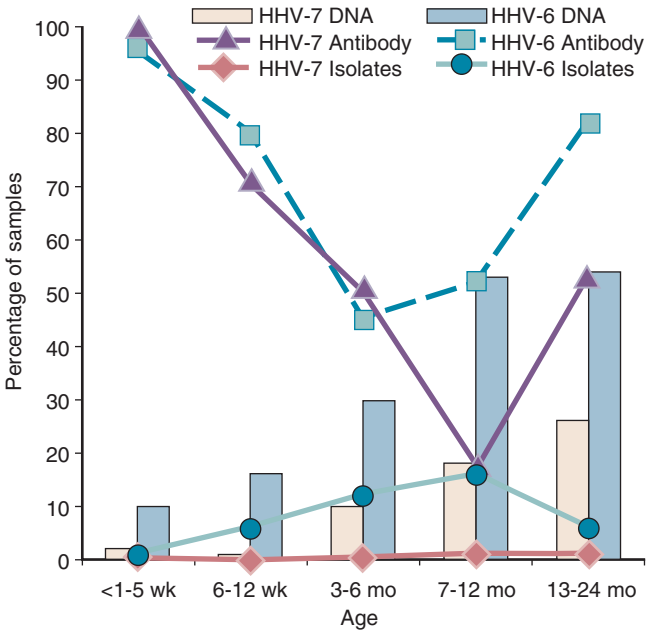

FIG. 139.1 Percentages of blood samples from children 2 years of age or younger with antibody, virus culture, and virus polymerase chain reaction for human herpesvirus *(HHV)*-6 and HHV-7. *(From Hall CB, Caserta MT, Schnabel KC, et al. Characteristics and acquisition of human herpesvirus [HHV] 7 infections in relation to infection with HHV-6.* J Infect Dis. *2006;193:1063–1069.)*

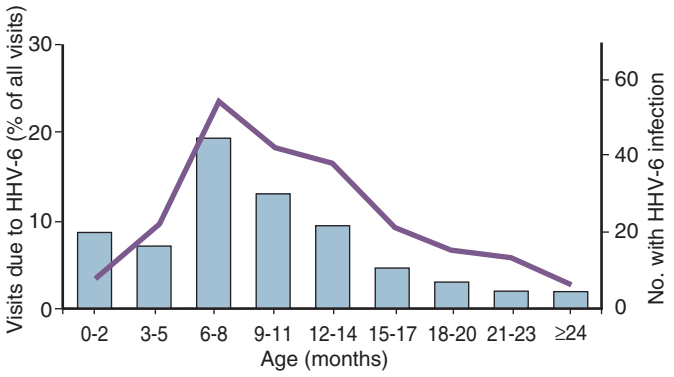

FIG. 139.2 Percentage of visits to the emergency department for febrile illnesses associated with human herpesvirus 6 *(HHV-6) (line)* and the number of children with primary HHV-6 infection *(bars).* *(From Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus-6 infection in children: a prospective study of complications and reactivation.* N Engl J Med. *1994;331:432–438.)*

Pathogenesis

In addition to infecting its primary target, $CD4^+$ T cells, HHV-6 infects other T cells, B cells, natural killer (NK) cells, monocytes, macrophages, epithelial cells, and neural cells. HHV-6A has a greater predilection to infect neural cells than does HHV-6B, whereas HHV-6B is more commonly detected in PBMCs than HHV-6A. Detection of HHV-6 DNA at high levels in the olfactory bulb or tract relative to other portions of the brain and in nasal mucous specimens suggests that HHV-6 may enter the CNS through the olfactory pathway.[18] Infection of lymphocytes with HHV-6 results in ballooning of the cells with intranuclear inclusions, followed by cell death. HHV-6 establishes a latent infection in $CD34^+$ hematopoietic stem cells, monocytes, and macrophages, and a persistent infection in salivary glands. HHV-6 is detected in saliva, but at a much lower frequency than HHV-7. Cellular immunity is more important than humoral immunity for controlling HHV-6 infection.

HHV-6 induces expression of CD4 on the surface of T cells, which can increase susceptibility to HIV infection. However, infection of cells with HHV-6 results in reduced expression of the HIV coreceptor CXCR4 on the surface of cells and increased expression of the RANTES (regulated on activation, normal T-cell expressed and secreted) chemokine, which can inhibit replication of CCR5 tropic HIV strains in HHV-6-infected cells. HHV-6A inhibits expression of major histocompatibility complex (MHC) class I on dendritic cells.

Clinical Manifestations

Infantile Fever and Seizures

The incubation time for HHV-6 infection is estimated to be 1 to 2 weeks. Infantile fever is the most common manifestation of HHV-6 infection. More than 90% of children infected with HHV-6 have symptoms, including fussiness, rhinorrhea, and fever in over half of patients; cough, diarrhea, and rash occur in about one-third of patients.[6] It is estimated that 5% to 25% of visits to emergency departments for fever in infants are due to HHV-6. Children older than 6 months are more likely to have fever than are younger children.[6] Of children younger than 3 years seen in emergency departments with fever, 10% had primary infection with HHV-6; this number increased to 20% for children 6 to 12 months old (Fig. 139.2).[19] The mean age of primary HHV-6 infection was 9.4 months, the median duration of illness was 6 days, and the mean temperature was 39.6°C. In another study of 243 children seen in the emergency department with HHV-6, more than 50% had fever of 40°C or higher, malaise, otitis, and nasal congestion.[20]

HHV-6 is responsible for about one-third of febrile seizures in children up to 2 years of age.[19] Of 160 children with acute HHV-6 infections, 13% had seizures; the median age of children with HHV-6 and seizures was 14 months. Primary infection with HHV-6 is more frequently associated with severe seizures, long seizures, and recurrent seizures than seizures not associated with HHV-6.[21] HHV-6 viremia was present in 32% of children with febrile status epilepticus; 22% of patients had primary HHV-6B infection, and 10% had virus reactivation.[22]

Exanthem Subitum (Roseola Infantum or Sixth Disease)

Exanthem subitum is caused by either HHV-6B or HHV-7. Approximately 25% of patients with HHV-6 infection in the United States have exanthem subitum at presentation[6]; in contrast, in Japan about 75% of primary HHV-6 infections result in exanthem subitum.[23] The disease begins with a high fever that usually lasts for 3 to 4 days.[6,23] At the time of defervescence, patients develop a macular or maculopapular rash that begins on the neck or trunk, spreads to the extremities, and persists for a few hours to 2 days (Fig. 139.3). The disease may be accompanied by cough, cervical and occipital lymphadenopathy, erythema of the tympanic membranes, conjunctivitis, eyelid edema, bulging fontanelles, lymphadenopathy, diarrhea, or Nagayama spots (red papules on the soft palate or base of the uvula). The median duration of symptoms is 9 days. Rare complications include febrile seizures, meningitis, and encephalitis.[24] Patients often have leukocytosis during the first day, followed by leukopenia with a relative lymphocytosis and in some cases thrombocytopenia.

Other Neurologic Symptoms Associated With HHV-6

HHV-6 is a rare cause of meningitis; the virus can also cause encephalitis in otherwise healthy children with an altered level of consciousness, seizures, psychosis, or cranial nerve deficits.[25] These children usually have a panencephalitis and can have persistent neurologic sequelae. Eight percent (13/156) of young children hospitalized in Britain and Ireland with encephalitis or fever and seizures were found to have acute HHV-6 infection.[26] HHV-6 DNA was detected in the cerebrospinal fluid (CSF) of 7% (9/138) of patients with encephalitis with a lymphocytic pleocytosis, but no specific differences were noted in patients with encephalitis attributed to HHV-6 versus that due to other causes.[27] HHV-6 may also cause encephalitis in otherwise healthy adults,[28] and HHV-6 DNA was detected in the CSF of 0.4% (4/1000) of persons in the California Encephalitis Project study.[29]

HHV-6 has been associated with multiple sclerosis based on detection of DNA in CSF and DNA and viral antigens in the brain; however, viral



FIG. 139.3 A child with exanthem subitum. *(Courtesy Professor K. Yaminishi, Osaka University Medical School, Osaka, Japan.)*

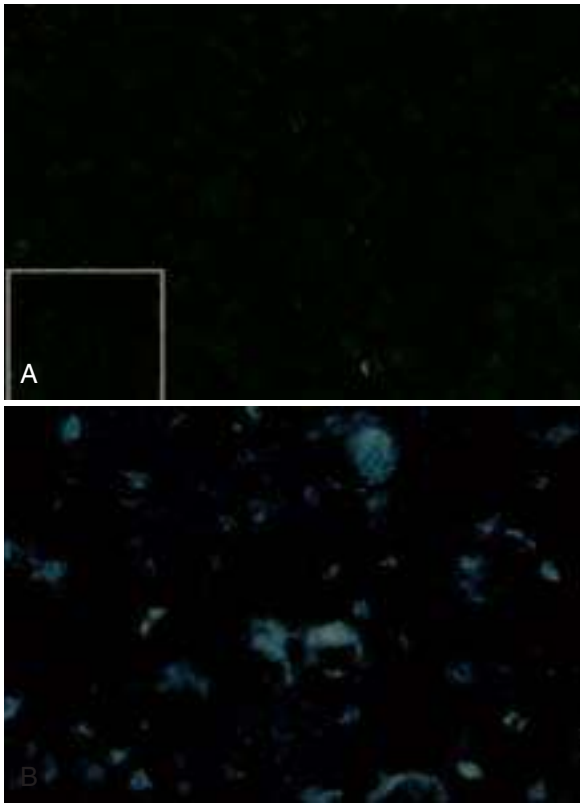

FIG. 139.4 Eosinophilic intranuclear and cytoplasmic inclusions with hematoxylin and eosin stain (A) and immunohistochemical staining with antibody to human herpesvirus 6 (HHV-6) envelope glycoprotein gp60 in viral inclusions (B), in a lymph node from a patient with human herpesvirus 6 infectious mononucleosis. *Inset* shows a cell with an intranuclear inclusion. *(From Maric I, Bryant R, Abu-Asab M, et al. Human herpesvirus-6–associated acute lymphadenitis in immunocompetent adults.* Mod Pathol. *2004;17:1427–1433.)*

DNA and proteins have also been detected in the brain of controls,[30,31] and the role of HHV-6 in multiple sclerosis is controversial.[32] HHV-6 antigen was detected in astrocytes cultured from the brain of patients with mesial temporal sclerosis (MTS).[33] A study of patients with MTS found HHV-6 DNA in 22% to 29% of studied tissues (hippocampus, amygdala, and mixed amygdala and uncus), and significantly greater levels of viral DNA were found in specimens from patients with MTS patients than from patients without MTS.[34] Markers of neuroinflammation have also been noted in association with HHV-6 infection of the CNS.[35]

Infectious Mononucleosis

Older patients who develop primary infection with HHV-6 may present with infectious mononucleosis with fever, lymphadenopathy, generalized rash, and atypical lymphocytes.[36] Lymph node biopsy specimens show intranuclear and cytoplasmic inclusions with HHV-6 antigens (Fig. 139.4).[37]

Other Complications in Healthy Persons

HHV-6 has been associated with chronic or fulminant hepatitis,[38] thrombocytopenic purpura, myocarditis,[39] and hemophagocytic syndrome in case reports. Although HHV-6 DNA has been found in some lymphomas, there is no compelling evidence that HHV-6 is associated with malignancy. HHV-6 is not a cause of chronic fatigue syndrome.

Congenital Infection

HHV-6 congenital infections (defined by detection of virus in cord blood) occurred in 1% (57/5638) of births and, unlike infections later in life, were asymptomatic.[40] In a follow-up study, neurodevelopmental scores were significantly lower at 1 year of age in infants who had HHV-6 congenital infection.[41]

Infection in the Immunocompromised Host

Because a large proportion of HSCT and organ transplant recipients reactivate HHV-6 with viral DNA in the peripheral blood (see "Epidemiology," earlier), it is often difficult to confirm that symptoms in transplant recipients are due to HHV-6.

HHV-6 is frequently associated with fever and rash early after transplant.[14,42] The virus has also been associated with delayed monocyte and platelet engraftment in HSCT recipients.[43] One study showed that high levels of HHV-6 DNA in the blood correlated with delayed platelet engraftment after transplant.[44] HHV-6 infects hematopoietic progenitor cells, inhibiting colony formation in vitro,[45] and has been associated with bone marrow suppression and delayed engraftment in some[13] but not other[46] studies. The association of HHV-6 reactivation with CMV infection and acute graft-versus-host disease[47] may make it difficult to confirm HHV-6 as a primary cause of marrow suppression or graft rejection. HHV-6 reactivation was associated with an increase in nonrelapse mortality in HSCT recipients.[47]

One of the best-documented manifestations of HHV-6 in immunosuppressed patients is limbic encephalitis.[48,49] The disease is especially common in transplant recipients receiving cord blood. In a meta-analysis, the prevalence of HHV-6 encephalitis was 8.3% in cord blood transplant recipients versus 0.5% in persons receiving another source of stem cells.[50] Other risk factors for HHV-6 acute limbic encephalitis include acute graft-versus-host disease, receipt of adult mismatched donor cells, and engraftment syndrome.[51,52] Interleukin-6 levels in the plasma were more often elevated in persons with HHV-6–associated neurologic complications compared with those without such complications.[53] In a study of 1344 HSCT recipients, 50% of cord blood recipients who developed HHV-6 limbic encephalitis died, whereas no adult donor cell recipients with HHV-6 encephalitis died.[51] Most cases of HHV-6 encephalitis in the literature were diagnosed through detection of viral DNA in the CSF[54]; HHV-6 antigen in the brain is more specific for diagnosis. Patients often present 2 to 6 weeks after HSCT with headache and confusion and nonfocal neurologic examination findings that may progress to seizures, psychosis, and cranial nerve deficits. Although the computed tomography (CT) scan is usually unremarkable initially, magnetic resonance imaging (MRI) shows abnormalities in 75% of patients with

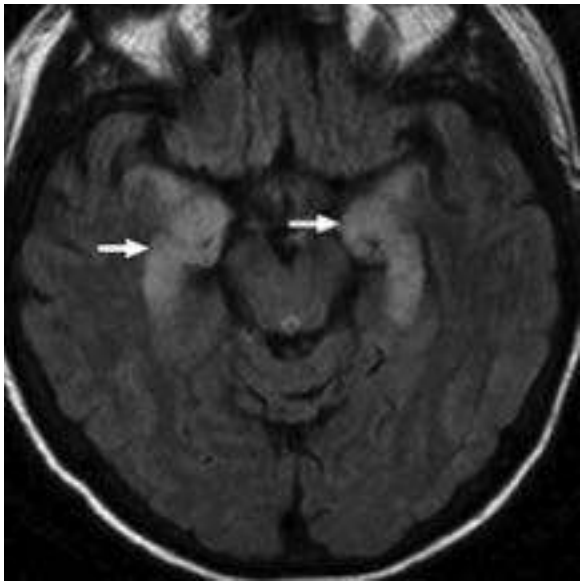

FIG. 139.5 Magnetic resonance image showing bilateral medial temporal lobe involvement *(arrows)* in a hematopoietic stem cell transplant recipient. *(Courtesy Alexander Freeman, National Institute of Allergy and Infectious Diseases.)*

changes in the gray matter of the temporal lobes, especially the medial aspect of these lobes (Fig. 139.5). The CSF shows an elevated protein in two-thirds of cases, with a mild lymphocytic pleocytosis in the minority of patients. Limbic encephalitis often manifests with short-term memory loss and insomnia, with HHV-6 proteins or RNA in the hippocampus and amygdala in HSCT recipients.[48,55,56] Nine transplant patients were reported to have limbic encephalitis with MRI changes involving the amygdala and hippocampus, inappropriate diuretic hormone secretion, and anterograde amnesia.[57] Survivors of HHV-6 often have atrophy of the hippocampus with persistent memory impairment and fatigue and frequently are unable to return to school or work.[51,58] HHV-6 reactivation in the blood is associated with delirium and cognitive decline in the first 3 months after HSCT[59] and cord blood transplantation.[60] HHV-6 myelitis has been reported during engraftment after cord blood transplantation.[61]

Although HHV-6 DNA was detected at high levels in the lungs of bone marrow transplant patients with idiopathic pneumonia compared with lower levels in the lungs of control immunocompetent patients,[62] other studies have not confirmed these findings.[46] The high frequency of virus reactivation in these patients emphasizes the importance of detecting viral proteins in tissues (see "Diagnosis," later).

Case reports have described HHV-6 associated with giant cell hepatitis[11] and other forms of hepatitis, colitis, and gastroduodenitis in transplant recipients. HHV-6 antigen was detected in PBMCs infiltrating biopsy specimens of gastroduodenal mucosa in 23% of liver transplant recipients, but also in 19% of immunocompetent patients with upper gastrointestinal symptoms; the number of HHV-6–positive cells tended to be higher in the transplant patients than in the immunocompetent patients.[63] HHV-6 proteins have been detected in tubular epithelia of kidneys undergoing rejection after transplantation.

HHV-6 has less commonly been associated with encephalitis and pneumonitis in acquired immunodeficiency syndrome (AIDS) patients. HHV-6 has generally not been shown to influence the rate of progression of HIV to AIDS.

Laboratory Diagnosis

Healthy Persons

The diagnosis of exanthem subitum is usually made clinically. The most frequently used diagnostic test for acute HHV-6 disease in children is comparison of acute and convalescent serum for seroconversion to HHV-6.[64] The observation of asymptomatic reactivations of HHV-6 in healthy persons indicates that detection of a fourfold or greater rise in titer alone may not be diagnostic of acute infection. HHV-6 immunoglobulin G (IgG) is usually present 1 week after infection, peaks in the second week, and persists for life. An alternative diagnostic test for acute disease in children is detection of HHV-6 DNA in sera or plasma at a time when antibody to the virus is absent, reflecting the transient viremia that occurs before the onset of antibody; however, this test is less specific than seroconversion.[64] Currently used serologic tests include immunofluorescent antibody, enzyme-linked immunosorbent assay (ELISA), and immunoblot assays. These tests cannot distinguish HHV-6A from HHV-6B, and there can be cross-reactivity of HHV-6 with HHV-7. HHV-6 IgM is present early in infection and persists for a few weeks; however, virus-specific IgM may not be detectable in some children, and this antibody has been detected in some adults, suggesting virus reactivation.

Culture of HHV-6 from PBMCs, serum, or plasma of patients with exanthem subitum during the febrile period is considered diagnostic[3] but is available only in research laboratories. Detection of HHV-6 DNA in plasma in children younger than 2 years is not considered sufficiently specific to differentiate infants with acute HHV-6 infection from those with serious bacterial infections.[65]

Immunocompromised Persons

Diagnosis of HHV-6 as the cause of symptoms in immunocompromised patients is often difficult. Because HHV-6 frequently reactivates in a large proportion of these patients, it is important to distinguish latent infections that are not associated with disease from productive HHV-6 infections that may cause disease. A high or a rising level of viral DNA, or detection of viral RNA (available only as a research test), in the blood is more likely to enable differentiation of productive infection associated with HHV-6 disease from latent viral DNA. In a study of HSCT recipients, the presence of more than 10^3 copies of HHV-6 DNA per 10^6 PBMCs was statistically associated with myelosuppression, pneumonitis, fever, and rash.[13] However, in a trial of liver transplant recipients, monitoring of HHV-6 viremia had no effect on outcomes.[66] Detection of viral DNA in the serum or plasma may also be more predictive of disease than the presence of HHV-6 in PBMCs. Detection of HHV-6 RNA, and especially viral proteins, in tissues is more specific for active disease than is detection of viral DNA. In addition to a rising level of HHV-6 in the blood, and preferably detection of HHV-6 in tissue, other potential causes of disease (including CMV, which is often associated with HHV-6) must be excluded before HHV-6 can be considered to have a causative role in disease.

Detection of HHV-6 in the CSF is strongly suggestive of CNS disease but is not absolutely diagnostic because some patients with HHV-6 in the CSF have had other CNS diseases documented at autopsy, and HHV-6 has been detected in the CSF of children years after acute infection.[67] In one study, HHV-6 DNA was detected in the CSF of 8/11 (73%) patients with neurologic dysfunction and 3/11 (27%) of asymptomatic HSCT recipients.[68] Although another study reported HHV-6 in the CSF of 23% of transplant recipients with encephalitis, compared with only 1% of transplant recipients without encephalitis, controls were not well matched for the level of immunosuppression.[69] Thus, other causes of encephalitis must be ruled out before diagnosis of HHV-6 encephalitis is accepted based on CSF findings. Detection of HHV-6 proteins in the brain is more specific than detection of DNA because viral DNA has been detected with polymerase chain reaction (PCR) in the brain of about one-third of healthy persons.[70]

The use of PCR for diagnosis of HHV-6 is complicated by the finding that HHV-6 is integrated into chromosomal DNA in 0.7% to 1.5% of persons.[71] Stem cells containing integrated copies of HHV-6 have transmitted viral DNA to recipients.[12] Therefore ciHHV-6, in addition to HHV-6 productive infection, must be considered when a diagnosis is based on detection of HHV-6 in leukocytes or in CSF that may contain leukocytes. A high level of HHV-6 DNA in the blood (>320,000 copies/mL in whole blood or ≥1 copy per leukocyte) that persists over time is suggestive of ciHHV-6.[72] Digital droplet PCR can enable accurate diagnosis of ciHHV-6 by showing that the HHV-6/cell ratio is 1.[73] Although ciHHV-6 can be reactivated in vitro by histone deacetylase inhibitors,[74] it is unknown if this can occur in vivo. The clinical importance of ciHHV-6 is unknown, although a large study of patients with ciHHV-6 found an increased incidence of angina pectoris but not other

cardiovascular disease.[75] Another study of liver transplant recipients with ciHHV-6 showed a higher rate of bacterial infections.[76] Grade 2 to 4 acute graft-versus-host disease was more common when HSCT donors or recipients had ciHHV-6, although there was no difference in chronic graft-versus-host disease, engraftment, or mortality.[77]

Therapy

HHV-6 is sensitive to ganciclovir, foscarnet, and cidofovir in vitro; the latter two agents are more active in cell culture.[78] Brincidofovir, the lipophilic prodrug of cidofovir that can be given orally, has also been shown to be active against HHV-6A and HHV-6B in cell culture.[79] HHV-6, like CMV, is not sensitive to acyclovir. HHV-6 U69 is a protein kinase that phosphorylates ganciclovir. HHV-6 DNA levels in the CSF and serum declined with ganciclovir or foscarnet therapy in one series; however, without a control group it is unknown whether this was treatment related.[68] Antiviral therapy has been used in some immunocompromised patients, but no controlled studies have shown that these drugs are effective. Some anecdotal reports have suggested that patients with HHV-6 encephalitis may have responded to a 7-day course of ganciclovir or foscarnet. Either drug, or the combination of both drugs, is recommended for immunocompromised patients with HHV-6 encephalitis.[80] Patients receiving full-dose therapy (foscarnet ≥180 mg/kg or ganciclovir ≥10 mg/kg) had a better response than those receiving lower doses; the difference in responses between the two drugs was not significant.[51] Ganciclovir-resistant HHV-6, due to a mutation in the viral U69 protein kinase, has been isolated from a patient with AIDS.[81] Prophylaxis with ganciclovir or foscarnet has been reported to reduce HHV-6 reactivation or encephalitis in small studies,[82–85] but this is not recommended owing to the toxicity of the drugs and the low incidence of disease in immunocompromised patients. Intensive prophylaxis (ganciclovir 5 mg/kg daily on days −8 to −2 during conditioning followed by valacyclovir 2 g every 8 hours for the first 100 days after transplant) reduced HHV-6 reactivation in a small prospective study of cord blood transplant recipients.[60]

A number of small molecules that inhibit HHV-6 are being investigated.[78] These include cyclopropavir[86] and benzimidazole analogues,[87] which have been shown to have activity against HHV-6. HHV-6–specific T-cell immunotherapy has been shown to reduce disease in HSCT recipients with HHV-6 reactivation.[88,89] Work on development of antivirals and on adaptive immunotherapy for HHV-6 and HHV-7 infections has been reviewed as part of the summary of the 9th International Conference on Human Herpesviruses 6 and 7.[90]

HUMAN HERPESVIRUS TYPE 7

History

HHV-7 was discovered by Frenkel and colleagues[91] in 1990 in a healthy person and was shown to be a cause of exanthem subitum.[92]

Description of the Virus

HHV-7, like HHV-6, is a member of the *Roseolovirus* genus and shares 20% to 75% amino-acid identity with HHV-6 in many of their viral proteins.[5] The HHV-7 genome contains about 145 kilobase pairs of DNA.

Epidemiology

HHV-7 infections occur at a later age than HHV-6 infections (see Fig. 139.1).[7] About 18% of children are infected with HHV-7 by 1 year of age and 53% by 2 years. Most children are infected between ages 2 and 5, presumably from infected saliva of parents and siblings.[93] HHV-7 DNA was detected in PBMCs from 67%, and in cervical swabs from 3%, of pregnant women.[8] About 50% of HSCT and 20% of solid-organ transplant recipients reactivate HHV-7 as indicated by viral DNA in the peripheral blood.[14,94]

Pathogenesis

HHV-7 has a narrower tissue tropism than HHV-6. HHV-7 infects $CD4^+$ T cells, epithelial cells in the salivary glands, and cells in the lungs and skin. HHV-7 is frequently shed in saliva at high levels throughout life in most adults and children.[95] The virus has been detected in breast milk and establishes latency in $CD4^+$ cells. HHV-7 induces degradation of MHC class I molecules.

Clinical Manifestations

Primary HHV-7 infection may be asymptomatic or associated with fever or febrile seizures. In a study of 30 children with HHV-7 viremia, the most common clinical presentation was seizures, which occurred at 12 to 63 months of age; 10 of 12 patients had febrile seizures.[7] HHV-7 viremia was present in 7% of children with febrile status epilepticus; 5% of patients had primary HHV-6B infection and 2% had virus reactivation.[22]

The second most common presentation of HHV-7 viremia was nonspecific fever with a mean temperature of 40.1°C. Less common symptoms were upper respiratory tract disease, vomiting, and diarrhea. Leukopenia was frequently noted. In a study of 496 children presenting to the emergency department, children with HHV-7 had a similar level of fever, rash, and gastrointestinal symptoms but were older and more likely to have seizures than those with HHV-6.[96]

HHV-7 is also a cause of exanthem subitum, although most cases are due to HHV-6. HHV-7 has been less frequently associated with CNS disease than HHV-6, but two cases of hemiplegia associated with HHV-7 have been described. HHV-7 has been associated with encephalitis in immunocompetent[97,98] and immunosuppressed[99] patients. Ten percent (15/156) of young children hospitalized in Britain and Ireland with encephalitis or fever and seizures were found to have an acute HHV-7 infection.[64] HHV-7 was not reported to cause congenital infection (defined as viral DNA in cord blood) in more than 5600 births.[40]

Laboratory Diagnosis

Like HHV-6, the most common diagnostic test for HHV-7 in children is seroconversion based on detection of antibody by indirect immunofluorescence assay or ELISA.[64] Detection of HHV-7 in serum or plasma is much less common than for HHV-6; therefore the presence of HHV-7 DNA in blood in the absence of antibody is more likely to be indicative of acute infection. HHV-7 has been cultured from PBMCs of patients with exanthem subitum, but this is done only in research laboratories.

Unlike HHV-6, levels of HHV-7 in the blood did not correlate with disease in immunocompromised patients.[13] Because HHV-7 DNA has been detected in the brain by PCR in 37% of adults,[100] detection of HHV-7 protein is more specific than viral DNA for the diagnosis of encephalitis.

Therapy

Like HHV-6, HHV-7 is most susceptible to foscarnet and cidofovir in vitro, although virus replication is also inhibited by ganciclovir. There are insufficient clinical reports to indicate whether these drugs are effective in vivo.

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The complete reference list is available online at Expert Consult.

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140 Kaposi Sarcoma–Associated Herpesvirus (Human Herpesvirus 8)

Kenneth M. Kaye

SHORT VIEW SUMMARY

Definition
- Kaposi sarcoma–associated herpesvirus (KSHV), or human herpesvirus 8 (HHV-8), is the etiologic agent of Kaposi sarcoma and primary effusion lymphoma and is tightly linked with multicentric Castleman disease.

Virology and Epidemiology
- KSHV establishes lifelong infection, primarily persisting in latently infected B lymphocytes.
- Replication occurs in oral epithelium, and infectious KSHV is present in the saliva of asymptomatic seropositive individuals.
- Transmission is predominantly the result of exposure to infected saliva.
- Primary infection is usually asymptomatic and rarely recognized.
- In contrast to other herpesviruses, seroprevalence of KSHV varies significantly throughout the world and is highest in sub-Saharan Africa and the Mediterranean region and in men who have sex with men in the United States.
- KSHV malignancy usually occurs in the setting of immune suppression.

Microbiology
- KSHV is a gamma-2 herpesvirus, genus *Rhadinovirus*.
- KSHV is an enveloped, double-stranded DNA virus.
- KSHV is also known as human herpesvirus 8 (HHV-8).

Diagnosis
- KS can be diagnosed by its clinical appearance and confirmed by biopsy.
- Primary effusion lymphoma and multicentric Castleman disease are diagnosed by biopsy.
- KSHV infection can be diagnosed serologically, although assays are not standardized.

Therapy
- Antiviral therapy is currently only available for the lytic stage of infection, and is of no proven benefit in the treatment of KSHV malignancies.
- Enhancing immunity with antiretroviral therapy in HIV infection, or through reduction of immune suppression in transplantation, can lead to KS regression.
- Cytotoxic approaches are often necessary for KSHV malignancy.

Prevention
- There is currently no KSHV vaccine.

Kaposi sarcoma (KS)–associated herpesvirus (KSHV), or human herpesvirus 8 (HHV-8), is the eighth and most recently discovered human herpesvirus. KSHV was discovered as a result of its connection with KS and is also linked with primary effusion lymphoma (PEL) and multicentric Castleman disease. The role of KSHV in malignancy has generated much interest in this virus.

HISTORY

Kaposi sarcoma was first described in 1872 by Moritz Kaposi, a prominent Hungarian dermatologist.[1] Kaposi described findings in five men of "idiopathic multiple pigmented sarcoma of the skin."[2] He noted aggressive disease and emphasized that the syndrome was incurable and rapidly lethal.[3] In fact, three of the men reported by Kaposi were dead within 16 months of presentation, and autopsy demonstrated disseminated disease. Despite the aggressive nature of the disease Kaposi described, KS subsequently came to be regarded as an indolent disease in elderly men of Mediterranean and eastern European descent. It is not clear what accounted for the evolution in the defining features of KS from the aggressive, rapidly fatal disorder described by Kaposi to a relatively mild one. During the 1950s, KS was recognized as an important disease in areas of sub-Saharan Africa.[4] Then, in 1981, Alvin Friedman-Kein reported on 50 young men who had had sex with men with KS of the skin, lymph nodes, mucosa, and viscera.[5] This report heralded the acquired immunodeficiency syndrome (AIDS) epidemic. The similarity of the original syndrome described by Kaposi and that seen in human immunodeficiency virus (HIV) infection is striking and raises the question of whether AIDS-like immune suppression was present in the men initially described.[3]

Discovery of KSHV

KSHV was identified in 1994 by Chang and Moore and coworkers[6] in KS lesions by using a polymerase chain reaction (PCR)–based technique termed *representational difference analysis*. This technique searches for DNA, such as from a virus, that is present in diseased tissue and absent in normal tissue.[7] These investigations were based on epidemiologic observations suggesting that an infectious agent may have an etiologic role in KS. KS occurred at a 20-fold higher rate in men who had had sex with men who had AIDS, compared with those who contracted AIDS by other means, such as by a bloodborne route. Subsequent to this seminal discovery, work by many groups worldwide has elucidated much about this virus.

CLASSIFICATION AND BIOLOGY

KSHV is the only known human rhadinovirus (gamma-2 herpesvirus) and is related to other rhadinoviruses, including those that infect New World (South American) and Old World (African) monkeys and rodents (murine gammaherpesvirus 68).[8–12] Two Old World monkey rhadinoviruses (RFHVMm and RFHVMn) are found in retroperitoneal fibromatosis in monkeys. This entity has histologic similarities to KS. *Herpesvirus saimiri* (HVS), a New World virus, can cause T-cell lymphoma when infecting New World monkeys that are not its natural host.[13] Epstein-Barr virus, a gamma-1 herpesvirus, is KSHV's closest human relative.

Virus Description

KSHV is an enveloped virus that measures 140 nm in diameter, and its appearance by electron microscopy is indistinguishable from that of other herpesviruses.[14,15] The KSHV genome contains approximately 140 kb of unique sequence,[8,16] which encodes approximately 100 open reading frames (ORFs). The nomenclature of the ORFs is based on that of HVS because of high sequence and positional homology with those of HVS and the fact that HVS was the only fully sequenced gamma-2 herpesvirus before KSHV. ORFs without homology to those in HVS are numbered sequentially with K prefixes. A number of KSHV genes are homologues of human genes that were presumably "pirated" from mammalian cells during the evolution of the virus. The unique KSHV

sequence is flanked by approximately 40 copies[17] of 0.8-kb guanine- and cytosine-rich terminal repeat elements. This translates to the terminal elements comprising about 20% of the viral genetic sequence, a feature common to the gamma-2 herpesviruses. This large devotion of energy to the terminal repeats is likely due to their central role in KSHV persistence during latent infection.

KSHV Entry Into Cells

KSHV attaches to cells before entry by binding to cell surface heparin sulfate, integrins (including $\alpha_3\beta_1$), and cysteine transporter xCT.[18,19] The bound virus is then translocated to lipid rafts and binds to the EphA2 receptor, where internalization occurs.[20,21] Binding of these cell receptors activates signaling cascades that facilitate virus entry into the cell by endocytosis or macropinocytosis, and the virus enters the cell in endosomes. The virus envelope then fuses with the endosomal membrane to release the virus capsid, which traffics to the nuclear pore, where it delivers virus DNA into the nucleus.[1]

Lytic Virus Infection

KSHV is capable of both latent and lytic infection.[9,22,23] During lytic infection, many encapsidated viral progeny are produced in a cell and then released as the infected cell dies. Almost all of the nearly 100 KSHV genes are devoted to, and only expressed during, lytic infection. These genes encode proteins responsible for replication of the viral DNA and packaging the DNA into capsids. The viral genome is linear, with terminal repeats on each end when packaged in viral capsids. In addition to genes involved in virus replication, some genes expressed during lytic infection are involved in immune evasion, preventing the host from properly responding to and targeting the infected cells.[24–26]

Latent Virus Infection

Latent KSHV infection sharply contrasts with lytic infection.[22,23,27,28] Latent infection predominates over lytic infection in KSHV-infected tumors and cell lines, with only a small fraction of infected cells undergoing lytic infection. The primary reservoir for KSHV is likely circulating B lymphocytes. Because of its capacity for latent infection, KSHV persistence in its human host is lifelong, similar to other herpesviruses. In latently infected cells, the viral genome circularizes by fusing at its terminal repeat ends and persists as a multiple-copy (ranging in number from 10 to 50 copies) extrachromosomal episome (plasmid) within the nucleus. Only approximately five KSHV genes are expressed during latent infection. Rather than causing cell death, these genes encourage cell survival. Because promotion of cell survival is also a prominent feature of malignancy, it is not surprising that KSHV is associated with certain tumors.[29]

KSHV Gene Expression in Latent Infection

Genes expressed in latent infection have important roles in tumorigenesis.[30–34] To persist in latent infection in proliferating cells such as tumor cells, KSHV episomes must replicate and efficiently segregate to progeny nuclei. The viral latency-associated nuclear antigen (LANA, or ORF73) acts on a specific sequence in the virus terminal repeat DNA to mediate KSHV DNA replication and to tether episomes to chromosomes during mitosis to ensure efficient segregation to daughter cells. LANA also exerts effects on transcriptional regulation and cell growth. Viral cyclin D (ORF72) is a homologue of cell cyclin D and stimulates the G_1-to-S transition of the cell cycle. The viral cyclin D is resistant to the multiple inhibitors that normally inhibit cell cyclin D, resulting in unchecked cell growth. The KSHV viral FADD-like interleukin-1β–converting enzyme (FLICE)–inhibitory protein (vFLIP, or K13) activates nuclear factor kappa B (NF-κB) and inhibits apoptosis, thereby preventing the cell from eliminating itself once it is infected. Notably, LANA, the viral cyclin, and vFLIP are consistently expressed in all latently infected cells from a single promoter. The kaposin locus encodes overlapping ORFs, and this transcript and its protein products are induced in lytic infection. Kaposin A (K12) has been reported to exert transforming effects, and kaposin B acts to increase the expression of cytokines. Perhaps the most important function of the kaposin transcript is the expression of viral microRNAs. These microRNAs include an orthologue of miR-155, which affects B-cell differentiation, and other microRNAs that target inhibitors of NF-κB and of a cell cyclin–dependent kinase, thereby promoting NF-κB activity and cell cycle progression. Latency-associated membrane protein (LAMP, or K15) interacts with growth control proteins. LANA2 (vIRF3) is expressed in B cells, not in KS tissue, and inhibits apoptosis.

Possible Paracrine Effects of Lytic Virus Infection

Although only a small percentage (≈1%) of cells within tumors undergoes lytic infection, these cells may also have an important role in tumorigenesis. For instance, in lytic infection, a G protein–coupled receptor homologue (ORF74) that is constitutively active and has paracrine effects is expressed.[35] Therefore, although the cell with lytic infection will die, it can produce factors that have growth effects on nearby cells. In fact, transgenic mice expressing this viral protein have KS-like lesions.[36,37]

Laboratory Infection Models

Cell culture and transformation models for KSHV remain limited. Primary bone marrow endothelial cells can be infected and transformed, but only approximately 5% of the cells are infected, with paracrine effects stimulating growth in the other cells.[38] Primary rat mesenchymal precursor cells are efficiently transformed by KSHV and can serve as a useful tool to assess KSHV transforming function, although harvesting these cells requires specialized expertise.[39] Because of a lack of a cell line permissive for KSHV lytic replication, the mainstay of KSHV production is from cell lines derived from KSHV primary effusion lymphomas. The vast majority of cells in these lines are latently infected, but lytic infection can be induced by several methods, such as incubation with phorbol esters, to produce infectious virus, albeit at relatively low titers. The most tractable models so far used to study the effects of KSHV virus infection are in dermal microvascular cells.[23] KSHV induces phenotypic changes in these cells, such as spindle formation, but does not immortalize or fully transform them.

PATHOGENESIS

Suppression of Immunity as a Factor Leading to KSHV Malignancy

KSHV has an etiologic role in KS, PEL, and multicentric Castleman disease. Overall, KSHV is well adapted to its human host and usually does not cause disease. Such a situation is ideal from the point of view of the virus because a commensal existence without harm to its host enhances its long-term survival. Suppression of the immune system appears to disturb the delicate balance between KSHV and its human host and can lead to KSHV-associated malignancy. However, other poorly understood factors also contribute to tumorigenesis. For instance, the cause of the more frequent occurrence of KS in men rather than in women, despite a similar prevalence of KSHV infection in many instances, is not clear. Furthermore, before the HIV epidemic, KS occurred relatively frequently in Uganda and Cameroon but not in Botswana and the Gambia, despite KSHV infection being common to all these countries.[40] These findings argue for as yet unknown factors interacting with KSHV to induce KS.

KSHV and Inflammation

There is an interesting link between inflammation and KSHV pathogenesis despite the fact the depression of immunity is the typical scenario leading to KSHV malignancy. Inflammatory cell infiltrates composed of lymphocytes, plasma cells, and macrophages are often found in KS. In addition, KS can occur at sites of trauma (Koebner phenomenon). Most notably, the immune reconstitution inflammatory syndrome (IRIS) (see "KS-Associated IRIS After Institution of Antiretroviral Therapy" later), in the setting of antiretroviral therapy (ART) for HIV infection, can exacerbate or lead to KS. Cultured endothelial cells infected with KSHV express a number of inflammatory cytokines and chemokines, including interleukin (IL)-6.[2] Cyclooxgenase-2 (COX-2) is expressed in KS and in infected cultured cells and leads to chemokine secretion.[3] Notably, KSHV expresses a viral IL-6 homologue (vIL6) and also viral chemokines. vIL6 and IL-6 are both significantly elevated and believed to be an important component of multicentric Castleman disease (see

under "Clinical Manifestations" later).[4,5] KSHV-encoded chemokines and vIL6 are expressed during lytic virus infection, although vIL6 can also be expressed in latency. Despite the clear linkage between KSHV and inflammation, much remains to be elucidated regarding the role of inflammation in KSHV pathogenesis.

EPIDEMIOLOGY

Assays to Identify KSHV Infection

Assays to identify KSHV-infected individuals are still evolving.[9,23,41–43] Serologic assays for antibodies against specific KSHV antigens expressed during the latent or lytic phases of infection have been most commonly used. The assays differ in sensitivity and specificity, resulting in some that likely overestimate and others that underestimate seropositivity. With these limitations in mind, certain general conclusions regarding prevalence of KSHV infection can be made. Detection of KSHV DNA by PCR assay of blood is less sensitive than the serologic assays, reflecting highly variable levels of viremia occurring in both those with and those without KSHV-induced disease.

Geographic Variance of KSHV Seroprevalence in Contrast to Other Herpesviruses

KSHV differs from other herpesviruses in that it does not cause worldwide ubiquitous infection.[9,23,41] Instead, the prevalence of infection in the general population varies significantly in different areas of the world. Sub-Saharan Africa has the highest rate of infection, with approximately 50% of the population infected. Seroprevalence is approximately 10% in the Mediterranean region, although in certain areas of Italy it approaches 30%. Seroprevalence in the United States and northern Europe is approximately 5%, but only 0.2% of individuals in Japan are positive. Despite the low prevalence of KSHV in the general population in the United States, approximately 15% to 20% of HIV-negative and approximately 40% of HIV-positive men who have sex with men are KSHV seropositive.[44] In contrast to the general population, approximately 90% to 100% of individuals with KS are seropositive, consistent with KSHV's etiologic role in this disease.

KSHV Transmission

There are several patterns of KSHV transmission. In the United States, KSHV is spread predominantly through sexual contact among men who have sex with men. Among men who have sex with men, KSHV seropositivity is associated with high numbers of sexual partners, a history of sexually transmitted diseases, and the use of amyl nitrates.[44,45] In contrast to the well-documented sexual transmission among men who have sex with men, the evidence for heterosexual KSHV transmission is conflicting.[46–50] In areas of the world where KSHV infection is more prevalent, nonsexual transmission also occurs, and KSHV infection occurs among children before they are sexually active.[51,52] Intrafamilial clustering has also been documented as further evidence of nonsexual transmission.[53] Saliva is likely a unifying vehicle of both sexual and nonsexual KSHV transmission. Relatively high titers of KSHV DNA can be found in the saliva of infected individuals, likely produced from lytic infection in oral epithelial cells, whereas high levels of virus are not found at other sites. In one study of 50 KSHV-infected men who had sex with men without KS, 30% of oropharyngeal samples, compared with 1% of anal and genital samples, were positive for KSHV. KSHV from the oral cavity was 2.5 logarithms higher than the titer at other sites.[44] Interestingly, deep ("French") kissing was a risk factor for KSHV transmission among men who have sex with men.[44] Human leukocyte antigen alleles may influence the degree of KSHV shedding in saliva.[54] Solid-organ transplantation from a seropositive donor to a seronegative recipient has also been shown to transmit KSHV.[55] Vertical transmission from mother to infant can occur but appears to be rare.[56] Transmission by blood transfusion can also occur, and the risk is greatest in regions of high KSHV seroprevalence. Whether or not the blood supply should be screened in the United States, where the seroprevalence is low, remains controversial. However, there is currently no US Food and Drug Administration–approved diagnostic test for KSHV infection, limiting potential strategies to screen blood products for KSHV infection.[57–59]

CLINICAL MANIFESTATIONS

Primary Infection

A primary infection syndrome for KSHV has not been clearly described, and most infections are probably asymptomatic or unrecognized. In a prospective Egyptian study, 86 children ages 1 to 4 years presenting to the emergency department with fever of unclear origin were evaluated for KSHV infection. Six of the children likely had primary KSHV infection because they were seronegative but had KSHV DNA detected in saliva. In three of these subjects, follow-up serology was obtained, and all three seroconverted to KSHV. All but one of the six had a maculopapular rash that began on the face and gradually spread downward over the trunk and extremities. Five of the six also had associated upper respiratory tract symptoms. Fever persisted for a median of 10 days.[60] Primary KSHV infection was associated with mild symptoms of diarrhea, fatigue, localized rash (ankle and face), and lymphadenopathy (cervical and submental) in four of five HIV-negative men.[61] A 43-year-old HIV-infected man developed fever, arthralgia, cervical lymphadenopathy, and splenomegaly 5 weeks after KSHV seroconversion, and his illness spontaneously resolved within 10 weeks. Biopsies showed angiolymphoid hyperplasia and foci of KS. Neither lesions nor clinical symptoms had recurred after 8 years of follow-up (on ART).[62] Four months after transplantation, two renal allograft recipients developed primary KSHV infection from the same KSHV-positive donor. One recipient developed disseminated KS and the other a syndrome of fever, splenomegaly, cytopenia, and marrow failure with plasmacytosis. KSHV infection of immature progenitor cells from the aplastic bone marrow was noted for the patient with marrow failure.[63] Although these data are very limited, it appears that primary infection in immunocompetent hosts is self-limited, whereas primary infection in immunosuppressed hosts can be severe and have significant consequences.

Kaposi Sarcoma

KS typically involves the skin and manifests as lesions that enlarge from patches to plaques to nodules.[64,65] The lesions often begin as violaceous and later evolve into a brown color because of hemosiderin deposition (Fig. 140.1). KS lesions are composed of vascular spaces, extravasated erythrocytes, and several different types of cells (Fig. 140.2). These include the malignant spindle cells and infiltrating mononuclear cells, such as hemosiderin-laden macrophages. The highly vascular nature of KS gives it its purple color. In the nodular stage, nearly all spindle cells are KSHV infected (see Fig. 140.2). KS is typically not monoclonal, and different tumor nodules in individuals may have different origins.[2]

Four Epidemiologic Forms of KS

There are four variants of KS: classic, endemic, epidemic, and iatrogenic, which differ epidemiologically and clinically.[9,23,66,67] The occurrence of KS largely reflects the seroprevalence of the population, with KS more common in areas with high KSHV seropositivity. *Classic KS* occurs in elderly men of Mediterranean or eastern European descent, predominantly involves the skin of the lower extremities, and is indolent. *Endemic KS* occurs in certain sub-Saharan African countries. At least two forms of endemic KS occurred before the HIV epidemic. In adults, cutaneous KS occurred in an approximately 20:1 ratio of men to women; it clinically resembled classic KS in adults. However, in children younger than 10 years, KS caused an aggressive, multifocal, lymphadenopathic form, often without cutaneous lesions, that was frequently fatal.[64,65,68]

Epidemic KS, which refers to KS in HIV-infected individuals, tends to be aggressive, commonly involving the skin, gastrointestinal tract, and respiratory tract. KS in the lung often has lesions present in the bronchial mucosa but may be associated with a variety of radiographic manifestations, including nodules, adenopathy, and pleural effusions. In contrast to the lesions of classic KS, lesions in epidemic KS commonly involve the face (often the nose), genitalia, and oral cavity (palatal and gingival), in addition to the lower extremities.[69] This form is most common in the United States, where it predominantly affects men who have sex with men. However, KS largely occurs in heterosexual HIV-infected individuals in Africa. Since the start of the HIV epidemic in Africa, the ratio of men to women with KS has dropped 10-fold to



FIG. 140.1 Kaposi sarcoma (KS) of the foot (A) and leg (B) in two human immunodeficiency virus–positive patients. Lesions are highly vascular and often occur on the lower extremities. Newer KS lesions are typically violaceous (A) and evolve to a brownish color (B) over time because of hemosiderin deposition. (Numbered labels in A are present as part of a clinical treatment trial.) *(Courtesy Bruce Dezube, MD.)*



FIG. 140.2 Kaposi sarcoma *(KS)* involving a lymph node. (A) A spindle cell proliferation *(white arrows)* containing poorly formed vascular spaces with entrapped red blood cells *(black arrows).* Areas of uninvolved lymph node *(LN)* are seen at the top. (Hematoxylin and eosin stain.) (B) Immunohistochemical detection of Kaposi sarcoma–associated herpesvirus (KSHV) latency-associated nuclear antigen *(brown)* in the nuclei of many spindle cells indicates KSHV infection (×200). *(Courtesy Dan Jones, MD, PhD.)*

approximately 2:1. The number of childhood cases of KS has also significantly increased in Africa with the AIDS epidemic.[70,71] For instance, in Zambia in the early 1980s, KS accounted for 0% to 2% of childhood malignancies, but by 1992 it accounted for approximately 25% of childhood malignancies.[71–73] *Iatrogenic KS* occurs in individuals who are immunosuppressed, such as from organ transplantation, and tends to be aggressive. Kidney allograft recipients appear to be at higher risk for developing KS compared with other transplant recipients.[74]

The incidence of KS in HIV infection has decreased significantly in developed countries since the introduction of ART, but the standardized incidence rate remains very high for KS compared with other cancers in HIV infection.[75,76]

Diagnosis

Although KS can often be recognized by a trained observer, the diagnosis is easily confirmed by biopsy.[65,69] Early stages of KS can be more difficult to recognize. The differential diagnosis of KS includes bacillary angiomatosis, which is caused by *Bartonella* species. Skin lesions of bacillary angiomatosis are very vascular and may mimic those of KS.[65]

KSHV Viral Load Measurement

The measurement of KSHV viral loads has been performed on peripheral blood of patients with KS. Viral loads are performed by PCR assay of viral DNA either in plasma or in peripheral blood mononuclear cells (PBMCs), and studies vary as to which blood component is used. One study showed that detection of KSHV DNA in PBMCs of HIV-infected individuals without KS predicted the development of KS lesions.[77] Plasma KSHV DNA levels were greater in more advanced KS disease compared with less advanced disease; they were also greater in AIDS KS compared with classic KS.[78,79] KSHV levels were higher in PBMCs in patients with active KS compared with those with KS in remission,[79] and KSHV levels in buffy coat cells were higher in those patients with higher rates of eruptions of KS lesions.[80] A study comparing plasma and PBMC KSHV load in patients with KS found that there was generally a linear relationship between the two,[78] although there was significant variation in the correlation for many individuals. Despite the detection of KSHV in the blood of KS patients and apparent correlation with KSHV load and disease activity, the clinical use of viral loads for monitoring KS activity or as a guide for therapy is limited by the relatively low levels of KSHV viremia.[79] In contrast, the levels of KSHV viremia are significantly higher in multicentric Castleman disease.

Genetic Predisposition to KS

Rare genetic disorders can predispose to KS. Wiskott-Aldrich syndrome, caused by a mutation of the *WAS* gene, is X-linked recessive, results in susceptibility to many infections, and can lead to aggressive KS. Interferon-γ receptor 1 deficiency, caused by mutation in the *IFNGR1* gene, is autosomal recessive and also results in a generalized immunodeficiency that can lead to KS.[6] Mutation of *STIM1,* which encodes the stromal interaction molecule 1, results in an autosomal-recessive disorder, and had led to fatal KS in a 2-year old child.[81] STIM1 is an endoplasmic reticulum membrane protein, which regulates calcium stores in the cell, and defects in this gene also result in susceptibility to other infections.[82] Mutation of the *TNFRSF4* gene, encoding OX40, leads to an autosomal-recessive disorder that was described in a 14-year-old patient who developed aggressive KS.[7] This patient did not have a history of other infections. A rare heterozygous amino acid substitution in the *STAT4* gene was present in five individuals with KS in one family; these family members did not otherwise have a history of immunodeficiency.[8] The unifying theme for these genetic disorders is a defect in T-cell immunity, leading to loss of control of KSHV infection. Case-control studies have also identified genetic variants of *FCGR3A, CXCR2,* and *IL13* that may be linked with classic KS.[2]

Treatment

ART is generally recommended for HIV-infected patients with KS and often leads to regression of KS lesions as the immune system reconstitutes.[65,83] In iatrogenic KS, boosting immunity through reduction of immune suppression can lead to KS remission, again highlighting the critical role of the immune response to KSHV. KS regressed in renal transplantation patients with KS who were switched from cyclosporine to rapamycin (sirolimus) immunosuppression. Therefore immunosuppression with rapamycin (or one of its analogues) should be considered in transplant recipients with KS.[84,85]

Despite these approaches, targeted therapy of KS is often necessary. Such strategies are palliative and not curative.[65,69,75,83] Depending on the severity of disease, treatment options may include observation, topical therapy, or systemic therapy. Local therapy may include chemotherapeutic agents, laser treatment, cryotherapy, and irradiation. Systemic therapy is reserved for more severe disease and includes liposomal anthracyclines, paclitaxel, and vinorelbine. Importantly, corticosteroids have been associated with the appearance or worsening of KS lesions. Withdrawal or reduction of steroids can lead to regression of KS lesions.[9]



FIG. 140.3 Kaposi sarcoma–associated herpesvirus–infected primary effusion lymphoma cells from this pleural effusion have plasmacytoid features and deeply basophilic cytoplasm. Red blood cells are interspersed among the lymphoma cells. (Wright-Giemsa stain, ×1000.) *(Courtesy Dan Jones, MD, PhD.)*

KS-Associated IRIS After Institution of Antiretroviral Therapy

With the increased utilization of combined ART, the phenomenon of IRIS has also been recognized in the setting of KS. Manifestations have included worsening of KS that is already clinically present or "unmasking" of KS that was not apparent until then.[86–88] Therapy with ART should generally be continued in cases of KS-associated IRIS, and cytotoxic chemotherapy may also be necessary to control disease.[89–91]

Primary Effusion Lymphoma

PEL was first described in 1989 in HIV-infected patients.[92] It occurs in the potential body spaces of the pleural, pericardial, and peritoneal cavities.[9,93] Lymphoma cells (Fig. 140.3) grow in suspension with little or no contiguous solid mass component. Cells contain clonal immunoglobulin gene arrangements, indicating a B-cell origin, despite lacking most typical B-cell antigens. The malignant cells are infected with KSHV, and Epstein-Barr virus often coinfects the cells. PEL is rare, accounting for approximately 3% of AIDS-related lymphomas and only an estimated 0.4% of non–AIDS-associated large cell non-Hodgkin lymphomas.[94] The prognosis is poor, with death often occurring within months of diagnosis. Patients with PEL tend to have higher levels of KSHV viremia than those with KS but lower levels than patients with multicentric Castleman disease.[79,95] In HIV-associated PEL, ART appears to be beneficial and is typically administered with cytotoxic chemotherapy. Radiation can sometimes be used when chemotherapy is not possible or has failed.[10] A rare solid tumor variant of PEL that does not occur in potential body cavities has also been described in HIV-infected patients.[96]

Multicentric Castleman Disease

Castleman disease is a rare lymphoproliferative disorder, first described in 1956,[97] that occurs in two forms. Localized Castleman disease (hyaline vascular variant) is not associated with KSHV and has an indolent clinical course. Multicentric Castleman disease (plasma cell variant), first described in 1978,[98] is associated with KSHV and has a much more aggressive clinical course, frequently resulting in death. Multicentric Castleman disease is often associated with fever, hepatosplenomegaly, and generalized lymphadenopathy. Complications include infection (often a cause of death) and the development of either a plasmablastic lymphoma or KS.[99,100] KSHV is almost always linked to multicentric Castleman disease in HIV-infected individuals, and KSHV infection is linked to approximately 50% of cases in individuals without HIV infection.[101,102] IL-6, which induces B-cell differentiation, is expressed at high levels in the germinal centers of affected lymph nodes and may be responsible for the high numbers of plasma cells

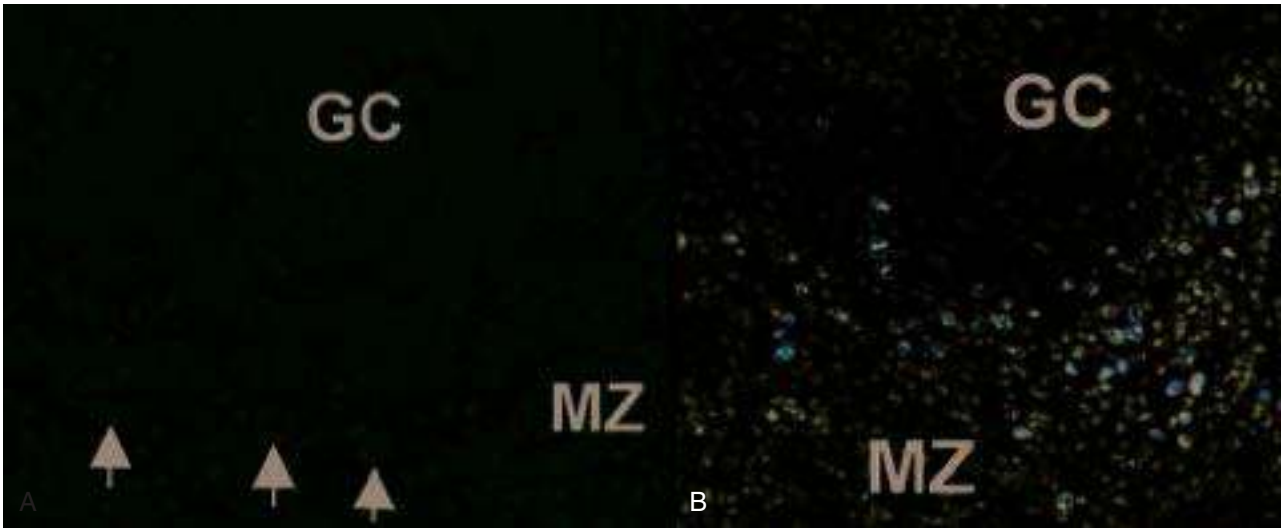

FIG. 140.4 Multicentric castleman disease in a lymph node of a human immunodeficiency virus–negative patient. (A) Regressed germinal center *(GC)* has atypical plasmablasts *(arrows)* concentrated in the follicle mantle zone *(MZ)*. (Hematoxylin and eosin stain, ×600.) (B) Immunohistochemical detection of Kaposi sarcoma–associated herpesvirus (KSHV) latency-associated nuclear antigen *(brown)* in the nuclei of plasmablasts indicates KSHV-infected cells (×400). *(Courtesy Dan Jones, MD, PhD.)*

present (Fig. 140.4A). Of interest, KSHV encodes a homologue of IL-6 that is expressed in latent and lytic infection and may have a role in disease.[103,104] KSHV-infected plasmablasts are typically seen in the mantle zone of affected lymph nodes (Fig. 140.4B).[105] Although the optimal therapy for multicentric Castleman disease is not clearly defined, treatment modalities include the anti-CD20 monoclonal antibody rituximab, a monoclonal antibody directed against the IL-6 receptor (tocilizumab) or against IL-6 (siltuximab), ganciclovir, and cytotoxic chemotherapy.[100,106–108]

Of interest, KSHV viral loads in peripheral blood are relatively high in multicentric Castleman disease (ranging up to 4 or 5 logarithms). High KSHV levels can be found in both PBMCs and plasma.[96] Furthermore, the presence of symptoms or active disease has been associated with higher levels of KSHV in PBMCs compared with the absence of symptoms or when disease is in remission.[79,109] For this reason, KSHV viral loads may be useful for monitoring activity of disease during treatment of patients with multicentric Castleman disease.[79] It is unclear why there are higher levels of KSHV DNA in multicentric Castleman disease compared with KS. It is possible that there may be increased levels of lytic replication occurring in patients with multicentric Castleman disease,[79,110] but this question remains open.

KSHV Inflammatory Cytokine Syndrome

Recently, a manifestation of KSHV has been described in HIV-infected individuals that appears to be distinct from multicentric Castleman disease.[111] This disease, designated KSHV inflammatory cytokine syndrome (KICS), clinically resembles multicentric Castleman disease in a number of respects, including the presence of systemic inflammation, but the pathologic lymph node findings are absent. KICS patients typically have KS and may also have PEL. In a prospective study, patients diagnosed with KICS had more severe symptoms, higher KSHV viral loads, and an increased risk of death compared with HIV-infected and HIV/KSHV-coinfected non-KICS control subjects.[112,113] Severe CD4 lymphocytopenia appears to be more common in KICS patients, and it is possible that the pathologic characteristics of multicentric Castleman disease are less likely to develop in the setting of very low CD4 T-cell counts.

Other Syndromes

A number of syndromes have been linked to KSHV infection but either are disputed in the literature or have not been confirmed. These include the skin diseases pemphigus and bullous pemphigoid, sarcoid, Kikuchi disease, multiple myeloma, hemophagocytic syndrome, and primary pulmonary hypertension.[9,114] An intriguing link between KSHV and ketosis-prone diabetes has been observed.[115]

THERAPY AND PREVENTION

Several agents have activity against KSHV lytic replication, but none has an established role in KSHV-associated diseases. Ganciclovir, foscarnet, cidofovir, and adefovir, but not acyclovir, inhibit KSHV lytic replication.[116–119] A likely reason for a lack of efficacy of these agents in KSHV-associated diseases is that they target lytic, rather than latent, replication of KSHV. The vast majority of KSHV-infected cells in KS, PEL, and multicentric Castleman disease are latently, not lytically, infected. Development of agents that target latent infection would therefore likely result in a major advance in treatment of KSHV-associated diseases.

Lytic KSHV infection has a role in the biology and transmission of KSHV. Of note, a study investigating cytomegalovirus retinitis in AIDS showed that ganciclovir reduced the incidence of KS.[120] Also, a randomized study showed that oral valganciclovir reduced oropharyngeal KSHV shedding,[121] indicating that interference with lytic infection might reduce rates of KSHV transmission. However, although valganciclovir was well tolerated in this study, the adverse effects of ganciclovir or valganciclovir would mitigate against either being used widely for the prevention of KSHV transmission or disease. The best prevention of KSHV-associated disease would be a vaccine to prevent infection or the development of malignancy, but to date no vaccine has been developed.

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141 Herpes B Virus

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SHORT VIEW SUMMARY

Definition
- Herpes B virus is a macaque virus that can cause fatal encephalitis in humans.

Epidemiology
- Herpes B virus naturally infects Old World macaques, including rhesus and pig-tailed macaques and cynomolgus monkeys.
- Humans are infected with herpes B virus after bites, scratches, needlesticks, or mucosal splashes with fluids from Old World macaques.

Microbiology
- Herpes B virus is an alphaherpesvirus and is the homolog of herpes simplex virus in macaques.

Diagnosis
- A positive polymerase chain reaction (PCR) or culture for herpes B virus in skin lesions, conjunctival swabs, or cerebrospinal fluid in the presence of symptoms is diagnostic for herpes B virus infection.
- A positive PCR or culture for herpes B virus of wounds or mucosa shortly after injury indicates exposure to the virus but not necessarily infection.
- Human specimens for PCR, culture, or antibody testing should be sent to the National B Virus Resource Center in Atlanta, Georgia (www2.gsu.edu/~wwwvir/index.html).

Therapy
- Individuals with signs or symptoms of B virus or positive cultures or PCR (other than wound or postcleansing PCR or culture) and exposure to Old World macaques should be treated.
- Intravenous acyclovir (12.5–15 mg/kg q8h) or ganciclovir (5 mg/kg every 12 hours) is recommended for patients without central nervous system diseases (CNS) disease.
- Intravenous ganciclovir (5 mg/kg every 12 hours) is recommended for patients with CNS disease.
- Treatment is continued until symptoms resolve and two cultures over a 2-week period are negative; oral valacyclovir or acyclovir is often given after intravenous therapy is discontinued to prevent reactivation of latent virus.

Prevention
- First aid, with thorough cleansing of wounds or exposed mucosa, is important after injuries or mucosal splashes with macaque fluids.
- Individuals who have high risk of exposure to herpes B virus (see Table 141.1) should receive postexposure prophylaxis within 5 days of exposure with valacyclovir, 1 g three times a day, or acyclovir, 800 mg five times daily, for 14 days.

Herpes B virus, whose taxonomic species name was formerly Cercopithecine herpesvirus 1 and is now Macacine herpesvirus 1,[1] causes a disease in macaque monkeys that is similar to that seen with herpes simplex virus (HSV) type 1 in humans; however, infection of immunocompetent humans with herpes B virus can result in fatal encephalitis. Individuals who are scratched or bitten or have splashes to mucosal surfaces with material from macaque monkeys should be evaluated for possible herpes B virus infection and, when appropriate, should receive postexposure prophylaxis or treatment.

HISTORY

Herpes B virus was first described in 1933[2] in a researcher who died after being bitten by a macaque. Sabin and Wright[3] isolated the virus and named it B virus after the patient's last name. About 50 cases of herpes B virus in humans have been reported in the literature, with 26 well-documented cases.[4]

DESCRIPTION OF THE VIRUS

Herpes B virus is an alphaherpesvirus in the same subfamily as HSV. The complete sequence of herpes B virus[5] shows that it is closely related to HSV with a conserved genomic structure, and the viral glycoproteins show about 50% amino acid identity between the two viruses.

EPIDEMIOLOGY

Herpes B virus is endemic in Old World macaques, and most macaques in captivity (unless separated from their parents at birth and reared apart from other animals) should be considered as possibly infected. Most macaques are infected during adolescence, and nearly 100% of adult (≥2.5 years old) macaques bred in captivity or in the wild are infected.[6] The virus naturally infects all types of Old World macaques including rhesus macaques *(Macaca mulatta),* cynomolgus monkeys *(Macaca fascicularis),* and pigtailed macaques *(Macaca nemestrina).* The virus has also been isolated from bonnet, Japanese, stumptail, and other macaques, but no other Old World or New World monkeys are naturally infected.[7] Herpes B virus has also been detected in free-ranging monkeys in Bali and other sites in Southeast Asia.[8]

Humans are inadvertent hosts. Humans have been infected by bites and scratches from macaques. Other exposures that have transmitted the virus are needlestick injury from a needle that was exposed to tissue around the eye of a macaque or a needle that was thought to be used to inject monkeys, contamination of wounds with macaque saliva, lacerations from bottles containing macaque cell cultures, scratches from cages, exposure to monkey nervous tissue at autopsy, and possible aerosol exposures.[4] One reported case was caused by a splash to the eye from material from a caged macaque.[9] A single case of human-to-human transmission of herpes B virus was reported in a woman who became infected after applying hydrocortisone cream to her contact dermatitis lesions and her husband's herpes B virus skin lesions.[10] Herpes B virus was reported in a primate worker who had not cared for primates for more than 10 years. The disease was presumed to be due to reactivation of the virus from latency in the worker[11]; however, this case is considered controversial, and the patient may have had an unrecognized exposure to herpes B virus more recently. All cases of herpes B virus except for the patient with mucosal splash have been due to percutaneous exposure. Although a large number of animal bites and scratches occur each year, cases of herpes B virus are rare; nonetheless, the potential for fatalities requires that each of these exposures be evaluated.

[a]All material in this chapter is in the public domain with the exception of any borrowed figures or tables.



FIG. 141.1 Macaque with a lesion on the upper lip due to herpes B virus infection. *(Courtesy J. Hilliard, Georgia State University, Atlanta, GA.)*

PATHOGENESIS

Animals are infected through the mucosa or skin from oral or genital secretions of other animals. Herpes B virus rarely causes disease in macaques,[12] although oral lesions can occur (Fig. 141.1). The virus is latent in the sensory ganglia of the animals and can reactivate with shedding. Sites of shedding include the genital tract and oral and conjunctival mucosa. One study reported oropharyngeal or urogenital shedding rates of 39% in macaques shortly after capture and shipping.[13] On a given day, about 2% of herpes B virus–seropositive healthy adult monkeys shed virus.[14] Shedding is more common in animals that are ill, immunocompromised, stressed, or breeding. Similar to HSV in humans, latently infected macaques shed virus intermittently and often in the absence of lesions. Peripheral blood of macaques has been reported to contain herpes B virus in animals that are ill[15]; viremia rarely, if ever, occurs in healthy macaques.[16]

Humans are infected from monkey oral, genital, or ocular secretions or monkey nervous system tissues, with a usual incubation period of 5 days to 3 weeks (range, 2 days to 5 weeks). The virus replicates at the site of infection and then ascends the peripheral nervous system in retrograde fashion before advancing to the central nervous system (CNS). Antibody to HSV does not protect humans from herpes B virus infection.

CLINICAL MANIFESTATIONS

Asymptomatic infection (i.e., seropositivity without disease) of human primate workers with herpes B virus, including most of those who had histories of bites and scratches, has not been detected.[17,18] Most human infections have been reported from animals without any symptoms. Infection of humans with herpes B virus can initially manifest in three different forms. First, patients may present with nonspecific flulike symptoms including fever, chills, myalgias, and malaise before presenting with CNS symptoms. Second, patients may present with symptoms including itching, tingling, numbness, and pain at the site of herpes B virus inoculation. Some patients have a vesicular rash at the inoculation site and may have lymphadenopathy in the draining lymph nodes. Third, patients may present directly with peripheral nervous system or CNS symptoms. Patients with the first two presentations may develop weakness or paresthesias involving the nerve at the site of infection before developing CNS symptoms. These symptoms include headache, nuchal rigidity, nausea, vomiting, confusion, dysphagia, dysarthria, ataxia, urinary retention, and cranial nerve palsies. The disease progresses from the upper spinal cord to the brainstem and then results in a global encephalitis manifested by seizures, ascending paralysis, hemiplegia, coma, and respiratory failure. Additional symptoms include sinusitis, conjunctivitis, hiccups, and abdominal pain. The mortality rate in untreated humans is estimated at 70%[7] and is considerably lower in patients treated at an early stage of the disease.

LABORATORY DIAGNOSIS AFTER EXPOSURE

Some authorities recommend obtaining baseline serum at the time of exposure to simultaneously test it with serum obtained about 3 to 6 weeks later to document seroconversion or a fourfold rise in titer. Patients receiving acyclovir may have delayed seroconversion; serum can be obtained from patients receiving postexposure prophylaxis 3 to 6 weeks after the exposure and at 12 weeks. Because asymptomatic infection has never been reported, other authorities do not recommend testing serum except to confirm a diagnosis in individuals with symptoms compatible with herpes B virus disease. Positive serologies are confirmed using competition enzyme-linked immunosorbent assay or Western blotting.[17]

Cultures of the wound or exposed mucosa should be obtained only after cleansing is performed so as not to delay first aid or removal of virus from the site. Some authorities believe that cultures are not especially helpful because decisions must be made regarding postexposure prophylaxis before the results are available. Any patient who has a positive culture for herpes B virus needs subsequent follow-up cultures to ensure that they are not shedding virus.

A polymerase chain reaction (PCR) assay for herpes B virus DNA can be performed on lesion swabs,[19] spinal fluid,[20] and other sites. A positive PCR in the setting of symptoms consistent with herpes B virus is considered diagnostic of infection.

Some authorities recommend testing the primate with which the patient was in contact for herpes B virus by culture or serologic test. However, the monkeys can be in the process of seroconverting at the time of the exposure, and a positive serologic test in a monkey does not indicate that it is actively shedding virus. Culture, serology, and PCR testing of humans and primates in the United States is performed by the National B Virus Resource Center in Atlanta, Georgia; the virus should be isolated only in a BSL-3 laboratory. Their website (www2.gsu.edu/~wwwvir/index.html) offers useful information on collecting and shipping specimens.

POSTEXPOSURE EVALUATION AND PROPHYLAXIS

First aid, with prompt, thorough irrigation of wounds and exposed mucosal tissues, is essential to reduce the likelihood of infection. Mucous membranes should be flushed with saline, and wounds should be irrigated with detergent (e.g., chlorhexidine or povidone-iodine) for 15 minutes.[4,21] A health care professional should evaluate the inoculation site and thoroughness of cleansing, document the type of exposure (including whether it involved a macaque), consider obtaining baseline serum samples, consider culturing the wound, educate the patient regarding signs and symptoms of herpes B virus, identify a local medical consultant if the need arises, and consider postexposure prophylaxis. The medical history of the monkey should be evaluated, including whether it is ill or immunocompromised or has lesions compatible with herpes B virus infection. All these factors increase the risk that the animal is actively shedding herpes B virus.

Postexposure prophylaxis with oral acyclovir or ganciclovir was shown to be effective in a rabbit model of herpes B virus infection[22,23] but has not formally been shown to be effective in humans. Nonetheless, although postexposure prophylaxis with antiviral therapy has been recommended only since 1995,[7] no cases of herpes B virus have been reported to date in people receiving postexposure prophylaxis within 3 days of exposure.[4]

A working group convened in 2002 by the US Centers for Disease Control and Prevention prepared a series of recommendations for postexposure prophylaxis of herpes B virus.[4] Certain types of exposure to macaques were considered to impart a much higher risk of herpes B virus infection in humans. These include inadequately cleansed wounds, deep puncture wounds (which are difficult to clean), bites to the head and face (in which virus can quickly travel to the CNS), exposures involving materials known or highly likely to be infected with herpes

TABLE 141.1 Recommendations for Postexposure Prophylaxis for Persons Exposed to Herpes B Virus

Prophylaxis Recommended
Skin exposure[a] (with loss of skin integrity) or mucosal exposure (with or without injury) to a high-risk source (e.g., a macaque that is ill, immunocompromised, or known to be shedding virus or that has lesions compatible with herpes B virus disease)
Inadequately cleaned skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury)
Laceration of the head, neck, or torso
Deep puncture bite
Needlestick associated with tissue or fluid from the nervous system; lesions suspicious for herpes B virus; eyelids or mucosa exposure
Puncture or laceration after exposure to objects (a) contaminated either with fluid from monkey oral or genital lesions or with nervous system tissues or (b) known to contain herpes B virus
A postcleansing culture is positive for herpes B virus
Prophylaxis Considered
Mucosal splash that has been adequately cleaned
Laceration (with loss of skin integrity) that has been adequately cleaned
Needlestick involving blood from an ill or immunocompromised macaque
Puncture or laceration occurring after exposure to (a) objects contaminated with body fluid (other than that from a lesion) or (b) potentially infected cell culture
Regimen for Prophylaxis
Valacyclovir, 1 g PO tid, or acyclovir, 800 mg PO 5 times daily × 14 days
Prophylaxis Not Recommended
Skin exposure in which the skin remains intact
Exposure associated with nonmacaque species of nonhuman primates

[a]Exposures include macaque bites or scratches or contact with ocular, oral, or genital secretions; nervous system tissues; or materials contaminated by macaques (e.g., cages or equipment).
PO, Per os (orally).
From Cohen JI, Davenport DS, Stewart JA, et al. Recommendations for prevention and therapy of persons exposed to B virus (Cercopithecine herpesvirus 1). Clin Infect Dis. *2002;35:1191–1203.*

B virus, and exposures involving ill or immunocompromised macaques or those with lesions consistent with herpes B virus disease. Recommendations concerning which patients should receive postexposure prophylaxis are presented in Table 141.1. Postexposure prophylaxis is given as early as possible and within 5 days of the exposure because animals given antiviral medication have benefited as late as 5 days after inoculation.[22,23] Postexposure prophylaxis is not a substitute for prompt and thorough cleansing of the infected site. Most authorities recommend either valacyclovir, 1 g three times daily, or acyclovir, 800 mg five times daily for 14 days, although these medications are not approved for this use by the US Food and Drug Administration. High doses of the oral drugs are used because the dose needed to inhibit virus replication by 50% for herpes B virus is 18 μg/mL,[23] which is about 10 times higher than that for HSV. Valacyclovir is the drug of choice because of the higher serum levels of acyclovir achieved with valacyclovir than with oral acyclovir. If symptoms compatible with herpes B virus disease occur while patients are receiving postexposure prophylaxis, treatment for herpes B virus should be started; thus it is important to follow up patients with a potential herpes B virus exposure whether or not they receive postexposure prophylaxis.

DIAGNOSIS OF HERPES B VIRUS DISEASE

A physical examination of the lesion site (looking for vesicles) and a complete neurologic examination should be performed in patients with herpes B virus disease. Cultures of conjunctiva, oropharynx, and the exposure site are recommended, along with obtaining serum for herpes B virus serologic testing. Magnetic resonance imaging of the brain should be performed, and cerebrospinal fluid should be sent for a PCR assay.[7,20,24,25] Electroencephalography may help differentiate herpes B virus, which initiates with upper spinal cord and brainstem involvement and results in a diffuse encephalitis, from HSV encephalitis, which usually involves one of the temporal lobes. Somatosensory evoked potentials can help identify early lesions in the brain or spinal cord.

PCR assay for herpes B virus has been reported using primers for glycoprotein G, which differs from glycoprotein G in HSV-1 and HSV-2.[25] A real-time PCR assay was as specific, but twice as sensitive, as culture to detect herpes B virus in human and monkey specimens. Regions on herpes B virus glycoproteins B and D have been identified that are recognized by antibodies in sera from infected macaques, and these are being studied for future peptide-based serologic assays to detect herpes B virus infection.[26]

THERAPY

Intravenous treatment rather than oral prophylaxis should be initiated in any patient with signs or symptoms of herpes B virus or a positive culture or PCR (not including a postcleansing culture or PCR from the wound) if the patient has had a documented exposure to a macaque. In the absence of CNS symptoms, either acyclovir, 12.5 to 15 mg/kg intravenously every 8 hours, or ganciclovir, 5 mg/kg intravenously every 12 hours, is recommended until symptoms resolve and two cultures over a 2-week period are negative for herpes B virus.[4] Because in vitro studies and animal models show that herpes B virus is more sensitive to ganciclovir than acyclovir,[23,27] most experts recommend ganciclovir for patients with CNS symptoms. If herpes B virus can establish latency and reactivate in humans, discontinuation of antiviral therapy could allow reactivation to occur. Therefore many authorities recommend that patients who survive herpes B virus infection be maintained on oral acyclovir or valacyclovir, initially at doses used for postexposure prophylaxis and later at suppressive doses, for a prolonged time after intravenous therapy is stopped.[4,7] Repeated cultures for herpes B virus are often recommended after intravenous therapy has been changed to oral therapy to confirm that herpes B virus shedding is not occurring or when antiviral therapy is discontinued.

Before antiviral therapy was available, about 80% of patients with herpes B virus infection died; with antiviral therapy, it is estimated that 80% of patients survive.[28] Although there have been relatively few cases of documented herpes B virus infection treated in the era of antiviral therapy, five patients with laboratory-confirmed infection with herpes B virus (some of whom had CNS symptoms) who were treated with intravenous acyclovir or ganciclovir had resolution of symptoms within 2 to 3 weeks of therapy.[10,18,29,30] As with HSV encephalitis, therapy for herpes B virus encephalitis is likely to be more effective when given earlier.

PREVENTION

Prevention of herpes B virus requires strict precautions when working with nonhuman primates. In view of a fatal case of herpes B virus occurring in a woman who received a splash to her eyes, primate workers exposed to macaques should wear goggles or glasses with side shields and a mask or a chin-length face shield and a mask to prevent infection of the eyes and oral mucosa.[4] Although only a single case of person-to-person transmission of herpes B virus has been reported,[10] individuals infected with the virus can shed infectious virus for more than 1 week even while receiving intravenous acyclovir[4]; therefore body fluids should be considered potentially infectious. Oral and genital secretions from individuals who have been exposed to herpes B virus, when it is not yet known whether they are infected, should be considered potentially infectious to others. If the incubation period for herpes B virus (generally 5 weeks in untreated individuals) has passed and the person is asymptomatic or serologies are persistently negative (at least 12 weeks after exposure in patients given antiviral prophylaxis), the likelihood of infection and virus transmission is exceedingly low.

It is essential that individuals exposed to macaques be educated regarding the importance of first aid and the need for rapid cleansing of wounds or mucosal exposures, the need to see health care personnel regarding evaluation for postexposure prophylaxis, and the signs and symptoms of herpes B virus disease so that early therapy can be initiated. Specific pathogen-free colonies of macaques have reduced the risk of herpesvirus B virus exposure almost 20-fold.[31]

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The complete reference list is available online at Expert Consult.

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142 Adenoviruses

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SHORT VIEW SUMMARY

Definition
- Human adenoviruses (HAdVs) are DNA viruses that can cause a broad range of clinical syndromes, including respiratory tract infections, ocular disease, gastroenteritis, diarrhea, and cystitis.

Epidemiology
- HAdVs are ubiquitous; most humans have serologic evidence of prior infection by age 10 years.
- Transmission is via respiratory droplets or fecal-oral transmission. Virus secretion may persist for prolonged periods after acute infection resolves.
- Typically, the disease is subclinical or mildly symptomatic and self-limited.
- HAdVs are a common cause of febrile illness, respiratory tract infections (types 1–7), and gastroenteritis (types 2–5) in young children; sporadic pediatric outbreaks are associated with daycare centers and summer camps.
- Acute respiratory disease, including pneumonia, is uncommon; sporadic outbreaks are associated with military recruits (types 4 and 7); a recent outbreak occurred in healthy adults (type 14).
- Epidemic keratoconjunctivitis (types 8, 19, and 37) has been linked to nosocomial transmission by infected instruments.
- HAdV is an emerging opportunistic pathogen in immunocompromised hosts, primarily hematopoietic stem cell transplants (HSCT) and solid-organ transplant (SOT) recipients; it can result in disseminated disease or target grafted organ.
- There is interest in using HAdVs as vectors for gene therapy, in vaccines being developed for infectious diseases (human immunodeficiency virus, Ebola, Zika); and as immunomodulatory treatments for solid tumors.

Microbiology
- HAdVs are nonenveloped, lytic DNA viruses, characterized by serologic responses to major capsid proteins and whole-genome analysis.
- HAdVs were originally isolated from adenoid tissues, leading to the virus name.
- HAdVs are classified into 7 species (A–G); 85 types have been detected in clinical specimens so far.

Diagnosis
- Diagnosis is not routinely pursued because most infections are mild and self-limited.
- HAdVs can be isolated by routine viral tissue culture (except types 40 and 41) and can be recovered from swabs, samples, and tissues; immunofluorescence assay, enzyme-linked immunosorbent assay, acute/convalescent serum titers can establish diagnosis.
- Polymerase chain reaction assays for HAdVs are highly sensitive and specific (96%–100%) and are now widely used.

Therapy
- There are no approved therapies available.
- Case series report partial clinical response but substantial toxicities to cidofovir in HSCT/SOT. Clinical trials of brincidofovir in transplant patients are underway.

Prevention
- Live oral vaccines (type 4 and 7) are administered to military personnel and are highly effective in preventing adenovirus-associated febrile respiratory diseases.

In 1953 Rowe and coworkers[1] isolated a novel cytopathic agent from surgical human adenoid samples undergoing spontaneous degeneration in tissue culture. Soon after, Hilleman and Werner[2] recovered similar viral agents from cases of acute respiratory disease (ARD) in military personnel. To denote their origin, these agents were designated adenoviruses. Subsequently, links to clinical disease were established by studies in which rising antiadenovirus antibody titers were detected in historic serum samples from World War II military personnel and from patients with ARD, exudative tonsillitis, and atypical pneumonia.[3] In 1955 adenovirus type 8 was identified as a cause of epidemic keratoconjunctivitis.[4]

In the 20 years after the discovery of adenoviruses by Rowe and coworkers, more than 30 different adenovirus types were identified and were shown to cause several clinical syndromes, including upper and lower respiratory tract infections, keratoconjunctivitis, and infantile gastroenteritis.[5] Epidemiologic studies in the 1960s and 1970s established that adenovirus infections are very common, causing 5% to 10% of all febrile illnesses in infants and young children.[6] Although clinically evident adenovirus infections are typically mild and self-limited in immunocompetent patients, outbreaks of severe respiratory disease associated with significant morbidity and occasional deaths have been observed in neonates and military recruits[7,8,9] and, more recently, in civilian populations.[7,10] Adenoviruses have also emerged as serious opportunistic pathogens in immunocompromised patients who have undergone hematopoietic stem cell or solid-organ transplantation (SOT).[11,12] With the advent of molecular diagnostics and application of whole-genome sequence analysis, at least 85 human adenovirus types have been identified.[13–15] The original 51 HAdV types were determined by serology, and types 52 to 85 were determined by whole-genome sequencing and phylogenomics, with serology as an adjuvant for some types.[16] The original 51 serotypes are also recognized as individual genotypes by whole-genome sequencing. Although several adenovirus types have not been linked to clinical disease, many have been shown to cause a broad range of clinical syndromes, including hepatitis, hemorrhagic cystitis, nephritis, myocarditis, and meningoencephalitis.

In 1962 adenovirus type 12 was shown to cause tumors in rodent cells.[17] This was the first description of a human virus that could induce malignant tumors in animals, and certain adenoviruses became model systems for studying oncogenesis. However, the oncogenic potential of adenoviruses has not been associated with any malignancies in humans. Adenoviruses also provided an important model system for studying viral and cellular gene expression and regulation, cell-cycle control, and DNA replication.[18] Recently, intense interest has focused on using

modified adenoviruses as vectors for gene therapy and vaccines for infectious diseases and cancers.

DESCRIPTION OF THE PATHOGEN

Adenoviruses are nonenveloped, lytic DNA viruses. Mature virions are 70 to 90 nm in diameter and contain a linear 36-kilobase double-stranded DNA core complex encased in an icosahedral capsid (Fig. 142.1). The adenovirus capsid is composed primarily of three major capsid proteins called *hexon, penton*, and *fiber* (Fig. 142.2). There are 252 subunits called *capsomeres*, including 240 hexon proteins and 12 penton proteins that form the 20 surfaces and 12 vertices of the capsid. At each vertex a penton protein is located at the base from which a fiber protein protrudes. The fiber protein interacts with primary cellular receptors and consists of a distal globular knob, a central shaft, and a tail that anchors the wandlike fiber to its penton base.[18–20] Although the overall structure of adenoviruses appears to be conserved across serotypes, the length of the fibers can vary and is specific for a given type.[21] Less well characterized are several minor proteins, including IIIa, VI, VIII, and IX, that contribute to stabilization of the capsid structure. Most of the epitopes recognized by group- and type-specific antibodies are present on the hexon and fiber proteins. Hexon proteins contain seven short hypervariable regions that are located on the solvent-exposed surface and that represent type-specific targets of dominant neutralizing antibodies. Fiber proteins also contain certain type-specific antigenic determinants that are responsible for in vitro hemagglutination characteristics.[22]

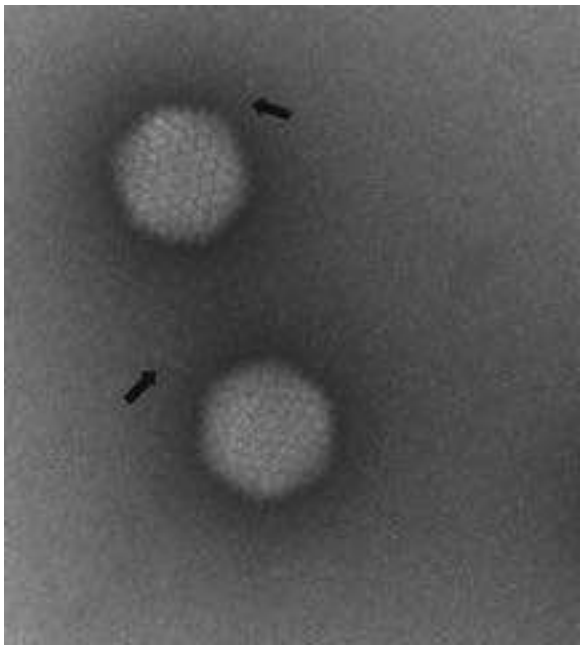

FIG. 142.1 Electron micrograph of adenovirus particles. Arrows indicate fibers.

More than 100 different adenoviruses have been isolated from vertebrates, ranging from reptiles to humans. Nonhuman adenoviruses have not been demonstrated to cause clinical disease in humans. Human adenoviruses belong to the genus *Mastadenovirus* (encompassing all mammalian adenoviruses) and are further divided into seven species (A–G) based on their hemagglutination characteristics (Table 142.1). Further characterization into types has been determined in part by their resistance to neutralization by antibodies to other known adenoviruses.[23] Revised criteria for typing human adenoviruses now include genome sequence data and computational analysis in addition to traditional serologic criteria, reflecting recent approaches to characterizing novel adenoviruses.[24,25] Adenoviruses have also been classified by their oncogenic properties, including their ability to transform cells in cultures and cause tumors in animals, and by the percentage of guanine and cytosine in adenovirus DNA.[18]

INTERACTIONS WITH THE HOST

Adenoviruses can cause a broad range of clinical syndromes, but it is not well understood why specific adenovirus types are often associated with particular syndromes. The portal of viral entry often appears to determine the primary site of disease, as seen in the spread of ARD by respiratory droplets or of infantile diarrhea by fecal-oral transmission, whereas other organ-limited diseases, such as hemorrhagic cystitis, likely result from a viremic phase of infection. Tissue tropism varies between different adenovirus species; species C, E, and some B viruses typically infect the respiratory tract; species D viruses can cause ocular and gastrointestinal (GI) infections; and species A, F, and G viruses target the GI tract. Viral tropism may be partially determined by differences in virus binding and host cell entry, which is typically initiated by binding of the fiber knob to a high-affinity receptor on the cell surface. Internalization of the virus particle is then mediated by association of the penton base with cell surface integrins.[26]

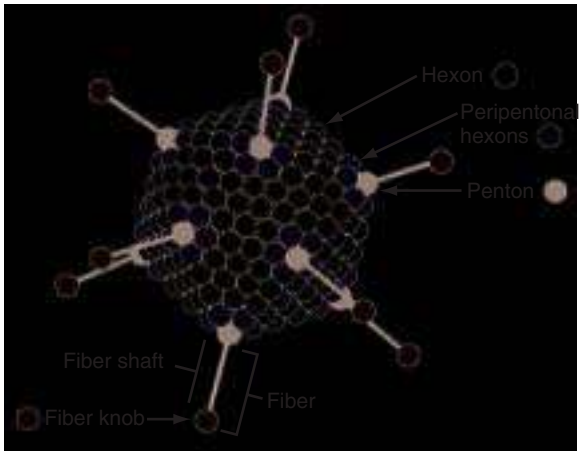

FIG. 142.2 Schematic of an adenovirus capsid.

TABLE 142.1 Classification of Adenoviruses

GROUP	HEMAGGLUTINATION GROUPS	TYPES	COMMON SITES OF INFECTION
A	IV (little or no agglutination)	12, 18, 31	GI tract, respiratory tract
B	I (complete agglutination of monkey erythrocytes)	3, 7, 11, 14, 16, 21, 34, 35, 50, 55	Respiratory tract, genitourinary tract
C	III (partial agglutination of rat erythrocytes)	1, 2, 5, 6, 57	Respiratory tract, liver
D	II (complete agglutination of rat erythrocytes)	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56, 58–60	Eye, GI tract
E	III	4	Respiratory tract
F	III	40, 41	GI tract
G	III	52	GI tract

GI, Gastrointestinal.

The primary cellular receptor for the majority of adenoviruses, including species A, C, E, and F adenoviruses, is the coxsackie B virus–adenovirus receptor (CAR), a transmembrane protein belonging to the immunoglobulin superfamily. CAR is a component of epithelial cell tight junctions and is abundantly expressed in heart, pancreas, the central and peripheral nervous systems, prostate, testis, lung, liver, and intestine.[27] In contrast, several species B and species D adenoviruses bind the transmembrane protein CD46, which is also widely expressed.[28] In addition, a number of other receptors, including CD80 and CD86,[29] sialic acid,[30] and heparan sulfate proteoglycans,[31] have been shown to contribute to attachment and internalization of specific adenovirus types into host cells. It has also been demonstrated that coagulation factor X mediates binding of the adenovirus type 5 hexon protein to hepatocytes, providing a rationale for the hepatic tropism of type 5.[32]

After internalization into endosomes, the virus capsid undergoes conformational changes and is released into the cytoplasm. The virion is then transported by microtubules to the nucleopore, where the adenovirus genome is transferred into the nucleus. Activation of viral transcription leads to expression of early proteins that result in deregulation of the cell cycle and modulation of host antiviral immune responses. These early regulatory proteins are under the control of early region 1A *(E1A)* genes, which in turn control expression from other early genes (*E1B, E2, E3,* and *E4*). The *E3* genes encode several proteins that modulate host immune responses, including inhibition of class I major histocompatibility complex expression and antigen presentation and downregulation of Fas, tumor necrosis factor (TNF), and TNF-related apoptosis-inducing ligand (TRAIL) receptors that lead to inhibition of apoptosis. Within several hours of infection, viral DNA synthesis is initiated, followed by production of viral structural proteins encoded by late genes. New virions are assembled in the nucleus of infected cells and are released by cell lysis.[18]

There is evidence that adenovirus can persist as a latent infection for years after an acute initial infection. Intermittent fecal excretion of species C adenoviruses has been demonstrated to persist for months after an initial acute respiratory infection.[6,33,34] Species D adenoviruses have also been isolated from asymptomatic children.[6,35] Persistent adenovirus secretion into tears has been documented up to 10 years after conjunctivitis.[36] Adenovirus persistence may also be more likely in the setting of immunocompromise. For example, adenoviruses have been identified in the stool of asymptomatic human immunodeficiency virus (HIV)-infected individuals.[37,38] T lymphocytes in tonsils and adenoids are the most likely potential reservoirs of adenovirus because they have been demonstrated to harbor adenovirus DNA for several years in the absence of infectious particles.[39] Although latency has been well described, the mechanisms for this phase of infection are not established.

EPIDEMIOLOGY

Adenovirus infections are ubiquitous. Most individuals have serologic evidence of prior adenovirus infection by age 10 years, often having experienced infection by several adenovirus types during early childhood.[5] Approximately 50% of all adenovirus infections result in subclinical disease, and most symptomatic infections are mild and self-resolving.[33] Therefore the majority of adenovirus infections remain undocumented, and epidemiologic data are derived from several surveillance studies and investigations of sporadic outbreaks. Epidemiologic studies conducted in the United States in the 1960s and 1970s demonstrated that 5% to 10% of all febrile illnesses in infants and young children are attributable to adenovirus infections, typically involving the respiratory tract and commonly caused by types 1, 2, 3, and 5.[6,33] According to a survey from the United Kingdom, 61% of documented adenovirus infections were present in children younger than 5 years.[40] Based on data from the US National Adenovirus Type Reporting System, begun in 2003, the most commonly reported types of HAdVs during 2003–16 in the United States were HAdV types 1, 2, 3, 4, 7, and 14, which accounted for 85.5% ($n = 1283$) of all types reported.[41] Although types that cause respiratory illness may be transmitted through aerosolized droplets, prolonged secretion after acute infection occurs through the GI tract such that fecal-oral transmission may account for a substantial number of infections in young children.[33] Sporadic outbreaks of pediatric infections have been documented in daycare centers, summer camps, and public swimming pools, among other settings.[41–47]

In contrast, acute adenovirus infections are less common in immunocompetent adults, with the notable exception of military recruits. In this population several epidemics of ARD caused by adenoviruses have been well documented and have led to significant morbidity.[48] Initial studies in military personnel demonstrated that these epidemics were most commonly caused by adenovirus types 4 and 7, leading to the development of a live oral vaccine that was administered to the military until 1999.[49] After discontinuation of this vaccine, outbreaks of adenovirus-related respiratory infections reemerged until a replacement vaccine was reintroduced in 2011, which led to dramatic and sustained decreases in ARD cases among US Army trainees by 2014.[9,50]

In subsequent studies of military personnel investigators have demonstrated that types 3, 4, and 21 are the most common adenovirus infections[10] and that several group B adenoviruses, including types 3, 7, 14, and 21, have caused ARD outbreaks.[10,51,52]

Severe or fatal adenovirus infections in immunocompetent adults are rare, but in 2006 and 2007, several clusters of severe ARD were caused by a virulent strain of adenovirus type 14 that affected military personnel, infants, and immunocompetent adults. The majority of hospitalized patients in these outbreaks were admitted to the intensive care unit, and several patients died, including previously healthy young adults.[7,51] A similarly severe outbreak of adenovirus type 7 causing ARD was reported among a nonmilitary population in Oregon in 2014.[53] In 2008 adenovirus type 55 was recognized as a cause of severe pneumonia among immunocompetent adults in China[54] and has remained an important pathogen in that population.[55] Outbreaks of adenovirus type 55 infection have also been described.[56,57] In addition, adenovirus type 8 was responsible for at least two recent outbreaks of epidemic keratoconjunctivitis in 2016; these outbreaks occurred in the Tibet Autonomous Region of China and the US Virgin Islands.[58,59]

Transmission of adenovirus infections typically occurs through respiratory droplets or the fecal-oral route from individuals with acute infection or asymptomatic viral shedding postinfection. Rare cases of transmission through cervical secretions have been documented in neonates.[8,60] Infections can also be spread by contact with contaminated fomites because adenoviruses can survive for extended periods on environmental surfaces. In one study an isolate responsible for epidemic keratoconjunctivitis (EKC) remained viable for 35 days on an inanimate surface.[61] Nosocomially acquired adenovirus infections have been documented in outbreaks of keratoconjunctivitis[62] and respiratory disease[63] on hospital wards. There is also serologic evidence that adenoviruses can be transmitted in the setting of organ donation.[64]

CLINICAL SYNDROMES

Most adenovirus infections are self-limited, although fatal infections can occur in immunocompromised hosts, neonates, and occasionally healthy children and adults. Severe disease is also associated with certain adenoviruses, including types 5, 7, 14, and 21. A broad spectrum of clinical adenovirus-associated disease exists, presumably as a result of the diverse types and tissue tropisms of adenoviruses (Table 142.2).

Respiratory Tract Disease

In children adenoviruses cause approximately 5% of upper respiratory tract infections and 10% of pneumonias.[65] Most commonly, upper respiratory tract disease presents as mild pharyngitis or tracheitis accompanied by coryza. The common types that cause these syndromes are adenovirus 1, 2, 5, and 6, and occasionally 3 and 7. Other systemic manifestations, including fever, malaise, headache, myalgia, and abdominal pain, are common.[66–68] Exudative tonsillitis and cervical adenopathy may be present and can be clinically indistinguishable from group A streptococcal infection.[66] In children younger than 1 year otitis media can also be a common presentation, and adenoviruses have been isolated from middle ear washes in children aged 2 to 12 years with otitis media with effusion.[67,68,69] Adenoviruses have also been associated with a pertussis-like syndrome in cases in which the bacteria were never cultured.[70,71] Several adenovirus types, including 1 to 5, 7, 14, and 21, can cause pneumonia in children and may occasionally result in sequelae such as bronchiectasis. Certain subgroup B adenoviruses (3, 7, 14, and 21) have been associated with

TABLE 142.2 Clinical Diseases Caused by Adenovirus Infection

CLINICAL DISEASE	POPULATIONS AT RISK	CAUSAL ADENOVIRUS TYPES
Pharyngitis	Infants, children	1–7
Pharyngoconjunctival fever	Children	3, 7
Pertussis-like syndrome	Children	5
Pneumonia	Infants, children	1–3, 21, 56
	Military recruits	4, 7, 14
Acute respiratory disease	Military recruits	3, 4, 7, 14, 21, 55
Conjunctivitis	Children	1–4, 7
Epidemic keratoconjunctivitis	Adults, children	8, 11, 19, 37, 53, 54
Gastroenteritis	Infants	31, 40, 41
	Children	2, 3, 5
Intussusception	Children	1, 2, 4, 5
Hemorrhagic cystitis	Children	7, 11, 21
	HSCT recipients, renal transplant recipients	34, 35
Meningoencephalitis	Children, immunocompromised hosts	2, 6, 7, 12, 32
Hepatitis	Pediatric liver transplant recipients	1–3, 5, 7
Nephritis	Renal transplant recipients	11, 34, 35
Myocarditis	Children	7, 21
Urethritis	Adults	2, 19, 37
Disseminated disease	Neonates, immunocompromised hosts	1, 2, 5, 11, 31, 34, 35, 40

HSCT, Hematopoietic stem cell transplant.

severe and complicated pneumonias, particularly in infants. Adenoviruses have also been identified as the cause of community-acquired pneumonia (CAP) in children, with up to 15% of children younger than 5 years with CAP requiring hospitalization, having adenovirus-positive respiratory samples in one study.[72,73] In retrospective studies from South America adenovirus type 7 infection resulted in substantial mortality rates in infants with pneumonia,[74,75] and in an outbreak of adenovirus type 30 infections in neonatal patients in the United States, pneumonia was associated with increased mortality.[76]

Several outbreaks of ARD have been documented in military recruits, most commonly caused by adenovirus types 4, 7, 14, and 21. The clinical syndrome is characterized by fever, sore throat, cough, hoarseness, and rhinorrhea and may progress to involve the lower respiratory tract. Symptoms usually last 3 to 5 days, and on examination pharyngitis, rales, and rhonchi may be present. One study of an outbreak in 2005 showed that pneumonias caused by adenovirus type 14 was associated with higher admission temperature, lower white blood cell count, and lower platelet count than pneumonias not caused by adenovirus type 14, but were not associated with any excess morbidity or mortality.[77] On chest radiographs bilateral patchy ground-glass opacities are consistent with the appearance of viral pneumonia. Rare extrapulmonary complications have been reported, including meningoencephalitis, hepatitis, myocarditis, nephritis, neutropenia, and disseminated intravascular coagulopathy.[78,79,80]

Ocular Disease

Pharyngoconjunctival fever is a common syndrome consisting of benign follicular conjunctivitis, fever, pharyngitis, and cervical adenitis, commonly caused by adenovirus types 3 and 7. Palpebral and bulbar conjunctivitis may be the sole finding and is typically bilateral. It is a common sporadic illness in children and has also been associated with outbreaks in children's summer camps, swimming pools, and lakes. The illness is usually mild and self-limited.[81]

In contrast, EKC is a more serious illness. Patients present with unilateral or bilateral follicular conjunctivitis, followed by corneal subepithelial infiltrates that are painful and can cause blurry vision. Prominent preauricular lymphadenopathy is common. The incubation period typically lasts 8 to 10 days, and virus can be isolated for up to 9 days after the onset of symptoms.[82] Adenovirus types 8, 19, 37, 53, and 54 have all been documented to cause outbreaks. Although usually self-limited, EKC can take up to 1 month to resolve and is associated with significant patient morbidity. Corneal opacities may persist for several months to years after infection.[83,84] EKC is highly contagious, and outbreaks have been documented in schools, military bases, and hospital wards. Transmission by instruments, eye drops, and skin has been documented in ophthalmic practices, neonatal intensive care units, and the community.[61,62,85–87]

Gastrointestinal Tract Disease

The detection of adenovirus isolates from the stool of patients with and without clinical disease confounded initial attempts to attribute diarrheal illnesses to adenoviruses. Subclinical infections, confirmed by positive stool cultures and antibody responses, appeared to account for the majority of infections. Furthermore, positive stool cultures without GI symptoms were often observed for weeks to months after respiratory adenoviral disease. Subsequently, the identification of "noncultivable" adenoviruses on electron microscopy examination of symptomatic patients' stools led to the discovery of enteric adenovirus types 40 and 41, which have been closely associated with infantile diarrhea. These viruses can be detected readily by polymerase chain reaction (PCR) and antigen detection assays, and they can be grown in special cell lines. Acute infantile gastroenteritis results in a watery diarrhea that lasts 8 to 12 days on average, accompanied by fever and vomiting.[88,89] In young children approximately 2% to 5% of acute diarrheal illnesses are caused by adenoviruses 40 and 41.[90] Although cases are generally acquired in the community, nosocomial infections have been reported as well. In addition to adenovirus types 40 and 41, types 2, 3, 8, and 31 have been associated with infantile diarrhea in some reports.[91] With the widespread availability of molecular detection techniques, a wide diversity of adenoviruses are now catalogued in many studies of diarrhea in children, including the description of high prevalence of species D in fecal specimens from children in sub-Saharan Africa.[46,47,92] Isolation of adenoviruses from outbreaks of gastroenteritis in adult patients has also been documented, including the initial report of type 52.[93]

Lower-type adenoviruses (1, 2, 5, and 6), but not adenoviruses 40 or 41, are associated with mesenteric adenitis, which can clinically mimic appendicitis and have been shown on occasion to cause intussusception. In these cases adenoviruses have been isolated from stool cultures and lymph nodes. In several studies of children with intussusception, evidence of adenovirus infection ranged from 22% to 61%.[94,95]

Genitourinary Tract Disease

In children adenoviruses can cause acute hemorrhagic cystitis, which is a benign, self-limited illness. Patients present with gross hematuria lasting 3 days on average, without fever or hemodynamic instability. Microscopic hematuria and dysuria may persist for several more days, but tests for renal function remain normal. Boys are two to three times more commonly affected than girls. In Japan several case series of hemorrhagic cystitis have attributed up to 70% of infections to adenovirus. In the United States only 20% of hemorrhagic cystitis cases can be linked to acute adenovirus infection. Adenovirus types 11 and 21 are most commonly isolated, although adenovirus type 7 has also been detected.[96,97]

Adenovirus types 11, 34, and 35 have caused cases of hemorrhagic cystitis and tubulointerstitial nephritis, reported in renal transplant and stem cell transplant recipients either as isolated syndromes or as part of disseminated disease. In immunocompetent adult males, adenoviruses have been detected in a significant number of cases of

nongonococcal urethritis, and have been associated with adenovirus types 19 and 37.[98,99,100]

Central Nervous System Disease

Adenoviruses have been associated with sporadic cases of meningitis and meningoencephalitis, either as a primary manifestation or as a complication of systemic or respiratory infection. In rare cases adenovirus has been cultured only from cerebrospinal fluid (CSF) in immunocompetent patients, including from a healthy infant who presented with signs and symptoms of bacterial meningitis,[101,102] and patients undergoing chemotherapy for lymphoma.[103] In one instance adenovirus type 26 was identified by PCR and immunohistochemical staining of a brain biopsy in a patient with medulloblastoma presenting with acute meningoencephalitis.[104] More commonly, meningoencephalitis has been reported as a complication of severe pneumonia, seen primarily with type 7 infection, although also reported with types 1, 6, and 12.[78] Spinal fluid cell counts and chemistries are variable in these cases.

Other Clinical Syndromes

Myocarditis caused by adenovirus has been described in several case series of acute myocarditis in children, based on detection of virus in myocardial tissue by PCR.[105,106,107,108] In one large study that included neonates and adults, adenovirus PCR of cardiac tissue was positive in 23% of patients with myocarditis, 12% of patients with dilated cardiomyopathy, and in none of the control patients, suggesting that adenovirus may be a common cause of myocarditis.[106]

Rare cases of myositis associated with rhabdomyolysis,[109] arthritis,[110] and pancreatitis[111] caused by adenovirus have been reported. Disseminated adenoviral disease has been best described in pediatric and immunocompromised patients, particularly in neonates, infants, and stem cell transplant recipients. Several adenoviruses, including types 3, 7, 21, and 30, have been isolated in these cases.[112,113]

INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

Adenoviruses have emerged as important opportunistic pathogens in immunocompromised hosts. Infections can range from asymptomatic shedding of virus to disseminated and potentially life-threatening disease. The majority of clinically significant adenovirus infections occur in hematopoietic stem cell transplant (HSCT) and SOT recipients. One study showed the median time to infection in these populations was 1.6 months posttransplantation, which suggests that these infections were from reactivation disease.[114] The incidence of adenovirus infections in these populations has increased in the past 20 years because of improvements in diagnostic methods, more aggressive conditioning regimens, and the institution of surveillance for adenoviral infection by PCR at some centers.[11,12] These populations are also more likely to have coinfection with more than one adenovirus type.[10]

Hematopoietic Stem Cell Transplant Recipients

The rates of adenovirus disease in HSCT recipients are difficult to assess because of variations in diagnostics and study inclusion criteria, but mortality rates with adenovirus disease in this population are significant, ranging from 6% to 70% in different case series.[11] In a study of 1050 HSCT recipients, 4.8% of patients were found to shed adenovirus asymptomatically, and 0.9% had invasive disease.[115] Pediatric HSCT recipients have a threefold higher risk of adenovirus infections and are more likely to have severe disease.[116,117] The increased risk of clinical disease in children is likely due to an increased risk of acquiring primary infection, although reactivation of latent infection or reactivation of infection in the transplanted cells may also occur. In addition to younger age, other risk factors for infection include unrelated donor, graft-versus-host disease (GVHD), T-cell depletion of the graft, cord transplants, aggressive immunosuppression, total-body irradiation, and low T-lymphocyte counts after transplantation.[118] Adenovirus is usually detected within the first 100 days posttransplant, with a mean of day 58 and ranging up to day 333 in one study.[119] The presence of adenovirus DNA in blood, a greater degree of immunosuppression, lymphocytopenia, and a rising viral load all increase the risk for serious adenovirus-related clinical disease.[120] In the pediatric HSCT population the most common adenoviral disease is diarrhea or gastroenteritis.[114] Cases of pneumonia, hemorrhagic cystitis, pneumonitis, tubulointerstitial nephritis, hepatitis, cholangiohepatitis, encephalitis, and disseminated disease have been reported as well, with several cases of fatal fulminant hepatic failure due to adenovirus infection reported.[11,121,122] Hemorrhagic cystitis can be severe and prolonged, leading to urethral obstruction by blood clots and occasionally requiring cystectomy for control. There is a wide diversity of adenovirus types that have been identified from samples from stem cell transplant recipients, without one clear type predominating.[123,124]

Surveillance of blood samples by adenovirus PCR to assess risk of infection has become a common practice in some pediatric and adult HSCT centers.[125,126] This practice is based on studies that have shown that adenovirus can be detected in blood 2 or 3 weeks before the development of clinical symptoms.[125] Studies have also shown that increasing viral load measurements have been associated with increased mortality once clinical disease is established. However, the potential use of preemptive therapy in these situations is unclear.[127,128,129,130]

Solid-Organ Transplant Recipients

In SOT recipients the transplanted organ is typically the primary site of disease.[131] Clinical adenovirus disease may be due to a primary infection or reactivation of latent virus in the transplanted organ because infections are more common in children and in patients with donor-positive/recipient-negative adenovirus status. Severe disease, which may include dissemination, is more common in the pediatric transplant population, particularly liver and lung recipients, and in patients who receive antilymphocyte antibodies.[11] In adults adenovirus infection may be less severe. In one prospective study that included adult liver, heart, and kidney recipients, viremia was documented by PCR in 7% of cases, and more than half of the patients remained asymptomatic and were able to clear the infection spontaneously.[132]

Adenovirus hepatitis has been well described in pediatric liver transplant recipients. In a case series rates of hepatitis ranged from 3% to 10% and frequently led to graft loss and death, with mortality rates up to 53%.[119] Most commonly, hepatitis is caused by adenovirus type 5, but cases caused by adenovirus types 1 and 2 have also been documented. Lung transplant recipients may develop adenovirus pneumonia in the early posttransplant period. One study of adults and children documented a 1.3% prevalence in this population, and subsequent graft failure, death, or bronchiolitis obliterans has been reported.[133,134] Renal transplant recipients can develop acute hemorrhagic cystitis, sometimes complicated by tubulointerstitial nephritis. Adenovirus types 11, 34, and 35 have been detected in these cases. In general, adenovirus infections are less common and less serious in renal transplant recipients, although cases of pneumonia and rare cases of fatal disseminated infections have been reported.[132,135] Adenovirus infections involving the grafted organ have also been reported in cardiac transplant and small bowel transplant recipients.[11] In one study of small bowel recipients, intensive immunosuppressive therapy was associated with progression of infection and systemic adenovirus dissemination.[136] In pediatric cardiac transplant recipients the detection of adenoviruses in myocardial biopsies was associated with reduced graft survival.[137]

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients

Most observations regarding adenovirus infections in HIV-positive patients were made before the availability of highly active antiretroviral therapy.[41] In these studies the risk of adenovirus infection was 28% in patients with acquired immunodeficiency syndrome (AIDS) and 17% in patients with CD4 counts greater than 200 cells/μL.[37] Although adenovirus can be isolated frequently from the stool and urine of patients with AIDS, no causative link with diarrhea, hematuria, or other clinical syndromes has been established. Several novel adenovirus types have been detected in specimens from AIDS patients, including many from group D in stool (types 43–51, 58) and respiratory secretions (type 59).[14,138] If symptoms are present, they are usually attributed to other opportunistic infections. Rare cases of fatal hepatic necrosis, fatal pneumonia, encephalitis, nephritis, and systemic infection caused by

adenoviruses have been reported, but adenovirus infections in AIDS patients are an uncommon cause of morbidity or mortality.[139–141]

DIAGNOSIS

Because most adenovirus infections in immunocompetent patients are mild and self-limited, diagnosis is not routinely pursued. However, establishing a diagnosis may be useful in the setting of outbreaks or for individuals who are immunosuppressed or seriously ill. A confirmed diagnosis can also be useful to help decide whether the use of antiviral medications is warranted or to exclude other treatable infections. Traditional methods of determining adenovirus infection include viral culture, antigen-specific assays, and serologies. Detection by PCR has become widespread because of greater sensitivity and specificity and also rapid turnaround.

With the exception of types 40 and 41, adenoviruses are detectable by routine tissue culture. They grow well in human epithelial cell lines, producing a typical cytopathic effect within 2 to 7 days, although some species D types can take up to 4 weeks to isolate. Viruses may be recovered from nasopharyngeal swabs or aspirates, throat swabs, conjunctival swabs or scrapings, bronchoalveolar lavage fluid, stool or rectal swabs, urine, CSF, and tissue. Viral shedding is detectable in the first 1 to 3 days in patients with pharyngitis, 3 to 5 days in patients with pharyngoconjunctival fever, and up to 2 weeks in patients with keratoconjunctivitis.[142] In immunocompromised patients adenoviruses can be detected in stool intermittently for a more prolonged period of time. For example, adenovirus excretion was detected in the stool of HIV-infected patients for up to 27 months, and prolonged excretion was associated with lower CD4 counts.[37] If culture is not available, direct antigen detection provides rapid diagnosis. The immunofluorescence assay (IFA) is useful for respiratory samples and tissue, and enzyme-linked immunosorbent assay is the test of choice to detect adenoviruses 40 and 41 in stool.[143] The sensitivity of virus detection by IFA of respiratory samples is 40% to 60% lower compared with culture.[144] Viral antigen assays are also less sensitive in immunocompromised patients.[145] Adenovirus infection may also be established by detecting a fourfold or greater rise in adenovirus-specific antibody titers in paired acute and convalescent sera. Detection of adenoviral DNA by PCR has become increasingly attractive for diagnosis, typing of adenovirus, and quantification of virus from a variety of clinical specimens, including fixed tissues, serum, and blood. Primers may be directed against conserved hexon or fiber genes, but more specific typing can be done by a multiplex PCR format, followed by sequencing to detect and identify multiple adenovirus types.[146,147] Real-time PCR has also permitted quantification of virus, which is sometimes used in monitoring viral loads in peripheral blood samples from immunocompromised patients, particularly pediatric HSCT patients. Adenovirus DNA quantification can also be used to measure response to antiviral therapy, and the detection of adenovirus DNA can also establish the diagnosis if found in tissue samples (e.g., endocardial biopsy).[105,129] The specificity of PCR in asymptomatic, immunocompetent adults is high, ranging from 96% to 100% in studies of urine, throat swabs, and peripheral blood.[11,148]

Typing of viral isolates is not routine but can be determined in a reference virology laboratory. Traditionally, typing has been determined by hemagglutination patterns and serum neutralization assays against a panel of type-specific sera. Recently, whole-genome sequencing and phylogenetic analyses of adenoviruses have shown that serum neutralization may be misleading, because neutralization is primarily directed against small portions of viral capsid proteins. In 2011 new criteria for characterizing and typing novel human adenoviruses were proposed and include analysis of the complete genome sequence, with continued use of serum neutralization as an additional criteria.[24]

When obtained, tissue should be sent for culture and pathologic examination. Histopathologic findings in the lung include diffuse interstitial pneumonitis, necrotizing bronchitis, bronchiolitis, and pneumonia with mononuclear cell infiltration and hyaline membrane formation.[149] Early postinfection, infected cells may display small eosinophilic inclusions. During late infection basophilic intranuclear inclusions surrounded by a thin, clear halo emerge and eventually enlarge to obscure the nuclear membrane. This produces "smudge cells," which are characteristic of adenovirus infections (Fig. 142.3). In contrast to cytomegalovirus, there are no intracytoplasmic inclusions or multinucleated giant cells. Further study by electron microscopy, adenovirus-specific immunohistochemical assays, and in situ DNA hybridization can be performed to make the diagnosis.

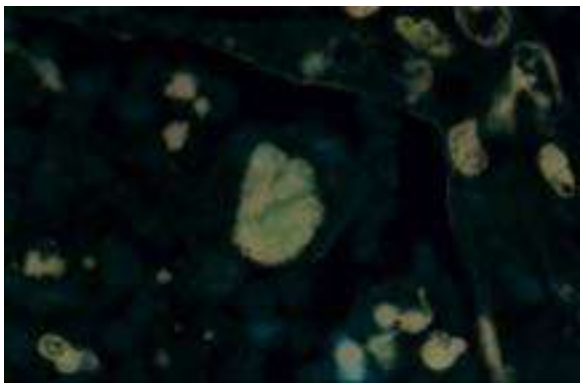

FIG. 142.3 Lung biopsy specimen from a patient with adenovirus pneumonia showing a characteristic "smudge cell" (hematoxylin and eosin stain, ×400). *(Courtesy Franz C. Lichtenberg, MD, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

THERAPY

There are currently no approved antiviral agents for the treatment of adenovirus infections. Clearance of adenovirus infection in HSCT and SOT patients is typically associated with immune reconstitution, particularly with improved absolute lymphocyte counts and CD4 T-cell counts.[150,151] There are few prospective, controlled trials of antiviral drugs for adenovirus infections, and thus clinical experience is mostly limited to retrospective case series and case reports, primarily in immunosuppressed patients. Cidofovir has good in vitro activity against adenovirus and has been reported to be useful in certain animal models of ocular adenovirus infections.[152] However, a large, multicenter trial to evaluate the potential efficacy of topical cidofovir for EKC was discontinued because of toxicity.[153] In several case series and case reports involving pediatric and adult HSCT recipients, with a variety of clinical adenovirus syndromes, treatment with cidofovir appeared to be associated with clinical improvement in a subset of patients, although fatalities still occurred and significant nephrotoxicity was noted.[117,154,155,156] Moreover, it was not clear if the clinical improvement in these individuals was due to the drug. Studies are currently evaluating an oral liposomal formulation of cidofovir (CMX001, brincidofovir) for severe adenovirus infection in immunocompromised patients[157] (also see Chapter 46). Retrospective case series have suggested that brincidofovir is well tolerated and that treatment can lead to decreases in adenovirus loads.[158,159] A phase II study of brincidofovir in pediatric and adult HSCT recipients with asymptomatic adenovirus viremia demonstrated that brincidofovir resulted in rapid and sustained virologic responses in patients with high viremia at baseline.[160] However, the drug was also associated with GI toxicity in some patients and more frequent incidence of acute GVHD. A phase III multicenter study of brincidofovir comparing treatment of early versus late adenovirus infection in HSCT or SOTs will hopefully clarify the safety and efficacy of brincidofovir; the study has been completed, but the results are not yet published.[161] Ribavirin use has been reported in several cases of adenovirus infection in HSCT recipients, but results in case series have been mixed[162,163] and may be explained in part by the observation that in vitro activity of ribavirin appears restricted to group C types.[164] Vidarabine and ganciclovir are reported to possess in vitro activity against adenovirus, but there are scant clinical data for these agents.[165] In some centers preemptive therapy has been proposed to treat patients who are at risk for adenovirus infection. In a study of 58 pediatric HSCT recipients in which patients were prospectively screened weekly for evidence of infection and then treated with cidofovir, symptoms and viremia resolved in the majority of recipients.[154] It is clear that larger prospective studies are needed to determine if any of these antiviral agents have clinical efficacy and whether their use is warranted in particular clinical settings.

Immunotherapy by adoptive T-cell therapy is being pursued by some groups but remains an investigative approach. The idea is that the restoration of virus-specific immunity in immunocompromised individuals could be more effective at suppressing adenovirus than antiviral drugs, while being less toxic and less likely to induce resistance. Small studies have shown that infusion of adenovirus-specific T cells into HSCT patients led to the induction of effective T-cell responses, reductions in adenoviral load, and instances of clinical improvement of adenoviral-related disease in some individuals.[166,167,168–170] These results were followed up by an open-label clinical trial of the safety and efficacy of ex vivo adoptive T-cell transfer with hexon-specific T cells in 30 patients with adenovirus disease or viremia. In this study immunotherapy led to in vivo antiviral immunity for up to 6 months with viral control, resulting in complete clearance of viremia in 86% of patients with antigen-specific T-cell responses, and the suggestion of a mortality benefit in responders.[171] Intravenous immune globulin has also been used in immunocompromised patients with mixed results.[156,172,173]

PREVENTION

Because of the morbidity seen with respiratory adenovirus infections in military recruits, successful live oral vaccines for types 4 and 7 were developed and administered in the military starting in 1971. The vaccine was packaged in enteric capsules that ensured that replication would occur in the GI tract and not the airways, resulting in subclinical infection and good neutralizing antibody responses. The sole manufacturer discontinued vaccine production in 1996, and vaccination stopped in 1999 when the supply was exhausted. Subsequently, ARD recurred in military recruits at rates similar to those of the prevaccination era, with several outbreaks affecting up to 80% of recruits, resulting in hospitalization rates ranging from 11% to 20% and producing occasional fatalities.[52,174] After a decade of development, the vaccine was restored to use in the military in 2011, and rates of adenovirus disease burden have fallen by more than 100-fold in military trainees, without an increase in diseases associated with adenoviruses not present in the vaccine.[175,176]

ADENOVIRUSES AS VECTORS FOR GENE THERAPY AND VACCINATION

There has been intense investigation during the past two decades into the capacity of adenoviruses to serve as a vector platform for delivery of genes for both gene therapy and vaccination. Typically, adenoviruses are rendered replication incompetent by deletion of the *E1* gene, which allows the insertion of a transgene expression cassette that encodes a gene of interest. Scores of human clinical trials using adenovirus vectors have been conducted, and others are currently in progress or are planned. Adenovirus vectors have been well studied and have several advantages over other available vectors, including their ability to be produced at high titers, to infect several cell types, including both dividing and nondividing cells, and to accommodate gene inserts stably.

Applications for gene therapy have primarily focused on delivery of a functional gene to replace a dysfunctional or absent gene product for diseases such as cystic fibrosis, ornithine transcarbamylase deficiency, hemophilia, and bilirubin uridine diphosphate glucuronosyl transferase deficiency. Although promising in several animal models, early human gene therapy studies have proven disappointing because of inefficient gene delivery.[177] Initial studies also demonstrated that adenovirus vectors at high doses elicit early innate immune responses, including production of proinflammatory cytokines that can lead to systemic toxicity and resulted in the death of a volunteer in a clinical trial.[178,179] Vector-specific immune responses also develop rapidly and limit the utility of repeat vector administration. Several strategies to minimize vector-specific immunity have been developed, including using different human adenovirus types, nonhuman adenoviruses, and structurally modified adenoviruses to create novel vectors.

Adenovirus vectors are also being developed as vaccines for both infectious diseases and cancer, primarily because of their ability to elicit robust cellular immune responses against encoded transgenes. These vectors contain genes encoding pathogen-specific antigens and elicit robust $CD8^+$ T-lymphocyte responses and polyfunctional antibody responses.[180] Adenovirus vector–based vaccine candidates are currently being explored for a variety of infectious diseases, including malaria, herpes simplex virus, tuberculosis, Ebola, Zika, and HIV-1 infections.[181–183] Clinical trials for adenovirus vectors are also being conducted in the tumor vaccine field. Strategies include directly delivering genes that control cell growth, apoptosis, and angiogenesis, or genes that express cytokines to induce antitumor responses. Another approach has been to modify adenovirus vectors to replicate and induce lysis in tumor cells but not normal cells. Approximately a dozen clinical trials are currently evaluating oncolytic adenoviruses for the treatment of ovarian, prostate, pancreatic, and other solid tumors, although this concept has yet to demonstrate clinical efficacy.[184,185]

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The complete reference list is available online at Expert Consult.

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143 Papillomaviruses

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SHORT VIEW SUMMARY

Definition

- Human papillomavirus (HPV) infects the squamous stratified epithelia of the body and causes tumors that can be benign (warts, condylomas, papillomas) or malignant (squamous cell carcinomas, uterine cervical adenocarcinoma).
- They cause two main groups of diseases: (1) cutaneous (hand, foot, flat) warts and (2) lesions of the mucosal or genital surfaces, such as genital warts; laryngeal papillomas; and cancers of the cervix, vagina, vulva, anus, penis, oropharynx, and their respective precursor lesions, called intraepithelial neoplasias (dysplasias).

Epidemiology

- Cutaneous warts are predominantly a disease of school-aged children. They are acquired from close contacts, predominantly in the family environment.
- Genital (or mucosal) HPV infections are mostly sexually transmitted, and their incidence peaks in late adolescence and early adulthood.
- Most of the sexually active population will have been exposed to genital HPV in a lifetime.
- Genital HPV infections in males and females are easily acquired, but most also disappear quickly. Persistence is a risk factor for the development of cancer.

Microbiology

- HPVs are small, nonenveloped DNA viruses, classified according to the nucleotide sequence of the gene coding for the major capsid protein. These viruses are not routinely cultivable.
- At least 210 types have been identified, but only a small number carry the bulk of the health burden.
- HPV types 1, 2, and 4 are the most common types found in cutaneous warts.
- HPV types 6 and 11 account for most genital warts.
- HPV types 16 and 18 cause the great majority of cancers of the anogenital tract and oropharynx and are defined as high-risk oncogenic.
- The more severe the grade of intraepithelial neoplasia, the more prevalent are high-risk oncogenic HPVs in the lesion.

Diagnosis

- The diagnosis of cutaneous warts and of genital warts is typically clinical. A biopsy is indicated when the diagnosis is in doubt or a malignancy or its precursor is a consideration.
- For the screening of cervical cancer, cytology (Pap smear) is the primary diagnostic approach.
- HPV DNA testing supplements screening cytology.

Therapy

- Many therapeutic modalities exist for the treatment of HPV-induced lesions, none of them fully satisfactory. They can be divided into medical and physical approaches.
- The chemical methods include salicylic acid solutions for cutaneous warts or podofilox or imiquimod for genital warts.
- The physical methods include cryotherapy, cold-blade excision, electrosurgery, and laser therapy, and they can be applied to most lesions.

Prevention

- Male condoms have some effectiveness in protecting against genital infections.
- Pap smears are essential for the prevention of cervical cancer.
- Vaccination is very effective and safe in preventing genital warts and intraepithelial neoplasias of the cervix, vagina, vulva, and the anus in males and females, and cervical cancer
- A 9-valent vaccine (Gardasil 9) is available in the United States. This vaccine is not infectious and protects against HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58. It has now replaced an earlier quadrivalent vaccine (Gardasil) that covered only the first four of these genotypes. It is administered intramuscularly in a two- or three-dose schedule. Cervarix, a bivalent vaccine covering only HPV-16 and HPV-18 is still available in some parts of the world.

Papillomaviruses have been detected in a variety of higher vertebrates. *Human papillomaviruses* (HPVs) are widespread throughout the population, produce epithelial tumors of the skin and mucous membranes, and have been closely associated with genital tract malignant diseases. HPVs are strictly species specific, and cross-species infections do not occur even in experimental conditions. The infectious nature of human warts was initially seen in the late 19th century when human wart extracts were shown to produce warts with injection into humans. Ciuffo[1] suggested that the infectious agent of warts was a virus, after he was able to transmit the infection through cell-free filtrates in 1907. Despite these early observations, HPVs have not been studied with standard virologic techniques because they have not been propagated successfully in tissue culture or in standard laboratory animals. For this reason, much of our knowledge of the biology of HPVs and the diseases with which they are associated has depended on the use of molecular biologic techniques and, more recently, of organotypic cultures and complex animal models. These techniques have led to an understanding of the genomic organization of these viruses, the functions of different viral genes, and the multiplicity of HPV types. Detailed reviews of these subjects are available.[2–6]

VIROLOGY

Papillomaviruses constitute the *Papillomavirus* genus of the Papillomaviridae family. They are nonenveloped viruses that are 55 nm in diameter

and have an icosahedral capsid composed of 72 capsomeres that enclose a double-stranded circular DNA genome. Virion particles contain at least two capsid proteins. The major capsid protein constitutes 80% of the virion by weight and has a molecular weight of about 56,000 daltons. The minor capsid protein has a molecular weight of approximately 76,000 daltons.

The HPV genome consists of approximately 7900 base pairs. All putative coding sequences (open reading frames [ORFs]) are arranged on one DNA strand, and all papillomaviruses share the same genomic organization.[6–13] Specific protein products are derived from these ORFs. However, analyses of viral messenger RNA (mRNA) transcripts suggest that most viral proteins derive from splicing of more than one ORF-specific mRNA. The genome is divided functionally into three regions. A noncoding upstream regulatory region contributes to the control of DNA replication and transcription of eight to nine ORFs that are divided into early (E1–E7) and late (L1 and L2) regions.[6–13] E1 is involved in viral plasmid replication.[10–12] The E2 product is an important modulator of viral transcription and also plays a role in viral replication.[10–12] E4 proteins form filamentous cytoplasmic networks and share the same cellular distribution as cytokeratin intermediate filaments, with which they may interact.[2,3,12] The E5 protein is located in the cellular membrane and prevents the acidification of endosomes.[12] It stimulates the transforming activity of the epidermal growth factor receptor and contributes to the oncogenicity of HPV.[3,9,11–13] The gene products of E6 and E7 of oncogenic HPV types have major transforming properties through the binding of various cellular factors and key tumor suppressor proteins.[1–3,7,9,11–13] The E6 protein binds to the p53 tumor suppressor gene product and abrogates its activity by accelerating its degradation. The E7 protein also binds to a tumor suppressor gene product, the retinoblastoma protein, and to related proteins, thus inhibiting their functions. Both E6 and E7 proteins can impede apoptosis. The L1 and L2 ORFs encode the major and minor capsid proteins, respectively.[1–3,7,13]

Although the genomes of several papillomaviruses can transform certain cell lines in tissue culture, only recently have both replication and propagation of HPV been possible in vitro, with use of organotypic culture systems.[8,14] In addition, HPV types 6, 11, 16, 40, and 59 have been propagated successfully in human skin grafted in the (nude) mouse or the mouse with severe combined immunodeficiency (SCID).[15] HPV-infected grafts recovered from these animals can maintain viral particle production in vitro.

Virions of most HPV types cannot be purified from naturally occurring lesions in significant quantities, and well-characterized type-specific antigens have not been available until recently.[16] Therefore types are determined according to the degree of nucleic acid sequence homology rather than with serologic techniques. Distinct HPV types share less than 90% of DNA sequences in the L1 ORF, subtypes share between 90% and 95%, and variants between 95% and 98%.[7,17] According to common nomenclature, HPVs belong to five genera of the 49 in the Papillomaviridae family: alpha, beta, gamma, mu, and nu. A genus may be further divided into species. For example, the alphapapillomavirus HPV-16 is the representative type of species 9, which also includes types 31, 33, 35, 52, and 67. At least 210 HPV types have now been characterized, and many others have been recognized. HPVs are host specific, and each type is, to a large extent, associated with a distinct histopathologic process (Table 143.1).

A broadly cross-reactive genus-specific antigenic determinant, located in the middle of the major capsid protein,[18] can be prepared with denaturation of viral particles, typically from bovine papillomavirus, with detergents and reducing agents. Antisera prepared against this papillomavirus common antigen have been used in the immunocytochemical diagnosis of HPV infections (see "Diagnosis").[19] The antigenic characteristics of native viral particles can also be studied with the use of virus-like particles (VLPs). These are obtained with the expression in eukaryotic systems of the L1 or L1 and L2 ORFs (see "Prevention").[14] A close correlation generally exists between genotype and serotype.[16]

TABLE 143.1 Human Papillomavirus Types and Their Disease Association

DISEASE	HPV TYPES[a] FREQUENT ASSOCIATION	HPV TYPES[a] LESS FREQUENT ASSOCIATION
Plantar warts	1, 2, 27	4, 26,[b] 28, 29, 41,[c] 57, 63, 65, 77,[c] 117,[b] 125,128, 129, 130, 131, 132, 133, 148, 149, 179, 184
Common warts	1, 2, 4, 27	
Common warts of meat, poultry, and fish handlers	2, 7	1, 3, 4, 10, 28
Flat and intermediate warts	3, 10	27,[b] 28, 38, 41,[c] 49,[b] 75, 76, 126[b]
Epidermodysplasia verruciformis	5,[c] 8,[c] 9, 12, 14,[c] 15, 17[c]	19, 20,[d] 21, 22, 23, 24, 25, 36, 37, 38, 47,[c] 49, 50, 75, 93
Condylomata acuminata	6, 11	16,[c] 18,[c] 26,[c] 31,[c] 33,[c] 35,[c] 40, 42, 43, 44, 45,[c] 51,[c] 52,[c] 53,[c] 54, 55, 56,[c] 58,[c] 59,[c] 66, 68,[c] 70, 153, 175, 178, 180, 200, 201, 202
Intraepithelial neoplasia, unspecified		26,[c] 30,[c] 34, 39,[c] 40, 53,[c] 57, 59,[c] 61, 62, 67,[c] 68,[c] 69, 71, 81, 83
Low grade	6, 11	16,[c] 18,[c] 31,[c] 33,[c] 35,[c] 42, 43, 44,[d] 45,[c] 51,[c] 52,[c] 54, 61, 70, 72, 74[b]
High grade	16,[c] 18[c]	6, 11, 31,[c] 33,[c] 34,[b] 35,[c] 39,[c] 42, 44, 45,[c] 51,[c] 52,[c] 56,[c] 58,[c] 66,[c] 67[c]
Cervical carcinoma	16,[c] 18[c]	26,[c] 31,[c] 33,[c] 35,[c] 39,[c] 45,[c] 51,[c] 52,[c] 56,[c] 58,[c] 59,[c] 66,[c] 67,[c] 68,[c] 73,[b,c] 82[c]
Recurrent respiratory papillomatosis	6, 11	16,[c] 18,[c] 31,[c] 33,[c] 35,[c] 39[c]
Focal epithelial hyperplasia of Heck	13, 32	18,[c] 33,[c] 45[c]
Conjunctival papillomas and carcinomas	6, 11, 16[c]	
Other cutaneous lesions[e]		26,[b,c]36, 37, 38,[c] 41,[c] 48,[b,c] 60, 72,[b] 88, 92, 93, 94, 95, 96, 107, 110, 111, 155, 174, 197[c]
Other genital lesions		26,[b,c] 30,[c] 84,[c] 85, 86,[c] 87, 89, 90, 91, 97, 101, 102, 103, 106, 175, 180, 199
Healthy cutaneous or mucosal tissue		80, 114, 115, 116, 118, 119, 120, 121, 122, 123, 124, 127, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 150, 151, 156, 157, 158, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 199, 205, 209

[a]The distinction between frequent and less frequent association is arbitrary in many instances. Large descriptive statistics of HPV type distribution by disease are not available for most HPV types. Moreover, many HPV types have been looked for or identified only once.
[b]Types first recovered from patients with immunosuppression or immunodeficiency.
[c]Types with high malignant potential or isolated in only one or a few lesions that were malignant.
[d]HPV-46 was found to be HPV-20, HPV-64 is a variant of HPV-34, and HPV-55 is HPV-44.
[e]Includes epidermoid cysts, keratoacanthoma, laryngeal carcinoma, and malignant melanoma.
HPV, Human papilloma virus.
Information on HPV DNA sequences and types is available at http://pave.niaid.nih.gov/#home and http://www.nordicehealth.se/hpvcenter/reference_clones/. Note that the sequence information on some of the newer genotypes is not yet available.

EPIDEMIOLOGY

Incidence and Prevalence

Although clinical HPV infections are the most recognizable and most important for the patient and practitioner, subclinical and asymptomatic

latent infections are probably most common, and past HPV infections represent an even larger group.[20–22] The study of these different types of infection poses different technical problems, and their respective interrelated epidemiologies are not equally well understood.

As Table 143.1 illustrates, HPV infections can also be divided according to predominant anatomic location of the lesions they cause. Thus the genital or mucosal infections are recognized as distinct from the nongenital infections, which include the cutaneous infections. The genotypes associated with asymptomatic infections are less restricted in their anatomic distribution.[23–25]

Three types of cutaneous HPV infections are widespread throughout the general population.[26] Common warts, which represent up to 71% of all cutaneous warts, occur frequently among school-aged children, with prevalence rates of 4% to 20%.[27,28] Although less common (34% of cutaneous warts), plantar warts are observed frequently among adolescents and young adults. Juvenile or flat warts are the least common of the three types (4%) and occur predominantly in children. Other groups at high risk for the development of cutaneous warts include butchers, meat packers, and fish handlers.[29] Epidermodysplasia verruciformis is a rare, typically autosomal recessive condition characterized by the appearance early in life of disseminated cutaneous warts and frequent malignant transformation.[30]

Large surveys in the United States have shown that the prevalence of any and high-risk genital HPV in 18- to 59-year-olds was 45.2% and 25.1% in males, and 39.9% and 20.4% in females, respectively.[31] Accordingly, 50 million Americans are likely infected and contagious. Peak prevalence was reached in the age-class 25 to 29 years in males and essentially remained unchanged in the older groups.[32] In females the peak prevalence was in the 20- to 24-years age-group, and declined by about one-fifth in the older cohorts.[33] Most of the sexually active population is likely to be infected in a lifetime.[20]

The prevalence rate of condyloma acuminatum (plural, condylomata acuminata) or anogenital warts (venereal warts) in the general population ranges from 0.2% to 5%, based on genital examination.[34] In a US survey of 18- to 59-year-olds, 4.0% of males and 7.2% of females reported having had genital warts.[34] The incidence of the disease has risen. The annual number of initial visits to physicians' offices for genital warts doubled between 2000 and 2014, from 220,000 to 465,000.[35] It has since remained stable.[36] However, with the introduction of a quadrivalent HPV vaccine in 2006, the prevalence of genital warts has dropped between 2006 and 2010 from 2.9 to 1.8 per 100 person-years in females aged 15 to 19 years, the cohort most likely to have been vaccinated.[36] HPV infection of the cervix gives rise to the most common cause of squamous cell abnormalities on Papanicolaou (Pap) smears and are found in two-thirds of 1000 females aged 15 to 39 years.[36] The incidence rate of recurrent respiratory papillomatosis, which is primarily a disease of the larynx, is estimated to be 4.3 per 100,000/year for the juvenile-onset form of the disease (peak prevalence age, 7 years) and 1.8 per 100,000/year for the adult-onset form (bimodal peak prevalence ages, 35 and 64 years).[37,38] Prevalence rates are about twofold to ninefold greater.[37] The prevalence of oral HPV infections is 7.5%, but that of associated lesions is 0.5%, although higher in human immunodeficiency virus (HIV)-infected subjects, particularly on highly active antiretroviral therapy.[39,40]

Transmission

Close personal contact, especially within the family and school class, is likely to be important for the transmission of most cutaneous warts.[29,41] Minor trauma at the site of inoculation may also be important, as suggested by the high frequency of disease among meat handlers.[29]

Evidence that anogenital warts are sexually transmitted includes the observations that the age of onset is similar to that in other sexually transmitted diseases (STDs) and that the disease develops in approximately two-thirds of sexual contacts of patients with anogenital warts.[42,43] In addition, patients with anogenital warts often have other concomitant STDs or a history of such infections. Also, as outlined in Table 143.1, particular HPV types are associated with these lesions. These types are rarely found in lesions at other sites. Finally, a large number of lifetime, present, or recent sexual partners; the frequency of sex or other intimate skin-to-skin contact; and the sexual histories or behavior of sex partners are risk factors of genital HPV transmission, whereas circumcision in some studies has been found to be protective, as with HIV and herpes simplex virus (HSV).[20,44–46] Despite these observations, in adults routes of transmission not involving the penis are possible, but their relative importance, probably small, remains unclear.[47] Young children may acquire genital warts from hand contact with nongenital lesions.[48] Approximately one-fifth of prepubertal children with condyloma acuminatum have HPV type 1 or 2 in the lesions.[49–51] Conversely, HPV-6 DNA has been identified in cutaneous warts of family contacts of children with anogenital warts.[50]

In adults estimates of the rate of HPV transmission do vary.[52,53] In one study these rates (expressed as number of events per 100 person-months) were 3.5 from penis to cervix and 4.0 from cervix to penis.[54]

Recurrent respiratory papillomatosis in young children is thought to be acquired via passage through an infected birth canal or through the placenta.[37] This hypothesis is based on the observations that HPV DNA is frequently recovered from placentas or cervicovaginal lavages of pregnant women, as well as in neonates.[55] Furthermore, similar HPV types are associated with both respiratory papillomatosis and anogenital warts and that a large percentage of the mothers of these children have a history of genital tract HPV disease.[37] In addition, neonates are more likely to harbor HPV DNA in the oral cavity if the cervix of the mother contains HPV DNA.[37] Many children with recurrent respiratory papillomatosis are first born babies who were delivered vaginally to young (often teenage) mothers. Although the median age of onset of recurrent respiratory papillomatosis is 3 years, cases have been documented at birth, even after cesarean section.[37] This observation suggests that the disease may be acquired in utero, probably via ascending infection from the mother's genital tract. The role of cesarean section, if any, in prevention of transmission is unknown, and the procedure is not recommended for that purpose.[37] Family members and others with close personal contact with these patients are not at risk for developing the disease. In the adult-onset form recurrent respiratory papillomatosis is associated with a higher-than-expected number of lifetime sexual partners and with oral-genital contact.[37]

The role of fomites in the transmission of HPV infection is uncertain.[47] However, nosocomial transmission appears possible because infectious virus can be recovered from the fumes released from lesions during treatment with a carbon dioxide (CO_2) laser or electrocoagulation.[56] In addition, HPVs are resistant to heat, and use of an autoclave is probably necessary for sterilization of contaminated instruments.[57,58]

Association Between Human Papillomavirus and Malignant Diseases

The oncogenic potential of animal papillomaviruses was shown many years ago.[59] Observations of patients with epidermodysplasia verruciformis provided the initial evidence that suggested that HPVs might also be carcinogenic. In these patients characteristic skin lesions induced by specific HPV types frequently undergo malignant transformation, particularly when they occur in sun-exposed areas.[30] Most research investigating the oncogenic potential of HPVs has focused on genital tract malignant diseases.[7]

The low prevalence of cancer of the uterine cervix among Catholic nuns,[60] the direct association of risk with number of sexual partners, and the increased risk of malignant disease that is associated with a male sexual partner whose previous consort had cervical cancer have been observations consistent with a sexually transmitted agent playing a role in the pathogenesis of cervical cancer.[3,7,12,21,61–63] Among several agents, HSV-2 was once strongly suspected. However, over the past 40 years a large and coherent body of biologic and epidemiologic observations has shown that HPV infection is the necessary, if not sufficient, cause of cervical cancer.[64] This evidence can be summarized as follows:

1. The association between those HPV types called high-risk oncogenic (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66, as classified by the International Agency for Research on Cancer) and cervical cancer is strong, with odds ratios (ORs) that range from 50 to 100.[7] For the most oncogenic of these viruses, HPV-16 for squamous cell carcinoma (SCC) and HPV-18 for

adenocarcinoma, the ORs range from 100 to 900. In a worldwide survey HPV DNA was found in 99.7% of cervical cancer samples.[65]

2. The association has been consistent through many studies done in different countries and populations.
3. The association has been specific, so that among the approximately 40 HPV types associated with the genitalia, or more broadly the mucosal surfaces, only a subset of the types (at least 15) are oncogenic for the cervix. In addition, the same HPV types, with an even greater predominance of HPV-16 or HPV-18, are found in other SCCs. The fraction of SCCs attributable to HPV is 69% for the vulva, 75% for the vagina, and 63% for the penis.[66] The figures are even higher if only warty and basaloid histologic variants of these tumors are considered.[67] HPV is found in 91% of anal SCCs and, for the period 2005–09, in 72% of oropharyngeal SCCs.[66,68] Before 2000, only 40.5% of oropharyngeal SCCs were associated with HPV.[68] HPV-associated oropharyngeal cancer is now more common than cervical cancer in the United States.[69] This sharp increase has not been seen with the nonoropharyngeal head and neck cancers; the percentage attributable to HPV has remained at 22%.[68]

   Largely on the basis of molecular epidemiologic studies, mucosal high-risk HPVs have been found in cancers of the esophagus, lung, and breast but also of the colon, urothelium, prostate, the ovary, and endometrium, thus raising a possible causal role in these tumors. However, the nature, consistency, and strength of these associations are controversial.[70–77] HPV-16 has been found in some SCCs of the conjunctiva and of the nail bed.

   The beta HPV types found in the SCCs of patients with epidermodysplasia verruciformis have also been found in about a third of keratinocyte carcinomas (SCCs and basal cell carcinomas) in immunocompetent hosts and in up to 80% of immunosuppressed hosts. There is growing evidence that this association is causal, but through mechanisms analogous yet distinct from those associated with the high-risk mucosal, alpha HPVs.[78,79]
4. The development of cervical abnormalities and cancer is preceded by HPV infection. Most HPV infections are transient and last a mean of 13.5 months for high-risk HPVs and 4.8 months for low-risk types. However, between 15% and 30% of women with normal cervical cytology but high-risk HPV infection have cervical intraepithelial neoplasia (CIN) grades 2 or 3 develop in the following 4 years. Conversely, CIN 2 or 3 is unlikely to develop in women with milder cytologic squamous abnormalities who are negative for high-risk HPV. Although clearance of HPV DNA appears to precede clearance of cervical lesions, persistence of HPV DNA after treatment for CIN 2 or 3 is a predictor of relapse.

   The temporal association between HPV and cervical premalignant lesions has proven to be useful for prevention strategies. Hence HPV testing is more sensitive than repeated cervical cytology in identification of women with atypical squamous cells of unknown significance (ASC-US) than those with CIN 2 or 3.

   The number of sexual partners, the age of first sexual intercourse, and the sexual behavior of the husband are risk factors for HPV infections and also for cervical cancer, which occurs later in life. This temporal sequence is consistent with a causal link between infection and cancer.
5. In some studies a direct association is found between viral load and the risk of cancer, which is consistent with a biologic gradient.
6. Several lines of biologic evidence support an oncogenic role for HPV. Virtually all neoplastic cells in cervical cancer tissue contain HPV DNA, including metastases. The *E6* and *E7* genes are expressed at higher levels in neoplasms than in benign lesions. When the *E6* or *E7* genes of high-risk HPV types are introduced in normal cells, they cause malignant transformation in cell culture. Transgenic animals carrying these genes develop SCCs. The role of E6 and E7 proteins is further discussed in the "Pathogenesis" section.
7. Papillomaviruses can cause cancer in animal experimental models, such as the cottontail rabbit papillomavirus in the domestic rabbit and bovine papillomaviruses in the cow. Moreover, human neonatal foreskin grafts infected with HPV-16 and placed in SCID mice develop intraepithelial neoplasias.[80]
8. Other alternative risk factors for cervical cancer, such as the use of oral contraceptives, high parity, tobacco smoking, nutrition (vitamins C and E, carotenoids, xanthophylls), immunosuppression, prior HSV-2 or *Chlamydia trachomatis* infection, have not reached the strength and coherence of the evidence gathered for HPV. Their contribution may be only secondary to the primary role played by HPV infection.
9. Finally, the clinical trials of the HPV vaccine (see "Prevention") have amply shown that immunization against HPV types 6, 11, 16, and 18 confers protection not only against subsequent infection but also against disease (warts and intraepithelial neoplasias of all grades) caused by the homologous genotype in the cervix, vagina, and vulva and in the male external genitalia and anus.

PATHOGENESIS

The pathogenesis of HPV disease has been reviewed by several authors.[1–4,6,7,9–12] The incubation period was established experimentally with inoculation of human subjects with extracts of cutaneous warts.[1,81] Most often, warts developed within 3 to 4 months, although lesions occasionally grew as early as 6 weeks or as long as 2 years after inoculation. A similar incubation period was observed for genital warts among wives of American soldiers returning from the Korean War.[82] All types of squamous epithelium may be infected by HPV, but with the exception of the cervical glandular epithelium, other tissues appear to be resistant to productive infection. Gross histologic appearances of individual lesions vary with the site of infection and the virus type. Fig. 143.1 is a schematic diagram of a typical exophytic cutaneous wart.

The virus replicative cycle is tightly dependent on epithelial differentiation. It begins with the entry of particles into the stratum germinativum (basale) because viral DNA is detected in the nuclei of the basal cells.[2,3,11,12,83] It requires a breach of the integrity of the epithelium so that the viral particle can bind the heparan proteoglycans present on the basement membrane and basal cell. It initiates a process that includes a modification of the capsid conformation and proteins that allows entry by endocytosis facilitated by several possible candidate host molecules.[83] As the basal cells differentiate and progress to the surface of the epithelium, HPV DNA replicates and is transcribed, and viral particles are assembled in the nucleus. Ultimately, complete virions are released, probably still tightly associated with the remnants of the shed dead keratinocyte shell.[11,84] In a wart or condyloma, viral replication is associated with excessive proliferation of all of the epidermal layers except the basal layer. This process produces acanthosis, parakeratosis, and hyperkeratosis. A deepening of the rete ridges, where normally present, produces the typical papillomatous cytoarchitecture. Some infected cells undergo the characteristic transformation of koilocytosis. With histology, koilocytes (from the Greek *koilos*, "cavity") are large, usually polygonal, squamous cells with a shrunken nucleus lodged inside a large cytoplasmic vacuole. Cytoplasmic keratohyalin inclusion bodies may also be observed. Excessive proliferation of the basal-like cells (basaloid proliferation) with a high nuclear/cytoplasmic ratio, accompanied by a high number of mitoses (some abnormal [dyskaryosis]), is a feature of incipient and malignant HPV disease.

Normal-appearing epithelium may contain HPV DNA,[85,86] and the presence of residual DNA after the treatment of warts may lead to recurrent disease. In benign lesions caused by HPV, viral DNA is located extrachromosomally in the nuclei of infected cells. However, when HPV DNA is detected in high-grade intraepithelial neoplasias and cancers, it is generally integrated.[2–4,8,9,12] Integration of HPV DNA may occur at preferential sites in host cell chromosomes,[3] and it specifically disrupts the E2 ORF. Interruption of E2 probably plays a role in the pathogenesis of malignant disease because expression of this ORF normally leads to downregulation of E6 and E7, whose products interfere with the p53

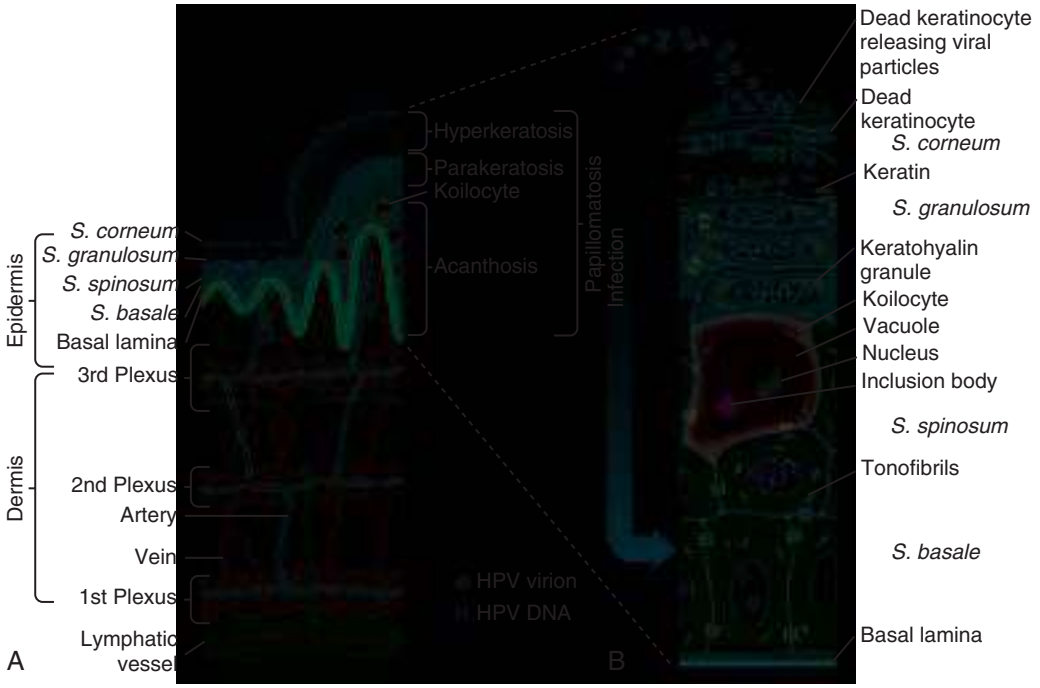

FIG. 143.1 Exophytic cutaneous wart: human papillomavirus *(HPV)* pathogenesis. (A) Histologic features. (B) Cytologic features (see text for details). *S.*, stratum.

and retinoblastoma tumor suppressor proteins (see "Virology").[1–4,7–9,12] Nevertheless, the frequency of viral integration varies with the HPV genotype and does not appear to be necessary for oncogenesis. Other events are also important, including hypermethylation of viral and cellular DNA; inhibition of apoptosis and telomerase activation, which both confer immortality; and cooperation with activated cellular oncogenes. The development of chromosomal instability and deletions (6p, 3p, 4p, 6q, 10p, and ultimately 11q) occur as the lesion becomes a high-grade intraepithelial neoplasia.[a]

Host defense responses to HPV infection are not fully understood.[87–93] Nevertheless, several clinical observations indicate that an effective immune system is important in the resolution of HPV infection. Epidermodysplasia verruciformis is a genodermatosis that results from the inactivation of two genes, *EVER1/TMC6* and *EVER2/TMC8*, that code for endoplasmic zinc transporter proteins, components of the nuclear factor kappa B cascade regulating the immune system. Primary immunodeficiencies, such as SCID, common variable immunodeficiency, Wiskott-Aldrich syndrome, and ataxia-telangiectasia, are known for their association with verrucosis, but there are many more.[94] For example, the WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome results from a gain-of-function mutation in the *CXCR4* chemokine receptor gene. Other entities include WILD (warts, immunodeficiency, lymphedema, and [anal] dysplasia) syndrome, XHIGCM1 (X-linked hyper–immunoglobulin M syndrome type 1), Netherton syndrome, and mutations in either the dedicator of cytokinesis 8 *(DOCKS8)* or of the *GATA2* genes.[95] Idiopathic CD4 lymphopenia has been associated with some of the most dramatic cases of profuse verrucosis, the so-called "treeman" patients. Severe frequent HPV disease is also seen in patients with lymphoproliferative disorders and in those with HIV infections.[96–102] The range of HPV-related diseases in HIV infection includes cutaneous warts, anogenital warts, CIN in women, and anal intraepithelial neoplasia and cancer in men who have sex with men (MSM).[96,102–104] HIV infection increases by 4-fold to 40-fold the incidence and prevalence rates of genital warts and CIN.[103] The prevalence of these conditions is greater with low counts of $CD4^+$ T lymphocytes and high HIV-1 RNA levels. Compared with subjects with HIV seronegativity, patients with acquired immunodeficiency syndrome (AIDS) have an increased risk for developing in situ or invasive SCCs of the cervix, vulva-vagina, anus (both genders), and penis.[100,105,106] Contrary to the expectation that led to the inclusion of cervical cancer as an AIDS-defining illness, the progression to AIDS does not appear to augment dramatically the risk of cervical cancer, even if a low $CD4^+$ T-lymphocyte count is a risk factor.[105–109] In contrast, the risk of invasive cancer of the anus in men and in situ and invasive cancers of the vagina or vulva do increase with AIDS progression, as well as with a low $CD4^+$ T-lymphocyte count at the time of AIDS onset.[108] The increased risk of anal cancer is greater than 40-fold.[110] Immunosuppressive therapy, notably in renal allograft recipients, has also been associated with high rates of extensive HPV infection.[98,99,111,112] Another indication of the role of the immune system comes from the observation that the regression of a wart may be promptly followed by the spontaneous regression of others.[113,114] Although the relative immunosuppression of pregnancy appears to be associated with an increased incidence and severity of HPV disease,[43,115] rates of HPV infection are not clearly found to be substantially higher in this population than in nonpregnant women.[116–118]

Evidence also shows that HPV-16 E6 and E7 proteins may inhibit Toll-like receptor 9 (TLR9), a component of innate immunity.[119]

The keratinocytes possess various TLRs, in particular TLR9, that recognize foreign pathogen-associated molecular patterns.[120] This causes the production of numerous cytokines and chemokines, including tumor necrosis factor-α; monocyte chemotactic protein-1; chemokines CCL2, CCL20, and CXCL9; CXCL10 vascular endothelial cell growth factor; interleukins 5 and 8; retinoic acid; transforming growth factor-β; interferons (IFNs) α, β, and γ; and IFN-γ–inducible protein-10.[2–4,8,9,12,121] Overall, this biochemical activity is associated with increased leukocyte trafficking and angiogenesis.

Concomitantly, at the histologic level, an alteration is seen in the number and function of the natural killer and helper T cells and cutaneous Langerhans cells.[122,123] These cells contribute to the local and systemic immune response, which becomes more apparent in regressing warts, as they show a clear lymphomononuclear cell infiltrate.[124]

A humoral and cellular immune response does develop after HPV infection, but its laboratory correlates are not necessarily uniform or constant. The E7 and L1 proteins are the strongest antigens.[125–127] The

[a]References 2, 3, 4, 8, 9, 12.

enhancement after immunization of neutralizing antibody formation against the L1 native protein has been exploited to produce the currently available vaccines (see "Prevention").

HPV also interacts actively with the immune response.[128] For example, HPV E7 reduces the expression of the chemokine CXCL14, which would otherwise trigger the recruitment of natural killer cells, T cells, Langerhans cells, and dendritic cells. HPV E6 and E7 downregulate the IFN response, the expression of TLR9, and alter the cGAS-STING (*c*yclic *G*MP-*A*MP *s*ynthase–*st*imulator of *in*terferon *g*enes) sensing pathway defending against DNA viruses. These viral proteins, along with E5, interfere with antigen processing at the level of intracytoplasmic transport and peptide loading on the cell surface major histocompatibility complex proteins.

Immunogenetic factors are also important. In a large case-control study of the association of classes A, B, and C human leukocyte antigen (HLA); DRB1 and DQB1 alleles; and cervical SCC, several low-magnitude (about twofold or less) associations were observed.[129] For example, class I HLA allele A*0301 increased the risk, and B*1501 decreased it. Similarly, class II HLA allele DQB1*0301 was a risk, and DRB1*1302 was protective. A particular combination of alleles, B*4402-DRB1*1101-DQB1*1302, increased the risk 10-fold. The same immunogenetic risk factors were noted with cervical adenocarcinoma and vulvar SCC. These results confirm the important role that helper and cytotoxic cell responses play in the development of HPV-associated genital cancer.[130]

CLINICAL MANIFESTATIONS

Cutaneous Warts

Cutaneous warts include deep plantar warts, common warts, and plane or flat warts.[131,132]

Deep plantar warts (verrucae plantaris), also called *myrmecia* (from the Greek, meaning "ant hill"), affect mostly adolescents and young adults. The lesions characteristically look like deep-seated, raised bundles of soft keratotic fibers 2 mm to 1 cm in diameter; shaving reveals punctate, bleeding blood vessels. When grouped in clusters they are called mosaic warts. These lesions are often painful and may also be located on the palms of the hands.

Common warts (verrucae vulgaris) appear as well-demarcated, exophytic, hyperkeratotic papules with a rough surface. They may occur on the dorsum of the hand, between the fingers, around the nails (periungual warts), on the palms or soles, or, rarely, on mucous membranes. Warts may coalesce and reach a diameter of 1 cm. Morphologic variants of common warts include mosaic warts, which appear as cobblestone-like patches of aggregated warts several square centimeters in diameter and barely rising above an indurated base. Filiform warts on the head and vegetating, hyperproliferative warts on the hands of butchers, fish handlers, and meat packers also occur.[29]

Plane warts (verrucae planae) are commonly found on children and appear as multiple, slightly elevated papules with an irregular contour and distribution and a smooth surface. They occur on the face, neck, and hands. When more protuberant, these lesions are called *intermediate warts*.

Cutaneous warts are usually asymptomatic, although they may bleed and can be painful when located over weight-bearing surfaces or points of friction. Rarely, cutaneous warts may degenerate into verrucous carcinomas.[133] The natural history of cutaneous warts is poorly characterized. Spontaneous resolution appears to occur in 50% and 90% of children within 1 and 5 years, respectively.[26] In a given patient two-thirds of the warts that resolve spontaneously do so within 2 months.[134]

Epidermodysplasia Verruciformis

Epidermodysplasia verruciformis is an autosomal-recessive genodermatosis linked to gene loci on chromosome 17.[2,30] The lesions are associated with a large array of HPV types, some linked to malignant transformation (see Table 143.1), most of which are specific for epidermodysplasia verruciformis.[2,30] These warts have several morphologic variants. They may resemble flat warts but more commonly resemble lesions of pityriasis versicolor, which cover the torso and upper extremities. Over extensor surfaces, these warts may become hypertrophic and coalescent. In most patients, warts appear in the first decade of life. Beginning in young adulthood, in about one-third of patients, the lesions undergo malignant transformation into invasive SCCs, particularly in sun-exposed areas. Although these patients may have depressed cellular immunity,[2,30] they appear to have normal resistance to other pathogens. Epidermodysplasia verruciformis does not appear to be contagious to healthy contacts. Of interest, lesions that resemble epidermodysplasia verruciformis have been observed in HIV-infected patients and solid-organ allograft recipients.[30]



FIG. 143.2 Vulvar condylomata acuminata. *(From Gagné H. Colposcopy of the vagina and vulva.* Obstet Gynecol Clin North Am. *2008;35:659-669.)*

Anogenital Warts

Anogenital warts are flesh colored to gray colored, hyperkeratotic, exophytic papules, either sessile on the skin or, more frequently, attached by a short, broad peduncle (Fig. 143.2). Lesions range from smooth, pearly papules to more jagged, acuminate growths. They vary in size from less than a millimeter in diameter to several square centimeters when they merge into plaques. In uncircumcised men the preputial cavity is involved in 85% to 90% of cases.[43,135,136] In the United States, where about 85% of the male population is circumcised, the penile shaft is the most common site of lesions. The urethral meatus is also involved in 1% to 25% of patients.[137] Urethral warts are clearly visible with eversion of the meatus or with the use of a pediatric nasal speculum. They are mostly confined to the fossa navicularis or, less frequently, to the distal 3 cm of the urethra. Involvement of the bladder or proximal urethra is exceptional.[137] Involvement of the perianal area varies according to sexual practice, from very high among MSM (about 10%, and double with HIV seropositivity) to low among heterosexual men.[138,139] Lesions are only occasionally observed on the scrotum, perineum, groin, or pubic area.

In women most lesions are distributed over the posterior introitus and, to a lesser degree, over the labia majora and minora and the clitoris (see Fig. 143.2). In order of decreasing frequency, the perineum, vagina, anus, cervix, and urethra each represent less than one-quarter of the sites of involvement.[43]

The use of the colposcope and prior soaking of examined tissues with 3% to 5% acetic acid has expanded the clinical spectrum of anogenital warts, particularly those caused by HPV types 16 and 18, which can be small acetowhite papules.[140] This technique was initially used to show the existence of flat condylomas on the uterine cervix. Typically, these lesions are shiny white patches with geographic borders



FIG. 143.3 Pigmented penile warts mimicking bowenoid papulosis. *(From Habit TP, ed.* Clinical Dermatology. *4th ed. London: Mosby; 2004.)*

and an irregular surface that contains characteristic capillary loops.[141] The presence of external genital warts may indicate the existence of cervical HPV squamous epithelial lesions, including CIN.[142,143] Morphologic differentiation among the grades of cervical squamous epithelial lesions is not sufficiently reliable, and biopsy is strongly recommended for diagnosis.[144,145]

In the vagina, in addition to flat condylomas, small white nodosities centered on a capillary loop, called *spiked condylomas*, have been described.[146] The vulvar introitus may display prominent, sometimes painful papillae whose relation to HPV infection is unlikely but controversial.[147,148] HPV infection of the vulva may also appear as white patches revealed or accentuated with the application of acetic acid, but acetowhitening lacks specificity.[149]

In men acetic acid soaking or examination with a colposcope has shown HPV-infected papules and macules to be up to two times more common than exophytic condylomas, particularly on the prepuce and scrotum.[140,150] With a range in size from minuscule to 1 cm in diameter, round sessile papules with brown to slate-blue pigmentation are encountered on both male (Fig. 143.3) and female external genitalia. These lesions and similarly colored macules are important to recognize because they may represent either HPV-6– or HPV-11–infected benign condylomas,[151,152] seborrheic keratoses,[153] or intraepithelial neoplasias associated with HPV type 16 or 18 infection.[152–154]

About three-quarters of patients with anogenital warts are asymptomatic. Otherwise, itching and burning, pain, and tenderness are encountered frequently. In addition, the disease can have serious psychological effects.[155] The natural history of genital warts, particularly of subclinical HPV disease, is poorly understood, but spontaneous remission may occur, as shown by the results of randomized, placebo-controlled therapeutic trials that indicate up to 10% to 20% spontaneous remission rates in untreated lesions over a 3- to 4-month period.[156–159]

Exophytic genital warts may rarely transform into invasive SCCs, including verrucous carcinoma. They may also reach considerable size, particularly during pregnancy or immunosuppression.[160] When large condylomas reveal histologic features of local destructive invasion without metastases, they may be called *Buschke-Löwenstein tumors*, a term that regroups verrucous carcinomas and giant condylomas.[161,162] A related lesion, condylomatous (warty) carcinoma, may metastasize.[162] Genital HPV infections may also belong to the spectrum of penile, anal, vulvar, vaginal, and cervical intraepithelial neoplasias (PIN, AIN, VIN, VAIN, and CIN, respectively).[163,164] For historical reasons, some variants of intraepithelial neoplasias are further recognized. Histologically, pigmented papules of the external genitalia may show condylomatous cytoarchitecture with evidence of intraepithelial neoplasia.[152] This clinicopathologic entity is called *bowenoid papulosis* (see Fig. 143.3).[165] *Bowenoid papulosis* can evolve to *Bowen disease*, which manifests as a flat red-to-brown plaque with well-demarcated borders and a scaly irregular surface.[166] On the glans penis the lesion is known as *erythroplasia of Queyrat*. Histologically, carcinoma in situ (CIS) is present. HPV-16 and HPV-18 have been recovered from both *bowenoid papulosis* and *Bowen disease*.[167] The natural history of intraepithelial neoplasias is best understood in cervical lesions.[168] Clearly, the outcome (regression, no change, or progression) is highly variable and depends on the histologic grade of the tumor, the HPV type, and the method of diagnosis (conization, punch biopsy, or scraping). CIN grade 1 lesions have an approximate probability of 60% to regress, 30% to remain unchanged, 10% to progress to CIN 3, and 1% to progress to invasive cancer.[168] For CIN 2 the figures are 40%, 40%, 20%, and 5%, respectively. The risk of progression to cancer is the highest with CIN 3 at 12%; only a third of these lesions disappear spontaneously.

Perianal warts are common among homosexual men, and up to two-thirds of patients with external anal warts also have internal lesions.[169] In consequence, the presence of perianal warts or anal symptoms in association with a history of anal sexual play or intercourse should prompt a digital rectal examination and an anoscopic evaluation. After the malignant transformation of anal condylomas was described,[170] the association between anorectal dysplasia or cancer and HPV infection was recognized in MSM.[171,172] Passive anal intercourse carries a risk of anal cancer in MSM, and heterosexual men and women with a history of anogenital warts have a 30-fold increased risk of disease compared with control populations.[173] The anus and the cervix have a different biology respective to HPV. HPV infections in women with and without HIV are 79% and 43% prevalent in the anus, respectively, but only 53% and 25%, respectively, in the cervix.[174] In the general population a history of anal warts increases by about 10 times the risk of anal cancer.[175] During pregnancy HPV shedding may increase, and condylomas may become so large as to impair normal delivery mechanically.[118,160,176] Anogenital warts in children should always raise the possibility of sexual abuse, but in very young children nongenital or possibly perinatal transmission may be the predominant mode of acquisition.[177–179]

Recurrent Respiratory Papillomatosis

Recurrent respiratory papillomatosis has been described by several authors.[37,180,181] Patients present with hoarseness or, in infants, with an altered cry. Sometimes these symptoms are accompanied by respiratory distress or stridor. The disease may spread to the trachea and lungs and lead to obstruction, infection, and respiratory failure. In young children rapid growth of lesions often threatens the upper respiratory tract and frequently necessitates surgical excision to avoid asphyxiation. In adults the course of the disease is usually less aggressive. Lesions may, however, undergo malignant transformation, particularly in patients who have received radiation therapy or in cases with lung involvement.

Other Human Papillomavirus Infections

Oral squamous cell papillomas (or squamous papillomas) are the most common HPV-related oral lesions. A closely related entity, with slightly different histologic features, is oral condyloma acuminatum. Both types of lesions are caused by mucosal HPV (mostly HPV-6, HPV-11, and HPV-16). Oral verrucae vulgaris are rarer and can be differentiated reliably only with histology. They are caused by cutaneous HPVs (HPV-2, HPV-4, HPV-57).[182] Focal epithelial hyperplasia of the oral cavity (Heck disease) is caused predominantly by HPV-3 and HPV-13 and tends to regress spontaneously.[183] Other HPV infections may also occur in the oral cavity.[183] The oropharynx is now the most common site for the development of HPV-associated cancer.[69] Conjunctival HPV-related papillomas and SCCs, and periungual SCCs have been described.[64] HPV DNA has also been identified in other skin lesions, such as epidermoid cysts, seborrheic keratoses (especially vulvar), skin squamous cell and basal carcinomas,[22] and aerodigestive carcinomas. The prevalence of HPV in these different lesions varies, which makes a causative link difficult to establish.

DIAGNOSIS

The diagnosis of warts is usually made clinically with physical examination. Exophytic warts have a characteristic appearance. Deep plantar warts may be confused with calluses, but paring usually reveals typical punctate, thrombosed capillaries. Nevi, seborrheic keratoses,

acrochordons, acanthomas, molluscum contagiosum, lichen planus, syringomas, and dermofibromas may be confused with cutaneous warts. Lesions of epidermodysplasia verruciformis may be similar to those of flat warts or pityriasis versicolor, but the patient's history should clarify the diagnosis.

Condyloma acuminatum of the external anogenital tract should rarely be confused with other STDs, such as condyloma latum of syphilis, nodular scabies, genital herpes, lymphogranuloma venereum, chancroid, or granuloma inguinale. Nevertheless, molluscum contagiosum, particularly in its more atypical presentations, may be difficult to distinguish from anogenital warts. In contrast to those of condyloma acuminatum, the lesions of molluscum contagiosum tend to predominate over the pubis and are rarely pedunculated, but rather appear as smooth, sessile domes, the color of the skin or lighter, often with a depressed center from which cheesy material can be expressed. In men a normal anatomic variant of the corona, hirsutoid papillomatosis (pearly coronal papules, papillae corona glandis), can be difficult to differentiate from small warts. A similar anatomic presentation exists in the vulvar introitus, where lesions may appear identical to those of HPV-related vulvar papillomatosis. On the keratinized vulva, hidradenoma papilliferum may be confused with a large wart. On the scrotum, epidermoid cysts and angiokeratomas should be easy to identify. Small and flat HPV lesions may sometimes be difficult to distinguish from lichen planus, lichen sclerosus et atrophicus, lichen nitidus, or syringomas, even with the help of the colposcope and acetic acid application. Finally, pigmented HPV lesions may be confused with nevi or seborrheic keratoses (see Fig. 143.3).

Although initially designed for the evaluation of the female internal genital tract, the colposcope, with prior application for 3 to 5 minutes of a 3% to 5% acetic acid solution, has become an important diagnostic tool for other HPV infections as well.[184] In studies of male partners of women with either cervical condylomas or dysplasias, biopsy-proven genital condylomas were detected in 65% to 88% of the patients, respectively. More significant, 43% to 73% of the lesions were seen only with a colposcope, whereas acetowhitening alone was used for the diagnosis in 22% of patients.[140,185,186] The same technique applied to the vulva revealed subclinical papillomavirus infection in 96% of women with vulvar warts and 80% of women who were partners of men with penile warts.[187] In the oral cavity 83% of HPV lesions are seen only with the colposcope.[188] The clinical significance of lesions that are detectable with acetowhitening only is unknown, and acetowhitening lacks specificity for the diagnosis of HPV infection, particularly for external anogenital warts.[153,189,190]

Lesions of the external genitalia that are pigmented (see Fig. 143.3), appear as plaques, bleed, or are large should have biopsies to establish the diagnosis and rule out malignancy.[152] Biopsy is also indicated to confirm the diagnosis of epidermodysplasia verruciformis and to determine the cause of lesions of the oral cavity and upper airways.

Anoscopic examination should be considered in patients with perianal warts, anal symptoms, or a history of receptive anal intercourse. Most intraanal lesions are below the pectinate line, and sigmoidoscopy is not routinely indicated.[191,192] The oral cavity should preferably be examined in all patients with anogenital warts because of the possibility of concomitant oral warts.[188]

Evaluation of the vagina and cervix, when appropriate, should include colposcopy and acetic acid application and should seek to rule out invasive cancer.[184] Internationally applicable colposcopic terminology should improve diagnostic accuracy and reliability.[141] Women with a history of anogenital HPV disease or whose sexual partners have had anogenital HPV disease should have a cytologic examination of a cervical smear (Pap smear), at least as part of regular screening (see "Prevention"). Koilocytes on a cytologic smear are the hallmark of HPV infection.[193] More important, diagnoses of dysplasia and cancer can also be made from the smear.[194] Depending on the patient's age and the location and nature of the HPV infection, the sensitivity of the Pap smear in detection of HPV infection ranges from 30% to 90%.[195]

The use of the colposcope during the anoscopic examination (high-resolution anoscopy [HRA]) combined with anal cytology has been applied with success to the diagnosis of intraanal HPV lesions.[196] It can be a screening tool for anal intraepithelial neoplasias in homosexual or bisexual males or in the female with HIV.[171,174,196–198] So far, only New York State recommends anal cytology and HRA for the management of women with HIV with high-grade intraepithelial neoplasia and of any patient with HIV with abnormal anal physical findings (www.hivguidelines.org). However, cytology turned out to have poor specificity in the given patient population, thus increasing the reliance on histology, and possibly HPV DNA testing, and high-grade AIN recurrence is high.[198,199] Nevertheless, any of these approaches have yet to be validated with long-term studies of outcomes (anal cancer), reliability, safety, and costs. In consequence, they have not been endorsed by broad public health policies.[200,201] Automated, liquid-based collection for cervical cytology has largely supplanted the conventional Pap smear in the United States.[194,202] Its only, but significant, advantage is that it allows, if need be, HPV DNA testing on the same sample (so-called reflex testing), which is now part of the screening strategy for cervical cancer.[202,203]

Cervical cytology has benefited from the development of the Bethesda system, last revised in 2002.[204] This interpretation scheme addresses the adequacy of the specimen, classifies its pathologic features, and provides guidelines for management and follow-up, updated in 2012 (http://www.asccp.org/asccp-guidelines).[204,205] HPV-related squamous cell abnormalities are regrouped in four categories: (1) atypical squamous cells "a" of undetermined significance (ASC-US) or "b," for which a high-grade squamous intraepithelial lesion (ASC-H) cannot be excluded; (2) low-grade squamous intraepithelial lesion (LSIL), a diagnosis that regroups the previous cytologic and histologic diagnoses of koilocytic or condylomatous atypia, mild dysplasia, and CIN 1; (3) high-grade squamous intraepithelial lesion (HSIL), previously including moderate and severe dysplasia, CIN 2 and CIN 3, and CIS; and (4) SCC.

The general histopathologic features of HPV infection are usually characteristic (see "Pathogenesis"). Therefore biopsy can be used to confirm most diagnoses. In addition, histologic examination can identify the presence of intraepithelial neoplasia or invasive cancer. Although histology is the gold standard, like cytology it suffers from lack of accuracy and reliability where disease grades are concerned.[194,206]

To enhance the sensitivity and specificity of cytohistopathology, two types of techniques are now available to the clinical laboratory. They rely on the demonstration in the cytologic or biopsy specimens of HPV nucleic acids or cellular antigens indicative of oncogenic risk.[207–209]

Among the approximately 200 commercially available tests, seven HPV nucleic acid detection tests are approved by the US Food and Drug Administration (FDA).[210] They have at least one of three purposes: the primary screening of cervical cancer, the reflex testing of specimens diagnosed as ASC-US for further identification of the women requiring colposcopy, and the management of women 30 years and older by doing contesting with cytology.

The tests are available on automated platforms. The Hybrid Capture II High Risk DNA (Quiagen, Germantown, MD) detects with RNA probes the DNA of 12 oncogenic HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, and 68). It does not differentiate the type(s) identified and does not check for the presence of human DNA. Cervista HPV HR (Hologic, Marlborough, MA) targets the HPV E6/E7 DNA of the same types as of the Hybrid Capture II as well as HPV-66. It relies on signal amplification, and assesses the presence of human cells in the specimen. The result does not differentiate the HPV type(s) present. A related test, Cervista HPV16/18 (Hologic), identifies whether types 16 or 18 are present. The COBAS 4800 HPV (Roche, Indianapolis, IN) is a polymerase chain reaction (PCR) assay targeting the L1 region of the same 13 types as the Cervista HPV HR assay. It incorporates a human β-globin control. Results are given as to the presence of HPV-16, HPV-18, or others. This assay is the only one approved for primary screening. The APTIMA HPV (Hologic) is an assay that detects the E6/E7 transcripts of the same HPV types as the COBAS assay, plus those of HPV-56, by transcription-mediated amplification. Detection of the amplification products is done by a hybridization protection assay. It includes a β-globin control. The result does not differentiate among the genotypes. A similar assay, the APTIMA HPV 16, 18/45 genotype assay does identify if HPV-16, HPV-18, and/or HPV-45 are present. A seventh assay, ONCLARITY HPV (Becton Dickenson, Franklin Lakes, NJ) detects the *E6/E7* DNA by real-time PCR. It has a β-globin control.

It reports the individual detection of HPV genotypes 16, 18, 31, 45, 51, and 52, and the group detection of 33/58, 35/39/68, or 56/59/66. These different assays, although not identical in their performance characteristics, appear equivalent in clinical effectiveness.[211–214]

Under the stimulus of HPV E6 or E7, lesions that progress to cancer also produce in greater quantity various proteins associated with the host cell-cycle regulation. These proteins can be detected by immunocytohistochemistry and provide the pathologist with additional prognostic tools.[215–218] Among these biomarkers, $p16^{INK4A}$ has received the most attention, and it has been integrated in the histologic diagnostic approach.[209,219] It is possible to identify on tissue sections the presence of the papillomavirus common antigen by immunocytochemistry.[19]

Virus cultivation techniques are not available for the clinical diagnosis of HPV infections. HPV infection may elicit a serologic response. In patients with cutaneous warts, condyloma acuminatum, or recurrent respiratory papillomatosis, antibodies directed against the viral capsid have been detected.[126] Recombinant VLPs based on the L1 or L1 and L2 proteins offer the same antigenic properties as viral capsids.[126] They have been used extensively to show, with enzyme-linked immunosorbent assay, that about one-half to almost 90% of patients with HPV infection have capsid antibodies.[126] Anti-HPV antibodies tend to disappear with disease resolution but can persist for several years in asymptomatic patients.[126] A fraction of the antibodies produced in response to HPV infection are neutralizing but in amounts offering at best limited protection sufficient to offer significant protection.[220,220a] No commercial assays are available for the serologic diagnosis of HPV infections because of insufficient sensitivity and clinical specificity. Such assays have been used for seroepidemiologic surveys. Moreover, assays that measure binding and neutralizing activity against the viral capsid have been useful in assessment of the immune response after HPV vaccination (see "Prevention").

THERAPY

Highly effective and safe treatments for HPV diseases are not yet available, and the current therapies are not designed to eradicate HPV infection. Rather, their purpose is to decrease or, if possible, eliminate clinical manifestations. The current therapeutic armamentarium has been largely developed empirically over decades and too often relies on the physical or chemical destruction of lesions. Newer approaches are directed at molecular viral targets and immunomodulation.[13,221–223]

Cutaneous Warts

The choice of treatments for cutaneous warts is complicated by the existence of weak and confusing evidence.[224–226] Nevertheless, the most common approach, the topical application of preparations that contain salicylic acid, a keratolytic agent, is effective for the treatment of common warts.[224–226] A meta-analysis of five placebo-controlled clinical trials revealed a complete response rate of 71% (117 of 165) in the cases, compared with 47% (78 of 166) in the control groups.[224,225] A widely available over-the-counter preparation for self-treatment is a salicylic acid and lactic acid paint (salicylic acid, lactic acid, collodion, 1:1:4 [SAL]) that is typically applied daily for up to 12 weeks. The cornified layer that typically covers skin warts may need to be removed. This removal is done with a hot water soak, followed by abrasion with a pumice stone, sandpaper, or an emery board. Occlusive bandages seem to increase treatment effectiveness. Mosaic warts tend to be more resistant to treatment than myrmecia.

Cryotherapy is a popular treatment, but it requires a health practitioner. It is typically accomplished with cotton wool buds dipped in liquid nitrogen and applied to the lesion or with spraying of liquid nitrogen.[227,228] Randomized, placebo-controlled studies have been inconclusive on the efficacy of cryotherapy, but when compared with salicylic acid preparations, cryotherapy appears to be equivalent.[224,225] Variations in technique may account for these confusing results. However, aggressive cryotherapy, a 10-second sustained freeze, is more effective than briefer traditional cryotherapy, despite a higher incidence of pain and blisters.[224,225,229] More than one treatment is often needed. A 2-week interval offers the best balance between the occurrence of side effects and brevity of treatment.[230] Treatment beyond 3 months, or about four cryotherapies, presents little advantage.[231]

A randomized study with blind evaluation compared cryotherapy with duct tape application, an occlusive treatment that has long been in the medical lore, for the treatment of common warts.[232] Complete clearance of the warts occurred in 85% (22 of 26) of the patients who received occlusive therapy but in only 60% (15 of 25) of the patients treated with cryotherapy. However, this trial had limitations with blinding and follow-up. Two subsequent placebo-controlled trials of duct tape application produced negative results.[233,234]

Other treatment methods that are less often used include glutaraldehyde, formaldehyde, podofilox, and cantharidin. Their use is empirical. Intralesional bleomycin has been better studied, but when it is compared with placebo, the results are inconclusive.[224] It is usually reserved for the treatment of periungual warts. Other treatments reportedly superior to placebo include silver nitrate sticks and topical zinc sulfate preparations.[226,235,236]

Allergic sensitization with dinitrochlorobenzene (DNCB), followed by direct application of DNCB on the lesions, has been found to be twice as effective as placebo.[224,237,238] However, the use of DNCB is risky, and other sensitizing agents, such as 2,3-diphenylcyclopropenone, squaric acid dibutyl ester, and 10% masoprocol cream (Actinex, Schwarz Pharma, Mequon, WI), appear to be safer and as effective.[237,238]

Imiquimod is an immunomodulator that is approved by the FDA for the topical treatment of genital warts (see "Anogenital Warts"). When used off label in an open study, imiquimod 5% cream applied once a day, 5 days per week, for up to 16 weeks on varied common warts resulted in a complete response.[238]

Cimetidine, an H2 blocker that has immunomodulatory properties, has been widely publicized as an effective treatment for cutaneous warts on the basis of uncontrolled studies. Yet several placebo-controlled, double-blind studies have failed to confirm that claim.[237,238]

Electrosurgery and laser surgery are used, but they can be expensive and have not been rigorously evaluated.[224,237,239,240] Electrosurgery is relatively contraindicated for the treatment of plantar warts because of the risk of permanent and painful scarring. Laser surgery is also not scar-free, but it may be useful for the treatment of periungual and subungual warts.

Photodynamic therapy, which relies on laser light to activate locally the cytotoxicity of a compound administered systemically or applied topically, is superior to placebo for the treatment of cutaneous warts.[224] However, the technique is costly and not widely available.

Suggestion, hypnosis, homeopathy, and distant healing are among alternative approaches that have been proposed for the treatment of cutaneous warts.[241–245] More rigorous evaluations of these interventions showed little, if any, promise.

Particular treatment methods have been proposed for some specific types of warts. For example, flat warts rarely need treatment, but when they do, cryotherapy or electrosurgery (electrodesiccation) is used. Cryotherapy may also be used for the treatment of eyelid and periungual warts, and electrodesiccation is useful to remove flat or filiform warts.

Anogenital Warts

The treatment methods for condyloma acuminatum are numerous yet unsatisfactory, but guidelines that attempt to optimize the therapeutic approach have been published.[201,246–250] Because no or scant evidence shows that treatment directly affects eradication of HPV, transmission of infection, or prevents the uncommon development of neoplasms, the rationale for treatment is restricted.[85,251,252] It includes cosmesis, relief of local symptoms, alleviation of the adverse psychological impact caused by the presence of anogenital warts,[155,253–257] and restoration of normal physiologic function (e.g., debulking of lesions that obstruct the birth canal). Before treatment is initiated, the goals of therapy, alternatives, costs, and potential side effects should be discussed with patients. Also, within 3 to 4 months, approximately 10% to 20% of patients have spontaneous resolution of the disease.[156–159,246] Independent of treatment, patient counseling is part of management.[136,258]

None of the available treatment methods is dramatically superior to the others, but each may have its particular advantages. Because convenience is one of the greatest advantages, the availability over the past few years of patient-applied therapies—podofilox, imiquimod, and Veregen (polyphenon E)—has been of considerable interest.

Podofilox (podophyllotoxin) is a derivative of podophyllin, which was long the mainstay of genital wart treatment by practitioners. Podophyllin, a resin extract from the rhizome of *Podophyllum peltatum* (podophyllum resin [US Pharmacopeia]) or *Podophyllum emodi*, has been the principal mode of therapy for many years.[259,260] The active molecules are lignans, particularly podophyllotoxin. Although podophyllin is a mitotic poison, its mode of action in warts is unknown. The compound is usually applied as a 10% to 25% solution in benzoin, directly on the wart, once weekly. Washing of lesions within 12 hours is recommended to minimize local reactions. Lack of regression after four applications suggests the need for alternative therapy. Podophyllin has never been compared with a placebo. Its effectiveness has been evaluated in a series of randomized controlled trials against other treatment methods; complete clearance rates ranged from 20% to 40%, taking into account frequent recurrences.[135,259] Side effects are both local and systemic.[259,260] Chemical burns are seen in one-third to one-half of the patients. Transient pseudoneoplastic histopathologic changes have also been reported. Neurologic, hematologic, and febrile complications, sometimes leading to death, and allergic sensitization have been associated with administration of topical podophyllin. Therefore areas larger than 10 cm^2 should not be treated. The drug is contraindicated in pregnancy.

Podophyllotoxin is available in the United States under the generic name podofilox. It offers distinct advantages over podophyllin and is preferentially recommended.[261] It is chemically uniform and of standardized potency. Podofilox is also more efficacious and less toxic than podophyllin.[135,260,262,263] Finally, it does not need to be washed off. Randomized controlled studies have shown that 0.5% podofilox solution applied twice daily for 3 consecutive days every week for up to 4 weeks results in rates of complete response from 45% to 58%.[246,250,263–266] A gel formulation is easier to apply without spillover. Side effects are mostly mild and similar in nature to those of podophyllin. As with podophyllin, relapses are common and occur in 33% to 91% of patients.[246,250,263–266] Application of podofilox to prevent recurrences is effective and well tolerated, but the long-term outcome after cessation of treatment is unknown.[267] In addition to podofilox 0.5% (Condylox; Actavis, Parsippany, NJ) solution, a 0.5% gel is also available. It yielded a 45% (81 of 181) complete clearance rate after 8 weeks in a large randomized controlled trial, as opposed to 4% (5 of 93) for the vehicle only.[268]

Imiquimod is an imidazoquinoline amine that induces the production of IFN-α and other cytokines. It appears to exert its unique antitumor and antiviral action by binding to the TLR7, and possibly TLR8, of dendritic cells.[269] It is available as a 5% cream (Aldara; 3M Pharmaceuticals, St. Paul, MN) and, since 2011, as a 3.75% cream (Zyclara; Graceway Pharmaceuticals, Bristol, TN) for the self-treatment of condyloma acuminatum.[270,271] Imiquimod 5% cream was compared with vehicle alone in a randomized double-blind trial and was given three times per week, on alternate days, for up to 8 weeks.[272] At the end of the treatment period, 108 patients were evaluable, and the complete response rate was 37% in the imiquimod group compared with 0% in the control group ($P < .001$). Nineteen percent of the patients had a recurrence during the 10 weeks of follow-up. In a similar study the treatment duration was extended up to 16 weeks, and imiquimod 5% cream was compared with a 1% cream and with vehicle.[273] At the end of treatment the complete response rates were 50%, 21%, and 14% in the three respective groups. Imiquimod 5% cream was significantly superior to either of the two other preparations ($P < .001$). In the 5% imiquimod group, 72% of women had a complete response, compared with 33% of the men. During the 12 weeks of follow-up, recurrences were noted in 13%, 0%, and 10% of the subjects in the three groups, respectively. The adverse reactions were local and included itching and burning sensations, erythema, erosions, and swelling; they were well tolerated. The daily administration of imiquimod 5% cream offers some enhancement of efficacy, mostly in men, but a substantially higher incidence of side effects.[274] Therefore Aldara is approved for three-times-weekly use only. Additional clinical trials have complemented and supported the results of these pivotal studies.[249,275,276]

The 3.75% cream formulation (Zyclara) is designed to be administered daily for up to 8 weeks.[271] This formulation was compared with a 2.5% formulation and a placebo in two randomized clinical trials that included 534 women. At 12-week follow-up, the complete response rates were 43.1% for the 3.75% cream, 35.1% for the 2.5% cream, and 16.1% for the placebo ($P < .003$ when comparing each of two active groups with the placebo). Rates of drug discontinuation for safety reasons were 2.3%, 1.4%, and 0.9%, respectively, with decreasing drug concentration.

Imiquimod also appears to be useful for the treatment of other possibly HPV-related conditions, such as actinic keratoses, basal cell carcinomas, and SCCs in situ.[222,277]

Veregen (Doak, Fairfield, NJ) is a botanical derived from green tea. It contains sinecatechins (polyphenon E), compounds with cytotoxic and apoptotic properties.[278,279] It is available as a 15% ointment that is self-applied three times per day on the lesions until complete disappearance, but for no more than 16 weeks. Three randomized controlled clinical studies have been conducted in men and women with genital warts, with a total of 477 subjects in the 15% ointment arm and 290 controls.[280] In the aggregate the complete clearance rate was 56% with the active compound and 37% in the placebo arm. Efficacy was better in women than men. The side effects were local and included erythema (18%), pruritus (14%), pain (14%), and ulceration (12%).[281] The drug is contraindicated in pregnancy. The red stain of the substance and its frequency of administration are potential drawbacks.

Various provider-applied therapies are available. They can be divided into nonsurgical and surgical treatments, which are as follows.

Podophyllin resin (see previous discussion) is still used widely where cost is an issue, although podofilox 0.5% solution or gel is more effective and safer to use.[260]

Trichloracetic acid (TCA) and, to a lesser extent, bichloracetic acid have been favored by gynecologists for the treatment of genital warts.[282] They can both be used during pregnancy. TCA in a 10% to 90% solution is used topically at weekly intervals. The application is painful and can cause ulcers. The unreacted acid should be removed with talcum powder or bicarbonate of soda. In one comparative trial TCA therapy appeared to be equivalent to cryotherapy, with complete response and relapse rates of 81% and 36%, respectively.[283] Another study was also unable to detect any differences, with complete response rates of 64% for cryotherapy and 70% for TCA.[284] TCA at 50% does not add to the effects of podophyllin alone and is ineffective in the treatment of vaginal and cervical warts.[285,286]

Cryotherapy is administered with a liquid nitrogen spray or cryoprobe. Lesions are frozen every 1 or 2 weeks. Cryotherapy is regarded as an effective treatment, with cure rates in the 50% to 100% range, and it is safe even during pregnancy.[283,287] One comparative study suggested that cryotherapy is more effective than podophyllin but probably less effective than electrosurgery.[288–290] Side effects are tolerable and include burning, which resolves within a few hours, and ulceration, which heals in 7 to 10 days with little or no scarring.

Other surgical techniques are available for the treatment of anogenital warts.[201,249,250] Conventional surgery with scissors offers the advantage of immediate eradication of visible lesions. This technique has been reserved mainly for the treatment of perianal warts, but it can be advantageously applied to other genital warts if they are limited in number. Up to one-third of patients have recurrences, and scarring, typically limited to some skin discoloration, is the most common complication.[291–294] Electrosurgical techniques have often been applied for the treatment of external genital warts, with results probably superior to those of cryotherapy, but scarring may occur.[289,290] Complete response rates of 80% to 90% have been reported with CO_2 laser therapy.[295–297] In a comparative assessment, however, laser therapy was not deemed to be superior to conventional surgery,[292] and subsequent better-designed studies indicated a long-term complete response rate of 19% to 39%.[298,299] Laser therapy is expensive, may require general anesthesia, and is frequently accompanied by pain and scarring.

The availability of lidocaine-prilocaine (EMLA) cream, which should be applied about 1 hour before the procedure, has facilitated local anesthesia before cryotherapy and laser surgery.[300–303]

Two treatments that are now rarely used but still deserve mention are 5-fluorouracil (5-FU) and intralesional IFN. 5-FU, used topically as a 5% cream applied daily, has been reported to have cure rates of 30% to 95%; the best results have been obtained with intraurethral

warts.[135,304,305] In a comparative trial in men, 5-FU appeared to be equivalent in efficacy to podophyllin.[306] In addition, prophylactic activity of 5-FU has been reported for vulvar warts.[307] This drug is not widely used because it often produces substantial pain, ulceration, and, if applied in the urethra, dysuria.[135] Like other antimetabolites, 5-FU is contraindicated during pregnancy.

IFNs have antiviral, immunomodulatory, and antiproliferative properties.[308,309] Encouraging in vitro and preliminary clinical studies were confirmed by four randomized, double-blind trials that showed the efficacy of intralesionally administered IFN-α and IFN-β compared with placebo.[113,310–312] Parenterally administered IFNs have also been evaluated for treatment of condyloma acuminatum but have generally been ineffective.[156–158,313] IFN, in the doses used, has been generally well tolerated. Side effects (influenza-like symptoms, neutropenia, and thrombocytopenia) are usually mild and are seen more frequently with higher doses. Imiquimod, an IFN-α inducer, is a more practical and cheaper substitute for IFN. No published experience is available with pegylated IFNs.

Cidofovir is an acyclic nucleotide that is a potent inhibitor of the DNA polymerase of cytomegalovirus (CMV) and other herpes viruses and is licensed for the intravenous treatment of CMV retinitis. Although HPVs do not possess a DNA polymerase, this compound triggers the apoptosis of HPV-infected cells.[222,314] In a randomized, vehicle-controlled trial of a compounded 1% gel applied daily to genital warts for 5 consecutive days every other week, at 12 weeks the treated group had 47% (9 of 19) complete clearance compared with 0 (0 of 11) in the vehicle group ($P = .006$).[315] Pain, pruritus, rash, erosions, and ulcerations were frequently noted but equally in both groups. The cost, the risk of nephrotoxicity, neutropenia, and carcinogenesis associated with cidofovir, and the absence of long-term data are reservations about this non–FDA-approved treatment.[222]

Although guidelines are helpful, firm recommendations on the proper treatment strategy for condyloma acuminatum are not always possible. The divergent results of several cost-benefit analyses reinforce this point.[260,316–318] Costs may vary widely for a given therapy, recurrences are common, yet long-term outcomes are not well studied, and the significance of the antecedent genital wart history and treatment is poorly known. Furthermore, the importance of factors such as gender, wart location, size, and number is largely unknown with respect to each treatment. Nevertheless, the duration of lesions (>1 year), their number (>10), and their location on dry rather than moist skin are adverse predictors of treatment response.[249,319,320] Treatment response may improve with the discontinuation of oral contraceptive use, pubic hair shaving, and tobacco smoking.[321,322]

In practice, availability, convenience, adverse reactions, location of lesions, and characteristics of the patient are determinant in the treatment choice. Patient-applied therapies should receive preference. Warts of the urinary meatus can be treated with careful application of podophyllin, podofilox,[135] or cryotherapy.[323] 5-FU cream may also be used.[135,324] Laser surgery and instillations of IFN-α can also be used with intraurethral warts.[150,325] Perianal and anal warts may be treated with scalpel removal,[291–293] cryotherapy,[326] laser surgery,[327] trichloracetic or bichloracetic acid,[201] or even, as adjunctive therapy, with imiquimod-soaked anal tampons.[328]

For vaginal warts, cryotherapy (sprays), TCA, and podophyllin are simple options[329]; laser therapy[330,331] and cryotherapy[332] have the advantage of being relatively safe during pregnancy, and they may be used for treatment of cervical warts also. Although intralesional IFN may be indicated for the treatment of single, very large warts, laser therapy seems to be better suited for large, extensive lesions.

Although HPV can be transmitted to the neonate and may lead to the development of recurrent respiratory papillomatosis, the presence of genital warts is not an indication for cesarean section because laryngeal papillomatosis is rare and when the transmission of the infection occurs remains uncertain.[37,201]

The genital warts of immunocompromised patients, including those with HIV, may still spontaneously regress, but they tend to be relatively refractory to treatment and frequently relapse.[333–335] Thus podophyllin, podofilox, intralesional IFN, and imiquimod alone have been largely ineffective.[336–340] Combination therapy appears more successful, such as electrosurgery plus cold-blade excision[336,341] or plus intralesional IFN for anal warts.[342] Imiquimod may also be used as adjunctive therapy.[343] Nevertheless, single therapy, especially for small (<1 cm^2) intraanal lesions, may be effective, as shown with TCA, liquid nitrogen, or the use of an infrared coagulator.[199,344] Lesion healing is generally not a problem after surgery.[345] Further recommendations for the management of HIV patients with HPV infections have been issued jointly by the Centers for Disease Control and Prevention (CDC), the National Institutes of Health, and the Infectious Diseases Society of America (http://aidsinfo.nih.gov/contentfiles/lvguidelines/Adult_OI.pdf), and the New York State Department of Health has issued, in January 2018, its own guidelines for the screening of cervical cancer (www.hivguidelines.org/adult-hiv/preventive-care-screening/cervical-dysplasia-cancer/#tab_0). The effects of active antiretroviral therapy on HPV diseases have been inconsistent but generally modest when present.[346–350]

Because internal genital warts are often associated with genital dysplasias and malignant diseases and because of the special skills and technical resources necessary for proper diagnosis and management, patients with internal lesions should be referred to a qualified specialist.

Other Warts

The lesions of epidermodysplasia verruciformis should be carefully observed, and any malignant changes should be treated with surgical techniques (cold blade or laser), cryotherapy, or 5-FU ointments.[30] Retinoids in combination with intralesional IFN or calciferol help with the management of the lesions of epidermodysplasia verruciformis.[30,351]

The management of recurrent respiratory papillomatosis is complex.[37,180,352–354] For the primary debulking of lesions, most surgeons use the CO_2 laser. Mechanical devices such as a microresector are also used. Photodynamic laser therapy is gaining acceptance. The recurrent nature of the disease requires a careful balance between the risks and benefits of the surgery, which can be achieved only by experienced and skilled operators. Tracheostomy should be avoided because the papillomatosis could then extend to the tracheostomy site and further down the respiratory tree. Radiotherapy is contraindicated because of the known risk of malignant transformation. Different adjuvant therapies are available. Parenteral IFN-α may yield long-term complete responses in a quarter of patients. The interest moved to the intralesional injection of cidofovir.[222,314,355] However, the excellent results of the early case series precluded the completion of a properly designed study.[314] More recently, severe side effects of nephrotoxicity and neutropenia, and a possible oncogenic risk, have tempered the enthusiasm for use of cidofovir in this setting. Indole-3-carbinol (I3C) and its main active metabolite, diindolylmethane, are derivatives of cruciferous vegetables (e.g., broccoli, cabbage, cauliflower) that are widely used by patients with recurrent respiratory papillomatosis. By increasing the 2-hydroxylation of estradiol, these compounds favor the formation of 2-hydroxyestrone, a nonestrogenic, antiproliferative, antiangiogenic, and apoptotic molecule, instead of 16α-hydroxyestrone. A randomized, placebo-controlled clinical trial has shown the ability of I3C to induce regression of biopsy-proven CIN 2 or 3.[356] A similar trial has not been conducted for recurrent respiratory papillomatosis. Oral warts (squamous papillomas, condylomata acuminata, and verruca vulgaris) can be treated with surgical excision, cryotherapy, laser surgery, or podophyllin application.[357] Because of its benign natural history, focal epithelial hyperplasia should not be treated.

PREVENTION AND VACCINATION

At present, no effective methods of prevention for cutaneous warts are available, other than avoiding contact with infectious lesions. In the case of plantar warts, empirical evidence suggested that the wearing of protective foot equipment (verruca socks) would be effective, but more recent work argues that the family environment is the more likely source of infection.[41,358–361]

Male condoms offer an imperfect protection against female acquisition of HPV infection.[362] A prospective study of 82 college-aged women, virgins at enrollment, showed protection against HPV cervical infection in direct relationship with frequency of condom use during intercourse.[363] Two randomized trials make a more dramatic argument in favor of condoms by showing that male condom use for at least 3 months

promoted regression of CIN and clearance of HPV DNA in the female sexual partners and regression of HPV-associated penile lesions in the patient.[364,365] This occurred only in couples with concordant HPV types.[366] Therefore reinfection is clinically important. Other epidemiologic data show that consistent condom use halves the risk of HPV acquisition in males.[367]

Examination of the partners provides an opportunity to educate, counsel, and screen for HPV disease and other STDs.[201]

The Pap smear is an essential tool for the screening and prevention of cervical cancer. The latest guidelines for cervical cancer screening from the American Cancer Society (in concert with the American Society for Colposcopy and Cervical Pathology [ASCCP] and the American Society for Clinical Pathology), the US Preventive Services Task Force, and the American College of Obstetricians and Gynecologists are summarized in Table 143.2.[368–370] Consensus guidelines for the management of the cytologic and histologic abnormalities have been issued by the ASCCP (http://www.asccp.org/asccp-guidelines).[201,205,370] Using the cervix as a model, screening for anal cancer using anal cytology has been proposed for populations at risk, such as HIV-infected individuals who are MSM, who have a history of anogenital condylomas, or women with abnormal cervical and/or vulvar histology.[371,372] Unfortunately, anal cytology alone has poor performance characteristics to detect high-grade anal intraepithelial neoplasia (HGAIN) and needs to be augmented by other, more costly tests.[373] Furthermore, there is presently no study results on the impact of HGAIN treatment on anal cancer mortality in HIV patients and its cost. In consequence, no national anal cancer screening guidelines has been issued.[171,374] It is nevertheless recommended to evaluate any symptomatic patient and to perform digital annual rectal examinations in HIV-positive patients and HIV-negative MSM[201] (https://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf).

Recurrent respiratory papillomatosis of children may be acquired by the infant during passage through the birth canal, as discussed previously. Cesarean section has probably only a limited role, if any, in the prevention of respiratory papillomatosis.[37]

Vaccination

The introduction starting in 2005 of a series of HPV vaccines has marked a great advance in the field of genital HPV diseases and in cancer vaccines in general. These vaccines are all based on VLPs. They are obtained by the expression in eukaryotic vectors of the gene coding for HPV L1, the major capsid protein. The viral proteins self-assemble into noninfectious viral capsids sharing the size, shape, and immunologic characteristics of native, infectious virions. HPV VLPs are capable of inducing neutralizing antibodies at titers sufficiently high and superior to those generated after natural infection, to block HPV infection.[375,376] Protection is entirely dependent on these antibodies and does not rely on cellular immunity.[252,376]

Three HPV vaccines have been licensed. Cervarix (GlaxoSmithKline, London, UK) is made in insect cells and is directed against HPV-16 and HPV-18.[377] This bivalent vaccine is FDA-approved for the prevention of CIN, adenocarcinoma in situ (AIS), and cancer of the cervix associated with these two HPV types. Gardasil (Merck, West Point, PA), which came out first, is made in baker's yeast and is directed at HPV-6, HPV-11, HPV-16, and HPV-18.[378] Since 2016 it has been progressively replaced by Gardasil 9, a nonavalent vaccine that targets five additional high-risk HPV types—31, 33, 45, 52, and 58.[379] Gardasil and Gardasil 9 extend the indications of Cervarix to the prevention of VIN2/3, VAIN2/3, and cancers of the vulva and vagina, and in males to the prevention of AIN and anal cancer. These vaccines differ in their adjuvants. Gardasil and Gardasil 9 contain amorphous aluminum hydroxyphosphate sulfate, whereas Cervarix uses AS04, a combination of aluminum hydroxide and 3-*O*-desacetyl-4′-monophosphoryl lipid A. They are stored by refrigeration but are not frozen, and each dose is given intramuscularly (deltoid muscle) in a volume of 0.5 mL. With the withdrawal of Cervarix, only Gardasil 9 is available in the United States.

Clinical trials, each totaling about 15,000 women aged 16 to 26 years, with CIN2+ and AIS as end point and using a per-protocol population analysis, have shown an efficacy of 98.2% (95% confidence interval [CI], 93.3% to 99.8%) for Gardasil, 42 months after the first dose, and 92.9% (CI, 79.95% to 98.3%) for Cervarix, 35 months after the first dose.[380] The cancer indication has been long supported by the strongly established link between CIN 2/3 and cancer. There is now early direct evidence. In Finland, the incidence of HPV-associated cancers has decreased only in the vaccinated population.[380a] Gardasil also has indications for the protection against VIN/VAIN 2/3, with an efficacy of 100% (CI, 82.6 % to 100%), and against external genital warts, with an efficacy of 99% (CI, 96.2% to 99.2%).[380]

To show that the vaccines are likely effective if administered to children aged 9 or 10 to 15 years, who otherwise are difficult to study because of their very low rate of HPV disease, neutralizing antibody levels were used as a surrogate marker of efficacy and were shown to be higher in boys and girls than in women.[377,378] Intention-to-treat population analyses that allowed the inclusion of women who at entry were not necessarily seronegative and/or HPV DNA negative for the vaccine types showed mediocre or absent vaccine efficacy for disease related to a given HPV type, if the subject was either seropositive or HPV DNA positive for that type.[381,382] However, full efficacy was retained for diseases caused by the other vaccine HPV types. Clearly, vaccination should occur before the onset of sexual intercourse to confer its fullest protection.

Gardasil has now received indications for males up to age 26 years in some populations. In studies conducted in HIV-negative MSM, the vaccine intention-to-treat efficacy was 68% (CI, 48.8% to 80.7%) for external genital warts and 54.2% (CI, 18.0% to 75.3%) against AIN 2/3 caused by HPV-6, HPV-11, HPV-16, or HPV-18. In the HIV-infected population the vaccine is safe and did not affect the $CD4^+$ T-lymphocyte count or the HIV viral load in either adults or children aged 7 to 12 years.[383,384] In these individuals the vaccine induces neutralizing antibodies, albeit at titers about 30% to 50% lower than in non–HIV-infected males. Vaccine efficacy in the HIV population was evaluated in 575 patients (472 MSM and 103 women) aged 27 years or older after immunization with three doses.[385] Although the vaccine was safe and immunogenic, the study was stopped at 2.4 years because it failed to demonstrate benefits regarding persistent anal HPV infections or anal HSIL. This failure was likely caused by the high rates of baseline anal HPV positivity, 60%, and anal HSIL, 33%. This reinforces the need to vaccinate populations early.

Vaccine immunogenicity decreases when administered to older women, both with Gardasil (up to 45 years of age) and Cervarix (up to 55 years of age).[386,387] Gardasil clinical efficacy was also reduced in women aged 24 to 45 years and could only be demonstrated by combining external genital warts and CIN as an end point.[386] Canada has approved Gardasil in women up to age 45 years, but the US Advisory Committee on Immunization Practices has not. This may change now that in October 2018 the FDA has approved Gardasil 9 up to 45 years for both sexes.[386a]

Gardasil 9 registration clinical trials included more than 12,000 women and were done using Gardasil, the quadrivalent formulation, as a control. They showed that, against HPV-6, HPV-11, HPV-16, and HPV-18, Gardasil 9 induced neutralizing antibodies levels comparable to those of Gardasil, which implied equivalent disease-prevention efficacy.[388] Gardasil served as a placebo to assess vaccine efficacy against diseases caused by HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58. Against CIN2+ and all CIN, vaccine efficacy was 96.3% (CI, 79.5% to 99.8%) and 97.7% (CI, 92.2% to 99.6%), respectively. Against vulvar and vaginal diseases, the figures for VIN2/3+ or VaIN2/3+ and all VIN or VaIN were 100% (CI, 71.5% to 100%) and 93.8% (CI, 61.5% to 99.7%), respectively. The efficacy of Gardasil 9 in reducing the number of genital warts or of cervix biopsies was overall 96.9% (CI, 93.6 to 98.6%).[388] Therefore the addition of five strains in Gardasil is expected to broaden the protection not only against cancer but also, and to a greater degree, against the more common CIN1 and CIN2/3, thus offering the prospect of simpler cervical cancer screening guidelines. Full vaccine efficacy is still present for at least 6, 8, and 9 years with Gardasil 9, Gardasil, and Cervarix, respectively.[389–391] A minimal protective antibody threshold has not been established yet.

All the HPV vaccines were originally licensed for a three-dose schedule of immunization. Several studies have shown that a two-dose schedule is as effective and may be advantageous in resource-limited countries.[392–397] It is unknown at this time if beyond 5 years the two-dose schedule will remain effective or if a booster will be necessary.[392]

TABLE 143.2 Summary of Cervical Cancer Screening Guidelines

When to Begin Pap Test Screening	
USPSTF, ACS, ACOG	Age 21 years
How Often? ***Cytology (21- to 65-Year-Olds)***	
USPSTF, ACS, ACOG	Every 3 years, regardless of the cervical cytology technique used
HPV DNA Co-test	
21- to 29-Year-Olds	
USPSTF, ACS, ACOG	No
30- to 65-Year-Olds	
USPSTF	Every 5 years is optional
ACS, ACOG	Every 5 years is recommended
When to Discontinue Screening	
USPSTF, ACS, ACOG	At age 65 years
	INCLUDE:
USPSTF, ACS, ACOG	Women with adequate screening history defined as three consecutive negative cytology results or two consecutive negative HPV DNA co-tests within 10 years of cessation of screening, with the most recent test performed within 5 years
	EXCLUDE:
ACS	Women age 65 years or older with a history of CIN 2, CIN 3, or adenocarcinoma in situ should continue screening for at least 20 years after spontaneous regression or proper management
ACOG	Women with a history of: (a) HIV infection, (b) CIN 2 or higher, (c) immunocompromised, (d) in utero exposure to diethylstilbestrol
Screening After Hysterectomy	
USPSTF, ACS, ACOG	Not necessary if it was a total (uterus + cervix) hysterectomy
Screening Among Those Immunized Against HPV16/18	
USPSTF, ACS, ACOG	No change in the screening guidelines at present
Screening of HIV Seropositive Women CDC/NIH/IDSA ***Women Younger Than 30 Years***	
	• Start within 1 year of onset of sexual activity or at the time of HIV diagnosis, but not later than age 21 years • Screening is done by cytology alone, not by co-testing • If initial testing is negative, repeat 12 (possibly 6) months later • If the results of three consecutive tests are normal, then screen every 3 years
Women Aged 30 Years or More	

	• Start at the age of HIV diagnosis if not started earlier
	• Screening is done either by cytology or co-testing
	• If screening is done by ***cytology***, the testing frequency guidelines as the same as for younger women. If cytology shows more than ASC-US, refer for colposcopy. If cytology shows ASC-US, repeat it in 6–12 months. If the result is ASC-US or worse, refer for colposcopy
	• If screening is done by ***co-testing***:
	  • Both tests (cytology + HPV) are entirely negative, then repeat in 3 years
	  • Cytology is negative but HPV is positive (but not for HPV-16/18), then repeat screening in 1 year. If at that time either test is abnormal, refer patient to colposcopy
	  • Cytology is negative and HPV is positive for types 16 or 18, refer the patient to colposcopy
	  • Cytology is abnormal for ASC-US and HPV is positive or cytology is abnormal for worse than ASC-US, refer the patient to colposcopy. If the cytology is positive for ASC-US and HPV is negative, repeat cytology in 6–12 months. If the result is ASC-US or worse, refer the patient to colposcopy

ACOG, American College of Obstetrics and Gynecology; *ACS,* American Cancer Society; *ASC-US,* atypical squamous cells of unknown significance; *CDC,* Centers for Disease Control and Prevention; *CIN,* cervical intraepithelial neoplasia; *HIV,* human immunodeficiency virus; *HPV,* human papilloma virus; *IDSA,* Infectious Diseases Society of America; *NIH,* National Institutes of Health; *USPSTF,* US Preventive Services Task Force.

From American Cancer Society (ACS)[368]: http://onlinelibrary.wiley.com/doi/10.3322/caac.21139/pdf; US Preventive Services Task Force (USPSTF)[369]: https://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/cervical-cancer-screening; and American College of Obstetrics and Gynecology (ACOG) [370]; Centers for Disease Control and Prevention/National Institutes of Health/Infectious Diseases Society of America (CDC/NIH/IDSA): https://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf.

The effect of HPV vaccination has already been substantial. Even in the United States, where the vaccine uptake has been poor, the cervicovaginal prevalence of the vaccine HPV types has decreased from 11.5% to 4.3% among females aged 14 to 19 years, within 4 years of the vaccine introduction.[398] In Australia, where vaccine uptake has been high, comparing cervical HPV prevalence in 18- to 24-year-olds before (2005–07) and after (2007–09) the introduction of immunization, the adjusted prevalence ratio has been an impressive 0.07 in immunized women and 0.65 in nonimmunized women, which suggests the existence of herd immunity.[399] In Costa Rica vaccination with HPV-16/18 or placebo of 7466 women led to, 4 years later, a prevalent oral HPV-16/18 infection in 1 subject who received the vaccine and in 15 placebo recipients, thus strengthening the expectation of an ultimate reduction of oropharyngeal cancer incidence.[400] More significant, HPV-associated disease incidence has also decreased after HPV vaccination in many population-based studies conducted both in the United States and abroad. A meta-analysis of 20 vaccine studies has shown a greater than 90% decrease of the relative risk of genital warts in women aged 15 to 19 years in countries with vaccine coverage superior to 50%.[401] This decrease was about 30% in countries with less than 50% vaccine coverage. High vaccine coverage was associated with herd immunity in the older age classes of women but also in all age classes of men. There is now

well-established evidence of a beneficial effect of HPV vaccination on the incidence and prevalence of CIN.[402–405] This gives further assurance that in a few years the effect of vaccination on HPV-associated cancers will be demonstrable beyond the early evidence already observed in Finland.[380a]

The HPV vaccines appear to be very safe (https://www.cdc.gov/vaccinesafety/vaccines/hpv-vaccine.html). Local reactions to immunization are common and include pain, redness, and swelling, with the corresponding rates of 89.9%, 34.0%, and 40.0% for Gardasil 9; 83.5%, 25.6%, and 28.8% for Gardasil; and 92.9%, 44.3%, and 36.5% for Cervarix.[406] These differences reflect mostly the nature and quantities of adjuvants. Systemic adverse reactions of any grade include headache, fatigue, and arthralgia in less than half of the recipients.[406] Vaccine safety has also been monitored extensively through several public and industrial surveillance programs in place in the United States and elsewhere.[407,408] Other than the rare occurrence of allergy and anaphylaxis, there has been no consistent evidence that the HPV vaccines increase the rate of autoimmune disorders, neurologic conditions, or thromboembolic events.[407–413] Syncopes may occur after vaccination, mandating a 15-minute period of observation before discharging the patient, but this appears to be an age-related rather than a vaccine-related complication.[408]

The current (May 2018) HPV vaccination guidelines of the CDC's Advisory Committee on Infection Prevention are presented in Table 143.3 (https://www.cdc.gov/vaccines/vpd/hpv/hcp/recommendations.html). Routine immunization of males and females is recommended at 11 or 12 years (it may be started at age 9 years and should be started this early in children with a history of sexual abuse). Vaccination is recommended in females aged 13 through 26 years and in males aged 13 through 21 years who have not been adequately vaccinated previously.[414–416] The vaccination window should begin at age 9 years but can be extended until age 26 years for gay and bisexual men, MSM, transgender persons, and for immunocompromised persons (including HIV infection) not adequately vaccinated previously. For maximum benefits, adolescents should be immunized before exposure to HPV, but previous infection to one or more HPV types still allows full protection against the other types in the vaccine. Two doses of vaccine (the second dose is to be given 6–12 months after the first) are given to most persons starting the series before their 15th birthday. Adolescents who receive two doses less than 5 months apart will require a third dose. Three doses (0, 1–2, and 6 months) are recommended for teens and young adults who start the series at ages 15 through 26 years and for immunocompromised persons (including HIV infection) ages 9 through 26 years. If the immunization series is interrupted, there is no need to restart; one just completes the series. Persons are adequately vaccinated if they previously received Cervarix, Gardasil, or Gardasil 9, before age 15 years as two doses (at 0, 6–12 months) or three doses (at 0, 1–2, 6 months), or at age 15 years or older in three doses (at 0, 1–2, 6 months). Gardasil 9 is the only vaccine available in the United States at present. It is administered intramuscularly.

TABLE 143.3 ACIP Recommendations for HPV Immunization

HPV Vaccine Recommendations

- HPV vaccine is routinely recommended for adolescents at age 11 or 12 yr.
- Vaccination is also recommended for females ages 13–26 yr and males ages 13–21 yr who are not adequately vaccinated when they were younger.
- Vaccination is also recommended for gay, bisexual, and other men who have sex with men; transgender persons; and persons with certain immunocompromising conditions ages 22–26 yr who were not adequately vaccinated when they were young.

HPV Vaccine Safety

- 9-valent HPV vaccine was studied in more than 15,000 males and females.
- Quadrivalent HPV vaccine was studied in more than 29,000 males and females.
- Bivalent HPV vaccine was studied in more than 30,000 females.
- Each HPV vaccine was found to be safe and effective.

ACIP, Advisory Committee on Immunization Practices; *HPV,* human papillomavirus. *From Centers for Disease Control and Prevention (CDC). HPV vaccine information for clinicians. https://www.cdc.gov/hpv/hcp/need-to-know.pdf. Accessed May 22, 2018.*

The contraindications include a severe allergic reaction (e.g., anaphylaxis) to a vaccine component (Gardasil 9 is produced in *Saccharomyces cerevisiae* [baker's yeast]) or after a prior dose of the HPV vaccine. Although the vaccines are contraindicated during pregnancy, both during the clinical trials and the postmarketing surveillance, many women became pregnant, and no excess of congenital malformations or miscarriages has been noted.[417] Gardasil 9 is safe to be administered concomitantly with the other CDC-recommended routine immunizations. HPV vaccination status does not change cervical cancer screening (Pap smear) recommendations.

Cervical cancer screening guidelines are not changed if the woman is vaccinated.

The HPV vaccine is strictly prophylactic and has no impact on the evolution of existing lesions. However, there is evidence that vaccination reduces the recurrence rate of high-grade AIN in males and of HPV-related genital disease in females.[418,419]

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144 JC, BK, and Other Polyomaviruses: Progressive Multifocal Leukoencephalopathy (PML)

C. Sabrina Tan and Igor J. Koralnik

SHORT VIEW SUMMARY

JC VIRUS

Definition
- JC virus (JCV) is a ubiquitous human polyomavirus that causes several central nervous system diseases in immunocompromised patients, including progressive multifocal leukoencephalopathy (PML), JCV granule cell neuronopathy, JCV encephalopathy, and JCV meningitis.

Epidemiology
- JCV infects 40% to 86% of the general population worldwide.
- JCV can be detected in the urine of one-third of healthy and immunosuppressed individuals.
- PML can occur in up to 5% of untreated patients with acquired immunodeficiency syndrome.
- Up to 82% of PML patients are infected with human immunodeficiency virus.

Microbiology
- JCV is a member of the Polyomaviridae family, *Orthopolyomavirus* genus.
- It is a double-stranded DNA virus without an envelope.
- After primary infection, JCV remains latent in the kidney tubular epithelial cells.
- Reactivation of JCV causes a lytic infection of oligodendrocytes in the brain, leading to PML.

Diagnosis
- Definitive Diagnosis of PML
  - JCV DNA is detected in cerebrospinal fluid by polymerase chain reaction (PCR) assay, or JCV proteins are detected in brain tissues through histopathologic assessment.
- Possible Diagnosis of PML
  - Magnetic resonance imaging findings and clinical presentation are consistent with PML in the absence of detection of JCV in cerebrospinal fluid.

Therapy
- There are no effective antiviral medications.
- Immune reconstitution can boost host cellular immune response to better control JC viral replication.

Prevention
- Measures should be taken to prevent immunosuppression.

BK VIRUS

Definition
- BK virus (BKV) is a ubiquitous human polyomavirus that causes hemorrhagic cystitis in hematopoietic stem cell transplantation patients and nephropathy in kidney transplantation recipients.

Epidemiology
- BKV infects 82% to 90% of the general population worldwide.
- BKV can be detected in the urine of up to 20% of asymptomatic healthy individuals.
- BKV nephropathy occurs in up to 10% of kidney transplant patients.
- BKV-associated disease can occur in up to 15.9% of allogeneic stem cell transplant recipients.

Microbiology
- BKV is a member of the Polyomaviridae family, *Orthopolyomavirus* genus.
- It is a double-stranded DNA virus without an envelope.
- After primary infection, BKV remains latent in the kidney tubular epithelial cells.
- Reactivation of BKV causes hemorrhagic cystitis and nephropathy.

Diagnosis
- BKV DNA is detected through PCR assay in blood and sustained viruria in urine.
- Cytopathologic changes may be detected in a kidney biopsy specimen.

Therapy
- There is no effective antiviral medication.
- Reduction in immunosuppression can boost host cellular immune response to better control BKV replication.

Prevention
- Early detection of viral reactivation in either urine or blood with PCR assay is an indicator for a preemptive reduction in immunosuppression to help reduce occurrence of renal disease.

The human polyomaviruses, JC virus (JCV) and BK virus (BKV), are ubiquitous in most human populations throughout the world and do not cause disease in immunocompetent individuals. As much as 90% and 86% of the general adult population are seropositive for BKV and JCV, respectively.[1,2] In individuals with immunosuppression, JCV is the etiologic agent of a demyelinating disease of the central nervous system (CNS)—progressive multifocal leukoencephalopathy (PML)[3,4]—whereas BKV causes nephropathy, hemorrhagic cystitis, and ureteral stenosis. PML is an acquired immunodeficiency syndrome (AIDS)–defining opportunistic infection.[5] BKV nephropathy is a cause of allograft loss in kidney transplant recipients. The polyomavirus family has expanded with the discoveries of new viruses and their associated diseases.

VIROLOGY

History

Polyomaviruses are small (45-nm) nonenveloped viruses that are composed of 72 capsomeres with icosahedral symmetry containing a circular double-stranded DNA. The Polyomaviridae family is composed of three genera: two mammalian genera, *Orthopolyomavirus* and *Wukipolyomavirus;* and one avian genus, *Avipolyomavirus*.[6] The polyomaviruses are ubiquitous in nature and are species specific, including humans (JCV, BKV), monkeys (simian virus 40 [SV40]), and mice (murine polyomavirus). In humans, JCV was first isolated from the brain of a patient with PML whose initials were J.C.[7] Similarly, B.K. were the initials of a kidney transplant patient in whom BKV-associated ureteral stenosis was first described.[8] Since 2007, the Polyomaviridae family has expanded to include newly discovered viruses. These were named after the site of discovery—WUPyV (Washington University) and KIPyV (Karolinska Institute)[9,10]; their geographic origins—MWPyV (Malawi)[11] and STLPyV (St. Louis)[12]; the diseases that they cause—MCV (Merkel cell carcinoma)[13] and TSPyV (trichodysplasia spinulosa)[14]; or their order of discovery—HPyV6, HPyV7, HPyV9, HPyV12, and HPyV 13 (human polyomaviruses 6, 7, 9, 12, 13).[15–17]

Epidemiology

Both JCV and BKV have worldwide distribution, including geographically isolated populations with little exposure to other infections.[18,19]

Although seroprevalence for both BKV and JCV increases rapidly with age,[20] primary infection of each virus occurs independently.[21] Acquisition of both viruses through vertical transmission has been documented.[22] After primary infection, BKV and JCV can be detected in renal tubular epithelial cells, where they may remain. Although JC viruria occurs independent of the host's immune status, JC viremia is usually detected only in individuals with immunosuppression. JCV is detected in the urine of 20% to 30% of healthy and immunosuppressed individuals alike, with or without PML.[23,24] However, JCV viremia has been detected in 20% to 40% of individuals who are human immunodeficiency virus (HIV) positive without PML and in 60% to 80% of patients with PML.[25,26] Conversely, BK viruria occurs in 0% to 20% of asymptomatic immunocompetent individuals,[1] but viral shedding in the urine is higher in individuals with immunosuppression (10%–60%),[27–29] correlating to the degrees of immunosuppression.[30] Specifically, in HIV-positive patients, BK viral load in the urine increases with decreased $CD4^+$ T-cell counts.[31,32] Although BKV is usually not detected in the peripheral blood of patients with either immunocompetence or immunosuppression, the detection of BKV DNA in the plasma of renal transplant patients is indicative of an increased risk of development of BKV-induced nephropathy.[27,33] In a cohort analysis of 491 patients undergoing hematopoietic stem cell transplant (HSCT), investigators reported that BKV-associated disease occurred in 15.9% and was associated with significant and prolonged morbidity.[34] Patients who underwent reduced-intensity, double umbilical cord blood cell transplantation had BK viremia frequently detected (15/27) within 100 days of transplantation.[35]

Genome

Polyomaviruses have a circular double-stranded genomic DNA of approximately 5 kb. The genomes of BKV and JCV have been used extensively in human population migration studies because of their extremely conserved coding regions. JCV and BKV share approximately 70% genome homology,[36] and all polyomaviruses maintain the feature of coding DNA from both strands. The early proteins, large T and small t, are transcribed counterclockwise from one strand, whereas the late proteins, VP1, VP2, VP3, and agnoprotein, are transcribed clockwise from the opposite strand (Fig. 144.1). The genome can be divided into three distinct areas: (1) the early genes region, which includes the large T and small t antigens, regulatory proteins responsible for viral transformation, replication, and regulation of gene expression; (2) the late genes region, which encodes the capsid proteins VP1, VP2, and VP3 and the agnoprotein; and (3) the noncoding regulatory region. The agnoprotein is important both in regulation of JCV transcription and translation and in dysregulation of host cell cycle and DNA repairs.[37,38] Similar to the origin of DNA replication, the noncoding regulatory region also contains several binding sites for nuclear factors, which play important roles in JCV transcription. Deletions, insertions, and rearrangements in the regulatory region are associated with tissue tropism and virulence. The archetype regulatory region is mainly found in JCV isolated from kidney cells and consequently from urine. It is thought to be the type from which all other JCV regulatory region sequences have evolved. The rearranged type of regulatory region of JCV is principally detected in the brain and cerebrospinal fluid (CSF) of patients with PML.[39] Whereas the rearranged type of regulatory region is found in the peripheral blood of patients with PML, a mixture of both types is present in the bone marrow.[25,40] Which type of regulatory region is associated with primary infection is not known.

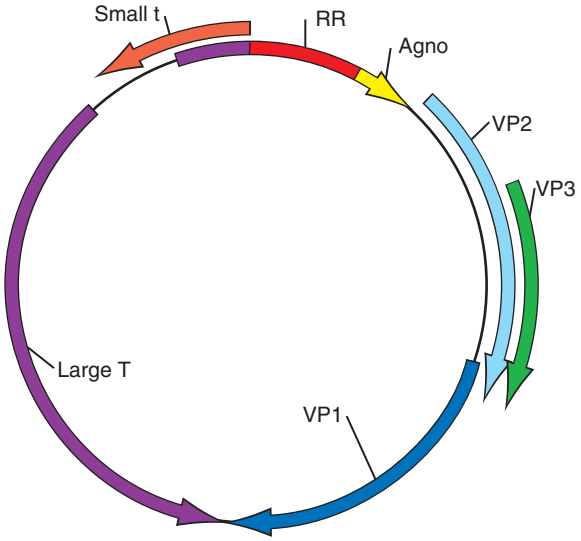

FIG. 144.1 Polyomavirus genomic map (~5 kb). *RR,* Regulatory region. *(Courtesy Dr. Yiping Chen.)*

Receptors and Cell Entry

JC Virus

Data suggest that *N*-linked glycoprotein containing an α(2,6)-linked sialic acid may act as a JCV receptor.[41] In addition, evidence from cultured cell lines shows that JCV uses the serotonergic 5-HT2A receptors for cell entry.[42] Pharmacologic blockade of the serotonergic receptors restricts JCV infection in vitro. The 5-HT2A receptor is present on glial cells and astrocytes and on B lymphocytes, platelets, and kidney epithelial cells. JCV also uses clathrin-coated pits on cell surfaces after binding and adsorption, mediated by the capsid protein VP1. Cell entry occurs via endocytosis and results in fusion of virus-carrying vesicle with the nuclear membrane.[43]

BK Virus

BKV uses an *N*-linked glycoprotein containing an α(2,3)-linked sialic acid as a receptor. Entry into the cell occurs via caveolae-mediated endocytosis.[44]

New Human Polyomaviruses

Seroprevalences for the newer human polyomaviruses range from 25% to 90%.[15,45–48] The KIPyV and WUPyV polyomaviruses were first isolated from respiratory samples with high-throughput screens.[10,49] Subsequently, molecular evidence indicated a worldwide distribution for both viruses in the respiratory secretions of children, with incidence rates as high as 2.5% in Australia[50] for KIPyV and 7% in South Korea[51] for WUPyV. A study analyzing nasopharyngeal aspirates in the United Kingdom showed a bimodal age distribution of KIPyV and detection of the WUPyV in samples from patients younger than 15 years old.[52] Although both WUPyV and KIPyV could be colonizers of the respiratory tracts, KIPyV was associated with respiratory symptoms in transplant recipients.[53] HPyV6, HPyV7, MWPyV, and STLPyV were also all recovered from nonsterile sites such as skin and stool. Therefore the proof of their existence as bona fide infectious agents remains to be established. Like KIPyV, these viruses could be part of the human microbiota that causes problems only in immunosuppressed patients. For example, HPyV7 was found to be the cause of a pruritic rash in a lung transplant recipient.[54] Lastly, the detection of HPyV9 in normally sterile blood distinguished this virus as a possible human pathogen.

The Merkel cell polyomavirus (MCV), discovered in 2008, and trichodysplasia spinulosa polyomavirus (TSPyV), discovered in 2010, are both associated with human skin diseases. MCV can be detected in up to 80% of tissues with Merkel cell carcinoma, an aggressive and deadly neuroectodermal tumor that principally affects patients with immunosuppression.[13] Molecular studies indicate that MCV shares a similar genome with the other human polyomaviruses and that integration of MCV into the tumor genome most likely takes place before clonal expansion of tumor cells. MCV is most similar to the African green monkey lymphotropic polyomavirus. TSPyV viral particles were detected with electron microscopy on a hair follicle from a heart transplant patient with trichodysplasia spinulosa, a rare skin disease affecting immunocompromised patients.[14]

Interesting to note, unlike JCV and BKV, these newer human polyomaviruses do not contain a gene that encodes the agnoprotein.

The agnoprotein was initially named because its function was unknown. However, this protein appears to have important regulatory functions of both polyomaviruses and host cells. The significance of the agnoprotein deletion in these polyomaviruses remains to be determined.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY AND OTHER JC VIRUS–ASSOCIATED SYNDROMES

Epidemiology

PML was initially described in 1958.[55] The disease was estimated to occur in 0.07% of individuals with hematologic malignant diseases,[56] such as leukemia and lymphoma, or rarely in patients with solid-organ cancer or organ transplants. Since the 1980s, in the era of HIV infection, PML has been recognized as a major opportunistic infection that affects up to 5% of patients with AIDS.[5] Despite the advent of combined antiretroviral therapy (ART), PML still occurs in 0.6 to 1.3 per 1000 HIV-positive person-years.[4] Currently, 82% of patients with PML have AIDS, 8.4% have hematologic malignant diseases, 2.8% are solid-organ transplant recipients, 0.95% have chronic inflammatory diseases, and 6% have no defined risk factor for PML.[4] Indeed, 38 cases of PML have been reported in HIV-negative individuals with minimal or occult immunosuppression.[57] In HIV-negative individuals, the incidence rate of PML per 100,000 person-years is 0.3 in the general population, 1.0 in patients with rheumatoid arthritis, 2.4 in those with systemic lupus erythematosus, 10.8 in those with autoimmune vasculitis, 8.3 in those with non-Hodgkin lymphoma and chronic lymphocytic leukemia, and 35.4 in bone marrow transplant recipients.[58] Lastly, the newest group of patients with PML has emerged among patients treated with immunomodulatory medications for malignant diseases or autoimmune diseases, such as patients treated with natalizumab and dimethyl fumarate for multiple sclerosis (MS) or Crohn disease,[59–63] rituximab for lymphoma[64,65] or lupus,[66] and efalizumab or fumaric acid for psoriasis.[67,68]

Natalizumab-treated MS patients are at increased risk of developing PML. As of November 30, 2017, 756 cases of natalizumab-related PML had been documented worldwide. Risk of PML increases in patients with longer natalizumab treatment duration, prior use of other immunosuppressants, and seropositivity for JCV. The incidence of natalizumab-associated PML is highest after 49 to 72 months of treatment in JCV-seropositive patients with a prior history of immunosuppressive treatment, where it reached 1.3%.[69]

Pathogenesis

Primary infection with JCV is asymptomatic, after which the virus becomes clinically latent. JCV DNA is detected with polymerase chain reaction (PCR) assay in the tonsillar tissues of 39% of healthy individuals, which suggests a possible oral or respiratory route of infection.[70,71] Detection of JCV in urine of healthy adults indicates that the kidney is a site of latency. Immunohistochemical staining shows viral proteins in the kidney tubular epithelial cells. Detection of JCV DNA in the bone marrow[72] and brain[73] of patients without PML suggests that these are also possible additional sites of latency.

In patients with PML, JCV infects oligodendrocytes and astrocytes in the brain and rarely the spinal cord, which causes a lytic infection that results in the destruction of the myelin sheath. Pathologic examinations of PML lesions show extensive demyelination of the affected areas. Because of the presence of oligodendrocytes and astrocytes in the hemispheric cortex, demyelination can also occur within the cortical gray matter.

Clinical Manifestations

Classic Progressive Multifocal Leukoencephalopathy

Because PML causes multifocal demyelination of the white matter of the CNS, the resulting neurologic deficits correspond to the location of the lesions. Although initial symptoms of PML can vary greatly, depending on the affected anatomic brain region, the predominant symptoms include coordination difficulties, gait imbalance, cognitive dysfunction, visual problems, and limb paresis. The optic nerves and the spinal cord of the CNS are usually spared, but incidental postmortem findings of PML lesions have been discovered in the spinal cord of a patient who was HIV positive with hemispheric PML.[74] Furthermore, although PML lesions are generally located in the white matter, seizures, which are usually considered to be of cortical origin, can occur in up to 44% of PML survivors and are associated with juxtacortical demyelinated lesions and with hyperintense cortical signal (HCS) demonstrated on T1-weighted magnetic resonance images. The histologic correlate of HCS is leukocortical encephalitis, which recapitulates the triad of PML (demyelination, astrogliosis, and infiltration with phagocytic macrophages, albeit in the cortical gray matter) (Table 144.1).[75–77]

Progressive Multifocal Leukoencephalopathy–Immune Reconstitution Inflammatory Syndrome

Inflammatory PML has been frequently detected when the immune response is rapidly restored with ART in patients with HIV. Patients usually have an increasing $CD4^+$ cell count and a decreasing plasma HIV viral load. Unlike classic PML, up to 57% of the PML–immune reconstitution inflammatory syndrome (IRIS) lesions may display contrast enhancement with magnetic resonance imaging (MRI),[4,78] which indicates a breakdown of the blood-brain barrier, along with worsening of the initial presenting symptoms. Conditions may stabilize after the initial worsening of symptoms, but fatal outcome has been reported.[79,80] Magnetic resonance spectroscopy (MRS) helped define a unique metabolic pattern in PML lesions that showed an increased ratio of lipids to creatine consistent with inflammation. Combined with the presence of contrast enhancement in PML lesions, this finding yielded a 79%

TABLE 144.1 JC Virus–Associated Diseases

CLINICAL PRESENTATION	CLASSIC PML	PML-IRIS	JCV GCN	JCV E	JCV MENINGITIS
Onset	Subacute	Immune recovery	Chronic	Subacute	Chronic
Magnetic resonance imaging	Asymmetrical, well-demarcated, nonenhancing subcortical white matter lesions; hyperintense on T2-weighted and FLAIR images; hypointense on T1-weighted images	Contrast-enhancing lesions and possible mass effect	Cerebellar atrophy	Cortical lesions	Enlarged ventricles
Neurologic symptoms	Based on location	Based on location and inflammation	Cerebellar syndrome	Encephalopathy	Headache
Histology	Demyelinating lesions often at gray-white matter junction; JCV detected in enlarged oligodendrocytes; bizarre astrocytes; presence of $CD8^+$ T cells near JCV-infected cells	Demyelination similar to classic PML but with marked inflammatory infiltrates	Focal areas of cell loss in granule cell layer of cerebellum; JCV detected in enlarged granule cell neurons	Focal areas of cell loss in hemispheric cortex; JCV detected in enlarged pyramidal neurons	Productive infection of meningeal and choroid plexus cells

E, Encephalopathy; *FLAIR,* fluid-attenuated inversion recovery; *GCN,* granule cell neuropathy; *IRIS,* immune reconstitution inflammatory syndrome; *JCV,* JC virus; *PML,* progressive multifocal leukoencephalopathy.

probability of IRIS compared with 13% in the absence of these criteria.[81]

In autopsy samples of HIV-positive patients with PML-IRIS, mainly $CD8^+$ lymphocytes were detected in the lesions, along with a paucity of $CD4^+$ lymphocytes.[80,82,83] The reported time at onset of PML-IRIS ranged from 4 to 108 weeks after start of ART.[79,80] In some patients, ART was started as treatment for PML[79,80,82–86]; in others, PML was diagnosed at the time of IRIS presentation.[79,87]

PML-IRIS has also been reported in HIV-negative patients, such as in natalizumab-treated MS patients after plasma exchange to remove natalizumab.[88]

JC Virus Granule Cell Neuronopathy

Demyelination of white matter in the cerebellum is well described in patients with PML. In addition to oligodendrocytes and astrocytes, JCV can also infect and destroy cerebellar granule cell neurons. This neuronal infection can result in a novel syndrome characterized by cerebellar atrophy, gait ataxia, and incoordination, without associated demyelination.[89] This novel syndrome, distinct from PML, is called JCV granule cell neuronopathy (GCN).[90,91] JCV GCN appears to be caused by a JCV variant harboring a 10-bp deletion in the carboxyl terminus of the *VP1* gene.[92] A histologic survey indicated that infection of granule cell neurons may be found in up to half of patients with PML.[93] This finding was confirmed in other JCV GCN isolates from various geographic origins, and other mutations occurring in this area of the *VP1* gene were characterized, strengthening its association with infection of GCN.[4,94] More recently, JCV GCN has also been diagnosed in natalizumab-treated MS patients.[95,96]

JC Virus Encephalopathy

JCV can also infect hemispheric cortical pyramidal neurons. The clinical presentation differs from both classic PML and JCV GCN. Brain lesions are initially restricted to the gray matter at MRI, and the patient presents with a global cognitive decline and aphasia, rather than with focal neurologic deficits such as sensory or motor dysfunction.[97] The JCV strain isolated from cortical samples had a unique deletion in the agnoprotein gene.[4,98]

This deletion impairs VP1 production and virion expression, which may explain a restrictive infection of JCV in cortical pyramidal neurons.[99,100]

JC Virus Meningitis

Although JCV is not routinely tested for in the CSF of patients with meningeal symptoms, several studies have documented JCV as the only pathogen present in the CSF of patients with typical meningitis symptoms, including neck stiffness and diplopia.[101–103] Whether these cases result from JCV primary infection or reactivation is unclear. A case report of JCV meningitis in an HIV-negative patient had reported productive JCV infection of meningeal and choroid plexus cells with limited infiltration of the cerebral parenchyma.[104,105] Because JCV PCR is not routinely performed in the CSF of patients with meningitis who do not have CNS lesions, the exact incidence of JCV meningitis is unknown.

Diagnosis

The diagnosis of PML can be established through either histopathologic or clinical pathways. Whereas the first requires demonstration of JCV infection in the brain, the latter is based on clinical and radiologic findings consistent with PML and not better explained by other disorders, coupled with demonstration of JCV DNA by PCR in CSF. Diagnostic classification includes certain, probable, possible, and not PML.[106]

Because PML remains a relatively rare disease, it is often misdiagnosed, with a median time from initial symptoms to diagnosis of 74 days.[107]

Imaging

Radiographically, PML brain lesions typically appear as multiple white matter lesions, usually sparing the cortex, that do not conform to particular vascular territories. No associated mass effect or contrast enhancement is generally seen. Both computed tomography (CT) and MRI can be used in the diagnosis of PML. Lesions appear in the white matter as hypodense or patchy on CT scans; at MRI, they are areas of hyperintense (bright) signal on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images and show as hypointense (dark) signal on T1-weighted images. MRI is more sensitive than CT and is the imaging method of choice for diagnosis of PML. As the name *progressive multifocal leukoencephalopathy* suggests, multiple lesions are usually present; they are often located in the subcortical white matter or in the cerebellar peduncles.[108,109] Lesions may also be found in gray matter structures, such as the basal ganglia or thalamus, which also contain myelinated fibers. Furthermore, HCS can also be seen overlying white matter lesions of PML.[77] In addition, PML may have atypical presentations, including unifocal and miliary patterns.[110,111] Although features of MRI appearance are not linked to survival time, the rare detection of mass effect predicts poor prognosis.[112] Lastly, atypical PML lesions, including ones with contrast enhancement, are seen in natalizumab-associated PML[113] and in inflammatory PML that results from IRIS (Fig. 144.2).[79]

Brain Biopsy

Brain biopsy is the gold standard for the diagnosis of PML. This test has a sensitivity that ranges from 64% to 96% and a specificity of 100%. Histologic examination shows demyelinated areas, with reactive gliosis and enlarged and bizarre astrocytes, and macrophages that contain phagocytosed myelin and cellular debris. These lesions are located in both cortical and subcortical regions of the brain.[114] JCV can be detected in infected oligodendrocytes with enlarged amphophilic nuclei located at the periphery of the lesions. Intraparenchymal and perivascular infiltrates by $CD8^+$ T cells are usually present in PML-IRIS (Fig. 144.3).[80]

Cerebrospinal Fluid Polymerase Chain Reaction Assay

Brain biopsy may not be feasible in some patients, depending on the location of the lesions and the disability of the patient. In addition, biopsy carries an inherent morbidity and mortality risk. Therefore CSF examination is more commonly used as an alternative method of diagnosis. Patients with PML generally have a nonspecific CSF profile, including mild pleocytosis, with slightly elevated protein and normal glucose levels. The detection of JCV DNA with PCR assay of CSF had a sensitivity of 72% to 92% and a specificity of 92% to 100%[115] before extensive availability of ART. However, in the post-ART era, the sensitivity of PCR detection of CSF has decreased significantly to 58% with no changes in the specificity in patients with HIV presenting with PML while on ART.[116]

Prognosis

PML is a fatal disease in most cases. Before the ART era, only 10% of patients who were HIV positive with PML lived longer than a year after diagnosis.[117,118] After the extensive use of ART, the 1-year survival rate has increased to 50%.[118] A number of studies have delineated several prognostic markers, which include patients who present with a lower JCV CSF burden,[119] detectable JCV-specific immune response in blood and CSF,[120–123] and development of inflammatory immune response in the CNS.[124,125] Because positive JCV serology is detected in most individuals, including those with PML, the humoral immune response alone is not able to prevent viral reactivation. However, cellular immune response to JCV has been detected in patients with PML, and a strong response is associated with better prognosis. Studies have documented the presence of cytotoxic T lymphocytes (CTLs) that recognize two human leukocyte antigen (HLA) A*0201–restricted epitopes of the major capsid protein VP1 in patients with PML who survived more than 1 year.[121,126–128] Presence of JCV-specific CTLs was associated with an increase in 1-year survival of PML from 46% to 73%.[129] In a study of 66 patients, PML survivors were 22.2 times more likely to have both $CD4^+$ and $CD8^+$ T-cell responses against JCV early after disease onset than PML progressors.[130] Furthermore, elevation of myoinositol, a marker of inflammation detected with MRS in PML lesions, was associated with a better prognosis.[125,131] Finally, hyperperfusion within PML lesions, measured with arterial spin labeling MRI, was associated with a worse prognosis.[132]

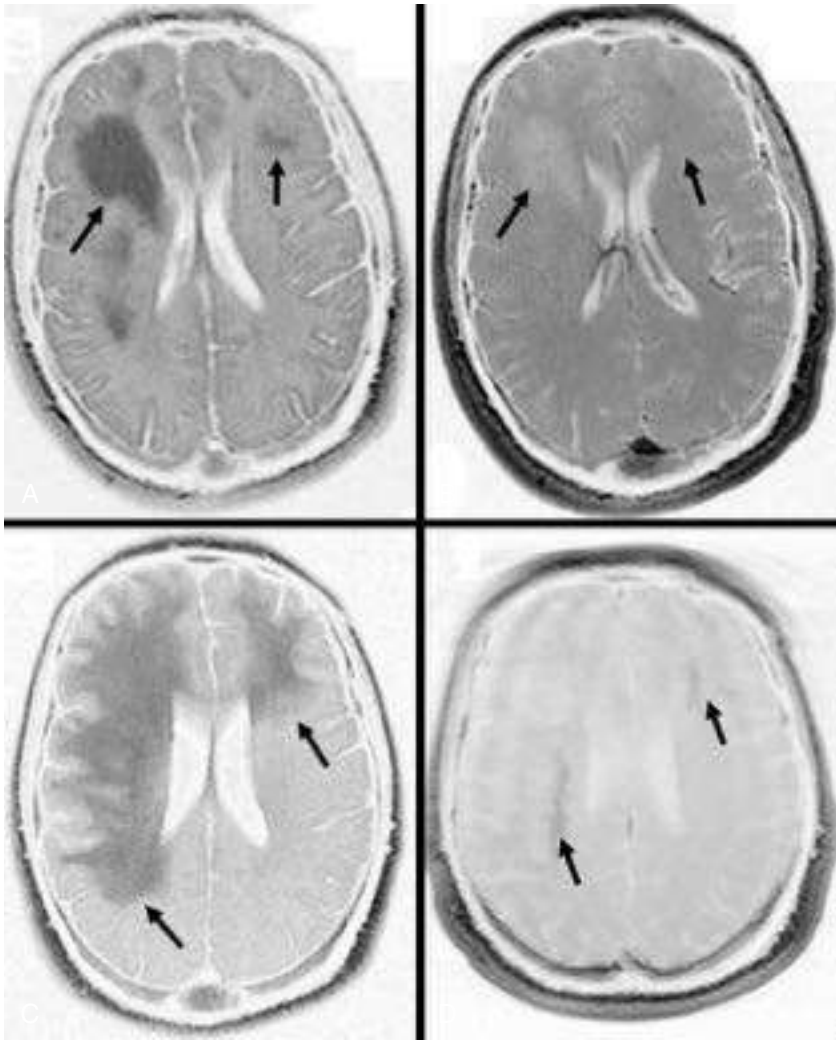

FIG. 144.2 Magnetic resonance images of patient with human immunodeficiency virus infection with classic progressive multifocal leukoencephalopathy at presentation. (A) Fluid-attenuated inversion recovery (FLAIR) image shows lesions in right and left frontal lobes *(arrows).* (B) No enhancement is evident after gadolinium injection on a T1-weighted image *(arrows).* (C) FLAIR image after antiretroviral therapy shows progressive multifocal leukoencephalopathy–immune reconstitution inflammatory syndrome displaying progression of lesions in both hemispheres *(arrows).* (D) Peripheral enhancement can be seen in T1-weighted image after gadolinium enhancement *(arrows).*

Therapy

No specific treatment exists for PML. However, in patients infected with HIV, initiation or optimization of ART has been associated with an increase in PML survival rate at 1 year from 10% to 50%.[118] For patients who are HIV negative, improvement of the immune status through reduction of immunosuppression may allow for the adaptive immune system to take control of the infection. Because a strong cellular immunity against JCV has been associated with a better clinical outcome, immunotherapies aimed at boosting this immune response may become a treatment option for PML.[133] Immune reconstitution after allogeneic HSCT has been associated with selective control of JCV reactivation.[134] Cytarabine has shown an in vitro effect in controlling JCV replication and multiplication.[135] One randomized controlled clinical trial with patients who were HIV positive with PML did not show efficacy,[136] but one small retrospective study did show stabilization after intravenous treatment with cytarabine[137] in 7 of 19 HIV-negative patients (37%) with PML who had leukemia or lymphoma. Cidofovir is another potent antiviral agent that has shown in vitro activities against murine polyomavirus and SV40, but it has not been tested against JCV or BKV. However, multiple studies in different centers with cidofovir in patients both with and without HIV have not shown a significant effect in changing the disease course.[118,138–140] Lastly, because in vitro studies revealed that JCV infects cells via the serotonin receptor 5-HT2A, mirtazapine, a serotonin receptor blocker, has been considered as a potential candidate for containment of JCV infection.[42] However, clinical improvement of PML with mirtazapine remains anecdotal so far.[141] A large pharmacologic screening study suggested that mefloquine, an antimalarial medication, has the ability to inhibit JCV replication in cell culture.[142] A multicenter study examining the role of mefloquine in the treatment of PML was terminated ahead of schedule because of lack of efficacy.[143]

Immunotherapy against JCV is a potential future option. This includes administration of interleukin 7 (IL-7) which boosted both $CD4^+$ and $CD8^+$ T-cell counts in a patient with idiopathic lymphocytopenia who survived PML.[144]

Another option is adoptive transfer therapy, in which virus-specific T cells are stimulated either from patient's own lymphocytes or from a matching donor's lymphocytes. Broadly neutralizing antibodies against multiple JCV VP1 capsid mutations could potentially be infused as therapy.[144a] JCV-specific vaccine against a specific strain was given to a PML patient and induced cognate neutralizing antibodies.[145]

In treatment of patients with inflammatory PML, in whom brain swelling and mass effect can be present, a short course of corticosteroids including prednisone, dexamethasone, and methylprednisolone may be used sparingly for mitigation of the inflammation surrounding the



FIG. 144.3 Histologic features of JC virus (JCV) infection in the central nervous system. (A) Classic progressive multifocal leukoencephalopathy (PML), with demyelinating lesion in white matter *(arrow)* surrounded by multiple JCV-infected glial cells *(dark blue, arrowheads).* (B) PML–immune response inflammatory syndrome, with marked lymphocytic perivascular infiltrates *(arrows).* (C) JCV granule cell neuronopathy in a patient with human immunodeficiency virus infection with focal JCV infection of granule cell neurons *(dark blue, arrows).* (D) JCV encephalopathy with hemispheric cortical neurons *(brown)* infected with JCV *(blue, arrows). (A, C, and D courtesy Dr. Christian Wühtrich; B courtesy Dr. Françoise Gray.)*

lesion, especially with a risk for brain herniation. However, the use of these medications in PML-IRIS cases remains a matter of debate.[146,147] In patients with HIV, no evidence exists that interruption of ART during corticosteroid treatment is beneficial. In published case studies, most clinicians chose to continue ART after the diagnosis of PML-IRIS. Short-term interruption of ART can be used temporarily to reduce the uncontrolled immune reaction. However, clinicians need to be aware that disruptions in ART have been associated with higher HIV viral load rebound and lower CD4 counts.[148,149] Furthermore, whether a recurrence of IRIS may develop when ART is restarted is also unclear. Corticosteroids, including dexamethasone, prednisone, and hydrocortisone, have been used anecdotally for their antiinflammatory and immunosuppressive effects.[150] However, no clear guidelines exist for corticosteroid treatments of patients with PML-IRIS. Therefore these medications should be used only in cases with clear clinical or radiologic worsening attributable to IRIS, including life-threatening situations associated with cerebral edema and impending brain herniation.

NEPHROPATHY AND OTHER BK VIRUS–ASSOCIATED DISEASES

Epidemiology

Although BKV-induced nephropathy rarely occurs in a native kidney,[151,152] the prevalence rate of this condition in kidney transplant recipients ranges from 1% to 10%.[153,154] Diagnosis is made an average of 44 weeks after transplantation, with a peak around 24 weeks.[27] With the use of potent immunosuppressive medications, such as tacrolimus and mycophenolate, a trend has been seen toward increasing prevalence of BKV-induced nephropathy in renal transplant patients.[155,156] Incidences of BK viremia and viruria decrease significantly 1 year after transplantation, diminishing the risk for BKV reactivation.[157] In addition, the incidence rate of BKV-induced ureteral stenosis in renal transplant patients is estimated to be 3%.[158] Lastly, hemorrhagic cystitis is the most prevalent BKV-associated complication and occurs in 10% to 25% of bone marrow transplant recipients.[159,160]

Pathogenesis

BKV primary infection occurs in childhood and is asymptomatic. Thereafter, the virus remains latent in the kidney. Most cases of asymptomatic BK viruria are not associated with nephropathy or hemorrhagic cystitis. Therefore BKV pathogenesis is the result of several factors, including host predisposition, target organ damage, and immunosuppression. During the initial posttransplant period, severe therapeutic immunosuppressive conditioning regimens are a trigger for reactivation of BKV in renal cells. Such reactivation may lead to productive infection and lysis of kidney tubular epithelial cells. Although the ability of the host to mount BKV-specific immunity can be limited by immunosuppressive medications, the resulting cell lysis and necrosis mediated by the immune cells may trigger further renal dysfunction. In patients with renal transplantation, BK nephropathy is associated with risk factors including age, sex, race, BK serostatus of the recipients, ABO incompatibility, and types of immunosuppression used.[161–164] In HSCT patients, BKV seropositivity, older age, high anti-BKV immunoglobulin G (IgG) levels before transplant, and lower total lymphocyte count have been associated with increased risk for BKV reactivation.[165,166] Furthermore, in the late engraftment period, the recovery of immune function may produce additional injury from inflammation caused by immune reconstitution, resulting in hemorrhagic cystitis.[167] Lastly, alloimmune dysregulation may play an important role because BKV-induced hemorrhagic cystitis is rarely detected in autologous transplant recipients who received similar conditioning regimens.[168]

Clinical Manifestations

Nephropathy

BKV has a tropism for cells of the genitourinary tract. Infection with BKV in patients with immunocompromise can result in asymptomatic

hematuria, both hemorrhagic and nonhemorrhagic cystitis, and ureteral stenosis. Furthermore, BKV can induce interstitial nephritis in patients with HIV or renal transplantation. However, in patients with a renal allograft, BKV-induced renal tubular epithelial infection results in nephropathy, which is not associated with any specific immunosuppressive medication. BK viruria and viremia usually precede nephropathy. The factors associated with detection of BKV in urine and blood include high donor BK antibody titer and, possibly, absence of the HLA-C7 class I allele in both donor and recipient.[169] In addition, a higher risk for development of BKV nephropathy is associated with older age, male gender, comorbidity of diabetes mellitus, white race, and placement of ureteral stent.[154,170] Onset of BKV nephropathy after transplant ranges from 6 days to 5 years, with a mean time of 10 to 13 months.[171,172] Clinical manifestations of BKV nephropathy are similar to that of graft rejection, with slowly increasing serum creatinine levels without symptoms. Hematuria and fever can be detected in some patients.[173,174] Laboratory findings suggest renal insufficiency and urinary abnormalities. A rise in quantitative BKV serology can be seen with reactivation of the virus; however, this increase does not prevent progression of disease.[169]

Ureteral Stenosis

Renal transplant patients with ureteral stenosis do not usually present with pain or discomfort because the transplanted kidney is not innervated. However, patients can present with urinary obstruction and laboratory findings of elevated serum creatinine levels.

Hemorrhagic Cystitis

BK viruria in HSCT patients is associated with increased mortality.[175] Because of BKV tropism for renal urinary tract cells, up to 50% of HSCT patients develop BK viruria within 2 months of transplant,[36,159] usually after engraftment.[176] The rate of BK viruria is no different between allogeneic versus autologous grafts.,[177] but only a portion of those with viruria develop hemorrhagic cystitis (10%–25%), ureteral stenosis, and interstitial nephritis. Furthermore, most cases of hemorrhagic cystitis occur in allogeneic HSCT recipients who also have graft-versus-host disease,[168] which indicates that immune reconstitution is part of the pathogenesis. Myeloablative conditioning regimen and graft-versus-host diseases have been identified as risk factors that predisposeHSCT patients to BK viruria and hemorrhagic cystitis.[178–180] Diagnosis of BKV-induced hemorrhagic cystitis is considered when postengraftment HSCT patients develop hematuria, dysuria, urgency, frequency, or suprapubic pain. Severe bleeding and clot formation can lead to complications, including urinary tract obstruction and renal failure.

Infections Outside the Renal System

BKV has been reported to disseminate to extrarenal organs. Multiple case reports have described encephalitis in immunosuppressed patients with detection of BKV in their CSF and brain tissues with PCR assay.[181–185] Furthermore, BKV-associated pneumonia and pneumonitis are also reported in HSCT patients after hemorrhagic cystitis.[186–188] Detection of BKV in the urine has also been associated with genitourinary tumors.[189]

Diagnosis

Urine Polymerase Chain Reaction Assay

In the appropriate clinical setting, detection of BK viruria and renal insufficiency is diagnostic of BKV-induced nephropathy. Detection of BKV in urine often precedes viral detection in the blood, and sustained viruria, defined as detection in two or more consecutive urine samples, can be 100% sensitive and 94% specific for BK viremia.[190] Urine cytologic evaluation that reveals decoy cells (enlarged nucleus with a single large basophilic intranuclear inclusion) is an indication of viral infection, but is not specific to BKV infection because these cells are also associated with CMV and adenovirus infections.[191,192] Although detection of BKV DNA with PCR assay in the urine of patients with hemorrhagic cystitis is sensitive, it is nonspecific because asymptomatic BK viruria is common. However, the presence of an increased BK viral load in urine along with hematuria supports the diagnosis.

Renal Biopsy

Renal biopsy is essential in the diagnosis of nephropathy. Histologic examination of renal biopsy shows viral replication in the tubular epithelium cells with large intranuclear inclusions and cell detachment (Fig. 144.4).[193] These cytopathic changes are initially localized to the medulla and distal tubules, with progression to the proximal tubules. However, renal biopsy is associated with a false-negative rate of up to 30% because of the focal nature of the disease.

Plasma Polymerase Chain Reaction Assay

BKV DNA can be detected in the plasma of kidney transplant recipients.[194] However, this test is more useful in ruling out BKV nephropathy than in diagnosing it. BKV PCR in blood has a negative predictive value of 100% but a positive predictive value of only 50% for BKV nephropathy.[36] One study, however, indicated that plasma BK viral load above 10^4 copies/mL has a sensitivity and specificity of 93% in prediction of histologic manifestations of nephropathy.[195] Unlike in nephropathy, BKV is usually not detected in the blood of patients with hemorrhagic cystitis or ureteral stenosis. However, one study found that BK viremia in HSCT patients is an independent predictor for development of post-HSCT renal impairment.[196]

Prognosis

BKV-induced nephropathy is associated with irreversible graft failure in 1% to 10% of cases.[70] Histologic findings of mild viral cytopathic changes with minimal inflammatory infiltrates or fibrosis correlated with better prognosis for allograft outcome.[195] Studies have shown[197] decreased incidence of nephropathy with regular and early screening for BK viruria, viremia, and urine cytologic detection of virus-infected decoy cells and subsequent reduction of immunosuppressive agents.[33,198–207] Poor outcome from BKV–induced nephropathy is influenced by higher BKV serum viral load, immunosuppression with tacrolimus, and delayed diagnosis.[208] In post–renal transplant patients with BKV reactivation, those patients with BKV-specific cellular responses curtail viral replication within a median duration of 1 month without other interventions.[209] Furthermore, in patients who developed BKV nephropathy, a robust BKV-specific cellular immune response correlates with decreased viruria and viremia, whereas the humoral immune response correlated with decreased viremia only.[210]

Prevention and Therapy

Nephropathy

Systematic screening for BKV reactivation in post–renal transplant recipients is effective in preventing nephropathy. Detection of BKV in blood is usually followed by reduction of immunosuppressants to revive adaptive immune responses against BKV.[211] Screening for BKV in urine can detect reactivation earlier because viruria almost always occurs before viremia. However, not all patient with viruria will progress to develop viremia.

Treatment of BKV-induced nephropathy is reduction of immunosuppression. Multiple medications, including cidofovir, leflunomide, quinolones, and intravenous immune globulin (IVIG), have shown varying success in small clinical studies.[212–216] However, patients in these studies also had concomitant decrease of immunosuppressive medications. Because no specific antiviral therapy exists for BKV, reduction of immunosuppression is the therapy of choice[200] and has to be balanced with the increase in risk for graft rejection. The clearance of BKV from plasma is a surrogate marker for resolution of renal tissue pathologic conditions. Fluoroquinolones, such as ciprofloxacin, can inhibit BKV replication in vitro by inhibiting BKV-encoded DNA gyrase.[217] Two small retrospective studies demonstrated decreased BKV infection after renal transplantation in the group with exposure to fluoroquinolones.[218,219] However, two recently conducted randomized, placebo-controlled studies showed that 3 months of treatment with levofloxacin did not reduce the rate of BK viruria, but did result in increased resistance to levofloxacin in bacterial isolates.[220,221] Antibody-based treatment of BKV was based on the finding that neutralizing antibodies against BKV are specific to viral capsid sequences.[222,223] Several case series have demonstrated a potential role for IVIG in

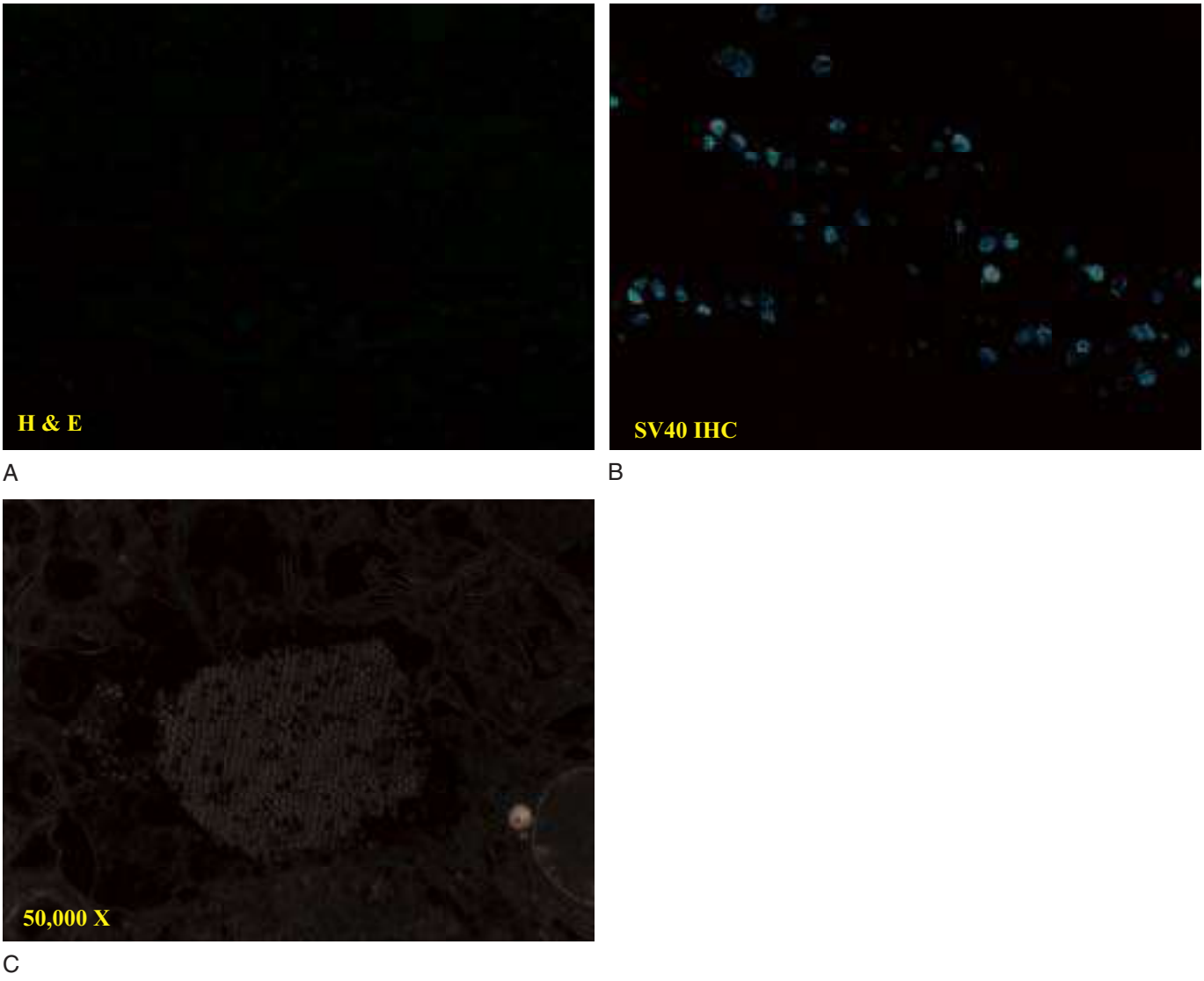

FIG. 144.4 Histology of BK virus nephropathy. (A) Hematoxylin and eosin stain demonstrating tubular epithelial cells with viral inclusions and interstitial inflammation. (B) Simian virus 40 (SV40) antibody stain showing BKV-infected cells. (C) Electron microscopy image showing BK virus. *(Courtesy I. Stillman, Department of Pathology, Beth Israel Deaconess Medical Center, Boston MA.)*

reducing BK viral load in the plasma of renal transplant recipients.[224–226] A randomized double-blinded study is currently underway to determine the efficacy of IVIG in treatment of BK viremia in renal transplant recipients. Lastly, potential future treatments include switching immunosuppression to mTOR inhibitors and changes in T-cell transfer of immunity.

Ureteral Stenosis

Post–renal transplant patients with the occurrence of BKV-associated ureteral stenosis can benefit from reduced immune suppression. Further treatments primarily involve surgical interventions that relieve the obstruction.

Hemorrhagic Cystitis

Treatment of hemorrhagic cystitis is symptomatic and includes continuous bladder irrigation, analgesia, hyperhydration, forced diuresis, and transfusion to maintain platelet levels above 50,000 cells/mm^3 and hematocrit values greater than 25%. Intravesicular cidofovir has been used in treatment of hemorrhagic cystitis in several case reports and retrospective studies.[227–232] Although this agent may provide some symptomatic relief, there is no definitive decrease of urine BKV load with treatment.[233] Randomized controlled trials are needed to clarify benefits of intravesicular cidofovir use in hemorrhagic cystitis. Prophylaxis with ciprofloxacin resulted in reduced incidence of BKV-associated hemorrhagic cystitis after HSCT in a small retrospective study.[234]

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145 Hepatitis B Virus

Chloe Lynne Thio and Claudia Hawkins

SHORT VIEW SUMMARY

Definition
- Hepatitis B virus (HBV) causes chronic hepatitis B, which can lead to progressive liver disease, including cirrhosis and hepatocellular carcinoma.

Epidemiology
- Nearly 5% percent of the world's population has chronic hepatitis B, but prevalence varies widely (see Table 145.1).
- The highest HBV prevalence is in Asia, Africa, and parts of the Middle East.
- Chronic hepatitis B is the leading cause of end-stage liver disease worldwide.
- Transmission of HBV is through percutaneous, sexual routes, or perinatal. Perinatal transmission is the leading cause of HBV worldwide.

Microbiology
- HBV is a partially double-stranded DNA virus in the Hepadnaviridae family that primarily infects hepatocytes.
- HBV replication is through an RNA intermediate, so it has a reverse transcriptase.

Clinical Presentation and Diagnosis
- Clinical presentation of HBV varies from asymptomatic to fulminant hepatitis with liver failure.
- The risk of developing chronic hepatitis B is inversely proportional to age of acquisition of infection. Chronic hepatitis B is also more likely in high-risk patient groups, such as injection drug users, men who have sex with men, and human immunodeficiency virus–infected individuals.
- Laboratory diagnosis of HBV is by enzyme immunoassays, which detect various HBV antigens and antibodies, and by real-time polymerase chain reaction to detect HBV DNA (see Table 145.2).

Therapy
- Anti-HBV agents significantly reduce complications of chronic hepatitis B, including liver cirrhosis and hepatocellular carcinoma. These therapies are also effective in reducing the recurrence of HBV infection in transplant recipients and other immunocompromised hosts.
- HBV is treated with 180 μg of pegylated interferon-α (PEG IFN-α) weekly for 48 weeks or with nucleos(t)ide analogues, often indefinitely (see Table 145.4).
- The first-line nucleos(t)ide agents are entecavir 0.5 mg daily or tenofovir disoproxil fumarate (TDF) 300 mg daily or tenofovir alafenamide 25 mg daily.
- New drugs are in early phase trials

Prevention
- Vaccination with the hepatitis B vaccine is recommended in all infants and children and for nonimmune adults at high risk for infection (see Table 145.6).
- Infants born to mothers with chronic hepatitis B should receive the hepatitis B vaccine and hepatitis B immune globulin (HBIG) at birth. Mothers with HBV DNA >1962 IU/mL should receive TDF in the third trimester of pregnancy to reduce the risk of transmission to the infant.
- Nonimmune individuals who have percutaneous, sexual, ocular, or mucous membrane exposure to HBV-infected fluids should receive postexposure prophylaxis with HBIG and hepatitis B vaccine.

OVERVIEW

Hepatitis B virus (HBV) infects more than 200 million people worldwide. It is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), and these sequelae of chronic infection account for more than 800,000 deaths annually. The outcome of infection and spectrum of illness varies widely. During the acute phase, infections range from asymptomatic hepatitis to icteric hepatitis, including fulminant hepatitis. Once chronic infection is established, the spectrum of illness ranges from the asymptomatic, inactive hepatitis to progressive liver disease that results in the sequelae of end-stage liver disease, including cirrhosis and HCC. Even though HBV cannot be cultured easily, enough is known about the viral life cycle that specific antivirals are available to control viral replication in patients; however, no cure is available yet for hepatitis B. Identification of the correlates of protective immunity has facilitated the widespread use of a highly effective vaccine, which by preventing chronic carriage of the virus and the subsequent development of HCC, represents the first true cancer vaccine.

Historical Background and Classification

Hippocrates recognized the spread of jaundice by infectious agents as early as 4000 BC. Recently, the oldest known HBV was isolated from the mummified remains of a child discovered in the 16th century from Naples, Italy.[1] The early modern cases of HBV infection were linked to the use of conventional viral vaccines, which were prepared from or contained human serum. In 1885 Lurman[2] described the appearance of jaundice in 15% of 1289 shipyard workers who received smallpox vaccine prepared from human lymph. Epidemics of hepatitis were also recorded after the administration of yellow fever vaccine, which was stabilized with human serum.[3] In the early part of the 20th century the increasing use of contaminated syringes and needles by diabetics on insulin and by patients treated for syphilis at venereal disease clinics elevated the importance of serum hepatitis.[4,5] This led to the association of hepatitis B with blood and blood products, as well as to its distinction from infectious hepatitis, caused by hepatitis A virus, a member of the Picornaviridae family.[6] The first hint of viral etiology came from the studies of Blumberg,[7] who reported the discovery of a human antigen in Australian Aboriginals termed Australian (Au) antigen. Subsequently, the Au antigen came to be known as hepatitis B surface antigen (HBsAg), and its association with acute hepatitis was established. For this discovery, Dr. Baruch Blumberg received the Nobel Prize in Physiology and Medicine in 1976. In 1971 Dane, an electron microscopist, visualized the presence of 22-nm HBsAg subviral particles along with the complete 42-nm virus particles in the blood of hepatitis B patients (Fig. 145.1).[8]

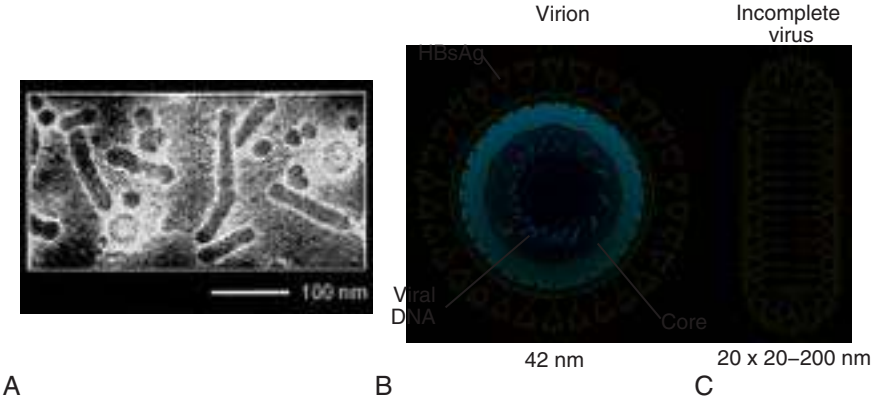

FIG. 145.1 Structure of hepatitis B virus (HBV) and hepatitis B surface antigen (HBsAg) particles. (A) Electron micrograph of negatively stained HBV. (B) Diagram of 42-nm HBV showing partially duplex DNA genome with a covalently linked protein at the 5′ end of the complete positive strand. (C) Diagram of 22-nm HBsAg filament. *(Modified from Flint SJ, Enquist LW, Racaniello VR, et al.* Principles of Virology. *2nd ed. Washington, DC: American Society for Microbiology Press; 2004:808.)*

Due to their unique biologic and molecular characteristics, as well as their liver tropism, HBV and HBV-like animal viruses were given the status of a new family designated Hepadnaviridae (*hepa*totropic *DNA vir*uses). HBV, the human pathogen, and other mammalian hepatitis viruses with sequence homology and similar genome organization are grouped in the genus *Orthohepadnavirus.* The genus *Avihepadnavirus* includes viruses that infect ducks, geese, and herons. The hepadnavirus animal models, which include duck hepatitis B virus,[9] woodchuck hepatitis virus, and ground squirrel hepatitis virus, have been extensively studied and have contributed to the current knowledge of the molecular biology of HBV infection and replication. However, these viral genomes are considerably divergent from human HBV, and there are limited tools to study the immune reaction to the virus in these animals. The primary host for HBV is human, but HBsAg has been detected in other primates, such as chimpanzees, gibbons, orangutans, African green monkeys, and squirrel monkeys. However, the use of these animals for the study of pathogenesis is both difficult and expensive. Thus a convenient animal model for HBV remains unavailable; however, the discovery of the HBV receptor on hepatocytes has led to development of a more efficient tissue culture system that should facilitate new drug discovery. HBV was molecularly cloned from patients' sera in 1979 and its complete DNA sequence determined.[10–12]

Virology

HBV is a small DNA virus, whose 3200-kilobase (kb) partially double-stranded DNA genome is maintained in a circular conformation.[13] Partially duplex DNA molecules represent incomplete synthesis of the viral DNA during morphogenesis (Fig. 145.2).[14] One of the unique features of HBV infection is the production of large quantities of subviral spherical and filamentous HBsAg particles, which are devoid of HBV DNA and so are noninfectious, in addition to complete virus particles. Under electron microscopy, these are distinguished by a diameter of 42 nm for complete virus (Dane) particles and 22-nm spherical and filamentous structures for subviral particles (see Fig. 145.1).[8] HBsAg is expressed on the exterior of all these particles. HBsAg subviral particles reach a titer of 10^{13}/mL, whereas viral particles range in titer from 10^{4}/mL to 10^{9}/mL. All of these particles circulate in blood and permit convenient diagnosis of viral antigen by enzyme-linked immunosorbent assay or radioimmunoassay.[15] HBsAg forms the viral component of the lipoprotein envelope, which encloses a core shell containing the viral DNA genome and the virus-encoded polymerase protein. The nucleocapsid or core is composed of a 21-kilodalton (kDa) basic phosphoprotein commonly known as hepatitis B core antigen (HBcAg).[16] A cell-derived kinase activity has been shown to be associated with the virion particles,[17] but the functional significance of this enzyme is not understood.

The subviral particles are composed of surface antigen polypeptides and lipids derived from hepatocyte membranes. The lipid content is approximately 30% by weight and includes phospholipids, cholesterol, cholesterol esters, and triglycerides.[18,19] Because of their high immunogenicity, purified HBsAg particles can be used as an HBV vaccine. HBsAg elicits neutralizing antibodies, which offer protection from reinfection.[20] The high titers of HBsAg in patients during natural infection can potentially serve to adsorb neutralizing antibody or promote T-cell exhaustion, thus protecting the virus from host defenses.[21]

Attachment, Entry, and Hepatotropism

HBV primarily infects hepatocytes. The pre-S1 domain of the large HBsAg protein promotes attachment and entry of HBV into the hepatocyte via a liver cell–specific receptor recently identified as Na (sodium) taurocholate cotransporting polypeptide (NTCP), which is an integral membrane protein used in bile acid transport.[22,23] There is evidence that hepatocyte entry may be a multistep process, including binding to heparan sulfate proteoglycans,[24] which are found on a variety of cells, and clathrin-mediated endocytosis.[25] The viral envelope fuses with the endosomal membrane and delivers core particles into the cytoplasm, which are transported to the nuclear pore complex, allowing viral DNA to enter the nucleus.[26] In the nucleus the partial strand is repaired, forming a double-stranded covalently closed circular (ccc) DNA form (Fig. 145.3), which is the template for replication (see "Replication").[9] Liver specificity is also displayed at the level of viral gene expression, which is controlled largely by the promoters and enhancers (see Fig. 145.2).

Viral Genome

HBV uses unique transcriptional and translational strategies to maximize the limited coding capacity of its genome. The HBV DNA consists of the complete (negative) strand, which contains a protein covalently linked to its 5′ terminus, and the incomplete (positive) strand, displaying variable length and bearing a 5′ capped oligoribonucleotide (see Figs. 145.1 and 145.2).[27,28] The asymmetry of the DNA strands reflects the incomplete synthesis of DNA during maturation of viral particles. HBV DNA codes for four overlapping open reading frames (ORFs): S, for the surface antigen or envelope gene (HBsAg); C, for the nucleocapsid (core), and "e" antigen gene (HBcAg, HBeAg); P, for the polymerase gene; and X, for the HBx gene (see Fig. 145.2). The surface antigen ORF contains three in-frame initiator codons from which small or major (S), middle (M), and large (L) kDa HBsAg polypeptides are synthesized, respectively. These polypeptides are variably glycosylated to yield several species: S, p24/gp26; M, p30/gp33/gp37; and L, p39/gp42 kDa HBsAg polypeptides, respectively. L and M HBsAg contain characteristic pre-S1 and pre-S2 domains, and hence these proteins are also referred to as pre-S1 and pre-S2 proteins, respectively. The subviral particles are composed of mostly S and lesser amounts of M polypeptides and few or no L polypeptides. The Dane particles, which represent the complete virus, contain all three HBsAg forms.

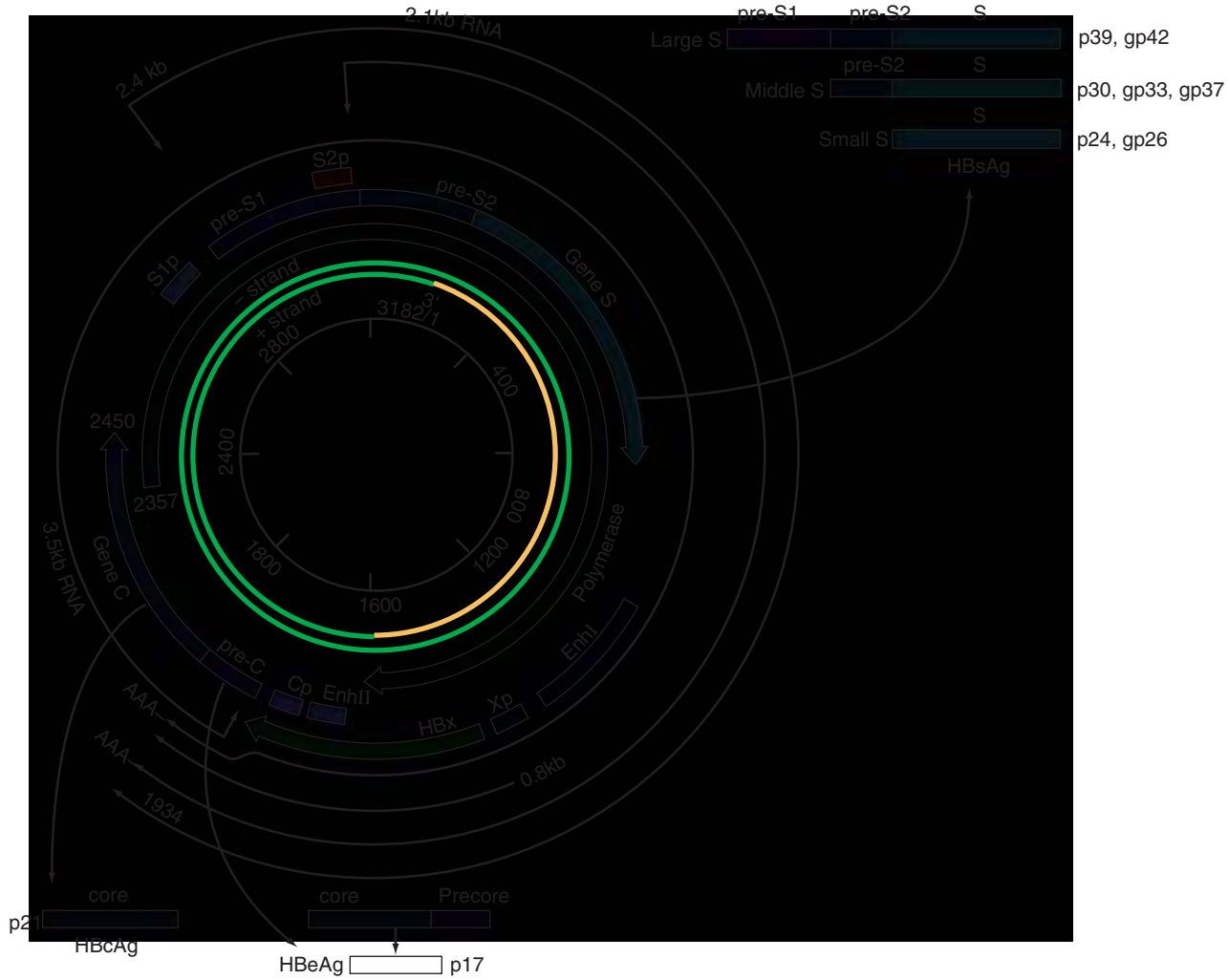

FIG. 145.2 Hepatitis B virus (HBV) genome organization, map of viral transcripts, and proteins. The partially double-stranded 3.2-kilobase viral DNA is shown in the inner circle. The single-stranded (ss) region is indicated in yellow-orange. The extent of the ss region varies from molecule to molecule. HBV-encoded overlapping genes are indicated in the outer circles in various colors. Four promoter regions preceding a corresponding gene are indicated as S1p, S2p, Cp, and Xp. Two enhancer elements (I and II) are also shown. Viral transcripts are indicated in the outermost circles *(thin lines).* The three forms of HBsAg, HBcAg, and HBeAg (surface, core, and e antigen, respectively) polypeptides are also shown. *(Modified from Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Hepatitis viruses. In: Murray PA, Rosenthal KS, Kobayashi GS, et al, eds.* Medical Microbiology. *4th ed. St. Louis: Mosby; 2002:591–605.)*

The C region, or the core ORF, contains two in-frame initiator codons, with the first adenine-thymidine-guanine (ATG) start codon responsible for e antigen polypeptide synthesis (HBeAg) (see Fig. 145.2). The second ATG serves as an initiation codon for the HBcAg polypeptide. HBeAg is secreted from cells and accumulates in serum as an immunologically distinct soluble antigen and serves as a marker of ongoing viral replication. Both gene products (core and e polypeptides) are made from the same reading frame. The P and X ORFs encode the polymerase and the HBx proteins, respectively.

Before the advent of current molecular methods, HBV was divided into serotypes on the basis of the antibody response to HBsAg. However, HBV is now divided into 10 genotypes (A–J), based on genetic diversity of at least 8% in the HBV genome.[29,30] Within these genotypes are subgenotypes, which differ by a minimum of 4%. These genotypes and subgenotypes are distributed geographically, with genotypes A in North America, Europe, and parts of Africa; genotypes B and C in Asia; genotype D in India, the Middle East, the Mediterranean region, and parts of Africa; genotype E in Africa; genotype F in Central and South America; genotype G in France, Germany, and North America. Genotype H is found in Central America. Genotype I, a novel recombination between genotypes A, C, and G, is found in Vietnam and Laos.[31] Genotype J was identified in Japan.[32] There are accumulating data that these genotypic differences are associated with disease and treatment outcomes. Genotypes G and C are associated with more severe liver disease and higher HCC risk.[33] Genotype C also has slower HBeAg seroconversion compared with genotype B.[34,35] Genotypes A and B are more responsive to pegylated interferon-α (PEG IFN-α) therapy than are genotypes C and D.[36]

Transcription

The HBV genome maximizes the use of its limited genomic size by using multiple overlapping reading frames and multiple initiation codons to generate antigenically different proteins. The cccDNA is a stable episomal form of HBV in the nucleus that is associated with histones and other proteins, serves as the substrate for viral transcription, and uses host polymerase II (pol II) in the synthesis of viral transcripts (see Fig. 145.3). The expression of the four different genes, designated C, S, P, and X, is regulated by four promoter elements (S1p, S2p, Cp, and Xp) and two enhancer elements (enhancer I and II; see Fig. 145.2).[15] These transcriptional regulatory elements direct the synthesis of multiple viral transcripts that are approximately 3.5, 2.4/2.1, and 0.8 kb in length, respectively. All viral transcripts

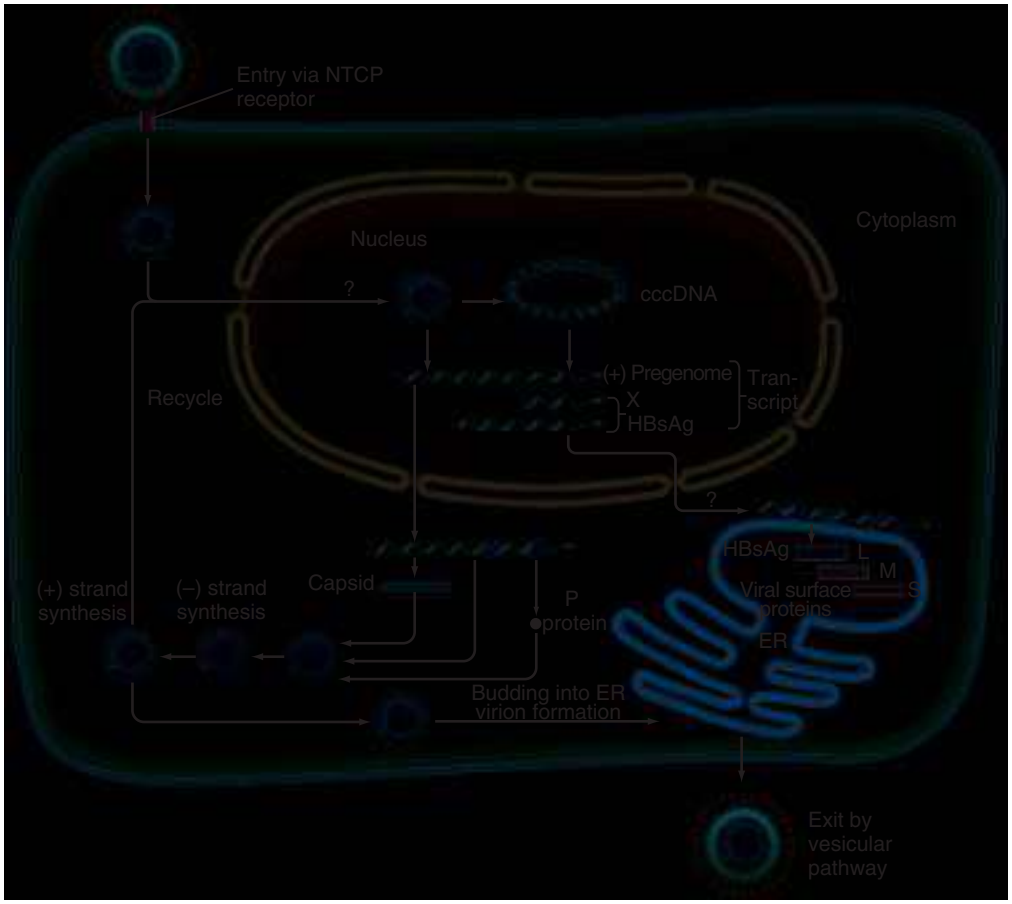

FIG. 145.3 Hepatitis B virus life cycle. The virion attaches to the hepatocyte through the Na (sodium) taurocholate cotransporting polypeptide *(NTCP)* receptor. It is brought into the cytoplasm via clathrin-mediated endocytosis. The surface protein binds to the endosomal membrane, allowing release of the capsid, which is transported to the nuclear pore complex, where the capsid dissociates and the DNA is translocated to the nucleus. In the nucleus the partial double strand is completed, forming a covalently closed circular form called *cccDNA*. The negative strand of such cccDNA is the template for transcription by cellular RNA polymerase II of a longer-than-genome-length RNA, called the *pregenome,* and shorter subgenomic transcripts. Viral messenger RNAs (mRNAs) are transported from the nucleus. The hepatitis B surface antigens *(HBsAgs)* encoding viral mRNAs are translated by ribosomes bound to the endoplasmic reticulum *(ER),* and the proteins enter the secretory pathway. The pregenome RNA is translated at low efficiency to produce a 90-kilodalton polymerase protein, *P,* which possesses reverse transcriptase activity. This protein then binds to a specific site at the 5′ end of its own transcript, where viral DNA synthesis is eventually initiated. The pregenomic RNA also serves as mRNA for the capsid protein. Concurrently with capsid formation, the RNA-P protein complex is packaged and reverse transcription begins with synthesis of negative-strand DNA, followed by positive-strand DNA synthesis. Before the completion of +/– strand synthesis, core particles mature, and these structures acquire envelopes by budding into the ER, where viral morphogenesis is completed. Progeny-enveloped virions are released from the cell by exocytosis or are recycled to the nucleus, where the process is repeated. *(Modified from Flint SJ, Enquist LW, Racaniello VR, et al.* Principles of Virology. *2nd ed. Washington, DC: American Society for Microbiology Press; 2004:809.)*

are unspliced, capped, and polyadenylated. All the transcripts are encoded on one strand of the DNA and coterminate at an identical polyadenylation site.[37]

The pre-S1 (S1p) promoter directs the synthesis of the 2.4-kb transcripts, which code for the large envelope (L HBsAg) polypeptide. This promoter contains binding sites for two liver-enriched transcription factors, hepatocyte nuclear factor 3 (HNF-3) and HNF-1, which are primarily responsible for the liver-specific activity of this promoter.[38] The pre-S2 or S (S2p) promoter directs the transcription of multiple species of messenger RNAs (mRNAs) coding for pre-S2/M and S HBsAg polypeptides. Collectively, these RNA species are approximately 2.1 kb in length with 5′ heterogeneous ends. The mRNAs, which initiate downstream of the pre-S2 initiator ATG, code for the S, or the major polypeptide. The S2p promoter also displays liver specificity and is controlled by the enhancer II element located about 2000 base pairs away (see Fig. 145.2).[39] The S2p promoter is stronger than the S1p promoter, which results in the synthesis of an excess of the major S over the L (pre-S1) and M (pre-S2) forms of the HBsAg. This regulation is especially critical for synthesizing the appropriate levels of the three forms of surface proteins within the cell. The basis for the differential regulation of these promoters is governed by mechanisms that involve both positive and negative *cis*-acting elements and the *trans*-acting transcriptional factors.[15,40]

The core/pregenomic promoter (Cp) governs the expression of two longer-than-genome-length transcripts (3.5 kb) designated precore (pre-C) and core (C) RNAs. The slightly longer precore RNA directs the translation of HBeAg polypeptide. The shorter C RNA is used for the translation of core and polymerase proteins. The ATG for the polymerase (reverse transcriptase) is located several hundred nucleotides from the 5′ end (see Fig. 145.2). The C RNA, after its translation into core polypeptides, packages its own RNA, which then functions as a pregenome RNA (pregenomic RNA). The Cp promoter contains binding sites for several liver-enriched and ubiquitous transcription factors.[40] Immediately juxtaposed to this lies enhancer II, which, in concert with enhancer I, plays a regulatory role in the overall HBV gene expression. A complex scheme of transcriptional regulation appears to operate within these control elements.[41]

The X promoter (Xp) is located immediately downstream of enhancer I and regulates the synthesis of the low abundance 0.8-kb X transcripts that correlate with similar levels of HBx protein synthesis in infected

cells.[42,43] Both ubiquitous and liver-enriched factors binding to enhancer I regulate the biosynthesis of X transcripts.

Translation

HBV, which encodes three major viral transcripts, also uses unique translational strategies to maximize the limited coding capacity of its genome. The S region contains three in-frame translational start codons from which the synthesis of three distinct surface antigen polypeptides is regulated (L/pre-S1, M/pre-S2, and S). These proteins exhibit distinct amino termini and differ with respect to the extent that they are post-translationally modified by glycosylation.[15] The S protein is referred to as the major surface antigen because it represents approximately 85% of the HBsAg that is produced by the virus. The pre-S1 (L) and pre-S2 (M) proteins are present at an abundance of approximately 15% and 1% to 2%, respectively.

The pre-C and C mRNAs are translated into e and core polypeptides (HBe/cAg), respectively. The pre-C protein contains a signal peptide sequence encoding 19 amino-acid residues, which targets this protein to the secretory pathway in the endoplasmic reticulum (ER).[44] Further processing of the protein includes cleavage of the signal peptide, and several amino-acid residues at the carboxyl terminus, leading to the production of a 17-kDa HBeAg (see Fig. 145.2). HBeAg is secreted and found in the serum of patients and serves as a marker of active replication in chronic hepatitis. Although the function of HBeAg is not clearly understood, one study demonstrated that it downregulated Toll-like receptor 2 expression on hepatocytes and monocytes, leading to a decrease in cytokine expression.[45] These data suggest a role for HBeAg in modifying the innate immune response to HBV. HBeAg is dispensable for replication, as HBV carriers with pre-C mutant viruses arise spontaneously and are both infectious and pathogenic.[46,47] The C RNA initiates downstream of the pre-C ATG and translates into HBcAg and less frequently into polymerase. Because polymerase ATG is located several hundred nucleotides downstream of the 5′ end (see Fig. 145.2), the ribosomes must access this initiator codon by a mechanism different from ribosome scanning. Because this process is inefficient, one polymerase protein is translated for every 200 to 300 molecules of core polypeptides.

HBV polymerase protein consists of 832 amino acids and is composed of four domains: terminal protein (TP), spacer, reverse transcriptase (RT), and ribonuclease H (RNase H).[15] The TP domain represents the portion of the polymerase protein that is covalently bound to negative-strand DNA. The spacer domain or tether region connects the TP domain to the RT, which contains the catalytic domain for the polymerase. The RT domain is followed by an RNase H domain, which functions to hydrolyze the pregenomic RNA template after reverse transcription within the nucleocapsid.[48]

Replication

Although HBV is a DNA-containing virus, the replication occurs via an RNA intermediate, a characteristic that places hepadnaviruses close to retroviruses. Much of our understanding of HBV replication strategy comes from the classic experiments of Summers and Mason,[49] which demonstrated that HBV amplifies via reverse transcription of an RNA intermediate and that these events occur within the subviral core particles in the cytoplasm. The complex mechanism by which pregenomic RNA is converted to partially double-stranded virion DNA begins with the encapsidation of pregenomic RNA by the core polypeptide along with the polymerase (Fig. 145.4).[50,51] The pregenomic RNA is greater than genome length because it contains terminally redundant 200-nucleotide sequences, which include an epsilon (ε) stem-loop structure, and a short sequence of 11 to 12 nucleotides termed DR1 (see Fig. 145.4).[15] However, only the 5′ end ε stem-loop structure contains a binding site for the HBV polymerase to bind to its own molecule. The interaction between the ε signal of the pregenomic RNA and the polymerase leads to the formation of a three-base DNA priming sequence. This primer sequence then transfers to the DR1 near the 3′ end of the pregenomic RNA (pgRNA) to initiate elongation of the negative-strand DNA and concurrent digestion of pgRNA by RNase H of the polymerase. After this, positive-strand synthesis occurs. The positive strand is extended to variable lengths to yield mature HBV DNA. The cessation of the positive-strand synthesis at various stages of its synthesis coincides with the maturation of the core particles and their entry into the ER, leading to the packaging of partially duplex genomic DNA found in hepatitis B (HB) virions (see Fig. 145.3). This entry prevents access to cytoplasmic deoxynucleotide pools needed for completion of DNA synthesis. Matured core particles face two options at this stage: They either enter the ER, undergo virion packaging with HBsAg polypeptides, and bud from the membrane as 42-nm Dane particles; or they enter the nucleus and deliver the partially duplex DNA and repeat the cycle of replication (see Fig. 145.3). cccDNA serves as a substrate of all viral mRNA synthesis; it is the stable form of HBV DNA that is most resistant to antiviral treatment and the host immune response.[15] In summary, the salient features of HBV replication are cccDNA as the replication template for all viral RNAs, the use of pregenomic RNA as a template for reverse transcription occurring within core particles; incomplete positive-strand synthesis that results in a partially duplex DNA genome found within the hepatitis B virion particles; and mature core particles either enter nucleus or are secreted out of the hepatocyte.[52]

HBx

HBx is a regulatory protein whose primary role is to enhance transcription of the HBV genome by promoting degradation of a host restriction factor, Smc5/6.[53] It is also involved in several cellular pathways. The cellular targets are numerous, and the list of the cellular functions HBx has been shown to modulate continues to grow.[15] Overall, the functions of HBx are characterized as transcriptional *trans*-activators. Nuclear factor kappa B (NF-κB) was one of the first HBx-responsive elements identified.[54] Because the NF-κB site does not exist within the HBV genome, it is believed that the *trans*-activating potential of HBx extends to cellular target genes involved in inflammation. Other HBx-responsive transcription factors include *a*ctivating *t*ranscription *f*actor 2/*c*AMP *r*esponse *e*lement *b*inding protein (ATF-2/CREB), activation protein 1 (AP-1), signal transducer and activator of transcription (STAT)-3, and nuclear factor of activated T cells (NF-AT).[55,56] Within the cytoplasm, it also localizes to the mitochondria and induces oxidative stress via calcium signaling.[57,58] These activities may mediate mitochondria-mediated liver injury. HBx has also been shown to modulate cellular signal transduction pathways.[59,60] It was shown to activate sarcoma (Src) kinase and stimulate the Ras-Raf–mitogen-activated protein kinase (MAPK) signal transduction pathway.[61] The physiologic significance of any of these observations remains to be established in the context of human infections. HBx is not known to be directly oncogenic, but on the basis of its role in activation of transcription factors, elevation of reactive oxygen species, and inhibition of apoptosis,[62] it is considered a potential contributor to the processes of liver oncogenesis.[63]

Morphogenesis and Assembly

Envelopment of the nucleocapsid occurs in the ER or ER-Golgi intermediate compartments.[15] All three forms of the surface proteins are synthesized in the ER as integral transmembrane proteins. M protein is not important for morphogenesis but is required for infectivity. The N-terminal domain of L is critical for virion morphogenesis, serving to translocate the newly-synthesized viral cores across the ER membranes[64] After budding from the ER the virions are secreted via vesicular transport through the remaining compartments of the secretory pathways and are eventually released into the bloodstream. During chronic hepatitis, accumulation of the L surface protein–containing particles has been associated with the pathologic phenotype of ground-glass hepatocytes.[15]

NATURAL HISTORY AND PATHOGENESIS OF DISEASE

Natural History

If HBV is acquired in adulthood, ~95% mount a successful immune response that results in recovery and production of protective antibodies (anti-HBs). In contrast, this only occurs in 5% to 10% of perinatally acquired infection. However, recovery is not synonymous with viral clearance because cccDNA is still present in some hepatocytes, so reactivation can occur (see "Reactivation").

The phases of chronic hepatitis B are in flux, with the newest guidelines delineating phases based on serologic markers. These phases result from the interplay between the virus and the host; however, it is not necessary

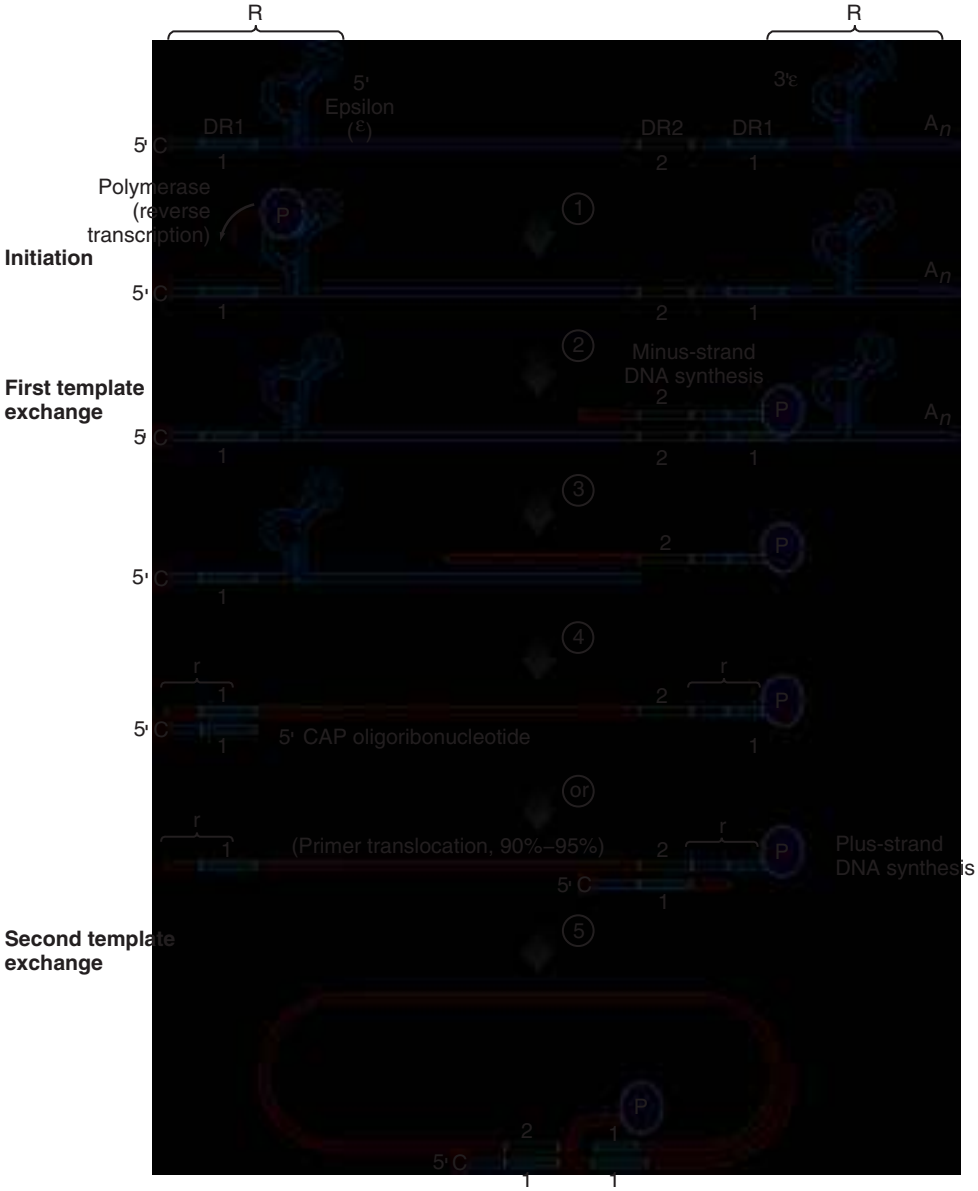

FIG. 145.4 Hepatitis B virus replication pathway. The terminally redundant pregenomic RNA (pgRNA) *(top line)* is capped and polyadenylated. Boxes 1 and 2 represent *DR1* and *DR2*, respectively. The horizontal bracket labeled *R* represents the terminally redundant region of the RNA. The epsilon (ε) stem-loops are indicated at the termini. pgRNA packaging into cores is initiated by the interaction of polymerase *(P)* protein with the 5′ copy of ε, which leads to formation of 3–base pair priming sequence. This primer sequence translocates to the DR1 of the 3′ end of the pgRNA, initiating positive synthesis (step 2). As DNA synthesis continues the pgRNA template is degraded by the associated ribonuclease H activity of the polymerase (step 3). Elongation of negative-strand DNA is finished on complete copying of the pgRNA template (step 4). The product is a terminally redundant complete negative-strand DNA species. The redundancies (8–10 nucleotides) are labeled *r*. At this time the primer for positive-strand synthesis is generated from the 5′-terminal 15 to 18 nucleotides of the pregenomic RNA. The primer is capped *(CAP)* and includes the sequence to the 3′ end of DR1. Elongation of the positive strand results in a duplex linear genome. In the majority of cases the primer is translocated and base pairs with the DR2 sequence near the 5′ end of negative-strand DNA. The positive-strand synthesis is initiated, and then elongation begins. On reaching the 5′ end of negative-strand DNA, an intramolecular template exchange occurs, resulting in a circular DNA genome (step 5). This exchange is facilitated by the short terminal redundancy in negative-strand DNA. The positive-strand DNA synthesis then continues for a variable distance, resulting in the relaxed circular partially duplex form of the genome found in mature virions. *(Modified from Tavis JE. The replication strategy of the hepadnaviruses.* Viral Hepatitis Rev. *1996;2:205–218.)*

to go through all of the phases.[65] The first phase, the HBeAg-positive HBV infection phase (also known as the immunotolerant phase), is characterized by limited immune response against the virus, so there is minimal elevation of serum aminotransferases and minimal liver inflammation despite circulating HBsAg, HBeAg, and high levels of HBV DNA. In cases of perinatal infection, this phase may last for decades, during which there are low rates of spontaneous seroconversion to anti-HBe. The cumulative rate of spontaneous HBeAg clearance during this phase is estimated to be approximately 2% during the first 3 years of life and only 15% 20 years after perinatal infection.[66,67] In contrast, among immunocompetent adults, this phase is typically present only during the incubation period.

During the second phase of the infection the HBeAg-positive chronic hepatitis B phase (also known as the immune active phase), there is an increase in immune response, accompanied by increased aminotransferases and liver inflammation and high HBV DNA levels. This is thought to be the period in which there is augmentation of both innate and acquired HBV immunity, leading to cytolytic destruction of hepatocytes, as the peak of the immune response coincides with the aminotransferase elevations.[68] The result of this increased immune response is usually

conversion from HBe antigenemia to anti-HBe. In perinatally acquired infection, this period usually occurs in the second to third decade of life, and HBeAg seroconversion rates may approach 10% to 20% per year.[66,69] Individuals with active chronic hepatitis B remain in this phase because the immune reaction is not sufficiently vigorous to result in production of protective antibodies and viral control. Some individuals with chronic hepatitis B can eventually experience HBeAg seroconversion. In a study of 1536 Alaskan natives who acquired HBV as adults and did not receive treatment, two-thirds of subjects cleared HBeAg and developed anti-HBe during 12 years of follow-up without reversion to the HBeAg-positive state.[70] The likelihood of seroconversion is inversely related to the serum aminotransferase. Spontaneous seroconversion occurs in 50% of those with serum aminotransferases greater than five times the upper limit of normal (ULN), compared with <10% in those with lower aminotransferases.[71] Spontaneous seroconversion is marked by the appearance of HBcAg-specific $CD4^+$ and $CD8^+$ T cells,[72] but the precise factors preceding this event are unknown, and seroconversion cannot be linked to specific viral mutants, as was previously believed.[73] Males and older individuals are also more likely to have spontaneous seroconversion, and viral genotype may play a role.[35] Because immunoglobulin M (IgM) anti-HBc may increase with alanine aminotransferase (ALT) flares during this phase, acute hepatitis B may be erroneously diagnosed.

The third phase, known as the HBeAg-negative HBV infection phase (also known as the inactive phase), is characterized by absence of HBeAg, presence of anti-HBe, normal aminotransferases, and low or undetectable HBV DNA. Termination of virus replication is associated in most patients with biochemical and histologic regression of inflammatory activity.[74] Some individuals will also clear HBsAg, although this is unusual, occurring in <1% of patients per year in adults and 0.05% to 0.8% in infection acquired in infancy or childhood.[70] Individuals who remain in this phase have excellent prognosis and minimal liver injury. During this phase, seroreversion to HBeAg positivity and return to the second phase occurs 10% to 40% of the time.

The fourth phase is the HBeAg-negative chronic hepatitis B phase, where HBeAg remains negative but viral replication increases, resulting in high levels of HBV DNA and elevations of ALT. During this phase the HBV DNA levels are moderately high but generally lower than in those with HBeAg-positive chronic hepatitis B.

Pathogenesis

It has been difficult to understand the mechanism for viral persistence and immune-mediated liver injury because research in this area is hampered by the lack of a robust culture system or a small animal model. Most studies suggest that HBV is not directly cytopathic to the hepatocyte,[76,77] which is also supported by the existence of asymptomatic hepatitis B carriers with normal liver histology and function. Both human and animal studies have now shown that the liver injury mediated by HBV is initiated by a virus-specific cellular and humoral immune response. In >95% of immunocompetent adults the immune response is vigorous, polyclonal, and multispecific and results in acute, self-limited hepatitis with reduction of viral load and the development of long-lasting humoral and cellular immunity. Persistent infection is associated with necroinflammatory activity, which eventually leads to cirrhosis.

Acute Hepatitis B

Natural recovery from acute hepatitis B probably depends on multiple components of cellular immune responses, including natural killer (NK) cells, natural killer T (NK-T) cells, and virus-specific $CD4^+$ T cells and $CD8^+$ cytotoxic T lymphocytes (CTL). However, a hallmark of acute hepatitis B is failure to induce an innate cytokine response mediated by pattern recognition receptors.[78,79] In self-limited infections the noncytopathic response leads to lowering of the HBV DNA by >90% within a few weeks of peak HBV replication and before liver damage occurs.[80] Both NK and NK-T cells also contribute to clearance through production of IFN-α/β.[81] Acute HBV infection is also accompanied by a strong and transient expansion of $CD4^+$ T cells directed against multiple epitopes within the HBV. HBc is the dominant antigen recognized by $CD4^+$ T cells in most cases of acute, resolving HBV infection.[82] These HBc-specific $CD4^+$ T cells provide help for the production of antibody to HBsAg and are also associated with the development of a vigorous and polyclonal cytotoxic CTL capable of recognizing different epitopes within the HBV genome.[83] Individuals who successfully resolve an HBV infection, either spontaneously or after IFN therapy, maintain these broad and strong peripheral CTL responses.[84] $CD4^+$ and CTL memory in the presence of low levels of persisting HBV DNA has been shown to persist up to 23 years after infection despite markers of serologic recovery. Both virus-specific $CD4^+$ and CTL contribute to control of HBV replication through both direct cytolysis of infected cells but, more important, through production of cytokines that control viral replication.[85] Analysis of the NK, CTL, and $CD4^+$ T-cell responses in the incubation phase has demonstrated that CTL response increases in parallel with ALT, consistent with the previously observed notions that CTL activity is responsible for liver injury, and peaks about the time that the HBV DNA titers begin to fall.[86] Recently, regulatory T cells were found to influence the immune response during acute HBV infection by decreasing cytokine production of effector T cells, thereby down-regulating the antiviral activity. This limits the liver injury during acute infection but, as a consequence, prolongs viral clearance.[87]

The development of surface antibody (anti-HBs) follows the disappearance of HBsAg and marks recovery from acute hepatitis B, which occurs in 95% of adults after acute infection. Anti-HBs is sufficient for protection against HBV infection, as demonstrated by the success of the current HBV vaccines, even if it is not the sole operative mechanism clearing acute infection. The "a" determinant is the predominant B-cell epitope common to all HBV subtypes. Antibodies against this epitope confer immunity to all HBV subtypes. Coexistence of HBsAg and anti-HBs is reported in up to 24% of chronically infected individuals, in which case the anti-HBs is directed against one of the subtypic determinants, and a mutation preventing neutralization of the virus has occurred. Other surface antigens that stimulate antibody responses include the pre-S1 and pre-S2 antigens. Antibody to these develops during recovery and can be detected before anti-HBs; however, routine serologic assays are not readily available. Mutation in the S gene usually occurs in the "a" determinant, which allows the virus to escape antibody neutralization. This may occur after the use of hepatitis B immune globulin (HBIG) for passive immunization of newborns and transplant recipients.

Chronic Hepatitis B

Persistent HBV infection may result because of the failure of initial innate and adaptive immune responses. In animal models HBeAg may be tolerogenic,[88] and because HBeAg and HBcAg are cross-reactive at the T-cell level, deletion of the $CD4^+$ HBc-specific T-cell responses results in ineffective CTL responses to HBcAg.

Adult immunocompetent individuals who fail to resolve acute HBV infection also have less vigorous $CD4^+$ T-cell and CTL responses. In contrast to acute resolving infection, peripheral $CD4^+$ T-cell and CTL responses in those individuals who develop chronic infection are weak and more narrowly focused.[89] Despite these weak CTL responses, HBV-specific $CD4^+$ and $CD8^+$ T cells can be isolated from the livers of chronic hepatitis B patients that are capable of ex vivo, class-I–restricted cytolytic activity in response to envelope and core peptides. The cytotoxicity mediated by these cells appears to be sufficiently strong to cause liver injury but not strong enough to eradicate virus and cccDNA from all hepatocytes.[90,91] In addition to the virus-specific cells, the inflamed liver contains other cells that may participate in hepatocyte damage.[92] $CD8^+$ T cells from patients with chronic hepatitis B exhibit multiple coinhibitory molecules, including programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4). Upregulation of these exhaustion markers may occur in the liver microenvironment, where high HBV antigen concentrations exist, because one study found that HBV-specific $CD8^+$ T cells in the liver express higher levels of PD-1 compared with circulating T cells.[93]

The mechanisms by which HBV evades the immune response and results in chronic infection in these adults remain obscure. In part this is determined by host genetic factors because persons with certain genetic polymorphisms and human leukocyte antigen (HLA) alleles appear to be more susceptible to chronic infection,[94–97] and certain HLA-DP alleles have been associated with recovery from acute HBV infection.[98] It has

also been suggested that the size of initial viral inoculum and viral kinetics may be such that the immune system is overwhelmed by the virus and becomes "exhausted."[99]

Hepatocellular Carcinoma

Although epidemiologic evidence supports the role of HBV as a causal agent of liver cancer (see "Epidemiology of Hepatitis B" later),[100] the molecular mechanisms addressing the link between the viral infection and the development of HCC have been highly debated.[101] Despite a large body of work on this subject, a clear view of how HBV infection triggers events that lead to liver oncogenesis remains elusive. HCC development, like other cancers, proceeds in multiple steps that correlate with specific lesions associated with livers of patients with HCC. These include altered hepatic foci, dysplastic (neoplastic) nodules, and low- and high-grade HCCs. These lesions are characterized as exhibiting different levels of cell differentiation. Similar to other well-characterized human cancers, HCC progresses through these individual stages. The molecular switches associated with each step of HCC development still need to be identified.

HBV-related HCCs are derived from the clonal expansion of a single transformed cell or cancerous cells.[100] Whereas 80% of HBV-associated HCC tumors contain integrated viral DNA, most of the HBV genes are either truncated or transcriptionally inactivated.[15] After integration into the host chromosomes, the HBV DNA control elements, such as enhancers or promoters, can act in *cis* to activate a cellular oncogene. HBV integration was initially thought to be a random event, but recent next-generation sequencing has identified recurrent sites for integration alteration of the *TERT, MLL4, CCNE1, NTRK2, IRAK2,* and *p42MAPK1* genes, all of which are important for cell growth and differentiation.[102] It is thought that the random but multiple integration events might frequently lead to genomic instability by causing chromosomal aberrations, such as amplifications, translocations, and deletions, all of which have the potential for activating pathways that lead to liver neoplasia. Indeed, in HBV-associated liver tumors, the HBV DNA integrants are highly rearranged with deletions, inversions, and sequence reiterations.[103] HBV DNA insertion into cellular genes encoding for proteins that are necessary for control of cell signaling and proliferation has been demonstrated.[104]

The pleiotropic functions of HBx in HCC have been implicated in the processes of liver oncogenesis.[105] HBx modulates the expression and activities of numerous genes, as well as epigenetic molecules (e.g., microRNAs) and events (e.g., methylation and acetylation), leading to deregulation of various pathways and functions. Other functions include its effects on DNA repair processes, antiapoptotic properties, and potential to cause direct oxidative damage to host DNA by elevating the intracellular levels of reactive oxidant species in cells.[15,106] HBx activates a number of nuclear transcription pathways and can modulate cytoplasmic signal transduction pathways, including Ras-Raf-MAPK, Janus kinase/STAT (Jun kinase [JNK]/STAT), resulting in hepatic cell proliferation. In addition, HBx can directly inactivate or indirectly downregulate various tumor suppressors, such as p53, or senescence-related factors.[107] Because most of the HBV genes are extinguished in advanced tumors, the viral role in the development of HCC is most likely to be at the stage(s) of initiation or promotion of hepatocarcinogenesis. This would preclude the presence of HBx in maintaining the transformed phenotype.

Finally, HBV-associated HCC may be due to the repeated cellular division associated with the inflammatory response.[15,108] HCC associated with HBV is most common in the context of cirrhosis, although it can occur in all stages of liver disease. Cirrhosis is the result of years of inflammation and associated repair processes, during which there is considerable cell killing and repeated hepatocyte regeneration. In all types of liver damage, there is evidence of enhanced production of free radicals or significant decrease of antioxidant defense, or both.[109] Oxidative stress induced by inflammation and accumulation of viral surface proteins over a long period may give rise to high mutation rates in infected hepatocytes.

EPIDEMIOLOGY OF HEPATITIS B

Approximately 3.6% of the world's population (≈257 million people) is chronically infected with HBV, and more than 800,000 deaths due to HBV occur annually.[110,111] The prevalence of chronic HBV varies widely, ranging from 0.01% to 2% in the low-prevalence areas (United Kingdom, United States, Canada, Western Europe, and Japan), 2% to 5% in the lower intermediate areas (parts of South America, North Africa, Russia), 5% to 8% in the higher intermediate areas (South Africa, China, and Thailand), and >8% in areas of high prevalence (most of sub-Saharan Africa and a number of countries in the Western Pacific region; Fig. 145.5 and Table 145.1).The largest number of people living with chronic HBV live in the Western Pacific region (>95 million) and the African region (>75 million).[110] In some parts of Europe, typically considered a low-prevalence area, high HBV prevalence rates have been observed in persons migrating from countries with intermediate/high HBV prevalence.[112] Specific patient groups, such as injection drug users, men who have sex with men (MSM), and human immunodeficiency virus (HIV)-infected patients have been shown to have significantly higher rates of chronic HBV infection than the general population.[113] The risk of chronic infection is inversely proportional to age of acquisition of infection. In areas of high seroprevalence, HBV is more likely to be acquired perinatally, with an attendant high risk of chronic infection of 90%. For infections acquired between the ages of 6 months and 5 years, the risk of chronic infection is between 20% and 60%, and for infections acquired by immunocompetent adults the risk is approximately 5%.[114]

The epidemiology of HBV is changing with the advent of universal vaccination programs adopted by many countries. Over the past 40 to 50 years decreases in HBV prevalence in countries of South-East Asia, the Western Pacific, and the Eastern Mediterranean have been observed, whereas increases have been observed in parts of Eastern Europe and Africa (Nigeria) (8.55%–9.8%). In the United States the estimated number of new symptomatic infections per year was more than 10 per 100,000 in the mid-1980s, whereas after the adoption of universal vaccination for infants and "catch-up" vaccination for older children in 1991, this number was estimated to be about 1.1 per 100,000 in 2015.[115] The overall incidence of acute hepatitis B has declined in all age groups over this period, although it remains highest in persons age 30 to 39 years (2.6 cases/100,000 population) (Fig. 145.6).[115] Despite these declines, there are still between an estimated 850,000 and 2.2 million HBV carriers in the United States, of whom about 25% will develop serious sequelae during their lifetime, and 1800 will die annually due to complications of chronic liver disease, including HCC.[115,116]

ROUTES OF TRANSMISSION

Hepatitis B replicates to high titer in the blood (10^8–10^{10} virions/mL), especially during the acute phase of illness. Any parenteral or mucosal exposure to infected blood thus represents a potential risk for acquisition of hepatitis B and accounts for the 100 times more efficient transmission of HBV compared with HIV after needlestick exposure.[117] HBV is also found in other body fluids to a variable degree, including semen, saliva, cervical secretions, and tears, and can survive up to 7 days on environmental surfaces.[117a] Thus exposure to even minute amounts of blood or contaminated secretions may transmit virus, and infection can occur in settings of prolonged, close personal contact, such as occurs between children, or among residents of institutions for the developmentally disabled, probably due to inapparent contact of infected secretions with nonintact skin. HBV is not found in urine, sweat, or stools. The typical mode of transmission of HBV varies in part with the prevalence of infection (see Table 145.1). In high- and intermediate-endemicity areas, such as sub-Saharan Africa and South East Asia, the predominant route of transmission is perinatal or horizontal during childhood (i.e., close contact excluding parenteral, sexual, or perinatal exposure), whereas in more low-prevalence areas, transmission occurs via injection drug use and high-risk sexual behaviors. Persons at increased risk of acquiring HBV infection include members of the following groups: illicit drug users (injecting, inhaling, snorting, pill popping), heterosexual men and women and MSM with multiple partners, household contacts and sexual partners of HBV carriers, infants born to HBV-infected mothers, patients and staff in custodial institutions for the developmentally disabled, recipients of certain plasma-derived products (including patients with congenital coagulation defects) before 1992, hemodialysis patients, health and public safety workers who have contact with blood, travelers



FIG. 145.5 Global prevalence of hepatitis B surface antigenemia. *(From Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013.* Lancet. *2015;386:1546–1555.)*

TABLE 145.1 Global Seroprevalence Rates and Modes of Transmission of Hepatitis B

CHARACTERISTIC	HIGH	INTERMEDIATE	LOW
Carrier rate (%)	≥8	2–7.99	<2
Distribution	Alaskan Eskimos, sub-Saharan Africa, Somalia, Yemen, Djibouti Haiti, Kyrgyzstan, Papua New Guinea, Solomon Islands, Nauru, Vietnam,	Eastern and southern Europe except Kyrgyzstan, Eastern Mediterranean, Western Pacific region except Australia, Japan, Papua New Guinea, Vietnam, Southeast and Central Asia, except Nepal and India	United States, Canada, Western Europe, Australia, Mexico, South and Central America except Peru, Suriname, Columbia and Belize; Nepal, India; Japan
Age at infection	Perinatal and early childhood	Childhood	Adult
Mode of transmission	Maternal and perinatal	Percutaneous, horizontal	Sexual, percutaneous

to regions with intermediate or high rates of HBV (≥2%), and persons born in areas of high HBV endemicity and their children. Persons at risk of exposure to HBV should be screened for markers of HBV infection and receive hepatitis B vaccine if seronegative (see "Prevention of Hepatitis B Virus Infection" later).

CLINICAL MANIFESTATIONS AND PROGNOSIS

The spectrum of clinical manifestations of HBV infection varies in both acute and chronic infection. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis. During the chronic phase, manifestations range from an asymptomatic carrier state to the signs and symptoms of cirrhosis and HCC. Extrahepatic manifestations can also occur with both acute and chronic infection.

Acute Hepatitis B

After exposure to HBV, there is an incubation period of 1 to 4 months.[118] Acute hepatitis B is a clinical syndrome indistinguishable from other acute hepatitides and often consists of a flulike syndrome with malaise, fatigue, anorexia, nausea, vomiting, and right upper quadrant discomfort. Serum sickness–like manifestations may be present before the onset of jaundice. Physical signs include jaundice (present in almost all patients) and tender hepatomegaly. The likelihood of developing icteric illness is inversely proportional to age. Symptomatic hepatitis rarely develops in children younger than 1 year, in 10% of children younger than 5 years, and between 30% and 80% of adults.[114] Most reported cases of HBV are the result of icteric illness, but it is believed that approximately two-thirds of cases either result in subclinical illness or are only diagnosed in retrospect. The acute illness may be more severe in the setting of other coinfections, such as simultaneous acquisition of hepatitis D (see

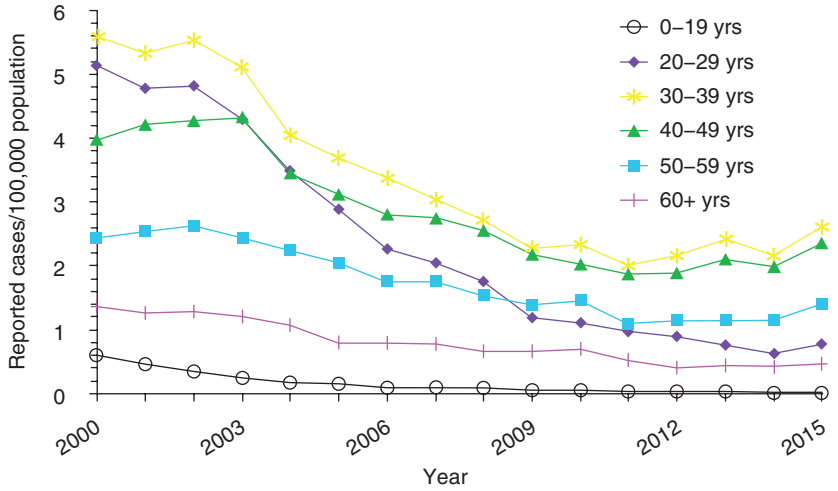

FIG. 145.6 **Incidence of symptomatic, acute hepatitis B per 100,000 population by age group: United States, 2000–2015.** *(Modified from Centers for Disease Control and Prevention.* Surveillance summaries: surveillance for viral hepatitis—United States, 2015. https://www.cdc.gov/hepatitis/statistics/2015surveillance/pdfs/2015HepSurveillanceRpt.pdf. *Accessed December 21, 2017.)*

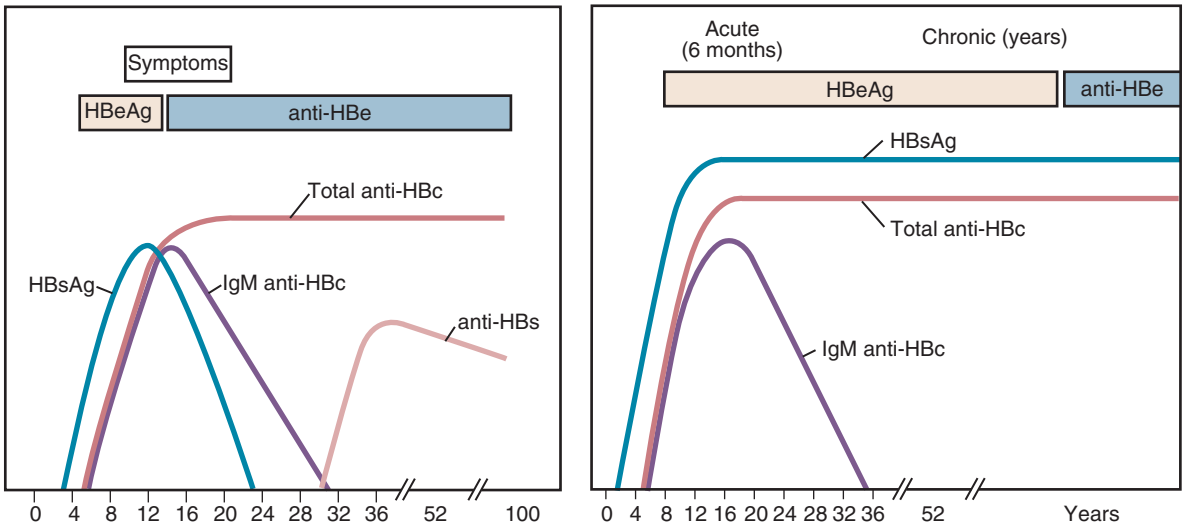

FIG. 145.7 **Typical course of hepatitis B.** *Left,* Typical course of acute hepatitis B. *Right,* Chronic hepatitis B. *HBc,* Hepatitis B core; *HBe,* hepatitis B envelope; *HBsAg,* hepatitis B surface antigen; *IgM,* immunoglobulin M.

Chapter 146), or with underlying liver disease, such as alcoholic liver disease. The symptoms and jaundice generally disappear in 1 month, but some patients have prolonged fatigue even after resolution of the elevated serum aminotransferases. See Fig. 145.7 for an illustration of a typical clinical and laboratory course of acute hepatitis B compared with that of chronic hepatitis B. In acute HBV, elevations in the concentration of ALT and aspartate aminotransferase (AST) levels with values up to 1000 to 2000 U/L can be seen, with ALT being higher than AST. The serum bilirubin concentration may be normal in patients with anicteric hepatitis. The prothrombin time (PT) is the best indicator of prognosis, with a high PT indicative of fulminant liver failure.[119] Among patients who recover, normalization of serum aminotransferases usually occurs within 1 to 4 months. Persistent elevation of serum ALT for more than 6 months indicates progression to chronic hepatitis. Rates of progression to chronic disease are similar whether or not there is symptomatic acute disease.

Acute HBV is characterized by the development of IgM antibody against the core antigen, followed by the development of immunoglobulin G (IgG) anti-HBc (see "Screening and Diagnosis of HBV Infection" later). Markers of viral replication, such as HBsAg and HBV DNA in serum, are present at the same time as anti-HBc. Loss of HBV DNA, HBeAg, HBsAg, and IgM anti-HBc and the development of anti-HBe and anti-HBs all characterize immunity. In a recent longitudinal study of Chinese patients with acute HBV, the median HBsAg clearance time from the onset of symptoms was 6 weeks, and 90% of the patients achieved HBsAg seroconversion in 19 weeks.[120] Patients who recover from acute hepatitis B are usually not cured of infection because a significant number of patients will have HBV DNA detectable by polymerase chain reaction (PCR) many years after clinical recovery.[121] They generally have lifelong protection from disease unless there is significant immunosuppression with loss of protective immune responses, such as in the setting of HIV, chemotherapy, or bone marrow transplantation.[122,123]

Fulminant Hepatitis

Fulminant hepatitis B is rare, occurring in about 1% of patients, and causes <10% of fulminant liver failure in the United States.[124,125] Patients typically present with rapidly progressive acute hepatitis <28 days from the time of symptom onset, accompanied by signs of liver failure, such as coagulopathy, encephalopathy, and cerebral edema. Mortality rates from fulminant HBV as high as 70% have been reported, although the rates have decreased substantially with advances in liver transplantation and critical care management.[126–128] Poor prognostic factors for transplant-free survival include a lower mean arterial pressure on admission and low platelet count.[124] Laboratory testing may not reveal HBsAg due to early clearance but will have IgM anti-HBc and a positive HBV DNA.

Recently, there have been increasing reports of fulminant HBV in the setting of HBV reactivation with chemotherapy or immunosuppressants, with an associated high mortality (5%–22%).[129–131] Fulminant hepatitis is also more common in the setting of coinfection with hepatitis D virus (HDV).[132]

The pathogenesis of fulminant hepatic failure is not clear but may be related to massive immune responses against the virus. Staining of the liver in fulminant HBV often reveals few copies of HBV, suggesting that it is the exuberant immune response that is primarily responsible for the pathogenesis.[133] In addition to host factors relating to immune status, several variants of HBV have also been implicated in several outbreaks of fulminant HBV, including pre-S and S gene variations; BCP; and precore and core gene variations, which result in the decrease or absence of HBeAg; *pol* gene; and *X* gene variatons.[134] Particular HBV genotypes, such as B1 and F (in the context of HDV infection), have also been shown to be associated with fulminant HBV.[135]

Chronic Hepatitis B

Chronic hepatitis B is defined by at least 6 months of persistent HBsAg. Many patients with chronic hepatitis B are not diagnosed as a result of follow-up after a case of icteric illness or due to specific symptoms, but rather as a result of incidental elevations in serum aminotransferases or due to membership in a specific risk category. Symptoms, if present, can be as nonspecific as fatigue, unless cirrhosis or HCC is present. Other less common symptoms include nausea, right upper quadrant tenderness, anorexia, myalgias, and arthralgias. Symptoms often do not correlate with severity of disease, levels of serum aminotransferases, or hepatic injury on liver biopsy. The physical examination may be normal, or there may be an enlarged liver. The presence of jaundice, splenomegaly, ascites, encephalopathy, or pedal edema suggests cirrhosis.

In patients with chronic hepatitis B, serum aminotransferases may be normal, although most patients with HBeAg-positive or HBeAg-negative chronic hepatitis B have at least mild-to-moderate elevations. Patients will have markers of viral replication, including HBsAg and often HBeAg or HBV DNA as well (see "Screening and Diagnosis of HBV Infection" later). During flares of disease activity or just before seroconversion to anti-HBe status, there may be marked elevations in the serum aminotransferases to more than 20 times normal. Flares of disease activity may be due to changes in the level of baseline HBV replication, followed by an immune response against the virus. In some patients this results in repopulation of the viral species with a new variant.[136] Patients with flares of disease activity should be followed for the development of anti-HBe, which signals control of viral replication. Cirrhosis should be suspected if there is evidence of hypersplenism, manifested as decreased platelet count, or impaired hepatic synthetic function, indicated by hypoalbuminemia, hyperbilirubinemia, or decreased albumin. Similar to the range of findings observed in other laboratory tests, findings on liver biopsy range from minimal inflammation to cirrhosis. About 2% to 5% of patients with HBV-related compensated cirrhosis develop decompensated disease each year. Decompensation can occur over time or can result from hepatitis flares.[137] Clinical signs and symptoms of decompensated disease include ascites, jaundice, hepatic encephalopathy, or variceal bleeding.

There are no characteristic pathologic findings that can distinguish hepatitis B from other forms of viral hepatitis, although liver biopsies can be stained for the presence of HBsAg and HBcAg. The most characteristic feature of chronic hepatitis B is the ground-glass hepatocyte, which is thought to be due to accumulation of HBsAg within the ER.[138] Differentiation from alcoholic liver disease can be made by characteristic patterns of steatosis, Mallory bodies, and micronodular cirrhosis, whereas chronic hepatitis C is marked by steatosis and characteristic lymphoid follicles within portal tracts. Immunosuppressed hosts may have a variant of HBV known as fibrosing cholestatic hepatitis, which reveals periportal fibrosis, hepatocyte ballooning, bile stasis, and mild or absent inflammation.[139]

Precore or HBe-Negative Mutants

Early reports described patients in whom HBeAg was absent, although HBV DNA continued to be present at a high level, typically in association with severe liver disease.[140–143] This is due to the development of HBV pre-C or core promoter mutants that cannot produce HBeAg (see "Translation" earlier).[144] The most frequent pre-C mutation is a guanine (G) to adenine (A) change at nucleotide 1986, which produces a translation stop codon at amino-acid position 28 in the pre-C region and abolishes production of HBeAg.[145] A1762T and G1764A are frequently occurring mutations that take place in the basal core promoter (BCP) region. All these mutations abrogate HBeAg synthesis without affecting the replication of the virus, which persists and results in chronic inflammation. Several large cohort and case-control studies have demonstrated an association between BCP A1762T/G1764A mutations and an increased risk of liver disease progression and HCC.[146] At the time of initial presentation, 29% to 38% of HBeAg-negative chronic hepatitis B patients have cirrhosis.[147,148] These patients have very low rates of spontaneous seroconversion of <0.5% per year[149] and are more difficult to treat.[141] Originally, HBeAg-negative hepatitis was thought to be predominantly a geographic phenomenon because it is more prevalent in certain parts of the world, especially Asia and the Mediterranean. However, studies suggest that the likelihood of HBeAg-negative chronic hepatitis B is related to duration of infection, as suggested by the older age at presentation,[147] which would make it appear to be of higher prevalence in areas where perinatal transmission predominates. HBeAg-negative disease is also more frequent with genotypes C, D, and F than with genotype A and B infection and presumably contribute to genotype specific immunopathogensis.[135,150]

Prognosis of Untreated Chronic Hepatitis B

The likelihood of morbidity and mortality in chronic hepatitis B is directly related to the development of cirrhosis. Among chronic HBsAg-positive patients, the risk of developing cirrhosis ranges from 1 to 5.4 per 100 person-years, with a 5-year cumulative probability of progression ranging from 8% to 20%.[147,151,152] For persons who clear HBsAg, the prognosis is good, although, perhaps surprisingly, not entirely benign. In one study of 189 patients who were noncirrhotic at the time of HBsAg clearance, 3 (1.6%) developed cirrhosis, 2 (1.1%) developed HCC, and 1 died of HCC. These complications all developed in patients with concurrent hepatitis C virus (HCV) or HDV infection, however.[153] In the absence of cirrhosis the long-term prognosis even of HBsAg-positive patients is good. In a 16-year follow-up study of 317 HBsAg-positive blood donors from Montreal, for example, only 3 died from HBV-related cirrhosis and none developed HCC.[154] In a US-based study of 6689 patients with a chronic hepatitis B who did not receive therapy and who were followed for 10 years, 3.1% died from HBV-related causes, with the highest percentage (15.5%) in those older than 65 years.[155] However, in another study of HBsAg-positive patients in England and Wales, during a mean of 22 years of follow-up, 17.4% of deaths were due to HCC or liver disease.[156]

Multiple variables account for the wide estimates of risk. A study from Taiwan (REVEAL [*R*isk *E*valuation of *V*iral Load *E*levation and *A*ssociated *L*iver Disease/Cancer]–HBV) of 3582 people with chronic hepatitis B followed for 11 years found that the strongest predictor of progression to cirrhosis was the HBV DNA level, which was independent of the HBeAg status, and inflammation as represented by ALT values.[157] Individuals with $>10^6$ copies/mL of HBV DNA had a 36.2% cumulative incidence of cirrhosis compared with 4.5% in those with <300 copies/mL. Most of the people in this study acquired HBV in infancy or early childhood; thus this study may not be applicable to adult-acquired HBV. The rate of progression to cirrhosis may be higher in patients who are HBeAg negative compared with those who are HBeAg positive,[141] although these findings may be confounded by a longer duration of disease in HBeAg-negative patients and presence of precore and BCP mutations.[158,159] Patients with more severe inflammation and fibrosis at the time of presentation are also more likely to progress to cirrhosis. Fattovich and colleagues[71] found that 30% of patients with moderate, chronic active hepatitis developed cirrhosis on histology after 6 years, whereas 50% of patients with severe, chronic active hepatitis (bridging necrosis) developed cirrhosis on histology after 4 years. Consumption of alcohol also increases both the risk of cirrhosis and HCC.[160] Infection with HCV, when accompanied by active replication of

both viruses, also increases the rate of progression to cirrhosis,[161,162] as does HDV.[163]

For patients with compensated cirrhosis, survival is 84% at 5 years and 68% at 10 years without treatment.[164] Once cirrhosis develops the risk of decompensation is 20% to 25% per year without treatment.[71,165] High HBV DNA levels and HBeAg seropositivity and persistence have been found to be independently associated with decompensated disease and death.[166–168] The prognosis is poor after the development of decompensation, with estimated survival rates of only 55% to 70% at 1 year and 14% to 35% at 5 years.[151,169]

Hepatocellular Carcinoma

Another major cause of mortality in chronic hepatitis B is HCC, which has a poor prognosis unless caught in a small, surgically resectable stage. HCC is the sixth most common cancer and the fourth leading cause of cancer-related deaths worldwide. An estimated 800,000 new cases occur each year, with chronic hepatitis B accounting for 18% (Europe) to 65% (China) of total cases.[170] The lifetime risk for untreated HBV chronic carriers is currently estimated to be about 20% to 40% and about 10- to 25-fold higher compared with non–HBV-infected controls.[171,172] In a recent systematic review of 66 studies of HCC in HBV-infected persons from Europe and North America, summary estimates of incidence rates according to liver disease status (95% confidence intervals [CIs]) were 0.05 (0.03 to 0.08), 0.31 (0.22 to 0.41), 0.42 (0.27 to 0.56), and 2.97 (2.35 to 3.59) in inactive carriers, asymptomatic carriers, subjects with chronic hepatitis, and with compensated cirrhosis, respectively.[173] Studies suggest that the interval between acquisition of HBV and development of HCC spans several decades (average, 30 years).[174] However, that interval is much shorter in parts of sub-Saharan Africa, where 43% of HBV-related HCC has been shown to develop in adults younger than 40 years.[174a] Coinfection with HDV, HCV, or HIV augments the risk of HCC development in HBV infection. More recently, patients with genotype C (Asians) and A1 (Africans),[175] as well as those with the A1762T and G1764A basal core promoter and pre-S gene mutations, have been shown to have a higher risk of HCC.[135,176] Other risk factors include older age, male gender, heavy alcohol consumption, metabolic syndrome, exposure to aflatoxin B, and family history of HCC.[177–180] Levels of HBV DNA are also a risk factor, with levels $>10^5$ copies/mL (≈20,000 IU/mL) being strongly linked to the development of HCC.[177] In a recent study the importance of serum HBV DNA level as a predictor of HCC was even greater in HBeAg-negative patients with normal ALTs and no cirrhosis at baseline.[181] HBsAg levels have also been shown to positively correlate with the development of HCC and better predict HCC in HBeAg-negative patients with low HBV DNA. In the Taiwanese *E*lucidation of *R*isk F*a*ctors for *Di*sease *C*ontrol or *A*dvancement in *T*aiwanese H*e*patitis *B* Carriers (ERADICATE-B) study, among a subset of 1068 HBeAg-negative individuals with HBV DNA levels ≥2000 IU/mL, the risk of HCC was significantly increased in these patients with an HBsAg level ≤1000 IU/mL compared with those with a level <1000 IU/mL (hazard ratio, 5.4; 95% CI, 2.1 to 14.2).[182] Clearance of HBsAg by the age of 50 years appears to decrease the risk of HCC.[183] Finally, treatment with nucleoside analogues decreases the risk of HCC about 30% to 80%.[180] To date, no studies have shown convincing data for an association between occult HBV, defined as the presence of HBV DNA without serologic markers of HBV infection, and the presence of HCC.

Extrahepatic Manifestations of Hepatitis B

Extrahepatic manifestations can occur in approximately 20% of patients with acute and chronic hepatitis B and are thought to be mediated by circulating immune complexes. Acute hepatitis may be manifested in 10% to 20% of patients as a serum sickness–like illness with fever, skin rash, arthralgias, and polyarthritis, typically occurring just before the onset and subsiding with the development of jaundice. The skin rash can be of virtually any type, including erythematous, macular, maculopapular, urticarial, or petechial. The most common extrahepatic clinical manifestations of chronic hepatitis B include sensorimotor neuropathies, myalgias, arthralgias, Sjögren syndrome, glomerulonephritis, uveitis, and Raynaud syndrome.[184] A number of autoantibodies can also be seen, including cryoglobulinemia and rheumatoid factor. Polyarteritis nodosa (PAN) is a rare complication of chronic hepatitis B, which usually manifests within the first 6 months of infection. It is a vasculitis of small- to medium-size arteries and typically presents with fever, rash, hypertension, eosinophilia, abdominal pain, renal disease, and polyarthritis. In one review the frequency of HBV infection in PAN cases was 33.7%; however, the overall incidence is declining.[185] Treatment of PAN usually includes the combination of plasma exchanges, anti-HBV therapies, and a short course of steroids in cases of life-threatening organ involvement.[186–188] Recovery from vasculitis can occur in up to 81% when HBeAg seroconversion and decreased viral replication are achieved.[189] HBV-related cryoglobulinemic vasculitis has been successfully treated with nucleoside therapies.[190,191] Glomerular disease also occurs as a manifestation of HBV infection. Nephrotic syndrome secondary to membranous or membranoproliferative glomerulonephritis is a rare complication of HBV infection occurring predominantly in children with active viral replication. The typical presentation is with nephrotic-range proteinuria. Approximately 30% to 60% of children with HBV-related membranous nephropathy undergo spontaneous remission, usually in association with HBeAg to anti-HBe seroconversion.[192] Progression to renal failure can occur, particularly in adults. The prognosis of HBV-related membranous nephropathy is variable. In adults it may lead to progressive renal insufficiency[193]; however, it can also be successfully treated. In a recent meta-analysis of 82 patients, treatment with IFN-α (five studies) or lamivudine (one study) resulted in proteinuria remission in 65.2% of the patients.[194]

CLINICAL MANIFESTATIONS AND NATURAL HISTORY IN SPECIAL HOSTS

Individuals With Human Immunodeficiency Virus Infection

Because of common parenteral routes of transmission, HBV and HIV are frequently seen in concert. Since the introduction of antiretroviral therapies (ARTs), the incidence of acute HBV infection in persons living with HIV has been declining. Earlier studies reported the incidence of HBV as high as 12.2 cases per 1000 person-years, although lower in those receiving either ART with lamivudine, ART without lamivudine, or one or more doses of HBV vaccine.[195] In a recent study of 2375 HBV-uninfected MSM enrolled in the Multicenter AIDS Cohort Study, the overall unadjusted HBV incidence rate was 9.6 per 1000 person-years. Incidence rates were significantly lower in the highly active antiretroviral therapy (HAART) era than in the pre-HAART era among both those infected with HIV (incidence rate ratio (IRR), 0.2 [95% CI, 0.1 to 0.4]) and not infected with HIV (IRR, 0.3 [95% CI, 0.2 to 0.4]).[196] Of interest, this study also found that receiving effective ART (HIV RNA < 400 copies/mL) was protective against acute hepatitis B, whereas men receiving ART who had HIV RNA ≥400 copies/mL were not protected. In fact, the incidence rate of acute hepatitis B in HIV-infected men with effective ART was equivalent to HIV-uninfected men.[196] Current estimates are that between 40% and 60% of individuals who are HIV seropositive in the United States are positive for some marker of past HBV infection, and 4% to 10% are HBsAg positive.[197] In the regions with high HBV endemicity, up to 25% of HIV-infected persons are coinfected with chronic HBV.[198] Patients with HIV and acquired immunodeficiency syndrome are more likely to have chronic HBV infection, increased replication manifested as higher HBV DNA, and increased likelihood of HBeAg positivity.[199] They are also more likely to have occult HBV; therefore HIV patients should be screened for both HBsAg and anti-HBc. The availability of ARTs with activity against both HIV and HBV has resulted in improved outcomes for patients with HIV/HBV coinfection. However, the risk of liver disease and overall mortality remains higher compared with those with either infection alone, even with effective virologic suppression.[200–202] In fact, as ART has reduced HIV-related deaths, liver-related deaths have now emerged as one of the leading causes of deaths in persons living with HIV. Many of these liver-related deaths are due to HCC, which among HIV/HBV coinfected persons is approximately fivefold to sixfold higher than in the general population, although less in the era of ART.[203] Other factors likely contribute to this increased risk of HCC, such as obesity, age,

HCV coinfection, and metabolic factors. Lower CD4 counts in HIV-HBV coinfected persons have also been associated with high HBV DNA levels and an increased risk of HCC and mortality.[204–206] Finally, isolated anti-HBc is found in approximately 16% to 20% of HIV-infected patients.[207] One study demonstrated that the isolated anti-HBc serologic pattern is usually stable, but when changes from isolated anti-HBc occur, the transition is usually to anti-HBc and anti-HBs positivity, suggesting that isolated anti-HBc is due to intermittently low levels of anti-HBs.[207] Rates of occult hepatitis B in those with isolated anti-HBc are variable, ranging from <5% up to 90%.[207–210]

Hepatitis B After Liver Transplantation

Reinfection of the allograft accounts for the majority of cases of HBV after liver transplantation in patients with chronic HBV. HBV can also be acquired de novo in patients who are HBsAg negative from anti-HBc–positive donors, with rates of infection as high as 77% in the absence of HBIG or antiviral prophylaxis.[211–213] This includes previously vaccinated recipients in whom HBV acquisition can also occur.[214,215] Recipients who are anti-HBs positive and anti-HBc positive are at the least risk of de novo infection occurring in 0% to 4%. Reactivation of HBV has also been reported in recipients who are HBsAg negative and anti-HBc positive pretransplant. Before the use of prophylactic HBIG and nucelos(t)ide therapy at the time of transplantation, graft reinfection rates approached 80% to 100% and was a major cause of allograft dysfunction, cirrhosis, and graft failure.[216] Reinfection was strongly correlated with high levels of HBV DNA, antiviral resistance, and HBeAg seropositivity.[217] The use of HBIG and nucleos(t)ide therapy, considered the standard of care in transplantation programs, has resulted in significant reductions in recurrence of HBV infection in the allograft, and post-transplant survival has improved markedly.[218] In a large study conducted between 1988 and 2002, the 1-, 5-, and 10-year patient survival rates were 91%, 81%, and 73%,[219] respectively, which are comparable with other conditions leading to transplantation. Specific HBV therapies used in liver transplantation are discussed in more detail under "Management in Special Populations" and "Liver Transplantation."

Hepatitis B After Other Types of Transplantation

HBV is associated with high morbidity and mortality in renal transplant recipients, which is thought to be due to persistent viral replication and reduced HBsAg seroconversion as a result of the effects of immunosuppression. HBV reactivation has occurred in renal transplant patients with previously resolved HBV infection characterized by negative HBsAg and positive anti-HBc, with or without anti-HBs, but the overall risk is low.[220] Reactivation is higher among those without anti-HBs compared with those with anti-HBs.[221] All renal transplant recipients who are HBsAg positive should receive antiviral therapy with nucleos(t)ide therapy (entecavir or tenofovir alafenamide [TAF] preferred) before or at the time of transplantation and continue indefinitely. Renal transplant recipients with anti-HBc with or without anti-HBs should have HBV DNA measured every 3 to 6 months after renal transplantation and receive nucleos(t)ide therapy if viral reactivation occurs but before onset of clinical hepatitis.[222] The risk of de novo infection in HBV naïve kidney recipients depends on use of prophylaxis and HBV immunity in the recipient and ranges from 0% to 27%. HBV reactivation is low among patients receiving transplants from anti-HBc–positive donors (1%–4%) and may present subclinically.[223] HBV prophylaxis before transplantation protects against HBV acquisition in nonimmune renal transplant recipients whose donors are anti-HBc positive. Recipients of bone marrow transplantations are also at risk of hepatitis B reactivation, which may present as a severe flare at the time of withdrawal of immunosuppression[224] or as progressive chronic liver disease.[225] HBV reactivation has been reported in 10% to 21% of allogeneic hematopoietic stem cell transplantation (HSCT) recipients with resolved HBV infection and without antiviral prophylaxis and 45% in HBsAg-positive auto-HSCT patients.[226,227] Lamivudine in these patients has been shown to reduce the incidence and severity of HBV reactivation.[228,229] Of interest, there have been case reports of hepatitis B cure after bone marrow transplantation due to the transfer of HBV-specific immune cells in the graft.[230,231]

Coinfection With Hepatitis C

Rates of HCV coinfection among patients with HBV range from 3% to 18%, depending on the geographic area and the epidemiologic risk group.[232] HCV infection is especially frequent in HBV endemic areas, in intravenous (IV) drug users, and among persons infected with HIV.[233] Superinfection and acute infection with HCV can result in suppression of HBV replication and clearance of HBsAg and HBeAg.[75,234–236] In turn, inhibition of HCV replication has been observed in patients with chronic HCV superinfected with HBV and higher rates of HCV RNA clearance have been reported in HBV/HCV coinfected compared with HCV monoinfected individuals.[237,238] However, when both viruses are replicating, the liver disease is usually more severe than in patients infected by HBV or HCV alone.[239–241] Patients with dual HBV and HCV infection may also have a higher rate of HCC compared with patients infected by either virus alone.[242] Treatment of HCV in HCV-HBV coinfected individuals has resulted in significant declines in HCC, all-cause mortality, and liver-related mortality.[243] In the past, treatment of HCV with PEG IFN was associated with both relatively high rates of HBsAg seroclearance (≈11%) and HBV reactivation (≈62%).[244,245] HBV reactivation has also been demonstrated in HBV-HCV coinfected patients during and after HCV treatment, with directly acting antivirals (DAA) in patients who are HBsAg positive with undetectable or low HBV DNA and in patients who are isolated anti-HBc positive.[246,247,248] The risk of reactivation is highest among HBsAg-positive individuals; reactivation among patients with isolated anti-HBc is limited to case reports.[249] Current Infectious Diseases Society of America/American Association for the Study of Liver Disease (AASLD) guidelines recommend all patients initiating HCV DAA therapy should be assessed for HBV coinfection with HBsAg testing and for evidence of prior infection with anti-HBs and anti-HBc. In HBsAg-positive patients HBV therapy should be initiated for patients who meet criteria for treatment (see "Treatment"); for patients whose HBV DNA level is below treatment initiation criteria, either prophylactic antiviral therapy or monitoring of HBV DNA levels during and immediately after DAA therapy with preemptive therapy is recommended.[250]

SCREENING AND DIAGNOSIS OF HBV INFECTION

Infection with HBV is associated with characteristic patterns of hepatitis B antigens and antibodies. In addition to confirming the stage of HBV infection, proper interpretation of the available tests will aid in the monitoring of patients and selection for antiviral therapy.

Acute Hepatitis

The diagnosis of acute hepatitis B is based on the detection of HBsAg and IgM anti-HBc (Table 145.2; see Fig. 145.7). During the replicative phase of infection, HBeAg and HBV DNA are also present. Recovery is accompanied by the disappearance of markers of HBV replication and the appearance of antibodies to these proteins.

HBsAg is the serologic hallmark of HBV infection and is the first marker to appear after acute infection. HBsAg appears in serum 1 to 10 weeks after an acute exposure to HBV, before the onset of symptoms or elevation of serum ALT. In patients who subsequently recover, HBsAg usually becomes undetectable after 4 to 6 months, followed by development of anti-HBs, as early as 8 months after infection. In most immunocompetent patients who recover, anti-HBs persists for life, thereby conferring long-term immunity.

HBcAg is an intracellular antigen that is not detectable in serum. Anti-HBc can be detected throughout the course of HBV infection, and its presence signifies natural infection. During acute infection, anti-HBc is detectable at the time symptoms appear (9–21 weeks after exposure) and is predominantly IgM class. It can be the sole marker of HBV infection during what is called the *window period* between the disappearance of HBsAg and the appearance of anti-HBs. Although IgM anti-HBc is usually an indicator of acute HBV infection, it may remain detectable up to 2 years after the acute infection and can be detectable during HBV flares. IgG anti-HBc persists even in individuals with HBs antigenemia, indicating that the presence of this antibody does not confer protection against viral replication.

TABLE 145.2 Interpretation of Serologic Tests in Hepatitis B

TEST	ACUTE HEPATITIS B	IMMUNITY THROUGH INFECTION[a]	IMMUNITY THROUGH VACCINATION	CHRONIC HEPATITIS B
HBsAg	+	–	–	+
Anti-HBs	–	+	+	–
HBeAg	+	–	–	±[b]
Anti-HBe	–	±	–	±
Anti-HBc	+	+	–	+
IgM anti-HBc	+	–	–	–
HBV DNA	+	–	–	+
ALT	Elevated	Normal	Normal	Normal or elevated

[a]On occasion, individuals with past infection have isolated anti-HBc only. The presence of an isolated immunoglobulin G anti-HBc may indicate a window period during acute infection or remote prior infection with loss of HBsAg or anti-HBs. In such cases an HBV DNA test may prove useful.
[b]Chronic hepatitis B with a precore mutant is $HBeAg^-$ and anti-HBe^+.
ALT, Alanine aminotransferase; *anti-HBc,* antibody to hepatitis B core antigen; *anti-HBe,* antibody to hepatitis B e antigen; *anti-HBs,* antibody to hepatitis B surface antigen; *DNA,* deoxyribonucleic acid; *HBeAg,* hepatitis B e antigen; *HBsAg,* hepatitis B surface antigen; *HBV,* hepatitis B virus; *IgM,* immunoglobulin M.

Past Hepatitis B Infection and Vaccination

Anti-HBs is the protective antibody and is present both after recovery from acute infection and successful vaccination. These two can be distinguished by testing for total anti-HBc (Table 145.3). Recovery from an HBV infection is characterized by the presence of both anti-HBs and total anti-HBc. Immunity to HBV after vaccination is indicated by the presence of anti-HBs only.

Chronic Hepatitis B Infection

The diagnosis of chronic hepatitis B is based on the persistence of HBsAg for more than 6 months. Additional tests for HBV replication—HBeAg and serum HBV DNA—should be performed in those with chronic hepatitis B to further classify the disease and to determine the need for treatment. In adults there are two groups of patients with chronic hepatitis B who do not need treatment but who should be monitored. The first are those who are HBeAg negative with an undetectable HBV DNA and normal serum aminotransferases. These patients are in the inactive phase of chronic hepatitis B (previously known as "inactive carriers") and should have aminotransferases tested every 6 to 12 months to detect reactivation. The annual rate of reactivation is about 4%, with pre-C mutations, male sex, and age older than 30 years being predictors of reactivation.[251] The second group is patients who are in the immune-tolerant phase of infection (see "Natural History" earlier) with HBeAg and high HBV DNA levels but normal or minimally elevated serum aminotransferases. They should also be monitored every 6 to 12 months with aminotransferase testing.

Those with progressive liver disease who should be considered for treatment can be either HBeAg negative or positive, have detectable HBV DNA, and usually have elevated serum aminotransferase concentrations. The HBV DNA level in HBeAg-positive chronic hepatitis B patients tends to be higher than in those with HBeAg-negative chronic hepatitis B.

In HBeAg-positive patients, development of anti-HBe can occur at any point during chronic hepatitis B infection; it occurs spontaneously in a small percentage of patients per year. It is often accompanied by an increase in serum aminotransferases, followed by a disappearance of HBeAg and HBV DNA from the serum and improvement in liver inflammation. However, some patients continue to have active liver disease and detectable HBV DNA in serum after HBeAg seroconversion.[252]

Measures of Hepatitis B Replication

PCR-based assays are the standard way to quantify HBV replication. Several real-time PCR assays are available now and have lower limits of sensitivity of approximately 10 IU/mL and upper limits of 4×10^9

TABLE 145.3 Selection of Patients for Treatment in Chronic Hepatitis B

HBeAg	HBV DNA[a]	ALT[b]	TREATMENT STRATEGY
+	>20,000 IU/mL	<2 × ULN	Observe patient Consider liver biopsy or noninvasive measurement of liver disease if age > 40, ALT > 1 but <2 × ULN, family history HCC, HIV positive; treat if moderate-to-severe inflammation or fibrosis
+	>20,000 IU/mL	>2 × ULN	Observe 3–6 mo for spontaneous HBeAg seroconversion before treatment Treatment with PEG IFN-α (48 wk) or NUC (indefinite in most; minimum 6–12 mo after HBeAg seroconversion)
–	>20,000 IU/mL	>2 × ULN	Treatment with NUC (end point not defined; likely indefinite) PEG IFN-α (48 wk) second line
–	>2000 IU/mL	1 to <2 × ULN	Consider liver biopsy or noninvasive measurement of liver disease and treatment if moderate-to-severe inflammation or fibrosis
–	>2000 IU/mL	<ULN	Observe; treat if HBV DNA or ALT increase
+/–	+	Cirrhosis	Compensated: treat with NUC if HBV DNA detectable Decompensated: NUC; consider liver transplantation
+/–	–	Cirrhosis	Compensated: observe Decompensated: consider liver transplantation

[a]Conversion factor to copies/mL = 5.6 (20,000 IU/mL is approximately 10^5 copies/mL).
[b]Also use moderate-to-severe necroinflammation on liver biopsy as guide.
ALT, Alanine aminotransferase; *anti-HBe,* antibody to hepatitis e antigen; *DNA,* deoxyribonucleic acid; *HBeAg,* hepatitis B e antigen; *HBV,* hepatitis B virus; *HCC,* hepatocellular carcinoma; *HIV,* human immunodeficiency virus; *NUC,* nucleoside or nucleotide; *PEG IFN-α,* pegylated interferon-α; *ULN,* upper limit of normal.
Modified from Terrault N, Bzowej NH, Chang KM, et al. AASLD Guidelines for the treatment of chronic hepatitis B. Hepatology. *2016;63;261–283.*

IU/mL. The major role of HBV DNA assays is to establish the phase of the infection and assess candidacy for treatment. In those who are treated, monitoring HBV DNA is essential for assessing the response. HBV DNA is also useful in several other circumstances. The first is distinguishing the window period of acute infection from chronic infection in those patients who are IgG anti-HBc only. HBV DNA is also useful in cases of fulminant hepatitis, where there may be undetectable levels of HBsAg on presentation. HBV DNA testing is also indicated in patients in whom there may be a false-negative HBsAg test, which occurs due to mutants in the surface protein that are not detected by all HBsAg assays.[253]

The enhanced sensitivity of the newer tests, especially PCR-based methods, has also raised questions about the significance of low-level viremia. Recovery from acute infection was formerly thought to be accompanied by complete clearance of HBV from serum. However, use of PCR-based methods has demonstrated that some individuals may have very low-level replication in the absence of any biochemical or histologic markers of liver injury.[254] The significance of this is not known, although some studies have suggested that such "occult" hepatitis B is associated with progressive liver disease. The threshold level that is associated with a risk of progression of chronic liver disease is not known. An arbitrary value of ≥20,000 IU/mL or 10^5 copies/mL has been suggested as a diagnostic criterion for chronic hepatitis,[254] but lower levels may be seen in patients with ongoing liver injury, especially in HBeAg-negative patients. Because levels of HBV DNA may fluctuate over time in a given individual, the use of HBV DNA testing as a sole determination of future prognosis is not recommended.[255] Recently there has been increasing interest in alternative biomarkers of HBV infection, such as hepatitis B core-related antigen (HBcrAg) or HBV RNA, that may better define the activity of chronic HBV infection, monitor infection, and predict long-term outcomes.[256–258] However, more data are needed on these markers before they can be incorporated in routine clinical care.

Isolated anti-HBc

The isolated presence of anti-HBc without other HBV serologic markers is not uncommon, being present in 0.4% to 2.3% of blood donors in low-prevalence areas[259,260] and in 10% to 20% of the population in endemic countries.[261] Isolated anti-HBc can be found in patients during the window period of acute hepatitis, many years after resolution of acute hepatitis due to a decline of anti-HBs to undetectable titers, or rarely, after years of chronic infection with decline of HBsAg to titers below the limit of detection. Individuals with isolated anti-HBc should have repeated testing done to exclude false-positive results and IgM anti-HBc to exclude acute hepatitis if that is suspected. If there are unexplained aminotransferase elevations in a patient with isolated anti-HBc, HBV DNA testing is recommended. Isolated anti-HBc is more common in people coinfected with HIV and in those with both chronic and cleared HCV.[207] The clinical significance of isolated anti-HBc with or without a low-level positive HBV DNA test is not clear; however, such patients should be considered potentially infectious because transmission of HBV from blood and organ donors with anti-HBc alone has been reported.[262,263]

MANAGEMENT OF HEPATITIS B

Chronic Hepatitis B

The goals of antiviral therapy in hepatitis B are reduction of the morbidity and mortality due to liver disease. The beneficial effects of antiviral therapy on reducing the incidence of cirrhosis and HCC have been shown for all of the approved anti-HBV medications. In a meta-analysis the risk reduction for cirrhosis with antiviral treatment was 0.39 (95% CI; 0.20 to 0.75) in observational studies and was 0.55 (95% CI; 0.38 to 0.78) in randomized clinical trials.[250] Reductions in death, HCC, and hepatic decompensation were also demonstrated.

Because reduction in morbidity and mortality is challenging to study, a variety of end points have been used as surrogates to define response to treatment in clinical trials, including normalization of serum aminotransferases (biochemical response), improvement in liver histology (histologic responses), achieving an undetectable HBV DNA in the serum (virologic response), and loss of HBeAg with or without anti-HBe (serologic response).[250] Elimination of HBsAg is rare but important because the risk of HCC continues to be elevated above baseline among patients with persistent HBs antigenemia.[183] The rate of HBsAg loss with IFN or tenofovir disoproxil fumarate (TDF) alone after 48 weeks of treatment is in the range of 3% to 4%.[36,264,265] Because long-term studies have demonstrated that either seroconversion to anti-HBe or durable suppression of HBV DNA is associated with reduction in cirrhosis,[250,266–268] an improvement in survival,[269–272] and reduction of the rates of HCC,[273] these are the most common surrogate markers used in both clinical practice and clinical trials to monitor the efficacy of therapy. Quantitative HBeAg and HBsAg have also been studied, but they are not clearly superior to the existing markers in terms of predicting long-term outcome. Markers such as circulating HBcrAg and pregenomic RNA are being assessed as additional potential markers (see "Measures of Hepatitis B Replication").

Acute Hepatitis B

Treatment in acute hepatitis B is generally supportive. Medication lists should be reviewed, and patients should be reminded to avoid medications metabolized by the liver if possible or limit the doses. This is particularly true for agents such as acetaminophen, which patients may be taking to minimize discomfort and fever. Treatment of fulminant hepatitis is also supportive, including liver transplantation for those patients who do not appear to have spontaneous recovery. Treatment with antiviral agents is not indicated for acute hepatitis B because approximately 95% will spontaneously seroconvert to anti-HBs; however, there is some evidence of benefit for cases of acute fulminant HBV. One randomized controlled trial of lamivudine versus placebo in acute severe hepatitis B showed greater decline in HBV DNA at 4 weeks with lamivudine but did not show long-term clinical benefit.[274] Several case series support that lamivudine, entecavir, and TDF may be beneficial when given before the onset of liver failure.[275,276] TAF should also be effective, but it has not been studied in fulminant, acute hepatitis B. If given, treatment for fulminant hepatitis B should be continued for 3 months after HBsAg seroconversion or for at least 12 months after HBeAg seroconversion if HBsAg loss does not occur. Treatment is also recommended for protracted severe acute hepatitis when increase in international normalized ratio and jaundice last for more than 4 weeks.[250]

Selection of Patients for Treatment

(Also see Chapter 117.)

The currently available treatments all have limited short- and long-term efficacy, as well as substantial costs, both in terms of side effects (e.g., as in the case of IFN) and financial considerations. Thus not all patients warrant treatment. Current recommendations are to treat all cirrhotic patients with detectable HBV DNA. For noncirrhotic patients, treatment should be initiated with detectable HBV DNA—either HBeAg positive with a HBV DNA >20,000 IU/mL or, in the case of HBeAg-negative disease, with HBV DNA >2000 IU/mL—and evidence of liver disease (serum aminotransferases greater than two times the ULN [30 U/L for men and 19 U/L for women] or moderate-to-severe necroinflammation on biopsy) (see Table 145.3).[250] These HBV DNA cutoffs were initially arbitrary, but there is evidence that at these levels the risk of progressive liver disease and HCC increases.[157] Because HBV DNA levels can fluctuate, serial monitoring of levels is important if treatment is not initiated.

Interferon

IFN-α is the recombinant version of one or more proteins that are naturally produced by the body in response to viral infection and has been used in the treatment of hepatitis B for more than 25 years. The precise mechanism of action as an anti-HBV therapeutic has not been established. One study demonstrated that IFN-α decreases transcription of pregenomic RNA and subgenomic RNA from cccDNA.[277] PEG IFN-α, given as a once-a-week injection of 180 μg, has supplanted standard IFN-α for treatment of hepatitis B. Factors predictive of a high response to PEG IFN-α are low HBV DNA, aminotransferase elevation greater than twice the ULN, age, female sex, and genotype A or B (compared with genotype C or D).[278] There is also some evidence that IL28B genotype may be associated with response to PEG IFN-α.[279] Treatment of patients

with normal ALT results in response rates of <10%,[280] suggesting an important contribution of the immune response in IFN-based therapy.

PEG IFN-α is administered as subcutaneous injections. Patients should be treated for at least 48 weeks, and there is some evidence that up to 72 weeks will increase the rate of sustained response in those with HBeAg-negative disease.[281] In HBeAg-positive patients, 1-year rates of HBeAg seroconversion are 27%[36,278]; the durability of the response is improved if PEG IFN-α is continued for at least 24 weeks after seroconversion. Quantitative HBeAg is a predictor of response to PEG IFN-α in HBeAg-positive disease, with levels >20,000 IU/mL at 24 weeks being associated with decreased likelihood of HBeAg seroconversion.[282] If HBeAg or HBsAg loss occur, the durability of the response in one study of 172 HBeAg-positive patients followed for 3 years was 81% and 30%, for HBeAg and HBsAg, respectively.[283] In HBeAg-negative disease, the rate of sustained undetectable HBV DNA, measured 24 weeks after 48 weeks of treatment, is approximately 19%. Given the toxicity of IFN-α, most providers do not use it in HBeAg-negative disease as the tolerability of long-term nucleos(t)ides is better.

Acute side effects of PEG IFN-α therapy include flulike illness, fever, myalgias, headache, and fatigue. Other side effects seen after prolonged dosing include leukopenia and thrombocytopenia, hair loss, and changes in mood, including depression, which can be severe. IFN therapy can also lead to the development of autoantibodies, such as antithyroid antibodies, and worsening of other autoimmune disorders.[284] PEG IFN-α is contraindicated in decompensated cirrhosis because it may precipitate flares with subsequent decompensation.[285]

Nucleoside and Nucleotide Analogues

Recognition that HBV, like HIV, has an RT step in its life cycle led to the testing of many nucleoside agents (Table 145.4). Because these agents are better tolerated than IFN-α, they are used long term for both viral suppression and improvement in histologic disease. Unlike IFN-α, nucleos(t)ide agents can be used in the setting of hepatic decompensation[286] and may even prevent or delay the need for transplantation.[287,288] However, resistance to some of these agents develops over time (see "Viral Resistance" later). The currently approved agents in this category include lamivudine, adefovir dipivoxil, telbivudine, entecavir, TDF, and TAF (also see Chapter 47). Emtricitabine, which is coformulated with TDF and TAF as an anti-HIV therapeutic, is also active against HBV but has not been approved for HBV treatment. These agents all suppress HBV replication but cannot directly eliminate cccDNA; thus, once therapy is discontinued, relapse of viremia occurs in the majority of HBeAg-positive patients who do not achieve HBeAg seroconversion and in nearly all HBeAg-negative patients.

Lamivudine

Lamivudine is the negative enantiomer of 2′,3′dideoxy-3′-thiacytidine (see Chapter 47). It is phosphorylated by host enzymes, and the incorporation of the triphosphate form into DNA results in premature chain termination, which inhibits HBV DNA synthesis. Lamivudine is administered at 100 mg/day in those without HIV infection and is well tolerated, with side effects no different than placebo. In patients coinfected with HIV, 300 mg/day is given to inhibit both HIV and HBV replication. Dose reduction is necessary for patients with renal insufficiency (creatinine clearance [CrCl] < 50 mL/min). Several large, randomized clinical trials in patients with HBeAg-positive and HBeAg-negative chronic hepatitis B demonstrate the efficacy of lamivudine (see Table 145.4).[289,290] A multinational study of Asian patients showed that HBeAg seroconversion rates increased over time from 17% to 27% at 2 years to 50% by 5 years.[291,292] An important predictor of HBeAg seroconversion at 1 year is the pretreatment aminotransferase level, ranging from 5% in patients with ALT less than two times the ULN to 64% in those with ALT greater than five times the ULN.[293] Patients with HBeAg-negative hepatitis B appear to have similar response rates,[294–296] as do patients who have previously failed IFN-α therapy.[297] Genotype, as with all the oral agents, is not a known predictor of response. Resistance to lamivudine develops when used as monotherapy limiting the durability of response; thus it is not a recommended first-line agent (see "Viral Resistance" later).

Adefovir

Adefovir dipivoxil is the oral prodrug of adefovir, a phosphonate nucleotide analogue of adenosine monophosphate (see Chapter 47). It is effective against both wild-type and lamivudine-resistant HBV[298–300]; however, it is the least potent of the oral agents (see Table 145.4) and is not a recommended first-line agent. Adefovir is administered at 10 mg daily with adjustment for renal insufficiency, and it should not be given with TDF or TAF.

In clinical trials, headache and abdominal pain are the most common side effects. Nephrotoxicity occurs in 3% of patients with compensated liver disease after 4 to 5 years of continued adefovir and in 47% of patients who underwent a liver transplant.[301,302]

Entecavir

Entecavir is a guanosine analogue that inhibits all three functions of the HBV polymerase: priming, negative-strand reverse transcription, and synthesis of the positive-strand HBV DNA (also see Chapter 47). It received US Food and Drug Administration (FDA) approval for the treatment of HBeAg-negative and HBeAg-positive chronic

TABLE 145.4 Approved Agents for Treatment of Chronic Hepatitis B With 48-Week Response

	PEG IFN-α	LAMIVUDINE	ADEFOVIR	ENTECAVIR	TELBIVUDINE	TENOFOVIR DF/AF
Route	Subcutaneous	Oral	Oral	Oral	Oral	Oral
Dose	180 μg/wk	100 mg/d[b]	10 mg/d[b]	0.5 mg/d[b] (1 mg/d if lamivudine resistant)	600 mg/d[b]	300 mg/d[b] for TDF and 25 mg/d for TAF
Duration (wk)	48	≥48	≥48	≥48	≥48	≥48
Tolerability	Fair-poor: flulike symptoms	Good	Good: follow renal function	Good	Good	Good: follow renal function and bone mineral density on TDF
HBeAg seroconversion	27%	16%–21%	12%	21%	22%	21%
Undetectable HBV DNA[c]	25%–63%	40%–73%	21%–51%	67%–90%	60%–88%	76%–93%
ALT normalization	38%	41%–75%	53%	72%	65%	74%
HBsAg loss	3%	<1%	0%	2%	<1%	3%
Viral resistance	None	15%–30%	Minimal	None[d]	6%	0%

[a]All data are for 1 year unless otherwise noted.
[b]Dose adjustment for creatinine clearance.
[c]Higher end of range for HBeAg-negative disease.
[d]None; otherwise, 7% if preexisting lamivudine resistance after 48 weeks and 50% after 5 years.
ALT, Alanine aminotransferase; *anti-HBe,* antibody to hepatitis B e antigen; *HBV,* hepatitis B virus; *HBsAg,* hepatitis B surface antigen; *PEG IFN-α,* pegylated interferon-α; *TAF,* tenofovir alafenamide; *TDF,* tenofovir disoproxil fumarate.
Modified from Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. *2009;50:661–662.*

hepatitis B in 2005. As one of the most potent anti-HBV agents, it is one of the first-line therapies for chronic hepatitis B in patients without lamivudine-resistant HBV. The recommended dose of entecavir is 0.5 mg/day for those who are treatment naïve and 1 mg/day in those with prior lamivudine experience because the activity is lower against lamivudine-resistant HBV. The dose should be adjusted for those with CrCl <50 mL/min. In a double-blind controlled trial of 709 HBeAg-positive patients, the 354 who received 48 weeks of entecavir had a decline in HBV DNA of 6.9 $\log_{10}$ copies/mL, which was superior to the 5.4 $\log_{10}$ copies/mL in the 355 who received lamivudine.[303] Furthermore, 67% in the entecavir group compared with 36% in the lamivudine group achieved HBV DNA that was <300 copies/mL, and histologic improvement occurred in 72% and 62% in the entecavir and lamivudine groups, respectively. However, HBeAg seroconversion was similar between the groups. Results after 96 weeks of treatment showed similar efficacy rates.[304] Similarly, in the HBeAg-negative patients, 70% of the 331 in the entecavir group had histologic improvement compared with 61% of the 317 in the lamivudine group. An undetectable HBV DNA was achieved in 90% in the entecavir group compared with 72% in the lamivudine group. In a study from China, after 7 years of entecavir undetectable HBV DNA was achieved by 96.8% of the HBeAg-positive patients and 100% of the HBeAg-negative patients.[305] Side effects to entecavir include headache, abdominal pain, and diarrhea.

Entecavir inhibits HIV-1 replication and can select for the lamivudine-resistant HIV mutation M184V when given as monotherapy[306]; thus HIV status should be checked before initiating therapy with entecavir. Furthermore, it should not be given to an HIV-coinfected patient without a suppressive HAART regimen.

Telbivudine

Telbivudine is an L-nucleoside analogue that is structurally related to lamivudine (see Chapter 47) and thus has overlapping resistance patterns at the rtM204 position. Telbivudine is administered at 600 mg daily and has potent activity. However, due to relatively high rates of resistance and cross-resistance with lamivudine, it is not a first-line agent. It is controversial as to whether it inhibits HIV-1 replication. The GLOBE trial, the registration trial for telbivudine, was an international study randomizing patients to telbivudine ($n = 680$) or lamivudine ($n = 687$) daily.[307] At 52 weeks the proportion with undetectable HBV DNA (<300 copies/mL) was greater in the telbivudine than the lamivudine group (60% vs. 40%, respectively). At week 104 telbivudine continued to be superior, but the proportion with undetectable HBV DNA decreased to 56%. These changes resulted from the development of resistance at rtM204I, which reached 25% at week 104. Results of the HBeAg-negative group also showed superiority of telbivudine over lamivudine and demonstrated poor durability of response due to development of resistance, which occurred in 8.6% of the telbivudine group.

Tenofovir Disoproxil Fumarate (TDF) and Tenofovir Alafenamide (TAF)

TDF was approved for treatment of hepatitis B in 2008 and is also approved for treatment of HIV-1 (see Chapters 47 and 128). TDF is potent, and resistance has not clearly been identified; thus it is a recommended first-line agent in patients with and without lamivudine-resistant HBV. TDF is dosed at 300 mg daily and needs to be adjusted for renal insufficiency. In two phase III trials comparing TDF with adefovir, viral suppression occurred more often in those receiving TDF than adefovir dipivoxil in both HBeAg-negative (93% vs. 63%, $P < .001$) and HBeAg-positive patients (76% vs. 13%, $P < .001$) after 48 weeks.[264] Of significance, more HBeAg-positive patients treated with TDF than those treated with adefovir dipivoxil had normalized ALT levels (68% vs. 54%, $P = .03$) and loss of HBsAg (3% vs. 0%, $P = .02$). TDF produced a similar HBV DNA response in patients regardless of prior lamivudine use. Continued use of TDF up to 7 years demonstrates that 99% of the HBeAg-positive and HBeAg-negative patients on treatment have HBV DNA <29 IU/mL.[308] No signature TDF-resistance mutations have been identified. The major side effects of TDF are renal insufficiency with increases in serum creatinine and decrease in bone mineral density.

TAF has equivalent anti-HBV activity to TDF but has less kidney and bone toxicity.[309,310,311] It was approved for treatment against hepatitis B in November 2016 and is given at a dose of 25 mg once daily. Two randomized, double-blinded studies compared TAF with TDF for the treatment of HBeAg-positive and HBeAg-negative chronic hepatitis B. After 48 weeks of treatment, HBV DNA of <29 IU/mL was achieved in 94% of the TAF group and in 93% of the TDF group in the HBeAg-negative study.[310] The proportions were 64% and 67% for TAF and TDF, respectively, in the HBeAg-positive study.[311] In both studies the patients receiving TAF had significantly smaller declines in bone mineral density and lower changes in glomerular filtration rate.

Other Agents and Combination Therapy

The other agent that is approved for HIV treatment but is also active against HBV is emtricitabine. Emtricitabine is similar to lamivudine, but it has a longer half-life. Mutations that confer resistance to lamivudine also confer resistance to emtricitabine (see Chapters 47 and 128).

Combination therapy for hepatitis B is attractive because it may increase potency and decrease the rates for developing resistance; however, these concepts have not been clearly demonstrated to date, so combination therapy is not generally recommended. PEG IFN-α and lamivudine for 48 weeks had greater HBV DNA decline compared with monotherapy with either one alone; however, the relapse rate in the combination group was higher, so 24 weeks after the end of treatment, there was no difference between the PEG IFN-α group alone or with lamivudine.[36,265] Another study showed that adding PEG IFN-α for 24 weeks after 24 weeks of entecavir improved HBV DNA, HBeAg, and HBsAg responses compared with continuing entecavir for 48 weeks; however, this study did not have a PEG IFN-α monotherapy arm.[312] Other studies have not shown a benefit of PEG IFN-α added after 12 weeks of entecavir compared with PEG IFN-α alone.[313,314] In a randomized, open-label study, the combination of TDF and PEG IFN-α demonstrated greater HBsAg loss (9%) compared with either TDF or PEG IFN-α alone (0% and 2.8%, respectively); however the rates were low in all groups.[315] Another randomized open-label study of HBeAg-positive patients on TDF or entecavir for more than12 months with HBV DNA <2000 IU/mL found that adding PEG IFN-α compared with continuing the nucleoside alone for 48 weeks had greater HBeAg seroconversion, but it was not statistically significantly different (18% vs. 8%, $P = .31$).[316]

Given the expansion in the number of oral agents available, it is desirable to consider combining these as is done in HIV therapy. The oral anti-HBV drugs all target the same part of the HBV life cycle; thus the situation is not entirely analogous to HIV, in which drugs target different aspects of the viral life cycle. In a randomized, open-label study combining the two most potent agents, entecavir and TDF compared with entecavir alone, there was no difference after 96 weeks of therapy between the 197 subjects receiving combination therapy and the 182 subjects receiving entecavir alone for the primary end point (<50 IU/mL) of HBV DNA (83.2% and 76.4%, respectively, $P = .088$).[317] However, when stratified by HBeAg status, the HBeAg-positive patients who received combination therapy were more likely to have a virologic response compared with the monotherapy group ($P = .046$), which was entirely attributable to those with baseline HBV DNA $>10^8$ IU/mL. There were no differences in HBeAg or HBsAg seroconversion in any groups. Thus this study suggests that combination therapy may be most beneficial in those with high HBV DNA levels, but more studies of longer duration are needed to confirm this.

Viral Resistance

The major limitation in the use of oral agents for hepatitis B is the development of resistance, which has been observed with the use of all approved oral agents except tenofovir (DF and AF), albeit at different times and frequencies. Resistance mutations differ based on the structural group of the drug, which include: L-nucleosides, D-cyclopentanes, and acyclic nucleotides. Cross-resistance occurs between agents in the same group and may decrease sensitivity between groups. The L-nucleosides group consists of lamivudine, emtricitabine, and telbivudine, with the primary resistance mutation occurring at position rt204 of the HBV polymerase, which is essential for polymerase activity, and leads to

substitution of isoleucine (I) or valine (V) or serine (S) for methionine.[291,318,319] Of the L-nucleosides, genotypic resistance develops most rapidly to lamivudine, with 14% to 32% of patients having evidence of mutations after 1 year of treatment.[289,290] Cumulative resistance increases over time, with one study demonstrating rates of 15%, 38%, 55%, 67%, and 69%, at 1, 2, 3, 4, and 5 years, respectively.[291] HBV mutants with the rtM204V/I appear to have reduced replication capacity in vitro and in vivo compared with wild-type HBV.[320] However, the development of a compensatory mutation at rtL180M restores replication capacity, at least in vitro.[320] The rates of lamivudine resistance in patients treated for HBeAg-negative chronic hepatitis B appear to be more variable (0%–27% at 1 year and 10%–56% at 2 years).[296,321]

Although the rates of resistance to telbivudine are lower than to those of lamivudine (11% vs. 26% at 2 years), resistance is high enough to preclude its widespread use.[322] Telbivudine is not active against HBV strains bearing lamivudine mutations rtL180M/rtM204V/I but remained active against the rtM204V single mutant in vitro, potentially explaining the difference in resistance profiles between telbivudine and lamivudine. Against HBV genomes with known telbivudine-resistance mutations, rtM204I and rtL80I/M204I, telbivudine, lamivudine, and entecavir lost 353- to >1000-fold activity, whereas adefovir and TDF exhibited no more than threefold to fivefold change.[323]

The second group, D-cyclopentanes, consists of entecavir, which has a high genetic barrier to resistance as it requires the presence of multiple mutations, including the rtM204V and rtL180M mutations, along with either the rtT184S/A/I/L or rtC202G/C or rtM250 mutations. In patients who are treatment naïve and do not have a preexisting rtM204V or rtL180M mutation, the rates of resistance are <1% per year with a 5-year cumulative rate of 1.2%.[324] However, in patients with the characteristic lamivudine mutations rtM204V and rtL180M, the cumulative incidence is 51% after 5 years; therefore treatment with tenofovir (DF or AF) is preferred over entecavir in patients with lamivudine-resistant HBV. Due to overlapping resistance mutations with lamivudine, lamivudine should be discontinued when entecavir is started, to theoretically decrease the risk for developing entecavir resistance.

The third group, acyclic nucleotides, consists of tenofovir (DF and AF) and adefovir dipivoxil. Resistance to adefovir dipivoxil occurs at residue rtN236T and rtA181V/T of the HBV polymerase. After 240 weeks of treatment the cumulative probability of resistance was 29% for HBeAg-negative patients. In patients with prior lamivudine-resistant mutations, cumulative incidence of adefovir dipivoxil resistance was higher—43% at 4 years.[325] In HBV cell lines expressing adefovir dipivoxil resistance mutations rtN236T and rtA181V, telbivudine remained active as shown by respective changes of 0.5-fold (rtN236T) and 1.0-fold (rtA181V and rtA194T).[323]

TDF resistance rates appear low, but precise mutations with confirmed resistance to TDF have not been characterized. Through 5 years of therapy, TDF maintains HBV DNA suppression, and there is no clear evidence of resistance.[326] The adefovir resistance mutations rtA181T/V and rtN236T confer decreased sensitivity to TDF. In one retrospective study, patients on TDF with these mutations were less likely to achieve HBV DNA <400 copies/mL than those without the mutations.[327] Because TAF is a different prodrug than TDF, the resistance patterns will be the same.

Resistance is suspected with the reappearance of HBV DNA in a patient who was previously undetectable or with a >1 $\log_{10}$ increase in HBV DNA in a patient who is compliant with therapy. Serum aminotransferases may or may not become elevated, and there are rare instances of acute exacerbations and hepatic decompensation.[328] Commercial assays are available to detect resistance mutations. If resistance occurs, current recommendations are to change therapy to the most effective agent that does not share cross-resistance. In general, the L-nucleosides (lamivudine, telbivudine, and entecavir) are not cross-resistant with the acyclic nucleotides (adefovir, tenofovir DF/AF), with tenofovir DF or AF preferred. In cases of multidrug resistance a combination of entecavir and tenofovir DF or AF is recommended.[329,330]

Given the overlapping reading frames, mutations in the HBV polymerase can lead to changes in the surface antigen, including in the immunogenic "a" determinant. One study demonstrated that a triple mutant that occurs on lamivudine monotherapy (rtV173L/rtL180M/rtM204V) leads to the surface changes sE164D/I195M. These changes in the surface antigen lead to reduced binding of anti-HBs in vitro, and thus the virus behaves as a vaccine escape mutant.[331]

Monitoring and Treatment Duration of Nucleos(t)ide Therapy

Virologic response to nucleos(t)ide analogue therapy is based on HBV DNA levels and should be evaluated every 3 months after initiating or changing therapy until the HBV DNA is undetectable and then monitored every 3 to 6 months. In addition to HBV DNA, HBeAg (in HBeAg-positive patients), and ALT should also be monitored (Table 145.5). If there is less than a 1-log IU/mL decline in the first 3 months, this is defined as primary nonresponse, which is most commonly due to noncompliance, especially if a patient is on entecavir, TDF, or TAF. If a patient with a primary nonresponse is compliant, then resistance testing is appropriate to determine a change in the treatment strategy. After 12 months of therapy a virologic response is defined as having an undetectable HBV DNA by a sensitive PCR assay. However, if after 12 months of therapy in a patient who is compliant, the HBV DNA is detectable but has declined more than 1 $\log_{10}$, then this is defined as a partial virologic response. In this case a treatment-naïve patient receiving entecavir, TDF, or TAF can continue the same therapy because there is a low rate of resistance. In a patient receiving a less potent drug, changing to entecavir, TDF, or TAF is recommended. In patients with low-level persistent viremia on entecavir, TDF, or TAF, there is little evidence that adding a second drug is beneficial. Virologic breakthrough can occur at any time and is defined as >1 log IU/mL increase in HBV DNA from the nadir value on therapy. The main causes of virologic breakthrough are noncompliance with therapy or viral resistance (see "Viral Resistance" earlier).

The duration of therapy for most individuals is lifelong, but there are some patients for whom discontinuation of therapy can be considered. For patients with HBeAg-positive chronic hepatitis B who have HBeAg seroconversion, current recommendations are to treat for 1 year after seroconversion and then consider discontinuing therapy if HBV DNA is undetectable and ALT is normal.[250] The exception

TABLE 145.5 Virologic Response to Nucleos(t)ide Treatment

RESPONSE	EVALUATION TIME (MONTHS)	CRITERIA	THERAPEUTIC STRATEGY
Primary nonresponse	3	<1 $\log_{10}$ decline	Assess compliance. If compliant, obtain resistance test and change treatment.
Partial virologic response	12	HBV DNA detectable but >1 $\log_{10}$ decline from baseline	If treatment naïve and taking entecavir or tenofovir DF/AF, then continue. If treatment naïve and taking less potent drug, change to entecavir or tenofovir DF/AF. If treatment experienced on entecavir, either change to or add tenofovir DF/AF.
Virologic response	12	HBV DNA undetectable by sensitive PCR assay	Continue treatment and monitor every 3–6 mo.
Virologic breakthrough	Any time	>1 $\log_{10}$ increase in serum HBV DNA level from on therapy nadir	Obtain resistance test and change therapy (see "Viral Resistance" in text).

AF, Alafenamide; *DF,* disoproxil fumarate; *DNA,* deoxyribonucleic acid; *HBV,* hepatitis B virus; *PCR,* polymerase chain reaction.

to this is that patients with cirrhosis should not be considered for discontinuation of therapy. Once HBeAg seroconversion has occurred, the rate of durable response after discontinuation ranges from 30% to 80%.[332] However, due to the possibility of relapse, some recommend continuing therapy until HBsAg seroconversion, which is infrequent. In one study recurrent viremia occurred in 90% of the 39 patients who stopped therapy a median of 12 months after seroconversion, compared with 0% in those who continued therapy, so monitoring every 3 months with serum aminotransferases and HBV DNA is important after discontinuation of therapy.[250,333] There is some evidence that quantitative HBsAg may be useful to predict who will relapse after therapy discontinuation.[334] If a patient does not have HBeAg seroconversion but has suppressed HBV DNA replication, then therapy should be continued indefinitely because there is evidence of progressive enhancement of the HBeAg seroconversion rate with longer duration of treatment, and patients may continue to derive histologic benefit.[267] In HBeAg-negative patients therapy should be continued indefinitely because relapse occurs in the majority of cases except in rare instances of HBsAg seroconversion. However, the European guidelines suggest that, in selected noncirrhotic HBeAg negative patients with undetectable HBV DNA for at least 3 years, discontinuation of therapy could be considered with close monitoring.[65] In support of this, one study of HBeAg-negative patients with undetectable HBV DNA for ≥3.5 years found that 13 of 21 people who discontinued TDF remained off therapy through 144 weeks.[335] In both HBeAg-negative and HBeAg-positive disease, therapy discontinuation can be considered 6 to 12 months after HBsAg seroconversion, which only occurred in 8% after 6 years of TDF.

MANAGEMENT IN SPECIAL POPULATIONS

Liver Transplantation

Recurrent hepatitis B after liver transplantation can occur in patients with a history of acute or chronic hepatitis B before transplantation or in HBsAg-negative recipients of anti-HBc–positive donors. Historically, rates of recurrence of HBV infection were high (90%), and the consequences of reinfection were devastating, such that HBV was considered a relative contraindication to liver transplantation. HBIG was introduced in the early 1980s and nucleosides in the early 1990s, with progressive reduction in likelihood of HBV recurrence with each intervention. Using HBIG alone, recurrence rates ranged from 20% to 25%, depending on the schedule and trough anti-HBs titer. The addition of oral nucleos(t)ide therapy (lamivudine) to HBIG was shown to further decrease recurrence rates to as low as 10% and improve mortality.[336,337] Similar to nontransplant patients, however, treatment with lamivudine alone resulted in the emergence of lamivudine resistance and severe and even fatal posttransplantation disease.[338] Tenofovir (DF or AF) or entecavir are now the preferred nucleos(t)ide analogues and are >95% effective in protecting the grafts from recurrent disease. Both have been shown to suppress lamivudine-resistant variants[298] and even resolution of graft failure in those patients who have them.[339] In a recent study evaluating long-term outcomes in 265 chronic HBV liver transplant recipients treated with entecavir without HBIG, followed for a median of 59 months, 92% were negative for HBsAg at 8 years, and 100% had undetectable HBV DNA. Eighty-five percent of the patients were alive at 9 years.[340] In a randomized controlled trial comparing TDF and emtricitabine with and without HBIG in orthotopic liver transplant recipients, followed for a median of 3.4 years, no recurrences of HBV occurred in either arm, suggesting therapy with TDF and emtricitabine without HBIG is effective at reducing HBV recurrence.[341] Although an increasing number of transplant centers are using nucleos(t)ide analogues alone for post-transplant prophylaxis, HBIG continues to be recommended in certain high-risk patients, including individuals with high HBV DNA levels, known antiviral resistance, higher risk for recurrence of HCC, HIV coinfection, and HDV coinfection.[342] Finally, although TDF is most studied in posttransplant patients, TAF is a better alternative due to its safer renal profile. In summary, with currently available combination therapy, survival is excellent in patients undergoing liver transplantation for chronic hepatitis B, even in those with active viral replication pretransplantation.

Vaccination after transplantation has variable success; in one study, active immunization after liver transplantation induced anti-HBs titers (>10 UI/mL) in 82% of liver transplant candidates with inactive HBV disease[343]; however, in a later study of liver transplant recipients with cirrhosis who received conventional HBV vaccine after transplantation, only 18% achieved protective anti-HBs titers.[344] Other reports of conventional vaccine failure after liver transplantation have been reported.[345,346] The use of repeated immunization with recombinant HBV vaccine emulsified in novel adjuvants in patients who were HBV DNA negative after orthotopic liver transplantation resulted in anti-HBs levels ranging from 721 to 83,121 IU/L, depending on the concentration, in 80%, which remained stable after discontinuation of HBIG.[346] However, in a more recent study of eight liver transplant recipients with similar characteristics but in whom HBIG was stopped, the use of an adjuvant containing HBV vaccine resulted in development of anti-HBs in only one patient.[347]

Human Immunodeficiency Virus

The efficacy of the nucleos(t)ide agents does not seem to be compromised by coinfection with HIV.[348] However, with lamivudine monotherapy, drug resistance develops more rapidly than in HBV monoinfection.[349] Lamivudine-resistant HBV is common in HIV coinfection because it has been a backbone agent for HIV therapy for years. Tenofovir (DF and AF) has potent activity against both HIV and HBV[350] and is also effective against lamivudine-resistant HBV.[351–353] The combination of TDF or TAF and emtricitabine (or TDF and lamivudine) is therefore now recommended in the treatment of chronic HBV in HIV-infected individuals.[354] TAF is less likely to adversely impact renal function and bone mineral density without compromising efficacy and thus is preferred over TDF when CrCl > 30 mL/min. The three approved fixed-dose TAF-containing regimens include elvitegravir/cobicistat/emtricitabine/TAF; emtricitabine/rilpivirine/TAF; and emtricitabine/TAF. Current Department of Health and Human Services (DHHS) HIV treatment guidelines recommend initiation of ART in all HIV-infected patients, including those with HIV/HBV coinfection.[354] If TDF or TAF cannot be safely used, entecavir should be used with a fully suppressive ART regimen because entecavir can also inhibit HIV replication and select for the HIV drug-resistance mutation M184V.[306] A higher dose of entecavir (1 mg) should be used in patients who have already received lamivudine or have known lamivudine resistance. However, due to the relatively high rate of resistance of entecavir in those with lamivudine resistance (see "Viral Resistance" earlier), HBV DNA should also be monitored frequently.[354] If HBV needs treatment and a fully active anti-HIV regimen cannot be given, then drugs that are active only against HBV and do not lead to HIV drug resistance, such as adefovir dipivoxil or PEG IFN, are recommended. Telbivudine is not recommended, given its intermediate rate of resistance in HBV monoinfection and unknown rates in HIV coinfection. Treatment is indefinite unless HBsAg seroconversion occurs.

Recipients of Immunosuppressive Therapies

A number of cytotoxic chemotherapeutic agents, immunomodulators, and immunosuppressants have been associated with reactivation of HBV. Agents that have been most commonly associated are prednisone, anthracyclines, and anti-CD20 blockers, specifically rituximab or ofatumumab as part of chemotherapy.[355] The frequency of reactivation in patients undergoing cytotoxic chemotherapy ranges from 14% to 72% in different studies and case series, depending on serologic status of the patient and potency of immunosuppressants. Patients at risk for reactivation include those with resolved infection (HBsAg negative, anti-HBs, and anti-HBc positive), those with inactive chronic hepatitis B (HBsAg positive, HBV DNA undetectable), and those with untreated chronic active hepatitis B (HBsAg positive, HBV DNA detectable). Tumor necrosis factor-α (TNF-α) inhibitors, including infliximab, etanercept, and adalimumab and low-dose steroids, have a low-to-moderate risk of reactivation. Very few cases of reactivation have been reported with the use of immunosuppressants, including azathioprine and methotrexate.[355] Anthracyclines carry a moderate risk for HBV reactivation in HBsAg-negative, anti-HBc–positive patients and a high

risk in HBsAg-positive patients. The risk of reactivation of HBV associated with rituximab, a monoclonal antibody directed against B-cell marker CD20 and increasingly used in standard chemotherapy regimens for many rheumatologic disorders, carries the highest risk of reactivation in patients who are anti-HBc positive, with or without anti-HBs. In a recent meta-analysis of case series and reviews of HBV reactivation in lymphoproliferative disorders associated with rituximab, there was a more-than-fivefold higher rate of HBV reactivation in patients with a history of prior HBV exposure (anti-HBc positive, with or without anti-HBs) who received rituximab-based therapy, compared with those who did not.[356] In a study of 46 HBsAg-negative/anti-HBc–positive patients (the majority of whom were anti-HBs positive), 25% of patients receiving rituximab and cyclophosphamide-hydroxydaunorubicin-Oncovin (vincristine)-prednisone (CHOP) for diffuse large B-cell lymphoma reactivated HBV (became HBsAg positive) compared with 0% who received CHOP alone.[357] In 2013 the FDA issued a black box warning concerning the risk of HBV reactivation in patients receiving anti-CD20 monoclonal antibodies after more than 100 cases of fatal HBV-related liver failure associated with rituximab or ofatumumab were reported in postmarketing data from the FDA adverse-event report system between 1997–2012.[357a]

Prophylaxis is recommended in HBsAg-positive patients at highest risk of reactivation, if untreated, especially if they are taking B-cell–depleting drugs, anthracycline derivatives, or high-dose steroids or receiving bone marrow transplants.[358] Anti-HBc–positive patients who are HBsAg positive or negative and at moderate risk of reactivation, that is, patients on TNF-α inhibitors, low-dose steroids, and anthracycline inhibitors (if HBsAg negative) can be closely monitored or receive prophylaxsis.[355] Although lamivudine is the most widely studied antiviral agent (associated with reductions in risk of HBV reactivation of 79%–100%), tenofovir (DF or AF) and entecavir are now preferred, especially when the duration of prophylaxis is expected to be longer than 1 year. Entecavir and TDF have higher antiviral potency and lower rate of resistance with long-term use. In a randomized trial in patients with diffuse large B-cell lymphoma receiving chemotherapy with rituximab-CHOP (R-CHOP), entecavir was found to be superior to lamivudine for the prevention of HBV reactivation and HBV-related hepatitis in those with prechemotherapy HBV DNA ≥2000 IU/mL. TDF has also shown to be effective in reducing HBV reactivation in anti-HBc–positive patients with hematologic malignancies treated with rituximab.[359] The optimal duration of prophylactic therapy is unknown; current recommendations are to start preferred antiviral drugs with immunosuppressive medication and continue 6 to 12 months after the last dose of treatment. For patients receiving B-cell–depletion agents, therapy should be continue 12 to 18 months after the last dose, as reactivations as late as 17 months after completion of therapy have been reported.[250,360,361] Among patients with higher levels of baseline HBV DNA (>2000 IU/mL), the American Association for the Study of Liver Disease (AASLD) recommends continuing antiviral therapy until they reach treatment end points for HBV infection.[250] Among bone marrow transplantation recipients, the risk of reactivation of HBV in HBsAg-positive patients is up to 54%.[358] The use of preemptive antivirals therefore, in these situations, is also recommended. Of interest, a donor bone marrow with natural immunity (anti-HBs and anti–HBc positive) may result in clearance of HBsAg.[362]

Treatment of Hepatitis B Virus During Pregnancy

All women presenting for prenatal care should be routinely tested for HBsAg early in their pregnancy. There are at least three indications for treatment of HBV during pregnancy: (1) to treat active liver disease in situations when therapy cannot be delayed, (2) the continuation of HBV treatment in a mother who becomes pregnant while on HBV therapy, and (3) to prevent transmission of HBV from mother to child in mothers with high circulating viremia. All treatment decisions need to take into consideration the risks and benefits for mother and fetus (potential exposure to teratogenic drugs). TDF (category B) and lamivudine (category C) have been studied most extensively in pregnancy. In the Antiretroviral Pregnancy Registry and the Development of Antiretroviral Therapy study (DART), birth defects among women exposed to HBV therapy (mostly TDF and lamivudine) were similar to that in the general population in the first and second trimesters and to other classes of antiretrovirals.[363] Similarly, to date no adverse outcomes have been reported with telbivudine (category B).[364] A recent systematic review and meta-analysis of antiviral therapy in chronic HBV found no evidence of fetal adverse effects or congenital defects associated with TDF.[364] There are minimal data on the safety of adefovir or entecavir or TAF (category C) in pregnancy; thus they should be avoided in pregnancy until more clinical data are available. Interferon is contraindicated in pregnancy. Based on data demonstrating a decrease in perinatal transmission, current guidelines recommend treatment of pregnant women in the third trimester when HBV DNA > 200,000 IU/mL (see also "Prevention of Perinatal Transmission" later). The guidelines also recommend that pregnant women in the immunoactive phase are managed the same as nonpregnant women, with the caveat that only drugs tested in pregnant women should be used.[250]

OTHER MANAGEMENT ISSUES IN CHRONIC HEPATITIS B

Patients with chronic hepatitis B should be counseled about disease-modifying factors, as well as means to prevent spread of HBV to other persons. For example, patients should be counseled about the means of spread of delta hepatitis and hepatitis C to avoid superinfection with these viruses. Patients should also be counseled to consume minimal, if any alcohol, in the absence of data regarding safe levels of consumption and because consumption of large amounts of alcohol is clearly a risk factor for more rapid progression to cirrhosis. Other major issues in the management of patients with chronic hepatitis B include prevention of hepatitis A, prevention of spread of HBV, and surveillance for HCC.

Hepatitis A Vaccination

The official recommendation of the Advisory Committee for Immunization Practices (ACIP) in the United States is that all persons with chronic liver disease be vaccinated against hepatitis A virus (HAV).[365] The data supporting this recommendation are not strong[366] because at least one study revealed that the risk of fulminant HAV is significantly increased only in patients with underlying hepatitis C and not hepatitis B.[367] However, the guidelines of the AASLD call for immunization of all patients with chronic HBV against HAV.

Screening and Vaccination of Contacts

Sexual and household contacts of persons with HBV are at increased risk of infection. All sexual partners and household contacts should be tested for HBV and vaccinated if seronegative. Until the immunization series is complete, sexual partners should use barrier methods. Both patients and contacts should be counseled regarding the modes of transmission and advised on methods to prevent household transmission, including avoiding sharing of items that might be contaminated with small amounts of blood, such as toothbrushes, and the need to cover open wounds. Pregnant women or women who want to become pregnant and are infected with HBV should also be counseled on the risk of transmission to the newborn and the method to prevent such transmission. Postexposure prophylaxis with HBIG and hepatitis B vaccine should be given to infants born to HBsAg-positive mothers, unvaccinated infants whose mothers or primary caregivers have acute hepatitis B, sexual contacts of persons with hepatitis B, and health care workers after occupational exposure to HBsAg-positive blood depending on their vaccination and vaccine response status. Household and sexual contacts of persons with chronic HBV infection should be vaccinated.

Recommendations for the health care worker infected with HBV vary from country to country. Although there is general agreement that individuals with HBeAg or HBV DNA >10,000 IU/mL pose the greatest risk of transmission,[368] there have been documented cases of transmission from health care workers in the absence of HBeAg and during "low-risk" procedures.[369,370] Updated Centers for Disease Control and Prevention (CDC) guidelines published in 2012 recommend that most chronically HBV-infected providers and students who adopt standard infection control precautions do not require curtailing their practices. Previous recommendations were updated to also include the following: (1) prenotification of a health care provider's or student's HBV status is no longer required; (2) the use of HBV DNA serum levels rather than

HBeAg status is recommended to monitor infectivity; and (3) for those health care professionals requiring oversight (i.e., health care worker performing highly exposure-prone procedures), there are specific suggestions for composition of expert review panels and threshold value of serum HBV DNA considered "safe" for practice (<1000 IU/mL).[371]

Surveillance for Hepatocellular Carcinoma

In multiple longitudinal studies, carriers of HBsAg have been shown to be at increased risk of developing HCC.[372] The risk is higher among males and those who are older, have a family history of HCC, consume alcohol regularly, have elevated serum ALT levels, have positive HBeAg status, have hepatitis B DNA >10^4 copies/mL (≈2000 IU/mL), have genotype C virus, have cirrhosis, and have a longer duration of infection.[177,373,374] The goal of HCC screening is to detect small, surgically resectable tumors because the prognosis for more advanced lesions is poor. Current recommendations are that HBsAg-positive persons at high risk for developing HCC should be periodically screened; these include Asian males older than 40 years, Asian females older than 50 years, African/North American blacks older than 20 years, those with a family history of HCC, those with cirrhosis, and persons with ALT elevations or HBV DNA >2000 IU/mL, or both.[250] In a single, randomized, controlled trial conducted in China comparing surveillance using ultrasonography and α-fetoprotein levels with no surveillance, the incidence of HCC-related mortality was reduced by 37%.[375] However, there are no randomized trials of HCC surveillance in US populations, and several studies show that α-fetoprotein determination lacks adequate sensitivity and specificity. Therefore the current recommendation for HCC screening is with ultrasonography alone every 6 months.[375] The role of other imaging technologies, such as computed tomography, in screening have not been adequately assessed. Although the risk of HBV is decreased in patients achieving viral suppression, it is not eliminated, especially in the setting of cirrhosis. A study following Chinese patients with chronic hepatitis B on adefovir for 10 years found that the incidence of HCC in cirrhotic patients was 43.1% compared with 7.1% in noncirrhotics.[376] Furthermore, cirrhotic patients who achieved virologic suppression had a lower incidence of HCC than those who did not (27.8% vs. 62.2% 10-year incidence), and the same pattern was seen in noncirrhotic patients (4.0% vs. 13.4%). Thus, even with virologic suppression, HCC occurs and surveillance every 6 months is recommended.

PREVENTION OF HEPATITIS B VIRUS INFECTION

The HBV vaccine is effective in preventing HBV and its complications. It has been shown in multiple studies to be safe, efficacious, and immunogenic, with seroconversion rates of >90% in healthy adults and seroprotection rates in infants born to HBV-infected mothers of 88.5% to 95.8%.[377–379] Successful vaccination prevents HBV infection and therefore reduces HCC-related mortality.[380] In Taiwan, which was an early adopter of universal HBV vaccination in children, the average annual incidence of HCC in 6- to 14-year-olds declined from 0.70 per 100,000 children between 1981 and 1986, before widespread vaccination, to 0.36 between 1990 and 1994, after initiation of widespread vaccination ($P < .01$).[381] The corresponding rates of mortality from HCC also decreased.

Active Immunization

Vaccines against hepatitis B have been available since 1982. The first vaccines were plasma derived and have now been completely replaced by recombinant vaccines. The vaccines contain small HBsAg as the major component produced in transfected yeast cells. Hepatitis B vaccines are available in monovalent forms for use in adults and for birth doses (Engerix-B [GSK; Philadelphia, PA] and Recombivax HB [Merck; Kenilworth, NJ]) or in combination with other vaccines for infant vaccination, including diphtheria-tetanus-pertussis (DTP), *Haemophilus influenzae* type b (Hib), and inactivated polio vaccine (IPV) (Pediarix; GSK). Pediarix can only be administered before age 6 weeks through 6 years of age. A combination vaccine (TWINRIX R; GSK), which expresses both HBsAg and hepatitis A, is also available and is approved for use in persons ≥18 years of age in the United States and Europe (see Chapter 316). Despite high efficacy of recombinant vaccines, certain populations remain at risk of suboptimal or nonresponse to these vaccines (see "Efficacy"). Recently, the first and only two-dose hepatitis B vaccine, Heplisav-B, was approved for use in adults 18 years of age and older. This novel vaccine uses HBsAg adjuvanted with immunostimulatory phosphorothioate oligodeoxyribonucleotide sequences that stimulate the innate immune system through Toll-like receptor 9 (TLR-9). The approval of Heplisav-B was based on data from three phase III noninferiority trials of nearly 10,000 adult participants who received the vaccine.[382,383] Results from the three trials have demonstrated high levels of protection early after administration with the largest trial, which included 6665 participants, demonstrating a statistically significantly higher rate of protection of 95% compared with 81% for Engerix-B. In a subgroup analysis of 961 participants with type 2 diabetes, Heplisav-B demonstrated a statistically significantly higher rate of protection of 90% compared with 65% for Engerix-B.[384] Additional subgroup analyses revealed significantly higher rates of protection among smokers, persons with body mass index ≥30 kg/m^2, and older age groups who are typically at risk of suboptimal responses with recombinant vaccines. Adults younger than 40 years had a similar response.[385] Heplisav-B has not been studied in persons infected with HIV.

Indications for Vaccination

All persons at high risk of acquiring HBV (see "Routes of Transmission" earlier) should be offered vaccination if nonimmune. Recent best-practice advice from the American College of Physicians and CDC recommends the following at-risk patient groups should be vaccinated: (1) all unvaccinated adults (including pregnant women) at risk for infection due to sexual, percutaneous, or mucosal exposure; (2) health care and public safety workers at risk for blood exposure; (3) adults with chronic liver disease and end-stage renal disease (including hemodialysis patients); (4) adults with HIV infection; (5) travelers to HBV-endemic regions; and (6) any adults seeking protection from HBV infection.[386,387] In 2011 the ACIP updated their guidelines to recommend vaccination in all adults age 19 to 59 years with diabetes. Universal vaccination of all infants has been recommended in the United States since 1991, and has since been adopted by many other countries and incorporated into routine childhood immunization programs. As of 2015, 185 (95%) of World Health Organization (WHO) member states vaccinated infants against hepatitis B as part of their vaccination schedules; global coverage with three doses of HBV vaccine during infancy reached 84%. Since the introduction of the HBV vaccine (ranging from the 1980s–2000s, varying by country) and 2015, the prevalence of HBV infection among children younger than 5 years has fallen from 4.7% to 1.3%[388] In the United States in 2014, 92% of infants had been fully vaccinated with three doses of HBV vaccine.[389] In October 2016 ACIP recommended that all US-born infants who weigh at least 2000 g receive a dose of HepB vaccine before 24 hours of age.[390]

Dose Regimen

Two single-antigen hepatitis B vaccines are currently licensed in the United States: Engerix-B and Recombivax HB. Engerix-B is formulated to contain 20 μg HBsAg/mL, and Recombivax HB contains 10 μg HBsAg/mL (Table 145.6). The recommendation for adults is to administer 1 mL of either Engerix-B or Recombivax HB in three doses at months 0, 1 to 2, and then at 6 to 12 months. In infants, three doses of 0.5 mL of vaccine are required to complete the course. For adolescents (11–19 years of age), three doses of 0.5 mL of Recombivax HB or 1 mL of Engerix-B is recommended.[391] Either vaccine can be interchanged during the series of injections. An optional two-dose regimen of 1 mL Recombivax HB has also been approved for adolescents age 11 to 15 years, with a second dose given 4 to 6 months after the first dose.[392] The recently approved Heplisav-B is administered in two doses over 1 month at 0 and 4 weeks, although there are no formal guidelines currently regarding who should receive this vaccine over the other two. However, since safety in pregnancy has not been assessed, pregnant women should receive either Engerix-B or Recombivax HB. Vaccines should be administered intramuscularly because deposition of the vaccine into adipose tissue results in a lower seroconversion rate.[393] Adverse reactions are uncommon, and most consist of soreness at the

TABLE 145.6 Doses and Schedules of Licensed Hepatitis B Vaccines

HEPATITIS B VACCINES	AGE	DOSE	VOLUME	SCHEDULE
Engerix-B	<20 yr	10 µg	10 µg/0.5 mL	Infants[b]: 1st dose within 24 h of birth, 1–4, 6–18 mo Older children: 0, 1–2, 4 mo
	>20 yr	20 µg	20 µg/1 mL	0, 1, 6 mo
	Diabetes 19–59 yr[c]	20 µg	20 µg/1 mL	0, 1, 6 mo
	Dialysis and other immunocompromised	40 µg	2–20 µg/1 mL doses	0, 1, 2, 6 mo
Recombivax HB	<20 yr	5 µg	5 µg/0.5 mL	Infants[b]: 1st dose within 24 h of birth, 1–4, 6–18 mo Older children: 0, 1–2, 4 mo
	11–15 yr	10 µg	10 µg/1 mL	0, 4–6 mo
	>20 yr	10 µg	10 µg/1 mL	0, 1, 6 mo
	Diabetes 19–59 yr[c]	10 µg	10 µg/1 mL	0, 1, 6 mo
	Dialysis and other immunocompromised	40 µg[d]	40 µg/1 mL	0, 1, 6 mo
Heplisav-B[e]	>18 yr	20 µg	20 µg/0.5 mL doses	0, 1 mo
COMBINATION VACCINES	**AGE[f]**	**ANTIGEN**	**VOLUME**	**SCHEDULE**
Pediarix	6 wk–6 yr	Engerix-B, Infanrix (DTaP), and IPV	0.5 mL	For newborns, give monovalent hepatitis B vaccine within 24 h of birth, then give Pediarix at 2, 4, 6 mo
Twinrix	>18 yr	Havrix (HAV) and Engerix-B (20 µg)	1 mL	0, 1, 6 mo

[a]All vaccines should be administered intramuscularly in the deltoid. https://www.cdc.gov/hepatitis/hbv/hbvfaq.htm#vaccFAQ.
[b]Infants born to hepatitis B surface antigen (HBsAg)-positive mothers should have hepatitis B immune globulin (HBIG) within 12 h of delivery, along with vaccine at a separate site. If mother's HBsAg status is unknown, administer vaccine within 12 h and test mother. If mother is HBsAg positive, administer HBIG within 1 wk.
[c]Hepatitis B vaccination may be administered at the discretion of the treating clinician to unvaccinated adults with diabetes mellitus who are age ≥60 yr (see http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6050a4.htm).
[d]Special formulation.
[e]Safety and effectiveness of Heplisav-B have not been established in adults on hemodialysis.
[f]Birth dose should be monovalent vaccine only; subsequent doses can be combination.
DTaP, Diphtheria, tetanus, acellular pertussis (vaccine); *IPV,* inactivated polio vaccine.

injection site. Low-grade fever, malaise, headache, and myalgias are seen in <1% of vaccinees. The vaccine can be administered during pregnancy.

Efficacy

A protective level of immune response after vaccination is defined as a titer of anti-HBs of >10 IU/L. Although this was somewhat arbitrary, clinical studies suggest that a decrease in titer below this level is associated with a risk of infection. Both Recombivax HB and Engerix-B administered over three doses, as described previously, result in a protective antibody response in >95% of adults, infants, and children. In persons receiving the newly approved Heplisav-B, 95% of patients exhibited a seroprotective response 8 weeks after the second dose of vaccine.[382] Because the response rate in healthy individuals is so high, routine postvaccination testing in persons receiving Recombivax HB and Engerix-B is not recommended, except among immunocompetent individuals who are at high risk of repeated exposure to HBV, such as health care workers, IV drug users, chronic hemodialysis patients, and individuals who are at risk for recurrent exposure to HBV (e.g., sexual partners of carriers, MSM). Testing should be performed 1 to 2 months after the vaccine series in these individuals. For these individuals who have no or inadequate anti-HBs titers after a primary series, the recommendation is to administer one or more doses of vaccine. After one to two doses, up to 25% of previous nonresponders or hyporesponders may have adequate titers. Almost all healthy adults who do not respond to a primary three-dose series with anti-HBs concentrations of >10 IU/L will respond to a three-dose revaccination series.[394] Postvaccination testing is also recommended in infants born to HBsAg-positive mothers, which should be performed at age 9 to 12 months to avoid detection of anti-HBs from HBIG administered during infancy.[395] Vaccination response is decreased in certain populations, including those older than 40 years; those who are obese, have particular genetic polymorphisms, and have a lack of sleep; and those who smoke, have HIV or any form of immunosuppression, diabetes mellitus, renal failure, chronic liver disease, improper administrations (e.g., administration into the buttock or subcutaneous injection), and freezing of the vaccine.[396,397]

Efficacy in Patients With Human Immunodeficiency Virus Infection

In patients with HIV response rates vary between 18% and 71%.[398–400] Although a strict correlation between response and immune status is not always found, response is better with CD4 T-cell counts >500 cells/mL.[401,402] US guidelines do not recommend delaying HBV vaccination in patients with HIV infection. HIV-infected persons who are nonresponders to HBV vaccine are not protected from infection and have similar rates of HBV infection as unvaccinated individuals with HIV, unlike immunocompetent individuals who may have some level of protection from the vaccine, even if anti-HBs titers fall below protective levels.[403] This is thought to be due to decreased numbers of antigen-specific memory B cells in HIV-infected individuals. A number of vaccination strategies to boost immunity in HIV-infected individuals have been examined, including accelerated schedules (four vaccine doses at 0, 1, 2, and 6 months),[404] intradermal administration, and rescue vaccination after primary vaccination, with variable success. Another strategy is use of adjuvants as stimulators of immunogenicity of the vaccine, although studies show mixed results.[405–407] Currently DHHS recommends the standard three-dose HBV vaccination, although other vaccines strategies are recommended by some experts, including the use of double-dose vaccine at 0, 1, and 6 months, with or without additional doses.[408]

Efficacy in End-Stage Renal Disease

In patients on chronic hemodialysis the response rate to recombinant vaccines is 50% to 60%.[409] In addition, anti-HBs levels decline more rapidly compared with healthy individuals.[410] Intradermal injection has been shown to be associated with higher seroconversion rates than intramuscular administration.[411,412] The CDC recommends all end-stage renal disease patients receive double doses of conventional recombinant HBV vaccine, preferably before the onset of dialysis; administration of a fourth vaccine dose may be necessary to increase the seroprotection rate (see Table 145.6). Testing 1 to 2 months after vaccination is recommended for hemodialysis patients to determine their response to the vaccine.[413] They should also undergo yearly anti-HBs

testing and receive a single booster dose of vaccine if the titer falls below 10 IU/L.

Durability of Response

There is excellent durability of response after a successful primary HBV vaccine series with Recombivax HB and Engerix-B. No long-term durability data are available from Heplisav-B. In a study from Alaska, one of the longest post-HBV vaccine cohorts, 94% of Native Alaskan children and adults who had received plasma-derived HBV vaccine at a minimum of 6 months of age in the early 1980s had evidence of immunity at 22 years of follow-up, even though only 60% of the vaccinated cohort retained detectable levels of anti-HBs. Complete protection was conferred for up to 22 years in those immunized as children and adults, and for up to 15 years in those immunized as infants.[414] A recently published study extended follow-up to 30 years and indicated that >90% of vaccines had evidence of protective immunity, based on anti-HBs levels ≥10 IU/L or responses to booster doses of vaccine.[415] A companion study indicated that evidence of T-cell immunity to HBV was present even if anti-HBs levels were not detected.[416,417] Long-term data from Taiwan, where 20% of the population is infected with chronic hepatitis B, indicate that the vaccine provides long-term protection up to 20 years in adults.[418] Among HIV-infected individuals the durability of a response to HBV vaccination is significantly associated with higher anti-HBs titers after primary vaccination, with anti-HBs responses >100 IU/L associated with the most prolonged seroprotection.[419] Breakthrough infections have been reported in successfully vaccinated individuals who were subsequently exposed to HBV after a full vaccine series; however, the number of such cases is few, and clinically significant infections are rare.

Postexposure Immunoprophylaxis

Postexposure prophylaxis with HBIG and vaccine is recommended for all nonimmune individuals who have percutaneous, sexual contact, ocular, or mucous membrane exposure to blood, including human bites that penetrate the skin (see Chapter 316), where the source is known to be at high risk of being HBsAg positive. The first dose of 0.06 mL/kg should be administered as soon as possible, preferably within 12 hours, although there is a window period of up to 24 hours.[420] The first vaccine dose should be given at the same time, although in a different site, followed by the remainder of the series. For individuals who are vaccinated but who do not have documentation of adequate titers of anti-HBs, recommendations are to administer both HBIG and a vaccine booster dose pending documentation of adequate anti-HBs. Individuals who have failed to respond to a vaccine series require two doses of HBIG 1 month apart.

Prevention of Perinatal Transmission

In women with chronic HBV infection, the risk of transmission of HBV infection to their child without prophylaxis is high and varies according to HBeAg status of mothers from 12% for HBeAg-negative/anti-HBe–positive mothers, to 25% for HBeAg-negative/anti-HBe–negative mothers, to 70% to 90% for HBeAg-positive mothers.[421–423] Maternal serum HBV DNA levels also correlate with the risk of transmission.[424] It is recommended that all neonates born to mothers with chronic hepatitis B receive HBIG 0.5 mL IM at birth and hepatitis B vaccine within 12 hours of birth, followed by vaccine dose at 1 and 6 months, which together are 85% to 95% effective in preventing perinatal HBV infection.[422,423] In a large systematic review and meta-analysis the addition of HBIG decreased risk of transmission from mother to infant compared with HBV vaccine alone[425]; however, it may not be necessary in HBsAg-positive/HBeAg-negative mothers.[426,427] Administration of a "birth-dose" HBV vaccine (within 12 hours of birth), compared with administration after 12 hours of birth, is associated with further reductions in HBV transmission, although birth-dose coverage remains low in many developing countries (39% in 2016).[428] As of 2016, 97 WHO member states have introduced the hepatitis B birth-dose vaccine.[429] Despite the relative success of immunoprophylaxis, a significant proportion of infants remain at risk for HBV transmission. Ten percent to 30% of infants develop HBV infection when mothers have high levels of viremia (defined as HBV DNA $>2 \times 5 \log_{10}$ IU/mL)[430,431,432]

Updated 2016 AASLD HBV treatment guidelines now recommend antiviral therapy beginning in the third trimester, to reduce the risk of perinatal transmission of hepatitis B in HBsAg-positive pregnant women with an HBV DNA level >200,000 IU/mL.[250] TDF remains the first-line recommended therapy based on its safety and efficacy, although telbivudine and lamivudine are also recommended. In a recent randomized, placebo-controlled trial, Pan and colleagues[433] evaluated 200 HBeAg-positive mothers with HBV DNA levels $>2 \times 5 \log_{10}$ IU/ mL and compared TDF therapy (300 mg daily) from 30 to 32 weeks of gestation to 4 weeks postpartum versus no drugs (control subjects). All infants received standard immunoprophylaxis at birth. A reduction in mother-to-child transmission for TDF compared with control subjects was found (0% vs. 7%; $P = .01$).[433] Other studies with lamivudine and telbivudine have shown similar effects.[434–436] In a recent systematic review of safety studies with TDF, many found no difference in fetal or maternal adverse effects.[436] Resistant variants have been shown in up to 19% of women receiving lamivudine in prior studies,[437] and these resistant variants rarely occur in women receiving telbivudine.[438] More data are needed on the efficacy and safety of entecavir and TAF in pregnancy before they can be recommended. Additional information is also needed on optimal timing of antiviral therapy during pregnancy, the optimal HBV DNA thresholds to treat patients with antivirals, and resistance patterns in infants with nucleos(t)ide therapy. There is no evidence that cesarean section prevents maternal-infant transmission, and thus routine cesarean section is not recommended.[439] Neither breastfeeding nor amniocentesis appears to increase the risk of transmission.[440]

Hepatitis B Surface Antigen Escape Mutants

HBs mutants have been described in infants infected with HBV after passive-active vaccination,[441] in vaccinated adults, in liver transplant recipients who have received prolonged courses of HBIG to prevent recurrence of HBV in the allograft,[442,443] and in immunocompromised patients with resolved HBV infection receiving dasatanib.[444,445] The most common mutations are the glycine-to-arginine substitution at codon 145 (G145R) and the sD144A in the "a" determinant sequence of HBsAg, which decrease binding of HBsAg to anti-HBs. The prevalence of these escape mutants is increasing over time, but the clinical and epidemiologic importance and the impact on current vaccination strategies are unclear. Selection of these HBs antigen mutants appears to be a rare event, and their prevalence even in HBV endemic areas is low.[446,447] In a chimpanzee model of infection, the current vaccines appear to protect against the spread of HBs mutants.[448]

ACKNOWLEDGMENTS

We want to thank Aleem Siddiqui for his contributions to this chapter in the sixth edition of this book and Margaret Koziel for her contributions to the seventh edition.

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The complete reference list is available online at Expert Consult.

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146 Hepatitis Delta Virus

Chloe Lynne Thio and Claudia Hawkins

SHORT VIEW SUMMARY

Definition
- Hepatitis delta virus (HDV) occurs as a coinfection or superinfection with HBV, establishing a chronic infection in hepatocytes.
- About 5% of people with chronic hepatitis B globally have evidence of exposure to HDV.
- HDV prevalence generally mirrors hepatitis B virus (HBV) prevalence, although there are geographic differences.
- HDV is primarily transmitted via the parenteral route, but there is some sexual transmission. Perinatal transmission is uncommon.

Microbiology
- HDV is a small, defective RNA virus that relies on host cell machinery for replication.
- HDV requires the envelope of HBV for viral assembly and transmission.

Clinical Presentation and Diagnosis
- Clinical presentation of HDV is variable, but both chronic and superinfection can be present as acute severe hepatitis.
- Superinfection of HDV in individuals with chronic hepatitis B results in chronic infection in greater than 90% of cases. The risk of cirrhosis and hepatocellular carcinoma is significantly elevated in these patients.
- Laboratory diagnosis of HDV is with enzyme immunoassay for HDV antibodies and HDV RNA by real-time polymerase chain reaction.

Therapy
- HDV is treated with pegylated interferon-α weekly for a minimum of 48 weeks.
- Newer therapies are currently under development.

Prevention
- Vaccination against hepatitis B (see Chapter 145) can be preventative because HDV needs HBV to replicate.

HEPATITIS DELTA VIRUS

Delta agent was identified by Mario Rizzetto in 1977 as a nuclear antigen distinct from hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg) in hepatocytes of some HBsAg carriers in Italy. It soon became clear that this passenger virus, termed *hepatitis D virus* (HDV), accompanied hepatitis B virus (HBV) infection and required the HBsAg for transmission. This unique HDV RNA genome is circular and resembles plant pathogens, including viroids and virusoids.[1] HDV is the only member of the genus *Deltavirus*.[2] In nature HDV is only found in patients who are also infected with HBV.

HDV is an enveloped virus of 36 nm that is distinct from 22-nm HBsAg or 42-nm HBV particles. Because HDV derives its viral envelope from HBV, the outer coat consists of the three forms (L, M, and S) of HBsAg and host lipids that surround the nucleocapsid, which includes the HDV genome in a complex with hepatitis delta antigen (HDAg). The genome is a small, single-stranded, circular RNA genome of approximately 1680 nucleotides, which has a high guanine-cytosine content, allowing it to fold into a rodlike structure.[3] Similar to other RNA viruses, there is no proofreading ability of the RNA polymerases, so the mutation rate is high, and the virus exists as a swarm of closely related quasispecies in an individual. The C-terminal region of the large-HDAg (L-HDAg) is the most variable.

Hepatitis Delta Antigen

The HDAg is the only known protein encoded by HDV and consists of two isoforms: small S-HDAg (24 kilodalton [kDa]) and large L-HDAg (27 kDa) (Fig. 146.1). Both are initiated from the same adenine-thymine-guanine (ATG) codon on the antigenomic RNA and are identical except that the L-HDAg has an additional 19 amino acids at the end of the C-terminus due to a unique RNA editing event during replication via the cellular enzyme adenosine deaminase acting on RNA 1 (ADAR1).[4–6] Each is translated from a distinct species of RNA. Both L- and S-HDAgs are phosphorylated and contain a nuclear localization signal that imports them into the nucleus.[7,8] L-HDAg acts as a dominant-negative inhibitor of replication and is necessary for binding HBsAg for virion assembly.[9] Binding of the L-HDAg to HBsAg is facilitated by prenylation of the C-terminal cysteine residue, which is a current target for new therapeutics (see "Treatment"). S-HDAg is involved in the initiation of RNA replication and is believed to have RNA chaperone activity.[10] Posttranslational modification of the S-HDAg affects the synthesis of genomic RNA and is a potential novel therapeutic target. Of note, L-HDAg and S-HDAg downregulate HBV replication through repression of two HBV enhancer regions and by activating the myxovirus resistance A (MxA) gene, which reduces export of HBV messenger (m)RNA from the nucleus.[11]

Hepatitis D Virus Life Cycle

Hepatocyte Entry

HDV infects only hepatocytes with no evidence of extrahepatic sites of its replication. The mechanism of HDV entry is similar to HBV because it requires L-HBsAg for entry through binding the sodium taurocholate cotransporting polypeptide receptor, followed by internalization through endocytosis (see "Attachment, Entry, and Hepatotropism" in Chapter 145). Once in the hepatocytes, the HDV nucleoprotein is released and translocated by HDAg to the nucleus via a nuclear localization signal.[3] The host range of HDV is limited to those species that can support the replication of hepadnaviruses and supply, in *trans*, the HBsAg envelope. For instance, HDV can infect woodchucks and can be packaged with HBsAg derived from woodchuck hepatitis virus. Chimpanzees are susceptible to HDV infection, and the infection is similar to that in humans.[12]

RNA Genome and Replication

The HDV RNA genome does not encode protein. Instead, the complementary antigenomic HDV RNA codes for HDAg and contains a sequence of approximately 85 nucleotides with an intrinsic ribozyme activity.[13,14] It is estimated that about 300,000 copies of genomic RNAs can be found in an infected hepatocyte during replication and 10-fold–less antigenomic RNA. S-HDAg is believed to be involved in RNA replication and may serve as an RNA chaperone.[15]

HDV replication involves the transcription of HDAg-encoding RNA from genomic RNA, the synthesis of antigenomic RNA from genomic RNA, and the synthesis of genomic RNA from antigenomic template via a double rolling circle mechanism (Fig. 146.2). Because HDV does

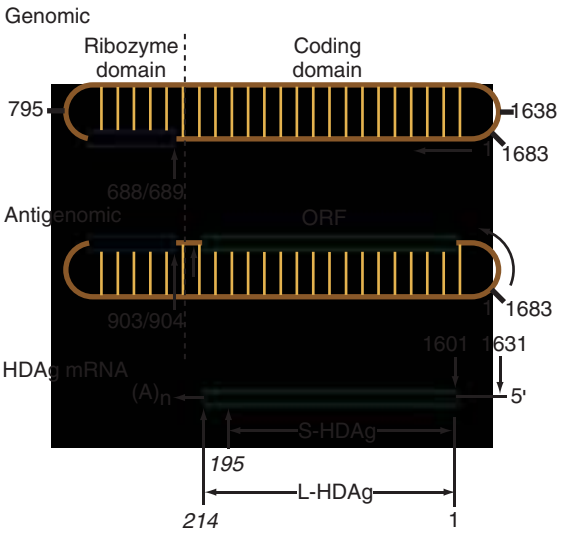

FIG. 146.1 Schematic structures of hepatitis D virus genomic and antigenomic RNA and hepatitis D antigen *(HDAg)*-encoding messenger RNA *(mRNA).* The minimum ribozymes are indicated by light boxes. The italicized numbers for HDAg are amino-acid residues. All other numbers are nucleotide positions on the genomic-sense RNA. The L-HDAg and S-HDAg are encoded by the antigenomic strand indicated by the open reading frame *(ORF). (Modified from MacNaughton TB, Lai MC. The molecular biology of hepatitis delta virus. In: Ou JE, ed.* Hepatitis Viruses. *Norwell, MA: Kluwer; 2002:109–128.)*

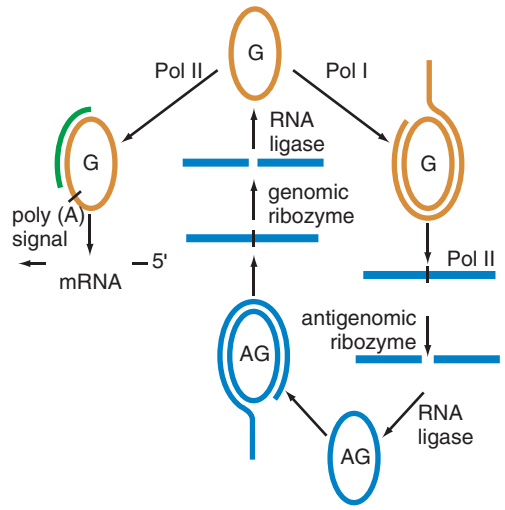

FIG. 146.2 Proposed model of hepatitis D virus RNA replication. The hepatitis D antigen-encoding messenger RNA *(mRNA)* is synthesized by polymerase II *(Pol II)* from the genomic RNA template independently of RNA replication. RNA Pol I synthesizes antigenomic RNA, and RNA Pol II synthesizes the genomic RNA. *AG,* Antigenomic strand; *G,* genomic strand. *(Modified from MacNaughton TB, Lai MC. The molecular biology of hepatitis delta virus. In: Ou JE, ed.* Hepatitis Viruses. *Norwell, MA: Kluwer; 2002:109–128.)*

not encode a polymerase, it recruits the host RNA polymerase II (Pol II) for transcription of HDV mRNA and for genomic RNA synthesis and likely uses host Pol II, and possibly also host Pol I, for antigenomic RNA synthesis.[10,16] Because of the rolling circle mechanism of RNA synthesis, multimeric linear transcripts are produced that are cleaved into monomers by the ribozyme activity. The linear product is ligated to form the antigenomic molecule, which then serves as a template for the synthesis of genomic RNAs through a rolling circle mechanism; HDV then interacts with the clamp of RNA Pol II, a cellular structure that holds RNA and DNA in place. HDAg loosens the clamp, which facilitates forward translocation of RNA Pol II while sacrificing fidelity.[17] HDV envelopment follows the scheme of virion assembly similar to HBV. Because HDV assembly can also occur in an L-HBsAg–independent fashion,[18] noninfectious HDV virions can be produced in abundance (≈90%). These particles are noninfectious because of the absence of pre-S1 domain, which is required for infectivity.

Pathogenesis

HDAg is the only viral protein known to be expressed during HDV infection. A high titer of immunoglobulin (Ig)M anti-HDV is strongly associated with elevated hepatitis D viremia and the severity of liver injury and alanine aminotransferase (ALT)/aspartate aminotransferase levels, whereas a more favorable course to HDV infection is found in individuals with IgG anti-HDV.[19] Although these antibody responses were present during acute and chronic infection, there was no association of IgM anti-HDV levels with HDV RNA, suggesting there is no protective role of anti-HDV antibodies. Woodchucks immunized with recombinant HDAg are only partially protected from subsequent challenge with HDV, which suggests that other mechanisms are responsible for immunity.[20]

The mechanisms of liver damage in HDV infection are unclear. There is conflicting evidence regarding whether HDV has a direct viral cytopathic effect that contributes to hepatocyte injury. In cell lines, expression of HDAg led to cytotoxic changes[21]; however, in transgenic mice, HDAg expression had no cytopathic effect.[22] HDV RNA levels have not been associated with severity of liver disease in 80 individuals with genotype 1 disease, supporting the concept that HDV does not directly lead to hepatocyte injury.[23] It is likely that the adaptive immune response plays a role in liver injury; however, the data are also conflicting. In one study HDV-specific T-cell responses correlated with lower ALT levels, suggesting that immune control of viral replication leads to lesser degrees of liver injury.[24] However, in another study histologic assessment demonstrated that the degree of cellular infiltration in the portal tracts and lobules correlates with the degree of staining for HDAg in the liver, suggesting that the immune response contributes to hepatocellular injury.[25] Thus the etiology of liver injury in HDV infection is unclear.

The adaptive immune response plays an important role in control of HDV infection, but the precise mechanisms are not understood. HDV-specific CD8 T-cell responses were detectable in those with previous infection but not in chronic, infection.[9,26] Another study demonstrated that chronic HDV was associated with a Th-2 response and secretion of interleukin-10. Taken together, these suggest that control of an HDV infection require Th1 CD4 and CD8 T-cell responses.

Epidemiology

An estimated 15 to 20 million people have serologic evidence of exposures to HDV, with an overall global prevalence of approximately 5% of HBV-infected persons.[27] Since the 1980s the prevalence of HDV has declined substantially as a result of better control of HBV due to more widespread vaccination practices and improved socioeconomic conditions. Infection with HDV has a worldwide distribution, although there are considerable geographic differences that do not entirely mirror the prevalence of HBV infection. Prevalence in the United States and Northern Europe are about 5% to 15% in HBsAg-positive carriers.[28] Immigrants from the Middle East, Africa, and Eastern Europe, and older patients from the Mediterranean Basin (most acquired parenterally), as well as young injection drug users account for the largest proportion of cases.[29–31] In the developing world, where HBV is highly endemic, such as Asia and sub-Saharan Africa, as many as two-thirds of the HBV-infected population is infected with HDV, although data in several countries are lacking. Some areas with the highest prevalence include parts of Central and West Africa (33% in Mauritania, 67% in Gabon), parts of the Amazon Basin (42%), the Middle East (33%–47%), India, Pakistan (24%–89%), and Northern Vietnam (15%–43%). In Mongolia greater than 60% to 88% of people with chronic hepatitis B have evidence of chronic HDV.[32] Higher HDV prevalence is also seen in patients with HIV-HBV coinfection compared with HBV monoinfection.[33] It should be noted that many of the estimated prevalence data are based on anti-HDV alone because commercial assays for HDV RNA are not readily available in many countries. In a recent study from Tanzania the prevalence of anti-HDV positivity was 5%; however, HDV RNA was not detectable in any of the anti–HDV-positive patients, and a

second serologic test (HDV-Ab kit; DiaPro, Milan, Italy) was negative in all the samples.[34]

There are eight HDV genotypes that have been identified by phylogenetic analyses. Divergence between genotypes ranges from 20% to 40%. Some of the genotypes are more aggressive than others.[35] HDV-1 strain is the most common worldwide, present in almost all continents. Four HDV-1 subgenotypes have been identified, and most have been associated with severe disease, including cirrhosis, fulminant hepatitis, and death. HDV-2 and HDV-4 strains are found in Asia and are associated with milder disease. HDV-3 is found in northern South America, especially the Amazon Basin, and is associated with the most severe form of HDV.[36,37] HDV-5 to HDV-8 are present only in Africa. HDV-5 is the next most common genotype after HDV-1 and originates from western and sub-Saharan Africa[35] (Fig. 146.3).

Diagnosis

HDV is diagnosed with detection of anti-HDV IgG, and because this remains positive with HDV clearance, chronic infection can be established using real-time polymerase chain reaction.[38] HDV IgM antibodies have a role if the HDV RNA is negative, and there is still a high suspicion for chronic HDV because viremia can fluctuate over time, and the variability of the HDV sequence can produce false-negative results.

Transmission and Clinical Manifestations

Similar to HBV, HDV is spread by blood, blood products, and bodily secretions and only requires a small inoculum to transmit.[9] Unlike HBV, perinatal transmission is uncommon. As a result of universal blood screening for HBsAg and widespread HBV vaccination, HDV has virtually disappeared in multiply transfused subjects and hemophiliacs in developed countries.[39] After exposure there is a short incubation period of 3 to 7 weeks. Because HDV requires the surface antigen from HBV, disease may occur as an acute coinfection with HBV or as a superinfection of a chronic HBV infection.

As with acute hepatitis B monoinfection, the clinical presentation and natural history of acute coinfection with HBV and HDV are highly variable. Acute coinfection usually occurs after an incubation period of 45 to 160 days. The incubation period during coinfection may display a biphasic pattern of ALT levels because the titers of the infecting inoculum for the two viruses may be different or because the two viruses are expressed sequentially.[12,40] In animal models the incubation period of each virus has been shown to be inversely proportional to the dose of the virus.[41] Usually the first episode is due to hepatitis B replication and immune response, followed by that of hepatitis D. Clinically, coinfection presents as an acute self-limited hepatitis with complete recovery with only 4% to 5% progressing to chronic HBV and HDV coinfection.[42–44] In rare instances a severe or fulminant hepatitis can occur.[45]

Superinfection of HDV in individuals with chronic hepatitis B generally results in acute severe hepatitis with a relatively short incubation period (2–8 weeks), followed by chronic hepatitis D in 90% of the cases. Of interest, when HDV clearance occurs after superinfection, there is increased HBsAg loss compared with HBV monoinfection.[46] Superinfection with HDV can be associated with fulminant hepatitis and chronic active hepatitis with cirrhosis. Fulminant hepatitis, a severe form of acute hepatitis, is 10 times more common in HBV-HDV coinfection than in chronic HBV infection alone. The prognosis with fulminant hepatitis is very poor. Massive liver necrosis can occur with mortality approaching 80% in the absence of liver transplantation. In patients with chronic hepatitis D, HDV is the dominant virus because the L-HDAg suppresses HBV replication (see "Hepatitis Delta Antigen"). Thus most HBV-HDV coinfected patients have low serum HBV DNA levels, and most are HBeAg negative.

Once chronic HDV infection is established, the clinical course of hepatitis is accelerated compared with HBV monoinfection. Cirrhosis occurs in 60% to 80% of chronic hepatitis D patients within 5 to 10 years, and the risk of hepatocellular carcinoma (HCC) is about threefold higher than in HBV monoinfection.[47–49] In one study where the HDV seroprevalence was 6%, the relative risk of cirrhosis and HCC was 2.58 and 2.87, respectively, in patients with both infections compared with those with HBV alone.[50] The high risk of HCC may reflect the higher rate of cirrhosis. Once cirrhosis is established, the risk for clinical decompensation is twofold higher than in HBV monoinfection.[47] Splenomegaly along with elevated aminotransferases and high levels of viremia are common in chronic hepatitis D.[47] However, unlike HBV, the level of HDV viremia does not seem to correlate with severity of disease.[48,51] The clinical course is affected by both the HDV genotype (see "Epidemiology") and the HBV genotype.[47,52] Liver disease is known to be accelerated in HIV-infected patients with triple coinfection (HIV/

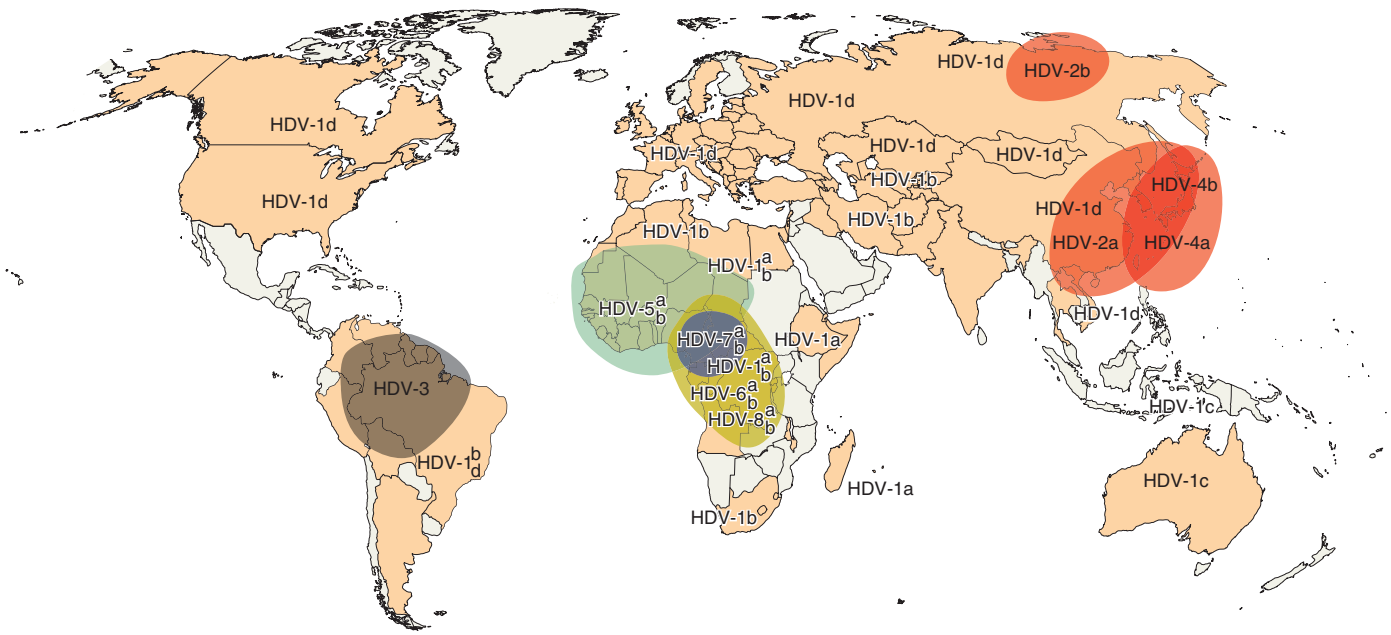

FIG. 146.3 Worldwide distribution of hepatitis D virus *(HDV)* genotypes and subgenotypes. *(From Le Gal F, Brichler S, Drugan T, et al. Genetic diversity and worldwide distribution of the* Deltavirus *genus: a study of 2,152 clinical strains.* Hepatology. *2017;66:1826–1841.)*

HBV/HDV), and the incidence of HCC and mortality is higher than in patients with HIV alone.[53,54]

Up to 30% of HBV-HDV coinfected individuals also have hepatitis C virus (HCV) antibodies; however, in one study 84% of them had undetectable HCV RNA, suggesting that HDV is the dominant virus in this triple infection.[55]

Treatment

Interferon-α (IFN-α) and pegylated (PEG) IFN-α have been shown to have antiviral activity against HDV. However, the efficacy of IFN-α in the treatment of HDV is limited unless high doses (9 million units three times a week) are used as in the reported studies. In one of the largest trials to date, treatment with IFN-α was only successful in achieving sustained HDV virologic response in 25% to 30% of individual patients.[56] With PEG IFN-α, sustained virologic response has been variable, occurring in 17% to 43% of patients in clinical trials. The current recommended treatment for chronic hepatitis D is weekly PEG IFN-α for a minimum of 48 weeks. The ultimate duration of therapy for HDV is still not known (also see Chapter 47), and late relapses have been reported.[57] In the long term, biochemical and histologic improvements with IFN-α can occur, even among those who relapse. In a previous study, improvement in liver histology was maintained 10 years post-treatment among the patients who received high-dose IFN-α.[58] Nucleoside reverse-transcriptase inhibitors with activity against HBV, including lamivudine and entecavir, have been shown to be ineffective at controlling HDV replication, which is not surprising because HDV does not have its own polymerase.[59] HDV RNA declines, and virologic suppression have been reported in HIV/HBV/HDV coinfected patients receiving long-term tenofovir DF, correlating in one study with declines in HBsAg and reduction in fibrosis.[60] This decline may have been related to the longer duration of therapy that led to decrease in HBsAg.

Newer therapies are under development and fall into three categories: entry inhibitors, farnesyltransferase inhibitors, and nucleic acid polymers. Blocking HDV entry with Myrcludex B ± PEG IFN-α has shown promise in phase II clinical trials.[61] Farnesyltransferase inhibitors (lonafarnib) prevent virion assembly, and in early phase II clinical trials, a reduction in HDV RNA was observed over 28 days.[62] Longer studies up to 24 weeks with and without ritonavir as a boosting agent and/or PEG IFN-α have led to HDV RNA suppression at the end of treatment but not necessarily off treatment. Gastrointestinal toxicity is a dose-limiting side effect of lonafarnib. The mechanism of action of nucleic acid polymers is believed to be decreasing viral secretion from hepatocytes. One example is intravenous REP 2139 (Replicor; Montréal, Quebec, Canada), which was given in combination with PEG IFN-α to 12 HBV-HDV coinfected patients in a phase II clinical trial.[63] Patients received 30 weeks of REP 2139 and 48 weeks of PEG IFN-α with a 15-week overlap of the two drugs. One year after the end of treatment, 7 were HDV RNA negative and 8 were HBV DNA negative. Four individuals had a serious adverse event, which was attributed to the PEG IFN-α, but only 1 discontinued therapy. Other treatments under investigation include PEG IFN-γ, which is being tested at two doses for 48 weeks in 33 individuals. Interim 24-week results from 10 of the 33 participants showed that five had a ≥2-log decline in HDV RNA, and 4 became HDV RNA negative.[64] Further studies with these and other agents in development are needed to determine if they are superior to PEG IFN-α.

Transplantation

Liver transplantation is reserved for patients with end-stage liver disease from HBV-HDV coinfection. Reinfection of the graft is prevented with administration of hepatitis B immunoglobulin because prevention of HBV reinfection stops propagation of HDV. There is no evidence for latency of HDV outside the liver. The 5-year survival of liver transplantation for HDV is >80%.

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147 Human Parvoviruses, Including Parvovirus B19V and Human Bocaparvoviruses

Kevin E. Brown

SHORT VIEW SUMMARY

Definition
- At least five different types of parvoviruses infect humans.
- Parvovirus B19 (B19V) can cause erythema infectiosum (slapped cheek disease or fifth disease), transient aplastic crisis, and pure red cell aplasia or fetal hydrops.
- Human bocaparvoviruses (HBoVs) can cause respiratory infections and may be associated with some cases of gastroenteritis.
- Human dependoparvovirus infections (adeno-associated viruses) are asymptomatic, and modified dependoparvoviruses are used as vectors for gene therapy.
- Disease associations with the human protoparvoviruses and Parv4 infection are not clear.

Epidemiology
- Parvovirus B19 (B19V) infection is a common infection in children and young adults. By age 15 years, 50% of children in America and Europe will have been infected and have immunoglobulin G against B19V.
- B19V is mainly spread through the respiratory route, although it may also be transmitted through blood and blood products.
- B19V infections in temperate climates are more common in late winter, spring, and early summer, with increased rates of infection every 3 to 5 years.
- HBoV infections are ubiquitous in young children, with most, if not all, children being infected with HBoV1 by age 6 years.

Diagnosis
- Although the slapped-cheek rash of B19V infection is classic, it is difficult to accurately diagnose outside of the context of an outbreak.
- Diagnosis of B19V rash is by detection of B19V immunoglobulin M in serum.
- Hematologic disease due to B19V can be diagnosed by detection of high-titer B19V DNA (>10^6 IU/mL) in blood samples.
- After infection, low levels of B19V DNA may be detected lifelong. Detection of low-level B19V DNA in samples therefore does not indicate recent or current infection.
- Similarly, long-term persistence of bocaparvovirus DNA in respiratory and fecal samples indicates that detection of viral DNA alone does not correlate with active infection. Respiratory HBoV1 infection should be diagnosed by detection of viral DNA in serum or serology, or both.

Therapy
- Treatment for all parvovirus infections is mainly symptomatic.
- Intravenous immunoglobulin can be used for treatment of chronic anemia or pure red aplasia due to high-titer B19V infection.

Prevention
- A vaccine for B19V is in development.

Parvum is Latin for "small," and the Parvoviridae are among the smallest known DNA-containing viruses that infect mammalian cells. The virions are nonenveloped particles about 22 nm in diameter with icosahedral symmetry. The Parvoviridae are divided into two subfamilies, Parvovirinae and Densovirinae, on the basis of their ability to infect vertebrate or invertebrate cells, respectively. The Parvovirinae are further subdivided into eight genera on the basis of their transcription map, their ability to replicate efficiently either autonomously or with a helper virus, and their sequence homology. The eight genera are *Protoparvovirus* (previously *Parvovirus*), *Dependoparvovirus, Erythroparvovirus, Bocaparvovirus, Amdoparvovirus, Aveparvovirus, Copiparvovirus,* and *Tetraparvovirus*.[1]

At least five different parvoviruses are known to infect humans. Parvovirus B19 (B19V) is the best characterized and is classified as a member of the *Erythroparvovirus* genus, of which it is the type species. The other human viruses are the human adeno-associated viruses (AAVs, or dependoparvoviruses), human bocaparvovirus (HBoV), human Parv4 virus, a member of the newly created *Tetraparvovirus* genus, and the human protoparvoviruses—human bufavirus (BuV), tusavirus (TuV), and cutavirus (CuV).[2]

PARVOVIRUS B19

Parvovirus B19 (B19V) was discovered in 1975 during evaluations of assays for hepatitis B surface antigen using panels of serum samples.[3] Sample 19 in panel B (hence B19) gave an anomalous result, a "false positive" in the relatively insensitive counterimmunoelectrophoresis assay, and when the precipitin line was excised, electron microscopy showed the presence of 23-nm particles resembling parvoviruses. Although originally labeled serum parvovirus-like particle or human parvovirus, in 1985, the virus was officially recognized as a member of the Parvoviridae, and the International Committee on Taxonomy of Viruses recommended the name B19V to prevent confusion with other viruses.

An association of B19V with significant clinical disease was not made until 1981, but it is now known that B19V infection has a wide variety of disease manifestations dependent on the immunologic and hematologic status of the host (Table 147.1). In normal immunocompetent children, B19V is the cause of erythema infectiosum (EI), also called fifth disease or "slapped cheek" disease, which is an innocuous illness with rash. On occasion, especially in women, fifth disease leads to an acute symmetrical polyarthropathy, which can mimic rheumatoid arthritis. In persons with underlying hemolytic disorders or increased erythropoiesis, or both, infection leads to a temporary failure of red blood cell production and transient aplastic crisis (TAC). In the immunocompromised host, persistent B19V viremia manifests as pure red cell aplasia (PRCA) and chronic anemia, and in the fetus, in which the immune response is immature, infection may lead to fetal death in utero, hydrops fetalis, or, rarely, the development of congenital anemia.

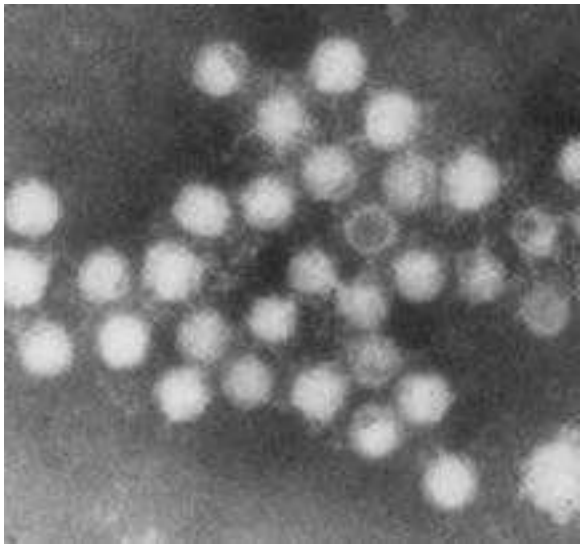

FIG. 147.1 Electron micrograph of parvovirus B19 particles showing icosahedral symmetry. *(Courtesy Dr. Anne Field.)*

TABLE 147.1 Disease Manifestations and Persistence of Parvovirus B19 Infection in Different Host Populations

DISEASE	ACUTE OR CHRONIC	HOST
Fifth disease	Acute	Normal children
Polyarthropathy syndrome	Acute or chronic	Normal adults
Transient aplastic crisis	Acute	Patients with increased erythropoiesis
Hydrops fetalis or congenital anemia	Acute or chronic	Fetus (<20 wk)
Persistent anemia	Chronic	Immunodeficient or immunocompromised patients

VIROLOGY

By electron microscopy, B19V particles have the typical parvovirus morphology (Fig. 147.1). Mature infectious particles have a molecular weight of 5.6×10^6 and a buoyant density in cesium chloride gradients of 1.41 g/mL. As a consequence of the lack of envelope and limited DNA content, B19V is resistant to physical inactivation. Although the virus can be inactivated by heat in low concentrations of protein,[4] the virus resists inactivation at 56°C for more than 60 minutes, and at high viral concentrations, it resists 80°C for 72 hours in clotting factor concentrates.[5] B19V is stable in lipid solvents such as ether and chloroform but can be inactivated by formalin, β-propiolactone, oxidizing agents, and γ-irradiation.

The B19V genome size is limited, consisting of a single strand of DNA of approximately 5596 nucleotides, with identical inverted, 365-nucleotide-long terminal repeat sequences at each end. The transcription map of B19V distinguishes it from other Parvovirinae. There is a single strong promoter at the far left side of the genome and unusual polyadenylation signals in the middle of the genome.[6] The three major viral proteins, one nonstructural (NS) protein and two capsid proteins, are produced by alternative splicing from the promoter and its accompanying leader sequence. The relative quantities of the major and minor capsid proteins are in part regulated by the presence of multiple upstream adenine-uridine-guanine (AUG) codons situated before the authentic transcription initiation codon.[7] In addition, there are transcripts for several smaller proteins of 7.5 and 11 kilodalton (kDa). Although the 11-kDa protein is known to be required for producing infectious virus, the role of the 7.5-kDa protein is unknown.[8]

The only unspliced transcript encodes the NS protein, a 78-kDa phosphoprotein.[9] Consistent with its role in viral propagation, the protein has DNA-binding properties and adenosine and guanosine triphosphatase activity.[10] Expression of the NS protein causes host cell death through induction of apoptosis.[11]

The B19V virion is an icosahedron consisting of 60 copies of the capsid proteins. Most of the capsid is VP2, a 58-kDa protein with 5% or less of the larger 84-kDa VP1 protein. VP1 protein differs from VP2 by an additional 227 amino acids at the amino terminus. Using genetic engineering techniques, the capsid proteins can be expressed in a variety of both mammalian,[12] insect,[13,14] and yeast[15] cell lines. Capsid proteins self-assemble in the absence of B19V DNA, and in these systems, protein expression leads to formation of recombinant empty capsids; VP1 is not required for capsid formation.

The atomic structure of both B19V VP2 empty capsids and infectious virus has been resolved.[16,17] The virion surface has a major depression encompassing the fivefold axis, similar to the canyon structure found in RNA-containing icosahedral viruses. In B19V capsids there is also a hollow cylindrical structure around the fivefold axes that appears to penetrate to the inside of the virion. The structural distribution of VP1 in the B19V capsid structure cannot be inferred from the crystallographic structures, but on the basis of antibody-binding and structural studies, in infectious B19V the VP1 unique region appears to be exposed on the viral surface adjacent to the fivefold axis cylinder.[17] It has been shown that the VP1 unique region of all parvoviruses, including B19V, has a phospholipase A_2 motif.[18] Infection studies with B19V and other parvoviruses show that this motif is required for viral infectivity.[18,19]

It is now recognized that there are three different genotypes, with approximately 10% variability at the DNA level between them.[20] Most of the B19V identified is genotype 1,[21] the original B19V genotype, and is distributed worldwide. Genotype 3 seems to be the predominant B19V genotype in Ghana, representing more than 90% of the sequences identified.[22] Genotype 2 has been primarily identified in tissues of older patients (born before 1973), suggesting that it may have circulated more frequently before the 1970s.[23,24] However, blood samples or donations containing high-titer genotype 2 are occasionally identified,[25] and genotype 2 and 3 sequences have been identified in blood and tissues from many different parts of the world,[26–29] suggesting a more widespread distribution than originally assumed. However, the true prevalence of these different genotypes is currently unknown.

Despite the differences in the DNA sequences, the capsid protein sequence is conserved between the different genotypes, and there is evidence for both serologic and cross-neutralization.[25,30,31]

PATHOGENESIS

B19V, like the other autonomous parvoviruses, is dependent on mitotically active cells for replication. However, B19V has a narrow target cell range and can be propagated efficiently only in human erythroid progenitor cells. The virus cannot be easily cultivated in the laboratory, apart from in primary erythroblasts.[32] Humans are the only known host of B19V, although primates have their own related erythroparvoviruses.[33]

In human erythroid cells derived from bone marrow, susceptibility to B19V increases with differentiation; the pluripotent stem cell appears to be spared, and the main target cells are erythroid progenitors (cells capable of giving rise to erythroid colonies in vitro) and CD36-positive erythroblasts.[32,34] Infection with B19V is cytotoxic[35] because of expression of the NS protein in infected cells.[11] Infected cultures are characterized by the presence of giant pronormoblasts, 25 to 32 μm in diameter, with cytoplasmic vacuolization, immature chromatin, and large eosinophilic nuclear inclusion bodies (Fig. 147.2). By electron microscopy, virus particles are seen in the nucleus and cytoplasmic membrane lining, and infected cells show marginated chromatin, pseudopod formation, and cytoplasmic vacuolation,[36] all of which are typical of cells undergoing apoptosis.

Erythroid specificity of B19V is, in part, due to the tissue distribution of the virus's cellular receptor globoside, also known as blood group P antigen.[37] P antigen is found on erythroid progenitors, erythroblasts, and megakaryocytes.[38] It is also present on endothelial cells, which may

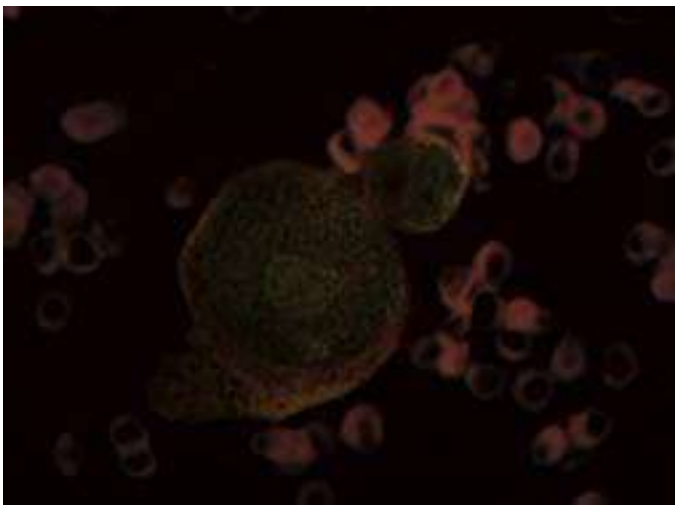

FIG. 147.2 Giant pronormoblast in patient with parvovirus B19 infection.

be targets of viral infection involved in the pathogenesis of transplacental transmission, possibly vasculitis, and the rash of fifth disease and on fetal myocardial cells. Rare individuals who genetically lack P antigen on erythrocytes are resistant to B19V infection, and their bone marrow cannot be infected with B19V in vitro.[39] However, although P antigen is required for infection, it is not sufficient for viral entry, and additional B19V receptors have been proposed as putative cellular receptors.[40–42] In addition, the erythroid specificity may also be modulated by specific erythroid cell transcription factors.

Studies in healthy volunteers showed that B19V infection led to an acute but self-limited (4–8 days) cessation of red cell production and a corresponding decline in hemoglobin level.[43] In patients with normal erythroid turnover, this short interruption of red cell production does not lead to anemia, but in patients with high red cell turnover related to hemolysis, blood loss, or other causes, the temporary failure of erythropoiesis can precipitate an aplastic crisis. The anemia improves as the immune response develops. In patients who are immunocompromised, infection may persist and produce chronic PRCA.

The infected fetus may suffer severe effects because red blood cell turnover is high and the immune response deficient. During the second trimester there is a great increase in red cell mass. Parvovirus particles can be detected by electron microscopy within the hematopoietic tissues of the liver and thymus.[44] B19V DNA and capsid antigen have been detected in the myocardium of infected fetuses,[45] and there is evidence that the fetus may develop myocarditis,[46] compounding the severe anemia and secondary cardiac failure. By the third trimester, a more effective fetal immune response to the virus probably accounts for the decrease in fetal loss at this stage of pregnancy.

The pathogenesis of the rash in EI and polyarthropathy is almost certainly immune complex mediated (Fig. 147.3). In volunteer studies these appeared when high-titer viremia was no longer detectable and coincident with a detectable immune response.[43] Similar findings have been reported in chronically infected individuals who received immunoglobulin therapy.[47] However, in vitro studies have shown that the B19V NS protein induces not only apoptosis in host cells but also activation of interleukin-6,[48] and the phospholipase motif in the VP1u region is functional[49,50]; both of these findings could contribute in vivo to the B19V-induced arthropathy.

EPIDEMIOLOGY

Prevalence and Incidence

B19V infection is common in childhood, and by age 15 years, approximately 50% of children have detectable immunoglobulin G (IgG) against B19V. Infection also occurs in adult life, and greater than 80% of elderly people have detectable antibody.[51] Women of childbearing age in the United States and Europe have an annual seroconversion rate of approximately 1%.[52,53] Studies in different countries (France, Germany,

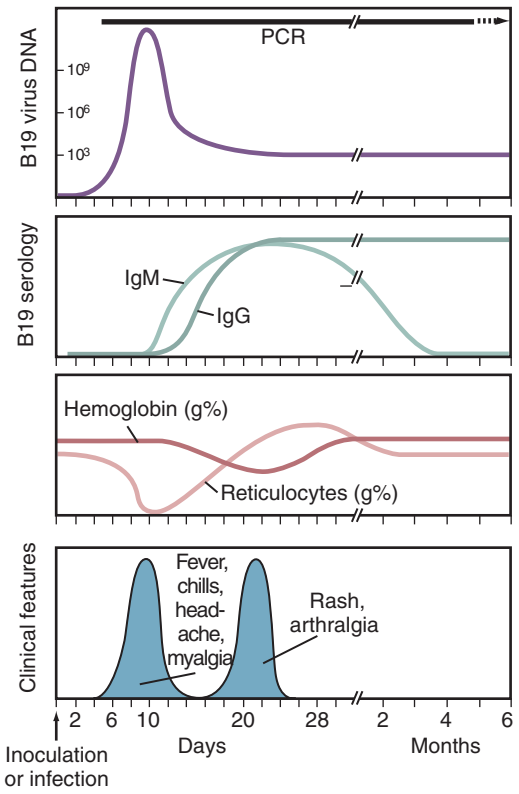

FIG. 147.3 Virologic, immunologic, and clinical courses after acute parvovirus B19 infection in a healthy, immunocompetent individual. *DNA,* Deoxyribonucleic acid; *PCR,* polymerase chain reaction; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M. *(From Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans.* J Infect Dis. *1985;152:257–265; and Patou G, Pillay D, Myint S, Pattison J. Characterization of a nested polymerase chain reaction assay for detection of parvovirus B19.* J Clin Microbiol. *1993;31:540–546.)*

Japan, United Kingdom, and United States) show similar patterns of seroprevalence, with significantly higher levels in parts of Africa and Papua New Guinea, with greater than 80% of 10-year-olds having detectable antibody.[54] In contrast, parts of Asia[55] and some isolated tribal populations have a much lower prevalence.[56]

Although antibody is prevalent in the general population, very high-titer viremia (>10^9 IU/mL) is rare: Approximately 1 per 20,000 to 1 per 40,000 units of blood during epidemic seasons contain high-titer B19V.[52] However, estimates of the prevalence of lower levels of B19V DNA vary widely depending on the sensitivity of the method used,[57] with approximately 1% of donations having detectable B19V DNA by sensitive polymerase chain reaction (PCR).[58] The significance of these low levels of B19V DNA in blood samples is unknown.[57]

Mechanism and Routes of Transmission

B19V infections in temperate climates are more common in the late winter, spring, and early summer months.[59] Rates of infection may also increase every 3 to 4 years, as reflected by corresponding increases in the major clinical manifestations of B19V infection, TACs, and EI.[60,61]

B19V DNA has been found in the respiratory secretions of patients at the time of viremia,[62] suggesting that infection is generally spread by a respiratory route of transmission. The virus can be readily transmitted by close contact, and the secondary attack rate has been calculated in various settings; in one study the rate of secondary attack from symptomatic TAC or EI patients to susceptible (IgG-negative) household contacts was approximately 50%.[62] For school outbreaks, serologic studies are generally not available, but 10% to 60% of students may develop a rash consistent with B19V infection.[63,64] The highest secondary attack rates and annual seroconversion rates, even in the absence of known community outbreaks, are for workers in close contact with affected

children, such as daycare providers and school personnel.[65] Nosocomial transmission in hospital situations has been described[66] but is probably infrequent, especially from patients with chronic infection. Nevertheless, patients with TAC or persistent disease should be considered infectious and appropriate precautions taken to limit interaction with other patients and susceptible staff.

The virus can be found in serum, and infection can be transmitted by blood and blood products,[67] including albumin and plasma.[68,69] As described previously, parvoviruses, including B19V, are very heat resistant, and they can withstand the usual thermal treatment aimed at infectious agents in blood products. In addition, solvent-detergent methods, which inactivate only lipid-enveloped viruses, are ineffective. B19V infection has been transmitted by steam-treated, dry-heated, and solvent-detergent-treated factors, although hemophiliacs who received heat-treated factor VIII alone had a lower prevalence of B19V antibody and lower rates of seroconversion than those receiving non–heat-treated factor.[70]

CLINICAL MANIFESTATIONS

Erythema Infectiosum

Manifestations of B19V infection vary, even in the normal host, from asymptomatic or subclinical infection (most people with B19V-specific antibody have no recollection of any specific symptoms) to a biphasic illness with symptoms during the viremic and immune complex–mediated stages of the disease, but EI is the major manifestation. EI was well characterized clinically before the discovery of B19V.[71] This exanthematous rash illness of childhood was probably first described by Robert Willan in 1799 and illustrated in his 1808 textbook. The disease was rediscovered in Germany, where in 1899 Sticker termed it *erythema infectiosum,* and 6 years later Cheinisse classified it as the "fifth rash disease" of the six classic exanthems of childhood.[72] Often the epidemiologic data suggested "a common-source exposure to a highly effective transmitter," and an atypical rubella virus or echovirus was thought to be responsible. However, neither virus could be reproducibly isolated from patients with fifth disease. In 1983, after an outbreak of EI in London, all 31 affected children or adolescents had anti–B19-specific IgM.[73] Similar results were obtained in other epidemics of fifth disease, and B19V is now recognized as the etiologic agent.

Clinical symptoms begin with a nonspecific prodromal illness, which often goes unrecognized; there may be symptoms of fever, coryza, headache, and mild gastrointestinal distress, including nausea and diarrhea. In 2 to 5 days the classic "slapped cheek" rash appears, a fiery red eruption on the cheek, accompanied by relative circumoral pallor (Fig. 147.4). There may be a second-stage rash within a few days and an erythematous maculopapular exanthem on the trunk and limbs; as this eruption fades it produces a typical lacy appearance. There is great variation in the dermatologic symptoms: The classic slapped cheek is much more common in children than adults; the second-stage eruption may vary from a faint, barely perceptible erythema to a florid exanthem; and the rash may be transient or recurrent for weeks. Rarely, other dermatologic presentations are seen: vesicopustular rash,[74] papular-purpuric glove and sock syndrome,[75] other purpuric rashes with or without Koplik spots,[76] and erythema multiforme.[77] Pruritus, especially on the soles of the feet, can be the dominant symptom.[78]



FIG. 147.4 Slapped-cheek appearance of a child with fifth disease.

Arthropathy

Although B19V infection in children is usually mild and of short duration, a large proportion of adults, especially women, suffer arthralgia or frank arthritis, with painful joints often accompanied by swelling and stiffness.[64] The arthralgia is usually symmetrical, with mainly the small joints of hands and feet involved, and generally lasts for 1 to 3 weeks, although it may persist or recur for months or even years. In the absence of a history of rash the symptoms may be mistaken for those of acute rheumatoid arthritis, especially because prolonged symptoms do not correlate with serologic studies, such as the duration of B19V IgM response, or persistent viremia. In addition, B19V infection can be associated with transient rheumatoid factor production.[79] In one large study of patients attending an "early synovitis" clinic in England, 12% had evidence of recent infection with B19V.[80] Three patients would have fulfilled the American Rheumatism Association's diagnostic criteria for definite rheumatoid arthritis. B19V infection should be considered as part of the differential diagnosis in any patient presenting with acute arthritis.

It has been postulated that B19V is involved in the initiation and perpetuation of rheumatoid arthritis leading to joint lesions,[81] but these results have not been reproducible by other groups. In contrast, parvoviral B19V DNA is frequently found in synovial tissue of patients with rheumatoid arthritis, chronic arthropathy, and control subjects. In one carefully performed controlled study, although B19V DNA was indeed detected in synovial tissue of 28% of individuals with chronic arthritis, it was also found in 48% of nonarthropathy controls,[82] indicating that PCR-detectable DNA may persist in synovial tissues for months or years. In addition, in one study with long-term follow-up, none of the 54 patients with B19V-associated arthralgia reported persistence of joint swelling or restricted motion, and no evidence of inflammatory joint disease was found.[83] Therefore it seems unlikely that B19V plays a role in classic erosive rheumatoid arthritis. The association of B19V and juvenile rheumatic disease is more convincing,[84] but whether it is the cause of the disease or one of many potential triggers is less clear.

Transient Aplastic Crisis

TAC, the abrupt cessation of erythropoiesis characterized by reticulocytopenia, absent erythroid precursors in the bone marrow, and precipitous worsening of anemia, was the first clinical illness associated with B19V infection. When stored sera from children admitted to a London hospital were examined for B19V, samples from six Jamaican immigrants with sickle cell disease presenting with aplastic crisis showed evidence of recent infection with B19V (either antigenemia or seroconversion).[85] Retrospective studies of sera from Jamaican patients with sickle cell disease showed that 86% of TACs were associated with recent parvovirus infection.[86]

TAC caused by B19V has now been described in a wide range of patients with underlying hemolytic disorders, including hereditary spherocytosis, thalassemia, red cell enzymopathies such as pyruvate kinase deficiency, and autoimmune hemolytic anemia.[87] TAC can also occur under conditions of erythroid "stress," such as hemorrhage, iron deficiency anemia, and kidney or bone marrow transplantation. Acute anemia has been described in hematologically normal persons,[88] and a drop in red cell count (within the normal range) and reticulocytes was seen in healthy volunteers.[43]

Although suffering from an ultimately self-limiting disease, patients with aplastic crisis can be severely ill. Symptoms may include dyspnea, lassitude, and even confusion related to the worsening anemia. Congestive heart failure, severe bone marrow necrosis,[89] and cerebrovascular complications[90] can develop, and the illness may be fatal. Aplastic crisis can be the first presentation of an underlying hemolytic disease in a well-compensated patient.

Community-acquired aplastic crisis is almost always due to B19V,[91] and B19V infection should be the presumptive diagnosis in any patient

with anemia related to abrupt cessation of erythropoiesis as documented by reduced reticulocytes and bone marrow appearance. In contrast to patients with EI, patients with TAC are often viremic at the time of presentation, with concentrations of virus as high as 10^{15} genome copies/mL (IU/mL); thus the diagnosis is readily made by detection of B19V DNA in the serum. As B19V DNA levels fall in serum, B19V-specific IgM becomes detectable. TAC is easily treated by blood transfusion. After acute infection, immunity is lifelong.

TAC and B19V infection in hematologically normal patients are often associated with changes in other blood lineages, varying degrees of neutropenia,[92] and thrombocytopenia.[93] Some cases of idiopathic thrombocytopenic purpura and Henoch-Schönlein purpura[94] have been reported to follow B19V infection. Transient pancytopenia after parvovirus infection is rare. Although some cases of chronic neutropenia of childhood have also been ascribed to B19V infection,[95] other studies have not confirmed an association.[96]

B19V does not appear to be the cause of true (chronic) aplastic anemia[97] or transient erythroblastopenia of childhood (TEC),[98] the temporary failure of red cell production in normal children. Sporadic cases of TEC with thrombocytopenia with evidence of recent B19V infection have been described, whereas "classic" TEC is associated with *high* platelet counts.

Pure Red Cell Aplasia

Persistent B19V infection that results in PRCA has been reported in a wide variety of immunosuppressed patients, including patients with congenital immunodeficiency, acquired immunodeficiency syndrome (AIDS), and lymphoproliferative disorders and transplant recipients.[99] The stereotypical presentation is with persistent anemia rather than immune-mediated symptoms of rash or arthropathy. Patients have absent or low levels of B19V-specific antibody and persistent or recurrent parvoviremia as detected by B19V DNA in the serum. Bone marrow examination generally reveals the presence of scattered giant pronormoblasts. Administration of immunoglobulin can be beneficial and ameliorative, if not curative.[100]

The prevalence of B19V-induced anemia in human immunodeficiency virus (HIV)-seropositive patients is probably higher than that recognized. In one early study of 50 patients with AIDS, no patients with B19V viremia were identified. In a larger cohort study, B19V DNA was found in only 1 of 191 (0.5%) HIV-seropositive homosexuals. However, B19V DNA was found in 5 of 30 (17%) transfusion-dependent HIV-seropositive homosexuals, and when a hematocrit of less than 20 mL/dL was used as a criterion, 4 of 13 (31%) were positive.[101] In contrast to the earlier studies the marrow morphology need not be suggestive of PRCA, and giant pronormoblasts may not be present.

In less severely immunosuppressed patients (e.g., patients with systemic lupus erythematosus receiving steroid therapy), prolonged anemia after B19V infection has also been described.[102] However, in these patients there was a spontaneous, albeit delayed, development of antibodies, and viremia resolved without therapy. Such patients represent one end of the spectrum of disease manifestations of B19V in patients with a compromised immune system.

Virus-Associated Hemophagocytic Syndrome

Virus-associated hemophagocytic syndrome (VAHS) is characterized by histiocytic hyperplasia, marked hemophagocytosis, and cytopenia in association with a systemic viral illness.[103] In contrast to malignant histiocytosis, VAHS is usually a benign, self-limiting illness in which histiocytic proliferation is reversible. Hemophagocytosis is not uncommon and occurs in the setting of a wide range of infections, not only viral but also bacterial, rickettsial, fungal, and parasitic.[104] However, in many patients there is underlying immunosuppression, usually iatrogenic, so the role of the incriminated pathogen as an etiologic agent or coincidental opportunistic infection remains unclear.

B19V infection has been detected in 15 cases of hemophagocytosis syndrome among children and adults.[105] The majority of patients were previously healthy, but four patients were immunosuppressed by drug therapies. In all but 1 case there was a favorable outcome (one immunosuppressed patient died of fulminant aspergillosis). Further studies are required to determine whether B19V is a major cause of VAHS and what the rate of VAHS is in otherwise uncomplicated B19V infection.

Fetal Infection (Hydrops Fetalis and Miscarriage)

B19V causes 10% to 15% of all cases of nonimmune hydrops fetalis.[106] Nonimmune hydrops fetalis is rare (1 per 3000 births), and in approximately 15% of cases the etiology is unknown. In a study of 63 cases, 8 were due to B19V infection. When pathologic studies have been undertaken, B19-infected fetuses showed evidence of leukoerythroblastic reaction in the liver and large pale cells with eosinophilic inclusion bodies and peripheral condensation or margination of the nuclear chromatin. B19V DNA can be detected by PCR and in situ hybridization, and viral particles by electron microscopy.

Even in the absence of treatment, an adverse fetal outcome is not typical after maternal B19V infection. In a prospective British study of more than 400 women with serologically confirmed B19V during pregnancy, the excess rate of fetal loss was confined to the first 20 weeks of pregnancy and averaged only 9%.[107] No abnormalities were found at birth in the surviving infants, even when there was evidence of intrauterine infection by the presence of B19V IgM in the umbilical cord blood, and there were no long-term sequelae in the 129 children observed for more than 7 years. Similar findings have been found in studies in other countries.[108,109]

No systematic studies have shown evidence for congenital abnormalities after B19V infection,[107,110] although there are case reports of congenital ocular and neurologic abnormalities after maternal B19V infection. Rare cases of congenital anemia after a history of maternal B19V exposure have been reported.[111,112] In these cases the virus load is generally low, and the anemia does not respond to immunoglobulin therapy. The B19V infection may mimic Diamond-Blackfan anemia,[113] and investigation of erythrocyte enzyme activity and ribosomal protein genes may be needed to distinguish the two.[114]

Other Disease Manifestations

B19V infection has been associated with a range of other disease manifestations, including neurologic disease, myocarditis, kidney disease, hepatitis, and vasculitis. However, most of these are case reports or limited PCR-based studies with poorly documented controls. Determining the role of B19V in these diseases is often difficult; the diseases are rare, and B19V may not be the only cause. In addition, with sensitive PCR-based assays, B19V DNA can be detected in many tissues, including bone marrow, synovial, and other tissues from healthy individuals, probably lifelong after infection.[23,115] If the disease is rare, large multicenter trials may be required to substantiate or disprove the causal relationship.

Encephalitis and, more often, aseptic meningitis have been described in serologically confirmed B19V infection[116] and detection of B19V DNA in cerebrospinal fluid. The long-term outcome of infection was generally favorable, and only rarely did long-term sequelae occur. Brachial plexus neuropathy with weakness and sensory loss has also been described in patients with B19V infection,[117] and in one study, 50% of patients with classic fifth disease (confirmed serologically) experienced neurologic symptoms (tingling and numbness in the fingers or toes).[118]

There have been several case reports of myocarditis associated with B19V infection in both children and adults.[119,120] In many of the case reports the diagnosis of B19V as the cause is made simply on the detection of the B19V DNA genome, and given the known persistence of B19V DNA in tissues, this may be erroneous. However, the putative role of B19V in the pathogenesis of myocarditis warrants further investigation, particularly because P antigen is found on fetal myocardial cells, and B19V appears to cause myocarditis in the fetus.[45,46]

Similarly, a number of case reports have described an association of B19V infection and glomerulonephritis in both children and adults.[121]

The role of B19V in both hepatitis and vasculitis remains unclear. Although transient elevation of liver transaminases is not uncommon in B19V infection, frank hepatitis associated with B19V infection has rarely been reported.[122] B19V has been suggested as a possible causative agent of fulminant liver failure and associated aplastic anemia on the

basis of PCR studies.[123] However, the detection of B19V DNA in control liver tissue is not uncommon, and with appropriate controls we have been unable to confirm this putative relationship.[26,124]

Several case reports have described positive B19V serology in patients with vasculitis or polyarteritis nodosum, systemic necrotizing vasculitis, and Kawasaki disease, a multisystem vasculitis of early childhood. However, other studies have failed to confirm a relationship between B19V and vasculitis[125] or Kawasaki disease.[126]

IMMUNE RESPONSE

Both virus-specific IgM and IgG antibodies are made after experimental[43] and natural B19V infection (see Fig. 147.3). After intranasal inoculation of volunteers, virus can be detected first at days 5 to 6, and levels peak at days 8 to 9. IgM antibody to virus appears about 10 to 12 days after experimental inoculation, and IgG antibody appears at about 2 weeks (see Fig. 147.3). The time course is similar in natural infections. In patients with TAC, 10^9 to 10^{14} genome copies per milliliter of viral DNA may circulate. IgM antibody may be present in patients with TAC at the time of reticulocyte nadir and during the subsequent 10 days; IgG usually appears during the period of hematopoietic recovery. High-titer viremia is not detectable in patients with clinical fifth disease (the manifestations are secondary to immune complex formation).

IgM antibody may be found in serum samples for several months after exposure.[127] IgG persists for life, and levels rise with reexposure.[43] Measurable IgA antibodies specific to B19V may play a role in protection against infection by the nasopharyngeal route.[128]

In immunocompetent individuals the early antibody response is to the major capsid protein VP2, but as the immune response matures, reactivity to the minor capsid protein VP1 dominates. Sera from patients with persistent B19V infection typically contain antibody to VP2 but not to VP1.[129] The importance of an immune response to VP1 for protective immunity has been confirmed in animal experiments using recombinant capsids. Rabbits immunized with capsids containing only VP2 produced a strong antibody response, as measured by enzyme-linked immunosorbent assay (ELISA), but the sera had low neutralization titers. In contrast, rabbits immunized with capsids containing VP1 produced antibody with neutralizing titers comparable with those produced in humans after acute B19V infection.[130] In addition, the importance of the humoral arm of the immune response is shown by recovery from infection with the appearance of circulating specific antivirus antibody, and administration of commercial immunoglobulins can cure or ameliorate persistent parvovirus infection in immunodeficient patients.

The role of the cellular immune response in limiting B19V infection has been studied less intensively. Using a combination of recombinant capsids and peptides from the NS and capsid proteins, it is now clear that B19V infection induces profound $CD8^{+}$[131] and $CD4^{+}$[132–134] responses, both of which are required for viral clearance.

Persistent B19V infection is the result of failure to produce effective neutralizing antibodies by the immunocompromised host. Perhaps because of the limited numbers of epitopes presented to the immune system by B19V, the congenital immunodeficiency states associated with persistent infection may be clinically subtle, with susceptibility largely restricted to parvovirus, although multiple immune system defects are apparent when direct testing of T- and B-cell function is performed.

DIAGNOSIS

There is no suitable method for virus isolation from clinical specimens, and the detection of virus relies on the detection of B19V DNA by quantitative PCR. In immunocompetent individuals, B19V DNA is detectable at very high titer (>10^9 IU/mL) for 2 to 4 days (see Fig. 147.3) and then drops to between 10^5 and 10^6 IU/mL as the immune response develops. The development of the antibody response correlates with the appearance of the rash, and therefore the diagnosis of acute infection due to B19V infection is generally based on IgM assays, ideally performed by the capture technique.[135] In an ELISA, antibody can be detected in greater than 90% of cases by the third day of TAC or at the time of rash in EI. IgM antibody remains detectable for 2 to 3 months after infection.

B19V IgG can be detected by capture assay or indirect assay. IgG is usually present by the seventh day of illness and is probably present for life thereafter. Because greater than 50% of the population has IgG antibody to B19V infection, this test is not helpful for the diagnosis of acute infection.

Immunocompromised or immunodeficient patients with hematologic disease due to chronic infection typically do not mount an immune response to the virus, and testing for B19V DNA is necessary to document recent infection. However, this needs to be done by quantitative PCR because even in immunocompetent persons, low levels of B19V DNA may be detectable by PCR for more than 4 months in serum after acute infection[136,137] and for years in bone marrow, synovium, liver, heart, and other tissues.[23,115] As noted earlier in this chapter, detection of B19V DNA in tissues does not prove that the disease is due to B19V infection. In general, the diagnosis of acute or chronic infection can be made on the basis of quantitative (real-time) PCR in combination with serologic assays for B19-specific IgG or IgM, or both.[138]

Investigation of B19V fetal or congenital infection should be accompanied by serologic studies of the maternal serum. At the time of fetal infection the mother should have evidence of recent B19V infection with detectable IgG and possibly IgM. If the IgM titer is low or absent, recent infection can be documented using IgG avidity studies or measuring B19V DNA levels.[139] Fetal infection can be confirmed by amniotic fluid sampling, by fetal blood sampling, or from postmortem tissue.

THERAPY

In the overwhelming majority of children and adults, B19V infection is a benign and self-limiting infection that results in lifelong immunity and requires no treatment other than symptomatic relief. Patients with arthralgia and arthritis usually respond to nonsteroidal antiinflammatory drugs, although in some patients symptoms can persist for months and even years.[80] In patients with hematologic disease or persistent infection, specific treatment may be necessary. Cidofovir, an acyclic nucleoside phosphonate that is approved for treatment of herpes simplex virus and cytomegalovirus infections, has been shown to have activity against B19V in vitro, but in vivo studies have not been carried out.[140]

Immunocompetent patients with TAC have a self-limiting illness, and typical TAC is readily treated by blood transfusion and supportive therapy alone. In one study of sickle cell patients with aplastic crisis, 87% required blood transfusions, and 61% required hospitalization for their symptoms. One death occurred before transfusion could be given,[141] which underscores the importance of prompt medical intervention.

In immunosuppressed patients with documented, persistent B19V infection, temporary cessation of immunosuppression may be sufficient to allow the host to mount an immune response and resolve the B19V infection, and no additional treatment is required. In cases in which cessation of immunosuppression is not feasible or is ineffective, administration of immunoglobulin can be beneficial.[47,100,142] The usual regimen is intravenous IgG at a dose of 0.4 g/kg for 5 days. Patients often respond with a marked reduction in the level of B19V viremia, reticulocytosis, and resolution of the anemia within 1 to 2 weeks of treatment. However, monitoring for relapse is important, by observation of the reticulocyte counts and quantitative assays for B19V DNA when indicated. If relapse occurs less than 6 months after the initial treatment, especially in HIV-positive patients, an empirical maintenance treatment with a single-day infusion of 0.4 g/kg IgG every 4 weeks may control the B19V viremia.

The role of intrauterine blood transfusions in the treatment of hydrops fetalis related to maternal B19V has been shown to be beneficial.[143,144] However, intrauterine blood transfusions have risks. B19V-associated hydrops is known to resolve spontaneously, and the fetus can be normal at delivery. In addition, there remains the theoretical risk that treatment may be confounded by an increased incidence of antibody-enhanced infection and damage, especially to myocardial cells and the immune system.[111]

PREVENTION AND VACCINATION

The only measures currently available to prevent B19V infection are those designed to interrupt virus transmission. However, because patients

are viremic and infectious before the symptoms of EI, isolation of patients with fifth disease is not rational. Patients with TAC and PRCA are both viremic and infectious and should be appropriately separated from high-risk contacts. The Centers for Disease Control and Prevention recommend that patients with TAC have droplet isolation precautions for 7 days, and for patients with chronic infection, isolation should be continued for the duration of their hospitalization.[145]

The humoral immune response plays the major role in the normal immune response to parvovirus. Although antibodies appear protective in both passive and active immunizations, insufficient data are available to assess the efficacy of immunoprophylaxis.[146,147]

Prospects for vaccination are favorable, with a B19V empty capsid vaccine currently under development. The presence of VP1 protein in the capsid immunogen appears critical for the production of antibodies that neutralize virus activity in vitro, and capsids with supranormal VP1 content are even more efficient in inducing neutralizing activity in immunized animals. Although phase I trials of a VP1-enhanced baculovirus-produced B19V vaccine looked promising,[148] the most recent study has been halted due to unexplained cutaneous reactions in 3 of the 43 patients enrolled, and further studies are on hold.[149] A candidate virus-like particle (VLP) vaccine has been generated in yeast, consisting of both VP1 and VP2, which is phospholipase negative and can be highly purified, with the intent to reduce reactogenicity.[150] Even with an effective and safe vaccine, the target populations for such a vaccine remain to be determined. Should only those at high risk for severe or life-threatening disease, such as sickle cell patients, be protected? Or, in view of the wide variety of disease manifestations affecting all strata of the population, should a universal vaccine policy be pursued?

OTHER HUMAN PARVOVIRUSES

Human Dependoparvoviruses

At least nine different primate dependoparvoviruses have been described,[151] and AAVs 1, 2, 3, 8, and 9 are common agents of human infections.[151] Although AAV DNA has been detected in a wide range of tissues, including some fetal abortion tissues, none of the dependoparvoviruses have been definitively linked with disease in either humans or animals. This lack of pathogenicity, plus the ability of the genome to infect both dividing and nondividing cells and to assess transgenes for extended periods with or without integration into the human genome, has made the dependoparvoviruses popular choices to modify and use as gene therapy vectors in a number of different clinical settings, including treatment for monogeneic diseases and against infectious diseases.[152,153]

Human Bocaparvoviruses

Bocaparvoviruses are parvoviruses that infect the respiratory and gastrointestinal tracts of young animals and humans. In 2005 sequences of the first human bocaparvovirus (HBoV1) were identified in respiratory samples of Swedish children with lower respiratory tract infections using a molecular screening method.[154] The nucleotide sequence of HboV1 was determined and shown to have the same genomic organization as other members of the bocaparvovirus genus, with a third middle open reading frame. However, the sequence was significantly different from the other known animal bocaparvoviruses at the time. Subsequently, related viruses—human bocaparvoviruses 2 to 4 (HboV2, HboV3, and HboV4)—have been detected in fecal samples.[155–157]

Human bocaparvoviruses have a worldwide distribution and have been identified in all countries that have looked for them. HboV1 is predominantly found in respiratory secretions and is found in 2% to 20% of respiratory secretions in prevalence studies primarily from children with acute respiratory disease.[158] Although HboV1 DNA can be detected in respiratory secretions throughout the year, primary infection is predominantly in the winter and spring months, as for many other respiratory infections. HboV-specific antibodies can now be detected using binding assays with recombinant viral capsid proteins,[159] and seroepidemiology studies indicate that HboVs are a common infection of early childhood, with most acquiring antibodies in the first 4 years of life.[160] In contrast to HboV1, HboV2 to HboV4 are predominantly found in fecal samples of both children and adults. Seroprevalence studies in adults indicate that 30% to 50% of adults have antibodies to HboV2, 8% to 38% to HboV3, and 1% to 4% to HboV4.[158]

Despite the observation that HboV1 is often found with other pathogens, there is increasing evidence that the virus itself is pathogenic and especially associated with wheezing and respiratory disease in young children.[161] Several studies have reported that HboV1 is more frequent in patients with respiratory tract symptoms than in asymptomatic individuals.[162–165] A variety of respiratory diseases have been implicated in HboV1 infection, including lower and upper respiratory tract illnesses,[159,166] primarily in young children. HboV1 infections in otherwise normal adults with respiratory illnesses appear to be uncommon but have been reported mainly from immunocompromised individuals.[165–168]

The association of HboV1 infection with respiratory illness is confounded not only by the frequent coinfection with other respiratory viruses but also by the persistence of detectable HboV1 DNA in respiratory and fecal samples and the long period of HboV1 shedding after infection. It is now recognized that diagnosis of HBoV1 infection should not be based on detection of HboV1 DNA alone in respiratory or fecal samples, but on detection of high-titer viral load ($>10^4$ genome copies/mL or respiratory secretion), viral DNA in serum, serologic evidence of recent infection, or a combination of these.[155,169] Without this combination of data the etiologic significance of detection of bocavirus DNA remains uncertain,

Currently there is no specific treatment for any of the bocaparvovirus infections.

Parv4

Using similar methods to those used to identify HBoV1, a fourth group of parvoviruses, human parvovirus 4 (Parv4),[170] was also found in 2005, with three different genotypes of Parv4 identified to date.[171] The virus has not been grown in culture, but studies of the viral sequence and transcription map[172] suggest that Parv4 does not group with any of the other parvovirus genera and has now been placed in the new parvovirus genus, *Tetraparvovirus*. The viral sequences are commonly found in pooled serum samples[173] and in bone marrow and lymphoid tissue of injection drug users and hemophiliacs.[174] Seroprevalence studies indicate that although antibody is rarely found in blood donors in Europe,[175,176] it is found in those who share needles or receive blood products[177] and at significantly higher rates in parts of Africa.[178,179] It is still unclear which is the main route of transmission, with data suggesting parenterally in Europe, but by fecal-oral routes or even respiratory routes in Africa.[180] Seroconversions to Parv4 have rarely been documented, but primary infection may be associated with a rash.[181] Further information about the pathogenicity of this virus is necessary.

Human Protoparvoviruses

Metagenomic next-generation sequencing has also been used to identify three groups of human protoparvoviruses in human fecal samples: BuVs in 2012, TuV in 2014, and CuV in 2016.[2] Limited information is known about any of these viruses, although BuV has only been found in samples from patients with diarrhea, suggesting that it may be a cause of gastroenteritis. CuV has been found in skin biopsies and fecal material, but the significance of this is unknown.

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The complete reference list is available online at Expert Consult.

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148 Orthoreoviruses and Orbiviruses

Kenneth L. Tyler

SHORT VIEW SUMMARY

Definition
- Reoviruses are linear double-stranded RNA viruses with broad host ranges.
- The term *reovirus* is an acronym for respiratory enteric orphan virus, which emphasizes the anatomic site from which these viruses were initially isolated.

Epidemiology
- Infection of humans is common but is rarely associated with significant disease.
- Asymptomatic infection is common; symptomatic infection usually consists of mild, self-limited upper respiratory tract and gastrointestinal illness.
- Reoviruses have been identified as the causative agent in rare human cases of meningitis, encephalitis, pneumonia, and myocarditis and are potentially associated with biliary atresia and choledochal cysts.
- Orthoreoviruses are USED in human clinical trials as oncolytic agents.

Microbiology
- Five genera of the Reoviridae have been etiologically linked with diseases of humans: *Orthoreovirus, Orbivirus, Rotavirus, Coltivirus,* and *Seadornavirus*.
- Double-stranded RNA is organized into 10 segments (orthoreoviruses and orbiviruses), which are capable of reassortment and resultant generation of novel viruses.

Diagnosis
- Laboratory diagnosis can be made: (1) serologically by demonstrating a fourfold rise in virus-specific antibody titer between acute and convalescent serum, (2) by virus isolation from serum, stool, respiratory secretions, or cerebrospinal fluid (CSF), (3) by nucleic acid amplification from body fluids or tissues using reverse-transcriptase polymerase chain reaction (RT-PCR) techniques, or (4) by detecting viral antigens in tissues from biopsy or autopsy specimens with immunocytochemistry using virus-specific antibodies.

Therapy
- No specific therapy is available.

Prevention
- No specific preventative measures are recommended; reoviruses are ubiquitous.

The Reoviridae family of viruses consists of 15 genera, whose members have a widely varied host range, including plants and invertebrate (insects, crustaceans) and vertebrate (mammalian, reptilian, avian) animals. Five genera have been etiologically linked with diseases of humans: *Orthoreovirus, Orbivirus, Rotavirus, Coltivirus,* and *Seadornavirus*; the first two are covered in this chapter and the others in additional chapters. The genome of all Reoviridae consists of linear double-stranded RNA surrounded by a nonenveloped icosahedral capsid 60 to 80 nm in diameter. Genomic material is organized into 10 to 12 segments, which are capable of reassortment and resultant generation of novel viruses.[1] In this chapter, clinically relevant information is presented pertaining to human infection due to orthoreoviruses and orbiviruses. Rotaviruses, coltiviruses, and seadornaviruses are discussed in Chapters 149 and 150.

ORTHOREOVIRUSES

Background and Epidemiology

Reoviruses were first discovered in the 1950s after isolation from human enteric specimens, and they were subsequently documented to infect a wide range of hosts including mammals, birds, reptiles and fish. Mammalian *Orthoreovirus* serotypes 1, 2, and 3 are found ubiquitously in the environment, and their sources include stagnant and river water and untreated sewage. The term *reovirus* is an acronym for respiratory enteric orphan virus, which emphasizes the anatomic site from which these viruses were initially isolated and the fact that infection of humans, although common, is only rarely associated with significant disease. Human infection primarily results in either asymptomatic infection or mild, self-limited symptoms such as upper respiratory tract illness and gastroenteritis.[2,3] Rarely, reoviruses have been identified as the causative agent in human cases of meningitis, encephalitis, pneumonia, and myocarditis and are potentially associated with biliary atresia and choledochal cysts (discussed later).[2–4] Serologic studies of reovirus prevalence have documented steady increases from infancy (5%–10% seropositivity at 1 year of age; 30% at 2 years of age; 50% at 5 years of age) through adulthood (50% at 20–30 years of age; >80% by 60 years of age),[4,5] reflecting immunity acquired as a consequence of natural infection. Despite this, it has been difficult to definitively correlate seroconversion and primary acute infection with specific diseases. Natural transmission likely occurs by the fecal-oral and airborne routes in humans. Reovirus infection has been used as an experimental animal model system to study viral CNS infections, myocarditis, and hepatobiliary and respiratory diseases.[6]

Clinical Disease

Respiratory Tract Manifestations

Mild upper respiratory tract illness consisting primarily of rhinorrhea and pharyngitis accompanied by low-grade fever, headache, and malaise (35%–80%), with or without mild diarrhea (15%–65%), has been described in children during outbreaks and was produced in experimental reovirus infections of adult volunteers.[7,8] In children, a maculopapular and very rarely a vesicular exanthem has been described,[4] as have coryza and otitis media. Rare reports of lower respiratory tract disease have included interstitial or confluent pneumonia, including fatal cases.[9,10] A novel reassortant reovirus designated as BYD1 strain was isolated in 2003 from five patients with severe acute respiratory syndrome (SARS) who were coinfected with SARS-associated coronavirus. Subsequent characterization in animal experimental models has suggested a possible copathogenic role for this virus in SARS.[11,12]

At least a dozen orthoreoviruses have been isolated from bats (pteropine reoviruses) beginning (1970) with Nelson Bay virus, which serves as the prototypical species. Two closely related pteropine orthoreoviruses (Melaka and Kampar viruses) were identified in 2006 and linked with acute upper respiratory disease in six adult and pediatric Malaysian patients.[13,14] Both viruses are presumably of bat origin with transmission to humans by exposure to bat droppings or contaminated fruits. Serologic studies have suggested that pteropine orthoreovirus infection is ubiquitous in Southeast Asia, and possibly other areas, and that bats can serve as reservoirs for transmission of infection to humans.[15] Index adult cases of pteropine-derived orthoreovirus infection occurred in patients with high fever, chills and rigors, sore throat, headache, and myalgia[13,14]. Human-to-human transmission has been substantiated through serologic analysis of secondary cases, including children. The human seroprevalence rate is unknown, but one study from Vietnam found that 4% of adult sera contained immunoglobulin G (IgG) antibodies against pteropine orthoreovirus, detected with enzyme-linked immunosorbent assay (ELISA); half of these individuals also had neutralizing-antibodies.[16] Other studies from Southeast Asia have suggested higher seroprevalence rates, with results up to 17% among outpatients presenting with upper respiratory infections.[17,18] At least six cases of acute respiratory tract infection, similar to those described earlier, have been associated with Nelson Bay virus, the prototypical pteropine orthoreovirus.[19] Isolated cases of human disease linked to other pteropine orthoreovirus isolates (Hong Kong HK23629/07, HK46886/09, and HK50842/10; Sikamat virus) have been reported.[17,20,21]

Gastrointestinal and Hepatobiliary Manifestations

A long-term study of children with diarrhea implicated reoviruses in only 0.1% of cases, and those occurred mainly in infants younger than 1 year.[2] A role for reovirus infection in the pathogenesis of extrahepatic biliary atresia and choledochal cysts has long been proposed based on similarities between pathologic changes observed in pediatric patients with these diseases and reovirus-infected mice.[22] However, results from serology-based and more recent molecular-based studies are conflicting.[22,23] In one study, reovirus RNA was detected in hepatic or biliary tissues from 55% of patients with extrahepatic biliary atresia and 78% of patients with choledochal cysts, compared with 21% of patients with other hepatobiliary diseases and 12% of autopsy cases.[22] However, a subsequent study failed to detect reovirus genome in hepatobiliary tissues taken from 26 patients with extrahepatic biliary atresia and 28 patients with congenital dilation of the bile duct.[23] No convincing data exist to support an etiologic role for reovirus infection in the setting of idiopathic cholestatic liver diseases in adults.

Central Nervous System Manifestations

Viruses belonging to orthoreovirus serotypes 1, 2, and 3 have been isolated from cerebrospinal fluid (CSF) of infants with meningitis, systemic illness, or both. An orthoreovirus serotype 1 was isolated from a previously healthy 3-month-old child with symptoms of meningitis, diarrhea, vomiting, and fever.[24] Orthoreovirus serotype 2 (subsequently identified as a new mammalian orthoreovirus type 2 Winnipeg) was isolated from the CSF of an 8-week-old child with active varicella-zoster virus infection, *Escherichia coli* sepsis, intermittent fever, diarrhea, and feeding intolerance.[25,26] A novel orthoreovirus belonging to serotype 3 reovirus (T3C/96) was isolated from a 6-week-old child with meningitis and was subsequently shown to be capable of producing lethal encephalitis in neonatal mice.[27] A novel orthoreovirus strain belonging to serotype 2 (MRV2Tou05), was implicated in two cases of acute necrotizing encephalopathy (ANE) in children from the same family.[28] These reports illustrate the rare but possible neuroinvasive potential of reoviruses in the human host.

Reovirus as an Oncolytic Agent

Orthoreoviruses induce apoptosis in multiple cell types.[6] They replicate preferentially in cells with activated *ras* genes or ras signaling pathways, which is the case in as many as 60% to 80% of human malignancies and 90% of metastatic cancers.[29] A number of preclinical studies have demonstrated the potential application of serotype 3 orthoreovirus as an anticancer oncolytic agent.[30] Multiple tumor types have been shown to be susceptible to orthoreovirus infection and oncolysis in vitro, including breast, ovarian, brain (glioma, medulloblastoma), colon, bladder, pancreatic, prostate, and lung cancers and melanoma; childhood sarcoma; and head and neck tumors.[31,32] Human clinical trials with proprietary formulations of mammalian reovirus serotype 3 Dearing (pelareorep [Reolysin]; Oncolytics Biotech, Calgary, Alberta, Canada) began in 2002. Patients have been treated in phase I, II, and III trials with pelareorep (Reolysin) intratumorally or intravenously, alone or in combination with other chemotherapeutic agents or radiation therapy.[33,34] The most recently published findings have included lack of efficacy of adjunctive orthoreovirus therapy to chemotherapy alone in trials involving recurrent ovarian, tubal, or peritoneal cancer[35] and metastatic pancreatic cancer,[36] but a possible added benefit against metastatic or recurrent small cell lung cancer[37] and potential efficacy in advanced malignant melanoma.[38] A phase II study in metastatic breast cancer suggested that addition of oncolytic reovirus (pelareorep) to chemotherapy did not enhance progression-free survival or recurrence rate but did increase overall survival from 10.4 months to 17.4 months, although the result did not reach statistical significance ($P = .1$).[39] In May 2017, the FDA granted pelareorep "fast track" designation for studies in treatment of metastatic breast cancer.

Although reovirus-based oncotherapy primarily targets cancer cells through direct killing by apoptosis (oncolysis), additional immune-based mechanisms of oncolysis including enhancement of antibody-dependent cellular cytoxicity have also been reported.[40,41] Synergistic antitumor effects of reovirus in combination with radiation or chemotherapy have been demonstrated.[41,42] In general, efficacy occurs in tumors with activated Ras signaling pathways. Characterization of immune responses to intravenous Reolysin have been reported[43]; in most clinical trials to date, the antireovirus immune response has been observed to correlate with decreased efficacy. Investigation into the use of reovirus as an immune adjuvant is underway with the goal of redirecting immune responses to target tumors.[42]

ORBIVIRUSES

Background and Epidemiology

The genus *Orbivirus* contains more than 130 subspecies classified within 19 species and/or antigenic serogroups, infecting a broad range of arthropod and vertebrate hosts. Orbiviruses are named based on their characteristic doughnut-shaped capsomers. The bulk of disease due to orbiviruses occurs in nonhuman vertebrates; the most frequently identified are bluetongue virus (sheep, cattle, goats, and wild ungulates),[44] African horse sickness virus (horses, donkeys, and dogs), and epizootic hemorrhagic disease virus (deer).[45] Disease in humans has been reported infrequently (fewer than 100 cases reported in the literature worldwide). Only one primary orbivirus-linked human disease, Oklahoma tick fever, occurs in the continental United States. This syndrome is associated with elevated antibody titers against Sixgun City and Lipovnik viruses, suggesting a likely causal association with these or a closely orbivirus species. Humans typically serve as an incidental host during the maintenance cycle of vector-borne transmission between nonhuman vertebrate hosts, and human-to-human orbivirus transmission has not been reported. Vectors for disease include mosquitoes, midges, gnats, sand flies, and ticks.[44–47]

Clinical Disease

Only four orbivirus serogroups have been linked to disease in humans; these are the viruses belonging to the Kemerovo antigenic complex, including Kemerovo, Lipovnik, and Tribec viruses (Russia and Eastern Europe; Oklahoma tick fever virus in the United States), Orungo virus (sub-Saharan Africa), Lebombo virus (South Africa and Nigeria), and Changuinola virus (Central America).[45] The spectrum of reported human disease includes neurologic infection (encephalitis, meningitis, meningoencephalitis, polyradiculitis) and acute febrile illnesses. Many orbiviruses preferentially infect vascular endothelial cells; thus clinical and laboratory manifestations can mimic those seen in the setting of rickettsial illnesses. No deaths have been reported due to human orbivirus infection, and patients generally recover without long-term sequelae of infection. All age groups may be infected; however, the pediatric population is

overrepresented in seroprevalence studies. In animals, orbivirus infection has been linked to congenital abnormalities such as hydranencephaly, arthrogryposis, and deafness, but these syndromes or illnesses have not yet been reported in humans.[45]

Clinical Manifestations of Specific Agents

Kemerovo Virus Antigenic Complex

Viruses of the Kemerovo complex (Kemerovo, Tribec, and Lipovnik) are transmitted by *Ixodes* spp. ticks in Russia and Eastern Europe and were first isolated in 1963 from ticks and patients with meningitis and meningoencephalitis. Meningoencephalitis and polyradiculitis have been linked to Lipovnik virus in the present-day Czech Republic. Seroprevalence studies in healthy residents of the former Czechoslovakia indicate up to 18% seropositivity; additional serologic evaluation of patients from Central Europe with tick-borne encephalitis virus infection and neurologic symptoms demonstrated the presence of concurrent Lipovnik virus antibodies in more than 50% of patients.[48]

Oklahoma Tick Fever

In the United States, cases of acute febrile illness, subsequently designated as Oklahoma tick fever, have been reported in Oklahoma and Texas and attributed to orbivirus infection, likely by a Kemerovo serogroup–related virus. Clinical features of infected patients included myalgia, vomiting, and severe abdominal pain. Laboratory features included transient leukopenia, thrombocytopenia, and anemia, suggesting possible rickettsial disease; however, serologic analysis for Rocky Mountain spotted fever, Colorado tick fever, and Powassan virus was negative.[44] Diagnosis was based on positive serology for Kemerovo group–related orbiviruses (Sixgun City and Lipovnik viruses). Viremia was not present in these patients. Transmission of Kemerovo-related viruses in rabbit and large animal populations has been documented in states in the Midwest, but no human cases have been reported to date.

Orungo Virus

Orungo virus is transmitted primarily by *Aedes* species but also by *Culex* and *Anopheles* species mosquitoes in regions of sub-Saharan Africa. Seroprevalence rates in that region are as high as 24% to 34%.[49] Acute febrile illness, including fever, headache, and myalgia, has been reported, as has one case of encephalitis in a child with convulsions and flaccid paralysis.[50,51] Coinfection with yellow fever virus has been documented.[52]

Lebombo Virus

Transmission of Lebombo virus occurs from *Aedes* and *Mansonia* species mosquitoes in South Africa and Nigeria. A case of nonspecific acute febrile illness has been reported in a Nigerian child.[50]

Changuinola Virus

Acute febrile illness due to Changuinola virus has been reported in a single human case from Panama. Seroprevalence studies indicate high rates of seropositivity in parts of South America, but the infection-to-disease ratio is unknown. Transmission may occur via phlebotomine flies.

African Horse Sickness Virus

Naturally occurring infection of humans with African horse sickness virus has not been reported. However, four workers in a South African veterinary office were infected in 1989 with accidentally aerosolized freeze-dried virus present in a vaccine containing attenuated viral strains. Illness was severe in all exposed workers; three developed frontotemporal encephalitis, and all four developed uveochorioretinitis. Diagnosis was confirmed serologically.[53]

Diagnosis

Laboratory diagnosis of orbivirus infection is made by (1) a fourfold rise in acute and convalescent serum antibody response as detected by complement fixation, enzyme immunoassay, or neutralization; or (2) virus isolation from serum or CSF by inoculation of suckling mice or cell cultures (Vero or BHK021). Virus-specific immunoglobulin M (IgM) testing is available at reference laboratories, including the Centers for Disease Control and Prevention and the US Army Medical Research Institute of Infectious Diseases. A variety of reverse-transcriptase polymerase chain reaction (RT-PCR) nucleic acid amplification techniques have been developed for research purposes and can be used for identification and molecular typing of veterinary pathogens including bluetongue viruses and epizootic hemorrhagic disease viruses. A consensus orbivirus RT-PCR using primers specific for the viral RNA-dependent RNA polymerase is also available as a research tool.[54] No specific therapy is available.

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The complete reference list is available online at Expert Consult.

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149 Coltiviruses (Colorado Tick Fever Virus) and Seadornaviruses

Daniel M. Pastula and Kenneth L. Tyler

SHORT VIEW SUMMARY

Overview and Virology

- *Coltivirus* and *Seadornavirus* are two of five genera of the Reoviridae virus family that have been documented to cause human disease.
- Coltiviruses and seadornaviruses are nonenveloped double-stranded RNA viruses with a segmented genome (12 gene segments enclosed within two concentric capsid shells).

Epidemiology

- Coltiviruses associated with human disease include Colorado tick fever virus (CTFV; North America), Salmon River virus (unclear if a distinct virus or just a serotype of CTFV; North America), and possibly Eyach virus (Europe).
- The only seadornavirus implicated in human disease to date is Banna virus (southeast Asia and China). Several other novel seadornaviruses have also been identified, though none are known to cause human disease.
- Ticks are thought to be the principal vectors of coltiviruses; mosquitoes are thought to be the principal vectors of seadornaviruses.
- CTFV is endemic to high-elevation (>4000 feet above sea level) western North America, where its principal tick vector—*Dermacentor andersoni,* or the Rocky Mountain wood tick—is found.

Clinical and Laboratory Features

- CTFV infection can cause a febrile illness with chills, headache, myalgias, and/or fatigue. Sore throat, nausea, vomiting, abdominal pain, arthralgia, neck pain or stiffness, and/or rash (either maculopapular or petechial) can also occur.
- Complications of CTFV disease can rarely include meningitis, encephalitis, disseminated intravascular coagulation, myocarditis, pericarditis, pneumonia, hepatitis, and epididymo-orchitis.
- Leukopenia is common in CTFV disease, and thrombocytopenia can occur. Anemia is rare despite CTFV infecting erythrocyte progenitor and red blood cells. Cerebrospinal fluid analysis may show a lymphocytic pleocytosis with mildly elevated protein and near-normal glucose.
- Eyach virus antibodies have been found in patients with polyradiculoneuritis and meningoencephalitis in Europe, but it is unclear whether Eyach virus itself was the cause.
- Banna virus disease can include fever, myalgia, arthralgia, and/or encephalitis.

Diagnosis

- CTFV should be considered in anyone presenting with a febrile illness following tick exposure in high-elevation western North America.
- CTFV nucleic acid can often be detected in the serum for 2 weeks after symptom onset by reverse-transcriptase polymerase chain reaction (RT-PCR).
- CTFV antibody development is often delayed for 2 to 3 weeks; CTFV-specific immunoglobulin M and confirmatory neutralizing antibody tests are available.
- Contact your state or local health department for assistance with CTFV testing.
- Eyach virus– and Banna virus–specific RT-PCR and antibody tests have been developed.

Therapy and Prevention

- No specific therapy is available for CTFV or Banna virus disease.
- Tick-bite and mosquito-bite prevention strategies are important.
- CTFV-infected patients should not donate blood or bone marrow for at least 6 months given the virus's persistence in red blood cells.

Coltivirus and *Seadornavirus* are two of five genera of the Reoviridae virus family that have been documented to cause human disease; *Orthoreovirus, Orbivirus,* and *Rotavirus* genera are discussed in Chapters 148 and 150. Like all Reoviridae, coltiviruses and seadornaviruses are nonenveloped double-stranded RNA viruses with a segmented genome (both have 12 gene segments enclosed within two concentric capsid shells). Coltiviruses were classified within the *Orbivirus* genus until 1991, at which time their distinct molecular identity was established and a unique genus was proposed. Coltiviruses were initially subclassified into the tick-borne subgroup A (North American and European distribution) and mosquito-borne subgroup B (Asian distribution) viruses. In the year 2000, subgroup B coltiviruses were reclassified into the newly designated genus *Seadornavirus* (an acronym for Southeast Asian dodeca RNA virus) based on sequence data and antigenic properties.[1–5]

Coltiviruses associated with human disease include Colorado tick fever virus (CTFV; North America), Salmon River virus (unclear if a distinct virus or just a serotype of CTFV; North America), and possibly Eyach virus (Europe).[5–11] The only seadornavirus implicated in human disease to date is Banna virus (southeast Asia and China).[3,5,12–16] Other novel seadornaviruses such as Liao Ning, Kadiporo, Mangshi, and Balaton viruses have recently been identified, but it is not clear whether they cause human disease.[3,5,17–19]

COLTIVIRUSES

Colorado Tick Fever Virus

Epidemiology

CTFV is endemic to high-elevation (>4000 feet above sea level) western North America, where its principal tick vector—*Dermacentor andersoni,* or the Rocky Mountain wood tick—is found (Fig. 149.1). CTFV is maintained in an epizootic cycle between *D. andersoni* and small, high-elevation mammals such as porcupines, squirrels, chipmunks, and mice.[6,20–23] People are typically infected after being bitten by a CTFV-infected tick; approximately 90% of CTFV disease cases in a recent epidemiologic review reported tick exposure prior to illness onset.[24] Hikers, campers, forestry workers, ranchers, and outdoor laborers in endemic areas are thought to be at particular risk. Rarely, CTFV transmission has also been documented by blood transfusion and laboratory exposure.[25,26]

Because CTFV disease has not been reportable on a national level, exact incidences across states are difficult to calculate, and CTFV disease cases may be underrecognized and underreported.[21,24,27] Two recent epidemiologic reviews suggest that Wyoming currently has the highest incidence of reported CTFV disease cases (3.4–7.2 cases per million people per year), followed by Montana (1.5–3.4) and Utah (0.5–1.4).[21,24] Colorado had the most reported CTFV disease cases

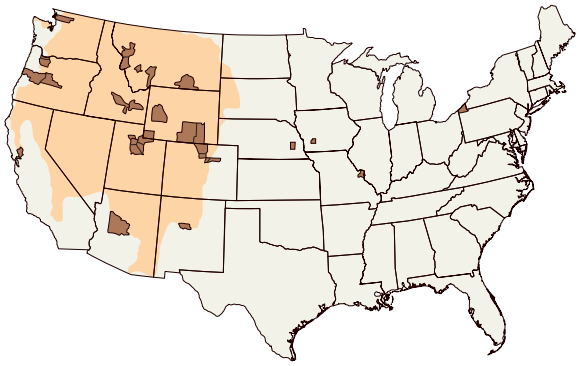

FIG. 149.1 Approximate geographic distribution of *Dermacentor andersoni* ticks and counties of residence for confirmed and probable Colorado tick fever virus (CTFV) disease cases, United States, 2002–2012. *All cases were acquired in states where local transmission of CTFV has been reported previously. Two additional cases were reported from Colorado with unknown county of residence. **Derived from James AM, Freier JE, Keirans JE, et al. Distribution, seasonality, and hosts of the Rocky Mountain wood tick in the United States. *J Med Entomol.* 2006;43:17–24. *(Courtesy Centers for Disease Control and Prevention. Colorado tick fever: statistics and maps.* https://www.cdc.gov/coloradotickfever/statistics.html.*)*

from 1987 to 2000, but that number has declined since for unknown reasons (possibly related in part to changes in testing and reporting practices).[21,27] CTFV disease cases typically occur from March through September, peaking in May or June. Males make up a higher percentage of reported cases than females. All age groups can be affected, but the highest incidence of reported CTFV disease appears to be in those of age >40 years.[21,24]

Clinical and Laboratory Features

CTFV has been isolated from patients with acute febrile syndromes, meningitis, and encephalitis in North America. The first published clinical descriptions of CTFV appeared as early as 1855, with identification of the virus in 1946 after isolation from human serum.[28,29]

Onset of symptoms typically occurs after an incubation period of 1 to 14 days, usually within 3 to 4 days. The most common CTFV disease symptoms include abrupt fever, chills, headache, myalgias, and/or fatigue. Sore throat, nausea, vomiting, abdominal pain, arthralgia, neck pain or stiffness, and/or rash (either maculopapular or petechial) can also occur. Approximately half of CTFV disease cases have a biphasic "saddleback" illness with a remission for 1 to 3 days followed by recurrence of fever and other symptoms. Neurologic complications such as meningitis and encephalitis can rarely occur, especially in children. Other rare complications of CTFV disease include disseminated intravascular coagulation, myocarditis, pericarditis, pneumonia, hepatitis, and epididymo-orchitis.[6,20,22,29–31]

In terms of laboratory findings, leukopenia (<4500 cells/mm^3) is common with a relative lymphocytosis and atypical lymphocytes. Moderate thrombocytopenia may also occur. Anemia is rare, though CTFV can infect both erythrocytic precursors and red blood cells, potentially for weeks. For those with meningitis or encephalitis, cerebrospinal fluid analysis can show a lymphocytic pleocytosis with mild elevation of protein and near-normal glucose.[6,20,22,29–33]

Most people with CTFV disease recover after 1 week, but prolonged fatigue (weeks to months) may occur in those over age 30 years. Up to 30% of CTFV disease cases may be hospitalized at some point in their illness. Death is rare, often being associated with disseminated intravascular coagulation or meningoencephalitis in children.[6,20,22,29–31]

Diagnosis

Suspicion for CTFV disease is warranted for any person with a CTFV disease–like illness with recent travel to and exposure in CTFV-endemic areas. CTFV infection can be diagnosed either molecularly by detection of CTFV nucleic acid (through reverse-transcriptase polymerase chain reaction [RT-PCR]) or serologically by detection of CTFV-specific immunoglobulin M (usually through indirect fluorescent antibody tests) and confirmatory CTFV neutralizing antibodies (usually through plaque-reduction neutralization testing). Given CTFV's ability to infect red blood cells for weeks, RT-PCR can often detect CTFV nucleic acid in the serum reliably for the first 2 weeks after symptom onset. On the other hand, antibody production is often delayed in CTFV infection, and CTFV antibody tests may not be positive until 2 to 3 weeks after onset of symptoms.[6,20,34,35] CTFV testing is available from some commercial laboratories and some state health departments, and at the US Centers for Disease Control and Prevention. State and local health departments can often assist with testing.

The differential diagnosis of CTFV-like illness includes other vector-borne diseases such as Rocky Mountain spotted fever, tick-borne relapsing fever, tularemia, and other arboviral diseases.

Treatment and Prevention

No specific antiviral therapy is available for CTFV disease, and treatment is supportive. Aspirin should be avoided because it may exacerbate the potential for hemorrhage associated with thrombocytopenia. Patients with severe symptoms may need to be hospitalized for rehydration and symptom management.[6,20,29] Ribavirin inhibited the growth of CTFV in vitro and ribavirin triacetate increased the survival of mice infected with CTFV, but no studies have been completed to determine these agents' efficacy or safety in humans.[36]

Tick-bite prevention strategies are most important in limiting CTFV disease in humans. People outdoors in endemic areas should use insect repellent on exposed skin, wear long-sleeved shirts and pants when feasible, avoid walking through high-brush areas, and perform tick checks once inside.[6,20]

Given the persistence of CTFV in bone marrow and red blood cells, persons infected with CTFV should not donate blood or bone marrow for at least 6 months after their illness.[6,20]

Salmon River Virus

It is unclear whether Salmon River virus is a distinct coltivirus or just a serotype of CTFV. It was isolated from a viremic patient with a CTFV-like illness and named for the area where infection was likely acquired: Idaho's Salmon River. Little is known about its epidemiologic, ecologic, or clinical features.[5]

Eyach Virus

Eyach virus is an antigenically related but distinct coltivirus from CTFV that was first isolated from *Ixodes* sp. ticks in France and Germany. Its ecologic cycle is not fully known, though its amplifying host is thought to be the European rabbit, and serosurveys have found Eyach virus–specific antibodies in rodents, sheep, goats, and deer in parts of Europe.[5–11] Eyach virus has been suspected of causing human neurologic disease (specifically polyradiculoneuritis and meningoencephalitis) because Eyach virus–specific antibodies were recovered in the serum of affected patients.[8] However, the actual virus was never isolated from these patients so causation is difficult to prove. Eyach virus–specific RT-PCR and antibody tests have been developed.

SEADORNAVIRUSES

Banna Virus

Banna virus is a presumably mosquito-borne seadornavirus endemic to Southeast Asia and China, and is the only known seadornavirus implicated in human disease to date.[3,5,12–16] It has been isolated from multiple mosquito species (e.g., *Aedes, Anopheles, Culex* spp.) throughout the region. Not much is known about its ecologic cycle or epidemiology. Banna virus was first isolated from the sera of febrile patients and the cerebrospinal fluid of encephalitic patients in the Xishuangbanna Prefecture of China's Yunnan Province in 1987.[12] Banna virus was later isolated from the sera of several febrile patients in China's Xinjiang Province, and Banna virus–specific antibodies were detected in several patients suspected of having Japanese encephalitis virus disease throughout eastern China.[13] Clinically, symptoms of Banna virus disease appear to include fever, myalgia, arthralgia, and/or encephalitis. However, the

full range of clinical disease and subsequent outcomes is not known. Banna virus–specific RT-PCR and antibody tests have been developed. There is no specific known treatment for Banna virus disease other than supportive care. Mosquito-bite prevention strategies such as applying insect repellent when outdoors, wearing long-sleeved shirts and pants when feasible, and using window screens or air conditioning when indoors may help prevent infection in endemic areas.

ACKNOWLEDGMENT

Dr. Roberta DeBiasi contributed to earlier editions of this chapter.

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150 Rotaviruses

Philip R. Dormitzer[a]

SHORT VIEW SUMMARY

Definition
- Rotavirus is a nonenveloped, double-stranded (ds)RNA virus that is the most important cause of dehydrating infant and childhood gastroenteritis.

Viral Structure and Replication
- The viral particle is a triple-layered icosahedron.
- Genome consists of 11 segments of dsRNA.
- The outer-layer glycoprotein VP7 and the spike protein VP4 mediate entry into cells and are the neutralization determinants.
- The virus binds cell surface carbohydrates.
- Cells are entered by viral penetration of cellular membranes.
- After the outer layer is removed, a subviral particle transcribes and extrudes messenger RNA.
- New subviral particles assemble in viroplasms in the cytoplasm, and virions are completed by addition of the outer-layer proteins in the endoplasmic reticulum.

Clinical Manifestations
- Gastroenteritis due to rotavirus is not readily distinguished from gastroenteritis caused by other agents on clinical grounds alone.
- Main symptoms are diarrhea and vomiting. Severe dehydration and death can result.

Pathogenesis
- The virus primarily infects epithelial cells at the tips of the intestinal villi.
- Multifactorial pathogenesis of rotavirus diarrhea includes malabsorption due to loss of intestinal epithelial cells, an enterotoxin, and enteric nervous system signaling to create a secretory state.

Serologic Classification
- Most rotaviruses that cause human disease are in group A.
- There is extensive diversity of antigenic types.
- Within group A, rotavirus has a dual serologic classification system, with G type based on VP7 and P type based on VP4.
- Genetic classification is starting to replace serologic classification.

Epidemiology
- Infection is universal in the first years of life.
- Infection may be symptomatic or asymptomatic.
- Severe illness is most common between 6 months and 2 years of age.
- Reinfection occurs throughout life.
- There is a large burden of illness in all socioeconomic settings, but mortality is concentrated in developing countries.
- As of 2016 rotaviruses caused approximately 128,500 deaths of children younger than 5 years each year, a reduction from approximately 538,000 in 2000 due, in part, to implementation of vaccines.[1]
- Exchange of strains and genome segments between human rotaviruses and animal rotaviruses maintains the diversity of circulating strains.

Immunity
- Partial protection from reinfection is provided by previous infection, with generally decreasing severity of subsequent infections.
- Serotype influences but does not determine protection from reinfection.
- VP4 and VP7 each contain serotype-specific and heterotypic neutralizing epitopes.
- Neutralizing antibodies block the functions of the outer capsid proteins in cell entry.
- Nonneutralizing immunoglobulin A against the middle-layer protein, VP6, can interfere with intracellular virus replication, as the antibody is transcytosed across intestinal epithelial cells.
- Humoral immunity appears to be the most important determinant of protection from infection.
- Innate and cellular immunity contribute to clearance of infection.
- Rotavirus interferes with the innate immune response by several mechanisms.

Diagnosis
- Specific virologic diagnosis is not necessary for routine clinical care but is useful for epidemiologic study, in complicated or prolonged cases, and to reduce unnecessary antibiotic use.
- Antigen can be detected readily in the stool, most commonly by enzyme-linked immunosorbent assay.
- There is increasing use of nucleic acid–based testing for epidemiologic investigations.

Therapy
- Therapy is primarily supportive and aimed at maintaining hydration until the infection resolves.
- No specific antiviral therapy is available.
- Mild and moderate dehydration can be treated effectively with oral rehydration solutions.
- Treatment of patients who have severe dehydration requires the use of intravenous fluids.
- In the context of malnutrition, zinc supplementation may be a useful adjunct to oral rehydration therapy.

Immunization
- Two live-attenuated, oral rotavirus vaccines have been licensed in multiple countries, another two have been licensed in India and achieved World Health Organization prequalification, and two more have national licenses in China or Vietnam.
- Where rotavirus vaccines have been introduced, they have dramatically decreased severe rotavirus gastroenteritis and substantially decreased severe dehydrating pediatric gastroenteritis overall.
- A rare complication of immunization, intestinal intussusception, led to the withdrawal of a previous rotavirus vaccine, but there is consensus that the benefits of immunization with the current rotavirus vaccines far outweigh the risk of intussusception.
- Progress is being made in distributing rotavirus vaccines in developing countries where mortality is concentrated, in part due to local production of new rotavirus vaccines.
- Despite lower efficacy in impoverished settings than in affluent settings, rotavirus vaccines have the potential for a large impact on infant mortality worldwide.

HISTORY AND OVERVIEW

Classification and Impact

Rotavirus is a genus within the Reoviridae, a family of nonenveloped, icosahedral animal viruses with double-stranded (ds), segmented RNA genomes. The family Reoviridae also includes reoviruses, which have occasionally been isolated from patients with respiratory illnesses but have not been established as a cause of human disease, and coltiviruses and seadornaviruses, which cause disease in humans (see Chapters 148 and 149). Rotaviruses are the most important cause of severe dehydrating gastroenteritis in children younger than 5 years in all socioeconomic groups and in all regions of the world. In 2016 they caused approximately

[a]Philip Dormitzer notes a potential conflict of interest because he is a Pfizer employee and shareholder.

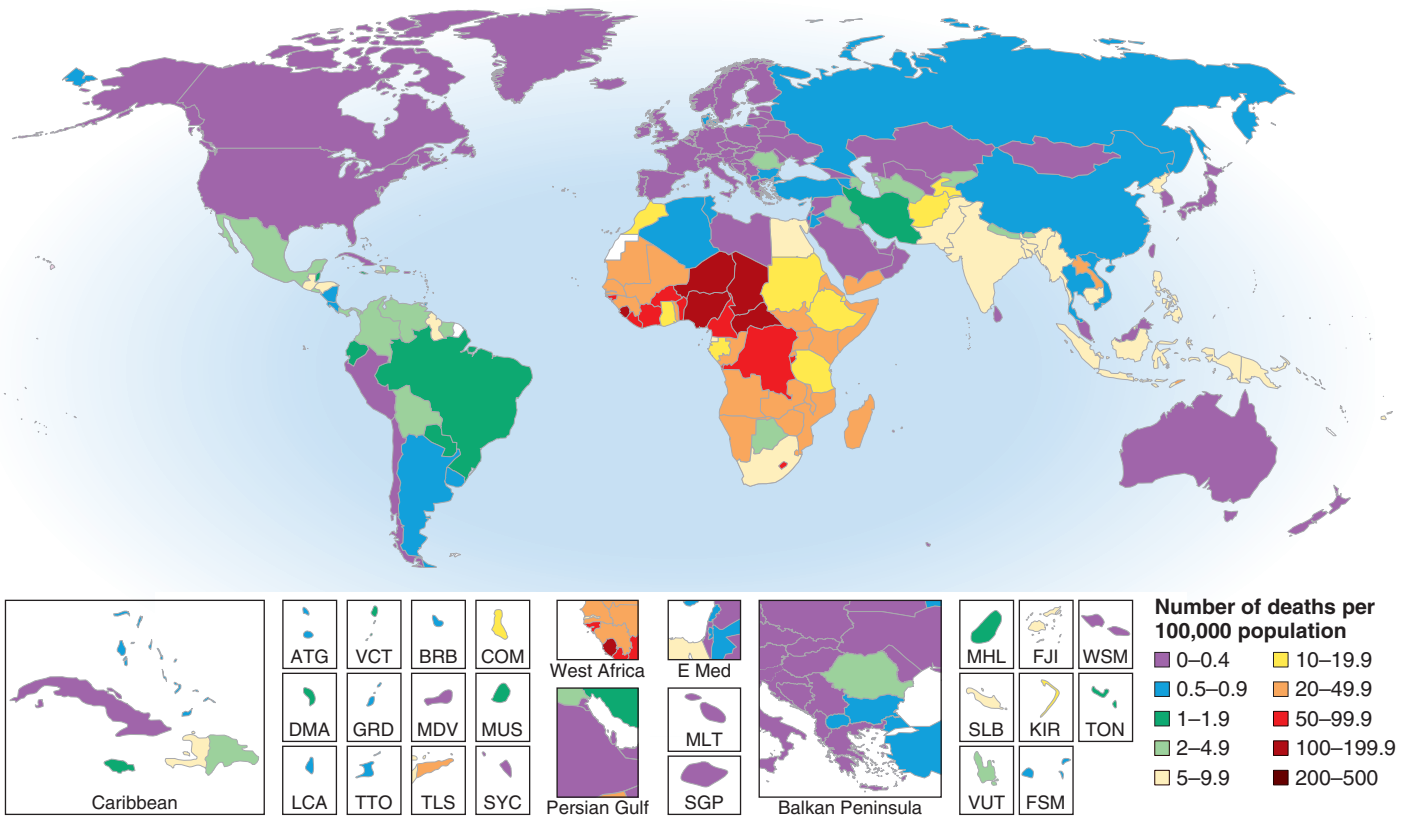

FIG. 150.1 Geographic distribution of rotavirus-associated mortality rates among children younger than 5 years in 2016. *(From Troeger C, Khalil IA, Rao PC, et al. Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years.* JAMA Pediatr. *2018;172:958–965.)*

238 million episodes of diarrhea and approximately 128,500 deaths of infants and children younger than 5 years, with approximately 104,733 of those deaths occurring in sub-Saharan Africa (Fig. 150.1).[1] Higher latitude and higher income correlate with a seasonal pattern of disease in cold and dry months; lower latitude and income correlate with less distinct seasonality, although neither geography nor income fully account for observed annual patterns.[2]

Discovery

Before the association of rotaviruses with human disease, etiologic agents were not identified in most cases of childhood gastroenteritis. In 1973 electron microscopic examination of duodenal biopsy specimens from six of nine children with acute gastroenteritis revealed similar viral particles, which were approximately 70 nm in diameter.[3] Morphologically, these particles were indistinguishable from viruses previously identified in specimens from mice and cows with diarrhea[4,5] and were designated "rotaviruses" because of their appearance in electron micrographs as wheels with spokes (Fig. 150.2). The antigenic similarity between the human and bovine agents was confirmed when an exchange of matched liquid stool samples and convalescent sera between a veterinary and a medical laboratory showed that antibodies in the sera of children and calves agglutinated the rotavirus particles in the stools of both species.[6] The human sera also neutralized the infectivity of the bovine agent, which had been adapted to growth in cell culture.[6]

Path From Basic Science to Vaccine Introduction

Since the discovery of the agent, understanding of rotavirus replication, pathogenesis, and immunity has relied on the study of strains that grow well in cell culture and of large and small animal models for rotavirus infection and disease. The ability to "mate" rotavirus strains by coinfecting cells to achieve reassortment of the 11 genome segments has allowed classic genetic studies. Extensive global epidemiology has relied on elaborate serology, now supplemented by nucleic acid–based diagnostics. This scientific background enabled the introduction of live-attenuated vaccines for human use. These vaccines greatly reduced the health impact of rotaviruses in the upper- and middle-income countries where they were first introduced, with evidence of both direct vaccine-elicited immunity and a herd effect.[7] The development of locally produced, less costly live-attenuated rotavirus vaccines promises more widespread rotavirus immunization for the most severely affected populations in developing countries.[8] The application of molecular and structural biology provides the scientific basis for the next generation of vaccines, which may overcome the lower effectiveness of the live-attenuated approach in low-income settings.[9]

VIRAL STRUCTURE AND REPLICATION

Structural Overview

The rotavirus virion is a nonenveloped icosahedral particle that is approximately 770 Å in diameter, excluding the VP4 spikes (Fig. 150.3A).[10] It consists of three concentric protein layers that encapsidate 11 segments of tightly packed dsRNA, together with polymerase and capping enzyme complexes. Each genome segment contains one or, in the case of genome segment 11, two open reading frames. These segments encode six structural proteins (VP1–VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP6).

The innermost protein shell consists of 120 copies of VP2, arranged in an icosahedral lattice (T = 1 with two VP2 molecules making up each icosahedral asymmetrical unit).[10] The VP1 polymerase and VP3 capping enzyme are anchored inside the VP2 shell adjacent to pores at the icosahedral fivefold vertices. The middle layer consists of 780 copies of VP6, which form thick trimeric pillars in an icosahedral lattice (T = 13 *levo*). Although not exposed on the virus surface, VP6 is the target of the most abundant antibodies elicited by rotavirus infection. The genome, VP1, VP3, and the inner two protein layers make up the transcriptionally active, double-layered subviral particle (DLP) (see Fig. 150.3C *right*).

The thin outermost layer consists of 780 copies of a coat glycoprotein, VP7, and 180 copies of a spike protein VP4, which forms asymmetrical trimers that protrude from the virion (see Fig. 150.3A, D *left*).[10] One hundred and thirty-two aqueous holes perforate the outer and middle layers. VP4 and VP7 translocate the DLP across a host membrane and into the cytoplasm (see Fig. 150.3C). Both outer-layer proteins are neutralization antigens.[11] VP4 is the major cell attachment protein, a membrane penetration protein, and a determinant of virulence.[11–14] VP7 forms the icosahedral lattice of the outermost layer, which is in register with the VP6 lattice of the middle layer.

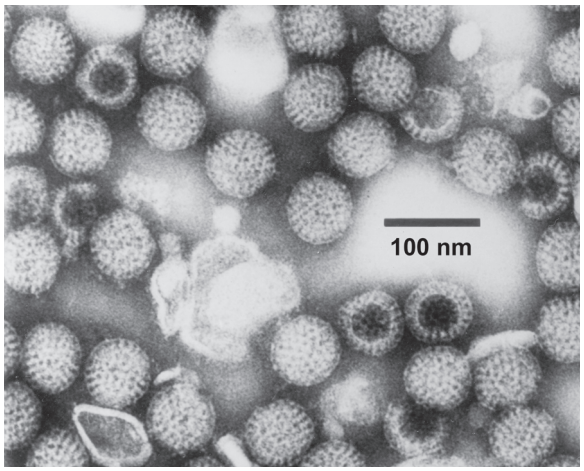

FIG. 150.2 Electron micrograph of negatively stained rotavirus virions in a stool filtrate from a child with gastroenteritis. The outer capsid appears as a thin rim surrounding stubby protrusions made up of VP6 trimers. "Empty" particles with dark centers lack genomic RNA. *(From Kapikian AZ, Kim HW, Wyatt RG, et al. Reoviruslike agent in stools: association with infantile diarrhea and development of serologic tests.* Science. *1974;185:1049–1053.)*

Inactivation

The rotavirus particle is physically hardy and resists inactivation by treatment with fluorocarbons, ether, and concentrations of chlorine typically used to treat sewage effluent and drinking water.[15] However, the particle is inactivated by calcium chelators and by antiseptic agents that contain relatively high concentrations of alcohols (>40%), free chlorine (>20,000 ppm), or iodophors (>10,000 ppm iodine).[16] A commonly used hand-sanitizing agent (Purell), which contains 62% ethanol, substantially reduces viable rotavirus carriage on fingertips,[17] and a disinfectant spray (Lysol), which contains 79% ethanol and 0.1% *o*-phenylphenol, prevents the experimental transmission of rotavirus from fomites to human volunteers.[18] Rotavirus survival in the environment is significantly decreased at high relative humidity.[15]

Priming for Infectivity

Efficient rotavirus infectivity requires priming of the virus by intestinal trypsin, which cleaves the spike protein, VP4, into an amino-terminal fragment, VP8*, and a carboxy-terminal fragment, VP5* (see Fig. 150.3B, C *left*, D *left*). The VP8* fragment contains a carbohydrate receptor-binding domain (lectin domain), and the VP5* fragment contains a β-barrel domain that interacts with membranes.[10] Cleavage into VP5* and VP8* triggers an initial rearrangement of VP4 trimers into an asymmetrical conformation (see Fig. 150.3D *left*) in which the three subunits form a "foot," buried under the VP7 shell with approximate threefold symmetry, and the β-barrel domain of VP5* of one subunit

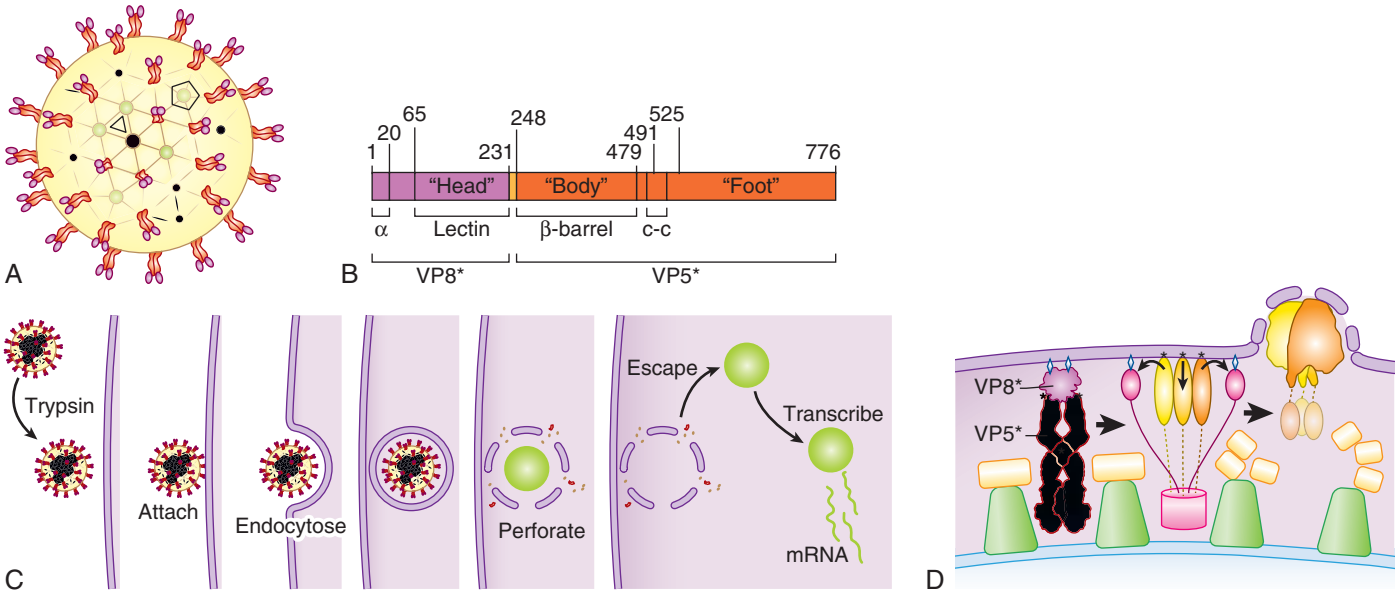

FIG. 150.3 The rotavirus particle and conformational transformations during entry. (A) Surface representation of the mature rotavirus virion. VP4 is in *magenta* (VP8*) and *red* (VP5*); VP7, in *yellow;* VP6 in *green*; *black triangle*—threefold symmetry axis; *black pentamer*—fivefold symmetry axes. (B) Domains of VP4. Numbers above the bar, amino-acid residues; lines immediately beneath the bar, substructures of cleaved VP4 (α: N-terminal helix, which anchors VP8* into the foot; *lectin*: receptor-binding domain; β-*barrel*: projecting domain of VP5*, which contains the membrane interaction loops; *c-c*: coiled-coil structure, which forms when VP5* folds back); *bottom lines:* trypsin cleavage fragments. (C) Entry pathway. The loss of VP4 and VP7 after endocytosis into a tightly fitting vesicle is concurrent with membrane perforation and converts the triple-layered virion into a double-layered, transcriptionally active particle with a VP6 *(green)* surface. (D) Proposed correlation of conformational changes in the mature VP4 spike with remodeling and breaking of a bilayer membrane. *Left:* mature spike, anchored into the VP6 layer *(green trapezoid)* by VP7 *(yellow rounded rectangle)*. VP4 is activated by cleavage to VP8* *(magenta)* and VP5* *(red*). *Black stars:* hydrophobic membrane interaction loops of the VP5* β-barrel domain; *blue diamonds:* glycolipid head groups to which the VP8* lectin domains attach; *blue curved bar:* VP2 layer; *purple curved bar*: cellular membrane. *Center:* Schematic diagram of the product of an initial conformational change, in which VP8* lectin domains dissociate from their positions covering the hydrophobic loops of VP5*, exposing them for interaction with the membrane. VP8* lectin domains are *magenta ovals;* VP5* β-barrel domains are *red, orange,* and *gold ovals* connected by flexible links to the foot *(pink cylinder). Right:* loss of Ca^{2+} causes VP7 *(yellow)* to detrimerize and dissociate from VP6, releasing VP5*, which folds back to its most stable, postcleavage conformation. The interaction between membrane and VP5* hydrophobic loops couples membrane distortion and perforation *(dashed lines)* to folding back of VP5*. VP5* coiled-coil and β-barrel domains are *red, orange,* and *gold irregular shapes;* VP5* foot domains are *light red, orange,* and *gold ovals. (Modified from Abdelhakim AH, Salgado EN, Fu X, et al. Structural correlates of rotavirus cell entry.* PLoS Pathog. *2014;10:e1004355. Courtesy Stephen C. Harrison.)*

forms a "stalk" that supports a "body," formed by the β-barrel domains of VP5* from each of the other two subunits.[10] The spike is topped by two "heads," formed by the VP8* lectin domains of two of the subunits.[10]

Receptor Binding and Human Susceptibility

The rotavirus cell entry pathway is complex, with entry-related conformational changes in VP4 and VP7, strain-dependent variation in use of cell surface receptors, and possibly strain-dependent variation in the use of different endocytic pathways.[19,20] The first rotavirus interaction with a cell generally occurs when the VP8* lectin domains bind a cell surface glycan (see Fig. 150.3B, C). Many rotavirus strains that cause human disease bind to the human histo-blood group antigens (HBGAs), which are present not only on the surface of erythrocytes but also on enterocytes and other cells.[21,22] The distribution of HBGAs in human populations is determined by polymorphism in glycosyltransferases, such as fucosyltransferase 2 (FUT2; for secretor status) and FUT3 (for Lewis type). The specificity of rotaviruses for different HBGAs varies with their P genotypes (determined by VP4).[22] Thus human populations vary in their susceptibility to infection by certain rotavirus strains. For example, those who are secretor negative appear to be resistant to disease caused by P8 rotaviruses and at lower overall risk of severe rotavirus gastroenteritis in the United States, where P8 rotaviruses are prevalent.[23–25] In Bangladesh nonsecretor status was found to protected against P4 rotavirus disease.[26] In contrast, many rotaviruses that infect nonhuman animals initially bind neuraminidase-sensitive terminal sialoside on cell membrane glycoproteins or glycolipids.[27] A variety of potential secondary receptors, such as heat shock protein 70 or integrins α2β1, αVβ3, or αXβ2, have been proposed for rotavirus, although the position of the putative binding sites for these ligands on VP4 and VP7 appear inconsistent with interactions before virus uncoating.[28]

Internalization Into Target Cells

Several rotavirus entry pathways have been proposed, such as direct plasma membrane penetration or uptake through dynamin-dependent, clathrin-dependent, or clathrin- and caveolin-independent endocytosis.[20,29,30] Rotavirus entry studies are complicated by many nonproductive internalizations accompanying every productive entry event and by potential differences in entry pathway based on virus strain or cell type. The entry of single, fluorescently labeled viruses has been studied in cultured monkey kidney epithelial cells, with the rapid cytoplasmic diffusion of rotavirus DLPs after separation from the viral outer layer used as a marker for productive entry. In these experiments, the VP4 spikes appear to mediate the invagination of virus adherent at the plasma membrane into tight-fitting vesicles (see Fig. 150.3C) that are not consistently associated with the endosomal marker Rab5, the clathrin-associated adaptor protein AP-2, or dynamin.[31]

Conformational Changes Linked to Membrane Perforation and Escape

Endosomal acidification is not required for rotavirus entry, as it is for many other viruses. Instead, loss of calcium from the tight-fitting vesicle formed around the virus as it is invaginated appears to be a key trigger for rotavirus molecular rearrangements that lead to cell entry (see Fig. 150.3C, D).[32] In low calcium environments, calcium is lost from the VP7 intratrimer interface, and the VP7 trimers dissociate, releasing clamplike contacts with VP6 so that VP7 is shed from the virion.[33,34] When VP7 uncoating occurs in the presence of membranes, cleaved VP4 rearranges to an inferred intermediate conformation that binds the membranes (see Fig. 150.3D *middle*).[35] This intermediate conformation resolves into a post–membrane-penetration state in which the VP5* fragments form an umbrella-shaped, threefold symmetrical, folded-back trimer (see Fig. 150.3D *right*).[36] During these conformational changes, the hydrophobic apex of VP5* is translocated from one end of the molecule to the other.[36] This motion resembles the fold-back rearrangements of enveloped virus fusion proteins, which mediate fusion between a virus envelope and a cellular membrane. Loss of membrane perforation by recoated rotaviruses, as measured by α-sarcin entry into cells, when the VP5* apices are mutated to reduce their hydrophobicity, suggests that the "jackknifing" of VP5* breaches the cellular membrane.[14] Escape of DLPs from the small vesicles that form around invaginating rotavirus virions also requires intact hydrophobic VP5* apices, suggesting that the VP5* rearrangement disrupts this small vesicle to release DLPs into the cytoplasm (see Fig. 150.3C).[14,31] The failure of particles with cross-linked VP7 to perforate membranes or escape from the small vesicles suggests that release of the VP7 "clamp" is required for the VP5* rearrangement to occur (see Fig. 150.3D).[31,37]

Transcription From Double-Layered Particles and Preferential Translation of Viral mRNA

The loss of VP7 leads to conformational shifts deeper in the particle, near the polymerase complex below the channels at the fivefold vertices, to activate the transcriptase activity of the rotavirus DLP so that, once in the cytoplasm, the DLP extrudes capped, nonpolyadenylated messenger RNA (mRNA) through channels at its fivefold icosahedral vertices (see Fig. 150.3C *right*).[34,38,39] The genome remains encapsidated throughout transcription. The translation of viral mRNAs is enhanced by circularization, mediated by the specific binding of the rotavirus nonstructural protein NSP3 to a four-nucleotide consensus sequence at the 3′ end of viral mRNA and to the cellular cap-binding protein eIF4G.[40–42] Thus NSP3 substitutes for a cellular polyadenylic acid–binding protein to favor the translation of the nonpolyadenylated viral mRNAs over polyadenylated host mRNAs.

Genome Packaging and Assortment

Rotavirus genome replication must overcome two major challenges: One copy of each of 11 different RNAs must be selected for packaging, and the newly synthesized dsRNA must be sequestered so that it does not induce an interferon (IFN) response. The mechanism of selective packaging remains obscure but may involve base-pairing interactions between the nascent genome segments; the mechanism of genome sequestration is better understood.[43] The viral polymerase, VP1, specifically binds rotavirus mRNAs by recognizing a consensus sequence (UGUG) near their 3′ ends and holds the mRNAs in inactive complexes.[44] The mRNA shifts to a position that permits negative-strand synthesis only when the polymerase binds to the inner face of a decamer of the inner-shell protein, VP2.[45] The dependence of negative-strand synthesis on VP1-mRNA complexes binding to the inner-shell protein ensures that double-stranded genomic RNA is synthesized within a nascent particle.

Viroplasm Formation

DLPs are assembled in cytoplasmic viral "factories," termed *viroplasms.*[43] Viroplasms are associated with intracellular lipid droplets and form through the interaction of rotavirus nonstructural proteins NSP2 and NSP5 and through lattice formation by NSP2 when both NSP2 and NSP5 are phosphorylated by a cellular kinase, casein kinase 1α, and by autokinase activity.[46,47] NSP2 is an octomeric doughnut-shaped structure with RNA-binding, helix-destabilizing, autokinase, nucleoside triphosphatase, and nucleoside diphosphate kinase–like activities.[47] Activities proposed for NSP2 include mediating specific interactions between rotavirus RNAs to drive genome segment assortment, acting as a molecular motor to drive replicating mRNA into the nascent particles, and maintaining nucleotide pools in viroplasms during replication.[48–50] NSP5 is a metalloprotein that binds an iron-sulfate cluster and also a phosphoprotein that has autokinase activity.[51,52] NSP5 probably has a regulatory role, competing with single-stranded RNA to bind in grooves of the NSP2 octamer, competing with VP6 for binding to VP2, and binding RNA.[53,54] Once VP6 is added to the nascent particle, the newly formed DLP can transcribe mRNA, amplifying virus yield. Newly transcribed rotavirus mRNA enters two pools: mRNA that remains sequestered within viroplasms is replicated and packaged as genome segments; mRNA that escapes viroplasms is translated.[55]

Reverse Genetics Systems

Despite the barrier imposed by the need to introduce transfected mRNA into viroplasms for replication into packaged rotavirus double-stranded genome segments, recent advances in rotavirus reverse genetics systems now allow the relatively efficient incorporation of recombinant genes

into infectious rotavirus genomes.[56] Previous rotavirus reverse genetics systems required the use of helper viruses or genes encoding accessory proteins to achieve reliable rotavirus rescue from DNA.[57] However, increasing the amounts of plasmids encoding NSP2 and NSP5 relative to the amounts of plasmids encoding the other genome segments in the transfection mixtures has allowed recovery of infectious rotaviruses expressing fluorescent markers after the transfection of plasmids encoding the rotavirus genome segments.[56] These advances in rotavirus reverse genetics promise accelerated basic discovery and, potentially, rationally attenuated vaccine strains.

Outer Capsid Assembly

After genome replication and DLP assembly, the outer capsid is added through an unusual maturation process. The DLP binds the cytoplasmic tail of NSP4, a rotavirus NSP that is resident in the membranes of the endoplasmic reticulum (ER) and viroplasm-associated vesicles, and then buds into the ER lumen, transiently acquiring an envelope.[58] VP7, also resident in the ER, mediates removal of the transient envelope and binds the DLP, locking the VP4 spike protein in place.[59,60] Thus rotavirus penetrates membranes twice. During entry rotavirus penetrates a cellular membrane through a mechanism involving rearrangements of cleaved VP4 as the outer capsid disassembles (see Fig. 150.3C, D); during maturation rotavirus penetrates an envelope derived from the ER through a mechanism that involves VP7 and possibly NSP4, as the outer capsid assembles. Rotavirus egress from infected enterocytes is accomplished by release from the apical surface after vesicular transport from the ER by a pathway that bypasses the Golgi apparatus.[61]

CLINICAL MANIFESTATIONS

Typical Clinical Illness

Infants and young children with diarrhea caused by rotavirus are more likely to have severe symptoms and become dehydrated than patients with diarrhea related to other common enteric pathogens.[62,63] The clinical manifestations of rotavirus gastroenteritis in humans have been studied in experimental infections in adults. In one such study 4 of 18 adult volunteers developed vomiting 1 to 3 days after oral administration of a virulent rotavirus strain.[64] This was followed by diarrhea lasting 1 to 4 days and associated with anorexia, crampy abdominal pain, and low-grade fever. Viral shedding in stool was detected for 6 to 10 or more days. Two-thirds of the adult volunteers developed serologic evidence of infection without disease. Similarly, most naturally occurring rotavirus infections of adults are asymptomatic, manifested only by a rise in antibody titer.[65] However, rotavirus infection of adults can also cause severe and even fatal disease.[66]

Observational studies of children in North America hospitalized with rotavirus gastroenteritis reveal a similar, although more severe, pattern of disease.[62,63,67] Rotavirus gastroenteritis in children generally begins with vomiting and fever, which lasts 2 to 3 days, and progresses to profuse watery diarrhea, which continues for 4 to 5 days. Vomiting is more common and prolonged with rotavirus gastroenteritis than with pediatric gastroenteritis caused by most other agents.[67]

Clinical Laboratory Findings

Laboratory findings in children hospitalized with rotavirus gastroenteritis reflect isotonic dehydration and include high urine specific gravity and metabolic acidosis.[62,67] Rotavirus gastroenteritis is not generally associated with leukocytosis but is sometimes accompanied by a mild elevation in aminotransferase and uric acid levels.[62,67] Liquid stools from children with rotavirus diarrhea usually do not contain blood or fecal leukocytes,[62,67] although the presence of fecal leukocytes does not exclude rotavirus as a cause.[63] Typically, virus is detected in stools of children by antigenic assays for 4 to 10 days after the onset of symptoms.[62] When a sensitive reverse-transcriptase polymerase chain reaction (RT-PCR) assay is used, shedding of viral RNA can be detected for up to 57 days from immunocompetent children, although this does not necessarily indicate shedding of infectious particles.[68]

Spectrum of Disease Severity

Dehydration and severe electrolyte abnormalities leading to cardiovascular failure are the most common proximate causes of death from rotavirus gastroenteritis.[69] Seizures and aspiration of vomitus may also lead to death.[69] The spectrum of illness after rotavirus infection of infants and children also includes mild gastroenteritis and asymptomatic infection. In some neonatal nurseries, difficult-to-eradicate endemic rotavirus strains asymptomatically infect neonates year round.[70,71] A common VP4 type (P[6]) and genetic stability over time suggest that these nursery strains may be less virulent than most circulating strains,[70] although maternally transmitted immunity or maturational resistance may also protect the neonates from disease. Because outbreaks of rotavirus diarrhea in neonatal nurseries also occur, any component of maturational resistance cannot be absolute.[72]

Extraintestinal Manifestations and Disease in Immunodeficiency

Rotavirus also has extraintestinal manifestations.[73] Viremia, which is common in symptomatic and asymptomatic rotavirus infection, has unknown clinical significance in immunocompetent children.[74] In immunocompromised children rotavirus infection has been associated with chronic diarrhea and extraintestinal infection. Conditions associated with chronic rotavirus infection include X-linked agammaglobulinemia, cartilage hair hypoplasia, and DiGeorge syndrome.[75–78] Rotavirus shed from chronically infected children often has altered genome segments.[76,78] Immunohistochemical staining for rotavirus structural and nonstructural proteins in autopsy specimens has demonstrated rotavirus replication in the hepatocytes and renal tubular cells of immunodeficient children with chronic rotavirus infection at the time of death.[75] Relatively prolonged rotavirus shedding and severe gastroenteritis have been noted in both children and adult inpatients with immunosuppression related to bone marrow or renal transplantation.[79–82] Rotavirus is not more common or severe in children with human immunodeficiency virus (HIV) disease, and rotavirus immunization is not contraindicated for infants infected with or exposed to HIV.[83,84]

Other Disease Associations

Rotavirus has been detected in association with a number of syndromes other than gastroenteritis, including respiratory tract infections,[85] necrotizing enterocolitis,[86] pneumatosis intestinalis,[87] myocarditis,[88] seizures,[89] and meningoencephalitis.[89] Because rotavirus infection is universal, these associations may be coincidental rather than causative, although an 18% to 21% decrease in the incidence of seizures requiring hospitalization or emergency room care during the year after rotavirus immunization suggests that the link between rotavirus infections and seizures could be causal.[90] Although intestinal intussusception has been temporally associated with immunization with rotavirus vaccines (see "Immunization"), intestinal intussusception does not appear to be associated with natural rotavirus infection.[91]

Proposed Links to Autoimmunity

Links between rotavirus infection and the subsequent development of autoimmune disease have been proposed. Although some strains of rotavirus can cause biliary atresia in mouse models, and group C rotavirus has been detected in liver tissue from biliary atresia patients, rotavirus has not been convincingly demonstrated to cause this condition in humans.[92] Serologic evidence of more frequent rotavirus infection has been associated with the development of celiac disease in predisposed children.[93] A potential for molecular mimicry between rotavirus peptides and pancreatic islet autoantigens led to a proposal that rotavirus infection can be a trigger for type I diabetes mellitus.[94] To date the introduction of effective rotavirus immunization has not provided evidence of a causal link between symptomatic rotavirus infection and subsequent type 1 diabetes mellitus, celiac sprue, or primary biliary atresia.[95,96]

PATHOGENESIS

Pathologic Findings in Infection

The pathogenesis of rotavirus diarrhea is complex and incompletely understood, with potential roles for a viral enterotoxin, malabsorption related to mucosal damage and depression of disaccharidases, and secretion mediated by the enteric nervous system (ENS). Postmortem examination of the gastrointestinal (GI) tract of gnotobiotic pigs with diarrhea after experimental infection with a virulent human rotavirus

strain demonstrated that virus replicates primarily in the villous epithelium of the small intestine.[97] This pattern is consistent with the patchy villous epithelial distribution of rotavirus antigen noted after immunofluorescent staining of duodenal biopsy specimens from children with severe gastroenteritis.[98] Light microscopic examination of such duodenal biopsy specimens reveals shortened and blunted villi with a cuboidal epithelium, crypt hypertrophy, and mononuclear cell infiltration of the lamina propria.[3,99]

Human Intestinal Enteroid Infection Model

Human intestinal enteroids (HIEs) derived from human intestinal crypt domains can be differentiated into three-dimensional cultures that recapitulate elements of small intestinal architecture, contain multiple intestinal cell types, and support the efficient replication of human rotaviruses.[100] Human rotaviruses can replicate in HIEs from the duodenum, jejunum, and ileum; infect HIEs with villus-like architecture in preference to HIEs with cryptlike architecture; and infect both enterocytes and enteroendocrine cells.

Correlations Between Pathologic and Clinical Findings

The severity of diarrhea in children with rotavirus gastroenteritis correlates with the degree of mucosal damage, which suggests that malabsorption related to loss of absorptive cells may contribute to rotavirus diarrhea late in infection.[99] However, in experimentally infected gnotobiotic pigs, diarrhea precedes villous atrophy.[97] Similarly, small intestinal biopsy specimens from children with relatively mild rotavirus gastroenteritis do not consistently display histologic changes, probably reflecting patchy epithelial injury.[101] These observations indicate that potentially absorptive villous epithelial cells remain despite net fluid losses and that factors other than destruction of the intestinal epithelium are also important in the pathogenesis of rotavirus gastroenteritis.

Physiology of Rotavirus Diarrhea

In several animal models, rotavirus infection induces a net secretion of fluid, sodium, and chloride from intestinal segments.[102] Glucose cotransport of electrolytes is inhibited,[103] although the degree of inhibition in humans does not prevent rehydration with oral solutions. Decreased disaccharidase activity makes less glucose available for cotransport.[3,103] Changes in the molecular-weight distribution of absorbed polyethylene glycol in children with rotavirus gastroenteritis indicate an increase in paracellular permeability.[104] Experiments in cell culture suggest that disruption of tight junctions by VP8* or NSP4 may mediate this increased permeability.[105,106]

Viral Enterotoxin

The rotavirus nonstructural glycoprotein NSP4 is an enterotoxin that causes diarrhea in some animal models.[107] The fragment of NSP4 associated with this activity forms oligomers through a tetrameric or pentameric coiled-coil and raises cytoplasmic calcium levels, leading to a secretory state by activating a calcium-dependent chloride channel of the TMEM16 (transmembrane proteins of unknown function 16A) family.[108–111] Several mechanisms have been proposed for NSP4's effect on cytoplasmic calcium levels. Most NSP4 is resident in the ER membrane, and residues 47 to 90 have a viroporin-like activity that could disrupt the ER membrane, releasing calcium stores in infected cells.[112] In addition, an NSP4 peptide corresponding to residues 112 to 175 and intact NSP4 in lipoprotein complexes are released from rotavirus-infected cells and could affect uninfected cells.[113,114] The application of an NSP4 fragment (residues 95–146) to HIEs leads to swelling of the HIE lumens, an indicator of fluid movement from the basolateral to the apical surfaces of the HIE cells.[100] Extracellularly applied NSP4 fragments stimulate phospholipase C to produce inositol-1,4,5-triphosphate, which leads to calcium influx and release of calcium from intracellular stores.[115] Other mechanisms proposed for NSP4 enterotoxin activity include inhibition of sodium absorption by the epithelial sodium channel ENaC and by the glucose-coupled sodium cotransporter SGLTI and interference with lactase function.[111,116,117] Orally administered antibodies against NSP4 protect mice from rotavirus diarrhea, although actively acquired anti-NSP4 antibody fails to protect gnotobiotic piglets from challenge, and the role of an extracellular enterotoxin in human rotavirus gastroenteritis is not yet established.[107,118]

The Enteric Nervous System in Rotavirus Gastroenteritis

The ENS has a role in the pathogenesis of rotavirus gastroenteritis. Treatment of rotavirus-infected mice with lidocaine (a sodium channel–blocking anesthetic), ondansetron or granisetron (serotonin receptor antagonists), or a vasoactive intestinal peptide receptor antagonist attenuates diarrhea.[119–121] The ability of racecadotril, an enkephalinase inhibitor, to attenuate rotavirus diarrhea in children confirms that ENS-mediated secretion contributes to the pathogenesis of diarrhea in humans and is a target for therapeutic intervention (see "Therapy").[122] Secretion of nitric oxide, a mediator of intestinal motility, from NSP4-exposed human epithelial intestinal cells (HT-29) in culture, secretion of serotonin from an NSP4-exposed human midgut carcinoid enterochromaffin cell line (GOT1), and reduction of serotonin secretion from rotavirus-infected GOT1 cells in the presence of small interfering RNA that inhibits NSP4 expression suggest a potential role for the ENS in rotavirus-mediated intestinal hypermotility and vomiting and a link between the roles of NSP4 and the ENS in rotavirus pathogenesis.[121,123,124]

SEROLOGY AND CLASSIFICATION

Overview

The diversity of rotaviruses, their ability to exchange genome segments encoding antigenic determinants, and changing diagnostic technology have led to evolving serology and classification systems. Rotavirus strains have been classified serologically into serogroups, subgroups, G serotypes, and P serotypes, and genetically into electropherotypes, species, genogroups, G genotypes, and P genotypes. To organize the increasing volume of whole-genome rotavirus sequence data, in 2008 the Rotavirus Classification Working Group (RCWG) developed a nucleotide sequence–based complete genome classification system for group A rotaviruses.[125]

Rotavirus Groups

The broadest designation is the group, also referred to as serogroup or species. Historically, groups have been defined serologically, based on the cross-recognition of rotavirus particles by serum antibody obtained from parenterally hyperimmunized animals.[126] Serogroup determinants are predominantly located on VP2 and VP6 (the innermost and middle-layer proteins). More recently, a VP6 amino-acid sequence identity cutoff of 53% has been proposed as an alternative criterion for differentiating rotavirus groups or species.[127] Genetic exchange has not been observed between members of different groups. To date nine rotavirus groups (A–I) have been identified.[128] Groups A, B, and C cause disease in humans and other animals; groups D through I have been described only in nonhuman animals. Group A is the most important clinically, as group A viruses cause the endemic gastroenteritis of children; groups B and C have been associated with epidemics of gastroenteritis affecting all ages. Group B includes the strain ADRV (adult diarrhea rotavirus), which has been associated with large outbreaks of severe diarrhea in adults in China.[129] Group C viruses have been associated with less severe gastroenteritis in both children and adults.[130,131]

Binomial Serology of Group A Rotaviruses

Within group A, rotavirus serotypes have been defined primarily on the basis of reciprocal cross-neutralization of rotavirus strains by antibodies from immunized mice. Initially, rotavirus serotypes were designated by a unitary system, which primarily reflected neutralizing antibodies directed against VP7. This system did not fully explain cross-neutralization patterns because VP4 also contains neutralization determinants.[132,133] As a result, group A rotaviruses are now classified into serotypes with a binomial nomenclature, in which neutralization by antibodies against VP7 defines "G" serotype (for glycoprotein antigen), and neutralization by antibodies against VP4 defines "P" serotype (for protease-sensitive antigen). Further complicating the serology, VP7

and VP4 are each targets both of homotypic antibodies that neutralize only within serotype and of heterotypic antibodies that neutralize several serotypes.[133,134] Serotype-specific epitopes of VP4 are predominantly found on the variable VP8* lectin domain, at the tips of the spikes, and heterotypic epitopes are predominantly found on the more conserved β-barrel domain, in the body and stalk of the spikes (see Fig. 150.3D *left*).[10,12,36] Immunodominant serotype-specific and heterotypic neutralizing epitopes of VP7 straddle the calcium-containing interface between the subunits of each VP7 trimer, and additional neutralizing epitopes are found at an interdomain boundary within each VP7 subunit.[33]

Genetic Classification of Group A Strains

Early attempts at genetic classification grouped rotaviruses according to RNA genome electropherotype, which distinguished group A strains as "long," "short," and "supershort" on the basis of the electrophoretic mobility of genome segments 10 and 11. Rotavirus whole-genome analysis indicates that there are two main genogroups of human group A rotaviruses, each of which contains viruses with overall genome similarity.[135] The Wa-like human rotavirus genogroup appears to share origins with porcine rotaviruses, and the DS-1–like genogroup appears to share origins with bovine rotaviruses.[135] In the RCWG genetic classification, a separate genotype is assigned to each of the 11 genome segments. Gx, P[x], Ix, Rx, Cx, Mx, Ax, Nx, Tx, Ex, and Hx designate the genes that encode VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5/6, respectively.[125] The RCWG has proposed a strain-naming convention in which strains are named "RV serogroup/species of origin/country of identification/common name/year of identification/G- and P-type."[136]

Diversity of Group A Strains

Because the sequence of the genome segment encoding VP7 accurately predicts G serotype, genotyping provides a practical surrogate for G serotype determination. As of December 2018 the RCWG listed 36 accepted G types (G1–G36) (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/rcwg). At the time of vaccine introduction, five G types (G1–4 and G9) comprised 88% of human rotavirus isolates.[137] Since vaccine introduction, G12 viruses have emerged in the United States and other countries.[138] Substantial geographic and temporal variation exists in strain distribution. For example, G8 rotavirus strains are prevalent in high-rotavirus-mortality countries in Africa and therefore have more clinical significance than their global prevalence would suggest.[138]

To date at least 14 P serotypes (with serotypes 1, 2, and 5 each divided into subtypes with letter designations, such as P1A) have been identified.[125,137] Immunologic reagents for determining P serotype are limited, and P serotyping is complicated by cross-reactivity between P serotypes. Therefore P genotyping is more commonly used for classification. As of December 2018, 51 accepted P genotypes (P[1] to P[51]) were listed in the RCWG database. Unlike G serotypes, which have a one-to-one correspondence with G genotypes, some P serotypes include more than one P genotype.

In strain descriptions the genotype is enclosed in brackets after the serotype designation. Thus G2P1B[4] refers to a virus of G serotype 2, P serotype 1B, and P genotype 4. Two P genotypes, P[4] and P[8], cause most human rotavirus disease. Reassortment between human and animal rotavirus strains, antigenic drift, and the occasional introduction of animal rotaviruses into the pool of viruses circulating among humans provide a continuous introduction of genetic diversity, necessitating ongoing surveillance to determine whether rotavirus vaccines will require strain changes in the future for continued efficacy.[135]

EPIDEMIOLOGY

Universality of Infection

Rotavirus infection is universal, and almost all children acquire serum antibody against the virus in the first 2 or 3 years of life.[139] Severe gastroenteritis caused by rotavirus most commonly affects infants and children between 6 months and 2 years of age,[140] although in lower socioeconomic populations the peak of illness may be somewhat earlier.[140–142] The age at greatest susceptibility to severe disease may be bracketed by the waning of maternally transferred passive immunity and the maturation of the GI tract at about 6 months and by the acquisition of active immunity related to natural infection later in childhood.

Mortality and Global Impact

As of 2016 rotavirus remained the most common cause of severe dehydrating diarrhea in infants and children younger than 5 years in low-, middle-, and high-income countries, responsible for approximately 28.8% of deaths due to diarrhea in this age group.[1] The estimated 128,500 childhood deaths in 2016 is a substantial decrease from the estimated 453,000 childhood deaths in 2008.[1,143] A number of factors may be responsible for the decrease, including improvements in sanitation and nutrition, better access to treatment, more widespread use of oral rehydration solutions, reduced rates of coinfections, and the introduction of rotavirus vaccines. Although rotavirus gastroenteritis is common in both high- and low-income countries, mortality is concentrated in low-income countries, with approximately 104,733 of the rotavirus-associated deaths in 2016 occurring in sub-Saharan Africa (see Fig. 150.1).[1]

Health Care Utilization and Impact in High-Income Countries

The physical hardiness of the rotavirus particle, the high particle concentration in stool (up to 10^{11} particles/mL),[144] and the small minimum infectious dose (ID_{50} = 10 focus-forming units)[145] account for the weak association between the incidence of rotavirus gastroenteritis and the level of economic development or hygiene. Before the introduction of rotavirus vaccines in 2006, rotavirus was a major cause of morbidity and health care costs in developed countries. For example, in the prevaccine era in the United States, approximately 3 million cases of childhood rotavirus gastroenteritis were responsible for approximately 735,000 physician visits, 27,000 hospitalizations, 20 to 40 deaths, $319 million in direct health care costs, and $893 million in total economic costs to society each year.[146,147] Since rotavirus vaccines were introduced in 2006, there has been a large and sustained decrease in pediatric rotavirus gastroenteritis. By 2017 US pediatric hospitalizations due to rotavirus had declined by 80%, with an estimated annual savings in US direct health care costs attributable to rotavirus and acute gastroenteritis of approximately $121 to $231 million per year.[148]

Seasonality

Rotavirus gastroenteritis has been considered a winter disease, based on its historic seasonality in many temperate regions such as North America, where annual rotavirus epidemics typically began in the fall in the Southwest, spreading north and east, reaching the eastern seaboard by late winter, and tapering off in the spring.[149] Since the introduction of rotavirus immunization, this pattern has been disrupted with delayed or absent onset and shorter duration of the rotavirus season in the western, midwestern, and northern regions of the United States.[148] In lower-income regions and the tropics, year-round infection is a more common pattern.[2] During a rotavirus season, multiple strains, representing several P and G types, usually circulate. In a single region the dominant strains differ from year to year; in the same year the dominant strains differ regionally.[150]

Transmission

The best-documented and dominant mode of rotavirus transmission is fecal-oral.[145] However, the more limited effect of hygiene on the incidence of viral gastroenteritis than bacterial gastroenteritis and the universality of rotavirus infection early in life are more typical of pathogens with respiratory spread, suggesting that transmission may not be exclusively fecal-oral. Experiments on the transmission of rotavirus gastroenteritis (epizootic diarrhea of infant mice) between cages of mice support the possibility of airborne spread.[151] Although rotavirus can be foodborne, it is not a major cause of foodborne illness.[152] Transmission by contaminated water has also been documented.[129]

Risk Factors for Disease

Attendance at daycare centers is a risk factor for pediatric hospitalization due to rotavirus gastroenteritis.[153] The illness can spread in families and

may cause gastroenteritis in the adult caregivers for small children.[154] It is likely that the large viral inoculum ingested by these caregivers is, in part, responsible for illness despite some level of acquired immunity. Rotavirus outbreaks have occurred in nursing home populations and sometimes have resulted in fatalities.[66] Rotavirus is an important cause of nosocomial infection; in addition to the asymptomatic infections associated with rotavirus endemics in neonatal nurseries, rotavirus causes symptomatic hospital-acquired outbreaks associated with circulation of rotavirus in the community.[155] Rotavirus also can cause traveler's diarrhea.[156]

Veterinary and Zoonotic Disease

Rotavirus is a major veterinary pathogen, causing disease in infant cattle, sheep, swine, and camels, as well as in adult chickens and turkeys and in domestic pets, such as cats and dogs. In addition to the remote shared ancestry of major human and nonhuman animal rotavirus lineages, there is evidence of low-level ongoing zoonotic rotavirus disease.[135,157] In addition, some human rotaviruses have individual genome segments that are closely related to those of animal rotaviruses, suggesting reassortment between viruses with different primary hosts.[135] Nevertheless, in nonnative hosts most rotavirus strains are attenuated and do not spread efficiently.

IMMUNITY

Protection From Reinfection by Natural Infection

The immune response to rotavirus is complex and redundant: innate, cellular, and humoral mechanisms contribute to clearance of infection, and humoral immunity has a dominant role in the prevention of reinfection. The observation that natural rotavirus infection, whether symptomatic or asymptomatic, provides partial protection from subsequent episodes of rotavirus gastroenteritis guided the development of the current generation of vaccines. For example, asymptomatic infection of neonates with nursery strains of rotavirus protects against subsequent severe rotavirus gastroenteritis but not against asymptomatic reinfection or mild-to-moderate disease.[158] In a prospective observational study, 200 Mexican infants were monitored for rotavirus infection from birth to 2 years of age. Protection from subsequent moderate or severe rotavirus diarrhea was 87% after one natural infection of any severity and 100% after two natural infections.[139]

The duration of immunity to rotavirus is limited, with repeated symptomatic rotavirus infections occurring (generally with decreasing severity) in both children and adults.[139,159] In isolated human communities with limited prior exposure to rotaviruses, explosive epidemics of rotavirus diarrhea with high attack rates in adults are seen.[66,160] Although some experimental animals, including mice, show maturational resistance to rotavirus disease,[151,161] these clinical observations suggest that maturational resistance is less significant in humans and does not fully account for the lower incidence of severe disease in older children and adults. Rather, repeated asymptomatic or mildly symptomatic episodes of rotavirus gastroenteritis throughout life appear to be important for maintaining immunity.[65,162]

Protection by Antibody

In experimental studies of passively transferred immunity to rotavirus in mice, circulating neutralizing antibody does not protect against disease; however, the presence of sufficient levels of neutralizing monoclonal or colostrum-derived antibody in the gut does protect.[163,164] In immunized mice the appearance of immunoglobulin A (IgA) in the gut correlates with clearance of infection.[165] Passive infusion of neutralizing antibodies into monkeys protects from experimental challenge and, at high dose, results in detectable antibody in stool.[166] These experimental results corroborate the observation that, in naturally exposed children, preexisting rotavirus-specific serum IgA and fecal IgA, which reflect duodenal IgA, are associated with protection from disease.[162,167,168] Postimmunization rotavirus-specific serum IgA levels also correlate with vaccine-mediated protection from rotavirus disease, although imperfectly.[169] Virus-specific serum antibody may correlate with protection in children because it signifies previous infection without necessarily being a primary effector of protection.[142,170]

Protection by Breastfeeding

The evidence that breastfeeding protects human infants from rotavirus diarrhea is mixed, with a meta-analysis of the literature showing no significant correlation between breastfeeding and rotavirus gastroenteritis.[171] In Bangladesh hospitalized children with rotavirus diarrhea are more likely to be breastfed than patients with diarrhea from other causes, which suggests that breastfeeding prevents rotavirus gastroenteritis less effectively than it prevents gastroenteritis caused by other agents.[172] In contrast, in some clinical studies of rotavirus gastroenteritis, breastfeeding is associated with reduced frequency of vomiting, less severe dehydration, or, for infants younger than 6 months, a lower risk for hospitalization.[153,173,174]

Role of Serotype in Protection

Serotype influences but does not determine the degree of protection from challenge. Protection after natural infection appears to be somewhat more reliable against viruses of the same G type as the infecting strain.[139,159] On the other hand, cross-protection between serotypes clearly also occurs, particularly after multiple infections.[139] In controlled clinical trials, immunization with a G1P[4] monovalent live-attenuated rotavirus vaccine provides detectable but diminished protection against G2P[8] circulating strains relative to protection against strains that share a VP7 or VP4 type with the vaccine, and the degree of heterotypic protection provided by monovalent or multivalent live-attenuated rotavirus vaccines exceeds the level that can be explained on the basis of G type–specific serum neutralizing antibody titers.[175-177] This finding correlates with the primarily homotypic neutralizing antibody response to a first infection or dose of a live-attenuated vaccine and the increasingly heterotypic responses to subsequent infections or doses.[178,179]

Heterotypic Neutralization by Antibodies

Monoclonal antibodies derived from adult human intestinal B cells, although limited in number, provide insight into potential mechanisms of heterotypic protection from rotavirus disease. Human-derived heterotypic neutralizing antibodies (those that neutralize multiple P types) are largely directed at VP5*, although heterotypic neutralizing antibodies directed against VP8* (neutralizing multiple P types) and VP7* (neutralizing multiple G types) are also found.[180] These results are similar to those obtained with monoclonal antibodies derived from mouse immunization except that most identified human monoclonals that recognize VP8* bind heterotypically without neutralizing in vitro, whereas many animal monoclonals that recognize VP8* bind and neutralize homotypically.[133,134,180,181] Therefore antibodies that recognize VP5* probably make important contributions to heterotypic protection of humans against rotavirus, with some potential contribution by a subset of antibodies that recognize VP8* and VP7 as well.

Mechanisms of Neutralization by Antibodies

The mechanisms of rotavirus neutralization have been examined in detail using mouse-derived monoclonal antibodies. Neutralizing monoclonal antibodies that bind the VP8* receptor binding domain, which forms the heads of the VP4 spikes, block the attachment of neuraminidase-sensitive rotaviruses to cells or trigger virus uncoating.[182–184] A neutralizing monoclonal antibody that binds the hydrophobic tip of the β-barrel domain of the VP5* fragment of VP4 acts after cell attachment and blocks the binding of rearranging VP4 to membranes.[35,182,183] Neutralizing antibodies that bind VP7 on the virion prevent VP7 trimer dissociation and virion uncoating in low calcium conditions.[185,186] Antibodies with epitopes that span the calcium-stabilized interface between the subunits of a VP7 trimer can cross link the virion surface and neutralize as monomeric Fab; those that bind within a single VP7 molecule require divalency.[33,37]

Protection by Antibodies Against VP6 and Intracellular Neutralization

Nonneutralizing antibodies that recognize the middle-layer protein VP6, which is buried under the VP7 shell on intact virions, may contribute to heterotypic protection from rotavirus gastroenteritis by neutralizing subviral particles intracellularly.[187] IgA monoclonals that bind in the

mRNA exit channel through the VP6 layer can block transcription by DLPs in vitro. These antibodies do not neutralize intact virions in cell culture but do protect mice from rotavirus infection when secreted into the serum by "backpack" hybridoma tumors.[187–189] Host J-chains are required for heterotypic protection after immunization of mice with recombinant virus-like particles (VLPs) containing only VP2 and VP6.[190] These observations suggest that anti-VP6 IgA can block infection by binding DLPs and interfering with viral transcription while the antibody is transcytosed across the intestinal epithelium. However, protection is not provided by immunogens that lack VP4 and VP7 in all animal models. For example, immunization of gnotobiotic pigs with VP2/6 VLPs boosts protective responses elicited by immunization with live virus, but primary immunization with these VLPs does not protect gnotobiotic pigs from diarrhea on challenge with a virulent human rotavirus strain.[191] Alternatively, llama single-chain antibody fragments that lack light chains and recognize VP6 can neutralize virions across serotypes, presumably by accessing VP6 through the pores in the VP7 shell.[192]

Mouse Studies on the Roles of T-Cell–Mediated, Antibody-Mediated, and Innate Immunity in Viral Clearance and Protection From Reinfection

Studies in an adult mouse model of rotavirus infection and an infant mouse model of rotavirus diarrhea have helped to elucidate the roles of T-cell–mediated, antibody-mediated, and innate mechanisms in the control and prevention of rotavirus infection. Mice that are T-cell deficient on the basis of an αβ/γδ T-cell receptor knockout clear primary infection but somewhat later than immunocompetent mice, mount a modest IgA response (primarily against VP6), and are resistant to reinfection on challenge.[193] In contrast, most B-cell–deficient mice (J_HD knockouts) with intact $CD8^+$ T cells also clear primary rotavirus infections but can be reinfected.[194] Thus humoral immunity appears to have a central role in protecting mice from infectious challenge. Innate responses to rotavirus are essential triggers for the humoral immune response. Depletion of plasmacytoid dendritic cells or abrogation of type I IFN production from these cells in rotavirus-infected mice diminished the production of rotavirus-specific IgG and IgA in serum and rotavirus-specific IgA in stool.[195]

Although some rotavirus-inoculated adult mice with severe combined immunodeficiency disease (SCID) persistently shed virus, 40% of SCID mice on a C57BL/6 background do clear the infection despite the lack of adaptive immunity.[193] Chronic shedding of rotavirus from *RagI*$^{-/-}$ mice, which lack mature T and B cells, can be completely eliminated by administration of flagellin, independent of both an adaptive immune response and an IFN response.[196] Flagellin induces a protective gene expression program in intestinal epithelial cells by its interaction with Toll-like receptor 5 (TLR5) on dendritic cells, with subsequent production of interleukin-22 (IL-22), and also induces an inflammasome response by its interaction with nucleotide oligomerization domain (NOD)-like receptor 4, with subsequent production of IL-18.[196] The innate immune-mediated clearance of rotavirus infection by flagellin administration can be completely reproduced by administration of IL-22 and IL-18.[196] Consistent with these findings, infant mice deficient in inflammasome components are more susceptible to rotavirus diarrhea and support higher levels of rotavirus replication than wild-type infant mice.[197] Absent the administration of flagellin or IL-18, inflammasome-mediated restriction of rotavirus infection is triggered when short stretches of rotavirus dsRNA trigger the intestinal epithelial cell-specific NOD-like receptor Nlrp9b via RNA helicase Dhx9.[197] Rotavirus antagonizes these innate immune responses by several mechanisms, including NSP1-mediated degradation of transcription factors IFN regulatory factor 3 (IRF3), IRF5, and IRF7; NSP3-mediated interference with host mRNA translation; and sequestration of rotavirus dsRNA in viroplasms.[198,199]

Evidence for the Role of Innate Immunity From Studies in Human Cells

Studies in human-derived intestinal cell lines, organoids, enteroids, and peripheral blood mononuclear cells (PBMCs) confirm the essential role of innate immunity in control of rotavirus infection that is indicated by studies in mice and in nonhuman mammalian cell lines. Treatment of a human intestinal cell line (Caco2 cells) and of human intestinal organoids with exogenous IFN reduces rotavirus replication.[200] Increasing endogenous IFN expression in human enteroids by knocking down stromal antigen 2 (STAG2) expression also reduces rotavirus replication.[201] Conversely, reducing basal expression of IFNs in human intestinal cells by silencing signal transducer and activator of transcription 1 *(STAT1), STAT2,* and *IFR9* genes increases rotavirus replication.[200] Although infecting human intestinal cells with simian rotavirus (strain SA11) stimulates the expression of RNAs encoding type I and III IFNs, no expression of IFN proteins results, consistent with the host translation interference mediated by NSP1.[200] Confirming the essential role for innate immunity in stimulating an adaptive antibody response, when human PBMCs are infected in vitro with rotavirus, B-cell activation requires the production of type I IFNs by plasmacytoid dendritic cells.[195]

DIAGNOSIS

Clinical Utility of Virologic Diagnosis

Rotavirus gastroenteritis is not clearly distinguished from acute gastroenteritis due to other agents on clinical grounds alone. Because the standard treatment for rotavirus gastroenteritis is rehydration and supportive care, a specific microbiologic diagnosis is not required in most cases. However, with prolonged diarrhea, in complicated cases, in immunocompromised hosts, when alternative diagnoses are considered, or when epidemiologic or infection control data are needed, it may be desirable to establish rotavirus as the etiologic agent. Definitively diagnosing rotavirus gastroenteritis may also prevent the unnecessary and potentially harmful use of antibiotics.

Diagnosis by Antigen Detection

Rotavirus can be detected by numerous techniques, including a variety of commercial antigenic assays, RT-PCR, electron microscopy, immune electron microscopy, polyacrylamide gel electrophoresis (PAGE) for viral genomic RNA, and viral culture. Detection of viral antigen in stool or rectal swabs, most commonly using enzyme-linked immunosorbent assay (ELISA) or latex agglutination formats, forms the basis for practical, commercially available, and widely used diagnostic kits.[202] Latex agglutination is particularly suitable for use in areas with limited resources, although a confirmatory technique is desirable to evaluate indeterminate results because of the limited sensitivity of the test.[202,203] Commercial antigenic assays primarily detect the VP2 and VP6 proteins of the subviral double-layered particle and detect only group A rotaviruses. Serotype-specific ELISAs, based on recognition of VP7 or VP4, allow determination of serotype without the need to perform neutralization assays.[204] Although there are various techniques for measuring serum, fecal, and salivary antibodies against rotavirus, the acute and generally self-limited nature of rotavirus infections limits the usefulness of these techniques for clinical decision making.

Diagnosis by Reverse-Transcriptase Polymerase Chain Reaction

Multiplexed RT-PCR has become a major diagnostic technique used in epidemiologic studies. RT-PCR allows determination of P and G types and permits finer definition of strain differences.[205,206] Real-time RT-PCR provides greater sensitivity and speed than conventional or nested PCR diagnosis.[207] DNA oligonucleotide microarray methods offer greater robustness to sequence drift, which can prevent PCR amplification if a primer binding site is affected, and greater ability to distinguish a mixed infection from a single-strain infection.[208] Micromass sequencing allows metagenomic analysis of the gut microbiome, which includes rotaviruses.[209]

Diagnosis by Electron Microscopy, Electrophoresis, and Viral Culture

Both electron microscopy and PAGE can detect unusual strains of rotavirus (such as non–group A rotaviruses) that might be missed by standard antigenic or nucleic acid–based assays. Electron microscopy of stool specimens negatively stained with phosphotungstic acid is rapid and, despite only moderate sensitivity, has high specificity because of the distinctive appearance of rotavirus particles (see Fig. 150.2).[210]

Electrophoresis of simply prepared stool suspensions on polyacrylamide gels, followed by silver staining for the pattern of 11 segments of genomic dsRNA, allows both diagnosis and tracking of rotavirus strains in molecular epidemiologic investigations.[211] Rotavirus can be detected, although with relatively low sensitivity, by growth in cell culture. Human rotavirus strains have proved more difficult to culture routinely than most animal rotavirus strains, but in many cases human rotavirus strains can be propagated in MA104 cells or primary green monkey kidney cells grown in roller tubes with the addition of trypsin to the cell culture medium.[144,212]

Future Diagnostic Trends

Although still an experimental tool, direct whole-genome sequencing of rotavirus obtained directly from stool without culture has potential as part of a general trend toward increased use of sequencing in viral diagnostics. To overcome the challenges posed by the multisegment RNA rotavirus genome, high sequence variability, and inhibitors in the stool matrix, capture of virus on standard commercial rotavirus immunoassay plates has been combined with RNA extraction, reverse transcription, amplification with random primers, and next-generation sequencing to produce reliably >90% sequence coverage from stool samples containing diverse rotavirus strains.[213]

THERAPY

Overview

No specific antiviral therapy is recommended for rotavirus gastroenteritis. Because rotavirus gastroenteritis is generally self-limited, and dehydration is the primary cause of morbidity and mortality, rehydration and restoration of electrolyte balance are the primary therapies. The Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition (FISPGHAN) Working Group has systematically reviewed and compared the multiple recommendations available for the treatment of pediatric gastroenteritis and developed a set of consensus universal recommendations for the management of acute diarrhea in nonmalnourished children.[214] World Health Organization (WHO) has published guidance on the treatment of acute diarrhea and dehydration in both well-nourished and malnourished children.[215,216]

Rehydration

Oral rehydration solution (ORS) is effective in treating dehydration related to rotavirus gastroenteritis, even in the presence of moderate vomiting, and is preferred over intravenous (IV) rehydration in cases of mild or moderate dehydration. The effectiveness of ORS is based on the solute-coupled cotransport of sodium by enterocytes, which continues to operate even in the damaged gut.[217] Effective solutes include glucose, amino acids, and short oligopeptides. In 2002 WHO revised the recommended standard ORS formula to a lower osmolarity (245 mOsm/L) solution, which is associated with less vomiting, lower stool output, and a reduced need for IV infusions. The low-osmolarity formulation is 75 mM sodium, 20 mM potassium, 65 mM chloride, 10 mM citrate, and 75 mM glucose (see reference for acceptable variations).[218] The lower sodium losses resulting from diarrhea due to enteric viruses than from diarrhea due to cholera or enterotoxigenic *Escherichia coli* allows lower sodium content in the oral rehydration solution.[219] In children with severe acute malnutrition and dehydration, WHO recommends an alternative oral rehydration solution, ReSoMal (see reference for formulation).[216]

In case of severe dehydration, initial IV hydration with an isotonic solution, such as Ringer's lactate or normal saline, is recommended, with oral rehydration instituted once the child can drink.[215] Failure of oral rehydration can be addressed by IV hydration or by enteral rehydration with ORS through a nasogastric tube, which is also effective and is associated with fewer side effects than IV hydration, particularly in malnourished children.[214–216]

Despite the depressed disaccharidase levels associated with rotavirus gastroenteritis, it is recommended that nursing infants continue to breastfeed during rehydration and that children resume a diet as soon as they can tolerate feeding.[214–216] The early reinstitution of an age-appropriate diet has nutritional benefits and shortens the duration of diarrhea by about half a day.[220]

Zinc Supplementation

Randomized controlled trials in developing countries have demonstrated that, for children older than 6 months, zinc supplementation during and for a short time after an episode of diarrhea can decrease the duration of diarrhea by approximately half a day on average and decrease the probability that diarrhea will last more than 7 days, but the treatment is associated with a small increase in the incidence of vomiting.[221,222] Because the benefits of zinc supplementation outweigh the risks in areas where zinc deficiency is common, WHO recommends zinc supplementation (10–14 days of 10 mg/day in children ≤6 months and 20 mg/day in children ≥6 months).[215] Zinc supplementation is not recommended in well-nourished populations, in which its benefits have not been established.[214]

Antidiarrheal Agents

Racecadotril (no commercial preparations available in the United States) is an enkephalinase inhibitor that reduces intestinal hypersecretion and has been evaluated as an adjunct to oral rehydration solutions. Although some randomized clinical trials indicate that the addition of racecadotril can decrease mean stool output in inpatients and the mean number of diarrheic stools in outpatients, two randomized, double-blind, placebo-controlled clinical trials in India showed no significant effect.[214,223] Smectite, a multilamellar aluminum-magnesium clay silicate does reduce the duration and volume of diarrhea in children, with trials in Peru and Malaysia showing a greater effect in rotavirus gastroenteritis than in nonrotavirus gastroenteritis, but it has not been demonstrated to reduce the need for IV hydration.[224,225] Although racecadotril and smectite are included in some regional guidelines, neither is recommended by the FISPGHAN Working Group.[214] Bismuth subsalicylate (Pepto-Bismol) has been shown to decrease the duration of diarrhea and the intake of oral rehydration solutions in children with gastroenteritis,[226] but the modest benefits observed, need for frequent administration, and a theoretical possibility of Reye syndrome related to salicylate absorption argue against routine use of this agent. Because of complications including ileus and respiratory depression, antimotility agents such as loperamide have no role in the treatment of childhood gastroenteritis.[214]

Antiemetics

Most antiemetics are not considered to have a favorable risk-benefit profile for self-limiting vomiting in acute pediatric gastroenteritis, although a single dose of the selective serotonin 5-hydroxytryptophan 3 receptor antagonist ondansetron may be considered in the emergency room context to enable oral rehydration (taking into account a warning for QT prolongation and cardiac arrhythmias).[214] Consistent with the role of the ENS in the pathogenesis of rotavirus gastroenteritis, a single dose of ondansetron has also been shown to reduce number of episodes of diarrhea and the duration of symptoms in pediatric gastroenteritis caused by rotavirus or norovirus.[227]

Probiotics

Evaluating the effectiveness of probiotic treatments as a supplement to ORS for rotavirus gastroenteritis is complicated by the variety of preparations available, the potential for differences in efficacy for viral and bacterial gastroenteritis, and a range of quality of the studies. The FISPGHAN Working Group concluded that selected probiotic strains, including *Lactobacillus rhamnosus* GG, *Saccharomyces boulardii*, and *Lactobacillus reuteri* DSM 17938 can be considered as a supplement to ORS.[214] However, recent, large, prospective, randomized, double-blind, placebo-controlled clinical studies of the efficacy of treating moderate-to-severe pediatric gastroenteritis with *L. rhamnosus* GG or with a combination of *L. rhamnosus* R0011 and *Lactobacillus helviticus* R0052 in the United States and Canada showed no evidence of any effect of the probiotic treatment on acute gastroenteritis signs, symptoms, or health care utilization outcomes.[228–229]

Passive Oral Immunotherapy and Immunoprophylaxis

Oral administration of immunoglobulins is not indicated for routine use but may have a role in treating chronic rotavirus diarrhea and may merit further evaluation for prophylaxis in high-risk settings, such as

immunodeficiency or severe prematurity, in which vaccination is unlikely to be effective. In case reports, feeding human serum immune globulin to children with chronic rotavirus diarrhea has been followed by resolution of diarrhea and viral shedding.[230] When given prophylactically to low-birth-weight infants, oral gamma globulin reduced the severity of diarrhea associated with neonatal rotavirus infections.[231] A potential rotavirus-specific therapy, llama single-chain antibodies that recognize VP6 and neutralize multiple rotavirus strains, when administered in a randomized, placebo-controlled trial to 6- to 24-month-old children with severe rotavirus diarrhea in Bangladesh, reduced stool output by 22.5%.[232]

IMMUNIZATION

Overview

Because lack of access to treatment is one of the major causes of childhood mortality from rotavirus, and improved sanitation has limited impact on rotavirus prevalence, prevention by immunization is a critical approach to decreasing the impact of this infection. As of 2018 two live-attenuated, oral rotavirus vaccines had been licensed in multiple countries, two more had been licensed in India and had achieved WHO prequalification, and two more had national licenses in China or Vietnam. Rotavirus immunization has led to substantial decreases in the burden of rotavirus disease in the regions where it had been implemented.

RotaShield and History of Intestinal Intussusception

The first human rotavirus vaccine, RotaShield (Wyeth Lederle Vaccines), was licensed in the United States in 1998. RotaShield was a reassortant, live-attenuated, oral vaccine containing four viruses, each with VP7 from the G types 1, 2, 3, or 4 presented on the genetic background of a simian rotavirus strain (RRV) that was attenuated for humans. In phase III trials RotaShield proved safe and highly effective against moderate-to-severe diarrhea in both developed (United States and Finland) and developing (Venezuela) countries.[177,233,234] However, in the year after launch, after more than 1 million doses had been administered, an increased incidence of intestinal intussusception (telescoping of the intestine) 3 to 14 days after administration of the first dose (odds ratio [OR], 21.7) and a smaller increase after the second dose (OR, 3.3) was detected, corresponding to a total excess risk for intussusception of approximately one case per 5000 to 10,000 children immunized.[235,236] As a result of these findings the manufacturer voluntarily withdrew the vaccine from the market.

The unknown mechanism of the temporal association between intussusception and immunization with RotaShield, combined with the failure to observe this complication in prelicensure clinical trials of RotaShield, increased the size of the phase III safety databases required for licensure of subsequent live-attenuated rotavirus vaccines to greater than 60,000 subjects each. Nevertheless, the development of two other live-attenuated rotavirus vaccines, Rotarix (GlaxoSmithKline, Research Triangle Park, NC) and RotaTeq (Merck & Co., Inc., Whitehouse Station, NJ), continued, with both vaccines achieving licensure with no intestinal intussusception observed in clinical trials.[175,176]

Rotarix

Rotarix, an oral, live-attenuated vaccine, is derived from a human rotavirus isolate (strain 89-12; P[8]G1) that was attenuated by serial passage in cell culture.[175] Because Rotarix is monovalent, breadth of coverage relies on cross-protection between serotypes, as has been observed after natural rotavirus infection.[139] In a phase III trial carried out in Finland and in middle-income Latin American populations, Rotarix proved effective (84.7%) against severe gastroenteritis. The vaccine was 87.3% effective against severe gastroenteritis caused by strains that shared the P[8] VP4 type with the vaccine and 91.8% effective against strains that shared both VP4 type and VP7 type (G1) with the vaccine.[175] Although efficacy criteria were not met for doubly mismatched G2P[4] strains in the initial phase III trial or after a second year of monitoring of a subset of infants from that trial, Rotarix achieved 85.5% protection against severe diarrhea caused by G2P[4] strains in a placebo-controlled, double-blind study in European infants.[175,237,238] Since Rotarix was first licensed in Mexico and the Dominican Republic in 2004, approvals have followed in more than 100 nations, including the European Union in February 2006 and the United States in April 2008. The Advisory Committee on Immunization Practices (ACIP) has recommended immunization with Rotarix at 2 and 4 months of age.[84]

RotaTeq

RotaTeq is a multivalent, orally administered, modified Jennerian vaccine. The five strains in RotaTeq are reassortants with a bovine rotavirus (strain WC3) background. Each of four strains has a VP7 from one of four prevalent human rotavirus G types (G1, G2, G3, or G4) combined with a VP4 from a bovine genotype, P[7]. The fifth strain has a VP7 from a bovine G type, G6, combined with a VP4 from the common human rotavirus P genotype, P[8].[239] In a phase III trial RotaTeq was highly effective (>98% protection) against severe rotavirus gastroenteritis and effective against any diarrhea caused by G1 or G2 rotaviruses (74.9% and 63.4% protection, respectively).[176] RotaTeq was licensed in the United States and the European Union in 2006 and has since been licensed in more than 90 countries. The ACIP has recommended RotaTeq for routine use in the United States, with doses given at 2, 4, and 6 months of age.[84]

Contraindications for Allergic Reactions, Severe Combined Immunodeficiency Disease, and Elevated Risk of Intestinal Intussusception

Since launch, Rotarix and RotaTeq have had good overall safety profiles, but two issues have led to the addition of new contraindications to the original contraindication of severe allergic reaction to a previous dose of rotavirus vaccine or to a vaccine component. First, the finding of prolonged shedding of vaccine virus associated with diarrhea in children with SCID led, in 2009 and 2010, to the addition of contraindications to the immunization of infants who have SCID.[240–241]

Second, although association of immunization with intestinal intussusception was not observed in clinical trials of Rotarix or RotaTeq, postlicensure surveillance in Australia, Mexico, and the United States detected temporal clustering of intestinal intussusception 3 to 7 days after administration of the vaccines, particularly after the first dose, with an excess risk of 1 to 5 cases per 100,000 infants immunized.[242–246] The Centers for Disease Control and Prevention (CDC) estimated that, if the risk exists, immunization could cause 50 to 60 excess cases of intussusception per year in the United States, while preventing more than 50,000 hospitalizations for rotavirus disease.[247] In 2011, to reduce the potential risk of vaccine-associated intussusception, a history of intestinal intussusception (Rotarix and RotaTeq) and an uncorrected congenital malformation of the GI tract that would predispose to intussusception (Rotarix) were added as contraindications to rotavirus immunization.[247] An analysis of the risk of intussusception after immunization with Rotarix in Africa found no clustering of cases in the week after the first dose.[248] The apparent lack of association between rotavirus immunization and intussusception in several lower-income African countries is reassuring, especially given the higher case fatality for intussusception in low-income countries.[248] Analysis of the RotaShield experience indicated that infants who received a first dose at ≥90 days of age contributed disproportionately to the cases of intussusception.[249] In accordance, for Rotarix and RotaTeq the ACIP recommends that the first dose of vaccine be given before 15 weeks of age[84] (see Chapter 316).

Porcine Circovirus in Vaccine Manufacturing

In March 2010 a manufacturing issue led the US Food and Drug Administration (FDA) to temporarily suspend use of Rotarix. Metagenomic analysis of a vaccine sample revealed porcine circovirus 1 (PCV-1) DNA.[250] Investigations by the manufacturer confirmed the presence of infectious PCV-1 in Rotarix cell banks, viral seeds, and vaccine lots, including those used in the large phase III safety studies.[251] PCV-1 and PCV-2 DNA fragments, but no infectious virus, were detected in RotaTeq samples, and irradiated porcine trypsin used in manufacturing was identified as the source of the PCV-2 DNA.[251,252] PCV-1 causes no known disease in animals or humans; PCV-2 is not known to cause human disease but is a cofactor for the development of pig wasting disease.[253]

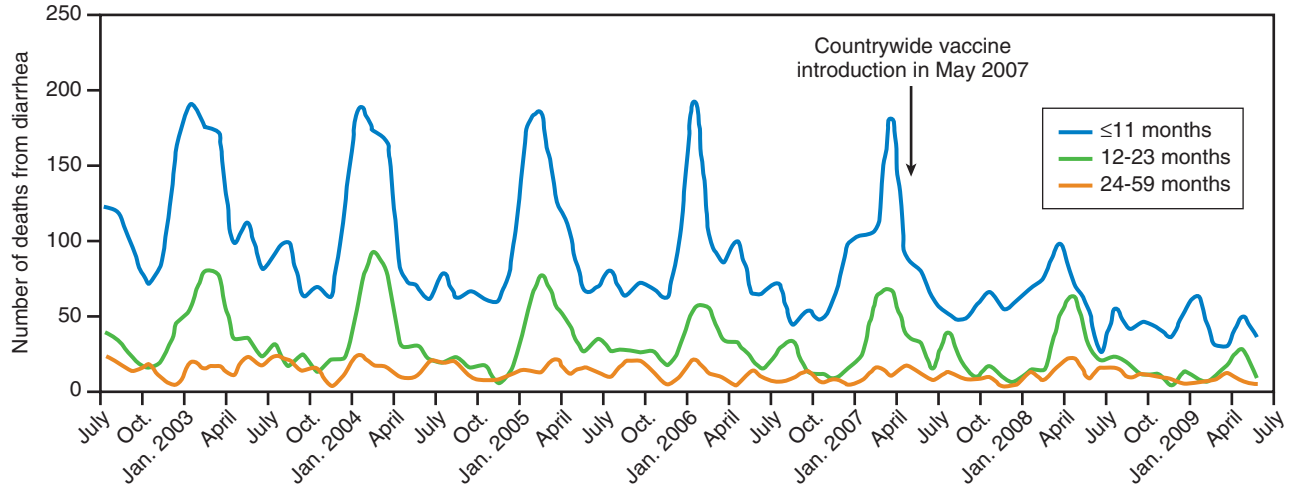

FIG. 150.4 Number of diarrhea-related deaths among children 59 months of age or younger from July 2002 through May 2009 in Mexico, according to age group. *(From Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico.* N Engl J Med. *2010;362:299–305.)*

Both PCV-1 and PCV-2 are commonly present in pork products and are often ingested by humans with no known health consequences.[251] In May 2010 the FDA concluded that the benefits of immunization with Rotarix and RotaTeq outweigh any theoretical risks based on the PCV findings, that immunization should continue, and that manufacturers should develop PCV-free vaccine preparations.[254]

Reduction in Rotavirus Disease Burden Resulting From Immunization

Rotarix and RotaTeq have had a major impact on hospitalizations due to rotavirus infection and gastroenteritis overall in the regions where they are widely distributed (see "Epidemiology" earlier). The estimated decrease of 55,000 acute gastroenteritis hospitalizations in the United States in 2008, shortly after vaccine introduction, corresponds to the elimination of 1 of every 20 hospitalizations among children younger than 5 years.[255] In the United States the threshold level of detection of rotavirus infections that would signal the start of the rotavirus season was never achieved on a nationwide basis in 2011–12.[256] In the decade since vaccine introduction (2006–17), with 71% to 75% vaccine coverage, effectiveness against rotavirus-associated hospitalization and emergency department visits in the United States has been 84% for RotaTeq and 83% for Rotarix.[148] In El Salvador, a low- to middle-income country where Rotarix was introduced, rotavirus hospitalization rates declined by 69% to 81% in 2008–09, which were years with vaccine coverage rates of 50% to 61%, and overall diarrhea-related health care visits during rotavirus season declined by 35% to 48% for children younger than 5 years.[257] In Mexico in 2008, at a first-dose Rotarix coverage rate of 74%, overall diarrheal deaths among children 59 months of age or younger decreased by 35%, and the reductions were sustained into 2009 (Fig. 150.4).[258] Studies in the United States, Australia, El Salvador, and Mexico have demonstrated decreases in rotavirus-associated disease in children outside the age range of rotavirus immunization programs, indicating substantial herd immunity.[148,255,257–259] Although dominant rotavirus strains have changed in the years since the introduction of the vaccines, effectiveness data indicate that both the monovalent and pentavalent vaccines provide substantial heterotypic protection, and there is no clear, sustained pattern of strain change that would allow a definitive conclusion that virus escape from the vaccine is driving the changes.[138,260]

Reduced Vaccine Efficacy in Low-Income Settings

Live-attenuated rotavirus vaccines have shown lower efficacy (typically ≤50%) against severe gastroenteritis in low-income settings than in middle-income settings (typically ≈75%) or in high-income settings (typically >85%).[b] No definitive cause for the disparity has been established. Hypotheses include the effects in low-income settings of overall nutritional deficiency, micronutrient deficiency, inhibition by high breast milk antibody levels of live-attenuated vaccine take, higher levels of transplacentally transferred antirotavirus IgG, a higher burden of other enteric infections, and more frequent and larger infectious challenges.[265,266] An emerging literature suggests that differences in the intestinal microbiome may affect rotavirus vaccine take, although geographic variation in microbiome composition and the instability of the microbiome before 2 years of age result in a high degree of variability between studies.[266] A similar trend of decreased vaccine performance in resource-poor settings is observed for the live oral polio vaccine (OPV), and, although coadministered rotavirus vaccines do not decrease the immunogenicity of OPV, coadministered OPV may decrease the immunogenicity of live-attenuated rotavirus vaccines.[267]

Benefits of Rotavirus Immunization in Low-Income Settings

Despite these challenges, the greatest benefits of rotavirus immunization are actually realized in low-income settings, where the burden of rotavirus disease is greatest. Postlicensure observational analysis of the impact of rotavirus immunization on children younger than 5 years in countries with high baseline rotavirus-associated mortality showed a 60% decrease in hospitalizations and emergency department visits due to rotavirus-associated gastroenteritis and a 36% decrease in mortality due to acute gastroenteritis regardless of the causative agent.[268]

Global Rotavirus Vaccine Introduction

Because rotavirus mortality is concentrated in impoverished settings, in 2009 the Strategic Advisory Group of Experts (SAGE) on Immunization of WHO recommended the inclusion of rotavirus vaccination in all national immunization programs, strongly recommending introduction of rotavirus immunization in countries in which diarrheal deaths account for 10% or more of the mortality of children younger than 5 years.[269] Initiatives to increase global rotavirus vaccine coverage involve the Global Alliance for Vaccines and Immunization (GAVI), the Bill and Melinda Gates Foundation, the Program for Appropriate Technology in Health (PATH), the CDC, and WHO, among other organizations. As of December 2016, of the 194 countries of the world, rotavirus immunization had been incorporated into the national immunization programs in 84, in which 31% of the world's children reside[270] (see Chapter 316). Efforts to introduce the vaccines have focused heavily on Africa, owing to the >80% of rotavirus infant deaths occurring in that region.[1,270]

[b]References 175, 176, 237, 238, 257, 261–264.

Regional Rotavirus Vaccines: Lanzhou Lamb Rotavirus Vaccine, Rotavac, Rotasiil, and Rotavin-M1

In addition to Rotarix and RotaTeq, which have been introduced in global markets, there are several live-attenuated rotavirus vaccines produced in emerging markets for local use. One of these, the Lanzhou lamb rotavirus vaccine, is a live-attenuated, oral, monovalent vaccine based on an ovine rotavirus (P[12]G10) strain. It was licensed in China in 2000 without having been tested in a prelicensure efficacy trial, and more than 30 million doses have been distributed. Although observational studies have been done, comparisons to other licensed rotavirus vaccines are difficult to make for methodologic reasons, including an unusual immunization schedule, with one dose given every year for 3 years between the ages of 2 and 35 months.[271,272]

Rotavac, a human neonatal strain 116E, oral, live-attenuated monovalent vaccine candidate, has been developed by Bharat Biotech International in Hyderabad, India in partnership with a consortium of international governmental, nongovernmental, and academic institutions and with a price commitment for the public sector at the time of launch of less than US$ 1.00 per dose.[273] Rotavac provides an example of the potential for rotavirus vaccines developed and manufactured in lower-income settings to be more affordable, which could enable increased, sustainable vaccine coverage in regions of high rotavirus mortality. The vaccine virus is a naturally occurring G9P[11] reassortant, with a bovine genome segment encoding VP4 on a background of human rotavirus genome segments, and it generally caused asymptomatic infection, even before adaptation to growth in cell culture.[273] The vaccine showed 53.6% efficacy against severe rotavirus gastroenteritis during the first year of life in a randomized, double-blind, placebo-controlled, multicenter phase III trial in India.[273] The vaccine was licensed in India in 2014 and prequalified by the WHO in 2018, enabling distribution beyond India by United Nations agencies.[274] A staged roll-out of the vaccine in India is underway.

Rotasiil, a pentavalent (G1, G2, G3, G4, G9) bovine-human reassortant, oral, live-attenuated vaccine developed by the Serum Institute of Pune, India, based on strains from the US National Institutes of Health, has been tested in Niger, where it showed 66.7% efficacy against severe rotavirus gastroenteritis and in India, where it showed 36% efficacy.[275,276] Rotasiil is heat stable, not requiring a cold chain for terminal distribution and administration. The vaccine was licensed in India in 2017 and prequalified by WHO in 2018.

Rotavin-M1, a monovalent G1P[8] live-attenuated vaccine manufactured by the Center for Research and Production of Vaccines and Biologicals (Hanoi, Vietnam), is based on a strain isolated from a Vietnamese child and was licensed in Vietnam in 2012 based on immunogenicity and safety data.[8,277] Additional live-attenuated vaccine candidates are in development.

Nonreplicating Rotavirus Vaccine Candidates

New approaches to rotavirus vaccines, including nonreplicating rotavirus vaccine candidates, could provide greater immunogenicity in impoverished settings, easier combination with other routine childhood vaccines, and a definitive solution to concerns over intestinal intussusception.[278] Such candidates include inactivated rotavirus particles[279]; recombinant noninfectious virus-like particles[280]; and recombinant constructs in which the stable and easily expressed VP8* lectin domain is mounted on multivalent display platforms to increase its immunogenicity.[281,282] A subunit vaccine candidate in which a P[8] VP8* receptor–binding domain is fused to the P2 epitope of tetanus toxin and adsorbed to aluminum hydroxide has advanced to safety and immunogenicity testing in human toddlers and infants.[283] Such nonreplicating rotavirus vaccines could complement live-attenuated rotavirus vaccines, much as inactivated polio vaccines complement OPV, to lower practical barriers to universal infant immunization in the most challenging settings.

ACKNOWLEDGMENTS

I thank Stephen C. Harrison for providing the schematic depictions of rotavirus structural rearrangements and entry into cells, Kathrin Jansen for review and helpful discussion, and the late Albert Z. Kapikian for providing the electron micrograph of rotavirus particles.

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151 Alphaviruses (Chikungunya, Eastern Equine Encephalitis)

Lewis Markoff

SHORT VIEW SUMMARY

Definition

- Alphaviruses constitute a genus of at least 29 viruses in the family Togaviridae. Alphaviruses are lipid-enveloped, and the genome is a positive-sense RNA approximately 12 kb in length.
- All human pathogenic alphaviruses are mosquito-borne.

Epidemiology

- Mosquitoes and a human or animal reservoir are required for the virus life cycle and for disease outbreak. Human-to-human transmission does not occur.
- New World alphaviruses include Eastern, Western, and Venezuelan equine encephalitis viruses (EEEV, WEEV, and VEEV, respectively), which are found in North and South America.
- Old World alphaviruses, especially Sindbis, Ross River, and O'nyong-nyong viruses, found in Europe, Africa, and Asia, cause a fever/arthralgia/rash syndrome. Chikungunya virus (CHIKV) originated in Africa and is grouped with Old World viruses, but is now found in North and South America, as well as in Africa and Asia.

Pathogenesis

- Alphaviruses enter cells by receptor-mediated endocytosis and exit by budding from the plasma membrane.
- After infection via mosquito bite, alphaviruses initially replicate in various cell types in the skin, resulting in viremia. Viremia permits invasion of the central nervous system (CNS) by alphaviruses that cause encephalitis or invasion of the joints and internal organs by viruses that cause fever, arthralgia, and rash. Infection may rarely be acquired via transfusion of infected blood or by aerosol.
- Alphaviruses suppress the innate immune response, primarily by inhibiting JAK/STAT signaling.
- In the encephalitis caused by New World alphaviruses and in the arthralgia caused by Old World alphaviruses, disease pathology is the result of the cell-mediated immune response to the infection.
- Recovery from the disease state is mediated by the combined effects of virus-neutralizing antibodies and cytotoxic T cells.
- In the CNS, virus replication in neurons may be suppressed indefinitely by antibody-secreting B cells.

Diagnosis

- Knowledge of the patient's travel history is of major importance in diagnosis.
- The fever/arthralgia/rash syndrome caused by Old World alphaviruses can be confused with many other viral exanthems.
- Alphavirus encephalitis is similar in clinical presentation to that caused by the flaviviruses West Nile virus and St. Louis encephalitis virus.
- Culture of virus from blood or detection by reverse transcriptase–polymerase chain reaction has a low sensitivity for diagnosis of alphavirus encephalitis but may occasionally yield positive results in the acute phase of fever/arthralgia/rash syndrome.
- Assays to detect virus-neutralizing antibodies in blood may be diagnostic in the late acute phase or the convalescent phase of disease.

Therapy

- At present, there are no products licensed for treatment.
- For encephalitis, supportive measures and intensive nursing care are currently indicated.
- For fever/arthralgia/rash syndrome, analgesics and nonsteroidal antiinflammatory drugs are the main treatment options.

Prevention

- Veterinary vaccines are available for horses, and their immunization against WEE, EEE, and VEE is required in the United States.
- There are no licensed vaccines for prevention of alphavirus diseases in humans, but unlicensed vaccines against VEE, EEE, WEE, and CHIK are available for at-risk laboratory workers.
- Vaccines are under development, particularly against CHIK fever.
- Disease prevention is best effected by mosquito eradication programs, avoidance of mosquito-infested areas, and use of protection from mosquito bites in locations where exposure to possibly infected mosquitoes is unavoidable.

Alphaviruses constitute a genus in the Togaviridae family of enveloped RNA viruses.[1] All the medically important alphaviruses are mosquito vector borne. In nearly all instances, the life cycles of alphaviruses require specific mosquito vectors and nonhuman mammal or bird species. Thus humans are innocent bystanders in virus spread. Three mosquito-borne alphaviruses have historically been associated with human disease in the United States: eastern equine encephalitis (EEE), western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE) viruses. However, the incidence rates of WEE and VEE are nil to low, respectively, thus far in the 21st century. EEE virus (EEEV), WEE virus (WEEV), and VEE virus (VEEV) are "New World" alphaviruses, defined by their antigenic and nucleotide sequence relatedness, as well as by their geographic distribution. "Old World" alphavirus species of major importance include chikungunya (CHIK) (in Africa, Asia, and tropical North and South America); O'nyong-nyong (Africa); Mayaro (South America); Ross River (Australia, Oceania); Sindbis (Africa, Scandinavia, the countries of the former Soviet Union, Asia); and Barmah Forest (Australia) viruses. The Old World alphaviruses primarily cause fever, rash, and arthropathy. Relevant information on medically important alphaviruses and some related species is presented in Table 151.1.

HISTORY

WEEV and EEEV were initially recovered from the brains of horses with encephalitis in California (1930) and New Jersey (1933), respectively. By 1938, both of these agents had been established as causes of encephalitis in humans.[2] Similarly, VEEV was first isolated from the brains of horses in Venezuela, during an epidemic of encephalitis in 1938.[3] The first reported cases of VEE in humans were attributable to aerosol spread of infectious virus from equine pathology specimens to laboratory personnel, in 1943. Naturally acquired VEE in humans was first reported from Colombia in 1952, in association with an epizootic disease in equines.[4] The first reports of human cases of VEE in the United States

TABLE 151.1 Medically Important Alphaviruses and Some Related Alphavirus Species

ANTIGENIC COMPLEX	VIRUS	GEOGRAPHIC DISTRIBUTION	ANIMAL RESERVOIR	HUMAN VECTOR[a]	HUMAN DISEASE (ANIMALS AFFECTED)
EEE	EEE	NA, SA, Caribbean	Birds	*Aedes*	Encephalitis (horses, birds)
WEE	WEE	NA, SA	Birds, horses	*Culex tarsalis*	Fever, encephalitis (horses, birds [especially emus])
	Aura	SA	Birds		
	Fort Morgan	Colorado	Birds		
	Highlands J	Eastern United States	Birds	*Culex, Aedes*	Encephalitis (turkeys, pheasants, partridges, ducks, emus, horses)
	Kyzylagach	Azerbaijan	Birds		
	Sindbis	AUS, AFR, EUR, Asia Minor	Birds	*Aedes*	Fever, arthralgia, rash
	Whataroa	AUS, NZ	Birds		
VEE	VEE	NA, SA	Horses and others	*Psorophora, Aedes*	Fever, encephalitis (horses)
	Cabassou	SA			
	Everglades	Florida	Mammals	*Ochlerotatus*	Encephalitis
	Pixuna	Brazil	Mammals		
SF	Semliki Forest	AFR	Mammals	*Aedes*	Fever, arthralgia, rash? (rare)
	Bebaru	Asia			
	Chikungunya	AFR, Southeast Asia, Philippines	Primates	*Culex, Aedes*	Fever, arthralgia, rash
	Getah	Asia	Mammals	*Culex, Aedes*	Fever? (horses)
	Mayaro	SA		*Haemagogus, Aedes*	Fever, arthralgia, rash
	O'nyong-nyong	AFR		*Anopheles*	Fever, arthralgia, rash
	Ross River	AUS, South Pacific	Mammals	*Aedes, Culex*	Fever, arthralgia, rash
BF	Barmah Forest	AUS	Birds	*Aedes*	Fever, arthralgia, rash

[a]The mosquito vector genus or species required for epizootic transmission of infection is shown. No epizootic vector is listed for viruses that rarely cause disease or for which there are no reports of disease.
AFR, Africa; *AUS,* Australia; *BF,* Barmah Forest; *EEE,* eastern equine encephalitis; *EUR,* Europe; *NA,* North America; *NZ,* New Zealand; *SA,* South America; *SF,* Semliki Forest; *VEE,* Venezuelan equine encephalitis; *WEE,* western equine encephalitis.

were published in 1968.[5] Retrospective analysis of historical accounts suggests that CHIK virus (CHIKV) caused epidemics of fever, rash, and arthralgia in Indonesia (1779); East Africa (1823, 1870); India (1824, 1871, 1901, 1923); the Far East (1901); West Africa (1925); and the southeastern United States (1827). The virus was first isolated during an epidemic in Tanzania in 1952 and 1953.[1] An epidemic of CHIK fever that originated in Africa in 2004 and then spread to islands in the Indian Ocean, India, and elsewhere in South Asia, Africa, Europe, and tropical areas of North and South America[6] is on the wane in at least some of these areas as of 2017.

MECHANISM OF REPLICATION

Alphaviruses are lipid-enveloped RNA viruses with a diameter of 60 to 70 nm. The genome is a capped, 11- to 12-kilobase, bicistronic positive-strand (or message sense) RNA. In virus particles, genome RNA is tightly associated with the virus-coded core protein, forming an ordered icosahedral nucleocapsid structure. Each virus particle consists of one nucleocapsid surrounded by the lipid envelope. Two envelope glycoproteins, E1 and E2, are inserted in the lipid membrane as heterodimers. Trimers of heterodimers of E1 and E2 form 80 "spikes" that are distributed regularly on the outer membrane surface.[7] Two additional small structural proteins, the 6K protein and the TF protein, share the same open reading frame and are also associated with membranes. 6K protein enhances membrane permeability by inducing pore formation and induces caspase-dependent apoptosis. TF is differentiated from 6K by ribosomal frame shifting within the C-terminus of the 6K protein coding sequence.[8] Palmitoylation of the TF protein is required for its incorporation into viral membranes, and abrogation of either TF synthesis or its palmitoylation inhibits virion morphogenesis.[9] E2 appears primarily responsible for attachment of virus to the cell surface. E2 antibodies, but not generally E1 antibodies, can neutralize virus infectivity. E1 contains fusion and hemagglutinin activities.[1]

Alphaviruses enter cells primarily by receptor-mediated endocytosis. Several different receptors have been identified for different alphaviruses in different cell types in vitro. Two such receptors are found on dendritic cells: DC-SIGN (*d*endritic *c*ell–specific *i*ntercellular adhesion molecule-3-*g*rabbing *n*onintegrin) and L-SIGN (*l*iver/*l*ymph node–specific *i*ntercellular adhesion molecule-3-*g*rabbing *n*onintegrin).[10] Their affinity for dendritic cells and other cell types central to the host immune response has much to do with ongoing interest in alphaviruses as vaccine vectors for foreign antigens. After the E2 envelope protein binds to the cellular receptor, the virus particle is engulfed in a clathrin-coated endocytic vesicle that bears markers of an "early endosome." Subsequent acidification of the endosome triggers disassembly of the E1-E2 heterodimer and a low-pH–dependent fusion reaction with the endosomal membrane, mediated by E1. Fusion of the virus lipid envelope with the membrane of the endosome ultimately results in the release of virus RNA into the cytoplasm. Among the different species of alphaviruses, there is some variation on this theme. Some alphaviruses traverse the early endosomal pathway to fuse with the membrane of late endosomes. Some older and some recent data suggest that Sindbis virus (SINV) (for one example) may bypass the endocytic pathway entirely and enter cells by direct fusion with the plasma membrane.[11]

Once virus RNA has entered the cytoplasm, the four viral nonstructural proteins (nsp1-4) are then derived by direct translation of the 5′-terminal two-thirds of genomic RNA and sequential cleavage of the resulting polyprotein. Nsps 1 through 3 contain various activities necessary for capping of viral RNAs (nsp1), protease cleavage of the polyprotein (nsp2), and accessory functions required for RNA synthesis (nsp2 and nsp3). Nsp4 contains RNA-dependent RNA polymerase activity. Partial cleavage products of the nonstructural polyprotein (P123 + nsp4) initiate negative-strand RNA synthesis after infection. Eventually, nsp1, uncleaved P23, and nsp4 form a replication complex in virus-induced cytoplasmic vacuoles that is active in both negative-strand and genome RNA synthesis. Finally, cleavage of P23 results in a complete switch from negative-strand RNA synthesis to synthesis of new positive-sense genomes exclusively.[1] Cleavage of P23 is also required to release nsp2, which mediates evasion of the cellular innate immune response.[12] Viral structural proteins are encoded by a subgenomic (26S) messenger RNA collinear with the 3′-terminal one-third of the genome.

Initial products of translation of the 26S messenger RNA include a 62-kDa E2 precursor polypeptide, PE2 or "p62"; E1; and the hydrophobic 6K and TF membrane proteins. PE2 and E1 glycoproteins form a heterodimer in the endoplasmic reticulum that is transported to the plasma membrane through the Golgi secretory pathway. PE2 is cleaved by cellular enzymes during exocytosis to produce E2, and the E2/E1

heterodimer is acquired in *trans* by nascent virus particles via budding of nucleocapsids at the plasma membrane.[13] Thus the lipid envelope in mature particles is derived from the plasma membrane of the cell. The cleavage of PE2 destabilizes the heterodimer with E1 such that it is more readily dissociated by low pH, which, as noted, activates the capacity of mature virions for fusion.[1]

Phylogeny

The alphavirus genus contains at least 29 different viruses classified into seven serocomplexes.[14] EEE, VEE, and WEE are prototype viruses for each of three antigenic complexes. The WEE genome and those of WEE-like viruses, except for Aura virus (see later), are intragenic recombinants: the nonstructural and core protein genes are derived from an EEE-like ancestral genome, whereas the structural glycoproteins E1 and E2 are derived from the genome of a Sindbis-like virus.[15,16] Because the serologic basis for speciation is largely dependent on antigenicity of E2 in the hemagglutination inhibition (HI) test, some of the Old World alphaviruses—SINV and Sindbis-like viruses—group with WEE complex viruses (see Table 151.1). This is also true for a phylogenetic analysis based only on sequences of the viral structural proteins.[16] CHIKV, O'nyong-nyong virus, Mayaro virus, and Ross River virus (RRV) are grouped with Semliki Forest virus in a fourth complex. Three additional alphavirus serogroups contain only a single virus species: Barmah Forest virus (BFV) (see Table 151.1) and Middelburg and Nduma viruses (not shown in Table 151.1).[14,16]

A phylogenetic study of alphaviruses, derived from a Bayesian analysis of whole-genome sequences, is in essential agreement with their serologic classification, although the resolution is slightly different.[17] All the medically most important alphaviruses group into two clades: the Semliki Forest clade, including CHIKV, O'nyong-nyong virus, and RRV; and the Sindbis–equine encephalitis clade, divided into three complexes including major members SINV, VEEV, WEEV, and EEEV. This analysis also suggested that alphaviruses arose from a primordial aquatic virus ancestor. Viruses that share medically important characteristics, in addition to their genetic and antigenic relatedness, are widely dispersed geographically. For example, the geographic distribution of the EEE complex viruses is quite different from that of the VEE complex viruses, yet members of these complexes share encephalitic potential in equines and humans. Similarly, viruses of the Semliki Forest virus complex, Mayaro (limited to Latin America), and O'nyong-nyong (limited to Africa) produce identical fever/arthralgia/rash syndromes.

EPIDEMIOLOGY

Encephalitis-Causing Alphaviruses

Eastern Equine Encephalitis Viruses

Alphaviruses are limited in their geographic distribution by the range of their respective arthropod vectors. EEEV infection occurs focally along the eastern and Gulf coasts of the United States, with a very few human cases reported also in central states that border the Mississippi River. Documented cases have occurred as far north as southern Canada and as far south as northern areas of South America and the Caribbean.[18] The EEE complex consists of four antigenically and genetically distinct lineages: one that circulates in North America and the Caribbean (NA) and three that circulate in Central and South America (SA).[18–20] One recent study of NA and SA EEEV isolates, based on nucleotide sequencing,[21,22] suggested that the NA and SA viruses are highly diverged and should be considered different species. Thus it is not surprising that NA and SA EEEVs differ radically in pathogenicity and ecology.

In North America, EEE is a summertime disease, occurring most frequently in children and in the elderly. Between 1965 and 2016 the maximum number of cases reported in any one year was 15, in 2012. However, the years between 2003 and 2016 were notable for both a trend toward an increase in the average number of cases per season and a slight increase in the ratio and number of cases reported in northern states compared to southern. These observations were attributed to the effects of climate change on vector spread and viability over winters.[19] From 2007 to 2016, 68 cases of neuroinvasive disease caused by EEEV were reported, typically during summer months.[20,23] Of these, 29 were reported in New England states or New York State. Although relatively rare, an outbreak is usually noteworthy because of the high case-fatality rate (50% to 70%). The incidence of disease in equids and swine (which constitute a second animal host) greatly exceeds that in humans, and outbreaks resulting in the deaths of hundreds of horses have been reported in the northeastern United States and especially Florida, among southern states. For the period 2007–2016, the US Department of Agriculture reported a total of 1724 cases of EEE in horses in the United States, with 118 cases in 2016 alone.[24] In horses, EEE can cause necrotizing infections involving multiple organ systems, including the heart, spleen, urinary tract, and gastrointestinal tract, as well as the entire central nervous system (CNS).[25]

In North America, the principal enzootic vector for EEE is the mosquito *Culiseta melanura,* which breeds in freshwater swamps and feeds on passerine birds. Infection of avian species may result in death in some cases and may be without apparent consequence in others. In either case, it results in viremia of sufficient magnitude and frequency to maintain a reservoir of infected mosquitoes.[19,26] *Culiseta melanura* has not been implicated in transmission of infection from birds to horses and humans, because this species is highly ornithophilic. However, this possibility cannot be ruled out because of the high population density of infected *C. melanura* mosquitoes during an enzootic cycle. Other possible bridge vectors include *Aedes* and *Coquillettidia* spp.[27] Horses and humans develop only low or undetectable levels of viremia and do not serve as reservoirs for further virus spread. Among birds, infection seems to be spread by the oral route as well as by mosquito bite.[22]

In summary, conditions for EEE epizootics include the presence of *C. melanura* and susceptible bird populations coincident with vector mosquitoes capable of feeding on both birds and the horses or humans in the vicinity. In temperate climates, maintenance of epizootics is theoretically interdicted by winter. However, EEEV can be isolated from the same endemic foci in successive years, and genome sequence data from several isolates suggest that the virus does persist or "overwinter" in cold climates and that EEEV strains migrate from south to north along the East Coast of the United States.[26] EEEV is highly infectious by the aerosol route.[28] However, this mode of infection poses a risk associated only with handling of infected birds or with laboratory exposure.

EEE is a rare disease in South America, with only two recent case reports.[29] Serologic studies of native populations exposed to EEEV showed that infections occur without subsequent illness, suggesting that South American strains are relatively avirulent in humans.[19] This difference in virulence between North and South American isolates may be due to differences in replication efficiency in neuronal cells and in interferon (IFN) sensitivity and is probably multigenic in origin.[19,30]

Western Equine Encephalitis Viruses

The WEE complex viruses—New World viruses WEE, Buggy Creek, Highlands J, Fort Morgan, and Aura, and Old World viruses SINV and the Sindbis subtypes—cause either arthralgic (SINV-like subgroup) or encephalitic (WEE and Highlands J virus subgroup) syndromes. Their bimodal disease potential probably reflects the relative genetic contributions to tissue tropism of the EEE-like ancestral virus core and nonstructural genes versus that of the Sindbis-like *E1* and *E2* genes, in each particular genome. WEEV is distributed primarily in the Americas. A subtype of WEE, isolated in Argentina, is presumed to be representative of endemic strains in South America.[31] In North America, WEE is a summertime disease of horses and humans in states west of the Mississippi River and in corresponding Canadian provinces. The vector is *Culex tarsalis.* Risk factors for infection include rural residence, outdoor employment in farming (because the vector favors irrigated areas), and male sex. During an epidemic, a high percentage of the adult population seroconverts, but the case-to-infection ratio ranges from less than 1:1000 in older adults to nearly unity in infants. Thus encephalitis is most frequent in infants younger than 1 year. However, encephalitis is most severe in older adults. The case-fatality rate is 3% to 4%. In contrast, EEE infection rates are low in an epidemic, but the case-to-infection ratio is higher than that for WEE and is highest in the young.[31] The near total absence of WEE case reports in the past 15 years may reflect the predominance of strains that are relatively avirulent in humans. Using a mouse model for WEE, eight different strains of WEEV displayed either high- or low-virulence phenotypes. These strains segregated into one of two genotypes that correlated with their pathogenicity.[32]

Between 1964 and 1998, 639 confirmed cases of WEE were reported to the Centers for Disease Control and Prevention (CDC). WEE cases occurred most commonly in Colorado (173 cases), Texas, North Dakota, and California, with additional significant numbers of cases in Missouri, South Dakota, and Kansas.[20] The fact that there were no new cases of WEE reported to the CDC between 1998 and 2016 suggests that control efforts and other natural variables, such as strain virulence, have had a dramatic negative effect on disease incidence. (A single case of probable WEE was reported from Uruguay in 2009.[34]) In contrast, EEE is currently estimated to cause about 1% of all cases of viral encephalitis in the United States.[19,20,35] Formalin-inactivated whole-virus vaccines against EEE and WEE are available for equines, and vaccination is currently recommended for all horses residing in North America as core prophylaxis. There are no licensed vaccines for humans.

Venezuelan Equine Encephalitis Viruses

Six antigenic subtypes of VEE are recognized. Subtype I virus strains, or variants AB, C, D, and E, are found in tropical America and are medically most important. Variants IAB and IC were associated with equine epizootics and human epidemics that occurred between the 1920s and the early 1970s. Variants ID and IE and subtype II (also known as Everglades virus) are less virulent and are associated with enzootic disease.[18] The complex genetic relationships among VEE complex viruses were recently reviewed.[36] A live virus vaccine derived from a 1AB subtype virus is thought to have been the cause of an outbreak of human disease after a 1943 vaccination campaign.

VEEV outbreaks in South America and Central America have been associated with tens of thousands of both equine and human cases.[22] For epizootic viruses, equines play an important role in maintenance because they develop high-titer viremia and are thus likely to transmit infection to mosquitoes. At least 10 mosquito species, including *Culex, Aedes, Mansonia, Psorophora,* and *Deinocerites,* have been identified as probable epidemic vectors. Epizootics have been documented in Mexico, Venezuela, Colombia, Ecuador, and Peru at intervals of 10 years or less since the 1930s. Typically, epizootics begin in areas of tropical forest during the rainy season. In the center of an epizootic, transmission usually continues until all horses are dead or immune. Spread may be to contiguous areas or may be sporadic. Major VEE outbreaks occurred in 1962, 1973, and 1995, the last outbreak involving as many as 100,000 human cases.[37,38] Nearly all viruses isolated during the 1995 outbreak were closely related to the 1C subtype viruses that had been isolated in 1973. Five years later, viruses isolated from a few scattered cases of VEE in Venezuela were related to those isolated in 1995, yet epizootic vectors were not plentiful. This suggested that epizootic viruses could persist in nature via a cryptic transmission cycle.[39] For example, a study of VEE endemicity among horses, cattle, dogs, and humans in the Gulf Coast region of Mexico during the period 2003–2010 demonstrated that the 1E subtype of VEEV circulating in that area is phylogenetically related to the 1E virus that caused a major outbreak in 1969–1971, although in a separate clade.[40]

Increased incidence of human disease typically follows outbreaks of equine disease by 1 to 2 weeks. Severe human disease with encephalitis is most common in children. Like horses, humans also develop a viremia of sufficient magnitude to infect mosquitoes. However, humans have never been implicated in epidemic transmission. Similarly, although VEE can be isolated from throat washings and is certainly infectious by the aerosol route, person-to-person transmission has not been documented.[41]

Studies comparing epizootic strains with commonly isolated enzootic ones originally suggested that these sets of VEE subtypes were highly unrelated. In fact, *Culex taeniopus,* the common vector for enzootic strains of VEE, is refractory to oral infection with epizootic virus.[42] However, comparisons of the nucleotide sequences of newly emerging (1C-like) epizootic viruses in South America suggest that they evolved from a group of enzootic 1D-like viruses by a spontaneous mutation or mutations within the *E2* gene sequence that increase the positive charge of the E2 ectodomain. A variety of additional observations implicate similar net positive charge in E2 as a major determinant of equine and human virulence.[43–45] Fig. 151.1 illustrates alternative general mechanisms by which viruses such as EEEV, WEEV, and VEEV might transition from an enzootic to an epizootic cycle.

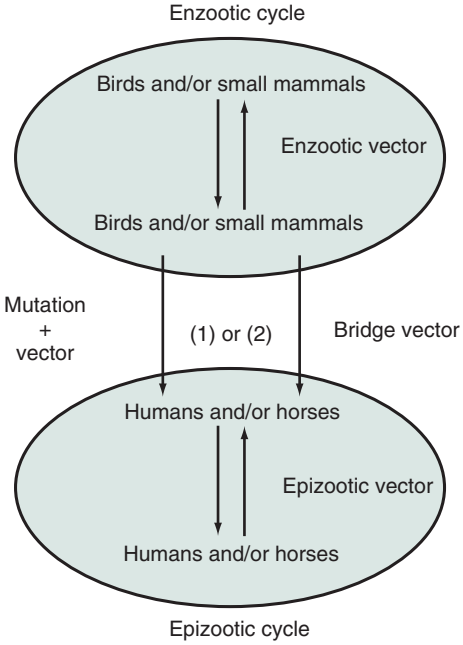

FIG. 151.1 Two different mechanisms leading to epizootic spread of alphavirus infections. The normal enzootic life cycle depends on the habits of mosquito vectors that are adapted to feed on the required small animal hosts, including birds and rodents. Virus infection spreads to horses or humans, or both, when either *(1)* a mutation occurs in the viral genome, adapting it to replication in large animals and in vector mosquitoes that are adapted to feed on them; or *(2)* a "bridging" vector transmits virus directly from animals involved in the enzootic cycle to humans or horses. Once the epizootic cycle is initiated, virus is spread by a different vector or set of vectors adapted to the large animal host. Venezuelan equine encephalitis virus is an example of an alphavirus that requires a mutation to initiate epizootic spread (mechanism 1). Eastern equine encephalitis virus is an example of an alphavirus that can spread to humans and horses by the action of a bridging vector mosquito (mechanism 2).

Horses are not amplifying hosts for enzootic strains of VEE. These viruses are principally maintained by their mosquito vector and rodents that thrive in tropical and subtropical swamps and forests. Humans living in these areas manifest a high prevalence of antibody. These viruses cause an illness resembling dengue fever and cause encephalitis sporadically, in Mexico and Central America (subtypes ID and IE) and Florida (subtype II).[42]

Alphaviruses Causing Fever, Polyarthritis, and Rash

Chikungunya Virus

Chikungunya means "to walk bent over," in reference to the crippling arthritic manifestations of the disease. *Aedes* mosquitoes of the subgenus *Stegomyia* are the principal vectors in Africa, and the virus seems to be maintained by transmission to nonhuman primates. Humans with high concentrations of virus in blood also provide a reservoir for the infection of mosquitoes. This permits epidemic spread even in urban settings, depending only on the presence of competent mosquito vectors. There are four known chikungunya virus (CHIKV) genotypes. These are the East-Central-South African, the West African, and the Indian Ocean lineages and the Asian genotype. The East-Central-South African and West African genotypes are mostly enzootic in Africa. The other two genotypes are found in Southeast Asia and India. The Asian genotype is implicated in the spread of disease to the New World.[46]

A major epidemic of CHIKV apparently started in Kenya in 2004, causing an estimated 500,000 cases in Africa, and spread initially in 2006 to the island of Réunion in the Indian Ocean. On Réunion, approximately 265,000 of 770,000 inhabitants experienced CHIK fever by the end of 2006, with 237 deaths.[47] After 2006, the outbreak spread to the southeast coast of India, the islands of Mauritius, Seychelles, and

Mayotte, and to Madagascar. At least 1.4 million cases of CHIK fever were reported in 2006–2007 in India. From 2006 at least up to 2016, the CHIK fever epidemic continued to spread into northern India, involving also neighboring areas of China in 2010[48] and countries of the Far East more distant from the epicenter. The causative strain was shown to be genetically related to the virus that initiated the epidemic in Réunion.[49]

The major vector involved in the outbreak on Réunion was *Aedes albopictus,* whereas *Aedes aegypti* is implicated as a vector in urban epidemics in India and elsewhere in Asia. Thus two lineages of CHIKV coexist: Asian strains adapted best to *A. aegypti* as a vector and Indian Ocean strains adapted to *A. albopictus.* A single amino-acid mutation in the E1 envelope glycoprotein was associated with adaptation of African CHIKV strains to *A. albopictus* mosquitoes.[50] Isolates obtained from India in 2011 contained at least two additional mutations in the E2 glycoprotein that further promote replication in *A. albopictus.*[51] Because *A. albopictus* is better adapted to temperate climates than is the *A. aegypti* species, the potential for epidemic spread to nontropical regions of the world was enhanced by these adaptive mutations. CHIK fever was imported to northern Italy by a traveler, and 205 cases occurred by autochthonous spread in that region between July and September 2007.[52] Similar data up to 2012 suggest limited spread in southern France. Most countries in Europe have by now documented cases of CHIK fever in travelers returning from epidemic regions.

Between 1995 and 2005, only four cases of CHIK fever among travelers returning to the United States were reported to the CDC. In contrast, 106 cases were identified from 2006 to 2009.[53] Sixty-two of these subjects had recently visited India, and 13 had CHIKV viremia on arrival in the United States; 12 of these were returning to states that reported the presence of potential CHIKV vector mosquitoes. No endogenous spread of CHIK fever had been reported in the United States or its territories up to 2012. However, in October 2013, Asian-linked CHIK virus was introduced into St. Martin, spread to Martinique and Guadeloupe, and affected nearly every island in the Caribbean, including Puerto Rico. In 2014, a peak of 4710 cases of CHIK fever were reported to the CDC from US territories and 2811 cases from the US mainland, with 12 endogenous cases in Florida. This seems to have been the peak of the outbreak in the United States and territories. Since then, case reports to the CDC have waned in number on a year-to-year basis; in 2017 only 104 cases, all thought to have been imported, were reported from 24 states, with only 33 cases reported in US territories.[46]

Asian lineage CHIKV also appeared in multiple Central and South American countries in 2013, probably imported from Micronesia.[46,54] As of April 2016, the Pan American Health Organization reported that local transmission of CHIK had occurred in 45 countries or territories throughout the Americas, with more than 1.7 million suspected cases in the affected countries, including Colombia, Venezuela, and Brazil, and with cases reported from nearly all other countries of the northern half of South America as well, most recently in Argentina.[54,55] Most of these countries were also experiencing simultaneous outbreaks of dengue virus, which is endemic to the area, and Zika virus. All three viruses are vectored by the same *Aedes* mosquitoes and cause very similar, nearly clinically indistinguishable, disease states in their simplest presentation.

Serologic survey results suggest that epidemics occur periodically when the youngest group of inhabitants of an endemic area is susceptible. The following factors suggest the potential for future major CHIK fever epidemics, especially in the Western hemisphere: (1) the increasing prevalence of Aedes species, especially *A. albopictus,* in North and South America and Europe[47]; (2) the increasing mobility of populations residing in endemic or currently epidemic areas; (3) the lack of a requirement for an animal host, other than humans, to support the CHIKV life cycle; and (4) the presence of large populations of immunologically naïve individuals in the United States. However, it can also be said that the threat of autochthonous spread of CHIK fever on the US mainland seems to have abated as of 2017, despite the apparently favorable conditions for outbreaks in eastern regions of the United States harboring *Aedes* mosquitoes.

O'nyong-Nyong Virus

O'nyong-nyong virus is closely related to CHIKV, both antigenically and genetically. It initially appeared in Uganda in the form of an epidemic that involved 2 million people in its final extent by the middle-to-late 1960s. The virus then disappeared until 1996, when it reappeared in the context of a second epidemic in southern Uganda. The reemerging virus was shown by nucleotide sequence analysis to be closely related to the 1959 strain.[56] During the fall of 2003, there was a third outbreak of O'nyong-nyong fever in the Ivory Coast that was initially mistaken for measles.[57] A 2010 serologic survey conducted in Cameroon demonstrated that O'nyong-nyong virus continues to circulate at a low level in the absence of a frank epidemic, suggesting that most infections are asymptomatic or only mildly so.[58] O'nyong-nyong virus and CHIKV are closely related to a third virus, Igbo-Ora, also found in Africa. Risk factors for O'nyong-nyong fever include residence in rural villages where the vector *Anopheles gambiae* mosquitoes congregate. A nonhuman primate reservoir of infection has not been identified.

Sindbis Virus

SINV was first isolated in Egypt in 1952. It is transmitted among birds by *Culex* and *Culiseta* mosquitoes. Studies in South Africa show that extensive human disease occurs in parallel with years of abundant rainfall in association with flooding of usually arid regions. Thus infected mosquitoes and susceptible humans are presumably brought into proximity. Infection rates may approach 15% during a major transmission season. SINV and the flavivirus West Nile virus share the avian–*Culex* mosquito hosts. In South Africa, the Nile Valley of Egypt, and Israel, individuals with antibodies to SINV frequently also have antibodies to West Nile virus.

Besides the indicated countries of Africa, SINV also causes a fever/arthralgia/rash syndrome in northern Europe, China, and Australia, as well as in the Philippines. In northern Europe, symptomatic SINV infection is recognized in the region between 60° and 65° north latitude in Sweden (as Ockelbo disease), Finland (as Pogosta disease), and the Commonwealth of Independent States (as Karelian fever). These are a single disease entity characterized by arthritis, pruritic rash, fatigue, mild fever, headache, and muscle pain. The causative agent is a northern European strain of SINV, genetically related to SINVs isolated in Africa.[59] Older adults who work or vacation in forested areas are most often affected. The virus has been isolated from *Culiseta, Aedes,* and *Culex* mosquitoes. The grouse is the major avian host of SINV in Finland.[60] Outbreaks of Pogosta disease occurred every 7 years, between 1974 and 2002, for reasons that may have to do with the migratory habits of grouse between South Africa and northern Europe. For example, 1301 and 597 cases were documented in 1995 and 2002, respectively. However, the expected outbreak in 2009 resulted in many fewer cases (105) than were expected based on the outbreaks recorded in 1995 and 2002.[61] Debilitating arthritis following acute Pogosta disease may persist for years. It is positively associated with the human leukocyte antigen subtype DBR1*01 and the presence of autoantibodies.[62]

Ross River Virus and Barmah Forest Virus

RRV is a cause of epidemic polyarthritis, myalgia, and fatigue in humid northern tropical areas of Australia and neighboring Pacific Islands. It is the most common and most widespread arboviral disease in Australia. Joint symptoms are especially intense and may last as long as 3 years after fever and rash have abated. RRV is transmitted by a wide variety of mosquito species; the life cycle principally requires mosquito vectors and marsupial hosts, especially the kangaroo and the wallaby, but dogs, cats, and possums are also implicated.[63] Human disease is seasonal in occurrence. A second alphavirus, BFV, is also found in northern Australia and shares mosquito vectors and animal hosts with RRV. BFV causes, in general, a less severe arthritis that is shorter in duration than that caused by RRV.[64] Both diseases were increasing in incidence as of 2013. RRV and BFV were implicated in 6546 disease reports between July 2009 and June 2010, probably due to flooding that enhanced vector mosquito replication.[65] In 2015, Queensland reported the worst outbreak of RRV in 20 years, with 2835 cases recorded in the first 3 months of 2015, mostly among young adults; more than 1000 cases had been reported in Victoria during the first 2 months of 2017.[66]

Mayaro Virus

Mayaro virus was first isolated in the Caribbean in the 1950s in association with an epidemic of febrile illness with rash and occasional arthropathy.

It has since caused epidemics in Brazil and Bolivia. The virus has been isolated from *Haemagogus* mosquitoes and from marmosets, as well as other nonhuman primates.[67] These may provide a reservoir for virus in the natural setting. As for other human pathogenic flavi- and alphaviruses, there is cause for concern regarding epidemic spread; a serosurvey in rural areas of Brazil recently identified 55% of febrile patients (15 of 27), in an outbreak thought to have been caused by CHIKV or dengue virus, as positive for Mayaro virus–specific immunoglobulin M (IgM), suggesting a cause-effect relationship.[68]

A long list of alphaviruses, including Bebaru, Cabassou, Getah, Kyzylagach, Middelburg, Nduma, Pixuna, Sagiyama, Semliki Forest, Una, and Whataroa viruses, either are not known to cause human disease or cause disease of the fever/arthropathy type that is rare.

PATHOGENESIS

Encephalitis

The locus of alphavirus replication in the mosquito is the midgut epithelium, which is targeted after the mosquito has taken a blood meal from a viremic host. In the mosquito, the infection is generally thought to be a lifelong, persistent, productive one, although there may be associated necrotic changes in the midgut.[69] In humans, the bite of an infected mosquito results in the deposition of virus in cutaneous tissues. Muscle cells and Langerhans cells are sites of replication. Infected Langerhans cells deliver virus to lymph nodes and ultimately to the blood.[1] The initial phase of infection is marked by viremia and a febrile response, signaling the replication of virus in nonneural tissues. Alphaviruses stimulate the innate immune response via interaction with Toll-like receptors and because the double-stranded RNA generated in the course of alphavirus RNA replication is a potent IFN inducer. The innate response and the capacity of the virus to suppress or evade it are major determinants of outcome for all alphavirus infections.

Although it does not commonly invade the human CNS, mouse brain–adapted SINV causes encephalitis in mice that has been intensively studied as a model for alphavirus encephalitides in humans. The degree of neurovirulence is different for different inbred strains and for different SINV strains, and is inversely related to age; disease is fatal in neonates.[70] The initial event in a fatal encephalomyelitis appears to be infection of capillary endothelial cells, after which virus quickly spreads throughout the CNS, especially involving neurons of the olfactory tract and hippocampus and motor neurons of the brainstem and spinal cord. Peak titers of virus are seen 2 to 3 days after infection in the brain and spinal cord. In neonates, neuronal cell death is by both the apoptotic and necrotic pathways. Apoptosis is triggered by the fusion event that occurs during virus entry, which induces sphingomyelinases that promote release of proapoptotic ceramide.[71] Mature neurons are more resistant to apoptosis than immature neurons, which may account for the increased susceptibility of very young mice to a fatal outcome.[72] Infected neurons also produce other proapoptotic products (e.g., BCL-2–associated X protein [BAX]), as well as antagonists of apoptosis (e.g., BCL-2, Beclin-1, certain protease inhibitors).[72] Locally produced type III and type I IFNs are important for control of virus replication early in infection, especially IFN-γ (type III) and IFN-β (type I). Conversely, differences in neurovirulence in mice of a highly neurovirulent and a nonneurovirulent strain of SINV correlated with the capacity of nsp1 to suppress the cellular response to IFNs, by disrupting Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling.[73] Mice deficient in IFN-β produce 10-fold higher titers of virus in brain, compared with normal mice. The virus-produced inflammatory response also stimulates interleukin (IL)-10 production from Th17 cells, which seems to protect from immune-mediated cell death without affecting virus replication. In mice infected by a nonlethal strain of SINV, absence of IL-10 led to a shift to Th1 type response, a reduction in the beneficial inflammatory response, and increased morbidity.[74] Other factors produced as part of the innate response include tumor necrosis factor (TNF); IL-1 and IL-6; chemokine (C-C motif) ligand 2 (CCL2), ligand 3 (CCL3), and ligand 5 (CCL5); and chemokine (C-X-C motif) ligand 9 (CXCL9) and ligand 10 (CXCL10).[75] These induce glial cell activation, expression of adhesion molecules on endothelial cells, and cell migration.[76]

Clearance of virus is initiated 4 to 5 days postinfection in nonlethal infection of mice. The adaptive immune response plays the major role in this process and is initiated in peripheral draining lymph nodes. Virus-specific $CD4^+$ and $CD8^+$ T cells rapidly increase, acquire effector functions, and enter the circulation. There is a two-phase B-cell response: a rapid extrafollicular response resulting in low-affinity IgM class antiviral antibody and a slower one emanating from the germinal center of affected lymph nodes, which eventually yields higher-affinity immunoglobulin G (IgG). Virus-specific cytotoxic $CD8^+$ T cells enter the brain within 3 to 4 days after infection and produce IFN-γ, followed by cytokine-producing $CD4^+$ T cells and antibody-secreting $CD19^+$ B cells.[77,78] Cytotoxic T cells do not clear virus-infected neurons in the brain, probably because neurons do not display human leukocyte antigens.[79] However, endothelial cells and ependymal cells in the brain are subject to T-cell surveillance, and cytotoxic T cells do play a major role in clearance of virus-infected cells from motor neurons of the spinal cord. IFN-γ both increases proinflammatory cytokines, leading to clinical disease in the early phase, and synergistically clears virus in the presence of SINV antibodies, partly by increasing chemokine production important for the infiltration of antibody-secreting B cells into the CNS, in late phases of disease.[80]

SINV E2-specific antibodies produced primarily by $CD19^+$ B cells entering the brain are primarily responsible for clearance of virus from neurons of the olfactory tract and hippocampus. After extracellular infectious virus has been eliminated in the brain, E2 antibodies (and IFN-γ) also suppress virus production from infected neurons by preventing the budding of nascent virions and shutting down viral RNA synthesis, although virus RNA persists in cells. Antibody and IFN-γ, operating via the JAK/STAT pathway rather than cytolysis, also restore host cell protein synthesis, membrane integrity, and the capacity to respond to IFN-α/β.[81] In the first several days after infection, the percentage of antibody-secreting B cells specific for SIN antigens increases in the brain, and these specialized B cells are maintained apparently for the life of the animal. Likewise, SINV RNA is detectable indefinitely in neurons of infected mice (>60 days), even though recovered from clinical manifestations of infection. In mice lacking an adaptive response, infectious virus is not cleared and the IFN-α and IFN-β responses are sustained.[77] Various chemical inhibitors of glutamate excitotoxicity protect mice from SINV encephalomyelitis paradoxically by reducing the entry of inflammatory cells into the CNS and delaying virus clearance.[80]

The pathogenesis of encephalitis caused by the New World viruses, EEEV, WEEV, and VEEV, is not nearly as well studied as is the SINV model, possibly because of the level of biosafety that must be observed when working with these viruses in the laboratory. However, available data do suggest there are parallels to the SINV system. For example, mortality due to VEEV is enhanced in mice lacking IFN regulatory factors (IRF-1 and IRF-2), IFN receptors, or type I IFN itself.[82,83] Moreover, VEEV can interdict the capacity of cells to respond to IFN by interrupting JAK/STAT signaling, as for SINV. The earliest adaptive immune response to VEEV is nonneutralizing antibody that mediates clearance of virus-infected cells in the periphery. This is followed by a neutralizing antibody response with E2 specificity. Before CNS invasion in experimental animals, VEEV replicates in lymphoid tissues, especially including dendritic cell types, resulting in necrotic changes, and in bone marrow, resulting in lymphopenia. Lymphoid infection in mice is followed by high-titer viremia, during which the peripheral CNS is seeded, mainly through the olfactory system.[84] VEEV also replicates in the pancreas and salivary glands of experimental animals but does not have a diabetogenic effect in humans who have survived encephalitis. Pathologic changes associated with encephalitis include neuronal cell loss due to apoptosis, mild-to-moderate neutrophilic infiltrate, gliosis, and perivascular cuffing with involvement of Purkinje cells.[85] EEEV causes lesions throughout the brain and spinal cord, most severely involving the cerebral cortex and basal ganglia. WEEV causes focal necrosis in the striatum, globus pallidus, cerebral cortex, thalamus, pons, and meninges. Transplacental spread of VEEV and WEEV may affect the fetus, resulting in massive cerebral necrosis.[86] Although persisting neurologic deficits may be among the sequelae of human infection with VEEV, WEEV, and EEEV, long-term persistence of either infectious virus or virus RNA in the CNS has not been documented.

Fever, Arthralgia, and Rash

During self-limited infection with a nonneurotropic alphavirus, such as CHIKV, viremia may precede the onset of symptoms by several days.

Illness is typically heralded by fever roughly coinciding with the peak of viremia, and the level of viremia closely parallels the fever curve at early times. The fever curve may be "saddleback"; fever may abate 4 to 8 days after onset in concert with the level of viremia and then recrudesce to peak readings for up to 2 weeks. IgM antibody titers rise during the acute phase and remain at a high plateau. As viremia fades and the first phase of the fever subsides, HI and neutralizing IgG antibody titers start to increase, suggesting that these immune responses curtail the acute phase of infection. However, the late and chronic sequelae of CHIKV and RRV occur in the presence of the vigorous humoral response, and pathology is consistent with an autoimmune etiology. (Likewise, symptoms and signs of encephalitis occur in the late phases of infection by EEEV and VEEV.) Biopsy of the maculopapular-to-macular rash in CHIK infection shows lymphocytic perivascular cuffing and extravasation of erythrocytes from superficial capillaries.[87] Arthralgia may accompany early symptoms of fever and malaise (especially in CHIK fever) or may be slightly delayed and quite severe, leading to frank arthritis (for both CHIK fever and RRV disease). Virus RNA and viral antigens can be detected in joint tissues as well as inflammatory cell infiltrates, consisting primarily of monocytes and virus-infected macrophages within synovial effusions.[88,89] Similarly, CHIKV antigens have been detected in muscle biopsy specimens exhibiting severe myositis with inflammatory macrophages, T lymphocytes, and myofibril pathology.[90] A general scheme for the time course of the pathogenesis of acute alphavirus diseases is shown in Fig. 151.2.

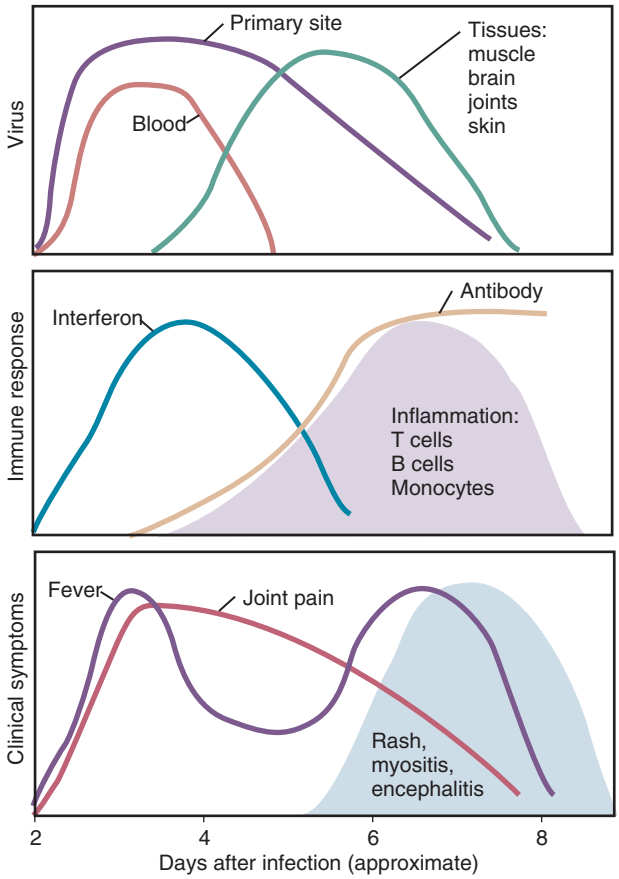

FIG. 151.2 Schematic diagram of the pathogenesis of alphavirus-induced disease. Viremia may be accompanied by production of interferon, other proinflammatory cytokines, and fever. Virus then spreads through the blood to other target tissues. As the immune response is induced, the viremia is terminated, but fever is renewed with the appearance of a mononuclear inflammatory response in the infected tissues. In infections that lead to rash and arthritis, joint pain usually appears early after infection and before the appearance of the rash. *(From Griffin D. The alphaviruses. In: Knipe DM, Howley PM, eds.* Fields Virology. *5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007:1031.)*

Using an established mouse model of RRV disease in humans, macrophage-derived factors were shown to be critical in the development of striated muscle and joint tissue damage. Histologic analyses of muscle and ankle joint tissues demonstrated a substantial reduction in inflammatory infiltrates in infected mice depleted of macrophages. Levels of the proinflammatory factors TNF-α, IFN-γ, and macrophage chemoattractant protein-1 were also dramatically reduced in tissue samples obtained from infected macrophage-depleted mice, compared with samples obtained from infected mice without macrophage depletion. These factors were also detected in the synovial fluid of patients with RRV-induced polyarthritis. Neutralization of these factors reduced the severity of disease in mice, whereas blocking nuclear factor kappa B by treatment with sulfasalazine ameliorated RRV inflammatory disease and tissue damage.[91] The balance between the innate response and the capacity of the virus to blunt that response as a major determinant of outcome seems to hold for RRV, as noted for other alphaviruses. Myeloid differentiation primary response gene 88 *(Myd88)*–dependent Toll-like receptor 7 signaling has a protective effect in RRV-infected mice. Higher viral loads, enhanced tissue damage, and reduced antibody affinity were seen in knockout mice.[92]

The outbreak of CHIK fever in East Africa and its spread into Asia and the New World has renewed interest in CHIK fever pathogenesis. Acute disease correlated with a dramatic increase in IFN-γ and IL-12, and chronic arthritis correlated with prolonged elevated levels of IL-12 in Réunion patients. IL-12 returned to normal in association with recovery.[93] A strong innate and adaptive immune response was associated with recovery among patients in Gabon as well, with elevated levels of IL-4, IL-10, and IFN-γ. A $CD8^+$ T-cell response preceded $CD4^+$ T-cell increases.[94] In vitro studies showed that CHIK (and SIN) nsp2 mediates the ubiquitin-dependent degradation of Rpb1, a component of RNA polymerase II, thus globally downregulating host gene expression, independent of autophagy, apoptosis, and inhibition of STAT1.[95] CHIK nsp2 also inhibits JAK/STAT phosphorylation, blocking IFN-I and IFN-II responses.[96] This is a common ancillary function for nsp2 of the Old World alphaviruses, including SINV and Semliki Forest virus. Other new information from in vitro studies suggests that CHIKV triggers autophagy to promote its replication; that CHIKV evades the host unfolded protein response by suppressing phosphorylation of the translation inhibitory factor eIF2α; and that CHIK nsp3 suppresses stress granule assembly in infected cells.

A mouse model for CHIK fever–related arthritis has been described in which the mice display evidence of arthritis, tenosynovitis, myositis, and virus persistence in the ankle joint distal to the virus-inoculated footpad.[97] In the mouse model, the dendritic cell immunoreceptor (DCIR) affects CHIKV pathogenesis. DCIR is a negative regulator of the host inflammatory response expressed on dendritic cells, monocytes, macrophages, B cells, and neutrophils.[98] The capacity of dendritic cells to express DCIR downregulated the inflammatory response and affected cytokine expression; IL-10 and IL-6 expression was increased, IL-12 expression was reduced, and virus replication was enhanced at late times in mice lacking DCIR. In a complementary study, $CD4^+$ T cells were seen to mediate joint inflammation in mice, independent of IFN-γ secretion.[99] In addition, IRF-3 and IRF-7 were shown to protect mice against CHIKV hemorrhagic fever and shock. IRF-3 and -7 knockout *($IRF3/7^{-/-}$)* mice developed high virus titers, edema, vasculitis, hemorrhage, fever and hypothermia, oliguria, and thrombocytopenia, indicating once again the importance to the host of the innate response as defense against pathogenic alphaviruses.[100] Recombinant activating gene 1 knockout *($RAG1^{-/-}$)* mice were recently shown to exhibit a chronic CHIKV infection and used to evaluate a candidate live-attenuated virus CHIK vaccine.[101]

CLINICAL MANIFESTATIONS

Alphaviruses Causing Encephalitis

EEE is heralded by a 5- to 10-day prodrome of headache, high fever, chills, nausea, and vomiting or diarrhea. In patients in whom CNS involvement develops, initial symptoms are followed by mental confusion and somnolence often accompanied by photophobia. Seizures or convulsions occur most often in younger patients. Seizures are usually tonic-clonic, but may also be of the complex partial type. Progression to frank

coma can occur rapidly. Physical examination may reveal nuchal rigidity, depressed or hyperactive reflexes, tremors, muscle twitching, spastic paralysis, bilateral papilledema, and cranial nerve palsies, which can be secondary to increased cerebrospinal fluid (CSF) pressure or directly related to inflammation. Cranial nerves VI, VII, and XII are most often affected. Infants may develop bulging fontanelles. Other occasional findings include cyanosis secondary to depressed respiratory drive and facial, periorbital, or generalized edema.[35,102]

Laboratory findings in acute EEE are as follows[35,102]: a polymorphonuclear leukocytosis is present in most cases to levels of 15,000 cells/mm^3 or higher. Hyponatremia, when noted, may be due to the syndrome of inappropriate antidiuretic hormone. CSF protein is increased, and 500 to 2000 cells/mm^3 are present, mostly lymphocytes. Red blood cells are occasionally noted as well. Hypoglycorrhachia is not present. Serologic tests are positive for antibodies to EEEV (see "Diagnosis" later).

Neurologic sequelae include mental retardation, behavioral changes, convulsive disorders, and paralysis. These may occur in 70% of patients recovering from EEE infection. Negative prognostic signs in EEE are age older than 40 years, rapid progression to coma, severe hyponatremia, and CSF cell counts higher than 500/mm.[35,102] Among patients who recover, sequelae are less frequent in adults.

WEE infection is also heralded by a short prodromal phase lasting 1 to 4 days. Signs and symptoms during this period are similar to those described for EEE. In adults, the prodromal phase may subside spontaneously with no neurologic complications. In subjects who do progress, the course of encephalitis resembles that of EEE as well, except that focal neurologic abnormalities may be less common.[35,102] Laboratory studies usually reveal a polymorphonuclear leukocytosis, but counts are significantly lower than those noted for EEE. The CSF protein is usually increased, and lymphocytes and red blood cells are present. Glucose is normal. Neurologic sequelae occur in 30% of young patients and are similar to those noted for EEE.[103] Parkinsonism is an occasional late sequela of WEE in adults.

The most common clinical manifestation of epizootic VEE infection is a febrile illness with malaise after an incubation period of 1 to 6 days. Chills, myalgia, and headache with or without photophobia, hyperesthesia, and vomiting are common. Occasionally patients report a sore throat. Fever may remit in a short time, with recrudescence the next day. Approximately 4% of children and less than 1% of adults progress to severe encephalitis, which usually occurs after a few days to a week of the prodromal illness.[37,38] Features of encephalitis include nuchal rigidity, ataxia, convulsions, coma, and paralysis, in ascending order of severity. Laboratory studies characteristically reveal lymphopenia, sometimes accompanied by neutropenia and mild thrombocytopenia within a day or two of onset. Serum glutamic-oxaloacetic transaminase (aspartate aminotransferase) and lactate dehydrogenase enzymes are typically increased. CSF examination reveals a few hundred lymphocytes. The overall case-fatality rate is less than 1% but approaches 20% in those who progress to encephalitis. Nearly all individuals in endemic areas contract enzootic VEE infection, as suggested by the results of serologic surveys.[38] Most seem to have experienced the influenza-like prodromal illness or to have had asymptomatic infection.

Alphaviruses Causing Fever, Polyarthritis, and Rash

CHIK fever is taken as the prototype of the diseases caused by this large group of alphaviruses. This is an acute viral infection characterized by a rapid transition from a state of good health to illness that includes severe arthralgia and fever.[104,105] The incubation period ranges from 1 to 12 days. Body temperature increases abruptly to as high as 40°C and is often accompanied by shaking chills. After a few days, fever may abate and recrudesce, giving rise to a "saddleback" fever curve (see Fig. 151.2). Arthralgia is polyarticular, favoring the small joints and sites of previous injuries, and is most intense on arising. Patients typically avoid movement as much as possible. Joints may swell without significant fluid accumulation. These symptoms may last from 1 week to several months and are accompanied by myalgia. The rash characteristically appears on the first day of illness, but onset may be delayed. It usually arises as a flush over the face and neck, which evolves to a maculopapular or macular form that may be pruritic. The latter lesions appear on the trunk, limbs, face, palms, and soles, in that order of frequency. Petechial skin lesions have also been noted. Headache, photophobia, retro-orbital pain, sore throat with objective signs of pharyngitis, nausea, and vomiting also occur in this setting. Laboratory test results may reveal a mild leukopenia with relative lymphocytosis. The erythrocyte sedimentation rate is usually markedly elevated, and the C-reactive protein is positive.[105,106] Severe arthritic involvement is most commonly seen in adults, whereas children occasionally present with symptoms referable to the CNS, including seizures and convulsions. Long-term joint involvement has been reported in association with human leukocyte antigen B27.[106]

As a result of the recent CHIKV pandemic, additional clinical aspects of the disease have become evident. In 88 patients on Réunion Island who were assessed a median of 18 months after onset of disease, 56 reported persistent arthralgia and half of those reported a resulting reduction in their ability to carry out daily activities. All had polyarticular involvement, and pain was continuous in more than half of those who reported arthralgia. Approximately 40% of the total had persisting CHIK-specific IgM antibodies. Transient ocular involvement was described among adult patients in South India, with the main manifestations including anterior uveitis, optic neuritis, retrobulbar neuritis, and dendritic lesions.[107] In another study emanating from Réunion Island, atypical cases of CHIKV infection were defined as those in which patients developed symptoms other than fever and arthralgia. A total of 610 such cases were studied, among which 222 were graded as severe. Of these, 65 patients died. Atypical manifestations included meningoencephalitis, hepatitis, bullous dermatitis, pneumonia, and diabetes.[108] According to a second report from Réunion, serious acute CHIKV infection resulted in encephalitis (14 cases), myocarditis, hepatitis, and Guillain-Barré syndrome among 33 patients who were admitted to an intensive care unit. There was a 48% fatality rate in this severely ill group.[109]

Mother-to-child transmission of CHIKV infection was demonstrated in a retrospective study of neonates. Clinical signs seen in 38 infants included fever (79%), rash (82%), pain (100%), and peripheral edema (58%). Laboratory abnormalities included thrombocytopenia, lymphopenia, decreased prothrombin, and elevation of alanine aminotransferase. Virus was detected in the CSF of 22 of 24 infants evaluated by polymerase chain reaction (PCR); 14 of these subjects had abnormal findings on brain magnetic resonance imaging with white matter lesions or intraparenchymal hemorrhages. Myocardial hypertrophy, ventricular dysfunction, pericarditis, and coronary artery dilatation were documented in a minority of this group, and one neonate died of necrotizing enterocolitis.[110] Among all patients, the case-fatality rate on Réunion was estimated at 1:1000. Higher rates were calculated for Mauritius and parts of India. Also on Réunion, a cohort of 180 patients with proven CHIK fever was followed for 36 months. Sixty percent of patients reported arthralgia or arthropathy with swelling, usually with episodic relapse and recovery, typically affecting several joints in a symmetrical distribution. In 77% of those reporting arthralgia, the symptoms were characterized as highly incapacitating. Age older than 35 years and presence of arthralgia at 4 months after the acute illness were risk factors for long-term arthropathy. These patients did not display biologic markers commonly associated with autoimmune disease/rheumatoid arthritis, in contrast to results reported earlier from a study of chronic persisting CHIK fever–related arthritis of 18 months' duration.[106,111]

Cases of CHIK fever among travelers returning to their home countries from the epidemic areas have been characterized. In one study from Germany, CHIKV infection was documented in 20 of 69 patients who initially reported CHIK-like symptoms, including fever and arthralgia. Two-thirds of the patients had persistent arthralgia for longer than 2 months, and 13% had it for longer than 6 months. Active viremia was reported in all patients who reported to the clinic within the first week of illness.[112] The recent experience with travelers returning from Africa, Réunion, or Asia to Italy or France was mentioned earlier.

DIAGNOSIS

The epidemiology of each of the disease entities caused by alphaviruses is highly specific and provides a major clue to diagnosis. Thus knowledge of the recent travel or outdoor exposure history of the patient is of vital importance. In certain locales, during epidemic spread of a disease, the diagnosis is obvious. In the United States, the initial signs and symptoms

of EEE or WEE infection may mimic those of enteroviruses. Encephalitis caused by the flaviviruses West Nile virus and St. Louis encephalitis virus may occur in the same setting as EEEV encephalitis. CDC criteria for the diagnosis of an arboviral encephalitis require the presence of an acute febrile illness with encephalitis during a time when virus transmission is likely, plus one of the following criteria: (1) greater than fourfold increase in viral antibody titer between acute and convalescent sera; (2) virus isolation from CSF, blood, or tissue; or (3) IgM positive to the virus in CSF.

EEEV can sometimes be isolated from serum during the prodrome,[35] but historically most cases are diagnosed by testing paired sera in HI tests or in a neutralization assay because only low-level viremia occurs in human subjects. Convalescing patients may manifest high complement fixation antibody titers, and IgM antibodies can be detected by enzyme-linked immunosorbent assay (ELISA).[113] Magnetic resonance imaging with specialized imaging techniques such as fluid-attenuated inversion recovery and T2 weighting is of value for both diagnosis and following the clinical course of encephalitis.[114] EEE caused focal radiographic changes involving the basal ganglia, thalamus, and brainstem, in descending order of frequency, in one study of 36 cases.[102]

Diagnostic testing for WEE follows a similar pattern except that viremia is usually not detectable. The presence of WEE in a specimen may be documented by inoculating suckling mice or embryonated eggs. In contrast, sera taken from patients with VEE infection within 48 hours of onset are usually positive for virus. However, sera from patients with full-blown encephalitis are usually negative, and the diagnosis may be made by complement fixation testing. ELISA for VEE-specific IgM in sera and CSF is available. IgM and IgG ELISAs using attenuated VEE as antigen are the most sensitive diagnostic tests but should be followed up with the plaque reduction neutralization assay to prove specificity.[115]

Sensitive nucleic acid amplification assays using the reverse-transcriptase PCR (RT-PCR) exist for rapid diagnosis of EEE, WEE, VEE, and West Nile virus infections.[116] Most recently, ELISA and RT-PCR have been combined in a single, rapid-detection system that uses biotinylated oligonucleotide probes complementary to unique amplified nucleotide sequences in the WEE, EEE, VEE, and Mayaro virus genomes.[117] Human monoclonal antibodies directed against epizootic VEEVs have been expressed in bacteriophage and are being used for rapid diagnostic assays.[118] When these assays are available, they constitute a rapid substitute for actual culturing of virus from a clinical specimen.

CHIK fever in its acute presentation or in mild disease may be clinically indistinguishable from disease caused by the alphaviruses (Mayaro virus, O'nyong-nyong virus, RRV, or SINV) or by the flaviviruses (dengue virus and Zika virus). Dengue virus and CHIKV can coinfect mosquitoes, and simultaneous transmission of both viruses to humans has been documented. In one clinical study conducted in Singapore, where dengue virus and CHIK now cocirculate,[119] CHIK fever was distinguished from dengue fever (DF) or dengue hemorrhagic fever (DHF) on the basis of clinical and laboratory criteria. Bleeding, sore throat, cough, nausea, vomiting, diarrhea, abdominal pain, anorexia, and tachycardia were more common in DF or DHF than in CHIK fever, whereas joint pain was more common in CHIK fever. Laboratory results showed that hemoconcentration, thrombocytopenia, lymphopenia, and liver enzyme elevations were also more common in DF or DHF than in CHIK fever. In addition to DF and DHF, parvovirus B19 infection, the prodrome of hepatitis B, juvenile rheumatoid arthritis, and rubella, may also be confused with CHIK fever or with the other alphavirus infections. Patients with CHIK fever are usually viremic for the first 48 hours, and the virus is easily isolated by in vivo or in vitro methods. Viremia may be so intense (titer $>10^7$ plaque-forming units/mL of blood) as to yield measurable amounts of hemagglutinating activity from sera.[120] Consequently, virus in sera can also often be detected by ELISA directly.[121] As previously mentioned, the decrease in levels of viremia parallels a rapid increase in titers of HI and neutralizing antibodies. ELISA testing for virus-binding antibodies may detect cross-reactive immune responses to other alphaviruses. The recent CHIK virus epidemics in Africa and Asia have stimulated the development of rapid diagnostic tests on the basis of variations of the real-time RT-PCR, with particular emphasis on their ability to distinguish dengue from CHIK.[121,122] A recent study of the antibody response to CHIK proteins in Singaporean patients revealed high-titer neutralizing activity and responses to E1, E2, E3, and nsp3 in six patients at 2 to 3 months postinfection. Only the response to E2 was still significant at 21 months postinfection, and it seemed to account for persisting capacity of sera to neutralize virus at high dilutions.[123]

THERAPY AND PREVENTION

There is no licensed product approved for treatment of any of the diseases caused by alphaviruses. Low-dose steroids have been used to treat arthritis following RRV infection, with the attendant risks and side effects. Chloroquine has proven ineffective in treatment of CHIK fever–related arthritis, but there has been some modest success with TNF blockers.[124] Harringtonin, a cephalotaxine alkaloid, ameliorates SIN arthritis in mice and inhibits CHIKV replication in vitro; it is under further study.[125] Monoclonal antibodies are currently of major interest as potential prophylactic or therapeutic agents in CHIK fever.[126] Because the clinical and laboratory findings of alphavirus arthritides bear some resemblance to rheumatoid arthritis, there is some hope for crossover effectiveness of drugs used to treat the latter.[124] For encephalitis, supportive measures and intensive nursing care are indicated. Analgesics and nonsteroidal antiinflammatory drugs remain the main treatment options for symptoms of arthritogenic alphavirus diseases.

Prevention of WEE and EEE depends primarily on control of vector mosquito populations. During outbreaks, susceptible individuals engaged in high-risk activities should be advised to avoid exposure as much as is practicable by using effective mosquito repellents and netting, wearing full-length trousers and long-sleeved shirts, and avoiding outdoor activities, at least during periods of maximal mosquito activity. Inactivated vaccines against EEE and WEE (and against VEE and CHIK) were developed at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) several decades ago, for use in laboratory workers and other personnel who might be exposed to infectious aerosols. Although acceptably safe, these vaccines induce a relatively short-lived immune response, as measured by an in vitro assay for virus neutralizing antibodies in sera. Periodic booster doses are necessary to maintain seropositivity.[127] There has been no attempt to license these vaccines for widespread use. An inactivated EEE/WEE veterinary vaccine, administered as a component of a multivalent vaccine, is used against EEE in horses and birds and against WEE in horses.

Both formalin-inactivated and live-attenuated vaccines to prevent VEE are in limited use in humans. The live-attenuated VEE vaccine strain TC-83 is used in the diagnostic ELISA, and large-scale vaccination of horses with this strain is the major approach to the prevention of VEE epizootics. A live-attenuated vaccine to prevent CHIK fever (strain TSI-GSD-218) has been shown to be safe in volunteers at the level of phase II clinical trials.[128] One study in which the experimental live-attenuated VEE and CHIK fever vaccines were sequentially administered to human volunteers indicated that preexisting alphavirus immunity interferes with a subsequent neutralizing antibody response to a heterologous vaccine virus.[129] Similarly, the inactivated EEE and WEE vaccines described above interfere with each other if administered on the same day, and with the live VEE vaccine if given shortly prior to a dose of the latter vaccine.[130]

The live VEE and CHIK vaccine candidates mentioned previously were derived in the early 1990s by serial passage of virulent human isolates in tissue culture, and neither one is licensed by any regulatory authority. Newer approaches to alphavirus vaccine development, stimulated especially by the worldwide spread of CHIKV, have involved the use of recombinant DNA technology. Attenuated mutant VEEVs have been developed with a goal to both prevent VEE and use VEE as a vector for presentation of foreign antigens, because of the propensity of VEEV to target dendritic cells. One viable mutant virus derived from VEE infectious DNA, which contains mutations affecting cleavage of the viral structural proteins, was avirulent in mice, produced solid mucosal immunity against VEE, and currently shows promise primarily as a vaccine for horses.[131] This genetic backbone provided the basis for a number of VEE DNA-based vaccines designed to induce immunity to foreign viral and nonviral antigens, especially when mucosal immune responses are considered to be of crucial importance for protection,[132] but there has been little progress in clinical development of these vaccines in recent years.

Novel vaccines to prevent EEE and several to prevent CHIK fever were recently described, and CHIK vaccine development was recently reviewed.[133] In one approach, the Sindbis structural protein genes were replaced by those of EEEV or CHIKV, in the context of a Sindbis infectious DNA.[134,135] In another, a live-attenuated CHIKV was derived by insertion of the internal ribosome entry site from the encephalomyocarditis virus genome at the 5′ terminus of the CHIK genome to control translation.[136] Modified vaccinia Ankara has recently been used to vector CHIKV antigen.[133] Two groups are developing CHIK vaccines based on virus-like particles.[137,138] Virus-like particles resemble native virus particles by structural and antigenic criteria, but they are nonreplicating and cannot cause disease. One of these two candidate vaccines was shown to protect monkeys from CHIK viremia and is currently in clinical trials.[136] A recombinant measles virus–based CHIK vaccine recently underwent a phase I clinical trial and was found to be immunogenic and to have a generally acceptable tolerability profile.[139] An alphavirus specific for insect cells, Eilat virus, was genetically modified to express the CHIKV surface antigen genes. The resulting chimeric virus can be produced in insect cells and is unable to replicate in vertebrate cells.[140] There is little doubt that one or more of these strategies can result in a safe, effective CHIK vaccine, if development is further pursued.

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152 Rubella Virus (German Measles)

Anne A. Gershon

SHORT VIEW SUMMARY

Definition
- Rubella is an infectious illness characterized by fever and maculopapular rash, which may be accompanied by arthritis.
- Many postnatal infections are asymptomatic.
- Postnatal rubella is a benign illness, but congenital rubella can result in a variety of serious medical problems.
- Manifestations of congenital rubella include deafness, cataract or glaucoma, congenital heart disease, autism, and mental retardation.
- Rubella vaccine was developed primarily to prevent congenital rubella from occurring.

Epidemiology
- In temperate climates, disease occurs mainly in the spring (if vaccine is not being used).
- Rubella is somewhat less contagious than measles.
- Widespread vaccination in the Americas led to the elimination of rubella as of 2009.
- Globally, rubella and congenital rubella remain serious medical problems, especially in Africa, some areas of Europe, Southeast Asia, and the Western Pacific.

Microbiology
- Rubella is an RNA virus, a member of the Togaviridae family and the genus *Rubivirus.*

Diagnosis
- Diagnosis can be made serologically (enzyme-linked immunosorbent assay) with acute and convalescent serum samples or by demonstration of rubella immunoglobulin M antibody on a single serum sample.
- A positive test for viral RNA using reverse-transcriptase polymerase chain reaction on throat swabs, cerebrospinal fluid, or amniotic fluid is especially useful for diagnosis of congenital rubella.

Therapy
- None is available.

Prevention
- Live-attenuated rubella vaccine is effective and safe, usually administered along with measles and mumps vaccines (MMR).

Rubella (German measles) is an acute exanthematous viral infection of children and adults. The clinical illness is characterized by rash, fever, and lymphadenopathy and resembles a mild case of measles (rubeola). Although many infections with the agent are subclinical, this virus has the potential to cause fetal infection, with resultant birth defects, and (uncommonly but especially in adults) various forms of arthritis.

Rubella virus was first isolated in 1962 by Parkman and colleagues[1] and by Weller and Neva.[2] Rubella virus is classified in the Togaviridae family[3,4] on the basis of its single-stranded, positive-sense polyadenylated RNA genome; replication strategy; icosahedral capsid; and lipoprotein envelope. Rubella virus is closely related to the alphaviruses, but in contrast to alphaviruses, no vector is required for its transmission, and it is serologically distinct from alphaviruses.[4] Therefore rubella virus alone has been placed in a separate genus, *Rubivirus.* There are 2 clades and 13 genotypes.[5,6] Only one antigenic type, however, is recognized.[6] On electron microscopy, rubella virus is roughly spherical. Its envelope, which has short surface projections, has a diameter of about 60 nm. The envelope surrounds the 20-sided nucleocapsid, which has a diameter of about 30 nm and comprises a helix of protein and RNA. Rubella virus matures by budding from the cell membrane.[7]

Three structural polypeptides associated with rubella virus are termed E1, E2, and C. There are also two nonstructural proteins that are related to replication and transcription. E1 and E2 are transmembrane glycoproteins, and C is the capsid protein that surrounds the RNA of the virion. Hemagglutinin and complement-fixing antigens are composed of varying proportions and mixtures of E1, E2, and C.[8,9] E1 is important in attachment, fusion, hemagglutination, and neutralization.

Rubella virus is relatively unstable. It is inactivated by lipid solvents, trypsin, formalin, ultraviolet light, and extremes of pH and heat, and it is inhibited by amantadine.[10] Cytopathic effects may not be noted in all cell lines in which rubella virus replicates. However, cytopathic effects are readily observed in the rabbit kidney cell line RK-13 and in primary African green monkey cells.[8]

EPIDEMIOLOGY

Rubella was not distinguished clinically from certain other exanthematous infections until the late 19th century. At one time it was termed *third disease,* when measles and scarlet fever were called *first disease* and *second disease,* respectively.[11] Because postnatal rubella is such a mild illness, the disease was considered to be of only minor importance for many years. However, in 1941, when the Australian ophthalmologist Gregg[12] recognized the link between maternal rubella and certain congenital defects, a more complete picture of disease caused by rubella virus began to emerge.

Before widespread vaccine use, the incidence of clinical cases of postnatal rubella in temperate climates was highest in the spring, and it was traditionally recognized to be most common in children 5 to 9 years of age.[13] Rubella is only a moderately contagious illness, in contrast to measles. Therefore in the prevaccine era, only 80% to 90% of adults were immune to rubella, whereas 98% were immune to measles.[14]

Epidemics of rubella of minor proportions occurred in the prevaccine era every 6 to 9 years, and large-scale epidemics occurred at intervals of up to 30 years. The most recent major epidemic in the United States occurred in 1964, during which some 12,500,000 people were infected.[15] Since the licensure of a live-attenuated rubella vaccine in 1969, there have been no large rubella epidemics in countries where the vaccine is widely used. However, limited outbreaks continued to occur in settings such as workplaces, schools, and military camps, where groups of susceptible individuals had close contact with each other.[14,16] In 2001, only 23 cases of postnatal rubella and 3 confirmed cases of congenital rubella syndrome were reported to the US Centers for Disease Control and Prevention (CDC).[17] An increase in susceptibility to rubella was noted among Hispanic young adults for some years, and there were increasing efforts to identify rubella-susceptible women before they became pregnant.[17,18]

In 2004 rubella was declared no longer endemic in the United States.[5,6,19–21] Since 2001, there had been fewer than 25 annual postnatal cases and only rare cases of congenital rubella syndrome. In 2017 the

CDC reported that about 10 annual cases of postnatal rubella occurred in the United States.[22] In 2012, three cases of congenital rubella were reported to the CDC, and all three were likely acquired through mothers who traveled early in their pregnancies in Africa.[23] Rubella was considered eliminated from the United States, as 95% of school-aged children were vaccinated, more than 90% of the population had immunity, there was adequate surveillance to detect outbreaks, and molecular evidence indicated that there were no circulating American genotypes of rubella virus.[24] This was a major advance in public health and occurred at a time similar to that when measles was no longer endemic in the United States, which was also a result of widespread immunization. Isolated cases of rubella may be imported into the United States from another country but with only the possibility of limited spread. Mathematical models indicate that transmission of rubella ceases at 90% immunization levels, which has been achieved in the United States as a whole,[5,25] although levels in individual states may be lower.[26] Measles, by comparison, is more contagious than rubella, and higher immunization rates may be required to prevent measles outbreaks.[27] Global efforts are underway to decrease the global burden of rubella and congenital rubella syndrome.[28] The expansion of vaccination programs worldwide has resulted in significant progress toward the control and ultimate eradication of the disease. A 95% decrease in cases of rubella was reported by the World Health Organization between 2000 and 2014.[29] In April 2015 North and South America became the first regions in the world to be declared free of endemic rubella transmission. As of December 2016, 152 (78%) of 194 countries had introduced rubella vaccination into their immunization schedules, which represented an increase of 53 countries since 2000 and an increase in 22 countries since 2012.[30] The CDC has established the target goal of eradication of rubella and measles to be accomplished by 2020.[31]

TRANSMISSION OF RUBELLA

Rubella virus is spread in droplets that are shed from respiratory secretions of infected individuals. Patients are most contagious while the rash is erupting, but they may shed virus from the throat from 10 days before until 15 days after the onset of the rash. Patients with subclinical cases of illness may also transmit the infection to others.[10]

Infants with congenital rubella shed large quantities of virus from body secretions for many months and therefore may transmit the infection to individuals who care for them. These infants continue to excrete rubella virus despite high titers of neutralizing antibody, a puzzling phenomenon that has yet to be explained.[32] The possibility of immune tolerance due to fetal infection has been raised.[9] Infants of recent immigrants from developing countries who have congenital rubella may infect susceptible individuals if the diagnosis is not made and contacts are not immunized.

People who receive rubella vaccine do not transmit rubella to others, although the virus may be transiently isolated from the pharynx. The quantity of virus shed may be too small to be infectious.[18]

MAINTENANCE OF IMMUNITY TO RUBELLA

After an attack of rubella, lifelong protection against the disease develops in most people. However, the factors responsible for this protection are not precisely understood. Antibody titers to rubella virus develop, but the significance of the decline of antibody titers with time remains unclear. Cell-mediated immunity to rubella virus associated with $CD4^+$ and $CD8^+$ T lymphocytes has also been detected by in vitro assays[8,33] months to years after an attack of rubella. The long-term persistence of humoral and cellular immunity to rubella in a group of cloistered nuns who had no opportunity for reexposure to rubella virus has been documented.[34] The persistence of specific antibody for 14 years after immunization has also been demonstrated.[35,36]

Nevertheless, despite the presence of specific immunity to rubella virus, reinfection with rubella virus can occur. This had been long suspected on clinical grounds alone.[37,38] Rubella reinfections have been documented by detection of a significant boost in rubella antibody titers in naturally immune individuals after reexposure to the virus. Most reinfections are asymptomatic.[39] It is likely that the virus can multiply locally in the upper respiratory tract, but that viremia occurs infrequently because the host's immune response eradicates the virus before it can invade the blood. However, in rare instances, patients have been reported to have proven rubella reinfection occurring years after naturally acquired rubella, with symptoms indicative of viremia (e.g., arthritis, rash).[40]

Rubella reinfection occurring months or years after the receipt of rubella vaccine has also been observed. Several investigators have documented reinfections in up to 80% of individuals who had received rubella vaccine previously and were subsequently exposed to rubella during an epidemic.[39,41] Most of these reinfections were not characterized by clinical illness but were identified only by a rise in antibody titer. Viremia is probably extremely rare in such cases,[41–43] although rubella virus has been recovered from throat secretions in reinfections.[39,42] In one study of eight seronegative adult vaccinees who were experimentally challenged with wild-type rubella virus, replication in the respiratory tract was found in seven subjects, and viremia was present in two.[44] However, these subjects experienced only a mild illness or remained asymptomatic.[44]

Reinfections are more common among vaccinees than among individuals who have experienced natural rubella, and they are most common among individuals with hemagglutination inhibition (HAI) antibody titers of 1:64 or less.[39,41,42] It has been suggested that there may also be qualitative differences in antibody between individuals with vaccine-induced immunity and those with natural immunity because in one study, even with similar HAI titers, vaccinees were 10 times more likely to be reinfected than individuals with natural immunity to rubella.[39]

Whether rubella reinfection that occurs during pregnancy can result in transmission of the virus to the fetus has been the subject of much debate. Several case reports in the older literature that ascribed fetal defects to maternal rubella reinfection likely actually involved primary maternal infections.[45,46] Viremia was documented in one woman with detectable rubella antibody before immunization.[47] Boué and coworkers[48] studied a small number of women with documented subclinical cases of rubella reinfection during pregnancy who carried their babies to term; all the babies were found to be normal. In a number of other case reports of rubella reinfection during pregnancy, infants born at term to the affected mothers had symptoms suggestive of congenital rubella.[49–54] Most of these reinfections occurred years after natural infection, although some occurred years after immunization.[53,54] However, these transmissions are acknowledged to be extremely rare, particularly considering the exceedingly low incidence of congenital rubella in the United States today.

It appears that individuals who are immune to rubella, either by having had the natural infection or by having received rubella vaccine, may be reinfected when reexposed. However, reinfection is usually asymptomatic and detectable only by serologic means. Viremia and congenital rubella in maternal reinfection appear to be very rare events.

The presence of large numbers of immune people in a community appears to be able to prevent rubella epidemics from occurring; this effect is termed *herd immunity.* Although it has been documented that herd immunity does not entirely eliminate the spread of rubella, it probably plays a major role in control of this infection, which is now rare in the United States.[5]

PATHOGENESIS

The incubation period for rubella ranges from 12 to 23 days (average, 18 days). As in measles (see Chapter 160), a primary and a secondary viremia are believed to accompany rubella. Rubella virus has been detected in leukocytes of patients 1 week before the onset of symptoms.[55] Also as in measles, the rubella rash appears as immunity develops and the virus disappears from the blood,[8] suggesting that the rash is immunologically mediated. Although circulating immune complexes are detectable during rubella, they do not appear to contribute to the development of rash.[8,56] Rubella virus has been isolated from involved skin,[57] but this does not preclude the possibility that the rash is secondary to an immune response to the virus.

CLINICAL MANIFESTATIONS

Age is the most important determinant of the severity of rubella. Postnatally acquired rubella is usually an innocuous infection, and, as



FIG. 152.1 Child with exanthem of acute rubella. *(Courtesy Centers for Disease Control and Prevention, Atlanta, GA. Vaccine Information. http://www.immunize.org/photos/rubella-photos.asp.)*

is true for many viral illnesses, children have milder disease than adults. In contrast, the fetus is at high risk for development of severe rubella, with serious sequelae if infected transplacentally in early pregnancy because of maternal rubella.

Postnatal Rubella

Many, if not most, cases of postnatal rubella are subclinical.[15,58] Among patients who are symptomatic, children do not experience a prodromal phase, but adults may have a prodrome of malaise, fever, and anorexia for several days. The major clinical manifestations of postnatal rubella are adenopathy, which may last several weeks, and rash. The lymph nodes involved include the posterior auricular, posterior cervical, and suboccipital chains. Splenomegaly also occasionally occurs.[59] These symptoms are not specific for rubella, and clinically the disease may resemble measles, toxoplasmosis, scarlet fever, roseola, parvovirus B19 infection, and certain enterovirus infections.

The rash of rubella begins on the face and moves down the body. It is maculopapular but not confluent, may desquamate during convalescence, and may be absent in some cases (Fig. 152.1). An enanthem consisting of petechial lesions on the soft palate (Forschheimer spots) has been described for rubella, but this enanthem is not diagnostic for rubella (in contrast to Koplik spots in measles). The rash may be accompanied by mild coryza and conjunctivitis. The rash usually lasts 3 to 5 days. Fever, if present, rarely lasts beyond the first day of rash.

Complications of Postnatal Rubella

Complications of postnatal rubella, in contrast to complications of measles, are uncommon. Bacterial superinfections after rubella are rare.

Arthritis or arthralgia has been reported in one-third of women with rubella; this complication is less common in children and in men.[60] The arthritis tends to involve the fingers, wrists, and knees, and it occurs either as the rash is appearing or soon afterward. It can be slow to resolve, taking 1 month. Chronic arthritis rarely develops.

The pathogenesis of rubella arthritis is not entirely understood. The frequency of detection and the quantity of circulating immune complexes are higher in rubella vaccinees who report joint complaints than in those with no joint involvement.[56,61] Rubella virus has been isolated from joint effusions in patients with acute or recurrent rubella arthritis associated with either previous natural infection or vaccination.[a] Rubella virus has been isolated from peripheral blood mononuclear cells in patients with chronic arthritis.[62,63] A persistent rubella virus infection of human synovial cells cultured in vitro was reported, and this was advanced as an explanation for the pathogenesis of chronic forms of rubella arthritis.[64]

Hemorrhagic manifestations occur as a complication in about 1 of every 3000 cases of rubella.[59,74] In contrast to other complications of rubella, hemorrhagic manifestations occur more often in children than in adults. This complication may be due to both thrombocytopenia and vascular damage, and it is probably immunologically mediated.[74] Some investigators have proposed that mild thrombocytopenia often goes undetected in apparently uncomplicated rubella.[75] Thrombocytopenia may last weeks to months and may cause serious problems if bleeding into vital areas (e.g., brain, kidney, eye) occurs.[59] Thrombocytopenic purpura as the single clinical manifestation of rubella in children has also been reported.[74]

Encephalitis is an extremely uncommon complication of rubella; its incidence during an epidemic was reported to be 1 in 5000 cases. It occurs more frequently in adults than in children, and it is associated with a mortality rate of 20% to 50%.[59,76,77] Survivors usually have no sequelae.[8] A fatal case of rubella encephalitis in a 2-month-old child whose mother had rubella in the last week of pregnancy has been reported.[78]

Mild hepatitis has been described as an unusual complication of rubella.[79]

Congenital Rubella Syndrome

Rubella can be a disastrous disease in early gestation and can lead to fetal death, premature delivery, and an array of congenital defects. The incidence of congenital rubella in a given population is quite variable, depending on the number of susceptible individuals, the circulation of virus in the community, and, in recent times, the use of rubella vaccine. The rubella epidemic of 1964 left 30,000 affected infants in its wake.[6] Between 1969 and 1979, however, an average of 39 cases per year were reported to the CDC.[80–82] Since the advent of the 21st century, congenital rubella has been essentially eliminated from the United States.[6,17,23,24]

The effects of rubella virus on the fetus depend to a large extent on the time of infection; in general, the younger the fetus when infected, the more severe the illness. During the first 2 months of gestation, the fetus has a 65% to 85% chance of being affected, with an outcome of multiple congenital defects or spontaneous abortion.[8] Rubella during the third month of fetal life has been associated with a 30% to 35% chance of developing a single defect, such as deafness or congenital heart disease. Fetal infection during the fourth month carries a 10% risk for a single congenital defect. Occasionally fetal damage (deafness alone) is seen if rubella occurs up to the 20th week of gestation.[83]

The specific signs and symptoms of congenital rubella may be classified as temporary (e.g., low birth weight), permanent (e.g., deafness), and developmental (e.g., myopia).[80] The most common manifestations are deafness, cataract or glaucoma, congenital heart disease, and mental retardation; the major clinical manifestations are listed in Table 152.1.[80]

Prospective studies of the congenital rubella syndrome suggest that it should not be considered a static disease. Some children whose mothers had rubella during pregnancy and who were considered normal at birth were found to have manifestations of congenital rubella when they reached school age.[84,85] Diabetes mellitus in late childhood has also been observed 50 times more frequently in children who had congenital rubella than in healthy children.[8,86] Insulin-dependent diabetes has been reported in 40% of adult survivors of congenital rubella from the 1942 epidemic.[87] In a follow-up study of 242 children who had congenital rubella, rubella virus–induced diabetes had genetic and immunologic features similar to those observed in other forms of insulin-dependent diabetes: the frequency of the human leukocyte antigen allele HLA-DR3 was increased, and the frequency of HLA-DR2 was decreased.[88] Antibodies to pancreatic islet cells or cytotoxic surface antibodies were present in 80% of patients with abnormalities in serum glucose concentration.[88] At autopsy of patients with congenital rubella, the virus was isolated from the pancreas, which was noted to have a subnormal number of glandular cells.[88] Progressive encephalopathy resembling subacute sclerosing panencephalitis was observed in children with congenital rubella.[89,90] In 1991, a group of 40 adults born with the congenital rubella syndrome between 1939 and 1943 were reexamined. Although they had multiple defects involving hearing, diabetes, growth retardation,

[a]References 62, 65, 66–68, 69, 70, 71, 72, 73.

TABLE 152.1 Congenital Rubella: Transient (T), Permanent (P), and Developmental (D) Manifestations

COMMON	UNCOMMON OR RARE
Low birth weight (T)	Jaundice (T)
Thrombocytopenic purpura (T)	Dermatoglyphic "abnormality" (P)
Hepatosplenomegaly (T)	Glaucoma (P)
Bone "lesions" (T)	Cloudy cornea (T)
Large anterior fontanelle (T)	Severe myopia (P, D)
Meningoencephalitis (T)	Myocardial abnormalities (P)
Hearing loss (P, D)	Hepatitis (T)
Cataract (and microphthalmia) (P)	Generalized lymphadenopathy (T)
Retinopathy (P)	Hemolytic anemia (T)
Patent ductus arteriosus (P)	Rubella pneumonitis (T)
Pulmonic stenosis (P, D)	Diabetes mellitus (P, D)
Mental retardation (P, D)	Thyroid disorders (P, D)
Behavioral disorders (P, D)	Seizure disorders (D)
Central language disorders (P, D)	Precocious puberty (D)
Cryptorchidism (P)	Degenerative brain disease (D)
Inguinal hernia (P)	
Spastic diplegia (P)	
Microcephaly (P)	

From Cooper LZ. Congenital rubella in the United States. In: Krugman S, Gershon A, eds. Infections of the Fetus and Newborn Infant. *New York: Alan R. Liss; 1975. Reprinted with permission of John Wiley & Sons. © 1975. This material is used by permission of Wiley-Liss, a subsidiary of John Wiley & Sons.*

and eye and heart abnormalities, most were well adjusted socially. There was no increased incidence of malignant disease in these 50-year-old survivors.[91]

Infants with congenital rubella develop high titers of neutralizing antibody that may persist for years.[92] However, these children may eventually lose detectable antibody.[93] Reinfection with rubella has also been documented in some of these children.[94] Impairment of cell-mediated immunity to rubella antigen was found in some children with congenital rubella.[95]

A number of pathologic mechanisms have been proposed to explain certain manifestations of congenital rubella. It has been suggested that persistent infection with rubella virus leads to a mitotic arrest of cells, which causes inhibition of cellular growth and, consequently, retarded organ growth.[96] Additional hypotheses put forth to explain the growth retardation associated with congenital rubella are that infection leads to angiopathy with placental and fetal vasculitis, which compromises growth,[97] and that tissue necrosis without inflammation or fibrotic damage leads to cellular damage.[98] Another possible explanation is that infection of various types of cells during gestation interferes with the normal balance of growth and differentiation, which leads to defects in organogenesis.[8] Human fibroblasts infected with rubella virus in vitro were found to produce a growth inhibitor, which might also account for fetal growth retardation.[99] An increased frequency of chromosomal breakage was found in cultured cells from children with congenital rubella compared with cells from healthy children.[100] Molecular evidence of cell cycle arrest caused by rubella virus has been obtained.[101] It has been postulated that lymphocyte abnormalities in patients with congenital rubella syndrome may predispose them to organ-specific autoimmunity.[102]

DIAGNOSIS

Because rubella is usually a mild disease with nonspecific symptoms, it is often difficult to diagnose clinically. The disease has been confused with other infections such as scarlet fever, mild measles, infectious mononucleosis, toxoplasmosis, roseola, erythema infectiosum, and certain enteroviral infections.[103,104] Routine laboratory studies are not helpful for diagnosis because they may reveal only leukopenia and atypical lymphocytes; more specific laboratory diagnostic techniques are usually necessary.

Virus isolation from throat swabs, urine, synovial fluid, or other body secretions is an acceptable method for diagnosis, particularly in the early days after rash onset.[6] The diagnosis of congenital rubella infection has been made by isolation of virus from amniotic fluid.[105] However, virus isolation is time-consuming and expensive,[8,10] and it is not used frequently today. Virus isolation for diagnosis of rubella and especially for congenital rubella has been largely supplanted by molecular methods such as reverse-transcriptase polymerase chain reaction.[6,9] Results of reverse-transcriptase polymerase chain reaction may be positive before the appearance of rubella immunoglobulin M (IgM) antibodies.

The laboratory diagnosis of postnatal rubella is most conveniently made serologically. At one time, HAI was the preferred means of measuring rubella antibody titers, but this technique has been supplanted by simpler, more accurate methods of similar sensitivity.[10,104,106–111,112] These include enzyme-linked immunosorbent assay, passive latex agglutination test, and radial hemolysis test. These tests have variable sensitivity. Most of these tests may be used to measure either IgG or IgM antibodies. A demonstration of specific IgG on one serum sample is evidence of immunity to rubella. Acute rubella infection may be diagnosed either by a demonstration of specific IgM in one serum sample or by a fourfold or greater increase in rubella antibody titer in acute and convalescent specimens assayed in the same test.[9,113] Positive rubella IgM antibody tests have been associated not only with primary infection but also with reinfection with rubella virus.[114] This phenomenon may explain, at least in part, why apparent false-positive results on IgM-rubella enzyme-linked immunosorbent assay testing in pregnant women have been reported.[115] Results of many of these serologic tests are available within a matter of minutes or hours and yield prompt, useful information. However, some commercial assays for rubella immunity may yield false-negative results (see "Vaccination Against Rubella").

For a serologic diagnosis of congenital rubella in the neonatal period, antibody to rubella virus should be measured in both infant and maternal sera. It may be necessary to perform several antibody determinations on serum from the infant to detect whether the titer of rubella antibody is falling, which indicates passively acquired maternal antibody, or rising, which suggests rubella infection. If rubella IgM is detected in a newborn infant's serum, transplacental rubella infection has occurred. Congenital rubella infection has been diagnosed by the following tests or procedures: placental biopsy at 12 weeks, demonstration of rubella antigen with monoclonal antibody, cordocentesis and detection of RNA by in situ hybridization,[116] and polymerase chain reaction.[117] It may also be diagnosed by the presence of specific IgM in fetal blood, but this may not be detectable until 22 weeks of gestation.[118,119] Because of public health concerns today, it is extremely important to confirm suspected congenital rubella by laboratory means.

THERAPY

There is no specific therapy, but for patients with fever and arthritis or arthralgia, the treatment of symptoms is indicated. At one time, immune globulin (IG) was advocated for the prevention or modification of rubella in susceptible pregnant women who were exposed to the infection. However, it was discovered that although IG might suppress symptoms, it would not necessarily prevent viremia.[120] Therefore indications for the use of IG for rubella prophylaxis are few. Possibly, IG may be given to a susceptible pregnant woman who is exposed to rubella and for whom abortion is not an option if she should develop the disease. With the advent of rubella vaccine, it is now recommended to immunize susceptible women of childbearing age against rubella before they become pregnant.

VACCINATION AGAINST RUBELLA

Rubella virus was isolated in 1962[1,2] and attenuated in 1966[121]; the live-attenuated vaccine was licensed for use in the United States in 1969. The rationale for use of the vaccine is to prevent congenital rubella by control of postnatal rubella (see Chapter 316). In the United States, the first strategy was to vaccinate prepubertal children to minimize exposure of susceptible pregnant women to rubella. More recently, there has been an emphasis on immunization of rubella-susceptible women of childbearing age who are not pregnant. Often, this is done just after delivery of an infant; nursing mothers who are vaccinated do not cause harm to their infants. In some other countries, the approach has been to vaccinate girls against rubella as they approach puberty.

Immunization programs in the United States have dramatically reduced the transmission of rubella in young children and prevented major epidemics of rubella. There have been no such epidemics for

almost 50 years, a phenomenon never previously observed in the United States. A mini-epidemic of congenital rubella in 21 infants occurred in 1990 in southern California. More than 55% of the mothers had a total of 22 missed opportunities for vaccination at the time of marriage or after previous delivery of a child; therefore more than half of these cases of congenital rubella were preventable.[122]

The incidence of postnatal rubella fell to an all-time low in 1988, but by 1991 it had increased threefold. There was a concomitant increase in cases of congenital rubella syndrome during the same period, although there was still a decline of more than 98% in cases of rubella compared with the prevaccine era. The observed increase in cases was attributed to failure to immunize rather than vaccine failure.[81] With improvements in vaccine delivery since 1991, the incidence of rubella in the United States again fell. As noted previously, endemic rubella has been eliminated from the United States. There remains, however, a continued need to emphasize the importance of immunization of susceptible women of childbearing age who are not pregnant, hospital employees, and infants and children.[121,122,123,124]

At the present time the only rubella vaccine available in the United States is RA 27/3. This vaccine has been widely used in Europe and is more immunogenic than the previously used vaccines. RA 27/3 vaccine stimulates the production of secretory and humoral IgA, which may account for its increased immunogenic potency.[125,126,127] Single antigen rubella vaccine is not available in the United States, so rubella vaccine is administered either as measles-mumps-rubella (MMR) vaccine or as measles-mumps-rubella-varicella (MMRV) vaccine. Because there is a second routine dose of measles vaccine in the United States, two doses of rubella vaccine are also routinely administered. Two doses of rubella are useful because they prevent primary vaccine failure. Second doses of MMR are not considered booster doses.

Presumptive evidence of rubella immunity according to the CDC is as follows: (1) written documentation of vaccination with one or more doses of live rubella virus vaccine on or after 12 months of age, *or* (2) birth before 1957, *or* (3) laboratory confirmation of disease or of rubella immunity by antibody testing. It is not uncommon for rubella antibodies to be undetectable using commercial antibody assays in immune individuals. Persons who do not have acceptable presumptive evidence of immunity should receive one dose of MMR vaccine.[128]

Complications of Vaccination

Rubella vaccine may cause viremia,[129,130] and therefore the main complications are fever, adenopathy, arthritis, and arthralgia. All the complications are more common in adults than in children, and they are most common in women older than 25 years.[131,132,133] In one study, 40% of such vaccinees developed joint complications[132]; however, all reactions were transient. It is uncommon for children to develop complications. In general, the incidence of joint complications, even in adults, is lower after vaccination than after natural rubella.[14,130] In 1991 a committee of the Institute of Medicine examined the issue of chronic arthritis after administration of RA 27/3 rubella vaccine and concluded that there is a rare causal relationship.[134] The risk for arthritis is increased in individuals with HLA alleles DR1, DR4, and DR6.[135] Evidence in vitro indicates that wild-type strains of rubella virus can be propagated more efficiently in joint tissues than vaccine strains.[136]

Efficacy of Vaccination

Since the introduction of rubella vaccine, the number of reported cases of clinical rubella has declined progressively. The vaccines available today, when properly administered, produce a seroconversion rate of about 95% after one dose.[14] Seroconversion in response to rubella vaccine is not impaired in children with upper respiratory tract infections.[137] Because antibody titers are lower after vaccination than after natural disease, the question has been raised as to whether the antibody titer, years after vaccination, will remain high enough to prevent clinical rubella. Only time and continued surveillance will provide an answer to this question, but at the present time there is little evidence of waning immunity,[35,36] as reflected by the low incidence of rubella in the United States. Therefore booster injections of rubella vaccine are not routinely indicated.

Effects of Rubella Vaccine on the Fetus

Since rubella vaccine was licensed in 1969, the CDC has monitored the outcome in infants born to women who were reported to have been inadvertently immunized against rubella during early pregnancy. As of late 1987, 812 such women who carried their infants to term had been included in the CDC study, with no cases of the congenital rubella syndrome attributed to rubella vaccine.[138] Therefore the observed risk for congenital rubella after immunization is reported as zero; however, the theoretical maximal risk could be 1% to 2%. This is in contrast to a 20% or greater risk after maternal rubella in the first trimester.[138] The vaccine-type virus can cross the placenta, and rubella virus has been isolated from both decidua and fetal tissue at abortion after inadvertent vaccination of pregnant women.[138–142] Rubella virus was isolated from the fetus of a woman given rubella vaccine 7 weeks before conception.[143] A single case of persistent infection of a fetus whose mother was inadvertently immunized in early pregnancy has been recorded; the infant had no signs or symptoms of congenital rubella syndrome.[144]

Based on an analysis of 293 normal infants born to rubella-susceptible mothers vaccinated 1 to 2 weeks before or 4 to 6 weeks after conception, for whom the theoretical risk to the fetus is 1.3%, the CDC recommends that women avoid pregnancy for 28 days after rubella vaccination.[145] Although it is not recommended that rubella vaccine be administered to women who are pregnant, the currently recognized minimal theoretical fetal risk does not mandate automatic termination of a pregnancy. Many, if not most, of such vaccinated women may wish to carry their baby to term.

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153 Flaviviruses (Dengue, Yellow Fever, Japanese Encephalitis, West Nile Encephalitis, Usutu Encephalitis, St. Louis Encephalitis, Tick-Borne Encephalitis, Kyasanur Forest Disease, Alkhurma Hemorrhagic Fever, Zika)

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SHORT VIEW SUMMARY

Definition

- The Flaviviridae family incorporates the *Flavivirus, Pestivirus, Pegivirus,* and *Hepacivirus* genera, which cause systemic febrile, central nervous system, and plasma leakage and hemorrhagic fever syndromes, or a combination of these.

Epidemiology and Clinical Features

- Transmission and disease may occur with endemic, hyperendemic, or epidemic patterns, or a combination of these.
- Yellow fever (YF) is found in tropical South America and sub-Saharan Africa. Disease is severe, with high morbidity and mortality. Recent outbreaks have demonstrated new areas at risk for sustained YF transmission and the inadequacy of the current global vaccine production and supply chain.
- Dengue is widely distributed throughout the tropics and subtropics. Severe disease is infrequent (~2%–4% of clinically apparent sequential infections) but potentially fatal. Nonsevere disease (outpatient) accounts for much of dengue's global socioeconomic impact.
- Japanese encephalitis virus (JEV) is a significant cause of encephalitis in Southeast and South Asia. Chronic morbidity and mortality are high in symptomatic cases.
- West Nile virus (WNV), St. Louis encephalitis (SLE) virus, JEV, Usutu virus (USUV), and tick-borne encephalitis virus (TBEV) are the major neurotropic members of the genus *Flavivirus.* Infection with WNV is found worldwide, SLE virus is found in the Americas, and USUV is found in Africa and Europe, whereas TBEV occurs from Western Europe to Russia, Japan, and China. Most infections are subclinical. Clinically apparent cases with neurologic manifestations have a predilection for older individuals.
- Zika virus (ZIKV) infections have been documented since the 1950s in Africa and Asia. In 2007, a large outbreak occurred on Yap Island in the Pacific. In 2012, ZIKV transmission was documented with increasing frequency in Southeast Asia and French Polynesia. Beginning in 2015, ZIKV experienced an epidemic explosion in Central and South America. ZIKV infection generally causes a mild self-limited illness consisting of fever, maculopapular rash, arthralgias, and conjunctivitis. Recent outbreaks have been associated with increased incidences of neurologic complications such as Guillain-Barré syndrome and a spectrum of perinatal complications and developmental disorders such as microcephaly and motor deficits.
- Alkhurma hemorrhagic fever virus (AHFV) and Kyasanur Forest disease virus (KFDV) infections are emerging or reemerging in the Middle East, India, and Africa/Asia, respectively. Infections initially have nonspecific clinical findings; persons infected with AHFV and KFDV may develop hemorrhagic manifestations.

Microbiology

- Flaviviruses are icosahedral, approximately 50 nm in diameter, approximately 11-kb single-stranded, positive-sense RNA viruses consisting of a lipid envelope covered densely with surface projections consisting of M (membrane) and E (envelope) glycoproteins. Nonstructural proteins make up the remainder of the genome.
- Mutations and replication errors are high.

Diagnosis

- Diagnostic approaches are dictated by the disease course.
- During the viremic period, viral isolation and nucleic acid or antigen detection from blood are possible.
- During the subacute period, early-phase immunoglobulin M antibodies may be detected in sera or cerebrospinal fluid using various assay platforms (e.g., enzyme-linked immunosorbent assay).
- During the convalescent period, a rise in immunoglobulin G between acute and convalescent specimens is measured to identify a seroresponse (plaque reduction neutralization test, hemagglutination inhibition).
- Potentially unique to ZIKV, measurement of RNA in urine, saliva, and blood during the acute and remote period from infection has been documented and may enhance testing strategies.
- Cross-reactivity among the Flaviviruses adds complexity to interpreting serologic test results from areas where numerous Flaviviruses cocirculate.

Therapy

- No licensed or specific antiviral therapies are available.

SHORT VIEW SUMMARY—cont'd

- Treatment is supportive, and outcomes are variable depending on the experience of the practitioners and available critical care resources.

Prevention

- Personal protective measures to avoid exposure to the vector and the potential for infection is the first line of prevention.
- US-licensed vaccines exist for YF and JE. Vaccines also exist for TBE and Kyasanur Forest disease.
- A single dengue vaccine (Dengvaxia) has been licensed in 20 countries, but a safety signal in vaccine recipients who were dengue naïve at the time of vaccination has limited its potential widespread use. Two other vaccines (National Institutes of Health and Takeda Vaccines) are in advanced clinical testing.
- ZIKV is unique (except for hepatitis C) in its potential for sexual transmission and maternal-fetal infection. Pregnant women should weigh the risks of traveling to areas with active ZIKV transmission. Numerous Zika vaccine candidates are in preclinical and clinical development, with two DNA constructs in phase II clinical testing.

The *Flavivirus* genus consists of more than 70 viruses, most of which are arthropod-transmitted or zoonotic viruses, including some 30 known to cause human disease.[1–4] Many of the remainder have an unknown pathogenic potential but have been implicated in laboratory infections; several are veterinary pathogens. The agents are classified in the family Flaviviridae (Latin *flavus,* for "yellow" and for *yellow fever virus,* the type species), together with viruses in the *Pestivirus* genus (which are of veterinary importance) and the *Pegivirus* genus (11 species that cause persistent virus infections of primates, bats, rodents, pigs, and horses), and those in the genus *Hepacivirus* (hepatitis C–like viruses) on the basis of similar morphologic characteristics and genomic structures.[1–4] There are no significant antigenic relationships between viruses across genera.

Currently (2014–2018), numerous Flaviviruses pose a significant global public health threat. Zika virus (ZIKV) infections have spread extensively throughout the tropical and subtropical regions of the world to include Central and South America, the Caribbean, Mexico, the Pacific Islands, Africa, and Southeast Asia. ZIKV has been associated with devastating congenital complications (microcephaly and intracranial calcifications) and neurologic illnesses, such as Guillain-Barré syndrome (GBS; discussed in detail later).[2,3] From a global perspective, the public health and socioeconomic burdens of flavivirus infections such as dengue, yellow fever (YF), Japanese encephalitis (JE), Zika, and tick-borne encephalitis (TBE) have been of sufficient magnitude to warrant the development of vaccines to control these diseases.[5–7] The licensure and distribution of effective vaccines for YF (>80 years), JE (>50 years), and TBE (>30 years) have led to significant reductions in incidence and, in some locations, the effective disappearance of disease.[8] Dengue vaccine candidates have been under development for more than 75 years, two are in clinical efficacy trials and one has completed efficacy trials and is licensed in 20 countries. Demonstrating long-term safety and efficacy sufficient for licensure has been challenging for this vaccine (discussed in detail in the section "Prevention and Therapy" later).[9] Many of the other flaviviruses have the ability to cause considerable morbidity, but they occur too sporadically to support a concerted approach to prevention and control.

Flavivirus infections are important considerations in the differential diagnosis (especially in returning travelers) of a systemic febrile illness with headache, bone pain, and rash; central nervous system (CNS) infection; plasma leakage or hemorrhagic complications, or both; birth defects; and GBS.[10,11] By evaluating the epidemiologic history, including the places and dates of travel, incubation time of the viruses, activities during travel, and immunizations, in conjunction with clinical features of the illnesses, the clinician can obtain important clues to pursue or exclude a diagnosis. The diseases of chief importance in this group are described later in this chapter.

HISTORY

Yellow Fever

Although the historical record and molecular taxonomic studies of viral strains have indicated an African origin of YF virus, the disease was first recognized in an outbreak that occurred in the New World in 1648 (Fig. 153.1A).[12] The consensus is that the virus was introduced to the New World by *Aedes aegypti*–infested slave-trading vessels from West Africa. Through the next 2 centuries, similar outbreaks spread to port cities in the New World and in Europe. The resulting calamities were exemplified by the 1793 Philadelphia epidemic, in which one-tenth of the city's population died, and by the 1878 Mississippi Valley epidemic of 100,000 cases, the cost of which equaled the national budget. Sanitary measures, especially the introduction of piped water, inadvertently served to diminish transmission of the disease, although its mosquito-borne route of spread was not demonstrated until 1900 and its viral cause not until after 1928. Theiler's development of the attenuated 17D vaccine strain in the 1930s was recognized by a Nobel Prize, but more than 80 years later, vaccine implementation (through the World Health Organization [WHO] Expanded Program on Immunization) in areas with endemic transmission remains incomplete and outbreaks recur periodically.[13]

Dengue

Because of dengue's nonspecific clinical features, the interpretation of historical records for evidence of past epidemics is open to speculation.[14–17] However, Benjamin Rush's description of a 1780 Philadelphia epidemic was the earliest description in English of so-called breakbone fever. Subsequently, sporadic outbreaks were reported throughout the tropics and subtropics. Mosquito-borne transmission of the infection by *A. aegypti* was demonstrated in 1903, and its viral etiology was proven in 1906.[14,15] Outbreaks were common in the continental United States through the early decades of the 20th century, the last occurring in Florida in 1934 and in New Orleans in 1945. Dengue was generally considered a relatively benign disease (certainly in comparison to YF) with few, if any, complications or deaths, although cases of dengue complicated by hemorrhage, shock, and death were described in outbreaks in Australia in 1897, in Greece in 1928, and in Formosa in 1931.[14,16,17] While isolating the virus in 1944, Sabin demonstrated the failure of two viral strains to cross-protect in humans, first establishing the existence of distinct dengue virus (DENV) types; Hammon characterized two more types in 1956.[18] After World War II, intensified transmission of multiple DENV types began in Southeast Asia, and were associated with outbreaks of severe disease, which was given the name dengue hemorrhagic fever (DHF).

The program to control YF in the Americas in the 1940s by eradicating *A. aegypti* mosquitoes was also successful in controlling DENV transmission. The ending of these programs in the 1970s resulted in *A. aegypti* becoming widely reestablished in the region and dramatic increases in the occurrence of dengue and severe disease.[19–21] The introduction to Cuba in 1981 of a novel DENV-2 viral strain from Southeast Asia produced the first major DHF epidemic in the hemisphere, resulting in 116,143 hospitalizations, including 10,000 for shock. Since 1989, DHF outbreaks have been reported in most of Central and South America.[12,21,22] With the reintroduction of DENV-3 into Central America in 1994, all four DENV types now circulate in the Americas.[23,24] The most dramatic ascendance of dengue and severe disease over the past several decades has occurred in the Caribbean and in Latin America.[19–21] DHF outbreaks result in several thousand DHF cases each year throughout most of Central and South America and the Caribbean.[12,21,22]

In the past 30 years, this pattern of hyperendemic transmission of all DENV types and increased DHF incidence has become well established in South Asia, the Americas, and Oceania. Increased transmission and

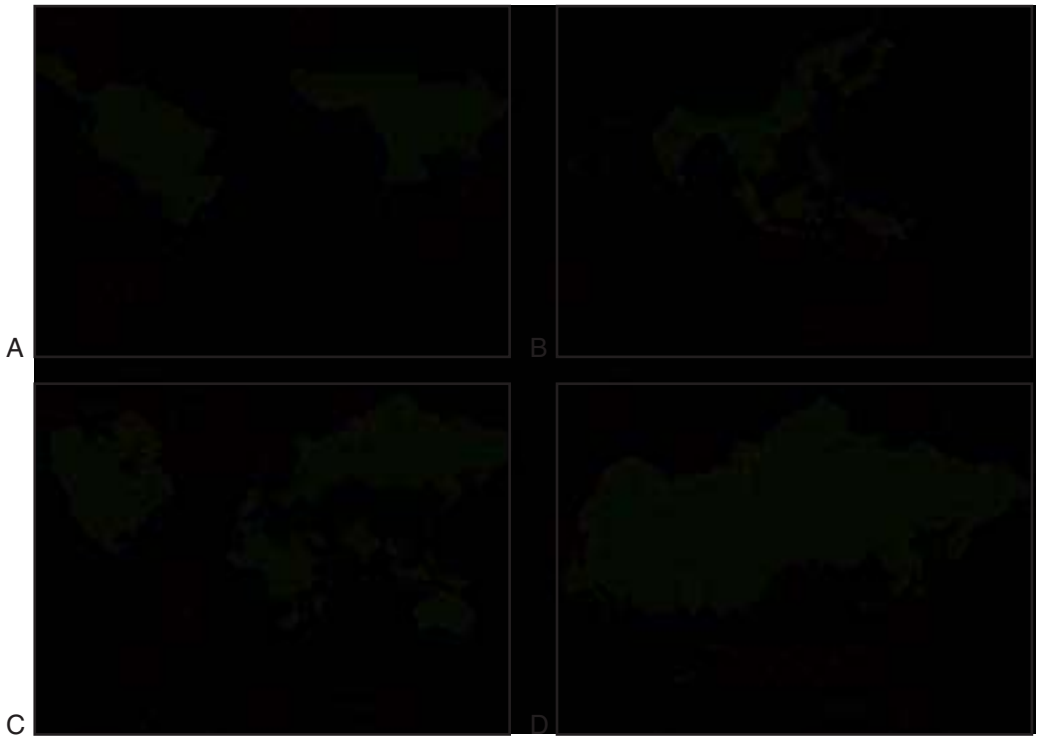

FIG. 153.1 Geographic distribution of medically important flaviviruses. (A) Regions with yellow fever virus transmission. (B) Regions with Japanese encephalitis virus transmission. (C) Countries with West Nile virus transmission. (D) Countries with tick-borne encephalitis transmission.

disease has been noted in Africa and the Middle East, as well as more temperate climates such as the southern parts of the continental United States and Europe.[25–29] DENV transmission and increasing global burden of disease is being fueled by population growth, urbanization, increasingly favorable ecologic and environmental habitats for *Aedes* mosquitoes compounded by poor vector control, and the ease of international travel.[30]

Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) was isolated from a fatal human case in Japan in 1934, but summertime encephalitis outbreaks leading to thousands of cases were described previously and were called *Japanese B encephalitis,* differentiating it from von Economo's encephalitis lethargica, which was known as *type A encephalitis* (the qualifying *B* has fallen into disuse). Elegant studies conducted on the Kanto Plains outside Tokyo, Japan, established the complex ecosystem of JEV transmission among migrating birds, *Culex* mosquitoes, local pig populations, and accidental human hosts.[31–40] The burden of annual epidemics led to the introduction in the 1960s of vaccines that effectively eliminated the disease in Japan, Korea, and Taiwan and reduced its annual incidence in China by 10-fold. Since the 1970s, the incidence of the disease has increased in countries in Southeast Asia, India, Nepal, and Sri Lanka, probably owing in part to changes in agricultural productivity and increased recognition (see Fig. 153.1B). Smaller outbreaks have been reported in the Philippines, Indonesia, and the northern tip of Queensland, Australia.[41–43] Reported human cases vastly underestimate the burden of infection in endemic areas. An estimated 35,000 to 50,000 cases and 10,000 to 15,000 deaths are reported annually, making JEV the most important cause of epidemic viral encephalitis worldwide.[44] JE vaccines are effective, and their routine distribution and use are increasing but are not maintaining pace with transmission. Childhood immunization programs have been established in Thailand and areas of Vietnam, India, and Sri Lanka, but a great deal more remains to be done.

West Nile Virus

West Nile virus (WNV) was isolated from the blood of a febrile woman in the West Nile region of Uganda in 1937, and mosquito transmission between vertebrate hosts (especially birds) was demonstrated soon afterward. Although not associated with neurologic disease at that time, the virus was shown by serologic cross-reactivity to be closely related to the then–recently identified neurotropic viruses, JEV and St. Louis encephalitis (SLE) virus. Later, sporadic cases and larger outbreaks of febrile disease were reported in the Middle East and Africa, in some instances in association with arthralgia and a rash. In the 1950s, meningeal irritation and meningoencephalitis were noted in a few patients in Israel. Outbreaks of equine and human meningoencephalitis occurred in southern France during the 1960s, and a subtype of WNV (Kunjin virus) was isolated in Australasia. Since the 1990s, the epidemiology of WNV has changed, with increasing frequency and severity of outbreaks in southern Europe, Russia, and the Middle East and spread of the virus to the Americas (see Fig. 153.1C). The first outbreak in the Western Hemisphere occurred in the northeastern United States in 1999. The virus rapidly spread across the continent, reaching the Pacific Coast in 2002. During 2002 and again in 2003, more than 2000 cases of CNS disease and 200 deaths were reported from the United States, with additional cases in Canada and enzootic transmission reported in the Caribbean and Central America. Since 2003, the number of cases reported in the United States has decreased, but there are still more than 1000 neurologic cases per year. WNV activity has not been reported in tropical South America, but there was an outbreak in Argentina in equines in 2003.[45] In 2012, the United States experienced the worst outbreak of WNV since its introduction in 1999.[46] As of December 11, 2012, there were 48 states that reported WNV infections in people, birds, or mosquitoes, with a total of 5674 cases of illness in humans, the highest number of WNV cases ever reported in the United States, and 286 deaths. Of the human cases, 80% occurred in 13 states (Texas, California, Louisiana, Illinois, Mississippi, South Dakota, Michigan, Oklahoma, Nebraska, Colorado, Arizona, Ohio, and New York); one-third of all cases were reported from Texas, with an epicenter around the large metropolitan area of Dallas. The US Centers for Disease Control and Prevention (CDC) has reported more than 2000 cases of WNV disease each year from 2013 to 2017. WNV is now the leading cause of mosquito-borne and epidemic encephalitis in the United States.[47]

Usutu Virus

Usutu virus (USUV) is a mosquito-borne flavivirus that was first isolated in South Africa in 1959 and was subsequently identified in a number of African countries (Burkina Faso, Central African Republic, Côte d'Ivoire, Morocco, Nigeria, Senegal, Tunisia, and Uganda). As with many flaviviruses, it was not regarded as a public health problem until it emerged in Austria in 2001, where it caused a large number of fatal infections in birds, especially Euroasian blackbirds (*Turdus merula*); however, retrospective analyses suggest birds died from USUV infection in Italy in 1996. Subsequently, the virus has become endemic in a number of European countries as determined by serologic or reverse-transcriptase polymerase chain reaction (RT-PCR) analyses, or both, of samples from Austria, Belgium, Czech Republic, Croatia, England, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Serbia, Spain, and Switzerland (for a review, see Gaibani and Rossini[48]).

The virus exists in an enzootic cycle involving ornithophilic mosquitoes and birds and is a member the JE serocomplex. The main mosquito vector is *Culex pipiens*. Over 60 avian species have been described as vertebrate hosts from African and European countries, including a number of migratory bird species that are thought to have transported the virus from Africa to Europe. As with other members of the JE serocomplex, humans are dead-end hosts. Phylogenetic studies suggest that there are at least eight genetic lineages of USUV (Africa 1–3 and Europe 1–5), which suggests USUV originated in Africa in the 16th century and emerged in Europe in the middle of the 20th century via multiple introductions.[48,49] The first hypothesized introduction was to Spain in the 1950s, followed by introductions to Austria and Italy in the 1970s, and a further introduction in to Spain at the end of the 20th century.

The incidence of human USUV infections is difficult to estimate, but examination of blood donors in Germany, Austria, and Italy generated seroprevalence rates of 0.02%, 0.058%, and 1.1%, respectively. However, it is clear that awareness of the virus is steadily increasing over time, and this corresponds to increased identification of the virus in birds and clinical cases of USUV infection. To date, 21 human cases of USUV infection have been reported.[48] Clinical symptoms vary from febrile infections to neurologic disorders, including encephalitis and meningoencephalitis. Historically, USUV was shown to be the cause of two human febrile infections in Africa, one in Central African Republic in 1981 and the other in Burkina Faso in 2004.[48,50] The first human cases in Europe were described in Italy in 2009 and involved two human cases of encephalitis in immunocompromised patients.

St. Louis Encephalitis Virus

SLE was first reported as an epidemic of unknown cause in St. Louis, Missouri, in 1933, although outbreaks compatible with SLE had been described from the 1920s. A US Public Health Service investigation identified the viral cause and, on the basis of epidemiologic features, surmised a mosquito-borne mode of transmission, which subsequently was proved by viral isolations from *Culex* spp. mosquitoes in outbreaks in the Yakima Valley, Washington. The occurrence of more than 10,000 cases in more than 50 outbreaks through 1990 revealed the disease to be the most important cause of epidemic viral encephalitis in the United States until WNV became established. In 1975, there were 2900 cases of SLE in 31 states, and more than 200 cases occurred during an epidemic in Florida in 1990. SLE continues to be seen in the United States, particularly among elderly people in the southern states.[51] According to the CDC, there were 6, 19, and 7 neuroinvasive cases reported in 2014, 2015, and 2016, respectively.[51a] Interestingly, there is evidence that WNV is displacing the SLE virus in its ecosystem in California and Texas.[52,53]

Tick-Borne Encephalitis Virus

Descriptions of a disease compatible with TBE appeared in Austria in the early 1930s, although isolation of the virus responsible for this disease (then known as *Central European encephalitis* [CEE]) was not reported until 1948. Investigation of similar cases in the far eastern part of Russia in 1932 had led to descriptions of so-called Russian spring-summer encephalitis (RSSE), and in 1937 the virus was isolated from the blood of patients and from *Ixodes* spp. tick vectors. It is now recognized that three closely related subtypes of TBE virus (TBEV) exist, whose names reflect the geographic areas that they principally affect: European (TBEV-Eu), Siberian (TBEV-Sib), and Far Eastern (TBEV-FE). Across this vast geographic area, the disease has been given a range of different names, including CEE, RSSE, Far Eastern encephalitis, and biphasic milk fever. This last name reflects the transmission of TBEV through ingestion of unpasteurized milk from infected livestock, first confirmed during a 1961 common source epidemic in Czechoslovakia leading to 660 cases.

Disease is found across large geographic areas from Western Europe to Russia, Japan, and China (see Fig. 153.1D). During the past 2 decades, both new endemic foci and an increase in cases have been reported in many European countries.[54,55] The incidence of TBE varies according to location and year. It is estimated that there are 10,000 to 12,000 clinical cases of TBE per year. In Austria, where there had been several hundred cases annually, a formalin-inactivated vaccine was introduced in the 1970s. Administration of a purified form of the vaccine in mass campaigns since the 1980s and in subsequent routine immunization campaigns has led to a dramatic reduction in the number of cases such that the disease has nearly been eliminated from Austria. The TBEV group serocomplex also includes viruses that are rare causes of human neurologic disease (e.g., Powassan virus [POWV], first isolated from a fatal case in Ontario, Canada, in 1958, and louping ill virus, first isolated from a sheep in Scotland in 1930) and viruses that produce a hemorrhagic fever syndrome (e.g., Omsk hemorrhagic fever virus, Alkhurma hemorrhagic fever virus [AHFV], and Kyasanur Forest disease virus [KFDV]). Between 2007 and 2016 the CDC reported 88 cases of neuroinvasive POWV disease, with most cases found in Minnesota, Michigan, and New York.[56]

Alkhurma Hemorrhagic Fever Virus

AHFV was originally isolated from a male butcher who died of an acute febrile hemorrhagic fever in the village of Alkhurma district in Jeddah, Saudi Arabia.[57,58] It was confirmed as a unique flavivirus related to KFDV and believed to have diverged from a common ancestor flavivirus about 700 years ago.[59] AHFV along with KFDV are members of the mammalian tick-borne virus subgroup of the genus *Flavivirus,* family Flaviviridae.[60,61] In 2003 there were 20 confirmed cases in the city of Makkah, which is 75 km from Alkhurma. Cases have been sporadically reported from the southern region of Najran, and since 2008 there has been an increase in the number of reported cases. AHFV appears to be isolated to the Arabian Peninsula and Egypt and associated with the camel ticks: the soft-bodied tick *Ornithodoros savignyi* and the hard-bodied tick *Hyalomma dromedarii.* Seroprevalence in Saudi Arabia appears to be around 1%, and the case-fatality rate appears to be around 10%.[60]

Kyasanur Forest Disease Virus

In 1957, there were reports of an epizootic outbreak of a fatal disease in monkeys located in the forest areas of Shimoga District, Karnataka State, India. Simultaneously there was an outbreak in humans of what was thought to be enteric fever but locally known as "monkey disease," owing to its association with dead monkeys. The virus was isolated from dead langur monkeys and humans and demonstrated to be a new virus named for the locality of its isolation, Kyasanur Forest. KFDV is now known to be a tick-borne flavivirus in the antigenic complex of mammalian tick-borne flaviviruses related to AHFV.

Zika Virus

ZIKV was originally isolated at a YF surveillance station in a caged febrile rhesus monkey located in the Zika Forest, Entebbe, Uganda in 1947 and subsequently isolated from an *Aedes africanus* mosquito in 1948.[62–64] Cross-neutralization tests revealed that this was a unique arbovirus, and a serosurvey demonstrated evidence of human infection.[63,64] ZIKV is now known as a member of the Spondweni virus complex. Spondweni virus was first isolated in 1955 from mosquitoes (*Taeniorhynchus uniformis*) collected in Tongaland, South Africa. The name was derived from the location in which the mosquitoes were collected, the district of Spondweni.[63,64] ZIKV transmission was documented in Southeast Asia and French Polynesia in 2012, and had an

epidemic spread in Central and South America beginning in 2015 (see discussion in the "Epidemiology" section later).

PATHOGENS

Flaviviruses are icosahedral, are about 50 nm in diameter, and consist of a lipid envelope covered densely with surface projections consisting of 180 copies of the M (membrane) and 180 copies of the E (envelope) glycoproteins.[65,66] The latter are organized as dimers, paired horizontally head to tail, on the virion surface. The viruses are unstable in the environment and are sensitive to heat, ultraviolet radiation, disinfectants (including alcohol and iodine), and acid pH. The nucleocapsid joins the capsid (C) protein to a single strand of positive-sense RNA of 11 kb. The viral RNA sequence includes a 10-kb open reading frame that is translated as a single polyprotein precursor, which is cotranslationally and posttranslationally processed to yield 10 proteins. The order of protein gene products from the 5′ end is C, premembrane (preM, a precursor of the mature M protein), E, and a series of seven nonstructural (NS) proteins needed in the viral replicative process: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The open reading frame is flanked by a short (about 100 nucleotides) 5′ noncoding region and a 3′ noncoding region that is variable in length (100–700 nucleotides).

The E protein exhibits important biologic properties, including attachment to host cell receptors, endosomal membrane fusion, and display of sites mediating hemagglutination and viral neutralization.[65] Its carboxyl terminus provides a membrane anchor, and the ectodomain is folded into three structural and corresponding antigenic domains (domains I, II, and III).[67] Domain II is involved in fusion, and domain III is involved in receptor binding. Viral-neutralizing epitopes are found in all three domains; because the protein is folded, they are nonlinear and conformationally dependent. PreM protein chaperones the E protein into the cell secretory pathway, preventing its misfolding, before it is cleaved to its M form in the mature virion. NS1 is expressed on the surface of infected cells and is also secreted as a complement-fixing antigen. Although antibodies to NS1 do not neutralize the virus, they contribute to protective immunity, probably by antibody-dependent cellular cytotoxicity and cell-mediated responses against infected cells.[68] Aside from their replicative functions, NS1, NS2A, NS3, and NS5 display epitopes mediating viral serotype and flavivirus cross-reactive human leukocyte antigen (HLA)–restricted lymphocytic responses.[69–72] Studies suggest that multiple NS proteins, including NS2A, NS2B, NS3, NS4B and NS5, are involved in interferon antagonism.[70,72]

Viral attachment to unidentified cellular receptors is followed by endocytic uptake of virus-containing vesicles. Acidic-induced changes of the viral envelope lead to fusion activity, uncoating of the nucleocapsid, and viral RNA release into the cytoplasm. Glycosaminoglycans and proteoglycans have been implicated as coreceptors in some studies, but proteinaceous receptors are believed also to be involved, and viral binding evidently varies with cell type.[73,74] The viral polyprotein is cotranslationally and posttranslationally processed by passage through the rough endoplasmic reticulum, providing the replicative complex for further viral RNA and protein synthesis and the assembly of nascent virions that mature through the Golgi complex and *trans*-Golgi network. Immature virions collect in the highly proliferated endoplasmic reticulum and secretory vesicles before release, although intracellular nucleocapsid accumulations have been observed in some virus cell systems.

Flaviviruses are adapted to grow in a wide variety of insect, tick, and vertebrate cells and at temperatures spanning the normal temperatures of their arthropod, reptilian, mammalian, and avian hosts. Cytopathologic changes and plaque formation develop in Vero, LLC-MK2 (rhesus monkey kidney), BHK-21 (baby hamster kidney), PS, and primary chick and duck embryo cells, whereas infection of mosquito cell lines (e.g., C6/36, AP61) is typically nondestructive and may persist.

A wide range of vertebrates, including mammals, birds, and reptiles, are naturally infected as amplifying hosts in the transmission cycle of alternating arthropod and vertebrate infection.[1] These infections are usually asymptomatic, but individual viruses may be pathogenic for domesticated or wild animals; for example, several neurotropic flaviviruses, including JEV, SLE virus, WNV, Kunjin virus, TBEV, and POWV, produce encephalitis in horses and certain TBEV strains are neurotropic for dogs, sheep, and goats. JEV is an important cause of swine abortion; louping ill virus is a manifestation of encephalitis in sheep, and YF virus and KFDV are lethal in some monkey species. Laboratory rodents are generally susceptible to neurotropic infection, with sensitivity inversely related to age.

With few exceptions, the flaviviruses can be classified by cross-neutralization assays into eight antigenic groups, of which the most important is the JE complex, which consists of JEV, SLE virus, WNV, USUV, and Murray Valley encephalitis virus; the dengue complex of DENV-1 through -4 viruses; the mammalian tick-borne virus complex, including TBEV, louping ill virus, POWV, KFDV, and Omsk hemorrhagic fever virus; YF virus; and a complex of non–vector-borne rodent- and bat-associated viruses.[1,75] Genetic studies largely support the antigenic classification and suggest the evolution of flaviviruses from viruses found only in insects to mosquito-borne viruses and subsequently non–vector-borne flaviviruses and tick-borne viruses.[76] Genomic sequencing studies of specific viruses have facilitated evolution studies and the tracking of viral movements historically and in epidemics. For example, YF virus strains have been divided into seven genotypes—three in East and Central Africa, two in West Africa, and two in South America—with a close relationship between West African and South American viruses supporting the hypothesis that YF virus was introduced to the Western Hemisphere from Africa.[77] Genotypic markers have been of particular help in understanding the emergence of dengue and JE epidemics in the wake of viral introductions from other regions.[78,79] Genotypes also have been correlated with viral biologic characteristics that underlie their transmission patterns. For example, SLE viral genotypes from the eastern and western United States exhibit distinct neurovirulence and transmission characteristics that are consistent with epidemiologic observations.[80–82] Four DENV types (also known as serotypes) are recognized and correspond to distinct virus species (DENV-1 to DENV-4) based on antigenic and genetic characteristics.[81,82] On a clinical level, structural distinctions between strains associated with classic dengue and with severe dengue (DHF) have been described, providing potential clues to molecular determinants of dengue viral virulence.[81,82]

EPIDEMIOLOGY

Yellow Fever

YF is transmitted in 48 countries in sub-Saharan Africa ($n = 34$) and tropical South America ($n = 14$) (see Fig. 153.1A). The disease has never been documented in Asia (although 11 infected travelers from Angola in 2016 had clinical disease in China, this did not lead to outbreaks in China), but, in principle, anthroponotic (vector-borne person-to-person) transmission of the virus could occur there and in other *A. aegypti*–infested locations, including the southern United States.[6,83–85] Epidemic ("urban") YF is transmitted by *A. aegypti* mosquitoes; the mosquitoes are infected after feeding on viremic humans and then spread the infection in subsequent feeding attempts. The threat of epidemic transmission arises when a person with a forest-acquired infection travels to an *A. aegypti*–infested location while viremic.

The fluctuating incidence of YF has been dominated by epidemics in Africa, with 90% of the 130,000 annual cases occurring there.[84,86] However, official reports considerably underestimate the true magnitude of those epidemics, which field studies estimated as 50-fold greater.[84–88] Epidemic attack rates ranged as high as 30 in 1000 persons, with case-fatality ratios of 20% to 50%. Since 1990, epidemics have been reported in a number of previously unreported areas, including Niger (1990), Kenya (1992), Gabon (1994), Central African Republic (2008), Cameroon (2012), and Sudan (2012), demonstrating why YF is classified as a reemerging disease. The variable size and frequency of epidemics in recent years may reflect cyclic changes in viral activity and in human immunity, acquired in recent epidemics and through vaccination in emergency campaigns and the WHO Expanded Program on Immunization. Over the last 15 years, a combination of mass vaccination campaigns followed by routine immunization have led to substantial reduction in cases of YF in West Africa, with no outbreaks reported in 2015, but the disease has resurged since then, including a large outbreak in Angola and the Democratic Republic of the Congo (DRC) in 2016. Currently, plans are underway to expand vaccination campaigns to other African countries and immunize a further 1.3 billion individuals through 2026 via the Eliminate Yellow Fever Epidemics (EYE) initiative.

In South America, an annual mean of about 100 cases has been reported in the past 25 years, reflecting the occurrence of forest-acquired infections in the greater Magdalena, Orinoco, and Amazon River basins.[89,90] However, as in Africa, YF has emerged in new areas in Brazil, with outbreaks spreading to Sao Paulo and Rio de Janeiro States for the first time in 2016 to 2018.[89,90] In its *jungle cycle,* the virus is transmitted from tree-hole *Haemagogus* and *Sabethes* spp. mosquitoes to forest monkeys in wandering epizootics that follow movements of the animals and of the virus to susceptible populations. Cases in humans predominate between January and March among men 15 to 45 years of age who are bitten incidentally by infected mosquitoes while employed as agricultural and forestry workers, soldiers, and settlers. Recent outbreaks frequently have occurred among nonimmune migrants from their coastal or Andean homes to at-risk locations in the tropical zone. An intensification of enzootic viral transmission frequently produces clusters of monkey deaths, indicating an increased transmission risk to humans. The last urban outbreak in the Western Hemisphere took place in Paraguay in 2008 after a period of 54 years since the penultimate urban outbreak in Trinidad in 1954. The growth of urban areas and their reinfestation by *A. aegypti* have renewed concern for the emergence of epidemics, especially in cities that border forested areas, which was exemplified by the outbreak in Paraguay in 2008. Since 1995, several patients with sylvatic disease have been hospitalized in Brazilian cities and in Santa Cruz, Bolivia, fortunately without urban spread. The imminent threat of epidemic transmission has prompted mass vaccination campaigns and other control measures, which have been very successful at controlling the disease.[89–91] In the moist savanna of Africa, a variety of tree-hole–breeding mosquitoes transmits infections among humans and monkeys during the end of the rainy season, leading to early infections in children and sporadic cases that are typically unrecognized. Infections are spread readily by migration, with the potential for involvement of *A. aegypti* in urban areas and in dry locations where stored water provides breeding sites. During the dry season, the virus survives in infected mosquito eggs that are resistant to desiccation.

Dengue

The four DENV types share similar mechanisms and patterns of transmission, with relatively small differences in the frequencies of different clinical manifestations.[92] They are transmitted in the tropics, in an area roughly between 35° north and 35° south latitude, corresponding to the distribution of *A. aegypti,* the principal mosquito vector, though this distribution is enlarging with global warming (Fig. 153.2).[93] *Aedes albopictus, Aedes polynesiensis,* and other species are also capable of transmitting the DENVs. Although enzootic transmission among forest monkeys in Asia and Africa has been described, anthroponotic viral transmission is sufficient to maintain the virus, and these animal infections could represent either epiphenomena or evidence for spread from sylvatic to endemic cycles.[94] The intensification of dengue transmission in tropical cities, where growing populations live under crowded conditions, can be understood in view of the close relationship of *A. aegypti* to humans.[95] After the female mosquito feeds on a viremic person, viral replication and dissemination must take place in the mosquito before it can transmit the virus on subsequent feeding attempts; this extrinsic incubation period is typically 1 to 2 weeks. Feeding attempts may occur several times a day over the insect's lifetime of 1 to 4 weeks. Mechanical transmission, without extrinsic incubation, has also been suggested. *A. aegypti* is adapted to breed around human dwellings, where the insects oviposit in uncovered water storage containers as well as miscellaneous containers holding water, such as vases, flower dishes, cans, automobile tires, and other discarded objects. Adult mosquitoes shelter indoors and bite during 1- to 2-hour intervals in the morning and late afternoon. In areas with endemic transmission, 1 of every 20 houses may contain an infected mosquito.[96,97] Cases often cluster in households, and human movements and the mosquito's peregrinations within a range of 800 m rapidly spread the infection.[98–103] DENV transmission between mosquitoes and humans appears closely linked to weather conditions in different regions, specifically rainfall, humidity, and temperature variation.[100,102,103]

When the virus is introduced into susceptible populations, usually by viremic travelers, outbreaks with significant infection and clinical attack rates are possible. Because cross-protective immunity among the four DENV types is limited, epidemic transmission recurs with the introduction of novel DENV types. Furthermore, because secondary infections predispose to severe disease (discussed in the "Pathogenesis" section later), the concurrent transmission of multiple DENV types establishes the conditions for endemic DHF. Under these circumstances, most DHF cases occur in individuals with secondary infections, with the relative risk for developing DHF, with shock in secondary compared with primary infection being as high as 100.[104–107]

DHF during primary DENV infection in the first year of life represents a special case. In dengue-endemic areas, infants passively acquire DENV-specific antibodies from their DENV-immune mothers, and the age distribution of DHF parallels the expected decline of maternal antibodies, a phenomenon taken to support a central role for antibodies in causing more severe disease.[108–111]

As noted earlier, intensified DENV transmission and epidemics of DHF evolved in Southeast Asia after World War II. Today, Asia and the Western Pacific bear nearly 75% of the global dengue disease burden, with the highest incidence during childhood.[112] In Thailand, for example, DENV infection rates have been documented in the range of 5% to 10% of schoolchildren per year, with DHF incidence rates of 10 to 300 per 100,000 persons per year, and dengue accounts for one-third of acute febrile illnesses in children seeking medical attention.[113,114] Although DHF still is principally a disease of children younger than 15 years of



FIG. 153.2 Approximate actual and potential distribution of *Aedes aegypti.* The band between the 10°C isotherms represents potential distribution. *(From World Health Organization.* Technical Guide for Diagnosis, Treatment, Surveillance, Prevention, and Control of Dengue Haemorrhagic Fever. *2nd ed. Geneva: World Health Organization; 1997.)*

age in hyperendemic areas, the peak age of risk has risen in recent years as DENV transmission has declined due to increased use of screens and air-conditioning and lowering of birth and death rates.[115–118] In contrast, dengue occurs in all age groups when relatively newly introduced to an area or reintroduced after a significant interval, and severe disease may occur only in previously immunologically primed adults.[119]

The dissemination of DENVs by viremic travelers has been facilitated by the increased mobility of people living within endemic areas and internationally by burgeoning air travel.[115–118] In several studies, DENV infection was documented in 7% to 45% of febrile returned travelers.[120–122] Small numbers of autochthonous cases acquired in Texas towns bordering Mexico were recognized in 1980, 1986, 1995, 2005, and 2013.[123,124]

Infection can be transmitted by accidental needlestick.[125–127] The high incidence of infection in endemic areas suggests that transfusion-associated DENV transmission may be a risk. Despite this being an infrequent occurrence, guidelines for blood product donation have been developed.[125–127]

The continental United States has regularly experienced autochthonous DENV transmission and occasional small outbreaks in Texas, along its border with Mexico.[128] In Key West, Florida, expansion of DENV has continued into previously DENV-free areas with establishment of a vector population.[129] Sequencing of DENV-1 strains isolated in Key West, Monroe County, Florida, indicated that the viruses were undergoing endemic transmission for more than 2 years before being recognized.[26] Southern areas of Europe have also experienced periodic DENV introduction and local transmission.[27,29,125]

Japanese Encephalitis

JE is transmitted in Asia over an area spanning one-third of the world's circumference, from Pakistan at the westernmost edge to far eastern Russia (Table 153.1; see Fig. 153.1B). The disease is endemic and periodically epidemic in Southeast Asia, China, and the Asian subcontinent.[130] During 2005 and 2006, there were large outbreaks in Northern India that caused several thousand deaths.[131] Sporadic cases are reported in

TABLE 153.1 Estimated Risk for Japanese Encephalitis by Country and Season

COUNTRY	AFFECTED AREAS	TRANSMISSION SEASON	COMMENTS
Australia[b]	Islands of Torres Strait	February–April peak; year-round transmission risk	Localized outbreak in Torres Strait in 1995 and sporadic cases in 1998 in Torres Strait and on mainland Australia at Cape York Peninsula
Bangladesh	Few data, but probably widespread	Possibly July–December, as in northern India	Outbreak reported from Tangail District, Dacca Division; sporadic cases in Rajshahi Division
Bhutan	No data	No data	—
Brunei	Presumed to be sporadic-endemic, as in Malaysia	Presumed year-round transmission	—
Cambodia	Endemic-hyperendemic countrywide	Presumed to be May–October	Cases in refugee camps on Thai border and from Phnom Penh
Democratic Republic of Korea	Presumed to be countrywide in rural areas <800 m altitude	July–October	Epidemics in 1970s; few recent data
India	Reported cases from all states except Arunachal, Dadra, Daman, Diu, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Orissa, Punjab, Rajasthan, and Sikkim	South India: May–October in Goa, October–January in Tamil Nadu, August–December in Karnataka Second peak: April–June in Mandya District Andhra Pradesh: September–December[b] North India: July–December	Outbreaks in West Bengal, Bihar, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Assam, Uttar Pradesh, Manipur, and Goa; urban cases reported (e.g., in Luchnow)
Indonesia	Kalimantan, Bali, Nusa, Tenggara, Sulawesi, Moluccas, Irian Jaya, and Lombok	Probably year-round risk; varies by island; peak risks associated with rainfall, rice cultivation, and presence of pigs Peak periods of risk: November–March, June and July in some years	Endemic on Bali, Java, and possibly in Lombok; sporadic cases recognized elsewhere
Japan[b]	Rare; sporadic cases on all islands except Hokkaido	June–September, except April–December on Ryuku Islands (Okinawa)	Vaccination not routinely recommended for travel to Tokyo and other major cities; enzootic transmission without human cases observed on Hokkaido
Laos	Presumed to be endemic-hyperendemic countrywide	Presumed to be May–October	—
Malaysia	Sporadic-endemic in all states of Peninsula, Sarawak, and probably Sabah	Year-round transmission; October–February in Sarawak	Most cases from Penang, Perak, Selangor, Johor, and Sarawak
Myanmar	Presumed to be endemic-hyperendemic countrywide	Presumed to be May–October	Repeated outbreaks in Shan State in Chiang Mai Valley
Nepal	Hyperendemic in southern lowlands (Terai); sporadic cases in Kathmandu Valley	July–December	Vaccination not recommended for travelers visiting only high-altitude areas
Pakistan	May be transmitted in central deltas	Presumed to be June–January	Cases reported near Karachi; endemic areas overlap those for West Nile virus; lower Indus Valley might be an endemic area
Papua New Guinea	Normanby Islands and Western Province	Probably year-round risk	Localized sporadic cases
People's Republic of China[b]	Cases in all provinces except Xizang (Tibet), Xinjiang, Qinghai Temperate areas: endemic to periodically epidemic Southern China: hyperendemic Hong Kong: rare cases in new territories	Northern China: May–September Southern China: April–October (Guangxi, Yunnan, Guangdong, and southern Fujian; Sichuan, Guizhou, Hunan, and Jiangxi provinces) Hong Kong: April–October	Vaccination not routinely recommended for travelers to urban areas only

Continued

TABLE 153.1 Estimated Risk for Japanese Encephalitis by Country and Season[a]—cont'd

COUNTRY	AFFECTED AREAS	TRANSMISSION SEASON	COMMENTS
Philippines	Presumed to be endemic on all islands	Uncertain; speculations based on locations and agro-ecosystems West Luzon, Mindoro, Negros, Palawan: April–November Elsewhere: year-round, with greatest risk April–January	Outbreaks described in Nueva Ecija, Luzon (including January 2004), and Manila
Republic of Korea[b]	Sporadic-endemic with occasional outbreaks	July–October	Last major outbreaks were in 1982–1983
Russia	Far eastern maritime areas south of Khabarovsk	Peak period July–September	Sporadic transmission in rural and sylvatic cycles
Singapore	Higher rates of enzootic transmission in western rural areas of island	Year-round transmission with April peak	Vaccination not routinely recommended; two sporadic cases in 2001
Sri Lanka	Endemic in all but mountainous areas; periodically epidemic in northern and central provinces	October–January; secondary peak of enzootic transmission in May–June	Outbreaks in central (Anuradhapura) and northwestern provinces
Taiwan[b]	Endemic, sporadic cases island-wide	April–October; June peak	Cases reported in and around Taipei and the Kaohsiung-Pingtung River Basins
Thailand[b]	Hyperendemic in north; sporadic-endemic in south	May–October	Annual outbreaks in Chiang Mai Valley; sporadic cases in Bangkok suburbs
Vietnam[b]	Endemic-hyperendemic in all provinces	May–October in the North, year-round in the South	Highest rates in and near Hanoi
Western Pacific	Two epidemics reported in Guam and Saipan since 1947	Uncertain; possibly September–January	Enzootic cycle may not be sustainable; epidemics have occurred after introductions of virus

[a]Assessments are based on publications, surveillance reports, and personal communications. Extrapolations have been made from available data. Transmission patterns may change.
[b]Locally reported incidence rates may not reflect risks to nonimmune visitors because high immunization rates in local populations may obscure ongoing enzootic transmission.
Modified from references 2 and 99 and updated from ProMED Mail (http://www.promedmail.org) and after the Global Alliance for Vaccines and Immunizations, Southeast Asia and Western Pacific Regional Working Group's Japanese Encephalitis Meeting: Setting the Global Agenda on Public Health Solutions and National Needs, Bangkok, Thailand, 2002.

tropical Asia, including the Indonesian and Philippine archipelagoes, but field studies suggest a higher incidence.[132,133] Twice, in 1947 and 1990, the virus was introduced to the western Pacific, resulting in outbreaks on Guam and Saipan. The virus invaded the Torres Strait Islands of Australia in 1995 and the Australian mainland in 1998.[79,134]

Worldwide, 160,000 cases of JE were reported to the WHO in 1966 and 16,000 cases in 1996, the 10-fold decline reflecting widespread childhood immunization in China, Japan, Korea, and Taiwan as well as regional economic development and the declining emphasis on agriculture. In the latter three countries, few cases are reported currently, although enzootic viral transmission persists. In areas with endemic transmission, an annual incidence of 2.5 per 10,000 children younger than age 15 years is estimated, with a case-fatality rate of 25% and disability in 45% of surviving patients.[6,130] Extrapolating this incidence to the population of 700 million children younger than age 15 years in the region, an estimated 175,000 JE cases, 45,000 deaths, and 78,000 cases of newly disabled children would occur annually in the absence of immunization.[132] Allowing for the countries where there is immunization, the expected number of cases annually is greater than 125,000. The fact that only one-fifth of these are officially recorded probably reflects the lack of reporting from many countries where no surveillance currently exists.[132] In an era in which polio has declined to the point of eradication, JE is now preeminent among causes of pediatric CNS infections in the region. The introduction of new WHO surveillance guidelines in many Asian countries should improve detection.[135]

Within temperate areas, JE is transmitted sporadically from July to September at a relatively low incidence and with periodic seasonal epidemics. In subtropical Asia, viral transmission extends from March to October in a hyperendemic pattern, resulting in cases throughout the year and the absence of easily detected seasonal epidemics. The geographic distribution of different JEV genotypes was postulated to explain the differences in clinical epidemiology, but it is now believed to be best explained by the virus's evolution in the Indonesia-Malaysia region and its subsequent spread as more recently evolved genotypes.[134,136]

The virus is transmitted by *Culex tritaeniorhynchus* and related ground- and pool-breeding mosquitoes to pigs and aquatic birds, which are the principal viral-amplifying hosts.[137] Viremic adult pigs are asymptomatic, but infected pregnant sows abort or deliver stillbirths. Infected horses and humans are symptomatic but incidental hosts. Because rice paddies provide favorable breeding habitats for vector mosquitoes, the risk for infection is highest in rural areas. However, both pigs and rice paddies are found at the edges of some Asian cities, resulting in isolated cases and, rarely, urban outbreaks. The mosquito vectors chiefly feed outdoors, in the evenings, and prefer animal to human hosts.

More than 99% of infections with JEV are subclinical; consequently, in areas with endemic transmission, infections acquired naturally at an early age result in immunity in more than 80% of young adults. Cases occur chiefly in children between 2 and 10 years of age, with a slight predominance of boys. In Japan, Korea, and Taiwan, children are protected by immunizations and cases occur principally in elderly people, reflecting waning immunity or other biologic factors associated with senescence.[138]

Expatriate and traveler cases have been recognized since 1932, and outbreaks among American, British, and Australian soldiers in World War II, the Korean War, and the Vietnam war were considered militarily important. Travelers of all ages without naturally acquired protective antibodies are at risk for acquisition of the illness. The risk is slight, estimated to be 1 in 150,000 person-months of exposure, reflecting low vector mosquito infection rates (0.5%) and the small case-to-infection ratio (0.3%).[139] However, cases of JE among travelers, even on short trips to Southeast Asia, serve as a reminder of the unpredictable risk for acquiring this disease.[140–142]

West Nile Encephalitis

WNV is one of the most widely distributed arboviruses, being found across much of Africa, southern Europe, the Middle East, Asia, Australia (Kunjin subtype), and, more recently, the Americas.[143,144] Recent outbreaks have included almost 400 confirmed cases in Romania in 1996, almost 200 cases in the Volgograd region of Russia in 1999, and more than 200 cases in Israel in 2000 (Table 153.2).[144] In 1999, the virus appeared in North America for the first time in the New York area, with 62

TABLE 153.2 Outbreaks of West Nile Virus Infection

YEAR OF OUTBREAK	COUNTRY	NO. OF SUSPECTED CASES	NO. OF CASES INVESTIGATED	NO. OF CONFIRMED CASES	NO. OF DEATHS	NOTES
1957	Israel	419	247	About 180	4	Included first naturally occurring encephalitis cases (12 patients)
1974	South Africa	18,000	558	307	0	Estimated 18,000 cases of WN fever
1962–1966	France (Camargue)	—	—	14	1	Many horses also affected
1994	Algeria	50	18	17	8	—
1996	Romania	835	509	393	17	Continuing cases in 1997–1999
1997	Tunisia	173	129	111	8	—
1998	Democratic Republic of Congo	35	35	23	0	Military personnel newly arrived in this area
1999	USA (New York)	719	719	62	7	—
1999	Russia (Volgograd region)	826	318	183	40	—
2000	Israel	—	—	233	33	91 nonhospitalized patients with WN fever also identified
2000–2001	USA	—	—	85	24	—
2002	USA	—	—	4156	284	2956 with CNS disease
2003	USA	—	—	9862	264	2866 with CNS disease
2004	USA	—	—	2539	100	1142 with CNS disease
2005	USA	—	—	3000	119	1294 with CNS disease
2006	USA	—	—	4269	177	1459 with CNS disease
2007	USA	—	—	3630	124	1217 with CNS disease
2012	USA	—	—	5674	286	2873 with CNS disease
2013	USA	—	—	2469	119	1267 with CNS disease
2014	USA	—	—	2205	97	1347 with CNS disease
2015	USA	—	—	2175	146	1455 with CNS disease
2016	USA	—	—	2149	106	1309 with CNS disease

[a]Only details of selected outbreaks are shown. Criteria for hospitalization, case definitions, and diagnostic methods varied among outbreaks, and some numbers are approximations.
CNS, Central nervous system; *WN,* West Nile.

confirmed human cases and 25 equine cases. The virus rapidly established itself in North America with a peak in 2003 of nearly 10,000 clinical cases, including 286 cases of neurologic disease. Since 2004, the number of cases has varied from 1300 to 2900 neurologic cases per year and there has been a consistent mortality rate of 10% among neurologic cases. As with JEV, many of the survivors of WNV neurologic infection suffer from neuropsychiatric impairment that may last as long as 3 to 4 years. Surprisingly, there are few reports of human disease south of the United States, with a few equine cases reported in Argentina.

WNV is transmitted in an enzootic cycle between birds by mosquitoes (Fig. 153.3). Recent studies in the United States have demonstrated infection in at least 300 different bird species and 62 mosquito species. In addition, at least 30 vertebrate species are infected, but they have insufficient viremia to infect a feeding mosquito and are considered "dead-end" hosts. Some, however, may have clinical infection (e.g., in humans). Members of the order Passeriformes (jays, blackbirds, finches, warblers, sparrows, and crows) appear to be important in transmission of the virus in nature; the family Corvidae (crows and blue jays) is particularly susceptible. In some areas, arrival of the virus was heralded by dying birds falling from the sky. The lack of resistance and fatal infection of avian intermediary hosts provided evidence for the novel introduction of the virus to the ecosystem of the Western Hemisphere, unlike the asymptomatic infection of autochthonous birds by the closely related SLE virus. Of the many mosquito species from which WNV has been isolated, *Culex* spp. appears to be important in the enzootic cycle, although the species varies by geographic location. Transovarial transmission of the virus in mosquitoes probably provides for viral overwintering. During the 2002 outbreak in the United States, it became clear that on rare occasions viral transmission could occur by transplantation of infected organs, from infected blood products, transplacentally, and, possibly, through breast milk.[145,146] During the height of the 2002 epidemic, the risk for infection by transfusion was estimated to be as high as 21 per 10,000 donations. Blood screening using real-time PCR assay has been instituted and has dramatically decreased the risk for contaminated blood.[145,146]

The means by which WNV is introduced to new areas are not completely understood. Migratory birds are thought to be important for movement of the virus from Africa into southern Europe. They may have been involved in its introduction into North America, although importation of viremic exotic birds or amphibians and inadvertent transport of mosquitoes are alternative explanations.[147] Molecular genetic evidence suggests a single introduction into the United States of a strain, possibly from the Middle East.[148]

Most human infections with WNV are asymptomatic. Epidemiologic surveys taken after the 1999 outbreak in New York suggested that about 1 in 5 people infected with WNV develops fever and that only about 1 in 150 develops CNS disease.[145,146] These rates are similar to the attack rates for the Romanian 1997 outbreak, but they appear to be much higher than those reported from Egypt and South Africa, most likely because of preexisting antibodies in people in Africa.[149–151] In New York, Romania, and Israel, the risk for neurologic disease increased with age, which may explain, in part, the different epidemiologic patterns seen in parts of Africa. In Egypt, most of the population is infected during childhood and neurologic disease is rare.[150] In South Africa, a large outbreak affected an estimated 18,000 people of all ages, yet only a single West Nile encephalitis (WNE) case was reported.[151]

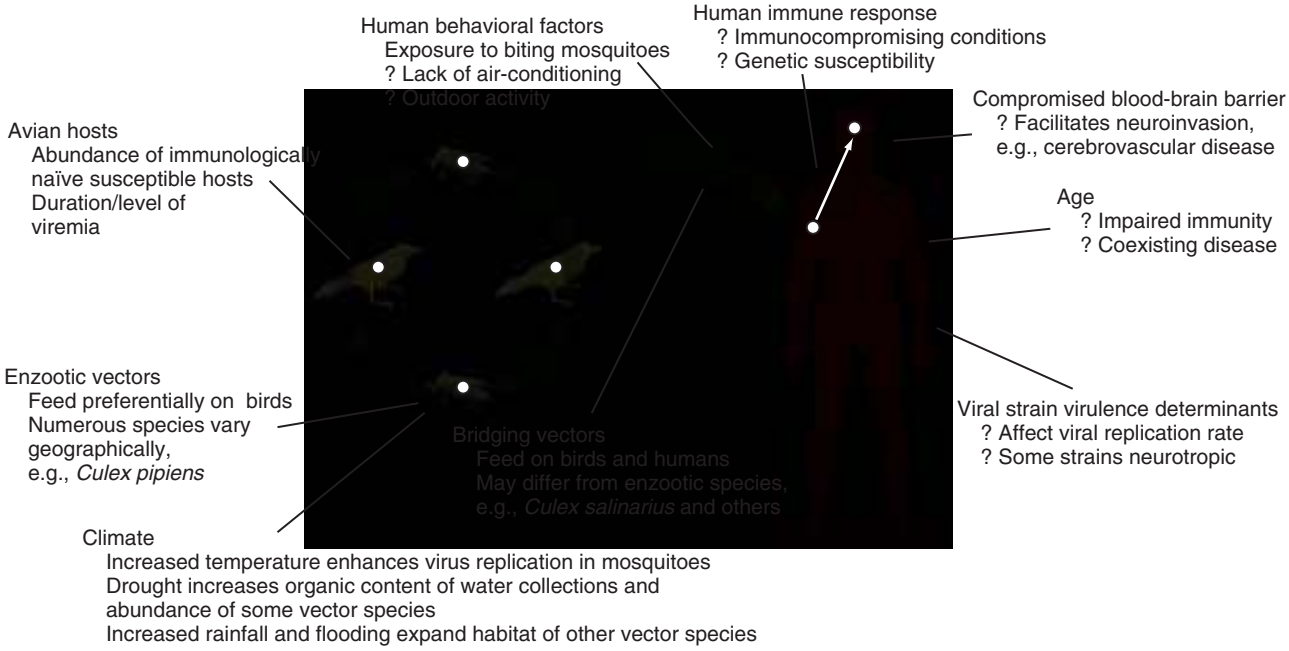

FIG. 153.3 **West Nile virus transmission cycle as an exemplar of mosquito-avian-virus transmission cycle and the ecologic, virologic, vector, and human factors impacting exposure, infection, and infection outcomes.**

St. Louis Encephalitis

Outbreak-associated cases of SLE have been reported from virtually all US states, the provinces of Ontario and Manitoba in Canada, and Sonora State in Mexico, whereas only sporadic cases have been reported from Argentina, Brazil, Panama, Trinidad, French Guiana, Surinam, Curaçao, Jamaica, and the Dominican Republic. Enzootic viral transmission has also been recognized in Alberta and British Columbia in Canada and in Ecuador, Guatemala, and Haiti and may occur elsewhere in the hemisphere.[152,153]

In the United States, the virus is transmitted to birds in three distinct cycles overlapping those of WNV: by *Culex pipiens* and *Culex quinquefasciatus* in the Midwestern and Eastern states, by *Culex nigripalpus* in Florida, and by *Culex tarsalis* in the Great Plains and farther west. Humans are infected incidentally from the enzootic cycle and, as with WNV infection, humans are dead-end hosts. Characteristics of the vectors and their respective transmission cycles define epidemiologic features in each location.

In the eastern states, SLE is transmitted periodically in localized and regional outbreaks at lengthy intervals without significant transmission in intervening years. Outbreaks in the late summer and fall occur in urban areas, often in older neighborhoods, where polluted wastewater provides a breeding habitat for *C. pipiens* and *C. quinquefasciatus,* the northern and southern house mosquitoes, respectively. More than 50 epidemics, ranging in the hundreds of cases, have been recognized in small towns or cities, including Houston, Dallas, Memphis, New Orleans, Chicago, and Detroit. The largest epidemic, in 1975, led to more than 3000 cases of neurologic infection nationally, similar in scale and geographic location to recent WNE outbreaks. In three outbreaks since 1991, disproportionate risk was reported among homeless persons infected with the human immunodeficiency virus (HIV), probably reflecting their increased vulnerability to mosquito bites in the evening, when the vectors are most active.[154] Between 1992 and 2000, much smaller outbreaks and sporadic cases occurred (median, 14 cases; range, 2–26 cases annually). The 2001 outbreak in Louisiana produced 71 cases, and was a reminder of the continued enzootic transmission and epidemic potential of this virus.[155]

Outbreaks in Florida occur more diffusely in suburban and urban locations, where swales and ground pools provide breeding sites for C. *nigripalpus.*[156] In the western states, SLE is transmitted perennially and at a low level in rural areas, frequently in association with irrigated farms and pastures. Forty years ago, outbreaks in agricultural areas occurred at regular intervals; more recently, cases have been more sporadic and frequently have involved vocational exposures or have occurred in proximity to cities. Small urban outbreaks have also occurred. The decline in cases has been attributed to secular changes in land use, air-conditioning of residences, and other factors leading to reduced exposure. Diminished exposure to infection has been confirmed in rural California populations, in whom seroprevalence rates now range from 0.1 to 11%.[153]

The risk for illness is associated most strongly with advanced age, but a slightly elevated risk is also seen in infants. The importance of age is reflected in the declining ratio of asymptomatic to symptomatic infection, which ranges from 800:1 in children to 85:1 in adults older than age 60 years.[157] Between 1999 and 2007, 75% of all patients were older than age 40 years, with an increased risk in males and in blacks.[51]

Tick-Borne Encephalitis

TBEV is classified as one species within the mammalian group of tick-borne flaviviruses and is further subdivided into three subtypes: Far Eastern (TBEV-FE; previously RSSE), Siberian (TBEV-Sib; previously west-Siberian), and European (TBEV-Eu; previously CEE). There is evidence that these three virus subtypes have overlapping geographic areas.

The three subtypes of TBEV, as well as other related mammalian tick-borne complex viruses, are transmitted across the Holarctic, with some evidence for their dissemination from an Asian source.[76,158] The TBEV-FE subtype is transmitted in eastern Russia, Korea, China, and parts of Japan and the Baltic states; the TBEV-Eu subtype and related viral strains are found in Scandinavia, Europe, and eastern states of the former Soviet Union; and the TBEV-Sib subtype is found in all TBE-endemic areas of Russia (see Fig. 153.1D). The geographic distributions overlap in Eastern Europe, where both the TBEV-Sib and TBEV-FE subtypes may be isolated.[54] Louping ill virus is found in the British Isles, and POWV is found in North America and northern Asia. Closely related tick-borne flaviviruses include Turkish and Spanish sheep encephalitis viruses, which are found in southern Europe, and three viruses that cause hemorrhagic fever—KFDV in India, AHFV (which is a subtype of KFDV) in Saudi Arabia and Egypt, and Omsk hemorrhagic fever virus in Siberia.[59,159,160]

Surveillance systems for TBE exist in 20 of the 30 European Union and European Free Trade Association countries. TBE is recognized throughout Europe, except in Portugal and the Benelux countries, but

endemic transmission is most intense in Austria, areas of Sweden, Switzerland, Slovenia, the Czech Republic, Estonia, Lithuania, Latvia, and Russia.[159,160] From 2000 to 2010, the overall number of reported TBE cases demonstrated increases in 2003, 2006, 2009 and 2010, with the Czech Republic reporting 25% of all cases, Lithuania 15%, Latvia 11%, Germany 11%, and Slovenia 10%. Incidence rates in unvaccinated populations have approached 20 in 100,000 persons. In Austria, national vaccination programs have reduced the incidence of disease to fewer than 1 in 1 million.[159–161] Sporadic cases are reported from France, Liechtenstein, Sweden, Switzerland, Italy, and Greece. In the Far East, TBE cases occur principally among people working or living in sylvatic locations in Russia, Korea, northern China, and Hokkaido Island, Japan. New models based on environmental factors and satellite data suggest that climate change is partially responsible for the increased incidence in Europe.[162] Longitudinal studies show the virus now circulates at greater altitudes in the Czech Republic and Switzerland.

The viruses are transmitted horizontally between ticks and vertebrates and through the winter by vertical transmission in the ticks and latent infections in hibernating animals. The virus passes transovarially and transstadially, from egg to larva, nymph, and adult, so all stages of the tick and both male and female ticks transmit infections to animals and humans. In addition, it appears that virus may be transmitted between ticks, as they feed on the skin of the same host, through infected host reticuloendothelial and inflammatory cells, without the need for host viremia.[163] Larval and nymphal ticks feed principally on birds and small mammals, and adult ticks on larger mammals such as roe deer, deer, domestic goats, sheep, cows, dogs, cats, and humans. Human infections are incidental to the transmission cycle. Animal movements can spread ticks and the virus to new foci.

Within the ranges of *Ixodes ricinus* and *Ixodes persulcatus* (the principal tick vectors of the TBEV-Eu and the TBEV-FE plus TBEV-Sib subtypes, respectively) and additionally *Ixodes ovatus* and *Haemaphysalis* spp. (vectors in Japan and Korea, respectively), the ticks are distributed focally in sheltered microhabitats with high humidity and moderate temperatures and can be found at elevations up to 2100 m. Landscape ecology studies have characterized forest ecotones to fields or meadows and low stands of deciduous trees and brush with a thick canopy as high-risk biotopes that correlate with foci of human cases.[164] Transmission foci tend to be highly stable but are subject to human environmental modifications and possibly climate change.[165] In central Europe, cases occur from April until November, peaking in June and July, with a secondary rise in October. Climate change has produced a dramatic change in the distribution of *Ixodes* spp. tick vectors and TBEV transmission.[166–169]

Cases occur mainly in adults 20 to 50 years of age, with a male predominance, reflecting occupational exposure in forestry and farming. Nevertheless, children at outdoor play and persons with vocational exposure while hiking, berry picking, or mushroom gathering also may be at risk, depending on the location and season. However, the risk for most persons with short-term exposures is low. Among American soldiers stationed in central Europe, no cases and a low rate of seroconversion (0.1%–0.4%) have been reported.[170] Louping ill is principally a rare occupational disease of veterinarians, sheepherders, and butchers.[171]

TBEV is stable at acid pH, and consumption of unpasteurized milk or milk products from infected goats, sheep, or cows previously accounted for 10% to 20% of cases in some parts of central Europe. The possibility that POWV can be transmitted from raw milk products in the United States has been suggested.[172] Slaughter or butchering of infected animals or meat is a principal mode of transmission for louping ill virus to humans and also has been reported in TBEV and in outbreaks of AHFV.[173] Infection has also been acquired from infected ticks carried to households on fomites.

In addition to TBEV, *I. ricinus* also transmits several *Borrelia* spp. responsible for Lyme disease (as well as *Anaplasma phagocytophilum, Babesia microti,* and several species of *Rickettsia*), and dual infections of ticks and humans are observed. However, in at-risk areas, Lyme disease is far more common than the other diseases. This difference reflects the low proportion of virus-infected ticks (0.1%–5%) and the 10-fold higher borrelial infection rates of ticks in the same location. This distinction may result from the brevity of viremia in animal hosts, which provides an opportunity for tick infection of only a few days; in contrast, persistent borrelial infections of rodents offer a higher likelihood of tick infection during feeding. An analogous situation occurs in the United States, where *Ixodes scapularis* transmits Lyme disease, babesiosis, human anaplasmosis, and a genotype of POWV represented by deer tick virus.[174] However, *Ixodes cookei* ticks (the principal vector of POWV) and *I. scapularis* differ somewhat in their host range, which may further limit opportunities for the viral and borrelial transmission cycles to intersect. The main enzootic cycles for *I. cookei* involve woodchucks and for *Ixodes marxi* involve squirrels (POWV lineage 1). For *I. scapularis,* they involve deer (POWV lineage 2).[55,56]

Alkhurma Hemorrhagic Fever

Since its original isolation in 1995, Alkhurma hemorrhagic fever has occurred in sporadic outbreaks in Saudi Arabia, usually in limited numbers and characterized by a mortality that ranged from 1.3% to 25%.[57] From August 2003 to December 2009, there were 148 suspected cases of Alkhurma hemorrhagic fever in Najran, Saudi Arabia, of which 78 (53%) were laboratory confirmed. There were 2 cases in 2003, 1 in 2004, 4 in 2005, 12 in 2008, and 58 cases in 2009.[57] Cases occurred year-round, although more than half occurred during the summer months. Transmission is thought to be tick-borne and occurs as a zoonotic disease in domestic livestock, with outbreaks associated with livestock contact and familial clustering. A seroprevalence study of 1024 Saudi soldiers deploying to Jazan Province was conducted in 2009.[175] Thirteen (1.3%) of the soldiers were seropositive for AHFV and came from Tabuk and Eastern Directorates not known for cases of AHFV, which suggests a wider geographic distribution of this virus in the Arabian Peninsula. This was confirmed in 2010 when two tourists from Italy on vacation in southeastern Egypt near the Sudanese border became infected with AHFV.[176] It is now believed to have wider geographic distribution than once thought, is considered an emerging infectious disease, and is potentially spread by the transportation of cattle, goats, and camels and their potentially infected ticks throughout the Arabian peninsula and Egypt.

Kyasanur Forest Disease

As previously noted, KFDV was first isolated from sick monkeys in the Kyasanur Forest of Shimoga district of Karnataka state of India. This is a heavily rain-forested region with a large nonhuman primate population of *Presbytis entellus* (black-faced langur) and *Macaca radiata* (red-faced bonnet) monkeys.[177] Monkeys become ill from KFDV presumably through the bite of infected ticks, primarily *Haemaphysalis spinigera,* although the reservoir is not known but thought to be a rodent species. KFDV was originally thought to be geographically localized to the Kyasanur Forest area. Between 1964 and 1973, 1046 wild monkeys had died of KFDV, with a seasonal activity between December to May coinciding with the immature stages of the *Haemaphysalis* tick.[177] The epizootic geographic areas have changed from the 1960 location of Kyasanur Forest to the Yakshi and Gudavi forests in the 1970s and to further distances in Shimoga district. Serologic surveys detected the presence of antibodies in humans located in Gujrat state, 1200 km from the Kyasanur Forest area.[177] Outbreaks in humans occur during epizootic outbreaks in wild monkeys and humans working in forested areas for lumber or clearing of areas for farming. From December 2011 to March 2012, 215 suspected cases from 80 villages (total population, 22,201) in Shimoga were identified as potential infections from KFDV, representing an attack rate of 9.7 cases/1000 persons.[178] Of these, 61 (28%) cases were laboratory confirmed. Cases began occurring in the last week of December 2011, peaked during the first 2 weeks of February, and then declined gradually. Risk factors for infection included handling of cattle, frequent visits to the forest, and piles of dry leaves within the compounds of their house. Of the 51 case-patients, 20 had received two doses of vaccine and 2 had received one dose. KFDV was discovered in Yunnan Province, China, in 1989, where it was originally called Nanjianyin virus and is now known as a subtype of KFDV.[179] A seroepidemiologic investigation in Yunnan Province from 1987 to 1990 demonstrated that residents in the region of the Hengduan Mountains had been infected with this virus. This suggests that KFDV may have a wider geographic distribution than previously appreciated and that migratory birds with

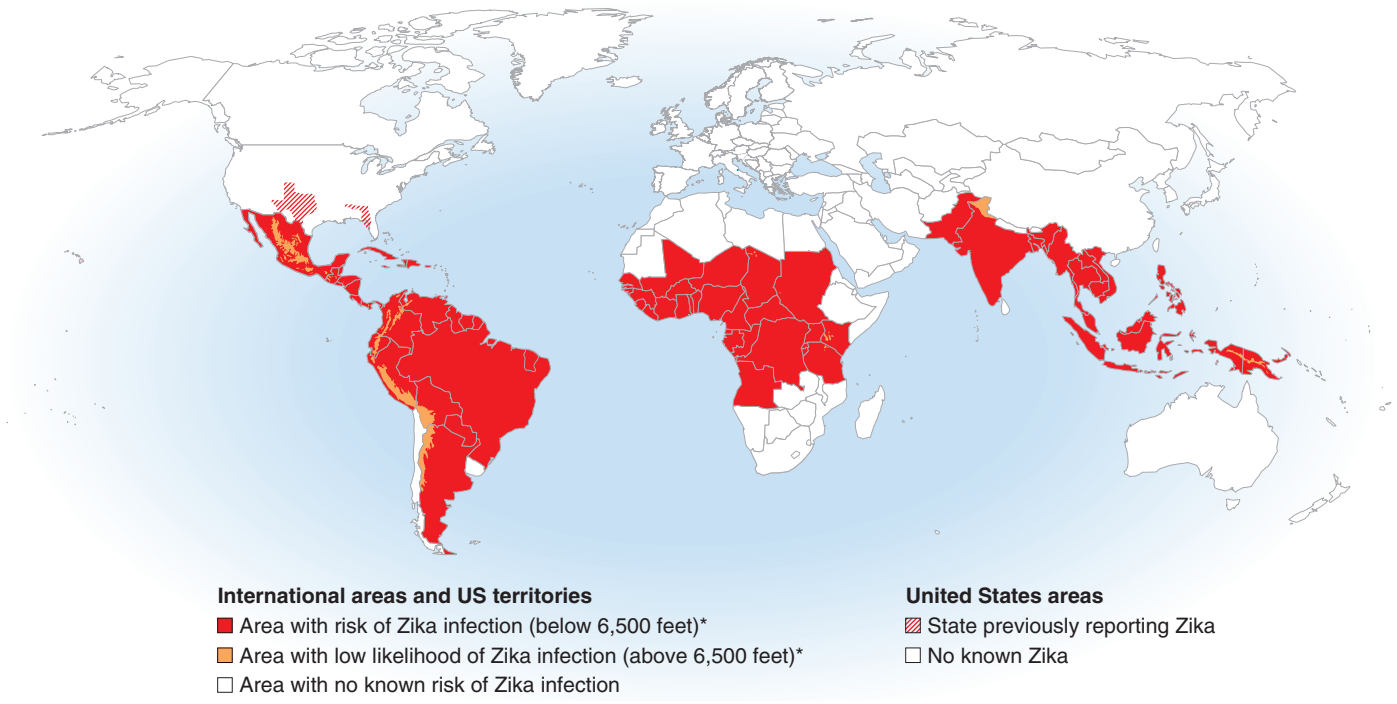

FIG. 153.4 World map of areas with risk of Zika as of March 2018. *Mosquitoes that can spread Zika usually live in locations below 6500 feet. The chances of acquiring Zika from mosquitoes living above that altitude are very low. *Data from the Centers for Disease Control and Prevention.*

attached infected ticks may be responsible for its geographic distribution.[179] A vaccine for KFDV is available and licensed in India. It is a formalin-inactivated virus vaccine produced in chick embryo fibroblasts with a vaccine efficacy of 79.3% with one dose and 93.5% with two doses.[177,180–182]

Zika

ZIKV was demonstrated to be a human infection soon after its discovery in a febrile monkey through serologic surveys and in a case of human infection in a field worker. An experimental infection of a volunteer resulted in a febrile illness associated with headache, myalgias, and symptoms of prostatitis.[183] Subsequently, ZIKV has been isolated from a variety of *Aedes* mosquitoes and is a cause of human infections throughout equatorial Africa, Indonesia, Malaysia, and Cambodia, with *Aedes aegyptii* being the primary vector in Southeast Asia.[184] In 2007, a large outbreak of ZIKV infection occurred in Yap Island, Micronesia.[185] In total, there were 49 confirmed and 59 probable cases of ZIKV illness. Patients developed rash, fever, arthralgia, and conjunctivitis. *Aedes hensilli* was the predominant mosquito species identified. A large outbreak of ZIKV infection occurred in French Polynesia in 2013 and 2014 and subsequently moved to the Western Hemisphere, first on Chile's Easter Island in February 2014 to June 2014, followed by detection in Brazil in May 2015.[186] Within a year, the Brazilian epidemic resulted in ZIKV spread through the majority of states within Brazil to a total of 205,578 reported cases in 2016; a total of 13,353 cases were reported in 2017 with a peak seroprevalence of 63% in Northeast Brazil.[187] By February 2016, ZIKV had spread from Brazil and was detected in 31 countries and territories in the Americas.[186] According to the WHO, as of March 2017 ZIKV had spread to 84 countries and territories where vectors are present, to include 61 areas with ongoing transmission throughout Central America, South America, the Caribbean, Africa, and Southeast Asia; 13 countries with person-to-person transmission; 31 countries with reports of microcephaly and other CNS abnormalities associated with congenital infection; and 23 countries reporting an increase in GBS.[188] In the United States ZIKV became a nationally notifiable infection in 2016.[189] According to the CDC, from 2015 through June 5, 2019 there have been 5,470 travel-related cases in the United States, 231 locally acquired cases, and 55 cases related to other routes of transmission such as sexually acquired Zika. In the United States and territories, there have been 148 travel-related cases and 37,206 locally acquired Zika cases with zero cases accounting for transmission via other routes. Clinicians should be aware of areas at risk of ZIKV transmission when conducting pretravel counsel or when seeing the ill returning traveler (Fig. 153.4).

ZIKV transmission appears to be primarily by *A. aegyptii* mosquitoes, but *A. albopictus,* which lives in temperate regions, may also be able to transmit the virus.[190] ZIKV has been associated with possible sexual transmission, transmission through blood transfusion, and perinatal transmission.[191,192] On February 5, 2016, the CDC issued interim guidelines for prevention of sexual transmission of ZIKV.[191,193] These include the recommendation that men who travel to or reside in an area of active ZIKV transmission and who have a pregnant partner should abstain from sexual activity or consistently and correctly use a condom. Men with such travel histories and nonpregnant sex partners should also consider abstinence or proper use of condoms.[191,192,194,195]

PATHOGENESIS

The early stages of infection are quite similar for all of the flaviviruses, with an initial stage of local replication followed by dissemination and viremia. Subsequent events in disease pathogenesis likely depend to a greater extent on the clinical syndrome than on the specific virus. As a result, the pathogenesis of infection by those viruses that have not been characterized in detail are presumed to be similar to one of the prototype pathogens; for example, hemorrhagic fevers caused by ALKV, KFDV, and Omsk hemorrhagic fever virus are expected to share characteristics similar to YF. ZIKV appears unique among the flaviviruses for its robust tropism for placenta, neuroprogenitor cells, and other cells, which results in a spectrum of congenital and other defects.[191,196] The tissue tropism of ZIKV is presented in Fig. 153.5.

Yellow Fever

Early stages of YF infection can be inferred from human vaccine studies and from experimental wild-type viral infections of primates. Two days after inoculation of the attenuated 17D vaccine, levels of tumor necrosis

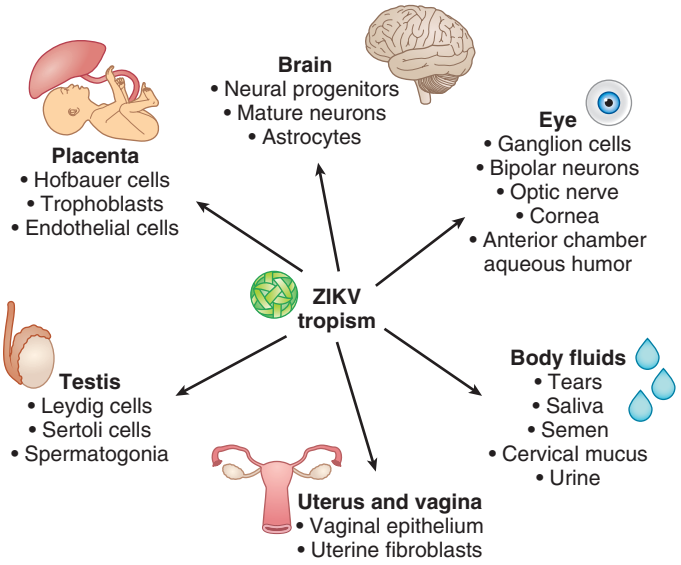

FIG. 153.5 Observed Zika virus *(ZIKV)* tissue and cell tropism. Human studies and studies in animal models (mice and nonhuman primates) have detected ZIKV in cells of the placenta, including Hofbauer cells (in vitro and in explanted human placental tissue), trophoblasts (mice, nonhuman primates, and humans), and endothelial cells (in vitro in explanted human placental tissue and in vivo in placenta of mice). Other ZIKV cellular targets include neuronal cell types, including neural progenitor cells and mature neurons (mice, nonhuman primates, and humans) and astrocytes (in vitro human cell cultures). In addition, ZIKV infects ocular tissues, including the cornea, neurosensory retina, and optic nerve (mice), as well as the aqueous humor of the anterior chamber (humans). ZIKV also targets cells of the reproductive tract, including spermatogonia, Sertoli cells, and Leydig cells (in the testis of mice); sperm (samples from mice and humans); and the vaginal epithelium (mice) and uterine fibroblasts (in vitro infection of human samples). The extensive tropism results in ZIKV detection in multiple body fluids, including conjunctival fluid or tears (mice and humans), saliva (nonhuman primates and humans), semen (mice, nonhuman primates, and humans), cervical mucus (humans), vaginal washings (mice and human), and urine (nonhuman primates and humans). *(Modified from Miner JJ, Diamond MS. Zika virus pathogenesis and tissue tropism.* Cell Host Microbe. *2017;21:134–142.)*

factor-α (TNF-α), interleukin (IL)-1 receptor antagonist, and, to a lesser extent, IL-6 increase, with a secondary TNF-α peak 7 days later.[6,83,197,198] The cytokines are synthesized in response to local spread of the vaccine and again as a response to viremia, which peaks between days 3 and 6.[6,83,199] TNF-α elevations correspond to declines in the lymphocyte count. After wild-type viral infection, the grippe phase of early YF presumably is associated with a similarly timed elaboration of cytokines. In experimentally inoculated rhesus monkeys, the virus replicates initially in local lymph nodes, followed rapidly by bloodborne infection of fixed macrophages, especially Kupffer cells in the liver, and further spread and replication in liver, lung, kidney, and adrenal glands, and most prolifically in regional lymph tissue, spleen, and bone marrow.[6,83] Infection by mosquito feeding, which introduces salivary proteins, is believed to differ from experimental needle inoculation in the outcome of local viral replication and distribution, but the importance of these factors in modulating human flavivirus infections is unknown.

Pathologic changes are most pronounced in the liver and kidneys, but widespread hemorrhages are found on mucosal surfaces, in the skin, and within various organs. Numerous petechial hemorrhages and erosion of the gastric mucosa contribute to the hematemesis that typically introduces the illness. Hepatocellular damage is characterized by a patchy mid-zonal distribution, sparing cells around the central vein and portal triad. The extent of lobular necrosis is variable, with an average of 60%, but even with confluent lobular necrosis, the reticular architecture is preserved. The preservation of the reticulin network, minimal inflammatory changes, and the morphology of degenerating hepatocytes are consistent with apoptosis as the principal pathway of cell death. Early changes in infected hepatocytes consist of glycogen depletion and cloudy swelling, followed by accumulations of fat and of ceroid pigment. Necrotic cells finally undergo coagulation, with the formation of characteristic eosinophilic Councilman bodies, which correspond to apoptotic cells. Viral antigen is identified initially in Kupffer cells and appears later in hepatocytes, Councilman bodies, and endothelial cells.[200–202] Healing occurs without fibrosis.

Albuminuria and renal insufficiency reflect prerenal factors, including vomiting and myocarditis, as well as parenchymal invasion and, in advanced illness, acute tubular necrosis.[83,203] Viral antigen can be identified in the kidney and also in the heart, in which degenerative fatty infiltration of the myocardium and of the conduction system contributes to decreased output and arrhythmias.[202] Neurologic findings probably reflect metabolic disturbances, cerebral edema, and hemorrhages rather than encephalitis. The cause of the bleeding diathesis is ill defined but probably represents a combination of reduced hepatic synthesis of clotting factors, intravascular coagulation, thrombocytopenia, and endothelial and platelet dysfunction. A combination of direct parenchymal damage and a systemic inflammatory response–like syndrome appears to contribute to shock and a fatal outcome. Neutralizing antibodies elaborated within the first week of illness clear the virus, and recovery is followed by lifelong immunity.

Heterologous flavivirus immunity (e.g., previous dengue) is believed to provide partial protection against infection, which may contribute to the absence of YF in Asia.[204] However, in contrast to the situation with DHF, there is no evidence of antibody-mediated immune enhancement. Genetic selection has been described in survivors of YF epidemics, and youth and advanced age have both been implicated as risk factors for symptomatic illness.[6,205] Hepatitis B carriage, which is prevalent in areas of Africa with endemic YF, is not a risk factor for symptomatic disease.[206]

Dengue and Dengue Hemorrhagic Fever

Most DENV infections are subclinical. Self-limited dengue fever is the usual clinical outcome of infection. The syndrome of DHF (Fig. 153.6) occurs in a small percentage of cases, apparently as an immunopathologic response to infection and usually in the setting of heterologous immunity from prior infection with a different DENV type or possibly another flavivirus.[207,208]

As with YF, early stages of DENV infection are inferred from experimental infection of humans or other primates. After an infectious mosquito bite, the virus replicates in local lymph nodes and within 2 to 3 days disseminates through the blood to various tissues. Virus circulates in the blood typically for 4 to 7 days in plasma and infected monocytes.[209] Viral RNA is also readily detected in circulating B cells.[210] Virus likely also replicates in skin, reactive spleen lymphoid cells, and tissue macrophages.[211,212] Viral antigen can be demonstrated more widely in liver Kupffer cells and endothelia, renal tubular cells, and alveolar macrophages and endothelia, although active viral replication at these sites has been difficult to document. Almost all patients are viremic at the point of clinical presentation with fever and clear the virus from the blood within days after defervescence.[213,214] The malaise and influenza-like symptoms that typify dengue probably reflect patients' cytokine response; however, myalgia, a cardinal feature of the illness, may also indicate pathologic changes in muscle, typified by a moderate perivascular mononuclear infiltrate with lipid accumulation.[215] Musculoskeletal pain (breakbone fever) conceivably could reflect viral infection of bone marrow elements.[216,217] Suppression of erythropoiesis, myelopoiesis, and thrombopoiesis is reflected in peripheral cytopenias. Histopathologic examination of skin from patients with rash discloses a minor degree of lymphocytic dermal vasculitis and, variably, viral antigen.[211,212] Elevated hepatic aminotransferase concentrations have been reported in most cases of dengue, with the aspartate aminotransferase (AST) level initially higher than that of alanine aminotransferase (ALT) and with levels higher in DHF compared with dengue fever.[114] In fatal cases, histopathologic findings resemble those of early mild YF, with hypertrophy of Kupffer cells, focal ballooning, and necrosis of hepatocytes in a mid-zonal distribution, with occasional Councilman body formation, mild fatty changes, and a scant periportal mononuclear cell response.[218] Neurologic complications have been attributed chiefly to metabolic

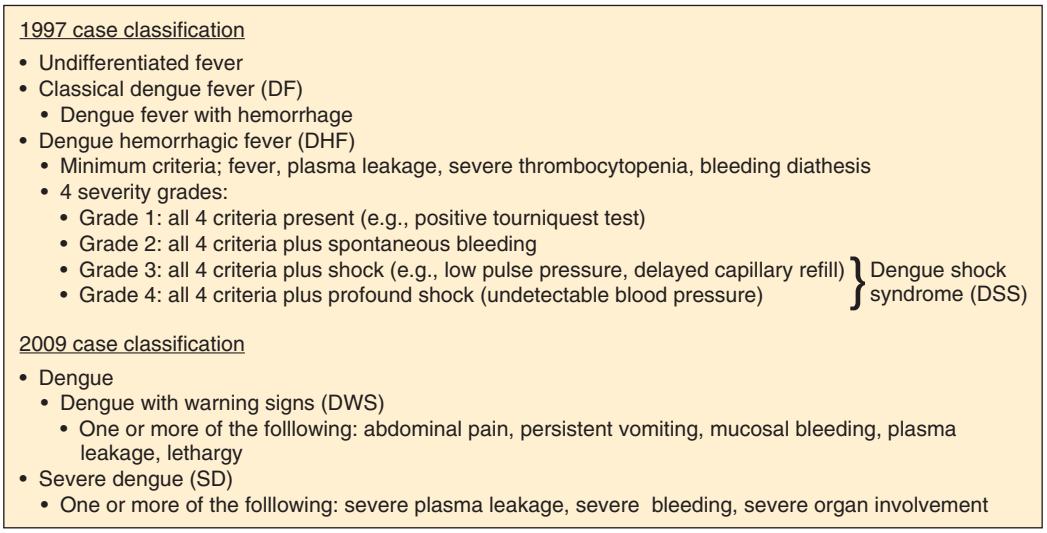

FIG. 153.6 Clinical spectrum, pathophysiology, and case classification of dengue hemorrhagic fever. For the 1997 case classification, severe thrombocytopenia refers to a platelet nadir of 100,000 cells/mm^3 or lower; evidence of bleeding diathesis includes at a minimum a positive tourniquet test; evidence of plasma leakage includes hemoconcentration of 20% or greater, pleural effusion, or ascites; and criteria for shock include a pulse pressure of 20 mm Hg or lower. For the 2009 case classification, evidence of severe plasma leakage includes either shock requiring fluid resuscitation or fluid accumulation impairing respiratory function; severe bleeding and severe organ involvement are determined by the clinician's judgment. *(From World Health Organization.* Technical Guide for Diagnosis, Treatment, Surveillance, Prevention, and Control of Dengue Haemorrhagic Fever. *2nd ed. Geneva: World Health Organization; 1997.)*

alterations and to focal, and sometimes massive, intracranial hemorrhages, but viral CNS invasion and encephalitis have been demonstrated to occur.[219,220]

Shock in dengue shock syndrome (DSS) occurs after the sudden extravasation of plasma into extravascular sites, mainly the pleural and abdominal cavities, usually coincident with the disappearance of fever.[207,221] The extensive increase in vascular permeability is associated with immune activation, as manifested by increased plasma levels of soluble tumor necrosis factor receptor (sTNFR/75), IL-8, interferon (IFN)-γ, and vascular endothelial growth factor, among other mediators.[222–225] In addition, immune complex formation activates the complement system, with increases in C3a, C5a, and soluble C5b-9 complexes.[226,227] The rapid, predictable reversibility of the syndrome within 48 hours and the paucity of histopathologic correlates—usually perivascular edema with diapedesis of red cells and widespread focal hemorrhages—suggest that the inflammatory response produces a vasculopathy. Reduced cardiac output may contribute further to shock.[228,229] The hemorrhagic diathesis is complex and not well understood, reflecting a combination of cytokine action and vascular injury, viral antibodies binding to platelets or cross-reacting with plasminogen and other clotting factors, reduced platelet function and survival, and a mild consumptive coagulopathy.[230–233]

The increased frequency of DHF associated with sequential DENV infections of different DENV types has suggested a role for adaptive immunity in the pathogenesis of severe dengue disease. Serotype cross-reactive "heterologous" antibodies from an earlier DENV infection have been shown to enhance viral uptake and replication in Fc receptor–bearing cells (antibody-dependent enhancement) in vitro and in a mouse model.[234,235,236–238a]

Passively acquired antibody has been proposed to have the same pathologic effect during primary DENV infection in infants born to dengue-immune women.[239] Simultaneously, memory cellular responses to heterologous antigens also may contribute to immunopathology during secondary infections.[240] Elevated levels of plasma-soluble CD8 and soluble IL-2 in patients with DHF and flow cytometry of circulating peripheral blood lymphocytes indicate an activation of memory CD4$^+$ and CD8$^+$ T cells in response to a second infection.[223,241] Cells responding to cross-reactive antigens predominate over primary responses to the infecting virus, following the paradigm of original antigenic sin, but may be ineffective in viral clearance, and instead may be a source of cytokines with a negative clinical effect.[241] The resulting production of IL-2, IFN-γ, TNF-α, and other lymphokines is reinforced by the increased abundance of infected target cells and flavivirus-induced expression of major histocompatibility complex type I and II molecules that further activate T lymphocytes.[242–246] Activated infected monocytes (and possibly endothelial cells) produce and release with their lysis TNF-α, IL-1, platelet-activating factor, IL-8, and RANTES (regulated on activation, normal T-cell expressed and secreted), which act in synergy with lymphokines, histamine, and C3a and C5a to produce the transient vascular endothelial dysfunction that leads to plasma leakage.[242,243] Additional direct and indirect effects on vascular endothelial function have been ascribed to secreted NS1 protein and to the activation of other cell types, including platelets and mast cells.[242,244–246]

Although infection with any of the four DENV types can produce DHF, there is some indication of a greater propensity after second infections with certain DENV types or with specific strains of putatively greater virulence in a given partially immune population.[214,247,248] Trends toward an increased or fluctuating severity of illness during prolonged outbreaks have been attributed to the evolution of viral quasispecies.[249] Various genetic polymorphisms in the human host also influence the risk for DHF, particularly in HLA and *MICB* genes, which control immune responses, as well as in the *PLCE1* gene, the mechanism for which is unknown.[250,251]

The induction of innate immune responses and a rise in levels of serum neutralizing antibodies are correlated with the clearance of viremia,[213,252] but protective immunity is associated with both humoral and cellular immune responses.[240,253–255] The cross-protective immunity induced by infection with one DENV type is limited and brief because infection with a second DENV type during the same transmission season is not uncommon. Illness after infection with two or more DENV types (i.e., a third or fourth clinically apparent DENV infection) occurs infrequently, which is thought to reflect the higher titers of anti-DENV antibody after secondary infection and a greater complement of cross-reactive antibodies.[256]

During outbreaks in Cuba in 1981 and 1997, DHF cases occurred only in the age cohorts exposed during the previous epidemic (older than 3 years and older than 17 years, respectively), further underscoring sequential/secondary infection as a critical precondition for DHF, at least for some DENV types.[119,257] However, DHF cases have been well documented in persons with primary infections, pointing to other

contributory factors such as viral strain and host factors.[258,259] Race and specific HLA haplotypes have been implicated in the risk for acquiring DHF, and a variable predominance of severe cases has been observed in girls and in children with good nutrition, indicating the contributions of both genetic and acquired host factors to susceptibility to the syndrome.[250,260–266]

Encephalitis

The variable and potentially lengthy incubation period of 4 to 21 days may reflect the interval for viral replication in the skin Langerhans cells and local lymph nodes, with a subsequent brief viremia before the virus invades the CNS.[267] Virus can rarely be recovered from blood, usually less than 1 week after the onset of illness and before the onset of neurologic symptoms, but sometimes later in an immunosuppressed patient; for WNV, detection by real-time PCR assay is more common.[268,269] The large proportion of infections that are asymptomatic, about 100 times the number of symptomatic cases, is striking and remarkably consistent among SLE, JE, and WNE. Subclinical infection presumably reflects the peripheral clearance of virus before neuroinvasion.

Innate responses are thought to be critical to this clearance. Genetic susceptibility to flaviviral encephalitis in laboratory mice was first associated with an allelic variant in the IFN-induced gene 2′-5′-oligoadenylate synthetase 1 (*OAS1*) that produced a truncated protein,[69,71] and a polymorphism in the same gene has been associated with WNV infection in humans.[270] Knockout mice deficient for other genes in the type I IFN-induced pathway similarly show increased susceptibility to lethal encephalitis.[271] Other host response factors have also been linked to susceptibility to flavivirus encephalitis. A functional Toll-like receptor 3–dependent inflammatory response has been associated with WNV neuroinvasion and neuronal injury in mice and with TBE in humans.[272,273] A defective allele in the chemokine receptor CCR5 (CCR5Δ32) was found to be significantly associated with symptomatic WNV infection and with severe disease in TBE.[274,275]

The mechanisms of viral neuroinvasion remain incompletely understood. In animal models of arboviral encephalitis, virus enters the CNS by crossing the vascular endothelium or through the olfactory epithelium, where the blood-brain barrier is impaired. However, in humans, the evidence suggests transmission across the vascular endothelium, either by passive transfer or by replication in endothelial cells.[267,271] Infected monocytes have also been proposed to carry virus across the blood-brain barrier.

Within the brain, virions spread from cell to cell. Pathologic changes consist of meningeal congestion and inflammation, brain edema, and widespread encephalitis with a predilection for the hippocampus and temporal cortex, thalamus, substantia nigra, cerebellum, periventricular areas of the brainstem, and anterior spinal cord.[267] Focal neuronal degeneration and necrosis with neuronophagia evolve to the formation of glial nodules and, with healing, spongiform changes. Viral antigen appears in neuronal bodies and their processes and later in phagocytic cells.[276] Perivascular inflammatory infiltrates consist of activated $CD4^+$ and $CD8^+$ T cells, macrophages, and B cells. Activated T cells predominate in the cerebrospinal fluid (CSF).[277]

A variety of processes may contribute to neuronal cell death, including apoptosis, cytoplasmic swelling, vacuolation, and membrane breakdown. In one study of TBE pathology, viral antigen was identified predominantly in large neurons; there was a poor topographic relation between inflammatory infiltrates, mainly T cells and macrophages, and distribution of antigen, suggesting an immune-mediated neuronal cell death rather than direct viral lysis.[278]

The rare recovery of virus from CSF, usually in patients with fulminant and fatal disease, is associated with the absence of intrathecal antibodies, indicating an important role of viral neutralization in recovery.[279] T cells are also essential for clearance of virus in experimental infections of mice, and low $CD4^+$ T-cell IFN-γ responses to JE viral NS3 protein correlated with more severe disease in one human study.[280–282] On the other hand, intrathecal immune complex formation and antineurofilament and anti–myelin basic protein antibodies have been reported in association with a poor outcome, suggesting immunopathologic injury.[283,284] Immunopathology is also implicated in animal models of flavivirus encephalitis and is supported by the observation that in some immunocompromised patients infected with WNV there is a delayed onset of clinical features despite high levels of viremia.[271,285] Regulatory T cells may play an important role in controlling this response to protect against severe disease.[286]

Subacute and progressive paralysis of the limb musculature and chronic epilepsy are well-known features of TBE, and CNS viral persistence has been demonstrated.[158] CSF neopterin, β_2-microglobulin, and intrathecal immunoglobulin G (IgG) synthesis remain elevated for weeks and pleocytosis persists considerably longer than in other CNS infections, consistent with a protracted inflammatory reaction.[287] In some studies, mutations in the virus NS1 gene and defective T-cell responses have been reported.[158,288] Delayed CNS clearance of JEV has also been suggested by the presence of infectious virus, antigen, or immunoglobulin M (IgM) antibodies in CSF several weeks after the onset of illness.[289]

Advanced age is preeminent among the risk factors for development of neurologic infection. The biologic basis for this age-related susceptibility is ill defined. Although immunosenescence affecting innate or adaptive immune responses or both is a likely scenario, other observations indicate roles for functional or structural CNS changes that facilitate neuroinvasion. As examples, in some studies, severe encephalitis has been associated with neurocysticercosis, hypertension, and concurrent viral, bacterial, or parasitic infections.[290–304]

Congenital Malformations and Guillain-Barré Syndrome

ZIKV is unique among the flaviviruses for its association with a range of congenital defects to include microcephaly and neurologic conditions such as GBS.[293–294] The pathogenesis of fetal infections and resulting congenital defects likely involves insult within the placenta or developing fetal neural tissue or both.[293,295,296] During the first trimester the placenta barrier is developing and the fetus is at its most vulnerable, as illustrated by rubella infection, when the risk of fetal infection during maternal rubella infection is 20% if it occurs during the first trimester.[292,297] In a postmortem pathology study of infants born with microcephaly, ZIKV was present in both neuronal and nonneuronal cells with high infectivity in intermediate progenitor cells and immature neurons with neuronal apoptosis.[293,298]

GBS is an autoimmune disorder affecting the peripheral nervous system, resulting in an ascending progressive polyradiculoneuritis and paralysis, and may require ventilator support.[294,299] GBS has been associated with the flaviviruses, including JEV and DENV, as well as an atypical polio-like syndrome with WNV.[300–302] ZIKV infections have also been associated with GBS and the pathophysiologic mechanisms have been evaluated.[294,299,303,304]

CLINICAL MANIFESTATIONS

Yellow Fever

YF illness ranges in severity from an undifferentiated, self-limited grippe to hemorrhagic fever that is fatal in 50% of cases.[6,83,203] In addition, between 5% and 50% of infections are inapparent. After an incubation period of 3 to 6 days, fever, headache, and myalgias begin abruptly, accompanied by few physical findings except conjunctival injection, facial flushing, a relative bradycardia (Faget sign), and, on laboratory examination, leukopenia. In most cases, resolution of this *period of infection* concludes the illness; in others, the remission of fever for a few hours to several days is followed by renewed symptoms, including high fever, headache, lumbosacral back pain, nausea, vomiting, abdominal pain, and somnolence (*period of intoxication*). Profound weakness and prostration ensue, compounded by poor oral intake and protracted vomiting, but the severe multisystemic illness is dominated by icteric hepatitis and a hemorrhagic diathesis with prominent gastrointestinal bleeding and hematemesis, epistaxis, gum bleeding, and petechial and purpuric hemorrhages. Albuminuria is a constant feature that aids in the differentiation of YF from other causes of viral hepatitis. Deepening jaundice and elevations in aminotransferase levels continue for several days, at the same time that azotemia and progressive oliguria ensue. Whereas direct bilirubin levels rise to 5 to 10 mg/dL, alkaline phosphatase levels are only slightly raised; not infrequently, AST may be elevated above ALT because of myocardial damage.[305] Ultimately, hypotension,

shock, and metabolic acidosis develop, compounded by myocardial dysfunction and arrhythmias as late events and acute tubular necrosis in some patients. Confusion, seizures, and coma distinguish the late stages of illness, but CSF examination discloses an increased protein level without pleocytosis, consistent with cerebral edema or encephalopathy. Death usually occurs within 7 to 10 days after onset. If the patient survives the critical period of illness, secondary bacterial infections resulting in pneumonia or sepsis are common complications. Recovery has not been followed by chronic hepatitis.

Clinically, severe YF resembles other viral hemorrhagic fevers occurring in Africa and South America, so laboratory confirmation is required to make the diagnosis. Early exclusion of other causes with the potential for person-to-person spread is important to prevent nosocomial transmission. Other forms of viral hepatitis, particularly hepatitis E (which frequently appears in outbreaks), leptospirosis, malaria, typhoid, typhus, relapsing fever, acute fatty liver of pregnancy, and toxin-related hepatitis, are alternative diagnoses.

Dengue Fever and Dengue Hemorrhagic Fever

The classification of dengue illness (see Fig. 153.6) has traditionally focused on the distinction between classic dengue fever and DHF, the latter reflecting more severe disease manifesting with plasma leakage, bleeding complications, or both.[306] In 2009, an alternative classification defined criteria for dengue, dengue with warning signs, and severe dengue; the revised classification has been adopted for epidemiologic reporting in most countries, but both systems continue to be used in the literature.[306,307] Classic dengue fever is an acute febrile disease with headaches, musculoskeletal pain, and rash, but the severity of illness and clinical manifestations vary with age and DENV type. Infection is often asymptomatic or presents as a nonspecific syndrome consisting of fever, malaise, pharyngeal injection, upper respiratory tract symptoms, and rash, particularly in children.[12,308–310] DENV-2 and DENV-4 may be more likely to cause inapparent infections in flavivirus-naïve persons.[214] Disease severity may be increased among infants and elderly people.[311] After an incubation period of 4 to 7 days, fever—often with chills, severe frontal headache, and retro-orbital pain—develops abruptly with a rapid progression to prostration, severe musculoskeletal and lumbar back pain, and abdominal tenderness. Anorexia, nausea, vomiting, hyperesthesia of the skin, and dysgeusia are common complaints. Initially, the skin appears flushed, but within 3 to 4 days and with the lysis of fever, an indistinct macular and sometimes scarlatiniform rash develops, sparing the palms and soles. As the rash fades or desquamates, localized clusters of petechiae on the extensor surfaces of the limbs may remain. A second episode of fever and symptoms may ensue ("saddleback" pattern). Recovery may be followed by a prolonged period of listlessness, easy fatigability, and even depression.

Although virtually all cases of dengue fever are uncomplicated, minor bleeding from mucosal surfaces (usually epistaxis, bleeding from the gums, hematuria, and metrorrhagia) is not uncommon, and gastrointestinal hemorrhage and hemoptysis can occur (see Fig. 153.6).[312] In patients with preexisting peptic ulcer disease, severe, even fatal, gastric bleeding can be precipitated.[313] Subcapsular splenic bleeding and rupture, uterine hemorrhage resulting in spontaneous abortion, and severe postpartum bleeding have also been reported.[314,315] It is important to differentiate these phenomena from the bleeding diathesis that accompanies the life-threatening syndrome of hypotension and circulatory failure in DHF-DSS.

Hepatitis frequently complicates dengue fever.[114,316] In Taiwan, transaminase levels raised 10-fold above normal were observed in 11% of cases, with rare deaths due to hepatic failure. Neurologic symptoms associated with dengue fever have been reported sporadically and attributed to hemorrhages or cerebral edema, but recovery of virus from the CSF, intrathecal viral-specific IgM, and immunohistochemical evidence of infection in the brain indicate the possibility of primary dengue encephalitis in some cases.[219,317–319] Myositis with rhabdomyolysis has also been reported.

Vertical transmission of DENV to neonates whose mothers had an onset of primary or secondary dengue fever up to 5 weeks before delivery has resulted in acute neonatal dengue manifesting as fever, cyanosis, apnea, mottling, hepatomegaly, and reduced platelet counts (as low as 11,000/mm^3).[320] One infant died of intracerebral hemorrhage, but others, although ill, did not have other signs of DHF, and they recovered without incident. DENV was isolated from the neonates in some cases. The outcome of infection acquired earlier in pregnancy has not been addressed satisfactorily. Anecdotal reports have described spontaneous abortion (see earlier discussion) and a variety of birth defects and, in a postepidemic investigation, an increase in neural tube defects.[321] Other investigations have found no increases in abnormal pregnancy outcomes.[320,322,323] In a study of cord blood samples from infants delivered 5 to 9 months after an outbreak, 4 of 59 samples had viral-specific IgM but all the infants appeared normal.[324]

The central clinical features of DHF-DSS are hemorrhagic phenomena and hypovolemic shock caused by increased vascular permeability and plasma leakage.[325–327] The early clinical features in children who ultimately develop DHF-DSS are indistinguishable from those of ordinary dengue fever, namely, fever, malaise, headache, musculoskeletal pain, facial flushing, anorexia, nausea, and vomiting. However, with defervescence, typically 3 to 7 days after illness onset, reduced perfusion and early signs of shock are manifested by central cyanosis, restlessness, diaphoresis, and cool, clammy skin and extremities. Abdominal pain is a common complaint. In cases with a benign course of illness, blood pressure and pulse may be maintained but a rapid and weak pulse, narrowing of the pulse pressure to less than 20 mm Hg, and, in the most extreme cases, an unobtainable blood pressure establish DSS. The platelet count declines, and petechiae appear in widespread distribution with spontaneous ecchymoses. Bleeding occurs at mucosal surfaces from the gastrointestinal tract and at venipuncture sites. The liver is palpably enlarged in up to 75% of patients, with variable splenomegaly. Increased amylase levels and ultrasonographic evidence of pancreatic enlargement are found in up to 40% of patients. Pleural effusions can be detected in more than 80% of cases if a decubitus film is taken; in combination with an elevated hematocrit and hypoalbuminemia, reflecting hemoconcentration, these studies provide objective measures of plasma loss. In some studies, ultrasonography has been more sensitive in detecting pleural effusions, ascites, and gallbladder edema in more than 95% of severe cases, and pararenal and perirenal effusions in 77%, as well as hepatic and splenic subcapsular and pericardial effusions.[328,329] The presence of pleural and peritoneal effusions is associated with severe disease. In contrast, pulmonary edema is uncommon except in cases with prolonged shock.[330] In untreated patients, hypoperfusion complicated by myocardial dysfunction and reduced ejection fraction results in metabolic acidosis and organ failure. However, with support through the critical period of illness, spontaneous resolution of vasculopathy and circulatory failure usually can be expected within 2 to 3 days, with complete recovery afterward. The duration of illness ranges from 7 to 10 days in most cases. Fatality rates have reached 50% in underserved populations, but in experienced centers, less than 1% of cases are fatal. Encephalopathy, prolonged shock, and hepatic or renal failure infrequently complicate the illness and are associated with a poor prognosis. As would be expected in areas where DENV infects 10% of children each year, concurrent infection with bacteria, parasites, and other viral pathogens occurs frequently. Dual infections, principally gram-negative sepsis, have been reported in 1 of 200 children hospitalized with dengue, resulting in prolonged fever and hospitalization.[331]

Attempts to differentiate dengue fever from other acute febrile illnesses on clinical grounds are unreliable, although laboratory findings of leukopenia, neutropenia, thrombocytopenia, or mildly elevated AST levels are supportive.[11,114,249] A positive tourniquet test, one of the criteria in the DHF case definition, is obtained more often in children with dengue than in children with other febrile illnesses, but its specificity is low. In comparison with chikungunya, another *A. aegypti*–borne viral infection, dengue patients are less likely to have conjunctivitis, rash, and joint pain.[332] The difficulty of differentiating dengue from rubella, measles, and even influenza has been underscored by the early misrecognition of entire epidemics. The clinical picture of DHF is more distinct, but overlaps with YF and other viral hemorrhagic fevers.

Clinical or laboratory differentiation, at the time of first presentation, of children destined to develop DHF would facilitate intervention

before the sudden onset of shock.[333] In one study, AST elevations greater than 60 U/mL, leukocyte counts less than 5000/mm^3, and absolute neutrophil counts less than 3000/mm^3 had higher predictive values than the tourniquet test in differentiating dengue from other febrile illnesses.[114,249] In another study, an elevated sTNFR/75 level had a sensitivity of 93% and a negative predictive value of 95% in foretelling shock.[222] Other cytokines, and combinations of markers, have been evaluated as predictors of mild versus severe disease, but results have varied and there have been no prospective validation studies. Furthermore, implementation of useful measures at the point of care may be a challenge.[208,334]

Japanese Encephalitis

Infection with JEV is symptomatic in less than 1% of cases of JE, but the illness is usually a severe encephalitis, leading frequently to coma and to a fatal outcome in 25% of cases. The spectrum of clinical illness is probably broader than is appreciated from an evaluation of hospitalized patients. JE cases are found among hospitalized children with acute pyrexia of undetermined origin, and, undoubtedly, many patients with febrile illnesses and headache or aseptic meningitis do not present to a hospital. Studies have drawn attention to patients presenting with spinal paralysis without encephalitic signs, initially misdiagnosed as poliomyelitis cases, and, conversely, acute behavioral changes mimicking psychosis without motor signs.[1,5,335–337] The earliest symptoms are lethargy and fever and, frequently, headache, abdominal pain, nausea, and vomiting. Lethargy increases over several days, when uncharacteristic behaviors associated with an agitated delirium, unsteadiness, and abnormal motor movements may develop, advancing to progressive somnolence and coma. Although the prodrome may evolve over several days to 1 week, some children present with a sudden convulsion after a brief febrile illness.

The chief findings are high fever and altered consciousness, ranging from mild disorientation or a subtle personality change to a severe state of confusion, delirium, and coma.[1,5,335] Mutism has been a presentation in some cases. Nuchal rigidity is a variable finding, present in one-third to two-thirds of the cases. Cranial nerve palsies resulting in facial paralysis and disconjugate gaze are detected in one-third of the cases. Muscular weakness can be associated with decreased or increased tone and can be generalized or asymmetrical, with hemiparesis or unusual distributions of flaccid and spastic paralysis. Hyperreflexia, ankle clonus, and other abnormal reflexes may be elicited. Disordered movements such as nonstereotypical flailing, ataxia, or tremor may be present initially. Not uncommonly, choreoathetosis, rigidity, masked facies, and other extrapyramidal signs appear later in the illness. Focal or generalized seizures develop in up to 85% of children and 10% of adults.[338] Multiple seizures and status epilepticus are associated with a poor outcome. Subtle motor status epilepticus, in which the only clinical manifestation might be the twitching of a finger or eyebrow, may also occur but is easily overlooked.[338]

Signs of increased intracranial pressure, such as papilledema and hypertension, are detected in a minority of patients, although some fatal cases show evidence of uncal or tentorial herniation, and clinical signs consistent with brainstem herniation syndromes are not uncommon.[338] More than one-third of patients in coma need ventilatory support. Fulminant cases may be rapidly fatal. More typically, improvement can be expected after 1 week with the defervescence of fever. Neurologic function is regained gradually over several weeks, with further recovery after hospital discharge over intervals of months to years.[339,340] Infections from stasis ulcers, urinary tract infection, pneumonia, and bacteremia frequently complicate the lengthy recovery from coma and paralysis and may be secondary causes of death. The virulence of the infection is underscored by contemporary fatality rates of 25% in locations with intensive care facilities. Neurologic abnormalities such as seizure disorders, motor and cranial nerve paresis, cortical blindness, and movement disorders persist in up to one-third of patients after 5 years. A greater proportion, perhaps even 75% of recovered children, exhibit behavioral and psychological abnormalities. Anecdotal cases of clinical relapse weeks after hospital discharge, with recovery of virus from peripheral blood, have alluded to delayed viral clearance or persistence, but the significance of these observations is uncertain.[341] In illness acquired during the first or second trimesters of pregnancy, the virus can infect the fetal-placental unit and precipitate abortion.[342] Cases acquired in the third trimester have not been reported to interrupt pregnancy. Congenital infections have been reported only when the virus was newly introduced to a susceptible adult population because almost all women in endemic areas have acquired immunity. Nonimmune travelers may have an increased risk.

Laboratory studies disclose peripheral leukocytosis, as high as 30,000/mm^3 with a left shift, and hyponatremia. The CSF opening pressure is elevated in about 50% of patients.[338] Pleocytosis ranges from less than 10 to several thousand cells per cubic millimeter, with a median of several hundred cells of a predominantly lymphocytic composition. CSF protein may be normal or elevated up to 100 mg/dL. The electroencephalogram discloses a pattern of diffuse slow waves (theta or delta) with superimposed seizure activity, including periodic lateralized epileptiform discharges.[338,343] Brain imaging reveals diffuse white matter edema and abnormal signals mainly in the thalamus (often with evidence of hemorrhage), basal ganglia, cerebellum, midbrain, pons, and spinal cord.[344,345] Electromyography shows changes of chronic partial denervation consistent with anterior horn cell destruction.

In rural Asia, tuberculosis, cerebral malaria, and bacterial meningitis (especially partially treated) are the principal alternative diagnoses.[11,132,346,347] Typhoid fever with tremors and ataxia, dengue infection with encephalopathy, lead poisoning, heat stroke, and enterovirus 71 encephalitis have all been confused with JE. In JE-endemic areas, any encephalitis outbreak is initially assumed to be JE, but the outbreak of the previously unknown Nipah virus in Malaysia in 1999 and outbreaks of Chandipura virus infection in India since 2003 have shown how easy it is to be misled.[348] WNV and JEV infections in particular may be mutually misrecognized because the viruses overlap in their distribution in Southwest Asia and can produce clinically indistinguishable illnesses in contemporaneous outbreaks, and because differentiation in laboratory tests may be difficult.

West Nile Virus Fever and West Nile Encephalitis

Most infections with WNV are asymptomatic; when symptoms do occur, they develop after an incubation period that typically lasts 2 to 6 days but may extend to 14 days, or even longer in immunosuppressed persons. In recent outbreaks, the syndrome of WNV fever occurred in about 20% of infected individuals, who developed a sudden onset of an acute, nonspecific, influenza-like illness lasting 3 to 6 days, with high fever and chills, malaise, headache, backache, arthralgia, myalgia, and retro-orbital pain, without overt neurologic signs.[143,144,335] Other nonspecific features include anorexia, nausea, vomiting, diarrhea, cough, and sore throat. In some epidemics, a flushed face, conjunctival injection, and generalized lymphadenopathy were common, and a maculopapular or pale roseolar rash was reported in about 50% of patients, more frequently in children. In one outbreak, 20% of patients with WNV fever were reported to have hepatomegaly and 10% had splenomegaly.[349] Myocarditis, pancreatitis, and hepatitis have also been described occasionally in severe WNV infection.

Neurologic disease occurs in less than 1% of infected individuals. Patients typically have a febrile prodrome of 1 to 7 days, which may be biphasic, before developing neurologic symptoms (Fig. 153.7). Although in most cases the prodrome is nonspecific, 15% to 20% of patients have features suggestive of WNV fever, including eye pain, facial congestion, or a rash; fewer than 5% have lymphadenopathy.[350,351] In recent outbreaks, approximately two-thirds of hospitalized patients had encephalitis (with or without signs of meningeal irritation), and one-third had meningitis.[149,352,353] Acute flaccid paralysis caused by virus infection of the anterior horn of the spinal cord (myelitis) has been recognized in recent outbreaks.[354–356] The clinical picture suggests poliomyelitis; paralysis is frequently asymmetrical and may or may not be associated with meningoencephalitis. Once paralysis is established, little long-term improvement has been described. Although convulsions occurred in about 30% of patients in the early descriptions of WNE, they did not appear to be an important feature in the more recent outbreaks.[353,356] Other neurologic features include cranial neuropathies, optic neuritis, and ataxia. Stiffness, rigidity, spasms, bradykinesia, and tremors,

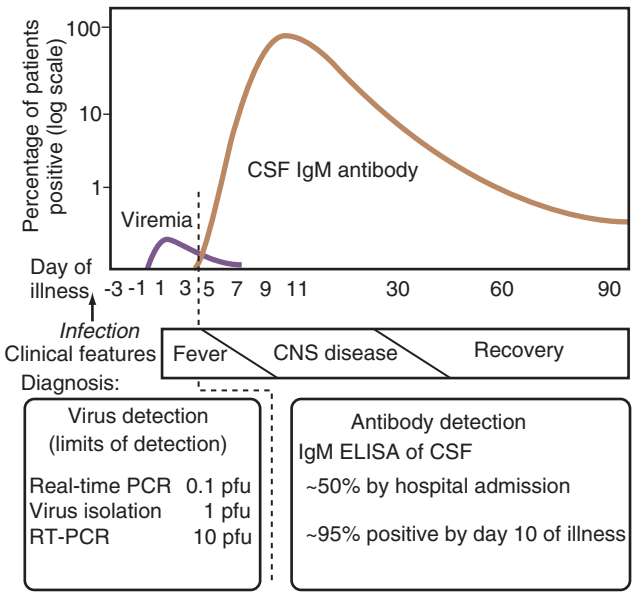

FIG. 153.7 Schematic representation of the clinical course of West Nile encephalitis. Schematic includes viremia, development of antibody, and implications for diagnosis (as approximate percentage of patients). Limits of virus detection are expressed as plaque-forming units *(pfu)* per 100 µL; human viremia is believed to be less than 10 pfu/100 µL. The first day of fever is taken as the first day of illness; most patients are not admitted to a hospital until day 3 to 5 of illness. *CSF,* Cerebrospinal fluid; *CNS,* central nervous system; *ELISA,* enzyme-linked immunosorbent assay; *IgM,* immunoglobulin M; *PCR,* polymerase chain reaction; *RT,* reverse transcriptase. *(From Solomon T, Ooi MH. West Nile encephalitis.* BMJ. *2003;326:865–869.)*

associated with basal ganglia damage, have also recently been recognized in WNE.[356,357]

In recent outbreaks, overall case-fatality rates for hospitalized patients ranged from 4% to 14% but were higher in older patients.[352,353,356] Other risk factors for death include the presence of profound weakness, deep coma, failure to produce IgM antibody, impaired immunity, and coexisting illness such as hypertension or diabetes mellitus.[353,358] Neurologic sequelae are common among survivors. In one study, one-half of hospitalized patients still had a functional deficit at discharge and only one-third had recovered fully by 1 year.[359]

About 50% of patients have a peripheral leukocytosis, and 15% have leukopenia.[353,359] Hyponatremia sometimes occurs in patients with WNE. Examination of the CSF typically shows a moderate lymphocytic pleocytosis, although sometimes there are no cells, or neutrophils may predominate. The protein is moderately elevated, and the glucose ratio is typically normal. Magnetic resonance imaging (MRI) may show high signal intensities in the thalamus in T2-weighted images and diffusion-weighted images in some patients, although this is a late finding and occurs in the more severely ill patients.[356,357] Electroencephalograms show diffuse slowing and, in some cases, focal seizure activity. Nerve conduction studies typically show the reduced motor axonal amplitudes consistent with anterior horn cell damage, although there may also be some slowing of conduction velocities and some changes to sensory nerves.[335]

St. Louis Encephalitis

SLE has been classified into three syndromes, characterized respectively by constitutional symptoms and headache (febrile headache), aseptic meningitis, and fatal encephalitis.[1,152,157] The proportion of cases in each category is age dependent, with increasing proportions of encephalitis and fatal cases in adults, especially in elderly people. The illness usually begins with a febrile prodrome of malaise, fever, headache, and myalgias, sometimes with upper respiratory or abdominal symptoms, that evolves over several days to more than 1 week with progressive lethargy, periods of confusion, and the onset of tremors, clumsiness, and ataxia. Vomiting and diarrhea are common, and some patients complain of dysuria, urgency, and incontinence.

Altered consciousness, marked by confusion, delirium, or somnolence, is the predominant presenting feature, and generalized motor weakness is more usual than are focal signs. Indications of meningeal irritation are inconstant and are elicited more often in children. Mental clouding may be subtle and manifested only by slight disorientation. Most patients do not progress to deep coma. Tremulousness involving the eyelids, tongue, lips, and extremities is usual, and cerebellar and cranial nerve signs are common.[155,360] Various abnormal movements may be present, including myoclonic jerks and nystagmus. Convulsions are infrequent and signal a poor prognosis, except in children; subtle motor seizures also have been reported. Most patients improve over several days; however, pneumonia, thrombophlebitis and pulmonary embolism, stroke, gastrointestinal hemorrhage, and nosocomial infection can complicate recovery. The mortality rate is 8% overall and 20% among patients older than 60 years of age. In recovered adults, asthenia, emotional lability, anxiety, irritability, forgetfulness, tremor, dizziness, and unsteadiness may persist for months, accompanied by tremor, asymmetrical deep tendon reflexes, and visual disturbances.[361] No cases of clinical relapse or progressive illness have been described. Infants and young children frequently exhibit significant neurologic sequelae when discharged, but psychomotor function is usually recovered on later follow-up.[362] Little is known of the risk or outcome of congenital infection. HIV-positive individuals appear to be at greater risk for acquisition of SLE, but whether this is related to their immune status or to other factors (e.g., increased risk for exposure due to homelessness) is not clear.[154]

The peripheral white cell count may be slightly elevated. In some patients, microscopic hematuria, proteinuria, and pyuria have been reported. Hyponatremia due to syndrome of inappropriate secretion of antidiuretic hormone (SIADH) occurs in more than one-third of patients, and the concentrations of ALT and creatine phosphokinase may be slightly elevated. One-third of patients have an increased CSF opening pressure, and there is typically a moderate mononuclear pleocytosis, with an elevated protein concentration. The electroencephalogram shows diffuse slowing and seizure activity, including periodic lateralized epileptiform discharges. MRI may show high signal intensity in the substantia nigra.[363] The diagnosis should be suspected if the case is one of a cluster in the summer or early fall, especially if the patient is an elderly or homeless person. A cerebral ischemic event, heat stroke, medication or drug toxicity, or other cause of delirium or encephalopathy has frequently been the initial diagnosis in confirmed cases. Other infectious causes of aseptic meningitis or acute encephalitis, including WNE, which can be transmitted contemporaneously, cannot easily be distinguished on a clinical basis.

Tick-Borne Encephalitis (Including Powassan Encephalitis)

Infection leads to symptoms of TBE in as many as 1 in 3 persons. Three-fourths of patients are able to recall a tick bite occurring a median of 8 days (range, 4–28 days) before symptoms developed.[364–366] The illness usually begins with a nonspecific grippe of fever, malaise, headache, nausea, vomiting, and myalgias that may be accompanied by fasciculation. Within 1 week, these symptoms resolve spontaneously. In most patients who have the "febrile form" of the disease, there are no further symptoms; in others who progress to more severe illness, the remission of symptoms is temporary, usually 2 to 8 days (range, 1–20 days), before high fever, headache, and vomiting resume.[158] The second phase may be limited to a "meningeal form" with aseptic meningitis (commonly in children), or it may manifest as a "meningoencephalitis form," a "poliomyelitic form" with poliomyelitis-like flaccid paralysis, or a "polyradiculoneuritic form" with a Guillain-Barré–like paralysis, which usually resolves spontaneously. Neurologic infections usually are benign in children, whereas severe disease occurs more often in elderly persons. The TBEV-FE form of TBEV is reported as more severe, with fatalities occurring in 20% of hospitalized patients and residual neurologic sequelae in up to 60% of recovered patients.[367] Six percent to 8% of the TBEV-Sib subtype and 1% to 2% of the TBEV-Eu subtype infections are fatal. Although differences in hospital admission rates may have confounded some of

these observations, intrinsic differences in the neurovirulence of different TBEV subtypes have been shown in experimental animal infections.

Early prodromal symptoms may be undetected in children, whose illness in more than two-thirds of cases consists of aseptic meningitis. Altered consciousness, ataxia, tremor, paresthesias, focal signs, and, less often, seizures characterize the presentation with encephalitis. Limb weakness and paralysis usually represent lower motor neuron lesions caused by myelitis or radicular neuritis; paresis may be transient, or it may evolve to permanent weakness and muscular atrophy. The shoulder girdle and upper limb musculature are affected most frequently, and urinary bladder continence and other autonomic functions can also be disturbed. Involvement of cranial nerves III, VII, IX, X, and XI produces gaze and peripheral facial paralysis and dysphagia. The outcome generally is good, especially in children, but the prognosis varies with age. A hemorrhagic syndrome has been reported in some cases and also in a laboratory-acquired louping ill infection.[368]

Sequelae are reported in up to 40% to 60% of patients, most frequently consisting of psychological disturbances such as asthenia, headache, memory loss and decreased concentration, anxiety, and emotional lability. Residual motor abnormalities include ataxia and incoordination, tremor, dysphasia, and in fewer than 5% of cases, specific cranial or spinal muscular paralysis.[364,369] Progressive motor weakness and epilepsia partialis continua (Kozhevnikov epilepsy) are specific syndromes that may reflect a chronic encephalitic process. Pneumonia, heart failure, and other complications associated with prolonged hospitalization have been reported.

Powassan encephalitis is a severe encephalitis with a case-fatality rate of less than 10%. Focal features have occurred in more than 50% of reported cases; in one patient, the clinical presentation with olfactory hallucinations and temporal lobe seizures mimicked herpes encephalitis.[370,371] Significant residua of hemiplegia, quadriplegia, or aphasia may result, and spinal paralysis with residual muscular wasting is similar to the myelitis associated with TBE. Powassan encephalitis continues to emerge; the CDC reported 1 human case in 2001 and 21 human cases in 2016.[55,56] The highest total number of cases and highest incidence continue for the northern United States, with the majority occurring in Minnesota, Wisconsin, and New York. In New York, POWV has spread to the lower part of the state in Westchester County. Mortality rates from neuroinvasive disease in 2015 and 2016 were 17% and 14%, respectively.

Examination of the peripheral blood in TBE discloses leukopenia in the initial phase of illness, and leukocytosis up to 20,000/mm^3 during the second phase, with a transition to leukopenia again before recovery. Thrombocytopenia can also occur in the initial viremic phase.[372] An elevated erythrocyte sedimentation rate and C-reactive protein concentration are common by the time patients present with neurologic disease, and elevated ALT and AST levels and electrocardiographic abnormalities have been reported anecdotally.[365] There is a moderate lymphocytic CSF pleocytosis (average, <100 leukocytes/mm^3). The CSF protein level, although normal at the onset of neurologic symptoms, rises during the next 6 weeks, with an increase in the IgG index, indicating intrathecal synthesis. The albumin CSF-to-serum ratio, indicating disturbed blood-brain barrier permeability, can remain abnormal for as long as 1 year.[364] The number and distribution of CSF lymphocytes and their cell markers differ in TBE and neuroborreliosis, a fact that may have diagnostic value in rapidly identifying patients for antibiotic treatment.[277] Abnormal MRI signals are found most consistently in the thalami and basal ganglia.[373] Electroencephalograms show diffuse slowing in 90% of patients with encephalitis, with or without focal abnormalities.[365]

Although a history of tick bite is not given in all cases, exposure to an endemic focus during the transmission season should trigger suspicion. TBEV is transmitted under the same circumstances as *Borrelia burgdorferi,* and clinically their radicular and aseptic meningitis syndromes can overlap. Anecdotal observations suggest that neurologic symptoms of Lyme disease may occur more often in the context of concurrent TBE, and, conversely, that TBE may be more severe in a dual infection.[292–304,374] TBE results in a more prolonged course of illness and hospitalization than do the other acute encephalitides of presumed viral origin; this pattern and the presence of spinal paralysis may aid in making the diagnosis.

Alkhurma Hemorrhagic Fever

The largest case series of AHFV occurred in patients from 23 districts in Najran Province, Saudi Arabia.[375] From August 2003 to December 2009, there were 148 cases that fulfilled the clinical case definition of AHFV infection. Of the clinical cases, 78 (52.7%) were laboratory confirmed. Sixty-nine (88.5%) patients were diagnosed as inpatients hospitalized for acute febrile illness, and 9 (11.5%) patients were identified by contact tracing. The mean age was 30 years (range, 4–85 years). The majority of patients were male (63%), and the duration of illness prior to hospitalization was 1 to 10 days (mean, 4.9 days). Hospital stay ranged from 1 to 19 days (mean, 6.2 days). Patients were febrile, and two had a biphasic febrile illness. Clinical symptoms included headache (85.9%), malaise (85.9%), arthralgia (83.3%), myalgia (82.1%), nausea and vomiting (71.8%), diarrhea (51.3%), abdominal pain (48.7%), and hemorrhagic manifestations (25.6%). CNS manifestations occurred in 23% and included altered sensorium, confusion, disorientation, and neck stiffness, with four in a coma. Of the 78 patients with laboratory-confirmed infection, 70 (89.7%) were positive for virus by PCR assay. Laboratory abnormalities include elevation in serum creatinine concentration, with one patient having acute renal failure, an increase in liver function test results, hyperbilirubinemia, thrombocytopenia, and leukopenia. The overall mortality in this case series was 1.3%, compared with a mortality of 25% in previous outbreaks.[57]

Kyasanur Forest Disease

The incubation period for Kyasanur Forest disease is believed to be 3 to 8 days, after which illness begins abruptly with fever, headache, chills, vomiting, myalgia, photophobia, and conjunctival suffusion.[376] Facial and conjunctival hyperemia, lymphadenopathy, hepatosplenomegaly, and petechiae are found on examination. Diffuse hemorrhages from the nares, gums, and gastrointestinal tract develop, with hemorrhagic pulmonary edema in 40% of cases and renal failure in severe cases. A biphasic course of illness can occur with neurologic manifestations during the second and third week of illness. Neurologic manifestations include severe headache, stiff neck, changes in sensorium, and CSF pleocytosis.[376] Laboratory findings are similar to those of DHF and Alkhurma hemorrhagic fever, with leukopenia, thrombocytopenia, and elevated hepatic transaminase levels. Patients have detectable viremia up to 12 days after the onset of illness, which is unusual and prolonged compared with other viral hemorrhagic fevers.[177] The case-fatality rate in KFDV infection is 2% to 10%, which is higher than in DHF and lower than was observed in Alkhurma hemorrhagic fever.[177] Convalescence in survivors can be prolonged—up to 4 weeks after acute illness.

Zika Virus Infection

The clinical presentation and course of infection with ZIKV is most consistent with a dengue fever–like illness without hemorrhagic manifestations. The first large outbreak of ZIKV infection occurred on Yap Island, Micronesia, in 2007.[185] During this outbreak, there were 49 confirmed and 59 probable cases of ZIKV illness. The median age was 36 years (range, 1–76 years), and the majority of patients were female (61%). Rash was common in all cases of acute infection, seen in 90%, followed by fever (65%), arthralgia (65%), nonpurulent conjunctivitis (55%), and headache (45%).[185] No deaths were associated with acute infection. Based on an experimental infection in a human volunteer in the 1950s, the incubation period of ZIKV infection was 3 days, with the first symptoms a frontal headache followed by fever.[377] Clinical illness lasted for 5 days and then resolved. Laboratory findings included a mild leukopenia and minimal changes in liver function test results and platelet counts. The clinical signs and symptoms of the majority of cases seen in the 2013 to 2015 outbreaks of ZIKV infection are generally similar to these.[377,378] Gastrointestinal symptoms and mucous ulceration have been observed less commonly. Generally, illness has been mild and self-limited, and lasted for several days to 1 week.[377,378] It is estimated that approximately 80% of ZIKV infections are asymptomatic.

The recent outbreaks of ZIKV infection appear to be more frequently associated with certain neurologic syndromes, particularly GBS.[63,64] The outbreak in French Polynesia had 74 patients who presented with various neurologic syndromes, 42 of whom were classified as having

GBS. In 2015 and 2016, increased rates of GBS were reported in patients with recent histories of ZIKV infection in Brazil and El Salvador.[63,64] The association between cases of ZIKV infection and GBS is currently being investigated in multiple other countries in Central and South America.

Perinatal complications have also been noted in association with the recent outbreaks of ZIKV infection,[63,64] as well as retrospectively in the Yap Island outbreak.[377,379] Most notably, a marked increase in microcephaly was first noted in northeast Brazil in October 2015. As of the first week of January 2016, 3530 cases of microcephaly had been reported in Brazil, compared to an average of 163 per year between 2010 and 2014.[63,64] Cases of microcephaly had extensive intracranial calcifications, lissencephaly, and pachygyria.[377,380] Macular abnormalities have also been noted in children with microcephaly.[63,64]

Vertical transmission with ZIKV has also been reported. ZIKV has been detected in amniotic fluid of live fetuses with microcephaly[377,381] and in tissue specimens from fetal losses,[377,382] including brain tissue.[377,383] The impact and potential pathogenic mechanisms of ZIKV fetal infection are incompletely understood at present. Important questions remain to be addressed, including the effect of pregnancy stage on the fetal risk, and these are now the subject of intense investigation. Based on information available in January 2016, the CDC[377,384] and the European Centre for Disease Prevention and Control issued guidelines for pregnant women during a ZIKV outbreak.[377,385]

As stated, an increase in cases of microcephaly was noted during the outbreak of ZIKV infection in Brazil in 2015. A comprehensive understanding of the mechanisms for this association remains elusive. ZIKV was detected by immunohistochemistry in chorionic villi of one case of spontaneous first-trimester miscarriage and in amniotic fluid sampled at gestational week 28 in two cases of microcephaly.[377,383,386] In two cases of microcephaly with death within the first day after birth and one case with medical termination of pregnancy at gestational week 32, ZIKV RNA was detected only in fetal brain tissue.[377,383,387] These data suggest that initial fetal infection via placental tissue is followed by localization of ZIKV replication in the fetal CNS. Much has been learned but much remains unknown about ZIKV's tissue tropism and pathophysiologic potential (see Fig. 153.5).

ZIKV is unique among the flaviviruses for being concentrated in semen, with documented sexual transmission and infection of the partner.[377,388,389] ZIKV was detected by RT-PCR in semen up to 62 days after acute infection.[377,388] Sexual transmission of ZIKV changes the nature of the transmission dynamics of this virus from a seasonal vector-borne disease to potentially a continuous sustained sexual transmission in both the heterosexual and the men who have sex with men populations.[377,390]

LABORATORY DIAGNOSIS

Viral isolation is relevant to the diagnosis of suspected flavivirus infection during the early illness phase when patients are viremic and viral infectivity titers in blood are sufficiently high. In the case of the flavivirus encephalitis-producing viruses such as JEV and WNV, mammalian hosts are considered dead-end hosts, because viral load is minimal and titers are insufficient for isolation. However, real-time RT-PCR has become the technique of choice to detect viremia for all the flaviviruses.[268,391,392] In the case of DENV, identifying the infective dengue viral type is important chiefly for public health reasons, but an individual patient also may benefit because future exposure to other DENV types places the patient at higher risk for DHF. In general, viral recovery from blood is successful only before an antibody response develops. Contrary to expectation, the isolation of neurotropic viruses from the CSF is usually unsuccessful except in the early stages of fulminant illness (Fig. 153.8).

Tissue samples, whether from biopsy or autopsy, ideally should be divided into aliquots that are frozen at −70°C for viral isolation and fixed in buffered formalin and glutaraldehyde for light and electron microscopy. Viscerotomy liver samples are frequently taken after death as a means of postmortem YF diagnosis, but because pathologic changes are not pathognomonic, a purely histologic diagnosis should be considered presumptive and should be supplemented with immunohistochemical staining using virus-specific antibodies. Liver biopsy should never be attempted from patients with suspected YF, because they are at risk for fatal hemorrhage. SLE virus, WNV, TBEV, and JEV have been isolated from brain, lung, liver, spleen, and kidney with varied success, depending on the duration of illness and the day of death. Diverse areas of the brain and spinal cord should be sampled. JEV is rarely cultured from peripheral blood using conventional techniques, but culture of virus from white blood cells separated from blood clots may be positive.[393] SLE virus was also isolated from vitreous humor in one case. Suckling mice and C6/36 or AP61 mosquito cell cultures are the most sensitive systems for viral isolation, but Vero, LLC-MK2, PS, and other continuous vertebrate cell lines are also used.[394]

Multiplex PCR assays in various formats that simultaneously identify the presence of DENV and its serotype in serum samples are used only in specialized laboratories in the United States and several Asian, African, and South American countries.[395] YF virus genomic sequences have also been detected in blood, but clinical experience is limited. In patients with flavivirus encephalitis, RT-PCR assay of the CSF has not proved very useful, although it may have a role in the detection of TBEV in acute serum. However, real-time (TaqMan) PCR assay has proved sensitive in detecting WNV in the CSF and serum and has been used for screening of blood products.[146,395] PCR assays have been developed

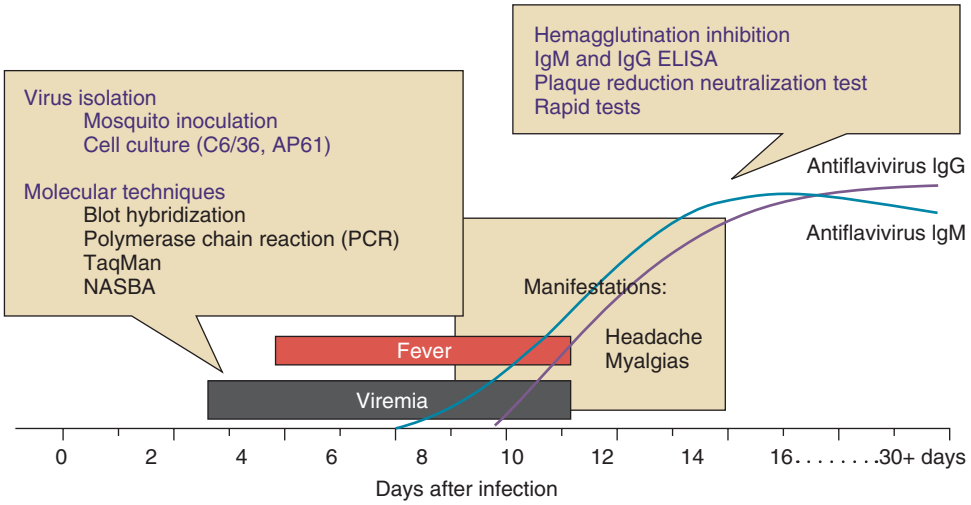

FIG. 153.8 Schema for the types of diagnostics appropriate for day after infection in most flavivirus infections. *ELISA,* Enzyme-linked immunosorbent assay; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *NASBA,* nucleic acid sequence–based amplification.

for the detection and quantification (viral load) of all the flaviviruses and in many ways are the preferred diagnostic tests of choice during the viremic period owing to speed (<4 hours in many assays) and high degree of sensitivity and specificity.[395]

Laboratory diagnosis of most cases, especially in travelers who come to clinical attention after viremia has cleared, depends principally on the serologic testing of serum and, in the case of neurologic infections, the CSF.[396] IgM detection by antibody capture enzyme-linked immunosorbent assay (ELISA) is the preferred technique for a specific diagnosis, although some laboratories successfully detect IgM and IgG antibodies by indirect immunofluorescence assay. The assay is more than 95% sensitive when serum specimens obtained between 7 and 10 days after the onset are tested. In secondary flavivirus infections, a combination of IgM and IgG ELISA is 100% sensitive as early as 4 to 5 days after the onset of illness. Both CSF and serum should be examined in cases of flavivirus encephalitis because IgM may appear earlier in the CSF. If both specimens are tested, positive results are obtained in almost all patients by 10 days after the onset of illness, with, in general, a 10% increase in cumulative positivity per day (see Fig. 153.7). However, some patients die without making an antibody response.[335] Serum IgM in DENV infection declines to undetectable levels within 60 days, but antibodies persist for up to 9 months in recovered SLE and TBE patients and for longer than 16 months in some WNE patients, potentially limiting the specificity of tests.[397]

Heterologous reactions with other flaviviruses are problematic where numerous flaviviruses cocirculate, owing to extensive serologic cross-reactivity between flaviviruses. In Asia, five flaviviruses—DENV, JEV, ZIKV, and in specific locations WNV and KFDV—may cocirculate with a high degree of serologic cross-reactivity; in Africa, YF virus and DENV cocirculate; and in South America, YF virus, DENV, and SLE virus cocirculate. Recent vaccination against JE or YF, or recent infection with dengue or SLE virus, may cause a false-positive WNV IgM antibody test result; neutralization assays such as the plaque reduction neutralization test and ELISA that detect antibody to NS5 are more specific.[398] Fractionation of IgM before hemagglutination inhibition testing and competitive epitope-blocking ELISA improve specificity. However, among sera from African patients, all serologic approaches frequently fail to resolve previous and recent infections. Heterologous flavivirus antibodies have become an issue even among specimens submitted in the United States for arboviral diagnosis. Previous DENV infection, reflecting prior exposures in persons who have resided abroad, is now a frequent finding that can interfere with interpretation of the serologic diagnosis of a recent flavivirus infection. These heterologous dengue antibodies frequently pose difficulties in the interpretation of tests for SLE, WNE, and Powassan encephalitis. Although neutralization tests provide the greatest specificity, they are time consuming, are expensive to perform, are offered only in specialized laboratories, and require control reference sera to obtain reliable results. Hemagglutination inhibition and complement fixation tests are now used infrequently, but they still have utility under some circumstances. Complement fixation antibodies, which recognize NS1 protein, are relatively specific in distinguishing between antigenic complexes, and because they rise rather late (often 4–6 weeks after the onset) and decline with a half-life of 3 years, positive reactions indicate infection in the intermediate period after the disappearance of IgM antibodies. Hemagglutination inhibition and neutralizing antibodies can persist for decades after infection. Rapid immunochromatographic tests formatted as small folders to detect dengue and JE IgM and IgG have demonstrated high sensitivity and specificity in field evaluations (100% and 90%, respectively, for the dengue test) and should facilitate laboratory confirmation of cases in clinical facilities.[399] NS1 protein is present in serum early in DENV infection and can be detected by antigen capture ELISA or rapid immunochromatographic tests.[400]

In a patient with a compatible illness, a case is confirmed by a fourfold change in the serum antibody titer or, alternatively, in encephalitis patients, by the demonstration of viral-specific IgM in CSF, reflecting intrathecal immune response. An elevated serum IgM antibody level alone is considered presumptive evidence of recent infection if high IgM prevalence rates in the population prevail because of frequent asymptomatic infections and because antibodies may persist beyond a single transmission season.

Serologic testing for SLE virus, WNV, DENV, and other selected arboviruses is performed at several private laboratories, most state laboratories, the CDC, the US Army Medical Research Institute of Infectious Diseases, and other reference laboratories. In addition, an indirect immunofluorescence assay kit for the domestic arboviruses that includes an SLE antigen can be purchased in the United States, and a TBEV ELISA kit can be obtained in Europe. Dengue immunochromatographic folders and ELISA kits are sold in Asia and Australia.[401–404]

A single IgM assay (ZIKV *Detect* 2.0 IgM Capture ELISA [InBios, Seattle, WA]) has been granted a marketing authorization by the US Food and Drug Administration (FDA) (May 2019).[404a] Laboratory testing is available through the CDC Arbovirus Diagnostic Laboratory, various state health laboratories, and the Pan American Health Organization/WHO. Some state health departments in the United States also perform ZIKV laboratory testing. Detection of ZIKV can be established by RT-PCR, and virus is present in the bloodstream within the first few days of illness.[401,402] ZIKV RNA is present in the blood for only a relatively short period after onset of illness (3–7 days). Saliva or urine samples collected 3 to 5 days after onset of illness may also be positive.[401,403] ZIKV may be present in urine samples up to 20 days or longer after the onset of illness.[401,404] ZIKV has also been detected in semen,[191,405] and possible sexual transmission has been reported.[191,406] Serologic tests can also be used to detect ZIKV infection and may become positive 4 or more days after onset of illness. IgM antibody determinations by immunofluorescence, ELISA, or neutralizing antibody assays are being used. Neutralizing antibodies should be greater than or equal to fourfold higher than anti-DENV antibodies to indicate the presence of specific anti-ZIKV antibodies.[401,404] Because of cross-reactions with other flaviviruses, antibody testing to DENV, chikungunya virus, and WNV should also be carried out.

PREVENTION AND THERAPY

Yellow Fever

Hospitalization in an intensive care facility where the patient can be sequestered from mosquitoes is recommended to provide close clinical monitoring and supportive care and to prevent anthroponotic transmission. Blood in the acute phase of illness is potentially infectious. No antiviral therapy is available, and specific supportive interventions have not been clinically evaluated. Exploratory studies in small animal models and nonhuman primates of various compounds (e.g., interferons, ribavirin) have had varied results.[407] Recent efforts to develop a therapeutic agent have focused on directly targeting viral proteins or key host proteins involved in viral replication.[408,409] Supportive care with oxygen, fluids, and pressors is indicated to treat and prevent hypotension and metabolic acidosis. Histamine type 2 receptor antagonists and sucralfate may be of value in preventing or ameliorating gastric bleeding. Avoidance of sedatives and drugs dependent on hepatic metabolism is prudent, and the medication dosing intervals should be adjusted with reduced renal function. Encephalopathy should be investigated for treatable metabolic causes, particularly hypoglycemia. Fresh-frozen plasma and vitamin K have been administered to replenish clotting factors. The effect of heparin therapy is unproven. Secondary infections should be pursued and treated.

YF is preventable with the attenuated 17D vaccine, which produces immunity in more than 95% of recipients and long-term (at least 10 years and possibly lifelong) protection with a single 0.5-mL subcutaneous dose.[6] The WHO updated the recommendations for vaccination in 2013. It was recommended for healthy immunocompetent individuals that a single dose of vaccine is sufficient to confer lifelong protective immunity against YF disease and that a booster dose every 10 years is not necessary. The recommendations for immunocompromised and other special groups were not changed. Subsequently, some countries have adopted this recommendation, including Brazil, which updated its policy in 2017. In 2015 the US Advisory Committee on Immunization Practices updated its recommendations so that most travelers do not require booster vaccinations. However, certain groups still require booster doses, including travelers who had received a vaccine dose more than 10 years previously and plan to spend a prolonged period in endemic areas or those traveling to an area with an ongoing outbreak. In addition, booster doses should be given to laboratory workers and immunocompromised

individuals, and women who were pregnant when they received their first dose of vaccine should receive a booster dose before they travel.[408,409]

Vaccination has been associated with anaphylaxis in 13 of every million doses distributed, but the relative roles of hypersensitivity to chicken eggs and gelatin are unclear because the vaccine is produced in chick embryos, and gelatin has been removed.[410,411] Equally serious, however, are rare but potentially fatal cases of vaccine-associated CNS infection or systemic illness, mimicking wild-type infection, which have been reported from multiple countries. To date, 100 suspected or probable cases of yellow fever vaccine–associated viscerotropic disease (YEL-AVD) have been reported. All known cases occurred in primary vaccinees who ranged in age from 10 months to 81 years (mean, approximately 45 years). Clinical signs of high fever, arthralgia, myalgia, headache, and vomiting usually occur within 2 to 5 days after immunization and are followed by elevated liver enzyme and bilirubin levels and thrombocytopenia and lymphocytopenia. Subsequently, combinations of fulminant hepatitis, shock, renal failure, and disturbances of coagulation, all consistent with YF, have been observed. When laboratory tests for virus have been undertaken, large quantities of vaccine virus are detected in tissues or blood. The clinical and laboratory picture, plus the histology and immunohistochemistry at autopsy, is similar to those seen with wild-type YF infection. The case-fatality rate is over 60%. There is little evidence in support of mutation in the vaccine virus as a cause of YEL-AVD; rather, host factors are thought to be responsible. Since 1989 there have been 113 cases of yellow fever vaccine–associated neurotropic disease (YEL-AND), including cases of meningoencephalitis, acute disseminated encephalomyelitis, and GBS, but only 1 has been fatal, and the majority of cases are aseptic meningitis. Clinical signs of headache and fever (>38.6°C [101.5°F]), focal neurologic dysfunction, and altered mental state are first seen from 2 to 30 days after immunization and are accompanied by CSF pleocytosis or elevated protein.[412–414]

The reported rates of YEL-AVD and YEL-AND vary in publications. In part, this is due to differences between travelers and those who live in endemic areas. In travelers, the rate of YEL-AVD is 2.5 to 4 cases per million doses and that for YEL-AND is 2.5 to 8 cases per million doses.[413,415] Both rates are low and have resulted in no change in vaccine recommendations for travelers. Not surprisingly, rates are lower in endemic areas at 0.13 to 3 cases per million doses for YEL-AVD and 0.16 to 11 cases per million doses for YEL-AND. Because of the well-established risk for vaccine-associated encephalitis in infants, YF vaccine is contraindicated in infants younger than 4 months of age and is recommended for 4- to 9-month-old infants only under situations of high risk. YF and measles vaccines are coadministered at the 9-month Expanded Program of Immunization visit under WHO–United Nations Children's Fund recommendations in 35 African countries. The potential risk of administration of vaccine in pregnancy is unclear. Cord blood IgM viral antibodies indicating congenital infection were reported in one case without evidence of birth defects.[416,417] In one study the vaccine was less immunogenic in pregnant women (39% seroconversion),[421] whereas in another it was as immunogenic (95% seroconversion)[417a] as in the general population. A recent study reported 65 of 78 (83%) HIV-infected adults develop neutralizing antibodies compared with controls (64 of 66; 97%), and this has been followed up with a study of the same cohort 10 years postvaccination that showed long-term immunity is largely dependent on whether or not HIV replication was controlled at the time of vaccination.[418,419] For those taking a successful combination antiretroviral therapy regimen (CARTR), the immune response up to 10 years was comparable to that of non–HIV-infected adults. Thus vaccine boosters were recommended after 10 years for individuals who are on a successful CARTR, while individuals whose HIV RNA levels are uncontrolled may need a booster earlier. No adverse events were reported in pregnant women, but a fatal CNS infection has been reported in one HIV-infected vaccinee.[416,417,420–426]

The YF vaccine is only manufactured by six producers worldwide and so periodically vaccine supplies are exhausted when large outbreaks of disease take place, requiring a response with large quantities of vaccine.[422,423] In 2016, there was a large outbreak of YF involving Angola and subsequently the DRC. During the first 6 months of 2016, the world's supply of vaccine was exhausted twice, and in the summer of 2016 the disease spread to the DRC, including urban YF in the capital Kinshasa. It was clear there was insufficient vaccine to immunize everyone and so fractional dosing of the vaccine was undertaken.[422,424] Previous studies with the 17DD substrain vaccine manufactured in Brazil had shown that fractional dosing would work. Seven hundred forty-nine adult males in the Brazilian army were recruited to a double-blind, randomized clinical trial to test for immunologic noninferiority, over 12 months of different doses of 17DD vaccine. A "full dose" of 27,476 international units (IU) was compared to five lower formulations: 10,447, 3013, 587, 158, and 31 IU, respectively.[422,425,426] No serious adverse events were reported from any groups. Seroconversion was 97% or higher, except in those who received fractions lower than 587 IU. Analysis of serum cytokines/chemokines in association with neutralization titers and viremia supported the use of a 10-fold lower subdose (3013 IU) of vaccine that would be equivalent to that given with a full dose of vaccine. Based on these data and availability of the Brazilian 17DD vaccine, fractional dose YF vaccination, an off-label use of the product, was considered in response to an emergency situation in which current vaccine supply was insufficient. A preventive vaccination campaign was launched in the DRC on 17 August 2016. The campaign aimed to immunize over 8 million people in 32 Health Zones in Kinshasa province using the fractionated dose strategy, in which the vaccine was administered at one-fifth of the standard vaccine dose; this strategy was only recommended for use in an emergency situation in the context of limited vaccine availability, and in those over 2 years of age and excluding pregnant women. One-fifth of a dose was selected for practicality because a full dose is given in a volume of 0.5 mL, and 0.1 mL could be given using tuberculin syringes without the need for dilution of the vaccine. The vaccination campaign proved successful and the outbreak was controlled.

The 17DD substrain vaccine is only produced in Brazil, and the other producers use 17D-204 or 17D-213 substrain vaccines. Consequently, clinical trials are ongoing to evaluate fractional dosing for vaccine made by all the producers to ensure that all vaccines can be fractional dosed in emergency situations.

As stated earlier, a large outbreak of YF took place during later 2016 to mid-2018 in Brazil, where the virus has spread to new regions where there are large population centers. Vaccine supply was exhausted and, for the second time in the history of the vaccine, a fractional dose vaccination regimen was employed. In January 2018, Brazil launched a mass immunization campaign to give fractional doses of 17DD vaccine to approximately 24 million residents of 69 municipalities in the states of Rio de Janeiro and São Paulo.

Travelers to at-risk South American and African countries should receive the vaccine either once or at 10-year intervals to meet current national requirements. The vaccine can be given concurrently with measles, oral polio, hepatitis A or B, meningococcal polysaccharide, oral or intramuscular typhoid, or oral cholera vaccines; chloroquine; or immune serum globulin.

Prevention of epidemic *A. aegypti*–borne YF follows the approach for dengue control, with the reduction of peridomestic breeding sites. In dry savanna and urban locations where drinking water frequently must be stored, the simple expedient of covering the containers or reservoirs eliminates a principal source of breeding. Surveillance of viral activity by monitoring of viral infection rates in sylvatic mosquitoes has been proposed as an early warning system for West and Central Africa, where outbreaks frequently emerge in a region-wide distribution. The discovery of intensified viral activity, even in a small number of sentinel sites, may be a sufficiently sensitive predictor of viral activity in a broader area to trigger timely and effective mass immunization. In South America, surveys to detect dead monkeys on the forest floor are conducted to monitor viral transmission and risk for its spillover to humans.

Dengue and Dengue Hemorrhagic Fever

Management of dengue is straightforward and highly effective at reducing morbidity and mortality if properly executed. The key is to recognize the disease phase the patient is experiencing at presentation or is transitioning to (i.e., febrile, critical, recovery) and to implement, in a judicious and controlled manner, the required interventions to comfort and address key pathophysiologic mechanisms, mainly plasma leakage

and hemorrhage.[112] When fatalities do occur, one or more of the following issues is often at fault: late presentation to medical care, delayed escalation of care, and overaggressive provision of intravenous fluids. Therefore it is essential that patients, parents or guardians of patients, and clinicians are cognizant of the warning signs and symptoms for plasma leakage and intravascular volume depletion, such as abdominal pain, nausea, vomiting, lethargy, mental status changes, decreased urine output, tachycardia, and decreasing pulse pressure, so that supportive therapy can be administered promptly. Clinicians must also follow the clinical and biochemical clues of volume status to avoid contributing to iatrogenic volume overload. Comorbidities such as hypertension, diabetes, and pulmonary and cardiac disease increase the risk for severe disease and a poor outcome.[427,428] Evidence-based management algorithms exist and are highly effective.[112,429]

Acetaminophen-based products may be used to reduce fever and treat myalgias and arthralgias. Acetylsalicylic acid (aspirin), ibuprofen, or other nonsteroidal antiinflammatory agents may aggravate hemorrhagic complications or induce Reye syndrome and should be avoided. Oral rehydration with fluids containing electrolytes and sugars is encouraged to counter vomiting and diarrhea.

Attentive clinical monitoring of patients with suspected severe dengue (DHF-DSS) and anticipatory and supportive care, especially at the time of defervescence, are lifesaving and have reduced fatality rates 50- to 100-fold. The critical activities are monitoring the adequacy of circulation and vascular leakage, by serial clinical assessments of pulse, blood pressure, skin perfusion, urine output, and hematocrit, to trigger intravenous fluid therapy. An increase in hematocrit of greater than 20% (e.g., from 35% to 42%) indicates a significant loss of intravascular volume and the urgent need for fluid resuscitation. Normal saline is administered to maintain circulation and, under continued monitoring, to treat recurrent shock. Shock necessitates rapid intervention with isotonic crystalloid or colloid solutions or, if needed, plasma or whole-blood transfusions.[430] As a result of the danger of acute respiratory distress syndrome (ARDS) due to excessive fluid administration, DHF-DSS has been reported to be the third most common cause of ARDS in hospitalized children in Malaysia.[330] Whole-blood, platelet, and fresh-frozen plasma transfusions may be needed if there is significant hemorrhage, but caution is indicated in the administration of heparin except in patients with clear signs of disseminated intravascular coagulopathy.[431] Preventive transfusions may be harmful and should be avoided, and invasive procedures should be minimized to avoid hemorrhagic complications. Treatment to end virus replication could be beneficial, although viremia levels usually are already decreasing dramatically at the time of presentation to health care providers.[432] Controlled clinical trials have shown no benefit for corticosteroids.[433,434] Numerous other antiviral and immunomodulatory drugs have been proposed for use, such as intravenous immune globulin, pentoxifylline, and chloroquine, and some have been in wide use in dengue-endemic areas, but none has been established to have clinical benefit.[435–442]

In the absence of a vaccine suitable for widespread use, dengue prevention relies on community-based *A. aegypti* control programs to remove and destroy mosquito breeding sites.[98] The ubiquity of containers that potentially provide breeding habitats in urban neighborhoods and individual houses makes this a formidable challenge. Although a combination of vector surveillance, area treatment, and monitoring can be effective, it has rarely been successful for prolonged periods. Insecticidal fogging is considered unhelpful, but indoor residual insecticidal sprays should be effective in sealed houses.[443–445] Insecticide-treated curtains may be effective; bed nets are not useful because the mosquitoes are most active during the day.[443–445]

A live-attenuated tetravalent vaccine against dengue, containing four recombinant chimeric flaviviruses each constructed from the YF virus 17D genome with PreM and E gene segments replaced by the corresponding segments of one DENV serotype (Dengvaxia), has been licensed in 20 countries for prevention of dengue in healthy individuals 9 to 45 years of age. In two large phase III clinical trials, the vaccine showed overall efficacy of 56.5% and 60.8% in Asia and Latin America, respectively, against virologically confirmed dengue during the first year after completion of the three-dose vaccination schedule (0, 6, and 12 months).[446–449] There were higher rates of efficacy for preventing hospitalized dengue (80.3%) or severe dengue (95.5%).[446,448] However, efficacy against DENV-2 (47%) was significantly lower than against DENV-1 (58%), DENV-3 (74%), or DENV-4 (83%), and overall efficacy was low in seronegative individuals. Furthermore, there was an increase in the relative risk of hospitalized dengue in the youngest vaccinated cohort (2- to 5-year-olds) during the second year following vaccination.[446,450] Further analyses have confirmed a lack of efficacy, and a higher risk for hospitalized dengue, among subjects in the targeted age group (9- to 16-year-olds) who were seronegative for dengue at baseline.[446,451] As a result, this vaccine is not recommended for use without laboratory confirmation of prior DENV infection.

Two other live-attenuated tetravalent dengue vaccines are currently in phase III trials. These vaccines differ in composition from Dengvaxia. One vaccine contains a recombinant attenuated DENV-2 strain and three recombinant chimeric viruses constructed from the DENV-2 genome with pre-M and E gene segments of the other DENV types. The second vaccine contains recombinant strains of DENV-1, DENV-3, and DENV-4 containing engineered attenuating mutations and a chimeric virus constructed from the DENV-4 genome with pre-M and E gene segments of DENV-2. It is hoped that inclusion of DENV nonstructural gene segments in these vaccines will eliminate the increased risk observed with Dengvaxia. Several vaccines based on other platforms, including purified inactivated viruses adjuvanted with novel adjuvants, recombinant proteins, and plasmid DNA, are in earlier phases of development.[9,452–457]

Zika Virus Infection

Vaccines or antivirals are not available against ZIKV infection, although several vaccines are in clinical development and undergoing human clinical trials.[458,459] DNA and purified inactivated Zika vaccine candidates that have reported phase I human data indicate the safety profile is acceptable, and immunogenicity, as measured by neutralizing antibody, is also supportive of advancing clinical development.[458,460–462] It is unclear, however, if the current diminution of Zika cases will support pursuit of a clinical end point study.

Treatment of acute Zika cases is generally supportive and may include rest, fluids, and use of analgesics and antipyretics. Fever should be treated with acetaminophen. Aspirin and nonsteroidal antiinflammatory drugs should be avoided until dengue can be ruled out, to reduce the risk of hemorrhage.

For prevention of infection, measures to avoid mosquito exposure in areas of potential transmission should be taken, including wearing long sleeves and pants, applying insect repellent, and staying indoors in areas well protected by windows, screens, and mosquito nets. Environmental control measures to eliminate mosquito breeding areas should be implemented, particularly reduction of standing water containers and tanks.

The CDC and the European Centre for Disease Prevention and Control have issued guidelines for pregnant women during ZIKV outbreaks.[377,384,385] The CDC recommends that pregnant women consider postponing travel to an area where ZIKV transmission is ongoing. If travel is necessary, steps to prevent mosquito bites should be taken.

Recommendations for pregnant women with a history of travel to an area of ZIKV transmission have also been formulated.[377,384] Women who traveled to such areas should be evaluated for ZIKV infection. Women who report a travel history and who have two or more symptoms consistent with ZIKV infection (fever, onset of rash, arthralgias, or conjunctivitis) should have laboratory tests for ZIKV infection (see "Laboratory Diagnosis" earlier). Women with a travel history and fetal ultrasound findings of microcephaly or intracranial calcifications should also be tested. If laboratory testing is inconclusive, an amniocentesis can be considered. Testing is not recommended for women with a travel history but no other findings.

For pregnant women with laboratory evidence of ZIKV infection, serial ultrasounds should be considered to monitor fetal anatomy and growth every 3 to 4 weeks. Referral to a maternal-fetal medicine or infectious disease specialist with expertise in pregnancy management is recommended.

For a live birth with evidence of maternal or fetal ZIKV infection, the following tests are recommended: histopathologic examination of

the placenta and umbilical cord, testing of frozen placental and cord tissue for ZIKV RNA, and testing of cord serum for ZIKV and DENV IgM and neutralizing antibodies. If a pregnancy results in fetal loss in a woman for whom laboratory testing is recommended, ZIKV RT-PCR and immunohistochemical staining should be performed on fetal tissues. Because of the potential sexual transmission of ZIKV, the CDC has formulated guidelines for prevention of sexual transmission (discussed earlier).[191,193]

Flavivirus Encephalitis

No specific antiviral therapy for flavivirus encephalitis has been developed; current treatment options are supportive. Anecdotal use of IFN-α in the prophylaxis and treatment of JE and SLE cases has been reported, but a phase III randomized, double-blind, placebo-controlled trial of IFN-α2a in children with JE showed that it did not improve the outcome at hospital discharge or at 3 months' follow-up.[463] A randomized controlled trial of oral ribavirin versus placebo in children with JE in Uttar Pradesh, India, failed to demonstrate a difference in outcome.[464] Ribavirin was employed during a WNV outbreak in Israel in 2000, with a high mortality (41%) observed in the ribavirin group.[352] Corticosteroid therapy for TBE resulted in a more rapid reduction of fever but prolonged hospitalization, whereas in JE a small trial of corticosteroid treatment failed to show benefit or harm.[465] In a small controlled study, tetracycline administered as an immunomodulator was shown to reduce elaboration of proinflammatory cytokines and to hasten recovery from TBE.[466] Antisense oligomers that inhibit WNV replication in vitro have been reported, and an RNA interference screen approach has examined in detail virus and human host cell interactions, identifying potential targets for antiviral treatments.[467,468] In addition, cell-based high-throughput assays have been developed to screen compound libraries for agents effective against WNV and other flaviviruses.[469]

A mixture of JEV-neutralizing monoclonal antibodies was reportedly beneficial in a clinical trial in China, but considerable experience with TBE immune globulin therapy has highlighted the potential hazards of immunotherapy.[470,471] Humanized monoclonal antibodies against WNV envelope protein have shown efficacy in mice and hamsters, even when given as a single dose on day 5 after infection and when the virus has reached the CNS.[472,473] A phase I safety study of a recombinant humanized monoclonal antibody targeting the E protein of WNV was well tolerated.[474] Hyperimmune gamma globulin has been used on a small scale in severe WNV infections with varied clinical outcomes.[475,476] Additional WNV treatment candidates are being explored in small animal disease models and early-phase human trials.[477–485]

Supportive care should focus on controlling seizures, providing ventilatory support in respiratory failure, and monitoring and reducing cerebral edema. In many parts of Asia, mannitol, corticosteroids, or both are given to patients with severe JE. Fluid and electrolyte administration should balance circulatory needs, the avoidance of cerebral edema, and SIADH. Secondary infections should be anticipated and treated, and careful nursing attention should be paid to minimize complications such as bedsores and contractures.

JE vaccines have been available since the 1950s (Table 153.3).[5] Inactivated mouse brain vaccines, containing either Nakayama-NIH

TABLE 153.3 Landscape and Other Details of Japanese Encephalitis Vaccines Licensed to Date

TYPE OF VACCINE	VIRUS STRAIN AND PHENOTYPE	SUBSTRATE	GENERIC NAME, IF ANY	MANUFACTURER AND TRADE NAME	REGIMEN	COUNTRY
Inactivated	Nakayama-NIH; wild-type	Mouse brain	JE-MB	BIKEN, Japan (Biken, JE-VAX); other local producers	Days 0, 7, 28 at 12–24 mo of age; booster after 12–14 mo, then every 3–5 yr	European Union, India, Japan, Malaysia, North Korea, South Korea, Sri Lanka, Taiwan, Thailand, United States, Vietnam
	Beijing-1 (P-1); wild-type	Mouse brain	JE-MB, BK-VJE	GCC, Korea (JEV GCC); other local producers	Days 0, 7, 28 at 12–24 mo of age; booster after 12–14 mo, then every 3–5 yr	European Union, India, Japan, Malaysia, North Korea, South Korea, Sri Lanka, Taiwan, Thailand, United States, Vietnam
		Vero cells	BK-VJE	BIKEN, Japan (JEBIK-V); KAKETSUKEN, Japan (ENCEVAC, JEIMMUGEN); Boryung Pharma, Korea (TC-JEV)	Days 0, 7, 28 at 12–24 mo of age; booster after 12–14 mo, then every 3–5 yr	Japan, South Korea
		Mouse brain		China	Days 0, 7 at 12 mo of age; then 2, 6 and 19 yr of age	China
	Beijing-3 (P-3); wild-type	PHK cells		China		China
		Vero cells		China		China
	SA-14-14-2; live attenuated	Vero cells	JE-PIV, IC51, JE-VC	Intercell/Novartis/Valneva, Austria (IXIARO); CSLB, Australia (JESPECT), BEL (JEEV)	Days 0, 28 as early as 2 mo of age; booster after 1 yr	Australia, Bangladesh, Bhutan, Canada, European Union, Hong Kong, India, Japan, Latin America, Nepal, New Zealand, Pacific Islands, Papua New Guinea, Singapore, South Korea, Switzerland, United States
	821564XY; wild-type	Vero cells		BBIL (JenVac)	Days 0, 28 as early as 6 mo of age	India
Live attenuated	SA-14-14-2; live attenuated	PHK cells	SA-14-14-2	CDIBP (CD.JEVAX)	Single dose at the age of 8–9 mo; if needed, booster 3–12 mo later, and at 6–7 yr of age	China, Hong Kong, India, Japan, Nepal, South Korea, Sri Lanka, Thailand
Chimeric	YFV 17D containing JEV proteins; live attenuated	Vero cells	ChimeriVax-JE; JE-CV	Acambis/Saofi-Pasteur (IMOJEV, THAIJEV)	A single dose for those >18 yr, with a booster, if needed, after 5 yr; for those ≥9 mo and ≤18 yr, primary dose followed by booster after 12–24 mo	Australia, South Korea, Thailand

BBIL, Bharat Biotech International Ltd., India; *BEL,* Biological Evans Ltd., India; *BIKEN,* The Research Foundation for Microbial Diseases of Osaka University, Japan; *CDIBP,* Chengdu Institute of Biological Products, China; *CSLB,* Commonwealth Serum Laboratories Biotherapeutics, Australia; *GCC,* Green Cross Corporation, Korea; *JEV,* Japanese encephalitis virus; *KAKETSUKEN,* Chemo-Sero-Therapeutic Research Institute, Japan; *NIH,* National Institutes of Health, Japan; *PHK,* primary hamster kidney; *YFV,* yellow fever virus.

Note: The mouse brain– and the PHK cell–derived inactivated vaccines have been discontinued.

From Hegde NR, Gore MM. Japanese encephalitis vaccines: Immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. Hum Vaccin Immunother. *2017;13:1–18.*

or Beijing-1 virus strains, were developed in Japan and produced by several manufacturers.[44] For several decades, this JE vaccine was used in the United States and Europe (BIKEN; distributed as JE-VAX by Sanofi Pasteur, Lyon, France).[486–490] Routine vaccination led to decreases in JE incidence in Thailand, India, Korea, Taiwan, Vietnam, and areas of Malaysia and Sri Lanka.[491,492]

The safety profile of mouse brain–derived JE vaccines has been questioned. Acute disseminated encephalomyelitis (ADEM) temporally related to vaccination in Japan prompted the Japanese government to suspend strong recommendations for routine childhood JE vaccination. However, ADEM has been infrequently reported as a severe drug reaction.[493] The Global Advisory Committee on Vaccine Safety found no definite evidence of an increased risk for ADEM temporally associated with JE vaccine.[488–490] Production of JE-VAX by the BIKEN laboratories in Japan has since ceased.[494]

A live-attenuated vaccine (SA-14-14-2) developed by Chinese researchers has been used extensively in China; because issues over the primary hamster kidney cells used in its production have been resolved, it has also been used in South Korea, Nepal, India, and Sri Lanka.[495] Two doses are administered during spring campaigns to children older than 1 year. The live vaccine, however, is highly efficacious after even one dose.[5,495,496] Since its licensure, more than 500 million doses have been produced and administered to more than 200 million Chinese children.[497]

IXIARO is a purified, formalin-inactivated, whole-virus JE vaccine. The product is licensed and distributed in the United States, Europe, Canada, Hong Kong, and Israel and distributed in Australia under the trade name JESPECT.[498] The vaccine construct, developed at the Walter Reed Army Institute of Research (Silver Spring, MD), is based on an SA14-14-2 virus strain passaged in primary dog kidney (PDK) cells, cultivated in Vero cells, and formulated with 0.1% aluminum hydroxide.[499] Several clinical studies established its safety and immunogenicity profile.[500–509] Booster doses given 11 or 23 months after primary vaccination series in recipients whose neutralizing antibody titers had waned to below detection led to 100% seroconversion.[505,506] The initial indication was for adults 17 years of age or older. In May 2013 the FDA extended the age range to include infants, children, and adolescents ages 2 months to younger than 17 years for active immunization.[504,510] The primary vaccination schedule is two doses delivered at day 0 and 28 in a 0.5-mL suspension given intramuscularly. The dosing series should be completed at least 1 week before travel or potential exposure.

Sanofi Pasteur has developed a JE vaccine (JE-CV; IMOJEV) by inserting pre-M and E genes from the SA14-14-2 JEV into the YF 17D viral strain "backbone" containing the YF nonstructural genes.[511–513] The vaccine is currently available in Australia; additional filings for approval have been submitted in Asia. IMOJEV has demonstrated safety and seroprotective immunogenicity after a single vaccine dose.[514–519]

The Advisory Committee on Immunization Practices suggests that physicians and others should counsel travelers to be aware of the low-level, but almost unavoidable, risk for acquiring JE when traveling to JE-enzootic countries.[498] JE vaccination should be considered as an important option to reduce this risk. At-risk individuals, even if exposure is short term (<1 month), should consider vaccination. High-risk activities include those that occur outdoors, near agricultural areas, or during evening hours, and where lodging is in the open without use of bed nets. Vaccination should be considered for travel to a JE-endemic area without a defined destination or to an area with a known JE outbreak. JE vaccine is not recommended for short-term travelers (<1 month) whose visit will be restricted to urban areas or outside the transmission season. Lastly, JE vaccine is recommended for laboratory personnel who work with live, wild-type JEV strains. At-risk laboratory personnel should receive booster doses of JE vaccine or be evaluated regularly for JEV-specific neutralizing antibody titers.[498,520] Although data are lacking, individuals partially or completely immunized with any first-generation inactivated JE vaccine should likely receive the complete immunization series of IXIARO to ensure a protective immune response.[497]

No human vaccines against WNE or SLE viruses are licensed; however, formalin-inactivated and canarypox-vectored WNV vaccines for horses are commercially available in the United States. A live-attenuated chimeric WNV/YF 17D vaccine has advanced to phase II clinical evaluation.[521–523] Chimeric vaccines of WNV combined with DENV-2 or DENV-4 have also been developed and provide complete protection against WNV in challenge experiments.[524]

TBE vaccines currently in use are FSME-Immun (Baxter, Austria), Encepur (Novartis Vaccines, Germany), EnceVir (Scientific Production Association Microgen, Russia), and TBE vaccine Moscow (Federal State Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides, Russian Academy of Medical Sciences, Russia).[525] FSME-Immun and Encepur are available in the European Union and have been authorized by the European Medicines Agency.[526] They are all safe and immunogenic with considerable uptake rates in areas that have a high transmission risk.[55,56,161] All four vaccines seem to give protective immunity against all three subtypes of TBE. Mass vaccination has been practiced in Austria since the 1990s.[527] Administration in three doses over a period of 1 year, with an additional booster 3 years later, has been highly effective in reducing rates of disease. An abbreviated 0-, 7-, and 21- or 28-day immunization schedule also is immunogenic.[528] Cross-protection against TBEV-FE has been shown in animals and in clinical trials. Chiefly, mild adverse events (fever and local reactions) are reported; however, neurologic adverse events, including GBS, have been noted, albeit without a proven causal association, in about 1 of every 1 million vaccinees. TBE vaccine is not licensed in the United States but is available in Canada. For most travelers, the risk for acquiring the disease is extremely low and personal protective measures (e.g., avoidance of risky habitats, wearing protective clothing, using repellents) are appropriate. Expatriates may choose to be immunized abroad. Live-attenuated and recombinant vaccines are under development.

Vaccines for KFDV infection have been in development since the 1960s using varied approaches.[182,529–531] Annual rounds of vaccination using formalin-inactivated tissue-culture vaccine have been conducted in India in the Karnataka State since 1990. Unfortunately, vaccine coverage and effectiveness appears lower than previously reported.[532]

OTHER FLAVIVIRUS INFECTIONS

Murray Valley Encephalitis

Murray Valley encephalitis virus is a member of the antigenic complex of JEV, SLE virus, and WNV, and, like them, it is transmitted in a mosquito-avian cycle, chiefly by *Culex annulirostris*. Foci of perennial viral transmission are maintained in Western Australia, where sporadic cases and small outbreaks occur. Most sporadic cases occur in Aboriginal children living in areas where they are exposed to the virus, but cases have also occurred among travelers to these areas, including a visitor from Europe.[533–535] At infrequent intervals since the initial recognition of the disease in 1917, the virus has spread to the heavily populated southeastern river valleys, where it has produced larger outbreaks, most recently in 1981. Sporadic cases also have been recognized in Papua New Guinea. About 350 cases have been reported in total, with a case-fatality rate of 20% in the most recent outbreak. The onset of encephalitis is preceded by a prodrome of headache, nausea, vomiting, photophobia, and neck stiffness, followed within 2 to 5 days by changes in sensorium, stupor, and motor signs. Coma, limb paralysis, and respiratory depression necessitating ventilatory support develop in severe cases. Recovery is followed by motor paralysis in severe cases and by milder motor disturbances and emotional and psychological symptoms in a higher proportion of survivors. Serologic diagnosis is potentially encumbered by cross-reactive antibodies to Kunjin, Kokobera, JE, Edge Hill, Alfuy, Sepik, dengue, and other flaviviruses in the region. Supportive treatment has reduced mortality and morbidity. Regional surveillance of sentinel chicken infections is maintained as an early warning system.

Rocio Encephalitis

Rocio virus was recognized to be the novel cause of a series of encephalitis outbreaks that occurred from 1975 to 1977 in the Ribiera Valley and Santista lowlands in coastal São Paulo and Paraná States, Brazil.[536] More than 1000 cases were identified, chiefly in fishermen and others with outdoor occupations. The virus was isolated from human brain, and its relationship to SLE virus was shown antigenically and, later, by genomic sequencing. The virus is transmitted from *Psorophora* spp.

TABLE 153.4 Less Commonly Recognized Flavivirus Infections

VIRUS	CLINICAL SYNDROME	GEOGRAPHIC DISTRIBUTION	TRANSMISSION CYCLE	MODE OF TRANSMISSION
Alkhurma	Hemorrhagic fever, encephalitis	Saudi Arabia, in Africa near the Egypt-Sudan border and Djibouti	*Ornithodoros savignyi* and *Hyalomma dromedarii*–camels	DC, V
Bussuquara	Fever, arthralgia	Argentina, Brazil, Colombia, Panama	*Culex melanoconion* spp.–rodent	V
Edge Hill	Fever, polyarthritis	Australia	*Aedes vigilax*–marsupial	V
Ilhéus	Fever, myalgia, encephalitis	Argentina, Brazil, Colombia, Guatemala, Panama, Trinidad	*Psorophora ferox*–bird	V, E
Kokobera	Fever, polyarthralgia	Australia, Papua New Guinea	*Culex annulirostris*–? marsupial	V
Koutango	Fever, rash, arthralgia	West and Central Africa	Tick–rodent	L
Kunjin	Fever, polyarthralgia, encephalitis	Australia, Malaysia, Thailand	*Culex annulirostris*–bird	V
Modoc	Aseptic meningitis	Western United States, Canada	Rodent–rodent	Z
Rio Bravo	Nonspecific febrile illness, meningitis	Western United States, Canada	Bat–bat	Z, L
Sepik	Nonspecific febrile illness	Papua New Guinea	*Mansonia* spp.–?	V
Usutu	Fever, rash	South and Central Africa, and Europe	*Culex* spp.–bird	V
Wesselsbron	Fever, arthralgia, rash, encephalitis	Sub-Saharan Africa, Thailand	*Aedes* spp.–?	V, L, DC

DC, Contact with infected domestic animals or livestock; *E,* experimental infection; *L,* laboratory-acquired infection; *V,* vector-borne; *Z,* zoonotic infection.

mosquitoes to birds, and human infections are incidental. Sporadic asymptomatic infections have been detected in field studies, but outbreaks have not recurred. In 1996, serologic evidence of infection was reported in Bahia State, far to the north, but the virus has not been isolated outside the original focus. A prodrome of fever, headache, malaise, vomiting, and conjunctivitis precedes the onset of altered consciousness, motor weakness, and, frequently, cerebellar signs. Neurologic infection progresses to coma in one-third of cases and death in 10%. Neurologic and psychological sequelae have been reported in 20% of survivors. Supportive treatment is potentially lifesaving. Emergency applications of insecticides have been implemented in outbreak control.

Omsk Hemorrhagic Fever

Omsk hemorrhagic fever virus is transmitted between *Dermacentor* spp. ticks and small mammals in forest-steppe zones of the Omsk, Novosibirsk, Kurgan, and Tyumen regions of western Siberia, but the disease emerged in significant form only after muskrats were introduced to the region to establish a fur industry.[458–462,537,538] Outbreaks between 1945 and 1958 led to muskrat epizootics and 1500 human cases, chiefly among trappers, their family members, and laboratory workers. Infection is transmitted directly from infected animal tissues or by tick bite, with a peak in spring or early summer and another peak in autumn. The illness resembles Kyasanur Forest disease, but neuropsychiatric sequelae have been reported more often. The case-fatality rate is less than 3%. Inactivated TBE vaccine (produced in Russia) has been reported to offer cross-protection against the disease.

Less Commonly Recognized Flavivirus Infections

Small numbers or even single cases of the diseases listed in Table 153.4 have been reported. In some instances, experimental human infection (evaluated as cancer therapy) provides the only knowledge of their pathogenicity.

ACKNOWLEDGMENTS

The authors recognize and commend Dr. David Vaughn and Dr. Tom Solomon, two of the authors of the *Flaviviruses* chapter from the 7th edition of this text. Drs. Vaughn and Solomon expertly crafted a comprehensive and state of the art flavivirus review, upon which the current chapter is based.

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154 Hepatitis C

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SHORT VIEW SUMMARY

Definition

- Hepatitis C virus (HCV) is an enveloped, positive-strand RNA virus that is a member of the Flaviviridae family, *Hepacivirus* genus.
- It is a major worldwide cause of chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC).

Microbiology

- HCV is roughly spheroid and approximately 65 nm in diameter (see Fig. 154.1).
- The RNA genome is 9.6 kb in length and contains a single large open reading frame, which is processed into at least 10 proteins, including 3 structural proteins and 5 proteins of the viral RNA replicase complex (NS3, NS4A, NS4B, NS5A, and NS5B (see Fig. 154.2).
- HCV has seven major genotypes and provisionally an eighth.
- Extensive quasispecies variation is present in each infected individual.

Epidemiology

- An estimated 71 million persons are actively infected (viremic) with HCV worldwide.
- Transmission occurs most commonly by percutaneous exposure to blood.
- In developed countries, two epidemics of incident HCV infections are occurring: one in persons who use intravenous drugs and a second in men who have sex with men (MSM).
- In the developing world, most transmission occurs from unsafe medical practices.
- HCV may be transmitted sexually, but this appears to be infrequent except in select high-risk populations such as human immunodeficiency virus (HIV)–positive MSM.

Clinical Manifestations

- Acute illness caused by HCV infection is unusual, but it is typical of acute hepatitis and consists of malaise, nausea, and right upper quadrant pain, followed by dark urine and jaundice.
- Fulminant hepatitis due to HCV infection is uncommon.
- Chronic infection with HCV occurs in approximately 75% of patients after acute infection with HCV.
- Once established, chronic infection persists indefinitely and can be associated with cirrhosis, metabolic disorders such as insulin resistance and steatosis, and HCC.

Diagnosis

- Diagnosis of HCV infection is usually made by means of assays of serum antibody to HCV followed by testing for HCV RNA.
- RNA tests are also used to assess the effects of treatment.
- Noninvasive liver tests have now replaced biopsy as the preferred methods to assess the stage of liver disease.

Treatment (Table 154.4)

- The primary aim of treatment is to eradicate HCV in blood and liver (cure) and thus prevent complications of HCV infection.
- The American Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America (IDSA) provide regularly updated treatment recommendations for hepatitis C (see the online guidance for recommendations for re-treatment and for treatment of special populations, including those who are HIV-infected or have renal failure).
  - Current recommendations for initial treatment were issued in September 2017.
  - See Table 154.4.
    - Genotype 1a
      - Elbasvir-grazoprevir for 12 weeks if no NS5A resistance-associated substitution (RAS) for elbasvir is detected (no cirrhosis or compensated cirrhosis)
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks in patients with compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
        - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 1b
      - Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
        - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 2
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 3
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis (treatment naive)
      - Sofosbuvir-velpatasvir for 12 weeks if no cirrhosis or if compensated cirrhosis; testing for NS5A RAS for velpatasvir is recommended and an alternative approach recommended if present
    - Genotype 4
      - Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
    - Genotype 5 or 6
      - Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
      - Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
      - Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)
  - Clinicians are urged to consult online guidelines for the latest recommendations on HCV treatment (www.hcvguidelines.org).

Prevention

- Strategies are primarily designed to reduce exposure to contaminated blood through screening of blood products, application of precautions in health care settings, and reduction of intravenous drug use risks.
- In settings where sexual transmission appears prominent (HIV-positive MSM), barrier protection methods should be recommended.

SHORT VIEW SUMMARY—cont'd

- High-coverage screening and treatment approaches in high-risk populations are emerging as another approach to prevention (so called "treatment as prevention").
- Development of vaccines against HCV is challenging because of extensive viral diversity.

Elimination

- The introduction of highly effective therapy has fostered the discussion, planning, and implementation of HCV elimination programs.
- The World Health Organization has put forth goals for HCV elimination by 2030, including 90% diagnosed and 80% of eligible persons treated, to reach 90% reduction in HCV incidence and 65% reduction in mortality.
- Localized examples of progress toward elimination are beginning to emerge in select populations (HIV-positive MSM) and countries (Georgia).
- Major barriers to elimination currently include suboptimal diagnosis rates, poor or variable access to direct-acting antiviral (DAA) therapy, and lack of governmental support and/or coordinated efforts.

NON-A, NON-B VIRAL HEPATITIS AND HEPATITIS C

When serologic tests for hepatitis A virus (HAV) and hepatitis B virus (HBV) were developed during the 1970s, it became evident that most cases of transfusion-associated hepatitis must be caused by yet another agent, leading to the term *non-A, non-B hepatitis* (NANB).[1,2] Studies in chimpanzees confirmed that bloodborne NANB hepatitis was transmissible and due to a relatively small, lipid-enveloped virus.[3,4] In the late 1980s, Michael Houghton's laboratory at Chiron Corporation, working with Daniel Bradley's laboratory at the Centers for Disease Control and Prevention (CDC), identified a virally encoded antigen associated with NANB hepatitis and called the agent *hepatitis C virus* (HCV).[5] This finding rapidly led to the molecular cloning of the complete viral genome[6] and other major discoveries, including the proclivity of this virus to establish persistent infection and its strong association with chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).

HEPATITIS C VIRUS

Virology and Classification

HCV is a roughly spherical, enveloped, positive-strand RNA virus approximately 65 nm in diameter with a broad size range (40–100 nm) (Fig. 154.1).[7–10,11] Its structure, genomic organization, and replication cycle support classification as a member of the family Flaviviridae, yet it is sufficiently distinct from the type genus *Flavivirus* (e.g., yellow fever virus and dengue virus) to merit classification within a separate genus, *Hepacivirus.* Sibling genera also include *Pestivirus* (e.g., bovine viral diarrhea virus and classic swine fever virus) and *Pegivirus* (human pegivirus [HPgV; formerly GBV-C] and related viruses).[12,13] In addition to HCV, the *Hepacivirus* genus includes GB virus B (GBV-B), and nonprimate hepaciviruses (NPHVs) found in rodents, dogs, horses, and bats.[14–16] Studies of infectious plasma, sera, and culture supernatants suggest that the HCV envelope may associate with and be partially masked by low-density lipoproteins in a "lipo-viro-particle" or "lipovirion."[17,18]

Organization of the Hepatitis C Virus Genome

The genome of HCV is a positive-sense, single-stranded RNA molecule approximately 9.6 kb in length. Unlike a typical eukaryotic mRNA, the HCV genomic RNA is neither 5′ capped nor polyadenylated and contains a large open reading frame (ORF) encoding a single large polyprotein (approximately 3010 amino acids). A frame-shifting event occurring near the 5′ end of the ORF has been suggested to shift some translating ribosomes into production of an alternative reading frame protein (ARFP) that is of uncertain function in the viral life cycle (see later). The large ORF is flanked by highly conserved 5′ and 3′ untranslated regions (UTRs) that function in both translation and viral RNA replication (Fig. 154.2).[6]

Nontranslated RNA Segments

The HCV 5′UTR is approximately 341 nucleotides in length, demonstrates extensive secondary and tertiary RNA structure (see Fig. 154.2), and contains two overlapping functional regions. The 5′ terminal 125 nucleotides are essential for viral RNA replication, probably for recognition of the RNA by the viral replicase, whereas the remainder of the 5′UTR appears to play an accessory role in this process.[19] An overlapping approximately 300-nucleotide segment acts as an internal ribosomal

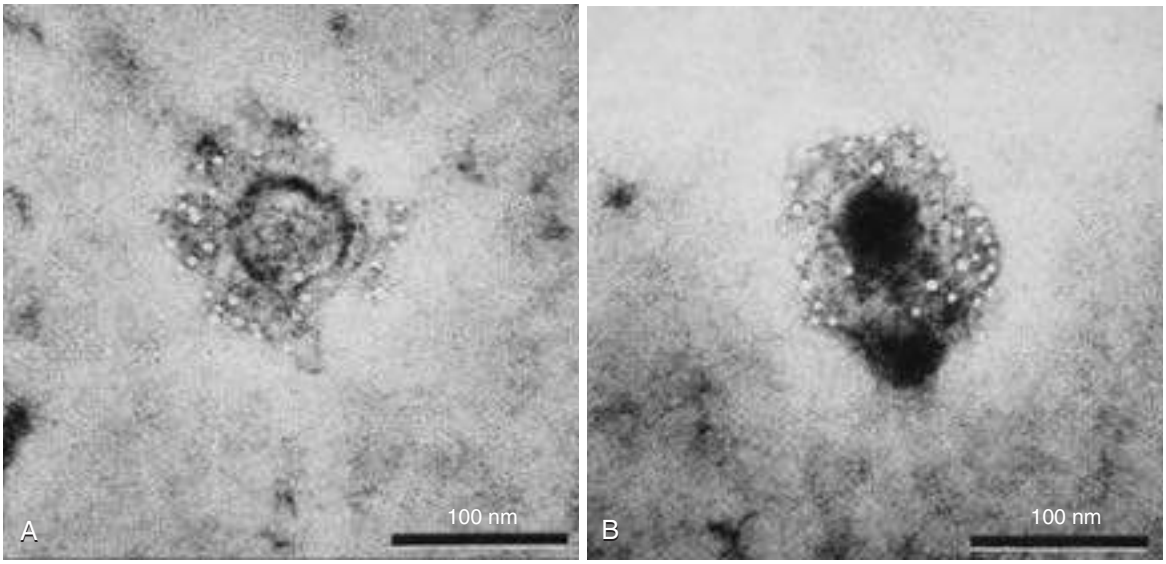

FIG. 154.1 (A and B) Electron microscopic images of hepatitis C virus (HCV) virions concentrated from human plasma by means of high-speed centrifugation. The virions are identified by staining with gold-labeled antibodies to the HCV envelope proteins. *(From Kaito M, Watanabe S, Tsukiyama-Koham K, et al. Hepatitis C virus particle detected by immunoelectron microscopic study.* J Gen Virol. *1994;75:1755–1760.)*

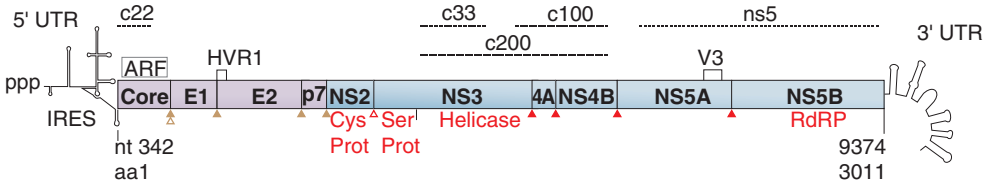

FIG. 154.2 Organization of the hepatitis C virus (HCV) genome and viral polyprotein. The 5′ and 3′ untranslated region *(UTR)* RNA structures *(curves)* flank the major open reading frame (ORF). Unlike eukaryotic mRNA, the viral RNA genome has 5′ triphosphate *(ppp)* and no 3′ polyadenylation. The 5′ UTR contains an internal ribosomal entry site *(IRES). Triangles* indicate sites of cleavage by host cellular signal peptidase (*brown,* open indicating additional processing by signal peptide peptidase), and viral (*red,* open indicating cleavage by the NS2/NS3 cysteine protease) proteases are shown as triangles. Putative structural *(purple)* and nonstructural *(blue)* mature proteins *(boxes)* generated by these cleavage events are labeled, below which in *red* are viral enzymatic functions NS2/NS3 cysteine protease *(Cys Prot),* NS3 serine protease *(Ser Prot),* NS3 helicase, and the NS5B RdRP. Positions of the first and last nucleotides *(nt)* and amino acids *(aa)* of the polyprotein ORF are shown, based on reference genome H77 (GenBank accession number AF009606). Above the ORF are positions of the alternate reading frame protein *(ARF),* variable regions HVR1 and V3, and (indicated by *horizontal lines*) the antigens included in the enzyme immunoassay serologic assay. See text for additional details.

entry site (IRES), directing the cap-independent translation of the viral ORF.[19–24] The HCV IRES and the closely related IRES elements of pestiviruses are unique among eukaryotic RNAs in that they are capable of binding directly to the 40S ribosome subunit in the absence of any protein translation initiation factor.[25–27] The binary complex formed by the 5′UTR RNA and the 40S subunit appears to involve specific macromolecular interactions around the initiator AUG codon of HCV.[28] Thus, HCV initiates translation of its proteins via a unique prokaryotic-like mechanism that may prove to be a useful target for future antiviral drug development. A highly unusual feature of HCV replication that involves the 5′UTR is the complementary binding of liver-specific microRNA 122 (miR-122), an interaction that has been found to enhance translation of HCV RNA, be necessary for HCV replication, prevent degradation of cytoplasmic viral RNA, contribute to the liver tropism of HCV, and represent a therapeutic target.[29,30–33] HCV RNA sequestration of miR-122, through a molecular sponge effect, derepresses endogenous mRNAs normally downregulated by miR-122 including those involved in inflammation and oncogenesis.[34–36] Although demonstrated in vitro, to what extent this occurs in vivo and contributes to fibrogenesis or HCC development clinically is unknown.[34]

The 3′UTR consists of a relatively variable 30- to 60-nucleotide segment downstream of the termination codon that is followed by a highly variable poly-U/UC tract of 50 to 100 nucleotides. Downstream of the poly-U/UC tract there is a highly conserved 98-base sequence (the "3′-X" region).[37–39] This highly structured 3′ terminal 98-base sequence is the most conserved segment of the HCV genome. Experiments in vitro (discussed later) indicate that "kissing loop" interactions between RNA structures in the 3′-X region and NS5B coding region, and 33 consecutive U residues in the poly-U/UC tract, are absolutely required for viral RNA replication.[40–42] The poly-U/UC tract may be the principal pathogen-associated molecular pattern (PAMP) of HCV sensed by the human cytoplasmic pattern recognition receptor (PRR) retinoic acid inducible gene-I (RIG-I, see "Viral Persistence").[43]

Outside of these well-defined untranslated structural regions, data increasingly point to a functional role of RNA structure in other areas of the HCV genome (core, NS4B, and NS5B). These structural elements appear to further enhance viral replication and aid in innate immune evasion and may undergo conformational changes which modulate replication.[44–46]

Polyprotein

The approximately 9-kb ORF encodes a polyprotein that is cotranslationally processed into at least 10 proteins. These include 3 "structural" proteins: the nucleocapsid protein, core (C), and two envelope proteins (E1 and E2); two proteins that are essential for virion production but not required for viral RNA replication (p7 and NS2); and 5 nonstructural proteins that form the viral RNA replicase complex (NS3, NS4A, NS4B, NS5A, and NS5B) (see Fig. 154.2). Processing of the polyprotein is directed by both cellular and viral proteases. Four distinct signal sequences within the amino third of the polyprotein direct the translocation of the nascent protein into the endoplasmic reticulum (ER), with the result being that signal peptidase cleaves the polyprotein at the C/E1, E1/E2, E2/p7, and p7/NS2 junctions. The nascent NS2/NS3 protein is a cysteine protease that cleaves the NS2/NS3 junction, whereas the NS3 protein contains a second (serine) protease activity that catalyzes the remaining in *trans* polyprotein cleavages among the nonstructural proteins. Full expression of NS3 protease activity requires formation of a complex with the NS4A protein.

Structural Proteins

The 191–amino acid segment at the amino terminus of the HCV polyprotein is cleaved from the nascent polypeptide by signal peptidase, forming the highly basic core protein, which has RNA-binding activity.[47–50] A second cleavage occurs just upstream of the signal peptide sequence, directed by signal peptide peptidase within the membranes of the ER, producing a mature core protein of 173 amino acids that is trafficked to lipid droplets, where it associates with NS5A.[51–53] The core protein is immunogenic; both core protein and antibody to it are typically present in the serum of infected individuals.

Many biologic activities have been associated with the core protein, including suppression of HBV replication, alterations in regulation of the cell cycle and transcription of cellular protooncogenes, either induction or suppression of apoptosis, and transformation of rat embryo fibroblasts.[54–64] The core protein has also been suggested to interfere with anti-HCV immune responses through a variety of mechanisms, including NK cell inhibition via upregulation of major histocompatibility complex (MHC) class I expression, inhibition of T-cell proliferation via interaction with complement receptor gC1qR, and interaction with the cytoplasmic tail of several cellular receptors belonging to the tumor necrosis factor (TNF) receptor family.[60,61,63,65,66] However, these data are derived largely from studies in which core has been overexpressed from recombinant cDNA. It is not clear whether core exerts any of these biologic effects when expressed by replicating virus within the liver, and in many cases there is contradictory evidence.

Yellow fever virus and other flaviviruses have a single major envelope protein and a glycosylated, cell-associated NS-1 protein that can elicit neutralizing antibodies. In contrast, hepaciviruses have two major envelope glycoproteins (E1 and E2) and no comparable NS-1 protein. Signal peptidases direct cleavage of the HCV polyprotein at amino-acid residues 383 and 746 (numbering based on the prototype strain H77), producing the E1 and E2 proteins, respectively (see Fig. 154.2).[48] These are secreted into the ER as type 1 transmembrane proteins, fold cooperatively, and remain anchored to the membrane by a hydrophobic carboxyl-terminal anchor sequence (Fig. 154.3). The E1 and E2 proteins are heavily glycosylated, with sugar moieties representing about 50% of the mature mass of each. Although initially associated as a noncovalent heterodimeric complex because of oligomerization determinants in the transmembrane segments, covalently linked complexes have been identified in mature, infectious virions.[67,68] In HCV cell culture, E1 and E2 are localized to the ER compartment, perhaps owing to ER-retention signals located in the transmembrane domain.[52,69–71] This suggests that, like other members of the Flaviviridae, HCV particles assemble and exit the cell by budding into intracytoplasmic vesicles and then follow the secretory pathway for release.

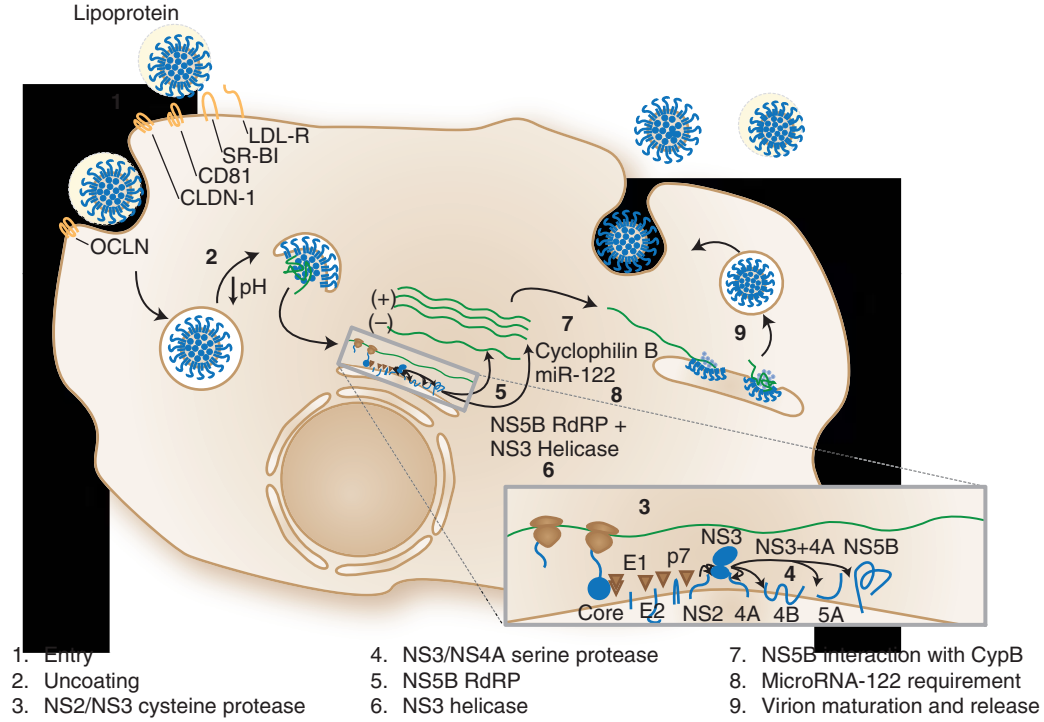

FIG. 154.3 Enhanced understanding of hepatitis C virus replication leads to new therapeutic targets. The life cycle has been labeled with key steps for which therapeutic agents are approved or have been investigated. *(Graphic art by Jocelyn Ray.)*

Detailed structural information regarding the HCV envelope proteins continues to be refined, with partial structures of the E1 and E2 ectodomains now resolved.[72–74] Although further details remain to be elucidated, some domains and putative functions are known. Major functions of the E1/E2 heterodimer include viral attachment through key interactions with cellular receptor and fusion with endosomal membranes. E1 modulates E2 interactions with CD81 and also appears to impact other coreceptor usages such as shifts from claudin-1 to claudin-6 entry dependence.[75,76] Recent data also implicate a role for E1 in recruiting viral genomic RNA during virion morphogenesis.[76] E2 is responsible for primary interactions with viral receptors such as SR-B1 and CD81. It also contains major epitopes for several highly neutralizing antibodies.[77,78] A highly variable segment approximately 30 amino-acid residues in length near the amino terminus of E2 has been called hypervariable region 1 (HVR1).[79–81] HVR1 is the most genetically variable segment of the envelope proteins, and the extent of sequence heterogeneity within HVR1 indicates that there are few sequence-related constraints on its function; however, some constraints have been observed (see "Humoral Immunity").[82–84] It has been suggested that HVR1 may function as an immunologic decoy during infection by masking a deeper, more highly conserved structure within the envelope, such as a recognition site for the cellular receptor.[85] Important to note, deletion of this region reduces but does not eliminate virus infectivity.[86] Downstream of HVR1 are HVR2 and the intergenotypic variable region (igVR), which in spite of their variability may be more directly involved in viral entry.[87] These regions are interspersed among determinants of binding to CD81 and other host molecules that are necessary for viral entry (see "Viral Replication").[88–90]

p7 and NS2 Proteins

The proteins p7 and NS2 are required for viral particle assembly or egress from the cell, but neither is required for viral RNA replication.[91] A signal peptidase cleavage near the carboxyl terminus of E2 generates the p7 protein (see Fig. 154.2). Hexamers of this small 63–amino acid hydrophobic polypeptide form a transmembrane viroporin that is cation selective.[92–95] This activity is essential for production of infectious virions and inhibited in vitro by amantadine and long-alkyl-chain iminosugar derivatives, thereby representing a possible therapeutic target.[91–93,96,97]

The NS2 protein is a membrane-associated dimeric cysteine protease with two composite active sites that mediate cleavage at the NS2/NS3 junction.[98–100] The transmembrane and protease domain structures of NS2 are essential for production of infectious virions in cell culture, whereas the protease activity is not.[91,101] Structural and in vitro analyses suggest that NS2 may serve as a lipid droplet–associated bridge linking the envelope proteins with p7 and NS3.[100,102–104]

Nonstructural Proteins Involved in RNA Replication

Proteins spanning the region within the polyprotein from NS3 to NS5B (see Fig. 154.2) are required for RNA replication and assemble into a membrane-associated replicase complex within the cytoplasm of infected cells. The NS3 protein possesses serine protease activity localized to its amino-terminal third and an RNA helicase with NTPase activity in its carboxyl-terminal domain. The mature, fully active NS3 protease requires the noncovalent association of NS3 with the NS4A protein, which becomes an integral part of the protease structure.[105–108] Atomic-level resolution structures of these domains have been solved separately and together, and these have been exploited for antiviral drug discovery (see "Treatment of Chronic Hepatitis C").[105,109,110] The NS3 serine protease is dependent on zinc and is responsible for the NS3/NS4A *cis* cleavage, and for the NS4A/NS4B, NS4B/NS5A, and NS5A/NS5B cleavages that follow in the processing of the polyprotein; all except the *trans* cleavage sites share a canonical (D/E)xxxxC∧(A/S) recognition motif (with "∧" indicating the site of cleavage). The carboxyl-terminal 465 amino acids of NS3 contain the NTPase and RNA helicase activities, which are likely to direct the unwinding of duplex RNA molecules during genome replication. The NS3 helicase can bind to the 3′ poly-U/UC sequence and has 3′ to 5′ directionality.[111,112] No cleavage site has been identified between the NS3 protease and helicase, and functional studies suggest that these domains are interdependent.[113] In addition, the NS3 helicase domain directly interacts with the core protein during virion production.[114]

NS3 protease activity has been shown to interfere with interferon (IFN)-mediated signaling by blocking the virus-activated phosphorylation of IFN regulatory factor 3 (IRF3) via cleavage of host TIR-domain-containing adapter-inducing IFN-β (TRIF) and mitochondrial antiviral

signaling (MAVS) proteins, providing a mechanism by which HCV might evade innate cellular antiviral defenses (see "Viral Persistence").[115,116] The multifunctional nature of NS3, including polyprotein processing, its role in the RNA replicase, and its contribution to immune evasion, is typical for the proteins of small, positive-stranded RNA viruses such as HCV.

The NS4A protein acts as a cofactor for the NS3 protease, as described earlier. An amino-terminal segment of the protein anchors the NS3/4A complex to intracellular membranes, while NS4A also interacts with NS5A as a critical component of the replicase complex. NS4B is a hydrophobic, membrane-associated protein. It appears to mediate modifications of the ER membranes that occur in association with replicase assembly, and in doing so may also inhibit normal ER-to-Golgi secretory pathways.[117–120]

NS5A is a membrane-anchored cytoplasmic RNA-binding phosphoprotein that appears to play a role in RNA replication, although its exact function remains obscure.[121–123] It contains an amino-terminal amphipathic alpha helical membrane anchor followed by three domains (D1–D3) separated by low-complexity sequences (LCS1 and LCS2). RNA replication depends on RNA binding and dimerization mediated by the region extending from D1 to D2; D3 interacts with core protein on lipid droplets and is essential for viral assembly.[124] Portions of D2 and D3 interact with multiple proteins, including cyclophilin A and a lipid kinase, and each of these functions has been targeted therapeutically (see "Medications").[125–127] Sequence polymorphisms within a short segment of NS5A (the "IFN sensitivity determining region" [ISDR]) have been correlated in some studies with resistance to IFN therapy for some HCV genotypes, and this may be mediated by the interaction of NS5A with the catalytic domain of IFN-induced dsRNA-activated protein kinase R (PKR).[128–131] Inactivation of PKR by NS5A could mitigate both the antiviral and antiproliferative activities of IFN, though this remains unproven. In addition, NS5A may induce expression of interleukin (IL)-8, which antagonizes expression of IFN-stimulated genes.[132]

NS5B is a membrane-bound protein that contains a Gly-Asp-Asp motif characteristic of RNA-dependent RNA polymerases and is considered to be the catalytic core of the replicase complex. As with the enzymatic activities of the NS3 protein, the NS5B RNA polymerase has proven to be a useful target for antiviral drug development with nucleoside and nucleotide analogues and nonnucleoside small molecule inhibitors, and cyclosporine A analogues (see "Medications").

In addition to the polyprotein described earlier, ribosomal frameshifting may generate the ARFP from a short ORF found within the core region of some genotype 1 isolates.[133–135] Some individuals with HCV infection, but not those with HBV monoinfection, have been found to have serum antibody and T-cell reactivity to in vitro synthesized ARFP, suggesting that the protein can be expressed in vivo. The role of this protein remains speculative, and it may be a related RNA element, rather than the ARF protein itself, which is required for infection in vitro and in vivo.[136]

Viral Replication

Details of the viral life cycle were initially examined through use of replication-competent subgenomic RNA replicons in cultured cells, and then more recently with a cell culture system that completes the viral life cycle from cell entry to release of infectious virions (see "Experimental Models"). These studies and reasonable analogies with other positive-strand RNA viruses suggest the following scenario (see Fig. 154.3). The virus enters the cell through interaction with multiple cell surface receptor molecules including CD81, the LDL receptor, NPC1L1, the EGF receptor, DC-SIGN, L-SIGN, human scavenger receptor SR-B1, and the tight junction proteins claudin-1 (CLDN1) and occludin (OCLN).[137–146] Following attachment, penetration, and uptake into a cellular endosome, acidification alters the conformation of the envelope proteins, resulting in fusion with the endosomal membrane. The viral RNA is released into the cytoplasm, where it acts as messenger RNA directing the cap-independent translation of the viral polyprotein.[147,148] Viral translation occurs in association with the rough ER by the process of internal ribosome entry, and the polyprotein undergoes a series of further cotranslational proteolytic cleavages, as described in the preceding section.

The core protein remains within the cytoplasm following cleavage by signal peptide peptidase from the signal sequence at its carboxyl terminus, while E1 and E2 are secreted into the lumen of the ER, remaining attached to the membrane and becoming heavily glycosylated. A replicase complex, composed of NS3, NS4A, NS4B, NS5A, and NS5B, forms cytoplasmic clusters of "membranous webs" derived from the ER associated with lipid droplets.[119,120] This replicase complex recognizes the 3′ end of the genomic RNA and subsequently directs the synthesis of a negative-strand copy of the genome. The resulting duplex RNA most likely serves as a template for the subsequent synthesis of multiple copies of the positive-strand genomic RNA, following recognition of the opposite end of the genome by the replicase. The genomic RNA is packaged into new viral particles, which are likely to be extruded into the ER, leading to the release of the virus via the vesicular secretory pathway.

Although the liver appears to be the primary source of virus present in blood, there are few data that directly support this conjecture. HCV-specific antigens and both negative- and positive-strand HCV RNA have been identified within hepatocytes, indicating that replication does occur in this cell type via a negative-strand intermediate as outlined earlier.[149–151] However, additional data suggest that the virus may also replicate within peripheral mononuclear cells of lymphoid or perhaps bone marrow origin (see next section).[152,153]

Mathematical models of viral kinetics suggest a half-life of approximately 45 minutes for virions in the bloodstream and that up to 10^{12} virions are produced each day in a chronically infected human.[154,155] Compared with human immunodeficiency virus (HIV), the rate of HCV production is 10- to 100-fold higher; however, similar half-lives suggest that the mechanism of clearance could be similar.[155,156]

Genetic Diversity

Quasispecies Variation

The high level of virion turnover, coupled with the absence of proofreading by the NS5B RNA polymerase, results in the rapid generation of viral mutations. Multiple HCV variants can be recovered from the plasma and liver of an infected individual at any time, and this "swarm" of closely related but distinct variants infecting a person is called a "quasispecies."[81,157] During RNA replication, mutations occur in a nearly random fashion throughout the genome, whereas fixation of a substitution within the quasispecies population (evolution) depends on how that substitution influences viral "fitness" as related to its effect on functional protein and RNA structures, the capacity of the virus for replication, and the host-viral interaction.

Strong selective forces such as immunologic responses drive HCV evolution in vivo. For example, spontaneous mutations within the HVR1 segment of the E2 protein may be favored for survival in the host when they reduce the binding of neutralizing antibodies to the viral envelope, whereas they occur slowly or not at all in those who do not generate antienvelope antibodies.[158–163] There is also evidence that cellular immune responses may drive the selection of specific quasispecies variants, although they may also incur a viral "fitness cost" such as reducing the efficiency of a viral enzyme.[84,164–170] Thus, quasispecies variants recovered from blood reflect the balance of production and selective forces.

Although the nucleotide substitutions identified in circulating virus represent only a fraction of all mutations generated during viral replication, these mutations are estimated to occur at an overall rate of 0.9 to 1.92×10^{-3} base substitutions per site per year during chronic infection.[171–173] Variation within an HCV quasispecies instantaneously and over time may be linked to the extent of disease and the duration of infection.[174,175] This is consistent with the hypothesis that immunologic responses affect both the extent of disease progression and the pace of sequence change. Differences in the HCV quasispecies present in the blood and liver have been described, suggesting that differences in tissue tropism may also influence genetic variation, though these studies have suffered from limited sampling.[153,176,177]

The extent of genetic diversity varies markedly throughout the HCV genome, being highest in the segment that encodes the amino terminus of the second envelope protein, E2, within HVR1, and lowest in the core gene and the 5′ and 3′ nontranslated segments of the genome.[178–182] High conservation at some loci suggests functional constraint (mutations

may be lethal or sufficiently disadvantageous to replication to be undetectable among surviving virus populations).

Hepatitis C Virus Genotypes

In addition to the impressive heterogeneity that often exists among HCV sequences present in a single infected individual (i.e., quasispecies variation), there is also remarkable genetic heterogeneity and divergence among HCV sequences recovered from different individuals (i.e., strain and genotype variation). Phylogenetic evaluation of HCV sequences recovered from multiple geographic regions suggests that there are at least seven major genotypes or clades, with provisional identification of an eighth genotype in a specimen originating from the Punjab region of India.[183–186] These genotypes are even more diverse than those causing the worldwide HIV-1 pandemic (Fig. 154.4), in part because of a much longer epidemic history for HCV.[187,188] Depending on the genomic region evaluated, HCV sequences assigned to different genotypes may have less than 50% nucleotide sequence identity. Significantly, this level of nucleotide sequence divergence usually correlates with substantial serotypic differences among other RNA viruses (e.g., poliovirus type 1 vs. poliovirus type 2, which are not cross-neutralizable in assays of antibody-mediated neutralization). The extent of serotypic variation among HCV strains is poorly understood, although some human monoclonal antibodies have broad neutralizing activity.[189–191] Despite extensive study, there is little evidence that HCV genotypes differ in transmissibility or level of replication. However, genotype-specific difference in the rate of progression of liver disease and complications such as HCC are now recognized, with genotype 3 in particular being associated with a more aggressive clinical phenotype (see "Natural History and Pathogenesis").[192–194] Genotype differences were also recognized in response to IFN-based treatments and early direct-acting antiviral (DAA) regimens. The arrival of highly potent pangenotypic regimens has negated much of the impact of genotype on response (see "Medications").

Within individual HCV genotypes, strains can be further grouped into subgenotypes (subtypes) that generally share 75% to 85% nucleotide sequence identity within the core-E1 and NS5B regions of the genome.[183,195] Currently, 86 confirmed subtypes have been described, with genotype 6 followed by genotype 4 having the most confirmed subtypes (https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/56/hcv-classification).[185] In contrast, the quasispecies variants that exist within a single person generally have 91% to 99% identity in these regions.[196] The phylogenetic grouping of HCV strains is largely independent of the segment of the genome that is analyzed (i.e., in contrast to HIV, recombination is rarely observed for HCV).[183,197–199]

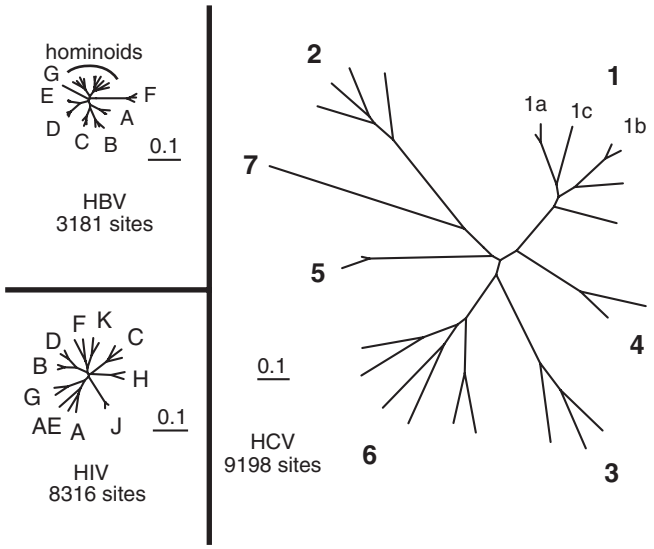

FIG. 154.4 Phylogenetic trees for hepatitis B virus *(HBV),* human immunodeficiency virus *(HIV),* and hepatitis C virus *(HCV)* shown to the same scale in terms of nucleotide genetic distance, based on full-length genome nucleotide sequences. Sequences of representative strains for major genotypes were obtained from GenBank and aligned using ClustalX with minor manual adjustment, and then sites containing gaps were removed, resulting in an alignment of 3181 sites for HBV, 8316 sites for HIV, and 9198 sites for HCV. Maximum likelihood trees were inferred using PAUP* version 4b10, with the model (GTR+I+G in all three cases) and parameters selected by ModelTest 3.7 using the AIC criterion.

Globally, HCV recombination is a rare event, with eight of the nine identified recombinants limited to a single isolate. The notable exception is the HCV 2k/1b recombinant virus, which has been well characterized and is increasingly recognized in parts of Eastern Europe, Georgia, and Russia.[185] The 2k/1b recombinant variant was first recognized in 2002 in samples from St. Petersberg.[200] Initial epidemiologic studies suggested a low prevalence of this recombinant (1%–2%) largely confined to the former Soviet Union; however, more detailed contemporary analyses have found the 2k/1b recombinant in up to 20% of HCV-infected persons in the country of Georgia.[201,202] Identification is complicated by the fact that line probe assays that rely on NS regions for genotyping (5′UTR with or without core) will misidentify this recombinant as genotype 2.[203,204] Detailed phylogenetic analyses from DAA treatment trials have indicated an increased spread, including isolation from patients in the Netherlands and the United States.[203] Patients harboring the 2k/1b virus respond to DAA therapy in a fashion similar to those with genotype 1 infection, with lower responses to sofosbuvir plus ribavirin therapy.[203,205] As pangenotypic therapies are more widely used, this distinction will be of less clinical importance.

Our understanding of the geographic distribution of HCV genotypes has improved significantly with the increased availability of sequencing technologies aided by detailed clinical virology studies accompanying DAA therapeutic trials. However, data are still biased by the large amount of sequence data from North American and Western Europe compared to the rest of the world. Genotype 1 is the most widely dispersed worldwide accounting for 44% of infections with subtype 1b predominating globally.[206] In the United States, roughly 70% of infections are genotype 1 with subtype 1a predominating (~60% of genotype 1 sequences) (Fig. 154.5).[207–209] Genotype 2 is widely dispersed but is most diverse (suggesting the location of its origin) in central and west Africa.[210] Genotype 3 is the second most prevalent globally (25%) and is most diverse in Asia but has been linked in other geographic regions, including North America, to illicit drug use.[206,211–213] Genotype 4 infections are most prevalent in northern Africa and the Middle East.[214,215] Types 5 and 6 have been reported in South Africa and Southeast Asia, respectively.[216–218] The four isolates of genotype 7 all originated from the Democratic Republic of Congo.[219] Differences in genotype nomenclature may cause some confusion in interpreting older studies; however, there is general acceptance of a revised nomenclature.[14,185,220]

Viral Tropism

HCV replicates within the hepatocyte, and the liver-specific expression of miR-122 may contribute to this specificity. Differential expression of receptors may also contribute to the hepatotropic nature of HCV, with LDL-r (nonspecific) and SR-B1 (specific) contributing to liver tropism. However, replication may also occur in other cell types. Some studies have suggested the presence of negative-strand (replicative intermediate) HCV RNA in T cells, B cells, and monocytes, especially in patients with chronic infection.[221–223] Others have suggested that this occurs rarely,[152] or not at all,[224,225] but, as mentioned earlier, differences in dominant quasispecies populations in these various compartments are supportive of extrahepatic infection. Substantial evidence also supports the ability of HCV to replicate at low levels in cultured human cells of T- and B-cell origin.[226]

HCV RNA also has been detected in cutaneous lesions of persons with HCV-related cryoglobulinemia and vasculitis,[227] in renal biopsy specimens of patients with HCV-associated membranoproliferative glomerulonephritis,[228] and (with variable success) in various body fluids including saliva, semen, tears, urine, cerebrospinal fluid, and ascitic fluid.[229–233] Unlike HBV, HCV does not replicate through a DNA intermediate and does not have the ability to integrate its genetic information into chromosomal DNA. Because of this, the detection of HCV sequences in these tissues and fluids may indicate the presence of infectious virus, although transmission via these fluids appears to be rare (see "Incidence and Transmission of Hepatitis C Virus").

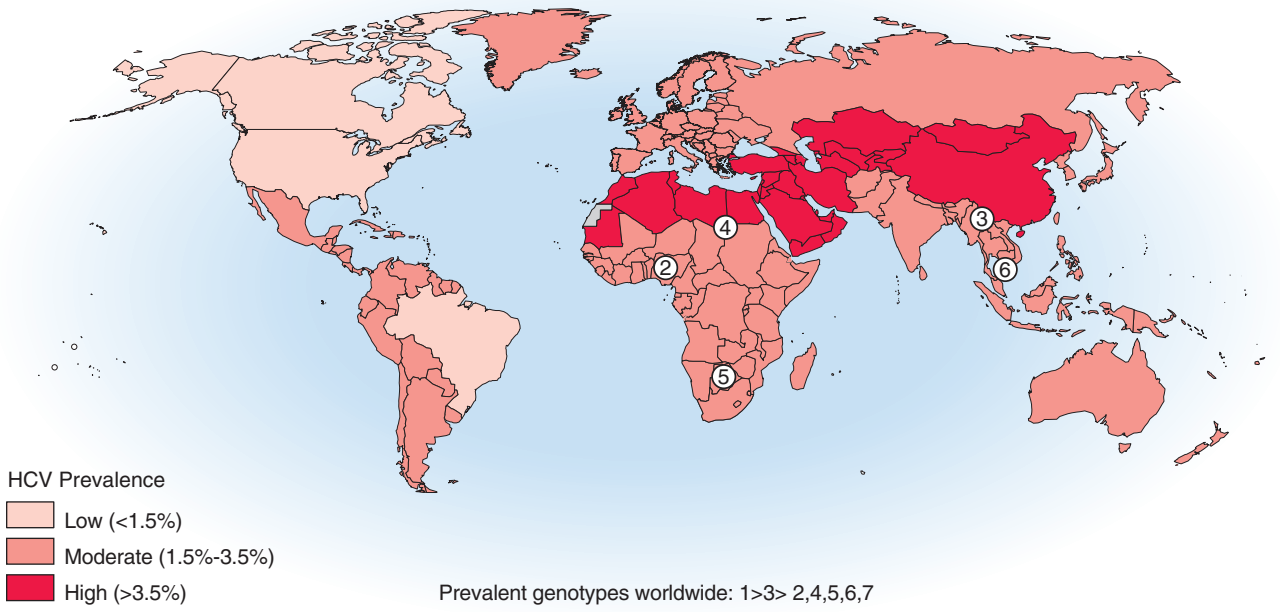

FIG. 154.5 World map indicating prevalence of hepatitis C virus *(HCV)* infection *(shading)* and subtypes *(numerals).* Genotypes 1, 2, and 3 are prevalent with variable dominance worldwide; genotypes particularly diverse (e.g., genotype 2 in Central and West Africa) or associated with a geographic region (e.g., genotype 5 in South Africa) are indicated by *circled numbers. (Prevalence estimates are modified from the Global Burden of Diseases, Injuries, and Risk Factors 2010 study [Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence.* Hepatology. *2013;57:1333–1342.])*

Experimental Models

Autonomously Replicating Viral Replicons and Genome-Length RNAs

Specially constructed subgenomic HCV RNAs ("replicons") and genome-length RNAs have been shown to undergo autonomous replication in certain cultured cells. These model systems have provided important information regarding HCV RNA replication mechanisms and virus–host cell interactions. The first such replicons were dicistronic RNAs derived from molecularly cloned cDNA.[234] The expression of a selectable antibiotic marker from the upstream cistron allowed the selection of stable cell clones supporting replication of the RNA following its transfection into the cells. The downstream cistron, encoding either the NS2-NS5B or NS3-NS5B nonstructural proteins, was placed under the translational control of a picornaviral IRES. Such RNAs demonstrate a surprisingly robust replication phenotype in cultured human hepatoma cells (Huh7 cells and their derivatives), but typically only after the accumulation of adaptive mutations within the HCV sequence (often in the NS5A protein) that enhance in vitro replication capacity.[235] Interesting to note, such mutations appear to substantially attenuate the ability of the virus to replicate in chimpanzees.[236] Some HCV RNAs have been further adapted to growth in HeLa cells and even cells of murine origin.[237]

Because subgenomic replicon RNAs typically lack the sequence encoding the structural proteins (core and E1, E2, and p7), they are not capable of producing infectious particles. However, replication-competent, selectable dicistronic RNAs that encode all of the viral proteins also did not produce infectious particles.[238] Although the first replicons were made from genotype 1b strains of HCV, RNAs from all other major genotypes (e.g., genotypes 1a, 2–6) have been adapted to highly efficient replication in Huh7 cells.[239,240] These subgenomic HCV replicons and genome-length RNAs recapitulate many of the natural mechanisms of HCV RNA replication and have been useful for discerning differences in the susceptibility of different viral strains and genotypes to candidate antiviral compounds and for studying mechanisms of resistance. Further enhancements to these replicons allow for insertion of patient-derived sequences facilitating in vitro phenotypic sensitivity testing to antivirals; however, this process remains largely a research tool.[241–243]

Propagation of Virus in Cell Cultures

The description in 2005 of a complete cell culture system for propagating HCV (designated HCVcc) opened a new era in HCV research, enabling in vitro study of a complete viral life cycle.[9,244,245] Key aspects of this system were the recognition that the JFH-1 (subtype 2a) genomic RNA clone replicated efficiently without the need for adaptive mutations and the isolation of highly permissive derivatives of the Huh7 hepatoma cell line (e.g., Huh 7.5.1) that are defective in some components of the innate immune response, including Toll-like receptor 3 (TLR3) and RIG-I.[246,247] The HCVcc system has been broadened to include chimeras of the JFH-1 strain with portions of other strains (e.g., J6/JFH-1) and derivatives of the subtype 1a strain H77 that are important because of the global importance of genotype 1; more recently recombinants containing nonstructural segments of interest (e.g., NS5A) from all major genotypes.[248–251] Full-length virus clones of other genotypes, which are prevalent globally (e.g., 3a and 6a) and replicate efficiently in vitro, have been established.[252,253]

Animal Models

The chimpanzee, *Pan troglodytes,* is the principal nonhuman animal species that has been demonstrated to be permissive for acute and chronic HCV replication. Percutaneous inoculation of HCV RNA-positive plasma, and in one instance saliva, resulted in HCV infection.[254–256] Also, chimpanzees have been infected by means of intrahepatic inoculation of synthetic genome-length RNA derived from cDNA clones.[147,148] Thus the chimpanzee was a valuable model for hepatitis C infection, although the use of this animal model is now extremely limited.[257] The recent discovery of NPHVs in dogs and then in their natural equine host and in rats offers potential models.[258–260] In particular, rodent hepacivirus (RHV-rn1) has been shown to have a similar genetic organization to HCV, results in chronic infection with gradual fibrosis progression, and responds to HCV antivirals such as sofosbuvir.[261] A more distantly related hepacivirus called GBV-B infects tamarins and marmosets, replicates within the liver of these animals, and can cause both acute and chronic hepatitis with many features resembling hepatitis C.[262] The related *Pegivirus* genus includes HPgV, which was previously called GBV-C and is quite distinct from HCV.[16]

Initially, mouse models of HCV infection were limited to transgenic mice that express transgenes encoding all or some HCV proteins under the control of liver-specific constitutive or inducible promoters.[263–265] Xenotransplantation of human hepatocytes to immunodeficient mice with liver disease resulted in a small-animal model that supports HCV replication.[266] For example, mice with severe combined immunodeficiency (SCID) transgenic for expression of plasminogen activator under control of the albumin promoter (resulting in liver injury) were transplanted with human hepatocytes and then used to test for protection in passive immunization using monoclonal antibodies specific for HCV envelope genes.[190,250] Other models have been generated with different combinations of immunodeficiency and liver injury, resulting in physiologic levels of albumin and lipoproteins.[267]

Immunocompetent mouse models of HCV infection include genetically humanized mice that express human variants of host entry molecules CD81 and OCLN.[144] Although these mice support studies of HCV entry determinants, they do not yet support significant replication.[191,268] Further enhancements to this model by introduction of defects in innate antiviral immunity through cross-breeding have resulted in mice that support completion of the entire HCV life cycle with replication levels suitable for testing of antiviral compounds.[269]

NATURAL HISTORY AND PATHOGENESIS

Viral Persistence

In experimentally infected chimpanzees and in humans, HCV RNA can be detected in plasma within days of exposure, often 1 to 4 weeks before liver enzyme levels rise.[254,270–274] Viremia peaks and may be quite variable in the first 8 to 12 weeks of infection and then plateaus or drops to lower levels and persists (Fig. 154.6).[275] In some instances, plasma HCV RNA becomes undetectable in the first few months and remains undetectable indefinitely (viral clearance); in other instances, viremia is inconsistently detected early and a stable pattern of recovery or persistence is not evident for more than 6 months.[254,273,276–280] Some instances of intermittent viremia may reflect reinfection, which has been observed in active injection drug users.[276] In other cases, rebounding viremia may represent escape from an initially successful immune response.[165,277,279,281] Overall, viremia persists in 50% to 85% of acutely infected persons (see "Acute Hepatitis C").[273,276,282,283]

Host genetic factors influence viral persistence. There are clinical and epidemiologic clues that suggest host genetic factors play an important role in the outcome of acute infection. In common source outbreaks in which persons were accidentally infected with the same HCV inoculum, some recovered and others had persistent infection.[284] In addition, HCV infection more often persists in African Americans than Caucasians and in persons infected with HIV infection compared with immunocompetent persons.[285,286] In one study, 90 persons with plasma HCV antibodies but not HCV RNA were compared with 722 with both HCV antibodies and RNA.[285] Viral persistence was found 5.1-fold more often in blacks compared with nonblacks after adjustment for HIV and other factors. These racial differences and differences in viral persistence after exposure to a common HCV inoculum point strongly to a host genetic basis for viral persistence.

Multiple studies have reported differences in the frequencies of various alleles in persons who have persistent infection compared with those with spontaneous resolution. The most consistent findings map to HLA and genes for λ-IFNs.[287–292] The association of HLA class I with HCV persistence was strengthened when genes for NK receptors were also considered.[287] Specifically, the most inhibitory interactions between *HLA C* and *NKIR* were most strongly linked to viral persistence.

A genome-wide association study compared the frequency of 792,721 alleles in 919 persons with spontaneous resolution of HCV infection with frequencies in 1482 with persistent infection.[293] Marked differences in allele frequencies were found on chromosome 6 for 117 single nucleotide polymorphisms (SNPs) spanning 1 million bp encompassing genes for *HLA* class II (Fig. 154.7). The strongest signals mapped to *HLA DQA1-DQB2* and *DQB1*0301* in particular. The chromosome 19 allele frequency differences mapped to a 55-kb region encompassing λ-IFN 3 (or *IL28B)*. The most significant association was a C/T at rs12979860, 3 kilobases from the *IL28B* start codon. Interesting to note, this allele (but not the chromosome 6 SNP) was initially identified as having predicted the response of persons with persistent HCV infection to IFN and ribavirin.[294] In addition, the global allele frequency correlated closely with the outcome of HCV infection, with the highest prevalences of the favorable allele found in Asia and the lowest in Africa.[290] In the cohort in which the racial difference in HCV persistence was first recognized, rs12979860 accounted for approximately 50% of the racial difference in viral persistence.

rs12979860 is located approximately 3 kb upstream from the gene for IFN-λ3, and a dinucleotide polymorphism was discovered (ss469415590) 367 bp upstream from rs12979860 that was associated with transient production of another protein called IFN-λ4.[291] That dinucleotide polymorphism is highly linked to rs12979860, but in some cohorts appears to be more predictive of both spontaneous resolution and response to IFN and ribavirin treatment, particularly in individuals of African ancestry.[291,295] The mechanisms through which genetic polymorphisms near the genes for λ-IFNs on chromosome 19 and near the HLA class II genes explain viral persistence remain unclear. An SNP (rs4803217) located in the 3′ UTR of IFN-λ3 mRNA was investigated for potential regulation of mRNA translation efficiency.[296] With an in vitro approach, it appeared that rs4803217 induced alterations in gene expression, influencing the global structure of IFN-λ3 mRNA, with the G allele producing a more stable form whereas the T allele mRNA was more dynamic. The hypothesis was that variation in translational efficiency could result in modulation of IFN-λ3 protein abundance and hence HCV clearance based on the genotype. The rs12979860 discovery SNP and rs4803217 were highly correlated with clinical phenotypes. However, in a comparison of rs4803217 and IFN-λ4-ΔG/TT, there was no indication that the rs4803217 allele improved HCV clearance, a finding that argues against this new allele as the causal variant.[297]

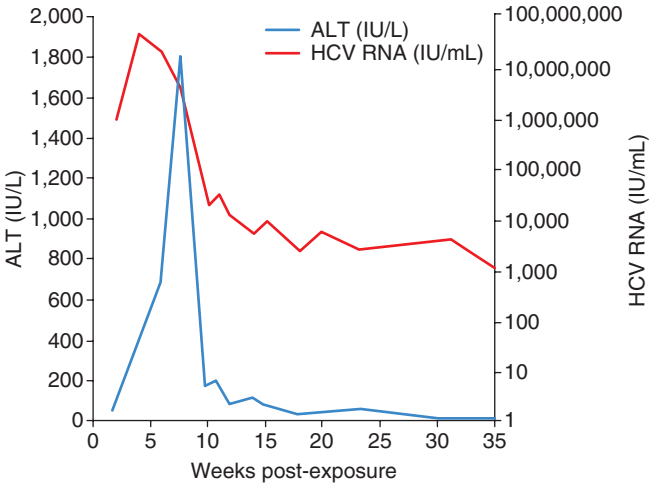

FIG. 154.6 Acute hepatitis C virus *(HCV)* infection in a health care worker following a needlestick accident (time zero). *ALT,* Alanine aminotransferase. *(From Sulkowski MS, Ray SC, Thomas DL. Needlestick transmission of hepatitis C.* JAMA. *2002;287:2406–2413.)*

Innate Immunity

HCV infection induces a wide range of innate host responses, and each may be balanced by one or more viral evasive strategies.[298] At the cell membrane, or more likely in endosomes, an extracytoplasmic PRR such as TLR3 may sense the viral genome from disrupted virions and trigger an antiviral program via the adaptor protein TRIF. Once in the cytoplasm, PAMPs such as the 3′UTR may trigger RIG-I, a cytoplasmic sensor of viral RNA (uncapped RNA that is double-stranded or contains certain homopolymers), and trigger inflammation via the mitochondria-associated membrane adaptor protein MAVS.[299] Signaling via TRIF or MAVS results in nuclear translocation of activated IRF3, IRF7, nuclear factor kappa B (NF-κB), or a combination of these, which trigger transcription of inflammatory genes including IFN-β. IFN-β, through subsequent autocrine and paracrine mechanisms, subsequently stimulates

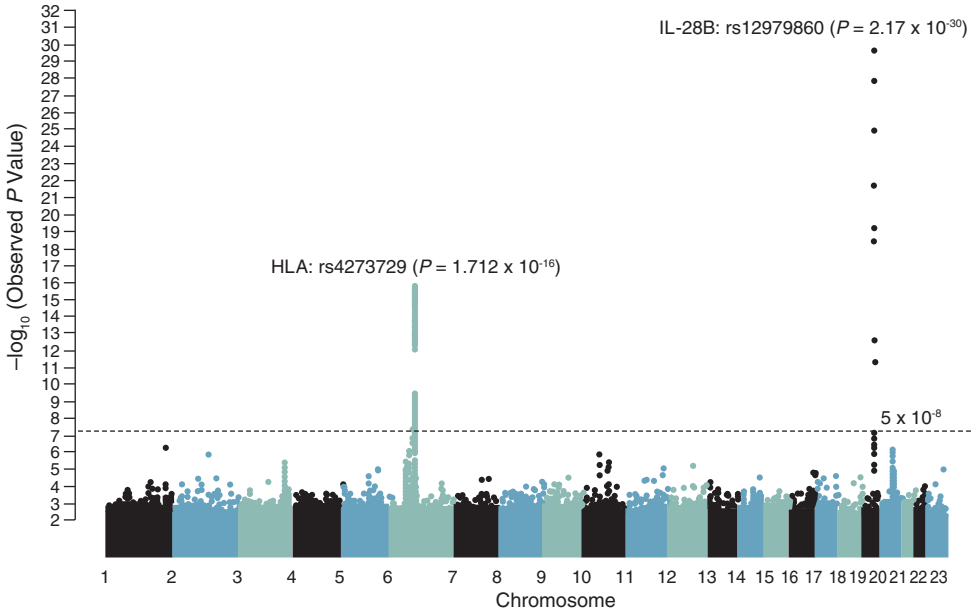

FIG. 154.7 Manhattan plot summarizing genome-wide association study results in 919 persons with spontaneous resolution of hepatitis C virus infection and 1482 persons with chronic hepatitis C virus infection. Each point corresponds to a *P* value from a test of association for an SNP. The $-\log_{10}$ *P* values are plotted by location of the single nucleotide polymorphism (SNP) across the genome for the 23 chromosomes. The *dashed line* represents an accepted level of genome-wide significance ($P = 5 \times 10^{-8}$). SNPs in the *HLA* and *IL-28B* region on chromosomes 6 and 19, respectively, exceed this threshold. *(From Duggal P, Thio CL, Wojcik GL, et al. Genome-wide association study of spontaneous resolution of hepatitis C virus infection: data from multiple cohorts.* Ann Intern Med. *2013;158:235–245.)*

the synthesis of IFN-α and a wide variety of other antiviral cytokines and chemokines that both inhibit viral replication and help to orchestrate the subsequent adaptive immune response.[115] That these responses are important is suggested by the cleavage of TRIF and MAVS, adaptor proteins for TLR3 and RIG-I, respectively, by the HCV protease NS3/4A.[300,301] Gene expression microarray studies have shown convincing, vigorous type 1 IFN responses within the livers of both acutely and chronically infected chimpanzees, indicating that the inhibitory effects of NS3/4A are incomplete.[302,303] This may be due to IFN production by uninfected cells or by recently infected cells in which NS3/4A is not yet active, or another mechanism.

During viral infections, a major source of type I IFNs is the plasmacytoid dendritic cell (PDC), but they are not activated by infectious HCV virions.[304] Further investigation revealed that PDCs are activated in the presence of HCV-infected cells and that this phenomenon required (1) replication, rather than the simple presence, of HCV RNA in the non-PDC, (2) cell-to-cell contact, and (3) expression on the PDC of TLR7. Supernatants from these activated PDCs suppressed HCV replication in an IFN receptor–dependent fashion.[305]

In addition to potentially blocking the induction of IFNs, HCV appears capable of impairing IFN-related effector functions. HCV-related dysfunction in the Jak/STAT signaling pathway in model systems has been ascribed to hypomethylation of STAT1 related to increased expression of protein phosphatase 2A (PP2A), stimulation of IL-8 production, and inhibition of PKR.[130,132,306,307] Confirmation of these findings in model systems expressing HCV proteins in a more physiologic context is necessary before any firm conclusions can be drawn.

NK and NKT cells are abundant in liver and, through production of IFN-γ and other cytokines, prime cellular immune responses.[308] Thus it is important that the binding of the E2 protein to CD81 has been associated with inhibition of NK cell activity.[309,310] Likewise, *HLA Cw*04* and its related haplotypes, which reportedly bind to inhibitory killer immunoglobulin-like receptors on NK cells, have been associated with viral persistence.[287,311] Notably, the least inhibitory *HLA-C-NKIR* haplotypes are most strongly associated with recovery. Furthermore, we have recently learned that these inhibitory effects are reversible with HCV viral suppression. The development of DAA therapy has provided a unique opportunity to understand the reversibility of the NK cell activation and liver inflammation. Because of the immunomodulatory effects of IFN, this was not feasible until the availability of IFN-free regimens. With DAA-mediated HCV viral clearance, there is rapid decrease in NK cell activation and a normalization of NK cell cytotoxic effector functions to levels reported in uninfected controls.[312] Thus the NK cell dysfunction noted in chronic HCV infection is normalized with DAA therapy. Furthermore, a similar study evaluated restoration of HCV-specific $CD8^+$ T-cell function in the setting of DAA-mediated HCV clearance.[313] Together these studies suggest that innate and adaptive immune systems may be in a better spot to respond to reexposure than after IFN-based therapies and may even confer partial protection against reinfection.

HCV may interfere with NK cell activation of dendritic cells (DCs), which facilitate T-cell priming.[314] In some studies, but not others, HCV infection has been associated with measurable impairment of peripheral DC function.[315,316] This impairment is one possible mechanism that might explain the arrested development and then collapse of cellular immune response observed during the transition from acute to chronic infection.[317] Whether or not intrahepatic DCs are infected with HCV is unknown, and studies have suggested that the effects of HCV on DCs do not require viral replication and may be mediated by viral proteins including core protein.[318] In addition, immune responses within the liver may inherently be biased toward tolerance because of the frequent exposure of the intrahepatic environment to antigenic material and cytokines from the gut.[319]

Humoral Immunity

Within months of infection, antibodies to multiple recombinant antigens that correspond to structural and nonstructural genes are detectable in blood.[320] Emergence of HCV-specific antibodies does not correlate temporally with viral recovery. Indeed, although virtually all immunocompetent persons develop antibody responses to some HCV antigens, most infections persist. Viral recovery has also been described in persons with congenital agammaglobulinemia.[321] On the other hand, humoral immune responses can neutralize HCV, at least partially. For example, there were fewer HCV infections in liver transplant recipients who received immune globulin before 1990, when it contained antibody to HCV.[322] Likewise, in a randomized controlled study, immune globulin administration was associated with a reduced incidence of sexual HCV transmission.[323]

Available evidence suggests that HVR1, located at the N-terminus of E2, harbors one or more neutralization epitopes and that it is a site of mutations causing immune escape during acute and chronic infection.[158,324–326] In the chimpanzee model, passively transferred anti-HVR1 antibodies could neutralize HCV infection in chimpanzees, whereas those same antibodies did not neutralize an inoculum collected later that had amino-acid changes in the envelope sequence.[324,327] Postexposure immune globulin administration prolonged the incubation of HCV infection, and in later studies was associated with early termination of infection.[328] Persons with reduced humoral immunity also accumulate fewer amino-acid mutations in the E2 sequence.[162,329] In persons who recover from HCV infection, HCV antibody responses decline, sometimes below the level of detection by commercial assays.[256,276,330] In contrast, $CD4^+$ lymphocyte responses often are maintained (see the following section). Antibodies to HCV envelope proteins appear later and at lower titers compared with antibodies directed against nonstructural proteins, suggesting that neutralizing antibodies may play only a minor role in spontaneous resolution.[331,332] However, negative results may be due to mismatch of poorly conserved envelope antigens.

Several functional expression systems have enhanced studies of humoral responses directed against the HCV envelope proteins, including the use of lentiviral HCV pseudoparticles (HCVpp) in cell culture–based assays for neutralizing antibodies.[332–334] Application of this system to samples from one well-characterized individual suggested that HCV escapes continuously from neutralizing antibody responses in vivo.[158] Studies using HCVpp have shown a relationship between the control of virus infection and a neutralizing antibody response in single-source outbreaks of acute HCV infections.[335,336] These studies showed that a strong and progressive neutralizing antibody response resulted in decreased viremia and that rapid and early induction of neutralizing antibodies was associated with viral clearance. Meanwhile, absent or low-titer neutralizing antibodies in the early phase resulted in chronic infection, regardless of later induction of cross-neutralizing antibodies. Studies using autologous HCVpp have also demonstrated sequential escape from neutralizing antibody responses and that this mechanism drives the high rate of E1/E2 evolution.[159,160,337] Collectively, the available data suggest that the humoral immune responses reduce the fitness of some HCV variants, can cross-neutralize genotypes, and possibly limit the severity or even risk of recurrent infection but are unlikely to be necessary or sufficient for primary viral clearance.[338]

The potential existence of broadly neutralizing antibodies that are not associated with virus escape are critically important in the search for a universal vaccine. Studies in human liver chimeric mice have had success in protecting against HCV infection by delivering neutralizing antibodies through a recombinant adeno-associated virus (AAV) vector.[339] This group also tested the ability of these antibodies to interfere with established HCV infection. With maintenance dosing of the antibodies every 3 days, HCV RNA fell below the limit of detection between 5 and 11 days after the antibody treatment was started.[339] These data support that neutralizing antibodies are able to protect uninfected hepatocytes from becoming infected and allow for clearance of HCV in already infected hepatocytes.

Cellular Immunity

Viral recovery has been associated with a vigorous, broad cellular immune response.[277,340–342] Experiments carried out in chimpanzees indicated that both $CD4^+$ and $CD8^+$ memory T cells play critical roles in protection against reinfection with HCV.[343,344] The control of second infections appears to be linked kinetically to rapid acquisition of cytolytic activity by $CD8^+$ T cells that are resident in the liver and is normally associated with an expansion of circulating $CD4^+$ and $CD8^+$ memory T cells.[344] Most people with acute HCV infection develop vigorous $CD4^+$ and $CD8^+$ T-cell responses in the first few months, such that there is overlap between those who will clear and those who will develop persistent infection.[317] Subsequent evolution to persistence is associated with a rapid loss of multifunctional and proliferative anti-HCV $CD4^+$ T cells.[345] Prolonged viremia is characterized by HCV-specific $CD8^+$ lymphocytes that are unable to produce IFN-γ (a so-called "stunned" phenotype) and often express counterregulatory molecules such as programmed death 1 (PD-1) that could contribute to their inability to eradicate infection.[346–348] Stronger polyclonal cytotoxic T-lymphocyte (CTL) responses in the peripheral blood and liver also have been associated with lower levels of circulating HCV RNA.[349,350] HCV-specific CTLs also have been found in persons who were exposed to HCV but never were known to have had HCV antibody or viremia.[351,352] Theoretically, inhibition of the programmed death pathway may result in reversal of this exhausted phenotype and clearance of HCV infection.[353] Although registration trials of PD-1 inhibitors excluded HCV-infected subjects owing to concern for immune reconstitution syndromes, there are reports of use of these checkpoint inhibitors in patients with HCV-associated liver disease. In one HCC therapeutic study with tremelimumab, a fully human monoclonal antibody that binds to cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), 12 of 14 subjects had a decline in HCV RNA from baseline, but no patients resolved their HCV infection.[354]

Still other mechanisms have been suggested to reduce the susceptibility of infected cells to cytolytic attack by immune cells. The HCV core protein may bind to the cytoplasmic tail of the TNF-α receptor and the lymphotoxin-β receptor, based on ectopic expression studies.[60,61,63] This binding occurs immediately adjacent to the death domain and may modulate signal transduction through the receptor. Although the relevance and biologic effects of this interaction remain controversial, it is possible that it protects the infected cell against TNF-α–mediated apoptotic cell death. Although this has not been observed in HCV transgenic mice, defects in Fas-mediated apoptosis have been documented in HCV-transgenic mice, both in vivo and in hepatocyte explant cultures ex vivo.[355]

Finally, HCV sequence variation and immune escape from both T and B cells may also contribute to viral persistence.[85,356] Mutation within the amino-acid sequence of a critical epitope may allow a new quasispecies variant to evade a previously suppressive immune response, either cellular or humoral.[149,158,326] In several studies, acutely infected persons who developed persistent infection had a more complex quasispecies.[85,356] In agreement with that finding, viral escape in CTL epitopes has been reported frequently in persistently infected persons even when examined during acute infection.[165] Consistent with this notion, probable escape mutations within class I MHC-restricted epitopes were observed in chimpanzees that had an inadequate $CD8^+$ memory T-cell response owing to antibody depletion of $CD4^+$ memory cells.[344] In summary, it appears likely that many factors contribute to HCV persistence and that HCV may have evolved a variety of redundant and overlapping mechanisms of immune evasion to ensure its long-term persistence in the majority of immunologically normal persons who become infected. It is probable that additional mechanisms of viral immune evasion will be identified in the future.

Disease Progression

Although HCV infection leads to hepatic inflammation and steatosis, the major consequence of persistent HCV infection is the development of hepatic fibrosis, which may progress to life-threatening cirrhosis and a greatly increased risk of HCC (see "Clinical Manifestations of Hepatitis C Virus Infection"). These long-term complications occur in persons with persistent infection and generally more than 20 years after the onset of infection, although more rapid progression has been reported in persons infected after the age of 40 years, with immunosuppression from HIV or organ transplantation, or agammaglobulinemia, or in the setting of other comorbidities including HBV coinfection, diabetes, insulin resistance, obesity, excessive alcohol consumption, and hepatic steatosis.[357–365]

There are wide estimates (5%–25%) of the probability of cirrhosis occurring within 10 to 20 years of infection and little information available on progression beyond 30 years (Fig. 154.8).[366,367] One of the longest follow-up studies comes from the Irish anti-D immunoglobulin cohort, wherein over 800 Rh-negative women in Ireland were infected with HCV genotype 1b via contaminated anti-D immunoglobulin from a single donor.[368] Infected in 1977–79, these women have been followed for almost 4 decades. The most recent report of liver disease outcomes included assessments through December 31, 2013. This comprehensive report found that 19% of chronically infected women developed cirrhosis and that the rate of cirrhosis development increased over the successive decades of follow-up. Risk factors associated with more rapid progression

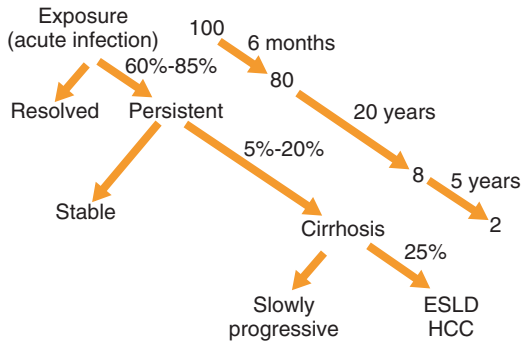

FIG. 154.8 Natural history of hepatitis C virus (HCV) infection. Estimates of the most common outcomes of HCV infection are provided, extrapolated to the hypothetical acute infection of 100 persons (numbers 100, 80, 8, and 2). *ESLD,* End-stage liver disease (e.g., esophageal varices, ascites, or hepatic encephalopathy); *HCC,* hepatocellular carcinoma. See text for expanded discussion.

of fibrosis included diabetes and high alcohol consumption. Once cirrhosis occurs, the rates of progression to liver failure (decompensated cirrhosis) and HCC are approximately 2% to 4% and 1% to 4% per year, respectively.[369–371] In the anti-D cohort, 1.9% and 4.9% of women developed HCC and died of liver-related disease, respectively.

Several studies are instructive for the range of outcomes and disease cofactors. In two separate studies of women infected by contaminated Rh immune globulin, the exact onset of HCV infection could be inferred, and the incidence of cirrhosis during 15 to 20 years of follow-up was very low (<5%); but, as noted earlier, in one of these cohorts with longer follow-up this risk increased over time, whereas in the other it remained low.[372–375] In another cohort of 1667 HCV-infected injection drug users, infected an average of 14 years and followed for an average of 8.8 years, the incidence of liver-related mortality was also low (3 per 1000 person-years).[285] Liver biopsies were performed on a random sample of 210 HCV-infected members of this cohort, and only 10% had evidence of serious liver disease (Ishak modified fibrosis scores of 3–6). There was little progression an average of 3 years later.[376,377] In a follow-up study of 1176 HCV antibody–positive participants in the same cohort approximately 10 years later, an increased risk of cirrhosis was noted and linked with increasing age, alcohol use, hepatitis B coinfection, high body mass index (BMI), and HIV infection.[378] These studies underscore the role of factors such as increasing age, comorbidities, and coinfection with HIV in progression of HCV-related liver fibrosis, and differences in cohorts with similar follow-up and exposures also emphasize unrecognized confounders.

In yet another study, Seeff and coworkers evaluated mortality and morbidity in a cohort of patients a mean of 18 years after development of posttransfusion hepatitis in comparison with control patients who were similarly transfused but had not developed recognizable hepatitis.[372,379] Overall mortality was high, reflecting the severity of underlying conditions, but not increased in HCV-infected patients. Liver-related mortality was slightly higher in patients with posttransfusion hepatitis (3%) than controls (1.5%), and it was estimated that approximately 10% of patients with posttransfusion hepatitis had cirrhosis 20 years later.[372] Other studies of posttransfusion hepatitis have found a higher rate of cirrhosis.[357,359]

This variability and uncertainty in the estimated frequency of life-threatening liver injury relate to limitations in basic research tools and the variable impact of environmental, host, and possibly viral factors for disease progression in different study cohorts (Table 154.1). Studies in tertiary care facilities generally predict higher rates of progression because they include a greater proportion of symptomatic participants (referral bias). Disease stage measurements are also relatively imprecise (see "Liver Fibrosis Staging"). However, much of the variability relates to environmental and host differences.

The leading environmental determinant appears to be alcohol ingestion.[380–382] Although excessive alcohol ingestion and HCV infection independently may cause cirrhosis, combined exposure has a synergistic

TABLE 154.1 Factors Associated With Cirrhosis in Persons With Hepatitis C Virus Infection

FACTOR	IMPACT	COMMENT
Environmental		
Alcohol use	+4	The importance of minimal alcohol ingestion (<20 g daily) has not been established.
Host		
HIV infection	+4	Increasingly important as HIV-related survival improves. May be masked by competing mortality.
HBV infection	+3	Strong effect when HBsAg positive; relatively uncommon.
Age	+4	Strong effect; increases as low as 40 yr. Hard to distinguish from infection duration.
Body mass index	+2	Associated with metabolic syndrome.
Duration of HCV infection	+3	Cirrhosis is rare before 10 yr.
HLA type	+1?	HLA B54 is correlated with increased risk of cirrhosis; DRB1*0301 with lack of cirrhosis.
Viral		
Quasispecies complexity	+1	Cross-sectional studies cannot assess causality, and complexity may be confounded by duration of infection.
HCV genotype 3	+3	Supported by multiple studies in different cohorts. Some are limited by cross-sectional assessment. Increased rates of steatosis with genotype 3 may contribute.
Quantitative measures of viremia (serum or plasma HCV RNA level)	+1	Not always detected or lost in multivariate analysis of age or HIV.

HBsAg, Hepatitis B surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen.

effect.[285,380,383] This is especially true with heavy alcohol ingestion (>50–125 g a day), which in one study increased the risk of cirrhosis approximately 100-fold.[380] The mechanism underlying this synergy with an environmental toxin remains obscure, but both alcohol and HCV infection may cause microvesicular steatosis, suggesting a common pathway involving mitochondrial injury.[384,385] Coinfection with HBV may also accelerate disease progression, whereas persistent HPgV (formally called GB virus C or hepatitis G virus) infection is likely not pathogenic and does not appear to affect hepatitis C.[386–390] A novel human virus, human hepegivirus (HHpgV-1), which shares features with HCV and HPgV, was discovered in blood transfusion recipients in 2015.[391] In an HIV-HCV coinfected cohort of people who use intravenous drugs, HHpgV-1 infection was reported in 10.9% of patients.[392] The medical importance of this virus remains unclear, as does whether it has any influence on the natural history of HCV infection. Schistosomiasis coinfection is associated with more rapid progression of HCV-related fibrosis in Egypt.[393] Increased progression of liver disease also has been reported in immunosuppression associated with agammaglobulinemia and transplantation.[394–396]

There is substantial evidence that disease progression is increased in persons infected at older ages, and this factor alone may explain much of the variability in studies because persons infected at older ages through transfusions appear to have the highest rates of progression to cirrhosis.[285,397,398] Although a few studies have detailed the natural history of HCV infection in children, the overall rate of disease progression appears quite slow, with few notable exceptions.[399–401]

Genotype has also been identified as a potential predictor of severe fibrosis. Early studies suggested an association with the genotype 3 infection and greater severity of fibrosis, but these retrospective studies

were limited by small cohorts, variability in patient characteristics such as presence of insulin resistance or BMI, HCV genotype distribution, and inconsistencies in methodology, particularly with respect to the grading of steatosis.[402,403] A retrospective analysis of the Swiss Hepatitis C study cohort ($N = 1189$) reported an independent association of genotype 3 infection with fibrosis.[193] A meta-analysis of 8 single biopsy studies ($N = 2349$) in patients with chronic HCV infection confirmed an association between fibrosis and genotype 3 (odds ratio, 1.52).[404] The same meta-analysis included 8 paired biopsy studies, which were underpowered and did not reveal a similar association. The primary limitation of these studies was that they did not account for steatosis. Studies that have considered steatosis as an independent risk factor for fibrosis have drawn different conclusions. For example, a large meta-analysis of 3068 patients with chronic HCV infection reported that genotype 3 infection was associated with steatosis, not fibrosis, and a multivariate analysis of fibrosis identified steatosis and level of inflammatory activity at histopathologic assessment as independent predictors of disease.[365] There are multiple studies supporting the association of steatosis with higher rates of fibrosis progression and studies supporting the concept that greater liver fibrosis risk is predominantly associated with steatosis in genotype 3 infection.[405]

Since the advent of active antiretroviral therapy, HCV-related liver disease has emerged as a major cause of death in HIV-infected persons.[406–408] In the large D:A:D study, 49,741 HIV-infected persons were followed from March 1999 through February 1, 2011, and 3909 died.[408,409] The primary causes of death were acquired immunodeficiency syndrome (AIDS; 29%), non-AIDS cancers (15%), and liver disease (13%). Although this was a decline in liver-related deaths from second highest to third from the prior publication from this cohort, the absolute decrease was minimal. Overall, all causes of death decreased, highlighting the significant positive impact of antiretrovirals (ARVs) on mortality, with one exception: non-AIDS cancers, which remained stable. Accumulating population data show that liver disease will attenuate the remarkable long-term benefits of antiretroviral therapy in the subset coinfected with HCV (see Chapter 128) until greater penetration of DAAs is seen.[410–412]

Hepatic Fibrosis

Liver fibrosis is a wound-healing response that occurs in the setting of chronic liver injury. Liver fibrosis is the net result of a complex, tightly regulated dynamic process in which collagen and other proteins are deposited and removed from a matrix in the subendothelial space between hepatocytes and the sinusoidal endothelium. Accumulation of liver fibrosis occurs in response to all forms of liver injury, and with viral hepatitis begins in the periportal zone and gradually extends as so-called septae into lobules toward the central veins.[413] As the matrix expands and changes its composition, normal liver physiology can be disrupted and the architecture of the organ altered, although it is not clear whether this process is clinically evident before development of cirrhosis. The hepatic stellate cell (Ito cell) appears to be the chief architect of this matrix, responding to a variety of stimuli and resulting in activation (see Friedman's excellent review[414]).

Cytokines, reactive oxygen species, and other mediators of inflammation can initiate stellate cell activation, which can be perpetuated by autocrine and paracrine stimulation. Kupffer cells can play an important role initiating and perpetuating fibrogenesis, through production of TGF-β metalloproteinases, and reactive oxygen species.[415–417] $CD8^+$ and $CD4^+$ lymphocytes also appear to influence the pathogenesis of fibrosis, chiefly through stellate cell activation. In the CCl_4 mouse model, fibrogenesis is enhanced when Th1/$CD8^+$ lymphocyte responses (in particular, IFN-γ) are depleted and by expression of Th2/$CD4^+$ lymphocyte-derived cytokines such as IL-4.[418] These data are interesting in light of the clinical observation of greater progression of fibrosis in persons coinfected with schistosomiasis in whom Th2-like responses predominated,[393] and the extremely rapid fibrosis progression in persons with congenital agammaglobulinemia.[394] Similarly, evidence suggests that alcohol-related liver injury and fibrosis may be mediated in part by microbial translocation products including lipopolysaccharide, which may trigger TLR4 on stellate cells, resulting in recruitment of Kupffer cells and sensitization of stellate cells to the profibrotic action of transforming growth factor-β (TGF-β).[419] The strong associations between progression of fibrosis and both older age and various forms of immunosuppression are consistently found. In the previously mentioned cohort of injection drug users, even after adjustment for other factors such as alcohol intake, hepatitis B coinfection, and BMI, persons with HIV had liver fibrosis stages comparable with HIV-uninfected persons an average of 9.2 years older.[378] However, it remains unclear whether age and immunosuppression affect liver fibrosis through similar mechanisms.

Likewise, it remains unknown why only some immunocompetent persons with chronic hepatitis C develop cirrhosis. There is little reason to believe that certain HCV variants are more virulent than others, although as previously discussed there are differences in fibrosis progression for some genotypes, in particular genotype 3. These differences in pathogenesis are less likely to be due to "virulence" and more likely related to differences in HCV-related steatosis. In acute infection, the virus replicates at high level for weeks with little or no evidence of liver damage, and large studies have not shown an association between the development of hepatic fibrosis and HCV viremia in chronic infection.[412]

Host genetic factors probably play a role in determining why some infected persons develop cirrhosis. Wiley and coworkers have reported that African-Americans have slower progression of fibrosis compared with Caucasians, a finding that appeared independent of other factors and that has also been noted by others.[420,421] Specific HLA alleles have been associated with differences in the progression of disease, as have polymorphisms in several genes believed to play a role in disease pathogenesis, including TGF-β.[422–424] With few exceptions, however, the link between these polymorphisms and the pathogenesis of disease remains unclear.

A genome-wide association study of the IDEAL study identified two SNPs—the *IL28B* SNP, previously discussed, which was also associated with treatment response and natural clearance, and an SNP in the *PNPLA3* gene—that were associated with steatosis on histopathology.[425] The *PNPLA3* gene codes for a hydrolase against triglycerides and retinyl esters in hepatic stellate cells. The polymorphism, rs738409 GG, was initially identified as a risk factor for nonalcoholic fatty liver disease and was associated with an increased risk of steatosis and fibrosis in the setting of chronic HCV infection.[425] Multiple candidate gene studies have now reported an association of PNPLA3 and severity of liver disease including fibrosis and HCC.[426] The I148M variant has been reported to modulate the fibrogenic phenotype of hepatic stellate cells, the primary cell type in fibrogenesis and matrix deposition.[427] Thus it is possible that the failure of some studies to show associations of polymorphisms and fibrosis is because they do not target the primary pathogenic mechanism, which may be steatosis.

Human Immunodeficiency Virus and Hepatitis C Virus Infection

HIV infection adversely affects all phases of the natural history of HCV, increasing the frequency of viral persistence after acute infection, the level of viremia among persistently infected persons, the rate of progression to cirrhosis, and the proportion of persons who will ultimately develop end-stage liver disease.[428,429] Darby and coworkers[430] studied mortality from liver disease and HCC among 4865 men with hemophilia who were exposed to HCV-contaminated blood products. At all ages, the cumulative risk of liver-related death after the presumed exposure to HCV was 1.4% (range, 0.7%–3%) for HIV-uninfected men and 6.5% (range, 4.5%–9.5%) for HIV-infected men.[430] Goedert[431] also found a significantly increased risk of liver disease in HIV- and HCV-coinfected members of the Multicenter Hemophilia Cohort Study. In studies done before effective antiretroviral therapy use was widespread, the impact of liver disease on mortality in HIV- and HCV-coinfected persons was lower in injection drug users than in patients with hemophilia. Thomas and colleagues[285] did not detect more end-stage liver disease in HIV-infected members of a study of 1667 HCV-infected current and former injection drug users. This apparent discrepancy was chiefly due to high competing causes of mortality in the HIV-infected drug users. In that same cohort, a more recent analysis of 1176 HCV antibody–positive participants, the prevalence of cirrhosis was higher in HIV- and HCV-coinfected persons (19.5%) than in those infected with just HCV (11%).[378] Similarly, Lo Re and colleagues investigated rates of hepatic decompensation among

veterans with HCV infection and found higher rates in patients with ARV-treated HIV and HCV coinfection (hazard ratio, 1.56).[432]

On the other hand, it remains unclear whether chronic hepatitis C affects the natural history of HIV disease. In the Swiss cohort study, Greub and colleagues[433] reported that among 3111 patients receiving antiretroviral therapy, HCV-infected persons had a modestly increased risk of progression to a new AIDS-defining event or death, even among the subgroup with continuous suppression of HIV replication. They also reported that the magnitude of the CD4 cell increase after effective anti-HIV therapy was significantly less than observed in HCV-uninfected persons, suggesting that HCV coinfection may blunt immune recovery.[433] Likewise, an analysis of 9164 HIV-infected persons with known dates of seroconversion, since 1997 when antiretroviral therapy was available, reported higher HIV/AIDS-related mortality among HIV- and HCV-coinfected persons than among those with HIV infection alone.[434] On the other hand, among 1742 patients in a Baltimore HIV clinic, differences in the progression to AIDS or death chiefly appeared to be attributed to lower exposure to highly active antiretroviral therapy among the HCV-coinfected injection drug users.[435] Similarly, Chung and coworkers failed to detect a difference in immune restoration in HIV- and HCV-coinfected participants in a well-controlled AIDS Clinical Trial Group study, suggesting that the effect of HCV infection on progression of HIV is not large.[436]

There is a complex relationship between antiretroviral therapy and liver disease progression in HIV-infected persons. On one hand, antiretroviral therapy can itself be hepatotoxic. Hepatotoxicity occurs in about 10% of persons given a new antiretroviral regimen, more often if the person is HCV coinfected.[437–439] In some cases, antiretroviral therapy–associated hepatotoxicity has been linked to liver failure and death.[440] However, in most cases it manifests with an elevation in liver enzymes (often greater than fivefold normal) in the absence of symptoms. Although there is no reason to withhold antiretroviral therapy from HCV-infected persons, close monitoring is advisable if there is cirrhosis. Treatment of HCV infection also may reduce the incidence of antiretroviral therapy–related hepatotoxicity.[441]

On the other hand, antiretroviral therapy may protect against the adverse effects of HIV infection on liver disease.[442–444] In one recent prospective study, antiretroviral therapy exposure and effectiveness was compared in 174 HIV- and HCV-coinfected persons who underwent at least two liver biopsies between January 1998 and July 2006, and 41 (24%) had significant progression (≥2-point increase in Ishak system score).[445] Little difference was detected in this study in the rate of fibrosis progression according to antiretroviral use between biopsies. However, most persons were already on antiretroviral therapy before the first biopsy, which might have diminished the effect in the relatively short interval between biopsies. Because antiretroviral therapy is widely advocated at all $CD4^+$ lymphocyte counts, the clinical implication of demonstrating whether antiretroviral therapy is beneficial in patients with HCV coinfection has diminished.

The pathogenesis of HIV and HCV coinfection is poorly understood. HIV infection is likely to affect the adaptive immune response to HCV. Similar to experimental depletion of $CD4^+$ lymphocytes in the chimpanzee model, HIV infection of $CD4^+$ lymphocytes activated to combat HCV could diminish $CD8^+$ lymphocyte responses and maturation of humoral immunity.[344] Kim and coworkers correlated the nadir of CD4 lymphocyte depletion with the magnitude of reduction in HCV-specific $CD8^+$ lymphocyte responses in HIV-infected persons (who, it is interesting to note, do not have lower Epstein-Barr virus– or cytomegalovirus-specific $CD8^+$ responses).[446,447] Likewise, Netski[448] found that HCV-specific humoral responses were indirectly correlated with $CD4^+$ lymphocyte suppression. Thus, HIV-related CD4 lymphocyte depletion probably contributes to the clinical observation that HIV infection reduces the likelihood of spontaneous HCV clearance.[285]

Whether HIV replication in hepatocytes, Kupffer cells, or stellate cells occurs and directly affects HCV replication or fibrogenesis is largely unknown. Likewise, HCV replication in monocytes and lymphocytes has been inferred, but only at low level and in a minority of cells.[224,436] Chung and coworkers have reported that HIV proteins such as p24 and virus (produced from other cells) can enhance HCV replication in vitro and increase TGF-β1 expression and that these processes can be blocked by antibodies to the CCR5 or CXCR5 HIV coreceptors.[449] These investigators also showed that HCV and HIV independently induce TGF-β1 through the generation of reactive oxygen species, which in turn induces p38 MAPK, JNK, ERK, and ultimately NF-κB.[450,451] Increased STAT1 activation and Fas ligand expression in HIV-infected persons may lead to increased hepatocyte apoptosis, an effect that appears to be enhanced by both HCV and HIV envelope proteins.[452,453]

HIV infection may also enhance liver fibrosis by increasing translocation of microbial products from the gut. HIV infection of gut lymphoid tissues is associated with enhanced microbial translocation, and these products are taken up near the portal vein by Kupffer cells.[454,455] As has been shown in an experimental model of alcohol-related liver disease, that process may lead to increased Toll cell 4 activation of stellate cells, especially if the Kupffer cell ability to take up bacterial products is diminished by HIV infection.[419] Cirrhosis itself, irrespective of the etiology, increases shunting of blood past the liver and might explain some of these findings.

The interaction between antiretroviral therapy and HCV infection is also poorly understood. Hepatotoxicity in HIV- and HCV-coinfected persons could reflect decreased drug metabolism, HCV-specific immune reconstitution, or increased susceptibility to mitochondrial dysfunction.[385,456–459] Thus, the mechanisms by which these viruses interact and the pathogenesis of coinfection remain important research topics.

CLINICAL MANIFESTATIONS OF HEPATITIS C VIRUS INFECTION

Acute Hepatitis C

Acute HCV refers to the initial 6 months of infection.[460] Although usually not associated with clinical symptoms (present in 15%–30%), acute HCV infection may cause malaise, nausea, and right upper quadrant pain, followed by dark urine and jaundice.[461] Symptoms usually occur within 5 to 12 weeks of the HCV exposure and are clinically indistinguishable in the individual patient from other types of acute viral hepatitis.[461] HCV RNA can be detected in blood within days of exposure (2–14 days), followed by elevations in serum levels of the liver-specific enzyme, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and in some cases bilirubin.[273,276,462] The most detailed information regarding the time course of acute infection comes from studies of HCV-contaminated transfusion recipients. Mosley and coworkers performed a look-back study of 94 persons infected through transfusion.[274] In 67 sera collected between the fourth and eighth days after transfusion, HCV RNA was detected in all but 2, underscoring the rapid onset of viral replication. There was wide variation in incubation (time to elevated ALT or symptoms), which ranged from 6 to 112 days (median, 46 days). Shorter incubation was associated with higher ALT, and jaundice was detected in only 21%.

The early HCV RNA kinetics have been well described and can be useful to determine the probability of natural clearance.[463,464] The early viral dynamics have been characterized in three phases: pre–ramp-up (first week after exposure), ramp-up (second week after exposure), and plateau phase (2–3 months after exposure).[463] Fluctuations in levels of HCV RNA are common (>1 log), and low levels of HCV RNA can be useful in differentiating acute from chronic infection and can help in predicting which patients are more likely to naturally clear the infection.[463–465] Factors associated with HCV RNA dynamics and natural clearance include sex, HIV coinfection, IFN-λ CC genotype, and HCV genotype 2.[466] As noted previously, in approximately 25% of patients, acute HCV infection is followed by spontaneous clearance, whereas in the rest it progresses to chronic infection. In addition to the innate and humoral immune response, host factors play a role in determining the risk of persistence, including sex, HIV coinfection, and genetics.[280,290,467]

Fulminant Hepatitis C

The frequency with which HCV causes fulminant hepatitis is controversial. HCV infection has been associated with 40% to 60% of fulminant NANB in Japan, but it is an uncommon cause of fulminant liver disease in Western countries.[468–470] This discordance might arise from variation in either host factors or viral strains, or both. There appears to be an increased likelihood of fulminant liver disease following acute HAV infection in persons with underlying chronic hepatitis C.[471]

Chronic Hepatitis C

Seventy-five percent or more of persons with acute hepatitis C infection develop persistent infection with long-term viremia, as described earlier (see "Viral Persistence") (see Fig. 154.8). Thus, although only one in six cases of symptomatic acute viral hepatitis is due to HCV infection, HCV is the leading infectious cause of chronic liver disease in the United States. Persistently infected individuals tend to have few symptoms (e.g., fatigue or malaise), resulting in lack of presentation to care and therefore lack of diagnosis. Because of this challenge and of failure of risk-based screening to identify the majority of people living with HCV infection in the United States, the CDC made the recommendation to expand screening to all persons born between the years of 1945 and 1965—that is, birth cohort testing.[472]

Once chronic, HCV infection persists indefinitely. Serum ALT levels typically fluctuate independent of symptoms, whereas serum HCV RNA levels remain fairly constant.[282,357,473,474] The degree of inflammation present at liver biopsies also varies over time.[358,475] Some individuals will develop fibrosis, which typically begins in portal triads but can bridge between triads or central veins and ultimately destroy the hepatic architecture, progressing to nodularity and cirrhosis.[413] There is a poor correlation among necroinflammatory liver injury, serum ALT levels, serum HCV RNA levels, and the extent of fibrosis.[476] The rate of hepatic fibrosis progression has been reported with serial liver biopsies in two large systematic reviews in populations with HCV monoinfection and HIV and HCV coinfection. The mean annual stage transition probabilities have been reported as 0.117 and 0.122 (F0 to F1), 0.085 and 0.115 (F1 to F2), 0.120 and 0.124 (F2 to F3), and 0.116 and 0.115 (F3 to F4), respectively.[428,477] Once established, 10% to 20% of HCV-infected persons with cirrhosis will decompensate clinically within 5 years, as evidenced by bleeding esophageal varices, ascites, encephalopathy, or HCC.[369–371] In a study of 214 persons with chronic hepatitis C and compensated (Child Pugh A) cirrhosis followed for an average of 114 months, 32% developed HCC, which occurred at a rate of 3.9% per annum.[478]

Hepatocellular Carcinoma

Primary HCC is typically a late complication of chronic hepatitis C, primarily occurring in patients with cirrhosis.[479,480] The rate of HCC development in people with HCV-associated cirrhosis is approximately 2% to 4% per year, with higher rates reported in some countries.[481–483] HCV-related liver cancer has been particularly evident in Japan and Italy and often follows 2 or more decades of infection.[480,484] The incidence is increasing in the United States, with new liver cancer cases increasing 38% from 2003 to 2012 and associated mortality increasing 56% during that same period.[485] An increasing incidence of HCC in Western countries has been attributed to prior increases in the prevalence of HCV infection and aging of those cohorts.[486,487] Worldwide, there is considerable variation in the fraction of HCC attributed to HCV infection and variability among HCV-infected persons in the proportion of mortality attributed to HCC versus cirrhosis. These differences probably are due to heterogeneity in the average ages of HCV-infected persons and the relative abundance of hepatitis B or other HCC cofactors.[479,480,488,489]

In Japan and Southern Europe, 50% to 75% of HCC is associated with HCV infection.[484] In one Italian study, HCV infection was found in 71% of patients with liver cancer, and HBV infection in only 15%.[480] In contrast, in a study from Taiwan, only approximately 15% of HCC was attributed to HCV.[490] In Japan, the mortality rate due to HCC increased approximately twofold during the 1980s and is chiefly linked to HCV.[491] Among HCV-infected persons in Japan, the mortality from HCC exceeds that due to cirrhosis. Reports from the Japanese Ministry of Health suggest that this increase in the HCV-related disease burden stems from widespread illicit injection of amphetamines in the 1950s and related spread of HCV within the Japanese population.

Overall, more than half of patients with cirrhosis untreated with antivirals die from cirrhosis complications before a diagnosis of HCC can be made.[492] With increasing access to DAA therapy, with which it is expected that eradication of HCV will decrease the risk of decompensating events, it is likely we will see a shift in HCV-attributable mortality from cirrhosis to HCC.[493] Modeling studies project a decrease in cirrhosis-related mortality by 21% to 27%.[493] This increased lifespan also returns a longer lifetime risk of developing HCC. These findings are based on the assumption that HCC incidence is not modified by DAA therapy, yet there is already emerging data that DAA will affect HCC incidence.[494] A meta-analysis of 18 studies from the IFN era suggested that sustained virologic response (SVR) was associated with a reduced risk of HCC (relative risk, 0.24; 95% confidence interval, 0.18–0.31).[495] Whether this remains true in the DAA era is an emerging area of investigation. Early studies suggested either minimal or no decrease in risk of HCC after DAA therapy, whereas others actually reported an increased risk of HCC after DAA exposure.[496–499] However, more recent and more comprehensive studies have reported a significant decrease in HCC incidence.[500,501] Although multiple groups have reported higher crude incidence rates for HCC in patients receiving DAA versus IFN-based regimens, there is no significant association between treatment regimen and HCC risk after adjustment for important confounders, including severity of cirrhosis, age, diabetes, and low platelet counts and albumin levels.[500]

As with cirrhosis, several cofactors have been proposed in the development of HCV-associated HCC. HBV coinfection appears to increase the risk of HCC in HCV-infected persons.[387,502] Infection with genotype 3 virus also has been associated with HCC.[479,503,504] In addition, alcohol and tobacco use, older age, and male gender are associated with HCC among HCV-infected persons.[387,479]

Relatively little is known about how chronic HCV infection leads to cancer. The HCV genome is not reverse-transcribed to DNA and thus cannot integrate into host cell chromosomes. Transforming activities have been associated with the core and NS3 proteins.[59,505] Strong evidence in favor of a direct or indirect transforming action of the core protein comes from studies of transgenic mice that develop such tumors in the absence of an immune response.[264,506] In addition, it is possible that NS5A promotes the development of tumors by repressing the antitumor activity of PKR.[130] However, in humans, chronic HCV replication is not by itself sufficient to cause HCC, even when accompanied by inflammation, because overall cirrhosis is evident when HCC develops. Even in the transgenic mouse model, liver cancer probably occurs as a result of increased hepatocyte turnover, dysregulation of proapoptotic and antiapoptotic cellular signaling pathways, or the generation of free hydroxyl radicals that are capable of damaging cellular DNA.[385,507]

Extrahepatic Manifestations of Hepatitis C Virus Infection

Although the most common end-organ disease associated with chronic HCV infection occurs in the liver, there are numerous extrahepatic manifestations attributable to HCV infection that result in morbidity and mortality. Increased all-cause mortality, as reported in patients with HCV infection, is thought to be related in large part to the extrahepatic manifestations of the chronic infection. With IFN-based therapies, SVR was associated with a decrease in all-cause mortality—evidence of the extrahepatic health impacts of chronic HCV infection.[194,508,509] Similarly, SVR achieved with DAA therapy is associated with significant decreases in all-cause mortality, and this is true in patients with and without severe liver disease at the time of treatment.[510,511] The range of extrahepatic manifestations of chronic HCV infection is broad and includes vasculitis, glomerulonephritis, nonhepatic malignancies, neurologic disease, and metabolic diseases such as diabetes, insulin resistance, and cardiovascular disease.[512–515]

HCV infection is associated with essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, and porphyria cutanea tarda. Up to half of HCV-infected persons have circulating cryoglobulins. However, only a small percentage develop the vasculitic syndrome of essential cryoglobulinemia (type II or type III, with circulating polyclonal immunoglobulin G [IgG] and IgM immune complexes).[516,517] HCV infection is also associated with B-cell lymphoproliferative disorders.[518–520] Clonal expansion of B cells in response to viral antigens and production of immune complexes is believed to be the primary pathogenesis of these extrahepatic diseases.[521] These clonal expansions are found in patients with HCV and non-Hodgkin lymphoma (NHL), suggesting a strong shared pathogenesis among these immune-mediated conditions.[522] HCV is also associated with aggressive diffuse large B-cell NHL and follicular lymphoma; however, the pathogenesis is likely different, with

a greater association with direct viral infection of B cells.[523] Membranoproliferative glomerulonephritis may also occur in association with HCV-related cryoglobulinemia, generally in the absence of vasculitis.[228,517] There is accumulating evidence that treatment of HCV infection stabilizes or reverses renal disease progression, including in the DAA era.[524,525] Chronic HCV infection has also been found in 60% to 80% of persons with sporadic (but not familial) porphyria cutanea tarda.[526,527] To a lesser extent, HCV infection has been associated with Mooren corneal ulcers, Sjögren syndrome, lichen planus, and idiopathic pulmonary fibrosis.[528] The pathogenesis of such conditions remains unknown. However, sialadenitis resembling that occurring in Sjögren syndrome has been observed in transgenic mice expressing HCV envelope proteins.[529] Thyroid autoantibodies, Hashimoto thyroiditis, and hypothyroidism have been associated with chronic hepatitis C in women.[530]

Chronic HCV infection is also associated with metabolic disorders such as insulin resistance, type 2 diabetes mellitus, and atherosclerosis.[384,531–533] As previously mentioned, there is strong evidence of a direct viral link between steatosis and genotype 3 chronic HCV infection (see also "Disease Progression"). The prevalence of steatosis is increased in patients with genotype 3 HCV infection, and steatosis improves with successful HCV treatment.[384,534] In patients with the more common genotype 1 HCV infection, there is also a correlation with insulin resistance and steatosis.[532,533] Several studies have shown that patients with chronic HCV infection have an increased risk of diabetes mellitus compared with both uninfected and HBV-infected controls.[134,535–538] The elevated risk is likely to be due to the association of HCV infection and insulin resistance. HCV infection causes both hepatic and extrahepatic insulin resistance. Patients with HCV infection have been found to have an increased homeostasis model for assessment of insulin resistance (HOMA-IR) as compared with both uninfected and HBV-infected controls.[539–541] Furthermore, HCV clearance with IFN-based and DAA antiviral therapies results in reduced levels of HOMA-IR, suggesting that clearance restores insulin sensitivity.[542,543] It can be difficult to tease out the impact of HCV on these metabolic diseases. One large observational study found that HCV infection was associated with diabetes mellitus until liver enzyme levels were included as confounders, attenuating the association.[544] It is possible that the liver enzyme elevation represented steatosis and is the true mediator of diabetes risk in patients with HCV infection. It is also possible that liver inflammation is the driver of insulin resistance and diabetes risk, and that the virus itself has less role to play in these metabolic disease complications.

Although the relationship of HCV infection and insulin resistance, steatosis, and diabetes is generally well accepted, whether HCV is an independent risk factor for cardiovascular disease is less clear. Studies focusing on markers of atherosclerosis have generally suggested a greater risk among patients with HCV infection; however, the outcomes of these studies have been highly variable.[545–548] Furthermore, studies assessing myocardial infarction and cardiovascular-related mortality have conflicted.[549–552] One difference in outcomes that may affect the results of such studies is differentiating type I (e.g., thrombotic or plaque rupture) from type II (supply-demand mismatch) myocardial events. Similar to the situation with other metabolic diseases, there is increasing evidence that HCV eradication with antivirals decreases cardiovascular events.[553–555]

DIAGNOSIS LABORATORY ASSESSMENT OF HEPATITIS C VIRUS INFECTION

Screening Serology

The diagnosis of HCV infection is based principally on detection of antibodies to recombinant HCV polypeptides and by assays for HCV RNA. There have been several "generations" of enzyme immunoassays (EIAs) that measure antibodies directed against NS4, core, NS3, and NS5 sequences.[556–558] The US Food and Drug Administration (FDA) has approved at least six assays for detection of HCV antibodies by commercial laboratories.[559] The sensitivity of the third-generation assay is estimated to be 97%, and it can detect HCV antibody within 6 to 8 weeks of exposure.[560,561] These assays are measures of either past or current HCV infection, not immunity. Direct testing for HCV RNA is necessary to distinguish between ongoing or prior infection in persons with HCV antibodies. Surrogate neutralizing tests based on pseudotyped lentivirus particles were described earlier ("Humoral Immunity"). Assays for IgM HCV antibodies are not clinically useful.

Point-of-care, "rapid" HCV tests may also have sensitivity and specificity for detection of HCV antibodies in a fingerstick-derived capillary blood specimen, similar to what is obtained with venous blood assayed in a commercial laboratory (>98%), and have the added value of providing the information quickly to the patient and provider.[562] The OraQuick HCV Rapid Antibody Test (OraSure Technologies) has been given a waiver by the FDA for use to detect HCV infection in nontraditional settings.

In the past, supplemental serologic testing such as with the recombinant immunoblot assay (RIBA) has been used to improve the specificity of the screening tests by demonstrating to which HCV antigen there was reactivity.[563,564] For this purpose, the FDA licensed the Chiron RIBA HCV 3.0 SIA (Chiron Corporation, Emeryville, CA). However, supplemental antibody assays are no longer commercially available in the United States. Accordingly, use of high signal/cutoff ratios has largely replaced RIBA testing to improve the specificity of EIA results.[559] In clinical laboratories, EIA-reactive samples with high optical density (e.g., >3.8 for the Ortho and Abbott EIA tests) are nearly always RIBA positive or contain HCV RNA.[565] Thus, HCV testing recommendations generally call for screening for HCV antibodies followed by RNA testing, as detailed later.[566,567]

According to US Public Health Service Guidelines, all HIV-infected persons should be screened for HCV infection at entry into health care.[568] HCV screening should be done as in persons without HIV infection, as described earlier. In HIV-infected persons, HCV antibody titers may decline below the level of detection, especially in those with advanced immunodeficiency (CD4 cell count <100/mm^3).[569–572] There are case reports of HCV *seroreversion* occurring in association with immunosuppression, and *seroconversion* associated with antiretroviral therapy.[457,573] Thus, although uncommon in other settings,[574] HCV antibody–negative persons with HIV infection who have unexplained liver disease should be tested for HCV RNA to exclude the possibility of seronegative infection.[575,576] In addition, patients with HIV infection and ongoing risk of HCV exposure (men who have sex with men [MSM], people who inject drugs [PWID]) should be tested at least annually.[577]

RNA Detection

HCV RNA can be detected in plasma and serum by means of a variety of molecular techniques, including reverse-transcriptase polymerase chain reaction (RT-PCR), real-time PCR, transcription-mediated amplification (TMA), and branched DNA (bDNA) methods. Initially, tests used to quantify the amount of virus were less sensitive than those used to detect HCV RNA and also did not precisely characterize HCV RNA in persons with levels estimated at more than 6 $\log_{10}$IU/mL. Accordingly, some tests are FDA approved specifically for detection, and others for quantification. More recently developed real-time PCR and TMA assays quantify virus across a wide range and have comparable sensitivity (10–50 IU/mL) to qualitative assays.[578–580]

A number of factors may affect the estimate of HCV quantity, including the assay used, time to serum separation, storage temperature, collection tube, and testing laboratory.[579,581] To improve the comparability of HCV RNA results, clinicians should use the same assay and laboratory to monitor a patient on treatment, and laboratories should report quantitative results in international units (IU), which correspond to a standardized amount of HCV RNA rather than viral particles.[582] Algorithms have been published for the conversion of proprietary unit values provided by commercially available assays to international units.[583] In persistently infected persons not receiving treatment, HCV RNA levels tend to remain stable (within one-half $\log_{10}$) over years.[584,585] In the absence of treatment, there is little need to repeat HCV RNA tests frequently.

Core Antigen Detection

HCV core antigen (p21) is a highly conserved protein that is a component of the viral nucleocapsid and present in plasma throughout infection. HCV core antigen detection represents another means of confirming active viral replication and offers several potential advantages to RNA detection in the current therapeutic era in which RNA quantitation is generally not required in order to optimize therapy. Current advantages

include shorter assay time with automated sample processing and lower costs compared with HCV RNA detection; however, a theoretical benefit is improved potential for low-cost, point-of-care assay development—a key component of HCV treatment rollout in resource-limited settings.[586,587]

Several commercial EIAs are available, with most clinical data limited to two assays (Abbott ARCHITECT HCV Ag [Abbott] and Ortho HCV Ag ELISA [Ortho Clinical Diagnostics]).[583,588] Sensitivity and specificity of the assays are high (90%–94% and >97%, respectively), although sensitivity falls off at HCV RNA levels below 3000 IU/mL.[587] Emerging clinical data indicate that core Ag assays are highly accurate in determining HCV treatment outcomes with DAA-based therapies when compared with quantitative HCV RNA detection.[586,589,590]

Genotype

Determination of the viral genotype played a large role in predicting the response to IFN-based therapy (see "Genetic Diversity" earlier). With the currently available DAA regimens, responses are now uniformly high, with several regimens providing roughly equivalent response rates regardless of genotype.[591,592] However, depending on the DAA regimen used, genotype and (in some cases) subtype determination (1a vs. 1b) remains critical for appropriate treatment, including the need for further genotypic resistance testing in select situations (see "Treatment of Chronic Hepatitis C," sections on "Initial Treatment of Chronic Hepatitis C Virus Infection," "Genotype 1," and "Antiviral Resistance").[593,594] The reference method for determination of HCV genotype and subtype is based on sequencing of the core/E1 and NS5B regions; however, this approach is rarely used in the clinic.[185,595]

Several assays are commercially available to determine HCV genotypes on the basis of direct evaluation of 5′ noncoding sequence (5′UTR; Trugene 5′NC HCV Genotyping kit; Bayer Healthcare Diagnostics Division, Tarrytown, NY) and hybridization to genotype-specific 5′ noncoding and core region oligonucleotide probes (INNO-LiPA HCV II [Innogenetics, Ghent, Belgium] and Versant HCV Genotyping Assay 2.0 [Bayer Healthcare Diagnostics Division].[596] The first-generation line probe assays that use only the highly conserved 5′UTR accurately identify the genotype but have limited ability to accurately determine subtype (1a vs. 1b) and may not be able to distinguish subtype 1b from certain genotype 6 subtypes.[597–599] Second-generation line probe assays that also include core sequences have been shown to have improved ability to discriminate between genotype 1 subtypes.[599] Genotyping assays that use oligonucleotide probes to the 5′UTR for genotype assignment and NS5B for subtype determination also have improved ability to discriminate between genotype 1a and 1b subtypes (RealTime HCV Genotype II; Abbott Molecular, Des Plaines, IL).[599,600] In settings in which subtype determination is critical for treatment decision making, a second-generation assay (either line probe or oligonucleotide probe) that targets two regions of the HCV genome should be used.

Other Laboratory Testing

Some clinicians routinely test persons with confirmed HCV infection for related conditions. Typical evaluations include testing for HIV, hepatitis B surface antigen, and susceptibility to HAV and HBV infections, and screening for other underlying causes of liver disease; for example, autoimmune liver disease, hemochromatosis, Wilson disease, and α_1-antitrypsin deficiency.[575] With the recent recognition of HBV flares during DAA-based HCV treatment in patients with prior HBV infection, including a few patients with isolated HBV core IgG antibody positivity, expanded baseline testing for prior HBV exposure is now recommended.[598–602] All patients with a negative hepatitis B surface antibody test result (<10 mIU/mL) should be screened for prior HBV infection with HBV core total or IgG antibody testing. Treatment of HCV in patients who have evidence of prior HBV exposure yet lack of serologic evidence of protective immunity (HBV surface antibody titer <10 IU/mL) should be monitored more closely for evidence of HBV reactivation.

Clinical Application of Tests for Hepatitis C Virus

Detection of HCV infection begins with testing. In 1998, the CDC recommended testing persons with various HCV risk factors such as injection drug use or receipt of blood transfusion before 1992 (Table 154.2). That recommendation had little impact on HCV testing because most studies suggested that no more than half of HCV-infected persons were aware of their status (see "Epidemiology of Hepatitis C"). Thus, both the CDC and the US Preventive Services Task Force (USPSTF; grade B recommendation) recommended that all persons born from 1945 to 1965 undergo at least one HCV test.[472,603] Because roughly 75% of all those with HCV infection in the United States are in that birth cohort, this practice could lead to recognition of nearly 1 million new cases. Efforts to integrate this recommendation into clinical practice have met with mixed results. For example, a nationwide survey of Medicare data demonstrated a 91% increase in testing from 2011 to 2014 after the USPSTF recommendations were made; however, the absolute screening rate remained at a very low level (3.26% in 2014).[604] In isolated settings, approaches such as electronic medical record prompts and even laws requiring HCV screening have been effective.[605–609] Given the inconsistent nature of screening practices and the recognition of an increasing HCV incidence and burden in those outside the birth cohort, universal screening for HCV in adults should be considered and has been modeled to be cost-effective and to increase the number of people diagnosed and treated.[610]

Chronic HCV infection is usually diagnosed by testing for HCV antibodies, then by testing reactive sera for HCV RNA to assess if the infection is ongoing. As HCV treatments have evolved, the importance of accurately quantitating baseline HCV RNA levels and determining changes during therapy have been dramatically diminished.[611,612] Still, in most settings a quantitative HCV RNA test with a sensitivity limit of less than 15 to 25 IU/mL and a wide linear range is used. A negative RNA test result (<15 or <25 IU/mL, depending on the specific assay) in a person found to have HCV antibodies with EIA most likely indicates that HCV infection has resolved. Other interpretations include that the EIA result is falsely positive, that the HCV RNA test result is falsely negative, or rarely that a person has intermittent or low-level viremia. The latter condition may occur transiently in the initial 2 years of infection but is rare with chronic hepatitis C.

In acute HCV infection or in immunosuppressed states, a negative EIA does not exclude HCV infection. HCV RNA testing is helpful if seronegative acute HCV infection is suspected because HCV RNA can be detected within 2 to 3 days after an exposure, whereas antibodies to HCV are detectable an average of 7 to 8 weeks later.[273,274] HCV RNA testing can also be used to screen for HCV infection in persons with negative HCV EIA results who are known to have conditions associated with diminished antibody production, such as HIV infection and hemodialysis.

Liver Fibrosis Staging

A number of approaches are available for the staging of liver fibrosis in patients with chronic HCV infection. As the toxicity and efficacy of

TABLE 154.2 Persons Who Should Be Screened for Hepatitis C Virus (HCV) Infection

HIGH PREVALENCE	POSTEXPOSURE TESTING
Persons who ever injected illegal drugs Persons born from 1945–1965 Persons with elevated aminotransferase levels Persons on hemodialysis Persons who received transfusions or organ transplants, including clotting factor concentrates produced before 1987 or either a transfusion or organ transplant before July 1992 Persons in settings with demonstrated high HCV prevalence and where risk factor ascertainment may be poor (e.g., inmates, patients attending inner-city clinics for sexually transmitted diseases, and some university emergency departments)	Persons with percutaneous or heavy mucosal exposure to HCV-positive blood Children born to HCV-infected women Sexual partners of HCV-infected persons should be considered for HCV screening, although the risk of transmission during sexual intercourse is low

[a]See 1998 US Public Health Service Guidelines for full recommendations regarding HCV screening.[739]

hepatitis C treatment have dramatically improved, preferred approaches for staging have shifted away from liver biopsy to serum fibrosis markers and radiographic modalities (e.g., elastography; see later), often in combination. Despite therapeutic advances, proper fibrosis staging remains critical to proper treatment regimen selection and optimal patient management (e.g., esophageal varices and HCC screening).

Liver Biopsy

Although liver biopsy remains the most definitive method for assessing the stage of liver injury associated with HCV infection, it is infrequently used in the current HCV treatment era due to high treatment efficacy rates regardless of fibrosis stage and the lack of frequent treatment-associated side effects or toxicity. Biopsy may identify other causes of liver disease, such as alcohol use or hemochromatosis, and also provides information on distinct HCV-related processes: periportal necrosis (piecemeal necrosis), parenchymal injury, portal inflammation, and fibrosis.[413,475,613] Various systems have been proposed for grading inflammation and staging fibrosis (Table 154.3).[613–615]

There are important limitations to the liver biopsy. Although interobserver variance has been described, the main concern is with the insensitivity of the test (failure to detect significant fibrosis), which is a function of the size of the sample taken.[616–618] Tissue samples shorter than 15 mm are especially likely to miss significant disease. However, even in carefully controlled clinical trials, the average size of a liver biopsy specimen is often less than 20 mm.[619] Complications such as serious bleeding may also occur, although infrequently (<1%).[616,617] The histologic "snapshot" of the current state of inflammation and fibrosis also does not predict the future course of disease with the level of certainty expected for an invasive screening test. Contraindications to liver biopsy include uncorrectable coagulopathy and clinical evidence of decompensated cirrhosis.

Noninvasive Blood Markers of Hepatic Fibrosis

A large number of serum fibrosis markers have been considered as surrogates for the liver biopsy.[620] Studies have considered liver-related enzymes such as ALT, AST, and γ-glutamyl transferase (GGT); direct and indirect measurements of molecules made or processed by the liver, such as the prothrombin time, platelet count, and levels of serum albumin, bilirubin, γ-globulin, and apolipoprotein A_1; and markers of inflammation, fibrinolysis, fibrogenesis, or stellate cell activation (YKL-40, hyaluronic acid, procollagen III N-peptide, TGF-β, α_2-macroglobulin, α_2-globulin). However, these individual test results are not sufficiently sensitive or specific to play a major role in clinical decision making.

The validity of blood tests for liver fibrosis has been improved through use of algorithms that combine multiple individual results.[620] The most accessible of these algorithms are those that use common laboratory parameters, with or without patient age, and include the fibrosis 4 index (FIB-4) and AST-to-platelet ratio index (APRI).[621,622] The APRI uses platelet count and AST to estimate the likelihood of advanced fibrosis or cirrhosis. Use of a cutoff of ≤0.5 provides a high negative predictive value (NPV) (86%–90%) for advanced fibrosis or cirrhosis; in contrast, the positive predictive value (PPV) for cirrhosis with use of a cutoff of 2.0 is not as robust (57%–65%).[621] As an example, in 192 patients with chronic hepatitis C, significant fibrosis could be predicted accurately in 51% of patients by using the APRI, which is calculated by dividing the AST level (divided by the upper level of normal) by the platelet count (10^9/L) and multiplying by 100.[623]

Similarly, the FIB-4 uses AST, ALT, and platelet count combined with patient age to estimate the likelihood of advanced fibrosis or cirrhosis. Typical cutoffs used include <1.45, which has a high NPV for advanced fibrosis (95%), and >3.25, with a PPV of 82% for advanced fibrosis or cirrhosis.[622] The FIB-4 has also been validated in HIV coinfected population and is predictive of clinical outcomes.[624] Other groups have reported variable success with these "multitest" fibrosis algorithms designed for detection of significant fibrosis (METAVIR 2–4) or cirrhosis (METAVIR 4) among persons with chronic hepatitis C, with or without HIV coinfection.[623,625–627] It is important to recognize that both of these indices are relatively specific, provided there is no other process independently affecting the markers being used; however, they possess only moderate sensitivity for advanced fibrosis or cirrhosis, and therefore patients with clinically significant fibrosis may be missed.[628]

The European MULTIVIRC group found that a combination of markers (α_2-macroglobulin, haptoglobin, apolipoprotein A_1, GGT, ALT, and total bilirubin) had relatively high NPVs and PPVs for significant liver fibrosis.[629] However, the high NPVs and PPVs required scores that pertained only to 12% and 34% of patients, respectively. In addition, the test does not appear to predict future fibrosis progression as well as it does contemporaneous disease.[377] The related assay is commercially available in the United States (FibroSURE; LabCorp, Burlington, NC).

Diagnostic accuracies of the various marker panels vary across studies. In one systematic review, Chou and coworkers reported average areas under the receiver-operating curves of 0.82 to 0.9 for detection of cirrhosis compared with biopsy when these algorithms were used.[620] The consistency of the apparent ceiling of the accuracy of these tests may reflect error in the biopsy reference standard as much as inaccuracy in the surrogate tests themselves.[630]

Radiographic Tests

Liver stiffness measurement (LSM) with transient elastography has been introduced as a noninvasive method of staging liver disease in persons with chronic hepatitis C.[631–633] Based on the premise that liver fibrosis can be predicted through measurement of liver stiffness, this ultrasonography-like device provides a series of measures (shots) of liver stiffness ranging from 2.5 to 75 kilopascals (kPa). Typically, 10 individual measurements are obtained and averaged. Results less than

TABLE 154.3 Comparison of Systems Used to Grade Liver Fibrosis in Hepatitis C Virus–Infected Persons

	LIVER BIOPSY			NONINVASIVE		
HISTOLOGIC FINDING	MODIFIED HAI[615]	METAVIR[614]	KNODELL[613]	FIBROSCAN[a,631]	FIBROTEST[629]	APRI[623]
No fibrosis	0	0	0		<0.21	
Expansion of some portal zones	1	1	1			
Expansion of most portal zones	2	1	1	<7.1	0.27–0.31	≤0.5
Expansion of most portal zones and occasional bridging	3	2[b]	3	7.1–9.4	0.48–0.58	
Expansion of most portal zones and marked bridging	4	3[c]		9.5–12.4	0.58–0.72	>1.5
Marked bridging and occasional nodules	5	3				
Cirrhosis	6	4	4	12.5	>0.74	

[a]FibroScan is a radiographic technique. FibroTest is marketed as FibroSURE in the United States. Other thresholds have been used for some of these noninvasive tests. See text, "Noninvasive Blood Markers of Hepatic Fibrosis."
[b]More than one septum.
[c]Portal-central septae.
APRI, Aspartate aminotransferase–to–platelet ratio index; *HAI,* histologic activity index.

7.5 and more than 12.5 kPa generally correlate strongly with no or minimal fibrosis or cirrhosis, respectively.[378,634] One limitation of the test is that approximately 20% of persons do not have a valid result, which can occur if the machine does not receive 10 valid shots, the proportion of valid shots is less than 60%, or the interquartile range of the shot results divided by the median is greater than median value.[634] Obesity, female sex, operator inexperience, and age older than 52 were associated with invalid results. Falsely high estimates of liver fibrosis have also been reported with acute inflammation and recent food intake.[635] A liver stiffness test (FibroScan) was approved for use in the United States in 2013 but has been used in Europe since 2003. An interesting aspect of elastography to diagnose cirrhosis is that additional information can be provided as the LSM increases above 12.5 to the limit of 75 kPa. Data suggest that an LSM ≥21 kPa corresponds closely to a hepatic venous pressure gradient (HVPG) of ≥10 mm Hg—the accepted gold standard for the diagnosis of portal hypertension.[636,637] These data have been adapted by the American Association for the Study of Liver Diseases (AASLD) into recommendations that patients with compensated cirrhosis, platelet count >150,000/mm^3, and an LSM <20 kPa may have screening for esophageal varices deferred.[637]

Significant liver disease can also be detected by hepatic imaging with ultrasound, computed tomography, and magnetic resonance imaging. These modalities may depict a small nodular liver, ascites, an enlarged spleen, intraabdominal varices, and HCC. However, although potentially useful in the management of persons known to have cirrhosis (e.g., in screening for HCC), such imaging methods are not sensitive for fibrosis detection and should not be used for initial staging of HCV infection. Modification of MRI with the addition of LSMs, magnetic resonance elastography (MRE), yields a test that performs as well as or better than ultrasound-based elastography.[638] The major limitations of this modality are cost and limited availability, given the requirement for specialized equipment.

Approach to Staging Liver Disease

There are two major clinical applications for liver disease staging. One is to ascertain the appropriate length of treatment, and the other is to determine if there is cirrhosis because there will be an indication for routine screening for portal hypertensive complications and HCC. In the current HCV treatment era, the primary application of staging is detection of cirrhosis. The relative sensitivity of LSMs (FibroScan) for detection of cirrhosis and high tolerability make that method particularly attractive where it is available in clinical practice. Some combine liver stiffness evaluation with fibrosis blood tests to achieve greater confidence.[631] In one study, the predictive value of LSMs was better than that of liver biopsy for predicting liver-related outcomes in HIV- and HCV-coinfected persons.[639] Accordingly, liver biopsy now has a diminishing role in liver disease staging, being reserved for instances in which cirrhosis cannot confidently be excluded by other methods or when other disease processes need to be excluded by direct tissue examination.

Persons with chronic hepatitis C and bridging fibrosis or cirrhosis (METAVIR 3 or 4) should be screened for HCC.[640] Elevations in serum α-fetoprotein (AFP) may indicate the development of HCC in persons with HCV-related cirrhosis. But AFP is neither sensitive nor specific, and liver ultrasound testing every 6 months is the preferred method to screen for hepatocellular cancer in patients with bridging fibrosis or cirrhosis from chronic hepatitis C.[640] Endoscopy is also recommended for most HCV-infected persons with cirrhosis to assess for esophageal varices and provide appropriate treatment and follow-up.[637]

EPIDEMIOLOGY OF HEPATITIS C

HCV is most often transmitted through percutaneous exposure to blood. However, the predominant modes of transmission may change over time and differ between, and even within, countries. In economically developed countries, most new HCV infections are related to illicit injection drug use, although blood transfusions were once important sources of infection. HCV may also be transmitted between sexual partners and from a mother to her infant, although this is relatively uncommon compared with HBV. HCV can also be transmitted by means of percutaneous medical procedures, when there is a breach in infection-control protocols. This transmission route is especially important in settings with insufficient resources to maintain infection-control standards. Worldwide, unsafe medical practices are the dominant transmission route.

Prevalence of Hepatitis C Virus

HCV infection has been reported in virtually every country where it has been carefully evaluated, suggesting that HCV, unlike HIV, has a long-standing global distribution. Estimates for the global prevalence of viremic HCV infection have been recently updated and now stand at 71.1 million persons (approximately 1.0% of the population); although infection is prevalent, this is a marked decrease from prior estimates of 185 million HCV antibody–positive persons in 2005 due to updated and lower than previously estimated prevalence in sub-Saharan Africa.[206,641] In developed nations, the HCV prevalence is typically 1% to 2% in the general population. Estimates of hepatitis C prevalence in the United States are available from serial National Health and Nutrition Examination Surveys (NHANES). A random sample of the households from 2003 to 2010 suggested that 3.6 million persons had HCV antibodies or 1.3% of the general population.[642] The peak age of infection spanned 2 decades, with 41% of infections in those 40 to 49 and 38% in those 50 to 59 years. Epidemiologic factors associated with HCV infections included male sex, non-Hispanic black race, less than high school education, and income less than two times the poverty level.[642] Estimates accounting for high-risk population, such as the incarcerated or homeless, suggest there are at least 5.2 million anti-HCV–positive persons in the United States.[643] In comparison to prior NHANES surveys, the overall prevalence of HCV was seen to be decreasing in the United States with the aging of the cohort with the highest HCV prevalence. Given the aging of the HCV epidemic in the United States, approximately 75% of all HCV-infected persons in the United States were born between 1945 and 1965.[472] This finding, the discovery that about half of HCV-infected persons are unaware of their status, and improved treatments led the CDC to call for all persons born between 1945 and 1965 to be tested for HCV antibodies, in addition to those individuals with apparent risk factors.[603] However, with the ongoing shift in HCV epidemiology in the United States, such screening approaches are no longer adequate to capture many new infections, and updated approaches (e.g., universal HCV screening) are needed.[610]

There is considerable heterogeneity in HCV infection prevalence around the world. In Egypt, for example, HCV infection occurs in 5% to 30% of the general population.[644,645] Similarly high rates of infection have been found in certain regions of Japan, Taiwan, and Italy. As in the United States, HCV infection is generally more prevalent among persons older than 40 years and uncommon in those younger than 20.[646–649] This cohort effect suggests that transmission occurred through a practice that has been discontinued, such as traditional folk remedies or reuse of needles for injection.[646,650,651] It is likely that a national campaign to treat schistosomiasis infections with frequent reuse of needles and syringes was responsible for many infections in Egypt, and decades before in Japan.[652–654] Very high HCV prevalence rates are also reported in Pakistan and linked to unsafe injections.[655,656] One study estimated a mean of 13 injections per person per year in Pakistan, the highest in the world.[656]

A high prevalence of HCV infection has also been reported in some urban areas of developed countries. In Baltimore, Maryland, HCV infection was found in 18% of patients attending an inner-city emergency department and in 15% of persons attending a nearby clinic for sexually transmitted diseases.[657,658] A high prevalence of HCV infection (20%–40%) also has been noted among incarcerated persons in California, Maryland, and Texas.[659] Undoubtedly, in these settings, prior illicit injection drug use is chiefly responsible.

Prevalence of Hepatitis C Virus in Those With HIV

Because of shared routes of transmission, HCV infection is found in persons with HIV much more frequently than in the general population. In the United States and Europe, 15% to 30% of HIV-infected persons are coinfected with HCV.[576,660] However, the prevalence of HIV and HCV coinfection varies markedly depending on the route of HIV infection, with 50% to 95% of HIV-infected injection drug users being

coinfected, compared with less than 10% of HIV-infected men who have sex with men.[661] Globally it is estimated that there are around 2 to 5 million persons living with HIV who are coinfected with HCV.[662,663]

Incidence and Transmission of Hepatitis C Virus

Starting around 2011 in the United States, a dramatic increase in acute HCV infections in young persons (≤30 years old) in nonurban settings was noted, with an 85% increase in incidence between 2010 and 2011.[664] Although over 50% of the states surveyed for the time period from 2006 to 2012 had an over 200% increase in HCV incidence, the epicenter of the outbreak appeared to be the Appalachian region of the United States, with injection drug use as the major risk factor.[664,665] Fueled by these changes, the number of estimated acute HCV infections in the United States has increased year after year since 2010, with an estimated 34,000 infections in 2015, which was likely an underestimate.[666,666a]

Biologic Basis of Transmission

HCV transmission requires that infectious virions contact susceptible cells that are permissive for replication. HCV RNA can be detected in blood (including serum and plasma), saliva, tears, seminal fluid, ascitic fluid, and cerebrospinal fluid.[230,231,667] HCV RNA-containing blood is infectious when inoculated intravenously (e.g., by transfusion). In addition, a chimpanzee has been infected by intravenous inoculation of saliva.[255] However, there is very little information available regarding the potential infectivity of HCV RNA–containing body fluids. Furthermore, it is not clear whether cells other than hepatocytes can be infected (and thus whether infection requires direct percutaneous inoculation into the bloodstream).

Transmission appears to be enhanced by the stability of virus in environmental conditions such as in syringes, gauze, or water bottles.[668,669] In one study, a high HCV inoculum remained infectious in cell culture after storage in bottled water for 3 weeks.[669]

HIV infection may also enhance the transmissibility of HCV. The most conclusive data derive from studies comparing the rate of perinatal transmission of HCV from HIV- and HCV-coinfected mothers with those infected with HCV only.[670,671] Whether the association of HIV infection with higher HCV RNA level is the reason for more frequent perinatal (and sexual) HCV transmission is unknown.[672,673] There are fewer studies investigating whether HIV- and HCV-coinfected persons are more likely to transmit HCV by means of sexual intercourse, but such persons appear to be much more likely to transmit HIV than HCV to their partners. In one study, HIV infection was detected in 13% of 162 female sexual partners of HIV- and HCV-coinfected hemophilia patients, whereas only 3% were HCV infected.[674] The greater transmissibility of HIV by sexual intercourse and related recommendations to prevent HIV transmission by using barrier precautions for every act of sexual intercourse are more than sufficient to prevent spread of HCV to sexual partners.

Percutaneous Transmission

Infection occurs in more than 90% of seronegative recipients who are transfused with blood from HCV-antibody positive donors.[675,676] Before the introduction of nonspecific surrogate tests (serum ALT and antibody to HBV core protein), and specific EIA assays for detection of HCV infection in blood donations, approximately 17% of HCV infections in the United States were caused by transfusion.[677] Use of HCV EIA test to screen donations reduced the risk of transfusion-transmitted hepatitis C substantially, and that risk is less than 1 : 1,000,000 in areas (such as the United States) where donations are also screened for HCV RNA.[678–680]

HCV also has been transmitted by intravenous administration of contaminated blood products including immune globulin.[681,682] In recent years, however, the risk of transmission by these products has been effectively eliminated by the introduction of solvent-detergent and other virus inactivation procedures. Transplantation of organs from HCV-infected donors almost always results in HCV infection in seronegative recipients,[683] and in seropositive recipients may lead to superinfection with a second distinct viral strain.[684]

Contaminated needles and perhaps other paraphernalia associated with illicit drug use account for the majority of HCV infections in most developed countries. Since 1992, at least two-thirds of new HCV infections in the United States have been associated with illicit drug use.[685] Worldwide, 50% to 95% of persons acknowledging drug use have HCV infection.[686–689] HCV infection generally occurs within months of initiating the illicit use of injected drugs. In one cohort, 80% of subjects acknowledging 2 or more years of injection use were infected with the virus, a prevalence that was higher than that of HIV or HBV infection.[686,690] Early acquisition of HCV is probably related to the practice of older (infected) intravenous drug users teaching new (uninfected) initiates by demonstrating on themselves and then on the new initiates.[691] In the United States, there appears to have been a significant reduction in the incidence of HCV associated with illicit drug use in the late 1980s.[685] However, incidences of 5% to 30% are still reported in many urban areas. There are also HCV outbreaks in persons 15 to 24 years of age in urban and rural areas, linked to transitions to injection drug use.[692,693]

HCV may be transmitted by other percutaneous exposures that are not associated with drug use but that occur too infrequently to be detected in many studies. For example, tattoos have been associated with HCV infection in some studies, with tattooing in prison appearing to be a particularly high-risk procedure.[694–696] Human bite and folk remedies such as acupuncture and scarification rituals may also be associated with HCV infection.[697]

Nosocomial Infection

Worldwide, unsafe medical practices are the predominant route of HCV transmission. In countries where there are adequate resources to observe universal precautions, nosocomial transmission is uncommon and associated with breaches in infection-control protocols.[698,699] Nonetheless, a report from Spain suggests that nosocomial spread is one of the more preventable forms of HCV transmission.[700] The investigators considered 109 cases of acute hepatitis diagnosed between 1998 and 2005. In the 6-month period preceding the diagnosis of acute hepatitis C, hospital admission was recognized in 73 (67%) of cases, whereas intravenous drug use was reported in only 9 (8%) and sexual contact in 6 (5%). Among the 73 patients in whom hospital admission was the only risk factor, 33 underwent surgery and 24 were admitted to a medical emergency unit or a medical ward; the remaining 16 patients underwent an invasive diagnostic or therapeutic procedure. In some instances, the transmission vector was identified (e.g., multidose saline containers, heparin flush vials, or colonoscope).[701–704] In one example, 16 persons were infected from a single source patient through preparation of radiopharmaceutical injections.[705] The nosocomial transmission of HCV within hemodialysis units is a particular concern related to contamination of surfaces and failure to adhere to hand hygiene and glove use in dialysis units.[706–708] Adherence to strict universal precautions can significantly lower the risk of transmission in dialysis units.[666,680,696,709]

In economically developing regions of the world, HCV transmission occurs from the widespread use (and reuse) of injection supplies and other percutaneous practices in both traditional and unconventional medical settings. For example, one analysis estimated that the annual ratio of injections per person ranged from 1.7 to 11.3 and that reuse of equipment without sterilization was as high as 75% in some areas such as in Southeast Asia.[710] As mentioned earlier, widespread use of injections for schistosomiasis appears to explain the approximately 20% population prevalence of HCV in Egypt.[652] Likewise, frequent unsafe injections explain most infections in Pakistan.[656]

Transmission of HCV to health care workers occurs after 1% to 2% of accidental needlestick exposures to HCV-infected patients.[711–713] Studies of such accidents indicate that the risk of HCV transmission is intermediate (approximately 3% per documented exposure to susceptible host) between that of HIV (approximately 0.3%) and HBV (approximately 30%).[711,713,714] Although hollow-bore needlestick exposures account for most documented instances of HCV transmission, HCV infection has also been reported from blood splashed on the conjunctiva, and from a solid-bore needlestick.[715] Despite these risks, the prevalence of HCV infection among dental and medical health care workers is less than or similar to that of the general population.[716–718]

HCV also may be transmitted from health care providers to patients.[719,720] Performance of procedures in which percutaneous injuries can occur (e.g., intrathoracic surgery) is a common feature. In

community-based studies in the United States, patients with acute HCV infection do not commonly report recent interaction with a health provider, and work restrictions are not routinely required for HCV-infected health care workers.[685] Thus, although nosocomial exposure is a leading cause of infection in developing countries, it is rare where resources permit adherence to universal precautions.

Sexual Transmission

Although transmission of HCV during sexual intercourse is difficult to prove, there is mounting circumstantial evidence that it occurs. HCV RNA has been detected in semen and saliva,[230,721] and persons with multiple sexual partners and commercial sex workers have a high HCV prevalence.[722,723] In multiple studies of families of HCV-infected patients carried out in Japan and in Europe, sexual partners generally have been the only household contacts at increased risk for infection with the same viral variant, and the odds increase with the duration of the relationship.[722,724–726]

On the other hand, there are data that indicate that sexual transmission of HCV is relatively infrequent in monogamous partnerships. Studies of long-term sexual partners of HCV-infected hemophiliacs and transfusion recipients generally show little or no evidence for HCV transmission, even if there was unprotected sexual intercourse.[727–730] Vandelli and coworkers studied 895 monogamous sexual partners of persons with chronic hepatitis C for more than 8000 person-years and found no convincing instances of HCV transmission, despite unprotected intercourse occurring an average of 1.8 times per week.[731] Likewise, Terrault and colleagues[732] studied 500 anti-HCV–positive subjects and their long-term heterosexual partners. On the basis of 8377 person-years of follow-up, the maximum incidence rate of HCV transmission by sex was 0.07% per year.[732] HCV prevalence among MSM is also generally lower than for other infections such as HIV, HBV, and syphilis, for which sexual transmission is well established; yet HCV prevalence in MSM has also been found to be higher than in the general population.[733,734] In one study, only 4.6% of a cohort of MSM were infected with HCV, whereas 81% had been infected with HBV.[735]

Transmission of HCV among HIV-infected MSM has also been reported, including several recent outbreaks.[360,736,737] In one recent outbreak, 60 HIV-infected men who acquired HCV had more high-risk sexual practices and were more likely to have shared drugs via a nasal or anal route in the preceding year in comparison with 130 controls who did not acquire HCV infection.[360,736,737] Similar reports have come from other European countries, Australia, and the United States. Although it is difficult to exclude the possibility of unacknowledged (or unrecalled) prior injection drug use in these instances, cumulatively the reports underscore that HCV infection is an important risk for MSM, especially those who engage in high-risk practices. In addition, there are multiple well-documented outbreaks of HCV among men who report no other risk factor than having sex with other men.[722,724,738] These outbreaks generally occur among HIV-infected persons and have been associated with high-risk practices such as fisting that might involve mucosal tears, and noninjection recreational drug use.

Thus, available data suggest that HCV may be transmitted during sexual intercourse, but it occurs rarely in most populations outside of HIV-positive MSM. One conjecture is that sexual transmission occurs more often from persons with acute HCV infection than from those with chronic hepatitis C. This might explain the high prevalence in settings in which exposure to someone with acute hepatitis C is likely (e.g., partner of an injection drug user) and the relatively low transmission incidence in long-term sexual partners of those with chronic hepatitis C. Nonetheless, individuals in long-term monogamous relationships should be informed of the low risk of future transmission and, according to US Public Health Service Guidelines, encouraged to discuss this risk and the use of barrier precautions with their sexual partners.[575,739]

Maternal-Infant Transmission

HCV is uncommonly transmitted from mother to infant. Estimates of the perinatal transmission frequency range from 0% to 4% in larger studies.[670,740–742] The timing of transmission is not known. However, HCV RNA has been detected within a month of birth in non-breastfed infants delivered by cesarean section, suggesting that transmission occurs in utero in at least some instances.[741] Because of the passive transfer of maternal HCV antibodies, the diagnosis of infant HCV infection must be based on detection of viral RNA or the persistence of antibody after 18 months of age. Cases of vertically transmitted HCV are poised to dramatically increase in the United States owing to the expanding HCV epidemic in young persons due to injection drug use. For instance, from 2011 to 2014, the rate of HCV detection in women of childbearing potential in Kentucky increased 213%; during the same time frame there was a 124% increase in infants born to mothers infected with HCV (1.6% of infants born in Kentucky).[743]

HCV RNA has been detected in breast milk.[744] However, in most studies the risk of HCV transmission is similar in breastfed and bottle-fed babies.[670,745,746] Neither the CDC nor the American Academy of Pediatrics recommends that HCV-infected mothers bottle-feed to prevent HCV transmission.[739,747] Likewise, although one study showed a reduction in perinatal HCV transmission in women who had elective cesarean sections, this measure is not routinely recommended for HCV-infected mothers.[671,739] A systematic review failed to identify any intervention that was clearly demonstrated to reduce the risk of mother-infant HCV transmission.[748]

Transmission Cofactors

Risk factors for HCV transmission chiefly relate to the probability of virus reaching recipient bloodstream. Donor HCV RNA level matters, especially at the extremes. In a review of 2022 parenteral, sexual, and perinatal HCV exposures, HCV was transmitted only by individuals with detectable viremia.[749] Moreover, nonparenteral (e.g., perinatal) transmission of HCV is rare when the level of viremia is low.[750] Some but not all studies indicate that infection with HIV may be an important cofactor for both sexual and maternal-infant transmission,[670,674,751] possibly because HIV infection is also associated with higher HCV RNA levels.[672,673,752]

TREATMENT OF CHRONIC HEPATITIS C

Treatment of chronic hepatitis C has undergone extensive evolution. More details of current recommendations are presented in the AASLD and Infectious Diseases Society of America (IDSA) online guidelines (www.hcvguidelines.org).

Over the past 5 years, the FDA has approved for the treatment of chronic HCV infection nine oral, IFN-free therapeutic regimens that are capable of inhibiting HCV replication, eradicating infection, and improving the natural history of the disease. These regimens have allowed expanded access to previous "difficult-to-treat" populations including patients with HIV coinfection, chronic kidney disease, or decompensated cirrhosis; liver and kidney transplant recipients; and adolescents. An improved understanding of HCV replication and atomic level resolution structures of several critical viral enzymes have provided targets for specific antiviral agents (see Fig. 154.3).

Treatment Responses

Virologic Responses

The primary aim of treatment is to prevent complications of chronic hepatitis C by eradication of infection. Treatment can permanently eradicate HCV infection such that HCV RNA is no longer detectable in blood or liver, titers of antibodies to HCV decline (although seldom to an undetectable level), and HCV-related liver pathology remits or improves.[753,754] Accordingly, treatment responses chiefly are characterized by the results of HCV RNA testing at various time points. In the setting of DAA therapy, the most relevant clinical time point test is termed *sustained virologic response,* which is defined as the absence of HCV RNA in serum 12 weeks after the end of therapy. Sometimes SVR is modified to indicate the number of weeks after treatment when HCV RNA is not detected (e.g., SVR_{12} means no HCV RNA detectable 12 weeks after stopping treatment). SVR_{12} has been accepted by the FDA as the marker of successful treatment. A relapse is defined when HCV RNA is detected after completion of therapy in persons who achieved an on-treatment undetectable viral load. Relapse typically occurs within the first 12 weeks after treatment completion. *Breakthrough* refers to a greater than 1 log increase in HCV RNA level in a patient who is still on treatment. Undetectable virus at the end of therapy is referred to as

an *end-of-treatment response* (ETR). Assessing virologic response at early time points (week 4 or 12) was essential in the IFN era. Given the potency of current DAA regimens, responses at early time points (week 4) are no longer useful to predict treatment outcome; despite this, an HCV RNA level at week 4 may be required by payers for completion of treatment.[611]

Histologic and Clinical Responses to Therapy

There is consistent evidence that liver histologic findings (chiefly inflammation but in some cases fibrosis) and long-term clinical outcomes are improved in patients treated with antivirals, including DAA therapies, particularly in those patients achieving an SVR.[755–763] One retrospective study considered outcomes of 479 persons with chronic hepatitis C a median of 2.1 years after they underwent IFN-α–based treatment. The risk of a clinical event (liver failure, liver cancer, or death) was substantially lower in the 30% who had achieved an SVR compared with the others.[760,764] Another study of 530 persons with advanced fibrosis or cirrhosis (Ishak fibrosis stages 4–6) followed over a median of 8.4 years after IFN-based HCV treatment reported a 10-year mortality rate among persons with SVR of 8.9% compared with 26% among those without SVR.[765] The 10-year liver-related mortality or transplant rate was 1.9% with SVR compared with 27.4% without SVR. Another study of more than 17,000 US veterans who started HCV treatment between January 2001 and June 2007 found that SVR was associated with a reduced hazard ratio for death of 0.51 to 0.70, depending on the HCV genotype.[766] New studies assessing the same benefit of SVR in the setting of DAA therapy show similarly impressive benefits with HCV eradication in patients with and without severe liver disease, including patients with decompensated liver disease.[501,510,572,767] Successful treatment of HCV infection can also improve various measures of quality of life and patient-reported outcomes, and the benefit is seen in patients with minimal or no hepatic fibrosis and those with severe liver disease.[768–772]

Medications

Interferon-α

Before the availability of DAAs, IFN-α was the mainstay of antiviral therapy for HCV. The type 1 IFNs (which include multiple types of IFN-α and IFN-β) comprise a heterogeneous group of cytokines that are expressed in response to viral infection. IFNs may alter the course of virus infections both directly and indirectly. With HCV, there is strong in vivo and in vitro evidence for a direct, IFN-mediated antiviral response, which in some cases the virus may be able to partially evade (see "Viral Persistence" earlier).[773,774] In 2018, neither AASLD nor IDSA recommended IFN-containing regimens in treatment of hepatitis C.

Ribavirin

Ribavirin is a guanosine analogue with high oral bioavailability and exceptionally broad, although not particularly potent, antiviral activity. At least four different mechanisms of action have been proposed to explain its efficacy in the treatment of HCV infection. First, ribavirin is a potent inhibitor of cellular inosine monophosphate dehydrogenase and thus may influence intracellular nucleoside pools; second, it has been suggested that it may weakly inhibit the NS5B-encoded RNA-dependent RNA polymerase; third, it has been shown to promote the mutagenicity of RNA viruses; and, fourth, ribavirin may possibly modulate the Th1/Th2 balance in the host immune response.[775–778] When administered alone, ribavirin therapy appears to reduce liver enzyme levels in 21% to 43% of patients.[779–781] However, HCV RNA levels were significantly reduced in only a few percent of patients receiving ribavirin monotherapy. Ribavirin has a multiple-dose half-life of 12 days and can be administered once or twice daily. Interesting to note, the clinical importance of ribavirin appears to be in preventing relapse and improving DAA efficacy in certain difficult-to-treat populations such as those with cirrhosis (including decompensated cirrhosis) and resistance-associated substitutions (RASs).[593,782–784]

Protease Inhibitors

The early direct-acting inhibitors of HCV replication targeted the NS3/4 serine protease.[785] In an initial phase I study, 31 patients with genotype 1 infection were treated with BILN 2061 given orally twice daily for 2 days, and reductions in HCV RNA level of 2 to 3 $\log_{10}$ copies/mL were noted.[785] Although later-phase testing of that compound was aborted owing to toxicity, additional compounds (boceprevir and telaprevir) were licensed in 2011 for use for genotype 1 HCV infection in combination with IFN and ribavirin.[786] These agents were limited by their frequent dosing, limited genotype scope, and relatively poor tolerability and efficacy and thus were rapidly replaced by IFN-free DAA combinations, which became available in 2013. Both are no longer manufactured in the United States. Additional first-generation protease inhibitors active against a broader range of viruses and administered once daily were approved as part of DAA combination therapies. For example, simeprevir was approved for treatment in genotype 1 infection in combination with an NS5B polymerase inhibitor (sofosbuvir; see later); grazoprevir was approved for the treatment of genotype 1 and 4 infection in combination with elbasvir (an NS5A inhibitor); and paritaprevir was approved for the treatment of genotype 1 infection in combination with ombitasvir (NS5A inhibitor) and dasabuvir (NS5B nonnucleoside inhibitor) with or without ribavirin, depending on the genotype, and for the treatment of genotype 4 infection in combination with ombitasvir and ribavirin.[251,787,788] The limitation of these first-wave protease inhibitors was primarily the lack of pangenotypic activity, low genetic barrier to resistance, and safety in patients with severe liver disease, particularly decompensated disease.

Second-generation NS3/4 protease inhibitors glecaprevir and voxilaprevir are both pangenotypic, have higher genetic barriers to resistance (particularly glecaprevir), and are safer in patients with cirrhosis, although both remain contraindicated in patients with decompensated cirrhosis. Glecaprevir is approved in combination with pibrentasvir (NS5A inhibitor) and is active versus RASs that may develop from exposure to first-generation protease inhibitors including substitutions at the 80, 155, and 168 positions.[789] Voxilaprevir is approved in combination with sofosbuvir (NS5B polymerase inhibitor) and velpatasvir (NS5A inhibitor) and similarly remains active versus the 155 and 168 substitutions.[790,791] Although the Q80K RAS does not confer an increase in 50% effective concentration (EC_{50}) to voxilaprevir, it was associated with lower response rates to 8 weeks of sofosbuvir-velpatasvir-voxilaprevir in genotype 1a infection.[790,792]

Nucleos(t)ide and Nonnucleoside Polymerase Inhibitors

The NS5B polymerase activity can be inhibited by nucleoside and nucleotide substrates that incorporate into the growing RNA chain and terminate elongation. For example, sofosbuvir is a chain terminator prodrug with broad pangenotypic activity and received initial FDA approval for once-daily administration in December 2013. It is now most commonly used as a component of several fixed-dose combination therapies.[793] Additional nonnucleoside compounds bind to allosteric sites in the palm or thumb regions of the enzyme and induce conformational changes that diminish enzymatic activity. Nonnucleoside inhibitors have a narrower genotypic activity range than the nucleoside or nucleotide inhibitors and a lower barrier to resistance (see "Antiviral Resistance"). The only approved nonnucleoside polymerase inhibitor is dasabuvir, a palm region substrate, which as described earlier is approved in combination with paritaprevir (NS3/4 protease inhibitor) and ombitasvir (NS5A inhibitor) for the treatment of genotype 1 infection.

NS5A Protein Inhibitors

As described earlier, NS5A is a nonenzymatic phosphoprotein required for RNA replication and assembly of infectious particles. The protein has no human analogue, and disruption of its function results in potent suppression of replication. As a class, NS5A inhibitors are pangenotypic but have a low genetic barrier to resistance. First-generation NS5A inhibitors were approved as part of DAA combination therapies in 2014, 2015, and 2016. Ombitasvir and ledipasvir were both approved in 2014 as fixed-dose combination regimens. Ombitasvir is approved as part of a triple–mechanism-of-action regimen with an NS3/4 protease inhibitor (paritaprevir) and a nonnucleoside NS5B palm region polymerase inhibitor for the treatment of genotype 1 infection and as part of a dual–mechanism-of-action regimen with paritaprevir for the treatment of genotype 4 infection.[794,795] In the case of genotype 1a and

genotype 4 infection, ribavirin is also required as part of the treatment regimen. Ledipasvir is approved as part of a fixed-dose combination regimen with sofosbuvir (NS5B nucleoside polymerase inhibitor) in genotypes 1, 4, 5, or 6 infection.[796] Daclatasvir was approved in 2015 as part of a combination therapy with sofosbuvir in the treatment of genotype 1 or 3 infection.[797] Although daclatasvir has activity versus genotypes 1 to 6, there was insufficient data for the FDA to provide a broader pangenotypic indication. Elbasvir was approved in 2016 in combination with grazoprevir (NS3/4 protease inhibitor) for the treatment of genotype 1 or 4 infection.[798]

In genotype 1a infection, substitutions at amino-acid positions 28, 30, 31, and 93 confer high-grade resistance to elbasvir; thus baseline resistance testing is recommended by the FDA before use of this combination regimen for the treatment of genotype 1a infection.

Velpatasvir is not a true next-generation NS5A inhibitor because the Y93H substitution still confers high-fold resistance, but it does retain activity versus other first-generation RASs.[799] Velpatasvir, in a fixed-dose combination with sofosbuvir, was the first FDA-approved pangenotypic regimen.[800] Velpatasvir is also approved as part of a pangenotypic, triple-mechanistic, fixed-dose combination therapy with sofosbuvir and voxilaprevir (NS3/4 protease inhibitor) for the salvage of patients in whom prior DAA therapies have failed.[791] Pibrentasvir, a second-generation NS5A inhibitor, is approved as a pangenotypic, fixed-dose combination formulation with glecaprevir (NS3/4 protease inhibitor). In vitro pibrentasvir has the highest barrier to resistance of the NS5A inhibitors and retains activity against all common single-position NS5A RASs in all HCV genotypes (<10-fold shift in EC_{50}).[801]

Treatment of Chronic Hepatitis C

History and Progress (Fig. 154.9)

The first large-scale clinical trials of IFN-α compared 3 million units of IFN-α2b given subcutaneously three times a week for 6 months versus placebo and achieved SVR rates less than 15%.[802,803] Extension of the length of therapy to 12 to 18 months improved the SVR rates to 20% to 30%.[804] Higher SVR rates have been achieved with the pegylated (PEG) IFN-α products compared with standard IFN-α, and even higher rates when PEG IFN-α was combined with oral ribavirin given twice daily.[805–808] In 2011, boceprevir and telaprevir were approved for use together with PEG IFN-α and ribavirin for genotype 1 chronic HCV infection and provided a significant increase in SVR rates to 80% to 90% when response-guided treatment based on on-treatment viral kinetics was incorporated into the medical decision making for futility versus continuation of therapy (see "Virologic Responses" earlier).[786] However, these regimens continued to carry significant adverse event profiles and prolonged courses of therapy. Owing to the adverse event profiles and the need for IFN and ribavirin, these regimens remained inaccessible for many special patient populations including patients with psychiatric disease, decompensated liver disease, chronic kidney disease and end-stage renal disease, children and adolescents, and patients with organ transplants.

The first all-oral DAA regimen, sofosbuvir in combination with ribavirin, was approved for use in genotype 2 and 3 infections.[797,798,809] Although this regimen included only a single DAA, it increased SVR rates to 97% with 12 weeks of therapy in genotype 2 infection and 84% with 24 weeks of therapy in genotype 3 infection.[810,811] Sofosbuvir was also approved in patients with HIV infection and in patients with HCC awaiting liver transplant. Owing to the lack of IFN, this regimen was also explored for the first time in patients with decompensated cirrhosis and in those with liver transplants.[812] In addition, phase II studies of sofosbuvir with a next-in-class, first-generation NS3/4 protease inhibitor, simeprevir, or with a first-in-class NS5A inhibitor, daclatasvir, provided early insights into the true potential of potent DAA combinations.[813,814] The regimens would later be approved by the FDA for the treatment of genotype 1 infection and genotype 3 infection, respectively.[797,815] From 2014 through 2017, a total of six fixed-dose combination DAA regimens were approved by the FDA. These approvals included the first approved therapies for patients with decompensated liver disease, with liver and kidney transplants, or with chronic kidney disease and end-stage renal disease and for adolescents, and would shepherd in pan-genotypic regimens and next-generation regimens that could be used to salvage patients in whom other DAA regimens had failed.[791,796,798,800,816] In 2018, we reached the pinnacle of HCV therapeutics; with approved regimens offering >95% SVR for the vast majority of patients and options for salvage, there is unlikely to be a significant change in the approach to or recommended regimens for chronic HCV infection.

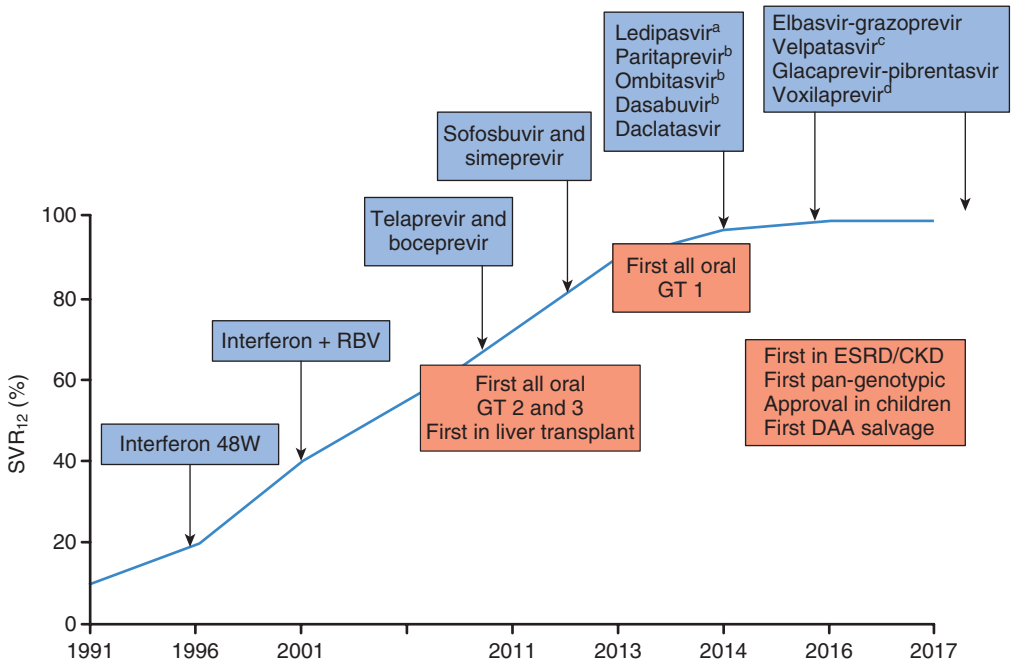

FIG. 154.9 Timeline of hepatitis C virus *(HCV)* therapeutic approaches indicating approval of new agents and approximate sustained virologic response (SVR_{12}). [a] Ledipasvir administered as fixed-dose combination with sofosbuvir. [b] Administered as a fixed-dose combination of paritaprevir/ritonavir/ombitasvir dosed with dasabuvir. [c] Velpatasvir administered as fixed-dose combination with sofosbuvir. [d] Voxilaprevir administered as fixed-dose combination with velpatasvir and sofosbuvir. From 2014 to 2017, multiple new HCV antiviral agents and fixed-dose combination treatment regimens were approved, revolutionizing the treatment of hepatitis C virus infection. *(Figure provided by Dr. Naggie.)*

Initial Treatment of Chronic Hepatitis C Virus Infection

Initial treatment of chronic HCV infection includes patients who have not been previously treated with any HCV antiviral regimen, including prior IFN-containing regimens. All recommended treatments for HCV infection include fixed-dose combinations of DAAs, and neither the AASLD/IDSA nor the European Association for the Study of the Liver (EASL) HCV treatment guidelines recommend IFN-containing regimens.[817–819] There are few recommended regimens that include ribavirin, and these are for use primarily in select, very difficult-to-treat populations (see "Response Indicators").

Choosing the most appropriate initial treatment DAA regimen includes considering the genotype and subtype (although there are examples where this may not be necessary), baseline HCV RNA, presence of cirrhosis, and, if the patient has cirrhosis, the Child-Turcotte-Pugh score, presence of renal dysfunction, and concomitant medications in order to manage drug interactions. All of these baseline characteristics will aid in selecting the safest DAA regimen and the appropriate length of therapy. In addition, owing to rapid realization that IFN-free therapies were significantly more efficacious and safe, the FDA allowed registration trials to include single-arm intervention studies with efficacy analyses in which a historical control was used. This resulted in a limited number of head-to-head comparisons of the currently available regimens.

Genotype 1

There are currently four recommended DAA regimens for the initial treatment of genotype 1 infection. There are minimal differences in approach to initial therapy for subtype 1a versus 1b, with the primary difference relevant only to the use of the elbasvir-grazoprevir regimen, a single-tablet fixed-dose combination regimen of NS5A and NS3/4 inhibitors. The phase III C-EDGE trial enrolled treatment-naïve patients, 91% with genotype 1 infection and 22% with cirrhosis, and randomized them to immediate or deferred 12-week treatment arms.[820] The SVR^{12} for patients with genotype 1a infection was 92% and with genotype 1b infection was 99%. Virologic failure was associated with baseline NS5A substitutions and emergent NS3 and/or NS5A variants (see also "Antiviral Resistance"). Based on these data, the FDA recommended baseline NS5A genotype testing before use of elbasvir-grazoprevir in patients with genotype 1a infection, and when NS5A substitutions associated with high-fold resistance are identified, recommendations include extension of therapy to 16 weeks plus the addition of ribavirin. The AASLD/IDSA HCV guidance similarly recommends baseline RAS testing for patients with genotype 1a infection, but owing to the longer course of therapy and the need for ribavirin, the 16-week regimen is listed as an alternative treatment option.

Otherwise, the treatment approach for the other three recommended regimens is the same across subtypes. Glecaprevir-pibrentasvir, a three-tablet fixed-dose combination of NS3/4 and NS5A inhibitors, is approved as an 8-week regimen in patients without cirrhosis and a 12-week regimen in patients with cirrhosis. The phase III ENDURANCE-1 trial randomized patients without cirrhosis and with genotype 1 infection to 8 or 12 weeks of therapy; the SVR_{12} in both arms was 99%.[592] The phase III EXPEDITION-1 single-arm, open-label trial investigated 12 weeks of glecaprevir-pibrentasvir in patients with chronic HCV infection (60% genotype 1) and cirrhosis; the SVR_{12} was 99%.[821] Ledipasvir-sofosbuvir, a single-tablet fixed-dose combination of NS5A and NS5B inhibitors, is approved as a 12-week regimen for initial treatment in patients with or without cirrhosis.[795,796] The phase III ION-1 trial randomized treatment-naïve patients with genotype 1 infection (16% with cirrhosis) to four different treatment arms: ledipasvir-sofosbuvir for 12 weeks, ledipasvir-sofosbuvir plus ribavirin for 12 weeks, ledipasvir-sofosbuvir for 24 weeks, and ledipasvir-sofosbuvir plus ribavirin for 24 weeks.[822] The SVR_{12} was 99%, 97%, 98%, and 99%, respectively, confirming the efficacy of the 12-week regimen. The phase III ION-3 study randomized treatment-naïve patients with genotype 1 infection and without cirrhosis to 8 versus 12 weeks of therapy.[823] Although the 8-week duration of therapy was noninferior to the 12-week duration, the 8-week arm with ledipasvir-sofosbuvir had a higher relapse rate (5%) versus the 12-week arm with ledipasvir-sofosbuvir (1%). Based on a post hoc analysis, a baseline HCV RNA of 6 million IU/mL was identified as a threshold under which the relapse rate in the 8-week arm was the same as in the 12-week arm (2%), whereas above this threshold it was much higher (10% versus 1%). Thus, shortening to 8 weeks of ledipasvir-sofosbuvir is an option for treatment-naïve patients without cirrhosis and with a baseline HCV RNA <6 million IU/mL.

Sofosbuvir-velpatasvir, a single-tablet fixed-dose combination of NS5B and NS5A inhibitors, is approved as a 12-week regimen for the treatment of chronic genotype 1 to 6 HCV infection with or without cirrhosis.[800] The phase III ASTRAL-1 double-blind randomized trial enrolled patients (19% cirrhosis) with genotype 1, 2, 4, or 6 infection to 12 weeks of sofosbuvir-velpatasvir versus placebo and enrolled patients with genotype 5 infection to 12 weeks of sofosbuvir-velpatasvir.[591] The overall SVR_{12} was 99%, with two relapses in patients with genotype 1.

Although there are other FDA-approved regimens for genotype 1, including sofosbuvir plus simeprevir and the fixed-dose combination ombitasvir-paritaprevir-ribavirin with dasabuvir (a nonnucleoside NS5B polymerase inhibitor), these are no longer recommended regimens owing to pill burden, side-effect profile, drug interaction risk, or some combination of these.

Genotype 2

There are two recommended regimens for the treatment of genotype 2 infection: glecaprevir-pibrentasvir and sofosbuvir-velpatasvir.[819] Both are pangenotypic regimens; the efficacy of either is ≥99%. The phase III ENDURANCE-2 double-blind, placebo-controlled trial randomized patients without cirrhosis and with genotype 2 infection to 12 weeks of therapy versus placebo and found a 99% SVR_{12}.[824] The phase II SURVEYOR-II, part 4 study was a single-arm study that investigated a shortened 8-week course of therapy among patients without cirrhosis and with genotype 2, 4, 5, or 6 infection. Among the treatment-naïve patients with genotype 2 infection, the SVR_{12} was 99%. For patients with genotype 2 infection, glecaprevir-pibrentasvir is recommended for 8 weeks in the absence of cirrhosis and 12 weeks when cirrhosis is present.[824] The phase III ASTRAL-2 study randomized patients with genotype 2 infection with or without cirrhosis to 12 weeks of sofosbuvir-velpatasvir or 12 weeks of sofosbuvir plus ribavirin, which was one of the previously recommended regimens.[825] The study showed superior efficacy of sofosbuvir-velpatasvir versus the comparator (SVR_{12}, 99% vs. 94%, respectively). The phase III ASTRAL-1 study also enrolled patients with genotype 2 infection with and without cirrhosis to receive 12 weeks of sofosbuvir-velpatasvir and found a 100% SVR_{12}.[591] For patients with genotype 2 infection, sofosbuvir-velpatasvir is recommended for 12 weeks regardless of the presence of cirrhosis.

Genotype 3

Two regimens are recommended for the treatment of genotype 3 infection: glecaprevir-pibrentasvir and sofosbuvir-velpatasvir. For treatment-naïve patients with genotype 3 infection and cirrhosis, there is a recommendation to check for NS5A RAS at baseline before starting therapy, when considering sofosbuvir-velpatasvir (discussed in more detail later). Phase III ENDURANCE-3 was a randomized trial comparing 12 weeks of glecaprevir-pibrentasvir versus 12 weeks of sofosbuvir plus daclatasvir (NS5A inhibitor), the prior recommended regimen, in treatment of patients with genotype 3 infection and without cirrhosis.[592] An 8-week arm of glecaprevir-pibrentasvir was later added as an amendment to the study. Both glecaprevir-pibrentasvir arms met noninferiority as compared with the comparator standard-of-care arm, with SVR_{12} of 95% for both versus 97%, respectively. For patients with genotype 3 infection, glecaprevir-pibrentasvir is recommended for 8 weeks in the absence of cirrhosis and for 12 weeks when cirrhosis is present. The phase III ASTRAL-3 study randomized patients with genotype 3 infection with or without cirrhosis to 12 weeks of sofosbuvir-velpatasvir or 24 weeks of sofosbuvir plus ribavirin, which was one of the previously recommended regimens.[825] The study showed superior efficacy of sofosbuvir-velpatasvir versus the comparator (SVR_{12} 95% vs. 80%). Of the 250 patients in the ASTRAL-3 study with cirrhosis, 16% had baseline NS5A RASs. Patients with baseline RASs had a lower SVR_{12} (88%) than did those without RASs (97%). This lower SVR_{12} was primarily driven by patients with the Y93H RAS at baseline. Currently, the guidelines recommend use of the

glecaprevir-pibrentasvir regimen or an alternative regimen, sofosbuvir-velpatasvir-voxilaprevir, if the Y93H RAS is present and the patient has cirrhosis. Sofosbuvir-velpatasvir-voxilaprevir is a triple-mechanism targeted, single-tablet, fixed-dose combination regimen that is approved by the FDA for the treatment of prior DAA failures.[791] Although it is approved by the FDA only for treatment of DAA failures, the phase III POLARIS-3 study randomized treatment-naïve patients with genotype 3 infection and cirrhosis to 8 weeks of sofosbuvir-velpatasvir-voxilaprevir versus sofosbuvir-velpatasvir for 12 weeks.[792] The SVR_{12} was 96% in both arms. Accordingly, this regimen is recommended as an alternative regimen to 12 weeks of sofosbuvir-velpatasvir when the Y93H RAS is present in patients with cirrhosis, because the Y93H RAS does not appear to affect the treatment response with the triple-drug regimen.

Genotype 4

The same four regimens recommended for genotype 1 infection are also recommended for genotype 4 infection, with only minor differences. One difference is that NS5A RASs do not affect the treatment response for elbasvir-grazoprevir in genotype 4 infection, and RAS testing at baseline is not recommended; therefore, 12 weeks of elbasvir-grazoprevir is recommended for genotype 4 infection regardless of presence of cirrhosis. Also, with genotype 1 infection there is the option to shorten ledipasvir-sofosbuvir to 8 weeks in select patients, but this is not an option for genotype 4 infection; therefore, 12 weeks of ledipasvir-sofosbuvir is recommended regardless of presence of cirrhosis. For the glecaprevir-pibrentasvir regimen, 8 weeks of therapy is recommended for patients without cirrhosis and 12 weeks is recommended for those with cirrhosis. Last, for sofosbuvir-velpatasvir, 12 weeks of therapy is recommended regardless of presence of cirrhosis.

The studies supporting the efficacy of these regimens in genotype 4 infection are primarily the same as those supporting the efficacy in genotype 1 because most studies included both genotypes. For sofosbuvir-velpatasvir, the ASTRAL-1 study included 64 patients with genotype 4 infection, with and without cirrhosis, and the SVR_{12} was 100%.[591] Another 57 patients with genotype 4 infection were enrolled as part of the sofosbuvir-velpatasvir comparator arm in the POLARIS-2 study, and SVR_{12} was 98%.[792] Both studies support the use of sofosbuvir-velpatasvir for 12 weeks in genotype 4 infection regardless of presence of cirrhosis. ENDURANCE-4 enrolled 76 patients with genotype 4 infection without cirrhosis to 12 weeks of glecaprevir-pibrentasvir and achieved 99% SVR_{12}.[824] In addition, SURVEYOR-2 part 4 investigated 8 weeks of glecaprevir-pibrentasvir in patients without cirrhosis, and 46 patients with genotype 4 infection were included; the SVR_{12} was 93%.[824] EXPEDITION-1 included 16 patients with genotype 4 infection and cirrhosis; all received 12 weeks of therapy, and all achieved SVR_{12}. These three studies support the recommendation of 8 weeks of glecaprevir-pibrentasvir in patients without cirrhosis and 12 weeks in patients with cirrhosis. Meanwhile, a pooled analysis of the phase II/III registration program found that a total of 101 patients with genotype 4 infection were treated with 12 weeks of elbasvir-grazoprevir and that the SVR_{12} was 96%.[826] Two small pilot studies support the use of ledipasvir-sofosbuvir for 12 weeks in patients with genotype 4 infection; the SYNERGY trial was an open-label study of 21 patients, and a second single-arm study enrolled 22 patients with genotype 4 infection.[827,828]

Genotypes 5 and 6

Three DAA regimens are recommended for treatment of patients with genotype 5 or 6 infection: glecaprevir-pibrentasvir, sofosbuvir-velpatasvir, and sofosbuvir-ledipasvir.[819] Because of the lower prevalence of these genotypes in North America and Western Europe, where many registration trials take place, the numbers of patients enrolled are limited. The studies supporting these regimens overlap with those supporting genotype 1 and 4 infection, as often the studies have enrolled across multiple genotypes. The approach to treatment is also similar to that for genotype 4, with glecaprevir-pibrentasvir recommended for 8 weeks in patients without cirrhosis and 12 weeks in patients with cirrhosis, and both sofosbuvir-velpatasvir and ledipasvir-sofosbuvir recommended for 12 weeks regardless of presence of cirrhosis. Across the registration program for glecaprevir-pibrentasvir, 30 patients with genotype 5 infection were treated, including 2 without cirrhosis who received therapy for 8 weeks and 2 with cirrhosis for 12 weeks; all patients achieved SVR_{12}.[824] Similarly, 36 patients with genotype 6 were treated across the same studies, including 10 without cirrhosis who received therapy for 8 weeks and 7 with cirrhosis for 12 weeks; SVR_{12} was 97% and there were no virologic failures.[824] Across clinical trials that included sofosbuvir-velpatasvir (ASTRAL-1 and POLARIS-2), 24 patients with genotype 5 infection received 12 weeks of the regimen and 96% achieved SVR_{12}. Similarly, 47 patients with genotype 6 infection received 12 weeks of the regimen and all patients achieved SVR_{12}.[591,792] For ledipasvir-sofosbuvir, several small pilot studies have supported the efficacy of 12 weeks of this regimen in patients with genotype 5 or 6.[829,830]

Response Indicators

Historically, the likelihood of SVR could be predicted according to viral and host factors before treatment. Aggregate response rates for almost all populations and genotypes are sufficiently high with current DAA regimens that response indicators are of significantly less value. Even in the presence of multiple negative predictors, response rates are sufficiently high that in no population would therapy be withheld; however, in the increasingly rare situation in which several of the most predictive factors are present, there may still be room for treatment optimization. In the DAA era, baseline predictors that can play a role in treatment response when present in combination with others include genotype, presence of cirrhosis (particularly decompensated), prior treatment experience (primarily although not entirely DAA experience), and presence of RASs (primarily but not entirely NS5A).

One of the most important predictors of the response to IFN-α–based HCV treatment was the viral genotype (Fig. 154.10). The lowest SVR rates were consistently reported in patients who had genotype 1 or 4 HCV infections; genotypes 3 and 5 were intermediate, and genotypes 2 and 6 were most responsive.[831–835] DAAs have now mostly leveled the playing field, although genotype 3 infection, particularly in patients in whom HCV therapy previously failed and/or who have cirrhosis, has emerged as a standout. This may at least in part be due to the higher rates of steatosis in genotype 3 infection, which was known to affect

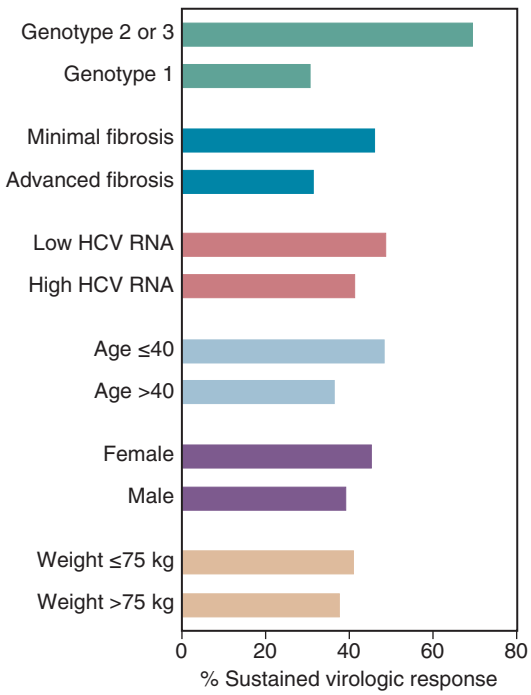

FIG. 154.10 Sustained virologic response rates in persons taking peginterferon and ribavirin according to duration of treatment and ribavirin dose, stratified by hepatitis C virus *(HCV)* genotype and HCV RNA level. *(From Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose.* Ann Intern Med. *2004;140:346–355.)*

treatment response in the IFN era, and the higher prevalence of naturally occurring NS5A RAS.[592,825]

The amount of HCV detected in the blood before treatment, the baseline HCV viral load, was also predictive of responsiveness to PEG IFN and ribavirin.[831,832] As previously discussed (see "Initial Treatment," "Genotype 1"), baseline HCV RNA may still be predictive of treatment response with some DAA regimens, although this primarily occurs with attempts to shorten therapy.

In addition to viral factors, there are several important host determinants. Interesting to note, as with spontaneous resolution of infection (see "Viral Persistence" earlier), the strongest host determinant of response to PEG IFN and ribavirin is the patient's DNA sequence near *IL28B,* the gene for λ-IFN 3. With use of a commercially available test (the C vs. T allele for rs12979860), response rates for persons with genotype 1 chronic HCV infection who were treated with PEG IFN and ribavirin were 69%, 33%, and 27% in Caucasians who had the CC, CT, and TT genotypes, respectively; among black patients, SVR rates were 48%, 15%, and 13% for CC, CT, and TT genotypes, respectively.[836] However, the predictive value of *IL28B* genotype diminished significantly with combination DAA therapy and is no longer used clinically in the setting of chronic HCV infection. Yet, there are examples that highlight the potential role, albeit small, of the host immune response in DAA-mediated clearance. Both examples include studies of DAA therapies that attempted to shorten the course of therapy from 12 weeks, a duration in which high efficacy was noted, to 8 weeks. The ION-3 study, as discussed previously (see "Initial Treatment," "Genotype 1"), randomized treatment-naïve patients with genotype 1 infection and without cirrhosis to 8 versus 12 weeks of therapy.[823] Although the 8-week duration of therapy was noninferior to the 12-week duration, the 8-week arm with ledipasvir-sofosbuvir had a higher relapse rate (5%). A post hoc multivariable analysis assessed predictors of SVR and identified female sex and *IL28B* favorable CC genotype as associated with treatment response. In addition, from pooled data of the ledipasvir-sofosbuvir program, baseline NS5A resistance to ledipasvir is also predictive of relapse in patients receiving 8 weeks of therapy.[837,838]

Cirrhosis remains a host predictor that increases the risk for treatment failure; hence there are differences in the treatment approach with some regimens in patients with cirrhosis. Although DAAs have significantly improved the SVR rates in patients with compensated cirrhosis, patients with decompensated cirrhosis have a lower SVR.[782,783] Because of the lower SVR in patients with decompensated cirrhosis, studies investigated the benefit of adding ribavirin to the DAA regimen and consistently showed higher SVR; therefore regimens for decompensated cirrhosis include ribavirin. Cirrhosis appears to be especially important with genotype 3 infection.[825,832,839,840] The reason for the higher relapse rate in patients with cirrhosis, especially decompensated cirrhosis, is unclear but may be related to penetration of fibrotic nodules, which may increase the risk of RAS development. In addition, historically, HIV infection was associated with a lower treatment response rate, but this is no longer true. With combination DAA therapies, multiple phase III trials have found a similar efficacy and safety of DAA regimens in patients with HIV infection.[841–844] Thus, guidelines recommend treating patients with HIV infection the same as those without HIV infection, with attention to the potential for drug interactions, which now is the only clinically relevant difference when treating patients with HIV infection.

Antiviral Resistance

Antiviral resistance is a new clinical consideration directly related to the use of DAA agents. From a virologic perspective, the potential for development of resistance in HCV is greater than in HIV, and mathematical modeling studies accounting for daily virion production and the error-prone nature of the HCV RNA-dependent RNA polymerase (RdRP) suggested that all possible single- and double-position resistant variants are generated daily in chronically infected individuals.[845] Clinical data with the first-generation NS3 protease inhibitors seemed to validate these concerns and demonstrated that high-level resistance was selected within 1 to 2 weeks with monotherapy, and most patients (60%–80%) in whom IFN-based therapy with boceprevir or telaprevir failed were found to have genotypic portease inhibitor (PI) resistance.[283,846–850] However, several phenomena have converged to transform the major clinical impact of antiviral resistance to only a modest effect in select groups of patients (see treatment section). Those factors are (1) the rapid pace of drug development, with approval of multiple highly potent DAAs with increasingly high barriers to resistance; (2) the widespread use of combination therapy, including the availability of regimens in which three drug classes are used, and (3) in stark contrast to HIV and HBV, the lack of a long-lived cellular reservoir or integrated form.

The key populations or clinical scenarios in which antiviral resistance appears to have a detrimental impact on responses to DAA therapy include genotype 1a infection (treatment-naïve and treatment-experienced patients) with elbasvir-grazoprevir and genotype 1a infection in treatment-experienced patients with ledipasvir-sofosbuvir; genotype 3 infection in treatment-experienced patients and/or in the presence of cirrhosis with sofosbuvir-velpatasvir and possibly with shortened glecaprevir-pibrentasvir therapy (8 weeks); and previously DAA-treated patients, particularly those exposed to NS5A inhibitors with genotype 1a or 3. Important to note, in many of these scenarios the occurrence is sufficiently rare or the absolute decrease in SVR is small enough (combined with lack of statistical significance) that routine testing or changes in clinical management may not be recommended (see treatment section).

Testing for genotypic resistance is widely available in the United States, with several commercial assays available for the NS3, NS5A, and NS5B regions. Testing approaches vary in terms of how the sequencing is carried out and include Sanger sequencing (also known as population sequencing) and ultra-deep sequencing with a detection threshold set at 10% for reporting variants. Correlative clinical data suggest that detection levels in the range of those expected for population sequencing (15%–25%) enable identification of the vast majority of clinically significant RASs and avoid "overdetection" of RASs present at low levels (1%–5% of the virus quasispecies) that do not appear to affect clinical outcomes.

The NS3 protease inhibitors were the first class of DAAs used in the clinic; however, currently available agents are quite different with regard to structure and resistance profile. Telaprevir and boceprevir are linear ketoamide inhibitors of the HCV protease and readily selected for resistance if therapy was unsuccessful. The major clinical resistance mutation seen was the R155K variant in genotype 1a.[849,851] This variant was almost exclusively seen in genotype 1a owing to a lower barrier to resistance resulting from different codon usage in 1a versus 1b, which required only a single nucleotide transition in 1a (AGG to AAG) versus a two-nucleotide change in 1b (CGG to AAG) including a transversion change.[851,852] Although of negligible clinical impact currently, efforts to develop protease inhibitors active in vitro against R155K spurred the development of modern, highly potent protease inhibitors (grazoprevir, glecaprevir, and voxilaprevir) that retain wild-type activity against this variant in vitro.[789,790,853] The resistance barrier of early protease inhibitors was significantly higher in genotype 1b, although variants at positions V36, T54, V55, A156, and V170 were selected.[849,851]

The enhanced activity of modern HCV protease inhibitors has significantly lessened the impact of resistance for this class of agents. First, there are no commonly occurring baseline polymorphisms (variants found in the absence of drug selective pressure) that have been demonstrated to have a clinically significant impact on responses with current DAA protease inhibitor–containing regimens. The most prevalent NS3 polymorphism is the Q80K variant in genotype 1a, which is found in around 40% of clade I genotype 1a isolates.[854,855] In vitro, it results in low-level resistance to simeprevir (7–10×) and paritaprevir (3×); clinically, it adversely affects responses to simeprevir plus IFN and simeprevir plus sofosbuvir when used for short durations (8 weeks) or in those with cirrhosis treated for 12 weeks.[856–860] The Q80K variant does not affect the antiviral activity of grazoprevir, glecaprevir, or voxilaprevir in vitro.[789,790,853]

On virologic failure with grazoprevir- or glecaprevir-containing regimens, the most commonly selected NS3 RAVs include Y56H/N, Q80R, A156G/T/V, and D168A/E (and other D168 variants) in genotype 1 and Y56H and Q168R in genotype 3.[861–863] Resistance selection during a 3-day monotherapy trial with voxilaprevir suggested a similar resistance profile.[790] In vitro high-level resistance to all current NS3 PIs is expected for A156G/T/V variants, although testing is difficult given the very poor replicative fitness of these variants.[789] D168 variants are more variable in their in vitro impact, with most conferring ≤20× shifts in EC_{50} to glecaprevir and voxilaprevir.[789,790] Despite selection with failure of therapy and demonstrable impact in vitro on antiviral activity, there

is no clear role for NS3 resistance testing after DAA failure with or without a PI. In the setting in which a two-drug regimen is attempted for re-treatment after DAA failure, resistance in NS3 along with NS5A does predict a poor response to re-treatment with glecaprevir plus pibrentasvir and is not recommended.[863,864] However, this determination is typically made on clinical grounds rather than viral resistance genotype sequencing. In the case of three-drug re-treatment regimens, NS3 PI resistance does not appear to impair responses.[865]

NS5A inhibitor resistance, both preexisting and selected, has the greatest impact on DAA treatment outcomes and is the area where clinical resistance testing is advocated for select populations. Naturally occurring NS5A RASs of potential clinical significance are found with relative frequency in both genotype 1 and genotype 3. In a survey of over 2000 patients infected with genotype 1, baseline NS5A class RASs (conferring >2.5× shift in EC_{50} to any NS5A inhibitor) were found in 15%.[837] A second study refined these estimates, looking at RAS prevalence based on GT 1 subtype and detection threshold.[838] Although NS5A RASs are more prevalent in genotype 1b (18%) as compared with genotype 1a (13%), they do not have clinical significance for genotype 1b in the non–DAA-exposed patient. Specific RASs of interested in genotype 1a include the Y93H and Q30H variants, which were each found in 1% to 2% of sequences.[837] The Y93H variant in genotype 1a confers high-level resistance to all NS5A inhibitors except pibrentasvir.[801,866] The Q30H variant confers moderate to high-level resistance to all NS5A inhibitors except velpatasvir and pribrentasvir.[801,866] Interesting to note, the Y93H variant is found in 9% of genotype 1b sequences yet does not appear to have a large clinical impact on treatment outcomes.[837,838] Excluding the M28V variant, which is frequently found (5%–6% of genotype 1a sequences) but does not confer resistance in vitro to ledipasvir (LDV), elbasvir (EBR), velpatasvir (VEL), or pibrentasvir (PIB), shifts the baseline prevalence of NS5A RASs in genotype 1a to <10%.[801,866–868] This is of particular importance for elbasvir, for which baseline testing is recommended in genotype 1a before treatment; although the label does not distinguish based on the specific M28 RAS detected, guidelines do not recommend extending therapy or adding RBV if the M28V variant is identified.[797,798,819]

In post hoc analyses of registrational clinical trial programs, baseline NS5A RASs have a demonstrable negative impact on treatment outcomes with LDV-SOF and EBR-GZR. In a pooled analysis of genotype 1 patients treated in phase II/III trials with LDV-SOF for 6 to 24 weeks with or without RBV, baseline RASs were associated with a significantly lower SVR_{12} of 93.5% (316 of 338) compared with 98.4% (1741 of 1770) without NS5A RASs ($P < .001$).[837] The effect of RASs was most pronounced in treatment-experienced patients treated for 12 weeks who harbored LDV RASs that conferred high-level resistance (>100×; SVR 64.7%, 11 of 17). Addition of RBV resulted in a numeric but not statistically significant increase in SVR_{12} in those treated for 12 weeks. A second global analysis of LDV-SOF–treated patients expanded on these findings by looking at the impact of RASs by genotype subtype only in those treated according to guideline recommendations.[867] Consistent with the prior study, the only group in which baseline RAS significantly affected SVR was treatment-experienced patients with genotype 1a infection (88% vs. 98% SVR). High-level resistance (>100×) in this group portended worse outcomes, with only 70% SVR (16 of 23). Despite these data, firm management recommendation cannot be made for the use of LDV-SOF in the setting of NS5A RASs, given the relatively small number of patients,the contribution of other factors (e.g., stage of liver disease), and lack of randomized data to guide alternative treatment approaches. Guidelines do advocate for NS5A resistance testing in genotype 1a treatment-experienced patients with cirrhosis in whom LDV-SOF is being considered.[819] If NS5A RASs, particularly those conferring high-level LDV resistance, are identified, alternative treatment regimens are recommended.

Baseline NS5A RASs also affect outcomes for EBR-GZR in genotype 1a infection. An integrated analysis across phase II/III EBR-GZR studies demonstrated lower SVR rates in genotype 1a patients with baseline EBR-specific RASs: 52% (16 of 31) with RASs versus 98% (464 of 475) without EBR RASs.[593] Numerically, the impact was larger in treatment-experienced patients. Two important considerations for these data are that population sequencing was used and M28V was not included because it does not confer resistance to EBR in vitro.[868] Given these considerations, EBR-specific RASs were present in 6% of the population studied. Based on a very limited number of patients ($n = 6$), extension of therapy to 16 to 18 weeks with RBV appeared to abolish the adverse impact of NS5A RASs on response (100% SVR).[797,798] Finally, a multivariate analysis of response factors found only baseline viral load above 800,000 IU/mL and the presence of EBR-RASs to be significant predictors of response in genotype 1a patients treated with EBR-GZR.[594] Based on these data, baseline testing is recommended (see "Initial Treatment of Chronic Hepatitis C" section and Chapter 117).

The NS5A RASs of clinical significance in GT3 are A30K and Y93H. In non–NS5A-exposed patients, each RAS is found in 5% to 10% of GT3 sequences.[592,869,870] In vitro, the A30K confers moderate resistance to DCV (40×) and VEL (50×), whereas little impact is seen with PIB (1.1×).[798,799,801,869] The Y93H RAS confers high-level resistance in vitro (>100×) to both DCV and VEL, but no appreciable shift is seen with PIB (<3.0×).[798,799,801,869] In other genotypes—for example, GT2—polymorphisms such as L31M are found with regularity (approximately 40%), yet do not have clinical impact. Furthermore, sequence-based resistance testing is not commercially available outside genotypes 1 and 3.

The impact of NS5A RASs in patients infected with GT3 not previously exposed to NS5A inhibitors is more nuanced. The presence of baseline NS5A RASs (A30K, L31M, Y93H) resulted in a 93% SVR rate with SOF-VEL (98% without RASs).[870] In addition, response rates were lower in those with the Y93H variant, at 86% (19 of 22). However, response rate is also modified by prior treatment experience and/or the presence of cirrhosis.[824,825] The regimen of SOF-VEL-VOX does not appear to be affected by baseline NS5A resistance in GT3 infection not previously treated with an NS5A inhibitor (100% SVR, 32 of 32).[871,872] For the regimen of GLE-PIB, baseline presence of the A30K RAS has been associated with numerically lower response rates to 8 weeks of treatment in patients without cirrhosis and GT3 infection. In the phase III ENDURANCE-3 study, SVR rate with 8 weeks of GLE-PIB was 75% in those with baseline A30K (12 of 16).[592] An integrated analysis of phase II and III studies did not demonstrate an impact of NS5A RASs on responses with 8 weeks of GLE/PIB in GT3.[873] Notably, all GT3 virologic failures in the integrated analysis came from the phase III ENDURANCE-3 study. Finally, an integrated GT3 analysis that specifically assessed the impact of the A30K variant found a numerically lower SVR rate of 84% (16 of 19) with 8 weeks versus 94% (17 of 18) with 12 weeks of GLE-PIB.[874] Given the small numbers, this difference was not statistically significant. Mechanistically, despite the fact that neither the A30K nor the Y93H variant results in a significant shift in in vitro potency for PIB, the A30K variant lowers the barrier to development of the dual A30K + Y93H variant to a single nucleotide change, and this combination results in a 69-fold decrease in PIB potency in vitro.[801] Conversely, the baseline presence of the Y93H variant in GT3 still requires two nucleotide changes to result in the double variant (A30K + Y93H), and clinically a baseline Y93H variant alone does not appear to affect GLE-PIB in GT3.[592,873,874]

After unsuccessful therapy containing an NS5A inhibitor, genotypic resistance is frequently selected. For instance, after 12 weeks of LDV-SOF therapy, NS5A resistance is selected in 95%.[866] A number of factors contribute to rates of resistance selection including duration and regimen composition. Treatment with highly potent triple-drug regimens containing a nucleoside appear to infrequently select for NS5A resistance (<10%).[871] Long-term follow-up data on persistence of NS5A RASs indicate that the majority (>80%) continue to have detectable resistance mutations for over 3 years despite the lack of a true archiving mechanism in HCV.[875]

The impact of selected NS5A resistance on re-treatment has been significantly mitigated with the arrival of potent double- and triple-drug regimens, some of which are now approved for re-treatment of HCV in the setting of prior DAA exposure.[791,800,816] A clear impact of resistance was seen when a longer duration of the same regimen was used for re-treatment. In a small study of LDV-SOF failures ($N = 41$) re-treatment outcome with LDV-SOF for 24 weeks was determined by baseline resistance. Sustained response rate was 100% in those without resistance and 60% in those with NS5A resistance.[876] In contrast, there is no impact of NS5A resistance on re-treatment efficacy when the triple combination of SOF-VEL-VOX is used. In the phase III POLARIS-1 study, SVR rate

was 100% in genotype 1-infected patients with baseline NS5A RASs (n = 59) and 97% in those with both NS5A and NS3 RASs (n = 71).[865,877]

In the current treatment era, resistance to NS5B polymerase inhibitors, either naturally occurring or selected, does not appear to have a clinically meaningful impact. Dasabuvir is the lone nonnucleoside HCV RNA polymerase inhibitor approved for clinical use. On failure with a dasabuvir-containing regimen, resistance is selected in approximately 60% of patients, with variants S556G/R being most prevalent.[878] Sofosbuvir, the lone NS5B nucleotide inhibitor currently approved for treatment of HCV, is unique among all HCV DAAs in that it has an extremely high barrier to resistance and infrequently selects for resistance after DAA failure. The signature resistance mutation for sofosbuvir is the S282T in NS5B, which in vitro results in a 5-fold to 20-fold increase in EC_{50} depending on additional mutations present.[879,880] Replicative fitness of replicons or viruses harboring the S282T mutation in vitro are significantly diminished (approximately 10% of wild-type). Combined, the modest impact on potency and significant decrease in viral fitness likely explain why resistance is rarely seen in the clinical setting. Multiple baseline sequencing studies have not detected the S282T variant down to a 1% level.[866,880] A combined analysis from phase II and III studies of SOF plus ribavirin with or without IFN found the S282T variant in <1% (1 of 300) of virologic failures.[880] After virologic failure with LDV-SOF, the S282T variant was found in a single patient (2% of sequenced patients).[866] Consistent with its poor replicative fitness, the S282T variant quickly becomes undetectable via sequencing after withdrawal of drug selective pressure.[866,880,881] This appears to occur via reversion to wild-type, as opposed to outgrowth of persistent wild-type populations, as evidenced by altered codon usage for serine at the 282 position after reversion.[866,881] A handful of other mutations have been identified after unsuccessful sofosbuvir-containing therapy, with the two most frequently selected being L159F and V321A. In vitro, these mutations do not result in a significant fold-shift in sofosbuvir activity (1.2-fold to 1.3-fold), and their impact, if any, on responses to SOF-based therapies is unclear. At this time there is no defined role for NS5B genotypic resistance testing either at baseline or after failure of DAA treatment including an NS5B nucleotide.

Re-treatment of Chronic Hepatitis C Virus Infection

Re-treatment of patients in whom prior HCV antiviral therapy has failed falls into several subgroups, including failure of IFN and ribavirin with or without a single DAA (first-generation NS3/4 protease inhibitor), DAA failure without NS5A, and DAA failure with NS5A. Patients in whom failure of IFN and ribavirin with or without a single DAA has occurred are treated in the same manner as treatment-naïve patients, with few exceptions. Patients in whom an all-oral DAA regimen (especially NS5A-inclusive regimens) has failed represent a new and challenging re-treatment population and are potentially difficult to re-treat owing to increased likelihood of resistance (see section on DAA resistance) and enrichment of multiple other traditional negative predictors (male, high BMI, cirrhosis, *IL28B* unfavorable genotype).

Failure of Interferon and Ribavirin With or Without NS3/4 Protease Inhibitor

The re-treatment of patients with genotype 1, 4, 5, or 6 infection who experienced failure of previous treatment with IFN and ribavirin with or without NS3/4 protease inhibitors (NS3/4 protease inhibitors were approved only for genotype 1 infection) is the same as the initial treatment of patients with these genotypes with a few exceptions. The 8-week option for ledipasvir-sofosbuvir is not applicable, because this applies only to patients who have never received therapy for their HCV infection. Furthermore, a meta-analysis of a phase II/III study of ledipasvir-sofosbuvir found that patients with genotype 1 infection who had experienced previous failure of IFN and ribavirin with or without an NS3/4 protease inhibitor and had cirrhosis had a lower SVR when treated for 12 weeks than those who received 12 weeks of therapy with ribavirin or 24 weeks of therapy.[a] The phase II SIRIUS trial randomized patients to ledipasvir-sofosbuvir with ribavirin for 12 weeks versus 24 weeks without ribavirin and reported similar SVRs (96% vs. 97%, respectively).[784] With multiple ribavirin-free, 12-week options available for this subgroup of patients, this regimen is listed as an alternative, not a recommended, treatment option. This same approach is applied in patients with genotype 4 infection, but for genotype 5 and 6 infections, 12 weeks of ledipasvir-sofosbuvir is recommended regardless of the presence or absence of cirrhosis. This is based on very limited data. In addition, the elbasvir-grazoprevir regimen was studied (and approved) with ribavirin in patients with prior failure of an NS3/4- and IFN-containing regimen; thus, this regimen is an alternative, not a recommended, regimen in this re-treatment population.

The re-treatment of patients with genotype 2 infection in whom previous treatment with IFN and ribavirin failed is the same as initial treatment. Both glecaprevir-pibrentasvir and sofosbuvir-velpatasvir offer high efficacy for this patient population. However, the re-treatment of patients with genotype 3 infection who experienced previous failure of IFN and ribavirin has unique recommendations. As previously mentioned in the "Initial Treatment of Chronic Hepatitis C Virus Infection" section, there are subpopulations of patients with genotype 3 infection that present a therapeutic challenge. Consistently, across multiple DAA regimens, prior treatment failure and/or presence of cirrhosis increases the risk of treatment failure. It is also in the setting of these multiple baseline negative predictors that the presence of NS5A RASs has more impact on the risk of treatment failure. The ASTRAL-3 study, which investigated 12 weeks of sofosbuvir-velpatasvir in patients with genotype 3 infection, included 71 patients in whom therapy had previously failed and 80 patients with compensated cirrhosis. Although the overall SVR_{12} was 95%, lower SVR was reported for patients with previous failure (90%) and with cirrhosis (91%).[825] As previously described, when a sofosbuvir plus NS5A (velpatasvir or daclatasvir) regimen is used, baseline NS5A RASs, especially when other negative predictors are present, affect DAA treatment response for genotype 3 infection, with the Y93H variant having the greatest impact. Meanwhile, glecaprevir-pibrentasvir, which is approved as an 8-week regimen for initial treatment of genotype 3 infection, regardless of presence of cirrhosis, had a lower SVR rate when studied for 12 versus 16 weeks among 44 patients without cirrhosis in whom prior therapy had failed (previously treated with IFN and ribavirin with or without sofosbuvir; those previously treated with NS5A or NS3/4A protease inhibitors were excluded).[883] Owing to small numbers, the role of baseline NS5A RASs is less clear, but all patients who experienced treatment failure had RASs at baseline. In particular, two subjects (one in each arm) had the A30K substitution at baseline, and treatment failure occurred with the A30K plus emergence of the Y93H variant, a double RAS that confers 69-fold resistance to GLE-PIB. The phase III POLARIS-3 study randomized treatment-naïve patients with genotype 3 infection and cirrhosis to 8 weeks of sofosbuvir-velpatasvir-voxilaprevir versus sofosbuvir-velpatasvir for 12 weeks.[792] The SVR_{12} was 96% in both arms.

Thus, for patients with genotype 3 infection who have previously experienced failure of treatment with IFN and ribavirin and who do not have cirrhosis, the recommendation per the AASLD/IDSA HCV treatment guidance is to check for baseline NS5A RASs. If the Y93H RAS is not present, the recommendation is to use sofosbuvir-velpatasvir for 12 weeks. If the Y93H RAS is present, the recommendation is to use sofosbuvir-velpatasvir-voxilaprevir for 12 weeks. If neither of these is possible, alternative regimens are glecaprevir-pibrentasvir for 16 weeks or daclatasvir and sofosbuvir for 12 weeks (without Y93H RAS only).[819] The re-treatment approach for genotype 3 infection in patients in whom treatment with IFN and ribavirin previously failed and who have cirrhosis is more challenging. Because of the addition of the negative baseline predictor in cirrhosis, there is a need to further optimize therapy, and both recommended regimens are off-label recommendations. As previously mentioned, although sofosbuvir-velpatasvir-voxilaprevir is not approved for this indication by the FDA, POLARIS-3 supports the efficacy of this combination for 8 weeks in patients with genotype 3 infection and cirrhosis.[792] Owing to the small number of treatment-experienced patients, the guidelines recommend 12 weeks of therapy, because of the number of negative baseline predictors in this difficult-to-treat patient population and the need to optimize treatment response. The other recommended regimen is the combination of sofosbuvir with elbasvir-grazoprevir. Although elbasvir-grazoprevir is not FDA approved for genotype 3 infection, the regimen does have activity versus this genotype. The C-ISLE

[a]References 852–854, 856, 861, 864–867, 869, 871, 873–879, 881, 882.

study investigated this triple-drug regimen for 12 weeks with or without ribavirin or for 16 weeks without ribavirin, in patients experienced with IFN plus ribavirin ($n = 53$) with genotype 3 infection and cirrhosis.[884] The overall SVR_{12} in this subgroup was 100% in a per protocol analysis. Because of the unmet need in this very difficult-to-treat subgroup, 12 weeks of treatment with either sofosbuvir with elbasvir-grazoprevir or sofosbuvir-velpatasvir-voxilaprevir is recommended.[819]

Failure of Non-NS5A Inhibitor, Sofosbuvir-Containing Regimens

In patients who have previously experienced treatment failure with a non-NS5A inhibitor, sofosbuvir-containing regimen, failure primarily occurred with sofosbuvir and ribavirin (primarily genotypes 2 and 3), sofosbuvir plus IFN and ribavirin (primarily genotypes 1 and 3), or sofosbuvir and simeprevir (primarily genotype 1). For genotype 1 infection, regardless of the presence of cirrhosis, there are three recommended regimens: sofosbuvir-velpatasvir-voxilaprevir for 12 weeks for genotype 1a, sofosbuvir-velpatasvir for 12 weeks for genotype 1b, and glecaprevir-pibrentasvir for 12 weeks, regardless of subtype.[819] The phase III POLARIS-4 randomized trial compared sofosbuvir-velpatasvir-voxilaprevir for 12 weeks versus 12 weeks of sofosbuvir-velpatasvir.[877] Cirrhosis was common—46% in both study arms. SVR_{12} rates for patients with genotype 1 infection were 97% and 90%, respectively. Overall, there was only 1 relapse in the sofosbuvir-velpatasvir-voxilaprevir arm, and 14 relapses in the sofosbuvir-velpatasvir arm. Eight of the 14 relapses occurred in patients with genotype 3 infection and 5 had genotype 1a infection; accordingly, sofosbuvir-velpatasvir is not recommended for these two genotypes or subtypes, and sofosbuvir-velpatasvir-voxilaprevir is recommended. However, only one relapse occurred in a patient with genotype 1b infection and none occurred for genotype 2 infection, suggesting that sofosbuvir-velpatasvir for 12 weeks is an efficacious regimen with these genotypes or subtypes and that the triple salvage regimen is not required. It is also notable that the single patient with relapse in the sofosbuvir-velpatasvir-voxilaprevir arm did not experience treatment-emergent RAS, whereas 9 of the 14 relapses in the sofosbuvir-velpatasvir arm occurred in patients who developed treatment-emergent NS5A RASs. The data to support the use of glecaprevir-pibrentasvir in this re-treatment population for genotype 1 or 2 are limited.

The ENDURANCE-1 and EXPEDITION-1 studies included a total of 14 patients with genotype 1 infection in whom a sofosbuvir-containing regimen had failed and who had received glecaprevir-pibrentasvir for 12 weeks.[592,821] For genotype 2 infection, the ENDURANCE-2 study, which investigated 12 weeks of glecaprevir-pibrentasvir, included six patients who had previously experienced failure of sofosbuvir plus ribavirin with or without IFN.[824] All six patients achieved SVR_{12}. Thus, glecaprevir-pibrentasvir for 12 weeks is recommended in the case of genotype 1 or 2, non-NS5A, prior sofosbuvir-containing regimen failures, regardless of the presence of cirrhosis. This regimen is not recommended for similar patients with genotype 3 to 6 infections (see "Failure of Direct-Acting Antiviral Regimens Containing NS5A Inhibitors" section).

Failure of Direct-Acting Antiviral Regimens Containing NS5A Inhibitors

There are only two regimens approved by the FDA for patients in whom an NS5A-containing regimen has previously failed: sofosbuvir-velpatasvir-voxilaprevir for 12 weeks for all genotypes, and glecaprevir-pibrentasvir for 16 weeks for genotype 1. Owing to either poor performance of other regimens or limited data, sofosbuvir-velpatasvir-voxilaprevir for 12 weeks is recommended for genotypes 3 to 6 in situations in which any DAA regimen, including NS5A-containing regimens, has failed. POLARIS-1, a placebo-controlled phase III trial, supports the use of 12 weeks of sofosbuvir-velpatasvir-voxilaprevir in patients with all genotypes in whom an NS5A-containing regimen has failed, regardless of the presence of cirrhosis.[877] In the majority of patients a regimen containing NS5A plus sofosbuvir had failed, and the overall SVR_{12} was 97%. There were seven virologic failures, six of which were relapses, and all occurred in patients with cirrhosis. Four of the relapses occurred in patients with genotype 3 infection (and cirrhosis). Because of the difficulty in re-treating patients with genotype 3 infection in whom a previous DAA therapy has failed and who have cirrhosis, the AASLD/IDSA HCV treatment guidance recommends adding weight-based ribavirin to the 12-week sofosbuvir-velpatasvir-voxilaprevir regimen in order to optimize treatment outcomes. The phase II, open-label, MAGELLAN-1 and MAGELLAN-2 studies investigated the efficacy of glecaprevir-pibrentasvir for 12 to 16 weeks with or without ribavirin in patients primarily with genotype 1 infection in whom a prior DAA-containing regimen, including NS5A-inhibitors, had failed.[864,885] Although limited by small samples sizes, these studies reported that patients who had been previously exposed to DAA regimens with NS3/4 protease inhibitors and NS5A inhibitors had the highest relapse rates, regardless of the length of regimen; 12 weeks appeared sufficient for patients who had experienced failure of a prior NS3/4-only regimen, and 16 weeks appeared necessary for patients who experienced failure of an NS5A-only regimen. Accordingly, this regimen was FDA approved for DAA salvage only in genotype 1 infection and for the aforementioned durations and subpopulations. Owing to the need for more NS5A-failure salvage regimens in these patients, who may not be able to take the sofosbuvir-velpatasvir-voxilaprevir regimen, the AASLD/IDSA guidelines identify glecaprevir-pibrentasvir as an alternative regimen in genotype 1 infection. It is not recommended for DAA salvage for other genotypes owing to limited data.

Adverse Reactions

Adverse reactions were extremely common and often treatment limiting with IFN-α-based regimens. Flulike symptoms were experienced by most persons, and fatigue, depression, and cognitive changes were also common. In addition, IFN-α commonly caused mild-to-moderate transient bone marrow suppression, manifesting with anemia, thrombocytopenia, and especially neutropenia. Ribavirin also added to the side-effect profile, with psychiatric-associated symptoms and anemia being the most common manifestations. The side-effect profile of these medications and the lower chance of response significantly limited the uptake and the access for patients with concomitant medical conditions including psychiatric disease, cardiovascular disease, and HIV infection. Although the first DAAs, telaprevir and boceprevir, significantly improved the treatment response rates for genotype 1 infection, they were still used in combination with PEG IFN–ribavirin, and they had their own adverse event profiles, including severe anemia and, in the case of telaprevir, rash and severe anal burning and pruritus.

With DAA combination therapies and, increasingly, the limited need for ribavirin as part of most regimens, the safety and adverse event profile has dramatically improved, and therefore discontinuation of therapy due to adverse events is extremely uncommon. Several DAA regimen registration trials have randomized participants to receive placebo, providing an opportunity to assess the safety of these regimens. In POLARIS-1, a phase III placebo-controlled trial of sofosbuvir-velpatasvir-voxilaprevir, patients receiving the intervention arm reported headache (25%), fatigue (21%), diarrhea (18%), and nausea (14%), as compared with 17%, 20%, 12%, and 8% in the placebo arm, respectively.[877] There were no differences in laboratory abnormalities, with only 1% reporting hemoglobin <10 g/dL in both arms and only two participants in the intervention arm reporting elevated liver enzymes. Liver enzyme elevation was more common in the placebo arm owing to active HCV infection. Rates of discontinuation of therapy due to adverse events were 2% in the placebo arm and <1% (single subject) in the intervention arm. The C-EDGE study randomized patients to 12 weeks of elbasvir-grazoprevir versus a delayed-start arm.[820] In the first 12 weeks of follow-up, reports of adverse events (>10% incidence) were similar between the two arms: headache (17% vs. 18%, respectively) and fatigue (16% vs. 17%, respectively). Thus, whereas with IFN-containing regimens the management of adverse events was an important part of treatment, this is no longer the case. The need for growth factors and blood transfusions, hospitalization, and symptom management are almost nonexistent.

Drug interactions are important complications of therapy, especially with NS3/4 protease inhibitors. Because this field is rapidly changing, clinicians are strongly urged to check updated information (e.g., www.hep-druginteractions.org) before prescribing any DAA regimen to patients who are taking other medications. There are several key drug interactions that are worth mentioning, including sofosbuvir and amiodarone; ledipasvir or velpatasvir and proton pump inhibitors; and multiple DAA regimens with lipid-lowering drugs and antiepileptics.

Recommended Treatments for Chronic Hepatitis C as of 2018

The AASLD and IDSA recommendations for treatment of hepatitis C are presented in Table 154.4. AASLD and IDSA provide regularly updated treatment recommendations for hepatitis C. (See the online guidance for recommendations for re-treatment and for treatment of special populations, including patients who are HIV infected—available at https://www.hcvguidelines.org.)

Selection of Patients for Treatment Initial Treatment and Re-treatment of Patients With Chronic Hepatitis C, Including Those With HIV Infection

Historically, the risk of treatment with IFN-containing regimens, which, as noted earlier, carried significant adverse event profiles, had to be weighed against the benefit of SVR. Owing to low SVR rates in the IFN era and high rates of adverse events, only select patients were considered appropriate for therapy. Now, however, with DAA therapy that is very well tolerated and quite safe when the appropriate DAA is used in the correct patient population, the risk of therapy is low. Furthermore, the potential benefit of cure, which is afforded the vast majority of patients who take therapy, is high.[495,508,886] Therefore the AASLD/IDSA HCV guidance recommends treatment "for all patients with chronic HCV infection, except those with a short life expectancy that cannot be remediated by HCV therapy, liver transplantation, or another directed therapy."[819] In addition, patient subgroups previously considered poor candidates for therapy, including patients who inject drugs, patients who abuse alcohol, or patients with psychiatric disease, can do well with DAA therapies and should not be excluded based on their comorbidities.[887–889] Finally, patients with HIV infection should not be considered any differently for DAA therapy than a patient without HIV. Although rates of adverse events in the IFN era were greater in studies of patients with HIV infection, this is no longer true. Multiple phase III trials have supported not only the same efficacy of DAAs in patients with HIV infection, but the same degrees of safety and tolerance as well, with similar treatment discontinuation rates across both patient populations.[842,844,890] With few exceptions, owing to the benefit of ARV therapy, patients with HIV infection should be started on ARVs first, and then HCV therapy should be started once HIV suppression has been attained.[891] Attention to the potential for DAA drug-drug interactions is critical in choosing the ARV regimen, and in patients on stable ARV therapy, the choice of the DAA regimen should include considerations of drug-drug interactions.

TABLE 154.4 Current AASLD and IDSA Recommendations for Initial Treatment (September 2017)

Genotype 1a

- Elbasvir-grazoprevir for 12 weeks if no NS5A RAS for elbasvir is detected (no cirrhosis or compensated cirrhosis)
- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
  - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 1b

- Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
  - 8 weeks for patients without cirrhosis and baseline HCV RNA <6 million IU/mL
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 2

- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 3

- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Sofosbuvir-velpatasvir for 12 weeks if no cirrhosis or if compensated cirrhosis
  - Cirrhosis: testing for NS5A RASs for velpatasvir is recommended
    - Consider alternative treatments if Y93H present

Genotype 4

- Elbasvir-grazoprevir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Genotype 5 or 6

- Glecaprevir-pibrentasvir for 8 weeks if no cirrhosis and 12 weeks if compensated cirrhosis
- Ledipasvir-sofosbuvir for 12 weeks (no cirrhosis or compensated cirrhosis)
- Sofosbuvir-velpatasvir for 12 weeks (no cirrhosis or compensated cirrhosis)

Clinicians are urged to consult online guidelines for the latest recommendations on HCV treatment (www.hcvguidelines.org).
[a]Regimens are listed in groups by level of evidence, then alphabetically. See www.hcvguidelines.org for alternative treatments, treatments in experienced patients, and special populations.
AASLD, American Association for the Study of Liver Diseases; *FDA,* US Food and Drug Administration; *IDSA,* Infectious Diseases Society of America; *RAS,* resistance-associated substitution.

Treatment of Acute Hepatitis C

In 2015, 1.75 million new HCV infections were reported by the World Health Organization (WHO), an epidemic that is widespread.[665,892,893] Because prevention of future infection is a mainstay of the elimination plan, early identification and treatment of incident HCV infections are critical. Currently, national guidelines for the management of HCV infection provide different recommendations for the treatment of acute HCV infection. EASL recommends an 8-week DAA regimen of an NS5A inhibitor–sofosbuvir combination.[818] Furthermore, EASL recommends that patients with HIV infection and/or those with HCV RNA >1,000,000 IU/mL should have therapy extended to 12 weeks. The AASLD/IDSA Guidance Panel recommends that acute infection be treated in the same manner as chronic infection, in which case, as with EASL, use of a shortened course of therapy to 8 weeks for persons with HIV infection is not recommended.[819]

Historically, with IFN-based regimens, treatment response was improved when patients were treated in the acute infection period, which allowed for shortening of the course of therapy from 12 to 6 months for genotype 1 infection.[894] Over the past 2 years, several pilot clinical trials of all-oral, DAA therapies have been reported in the setting of acute or early HCV infection.[895–899] These studies, which have investigated shortened courses of DAA therapy, show significant variability in study population, definitions of acute or early infection, presence or absence of symptoms attributable to HCV infection, frequency of the *IL28B* favorable genotype, DAA therapy potency, and/or duration of DAA therapy. Although small and variable, these studies have identified challenges in the treatment of acute infection including the potential impact of high baseline HCV RNA on treatment response.[896] Viral load in the setting of acute infection may correlate with the host immune profile, with higher viral load correlated with a more chronic immune profile. Because of the potential for natural clearance of acute HCV infection, there are some concerns that immediate treatment results in overtreatment of a group of patients who would otherwise naturally clear the infection. Natural clearance of HCV infection has been reported in 30% to 50% of patients, although this is lower in patients with HIV infection.[900–902] The majority of patients (two-thirds) will clear the infection in the first 6 months after exposure.[902]

The WHO targets for HCV elimination include a 90% reduction in incident infections by 2030. The epidemic of new HCV infection is reported primarily in PWID and MSM with HIV infection. Modeling indicates that HCV elimination among these two groups is achievable with scale-up of access to DAA therapy and thus cure.[903] In the Netherlands, universal access to DAA therapy among MSM with HIV infection resulted in >70% HCV eradication and a 50% decline in incident HCV

infections.[904] These studies suggest that treatment is prevention, and that the earlier a patient with HCV can be treated, the less likely it is that he or she will transmit the infection to another patient. This is a rapidly evolving area of investigation, and we are likely to see sustentative changes over the next few years, with a predicted movement to treat earlier and potentially to use abbreviated courses of DAA therapies.

PREVENTION

Preexposure Prevention

The key to reducing the incidence of HCV infection is decreasing exposure to contaminated blood. The incidence of posttransfusion HCV infection has been reduced to very low levels by screening blood donations for HCV antibody and surrogate markers of HCV infection.[678,905] Although the impact is more difficult to measure, nosocomial HCV transmission in developing countries should decrease with worldwide adherence to universal precautions. Among people with illicit drug use, harm-reduction interventions such as opiate substitution therapy (OST) and needle and syringe programs (NSPs) have been the traditional backbone of HCV prevention. A meta-analysis found that OST reduces risk of HCV acquisition by 50%, and when combined with high coverage of NSPs results in a 71% reduction in the risk of HCV acquisition.[906] In fact, harm-reduction interventions are viewed as a critical component of the push toward HCV elimination.

Efforts to develop an HCV vaccine are complicated by the extensive antigenic diversity existing among different HCV genotypes, in addition to the absence of solid immunity following natural infections, as discussed earlier. Nonetheless, there is evidence that immunity can be acquired that protects against *persistent* HCV infection, both in chimpanzees and in humans. Chimpanzees that responded immunologically to an initial HCV infection or that were immunized with an experimental vaccine have been readily infected on subsequent experimental challenge with the virus.[907] Significantly, however, infection appears to be attenuated following prior infection or immunization and rarely becomes persistent.[272,344,908–911] Lanford and coworkers have shown that such protection can be achieved even across HCV genotypes.[912] Likewise, injection drug users who recovered from an initial HCV infection were shown to be less likely to become viremic.[286,338] When viremia did occur, it often was at a low level and resolved. These data suggest that a vaccine might be capable of preventing viral persistence and the significant pathologic consequences of HCV infection. Although recent studies have greatly expanded the number of well-characterized broadly neutralizing human monoclonal antibodies (bNAbs) against HCV, there remain a paucity of human prevention studies.[913] More work is needed to identify optimal vaccine antigens and adjuvants. It remains unclear that anti-HCV antibody responses induced in animals are predictive of responses that would be induced by the same vaccine in humans.

Postexposure Prevention

Early studies provided conflicting data about the extent to which HCV infection is modified by administration of pooled human immune globulin.[328,914,915] However, because HCV-seropositive donations are no longer included in the plasma pools from which immune globulin is manufactured, no benefit would be expected from products on the market today. Administration of immune globulin is not recommended after exposure to HCV in US Public Health Service guidelines.[739]

An individual who has a documented exposure (e.g., a health care worker sustaining a needlestick derived from a patient who is known to be infected) should be screened for HCV antibodies as soon as possible after exposure to exclude prior infection.[275,739] Serology and ALT testing should be repeated at least once 6 months later. Some authorities also test for HCV RNA 2 to 4 weeks after exposure. Currently, postexposure prophylaxis (PEP) is not recommended for HCV exposures. The rationale for postexposure chemoprophylaxis is based on several core principles: (1) the pathogenesis and time course of early infection; (2) the biologic plausibility that infection could be prevented with antiviral drugs; (3) evidence of antiviral efficacy of the drugs being used for PEP; and (4) the risk to the health care worker from exposure to PEP.[916] The impact of the failure to prevent the development of a chronic infection also drives the clinical need for exploring PEP for infectious pathogens. For example, in the case of chronic HBV infection and HIV, there is no cure for chronic infection, and the long-term impact of infection may be substantial; on the other hand, chronic HCV infection is curable in the vast majority of patients. Therefore the impact on the health care worker of the failure to prevent chronic infection is less critical for HCV compared with HIV or HBV infection. Cost-analysis modeling, assuming a seroconversion rate of 1.9% and PEP with DAA daily for 4 weeks versus no PEP and treatment of patients who develop active infection, concluded that there is unlikely to be a scenario in which PEP is cost-effective compared with early HCV treatment.[917]

HEPATITIS C VIRUS ELIMINATION

Thus far in human history, eradication or elimination of an infectious disease has been achieved only when a highly effective vaccine was available (e.g., smallpox, polio) or through environmental measures to eliminate transmission such as vector control (e.g., malaria in North America) or improved sanitation (e.g., dracunculiasis).[918–920] The availability of multiple highly efficacious and well-tolerated HCV treatment regimens that can eradicate infection within an individual and prevent spread to others has spurred a movement toward elimination of HCV. Although many hurdles remain across all facets of HCV care, from screening and diagnosis to access and treatment to harm reduction and reinfection, the timing is right, and without aggressive goals true progress will languish.

World Health Organization Goals

WHO commissioned a mathematical model, which suggested that HCV could be eliminated as a public health threat by 2030 if the response reached coverage targets for core interventions. Core interventions identified for HCV include prevention and cascade of care.[890–892,906,916,917] Prevention interventions for HCV include blood safety (donations screened with quality assurance), injection safety (proportion of unsafe injections), and harm-reduction strategies (syringes and needles distributed among PWID per year). The "cascade of care" is a continuum of services that persons living with HCV should receive as they go through the progressive stages, from diagnosis to treatment to chronic care and ultimately to cure. Testing and treatment are the identified coverage indicators for WHO (i.e., the percent of HCV-infected persons diagnosed and the percent diagnosed with HCV started on treatment). The WHO targets for the core interventions for 2020 and 2030 are 95% and 100% for blood safety, 90% for injection safety, 200 and 300 needles and syringes per person who injects drug per year for harm reduction, 30% and 90% for testing, and 80% treated for HCV by 2030.

Current State: Hepatitis C Virus Treatment Cascade

Although the WHO targets are aggressive, with commitment from local and national stakeholders they are achievable. As of 2015, the baseline goals were 97% for blood safety, 5% for injection safety, 27 needles and syringes per PWID/year for harm reduction, 20% for testing, and 7% for treatment. For the current global cascade of care, in 2015 the gap for testing was 70%; for treatment, 73%; and for cure, greater than 90%. These gaps are not uniform across the globe because there are disparities in access to testing and to treatment. Although access to DAAs, which offer the greatest chance of cure, is increasing globally, there are many regions of the world where DAAs are not available, significantly limiting the ability to achieve cure. In order to reach sufficient coverage levels, there must be universal provision of a safe blood supply and safe injection equipment to ensure that HCV is not spread through unsafe medical practices; scaling up of harm-reduction services; provision of testing services; and secure access to safe, effective treatment for those with identified infection. This summary is theoretical, but there are emerging real-world examples of how such interventions translate to decreased incidence of HCV infection.

From Modeling to Real-World Examples

Taking into account current prevalence and incidence rates and factoring in different treatment efficacies and penetration rates, a time horizon for changes in HCV epidemiology can be modeled. After the introduction of modern DAA-based HCV therapies wherein extremely high efficacy can be expected, multiple modeling studies in specific populations (PWID,

individuals with HIV coinfection, and prisoners) have come to the same conclusion: Substantial reduction in HCV prevalence (>50%) can be achieved within 1 to 2 decades with relatively modest scale-up of DAA treatment (approximately 10% treated per annum).[921–924] Furthermore, the addition of harm-reduction measures such as OST or NSPs for PWID or safer sex counseling for high-risk MSM can significantly enhance responses compared with antiviral treatment alone.[921,924]

Treatment of individuals with acute or recent HCV infection will be a key component of elimination strategies because, by definition, these are persons at high risk for onward transmission and, largely theoretically, they may be more infectious to others. Although the prospect for spontaneous clearance of HCV has given some pause about rapidly initiating therapy in this setting, a cost and decision analysis convincingly demonstrated cost-effectiveness, and even cost savings, of acute HCV treatment when there was risk of onward transmission.[925] Identification of acute HCV infection is problematic, but individuals in specific populations, such as those with HIV, are more likely to be diagnosed and should be a particular focus for treatment of acute HCV infection.

Despite the convincing data from modeling studies, there are as yet no large-scale demonstrations of the impact of DAA treatment on HCV incidence and prevalence. Several ongoing or localized programs with impressive early results are highlighted.

The country of Georgia has a high HCV prevalence (approximately 7%) and committed early to a program to eliminate HCV.[926] In cooperation with the CDC, the elimination program was launched in April 2015 with initial efforts focused on developing an updated assessment of disease prevalence, building laboratory support and treatment capacity, and targeting initial treatment to those with more advanced liver disease. During the first 20 months of the program, nearly 26,000 persons initiated treatment for HCV with sofosbuvir plus ribavirin (with or without IFN) or, later, LDV-SOF. Sustained response to treatment has been assessed in over 6000 persons with an overall SVR_{12} of 79.5%, and 98% in those treated with LDV-SOF.[927] Based on current data, Georgia is on target to achieve a 90% reduction in HCV prevalence by 2020—a full decade ahead of WHO goals.

A high HCV prevalence combined with the ability to provide monitored treatment makes prisons an attractive venue to launch "micro-elimination" programs.[928,929] Dramatic results from a comprehensive diagnosis, treatment, and re-infection program in Queensland, Australia demonstrated a decrease in HCV prevalence from 12% to 1% in just 22 months.[930] In the United States, the fragmented nature of health care and significant funding challenges for HCV treatment in prisons precludes the use of such an approach at present.

Elimination of HCV in those with HIV is perhaps a more readily attainable goal because this is a relatively small population with levels of engagement in care that result in more effective diagnosis of HCV.[931] In addition, HIV coinfection may improve access to HCV medications because this is seen as a priority population for treatment. Modeling studies specific to this population have indicated that increased rates of treatment combined with harm-reduction measures could have a significant positive impact. Based on the HIV- and HCV-coinfected population of MSM in the United Kingdom, the impact of multiple different treatment approaches on HCV incidence and prevalence was modeled.[924] With the conservative assumption of 90% SVR with DAA treatment, maintaining 2015 treatment levels (approximately 50% of recent infections and 20% of chronic infections treated), there was little impact of DAA therapy on chronic HCV prevalence (8% with DAAs vs. 9% with IFN) or incidence (1.3 per 100 person-years vs. 1.5 per 100 person-years) in HIV- and HCV-coinfected MSM by 2025. As increased rates of treatment of recent and chronic infections were scaled up to 80% and 20%, respectively, dramatic decreases in prevalence and incidence were predicted; the largest impact was realized when 20% risk reduction was also included in the models (2.4% prevalence and incidence of 0.4 per 100 person-years in 2025). An important corollary to these findings is that although the number of treatments increases transiently during the rollout, within a few years the annual treatment rates drop below the base-case scenario as a significant net impact on new infections is realized in later years.[924]

In support of modeling predictions, real-world cohorts demonstrating the power of intense screening campaigns coupled with universal access to therapy are beginning to emerge. For example, in the Netherlands, universal access to DAA therapy arrived in late 2015; after this, data from the ATHENA HIV cohort indicated that by 2017, of the 1471 HIV- and HCV-coinfected persons (69% MSM) in care, 76% had been treated.[932] Notably, the bulk of treatment uptake occurred after removal of treatment restrictions. During this same time period (2014–16) the incidence of acute HCV infection dropped dramatically in HIV-positive MSM, from 1.12 per 100 person-years to 0.5 per 100 person-years, translating to a 51% decrease.[904] Concomitant diagnoses of sexually transmitted infections (e.g., syphilis and gonorrhea) increased during the same time frame, which suggests that it was not a change in risk behaviors that led to the decrease in acute HCV infections. Despite these encouraging data, the impact may not be universal because data from the French HIV cohort have not yet realized a decrease in HCV incidence despite similar rates of DAA treatment uptake.[933]

In addition to concerns about generalizability, the high rates of reinfection seen in some HIV-positive MSM cohorts coupled with a lack of universal, contemporary rollout of aggressive screening and treatment programs have raised concerns over reintroduction of HCV.[934–936] This is particularly relevant given the demonstration of international HCV transmission networks in HIV-positive MSM.[893]

ACKNOWLEDGMENTS

We thank Stanley Lemon, Stuart Ray, and David Thomas for their extensive contributions to earlier versions of this chapter. We also thank Stacey Horner for her expert review of this chapter.

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The complete reference list is available online at Expert Consult.

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155 Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)

Stanley Perlman and Kenneth McIntosh

SHORT VIEW SUMMARY

Definition
- The coronaviruses (CoVs) commonly cause mild but occasionally more severe community-acquired acute respiratory infections in humans. CoVs also infect a wide variety of animals, and several CoVs (e.g., severe acute respiratory syndrome [SARS], Middle East respiratory syndrome [MERS]) have crossed the species barrier, producing outbreaks of severe human respiratory disease. While SARS-CoV was eradicated, MERS-CoV continues to circulate in human and camel populations. As of March 10, 2019, 2374 cases of laboratory-confirmed MERS were reported to the World Health Organization, with 823 deaths.

Epidemiology
- Community-acquired CoV infections cause about 15% of common colds. They are typically epidemic in the winter months. MERS has occurred in patients in the Arabian Peninsula and those who recently traveled from this locale.

Microbiology
- CoVs are members of the Nidovirales order, single-stranded, positive-sense RNA viruses with a large genome. They mutate and also recombine frequently.

Diagnosis
- Laboratory diagnosis is best accomplished by finding viral RNA through polymerase chain reaction.

Therapy
- There are no accepted effective antiviral drugs for CoVs.

Prevention
- Prevention is through epidemiologic methods and the use of appropriate respiratory precautions in hospital settings. The SARS epidemic and MERS outbreaks were controlled through careful case identification, quarantine, and use of barrier precautions.

The family Coronaviridae, within the order Nidovirales, presently contains two subfamilies, the Coronavirinae and the Torovirinae. However, increased recognition of the genomic diversity of viruses within the Nidovirales order makes it likely that nidovirus, coronavirus, and torovirus taxonomy will require modification. Coronaviruses (CoVs) are a large group of viruses infecting mammals and birds and producing a wide variety of diseases. They have been divided into four genera, two of which contain viruses infecting humans (see later). All human coronaviruses (HCoVs) are primarily respiratory pathogens. During the winter of 2002–2003, an alarming new disease appeared: severe acute respiratory syndrome (SARS), which was quickly attributed to a new CoV, the SARS-CoV. The outbreak originated in southern People's Republic of China, with evidence that the virus was first derived from bats and was transmitted to humans through intermediate hosts, probably the palm civet *(Paguma larvata)* and raccoon dog *(Nyctereutes procyonoides)*.[1–3] The SARS epidemic was controlled through a massive effort at case identification and containment, and the last known case occurred in mid-2004. In retrospect, the emergence of SARS is consistent with what is known about CoVs as a group: They are important pathogens in animals causing a wide variety of diseases through a wide variety of pathogenic mechanisms, and they have been noted to mutate frequently and infect new species.[4,5]

More recently, a related but different CoV producing severe respiratory disease has emerged, the Middle East respiratory syndrome coronavirus (MERS-CoV). MERS-CoV was grown in June 2012 from a sputum sample obtained from a man in Saudi Arabia who died of overwhelming pneumonia. The virus was quickly identified as a new CoV most closely related to several bat CoVs.[6] This report was followed by a number of other reports identifying a total of 2374 infected individuals, most of whom had acute respiratory symptoms, severe in most and fatal in 823 (as of March 10, 2019).[7,8]

Human-to-human transmission has been documented but appears to be inefficient except in hospital settings.[9–11] The animal reservoir of MERS-CoV is believed to be camels, although evidence suggests that bats may be infected with related viruses.[12–14] Infection of camels on the Arabian peninsula and throughout Africa is widespread, and several cases of camel-to-human transmission have been reported, generally from juvenile camels.[12,15]

HISTORY

Community-Acquired Respiratory Coronaviruses, Severe Acute Respiratory Syndrome, and Middle East Respiratory Syndrome

In 1965, Tyrrell and Bynoe[16] cultured a virus obtained from the respiratory tract of a boy with a common cold by passage in human embryonic tracheal organ cultures. The media from these cultures consistently produced colds in volunteers. The agent was ether sensitive but not related to any known human virus. Subsequently, electron microscopy of fluids from infected organ cultures revealed particles that resembled infectious bronchitis virus of chickens.[17] At about the same time, Hamre and Procknow recovered a cytopathic agent in tissue culture from medical students with colds.[18] The prototype virus was named 229E and was found on electron microscopy to have a similar or identical morphology (Fig. 155.1).

Using techniques similar to those used by Tyrrell and Bynoe, McIntosh and colleagues[19] reported the recovery of several infectious bronchitis–like agents from the human respiratory tract, the prototype of

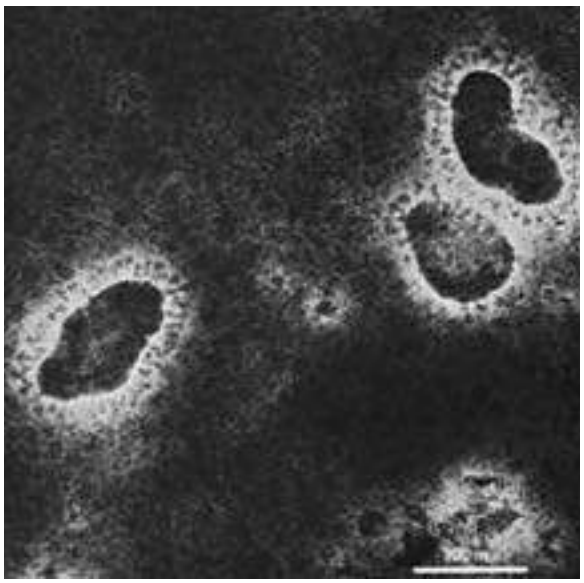

FIG. 155.1 Coronavirus strain HCoV-229E, harvested from infected WI-38 cells (phosphotungstic acid stain). *(From McIntosh K, Dees JH, Becker WB, et al. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease.* Proc Natl Acad Sci U S A. *1967;57:933–940.)*

which was named OC43 (OC for organ culture). At much the same time, mouse hepatitis virus and transmissible gastroenteritis virus of swine were shown to have the same morphology on electron microscopy.[20] Shortly thereafter, the name *coronavirus* (the prefix *corona* denoting the crownlike appearance of the surface projections) was chosen to signify this new genus.

The number of animal CoVs quickly grew, including viruses causing diseases in rats, mice, chickens, turkeys, various other bird species, cattle, several wild ruminants, beluga whales, dogs, cats, rabbits, and pigs, with manifestations in the respiratory and gastrointestinal tracts, central nervous system, liver, reproductive tract, and other locations. Through sequencing and antigenicity studies, the animal CoVs and HCoVs initially were divided into three groups: group 1, which contained HCoV-229E, as well as numerous animal viruses; group 2, which contained HCoV-OC43 plus the closely related animal viruses, bovine CoV and mouse hepatitis virus; and group 3, which included only avian viruses related to infectious bronchitis virus (Fig. 155.2). Current taxonomy divides the subfamily Coronavirinae into four genera: *Alphacoronavirus* (which includes viruses previously in group 1); *Betacoronavirus* (which includes viruses previously in group 2, most notably SARS-CoV and MERS-CoV); *Gammacoronavirus* (which includes viruses previously in group 3); and *Deltacoronavirus* (which includes several newly described avian and swine viruses).[21]

SARS was first identified in Guangdong Province of the People's Republic of China in November 2002 and spread from there to Hong Kong and then throughout the world.[22] A CoV was independently and

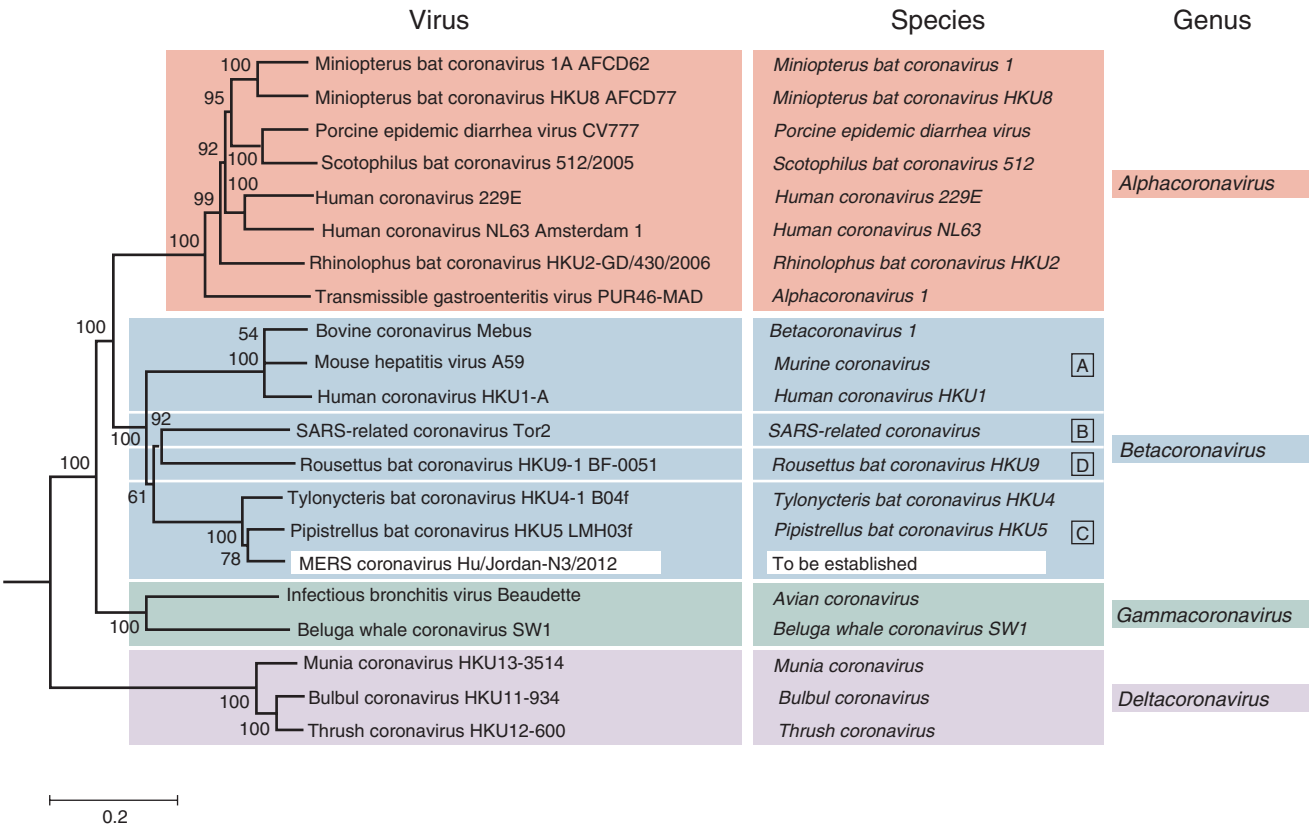

FIG. 155.2 Phylogenetic relationships among members of the subfamily Coronavirinae. A rooted neighbor-joining tree was generated from amino-acid sequence alignments of Coronaviridae-wide conserved domains in replicase polyprotein 1 (ADRP, nonstructural protein [nsp]3; Mpro, nsp5; RdRP, nsp12; Hel, nsp13; ExoN, nsp14; NendoU, nsp15; O-MT, nsp16) for 21 coronaviruses, each a representative of a currently recognized coronavirus species. Five of the six known human coronaviruses (HCoV-229E, HCoV-NL63, HCoV-HKU1, SARS-CoV, and MERS-CoV) are indicated. HCoV-OC43 is closely related to bovine coronavirus, which is shown in the figure. Equine torovirus Berne served as the outgroup. Virus names are given with strain specifications; species and genus names are in italics as per convention. The tree shows the four main monophyletic clusters, corresponding to genera *Alpha-, Beta-, Gamma-,* and *Deltacoronavirus* (color coded). Also indicated are betacoronavirus lineages A through D (corresponding to former CoV subgroups 2A through D). Bootstrap values (1000 replicates) are indicated at branch points. The tree is drawn to scale (scale bar, 0.2 amino-acid substitutions per site). *(From de Groot RJ, Baker SC, Baric RS, et al. Middle East respiratory syndrome coronavirus [MERS-CoV]: announcement of the Coronavirus Study Group.* J Virol. *2013;14:7790–7792.)*

almost simultaneously isolated from SARS patients by several laboratories and found by sequencing to be only distantly related to previously characterized CoVs.[23–26] The SARS outbreak stimulated a rapid and intense public health response coordinated by the World Health Organization (WHO), and by July 2003, transmission had ceased throughout the world. Despite this effort, however, 8096 probable cases had occurred in 29 countries, with 774 deaths.[22]

With the identification of the SARS-CoV, the HCoV field became much more active. Sensitive molecular methods were developed to detect RNA from viruses identical or closely related to HCoV-229E and HCoV-OC43 in the respiratory tract, and two new species were discovered: NL63, an alphacoronavirus, and HKU1, a betacoronavirus.[27–29] HCoV-NL63 was found independently by three groups, two in the Netherlands and, somewhat later, the third in New Haven, Connecticut.[30] In all three cases, positive samples were from infants and children with respiratory disease. Notably, HCoV-NL63 and HCoV-229E were estimated to have originated from a common bat precursor and diverged approximately 1000 years ago.[31,32] CoVs related to HCoV-229E have been isolated from camels.[33] All CoVs are susceptible to recombination, but HCoV-OC43 may be especially susceptible, with multiple recombinant strains identified in a confined geographical setting.[34] HCoV-HKU1 was found in Hong Kong in an adult with respiratory disease. These two new HCoV strains subsequently have been found worldwide and appear to have pathogenicity similar to that of HCoV-229E and HCoV-OC43, with the possible exception that NL63 is more frequently found in children with croup.[35,36]

The MERS-CoV was found when a man was admitted in June 2012 to a hospital in Jeddah, Saudi Arabia, with overwhelming acute pneumonia and renal failure. A sample of sputum grew a cytopathic virus that, on sequencing, proved to be a CoV, classified as a *Betacoronavirus* and most closely related to two bat CoVs, HKU4 and HKU5.[6] MERS-CoV continues to cause new infections, with all but a few of them sporadic or hospital-based and in individuals living or traveling in the Middle East.[37,38]

In the remainder of this chapter, the group of respiratory HCoVs first discovered in the 1960s and containing HCoVs 229E, OC43, NL63, and HKU1 are referred to as community-acquired respiratory (CAR) HCoVs to distinguish them from the SARS-CoV and the MERS-CoV.

Gastrointestinal Coronaviruses and Toroviruses

In view of the prominence of CoVs in animal enteric diseases, there have been extensive efforts to identify enteric HCoVs. There are numerous reports of CoV-like particles (CoVLPs) found by electron microscopy in human fecal matter, but these particles have been difficult to characterize further. Efforts to detect CoV RNA in feces using polymerase chain reaction (PCR) and primers for respiratory HCoVs have had limited success and have failed to associate CoVs with gastrointestinal disease.[39,40] In one instance, a human enteric CoV with high identity to bovine CoV was isolated from a child with diarrhea and shown to cause diarrhea when reintroduced into calves, demonstrating its pathogenic potential.[41]

Toroviruses were, like CoVs, first described in animals. They were first detected in the feces of cattle (Breda virus) and horses (Berne virus).[42,43] While previous publications suggested that particles resembling toroviruses could be detected in human fecal material using electron microscopy,[44] there are presently no reports definitively showing the existence of human toroviruses.

DESCRIPTION OF THE PATHOGENS

The CoV nucleic acid is RNA, approximately 30 kb in length, of positive sense, single stranded, polyadenylated, and infectious. The RNA, the largest known viral RNA (Fig. 155.3), codes for (in order from the 5′ end) a large polyprotein that is cleaved by virus-encoded proteases to form several nonstructural proteins, including an RNA-dependent RNA polymerase, methyltransferases, and a helicase, followed by either four or five structural proteins intermingled with a variable number of nonstructural and minor structural proteins.[4] The first of the major structural proteins is a surface hemagglutinin-esterase (HE) protein, present on HCoVs OC43 and HKU1 and some animal betacoronaviruses, that may play some role in the attachment or release of the particle, or both, at the cell surface. The gene for the HE protein contains sequences similar to the hemagglutinin of influenza C virus, likely evidence of an interfamily recombinational event that occurred many years ago. Notably, the HE receptor binding activity of HCoV-OC43 and probably also of HKU1 was progressively lost along with a decrease in HE-associated esterase activity during evolution in humans. These changes most likely reflected adaptation to the human sialic acid receptor after introduction into humans from zoonotic sources.[45] The next gene encodes the surface glycoprotein that forms the petal-shaped surface projections and is responsible for attachment and the stimulation of neutralizing antibody. This is followed by a small envelope (E) protein, a membrane glycoprotein, and a nucleocapsid protein that is complexed with the RNA. There are several other open reading frames, which are unique to each strain of CoV. While their coding functions are not clear, many of them are probably involved in immune evasion.[46,47] The strategy of replication of CoVs is similar to that of other nidoviruses, in that all messenger RNAs form a nested set with common polyadenylated 3′ ends, with only the unique portion of the 5′ end being translated.[4] As in other RNA viruses, mutations are common in nature, although the mutation rate is much lower, approximately 2×10^{-6} per site per replication cycle.[48] Unlike other RNA viruses, CoVs encode a 3′→5′ exonuclease that has proofreading activities, playing a critical role in maintaining replication fidelity in cell cultures and in animals.[49] CoVs are also capable of genetic recombination if two viruses infect the same cell at the same time.

All CoVs develop exclusively in the cytoplasm of infected cells (Fig. 155.4). They bud into cytoplasmic vesicles from membranes of the pre-Golgi endoplasmic reticulum. These virus-filled vesicles are then extruded by the exocytic secretory pathway with the small E protein critical for this process.[50] The resultant virus particles have a diameter of 70 to 80 nm on thin-section electron microscopy and 60 to 220 nm on negative staining. They are pleomorphic, with widely spaced, petal-shaped projections 20 nm long (see Fig. 155.1).

The cellular receptor for 229E and most other alphacoronaviruses is aminopeptidase N (APN).[51] Interestingly, NL63, the other known human alphacoronavirus, uses as its cellular receptor angiotensin-converting enzyme 2 (ACE2),[52] the same receptor as is used by the SARS-CoV.[53] Mouse hepatitis virus, a betacoronavirus related to strain OC43, uses as its receptor a member of the carcinoembryonic antigen family.[54] HCoV-OC43 and bovine CoV, which is closely related to HCoV-OC43, bind to 9-*O*-acetylated neuraminic acid as part of the entry process.[55] The host cell receptor for MERS-CoV is dipeptidyl peptidase 4, which, like ACE2 and APN, is an ectopeptidase that is abundantly expressed in the respiratory and enteric tracts.[56,57] This preferential usage of large host ectopeptidases for CoV entry is notable but not understood.

All the CAR HCoVs grow only with difficulty in tissue culture. Despite this, both 229E and NL63 were discovered because they produced a detectable cytopathic effect, the first in human embryonic kidney[18] and the second in LLC-MK2 cells.[27] Both the SARS-CoV and the MERS-CoV were initially isolated and grew readily in Vero cells.[6,25] HCoVs OC43 and HKU1 have been grown in tissue culture after laboratory adaptation or in primary ciliated human airway epithelial cells.[58,59] Detection of all these viruses in clinical specimens is most conveniently and sensitively achieved using PCR.

The enteric CoVs have been difficult to cultivate in vitro. All but a few strains have been detected only by electron microscopy of human fecal material. Two strains obtained from an outbreak of necrotizing enterocolitis in Texas were reported to contain four or five proteins with apparent molecular weights similar to those of other CoVs but not related antigenically to known human or animal strains.[60] The evidence favors the view that these isolates, as well as particles antigenically related to HCoV-OC43, are members of the family Coronaviridae, although their association with human disease is not proven.

EPIDEMIOLOGY

CAR Coronaviruses

Evidence of CAR CoV infections has been found wherever in the world it has been sought (e.g., Japan,[61] Ghana[62]). In temperate climates, CAR CoV infections occur more often in the winter and spring than in the summer and fall. The contribution of CAR CoV infections to the total

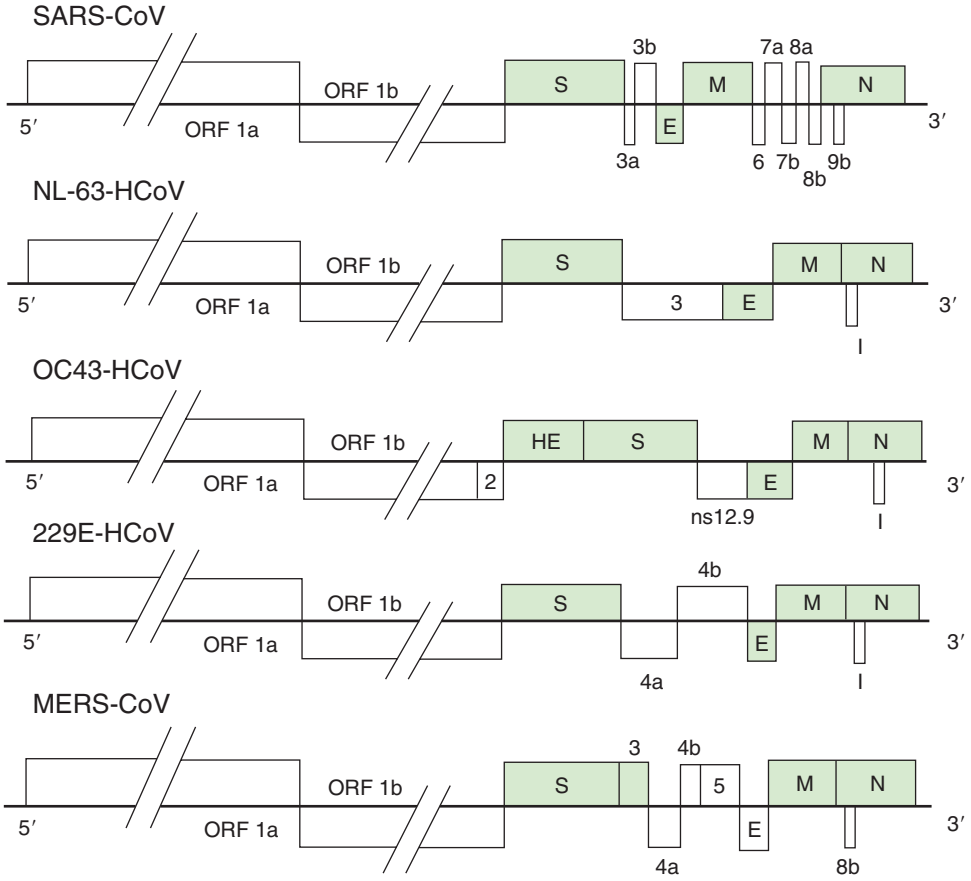

FIG. 155.3 Genome organization of representative human coronaviruses. All coronavirus genomes have the same basic structure and mechanism of replication.[4] The 5′ end of each genome encodes a leader sequence, which is attached to each virus-specific messenger RNA transcript by a novel mechanism of discontinuous replication. The first two-thirds of each genome encode replicase-associated genes. Gene 1 is translated as two large polyproteins, with the first expressed from ORF1a and the second from ORF1a/b following a −1 frameshift event. These polyproteins are then cleaved into individual proteins by two virus-encoded proteases. The major structural genes, the hemagglutinin-esterase *(HE),* surface *(S),* envelope *(E),* transmembrane *(M),* and nucleocapsid *(N)* proteins, are indicated in green. The nonreplicase, accessory genes located at the 3′ end of the genome are indicated with open boxes. The functions of these proteins are largely not known, and there is no sequence homology between accessory proteins of different coronaviruses. Some of these proteins are virion associated, but none is required for virus replication. The open reading frames (*ORFs*) encoding these proteins are numbered in order of appearance from the 5′ end of the genome, with the exception of ns12.9 of human coronavirus *(HCoV)*-OC43. I is an internal protein expressed from an alternative reading frame located within the N gene. It is equivalent to severe acute respiratory syndrome coronavirus *(SARS-CoV)*–specific protein 9b and the Middle East respiratory syndrome coronavirus *(MERS-CoV)*–specific protein 8b. *(Figure prepared by Rahul Vijay.)*

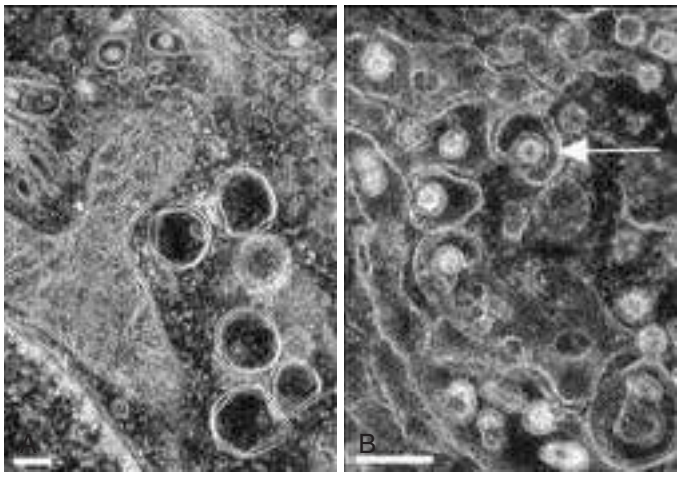

FIG. 155.4 Coronavirus strain 229E in Huh-7 cells. Huh-7 cells infected with HCoV-229E and fixed at 18 hours postinfection. (A) Double membrane vesicles, which are sites of virus replication, are shown. (B) Sites of virus assembly and budding *(arrow)* are shown. *(Images courtesy Drs. Eric Snijder and Montserrat Bárcena, Leiden University Medical Center, The Netherlands).*

number of upper respiratory illnesses may be as high as 35% during times of peak viral activity. Overall, the proportion of adult colds produced by CAR CoVs may be reasonably estimated at 15%,[63] with HCoV-NL63 and HCoV-OC43 being more common than HCoV-229E or HCoV-HKU1 in infants.[63,64]

Early studies of HCoV-OC43 and 229E in the United States demonstrated periodicity, with large epidemics occurring at 2- to 3-year intervals.[65] Similar studies of NL63 and HKU1 have not been done, but it seems from the available data that they also vary widely in incidence from year to year and place to place. Reinfection is common and may be due to the rapid diminution of antibody levels after infection.[66–68] Infection occurs at all ages but is most common in children. The ratio of symptomatic to total infections varies between 50% and 90%, depending on the age of the population studied, the method of virus detection, and the definition of "infection."[67,68] Among adult volunteers, 72% of those infected with HCoV-229E developed colds.[69]

MERS Coronavirus

Middle East respiratory syndrome (MERS) was first identified in 2012 in a man from Jeddah, Saudi Arabia, who developed pneumonia in June and died of respiratory and renal failure.[6] A virus was grown from a sputum sample that was subsequently sequenced and found to be a betacoronavirus most closely related to bat CoVs HKU4 and HKU5.

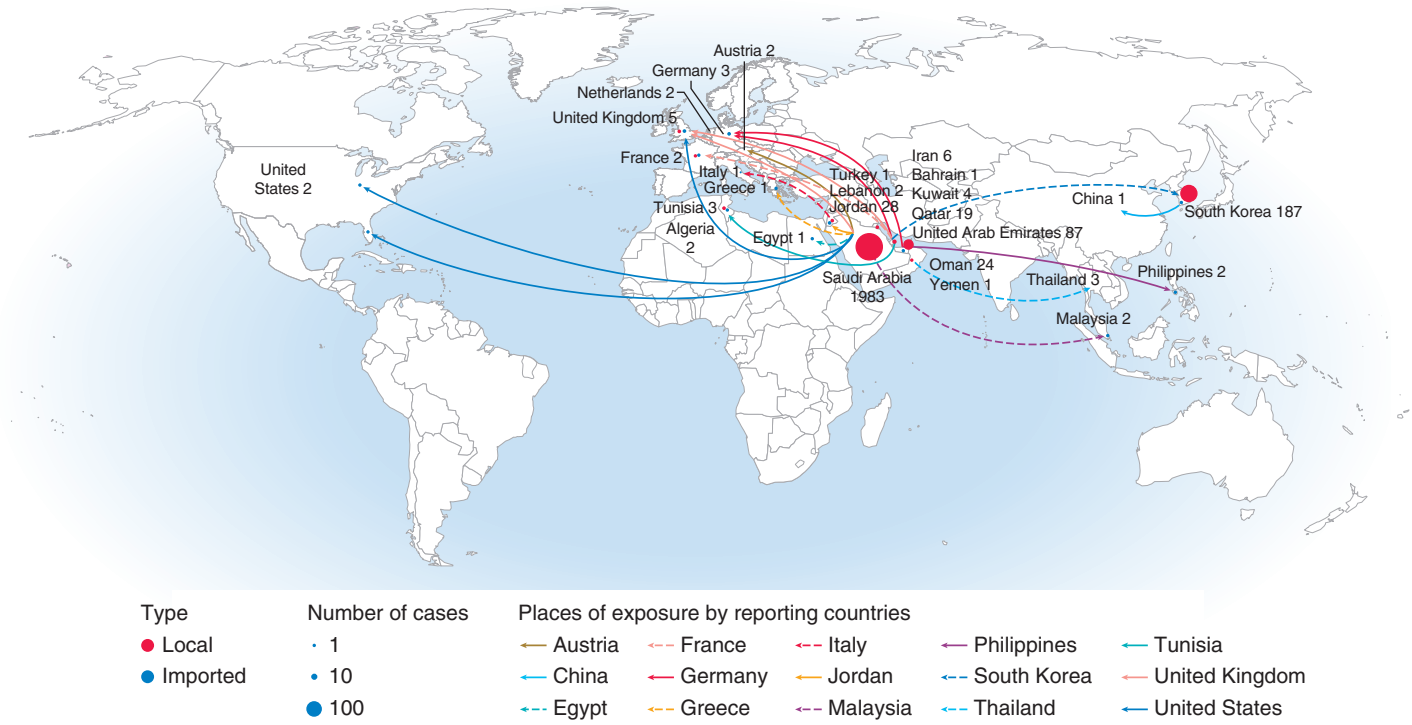

FIG. 155.5 Distribution of confirmed cases of Middle East respiratory syndrome coronavirus by reporting country, March 2012 to March 2019. This map is based on one published by the World Health Organization and shows the predominance of cases reported from the Kingdom of Saudi Arabia, with other cases arising in the Middle East, as well as the routes of travel during importation to other countries *(colored arrows). (Modified from World Health Organization. Middle East respiratory syndrome coronavirus [MERS-CoV].* http://www.who.int/emergencies/mers-cov/en/*. Accessed December 18, 2017.)*

Between then and March 10, 2019, a total of 2374 cases occurred, all infected by this virus, now termed the *Middle East respiratory syndrome coronavirus*.[70] The vast majority of these have been acquired and diagnosed in the Kingdom of Saudi Arabia, with most of the remainder in the United Arab Emirates, Qatar, Jordan, Oman, Lebanon, Iran, and Kuwait (Fig. 155.5). Cases originating in the Arabian peninsula have also occurred in travelers to Egypt, Tunisia, Germany, Italy, Great Britain, Greece, Malaysia, the Philippines, the United States, and the Republic of Korea, with secondary cases sometimes occurring in those locations through close family or hospital spread. In the United States, these include two unrelated MERS cases.[37,38,71]

WHO, the CDC, the Saudi Arabian Ministry of Health, and the Korean Centers for Disease Control and Prevention have published case definitions as well as surveillance instructions to aid in epidemiologic control of the MERS-CoV.[7,8,72,73] The majority of MERS-CoV transmission in the early years after virus identification reflected nosocomial spread.[74] Spread commonly occurred in emergency rooms and during aerosol-generating procedures. Environmental MERS-CoV contamination can be commonly detected in the vicinity of MERS patients, indicating that fomite and contact spread could occur and emphasizing the need for careful surface hygiene management.[75] However, as better infection control measures have been followed in hospitals, approximately 50% of cases are now believed to be primary cases, often acquired from camels.[76] (Of note, only a minority of presumptive primary cases describe camel contact.[11]) Virus may spread from camels through exposure to nasal or other body secretions, via the consumption of raw camel milk, or via environmental contamination[12] since virus can survive on hard surfaces for at least 48 hours.[77] Spread within family settings outside of the hospital is uncommon.[78]

In May and June 2015, a large outbreak of MERS occurred in South Korea; the index case was in a traveler returning from the Arabian Peninsula.[79] By the end of the outbreak, 186 cases, including 38 deaths, had been reported in Korea among household and hospital contacts. The large number and severity of cases resulted from several factors, including lack of timely diagnosis, "doctor-shopping," "super-spreading events," patterns of familial caregiving, and inadequate hospital infection control measures.[80,81]

Studies of MERS prevalence rely on virus detection at the time of acute infection or on measurements of anti–MERS-CoV antibodies. Prevalence studies may underestimate the numbers of exposed patients because antibody titers either do not develop (subclinical infection) or are transient (mild pneumonia).[78,82] Measurements of human MERS-CoV T-cell responses, although technically challenging, may provide better estimates of prevalence since they tend to decline less rapidly in both SARS and MERS survivors.[83,84]

Camels are almost certainly the major if not sole source for human infections. High percentages of dromedary camels that are currently or were previously infected have been detected throughout the Arabian peninsula, Africa, central Asia, and Pakistan.[12] Serologic studies demonstrated the presence of MERS-CoV antibodies in camels in Africa since at least 1983,[85] even though the first human infection was not diagnosed until 2012. MERS-CoVs have only been rarely detected in other animal species, with no evidence that they are involved in transmission to humans. Circulating MERS-CoVs with 98% to 99% identity were detected in camels and patients in the Kingdom of Saudi Arabia,[13] and in some cases virtually identical viruses were detected in patients and their contact camels.[15,86] While camels are the probable source for human MERS, bats may be the original source for the virus. Viruses related to MERS-CoV have been isolated from bats in Africa.[14,87]

SARS Coronavirus

The SARS epidemic began in Guangdong Province in the People's Republic of China in mid-November 2002.[22] It came to worldwide attention in March 2003 when cases of severe, acute pneumonia were reported to WHO from Hong Kong, Hanoi, and Singapore. Disease spread in hospitals to health care workers, visitors, and patients; among family members; and, on occasion, in hotels, apartment complexes, markets, and airplanes. Worldwide spread was rapid but focal. The

largest numbers of cases were reported from the People's Republic of China, Hong Kong, Taiwan, Singapore, and Toronto, Canada. The overall case-fatality rates in these locations ranged from 7% to 17%, but persons with underlying medical conditions and those older than 65 years of age had mortality rates as high as 50%. There was no mortality in children or in adults younger than the age of 24 years.[88]

In response to the global spread and associated severe disease, WHO coordinated a rapid and effective control program that included isolation of cases, careful attention to contact, droplet and airborne infection control procedures, quarantine of exposed persons in some settings, and efforts to control spread between countries through travel advisories and travel alerts. Presumably as a result of these efforts, global transmission ceased by July 2003. A few subsequent cases of SARS were detected, but all were either a result of laboratory spread or individual cases related to presumed contact with civet cats or other intermediate hosts. The last known case occurred in mid-2004.[89]

Spread of SARS to humans is thought to have occurred primarily through droplet or contact transmission, with a possible role for fomites. In most instances, an individual case transmitted to very few others, although super-spreading events were well documented,[90] likely involving small-particle airborne transmission.[91] Spread in hospital settings appeared to be surprisingly efficient, but it could be effectively suppressed with the enforcement of droplet and contact precautions and airborne precautions during aerosol-generating procedures.[92] Containment measures were efficacious, in part, because patients were most contagious only after lower respiratory disease developed.[93] The chain of spread was finally broken in the People's Republic of China, the last country to experience epidemic spread, in July 2003.

It now seems almost certain that the human epidemic began with the spread of a closely related bat virus first to palm civets or other animals sold in live wild game markets and then to humans in Guangdong Province in the People's Republic of China, and that the virus adapted itself through mutation and possibly recombination, until it transmitted readily among humans.[3,94–96] The virus that spread worldwide came largely from a single infected individual who traveled from Guangdong Province to Hong Kong and infected a large number of individuals before himself succumbing to the disease. In contrast, the virus that was epidemic in the People's Republic of China was more variable.

Gastrointestinal Coronaviruses

Although an etiologic role is not proven, enteric CoVLPs have been most frequently associated with gastrointestinal disease in neonates and infants younger than 12 months. Particles have been found in the stools of adults with the acquired immunodeficiency syndrome.[97,98] Asymptomatic shedding is common, particularly in tropical climates[99] and in populations living in poor hygienic conditions.[100] The particles can be detected for prolonged periods and without any apparent seasonal pattern.[101–103]

PATHOGENESIS

CAR Coronaviruses

CAR CoVs (HCoV-229E, OC43, NL63, HKU1) generally replicate in ciliated (HCoV-OC43, NL63, HKU1) and nonciliated (HCoV-229E) epithelial cells of the nasopharynx,[104] probably producing both direct cell degeneration[105] and an outpouring of chemokines and interleukins, with a resultant common-cold symptom complex similar to that produced by rhinovirus infection.[106] The incubation period is, on average, 2 days, and the peak of respiratory symptoms, as well as viral shedding, is reached at approximately 3 or 4 days after inoculation.[69]

The pattern of virus replication of CoVs is at least in part determined by virus-receptor interactions. The two best-defined receptors for the CAR CoVs are APN for strain HCoV-229E and ACE2 for NL63.[51,52]

MERS Coronavirus

Understanding of the pathogenesis and pathology of MERS has been hampered by a lack of surgical and autopsy specimens, largely for cultural and religious reasons. Autopsies from an immunocompetent patient in the United Arab Emirates and an immunocompromised patient in the Kingdom of Saudi Arabia revealed severe changes in the lungs, including hyaline membrane formation, alveolar fibrin deposition, and alveolar septal edema.[107,108] Hemorrhagic changes were observed in the lungs of the immunocompromised patient. Viral protein was detected by immunohistologic staining in the lungs of the patient from the United Arab Emirates. Particles resembling HCoVs were seen by electron microscopy in both lungs and kidney of the immunocompromised patient, but immunohistology was not recorded.

SARS Coronavirus

The pathogenicity of SARS includes systemic spread. Although the lung is the primary focus of the disease process, there are often signs of involvement in other organ systems, including diarrhea, leukopenia, thrombocytopenia, and, most notably, pan-lymphopenia.[109] Virus has been detected in respiratory secretions, blood, stool, and urine specimens and in lung, spleen and lymph nodes, brain, kidney, and intestine tissues when examined at autopsy.[110,111] On the basis of PCR testing, virus titer is highest during the second week of illness[112] and can often be detected in the stool into the third week of illness or longer.[26] Pulmonary symptoms may worsen late in the course of the illness, with the development of adult respiratory distress syndrome.[112] There may also be late evidence of liver and kidney involvement.

The pulmonary pathology of infection by the SARS-CoV has been described extensively,[25,111,113,114] but less has been published about the pathology in other organ systems.[110,111,115] The extrapulmonary pathologic changes found most consistently at autopsy are extensive necrosis of the white pulp of the spleen and a generalized small vessel arteritis. In the lung, there is hyaline membrane formation, interstitial infiltration with lymphocytes and mononuclear cells, and desquamation of pneumocytes in the alveolar spaces. Giant cells are a constant finding and usually have macrophage markers. In bronchoalveolar lavage, biopsy, and autopsy specimens, viral particles and viral RNA and protein have been noted in type I and II pneumocytes.[110,116]

CLINICAL MANIFESTATIONS

CAR Coronaviruses

Administration of antigenically distinct CAR CoV to volunteers produced illness with similar characteristics.[16,69,117] A summary of these characteristics is given in Table 155.1, in which a comparison is made with colds produced by rhinoviruses in similarly inoculated volunteers. The incubation period of CoV colds was longer and their duration somewhat shorter, but the symptoms were similar. Asymptomatic infection was sometimes seen and, indeed, has been a feature of both serologic surveys and PCR-based studies of natural infection of infants, children, and adults.[118,119]

More serious respiratory tract illness is probably also caused by all four strains of CAR HCoV. The evidence for this is not conclusive, but it seems likely that all strains can produce pneumonia and bronchiolitis in infants,[35,39,120,121] otitis and exacerbations of asthma in children and young adults,[122–124] pneumonia in healthy adults,[125] exacerbations of asthma and chronic bronchitis in adults,[126–128] influenza-like illness, serious bronchitis and pneumonia in the elderly,[63,67,129,130] and pneumonia in the immunocompromised host.[131,132] HCoVs are found in asymptomatic individuals of all ages, and, when accompanied by illness, are also sometimes accompanied by infections with other potential respiratory pathogens. Infection without disease and coinfection during disease are features of many respiratory pathogens, including rhinoviruses, adenoviruses, human metapneumovirus, human bocavirus, and parainfluenza viruses, but also (although less frequently) respiratory syncytial virus and influenza virus, making pathogenicity difficult to prove. Because infections with CAR HCoVs are so common, however, it is possible that they are responsible for a significant portion of these serious lower respiratory tract diseases, even though the basic pathogenicity of HCoVs (judging from volunteer studies) is similar to that of rhinoviruses, and clearly less than that of respiratory syncytial virus, influenza viruses, and certain adenovirus types. There is some evidence that HCoV-OC43 is more pathogenic in the elderly than HCoV-229E[133] and that NL63 differs from the other CAR HCoVs in preferentially causing childhood croup.[36]

MERS Coronavirus

MERS-CoV predominantly, if not solely, initiates infection via the respiratory tract. The median incubation period is 7 days, with a

TABLE 155.1 Clinical Features of Colds Produced by Experimental Infection With Four Viruses

	CORONAVIRUSES		RHINOVIRUSES	
FEATURE	**229E**	**B814**	**Type 2 (HGP or PK)**	**DC**
No. of volunteers inoculated	26	75	213	251
No. (%) getting colds	13 (50)	34 (45)	78 (37)	77 (31)
Incubation period (days)				
Mean	3.3	3.2	2.1	2.1
Range	2–4	2–5	1–5	1–4
Duration (days)				
Mean	7	6	9	10
Range	3–18	2–17	3–19	2–26
Maximum no. of handkerchiefs used daily				
Mean	23	21	14	18
Range	8–105	8–120	3–38	33–60
Malaise (%)	46	47	28	25
Headache (%)	85	53	56	56
Chill (%)	31	18	28	15
Pyrexia (%)	23	21	14	18
Mucopurulent nasal discharge (%)	0	62	83	80
Sore throat (%)	54	79	87	73
Cough (%)	31	44	68	56
No. (%) of volunteers with colds of indicated severity				
Mild	10 (77)	24 (71)	63 (80)	36 (47)
Moderate	2 (15)	7 (20)	12 (15)	28 (36)
Severe	1 (8)	3 (9)	4 (5)	13 (17)

From Bradburne AF, Bynoe ML, Tyrrell DAJ. Effects of a "new" human respiratory virus in volunteers. Br Med J. *1967;3:767–769.*

range of 2 to 17 days.[9,79] MERS-CoV causes a spectrum of illness ranging from subclinical disease to lethal pneumonia.[134–136] Although infections with severe respiratory involvement have occurred at all ages, the elderly and those with underlying conditions (diabetes, renal disease, immunosuppression) are the most often severely or fatally affected.[134,135,137] MERS requiring admission to the intensive care unit cannot be distinguished by clinical, radiologic, or standard laboratory criteria from other causes of severe pneumonia, so laboratory-based diagnosis is critical.[138,139] Outcomes are worse in critically ill MERS compared to non-MERS patients, with mortality rates of approximately 58% to 78% in patients admitted to the intensive care unit.[134,135,138,139] Health care workers typically develop mild disease, although MERS-CoV has also caused lethal disease in this group.[140] Patients usually present with nonspecific symptoms, including fever, shortness of breath, cough, fever, and diarrhea. Radiographic examination of MERS patients revealed a ground-glass appearance, initially located in the lung periphery. The presence of a pleural effusion was associated with a worse outcome.[141–143]

Acute renal injury occurs commonly in patients in the Arabian peninsula with severe disease, but less so in Korean patients, possibly reflecting differences in the extent of comorbidities such as diabetes and hypertension.[37,38,135,136,138] The MERS-CoV host cell receptor, dipeptidyl peptidase 4, is expressed at high levels in the kidney,[144] raising the possibility that direct infection of this organ contributes to renal disease. Particles resembling coronaviruses were seen by electron microscopy in the renal proximal tubular cells of a fatal case of MERS in a man who had recently received chemotherapy for T-cell lymphoma.[108] MERS patients often presented with hematologic abnormalities, including leukopenia, lymphopenia, and thrombocytopenia and elevated lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase, although this was less common than observed in patients with SARS.[37,138,145] As in patients with SARS, expression of proinflammatory mediators such as interferon-α, interleukin-6, and chemokine (C-X-C motif) ligand 1 is prolonged in severe compared to mild cases. Virus-specific antibody responses developed more rapidly and robustly in MERS patients who survived the infection than in those who died.[146]

Pediatric patients with MERS generally develop mild or subclinical disease, although fatal disease in patients with underlying disease has been reported. MERS in pregnancy has been associated with fetal and/or maternal demise.[147–150]

SARS Coronavirus

SARS is generally initiated through the respiratory tract. After an incubation period that is usually 4 to 7 days, but could be as long as 10 to 14 days, the disease begins, usually with fever and other systemic (influenza-like) symptoms, with cough and dyspnea developing a few days to a week later.[22,151] Approximately 25% of patients have diarrhea. Interestingly, upper respiratory symptoms such as rhinorrhea and sore throat usually do not occur.[112,151–154] The chest radiograph is frequently abnormal, showing scattered airspace opacification, usually in the periphery and lower zones of the lung.[155] Spiral computed tomography demonstrates both ground-glass opacification and consolidation, often in a subpleural distribution.[155–158]

Lymphopenia is common,[112,113,159] with normal or somewhat depressed neutrophils. Paradoxically, neutrophilia was associated with poor outcomes.[109] Creatine kinase is often abnormal, as are lactate dehydrogenase and aspartate aminotransferase. Levels of proinflammatory cytokines were elevated at early times during infection in patients with severe clinical disease[160] and decreased in those patients who resolved the infection.[161]

Approximately 25% of patients develop severe pulmonary disease that progresses to adult respiratory distress syndrome, most commonly in patients older than 50 years or with underlying disease such as diabetes, cardiac disease, and chronic hepatitis.[112,154,159,162] The overall mortality rate is between 9% and 12%, with the highest rates in the elderly and adults with underlying liver disease. In some patients, clinical deterioration occurred during the second week of illness, as virus levels decreased, suggesting that disease was partly immune mediated.[112,154,159,162] Clinical improvement was associated with the onset of a virus-specific antibody response.[161]

Pediatric disease is significantly less severe than adult disease, although the features are similar.[163] Disease during pregnancy is severe, with high mortality in both the mother and fetus.[164] Congenital transmission has not been described.

Gastrointestinal Coronaviruses

The nature of the illness associated with enteric CoV infection is much less clear. One study found a significant association of gastroenteritis in infants 2 to 12 months of age with the presence of CoVLPs in the stool.[165] Another study, confined to infants in a neonatal intensive care unit, found highly significant associations between the presence of CoVLPs in the stool and the presence of water-loss stools, bloody stools, abdominal distention, and bilious gastric aspirates.[103] Finally, CoVLPs have been associated with at least three outbreaks of necrotizing enterocolitis in newborns.[60,103,166] Efforts to detect HCoV RNA by PCR using primers that would detect the known CAR HCoVs in stool have been disappointing, with most HCoV-positive samples also containing rotavirus or norovirus.[39,40,167,168] Of note, all of the studies that associate CoVLPs with gastrointestinal disease were done before the development of diagnosis by PCR.

Neurologic Syndromes

Like many other viruses, CoVs have been sought as possible etiologic agents in multiple sclerosis. The search has been stimulated by the capacity of JHM, a well-studied strain of mouse hepatitis virus, to produce in mice and rats an immune-mediated chronic demyelinating encephalitis histologically similar to multiple sclerosis.[169] HCoV-OC43[170,171] and HCoV-229E[172] have been detected in brain tissue from multiple sclerosis patients using virus isolation,[170] in situ hybridization, immunohistology,[171] and PCR.[172] The strongest support for CoV-mediated infection of the brain comes from a study in which HCoV-OC43 was identified in neurons in an 11-month-old patient with severe combined immunodeficiency and lethal encephalitis.[173] Except for this one report, evidence is lacking to establish an etiologic or pathogenic association of CoVs with human central nervous system disease.

LABORATORY DIAGNOSIS

CAR Coronaviruses

Although some human CAR CoVs grow in tissue culture directly from clinical samples and although antigen detection systems have been developed for both HCoV-OC43 and HCoV-229E,[174,175] laboratory diagnosis of CoV respiratory infections is best accomplished by molecular methods. Reverse-transcriptase PCR (RT-PCR) systems have been developed using many different primers and detectors.[176–182] From a clinical point of view, a single generic test for respiratory CoVs would be desirable, and such tests have been developed. However, when tested side by side with specific systems, the generic systems have somewhat lower sensitivity.[120]

MERS Coronavirus

MERS-CoV was originally isolated in Vero and LLC-MK2 cells, and standardized methods using RT-PCR–based examination of respiratory and other clinical samples have been published.[183,184] These methods can detect as little as 10 to 15 copies of viral RNA. Peak virus titers were detected at approximately 14 days after infection, and viral RNA could be detected for greater than 21 days in patients with severe disease.[185] MERS-CoV infection can be diagnosed beginning 2 to 3 weeks after the onset of illness using serologic methods, enzyme-linked immunosorbent assay (ELISA) initially, with confirmation by indirect immunofluorescence assays (IFA) and neutralization assays.[186] The transient nature of the antibody response in MERS patients with subclinical or mild disease has diminished the diagnostic utility of this approach.[78,82]

SARS Coronavirus

Although SARS-CoV was grown from respiratory tract specimens in Vero E6 and fetal rhesus monkey kidney cells, the more sensitive and rapid RT-PCR assays were most widely used to detect infection. Virus was detected by RT-PCR in upper and lower respiratory tract, blood, stool, and urine specimens. Early in the illness, specimens were found positive only in approximately one-third of patients.[112] Use of samples from multiple sources increased the yield. Virus was detected most frequently during the second week of illness.[112,187]

Antibody tests have been developed using tissue culture–grown virus and ELISA and IFA. Immunoglobulin M antibody can be detected in most patients for a limited period of time, and immunoglobulin G antibody appears first approximately 10 days after onset of fever in patients with good outcomes and becomes essentially universal after 4 weeks.[112,161]

THERAPY

Given the severity of SARS, clinicians throughout the world empirically treated most patients with corticosteroids and intravenous or oral ribavirin.[188] It is now known that ribavirin has little activity against SARS-CoV in vitro, and corticosteroid usage may have resulted in worse outcomes.[189,190] Lopinavir-ritonavir and intravenous immune globulin were also used in some patients, without conclusive evidence that they were helpful or harmful. There is anecdotal evidence of the benefit of either interferon-α or interferon-β treatment. However, no therapy has proven efficacy, and therapy continues to be largely supportive.[190]

Similarly, treatment of MERS-CoV infection at present depends entirely on supportive measures. No antiviral drugs are recommended,[191,192] although several studies have indicated that MERS-CoV is more sensitive to interferon-α or interferon-β than is SARS-CoV.[193,194] Combinations of interferon alfa-2b and ribavirin inhibit MERS-CoV in vitro, and administration of the combination improved outcomes in MERS patients at 14 but not 28 days.[195] A new broad-spectrum drug, GS-5734, has demonstrated efficacy against SARS-CoV and MERS-CoV in cells and experiment animals and may be a useful therapeutic option.[196] Standard droplet precautions should be used, with aerosol precautions during certain high-risk procedures.[197]

PREVENTION

Rigorous application of hospital infection control procedures, particularly those directed at contact and droplet spread, was shown to have a major beneficial effect on the spread of the SARS-CoV.[92] The containment of the global SARS outbreak is a testament to the power of the cooperation and collaboration engendered by WHO to address a major public health threat. Similarly, standard droplet precautions are recommended for patients with suspected or confirmed MERS-CoV infections, with aerosol precautions during certain high-risk procedures.[196,198,199] Suitable barrier precautions are especially critical in the case of MERS patients since a large fraction of infections result from nosocomial transmission.[11,74]

Vaccines for animal CoVs have been developed and widely used with variable efficacy. Vaccines are likely to be key in disrupting MERS-CoV camel-to-human and interhuman transmission. A variety of vaccination strategies, including inactivated, subunit, live-attenuated, DNA, and nanoparticle vaccines, are being pursued.[200,201] Vaccines will need to be carefully evaluated because an inactivated SARS-CoV vaccine and a feline infectious peritonitis surface glycoprotein vaccine caused immunopathologic disease after challenge.[202–204] Monoclonal antibodies that neutralize MERS-CoV have been identified and proposed for use therapeutically.[205,206] Given the small number of human cases and the high prevalence of virus in camels, vaccination of juvenile camels may be the preferred approach to protecting human populations.[207] Such vaccines are under development.

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The complete reference list is available online at Expert Consult.

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156 Parainfluenza Viruses

Michael G. Ison

SHORT VIEW SUMMARY

Definition
- Parainfluenza virus (PIV) causes acute respiratory illness, including colds, croup, bronchiolitis, and pneumonia.

Epidemiology
- PIV-1 and PIV-2 cause seasonal outbreaks in the fall, with PIV-1 causing epidemics in odd-numbered years. Clinically, PIV-1 is strongly associated with croup in children.
- PIV-3 causes annual epidemics in the spring; in years in which PIV-1 does not circulate, the season is typically more active and prolonged. PIV-3 is associated with more severe infections, particularly among immunocompromised adults and children.
- PIV-4 is associated with milder disease, typically limited to the upper airway.
- Although antibodies are produced in response to clinical disease, reinfection is common throughout life.
- Disease, particularly associated with PIV-3, frequently progresses to the lower airway in immunocompromised adults and children and is associated with significant morbidity and mortality.

Microbiology
- PIV is a single-stranded, enveloped RNA virus belonging to the Paramyxoviridae family. It includes PIV-1, PIV-2, PIV-3, and PIV-4.

Diagnosis
- National PIV trends are available at https://www.cdc.gov/surveillance/nrevss/human-paraflu/index.html.
- Laboratory diagnosis can be made through culture or polymerase chain reaction (PCR) assay, with improved sensitivity with molecular diagnostic methods.

Therapy
- Croup is generally treated with glucocorticoids and nebulized epinephrine in young children.
- Although ribavirin and intravenous immunoglobulin have been used in the treatment of immunocompromised adults and children, their efficacy is uncertain.
- A number of novel antivirals are currently under development.

Prevention
- Standard and contact precautions are recommended to avoid nosocomial spread of PIV in the health care setting.
- A number of live-attenuated vaccine candidates are undergoing development.

Parainfluenza viruses (PIVs) are paramyxoviruses that are important causes of respiratory viral infections in adults and children. Although parainfluenza is a leading cause of croup in children, broader use of molecular diagnostics has refined our understanding of the impact of PIV on adults and children.[1] Infection is typically mild and self-limited, but life-threatening lower respiratory tract infections do occur, particularly in immunocompromised or elderly patients.[1–3]

VIROLOGY

PIVs are single-stranded, enveloped RNA viruses belonging to the Paramyxoviridae family (Fig. 156.1).[4,5] There are four major serotypes of human PIV (PIV-1, PIV-2, PIV-3, PIV-4), which are defined by complement fixation and hemagglutinating antigens.[1,6,7] PIV-1 and PIV-3 are members of the genus *Respirovirus,* whereas PIV-2 and PIV-4 are members of the genus *Rubulavirus.* Although the hemagglutinin-neuraminidase (HN) glycoproteins of PIV are more antigenically stable than those of influenza A viruses, antigenic differences have been reported over time.[8]

The virions are pleomorphic, range in diameter from 150 to 200 nm, and have a lipid bilayer envelope derived from the host cell.[7] The single strand of negative-sense RNA encodes at least six viral proteins: the nucleocapsid protein (NP), the phosphoprotein (P), the matrix protein (M), the fusion glycoprotein (F), the HN glycoprotein, and the RNA polymerase (L).[9] The HN and F proteins sit in the lipid envelope and form the major antigenic targets for neutralizing antibody.[10] The nucleocapsid core is composed of NP, P, and L proteins in association with viral RNA. NP proteins bind tightly to the viral genome, creating a template for the RNA-dependent RNA polymerase composed of the P and L proteins.[11] The HN glycoproteins are critical to attachment of the virus to the host cell wall at sialic acid residues on the cell surface.[12] Once attached to the cell, the F protein mediates virus–cell membrane fusion and subsequent infection of the host cell. The neuraminidase portion of the HN protein cleaves the progeny virions free from the surface of an infected cell.[12,13]

In addition to these essential proteins, each PIV expresses nonessential proteins: PIV-1 and PIV-3 RNA encode short C proteins, PIV-2 RNA encodes a V protein, and PIV-3 also expresses a D protein. Although the function of the D protein is unknown, the C and V proteins suppress the activity of type 1 interferons and likely play a role in the pathogenesis of disease.[14,15]

Viral replication takes place in the cytoplasm, where the NP protein assembles with the genomic RNA to form a helical structure. This NP-RNA complex is then joined by the P and L protein complexes to form the nucleocapsid. The envelope proteins are assembled preferentially on the apical surface of the cell. Finally, the matrix plays a role in the final assembly of the virus and subsequent release from the cell by budding. The neuraminidase activity of the HN is critical in facilitating release from the cell and preventing virus clumping.

PATHOGENESIS

PIVs preferentially infect ciliated epithelial cells that line the upper and lower respiratory tracts.[16] After exposure, replication begins in the epithelial cells of the nose and oropharynx with subsequent spread to the large and small airways.[17] Although replication can be first recognized about 24 hours after infection, peak replication occurs 2 to 5 days after infection.[18] Shedding typically begins to decrease after 7 days.[19] The extent of infection correlates well with disease; mild upper respiratory infections are associated with limited infection of the nasopharynx,

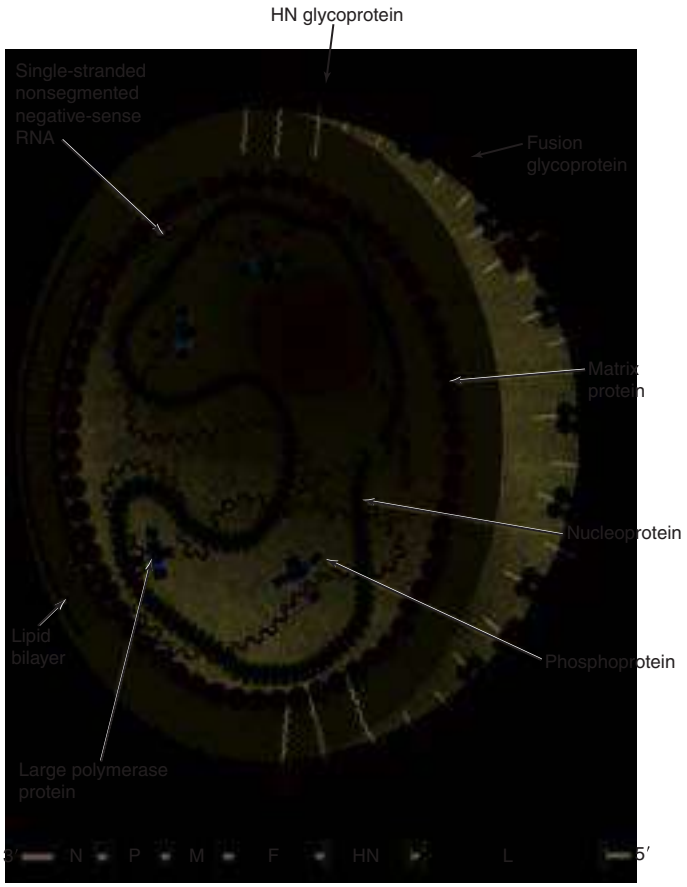

FIG. 156.1 Human parainfluenza virus virion and genome organization. *F,* Fusion; *HN,* hemagglutinin-neuraminidase; *L,* large; *M,* matrix; *N,* nucleocapsid; *P,* phosphoprotein. *(Modified from Schmidt AC, Schaap-Nutt A, Bartlett EJ, et al. Progress in the development of human parainfluenza virus vaccines.* Expert Rev Respir Med. *2011;5:515–526.)*

whereas more severe disease is generally associated with replication in the large and small airways.[20,21] Peak symptoms correlate with peak viral replication. Infection of the larynx and upper trachea is associated with croup, whereas bronchiolitis and pneumonia are associated with infection of the distal airways.[18]

Animal models of infection suggest that there is limited damage to infected cells because of direct viral effects.[19] Instead, the host immune response appears to play an important role in the pathogenesis of PIV infection.[18] Specifically, the key host immune responses that contribute to viral clearance, including the innate immune responses, $CD8^+$ and $CD4^+$ T-cell responses, interferon production, and local and systemic immunoglobulin A (IgA), IgE, and IgG responses, are also the key drivers of the clinical signs and symptoms of infection.[18] Stromal interleukin-11 production, enhanced acetylcholine release, and increased release of leukotrienes induced by PIV-3 infection, for example, appear to be associated with increased airway responsiveness during infection.[22–24]

There are two major components of the immune response to PIV: humoral and cellular immune responses. Both serum neutralizing antibodies and T-cell recognition are directed at the HN and F surface proteins of PIV.[7] Although there is a slight degree of antigenic variability in these proteins, reinfection appears to generally result from waning immunity instead of significant drift of antigenic epitopes.[25] Mucosal IgA immunity appears to be more important than systemic IgG-derived immunity in the prevention of infection.[7] Once PIV replication is ongoing, cytotoxic T cells play a critical role in the control and clearance of PIV infection. Lack of this cellular immune response is associated with progressive disease, as is demonstrated by the increased risk of progressive and fatal disease in hematopoietic stem cell transplant (HSCT) recipients.[26] Recently, the cellular immune response to PIV has been more carefully characterized.[27] Both $CD4^+$ and $CD8^+$ T cells were found to be capable of producing Th1-polarized effector cytokines and killing PIV3-expressing targets. The potential impact of this response was suggested by correlation between the presence of PIV3-specific T cells and viral control in allogeneic HSCT recipients.[27]

EPIDEMIOLOGY

PIVs are transmitted by direct person-to-person contact through large respiratory droplets and contact with fomites contaminated with respiratory secretions.[28] After initial exposure to the virus, clinical symptoms typically develop after a 2- to 6-day incubation period. Initial infection with parainfluenza typically occurs early in childhood, most commonly in children younger than 5 years. Serologic studies have demonstrated that PIV-3 affects up to 50% of children within the first year of life, with PIV-1 and PIV-2 causing initial infections later, generally between ages 3 and 5 years.[29,30] By adulthood, more than 90% of humans have antibodies to PIV.

The burden of PIV infection has been recognized to be more significant in children historically. In fact, PIV is responsible for 20% to 40% of lower respiratory tract infections in children. Parainfluenza is the second most common viral cause of hospitalization in children.[31] Hospitalization is required in 2.8 per 1000 children according to one report and is associated with 7600 to 48,000 pediatric hospitalizations annually in the United States.[32] Rates of hospitalization are higher in children younger than 1 year (1.9–12 per 1000 children) than in children aged 1 to 4 years (0.5–2 per 1000 children).[33] PIV-3 causes most infections necessitating hospitalization in children.[30,33] A recent study demonstrated that PIV was a common cause of hospitalization in children with pneumonia, with a rate similar to that of influenza.[34] With the advent of more contemporary assays, the impact of PIV in adults has also been appreciated.[2] In one recent study, PIV-3 was the third most commonly isolated viral isolate in individuals 16 to 64 years old who required hospitalization (0.7 per 1000 population).[35] In adults presenting with an influenza-like illness during the 2009 influenza pandemic, PIV-3 was the second most common cause of illness after influenza in adults.[36] Among adults hospitalized with pneumonia, PIV was a commonly identified pathogen, with about half the rate of influenza and a similar rate to respiratory syncytial virus (RSV).[37] In both adults and children, reinfection is common despite the presence of antibodies formed during prior infection.[38] Reinfection is typically associated with milder disease than initial infection and is typically limited to the upper airway.[38]

PIV-3 is the most prevalent serotype and is associated with pneumonia and bronchiolitis. PIV-1 and to a lesser extent PIV-2 are associated with croup in children. PIV-1 and PIV-2 are seen less frequently in adults and can be associated mostly with upper respiratory tract infection, although lower respiratory tract disease has also been described.[2,7,26,39] In adults hospitalized with pneumonia, PIV-3 was found in 3.1% of patients, PIV-1 in 2.5%, and PIV-2 in 0.2%.[40] PIV-4 causes only mild upper respiratory tract infection in both adults and children.

Although PIV-1, PIV-2, PIV-3, and PIV-4 cause disease in all parts of the world, the seasonal patterns of PIV infections depend on the specific locations. In tropical and subtropical regions, PIVs do not show seasonal variations.[41] In the United States, PIV-1 and PIV-2 cause seasonal outbreaks in the fall (September through December); of interest, PIV-1 epidemics occur every other year, typically in odd-numbered years (Fig. 156.2).[42] PIV-3 causes epidemics during the spring (April through June). In years when there is no PIV-1 circulating, PIV-3 activity is generally higher, with either a longer spring season or a small second period of increased activity in the fall.[42] There are fewer data available on PIV-4, in part because of the generally mild degree of illness caused by the virus. It appears that PIV-4 is more common in the autumn and winter months.[43]

In addition to age and geography, other factors may affect the presentation and severity of illness caused by PIV. Sex and ethnicity may play a role in the severity of PIV infection because PIV-associated bronchiolitis occurs most commonly in nonwhite males.[21] Breastfed infants have a reduced risk of severe infection with PIV. Pneumococcal vaccination is associated with a reduced risk of pneumonia in infants with PIV.[44] Last, patients with immune compromise may shed PIV for prolonged

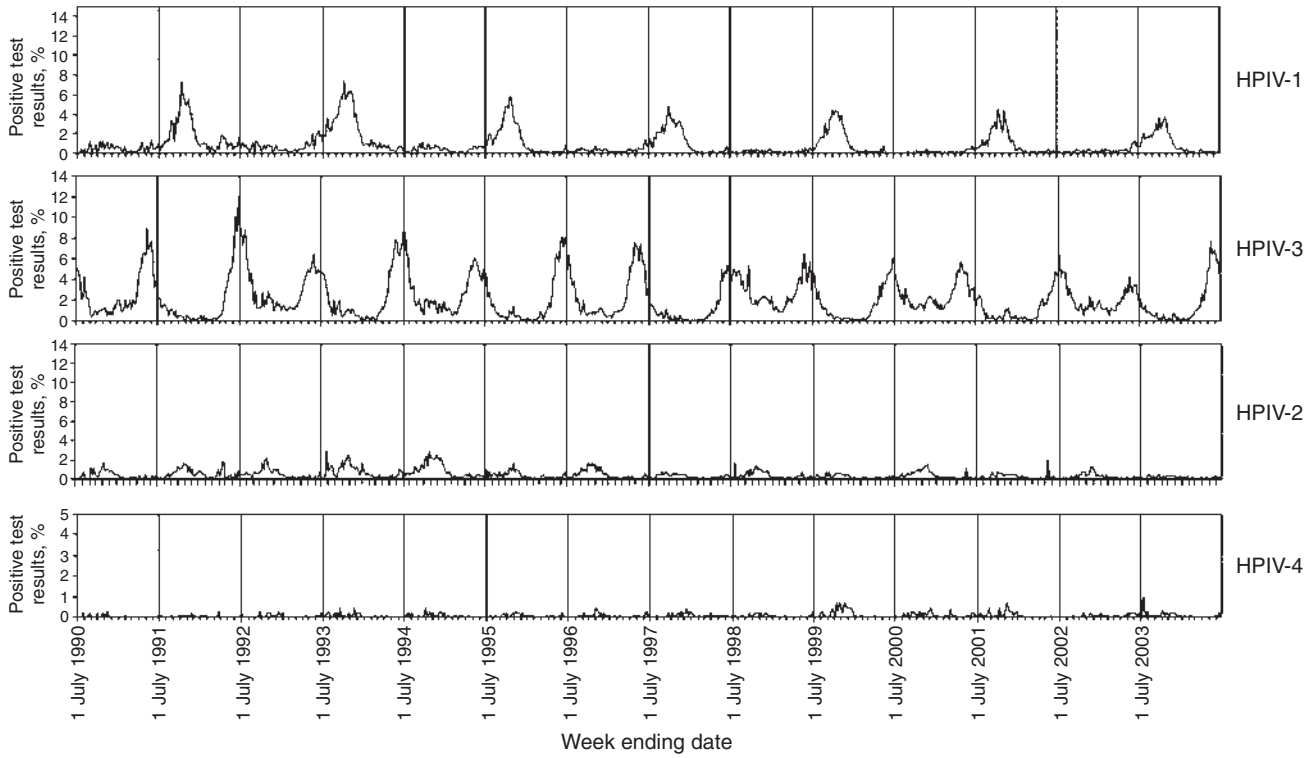

FIG. 156.2 Seasonal distribution of human parainfluenza virus *(HPIV)* serotypes 1, 3, 2, and 4 from July 1990 to June 2004. The percentage of tests positive for HPIV serotypes 1, 3, 2, and 4 reported to the National Respiratory and Enteric Virus Surveillance System in the United States, by week, July 1990 to June 2004. Note that HPIV-4 has different axis scales than the other HPIV serotypes. *(Modified from Fry AM, Curns AT, Harbour K, et al. Seasonal trends of human parainfluenza viral infections: United States, 1990–2004.* Clin Infect Dis. *2006;43:1016–1022.)*

periods of time, and infections may be associated with increased risk of progression to the lower airway and fatal outcomes. Parainfluenza is one of the more common causes of respiratory viral infections in patients with hematologic malignancies, HSCT, or solid-organ transplantation and is associated with a high rate of progressive disease involving the lower airway and an increased mortality rate.[26,45]

CLINICAL MANIFESTATIONS

PIVs cause a variety of upper and lower respiratory tract illnesses, ranging from mild coldlike syndromes to life-threatening pneumonias. Although PIV infection may require hospitalization, most infections are mild and self-limited and can be managed on an outpatient basis.

Pediatric Disease

Most infections in children are limited to the upper respiratory tract, with only about 15% involving the lower respiratory tract.[46] Upper respiratory tract disease is associated with otitis media and sinusitis in 30% to 50% of children and less frequently in adults; this complication can result from primary viral infection or secondary bacterial superinfection.[46–48] Although no one particular virus is associated with a classic clinical presentation, specific PIV serotypes are more strongly associated with certain clinical presentations. PIV-1 and PIV-2 are associated with croup, or laryngotracheobronchitis, in children.[1,49] PIV-induced croup generally manifests with fever, rhinorrhea, and pharyngitis, which is typically followed by a barking cough associated with stridor, dyspnea, and chest wall retractions.[1,49] Hospitalization for respiratory distress and hypoxemia occurs, particularly if there is significant involvement in the lower airway.[30] Disease caused by PIV-2 is generally less severe than that caused by PIV-1. Alternatively, PIV-3 is associated with more distal lung involvement, particularly in the first 6 months of life.[1,50] PIV-3 pneumonia and bronchiolitis in children are clinically difficult to differentiate from RSV infection. PIV-4 is generally associated with mild upper respiratory infections or asymptomatic infection. Rarely, particularly in children with underlying cardiopulmonary disease or immune compromise, more severe infections can occur because of PIV-4.[1,51]

Although PIV infection is generally limited to the respiratory tract, nonrespiratory complications, including meningitis, myocarditis, pericarditis, and Guillain-Barré syndrome, have been described in adults and children.[52–55]

Adult Disease

PIV infections in immunocompetent adults are usually asymptomatic or mild, self-limited upper respiratory tract infections.[2,56] PIV is responsible for 1% to 15% of acute respiratory illnesses in adults, with higher rates and more significant morbidity in older adults.[39] Up to 11% of elderly patients developed PIV pneumonia in one study, whereas up to 14% of nursing home residents developed respiratory illness attributable to PIV.[57,58] Fatal cases and outbreaks in long-term care facilities have been described.[39] With the wider use of more sensitive molecular assays, PIV has been demonstrated to be a significant cause of community-acquired pneumonia.[2,37,59–61] PIV is also increasingly associated with exacerbations of asthma and chronic bronchitis.[2,24,62–64]

In general, PIV-3 is associated with a higher risk of lower tract disease in adults, and PIV-4 is usually associated with milder upper respiratory tract disease. PIV-1 and PIV-2 have a broader spectrum of disease in adults, with a risk of lower respiratory tract disease between that of PIV-3 and PIV-4. Most adults present with fever, rhinorrhea, cough, and a sore throat that does not clinically distinguish it from other respiratory viruses.[2,39]

Parainfluenza Virus in Immunocompromised Patients

PIV causes significant direct and indirect morbidity and mortality among immunocompromised adults and children.[65,66] Most studies conducted to date have focused on patients admitted to the hospital, and as a result, data may underestimate the incidence and overestimate the severity of disease caused by PIV. Among HSCT recipients, the incidence of PIV is 4% to 7%, of which 13% to 43% have lower respiratory tract involvement.[45] Mortality ranges from 12% to 50%, with enhanced mortality in patients with lower respiratory tract disease and earlier

onset after transplant.[3,45,67,68,69–72] Although nosocomial outbreaks have been described, most cases appear to be community acquired, with PIV-3 being the most frequent type to cause disease.[69,73–75] PIV is associated with asymptomatic shedding among HSCT recipients and may contribute to the risk of nosocomial spread when only symptomatic patients are tested and isolated.[3]

At presentation, most HSCT recipients present with upper respiratory tract–only involvement (57%–87%), and 13% of patients with upper respiratory tract disease will progress to lower respiratory tract disease, usually within 1 week.[26,67,76,77] Steroids, in a dose-dependent manner, are associated with increased risk of progression from upper to lower respiratory tract disease and mortality.[69,77,78] Other risk factors for progressive disease include early onset after transplant, allogeneic (matched unrelated and matched related) donor, presence of lymphocytopenia, active graft-versus-host disease, and pediatric age group.[26,45,76,78] Reduced-intensity conditioning appears to be a risk factor for late-onset (≥30 days) PIV infection.[76] Mortality, even with treatment, in patients with lower airway disease remains high.[76,77] Copathogens are frequently found in patients with PIV and may contribute to the significant morbidity and mortality of infection.[79] In addition to the morbidity and mortality directly attributed to PIV infection, PIV infection is associated with 17.9-times-greater odds of developing severe airflow declines after infection.[80] This progressive decline in pulmonary function is associated with functional impairment and increased morbidity and mortality.

Parainfluenza has also been demonstrated to cause severe disease in patients undergoing chemotherapy.[81,82] Risk appears highest in children and patients being treated for hematologic malignancies.[82,83] In general, infection is acquired in the community and more frequently affects children younger than 2 years and those with acute lymphoblastic leukemia.[82] Lymphocytopenia appears to be a risk factor for lower airway disease; despite causing significant morbidity, it is infrequently fatal.[82] Children with severe combined immunodeficiency undergoing HSCT have developed a rapidly fatal giant cell pneumonia caused by PIV.[84]

A wide range of imaging findings, including interstitial infiltrates, ground-glass opacities, and/or airspace consolidations, have been demonstrated in HSCT patients with PIV pneumonia.[26] Rarely, small peribronchial nodules have been described, suggesting that PIV must be included in the differential diagnosis of nodular pneumonia in immunosuppressed patients.[85] Because these findings are not specific for PIV, appropriate studies should be done to rule out alternative pathogens, including bacterial and fungal pathogens that often complicate PIV infections in these patients.

Data on the impact of PIV on solid-organ transplant recipients are more limited, with most data coming from lung transplant recipients.[26,86,87] The incidence of PIV in lung transplant recipients is about 5%.[88,89] PIV infections among lung transplant recipients have been associated with significant short- and long-term pulmonary dysfunction. Animal models of a related virus (Sendai) have suggested that there is a complex interplay between direct viral cytopathic effects and local induction of inflammation that contributes to allograft rejection.[90,91] Although PIV may cause acute rejection, the pathologic picture could also be associated with immunologic response to viral infection, which makes interpretation of biopsy findings challenging.[88,92] There are more convincing data from lung transplant recipients that PIV, particularly with lower respiratory tract infection, is associated with development or progression of bronchiolitis obliterans or bronchiolitis obliterans syndrome.[87,88,89,92,93]

DIAGNOSIS

Local surveillance for PIV is not routinely available in most parts of the United States. National trends are monitored and available as part of the National Respiratory and Enteric Virus Surveillance System (https://www.cdc.gov/surveillance/nrevss/human-paraflu/index.html).

PIV can be diagnosed through culture, antigen detection, or nucleic acid testing. Swabs, aspirates, and washes from the nasopharynx or oropharyngeal secretions are appropriate specimens for making a diagnosis, with paired oropharyngeal and nasopharyngeal samples associated with the highest sensitivity. For patients with pneumonia or in whom lower airway involvement is suspected, bronchoalveolar lavage fluid may be used for diagnosis of PIV and may increase diagnostic yield. PIV is stable in viral transport medium at 4°C for up to 5 days. Freezing to −20°C does decrease infectivity of the virus, but long-term storage can be achieved easily by adding sucrose or glycerol to the holding media and freezing to below −70°C.[6] Historically, cell culture was considered the gold standard for the diagnosis of PIV. PIV is culturable on LLC-MK2 rhesus monkey kidney, Vero African green monkey kidney, and NCI-H292 human lung carcinoma cell lines with use of standard and shell vial techniques[7]; fixed-mixed cell lines, such as R-Mix (Diagnostic Hybrids, Athens, OH), have sensitivity that approaches that of standard cell culture lines for the detection of PIV and are more broadly used in many clinical laboratories currently.[6,94–96] Trypsin is required for the growth of PIV-1 and PIV-4 but not PIV-2 and PIV-3.[7] Hemadsorption-inhibition, hemagglutination inhibition, or immunofluorescence is used to identify the PIV in cultures.[6]

With broader availability and improved sensitivity, polymerase chain reaction (PCR)–based tests are now considered the gold standard for the diagnosis of PIV. Typically, these are directed toward the HN gene, although exact primers have differed among assays. Several studies have demonstrated the improved sensitivity of PCR-based assays over cultures for the diagnosis of PIV, particularly in immunocompromised patients.[97,98–100] Yield of detection may be increased by 1.5-fold or higher, compared with culture, for PCR systems in the diagnosis of PIV.[99] Because no one clinical syndrome is associated with a unique virus, assays that simultaneously detect multiple viruses are considered best for diagnosis. Despite the advantages of highly multiplexed PCR-based systems in detecting a wide range of viruses, the diagnostic yield for PIV is not consistent for all available systems.[101–103]

There are currently no approved rapid antigen kits available for the detection of PIV. There are several monoclonal antibody systems that allow identification and differentiation of the different PIV serotypes in primary patient samples and from cell cultures.[6,104]

THERAPY

There are currently no antivirals with proven efficacy that are approved for the treatment of PIV infections. For children with croup, glucocorticoids and nebulized epinephrine have been associated with improved clinical outcomes.[105,106] Glucocorticoids, generally dexamethasone and budesonide, are associated with improved Westley scores at 6 and 12 hours after administration, in addition to fewer clinic visits and admissions for croup.[106] Furthermore, glucocorticoids are associated with shorter emergency room and hospital visits and reduced use of nebulized epinephrine.[106] Use of nebulized epinephrine is associated with improvement in croup scores at 30 minutes but not at 2 and 6 hours after treatment, and also with shorter hospital stays compared with placebo.[105] There is generally no difference in early responses when racemic epinephrine is compared with L-epinephrine nebulization.[105]

Of the available data regarding the use of antiviral agents for treatment of PIV, many come from immunocompromised patients. The mainstay of therapy in these patients is reduction of immune suppression, particularly steroids, if possible.[65,66] Most publications have been case reports or small case series of HSCT and solid-organ transplant patients who have received aerosolized, oral, or intravenous ribavirin for the treatment of PIV.[a] In the largest study published, 31 of 55 PIV-infected HSCT recipients received aerosolized ribavirin with or without intravenous immunoglobulin. In this study, treatment was not associated with reduction in viral shedding or mortality.[69]

A number of antiviral agents with activity against PIV are currently being tested. (Also see Chapter 45.) Two HN inhibitors (BCX 2798 and BCX 2855) have been developed and have potent in vitro and in vivo activity against PIV; there are no human data yet on these novel inhibitors.[115,116] DAS181 is a novel antiviral agent that consists of a sialidase from *Actinomyces viscosus* attached to a respiratory epithelium anchoring domain.[117] The drug cleaves the terminal sialic acid residues from the surface of human respiratory epithelial cells, thereby reducing binding and therefore infectivity of PIVs.[117] DAS181 has been used in lung and stem cell transplant patients, with documented PIV-3 infection derived from clinical and radiologic evidence of lower airway involvement.[2,118–125] Although not all patients had improvement in symptoms, oxygenation, pulmonary function, and nasopharyngeal viral loads, those without

[a]References 2, 45, 55, 68–71, 76, 88, 107–114.

coinfections tended to have the best response to therapy.[2] Favipiravir, a pyrazine derivative that has antiviral activity against multiple RNA viruses, including paramyxoviruses, has been shown to have in vitro activity against PIV-3 in tissue culture (90% effective concentration [EC_{90}] of 36 μM).[126]

Preclinical data show promise for immunoglobulin and short interfering RNAs (siRNAs) for the treatment of PIV,[127] although there are currently no data in humans to prove efficacy of either of these interventions. Human PIV immunoglobulin and convalescent rat serum were associated with reduced PIV titers when delivered to rats intranasally.[128] Likewise, to date, siRNAs prevented infection in mice in one study.[129]

PREVENTION

Despite the significant clinical impact of PIVs, there is currently no licensed vaccine. Although natural immunity to PIV allows reinfection, subsequent infections are typically milder in nature.[5] Natural infection results in antibody development to all viral proteins, but the HN and F antibodies appear to be neutralizing.[7] Initial studies of candidate vaccines focused on inactivated mixtures of PIV-1, PIV-2, and PIV-3 that, although capable of producing robust antibody responses, were not effective in protecting subjects against challenge.[130] A subsequent vaccine candidate used a modified Ankara vaccinia virus vector that expressed HN or F proteins of PIV-3. This vaccine protected nonhuman primates from lower respiratory tract disease but was ineffective in preventing upper respiratory infection and therefore was not pursued further.[131]

Currently, most efforts at PIV vaccine development have focused on reverse genetic technology applied to live-attenuated intranasally administered vaccines.[5] A live-attenuated bovine PIV-3 was evaluated but noted to have only modest seroconversion rates to human PIV-3.[5,132] A live-attenuated, cold-adapted PIV-3 virus derived from the JS strain (human PIV [HPIV]-3-cp45) has been studied and found to be safe and immunogenic in seropositive and seronegative infants and children. It appears to require two to three doses to result in durable immunity (84% of seronegative vaccine recipients developed a fourfold or greater increase in antibody titers).[5,133,134] A combined HPIV-3-cp45/RSV vaccine was found to result in antibody responses similar to those of the HPIV-alone vaccine.[135] More recently, another chimeric virus based on the HPIV-3-cp45 virus has been studied and found to be well tolerated and immunogenic in children 6 to 36 months of age. Existing data suggest that a three-dose regimen may be required in order to provide protective responses in infants younger than 6 months.[136] Likewise, two different recombinant bovine (rB)–human PIV-3 (rB/HPIV-3) live virus vaccine candidates have been studied. The rB/HPIV-3 was more restricted in replication in seronegative children than was rHPIV3-N(B), but it was demonstrated to induce significantly higher titers of hemagglutination inhibition antibodies against HPIV-3.[137] Another chimeric rB/HPIV-3 that also expresses RSV F or both F and G proteins has been developed and is undergoing clinical trials for prevention of both RSV and PIV.[5,138,139]

Although there are fewer candidates, there are also efforts to create PIV-1 and PIV-2 vaccines. These have focused on viruses produced through reverse genetics and another approach that uses a live-attenuated Sendai virus.[5,140–145] A live murine Sendai virus that shares antigenic similarities with HPIV-1 was administered intranasally to HPIV-1 seropositive children in a recent study, as a potential jennerian vaccine candidate. It was well tolerated, augmented antibody titers to HPIV-1, and is being considered for further testing in HPIV-1–seronegative children.[146]

Infection-control measures are crucial to prevent transmission of PIV in the health care setting. Localized outbreaks of PIV have been demonstrated in pediatric hospitals, wards housing immunocompromised patients, and nursing homes. Hospitalized patients with PIV infection should be placed on standard and contact precautions and should have a private room whenever possible.[147] Respiratory precautions are not necessary, because the droplets are large and do not aerosolize. A single-center study in HSCT patients found that requiring all individuals with direct patient contact to wear a surgical mask was associated with a reduction in respiratory viral infections, particularly PIV-3, during the most vulnerable period after HSCT.[72]

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157 Mumps Virus

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SHORT VIEW SUMMARY

Definition
- Mumps is an acute viral infection that most commonly manifests as nonsuppurative swelling and tenderness of the parotid or other salivary glands caused by the mumps virus.
- Less common manifestations of mumps include meningitis, encephalitis, epididymo-orchitis, oophoritis, and pancreatitis.

Epidemiology
- Mumps is endemic throughout the world, and humans are the only natural hosts for the virus.
- Incubation period is usually 16 to 18 days, with a range of 2 to 4 weeks.
- Before the introduction of the mumps vaccine in the United States in 1967, epidemics occurred every 2 to 5 years, with peak incidence between January and May.
- Since 1967 there has been greater than a 99% decline in the annual US incidence of mumps.
- Outbreaks of mumps have been reported throughout the world, including the United States, even in populations who have received the recommended two-dose measles-mumps-rubella (MMR) series.

Microbiology
- Mumps is an enveloped, single-stranded RNA virus.
- Only one serotype of mumps virus exists, but there are 13 genotypes.

Diagnosis
- The clinical diagnosis is made on the basis of a history of exposure and of parotid swelling and tenderness.
- The diagnosis is confirmed by isolation of mumps virus or detection of mumps nucleic acid by polymerase chain reaction from clinical specimens or the presence of mumps-specific immunoglobulin M (IgM) antibodies or a fourfold rise in mumps IgG antibodies in serum.

Therapy
- Therapy for mumps is symptomatic and supportive.

Prevention
- Immunization with live-attenuated mumps virus vaccine as part of the standard MMR vaccine at 12 months and 4 to 6 years of age is recommended for all children; a two-dose series of MMR is recommended for individuals beyond childhood who have not received the childhood series.
- The Advisory Committee on Immunization Practices recommended that a third dose of MMR be administered to individuals at increased risk of mumps during an outbreak (2018).

Mumps is an acute, generalized viral infection that occurs primarily in school-aged children and adolescents. The most prominent manifestation of this disease is nonsuppurative swelling and tenderness of the salivary glands, with one or both parotid glands involved in most cases. The disease is benign and self-limited, with one-third of affected persons having subclinical infection. Meningitis and epididymo-orchitis represent the two most important of the less frequent manifestations of this disease. As is characteristic of many viral infections, mumps is usually a more severe illness in persons past the age of puberty than in children and more commonly leads to extrasalivary gland involvement in these older patients. Although the use of effective vaccines has markedly reduced the incidence of mumps, the occurrence of outbreaks of mumps in the United Kingdom, Canada, United States, and elsewhere in recent years has raised concerns regarding the possible resurgence of the disease.

HISTORY

Hippocrates described mumps and its contagious characteristics in the fifth century BCE. In the late 1700s Hamilton emphasized the occurrence of orchitis as a manifestation of mumps. The experimental production of the disease in monkeys by Johnson and Goodpasture in 1934[1] provided the evidence that a filterable virus was present in the saliva of patients with mumps. In 1945 Habel reported the cultivation of mumps virus in the chick embryo.[2] Enders and colleagues[3] described the skin test and development of complement-fixing antibodies after mumps in humans. A killed-virus vaccine used in the early 1950s on human subjects achieved limited success,[4] and in 1966 Buynak and Hilleman[5] reported the development of an effective live-virus vaccine.

The etymology of the word *mumps* is unclear. It may arise from the English noun *mump,* meaning a lump, or from the English verb *to mump*, defined as "to be sulky"—a description of the characteristic facial expression. Alternatively, the term *mumps* has been ascribed to the mumbling speech pattern of the affected person. In the older literature mumps may have been called "epidemic parotitis."

VIROLOGY

Mumps virus is a member of the Paramyxoviridae family, which includes the following genera: *Rubulavirus* (mumps virus, New Castle disease virus, human parainfluenza virus types 2, 4a, and 4b); *Paramyxovirus* (human parainfluenza virus types 1 and 3); *Morbillivirus* (measles); and *Pneumovirus* (human respiratory syncytial virus). The complete mumps virion has an irregular spherical shape, with a diameter ranging from 90 to 300 nm and averaging about 200 nm. The nucleocapsid is enclosed by an envelope that has three layers and is about 10 nm thick.[6] The external surface is regularly studded with glycoproteins possessing hemagglutinin, neuraminidase, and cell fusion activity. The middle component of the envelope is a lipid bilayer acquired from the host cell as the virus buds off the cytoplasmic membrane. The innermost surface of the envelope is a nonglycosylated membrane protein that maintains the outer structure of the virus. The genome of the virus is contained in a nucleocapsid that is a helical structure composed of a continuous linear molecule of single-stranded RNA genome surrounded by symmetrically repeating protein subunits. The genome codes for eight proteins—the hemagglutinin-neuraminidase protein (HN), fusion protein (F), nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), hydrophobic protein (SH), and L proteins.[7] The P protein contains two nonstructural proteins, V and I. Antibodies to the F and HN proteins appear to be the most prominent determinants of immunity. Although only one serotype of mumps virus is known, there are 13 genotypes (A–M) that have been determined on the basis of

sequencing of the SH protein, which is the most variable protein among mumps strains.[8–10]

Mumps virus is ether sensitive by virtue of its lipid envelope. It is stable at 4°C for several days and at −65°C for months to years; however, repeated freezing and thawing may diminish viral activity.

The virus replicates in a variety of cell cultures and in embryonated hens' eggs.[11] For primary viral isolation, monkey kidney, human embryonic kidney, or HeLa cell cultures are used for primary isolation. Cytopathic effects, such as the appearance of intracytoplasmic eosinophilic inclusions, rounding of cells, or the fusion of cells into giant multinucleate syncytia, may be noted.[12] The presence of mumps virus is usually confirmed by the hemagglutination inhibition (HAI) test, which uses convalescent serum after mumps infection to inhibit the adsorption of chick erythrocytes added to mumps-infected epithelial cells.

EPIDEMIOLOGY

Mumps is endemic throughout the world. In the United States, before the licensing of live-attenuated mumps vaccine in 1967, epidemics occurred every 2 to 5 years.[13] Although the disease occurred throughout the year, the peak incidence was between January and May.[14] Epidemics have been reported in military populations and other closed communities, such as prisons, boarding schools, ships, and remote islands.[15,16] Meyer demonstrated that mumps is spread throughout the community by children in schools, with secondary spread to family members.[17] There has been more than a 99% decline in the annual US incidence of mumps since 1967, with an average of only 265 cases/year reported to the Centers for Disease Control and Prevention (CDC) from 2001–05; the seasonal variation that was evident in earlier years is no longer apparent.[18] However, there have been outbreaks of mumps reported from various sites, including the Netherlands,[8] United Kingdom,[19] United States,[20] and Canada.[21] In the 2006 outbreak in the United States, 6584 cases of mumps were detected; 85% of patients were in Iowa and the seven contiguous states; the highest attack rate was for the age group of 18 to 24 years, which comprised 29% of all cases, and 83% of this group attended college.[20] The outbreak virus was of genotype G, the same virus genotype that had caused an outbreak in the United Kingdom during 2004–06.[19] Surprisingly, for those patients with known vaccine status, only 13% had not received vaccine, and 63% had received two or more doses of mumps containing vaccine. The reasons for the outbreaks and the apparent vaccine failures are not clear and may have been multiple.[21,22] These include possible waning immunity and exposure pressure from crowded conditions in dormitories, where susceptible individuals may have gathered. Investigators demonstrated that in the Iowa outbreak, preoutbreak mumps antibody titers were lower among mumps case patients than in exposed but asymptomatic classmates.[23,24] Genotypic differences between the vaccine Jeryl Lynn strain (A) and the circulating mumps strain in outbreaks in the United States and United Kingdom (G) were present. Genotype G has been the predominant circulating mumps strain in the United States since 2006.[25] However, the genotype A virus vaccines were apparently effective in controlling genotype G outbreaks, so the role of genotype differences in vaccine failure, if any, is unclear. The epidemic rapidly subsided, so by 2008 there were only 376 cases of mumps reported to the CDC. From 2008–10, 3502 cases of mumps occurred in an orthodox Jewish community in New York City and nearby counties,[26] and in 2011 the California Department of Public Health reported 29 mumps cases on a college campus.[27] In the New York outbreak, most of the cases had previously received two doses of measles-mumps-rubella (MMR) vaccine; a third dose of MMR vaccine resulted in a rapid decline of the epidemic, suggesting that waning immunity could be bolstered even in populations with high two-dose coverage of MMR.[28] A study of 15 clusters of mumps in France in 2013 indicated that the "odds" of mumps increased for twice-vaccinated individuals by 10% for every year that had passed since the second dose.[29] On the basis of this and other studies, the French High Council of Public Health recommended that a third dose of MMR be given during outbreaks of mumps for individuals who had received their second dose more than 10 years before the outbreak.

A recent study of outbreaks of mumps in Ireland in 2008–09 showed that immunoglobulin G (IgG) levels to the circulating genotype G5 mumps strain needed to be greater than 40 relative units/mL to be correlated with anti-G5 neutralization activity in vitro.[30] Lower titers of serum neutralizing activity were seen in males compared with females with acute mumps infection. This is consistent with previous observations that males are more likely to be infected in such outbreaks.

In 2014 a large community outbreak of mumps occurred in central Ohio and involved local residents and students at Ohio State University. Four-hundred eighty-two cases were reported, with ages ranging from 6 months to 80 years.[31] There were 6369 cases of mumps reported to the CDC in 2016, which was the highest number of cases in a decade.[25] From January 1 to November 3, 2018, 2002 cases of mumps in the United States were reported to the CDC.[32]

Mumps is uncommon in infants younger than 1 year. Resistance to infection in this age group is based on passive immunity acquired by the placental transfer of maternal antibody. In the prevaccine era, greater than 50% of cases occurred in the 5- to 9-year-old age group, and 90% of the cases occurred in children younger than 14 years. In 2001 49% of infections were reported in persons older than 15 years. In the prevaccine era 80% to 90% of US adults older than 20 years were immune to mumps on the basis of natural infection. At present, in the United States immunity to mumps in children and most young adults relies on prior vaccination. Men and women have the same frequency of development of parotitis with mumps infection.[33]

Humans are the only known natural host; however, monkeys and other laboratory animals have been experimentally infected.[1] Although persistent infections in cultured cells are commonly established by mumps virus,[34] a carrier state is not known to exist in humans.

PATHOGENESIS

The virus is naturally transmitted via direct contact, droplet nuclei, or fomites and enters through the nose or mouth. More intimate contact is necessary to transmit mumps than for measles or varicella. The period of peak contagion is just before or at the onset of parotitis.

Experimental mumps infection has been produced in humans and monkeys by direct instillation of the virus into the Stensen duct.[1] However, the incubation period in this experimental model is shorter than in naturally occurring disease, and initial infection of the parotid gland does not explain the fact that meningitis or other manifestations of mumps infection may occur before the onset of parotitis. It has been suggested that, during the incubation period, the virus proliferates in the upper respiratory tract epithelium and viremia ensues, with secondary dissemination and localization to glandular and neural tissue.[35,36]

PATHOLOGY

Salivary glands from patients infected with mumps are rarely available for pathologic examination because of the benign course in the great majority of the cases. When parotid glands have been examined, diffuse interstitial edema has been found, along with a serofibrinous exudate consisting primarily of mononuclear leukocytes. Neutrophils and necrotic debris accumulate within the ductal lumen, and the ductal epithelium shows degenerative changes. The glandular cells are relatively spared but may also be involved with edema and overflow of the inflammatory reaction from the interstitial tissues. The multinucleate syncytia and intracytoplasmic eosinophilic inclusions that are occasionally seen in mumps-infected tissue culture are not present in vivo. When the pancreas or the testis is involved, the microscopic picture is similar to that seen in the salivary glands, except that interstitial hemorrhage and polymorphonuclear leukocytes are more frequently noted in orchitis. Local areas of infarction may occur because the vascular supply is compromised by increased pressure caused by edema within an inelastic tunica albuginea. When the process has been particularly severe, atrophy of the germinal epithelium may result, with accompanying hyalinization and fibrosis.

The description of brain involvement in mumps encephalitis has most often been that of postinfectious encephalitis characterized by perivenous demyelinization, perivascular mononuclear cuffing, and a generalized increase in microglial cells, with relative sparing of neurons.[37] However, descriptions of what appears to be primary mumps encephalitis that show widespread neuronolysis but no evidence of demyelinization have been reported.[38]

CLINICAL MANIFESTATIONS

The incubation period of mumps averages 16 to 18 days, with a range of 2 to 4 weeks. Characteristically, the prodromal symptoms are nonspecific and include low-grade fever, anorexia, malaise, and headache. Within 1 day the nature of the illness becomes apparent when the patient complains of an earache, and tenderness can be elicited by palpation of the ipsilateral parotid. The involved gland is soon visibly enlarged and progresses to a maximum size over the next 2 to 3 days. The most severe pain accompanies the period of rapid enlargement. At its height, parotitis results in lifting of the ear lobe upward and outward. Lesser degrees of enlargement can more readily be appreciated by viewing the patient from behind. The enlarged parotid gland obscures the angle of the mandible, whereas cervical adenopathy does not hide this anatomic landmark. Usually, one parotid gland enlarges 1 or 2 days after the other; however, mumps results in unilateral parotitis alone in one-quarter of patients with salivary gland involvement. The orifice of the Stensen duct is frequently edematous and erythematous. Trismus may result from the parotitis, and the patient may have difficulty with pronunciation and mastication. Ingestion of citrus fruits or juices typically exacerbates the pain. During the first 3 days of illness the patient's temperature may range from normal to 40°C. After parotid swelling has reached its peak, pain, fever, and tenderness rapidly resolve, and the parotid gland returns to normal size within 1 week. Complications of parotitis are rare but are reported to include sialectasia resulting in recurrent acute and chronic sialadenitis.[39]

Involvement of the other salivary glands may occur in conjunction with parotitis in up to 10% of cases but is rare as the sole manifestation of mumps infection (Table 157.1). Submandibular gland involvement mimics signs of anterior cervical lymphadenopathy. The sublingual glands are the least frequently inflamed during mumps infection; when involvement occurs, it is usually bilateral and may be associated with swelling of the tongue. Presternal pitting edema develops in 6% of patients with mumps, most commonly in those who have submandibular adenitis.[40] The proposed mechanism for the involvement of the tongue and presternal area is obstruction of the lymphatic drainage of those regions by enlarged salivary glands.

Pharyngolateral edema with dyspnea is a rare complication of mumps and may result in airway stenosis.[41] It is most often associated with submandibular gland swelling and is thought to be caused by local inflammation and circulatory disturbance of lymphatic flow. Cases generally respond to administration of steroids, but some cases have required tracheotomy.[41]

Central nervous system (CNS) involvement is the most common extrasalivary gland manifestation of mumps. As documentation of the remarkable neurotropism of this virus, Bang and Bang[42] reported the presence of cerebrospinal fluid (CSF) pleocytosis in 51% of 255 patients with mumps but without other evidence of meningitis. Clinical meningitis occurs in 1% to 10% of persons with mumps parotitis,[43] but, on the other hand, only 40% to 50% of patients with mumps meningitis, confirmed by serology or viral isolation, have parotitis.[43–46] Meningeal symptoms, like any of the other manifestations of mumps infection, may occur before, during, after, or in the absence of parotitis. Its onset averages 4 days after the appearance of salivary gland involvement but may be as early as 1 week before or as late as 2 weeks after parotitis.[42–45] Men are afflicted three times as often as women,[43–46] but the age distribution is the same as for uncomplicated mumps. Ritter has noted that mumps meningitis with parotitis is most frequent in the spring, whereas meningitis without parotitis is most frequent in summer.[44] The typical clinical features associated with viral meningitis are present—that is, headache, vomiting, fever, and nuchal rigidity. Lumbar puncture yields CSF containing 10 to 2000 white blood cells (WBC)/mm^3. The predominating cells are usually lymphocytes, but 20% to 25% of patients have a polymorphonuclear leukocyte predominance.[45] Protein levels are normal to mildly elevated, and 90% to 95% of patients have a CSF protein content less than 70 mg/dL.[45,46] Hypoglycorrhachia (CSF glucose concentration <40 mg/dL) is reported in 6% to 30% of the patients[45–47] and appears to be more common than in other viral meningitides. These CSF abnormalities may persist for 5 weeks or longer.[44,47] The finding of a depressed CSF glucose level with a moderate-to-marked pleocytosis may cause the physician to consider bacterial meningitis in the differential diagnosis, especially if neutrophils predominate, as they may early in the disease. As in other cases of meningitis, when mononuclear cells prevail in the CSF, tuberculous and fungal disorders should be considered.

Abatement of fever by lysis and resolution of symptoms generally occur 3 to 10 days after the onset of illness. The meningitis is benign, with complete recovery and an absence of sequelae. Before the introduction of the live-attenuated mumps vaccine in 1967, mumps accounted for approximately 10% of cases of aseptic meningitis in the United States. At present aseptic meningitis is rarely attributed to mumps.

Encephalitis is reported to occur in from 1 in 6000[48] to 1 in 400[49] cases of mumps. The former ratio probably represents a more accurate estimate. There appears to be a bimodal distribution of cases according to the time of onset—an early group in which onset coincides with the presence of parotitis and a larger late group in which the condition develops 7 to 10 days after the onset of parotitis. As noted earlier in the section "Pathology," early-onset encephalitis represents direct damage to neurons as a result of viral invasion, whereas late-onset disease is a postinfectious demyelinating process related to the host response to infection. These two processes probably represent the ends of a continuum of disease. Some patients die after the primary viral invasion of the brain, and some of those who survive produce antibodies to the virus or neural breakdown products and develop an "autoimmune" reaction. The clinical features are generally those of nonfocal encephalitis; in addition to marked changes in the level of consciousness, neurologic findings may include convulsions, paresis, aphasia, and involuntary movements. CSF values are similar to those in uncomplicated meningitis. Fever is high and, characteristically, temperatures of 40° to 41°C are present. Neurologic manifestations and fever gradually resolve over a period of 1 to 2 weeks. Sequelae such as psychomotor retardation and convulsive disorders are reported,[44–46] but their frequency cannot be determined from the available data. Death occurs in 1.4% of reported cases.[49]

Through the mid-1960s, mumps was the leading recognized cause of viral encephalitis in the United States, being responsible for 20% to 30% of cases. However, by 1981 it represented only 0.5% of cases of viral encephalitis nationwide, and by the 1990s mumps encephalitis was rare. The major factor accounting for this change was an effective mumps immunization program.

The term *meningoencephalitis* is frequently used when describing patients with various degrees of CNS involvement.[38,43,44,47,50] This term should be eliminated in reference to mumps because it confuses a common and essentially benign condition (meningitis) with a relatively uncommon and serious illness (encephalitis) that might result in

TABLE 157.1 Frequency of Common Clinical Manifestations of Mumps

MANIFESTATION	FREQUENCY (%)
Glandular	
Parotitis	60–70
Submandibular and/or sublingual sialadenitis	10
Epididymo-orchitis[a]	25 (postpubertal men)
Oophoritis[a]	5 (postpubertal women)
Neural	
Cerebrospinal fluid pleocytosis	50
Meningitis	1–10
Encephalitis	0.1
Transient high-frequency deafness	4
Other	
Electrocardiographic abnormalities	5–15
Renal function abnormalities (mild)	>60

[a]Rare before puberty and usually unilateral.

neurologic residua or death. Clearly, many patients with mumps meningitis may have lethargy, as may a large percentage of those with any viral infection, such as influenza. However, the presence of profound changes in the level of consciousness or other findings suggestive of supratentorial involvement indicates the clear diagnosis of encephalitis as distinct from the ambiguous designation of meningoencephalitis. Although nuchal rigidity and CSF pleocytosis may be present in patients with encephalitis, the meningeal component is a trivial aspect of this illness.

Transient high-frequency-range deafness has been reported in 4.4% of cases of mumps in a military population.[51] Permanent unilateral deafness occurs in 1 in 20,000 cases of mumps.[52] The onset of otologic symptoms may be gradual or abrupt; vertigo is frequently present. On subsequent testing vestibular function has been normal.

Other neurologic syndromes rarely associated with mumps include cerebellar ataxia,[53] facial palsy,[54] transverse myelitis,[55] ascending polyradiculitis (Guillain-Barré syndrome),[56] and a poliomyelitis-like syndrome.[57] There are now several well-documented cases of aqueductal stenosis and hydrocephalus developing after CNS infection caused by mumps.[58–60] Experimental and clinical reports have clearly implicated mumps as the probable causative agent of this disorder.[61–63]

Epididymo-orchitis is the most common extrasalivary gland manifestation in the adult. It develops in 20% to 30% of postpubertal male adolescents with mumps infection and is bilateral in one of six of those with testicular involvement.[64,65] Although it has been reported in infancy, it is rare before puberty. Two-thirds of cases occur during the first week of parotitis and another 25% arise during the second week.[64] However, gonadal involvement may precede parotitis or occur as the only manifestation of mumps. The onset is abrupt, with temperatures in the range of 39° to 41°C, chills, headache, vomiting, and testicular pain. Genital examination reveals warmth, swelling, and tenderness of the involved testicle and erythema of the scrotum. Epididymitis is present in 85% of cases and usually precedes the orchitis, but it is rare without orchitis. The testis may be enlarged to three to four times its normal size. Constitutional complaints and fever generally parallel the severity of gonadal involvement. Fever resolves in 84% of patients within 5 days. Pain and swelling resolve shortly after defervescence. However, tenderness may persist for longer than 2 weeks in 20% of the cases.[64] Early in convalescence a loss of turgor may be appreciated. When testes are examined months to years later, some degree of atrophy is noted in 50% of patients.

The anxiety engendered by mumps orchitis is difficult to allay. The psychological fears of sexual impotence and sterility far outweigh the potential debility from testicular atrophy. Clearly, most men who have unilateral orchitis need fear nothing other than a possible cosmetic imbalance. Even those with bilateral involvement should be assured that impotence (other than psychogenic) is not a sequela and that sterility is rare. In large surveys of infertile men, mumps is infrequently implicated as the causative disorder. Twenty-eight cases of testicular malignancy in men with atrophy of the testis due to mumps orchitis have been reported.[66]

Oophoritis develops in 5% of postpubertal women with mumps. Symptoms include fever, nausea, vomiting, and lower abdominal pain. Impaired fertility and premature menopause have been reported as a consequence of ovarian involvement but must be considered to be rare.[67]

Joint involvement during mumps is noted infrequently in adults and rarely in children.[68,69] Migratory polyarthritis is the most frequently described clinical form. Monarticular arthritis and arthralgia have also been reported; both large and small joints are involved. Symptoms most commonly start 10 to 14 days after the onset of parotitis and may last up to 5 weeks. The process resolves spontaneously without residual joint damage.

Pancreatitis is manifested by severe epigastric pain and tenderness accompanied by fever, nausea, and vomiting. It is uncommon as a severe illness; however, many affected persons may complain of mild degrees of upper abdominal discomfort.

Electrocardiographic changes appear in up to 15% of patients with mumps; the most common abnormalities are depressed ST segments, flattened or inverted T waves, and prolonged P-R intervals.[70,71] Clinically manifested myocarditis is rare; however, deaths associated with myocarditis have been reported during the acute illness and after a chronically progressive deteriorating course.[70,71]

Utz and colleagues[72] have prospectively evaluated renal function in 20 young adult Navy servicemen admitted with mumps. These investigators discovered transient mild-to-moderate abnormalities of urinary concentration, creatinine clearance, and phenolsulfonphthalein excretion in most of this group. Hughes and colleagues[73] have reported two deaths related to mumps-associated nephritis.

A variety of other manifestations have accompanied mumps infection but must be considered extremely rare; these include thyroiditis,[74] mastitis,[75] prostatitis,[76] hepatitis,[77] and thrombocytopenia.[78]

COMPLICATIONS

Gestational viral infections were extensively investigated in a controlled cohort study by Siegel and colleagues.[79–81] They observed excess fetal deaths when mumps developed during the first trimester; second- and third-trimester mumps infections were not associated with increased fetal mortality.[79] Low birth weight (<2500 g) was identified in 7.7% of infants born to mumps-infected mothers, compared with 3.3% of a control group; this is not, however, a statistically significant difference. Although the number of cases was small, when the data were analyzed with respect to the onset of infection, the effect on birth weight was greatest when mumps occurred in the first trimester.[80] A variety of congenital malformations have been described in pregnancies complicated by maternal mumps[82]; however, these anomalies are described in single case reports without comparison with an uninfected control population. As reported by Siegel and colleagues,[81] occurrence rates of major congenital defects were equal in both mumps and control newborn populations; even when the data were analyzed by trimester, no trends could be established. Similar results were obtained by a British team after reviewing 500 pregnancies complicated by maternal mumps.[83]

St. Geme and colleagues[84] have suggested an "embryopathic" relationship between intrauterine mumps infection and endocardial fibroelastosis (EFE) on the basis of the presence of skin test reactivity to mumps antigen in a high percentage of the EFE patients. Experimentally induced infection of the chick embryo has added histopathologic support to this association.[85] Although some observers have disputed that mumps plays a causative role,[86] studies using polymerase chain reaction (PCR) techniques have demonstrated mumps viral RNA in greater than 70% of samples of myocardium from patients with autopsy-proven EFE.[87] There has been a marked decline in the incidence of EFE in the past 3 decades, corresponding to the declining incidence of mumps.

A similar controversy exists over the possible role of mumps in the etiology of juvenile diabetes mellitus. Diabetes, transient or permanent, which developed soon after mumps, has been the subject of a number of case reports.[88,89] However, it is not clear whether this is simply coincidental. Epidemiologic studies have demonstrated a 7-year periodicity in the incidence of both mumps and childhood diabetes, with a 3- to 4-year lag time between their respective peaks.[90] Coxsackievirus B4 has also been epidemiologically linked to diabetes.[91] Although the frequency of EFE has declined in recent years, there has not been a decline in the frequency of juvenile diabetes mellitus coincident with the decreasing frequency of mumps after introduction of the mumps vaccine.

IMMUNOLOGY

After clinical or subclinical mumps infection, a variety of immunologic responses can be demonstrated. Complement-fixing antibodies directed against the NP protein (historically, S antigen) appear rapidly; sometimes they are present at the onset of clinically apparent illness. Antibody titers against the HN protein (historically, V antigen) rise more slowly and peak at about 2 to 4 weeks after the beginning of disease.[92] However, anti-NP antibody titers decline rapidly over a period of several months to undetectable levels, whereas anti-HN antibody titers drop more slowly and persist for years. This pattern of response provides the possibility of a serologic diagnosis of mumps from a single serum specimen. An acute-phase serum demonstrating a high anti-NP and low anti-HN titer or a high anti-NP and high anti-HN titer can be interpreted as evidence of current or recent infection, respectively. The presence in

serum of only anti-HN antibodies would indicate a more remote infection with mumps. IgM antibodies to mumps are the earliest humoral responses and usually fall within 2 to 6 months. IgM anti-NP antibodies detected by capture or enzyme-linked immunosorbent assay (ELISA) are the most sensitive early serologic responses and are used by the CDC to detect acute or recent infection (see later).

Neutralizing antibodies appear during convalescence. They are directed against HN and F proteins, and detectable titers persist for years. Although assays for these antibodies constitute the most reliable test to determine whether a person is immune to mumps, such assays are cumbersome and not routinely performed. Assays for HAI antibodies, which also develop after the onset of mumps, are the simplest of the serologic studies, but results are unreliable because of potential cross-reaction with other paramyxoviruses. ELISAs for antibody to mumps have been developed[93,94] and are widely available.

Delayed hypersensitivity to an intradermally administered mumps skin test antigen develops between 3 weeks and 3 months after mumps.[3] The skin test was widely used as a measure of immunity to mumps and as a test for the competence of delayed hypersensitivity. The use of mumps skin test antigen to determine immunity to mumps has been abandoned because of the variability of lots of the skin test antigen and of the occurrence of false-positive and false-negative results.

Transplacental transfer of maternal mumps complement-fixing, HAI, and neutralizing antibodies has been demonstrated.[95] Titers in maternal and cord serum are almost identical. Neutralizing antibodies persist for several months and account for the rarity of mumps in young infants and for the lack of response to immunization in this age group. One attack of mumps, whether inapparent or clinically manifested, confers lifelong immunity.

DIAGNOSIS

Historically, the diagnosis of mumps has been made on the basis of a history of exposure and of parotid swelling and tenderness accompanied by mild-to-moderate constitutional symptoms.

The WBC and differential counts in mumps are normal, or there may be a mild leukopenia with a relative lymphocytosis. When meningitis, orchitis, or pancreatitis is present, leukocytosis with a shift to the left is most commonly encountered. The serum amylase level is elevated in the presence of parotitis and may remain abnormal for 2 to 3 weeks. Serum amylase levels may also be elevated in the absence of clinical salivary gland involvement. Mumps pancreatitis also increases amylase levels; differentiation from salivary gland amylase may be achieved by isoenzyme analysis or serum pancreatic lipase determinations.

The typical CSF findings in mumps meningitis have been described previously. Similar, although less marked, CSF abnormalities are present in half of patients with mumps parotitis but without apparent CNS involvement. In a patient with aseptic meningitis an elevated serum amylase level should suggest mumps infection.

Laboratory confirmation of typical mumps is unnecessary. However, when parotitis is absent or recurrent, when extrasalivary gland manifestations are prominent, or when documentation of the presence of a specific viral disorder is desired, a variety of diagnostic aids can be used.

The definitive diagnosis of mumps depends on serologic studies, viral isolation, or PCR assay. The presence of IgM antibodies, as determined by ELISA or a fourfold rise between acute and convalescent sera on complement fixation, HAI, ELISA, or neutralization testing, confirms the diagnosis. The HAI test can be affected by heterologous antibody responses to parainfluenza virus infection. Because parotitis can be caused by parainfluenza type 3 virus,[96] serologic testing and virus isolation studies for parainfluenza type 3 virus should be undertaken if the HAI test is used in the diagnosis of mumps. Immunity to mumps is usually assessed by ELISA. This assay combines ease of performance with reliability. Reverse transcriptase PCR assays that are highly sensitive and specific have been developed and appear to be significantly more sensitive than tissue culture isolation methods.[7,97,98]

Virus is usually present in saliva for about 1 week, from 2 to 3 days before to 4 to 5 days after the onset of parotitis.[99] However, virus has been isolated from saliva as early as 6 days before and as late as 9 days after the first signs of salivary gland involvement. In addition, virus may be recovered from the saliva of persons with inapparent infection or those who manifest only extrasalivary gland signs.[100] A recent review of viral shedding data by the CDC, American Academy of Pediatrics, and Healthcare Infection Control Practices Advisory Committee has concluded that virus shedding is relatively low by 5 days after onset of parotitis and recommended that isolation of patients is not necessary for more than 5 days after clinical illness in the hospital or community setting.[101] The virus is frequently isolated from the CSF in patients with clinical meningitis during the first 3 days of meningeal symptoms[43] and is present as late as the sixth day of CNS disease. Viruria has been detected during the first 2 weeks of illness; in one study 72% of urine specimens during the first 5 days of illness yielded a positive culture.[72] Viremia has rarely been detected and has been found only during the first 2 days of illness.[35,36] Mumps viral RNA has been detected by PCR assay in clinical specimens from patients with mumps infection and in throat swabs of healthy children after administration of mumps vaccine.[102–104]

DIFFERENTIAL DIAGNOSIS

A variety of entities may simulate mumps but can be easily differentiated from mumps on the basis of chronicity or associated symptoms. Infectious processes involving parotid glands are most likely to be confused with mumps because of their acute onset and associated fever. Parainfluenza 3 virus, coxsackieviruses, and influenza A viruses have been reported to cause acute parotitis.[96,105,106] These entities can be differentiated from mumps only by viral culture or serology. In a study of 101 cases of sporadic nonoutbreak cases of parotitis in the United States from 2009–11, no specimen was positive for mumps virus; 38 specimens were positive for other viruses, including Epstein-Barr virus, human herpesvirus 6, and parainfluenza; and 17% of cases tested were positive for mumps IgM antibody.[107] Bilateral parotid swelling is often seen in children with human immunodeficiency virus (HIV) infection. Suppurative parotitis, most often caused by *Staphylococcus aureus* or gram-negative organisms, usually occurs in the postoperative period, in premature newborns, or in debilitated patients with poor oral intake. The gland is warm, hard, and extremely tender; the overlying skin is erythematous. Massage of the parotid expresses purulent drainage from the Stensen duct.

Parotid enlargement caused by drugs or metabolic disorders is usually bilateral and asymptomatic. Phenylbutazone, thiouracil, iodides, and phenothiazines have been implicated in this condition.[76] Diabetes mellitus, malnutrition, cirrhosis, and uremia are among the metabolic disorders that can cause parotid swelling.[76]

Tumors, cysts, and obstruction caused by stones or stricture are usually unilateral. Rare conditions that may mimic mumps include Mikulicz syndrome, Parinaud syndrome, uveoparotid fever of sarcoidosis, and Sjögren syndrome.

THERAPY

Therapy for mumps parotitis is symptomatic and supportive. Treatment with analgesic-antipyretics, such as aspirin, acetaminophen, or nonsteroidal antiinflammatory drugs, relieves pain caused by salivary gland inflammation and reduces fever. Topical application of warm or cold packs to the parotid may also relieve discomfort. Intravenous fluid administration may be necessary for patients with meningitis or pancreatitis who have persistent vomiting.

Management of orchitis is purely symptomatic. Bed rest, narcotic analgesics, support of the inflamed testis with a "bridge," and ice packs make the patient feel more comfortable. An anesthetic block of the spermatic cord with 1% procaine hydrochloride may alleviate severe pain.[108] There is no convincing evidence that the use of steroids or diethylstilbestrol or incision of the tunica albuginea produces more rapid resolution of the orchitis or prevents subsequent atrophy. Interferon-α2b administered to four men with bilateral mumps orchitis resulted in prompt resolution of symptoms, with no evidence of testicular atrophy or oligospermia during follow-up study.[109] Further investigation to establish the efficacy of this treatment has not been carried out.

Gellis and colleagues[110] have shown that 20 mL of mumps immune globulin administered intramuscularly to adult men with mumps reduces the incidence of orchitis from 27.4% to 7.8%. However, mumps immune globulin is no longer commercially available.

PREVENTION

As noted, recommendations for the management of patients with mumps include isolation for 5 days after the onset of parotid swelling to prevent the spread of infection to susceptible persons.[101] This measure may be of little value, particularly in closed populations, such as schools or hospitals,[111] because virus is present in saliva days before parotitis develops and because those with clinically inapparent infection can shed virus.

Passive protection to exposed susceptible persons may have been afforded by mumps immune globulin, available in the past. However, Reed and colleagues[16] reported that use of mumps immune globulin during an epidemic in Alaska did not reduce clinical parotitis or inapparent infection rates and did not diminish the incidence of meningitis and orchitis.

Active immunization with the Jeryl Lynn strain of attenuated mumps virus vaccine has been available in the United States since December 1967. The vaccine is prepared in chick embryo cell culture.[5] A single subcutaneous immunization produces protective levels of mumps-neutralizing antibodies in greater than 95% of vaccinees.[5] Although the antibody levels produced are lower than after natural infection, adequate titers are maintained for at least 10.5 years.[112] Adverse reactions to the vaccine are uncommon; transient suppression of tuberculin-delayed hypersensitivity has been reported, and parotitis and orchitis have been recognized rarely. Vaccine virus is not present in secretions of immunized children. In Japan aseptic meningitis associated with mumps vaccine virus occurred in 0.05% to 0.3% of recipients of the Urabe AM 9 mumps vaccine; manifestations began 2 to 4 weeks after immunization.[113,114] US studies did not reveal evidence of an increased risk of aseptic meningitis after administration of the Jeryl Lynn strain of mumps vaccine.[115]

All children older than 12 months should be immunized. Vaccination should take place at 12 to 15 months and again at 4 to 6 years of age, as part of immunization with the combined live measles-mumps-rubella (MMR) virus vaccine. Most states now require evidence of immunity to mumps (i.e., documented immunization, physician-diagnosed disease, or antibody studies) for school entrance and attendance (see Chapter 316). A two-dose immunization regimen is recommended for all adolescents and health care personnel without evidence of mumps immunity. Other adults should receive at least one dose of vaccine. Immunization after exposure may not provide protection from natural infection.

Based on the results of a study on the effectiveness of a third dose of MMR vaccine to control a mumps epidemic at the University of Iowa in 2015–16, where 98.1% of students had previously received two doses of MMR,[116] the Advisory Committee on Immunization Practices recommended in 2018 that a third dose of MMR vaccine be administered to persons at increased risk of mumps during an outbreak.[25]

As with other live-virus vaccines, mumps vaccine should not be administered to pregnant women, patients receiving immunosuppressive therapy, or those with severe febrile illnesses, advanced malignancies, or congenital or acquired immunodeficiencies. Serious reactions to the mumps component of MMR have not been reported in limited studies in HIV-infected patients. However, a fatal case of measles pneumonitis occurred in a 21-year-old man with advanced HIV disease who was vaccinated with MMR vaccine; therefore it should not be administered to such patients (see Chapter 316).[117] Individuals with HIV infection who are not severely immunocompromised may be immunized with MMR vaccine.

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The complete reference list is available online at Expert Consult.

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158 Respiratory Syncytial Virus

Edward E. Walsh and Janet A. Englund

SHORT VIEW SUMMARY

Definition
- Respiratory syncytial virus (RSV) causes acute upper and lower tract respiratory illnesses.

Epidemiology
- RSV circulates annually during the winter months in temperate climates.
- RSV is the leading cause of bronchiolitis in infants.
- Reinfection is common throughout life and causes severe disease in elderly adults and immunocompromised individuals.
- RSV is primarily transmitted by direct contact with infected individuals or their secretions.

Microbiology
- RSV is an enveloped nonsegmented RNA virus in the *Pneumoviridae* family.
- RSV isolates can be classified into antigenically distinct groups, A and B.

Diagnosis
- Clinical diagnosis in infants is relatively accurate during the winter months; however, in older children and adults, laboratory confirmation by culture, antigen detection, or reverse-transcriptase polymerase chain reaction is necessary.

Therapy
- Treatment for most infants and adults is mainly supportive only.
- Aerosolized ribavirin, a nucleoside analogue, can be considered for administration to high-risk, highly immunocompromised patients; other therapies are under investigation.

Prevention
- Attention to infection control measures such as hand hygiene and contact precautions can reduce the spread of RSV.
- Palivizumab, a humanized neutralizing monoclonal antibody to RSV, is beneficial for certain high-risk infants including infants with underlying cardiac and pulmonary disease and low gestational age.
- New vaccines and monoclonal antibodies for the prevention of RSV are under active development.

Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract illness in young children.[1–8] All people will have experienced RSV infection within the first few years of life. However, immunity is not complete, and reinfection is common. Although serious infections most commonly occur during the first few years of life, RSV infections contribute substantially to the morbidity caused by acute upper and lower respiratory tract infections among older children and adults. Estimated national hospitalization charges for bronchiolitis in children less than 2 years of age exceeded $1.7 billion in 2009.[1] The mortality related to RSV in developing countries and the morbidity and costs associated with RSV infections in older adults are being increasingly recognized.[9–12]

HISTORY

RSV was first discovered in 1956 when Morris and coworkers[13] isolated a new virus from chimpanzees with colds and coryza, and was originally named *chimpanzee coryza agent* (CCA). Subsequently, Chanock and colleagues[14] confirmed that the agent caused human illness when they obtained isolates indistinguishable from CCA from two children with respiratory illness. When specific neutralizing antibody to CCA was found in most school-aged children, CCA was more appropriately renamed RSV to denote its clinical and laboratory manifestations.

VIROLOGY

Classification

Human RSV belongs to the order Mononegavirales, family Pneumoviridae.[15] The two genera within the Pneumoviridae are *Metapneumovirus,* containing human metapneumovirus and *Orthopneumovirus,* which contains human RSV and the morphologically and biologically similar bovine, ovine, and caprine RSV.

Viral Structure and Characteristics

RSV is an enveloped, medium-sized (120–300 nm) RNA virus with a nonsegmented, single-stranded, negative-sense genome (Figs. 158.1 and 158.2).[16,17] The viral envelope has transmembrane surface glycoprotein spikes 12 nm in length, and on electron microscopy the virus can be identified as early tubular structures and later more mature spherical structures. (Fig. 158.3).[16,17]

The viral RNA consists of approximately 15,222 nucleotides containing 10 genes (see Fig. 158.2).[16,18] Each gene encodes a single protein except for the *M2* gene, which possesses two overlapping open reading frames encoding two separate proteins (M2-1 and M2-2, the transcription processivity factor and a transcriptional regulatory protein, respectively). Four proteins, N (nucleoprotein), P (phosphoprotein), L (polymerase), and M2-1, are associated with the RNA-containing nucleocapsid complex. The envelope has three transmembrane surface proteins, F (fusion), G (attachment), and SH (proposed viroporin protein), that are important for viral infectivity.[16,19] In addition, a truncated secreted form of G (Gs) is transcribed from a second start codon. The M (matrix) protein accumulates at the inner surface of the envelope and is important in viral morphogenesis.[17] Two proteins, NS1 and NS2, are nonstructural proteins that inhibit cellular type I and III interferon activity and subsequently affect the adaptive immune response to RSV.[16,20]

The two glycosylated surface proteins, F and G, are major immunoprotective antigens and targets for antibody-mediated neutralization.[21] The G protein is the primary mediator of attachment of the virus by binding to the CX3C receptor on respiratory epithelial cells and immune cells, although the F protein also can facilitate viral attachment by binding to cell surface nucleolin.[22–24] After attachment in the prefusion form, F undergoes structural changes into a postfusion form that initiates viral penetration by fusing viral and host cell membranes.[21] The prefusion F displays a greater number of neutralizing epitopes than the postfusion F, some of which are highly potent.[21]

Laboratory Properties

RSV poorly withstands slow freezing, thawing, or changes in pH. At 55°C, infectivity rapidly diminishes; at room temperature, only 10% infectivity remains at 48 hours; at 4°C, only 1% remains after 7 days.[25] RSV is inactivated quickly at pH <5 or contact with ether, chloroform, and detergents. At room temperature, RSV in secretions of patients

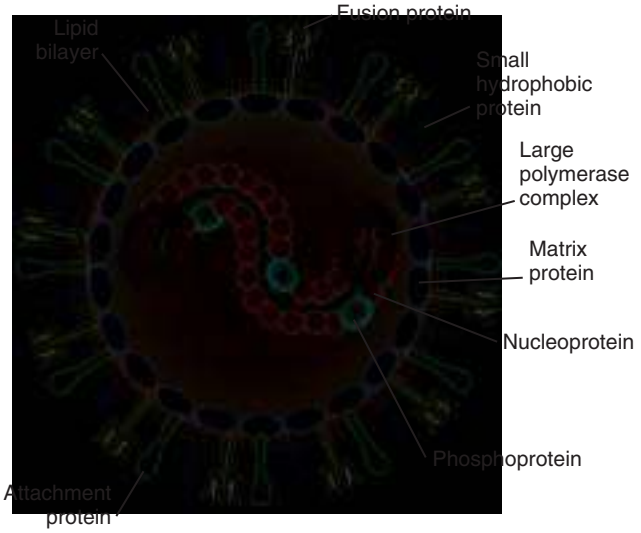

FIG. 158.1 Structure of respiratory syncytial virus. *(Modified from Hall CB. Respiratory syncytial virus and parainfluenza virus.* N Engl J Med. *2001;344:1917–1928.)*

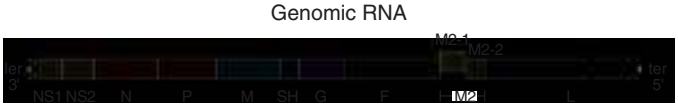

FIG. 158.2 Simplified representation of the negative-sense RNA genome of respiratory syncytial virus *(RSV)* and the encoded proteins. The genome is shown 3′ with the leader extragenic region *(ler)* and with the 5′ trailer extragenic region *(ter).* The viral genes are depicted by the divided bars. Each viral gene transcribes a single messenger RNA encoding a single protein with the exception of M2 messenger RNA, which transcribes two proteins, M2-1 and M2-2.

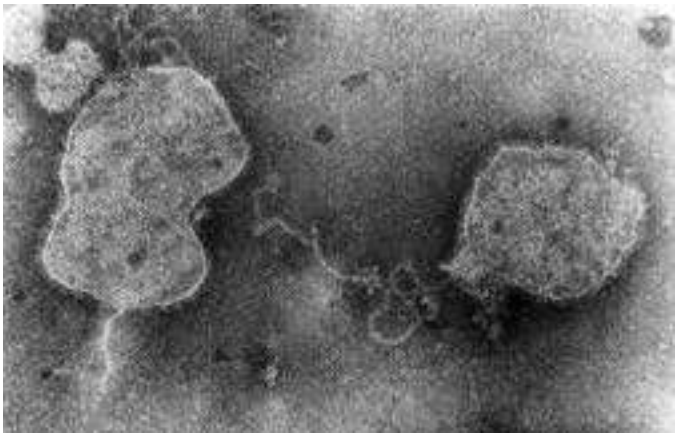

FIG. 158.3 Negative contrast electron micrograph of respiratory syncytial virus.

may survive on nonporous surfaces such as countertops for 3 to 30 hours and on porous surfaces such as cloth and paper tissue usually for less than 1 hour.[26] The infectivity of RSV on the hands is variable but is usually less than 1 hour.

Human heteroploid cell lines (HEp-2, HeLa, and A549) are usually preferred for primary isolation, but RSV may also be recovered in human kidney, amnion, and diploid fibroblastic cells and monkey kidney cells. Characteristic cytopathic effect may be first detected after an average of 3 to 5 days, and typical syncytia develop approximately 10 to 24 hours later. RSV can also infect macrophages, dendritic cells, and T and B cells.

Infection in Animals

Humans and chimpanzees are the only natural hosts for human RSV, although a variety of small animal species may be experimentally infected.[27] Although many animal models develop upper respiratory tract infection, their lack of symptomatic lower respiratory tract limits their utility. Rodents are the most commonly used models, particularly cotton rats and mice, but replication of RSV is only semipermissive. The ferret model has been used recently, as it demonstrates several characteristics of human illness and transmission.[28]

EPIDEMIOLOGY

Distribution and Seasonal Occurrence

In every geographic area studied, RSV infections are ubiquitous and clinically similar. However, the seasonality varies according to geography and climate.[29,30] RSV is singular in its ability to produce a major burden of disease every year.[1,6] In temperate climates, outbreaks occur primarily in late fall through early spring and spread across the United States over 20 or more weeks, generally from October to May. In warmer climates, RSV activity may be more prolonged or even present throughout the year.[29–32] In wet tropical climates, RSV peaks during rainy periods, whereas it peaks during cooler periods in drier climates.[33] Factors that initiate and terminate the recurring patterns of RSV activity remain elusive.[32,34,35] A complex interaction of local meteorologic conditions may explain part of the geographically variable epidemiologic patterns.[36] Because the only source of RSV infection is an infected individual, human behavior is an indefinable factor integral to its transmission.[37]

Antigenic Variation

Strain differences among RSV isolates may also affect the intensity, severity, and diversity of RSV outbreaks.[38–47] RSV isolates are divided into two major antigenic groups, A and B, each with multiple genotypes—up to 11 for group A and 23 for group B viruses.[41] The two major groups have 81% nucleotide identity, but some proteins between A and B strains vary appreciably, with the most diversity in the G protein, followed by M2-2 and SH proteins. This is reflected in antigenic relatedness between the two groups of only 1% to 7% for G proteins compared with 50% for F proteins.

Strains of both groups circulate simultaneously, but the proportions of A and B vary, as do genotypes.[39,40,42,43] Analyses of strains collected over decades and from diverse areas suggest that pressure of the population's immunity may result in a selective advantage for dominance of strains most divergent from those that have recently circulated. Recently emerged RSV viruses, containing unique 20–amino-acid and 26–amino-acid duplications in the group B and A G proteins, respectively, have spread throughout the world, suggesting transmission advantage over prior strains.[44,45] The relationship of circulating genotypes to illness severity and manifestations in young children has been inconsistent and thus inconclusive.[41] Several reports indicate that group A ON1 strains, originally isolated in Ontario, Canada, are less severe than strains lacking the 24-amino acid duplication.[41] The relationship between the substantial antigenic diversity of the G protein to reinfection with RSV has not been established.

Epidemiology Manifestations

RSV outbreaks may vary year to year in size and intensity.[1–3,48,49] Severe lower respiratory tract illness from RSV in previously healthy children occurs most frequently in the first year of life and is almost always associated with primary infection.[50,51] Essentially all children experience RSV infection within the first several years of life.[52–54]

Prevalence and Incidence

RSV is the most frequent cause of bronchiolitis and is estimated to cause 40% to 90% of bronchiolitis hospitalizations and up to 50% of pneumonia admissions among infants.[5,49–51,55] The remaining cases are caused by rhinovirus, parainfluenza viruses, human metapneumovirus, and influenza virus.[56] Of tracheobronchitis cases, 10% to 30% have been associated with RSV infection, but only 2% to 10% of croup cases have been associated with RSV infection. Bronchiolitis is the leading cause of all hospitalizations among infants in the United States. Overall, 172,000 children younger than 5 years of age are reported to be hospitalized

annually with RSV infection in the United States.[4,6,7] The yearly rates of RSV hospitalizations estimated from national databases have been variable, ranging from 2 to 44 per 1000 children within the first 2 to 5 years of life.[48,50,51,57,58] Children within the first year of life consistently had the highest rates of hospitalization for bronchiolitis and other RSV-associated illnesses, and the preponderance of admissions were in infants younger than 6 months.[7,51] Population-based studies in the United States indicated current hospitalization rates of 17 per 1000 children younger than 6 months and 3 per 1000 children younger than 5 years.[3,49,51] The highest hospitalization rates (25.9/1000) were in infants 1 month of age, slowly decreasing thereafter.[51] These rates are similar to rates derived from RSV-coded hospitalization data and are more than three times the rates from parainfluenza or influenza viral infections over 4 years of surveillance among the same population.[59,60] RSV hospitalization rates from European countries have been generally similar, ranging from 2.5 to 11 per 1000 children within the first 4 years of life and highest among infants younger than 12 months of age at 19 to 22 per 1000 children.[8,50,61–63]

Lower respiratory tract infections from RSV in developing countries are estimated to be twice as prevalent as in developed countries.[8] Worldwide, RSV is estimated to cause 33.1 million RSV acute lower respiratory infections annually and 3.2 million hospitalizations in children younger than 5 years, most of which are in developing and low-income countries.[8] Despite its frequency, mortality from RSV in developed countries is conspicuously less common.[64] US data indicate approximately 42 annual deaths in children with RSV, whereas estimates for developing countries suggest approximately 59,000 infant deaths annually are due to RSV.[8,64]

Many host, socioeconomic, and environmental factors have been associated with a greater likelihood of young children developing more severe RSV infection and requiring hospitalization (see "Patients at High Risk for Severe Infection").[2,6] Population-based surveillance of RSV hospitalizations and emergency department and outpatient visits in the counties surrounding Rochester, New York; Nashville, Tennessee; and Cincinnati, Ohio, during 2000 to 2004 indicated that outpatient visits constituted a major proportion of the health care burden attributable to RSV.[3] Of all visits for acute respiratory illnesses (ARI) among children within the first 5 years of life, RSV infection was documented among 20% of ARI hospitalizations, 18% of emergency department ARI visits, and 15% of office ARI visits. Estimated rates of ARI visits from RSV among children younger than 5 years in pediatric practices (80 per 1000 children) were approximately 3 times that observed among emergency department patients and 26 times hospitalization rates.

Fewer data exist on the health care burden imposed by RSV infection among older children and adults.[58,65] In the Houston family study of children followed from birth, the infection rate was 68.8 per 100 children in the first year of life, and at least half were reinfected during the second year of life.[52] In urban Rochester, 44% of families with young children became infected with RSV during winter months when RSV was prevalent.[54] Of exposed family members, 46% were infected with RSV. Although the attack rate was highest among infants, 38% to 47% of older children and adults developed RSV infection. Similar findings were noted in family studies in Finland and rural Kenya.[66,67]

The incidence of RSV infections among older adults and the resulting appreciable clinical and economic impact are increasingly recognized.[9,12,68–72] In one study spanning 4 years, rates of RSV infection ranged from 2% to 10% per year in elderly adults 65 years of age or older and in high-risk individuals with underlying cardiopulmonary disease.[9] RSV infection among elderly adults is remarkably similar to influenza infection with respect to clinical manifestations and as a cause of hospitalization.[68,73] The rate of hospitalization attributable to RSV among elderly adults ranges from 1.5 to 23 per 10,000 in various studies.

PATHOGENESIS

Infection with RSV is primarily acquired through close contact with an infected individual by direct inoculation of infectious secretions into the eyes and nose or by touching objects contaminated with infectious secretions.[37,74,75] Large-particle aerosols engendered by coughs and sneezes of an ill person may transmit RSV to others within a radius of about 3 feet. Longer-distance spread by small-particle (droplet nuclei) aerosols appears much less likely.[37] However, more recent studies found that small-particle aerosols collected in pediatric wards and clinics during RSV season contain infectious virus at relatively low levels, providing theoretical risk of transmission.[76–78]

Experimental infection occurred in adult volunteers after an incubation period of 2 to 5 days.[79–82] In naturally acquired infection, the average incubation period ranges from 2 to 8 days. In hospitalized infants with primary infection, peak nasal viral titers range from 10^1 to 10^7 plaque-forming units (pfu)/mL (mean, 10^5 pfu/mL) and decrease slowly.[83,84] Shedding duration is typically 7 to 10 days, but virus can occasionally be detected for 30 days.[85] Increased viral load has been associated with greater disease severity.[83,84,86,87]

RSV replicates in respiratory epithelium, primarily involving ciliated columnar cells, but additional cells, such as type I and II pneumocytes, may be involved.[88,89] During primary infection, lower respiratory tract infection usually is manifested as bronchiolitis, and initial pathologic findings are a lymphocytic peribronchiolar infiltration, predominantly $CD69^+$ monocytes, with edema of the walls and surrounding tissue.[88,90,91] Subsequently, the characteristic proliferation and necrosis of the epithelium of the bronchioles develop. Small airways become obstructed from sloughed epithelium and increased mucus secretion. The airway of the young infant is particularly vulnerable to any degree of inflammation because resistance to the flow of air is related inversely to the cube of the radius. Hyperinflation results from air trapping peripheral to the sites of partial occlusion. With complete obstruction, trapped air eventually is absorbed, producing characteristic multiple areas of atelectasis. Young infants are at increased risk for developing atelectasis because collateral channels that maintain alveolar expansion in the presence of airway obstruction are not yet well developed.

Infants with lower respiratory tract disease from RSV often have pathologic evidence of both pneumonia and bronchiolitis. Patients with pneumonia demonstrate an interstitial infiltration of mononuclear cells that may be accompanied by edema and necrosis that lead to alveolar filling.[88,90] Immunohistologic analysis of lungs from infants dying with RSV demonstrated lymphocyte populations expressing CD4, CD14, and CD75, but not CD8 or CD25.[90] Some histologic evidence of recovery is present in most children with bronchiolitis within the first week of illness and is marked by the beginning regeneration of the bronchiolar epithelium. However, ciliated cells may not be present for weeks.

Immunity and Pathogenesis of Disease

Much of the knowledge regarding immune responses to RSV is from in vitro studies and in animal models. In humans, the immune response is confounded by the influence of genetics, presence of maternal antibody, environmental exposures including concurrent respiratory tract microbiota, age, and the virus.[92–95] More severe disease in the youngest infants is thought to be related to decreased levels of maternally derived RSV-specific antibody as well as physical, immune, and viral factors. The severity of RSV infection in a young infant with augmented disease induced by the inactivated RSV vaccine developed in the 1960s first suggested a role of the immune response in pathogenesis of RSV in infants.[95–98]

The potential importance of the host's immune response to disease has been supported by the observation that RSV is not generally invasive or cytopathic.[95] However, one report of fatal RSV noted that RSV antigen was extensively present in pulmonary tissue, indicating abundant viral replication.[89] Cytokine production was nearly absent, and the expression of apoptosis was increased, with the conclusion that the patient had an inadequate immune response and unchecked viral replication.

Although viral load seems to correlate with disease severity in young children and adults, and disease symptoms decrease as viral load decreases, reducing viral replication by administration of neutralizing antibody to F protein has not ameliorated clinical disease in infants.[100,101] Data in murine models of RSV indicate that nonneutralizing antibodies to the centrally conserved CX3C chemokine motif of G protein can reduce inflammatory responses even after infection is established and in the absence of reduced viral load.[102–104] Whether innate or adaptive immune responses or both are enhanced or suppressed during more severe disease remains controversial.

The influence of the nasal microbiota and their distinct metabolic pathways on clinical manifestations of RSV infection has been appreciated.[105–107] It has also been recognized that RSV infection can influence the pathogenicity of *Streptococcus pneumoniae,* and RSV epidemics are associated with an increase in pneumococcal pneumonia in young children.[108] The presence of *S. pneumoniae* or *Haemophilus influenzae* increases the susceptibility and inflammatory response of airway epithelial cells in vitro.[109,110] Even in the absence of overt bacterial infection, the nasal microbiome may affect infant peripheral blood transcriptomic response to infection and is associated with illness severity.[106]

Maternally Derived Immunity

The first barrier of defense against RSV infection in infants is maternally derived RSV-specific serum antibody. Despite a relatively short half-life of 28 to 40 days, maternally derived antibodies have been correlated with protection in some, but not all, studies.[111–116] An early study noted that higher levels of cord blood neutralizing antibody were associated with reduced risk of hospitalization and were directly correlated with older age at hospitalization.[111] A similar analysis from Denmark calculated a 26% reduction in hospitalization during the first 6 months of life for every twofold increase in cord blood neutralizing antibody.[115] In addition, a study found that higher levels of antibody to prefusion-F and G proteins of RSV and neutralizing antibody were associated with less severe disease in the first few months of life.[113] Supplementing these observational studies, and perhaps the most compelling case for the beneficial effects of antibody, results from administration of RSV polyclonal or monoclonal antibody to high-risk infants have demonstrated protection against severe RSV disease.[99,117,118]

Innate Immunity

A rapid and vigorous innate immune response is initiated when infection of the infant's respiratory epithelium occurs.[95,98,119,120] Early events include RSV interaction with Toll-like receptors 2, 3, 4, and 7, which triggers secretion of inflammatory cytokines and chemokines such as interleukin (IL)-8, monocyte chemoattractant protein 1, macrophage inflammatory proteins MIP-1α and MIP-1β, RANTES (regulated on activation, normal T-cell expressed and secreted), and eotaxin with recruitment of macrophages, mononuclear cells, natural killer cells, and eosinophils.[98,121–123] RSV infection induces cellular production of innate interferons, which are counterbalanced by viral synthesis of the NS1 and NS2 proteins that are potent inhibitors of antiviral type I and III interferons (α, β, and λ).[20] An early and robust neutrophilic infiltration into the airway is correlated with a decline in viral load before the development of T-cell responses.[124–127] Dendritic cells also infiltrate the nasal mucosa early in infection and can be noted in lower airway secretions associated with a number of proinflammatory cytokines (e.g., IL-6, tumor necrosis factor-α, IL-8). Concentrations of many of these cell types as well as levels of cytokines and chemokines have been linked to clinical disease phenotypes and severity.[120] The variability in the endowed innate defense and susceptibility of the host are being increasingly correlated with polymorphisms in genes that are integral to various components of innate immunity.[94]

Adaptive Immunity

An effective immune response to RSV infection requires a fine balance of multiple components of immunity, a balance likely determined by both host and viral factors.[92,95,120] During primary infection, serum immunoglobulin M (IgM) antibody appears within several days but is transient and detectable usually only for a few weeks.[95] IgG antibody appears during the second week, peaks in the fourth week, and begins to decline after 1 to 2 months. An anamnestic response involving all three immunoglobulin classes occurs after reinfection, and after about three infections, titers reach levels similar to those in adults.

Primary and subsequent infections result in antibody production to many of the RSV proteins including the major immunoprotective surface glycoproteins F and G.[16] Both contain neutralizing epitopes, but those on the F protein are conserved between the two viral groups. The response to the variable G protein is group and genotype specific.[128] Most adults with RSV infection develop IgG responses to the centrally conserved chemokine motif of G, although their role in recovery or protection from infection or illness is not clear.[116,129]

Although young children are able to produce neutralizing antibodies directed against both the F and G proteins, neutralizing antibody responses are blunted in infants younger than 6 months of age owing to a dampening effect of maternal antibody.[130,131] In infants, antibody responses to F and G proteins mainly involve the subclasses IgG1 and IgG3. Adults respond to the G protein with both IgG1 and IgG2 subclass antibodies, and the adult response to the F protein is predominantly IgG1. After primary and recurrent infections, antibodies usually decline substantially within months. Following natural infection, 75% of adults demonstrate a fourfold or greater decrease in titer, returning to preinfection titers within 2 years in most cases.[132] Higher titers of antibody generally correlate with better resistance to infection, but no defined level of neutralizing antibody is predictive of the risk for infection, severity of illness, or recovery in children or adults.[10,111,133–135]

RSV-specific IgA antibody, produced in nasal secretions during primary and subsequent infection, is associated with protecting the upper respiratory tract from either natural infection or experimental challenge in adults and has been correlated with viral clearance in infants.[135–137] In the adult RSV challenge model, impaired induction of IgA-specific memory B cells was noted, which may account for the ease of reinfection.[138] Children with RSV infection may also produce transient specific IgE antibody responses in the respiratory tract. Higher levels of nasal RSV-specific IgE antibody and cysteinyl leukotrienes have been correlated with increased risk for more severe illness and wheezing and with later episodes of recurrent wheezing.[120,139,140]

Cell-mediated immunity is considered pivotal for clearance of virus and clinical recovery. Adults and children with deficiencies of cellular immunity have more severe disease and prolonged virus shedding.[141,142] Most data delineating the specific components of the cellular immune response induced by RSV are derived from rodent models and, to a lesser extent, from humans.[92,95,120] RSV infection has multiple inhibitory effects on the cellular immune response. Diminished in vitro lymphoproliferative responses during initial and repeated infections suggest impaired RSV-specific helper T-cell responses. Furthermore, RSV-infected dendritic cells have diminished ability to activate $CD4^+$ T cells, and enhanced apoptosis of $CD4^+$ and $CD8^+$ lymphocytes is observed among infants with bronchiolitis.[120,143,144]

The relationship between disease manifestations and the balance between Th1 and Th2 T-cell responses during infection has been an area of great interest. RSV infection in animals and humans engenders both Th1 and Th2 responses. Th1-dominant responses, characterized by the production of $CD8^+$ cytotoxic lymphocyte and Th1 $CD4^+$ cells secreting IL-2, IFN-γ, and tumor necrosis factor-α, are associated with viral clearance and minimal pulmonary cytopathology in animal models. In contrast, Th2 $CD4^+$ cells, with associated IL-4, IL-5, IL-10, and IL-13 secretion, impairs $CD8^+$ T-cell function and viral clearance.[145] An overexuberant memory $CD8^+$ T-cell response can mediate severe immunopathology in murine models of RSV infection.[146] IL-4 and IL-13 also augment isotype switching to IgE, and a Th2-biased response has been correlated with wheezing, more severe disease, and greater cellular inflammation and eosinophilia in the lung.[139]

Infants infected in the first several months of life have higher levels of Th2-type cytokines in nasal secretions than older infants.[147,148] Transcriptomic analysis of isolated circulating CD4 T cells during primary RSV infection in young infants suggested a pattern of activation consistent with a Th2 environment, including evidence of Th9 activation, that was associated with more severe disease.[149] Inactivated virus, as used in the initial formalin-inactivated vaccine and even in subunit vaccines, is more likely than live virus to induce a Th2-like response in experiments with unprimed animals.[145]

CLINICAL MANIFESTATIONS

Clinical observations have confirmed that naturally acquired immunity to RSV infection is incomplete. However, severe disease rarely occurs after primary infection in healthy children. Lower respiratory tract involvement and severe disease may occur during repeat infections but is generally confined to individuals with underlying conditions at either end of the age spectrum.[9,52,53]

Infection Among Young Children (Bronchiolitis and Other Lower Respiratory Tract Illnesses)

Primary infections frequently involve the lower respiratory tract, particularly in the first several months of life, and manifest most commonly as bronchiolitis, followed by pneumonia and tracheobronchitis.[3,52,53] Croup is uncommon, accounting for less than 2% to 10% of cases. Upper respiratory tract signs almost always accompany lower respiratory tract disease. Infection may be confined to the upper respiratory tract, which in young children is commonly associated with fever and otitis media. The first infection is rarely asymptomatic.[52–54] The risk for lower respiratory tract involvement with first infections is high; pneumonia or bronchiolitis has been estimated to occur in 30% to 71%, depending on the age and population.[3,52,53,150] Among infants younger than 6 months of age with underlying cardiopulmonary disease or in close contact with young children such as those attending child care, the proportion developing lower respiratory tract disease may be even higher.[53] Illness severity among preschool-aged children seeking outpatient care is considerable, with two-thirds manifesting wheezing and three-fourths having labored breathing.

RSV usually starts with upper respiratory tract illness with nasal congestion and cough. Hoarseness and laryngitis are not prominent features. Low-grade fever lasting 2 to 4 days occurs in most infants early in the illness. The height or duration of fever does not correlate with disease severity, and fever is frequently absent by the time of lower respiratory tract involvement. With progression to the lower respiratory tract, cough may become more prominent and productive, followed by increased respiratory rate, dyspnea, and retractions of intercostal muscles. With bronchiolitis, both expiratory and inspiratory obstruction may be evident. The infant may have crackles and wheezing on auscultation. The rapid variability in the presence and intensity of physical findings is characteristic of bronchiolitis, with marked transient swings in oxygen saturation often noted. Repeated observations are required for adequate assessment of clinical severity.[151]

Infants with lower respiratory tract infection commonly have impaired oxygenation[151]; however, hospitalization based on hypoxia alone is controversial. Mild degrees of desaturation may persist despite clinical improvement. Approximately 10% of infants who are hospitalized develop alveolar hypoventilation and hypercarbia, requiring respiratory support. Hospitalization, if required, averages 2 days, but prolonged hospitalization is not uncommon.[3] For most infants, however, the duration of the acute illness is 3 to 10 days.

Abnormalities on chest radiograph may be minimal, regardless of the severity of the child's illness. Hyperaeration is especially indicative of RSV infection and may be associated with peribronchial thickening.[152,153] Most children exhibit only airway disease. Less than 10% of bronchiolitis cases have evidence of both airway and airspace disease, and only about 1% have parenchymal consolidation.[152] Opacities are commonly misdiagnosed as bacterial pneumonia. These most common findings are subsegmental in right upper or middle lobes and result from atelectasis. Pleural fluid is rarely demonstrated.

Otitis Media

Acute otitis media is a common complication of RSV infection among young children.[154–156] Among children within the first 3 years of life who developed acute otitis media, 74% had RSV detected in middle ear fluid. Acute otitis media usually develops about 5 days after the onset of the respiratory illness and is more common among children older than 1 year. Otitis and earache are common complications even among older children and previously healthy adults with RSV infection; these complications also were noted in approximately half of hospitalized infants in one study.[54,65,156] RSV has been recovered from the middle ear fluid as the sole pathogen, with bacteria, or with another virus in 2% to 22% of cases.[154,156] The most frequent concurrent bacterial pathogens are *S. pneumoniae, H. influenzae,* and *Moraxella catarrhalis.*[156] Clinical and experimental evidence suggests that coinfection of RSV with a bacterial pathogen may prolong the duration and worsen the outcome of otitis media, resulting in a greater chance of treatment failure with antibiotics and persistent effusion.

Infections Among Older Children

The frequency of RSV infections at all ages is well illustrated among families with young children and among persons in contact with young children, as in schools and child care facilities.[52–54] Among children attending child care who had primary RSV infection in their first winter, 75% and 65% develop infection during their second and third years, respectively, using serologic and viral detection methods.[53] Recurrent infections commonly are upper respiratory tract illnesses, but 20% to 50% of recurrent infections among preschool-aged children involve the lower respiratory tract, including wheezing, although generally less severe than initial infection.[52] The overall burden of repeated infection in children younger than 5 years of age is substantial.[3]

Infections Among Adults

RSV infection in adults typically manifests with upper respiratory symptoms, nasal congestion, and scratchy throat preceding lower respiratory symptoms by several days. Wheezing is more common with RSV than with other respiratory viruses. Changes on the chest radiograph are seen in about half of hospitalized patients, with minimal lower lobe infiltrates or ground-glass appearance most common.[157] On computed tomography scan, ground-glass changes and bronchiole wall thickening are noted in 67%.[158] Adults also may have repetitive RSV infection occurring in sequential years, especially individuals living or working with children.[54,65,74,133] Despite the lack of durable immunity evoked by natural infection, both infection risk and severity of infection in adults are reduced by higher titers of serum and mucosal antibody in observational studies.[10,135]

The burden RSV places on the health care of this growing population has only recently been appreciated fully.[4,9,12,68,159–162] At greatest risk of severe illness are frail elderly patients; patients with underlying cardiopulmonary disease, especially chronic obstructive pulmonary disease; and severely immunocompromised patients.[10] Adults shed RSV at titers considerably lower than infants,[163] and thus estimates of RSV burden in this population have been hampered until more recently by lack of sensitive diagnostics. The incidence of hospitalization, found by using regression models using viral culture data from infants and adult hospital coding data, has been estimated at 86 per 100,000, or 28% of the incidence of influenza hospitalizations.[4] Using similar methods, RSV mortality in The Netherlands was 64% of that attributable to influenza.[12] Similar rates of RSV hospitalization and mortality have been reported by other authors.[120,161,164–167]

In long-term care facilities, 5% to 27% of respiratory infections have been reported to be caused by RSV, with attack rates estimated to be 1% to 15%, pneumonia rates of 10% to 20%, and mortality of 2% to 5% of infected individuals.[162] In a retrospective cohort analysis of residents of Tennessee nursing homes, RSV was associated with 15 hospitalizations, 17 deaths, and 76 antibiotic courses per 1000 people and 7% of hospitalizations and 9% of deaths from cardiopulmonary disease.[167] Among adults in daycare facilities, 10% of acute respiratory infections were due to RSV, a rate similar to influenza and coronaviruses.[159]

The highest risk for severe RSV infection is in patients with underlying cardiopulmonary disease, especially chronic obstructive pulmonary disease, and congestive heart failure.[9,68,164,168–170] The clinical illness caused by RSV in elderly adults is nonspecific and indistinguishable from other respiratory viruses, such that studies with active prospective diagnosis of RSV infection are required.[73,160] In a 4-year prospective outpatient study of 608 healthy individuals older than 65 years of age, 504 high-risk adults with cardiopulmonary conditions, and 1388 adults hospitalized with acute cardiopulmonary conditions, RSV infection was identified in 3% to 7% of the elderly cohort, 4% to 10% of the high-risk cohort, and 11% of the hospitalized cohort (Table 158.1).[9] These rates were similar to rates for influenza in the same groups. Among the hospitalized patients with RSV, 15% required intensive care and 8% died compared with 12% and 7%, respectively, of patients with influenza. In a large outpatient study of adults ≥50 years old with medically attended respiratory illness, RSV was identified in 8% compared with influenza identified in 17%.[160] The same investigators estimated the incidence of RSV medically attended respiratory illness to be 154 per 10,000, increasing with each decade of life.[11]

Peak nasal RSV titers in adults are substantially lower than in infants, ranging from 1 to 10^5 pfu/mL (mean, $10^{2.3}$ pfu/mL).[163] When simultaneously measured, virus titers in sputum are generally higher than in nasal secretions.[171] The duration of virus shedding in adults averages 10 days but can be 20 to 30 days. Higher viral load has been correlated with more severe disease.[172,173] Bacterial coinfection during RSV infection occurred in 31% of hospitalized patients, similar to the rate with influenza.[174] There are no controlled data on antiviral treatment for RSV in adults, and thus treatment is rarely considered except in immunocompromised patients (see "Therapy").

RSV severity among older adults is only partly explained by comorbidities and age-associated decline in pulmonary function, and it has been suggested that age-associated decline in immune function may be relevant. However, humoral immunity to RSV among elderly adults is equal to or greater than that among young adults.[134] In stable uninfected older adults, baseline RSV-specific $CD8^+$ T-cell function is diminished.[175–177] However, CD4 and CD8 T-cell responses during RSV infection were not age dependent, and more vigorous responses were noted in the most ill patients regardless of age.[178]

The burden of RSV among young healthy adults may be considerable and comparable to the burden influenza.[65] Among working healthy adults with RSV infection, 22% had lower respiratory tract manifestations. Compared with influenza, fever was less frequent, but earache, sinus pain, persistent productive cough, and wheezing were significantly more common with RSV (Table 158.2). In contrast to influenza, there are few descriptions of RSV infection during pregnancy or adverse impacts of maternal RSV on the fetus.[179–182] Reported rates of RSV infection in pregnant women are low (0.3–3.9/1000 person-years), but results are limited by lack of appropriate prospective viral surveillance. Severe disease with RSV during pregnancy has been reported, with one pregnant woman requiring mechanical ventilation.[182] No fetal adverse outcomes have been noted.[179]

TABLE 158.1 RSV Compared With Influenza Among Hospitalized Adults

CHARACTERISTICS	RSV (n = 132)	Influenza A (n = 144)
Age, years	76 ± 13	76 ± 12
Female sex	84 (64)	81 (56)
Chronic illness		
Any cardiac disease	71 (54)	71 (49)
Congestive heart failure	39 (30)	33 (23)
Any lung disease	77 (58)	79 (55)
Any heart or lung disease	106 (80)	113 (78)
Diabetes mellitus	35 (27)	28 (19)
Residence in long-term care facility	16 (12)	15 (10)
Smoking (current or past)	88 (67)	98 (68)
Influenza vaccination	99 (75)	98 (68)
Katz ADL score	1.2 ± 2.4	1.3 ± 3.0
IADL score	4.1 ± 4.1	3.3 ± 4.0
Length of hospital stay, days	14 ± 41	8 ± 5
Findings on chest radiography		
Infiltrate found	41 (31)	43 (30)
Congestive heart failure	17 (13)	15 (10)
Other	24 (18)	27 (19)
Admission to intensive care unit	20 (15)	17 (12)
Use of mechanical ventilation	17 (13)	15 (10)
Higher level of care at discharge than at admission	7 (5)	8 (6)
Death	10 (8)	10 (7)

[a]Values are reported as mean ± SD or number (%). Percentage may not sum to 100 because of rounding. Katz ADL score and the IADL score are functional assessments based on a 12-point scale, with 0 representing total independence and 12 representing total dependence.
ADL, Activities of daily living; *IADL,* instrumental activities of daily living; *RSV,* respiratory syncytial virus.
Modified from Falsey A, Hennessey PA, Formica MA, et al. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med. *2005;352:1749–1759.*

COMPLICATIONS

Patients at High Risk for Severe Infection

Young children most likely to require hospitalization are premature infants and children with underlying chronic lung disease, cyanotic or complicated congenital heart disease, immunosuppressive conditions, or other chronic diseases that affect the handling of respiratory secretions such as neuromuscular disease.[3,117,183,184] Children with Down syndrome and other genetic diseases also are at high risk for severe disease. About one-third of children who are hospitalized with RSV infection within the first 5 years of life have one or more underlying conditions, and the proportion is greater among children older than 2 years.[3]

Preterm gestation with or without associated chronic lung disease is a major risk for severe RSV disease.[3,117,184] Hospitalization rates in infants <36 weeks of gestational age are three or more times higher than rates for full-term infants. With gestational age less than 32 weeks, the admission risk with RSV infections is increased, and the need for intensive care significantly increases. A disproportionate economic and clinical burden is contributed by late preterm infants (gestational age 33–35 weeks) with RSV infections, who represent about three-fourths of all preterm infants,[117,185] and this burden may extend into their second year.[186]

TABLE 158.2 Clinical Characteristics of Illness Caused by Influenza or RSV Among 211 Previously Healthy Adults

CHARACTERISTIC	ADULTS WITH ILLNESS CAUSED BY RSV OR INFLUENZA, NO. (%)		
	RSV (n = 177)	Influenza (n = 59)	P
Sign or symptom			
Fever (temperature >37.8°C)	50 (58)	43 (73)	< .001
Nasal congestion, rhinorrhea	157 (89)	46 (78)	< .04
Sore throat	102 (58)	32 (54)	.65
Ear pain	35 (20)	3 (5)	< .01
Headache	70 (40)	48 (81)	< .001
Sinus pain	55 (31)	8 (14)	< .01
Cough			
Nonproductive	150 (85)	47 (80)	.36
Productive	92 (52)	14 (24)	< .001
Lower respiratory tract signs, wheezing	28 (16)	5 (9)	.16
Work absence	67 (38)	39 (66)	< .001
Duration of illness, mean days (range)	9.5 (1–20)	6.8 (39–66)	< .001

RSV, Respiratory syncytial virus.
From Hall CB, Long CE, Schnabel KC. Respiratory syncytial virus infections in previously healthy working adults. Clin Infect Dis. *2001;33:792–796. Copyright © Infectious Diseases Society of America.*

Congenital heart conditions, especially cyanotic heart conditions accompanied by pulmonary hypertension, are among the top three major conditions in infants hospitalized with RSV infection; up to one-third require intensive care, and one-fifth require mechanical ventilation.[187–189] Infants hospitalized in the first few months of life with uncorrected cyanotic congenital heart disease are at particular risk, with the risk persisting beyond infancy and appearing greatest during the second year of life.[187,189] Earlier surgical correction of cardiac defects has appreciably reduced the mortality from RSV infection from 30% in the 1970s to less than 2% currently.

Multiple demographic and environmental factors have been evaluated for augmenting the risk for more severe RSV infection.[3,190,191] These factors include male sex, crowded living conditions, lower socioeconomic status, exposure to other young children in the home or child care, tobacco smoke exposure, and lack of breastfeeding. However, the degree of risk these factors contribute to expression of RSV disease in children is difficult to quantify. Two independent risk factors most consistently important for RSV hospitalization are prematurity and young age, especially within the first 3 months of life.[74,117]

The genetic background of an individual has important, but mostly undefined, effects on susceptibility to more severe disease. Certain racial and ethnic backgrounds have notably increased rates of hospitalization and severe RSV infection. Aboriginal people such as Native American Indian and Alaskan Native infants, especially those living in the Yukon-Kuskokwim Delta region, have RSV hospitalization rates three to four times that observed for other infants in the United States.[117,192] Severe disease with primary RSV infection has been correlated with specific polymorphisms in certain genetic loci affecting the immune response including expression of cytokines and inflammatory chemokines.[94]

Immunocompromised Patients

RSV is a well-recognized cause of morbidity and mortality among immunocompromised patients. Greater awareness of RSV disease in this population is a result of increasing numbers of patients undergoing solid-organ transplant (SOT) and hematopoietic cell transplantation (HCT), the use of more prolonged and intensive chemotherapy regimens, and the wider availability of sensitive diagnostic techniques for respiratory viruses.[193,194] The reported frequency of RSV infection among immunocompromised patients varies widely from about 2% to 50%, depending on the type of surveillance, the population, and the degree of immunosuppression.[142,193–200] Although rhinovirus, not RSV, is the most common respiratory virus reported following HCT or SOT, RSV is one of the most serious viral causes of morbidity and mortality in pediatric and adult SOT and HCT recipients.[201–203] Reported rates of RSV-associated complications vary widely with reports of RSV progression from upper to lower respiratory tract disease up to 55% and mortality ranging from 7% to 33% in HCT recipients, with lower rates in SOT recipients.[204]

RSV may be introduced by medical staff or individuals in the community into both inpatient and outpatient settings where immunocompromised patients receive care. Viral spread may be rapid and difficult to control and accompanied by appreciable morbidity and mortality.[142,195,197–199,205–207] The severity of RSV infection among these patients is related to the duration and degree of immunosuppression, type of transplant or immunosuppressive therapy, and other risk factors including treatment for graft-versus-host disease or organ rejection.[142,198–200,208–211] Patients with severe combined immunodeficiency states, patients with infection early after HCT, and lung transplant recipients are particularly at risk for a poor outcome. Among transplant recipients, factors associated with poor outcomes include use of bone marrow cells (in contrast to peripheral stem cells), occurrence of RSV infection before engraftment, the presence of acute or chronic graft-versus-host disease, and lymphopenia.[209] Among children, young age also is associated with a poorer prognosis.

RSV infection in these patients may clinically mimic other opportunistic agents, and the correct etiology may not be suspected. Sinusitis, otitis media, and wheezing may be more indicative of RSV infection than other respiratory pathogens; fever is not universally present. Concurrent infections by other infectious agents including community-acquired respiratory viruses may further confound or delay the diagnosis of RSV infection. In adult HCT recipients with RSV, upper respiratory tract signs precede pneumonia in 80% to 90% of patients, with progression to pneumonia in 30% to 40% after a median of 7 days.[193,194] The need for oxygen supplementation at the time of presentation is associated with significantly higher rates of respiratory failure and mortality.[211,212] With lower respiratory tract involvement, radiographic findings range from focal interstitial infiltrates, sometimes with hyperinflation or with lobar consolidation, to generalized alveolar and interstitial infiltrates, or even to a picture of acute respiratory distress syndrome.[197] With high-resolution computed tomography, the characteristic findings are airspace consolidation; small centrilobular nodules; ground-glass opacities; and thickening of the bronchial walls, which may be asymmetrical.[213]

Long-term complications in the immunocompromised host include bronchiolitis obliterans syndrome in nearly 10%, although rates of 50% can be seen in lung transplant recipients with RSV lower respiratory infection.[214] In the past, overall mortality with RSV pneumonia was reported to be up to 45%, but current mortality rates appear to be substantially less than that (15% for HCT patients with upper respiratory tract presentation; 33% for patients with lower respiratory tract disease).[212] This has been attributed to better diagnostics, prospective screening before HCT to delay transplant in the face of active RSV infection, advances in supportive care, and potentially treatment with ribavirin with or without immunoglobulin (intravenous immune globulin [IVIG] or monoclonal antibody) (see "Therapy").

Among children with human immunodeficiency virus (HIV) infection, RSV has been the most frequently identified cause of viral respiratory disease.[195] The manifestations of RSV infection vary according to the stage and severity of the HIV infection, and although most patients develop lower respiratory tract involvement, disease is generally is not as severe as disease among highly immunosuppressed transplant recipients.[215] Patients with HIV infection may shed RSV for prolonged periods. Confounding this, however, is the observation that children with HIV infection and concurrent viral respiratory infections also have a higher rate of bacterial coinfections.[215] The clinical outcome of respiratory viral infections has not been consistently different between children with and without HIV infection.

Although treatment of severely immunocompromised patients with aerosolized or even oral ribavirin is recommended by some experts, definitive guidelines are not available because of the lack of highly effective antivirals and prospective controlled trials (see "Therapy").[216,217] The most important aspect of management of immunocompromised patients is prevention of RSV nosocomial infection by strict adherence to infection control policies; guidelines for preventing opportunistic infections in hematopoietic stem cell transplant recipients are available.[193,218,219] These guidelines emphasize preventing the introduction of community respiratory viruses, including RSV, onto units with immunocompromised patients and stress the importance of early diagnosis.

Acute Complications in Infants

Apnea is one of the most striking and serious acute complications in young infants with RSV. Up to 20% of infants hospitalized with RSV infection have been admitted with apnea.[220,221] Among infants evaluated in the emergency department with bronchiolitis, 3% had a diagnosis of apnea. Infants at highest risk for apnea are preterm infants with a gestational age ≤32 weeks, infants with a history of apnea of prematurity, and infants of postnatal age of less than 44 weeks after conception. Characteristically, apnea occurs at the onset of RSV infection and may precede respiratory symptoms. The pathophysiology of apnea is unclear, although it is nonobstructive. Prognosis is generally good after acute RSV infection with no subsequent episodes, even with respiratory infections.

Infants admitted with RSV lower respiratory tract disease may be at increased risk for aspiration, which can appear clinically similar to bronchiolitis with airway hyperreactivity.[222] In a 12-month follow-up of infants hospitalized with RSV bronchiolitis, 83% developed reactive airway disease if they received neither ribavirin nor therapy for aspiration. The decrease in reactive airway episodes was greater in infants receiving ribavirin and thickened feedings than infants who received either therapy alone.

Coexistent bacterial infection is a frequent concern in infants hospitalized with RSV, and many likely receive unnecessary antibiotics. This is

due in part to young age, the presence of fever, and the relatively frequent appearance on the chest radiograph of opacities from viral infiltrates and atelectasis commonly mistaken for bacterial pneumonia. Multiple studies have shown that secondary bacterial infection is an unusual complication of RSV infection.[223–225] A 9-year prospective study identified secondary bacterial pneumonia in less than 1% of subjects, and a multicenter Spanish study identified bacteremia in only the most seriously ill children.[223,226] Furthermore, antibiotic therapy has not been shown to improve recovery from RSV lower respiratory tract disease.[227] Urinary tract infections are the most frequently identified concurrent bacterial infections.[228] In developing countries, however, respiratory bacterial coinfections are more common and may contribute appreciably to the high mortality rate from RSV. The most common coinfection in infants is infection with another virus, most commonly rhinoviruses, adenoviruses, coronaviruses, parainfluenza viruses, human metapneumovirus, and bocavirus.[229] There is no definitive evidence that viral coinfection carries a worse outcome; studies indicate that RSV coinfections may be less severe than RSV infections alone.[230,231]

Long-Term Complications

Recurrent wheezing after RSV bronchiolitis in infancy has long been recognized as a frequent sequela, but a causal link between the two remains unclear.[231a] Approximately 30% to 50% of children hospitalized with RSV infection later develop repeated occurrences of wheezing.[232–234] For many children, the severity of the recurrent wheezing episodes decreases with age, although pulmonary function abnormalities may persist in some without clinical manifestations.[235,236] Others may have persistent wheezing into adolescence or have wheezing cease during childhood and recur in adulthood. The frequency of long-term sequela in the general population is confounded by most studies having focused on children with more severe illness.

Epidemiologic evidence indicates that atopy in the child or family is not a major cause of this link. However, in a murine model of allergen-induced airway inflammation and remodeling, previous RSV infection could induce airway abnormalities in mice exposed to allergen through the airway, even though these mice had not been previously sensitized to the allergens.[237] In addition, RSV infection has been shown both in vitro and in children infected with RSV to produce an immunologic response similar to that observed with allergic sensitization, one with a predominantly Th2 T-cell profile and release of proinflammatory mediators, IgE, and neuropeptides (see "Immunity and Pathogenesis of Disease").[92,238] However, a similar response may be produced by viruses other than RSV.[239,240] Relevant to the relationship between severe RSV infection and asthma are two studies suggesting that palivizumab prophylaxis of premature infants was associated with a decrease in incidence of recurrent wheeze in the first few years of life,[241,242] although this decrease did not persist at age 6 years.[242a]

DIAGNOSIS

RSV infection in young children is most often diagnosed clinically in the setting of the community's RSV season. Clinical findings are less specific in adults, and RSV is frequently not suspected. At the present time, laboratory diagnosis is generally made by reverse-transcriptase polymerase chain reaction (RT-PCR) testing but may be made by rapid diagnostic commercial antigen tests or immunofluorescent antigen detection. Less widely available methods include culture or serologic testing. The sensitivity of viral diagnostic testing depends on the viral load and the specimen type. Nasopharyngeal washes or tracheal secretions are generally better than nasal swabs. Combined throat and nares swabs may improve the rate of recovery compared with a single nasal swab,[243,244] but sensitive RT-PCR testing methods in young children have shown similar results for RSV detection in nasal swabs alone.[245,246]

RT-PCR assay for the diagnosis of RSV infections has consistently demonstrated much higher rates of specificity and sensitivity than rapid antigen diagnostic assays and are more widely used in clinical settings due to the availability of rapid, simple commercialized laboratory test kits.[243,247] In one study of 496 specimens obtained from children with RSV infection determined by viral isolation or duplicate positive RT-PCR assays, about 50% were positive by both RT-PCR and culture, and 50% were positive by RT-PCR alone. Less than 1% were positive only by viral isolation.[244] Sensitive and specific multiplex PCR panels for 12 or more respiratory viruses are available from many manufacturers, and development of simple tests evaluating influenza and RSV simultaneously are marketed for use in point-of-care testing in outpatient and inpatient settings. In adults, RT-PCR is considerably more sensitive than rapid antigen test.[248] Serologic diagnosis is primarily useful for epidemiologic studies, especially in older adults, who have the most vigorous antibody responses to RSV.[134]

THERAPY

Most children and adults with RSV infection require no more than the usual care given to ensure comfort, fever control, and adequate fluid intake. For bronchiolitis, the most commonly administered therapies used for exacerbations of hyperreactive airway disease include hydration and supplemental oxygen, if needed. Bronchodilators and corticosteroids continue to be unnecessarily used[151,153]; multiple studies and meta-analyses have shown neither bronchodilators nor corticosteroids are effective for RSV disease or bronchiolitis of unspecified cause among previously healthy young children and are not recommended.

Antibiotic therapy for children with RSV lower respiratory tract disease should be reserved for patients with specific evidence of a coexisting bacterial infection.[151] Preemptive administration of antibiotics to children with RSV infection or bronchiolitis has not been associated with an improved outcome. Furthermore, complicating or secondary bacterial infections, other than otitis media among children with RSV infection in developed countries, is unusual.[223,224]

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a synthetic nucleoside, is the only currently approved specific treatment for RSV lower respiratory tract disease in hospitalized infants, although it is currently used only in immunocompromised patients (see Chapter 45). The drug, administered as a small-particle aerosol, has shown modest clinical benefit and improved oxygenation in some studies, but an improvement in the duration of hospitalization or short-term outcome in infants was not consistently demonstrated.[151,249] Ribavirin has potential teratogenic properties, and precautions should be taken to guard against exposing women of potential childbearing capacity. In view of unclear benefit relative to the very high cost of aerosolized ribavirin, which more recently increased by more than 200%, ribavirin is currently reserved for the most seriously ill immunocompromised patients.[250]

Data supporting ribavirin treatment of RSV infection in severely immunocompromised patients with RSV infection is primarily drawn from retrospective case series.[142,193,251,252] In a large retrospective single-center analysis of 280 HCT patients with RSV infection, progression to lower respiratory disease and mortality was reduced significantly in patients in whom administration of inhaled ribavirin was started when symptoms were limited to the upper respiratory tract.[251] The only prospective placebo-controlled study was performed in 14 HCT patients with RSV upper respiratory tract infection, which demonstrated safety and a trend of decreasing viral load during therapy.[253] Inhaled ribavirin has been given either continuously (6 g over 18 hours per 24 hours) or intermittently (2 g over 2–3 hours three times per day). In a prospective randomized trial comparing these two dosing schedules in immunocompromised patients with RSV infection, the intermittent schedule appeared to be more effective in preventing development of lower respiratory disease.[254] The efficacy of systemic ribavirin, administered by either the oral or the intravenous route, has been reviewed in retrospective case studies of immunocompromised patients with RSV infection, with some suggestion of benefit.[255–258] Oral ribavirin also has the advantage of being able to be given to outpatients at substantially decreased cost, although concentrations of ribavirin in respiratory secretions are likely to be substantially lower than that found following aerosolized administration. Retrospective evaluation of oral ribavirin has been reported in multiple patient populations including HCT and lung transplant recipients.[259,260]

Immunoglobulin or anti-F monoclonal antibody therapy for the treatment of RSV infection in highly immunocompromised patients is based on retrospective observational studies. More recent studies in HCT patients have demonstrated the confounding influence in retrospective analyses based on whether or not subjects required oxygen at diagnosis or type of stem cell source used for transplant

(bone marrow vs. peripheral).[261] Although some studies suggest a trend toward diminished morbidity and progression to lower respiratory tract disease with IVIG/antibody use, other studies suggest that antibody-based therapies are not independently associated with improved outcome.[a] Overall, monoclonal antibodies appear safe and well tolerated by highly immunosuppressed patients. Prophylactic administration of palivizumab to immunocompromised patients is not routinely recommended,[117] although use of this drug in outbreak settings including adult HCT units and newborn intensive care units has been reported.[264] Palivizumab is extraordinarily expensive in dosages administered to adults and is approved only for intramuscular use, further complicating its potential use in adults undergoing transplant.

Multiple approaches are being investigated to develop new antiviral therapies specific for RSV disease.[265,266] New approaches include antisense/small interfering RNA (siRNA) inhibitors and inhibitors of attachment and fusion proteins of RSV including small molecule peptide fusion inhibitors, N protein inhibitors, and RNA-dependent RNA polymerase inhibitors.[192] Oligonucleotides that interfere with viral RNA, or siRNA inhibitors, have shown some promise, with a study showing inhaled RNA interference therapy in lung transplant recipients to be safe but not resulting in a reduction of viral load or symptom scores. However, per-protocol follow-up in this prospective trial demonstrated a reduction in the development of bronchiolitis obliterans syndrome.[214,266–269] A small-molecule fusion inhibitor, presatovir (GS-5806; Gilead, Foster City, CA), administered orally, reduced symptom scores and RSV loads in nasal washes in a placebo-controlled trial in healthy adult volunteers challenged with RSV.[270] Presatovir was evaluated in placebo-controlled trials in hospitalized adults and HSCT recipients with RSV-associated lower respiratory tract disease, but study endpoints were not met, and further studies with presatovir are not being conducted.[271] Another approach is the use of nucleoside analogues, which inhibit the RSV RNA polymerase. In an RSV challenge study in healthy adults, the oral cytidine nucleoside analogue, ALS-008176, was shown to decrease viral load and severity of symptoms compared with placebo.[269] Additional studies of orally administered ALS-008176 are underway in infants and adults infected with RSV.

TABLE 158.3 Infection Control Procedures, Both Standard Precautions and Contact Precautions, for Prevention of RSV Infection Recommended by the Centers for Disease Control and Prevention

RECOMMENDATION CATEGORY, PROCEDURE	COMMENTS
Category 1-B Recommendations[262,263]	
Hand washing	Water with soap or antibacterial agent or waterless antiseptic hand rub
Wearing gloves	Combined with hand washing before and after each glove change; may diminish self-inoculation
Wearing gowns	When direct contact with patient or patient secretions is likely
Wearing masks plus eye protection	Eyes and nose are major sites for inoculation
Housing patient in private room or in a cohort isolated from other patients	Patients with documented infection can be grouped and isolated from other patients; beds should be separated by >0.9 m
Using dedicated patient care equipment	Equipment, including toys, assigned to specific patients
Sometimes Recommended With Less or No Supporting Evidence	
Staff assigned according to patient's RSV status	Specific staff care only for patients with RSV infection
Visitor restrictions during RSV season	Some qualify by restricting young children only
Screening visitors for illness during RSV season	Visitor assessed by trained personnel or advised by use of an educational patient information list

RSV, Respiratory syncytial virus.
From Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics *2014;134:e1474–e150; and Hall CB. Nosocomial respiratory syncytial virus infections: the "Cold War" has not ended.* Clin Infect Dis. *2000;360:588–598.*

PREVENTION

Infection Prevention

Prevention rather than treatment is the preferable goal for control of RSV infection. Avoiding infection in the home setting through interruption of the transmission of the virus is difficult and unlikely to be truly effective. In child care settings with good infection control practices in place, spread of RSV to more than 50% of children within a week has been documented.[272] Nonetheless, general precautions including good hand hygiene; use of hand-rub antiseptic products; and regular care or disinfection of contaminated tissues, stethoscopes, toys, and other objects likely to be contaminated with secretions are widely recommended in medical settings.[273,274]

On hospital wards, RSV poses a particular hazard for nosocomial spread.[74,197,275–277] Annual outbreaks occur with widespread infection among both children and adults including medical personnel who may continue to work despite upper respiratory tract signs. Considerable morbidity and mortality have been associated with nosocomial RSV infection among patients with underlying conditions, especially prematurity, cardiopulmonary disease, and immunocompromising conditions. Strict adherence to recommended guidelines is essential and cost-effective.

RSV may be spread by close contact and by direct inoculation of large droplets from the secretions of an infected person as well as by indirect spread from hands that touch infectious secretions in the environment.[74,276] Careful hand hygiene by all personnel is integral to preventing nosocomial transmission (Table 158.3). Additional procedures aimed at preventing self-inoculation include wearing of eye-nose goggles and gloves. Procedures aimed at reducing the risk for the introduction and spread of RSV to other personnel and patients include wearing gowns for close contact with infected patients, isolation or cohorting of infected patients, and use of rapid diagnostic techniques to assess symptomatic individuals. In addition, during the RSV season, staff with signs of respiratory illness should not care for high-risk patients, and visitors should be screened for respiratory illness.[74]

Prophylaxis

Prophylaxis using the passive administration of RSV-specific antibody currently is available primarily for groups most at risk for developing severe or complicated RSV disease. In high-risk children administered monthly doses of IVIG containing high levels of RSV neutralizing antibody (RSV-IVIG) or intramuscular monoclonal antibody, reduced rates of hospitalization due to RSV infection have been shown. RSV-IVIG has been replaced by palivizumab, a humanized monoclonal antibody developed from a mouse monoclonal antibody directed against a protective epitope of the RSV F protein. Immunoprophylaxis with five monthly intramuscular doses of 15 mg/kg initiated 1 month before the RSV season demonstrated a 4.4% to 5.8% absolute reduction in hospitalization rates among selected high-risk infants.[117] It has consistently been demonstrated that preterm infants born before 29 weeks' gestation are at greatest risk for hospitalization as a result of RSV infection.[117] For preterm infants born after 29 weeks' gestation who are otherwise healthy, there is no distinct cutoff at which the benefits of prophylaxis are clear. Palivizumab administration does not prevent infection with RSV but is associated with decreased clinical severity, risk for developing lower respiratory tract disease, and need for hospitalization.

At the present time, palivizumab is recommended by the American Academy of Pediatrics for a small group of infants in the first year of life who are considered to have a high risk for developing severe RSV disease.[278] Included are infants with prematurity (<29 weeks' gestation), chronic lung disease, functionally important cardiac disease that has not been surgically corrected, and chronic conditions that interfere with the handling of respiratory secretions.[117] Palivizumab is not

[a]References 197, 198, 208, 253, 262, 263.

recommended in the second year of life except for preterm children who required at least 28 days of supplemental oxygen at birth and continue to require supplemental oxygen, long-term systemic steroid therapy, or diuretic therapy within 6 months of the start of the second RSV season. Palivizumab is administered at a dose of 15 mg/kg for a maximum of 5 monthly doses during the RSV season. Prophylaxis should be discontinued in any child who experiences a breakthrough RSV hospitalization.[117]

Controversy exists concerning the extent of the use of palivizumab prophylaxis based primarily on concern regarding its considerable cost relative to its benefit. Economic analyses in general have not shown an overall savings in health care costs for prophylaxis of all infants less than 32 weeks' gestation and with underlying high-risk conditions, but the benefit relative to the cost among those at highest risk increases.[279,280] Mathematical modeling has suggested that four monthly doses of palivizumab initiated later in the season could provide comparable protection to five doses and improve cost-effectiveness.[281] Although the mortality from RSV infection even among high-risk infants in the United States is very low, studies evaluating the impact of decreased palivizumab administration in infants >29 weeks' gestation have demonstrated increased rates of hospital admission in infants 29 to 34 weeks' gestation and demonstrated increased morbidity as well as mortality.[282,283] The effect of palivizumab on the long-term sequelae of RSV infection has not been adequately evaluated.[241,242,284]

Children with severe immunodeficiencies may benefit from RSV prophylaxis, particularly when used early in life to prevent pretransplant infections. The use of palivizumab during or after transplant remains controversial. Using a decision analysis model to evaluate palivizumab prophylaxis to prevent RSV mortality after HCT in children, the survival rate was estimated to increase by 10%, and 12 children would need to be treated to prevent one fatal RSV infection.[264]

Additional products have been and are being evaluated for the prevention of RSV disease in high-risk individuals. The enhanced potency humanized monoclonal antibody against the F protein, motavizumab, had a 20-fold higher rate in RSV neutralization compared with palivizumab and prevented RSV disease in preterm infants, but this agent was not licensed due to safety issues (primarily rash reactions).[285,286] A trial of motavizumab in healthy Native American infants demonstrated an 87% relative reduction in hospitalizations for RSV,[287]marking the first time an anti-RSV antibody prevented serious RSV disease in healthy term infants. A new potent monoclonal antibody, MEDI18897, also known as nirsevimab, has an active site directed against the prefusion form of the RSV F protein and has approximately 100-fold potency in vitro compared with palivizumab.[288,289] This antibody is a totally human recombinant IgG1 κ monoclonal antibody with an extended half-life due to a 3–amino acid mutation in the Fc region enhancing binding within the lysosome and preventing antibody degradation and increasing recirculation to the cell surface. Safety and tolerability in adults has been established, and this compound is in clinical trials in infants.[290,291] The increased half-life may potentially provide passive protection for infants throughout an entire RSV season following a single intramuscular injection. This compound is intended for use in healthy term and at-risk preterm infants entering their first RSV season in both developed and developing countries.

Immunization

No effective vaccine for prevention of RSV in any population is available. Challenges in vaccine development include concern that an unpredictable abnormal immune-mediated response to subsequent infection could occur, such as was observed during the trials with the initial formalin-inactivated RSV vaccine.[96] Advances in molecular technology have resulted in new RSV vaccine candidates including subunit, particle, vector-based, and live-attenuated/chimeric vaccines. Recent crystallographic characterization of the RSV F-protein has led to design of a structure-based vaccine consisting of a stable prefusion form of the F-protein. This immunogen induces high levels of neutralizing antibody in mice, hamsters, and macaques and is a promising vaccine candidate.[289]

Multiple approaches are being pursued for vaccines to protect two major groups at highest risk for severe RSV disease: very young infants and frail elderly adults.[292,293] Immunization for infants would optimally be initiated within the first weeks of life because most hospitalization for RSV occurs in the first several months of life. Another approach for protection of infants is maternal immunization with a subunit vaccine, similar to the approach taken with influenza virus and tetanus.[294,295] Boosting of maternal neutralizing antibodies using the RSV F protein could potentially reduce the severity of infection in the first few months of life or delay infection until the infant is older when disease is less severe.[296,297] A large international clinical trial with a nanoparticle F protein vaccine candidate given to pregnant women at 32 to 36 weeks of gestation is underway (Novavax, Gaithersburg, MD). Infants with decreased transplacental antibody transfer such as premature infants or infants born to mothers with decreased maternal antibody transfer due to HIV infection or maternal hypergammaglobulinemia would benefit less from this strategy.[298]

An approach for older infants or children is intranasal immunization with live-attenuated vaccine strains.[299,300] The use of reverse genetics has resulted in the generation of candidate strains that contain mutations specifically chosen for their attenuating, immunogenic, and other advantageous characteristics. These candidate designer gene vaccines are potentially safer and have increased breadth of antigenic expression.[300] An advantage of this approach is that immunization should induce nasal IgA and cellular responses as well as serum antibody. An alternative approach is the use of live vectored or chimeric vaccines to deliver RSV antigens.

Subunit F or G vaccines are being considered for older adults. Several subunit F protein vaccines have been shown to be safe and immunogenic in individuals seropositive from previous natural RSV infection and should theoretically boost neutralizing serum antibodies, especially in adults, although results from several trials in elderly adults have been disappointing. Such a vaccine may also offer the possibility of boosting immunity among older children and adults who have an increased risk for developing more severe RSV infection.

ACKNOWLEDGMENTS

We acknowledge the contribution of Caroline B. Hall to past editions of this chapter.

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159 Human Metapneumovirus

Angela R. Branche and Ann R. Falsey

SHORT VIEW SUMMARY

Definition
- Human metapneumovirus (hMPV) is a pneumovirus that causes acute respiratory tract infections.

Epidemiology
- Distribution is worldwide.
- Infection is most common in the late winter and spring in temperate climates.
- hMPV cocirculates with other seasonal viruses, including influenza and respiratory syncytial virus (RSV).
- Infection is universal by age 5 years.
- Reinfections occur throughout life.

Microbiology
- Virus is in the family Pneumoviridae, genus *Metapneumovirus;* has a nonsegmented single-stranded RNA (ssRNA).
- Diverged from avian metapneumoviruses 200 to 300 years ago.
- Two major genotypes (A and B) and four subgroups (A1, A2, B1, and B2).

Diagnosis
- Clinical syndrome is not distinct and ranges from common cold to acute respiratory distress syndrome.
- Bronchiolitis, asthma exacerbations, pneumonia occur in children.
- Diagnosis is best accomplished by reverse-transcriptase polymerase chain reaction (RT-PCR) of respiratory secretions.
- Immunofluorescence testing of respiratory secretions is less sensitive than RT-PCR but an acceptable method of diagnosis.

Therapy
- Treatment is supportive.
- Intravenous gamma globulin and ribavirin have been used in severely ill immunocompromised patients but efficacy, if any, is unclear.

Prevention
- No vaccine is available.
- Infection control measures include hand hygiene and use of masks, gowns, and gloves in outbreak situations.

Human metapneumovirus (hMPV) was isolated from the nasopharyngeal secretions collected over a 20-year period from 28 Dutch children with upper respiratory infections in the Netherlands in 2001.[1] This previously unidentified virus exhibited paramyxovirus-like morphology, and genetic analysis was most similar to respiratory syncytial virus (RSV). Serologic analyses indicate that infection with hMPV is nearly universal by age 5 years, and numerous studies show that hMPV can cause lower respiratory tract infections (LRTIs) resulting in substantial morbidity and cost.[2]

VIROLOGY

hMPV is a nonsegmented, single-stranded, negative-sense RNA virus belonging to the order Mononegavirales, family Pneumoviridae, genus *Metapneumovirus*.[1,3] hMPV particles are pleomorphic, spherical, or filamentous with a lipid envelope and projections on the surface as imaged by electron microscopy (Fig. 159.1).[1,4] Within the family Pneumoviridae are the genera *Orthopneumovirus* and *Metapneumovirus*.[5] Members of the *Orthopneumovirus* genus include human RSV and a number of animal pathogens, such as bovine, ovine, and caprine RSVs, and the pneumonia virus of mice. Until 2001, the only member of the *Metapneumovirus* genus was avian pneumovirus (APV), also known as *turkey rhinotracheitis virus. Orthopneumovirus* and *Metapneumovirus* were categorized into separate genera because of different gene numbers and order and because the viruses shared only 40% homology.

The original genetic analysis of hMPV by van den Hoogen and colleagues indicated a gene order of 3′-*N-P-M-F-M2-SH-G-L*-5′.[1] These eight genes encode for nine proteins and include nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), transcription elongation factor (M2-1), RNA synthesis regulatory factor (M2-2), small hydrophobic protein (SH), glycoprotein (G), and major polymerase subunit (L).[6] The lipid envelope of the virus is covered at the interior by the M protein and contains three surface glycoproteins (F, SH, and G) that form spikes of 13 to17 mm. Within the envelope lays a helical ribonucleoprotein complex, consisting of N, P, and L proteins and the hMPV genome.[7] The F protein of hMPV is a trimeric, type I membrane glycoprotein and serves both to bind cellular receptors and to mediate fusion.[8] Monomers of F are translated as an inactive precursor that is proteolytically cleaved by host proteases into two disulfide-linked subunits, F1 and F2, that remain covalently bound and create trimers of the metastable prefusion F conformation. During fusion, the F protein refolds through a series of unstable intermediates into a highly stable postfusion F conformation.[9] There is increasing evidence that multiple pathways may be involved in hMPV entry into cells, involving proteoglycan, integrin, and C-type lectin receptors as well as clathrin-mediated endocytosis.[10–13] For most hMPV strains fusion occurs at neutral pH, although a few strains demonstrate enhanced fusion at acidic pH that may be mediated by a pH sensor located on the prefusion F conformation.[7,9,14–16] The heptad repeat domain A in the F protein appears to be critical for fusogenecity, and recent work shows that cell-to-cell viral spread involves the creation of actin-mediated networks of branched cell–associated filaments.[17,18] Like RSV, the G protein of hMPV is heavily glycosylated and appears to serve similar functions in the viral life cycle. Current evidence suggests that the G protein helps tether virus particles to the cell surface and confers optimal infectivity but is not critical for viral attachment.[8] The absence of the nonstructural interferon-inhibiting genes *NS1* and *NS2* is the most striking difference between hMPV and RSV and confirmed its classification in the *Metapneumovirus* genus (Fig. 159.2).[19]

Sequence homology of *N, P, M*, and *F* genes of hMPV indicate the highest identity with APV serotype C (APV-C), one of four avian pneumovirus types.[19] Given the close relationship between hMPV and APV-C, it is speculated that the human virus originated from birds. Phylogenetic analyses of multiple hMPV gene sequences suggest that hMPV diverged from APV about 200 to 300 years ago.[20,21] Current evidence indicates that hMPV is a primary human pathogen rather than an avian pathogen that incidentally infects humans.[1,22]

Genetic variation among hMPV isolates clusters in two major genotypes (A and B) and five subgroups with two sublineages (A1, A2a, A2b, B1, and B2).[23,24] Novel sublineages with diversity in the *F* and *G*

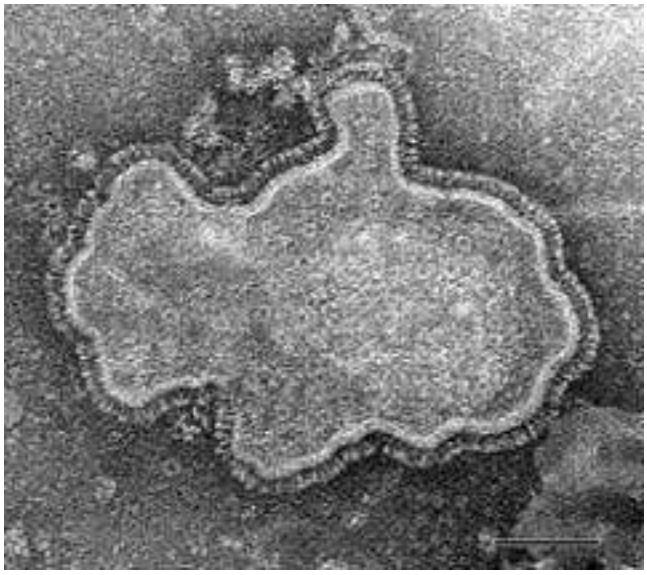

FIG. 159.1 Negative-stain electron micrograph of human metapneumovirus. This pleomorphic form of the virus is stain penetrated, thereby permitting visualization of portions of the virus envelope and nucleocapsid. A border composed of the surface projection proteins may also be seen around the virus periphery. Phosphotungstic acid negative stain, pH 6.5. Bar marker = 100 nm. *(Courtesy Charles Humphrey, PhD, research biologist, and Dean Erdman, PhD, Centers for Disease Control and Prevention.)*

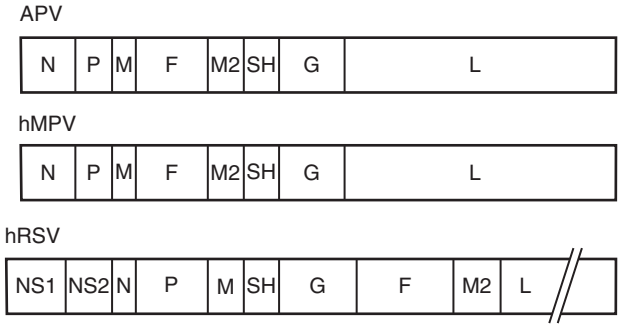

FIG. 159.2 Schematic representation of the gene order and sequence of avian pneumovirus (APV), human metapneumovirus (hMPV), and human respiratory syncytial virus (hRSV). Gene lengths are not drawn to scale.

genes have recently been described.[25,26] Isolates representing the two major genotypes have been completely sequenced, and amino acid identities between genotypes were 80% and 90%, respectively, similar to the differences found between RSV groups A and B. The greatest diversity is found in two of the surface glycoproteins, G and SH: 59% and 37% identity, respectively, which is considerably greater than the diversity observed in the RSV groups.

PATHOGENESIS AND HOST RESPONSE

hMPV infects the upper and lower respiratory tract. Type 2 alveolar and bronchiolar epithelial hMPV-infected cells have been observed in cynomolgus macaques.[7] In rodent models, hMPV replicates efficiently in the upper and lower airways with peak viral titers between days 3 and 5. Lung infection is characterized by alveolar and interstitial inflammation.[7] Limited human pathologic data indicate that bronchiolar epithelial cells are infected with hMPV, and prolonged inflammation may be observed. Bronchoalveolar lavage specimens from hMPV-infected children reveal epithelial degenerative changes, eosinophilic cytoplasmic inclusions, and multinucleated giant cells.[27] Animal data suggest that hMPV can persist in neuronal processes that innervate the lung, possibly explaining the prolonged pulmonary inflammation observed in children.[28] Lower airway involvement also occurs in adults with alveolar damage and hyaline membranes and hMPV antigens in bronchiolar epithelial cells demonstrated in autopsy samples.[29] Although hMPV and RSV are closely related viruses, the host immune responses to these two viruses are different.[7,30,31] hMPV lacks two nonstructural proteins found in RSV that are known to inhibit host interferon production, and likely uses other mechanisms to subvert innate immune responses.[32,33] In human volunteers, peripheral blood mononuclear cells stimulated with hMPV produce a stronger innate and weaker adaptive cytokine response than RSV.[34] In contrast, inflammatory cytokines measured in nasal secretions of babies infected with hMPV are less than in infants infected with RSV.[35]

Similar to RSV, immunity to hMPV is incomplete and reinfections occur throughout life in part due to poor development of T- and B-cell immunity.[36–39] The primary target of neutralizing antibody appears to be the F protein, and antibody directed against F is protective in animal models.[40] In contrast to RSV, where the most highly neutralizing antibodies are directed toward epitopes on prefusion F, pre- and postfusion hMPV F share most neutralizing epitopes.[9,41] Although serologic response is specific, several monoclonal antibodies to postfusion F neutralize both RSV and hMPV, raising the possibility of common therapeutic molecules.[41] The other surface proteins, G and SH, are weakly immunogenic and, unlike RSV, antibody to the G protein has been shown to be nonneutralizing.[42] Lymphopenia and receipt of cytotoxic therapy are risk factors for severe hMPV disease, suggesting that cellular immunity is important for hMPV illness resolution. Animal studies of CD4 and CD8 T-cell–depleted mice indicate that T cells are important for viral clearance but also contribute to disease pathogenesis.[43] Specific T-cell epitopes have been mapped to the M2 and N proteins.[7]

Because of the winter seasonality of hMPV, coinfection with other viral and bacterial respiratory pathogens ranges from 5% to 60%.[7] Several studies demonstrate that dual hMPV and RSV infection in children results in higher rates of ventilatory support, hypoxemia, and longer hospital stays, although others do not confirm these observations.[44–49] Importantly, the majority of hMPV infections are not associated with other viruses, indicating hMPV is the primary pathogen.[7] Similar to influenza, hMPV infection has been associated with bacterial pathogens such as *Streptococcus pneumoniae,* and bacterial coinfection is thought to contribute to severe disease and has been linked to increased mortality.[50–54] In children, epidemiologic studies have correlated hMPV activity with invasive pneumococcal disease, and introduction of the 7-valent pneumococcal conjugated vaccine has been shown to significantly reduce severe hMPV infections.[55,56] In one study of hospitalized adults, 39% of patients with hMPV infection had evidence of concomitant bacterial infection.[57] Animal models confirm that both influenza and hMPV predispose to severe pneumococcal infection but mechanisms of coinfection differ. The presence of the neuraminidases of influenza virus appears to promote the adherence of bacteria to the respiratory epithelium, whereas replication of hMPV is required to increase superinfection of pneumococcus in the respiratory tract.[58] In addition, hMPV infection appears to impair neutrophil recruitment to the airways, leading to delayed bacterial clearance.[59]

EPIDEMIOLOGY

hMPV is a ubiquitous pathogen that affects all age groups.[1,36,50,60] Seroprevalence studies indicate that by age 5 years, most children have been infected with hMPV. Illness caused by both RSV and hMPV appears to be common in young children, but primary infection with hMPV occurs at a slightly older age.[61,62] hMPV accounts for 2% to 3% of all symptomatic respiratory infections among children in the first 5 years of life.[60–66] In the United States, annual hospitalization rates are 3 per 1000 children younger than 6 months of age, 2 per 1000 children 6 to 11 months of age, and 1 per 1000 children younger than 5 years of age, resulting in approximately 20,000 to 27,000 yearly hospitalizations in children younger than 18 years of age.[2,60] As a cause of LRTI in children, hMPV ranks second to RSV with disease burdens similar to influenza and parainfluenza both in developed and developing countries.[53,60,67–72] A recently conducted study of community-acquired pneumonia in three cities in the United States (Nashville, TN; Memphis, TN; and Salt Lake City, UT) found that 13% of hospitalizations in children were caused

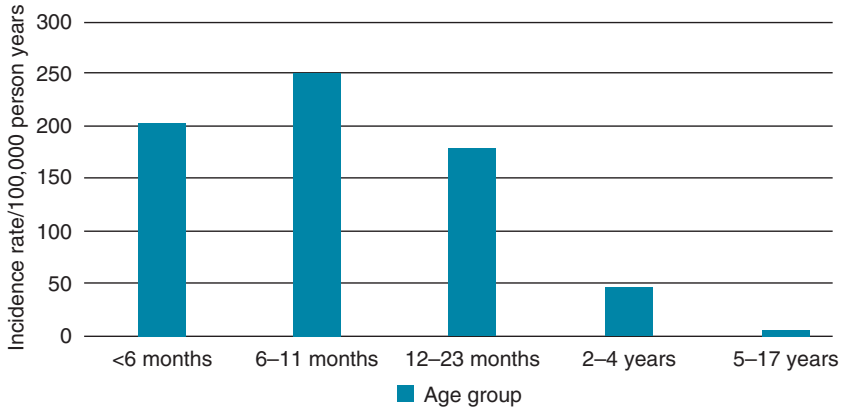

FIG. 159.3 Population-based incidence of hospitalization for human metapneumovirus (hMPV). hMPV infection rates per 100,000 person-years among children in Salt Lake City, Utah, from 2007 to 2013. *(Reproduced with permission of Journal of Pediatric Infectious Disease Society.)*

by hMPV infection.[73] A number of studies indicate that peak age for hospitalization with hMPV is somewhat older than for RSV infection, with highest rates noted between 6 and 11 months of age (Fig. 159.3).[2,60–62] hMPV also accounts for a significant number of outpatient and emergency room visits, with rates of 55 and 13 per 1000 children, respectively. Reinfection occurs throughout life, and about 2% of acute respiratory illnesses in the general adult population are due to hMPV.[36]

Numerous epidemiologic studies have now documented worldwide hMPV circulation.[1,56,61,65,74–78] In temperate climates, the virus circulates predominantly in the late winter and spring months, frequently overlapping with other seasonal respiratory pathogens and generally peaking 1 to 2 months later than RSV.[50,64,79,80] However, low levels of hMPV activity can occur during the summer months.[66,74] In the Southern Hemisphere, hMPV circulates in the summer, and in the subtropics, peak activity is in the spring and early summer.[61] Low temperature and vapor pressure and increased wind speed are associated with increased hMPV activity.[81] Most studies spanning multiple seasons indicate variation in intensity, with some showing a fairly regular biannual pattern of alternating large and small outbreaks and others showing less regular patterns of activity.[2,36,82,83] In a 5-year study from Sweden, the average incidence of hMPV infection was 2.9% but ranged from 0.8% to 5.9% depending on the year.[64] The two major genotypes of hMPV often circulate concurrently within the same community while the prevalence of genotypes and subgroups varies significantly each year, suggesting that immune pressure may play a role in the dominant circulating genotype.[65,79,83,84]

CLINICAL MANIFESTATIONS

The clinical manifestations of hMPV infection are similar to those of RSV and range from mild upper respiratory infection to bronchiolitis and severe pneumonia requiring mechanical ventilation.[6,50,85] The spectrum of disease depends on the age and the health of the host, but most infections are symptomatic.[69,83,86] The clinical syndrome is not distinct, with fever, cough, and coryza the most common symptoms. The incubation period is not precisely known, although cases of nosocomial transmission suggest an incubation period of about 5 to 6 days.[1]

Children

Most young children with hMPV infection exhibit fever, cough, sore throat, and rhinorrhea (Table 159.1).[61,62,87,88] Fever appears to be more common with hMPV than with RSV, and febrile seizures were noted in 16% of patients with hMPV compared with 3.1% in RSV-infected children in one study.[61] Wheezing is also common, with rates ranging from 22% to 83% depending on the age group studied.[62,63] In children younger than 3 years, acute otitis media has been documented in up to 60% of infected children, and hMPV RNA can be detected in middle ear fluid in some cases.[89,90] Conjunctivitis, pharyngitis, and laryngitis all occur with variable frequencies.[62,88,91] Less common symptoms include maculopapular truncal rash and diarrhea.[61] Neurologic complications

TABLE 159.1 Comparison of Signs and Symptoms in Children with hMPV, RSV, and Influenza A

	hMPV (%)	RSV (%)	INFLUENZA A (%)
Fever	52–80	47–57	78–81
Cough	90–100	99	96
Rhinorrhea	88–92	91	84
Retraction	65–92	95	82
Wheezing	22–83	23–65	5–57
Lacrimation	25	31	31
Diarrhea	8–17	17	9–27
Vomiting	10–25	8	10

hMPV, Human metapneumovirus; *RSV,* respiratory syncytial virus.
Data compiled from references 62, 63, and 67.

such as seizures, ataxia, and encephalitis appear to be approximately 10 times more common in children with hMPV compared to those infected with RSV.[92] hMPV RNA has been identified in postmortem brain tissue and cerebrospinal fluid in some cases, and a postinfectious reactive inflammatory demyelinating process has been postulated in others.[93,94]

Laboratory findings are relatively nonspecific, with lymphopenia and elevated hepatic transaminase values described.[61] Hypoxia and radiographic changes are common in hMPV-infected children, and abnormal chest radiographs have been found in 26% to 87% of hospitalized children.[61,88,95] Radiographic findings include perihilar infiltrates (87%), hyperinflation (69%), patchy opacities, and atelectasis (Fig. 159.4). Lobar consolidation occurs less frequently (18%) and may be due to bacterial complications.[56,95,96] Clinical diagnoses most frequently associated with hMPV hospitalization in children include bronchiolitis (47%–84%), asthma (11%–25%), and pneumonia (11%–17%).[60–62,91,97] The mean length of hospitalization for these children was 3 to 5 days.

hMPV has been strongly associated with bronchiolitis, pneumonia, and croup and has been detected in 4% to 8% of recurrent wheezing episodes.[98–100] The association of hMPV and asthma may in part depend on the age group studied. In older children, the dominant pathogen associated with asthma exacerbations was rhinovirus.[100] In contrast, in a case-controlled study of 133 children hospitalized with acute wheezing, the presence of hMPV was significant in children younger than 3 years.[101] In addition, infants hospitalized with hMPV bronchiolitis may be at increased risk for developing recurrent wheezing. In one study, hMPV bronchiolitis was associated with wheezing in 52% of patients at 2 years and 64% of patients at 5 years.[102]

LRTI during hMPV infection appears to be common, with hMPV RNA detected in 4% to 23% of children hospitalized with respiratory



FIG. 159.4 Chest radiograph of child with human metapneumovirus (hMPV) infection. This 23-month-old child admitted to an intensive care unit required mechanical ventilation for 4 days. A chest radiograph demonstrated patchy bilateral infiltrates. Nasal wash was positive for 5.5×10^6 copies/mL of hMPV by reverse-transcriptase polymerase chain reaction. The child recovered and was discharged to home off antibiotics on hospital day 6. *(Courtesy Janet A. Englund, MD, Professor of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, WA.)*

illnesses; the highest rates were found in children younger than 3 years.[103–106] In a report of 815 children hospitalized with hMPV in Salt Lake City, Utah, 18% were treated in the intensive care unit (ICU) and 6% required mechanical ventilation, with older children more likely to require ICU care.[2] Moreover, a recent cluster of severe respiratory illnesses, including deaths in the 2 of 6 children infected with hMPV, highlights the potential severity of hMPV.[107] The majority of these children had underlying medical conditions, including premature birth, congenital heart disease, and bronchopulmonary dysplasia. Overall, prematurity confers the greatest risk for severe disease, with an odds ratio of 13.97 compared to 3.08 for RSV.[108]

In some reports, a high viral load in respiratory secretions has been correlated with disease severity, including LRTI and hospitalization in young children infected with hMPV.[109–112] There are conflicting data regarding disease severity and the role of hMPV genotypes. Several studies suggest that severity or specific symptoms may vary with genotype.[68,113] However, the bulk of current evidence indicates that severity of illness associated with hMPV A is similar to hMPV B infection.[79,110] Other risk factors for severe disease include female sex, prematurity, esophageal reflux, underlying heart and lung disease, lack of breastfeeding, vitamin D deficiency, and household crowding.[68,74,96,114]

Adults

The clinical manifestations of hMPV infection in adults, like those in children, appear to depend on age and health status. In a 4-year prospective study of respiratory illnesses in New York, hMPV was detected by serology or reverse-transcriptase polymerase chain reaction (RT-PCR) in 2.2% to 10.5% of young, healthy elderly, and high-risk adult cohorts, depending on the year.[36] Healthy adults generally present with mild influenza-like illness and common cold syndromes, with some remaining asymptomatic.[36,50,115] In addition to respiratory illnesses, a mononucleosis-like syndrome due to hMPV has also been reported.[116] Rarely, severe acute respiratory distress syndrome (ARDS) has been associated with hMPV infections in young adults without underlying disease and during pregnancy.[117–119] Recent reports also describe hMPV-associated encephalitis after influenza-like illness in adults presenting with altered mental status, headache, seizures, and either lymphocytic or mononuclear pleocytosis.[120,121] In one case, contrast magnetic resonance imaging showed bilateral subcortical and external capsule fluid-attenuated inversion recovery and diffusion-weighted imaging hyperintensities without leptomeningeal or parenchymal enhancement.[120] Finally, two cases of myopericarditis have been reported, one case in a young previously healthy 25-year-old male and a second case in a 73-year-old woman with multiple comorbidities.[122,123] Both cases were associated with pulmonary edema and echocardiography demonstrating depressed systolic ejection fraction.

With increasing age, the impact of hMPV is greater. Although healthy older adults generally do not require medical attention, wheezing and dyspnea are more common than in young adults. In contrast, adults with underlying cardiopulmonary conditions are at high risk for hospitalization where illnesses are primarily characterized by severe cough and lack of fever. It is estimated that hMPV infection accounts for 4% to 12% of COPD, 7% of asthma, and 4% of community-acquired pneumonia admissions in adults.[51,124–126] Chest radiographs revealing patchy, multilobar infiltrates associated with small pleural effusions are noted in 50% of cases.[124] In studies of patients hospitalized in New York and Tennessee, 4.5% to 8.5% of illnesses were identified as hMPV related, with those infected having high rates of chronic cardiopulmonary conditions.[36,127] In a 4-year study of 128 patients hospitalized with hMPV, 31% required ICU admission, the majority of whom also required mechanical ventilation and half of whom had ARDS.[85] Solid organ transplant recipients (20%), chronic respiratory insufficiency (13%), congestive heart failure (25%), and severe COPD (20%) were the most common underlying comorbidities. Of note, 6 patients with ARDS had only minor comorbidities. Lastly, hMPV outbreaks have been reported in long-term care facilities and may result in high rates of LRTI and death.[29,128]

Immunocompromised Hosts

As is found with other common respiratory viruses, hMPV infection can be associated with severe illness and pneumonitis in immunocompromised patients.[129–131] hMPV infections have been described in patients receiving chemotherapy, recipients of solid organ transplants, and recipients of hematopoietic stem cell transplants (HSCTs).[132–135] In patients with cancer, hematologic malignancy, nosocomial infection, and hypoxia are risk factors for LRTI.[135] Patients receiving HSCTs are at highest risk of adverse outcomes. Symptoms of hMPV infection typically begin with nasal congestion and cough but may evolve into LRTI with diffuse pneumonia and respiratory failure in one-third of cases, with reported fatality rates as high as 80% (mean, 26%–28%).[129,136] In one report of five hMPV-infected HSCT recipients, four developed rapidly progressive respiratory failure and had a septic shock–like picture with pulmonary hemorrhage.[129] Risk factors for progression to LRTI include high-dose steroid use within 2 weeks prior to diagnosis, low lymphocyte count, timing since HSCT (before day 30), nosocomial infection, and hypoxia at presentation.[136,137] No correlation has been shown between viral load and progression to LRTI. Chest radiographs most often show bilateral airspace opacities, and computed tomography scan findings consist of patchy areas of ground-glass opacification and multiple nodules.[134,138] Along with diffuse alveolar damage, smudge cell formation has been found on histopathologic examination.[139] Although severe hMPV illness is often observed in HSCT recipients, mild disease and even persistent asymptomatic shedding have also been described.[132,133,140] Bronchiolitis obliterans and allograft rejection in lung transplant recipients have been linked to hMPV infection, although this association has not been conclusively proven.[131,141] Among HIV-infected children, hMPV has been associated with higher rates of bronchospasm, bacterial complications, and mortality compared with non–HIV-infected children, although the use of antiretroviral therapy may ameliorate severity.[56,70,142]

DIAGNOSIS

The virus remained unidentified for many years because the clinical syndrome is not distinct and isolation of the virus with standard cell culture techniques is difficult. hMPV replicates slowly in cell culture, does not display hemagglutinating activity, and is relatively trypsin dependent.[1] Four methods of diagnosis are currently used: viral culture, immunofluorescence assay (IFA), RT-PCR, and serology.

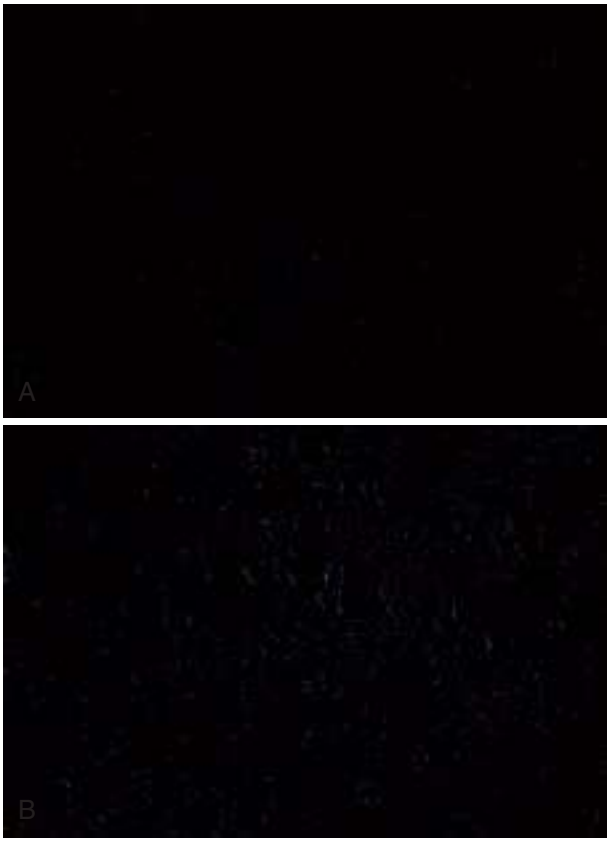

FIG. 159.5 Photomicrograph of human metapneumovirus (hMPV) (CAN97–83) in LLC-MK2 cells. LLC-MK2 cells in a semiconfluent monolayer were infected with hMPV (provided by Dr. Guy Boivan) and allowed to grow for 11 days. (A) Uninfected LLC-MK2 cells. (B) Infected LLC-MK2 cells. Granular cytopathic effects, without syncytia formation, are evident.

Viral Culture

The original isolations of hMPV were carried out on tertiary cynomolgus monkey kidney (tMK) cells or rhesus monkey kidney (LLC-MK2) cells in medium containing trypsin. Since that time a number of other cell lines have been found to support growth of hMPV, including Vero, BEAS-2B, A549, and Hep G2 cells.[7] The characteristic cytopathic effect in LLC-MK2 cells consists of small, round, granular, and refringent cells without large syncytia, and it is usually apparent after a mean of 17 days (range, 3–23 days) (Fig. 159.5).[4,50] Confirmation of hMPV infection requires either IFA with hMPV-specific antibodies or RT-PCR of the cell supernatant. Although use of shell vial cultures may speed time to identification of virus, delays in diagnosis limit clinical utility.[143]

Immunofluorescence Assay

Virus-specific monoclonal antibodies are now commercially available for direct detection of hMPV in respiratory secretions using IFA.[144,145] Although this technique is less sensitive than RT-PCR, it may be a useful alternative in microbiology laboratories that lack molecular diagnostic capabilities. Similar to RSV, rapid diagnosis of hMPV by IFA in adults may be insensitive owing to low viral load in secretions.

Reverse-Transcriptase Polymerase Chain Reaction

Because of difficulties with cell culture, molecular techniques for diagnosis of hMPV are favored as the optimal methods of diagnosis and typically use conserved regions of the *F* and *N* genes.[146,147] Detection of hMPV has been successfully incorporated into several commercially available multiplex PCR assays that simultaneously detect panels of common respiratory viruses.[148] Nasal washes and nasopharyngeal swabs are the most common respiratory samples tested, but bronchoalveolar lavage fluid and sputum can be used. Several papers suggest that, if sputum can be obtained, the yield for hMPV is higher than that of nasal secretions.[149,150]

Serology

Because seropositivity is nearly universal by age 5 years, a definitive serologic diagnosis requires a fourfold rise in antibody titer or seroconversion, and thus clinical utility is limited. Serologic diagnosis is most often accomplished by enzyme immunoassay using whole-virus lysates of the representative strains of the two major genotypes, recombinant F or N proteins as antigens, or by IFA using hMPV-infected cells fixed to slides.[6,36,151]

TREATMENT

Treatment of hMPV infection is supportive. Nebulized β-adrenergic agents, such as albuterol, have historically been administered to hospitalized children with a history of asthma or wheezing; however, current evidence suggests that this practice does not improve patient outcomes. In one recent study of children hospitalized with hMPV LRTI, the frequency of albuterol use was high (69%), although less than one-third of patients had a prior history of asthma and wheezing.[152] There were no differences in hospital length of stay or need for ICU admission between patients who received albuterol and those who did not. Similar questions regarding therapy arise in adult populations with virally induced acute exacerbation of COPD. While the benefit of bronchodilators and inhaled glucocorticoids in patients with acute exacerbation of COPD is generally accepted, one study has now demonstrated a detrimental effect of the latter therapy, whose antiinflammatory benefits may be offset by the blunting of immune responses that control viral replication.[153] Additionally, use of inhaled glucocorticoids enhanced hMPV replication in human primary bronchial epithelial cells and reduced viral apoptosis by induction of antiapoptotic genes from healthy subjects, asthmatics, and COPD patients.[153] The effect was suppressed by the use of adjuvant recombinant interferon therapy, raising potential therapeutic strategies.

Therapeutic antiviral agents or antibody preparations for the treatment or prevention of hMPV infection are not currently available but are subjects of active research[154] (see Chapter 45). Ribavirin, a nucleoside analogue with broad antiviral activity, is approved in the inhaled form for the treatment of serious RSV infections in young children (see Chapter 158). Oral ribavirin is currently licensed for the treatment of hepatitis C, and intravenous ribavirin has been administered on a compassionate-use basis for the treatment of viral hemorrhagic fevers. Polyclonal intravenous immune globulin (IVIG) was approved for the prophylaxis of RSV infections in high-risk children in 1996, although its use has been supplanted by palivizumab (see Chapter 158). Standard IVIG contains relatively high titers of neutralizing hMPV antibodies and thus may theoretically be useful for the treatment or prophylaxis of hMPV infection.[155] Ribavirin and IVIG inhibit hMPV and RSV in tissue culture and are found to have equivalent antiviral activity for both viruses.[156] In the mouse model, ribavirin combined with corticosteroid treatment was effective in reducing viral titers and histologic inflammation of the lung.[157] Ribavirin is also thought to have favorable immunomodulatory effects by upregulating CD4 and CD8 T-cell–derived interleukin-2, tumor necrosis factor-α, and interferon-γ and downregulating Th2 cytokines, thus controlling viral replication.[158] Ribavirin in various forms and IVIG have been used in seriously ill immunocompromised patients with anecdotal success.[155,159–161] Intravenous ribavirin carries a risk of intravascular hemolysis, and aerosolized ribavirin is extremely costly and can precipitate bronchospasm. An analysis using propensity scores to evaluate the effect of oral ribavirin to treat immunocompromised patients with paramyxovirus infections demonstrated no significant clinical benefit.[162] In view of lack of efficacy data and potential adverse effects, no recommendations for use of these agents have been made.[163]

hMPV-specific fusion inhibitors, humanized monoclonal antibodies, and small interfering RNAs are currently in development, and animal models indicate these agents may be useful for prophylaxis and preemptive treatment of hMPV infection.[164–169] Cross-reacting neutralizing antibodies to the RSV and hMPV fusion (F) proteins, such as MPE8 and 25P13, have been identified and may provide structural targets for

interventions.[170] DAS181 is a sialidase fusion protein that interferes with the binding of the F protein with the host cell sialic acid–containing receptor and has been explored for hMPV antiviral activity. A recent report demonstrated that DAS181 blocked hMPV infection of HEp-2 cells and inhibited binding of recombinant G attachment protein.[171] Finally, the small-molecule agent favipiravir (T-750) which is currently being evaluated for the treatment of influenza infections, has also demonstrated activity against hMPV in animal models and has broad antiviral activity.[172]

PREVENTION

Currently the major means of prevention of infection with hMPV is to interdict environmentally based or person-to-person transmission in hospital settings.

The mode of transmission of hMPV is similar to that of RSV. Efficient transmission of RSV occurs as a result of direct contact with infected secretions through fomites or large-particle aerosols. hMPV has been shown to be stable on nonporous surfaces for over 6 hours at room temperature and 4°C with minimal loss of infectivity.[173] Detection of hMPV by PCR may occur for up to 4 weeks in children with primary infection, although culture positivity is rare after 1 week.[74,83] Nosocomial transmission of hMPV has been documented in hospital settings and long-term care facilities among patients and health care workers.[37,61,91] Careful hand hygiene is of primary importance, and the use of gowns, gloves, and masks can be considered in outbreak situations.

No vaccine is currently available for the prevention of hMPV, but efforts to produce a vaccine are ongoing.[174,175] Vectored, recombinant, and chimeric vaccine candidates appear promising in small rodent and nonhuman primate models.[154,174,175] Because of concerns that inactivated vaccines may produce enhanced disease similar to RSV in immunologically naïve persons, live-attenuated vaccines are favored for development in children.[176] To date only one vaccine candidate has reached clinical trial stage; rHMPV-Pa, a live-attenuated chimeric virus vaccine candidate, was shown to replicate efficiently in vitro and be highly immunogenic in hamsters and nonhuman primates, but it proved to be overattenuated in hMPV-seronegative children in a phase I clinical trial.[177]

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160 Measles Virus (Rubeola)

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SHORT VIEW SUMMARY

Definition
- Measles is a highly contagious viral infection, usually of childhood.
- The disease presents with a nonpruritic rash that begins on the head and face and spreads down the body, with fever and malaise.
- Initially, before the rash appears, measles can resemble influenza.
- There is usually accompanying conjunctivitis, cough, and coryza.
- Complications include infections of the respiratory tract and central nervous system involvement.

Epidemiology
- Measles is very contagious before the rash appears, which enhances the chance of spread before the disease is identified.
- Measles is also contagious for a few days after rash onset.
- The virus spreads by droplets and also by the airborne route.
- Widespread vaccination with two vaccine doses in the Americas has reduced the incidence of measles so that the virus is no longer endemic in these regions.
- However, measles outbreaks continue to occur in the United States (34 outbreaks in 2013–14 and a large outbreak in 2015 related to exposure at Disneyland in California).
- When vaccination rates fall below 95%, measles outbreaks can occur if the virus is reintroduced into a population.
- Immunosuppressed patients are at high risk to develop severe measles without necessarily manifesting a rash.
- Measles vaccination has been shown to be unrelated to development of autism.

Microbiology
- Measles is caused by an RNA virus classified as a *Morbillivirus* of the Paramyxoviridae family.

Diagnosis
- Clinically, measles may be confused with Kawasaki disease.
- Measles virus is difficult to culture.
- Diagnosis is usually made clinically, particularly if Koplik spots on the oral mucosa are observed.
- Laboratory diagnosis can be made by reverse-transcriptase polymerase chain reaction on just about any body fluid or tissue.

Therapy
- No specific therapy has been proven to be useful.
- Administration of vitamin A once daily by mouth for 2 days should be considered for patients with measles.
- The mechanism of action of vitamin A is thought to be by immunomodulation.

Prevention
- Live-attenuated measles vaccine is administered to healthy children at 12 to 15 months of age and again at age 4 to 6 years.
- Passive immunization with immunoglobulin G should be given to high-risk children and adults exposed to measles but having no history of measles.

Measles, an acute infection caused by the rubeola virus, is highly contagious and usually seen in children. The illness is characterized by conjunctivitis, cough, coryza, fever, and a maculopapular rash that begins several days after the initial symptoms appear. There is a characteristic enanthem, Koplik spots, that is specific for measles and that precedes the onset of rash. Recovery from measles is the rule, but serious complications of the respiratory tract and central nervous system (CNS) may occur. Measles in the United States has been largely controlled since the introduction of live-attenuated measles vaccine in 1963; it remains a serious problem in developing countries, but successful efforts are now being carried out for improved control of the disease.[1]

Measles virus (MV) belongs to the genus *Morbillivirus* of the family Paramyxoviridae. It is closely related to the viruses causing canine and phocine distemper, rinderpest of cattle, peste des petits ruminants of goats and sheep, and morbilli of certain aquatic animals. Although these viruses are distinct agents, they share certain antigens.[2,3] Wild-type (WT) MV is pathogenic only for primates.

DESCRIPTION OF THE PATHOGEN

MV is an enveloped, nonsegmented, single-stranded, negative-sense RNA virus. The genome encodes at least eight structural proteins.[3]

Morphology

On electron microscopy measles virions are pleomorphic spheres with a diameter of 100 to 300 nm. Virions consist of an inner nucleocapsid that is a coiled helix of protein and RNA and an envelope that bears two types of short surface projections.[3,4] These projections include the hemagglutinin (H) and the fusion (F) proteins. The molecular weight of the single-stranded RNA is 4.5 kilodaltons. Because the entire genome has been sequenced, it is possible to differentiate between WT MV and vaccine-type virus.

Chemical and Antigenic Composition

MV encodes at least eight structural proteins. These have letter names and include the following: F, C, H, L (large), M (matrix), N, P, and V. Three of them, the nucleoprotein (N), the phosphopolymerase protein (P), and the large protein (L), are complexed with RNA. C and V interact with cellular proteins and also play roles in the regulation of transcription and replication of the virus. Three are associated with the viral envelope: the M protein, a nonglycosylated protein associated with the inner lipid bilayer, and the two glycoproteins, H and F.[5] The H glycoprotein is involved in attachment of the virus to host cells, and the F glycoprotein is involved in spread of the virus from one cell to another. A number of receptors for MV have been described. One is the signaling lymphocyte activation molecule (SLAM; CDw150); WT virus enters mainly using this reeceptor.[6,7,8] SLAM is a membrane glycoprotein that is expressed on T and B lymphocytes and antigen-presenting cells, which accounts for its lymphotropism and immunosuppressive effects. The complement regulatory protein CD46, which is widely distributed in primate tissues, also serves as a receptor for the MV and is particularly used by vaccine type virus.[3,9,10] A third receptor, extracellular matrix metalloproteinase inducer (CD147/EMMPRIN), on epithelial cells facilitates transmission by aerosol.[11,12] There are also additional receptors for MV. Multiple receptors probably enable MV to enter different types of cells during infection. The H glycoprotein constitutes the antigen that mediates hemagglutination. The hemagglutination inhibition (HI) test, using red

blood cells from Old World monkeys, is a historically important serologic test for measuring antibody to MV. The F glycoprotein causes hemolysis. Unlike many other paramyxoviruses, neuraminidase is not found on the envelope of MV.[13] Genetic and antigenic variations of MV are now recognized; the sequence of genes coding for H and N is the most variable.[14] Numerous genotypes have been described.[6,8,15,16] MV antigens and their role in human disease[5,17] are discussed later.

Growth of Measles Virus in Tissue Culture

MV was first successfully isolated in the laboratory by Enders and Peebles in 1954.[18] The virus was initially propagated in primary human renal cells but later was cultivated in cultured simian kidney cells. WT MV is rather difficult to propagate in vitro because it is slow growing, and only a limited number of types of cell cultures are permissive for the virus.[16] Typically, cytopathic effects produced by MV in tissue cultures consist of stellate cells with increased refractility and, especially on passage, multinucleated syncytial giant cells containing intranuclear inclusions. In the absence of cytopathic effects, virus replication can also be detected by hemadsorption of rhesus monkey erythrocytes. Presumptive isolates of MV are identified by typing with monoclonal antibodies by using immunofluorescence or plaque reduction tests.[3,19] Reverse-transcriptase polymerase chain reaction (RT-PCR) assays for MV are also available (see later).

Host Range

Humans are the only natural host for WT MV, but monkeys may also be infected. In general, illness caused by MV is milder in monkeys than that in humans.[20] It has not been possible to infect small laboratory animals, such as rodents, with WT MV. However, newborn and suckling rodents may be infected with vaccine strains administered by the intracerebral route.[21,22]

EPIDEMIOLOGY

Measles has been recognized as a disease for some 2000 years, but its infectious nature was not recognized until about 150 years ago. In 1846 Panum[23] studied an epidemic of measles in the Faroe Islands and noted that the disease was contagious, that there was an incubation period of about 2 weeks, and that infection appeared to confer lifelong immunity. The next major advance in the understanding of measles occurred in 1954, when Enders and Peebles[18] successfully propagated WT MV in primary human renal tissue culture cells. This was a prerequisite for the development of a live-attenuated measles vaccine, which was licensed for use in the United States in 1963.[24]

Measles is seen in every country in the world. Without a vaccine, epidemics of measles lasting 3 to 4 months could be predicted to occur every 2 to 5 years. Countries in which measles vaccine is widely used have experienced a marked decrease in the incidence of disease. For example, for many years, 200,000 to 500,000 cases of measles were reported annually in the United States. Since 1963, when the vaccine was licensed, the incidence of measles in the United States has decreased by almost 99%.[25,26] This decrease has been especially pronounced since the early 1980s, when state laws requiring proof of immunity to measles for school entry were enacted. The yearly incidence of measles in the United States reached its first nadir in 1983, when 1497 cases were reported to the Centers for Disease Control and Prevention (CDC) in Atlanta. In the late 1980s and early 1990s, however, there was an increase in the incidence of measles; this was brought under control by increasing the rate of immunization and by introducing a two-dose schedule of measles vaccine for all children.[27,28,29] In 1990 more than 25,000 cases of measles and 89 measles-associated deaths were reported to the CDC.[30] In 1991, however, the number of reported cases dropped significantly, to 9643.[31] Between 1993 and 1996 fewer than 1000 annual cases in the United States were reported to the CDC.[32] Between 2000 and 2007 an average of 63 annual cases were reported.[33] Since then, between 2010 and 2017 an average of about 200 cases were reported annually (range, 55–667); 2014 was a banner year, when 667 measles cases were recorded.[33a] Leaving out 2014, on average 130 measles cases were reported annually in the United States. Most cases occurred in the unvaccinated.

Using molecular techniques, it was demonstrated that transmission of indigenous measles largely ceased in the United States by 1993. Since that time most cases of measles in the United States have resulted from international importation of MV.[15]

In the first 6 months of 2008, 131 measles cases were reported to the CDC; 13% were associated with importations from Europe, Asia, and the Middle East. Of these cases, 99 (76%) were epidemiologically or virologically linked to importations. Most of these patients were younger than 20 years, and 91% were unvaccinated or had an unknown vaccination status. Of the unvaccinated individuals, many of whom declined vaccination for philosophic and/or religious reasons, 85% were eligible for vaccination. When vaccination coverage fell to 80% to 85%, measles once again became endemic in the United States. Although current vaccine coverage in the United States, which is more than 90%, is sufficient to prevent sustained outbreaks of measles, it is not sufficient to prevent imported cases, with resultant limited US spread. In the first 19 weeks of 2011, 118 cases of measles were reported to the CDC, the highest number of cases during that interval since 1996.[34] In 2011, moreover, a large epidemic of measles occurred in Quebec, Canada, with 725 reported cases, although 95% of 3-year-old children had been immunized with one dose and 90% with two doses. Among adolescents who contracted measles, 22% had received two doses. Thus, although measles vaccine is highly effective, further understanding on susceptibility in recipients of two doses is of interest.

In 2013, 11 outbreaks of measles were noted in the United States, and in 2014, 23 outbreaks were reported, including a large outbreak of 383 cases that occurred primarily among unvaccinated Amish individuals.[35] In early 2015 a multistate outbreak was noted that was related to exposure at Disney theme parks in California.[36] As of March 6, 2015, 173 cases were reported in 17 states.[35] Of the 110 cases in California, 45% were unvaccinated, and 43% had unknown or undocumented measles vaccination status. The measles isolate from this outbreak appears to be genotype B3, which has caused outbreaks in at least 14 other countries, including the Phillippines.[36] In the spring of 2017 a major outbreak of B3 measles occurred in Minnesota in a community with low vaccine coverage. There were more than 65 confirmed cases, 31% requiring hospitalization mostly for dehydration and pneumonia. Most of the cases were in infants younger than 12 months. Three children had received two doses of measles-containing vaccine. In this community the rate of measles vaccine had declined due to parental fears of measles vaccine causing autism. A major approach to control of measles in this population was to administer measles vaccine to infants younger than 1 year. For almost the past 20 years, more than half of all measles cases reported in the United States have occurred in individuals who were unvaccinated.[37,38]

Measles continues to be a worldwide problem that primarily affects children in developing countries. In 2000 it was estimated that more than 750,000 deaths attributed to measles occurred globally. With the advent of immunization programs supported by the World Health Organization and United Nations Children's Fund, the estimated deaths globally was reduced by 79% between 2000 and 2014.[39] The largest reduction in deaths was observed in Africa.[39] Measles continues to be a problem in Europe, where vaccine use may be spotty, and introduction of measles to the United States by air travel has resulted in measles outbreaks.[6,40] Despite the many challenges in controlling measles, however, eventual elimination of this infection continues to be a goal.

At present there is minimal published evidence that immunity induced by measles vaccine wanes significantly with time.[28,41–46] The major reasons why measles has not fully been eliminated from the United States are failure to immunize all persons who qualify for vaccination, primary vaccine failure, and importation of measles to the United States from other countries.[15,40–48] Based on the aforementioned recent Canadian experience, further studies on the possibility of waning immunity to measles, even after two doses, seem to be warranted.

Spread of Infection

The measles virion is very labile; it is sensitive to acid, proteolytic enzymes, strong light, and drying.[3] The virus, however, remains infective in droplet form in air for several hours, especially under conditions of low relative

humidity. This latter fact may account for the increased incidence of measles in winter.[49]

Measles is spread by direct contact with droplets from respiratory secretions of infected persons and also by the airborne route. It is one of the most communicable of the infectious diseases, most infectious during the late prodromal phase of the illness, when cough and coryza are at their peak[20]; however, the disease is probably contagious from several days before until several days after the onset of rash. MV has been isolated from respiratory secretions of patients with measles only until up to 48 hours after the onset of rash.[50] Airborne spread of measles in physicians' offices[51,52] and in a sports complex[53] has been observed.

OTHER DISEASES ASSOCIATED WITH MEASLES VIRUS

Subacute sclerosing panencephalitis (SSPE) is a chronic, degenerative, fatal neurologic disease that occurs on average 7 years after an attack of measles, particularly in children who had measles before 2 years of age. Possibly it is an autoimmune disease.[16] A few children who received measles vaccine and who had no prior history of measles have been observed to develop SSPE. It is thought that these children may have had a subclinical case of measles before receiving the vaccine. The incidence of SSPE in the United States has declined dramatically since the introduction of measles vaccine.[25,54] It is almost invariably caused by WT virus; a single case of inclusion-body encephalitis caused by the vaccine strain was reported in 1999.[55] Based on the number of cases of measles in children during 1989–91 and the number of cases of SSPE reported to the CDC after those years, it was estimated that the risk of SSPE after measles is 10 times greater than was originally thought, or 1 per 11,000 cases. Genotyping revealed that these SSPE cases were caused by the WT MV circulating during those years. It appears therefore that vaccination can prevent significantly more cases of SSPE than was originally projected.[56] More recent analysis of cases of SSPE in the current vaccine era indicate that this complication of natural measles (even mild or subclinical cases) continues to occur, underscoring the importance of measles vaccine in the US population.[57]

Patients with SSPE have unusually high measles antibody titers in their serum and their cerebrospinal fluid (CSF).[58] SSPE is caused by a persistent infection with a measles-related virus in the CNS that occurs despite a vigorous immune response on the part of the host. The pathogenesis of SSPE is extremely complex and has been ascribed to a combination of host factors and viral replicative phenomena. Although a measles-like virus has occasionally been isolated, using cocultivation techniques, from the brains of patients with SSPE at autopsy,[59,60] the infection is usually characterized by an inability to produce viral progeny.[61] This inability may be a result of defects in the formation of gene products arising from genomic mutations caused by errors of RNA replication. Originally, the inability to replicate was ascribed to failure of the infective virus to produce measles M protein.[62] Later, it was realized that this failure was related to mutations of the gene encoding this protein. Now it is recognized that defects in envelope gene products H and F also occur as a result of other genomic mutations of the causative virus. Host factors, such as defective cellular immunity and the ability of specific antibodies to confine the virus to intracellular multiplication, are also postulated to play a role in the pathogenesis of SSPE.[61–63,64–66] MV RNA was demonstrated by an in situ RT-PCR assay in neurons, astrocytes, oligodendrocytes, and vascular endothelial cells in the brain of a patient who died from SSPE.[67]

The evidence that multiple sclerosis, Crohn disease, and systemic lupus erythematosus are etiologically linked with MV is much weaker than that for SSPE,[68,69,70] and MV infection is probably unrelated to these diseases. A causative role for MV in Paget disease of bone has been raised as a possibility but as yet is unproven.[71,72,73,74]

PATHOGENESIS

MV was thought to infect by invasion of the respiratory epithelium from which it spreads to the local lymph nodes, blood, spleen, lymphatic tissue, lung, thymus, liver, and skin.[3,72,73] Recent studies in monkeys, however, have suggested that MV enters lymphoid cells of the upper respiratory tract by using the SLAM receptor. After replication in lymphoid cells, MV then invades epithelial cells in organs such as the respiratory tract, intestine, bladder, and skin. When MV infects epithelial cells, progeny are released into the airway, and thus the virus can be transmitted to new individuals.[11] Studies on volunteers inoculated with live MV have indicated that infection may occur after instillation of virus at any point from the nose to the lower parts of the respiratory tract.[71]

MV has been isolated from the leukocytes of patients with clinical measles.[75] The virus has also been propagated in vitro in human T and B lymphocytes and in monocytes.[76] The major infected cell in the blood is the monocyte.[3,77] Endothelial, epithelial, and dendritic cells are also infected. Infected tissues include the thymus, spleen, lymph nodes, liver, skin, conjunctiva, intestine, bladder, and lung.[3,6,11]

Infection of the entire respiratory mucosa accounts for the cough and coryza that are classic signs of measles. In addition, measles may directly cause croup, bronchiolitis, and pneumonia. Damage to the respiratory tract from edema and loss of cilia may predispose to secondary bacterial invasion, resulting in complications such as otitis media and pneumonia.[20]

Within a few days after generalized involvement of the respiratory tract has occurred, Koplik spots appear; subsequently rash develops. Both manifestations are believed to result from similar pathologic mechanisms. On microscopic examination of skin and mucous membranes, multinucleate giant cells and other similar histologic changes are observed in the epidermis and oral epithelium.[78] The appearance of the measles rash coincides temporally with the appearance of serum antibody and the termination of communicability of the disease. Therefore it has been postulated that the skin and mucous membrane manifestations of measles actually represent hypersensitivity of the host to the virus. MV antigen has been demonstrated in the involved skin and mucous membranes by immunofluorescence assay.[78,79,80] MV has also been isolated from the rash in its early stages.[73] If hypersensitivity is the actual cause of the rash, however, it is probably mediated by cellular rather than humoral immunity,[81] and therefore patients with agammaglobulinemia who contract measles develop a rash. Patients with deficiencies in cell-mediated immunity, on the other hand, may develop measles giant cell (Hecht) pneumonia without a rash after an exposure to measles or if measles vaccine is given.[82,83]

IMMUNITY

Immunity to measles after an attack of the disease appears to be lifelong. Rarely, second attacks of measles have been reported after natural infection. Similarly, after measles vaccination, immunity is of many years' duration and probably lifelong in most persons.[25] How measles antibody persists for years after infection is not understood. One possible explanation is that the virus becomes latent after acute infection and provides an immunologic stimulus to antibody formation. However, latent MV has not been demonstrated in humans or in experimental animals. An alternative explanation for the persistence of measles antibody is that reexposure to the virus results in persistent antigenic stimulation. Reinfection with measles can occur and is almost always asymptomatic, even though a boost in antibody titer can be detected.[84] Cellular immunity to MV probably also plays a role in the prevention of recurrent measles because patients with agammaglobulinemia do not have multiple attacks of measles. A cell-mediated response to measles antigen in the absence of detectable measles antibody was reported in two physicians in whom no disease developed despite repeated exposures to measles.[85] Therefore, when humoral antibodies to measles are absent or of low titer, cellular immunity to the virus may protect against subsequent illness. Cellular immunity to MV in peripheral blood of persons with a history of measles has been shown by in vitro lymphocyte stimulation after exposure to measles antigen[86] and by demonstration of measles-specific class I and II cytotoxic T cells.[87,88,89] A complex interplay of cellular immunity and cytokines occurs before, during, and after measles infection in healthy persons.[6,90]

During infection, CD8 and CD4 T cells are activated and probably participate in the clearance of virus and the development of rash. During recovery, suppression of cell-mediated responses occurs, with elevation of suppressive cytokines such as interleukin-4, which may be responsible for depressed delayed-type hypersensitivity to tuberculin.[3,91] Effects of

vaccine on the immune system that resemble the effects of naturally occurring measles have also been described.[92]

CLINICAL MANIFESTATIONS

The incubation period of measles is 10 to 14 days; it is often somewhat longer in adults than in children. A prodromal phase lasting several days begins after the incubation period. It is manifested by malaise, fever, anorexia, conjunctivitis, and respiratory symptoms, such as cough and coryza, and may resemble a severe upper respiratory tract infection. Measles has also been clinically confused with Kawasaki disease, especially in infants and young children. Other diseases that may mimic measles include rubella, meningococcemia, scarlet fever, toxic shock syndrome, human herpesvirus 6 and 7 infections, and parvovirus B19. Toward the end of the prodrome, just before the appearance of the rash, Koplik spots appear.

Koplik spots are pathognomonic of measles. First noted by Koplik in 1896, they consist of bluish gray specks on a red base.[93] They have been likened to grains of sand and, without examination of the buccal mucosa in good light, may be overlooked. Most often they appear on the mucosa opposite the second molars. However, in severe cases, the entire mucous membrane of the mouth may be involved. This enanthem persists for several days and begins to slough as the rash appears.

The rash of measles usually begins on the face and proceeds down the body to involve the extremities last, including the palms and soles (Fig. 160.1). During the healing phase, the involved areas (except palms and soles) may desquamate. The rash is erythematous and maculopapular; as it progresses, it becomes confluent, especially on the face and the neck. The rash usually lasts about 5 days and starts to clear first on the skin that was initially involved. The patient with measles is usually most ill during the first or second day of the rash. Several days after the appearance of the rash, the fever abates and the patient begins to feel better. The entire uncomplicated illness from late prodrome to resolution of the fever and rash lasts 7 to 10 days; cough may be the last symptom to disappear.

Complications

The most common complications of measles involve the respiratory tract and CNS. Involvement of the respiratory tract is part of the virus infection itself. In addition, bacterial superinfection may occur in any area of the respiratory tract, including the middle ear. Superinfection may be secondary to local tissue damage inflicted by the virus and depression of cellular immunity. Pneumonia accompanying measles may be caused by direct viral invasion of the lungs or by bacterial superinfection.[94] Radiographic evidence of pneumonia is common, even during apparently uncomplicated measles.[20] In infants who die of measles, pneumonia accounts for about 60% of deaths, whereas in children 10 to 14 years of age, death is more often observed to be from complications of acute encephalitis.[95,96]

Encephalitis after measles in normal hosts may be acute or chronic (e.g., SSPE). Acute measles encephalitis manifests with a resurgence of fever during convalescence and frequently with headaches, seizures, and changes in the state of consciousness. Up to 50% of patients with measles but no symptoms that suggest cerebral involvement may have abnormalities detected by electroencephalography,[97] so it is believed that viral invasion of the CNS is a common feature of measles. However, only 1 in 1000 to 2000 patients with measles develops clinical signs of encephalitis. Measles encephalitis ranges from mild to severe, and a high proportion of patients who recover are left with neurologic sequelae.

MV has been isolated from the brains of several persons dying of measles encephalitis.[98,99,100,101] However, virus isolation is uncommon and usually requires special virologic techniques such as cocultivation. It is hypothesized that acute measles encephalitis is caused by hypersensitivity to virus in brain tissue. Both viral and host antigens are present on the surface of measles-infected cells in vitro.[102] Therefore hypersensitivity may be directed against viral and host (brain) antigens, which accounts for the encephalitic symptoms. Demyelination, vascular cuffing, gliosis, and infiltration of fat-laden macrophages near blood vessel walls are noted in brain tissue from patients with measles encephalitis.[3] In a laboratory study of serum and CSF from 19 patients with postinfectious measles encephalitis, similarities between experimental allergic encephalomyelitis (e.g., immune responses to myelin basic protein, early destruction of myelin) were demonstrated in about 50%. There was no evidence of intrathecal synthesis of antibody against MV, which suggests that immunopathology, rather than viral multiplication, is involved in the pathogenesis of measles encephalitis.[103]

Transient hepatitis has also been reported during acute measles.[104]



FIG. 160.1 Typical rash on a patient with measles. *(From Kremer JR, Muller CP. Measles in Europe—there is room for improvement.* Lancet. *2009;373:356–358.)*

Special Considerations

Modified Measles

An extremely mild form of measles has been observed in persons with some degree of passive immunity to the virus. This includes some babies younger than 1 year who have passively acquired maternal antibody to MV and some susceptible persons who received immune globulin after an exposure to measles. The symptoms of modified measles are variable, and certain classic symptoms, such as the prodromal period, conjunctivitis, Koplik spots, and rash, may be absent. The incubation period may be prolonged. At times the infection is subclinical and, with a great degree of passively acquired immunity, may be prevented completely.[84]

Atypical Measles

The syndrome of atypical measles has been described in persons who received killed measles vaccine (or killed vaccine, followed soon afterward by live vaccine) and who, several years later, were exposed to WT MV.[105,106] Initially, these patients have an undetectable or a very low measles antibody titer. They then develop unusual manifestations of measles, followed by the appearance of extremely high measles antibody titers (e.g., 1:100,000) in their serum.[107] After a prodrome of fever and pain for 1 to 2 days, the rash appears. Unlike classic measles, it begins peripherally and may be urticarial, maculopapular, hemorrhagic, vesicular, or some combination of these types. The disease may be misdiagnosed as varicella, Rocky Mountain spotted fever, Henoch-Schönlein purpura, drug eruption, or toxic shock syndrome. The patient has a high fever, edema of the extremities, interstitial pulmonary infiltrates, hepatitis and, on occasion, a pleural effusion. The disease tends to be severe with a somewhat more prolonged course than regular measles. At least one fatality has been reported. No specific therapy is available. MV has not been isolated from these patients, and they do not appear to transmit measles to others.[106]

The pathogenesis of this syndrome is believed to be one of hypersensitivity to MV in a partially immune host. Whether cell-mediated

or humoral immune mechanisms, or both, are involved remains controversial.[106,108,109] One hypothesis concerning pathogenesis is that killed measles vaccine lacks the antigen that stimulates the immunity that prevents entry of MV into cells, thereby allowing measles infection to occur, despite the partial immunity derived from killed vaccine.[110,111] In an animal model the low avidity of measles antibodies induced by the inactivated vaccine fails to neutralize the WT virus, leading to deposition of immune complexes, vasculitis, and pneumonitis.[3]

Recurrences of atypical measles have not been reported. Therefore those who received killed measles vaccine (or killed vaccine, followed soon afterward by live vaccine) in the past may be reimmunized with live measles vaccine. It is important that persons who have received killed vaccine be made aware, however, that severe local reactions can follow an injection of live vaccine.[112,113] Usually, the reaction consists of tenderness and erythema around the injection site. However, severe local edema and high fever may also occur. Immunization with live vaccine should be strongly considered because the associated risk is lower than the risk of being exposed to the WT virus.[114] Because killed measles vaccine was not used after 1967, atypical measles is now extremely rare.

Immunocompromised Patients

Severe measles may occur in those with compromised or deficient cellular immunity, such as those being treated for malignant disease, after transplantation, and in individuals with acquired immunodeficiency syndrome (AIDS) or any form of congenital immunodeficiency.[82,115–117] In a report of measles cases occurring in immunocompromised patients in 1989–90, combined with some recorded in the literature, the case-fatality rate for severe measles in children and young adults was calculated to be 70% in 40 oncology patients and 40% in 11 patients infected with the human immunodeficiency virus (HIV).[118] Of the oncology patients, 40% had no rash, 58% had pneumonitis, and 20% had encephalitis. Of the HIV-infected patients, 27% had no rash, and 82% had pneumonia. Should immunocompromised patients be inadvertently exposed to measles, they may develop giant cell pneumonia without evidence of a rash.[82,115,118] In such cases the clinical diagnosis of measles may be difficult or impossible to establish. Because these children may also have poor antibody responses, virus isolation from infected tissue (or identification of measles antigen by immunofluorescence) may be the only means of diagnosis. A chronic form of encephalitis resembling SSPE, often with a concomitant pneumonia, has also been reported in those with deficient cellular immunity.[63,64] This entity has been classified as subacute measles encephalitis and may be confirmed by the presence of measles RNA or infectious virus in brain tissue.[119] Even in the era of molecular diagnostic techniques, however, this diagnosis may be difficult to establish, particularly if the person had no history of clinical measles in the past.[120] Malnourished children, especially in developing countries, have also been reported to develop severe measles. This may be related to poor cell-mediated immune responses resulting from malnutrition.[121] Intense exposure to the virus because of crowding may also play a role in the severity of measles in developing countries.[122,123]

Immunocompromised patients with no history of clinical measles who are exposed to the infection should be passively immunized with immune globulin, even if they have previously been immunized (see later).

Pregnant Women and Their Offspring

Rubeola during pregnancy, in contrast to German measles (rubella), is not known to cause congenital anomalies of the fetus.[124] However, measles in pregnancy has been associated with spontaneous abortion and premature delivery.[20] Measles can be severe in pregnancy. From 1988–91, when there was a resurgence of measles in the United States, a number of pregnant women developed measles. Of 13 such women hospitalized in Houston, 54% had respiratory complications requiring admission to the intensive care unit, and one died.[125] These women were thought to have primary measles pneumonia. Measles in the offspring of mothers with measles ranges from mild to severe.[126,127] It is therefore recommended that infants born to women with active measles be passively immunized with immune globulin at birth.

Persons With Tuberculosis

It has long been thought that tuberculosis is aggravated in persons who contract natural measles, presumably because of a depression of cell-mediated immunity by MV.[3] For example, the tuberculin test has been reported to become negative for about 1 month after measles or measles vaccination.[20] It seems prudent to defer measles vaccination in persons with known tuberculosis until antituberculosis therapy is underway. In geographic areas and populations where tuberculosis is rare, it is not mandatory to perform a tuberculin test on an infant before administering measles vaccine.[128]

Occurrence in Adults

Measles has long been regarded as an illness of childhood. When it occurs in adults, it is often a more severe illness. In a series of 3220 young adult military recruits with measles between 1976 and 1979, about 3% developed pneumonia requiring hospitalization. Bacterial superinfection of the respiratory tract occurred in 30%, and 17% had evidence of bronchospasm. In addition, 31% had laboratory evidence of hepatitis, 29% had otitis media, and 25% had sinusitis.[129] In patients with measles reported to the CDC in 1991, the incidence of complications was higher in those older than 20 years than in children.[30]

DIAGNOSIS

Classic measles with cough, coryza, conjunctivitis, Koplik spots, and a maculopapular rash beginning on the face is easily diagnosed clinically. Often, there is a striking leukopenia, perhaps related to the infection and death of leukocytes. A laboratory diagnosis of measles is helpful when the clinician is unfamiliar with the illness because of the decline in cases of clinical measles since the introduction of measles vaccine. A laboratory diagnosis may also be helpful in cases of possible atypical measles or when unexplained pneumonia or encephalitis occurs in an immunocompromised patient. The differential diagnosis of measles includes rubella, Kawasaki syndrome, scarlet fever, roseola, infectious mononucleosis and rickettsial, enteroviral, and adenoviral infections.

Measles may be diagnosed in the laboratory by virus isolation, identification of measles antigen or RNA in infected tissues, or demonstration of a significant serologic response to MV. Virus isolation is technically difficult, and facilities for isolation are not always available. It is particularly useful, however, for patients with fatal pneumonia and patients with an immunodeficiency, in whom an antibody response may be minimal. Immunofluorescent examination of cells from nasal exudates or from urinary sediment for the presence of measles antigen may be useful for rapid diagnosis of measles.[124,130] A sensitive RT-PCR amplification method to demonstrate MV RNA is available, and nucleotide sequencing can be used for precise characterization of diagnostic specimens.[16,131]

A commonly used laboratory diagnostic method is the serologic response to the virus. A fourfold or greater increase in measles antibody titer in acute and convalescent serum specimens is considered diagnostic for measles. SSPE may be diagnosed by the demonstration of high measles antibody titers in serum and CSF in the presence of a compatible illness.[130] A number of methods are available for measuring antibodies to measles, usually through hospital or state health department laboratories. Neutralization, which requires propagation of the virus in vitro, is technically difficult and infrequently used. Complement fixation lacks sensitivity and is rarely used. The HI test is not used frequently today because it has been supplanted by the enzyme-linked immunosorbent assay (ELISA), for which many diagnostic kits are commercially available.

The ELISA is sensitive and simple to perform and is now widely used.[132,133] This assay can also be adapted to detect specific immunoglobulin M (IgM) antibody[134] and is therefore useful for the diagnosis of acute measles on one serum sample. False-negative and false-positive results, however, may occur with this assay. Measles IgM persists for as long as 1 month after disease onset. Antibody tests that use capillary blood collected on filter paper from finger- or heel-stick specimens have been described and are used by some state health department laboratories.[135]

PREVENTION

Since the use of live measles vaccine, methods to prevent measles have changed dramatically. Prevention today is ideally carried out long before an anticipated exposure to measles by the administration of live vaccine during the early part of the second year of life. However, there are rare occasions when passive immunization against measles with immune globulin must be used.

Included in the group of persons for whom passive immunization is recommended are those who are at high risk for developing severe or fatal measles, are susceptible, and/or have been exposed to the infection. This includes children with malignant disease, particularly if they are receiving chemotherapy, radiotherapy, or both, and children with significant deficits in cell-mediated immunity, including patients with AIDS. Babies younger than 1 year (including newborns whose mothers have measles) are also at increased risk after an exposure to measles. Because measles has been reported even after vaccination in HIV-infected children, it has been recommended that they also be passively immunized with immune globulin after a recognized exposure.[117,118,128,136] To be effective, passive immunization must be given within 6 days after an exposure; administration after 6 days would not be expected to influence the course of the disease.

For a healthy infant who has been exposed to measles, the modifying dose of immune globulin is 0.5 mL/kg intramuscularly (IM). An infant passively immunized in this fashion should be given live measles vaccine at age 15 months.[128] Immunocompromised children should receive a similar dose of immune globulin, with a maximum of 15 mL.

Active immunization against measles was developed in the early 1960s. Live and killed measles vaccines were licensed for use in the United States in 1963. Killed vaccine was withdrawn from the market in 1967, after the recognition of atypical measles in recipients of this vaccine. The first marketed live measles vaccine was the Edmonston B strain. This vaccine was associated with a fairly high incidence of moderately severe side reactions, such as rash and fever, and it was therefore often administered along with a dose of immune globulin. Subsequently, more attenuated vaccines were developed from the Edmonston strain.[137] Because the incidence of vaccine reactions is low with these vaccines, immune globulin is no longer given along with measles vaccine.

In 1976 it was recommended that all healthy children be given live measles vaccine at 15 months. At present it is recommended that children be immunized between the ages of 12 and 15 months (usually given as measles-mumps-rubella [MMR] vaccine or measles-mumps-rubella-varicella [MMRV] vaccine).[128] A second dose is recommended later in childhood.[114,128] Properly administered measles vaccine has been associated with persistence of immunity to measles for many years.[137,138,139,140] In one study, although measles HI antibodies were no longer detectable in some subjects, antibodies were demonstrated by neutralization, and revaccination was associated with a classic booster antibody response.[138] The estimated rate of secondary immune failure was calculated as less than 0.2%. In the general population, 95% of properly immunized children can be expected to respond serologically to measles vaccine. MMRV has been licensed for use in healthy children (not adults) in the United States and in many other countries. After two doses this vaccine provides an adequate immune response to all four viral antigens with a single injection (see Chapter 316).[141]

Vaccination is not usually recommended for infants younger than 12 months because the induction of immunity may be suppressed by residual transplacentally acquired antibodies. In situations in which the incidence of natural measles before the age of 1 year is high, live measles vaccine may be given at 6 to 9 months of age but should be routinely followed by additional doses.[46] Measles antibody titers are lower in women vaccinated as children than in women who have had natural measles, and the offspring of vaccinated women often lose transplacentally acquired measles antibodies before they are 1 year of age.[142,143] Therefore vaccination can be routinely given as early as 12 months of age because most women in their childbearing years today were vaccinated as children. For individuals who were passively immunized after an exposure to measles, vaccination should not be performed for 6 months after a dose of 0.5 mL/kg.[128] Transient fever and rash develop about 1 week after vaccination in 5% to 15% of children. In a 1986 study of 1162 twins who were given either MMR or placebo, there were side effects (fever, irritability, drowsiness, conjunctivitis) in 0.5% to 4%.[144] Symptoms of CNS dysfunction after measles vaccine are exceedingly rare.[145] Because measles may be severe in adults, immunization of adults who were not vaccinated previously, who have no history of measles, and who were born after 1956 is recommended by the CDC.[114] A 1986 Chicago study of hospital employees, however, indicated that only 1 of 266 (0.03%) was susceptible to measles; about one-third were born after 1957.[146]

A number of reasons for apparent primary vaccine failures of measles vaccine have been proposed.[25] These include improper storage of vaccine at temperatures exceeding 4°C, failure to use the proper diluent for the lyophilized vaccine, exposure of the vaccine to light or heat, and vaccination in the presence of low levels of passive antibody. The latter may occur if infants are immunized at 12 months of age or younger, if children are vaccinated 1 or 2 months after receiving an injection of immune globulin, if the more attenuated vaccines are given with immune globulin, or if live measles vaccine is administered soon after killed measles vaccine. No deleterious effects have been associated with measles revaccination. Although it is probably unusual, sustained transmission of measles has been reported in secondary schools, even when 95% of the students were immune and greater than 99% were immunized.[147,148]

Live measles vaccine is contraindicated in persons with deficits in cell-mediated immunity and in pregnant women. Fatal measles in children with AIDS has been reported.[136,149] Although the potential risks of measles vaccine in these children are unknown, they are less than the disease itself. It is currently recommended that children with known asymptomatic HIV infection receive measles vaccine after the age of 12 months.[116,128] The use of measles vaccine should also be considered for children with known HIV infection who manifest symptoms if their CD4 T-cell levels are relatively well preserved, especially if they live in locations where there may be transmission of measles, such as certain inner city areas.[128] One case of fatal measles pneumonia resulting from vaccine virus in an HIV-infected vaccinated young adult has been described after a second dose of vaccine.[117,150] Children who have been treated for malignant disease may be given measles vaccine 3 months after they complete their course of therapy.[128] High-risk children, such as those described, may be given monovalent measles vaccine or MMR, but they should not be given MMRV vaccine, which contains a significantly higher dose of the varicella component. No safety data for MMRV in high-risk children are available.[151]

Serious hypersensitivity reactions to measles vaccine in persons allergic to egg protein have been reported. At one time it was recommended that persons with a history of anaphylactic reactions after the ingestion of eggs should be vaccinated only with extreme caution.[152] More recent studies, however, have indicated that it is safe to immunize such children.[153]

Susceptible persons who are exposed to measles, with the exception of young infants, pregnant women, and immunocompromised persons, may be given live measles vaccine to prevent disease, as an alternative to immune globulin. If the vaccine is given shortly after exposure, clinical cases of measles may be prevented because clinical manifestations associated with measles vaccine occur in about 7 days, compared with an incubation period of 10 days for clinical measles.[20]

An experimental measles vaccine, a derivative of the original Edmonston B vaccine strain termed *Edmonston-Zagreb vaccine*, administered at a dose 10 to 100 times higher than usual, proved to be immunogenic in 4- to 6-month-old infants.[154] Despite its short-term safety, however, the rate of mortality from causes other than measles in these vaccinees in Senegal was significantly higher than that in children who received standard vaccine.[155] Therefore this vaccine is no longer in use.

The possibility that an abnormal immune reaction to vaccine-type MV in MMR vaccine might cause autism in young children was raised in 1998 by Wakefield and coworkers.[156] This idea was never accepted by the vast majority of the scientific community, and 12 of the original 13 authors of the paper eventually withdrew their names from the article in retraction of its content, in part because of conflict of interest by Wakefield.[157] After extensive review, numerous national committees, including the Institute of Medicine, concluded that there is no evidence to support this hypothesis.[158–160] A recent large retrospective cohort

study of 95,727 children, including those with an elevated baseline risk for autism, found no association between receipt of the MMR vaccine and an increased risk of autism.[161] Numerous other scientific studies have also failed to identify an association between MMR vaccine and subsequent development of autism.[162–167] In the United Kingdom, where there has been extensive adverse publicity about MMR, the incidence of measles has increased as a result of suboptimal vaccination rates.[168]

THERAPY

Patients with measles should be given supportive therapy, such as antipyretics and fluids as indicated. Bacterial superinfection should be promptly treated with appropriate antimicrobials, but prophylactic antibiotics to prevent superinfection are of no known value and are therefore not recommended.

Vitamin A, 200,000 IU administered orally to children once daily for 2 days, has been reported to decrease the severity of measles, especially in those with vitamin A deficiency.[169–171] Children aged 6 months to 1 year should receive 100,000 IU for 2 days. Children younger than 6 months should receive 50,000 IU for 2 days. Children with clinical signs and symptoms of vitamin A deficiency should receive an age-specific dose (a third dose) 2 to 4 weeks later. Side effects include transient vomiting and headache.[172] WHO recommends vitamin A for all children with acute measles, regardless of their country of residence.[128] Administration of vitamin A has been reported to reduce seroconversion in vaccinees and should therefore be avoided at or after immunization.[173] The efficacy of ribavirin administered intravenously or by aerosol for treatment of severe measles is unproven.[125,136,174]

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161 Zoonotic Paramyxoviruses: Nipah, Hendra, and Menangle Viruses

Anna R. Thorner and Raphael Dolin

SHORT VIEW SUMMARY

Definition
- Hendra and Nipah viruses are highly pathogenic zoonotic paramyxoviruses that emerged during the 1990s in Australia and Southeast Asia, respectively, and cause meningoencephalitis in humans.

Epidemiology
- In 1994, in Queensland, Australia, Hendra virus caused two outbreaks of fatal illness in horses and their human caretakers. Additional outbreaks involving humans have occurred subsequently.
- Nipah virus was first recognized when it caused an outbreak of severe encephalitis in pig farmers in Malaysia and abattoir workers in Singapore in 1998 and 1999. Multiple outbreaks have been detected since then in Bangladesh and India, including cases that have involved human-to-human transmission. Drinking raw or fermented date palm sap has been identified as a risk factor in these outbreaks. An outbreak associated with exposure to sick horses occurred in the Philippines in 2014.
- Menangle virus, another paramyxovirus, caused decreased farrowing rates and stillbirths in pigs, and illness in two humans in Australia in 1997.
- The *Pteropus* genus of fruit bat, also known as the flying fox, is the reservoir of all three viruses.

Microbiology
- Nipah and Hendra viruses are members of the *Henipavirus* genus within the Paramyxoviridae family. They are single-stranded, negative-sense RNA viruses.

Clinical Manifestations
- Nipah virus causes a severe and often fatal meningoencephalitis. Clinical manifestations of Hendra virus infection range from a self-limited influenza-like syndrome to a fatal respiratory illness or encephalitis. Menangle virus caused an influenza-like illness in two people.

Diagnosis
- Diagnostic tests that can be used to detect Nipah and Hendra viruses include culture, electron microscopy, immunohistochemistry, serology, and real-time reverse-transcriptase polymerase chain reaction.

Therapy
- Because no specific antiviral therapies have been evaluated for the treatment of Nipah virus or Hendra virus infection, treatment involves only supportive care, such as intravenous hydration and mechanical ventilation, when indicated.
- A recombinant human monoclonal antibody, m102.4, directed against the *Henipavirus* glycoprotein protects animals from disease after inoculation with Hendra virus or Nipah virus.

Prevention
- Several vaccines have been developed against Nipah virus and Hendra virus, but none is currently available for use in humans.
- A Hendra virus subunit vaccine was shown to be highly effective at preventing Hendra virus infection in horses after a lethal challenge; it became available for use in horses in Australia in 2012.

Hendra and Nipah viruses are highly pathogenic zoonotic paramyxoviruses that emerged during the 1990s in Australia and Southeast Asia, respectively. In 1994, in Queensland, Australia, Hendra virus caused two outbreaks of fatal illness in horses and their human caretakers.[1,2] Additional outbreaks involving humans occurred in Australia in 2004, 2008, and 2009.[3–5] Nipah virus was first recognized when it caused an outbreak of severe encephalitis in pig farmers in Malaysia and abattoir workers in Singapore in 1998 and 1999.[6,7] Multiple outbreaks have occurred subsequently in Bangladesh and India,[8–18] and one outbreak has occurred in the Philippines.[19] In 1997 in Australia, another zoonotic paramyxovirus, Menangle virus, caused decreased farrowing rates and stillbirths in pigs, and an influenza-like illness in two people who had occupational exposure to infected pigs.[20,21] The *Pteropus* species of fruit bat, also known as the flying fox, is the reservoir of all three viruses.[9,22–24] Because Nipah and Hendra viruses are Biosafety Level 4 agents, research with these pathogens has been difficult to perform.[25,26] However, the development of pseudovirus and reverse genetics techniques has increased opportunities for their investigation.[25–27]

VIROLOGY

Classification

Nipah and Hendra viruses are species within the *Henipavirus* genus of the Paramyxoviridae family, which also includes the *Rubulavirus* genus in which Menangle virus is a species.[28–31] *Rubulavirus* also includes mumps; Newcastle disease; human parainfluenza types 2, 4A, and 4B; and Menangle viruses. Other genera in the Paramyoxviridae family are *Respirovirus* (human parainfluenza virus types 1 and 3), *Morbillivirus* (measles virus), and *Avulavirus*. Nipah and Hendra viruses are most similar to the viruses of the *Respirovirus* and *Morbillivirus* genera, but there is significantly less sequence homology between Nipah or Hendra virus and the members of either of these genera.[29] Nipah and Hendra viruses share a high degree of sequence homology and have similar genomic organization.[32]

Structure and Molecular Biology

Nipah and Hendra Viruses

Nipah and Hendra viruses have a single-stranded, nonsegmented, negative-sense RNA genome that is fully encapsidated by protein.[33] Nipah virus particles range in diameter from 120 to 500 nm,[29] and Hendra virus particles range from 40 to 600 nm.[34] The paramyxovirus envelope contains two transmembrane glycoproteins: an attachment protein or cell receptor-binding glycoprotein (G) and a fusion protein (F).[29] Thin-section electron microscopy (EM) of infected cells reveals filamentous nucleocapsids contained within cytoplasmic inclusions and incorporated into virions budding from the plasma membrane. Hendra virus has a double-fringed appearance caused by projections on the surface of the viral envelope, whereas Nipah virus has only a single layer of surface projections (Fig. 161.1).[29,35]

Henipaviruses cause the formation of syncytia in infected Vero cells.[35] An unusual feature of Nipah virus infection is that nucleocapsid aggregates form at the periphery of infected syncytial cells late in infection. In contrast, Hendra virus nucleocapsid aggregates form

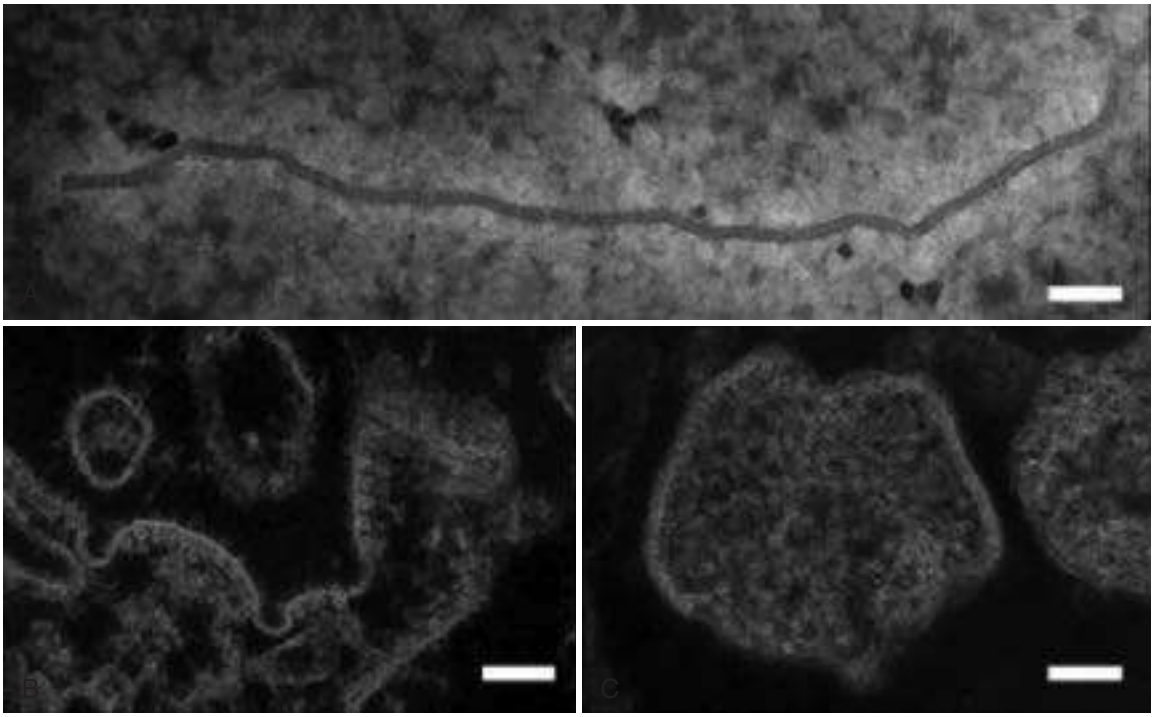

FIG. 161.1 Ultrastructural characteristics of Nipah virus isolate in cell culture as seen with negative stain (A) and thin-section (B and C) electron microscopy. (A) A single nucleocapsid with the typical herringbone appearance characteristic of the family Paramyxoviridae. (B) Viral nucleocapsids, as seen in cross and longitudinal sections, aligned along the plasma membrane of Nipah virus–infected Vero E6 cells. (C) Extracellular Nipah virus particle showing a curvilinear tangle of nucleocapsids enclosed within the viral envelope. Scale bars = 100 nm. *(From Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus.* Science. *2000;288:1432–1435.)*

randomly throughout the cytoplasm. Both Nipah and Hendra viruses have herringbone nucleocapsid structures.[29,34] Both viruses, but particularly Nipah virus, cause tubule-like structures to be present in the cytoplasm of infected cells.[35] These structures are unique to Hendra and Nipah viruses and Sendai virus, a paramyxovirus that infects animals such as mice. The attachment proteins (G) of Hendra and Nipah viruses lack hemagglutinin and neuraminidase activity, which most other paramyxovirus attachment proteins possess.[33,36,37] The attachment proteins of Nipah and Hendra viruses use ephrin-B2 and ephrin-B3 as receptors, which are present in neurons and arterial endothelial cells.[38–41]

The genomes of the henipaviruses have six transcription units that encode six major structural proteins.[33] The transcription units include the nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein or attachment protein (G), and large protein or RNA polymerase (L). This genome arrangement is most similar to those of the *Respirovirus* and *Morbillivirus* genera. The mRNA of the P genes of Nipah and Hendra viruses are cotranscriptionally edited by the insertion of G residues at editing sites, which leads to the translation of multiple gene products.[32,33]

Hendra and Nipah viruses share 68% to 92% amino-acid homology in the protein-coding regions and 40% to 67% nucleotide identity in the untranslated regions.[42–45] Both viruses are approximately 18.2 kb in length. The length of the genomes of the henipaviruses is in multiples of six nucleotides, a property that is important for efficient replication.[26,48]

A Nipah virus strain isolated during an outbreak in Bangladesh was six nucleotides shorter than the strain isolated in Malaysia and shared 92% sequence homology with it.[49] Although isolates from patients in Malaysia were nearly identical to one another, isolates from patients in Bangladesh differed by 0.9% in the nucleoprotein open reading frame.[16,44] In addition, genetically distinct sequences of Nipah virus were detected from patients in two adjacent districts of Bangladesh over a short period of time, indicating that multiple lineages circulated concurrently in a localized region.[50]

Menangle Virus

Like the other paramyxoviruses, Menangle virus consists of an enveloped, nonsegmented, negative-sense RNA genome that is tightly associated with nucleocapsid proteins.[31] EM reveals that the morphology of Menangle virus is similar to that of other members of the Paramyxoviridae family.[21] The viral particles appear spherical or pleomorphic and range from 30 to 100 nm in diameter. Virus isolated from the lungs, brains, and hearts of affected piglets caused cytopathic effects, including vacuolation and syncytia formation, when grown in baby hamster kidney (BHK21) cells. Menangle virus, like Nipah and Hendra viruses, contains herringbone nucleocapsids. It has an envelope with a single fringe of surface projections 17 ± 4 nm in length. Its genome encodes all six of the major structural proteins described previously for Hendra and Nipah viruses. Sequencing of the nucleoprotein (NP), P, M, F, and hemagglutinin-neuraminidase (HN) genes revealed that Menangle virus is a member of the *Rubulavirus* genus.[31] Ultrastructural analysis also supports this classification.[21] Unlike the other rubulaviruses, Menangle virus lacks hemagglutinin and neuraminidase activity.[31] The HN protein shares only 16% to 20% sequence homology with other *Rubulavirus* HN proteins. Menangle virus also lacks the hexapeptide NRKSCS, which is conserved in the other rubulavirus and the respirovirus HN proteins and is thought to be necessary for neuraminidase activity; only the last two amino acids in the sequence motif are conserved in the Menangle virus HN protein.

EMERGENCE OF HENIPAVIRUSES

Phylogenetic analyses show that Nipah and Hendra viruses are old viruses,[34,51] which suggests that their emergence in the 1990s was due to ecologic factors rather than virus mutations.[52] Ecologic change that drew flying foxes closer to horses, pigs, and humans was probably the largest contributor to the emergence of Hendra and Nipah viruses.[53,54] Deforestation has caused flying foxes to move into suburban and urban areas to use the trees in these regions for roosting. Climate change is likely to be causing an expansion of the geographic areas that are suitable for the bat host species of henipaviruses.[55]

NIPAH VIRUS

Epidemiology

An outbreak of severe encephalitis occurred in pig farmers in the Perak state of Malaysia, including the village of Nipah, in September 1998.[6,7,56]

By December, the outbreak had spread to pig farmers in the Negeri Sembilan state. In March 1999, 11 abattoir workers in Singapore who came into contact with pigs that were imported from Malaysia developed an encephalitis syndrome with associated pneumonia.[57] The last cases occurred in May 1999 in the Selangor state of Malaysia.[58] In total there were 276 cases of acute Nipah virus infection, 106 of which were fatal (case-fatality rate of 38%).[29,57,59] Of the cases in Malaysia, 70% occurred in individuals who worked directly with pigs,[58] and a case-control study showed that exposure to sick pigs was a risk factor for Nipah virus infection.[57] The pig farm that was the site of the initial outbreak in Malaysia was also an orchard, which meant that flying foxes were in close proximity to pigs.[52] The outbreak ended after several interventions were implemented, including the deployment of personal protective equipment to individuals in contact with sick pigs, restriction on movement of livestock, and culling of nearly 1 million pigs.[16]

Nipah virus was isolated from the respiratory secretions and urine of 8 of 20 patients with acute infection in Malaysia.[60] Despite evidence of Nipah virus shedding from infected patients during the outbreaks in Malaysia and Singapore, serologic studies of exposed health care workers did not show evidence of nosocomial transmission.[61,62] However, there was a single case report of a nurse who cared for patients with Nipah virus encephalitis who developed the characteristic lesions of Nipah virus encephalitis on magnetic resonance images despite having no signs or symptoms of infection.[63]

Subsequent outbreaks of Nipah virus encephalitis have occurred in Bangladesh nearly every year since 2001.[9–18,50,64,65] Outbreaks also occurred in regions of India neighboring Bangladesh in 2001 and 2007.[8,64] All of the outbreaks in this region occurred between December and May.[65] Several epidemiologic features distinguished the outbreaks in Bangladesh and India from those that occurred in Malaysia and Singapore, including the absence of an intermediate host, evidence of person-to-person spread, and higher case-fatality rates in Bangladesh and India. Some of the outbreaks in Bangladesh and India involved multiple cases of person-to-person transmission and clustering of cases within households.[8,9,14,17,50,64–68] During the outbreak in India in 2001, 45 of 60 patients (75%) had a history of exposure to individuals infected with Nipah virus.[8] Many of the affected individuals were health care workers, suggesting that nosocomial transmission played an important role in the outbreak. In an outbreak of 23 cases in Kerala State, India in 2018, all but the index case acquired the infection in the hospital; the index case transmitted Nipah virus directly to 19 contacts, whereas 3 cases were secondary cases, acquiring the infection from earlier cases.[66] Nipah virus has also been detected on environmental surfaces in hospitals where patients with Nipah virus infection were receiving care.[12,69] However, two studies did not demonstrate evidence of nosocomial spread during outbreaks in Bangladesh, although in one of them, individuals who lived with or cared for patients with Nipah virus were more likely to acquire Nipah virus infection.[9,70] In a case-control study in Bangladesh, subjects with Nipah virus were 7.3 times more likely to have had contact with a person with Nipah virus than controls (95% confidence interval [CI], 4.0–13.4).[71]

For at least two patients in Bangladesh, the only known exposure was contact with a deceased individual who had had Nipah virus infection.[50] The increased incidence of person-to-person spread in Bangladesh and India compared with Malaysia and Singapore may have been due to the higher rates of respiratory involvement in patients in Bangladesh and India, which could have facilitated transmission.[11,66] In ferrets that were exposed to a strain of Nipah virus from either Malaysia or Bangladesh, significantly higher levels of viral RNA were recovered from the oral secretions of ferrets infected with the Bangladesh strain, suggesting that higher levels of oral shedding could be an important factor for human-to-human transmission.[72]

Other risk factors associated with the outbreaks in Bangladesh or India, or both, included consuming raw date palm sap[13,71,73,74] or fermented date palm sap,[75] climbing trees,[10] exposure to sick cows,[9] exposure to pigs or goats,[65,67] and colder temperatures.[76] Flying foxes feed from pots that are used to collect date palm sap, which probably leads to contamination of the sap that is later consumed by people.[13] Although most patients affected by Nipah virus were adults, in two outbreaks in Bangladesh the median age of affected individuals was 12 years.[10,11] The lower median age and male predominance in one of these outbreaks were probably due to a behavior that increased the risk of infection, such as climbing trees.[10]

In 2014, an outbreak of severe neurologic and influenza-like illness caused by Nipah virus or a related virus occurred in humans and horses in two villages in the Philippines and included cases of horse-to-human, human-to-human, and foodborne transmission.[19] There were a total of 17 cases in humans, including 11 cases of encephalitis, 5 cases of influenza-like illness, and 1 case of meningitis; 9 of 11 patients (82%) with encephalitis died, whereas no patients with influenza-like illness or meningitis died. Of the 17 patients, 7 (41%) had been involved with horse slaughtering and horse meat consumption, and 3 (18%) had only consumed horse meat and had no history of slaughtering or meat preparation. Five patients (29%) had been exposed to other human cases, but not to horses. Of these, 2 were health care workers who did not visit the affected villages, had no contact with sick horses, and did not consume horse meat.

The case-fatality rate of Nipah virus infection was substantially higher in the outbreaks in Bangladesh and India than in Malaysia and Singapore. In a case series that included 92 patients from four of the outbreaks in Bangladesh, the case-fatality rate was 74% compared with 38% in the outbreak in Malaysia and Singapore.[11,29,57,59] During an outbreak in Bangladesh in 2005 that involved 12 individuals, the case-fatality rate was 92%.[13] In the outbreak in Kerala State, India in 2018 that involved 23 patients, the case-fatality rate was 91%.[66] It has been proposed that the increased mortality observed in the outbreaks in Bangladesh and India may have been due to increased virulence of the Nipah virus strains involved, although other factors, such as inadequate facilities for the care of critically ill patients and malnutrition, could have played a role.[11]

Researchers cultured a paramyxovirus from cerebrospinal fluid (CSF) that caused syncytia formation in Vero cells from two of the first three patients who died from Nipah virus.[56] The virus stained with anti–Hendra virus antibodies by indirect immunofluorescence, and an enzyme-linked immunosorbent assay (ELISA) for anti-Hendra immunoglobulin M (IgM) antibodies was positive in the CSF of all three patients. Given the epidemiologic and clinical differences between Hendra virus and the outbreak of encephalitis in Malaysia, it was proposed that a paramyxovirus that was related to but distinct from Hendra virus had caused the outbreak.[56] It was named Nipah virus after the village in which the first patient from whom the virus was isolated resided.[56,58]

Reservoirs and Intermediate Hosts

After the initial outbreak of Nipah virus in Malaysia, bats were screened for the presence of anti–Nipah virus antibodies because they were already known to be the reservoir of Hendra virus. Island flying foxes *(Pteropus hypomelanus)* and Malayan flying foxes *(Pteropus vampyrus)* were found to have neutralizing antibodies to Nipah virus.[24] Subsequently, viruses that caused Hendra virus–like cytopathic effect in Vero cells and stained strongly for Nipah and Hendra virus antibodies were identified from two urine samples from *P. hypomelanus* and from fruit that had been partially eaten by a fruit bat.[77] *Pteropus giganteus* is the reservoir of Nipah virus in Bangladesh[9] and India.[78] Motion sensor infrared cameras have documented flying foxes visiting date palm trees in communities where sap was collected for human consumption.[73,79]

Anti–Nipah virus antibodies have been detected in *Pteropus* species of bats in other countries, such as Cambodia, Thailand, Indonesia, and Madagascar.[80–83] In addition, anti–Nipah virus antibodies, Nipah virus RNA, or both, have been found rarely in non-*Pteropus* species of bats in several countries, including Malaysia, Thailand, and Ghana.[24,81,84] Specimens collected from western and southern Africa revealed a diverse array of paramyxoviruses in African bats, including 19 novel species of *Henipavirus*-like viruses distinct from the Nipah and Hendra viruses found in Southeast Asia and Australia.[85] In a study in Cameroon, Nipah virus cross-neutralizing antibodies were detected in 48% of bat specimens and 3% to 4% of human specimens; seropositive human samples were found almost exclusively in individuals who reported butchering bats for bushmeat.[86] This may represent an opportunity for cross-species infection of newly emerging henipaviruses from bats to humans in such regions.

Other animals may serve as reservoirs of related paramyxoviruses. In 2012, three people who had been working in an abandoned mine in China developed severe pneumonia of unknown etiology and died.[87] Anal swab specimens were collected from rats, bats, and musk shrews in the mine. A novel henipa-like virus, Mojiang paramyxovirus, was detected from anal swab specimens from the rats, but not from the bats or musk shrews, with polymerase chain reaction (PCR)–based sequencing techniques. A tissue specimen from a rat was also positive. The positive anal swab samples were cultured in various cell lines for virus isolation, but no cytopathic effect or viral replication was detected.

The only animals that are known to have served as intermediate hosts of Nipah virus are pigs during the initial outbreak in Malaysia and Singapore[6,7] and horses during the outbreak of Nipah virus or a related virus in the Philippines.[19] Nipah virus infection has been demonstrated serologically in cats, dogs, goats, and cattle[58,88,89]; among these species, only dogs have been shown to have clinical disease.[7]

Clinical Manifestations

The incubation period of Nipah virus in Malaysia ranged from several days to 2 months, although more than 90% of patients had an incubation period of 2 weeks or less.[90] In four of the outbreaks in Bangladesh, the incubation period ranged from 6 to 11 days, with a median of 9 days.[11] Although subclinical infection can occur, the ratio of symptomatic to subclinical infection was approximately 3:1 during the outbreak in Malaysia.[91]

In the outbreaks in Malaysia and Singapore, patients typically presented with fever, headache, dizziness, and vomiting.[58,90,92,93] More than 50% of patients had a decreased level of consciousness and brainstem dysfunction, including such signs as myoclonus, areflexia, hypotonia, hypertension, and tachycardia. Cerebellar signs were also common.[93] Some severely ill patients also had multisystem organ dysfunction, including sepsis, gastrointestinal bleeding, and renal failure.[90] Respiratory findings were uncommon in the outbreaks in Malaysia and Singapore, with only 14% of patients in Malaysia having a nonproductive cough[90] and 3 of 11 patients in Singapore having evidence of pneumonia.[57] In contrast, in a study of four of the outbreaks in Bangladesh, 62 of 90 patients (69%) had respiratory difficulty and at least 5 patients had acute respiratory distress syndrome.[11]

In the most severely affected patients in the outbreak in Malaysia, electroencephalogram (EEG) revealed bilateral temporal periodic complexes of sharp and slow waves occurring every 1 or 2 seconds.[58] The typical magnetic resonance imaging (MRI) findings were multiple 2- to 7-mm lesions best visualized in the T2-weighted images, without associated cerebral edema or mass effect; they were disseminated throughout the brain but were most commonly present in the subcortical and deep white matter of the cerebral hemispheres (Fig. 161.2A).[90,94] In a study of eight patients with Nipah virus encephalitis in Malaysia, all patients had multiple small bilateral foci of T2 prolongation in the subcortical and deep white matter, and in the periventricular areas and the corpus callosum.[95] Five patients also had cortical involvement, three had brainstem lesions, and one had a thalamic lesion. In five of the patients, diffusion-weighted images showed increased signal. Four patients had leptomeningeal enhancement, and four had enhancement of the parenchymal lesions. In a follow-up study 1 month after the outbreak in Malaysia, 5 of 12 patients had widespread small foci of high signal intensity on the T1-weighted images, especially in the cerebral cortex.[96] The diffusion-weighted images showed decreased prominence or disappearance over time. At the 6-month follow-up, there was no radiographic evidence of progression or relapse. In three patients who underwent MRI for acute Nipah virus encephalitis in Bangladesh, confluent high-signal lesions in both the gray and white matter were seen.[97] Relapsed or late-onset encephalitis, or both, occurred in some patients after the outbreaks in Malaysia and Bangladesh.[90,94,98,99]

A study performed 24 months after the outbreak in Malaysia, which included 160 patients who survived Nipah virus infection, found that 12 patients (7.5%) developed a relapse of encephalitis.[98] Of 89 patients who initially had nonencephalitic disease or asymptomatic infection, 10 patients (11%) had late-onset encephalitis, with a mean time to neurologic findings of 8.4 months. Four of the 22 patients (18%) with relapsed or late-onset encephalitis died. Brain MRI images in patients with relapsed disease usually showed patchy confluent cortical gray matter lesions (see Fig. 161.2B).[90,94,98]

In a study of long-term neurologic and functional outcomes that included 22 patients from Bangladesh who survived Nipah virus illness, 17 of whom had encephalitis and 5 of whom had febrile illness, all but 1 patient had disabling fatigue that lasted for a median of 5 months

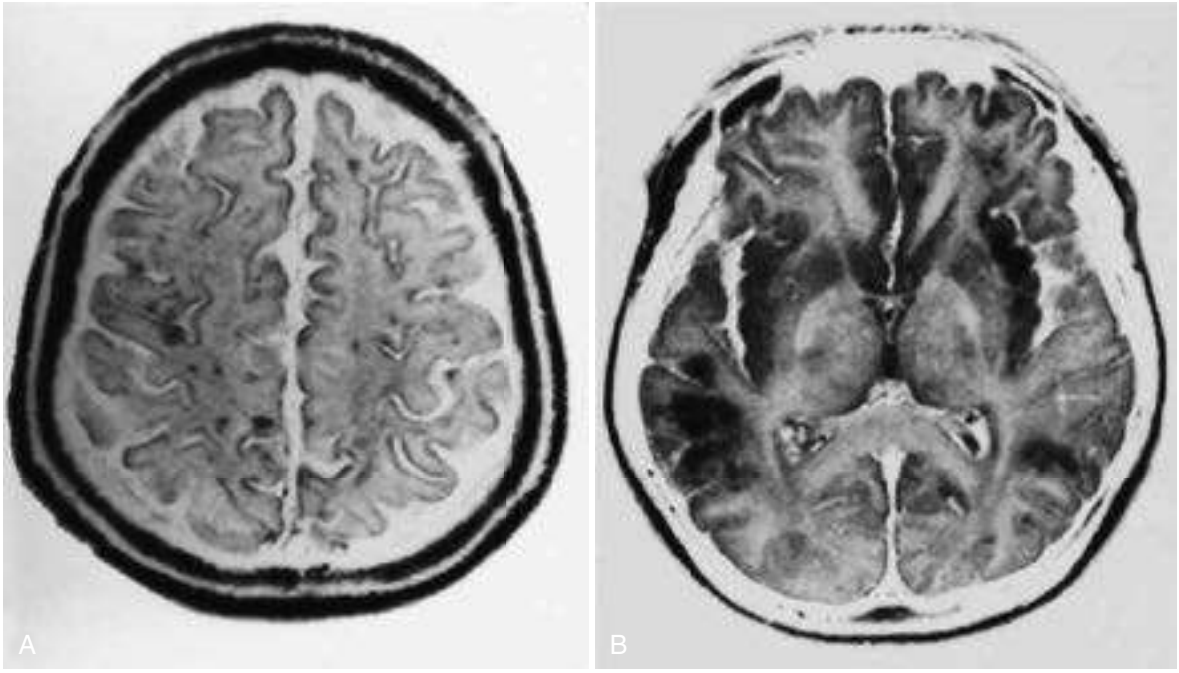

FIG. 161.2 Axial magnetic resonance imaging findings in patients with acute (A) and relapsed (B) Nipah virus encephalitis with use of fluid-attenuated inversion recovery. (A) Multiple discrete hyperintense lesions in the white and gray matter of a patient with acute Nipah virus encephalitis. (B) Confluent lesions primarily involving the cortical gray matter in a patient with relapsed Nipah virus encephalitis. *(From Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia.* N Engl J Med. *2000;342:1229–1235.)*

(range, 8 days to 8 months).[99] Seven of 17 patients (41%) with encephalitis, but none with febrile illness, had persistent neurologic deficits, including encephalopathy, ocular motor palsies, cervical dystonia, focal weakness, and facial paralysis. Four patients had late-onset neurologic abnormalities months after the acute illness.

Laboratory Abnormalities

Leukopenia (11%), thrombocytopenia (30%), and elevated levels of alanine aminotransferase (33%) and aspartate aminotransferase (42%) were the most common laboratory abnormalities in the outbreak in Malaysia.[90] The CSF was abnormal (elevated white blood cell [WBC] count or protein level, or both) in 75% of 92 patients evaluated; the mean CSF WBC count was 41 cells/μL (range, 0 to 842 cells/μL), and the mean protein level was 0.69 g/L (range, 0.12 to 2.15 g/L).[90] CSF glucose was normal in all patients. Unlike in the outbreak in Malaysia, all 18 patients evaluated during an outbreak in India and 4 of 6 patients evaluated during outbreaks in Bangladesh had a normal CSF WBC count (≤5 cells/μL).[8,11] Among patients with acute Nipah virus infection in Malaysia, virus-specific antibodies were present in the serum in more than 70% of samples but in less than one-third of CSF samples.[58] Isolation of Nipah virus from the CSF was strongly associated with mortality.[100] Stored CSF samples from 84 patients with Nipah virus encephalitis (27 fatal and 57 nonfatal) were cultured for Nipah virus. The virus could be cultured from the CSF in 17 fatal cases and 1 nonfatal case.[100]

Diagnostic Tests

Methods that can be used for the diagnosis of Nipah virus infection include culture, serology, EM, immunohistochemistry, reverse-transcriptase polymerase chain reaction (RT-PCR), and serum neutralization tests.[101–104] Real-time RT-PCR is more sensitive than conventional RT-PCR for the diagnosis of Nipah virus infection.[105] Nipah virus can be detected from urine and respiratory secretions by culture and PCR.[60] Serology is most useful for epidemiologic studies.

Pathology

Nipah virus causes a multiorgan vasculitis with a predilection for the central nervous system.[106,107] At autopsy, patients with Nipah virus infection from the outbreak in Malaysia exhibited widespread endothelial involvement characterized by vasculitis, thrombosis, ischemia, and parenchymal necrosis. This was most marked in the central nervous system, although the lungs, heart, and kidneys were also involved. Syncytial giant cell formation was present in affected vessels. Viral inclusions were detectable with both light microscopy and EM. Immunohistochemistry revealed the presence of Nipah virus antigens in the endothelial and smooth muscle cells of blood vessels, and in neurons and other affected cells.

In experimental infections in African green monkeys, a Nipah virus strain from Bangladesh was uniformly fatal, whereas a strain from Malaysia was fatal in only half of infected monkeys.[108] Histopathology of lungs and spleens from monkeys infected with the Bangladeshi strain showed more severe findings than from the monkeys infected with the Malaysian strain.

Therapy

The mainstay of therapy for Nipah virus infection is supportive care, including monitoring in the intensive care unit for patients with severe illness. In an open-label study of oral or intravenous ribavirin in patients with Nipah virus encephalitis in Malaysia, 45 of 140 patients (32%) in the ribavirin group died, compared with 29 of 54 (54%) in the control group.[109] Although these observational data suggest that ribavirin may be beneficial, it is not possible to draw conclusions about efficacy.

On the basis of its in vitro activity against Nipah virus, the antimalarial agent chloroquine was assessed, individually and in combination with ribavirin, for the treatment of Nipah virus infection in a golden hamster model.[110] Ribavirin delayed death in infected hamsters by approximately 5 days. Chloroquine did not protect hamsters when administered either alone or in combination with ribavirin. In another study, ribavirin delayed but did not prevent death in hamsters after a lethal challenge with Nipah virus.[111] A monoclonal antibody, m102.4, directed against the henipavirus G glycoprotein has been shown to have in vitro activity against Nipah virus and to protect ferrets and African green monkeys from a lethal challenge with Nipah virus, but it is not available for clinical use.[112–114] Delivery of antifusion lipopeptides via the respiratory route before and after a lethal challenge of Nipah virus in hamsters and nonhuman primates may reduce the mortality rate of Nipah virus infection.[115]

Prevention

Because many of the cases of Nipah virus infection in Bangladesh have been caused by people drinking raw date palm sap that has been contaminated with Nipah virus by flying foxes, it is important to avoid drinking it. It has been suggested that diverting a larger proportion of the supply of date palm sap to be used for making molasses will help reduce Nipah virus infections because the sap is heated to temperatures at which Nipah virus cannot survive during the manufacturing process.[16] Another preventive measure includes using barrier techniques to prevent access of flying foxes to date palm trees from which sap is being collected; this approach was shown to be effective in a randomized trial.[79] Infection control measures should include standard precautions.[16] Experts have noted that a higher level of infection control may not be feasible in Bangladesh, but that counseling family members to wash their hands with soap after caring for patients with Nipah virus infection can reduce the risk of human-to-human transmission.[12,16]

No vaccine is available for the prevention of Nipah virus, although several vaccines have shown promising results in animal models.[26,116–123] As an example, a Hendra virus G glycoprotein subunit vaccine afforded African green monkeys complete protection against subsequent Nipah virus infection, with no evidence of clinical disease, virus replication, or pathology observed.[118] The status of research and development of vaccines for Nipah virus was reviewed by the World Health Organization in a July 2015 report published in 2016.[124] Despite encouraging studies in animal models, clinical trials with candidate Nipah virus vaccines have not yet been carried out.

HENDRA VIRUS

Epidemiology

In September 1994, an outbreak of an acute respiratory illness occurred in thoroughbred horses in Hendra, a suburb of Brisbane in Queensland, Australia.[1,125] Affected horses had fever, facial swelling, severe respiratory distress, ataxia, and copious frothy nasal discharge, which was sometimes blood-tinged. The index case was a pregnant mare at pasture, which died after a 2-day illness. During the next 2 weeks, 13 more horses at the same stable died or were euthanized. There were four nonfatal cases, two of which had mild neurologic sequelae. Three more horses were found to have seroconverted without having had signs of clinical illness. Within 1 week of the death of the equine index case, a horse trainer and a stablehand became ill with a severe influenza-like illness. The trainer died after developing respiratory and renal failure, whereas the stablehand recovered. Infection with Hendra virus was demonstrated in both human patients with viral culture, immunoelectron microscopy, serology, and PCR using primers derived from other paramyxoviruses.[1,34,125]

In October 1995, a second outbreak of Hendra virus was retrospectively discovered after the death of a thoroughbred stud owner who had developed a relapse of encephalitis 13 months after a mild and self-limited episode of meningitis.[2] It was subsequently determined that two horses had died in August 1994 on his farm in Mackay, in central Queensland, Australia, approximately 1000 km from the original outbreak. The first horse was a pregnant thoroughbred that had developed severe respiratory distress, ataxia, and swelling of the cheeks and supraorbital fossa during a 24-hour period. The second horse had licked the face of the dead mare through a fence. The second horse, a 2-year-old colt, died 11 days later, after a 24-hour clinical course of aimless pacing, muscle trembling, and hemorrhagic nasal discharge. The etiologic agent was originally called equine morbillivirus, but the name was later changed to Hendra virus, after the suburb where the first outbreak had been identified.[52]

In 2004, a veterinarian in Cairns, Queensland, developed a fever, dry cough, sore throat, cervical lymphadenopathy, myalgias, and malaise 1 week after performing a necropsy on a horse that was later found to have Hendra virus.[3] She was diagnosed with Hendra virus by convalescent

serologies but recovered fully. An outbreak in five horses in Brisbane, Queensland, in July 2008 resulted in Hendra virus infection in two veterinary workers, one of whom died.[4] In August 2009, a veterinarian developed fatal encephalitis after close contact with the respiratory secretions of an infected horse.[5] In total, 7 cases have occurred in humans, including 4 deaths, and more than 60 cases have occurred in horses. Sporadic cases continue to occur in horses. In addition to Queensland, several cases have occurred in horses in New South Wales.

Reservoirs and Intermediate Hosts

A serologic survey of wildlife species that were present at the site of the outbreaks was performed.[22] A total of 168 animals from more than 16 species were tested, including rodents, marsupials, birds, amphibians, and insects, yet none were seropositive for Hendra virus. Nomadic birds and flying foxes (bats of the *Pteropus* genus) were then targeted as likely reservoirs, given their presence in the regions of both outbreaks and their ability to travel long distances. Anti–Hendra virus antibodies were found in several types of flying fox throughout Queensland. Hendra virus was subsequently isolated from the reproductive tract of a pregnant gray-headed flying fox that had become entangled on a wire fence and from tissue from aborted flying fox fetuses.[23] The bat isolates were identical to the isolate that infected the horses that died. Flying foxes are thought to have subclinical infection.[52,126,127] Pteropid bat species in geographic regions other than Australia, such as Papua New Guinea and Madagascar, have been found to have antibodies against henipaviruses.[83,128] In a survey of 2840 flying foxes from three of the four Australian mainland species (*Pteropus alecto, Pteropus poliocephalus,* and *Pteropus scapulatus*) captured from the wild in Queensland and New South Wales, specimens of a range of types (urine; serum; urogenital, nasal, oral, and rectal swabs) were collected from anesthetized bats and tested for Hendra virus RNA with a quantitative RT-PCR assay.[129] Hendra virus RNA was detected in *P. alecto* specimens, but not in *P. poliocephalus* or *P. scapulatus* specimens. Forty-two of 1410 *P. alecto* animals had Hendra virus RNA detected in at least one specimen and yielded a total of 78 positive samples, at an overall detection rate of 1.76% among all specimens tested from this species (78/4436). Urine was the most common specimen type to be positive (26 of 29 urine specimens).

Horses are the only animals known to have served as intermediate hosts.[5] Rarely, dogs have been found to have had natural infection after exposure to infected horses.[130–132] In an experimental model, beagles have been infected after oronasal exposure, but generally remain asymptomatic.[133]

Clinical Manifestations

Clinical manifestations of Hendra virus infection range from a self-limited influenza-like syndrome to a fatal respiratory illness or encephalitis. The incubation period is approximately 5 to 21 days.[1,3,5,37,125] One patient had an influenza-like illness characterized by fever, myalgias, headache, lethargy, and vertigo.[1] He was unwell for 6 weeks and then recovered. Another individual had a dry cough, sore throat, cervical lymphadenopathy, myalgias, fatigue, and fever that lasted for 4 days.[3] The illness lasted for a total of 8 days. Another patient presented with a similar syndrome but rapidly developed respiratory distress necessitating mechanical ventilation.[1,34] He also had acidosis, dehydration, an arterial thrombosis in his right lower extremity, and cardiac irritability. He died 6 days after the onset of illness from asystolic cardiac arrest. Two patients presented with an influenza-like illness, with subsequent progression to encephalitis; one of these patients recovered and the other one died.[5] Another patient presented with encephalitis immediately after completing a 5-day course of postexposure prophylaxis with intravenous ribavirin and hydroxychloroquine.[5] This patient had an incubation period of 21 days, compared with an incubation period of 5 to 16 days in the other patients.

Relapsed disease manifesting as encephalitis occurred in one patient 14 months after a horse that he had cared for died from Hendra virus.[2] The patient was a 35-year-old man who had aseptic meningitis in August 1994, after caring for two sick horses and assisting with their necropsies. He initially developed a sore throat, headache, drowsiness, vomiting, and neck stiffness, and he was found to have 560 WBCs/μL in the CSF, with a polymorphonuclear predominance, and negative bacterial and viral cultures. He recovered fully, but 13 months later he developed irritability, low back pain, and a seizure. During the following week he had a low-grade fever and recurrent seizures. By day 7 of hospitalization, he had a right hemiplegia, brainstem signs, and a decreased level of consciousness and required intubation. He remained febrile and unconscious and was found through the EEG to be having seizure activity despite control of clinically apparent seizures. He died 25 days after admission.

Laboratory Abnormalities

One of the patients who died from Hendra virus infection had thrombocytopenia and elevated levels of creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and glutamyltransferase.[34] He also had signs of dehydration and acidosis. In contrast, a patient who survived had no laboratory abnormalities.[37]

Diagnostic Tests

Diagnostic tests that can be used to detect Hendra virus include culture, EM, immunohistochemistry, serology, and RT-PCR.[5,134] For patients with possible Hendra virus infection, real-time RT-PCR is the diagnostic method of choice.[5,135,136] However, this method may not detect all variants because many different strains are circulating in the bat population in Australia.[5,137,138] Sample types that have yielded positive RT-PCR results include nasopharyngeal aspirates or swabs, urine, blood, and CSF.[139] The use of more than one technique improves the diagnostic yield.[5]

Hendra virus forms syncytia when grown in Vero cells.[35] Primary bat cells also support the growth of Hendra virus.[5,140] The typical EM findings of the herringbone nucleocapsid and the double-fringed appearance of the surface projections of the viral envelope aid in its identification.[34,35] Immunohistochemistry also helps confirm the diagnosis.[5] Serology is useful for epidemiologic and surveillance studies, but not for the diagnosis of acute infection.[5]

Pathology

The autopsy of the horse trainer who died of acute Hendra virus infection revealed interstitial pneumonia characterized by lung congestion, edema, and hemorrhage.[34,125] Histologic examination revealed focal necrotizing alveolitis with giant cells, syncytia formation, and viral inclusions. He also had mild chronic myocarditis and regions of inflammation with necrosis in the kidney, and a pulmonary embolism.[125] Kidney tissue inoculated in cell culture caused formation of syncytia, whereas lung, liver, and spleen tissue did not.[34,125]

The autopsy of the patient who died from relapsed encephalitis revealed a leptomeningitis with a cellular infiltrate that contained lymphocytes and plasma cells.[2] There were discrete foci of necrosis in the neocortex, basal ganglia, brainstem, and cerebellum, with sparing of the subcortical white matter. Rare multinucleated endothelial cells were detected in the brain, liver, spleen, and lungs. Immunohistochemistry of brain tissue was positive for Hendra virus. EM revealed aggregates of nucleocapsids in cell remnants. Hendra virus could not be cultured from the brain.

Therapy

Because no specific antiviral therapies have been proven effective for the treatment of Hendra virus infection, treatment involves only supportive care, such as intravenous hydration and mechanical ventilation, when indicated. Agents that have been evaluated or are being evaluated for the treatment of Hendra virus infection include ribavirin; chloroquine; LJ001, a broad-spectrum antiviral small molecule targeting entry of enveloped viruses[141]; the HIV-1–specific fusion inhibitor enfuvirtide; and a Hendra virus–specific monoclonal antibody.[5] Although ribavirin and chloroquine have activity in vitro, neither one has been effective in animal models of infection.[110,111,142,143] The most promising experimental agent is a recombinant human monoclonal antibody directed against the henipavirus G glycoprotein, m102.4, which protects African green monkeys from disease after inoculation with Hendra virus.[144] This agent was given to two individuals in 2010 and one in 2013 who had a "high-risk" exposure to Hendra virus. None developed Hendra infection, but the effect, if any, of the monoclonal antibody could not be determined.[145] It has also been given to several additional patients who remained well

and experienced no adverse effects.[146] A phase I clinical trial assessing the safety of m102.4 in healthy subjects is underway in Australia.[147]

Prevention

Measures to limit the spread of Hendra virus from infected horses to humans include the use of infection control precautions to prevent contamination of mucosal surfaces and nonintact skin.[5] An important strategy to reduce transmission from flying foxes to horses involves minimizing the opportunity for horses to contact potentially infected body fluids from flying foxes; this can be accomplished by preventing horses from grazing beneath trees in which flying foxes are roosting or feeding, locating yards and stables away from such trees, and placing feed and water stations away from trees and ideally under artificial cover.[5]

Several vaccines have been evaluated in animal models, but none is available for use in humans; vaccines that have shown promising results include a recombinant Hendra virus G glycoprotein–based subunit vaccine and a recombinant adeno-associated virus vector vaccine.[119,148,149] A Hendra virus subunit vaccine was shown to be highly effective at preventing Hendra virus infection in horses after a lethal challenge; it became available for use in horses in Australia in 2012.[150] The efficacy of the vaccine in naturally occurring infections in horses has not been formally tested. The increase in detection of cases of Hendra virus infection in horses in 2013 and 2014 has raised questions about the efficacy of the vaccine.[152] Another likely contributor is the limited acceptance of the vaccine by horse owners.[153]

MENANGLE VIRUS

Epidemiology

From April to September 1997, the farrowing rate at a commercial piggery in Menangle, a suburb of Sydney in New South Wales, Australia, decreased from 82% to 60%.[21] The number of live piglets declined, and the rate of mummified and stillborn piglets increased. Occasional abortions also occurred. The stillborn piglets had abnormalities that included severe degeneration of the brain and spinal cord, arthrogryposis, brachygnathia, and, rarely, fibrinous body cavity effusions and pulmonary hypoplasia. Of pigs at the affected piggery, 96% had neutralizing antibodies against the virus, whereas serum and plasma samples collected from pigs at the piggery before May 1997 were negative.[21,154]

A large colony of fruit bats was found to roost within 200 m of the affected piggery from October to April.[21] Given the proximity of the fruit bats to the piggery and the previous Hendra virus outbreak, fruit bats were immediate targets of the investigation. Forty-two (34%) of 125 serum samples from fruit bats in New South Wales and Queensland, Australia, were positive for neutralizing antibodies against Menangle virus. Antibodies were found in several species of fruit bat.[155] Other species in the area, including rodents, birds, cattle, sheep, cats, and a dog, were all seronegative.[21]

A total of 251 humans who had had potential exposures to infected pigs were tested serologically.[21] Only two of these individuals were found to have neutralizing antibodies, one of whom worked at the affected piggery and one of whom worked at a piggery that had received a shipment of pigs from the affected piggery. Both of these individuals had an influenza-like illness during the weeks after exposure to likely infectious material. They were also tested for a broad range of viruses, bacteria, and parasites that could have caused this illness, but none of the results suggested an alternative diagnosis.[20]

Pathogenesis

Respiratory spread has been proposed as the likely mode of transmission among pigs, but it is less clear how the virus spreads from pigs to humans.[20] Both of the humans who were infected had exposure to body fluids of infected pigs. The first individual helped to birth pigs, and he reported that splashes of amniotic fluid and blood often occurred. He also reported having frequent minor wounds of his hands and forearms. The second patient performed necropsies on pigs without wearing gloves or protective eyewear.

In a study of experimental infection and transmission in which pigs were infected with Menangle virus via intranasal inoculation, the incubation period was approximately 2 to 3 days in duration and was followed by viral shedding from nasal and oral secretions, feces, and urine, lasting for less than 1 week.[156] Cessation of shedding correlated with the development of neutralizing antibodies in sera. Secondary lymphoid organs and intestine were identified as major sites of viral replication and dissemination, as assessed with real-time RT-PCR and immunolabeling for viral antigen. Viremia occurred but was of short duration and low titer.

Clinical Manifestations and Diagnostic Tests

The first individual with Menangle virus reported that in early June 1997 he had the sudden onset of malaise and chills, followed by severe headache and myalgias.[20] He remained in bed for 10 days. Four days into the illness, he developed a spotty erythematous rash. His physician noted that in addition to the rash, he also had abdominal tenderness and lymphadenopathy. He returned to work after 2 weeks but continued to have fatigue. He lost 10 kg during the illness. An evaluation 2 months after the illness revealed mild right lower abdominal tenderness and an enlarged spleen on ultrasound. The liver size was at the upper limit of normal. Urinalysis findings, complete blood count (CBC), blood chemistries, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) level were all normal.

The second patient also became ill in early June 1997 with fever, chills, rigors, sweats, malaise, back pain, severe frontal headache, and photophobia.[20] The headache lasted for 4 or 5 days. On the fourth day of illness, he noticed a spotty erythematous truncal rash, which lasted for 7 days. His acute illness lasted for approximately 10 days, during which time he lost 3 kg. Two months after the illness, he underwent a clinical evaluation. Urinalysis findings, CBC, ESR, and CRP level were normal. Blood chemistries were normal, except for mildly elevated hepatic enzymes. However, he was found to have antibodies against hepatitis C, which could have explained these findings. Abdominal ultrasound examination revealed mild hepatomegaly and a spleen size at the upper limit of normal. Both patients had antibodies against Menangle virus, with titers of 1:128 and 1:512, respectively. As noted earlier, a real-time RT-PCR assay for Menangle virus RNA has been used in a study of experimental infection in pigs.[156]

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The complete reference list is available online at Expert Consult.

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162 Vesicular Stomatitis Virus and Related Vesiculoviruses (Chandipura Virus)

Steven M. Fine

SHORT VIEW SUMMARY

Definition
- Vesicular stomatitis virus (VSV) is a vesiculovirus that causes outbreaks of stomatitis, mainly in livestock but sometimes also in humans.

Epidemiology
- A large percentage of people who live in endemic areas are infected and usually have had mild or subclinical disease.
- Seasonal outbreaks among horses, cattle, and other livestock have the potential to cause large economic losses, particularly in the southwestern United States.
- Endemic disease occurs in areas of Central and South America.
- The mode of transmission is not known, although the virus is believed to be introduced to a herd by an insect vector, and it can then spread from animal to animal by direct contact.
- Related viruses, such as Chandipura virus, have caused outbreaks of encephalitis in India, mainly in children. Chandipura virus has been shown to be spread via mosquitoes and sand flies.

Microbiology
- VSV is a single-stranded, negative-strand RNA virus that is a member of the Rhabdoviridae family, genus *Vesiculovirus*.

Diagnosis
- Samples from lesions can be tested by enzyme-linked immunosorbent assay, complement fixation, or isolation of virus in tissue culture, as well as by polymerase chain reaction assays.
- The main purpose of diagnosis is to quickly distinguish the disease from the more dangerous foot-and-mouth disease in livestock.

Therapy
- Therapy in humans is generally not required.
- There is no known antiviral treatment, so therapy consists of supportive measures.

Prevention
- Quarantine of animals from infected ranches is commonly instituted.
- Protective clothing and gloves should be worn when handling infected animals.
- There are currently no licensed vaccines. Experimental vaccines tested during outbreaks showed immunogenicity but not efficacy.

Vesicular stomatitis virus (VSV) most prominently causes a vesicular disease in domestic animals that resembles foot-and-mouth disease. Outbreaks within domestic animal herds decrease production and result in restrictions on the transport and sale of animals and animal products, which results in significant economic losses. VSV infects a high percentage of people who live in endemic areas. VSV-associated disease in humans is generally mild, although significant morbidity can occur. In addition, because the VSV-G (glyco)protein can bind to numerous cell types, VSV has earned a major role in molecular biology research involving the transduction of genetic material into cells as a vaccine vector and as an antitumor agent.

VIROLOGY

Classification and Structure

Vesicular stomatitis virus is enveloped and contains a single strand of negative-sense RNA that encodes five structural proteins: the glycoprotein (G), membrane (or matrix) protein (M), nucleoprotein (N), and two internal proteins (L and P).[1,2] It belongs to the family Rhabdoviridae, genus *Vesiculovirus*,[3] and it assumes the bullet morphology characteristic of the Rhabdoviridae. Approximately 1200 identical copies of the G protein cover its surface in an ordered, densely packed array of spikes, which present only one antigenic determinant accessible to neutralizing antibodies.[4,5] Of the 9 confirmed and 22 tentative species of vesiculoviruses discovered thus far (Table 162.1), 6 cause animal or human disease: VS-New Jersey (VS-NJ), VS-Indiana (VS-I), VS-Alagoas, Chandipura, Isfahan, and Piry.[6–9]

MOLECULAR BIOLOGY

The VSV-G protein binds to the surface of most cell types. Thus molecular biologists often replace the envelope proteins in other viral vectors with VSV-G to expand the host range of the vector. Viruses produced in cell lines that express the VSV-G protein are thus *pseudotyped* with VSV-G on their surfaces. They can infect a large variety of cells and are therefore tremendously useful for gene transduction.[10,11] VSV is also used as an expression vector in candidate vaccines and can be reengineered to abrogate neurotoxicity and circumvent humoral immunity.[12–14] Live-attenuated VSV vectors are being tested for use in vaccines for human immunodeficiency virus,[15,16] Ebola disease,[17,18] malaria,[19] tuberculosis,[20] and avian influenza.[21]

EPIDEMIOLOGY

Epizootic

In North America VSV disease caused by VS-NJ or VS-I appears in sporadic, epizootic outbreaks in domesticated horses and cattle, mainly in the central and southwestern United States, Canada, and Mexico. Outbreaks of VS-I occurred in 1942, 1956, 1964, 1965, 1997, and 1998[22] and of VS-NJ in 1944, 1949, 1957, 1963, 1982, 1985, 1995, 1997, 2006, 2012, and 2014–15.[23–25] Outbreaks typically begin in late spring, spread to adjacent or remote herds, and abate after heavy frost. The vector is not known, although insects are suspected. VSV was isolated from a mosquito during an epizootic outbreak in New Mexico[26] and from biting midges and black flies that may be responsible for long-distance transport of the virus.[27–29] The 1995 epizootic episode in the southwestern

TABLE 162.1 Vesiculoviruses

SPECIES	LOCATION OF ISOLATION
VS-Indiana	United States
VS-New Jersey	United States
VS-Alagoas	Brazil[6]
VS-Carajas	Brazil[8]
Maraba	Brazil[8]
Piry	Brazil
Cocal	Trinidad[22]
Chandipura	India[74]
Isfahan	Iran[7]

Modified from Travassos da Rosa AP, Tesh RB, Travassos da Rosa JF, et al. Carajas and Maraba viruses, two new vesiculoviruses isolated from phlebotomine sand flies in Brazil. Am J Trop Med Hyg. *1984;33:999–1006.*

United States began in May, in horses in New Mexico, and by October had spread to 367 premises in Arizona, Colorado, New Mexico, Utah, and Wyoming. Seventy-eight percent of cases were in horses and 22% in cattle; one case was in a llama. Production losses, quarantines, and restrictions on livestock shows, auctions, and rodeos cost an estimated $50 to $100 million.[23] The 2015 outbreak of VS-NJ spread to 823 premises in eight states.[30]

Enzootic

In parts of Central and South America and in the United States on Ossabaw Island, Georgia, outbreaks of disease from enzootic VS-NJ predictably appear near the beginning of the dry season (November) and last through March. Farms located near forests and those with poultry experience higher rates of attack.[31] One year in Costa Rica, 9% to 11% of cattle on affected farms developed disease, which constituted 2.6% of cattle overall.[31,32] Lactation and a high acute VSV antibody titer increase the risk of disease for a given animal, but other diseases do not predispose to VSV disease.[31] The reservoirs and vectors for enzootic disease are not known, but phlebotomine sand flies harbor virus in enzootic areas.[6–8,33–35] They can transmit VSV to animals and transovarially to a new generation of sand flies, in which it can then replicate.[34,36,37] Mosquitoes can also harbor VSV and can transmit infection to animals in a laboratory setting.[37] In addition, VSV-infected black flies feeding on uninfected mice can horizontally infect other black flies feeding on the same mouse.[29] The high prevalence rate of VS-NJ antibodies in cows in enzootic areas of Costa Rica (82% for VS-NJ and 17.7% for VS-I)[32] indicates a high lifetime probability of infection, and many of these infections are probably subclinical. Wild animals in enzootic areas also have VS-I and VS-NJ antibodies, with VS-I mainly in arboreal and semiarboreal species and VS-NJ in bats, Carnivora, some rodents, and white-tailed deer.[38,39] Animals with high titers of neutralizing antibodies can become reinfected with other strains.[29]

Animal Disease

Vesicular stomatitis virus infection causes an acute vesicular disease in horses, cattle, swine, goats, llamas, and some wild animals.[23,40] Excess salivation, with fever and blisters or vesicles in and around the mouth, nose, hooves, or teats, appears after a 2- to 8-day incubation period. Vesicles may burst, and the epithelium may slough, leaving large contiguous areas exposed and irritated. Secondary bacterial infection that leads to mastitis may complicate the course, and lameness from foot lesions can develop. A debilitating, nonvesicular manifestation with systemic symptoms, such as fever and weight loss, sometimes occurs. Most animals recover after 2 to 3 weeks,[31,41] but viral sequences may persist.[42]

CLINICAL MANIFESTATIONS

Humans usually contract VSV during close contact with infected animals.[43,44] Most human VSV infections go unrecognized, which indicates either mild or subclinical illness; however, infection is not always benign. Of eight animal handlers who contracted VS-I during a 1965 epizootic episode in cattle, seven reported an illness that included fever, malaise, myalgias, emesis, and pharyngitis, and two had oral vesicular lesions develop in 24 to 48 hours. Although most had mild illness that quickly resolved, one otherwise healthy man had pharyngeal and buccal lesions, lymphadenopathy, and a 20-lb weight loss over 3 weeks.[41] In another case, 30 hours after self-inoculation with VS-I a laboratory worker developed fever, chills, retro-orbital pain, myalgias, nausea, emesis, and diarrhea, which resolved in 3 days.[45]

Vesicular stomatitis virus is neurotropic in baby mice,[46] and two cases of VSV meningoencephalitis have been reported in children. In one case a 3-year-old boy from Panama infected with VS-I had fever, chills, emesis, and generalized tonic-clonic seizures and remained neurologically impaired at discharge.[9]

A related vesiculovirus, Chandipura virus (CHPV), appears to infect humans more readily than VSV and has recently been implicated in outbreaks of encephalitis in parts of India. In 2003 an outbreak of encephalitis occurred in children in Andra Paresh, India, with a high fatality rate (183 of 329 cases, or 55%). CHPV was detected in 28 of 51 cases (51%) with either presence of viral RNA, immunoglobulin M antibodies, or both.[47] In 2004 an outbreak of encephalitis in children was noted in Eastern India and involved 26 cases, with a fatality rate of 78.3%. Laboratory studies in 9 of 13 patients[48] revealed CHPV RNA in serum specimens. CHPV has been isolated from sand flies,[49] which were believed to be the likely vector of spread in the first outbreak but were not detected in a small number of sand fly samples in the second outbreak. Mosquitoes have also been shown to be a vector. CHPV continues to be implicated in outbreaks of encephalitis in rural India,[50] and in 2016 an outbreak of CHPV-associated encephalitis was reported in the Bihar state and affected four children.[51–53]

In an area of Iran enzootic for the Isfahan vesiculovirus, all residents older than 5 years were seropositive in one study,[7] and in a VS-Alagoas–endemic area of Colombia, 62% to 83% of people were seropositive,[6] indicating previous infection. This relatively high rate of seropositivity, which increases with age, also occurs with other serotypes in their respective enzootic areas.[33,38,39,54]

DIAGNOSIS

VSV causes lesions that look like those of the more dangerous foot-and-mouth disease; therefore VSV outbreaks demand urgent diagnosis. Current diagnostic methods include complement fixation, serum neutralization, enzyme-linked immunosorbent assay, or viral isolation in tissue culture.[23] Recently developed assays, with reverse transcription (RT) and polymerase chain reaction (PCR), ease the collection of samples, can be used to identify viral RNA from lesions previously treated with toxic substances, and are being adapted for general use.[55]

Diagnosis of CHPV infection can be made by detection of virus through highly sensitive and specific RT-PCR. CHPV may also be detected in a variety of sand fly and mosquito tissue culture cell lines, and in vertebrate cell lines.[52]

HOST RESPONSE

Studies in mice showed that the presence of B cells and antibody responses was associated with recovery and the development of resistance to VSV; however, $CD4^+$ T cells also contribute to long-term survival, and secretion of interleukin-12 may be beneficial.[56–58] Antibody-mediated neutralization blocks virus-to-cell binding and requires 200 to 500 VSV-G protein–specific immunoglobulin G molecules per virus particle.[2,4] Neutralizing antibodies bind to the same G-protein epitope and protect against infection with the same strain.[5,59] However, in endemic areas, up to 10% of cattle with high titers of neutralizing antibodies become infected each year with clinical symptoms, presumably from mutant strains.[31]

THERAPY

Human infections with VSV are usually mild and generally do not necessitate treatment. No specific treatments exist, and antiviral agents have not been evaluated in vivo. Interferon (IFN)-α, IFN-β, and IFN-γ inhibit VSV growth in vitro[60–62] and protect newborn mice from lethal VSV infection.[63] Prostaglandins A_1 and A_2,[64,65] ribavirin,[66,67] and some experimental compounds[68–70] inhibit VSV in vitro. Doxycycline has been recently reported to inhibit VSV replication in vitro.[71] In animals

secondary bacterial infections of the mouth, teats, and hooves should be treated appropriately, and a mild antiseptic mouthwash may relieve pain from blisters.[72] Nutritional support may help animals that stop eating.

PREVENTION AND VACCINATION

Experimental vaccination with a recombinant vaccinia vector that expresses the VSV-G protein stimulated neutralizing antibody production and protected mice against lethal VSV disease after intravenous challenge. In cattle protection was incomplete, but it correlated with high antibody titer.[73] There are no licensed VSV vaccines for livestock available in the United States. Previously, vaccines used on a restricted basis during outbreaks showed immunogenicity but not long-term effectiveness.

Vaccines against CHPV are also under development.[52]

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The complete reference list is available online at Expert Consult.

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163 Rabies (Rhabdoviruses)

Brett Williams, Charles E. Rupprecht, and Thomas P. Bleck

SHORT VIEW SUMMARY

Definition
- Rabies is a zoonotic encephalitis caused by different species of neurotropic viruses in the *Lyssavirus* genus, Rhabdoviridae family.

Epidemiology
- Rabies is one of the oldest human diseases, with the highest case-fatality rate.
- Approximately 3.3 billion people live in regions where rabies is enzootic.
- An estimated 25,000 to 159,000 people die from rabies each year.
- Although all age groups are susceptible, rabies is most common in children younger than 15 years.
- Bites by rabid dogs cause 99% of human deaths globally.
- In the United States bats account for most human rabies cases.
- Rabies virus transmission may also occur through tissue or organ transplantation.

Microbiology
- Lyssaviruses are bullet-shaped, single-stranded RNA viruses of the order Mononegavirales, family Rhabdoviridae, and genus *Lyssavirus.*
- Rabies virus is the type species of the *Lyssavirus* genus.

Diagnosis
- Development of an acute neurologic syndrome after the bite of a rabid animal is recognized most often in developing countries.
- Encephalitic rabies occurs in the majority of patients, with fever, hydrophobia, aerophobia, agitation, autonomic overactivity with hypersalivation, coma, and paralysis.
- Paralytic rabies is characterized by ascending flaccid quadriparesis and coma.
- Antemortem diagnosis can be made by direct immunofluorescent staining of skin biopsy specimens, reverse-transcriptase polymerase chain reaction of saliva or skin biopsy specimens, or detection of antivirus antibodies in serum or cerebrospinal fluid.

Therapy
- There is no proven antiviral therapy.
- Most patient with rabies virus infection die within a few days to 2 weeks after the onset of illness.

Prevention
- Annually, more than 12 million people receive rabies postexposure prophylaxis.
- After exposure to a rabid animal, prevention consists primarily of prompt wound cleansing, infiltration of rabies immune globulin, and rabies vaccine according to the Advisory Committee on Immunization Practices and World Health Organization guidelines.
- Bat rabies causes the majority of human cases in the United States, and postexposure prophylaxis depends on the type of exposure and availability of the animal for rabies diagnosis.
- Preexposure prophylaxis should be targeted to high-risk groups, including veterinarians, laboratory workers, and certain travelers and children living in remote vampire bat–enzootic areas.

Rabies is a viral disease that produces an almost uniformly fatal encephalitis in humans and other mammals. This zoonosis has been present throughout recorded history and classical literature and likely predates the evolution of humans. Rabies remains one of the most common viral causes of mortality in the developing world. Exposure to the virus has profound medical and economic implications throughout the world, with as many as 12 million people annually receiving postexposure prophylaxis (PEP) to prevent rabies.[1] Although current technology can produce safe biologics for prevention, deviations from recommended regimens can lead to prophylaxis failure and fatal illness.

Rabies, Latin for "madness," derives from *rabere*, to rave, and is related to the Sanskrit word for violence, *rabhas.* The Greek term for rabies, *lyssa*, also means madness, and provides the genus name *(Lyssavirus)*. The Babylon Eshnuna code contains one of the first suggested mentions of rabies in the 23rd century BCE.[2] Democritus provided a clear description of animal rabies in about 500 BCE. Wound cauterization was the preferred treatment in the CE 1st century and was recommended for the management of rabid animal bites until the late 19th century.[2] This treatment remained the only real therapy until Pasteur introduced a vaccine based on an attenuated rabies virus in 1885.

Rabies in the Western Hemisphere likely predated Columbus but remained rare because of low canine population density.[3] Bats spread the disease among livestock and humans in the Americas during the early 16th century.[4] Rabies epizootics began in the northern and eastern United States in the 18th century, reflecting in part the introduction of rabid dogs and importation of foxes for hunting.[5] Globally, bites by rabid domestic dogs cause 99% of human deaths.[1]

Rabies was not diagnosed clinically until 1903, when Adelchi Negri described the cytoplasmic inclusions that bear his name.[6] These inclusions were the only pathologic marker before the development of the fluorescent antibody test in 1958.[7]

VIROLOGY

Classification

The Rhabdoviridae are rod-shaped, negative-sense, nonsegmented, single-stranded RNA viruses. Four major genera infect animals *(Lyssavirus, Vesiculovirus, Ephemerovirus, Novirhabdovirus)*. The genus *Lyssavirus* comprises multiple serotypes; rabies (serotype 1) is the type species of the genus,[5] vesicular stomatitis virus that of the *Vesiculovirus* genus, bovine ephemeral fever that of the *Ephemerovirus* genus, and infectious hematopoietic necrosis virus of the *Novirhabdovirus* genus. Rabies is enzootic, and sometimes epizootic, in a variety of mammals. Australian bat lyssavirus (ABLV) is genetically distinct from rabies virus but causes encephalitis in Australian flying foxes and insectivorous bats. Three human deaths in Australia have been reported due to encephalitis from ABLV infection acquired from animals.[8] The other members of the genus rarely cause human disease (Table 163.1). Current rabies biologics have excellent cross reactivity with members in phylogroup 1 but less so with other more disparate members of the genus. New putative *Lyssavirus* spp. are still emerging in bats around the world.[9,10]

Vesiculoviruses share many of the virologic characteristics of the lyssaviruses. They infect a large number of animal and insect species; humans are occasionally infected via contact with animals, typically via respiratory secretions.[11] Seven vesiculoviruses are known to

TABLE 163.1 Members of the *Lyssavirus* Genus

VIRUS	PHYLOGROUP	RESERVOIRS
Rabies	1	Bats and carnivores, found worldwide except for Antarctica, New Zealand, Sweden, Norway, Spain, Taiwan, Japan, and some other islands
Lagos bat	2	Probably enzootic in fruit bats; no reported human cases
Mokola	2	Probably an insectivore or rodent species limited to parts of Africa; a few domestic animal and two human cases reported
Duvenhage	1	Insectivorous bats; cases identified in South Africa, Zimbabwe, and Senegal
European bat lyssavirus 1 (EBLV1)	1	European insectivorous bats *(Eptesicus serotinus)*
European bat lyssavirus 2 (EBLV2)	1	European insectivorous bats (e.g., *Myotis dasycneme, Myotis daubentonii*)
Australian bat lyssavirus	1	Flying foxes (e.g., *Pteropus* spp.) and insectivorous bats (e.g., *Saccolaimus flaviventris*); three human deaths reported
Irkut	1	Siberian insectivorous bats (e.g., *Murina leurogaster*)
Aravan	1	Central Asian insectivorous bats (e.g., *Myotis blaythi*)
Khujand	1	Central Asian insectivorous bats (e.g., *Myotis mystacinus*)
West Caucasian Bat	3	Insectivorous bats (e.g., *Miniopterus schreibersi*), Caucasian Region
Shimoni	2	Bats (e.g., *Hipposideros commersoni*), Kenya
Ikoma	2	Thought to be African bats
Gannoruwa	1	Fruit bats (e.g., *Pteropus* spp.) in Sri Lanka
Lleida	3	Common bent-winged bat (e.g., *Miniopterus* spp.) in Spain

occasionally infect humans.[12] Another negative-sense single-stranded RNA virus, Borna disease virus, produces central nervous system (CNS) infection in birds and primates, and some authors have suggested that it is related to human psychiatric disorders.[13] However, in contrast to the rhabdoviruses, Borna disease virus replicates in the nucleus rather than in the cytoplasm. It is not a rhabdovirus and is classified within its own family.

EPIDEMIOLOGY

Human Rabies

Rabies is distributed worldwide except for Antarctica, New Zealand, Sweden, Norway, Japan, and some other islands. In 2007, the last year for which global data are available, at least 103 nations reported the presence of rabies, and 42 reported its absence.[14] In the United States an average of three human cases were reported annually during the past decade (Fig. 163.1).

World Health Organization (WHO) estimates that approximately 59,000 humans die of rabies annually. These deaths probably represent an underestimate of the worldwide incidence of the disease, which may cause as many as 159,000 deaths annually.[15,16] A disproportionate number of those affected are children younger than 15 years.[17] An estimated 12 million persons receive PEP annually, with some people still administered unsafe vaccines that carry a risk of neurologic complications.[1,18]

The epidemiology of human rabies reflects that of local animal rabies.[19] In developing areas where canine rabies remains common, most human cases result from dog bites. In regions where dogs are immunized, most human cases follow exposure to rabid wildlife.[20] In the United States 67 of the 22,478 (0.3%) dogs and 244 of 23,101 (1.1%) cats tested in 2015 were positive for rabies.[21] During 2015 (Table 163.2) such national rabies surveillance identified the four major animal reservoirs as bats, raccoons, skunks, and foxes, which accounted for 91% of the 5508 total cases of animal rabies reported.[21] Small rodents, such as chipmunks, squirrels, gerbils, rabbits, rats, and mice, are not a risk group for viral transmission.[20] A leading source associated with cases in the United States is the silver-haired bat *(Lasionycteris noctivagans)*. However, imported cases of canine rabies continue to be reported, and in 2013 a case of human rabies occurred in a Guatemalan immigrant detained at the Texas border, which was confirmed as a canine rabies virus variant.[22] The patient had a dog in Guatemala that died of unknown causes in 2011, but there were no reported bites.

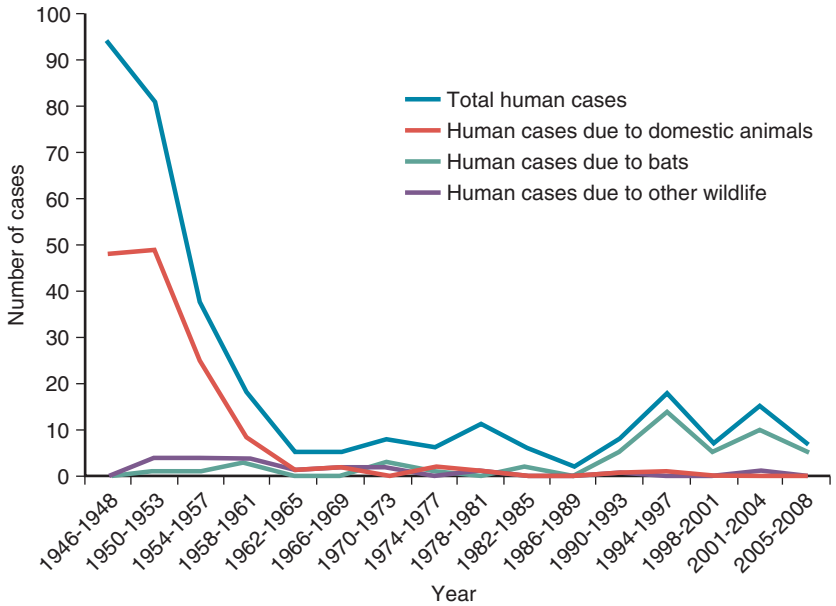

FIG. 163.1 Reported cases of human rabies in the United States by year, 1945–2008. *(From McNabb SJN, Jajosky RA, Hall-Baker PA, et al. Summary of notifiable diseases: United States, 2006.* MMWR Morb Mortal Wkly Rep. *2008;55:1–94.)*

TABLE 163.2 Cases of Rabies in Humans in the United States and Puerto Rico, January 2003–October 2018, by Circumstances of Exposure and Rabies Virus Variant

DATE OF ONSET	DATE OF DEATH	REPORTING STATE	AGE (YR)	SEX	EXPOSURE[A]	RABIES VIRUS VARIANT[B]
Feb. 10, 2003	Mar. 10, 2003	VA	25	M	Unknown	Raccoon, eastern United States
May 28, 2003	Jun. 5, 2003	PR	64	M	Bite, Puerto Rico	Dog-mongoose, Puerto Rico
Aug. 23, 2003	Sept. 14, 2003	CA	66	M	Bite	Bat, Ln
Feb. 9, 2004	Feb. 15, 2004	FL	41	M	Bite, Haiti	Dog, Haiti
Apr. 27, 2004	May 3, 2004	AR	20	M	Bite (organ donor)	Bat, Tb
May 25, 2004	May 31, 2004	OK	53	M	Liver transplant	Bat, Tb
May 27, 2004	Jun. 21, 2004	TX	18	M	Kidney transplant	Bat, Tb
May 29, 2004	Jun. 9, 2004	TX	50	F	Kidney transplant	Bat, Tb
Jun. 2, 2004	Jun. 10, 2004	TX	55	F	Arterial transplant	Bat, Tb
Oct. 12, 2004	Survived	WI	15	F	Bite	Bat, unknown
Oct. 19, 2004	Oct. 26, 2004	CA	22	M	Unknown, El Salvador	Dog, El Salvador
Sept. 27, 2005	Sept. 27, 2005	MS	10	M	Contact	Bat, unknown
May 4, 2006	May 12, 2006	TX	16	M	Contact	Bat, Tb
Sep. 30, 2006	Nov. 2, 2006	IN	10	F	Bite	Bat, Ln
Nov. 15, 2006	Dec. 14, 2006	CA	11	M	Bite, Philippines	Dog, Philippines
Sept. 19, 2007	Oct. 20, 2007	MN	46	M	Bite	Bat, unknown
Mar. 16, 2008	Mar. 18, 2008	CA	16	M	Bite, Mexico	Fox, Tb related
Nov. 19, 2008	Nov. 30, 2008	MO	55	M	Bite	Bat, Ln
Feb. 25, 2009	Survived	TX	17	F	Contact	Bat, unknown
Oct. 5, 2009	Oct. 20, 2009	IN	43	M	Unknown	Bat, Ps
Oct. 20, 2009	Nov. 11, 2009	MI	55	M	Contact	Bat, Ln
Oct. 23, 2009	Nov. 20, 2009	VA	42	M	Contact, India	Dog, India
Aug. 2, 2010	Aug. 21, 2010	LA	19	M	Bite, Mexico	Bat, Dr
Dec. 24, 2010	Jan. 10, 2011	WI	70	M	Unknown	Bat, Ps
Apr. 30, 2011	Survived	CA	8	F	Unknown	Unknown
Jun. 30, 2011	Jul. 20, 2011	NJ	73	F	Bite, Haiti	Dog, Haiti
Aug. 14, 2011	Aug. 31, 2011	NY	25	M	Contact, Afghanistan	Dog, Afghanistan
Aug. 21, 2011	Sept. 1, 2011	NC	20	M	Unknown (organ donor)[C]	Raccoon, eastern United States
Sep. 1, 2011	Oct. 14, 2011	MA	40	M	Contact, Brazil	Dog, Brazil
Dec. 3, 2011	Dec. 19, 2011	SC	46	F	Unknown	Bat, Tb
Dec. 22, 2011	Jan. 23, 2012	MA	63	M	Contact	Bat, My sp.
Jul. 6, 2012	Jul. 31, 2012	CA	34	M	Bite	Bat, Tb
Jan. 31, 2013	Feb. 27, 2013	MD	49	M	Kidney transplant	Raccoon, eastern United States
May 16, 2013	Jun. 11, 2013	TX	28	M	Unknown, Guatemala	Dog, Guatemala
Sept. 12, 2014	Sept. 26, 2014	MO	52	M	Unknown	Bat, Ps
Jul. 30, 2015	Aug. 24, 2015	MA	65	M	Bite, Philippines	Dog, Philippines
Sept. 17, 2015	Oct. 3, 2015	WY	77	F	Contact	Bat, Ln
Nov. 25, 2015	Dec. 1, 2015	PR	54	M	Bite	Dog-mongoose, Caribbean
May 5, 2017	May 21, 2017	VA	65	F	Bite	Dog, India
Oct. 6, 2017	Oct. 21, 2017	FL	56	F	Bite	Bat, Tb
Dec. 28, 2017	Jan. 14, 2018	FL	6	M	Bite	Bat, Tb
Jul. 15, 2018	Aug. 23, 2018	DE	69	F	Unknown	Raccoon, eastern United States

[A]Data for exposure history are reported when plausible information was reported directly by the patient (if lucid or credible) or when a reliable account of an incident consistent with rabies virus exposure (e.g., dog bite) was reported by an independent witness (usually a family member). Exposure histories are categorized as bite, contact (e.g., waking to find bat on exposed skin) but no known bite was acknowledged, or unknown (i.e., no known contact with an animal was elicited during case investigation).

[B]Variants of the rabies virus associated with terrestrial animals in the United States and Puerto Rico are identified with the names of the reservoir animal (e.g., dog or raccoon), followed by the name of the most definitive geographic entity (usually the country) from which the variant has been identified. Variants of the rabies virus associated with bats are identified with the names of the species of bats in which they have been found to be circulating. Because information regarding the location of the exposure and the identity of the exposing animal is almost always retrospective, and much information is frequently unavailable, the location of the exposure and the identity of the animal responsible for the infection are often limited to deduction.

[C]Infection was not identified until 2013, when an organ recipient developed rabies.

Dr, Desmodus rotundus; *Ln,* Lasionycteris noctivagans; *My sp., Myotis* species; *Ps,* Perimyotis subflavus; *Tb,* Tadarida brasiliensis.

From Ma X, Monroe BP, Cleaton JM, et al. Rabies surveillance in the United States during 2017. J Am Vet Med Assoc. *2018;253:1555–1568.*

Transplant-Associated Rabies

In 2004 rabies developed in four recipients of kidneys, an arterial segment, and the liver from a man in Texas who died of encephalitis of unknown cause.[23] Immunohistochemical and direct fluorescent antibody (DFA) staining demonstrated rabies virus in multiple tissues from all recipients, and Negri bodies were also seen in brain tissue of all recipients. Investigations conducted after the rabies diagnosis revealed that the infected donor had reported being bitten by a bat. A series of transplant-associated rabies cases in Germany was reported from an infected donor.[24] All six transplant patients received PEP with rabies immune globulin (RIG) and vaccine. There were three deaths among recipients of lung, kidney, and pancreas transplants. Two of the survivors had received corneal transplants, and one liver transplant recipient had received rabies immunization as a child and had detectable rabies virus antibodies on a pretransplantation blood sample. Transmission of rabies virus had been previously documented in corneal transplantation recipients.[25,26] One recipient received standard PEP plus interferon and did not develop rabies.[26] During 2013 four patients in the United States received organ or tissue donation from a man who had been infected with a raccoon rabies virus variant. One of the recipients subsequently died of rabies. The other recipients, all of whom received PEP, have not developed symptoms of rabies.[27] Two kidney transplant recipients in China died of rabies after receiving allografts from a boy who died of encephalitis in 2015; two recipients of corneal transplants from the boy remain well after receiving PEP.[28] The virus in the Chinese cases was found to be a recently described viral variant (Chinese lineage II).[29] These cases, in addition to transplant-associated West Nile encephalitis and lymphocytic choriomeningitis, have led guidelines to recommend rejection of potential organ donors with encephalitis of unknown cause.[30,31]

Animal Rabies

In the developing world rabies is predominantly a problem of domestic animals. As all developed nations have eliminated rabies from dogs, wildlife is the major affected group. Wildlife accounted for approximately 92% of the reported cases of rabies in the United States in 2015.[21] The prevalence of wildlife rabies waxes and wanes dependent upon surveillance bias and has been decreasing in most animal species except in bat populations (Fig. 163.2).[21] Bats are increasingly the source of human rabies in the United States.[32] The epizoology of bat rabies is changing from typical reservoirs (e.g., the common big brown bat) to include previously rarely affected species (e.g., the silver-haired bat).[33] Most of the human rabies cases known to have been contracted from bats in the United States since 1980 involve the silver-haired/eastern tricolored bat rabies virus variants.[34] Most cases of rabid skunks during 2015 were reported in south-central and eastern states.[21] An outbreak of raccoon rabies in the United States began in 1977 near the Virginia–West Virginia border, and in the ensuing 2 decades the territory expanded to involve most of the eastern states. More than 20,000 cases of raccoon rabies have been reported to date, with several thousand secondary cases in dogs and other animals.[35] Raccoon rabies remains enzootic in 19 states and the District of Columbia, but in 2015 cases were declining.[21]

Rabies reports had also been increasing among bats, but appears to have plateaued.[21,36] Although there was concern that in some wildlife species (e.g., spotted hyenas) rabies virus infection may not lead to clinically apparent disease, such speculation has not been supported.[37] In at least one bat species, *(Eptesicus serotinus)*, salivary samples may contain viral RNA without detectable RNA in concurrent brain samples, but the relevance of amplicon detection in lieu of viral isolation remains questionable.[38] Barring alternative explanations, the potential implications of this finding for routine screening of wildlife after potentially infectious human contact remain to be determined.

Previously unidentified viral variants from potential cross-species transmission are still emerging around the world.[39,40] Molecular epidemiologic evidence reinforces the importance of avoiding contact with downed bats or other ill wildlife.[41,42]

Composition

Lyssaviruses are bullet-shaped, with an average length of 180 nm and an average diameter of 75 nm.[43] The complete virion includes a helical nucleocapsid with 30 and 35 coils between 4.2 and 4.6 nm in length.[44] This is enclosed in a lipoprotein envelope 7.5 to 10 nm thick, from which glycoprotein (G protein) spikes project 10 nm.[45] These spikes cover the surface of the virus except at the blunt end (Fig. 163.3).

The *Lyssavirus* genome encodes five genes: *N, P (NS), M* (or M_2), *G,* and *L* (Table 163.3).[46] Phosphorylation of the nucleoprotein is necessary for efficient replication of the viral RNA,[47,48] and the nucleoprotein is potentially immunogenic.[49] It is needed to switch from the transcription of gene products to the production of full-length positive-strand RNA.[50] Use of monoclonal antibodies directed against the N protein is used routinely for antigenic typing of virus variants. Molecular sequence analysis of the *N* and, increasingly, *G, P,* and *L* genes, adds resolution and identifies virus lineages among virus variants. The P protein may control the L protein, an RNA-dependent RNA polymerase.[51,52] The M, or matrix, protein is located between the nucleocapsid and the lipoprotein envelope,[53] and, in concert with the G protein, is responsible for the

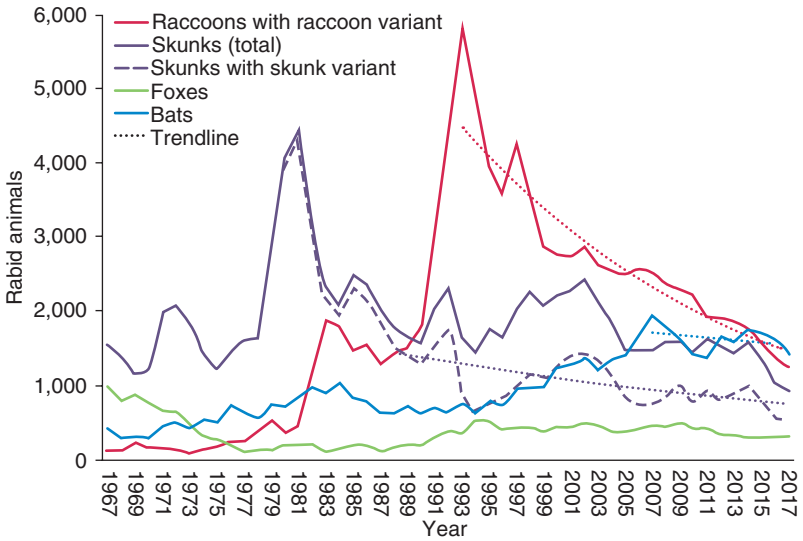

FIG. 163.2 Cases of rabies among wildlife in the United States by year and species, 1967–2017. *(From Ma X, Monroe BP, Cleaton JM, et al. Rabies surveillance in the United States during 2017.* J Am Vet Med Assoc. *2018;253:1555–1568.)*

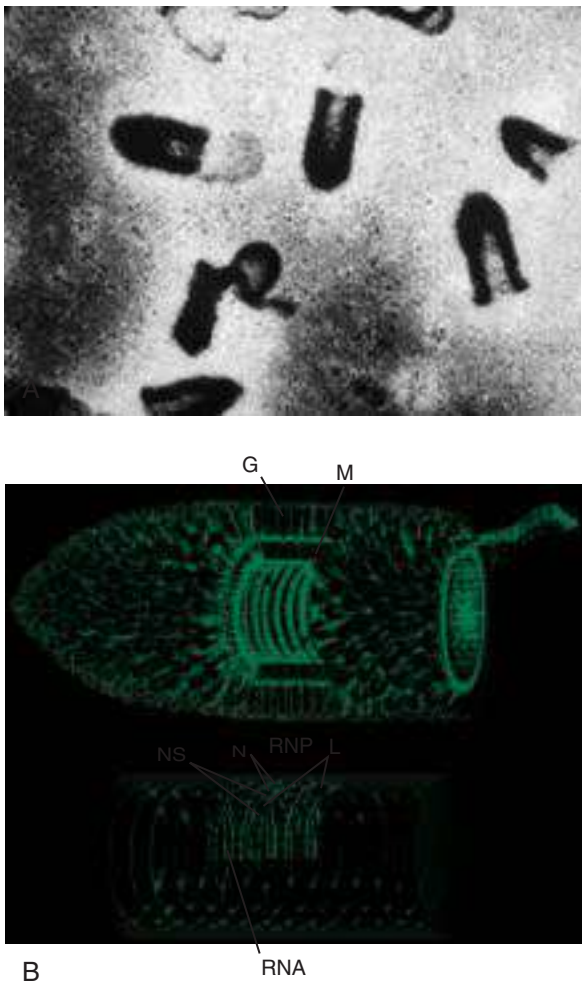

FIG. 163.3 Rabies virus morphology. (A) Electron micrograph of rabies virus. Original magnification, ×55,000. (B) Schematic illustration of rabies virus *(top)*, showing surface glycoprotein *(G)* projections extending from lipid envelope surrounding ribonucleoprotein *(RNP)* and matrix protein *(M)* lining envelope. Helical RNP *(bottom)* comprises single-stranded *RNA* genome, plus nucleoprotein *(N),* phosphoprotein *(NS),* and transcriptase *(L). (From Dietzschold B, Rupprecht CE, Fu ZF, Koprowski H. Rhabdoviruses. In: Fields BN, Knipe DM, Howley PM, et al, eds.* Fields Virology. *3rd ed. Philadelphia: Lippincott Raven; 1996:1137–1159.)*

TABLE 163.3 Rhabdovirus Genes and Products

GENE	SIZE (kDa)	FUNCTION
N (nucleoprotein)	50	Encapsidates the viral RNA and major constituent of the nucleocapsid
P (phosphoprotein)	40	Originally thought to encode a nonstructural (NS) protein but now known to produce a structural protein that is phosphorylated by kinases in the host cell and that joins with L
M (matrix)	26	Responsible for the assembly and budding of bullet-shaped particles, in concert with the G protein
G (glycoprotein)	65	Attachment to host cell receptors
L (large polymerase)	160–190	RNA-dependent RNA polymerase; required for transcription of the negatively stranded viral RNA; appears to form a complex with NS

Modified from Rupprecht CE, Smith JS, Fekadu M, Childs JE. The ascension of wildlife rabies: a cause for public health concern or intervention? Emerg Infect Dis. *1995;1:107–114.*

assembly and budding of bullet-shaped virions.[54] The N and M proteins determine a balance between transcription and replication.[55,56]

The G protein is involved in cellular reception and is the antigen that induces virus-neutralizing antibodies. Variability in this protein is responsible for serotypic differences among lyssaviruses,[57] and mutations at one position, 333 (with substitution of glutamine or isoleucine for arginine), can disrupt virulence.[58] This arginine residue appears to be essential for fusion of the viral envelope with neurons.[59] Molecular modifications of the G protein can increase its antigenicity.[60]

PATHOGENESIS

Natural rabies infection appears to require a period of local viral replication, perhaps to increase the inoculum, before nervous system infection occurs. Timely administration of RIG and active immunization can prevent spread of the virus into the nervous system, thereby preventing disease. The virus does not tolerate a pH below 3 or above 11 and is inactivated by ultraviolet light, sunlight, desiccation, and various chemicals, such as formalin, phenol, ether, trypsin, β-propiolactone, and many detergents.

Once the virus has entered peripheral nerves, current therapeutic techniques probably do not readily prevent subsequent replication and spread, and the virus quickly moves centrally. After the virus enters through a break in the skin, across a mucosal surface, or through the respiratory tract, it may replicate in muscle cells and, in so doing, infects the muscle spindle. In muscle the virus binds to the nicotinic acetylcholine receptor.[61] Some studies suggest that the neuromuscular junction is a major site of neuronal invasion,[62] and blocking acetylcholine receptors inhibits viral attachment.[63] Partial sequence homology exists between rabies virus G protein and several snake neurotoxins that bind to this receptor.[64] However, rabies virus can enter neurons that do not express acetylcholine receptors,[65] probably by G protein binding to nerve growth factor P75NTR or neural cell adhesion molecule (CD56).[66,67] Once bound to the receptor, virus is internalized with receptor-mediated endocytosis via a clathrin coated pit.[68] Rabies virus, after internalization into endosomes, ascends via endosomic trafficking along microtubules.[68] Endosomes acidify upon arrival at the soma, releasing viral particles into the cytoplasm.[68] Herpes simplex virus and tetanus toxin also make use of the microtubular transport systems.[69] Replication occurs in neurons but not usually in glia, either peripheral or central. The viral envelope forms from host membranes into which the G and M proteins are inserted.[70] The envelope includes small amounts of host proteins.[52]

Virus may be present in dorsal root ganglia within 60 to 72 hours of inoculation and before its arrival in spinal cord neurons, which confirms its transport within sensory neurons. After reaching the spinal cord the virus spreads throughout the CNS, following established patterns of synaptic connectivity.[71] Virtually every neuron is infected.[72]

After CNS infection, virus spreads to the rest of the body via peripheral nerves.[73] The high concentration of virus in saliva results from viral shedding from sensory nerve endings in the oral mucosa[70] and also reflects substantial replication in the salivary glands.

The mechanisms by which rabies virus damages the CNS are obscure because pathologic evidence of neuronal necrosis is frequently minimal or absent.[74] Animal models suggest that rabies virus may interfere with neurotransmission[75] and with endogenous opioid systems,[76] and the almost 30-fold increase in local nitric oxide production[77] suggests an excitotoxic mechanism. Analysis of CSF metabolites from rabies patients, including two survivors, suggests that production of quinolinate, an *N*-methyl-D-aspartate receptor agonist, is overproduced, potentially leading to excitotoxicity.[78] Cerebrospinal fluid (CSF) quinolinate levels fell with time in survivors but rose with time in those who died.[78] Rabies virus P protein also appears to increase production of reactive oxygen species by interaction with mitochondrial complex I.[79] This oxidative stress and resultant excitotoxicity leads to axonal swellings, degeneration, and impaired axonal growth but few morphologic changes or neuronal death.[80] Rabies virus antigen concentrates in the red nucleus and midbrain raphe, areas where lesions are known to cause aggression; this, along with interference with endogenous opioid receptors, may be the basis for the characteristic behavioral changes.[76,81,82]

An inverse relationship is found between the concentration of G protein produced and the pathogenicity of different viral variants, and

a monotonic relationship is found between pathogenicity and the induction of neuronal apoptosis.[83]

Pathology

The brain in encephalitic rabies (see subsequent discussion) usually appears unremarkable grossly,[84] except for the vascular congestion. The microscopic pathology of rabies is typically encephalitis with Negri bodies (Fig. 163.4). However, not all autopsy specimens show the perivascular lymphocytic cuffing and necrosis that characterize encephalitis, and some cases look histologically like meningitis.[85] Negri bodies are concentrated in hippocampal pyramidal cells and less frequently in cortical neurons and cerebellar Purkinje cells.[84] They are round or oval, usually eosinophilic, cytoplasmic inclusions between 1 and 7 µm across, and they contain viral nucleocapsids.[86] The acidophilic lyssa body is ultrastructurally identical to the Negri body.[87] Negri bodies and lyssa bodies are detected in only a relatively small percentage of the cells that are infected (as determined with immunohistochemistry).[88]

Paralytic rabies affects primarily the spinal cord, with severe inflammation and necrosis.[89] The brainstem is involved to a lesser extent. A few patients have cortical Negri bodies. Segmental demyelination occurs in the peripheral nerves and resembles acute inflammatory polyneuropathy (Guillain-Barré syndrome).

Systemic pathology is most remarkable for the presence of myocarditis.[90] This cardiac disorder resembles the myocarditis that occurs in hypercatecholaminergic states, such as pheochromocytoma, subarachnoid hemorrhage, and tetanus.[69] Negri bodies are found in the hearts of some patients, which suggests a viral role in this condition.[91] Atrial ganglioneuritis suggests that the virus reaches the myocardium via spread from the nervous system.[92]

Immune Responses

The immune response to natural rabies virus infection is generally insufficient to prevent disease. Rabies can produce immunosuppression,[93] potentially by replicating in and inducing apoptosis in T lymphocytes.[94,95] Unvaccinated patients who develop a measurable antibody response develop it late in the course.[96] Rabies virus may evade immune responses by using cell-to-cell transfection, disseminating through dendritic cells carrying infectious viral nucleic acid.[97] Persistence in macrophages may explain some cases of very long incubation periods.[98] The rabies *P* gene appears to prevent cellular immune response to infected muscle cells by suppressing expression of the interferon-β gene.[99] Patients with development of a cellular immune response tend to have the encephalitic form rather than the paralytic form, and they die faster than those who do not have such a response.[100] Differences in the host immune response appear more likely to explain whether encephalitic or paralytic rabies develops than do differences in the strains of virus that cause the natural infection.[101] Some investigators believe that interleukin-1 production in the CNS may explain the immunosuppressive effect of the virus.[102]

CLINICAL MANIFESTATIONS

Human Rabies

Several variables affect the risk of rabies and the rate of clinical disease development after exposure to a rabid animal.[103] The viral inoculum is important, reflected by the relationship between the extent of exposure to the saliva and the rapidity of progression. A bite with prominent salivary contamination (e.g., through exposed skin) is more likely to produce rabies than a bite through thick clothing, which removes saliva from the animal's teeth. Multiple bites are more likely to transmit the disease than a single bite. The location of the bite also influences the risk of rabies: Bites on the face are more likely to result in disease than those on the extremities. Salivary contamination of a preexisting wound can transfer virus, as can exposure of mucous membranes or the respiratory tract to aerosolized virus.[104]

Incubation Period

The reported incubation period for rabies varies from a few days to more than 19 years, although 75% of patients become ill in the first 90 days after exposure. Moreover, very long incubation periods in hyperendemic regions are quite difficult to document when one considers more likely, subtle routes, such as unrecognized nonbite exposures to puppies or kittens that may have occurred more recently. The solid-organ transplant patients described previously generally became ill within 45 days of transplantation, although one patient became ill 18 months after kidney transplantation.[27] The shorter period of incubation in some patients is theorized to be related to their immunosuppressed state.[23]

Prodromal Symptoms

The initial symptoms of rabies resemble those of other systemic viral infections, including fever, headache, malaise, and disorders of the upper respiratory and gastrointestinal (GI) tracts (Table 163.4).[105] Initial neurologic symptoms may include subtle changes in personality and cognition and paresthesias or pain near the exposure site. Rabies is rarely considered early in the differential diagnosis. In one series physicians considered rabies in only 3 of 21 patients on the first visit, despite an exposure history in many.[105] The prodrome typically lasts about 4 days, but up to 10 days may elapse before more specific symptoms and

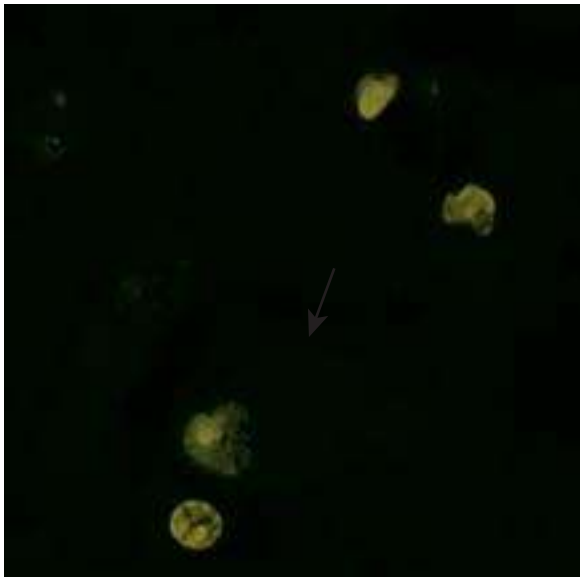

FIG. 163.4 Negri body *(arrow).* Original magnification, ×400. *(Courtesy Maria-Beatriz Lopes, MD, Division of Neuropathology, University of Virginia.)*

TABLE 163.4 Durations of Different Stages of Rabies

Stage	DURATION (% OF CASES)	ASSOCIATED FINDINGS
Incubation period	<30 days (25%) 30–90 days (50%) 90 days to 1 year (20%) >1 year (5%)	None
Prodrome and early symptoms	2–10 days	Paresthesias or pain at the wound site; fever; malaise; anorexia; nausea and vomiting
Acute neurologic disease; furious rabies (80% of cases)	2–7 days	Hallucinations; bizarre behavior; anxiety; agitation; biting; hydrophobia; autonomic dysfunction; syndrome of inappropriate antidiuretic hormone (SIADH)
Paralytic rabies (20% of cases)	2–7 days	Ascending flaccid paralysis
Coma, death[a]	0–14 days	—

[a]Rare recoveries have been reported.

Data modified from Fishbein DB. Rabies in humans. In: Baer GM, ed. The Natural History of Rabies. *2nd ed. Boca Raton, FL: CRC Press; 1991:519–549.*

signs supervene.[105] Myoedema (mounding of part of the muscle struck with a reflex hammer, which then disappears in a few seconds) is present during the prodrome and persists throughout the disease.[106]

Human rabies virus infections are divided into two forms: encephalitic ("furious") and paralytic ("dumb"). The encephalitic form presents with the hydrophobia, delirium, and agitation that form the common picture of rabies. About a fifth of patients present with the paralytic form and have little clinical evidence of cerebral involvement until late in the course. The spinal cord and brainstem bear the brunt of the illness in the paralytic form. The pathogenetic distinction between the two types of rabies is unclear; it does not appear to be based on virologic or antigenic differences.[107] In either form the symptomatic course usually runs 2 to 14 days before coma supervenes. In one series of 32 patients the median duration of illness was 19 days.[108] On occasion, atypical features have been described that do not fit into these classic forms of rabies.[109] Atypical cases can present with sensory or motor deficits, choreiform movements of the bitten limb during the prodromal phase, focal brainstem signs, cranial nerve palsies, myoclonus, and seizures.[109,110]

Not all infections may lead to death or even clinical illness as suggested by a study of Peruvians in the Amazon with a high incidence of bat bites[111]; 6 of 63 residents without history of rabies vaccination were seropositive for rabies virus–neutralizing antibodies, suggesting prior subclinical infection.

Encephalitic (Furious) Rabies

Hydrophobia is the symptom most identified with *encephalitic rabies*. Sir William Gowers[112] provides a seminal depiction of hydrophobia and its sequelae, in which he described:

> "…some discomfort about the throat, an occasional sense of choking, or a little difficulty in swallowing liquids… The attempt to drink occasions some spasm in the pharynx, which increases in the course of a few hours, and spreads to the muscles of respiration, causing a short, quick inspiration, a 'catch in the breath…' This increases in severity to a strong inspiratory effort, in which the extraordinary muscles of respiration, sterno-mastoid, scaleni, etc., and even the facial muscles, take part; the shoulders are raised, and the angles of the mouth drawn outwards. As the intensity of the spasm increases, so does the readiness with which it is excited. It may be caused by the mere contact of water with the lips, and a state of cutaneous hyperæsthesia develops, so that various impressions, such as a draught of air, which normally excite a respiratory effort, bring on the spasm. The mere movement of air caused by raising the bedclothes may be sufficient. The patient is often unable to swallow the saliva, which is usually abundant and viscid, so that it hangs about the mouth and is expelled with difficulty… Vomiting is common… The attacks of spasm are very distressing to the patient; the mental state which they occasion increases the readiness with which they are produced; and in some cases the mere sight of water or the sound of dropping water will cause an attack. It may even be excited by visual impressions which cause a similar sensation, as the reflection from a looking glass, or even a strong light. The sufferer's horror and dread of these excitants becomes intense. Thus the disturbance in the act of swallowing liquids, which constitutes… the first symptom and keynote of the disease, spreads, on the one hand, to mental disturbance, and on the other to extensive muscular spasm. In each of these directions further symptoms develop. The spasm, at first confined to the muscles of deglutition and respiration, spreads to the other muscles of the body, and the paroxysms, at first respiratory, afterwards become general, and assume a convulsive character, although still excited by the same causes. The convulsions may consist of general muscular rigidity, sometimes tetanoid in character, with actual opisthotonus… Actual delusions occasionally supervene, and there may even be wild delirium. The mental derangement is most intense during the paroxysms of spasms, and the frenzied patient may spit his saliva at those about him, and often attempts to bite them with his teeth, making occasional strange sounds in his throat which have been thought to resemble the barking of a dog."

Hydrophobia represents an exaggerated irritant reflex of the respiratory tract, possibly arising from the nucleus ambiguus.[113] Other findings include episodic hyperactivity, seizures, and aerophobia. Hyperventilation is frequently present. Along with coma, evidence of pituitary dysfunction often develops, especially disordered water balance (either inappropriate antidiuresis or diabetes insipidus). Hyperventilation gives way to forms of periodic and ataxic respiration,[113] and eventually apnea supervenes. Cardiac arrhythmias are common, predominantly supraventricular tachycardias, and bradycardias, and they reflect either brainstem dysfunction or myocarditis.[114] Autonomic dysfunction is observed, including pupillary dilation, anisocoria, piloerection, markedly increased salivation and sweating, and, rarely, priapism[115] or spontaneous ejaculation.[116] Two cases are reported of rabies-associated cerebral artery vasospasm that was treatable with drugs directed at the nitric oxide synthase pathway.[117] This vasospasm may relate to a deficiency of tetrahydrobiopterin and might be ameliorated by its replacement.[118]

With exceptions in some rare reports, patients entering coma generally die within 1 to 2 weeks, or earlier, despite maximal supportive care. Patients with encephalitic rabies who receive maximal intensive care support may survive for a longer-than-expected period and appear to pass through the paralytic phase before death.[107]

Paralytic (Dumb) Rabies

Patients with *paralytic rabies*, unlike those with the furious form, do not have hydrophobia, aerophobia, hyperactivity, or seizures. Their initial findings suggest an ascending paralysis, including hypophonia, resembling acute inflammatory polyneuropathy (Guillain-Barré syndrome), or a symmetrical quadriparesis. Paralysis is usually more severe in the bitten limb. Meningeal signs (headache, neck stiffness) may be prominent despite a normal sensorium. As the disease progresses the patient becomes confused and then declines into coma.

Nonneurologic Findings

In addition to the cardiac arrhythmias already mentioned, the systemic complications of rabies are similar to those of other critically ill patients. The virus disseminates to many organs,[119,120] but proof of its role in other organ dysfunction is lacking. GI disturbances include bleeding, vomiting, diarrhea, and ileus.[121] Death is usually from cerebral edema or myocarditis, with cardiac arrhythmia or congestive heart failure as mechanisms.[122]

Animal Rabies

A complete description of the effects of rabies on behavior in all of the species that can be infected is beyond the scope of this text. Prior WHO studies have established a crude ranking of rabies virus susceptibility, which is summarized in Table 163.5.[123] However, overt susceptibility is related in part to viral dose, route, and variant and host attributes. Descriptions of the behavioral changes of rabid animals are available elsewhere.[111,124]

DIAGNOSIS

The diagnosis of rabies poses little difficulty in a nonimmunized patient with hydrophobia after a bite by a known or suspected rabid animal. The presentation in areas where domestic animals are immunized is seldom this straightforward. During the incubation period, no diagnostic studies in the patient are useful; recognition of an exposure to a potentially rabid animal should prompt PEP before illness. When symptoms begin, standard laboratory testing does not reliably distinguish rabies from other encephalitides. The CSF may be normal; however, a mild lymphocytic pleocytosis and a modest increase in protein are often described.[125,126]

Because of limited sensitivity of any one methodology, antemortem diagnosis of rabies requires several specimens, for instance, skin biopsy, saliva, serum, or CSF, and multiple testing methods, including virus-specific immunofluorescent staining, antibody detection in serum or CSF, and use of reverse-transcriptase polymerase chain reaction (RT-PCR)

TABLE 163.5 Generalized Susceptibility of Various Animal Species to Rabies

VERY HIGH	HIGH	MODERATE	LOW
Wolves	Hamsters	Dogs	Opossums
Foxes	Skunks	Primates	
Coyotes	Raccoons		
Kangaroo rats	Domestic cats		
Cotton rats	Rabbits		
Jackals	Bats		
Voles	Cattle		

Data modified from World Health Organization. Sixth Report of the Expert Committee on Rabies: Technical Report Series 523. *Geneva: World Health Organization; 1973.*

in saliva or skin biopsy.[127] DFA staining of biopsy or necropsy (animal or human) tissue remains one standard for the real-time diagnosis of rabies. In humans the procedure of choice is DFA analysis of a full-thickness skin biopsy (5–6 mm in diameter containing approximately 10 hair follicles) obtained from the nape of the neck above the hairline.[128] The virus tends to localize in cutaneous nerves at the base of hair follicles. During the first week of symptoms, about 50% of samples reveal rabies virus, with an increasing percentage thereafter.[129] RT-PCR on biopsy specimens and saliva is another diagnostic procedure of choice in suspected human rabies.[130,131] RT-PCR of skin biopsy specimens has a high (98%) sensitivity and (100%) specificity, and saliva samples can be similarly sensitive if three successive samples are tested.[127] RT-PCR and sequencing allows more specific determination of the geographic and host species origin of a particular rabies virus.[132,133] It may in some cases be successfully performed on decomposed brain material,[134] although the use of fresh CNS tissue is much preferred.[131] Current recommendations for diagnostic testing in animals and humans, with instructions for sample collection and submission, are available at www.cdc.gov/rabies or by calling the rabies laboratory of the Centers for Disease Control and Prevention (CDC) at 404-639-1050. In the United States the state health department should be consulted whenever the diagnosis of rabies is suspected. The state rabies consultation contact phone numbers are also available at the CDC website.

The rapid fluorescent focus inhibition test (RFFIT) is the reference standard serologic test for neutralizing antibodies to the rabies virus G protein.[135] A few untreated patients have detectable antibody by day 6 of clinical illness, 50% by day 8, and usually 100% by day 15. Antibodies in CSF appear later but are diagnostic of infection, even in patients who have received PEP.[136] Immunofluorescent antibody tests that detect rabies virus–specific IgM and IgG antibodies, predominantly to the virus ribonucleoprotein, represent sensitive serologic methods that can be used in serum and CSF.[108]

Computed tomographic scan results of the brain are usually normal early in the course,[26] unless hypoxia has supervened. Later, evidence of cerebral swelling may supervene if the patient receives prolonged critical care support (Fig. 163.5). Magnetic resonance (MR) images show areas of increased T_2 signal in the frontal lobes, hippocampi, hypothalamus, brainstem, and sometimes other areas.[137,138] Fig. 163.6 shows a T_2 image, with involvement of the thalamus and hypothalamus highlighted. Late in the course, gadolinium enhancement may occur in the most profoundly involved areas, indicating breakdown of the blood-brain barrier. In paralytic rabies MR imaging of the spinal cord and nerve roots may be useful.[139] No imaging finding is pathognomonic for rabies.

Differential Diagnosis

With encephalitic rabies, the major differential consideration is another viral encephalitis.[140] However, rabies patients are significantly more likely to develop aerophobia, hydrophobia, dysphagia, and paresthesias or localized pain and weakness than nonrabies cases.[136] Because the CSF and electroencephalographic (EEG) findings in rabies may mimic those of herpes simplex encephalitis, some patients receive empirical therapy with acyclovir while awaiting a more secure diagnosis (e.g., with PCR). Tetanus is occasionally confused with rabies because opisthotonic posturing may be seen in either.[69] However, the other symptoms of rabies, such as hydrophobia, are not seen in tetanus, and CSF and EEG results are normal in tetanus. Rabies may be misdiagnosed as cerebral malaria and vice versa: At least one atypical case of the latter presented with phobic spasms.[141] Strychnine poisoning should be considered and can be excluded with laboratory testing.

Paralytic rabies may resemble acute inflammatory polyneuropathy, transverse myelitis, or poliomyelitis. Electromyographic studies may be useful in distinguishing rabies from polyneuropathy. In transverse myelitis a finding of pain at the level of the lesion may be helpful, as may the finding of a high T_2 signal lesion. A sensory level is characteristic of transverse myelitis, whereas in rabies, sensory function is typically normal.[115] Fever usually precedes weakness in poliomyelitis, and the resolution of fever with the onset of neurologic findings favors this diagnosis. A history of poliomyelitis immunization should be sought.

The sometimes prolonged incubation period of rabies recalls the slow infections of the CNS caused by conventional viruses (e.g., progressive multifocal leukoencephalopathy).[142] However, rabies requires neither a defect in host immunity nor a mutation in the virus to produce disease, which distinguishes it from other neurotropic viruses. Spongiform changes in brain tissue in rabies[143] may resemble those seen in the prion diseases.[144]



FIG. 163.5 **Noncontrast computed tomographic scan showing areas of both severe cerebral edema *(arrows)* and more widespread swelling.**

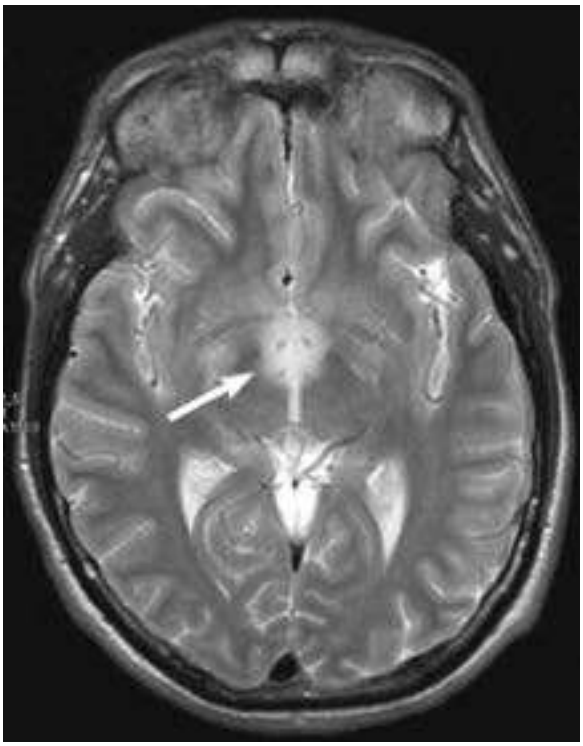

FIG. 163.6 **T2-weighted magnetic resonance image showing increased signal in diencephalon *(arrow).***

Although CNS reactions to the rabies vaccines available in developed countries are exceptionally rare, patients who receive older vaccine forms that contain myelin determinants occasionally have development of acute disseminated encephalomyelitis (ADEM; also called postvaccinial encephalomyelitis; see "Prevention"). ADEM is a syndrome with many precipitants other than rabies vaccine. It resembles encephalitis, or occasionally it presents as a mass lesion that resembles a brain abscess. It typically begins 10 to 14 days after vaccine exposure, which would constitute an unusually brief incubation period for rabies. Spinal fluid serology, viral isolation, or RT-PCR can differentiate rabies from ADEM after immunization.[145] ADEM produces high T_2 signal lesions visible with MR imaging.[146] However, differences in the distribution of the MR lesions in rabies and ADEM may aid in the differential diagnosis.[147]

Patients potentially exposed to suspect animals may have a psychological reaction termed *rabies hysteria*.[148] They may refuse to drink water; in contrast, the patient with rabies attempts, at least initially, to drink but is halted by pharyngeal spasms.

PREVENTION

Preexposure Prophylaxis

Although management of animal rabies is central to prevention of human disease, very few nations have truly eliminated it, and those that have been successful, in lieu of enzootic wildlife rabies, usually maintain quarantine procedures lest the disease reappear.[149] Therefore prophylactic procedures for domestic animals and humans remain essential. Prophylaxis for cats and dogs in many countries is required by law; in the United States the use of 1-year or 3-year vaccines is common, although only the 3-year vaccines are recommended.[150] Vaccination should be performed or supervised by a veterinarian; improper administration can lead to lack of immunity.[151] Measurement of animal seroconversion rates may be considered for exportation but is unnecessary to ensure protection,[152] and immunization of horses or valuable livestock is recommended in areas of increasing rabies prevalence or in petting zoo settings.

Rather than culling, vaccination of wildlife is an effective public health measure.[153] The use of vaccines effective after oral bait delivery allows immunization of wild animals.[154] One intensive 4-year campaign in Belgium eliminated rabies from the fox population.[155] This approach may also be effective in free-ranging dogs.[156] Veterinary vaccines cost about $0.50 or less per dose in the United States. In contrast, Semple-type (grown in sheep brain cultures) human vaccines cost about $5 per course, Vero cell vaccine in France about $160 per course, and human diploid cell rabies vaccine (HDCV) or purified chick embryo vaccine (PCEC) in the United States more than $500 per course.[157]

Preexposure prophylaxis should be targeted to people at high risk of rabies virus exposure, such as veterinarians, laboratory workers who handle rabies virus, cavers, and certain international travelers to countries with high rabies prevalence and poor access to medical care and PEP. For example, in some remote parts of Amazonia, routine exposure to vampire bat predation may warrant consideration of preexposure vaccination, particularly for pediatric populations at risk.[158] Even where PEP is available, it is important to note that patients often do not receive optimal PEP under real-world conditions, and shortages of RIG are common.[159] Current recommendations for international travelers are available at the CDC website (www.cdc.gov/travel/). A series of three intramuscular (IM) or intradermal injections (days 0, 7, and 21 or 28) is sufficient; antibody response determination is not required in healthy hosts. Preexposure vaccination does not eliminate the need for medical attention after a suspect exposure but eliminates the need for RIG and decreases the doses of vaccine required (two postexposure injections at days 0 and 3). Serology and booster doses every 2 to 3 years, if the titer is inadequate, are recommended for individuals frequently at risk of exposure. An adequate antibody response is generally considered to be complete neutralization at the 1:5 serum dilution level (0.1 IU/mL) with the RFFIT, which is in contrast to the 0.5 IU/mL antibody value suggested by WHO.

Postexposure Prophylaxis

Although the incidence of human rabies is low in the United States, animal rabies remains common, and approximately 16,000 to 39,000 people with contact to potentially rabid animals receive rabies PEP annually.[160] The estimated annual expenditure for such rabies prevention is approximately $584 million, with PEP accounting for almost half of this expenditure.[161] Despite the relative high costs, the recent cost-effectiveness analysis of the Advisory Committee on Immunization Practices (ACIP) showed that it is always cost saving to administer PEP if a patient is bitten by a animal that has tested positive for rabies or if a patient is bitten by a reservoir species, even if the animal is not available for testing.[160]

The cornerstone of rabies PEP is proper wound care, which potentially reduces the risk of rabies by 90%.[162] Thorough washing with a 20% soap solution is as effective as the formerly recommended quaternary ammonium compounds.[163] Irrigation with a virucidal agent, such as povidone-iodine, is also used.[164] After wound care the clinician must decide whether to institute specific PEP. Prompt consultation with public health officials is advised because this decision is based in part on the current prevalence of rabies in the animal species involved in the exposure.[165] In countries of low prevalence a healthy dog or cat that has bitten or otherwise transferred saliva to a human is observed for 10 days. If the animal's behavior remains normal, the patient need not receive PEP beyond proper wound care. If the animal's behavior changes, it should undergo immediate pathologic examination for evidence of rabies virus infection. If infection is confirmed, there is adequate time to institute PEP. Wild mammal exposure, especially if the animal exhibits uncharacteristic behavior, warrants PEP in most circumstances. If the animal is available for pathologic examination, and if pathologic examination of the brain does not indicate the presence of rabies virus, PEP may be discontinued (Fig. 163.7). Discovery of a bat in a room with an infant or a sleeping individual or mentally disabled person, who cannot report the occurrence of a bite reliably and on whom no bite is found, raises the issue of PEP. If the bat is captured and tests positive for rabies, PEP is indicated; if the bat is not available, the decision about PEP must be individualized. In a recent 17-year population survey of 14,453 households with bedroom bat exposure without a recognized bite, the number of individuals needed to be treated to prevent a single case of bat rabies ranged from 314,000 to 2.7 million persons at a cost of CAD$228 million to CAD$2 billion.[166] PEP appears to be safe in pregnant women and should not be withheld when an indication exists.[167]

Modern PEP should always include administration of both passive antibody (RIG) and vaccine for both bite and nonbite exposures in persons with no previous vaccination for rabies. Rabies immune globulin is available in human (HRIG) and equine forms (pooled antirabies antiserum [ARS] of equine origin and purified antirabies serum of equine origin [ERIG]). These immune globulins are purified from the sera of hyperimmunized donors. Two HRIG preparations are available in the United States: Imogam Rabies-HT (Sanofi Pasteur; Swiftwater, PA) and HyperRab S/D (Grifoils; Barcelona, Spain). HRIG is given only once in a dose of 20 IU/kg and is applicable to all age groups, including children. The most recent WHO and ACIP recommendations call for the entire dose to be infiltrated into the wound if anatomically feasible.[160] Any remaining volume should be administered IM at an anatomic site distant from that used for the active vaccine. The recommended dose of ERIG is 40 IU/kg. Failure to infiltrate wounds with RIG or surgical closure of wounds before RIG infiltration has been associated with the development of rabies in patients despite otherwise proper PEP.[168] If RIG is not administered when active vaccination is begun, it can be administered through day 7 of the PEP series.[160] Human monoclonal antibodies to rabies have been successfully administered as PEP during clinical trials and have demonstrated favorable side effect and pharmacokinetic profiles, suggesting that RIG may be replaced in the future by a more readily available and safer product.[169,170]

Many different forms of rabies vaccine have been produced since Pasteur's original success in 1882. In some developing nations, such as Ethiopia and Bolivia, nervous tissue–type vaccine (NTV) is still used, but it carries a risk of central and peripheral neurologic complications in the range of 1 per 200 to 1600 vaccinees.[105] Production of vaccine in sheep CNS, a common method of Semple-type vaccine production, also carries the theoretical risk of transmitting the scrapie prion.[171] Suckling mouse brain vaccine is effective and safer, with a neurologic complication rate of approximately 1:8000, but all NTVs should be discontinued in favor of much safer alternatives.

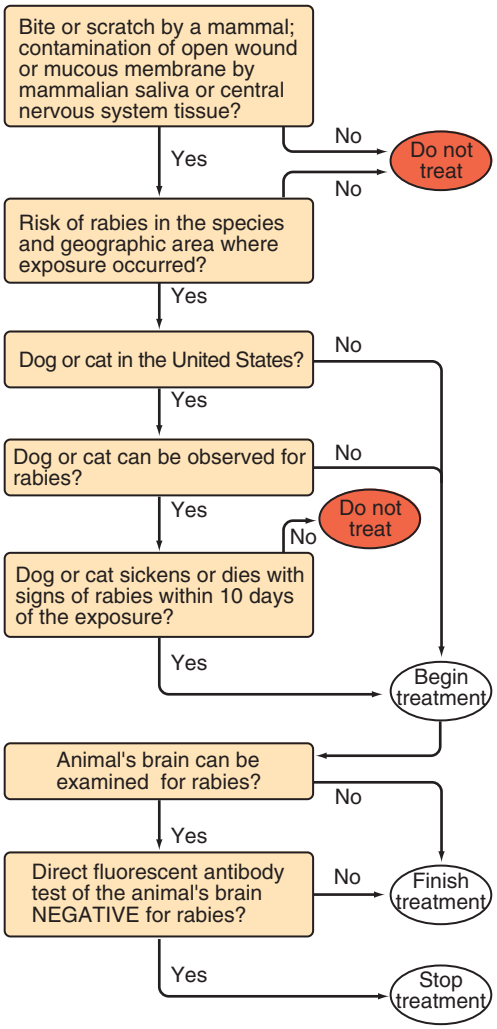

FIG. 163.7 Algorithm for human rabies postexposure prophylaxis. In highly suspect animals, prophylaxis should be started immediately and discontinued if fluorescent antibody test results of the animal brain are negative. In some cases with low risk, treatment may be delayed for up to 48 hours, pending the result of fluorescent antibody testing.

The currently available vaccines licensed for human use in the United States include HDCV (Imovax; Sanofi Pasteur) and PCECV (RabAvert; Novartis; Basel, Switzerland). Both are inactivated virus vaccines that are remarkably safe and immunogenic. Local reactions (pain, swelling, or induration) are common, but systemic symptoms (fever, headache, malaise, nausea, abdominal pain, or adenopathy) occur in only a minority of patients. Serious reactions have been exceedingly uncommon, with suggested Guillain-Barré syndrome reported rarely.[172] To report a vaccine reaction, call the appropriate number for each manufacturer. Corticosteroids should be given only to patients with a life-threatening vaccine reaction because they interfere with the development of immunity. Patients with immunocompromise may not respond adequately to vaccination, and antibody titers should be measured 2 to 4 weeks after immunization.[166]

In other countries other PEP vaccines (e.g., vaccines grown in duck embryos or Vero cell cultures)[173] and regimens are often used. Consultation with the rabies officer of the state health department may be helpful for the management of patients in whom PEP has been initiated with a vaccine not approved for use in the United States.

Cross-protection is provided by rabies vaccines to some other lyssaviruses, such as ABLV.[174] Protocols for potential exposure to ABLV encourage thorough cleaning of the wound, use of the same vaccine doses and schedules as recommended for rabies virus, and testing the bat, if possible, for ABLV. Protocols differ in the specific recommendations regarding the influence of both bat testing and type of exposure on PEP.[175]

The schedule for PEP vaccine administration in an unvaccinated individual changed in 2010 to a four-dose schedule (1.0 mL IM), starting as soon as possible after exposure (day 0) and repeated on days 3, 7, and 14.[176] However, immunocompromised individuals should continue to receive the five-dose schedule.[160,176] Patients who have been previously vaccinated receive 1.0 mL IM on days 0 and 3 only, without RIG.[160]

For adults the vaccination should always be administered in the deltoid area rather than the gluteal area. Vaccine injections into the gluteal result in lower neutralizing antibody titers.[177] In small children vaccination may be given in the lateral thigh. Other schedules are available for use; physicians not familiar with their use should consult local public health authorities and review the most recent WHO and ACIP recommendations.[1,160] The vaccine must not be given in the same region as the immune globulin. Because of the high cost by volume for the IM route of vaccination, intradermal vaccine (uses at least 60% less than IM vaccine) administration is recommended by the WHO as an alternative in developing countries.[1] A single case of transient false-positive enzyme-linked immunosorbent assay results for human immunodeficiency virus after HDCV immunization was reported in 1994.[178] Subsequent screening of samples from people recently immunized against rabies virus revealed no similar cases,[179] but in view of similar phenomena with other vaccines, physicians should be aware of this possibility.

Personnel who care for patients with rabies should practice standard infection control precautions, including use of gloves, gowns, masks, and eye protection, when there is risk of aerosols.[180] PEP should be offered to health care workers or family members if mucous membrane or nonintact skin comes in contact with potentially infectious body fluids such as saliva.

TREATMENT

There is no proven therapy for rabies.[181] An experimental protocol called the Milwaukee protocol (www.mcw.edu/Pediatrics/InfectiousDiseases/PatientCare/Rabies.htm) has been used in patients with rabies. The original patient was a 15-year-old girl from Wisconsin who had a bat bite 1 month before symptom onset.[182] The patient was admitted with fever, obtundation, ataxia, myoclonus, and dysarthria. The patient was placed into electrographic burst suppression with ketamine and midazolam. Antiviral therapy commenced with ribavirin and amantadine. Neither rabies vaccine nor RIG was administered because rabies was not suspected at onset, and during antemortem testing, the patient had an immune response in both the serum and the CSF. The patient was discharged to home on the 76th day after admission with persistent choreoathetosis and ballismus. Twenty-seven months after exposure the patient was taking college courses and attending to her own activities of daily living, with only mild neurologic deficits.[183] To date, she remains well. Since the description of the Milwaukee protocol, there have been 29 reported cases of rabies in the United States, United Kingdom, and Canada; two of those patients survived, and one received components of the Milwaukee protocol.[184,185,186] Eleven other patients received major components of the Milwaukee protocol (therapeutic coma, ketamine) but did not survive.[186] The routine use of this treatment protocol is not recommended, pending further data.

Despite excellent intensive care, the case-fatality rate approaches 100%, and most patients die within a few days to weeks of illness onset. All three documented surviving patients had evidence of rabies virus–specific antibodies in serum and CSF at clinical presentation, but no viral antigens or RNA were identified. This finding may represent a favorable prognostic factor.

Favipravir, a purine analog with activity against a broad range of RNA viruses, was effective in a mouse model as PEP and may be effective in a mouse model of CNS infection, but efficacy in humans is yet to be documented[187,188] (see also Chapter 164). During 2017 the drug was used in a Virginia resident who acquired the infection in India, but the patient succumbed (www.richmond.com/life/health/virginia-health-officials-investigating-rare-case-of-human-rabies-in/article_0aa5bfc3-3875-570b-8d4e-eadbab253b5d.html). A better understanding of basic viral pathobiology, the rational design of antiviral drugs, and development of relevant animal models are necessary for progress in therapeutic interventions.

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The complete reference list is available online at Expert Consult.

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164 Marburg and Ebola Virus Hemorrhagic Fevers

Thomas W. Geisbert

SHORT VIEW SUMMARY

Definition
- Marburg hemorrhagic fever and Ebola hemorrhagic fever (HF) are severe and often fatal diseases characterized by fever, headache, malaise, myalgia, coagulation disorders, and multiorgan failure.

Epidemiology
- Human outbreaks occur sporadically in regions of Central Africa.
- An unprecedented epidemic of Ebola hemorrhagic fever involving more than 28,000 cases and 11,000 deaths occurred in West Africa in 2013–16.
- Recent evidence suggests that bats may play a role as a reservoir host.
- The manner in which filovirus outbreaks are initiated is unknown; however, it is thought that the initial cases occur as a result of contact with an infected animal.
- Nosocomial transmission has occurred frequently during outbreaks of filovirus HF in endemic areas.

Diagnosis
- Clinical symptoms are nonspecific, but a constellation of symptoms, including fever, headache, malaise, myalgia, sore throat, vomiting, diarrhea, and, in particular, the appearance of a maculopapular rash, may indicate infection with a filovirus.
- Antigen-capture enzyme-linked immunosorbent assay and polymerase chain reaction are the most frequently used assays to diagnose filovirus infection.

Treatment
- There are no approved postexposure treatments for filovirus infections.
- Treating patients infected with marburgviruses or ebolaviruses consists primarily of intensive supportive care, which is directed toward maintaining effective blood volume and electrolyte balance.
- Several experimental treatments have shown promise in nonhuman primate models of filovirus infection, including small interfering RNAs, antisense oligonucleotides, RNA polymerase inhibitors, and pools of monoclonal antibodies; of these interventions, ZMapp, a pool of three monoclonal antibodies, showed promise in humans when used in phase II/III trials during the 2013–16 Ebola epidemic.

Prevention
- There are no approved vaccines against marburgviruses or ebolaviruses, although a vesicular stomatitis virus–based vaccine expressing the *Zaire ebolavirus* glycoprotein showed remarkable success in a ring-vaccination cluster randomized trial in Guinea during the 2013–16 Ebola epidemic.
- Barrier nursing procedures include the donning of protective clothing, masks, and eye shields.
- Infected patients and close contacts should be isolated.
- Avoid contact with bush meat and sick animals, particularly nonhuman primates, in endemic regions.

Viral hemorrhagic fever (VHF) is a syndrome characterized by fever, malaise, myalgia, and blood coagulation disorders that can progress to multiorgan failure, shock, and death in many cases. VHF is caused by members of four different families of RNA viruses. Among the VHFs members, the genera *Marburgvirus* and *Ebolavirus* in the family Filoviridae are the most feared because of their dramatic clinical presentation and unusually high case-fatality rates of up to 90%, and because their natural history remains a mystery. In addition to concerns of natural outbreaks and epidemics in regions of Central and Western Africa, ebolaviruses and marburgviruses are known to have been the subjects of former biological weapons programs and have the potential for deliberate misuse.[1,2] Currently, there are no filovirus vaccines or treatments approved for human use. For these reasons, ebolaviruses and marburgviruses have recently been included as only 2 of 11 human pathogens and only 2 of 4 viruses on the United States Department of Health and Human Services Tier 1 list of Category A select agents.[3] In addition to causing significant disease in humans, filoviruses have decimated populations of great apes in the Congo basin, further impacting an already endangered species.

VIRUS CHARACTERIZATION

The family Filoviridae is divided into two genera: *Marburgvirus* and *Ebolavirus*. The *Marburgvirus* genus contains a single species, *Marburg Marburgvirus,* which contains two genetically distinct members: Marburg virus (MARV) and Ravn virus (RAVV). The *Ebolavirus* genus consists of five distinct species: *Bundibugyo ebolavirus* (BDBV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SUDV), *Tai Forest ebolavirus* (TAFV) (more commonly known as *Ivory Coast ebolavirus*); and *Zaire ebolavirus* (EBOV).[4] Nucleotide and amino-acid differences between the genera *Marburgvirus* and *Ebolavirus* are each approximately 55%, and there is no serologic cross-reactivity between these viruses. In comparison, ebolavirus species show 37% to 41% differences in nucleotide and amino-acid sequences, and there is varying degrees of cross-reactivity among the ebolavirus species.

Filoviruses are enveloped, nonsegmented, negative-stranded RNA viruses. Filovirus particles take on a variety of forms, from circular or "6"-shaped to prototypical straight filaments, for which the virus family is named (Fig. 164.1). Although the length of the virions is variable, with marburgvirus particles averaging close to 800 nm and ebolavirus virions measuring about 1 μM, the diameter of all filovirus particles uniformly measures about 80 nm.[5] Filovirus particles contain an approximately 19-kilobase noninfectious genome that encodes seven structural proteins, with a gene order of 3′ leader, nucleoprotein (NP), virion protein (VP) 35 (VP35), VP40, glycoprotein (GP), VP30, VP24, RNA-dependent RNA polymerase L protein, and 5′ trailer. Four of these proteins are associated with the viral genomic RNA in the ribonucleoprotein complex: NP, VP30, VP35, and the L protein. Some proteins of the ribonucleoprotein complex have additional functions.

For example, VP35 has been shown to act as an interferon antagonist.[6] VP40 serves as the matrix protein and mediates particle formation and, in the case of marburgviruses, has also been shown to interfere with host innate immune responses.[7] VP24 is another structural protein associated with the membrane and for ebolaviruses also interferes with interferon signaling.[8]

The GP is the surface glycoprotein that forms the spikes on the virion and is the effector for receptor binding and membrane fusion. An important distinction of ebolaviruses from marburgviruses is that although the marburgvirus GP is encoded in a single open reading frame (ORF), the ebolavirus GP is encoded in two ORFs.[9,10] The single marburgvirus ORF translates into the structural surface GP. In contrast, the two ebolavirus ORFs are linked together by slippage of the L polymerase at an editing site (a string of seven consecutive template uracil residues) to insert an eighth uracil. This process results in the production of a messenger RNA (mRNA) transcript that permits read-through translation of full-length GP. However, only about 20% of the mRNA transcripts are edited and translate into structural surface GP. The remaining 80% of unedited mRNA transcripts result in the production of a truncated soluble GP (sGP) that is secreted in large quantities from infected cells. Although the function of sGP has not been fully elucidated, it has been postulated that sGP subverts the host immune response by both passively absorbing antibodies directed at the full-length structural GP[11,12] and by triggering the proliferation of B cells that preferentially bind sGP.[13]

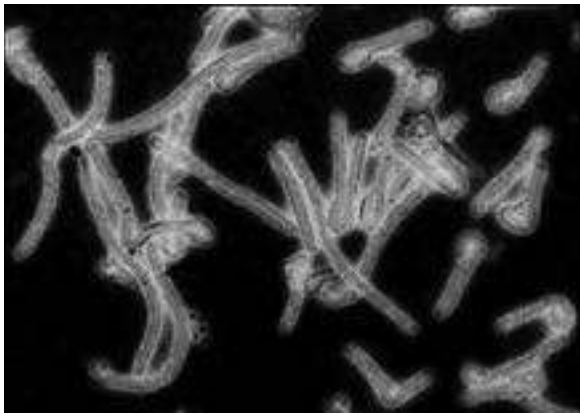

FIG. 164.1 Electron micrograph of *Sudan ebolavirus* virions. Negatively contrasted filovirus particles obtained from culture fluids from infected Vero cells (original magnification ×12,000).

EPIDEMIOLOGY

Marburg Hemorrhagic Fever

The first documented outbreak of VHF caused by a filovirus occurred in 1967, when there were three concurrent episodes of lethal marburgvirus infection in Marburg and Frankfurt, Germany and in Belgrade (in the former Yugoslavia) among laboratory workers exposed to blood and tissue products of African green monkeys imported from Uganda (Fig. 164.2).[14] Secondary transmission to medical staff and family members was also documented. In total, 31 patients became infected, and seven of these patients died. During the next 2 decades, marburgvirus was associated with sporadic, isolated, usually fatal cases among residents and travelers in southeast Africa.

In 1998–2000 there was a prolonged outbreak involving 154 cases of Marburg hemorrhagic fever (HF) in Durba, Democratic Republic of the Congo (DRC), which was associated with individuals working in an underground gold mine.[15] Case-fatality rates from this outbreak are unclear but may be up to 83%. This outbreak was unique and complicated by the fact that it had multiple introductions of marburgviruses of different phylogenetic lineages and included variants that are thought to be more pathogenic (Angola)[16,17] than others. The largest and most lethal marburgvirus outbreak to date occurred in 2004–05 in northern Angola.[16] This outbreak involved 252 cases, with a case-fatality rate of 90%. The epidemic was driven largely by nosocomial transmission;

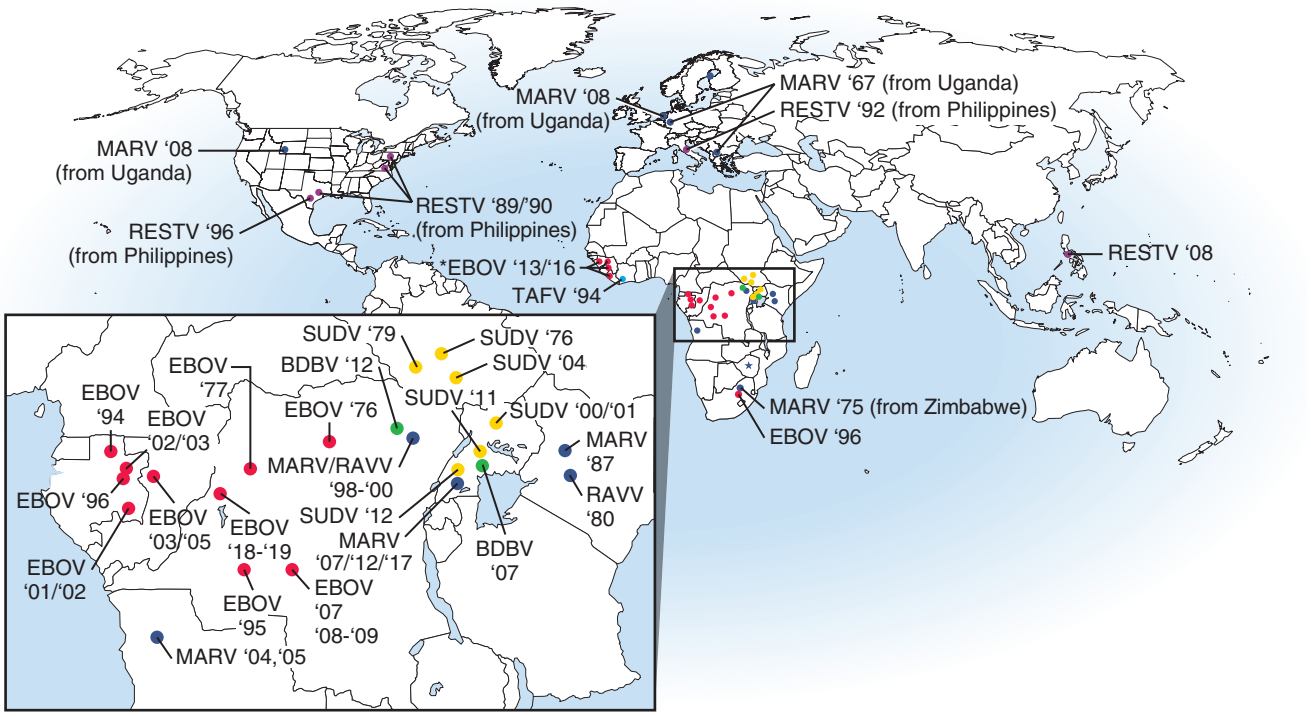

*In regard to the 2013-16 West African outbreak of EBOV, several cases in the US and Europe occurred that were associated with direct contact with medically evacuated patients or imported cases.

FIG. 164.2 Locations of filovirus infections and outbreaks. *BDBV, Bundibugyo ebolavirus; EBOV, Zaire ebolavirus; MARV,* Marburg virus; *RAVV,* Ravn virus; *RESTV, Reston ebolavirus; SUDV, Sudan ebolavirus; TAFV, Tai Forest ebolavirus.*

however, community-acquired infection was documented toward the end of the outbreak. Between 2007 and 2017 several small episodes of Marburg HF were reported in Uganda, with two cases being exported to the United States[18] and the Netherlands,[19] respectively.

Ebola Hemorrhagic Fever

Ebolavirus was first recognized during near-simultaneous explosive outbreaks in 1976 in small communities in the former Zaire (now the DRC) and Sudan (see Fig. 164.2).[20,21] There was significant secondary transmission through the reuse of unsterilized needles and syringes and nosocomial contacts. These independent outbreaks involved the serologically distinct species EBOV and SUDV. The EBOV outbreak consisted of 318 cases and 280 deaths (88% mortality), whereas the SUDV outbreak involved 284 cases with 151 deaths (53% mortality). Since 1976 EBOV has appeared sporadically in Central Africa, causing several small- to middle-size outbreaks between 1976 and 1979. In 1995 there was a large epidemic of Ebola HF involving 315 cases of EBOV, with an 81% case-fatality rate, in Kikwit, a community in the former Zaire.[22] Meanwhile, between 1994 and 1997 there were smaller outbreaks caused by EBOV in Gabon. Since 2000 there have been near-yearly occurrences of EBOV in Gabon, DRC, or the Republic of Congo. These EBOV outbreaks have also involved a catastrophic decline in populations of great apes.[23,24] Before 2013 the largest EBOV outbreak on record involved 425 cases, with a 53% case-fatality rate.[25] This outbreak occurred in 2000–01 in Sudan and was caused by SUDV. Smaller outbreaks of SUDV have occurred in Sudan in 2004 and in Uganda in 2011 and 2012. In December 2013 an unprecedented outbreak caused by EBOV began in the West African countries of Guinea, Liberia, and Sierra Leone. The epidemic continued unabated for more than 2 years, finally ending in January 2016 with 28,616 cases and 11,301 deaths.[26] In May of 2018 an EBOV outbreak was reported in the DRC, and as of June 20, 2018, there have been 61 cases and 28 deaths reported.[27] World Health Organization reported on June 22, 2018 that they believe that outbreak had been largely contained.[28]

In 1989–90 a third species of the genus *Ebolavirus,* RESTV, appeared in Reston, Virginia in association with an outbreak of VHF among cynomolgus macaques imported to the United States from the Philippine Islands.[29] Hundreds of monkeys were infected (with high mortality) in this outbreak, but no human cases occurred. Four animal caretakers seroconverted to REBOV with no overt disease. Epizootics in cynomolgus monkeys recurred at other facilities in Europe and the United States through 1992 and again in 1996. Subsequently, RESTV has been found in the Philippines on several occasions, with surprising reports documenting infections in domestic pigs.[30]

A fourth species of the genus *Ebolavirus,* TAFV, was identified in Côte d'Ivoire in 1994.[31] The virus was isolated from an ethnologist who had worked in the Tai Forest reserve and became infected after a necropsy on a chimpanzee. The individual became ill with symptoms consistent with filovirus infection and survived infection. The chimpanzee originated from a troop that lost several members to an illness that was subsequently identified as being caused by TAFV.

The latest and fifth species of the genus *Ebolavirus,* BDBV, was discovered in Uganda late in 2007 during an outbreak that involved 56 confirmed cases and an approximate 40% case-fatality rate.[32] A more recent outbreak of BDBV occurred late in 2012 in the DRC and involved 52 probable cases and a 48% case-fatality rate.[33]

Natural History

Human and nonhuman primates are susceptible to filovirus infection and are considered to be end hosts rather than potential reservoirs. Surveys to identify animal reservoirs and arthropod vectors have been aggressively undertaken in endemic areas, particularly after most large filovirus outbreaks. Until recently these efforts have been unsuccessful. Ecologic studies in 2003–06 in Gabon and the Republic of Congo demonstrated the initial evidence for the presence of EBOV in three different species of fruit bats.[34] These studies showed the presence of viral RNA and antibodies, although the investigators were unable to isolate infectious EBOV. Subsequent studies in 2007 detecting MARV RNA and isolating infectious MARV from cave-dwelling fruit bats in Uganda further support the view that bats may serve as a reservoir for filoviruses.[35] More recently, antibodies against RESTV were detected in fruit bats in the Philippines.[36] Although current data suggest a role for bats in maintaining filoviruses in nature, it remains unclear whether bats serve as the primary reservoir or whether other species are involved.

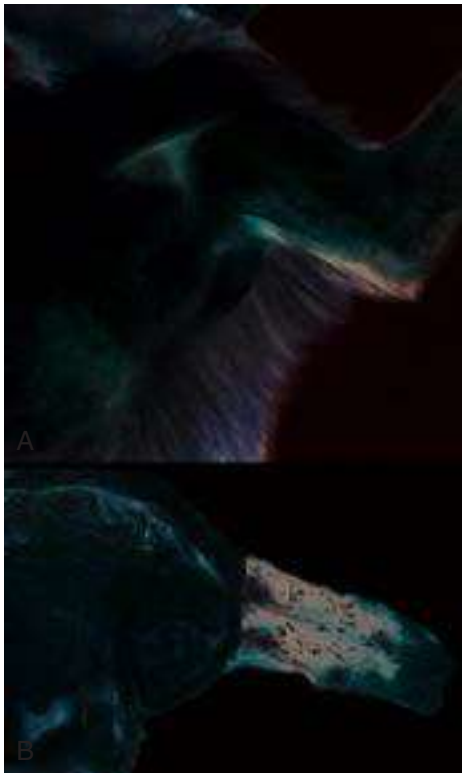

FIG. 164.3 Representative gross necropsy lesions from nonhuman primates experimentally infected with filoviruses. (A) Typical petechial rash of the left arm and chest of a rhesus macaque 11 days after infection with Marburg virus. (B) Marked congestion of the duodenum at the gastroduodenal junction of a rhesus monkey 9 days after infection with *Zaire ebolavirus*.

Clinical Manifestations and Diagnosis

Clinical and laboratory features of marburgvirus and ebolavirus infection are nonspecific and include an incubation period of 2 to 21 days (mean, 4–10) with a sudden onset of fever, malaise and/or myalgia, and may include a variety of other nonspecific symptoms (reviewed in references 4 and 37). The presence of an erythematous, maculopapular rash may be observed (Fig. 164.3). A constellation of other coagulation disorders may occur, including bleeding from venipuncture sites and the gastrointestinal tract (see Fig. 164.3). Clinical pathology findings include leukopenia and lymphocytopenia with increased levels of neutrophils, thrombocytopenia, and increased serum levels of the liver-associated enzymes aspartate aminotransferase and alanine aminotransferase. Prolonged blood coagulation times and increased circulating levels of D-dimers are also associated with filovirus infections.[38,39]

Confirmation of filovirus infection requires detection of virus in blood or other tissues or the demonstration of filovirus-specific antibody. Assays most frequently used to diagnose filovirus infections include immunofluorescent antibody tests, the enzyme-linked immunosorbent assays for filovirus antigen and specific immunoglobulin M (IgM) and IgG antibodies, and reverse-transcriptase polymerase chain reaction.[25,40–43] Rapid tests to detect antigen were optimized for point-of-care diagnosis during the 2013–16 EBOV epidemic.[44] Other assays that have been used to confirm filovirus infection included immunohistochemistry of skin and other tissues, virus culture, and electron microscopy.[40,45,46]

PATHOGENESIS

Filoviruses are thought to enter the host through mucosal surfaces, small abrasions and/or tears in the skin, or by parenteral introduction.

Both marburgviruses and ebolaviruses have a broad cell tropism, infecting a wide variety of cell types. Ultrastructural examination of tissues from fatal human cases and from experimentally infected nonhuman primates show that monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells, and several types of epithelial cells all lend support to replication of these viruses.[47,48,49–52,53] Systematic studies in nonhuman primates experimentally infected with MARV or EBOV suggest that monocytes, macrophages, and dendritic cells are the early and preferred replication sites.[51,52] Filovirus infection of mononuclear phagocytes appears to trigger a cascade of events involving the production and release of the procoagulant protein tissue factor,[38] as well as a variety of proinflammatory cytokines/chemokines and oxygen free radicals.[54,55] It is thought that the triggering of this chain of events is equal, or in fact more critical, to the development of the observed pathology than is any structural damage induced directly by the actual process of viral replication in host cells and/or tissues.

During filovirus infection, lymphoid depletion and necrosis are frequently observed in spleen, thymus, and lymph nodes of patients with fatal disease and in nonhuman primates that are experimentally infected (Fig. 164.4). Although lymphoid tissues are primary sites of filovirus infection, there is usually little inflammatory cellular response in these or other infected tissues. Despite the large die-off and loss of lymphocytes during the disease course, the lymphocytes themselves do not support the production of progeny virus. Large numbers of lymphocytes undergo apoptosis in man[56] and in experimentally infected nonhuman primates,[57] in part explaining the progressive lymphopenia and lymphoid depletion at death. Other morphologic lesions include focal necrosis in a number of organs, particularly liver, where Councilman bodies are a prominent finding (see Fig. 164.4).

Coagulation disorders are a hallmark feature of filovirus infection, and results from many studies have shown biochemical and histologic evidence of disseminated intravascular coagulation in both humans and experimentally infected nonhuman primates.[a] The mechanism(s) responsible for triggering the coagulation disorders that typify filovirus infection are not completely understood. Results from several studies strongly suggest that expression or release of tissue factor from monocytes and macrophages infected with filoviruses plays a pivotal role in inducing the development of coagulation irregularities reported in filovirus HF.[38] However, coagulopathy noted during filovirus HF could be caused by several factors, especially during the later stages of disease.



FIG. 164.4 Histopathology of nonhuman primates experimentally infected with filoviruses. (A) Necrosis and apoptosis of lymphocytes with concomitant lymphoid depletion in spleen of a rhesus monkey 9 days after infection with *Zaire ebolavirus* (hematoxylin and eosin stain; original magnification, 20×). (B) Councilman-like bodies *(arrows)* in the liver of a rhesus monkey 9 days after infection with *Zaire ebolavirus* (hematoxylin and eosin stain; original magnification, 20×) *(Photographs courtesy Karla Fenton, University of Texas Medical Department–Galveston, TX.)*

COUNTERMEASURES

Prevention

In the past there has been little commercial interest for developing vaccines against filoviruses, primarily because of the geographic location of epidemic areas and the small global market. However, the classification of filoviruses as important biological defense pathogens, bolstered by the increased press coverage of the recent EBOV epidemic in West Africa, has dramatically changed perspectives regarding the need for vaccines against marburgviruses and ebolaviruses. Effective and fast-acting filovirus vaccines would be valuable for at-risk medical personnel, first responders, military staff and researchers, and also for targeted vaccination in the most affected populations (e.g., primarily health care workers and family members of confirmed or probable cases).

Although there are no approved vaccines or postexposure treatment modalities available for preventing or managing filovirus infections, there are at least nine different vaccine systems that have shown promise in completely protecting nonhuman primates against either Ebola or Marburg HF, with five of these vaccines protecting animals against both ebolaviruses and marburgviruses (reviewed in references 60–62). Several of these vaccines require multiple injections to confer protective efficacy. However, in the setting of pathogens such as marburgviruses and ebolaviruses, which are indigenous to Africa and are also potential agents of bioterrorism, a single-injection vaccine is preferable. In the case of preventing natural infections, multiple-dose vaccines are both too costly and not practical (logistics and compliance) in developing countries. In the case of a deliberate release of these agents, there would be little time for deployment of a vaccine that requires multiple injections over an extended period of time. Thus, for most practical applications, a vaccine against the filoviruses necessitates a single immunization or, at the most, two injections within a very short time frame. Of the prospective filovirus vaccines, only two systems—one based on replication-defective adenoviruses (Ad5 or ChAd3) and the other based on the recombinant vesicular stomatitis virus (rVSV)—have been shown to provide complete protection to cynomolgus macaques when administered as a single-injection vaccine.[63–66]

Substantial progress was made in the advancement of candidate preventive vaccines during the 2013–16 EBOV epidemic. Several candidate EBOV vaccines were used in phase I and/or II studies during this time. These included rVSV-EBOV,[67–71] Ad5-EBOV,[72–74] rAd5-EBOV in combination with rVSV-EBOV,[75] ChAd3-EBOV either with or without modified vaccinia Ankara (MVA)-EBOV,[76–79] Ad26-EBOV combined with MVA-EBOV,[80] and a DNA vaccine that encoded multiple filovirus GPs.[81,82] Overall, these EBOV vaccines generated good immunogenicity against the EBOV GP, with no serious adverse events reported. Although at least 16 phase I and/or II trials were conducted during the 2013–16 EBOV epidemic, there were few opportunities to conduct phase III trials, and the results of only a single phase III trial were reported. Final results of the phase III rVSV-EBOV vaccine, which was used in a ring-vaccination, open-label, cluster-randomized trial in Guinea, were published in 2017.[83] This study demonstrated statistically significant protection, with no cases of EBOV among individuals from day 10 after vaccination in both randomized and nonrandomized clusters. The remarkable success of this phase III trial of the rVSV-EBOV vaccine should pave the way for future licensure.

Although promising results have been obtained in regard to EBOV vaccines in humans, many challenges remain. Among the most significant obstacles are the identification of a seemingly more pathogenic variant of MARV (variant Angola) in 2004–05 and the identification of a new

[a]References 21, 38, 39, 49, 58, 59.

species of EBOV, BDBV, in 2007. Until recently, filovirus vaccine development has primarily focused on two EBOV species, SUDV and EBOV, and one MARV variant (variant Musoke). Future efforts focusing on the filoviruses other than EBOV are needed. Preclinical studies in nonhuman primates suggest that multivalent filovirus vaccines are possible.[60–62]

Treatment

Before the 2013–16 EBOV epidemic, treating patients infected with MARVs or EBOVs in endemic areas consisted primarily of intensive supportive care directed toward maintaining effective blood volume and electrolyte balance. Several interventional therapies, including interferons, heparin, convalescent serum, and equine anti-EBOV IgG were used to treat natural and laboratory-acquired filovirus infections with little to no success.[59,84–86]

During the 2013–16 EBOV epidemic a number of different postexposure treatment approaches were used (reviewed in references 87 and 88). These primarily consisted of the interventions that showed the greatest promise as therapeutics in preclinical nonhuman primate models. These treatments included the anti-EBOV monoclonal antibody pool known as ZMapp (Mapp Biopharmaceutical; San Diego, CA), small interfering RNAs, and the small molecules favipiravir and GS-5734. Most of these interventions showed the ability to completely protect nonhuman primates against EBOV even when treatment was not initiated until the onset of illness or later. Clinical trials conducted during the West African EBOV epidemic using some of these drugs were unable to enroll enough patients to achieve statistically significant data; however, ZMapp showed promising results.[89] It was clear from all of the trials that patients with very high viral loads at the initiation of antiviral treatment were unlikely to survive, which is not surprising given that the case-fatality rate for EBOV is greater than 90% when the viral load exceeds 10^7 genome copies per mL of blood. Although most EBOV patients were treated in resource-poor settings in West Africa, some patients were treated in Europe or the United States. In these cases advanced supportive care, including real-time electrolyte balance, ventilation, and dialysis, appeared to be critically important successful treatment.[87,88,90,91] Specifically, only 2 of the 12 EBOV patients (17%) treated in the United States died. One patient who died shortly after arriving in the United States was at a very advanced stage of disease when treatment was initiated. There were also reports of 15 EBOV cases treated in Europe during the epidemic, of whom three died (20%). By comparison, mortality for EBOV reported in treatment centers in West Africa ranged from 37% to 4%.[92–96] It is evident from these cases that advanced supportive care clearly improved patient survival. It is also important to note that many EBOV patients who were medically evacuated to the United States or Europe also received various experimental antiviral therapies. Thus the combination of experimental antiviral interventions and advanced supportive care likely contributed to the improved outcome.

As with the development of preventive vaccines as a result of the 2013–16 EBOV epidemic, progress on the development of treatments for EBOV has outpaced the advancement of treatments for other filoviruses. However, the recent development of human monoclonal antibody MR-191, which completely protected nonhuman primates against both MARV and RAVV when treatment was delayed until after the onset of illness,[97] offers hope for other filoviruses. In addition, the recent investment in the development of panhuman monoclonal antibodies with antiviral activity against all species of EBOVs is encouraging.[98–100]

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165 Influenza Viruses, Including Avian Influenza and Swine Influenza

John J. Treanor

SHORT VIEW SUMMARY

Definition
- Influenza viruses are enveloped, negative-sense, single-stranded RNA viruses whose genome is segmented. They cause epidemic acute respiratory disease characterized by fever, cough, and systemic symptoms. Three types (A, B, and C) are recognized, with many subtypes within the type A viruses.

Epidemiology
- Influenza viruses are transmitted by the respiratory route and cause large epidemics, which generally occur during the winter in temperate climates. In addition to infecting humans, influenza A viruses infect a wide variety of animals, particularly migratory waterfowl. New influenza A virus subtypes sporadically emerge in humans to cause widespread disease, or pandemics.

Microbiology
- Influenza viruses are readily isolated in eggs or mammalian cell culture at 33°C. They undergo constant antigenic evolution, referred to as antigenic drift or shift, which allows them to reinfect individuals who have had previous infections.

Diagnosis
- In the context of recognized epidemics, influenza is usually diagnosed clinically on the basis of characteristic symptoms of fever and cough. Rapid detection of virus in respiratory secretions also can be accomplished through antigen detection or molecular techniques such as polymerase chain reaction assay.

Therapy
- Antiviral therapy with oseltamivir, zanamivir, or peramivir is available and may shorten the duration of illness and reduce the rate of complications. Therapy is most effective when used early in the course of illness (Table 165.1).

Prevention
- Influenza vaccines are effective in the prevention of influenza illness, although improved vaccines are needed.
- Inactivated and live-attenuated vaccines are available in trivalent and quadrivalent formulations (Table 165.2).
- The objectives of vaccination include protection of the individual and protection of the population through herd immunity. Antiviral drugs can also be used prophylactically in selected circumstances.

Influenza is an acute, febrile illness caused by infection with influenza type A or B virus that occurs in outbreaks of varying severity almost every winter in temperate climates, and year-round in tropical climates. The most common clinical manifestations are fever, malaise, and cough. Two unique features of influenza are the epidemic nature of the disease and the mortality that results in part from its pulmonary complications.

HISTORY

Influenza virus has been causing recurrent epidemics of febrile respiratory disease every 1 to 3 years for at least the past 400 years. Although the disease is not associated with a characteristic manifestation such as rash, the high attack rate, the explosive nature of the epidemic, and the frequency of cough allow the identification of past epidemics. For example, Hirsch tabulated 299 outbreaks occurring at an average interval of 2.4 years between 1173 and 1875.[1] The greatest pandemic in recorded history occurred in 1918–1919 when, during three "waves" of influenza, 21 million deaths were recorded worldwide, among them 549,000 in the United States.[2]

The modern understanding of influenza was ushered in with the isolation by Smith and associates of influenza A virus in ferrets in 1933.[3] Influenza B virus was isolated by Francis in 1939[4] and influenza C virus by Taylor in 1950.[5] The discovery by Burnet in 1936 that influenza virus could be grown in embryonated hens' eggs allowed extensive study of the properties of the virus and the development of inactivated vaccines.[6] Animal cell culture systems for the growth of influenza viruses were developed in the 1950s.[7] The phenomenon of hemagglutination, which was discovered by Hirst in 1941, led to simple and inexpensive methods for the measurement of virus and specific antibody.[8]

Evidence of the protective efficacy of inactivated vaccines was developed in the 1940s.[9] The use of live vaccines for influenza was first suggested shortly after the virus was discovered,[10] but the first live vaccine was not licensed in the United States until 2003, approximately 70 years later. Finally, antiviral agents in two classes have been approved for prevention and treatment of influenza. These include the so-called M2 inhibitors, amantadine in the mid-1960s, rimantadine in 1993, and the neuraminidase inhibitors (NIs) zanamivir, oseltamivir, and most recently peramivir.

THE VIRUSES

Classification

Influenza viruses belong to the family Orthomyxoviridae and are classified into three distinct types: influenza A, influenza B, and influenza C virus. There are significant differences in genetic organization, structure, host range, epidemiology, and clinical characteristics among the three influenza virus types (Table 165.3). However, all three viruses share certain features, including the presence of a host-cell–derived envelope, envelope glycoproteins of critical importance in virus entry and egress from cells, and a segmented genome of negative-sense (i.e., opposite of message sense), single-stranded RNA. The standard nomenclature for influenza viruses includes the influenza type, place of initial isolation, strain designation, and year of isolation. For example, the influenza A virus isolated by Francis from a patient in Puerto Rico in 1934 is given the strain designation A/Puerto Rico/8/34, sometimes referred to as PR8 virus. Influenza A viruses are further divided into subtypes based on the hemagglutinin (H, or HA) and neuraminidase (N, or NA) antigens (e.g., H1N1 or H3N2).

TABLE 165.1 Antiviral Chemotherapy and Chemoprophylaxis for Influenza

INDICATION	DRUG	ROUTE	DOSAGE
Influenza A and B: treatment	Oseltamivir	Oral	Adults: 75 mg bid ×5 days Children aged 1–12 yr: 30–75 mg bid, depending on weight,[a] ×5 days
	Zanamivir	Inhaled orally	Adults and children aged ≥7 yr: 10 mg bid ×5 days
	Peramivir	Intravenous	Adult: 600 mg IV once Children 2–12 yr: 12 mg/kg once
	Baloxavir	Oral	Adults: 80 mg PO once Children ≥12 yr and <80 kg: 40 mg PO once
Influenza A: treatment	Amantadine[b]	Oral	Adults: 100 mg qd or bid ×5–7 days Children aged 1–9 yr: 5 mg/kg/day (maximum, 150 mg/day) ×5–7 days
	Rimantadine[b]	Oral	100 mg qd or bid ×5–7 days in adults
Influenza A and B: prophylaxis	Oseltamivir	Oral	Adults: 75 mg/day Children aged ≥1 yr: 30–75 mg/day, depending on weight[a]
	Zanamivir	Inhaled orally	Adults and children aged ≥5 yr: 10 mg/day
	Peramivir	Intravenous	Not FDA approved for prophylaxis
Influenza A: prophylaxis	Amantadine[b] or rimantadine[b]	Oral	Adults: 200 mg/day Children aged 1–9 yr: 5 mg/kg/day (maximum, 150 mg/day)

[a]For detailed dosage recommendations in children aged <1 yr, see www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm.
[b]Amantadine and rimantadine are not considered for use because of widespread resistance in influenza A/H3N2 and A/H1N1 viruses currently circulating. They may be considered if sensitivities become reestablished.
FDA, US Food and Drug Administration.

TABLE 165.2 Influenza Vaccines Available in the United States, 2017–2018 Influenza Season

VACCINE (MANUFACTURER)	DOSE	AGES
IIV Quadrivalent, Standard Dose (SD-IIV4)		
Afluria Quadrivalent (Seqirus)	15 µg	≥5 yr
Fluarix Quadrivalent (GSK)	15 µg	≥3 yr
FluLaval Quadrivalent (ID Biomedical)	15 µg	≥6 mo
Fluzone Quadrivalent (Sanofi)	15 µg	≥6 mo
IIV Quadrivalent, Standard Dose, Cell Culture-based (ccIIV-4)		
Flucelvax Quadrivalent (Seqiris)	15 µg	≥4 yr
Recombinant Influenza Vaccine, Quadrivalent (RIV-4)		
Flublok Quadrivalent (Protein Sciences/Sanofi)	45 µg	≥18 yr
IIV Quadrivalent, Standard Dose, Intradermal (ID IIV-4)		
Fluzone Intradermal Quadrivalent (Sanofi)	9 µg	18–64 yr
IIV Trivalent, Standard Dose (SD-IIV-3)		
Afluria (Seqirus)	15 µg	≥5 yr
Fluvirin (Seqirus)	15 µg	≥4 yr
IIV Trivalent, High Dose (HD-IIV-3)		
Fluzone High Dose (Sanofi)	60 µg	≥65 yr
Adjuvanted IIV Trivalent, Standard Dose (aIIV-3)		
Fluad (Seqirus)	15 µg	≥65 yr
Live-Attenuated Influenza Vaccine, Quadrivalent (LAIV-4)		
FluMist Quadrivalent (MedImmune)	10^7 FFU	2–49 yr

For adults and older children, the recommended site of vaccination is the deltoid muscle. The preferred site for infants and young children is the anterolateral aspect of the thigh. Specific guidance regarding site and needle length for intramuscular administration may be found in the Advisory Committee on Immunization Practices General Recommendations on Immunization.
[a]Immunization providers should check US Food and Drug Administration–approved prescribing information for the most complete and updated information, including (but not limited to) indications, contraindications, and precautions. Package inserts for US-licensed vaccines are available at http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm.
FFU, Fluorescent focus units; *ID,* intradermal; *IIV,* Inactivated influenza vaccine; *IIV-3,* inactivated influenza vaccine, trivalent; *IIV-4,* inactivated influenza vaccine, quadrivalent; *IM,* intramuscular; *IN,* intranasal; *LAIV,* live-attenuated influenza vaccine; *RIV,* recombinant influenza vaccine.
Modified from Centers for Disease Control and Prevention. Summary recommendations: prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP)—United States, 2017–18. http://www.cdc.gov/flu/professionals/acip/summary-recommendations.htm#table1.

TABLE 165.3 Differences Among Influenza A, B, and C Viruses

	INFLUENZA A	INFLUENZA B	INFLUENZA C
Genetics	8 gene segments	8 gene segments	7 gene segments
Structure	10 viral proteins M2 unique	11 viral proteins NB unique	9 viral proteins HEF unique
Natural host range	Humans, swine, equine, birds, marine mammals[a]	Humans only	Humans and swine
Epidemiology	Antigenic shift and drift	Antigenic drift only; two main lineages cocirculate	Antigenic drift only; multiple variants
Clinical manifestations	May cause large pandemics with significant mortality in young persons	Severe disease generally confined to older adults or persons at high risk; pandemics not seen	Mild disease without seasonality

[a]Influenza A viruses have also been isolated from mink, dogs, and cats.

Virology

Influenza viruses are enveloped viruses that may exist in spherical or filamentous forms of 80 to 120 nm (Fig. 165.1), with surface projections consisting of HA and NA spikes. A schematic diagram of an influenza A virus is shown in Fig. 165.2.

The HA is the viral attachment protein, and the receptor binding site is located in the globular head of the molecule. Each rod-shaped HA spike measures approximately 4 nm in diameter by 14 nm in length. They can be removed from the intact virion by sodium dodecyl sulfate, by bromelain, or by chymotrypsin. Each spike is a trimer composed of three HA polypeptides, each with a molecular weight of 75,000 to 80,000, resulting in a trimer with a molecular weight of approximately 224,640. The HA is synthesized as a monomer (HA_0), which is cleaved by host-cell proteases into HA_1 and HA_2 components that remain linked together.

In birds, the ability of the HA to be cleaved by proteases plays an important role in pathogenesis. Proteases capable of cleaving the HA of avirulent viruses, such as tryptase Clara,[11] are restricted in distribution to cells of the respiratory and gastrointestinal mucosa, thereby limiting replication to these areas. However, addition of several basic amino acids to the cleavage site[12] renders the HA capable of being cleaved by ubiquitous cellular furin-like proteases[13] and allows these viruses to escape the confines of the mucosa and replicate systemically in chickens.[14] However, the potential role of HA cleavability in pathogenesis in humans is unknown.

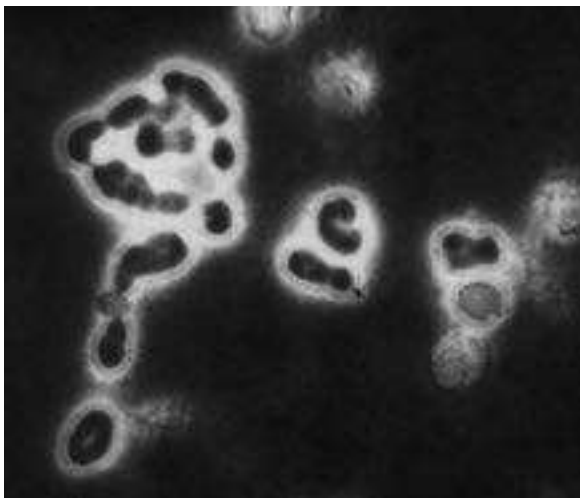

FIG. 165.1 Electron micrograph of influenza A/USSR/77 H1N1 (×189,000).

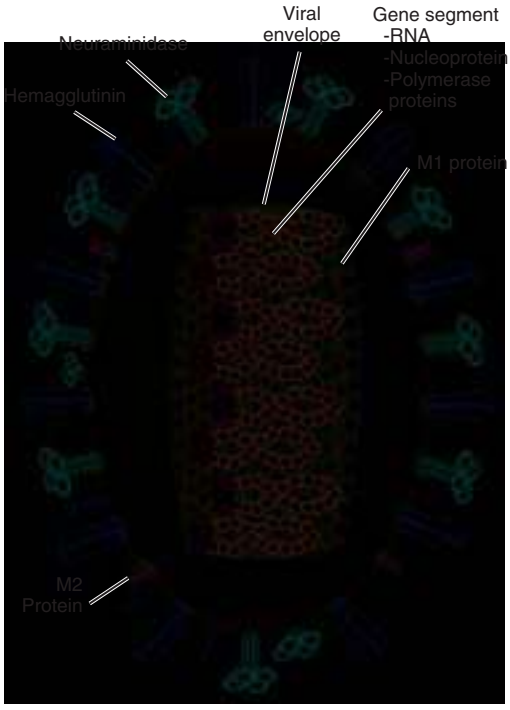

FIG. 165.2 Schematic model of an influenza A virus.

The viral NA is an enzyme that catalyzes the removal of terminal sialic acids (*N*-acetyl neuraminic acid) from sialic acid–containing glycoproteins. The NA spike is shaped like a mushroom rather than a rod and has a molecular weight of 240,000. The intact NA consists of a tetramer of NA polypeptides, each with a molecular weight of 58,000. The enzyme active site is located in the mushroom-shaped head.

At least 16 highly divergent, antigenically distinct HAs have been described in influenza A viruses (H1 to H16), in addition to at least nine distinct NAs (N1 to N9). In addition, two distinct HA subtypes have been detected in bats,[15,16] and two possible neuraminidase subtypes, although without actual neuraminidase activity.[17] Based on sequence comparisons, the HA subtypes of influenza A viruses can be grouped into two subgroups: group 1, containing H1 and H5 among others, and group 2, containing H3 and H7. A third integral membrane protein, the M2 protein, is also present in small amounts on the viral envelope.

Interior to the envelope is the matrix, or M1, protein. This protein provides structure to the virion and is important for virus assembly. Within the virion are eight physically discrete nucleocapsid segments (Table 165.4). Each nucleocapsid is composed of a single segment of genomic RNA intimately associated with the viral nucleoprotein (NP), with the three polymerase proteins PB1, PB2, and PA bound to one end. Two nonstructural viral proteins, NS1 and NS2 (also referred to as the nuclear export protein, or NEP), are also found within infected cells. Small amounts of NEP are present within virions.

Although influenza B viruses have a similar structure, they do not exhibit the same type of antigenic and genetic variation in the HA and NA, and therefore do not have subtypes. However, since 2001, two antigenically distinct lineages of influenza B viruses, termed the "Victoria" lineage and the "Yamagata" lineage, have cocirculated in humans.[18]

Influenza viruses enter the cell by attachment of the viral hemagglutinin to sialic acid–containing receptors on the cell membrane, followed by internalization of the virus into an acidic endosome. In the acidic environment of the endosome, the HA undergoes a conformational change that liberates a fusion peptide and results in fusion of the viral envelope with the endosomal membrane. At the same time, the third envelope protein, the M2 protein, acts as an ion channel allowing H^+ ions to enter the virion from the endosome. This in turn allows the viral gene segments to leave the virion and enter the cytoplasm, a process known as uncoating.

Viral gene segments are transported to the nucleus, where the viral polymerase complex, composed of the proteins PB1, PB2, and PA, directs the synthesis of the plus-sense messenger RNA and synthesis of negative-sense copies that will serve as progeny genomic RNA. The polymerase proteins also play a role in disruption of host cell protein synthesis. Because replication takes place in the nucleus, some mRNAs are spliced, giving rise in the case of influenza A viruses to the M2 protein from the M gene segment, and the NS2 or NEP (nuclear export protein) from the NS gene segment. Alternative start codons also give rise to a number of additional proteins from the polymerase genes of influenza A viruses including PB1-F2[19] and PA-X,[20] which may play roles in pathogenesis.[21]

TABLE 165.4 Genes and Protein Products of Influenza A Virus

RNA SEGMENT	GENE PRODUCT	NAME	PROPOSED FUNCTIONS
1	PB2	Basic polymerase 2	Cap-binding of host mRNA molecules
2	PB1 PB1-F2	Basic polymerase 1	RNA-dependent RNA polymerase Proapoptotic, immune evasion, proinflammatory
3	PA PA-X	Acidic polymerase	Endonuclease activity Multiple functions, inhibits host innate response
4	HA	Hemagglutinin	Viral attachment to cell receptor, membrane fusion
5	NP	Nucleoprotein	Encapsidates RNA, forms ribonucleoproteins
6	NA	Neuraminidase	Releases virus from cell, prevents aggregation of virions
7	M1 M2	Matrix protein 1 Matrix protein 2	 Ion channel, required for uncoating
8	NS1 NEP	Nonstructural protein 1 Nuclear export protein	Antagonizes interferon Transport of newly assembled ribonucleoproteins from nucleus to cytoplasm

Negative-sense daughter virion RNAs are encased in nucleoprotein, associated with one copy of the polymerase complex, and transported to the cytoplasm for assembly at the cell surface. Envelope proteins are glycosylated and transported to the cell surface. Virions bud from selected lipid rafts at the cell surface, acquiring an envelope derived from the cell membrane and decorated with HA, NA, and small amounts of M2 protein. Finally, the NA removes sialic acid from receptors on the cell surface or on the viral envelope, allowing the progeny viruses to leave the infected cell.

Because the genome of influenza virus is segmented, segments can be exchanged between viruses infecting the same cell, a process referred to as reassortment. Genetic reassortment plays an important role in the generation of pandemic influenza A viruses, and has also been taken advantage of for the construction of attenuated live influenza vaccines.

EPIDEMIOLOGY AND DISEASE IMPACT

Disease Impact

Influenza epidemics are regularly associated with excess morbidity and mortality, usually expressed in the form of excess rates of pneumonia and influenza-associated hospitalizations and deaths during epidemics. In order to estimate the mortality burden of influenza, observed pneumonia- and influenza-related deaths during periods of influenza epidemic activity are compared with an expected seasonal baseline derived from a time-series regression model, and the excess mortality attributable to influenza is calculated. A historical tabulation of levels of excess pneumonia and influenza deaths attributable to influenza epidemics[22] is shown in Table 165.5, along with the estimated percentage of isolates that were typed as influenza A (H3N2), A (H1N1), or B in each year. In general, the level of excess mortality is highest in years when influenza A (H3N2) viruses predominate, but influenza B and to a lesser extent H1N1 viruses also can be associated with excess mortality. Because not all influenza-related deaths manifest as pneumonia, the pneumonia and influenza mortality statistics probably underestimate the true impact of influenza on the population.[23] Table 165.5 also lists the all-cause excess mortality, defined as deaths due to any cause, above a similarly derived baseline, that occur during periods of influenza epidemic activity. As many as 51,000 deaths annually in the United States can be attributed to influenza.[24] After correction for underreporting by assessing the fraction of cases with diagnostic testing, it has been estimated that there were between 155,000 and 624,000 hospitalizations, 18,000 to 95,000 intensive care unit (ICU) admissions, and 5000 to 27,000 deaths per year in the United States attributable to influenza.[25] Surveillance data have suggested that pneumonia and influenza hospitalizations and inpatient deaths in the United States may have declined from 1996 to 2011, coincident with expanded recommendations for influenza vaccination and the introduction of conjugate pneumococcal vaccines.[26]

Mortality is only the most severe manifestation of influenza impact, and similar techniques can be used to estimate excess morbidity due to influenza epidemics.[27] Data from the Tecumseh Community Health Study have been used to estimate that influenza is responsible for 13.8 to 16.0 million excess respiratory illnesses per year in the United States among individuals younger than 20 years, and for 4.1 to 4.5 million excess illnesses in older individuals.[27] Much of the impact of influenza is related to the malaise and consequent disability that it produces, even in young, healthy individuals. It has been estimated that a typical case of influenza, on average, is associated with 5 to 6 days of restricted activity, 3 to 4 days of bed disability, and about 3 days lost from work or school.[28] Direct medical costs of illness account for only about 20% of the total expenses of a case of influenza, with a major proportion (30%–50%) of the economic impact due to loss of productivity.[29] In one study, influenza in schoolchildren resulted in 37 missed school

TABLE 165.5 Estimated Excess Pneumonia- and Influenza-Related Deaths and Excess Mortality of All Causes During Influenza Epidemics

YEAR	PERCENT OF ISOLATES THAT WERE OF THE FOLLOWING (SUB)TYPE			PNEUMONIA- AND INFLUENZA-RELATED EXCESS DEATHS (RANGE)	ALL-CAUSE EXCESS DEATHS (RANGE)
	H3N2	H1N1	B		
1972/73	90	0	10	7900 (5500–10,300)	18,300 (1200–35,000)
1973/74	20	0	80	0	0
1974/75	100	0	0	6500 (4100–8900)	15,100 (0–32,100)
1975/76	70	0	30	11,800 (9200–14,400)	24,600 (3400–45,900)
1976/77	5	0	95	0	0
1977/78	60	26	14	8300 (6000–10,500)	46,200 (19,800–72,700)
1978/79	0	98	2	0	0
1979/80	2	1	97	5100 (3500–6700)	17,300 (600–34,100)
1980/81	77	23	0	11,700 (9,100–14,200)	47,200 (27,800–66,600)
1981/82	1	24	75	2100 (600–3700)	0
1982/83	79	10	11	4700 (2800–6700)	9600 (0–19,200)
1983/84	5	50	45	3500 (1600–5400)	8200 (0–17,600)
1984/85	97	0	3	8100 (6600–9600)	36,200 (17,700–54,700)
1985/86	24	0	76	6700 (4900–8500)	34,000 (6800–61,200)
1986/87	—	—	—	1800 (1100–2500)	16,800 (1900–31,700)
1987/88	0	80	20	7400 (5600–9100)	33,400 (12,900–53,800)
1988/89	45	45	10	5100 (3600–6600)	10,500 (800–20,200)
1989/90	90	1	9	10,100 (8500–11,700)	43,600 (27,600–59,600)
1990/91	4	3	93	4200 (2400–6100)	23,000 (0–46,000)
1991/92	19	81	0	6600 (5600–7700)	41,700 (19,600–63,700)

Modified from Simonsen L, Clarke MJ, Williamson DW, et al. The impact of influenza epidemics on mortality: introducing a severity index. Am J Public Health. *1997;87:1944–1950.*

days by children and 20 days of missed work by parents, per 100 children.[30] Influenza is also associated with decreased job performance in working adults[31] and reduced levels of independent functioning in older adults.[32]

Influenza is usually associated with a U-shaped epidemic curve. Attack rates are generally highest in the young, whereas mortality is generally highest among older adults (Fig. 165.3).[33] In some studies, up to three-fourths of all influenza-related deaths occur in those older than 65.[25] Excess morbidity and mortality are also high in those with certain high-risk medical conditions, including adults and children with cardiovascular and pulmonary conditions such as asthma, or those requiring regular medical care because of chronic metabolic disease, renal dysfunction, hemoglobinopathies, or immunodeficiency, and in individuals with neurologic conditions that compromise the handling of respiratory secretions.[34] A summary of these conditions is shown in Table 165.6. Influenza-related death rates in nursing home residents with comorbid conditions are as high as 2.8% per year.[35]

The increased risk of influenza during pregnancy was dramatically demonstrated during the 2009 pandemic.[36] Previous studies had identified an increased risk of hospitalization associated with influenza epidemics during pregnancy, especially in the second and third trimesters and in the immediate postpartum period.[37] During the 2009 H1N1 pandemic, women in each stage of pregnancy, or in the immediate postpartum period, were significantly overrepresented among those admitted to hospitals and ICUs.[36,38] A meta-analysis has suggested that the overall risk of influenza-related hospitalization was more than twice as great during pregnancy.[39] The mechanism(s) by which pregnancy enhances the risk of influenza are not clear but might include the increased cardiovascular demands of pregnancy, and hormonally mediated changes to the innate and adaptive immune response.[40]

Obesity also emerged as a risk factor for influenza morbidity and mortality during the 2009 pandemic that had not been recognized in previous seasonal epidemics or pandemics.[41,42] Although compromise to respiratory mechanics as a direct result of extreme obesity undoubtedly plays a role, there is also evidence to support a detrimental role of adipose tissue in the inflammatory response that might also enhance the influenza disease process.

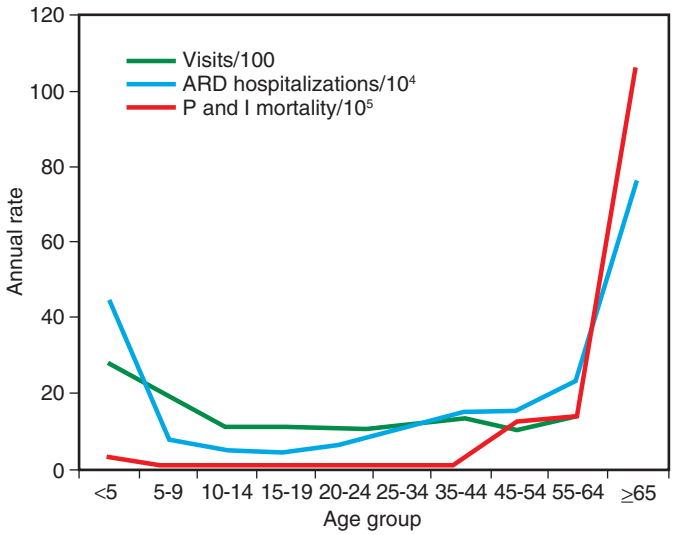

FIG. 165.3 Typical epidemic curve in the interpandemic era. Shown are the rates of medically attended illness (*green line,* rate per 100), hospitalizations for acute respiratory disease (ARD, *blue line,* rate per 10,000), and pneumonia *(P)*- and influenza *(I)*-related mortality (*red line,* rate per 100,000) by age for several seasons of influenza in Houston, Texas. Attack rates and hospitalizations occur at both extremes of age, but mortality occurs largely in those older than 65 years. *(Data from Glezen WP, Keitel WA, Taber LH, et al. Age distribution of patients with medically attended illnesses caused by sequential variants of influenza A/H1N1: comparison to age-specific infection rates, 1978–1989.* Am J Epidemiol. *1991;133:296–304.)*

Influenza is recognized as an important health problem in young children. Rates of influenza-related hospitalizations are particularly high in healthy children younger than 2 years, in whom rates approach those of older children with high-risk conditions.[43–45] In addition, a high rate of secondary complications, particularly otitis media and pneumonia, occur in children with influenza infection.[46] Rates of outpatient clinic visits for laboratory-documented influenza have been observed at 50 to 95 per 1000 person-years, and of emergency room visits at 6 to 27 per 1000 person-years in children younger than 5.[47] Although rare, influenza-related deaths occur each year in previously healthy children.[34] Notably, many of these deaths occur in children who were not recognized to have high-risk conditions before their illness.

Epidemic Influenza

An epidemic is an outbreak of influenza confined to one location, such as a city, town, or country. A graphic description of a typical and well-characterized epidemic due to influenza A (H3N2) in 1976 is shown in Fig. 165.4. The epidemic began abruptly, reached a sharp peak in 2 to 3 weeks, and lasted 5 to 6 weeks.[48] The first indicator of influenza activity was reports of increased numbers of children with febrile respiratory illness, followed by the occurrence of influenza-like illnesses among adults. These reports were subsequently followed by increased hospital admissions for patients with pneumonia, exacerbation of chronic obstructive pulmonary disease, croup, and congestive heart failure, and finally by increased school and industrial absenteeism. However, individual seasonal epidemics do not always follow this pattern.

During epidemics, attack rates in unvaccinated populations are estimated to be 10% to 20%, but rates as high as 40% to 50% have been reported.[49] The factors that lead to termination of an outbreak in any given location are unclear, but often the outbreak ends before the theoretical supply of susceptible individuals is exhausted.

In temperate climates in either hemisphere, epidemics occur almost exclusively in the winter months (generally November to April in the Northern Hemisphere and May to September in the Southern Hemisphere). Seasonal periodicity is also observed in tropical climates, with increased activity during periods of low absolute humidity, although influenza can occur throughout the year and seasonal fluctuations are not as marked.[50] The reasons for these seasonal changes are not entirely clear but might be the result of more favorable environmental conditions for virus survival.[51] Using a statistical model called convergent cross mapping, temperature and absolute humidity were shown to be important

TABLE 165.6 Groups at Higher Risk for Influenza Complications

Children and Adolescents at Higher Risk for Influenza Complications
Children younger than 4 years
Children with chronic pulmonary (including asthma), cardiovascular (except hypertension), renal, hepatic, hematologic, or metabolic (including diabetes mellitus) disorders
Children who are immunosuppressed, including children infected with human immunodeficiency virus and those taking immunosuppressive medications
Children with a condition that can compromise respiratory function or handling of respiratory secretions that can increase the risk for hypertension (e.g., cognitive dysfunction, spinal cord injuries, seizure disorders, or other neuromuscular disorders)
Children who are receiving long-term aspirin therapy and who therefore might be at risk for developing Reye syndrome
Children who are residents of chronic-care facilities
Those who will be pregnant during the influenza season
Adults at Higher Risk for Influenza-Related Complications
Persons aged 65 years or older
Women who will be pregnant during the influenza season
Persons with chronic pulmonary (including asthma), cardiovascular, renal, hepatic, hematologic, or metabolic disorders (including diabetes mellitus)
Persons who have immunosuppression (including immunosuppression due to medication or human immunodeficiency virus infection)
Persons with any condition that can compromise respiratory function or the handling of respiratory secretions or increase the risk of aspiration.
Persons with obesity, defined as a body mass index greater than 40
Residents of nursing homes and other chronic-care facilities

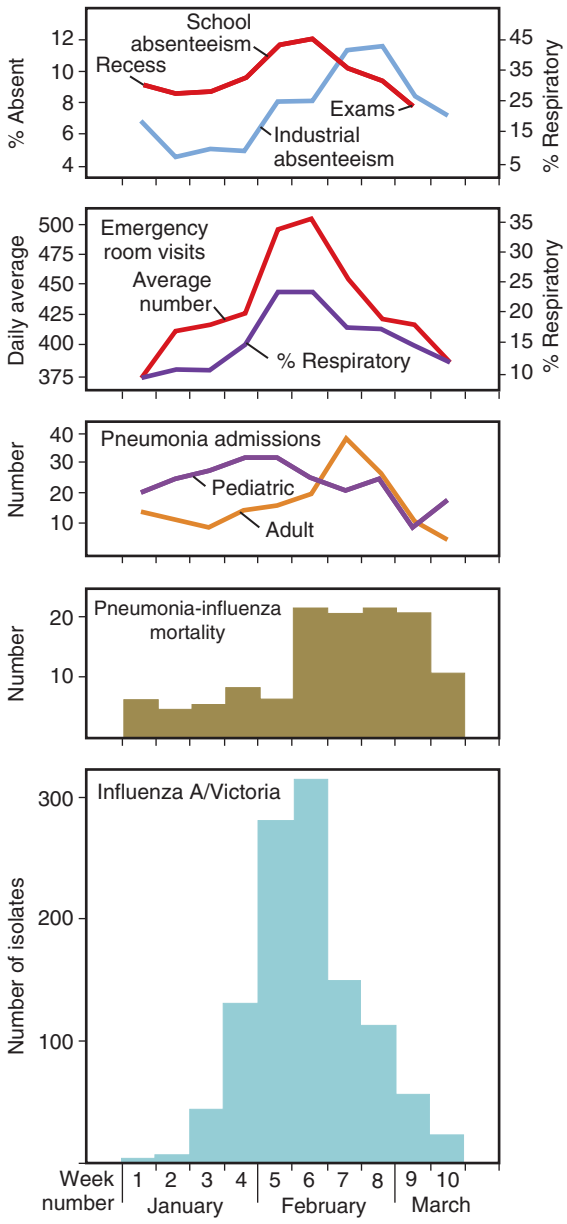

FIG. 165.4 Correlation of the nonvirologic indexes of epidemiologic influenza with the number of isolates of A/Victoria virus according to week, Houston, 1976. Industrial absenteeism is determined by the percentage with respiratory complaints. *(From Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974–1976.* N Engl J Med. *1978;298:587–593.)*

drivers of seasonal epidemicity.[52] Studies in a model of transmission of influenza in guinea pigs have also supported a role for conditions of cold temperature and dry humidity in facilitating transmission.[53] Seasonality may also be associated with behavioral changes that may increase transmission, such as indoor crowding or school attendance. Possibly for this reason, the effects of weather variability may be greater in young children.[54] Modeling studies based on medical claims data have suggested that winter holidays delay seasonal epidemic peaks and shift disease visits toward adults.[55]

In general, a single strain of influenza virus will be the predominant cause of cases during an epidemic. However, this is not always the case, and in some seasons two different lineages or strains within a single subtype or two different influenza A subtypes (H1N1 and H3N2) or concomitant outbreaks of influenza A and B have occurred. In some years, the end of the influenza epidemic season is characterized by cases due to a new strain. These limited outbreaks, which have been referred to as a "herald wave," sometimes predict the predominant strain in the next influenza season.[56]

Transmission

Influenza viruses are transmitted from person to person via the respiratory route. Three potential modes of transmission have been suggested.[57] Coughing and sneezing could generate small-particle aerosols (<10 μm mass diameter) that can remain suspended in air for many hours and could transmit infection to individuals at a substantial distance. Larger particles or droplets will typically fall to the ground within 3 meters of the infected person and would be expected to infect individuals in direct contact. Finally, viral particles could land on surfaces, where influenza viruses remain infectious and could infect others through indirect contact. There is substantial evidence for all three modes of transmission in experimental studies and epidemiologic observations, but the relative roles of each mode of transmission are uncertain and remain controversial, with obvious implications for infection control practices and for potential interventions to mitigate pandemics.

Small-particle aerosols are generated by infected humans, and influenza genome can be detected in these small particles by polymerase chain reaction (PCR) techniques.[58,59] It has not been proven that these aerosols contain significant amounts of infectious virus, but experimental studies in humans have shown that very small amounts (approximately five infectious particles) may be sufficient to infect humans by the aerosol route.[60,61] Aerosol transmission has also been demonstrated in animal models in which infected and exposed ferrets or guinea pigs are separated by several meters, with transmission occurring in the direction of airflow.[53,62]

Airborne transmission has also been implicated in multiple observations of outbreaks in which an airborne route of transmission appears to be the most plausible explanation for the characteristics of the outbreak. The most often cited such outbreak occurred in a commercial airliner that was delayed for approximately 4½ hours with a poorly functioning ventilation system. The risk of transmission of influenza A from the index case to other passengers was related to the amount of time passengers spent on the aircraft, and not on their seating proximity to the index case. Because most of the passengers did not have direct contact with the index case, airborne transmission appears to be likely.[63] In a well-investigated hospital outbreak, nosocomial cases occurred significantly less frequently in a hospital ward where the air was treated with ultraviolet (UV) light than in an otherwise similar ward without UV light treatment.[64] In an outbreak in a long-term care facility, there appeared to be an association between the risk of nosocomial influenza and the air-handling systems in several wards.[65]

Additional observations consistent with airborne transmission were made during an early study of zanamivir prophylaxis of influenza in families. In this study,[66] subjects who received short-term prophylaxis with inhaled zanamivir were protected compared with placebo recipients, but recipients of zanamivir administered by nasal spray were not. In addition, the combination of nasal and inhaled zanamivir was no better than inhaled zanamivir alone.

In most of these outbreaks, there were alternative explanations for the observations that could at least partially explain the epidemic behavior without requiring aerosol transmission,[67] and the real role of aerosol transmission remains controversial. If aerosol transmission plays a dominant role in influenza, then health care workers would need to wear filtering face masks, and patients would require negative pressure isolation, to prevent nosocomial transmission of influenza. This has prompted several studies that have attempted to evaluate the role of face masks in infection prevention in hospitals. In one large randomized trial, nursing staff who were randomly assigned to wear N95 respirators had the same rate of influenza as staff assigned to wear simple surgical masks while caring for patients with influenza.[68] This study suggests that airborne transmission does not play a major role at least in nosocomial influenza, although it has been pointed out that cases in the N95 group could have been acquired outside the hospital, and that compliance with use of these masks is frequently poor. In contrast, hand hygiene and simple surgical masks were reported to be modestly effective in the prevention of influenza transmission in households,[69] suggesting that in this setting, droplet spread was the predominant modality.

Pandemic Influenza

Pandemics are typically associated with the emergence of an antigenically variant influenza virus toward which the population has little or no prior immunity. The World Health Organization (WHO) has defined four levels of influenza pandemic activity (interpandemic, alert, pandemic, and transition), and currently defines pandemic influenza as the period of global spread of human influenza caused by a new influenza subtype.[70]

The epidemiologic behavior of pandemics differs in many ways from that of epidemic, seasonal influenza. In addition to higher attack rates overall with substantially greater disease impact, pandemics are typically associated with a different age distribution of cases, with greater impact in younger persons and relative sparing of the elderly. The reasons for this are unclear, although in some pandemics older segments of the population may have been exposed to antigenically related viruses in childhood, sometimes referred to as "antigenic recycling." Pandemics also often occur outside of the usual window of seasonality, possibly because their inherently greater transmissibility allows them to spread even under conditions that would not be favorable for transmission of seasonal influenza viruses. Many pandemics are characterized by multiple waves of activity during the pandemic period, such as the H1N1 pandemic, with an initial wave in the spring followed by a more severe wave of cases in the early fall in much of the United States. The intervals between pandemics are quite variable and unpredictable, but it is likely that pandemics of influenza will continue to occur in the future.

ANTIGENIC VARIATION

One of the unique and most remarkable features of influenza virus is the frequency with which changes in antigenicity occur, collectively referred to as antigenic variation. Alteration of the antigen structure of the virus leads to infection with variants to which little or no resistance is present in the population at risk. The phenomenon of antigenic variation helps explain why influenza continues to be a major epidemic disease of humans. Antigenic variation involves principally the two external glycoproteins of the virus, HA and NA, and is referred to as antigenic drift or antigenic shift, depending on whether the variation is small or great.

Antigenic Drift

Antigenic drift refers to relatively minor antigenic changes that occur frequently within the HA and/or NA of the virus. Drift has been studied most intensively for the HA and is the result of gradual accumulation of amino-acid changes in one or more of the five identified major antigenic sites on the HA molecule.[71] Because antibody generated by exposure to previous strains does not neutralize the antigenic variant as effectively, immunologic selection takes place and the variant supplants previous strains as the predominant virus in the epidemic. Antigenic variants can be produced in cell culture in the presence of limiting amounts of antibody, and these variants have similar single amino-acid sequences in the HA similar to those of drift variants.[72]

Although not studied as intensively, antigenic drift also occurs by amino-acid substitutions in the antibody epitopes in the NA.[73] A phenomenon analogous to antigenic drift has also been described for some epitopes recognized by cellular immune effectors.[74] Fitness costs to the virus, and the diversity of HLA types in the population, have been speculated to limit the extent of such variability.[75]

Comparison of the HA gene sequences of influenza viruses isolated in successive years reveals differences in the patterns of HA evolution among influenza A, B, and C viruses. In general, a single lineage, or relatively few lineages, of influenza A virus circulate in humans, and the accumulation of point mutations in the HA is linear, with each strain replacing the previously circulating one.[76] In contrast, multiple lineages of influenza C virus cocirculate, as shown by sequence comparisons of the HEF gene. The evolution of influenza B viruses is somewhere between these two examples, with relatively few lineages of the HA gene (but more than one) cocirculating.[77] Currently, two antigenically distinct lineages of influenza B viruses, referred to as the "Yamagata" lineage and the "Victoria" lineage, are cocirculating worldwide.

Although there is generally a very tight correlation between genetic changes and antigenic distance[78] the antigenic significance of any individual mutation can be more difficult to assess.

Antigenic Shift

At unpredictable intervals, influenza A viruses with more radical changes in the antigenicity of the HA and/or NA have emerged to cause widespread disease, or pandemics. These major antigenic changes are referred to as antigenic shifts, and result in viruses toward which the population has little or no prior immunity and is therefore highly susceptible. Fig. 165.5 ties together the concepts of antigenic shift and antigenic drift in relation to population immunity.[79] When a new virus, here called HxNx, to which immunity is lacking, is introduced into a population, pandemic influenza results. After one or more waves of pandemic influenza, the proportion of immune individuals in the population increases. Subsequent epidemics of seasonal epidemics due to strains of influenza A/HxNx that exhibit antigenic drift occur, with selection of new variants. After an unpredictable period of circulation of antigenic variants of the HxNx virus, a new virus, HyNy, will emerge, and the process repeats itself.

The pattern of replacement of HA and NA subtypes during the most recent century of pandemics is shown in Fig. 165.6. In addition, serologic studies have suggested that H3 subtype viruses circulated before 1918.[80] Virologic and PCR studies have shown that the "Spanish" pandemic of 1918 (mentioned previously) was caused by an H1N1 virus, which in turn was supplanted in the "Asian" pandemic of 1957 by H2N2 viruses. In 1968, the "Hong Kong" pandemic was caused by viruses of the H3N2 subtype. In 1977, viruses of the H1N1 subtype were reintroduced through an unknown mechanism. These viruses are genetically identical to the H1N1 viruses that were circulating in 1950. From 1977 to 2009, these viruses cocirculated with those of the H3N2 subtype. In 2009, a new variant of H1N1 viruses, referred to as pH1N1, emerged from pigs and replaced the previous H1N1 viruses.

The origin of new pandemic strains has been the subject of intense interest and study, for obvious reasons. The most plausible explanation for their origin takes into account three features of this phenomenon: that the virus has a segmented genome, that pandemics occur only with influenza A viruses, and that influenza A viruses, but not other influenza viruses, maintain a large reservoir of genetic diversity, primarily in birds.

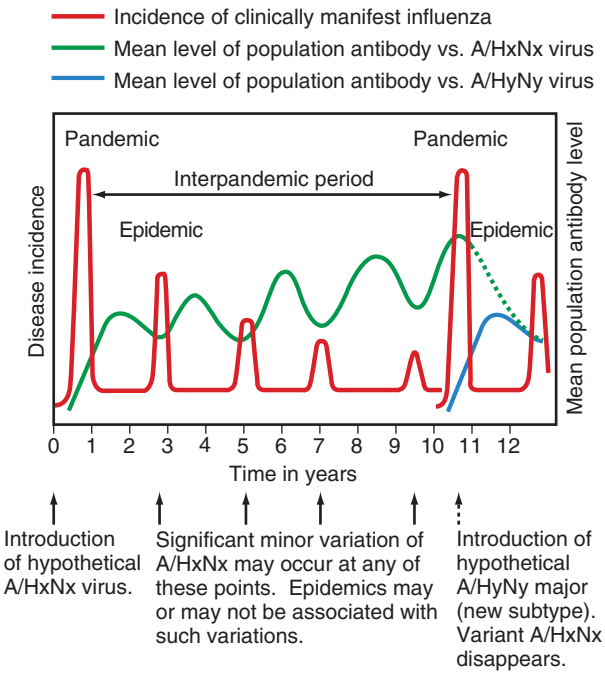

FIG. 165.5 Schema of the occurrence of influenza pandemics and epidemics in relation to the level of immunity in the population. A/HxNx and A/HyNy represent influenza viruses with completely different hemagglutinins and neuraminidases. *(Modified from Kilbourne ED. The epidemiology of influenza. In: Kilbourne ED, ed.* The Influenza Viruses and Influenza. *New York: Academic Press; 1975:483.)*

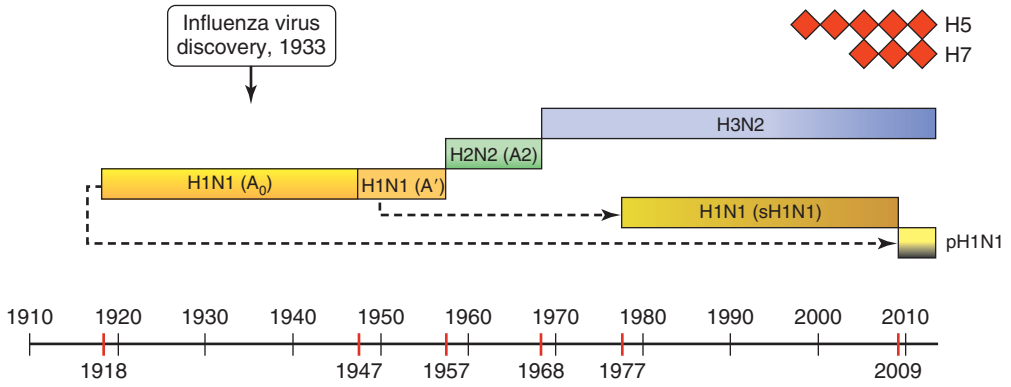

FIG. 165.6 Recent pandemics of influenza. The duration of circulation of viruses of various subtypes is shown by the boxes. Because the nature of influenza epidemics prior to 1918 is known only by serologic means, those boxes are *shaded tan*. Below the time line are the ages of individuals in 2004 who were alive during the various epidemic periods of earlier influenza subtypes. For example, individuals currently living who are between the ages of 47 and 86 probably experienced their first influenza A infection as an H1N1 virus. Individuals who are 36 years of age or younger have never been infected with H2N2 viruses.

Emergence of Pandemic Viruses From Birds

Extensive surveillance studies have identified influenza A viruses of 16 HA subtypes and all 9 NA subtypes in migratory waterfowl. In these birds influenza A causes mild illness or may be shed asymptomatically at high levels and for long duration in the feces. These birds may transmit influenza to other animals, including domestic poultry, horses, swine, and marine mammals, which may in turn transmit these viruses to humans. Comparisons of sequence data from animal and human influenza viruses isolates has suggested that the 1918 virus was introduced into humans from such an animal population. In contrast, the 1957 and 1968 pandemic influenza viruses were reassortant viruses that derived some genes from previously circulating human viruses, while deriving the HA and sometimes NA genes from an avian influenza virus.[81]

Because of the likely role of avian influenza viruses in the generation of emerging pandemics, there has been intense interest in recent outbreaks involving transmission of avian influenza viruses to humans, with resulting disease. Most of these transmission events have been quite limited, with small numbers of persons affected, relatively mild disease, and little or no evidence of person-to-person transmission. In most cases, virus has been transmitted to humans from infected domestic poultry, but cases have also occurred in association with marine mammals and possibly wild birds. These infections have involved primarily viruses of the H7, H5, H9, and H10 subtypes, with H7 and H5 viruses being most common.

Human Infections With H5 Viruses

H5N1 viruses were first recognized in humans in 1997[82] and have continued to cause substantial numbers of human cases since that time. The status of H5N1 infection is updated monthly by WHO. From 2003 until 2018, a total of 860 laboratory confirmed human cases of H5N1 infection, of which 454 (53%) cases were fatal, and 19 cases of H5N6 infection had been reported to WHO. Patients have ranged in age from 3 months to 75 years, with the median age being 20 years. Half of all cases have been in people younger than 20 years, and 90% of cases have been in people younger than 40 years. The median duration from onset of illness to hospitalization has been 4 days (range, 0–18 days). The case-fatality rates have been the highest for those in the 10- to 19-year-old age group, lowest for people 50 or older, and in between for children aged <10 years.[83]

Most cases have had close contact with ill poultry in the week before the onset of illness. Activities such as plucking and preparing diseased birds; playing with birds, especially asymptomatically infected ducks; and handling fighting cocks are risk factors for infection.[84] Other apparent modes of acquisition have included eating undercooked poultry or drinking raw duck blood, or exposure to contaminated water.[85] Person-to-person transmission has been rare, although family clusters of infection involving two or more family members have been documented.[86,87] Sequence comparisons of viruses from human cases and those of concurrent avian cases have established that at least some of the cases in the cluster represented person-to-person transmission.

Descriptions of the signs and symptoms of H5N1 infection are mostly from hospitalized patients. Most patients have presented with nonspecific complaints of a fever, cough, and shortness of breath. In many of the patients, there is a progression of symptoms leading to respiratory failure necessitating ventilation and other supportive measures. Atypical symptoms such as nausea, vomiting, encephalopathy, and bleeding gums and nose have been reported.[88] Watery diarrhea may be present before the onset of respiratory symptoms. The majority of patients have an abnormal chest radiograph with diffuse and multifocal or patchy infiltration, but pleural effusions are rare.

Laboratory abnormalities include significant lymphopenia and leucopenia, mild-to-moderate thrombocytopenia, and elevated transaminases. These abnormalities are poor prognostic signs.[89] Most patients have had negative bacteriologic cultures of sputum and blood. Pathologic changes include diffuse alveolar damage in the lungs, reactive hemophagocytosis in the marrow, and lymphoid depletion with atypical lymphocytosis in the spleen and lymphoid tissues.[90] Centrilobular hepatic necrosis and acute tubular necrosis have been reported.

Highly pathogenic avian H5N1 viruses have undergone significant genetic diversification and dissemination throughout Asia, the Middle East, and Europe since their initial detection, especially after a large outbreak in birds at Qinghai lake in Western China in 2005.[91] These viruses are currently classified into 10 clades (clades 0–9), of which viruses in clade 0, 1, 2, and 7 have been responsible for disease in humans.[92] Clade 2 viruses have been further classified into subclades, which are antigenically distinct. Current human infections have been mainly due to clade 2 viruses, including subclades 2.1, 2.2, 2.3, and 2.4.

Human Infections With H7 Viruses

Sporadic human infections with H7 viruses have been detected for many years, and early infections were typically relatively mild and limited. A characteristic of these infections has been the presence of conjunctivitis.[93,94] Human cases have typically been associated with outbreaks in birds, although one case of human H7 infection was reported in a laboratory worker who was sneezed on by a seal that was infected with an H7N7 influenza virus.[95] A large cluster of H7N7 infections of humans occurred in 2003 in association with an outbreak of highly pathogenic avian influenza in commercial poultry farms in the Netherlands.[96,97] Although almost all cases in this outbreak were mild or subclinical, there was one confirmed fatal case in a 57-year-old otherwise healthy veterinarian, who developed pneumonia.[97] Subsequent serologic surveys have suggested that there were substantial numbers of asymptomatic infections in this outbreak.[98] Since that time, several additional, small outbreaks of human infections with H7N7 and H7N3 viruses have been reported.[99–101]

The spectrum of disease associated with H7 viruses was radically altered when major outbreaks of severe human disease due to viruses

of the H7N9 subtype began to occur, first noted in Shanghai and with subsequent cases throughout a large area of eastern China. These outbreaks occurred annually between 2013 and 2017, during the winter with noted cocirculation of seasonal human H3N2 viruses. Coinfection with both H3N2 and H7N9 viruses has been reported,[102] but does not appear to have spread reassorted viruses.

Infection was associated with severe respiratory illness resulting in acute respiratory distress syndrome (ARDS) and ICU admissions, with a case-fatality rate of 27% among recognized cases.[103] Lymphocytopenia was seen in almost all of the patients, and the majority had extensive consolidation on chest radiographs, with ground-glass opacities. In contrast to previous outbreaks of avian influenza in humans, the mean age of the affected patients (61 years) was substantially higher, and 42% of those affected were 65 or older. Older age and the presence of chronic medical conditions have been demonstrated to be risk factors for severe illness, somewhat similar to the findings in seasonal influenza.[103,104] H7N9 viruses frequently have mutations (H279Y) in the NA gene, suggesting resistance to oseltamivir.

Exposure to infected birds in live bird markets has been identified as a major risk factor for infection.[103,104] However, control of outbreaks of H7N9 in domesticated poultry has been more difficult than H5 outbreaks because the initial H7 viruses did not exhibit high pathogenicity in birds, making infected flocks much more difficult to recognize. It has been speculated that the older age distribution of human cases may reflect social differences by age in patronizing these markets.[105] However, an alternative explanation may be related to the age-related previous exposures to influenza viruses. Individuals born after 1968, whose primary influenza exposure was due to H3N2 viruses, might be relatively resistant to H7 viruses compared with older individuals whose primary exposure was to an H1N1 virus.[106]

In 2016–2017 there was a fifth wave of human cases, associated with a high-pathology variant of the H7 virus.[107] The HA of the fifth wave virus was antigenically distant from the viruses isolated in the first four waves, and sera from humans immunized against earlier H7N9 viruses did not recognize this new variant. However, only three human cases of H7N9 infection were detected in 2017–2018, bringing the total number of cases to 1567. Because of the potential for person-to-person transmission and intrinsic virulence, H7 viruses are currently considered the most threatening pandemic risk as assessed by the Centers for Disease Control and Prevention (CDC) Influenza Risk Assessment Tool (IRAT).[108]

Human Infections With H9 Viruses

To date, human infections with H9 viruses have been rare. Influenza H9N2 virus was isolated from two children in Hong Kong with mild febrile pharyngitis in 1999.[109] Subsequently, H9N2 infection has been detected from five individuals with typical influenza in China[110] and from a child with relatively severe influenza in Hong Kong. Although these isolated incidents have been uncommon, H9 viruses remain a high priority for human surveillance because other threatening avian viruses such as H5 and H7 are often reassortants with H9 viruses.

Factors Controlling Host Range

Fortunately, avian influenza A viruses appear to be relatively restricted in their ability to replicate in humans.[111] The precise molecular mechanisms responsible for the host-range preferences of avian influenza A viruses are not completely known, but several factors probably play a role.[112] The divergent evolution of the genes of these viruses in avian hosts could have resulted in less efficient interactions between undefined viral and mammalian host cell components. The relative attenuation of avian-human influenza reassortants for man[113] supports a role for non-HA genes in this restriction. In addition, the HAs of avian and mammalian influenza viruses display a different host-cell receptor specificity, with avian viruses preferring receptors containing sialic acid–galactose linkages of the α2-3 variety, and mammalian viruses tending toward α2-6 linkages.

Extensive sequence analysis has suggested at least two mechanisms by which avian viruses can circumvent these barriers to interspecies transmission. These studies have shown significant sequence similarity between the HA, NA, and PB1 gene segments of the pandemic H2N2 virus and avian viruses,[81] and between the H3 and PB1 gene segments of the pandemic H3N2 virus and avian viruses,[81,114] suggesting that in some circumstances, new pandemic viruses arise by reassortment between avian viruses, which provide novel surface glycoproteins, and human viruses, which provide genes allowing efficient replication in humans. Reassortment would be facilitated by the presence of a third species that is susceptible to infection with both avian and human viruses, such as the pig, which contains both types of receptors.[115] However, there are likely to be constraints on what types of reassortants are viable; in particular, it has been suggested that the hemagglutinins of recent human influenza A viruses are not compatible with the matrix genes of current avian viruses,[116] and phenomena of this type may limit the possibilities for generation of pandemic viruses by reassortment.

A second mechanism would involve adaptation of avian viruses to the human host by evolution in swine, and this is supported by sequence analysis showing that the 1918 pandemic was most likely the result of direct introduction of an avian or swine influenza A virus into humans.[117]

The finding of limited transmission of H5N1 in humans is consistent with experiments in the ferret model, in which aerosol transmission over distances can be shown with seasonal H3N2 viruses, but not with human isolates of H5N1 viruses. A series of studies have been reported in which various manipulations were attempted to determine whether H5N1 viruses were capable of adapting to ferrets and eventually acquiring the ability to transmit from ferret to ferret.[118,119] These studies showed that several mutations are required for H5 viruses to gain the ability to transmit from ferret to ferret, including changes in the polymerase, changes in the receptor binding specificity of the HA, and changes that alter the stability of the HA under acidic conditions. Sequence analysis has suggested that some of these changes may already be present in naturally occurring H5 viruses,[120] and it will be important to continue monitoring isolates for sequences suggestive of successful human adaptation.

Emergence of Pandemic Viruses From Swine

Domestic swine have also been recognized as a potential source of pandemic influenza viruses in man. Genomic data have suggested that influenza A (H1N1) viruses were introduced into swine populations at around the same time that H1N1 viruses emerged in man in 1918.[121] Since that time these H1N1 viruses, or classic swine viruses, continued to be maintained in domestic swine, where they caused minor illnesses and underwent relatively little antigenic evolution. During this time, swine were also occasionally infected with influenza A viruses from humans and from birds, and have always been considered to represent a potential "mixing vessel" in which reassortment between human and avian influenza viruses could occur. This concept was strengthened by the recognition that the swine respiratory tract contains abundant receptors of both the α2-3 and α2-6 types favored by avian and human viruses, respectively.[115]

In 2009, a novel virus in which gene segments derived from four different sources (the so-called "quadruple reassortant") emerged from pigs to cause widespread disease in humans with sustained person-to-person transmission. This virus, the pandemic H1N1 virus, was derived from the classic swine triple reassortant virus (HA, NP, and NS from classic swine; PA and PB2 from avian influenza virus; PB1 from human virus) but with a substitution of the NA and M gene segments from a Eurasian swine lineage. The HA of the pH1N1 virus was closely related to the HA of human viruses circulating in the early 20th century and was not recognized well by antibody to recent seasonal H1N1 viruses. This virus went on to cause a pandemic of influenza with a major impact in children and adolescents, and replaced previous H1N1 viruses as the new seasonal H1N1. Subsequent studies in guinea pigs have identified the Eurasian swine M gene segment as playing a critical role in transmission phenotype of this virus.[122]

In the late 1990s, H3N2 viruses emerged in swine as a result of transmission of H3N2 viruses from humans to pigs, and of genetic reassortment between human and swine influenza viruses. Three different genotypes of H3N2 viruses have been observed in swine: nonreassortant human H3N2 viruses; reassortants bearing the HA, NA, and PB1 genes from human H3N2 viruses and the remaining gene segments from classical swine influenza viruses; and the "triple reassortant" viruses

that derived their HA, NA, and PB1 genes from human H3N2 viruses, their M, NP, and M gene segments from classical swine viruses, and their PA and PB2 gene segments from AI viruses.[123,124]

More recently, a number of cases of human infection with quadruple reassortant H3N2 viruses were reported. These viruses are genetically identical to pH1N1 viruses, except that the HA and NA genes are derived from circulating swine H3N2 triple reassortant viruses.[125] Important to note, the M gene segment is derived from the Eurasian swine lineage, perhaps increasing the likelihood of sustained transmission of these viruses in humans. Influenza viruses that circulate in swine are referred to as "variant" viruses when isolated in humans, designated H3N2v virus.

During the period from July to September 2012, a total of 306 cases of human infection with H3N2v influenza viruses were reported.[126] H3N2v has been associated with typical influenza illness, and there were 16 H3N2v-associated hospitalizations and one death. Almost all cases have documented histories of swine exposure, and the majority of cases were associated with attendance at state fairs.[127] However, there are some cases that have suggested the presence of limited person-to-person transmission. The H3N2v viruses isolated from humans are phylogenetically most closely related to human influenza viruses from the mid 1990s, particularly the A/Wuhan/95 and A/Sydney/99 viruses.[125,128] Clinical cases of H3N2v have occurred primarily in children 12 years of age and younger—that is, individuals born after these viruses last circulated in humans. Studies based on serosusceptibility have also suggested that children younger than 10 years would be largely susceptible to infection.[128,129]

CLINICAL FINDINGS

Uncomplicated Influenza

Typical uncomplicated influenza often begins with an abrupt onset of symptoms after an incubation period of 1 to 2 days. Many patients can pinpoint the hour of onset. Initially, systemic symptoms predominate, including feverishness, chilliness or frank shaking chills, headaches, myalgia, malaise, and anorexia. In more severe cases, prostration is observed. Usually, myalgia or headache is the most troublesome symptom, and the severity is related to the height of the fever. Myalgia may involve the extremities or the long muscles of the back. In children, calf muscle myalgia may be particularly prominent. Severe pain in the eye muscles can be elicited by gazing laterally, and arthralgia but not frank arthritis is commonly observed. Other ocular symptoms include tearing and burning. The systemic symptoms usually persist for 3 days, the typical duration of fever. Respiratory symptoms, particularly a dry cough, severe pharyngeal pain, and nasal obstruction and discharge, are usually also present at the onset of illness but are overshadowed by the systemic symptoms. The predominance of systemic symptoms is a major feature distinguishing influenza from other viral upper respiratory infections. Hoarseness and a dry or sore throat may also be present, but these symptoms tend to appear as systemic symptoms diminish, and thus they become more prominent as the disease progresses, persisting 3 to 4 days after the fever subsides. Cough is the most frequent and troublesome of these symptoms and may be accompanied by substernal discomfort or burning. Older adults may simply present with high fever, lassitude, and confusion without the characteristic respiratory complaints, which may not occur at all. In addition, there is a wide range of symptomatology in healthy adults, ranging from classic influenza to mild illness or asymptomatic infection.

Fever is the most important physical finding. The temperature usually rises rapidly to a peak of 100°F to 104°F, and occasionally to 106°F, within 12 hours of onset, concurrent with the development of systemic symptoms. Fever is usually continuous but may be intermittent, especially if antipyretics are administered. On the second and third days of illness, the temperature elevation is usually 0.5°F to 1.0°F lower than on the first day, and as the fever subsides, the systemic symptoms diminish. Typically, the duration of fever is 3 days, but it may last 4 to 8 days.

Early in the course of illness, the patient appears toxic, the face is flushed, and the skin is hot and moist. The eyes are watery and reddened. A clear nasal discharge is common, but nasal obstruction is uncommon. The mucous membranes of the nose and throat are hyperemic, but exudate is not observed. Small, tender cervical lymph nodes are often present. Transient scattered rhonchi or localized areas of rales are found in less than 20% of cases. A convalescent period of 1, 2, or more weeks to full recovery then ensues. Cough, lassitude, and malaise are the most frequent symptoms during this period.

At the extremes of age, there are prominent differences in influenza. Influenza attack rates are higher in children than in adults. Maximal temperatures tend to be higher among children, and cervical adenopathy is more frequent among children than among adults. Croup associated with influenza virus infection occurs only among children.[130,131] Among older adults, fever remains a very frequent finding, although the height of the febrile response may be lower than among children and young adults. Pulmonary complications are far more frequent in older adults than in any other age group.

Complications of Influenza

Pulmonary Complications

Two manifestations of pneumonia associated with influenza are well recognized: primary influenza viral pneumonia and secondary bacterial infection. In addition, less distinct and milder pulmonic syndromes often occur during an outbreak of influenza that may represent tracheobronchitis, localized viral pneumonia, or possibly mixed viral and bacterial pneumonia. Comparative features of these clinical syndromes are shown in Table 165.7.

Primary Influenza Viral Pneumonia

The syndrome of primary influenza viral pneumonia was first well documented in the 1957–1958 outbreak. However, many of the deaths of young healthy adults in the 1918–1919 outbreak may have been a result of this syndrome. In outbreaks since 1918, primary influenza viral pneumonia has occurred predominantly among persons with cardiovascular disease, especially rheumatic heart disease with mitral stenosis, and to a lesser extent in others with chronic cardiovascular

TABLE 165.7 Comparative Features of Pulmonary Complications of Influenza

	PRIMARY VIRAL PNEUMONIA	SECONDARY BACTERIAL PNEUMONIA	MIXED VIRAL AND BACTERIAL PNEUMONIA
Setting	Cardiovascular disease; pregnancy; young adult (pH1N1)	Adults and children	Any associated with A or B
Clinical history	Relentless progression from classic 3-day influenza	Improvement, then worsening after 3-day influenza	Features of both primary and secondary pneumonia
Physical examination	Bilateral findings, no consolidation	Consolidation	Consolidation
Sputum bacteriology	Normal flora	*Pneumococcus, Staphylococcus, Haemophilus influenzae*	*Pneumococcus, Staphylococcus, H. influenzae*
Chest radiography	Bilateral findings	Consolidation	Consolidation
Detection of influenza virus	Yes	Not always	Yes
Response to antibiotics	No	Yes	Often
Mortality	High	Variable	Variable

and pulmonary disorders. The illness begins with a typical onset of influenza, followed by a rapid progression of fever, cough, dyspnea, and cyanosis. Physical examination and chest radiographs reveal bilateral findings consistent with the adult respiratory disease syndrome but no consolidation. Blood gas studies show marked hypoxia, Gram stain of the sputum fails to reveal significant bacteria, and bacterial culture yields sparse growth of normal flora, whereas viral cultures yield high titers of influenza A virus. Such patients do not respond to antibiotics, and mortality is high. At autopsy, findings consist of tracheitis, bronchitis, diffuse hemorrhagic pneumonia, hyaline membranes lining alveolar ducts and alveoli, and a paucity of inflammatory cells within the alveoli (see Figs. 165.9 and 165.10).

Secondary Bacterial Pneumonia

Secondary bacterial pneumonia often produces a syndrome that is clinically indistinguishable from that occurring in the absence of influenza.[132,133] The patient has a classic influenza illness followed by a period of improvement lasting usually 4 to 14 days. Recrudescence of fever is associated with symptoms and signs of bacterial pneumonia such as cough, sputum production, and an area of consolidation detected at physical examination and on a chest radiograph. The two pathogens that are currently most commonly associated with influenza are *Streptococcus pneumoniae* and *Staphylococcus aureus,* which is otherwise an uncommon cause of community-acquired pneumonia. Community-acquired methicillin-resistant *S. aureus* (MRSA) has been seen in children following influenza,[134] and a study has suggested that the addition of a second active antibiotic may be important in children with MRSA complicating infection.[135]

During an outbreak of influenza, many patients do not clearly fit into either of the aforementioned categories. The disease is not relentlessly progressive, and yet the fever pattern may not be biphasic. These patients may have primary viral, secondary bacterial, or mixed viral and bacterial infection of the lung. In addition, milder forms of influenza viral pneumonia involving only one lobe or segment have been described that do not invariably lead to death, and that are more likely to be confused with pneumonia caused by *Mycoplasma pneumoniae* than to pneumonia produced by bacterial infection. Some studies have suggested that elevated levels of procalcitonin or C-reactive protein can be helpful in distinguishing secondary bacterial from primary viral pneumonia.[136,137]

Pulmonary Complications in Immunosuppressed Patients

Influenza has been noted to cause severe disease with an increased incidence of pneumonia in immunosuppressed children with cancer compared with age-matched individuals without immunosuppression.[138] Severe disease associated with pneumonia and death has been reported, particularly in bone marrow transplant recipients and leukemic patients.[139,140] Relatively more immunosuppressed individuals early after transplantation appear to be at greater risk. Influenza virus shedding can be quite prolonged in immunosuppressed children,[141] particularly those with human immunodeficiency virus (HIV) and low $CD4^+$ counts.[142] Because of the prolonged, unchecked replication of influenza viruses in these individuals, resistance to antiviral drugs eventually occurs in many treated patients.[143,144]

Other Pulmonary Complications

In addition to pneumonia, other pulmonary complications of influenza have been recognized. Bronchiolitis may also occur as a result of influenza A or B virus infection, but respiratory syncytial virus and parainfluenza virus type 3 are more important causes of bronchiolitis. Significant numbers of cases of croup occur in influenza A and B outbreaks.[130] Croup associated with influenza A virus appears to be more severe but less frequent than that associated with parainfluenza virus types 1 or 3 or respiratory syncytial virus infections (see Chapters 156 and 158). Acute exacerbation of chronic bronchitis is a common complication of influenza and may result in a permanent loss of pulmonary function.[145] Exacerbations of asthma and worsening pulmonary function in children with cystic fibrosis may also occur.[146]

Nonpulmonary Complications

Myositis

Myositis and myoglobinuria with tender leg muscles and elevated serum creatine phosphokinase (CPK) levels have been reported, mostly in children, but they can also occur in adults.[147] Symptoms may be sufficiently severe to interfere with walking.

Cardiac Complications

Both myocarditis and pericarditis have been rarely associated with influenza A or B virus infection,[148,149] although not observed at autopsy among those who died of primary influenza viral pneumonia.[150] Myocardial infarction may also be triggered by influenza infection,[151] possibly as an effect of platelet aggregation.[152] Studies have shown a substantially increased risk of myocardial infarction in the 7 days after hospitalization for influenza.[153]

Toxic Shock Syndrome

In recent outbreaks of influenza A or B, a toxic shock–like syndrome has occurred in previously healthy children or adults, presumably because viral infection changed colonization and replication characteristics of the toxin-producing staphylococci.[154–156]

Central Nervous Complications

Guillain-Barré syndrome (GBS) has been reported to occur after influenza A infection, as it has after numerous other infections, but no definite etiologic relationship has been established. In addition, cases of transverse myelitis and encephalitis have occurred rarely.[157] Influenza has also been associated with acute encephalitis and encephalopathy,[158] which have a wide variety of manifestations.[159] Most cases occur in children, but associated morbidity is higher in adults.[160] The specific pathogenesis of influenza-associated encephalitis is unclear but may be related to the cytokine response.

Reye Syndrome

Reye syndrome is associated with many viral infections, prominently including influenza and varicella in children. The classic manifestation is a change in mental status occurring several days after a typical respiratory illness. Manifestations range from lethargy to delirium, obtundation, seizures, and respiratory arrest. Lumbar puncture reveals normal protein values and normal cell counts, confirming the presence of encephalopathy rather than encephalitis or meningoencephalitis. The most frequent laboratory abnormality is elevation of the blood ammonia value, which occurs in almost all patients. Reye syndrome is almost exclusively seen in children who have been given aspirin for treatment of febrile illnesses due to influenza and other viruses, and it is important to use other antipyretics such as nonsteroidal antiinflammatory drugs in this situation. Children who require continuous aspirin therapy are an important target group for influenza vaccination to reduce the risks of Reye syndrome.

PATHOGENESIS

Cellular Pathogenesis

Influenza virus infection is acquired by means of a mechanism involving the transfer of virus-containing respiratory secretions from an infected to a susceptible person. Once virus is deposited on the respiratory tract epithelium, it can attach to and penetrate columnar epithelial cells if not prevented from doing so by specific secretory antibody (immunoglobulin A [IgA]), by nonspecific mucoproteins to which virus may attach, or by the mechanical action of the mucociliary apparatus. After adsorption, virus replication begins, leading to cell death through several mechanisms. There is a dramatic shutoff of host-cell protein synthesis that occurs at several levels. Newly synthesized cellular mRNAs are degraded (probably because cleavage by the virus cap endonuclease renders these transcripts susceptible to hydrolysis by cellular nuclease), whereas translation of already-synthesized cytoplasmic mRNAs is blocked at both initiation and elongation. Finally, expression of the influenza virus PA protein has been shown to induce generalized degradation of coexpressed proteins.[161] Ultimately, the loss of critical cellular proteins very likely contributes to cell death.

In addition to effects leading to cell necrosis, infection of cells with influenza A and B viruses causes cell death by apoptosis.[162] Bronchiolar

epithelial and alveolar cells harvested from experimentally infected mice also exhibit apoptotic changes, suggesting that this mechanism of cell death may be important in the pathogenesis of influenza in vivo.[163] The specific mechanism by which influenza virus induces apoptosis is unclear, but it may be related to induction of Fas antigen by double-stranded RNA during virus replication.[164] An unusual viral protein of influenza A viruses, encoded by a second open reading frame in the *PB1* gene and therefore referred to as PB1-F2, also plays a role in induction of apoptosis by poisoning mitochondria.[19]

Virus release continues for several hours before cell death ensues. Released virus then may initiate infection in adjacent and nearby cells, so within a few replication cycles a large number of cells in the respiratory tract are releasing virus and dying as a result of the virus replication. The time between the incubation period and the onset of illness and virus shedding varies from 18 to 72 hours depending in part on the inoculum dose.[165]

Influenza virus infection of peripheral blood mononuclear cells (PBMCs), including polymorphonuclear leukocytes (PMNs), lymphocytes, and monocytes, is nonproductive, but it is associated with measurable defects in cellular function that may be relevant to the pathogenesis of influenza-related infectious complications. These include defects in PMN chemotaxis and phagocytosis,[166] and decreased proliferation and costimulation by mononuclear cells.[167] The effects are mediated by virus replication and possibly by a direct toxic effect of certain virus proteins, including hemagglutinin, neuraminidase, and nucleoprotein.[168] It has been noted that the short portion of the sequence of the influenza A virus NP is homologous to a naturally occurring peptide found in normal bronchoalveolar lavage fluid that inhibits PMN chemotaxis and oxidative burst,[169] and that exposure of PMNs to influenza virus suppressed endocytosis by these cells.[170]

Virus Shedding

Quantitation of virus in respiratory tract specimens reveals a characteristic pattern (Fig. 165.7). Virus is first detected just before the onset of illness (within 24 hours), rapidly rises to a peak of 3.0 to 7.0 $\log_{10}$ tissue-culture infective dose [$TCID_{50}$]/mL, remains elevated for 24 to 48 hours, and then rapidly decreases to low titers.[171] Usually, virus is no longer detectable after 5 to 10 days of virus shedding. However, because of the relative lack of immunity in the young, more prolonged shedding of higher titers of virus is seen in children. Children also appear to exhibit heterogeneous shedding, with the top 20% of shedders estimated to represent 90% of infectiousness.[172]

The severity of illness correlates temporally with quantities of virus shed in experimental influenza in volunteers, thus suggesting that a major mechanism in the production of illness is cell death resulting from viral replication. Although the clinical manifestations of influenza are dominated by systemic symptoms, viral replication is limited to the respiratory tract. However, virus can be detected with PCR in peripheral blood in immunocompromised patients and may be a marker for more severe outcomes.[173]

Cytokine Response

Systemic symptoms have been linked to the release of potent cytokines, such as type I interferons (IFNs), tumor necrosis factor, and interleukins (ILs), by infected cells and responding lymphocytes.[171] In fact, it has been suggested that an overly vigorous cytokine response to infection may contribute to the high fatality rate seen with H5N1 influenza,[174,175] sometimes referred to as a cytokine storm.

Studies have suggested that defects in the cytokine response may increase the risk of severe influenza. Single nucleotide polymorphisms (SNPs) in the gene for IFN-inducible transmembrane 3 (IFITM-3) have been associated with cytokine dysfunction and increased severity of influenza.[176] Similar SNPs have also been identified as associated with increased severity of human H7N9.[177] Carriers of the risk allele were shown to have decreased levels of $CD8^+$ T cells in their airways during influenza infection, suggesting that a critical function of this protein may be to promote immune cell persistence at mucosal sites.[178] IFN deficiency related to mutations in IRF7 have also been contributory to severe influenza in a human child.[179] Mutations in TLR3 and CD55 have also been associated with influenza hospitalizations or death.[180]

Therapies designed to reduce the cytokine response have been beneficial in severe influenza in animal models,[181] although this has not been tested rigorously in humans. However, a meta-analysis suggested that steroid therapy for severe influenza in humans is associated with increased mortality.[182] Commonly used statins also have antiinflammatory properties, and an observational study suggested that use prior or during hospitalization may be associated with decreased mortality.[183] Because these drugs are very common, it has been suggested that they may be useful in a pandemic response.[184]

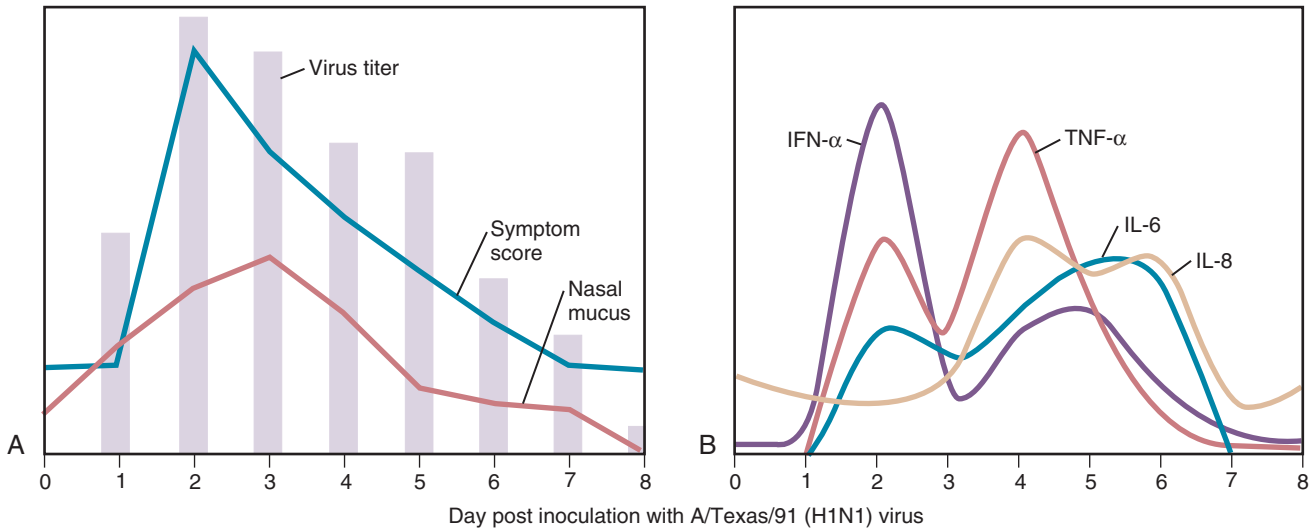

FIG. 165.7 Time course of virus shedding, symptoms, and cytokine responses of healthy adults after experimental inoculation with wild-type A/Texas/36/91 virus by nasal drops. (A) Mean $\log_{10}$ virus titer (tissue-culture infective dose [$TCID_{50}$] per milliliter of nasal secretions), clinical symptom scores, and nasal mucus weights (in grams). (B) Nasal cytokine levels measured by enzyme-linked immunosorbent assay (ELISA) (pg/mL lavage fluid, corrected for collection efficiency). In both graphs, multiple measurements have been combined for illustration, so that the *y*-axes are relative values only. Peak values reported in each assay are approximately as follows: virus titer, 3.6 $\log_{10}$ $TCID_{50}$/mL nasal secretions; symptom score, 7.0; nasal mucus weight, 7.0 g; interleukin (IL)-6, 450 pg/mL; interferon *(IFN)*-α, 150 pg/mL; tumor necrosis factor *(TNF)*-α, 270 pg/mL; IL-8, 9000 pg/mL. *(Data from Hayden FG, Fritz R, Lobo MC, et al. Local and systemic cytokine responses during experimental human influenza A virus infection: relation to symptom formation and host defense.* J Clin Invest. *1998;101:643–649.)*

Histopathology

Bronchoscopy of individuals with typical, uncomplicated acute influenza has revealed diffuse inflammation of the larynx, trachea, and bronchi, with mucosal injection and edema.[185,186] Biopsy in these cases has revealed a range of histologic findings, from vacuolization of columnar cells with cell loss, to extensive desquamation of the ciliated columnar epithelium down to the basal layer of cells (Fig. 165.8).[186] Individual cells show shrinkage, pyknotic nuclei, and a loss of cilia. Viral antigen can be demonstrated in epithelial cells.[187] In general, the tissue response becomes more prominent as one moves distally in the airway.[186] Epithelial damage is accompanied by cellular infiltrates primarily composed of lymphocytes and histiocytes. Histologic findings on autopsy in more severe cases show extensive necrotizing tracheobronchitis, with ulceration and sloughing of the bronchial mucosa,[188] extensive hemorrhage, hyaline membrane formation, and a paucity of PMN infiltration (Fig. 165.9). Patients with secondary bacterial pneumonia have the changes characteristic of bacterial pneumonia in addition to the tracheobronchial findings of influenza (Fig. 165.10). Recovery is associated with rapid regeneration of the epithelial cell layer and with pseudometaplasia.

Pathophysiology

Abnormalities of pulmonary function are frequently demonstrated in otherwise healthy, nonasthmatic young adults with uncomplicated (nonpneumonic) acute influenza. Demonstrated defects include diminished forced flow rates, increased total pulmonary resistance, and decreased density-dependent forced flow rates consistent with generalized increased resistance in airways less than 2 mm in diameter,[189,190] along with increased responses to bronchoprovocation.[189] In addition, abnormalities of carbon monoxide diffusing capacity[191] and increases in the alveolar-arterial oxygen gradient[192] have been seen. Of note, pulmonary function defects can persist for weeks after clinical recovery. Influenza in asthmatics[193] or in patients with chronic obstructive disease[194] may result in acute declines in forced expiratory vital capacity (FVC) or forced expiratory volume in 1 second (FEV_1). Individuals with acute influenza may be more susceptible to bronchoconstriction from air pollutants such as nitrates.[195]

Primary viral pneumonia occurs when virus infection reaches the lung either by contiguous spread from the upper respiratory tract or by inhalation. The trachea and bronchi contain bloody fluid, and the mucosa is hyperemic.[150] Tracheitis, bronchitis, and bronchiolitis are seen, with loss of normal ciliated epithelial cells. Submucosal hyperemia, focal hemorrhage, edema, and cellular infiltrate are present. The alveolar spaces contain varying numbers of neutrophils and mononuclear cells admixed with fibrin and edema fluid. The alveolar capillaries may be markedly hyperemic with intraalveolar hemorrhage. Acellular hyaline membranes line many of the alveolar ducts and alveoli.[150] Pathologic findings seen at biopsy of lung in nonfatal cases are similar to those described in fatal cases.[196]

The pathophysiology of bacterial superinfection has been studied intensively,[197] and a number of factors have been identified that could play a role. Uncomplicated influenza is associated with significant abnormalities in ciliary clearance mechanisms,[198] resulting in reduced clearance of bacteria from epithelial surfaces.[199] Alterations in PMNs and mononuclear cells may also contribute to enhanced bacterial infection. In mouse models, the inflammatory response to influenza, particularly the type I IFN response, also contributes to the downregulation of host antibacterial defense mechanisms.[200,201] The cytokine IL-10 plays an important role in this regard.[202] Influenza infection also reduces clearance by alveolar macrophages[203,204] and weakens NK responses.[205] In humans with H1N1 influenza, the development of pneumonia was associated with an early increase in T-regulatory cells (Tregs) in peripheral blood.[206] All of these observations are consistent with the concept that the inflammatory response to influenza infection leads to disruption in the normal host defense against bacterial pathogens, although direct demonstration of this in humans is lacking.

Host Response

Infection with influenza virus induces both systemic and local antibody, in addition to $CD4^+$ and $CD8^+$ T-cell responses, each of which plays a role in recovery from infection and resistance to reinfection. Epidemiologic and experimental observations in humans have shown that infection with influenza virus results in long-lived resistance to reinfection

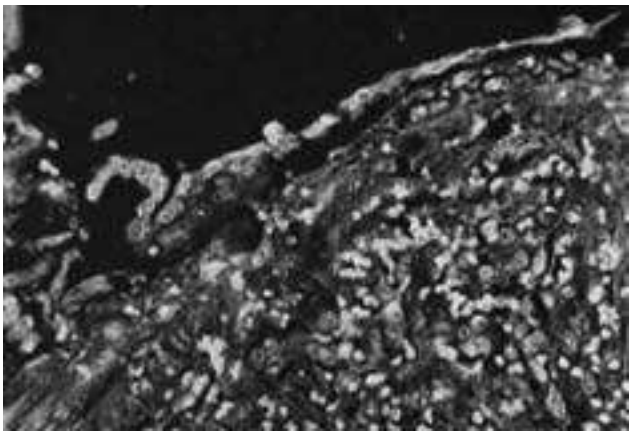

FIG. 165.8 A small bronchus in acute influenza A infection shows ulceration and attempted regeneration of epithelium. (Hematoxylin-eosin stain, ×100.) *(Courtesy I.D. Stuard, Reading, PA.)*

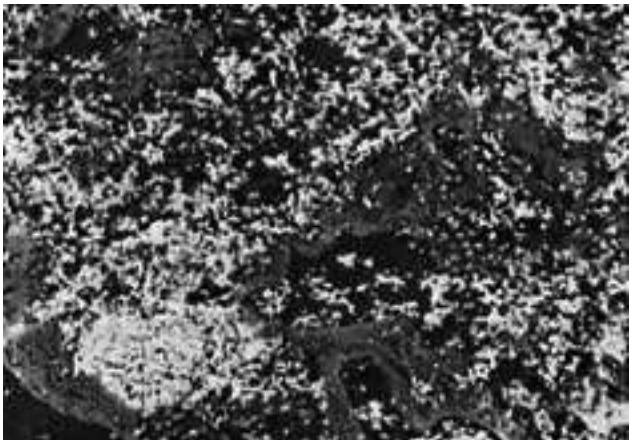

FIG. 165.9 Lung parenchyma in primary influenza viral pneumonia shows extensive hemorrhage, acellular hyaline membrane lining alveolar ducts and alveoli, and a paucity of inflammatory cells within the alveoli. (Hematoxylin-eosin stain, ×400.) *(Courtesy I.D. Stuard, Reading, PA.)*

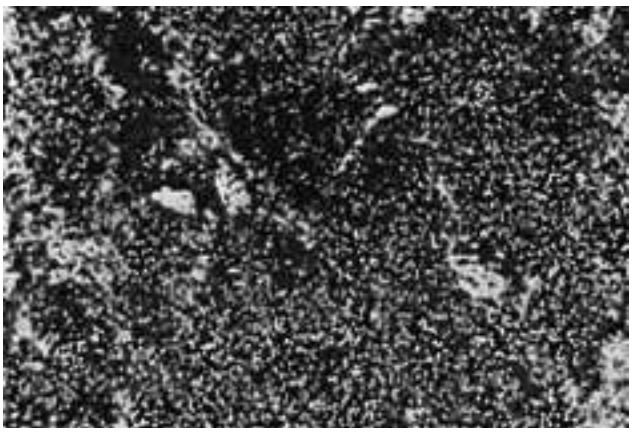

FIG. 165.10 Lung parenchyma in secondary bacterial infection *(Streptococcus pneumoniae)* complicating influenza A virus infection. Note the marked intraalveolar polymorphonuclear cell exudate. (Hematoxylin-eosin stain, ×400.) *(Courtesy I.D. Stuard, Reading, PA.)*

with the homologous virus. In addition, variable degrees of cross-protection within a subtype have been observed. Some epidemiologic data have raised the possibility of heterosubtypic protection in humans,[207] and identification of a variety of potentially cross-protective immune mechanisms is being explored as a strategy to increase the breadth of protection afforded by vaccination (see later).

Antibody Responses

Systemic Antibody Responses

Infection with influenza virus results in the development of antibody to the influenza virus envelope glycoproteins HA and NA, and to the structural M and NP proteins. Some individuals may also develop antibody to the M2 protein.[208] As measured with enzyme-linked immunosorbent assay (ELISA), serum IgM, IgA, and IgG antibody to the HA appear simultaneously within 2 weeks of inoculation of virus. The antibody response is more rapid after reinfection. The development of anti-NA antibodies parallels that of anti-HA antibodies.[209] Peak antibody responses are seen at 4 to 7 weeks after infection and decline slowly thereafter; titers can still be detected years after infection even without reexposure.[210]

Antibody to the HA can prevent attachment of the virus to the cell, and neutralizes virus infectivity.[211] A surrogate measurement of the neutralizing activity of serum is the hemagglutination-inhibition (HAI) assay, in which sera is tested for the ability to prevent agglutination of red blood cells by a virus preparation. An increased risk of laboratory-documented influenza among those with the lowest titers of preexposure HAI or neutralizing antibody is a consistent finding of most but not all studies. However, there is considerable uncertainty about the actual level of HA antibody that is the best predictor of protection, with estimates ranging from HAI titers of 1:8 to 1:160 or higher.[212] Given the substantial variation from laboratory to laboratory in the estimation of the HAI titer on the same set of samples,[213] the inability to use an absolute value for protection is not unexpected. In addition, the amounts of antibody needed to mediate protection could vary by population, degree of exposure, age, and specific influenza type or subtype, although this has not been analyzed comprehensively.

Studies evaluating the B-cell response of patients infected with novel influenza viruses, such as H5N1 or primary infections with pandemic H1N1, have identified B cells directed against epitopes on the stalk of the HA molecule.[214,215] The monoclonal antibodies produced by these B cells are typically highly cross-reactive among HA molecules within the same group; for example, group 1 specific antibodies react with H1, H2, and H5 viruses,[216] and group 2 specific antibodies react with H3 and H7 viruses.[217] Some levels of stalk-specific antibody are present in the sera of most adults, and the role of these antibodies in protection in humans has not been defined. However, there is considerable interest in exploiting this observation in the development of broadly cross-protective influenza vaccines.[218]

Antibody to the NA can be measured with NA inhibition or ELISA. In contrast to anti-HA antibody, anti-NA antibody does not neutralize virus infectivity but instead reduces efficient release of virus from infected cells, resulting in decreased plaque size in in vitro assays[219] and in reductions in the magnitude of virus shedding in infected animals.[220,221] Observations of the relative protection of those with anti-N2 antibody during the A/Hong Kong/68 (H3N2) pandemic,[209,222] and in experimental challenge studies in humans,[223,224] have shown that anti-NA antibody can be protective against disease and results in decreased virus shedding and severity of illness, but that it is infection permissive.[225]

Antibody to other influenza viral proteins have also been evaluated for potential protection. Antibody to M2 reduces plaque size in vitro, and passive transfer studies in mice have also suggested that antibody to the M2 protein of influenza A viruses may be partially protective if present in large enough amounts.[226] The mechanism of protection in vivo is related to mediation of antibody-dependent cytotoxicity.[227] Antibody to internal viral proteins such as M or NP are also cross-reactive among type A viruses, but they are nonneutralizing. Studies in mice have suggested that such nonneutralizing but cross-reactive antibody may mediate protection under some circumstances.[228] The mechanism by which antibody to viral proteins that are not exposed on the surface can mediate protection is unclear.

Mucosal Antibody Responses

The majority of studies of mucosal responses to influenza in humans have concentrated on measurement of HA responses with ELISA or neutralization tests, because nonspecific inhibitors of hemagglutination present in nasal mucus interfere with the standard HAI test. These studies have demonstrated significant mucosal responses to infection with wild-type virus or live-attenuated influenza vaccines (LAIVs). Both IgA and IgG are found in nasal secretions. Nasal HA-specific IgG is predominantly IgG1, and its levels correlate well with serum levels of HA-specific IgG1, suggesting that nasal IgG originates by passive diffusion from the systemic compartment.[229] Nasal HA-specific IgA is predominantly polymeric and IgA1, suggesting local synthesis.[230]

Studies in mice and ferrets have emphasized the importance of local IgA antibody in resistance to infection, particularly in protection of the upper respiratory tract. Polymeric IgA was shown to be specifically transported into the nasal secretions of mice and to protect against nasal challenge. Protection could be abrogated by intranasal administration of antiserum against IgA but not IgM or IgG.[231] Local antibody has also been shown to play a role in protection against antigenic variants in mice.[232] Studies in humans have also suggested that the resistance to reinfection induced by virus infection is mediated predominantly by local HA-specific IgA, whereas that induced by parenteral immunization with inactivated virus depends also on systemic IgG.[233] Important to note, either mucosal or systemic antibody alone can be protective if present in high enough concentrations, and optimal protection occurs when both serum and nasal antibodies are present.[234,235]

Cellular Responses

The induction of cellular immune response to influenza virus infection has been studied intensively in murine models, and such studies suggest that B cell, $CD4^+$ T cell, and $CD8^+$ T-cell responses all can play a role in protection against disease and recovery from infection. A large number of HLA class I restricted ($CD8^+$ T cell) and HLA class II restricted ($CD4^+$ T cell) epitopes have been described, and in situations where those epitopes are on relatively well conserved influenza proteins such as the polymerase, NP, and M proteins, the cellular responses are cross-reactive between subtypes, although not between types A and B.

Cellular immune responses to influenza vaccination and infection have not been studied as extensively in humans, but both B-cell (memory B-cell and antibody-secreting cell [ASC]) and $CD4^+$ T-cell and $CD8^+$ T-cell responses in peripheral blood have been described after infection or vaccination.[236] It can be difficult to capture the peak of the response, and detectable increases in antigen-specific cells may be seen on only a few days after exposure. In general, the peak cellular response occurs somewhere between 5 and 14 days depending on the status of the subject and the nature of the response. As seen in murine models, a major component of the cellular response is directed at conserved peptides to which the subject has already been exposed during previous infections or vaccinations.

ASCs appear in blood and tonsils as early as 2 days after vaccination, and are detected in the blood of adults and older children more frequently than in young children after immunization.[237] An increase in cytotoxic T lymphocytes, directed primarily at the conserved internal proteins, has been shown in healthy adults with a peak at 14 and 21 days after vaccination and return to baseline at 6 months. An increase in HA-specific $CD8^+$ T cells on day 7 after vaccination has also been detected by means of tetramer staining in adults receiving inactivated influenza vaccine (IIV).[238]

An important role of the cellular immune response in recovery from influenza infection in humans is strongly supported by the observation of prolonged illness and viral shedding in individuals who are lymphopenic as a result of disease or chemotherapy.[239] However, it has been difficult to develop specific markers of T-cell immunity as correlates of protective immunity. Activated T cells, in the form of granzyme B–positive T cells have been associated with protection in older subjects.[240] In the human challenge model, early studies identified virus-specific cytotoxic T cells as correlated with reductions in the duration and level of virus replication in adults.[241] In a subsequent study done many years later by the same group, prechallenge $CD4^+$ T cells, but not $CD8^+$ T cells, correlated with relatively lower levels of viral shedding and

symptoms after experimental infection.[242] In a large study of the efficacy of live-attenuated vaccine in children, it was shown that the presence of >200 influenza-specific T cells assayed by IFN-γ ELISPOT was associated with a decreased risk of PCR-documented influenza.[243]

In community surveillance of healthy adults during the pandemic of 2009, and in a setting of low baseline antibody to the pandemic virus, $CD8^+$ T cells were strongly correlated with protection against severe illness.[244] Under similar circumstances, the presence of NP-specific, predominantly $CD8^+$ T cells was correlated with less symptomatic influenza.[245] In addition, an early $CD8^+$ T cell response is associated with successful recovery from severe H7N9 illness in humans.[246] The development of more sophisticated markers that can specifically identify reactive cells in peripheral blood will help to define the role of cellular immunity in protection, but the field remains limited by the lack of convenient access to compartments other than the peripheral blood in humans.

DIAGNOSIS

The majority of cases of influenza are diagnosed on clinical grounds. In selected circumstances as discussed here, the diagnosis of influenza can be confirmed with laboratory diagnostic tests in which significant advances have been recently made in sensitivity and availability. These approaches are described in the following sections.

Clinical Diagnosis

Most cases of influenza are diagnosed based on compatible clinical symptoms and seasonal epidemiology. That is, when the presence of influenza virus is confirmed in a region or community, healthy adults with acute influenza-like illness most commonly have influenza. In fact, several studies have shown that the accuracy of a clinical diagnosis in healthy adults in the setting of an influenza outbreak is as high as 80% to 90%.[247–249] In an analysis of symptoms in young adults being assessed for entry into studies of influenza virus treatment, the best multivariate predictors of laboratory-confirmed influenza virus infection were cough and fever,[248] with an increasing predictive value with increasing levels of fever. However, the predictive value of such a symptom complex may be less in older adults[250] and in children.[251] In nursing homes, the presence of cocirculating pathogens (such as respiratory syncytial virus) that can result in identical symptoms can clearly complicate the ability to make a clinical diagnosis of influenza specifically.[252,253]

Laboratory Diagnosis

A wide variety of laboratory diagnostic approaches have been developed and are reviewed here. Available diagnostic testing information is updated frequently by the CDC on the website http://www.cdc.gov/flu/professionals/diagnosis/table-testing-methods.htm.

Polymerase Chain Reaction–Based Tests

The most widely used method in clinical laboratories to detect influenza infection is PCR tests. PCR-based tests have the advantage of being potentially more sensitive than other available tests and allow detection in samples in which the virions have lost viability. In addition, it is possible to devise multiplex techniques so that a single test can detect a number of different agents,[254] and many PCR methods also allow rapid subtyping of the virus. For these reasons, PCR and particularly real-time reverse-transcriptase polymerase chain reaction (rtRT-PCR) has become the gold standard of influenza diagnostic testing and is generally available at clinical laboratories. Diagnostic sensitivities of the tests depend on the level of virus present in various specimens, and generally samples obtained by nasopharyngeal swabs have slightly higher sensitivity, similar to the findings with antigen detection tests. In some patients with lower respiratory tract disease, sputum samples or tracheal aspirates have been positive when nasal swabs were not. Swabs with wooden handles should be avoided for PCR diagnostic testing, because these can interfere with the chemistry of the assay.

Improvements have been made in PCR techniques that have reduced the time to carry out assays in the laboratory to as little as 45 to 80 minutes. However, specimens still require transport from the point of care to the laboratory. Thus, despite their high accuracy and sensitivity, this may limit their usefulness for patient management. Therefore there has been considerable effort to develop simple, rapid nucleic acid–based detection methods that could be used at the point of care.

Rapid Nucleic Acid Amplification Tests

An alternative method of nucleic acid detection is isothermal amplification, which does not require a thermal cycler. A variety of isothermal amplification techniques have been developed, with the most frequently used approach being loop-mediated isothermal amplification (LAMP).[255] These assays are highly accurate, on a par with PCR-based tests, and are rapid, giving results in less than 30 minutes.[256] Because they are simple to operate, they can be approved for use at the point of care, which greatly improves their potential usefulness. However, these assays are currently expensive and not generally designed to identify other respiratory viruses.

Rapid Influenza Diagnostic Tests

The tests widely referred to as "rapid diagnostic" tests are based on immunologic detection of viral antigen in respiratory secretions. In this approach, the sample is treated with a mucolytic agent and then tested with specific antibody that results in a color change or similar end point that is read visually. All of these rapid tests are relatively simple to perform and can provide results within 30 minutes; many are eligible for Clinical Laboratory Improvement Amendments (CLIA) waiver and can be performed at the point of care. The reported sensitivities of each test in comparison to cell culture have ranged between 40% and 80%, and they are somewhat dependent on the nature of the samples tested and the patients from whom they were derived. In general, sensitivities in adults and older adult patients tend to be lower than those reported in young children, who shed much larger quantities of virus in nasal secretions and therefore have much higher concentrations of antigen in their samples. Similarly, sensitivity is likely to be higher early in the course of illness, when viral shedding is maximal. Although all types of respiratory samples can be used in such tests, the sensitivity appears to be better with nasopharyngeal swabs and aspirates than with throat swabs or gargles.[257,258] In some tests, the use of a digital readout can improve sensitivity compared with visual inspection.[259] However, because of the relatively low sensitivities of these tests, their usefulness in clinical decision making may be limited.

Virus Isolation

Isolation of influenza virus in cell culture has historically been the definitive laboratory test to diagnose influenza infection. Virus can be isolated readily from nasal swab specimens, throat swab specimens, nasal washes, or combined nose and throat swab specimens, or sputum samples. Samples should be placed into containers of viral transport medium and transported to the laboratory as soon as possible. Virus can be detected in a variety of cell lines by means of cytopathic effects, hemadsorption, or immunologic testing. Over 90% of positive cultures can be detected within 3 days of inoculation[260] and the remainder by 5 to 7 days. However, virus isolation requires specialized expertise and equipment and does not provide diagnosis within the time frame of clinical decision making.

Role of Viral Diagnosis in Clinical Decision Making

Most cases of influenza, occurring in otherwise healthy individuals with typical symptoms during the course of a recognized influenza epidemic, do not need specific viral confirmation. However, diagnostic testing should be used if the results of the test will influence subsequent clinical management. This would include decisions regarding the use of antiviral agents, the need for antibacterial drugs, and considerations for infection control.[261]

During the influenza season, certain groups should be considered for diagnostic testing if the results will influence decision making. Outpatients with higher risk for influenza complications (see Table 165.6) should be considered for testing if they are presenting within 5 days of symptom onset. Testing beyond this window may not be useful because the yield would be low and the patient would be outside the window for antiviral therapy. In contrast, outpatients who are immunocompromised, elderly, or infants may have prolonged shedding and could be considered for testing even beyond the 5-day window.

Hospitalized patients with febrile illness during influenza epidemics should also be tested regardless of duration of symptoms, as should patients who develop febrile illnesses in hospital during influenza epidemics, to determine nosocomial transmission. In addition, patients who are not in one of these groups might undergo diagnostic testing as part of epidemiologic surveillance in the community.

Outside of influenza epidemics, diagnostic testing should usually be restricted to patients with influenza-like symptoms who are epidemiologically linked to suspected outbreaks of influenza. Examples would include travelers returning from a region with influenza activity or individuals exposed during institutional outbreaks.

The choice of diagnostic test strategy will depend on local practice and availability of specific tests. Interpretation of rapid diagnostic test results, and, to a lesser extent, molecular assays, should take into consideration the known sensitivity and specificity of the test, and the a priori likelihood of influenza. Most positive results of rapid diagnostic tests obtained outside of the influenza season will be false positives. Important to note, if influenza is strongly suspected in a high-risk patient, antiviral therapy should be initiated without waiting for the results of diagnostic testing.

PREVENTION

Currently Available Vaccines

Multiple forms of influenza vaccine are currently available both in the United States and elsewhere, including virion- or protein-based IIVs administered intramuscularly, and LAIVs administered intranasally. Both types of vaccines are thought to work primarily by inducing antibody against the viral hemagglutinin, although other mechanisms probably also play a role.

As described earlier, influenza viruses undergo continual antigenic evolution to escape from prior immunity. As a result, the match between the viruses chosen as components of the vaccine and the viruses that circulate during the season is an important determinant of the efficacy of that season's vaccine.[262] Successfully matching the vaccine to the epidemic requires a considerable effort devoted to worldwide surveillance and subsequent antigenic characterization of emerging new variants. A final decision regarding strain selection for next year's vaccine generally must be made at least 6 months before expected vaccination—that is, in February for the Northern Hemisphere and September for the Southern Hemisphere. The need to constantly update vaccine formulations and to readminister vaccine on a yearly basis represents some of the biggest challenges in effectively controlling the impact of influenza.

The composition of the vaccine has changed over time to reflect changes in the epidemiology of influenza viruses. Since 1977, influenza vaccines have contained three strains representing the most up-to-date antigenic variants of A/H3N2, A/H1N1, and B viruses. Since approximately 2004, two antigenically distinct lineages of influenza B viruses, the Victoria lineage and the Yamagata lineage, have cocirculated. Therefore, quadrivalent formulations of vaccine containing both lineages were licensed beginning in the 2013–2014 influenza season. The nomenclature of influenza vaccines has changed to reflect this difference, with trivalent preparations now termed inactivated influenza vaccine-3 (IIV-3) and quadrivalent IIV-4.

Each of the vaccine types currently available for prevention of influenza are described briefly here.

Egg-Grown Inactivated Influenza Vaccines (IIV-3, IIV-4)

The original influenza vaccine, which consisted of formalin-inactivated whole virions grown in embryonated chicken eggs, was demonstrated to have a protective efficacy of 70% in healthy adults[9] and was licensed in 1945. Since then, although there have been several important advances in the techniques for producing vaccine, the basic vaccine strategy has remained the same. The development of the zonal gradient centrifuge allowed more efficient production and more highly purified vaccines from which reactogenic contaminants had been removed. Treatment of the whole virus with solvents to create "split" vaccines, or with detergents to create "subunit" vaccines, resulted in a vaccine with fewer adverse reactions, particularly fever, than the whole-cell vaccine. The efficiency of vaccine production was also improved through the development of techniques to create reassortant viruses adapted to provide high yield from hens' eggs.[263] Since the late 1970s, egg-grown vaccines have been standardized to contain at least 15 μg of each hemagglutinin (HA) antigen as assessed with single radial immunodiffusion (SRID) using antibody reagents prepared against the components of the vaccine. Embryonated hens' eggs are an extremely efficient substrate for the growth of most influenza viruses, and consequently egg production remains the primary mode of production for current influenza vaccines. However, although eggs are an efficient substrate for vaccine production, the process of adapting to growth in an avian substrate can result in mutations in the hemagglutinin protein that result in significant antigenic differences from the original starting material and adversely affect vaccine performance in unpredictable ways.[264–267]

Cell Culture–Derived Inactivated Vaccine (ccIIV-4)

One strategy to avoid the problems associated with egg adaptation would be to produce the vaccine by propagating the viruses in mammalian cell culture. Currently, a vaccine produced in a qualified line of Madin-Darby canine kidney (MDCK) cells is licensed for use in individuals aged 4 years and older in the United States. Other cell lines such as Vero cells and PerC.6 cells have also been explored for influenza vaccine production.

Recombinant Hemagglutinin Expressed in Insect Cells (rIV-4)

Another alternative is to express the hemagglutinin using an appropriate expression system. The expression of proteins in insect cells using recombinant baculovirus expression vectors can be achieved rapidly and results in proteins with mammalian-like glycosylation. A recombinantly expressed hemagglutinin vaccine is currently licensed in the United States for ages 18 and above. This vaccine uses a higher dose of HA antigen per component (45 μg), although the reagents used for release criteria are different from those used for egg-derived vaccine.

High-Dose Egg-Grown Inactivated Vaccine (HD-IIV3)

It has long been recognized that the immune response to influenza vaccine may be decreased in older adults. The use of higher doses of vaccine may be able to circumvent this problem, and a vaccine that uses four times the usual dose (60 μg per component) has been developed and shown to induce higher levels of antibody and improved protection against H3N2 viruses in older adults. The vaccine is produced in eggs and contains a single B-virus lineage, and is licensed for use in adults aged 65 and older.

MF-59 Adjuvanted Inactivated Influenza Vaccine (aIIV-3)

Another approach to improving vaccine performance is the use of adjuvants, or immune stimulators designed to improve the response to a coadministered antigen. The squalene-based oil-in-water adjuvant MF59 has been shown to increase the immunogenicity of egg-derived influenza vaccine in older adults, and has been licensed in the United States and elsewhere in this age group. MF59 adjuvanted vaccine is also currently being evaluated for potential improved efficacy in children.

Intranasal Live-Attenuated Influenza Vaccine (LAIV-4)

Live-attenuated vaccines have a long track record of success against a wide variety of viral diseases, such as smallpox, measles, polio, and others, in part because they generate a diverse immune response that mimics the immune response to the pathogen. Developing live vaccines for influenza represents a special challenge because of the frequent antigenic changes in the virus. Creation of reproducibly attenuated antigenically variant LAIVs takes advantage of the principle of reassortment to generate attenuated vaccines for new antigenic variants using a vaccine master donor virus (Fig. 165.11). The master donor viruses used in the LAIV currently licensed in the United States are the cold-adapted influenza A/Ann Arbor/6/60 (H2N2) and B/Ann Arbor/1/66 viruses, developed by Dr. John Maassab at the University

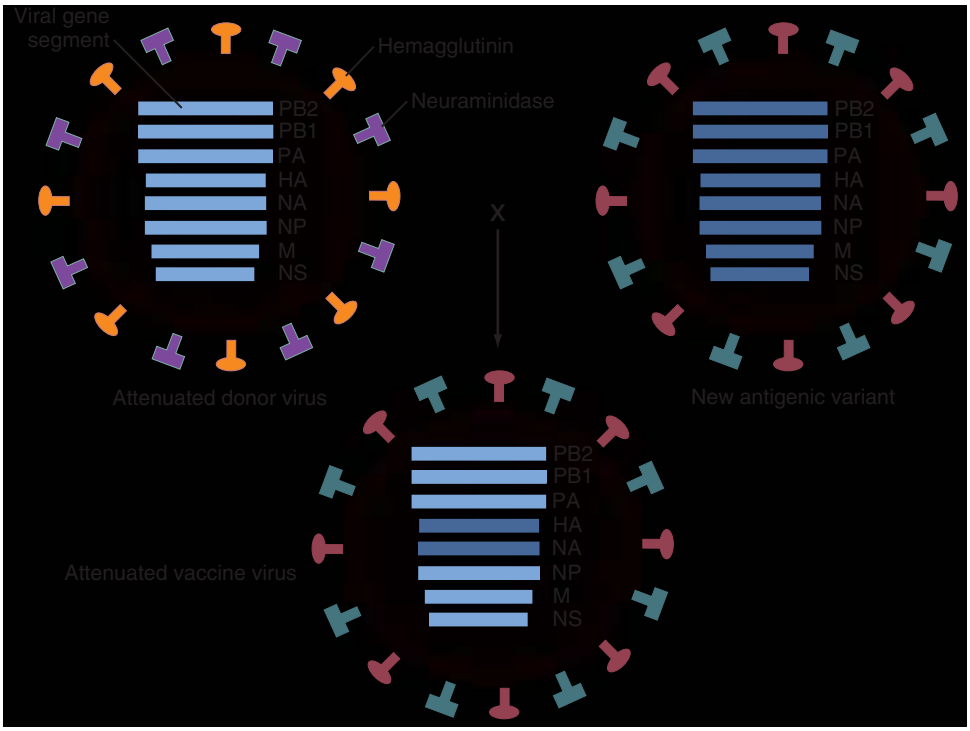

FIG. 165.11 Genetic reassortment is used to generate new live-attenuated vaccine viruses. The genetic basis of attenuation of the "master donor virus" is encoded in gene segments other than hemagglutinin (HA) or neuraminidase (NA). Using either mixed infection in cell culture or reverse genetics techniques, the genes encoding HA and NA of new antigenic variants can be inserted into the background of the master donor virus to rapidly create a new attenuated vaccine virus.

of Michigan in the 1960s.[268] A quadrivalent formulation of these viruses (LAIV-4) is currently licensed in the United States and other countries for use in individuals ages 2 to 49. A second type of LAIV, based on the cold-adapted A/Leningrad/60 and B/Leningrad/66 master donor viruses, is also licensed in some countries.

Safety

Inactivated Vaccines

Hundreds of millions of doses of IIV are administered to adults, elders, and children each year, and the safety of these vaccines has been repeatedly confirmed. For example, no increase in clinically important medically attended events has been noted among over 251,000 children <18 years of age who were enrolled in one of five health maintenance organizations within the Vaccine Safety Datalink, the largest published postlicensure population-based study of vaccine safety.[269] The most common adverse events reported after immunization with IIV are tenderness and/or pain at the injection site. Most injection site reactions are mild and rarely interfere with daily activities. Systemic reactions after immunization of adults with inactivated vaccine are uncommon. In placebo-controlled clinical trials in younger and elderly adults, rates of systemic reactions were similar among groups given inactivated vaccine or placebo.[270,271]

Immediate hypersensitivity reactions (hives, wheezing, angioedema, or anaphylactic shock) after vaccination with inactivated vaccine can also occur, and vaccine is considered contraindicated for persons who experienced a previous anaphylactic reaction following vaccine administration. However, recent studies support the safety of IIV among persons who experience mild allergic reactions to eggs, such as hives.[272] Current recommendations[273] for persons with other forms of egg allergy such as history of angioedema or respiratory distress are that any inactivated vaccine may be used, but vaccine should be administered in a medical setting where emergent severe allergic reactions can be treated.

Guillain-Barré syndrome (GBS), an acute inflammatory demyelinating polyneuropathy, was associated with the 1976 swine influenza vaccination campaign, with an increased risk of approximately 1 per 100,000 vaccinees.[274] Subsequent studies have suggested a statistically significant but very slight increased relative risk of GBS within 7 weeks of influenza vaccination.[275] A meta-analysis of 39 observational studies conducted from 1981 to 2014 confirmed a slightly increased risk of GBS after influenza vaccination.[276]

The oculorespiratory syndrome (ORS) is a syndrome of red eyes, facial edema, and/or respiratory symptoms such as coughing, wheezing, sore throat, hoarseness, difficulty breathing, or chest tightness that develop within 2 to 24 hours after vaccination, associated with a specific influenza vaccine used in Canada, but not elsewhere.[277] The specific mechanism underlying this phenomenon is unknown.

Pregnancy is recognized as a risk factor for more severe influenza, and pregnant women are an important target group for immunization. Most studies of pregnancy outcomes have found no association between vaccination and adverse pregnancy outcomes, although relatively less information is available regarding first trimester vaccination. Influenza vaccine is currently considered safe in all trimesters.[278]

During the response to the 2009 pandemic, monovalent H1N1 inactivated vaccines were administered in several countries adjuvanted with the squalene-based adjuvant AS03. The use of AS03 with pandemic H1N1 vaccine was associated with an increased risk of narcolepsy during vaccine campaigns in Europe. Initially noted in Finland,[279] this association has been established in multiple other locations where AS03-adjuvanted pH1N1 vaccines were used routinely in young children.[280,281] The pathogenesis of this disorder involves presumably immune destruction of neurons in the hypothalamus responsible for synthesis of the neurotransmitter hypocretin.[282] The mechanism that might link the use of influenza vaccine to the development of narcolepsy is unclear. Almost all cases that have been HLA typed are positive for the narcolepsy risk allele DQB1*0602.[283] An increased incidence of narcolepsy was also reported after H1N1 infection in China,[284] suggesting that narcolepsy may be linked in some way to the specific antigen in the vaccine. Narcolepsy after influenza vaccination has not been reported in other countries[285] and it was not reported after use of influenza vaccines containing the similar squalene-based adjuvant MF59.[286] However, the age distribution of adjuvant vaccine use also differed among countries and vaccines, and the role and potential mechanisms of AS03 in narcolepsy remain undetermined.

Live-Attenuated Vaccines

LAIVs based on the cold-adapted A/Ann Arbor and B/Ann Arbor viruses display three characteristic phenotypes: effective replication at low temperatures (25°C), reduced replication at high temperatures (38°C–39°C), and attenuation in a variety of animal models.[287] Multiple mutations in the internal gene segments contribute to these phenotypes.[288] Studies using single gene reassortants demonstrated that at least three of these gene segments (PB1, PB2, and PA) independently participate in attenuation in both animals and human subjects.[289,290] For the influenza B/Ann Arbor virus, the PA, PB2, NP, and M gene segments contribute to the cold-adapted and attenuation phenotypes[291,292] Because both donor viruses are attenuated at multiple sites, it would be predicted that the vaccines should be phenotypically stable even after prolonged replication in seronegative children, and this has been shown in clinical studies.[293]

Cold-adapted LAIVs have been well tolerated in adults, with mild nasal symptoms and sore throat occurring at rates slightly in excess of those in placebo recipients. These vaccines have also been shown to be safe and well tolerated in children, although children younger than 8 years have had slightly increased but variable rates of low-grade fever, runny nose, and abdominal symptoms in the 7 days after vaccination compared with placebo recipients. However, when considering all the pediatric studies in aggregate, no consistent symptom was significantly more common in LAIV recipients compared with placebo recipients. In older children, 11 to younger than than 16 years, sore throat was observed slightly more frequently in LAIV recipients than in recipients of inactivated influenza.

In larger studies, wheezing has been consistently identified as a vaccine-associated side effect in young children, although occurring at low rates. In the largest trial, medically significant wheezing within 42 days of vaccination was reported in 3.8% of children <2 years old after receipt of LAIV compared with 2.1% in those who received IIV.[294] Wheezing generally occurs in the youngest, previously unvaccinated children after the first dose of vaccine. Because of this observation, LAIV is currently approved for use in the United States for children ≥2 years old who do not have a history of asthma.

Safety of LAIV has also been demonstrated in some high-risk patient groups. No significant vaccine-related adverse events were seen in studies of children with cystic fibrosis or asthma, and vaccinated children with asthma did not experience significant changes in FEV_1, use of β-adrenergic rescue medications, or asthma symptom scores compared with placebo recipients.[295] LAIV has also been well tolerated in adults with chronic obstructive airway disease.[296,297] and older adults,[298] although the vaccine is not recommended for use in these populations.

Young children with advanced HIV infection may have difficulty clearing wild-type influenza virus from the respiratory tract, and there have been several reports of very prolonged virus shedding in highly immunosuppressed children with AIDS. However, in small studies in adults[299] and children[300] with HIV, LAIV was well tolerated and not associated with prolonged shedding.

Transmission of LAIV to susceptible contacts does not appear to happen frequently. LAIV can be recovered from nasal secretions of about half of adult recipients, although generally shedding of LAIV by adults is of low titer and short duration.[301] Transmission of LAIV from vaccine recipients to susceptible contacts has been rarely detected in studies of young children involved in daycare-like settings. In the largest study, 197 children between 8 and 36 months of age in a daycare setting were randomized to receive LAIV or placebo, and LAIV was detected in one placebo recipient. The estimate of transmissibility in this age group was 0.6% to 2.0%.[302] However, because of the possibility of transmission, LAIV is not recommended for close contacts of individuals who have levels of immunocompromise that necessitate a protected environment.[273]

Immune Responses to Vaccination

Intramuscular Protein Vaccines

Increases in serum HAI antibody are seen in about 90% of healthy adult recipients of vaccine. Only a single dose of vaccine is required in individuals who were previously vaccinated or who experienced prior infection with a related subtype, but a two-dose schedule is required in unprimed individuals.[303,304] Primed individuals also generally respond with antibody that recognizes a broader range of antigenic variants than do unprimed individuals.[305] Serum antibodies peak between 2 and 4 weeks after vaccination but fall quickly, reaching near baseline before the next influenza season.[306] Despite evidence that NA-specific antibody responses contribute to protection, neither the NA content nor the enzymatic activity of the vaccine is standardized, and NA-specific antibody responses are not routinely assessed. Antibody responses to the NA develop, albeit at a lower rate than those to the HA.

Mucosal HA-specific antibody responses are generally not a major component of the response to parenterally administered inactivated vaccine.[307,308] However, dose-related increases in mucosal HA-specific antibody responses have been observed following IM immunization with increasing doses of a monovalent influenza A/H1N1 vaccine.[309]

HA-specific ASCs peak in peripheral blood peak approximately 7 to 8 days after IIV administration in adults.[310–312] ASCs may be less frequently detected in the blood of children.[237]

Cellular responses, including $CD4^+$ T-cell responses, are also noted after IIV,[313] and there is a strong correlation between the CD4 T-cell response and the antibody response.[314,315] Baseline frequencies of influenza-specific, IFN-γ–producing memory $CD4^+$ T cells are higher in children who received more previous vaccinations.[316] An increase in HA-specific $CD8^+$ T cells on day 7 after vaccination has also been detected by means of tetramer staining in adults receiving IIV.[238] Although the induction of HAI antibody is generally accepted as a correlate of vaccine protection, in some populations such as the elderly it has been suggested that induction of cellular responses may also correlate with protection.[317]

Unimmunized young children generally require two doses of IIV given at least 4 weeks apart to generate substantial serum antibody responses. Once a child has been primed with two doses of vaccine, a single dose of vaccine is recommended in subsequent seasons regardless of age. However, predicting immune responses of young children who have received single doses in two consecutive seasons is more complex. In studies performed in immunologically naïve children, responses among children who received the first dose of vaccine in the spring and the second dose in the fall were similar when compared with those who received both doses in the fall when vaccine antigens did not change.[318] However, in a second study done when the influenza B component changed between seasons, the response in children who received a dose in the spring and then an antigenically different influenza B in the fall was inferior to the response to two doses of the same B antigen in the fall.[319] Whereas most children 6 months of age or older respond to vaccine after receiving the recommended number of doses, antibody responses in infants are reduced compared with older children. Reduced responses among very young children may be related to a combination of immaturity of the immune system and a lower degree of priming.

When compared with younger adults, the proportions of elderly subjects who achieve a putative protective HAI antibody titer are lower. Both age and underlying conditions may contribute to these lowered responses. Unlike in children, the administration of a second dose of vaccine in elders is not associated with improved responses.[320] In some studies, the history of multiple prior vaccinations was better correlated to reduced vaccine responses than was age.[321] Although the dose-response curve for seasonal influenza vaccine is rather flat, the administration of a fourfold higher dose (60 μg/HA) is associated with an improved serum HAI response in elders.[322] High-dose vaccine has also been reported to induce significantly higher frequencies of response and higher levels of antibody to the NA in elders when compared with a standard dose.[323] The high-dose vaccine is licensed in the United States for use in adults older than 65.

The MF59 adjuvant is also associated with improved antibody responses in older adults. Although there is relatively little effect on the immune response to seasonal vaccines in healthy young adults,[324] the oil-in-water emulsion results in an approximately 50% increase in antibody titers in older adults,[325] and MF59 adjuvanted seasonal inactivated vaccines have been licensed for use in elders in Italy for several years. Recently, MF59 adjuvanted standard-dose inactivated vaccine was licensed for elders in the United States based on studies showing noninferiority of the immune response to that of standard-dose inactivated vaccine, although the adjuvanted vaccine was not superior.[326]

In addition, individuals on immunosuppressive therapy, those with renal disease, and transplant recipients have impaired responses to vaccination. To be maximally effective, immunizations should be given before transplantation, should avoid the nadir of white counts, and should include vaccination of close contacts.[327] In HSCT, these have included better responses in autologous compared with allogeneic transplants, the time since transplantation, the use of myeloablative therapy as opposed to reduced intensity, the presence of graft-versus-host host disease,[328] and the specific immunosuppressive chemotherapy being used. Solid organ transplant recipients have similarly decreased immune responses. In addition to the time since transplantation and the general state of immunosuppression, mycophenolate is associated with decreased responsiveness to influenza vaccine,[329,330] and sirolimus may associated with relatively better responses.[331] In both allogeneic hematopoietic stem cell transplant (HSCT) recipients and solid organ transplant types of transplant recipients, there is concern regarding whether the immune stimulus of vaccination, with or without an adjuvant, might nonspecifically stimulate increased severity of graft-versus-host disease or organ rejection. However, the weight of evidence does not support a significant association between vaccination and the development of autoimmune adverse events.

The responsiveness to influenza vaccination in HIV-infected individuals is related to the degree of immunosuppression.[332,333] Most patients with chronic lung disease respond reasonably well to vaccination, and steroids at doses commonly used to treat reactive airway disease do not appear to preclude vaccine responses.[334,335]

Intranasal Live-Attenuated Vaccine

Studies of the immunogenicity of cold-adapted reassortant vaccines have been carried out in children, adults, and older adults. The results of these studies are consistent with the hypothesis that the replication of cold-adapted vaccines in the upper respiratory tract, and hence their immunogenicity, is influenced by the susceptibility of the host at the time of vaccination. The frequency and magnitude of immune responses to vaccination are therefore highest in young children, intermediate in adults, and lowest in older adults who have been repeatedly infected with influenza viruses throughout their lifetime. In addition, the mucosally administered LAIV is generally more effective than parenterally administered IIV at inducing nasal HA-specific IgA, whereas inactivated vaccine usually induces higher serum titers of HAI and HA-specific IgG antibody.[336]

Most susceptible children demonstrate measurable serum and mucosal HA-specific antibody responses. Mucosal responses have been demonstrated in up to 85% of young children after LAIV.[337] In contrast, adults generally have a low rate of serum antibody response after LAIV,[338,339] and relatively lower rates of mucosal responses.[340] Even in those prescreened to have low prevaccination vaccine-specific influenza antibody, the rates of serum antibody responses to intranasal LAIV in adults and older adults are low.[339] Immune responses in elderly subjects are relatively rare.[341,342]

Influenza-specific IgA and IgG ASCs peak on days 7 to 12 after either LAIV or IIV in both adults and older children.[237] In contrast to children, IgG ASCs were significantly higher in adults after IIV than LAIV. Antibody responses were also significantly lower after LAIV than IIV in both adults and children, and in general the development of ASCs seemed to be a more sensitive indicator of vaccine take after LAIV than was antibody response. The levels of prevaccination memory B cells were low in all age groups, but numbers of prevaccination memory B cells were higher in adults than in children. IIV, but not LAIV, increased the numbers of circulating memory B cells at 1 month.

Influenza-specific IFN-γ producing $CD4^+$ and $CD8^+$ lymphocytes have also been detected after LAIV or IIV administration.[343,344] In children 5 to 9 years of age, IIV resulted in increases in the numbers of $CD4^+$ but not in the numbers of $CD8^+$ cells on day 10 after vaccination. There were no real changes in natural killer (NK) cells after IIV administration. In contrast, administration of LAIV resulted in increases in both IFN-γ–producing $CD4^+$ and $CD8^+$ cells, in addition to NK cells. In adults, there were no consistent changes in any subset after either IIV or LAIV, although there was much variability in the responses.

The mechanism of protection induced by cold-adapted vaccine has mostly been evaluated in experimental infection studies. Cold-adapted vaccine is protective in these experiments in the absence of significant serum HAI responses, suggesting that the main protective effect is induction of mucosal antibodies.[234,235] Finally, an analysis of data collected during a large field trial evaluation of LAIV concluded that the postvaccination number of influenza virus–specific IFN-γ–producing T cells was the best correlate of vaccine-induced protection.[243] However, no clear correlate of immune protection afforded by LAIV has been identified.

Markers of Immune Responsiveness

A number of methods have been developed that allow an extensive, unbiased assessment of the state of the immune system, which, combined with sophisticated bioinformatics analysis, has provided the opportunity to assess the genetic and metabolic networks involved in generating an immune response. For example, previous studies of inactivated vaccines suggested that genes in the IFN response pathway are highly upregulated in healthy males who mount the highest serum HAI responses to IIV, in comparison with those who have low or absent responses.[345] In another study done in both males and females, the PBMCs of responders collected on the day after vaccine were enriched for genes involved in cell-mediated and inflammatory responses.[346] Of interest, similar gene expression signatures have been identified as correlates of protective immunity induced by 17D yellow fever vaccine.[347]

Because the correlates of immunity induced by LAIV have not been clearly defined, gene signatures predictive of potentially protective immunity cannot be generated. However, vaccination of adults with seasonal LAIV was associated with upregulation of a variety of IFN-related genes, particularly on day 3 after vaccination,[346] suggesting that vaccine virus replication was required for induction. Upregulation of type 1 IFN responses have also been documented after LAIV in children.[348] Although these signatures may not allow prediction of vaccine efficacy, they may provide more sensitive indications of vaccine take than conventional measures of cell culture isolation and development of serum antibody responses.

Efficacy (Results of Randomized Prospective Studies)

The ability of influenza vaccines to prevent influenza has been assessed in numerous clinical studies that have varied greatly in design, populations, and end points. These studies have included prospective, randomized controlled studies, in which case they are referred to as efficacy studies, and a wide variety of nonrandomized cohort and retrospective studies that have assessed vaccine effectiveness (VE). End points evaluated in these studies have included both laboratory-confirmed influenza and non–laboratory-confirmed respiratory illnesses. In this regard, it has been recognized that studies that use a serologic definition of influenza infection may overestimate the efficacy of influenza vaccine, because it will be harder to demonstrate postvaccination to postseason antibody increases in the vaccinated group.[349]

Intramuscular Protein Vaccines

Randomized studies of inactivated vaccine efficacy against laboratory-confirmed influenza have mostly been conducted in healthy adults. These studies have shown a wide range of efficacy, from approximately 40% to 80%, with lower levels of efficacy typically seen in years with apparent antigenic mismatch. For example, the efficacy of IIV for preventing culture-proven influenza A illness in adults was 76% (95% confidence interval [CI], 58%–87%) for H1N1 and 74% (95% CI, 52%–86%) for H3N2 in a controlled trial comparing live and inactivated vaccines.[350] Vaccination of adults is also associated with decreased absenteeism from work or school and is significantly cost-effective,[29] but these benefits may not be seen in years when there is not a good match between vaccine and circulating viruses.[351]

A meta-analysis of eight randomized, controlled trials in healthy adults during 2004–2008 estimated the pooled efficacy of IIV against culture-confirmed influenza to be 59% (95% CI, 51–67) among those aged 18 through 64 years.[352] The role of antigenic mismatch in the efficacy observed in these trials is unclear, and some studies in young

adults have demonstrated high levels of efficacy (76%) despite a degree of antigenic mismatch. Recent studies using virus culture and/or PCR end points have demonstrated similar levels of efficacy for both egg-grown (78%) and cell culture–grown IIVs (84%).[353] The protective efficacy of the recombinant HA vaccine in healthy adults was approximately 47% in healthy adults in a study done in a single season with significant antigenic mismatch.[354]

Although annual vaccination of elders and other high-risk persons has been recommended for many years, there are few randomized trials demonstrating absolute efficacy in these groups, in part because the existing vaccine recommendations make it difficult to do studies using a placebo group. In the most commonly referenced study, IIV was 52% (95% CI, 29–67) efficacious in preventing serologically documented influenza illness in a population of adults 60 years of age and older.[270] When the groups were further stratified by age, efficacy estimates against serologically documented influenza illness were 57% (95% CI, 33%–72%) in those 60 through 69 years and 23% (95% CI, −51 to 61%) in those ≥70 years old. However, interpretation of this study is complicated by the use of postvaccination to postseason serologic response as definition of infection.

Use of higher doses of vaccine induces a stronger antibody response in older subjects, and in a large randomized study the relative efficacy of high-dose vaccine was 24% compared with standard-dose vaccine,[355] with reductions in small numbers of more severe end points, as well. As described earlier, rHA vaccine is also administered at a higher HA dose than standard-dose egg vaccine, and was also recently shown to have approximately 30% improved efficacy compared with standard-dose egg vaccine during a season with substantial H3N2 vaccine mismatch.[356] Standard-dose inactivated vaccine has been shown to be protective in limited studies in other high-risk groups, including those with HIV infection.[357,358]

Relatively few prospective trials have assessed inactivated vaccine efficacy in children. In one randomized, controlled trial in healthy children aged 6 through 23 months, vaccine efficacy was 66% (95% CI, 34–82) in the first year, but efficacy could not be assessed in the second year owing to a very low influenza attack rate.[359] In an early efficacy study comparing LAIV and IIV over 5 years in children, IIV3 had 77% efficacy against culture-confirmed H3 and 91% efficacy against H1.[360] In a large field trial conducted at multiple international sites in children 3 to 8 years old, IIV-4 was show to have efficacy of 59% in prevention of PCR-confirmed influenza, and 74% efficacy against moderate-to-severe influenza.[361]

Two large studies have evaluated the addition of MF59 to inactivated vaccine in children. In the first randomized placebo-controlled study done in children 6 to 72 months of age, the efficacy of IIV-3 against PCR-confirmed influenza was 43%, and that of the MF59 adjuvanted vaccine was 86%.[362] In a second study done in a population in which most children had previously been vaccinated, MF59 adjuvanted vaccine efficacy was similar to that of unadjuvanted vaccine in the entire study population, but was 31% better than standard vaccine in the subpopulation 6 to 23 months of age,[363] consistent with observations of greater adjuvant effect in immunologically naïve populations.

Intranasal Live Vaccines

LAIV was demonstrated to be efficacious in the prevention of influenza in a 2-year, randomized, placebo-controlled trial conducted in 1314 children 15 to 74 months of age. Efficacy against culture-confirmed influenza illness in the first year of this trial was 95% against influenza A/H3N2 and 91% against influenza B.[364] In the second year of the trial, the H3 component of the vaccine (A/Wuhan/93) was not a close match with the predominant H3 virus that season, A/Sydney/95. However, the efficacy of LAIV against this variant was 86% (95% CI, 75%–92%),[365] suggesting that LAIV can induce protective immunity against drift variants. Overall, the efficacy of LAIV to prevent any influenza illness during the 2-year period of surveillance in this field study was 92% (95% CI, 88%–94%). The overall efficacy of LAIV against culture-confirmed influenza among children 6 to <36 months who were attending daycare was shown to be 85% and 89% in the first and second years of the study, respectively.[366] Significant protection against flu-associated acute otitis media also was demonstrated (>90% in both years). Studies done in Asia have reached similar conclusions, with an efficacy of LAIV compared with placebo of between 64% and 84% over multiple seasons, depending on the antigenic match with the vaccine.[367]

Relatively few placebo-controlled trials of the efficacy of LAIV have been conducted in adults. In the human challenge model, LAIVs and IIVs were of approximately equal efficacy in prevention of experimentally induced influenza A (H1N1), A (H3N2), and B. The combined efficacy in preventing laboratory-documented influenza illness due to the three wild-type influenza strains was 85% for LAIV.[339] In a randomized, controlled study in healthy persons aged 1 through 64 years, of whom most of the participants were adults, the efficacy of a prelicensure, bivalent preparation of LAIV for preventing culture-confirmed influenza A illness in adults was 85% (95% CI, 70%–92%) for H1N1 and 58% (95% CI, 29%–75%) for H3N2.[350] LAIV was also evaluated in a large study against clinical end points performed in 4561 healthy working adults.[368] In this study, the effectiveness of LAIV in preventing severe febrile respiratory illness of any cause during the influenza season was 29%.

In a randomized, double-blind, placebo-controlled clinical trial of LAIV among community-dwelling ambulatory adults ≥65 years old, the overall efficacy of LAIV against viruses that were antigenically similar to the vaccine was 42%.[369] However, LAIV is not currently approved for use in adults older than 49.

Effectiveness (Results of Observational Studies)

Many recent studies have used a test-negative, case-control design, in which individuals meeting a particular case definition are tested for influenza with a highly sensitive and specific diagnostic test, and the vaccination exposure of test-positive cases and test-negative controls is determined.[370] Large surveillance networks for this purpose have been established in Canada, the United States, Europe, and Australia for purposes of making interim and end-of-season estimates of VE. Studies using this design have shown variable results, with estimates generally ranging from as low as 20%, or in some cases, no effectiveness, to as high as 60% to 70%. Although the various networks vary in their study design and the specific selection criteria for subject inclusion, a few overall generalizations can be stated. Failure to detect VE has typically occurred in studies with very low prevalence of influenza in the study population, or in years with substantial antigenic mismatch between the vaccine and circulating strains; but even in situations of antigenically matched viruses, VE remains in the 50% to 60% range.[371] In some cases, these viruses have been shown to have substantial changes on an HA sequence level despite appearing well matched according to traditional HAI tests.[372]

Most studies have not enrolled enough subjects in a single season to make age-specific estimates of VE. However, there is a trend toward decreased VE in elderly, not surprising given their diminished immune response to vaccination. After accumulating cases over several seasons, it is possible to use the same test-negative case-control design to demonstrate VE of approximately 60% against influenza-related hospitalization in a population of community dwelling older adults.[373] Among hospitalized patients, vaccinated individuals have lower rates of ICU admission and in-hospital deaths.[374]

Observational studies to compare different types of vaccine are dependent on market share, so complete information is not always available. In a cohort study based on Medicare beneficiary data in the United States, the use of high-dose vaccine was associated with a significantly greater reduction in influenza-related deaths among seniors in a year in which H3N2 viruses predominated, but not in a year of primarily pH1N1 virus.[375] In a case-control study among elderly individuals in Canada, MF-59 adjuvanted vaccine also appeared to be more effective than standard-dose unadjuvanted vaccine.[376]

Although the use of a study design in which testing is performed without knowledge of vaccination status may eliminate some biases related to health care access and health-seeking behavior, the results are influenced by the accuracy of the diagnostic testing, because errors in assignment to the case or control group will bias VE toward nil.[377] In a study done in children, it was demonstrated that with use of the test-negative case-control approach, estimates of VE were substantially

higher when children with documented infections with viruses other than influenza were used as a control group, rather than all children who were test-negative for influenza.[378]

Effects of Prior Vaccination

Studies of influenza vaccine effectiveness using the test-negative design, in addition to other study designs, have suggested that vaccine effectiveness is decreased in those who are yearly vaccine recipients, compared with those who receive vaccine for the first time in a given year. These findings would be consistent with very early observations made in a boys' boarding school, referred to as the "Hoskins effect." However, not all studies have shown a negative effect of prior vaccination. The effects seem to be greatest when the current season and the previous season vaccine components are similar to each other, but the circulating virus is a mismatch.[379]

The immunologic mechanisms that might be responsible for the negative effects of prior vaccination are not known. Individuals with higher prevaccination antibody levels tend to have lower-magnitude responses to vaccination, although still typically achieving very high levels of antibody. Prior vaccination might result in memory responses that preferentially recognize shared epitopes and not new epitopes that are present in antigenically drifted viruses, representing a form of back-boosting.[380]

Because at least one component of the vaccine changes each year, there is not a practical solution to influenza control using current vaccine strategies other than annual vaccination. Further research to understand the magnitude and mechanisms of putative negative effects of prior vaccination will clearly be important in improving influenza control.

Comparisons of Live and Inactivated Vaccines

Although relatively few randomized direct comparisons of the efficacy of live and inactivated vaccines have been performed, the available studies are consistent with the observed effects of age and prior influenza experience on immunogenicity. When these vaccines have been compared in young children 12 months through 59 months of age, LAIV has shown consistently superior protection, with an approximately 50% greater protective efficacy than inactivated vaccine.[294,381]

In contrast, studies that directly compared the vaccines in adults have suggested that the vaccines have similar efficacy, or that IIV vaccine is slightly more efficacious than live vaccine. In one three-armed study, the efficacy of LAIV compared with placebo for prevention of laboratory-confirmed influenza in healthy adults was 57%, whereas the efficacy of the IIV was 77%, but the difference between the two vaccines was not statistically significant.[382] In a subsequent season in the same population, the absolute efficacies of IIV and LAIV were 68% and 36%, respectively.[383] In an effectiveness assessment in the US military, the effectiveness of IIV against medical visits for pneumonia or other influenza related diagnoses was higher than that of LAIV, except for personnel who had not been vaccinated in previous years.[384]

When the H1N1 component of LAIV was replaced by a virus with the HA and NA of an A/California/09 virus after the pandemic, there was a substantial decrease in effectiveness of LAIV, particularly against H1N1.[385] This poor effectiveness was repeatedly demonstrated in the United States, and to a lesser extent in other countries. The reasons for the diminished effectiveness of this formulation are not completely known but may be related to the replication fitness of the specific H1N1 virus, increasing levels of background immunity in the vaccinated population, or other factors. However, as a result, the use of LAIV in the United States was not recommended by the Advisory Committee on Immunization Practices (ACIP) for several years. Subsequently, the H1N1 component has been updated with a virus (A/Slovenia) that appears to generate stronger immune responses, and use of LAIV has resumed. No information is available regarding the efficacy or effectiveness of this new formulation at this time.

Secondary Protection

In general, individuals at the highest risk for influenza complications may also be compromised in their ability to respond to vaccination. There has been considerable interest in potential strategies to protect such individuals indirectly by preventing illness and viral transmission within highly susceptible populations that probably play a role in community transmission, such as schoolchildren. Several studies have suggested that this may be possible. During the 1968 pandemic, it was observed that the incidence of respiratory illness during the period of influenza A circulation among unvaccinated adults was substantially lower in a community in which schoolchildren were immunized than in a comparison community with no school immunization. Influenza B was not contained in the vaccine, and during a subsequent influenza B epidemic there was no difference in adjusted respiratory illness rates in adults in the two communities.[386] In a recent study, closed agricultural communities of Hutterites were randomized to vaccination of schoolchildren with influenza vaccine, or with hepatitis A vaccine as a control. In the subsequent influenza A epidemic, the rate of laboratory-documented influenza A in unvaccinated adults residing in school-vaccinated communities was reduced by 61% (95% CI, 8%–83%) compared with adults in unvaccinated communities.[387] Vaccination of children in daycare has been reported to reduce the rates of febrile respiratory illnesses in unvaccinated household contacts.[388] Observations in Japan, where it appeared that substantial fluctuations in overall influenza-related mortality (occurring mostly in the elderly) were directly related to the rate of influenza vaccination in school-aged children, also support a potential role for school vaccination in protection of elders.[389] A retrospective study conducted in Japan demonstrated that universal vaccination of schoolchildren reduced the number of class cancellation days and absenteeism when compared with years during which the immunization program was abandoned.[390]

A critical target group for vaccination is health care workers.[391] At a minimum, universal vaccination of heath care workers will reduce workplace absences and prevent disruptions in care.[392] In addition, there is supportive evidence that vaccination of health care workers reduces the risk of nosocomial influenza in hospitals.[393] In nursing homes, vaccination of staff reduces mortality in residents independently of the vaccination status of the residents themselves.[394,395]

Maternal Immunization

Infants younger than 6 months are at substantial risk for influenza-related morbidity but are too young to receive influenza vaccine. One strategy to protect vulnerable infants is maternal immunization, with protection mediated both by transfer of maternal antibody as by reduced potential for contact with an influenza-infected mother. In multiple randomized studies of maternal immunization, infants born to mothers immunized with influenza vaccine have had substantially lower rates of laboratory-documented influenza in the first 6 months of life than did infants born to mothers immunized with control vaccine.[396–398] Similarly, in a retrospective case-control study, the frequency of influenza immunization was substantially lower in the mothers of infants hospitalized with PCR-confirmed influenza than in mothers of hospitalized infants who were PCR negative, with an estimated protective effect of 92%.[399]

Recommendations for Vaccine Use

Current US recommendations are for routine annual influenza vaccination of all individuals aged 6 months and older. Ideally, vaccine should be administered by October, but later vaccination can still be beneficial if the seasonal epidemic has not yet occurred. Recommendations for influenza vaccine in the United States are updated annually by the CDC. (Also see Chapter 316.)

Vaccines for Pandemic Influenza

As described earlier, there have been multiple observations of human infection with avian or swine influenza viruses with novel HA and/or NA subtypes, and there continues to be concern regarding the potential of such viruses to acquire the ability to transmit efficiently from person to person and result in the next human influenza pandemic. In addition to active ongoing surveillance, there have been substantial efforts to prepare for such a pandemic by developing potential pandemic vaccines in advance. Because seasonal vaccines are licensed for use in all age groups and have substantial evidence of safety and efficacy, pandemic preparedness efforts have generally focused on development of pandemic formulations of seasonal vaccines, including inactivated and live varieties. Several of these have undergone advanced clinical development and

have been licensed or approved for emergency use based on a favorable safety and immunogenicity profile.

A large number of inactivated H5 and H7 vaccines have been developed and tested in humans. These vaccines have included egg-derived, cell culture–grown, and recombinant HA vaccines similar to the approaches taken for seasonal influenza. Extensive clinical trials have generally shown that these vaccines are well tolerated, but when administered without an adjuvant, poorly immunogenic. Generation of potentially protective immunity, as measured by serum HAI and neutralizing antibody responses, requires a two-dose schedule and the use of doses substantially higher than those used for seasonal vaccines.[400–402] When responses are seen, they are typically limited in breadth and predominantly focused on the homologous antigen.[403] Limited studies of whole-virion vaccines have suggested slight improvements in immunogenicity.[404]

Oil-in-water emulsions such as MF59, AS03, and stable emulsion (SE) have allowed much lower doses of pandemic vaccines to be used successfully.[405,406] With these adjuvants, substantial immune responses have been seen at doses as low as 3.75 μg of antigen, compared with the need to use 45 μg or 90 μg of unadjuvanted vaccine, allowing for the generation of a much larger supply of vaccine in the event of a pandemic. In addition, responses after adjuvanted vaccine are broader, with higher titers of antibody recognizing antigenic variants of the vaccine subtype.[407,408] A number of other adjuvants have also been tested with pandemic vaccines and also show effective improvement in response. Limited studies have suggested that the use of an adjuvant is much more important with the first dose of vaccine.[409] However, even with adjuvants, inactivated pandemic vaccination will likely require a two-dose schedule, imposing significant operational challenges to a pandemic response.

Despite the relatively poor immunogenicity of these vaccines, multiple studies have shown that even unadjuvanted pandemic vaccines may induced long-lasting immune memory, demonstrated by vigorous responses to subsequent booster doses many years later.[410,411] Such responses can be demonstrated even in subjects who did not respond to the primary series. Markers of immune priming have not been identified in these studies.

Live-attenuated pandemic vaccines have also been evaluated in clinical trials. Evaluation of pandemic live vaccines is complicated by the concerns regarding potential transmission of the vaccine virus, possible reassortment with cocirculating human influenza viruses, and potential generation of virulent human viruses with novel HA or NA surface proteins. Therefore, these studies have been conducted under isolation conditions to prevent possible transmission outside the study, and consequently have been somewhat limited in size.

Surprisingly, all of these studies have shown very limited replication of the pandemic vaccine viruses on either the A/Ann Arbor or the A/Leningrad backbone.[412,413] Vaccine virus shedding occurs in a minority of susceptible recipients, and when it is detected, is almost invariably of limited magnitude and short duration. Because immune correlates of protection afforded by seasonal live vaccines have not been determined, it is not possible to draw definitive conclusions regarding the relevant immunogenicity of pandemic formulations of these vaccines. However, measurable serum or mucosal immune responses occur rarely.

Similar to the findings with inactivated vaccines, pandemic formulations of LAIV appear to induce long-lasting immune memory despite the lack of measureable immune response to the initial immunization.[414–416] These observations have caused some to suggest that the most effective use of vaccines for pandemic control would be prepandemic vaccination,[417] in which the population would be vaccinated with a prototypic H5, H7, or other threat subtype prior to a pandemic, and then boosted with an antigenically matched virus should a pandemic occur.

Strategies for More Broadly Protective Vaccines

For a variety of reasons previously outlined, there is considerable interest in the development of influenza vaccine approaches that could generate more broadly protective immunity, potentially providing protection against multiple antigenic variants within a subtype, or possibly even providing protection against multiple subtypes. Several approaches have emerged in pursuit of this goal.

In contrast to the globular head of the HA, the stalk, or HA2 region, remains relatively conserved from season to season. Stalk-directed antibody or stalk-reactive B cells are seen most frequently when individuals are exposed to a novel influenza virus, such as after infection with H5N1[214] or pH1N1 viruses in 2009.[215] Stalk-specific antibodies are capable of mediating virus neutralization by inhibiting HA mediated fusion.[418] In addition, these antibodies can mediate antibody-dependent cellular cytotoxicity. Therefore development of vaccines to induce stalk antibodies is one of the prime strategies for more broadly protective vaccines.[218] Both chimeric approaches, wherein several doses of a vaccine in which the HA1 domain is derived from a novel subtype and the stalk remains the same,[419,420] and stalk-only constructs[421–423] are in development.

The influenza NA has also been explored as a potential target for more broadly protective vaccines. The rate at which mutations accumulate in the NA appears to be less than that in the HA, [424] suggesting that vaccines that induced substantial NA specific immunity would continue to provide protection against drifted viruses, and would need updating less often than HA-centric vaccines.

The third envelope protein, M2, has also been identified as a potential target because the extracellular domain (M2e) is fairly well conserved among human influenza A viruses. The mechanism of protection by M2e antibodies also involves antibody-dependent cellular cytotoxicity in M2e antibody-mediated protection.[227] Multiple platforms have been used to induce antibody specific for M2e, which have resulted in variable levels of M2e antibodies in humans.[425]

Vaccines designed primarily to elicit cellular antibodies often contain mixtures of peptides computationally identified as cellular target epitopes. Immunization of humans with a mixture of linear peptides has been reported to induce cellular immunity and prime for subsequent vaccine responses.[426] Such epitopes could be delivered by mixtures of peptides, or by live viral vectors, such as vaccinia.[427,428] Virus-like particles,[429] in addition to gamma-irradiated whole virus,[430] have also been evaluated for their ability to induce cellular immune responses.

ANTIVIRAL AGENTS (Also See Chapter 45)

Five antiviral drugs in two classes are currently available for the prevention and treatment of influenza, and several more are in the advanced stages of development. A comparison of the basic pharmacology and antiviral activity of the available agents is given in Table 165.8, and they are described in detail later. It is important to recognize that individuals with an intact immune system who have had previous influenza infections rapidly limit the replication of these viruses. Therefore the opportunity to affect viral replication with antiviral agents is limited, and effective use of these agents requires early initiation of therapy.

M2 Inhibitors: Amantadine and Rimantadine

Mechanism of Action and Activity

Currently, all circulating influenza viruses are resistant to the M2 inhibitors amantadine and rimantadine. However, they are described briefly because changes in susceptibility are unpredictable and they may become useful in the future. The M2 inhibitors amantadine and rimantadine are related primary symmetrical amines and are active against influenza A virus, but not influenza B or C virus, in a variety of cell culture systems and animal models. The antiviral activity of these drugs is the result of inhibition of the M2 ion channel activity of susceptible viruses. The function of the M2 ion channel in viral replication is to acidify the interior of the virion, allowing the ribonucleoproteins to be transported to the nucleus.[431] Thus, the antiviral effect primarily manifests in cell culture as inhibition of virus uncoating. Currently available M2 inhibitors are active against only influenza A virus.

Pharmacology and Side Effects

Although the mechanism of action and spectrum of activity for amantadine are similar to those for rimantadine, there are important pharmacokinetic differences between the two drugs. Amantadine does not undergo metabolic change and is excreted unchanged in the urine

TABLE 165.8 Antiviral Agents for Influenza

	AMANTADINE[a]	RIMANTADINE[a]	ZANAMIVIR	OSELTAMIVIR	PERAMIVIR	BALOXAVIR
Protein target	M2	M2	Neuraminidase	Neuraminidase	Neuraminidase	Cap-dependent endonuclease
Activity	A only	A only	A and B	A and B	A and B	A and B
Side effects	CNS (13%) GI (3%)	GI (6%) GI (3%)	Bronchospasm	GI (9%)	GI (8%)	Diarrhea, bronchitis
Metabolism	None	Multiple (hepatic)	None	Hepatic	None	UGT1A3, CYP3A4
Excretion	Renal	Renal + others	Renal	Renal (tubular secretion)	Renal	Fecal (80%): urine (15%)
Drug interactions	Antihistamines, anticholinergics	None	None	Probenecid (increased levels of oseltamivir)	—	Antacids and laxatives reduce concentration
Dose adjustments needed	≥65 yr old CrCl <50 mL/min	≥65 yr old CrCl <10 mL/min	None	CrCl <30 mL/min Severe liver dysfunction	CrCl <30 mL/min	—
Contraindications	Acute-angle glaucoma	Severe liver dysfunction	Underlying airway disease			—
FDA-Approved Indications						
Therapy	Adults and children aged ≥1 yr	Adults only	Adults and children aged ≥7 yr	Adults and children aged ≥2 wk	Adults who cannot take oral or inhaled medications	Adults and children ≥12 yr of age[b]
Prophylaxis	Yes	Yes	Adults and children aged ≥5 yr	Adults and children aged ≥1 yr	—	—

[a]Currently circulating influenza viruses are uniformly resistant to amantadine and rimantadine.
[b]Approved for acute, uncomplicated influenza of ≤48-hr duration.
CNS, Central nervous system; *CrCl,* creatinine clearance; *FDA,* US Food and Drug Administration; *GI,* gastrointestinal.

with a half-life of 12 to 18 hours. Rimantadine undergoes extensive metabolism, and less than 15% of the drug is excreted in the urine unchanged.

The most common side effects of amantadine are minor and reversible central nervous system (CNS) side effects such as insomnia, dizziness, and difficulty in concentrating. These side effects may be more troublesome in older adults. Rimantadine is associated with less CNS effect, and in comparative studies of long-term administration the rate of CNS side effects was not significantly different from the rate with placebo.[432]

Efficacy

Both amantadine and rimantadine are effective in the therapy of experimentally induced and naturally occurring influenza A. Amantadine treatment within the first 48 hours of illness is associated with decreases in the duration of fever and more rapid resolution of clinical symptoms, and more rapid improvement in small airway dysfunction in healthy adults with uncomplicated influenza. Studies of rimantadine therapy of acute influenza in otherwise healthy adults with uncomplicated influenza have shown levels of benefit essentially identical to those seen with amantadine.[433,434] Rimantadine has also been evaluated in the treatment of influenza A in children, with variable effects on clinical symptom scores; one study showed a decrease in scores and fever compared with acetaminophen,[435] and the other showed no significant difference.[436]

Drug Resistance

Drug resistance viruses emerge frequently in treated individuals, particularly children, in whom subpopulations of resistant virus can be detected after treatment in virtually all cases.[437] Resistance is the result of single point mutations in the membrane-spanning region of the M2 protein, and it confers complete cross-resistance between amantadine and rimantadine.[438] Resistant virus can be transmitted to, and can cause disease in, susceptible contacts. Prolonged shedding of resistant viruses may occur in immunocompromised patients, particularly children, and may continue even after therapy is terminated,[439] consistent with the relative fitness of these resistant viruses. Although previously rare, a rapid increase in the prevalence of de novo resistance to M2 inhibitors was noted in 2005,[440,441] and the emerging pH1N1 viruses are also completely resistant to the M2 inhibitors.

Neuraminidase Inhibitors: Zanamivir, Oseltamivir, and Peramivir

Mechanism of Action and Activity

The NIs act by inhibiting the functioning of the influenza virus NA. This enzyme cleaves terminal sialic acid from sialic acid–containing glycoproteins that serve as host cell receptors for attachment of influenza viruses. As virus replication proceeds within the cell, NA is synthesized and transported to the cell surface, where it removes the sialic acid from these cell surface glycoproteins. Destruction of these receptors by NA is critical in allowing newly formed viruses to subsequently egress from the cell and spread to other cells. Studies with mutant, NA-deficient viruses have shown that in the absence of a functional NA, virus remains attached to the host cell and to other virions.[442] In addition, NA may be important in facilitating the penetration of virus through secretions in the respiratory tract, which are rich in sialic acid–containing macromolecules.[443]

NIs are active against influenza viruses at millimolar concentrations or less. Activity against clinical isolates assessed in plaque inhibition tests ranges from concentrations of 0.01 to 16 μM. Influenza B viruses are approximately 10-fold less sensitive than influenza A viruses, but they are still sensitive well within clinically achievable concentrations. Among the influenza viruses sensitive to NIs are avian viruses with all nine known NA subtypes.

Pharmacology and Side Effects

Although all three drugs have identical mechanisms of action and similar profiles of antiviral activity, they have different pharmacologic properties. Oseltamivir is rapidly absorbed from the gastrointestinal tract and is converted in the liver by hepatic esterases to the active metabolite, oseltamivir carboxylate. The metabolite is excreted unchanged in the urine by means of tubular secretion, with a serum half-life of 6 to 10 hours. Administration of the drug with food may improve tolerability without affecting drug levels. Zanamivir is not bioavailable by the oral route and must be administered topically to be effective. The drug is supplied in blister packs in which each blister contains 5 mg of zanamivir and 20 mg of lactose carrier. The standard dose is therefore two inhalations twice a day. Peramivir was initially studied as an orally active agent, but pharmacodynamic evaluation determined that oral absorption was not sufficient for this mode of administration.[444] The drug is now

licensed as an intravenous, long-acting formulation that can be administered as a single dose.[445]

The major adverse effects reported for oseltamivir have been gastrointestinal upset, probably irritation due to rapid release of the drug in the stomach. Rates of nausea can be substantially reduced if the drug is taken with food. The most commonly reported adverse effects in individuals treated with zanamivir have been diarrhea, nausea, and nasal signs and symptoms, which have occurred at essentially the same rate as in placebo recipients. Peramivir has a similar safety profile in studies conducted to date. In one study in which zanamivir was used in influenza-infected patients with asthma or chronic obstructive pulmonary disease, the frequency of significant changes in FEV_1 or peak flow rates was higher in zanamivir than in placebo recipients. For this reason, individuals with these pulmonary conditions should have ready access to a rapidly acting bronchodilator when using zanamivir, in the event that the drug precipitates bronchospasm.

The dose of oseltamivir should be reduced to 75 mg once daily in individuals with renal impairment (i.e., with creatinine clearance of less than 30 mL/min). No data are available regarding the use of the drug in individuals with more significant levels of renal impairment. Likewise, no information is available regarding the use of oseltamivir in individuals with hepatic impairment. Clinically significant drug interactions have not been reported. Because oseltamivir is eliminated by means of tubular secretion, probenecid increases serum levels of the active metabolite approximately twofold. However, dosage adjustments are not necessary in individuals taking probenecid. Dosage reductions are also recommended in renal impairment for peramivir.

Although significant increases in the serum half-life of zanamivir are seen in the presence of renal failure, the small amounts of the drug that are absorbed systemically suggest that dosage adjustments would not be necessary. Studies of the pharmacokinetics of the drug in the presence of impaired hepatic function have not been reported.

Efficacy

In studies of naturally occurring, uncomplicated influenza in healthy adults, therapy with oseltamivir initiated within the first 36 hours of symptoms resulted in 30% to 40% reductions in the duration of symptoms and severity of illness and reduced rates of prolonged coughing.[446,447] In addition, early therapy is associated with a significantly earlier return to work or other normal activities. Similarly, in healthy adults, early therapy of uncomplicated influenza A or B with inhaled zanamivir has been shown to result in a reduction of approximately 0.8 to 1.5 days in the duration of influenza symptoms, and an earlier return to normal activities.[448,449] A study of a single dose of intravenous peramivir in uncomplicated influenza in otherwise healthy adults demonstrated an approximately 21-hour decrease in the duration of symptoms[450] However, in a study of hospitalized adults, peramivir offered no benefit over standard of care.[451]

Both oseltamivir and zanamivir have been evaluated as therapy for children. Administration of oseltamivir liquid at a dose of 2 mg/kg per dose twice daily for 5 days was well tolerated and resulted in a 36-hour reduction in the duration of symptoms in children with influenza A.[452] In addition, the use of oseltamivir was associated with a 44% reduction in the frequency of otitis media complicating influenza, and with reductions in antibiotic prescriptions in influenza-infected children. Similarly, therapy of children 5 to 12 years old with symptomatic influenza A and B virus infection who were treated within 36 hours with inhaled zanamivir (10 mg twice a day) resulted in relief of symptoms 1.25 days earlier than in placebo recipients, and a more rapid return to normal activities.[453]

NI therapy of influenza in adults with risk factors for influenza complications has not been evaluated extensively. However, both drugs have shown trends toward efficacy in such populations.[449,454] The results of meta-analyses of the pooled data from phase III studies have indicated that early treatment with inhaled zanamivir is associated with a median reduction of illness of 2.5 days in older adult and high-risk subjects, and a 3-day earlier return to normal activities.[455]

Initial placebo-controlled trials of NI therapy conducted primarily in otherwise healthy adults did not capture substantial numbers of complications. However, pooled analyses of these studies of early therapy with zanamivir[455] and oseltamivir[456,457] demonstrated a significant reduction in the rate of influenza complications in treated individuals. The subsequent experience in the emerging epidemic of pH1N1 virus has also suggested a beneficial effect of early therapy on complications. These include observations in hospitalized patients[458,459] and surveillance data suggesting that therapy as late as 5 days improved survival of hospitalized patients.[460] Surveillance data have also suggested that treated children had lower rates of complications.[461]

Drug Resistance

Analysis of drug-resistant viruses has revealed two basic mechanisms of resistance that illustrate the interactive roles of the viral HA and NA in binding to and release from infected cells. Mutations within the catalytic framework of the NA that abolish binding of the drugs are the most common drug resistance mutations.[462,463] The mutations conferring resistance are dependent on the specific NA; that is, common resistance mutations in N1 (e.g., H274Y) are different from the ones seen in the N2 (e.g., R292K or E119V) or influenza B (e.g., D198N). In addition, depending on the mutation, these viruses may be specifically resistant to only one inhibitor.[464]

A second type of mutation associated with cell-cultured resistant viruses involves mutations in the receptor binding region of the hemagglutinin. HA mutations associated with resistance to NIs reduce the affinity of the HA for its receptor, allowing cell-to-cell spread of virus in the absence of NA activity.[462,465] Inhibitor resistance due to HA mutations has not been identified as a clinically significant issue to date, but does illustrate the importance of balance between HA and NA activities in viral replication.

It is even possible to generate inhibitor-dependent viruses, in which the affinity for the receptor is apparently so low that NA activity must be inhibited to allow the virus to bind at all. Resistant viruses with HA mutations exhibit cross-resistance to these drugs in cell culture but may retain susceptibility in animal models. Many of these viruses also exhibit reduced virulence in animals.

Drug-resistant viruses were isolated infrequently from oseltamivir-treated individuals in clinical trials, being seen in less than 2% of treated adults and detected in 5.6% of children.[452] Subsequently, it was demonstrated that resistant viruses could be detected in up to 18% of treated children with use of sensitive PCR techniques to pick up minor subpopulations.[466] However, development of resistance has not generally been associated with more severe illness or failure of therapy. Antiviral therapy in highly immunocompromised individuals who may have difficulty clearing virus is frequently associated with development of resistance.

Some resistant viruses appear to have reduced fitness, with reduced levels of replication, attenuation in animals, and reduced ability to be transmitted from animal to animal.[467–470] Therefore, there had been hope that resistance would not be the same limiting issue with NA inhibitors as it has been with the M2 inhibitors. However, other resistance mutations, particularly the H274Y mutation, do not affect the fitness of the virus or transmission in animal models.[471] Beginning in 2006, spontaneously resistant H1N1 viruses carrying the H274Y mutation began to be detected in viruses from individuals who did not have a history of exposure to oseltamivir.[472] By 2008, all H1N1 viruses isolated in the United States were resistant to oseltamivir.

The emerging pH1N1 viruses, which replaced previously circulating H1N1 viruses, have largely remained sensitive to NIs to date.[473] Localized outbreaks of pH1N1 viruses with the H274Y resistance mutation have been reported[474] and these viruses clearly have the capacity to spread and become dominant just as their predecessor seasonal H1N1 viruses did. However, current worldwide surveillance indicates that the majority of seasonal influenza A and B viruses remain sensitive to NIs.

Antiviral Agents in Development

The novel antiviral drug pimodivir is a nonnucleoside inhibitor of the endonuclease cap-stealing function of the PB2 polymerase, and is therefore active against viruses resistant to adamantanes or NIs. However,

this drug is active against only influenza A viruses. In the challenge model, administration of pimodivir for 5 days beginning 24 hours after challenge with A/Wisconsin/05 (H3N2) virus resulted in reduced viral shedding and clinical symptom scores.[475] In a subsequent trial in uncomplicated influenza, pimodivir reduced viral shedding compared with placebo but did not reduce symptoms.[476] Baloxavir marboxil (Xofluza) is a cap-dependent endonuclease inhibitor of influenza A and B viruses. It has been approved in Japan (December 2017) and the United States (October 2018) as a single-dose treatment for uncomplicated influenza in adults and children ≥12 years of age.[476a] Controlled trials of baloxavir in naturally occurring influenza A (H3N2) infection in outpatients showed alleviation of symptoms and a reduction in viral shedding within 24 hours of administration.[476b] (Also see Chapter 45.)

Other agents in development include the long-acting topically applied NI laninamivir,[477] the broadly acting polymerase inhibitor favipiravir,[478] and nitazoxanide,[479] an inhibitor of HA maturation that also affects the host response. (Also see Chapter 45.)

The combination of amantadine, ribavirin, and oseltamivir has been assessed in the treatment of influenza based on encouraging preclinical results suggesting synergistic effects even against viruses resistant to amantadine.[480] Uncontrolled observations in humans had also suggested potentially improved effectiveness compared with oseltamivir alone.[481] However, a large prospective randomized trial showed no clinical benefit of combined therapy compared with oseltamivir monotherapy, although there was slightly faster viral clearance in the group receiving combined therapy.[482] Because of concerns regarding development of resistance, combination therapy may become a very important approach as additional effective antiviral agents with new mechanisms of action become available.

Recommendations for Therapeutic Use of Antivirals

There are several general features that guide the use of antivirals for influenza. First, the bulk of viral replication in persons with normal immune systems takes place very early in the course of illness. Not surprisingly, repeated studies have demonstrated that the effectiveness of therapy primarily depends on the rapidity with which treatment is initiated. However, in persons with severe illness or prolonged viral replication, even late therapy can occasionally be of benefit. Second, most healthy persons with uncomplicated influenza will recover completely without therapy. Thus, therapy is not necessarily universally indicated in all persons with influenza, but can be lifesaving in some persons. Third, resistance to antivirals has been observed frequently with almost all influenza antivirals, and the potential for generation of resistant viruses is an important component of therapy recommendations.

Guidelines for the therapy of influenza have been published[261] and are frequently updated as new information regarding susceptibility and the availability of new agents becomes available. Recommendations are also available from multiple online sources, particularly the CDC influenza website (http://www.cdc.gov/flu). In general, therapy should be started as soon as possible, and should be primarily used in individuals with severe influenza or who have a higher risk for influenza complications (see Table 165.6). Diagnostic tests for influenza, particularly nucleic acid tests such as PCR assays, are increasing in availability and sensitivity. However, in select situations, treatment based on a high degree of clinical suspicion may be appropriate.

There is general agreement that persons who are hospitalized with laboratory-documented influenza, or in whom the degree of clinical suspicion of influenza is high, should receive antiviral therapy. Although the greatest benefit is seen when therapy is initiated within 48 hours of symptom onset, the experience during the H1N1 pandemic supports the benefit of treatment of individuals who remain influenza positive, even if therapy is initiated later in the course of illness.[460,483] Therapy should also be administered to individuals with laboratory-confirmed or highly suspected influenza who are at high risk of influenza complications, if therapy can be initiated within 48 hours. The benefits of therapy in high-risk patients after 48 hours are not clear, but antiviral therapy should be strongly considered if the illness is progressing, particularly if they remain influenza positive.

Therapy can also be considered in otherwise healthy persons with laboratory-documented or highly suspected influenza who present within 48 hours of the onset of symptoms, and who wish to reduce the duration of symptoms. However, the decisions regarding treatment should consider the relatively small benefit demonstrated in clinical trials and the overall low risk of complications. The benefits of later therapy in otherwise uncomplicated influenza are unclear.

Decisions regarding the specific antiviral agent will depend greatly on the most current information regarding the resistance patterns of circulating influenza viruses. At the current time, almost all seasonal influenza A and B viruses would be expected to be resistant to the M2 inhibitors and sensitive to the NIs oseltamivir and zanamivir.

Chemoprophylaxis

Several schemes for prophylaxis have been evaluated, including seasonal prophylaxis, wherein drug is administered throughout the influenza epidemic season, generally 4 to 6 weeks; family prophylaxis, wherein drug is administered to family members for a short period of time after recognition of an index case in the family; and outbreak-initiated prophylaxis in institutions, which could be considered to be a variation on the theme of family prophylaxis. In addition, short-term antiviral prophylaxis can be considered for high-risk individuals who are vaccinated during the influenza season.

Seasonal Prophylaxis

Seasonal prophylaxis with amantadine and rimantadine has been shown to provide similar levels of protection against influenza A, ranging from 70% to 90%[432] Both zanamivir and oseltamivir have also been shown to be protective in seasonal prophylaxis. In healthy adults, inhaled zanamivir was shown to have about 67% efficacy for prevention of confirmed influenza,[484] and in a similar study the efficacy of oral oseltamivir was 74%.[485] Both drugs were well tolerated on prolonged use.

Relatively less information is available about the use of any of the influenza antivirals for prophylaxis in older adult or high-risk populations. In one study, seasonal prophylaxis was highly effective in preventing laboratory-documented influenza in older adult residents of retirement communities.[486] Important to note, 80% of the subjects had previously been vaccinated, and prophylaxis resulted in a 91% reduction in influenza in this group. Thus, vaccine and chemoprophylaxis had an additive protective effect in older adults.

Family Prophylaxis

Results of outbreak prophylaxis in the family setting with amantadine or rimantadine have depended on whether both the index case and the contacts are treated. When the index case was not treated with amantadine, protection of family contacts has been seen, but if the index case was treated with amantadine at the same time that contacts received prophylaxis, no protection was seen, because of the rapid generation and transmission of resistant virus. This problem has not been seen in studies of family prophylaxis with oseltamivir[487] or zanamivir.[488] In general, drug is administered to contacts for 5 to 7 days after recognition of the index case. It is important to realize that treated individuals remain susceptible to infection from outside the family after such prophylaxis is discontinued.

Outbreak Prophylaxis

Probably one of the most common uses of antiviral agents for influenza is to terminate the transmission of influenza within institutions such as nursing homes during outbreaks. Both adamantanes and NIs have been used to control outbreaks in institutional settings, combined with other infection control practices. Prophylaxis should begin as early as possible within the institution, and continue for 7 to 10 days.[261] If possible, individuals who are receiving treatment should be isolated from those who are receiving prophylaxis to reduce the likelihood of generation and transmission of resistant viruses within the institution.[489,490]

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The complete reference list is available online at Expert Consult.

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166 California Encephalitis, Hantavirus Pulmonary Syndrome, Hantavirus Hemorrhagic Fever With Renal Syndrome, and Bunyavirus Hemorrhagic Fevers

Raphael Dolin

SHORT VIEW SUMMARY

Definition
- Bunyavirales is a large order of RNA viruses consisting of 10 families and more than 350 named species. They are enveloped, single-stranded RNA viruses with a segmented genome. Bunyavirales members can be found worldwide and are able to infect invertebrates, vertebrates, and plants.

Epidemiology
- Bunyaviruses are significant human pathogens with the ability to cause severe disease, ranging from febrile illness, encephalitis, and hepatitis to hemorrhagic fever.
- With exception of hantaviruses, bunyaviruses are transmitted by hematophagous arthropods, including ticks, mosquitoes, and phlebotomine flies.
- Hantaviruses are maintained in nature through persistent infection of rodents.

Major Causes of Human Diseases (See Table 166.1)
- California encephalitis group:
  - La Crosse virus (LACV)
  - Jamestown Canyon virus (JCV)
- Rift Valley fever virus (RVFV)
- Crimean-Congo hemorrhagic fever virus (CCHFV)
- Hantaviruses
  - Hemorrhagic fever with renal syndrome (HFRS)
  - Hantavirus pulmonary syndrome
- Severe fever with thrombocytopenia syndrome virus (SFTSV)

Diagnosis
- In most cases the diagnosis is based on serologic investigation of acute and early convalescent sera. Early diagnosis can be based on reverse-transcriptase polymerase chain reaction assays of blood samples. Diagnostic tests are typically performed in reference laboratories.

Therapy
- Treatment is primarily supportive because specific antiviral therapy is not available. Ribavirin has been studied in some bunyavirus infections, and data from in vitro and in vivo models hold promise. Ribavirin has shown clinical benefit in HFRS and in CCHF. However, comprehensive clinical trials have not been conducted.

Prevention
- No specific preventive measures are available, but experimental vaccines for some bunyaviruses have been developed and are undergoing study. Individual protective measures, such as arthropod repellents and insecticide-impregnated mosquito bed nets, are recommended in endemic areas.

Bunyaviruses are an extraordinarily diverse and numerous group of viruses whose taxonomic complexity has led to a recently reorganized classification by the International Committee on Taxonomy of Viruses (ICTV). In 2017 the ICTV reclassified the family Bunyaviridae to that of an order, Bunyavirales, that comprises 10 families: Arenaviridae, Cruliviridae, Fimoviridae, Hantaviridae, Mypoviridae, Nairoviridae, Peribunyaviridae, Phasmaviridae, Phenuiviridae, and Wupedeviridae. The families comprise 35 genera, with more than 350 named viruses identified as species.[1,2] Classification into separate families and species is based on genomic, structural, biochemical, and antigenic chracteristics,[3] although limited data are available for a number of these viruses. The distribution of these viruses is via arthropod-vertebrate cycles or by chronic infection of vertebrates, which lead to epidemiologic patterns that are ecologically determined and can be focal or diffuse depending on climatic variables. Table 166.1 presents salient features of infections caused by Bunyavirales viruses, including vectors, transmission, epidemiology, and clinical expression. Details of these infections are discussed further as follows.

VIROLOGY

Structure, Replication, and Antigenic Relationships

Bunyavirales members are spherical, enveloped RNA viruses, 80 to 120 nm in diameter and that display surface glycoproteins of 5 to 10 nm.[3] The RNA is of negative sense or ambisense, and codes for six or fewer proteins. The genome consists of two to six segments, although the majority of bunyaviruses studied have a trisegmented genome (Fig. 166.1).[3–5] The large RNA segment (L) encodes an RNA-dependent RNA polymerase; the medium segment (M) encodes viral glycoprotein precursors, which are cleaved by the host protease to yield mature glycoproteins, Gn and Gc, that are involved in receptor binding and endomembrane fusion. The small genome segment (S) encodes the nucleocapsid proteins.[3–5] The molecular weights of the proteins and RNAs vary by genus. In general, the Gn or Gc proteins, or both, are targets for neutralizing antibodies.[3] The nucleocapsid protein is believed to be the most important source of immunologic relationships observed within and across families and genera. In general, the fluorescent antibody test is the most cross-reactive, with hemagglutination inhibition

(HI), and although the neutralization tests provide greater specificity, the latter is of greatest use in distinguishing individual viruses. Enzyme-linked immunosorbent assays (ELISAs) increasingly are used for diagnosis of acute or resolving (immunoglobulin [Ig]M) or retrospective (IgG) infections (see later).

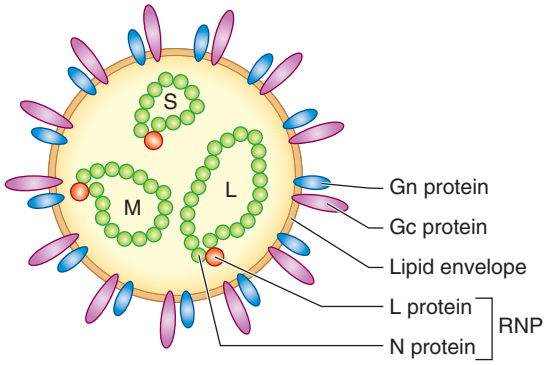

FIG. 166.1 Bunyavirus structure. The three genome segments (*L, M,* and *S*) are encapsidated by nucleocapsid protein to form ribonucleoprotein complexes *(RNPs),* and together with the viral L protein (RNA-dependent RNA polymerase) are packaged within a host cell-derived lipid envelope modified by insertion of the viral glycoproteins *Gn* and *Gc*. *(Modified from Elliot RM, Schmaljohn CS. Bunyaviridae. In: Knipe DM, Howley PM, eds.* Fields Virology. *Philadelphia: Lippincott Williams & Wilkins; 2013:1245.)*

Virus replication occurs in the cytoplasm, and virions mature by budding from the Golgi complex and endoplasmic reticulum into vesicles. Some bunyaviruses are exceptions, such as Rift Valley fever virus (RVFV), which buds through the outer cell membrane of hepatocytes, and Sin Nombre virus (SNV), which matures at the cytoplasmic membrane.[3]

EPIDEMIOLOGY

Ecology and Distribution

With the exception of hantaviruses, all bunyaviruses are transmitted by arthropods (mosquitoes, ticks, and sand flies). Hantaviruses are transmitted through feces from rodent species, such as deer mice or the striped field mouse.

California Encephalitis Viruses

Among the California encephalitis (CE) virus group, La Crosse virus (LACV) is the most medically significant virus, causing an average of 79 cases per year, predominantly in children younger than 15 years.[6] Its principal vector is *Aedes triseriatus,* a forest-dwelling, tree-hole–breeding mosquito of the north-central and northeastern regions of the country. LACV is maintained in this mosquito via transovarial transmission supplemented by intraspecific venereal transmission and amplification during summer by mosquitoes feeding on viremic chipmunks, squirrels, foxes, and woodchucks.[7,8]

Female mosquitoes infected by any of these mechanisms are capable of transmitting virus via a bite. The virus survives during the winter in mosquito eggs.[9] LACV and human encephalitis were first recognized in the upper Mississippi and Ohio River valleys. Most cases have

TABLE 166.1 Some Characteristics of Severe Diseases Caused by Bunyavirales

DISEASE	GENUS AND VIRUSES	VECTOR	TRANSMISSION TO HUMANS	DISEASE PATTERN AND ANNUAL INCIDENCE	MAJOR CLINICAL FEATURES
California encephalitis	*Orthobunyavirus* La Crosse Jamestown Canyon	*Aedes triseriatus* Transovarial transmission; amplification by chipmunks	Mosquito bite	Summer-fall; northern United States: 60–130 cases	Meningoencephalitis, seizures, cerebral edema
Rift Valley fever	*Phlebovirus* Rift Valley fever	*Aedes mcintoshi* Transovarial transmission; horizontal transmission in other arthropods	Mosquito bite; aerosol or contact with fresh carcasses, domestic animals	Endemic in rainy season sub-Saharan Africa: hundreds of cases; occasional epidemics associated with exceptional rainfall	Acute febrile illness with occasional retinitis, hemorrhagic fever, or encephalitis
Crimean-Congo hemorrhagic fever	*Orthonairovirus* Crimean-Congo Hemorrhagic Fever	*Hyalomma* ticks Amplified by hares, domestic animals	Tick bite, contact with blood of humans or domestic animals	Spring-summer; regions of former Soviet Union, Middle East, Africa: 50–200 cases	Severe hemorrhagic fever
Hemorrhagic fever with renal syndrome	*Orthohantavirus* Hantaan Dobrava Seoul Puumala	Chronic infection of striped field mouse, yellow-necked mouse, rat, or bank vole	Aerosols from rodent excreta	Endemic and epidemic; season depends on local conditions. Asia, Europe: 100,000 cases	Fever, shock, bleeding, renal failure
Hantavirus pulmonary syndrome	*Orthohantavirus* Sin Nombre Bayou Black Creek Canal	Chronic infection of deer mouse and other rodents	Aerosols from rodent excreta	Discovered in 1993; dozens of cases annually in North and South America	Fever, shock, pulmonary edema
Tropical Fever	*Orthobunyavirus* Oropouche	Biting midge *Culicoides paraensis*	By biting midges	Epidemic and sporadic disease in tropical areas of Brazil, Peru, and Panama	Acute febrile illness with occasional hemorrhagic complications
Sand fly fever	*Phlebovirus* Toscana Sand fly fever	*Phlebotomus perniciosus, Phlebotomus papatasi*	Mosquito bite	Circum-Mediterranean distribution in Southern Europe	Fever, headache, myalgia, meningitis
Severe fever with thrombocytopenia syndrome (SFTS)	*Phlebovirus* SFTS	*Haemaphysalis longicornis, Ixodes nipponensis, Amblyomma testudinarium*	Tick bites, close person-to-person contact	Central and eastern China, South Korea, western Japan	Fever, multiorgan dysfunction, bleeding diathesis
Heartland virus disease	*Phlebovirus* Heartland	Tick-borne Lone Star tick *Amblyomma americanum*	Probably tick-borne	Midwestern and southern United States: 30 cases since 2012	Fever, headache, cough, confusion, myalgia, multiorgan dissemination, thrombocytopenia

been reported from Wisconsin, Minnesota, Iowa, Indiana, Ohio, and Illinois.[10,11] However, recognition of the disease in West Virginia and Georgia[10] has led to an understanding that viral transmission occurs throughout the eastern United States; during 2003–07 West Virginia had the greatest number of cases (95) in the United States.[12] Studies in Tennessee suggest recent extension into that state.[11] Other vectors are not of major importance except focally. The Asian mosquito *Aedes albopictus* is an efficient vector and is capable of horizontal and vertical transmission in the laboratory.[13] Its strongly anthropophilic biting habits and its documented extension into areas where LACV is endemic raise concern, particularly because the virus has been isolated from field collections of *A. albopictus*.[14]

Other CE viruses have distinct ecologic cycles based on an element of transovarial transmission in mosquitoes, and human disease is uncommon and usually, but not always, mild.[15,16]

Rift Valley Fever

RVFV is maintained in sub-Saharan Africa via transovarial transmission in certain floodwater-breeding *Aedes* mosquitoes, notably *Aedes mcintoshi*.[17] Infected eggs can remain dormant but viable in soil for years while awaiting heavy rains for subsequent hatching. Other mosquitoes are important during epizootics and epidemics; large domestic ungulates, such as sheep or cattle, serve as amplifiers because they experience high viremia during infection.[18] In 1977 the virus was introduced into Egypt, producing widespread epidemic disease in humans and domestic animals; it reappeared in the 1990s. After an extensive epidemic in Kenya in 1997–98, it was introduced into the Arabian peninsula, where it also caused epidemic disease in animals and humans.[19,20] Heavy rainfall in East Africa resulted in a major recurring epidemic in 2006–07.[21] It is likely that other receptive areas, such as North America, might experience the same fate if an introduction should occur.[22]

Crimean-Congo Hemorrhagic Fever

Crimean-Congo hemorrhagic fever virus (CCHFV) is transmitted by ticks. It was first described in Russian soldiers in the Crimea in 1944 and named Crimean fever. Subsequently, an identical virus was isolated from an ill child in the Congo in 1956 and was named Congo virus. After the viruses were shown to be identical, they were renamed Crimean-Congo hemorrhagic fever virus.[23–25] The principal vectors belong to the genus *Hyalomma*. Immature stages feed on hares, hedgehogs, and ground-feeding birds, whereas adults parasitize large wild and domestic animals. This virus is widely distributed in southwestern Russia, the Balkans, the Middle East, central Asia, western China, and Africa, and between 1998 and 2013, CCHF was reported most frequently in Turkey, Russia, Iran, Pakistan, and Afghanistan.[26,27] The incidence of CCHF appears to be increasing, and possible causes include alterations in agricultural and domestic animal practices and climate changes.[28]

Hantaviruses

Hantaviruses cause two forms of severe acute illness: hemorrhagic fever with renal syndrome (HFRS), caused by hantaviruses of the Old World, and hantavirus cardiopulmonary syndrome (HCPS), also known as hantavirus pulmonary syndrome (HPS), caused by hantaviruses of the New World. These agents are fundamentally parasites of wild rodents and insectivores.[29] As such, hantaviruses are the exception to the general rule that bunyaviruses are arthropod-borne viruses. Although many rodent species worldwide have been shown to be infected, each of the presently recognized viral species has a single major rodent host species. This species becomes chronically infected despite an immune response that eliminates viremia, and its members excrete virus in urine and saliva for weeks or months.[30,31] Mechanisms of intraspecific transmission depend largely on horizontal transmission between sexually mature animals.[31] Hantaan virus (HTNV) is the cause of HFRS in Korea, China, and eastern Russia and is carried by the striped field mouse, *Apodemus agrarius*. *A. agrarius* is found in or near cultivars of humans; rodent breeding seasons and human agricultural practices result in fall and spring disease peaks.[32,33] Dobrava virus, associated with *Aedes flavicollis,* is the major cause of severe HFRS in the Balkans, and related viruses cause similarly severe disease in other areas of the former Soviet Union. Another hantavirus, Seoul virus (SEOV), is found worldwide in *Rattus norvegicus*. Although the virus is found wherever the reservoir sewer rat occurs, disease has rarely, if ever, been identified in the United States.

Bank voles, *Clethrionomys glareolus,* are the reservoir-vectors of Puumala virus, the cause of a milder form of HFRS termed *nephropathia epidemica* in Scandinavia, the western former Soviet Union, and Europe. These small rodents are found in forests and agricultural hedgerows, have highly fluctuating populations, and disperse into rural and suburban gardens and dwellings, particularly in the fall and winter of years when their populations reach peaks.[32,33]

Many native North and South American rodents (family Muridae, subfamily Sigmodontinae) host phylogenetically distinct hantaviruses associated with HPS.[34] HPS is a disease of the Americas and is probably more important in South America than in North America. The most important North American hantavirus is SNV. The reservoir of SNV is the deer mouse, *Peromyscus maniculatus,* a species that is widespread in the United States and readily enters homes and other structures. On the East Coast, the closely related New York virus causes chronic infection of the white-footed mouse, *Peromyscus leucopus*. Somewhat more distantly related viruses are Bayou and Black Creek Canal viruses found in the southern United States and Florida, respectively, and are associated with a degree of renal failure in their clinical picture. The most important South American virus is Andes virus, which is a common cause of disease in Argentina and Chile and is the only hantavirus that has caused person-to-person transmission.[34]

Transmission to Humans

California Encephalitis Virus Group

LACV transmission occurs through the bite of female mosquitoes that have viral infection of their salivary glands. Human infection occurs mainly during the summer and early fall in persons entering forested areas for recreation or those living near forests. Members of *A. triseriatus* (eastern tree-hole mosquito) range a considerable distance from forest across open terrain in search of a blood meal and breed effectively in some manufactured containers, such as abandoned tires, bringing the mosquito range closer to human habitation.[35] Jamestown Canyon virus has also been isolated from multiple mosquito species and various mammals (see later).

Rift Valley Fever

RVF in Africa has two main modes of transmission. It is recognized as a disease of farmers, veterinarians, and abattoir workers who have close contact with blood shed from sick domestic livestock or fresh carcasses containing a high concentration of virus.[18] Another major route of transmission to humans is from mosquito bites, particularly during epidemics. Infrequent years of heavy precipitation trigger the dormant transovarially infected eggs, and other secondary vectors widely disseminate virus.[18,22]

Crimean-Congo Hemorrhagic Fever

CCHFV infects humans principally by the bite of adult *Hyalomma* ticks. Milkers and shepherds are frequent victims. Asymptomatically viremic sheep and cattle have been implicated in transmission to abattoir workers, even outside known endemic areas,[36] and it is also hazardous to crush infected ticks. Highly infectious blood from patients also has caused several alarming nosocomial outbreaks with fatalities in medical personnel, particularly when the correct diagnosis of the index case was not suspected.[37–39] These have followed direct contact with blood and body fluids[40,41] and with aerosol generating procedures.[42]

Hantaviruses

Aerosols of virus-contaminated rodent urine or perhaps feces are thought to represent the principal vehicle for the transmission of hantaviruses; disease has also followed the bite of infected rodents because saliva contains virus, although this appears to be much less frequent.[43,44] Infections from *Apodemus* or *Clethrionomys* are acquired principally by persons visiting or working in forests and on farms. Depending on the circumstances, the incidence may be highest in summer or in fall and early winter. Disease is maximal in "high-rodent" years, when suburban residents may be exposed to disposing of infected rodents.[32–34,45]

Deer mice are numerous and readily enter human dwellings and outbuildings, particularly when mouse populations are high or in autumn when food and cover are scarce. Abundant rodent populations led to a large number of cases in the southwestern United States in the summer of 1993 and resulted in the first discovery of the virus. Most hantavirus epidemic years have been associated with increased rodent populations.[33,34]

CLINICAL MANIFESTATIONS

California Encephalitis Virus Group

Infection of humans by CE viruses is most commonly asymptomatic. After an incubation period of 3 to 7 days, however, individuals may experience mild febrile illness, encephalitis, or meningoencephalitis. Greater than 90% of acute central nervous system (CNS) disease caused by LACV occurs in children younger than 15 years; males are affected more often than females, and the mortality in acute CNS disease is about 1%.[1,10,46–48] LACV infection has caused encephalitis in an immunocompromised adult with a presentation resembling herpes simplex encephalitis.[47] Clinically and pathologically, CE is difficult to distinguish from other acute viral infections of the CNS. It can range in severity from mild aseptic meningitis to a severe disease mimicking herpes simplex encephalitis. Computed tomographic scans are abnormal in a minority of cases, magnetic resonance imaging is sometimes positive, and either can yield focal images.[10] The electroencephalogram is abnormal in two-thirds of patients, often with focal findings that include periodic lateralizing epileptiform discharges (PLEDs) that lead to a suspicion of herpes simplex encephalitis.[48] PLEDs often localize to the temporal lobe and are associated with more severe disease (e.g., convulsions, intubation, prolonged intensive care unit stay) as well as sequelae (e.g., epilepsy, cognitive and memory deficits).[49] Fever, headache, nausea, and vomiting are present in most patients. Lethargy, aphasia, incoordination, and focal motor abnormalities, even paralysis, may be present, but the outstanding serious finding is convulsions, which occur in about one-half of cases. The cerebrospinal fluid (CSF) generally shows a modest pleocytosis (<100 white blood cells [WBCs]/mm^3) that occasionally is largely granulocytic and exhibits a normal or slightly increased protein concentration. Peripheral leukocytosis in excess of 15,000 WBCs/mm^3 is not uncommon. Although most patients make uneventful recoveries, abnormal electroencephalographic findings 1 to 5 years later are present in 75%, emotional lability is persistent in 10%, and epilepsy is a chronic problem in 6% to 10% of all diagnosed cases. Frank neurologic deficit is uncommon but does occur.[46] Thus the residua of LAC encephalitis may be more serious than is generally appreciated. A possible congenital infection with LACV has been reported, without apparent abnormalities to the neonate. LACV infection during pregnancy has been associated with teratogenic effects in rabbits, gerbils, and sheep.[50–52]

Rift Valley Fever

RVFV infection in humans causes undifferentiated febrile disease in the great majority of instances. The incubation period lasts 2 to 6 days and is followed by fever and malaise, often accompanied by headache, photophobia, and back and joint pain.[53,54] Perhaps 10% of patients experience macular and perimacular retinitis, and vasculitis that may cause a permanent loss of vision. In as much as 1% of infections, fulminant disease with hemorrhage, jaundice, and hepatitis may develop at the end of a 3- to 6-day febrile episode, with high mortality.[55] Other infections (<1%) lead to severe, frequently fatal, encephalitis directly related to viral invasion of the CNS.

Crimean-Congo Hemorrhagic Fever

CCHF is generally a mild febrile illness but one that may progress to severe and fatal hemorrhagic disease.[56] CCHF has a distinct course of infection in humans with four different phases: incubation, prehemorrhagic, hemorrhagic, and convalescence period. After infection via tick bite, the incubation period is usually short, ranging on average between 1 and 5 days. The incubation period after contact with infected blood or tissues is usually 5 to 6 days, with a documented maximum of 13 days.[57] The first evidence of disease is usually a flushing of the face and the pharynx and a rash that progresses to petechiae, ecchymoses, hemorrhage of mucous membranes and conjunctiva, hematemesis, melena, epistaxis, hematuria, and hemoptysis.[57] Clinical manifestations in the prehemorrhagic period include fever, malaise, myalgia, dizziness, and, in some patients, diarrhea, nausea, and vomiting lasting for an average of about 3 days. The hemorrhagic phase usually begins on days 3 to 5 after the onset of illness and is usually short, lasting on average 2 to 3 days. Cerebral hemorrhage, gingival bleeding, and bleeding from the nose, vagina, uterus, or urinary tract may occur, as well as internal bleeding in abdominal muscles. Cerebral hemorrhage and massive liver necrosis indicate a poor prognosis. Hepatomegaly and splenomegaly may occur in up to 40% of the patients. Death generally occurs on days 5 to 14 of illness and is attributed to hemorrhages, hemorrhagic pneumonia, or cardiovascular disturbances. Lethal cases typically do not develop an antibody response. The mortality rate has been reported to average around 30% but may range from about 5% to greater than 80%.[58]

Hantaviruses

Hemorrhagic Fever With Renal Syndrome

The hallmarks of clinical infection by HTN, Dobrava, SEO, and Puumala viruses and other Eurasian hantaviruses are fever, thrombocytopenia, and acute renal insufficiency, pathologically typical of acute interstitial nephritis. The incubation period, typically 2 weeks, may vary from 5 to 42 days. In the severe form of HFRS exemplified by HTNV infection or Dobrava virus in Europe, patients who survive full-blown disease progress through febrile (toxic), hypotensive, oliguric, and polyuric clinical stages and may require weeks or months to recover from general asthenia.[59–61]

In the toxic phase patients complain of headache, abdominal and lower back pain, dizziness, and often blurred vision.[56–59] Conjunctival injection and petechiae occur over the upper trunk and soft palate. An erythematous flush that blanches on pressure is characteristically seen on the torso and face. Leukocyte levels are normal or more likely elevated, often exceeding 20,000/mm^3. The differential count shows a left shift, immature myeloid cells, and atypical lymphocytes as well, confirming the decreased thrombocyte count. At the end of the febrile period (4–7 days), many patients experience severe clinical shock. Those surviving then endure varied grades of renal insufficiency that can include anuria, oliguria, mucosal bleeding diathesis, electrolyte and acid-base abnormalities, hypertension, and pneumonitis complicated by pulmonary edema. After 3 to 10 days, polyuria begins with attendant stresses on the fluid and electrolyte balance. The fatality rate in severe HFRS caused by Hantaan or Dobrava viruses averages about 5%: one-third during the shock phases and two-thirds (cerebrovascular accidents and pulmonary edema) during the renal phases of illness.

Pathogenic hantaviruses enter endothelial cells via β3 integrins, which can result in disruption of cell-to-cell adhesion and in permeability alterations. This may result in an acute capillary leak syndrome, which accounts for varied clinical manifestations that are seen in hantavirus-associated diseases,[62,63] including hemodynamic changes. The renal lesions, predominantly in medullary tubules, may be related to both systemic and intrarenal hemodynamic factors and to the influence of immunopathologically released kinins and cytokines.[64] Bradykinin has been implicated in this regard,[65,66] and complement activation has been noted, involving both classical and alternative pathways.[67,68] Complement components C3 and C5 appear to be linked to observed disorders of coagulation and fibrinolysis cascades.[69]

The milder form of HFRS caused by Puumala virus, often referred to as *nephropathia epidemica*, is rarely hemorrhagic and is fatal in less than 1% of clinical cases. Abdominal pain and renal dysfunction may be manifestations. Up to 90% of Puumala virus infections are asymptomatic. Proteinuria, creatinine elevation, and leukocytosis are common but are much less severe than in HTNV infection.[70,71]

SEOV also causes a mild to moderately severe HFRS in Eurasia, with more prominent hepatic involvement than classic HFRS.[59,72]

Hantavirus Pulmonary Syndrome

HPS begins with a febrile prodrome, followed by a severe increase in pulmonary vascular permeability and shock.[64,73,74] If hypoxia is managed and shock is not fatal, the vascular leak reverses in a few days and recovery is virtually complete, although renal and other sequelae have

been suggested.[75] The first symptoms are fever of sudden onset and generalized myalgia. This prodrome resembles the initial phases of HFRS and may also be accompanied by abdominal pain and gastrointestinal (GI) disturbances.[73] About 4 to 5 days later (range, 1–10 days) the patient presents with respiratory symptoms, which usually consist of modest cough and dyspnea. Examination may be unrevealing, but in general fever, tachycardia, and tachypnea are present, perhaps with mild hypotension. Laboratory abnormalities commonly found at this time or developing within 1 to 2 days thereafter are an elevated hematocrit; leukocytosis, left shift, or both; abnormal (atypical) lymphocytes and immature myeloid cells on smear; mild thrombocytopenia; a prolonged activated partial thromboplastin time; and mildly elevated aspartate aminotransferase or lactate dehydrogenase (LDH) levels. Mild increases in serum creatinine levels and proteinuria occur in some cases,[34] but the severe renal lesions seen in HFRS are not a regular feature of this syndrome.[64,76] Respiratory involvement can progress from mild desaturation and interstitial pulmonary edema to florid pulmonary edema with respiratory failure in a matter of hours.[74,77] HPS should be suspected when an otherwise healthy adult develops unexplained pulmonary edema or is suspected of adult respiratory distress syndrome without one of the known causes of this syndrome being present; thrombocytopenia or a decreasing platelet count is a particularly useful finding early in the course.[74] Extracorporeal membrane oxygenation may improve survival but has never been tested in a controlled trial.[78]

The histopathologic findings of interstitial infiltrates of T lymphocytes and alveolar pulmonary edema without marked necrosis or polymorphonuclear leukocyte involvement, plus the rapid resolution of the lesion, suggest that the major abnormality may be the induction of a functional vascular permeability increase via an immunopathologic mechanism.[76,79]

DIAGNOSIS

A wide variety of laboratory tests have been developed to establish a diagnosis of infections with bunyaviruses. The applicability and availability of laboratory tests vary with the ecology and pathobiology of individual bunyaviruses. Serologic diagnosis is most frequently used for many bunyavirus infections, and most patients will have serum immunologic evidence of infection once they present clinically. The presence of virus-specific IgM may provide evidence for a recent infection but is generally less specific than a rise in IgG titers in acute and convalescent serum specimens.

Virus detection by conventional tissue culture techniques is difficult for most bunyaviruses. However, viral detection by highly sensitive and specific molecular techniques for viral detection have now been developed for many bunyaviruses.

Availability of and expertise with individual bunyavirus laboratory tests vary with geographic locale and frequency of infections encountered. Readers are urged to consult local public health authorities if questions arise.

Laboratory diagnosis for individual groups of bunyaviruses are described later.

California Encephalitis

The diagnosis of CE is by serologic means because virus is usually not present in blood or secretions by the time patients present with the clinical phase of CNS disease. The diagnosis can be rapidly and specifically achieved with ELISAs for antiviral IgM antibodies in blood and CSF, which are usually, but not always, positive at the time of admission.[10,80] A licensed indirect fluorescent antibody test is available for IgG and IgM antibodies to LACV and may be useful in the diagnosis.[11] An HI assay is also available and may be more sensitive but may require neutralization assays for confirmation.[81]

Viruses may also be detected by reverse-transcriptase polymerase chain reaction (RT-PCR) and multiplex nucleotide sequencing.[3] Virus isolation can be attempted by intracerebral inoculation of suckling mice or tissue culture in Vero cells.[3]

Riff Valley Fever and Congo-Crimea Hemorrhagic Fever Viruses

RVFV and CCHFV are readily recovered from the blood of acutely ill patients in cell cultures or suckling mice. Antigen-detection ELISA is useful in diagnosis, particularly in severe cases. The RT-PCR assay provides additional sensitivity with no loss of specificity. Antibodies detectable by a variety of methods generally appear within 5 to 14 days of onset and coincide with clinical improvement. ELISA detection of IgM antibodies is a reliable, definitive method.[18,36,37,82] Because of the aerosol hazard to laboratory personnel, acute samples must be handled with care, and attempts to isolate these two agents should be restricted to facilities with maximal containment.

Hantaviruses

Virtually all hantavirus patients have both IgM and IgG ELISA antibodies present when admitted to the hospital.[60] Hantaviruses can be recovered only with difficulty in cell culture or animal hosts,[31] but the agent can be detected in blood or tissues by RT-PCR or in tissues by immunohistochemical staining.[76,79,83]

THERAPY

Therapy with the antiviral drug Ribavirin, a guanosine analogue, has been shown to be effective in the treatment of HFRS in a double-blind placebo-controlled study in China using the intravenous dosing regimen established for Lassa fever (see Chapter 167). Intensive monitoring and frequent dialysis, even without ribavirin, result in very low mortality. An open-label trial of ribavirin failed to show any efficacy in HPS patients, perhaps because death typically occurs within 24 to 48 hours of hospitalization.[75]

Studies in vitro and in laboratory animals suggest that ribavirin also might be effective in the treatment of severe RVF and CCHF, and observational clinical experience with the drug in CCHF supports its use.[84,85] However, a recent Cochrane Database review states that there is "insufficient evidence to show whether ribavirin is effective in treating CCHF" and that appropriately designed clinical trials are necessary.[86,87] Ribavirin also has activity in vitro against LACV, and treatment of one unusual case of LACV infection diagnosed with brain biopsy has been reported.[88]

The possible effectiveness of treatment with immune plasma was examined in HPS in 29 confirmed cases in Chile (2008–12). The case fatality rate was 4 per 29 (14%) in cases treated with convalescent plasma, compared with 18 per 66 (27%) in HPS cases who were not treated with plasma at the same study sites ($P = .15$).[89] Further studies are required to investigate this observation.

Effective supportive care is important in all of the severe bunyavirus diseases. Careful management of coma, cerebral edema, and seizures is critical in CE patients; there is danger in too vigorous use of phenobarbital in children with status epilepticus.[10,48,88] Early management of hantavirus patients should avoid excessive administration of fluids in these febrile, hemoconcentrated, hypotensive patients. Vascular leak leads to extravasation into retroperitoneal tissues (HFRS) or lung (HPS); cardiotonic drugs should be used early because of the hemodynamic profile of decreased cardiac output and increased systemic vascular resistance.[64] Patients with severe HFRS may require hemodialysis or peritoneal dialysis during the oliguric phase, and plasma protein or whole blood, or both, may be useful in treating hemorrhage or shock, or both, in this and other hemorrhagic fevers. Heparin is not recommended for the treatment of presumptive or incipient disseminated intravascular coagulation in HFRS. Patients with mild HFRS due to Puumala virus rarely require dialysis.

PREVENTION

Prevention of bunyavirus diseases currently is accomplished primarily by personal means (e.g., avoidance of rodent contact, use of mosquito and tick repellents), and perhaps, in the case of LACV, by the elimination of manufactured containers leading to mosquito breeding, together with aerial spraying of slow-release insecticides over forested areas of known high *A. triseriatus* reproduction.[90]

A variety of vaccines against a number of bunyaviruses have been developed and studied, but none have received widespread usage. Immunization of livestock against RVFV is carried out with formalin inactivated and live-attenuated vaccines in parts of Africa.[91,92] An inactivated suckling mouse–derived vaccine against CCHFV has been developed in Eastern Europe,[93] and CCHFV glycoprotein vaccines are

also under study.[94] Several inactivated virus vaccines have been developed against SEOV and HTNV in Asia,[95] and DNA gene–based vaccines against hantaviruses are also under study.[3]

OTHER BUNYAVIRALES OF CONCERN

Jamestown Canyon Virus

Jamestown Canyon virus (JCV) is a member of the CE group of bunyaviruses that have been a likely unrecognized cause of infections and CNS disease in North America.[96,97] JCV was first identified in Jamestown Canyon, Colorado in 1961[98] and has been recovered from various mosquito species: *Aedes, Coquillettidia, Culex,* and *Culiseta*. Burgeoning populations of whitetail deer have been suspected as a vertebrate amplifier. Serologic surveys in humans and animals indicate that JCV is widespread through North America. A recent analysis of 31 laboratory-confirmed infections of JCV indicated that most infections occurred in spring to early fall and involved all age groups.[96] Clinically recognized infections present as an undifferentiated febrile illness, meningitis, or meningoencephalitis. Approximately half of identified infections required hospitalization, and deaths have not been reported to date.[96] The rate of asymptomatic infections with JCV is not known.[97]

Partial cross-reactions exist between LACV and JCV IgM responses and may require plaque neutralization or RT-PCR tests to differentiate between infections with these.

Oropouche Virus

Oropouche virus has been associated with epidemics and sporadic cases of febrile illness in tropical areas of Brazil, Panama, and Peru.[99–101] It is transmitted by biting midges *(Culicoides paraensis).* In rural areas transmission may also involve nonhuman primates, sloths, birds, and mosquitoes. In urban areas Oropouche virus can be transmitted between viremic individuals and biting midges.[101–104] The risk of transmission appears to be highest during the rainy season, during which breeding of midges is increased.[105] *C. paraensis* has been identified in states east of the Mississippi River, but the likelihood of establishment of ecologic factors for virus transmission in the United States is low.

The incubation period of disease in human ranges from 3 to 12 days.[99,100,106] Infection results in an abrupt onset of fever, chills, headache, myalgia, and often vomiting and arthralgia. Aseptic meningitis has been reported in some cases. Symptoms last for 4 to 5 days and are usually self-limiting. However, prolonged asthenia and arthralgia can occur, and recurrences 10 days after recovery have been observed.[101]

Toscana Virus

The classic sand fly fevers (Sicilian and Naples viruses) are acute febrile illnesses with headache and myalgias and were common in the broad European and Asian range of their vector, *Phlebotomus papatasi,* until dichlorophenyltrichloroethane (DDT) campaigns against malaria virtually eliminated this sand fly in much of Europe. Another sand fly, *Phlebotomus perniciosus,* spreads the related but distinct Toscana virus, which appears to be a common cause of infections in Tuscany and areas of Southern Europe.[107–110] High seroprevalence rates to Toscana virus in endemic areas suggest that most infections are asymptomatic or are of minimal symptomatology. However, Toscana virus can cause febrile disease, aseptic meningitis, or mild encephalitis. Overall prognosis is good, although severe cases have been reported. CNS infections are common in the circum-Mediterranean distribution of the vector in Europe, from Cyprus to Portugal and Spain, and are often causes of disease in returning travelers.[107,111,112] Diagnosis can usually be made serologically by detection of virus-specific IgM by ELISA and immunofluorescence assays, or by virus neutralizing antibody rises.[113]

Ngari Virus

During the 1997–98 RVF outbreak in Kenya, two viruses belonging to the *Orthobunyavirus* genus were isolated from putative hemorrhagic fever cases, and RT-PCR was positive in 12 sera. The virus was found to have the S and L RNA segments of Bunyamwera virus, but the M segment was derived from another bunyavirus, subsequently identified as Ngari virus.[114] Subsequent complete genomic sequencing of Ngari virus indicated that Ngari virus was a naturally occurring reassortant between two orthobunyaviruses, Bunyamwera and Batai viruses.[115,116] S and L segments were derived from Bunyamwera virus, and M segments were derived from Batai virus. Thus this virus should be considered in other viral hemorrhagic fever epidemics and is a cautionary example of the emergence of novel pathogens through viral reassortment.[117]

Severe Fever With Thrombocytopenia Syndrome Virus

A new member of the *Phlebovirus* genus was isolated in China (Hubei and Henan provinces) from patients who presented with fever, thrombocytopenia, leukocytopenia, and multiorgan dysfunction and named severe fever with thrombocytopenia syndrome virus (SFTSV).[118] The clinical symptoms were initially considered to resemble those of human granulocytic anaplasmosis.[119] The majority of affected patients were farmers living in wooded and hilly areas, and they were working in the fields before onset of clinical signs of disease. SFTSV RNA has been detected in ticks of the Ixodidae *(Haemaphysalis longicornis, Ixodes nipponensis, Amblyomma testudinarium)* that were collected from domestic animals where patients lived. Initially, no epidemiologic evidence of human-to-human transmission of SFTSV was detected.[118] However, subsequent publications indicated that the virus can be transmitted from person-to-person through close personal contact with the index patient without known exposure to suspected animals or vectors.[120–122] Nosocomial transmission in hospital settings has also been reported.[123]

SFTSV appears to be most closely related to the Bhanja serogroup among members of the *Phlebovirus* genus.[124] SFTSV has been detected in central and eastern China, rural areas of South Korea, and Western Japan.[125,126]

SFTS has an incubation period of 7 to 14 days and begins with a febrile illness, malaise, headache, GI disturbances, myalgias, and arthralgias. During the second week of illness, multiorgan dysfunction can develop, with renal dysfunction, myocarditis, and CNS involvement.[127–130] Bleeding manifestations, including disseminated intravascular coagulation and mucosal hemorrhage, can ensue. Disease manifestations may begin to resolve over 1 to 2 weeks, but cases can continue to worsen, and overall case fatality rates have been estimated to be 6% to 21%.[129,131,132] Laboratory abnormalities include leukopenia, thrombocytopenia, elevated liver function tests, and elevated LDH and creatine phosphokinase levels.

The pathogenesis of SFTS appears to be related to high viral loads, which results in a systemic inflammatory response. Marked upregulation of multiple cytokines has been correlated with disease severity.[133–135]

Laboratory diagnosis during the first week of illness can be made by detection of SFTSV in blood by RT-PCR[134] or by loop-mediated isothermal amplification.[135]

Therapy consists of general medical support and measures to treat bleeding diathesis. Ribavirin has in vitro activity against SFTSV and has been used to treat patients with SFTS in China, without apparent effectiveness.[136] Favipiravir has been shown to be effective in vitro and in a mouse model against SFTSV (also see Chapter 45).

Heartland Virus

Heartland virus is a newly recognized pathogenic bunyavirus in the *Phlebovirus* genus, first reported from two cases in Missouri in 2012.[137] As of 2017 30 cases have been reported to the Centers for Disease Control and Prevention, all from the midwestern and southern United States.[137,139] Transmission appears likely to be tick-borne, and Heartland virus has been detected by PCR and culture in nymphs of the Lone Star tick *(Amblyomma americanum),* which can be found in the United States from Texas to Maine.[140] Neutralizing antibodies to Heartland virus have been found in a wide variety of vertebrate life, including opossums, dogs, raccoons, deer, moose, horses, and coyotes,[141,142] but the major vertebrate host has not been identified. Human-to-human transmission has not been reported, although such has been observed in infection by a related *Phlebovirus* (SFTSV) (see earlier).

Clinical manifestations of Heartland virus–associated disease consist of fever, headache, GI disturbances, dry cough, confusion, myalgias, and arthralgias.[138] Fatigue and memory loss lasting weeks to months has been described.[138] Widespread dissemination[143] and fatal cases have been reported.[144,145] Laboratory findings include leukopenia, thrombocytopenia, and elevated liver enzymes.[143,146] A fatal case of Heartland virus infections

with hemophagocytic lymphohistiocytosis in an immunocompromised patient has been described.[147]

Laboratory diagnosis of Heartland virus infection can be made by virus detection by RT-PCR or tissue culture, or serologically by ELISA or plaque reduction neutralization tests.[148]

ACKNOWLEDGMENT

This chapter is based on and updated from chapters on Bunyaviridae written by C.J. Peters and D.A. Bente for the previous editions of *Principles and Practice of Infectious Diseases*.

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The complete reference list is available online at Expert Consult.

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167 Lymphocytic Choriomeningitis Virus, Lassa Virus, and the South American Hemorrhagic Fevers (Arenaviruses)

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SHORT VIEW SUMMARY

Epidemiology
- Lymphocytic choriomeningitis virus (LCMV) is worldwide in distribution, Lassa fever is found in Africa, and the New World complex viruses circulate in South and North America and can cause hemorrhagic fevers.
- Transmission is by rodents, with generally high species specificity of reservoir vectors.

Microbiology
- These pleomorphic viruses are 50 to 300 nm in diameter with two single RNA segments with ambisense gene organization.

Clinical Manifestations
- *LCMV:* Febrile illness is accompanied by headache and systemic symptoms; the disease course may be biphasic with meningitis manifesting during the second phase in 5 to 10 days; full recovery is usual, but immunosuppression may result in disseminated and fatal disease.
- *Lassa fever:* The illness is usually mild, but severe multisystem disease may develop in 5% to 10% of cases, resulting in death in 15% to 25% of hospitalized patients; fever, pharyngitis, retrosternal pain, and proteinuria are the most common features; fatal cases usually occur in the second week of illness, with hypotension, vasoconstriction, mucosal hemorrhages, and capillary leak syndrome.
- *South American hemorrhagic fevers:* Argentinean, Bolivian, and Venezuelan hemorrhagic fevers have progressive fever, malaise, and myalgias, most frequently affecting the lower back; petechiae and small vesicles are frequent; vascular disease (capillary leak), vasoconstriction, and shock may occur; various blood and neurologic abnormalities can be seen; overall mortality is 15% to 30%; convalescence may take weeks, but recovery is usually without sequelae.

Diagnosis
- Detection of virus is evident in acute blood specimens by reverse-transcriptase polymerase chain reaction assay or virus culture, depending on the particular viral agent.
- Immunoglobulin M antibodies may be detected by serology, and increases in antibody titers (seroconversion) can be noted by comparing acute and convalescent serum specimens.

Treatment
- Supportive treatment may be lifesaving.
- Convalescent human plasma has been shown to be effective in Argentinean hemorrhagic fever but has not been as successful in Lassa fever.
- Ribavirin is beneficial in treatment of patients hospitalized with Lassa fever and in patients with Argentinean and Bolivian hemorrhagic fevers.

Prevention
- Reduction of transmission from rodents to humans is needed.
- Person-to-person transmission in hospitals is a problem with Lassa fever, and use of gloves and gowns and careful disposal of patients' wastes and fomites should be implemented; if available, single rooms with negative pressure should be used.
- Steps should be taken to avoid infection in laboratory workers who examine samples that may contain arenaviruses.
- A live-attenuated vaccine is available against Argentinean hemorrhagic fever and is licensed in Argentina.

Arenaviruses are transmitted by rodents and comprise several medically important pathogens that cause hemorrhagic fevers in humans (Table 167.1). All arenaviruses can be divided based on their phylogenetic and antigenic relations into the Old World and New World virus complexes. The Old World complex includes arenaviruses that circulate worldwide and in Africa (i.e., lymphocytic choriomeningitis [LCMV] and Lassa [LASV] viruses). The New World complex includes viruses that circulate in South and North America (i.e., Junin [JUNV], Machupo [MACV], Guanarito [GTOV], and Sabia [SABV] viruses) and is further subdivided into three distinct clades designated as A, B, and C. The prototypic arenavirus LCMV has a worldwide distribution due to its association with *Mus domesticus* and *Mus musculus* and can cause febrile disease sometimes with central nervous system involvement as well as congenital infections. LASV is enzootic among rodents of the Murinae subfamily of the family Muridae inhabiting sub-Saharan Africa and is a causative agent of Lassa fever (LF), a severe disease in humans. There are at least four pathogenic South American arenaviruses, JUNV, MACV, GTOV, and SABV, that are transmitted by rodents of the Sigmodontinae subfamily of the family Muridae and are known to cause hemorrhagic fever in humans in endemic areas of Argentina, Bolivia, Venezuela, and Brazil.

VIRAL CHARACTERIZATION

Arenaviruses (family Arenaviridae) have pleomorphic virions ranging in diameter from 50 to 300 nm with an average diameter about 110 to 130 nm.[1] Electron micrographs reveal a granular appearance of the virion interior that is characteristic for all arenaviruses and is attributed to the presence of the host cell ribosomes (Fig. 167.1). This appearance gave the name to this family of viruses (Latin term *arenosus* means "sandy").

The genome of arenaviruses consists of two single-stranded RNA segments, large (L) and small (S). Each segment uses an ambisense gene organization to drive expression of two genes in opposite directions. The L RNA segment (approximately 7.1 kb) encodes the viral RNA–dependent RNA polymerase (L) and a small RING finger protein (Z) that is the arenavirus counterpart of the matrix proteins (M) of negative-sense RNA viruses. The S RNA segment (approximately 3.4 kb) encodes the glycoprotein precursor protein and the nucleoprotein (NP). Glycoprotein precursor protein is posttranslationally cleaved to yield two envelope glycoproteins, GP1 and GP2, and the stable signal peptide, which stays associated with the glycoprotein complex on mature virions.

The 250-kDa L polymerase uses the viral ribonucleoprotein consisting of genome RNA encapsidated by NP as a template to synthesize

TABLE 167.1 Arenaviruses and Human Disease

VIRUS	DISEASE	GEOGRAPHY	RESERVOIR	PATHOGENESIS	SPECIFIC THERAPY	PREVENTION
Lymphocytic choriomeningitis	Aseptic meningitis; other organ involvement	North and South America, Europe, and wherever *Mus* is introduced	*Mus domesticus* and *Mus musculus* (house mice)	Systemic infection; when CNS invasion occurs, immunopathologic CNS disease follows	None	House mouse control and avoidance, particularly by pregnant women; monitor mouse and hamster suppliers
Lassa	Lassa fever	West Africa, particularly Sierra Leone, Guinea, Liberia, and Nigeria	*Mastomys* (multimammate mouse)	Vascular leak, multiorgan dysfunction, shock; bleeding and CNS involvement occur but not as common as in South American diseases	Intravenous ribavirin	Rodent avoidance and control in houses may be of ancillary benefit; strict isolation of hospitalized patients
Junin	Argentinean HF	Argentine pampas	*Calomys musculinis*	As Lassa fever except encephalopathy and thrombocytopenia are common, as is hemorrhage	Convalescent plasma; ribavirin probably efficacious	Effective live-attenuated vaccine
Machupo	Bolivian HF	Bolivia (Beni Department)	*Calomys callosus*	As Argentinean HF	Ribavirin or convalescent plasma	Elimination of rodents from home; laboratory evidence for cross-protection by Junin vaccine
Chapare	Chapare HF	Bolivia (Cochabamba Department)	Unknown	Unknown, resembles Argentinean HF or Sabia infection	Unknown; ribavirin suggested	Unknown
Guanarito	Venezuelan HF	Venezuela (Portuguesa State)	*Zygodontomys brevicauda*	As Argentinean HF	Unknown; ribavirin or convalescent plasma suggested	Unknown; rodent control?
Sabia	Brazilian HF	Brazil	Unknown	Resembles Argentinean HF; a patient in single naturally occurring case had severe hepatitis	Unknown; ribavirin suggested	Unknown

CNS, Central nervous system; *HF,* hemorrhagic fever.

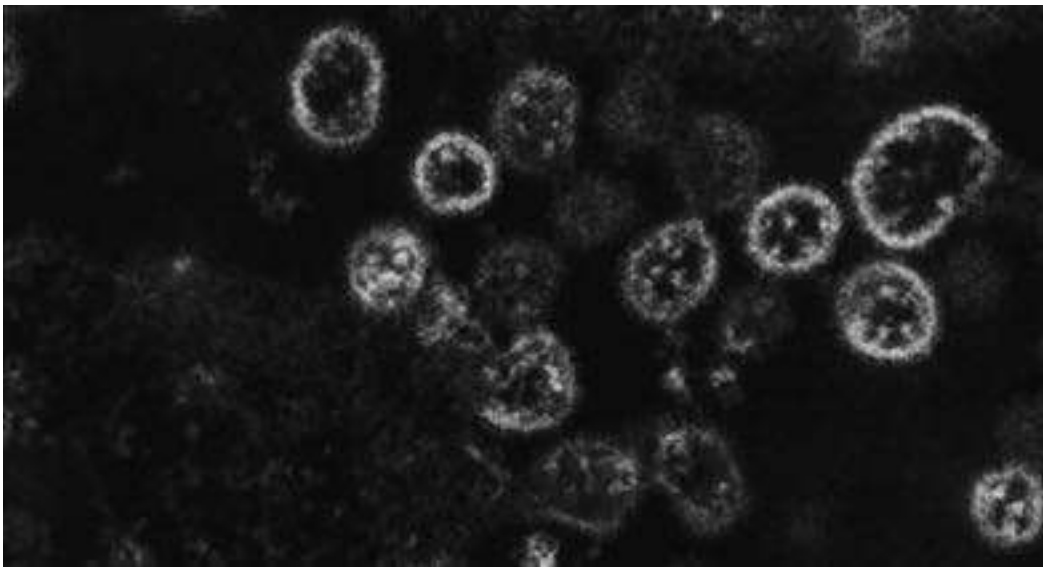

FIG. 167.1 Lassa virus. Electron micrograph of Lassa virus in the first Vero cell passage envelope; electron-dense interior granules can be seen (original magnification, ×121,000).

antigenomic copies of both RNA segments and capped nonpolyadenylated messenger RNA (mRNA) of the four viral genes.[2] Synthesis of viral RNA is initiated from the promoter regions located at the 3′-end of genomic and antigenomic segment RNAs. Viral transcription terminates at the distal side of the stem-loop structures within the intergenic regions that separate viral genes on both L and S RNA segments. These stem-loop structures have been proposed to stabilize the 3′-termini of the viral mRNAs.[3,4] Arenaviruses acquire cap structures of host cell mRNA by a cap-snatching mechanism mediated by the cap-binding activity of NP and endonuclease activity of the L polymerase.[5–7] The matrix protein Z is not required for the activity of the viral replication complex. On the contrary, Z has a dose-dependent inhibitory effect on viral RNA synthesis by locking the L polymerase in a promoter-bound state. This function of Z has been proposed to contribute to the switch between

active expression and replication of the viral genome and virion packaging, ensuring inclusion of the L polymerase into the maturing viral particles.[8–10] This inhibitory effect of Z is highly conserved throughout the *Arenaviridae* family, as it has been demonstrated for both New World[11] and Old World[12] arenaviruses.

Old World and clade C New World arenaviruses use α-dystroglycan, a cellular receptor for proteins of the extracellular matrix, to direct entry into cells.[13] Transferrin receptor 1 (TfR1) is the primary receptor used by the glycoprotein complexes of pathogenic New World arenaviruses; however, cell entry of nonpathogenic viruses is TfR1 independent. Glycoproteins of pathogenic members of the New World group can also use TfR1-independent entry pathways, although less efficiently.[14]

In addition to the functions required to support virus replication and formation of infectious progeny, viral proteins are also involved in the modulation of the host cell response to infection. Thus NP has been demonstrated to contribute to virus-induced inhibition of type I interferon signaling, which has been attributed to the 3′–5′ exoribonuclease activity mapped to the C-terminal domain of NP.[5,15–18] Besides being the main driving force of arenavirus budding and an essential regulator of the viral replication complex, the matrix protein Z has been shown to interact with a number of cellular factors and contribute to repression of cellular translation, evasion of infection-induced apoptosis, and inhibition of type I interferon induction.[19–21]

EPIDEMIOLOGY

Arenaviruses are transmitted by rodents and have high species specificity, with one species usually being the reservoir for one given virus. There are three possible outcomes for infected animals: rapid clearance of virus, development of an acute lethal disease, and persistent inapparent infection with the release of virus into excreta, specifically urine, which is believed to be the main cause of human infections through inhalation of aerosolized viral particles. Both vertical transmission and horizontal spread contribute to the maintenance of virus in the rodent population.

Lymphocytic Choriomeningitis

Although LCMV has potential for worldwide distribution, human cases have been reported and documented only in Europe and the Americas. Moreover, the prevalence of LCMV infection in both nonoverlapping reservoir species, *Mus domesticus* and *Mus Muscullus,* is highly focal, as was evident from surveillance studies conducted in various areas of the United States and Germany.[22] Thus distribution of mice that tested positive for antibody to LCMV in Baltimore, Maryland, and Washington, DC, was found to be significantly clustered within blocks and even households.[23] Similarly, much higher LCMV infection rates among mice were detected in west-central Germany than in the southern or northern parts of the country.[24] This phenomenon can be explained by the highly structured social system of mice, which promotes inbreeding and therefore limits dispersal.

Human cases of lymphocytic choriomeningitis (LCM) most commonly occur in autumn. This pattern of infection activity is associated with seasonal increase of rodent population density and migration of mice into homes and barns caused by cold weather. Additionally, greater stability of LASV virions was demonstrated at low relative humidity and moderate temperatures in the natural environment.[25] Situations associated with wild mouse infection of humans include substandard housing such as mobile homes or inner-city dwellings, the cleaning of rodent-infested barns or outbuildings, and the autumn entry of wild mice into dwellings. Most human infections occur among young adults, likely due to increased professional and personal activities associated with the maintenance of houses and surrounding properties, although people of all ages have been affected. The exact route of transmission of LCMV from infected mice to humans in still not known. Epidemiologic and experimental data suggest aerosolized virions, direct contact, and bites of infected rodents as most likely causes of human infections.[26–28]

The incubation period for LCM in humans ranges from 5 to 10 days. The disease begins as a febrile illness that in severe cases can progress to acute meningitis in 2 to 3 weeks after exposure. Most sporadic LCM cases have been attributed to the handling of laboratory mice or Syrian hamsters; however, several human exposures resulted from contact with hamsters injected with LCMV-infected tumor cell lines.[28] In addition, an epidemic of laboratory-acquired LCM resulted from handling of nude, athymic mice infected through hamster tumor cell lines.[29] Other outbreaks in the United States and Europe resulted from exposure to pet hamsters obtained from breeders with infected stocks.[30,31] LCM has also been associated with organ transplantation as discussed in "Clinical Manifestations."

Lassa Fever

LASV was first isolated in 1969 from a missionary nurse who worked in a clinic in a small town, Lassa, in northeastern Nigeria.[32] Presumably the nurse acquired infection from an obstetric patient who lived in Lassa. The nurse died approximately 1 week after the onset of clinical symptoms. Subsequently, two more nurses who provided care to the first nurse contracted the disease, which resulted in the death of one of them. Virus isolates were recovered in cultured cells from all three cases, and the disease was named Lassa fever.[33]

Several countries in West Africa were initially identified as endemic for LASV including Sierra Leone,[34,35] Guinea,[36,37] Liberia,[38–40] and Nigeria.[41–43] However, after a serologic survey conducted among hospitalized patients with fever and missionaries with a history of a febrile illness, LASV was concluded to be also present in Ivory Coast, Mali, and Central African Republic.[44] The results of an investigation of a case of LF imported to Germany that revealed the index patient had traveled during the incubation period through Ghana, Ivory Coast, and Burkina Faso, further supported the notion that LASV was endemic in larger areas of West Africa.[45] Later, LASV was demonstrated to circulate in Burkina Faso, Ivory Coast, Ghana, Senegal, Gambia, and Mali.[46,47]

The multimammate mouse, *Mastomys natalensis,* was originally identified as the primary reservoir for LASV.[48] Moreover, newborn *M. natalensis* mice infected intraperitoneally develop persistent asymptomatic infection, which suggests the importance of this species for the maintenance of the virus in nature.[49] However, because the taxonomy of the genus *Mastomys* is poorly understood, it still is uncertain which species and particular subspecies can serve as a host for LASV.[50]

LASV has been estimated to cause 100,000 to 300,000 infections and approximately 5000 deaths each year.[51] However, given that up to 55% of the general population living in endemic areas test positive for the presence of LASV-specific antibodies, most infections probably result in a mild disease or are even asymptomatic and do not require hospitalization.[34,36,42,52] This is also supported by the high incidence of seroconversion, from 5% to 20%, of the nonimmune population per year.[34] Infection with LASV presumably occurs through either inhalation of aerosols or contact with body fluids or excreta produced by infected animals. The viral particles are stable in aerosol, and horizontal animal-to-animal transmission has been demonstrated experimentally.[25,53] Hunting and consumption of meat of peridomestic rodents have also been suggested to be an important route of LASV transmission to humans.[37]

The incubation period of LF ranges from 7 to 21 days.[32] The clinical disease begins as a flulike illness characterized by fever, general weakness, and malaise, which may be accompanied by cough, sore throat, and severe headache. Common complications include gastrointestinal manifestations such as nausea, vomiting, and diarrhea.[54] High prevalence of many other acute diseases in West Africa can significantly complicate differential diagnosis of LF based on presenting symptoms.[54,55] Hemorrhagic manifestations may not always be present in LF; however, perturbation of vascular function is likely to play an essential role in LASV infection–induced pathologic changes. In severe cases, the condition of the patient rapidly deteriorates between the 6th and 10th day of illness with severe pulmonary edema; acute respiratory distress; clinical signs of encephalopathy, sometimes with coma and seizures; and terminal shock.[54] Recovery from LF generally begins 8 to 10 days after disease onset. Sensorineural deafness, a common neurologic manifestation, can be observed in the late stages of the disease or in early convalescence in survivors.[56]

Other African Arenaviruses

Several other Old World arenaviruses circulate in Africa; however, none of them have been demonstrated to be pathogenic in humans. A safari-booking agent in Zambia contracted a disease that resembled a viral hemorrhagic fever. The index patient and three secondary cases died

of the disease. A single tertiary case was administered ribavirin and survived. The isolated virus has been identified as a new arenavirus with potentially high case-fatality rate and named Lujo after the geographic locations where the infection was discovered and investigated (Lusaka, Zambia, and Johannesburg, South Africa).[57]

South American Hemorrhagic Fevers

Viral hemorrhagic fevers caused by the arenaviruses JUNV, MACV, and GTOV that circulate in South America pose serious public health problems in Argentina, Bolivia, and Venezuela. Another arenavirus that can induce a lethal disease in humans, SABV, was discovered in Brazil in 1990; however, its rodent reservoir has not been identified, and its impact on public health is currently unclear.[58] A sudden appearance of a new viral hemorrhagic fever was detected in December 2003–January 2004 near Cochabamba, Bolivia, 400 km away from the epicenter of MACV activity. The virus isolated from the specimens collected from a fatal case was named Chapare. However, the characterization of this viral agent is still incomplete and missing vital pieces of information such as the natural rodent host and well-defined epidemiology and clinical picture.[58]

JUNV, the causative agent of Argentinian hemorrhagic fever (AHF), causes chronic infections of rodents that belong to the species *Calomys musculinus*.[59] AHF initially occurred in the region of humid pampas of northern Buenos Aires province and then spread to a larger area encompassing two more provinces and putting 5 million people at risk. The population of reservoir rodents in cornfields peaks between February and May, and therefore the disease primarily affects men engaged in harvesting corn. Human infections are believed to occur through either inhalation of aerosolized viral particles, which is considered the most common route of transmission, or direct contact of abraded skin with blood and tissues of infected rodents crushed by agricultural machinery. Person-to-person transmission is very rare and may occur via direct contact with body fluids of a viremic patient; nosocomial infections have also been reported.[60] Before the introduction of a specific vaccine, approximately 200 to 2000 cases of AHF were reported annually, and mechanization of the harvesting process did not result in a substantial decrease of AFH cases. Development of a live-attenuated vaccine, Candid 1, by the US Army Medical Research Institute of Infectious Diseases led to a significant reduction in the incidence of AFH and reduced the number of reported cases to less than 100 per year.[61]

Circulation of MACV, the causative agent of Bolivian hemorrhagic fever (BHF), is restricted to the tropical savanna of the Beni Department in the northeastern part of Bolivia. The large vesper mouse, *Calomys callosus,* is the rodent host for MACV. In this region the mice can freely enter houses, and human exposure is believed to occur by aerosols from infected animals or consumption of food contaminated with urine of infected rodents. The incidence of infection is greatest from the late rainy (April) to the early dry (July) seasons. The hallmark feature of BHF is that occurrence of an epidemic is usually followed by several years of quiescence. In town epidemics, where people of all ages and both sexes are equally exposed, the disease equally affects all these categories. On remote ranches and in fields, men (usually agricultural workers) are a predominant target. Person-to-person as well as nosocomial infections have been reported; however, this route of transmission is not common.[62,63] For both AHF and BHF, the incidence of human infections and horizontal spread of JUNV and MACV among reservoir rodents correlate with the fluctuations in the rodent population density.[64]

In 1989 in the Guanarito municipality of Portuguesa State and adjacent regions of Barinas State of Venezuela, human cases of a new viral hemorrhagic fever, Venezuelan hemorrhagic fever, were discovered. The new virus was named Guanarito (GTOV) after the geographic location where most human cases were detected. The cane mouse *Zygodontomys brevauda* was identified as the reservoir. Venezuelan hemorrhagic fever also has a cyclic behavior, and epidemic periods of high incidence occur every 4 to 5 years mainly affecting male agricultural workers between 14 and 54 years of age.[65]

Based on clinical observations of patients with AHF and BHF, the incubation period after natural exposure to South American arenaviruses has been determined to range from 5 to 19 days, with a median of 7 to 12 days. The incubation period of South American hemorrhagic fevers can be significantly shortened after parenteral infections, and the onset of symptoms can begin 2 to 6 days after virus exposure.

Other Arenaviruses

Several other arenaviruses related to LASV and LCMV and belonging to the Old World group have been discovered, such as Mopeia, Mobala, and Ippy, but no human disease has been found to be associated with these viral agents. Moreover, it is unclear if these arenaviruses can productively, if at all, infect humans. In addition, these viruses are attenuated in the two most relevant to human disease animal models of infection, nonhuman primates and guinea pigs.[26,53] Similarly, there are many arenaviruses that circulate in both Americas and belong to the New World group (this group is larger in general) that have not been described as human pathogens.[2] However, some of these viruses have caused infections of laboratory personnel without inducing disease (Pichinde virus), and many are currently being monitored, for example, Whitewater Arroyo virus, which is believed to have caused fatal human disease in the western United States.[66]

PATHOGENESIS

Rodents

Arenaviruses can cause chronic infections of their reservoir rodents that are clinically benign. Important variables such as the age of animals, route of infection, and the strain of virus are used to determine the outcome of infection and pathologic manifestations.

Natural infection with LCMV of newborn or fetal mice results in a chronic infection disseminated to many organs with high levels of viremia that apparently does not affect the growth and fertility of these animals and fails to induce an inflammatory response. However, peripheral infection of adult mice results in a transient immunizing infection. The use of inbred mice led to significant achievements in understanding of the mechanisms of T cell–mediated immunopathology, as pathogenicity of LCMV strains with experimentally manipulated genomes significantly differs in these animals. Although newborn mice develop chronic infection with viremia, T-cell immunity is suppressed. However, depending on the particular genotype of infected mice, different quantities of virus-specific antibodies are produced and form complexes with viral antigens. On one hand, trapping of circulating virus-antibody complexes in the glomerulus ultimately leads to the development of chronic glomerulonephritis.[67] On the other hand, intracerebral injection of adult mice produces acute fatal choriomeningitis that is characterized by extensive infiltration of cytotoxic T cells.[68]

The results of laboratory infections of natural reservoir *Calomys* rodents with JUNV or MACV resemble the outcome observed in LCMV-infected mice. However, the infection is never lethal in these animals by any route at any age, formation of antigen-antibody complexes have never been demonstrated, and some mice inoculated as adults develop chronic viremic infections, which completely mimic that observed in very young mice infected with LCMV. Also, chronic MACV infections can induce a microcytic hemolytic anemia that would result in chronic splenomegaly, which can be used as a marker for infection.[26,59,69]

Infection of *Mastomys* rodents with LASV similarly results in a chronic infection with no acute response and absence of inflammatory responses. Alternatively, some animals, depending on age, can mount an effective immune response and clear infection.[49] According to field studies, permanently infected animals have little or no specific antibodies but carry significant amounts of virus in tissues and body fluids, particularly in urine.[70]

Arenavirus infections of nonreservoir rodents result in benign, self-limited infection and immunity. Guinea pigs, however, may experience severe acute disease and serve as useful models of arenaviral hemorrhagic fever, in particular, AHF.[53]

Nonhuman Primates

Monkeys represent useful, although not optimal, models for studying pathogenesis and development of vaccines and therapeutic interventions to combat arenavirus infections in humans. LCMV readily infects macaque monkeys by inhalation of aerosolized viral particles, but infection results in a hemorrhagic fever rather than a central nervous system disease. Marmosets are also susceptible to lethal LCMV infection. Of note, feeding

of zoo monkeys with infected newborn mice caused sporadic fatal hepatic disease.[71] Several nonhuman primate species have been evaluated as potential models for LF, including squirrel monkeys, capuchin monkeys, marmosets, hamadryas baboons, African green monkeys, cynomolgus monkeys, and rhesus monkeys.[49,53,72–77] Experimental LASV infection of common marmosets results in systemic disease that has clinical and pathologic features highly similar to features observed in fatal cases of LF in human patients.[76] Small-particle aerosol infection induces a lethal disease in rhesus monkeys with pathologic findings similar to findings in humans.[25] MACV and JUNV are also pathogenic for nonhuman primates; however, the clinical and pathologic disease presentation is highly species specific. The use of nonhuman primates as well as guinea pig models that are more accessible for experimental work has led to significant advancements in our understanding of pathologic mechanisms during hemorrhagic fever diseases in humans.[53,78–80]

Humans

Limited histopathologic data are available from fatal human cases of LCM. However, a detailed description of a single case of human encephalitis shows a marked neuronal pattern of LCNV infection.[81]

The current knowledge of LF pathogenesis permits reliable re-creation of the chain of events that leads to disease development and fatal outcome. Considering the high mortality and dramatic course of the disease, the pathologic findings do not provide the basis that would explain the mechanism of disease progression. Very little or even absence of inflammatory response and variable levels of necrosis have been observed in liver, spleen, and adrenal glands of LASV-infected patients.[55,82] Although focal hepatic necrosis is commonly observed, the histopathologic changes in the liver are not sufficient to explain the lethal outcome. Data from experimentally infected animals suggest direct viral infection of endothelial cells and release of inflammatory mediators, possibly from infected macrophages, to be a central mechanism causing vascular dysfunction and, ultimately, shock in arenavirus hemorrhagic fevers.[82,83]

Patients who die of AHF and BHF also show very limited histopathologic changes in tissues. No vasculitis and virtually no inflammatory response is observed in the organs. However, small focal hemorrhages are commonly detected primarily in mucosal surfaces. On average, hepatic necrosis is more pronounced in cases of LF than AHF and BHF, but Councilman-like bodies are distinctly detectable in all three diseases. In addition, bronchopneumonia of either primary viral or secondary bacterial origin, which is more common, is often present. Concentrations of circulating endogenous interferon-α are highly predictive of the disease outcome in patients with AHF. The highest levels are reached 6 to 12 days after the onset of specific symptoms and indicate a poor prognosis for survival.[84] Moreover, experimental and clinical data suggest that interferons and high levels of proinflammatory cytokines may have a detrimental rather than beneficial effect in arenavirus infections.[85,86]

High titers of infectious virus and large amounts of viral antigens are detected in tissues of fatal cases of human infections and monkeys experimentally infected with LASV, JUNV, and MACV. Major replication sites of New World viruses JUNV and MACV are spleen, lymph nodes, and bone marrow, whereas LASV is found in many visceral organs and, notably, in placentas of pregnant women. Mesothelial surfaces are markedly infected with LASV and thus may further facilitate the development of serous effusions.[83]

CLINICAL MANIFESTATIONS

Clinically apparent infections with all the arenaviruses are similar in presenting manifestations. Fever is typically insidious in onset and is accompanied by headache and significant myalgia and malaise. Relative bradycardia is common, as is dysesthesia, particularly hyperesthesia of the skin. Thereafter the various diseases pursue different courses.

Lymphocytic Choriomeningitis Virus

LCMV infections are most commonly febrile illnesses with headache and systemic symptoms and are associated with leukopenia and thrombocytopenia.[26–31,87] After 3 to 5 days of nonspecific illness, occasionally with lymphadenopathy and a maculopapular rash, the fever subsides, but it frequently recurs in 2 to 4 days with several days of even more severe headache. Patients may exhibit frank meningitis during this second febrile period. Cerebrospinal fluid pressure usually is elevated, occasionally even with papilledema; the protein concentration ranges from 50 to 300 mg/dL; and several hundred lymphocytes per cubic millimeter are commonly observed. Hypoglycorrhachia is found in fewer than one-third of patients. Encephalomyelitic infection may manifest as encephalitis; psychosis; paraplegia; or disturbances of cranial, sensory, or autonomic nervous function. Ependymal inflammation has resulted in transient aqueductal stenosis.

Occasionally, patients develop orchitis, myocarditis, arthritis, or alopecia. Orchitis develops 1 to 3 weeks after the onset of the illness; it is usually unilateral and painful and resolves within 2 weeks. Myocarditis is detected by electrocardiographic changes and labile tachycardia during and after the second febrile period. Arthritis occurs occasionally during convalescence, principally affects the metacarpophalangeal and proximal interphalangeal joints, and is marked by minimal swelling and redness; it generally resolves within a few weeks.

The second febrile episode and some of the complications of convalescence have long been believed to represent immunopathologic phenomena. Antibodies detectable by immunofluorescence appear at about this time, and the lymphocytes in the cerebrospinal fluid presumably are analogues of the T lymphocytes that cause lymphocytic choriomeningitis in the intracerebrally inoculated adult mouse.

Additional data supporting this idea have gained support from unexpected findings in renal transplantation. Thirteen recipients of kidney, lung, or liver transplants from four organ donors developed severe disease with multiorgan involvement and clear evidence of LCMV infection.[88–90] None of the transplant recipients developed classic LCMV aseptic meningitis. All except one died with severe disease and disseminated infection similar to that seen in LF. This pattern has also been described in patients with tumors receiving LCMV as a supposed oncolytic therapy.[91] The only survivor had decreased immunosuppression and received intravenous ribavirin. Only one of the donors had documented contact with a rodent, a hamster shown to be infected with LCMV and coming from LCMV-infected pet suppliers.[92]

Lassa Fever

Most LASV infections result in either mild or subclinical disease manifestations and do not require hospitalization.[34] Severe hemorrhagic fever that involves multiple organs occurs in only 5% to 10% of cases of infection. Case-fatality rates among hospitalized patients consistently range from 15% to 25%. The incubation period of LF usually lasts 7 to 21 days.[32,54] The onset of clinical disease is characterized as a flulike illness with fever, general weakness, and malaise and may be accompanied by cough, sore throat, and severe headache. Common symptoms are nausea, vomiting, and diarrhea. The differential diagnosis of LF based on the presenting symptoms can be problematic due to the many other acute undifferentiated febrile illnesses circulating in West Africa.[32,44,54,93] A case-control study in patients hospitalized with LF was performed in Sierra Leone: 74% reported retrosternal chest pain; 60%, sore throat; 62%, back pain; 62%, cough; 50%, abdominal pain; 49%, vomiting; 26%, diarrhea; 25%, conjunctivitis; and 10%, facial edema. In addition, 43% of patients developed proteinuria. Mucosal bleeding at any time was observed in only 17% of LF cases. In this study, the combination of fever, pharyngitis, retrosternal pain, and proteinuria predicted 81% of laboratory-confirmed cases of LF. Fatal outcome was best predicted by the combination of fever, sore throat, and vomiting.[54] Involvement of the central nervous system is commonly characterized by encephalopathy, encephalitis, meningeal signs, and convalescent cerebral syndromes.[54,94]

The level of viremia is highly predictive of the disease outcome. In one study that involved 137 patients with LF, patients who developed viremia of $<10^3$ median tissue culture infectious dose ($TCID_{50}$)/mL on the day of hospitalization had 3.7 times greater chance of survival than patients admitted with higher virus loads in blood. Similarly, the probability of fatal outcome in patients with serum titers $>10^3$ $TCID_{50}$/mL and serum levels of aspartate aminotransferase (AST) ≥150 IU/L was 21 times higher than that in patients not meeting either of these criteria. In virtually all fatal cases, patients had high levels of viremia at the time of death, ranging from 10^3 to 10^8 $TCID_{50}$/mL.[95] Virus burden in blood reaches its maximum peak between day 4 and 9 after the onset of

symptomatic disease, which is followed by pronounced clinical manifestations. Recovering patients with LF clear virus from the bloodstream about 3 weeks after the beginning of illness.[39,95–97]

The clinical manifestations in fatal cases of LF generally occur during the second week of illness and include hypotension, peripheral vasoconstriction, reduced urinary output, facial and pulmonary edema, and occasionally pleural effusions and ascites. Minor hemorrhages are often observed in mucosal surfaces, which strongly suggests the involvement of diffuse capillary leakage. Moreover, these patients often experience myocardial depression that can potentially contribute to the increase in vascular permeability.[54] Patients who do not develop vascular leakage may have other complications during the second and third weeks of illness. Unilateral or bilateral eighth cranial nerve deafness is most commonly observed and was observed in almost one-third of hospitalized patients.[56] Some cases of sensorineural deafness are mild or improve with time; however, LF is a major cause of hearing loss in endemic areas.

South American Hemorrhagic Fevers

The South American hemorrhagic fevers resemble one another. The onset of the clinical disease is insidious, with anorexia, malaise, headache, chills, myalgias, and fever (38°C–39°C). A few days later the disease may progress and patients develop gastrointestinal, cardiovascular, and potentially neurologic signs and symptoms. Patients may also report retro-orbital pain, nausea or vomiting, epigastric pain, photophobia, dizziness, and constipation or mild diarrhea. Common absence of productive cough or nasal congestion is helpful to exclude initial symptoms of conditions caused by influenza or other acute respiratory infections.[98–100]

The first clinical manifestations of South American hemorrhagic fever are nonspecific, so the history of potential exposure to infected rodents is crucial. Among the infectious diseases, the differential diagnosis includes typhoid fever, hepatitis, infectious mononucleosis, leptospirosis, hantavirus pulmonary syndrome, dengue, dengue hemorrhagic fever, and rickettsioses. Malaria should also be considered in endemic areas. In the respective endemic areas or in patients with a history of travel to such areas, a febrile syndrome with proteinuria, leukopenia, and thrombocytopenia is suggestive of one of the South American hemorrhagic fevers.[101,102]

Intrauterine Infection

Arenaviruses readily invade the fetus, whether in their natural reservoir, in laboratory animals, or in humans. Pregnant women with LF often miscarry and have a high mortality rate,[103] and similar observations have been made in pregnant women with AHF and BHF. LCMV infection in pregnant women can lead to fetal infection, hydrocephalus, microcephaly, or chorioretinitis, or all of these disorders.[104–107] Because viral antibody rates indicate that about 5% of adults in large cities of the United States have been infected with LCMV, congenital infection may be more common than appreciated.[108,109]

DIAGNOSIS

The specific diagnosis depends on demonstration of the infecting virus or one of its products in acute serum samples. Reverse-transcriptase polymerase chain reaction is the most sensitive and fastest diagnostic method. In general, viremia and antigenemia are readily detected during the acute phase, when patients present with fever, and disappear as the patient recovers. The presence of viral nucleic acid can be detected during the same period and perhaps 1 or 2 days longer. Seroconversion, notably immunoglobulin M antibodies, may be detectable in hospitalized patients; however, antibodies usually appear early in convalescence. Diagnosis of initial patients in any outbreak benefits from study of virus isolates and classic serology. Most hemorrhagic fever viruses are hazardous and should be isolated or studied only under Biosafety Level 4 containment. Ideally, collected blood samples from patients with hemorrhagic fever should be collected early in the course of illness. Both serum and blood clot should be frozen as soon as possible and kept frozen until virology assays can be performed. A second serum sample must be collected before discharge for comparative serology. In fatal cases, a full autopsy should be performed, if possible, with a complete set of organs collected in formalin for diagnostic studies; spleen, liver, and lymph nodes should be collected frozen for virus isolation. Classic histopathology is often useful when yellow fever, Rift Valley fever, or a filovirus infection is suspected and when diagnosing some of the confounding diseases. Immunohistochemistry on fixed tissues can usually make a definitive diagnosis possible.

TREATMENT

Most information about treatment of hemorrhagic fevers in the Americas comes from reports from Argentina. AHF has a 6- to 14-day prodromic phase, and 8 to 12 days following its onset, about 20% to 30% of patients advance to the neurologic and hemorrhagic phase involving symptoms such as confusion, convulsions, coma, and bleeding from body orifices. Current recommended therapy of AHF involves treatment with immune plasma from convalescent patients that is very effective if initiated in the first week of disease.

Immune plasma therapy can ameliorate the immediate disease symptoms of AHF and significantly reduce mortality if administered early. However, in about 10% of cases, this therapy results in a late neurologic syndrome due to unknown mechanisms. Further limitations to the use of this treatment are dictated by a short supply of plasma. In vitro and in vivo studies have documented the prophylactic and therapeutic value of the nucleoside analogue ribavirin (Rib) against several arenaviruses including JUNV.[101,102,110,111] (Also see Chapter 45.) Importantly, Rib reduced both morbidity and mortality associated with JUNV infections in humans[101] when given early in the course of clinical disease. Some limitations of the use of Rib are its frequent and sometimes significant toxicities, including anemia and congenital disorders, and the need for intravenous administration for optimal efficacy.

The intravenous administration of Rib to patients with LF admitted to the hospital in Sierra Leone with AST elevations of at least 150 IU reduced the mortality rate from 55% to 5% when treatment was begun before day 7 of the disease.[112] However, a positive effect on survival was achieved at all stages of infection. Ribavirin has been used with apparent success in aborting a Sabia virus laboratory infection[113] and in treating patients with BHF.[114] Given the similarities to LASV and similar preclinical test efficacy, the drug should be considered for use in the treatment of any serious arenaviral infection. Close contacts of patients with arenaviral infections or possible bioterrorist exposures should not be given prophylactic ribavirin, but rather should be monitored for the appearance of fever.[115] Ribavirin therapy should be begun expectantly if fever is confirmed. Intravenous ribavirin is not licensed in the United States, and arenavirus treatment is not a US Food and Drug Administration–approved indication for the oral drug.

Supportive treatment is also highly recommended in hospitalized patients and consists of adequate hydration and effective management of the neurologic alterations, blood losses, shock, and additional superimposed infections, if acquired. In the case of AHF, pneumonia is the most common secondary bacterial infection. It can be accompanied by radiographic changes and an increase in fever, but not by leukocytosis; pneumonia usually responds to antibiotics. However, most of the severe reported clinical manifestations are neurologic, and it may be necessary to sedate agitated patients with diphenhydramine or diazepam; diazepam also gives some protection against seizures.

PREVENTION

Prevention of arenaviral infection may be approached by interdicting transmission from rodents to humans, from person to person, and from infected specimens to laboratory workers or by passive or active immunization. Community rodent control completely halted a major outbreak of BHF[116]; elimination of infected laboratory hamsters controlled LCMV outbreaks.[28,117] However, a household rodent control study of LASV infection yielded disappointing results.[118]

Person-to-person spread within hospitals has been a problem with LF. In endemic situations such as in Sierra Leone, partial spatial isolation of patients and the use of enteric precautions such as gloves, gowns, and careful disposal of patient wastes and fomites have generally served to prevent nosocomial outbreaks. Segregation of patients on the basis of the risk for death and likely content of virus in blood and body fluids by measuring AST levels should be of value. Rarely, hospital outbreaks appear to have been caused by infectious aerosols.[119,120] Thus wherever

practical, it is advisable to place patients in single rooms with isolated negative-pressure airflow and to provide medical staff with goggles and positive-pressure filtered air respirators or absolute filter respirators.[121] All potentially contaminated refuse or specimens should be double-bagged and the outer bag rinsed with 0.6% sodium hypochlorite before removal from the patient's room. Isolation of patients should continue until multiple blood and urine specimens are virus negative. Condoms should be used for a period in convalescence because of the occasional sexual transmission of arenaviral hemorrhagic fevers. In the recognized cases of LF that have been exported from Africa, no secondary transmission has yet been detected in contacts or in medical staff, indicating that a conservative approach to isolation provides a reasonable standard of safe care in most cases.

Laboratory-acquired infection is a major problem because all arenaviruses are infectious as aerosols. Several infections, some fatal, have occurred in laboratory workers. Thus it is imperative that work with all arenaviruses except LCMV be conducted in special laboratories with Biosafety Level 4 containment. Clinical pathologic tests, particularly in LF patients, also present problems. Aerosols must be minimized or contained. Acid treatment inactivates virus for leukocyte counts, and alcohol fixation is useful for blood smears; heating serum at 60°C for 1 hour is feasible for measuring heat-stable substances.[122]

There are no arenavirus vaccines licensed in the United States. Human vaccine against AHF is available in Argentina. The vaccine was produced by serial passages prototype XJ strain of JUNV in newborn mice. This resulted in the generation of Candid 1 vaccine, which was generated from the 44th mouse brain passage of XJ strain of JUNV and found to be attenuated in guinea pigs. Preclinical studies at the US Army Medical Research Institute of Infectious Diseases supported the safety, immunogenicity, and protective efficacy of Candid 1 in both guinea pigs and rhesus macaques. Subsequent clinical studies performed in Argentina have shown Candid 1 to be an effective and safe vaccine in humans.[123] This vaccine received approval for human use in 2006 by the regulatory agency of Argentina for use exclusively in Argentina. Experimental data from animal models indicate cross-protection of this vaccine against Machupo virus.

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168 Human T-Cell Leukemia Viruses (HTLV-1, HTLV-2)

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SHORT VIEW SUMMARY

Definition

- Human T-cell leukemia virus (HTLV) is a human retrovirus of the subfamily Retroviridae and genus *Deltaretrovirus*.
- It causes chronic human infection by integration of proviral DNA into the somatic DNA of host T lymphocytes, with expansion of infectious burden by proliferation of infected lymphocytes more importantly than by production of free virus particles.
- To date, four types of the virus have been discovered: HTLV-1 and HTLV-2 infections are estimated in millions of humans, and HTLV-3 and HTLV-4 have occurred in only a few isolates since their discovery in the past decade.
- HTLV-1 causes adult T-cell leukemia-lymphoma (ATL), and both HTLV-1 and HTLV-2 cause HTLV-associated myelopathy (HAM).

Epidemiology

- HTLV-1 is endemic to central Africa, the Caribbean basin, parts of South America, and southwestern Japan, and in other discrete geographic areas. There is limited genetic variation and close homology to primate T-lymphotropic viruses, suggesting several instances of cross-species transmission, likely by bites from apes and monkeys sustained by humans.
- HTLV-2 is endemic to Amerindian tribes in North, Central, and South America and to Central African pygmies. It is hyperendemic among injection drug users in North America and Europe.
- Both HTLV-1 and HTLV-2 are transmitted by mother-to-child transmission predominantly by breastfeeding, by sexual transmission, and by parenteral infection.
- In populations in which they are endemic, both retroviruses show an increased prevalence in older individuals, and prevalence is frequently higher in females than in males.

Diagnosis and Pathogenesis

- HTLV antibodies may be detected with screening enzyme immunoassays and supplemental Western blot/recombinant immunoblot, with differentiation of HTLV-1 from HTLV-2 through use of type-specific antigens in supplemental assays.
- Polymerase chain reaction (PCR) assay to detect HTLV-1 or HTLV-2 proviral DNA in peripheral blood mononuclear cells may be performed if serology is inconclusive. PCR assay is also useful in detecting infection in infants carrying passive maternal antibodies.
- Most infected individuals are asymptomatic. Two percent to 4% of those infected with HTLV-1 will develop ATL, characterized by monoclonal integration of HTLV-1 provirus into a T-lymphocytic malignancy.
- One percent to 4% of those infected with either HTLV-I or HTLV-2 will develop HAM, a spastic paraparesis affecting predominantly the lower limbs and hyperactive bladder in a person infected with HTLV.

Therapy

- No treatment is currently recommended for persons with asymptomatic HTLV-1 or HTLV-2 infection. Periodic follow-up should include medical history, physical examination, and complete blood cell count to detect ATL or HAM.
- The lymphoma type of ATL is often treated initially with vincristine, cyclophosphamide, prednisolone, and doxorubicin with the addition of methotrexate, more complex 9- and 10-drug regimens. These have shown some success without prolonging long-term survival. Chronic, smoldering, and leukemia types of ATL often respond better to a combination of zidovudine and interferon-α.
- Therapy for HAM is currently unsatisfactory. Corticosteroids may produce improvement, but side effects limit the duration of therapy. Interferon-α and interferon-1β have been used with some success.

Prevention

- To interrupt mother-to-child transmission, HTLV-infected pregnant women in middle- and high-income countries should not breastfeed their infants if this can be accomplished safely.
- The use of condoms should be recommended to prevent sexual transmission.
- Injection drug users with HTLV infection should be instructed not to share needles.
- The US Food and Drug Administration recommends HTLV testing of all donated blood but not currently of donated organs. No vaccine is currently available.

Human T-cell leukemia virus type 1 (HTLV-1), the first recognized human retrovirus, was described in 1979 by Poiesz and coworkers,[1,2] who isolated retroviral particles with type C morphology budding from fresh cultured lymphocytes of a 28-year-old American man with a diagnosis of cutaneous T-cell lymphoma similar to cases seen in persons residing in Caribbean countries. Soon thereafter, Yoshida and associates[3] and Watanabe and colleagues[4] similarly isolated a new retrovirus from cases of adult T-cell leukemia-lymphoma (ATL), which was common in southwestern Japan at that time, and the two isolates were subsequently determined to be the same virus. The long-standing search for a human homologue to cancer-causing retroviruses of animals, first discovered at the beginning of the 20th century, ended at a time when most researchers had abandoned this quest and focused instead on viral transforming genes that occur as oncogenes in human tumors. HTLV type 2 (HTLV-2) was identified 2 years later from a case of variant T-cell hairy cell leukemia and has a different epidemiology and disease profile compared with HTLV-1.[5] More recently, rare isolates of two new HTLV types have been found among central African hunters and were designated HTLV-3 and HTLV-4 in 2005.[6,7] The techniques used to isolate and characterize these viruses provided the intellectual and technical basis for the discovery of the human immunodeficiency virus (HIV) in 1983, which was shown to be the cause of acquired immunodeficiency syndrome (AIDS) in 1984.

Within the taxa of DNA and RNA reverse-transcribing viruses, the HTLV viruses, along with bovine leukemia virus, are classified in the subfamily Retroviridae within the genus *Deltaretrovirus* (formerly *Oncovirus*).[8] The oncogenic properties of these viruses and their molecular

structure distinguish them from retroviruses HIV type 1 (HIV-1) and HIV type 2 (HIV-2), which are members of the genus *Lentivirus*. Both oncoviruses and lentiviruses are capable of prolonged asymptomatic infection.[8] In vitro, however, HIV-1 and HIV-2 have cytopathic effects on human T cells, whereas HTLV-1 and HTLV-2 are capable of transforming T cells, resulting in immortalized cell lines.

HTLV-1 has been shown to be associated with ATL and with a unique form of progressive neurological disease known as HTLV-associated myelopathy (HAM), also known as tropical spastic paraparesis (TSP). HTLV-2 is associated with HAM but is not known to cause leukemia or lymphoma. Although most HTLV-1 and HTLV-2 infections are asymptomatic, chronic infection with either retrovirus may cause other, more unusual immunologic and infectious complications.[9]

STRUCTURE AND MOLECULAR ORGANIZATION

The HTLV viruses are approximately 100 nm in diameter with a thin electron-dense outer envelope and an electron-dense, roughly spherical core (Fig. 168.1). Their genomic structure is illustrated in Fig. 168.2. The total provirus genome consists of roughly 9000 nucleotides with two flanking identical sequences. Long terminal repeats (LTRs) at the 5′ and 3′ ends of the genome are organized into three identical regions (U5, R, U3) that control gene expression through the requisite signals: enhancer, promoter, transcription initiation, and transcription termination and polyadenylation. Overall, there is 65% nucleotide homology between sequenced HTLV-1 and HTLV-2 isolates, 60% between HTLV-1 and HTLV-3,[10] and 62% to 71% between HTLV-4 and HTLV-1, HTLV-2, and HTLV-3, respectively.[11] Retroviral genes generally code for large overlapping polyproteins that are processed by a virally encoded protease and cellular proteases into functional peptide products. The HTLV viruses share with other replication-competent retroviruses the main genomic open reading frames (ORFs) of *gag* (group-specific antigen), *pro* (protease), *pol* (polymerase), and *env* (envelope) encoding for structural and enzymatic proteins (Table 168.1). However, unlike other vertebrate leukemia viruses, these deltaretroviruses have an additional region called *pX*. In HTLV-1, the *pX* region has six ORFs. Five of these reside on the plus strand, and the sixth, HBZ, resides on the minus strand. Of these six *pX* genes, three (*tax, rex,* and *HBZ)* have been implicated as crucial in sustaining HTLV infection by modulating replication and expression of regulatory proteins. As shown in Fig. 168.2, two overlapping reading frames are involved in the expression of both of these gene products translated from a doubly spliced messenger RNA (mRNA) involving the initiation codon from *env* and the remaining sequences from the *pX* region. In addition, ORFs encoding APH-2, APH-3, and APH-4 (antisense protein of HTLV) transcribe from the minus strand of the 3′ LTR in HTLV-1, HTLV-2, HTLV-3, and HTLV-4, respectively.[12] The detailed roles of these proteins are still being elucidated but may involve transcriptional regulation.

gag, pro, pol, and *env*

The *gag* gene encodes structural proteins of the nucleocapsid, capsid, and matrix, also named p15, p24, and p19 GAG proteins, respectively. The *pro* gene slightly overlaps *gag* and *pol* and encodes protease, which cleaves GAG and GAG/POL polypeptides into proteins of the mature virion. The *pol* gene encodes the enzymes reverse transcriptase, which generates a double-stranded DNA from the RNA genome, and integrase, which integrates viral DNA into the host-cell chromosomes. The polymerase region contains the largest ORF in the HTLV genome, potentially able to encode an 896–amino-acid product for HTLV-1 and a 982–amino-acid product for HTLV-2. The polymerase genes of HTLV-1 and HTLV-2 share only 56% homology based on their predicted amino-acid sequences. The *env* gene encodes the major components of the viral coat: the surface glycoprotein of 46-kDa (gp46) and the transmembrane glycoprotein of 21-kDa (gp2l). Although HTLV-1, HTLV-2, HTLV-3, and HTLV-4 share the same overall genetic organization, they show some diversity at the nucleotide level, exhibiting a variable degree of amino-acid homology between viral capsid and envelope proteins.

Production of the GAG proteins derives from the translation of the full-length mRNA, which yields a large precursor polypeptide that is subsequently cleaved by the virally coded protease. POL proteins are expressed as polyproteins, both GAG-PRO and GAG-PRO-POL, and production is mediated by ribosomal frameshifts between *gag* and *pro* ORFs. For the POL proteins, production depends on translation made possible when the stop codon of the *gag* gene is bypassed, leading to a large polypeptide including GAG- and POL-related proteins, which are subsequently cleaved into functional proteins by the viral protease. Production of the ENV surface and transmembrane proteins involves translation of a spliced message (see Fig. 168.2), which results in an envelope precursor cleaved into the subunits. The precursor proteins have characteristic molecular weights that Western blot (WB) analysis can detect immunologically.

TAX

HTLV-1 TAX is a 40-kDa protein (p40), and HTLV-2 TAX is a 37-kDa protein (p37).[13,14] These proteins localize primarily to the nucleus of

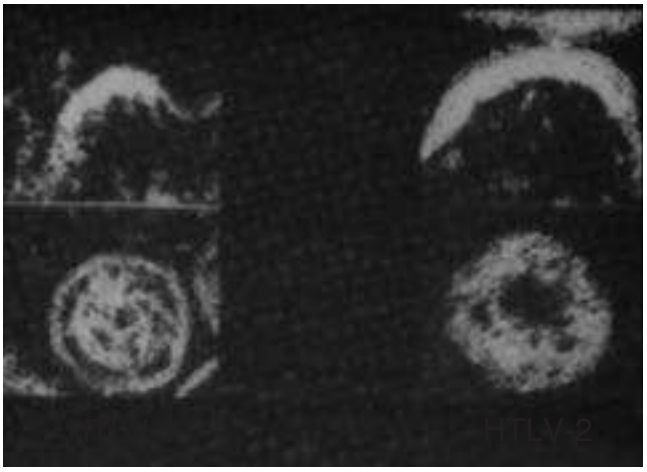

FIG. 168.1 Electron micrographs of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2. HTLV-1 and HTLV-2 have a diameter of approximately 100 nm. The budding particles are shown for each virus *(top)* and the mature virion *(bottom).* Both types of viruses have a roughly spherical electron-dense core.

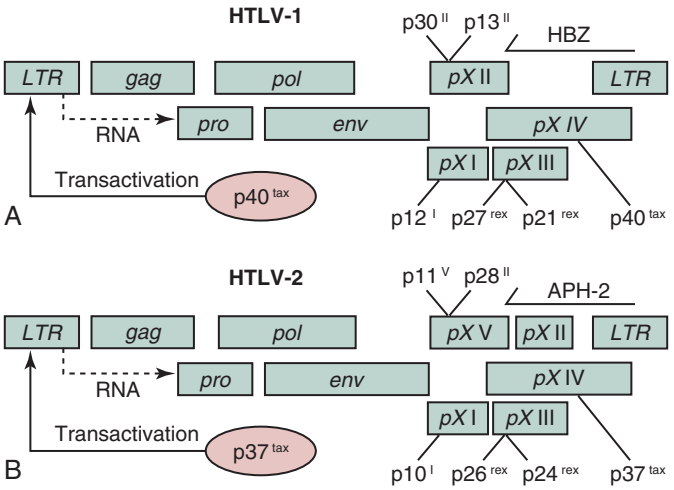

FIG. 168.2 Genomic structures of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2. (A) HTLV-1. (B) HTLV-2. *APH-2,* Antisense transcribed antisense protein of HTLV-2 gene involved in transcription regulation; *env,* envelope gene; *gag,* group-specific antigen whose products form the skeleton of the virion (matrix, capsid, nucleocapsid, nucleic acid–binding protein); *HBZ,* antisense transcribed HTLV-1 basic zipper protein involved in cell proliferation; *LTR,* long terminal repeat; *pol,* gene for reverse transcriptase and integrase; *pro,* gene for protease; *rex,* viral regulatory gene involved in promoting genomic RNA production; *tax,* transactivator gene.

TABLE 168.1 Major Structural and Regulatory Proteins of HTLV-1

VIRAL GENE	GENE PRODUCT (PROTEIN SIZE [kDa])	FUNCTION
5′ *LTR*		Regulation of viral gene expression and regulation of viral expression
5′ *LTR* and 3′ *LTR*		Integration points for provirus into host genome
hbz	HBZ	*hbz* mRNA is found in ATL tumor tissue and is responsible for lymphocyte proliferation
		HBZ protein prevents or suppresses Tax-mediated viral transcription
gag	p15	Nucleocapsid (NC) is a small basic protein found in the virion in association with genomic RNA
gag	p19	Matrix (MA) protein is myristoylated and anchored in the plasma membrane
gag	p24	Capsid (CA) protein forms the major internal structural feature of the core shell of the virion
gag	p53	Gag precursor
pro	Protease	Cleaves GAG precursor into CS, MA, NC, and Pol precursor in reverse-transcriptase and integrase proteins
pol	Integrase	Integrates viral DNA into the host-cell genome
pol	Reverse transcriptase	Reverse transcriptase generates a double-stranded DNA from the single-stranded RNA genome
env	gp21 and gp46	Envelope transmembrane and surface glycoproteins
pX	TAX (p40)	Transactivator that enhances transcription of viral promoter and alters transcription from cellular gene promoters
pX	REX (p27)	Regulator of unspliced and singly spliced viral mRNA transport from the nucleus to the cytoplasm
pX	$p12^{I}$	Downregulates major histocompatibility complex class I trafficking to the surface to evade CTLs; elevates cytoplasmic calcium, increases T-cell activation, increases NFAT activation, enhances LFA-1–mediated T-cell adhesion, and activates STAT5b
pX	p8	Cleavage product of p12; increases formation of intercellular conduits and viral transmission
pX	$p30^{II}$	Interferes with TLR4 signaling, alters cell cycle and DNA repair, and promotes viral latency by interfering with *tax/rex* RNA export
	$p13^{II}$	Induces apoptosis; binds to and interferes with *tax* transactivation

ATL, Adult T-cell leukemia; *CTL,* cytotoxic T lymphocytes; *LFA-1,* lymphocyte function–associated antigen 1; *NFAT,* nuclear factor of activated T cells; *TLR4,* Toll-like receptor 4.

infected cells, although small amounts of TAX have been found in the cytoplasm. The TAX proteins are responsible for enhanced transcription of viral and cellular gene products and are essential for transformation of human T lymphocytes.[15] The TAX viral regulatory protein for HTLV-1, like its counterpart TAT of HIV-1, plays an important role in promoting viral growth and disease pathogenesis. Both promote *trans*-acting, transcriptional activation of the *LTR,* but the effect of TAX appears to be mediated via expression of cellular growth factors that are abundantly activated by TAX through its *trans*-activation properties. This *trans*-activation of cellular genes by TAX not only facilitates viral replication but also has emerged as a cofactor in disease pathogenesis. The *tax* gene is responsible for the *trans*-activation of virus transcription via *tax*-responsive elements of a number of regulatory enhancers, such as the 21-bp enhancer, the nuclear factor-κB (NF-κB) binding site, and serum-responsive element. Such promoter interactions lead to the activation of a number of cellular genes, such as those encoding interleukin (IL)-2 and the IL-2 receptor (IL-2R), which promote cell proliferation. In addition, TAX activates the proto-oncogenes *fos* and *erb*, and the gene for granulocyte-macrophage colony-stimulating factor, an array of early response genes, the human lymphotoxin gene, and parathyroid hormone–related protein gene, whereas it *trans*-represses the β-polymerase gene. Overproduction of interferon (IFN)-γ via this pathway has been implicated in promoting the chronic inflammation that characterizes diseases such as HAM.[16] The mechanism by which TAX interacts with a variety of cell regulatory elements involves nuclear regulatory elements (the NF-κB pathway). Both Tax-1 and Tax-2 have been found to inhibit expression of certain tumor suppressor genes in vitro, but because HTLV-2 has not been shown to be oncogenic, the effect or implication of this has not been determined in vivo.

HBZ and APH

The antisense proteins HBZ in HTLV-1 and APH in HTLV-2, HTLV-3, and HTLV-4 may also be involved in HTLV pathogenesis, with effects on proviral load and transcriptional regulation. Analyses of T-cell lines transfected with mutated *hbz* genes showed that *hbz* promotes T-cell proliferation in its RNA form, whereas HBZ protein suppresses TAX-mediated viral transcription through the 5′ LTR.[17] Thus far, HBZ has been shown to inhibit TAX-mediated activation of the HTLV-1 LTR, activate cellular transcription, and promote T-lymphocyte proliferation, whereas all APH proteins have demonstrated the ability to repress TAX-mediated viral transcription.[12,18–21] Both HBZ protein and mRNA are required for the propagation of virus and/or replication of infected T cells in vivo, as demonstrated in a humanized mouse model.[22] In HTLV-2, although APH-2 is associated with higher proviral load, it has not been shown to promote cell proliferation or lymphocytosis.[21,23] Recent work has demonstrated that the HBZ protein and HBZ mRNA act in concert to induce cellular proliferation through modulation of the apoptotic process,[24] stimulation of telomerase,[25] and suppression of a T-cell–specific protein (THEMIS).[26] In addition, the ability of the HBZ protein to bind a tumor suppressor (Rb) demonstrates possible oncogenic pathogenesis.

REX

The *rex* gene of HTLV-1 and HTLV-2 encodes two protein species in each virus. In HTLV-1, a 27-kDa protein (p27) and a 21-kDa protein (p21) seem to result from the use of alternative initiator methionine codons. In HTLV-2, however, a 26-kDa protein appears to be formed by phosphorylation of a serine residue in a 24-kDa protein.[27] Unlike the products of the *tax* gene, REX does not directly regulate RNA transcription, but instead appears to act chiefly at a posttranscriptional level to regulate viral gene expression. REX (regulator of expression of virion proteins for HTLV) stabilizes viral mRNA and is essential for export of full-length *gag/pol* and single-spliced *env* mRNA from the nucleus to the cytoplasm.[28] REX localizes to the nucleus and specifically to the nucleoli of infected cells.[29] Phosphorylated REX binds with high affinity to *cis*-acting RNA sequences, called REX-response elements (RxRE), in the viral mRNA.[30–32] This interaction facilitates the export of mRNA. REX binding may also inhibit mRNA splicing by preventing early steps in spliceosome assembly.[33] As a consequence of the accumulation of REX in the cell, there is an accumulation of unspliced and

single-spliced mRNA, favoring the production of structural proteins (GAG and ENV). This is accompanied by a decrease in the levels of double-spliced mRNA encoding TAX and REX. REX accumulation may also inhibit TAX, thus slowing viral transcription.[34] A fine balance between TAX and REX expression and function may dictate the rate of viral replication within infected cells.

Other Proteins Encoded by the *pX* Region

pX ORF I, produced by a double-splicing mechanism similar to that of the gene products of *pX* ORF III and IV, codes for a hydrophobic 12-kDa protein, $p12^{I}$, and *pX* ORF II results in the production of two nuclear proteins, $p13^{II}$ and $p30^{II}$.[35] In addition to activating nuclear factor of activated T cells,[36] $p12^{I}$ localizes in the endoplasmic reticulum and *cis*-Golgi apparatus and elevates cytoplasmic calcium that is antecedent to T-cell activation and essential for establishing persistent infection.[37] Other major structural and regulatory proteins of HTLV-1 are summarized in Table 168.1. The gene products of *pX* ORF I and II also appear to affect cell proliferation and modulate host immune responses to HTLV-1 infection. TAX and the ORF I and II gene products may play an integral role in the pathogenesis of HTLV-associated diseases through their effects on cyclins, which regulate cell growth.

BIOLOGY

The replication strategy of HTLVs involves a life cycle typical of all members of the Retroviridae (Fig. 168.3), whereby the RNA genome undergoes reverse transcription into a DNA provirus that integrates into the host genome. Subsequently, new virions are produced via this integrated DNA template under the regulation of viral regulatory genes. The HTLV-1 receptor was determined to be the ubiquitous glucose transporter GLUT-1,[38] consistent with the infection by HTLV-1 of a wide range of cells in vitro, including endothelial cells and fibroblasts.[39] In addition, a number of animal species can be infected either experimentally (mice, rats, rabbits, and New World primate species) or naturally (Old World primates).[40] The natural host range for HTLV is therefore humans and Old World nonhuman primates.

Although HTLV-1 can infect a number of different cell types in vitro, its growth and propagation in vivo are supported mainly by $CD4^{+}$ cells and, to a lesser extent, $CD8^{+}$ cells, as determined with real-time quantitative polymerase chain reaction (PCR) assay performed on flow cytometry–sorted T cells from patients with HAM.[41] In contrast, HTLV-2 preferentially infects $CD8^{+}$ cells over $CD4^{+}$ cells.[42] HTLV-1 also infects dendritic cells, although different subsets of dendritic cells encountered by HTLV-1 during primary infection may either favor or inhibit subsequent infection of CD4+ lymphocytes.[43]

Clonal expansion of infected lymphocytes, rather than viral replication and infection of new lymphocytes, has been demonstrated as the primary mechanism of viral expansion within chronically infected humans, especially in HTLV-1.[44] Limited numbers of large clones have been found in HTLV-1 carriers and in patients with HAM/TSP,[45] and the relationship between number and size of clones may be related to mode of transmission and to disease pathogenesis.[46,47]

Based on epidemiologic data demonstrating that HTLV-1 transmission is strongly cell associated and through in vitro studies in which cocultivation is required for efficient infection of target cells, transmission of HTLV is mediated by live cells and not via cell-free body fluids. For this reason, HTLV-1 is not an easily transmitted virus, and universal biohazard precautions are adequate for the inactivation of potentially infectious blood or bodily secretions. Procedures that remove or kill lymphocytes, such as leukoreduction or refrigerated storage of blood products and freeze/thawing of expressed breast milk, have been shown to reduce but not eliminate the risk for HTLV-1 transmission.[48,49,50]

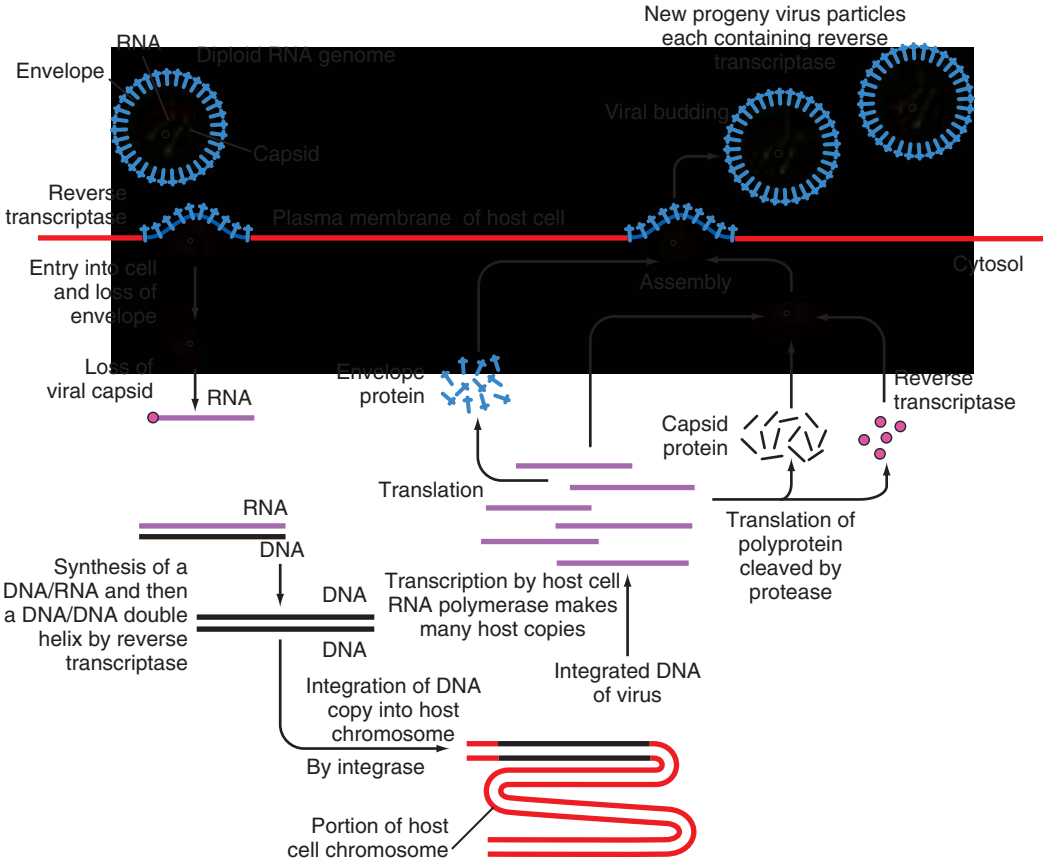

FIG. 168.3 Life cycle of human T-cell leukemia virus type 1 (HTLV-1). Virus infection involves initial binding to cell surface of target CD4 cell, uncoating, and release of viral genetic material. Virally encoded reverse transcriptase creates a DNA copy (provirus) that is integrated into the host genome under the influence of viral integrase. Viral replication involves production of both genomic RNA and polyproteins that are cleaved by the viral protease, resulting in virion assembly at the cell surface. See text for details. *(Modified from Alberts B, Bray D, Johnson A, et al, eds.* Essential Cell Biology: An Introduction to the Molecular Biology of the Cell. *New York: Garland Science Publishing; 2004.)*

Because HTLV-1 is highly cell associated, the means for viral attachment are not well characterized, but similar to other retroviruses, fusion of the virion with the cell membrane results in uncoating of the diploid RNA genome of the virus (see Fig. 168.3). Igakura and coworkers[51] found that additional cell surface–adhesion proteins and cell-cell contacts/interface (virologic synapse) are important for facilitating virus transmission. Cytoskeletal reorganization in the infected cells and segregation of virus particles to the interface between the infected and uninfected cells can be observed with immunofluorescence microscopy.[51,52,53]

Once in the cell, the virally encoded RNA-dependent DNA polymerase (reverse transcriptase) complexed to the genomic RNA of the virus transcribes viral RNA into double-stranded DNA. This double-stranded viral complementary DNA (cDNA) is transported to the nucleus as a ribonucleoprotein complex that includes the p24 capsid protein, and integrase and reverse transcriptase. Once there, the cDNA, through a complex process mediated by the viral integrase, is inserted into the host genome. The genomic integration of HTLV-1 establishes a lifelong infection and is integral to both the virus replication cycle and amplification of provirus.

Elements in the viral LTR are essential to integration and replication, because they form the sites for covalent attachment of the provirus to cellular DNA and provide important regulatory components for transcription. Additional key regulatory elements of HTLV are *tax,* which activates transcription of the viral genome, and *rex,* which modulates the processing of the viral RNA expressing unspliced forms of the viral mRNA. When the DNA provirus is expressed (i.e., transcribed by a cellular RNA polymerase), viral genomic RNA, mRNA, and, subsequently, viral proteins are made by the cell. Under the influence of REX, which stabilizes viral mRNAs and regulates their splicing and transport, new genomic RNA is assembled at the cell membrane and packaged for release. During the budding process, the envelope incorporates some of the cell's lipid bilayer, producing an infectious virion of about 100 nm (see Figs. 168.1 and 168.3).

LABORATORY DETECTION

Because HTLV-1 and HTLV-2 cause chronic infections and stimulate antibody production in most infected hosts, laboratory diagnosis can rely on detection of virus by culture of lymphocytes, anti-HTLV antibody, or HTLV genetic sequence by nucleic acid testing. Each approach has strengths and limitations, and diagnosis needs to be tailored to the specific clinical or epidemiologic setting.

Virus Isolation

Direct detection of virus through culture is intensive, expensive, and time-consuming; often several weeks are required for results to become available, and even then the result may be negative in infected persons. It is therefore impractical for clinical diagnosis in most clinical situations and is reserved for research. The ability to culture retroviruses has been improved by cocultivation of patients' T cells with human peripheral blood mononuclear cells (PBMCs) that have been stimulated in vitro with mitogens (e.g., phytohemagglutinin) and growth factors (e.g., IL-2), and by the removal of patients' $CD8^+$ suppressor cells from the coculture. The number of infected cells present in the blood of an infected individual is generally relatively low; mean proviral loads are 3.28 $\log_{10}$ copies per million PBMCs (range, 0.5–5.3) for HTLV-1 and 2.60 $\log_{10}$ copies per million PBMCs (range, 0.05–5.95) for HTLV-2.[54] The ability to isolate HTLV is dependent on viral load, immune status, and stage of disease. In infants and children, the small volume of blood available for culture and low viral load make virus isolation especially challenging.

Serologic Assays and Antigen Detection

The most common test for HTLV-1 and HTLV-2 infection is detection of antibody. Varieties of techniques are used to detect antibodies to HTLV-1. Because resolution of HTLV infection has not been described, confirmed HTLV-1 antibody positivity can be interpreted as infection with HTLV provirus. Samples are first screened with one of several assays that use whole-virus lysates or recombinant HTLV-1 and HTLV-2 antigens. The most widely used assay for the detection of HTLV-1 in the United States is the enzyme immunoassay (EIA), which uses whole disrupted virus and recombinant antigens.[55] This assay has demonstrated high sensitivity and specificity in the clinical setting but does not discriminate between HTLV-1 and HTLV-2 because of cross-reactive antibodies. Nevertheless, because false-positive results are common when EIAs are used in low-prevalence populations such as blood donors, repeating the EIA and retesting repeatedly reactive EIAs with a different supplemental test is strongly recommended before informing the patient.

For HTLV-1 and HTLV-2 infection, a combination of a screening EIA followed by a confirmatory WB is the standard approach, although some investigators and blood banks prefer alternative strategies incorporating a particle agglutination test for screening or an immunofluorescence assay for confirmation.[56] In December 2014, a test using a WB recombinant envelope protein became the first HTLV-1/HTLV-2 confirmatory test to be approved by the US Food and Drug Administration (FDA).[57] The line EIA, or LIA, uses well-defined antigens derived from HTLV-1 and HTLV-2 immunodominant proteins that are either recombinant proteins or synthetic peptides, highly purified and fixed on a nylon membrane strip. The sequences are selected to allow the detection of antibodies with a wide specificity to all known isolates of the HTLV strains. The antigenicity exhibited by these proteins and peptides is either common to both HTLV-1 and HTLV-2 or type specific to one to allow confirmation and discrimination in a single assay. Two *gag* (p19-1/2, p24-1/2) and two *env* (gp46-1/2, gp21-1/2) bands are applied as non–type-specific antigens, which are used to confirm the presence of antibodies against HTLV-1/2. The type-specific antigens for HTLV-1 (*gag* p19-1, *env* gp46-1) and for HTLV-2 (*env* gp46-2) are applied to differentiate between HTLV-1 and HTLV-2 infections.[58]

The radioimmunoprecipitation assay, a more difficult procedure based on radiolabeled virus-infected whole cells, is more sensitive for ENV antibodies but rarely available. Confirmatory testing with an immunofluorescence assay is also possible, although this assay is not suited for high-throughput operations because it is labor-intensive and subjective. Titration to allow quantification of antibody is possible by testing serial dilutions of serum with EIA or particle agglutination or immunofluorescence assays.

In tropical countries, particularly Africa, repeatedly reactive EIA-reactive samples exhibit a high frequency of indeterminate WB results. Typically, these indeterminate sera show an HTLV GAG indeterminate profile: GAG p19, p26, p28, and p30 without p24 or ENV gp21 and gp46, which may be related to endemic *Plasmodium falciparum* infection.[59] To avoid overestimating the rate of HTLV-1/HTLV-2 seroprevalence in these regions, PCR assay may be useful.[60,61]

Nucleic Acid Detection

PCR assays have been developed to distinguish virus type and to quantify viral presence. In this assay, proviral DNA is amplified enzymatically and subsequently detected through use of a system of specific nucleotide primers and probes.[62] The limit of detection is approximately one infected cell per million PBMCs.[63] With the addition of a reverse-transcription step before amplification, the PCR assay has been used in the research setting to detect viral RNA in infected cells, which helps to identify actively replicating virus, although RNA has not been convincingly demonstrated in ex vivo plasma samples. Although exquisitely sensitive in the best laboratories, the PCR assay remains a research technique; it is under consideration for use as a screening assay as new technologies are evolving. PCR assay has proved valuable in enigmatic situations such as cases of suspected HTLV disease in the setting of a seroindeterminate WB result. If used with degenerate primers capable of detecting a variant of HTLV, the PCR assay can also reveal the presence of a new exogenous retrovirus.[59] However, the PCR assay has only occasional clinical usefulness in the diagnosis of HTLV infection in low-risk populations because it also is subject to false-positive results. With a low pretest probability (nonendemic area, no risk factors), individuals with positive EIA and indeterminate WB results have only a 1% to 2% probability of true HTLV infection, so the PCR assay is not recommended.[64] Conversely, the PCR assay is useful in the diagnosis of neonatal HTLV infection because passively transferred maternal antibody makes antibody testing unreliable.[65]

The PCR technique has also been useful to facilitate epidemiologic research studies.[62] Theoretically, virus-positive, antibody-negative individuals could be missed with antibody tests, and the true prevalence

of virus may be underestimated. In fact, several surveys using the PCR assay have not detected more than the occasional virus-positive, antibody-negative individual.[66–68] Quantitative real-time PCR has allowed the measurement of HTLV proviral DNA in PBMCs from infected individuals in the research setting but is not readily adaptable to large-scale screening methods.[44,54,63,69] Neither has proviral load been fully characterized as a useful prognostic marker, and these assays are not available clinically.[70,71,72]

SEROLOGIC EPIDEMIOLOGY

Geographic Distribution of Prevalence

Fig. 168.4 shows the different geographic distributions of HTLV-1 and HTLV-2. Although precise estimates of the global burden of HTLV are difficult, a review of worldwide prevalence of HTLV-1 has placed the estimate at 5 to 10 million persons.[73] The largest endemic area for HTLV-1 is most probably Africa, with seroprevalence ranging from 0.5% to 5.5%. Central Africa appears to be the most highly endemic, although seroprevalence surveys have not been done in many African countries and the studies that have been done focused primarily on blood donors or pregnant women. Surveys from the Ivory Coast, Ghana, Nigeria, Democratic Republic of Congo, Kenya, and Tanzania document that rates of HTLV-1 seropositivity are similar to those in the Caribbean region (5%–14%), but more and better data from sub-Saharan Africa are needed.[74,75]

In Asia, endemic clusters of HTLV-1 infection are present in southern Japan, the islands of the Ryukyu chain including Okinawa, and some isolated villages in the north of Japan among aboriginal Ainu populations; most of the seropositives in northern Japan are among immigrants from endemic areas in the south.[76,77,78] Rates of infection among persons older than 40 years exceed 15% in these areas, and in some highly endemic areas they reach 30% to 40% in adults older than 50 years.[73,77,78] Inhabitants of the People's Republic of China and Vietnam are largely free of infection,[79] whereas those living in Hong Kong and Korea have very low seroprevalence rates (0.0041% and 0.007%–0.25% in blood donors, respectively).[73,80] The rates in Taiwan are slightly higher, with 0.058% in blood donors[73] and 0.82% to 1.63% in adults older than 30 years.[81]

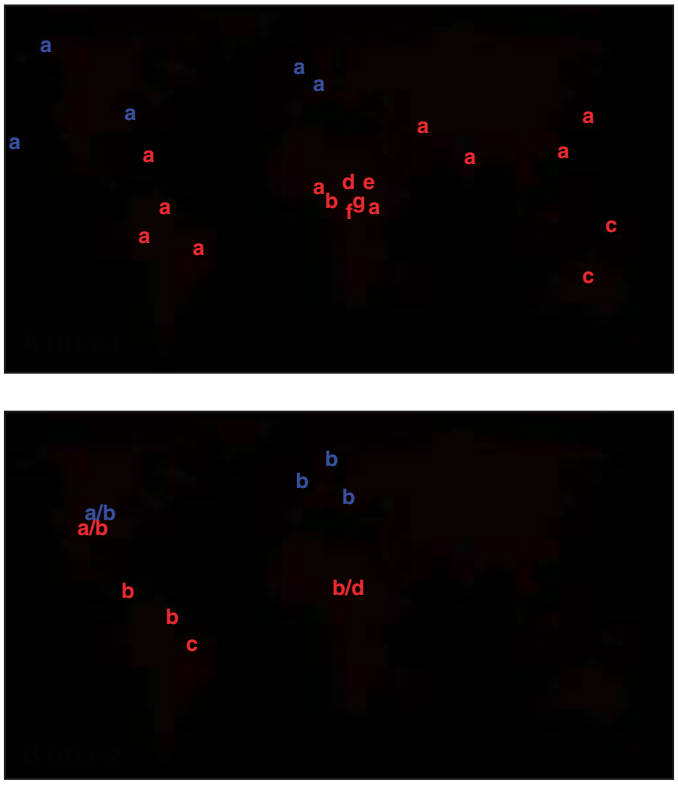

FIG. 168.4 Geographic distribution of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2. (A) Geographic distribution of HTLV-1 infection. HTLV-1–endemic populations are shown in *red,* and areas with sporadic cases of HTLV-1 often due to immigration from endemic areas are shown in *blue.* (B) Geographic distribution of HTLV-2 molecular subtypes 2a, 2b, 2c, and 2d. Areas with endemic HTLV-2–infected Native American and African populations are shown in *red,* and areas with HTLV-2 infection among intravenous drug users and their sexual partners are shown in *blue. (From Beilke MA, Murphy EL. The human T-lymphotropic leukemia viruses 1 and 2. In: Volberding PA, Palefsky J, eds.* Viral and Immunological Malignancies. *Hamilton, Ontario: BC Decker; 2006:328, 330.)*

In Australia, HTLV-1 is highly endemic (>30%) in aboriginal groups of inland Australia but occurs at very low rates in white nonaboriginals.[82–84] HTLV-1 seroprevalence is 0.6% in New Caledonia, 1% to 3% in the Solomon Islands, and very high (>15%) in Papua New Guinea.[73,85,86]

In the Americas, a major endemic focus of HTLV-1 infection occurs in the Caribbean, where rates of seropositivity in Jamaica, Trinidad and Tobago, Barbados, Haiti, and the Dominican Republic range between 5% and 14%, most in older persons and those of African descent.[87,88] In Jamaica, varying rates of seropositivity occur in different regions, with the highest rates (10%) of positivity observed in the lowland, high-rainfall areas.[89] In Trinidad and Tobago, seropositivity (5%–14%) is restricted almost exclusively to persons of African descent, even though individuals of Indo-Asian ethnic background have shared a common environment for more than a century. Seropositivity is found more frequently in persons of lower socioeconomic class and those who lack formal education.[81,87,88,90,91] Men and women attending clinics for sexually transmitted infections have the highest rate of seropositivity (>15%).[91]

Low rates of seropositivity are present in Central America, including Panama, Honduras, Costa Rica, and Nicaragua.[73] Of note is the higher rate in the non-Mestizo population compared with the Mestizo ethnic population, especially in coastal areas, in Honduras (8.1% vs. 0.5%).[73] In the entirety of South America, HTLV-1 is considered endemic, but several areas are foci of high endemic rates, primarily in indigenous Amerindians (Peru, Colombia, Chile, and Brazil) and, occasionally, specific groups of African origin (French Guiana, Surinam, Guyana, Colombia, and Brazil).[73,92,93] Brazil is considered one of the highest areas of endemic HTLV-1 infection (especially in Bahia and the northeast), whereas rates are very low in Venezuela, Paraguay, and Argentina.[73,94]

In North America, large-scale screening of blood donors in the United States has documented rates of HTLV-1 or HTLV-2 of up to 0.3 to 0.4 per 1000. Of the donors who test HTLV seropositive, one-half to two-thirds are HTLV-2 infected.[95,96,97] In a significant proportion of HTLV-1–positive cases, the donor has either links to an endemic area or a history of risk behaviors, such as blood transfusion or multiple sexual partners.[95] Populations that migrated from Okinawa to Hawaii, from the Caribbean to the United States, and from the Caribbean to the United Kingdom are at risk for HTLV positivity, as are those who experience exposure through sexual contact or blood transfusion in viral endemic areas.[90,98] HTLV infection is rare in Canada; when it is found, it is usually in migrants from endemic areas. However, several studies have documented HTLV-1 infections in First Nations peoples in coastal British Columbia. Little information is available on HTLV prevalence in Mexico, but the few studies that have been done indicate a near absence of infection.[73]

In Europe, small numbers of HTLV-1 infections are detected among emigrants from endemic areas to the United Kingdom, France, Spain, and other countries.[99–101] Middle East survey results have been largely negative, with the exception of Iranian Jews from northeastern Iran (Mashhad) and emigrants from that area now residing in Israel and New York.[102,103] Surveys in southern India and Indonesia have identified some HTLV-1–infected individuals but not endemic foci; the Seychelles in the Indian Ocean are highly endemic for HTLV-1 (>15%).[104]

HTLV-2 has a more restricted distribution than HTLV-1, primarily occurring in the Americas and parts of West and Central Africa.[105,106,107] A major reservoir exists in injection drug users in the United States and southern Europe, with rates ranging from 10% to 15% and higher.[108,109] Amerindians residing in North, Central, and South America have varying rates of positivity for HTLV-2 (5%–30%). Pockets of infection are present among the Seminoles in south Florida, the Pueblo and Navajo in New Mexico, and the Athapaskan in northwestern United States and Canada but not among various tribes in Alaska.[110,111] In Central America, the Guaymi Indians residing in northeastern Panama near the Costa Rican

border have high seropositive rates (>15%), but this does not hold true for the Guaymi living in southwest Panama. In South America, HTLV-2 is endemic in some indigenous Amazonian tribes of Peru and Brazil, and in the Chaco Indians of Argentina, with secondary spread to other population groups in those countries.[94] Finally, HTLV-2 has been identified in Africa, primarily among pygmy populations living in Central and Western rain forest areas.[106,112]

Demographic Patterns in Prevalence

HTLV-1 seroprevalence rates are strongly dependent on age and sex, with higher rates associated with older age and with female sex (Fig. 168.5).[76,88,113] The increasing prevalence with age may be due to the accumulation of infections over the lifetime of the individuals surveyed or birth cohort effect due to declining HTLV-1 seroprevalence over the past decades. The higher prevalence in females may be the result of more efficient male-to-female sexual transmission, as discussed later, or differences in sociodemographic or behavioral factors, such as duration of breastfeeding or frequency of condom use.

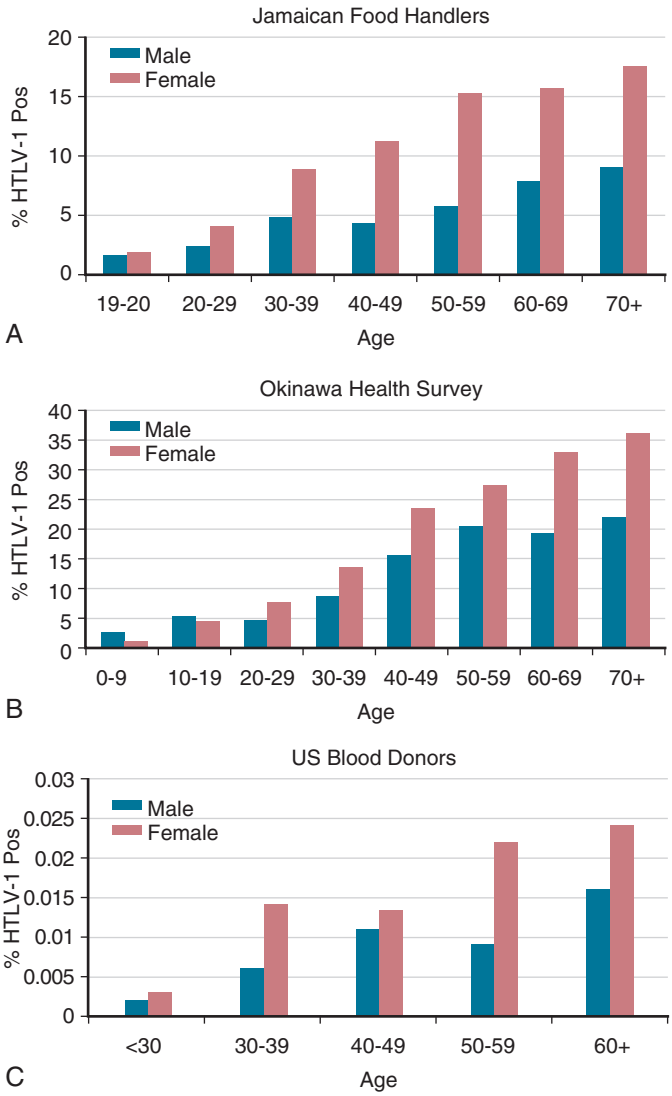

FIG. 168.5 Age- and sex-specific seroprevalence of human T-cell leukemia virus type 1 (HTLV-1) in several population groups. Graphs show the characteristic higher female prevalence and increasing prevalence with age. (A) Jamaicans employed in food-handling occupations (data from Murphy and colleagues[88]). (B) Participants in a community health survey in Okinawa, Japan (data from Kajiyama and colleagues[76]). (C) US blood donors with very low HTLV-1 seroprevalence (data from Murphy and colleagues[97]). *(From Beilke MA, Murphy EL. The human T-lymphotropic leukemia viruses 1 and 2. In: Volberding PA, Palefsky J, eds.* Viral and Immunological Malignancies. *Hamilton, Ontario: BC Decker; 2006:329.)*

For HTLV-2, there is also a characteristic age-dependent increase in seroprevalence in endemic Amerindian populations (Fig. 168.6).[114] In contrast, prevalence among US blood donors shows an HTLV-2 age-specific prevalence that peaks at age 40 to 50, consistent with a birth cohort effect due to injection drug use in the 1960s and 1970s, with secondary sexual transmission.[97]

Incidence

As might be expected from its relatively low infectivity, the incidence of HTLV-1 and HTLV-2 infections is quite low, even in endemic areas. Studies in large populations of repeated blood donors have measured seroconversions and thereby estimated incidence in the general population. Incidence rates in blood donors in HTLV-1–endemic Brazil and Japan were 3.6 and 3.8 per 100,000 person-years, respectively.[115,116] In contrast, studies in blood donors in the nonendemic United States ranged from 0.2 to 1.6 per 100,000 person-years.[117,118]

MOLECULAR EPIDEMIOLOGY

HTLV-1

Phylogenetic analysis has been used to classify HTLV-1 into seven major molecular and geographic subtypes: the Cosmopolitan subtype (a), with worldwide distribution, the Australo/Melanesian subtype (c), and five African subtypes (b, d, e, f, g)[119]; HTLV-1 isolates from different parts of the world show a high degree of nucleotide sequence conservation, in contrast to HIV-1 and HIV-2, in which considerable genomic variability occurs. Isolates of HTLV-1 from Japan, the West Indies, the Americas, and Africa share 97% or greater homology.[120–122] Even the most divergent HTLV-1c, isolated in Melanesia, is 92% homologous with the prototypic Japanese HTLV-1a isolate.[123] The majority of the nucleotide differences are single point mutations that do not correlate with specific disease patterns. Studies of the LTR by restriction fragment length polymorphisms

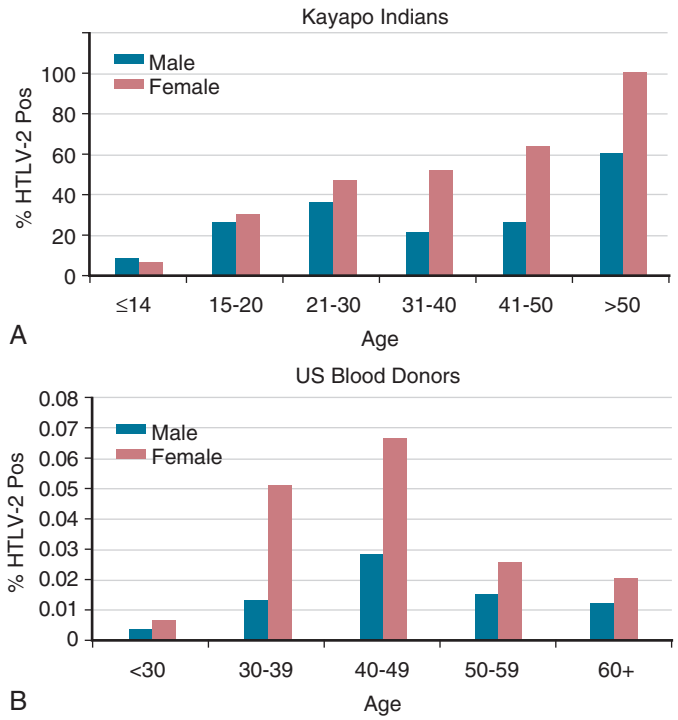

FIG. 168.6 Age- and sex-specific seroprevalence of human T-cell leukemia virus type 2 (HTLV-2). (A) Prevalence of HTLV-2 in the highly endemic Kayapo Indians of Brazil, consistent with ongoing mother-to-child and sexual transmission within a closed population (data from Maloney and colleagues[114]). (B) US blood donors with very low HTLV-2 seroprevalence show a pattern that suggests a birth cohort effect due to intravenous drug use in secondary sexual transmission in the 1960s and 1970s (data from Murphy and colleagues[97]). *(From Beilke MA, Murphy EL. The human T-lymphotropic leukemia viruses 1 and 2. In: Volberding PA, Palefsky J, eds.* Viral and Immunological Malignancies. *Hamilton, Ontario: BC Decker; 2006:332.)*

covering the major HTLV-1–endemic areas have demonstrated that variations are more linked to the geographic origin of the infected individuals than the patient's clinical status.[124,125] In addition, simian T-lymphotropic virus type 1 (STLV-1) isolates cluster phylogenically more closely with human isolates in the same geographic region than with STLV-1 isolates from different geographic regions, suggesting multiple instances of transspecies transmission.[126–129] Thus the genetic variability of HTLV-1 appears to reflect a combination of multiple transspecies transmission episodes and migration patterns of ancient populations carrying the virus.

An African origin of most HTLV-1 subtypes is supported by the occurrence of many HTLV-1 clusters in Africa[61,73,130] and among persons of African descent residing in the Caribbean, but not among other migrant populations residing in the region. The cosmopolitan subtype was likely dispersed throughout the world during the slave trade or early maritime explorations of European countries. The high prevalence of this HTLV subtype in southwestern Japan remains unexplained and could be due either to importation by maritime trade with Portugal and Holland starting in the 16th century or to prehistoric dissemination of the cosmopolitan subtype among some ancient ethnic people of Japan.[131–133]

HTLV-1 subtype c was first isolated from seropositive individuals in various parts of Melanesia.[134] The original isolate was obtained from an unacculturated hunter-gatherer tribe, the Hagahai, residing in the highlands of Papua New Guinea. More recent work has identified a high prevalence of HTLV-1c among Australian aborigines, with differences in viral sequence between northern and southern Australia.[119] HTLV-1 subtype c clusters closely with STLV-1 isolated from a stump-tailed macaque *(Macaca arctoides),* providing strong evidence of multiple cross-species transmissions.[135] In some cases, these strains have been isolated from persons with ATL[124] and HAM.

HTLV-2

Compared with HTLV-1, the geographic origin of HTLV-2 is less certain. HTLV-2 was first documented in and is highly prevalent among intravenous drug users in the United States and Italy.[109,136–138] Endemic foci of HTLV-2 were subsequently discovered among various Amerindian populations residing in North, Central, and South America.[114,139,140] The current distribution of HTLV-2 in the New World probably represents an original transmission from an endemic Amerindian to an intravenous drug user, followed by amplification of prevalence by the efficient transmission within the latter group. The presence of HTLV-2 infection in culturally and geographically distinct Indian groups in North America, Central America (Panama), and South America (Argentina, Brazil, Colombia, and Chile) led to the speculation that HTLV-2 may have originated in the New World. However, New World monkeys do not harbor STLV-2, and the discovery of HTLV-2 among Central African pygmies has discounted this hypothesis.[106,112]

Based on the relative divergence of nucleotide sequences of the *env* and *LTR* regions, HTLV-2 is classified into four subtypes: HTLV-2a (previously known as HTLV-2 Mo), HTLV-2b (formerly HTLV-2 NRA), HTLV-2c, and HTLV-2d.[141,142] Molecular epidemiologic studies have shown that HTLV-2a is the predominant infection among intravenous drug users in urban North America and among some North American Amerindians.[143] HTLV-2b is the predominant subtype in Indian groups in Panama, Colombia, and Argentina but is also found among Bakola pygmies in Cameroon and European intravenous drug users.[106] HTLV-2c appears to be confined to Brazilian Indian and urban populations and is supported as a separate subtype by *LTR* region phylogeny but not *env* gene phylogenic analyses. HTLV-2d was found in an Efe pygmy in the Democratic Republic of Congo and is the closest relative to STLV-2 isolated from Bonobo chimpanzees *(Pan paniscus).*[112]

Virus isolates from these populations have revealed that HTLV-2a and HTLV-2b differ molecularly by 2% to 4%.[144] The TAX-2 protein of HTLV-2b is 25 amino acids longer and is a more potent transactivator of the HTLV-2 LTR than the corresponding HTLV-2a protein.[142] The in vivo significance of this functional difference is unknown. Conversely, HTLV-2c has LTR and *env* sequences related to HTLV-2a and *tax* sequences similar to those of HTLV-2b. The identical sequence of the 456-bp *tax* gene of HTLV-2b isolates from a Cameroonian pygmy and a Colombian Wayuu Indian support an African origin for HTLV-2.[141] This, taken together with the close similarity of HTLV-2d and STLV-2, supports a scenario wherein HTLV-2 evolved into subtypes a, b, and d in Africa and was then transported to the New World, whereas HTLV-2c represents a more recent variant within subtype a.

HTLV-3 and HTLV-4

Two additional HTLV types have been isolated from Central Africans living in close contact with nonhuman primates. These viruses were discovered simultaneously in 2005 by two teams working on samples derived from two inhabitants of the rain forest area in South Cameroon. Wolfe and colleagues[6] described two different viral isolates from bushmeat hunters, which they named HTLV-3 and HTLV-4. Calattini and colleagues[7] described an HTLV-3 isolate that seems to be identical to the HTLV-3 described by Wolfe and colleagues.[6] Only three additional instances of HTLV-3 human infections and two additional instances of HTLV-4 human infections have been reported, all from Cameroon.[11,145,146] Simian STLV-4 isolated from two gorillas show nucleotide sequences virtually identical to human HTLV-4 isolates.[147] Therefore, reported isolates of HTLV-3 and HTLV-4 probably represent limited instances of simian-to-human transmission that have not become established in the human population.[148] There has been no evidence that either new HTLV type is widely prevalent in Africa or present at all outside Africa, and current commercially available tests have not yet been adapted to detect these new HTLV types.

ROUTES OF TRANSMISSION

Table 168.2 summarizes the routes, mechanisms, and cofactors associated with HTLV-1 and HTLV-2 transmission. The three major routes of HTLV-1 transmission are mother-to-child transmission, sexual transmission, and parenteral transmission. Although the routes of HTLV-2 transmission have been less well studied, the available evidence suggests that they are similar to those of HTLV-1. Transmission involving casual contact does not seem to occur. Zoonotic transmission of HTLV-1 continues to be reported in association with nonhuman primate bites in Central Africa.[149,150] These include, most frequently, bites from gorillas but also chimpanzees and small monkeys.

TABLE 168.2 Transmission of HTLV-1 and HTLV-2

	HTLV TYPE 1	HTLV TYPE 2
Route of Transmission		
Mother to child		
Transplacental	Low efficiency	Probable, but not quantified
Breast milk	Efficient	Probable, but not quantified
Sexual		
Heterosexual	Efficient	Efficient
Male to male	Efficient	Unknown
Parenteral		
Blood transfusion	Very efficient	Very efficient
Injection drug use	Efficient	Efficient
Cofactors of Transmission		
Elevated virus load		
Mother to child	Increased	Unknown
Heterosexual	Increased	Increased
Sexually transmitted diseases	Increased	Unknown
Cellular versus plasma transfusion products	Increased	Increased
Cold storage of blood	Decreased	Decreased

HTLV, Human T-cell leukemia virus.

Mother-to-Child Transmission

In contrast to mother-to-child transmission of HIV-1, wherein as many as 25% of offspring of positive mothers can acquire infection without breastfeeding,[151] breastfeeding is the predominant route of mother-to-child HTLV-1 and HTLV-2 transmission[152,153] and occurs through ingestion of infected milk-borne lymphocytes.[154] Both HTLV-1 and HTLV-2 viruses have been detected in breast milk.[155,156] During the first 6 months of life, maternal antibodies are present in infants; in serial WBs, all bands often disappear before new bands appear as a result of neonatal infection. In some cases, breastfeeding had ceased up to several months before seroconversion, but a study of cells from exposed but nonseroconverting children identified none with latent HTLV-1 viral infection.[66] In Japanese intervention trials,[152] 20% of breastfed infants seroconverted to HTLV-1, whereas only 1% to 2% of bottle-fed infants of HTLV-1–positive mothers became infected.[152] In prospective studies from Jamaica, a similar rate of transmission has been documented.[155]

The major predictor of maternal-to-child transmission is the proviral load of the mother as measured by means of antibody titer and viral antigen level on short-term culture.[71,157] The presence of antibody to HTLV Tax and/or Env has also been associated with transmission.[155,158] Furthermore, the duration and timing of breastfeeding were strongly associated with the efficiency of transmission.[154,159,160] In a prospective study conducted in Jamaica, among children born of HTLV-1–positive mothers in follow-up for more than 2 years, 32% of children breastfed for 12 months or longer were HTLV-1 seropositive compared with 9% of those breastfed for less than 12 months.[154] These data suggested that limiting the duration of breastfeeding to less than 6 months may reduce mother-to-child transmission of HTLV-1, and such a recommendation has been included in public health advisories. However, a study documented HTLV-1 transmission in 2 of 25 infant-mother pairs with short-term (<3 months) breastfeeding.[161] The same study reported that once initiated, breastfeeding was difficult to stop at 3 months. Follow-up studies indicated that seroconversion typically occurs in infants at the age of 1 to 3 years, with 2% to 5% of HTLV-1 infections resulting from mother-to-child transmission in the first few years of life.[162,163] In many studies, no infants or children became newly infected after 2 years of age.[153] Infection in early life may have considerable significance for subsequent risk for disease, particularly ATL.[164,165]

HTLV-2 transmission from mother to child also appears to occur through breastfeeding but has been less studied.[166] In a small study of infected mothers, 1 of 7 breastfed children was seropositive for HTLV-2, whereas 1 of 28 nonbreastfed children was seropositive.[167] In another study, no HTLV-2 transmissions were found among 19 infected mothers and 20 nonbreastfed children.[168] Among the Gran Chaco Indians of Argentina and the Kayapo Indians of Brazil, high rates of mother-to-child transmission have been observed (30% and 46%, respectively), suggesting the predominant role of breastfeeding in maternal-to-child transmission in these populations.[169] In addition, among the Guaymi Indians of Panama, there is a 1% to 2% prevalence among preadolescent children.[170] This is consistent with early-life infection and an excess of seropositive children when the mother is seropositive compared with the virtual absence of seropositive children when the mother is seronegative.[162,165]

Sexual Transmission

Several markers of sexual activity have been associated with transmission of HTLV-1 infection, including the number of lifetime sexual partners[91,171] and ulcerative (syphilis, herpes simplex virus type 2, and chancroid) and nonulcerative (gonorrhea and *Chlamydia*) sexually transmitted diseases.[91,172–174] Virus-positive mononuclear cells have been detected in semen, which suggests that seminal fluid is a likely vehicle for transmission. The presence of HTLV-1 DNA has also been found in cervicovaginal secretions from infected commercial sex workers in Peru.[175,176] Although some studies have suggested that male-to-female transmission occurs more efficiently than female-to-male transmission,[177,178,179] this difference may be less than previously thought.[180] In a 10-year follow-up study of 30 discordant heterosexual couples, the incidence of HTLV-1 infection was 0.9 per 100 person-years (95% confidence interval [CI], 0.1–3.3), with one male-to-female and one female-to-male transmission.[180] Male-to-male sexual transmission may also occur and is supported by the higher prevalence of HTLV-1 among men who have sex with men (15%) in Trinidad compared with the general population prevalence of 2.4%.[171] HIV-1 shares these routes of infection but appears to be an order of magnitude more infectious than HTLV-1.[171,172] This may reflect differences in viral load or that HTLV-1 is highly cell associated, whereas HIV-1 is both cell associated and cell free.

There are also other risk factors associated with sexual transmission of HTLV-1. In a study of married couples, there was a nearly 12-fold higher risk for infection in wives of seropositive husbands older than 60 years, possibly because of increased viremia with age or postmenopausal changes in the vaginal epithelium.[177] Higher proviral load and duration of relationship are also associated with increased transmission. In a prospective study of HTLV-infected men and their female sex partners, transmitters had higher proviral loads and had been in their relationships longer than nontransmitters.[178] Another prospective study demonstrated that HTLV-1 proviral loads were two $\log_{10}$ lower in newly infected partners than in their positive partners who transmitted HTLV, suggesting that a small dose of sexually transmitted HTLV produces a lower proviral load setpoint.[180] In addition, the presence of anti-TAX antibody has been shown to be associated with heightened transmission, possibly related to a state of virus proliferation induced by TAX and measured indirectly by anti-TAX antibody. Host-related factors may also play an important role in determining HTLV-1 proviral load. A study evaluating the sequence of the gp46 coding region among 13 infected patients and their partners revealed that, although the gp46 sequences were identical in each married couple, the level of HTLV-1 proviral DNA in the spouses often differed.[181]

Sexual transmission of HTLV-2 has been difficult to study because of the frequent coincidence of injection drug use in the study populations. However, a 10-year cohort study of 55 serodiscordant heterosexual couples found two HTLV-2 transmissions, with one male-to-female and one female-to-male transmission (0.5 per 100 person-years), which was not statistically different from rates seen for HTLV-1.[180] In this study, all newly infected sex partners denied any history of intravenous drug use or other parenteral exposure. Previous studies have documented an association between a history of receiving money for sex, total lifetime sex partners,[182] and length of sexual relationships with HTLV-2 seropositivity.[178,179,182] In one study of female prostitutes, intravenous drug use was the major risk factor for seropositivity.[183] In a serosurvey of Guaymi Indians, it was demonstrated that among women, early age at first intercourse (<13 years), higher number of lifetime sexual partners, and higher number of long-term sexual relationships were significantly associated with HTLV-2 positivity.[184] Among men, intercourse with prostitutes was associated with HTLV-2 seropositivity.

Parenteral Transmission

Parenteral transmission, either through transfusion or injection drug use, is another major route of HTLV transmission. Because the HTLV viruses are cell associated, transmission via transfusion of cellular blood components (e.g., whole blood, packed cells, platelet concentrates) is highly efficient. Seroconversion rates of 44% to 63% have been reported in recipients of HTLV-1–infected cellular blood components in endemic areas,[185,186–188,189] but seroconversion has not been associated with plasma or cryoprecipitate. Donor units of whole blood or packed cells are less likely to be associated with transmission the longer they are stored in the blood bank, presumably because of the loss of white blood cell viability at refrigerator temperature. In retrospective surveys, the rate of transmission decreased to near zero when blood components were stored for more than 14 days compared with 47% transmission for a storage period of 14 days or less.[48,186] Removal of leukocytes from blood products via filtration or leukoreduction also appears to substantially decrease but not eliminate the risk of HTLV-1 transmission by transfusion.[190] There is only rare evidence for transmission of HTLV-1 by needlesticks or other bloody occupational exposures.[191,192]

Transmission of HTLV-2 has been documented in 50% of the recipients of known units of positive blood.[193] Parenteral drug abuse has been associated with transmission of both HTLV-1 and HTLV-2, but most HTLV-positive drug abusers are HTLV-2 infected.[194] Risk factors for seroconversion include sharing of drug abuse paraphernalia and "booting"—that is, aspirating blood into the drug-filled syringe

before injection. This circumstance may help to explain the exceptionally high rates of seropositivity in older heroin-injecting drug users, suggesting a birth cohort effect linked to an HTLV-2 epidemic among US injection drug users in the 1960s and 1970s.[194]

Although rare, HAM and ATL have both been linked to HTLV-1 transfusion transmission.[195] In recognition of the risk for these diseases, testing of donated blood for HTLV viruses has been routine in the United States since 1988.[55] HTLV seroprevalence is generally low among hemophiliacs unless they were multiply transfused with cellular blood products before the institution of routine screening. In developing countries, however, blood transfusions remain a major risk factor for HTLV infection. Several cases of HTLV-1 infection in transplant recipients have been documented.[196] These infections have resulted in T-cell lymphomas[197] and in myelopathy.[198] In a cohort in which the diagnosis of HTLV-1 was made soon after transplantation, early treatment with zidovudine and raltegravir was ineffective at controlling viral replication.[199]

IMMUNOLOGY OF HTLV INFECTION

Humoral Immune Responses

Antibodies to the various antigens of HTLV-1 occur at high levels in carriers and among patients with ATL and HAM. During primary infection, the pattern of antibody responses (Fig. 168.7) demonstrates that the first specific antibodies to emerge after primary HTLV infection are directed against the gp21 transmembrane ENV protein and various regions of the GAG polyprotein. Over several weeks to months, anti-gp46 ENV antibodies appear, and about 50% of infected individuals develop detectable antibodies to p38/40 TAX protein.[200] Antibody titers vary from patient to patient and are significantly higher in patients with HAM and among those at risk for this disease. Antibody titers correlate with the proviral burden, which explains the HAM finding and the paradoxical finding of high antibody titers among women who transmit HTLV-1 to their infants through prolonged breastfeeding.[155] Transplacental maternal antibodies apparently protect the infant from infection in the first months of life, but subsequently the infant becomes infected via maternal virus in the breast milk. Miyoshi and coworkers[201] have shown that passive immunization with high-titer human anti-HTLV globulin is protective against experimental HTLV infection in rabbits. Antibody response is also elicited by immunization with experimental DNA or peptide HTLV-1 vaccines.[202] However, there is little evidence that humoral immune response controls HTLV-1 proliferation or prevents disease in humans.

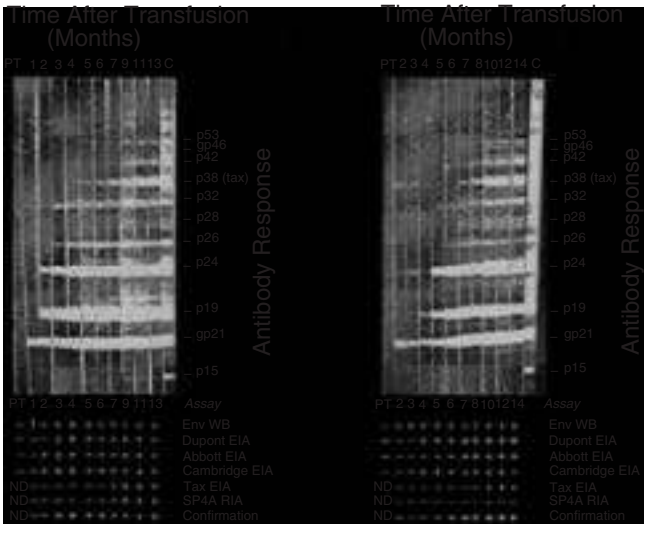

FIG. 168.7 Sequential serum samples from two transfused patients after exposure to human T-cell leukemia virus type 1 (HTLV-1)–infected blood products. The time course (in months) of seroconversion is revealed by the sequential appearance of bands on a standard HTLV-1 Western blot *(top)* and reactivity (+/–, ND) *(bottom)* on a battery of other Western blot *(WB),* enzyme immunoassay *(EIA),* and radioimmunoassay *(RIA)* tests. *ND,* Not done; *PT,* pretransfusion.

Cellular Immunity

Cytotoxic T lymphocytes targeting viral antigens play an essential role in the regulation of HTLV-1 viral burden.[203] Among chronic carriers, infected individuals mount a strong cell-mediated immune response to the virus, and as many as 1% of $CD8^+$ cytotoxic T lymphocytes can recognize at least one epitope of HTLV.[204–209] Freshly isolated cells have substantial expression of activation markers, indicating that these cells have recently encountered the TAX antigen.[203,205,206] A proposed model of this viral interaction with the host cell–mediated immune response is shown in Fig. 168.8. In this model, the dynamic equilibrium between viral replication and immune destruction is mediated through TAX overexpression, causing $CD4^+$ target cell proliferation and a robust cell-mediated response to the antigen in particular, resulting in cytotoxic T-lymphocyte–mediated lysis of these HTLV-1–infected $CD4^+$ cells.[205,206,209,210] As a consequence of ongoing TAX proliferation, there is an expansion of HTLV-1–infected $CD4^+$ cells and a compensatory expansion of $CD8^+$ cytotoxic T lymphocytes. As the number of infected $CD4^+$ cells expands, HTLV-1 antigens are expressed on the cell surface and become targets for $CD8^+$ T-cell–mediated cytotoxic killing. The role of $CD8^+$ T-cell–mediated killing as the primary means of viral suppression may explain the epidemiologic observation that recipients of HTLV-1–infected blood products who are also receiving exogenous immunosuppressive medications are more susceptible to infection, likely because a diminished cell-mediated immune response is unable to clear the initial virus infection.[189]

The role of $CD4^+$-mediated Th1 responses in upregulating the cytotoxic T-lymphocyte response is not well characterized. However, an association between class I human leukocyte antigen (HLA) haplotypes and protection against HAM suggests that carriers of certain antigen-presenting motifs augment the efficient control of HTLV-1–containing cells. HTLV-1 carriers with the HLA-A02 haplotype have lower HTLV-1 proviral loads and are less likely to develop HAM, most likely because they are able to mount a stronger cell-mediated immune response to HTLV-1 infection.[209,211,212,213] Recent work has focused on genetic polymorphisms of HLA alleles and associated immune responses with the hypothesis that genetic polymorphisms determine HTLV-1 clinical outcome; low immune responder HLA alleles exhibit no positive immune responses and are permissive to HTLV-1, and high immune responder alleles are nonpermissive to HTLV-1 and exacerbate host inflammatory responses. Macrophage function does not seem to be affected by HTLV-1 infection, although macrophages from HTLV-1–infected subjects produced more CXCL9 and CCL5 and less IL-10 than cells from uninfected controls.[214]

A review of HTLV pathogenesis suggested that a combination of insufficiency of host HTLV-1–specific T-cell response and elevated proviral load might identify persons with high propensity to progress to ATL.[215] The balance of host immune response with HTLV-driven clonal expansion of infected lymphocytes, possibly with aggravating chronic IFN production, may play a role in the pathogenesis of HAM.[216]

CLINICAL MANIFESTATIONS OF HTLV INFECTIONS

Clinical disease associated with HTLV-1 is rare. ATL develops in 2% to 4% and HAM in 1% to 4% of carriers over their lifetime.[217,218] The risk for the development of disease is related to age and route of infection and the immune competency of the host. Acute seroconversion is associated with no recognized clinical syndrome; the time from infection to seroconversion was about 50 days or perhaps shorter with more contemporary assays, as seen with transfusion cases. The time from seroconversion to disease can vary from years to decades, with rare cases of HAM occurring soon after blood transfusion.[200] As summarized in Table 168.3, there is a wide range of clinical conditions linked to HTLV-1, with some caused by virally induced cell transformation and others mediated by immunologic response to the virus.

HTLV-1–Associated Malignancies

ATL was first recognized in Japan in 1977, before the discovery of HTLV-1, as an aggressive leukemia or lymphoma of mature T lymphocytes with varied clinical manifestations: generalized lymphadenopathy, visceral involvement, hypercalcemia, cutaneous involvement, lytic bone lesions, and peripheral blood involvement with cells manifesting

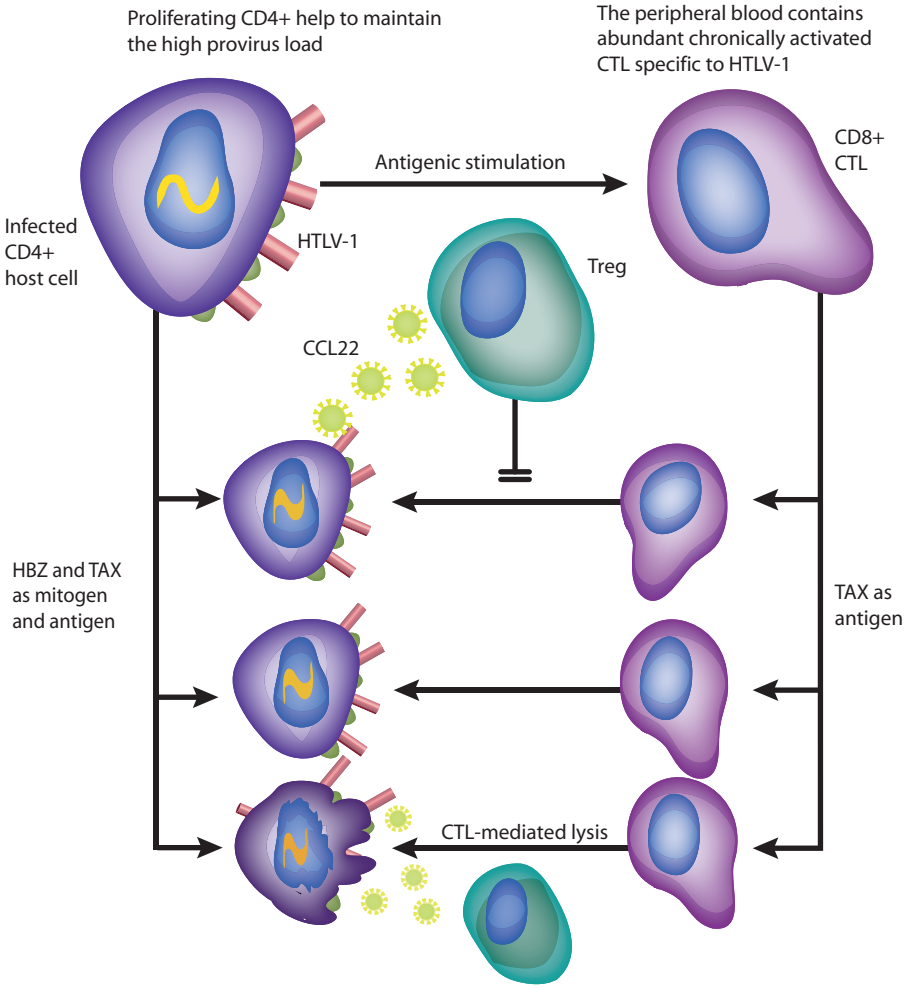

FIG. 168.8 A model of CD8⁺ cytotoxic T-lymphocyte *(CTL)*–mediated control of human T-cell leukemia virus type 1 *(HTLV-1)* infection. HTLV-1 infects CD4⁺ T lymphocytes with expansion of infection primarily via cell replication. As HTLV-1–specific antigens, particularly TAX, are expressed, a robust CD8⁺ cytotoxic T-lymphocyte response is generated. The inability of some persons to control HTLV-1 expansion is believed to contribute to disease pathogenesis. See text for details. *(From Bangham CRM. HTLV-1 infections.* J Clin Pathol. *1999;53:581–586.)*

TABLE 168.3 HTLV-Associated Diseases

	HTLV-1	HTLV-2
Children		
Infective dermatitis	++++	No
Lymphadenopathy	++	++
Adults		
Adult T-cell leukemia/lymphoma	++++	No
HTLV-associated myelopathy	++++	+++
Infective dermatitis	+++	No
Polymyositis	++	Unknown
Uveitis	+++	Unknown
HTLV-associated arthritis	++	++
Sjögren syndrome	++	Unknown
Strongyloidiasis	++	Unknown
Pulmonary infiltrative pneumonitis	++	++
Invasive cervical cancer	+	Unknown

++++, Very strong evidence; +++, strong evidence; ++, possible association; +, weak association; *HTLV,* Human T-cell leukemia virus; *No,* evidence does not support association; *Unknown,* no data to support association or lack of association.

pleiotropic features ("flower cells") in a large number of cases.[219] The skin lesions seen in ATL are varied and include localized or diffuse papules, nodules (Fig. 168.9A), plaques (see Fig. 168.9B), erythematous patches, and diffuse erythroderma. Biopsy specimens of skin lesions reveal dermal or epidermal infiltration with malignant lymphocytes. So-called Pautrier microabscesses may also be noted in the dermis, as in mycosis fungoides. Biopsy specimens of bone lytic lesions reveal osteoclast activation and bone resorption (Fig. 168.10), often without infiltration by ATL cells. It has been suggested that TAX transactivation and production of parathyroid hormone–related protein and other cytokines are responsible for the hypercalcemia, osteoclast activation, and lytic bone lesions seen in this disorder.[220] The lifetime risk for development of ATL in HTLV-1 carriers is estimated at 2% to 4%, and the latent period from infection to development of disease is estimated to be 30 to 50 years.[217,221]

The Lymphoma Study Group in Japan[222] has classified ATL into four clinical types based on clinical features and cell morphology: smoldering (5%), chronic (19%), lymphoma/leukemia (19%), and acute (57%) types. Transformation from the smoldering or chronic phase to the acute form can occur at any point during the course of the disease progression. Fig. 168.11 shows the characteristic morphologic features of the leukemia cells observed in ATL.

Smoldering ATL is characterized by 5% or more abnormal T cells in the peripheral blood with a normal total lymphocyte count, the presence of skin lesions, and, occasionally, pulmonary involvement. There is no hypercalcemia, lymphadenopathy, or other visceral

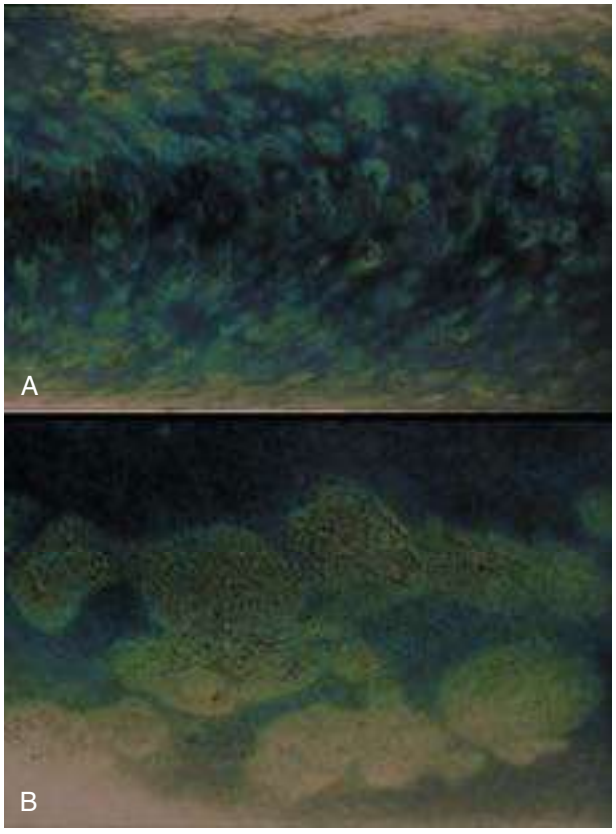

FIG. 168.9 Cutaneous manifestations in patients with adult T-cell leukemia. (A) Multiple skin papules and nodules. (B) Red plaques.



FIG. 168.10 Biopsy specimen of lytic bone lesions from a patient with adult T-cell leukemia reveals osteoclast proliferation and bone resorption.

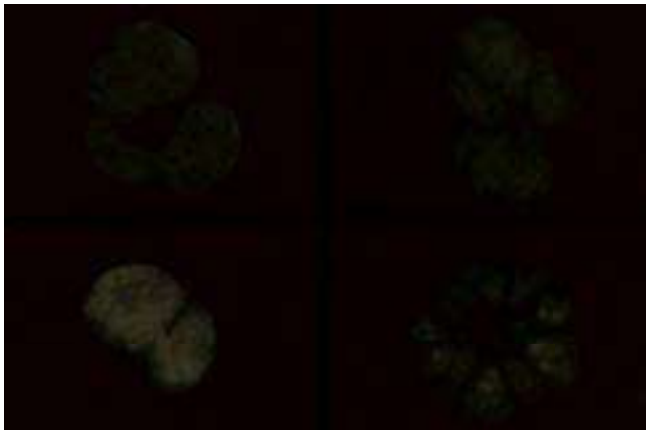

FIG. 168.11 Photomicrographs of typical peripheral blood lymphocytes from patients with adult T-cell leukemia. The characteristic cleaved nucleus can result in either a bilobed shape or the archetypical "flower cell" appearance. Low numbers of flower cells can also be seen in occasional HTLV-1–seropositive patients without any sign of leukemia or lymphoma.

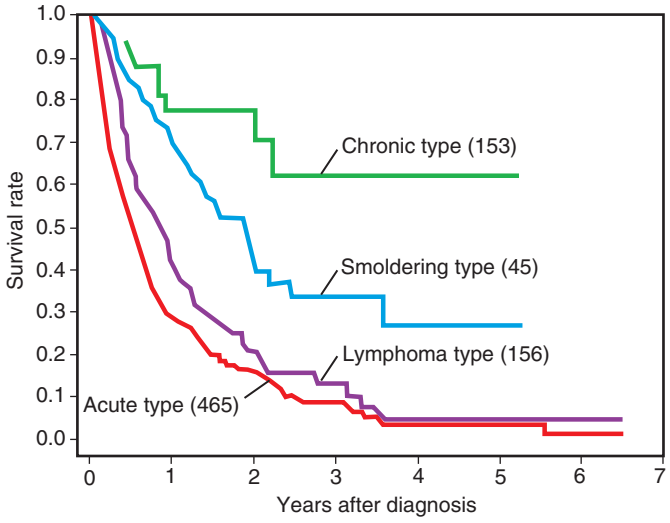

FIG. 168.12 Survival patterns of adult T-cell leukemia (ATL) subtypes after standard chemotherapy. Acute and lymphoma-type ATL have the poorest prognosis (see text). *(From Bazarbachi A, Plumelle Y, Ramos JC, et al. Meta-analysis of the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes.* J Clin Oncol. *2010;28:4177–4183.)*

involvement. Serum lactate dehydrogenase levels may be elevated. This phase is often indolent and may last for years. Smoldering ATL may clinically resemble mycosis fungoides or Sézary syndrome, with cutaneous involvement manifesting as erythema or as infiltrative plaques or tumors; Pautrier microabscesses may be observed.

Chronic ATL is characterized by an absolute lymphocytosis ($\geq 4 \times 10^9$/L) with a T-cell lymphocytosis ($>3.5 \times 10^9$/L) that resembles chronic T-lymphocytic leukemia. Lactate dehydrogenase level may be increased as much as twice the normal limit. Cells from chronic ATL patients are relatively uniform in size and nuclear configuration. Patients may have lymphadenopathy, hepatomegaly, splenomegaly, and skin and pulmonary involvement. No hypercalcemia, ascites, pleural effusion, or involvement of the central nervous system (CNS), bone, or gastrointestinal tract is present. The median survival time for patients with chronic ATL is 24 months.

Lymphoma/leukemia ATL is characterized by lymphadenopathy in the absence of lymphocytosis. Lymph node involvement with ATL must be histologically proved. The median survival time is approximately 10 months.

Acute ATL is distinguished by increased numbers of leukemic T cells with characteristic pleomorphic morphology, skin lesions, systemic lymphadenopathy, hepatosplenomegaly, and metabolic disorders, especially hypercalcemia. Lytic bone lesions and visceral involvement are common. Acute ATL has a poor prognosis, with a median survival time of 6.2 months.

Most patients with the acute and lymphoma types die within 6 months of diagnosis (Fig. 168.12), particularly if hypercalcemia is a presenting sign.[223] In general, the smoldering type is the least aggressive; the chronic type has a relatively poor prognosis, with death occurring within a few years of diagnosis.[222] The cause of death is usually an explosive growth of tumor cells, hypercalcemia, bacterial sepsis, and other infections observed in patients with immunodeficiency. Sometimes ATL manifests

as a T-cell non-Hodgkin lymphoma with no other clinical features of ATL except for monoclonal integration of HTLV-1 in the proviral DNA of the tumor cells. These cases are termed *lymphoma-type ATL* and are indistinguishable from peripheral T-cell lymphomas.

The malignant T cells of ATL are mature (terminal deoxynucleotide transferase negative) $CD4^+/CD8^-$ and have increased IL-2R α-chain (CD25/TAC antigen) expression.[224] All subtypes have a monoclonal integration of HTLV-1 proviral DNA into the cellular genome, indicating that the malignant T cells are monoclonal and originated from a single HTLV-1–infected T cell.[225] Southern blot analyses show frequent detection of defective provirus but fail to demonstrate consistency of HTLV-1 integration site in tumors from different patients, suggesting that integration site does not determine leukemogenesis.[226] More recently, reverse-transcriptase polymerase chain reaction (RT-PCR) assay has been used to determine the specific genomic location of integration, because HTLV-1 integration sites are more likely to occur in gene-coding than non–gene-coding regions of the genome and near transcriptional start sites in leukemic cells.[227–229] However, no specific proviral integration sites have been linked to the development of ATL.[230] Tanaka and colleagues[46] showed multiple, small HTLV-positive T-cell clonal populations in recently infected persons compared with fewer, larger clonal populations in persons believed to have been infected since birth. The dominant theory is that HTLV-1–driven lymphocyte proliferation results in the accumulation of additional somatic DNA mutations at one or more leukemogenic sites, either flanking the HTLV-1 proviral integration site or elsewhere.[231,232] Chromosomal additions and deletions are common in ATL and may be related to prognosis.[233] A large, multicenter study in Japan has revealed that specific mutations and copy number alterations were associated with both ATL subtype and prognosis.[234] Older age and *PLCG1* and *IRF4* mutations were associated with worse prognosis in aggressive ATL, and *TP53* and *IRF4* mutations, *CDKN2* deletions, and *PD-L1* amplifications were associated with shorter survival in indolent ATL. Consistent with this, a second laboratory's analysis of ATL tumors observed increased *IRF4* mutations, and emergence of *K59R* mutations at relapse suggests that *IRF4* for is a driver mutation and potential therapeutic target.[235]

The transcription factor TP53 guards against DNA damage, and mutations of the *TP53* gene have been reported in more than 50% of human cancers. Mutations of *TP53* are not common in ATL, but TAX inhibits the function of *TP53* through other mechanisms.[236–238] Ubiquitination of TAX attenuates its transcriptional activity.[239,240] As opposed to in vitro models of transformation, TAX is not expressed in approximately 60% of ATL cases.[241] Analyses of HTLV-1 provirus in ATL cases showed three mechanisms to inactivate TAX expression: (1) genetic changes of the *tax* gene,[241,242] (2) DNA methylation of the 5′ LTR,[241,243,244] and (3) deletion of the 5′ LTR.[245,246]

Pneumocystis jirovecii pneumonia, cryptococcal meningitis, disseminated fungal infections, and other opportunistic infections are often present and contribute to a rapid progression to death for patients with acute and lymphoma-type ATL. The fact that *Strongyloides stercoralis* seems to be a common concurrent infection[247] has led to speculation that *Strongyloides* infection may be a cofactor in the development of ATL.[248] The state of immune compromise resulting from HTLV-1 infection is not due to the type of immune ablation observed in HIV-1, even though CD4 cells are infected by HTLV-1; rather, the immunodeficiency is associated with rapidly proliferating malignancy, and the pattern of opportunistic infections is typical for those reported in patients with other types of aggressive non-Hodgkin lymphomas. In nonendemic regions, the diagnosis of ATL is suggested by the presence of HTLV-1 antibodies in serum from patients with the characteristic features of T-cell malignancy and is confirmed by the demonstration of monoclonal integration of HTLV-1 provirus in tumor tissue or leukemic lymphocytes. Measurement of HTLV-1 proviral load is not helpful because levels in ATL are not consistently elevated as they are in HAM and therefore do not discriminate among ATL, HAM, or a subset of healthy carriers. In some cases of ATL in patients from high-risk areas with typical clinical features, antibody is absent but a defective integrated virus with retained *tax* function can be detected with PCR.[249]

There is little evidence that HTLV-1 is associated with solid tumors or hematologic malignancies other than ATL.[250] Arisawa and colleagues[251] reported a 6-year prospective study in which HTLV-1 was not associated with an increased risk for cancer mortality. Previous case-control studies that showed associations between HTLV-1 infection and cancer may have been confounded by blood transfusion or genetic predisposition.[252,253] An inverse relationship between HTLV-1 and *Helicobacter pylori* infections has been noted in Japan, suggesting that there may be an interrelationship with gastric cancer in this setting. However, a recent study found a lower risk for gastric carcinoma in HTLV-1 patients.[254]

The differential diagnosis of ATL includes other T-cell malignancies, such as non-Hodgkin lymphoma, mycosis fungoides, and Sézary syndrome. ATL should be suspected in any patient with a T-cell malignancy who is from an endemic population or has risk factors for HTLV-1. The presence of circulating flower cells, hypercalcemia, and skin lesions is highly suggestive. Leukemic cells are characteristically terminal deoxynucleotide transferase negative $CD4^+$ and $CD25^+$. Laboratory detection and confirmation can be based on testing for anti–HTLV-1 antibody or demonstration of monoclonal integration of HTLV-1 proviral DNA in the malignant cells by means of Southern blot or RT-PCR.

HTLV-Associated Myelopathy

HAM, otherwise known as tropical spastic paraparesis, is a chronic progressive demyelinating disease that affects the spinal cord and white matter of the CNS.[255,256,257] The incidence of HAM in HTLV-1 carriers is estimated to be less than 4%.[218] HAM has been linked to blood transfusion, and some cases are acutely progressive.[195,258] Familial clusters have also been reported and may be associated with younger age of onset and slower disease progression.[259] Although HAM mainly affects adults, particularly women, cases occasionally occur in children younger than 10. The overrepresentation of females may reflect a higher occurrence of HAM after sexually acquired rather than vertical infection.[260]

The typical time of disease onset is in the fourth decade of life. The onset is often subtle, and the florid clinical picture of HAM is not always seen at first presentation. A single symptom or physical sign may be the only evidence of early HAM. Patients often present with a stiff gait,[261] progressing (usually slowly) to increasing spasticity and lower extremity weakness,[262] back pain, urinary incontinence,[263] and impotence in men. Patients may report sensory symptoms such as tingling, "pins and needles," and burning. Vibration sense is frequently impaired.[264] Hyperreflexia of lower limbs, often with clonus and Babinski sign, is frequently found. Hyperreflexia of the upper limbs is less common but may occur in severe cases, whereas upper limb weakness is rare. Exaggerated jaw jerk is seen in some patients, and ataxia may develop.

Nuclear magnetic resonance images may be normal or show atrophy of the spinal cord and nonspecific lesions in the brain.[263,265–267] The syndrome is significantly different from classic multiple sclerosis. HAM follows a slow course without the waxing and waning of symptoms, changes in affect, or multiple nuclear magnetic resonance scan abnormalities characteristic of multiple sclerosis. Varying degrees of brain parenchymal degeneration have also been described, with reactive astrocytosis and perivascular mononuclear cell infiltration. These mononuclear cells are predominantly $CD8^+$ lymphocytes,[268] suggesting that an immune mechanism may play a role in the development of HAM. High frequency of cytolytic T cells with specificity directed against major histocompatibility class I–restricted epitopes derived from the TAX protein has also been observed.[269]

It is possible that HTLV-1 induces an autoimmune-like process through molecular mimicry (an autoimmune model) or through indirect effects on immune function (a cytotoxic model). In the former model, it is postulated that HTLV-1 infection activates autoreactive T cells, which then cause autoimmune destruction within the CNS.[270,271] In the latter model, it is postulated that HTLV-1 infects glial cells, which subsequently induces a cytotoxic immune response against these cells, leading to demyelination.[268]

Patients with HAM characteristically have HTLV-1 antibodies or antigens in the blood and cerebrospinal fluid (CSF). The CSF may show mild lymphocytic pleocytosis; lobulated lymphocytes with morphologic similarity to ATL cells (flower cells) (see Fig. 168.11) also may be present in the blood and CSF.[262] Mild-to-moderate increases in protein may be observed in the CSF, and oligoclonal bonds with specific reactivity to

HTLV-1 antigens have been detected.[261,262] Lymphokines such as IL-6, tumor necrosis factor-α, and IL-2 are increased in the CSF. However, attempts to document the presence of HTLV-1 in the demyelinated lesion have not demonstrated a direct role of the virus in neurons.

The differential diagnosis of HAM includes multiple sclerosis, spinal cord tumors or extrinsic compression, toxic neuropathies, malnutrition, and HIV infection or syphilis. The diagnosis is suspected in unexplained CNS disease with loss of pyramidal tract functions and is confirmed by testing sera for HTLV-1 antibodies. In contrast to lymphocytes in ATL, HTLV-1–infected lymphocytes in HAM are oligoclonal or polyclonal rather than monoclonal.[272]

Neurologic abnormalities other than HAM have also been reported with HTLV-1 infection, including sensory neuropathy, gait abnormality, bladder dysfunction, erectile dysfunction, amyotrophic lateral sclerosis, mild cognitive deficit, and, rarely, motor neuropathy. In a cohort of asymptomatic HTLV-1–infected individuals followed for up to 8 years, 30% developed neurologic signs or symptoms.[273] It is unclear whether these abnormalities indicate future progression to HAM or that HAM is simply the "tip of the iceberg" of a broader spectrum of neurologic manifestations associated with HTLV infection.[274]

Other Diseases Associated With HTLV-1

The infective dermatitis syndrome was first shown to be HTLV-1 associated in Jamaica.[275] It has also been seen in Trinidad and Tobago,[276] Colombia,[277] the Dominican Republic,[278] Brazil,[279] and central and southern Africa,[280,281] but very rarely in Japan, despite the high frequency of HTLV-1 carriers.[282] It seems to represent the first childhood HTLV-1 syndrome. Patients are born of HTLV-1–positive mothers and experience failure to thrive. They are prone to refractory generalized eczema and infection with *Staphylococcus* and *Streptococcus* bacteria that are suppressed by long-term antibiotic therapy and recur when the therapy is stopped. This syndrome usually emerges early in life, in the first few years after birth, and may persist into adulthood. Anecdotal cases emerging in adolescence suggest that some infective dermatitis cases may result from infection at an older age. It is postulated that infective dermatitis is an immunodeficiency syndrome induced by HTLV-1. Interesting to note, some patients go on to develop ATL and HAM.[282,283] Further study of the pathogenesis of this syndrome should provide valuable insights into the pathogenesis of HTLV-1–associated diseases.

Polymyositis, also known as HTLV-1–associated inflammatory myopathy of skeletal muscle, is rarely associated with HTLV-1 seropositivity in viral-endemic areas.[284] These cases are indistinguishable from polymyositis seen in HTLV-1–nonendemic areas. A large joint polyarthropathy has been reported in Japan among elderly patients.[285,286] A distinguishing feature of these cases is the presence of HTLV-1–producing cells in the synovial infiltrate.

A unique form of uveitis has been observed in HTLV-1–positive individuals in Japan and Australia.[287,288] The first evidence of an association of HTLV-1 infection with uveitis was reported by Ohba and colleagues in Japan, who detected ocular involvement in patients with ATL and HAM and asymptomatic carriers.[289] The ocular manifestations were then classified into three groups: (1) opportunistic infections and tumor infiltration in ATL patients; (2) ocular alterations in HAM patients, including Sjögren syndrome, retinal pigmentary degeneration, optic atrophy, vitreous opacities, cotton-wool spots, and retinal vasculitis; and (3) HTLV-1 uveitis in asymptomatic carriers. Proviral DNA of HTLV-1 was identified in 60% of T cells from intraocular fluid of these patients.[289,290]

HTLV-1–associated infiltrative pneumonitis has also been reported in some individuals in Japan.[291,292] Recently, HTLV-1 infection has been linked to increased occurrence of bronchiolitis, chronic bronchitis, and bronchiectasis in indigenous Australians.[293,294] The risk of pulmonary disease and bacteremia was associated with higher HTLV-1 proviral load in this HTLV-1c–endemic population. A Brazilian study using chest computed tomography (CT) found evidence of bronchiectasis and other structural abnormalities in patients with HAM but not asymptomatic HTLV-1 carriers.[295] The HAM patients also had more pulmonary function abnormalities, but cigarette smoking histories were not reported.

The association of HTLV-1 with parasitic infestations (e.g., *Strongyloides* or crusted scabies) refractory to treatment has also been interpreted to suggest that HTLV-1 may have immunosuppressive effects.[296,297] A variety of subclinical perturbations in hematologic markers, such as depressed hemoglobin and lymphopenia, have been reported in healthy HTLV-1 carriers.[298] In addition, a study showed that HTLV-1 subjects had higher platelet counts (+16,544 cells/mm^3) than seronegative subjects.[299]

Other possible consequences of HTLV-1 infection include persistent lymphadenopathy in offspring of HTLV-1–positive women (including HTLV-uninfected children)[155] and waxing and waning lymphadenopathy seen in adult carriers. This lymphadenopathy may be a manifestation of a primary or secondary immune response to HTLV infection. The influence of HTLV-1/HIV-1 on the progression to AIDS is controversial because soluble factors produced by HTLV-1–infected cells can either enhance or inhibit replication of HIV-1. The findings of several epidemiologic and in vitro studies have been mixed,[171,300,301–306] which suggests that differences in study outcomes may be due to phenotypic differences in HIV-1 clinical disease.[307] It is noteworthy that HTLV-1/HIV-1–coinfected persons have increased CD4$^+$ counts but still develop opportunistic infections.[308]

Diseases Associated With HTLV-2

Although the disease associations with HTLV-2 are less clear than those with HTLV-1, there is accumulating evidence showing a link to HAM and to other neurologic abnormalities.[9] Prospective studies of former blood donors who are HTLV-2 infected have shown that they are at increased risk for developing HAM,[309] although their incidence is lower than that reported for HTLV-1 carriers.[310] Among these infected individuals, HTLV-2 proviral loads appeared to be lower than HTLV-1 proviral loads, which may explain why HTLV-2 has been less associated with the development of disease.[311] There have been four cases of HTLV-2 HAM in the HTLV Outcomes Study,[218,312] and there are a number of HTLV-2 HAM case reports in the literature.[313–319] Sensory neuropathy has been observed with HTLV-2 alone[320] and with HIV coinfection.[320–322] In addition, a spinocerebellar syndrome has been documented in HTLV-2–infected patients.

HTLV-2 infection has also been diagnosed in a few cases of hematologic malignancies, including atypical hairy cell leukemia,[5,323] and with some cases of large granular lymphocytic leukemia[324] and mycosis fungoides.[325] However, systematic surveys have not identified a clear association of these lymphoid malignancies with HTLV-2, suggesting that these patients may be coincidentally infected. One retrospective study of HTLV-2–positive drug users showed an excess of asthma-related deaths and an increased frequency of skin and soft tissue infections.[326] In a prospective cohort study, HTLV-2 has been associated with pulmonary disease, arthritis, and urinary symptoms.[63] HTLV-2 subjects also had higher mean lymphocyte counts (+7%) and platelet counts over 14 years of follow-up.[299] Finally, HTLV-2 has been associated with increased overall mortality and cancer mortality.[327,328]

In contrast to the possible association between HTLV-1 and accelerated HIV-1/AIDS progression, some studies of HTLV-2/HIV-1 coinfected persons suggest a neutral or protective effect, especially among those with high HTLV-2 proviral loads, possibly as a result of chemokine overproduction.[329,330] Further studies are needed to confirm these findings.

THERAPY

Although first-generation nucleoside analogues, such as zidovudine and lamivudine, have been recognized to have activity against HTLV reverse transcription in vitro, there is little clinical evidence of their efficacy in vivo.[331] Research with raltegravir, an antiviral agent approved for treatment of HIV-1, has shown promise in reducing proviral load in vitro, but a small clinical trial showed no significant reductions in infected individuals.[332,333] However, until further research is completed, the asymptomatic nature of HTLV-1 and HTLV-2 and the low penetrance of HTLV diseases mean that treatment of asymptomatic HTLV carriers is not currently indicated. Furthermore, the exact role of HTLV-1 in disease pathogenesis has not been clearly defined. In ATL, active viral replication does not appear to play a role in established malignant disease,

and tumor cells harbor oncogenic mutations in cell-regulatory genes that may not be reversible by treating the virus. HAM, with its high viral load and substantial cell-mediated immune response to HTLV-1, would appear to be a better candidate for antiviral treatment. A combination of zidovudine and lamivudine was used in a clinical trial of HAM treatment, but no clinical improvement was seen and there was no effect on HTLV-1 proviral load or immunologic markers.[334] Therapy that targets the immune response itself may afford an equally attractive avenue for the experimental treatment of HAM. Because of the shared routes of exposure, there is a potential opportunity to investigate the impact of antiviral therapy on persons coinfected with HTLV-1 or HTLV-2 and HIV-1. In fact, HTLV-2 proviral load actually increased after highly active antiretroviral therapy in patients with HIV-1/HTLV-2 coinfection.[335] The increase in HTLV-2 proviral load appeared to correlate with the magnitude of $CD4^+$ lymphocyte proliferation after successful control of HIV viral load, which suggests a clonal rebound of HTLV-2–infected lymphocytes.

Asymptomatic HTLV-1 and HTLV-2 Carriers

Evaluation and treatment of asymptomatic HTLV-1 and HTLV-2 carriers are the most frequently encountered clinical situations and generally follow HTLV diagnostic screening of asymptomatic blood, tissue, sperm, or oocyte donors. The first step is to confirm HTLV infection, either by review of positive screening EIA and confirmatory tests performed by a reputable testing laboratory or by submission of another specimen. False-positive results are common when EIA is used alone in low-risk patients; these patients may be relieved of a false diagnosis by the simple performance of a confirmatory assay. Typing of the infection as HTLV-1 or HTLV-2 is important because of the different disease outcomes associated with the two viral types. This can be done through type-specific WB or line immunoassay, differential titration on an immunofluorescence assay, or PCR assay. A clinical history regarding risk factors for HTLV infection is important in establishing the pretest probability of infection and can be helpful in typing the infection. Familial or sexual contact with people from HTLV-1–endemic areas favors that infection, whereas a history of injection drug use or sex with an injection drug user is more consistent with HTLV-2 infection. As mentioned earlier, patients with indeterminate WB results are also unlikely to be infected if they have no risk factors for HTLV infection.

Asymptomatic seropositive patients should be followed by their primary care or infectious disease physician with annual to biannual return visits. Medical history should elicit symptoms of leukemia, lymphoma, or neurologic disease. Physical examination is directed at the skin, lymph nodes, and neurologic system to detect manifestations of HTLV dermatitis, ATL, or HAM. Laboratory evaluation may be limited to a complete blood and white blood cell differential count. Although increases in the absolute lymphocyte and platelet counts have been described in prospective studies of HTLV-1 and HTLV-2 carriers, there is no indication that these have clinical significance.[299] It is more important to rule out subclinical leukemia by means of a normal lymphocyte count and absence of flower cell morphology. In general, asymptomatic carriers or those with nonspecific symptoms should be reassured with reminders of the low penetrance of hematologic and neurologic disease. Attention should be devoted to counseling regarding the prevention of further HTLV transmission through the use of condoms and safe sexual practices and the avoidance of breastfeeding neonates born to HTLV-infected women (at least in developed countries). There is also substantial psychological and social morbidity associated with chronic HTLV infection, and the physician needs to probe for and address these issues.[336–338]

Adult T-Cell Leukemia

Treatment of ATL patients depends on the type and extent of disease and has evolved with the discovery that some types of disease are better managed with zidovudine and IFN-α instead of traditional lymphoma chemotherapy.[339] The lymphoma type of ATL is an aggressive high-grade lymphoma with a generally poor prognosis, although prolonged remissions are seen.[222,340] In Japan, large trials of vincristine, cyclophosphamide, prednisolone, and doxorubicin with the addition of methotrexate, and more complex 9- and 10-drug regimens, have shown some success without prolonging long-term survival.[340,341] Initial response rates, even for the poorest risk categories, are more than 50%, and complete remissions are achieved in 20% of patients, but these responses can be short-lived, with relapses occurring in weeks to months.[340] Poor prognostic factors include poor performance status at diagnosis, age older than 40 years, extensive disease, hypercalcemia, and high serum lactate dehydrogenase level. Thirteen percent to 15% of patients with such aggressive cases experience long-term survival (>2 years), which in one study was associated with several factors: complete remission, longer time to remission, and total doxorubicin dose. Relapses in these long-term survivors often occurred in the CNS and proved refractory to subsequent therapy. Studies using combinations of doxorubicin and etoposide have demonstrated complete remission rates of 40%.

Substantial improvements in therapy for other ATL types (i.e., smoldering, chronic, and leukemia-type) have been achieved with newer regimens combining zidovudine and IFN-α.[342] Although its mechanism of action is not well defined, this combination produces a high rate of complete responses and prolongs survival.[343–346] Measurement of HTLV reverse transcriptase activity has suggested that ongoing HTLV replication might promote ATL and explain the mechanism of zidovudine and IFN-α action.[347] A meta-analysis has demonstrated superiority of this regimen for all ATL types except the lymphoma type.[348]

Therapy approaches for high-grade lymphomas including allogeneic bone marrow transplantation and autologous stem cell transplantation have resulted in remission and should be strongly considered in younger patients.[349] One case showed reappearance of cells harboring the integration of HTLV-1 previously observed in his leukemia cells, but the patient continued in clinical remission, suggesting a possible reversion to the preleukemic carrier state.[350] Experimental approaches that use arsenic trioxide or monoclonal antibodies targeting the IL-2R specific to ATL cells are being tested, with some evidence of at least partial responses.[224,351–353] Current trials for ATL include ruxolitinib, a Janus kinase 1 and 2 inhibitor, and for relapsed or refractory lymphoma include subcutaneous recombinant human IL-15 plus alemtuzumab, and nivolumab (www.ClinicalTrials.gov). A consensus report on ATL treatment has been published recently.[353a]

HTLV-1–Associated Myelopathy

Advances in the treatment of HAM have been hindered by the difficulty of performing randomized clinical trials because most cases occur in resource-limited countries. Accurate measurement of disease severity has also been a challenge in such trials, but the Osame disease severity scale[354] provides clinical staging and the 10-meter and 6-minute walk tests may be more quantitative.[355] Traditionally, immunosuppressive therapy with corticosteroids,[256,356] cyclophosphamide,[357] or both has been used to some benefit, particularly in acutely progressive cases. Toxicity limits the long-term use of these drugs, but short-term pulse therapy with intravenous methylprednisone may be of benefit for pain if not functional capacity.[358] More recently, IFN-α and IFN-β1a have shown some clinical benefit but fall short of definitive treatment.[359–361] Danazol, an androgenic steroid, has been used for symptomatic treatment of bladder and bowel symptoms resulting from spinal cord involvement in HAM, but it does not reverse the underlying neurologic deficit. The heparinoid pentosan polysulfate and the thiamine derivative prosultiamine were shown to improve motor function in some patients in small, open-label trials.[362,363] Given the emerging picture of disease pathogenesis with the inability to control high viral expression, therapy with antiviral drugs would seem a promising avenue for research, but initial clinical trials of antiretroviral therapy have not been successful.[334] Globally, current clinical trials for the treatment of HAM/TSP include mogamulizumab—a humanized antibody engineered to promote cellular cytotoxicity—raltegravir, and natural killer (NK) cell immunotherapy. Other studies underway are supportive and adaptive in nature, including sensorimotor exercises using a virtual reality platform, and urologic physical therapy for those with urinary symptoms. Recently completed trials include Mik-Beta-1 monoclonal antibody (Hu Mik-Beta-1), infliximab, anti-Tac antibodies directed against CD25 IL-2R α-chain

concurrently with zidovudine, pentoxifylline, zidovudine plus lamivudine, cyclosporine, and bortezomib plus raltegravir (www.ClinicalTrials.gov). The International Retrovirology Association publishes current treatment recommendations on its website (www.htlv.net/HAMpdf).

PREVENTION

Guidelines for HTLV-1 and HTLV-2 prevention and counseling were developed in 1993 by a Centers for Disease Control and Prevention working group; an update of these guidelines to incorporate more recent evidence would be welcome.[364] Standard prevention approaches address each of the routes of transmission and are similar for both viruses: screen blood donations before transfusion, eliminate breastfeeding by known infected mothers, and advise the use of condoms.

The value of blood donor screening has been well documented in highly endemic regions of Japan. In areas where the infection is not endemic, such as the United States, the cost-effectiveness of such screening has been questioned, but current FDA guidelines recommend HTLV testing of all blood donors but not currently of organ donors.

In high-income countries, it is recommended that pregnant women who are HTLV-1 positive should not breastfeed their infants. In developing countries where safe alternatives to breastfeeding may not be available, limiting breastfeeding to the first 3 months may afford some protection because of passively transferred maternal antibodies, although the safety of this approach has been cast in doubt.[161] The use of condoms is recommended for sexually active HTLV seropositive individuals and for couples who are serodiscordant for HTLV infection. Given the relatively low frequency of sexual transmission for each sexual encounter, couples who desire a pregnancy could plan to have unprotected sex during periods of maximal fertility. Techniques such as sperm washing, developed to allow assisted reproduction for HIV-infected persons, may also be used in the HTLV setting.

Population-based screening for HTLV infection (except for blood donors) is generally not indicated because of the low penetrance of disease and lack of effective therapies. The one exception in some countries may be HTLV-1 screening of pregnant women followed by interdiction of breastfeeding; such screening has been shown to be cost-effective in the United Kingdom.[365] Counseling seropositive patients should involve a clear discussion of the distinction of HTLV from HIV. In addition, HTLV type should be defined by serologic methods, and the distinctions in disease associations of the two virus types should be emphasized. Because the populations at risk for HIV are also at risk for HTLV-1 infection in viral endemic areas (e.g., persons at risk for sexually transmitted diseases, persons with high rates of partner exchange, commercial sex workers), HIV prevention guidelines also benefit those at risk for infection with HTLV-1. Thus, prevention measures that promote condom use, treatment of sexually transmitted infections, and decrease of high-risk exposures also prevent HTLV-1 infection.

There is no therapy for HTLV-1 infection and thus no chemoprophylaxis. Passive immunoprophylaxis is hypothetically effective, as noted earlier in animal studies, but has no practical clinical application, given the low risk for transmission, except through sexual contact, breastfeeding, and transfusion exposure, wherein other prevention methods are more applicable.

Although vaccines against HTLV-1 are potentially feasible, owing to the low penetrance of disease there has been little impetus to develop or market an HTLV-1 vaccine. Experimentally, vaccines containing whole-virus and recombinant HTLV-1 envelope antigens have successfully prevented HTLV-1 infection in monkey and rabbit models.[366] Protection correlates with the presence of neutralizing antibodies, indicating that humoral immunity can be an effective barrier against infection even when the challenge is cell associated.[202,367] The HTLV-1 envelope is relatively highly conserved, and neutralizing antibody appears to protect against challenge with even major strain variants, consistent with the conclusion that a single serotype protects against all variants. Thus, a synthetic vaccine against one HTLV-1 isolate could protect against other HTLV-1 isolates. A vaccine that induces cell-mediated immune responses in nonhuman primate studies has also been shown to be effective.

CONCLUSION

The study of HTLV-1 and HTLV-2 is important for public health, clinical, and scientific reasons. In the years since the discovery of this first human leukemia virus, significant progress has been made in understanding the biology and epidemiology of HTLV. Routes of transmission for HTLV-1 and HTLV-2 are well defined, and sexual and parenteral transmission may be controlled through appropriate public health interventions, including those directed at the HIV epidemic. Knowledge of the clinical outcomes of HTLV infection has expanded since the discoveries of the relationships between HTLV-1 and ATL and between HTLV-1 and HTLV-2 and HAM. A growing array of syndromes related to the virologic or immunologic effects of these retroviruses has been recognized, and prospective observational cohorts of persons infected with HTLV-1 and HTLV-2 will yield additional data on their clinical outcomes. Among those infected with HTLV-1, the incidence of either ATL or HAM is less than 5%, although there is substantial mortality in the former and morbidity in the latter. With millions of people estimated to be infected by HTLV-1 worldwide[73] and a lack of effective therapy,[368] prevention efforts are important. Blood transfusions need to be screened, sexual transmission prevented with condom use, and breastfeeding by HTLV-infected mothers limited, although the last approach may not be feasible in developing countries. In the United States, public health guidelines for the control and management of HTLV-1 and HTLV-2 infections are outdated and need to be revised.

From a scientific viewpoint, the HTLVs offer interesting models for understanding the interplay between chronic retroviral infection and the human immune system that determines the latent proviral reservoir and potential approaches for its eradication and cure of HTLV and HIV infections. Understanding the virology, immunology, and pathogenesis of HTLV infection may be relevant to the study of leukemia, lymphoma, and multiple sclerosis. It is also conceivable that additional viruses of this class with long latency, low-level replication, and specific cellular tropism may be discovered in unexplained autoimmune, neurologic, and malignant diseases.

ACKNOWLEDGMENT

The authors wish to thank Jessica Bailey for assistance with rendering figures in this chapter.

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The complete reference list is available online at Expert Consult.

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169 Human Immunodeficiency Viruses

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SHORT VIEW SUMMARY

Definition
- Human immunodeficiency virus (HIV) is a *Lentivirus*, family Retroviridae, that causes acquired immunodeficiency syndrome (AIDS). There are two types, HIV-1 and HIV-2, of which HIV-1 is the more widely distributed and more pathogenic.

Epidemiology
- HIV infection is worldwide in distribution and is transmitted by sexual activity and intravenous drug use.

Microbiology
- HIV is a single-stranded positive-sense RNA virus that contains three structural genes *(gag, pol, env)* and six regulatory genes. HIV infects $CD4^+$ T cells and uses its reverse transcriptase (RT) to transcribe its RNA genome into double-stranded DNA, which is integrated into the host cell genome.

Diagnosis
- Infection is identified by detection of viral RNA (virus load) or by immunologic responses to viral proteins (Western blot) (see Chapter 120).

Therapy and Prevention
- Antiretroviral therapy (ART) has been developed using nucleoside and nonnucleoside RT inhibitors, protease inhibitors, integrase inhibitors, and entry inhibitors (see Chapter 128). ART can also decrease the rate of transmission of infection. Vaccine development seeks to stimulate immune responses to HIV antigens that will inhibit transmission and/or replication of virus (see Chapter 119).

OVERVIEW OF HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME

Infection with human immunodeficiency virus type 1 (HIV-1) and its end stage, acquired immunodeficiency syndrome (AIDS), is one of the major public health challenge of modern times, with more than 25 million persons already dead and 30 to 40 million living with HIV/AIDS, many of whom are without access to therapy. AIDS was first recognized in the United States in 1981 with reports of unexplained opportunistic infections, including *Pneumocystis jirovecii* pneumonia and Kaposi sarcoma (KS), among homosexual men in New York and San Francisco.[1–3] On the basis of the epidemiologic features, association with the loss of $CD4^+$ lymphocytes and immunosuppression, and likely infectious cause, a new human retrovirus was postulated as a causal agent. The field of retrovirology had markedly advanced just a decade earlier with the description of reverse transcriptase (RT) and with the discovery of human T-cell lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2), the first two known human retroviruses, in 1979 and 1981 (reported in 1980 and 1982, respectively).[4,5] The discovery of interleukin-2 (IL-2), or T-cell growth factor,[6,7] allowed the culture of blood T lymphocytes from early cases of AIDS; by 1984 the detection, isolation, and propagation of HIV-1, the third human retrovirus,[8–12] had led to the development of an immunodiagnostic test, an increasingly detailed understanding of the molecular biology of this virus, and, most important, the introduction of a rational basis for antiviral therapy.[13] Eventually new therapeutic combinations (RT and protease inhibitors), combined with the ability to measure circulating viral RNA and resistance to drugs, led to a dramatically improved clinical course for those with access to therapy. More recently, inhibitors of virus-cell fusion, viral entry into the host cell, and integration of viral DNA into host chromosomal DNA have become available. Within a brief period, technologic advances provided a clearer understanding of viral dynamics and the disease process, focusing attention on viral replication, host immune responses, and T-cell dynamics while confirming and elaborating the causal role of the virus. Equally dramatic has been the elucidation of how HIV enters cells, using both the CD4 molecule and a chemokine receptor as a dual-receptor system, as well as the mapping of the three-dimensional structure of the viral envelope protein. Contemporary retrovirology is largely devoted to the study of HIV-1 and of HIV-associated diseases. The molecular and cellular biology of this virus is now better understood than that of almost any other in history. Research on HIV showed that rational antiviral therapy is possible and thereby pointed the way toward therapy for other viral diseases. Although this information has yet to be completely translated into greater progress in the areas of prevention, therapy, vaccines, and immune reconstitution for much of the developing world, where most HIV is transmitted, the Presidential Emergency Plan for AIDS Relief (PEPFAR) and other programs have greatly facilitated the institution of effective therapies in many parts of Africa.

Viruses are obligate intracellular parasites, and every aspect of the virus is in some way relevant to virus-host relationships. This chapter outlines the life cycle, molecular and cellular biology, structure, and regulation of HIV-1 and includes some discussion of pathogenesis and outcomes of infection. Although the division of the chapter into sections is useful for organization of the information presented, in reality these subjects cannot be separated from one another; from the perspective of both virus and host, the process of infection is a continuous series of connected interactions.

ORIGIN AND CLASSIFICATION OF HUMAN RETROVIRUSES

Current knowledge has placed retroviral infection of humans as zoonoses that originated in primate-to-human species-jumping events. For HIV-1 and HIV-2, these events occurred in Central and West Africa, most likely at multiple times, with the more recent attaining major epidemic significance. Simian immunodeficiency virus of chimpanzees (SIV_{cpz}) is the immediate precursor to HIV-1.[14] It is likely that similar species-jumping events occurred previously between certain types of monkeys and chimpanzees.[15]

Retroviruses are classified by a number of different biologic features into at least seven genera. Oncogenic retroviruses occur in all classes of vertebrates. The first identified infectious agents that produced cancer in chickens were isolated by Ellerman and Bang (1908)[16] and by Peyton Rous (1910).[17] These workers were considerably ahead of their time, and biologic systems to culture and study these viruses had not yet been described. Rous eventually won a Nobel Prize in 1966 for his work. The pioneering work of Ludwig Gross in the 1950s stimulated

renewed interest by demonstrating that oncogenic viruses could produce tumors in mammals,[18] but for the next 3 decades most scientists believed that human retroviruses did not exist. We now know that the pathogenic human retroviruses include lentiviruses (HIV-1 and HIV-2) and oncoviruses (HTLV-1 and HTLV-2). A variety of human endogenous retroviruses, present in the human germline, are often, but not always, replication defective and have not yet been shown to cause any disease.

As their major replication strategy, retroviruses reverse transcribe viral RNA into linear double-stranded DNA (dsDNA), with subsequent integration into the host genome. The characteristic enzyme used for this process, an RNA-dependent DNA polymerase that reverses the flow of genetic information, is known as RT. Its discovery altered the "central dogma" of molecular biology—namely, that genetic information only flows from DNA to RNA[19,20]—and helped initiate the modern era of molecular biology. RT is error prone; with the massive turnover of virions in the infected host, these errors accumulate in the viral DNA, accounting for the high mutability of HIV-1. The lifestyle of the retrovirus thus involves two genomic forms, a DNA provirus and an RNA-containing infectious virion.

The discovery of HTLV-1 and its etiologic association, first with adult T-cell leukemia, an aggressive T-cell lymphoma,[21-23] and later with a neurologic disease, tropical spastic paraparesis/HTLV-1–associated myelopathy (TSP/HAM),[24] were pivotal events in modern medicine (see Chapter 168). In an absolute sense, HTLV-1 is not very efficient in inducing lymphoma (~10% lifetime risk), but it is quite efficient compared with other human carcinogens, including other viruses. Although there is relatively little variation among HTLV-1 isolates, HTLV-2, the second human retrovirus, is only 50% identical to HTLV-1 at the genomic level.[4] Similarly, HIV-2, the fourth human retrovirus, was identified as a serologic variant of HIV-1, the third human retrovirus, and was isolated from patients in western Africa.[25,26] Some types of SIV are so closely related to HIV-2 that they may form an overlapping continuum with recent common ancestors. HIV-2 is known to infect several monkey species, including the sooty mangabey, its original host, and SIV has been transmitted, albeit rarely, to laboratory workers. SIVs and SIV/HIV hybrids (SHIVs) have been used extensively to study animal models of immunodeficiency. Other species, including cats (feline leukemia virus [FeLV] and feline immunodeficiency virus [FIV]) and cattle (bovine leukemia virus [BLV] and bovine immunodeficiency virus [BIV]), harbor retroviruses analogous to those of humans and some African primates. HIV-related retroviruses, known as lentiretroviruses, also include the ungulate viruses, maedi-visna virus of sheep, caprine arthritis-encephalitis virus, and equine infectious anemia virus.

As RNA viruses, retroviruses have the survival advantage of great genetic diversity. As viruses with a DNA intermediate in their replication cycle, they also have the advantage of latency, as do many DNA viruses, but even more so because the DNA provirus is integrated into the chromosomal DNA of the infected cell. As a $CD4^+$ T-cell and macrophage-tropic virus, HIV also has the advantage of reducing the effectiveness of the host immune response.

Retroviruses are typically approximately 100 nm in diameter and contain two single strands of RNA, which permits recombination between the strands (Fig. 169.1). The typical genome is approximately 10 kilobases (kb) or less in size and contains three major structural genes—namely, *gag* (group-specific antigen; core structural proteins), *pol* (polymerase; RT and other enzymes), and *env* (envelope proteins). HIV-1 also contains several additional genes; similar "extra" genes were first described in HTLV-1. More recently, antisense transcripts and proteins, of unclear function, have been described for both HIV-1 and HTLV-1.[27,28] In both viruses some of the extra genes are essential to viral replication, whereas others may modulate interactions of the virus with its host. Fig. 169.2 outlines the genome composition of HIV-1 and HTLV-1.

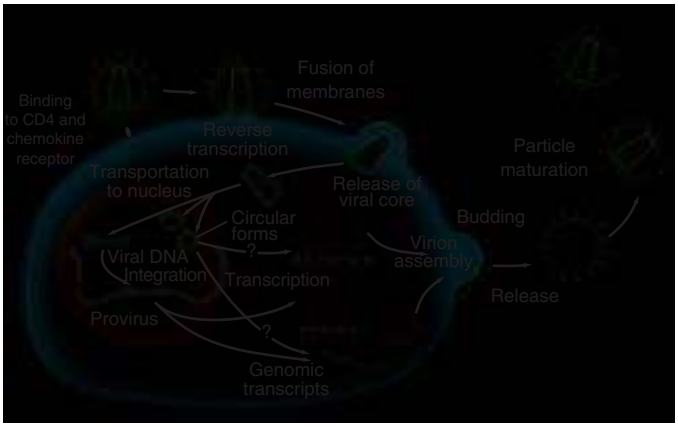

FIG. 169.1 The life cycle of human immunodeficiency virus type 1.

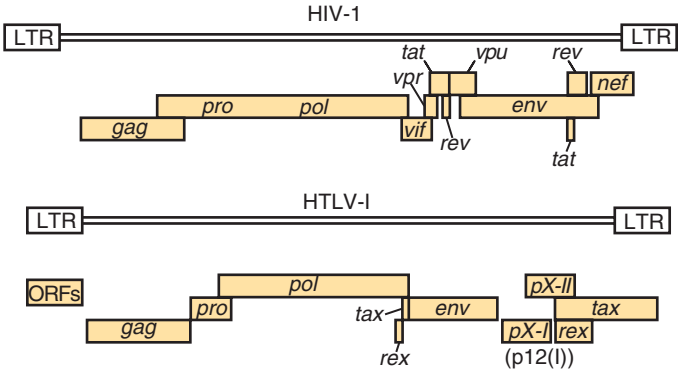

FIG. 169.2 Genomic organization of human retroviruses. A comparison of the genomes of human T-cell lymphotropic virus type 1 *(HTLV-1)* and human immunodeficiency virus *(HIV)* is shown. Studies of HTLV genes and gene products laid the foundation for an understanding of their functional homologues subsequently found in HIV (e.g., *tat* and *rev*), although there is little sequence homology of these genes between HTLV and HIV. *env,* Envelope; *gag,* group-specific antigen; *LTR,* long terminal repeat; *nef,* negative regulatory factor; *ORFs,* open reading frames; *pol,* polymerase; *pro,* protease; *rev,* regulator of viral expression; *rex,* regulator of viral expression; *tat,* trans-activator; *tax,* trans-activator of transcription; *vif,* virion infectivity factor; *vpr,* viral protein R; *vpu,* viral protein U.

VIRAL TRANSMISSION AND LIFE CYCLE

Biology of Transmission

The infectious life cycle of HIV can be described both at the molecular, single-cell level and at the level of a host organism infected with a "swarm" of closely related viral species. Multiple variables, including route of exposure, dose, immunogenetic background, and concomitant infections, influence the probability of transmission. The biologic events during exposure and successful infection of the host are only partially elucidated. The most common modes of infection are sexual transmission at the genital or colonic mucosa; exposure to other infected fluids, such as blood or blood products; transmission from mother to infant; and occasionally accidental occupational exposure, such as needlesticks. Transmitted viruses typically use the interaction of the viral glycoprotein gp120 with the cellular receptor CD4 and the chemokine receptor CCR5 to gain cell entry, thus selecting for macrophage-tropic *non–syncytia-forming* variants. This finding explains why persons who lack a functional CCR5 receptor are relatively resistant to infection by sexual transmission. Non–macrophage-tropic strains, also called *syncytia-inducing*, are typically found late in infection. These strains use another chemokine receptor, CXCR4, to facilitate entry and are apparently not readily transmitted from person to person. In a model of acute infection in the macaque, the first cellular targets of intravaginal inoculation of the virus are Langerhans cells, tissue dendritic cells in the lamina propria that then fuse with lymphocytes. However, direct infection of T cells has also been described. Infected cells can be found in draining lymph nodes within 2 days and in plasma after 5 days.[29]

In HIV-1–infected persons, there is a rapid rise in plasma viremia within days, with high viral titers and widespread dissemination, probably targeting lymphoid organs and the central nervous system (CNS). This acute stage of HIV infection is sometimes manifested as a transient

symptomatic illness characterized by a maculopapular rash and flulike symptoms. This phase is followed by a marked reduction in virus to steady-state levels, probably because of vigorous antivirus cellular responses. The immune response probably accounts for the mononucleosis-like acute syndrome seen in approximately half of patients. Initially, perhaps within hours of infection, at least three HIV inhibitory chemokines (notably macrophage inflammatory protein-1α [MIP-1α], MIP-1β, and RANTES [regulated on activation, normal T-cell expressed and secreted]) may be produced. As is the case for many infections with viruses that establish chronic infections, this response is at least partially successful in controlling replication. Levels of HIV-1–specific cytotoxic T lymphocytes (CTLs) are inversely correlated with plasma viral RNA levels.[30] High levels of potent CTL virus-specific cells targeted to the viral Env protein and soluble factors produced by these cells early in infection may correlate with the decline of virus, even before a neutralizing antibody can be detected.[31]

Great variability in peak viral RNA plasma levels is seen during the first 120 days after infection. By 3 to 6 months after infection, viral levels reach a temporary steady state, sometimes called a viral set point. This level positively correlates with subsequent disease progression. Thus, early in the course of infection, virus-host interactions are established that are predictive of subsequent disease.[32]

Intervention to control infection during this initial period has been shown to decrease the risk of subsequent infection in health care workers after needlestick exposure.[33] Treatment of infected mothers and exposed neonates has also shown a dramatic effect in decreasing the incidence of maternal-fetal transmission.[34] Likewise, treatment with antiretrovirals, such as Truvada, greatly reduce sexual transmission of HIV-1,[35] although drug-resistant variants may still be transmitted.

Replication Cycle: Cell Entry and Integration

The replication cycle of HIV-1 can be considered in two distinct phases (see Fig. 169.1). The first phase includes viral attachment, entry into the cytoplasm, reverse transcription, entry into the nucleus, and integration of the dsDNA (the provirus). The second phase occurs over the lifetime of the infected cell as viral and cellular proteins regulate the production of viral proteins and new infectious virions.

Infection is initiated by the binding of the virion gp120 Env surface protein to CD4, found on some T cells, macrophages, and microglial cells. Both SIV and HIV-2 also use CD4. CD4 was first identified as a viral receptor in a number of studies showing the susceptibility of CD4-bearing cells to infection and the ability to block infection with anti-CD4 monoclonal antibodies in culture. Transfection of human $CD4^-$ HeLa cells with CD4 DNA rendered them permissive for infection.[36] Successful in vitro experiments blocking this interaction with soluble CD4 used laboratory strains adapted to cell lines and led to therapeutic attempts using immunoglobulin CD4, which were unsuccessful. Subsequent experiments showed that primary isolates are insensitive to soluble CD4 and highlighted the necessity of using primary rather than laboratory-adapted isolates in studying virus-host interactions.

As with other lentiretroviruses, macrophages could also be infected with HIV, but strains differed in their ability to infect T-cell lines or monocytes.[37] Binding to CD4 is not sufficient for cellular entry of HIV, and the fact that small changes in the V3 loop of envelope gp120 (see later) could determine tropism of the virus for either macrophage or T-cell lines suggested that a second receptor was present. The first important clue for the basis of this tropism was the unexpected finding that a group of chemokines (RANTES, MIP-1α, and MIP-1β), small extracellular proteins naturally produced by $CD8^+$ T cells, inhibited macrophage-tropic but not T-cell line–adapted strains.[38] This discovery at once explained the nature of a long-sought CD8 viral suppressor factor and suggested a previously unexpected role for chemokine receptors. The independent identification of an orphan chemokine receptor, CXCR4, as the second receptor for T-cell line–tropic strains[39] was followed by a rapid series of reports demonstrating CCR5 to be the principal second receptor for macrophage-tropic strains and CXCR4 for T-cell line–adapted strains.[40–43] Crystallographic evidence indicates that CD4 binds in a recessed pocket on gp120, which includes a deep cavity that binds to phenylalanine-43 of CD4. Previous mutagenesis studies had shown this phenylalanine to be crucial for binding.[44] Other studies have also indicated a role for sugar molecules, glycosaminoglycans, in the binding of gp120, which influences interactions with the chemokine receptor.[45] CD4 binding triggers a conformational change in gp120 that allows it to then bind to CCR5 or CXCR4. This second binding event exposes the fusion domain of the gp41 transmembrane protein, allowing fusion of the viral and cellular membranes, followed by viral entry into the target cell.

Events that occur immediately after viral entry—collectively, the disassembly process—are highly regulated. For example, HIV-1 must incorporate a cellular protein, cyclophilin A, which binds to the viral capsid protein p24. Failure to incorporate this cellular protein results in a profound postentry block during the next viral entry. Coincidentally, cyclophilin (peptidyl-prolyl isomerase) is the binding protein for cyclosporine, an inhibitor of T-cell activation, suggesting that activation-related cellular processes are involved in viral disassembly.[46,47] In addition, virion infectivity factor (Vif) and "negative" regulatory factor (Nef; a misnomer), accessory viral proteins, may also be required.[48]

Reverse transcription begins in the cytoplasm, as DNA synthesis is initiated from a cellular transfer RNA (tRNA) primer bound to the viral genomic RNA just downstream of the 5′ long terminal repeat (LTR). Reverse transcription proceeds in a similar orderly fashion in all retroviruses. Briefly, the transcription complex begins at the 5′ end, copies the U5 and R regions of the 5′ LTR, and then jumps to the 3′ end of the RNA, where the newly synthesized R region DNA binds to the R region of the 3′ LTR. Reverse transcription continues through the U3 region of the 3′ LTR and then through the remainder of the viral RNA, which gives a complete minus strand of DNA. The RNA is degraded by the viral ribonuclease H, except for two resistant purine-rich tracts in the middle and toward the 3′ end of the viral RNA. These then serve as the primers for formation of the DNA plus strand.[49] Because reverse transcription takes place in the cytoplasm, local concentrations of nucleotides may be a limiting factor, particularly in nondividing cells. The cellular protein sterile alpha motif and HD-domain–containing protein 1 (SamHD1) dephosphorylates nucleotide triphosphates, reducing the precursor pool and inhibiting reverse transcription. SamHD1 is targeted for degradation by viral protein X (Vpx) of HIV-2 and some SIVs, but HIV-1 lacks Vpx and therefore has a reduced ability to infect dendritic cells, macrophages, and quiescent T cells.[50–52]

During the formation of dsDNA, the uncoated nucleoprotein complex, termed the *preintegration complex*, is imported into the nucleus. This is an energy-requiring process that uses nuclear localization signals present on viral Gag, viral protein R (Vpr), and integrase (IN) proteins. Unlike most retroviruses, which integrate into the host cellular DNA as the nuclear membrane is disrupted during cell division, HIV-1 can enter the nucleus and integrate into nondividing cells. This may be critical in the infection of monocytes and macrophages, which are essentially nondividing cells.

IN-negative mutants of HIV do not integrate and do not produce infectious virus.[53,54] Although integration is not site directed, HIV preferentially integrates into or near active genes, particularly those that are activated after infection by HIV-1.[55] Unintegrated viral DNA may survive, however, particularly in quiescent cells. This may provide a stable intermediate form in cells that are temporarily not permissive for infection; if cell activation occurs when these forms are present, viral infection may then proceed to completion. Integration of viral DNA establishes a linear copy of the viral genome within the genome of the cell, and replication of the virus then occurs with cell replication. Integration is generally for the life of the cell and, with the cell and its progeny, for the life of the organism.

Replication Cycle: Virus Expression and Production of New Virions

Synthesis of new viral RNA genomes and proteins is accomplished in a highly regulated manner by using host cell proteins. A high level of viral production from several different cellular compartments can be maintained throughout the course of infection. The high number of replication cycles allow the generation of variants and selection by drugs or the immune system. The half-life of virus-producing $CD4^+$ T cells is approximately 0.7 days, and the generation time of HIV-1 in vivo is

approximately 2 days.[56] HIV-1 pathogenesis is the result of a complex interplay between the virus and the immune system, particularly the mechanisms responsible for T-cell homeostasis and regeneration. Protracted loss of $CD4^+$ T cells results from early viral destruction of selected memory T-cell populations, followed by a combination of profound increases in overall memory T-cell turnover, damage to the thymus and other lymphoid tissues, and physiologic limitations in peripheral $CD4^+$ T-cell renewal.[57] Equally important are indirect hematopoietic and immunoregulatory effects of viral components (see later).

Once integration has occurred, virus production depends on the presence of cellular and viral factors required for activation of viral promoters. External factors, including coinfection with other agents, production of inflammatory cytokines, and cellular activation, may enhance viral replication.[58] The molecular mechanisms regulating virus production include cellular pathways involving factors, such as the nuclear factor kappa B (NF-κB) family of inducible transcription factors, that result in a cascade of events leading to viral genome expression.[59]

An important feature of HIV-1 is that expression of different viral RNA species is temporally regulated. Using cellular enzymes, such as RNA polymerase II, transcription of the provirus is initiated at the viral promoter, at the junction of the U3-R regions in the LTR, as a single complete message. The viral messenger RNA (mRNA) and genomic RNA transcripts, processed by cellular machinery, are spliced, capped, polyadenylated, and transported to the cytoplasm for translation into viral proteins. Differential splicing of this complete RNA, controlled in part by the viral protein Rev, determines the type of message and protein that is produced. Early after infection, activated cells produce 2-kb mRNAs for viral regulatory proteins that can be detected by Northern blot analysis[60]; using even more sensitive reverse-transcriptase polymerase chain reaction techniques, expression can be detected within 6 hours.[61] These messages include the unique double-spliced RNA for Tat, Rev, and Nef proteins.[62] Tat protein induces a markedly enhanced activity of the viral promoter, chiefly by facilitating the elongation of nascent short viral transcripts, resulting in greatly increased RNA and protein production. The Rev protein serves to decrease the production of double-spliced messages. With the accumulation of Rev protein, there is a switch to enhanced expression of unspliced and single-spliced mRNAs that encode the late viral proteins, including the virion proteins Gag, Pol, and Env and viral proteins Vpu, Vpr, and Vif, as well as genomic RNA. The delayed transit from early to late viral genes probably reflects a requirement for threshold levels of Rev needed to bind and form multimers of the protein complexed with the Rev regulatory element (RRE), located in incompletely spliced mRNAs. Packaging of the genomic RNA within a virus particle requires the presence of a specific packaging signal located between the major splice donor at the 5′ end of the genome and the initiation codon for the Gag precursor polyprotein. In the absence of this signal, mature particles are formed that are devoid of RNA. The incorporation of genomic RNA requires two zinc-finger domains found in the p7 Gag nucleocapsid protein. Assembly of mature viral particles occurs at the cell membrane with the association of the Gag matrix protein p17 with the cytoplasmic domain of the envelope transmembrane protein gp41, which in turn binds to the viral gp120 on the outer surface. Assembled virions include the viral envelope proteins, cell membrane and associated cellular proteins, a matrix composed mainly of Gag p17, and a capsid composed of the p24 capsid protein and RNA, RT, IN, Gag proteins p6 and p7, and Vpr. The mature viral particle characteristically buds from the cell surface into the surrounding media, completing the life cycle of the virus. Budding of HIV-1 virions occurs at highly specialized membrane microdomains known as lipid rafts.[63] These domains are characterized by a distinct lipid composition that includes high concentrations of cholesterol, sphingolipids, and glycolipids.

THE VIRION

The mature infectious virus particle buds from a cell membrane, forming a sphere with an outer lipid bilayer and a nucleocapsid with a dense, cone-shaped core (Fig. 169.3). The core appears to be attached to the viral outer envelope at its narrow end.[64] The outer membrane contains up to 72 spiked knobs, which are assembled as trimers of the outer envelope protein gp120 bound to the transmembrane portion gp41. The viral membrane is cholesterol rich and includes cellular proteins.[65]

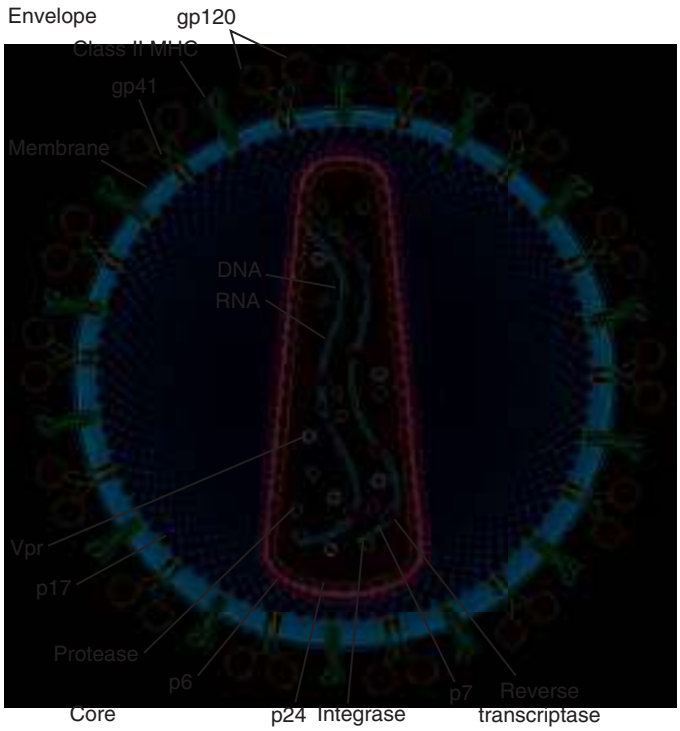

FIG. 169.3 Structure of the human immunodeficiency virus type 1 (HIV-1) virion. The viral envelope is formed from the host cell membrane, into which the HIV-1 envelope proteins gp41 and gp120 have been inserted, and may include several host cell proteins, most significantly the class II major histocompatibility complex *(MHC)* proteins. The matrix between the envelope and the core is formed predominantly from group-specific antigen (Gag) protein p17. The core contains the viral RNA, closely associated with Gag protein p7, in addition to reverse transcriptase (RT) and integrase. It has also been shown that virions contain complementary DNA, as shown, synthesized by the RT. The major structural proteins of the core are Gag proteins p24 and p6. Also present within the virion are the protease and two cleavage products from the Gag precursor protein (p1 and p2, not shown) of undetermined position within the virion. Viral protein R *(Vpr)* is also packaged in the virion and is thought to be localized within the core, as shown.

Each mature virion contains two molecules of single-stranded RNA surrounded by a capsid containing three *gag* gene cleavage products: the p17 matrix protein; the p24 major capsid protein, which forms the capsid shell; and the p7 nucleoprotein, which binds tightly to the viral RNA. The matrix contains the myristoylated matrix protein p17, which is critical for virion formation and is localized between the capsid protein p24 of the viral core and envelope. The p7 protein binds the two positive-strand copies of complete viral RNA attached at the packaging site, and it also binds to p24. Other viral proteins required for the early phases of infection are incorporated with the virion and include protease, which is essential for viral assembly; RT and IN, which are needed after entry for viral DNA synthesis and integration; $tRNA^{lys}$ at the 5′ end of the RNA, which serves as the primer for initiation of negative-strand DNA synthesis; and Vpr, a small protein that contains a nuclear localization signal and is associated with the nucleocapsid in large quantities. See Fig. 169.4 for a schematic view of the preintegration complex. The virus encodes at least five other regulatory and/or accessory genes of diverse function. Some have been discussed already, and most are present in the infected cell but not in the mature virion. A list of HIV genes and associated proteins is presented in Table 169.1.

Genomic Organization

The HIV-1 proviral DNA integrated into the host cell is 9.7 kb in length and follows the basic genomic structure common to most retroviruses:

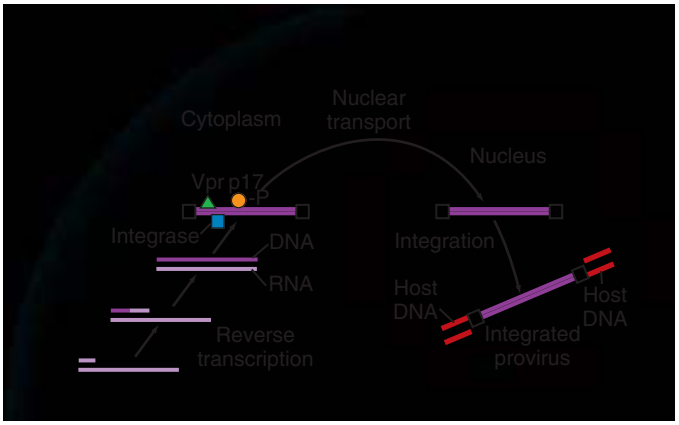

FIG. 169.4 Viral proteins required for nuclear transport and integration. After entry, formation of the reverse transcription complex and reverse transcription, human immunodeficiency virus (HIV) infection involves the formation of the preintegration complex, consisting of the newly synthesized viral DNA and several HIV proteins: the matrix protein *(p17),* integrase, reverse transcriptase, and viral protein R *(Vpr).*

TABLE 169.1 Genes and Gene Products of HIV-1 and HIV-2

GENE	PROTEINS	SIZE (kDa)	FUNCTION/PROPERTIES
gag	p17 p24 p6 p7 P1 p2		Matrix protein; interacts with gp41 Core protein Core protein; binds to Vpr Nucleocapsid; binds to RNA
pol	Protease Reverse transcriptase Integrase	10 66, 51 32	Proteolytic cleavage of Gag and Pol Polymerase and RNase H activity (p66 only) Integration into chromosome
env	gp120 gp41		Envelope; viral entry into cell Transmembrane protein; cell fusion
vif	Virion infectivity protein	23	Efficient cell-free transmission
vpr	Viral protein R	18	Enhances viral replication in primary cells, virion-associated protein; G_2/M phase arrest; nuclear localization
tat	Trans-activator of transcription	14	Major viral trans-activator, immune suppression
rev	Regulator of expression of virion protein	19	Enhances expression of unspliced and single-spliced RNAs
vpu[a]	Viral protein U	15–16	Enhances virion release from cells; downregulates CD4 and class I MHC surface expression
nef	Negative regulatory factor	27	Inhibits or enhances viral replication depending on strain and cell type Downregulates CD4; class I MHC Antiapoptosis
vpx[b]	Virion protein X	25	Packaged into the virion

[a]HIV-1 only.
[b]HIV-2 only.
HIV, Human immunodeficiency virus; *kDa,* kilodaltons; *MHC,* major histocompatibility complex; *RNA,* ribonucleic acid; *RNase H,* ribonuclease H.

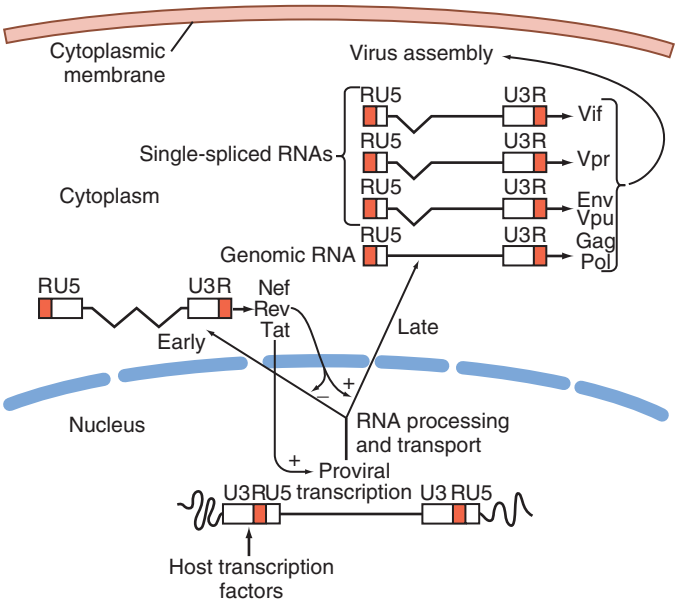

FIG. 169.5 The role of RNA splicing in the life cycle of human immunodeficiency virus type 1 (HIV-1). The early messenger RNA (mRNA) transcripts of HIV-1 are doubly spliced and produce viral regulatory proteins Tat, Rev, and Nef. The function of HIV Rev is to facilitate the expression of the late transcripts of HIV-1. These can be divided into two categories, unspliced and singly spliced. The unspliced HIV-1 mRNA has two functions: It is translated into the structural precursor polyproteins for the *gag* and *pol* gene products, and it is incorporated into virions as genomic RNA. The different single-spliced mRNAs of HIV-1 are translated into the envelope proteins gp120 and gp41, as well as Vif, Vpr, and Vpu. *Env,* Envelope; *Gag,* group-specific antigen; *Nef,* negative regulatory factor; *Pol,* polymerase; *Rev,* regulator of viral expression; *Rex,* regulator of viral expression; *Tat,* trans-activator; *Vif,* virion infectivity factor; *Vpr,* viral protein R; *Vpu,* viral protein U.

gag-pol-env genes flanked by two complete viral LTRs (see Fig. 169.2). The provirus is symmetrically flanked at either end by the viral LTR and by cellular sequences derived from the site of integration. These LTRs contain transcriptional regulatory sequences, RNA processing signals, packaging sites, and the integration sites. The 5′ end begins with the *gag* gene, which encodes core and matrix proteins; the *pol* gene, which begins in a partially overlapping reading frame encoding viral protease, RT, and IN; and then the *env* gene, which encodes the outer and transmembrane envelope proteins. A complex series of open reading frames encode the accessory proteins. The *vif* gene is contained partly within the *pol* coding region and partly downstream, and *vpr* is located downstream from *vif.* The first coding exons for *tat* and *rev* are colinear and located between *vpr* and *env*, and their second exons, located in the *env* gene, are joined to the first exons by RNA splicing. The *vpu* gene is colinear with the 5′ region of the *env* gene; *nef* is located downstream from the *env* gene and extends into the downstream LTR sequence.

Transcription of a single unspliced RNA is initiated at the 5′ end by the cellular RNA polymerase II. The unspliced mRNA serves as a template for translation of the Gag and Gag-Pol precursor polypeptides. This message is also spliced to produce single-spliced transcripts for Vif, Vpr, and Vpu proteins and the Env precursor polypeptide, and double-spliced transcripts for Tat, Rev, and Nef. The structures of these mRNAs are shown schematically in Fig. 169.5. The precursor polyproteins are then cleaved by cellular or viral enzymes; the Gag and Gag-Pol precursors are cleaved by the viral protease, which is itself transcribed from the unspliced viral message. The Env precursor polypeptide is cleaved by cellular proteases.

VIRION STRUCTURAL PROTEINS

Gag Proteins

The cleavage of the Gag precursor protein by viral protease produces the structural components of the viral core and matrix. To form the virus capsid structure, a large polyprotein is translated from the viral mRNA. This 55-kilodalton (kDa) Gag precursor protein (sometimes called p55) is cleaved into at least five structural proteins by the viral 34-kb protease encoded at the 5′ end of the *pol* gene. Lack of protease function, either through inhibition by antiretroviral drugs or after

transfection of the *p55* gene into a cell that lacks protease, results in the formation of noninfectious viral particles.[66]

The p55 protein is seen on Western blot preparations made from whole-cell lysates but not on those made from mature virions. During or shortly after self-assembly, the viral protease is activated, and the precursor is cleaved into three principal proteins and two smaller peptides. These proteins undergo extensive posttranslational modification by cellular enzymes. After translation, the initiating methionine residue is removed, p17 is myristoylated, p17 and p24 are phosphorylated by cellular kinases, and p7 (the nucleocapsid protein, or NC) binds to two zinc ions to form the zinc fingers that bind to RNA. Gag proteins are sufficient to self-assemble into noninfectious particles when expressed from transfected cells. The p17 matrix protein, MA (molecular weight, 17 kDa), contains about 130 amino acids and is myristoylated on a glycine at its amino terminus by the host cell enzyme *N*-myristoyl transferase.[67,68] The first 31 amino acids target the myristoylated p17 to the cell membrane, and virions with nonmyristoylated p17 are not infectious. In addition to viral assembly at the cell surface, MA functions as part of the preintegration complex targeting viral DNA to the cell nucleus and enabling HIV-1 to infect and integrate into nondividing cells, such as macrophages.[69] p17 has extracellular functions via binding to the IL-8 receptors CXCR-1 and CXCR-2 expressed on peripheral blood mononuclear cells (PBMCs),[70] increasing the production of proinflammatory cytokines and counteracting the inhibitory activity of IL-4 on these cells.[71]

The p24 capsid protein, CA, is produced by two cleavages to form a 240–amino-acid hydrophobic protein that forms the major subunit of the viral capsid and self-associates to form dimers and higher-order structures. This protein binds the cellular cyclophilins, a process that may be important for viral replication.[72] A major homology region of 29 amino acids is shared with many retroviruses.[73,74] p24 Gag is typically the easiest protein to detect using sera from infected patients, and serologic detection in retrovirus-infected animals gave the general name *group-specific antigen* (Gag) to these proteins. The carboxyl-terminal sequences encode a 70–amino-acid hydrophilic protein, NC, that binds both viral RNA and the capsid p24 protein, intertwining approximately one molecule with four to six nucleotides of RNA[75] and another small protein, p6. The p7 zinc-finger domain binds RNA and recognizes the packaging site on Gag.[76,77] The p6 protein appears to mediate the incorporation of Vpr into the virion and facilitates release of the virion from the cell surface, perhaps because of its binding to tumor susceptibility gene 101 (Tsg101) and ALIX (ALG2-interacting protein X), two cellular budding factors.[78] Two small peptides of unknown function, p2 and p1, are also found in the viral core.

An intracellular antiviral mechanism targets the capsid protein and is mediated by the tripartite motif (TRIM) family.[79] The TRIM motif consists of a RING (really interesting new gene) domain at the N-terminus, a B box-2 domain, and a "coiled coil" domain[80] and apparently represents a widespread and ancient innate host defense mechanism. TRIM5α appears to be functionally similar to the Friend virus susceptibility-1 (Fv1) restriction element, which regulates permissivity of murine cells of different genetic backgrounds for infection with different strains of tropic murine leukemia viruses and was identified more than 40 years ago.[81] HIV-1, however, is only partially sensitive to human TRIM5α (depending on allelic variants),[82] perhaps helping to account for its successful cross-species transmission from chimpanzees, although macaque TRIM5α strongly restricts HIV-1.[79,83] TRIM5α binds to viral capsid hexamers[84] and inhibits replication at a postentry step. The precise mechanism is not clear and may be mediated at more than one level, but may dysregulate viral capsid uncoating.[84,85]

Viral Enzymes

pol Gene Products

The *pol* gene encodes three enzymes, protease, RT, and IN. These proteins are synthesized from the same mRNA as the Gag proteins through a ribosomal translational frameshift. The cleavage of the 160-kDa precursor polyprotein is essential for viability. Because of the inefficiency of the frameshift, which is important in regulating the relative levels of viral proteins, there are about 2000 copies of each Gag protein and only 100 copies of each Pol protein per virion.

Protease

Protease is a 10-kDa 99–amino-acid protein that is fully active as a dimer. It is autocatalytically cleaved from the precursor protein during the viral assembly process. Site-specific mutagenesis has demonstrated that noninfectious particles containing uncleaved Gag and Gag-Pol proteins are produced if this enzyme is inactivated. The similarity of viral protease to other aspartyl proteases, such as angiotensin-converting enzyme, has greatly facilitated the design of potent antiviral drugs, including inhibitors of dimerization and molecules that bind to the active catalytic site.[66,86]

Reverse Transcriptase

Viral RT is an RNA-dependent DNA polymerase. This highly versatile enzyme synthesizes DNA copies from both RNA and DNA templates and degrades viral RNA from RNA-DNA hybrids. RT and its RNase H activity are required for viral replication. The protein is first cleaved from the precursor polyprotein to form a p66 homodimer and, after a second cleavage, forms a p66-p51 heterodimer with identical amino-terminal ends.

The structure of the RT heterodimer revealed the enzymatic mechanism of reverse transcription and the molecular basis of resistance to antiviral drugs.[87] The p66 and p51 assemble in an unusual head-to-tail heterodimer. Four domains of the p66 protein are similar in shape to a clenched right hand and therefore are designated as the fingers, palm, and thumb. These are joined to the RNase H domain. The cleft between them contains the highly conserved catalytic site Tyr-Met-Asp-Asp. Although the p51 subunit is derived from the same protein, it maintains a different conformation.[88,89]

RT plays a major role in the generation of genetic diversity in retroviruses. The fidelity of the enzyme has been determined for a variety of retroviruses by measuring misincorporation rates on defined templates. For HIV-1, this rate ranges from 1:1700 to 1:4000 misincorporations per nucleotide per replication, somewhat higher than for other retroviruses, and considerably greater than for host cell polymerases. For the 9.7-kb HIV-1 genome, the in vivo error rate is estimated to be one misincorporation per replication cycle.[90] Variants produced by RT generate sequence diversity that may resist immune responses or antiviral drugs or allow the virus to change cell tropism. However, the same lack of fidelity also allows nucleoside analogues to be preferentially incorporated into viral rather than cellular DNA, providing the basis for the effectiveness of RT inhibitors.

Integrase

IN is a 288–amino-acid, 32-kDa viral enzyme that mediates the linkage of double-stranded viral DNA into the host cell genome. Integration occurs after the translocation of a large complex derived from the viral core from the cytoplasm into the nucleus.[91] IN is part of this complex and catalyzes the cleavage of viral DNA and ligation to host cell DNA. A large central acidic domain of IN is highly conserved in retroviruses and retrotransposons. Once integrated, the provirus can be considered for most purposes to be a stable genetic element remaining for the life of the cell and, through cellular replication, for the life of the individual. Integrase is required for viral replication, and integrase inhibitors, such as raltegravir, are a relatively recent class of antiretroviral drugs entering clinical use (see Chapter 128).

Envelope Glycoproteins and Viral Fusion

The *env* gene of HIV-1 encodes a single-spliced viral RNA transcript that encodes both Vpu and a 160-kDa Env precursor that is synthesized in the late stages of viral replication. This 850–amino-acid Env polyprotein is cleaved by cellular proteases between amino acids 512 and 513 to form the external surface gp120 and transmembrane gp41 proteins. Proteolytic cleavage is essential for viral infectivity. There is extensive *N*-linked glycosylation on asparagine residues with high-mannose complex oligosaccharide groups. Selective removal of glycosylation sites reduces infectivity. These proteins, especially gp120, are the targets of neutralizing antibodies. The extensive variation among different strains of HIV-1, especially in gp120, and the ability of the virus to evolve during the course of a single infection and to adapt to drugs and immunologic attack rapidly present problems in therapy and vaccine

development. Most of the variability among strains of HIV occurs in the envelope sequence in five variable domains of gp120, designated V1 through V5 (comprising amino acids 128–152, 182–195, 300–330, 395–415, and 460–467, respectively).[92] The third variable region, called the V3 loop (formed by joining two cysteine residues), is a dominant antibody-neutralizing domain of gp120 and plays an important role in determining viral tropism. Four regions that are relatively invariant have been designated C1 through C4 (amino acids 33–60, 87–126, 231–276, and 460–467). These regions presumably maintain essential viral structures.

Although some structure-function relationships have been deduced from secondary structure, biochemical, mutagenic, and immunologic analyses, the solution of the crystal structure of the gp120 protein and of part of the gp41 protein has literally put a new face on the virus, with implications for cell fusion mechanisms and immune evasion. Significant progress has been made in elucidation of the structure of the intact gp120 trimer[93] by crystallization[94,95] and cryoelectron microscopy,[96] which shows a cavity-laden gp120-CD4 interface and a conserved binding site for the chemokine receptor, with evidence for a conformational change upon CD4 binding. The V3 loop, together with conserved regions that remain unexposed until CD4 binding occurs, is a principal determinant of chemokine receptor variability. An understanding of the structural basis that enables HIV to evade humoral responses, while maintaining function, may help in vaccine design.[97,98] One of the most daunting issues in designing vaccines to elicit neutralizing antibodies is the extreme variability of the outer surface of the envelope. Binding to CD4, however, exposes the coreceptor binding site, which is structurally constrained by its functional requirements. Efforts to target this region have resulted in the generation of remarkably broadly neutralizing antibodies, suggesting that this approach has promise.[99] A more recent vaccine trial[100] showed moderate efficacy that appeared to correlate with antibodies against a conserved structure in V2. Antiviral drugs, such as maraviroc, have been developed that target the gp120-CCR5 interaction.

Virus-Cell Fusion

The viral envelope is fundamentally a fusion machine that allows viral entry into target cells. Fusion depends on sequential binding of gp120 to CD4 and the chemokine receptors, but the fusogenic domain is located in gp41. The fusion peptide that is inserted into the target cell membrane is formed at the new amino terminus created by proteolytic cleavage of the gp160 precursor protein.[101] However, this hydrophobic tip must be kept in an inactive state until juxtaposed to the target cell membrane. Premature triggering of the fusion peptide would result in an inactive virus. The core structure of gp41 has been crystallized from peptide fragments.[102]

The core structure that mediates the fusion-active state between virus and cell is formed as a trimer of gp41 molecules composed of two α-helical regions within each gp41 that form a six-helix bundle characteristic of coiled coils. The crystallized complex shows striking structural homology with the low pH-induced fusogenic conformation of the influenza virus hemagglutinin protein (HA), which contains three antiparallel helices packed in a central trimeric coiled coil. The conformational change in HIV-1 is not mediated by endocytic uptake into the low pH compartment as it is for HA, however. The binding of gp120 to the second receptor likely triggers the conformational change that leads to cell fusion and virus uptake. The transition from a loop structure to a coiled coil state is the basis for the spring-loaded model of activation for membrane fusion. The fusion-active state has been identified using synthetic viral peptides to block fusion after triggering (Fig. 169.6).[103] Synthetic peptides that span all or part of these domains can inhibit HIV cell fusion and infection.[104]

Viral Regulatory and Accessory Genes

In addition to the structural genes, HIV has six accessory genes: *tat* (coding for the trans-activator of transcription), *rev* (encoding the regulator of viral expression), *vif* (encoding the virion infectivity factor), *vpr* (encoding viral protein R), *vpu* (encoding viral protein U), and *nef* (encoding the "negative" regulatory factor, a misnomer). HIV-2 and some SIV strains lack *vpu* but have *vpx*, which encodes a unique protein

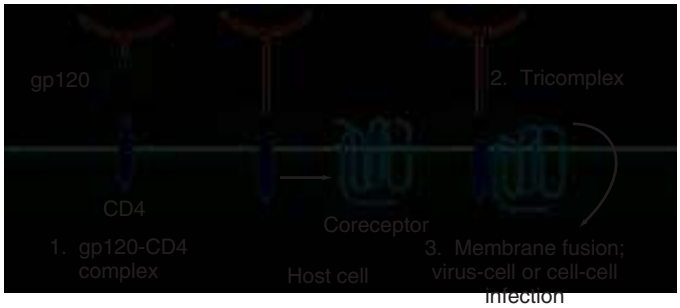

FIG. 169.6 The human immunodeficiency virus type 1 (HIV-1) infection process at the cell surface. On first infection and after initial binding of gp120 of HIV to the cell surface CD4 molecule, an envelope conformational change occurs that fosters binding to the chemokine coreceptor (e.g., CCR5 or CXCR4). This interaction involves specific portions of gp120, including the V3 region.

Vpx (viral protein X). Vpx, as mentioned earlier, targets the antiviral protein SamHD1. These genes enable the virus to usurp host cell processes and to achieve efficient replication under host-selective pressures, thus contributing to disease progression.[48] Expression of Nef in infected macrophages activates T cells through the CD2 stimulatory pathway, rendering the T cells susceptible to infection.[105] Of these genes, only *tat* and *rev* are necessary for high levels of viral expression in culture. Tat augments viral RNA levels by greatly increasing transcription, primarily by permitting elongation of otherwise blocked short nascent RNA chains. Rev regulates the splicing and transport of RNA. Vif is required for efficient cell-free transmission of virus and acts as an antagonist against APOBEC3G (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G),[106] a deoxycytidine deaminase that acts on newly synthesized viral DNA to induce its degradation and hypermutation and that may represent a general antiretroviral defense mechanism.[107] APOBEC3G may also inhibit reverse transcription. Nef has been found to be crucial to virulence and immune evasion and downregulates surface expression of CD4, presumably to allow efficient expression of gp120 on the cell surface. As noted, expression of Nef in macrophages may be important in rendering neighboring T cells permissive to infection. Vpr also downregulates surface expression of CD4; it is the only accessory protein found abundantly in the mature virion.

Early in infection a *rev*-independent pathway removes introns from the viral transcript. The multiply spliced mRNAs for *tat* and *rev* are transported and translated and, as proteins, are shuttled back to the nucleus. As Tat acts and Rev accumulates in the nucleus, unspliced and single-spliced mRNAs for *gag, gag-pol*, and *env* and viral accessory genes *vpu, vpr*, and *vif* are transported out of the nucleus[108] (see Fig. 169.5).

The Tat protein is translated from a transcript that contains three exons, the first of which is noncoding. The *tat* reading frame overlaps both the *rev* and *env* genes. Tat is a small nuclear (14- to 16-kDa) protein that varies in size, containing 86 to 102 amino acids, depending on the strain of HIV-1. The first 72 amino acids contained in the second exon are required for full activity, whereas all the length variation occurs at the carboxyl-terminal end of the protein.[109] Tat binds to a Tat activation response element (TAR) region found in all HIV-1 mRNAs, in conjunction with cellular factors, to stabilize the nascent mRNA and enhance its rate of elongation up to 1000-fold. However, Tat activation is downregulated after periods of several hours, suggesting that these cellular cofactors may be present in limited amounts and that negative regulatory factors may downmodulate Tat function.

The Tat protein has three functional domains. A highly conserved cysteine-rich domain between amino acids 22 and 37 contains seven cysteine residues and two zinc-finger motifs, as is characteristic of a number of DNA-binding proteins. Loss of any one of six cysteines results in loss of activity. The interaction of Tat with TAR is mediated through a basic domain between nucleic acids 48 and 56. One requirement for Tat activity is the CDC2-like kinase CD9, which phosphorylates the carboxyl-terminal domain of RNA polymerase II. However, Tat must first interact with a host cell factor, which has been identified as a novel 87-kDa cyclin C–related protein named cyclin T.[110] Cyclin T is

cell specific and limits Tat activation in nonhuman cells. Tat also contains an arginine-glycine-aspartate (RGD) motif at amino acids 78 to 80 that is common to proteins that bind to integrin receptors. This feature is consistent with the finding that Tat may be secreted by cells into the medium and induces extracellular effects. Binding of Tat to cells derived from KS lesions, mediated in part through the RGD motif, enhances the growth of these cells. Tat is released from infected cells,[111] and extracellular Tat has been shown to induce KS-like lesions in nude mice.[112] Tat contains a stretch of basic amino acids that functions as a protein transduction domain, conferring the ability to be rapidly taken up into uninfected cells from the medium.[111,113] Fusion of this domain with other proteins confers the ability of those proteins to internalize into cells.[114]

All three functional domains of Tat are highly conserved among HIV-1 isolates. HIV-2/SIV Tat has an additional 30 amino acids in the amino-terminal sequence but lacks the RGD region. Tat is theoretically an inviting target for blocking viral replication, although in some situations, cellular factors may allow virus to be expressed without Tat.[115] Tat can affect the expression of heterologous and cellular genes, including the promoters of the human polyomaviruses, human papillomaviruses, and cytokines, such as tumor necrosis factor-α (TNF-α) and IL-2. The first exon of Tat downregulates class I major histocompatibility complex (MHC) expression. Another viral protein, Nef, also downregulates this protein, illustrating that single viral proteins can have multiple or complex functions that contribute to immunosuppression.

The *rev* gene encodes a serine-phosphorylated protein, Rev, that ranges in size from 106 to 123 amino acids but is most commonly 116 amino acids, with a molecular weight of 19 kDa. This protein is translated from a unique double-spliced mRNA, with the second splice acceptor located downstream from the Tat translation initiation site.[116] Expression of Rev results in the accumulation of viral structural proteins encoded by the Gag, Pol, Env, Vpu, Vpr, and Vif mRNAs. In contrast, multiple-spliced viral messages for Tat, Rev, and Nef are efficiently expressed without Rev protein. In the absence of Rev most viral mRNA is processed to multiple-spliced forms, thereby limiting the production of virions. Rev protein accumulates in the nucleolus and shuttles back and forth to the cytoplasm. Although it binds to unspliced genomic RNA, it does not appear in the mature virion. Rev binds to a unique RNA element located in the Env coding region of HIV-1 RNA. This RRE is found in all unspliced and single-spliced mRNAs.

Rev contains at least two functional domains: an arginine-rich region at amino acids 35 to 50, which is conserved and required for nucleolar localization and specific RRE binding, and a multimerization domain. After initial RRE binding, multiple additional Rev molecules bind to each other, and this oligomerization is required for activity.[117]

As a presumed example of convergent evolution, *tax* and *rex* genes present in HTLV-I (and related viruses) encode two proteins, Tax (trans-activator of transcription) and Rex (regulator of viral expression); these genes function analogously to *tat* and *rev* in HIV-1 and HIV-2. Accessory genes formed by complex splicing arrangements are not generally found in all retroviruses, suggesting that retroviruses can be classified as simple or complex, depending on the presence or absence of these genes.

The *nef* gene product is a 206–amino-acid myristoylated protein that inserts into the cell membrane. The Nef protein may have many different properties that depend in part on the experimental methodology used to analyze them. The original observation that T-cell–tropic, Nef-deleted viruses replicated to high levels led to the term *negative factor*. Downregulation of CD4 requires myristoylation, and membrane targeting of Nef to the cytoplasmic domain of CD4 increases CD4 endocytosis. The protection of HIV-1–infected cells by Nef against killing by CTL correlates with downregulation of class I MHC.[118] Nef appears to be important in maintaining high virus loads associated with rapid progression to immunodeficiency. As mentioned earlier, Nef leads to activation of T cells when it is expressed in macrophages.

The immune response to Nef may exert considerable selection pressure. When T cells capable of CTL activity against Nef were transferred to HIV-infected patients, a Nef-deleted variant emerged and, although there was apparent successful immunologic intervention, the patient's disease progressed.[119] Nef-deleted viruses detected in several human clusters were originally associated with little or no disease, raising the possibility that they represented less virulent viruses.[120] However, long-term follow-up of these cases has demonstrated that definite, albeit slower, disease progression does indeed occur.[121]

Vif is a 193–amino-acid viral protein of 23 to 27 kDa with no *N*-linked glycosylation sites. The infectivity of Vif deletion mutants is decreased up to 1000-fold in some cell lines compared with that of wild-type virus. The deleted virus is capable of cell entry and initiating reverse transcription, but dsDNA is not produced. As noted, Vif blocks the antiviral activity of deoxycytidine deaminase APOBEC3G.[106]

Vpr is a 96–amino-acid protein translated from a single-spliced mRNA and, like Vif, its expression is dependent on Rev function. Vpr is abundantly present in the mature virion associated with capsid protein. Expression of p55 Gag and Vpr in transfected cells is sufficient for incorporation and export of viral proteins. Vpr plays a role in the nuclear localization of the preintegration complex.[122,123] In addition, in transfected human muscle cells, Vpr blocks proliferation and induces differentiation, suggesting a nuclear role for Vpr in regulation of gene expression. Vpr causes arrest of cell-cycle progression at the G_2/M interface, presumably through an effect on cyclin CDC2 activity, which correlates with the ability to activate HIV transcription.[124,125] In addition, Vpr causes massive ruptures in cell nuclei.[126]

Vpu is an amphipathic integral membrane protein.[127] The first 27 amino acids are hydrophobic, whereas the remainder of this small, 81–amino-acid 16-kDa protein is hydrophilic. A single-spliced message overlaps with the *env* gene in a different reading frame.[128] The protein forms oligomeric complexes localized to the perinuclear region. Cells infected with Vpu-defective mutants show large accumulations of intracellular vesicles, in contrast to those infected with wild-type virus. Vpu, along with Nef, is associated with the rapid degradation of CD4, which may in part eliminate CD4-gp160 intracellular complexes that interfere with virus production. The CD4 cytoplasmic tail is required for targeting the degradation of CD4 by Vpu. Vpu has structural similarities to the influenza virus M2 protein, an ion channel protein that modulates the pH of the trans-Golgi.[129] Perhaps more important, Vpu causes the ubiquitination and degradation of tetherin, an IFN-dependent antiviral protein that impedes viral release from the cell surface.[130]

Virus Regulation and the Long Terminal Repeat

The LTR of all retroviruses, located at each end of the provirus as a direct repeat containing the U3, R, and U5 regions, functions as an eukaryotic transcription unit. The U3 region contains the viral promoter and enhancer elements. The R region includes the mRNA initiation site (+1) and ends at a polyadenylation termination site. The function of the U5 region is not well understood. It separates the R region from the tRNA primer binding site used to initiate reverse transcription. HIV-1 uses $tRNA^{lys}$ as a primer. Once the virus has formed an integrated DNA copy, it depends on cellular machinery for transcription and translation. However, the control of virus expression results from a complex set of interactions between viral elements and cellular proteins. Small changes in these regions may result in profound differences in virus behavior. Cis-acting control elements of the virus (TAR, TATAA) and enhancer and negative regulatory regions (located within the U3 and R regions) interact with cellular and viral proteins. These interactions, which occur at both the DNA and the RNA levels, are crucial in controlling the level of viral expression in both resting and activated cells.

The TATAA box, located at –27 (relative to the RNA initiation site), binds the critical cellular transcription factor IID (TFIID) to initiate transcription. The promoter region, the binding site of the cellular polymerase, lies further upstream 5′ (between –45 and –77) and contains three binding sites for the cellular specificity protein 1 (SP1) transcription factor.[131] An enhancer element is still further upstream 5′, mapping to nucleotides –82 to –105, and contains a consensus sequence also found in the immunoglobulin κ, IL-2, and IL-2R enhancer regions. This region binds an inducible cellular transcription factor, NF-κB. Although originally described in B lymphocytes, this factor or family of factors is also expressed in activated T cells and stimulates HIV expression.[59,132] In addition to NF-κB, other factors can increase HIV-1 promoter activity by interactions in the region. These include cellular cytokines, such as

TNF-α and IL-1, and heterologous viral proteins, such as HTLV-I Tax. Such observations indicate molecular mechanisms whereby other viruses could interact with HIV; however, the relevance of such interactions in vivo is unknown.

Further upstream 5′ are the binding sites for additional cellular factors (activating protein-1 [AP-1], nuclear factor for activated T cells-1 [NFAT-1]) that lie within a negative regulatory element (NRE). Removing the NRE from a functional provirus enhances virus expression, again suggesting that viral production is carefully modulated both negatively and positively.

The R region of the LTR codes for the 5′ untranslated leader sequence shared by all HIV-1 mRNAs and for the TAR, an essential binding site for the potent virally coded HIV-1 trans-activating protein, Tat. The TAR in the LTR transcript forms a unique stem-loop structure. Of interest, the structure of the HIV-2 LTR is significantly different from that of the HIV-1 LTR and may contribute to the distinctly different biologic activities of these two human retroviruses.

VIRUS-HOST INTERACTIONS

Viral Receptors, Chemokines, Receptors, and Tropism

Chemokines and their receptors constitute a complex signaling system essential for orchestrating angiogenic, inflammatory, and chemotactic responses. More than 40 chemokines are grouped into two principal families, C-C and C-X-C; there are at least 14 known seven-transmembrane–spanning G protein–coupled chemokine receptors. These molecules have been exploited by some bacterial and viral pathogens as their receptors to gain entry to or activate cells, and virally encoded antagonists often subvert chemokine function.[133,134]

The revelation that chemokine receptors are essential for HIV cell fusion has brought together several distinct areas of viral research: how $CD8^+$ T cell–derived factors suppress HIV-1 replication, the mechanisms of cell entry and viral tropism, and host genetic determinants of infection. Three CC (or β) cytokines released by $CD8^+$ T cells—RANTES, MIP-1α, and MIP-1β—bind to the CCR5 receptor and potently suppress HIV macrophage-tropic virus.[135,136] The first coreceptor identified, the CXCR4 molecule, known at the time only as an "orphan receptor," was discovered using a complementary DNA screening approach for receptor activity mediating cell-cell fusion.[39] A group of reports rapidly followed, showing that a recently identified receptor, CCR5, which could use the identified suppressor molecules as ligands, was the main coreceptor for primary isolates and macrophage-tropic strains.[40–43] Dual-tropic strains can use both coreceptors.[137] Chemokine coreceptor use may be a determinant of viral virulence and disease progression.[138] In addition, studies have pointed to the critical role of cholesterol in the cell membrane in HIV-1 coreceptor function; removal of cellular cholesterol rendered primary cells and cell lines highly resistant to HIV-1–mediated syncytium formation and to infection by CXCR4- and CCR5-specific viruses.[139]

A mutant CCR5 gene *(CCR5delta32)* that encodes a defective protein and is unable to bind virus has been found in exposed but uninfected persons, strongly suggesting that a functional CCR5 protein is required for infection.[140] Homozygosity for this mutant[141] is a strong protective factor, although rare infections by CCR5-independent viruses have been documented in a person with hemophilia and in another person after sexual transmission. Of interest, the lack of CCR5 does not have apparent major negative health consequences. Heterozygous adults and children are not protected from infection but may take a longer time to develop disease.[142,143] An interesting example of the potential for CCR5-negative cells to resist infection is the so-called "Berlin patient,"[144] who was HIV infected and developed leukemia as well. Myeloablation was performed, followed by transplantation with hematopoietic stem cells from an individual with a homozygous *CCR5delta32* genotype. He has remained free of virus, perhaps due to a combination of nonpermissivity of donor cells and graft-versus-host killing of residual infected host cells. This suggests a potential mechanism for achieving a "viral cure" (see later).

Pathogenesis, T-Cell Depletion, and Viral Load

Understanding the rates of HIV-1 production and associated loss of T cells has been dramatically advanced by the ability to measure changes produced by potent new drug combinations. HIV-1 production and T-cell turnover constitute a continuous dynamic process.[145,146] Mathematical modeling of virus production has suggested that a continuous battle is being waged between the virus and host. Production of billions of virions and T-cell turnover, estimated at 1 billion cells/day, may help account for the very rapid emergence of viral variants and the fluctuating and progressive nature of T-cell depletion. Virus may be distributed across different cellular compartments, each with different rates of turnover and production. Even in the steady state that may occur during periods of clinical latency, large numbers of virus freshly infecting T cells lead to a highly activated immune system that is attempting both to control virus replication and to renew itself.[147] The increase in $CD4^+$ T cells with antiretroviral therapy (ART) is probably due to the result of a combination of initial redistribution of memory T cells and a continuous but slow repopulation with newly produced naïve T cells.[148] The mathematical models that have been proposed, however, grossly oversimplify the real dynamics of infection and are no longer widely accepted as a valid major explanation for HIV pathogenesis and T-cell loss. Several other pathologic mechanisms, including indirect viral killing and activation-induced apoptosis, have been suggested to play a role. Uninfected $CD8^+$ T cells turn over as rapidly as $CD4^+$ T cells but are not initially depleted.[149] The eventual loss of noninfected $CD8^+$ T cells may be mediated by gp120 binding to CXCR4.[150] Eventually, viral escape from immune control and emergence of T-cell–tropic viruses using CXCR4 may lead to immunodeficiency,[151] although progression to AIDS often occurs in the absence of detectable variants using CXCR4. All these models incorporate two common assumptions—a demonstrated quantitative association between virus production and T-cell depletion, and a compartmentalization of virus production and cell turnover at different rates.

The significance of virus integration for the natural history of HIV-1 infection has been vividly demonstrated by the effects of combination therapy on virus production from different populations of cells and has helped define what is meant by latent infection. Potent antiretroviral regimens that include a combination of RT and protease inhibitors can produce sustained reductions of plasma viral RNA to below detectable limits.[152] Treated patients with detectable viral plasma RNA, even if the level is greatly reduced, have viral loads in their lymph nodes similar to those in the nodes of patients who do not receive treatment, reflecting ongoing viral replication and emergence of drug resistance.[153] However, even in those with no detectable plasma RNA, viral DNA could still be detected in lymph nodes and PBMCs, and virus could be grown from PBMCs after removal of $CD8^+$ cells and activation. Furthermore, no new mutations associated with drug resistance were detected from these isolates recovered after 2 years of therapy. This strongly suggests that virus persists in a long-lived and latently infected T-cell population. This pool of latently infected cells is probably established very early during primary HIV-1 infection. Even though plasma viremia could be suppressed, initiation of ART as early as 10 days after onset of symptoms did not prevent generation of latently infected $CD4^+$ lymphocytes.[154] Although the frequency of these cells is low, on the order of 16/million PBMCs, the fact that hidden virus may survive in these cells may represent a significant factor in long-term therapy and may shape strategies to eliminate virus.[155] Indeed, therapy may need to be continued for 60 to 70 years to eradicate the reservoir of long-lived latently infected cells. One alternative has received consideration with the advent of convenient gene editing tools, such as clustered regularly interspaced short palindromic repeats (CRISPR), along with the example of the Berlin patient. In principle, ex vivo editing to cripple both copies of the *CCR5* gene from hematopoietic stem cells, followed by transplantation into patients, could result in a virologic cure. Some success in this approach has been reported in humanized mouse models of HIV infection,[156] but there are obviously formidable technical hurdles, such as off-target effects, efficiency, and expense to be overcome.

Similar to the impairment or killing of infected cells, there are several distinct mechanisms whereby bystander cells can be affected in vitro. For example, HIV-1–infected PBMCs taken directly from patients or obtained through infection of normal PBMCs with HIV-1 in vitro have shown a marked impairment of proliferative responses, even though only a small fraction of T cells are infected.[157–159] Other possible indirect

(bystander) effects include impaired hematopoiesis, leading to cytopenias of several blood cell lines, impaired thymopoiesis, and anergy; apoptosis of uninfected immune cells, likely through increased levels of cytokines, such as TNF-α, IFN-α, and transforming growth factor-β; and hyperactivation and apoptosis of uninfected immune cells by the effects of extracellular Tat.

Viral Variation: Genetic and Phenotypic Variation

On the basis of phylogenetic analysis, HIV strains have been separated into major (M) subgroups and more distant groups N and O. Within the M group are multiple subtypes A, B, C, D, F, G, H, J, and K and ever more numerous circulating recombinant forms, including CRF 01 AE/B and CRF 02 A/G.[160] A subspecies of chimpanzees *(Pan troglodytes troglodytes)* has been identified as the likely source of HIV-1.[161,162] Zoonotic transmission appears to have occurred on at least three occasions, with one such transmission in southeastern Cameroon being the origin of the most widespread HIV-1 group, HIV-1(M). It is likely that this has happened on other occasions, but that the transmitted viruses failed to survive as infectious human agents, and the three reported cross-species transmissions are the only ones that have been successful. Data has been recently presented[163] suggesting that after its introduction into humans in Africa, HIV-1 was introduced into the United States in the 1960s, from where it spread to many other countries, giving rise to clade B HIV-1(M), which is highly prevalent in the United States, Europe, and other areas. Clade C is the most prevalent in many parts of Africa and in India.

One of the most striking characteristics of HIV-1 is its remarkable variability, contributing to phenotype diversity and resulting in altered cell tropism, immune escape, and resistance to RT and protease inhibitors. As a consequence of the underlying variation by mutation, high rates of virus turnover and selection of viral variants cause viral evolution in individual hosts with time, as well as among populations of infected individuals. Most affected persons were thought to be infected with a single strain that evolves into a swarm of related viruses or quasispecies during the course of infection.[164] Recently, however, deep sequencing and phylogenetic analyses of viruses from recently infected individuals have suggested that only about two-thirds of individuals are infected with a single virus, with the remainder being infected with from three to five genetically distinct viruses, called transmitted/founder viruses.[165]

Most of the variation is neutral and not adaptive. Although the mutation rate per base pair per cycle is presumed to be equal throughout the genome, diversity is greatest within distinct regions of the envelope gp120, presumably because of selection and relatively low fitness costs. HIV is going through rapid epidemiologic changes, even as the virus is being studied. Thus genetic relatedness of viruses can be used to track transmission of the virus as well as the relatedness of different viruses. This information is often presented in the form of viral phylogenetic trees.[166]

MALIGNANCIES AND HUMAN IMMUNODEFICIENCY VIRUS

Although cancer is not common in the young adults most frequently exposed to HIV, KS has been a defining clinical presentation of AIDS (see Chapter 140). Previously known as a rare and usually indolent vascular tumor, the incidence of KS in patients with HIV infection early in the epidemic was 10% to 20%, more than 10,000 times that in the general population. Several forms of KS have been described: classic KS, endemic KS, non–HIV-associated KS, transplantation-associated KS, and AIDS-associated KS. The tumor appears as a vascular proliferation characterized by the presence of spindle cells, vascular channels, and a mixed cellular infiltrate. The "malignant" cell is most likely a population of activated endothelial cells, which are sometimes clonal and sometimes not. These cells themselves and factors produced from them, such as basic fibroblast growth factor and vascular endothelial cell growth factor, can induce vascular lesions in nude mice. The HIV protein Tat can bind to KS spindle cells and stimulate their growth.[167] Moreover, Tat can cooperate synergistically with a G protein–coupled receptor encoded by human herpesvirus 8 (HHV-8; see later) in inducing KS-like lesions in nude mice.[168] Although the importance of immunodeficiency and viral proliferation in the etiology of KS is well established, the mechanism(s) is not certain, and KS may occur early in the course of HIV infection.

Within the group of HIV-infected individuals, KS occurs predominantly in homosexuals and bisexuals, leading to the hypothesis that a previously undescribed infectious agent might cause KS. In 1994, using DNA subtraction hybridization, DNA representing a novel herpesvirus was obtained from KS lesions, which led to the isolation of HHV-8[169] (see Chapter 140). Also known as KS herpesvirus, it is related to other gammaherpesviruses such as Epstein-Barr virus (EBV). HHV-8 has also been identified in an EBV-transformed B-cell line and from a unique form of B-cell lymphoma known as body cavity lymphoma. This large virus contains many genes that interact with host cells, including several chemokine and cytokine homologues that can induce vascular growth.[170] Seroepidemiologic studies have made a strong association between this virus and KS. HHV-8 was found to be associated with all forms of KS, and seroconversion precedes the appearance of KS in HIV-infected persons. The prevalence of HHV-8 is far greater than the incidence of KS, however, and the relationship of the virus to the disease is not likely to be simple. HHV-8 thus appears to be necessary but not sufficient to cause KS. A review discusses this and other aspects of KS that are not clear at present.[171] The incidence of KS in AIDS in developed countries continues to decrease.

B-cell lymphoma, often associated with EBV, is the second most common cancer associated with AIDS, occurring in approximately 3% to 4% of individuals as their first AIDS-defining diagnosis. One type, primary effusion lymphoma, is caused by HHV-8. The most common lymphoma is that of the brain (primary brain lymphoma). However, in contradistinction to KS, lymphoma appears to be a late manifestation of HIV disease, with rates rising directly with length of time infected. Up to 16% of AIDS patients eventually die from this condition. In 53,042 AIDS patients from 21 European countries, approximately 2.6% of injection drug users, 3.2% of transfusion recipients, 3.4% of homosexual men, 3.9% of hemophiliacs, and 2.6% of those who acquired HIV through heterosexual contact eventually developed AIDS-related lymphoma.[172]

In the United States, data from the Multistate AIDS/Cancer Match Registry have demonstrated a significantly increased risk of lymphoma in patients with a prior AIDS diagnosis, compared with population control subjects who had not been diagnosed with AIDS.[173] The relative risk of all non-Hodgkin lymphoma (NHL) was increased approximately 113-fold. Although the greatest risk is for NHL, there is a 10-fold increase of Hodgkin disease in the infected group as well.

Because of its persistent presence in lymph nodes, even in the absence of detectable viral RNA,[174] and because it activates signal transduction in B cells,[70] the viral p17 has attracted interest as a possible contributor to lymphomagenesis. These tumors are predominantly extranodal and often of high grade, with small cell noncleaved histologic features.[175] EBV is found in almost all primary CNS tumors, but high rates of dysregulated B-cell turnover may predispose to lymphoma. Rates of EBV-associated primary brain lymphoma have fallen off markedly in the era of ART, but the same cannot be said for systemic lymphoma, which appears to be increasing in frequency as people live longer with HIV.[176]

Several other EBV-associated rare tumors, including leiomyosarcoma, have also been reported.[177] Cervical cancer, almost universally associated with human papillomavirus (HPV) infection, is accepted as an AIDS-defining illness.[177] High rates of HPV-associated anal intraepithelial neoplasia (AIN) have been documented among HIV-infected homosexual men, and routine screening for AIN is being considered, using the same methodology as Pap smears, with samples obtained through anoscopy.[178] The incidence of many common cancers does not appear to be greatly increased among HIV patients, and many of the incidence increases are among cancers associated with other viruses, although it should be noted that non-AIDS cancers are increasing as the infected population ages.

CONCLUSION

The widespread and successful use of ART directed at HIV-1 has continued to develop rapidly (see Chapter 128). Significant advances in the ability to treat individual cases, propelled by an understanding of the biology of the virus, have led to the control of viral replication and have altered the course of disease progression. At present, patients have been maintained on combination therapy for years, with a low

rate of relapse because of the development of resistance and low viral burdens. Immune restoration and long-term biologic control or complete eradication of the virus with minimal toxicity remain elusive goals, but intense efforts continue to focus on new anti-HIV therapies. As we have learned, retroviral disease has important social aspects that, as much as viral biology, determine the extent of viral transmission and the dimensions of the epidemic; this results in a series of epidemics, each with a unique sociobiology. Moreover, despite remarkable progress, social and economic effects of the global HIV epidemic have materialized vividly in sub-Saharan Africa, with devastating losses of population life expectancy and productivity. Renewed emphasis on the development of an effective anti-HIV vaccine that might stem an ongoing epidemic or ameliorate the course of disease has been the subject of intensive study in nonhuman primates and in clinical trials (see Chapter 119). The history of retrovirology has been filled with remarkable, often serendipitous, discoveries based on scientific imagination supported by technologic advances. There is every reason to expect that continued advances will deepen our understanding of fundamental biologic processes and that we will meet the challenges presented by HIV and the inevitability of the emergence of new pathogens.

ACKNOWLEDGMENT

This updated chapter is partially based on a chapter in an earlier edition of *Principles and Practice of Infectious Diseases,* coauthored by Dr. Mika Popovic and Dr. Farley Cleghorn of the Institute of Human Virology. We gratefully acknowledge their contributions.

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170 Introduction to the Human Enteroviruses and Parechoviruses

José R. Romero

Members of the genera *Enterovirus* and *Parechovirus* are picornaviruses. Picornaviridae is a large family of morphologically identical, single-stranded, positive-sense RNA viruses that share similar genomic and structural organizations. Originally the human enteroviruses were divided into five species on the basis of differences in host range and pathogenic potential: polioviruses, group A coxsackieviruses (CV-A), group B coxsackieviruses (CV-B), echoviruses (E), and newer enteroviruses (EVs) (Table 170.1).[1,2] All enteroviruses discovered since 1970 have been assigned to the enterovirus species.

A classification scheme based on RNA homology within the VP1 capsid protein coding region that replaces the traditional classification divides the genus *Enterovirus* into four species, designated enterovirus A through D (Table 170.2).[2,3] Isolates of the same serotype characteristically diverge in the VP1 region by less than 25% within corresponding nucleotide sequences and by less than 12% within amino-acid sequences.[3] Of the original 72 serotypes identified, 64 remain after recognition of redundant serotypes and reclassification of others (see Table 170.1).[2] Today more than 110 unique enterovirus serotypes known to infect humans have been identified, each distinguished from one another on the basis of phylogeny of the VP1 coding region.[3] Parenthetically, the rhinoviruses, once a genus unto themselves, have been reassigned as species within the genus *Enterovirus* (see Chapter 175).[2]

A more recently established genus, *Parechovirus,* includes four species (parechovirus A [formerly human parechovirus], parechovirus B [formerly Ljungan virus, a rodent picornavirus], parechovirus C, and parechovirus D).[4] Two serotypes formerly considered to be in the echovirus species, E-22 and E-23, have been reclassified as members of the parechovirus A species, human parechovirus 1 and 2 (HPeV1 and HPeV2),[4,5] on the basis of certain biophysical and biochemical properties that differ from those of enteroviruses (see later discussion). In addition, at least 19 new serotypes of HPeV have been described and are discussed in Chapter 173.

ENTEROVIRUSES

Virology

Physical Characteristics

Enterovirus virions are nonenveloped, icosahedral capsids of approximately 30 nm composed of 60 structural subunits; the subunits are formed from four polypeptides, with an aggregate molecular weight of 80 to 140 kDa, that surround the single-stranded RNA genome.[6] In contrast to other picornaviruses (e.g., rhinoviruses), the human enteroviruses are stable over a wide range of pH (3–10), permitting them to retain infectivity during transit through the gastrointestinal tract. Lacking a lipid envelope, enteroviruses are resistant to ether, chloroform, and alcohol. However, they are readily inactivated by ionizing radiation, formaldehyde, or phenol.[6] Molar magnesium chloride reduces the thermolability of enteroviruses across a wide range of temperatures; this feature allows live-attenuated oral poliomyelitis (OPV) vaccines to maintain potency when refrigeration is suboptimal or unavailable.

The capsid encloses a linear RNA genome of approximately 7.5 kb that is divided into four regions: a 5′ nontranslated region of approximately 740 nucleotides, a continuous-coding region of approximately 6625 nucleotides, a 3′ nontranslated region of approximately 70 nucleotides, and a 3′ poly(A) tail of 70 to 100 nucleotides in length.[7,8] The 5′ terminus is covalently linked to a small virus-coded protein (VPg), which is required for the initiation of RNA synthesis. The 5′ nontranslated region contains genomic elements and sequences essential for viral RNA replication, translation, and, for some enteroviruses, virulence. The most conserved region of the genome among the human enteroviruses is the 5′ nontranslated region. The regions coding for the structural proteins show the greatest intraserotypic sequence variability, in particular, within the coding regions for epitopes that bind neutralizing antibody. The 3′ nontranslated region plays a role in viral RNA replication. Removal of the poly(A) 3′ terminus renders the RNA noninfectious.

Molecular Biology

The RNA genomes of naturally occurring polioviruses, attenuated polioviruses, and many nonpolio enteroviruses have been fully sequenced, and the replication of polioviruses in primates and transgenic mice has been studied in extensive detail.[7,8] Full-length genomic clones of the polioviruses and several enteroviruses have provided the tools for the dissection of many of the molecular events of infection.[7,8,9] The molecular structure and intracellular replicative events appear to be similar for all four enteroviruses species A through D.

Host cell susceptibility to enteroviral infection is defined in large part by the presence of specific membrane receptor proteins that serve as cellular receptors or coreceptors that bind enteroviruses generally along taxonomic lines (Table 170.3).[10,11] The three poliovirus serotypes share a common receptor (poliovirus receceptor [PVR] or CD155), a member of the immunoglobulin superfamily that is coded on human chromosome 19.[12,13,14] The receptor and coreceptor proteins have been identified for many of the human enteroviruses. Both decay-accelerating factor (DAF, or CD55), a complement regulatory protein, and intercellular adhesion molecule 1 (ICAM-1) play a role in CV-A21 cell entry.[15] The CV-B serotypes also interact with two different cell membrane proteins, the 49-kDa coxsackievirus-adenovirus receptor (CAR) and DAF.[16,17] The presence of CAR permits binding and cell entry by all six CV-B serotypes,[17] whereas antibodies to DAF block binding and infection by serotypes 1, 3, and 5.[16,18,19] DAF also appears to be a major echovirus receptor, binding multiple echovirus serotypes,[11,20] whereas serotypes E-1 and E-8 bind to the α_2-subunit of very late antigen 2 (VLA2) integrin molecule.[21,22] P-selectin glycoprotein ligand 1 (PSGL-1) and the scavenger receptor class B member 2 (SCARB2) are both used as receptors for EV-A71.[23,24] The receptor for EV-D68 has been identified as ICAM-5/telencephalin.[25]

The processes of penetration, uncoating, and release of the nucleic acid into the cytoplasm occur within minutes at 37°C. Binding of poliovirus with its cellular receptor leads to conformational changes in the viral capsid that allow for extrusion of VP4 and the formation of a channel through which the RNA genome enters the cytoplasm of the cell.[8] RNA synthesis begins within 30 minutes, leading to an exponential increase of minus-strand complementary and plus-strand progeny RNA until 2.5 hours after infection, when there is a switch to a linear accumulation of mainly progeny RNA.[8] The full-length RNA functions as a monocistronic messenger whose translational product, a polyprotein of 250-kDa molecular weight, is encoded by a single open reading frame

TABLE 170.1 Conventional Classification and Host Range of Human Enteroviruses

SUBGENERA	NO. OF SEROTYPES	HOST RANGE[a]		
		Primates	Newborn Mice	Cell Culture
Polioviruses[b]	1–3	++	0	++
Group A coxsackieviruses[c,d]	1–24	0	+++	±
Group B coxsackieviruses[c,e]	1–6	0	+++	++
Echoviruses[f]	1–34	0	0	++
Enterovirus[g,h]	68–72	Variable	Variable	Variable

[a]Replicative capacity.
[b]Polioviruses generally replicate only in primates or primate cell cultures, although rare strains such as the type 2 Lansing strain have been adapted to rodents. Although poliovirus multiplies in the alimentary tract of some nonhuman primates, the hallmark of these viruses is the characteristic histopathologic lesions produced by direct inoculation of the central nervous system.
[c]The coxsackieviruses were first recovered from the feces of children with poliomyelitis in the town of Coxsackie, New York.[144] In contrast to polioviruses, they produce paralysis and death in experimentally infected suckling mice.
[d]All group A coxsackieviruses produce generalized myositis of skeletal muscle and flaccid hind limb paralysis in suckling mice,[145] and coxsackievirus A7 is pathogenic for the primate central nervous system. However, most group A coxsackieviruses except serotypes A9 and A16 grow poorly in cell culture. Coxsackievirus A23 has been reclassified as echovirus 9, leaving 23 coxsackievirus serotypes.
[e]Group B coxsackieviruses are distinguished by their ability to produce focal myositis and generalized infection of the myocardium, brown fat, pancreas, and central nervous system in suckling mice, resulting in spastic paralysis. The group B coxsackieviruses are commonly isolated in cultured primate cells.
[f]The echoviruses (enteric cytopathic human orphan viruses) were originally discovered in fecal specimens of healthy children.[146,147] They cause cytopathic effects in primate cell culture but are generally nonpathogenic for suckling mice (except for echovirus 21) and primates. Echovirus 10 has been reclassified as reovirus 1, and echovirus 28 has been reclassified as rhinovirus 1. Echovirus 34 is a variant of coxsackievirus A24. Echoviruses 22 and 23 have been assigned to the genus *Parechovirus* as parechovirus serotypes 1 and 2, respectively.[89] Therefore, 29 of the original 34 serotypes of echovirus remain.
[g]The human enteroviral serotypes recognized since 1970 are designated by serial numbers only.[148]
[h]Hepatitis A virus was briefly classified as enterovirus 72 until genetic sequence data led to its reclassification as a hepadnavirus.[149]

TABLE 170.2 Classification of Human Enteroviruses by Partial Sequencing of VP1

SPECIES	SEROTYPES
Enterovirus A	CV-A2, CV-A3, CV-A4, CV-A5, CV-A6, CV-A7, CV-A8, CV-A10, CV-A12, CV-A14, CV-A16 EV-A71, EV-A76, EV-A89, EV-A90, EV-A91, EV-A92, EV-A114, EV-A119, EV-A120, EV-A121
Enterovirus B	CV-A9, CV-B1, CV-B2, CV-B3, CV-B4, CV-B5, CV-B6 E-1, E-2, E-3, E-4, E-5, E-6, E-7, E-9, E-11, E-12, E-13, E-14, E-15, E-16, E-17, E-18, E-19, E-20, E-21, E-24, E-25, E-26, E-27, E-29, E-30, E-31, E-32, E-33 EV-B69, EV-B73, EV-B74, EV-B75, EV-B77, EV-B78, EV-B79, EV-B80, EV-B81, EV-B82, EV-B83, EV-B84, EV-B85, EV-B86, EV-B87, EV-B88, EV-B93, EV-B97, EV-B98, EV-B100, EV-B101, EV-B106, EV-B107, EV-B110, EV-B111, EV-B112, EV-B113
Enterovirus C	PV-1, PV-2, PV-3 CV-A1, CV-A11, CV-A13, CV-A17, CV-A19, CV-A20, CV-A21, CV-A22, CV-A24 EV-C95, EV-C96, EV-C99, EV-C102, EV-C104, EV-C105, EV-C109, EV-C113, EV-C116, EV-C117, EV-C118
Enterovirus D	EV-D68, EV-D70, EV-D94, EV-D111, EV-D120

CV-A, Group A coxsackievirus; *CV-B,* group B coxsackievirus; *E,* echovirus; *EV,* enterovirus; *PV,* poliovirus.
Modified from King AMQ, Brown F, Christian P, et al. Picornaviridae. In: King AMQ, Adams MJ, Carstens EB, et al, eds. Ninth Report of the International Committee on Taxonomy of Viruses. *New York: Academic Press; 2012:855.*

TABLE 170.3 Enterovirus and Parechovirus Cell Membrane Receptors

	RECEPTOR PROTEIN
Enteroviruses (Serotype)	
Polioviruses 1–3	Poliovirus receptor (PVR)
Coxsackieviruses A13, A18, A21	Intercellular adhesion molecule 1 (ICAM-1)
Coxsackieviruses B1–B6	Coxsackie-adenovirus receptor (CAR)
Coxsackieviruses B1, B3, B5	Decay accelerating factor (DAF)
Echoviruses 1, 8	Very late antigen 2 ($\alpha_2\beta_1$)
Echoviruses 6, 7, 11–13, 20, 21, 29, 33	DAF
Enterovirus C70	DAF
Enterovirus A71	P-selectin glycoprotein ligand 1 (PSGL-1) and scavenger receptor class B member 2 (SCARB2)
Parechoviruses	
Human parechovirus 1	$\alpha_v\beta_1$, $\alpha_v\beta_3$, integrins

involving about 90% of the entire genome. By convention, the enteroviral polypeptide is organized into three large regions, organized 5′ to 3′ as P1, P2, and P3.[26] The nascent polypeptide is cleaved as it emerges from the host ribosome by viral proteins acting initially in *cis* and later in *trans* to yield the proteins, and protein intermediates are essential to the viral life cycle.[8,26] Cleavage of the P2 and P3 regions results in eight nonstructural proteins and several protein intermediates, whose known functions include polymerase activity, proteolytic cleavage of the translational products, inhibition of host cell protein synthesis, and cellular remodeling.[8]

Following cleavage of P1 from the nascent polypeptide, it undergoes further cleavages to form the viral capsid, which proceeds by aggregation of five copies each of VP1, VP3, and VP0 (the precursor of VP2 and VP4) into subunits and assembly of 12 of these pentamers into the complete dodecahedral capsid shell. Encapsidation of the viral RNA is associated with a final cleavage of the VP0 protein to VP2 and VP4. The latter is an internal protein closely associated with the RNA. The complete virion contains 60 copies of each of the four structural proteins.[8]

Host protein and RNA synthesis are severely compromised by 3 hours after infection. After about 6 to 7 hours, virions are visible by electron microscopy within the cytoplasm, and they are subsequently released by lysis of the cell, resulting in a yield of 10^4 to 10^5 virions per cell. The number of infectious virions is 10- to 1000-fold lower.[8]

Pathogenesis and Immunity in Enteroviral Infections

Pathogenesis

The pathogenesis of poliovirus infection has been extensively investigated in primates experimentally infected with neurovirulent strains and in humans infected with vaccine strains,[27–30] and it is widely assumed that the early pathophysiologic events of nonpolio enterovirus infections are similar. Studies of coxsackievirus infection in mice have produced much information about the influence of various host and environmental

factors on the ability of the virus to replicate in the heart, brain, and other organs and about the mechanism of vertical transmission of enteroviruses from infected pregnant animals to their offspring.

Enteroviruses infect humans via direct or indirect contact with virus shed from the gastrointestinal tract or upper respiratory tract. Whereas ingested virus implants and replicates in the pharynx and the distal small bowel, volunteer studies have shown that attenuated polioviruses replicate most efficiently in the distal small intestine.[27] The precise site of viral entry has long been the subject of conjecture. Studies have demonstrated that microfold cells (M cells) expressing the PVR serve to transport polioviruses across the intestinal mucosa.[31,32] Enteroviral replication in ileal lymphoid tissue is detectable 1 to 3 days after the ingestion of virus. The quantity of virus recoverable from the tonsils is much less than that in Peyer patches, where it may reach 10^7 to 10^8 tissue culture median infective doses ($TCID_{50}$) per 1 g. In healthy individuals, the duration of viral excretion is typically less than 3 to 4 weeks from the pharynx and 5 to 6 weeks in the feces. Longer periods are reported in individuals with B-cell immunodeficiencies and, occasionally, in healthy children.[33,34,35,36]

After multiplication in submucosal lymphatic tissues, enteroviruses pass to regional lymph nodes and give rise to a transient minor viremia. This leads to infection and viral replication in reticuloendothelial tissue including liver, spleen, and bone marrow. The most common result is a subclinical infection, in which viral replication is contained by host defense mechanisms. In a minority of infected persons, however, further replication of virus occurs in these reticuloendothelial sites, leading to a sustained major viremia that coincides with the onset of the minor illness of poliomyelitis and probably of the nonspecific febrile illnesses associated with other enterovirus infections. Prodromal viremia has been demonstrated with wild strains of poliovirus[30,37] and E-9[38] but is uncommon with Sabin OPV strains except for type 2.[39]

The major viremia results in dissemination to target organs such as the central nervous system, heart, and skin, where tissue necrosis and inflammation occur in proportion to the level of viral replication. Histopathologic lesions are usually not seen in the gastrointestinal tract, even though the small bowel is the site of initial viral replication. The severity of infection in experimental animals can be enhanced by induced exercise, cold exposure, malnutrition, pregnancy, and immune suppression with corticosteroids or radiation.

Viral Mutation During Natural Infection

Enteroviruses undergo a high rate of mutation during replication in the human gastrointestinal tract, and transcription errors occur with a frequency of 1 per 10^4 bases, approximately one error per genome. As a result, single-site mutations are commonly observed in the 5′ noncoding region of attenuated polioviruses within days after feeding to young infants, and such changes are associated with longer excretion and increased neurovirulence.[40,41] Serial isolates of the same enterovirus serotype excreted over many years by patients with B-cell immunodeficiency syndromes represent a heterogenetic "swarm" with continuous genetic variation, which can be characterized by oligonucleotide fingerprinting[42,43] and RNA sequencing[44] and permits estimation of the duration of infection. In addition, RNA sequencing has led to the discovery of virulent circulating vaccine-derived polioviruses (cVDPVs) that have caused outbreaks of paralytic disease among underimmunized populations in a number of countries previously free of poliomyelitis.[45,46,47,48]

Dual infection with different enteroviral and parechovirus strains may produce recombinant progeny virus if the parent strains are in the same species.[49] Intertypia can be demonstrated in 1 in 10^4 to 1 in 10^5 infectious virions in vitro[50] and in the feces of infants given trivalent OPV. Most cVDPVs isolated to date are OPV viruses that have recombined with the nonpolio serotypes in the enterovirus C species.[46]

Immunity and the Immune Response

Immunity to enteroviral infections is serotype specific. Antibody-mediated immune mechanisms operate in the alimentary tract to prevent mucosal infection and in the blood to prevent dissemination to target organs. Neutralizing antibodies target epitopes primarily located on VP1 and less so on VP2 and VP3.[51] VP1 possesses the largest number of epitopes as a result of being the dominantly exposed viral capsid protein.[8] As early as 1 to 3 days after enteroviral challenge, immunoglobulin M (IgM) humoral antibodies are produced; they predominate in serum during the first month and disappear within 2 to 3 months.[29] IgG antibody, which is typically detected 7 to 10 days after infection, is mostly of the IgG_1 and IgG_3 subtypes.[52] Neutralizing IgG antibodies in serum persist for life after natural infection with enteroviruses.

Small concentrations of humoral type-specific neutralizing antibodies prevented poliovirus viremia and paralysis in experimentally infected primates,[53] and passive immunity to paralytic disease in humans can be achieved by the administration of immune serum globulin before exposure to neurovirulent polioviruses.[54] However, passively administered immune globulin does not modify the outcome of established central nervous system poliovirus disease[55]; at this late stage of infection, patients have detectable serum antibody. There is no proven role for immune globulin treatment of other systemic enterovirus infections.

IgA antibody appears in nasal and alimentary secretions 2 to 4 weeks after the administration of live-attenuated OPV and persists for at least 15 years.[29] However, mucosal immunity is relative: On reexposure to infectious virus, high titers of secretory IgA antibodies prevent or substantially reduce poliovirus shedding, whereas lower titers are associated with more extensive oropharyngeal replication of virus and longer viral shedding.[29] The elaboration of virus-specific IgA antibodies by the small intestine depends on local immunocompetent tissues, as was demonstrated in experiments in infants with double-barrel colostomies who were fed live-attenuated poliovirus through the colostomy and generated secretory IgA antibodies only in the distal loop of the colostomy, not in the pharynx or the proximal loop.[56] Antibodies are present in the colostrum and milk of immune women who are nursing and may interfere with the replication of OPV given to breastfed neonates.[55] Maternal antibodies passively acquired transplacentally or via milk prevent or modify enteroviral infections of early infancy.[57,58] Humoral antibodies have an important role in recovery from enteroviral infection, as evidenced by the development of persistent infections in individuals with significant B-cell immunodeficiency.[59]

There is both clinical and laboratory evidence that humoral antibody alone is not sufficient to limit enteroviral replication in vivo. It has been known that macrophage function is critical for viral clearance and that macrophage ablation enhances the severity of CV-B infections.[60] Host innate immunity appears to play a crucial role in limiting the severity of enteroviral infections. Findings in transgenic mice expressing PVR have shown that the host interferon (IFN) response inhibits poliovirus replication in extraneural tissues and limits the spread of poliovirus in the host.[61] This activity is likely mediated via interaction with Toll-like receptor 3 (TLR3) and the Toll/interleukin-1 receptor domain-containing adaptor-inducing IFN-β pathway.[62–65] The TLR3 pathway appears to have a central role in the immune response to human enteroviral infections as well. TLR3 variants with reduced responsiveness to double-stranded RNA have been associated with enteroviral myocarditis or dilated cardiomyopathy in humans (see Chapter 84).[66] A report examining the role of TLR3 gene polymorphism (TLR3c.1377C/T) in EV-A71 disease severity among Chinese children showed that the TT allele was found more frequently in children with severe rather than mild infection. Children possessing TT allele demonstrated decreased plasma IFN-γ levels and decreased IFN-γ/interleukin-4 ratio compared with children with mild disease.[67]

Inhibition of T-lymphocyte function has little effect on virus replication in vivo,[68] and persons with abnormal cell-mediated immunity are not predisposed to serious or prolonged enterovirus infections unless they have accompanying B-cell dysfunction. Although T lymphocytes do not contribute to the inhibition of enteroviral replication, there is evidence that certain immunopathologic events after enterovirus infection are mediated by T-cell activity. In the murine myocarditis model, expression of proinflammatory cytokines and acute inflammatory infiltration follow peak viral replication,[69–71] and induction of natural killer cell activity and T-lymphocyte immune responses contributes to necrosis of infected cardiac myocytes (see Chapter 84).[68,72,73] An inflammatory response may persist long after viral replication has ceased, and ongoing cardiac damage may be mediated by virus-induced antibodies against cardiac antigens[74] or by cytotoxic T-lymphocyte–mediated myocyte lysis.[70,72,75]

Epidemiology of Enteroviral Infections

Enteroviruses are distributed worldwide. Infection rates vary with season, geography, and age and socioeconomic status of the population sampled. Enteroviral infections occur throughout the year, but in temperate climates infections are more prevalent in the summer and autumn months (June to October in the Northern Hemisphere).[76,77] This seasonal periodicity is less pronounced in southern latitudes and disappears altogether in the tropics, where enteroviruses are endemic the year round. The seasonal pattern of incidence in the United States increased from south to north with the mean occurrence of nonpolio enteroviruses occurring earlier in southern states and northern states.[76] Additionally, the dispersion of cases throughout the year decreased from south to north. Southern states demonstrated a more even distribution of cases within the season, whereas northern states showed an increased peak in distribution.[76]

Age and Socioeconomic Status

The overwhelming majority of enteroviral infections occur in infants, children, and adolescents.[76,77] In the United States, attack rates for both infection and illness with nonpolio enteroviruses are highest in infants during their first year of life.[76,77,78] Surveillance data from 2000–2014 documented that 34% of infections reported to the US Centers for Disease Control and Prevention (CDC) occurred in this age group.[76] Among nearly 26,737 enteroviral detections reported to the CDC for the years 1983–2003, 11.4% occurred in neonates.[77] Rates of symptomatic enteroviral infection decrease after the second month of life[79] but remain higher for infants and toddlers compared with older children and adults.[77,80] Enteroviral infections are more prevalent among children of lower socioeconomic status,[81] probably because of crowding, poor hygiene, and opportunities for fecal contamination. Simultaneous infection by more than one serotype is common under these circumstances.[82]

The frequency with which different enterovirus serotypes cause infection varies markedly. In the United States, one to three enteroviral serotypes predominate in a given location in each season, although there is variation from one region to another and from year to year. Some prevalent serotypes are continuously isolated from year to year,[77] whereas others may emerge for the first time or reemerge after years of relative inactivity.[77,83–85] Wild-type polioviruses now circulate only in three countries (see Chapter 171), whereas vaccine strains are commonly isolated in countries that continue to use OPV for poliomyelitis prevention. Global epidemics of nonpolio enteroviruses occasionally occur, such as the worldwide outbreak of E-9 disease in the late 1950s and the explosive pandemics of acute hemorrhagic conjunctivitis due to EV-C70 and CV-A24 in the 1980s. Infection with some serotypes such as CV-B6 and EV-69 is infrequently or rarely recognized.

The reasons why individual serotypes of enteroviruses appear and disappear and behave as either endemic or epidemic pathogens are not well understood. Some epidemic echovirus strains spread rapidly and exhaust susceptible individuals in the population beyond a critical mass necessary for continued transmission, whereas endemic strains that are recovered over a number of years may be associated with lower attack rates due to a moderate level of herd immunity in the population. Indeed, time-series analysis and mathematical modeling of enterovirus infections in Japan over 15 years supports the theory that the number of susceptible individuals within the population determines when outbreaks occur.[86] In addition, periodic reappearances of the same enteroviral serotype may occur because the new enterovirus strain is poorly neutralized by antibodies raised in response to earlier strains.[87–89] Over the 11-year period between 2006 and 2016, 14 serotypes represented 81% of all enterovirus isolates submitted from state and local public health laboratories to the National Enterovirus Surveillance System of the CDC (Table 170.4).[90–92]

Molecular Epidemiology

Southern blotting, two-dimensional oligonucleotide gel RNA electrophoresis, and amplification and sequencing of defined RNA sequences have been used to differentiate between live vaccine and naturally occurring poliovirus strains[93,94] and trace the routes of spread of polioviruses[95] and other enteroviruses by comparison of RNA relatedness among epidemiologically distinct isolates.[96–98] Genomic RNA sequencing has proved to be the most adept technique for characterizing the evolutionary relationships among poliovirus isolates of the same serotype. Genotypes are distinguished from one another by greater than 15% divergence among the RNA nucleotides in the homologous portions of the genomes that are sequenced.[94] The application of genomic sequencing has detected cVDPVs in Haiti and other locations[45,46]; traced the pandemic spread of acute hemorrhagic conjunctivitis caused by EV-D70 and CV-A24 in the 1980s[99]; and documented the rise of new clades of EV-D68, a cause of severe respiratory infection.[83,100]

TABLE 170.4 Most Common Enterovirus Serotypes Submitted by State and Local Public Health Laboratories to the Centers for Disease Control and Prevention, 2006–2016

ENTEROVIRUS SEROTYPE	% OF ENTEROVIRUS ISOLATES
Enterovirus D-68	28.1
Coxsackievirus A6	6.6
Echovirus 18	5.6
Echovirus 30	5.3
Echovirus 11	4.8
Echovirus 9	4.6
Coxsackievirus B1	4.5
Echovirus 6	4.5
Coxsackievirus B4	3.9
Coxsackievirus A9	3.8
Coxsackievirus B3	3.2
Coxsackievirus B5	3.1
Coxsackievirus A16	1.7
Coxsackievirus B2	1.6
Total	81.3

Data from references 90–92.

Transmission

Enteroviruses are shed in feces and respiratory tract secretions. Transmission occurs from direct person-to-person contact and indirectly from environmental sources contaminated by enteroviruses. The relative importance of each route of transmission depends on viral factors, hygienic conditions, and, possibly, climatic conditions. Computational analysis of the seasonality of enteroviral infections in the United States indicates that dew point temperature was a main predictor of intensity of transmission.[76] Direct transmission from a respiratory source has been demonstrated in volunteer studies using CV-A21 as a challenge virus,[101] and it is likely that other enteroviruses frequently shed simultaneously from the upper respiratory tract and feces are also transmitted in this manner.[102] However, fecal contamination of the household environment is responsible for the high rates of infection demonstrated with polioviruses and many other enterically shed enteroviruses in poor hygienic conditions. Sampling of sewage in most cities, especially in summer months, usually yields several enteroviral serotypes.[103] Clams in seawater polluted by sewage concentrate enteroviruses 10- to 60-fold, and swimming in contaminated seawater was the apparent cause of an outbreak of enterovirus infection among tourists returning from Mexico.[104] Enterovirus D70, the agent of acute hemorrhagic conjunctivitis, is spread by fomites, fingers, and ophthalmologic instruments contaminated with virus in tears.[105]

Longitudinal studies have shown substantial clustering of enterovirus infections in families.[102] Once the virus has been introduced into the household, secondary attack rates for infection among seronegative family members are 90% to 100% for wild-type polioviruses and approximately 75% for coxsackieviruses.[102] Secondary attack rates for echoviruses range from less than 50% to 80%,[106] probably because these viruses tend to be shed only in feces and for shorter periods. Infants

in diapers who shed virus in the feces are the most efficient disseminators of infection. Mothers and infant siblings are at greater risk of acquiring infection than are fathers and teenaged siblings.[102] For all enteroviruses, the period of maximal contagiousness corresponds to the period of maximal viral excretion in the feces. When reinfection with the same enterovirus serotype occurs, the duration of excretion of virus is considerably shorter than in the primary infection.[102]

Incidence of Infection and Illness

Approximately 95% of infections caused by wild-type polioviruses and at least 50% to 80% of nonpolio enteroviral infections are asymptomatic. When illness occurs, it usually takes the form of an undifferentiated febrile illness lasting only a few days, often accompanied by symptoms of upper respiratory tract infection.[107] These illnesses may be caused by virtually any enteroviral serotype and are clinically indistinguishable from infection by many other viruses. Disease syndromes characteristic of enteroviruses (e.g., aseptic meningitis, pericarditis) are infrequent manifestations of infection. A 4-year longitudinal family-based study in New York City detected 291 enteroviral infections, none producing characteristic illnesses and only 6 with exanthems.[102]

The risk of some enterovirus-related clinical syndromes varies with age and sex. Aseptic meningitis is most commonly recognized in very young infants, whereas some other illnesses such as pleurodynia and myopericarditis are seen predominantly in adolescents and young adults. Symptomatic enteroviral infections in elderly adults are uncommon. Among young children, boys are at greater risk of illness (but not infection) than girls.[77,78] Aseptic meningitis and poliomyelitis occur almost twice as often in boys. After puberty, the reverse is true, perhaps because women have greater exposure than men to children shedding virus.[102,108] Pregnancy may enhance the severity of enteroviral infections. The incidence of paralytic poliomyelitis was two to three times higher in pregnant women than in age-matched nonpregnant women in Boston before the control of poliomyelitis.[109] Also, clinical and epidemiologic data demonstrate that enteroviral illnesses are more frequent[110] and more severe[111] in persons who exercise vigorously before the onset of symptoms. Although these data are anecdotal, they are supported by evidence that exercise enhances the severity of CV-B infection in the murine model.[112]

Although the incidence of nonpolio enteroviral disease has been measured in selected populations, the overall incidence in the United States is unknown. Serologic surveys encompassing all known enteroviral serotypes are not feasible. Antibody prevalence rates measured for a few serotypes indicate that following the decline of passively acquired maternal antibodies after the age of 6 months, the percentage of immune persons in the population rises progressively with age, so that 15% to 90% of the adult population have type-specific neutralizing antibodies for each serotype tested, depending on the serotype and the characteristics of the population surveyed.[78,102]

Incubation Period and Period of Communicability

The incubation period for illness due to enteroviral infections can rarely be determined precisely. Because the source of infection is often an asymptomatic person who transmits virus as readily as one who is ill, the time of exposure is usually unknown. Although the incubation period may range from 2 days to 2 weeks, it is usually 3 to 5 days. Patients with enteroviral illnesses typically excrete virus in throat secretions or feces for several days before the onset of symptoms and continue to excrete virus in feces for several weeks thereafter. Although the period of communicability is potentially long, maximal communicability probably occurs early in illness, when viral shedding is greatest.

Laboratory Diagnosis of Enteroviral Infections

The laboratory diagnosis of enteroviral infection is accomplished by virus isolation in cell culture, nucleic acid amplification testing (NAAT) (i.e., reverse-transcriptase polymerase chain reaction [RT-PCR] or nucleic acid sequence-based amplification), or retrospectively by serologic methods.[113] Cell culture is still performed by some academic medical centers and public health laboratories. However, because cell culture is laborious and relatively slow, it has been supplanted by more rapid and sensitive NAAT.

Viral Isolation

A presumptive diagnosis of enteroviral infection can usually be reported by the laboratory within 2 to 5 days by identification of a characteristic cytopathic effect in any of three or four appropriately chosen cell lines or cell line mixtures.[113,114] CV-As grow poorly in routinely used cell lines. Inoculation of newborn mice remains the method of choice for their recovery but is rarely used today because of its laborious nature.[113]

Isolates demonstrating a cytopathic effect may be preliminarily screened for enterovirus species or serotype with the use of monoclonal antibody blends.[113] Traditionally, the serotype of an enterovirus isolated in cell culture was determined by neutralization in cell culture using type-specific antisera. However, this has been largely replaced by molecular methods based on sequencing of the VP1 coding region.[113] Serotype identification by neutralization requires the use of Lim-Benyesh-Melnick intersecting antiserum pools.[115,116] The equine sera that constitute the Lim-Benyesh-Melnick pools were harvested against enterovirus strains prevalent more than 40 years ago, which explains their diminishing ability to identify contemporary isolates as well as their failure to identify newer human enteroviruses.

The opportunity to recover a virus in cell culture is optimized by sampling of multiple sites. Late in the course of enteroviral illnesses, viral cultures of feces are useful because the lower intestine may be the only site from which the agent is still being excreted. An etiologic diagnosis can be confirmed by the isolation of virus from cerebrospinal fluid, pericardial fluid, tissue, or blood, depending on the clinical syndrome. Isolation of virus from the upper respiratory tract or stool is considered to be less definitive because intercurrent asymptomatic enterovirus infections etiologically unrelated to the observed illness may produce a false-positive result.

Nucleic Acid Amplification Testing and Genomic Sequencing

RT-PCR and nucleic acid sequence-based amplification are rapid, sensitive, and specific methods of detecting enterovirus RNA in clinical specimens.[113] Most reported NAAT protocols amplify a highly conserved portion of the 5′ nontranslated region of the genome, enabling the detection of most enteroviruses.[117] Group, serotype, vaccine, and vaccine-related specific primers distinguish naturally occurring vaccine and vaccine-derived polioviruses from other enteroviruses.[118–120] With cerebrospinal fluid specimens from patients with suspected aseptic meningitis, NAAT has been shown to be superior to cell culture for the detection of enteroviruses, increasing yields from 7% to 61% in various studies.[113,117] Experience with specimens other than cerebrospinal fluid is more limited. NAAT has detected enteroviral RNA from throat swabs, serum, myocardium, urine, and stool, although the sensitivity with urine specimens is lower than with other specimens.[113,117,121,122] NAAT detected enteroviral RNA in approximately 15% of endomyocardial biopsy specimens from patients with acute myocarditis or dilated cardiomyopathy.[123] Further characterization of the species and serotype of enteroviruses isolated in cell culture or detected by RT-PCR can be accomplished by sequencing a portion of the VP1 capsid coding region of the viral genome.[2,3,123,124]

Serology

Type-specific, homotypic, and heterotypic immunoassays have been developed for the serologic diagnosis of enterovirus infections.[125–129] However, the utility of these assays as clinically relevant tools is limited by a number of issues. Serum IgM antibody to the enteroviruses may be nonspecific, leading to false-positive results, cross-reactivity with other *Picornaviridae,* and less sensitivity than with NAAT. Attempts to improve the sensitivity of the serologic assays have led to their enhancement using RT-PCR.[128] However, the latter are generally technically challenging to implement. Until identification of a common antigen sufficiently immunogenic to form the basis of a broadly reactive assay, serologic diagnosis will have limited use in the clinical management of enteroviral infections.

Treatment and Prevention of Enteroviral Infections

Most enterovirus infections are self-limited and do not require antiviral therapy. Exceptions include encephalitis, acute myocarditis, and infections in neonates and B-cell–deficient hosts that may be life threatening. The therapeutic options for these more serious infections are quite limited. Serum immune globulin and intravenous immune globulin have been given to persistently infected B-cell–deficient patients with mixed results[59] and have been used in nonrandomized trials in children with myocarditis and neonates with uncertain effect.[130,131]

An effective antiviral drug to treat serious enterovirus infections has not been licensed. The most promising of the compounds to undergo clinical trials has been pleconaril, an antiviral that binds to a pocket in the viral capsid, altering virus attachment and uncoating.[132,133] Pleconaril was found to inhibit replication of most enterovirus serotypes at concentrations of less than 0.1 μg/mL in vitro.[133] In a multicenter, placebo-controlled study, neonates with enteroviral sepsis (hepatitis, coagulopathy, or myocarditis) treated with pleconaril had greater survival and shorter times to PCR and culture negativity than infants who received placebo.[134] A post hoc analysis of two multicenter, double-blind, placebo-controlled trials of pleconaril in adults with enterovirus meningitis documented reduction in the duration of headache for patients with moderate to severe nausea.[135] Several additional non–peer-reviewed presentations of the results from pleconaril clinical trials also indicated benefit.[136] Uncontrolled experience with pleconaril for B-cell–deficient patients with persistent enterovirus infections and patients with potentially fatal infections including neonates and patients of all ages with acute myocarditis suggests possible clinical benefit.[137,138] Pleconaril has also shown mild benefit in the treatment of rhinovirus-induced colds,[139] but it was not approved by the US Food and Drug Administration for this indication. The drug induces cytochrome P-450 3A isoenzymes[139] and therefore has raised concern for multiple drug interactions.[138] Pleconaril is currently unavailable for clinical use (see Chapter 48).

The preexposure administration of immune globulin is known to reduce the risk of paralytic poliomyelitis.[54] It is likely that immune globulin would also prevent nonpolio enteroviral disease, but this strategy is rarely applicable to clinical practice. The successful vaccine approach against paralytic poliomyelitis is detailed in Chapter 171. In the setting of a community epidemic or a patient hospitalized with enteroviral illness, simple hygienic measures such as hand washing[140] and careful disposal or autoclaving of potentially infected feces and secretions should be practiced. For hospitalized patients, infection control measures using standard precautions are sufficient. Gown and mask procedures or isolation of the patient is not warranted except in the newborn nursery. In nursery outbreaks of enteroviral infection, infected infants should be cohorted. Pregnant women, especially those near term, should be advised to avoid contact with people suspected to have an enteroviral illness.

PARECHOVIRUSES

The human parechoviruses (HPeV) have many properties in common with other picornaviruses, but they also have biochemical and biophysical differences that have led to their designation as a separate genus of *Picornaviridae*.[2] The properties of HPeV are reviewed in the following paragraphs and discussed in more detail in Chapter 173.

Virology

Parechoviruses have the typical morphology of picornaviruses. They are small (28 nm), are nonenveloped, and have icosahedral symmetry. The capsid consists of 12 pentamers. Virions have a buoyant density in 1.34 g/cm of cesium chloride.[2,8,141] The genome of HPeVs is contained in a nonsegmented, linear, single-stranded RNA of positive sense. The complete genome is approximately 7350 nucleotides long, the organization of which is similar to the human enteroviruses: a 5′ nontranslated region of approximately 740 nucleotides, a coding region of approximately 6540 nucleotides, a short 3′ nontranslated region, and a 3′ polyadenylated tail.[141]

The genome encodes a single polyprotein, which undergoes cleavage into three capsid proteins (VP0, VP1, VP4) and seven nonstructural proteins and protein intermediates. However, in contrast to enteroviruses, cleavage of VP0 into VP4 and VP2 does not occur, resulting in the parechoviruses having only three structural proteins: VP0, VP1, and VP3.[8,141] The nonstructural proteins include an RNA-dependent RNA polymerase, VPg, and a probable helicase.[141] Receptors for HPeV1 appear to be the $\alpha_v\beta_1$, $\alpha_v\beta_3$ integrins (see Table 170.3).[8,142]

Virus Types

Because HPeVs replicate poorly in cell culture and owing to the relatively laborious and difficult process of determining neutralization of parechoviruses in tissue culture, sequencing of the VP1 capsid coding region is employed to designate types. There have been 17 types identified.[4]

Viral Diagnosis

Commercially available NAAT is available for detection of the HPeV. Serologic responses are infrequently used, but determination of virus-specific IgG or IgM neutralizing responses is occasionally used to establish the occurrence of infection.[141]

Epidemiology

HPeV1 (formerly known as echovirus 22) appears to be widely distributed and mainly infects young children. Infection with HPeV2 (formerly echovirus 23) appears to be much more limited.[141] HPeV3 is found worldwide and is the major cause of central nervous system infections due to the human parechoviruses. Infections occur primarily in infants younger than 6 months of age.[143] The epidemiology and clinical manifestations of parechovirus infections are discussed in Chapter 173.

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The complete reference list is available online at Expert Consult.

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171 Poliovirus

José R. Romero

SHORT VIEW SUMMARY

Definition

- Polioviruses are the cause of poliomyelitis, a systemic viral infection that predominantly affects the central nervous system, causing paralysis. Only three serotypes exist (1 to 3). The name of the disease (*polios,* "gray"; *myelos,* "marrow" or "spinal cord"), now commonly shortened to polio, is descriptive of the pathologic lesions that involve neurons in the gray matter, especially in the anterior horns of the spinal cord.

Epidemiology

- Wild-type poliovirus type 2 no longer circulates worldwide and was declared eradicated in 2015. Serotype 3 has not been isolated since November 2012. Serotype 1 remains endemic in three countries (Afghanistan, Nigeria, and Pakistan). In 2017, the annual number of cases of poliomyelitis (19) was the lowest since the initiation of the global poliovirus eradication effort in 1988.

Microbiology

- The polioviruses are members of the genus *Enterovirus,* family Picornaviridae. Morphologically, they are small (30 nm in diameter), nonenveloped, icosahedral-shaped viruses that possess a single-stranded, positive-sense RNA genome.

Diagnosis

- Polioviruses can be isolated from throat secretions and from feces and rarely from the cerebrospinal fluid. Characterization is accomplished by sequencing of the major capsid protein VP1 and is available only in public health reference laboratories.

Therapy

- No specific therapy is available. Supportive measures to ensure airway patency, adequate respiratory effort, and clearance of secretions are the mainstay of treatment for severe cases of paralysis.

Prevention

- Vaccination with inactivated or live-attenuated oral vaccines has been essential in the elimination of the polioviruses. In preparation for the global eradication of the polioviruses, a worldwide switch to bivalent oral polio vaccine containing poliovirus types 1 and 3 occurred in 2016. Public health measures, such as provision of potable water and proper sewage disposal, play major roles in the community-wide control of the polioviruses.

Polioviruses are the cause of poliomyelitis, a systemic viral infection that predominantly affects the central nervous system (CNS), causing paralysis. The name of the disease (*polios,* "gray"; *myelos,* "marrow" or "spinal cord"), now commonly shortened to polio, is descriptive of the pathologic lesions that involve neurons in the gray matter, especially in the anterior horns of the spinal cord. Paralytic poliomyelitis has been completely controlled in the United States and other developed countries[1] through routine childhood immunization with either inactivated poliovirus vaccine (IPV), live-attenuated oral poliovirus vaccine (OPV), or both. However, as of 2017,[1] the goal of worldwide poliomyelitis eradication has not yet been achieved, having been hampered by unforeseen scientific challenges, geopolitical unrest, and social disruption, among other factors.

HISTORY

Perhaps the earliest representation of poliomyelitis is found in a funerary stele from the Ramesside period (1300 BC) of Ancient Egypt, housed in the Carlsberg Museum, Copenhagen. It depicts the priest Rom with an atrophic lower extremity and foot held in a talipes equinus position.[2,3] Sporadic poliomyelitis cases were published as early as 1840, and the first descriptions of the natural history and neurologic complications of poliomyelitis were recorded in Sweden by Karl Oskar Medin in 1890.[4] There is little record of epidemic poliomyelitis until the late 19th century, when outbreaks were first recorded in Scandinavia, Western Europe, and the United States. Charles Caverly wrote the first description of epidemic poliomyelitis in the United States, an outbreak of 132 cases near Rutland, Vermont, in 1894.[5] Thereafter, sporadic epidemic disease occurred during the first half of the 20th century, and by the 1950s, epidemic polio occurred regularly, with approximately 25 cases per 100,000 population reported annually in the United States. Accompanying the increased incidence was a shift in the peak-affected age group from infants to school-aged children and young adults. Both the appearance of epidemic disease and the rising age incidence have been attributed to rising standards of hygiene, which delayed the age of infection beyond infancy and loss of maternal antibody, thus creating a pool of susceptible people large enough to permit the spread of epidemic disease.[6]

In 1908, Landsteiner and Popper[7] demonstrated that polio was caused by a "filterable virus" when they transmitted disease to monkeys from human spinal cord homogenates. Scientific progress remained somewhat limited until the landmark discovery in 1949 by Enders, Weller, and Robbins[8] that poliovirus could be propagated in vitro in cultures of human embryonic cells of nonneural origin. This discovery facilitated experimental investigation of the pathogenesis of the disease in primates and the development of vaccines. Bodian and associates[9] first recognized the three distinct serotypes of poliovirus. By 1952, Bodian[10] and Horstmann[11] had independently discovered that viremia occurred early in infection, which explained the systemic phase of the illness.

Salk[12] reported in 1953 that human subjects could be successfully immunized with formalin-inactivated poliovirus, a discovery that rapidly led to an extensive field trial and licensure of IPV in 1955. The introduction of IPV led to a sudden dramatic reduction in the incidence of paralytic poliomyelitis in the United States to 0.4 cases per 100,000 population in 1962, when OPV replaced IPV for routine use. The last case of endemic wild-type poliomyelitis occurred in 1979, indicating complete cessation of transmission of naturally occurring polioviruses.[13] However, in addition to rare cases of wild-type disease imported from other countries, the United States continued to experience 6 to 10 cases of OPV vaccine-associated paralytic poliomyelitis (VAPP) each year until 1997, when IPV was reintroduced into the routine childhood immunization schedule in combination with OPV. Since 2000, only IPV has been used.

Under the leadership of the Pan American Health Organization, the entire Western Hemisphere became free of paralytic polio by 1991. Poliovirus type 2 was certified as globally eradicated in 2015. As of the end of 2017, worldwide, endemic circulation of polioviruses had been controlled by all countries with the exception of Pakistan, Afghanistan, and Nigeria.[1] No naturally occurring polioviruses have been identified from any other country since 2014.[1] Virulent polioviruses derived from OPV strains (circulating vaccine-derived polioviruses [cVDPVs]) also cause periodic outbreaks of paralytic polio in some regions with low population immunity.[14]

PATHOPHYSIOLOGY

Virology

Polioviruses are prototypic members of the genus *Enterovirus*[15] (see Chapter 171). Three poliovirus serotypes exist, and infection with each confers serotype-specific, lifelong immunity to disease but little or no immunity to infection or disease caused by heterologous serotypes.[16] Before the introduction of poliovirus vaccines, most paralytic disease was caused by type 1.[17] Polioviruses are categorized into three groups: wild-type (naturally occurring) polioviruses (WPVs), vaccine-related poliovirus (VRPV) strains (live-attenuated OPV viruses), and vaccine-derived polioviruses (VDPVs) arising from OPV strains. The VDPVs are further classified as cVDPV when evidence of person-to-person transmission exists; immunodeficiency-associated VDPV (iVDPV) when isolated from persons with primary immunodeficiencies; and ambiguous VDPV (aVDPV) when isolated from individuals without immunodeficiency and evidence of transmission, or in the case of sewage isolates unrelated to other known VDPVs and whose source is unknown.[14,18] WPV, VPDV and VDPD may be encountered in different populations, depending on whether endemic transmission of WPV has been eliminated, on whether OPV is used, and on the vaccine-induced immunity rates in the population.

Humans are the only natural host and reservoir of polioviruses. Experimental infections and paralysis can be produced in other primates, polioviruses can be adapted to replicate in subprimate mammals, and transgenic (tg) mice expressing the poliovirus receptor (PVR) can be paralyzed by injection into the CNS or peripheral muscle but not by the oral route.[19] Recently, tg mice expressing PVR under the control of the murine promotor Tage4 have been demonstrated to be moderately susceptible to oral infection by poliovirus.[20] Naturally occurring strains vary over a 10^7-fold range in neurovirulence.[21] Polioviruses are more infectious for the human gut than for the gut of lower primates.

Attenuated OPV strains are occasionally able to paralyze rhesus and cynomolgus monkeys but only when injected in high doses directly into the CNS. In addition to low neurovirulence, vaccine strains can often be distinguished from WPV strains by their temperature sensitivity and by subtle antigenic differences. The RNA sequences of the vaccine strains differ from the sequences of their naturally occurring parents by less than 0.2% across the full genome, with the smallest difference occurring between the type 2 vaccine and parent strains. For all three serotypes, analogous nucleotide substitutions in the 5′-noncoding region are associated with reduced neurovirulence.[22–25] Attenuating mutations also map to capsid proteins for individual serotypes.

In contrast, cVDPVs have been permitted to circulate because of low population immunity for long periods of time and, by continuous mutation, acquire biologic properties indistinguishable from those of WPVs.[14,17,26] The cVDPVs isolated have RNA sequences that vary by more than 0.6% to 1.0% from the corresponding OPV parent strain within the region encoded for the VP1 capsid protein, have undergone genomic recombination with other C species enteroviruses, and have acquired virulence markers characteristic of WPV strains.[18]

Pathogenesis

The early events in the pathogenesis of poliomyelitis are similar to those in other enterovirus infections described in Chapter 171. After implantation at a mucosal site and replication in the gut, or less frequently the throat and adjacent lymphoid tissues, polioviruses disseminate to susceptible reticuloendothelial tissues via a minor viremia. In asymptomatic infections, the virus is contained at this point and elicits the formation of type-specific antibodies. In a few infected persons, replication in the reticuloendothelial system gives rise to a major viremia, which corresponds temporally with the minor illness and causes the symptoms associated with abortive poliomyelitis. At this point, the course of poliomyelitis deviates from that of other enteroviral diseases in the ability of polioviruses to infect neurons in the gray matter of the brain and spinal cord. Although the preponderance of evidence indicates that viremia precedes paralysis in both experimental primates and humans, the exact routes whereby the CNS becomes infected remain uncertain.[27,28] Studies in the PVR tg mouse suggest that polioviruses may enter the CNS via the bloodstream or by retrograde axonal transport in peripheral nerve fibers.[29–31] Neuropathologic studies and animal experiments have indicated that spread is neural once the virus reaches the CNS.[32,33]

Poliovirus principally affects motor and autonomic neurons. Neuronal destruction is accompanied by inflammatory lesions consisting of polymorphonuclear leukocytes, lymphocytes, and macrophages that are distributed throughout the gray matter of the anterior horns of the spinal cord and the motor nuclei of the pons and medulla.[32,34] The mesencephalon, cerebellar roof nuclei, and precentral gyrus of the cerebral cortex are less severely involved.[34] Clinical symptoms depend on the severity of lesions rather than on their distribution, which is similar in essentially all cases; almost all fatal cases have involvement of both the spinal cord and cranial nerve nuclei and brainstem, even in the absence of bulbar signs. The dorsal root ganglia are commonly involved pathologically, but this does not result in sensory deficits. Polioviruses can be isolated from the spinal cord for only the first few days after the onset of paralysis, but the inflammatory lesions may persist for months.

CLINICAL MANIFESTATIONS

Incubation Period

Best estimates of the incubation period of poliomyelitis are 9 to 12 days (range, 5–35 days), measured from presumed contact until the onset of the prodromal symptoms, and 11 to 17 days (range, 8–36 days) until the onset of paralysis.[35]

Clinical Manifestations of Infection

The manifestations of infection by polioviruses range from unapparent illness to severe paralysis and death. Usual estimates of the ratio of unapparent to clinically recognized polio infection, which vary by serotype, range from 60:1 to 1000:1.[6,36] Fig. 171.1 depicts the time

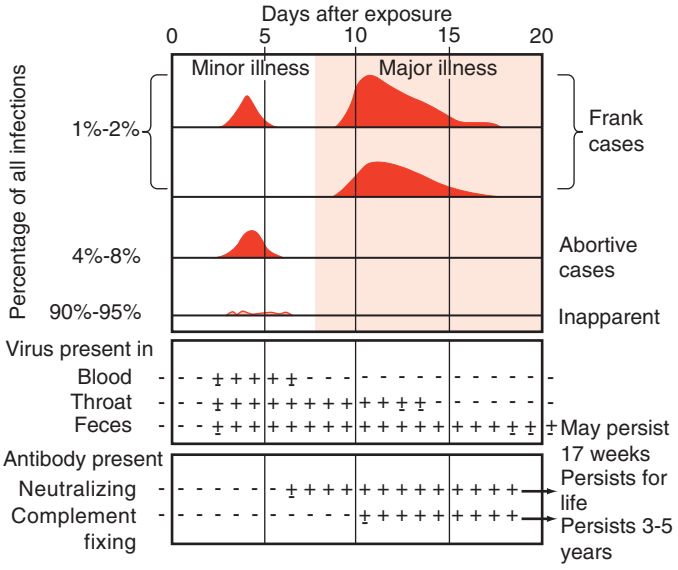

FIG. 171.1 Schema of the clinical and subclinical forms of poliomyelitis. This graphic representation shows the presence of virus and antibodies in relation to the development and persistence of the infection. *(From Paul JR.* History of Poliomyelitis. *New Haven, CT: Yale University Press; 1971.)*

course for the clinical manifestations of poliovirus infection. At least 95% of infections are asymptomatic or unapparent and can be recognized only by the isolation of poliovirus from feces or oropharynx or by a rise in antibody titer. *Abortive poliomyelitis,* which occurs in 4% to 8% of infections, is characterized by a 2- to 3-day period of fever, which may be accompanied by headache, sore throat, listlessness, anorexia, vomiting, or abdominal pain. Because the neurologic examination findings are normal, abortive poliomyelitis cannot be distinguished from other viral infections and can be clinically suspected only during an epidemic. *Nonparalytic poliomyelitis* differs from abortive poliomyelitis by the presence of signs of meningeal irritation. The disease is identical to meningitis caused by other enteroviruses. The systemic manifestations of nonparalytic poliomyelitis are generally more severe than in abortive poliomyelitis.

Spinal Paralytic Poliomyelitis

Frank paralysis occurs in roughly 0.1% of all poliovirus infections. A biphasic course with minor and major illnesses is observed in approximately one-third of children with paralytic poliomyelitis. The minor illness, coinciding with viremia, corresponds to the symptoms of abortive poliomyelitis and lasts 1 to 3 days. The patient appears to be recovering and remains symptom free for 2 to 5 days before the abrupt onset of the major illness, which is heralded by symptoms and signs of meningitis, including fever, chills, headache, fever, malaise, vomiting, neck stiffness, and cerebrospinal fluid (CSF) pleocytosis. Adults usually experience a single phase of illness, with a prolonged prodrome of symptoms preceding the gradual onset of paralysis.[37,38] The major illness begins with severe myalgias and occasionally localized cutaneous hyperesthesia, paresthesias, involuntary muscle spasm, or muscular fasciculations. The meningismus and muscle pain are present for 1 to 2 days before frank weakness and paralysis ensue. The severity of the disease varies from weakness of a single portion of one muscle to complete quadriplegia. The paralysis is flaccid; deep tendon reflexes are initially hyperactive and then become absent. The most characteristic feature of the paralysis is its asymmetrical distribution, which affects some muscle groups while sparing others. Proximal muscles of the extremities tend to be more involved than distal muscles, the legs are more commonly involved than the arms, and the large muscle groups of the hand are at greater risk than the small ones. Any combination of limbs may be paralyzed, but the most common pattern is involvement of one leg, followed by one arm, or both legs and both arms. Quadriplegia is almost never observed in infants.[38] Although occasional cases progress from the onset of weakness to complete quadriplegia and bulbar involvement in a few hours, more commonly the paralysis extends over 2 to 3 days. Progression of paralysis stops when the patient becomes afebrile.[37] Paralysis of the bladder is usually associated with paralysis of the legs. It occurs in about 25% of adults but is uncommon in children. Sensory loss in poliomyelitis is very rare,[39] and its occurrence should strongly suggest some other diagnosis (e.g., Guillain-Barré syndrome).

Bulbar Paralytic Poliomyelitis

Bulbar poliomyelitis results from paralysis of muscle groups innervated by the cranial nerves, especially those of the soft palate and pharynx, which may manifest as dysphagia, nasal speech, and sometimes dyspnea. The frequency of the bulbar form of the disease has varied in different epidemics from 5% to 35% of paralytic cases. The ninth and tenth cranial nerves are most commonly involved, and pharyngeal paralysis with pooling of secretions often is the only obvious sign.[40] Patients usually are extremely anxious and agitated about their inability to swallow and breathe. Involvement of the circulatory and respiratory centers of the medulla represents the most serious form of bulbar poliomyelitis.

Polioencephalitis

Encephalitis, manifesting primarily with confusion and disturbances of consciousness, is an uncommon form of poliomyelitis occurring principally in infants. It is the only type of poliomyelitis in which seizures are common. In contrast to spinal paralytic polio, there may be spastic paralysis, indicating the presence of upper motor neuron involvement. The illness is not clinically distinguishable from other forms of viral encephalitis.

Complications

The most important complication of paralytic poliomyelitis is respiratory compromise caused by paralysis of the respiratory muscles, including the diaphragm and intercostal muscles, by airway obstruction from involvement of the cranial nerve nuclei, or by lesions of the medullary respiratory center.[41] Myocarditis has been documented by the presence of inflammatory infiltrates in cardiac tissue obtained postmortem and isolation of poliovirus from myocardium,[42,43] but myocardial dysfunction is rarely recognized clinically. Gastrointestinal events such as hemorrhage, paralytic ileus, and gastric dilatation may complicate acute paralysis.[44] Paresis of the detrusor muscle can lead to bladder paralysis and urinary retention in the acute phase of paralytic poliomyelitis.[45,46] Patients with prolonged recumbency are at risk for the development of renal calculi.

RISK FACTORS

Several preexisting factors and provocative events are known to influence the risk of poliovirus infection and, after infection with poliovirus, the risk of developing paralysis. Before puberty, poliovirus infections occur equally in boys and girls, although paralysis is more common in boys. Among adults, women are at greater risk of infection but are not necessarily at greater risk of paralysis.[47,48] Both the incidence and severity of poliomyelitis may be increased in pregnant women.[48,49] Not only are women of childbearing age more likely to be exposed to infections in young children,[47] but also, late pregnancy may be associated with increased susceptibility to more serious illness.

Strenuous exercise increases both incidence and severity of paralytic poliomyelitis.[50,50a] Exercise during the minor illness or the prodrome has no effect but is detrimental when it occurs during the first 3 days of the major illness.[50]

Both epidemiologic[51,52] and experimental[53,54] studies have confirmed that poliomyelitis tends to localize in a limb that has been the site of an intramuscular injection or injury within 2 to 4 weeks before the onset of infection. Provocation poliomyelitis has been observed with both wild-type and OPV viruses.[55] Skeletal muscle injury in viremic PVR-expressing tg mice induces retrograde axonal poliovirus transport to the CNS.[54]

Tonsillectomy increases the risk of bulbar poliomyelitis whether the procedure is performed just before poliovirus infection or was performed in the remote past.[56,57] Because the ninth and tenth cranial nerves supply the fauces, the spread of virus from damaged nerve endings may explain this effect.

DIFFERENTIAL DIAGNOSIS

Sporadic cases of acute motor neuron disease that is indistinguishable from poliomyelitis may be caused by other enteroviruses, especially enteroviruses A-71 and D-68 (see Chapter 172) or West Nile virus infection[58] (see Chapter 153). Few other diseases are confused with paralytic poliomyelitis, except Guillain-Barré syndrome, which, unlike poliomyelitis, produces symmetrical, bilateral ascending paralysis, with loss of sensation that in most cases may progress over a period of 1 to 2 weeks. In acute poliomyelitis, pleocytosis and a minimally elevated protein concentration are present, whereas in Guillain-Barré syndrome the protein level is elevated with absent or minimal pleocytosis (the albuminocytologic dissociation).

LABORATORY DIAGNOSIS

Abnormalities of the CSF are not distinguishable from those of other viral diseases that cause aseptic meningitis. Polioviruses usually can be isolated from throat secretions in the first week of illness and from feces, often for several weeks. Unlike other enteroviruses that cause aseptic meningitis, polioviruses rarely are isolated from the CSF. In sporadic cases of poliomyelitis occurring in areas of low incidence, it is important to characterize virus isolates as wild-type (naturally occurring strains), OPV virus, or cVDPVs that have evolved from OPV virus. In all cases, this is accomplished by genomic sequencing, available only in public health reference laboratories.[59,60] Isolation from CSF (or brain and spinal cord in fatal cases), although uncommon, is especially valuable in evaluating VAPP, because recovery of fecal virus is expected for several weeks after receipt of OPV, and only a CNS virus isolate with

vaccine characteristics provides conclusive evidence of the causative association.

In the absence of a viral isolate, the diagnosis of poliovirus infection can be established serologically by testing paired acute and convalescent sera for neutralizing antibodies to each of the three poliovirus serotypes. Serologic tests cannot distinguish between wild-type virus and vaccine virus infection.

PROGNOSIS

Muscle paralysis usually progresses or extends for only 1 to 3 days after its onset but occasionally for as long as 1 week.[61] Permanent weakness is observed in approximately two-thirds of patients with paralytic poliomyelitis. Complete recovery is less likely when acute paralysis is severe and when patients require mechanical ventilation. An estimate of the eventual outcome can be made after 1 month, when most reversible damage has disappeared. Very little additional return of function can be expected beyond 9 months. Recovery from pharyngeal paralysis usually is evident by 10 days and is eventually complete. Bulbar poliomyelitis is rarely responsible for permanent sequelae in surviving patients.

Available mortality figures date from the era of epidemic poliomyelitis, a period when critical care medicine was less advanced than it is today. The reported overall mortality for acute paralytic poliomyelitis during this period was approximately 5% to 10%.[62] Mortality rates are higher among older individuals, who are more likely to have combined spinal respiratory muscle and bulbar involvement.[62,63] A poliovirus type 1 outbreak in the Republic of the Congo was associated with a case fatality rate of 32% and 48% in persons younger than 15 and those 15 years of age and older, respectively.[64]

THERAPY

Although antiviral drugs for the treatment of poliovirus-infected persons are under development,[65,66] they are not yet available, and therefore management is supportive and directed at relief of symptoms.[67] The most advanced antiviral in terms of clinical study is pocapavir (V-073), a capsid inhibitor with in vitro activity against polioviruses.[68,69] It was studied in a placebo-controlled challenge of 144 volunteers who received oral poliovirus type 1 vaccine, where it accelerated clearance of virus but was associated with frequent emergence of virus resistant to pocapavir.[66] Antivirals against polioviruses and other enteroviruses are further discussed in Chapter 48. In the acute phase of paralytic poliomyelitis, patients should be hospitalized. Bed rest may prevent augmentation or extension of paralysis. Hot moist packs applied to muscles are helpful for relieving pain and spasm. Physical therapy should be initiated once the progression of paralysis has ceased.

Paralysis of the respiratory muscles necessitates mechanical ventilation before hypoxia develops, generally when the vital capacity falls to less than 50%. Pooling of secretions in the pharynx in mild bulbar poliomyelitis, if unaccompanied by spinal respiratory paralysis, can be managed with postural drainage and suction. Severe bulbar paralysis necessitates tracheal intubation. Weakness or paralysis of the bladder may necessitate catheterization.

Management of the long-term physical and psychiatric sequelae of paralytic poliomyelitis is beyond the scope of this text. The reader is referred to excellent references on these topics.[70–72]

POSTPOLIOMYELITIS SYNDROME

Some patients who partially or fully recover from paralytic poliomyelitis experience a new onset of muscle weakness, pain, atrophy, and fatigue many years after the acute illness.[73] Typically, the involved muscles are the same as those affected during the original illness, but weakness may also occur in previously unaffected limb muscles. Progression of new symptoms is gradual, and affected persons are seldom severely disabled.[74,75] However, it may be dangerous in those with respiratory dysfunction or dysphagia. Population-based studies have suggested that the syndrome affects 20% to 85% of previously paralyzed patients.[72,75] The mean interval between acute poliomyelitis and the onset of postpoliomyelitis syndrome is 36 years. Although the cause is unknown, the leading theory is that late progression of muscle weakness is a result of physiologic attrition of motor units innervating muscles and muscle groups already less innervated as a result of earlier acute poliomyelitis.[72,75]

POLIOVIRUS IMMUNIZATION

Poliovirus Vaccines

Both IPV and live-attenuated OPV have been used effectively for more than 50 years to control polio. The introduction of Salk IPV in 1955 led to an immediate and dramatic reduction in both epidemic and endemic poliomyelitis in the United States and other Western countries. However, once the vaccine became generally available, it was observed that as many as 17% of children with paralytic poliomyelitis had received three or more doses of IPV, suggesting that the original formulation lacked optimum potency.[73] Meanwhile, live-attenuated OPV strains were developed by multiple passages of polioviruses in monkey kidney cell cultures and selection of mutants with low virulence for primates.[76] Successful field trials of the Sabin OPV vaccine strains were carried out in the United States and elsewhere from 1955 to 1959, and OPV was introduced for routine use in 1962 as separate monovalent vaccines; the trivalent product became available in 1964. OPV was quickly accepted by the pediatric and public health community because of superior immunogenicity compared with the original IPV formulation; lower cost; ease of administration; spread of vaccine virus to unimmunized, susceptible persons; and induction of gastrointestinal immunity. These properties have made OPV the logical choice for continued use in developing countries. However, because OPV also carries the risk of causing rare cases of VAPP, the United States and other developed countries have moved to exclusive use of a more potent IPV.[77]

Inactivated Poliovirus Vaccine

The majority of available IPV formulations worldwide are prepared by inactivation of wild-type, virulent poliovirus seed strains by the method originally developed by Jonas Salk (i.e., 1:1000 formalin treatment for 12–14 days at 37°C). All IPV preparations derived from WPVs currently available contain 40, 8, and 32 D-antigen units, respectively, for poliovirus serotypes 1, 2, and 3, formulated as either stand-alone trivalent IPV or together with other routinely administered childhood vaccines. In the United States, four doses of IPV are recommended at 2 months, 4 months, 6 to 18 months, and 4 to 6 years of age.[78]

Seroconversion rates are equal to, and mean antibody titers are superior to, those of OPV when given according to the same schedule. Studies conducted in the United States demonstrate that neutralizing antibodies are detectable to all three types in 99% of recipients after two doses and in 100% after the third dose.[79] A large boost in antibody titer follows the third dose.[79] After three doses, mean titers to types 1 and 3 are higher than in OPV-immunized children, whereas mean titers to type 2 are equivalent.

In tropical countries such as Cuba and in Puerto Rico, IPV administered at 3-dose schedules of 6, 10 and 14 weeks resulted in seroconversion in 94%, 83%, and 100% and 85.8%, 86.2%, and 96.9%, respectively, for poliovirus serotypes 1, 2, and 3, respectively.[80,81] In the latter country, a three-dose series similar to that used in the United States yielded seroconversion rates to poliovirus types, 1, 2, and 3 of 99.6%, 100.0%, and 99.1%.

Based on information derived from high-income countries, circulating antibody persists for decades and possibly for life. After a primary immunizing series of three or four doses, neutralizing antibodies are a usually found in all individuals at 5 years after immunization.[82] Although titers may decrease over time and fall below detectable levels, there is no evidence that this results in an increased susceptibility to paralytic poliomyelitis.

Several countries and the World Health Organization (WHO) have undertaken the development of IPV from the attenuated Sabin OPV strains.[83] Sabin-derived IPV (sIPV) is currently licensed for use in Japan and China. Possibly because of the vaccine strains used in creation and the reagents used to determine D-antigen concentration, the D-antigen content of each of the poliovirus serotypes varies among the various sIPV vaccines.[84,85] Furthermore, direct comparison of the D-antigen concentration of wild-type–derived IPV and sIPV is not possible. Protective antibodies against reference poliovirus strains, currently circulated wild poliovirus strains, and virtually all VDPV strains can be induced by sIPV.[83,86] The sole exception was a type 2 iVDPV that was not neutralized by sIPV or traditional IPV.[86] After administration

of a primary series vaccination in infants and a booster dose in adults, no differences in the decay of neutralizing antibodies were observed in those receiving sIPV when compared with individuals receiving traditional IPV.[87–89]

IPV-immunized children develop little or no measurable secretory antibody.[90,91] When challenged with live polioviruses, IPV-immunized children shed the challenge virus in their feces at a higher rate and a higher titer and for a longer period than OPV-immunized children, indicating a greater potential for asymptomatic infection and transmission of circulating polioviruses to unimmunized contacts.[91–93] Although this is widely considered to be a disadvantage of IPV, there is evidence that the universal use of IPV results in partial protection that extends to unvaccinated persons in the community, albeit less than the protection provided by OPV.[94,95]

Live-Attenuated Poliovirus Vaccine

OPV is no longer distributed in the United States.[77] Most trivalent OPV preparations made in other countries meet the WHO Expanded Program on Immunization minimum potency standards of $10^{6.0}$, $10^{5.0}$, and $10^{5.5}$ cell culture infectious dose ($CCID_{50}$) for poliovirus types 1, 2, and 3, respectively.[96]

Because the more efficient replication of type 2 OPV virus regularly interferes with the replication of types 1 and 3, a primary series of three doses is required to ensure seroconversion to all three serotypes.[97] Under normal conditions, infants respond to the first OPV with seroconversion rates of 50%, 85%, and 30% to serotypes 1, 2, and 3, respectively.[98] Two months after the second dose, more than 86% of infants have serum antibody to all three poliovirus serotypes, and 2 months after the third dose, the prevalence of antibody to all three types is more than 96%.[79,99] However, the excellent seroconversion rates observed in infants from industrialized nations are not seen in those of developing countries.[82,100] Reduced antibody response in these settings may be the result of a complex interaction among multiple factors including the vaccine and its delivery, the host, and their environment.

Detectable serum antibody to all three types persists in 84% to 98% of vaccinees 5 years after primary immunization,[101] and reexposure to vaccine viruses likely contributes to the maintenance of antibody levels in the population.[102,103] Secretory immunoglobulin A poliovirus antibody appears in oropharyngeal and duodenal secretions 1 to 3 weeks after OPV immunization and persists for at least 5 to 6 years.[90,104] Challenge studies have suggested that the intestinal immunity induced by OPV is similar to intestinal immunity after natural poliovirus infection.[105] The efficacy of OPV was never tested when WPVs circulated in the United States. However, data obtained during a type 1 poliovirus outbreak in Taiwan demonstrated estimated efficacy of 82%, 96%, and 98% for one, two, and three or more doses, respectively.[106]

Nonimmune OPV recipients shed vaccine viruses in the feces for 1 to 6 weeks and from the oropharynx for 1 to 3 weeks. The spread of OPV virus to unimmunized children is considered to be advantageous, especially in areas in which vaccine acceptance levels are low. The importance of this "backdoor" method of protecting the community is uncertain. A seroprevalence study in Houston and Detroit has found that 11% to 42% of 11- to 35-month-old children had antibody, despite receiving no prior OPV.[107]

Monovalent and Bivalent Oral Poliovirus Vaccines

The Sabin OPV was originally introduced in 1961 to 1962 as three individual monovalent vaccines that were administered separately. These were replaced in 1963 by the trivalent vaccine. The monovalent and bivalent types 1 and 3 OPV vaccines are more immunogenic than trivalent OPV for the corresponding serotype because of the absence of heterotypic interference from type 2 Sabin vaccine virus in the trivalent formulation. Monovalent and bivalent vaccines play major roles in the supplementary immunization activities (SIAs) of global poliovirus eradication effort in resource-limited settings.[108–111]

In 2014, the World Health Assembly recommended the worldwide withdrawal of the type 2 component from trivalent OPV.[112] This recommendation was based on the fact that indigenous wild poliovirus type 2 had not been detected since 1999, and up to 40% of VAPP cases are associated with the type 2 component in trivalent OPV. The recommendation took effect in April 2016 when all nations using trivalent OPV switched to bivalent OPV containing polio types 1 and 3. Successful global withdrawal of trivalent OPV was accomplished by mid-May 2016. Bivalent OPV has replaced trivalent OPV in all countries using OPV in their national immunization programs.[113]

Vaccine-Associated Paralytic Poliomyelitis

The only adverse reaction associated with OPV is the rare occurrence of VAPP. The WHO has estimated the incidence of VAPP to be 2 to 4 cases per 1,000,000 birth cohort per year in countries using OPV.[82] In the United States, it affected approximately 1 person per 2.6 million OPV doses distributed.[114] An average of 8 cases of VAPP were reported annually in the United States before OPV was removed from the immunization schedule in the late 1990s.[114] Estimates based on countries in which OPV was used recently place the risk of VAPP to be 3.8 cases per million births (range, 2.9–4.7).[115] In a review of the global burden of VAPP, the distribution of the Sabin strains most frequently associated with VAPP in recipient cases was as follows: type 3, 42%; type 2, 26%; type 1, 17%; and multiple serotypes, 15%.[115] Among contact cases of VAPP serotypes identified were type 3 in 37%, type 2 in 31%, type 1 in 20%, and multiple serotypes in 12%. Of recipient cases of VAPP, 71% occurred in patients younger than 1 year; 14%, 1 to 4 years old; 5%, 5 to 19 years old; and 10%, age 20 years or older. Approximately 75% of recipient VAPP cases are associated with the first dose of OPV. In developing countries, VAPP may result after the second or subsequent doses of OPV, with a concentration of cases in children 1 to 4 years old.[82] Among contact cases, 35% of patients were younger than 1 year; 33%, 1 to 5 years old; 8%, 5 to 19 years old; and 24%, age 20 years or older.[115] Fifty-two percent of contact VAPP patients had never been vaccinated. In the majority of recipient cases of VAPP, patients develop paralysis 7 to 21 days after the first feeding of OPV.[114] Contact VAPP cases (parents, family members, babysitters, or other household contacts) develop paralysis 20 to 29 days after the administration of OPV to a close contact. For immunocompetent patients, the clinical features and outcome of VAPP differ little from disease caused by WPVs.

Approximately 25% of reported VAPP cases occur in children and adults who are immunodeficient.[116] Most of these patients have transient or hereditary B-cell immunodeficiency, severe combined immunodeficiency syndrome, or common variable immunodeficiency. The risk of VAPP in newborn infants with a congenital B-cell immunodeficiency disorder is approximately 2000-fold higher than for immunocompetent infants. There is little evidence that immunodeficiency states that predominantly affect T-cell function increase the risk of VAPP. Similarly, there is little evidence that HIV infection, hematopoietic malignancy, or solid-organ transplantation increases the risk of VAPP.[117,118] Clinical features that distinguish VAPP in B-cell immunodeficient patients include an unusually long interval between the last OPV dose and onset of neurologic disease, with a typical range of 1 to 8 months, and as long as 12 years.[119,120] The illness is protracted and characterized by chronic meningitis; progressive neurologic dysfunction, suggesting involvement of both upper and lower motor neurons; and progression of paralysis over several weeks.[121,122] Immunodeficient patients have a much higher risk of dying from VAPP than immunocompetent patients. Although fewer than 20% of surviving VAPP patients excrete polioviruses for longer than 6 months,[119] fecal excretion of virus was estimated to occur for as long as 19 years in one immunodeficient patient.[123] Most VAPP cases in immunocompromised patients have been associated with type 2 OPV virus.[116]

The mechanism whereby the OPV viruses cause rare cases of paralytic disease is not completely understood. It is well known that OPV virus readily undergoes mutation during the brief period of intestinal replication and that isolates can be recovered that are neurovirulent for primates.[21] Most OPV recipients shed polioviruses that have reverted to the naturally occurring genotype in an analogous stem-loop structure in the 5′ noncoding region of the genome that affects initiation of transcription, which is strongly associated with attenuation for each of the three OPV serotypes.[22,124] Additional mutational events, mostly

within the protein coding region, probably contribute to reversion to neurovirulence.[22,124]

Use of Poliomyelitis Vaccines in the Developing World

Previously trivalent OPV and now bivalent OPV containing types 1 and 3 are used exclusively in developing nations for routine immunization because of lower cost, ease of administration, and superior secretory immunity in the gastrointestinal tract. In addition, OPV viruses are transmitted by immunized children to nonimmune contacts, a circumstance that is thought to be compounded by poor sanitation and crowded living conditions.

The WHO position paper on polio vaccines and polio immunization[83] recommends the inclusion of at least one dose of IPV for all countries using OPV, including all polio-endemic countries and countries at high risk for polio importation. The primary purpose of the IPV dose is to induce immunity to type 2 poliovirus, which could be rapidly boosted should an outbreak of type 2 disease occur after the global withdrawal of trivalent OPV. OPV should be administered according to the schedules of national immunization programs (e.g., at 6, 10, and 14 weeks or 2, 4, and 6 months). The dose of IPV can be given with the second or third dose of OPV. In addition, a dose of OPV should be administered as soon as possible after birth. Passively acquired maternal antibody present in the infant's circulation and in maternal colostrum blunts the immune response to the birth dose in some infants. However, infants who receive OPV at birth are more likely to have antibody to all three poliovirus types at 4 months of age.[125]

Unfortunately, many infants in tropical countries are left unprotected after receipt of the recommended number of OPV doses. Low seroconversion rates to three OPV doses have been widely documented,[125–130] averaging 73%, 90%, and 70% for types 1, 2, and 3, respectively.[100] This poor response appears to have contributed to poliomyelitis outbreaks in some countries with relatively high immunization rates.[130–132] Although the reasons for the lower potency of OPV in tropical areas remains incompletely understood, the effect of concurrent diarrheal illnesses has emerged as an important factor. Studies conducted in Brazil and Bangladesh have shown reduced seroconversion rates to types 2 and 3 OPV in infants with diarrhea at the time of OPV feeding, whereas the response to type 1 was not affected.[133,134] The impact of diarrhea on seroconversion persists despite the administration of three or four OPV doses. Similar effects of diarrheal disease have been observed on the immunogenicity of bivalent OPV in Nepalese children.[135] Malnourishment may be an additional factor influencing seroconversion. A study of Moroccan children found that seroprevalence rates for poliovirus types 1 to 3 were significantly lower among malnourished children.[136]

The low effectiveness of trivalent OPV in the presence of intercurrent diarrheal diseases has led to the introduction of other strategies to reduce poliovirus transmission in resource-poor settings. Monovalent and bivalent types 1 and 3 OPV vaccines have been introduced via supplemental immunization activities into many areas where WPVs continue to circulate. Studies in Egypt and Africa have confirmed the superior immunogenicity of monovalent OPV vaccines for newborn infants[108,111]; case-control studies in India and Nigeria have demonstrated that monovalent OPV type 1 vaccine is three to four times more effective per dose than trivalent OPV in protecting against paralytic poliomyelitis.[109,137] Comparisons of bivalent type 1 and 3 OPV vaccine to monovalent OPVs and trivalent OPV have demonstrated their noninferiority to the former and superiority to the latter,[110,138] resulting in WHO recommendations for their use in the global polio eradication program.[139] The effectiveness of monovalent and bivalent OPVs can be seen in India, where SIAs with these vaccines resulted in interruption of WPV circulation in January 2011.[1]

Historically, the costs associated with production and delivery and the requirements for injection have made the sole use of IPV an undesirable alternative for developing countries. WHO currently recommends the inclusion of at least one dose of IPV for all countries using OPV. In countries with a high vaccination coverage (90%–95%) and low importation risk, an IPV-OPV sequential schedule can be used. The purpose of this schedule is to provide protection against VAPP. For this schedule, WHO recommends one or two doses of IPV, followed by at least two doses of OPV.[82]

In general, IPV is highly immunogenic for infants in developing areas, although seroconversion and antibody titers are diminished, with short intervals between doses (e.g., 1 month), and also reduced in the presence of high titers of maternal antibody in young infants.[140–142] Studies have better defined the use of IPV in resource-poor areas of the world. In the 1980s, IPV was used as a supplement to OPV immunization in Israel, where type 1 poliovirus continued to cause epidemic disease in the Gaza Strip, despite relatively good rates of OPV coverage.[130,143] Israel switched exclusively to IPV in 2005, but in 2013 a dose of bivalent OPV was administered to all children born after 2005 to halt the circulation of a WPV detected in the sewage system.[144] IPV administered at 6 to 9 months of age has been shown to produce markedly enhanced seroconversion rates in infants given OPV at 2, 3, and 4 months of age in Côte d'Ivoire[145] and in Indian infants[146] for whom vaccination history could not be validated by clinic records. Last, a study conducted in Cuba documented that a single fractional dose of IPV administered intradermally can induce priming and seroconversion in more than 90% of immunized infants.[147]

Once the goal of polio eradication is achieved, the use of all live polio vaccines will cease in order to prevent outbreaks of VDPVs. IPVs will be required in order to maintain protection against reemergence of polio from immunodeficient individuals excreting virus, laboratory mishaps, or intentional reintroduction.

POLIOMYELITIS IN DEVELOPING NATIONS AND GLOBAL ERADICATION

Even after the introduction of polio vaccines, poliomyelitis was regarded as an epidemic disease of wealthier nations and was ignored in developing countries. However, surveys regarding lameness in schoolchildren in the 1960s and 1970s in more than 20 nations revealed lower limb paralysis prevalence rates of 2 to 11 per 1000 population, figures that reflect poliomyelitis incidence rates that equal or exceed those of the peak epidemic years in the United States.[148,149] Most cases of paralytic poliomyelitis in developing countries are caused by type 1 poliovirus and occur in children between the ages of 6 months and 2 years.

In 1974, WHO founded the Expanded Program on Immunization, which provided monetary and technical support for basic immunization against several childhood diseases, including polio, and created a worldwide standard polio immunization policy.[149] However, polio vaccines still failed to reach many children because of interrupted supplies, disruptions in the cold chain necessary to maintain the potency of OPV, civil strife, and poor political support. In 1983, an international conference held in Bellagio, Italy, soon after smallpox eradication, articulated the feasibility of worldwide poliomyelitis eradication,[150] and in 1988 the World Health Assembly set a goal of global eradication of poliomyelitis by the year 2000. Despite major progress, this goal has not yet been achieved.[1]

The WHO Global Poliomyelitis Eradication Initiative uses several major strategies to control and ultimately eradicate polio from most regions of the world, including encouragement of routine childhood immunization, SIAs, improvement of laboratory capabilities, intensified surveillance, and rapid response to identified outbreaks.[151] SIAs are highly coordinated nationwide or region-wide events in which all persons within a targeted age group receive two doses of OPV within a short interval.[152] Seroconversion rates during these mass campaigns are higher than for routine immunization, possibly because of the spread of OPV virus or because they are conducted during the dry season, when diarrheal disease is less prevalent.[153]

Progress toward eradication has been hampered by a number of unanticipated events, including a temporary but disastrous suspension of all immunization in northern Nigeria in 2003 to 2004 that led to importation of polio to 27 previously polio-free nations,[154] civil unrest in some locations leading to inadequate surveillance and vaccination activities,[155] and the emergence of cVDPV in many regions.[18,22] Renewed efforts and newly available resources have led to a dramatic reduction in global paralytic poliomyelitis cases to a low of 19 cases in 2017.[1]

There has been a complete disappearance of all cases caused by wild-type 2 poliovirus,[156] and the last known case of wild-type 3 poliovirus occurred in 2012.[1,156] In 2017, paralytic polio cases were reported from three nations where polio is considered to be endemic (Pakistan, Afghanistan, Nigeria).[1] In April 2013, WHO introduced the Polio Eradication and Endgame Strategic Plan 2013–2018, a 6-year plan for the global eradication of polio.[157,158] The four major objectives of the plan are outlined in Table 171.1.[157,158]

Vaccine-Derived Poliomyelitis Viruses

During 2000 and 2001, an outbreak of 21 cases of paralytic poliomyelitis on the island of Hispaniola occurred. This outbreak was caused by a virulent strain genetically related to the type 1 Sabin OPV vaccine strain.[26] Since then, at least 22 other outbreaks of paralytic disease caused by VDPVs have been discovered in underimmunized children living in economically deprived regions.[18,22] The low immunization rates in these areas have permitted these viruses to circulate for long periods and, by continuous mutation, acquire biologic properties indistinguishable from those of WPVs.[26]

The emergence of VDPV has profoundly influenced plans for the eventual cessation of poliovirus immunization after eradication of poliomyelitis, which will now include a strategy to discontinue OPV use worldwide, introduce IPV into as many countries as feasible, develop an OPV vaccine stockpile for use anywhere in the world, and develop a plan for containment of laboratory stocks of WPV and attenuated polioviruses.[22,157–159]

TABLE 171.1 World Health Organization (WHO) Polio Eradication and Endgame Strategic Plan 2013–2018

OBJECTIVE	PRIMARY GOAL
Poliovirus detection and interruption of its transmission	Stop all wild-type poliovirus transmission by the end of 2014
Strengthening of routine immunization programs and the withdrawal of OPV	Withdrawal of the type 2 component of OPV in all routine immunization programs by mid-2016 and use of bivalent OPV in 2019–2020
Containment and certification	Certification of all six WHO regions as having eradicated wild-type polioviruses by the end of 2018
Legacy planning	Have a strategy in place by the end of 2015 to ensure that the world remains permanently free of polio as well as to transition the lessons learned, with assets and infrastructure built to support this effort so that they may benefit other development goals and global health priorities

OPV, Oral polio vaccine.
From World Health Organization. Poliomyelitis: intensification of the Global Eradication Initiative. Report by the Secretariat. http://apps.who.int/gb/ebwha/pdf_files/WHA66/A66_18-en.pdf. Accessed July 31, 2018; and World Health Organization. Executive summary of the Polio Eradication and Endgame Strategic Plan 2013–2018. http://www.polioeradication.org/Portals/0/Document/Resources/StrategyWork/PEESP_ES_EN_A4.pdf. Accessed July 31, 2018.

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172 Coxsackieviruses, Echoviruses, and Numbered Enteroviruses (EV-A71, EVD-68, EVD-70)

José R. Romero

SHORT VIEW SUMMARY

Definition
- The coxsackieviruses, echoviruses, and numbered enteroviruses (EVs) are members of the genus *Enterovirus.*
- The four species, human EV-A to EV-D, within the genus *Enterovirus* total more than 100 serotypes.
- EVs are responsible for a wide array of clinical syndromes involving multiple organ systems, including acute meningitis, encephalitis, paralysis, exanthems, hand-foot-and-mouth disease, herpangina, myopericarditis, pleurodynia, and acute hemorrhagic conjunctivitis.

Epidemiology
- The EVs are found worldwide.
- In regions with temperate climates the majority of EV infections occur during the summer and early autumn months. In tropical regions infections occur year-round, with increased frequency during the rainy season.
- A large outbreak of EV-D68 occurred in the United States in 2014.

Microbiology
- Morphologically, they are small (30 nm in diameter), nonenveloped, icosahedral-shaped viruses that possess a single-stranded, positive-sense RNA genome.

Diagnosis
- EVs can be isolated, or their genomic RNA detected, from throat secretions, feces, cerebrospinal fluid, blood, and various tissues (heart, brain) and fluids (urine, pericardial, vesicle).
- Nucleic acid amplification testing has been shown to be quicker and more sensitive than cell culture for EV detection.
- Serotype identification is accomplished by sequencing of the major capsid protein VP1.

Therapy
- Therapy is supportive.
- No specific antiviral therapy is currently available for the treatment of EV infections.

Prevention
- Contagion can be prevented by hand washing and, in the case of certain serotypes, avoidance of EV-contaminated fomites.
- Vaccines for the prevention of human EV-A71 infection are available in Asia.

This chapter covers human disease caused by the group A coxsackieviruses (CVs), group B CVs, echoviruses (Es), and the numbered enteroviruses (EVs), which are distributed among four species, EV-A to EV-D, of the genus *Enterovirus.* Viral diseases caused by the closely related and newly designated genus *Parechovirus*, of the Picornaviridae, are discussed in Chapter 173. These viruses have many physical, epidemiologic, and pathogenetic characteristics in common, as described in Chapter 170. Greater than 90% of infections caused by the nonpolio EVs are asymptomatic or result only in undifferentiated febrile illness.[1] When disease occurs, the spectrum and severity of clinical manifestations vary with the age, gender, and immune status of the host and with the subgroup, serotype, and even the intratypic EV strain.

Some clinical syndromes (viral meningitis and some exanthems) are caused by many EV serotypes, some are predominately caused by certain EV serotypes (e.g., pleurodynia and myocarditis by the group B CVs), and other diseases are mostly associated with individual EV serotypes. Infections caused by some of the more recently recognized serotypes are discussed at the end of this chapter.

CENTRAL NERVOUS SYSTEM INFECTIONS

Acute Viral Meningitis

Viral infection is the dominant cause of the aseptic meningitis syndrome, and the EV-B species, which encompass all of the group B coxsackievirus (CV-B) and echovirus (E) serotypes, cause most acute viral meningitis cases in both adults and children.[2,3] Group A coxsackieviruses (CV-A) cause relatively fewer cases.[2] Historically, CV-B2 to CV-B5 and E-4, -6, -7, -9, -11, -13, -16, -18, -30, and -33 are the most frequently implicated serotypes. On occasion a single E serotype may cause widespread outbreaks; for example, E-13 caused outbreaks of aseptic meningitis throughout Europe and the United States in 2000 and 2001,[4] and E-33 caused widespread disease in New Zealand during the winter of 2000.[5]

Clinical Manifestations

Infants younger than 3 months have the highest rates of clinically recognized aseptic meningitis, in part because lumbar punctures are routinely performed for evaluation of fever in this age group.[6] Only a minority of these infants have clinical manifestations suggestive of neurologic disease.[7]

The severity of disease in older children and adults with aseptic meningitis varies widely. The onset may be gradual or abrupt, and the typical patient has a brief prodrome of fever and chills. Headache is usually a prominent complaint. Meningismus, when present, varies from mild to severe. Kernig and Brudzinski signs are present in only about one-third of patients. Signs of meningeal irritation are less frequently observed in young infants than adults.[6,8] Pharyngitis and other symptoms of upper respiratory tract infections are often present. The illness is sometimes biphasic, as in poliomyelitis; these patients present with a prodromal illness with fever and myalgias, followed by defervescence and absence of symptoms for a few days, and then experience abrupt recurrence of fever with headache and other signs of meningismus. Complications such as febrile seizures, complex seizures, lethargy, coma, movement disorders, and development of a syndrome of inappropriate antidiuretic hormone secretion occur early in the course of aseptic meningitis in 5% to 10% of patients.[6,9,10] Adults may experience a more prolonged period of fever and headache than infants and children, and some adult patients may take weeks to return to normal activity.[8,11]

Laboratory Diagnosis

The clinical diagnosis of viral meningitis depends on routine examination of cerebrospinal fluid (CSF). The CSF is clear and under normal or mildly increased pressure. The total CSF cell count is usually 10 to 500/mm^3 but may occasionally exceed 1000/mm^3. Cell counts less than 10 cells/mm^3 may occur.[2,12,13] Absence of pleocytosis may occur in 18% to 30% of infants and children with EV meningitis detected by nucleic acid amplification testing (NAAT) and occurs more frequently in neonates

and young infants.[14,15] Differential cell counts of the CSF often first reveal a high proportion of neutrophils, but the differential typically shifts to a predominance of lymphocytes during the initial 1 to 2 days of illness.[16–18] In general, the CSF glucose concentration is normal, and the CSF protein concentration is normal or slightly elevated. However, the glucose content may be lower than normal in 18% to 33% of cases,[19–21] and values less than 40 mg/dL may occur.[6,20] Uncommonly, it may be difficult to exclude bacterial meningitis on the basis of the CSF profile alone. In some cases the CSF findings may closely mimic those of tuberculous meningitis.[22]

NAATs (e.g., reverse-transcriptase polymerase chain reaction [RT-PCR] and nucleic acid sequence-based amplification [NASBA]) have replaced cell culture as the primary means of detection of EVs in CSF and other specimens.[23,24] Most PCR protocols amplify a highly conserved portion of the 5′ nontranslated region of the genome, which enables the detection of the majority of EVs. For confirmed or suspected enteroviral meningitis cases, PCR sensitivity ranges from approximately 70% to greater than 90%.[2] The overall sensitivity of virus isolation from the CSF of patients with viral meningitis is typically 30% to 35%,[2,25–28] although higher figures have been reported during some E outbreaks.[12,29] Concomitant testing of serum, upper respiratory secretions, urine, and stool enhances the likelihood of virus detection by either PCR or cell culture.[2,30,31]

Differential Diagnosis

Bacterial meningitis is the most important disease to be distinguished from EV aseptic meningitis. Although some clinical features of bacterial meningitis that is incompletely treated with antibiotics may overlap those of EV aseptic meningitis, when therapy has been instituted before lumbar puncture, several studies have demonstrated that pretreatment of bacterial meningitis alters the CSF minimally. Even when some laboratory indicators are altered by therapy (i.e., change from polymorphonuclear to lymphocytic pleocytosis), others continue to indicate bacterial disease (i.e., low glucose or high protein concentration).[32–34] Arboviruses, lymphocytic choriomeningitis virus, leptospirosis, Lyme borreliosis, and acute human immunodeficiency virus infection account for most of the remaining cases of infectious aseptic meningitis. Mumps virus infection was a common cause of aseptic meningitis before the introduction of mumps vaccine in the United States. Aseptic meningitis also occurs with other infectious and noninfectious diseases (see Chapter 88), but the etiology is usually suggested by other clinical features.

Management and Prognosis

Although hospitalization is not necessary for all cases in adolescents and adults and, indeed, may not be feasible during summer epidemics of EV infections, it is advisable when disturbances in consciousness, muscle weakness, or a petechial or purpuric rash suggest the possibility of a more serious illness. Pyogenic bacterial meningitis should be excluded by lumbar puncture. When bacterial meningitis cannot be excluded because of prior antibiotic treatment, administration of appropriate antibiotics is advisable after performing Gram stains and bacterial cultures. CSF EV NAAT may be useful in deciding whether to continue administration of antibiotics and hospitalization if the test can be reported within 1 to 2 days.[35,36]

In most cases treatment consists only of relief of symptoms. Analgesics are usually given to older children and adults to alleviate headache and lassitude, and easy fatigability may be present for weeks after acute illness.[8] Pleconaril, an experimental orally administered EV capsid-stabilizing drug, modestly reduced the duration of headache and other symptoms in clinical trials but has not been further developed for this purpose.[11] Treatment studies of infants and young children, who generally experience a shorter duration of symptoms, have been inconclusive.[37]

In one large study of EV aseptic meningitis, subtle disturbances in motor function (limitation of passive motion, muscle spasm, and poor coordination) were observed during convalescence.[38] These abnormalities slowly resolve and are rarely detectable 1 year after infection. In young children fever and signs of meningeal irritation subside in a few days to 1 week. Infants younger than 3 months may have fewer symptoms of illness and fewer complications than older infants.[6] Although some investigators have suggested that EV meningitis in the first year of life may result in permanent neurologic sequelae,[39,40] studies of larger numbers of children using more rigorous methods indicate that the long-term prognosis for the youngest infants is also excellent.[41,42]

Encephalitis

Encephalitis is a well-documented manifestation of nonpolio EV central nervous system (CNS) infection. In industrialized nations the EVs account for less than 5% of all encephalitis cases but may contribute to a larger proportion in developing nations.[25,43–46] EVs account for 11% to 22% of encephalitis cases that are proved to be viral.[25,43–46] Numerous serotypes have been implicated as causes of encephalitis; CV-A9 and CV-A16, CV-B2 and CV-B5, E-6 and E-9, and EV-A71 are the serotypes reported most often, but the evidence linking each of these serotypes to encephalitis is highly variable. A notable exception is that of EV-A71, where it has unambiguously been linked to encephalitis. In a minority of cases a specific etiology has been proved by isolating virus or detecting its genome in brain tissue or CSF; in others the cause of encephalitis has been inferred by isolating virus from a nonneurologic site or by serology.

In perinatally acquired EV infection, encephalitis is often only one manifestation of generalized viral disease, but beyond the neonatal period, signs and symptoms are generally limited to the CNS. Children and young adults are most frequently affected. CNS disease varies from relatively mild encephalopathic symptoms in patients with EV meningitis to severe generalized encephalitis with seizures, paresis, and coma. Children with focal encephalitis present with partial motor seizures, hemichorea, and acute cerebellar ataxia,[47–49] features that in some cases have suggested a diagnosis of herpes simplex virus (HSV) encephalitis.[50,51]

EV-A71 and, rarely, other EV serotypes are the cause of a severe, often fatal form of brainstem encephalitis (rhombencephalitis) with secondary cardiopulmonary manifestations, including noncardiogenic pulmonary edema.[51–54] EV-A71 encephalitis has a strikingly high prevalence in countries of the Asia–Pacific Rim. Multiple large outbreaks have occurred in this region of the world in the last 10 to 15 years.[55] The disease affects principally infants and toddlers. CNS disease is usually preceded by hand-foot-and-mouth disease (HFMD) or herpangina.[51,53] In addition to the neurologic signs of encephalitis mentioned previously, myoclonus occurs frequently.[55] The use of glucocorticoids and/or pyrazolones may be risk factors for the development of life-threatening disease.[56]

The CSF findings in EV encephalitis are similar to those in aseptic meningitis. Magnetic resonance imaging (MRI) of the brain and electroencephalography may demonstrate either generalized or localized abnormal signals, reflecting the extent and severity of brain involvement. Most patients with CV and E encephalitis beyond the neonatal period recover fully, although permanent neurologic sequelae and rare deaths occur.[25,54,57–59] EV-A71 encephalitis may be associated with such sequelae as limb atrophy and weakness, as well as long-term behavior problems in children.[52,60]

Acute Flaccid Paralysis/Myelitis and Other Neurologic Complications

The clinical presentation of acute flaccid paralysis (AFP) mimics that of poliomyelitis. For some EV serotypes the link with AFP has been established through detection of the virus or its nucleic acid in the CSF or CNS tissue of affected individuals.[61,62] In the majority, however, the link is inferential by viral detection in stool, respiratory tract, or by an increase in virus-specific antibodies.[63–66] The detection of AFP for monitoring the progress of polio eradication has led to an increase in the identification of serotypes inferentially associated with this syndrome. EV-A71 has been associated with large outbreaks of AFP in Russia, Eastern Europe, Thailand, and Taiwan.[52,67,68] CV-A7, which is neuropathogenic in monkeys, has also been associated with outbreaks of AFP.[69,70] Sporadic cases of AFP have been associated with multiple serotypes from EV-A, EV-B, and EV-C spp., in decreasing order of frequency.

Recently, a cluster of acute flaccid myelitis (AFM) temporally associated with an outbreak of EV-D68 respiratory disease characterized by asymmetrical limb weakness/paralysis, bulbar weakness, and cranial nerve dysfunction drew an inferential link between the two.[71] Nationwide

surveillance for cases of AFP further pointed to a possible association between EV-D68 and AFP.[72] Although EV-D68 has rarely been identified from the CSF, evidence for the causality of EV-D68 and AFP was further supported by the Bradford Hill criteria.[72] As a result of the bulbar weakness and paralysis of the muscles of respiration, inability to protect the airway and respiratory failure secondary are frequently seen. EV-D68 is rarely isolated from the CSF of cases of AFM but can be found in the nasopharynx and stool of infected individuals. Cytochemical analysis of the CSF demonstrates a lymphocytic pleocytosis in the majority of cases.[72] CSF protein and glucose concentrations are generally increased and normal, respectively. MRI findings included longitudinally extensive nonenhancing lesions of the gray matter in the spinal cord or brainstem,[71–73] the cervical spinal cord being the most commonly involved segment. Electrodiagnostic studies of affected limbs demonstrate motor neuropathy or neuronopathy without sensory abnormalities.[74] The majority of patients with AFM will experience persistent sequelae.[71,72,74] A national survey of AFP documented that only 5% of respondents reported complete recovery of strength, and only 18% were fully functional.

Treatment of EV-D68 associated AFM is supportive. Antiviral agents that inhibit EVs, such as pleconaril, pocapavir, and vapendavir, do not have in vitro antiviral activity against EV-D68. Fluoxetine, a selective serotonin reuptake inhibitor, has been shown to be active in vitro against EV-B and EV-D spp., including EV-D68, failed to show activity against EV-D68 in a murine model[75,76] (also see Chapter 48).

Paralytic disease caused by the nonpolio EVs, other than EV-A71 and EV-D68, is characteristically less severe than poliovirus-associated paralysis.[77,78] Prodromal fever or presence of fever at the time of onset of paralysis and residual paralysis or atrophy are less frequently encountered in paralytic disease due to the nonpolio EVs. Muscle weakness is more common than flaccid paralysis.

Cranial nerve involvement has occasionally resulted in complete unilateral oculomotor palsy.[79,80] Guillain-Barré syndrome has been reported in a small number of patients in association with CV-A2, CV-A5, and CV-A9 and with E-6 and E-22.[26,81,82] In a few cases the implicated virus has been isolated from CSF or the brainstem.[82] Transverse myelitis caused by CV-B4, CV-A9, and E-18, and has been reported in patients who had rises in neutralizing antibody and in patients who had E-5 and CV-B5 recovered from CSF.[83,84] Systemic CV-B2 disease has been reported with many of the clinical features of Reye syndrome.[85] Furthermore, several children with well-documented Reye syndrome have had a variety of EVs isolated concurrently from multiple sites, including the brain and CSF.[86,87] However, a clear etiologic or epidemiologic link between EV infection and Reye syndrome has not been established. Opsoclonus-myoclonus, or the "dancing eyes" syndrome, has been reported in two children with concurrent CV-B3 infection and in an adult with EV-A71 infection.[88,89]

EXANTHEMS

CVs and Es cause a variety of exanthems, which are sometimes associated with enanthems. With the exception of HFMD, these rashes are not sufficiently distinctive to permit a reliable etiologic diagnosis on clinical grounds alone. Virus can be isolated from the vesicular lesions of patients with HFMD, and therefore these lesions appear to be a direct result of viral invasion of the skin after viremia. No attempts at isolation of virus from the skin in cases of maculopapular and petechial exanthems have been reported; in consequence, it is not known whether these lesions are also caused by the virus directly or by immunopathologic mechanisms.

EV exanthems themselves cause little morbidity. They are important as sentinels of the prevalence of CVs and Es in the community and because they are often confused with other infective exanthems, some of which have more serious implications. Rashes caused by EVs may be grouped according to the type of exanthem that they mimic: rubelliform or morbilliform, roseoliform, vesicular, or petechial. Some overlap between these types of exanthems may be observed in different patients infected with the same EV or even among different morphologic lesion types in the same patient.

Rubelliform and Morbilliform Exanthems

Overall, EVs account for about 5% of acute morbilliform exanthems that occur in populations with high measles and rubella vaccine coverage.[90] Maculopapular rashes resembling rubella commonly occur during summer E epidemics. High attack rates have been noted with E-9, the most common serotype associated with rubelliform rash. In one epidemic 57% of persons younger than 5 years with illness caused by E-9 had rash, 41% of those 5 to 9 years of age had rash, but rash affected only 6% of those older than 10 years.[29] The rash, which characteristically appears simultaneously with fever, begins on the face and then spreads to the neck, chest, and extremities. The illness may be distinguished from rubella by the absence of pruritus and posterior cervical lymphadenopathy.[91,92] In occasional patients with an enanthem resembling Koplik spots and a blotchy eruption, the disease may be confused with measles, but the coryza and conjunctivitis characteristic of that disease are absent.[93]

Roseoliform Exanthems

These EV exanthems are distinctive, not in their appearance but in their timing; as in roseola, the rash does not appear until defervescence. The prototype is the "Boston exanthem," the first of the EV exanthems to be recognized and now known to be caused by E-16.[94,95] Multiple cases often occur sequentially in families, with rash developing in as many as one-fourth of the children in a household who are mildly ill with low-grade fever and pharyngitis. The fever lasts 24 to 36 hours and then declines simultaneously with the appearance of discrete, nonpruritic, salmon-pink macules and papules about 1 cm in diameter on the face and upper part of the chest. The extremities are less commonly involved. The duration of the rash is 1 to 5 days. Other EV serotypes (CV-B1 and CV-B5, E-11 and E-25) have also been associated with roseola-like illness.[94,96,97] Exanthem subitum (roseola infantum), a common nonseasonal exanthem in which the rash typically develops as the fever declines, is caused by human herpesvirus 6 (see Chapter 139).

Herpetiform Exanthems

Hand-Foot-and-Mouth Disease

HFMD is primarily associated with EV-A serotypes (CV-A4, -A5 to -A7, -A10, -A16, -A24; EV-A71), although EV-B serotypes (CV-A9, CV-B2 to CV-B5; E-4, E-18, E-19) are also associated with sporadic cases.[51,53,98–101] CV-A16 and EV-A71 are the most common causes of this distinctive vesicular eruption, also known as vesicular stomatitis with exanthema (Fig. 172.1A–C). In Southeast Asia the latter serotype has caused large outbreaks associated with severe CNS disease and deaths.[51–53] In recent years reports of outbreaks of HFMD caused by CV-A6 and CV-A10 have come from Europe, Asia, and the United States.[102–105]

Children younger than 10 years are often affected, and spread to other family members occurs commonly. Most patients complain of



FIG. 172.1 Hand-foot-and-mouth disease caused by enterovirus A71 in a young child. *(From Goksugur N, Goksugur S. Hand, foot, and mouth disease.* N Engl J Med. *2010;362:e49.)*

sore throat or sore mouth, and affected young children may refuse to eat. Temperatures of 38°C to 39°C last 1 to 2 days and are accompanied in essentially all cases by vesicles in the oral cavity, occurring chiefly on the buccal mucosa and tongue. Several lesions may coalesce to form bullae and ulcerate. Peripherally distributed cutaneous lesions occur in roughly 75% of patients, commonly on the extensor surfaces of the hands and feet and sometimes on the buttocks or genitalia.[106] The lesions are tender and consist of mixed papules and clear vesicles with a surrounding zone of erythema. Skin biopsy demonstrates subepidermal lesions with a mixed lymphocytic and polymorphonuclear inflammatory response and acantholysis of the overlying epidermis.[107] Eosinophilic nuclear inclusions and intracytoplasmic picornavirus particles can be seen microscopically within cells surrounding dermal vessels.[108]

The vesicular lesions of HFMD disease superficially resemble those caused by herpes simplex or varicella-zoster virus (VZV). Patients with HFMD invariably have lesions of the oral mucosa. In contrast, oral lesions are less common in patients with chickenpox; moreover, these patients generally appear more ill, and their cutaneous lesions are more extensive and centrally distributed, generally with sparing of the palms and soles. Patients with primary herpetic gingivostomatitis also usually appear more ill and have a higher fever and cervical lymphadenopathy; lesions are usually confined to the oral cavity and do not involve the extremities. The enanthem of herpangina also resembles HFMD, but it occurs in the posterior oropharynx and typically involves the fauces and soft palate.

An atypical form of HFMD, caused by a novel CV-A6 strain, has been observed since 2008 in multiple locations globally and is characterized by a wider distribution of skin lesions that enlarge and vesiculate, especially in areas of eczematous skin.[104,105,109,110] Generalized varicella form–like eruptions have also been reported in association with CV-A6 infection.[111] The acute disease is associated with a higher rate of hospitalization than typical HFMD, but it has an invariably benign outcome, with the exception of onychomadesis (loss of fingernails), which occurs in many cases 1 to 2 months after the infection. Generalized vesicular eruptions occurring in crops of lesions are also reported in association with CV-A9[112] and E-11 infection, and one case with disseminated lesions has been described in an infant with preexisting atopic eczema; the condition was given the sobriquet "eczema coxsackium," by analogy with eczema herpeticum and eczema vaccinatum.[113,114] Unlike HSV and VZV infection, the vesicles do not evolve to form pustules and scabs. Vesicular eruptions caused by E-11 have occurred in immunocompromised adult patients.[114] An acute eruption resembling dermatomal zoster, in which E-6 was isolated from the bullous lesions, has been reported.[115]

Petechial Exanthems and Other Cutaneous Manifestations

Petechial and purpuric rashes have been described with E-9[29,116] and CV-A9[117] infections. When these rashes have a hemorrhagic component, the illness is easily confused with meningococcal disease, especially if aseptic meningitis occurs simultaneously. On occasion, cutaneous eruptions of CV-A9 disease have an urticarial nature.[112] One child was reported to have papular acrodermatitis (Gianotti-Crosti syndrome) in association with CV-A16 infection.[118]

ACUTE RESPIRATORY DISEASE (EV-D68)

EVs account for most viruses recovered from children with summertime upper respiratory tract infections, including undifferentiated febrile illnesses ("summer grippe") with sore throat and on occasion cough or coryza.[119,120] EV upper respiratory tract illnesses are generally clinically indistinguishable from disease caused by rhinoviruses, *Mycoplasma pneumoniae* and other respiratory tract agents, unless accompanied by aseptic meningitis, exanthem, or other clinical features suggesting EV infection.

Many EV serotypes are associated with upper respiratory tract disease. Among the best-characterized EV respiratory viruses are CV-A21 and CV-A24, which produce illness resembling the common cold, except for a higher incidence of fever.[121,122] Outbreaks of CV-A21 illness are reported in military populations. Although epidemics in civilians have not been recognized, sporadic infections presumably account for the observed high antibody prevalence rates in the general population.[121] Unlike most EVs, CV-A21 is more readily recovered from throat swabs than from feces. In volunteers receiving small-particle aerosols of the virus, illness has included not only coryza and sore throat but also tracheobronchitis and pneumonia.[123] EV-D68 emerged in 2008–10 as a cause of clusters of upper and lower respiratory tract disease in Europe, Asia, and the United States.[124–129] EV-D68 has been isolated primarily from respiratory specimens during the autumn months. Cases have been reported primarily in children but also occur in adults.[130] Symptoms include no to low-grade fever, cough, wheezing, shortness of breath, dyspnea, tachypnea, retractions, and hypoxia.[130] Clinical syndromes associated with infection have included bronchiolitis, bronchitis, encephalitis, and pneumonia. In some, disease has been severe enough to warrant admission to the intensive care unit.[131] Deaths have been reported.[130] In 2014 the United States experienced a nationwide outbreak of EV-D68. From mid-August 2014 to January 2015, the Centers for Disease Control and Prevention (CDC) or state public health laboratories reported 1153 cases with respiratory illnesses caused by EV-D68 in 49 states and the District of Columbia.[132] Almost all of the confirmed cases were in children, many of whom had asthma or a history of wheezing. There were likely many thousands of mild EV-D68 infections in individuals who did not seek medical attention and who were not tested for EV-D68. Of the 2600 specimens sent to the CDC for EV testing, approximately 36% tested positive for EV-D68, and 33% were positive for another EV or for rhinoviruses.[130] Serious respiratory disease has also been reported in EV-D68 infection in adults,[130] including infections in patients with hematalogic malignancies.[133] The clinical syndromes seen in the 2014 outbreak of EV-D68 were primarily respiratory illnesses, typical of those seen in earlier outbreaks.

The CDC used a "real-time" reverse-transcriptase PCR that identifies all strains of EV-D68 that circulated in 2014. Genomic sequence of 2014 strains of EV-D68 showed that they are related to strains that circulated previously in the United States, Europe, and Asia.[132] The majority of strains in the 2014 outbreak were classified as clade B, but interclade variations were also seen, which led to identification of strains that comprised a new clade D among EV-D68 strains.[134]

E serotypes 4, 8, 9, 11, 20, 22, and 25 are also common causes of respiratory disease.[121] E-11 produces sore throat, coryza, and cough and has also been associated with croup. The spectrum of CV-B respiratory disease includes coryza, laryngotracheobronchitis, bronchiolitis, and pneumonia.[81,135] Pneumonia, which may be interstitial or a patchy bronchopneumonia, has occurred in children[136] and rarely in adults.[137] Severe lower respiratory tract EV infections are uncommon, although some EVs, notably E-6, -9, -11, and -33 and EV-A71, have been isolated after death from infants and young children with severe pneumonia.[5,53,138–140]

Herpangina

Herpangina (herpes: vesicular eruption; angina: quinsy, or inflammation of the throat) is a well-characterized vesicular enanthem of the fauces and soft palate that is accompanied by fever, sore throat, and pain on swallowing (Fig. 172.2). Despite the name, it has no relationship to HSV. Summer outbreaks of herpangina typically affect children age 3 to 10 years and, less commonly, adolescents and young adults. CV-A1 to -A10, -A16, and -A22 have been the most common viruses recovered from herpangina patients. Other serotypes include CV-B1 to CV-B5; CV-A6; E-3, -6, -9, -16, -17, -25, and -30; and EV-A71.[51,54,141]

Clinical Manifestations

Herpangina begins suddenly with fever, vomiting, myalgia, and headache. Sore throat and pain on swallowing are prominent symptoms that precede appearance of the enanthem by several hours to a day. Examination of the throat reveals erythema and mild exudate of the tonsils and the characteristic enanthem, which begins as punctate macules and evolves over a 24-hour period to 2- to 4-mm erythematous papules that vesiculate and then ulcerate centrally. The moderately painful lesions, which are usually small in number, are located on the soft palate, uvula, and less commonly, on the tonsils; the posterior pharyngeal wall; or the buccal mucosa. The fever subsides in 2 to 4 days, but the ulcers may persist



FIG. 172.2 Herpangina on the soft palate in a teenager with severe throat pain. *(From Cohen J, Powderly WG:* Infectious Diseases. *2nd ed. St Louis: Mosby; 2004.)*

for up to a week. Patients with herpangina do not appear very ill and require only symptomatic treatment for sore throat.

A variant of the herpangina, termed acute lymphonodular pharyngitis, has been described in association with CV-A10 infection.[142] Lesions occur in the same distribution as herpangina but consist of tiny nodules of packed lymphocytes that eventually recede without undergoing vesiculation or ulceration.

Differential Diagnosis

Herpangina is most often confused with bacterial tonsillitis or other viral causes of pharyngitis, herpetic gingivostomatitis, HFMD, and aphthous stomatitis. HSV gingivostomatitis occurs in the anterior oral cavity, especially on the inner aspects of the lips, the buccal mucosa, and the tongue. Gingivitis, prominent systemic toxicity, and cervical lymphadenitis are additional features of primary herpes simplex infection that are not seen in herpangina. In HFMD lesions also occur on the extremities in most cases. Aphthous stomatitis is characterized by recurrent large ulcerative lesions of the lips, tongue, and buccal mucosa among older children, adolescents, and adults.

MYOSITIS

Pleurodynia

Pleurodynia (Bornholm disease, "devil's grippe") is an acute EV infection of skeletal muscle, characterized by fever and sharp, spasmodic pain in the chest or upper part of the abdomen, which has been usually observed during infrequent outbreaks. Pleurodynia was first described in 1872 in Norway by Daae and by Homann as an outbreak of "acute muscular rheumatism spread by contagion." Subsequent reports from Scandinavia included a paper by Ejnar Sylvest, a Danish general practitioner, in 1933, in which he described his experience with the disease on the island of Bornholm in the Baltic Sea. This monograph received worldwide attention after it was translated into English in 1934,[143] and little has been added to Sylvest's descriptions of the disease and its epidemiology, pathogenesis, and complications. The etiologic role of CV-B, the most important cause of epidemic pleurodynia, was established in 1949.[144,145] Other agents rarely implicated in pleurodynia include E-1, -6, -9, -16, and -19 and CV-A4, -A6, -A9, and -A10.[146,147]

Epidemiology

Published reports of major epidemics have come primarily from Europe and North America. These epidemics have been reported at infrequent intervals, often 10 to 20 years, and attack rates have been higher in sparsely populated areas than in cities. Persons with pleurodynia are somewhat older than those with most other diseases caused by CVs and Es. Multiple family members may be attacked almost simultaneously or in rapid succession, separated by several days.

Pathogenesis

Although pleurodynia probably results from direct viral invasion of thoracic and abdominal muscles after viremia, direct virologic evidence supporting this hypothesis is lacking. Tenderness mimicking spontaneously occurring pain can be elicited by pressure on affected muscles in most cases; in addition, palpable, often visible muscle swelling is a subtle finding in some cases.[143] A pleural friction rub has been rare or absent in most epidemics, although this sign has occasionally been noted in 7% or greater of patients.[148,149]

Clinical Manifestations

Pleurodynia begins abruptly with an onset of spasmodic pain, typically over the lower part of the rib cage or the upper abdominal region. Fever, sore throat, and headache may occur, but cough and coryza are notably absent. Aseptic meningitis and orchitis occur in a small number of patients with pleurodynia, in general less than 10%.[143,150,151] Pericarditis and pneumonia are rare.[148]

The pain, which varies in intensity, is variously described as lancinating, stabbing, constricting, or viselike. Patients asked to localize the pain are likely to indicate a broad area with the palm of the hand rather than a specific point with the finger. The most common location is the vicinity of the costal margin on one or both sides or occasionally the subxiphoid region. About half the patients have pain primarily in muscles of the thorax, especially the intercostals, the trapezius, and occasionally the erector spinae or pectoralis major. In the remainder pain is primarily in the upper part of the abdomen, especially the hypochondrium (internal and external obliques and transversus abdominis) or the epigastrium (rectus abdominis). Periumbilical pain and pain in the lower abdominal quadrants are also seen, especially in children, in whom abdominal localization of pain is the rule.[122,149] A few patients experience pain in neither the chest nor the abdomen but instead in the neck or limbs[150]; in these cases the diagnosis can be made only by association with other typical cases in the family. Whatever the localization of the pain, it is usual for an individual patient to experience this pain in only one or two areas of the body.

Although the location and severity vary, it is the spasmodic and paroxysmal character of the pain that is its hallmark. If the pain is mild and the patient ambulatory, the patient stoops forward or leans to the side to splint the chest. With more severe pain, the patient lies still in bed and appears acutely ill and apprehensive. Motion produces pain, and patients resist being turned in bed. Chest pain limits deep inspiration, and respirations are shallow and rapid. Auscultation of the chest reveals no abnormalities. Pain can be elicited by pressure on the involved muscles in most patients. Swelling is seen or felt only occasionally and by careful, sequential observations.

Most patients are ill for 4 to 6 days. Children have milder disease than adults, who are often confined to bed. The first paroxysm is the most severe, and subsequent paroxysms are shorter and accompanied by less fever. Although dull aching of involved muscles usually persists between bouts of sharp pain, the patient may look and feel entirely healthy between paroxysms. About one-fourth of patients experience multiple recurrences, often after they have been free of pain for a day or more and have felt well enough to return to work or school.[143,150] In about half of these persons recurrence of pain is at the same site; in the remainder a new site is attacked. Late relapses occur in some patients after they have been free of symptoms for a month or longer.[150]

Diagnosis

The severity, location, and other characteristics of the pain are so protean that pleurodynia is readily confused with other illnesses. Pain in the chest may mimic pneumonia, pulmonary infarction, myocardial ischemia, and the preeruptive phase of zoster. Abdominal pain in epidemic pleurodynia may resemble that in acute abdomen of a variety of causes. Normal auscultatory examination of the chest, together with the characteristic spasmodic and relapsing character of the pain, is helpful in excluding pneumonia. A negative chest radiographic film is also helpful, although pleural effusions may rarely be present.

Management and Prognosis

Analgesics and the application of heat to affected muscles are useful in relieving pain in most cases; in some, opiate analgesics are required for adequate pain control. Despite the distressing tendency of the disease to relapse, all patients eventually recover completely. Debility out of all proportion to the apparent severity of the illness is occasionally observed for several months during convalescence.[143,151]

Other Skeletal Myositis

EVs have been implicated as a cause of acute myositis, not confined to the torso in some patients,[152–159] although the diagnosis has rarely been proved virologically. E-11 has been recovered from clinically involved skeletal muscle of a 3-month-old infant with a fatal systemic infection.[152] In other cases CV-A9, CV-B2 and CV-B6, and E-9 have been etiologically linked to myositis on the basis of serology, recovery of virus from the throat or feces, or demonstration of viral antigen in muscle by immunofluorescence. Both generalized polymyositis and focal myositis have been noted, the latter sometimes localized to the thighs. Clinical myositis is manifested by fever, chills, weakness, hypotonia, tenderness, and edema of the involved muscle groups. Myoglobinemia, myoglobinuria, and an elevated creatine phosphokinase level are often found. Most reported patients have recovered rapidly.

A dermatomyositis-like illness occurs in B-cell–deficient immunocompromised patients with persistent EV infections (see later).

MYOPERICARDITIS

Because EVs rarely, if ever, infect the pericardium alone without involving the subepicardial myocardium, the term *myopericarditis* best describes the disease caused by these viruses when they affect the heart (see Chapter 84).[160] In the clinical setting, however, signs of either myocarditis or pericarditis often predominate. In older children and adults the severity of myopericarditis varies from asymptomatic cardiac involvement to severe disease with intractable heart failure and death. Myocarditis that occurs with generalized EV infection in the newborn is discussed separately in the section on neonatal infections.

An epidemic of CV-B5 myopericarditis occurred in Finland in autumn 1965, when 18 patients were admitted to a single hospital.[161] Epidemic myopericarditis appears to be exceptional, however, and most reported cases beyond the neonatal period have been sporadic, probably because involvement of the heart is a relatively uncommon manifestation of illness even during substantial EV epidemics.

Etiology and Pathogenesis

EVs account for at least one-third of all cases of acute viral myopericarditis.[143,162,163] However, the strength of the evidence linking a given EV serotype with myopericarditis varies considerably. Proof of causation exists for all CV-B serotypes; CV-A4 and CV-A16; and E-6, -7, -9, -13, and -22 by demonstration of infectious virus, viral genome, or viral antigen in the myocardium or pericardial fluid.[164–168] The evidence is less substantive for CV-A1, -A2, -A5, -A8, and -A9 and E-1 to -4, -7, -8, -11, -14, -19, -25, and -30.[163,165–167,169–178] These serotypes have been recovered from noncardiac sources during an episode of acute myopericarditis, some with a significant increase in neutralizing antibody titer to the isolated virus.

Many other viruses and bacteria have been associated with myopericarditis, although adenovirus,[179,180] influenza A virus,[162,181] parvovirus B19,[162,182] mumps virus,[183] cytomegalovirus,[162] HSV,[162] respiratory syncytial virus,[162] Epstein-Barr virus,[162] and vaccinia virus[184] are the principal non-EV agents that have been detected directly in pericardial fluid or myocardial tissue. The weight of clinical evidence suggests that *M. pneumoniae*, VZV, and measles virus also cause myopericarditis.

CV-B and other EVs reach the heart during the viremia that follows replication in the gastrointestinal (GI) or respiratory tract (see Chapter 170). Experimental studies in a murine model demonstrate that virus replication of myocytes results in scattered myocyte necrosis, followed by focal infiltration of polymorphonuclear leukocytes, lymphocytes, plasma cells, and macrophages.[185] The chronic inflammatory response that may persist for weeks to months has been a subject of widespread interest. Some investigators have demonstrated the presence of EV RNA in biopsy samples of cardiac tissue for months after the acute episode, whereas others have reported negative results from similar experiments. Other investigators consider the late-phase inflammatory response to be due to virus-induced, cytotoxic T-lymphocyte destruction of myocytes,[186] whereas still others have postulated the development of a myocardial neoantigen or cross-reactivity between viral and myocardial cell antigens.[187] Healing is accompanied by a variable degree of interstitial fibrosis and evidence of myocyte loss.

Clinical Manifestations

EV myocarditis occurs at all ages but has a special predilection for physically active adolescents and young adults. The incidence in males is at least twice that in females.[163] In two-thirds of cases an upper respiratory tract illness precedes the onset of cardiac manifestations by 7 to 14 days.[163] Common initial symptoms include dyspnea, chest pain, fever, and malaise.[161,163,188–190] Pain in the precordial area is usually dull, but it may resemble angina pectoris or be sharp, pleuritic, and exacerbated by recumbency when pericarditis is present. A pericardial friction rub, often transient, has been observed in 35% to 80% of cases. Enlargement of the cardiac silhouette on chest radiograph films, present in about 50%, may be due to either pericardial effusion or cardiac dilation. A gallop rhythm and other signs of frank congestive heart failure are observed in roughly 20%.[189,190]

Electrocardiographic abnormalities, including ST-segment elevation or nonspecific ST-segment and T-wave abnormalities, are invariably present. More severe myocardial disease leads to the development of Q waves, ventricular tachyarrhythmias, and all degrees of heart block. Echocardiography may confirm the presence of acute ventricular dilation or a diminished cardiac ejection fraction. Serum levels of myocardial enzymes are frequently elevated. Other clinical manifestations of systemic EV disease sometimes occur with myopericarditis and include aseptic meningitis, pleurodynia, hepatitis, and orchitis. Cardiac MRI has been shown to be useful in the diagnosis of myocarditis.[191]

Acute myocardial infarction associated with chest pain, arrhythmias, and congestive heart failure may be difficult to distinguish from myopericarditis. Patients suspected of having acute myocardial infarction sometimes have evidence of concurrent CV-B infection,[192–194] and focal myocarditis has been proved in at least one case of acute CV-B5 infection.[195] Some patients presenting with suspected myocardial infarction who have normal coronary angiographic studies have been shown to have myocarditis by radiolabeled antimyosin antibody cardiac scanning.[196]

Diagnostic Virology

Although CVs have been isolated on numerous occasions from pericardial fluid or heart muscle at autopsy or by open biopsy procedures,[197] in practice, these specimens are rarely available. Cardiac tissue infrequently yields a viral isolate when cultured. The likelihood of a positive PCR result may depend on the number of potential viral pathogens included in the assay.[160,182,198,199] In the absence of identification of virus in cardiac tissue, the diagnosis often rests on circumstantial evidence provided by recovery of the agent from the oropharynx or feces or on serologic evidence of recent infection by a CV-B.

Management

Supportive treatment consists of bed rest, pain relief, and medical management of arrhythmias and heart failure.[200] Although one study reported improved cardiac function and a trend toward increased survival for children with acute myocarditis who received intravenous immune globulin (IVIG), compared with historical controls,[201] randomized trials of immunosuppressive therapy, including IVIG, prednisone, azathioprine, and other drugs, have failed to show any consistent treatment effect.[202–204] In cases of fulminant myocarditis with severe heart failure refractory to medical management, extracorporeal membrane oxygenation or use of a ventricular assist device may be beneficial in recovery.[205,206]

Course and Prognosis

Persistent electrocardiographic abnormalities (10%–20%), cardiomegaly (5%–10%), and chronic congestive heart failure are indications of permanent myocardial injury that occur overall in about one-third of adult patients identified with acute myopericarditis; these abnormalities

may ultimately lead to a diagnosis of dilated cardiomyopathy.[163,189,190] Chronic constrictive pericarditis has occurred after intervals of 5 weeks to 1 year.[207–09]

The prognosis for children with acute myocarditis is better than for adults. Less than 15% of children die during the acute illness from intractable heart failure or uncontrolled arrhythmias, and fewer than 10% develop persistent or recurrent compromise from dilated cardiomyopathy requiring cardiac transplantation.[210] The risk for developing long-term cardiac sequelae may be higher in children with less severe acute myocarditis.[211]

Dilated Cardiomyopathy

Chronic dilated cardiomyopathy, which is second only to ischemic heart disease as a cause of chronic congestive heart failure, is the final result of multiple infectious and noninfectious cardiac insults,[212] including up to one-third of cases of acute myopericarditis and, in some instances, unrecognized past EV infection.[163,189,190] Persistence of EV in cardiac tissues has been proposed as a mechanism for the development of chronic dilated cardiomyopathy.[213] Some investigators have detected positive- or negative-strand EV RNA or EV protein in cardiac tissue months to years after the onset of dilated cardiomyopathy.[203] However, others using similar methods have not.[214–216]

COXSACKIEVIRUS AND ECHOVIRUS DISEASE IN THE NEWBORN

The human neonate is uniquely susceptible to EV disease. Although many EV serotypes cause the same self-limited clinical syndromes in neonates as in older persons (e.g., aseptic meningitis, exanthems), some serotypes are capable of producing fulminant, frequently fatal disease in the newborn infant. CV-B1 to CV-B5 and E-11 are most frequently associated with overwhelming systemic neonatal infections. Rare cases of serious neonatal disease are reported with CV-A3, CV-A9, and CV-A16.[217–219]

Epidemiology

Although most neonatal EV infections are directly transmitted from the mother, some infections are acquired by a nosocomial route. The first description of CV-B disease in newborn infants followed outbreaks occurring in nurseries in South Africa, Zimbabwe, and the Netherlands.[220] Numerous nursery outbreaks of neonatal E infection have been recorded, with the severity of neonatal disease varying according to the viral serotype.[221,222] Introduction of infection into the nursery has been traced to an infected parent or to hospital personnel. Infant-to-infant spread within nurseries probably occurs by the hands of personnel engaged in mouth care, gavage feeding, and other activities requiring close direct contact.[223]

Because most neonatal EV infections are sporadic rather than nosocomial, the incidence and severity of neonatal EV infection generally reflect the occurrence of EV disease in the community. Although many cases occur sporadically during the enterovirus season, clusters of vertically transmitted neonatal infection sometimes occur during community outbreaks with a single EV serotype.[224,225] During the 20-year period between 1983 and 2003, CV-B1 to CV-B4, E-11, and E-25 were reported to the CDC significantly more commonly among neonates than among persons older than 1 month.[226] In 2007 and 2008 a markedly increased number of cases of serious neonatal disease caused by CV-B1 were reported to the CDC, reflecting both widespread circulation of this virus nationally and a higher attack rate in infants, compared with other circulating enteroviruses.[227]

Pathophysiology

Most newborns with life-threatening EV disease are infected by vertical transmission from the infected mother in the perinatal period.[221,222] About 60% to 70% of women who give birth to infected infants have a febrile illness during the last week of pregnancy.[13,221] Experimental evidence indicates that the fetus is relatively protected by the placenta during maternal infection,[222,228] but the newborn has a high risk for infection,[141,229] perhaps as a result of exposure to either virus-positive cervical secretions[230,231] or viremic maternal blood.[232] Although most vertically transmitted EV infections are probably acquired during delivery, some infants are infected before delivery, as evidenced by the recovery of virus from cord blood[230] and the development of disease within the first 2 days of life.[221,232]

In the infected neonate EV spread systemically through the bloodstream. Tropism for and replication within specific organs of the neonatal host depend on both virus and host factors. Experimental evidence suggests that some neonatal tissues are innately more susceptible to infection with some EV than the corresponding tissues from an adult host.[233] In addition, the neonatal immune system is insufficient to control the replication and spread of virulent EV. Both premature and term human infants respond adequately to EV infection with humoral neutralizing antibody.[234] However, macrophage function, which does not mature sufficiently until several weeks of age in the human neonate, is necessary to limit initial EV replication.[235,236]

The outcome of neonatal infection is also strongly influenced by the presence or absence of passively acquired maternal antibody specific for the infecting EV serotype.[228,229,237] Thus the timing of maternal infection in relation to the development of maternal immunoglobulin G (IgG) antibody and delivery of the infant may be the most critical factor in determining the outcome of neonatal EV infection.

Clinical Manifestations

Symptoms develop in most neonates with generalized EV disease between 3 and 10 days of life.[231,232] A small number have signs of illness in the delivery room or within the first 1 to 2 days of life[221,232]; conversely, the onset of fatal infection has been documented in infants as old as 3 months.[152] Male infants and premature infants are overrepresented among infants with serious illness. Early symptoms are generally mild and nonspecific and include listlessness, anorexia, and transient respiratory distress. Fever may or may not be present. About one-third of patients have a biphasic illness with a period of 1 to 7 days of apparent well-being interspersed between the initial symptoms and the appearance of more serious manifestations.

Generalized EV disease in the newborn most often occurs in one of two characteristic clinical syndromes: either myocarditis or fulminant hepatitis. Neonatal myocarditis, which is often accompanied by encephalitis (encephalomyocarditis syndrome) and sometimes by hepatitis,[238] is characteristically a manifestation of CV-B infection[220,226,239] and, less commonly, E-11 infection.[177,240] Fulminant hepatitis is characterized by hypotension, profuse bleeding, jaundice, and multiple organ failure (hemorrhage-hepatitis syndrome).[226] E-11 is responsible for a large proportion of cases, but well-documented cases of severe hepatitis in neonates have resulted from E-4, -6, -7, -9, -12, -14, -19 to -21, -31, and -33.[221,232–235,237–247]

Myocarditis

Signs of neonatal myocarditis include rapid onset of heart failure; respiratory distress; tachycardia, often exceeding 200 beats/min; cardiomegaly; and electrocardiographic evidence of myocardial injury and arrhythmias. Cyanosis and circulatory collapse develop rapidly in severely affected infants. Fatal cases are often accompanied by disseminated viral infection involving other organs (in order of frequency), such as the CNS, liver, pancreas, and adrenal glands. Most affected neonates are lethargic, and seizures, a bulging fontanelle, and CSF pleocytosis indicate the presence of meningoencephalitis. Enlargement of the liver is more often due to congestive heart failure than to viral hepatitis.

The overall mortality rate for neonatal myocarditis is 30% to 50%, and death usually occurs within a week of onset. Myocardial function rapidly improves in surviving infants after defervescence, generally by 1 week, although in a few infants, convalescence is prolonged for several weeks. Some surviving infants develop dilated cardiomyopathy with ventricular aneurysm formation.[238] Pathologic data are limited to information obtained at postmortem examination. Infants dying of myocarditis have enlarged, dilated hearts, extensive myonecrosis, and a variable degree of cardiac inflammation.

Hepatitis

The initial symptoms of severe neonatal hepatitis are lethargy, poor feeding, and increasing jaundice that progress in many infants, within 1 to 2 days, to jaundice, ecchymoses, bleeding from puncture sites, and

metabolic acidosis. Hepatic transaminases may rise rapidly to extremely high levels, and markedly prolonged prothrombin times and partial thromboplastin times confirm profound hepatic failure.

More than half of infants with severe neonatal E hepatitis die within days after the onset of symptoms despite therapy with blood products and intensive supportive care. Some ultimately fatal cases survive for 2 to 3 weeks with supportive care.[224] Postmortem findings include massive hepatic necrosis and extensive hemorrhage into the cerebral ventricles, pericardial sac, renal medullae, and interstitial spaces of many solid organs.[248] Inflammation is commonly limited to the liver and adrenal glands, with sparing of the heart, brain, meninges, and other organs. The long-term prognosis for surviving infants is not well known, although hepatic fibrosis and chronic hepatic insufficiency develop early in life in some.

Pneumonia

Several cases of EV pneumonia occurring in the first few days of life have been reported, all of them fatal and caused by E-6,[138] E-9,[139] and E-11,[140] and CV-A3.

Diagnosis and Differential Diagnosis

The diagnosis of neonatal CV and E infection depends on detection of viral RNA by PCR or isolation of virus in cell culture. Because virus is usually present in the infected neonate in high titer, isolation from oropharyngeal secretions, feces, and urine is relatively rapid; virus may also be recovered from blood, CSF, ascitic fluid, and multiple tissues obtained at biopsy or autopsy.

Neonatal myocarditis is sometimes mistaken for congenital heart disease because, in both conditions, murmurs and evidence of congestive heart failure may be present. However, fever and electrocardiographic evidence of acute myocardial injury are absent in patients with congenital heart disease. The early features of myocarditis and severe hepatitis resemble those of bacterial sepsis. Because of liver and CNS involvement in either syndrome, visceral dissemination with perinatally acquired HSV in the absence of cutaneous lesions may be suspected.

Management

Management of neonatal EV disease is supportive. Infants in congestive heart failure require judicious fluid management and administration of inotropic agents and diuretics. The profuse bleeding that results from hepatic failure necessitates frequent replacement therapy with packed red blood cells, platelets, and fresh-frozen plasma. Vitamin K should be administered intravenously in pharmacologic doses. Large doses of IVIG, which have been reported to improve outcome in at least one case,[249] may be justified, given the extremely poor prognosis, although there is not reliable evidence of efficacy. A phase II clinical trial using the experimental antipicornavirus drug pleconaril demonstrated shorter times to culture and PCR negativity along with greater survival among pleconaril recipients.[250]

CHRONIC MENINGOENCEPHALITIS IN AGAMMAGLOBULINEMIC AND OTHER IMMUNOCOMPROMISED PATIENTS

The EVs have been responsible for persistent, severe, sometimes fatal infections of the CNS in patients with hereditary (X-linked agammaglobulinemia, common variable immunodeficiency, severe combined immunodeficiency syndrome, and hyper-IgM syndrome)[251–254] or acquired defects in B-lymphocyte function (recipients of bone marrow transplants and of rituximab and obinutuzumab).[255–258] Most cases have been caused by Es; single cases caused by CV-A4, CV-A11, and CV-A15 and by CV-B2 and CV-B3 have been reported.[251,259]

Clinical Manifestations

Nervous system manifestations may be totally absent, or mild nuchal rigidity, headache, lethargy, papilledema, seizure disorders, motor weakness, tremors, and ataxia may be present. These neurologic abnormalities may fluctuate in severity, disappear, or steadily progress. The CSF exhibits lymphocytic pleocytosis and a higher protein concentration than is usually seen in cases of acute EV aseptic meningitis. An EV can be repeatedly recovered from the CSF over a period of months to years, usually in high titer. In some cases virus is isolated only intermittently from the CSF or detected only by PCR. For unknown reasons it is usually more difficult to isolate virus from the feces than from the CSF. Persistent skeletal muscle involvement causes a dermatomyositis-like syndrome in more than half of these patients, and some also have chronic hepatitis.

EVs have been recovered from many other sites in these patients, including the brain, lung, liver, spleen, kidney, myocardium, pericardial fluid, skeletal muscle, and bone marrow.[251] Some patients have been infected with more than one EV serotype, either concurrently[260] or sequentially.[251,259] The pathogenesis of the chronic muscle and soft tissue inflammation is not fully understood, but isolation of E from muscle in one case suggests a role for direct virus infection.[261]

In many patients, possibly most, the disease ends fatally. Autopsy findings have included chronic meningitis and encephalitis, with lymphocytic perivascular cuffing, focal loss of neurons, and gliosis of both gray and white matter. However, widespread destruction of motor neurons, such as that seen in poliomyelitis, has not been observed.

Prophylaxis and Therapy

Prophylactic use of IVIG serum globulin reduces the risk for acquiring chronic EV infection by these patients. However, IVIG has been less effective in the treatment of chronic EV meningitis, even when using IVIG lots with relatively high concentrations of specific antibody. Some patients have experienced clinical improvement when IVIG has been injected directly into the ventricles,[251] but relapse of infection may occur even after long-term intraventricular IVIG therapy. The experimental antiviral drug pleconaril has been used in this setting, but the reported experience with it is uncontrolled and limited to a small number of patients.[254,262]

Infections in Other Immunocompromised Patients

Hematopoietic cell allograft recipients have profoundly suppressed immunologic responses during the immediate posttransplantation period, including suppression of the ability to mount a humoral immune response. In some recipients EV infections have developed in the posttransplantation period that were disseminated, prolonged, and contributed to fatal outcomes.[255,262–265] One outbreak of CV-A1 diarrheal illness was observed in a bone marrow transplantation unit in which virus-induced diarrhea was difficult to distinguish from graft-versus-host enteritis.[266] Some patients receiving rituximab (anti-CD20) monoclonal antibody, which profoundly suppresses B-lymphocyte function, have developed paralysis, myocarditis, and other complications of CNS infection.[256,257,267]

ACUTE HEMORRHAGIC CONJUNCTIVITIS

Acute hemorrhagic conjunctivitis (AHC) is a contagious ocular infection characterized by pain, swelling of the eyelids, and subconjunctival hemorrhage that generally resolves spontaneously within a week. Epidemic or pandemic disease has now occurred in most parts of the world, resulting from EV-D70, which has been responsible for tens of millions of cases of AHC, and CV-A24 has caused a similar but geographically more restricted outbreak of disease that afflicted hundreds of thousands of persons. Some epidemics of conjunctivitis in the Far East have involved both viruses sequentially or concurrently. Although the relative contribution of these two agents has not always been defined, it is clear that EV-D70 has accounted for greater total morbidity.

Epidemiology

AHC appeared to emerge as a new disease in 1969 with explosive, pandemic spread from simultaneous foci in Ghana and Indonesia.[268] The initial epidemic caused by EV-D70 spread along the coast of West Africa and ultimately involved many countries on the African continent by 1973, as well as England, the former Soviet Union, Holland, France, and Yugoslavia.[269,270] CV-A24 was identified as the etiology of more than 60,000 cases of AHC in Singapore in 1970.[271–273]

Subsequently, both viruses circulated in Southeast Asia and the Indian subcontinent, causing large seasonal outbreaks.[274–277] Although the geographic distribution of AHC is wide, large-scale epidemics have occurred predominantly in crowded coastal areas of tropical countries during the hot, rainy season.[278] A 2012 outbreak of CV-A24 AHC on the French island of Mayotte, in the Indian Ocean, affected greater than 6% of the population and spread to neighboring islands of the Union of Comoros.[279,280]

Outbreaks in economically developed countries and temperate climates have been much more limited. AHC in the West has been mostly confined to seasonal outbreaks in Central America and the Caribbean. The disease did not appear in the United States until September 1981, when EV-D70 conjunctivitis was first reported in Key West, Florida. Within weeks, about 2500 cases occurred, largely among disadvantaged persons living in Miami.[281] With the exception of a few imported cases, AHC activity has not since been noted in the United States.[282] CV-A24 AHC cases first appeared in the Western Hemisphere in Trinidad, Jamaica, St. Croix, Panama, and Mexico in 1986.[283] Two outbreaks in Cuba in 2008 and 2009 resulted in more than 72,000 cases.[284]

Patterns of Transmission

AHC is highly contagious and spreads rapidly. Unlike most other EV infections, AHC is transmitted primarily from fingers or fomites directly to the eye rather than by respiratory secretions or fecal contamination. Both EV-D70 and CV-A24 can be regularly recovered from the conjunctiva early in the illness but only infrequently recovered from respiratory secretions or feces. Both serotypes appear to be naturally occurring, temperature-sensitive viruses whose optimal replication is at 33° to 35°C and reflects their adaptation to the temperature of the conjunctiva.[285,286] The observed rapid serial transmission at about 24-hour intervals is consistent with direct spread of virus from hand to eye. During a 1980 EV-D70 outbreak in Singapore, the secondary attack rate within affected households was 73%.[287] Contagion is favored by crowding and unsanitary living conditions.[280] AHC occurs substantially more often among the poor than among others living in the same country.[288,289] Reuse of water for bathing and sharing of towels are implicated as factors contributing to the spread of infection. Limited outbreaks of AHC in Europe have been primarily nosocomial, particularly in ophthalmology clinics, where infection appears to have been spread directly by physicians' fingers or by instruments.

Postepidemic antibody prevalence rates of nearly 50% have been observed in Ghana and Indonesia but only in 6% of affected populations of Japan. These findings are consistent with less intense spread of AHC in economically developed regions. Antibody prevalence rates are highest in children younger than 10 years, whereas attack rates for clinical disease are greatest in young adults, which indicates that many infections in children must be inapparent or mild.[290,291]

Clinical Manifestations

The clinical manifestations of AHC begin abruptly and peak within 24 hours. Burning, foreign body sensation, ocular pain, photophobia, eyelid swelling, and watery discharge begin in one eye and rapidly progress to the other eye.[281] Constitutional symptoms, such as fever, malaise, and headache, are observed in 20% of cases. The most distinctive sign is subconjunctival hemorrhage, which is present in 70% to 90% of patients with AHC caused by EV-D70,[291] but it is less frequent in cases caused by CV-A24.[272,273,275] The hemorrhages may be pinpoint or occupy the entire bulbar conjunctiva and are precipitated by everting the upper lid or by rubbing the eyes. Conjunctival edema is said to be more common in elderly people; hemorrhage is more profuse in young patients.[291] Small follicles appear on the tarsal conjunctiva after 3 to 5 days in 90% of patients. In most cases corneal erosion or a fine punctate epithelial keratitis can be demonstrated by slit-lamp examination after staining with fluorescein. The ocular discharge is serous or seromucoid and contains abundant neutrophils in the first 24 hours. Preauricular lymph nodes are often enlarged and tender by the second day of illness. Recovery is usually noticeable by the second or third day and is complete in most cases in 10 days, although discoloration from the hemorrhages sometimes persists for many days.

Complications

In severe cases of AHC, keratitis occasionally persists for several weeks but almost never leads to permanent scarring. Uveitis has not been reported. Conjunctivitis may be complicated by secondary bacterial infection.

More than 200 cases of acute motor paralysis have been reported as a complication of EV-D70 AHC in India, Thailand, Taiwan, and Senegal.[276,292–295] The paresis is clinically indistinguishable from poliomyelitis except for its temporal association with AHC, which it generally follows by 2 to 5 weeks. Neuroparalytic disease has been reproduced clinically and pathologically in monkeys by inoculation of EV-D70 into the spinal cord.[268,296]

Differential Diagnosis

Small outbreaks or sporadic cases may be mistaken for adenovirus infection causing epidemic keratoconjunctivitis (see Chapter 142), which has a longer incubation period (5–7 days), peaks several days after onset, and persists for up to 2 or 3 weeks, compared with AHC.

Laboratory Diagnosis

EV-D70 and CV-A24 can be recovered from conjunctival swabs or scrapings of patients with AHC during the first 3 days of illness.[271,297] Isolation rates exceeding 90% from conjunctival scrapings have been reported for CV-A24, but recovery rates for EV-D70 have been somewhat lower.[288] NAAT and sequencing of the VP1 coding region can readily identify the causative agent.[280,283] EV-D70 has not been recovered from the CSF in persons with paralysis, but high titers of specific neutralizing antibody to EV-D70 have been demonstrated in the CSF in virtually all affected patients.[295]

Treatment and Prevention

Treatment of conjunctivitis is symptomatic. Antimicrobial agents are not indicated. Contagion can be prevented by careful hand washing, use of separate towels, and sterilization of ophthalmologic instruments.

ILLNESSES IN WHICH THE ETIOLOGIC ROLE OF ENTEROVIRUSES IS MINOR OR POORLY DEFINED

Gastrointestinal Diseases

Acute hepatitis occurring beyond the neonatal period is described in association with CV-B and echovirus infections.[236,298–300] Most cases have been mild and self-limited. Prospective studies indicated that 2% to 20% of patients with acute pancreatitis have concurrent EV infection.[301,302] CV-B1 to CV-B5 and E-6, E-11, E-22, and E-30 are all reported to cause acute pancreatitis.[301–303]

CVs and echoviruses, possibly as a result of their replication in the small bowel, are frequently cited as causes of nonbacterial diarrhea or gastroenteritis. However, conflicting results have been obtained in several studies that compared rates of EV isolation from children with acute diarrheal illness with matched healthy control subjects.[304–307] Overall, the data favor a variable, generally small excess of EV infections in subjects with diarrhea. Evidence is somewhat stronger that certain Es, particularly E-11, E-14, and E-18, have occasionally been responsible for epidemic diarrhea in young infants.[304,306,308] Most of these studies were performed before the discovery of toxigenic *Escherichia coli*, rotaviruses, enteric adenoviruses, and caliciviruses, now established as major causes of diarrheal illness. In light of this knowledge, additional epidemiologic investigations encompassing all these agents are required before the contribution of EVs to diarrheal disease can be accurately assessed. Nonetheless, their role is probably minor.

Similarly, hemolytic-uremic syndrome (HUS) has been temporally associated with CV-A4, CV-B2, and CV-B4,[309,310] and CV-B5 has been reported associated with acute renal failure.[311] The well-established link between enterohemorrhagic *E. coli* infection and HUS renders the relationship between EV infection and acute renal disease questionable at best.

Other Diseases

Orchitis has been observed in adolescent boys during infection with CV-A9; CV-B2, CV-B4, and CV-B5; and E-6,[312–315] including CV-B5

isolated from a testicular biopsy specimen in one case.[312] Splenomegaly and a heterophile-negative mononucleosis-like syndrome have also been reported.[304] Es have been associated with acute arthritis, including E-11, which was recovered from synovial fluid in one case.[316,317] In separate case reports, E-25[318,319] and an untyped EV resembling a CV-A[319] have been recovered from the GI tracts of children with acute infectious lymphocytosis; however, further evidence of an etiologic association is lacking. Multiple reports of EV-induced lymphocytic hemophagocytosis occurring in infants, children, and adults have been published.[320–322]

Diabetes Mellitus

An accumulating body of epidemiologic, clinical, and experimental evidence suggests an intriguing link between the enteroviruses, particularly the CV-Bs, and type 1 diabetes mellitus (T1DM). However, the role of the EVs in the pathogenesis of T1DM remains controversial. Knowledge of the potential interactions of enteroviruses with beta cells in the islets of Langerhans, as well as the host's innate and adaptive arms of the immune system, is complex and evolving.[323] The reader is referred to several excellent reviews for more detailed analyses.[323–325]

The hygiene hypothesis of TIDM[323] contends that in areas where EV infections are frequent, infants develop early infection and immunity to them or are born with maternal antibodies to the common EV serotypes that partially protect the infant from severe infection. In areas of low prevalence, such as industrialized nations where sanitary conditions are better, EV infections may occur at older ages. Thus EV infection in infancy or early childhood may protect individuals from the risk of developing T1DM.

The observation that new-onset T1DM cases occur in seasonal patterns,[326] and sometimes in clusters or small outbreaks, has been cited as evidence for the role of viral disease in the pathophysiology of T1DM. The peak occurrence of new T1DM cases is late in the calendar year, 1 to 2 months later than peak EV activity. However, the occurrence of EV infection and T1DM during the same season could be independent, and several studies found no increase in new onset of T1DM after outbreaks of CV-B disease.[324,325,327,328] Evidence for direct infection of beta cells by EVs of patients with T1DM exists. Murine studies have documented that EVs cause specific destruction of beta cells in the islets of Langerhans.[329] Detection of EV RNA in serum or viral VP1 in the islets at the onset or recent onset of T1DM[330,331]; postmortem isolation of CV-B4[332,333] and CV-B5[334] from the pancreatic tissue of children dying of ketoacidosis, as their initial manifestation of T1DM; and the demonstration of CV-B IgM antibody in the serum of children with recent-onset T1DM all support the direct-infection hypothesis. However, inconsistency regarding this finding has been noted across different studies.[335–337]

Direct infection may result in viral-induced cytolytic death of islet cells or set the stage for an autoimmune response by the host or both.[323,338] EV infection of islet cells might activate the innate immune system, leading to inflammation. Human islet cells infected with CV-B5 results in the expression of Toll-like receptor 3, retinoic acid–inducible protein 1, and interferon (IFN)-induced helicase 1 pattern-recognition receptors that can detect EVs. Replication of the EVs results in the generation of double-stranded RNA that can bind to pattern-recognition receptors, leading to production of IFN-α and IFN-β, apoptosis, expression of major class I histocompatibility complex antigens, and production of cytokines. These innate immune responses serve to control the EV infection but can also be harmful to beta-cell function.

Evidence for "bystander activation" of preexisting autoreactive T cells exists in a transgenic mouse model overexpressing a T-cell receptor specific for an autoantigen in islet cells infected with CV-B4.[339] The resulting inflammation leads to the development of diabetes mellitus. Some investigations suggest that molecular mimicry between an EV protein (2C) and islet autoantigens or other cellular proteins may result in autoimmune destruction of pancreatic islet cell tissue.[340]

ENTEROVIRUS A71 INFECTIONS

EV-A71 is closely related to CV-A16, and both viruses cause skeletal myositis in suckling mice and myelitis with paralysis in cynomolgus monkeys.[341,342] Genomic analysis of strains from around the world has revealed the existence of three lineages, genogroups A to C, with several genotypes within the B and C genogroups.[343] The B lineage predominates in Singapore and Malaysia, whereas C lineage viruses predominate in east Asia.[55]

EV-A71 was first isolated in California in 1969 from young children with encephalitis and aseptic meningitis[344,345] and has since been found to cause infection globally, including large outbreaks of HFMD, which have been associated with aseptic meningitis and serious CNS complications in young children.[51,55,56,345–348] The largest recorded outbreak of EV-A71 HFMD occurred in Taiwan in 1998, where approximately 1.5 million individuals were infected.[53] In that epidemic 405 children were hospitalized because of neurologic complications, 78 of whom died. Infants and young children who develop a rhombencephalitis have a high mortality related to rapid cardiovascular collapse and pulmonary edema.[51–53]

EV-A71 is also unique among the nonpolio EVs as a cause of epidemic AFP, in which localized outbreaks have involved small numbers of patients over several years,[344,345] and regional epidemics have involved hundreds to thousands of persons within a single season.[51,346] Other, less common manifestations attributed to EV-A71 infection include generalized maculopapular rash,[345] interstitial pneumonia,[54] and myocarditis.[53] EV-A71 can be isolated from vesicle fluid, feces, oropharyngeal secretions, urine, and CSF. Primary isolation is most successful in African green monkey kidney cell culture, although a cytopathic effect may take 5 to 8 days to develop. Isolation rates are highest from vesicle swabs and lowest from the CSF.[347] Detection using NAAT facilitates and speeds the identification of the virus.[349] However, NAAT detection of EV-A71 from the CSF is also low.[350]

Treatment of EV-A71 infection is symptomatic and supportive. The large-scale epidemics occurring in the Far East have led to the development of an EV-A71 vaccine that has recently undergone a successful phase III trial, with greater than or equal to 90% efficacy against EV-A71–associated HFMD.[351–353] It remains to be determined if the vaccine will confer cross-protection against other C genotypes or strains from the A and B genogroups.

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173 Parechoviruses

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SHORT VIEW SUMMARY

Definition
- The human parechoviruses (HPeVs) are members of a relatively newly created genus designated *Parechovirus* in the Picornaviridae family. Four species (*Parechovirus* A through D) comprise the genus. Only serotypes within species A and B infect humans; A is the most important, with 19 serotypes currently identified.
- HPeVs are responsible for multiple clinical syndromes involving many organ systems, including undifferentiated febrile illness, sepsis-like syndrome, meningitis, encephalitis, paralysis, and respiratory illnesses.

Epidemiology
- HPeVs are found worldwide.
- The majority of HPeV infections occur during the summer and autumn months in infants and young children.

Microbiology
- Morphologically they are small (28 nm in diameter), nonenveloped, icosahedral-shaped viruses that possess a single-stranded, positive-sense RNA genome.

Diagnosis
- HPeVs can be isolated, or their genomic RNA can be detected from oral secretions, feces, cerebrospinal fluid, and blood.
- Nucleic acid amplification has been quicker and more sensitive than cell culture for their detection.
- Type identification is accomplished by sequencing of the major capsid protein, VP1.

Therapy
- Therapy is supportive. No specific antiviral therapy is available.

Prevention
- Contagion can be prevented by hand washing.

HISTORICAL BACKGROUND

The initial two serotypes of the genus *Parechovirus* were isolated in 1956 from children with diarrhea.[1,2] On the basis of the scheme used at that time for the classification of the enteroviruses, they were classified as echovirus (E) serotypes 22 (Harris strain) and 23 (Williamson strain).[2–4] After their classification as enteroviruses, it became evident that they differed from other serotypes within the genus. Their growth in cell culture produced a cytopathic effect that differed from that of the enteroviruses.[5] E22 and E23 produce incomplete cytopathic effects of the cell monolayer and a distinctive nuclear cytopathology consisting of thickening of the nuclear membrane and the disappearance of the nucleolus, and of nuclear chromatin.[2,5] As molecular methods for the evaluation of viral replication became available, additional discordances were identified. Salient among these were the inability of E22 and E23 to cleave the eukaryotic translation initiation factor 4G (eIF4G) and thereby shut off host cell protein synthesis—a notable characteristic of the enteroviruses—and the efficient translation of their genome in rabbit reticulocyte lysates, something not observed in the enteroviruses.[6,7] Furthermore, the cleavage of the nascent capsid proteins of E22 resulted in three rather than four mature capsid proteins, as with the enteroviruses.[8] With the advent of tools for direct and computational analysis of the viral genome, it became evident that E22 and E23 were clearly dissimilar to other members of the genus. Nuclease digestion of the RNA genome of E22 revealed that it possessed a greater mean relative resistance to digestion than that of poliovirus type 1, suggesting that it contained significantly more secondary structure (i.e., double-stranded or base-paired regions).[9] Failure to hybridize subgenomic probes to the E22 genome, known to be reactive with other serotypes of the *Enterovirus* genus, or amplify subgenomic segments using pan-enterovirus reactive primers, added further support that it was not a true enterovirus.[6,10,11] Ultimately, the sequencing and phylogenetic analysis of the complete genomes of E22 and E23 confirmed that they were indeed genetically distinct from the enteroviruses and led to their assignation to a new genus: *Parechovirus,* members of the Picornaviridae family.[12–14] The genus is currently comprised of 4 (A—D). Only members of species A and B infect humans.

Human parechoviruses (HPeVs) 1 and 2 (formerly E22 and E23, respectively) were soon joined by 17 newly identified members of the species bringing the total number of types to 19.[15,16] Potentially new types continue to be identified.[17]

VIROLOGY

Only the major differences between HPeVs and enteroviruses are discussed here. Several reviews of HPeVs have been published in recent years.[18,19] Readers are directed to those sources, and to Chapter 170 in this text, for a more detailed discussion of the virology of HPeVs and enteroviruses.

HPeVs are small (28 nm in diameter), nonenveloped viruses possessing a single-stranded, positive (messenger)-sense RNA genome. The external appearance of the virion is much smoother than that of the enteroviruses owing to shorter surface loops on the capsid protein, VP1, resulting in a less pronounced plateau at the fivefold axis of symmetry and a shallower canyon (Fig. 173.1).[20] The HPeV genome is approximately 7.35 kb in length and organized in a manner comparable with that of enteroviruses.[12–14,16,21–27] The open reading frame (ORF) codes for a polyprotein of approximately 2180 amino acids, depending on HPeV type and strain, that is shorter than that of enteroviruses.[16,17]

Computational support has been generated for the existence of linear regions of RNA and higher-order RNA structures within the HPeV 5′ and 3′ nontranslated regions (NTRs) analogous to those of enteroviruses (see Chapter 172).[14,25,27] Analysis of the HPeV internal ribosome entry site (IRES), a region essential for efficient translation of the picornavirus genome, reveals it to be more closely related to that of the *Cardiovirus* and *Aphthovirus* genera.[27] Conserved regions of nucleotide identity exist among the 16 types within the HPeV 5′ NTR and are used in nucleic acid amplification tests (NAATs) for their detection.[28,29]

Translation of the ORF yields three capsid (VP0, VP3, and VP1) and seven nonstructural proteins essential for replication and viral assembly. The structures and functions of all of the HPeV proteins have not been extensively studied. Many have functions similar to cognate enterovirus proteins, but differences are emerging. Unlike the enteroviruses, the HPeV genome codes for three, rather than four, capsid proteins as a result of failure to cleave the precursor protein, VP0, of capsid proteins

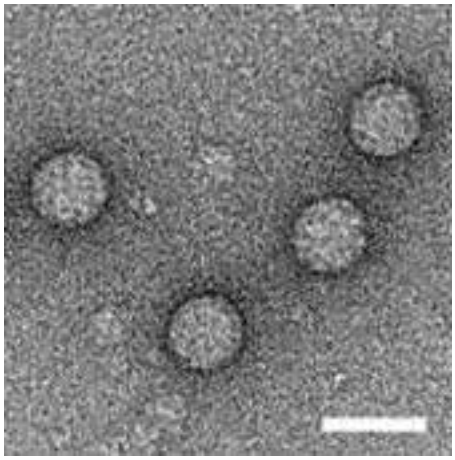

FIG. 173.1 Electron micrograph of parechoviruses (bar = 50 nm). *(From Stanway G, Williams C, Hyypia T. Parechovirus. In: Tidona C, Darai G, eds.* The Springer Index of Viruses. *2nd ed. New York: Springer; 2011.)*

VP2 and VP4.[8] The result is a capsid composed of VP0, VP3, and VP1.[12,14,23–26] The VP0 coding sequence contains a stem-loop structure that may function as a *cis*-acting replication element (CRE) critical for replicaton.[25] The C-terminal region of the VP1 protein of several HPeVs contains an arginine-glycine-aspartic acid (RGD) motif functional in binding to host cell integrins.[a] Evidence points to the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_6$ as receptors for some HPeVs.[20,30] The cell receptors used by HPeVs lacking the RGD motif are currently unknown. The VP3 of HPeV varies from that of the enteroviruses as the result of the N-terminal extension of 28-34 amino-acid residues.[24]

Among the nonstructural proteins, 2A protein differs functionally from that of the enteroviruses in that it lacks proteolytic activity and is incapable of mediating the cleavage between the 1D and 2A proteins.[31] The HPeVs fail to shut off host cell protein synthesis, a function mediated in enteroviruses by 2A cleavage of eIF4G.[6,7,8] The 2A protein also binds to RNA and may play a role in RNA replication.[32] The HPeV 2C protein also appears to differ from its cognate enterovirus protein. Like the enterovirus 2C, it localizes to endoplasmic reticulum membrane–associated replication complexes, supporting a role in viral replication. However, it is also associated with cellular structures not directly involved in replication.[33,34] 2C also has AMP (adenosine monophosphate) kinase activity, a property whose significance is unknown.

EPIDEMIOLOGY

Our understanding of the epidemiology of the HPeV is lacking for multiple reasons: continued discovery of new types[17]; inability of all HPeV types to be identified through cell culture[35]; and an NAA that is pan-amplifying for all HPeVs.[28]

HPeVs have been identified worldwide. HPeV infections occur throughout the year; however, they exhibit seasonal epidemiology. Worldwide, the peak incidence for HPeV infection is during the summer and autumn months.[24,36–50]

In the case of HPeV3, a biennial pattern has been reported,[44,51,52,53] but several reports specifically have not supported this finding.[26,37,46,54,55] Cocirculation of multiple types within a community or geographic region is common.[b] The HPeVs have been associated with community[45] and nosocomial outbreaks in pediatric and neonatal units.[36,58,59,60,61]

The contribution of the HPeV to the disease burden, and the relative proportion due to each of the types, is yet unclear. With use of traditional cell culture methods, the HPeVs accounted for approximately 2% of all "enteroviruses" isolated by clinical laboratories. HPeV1 is the most frequently identified member of the genus, followed by HPeV3. In the United States, HPeV1 (E22) accounted for 1.8% and HPeV2 (E23) for 0.1% of all typed enterovirus isolates reported to the Centers for Disease Control and Prevention (CDC) over a 39-year period (1970–2008) and ranked among the top 15 isolates isolated for 74% all years.[62,63] Reports from two clinical laboratories in Japan found that HPeV accounted for 2.2% to 2.8% of all enteroviruses isolated over a period of 15 and 9 years, respectively.[23,26] Typing revealed that HPeV1 and HPeV3 accounted for 85% of the isolates, whereas types 6 and 4 accounted for the remainder in decreasing order of frequency. A Canadian report found that over a 20-year period, of 28 HPeV isolates that were identified, types 1, 2, and 3 accounted for 71.4%, 10.7%, and 17.9%, respectively, of the total number.[24]

However, with use of molecular techniques for the detection of these viruses, it appears that HPeV may play a more significant role in disease burden than previously was thought. Surveillance by the CDC (2009–2013) found that HPeV3 accounted for 12.3% of 1819 reports with a known enterovirus or HPeV type.[64] However, most HPeV3 detections originated from a single hospital that routinely tested for HPeVs. A report from Northern Italy documented that HPeVs were detected in 5.2% of 812 samples collected from children younger than 5 years over a 4-year period. For comparison, enteroviruses were identified in 16.1% of samples. HPeV1 accounted for 66.7% of the HPeVs identified, followed by HPeV3 (20%) and HPeV6 (13.3%).[50]

HPeV infections occur early in life. Seroprevalence data and longitudinal studies of the shedding of HPeV in stools of healthy infants suggest that approximately 70% to 80% of infants have evidence of at least one HPeV infection by 2 years of age, increasing to approximately 90% by 5 years in some studies.[39,54,65–68,69] In the United States, approximately 70% of cases of HPeV1 and HPeV2 infection reported to the CDC occurred in infants younger than 1 year.[62] Children younger than 5 years accounted for 95.6% and 88.2% of all HPeV1 and HPeV2 isolates, respectively.[62] The seroprevalence for some HPeV types approaches 100% in adults.[69] However, in some geographic areas, certain HPeV types fail to circulate or do so at low levels.[41] A limited serologic survey of the prevalence of HPeV3 among adults in the Milwaukee, Wisconsin, area failed to detect any seropositive individuals[70]

CLINICAL MANIFESTATIONS

Transmission of HPeV occurs primarily by the fecal-oral and, less frequently, the respiratory routes. Fecal-oral transmission is favored by high viral titers in stool.[42] After infection, HPeVs are shed from the gastrointestinal and upper respiratory tracts.[37,42,71] The duration of shedding is still not well defined but may range from less than 2 weeks to as long as 5 months in stool and 1 to 3 weeks from the upper respiratory tract.[37,42,71] In one report, after symptomatic infection, shedding of HPeV lasted for 2 to 24 weeks (median, 58 days) and most of the shedding occurred while infants were asymptomatic.[72] Reports of HPeV infections occurring in the first 2 days after birth may support the possibility of in utero transmission.[44,73] Identification of genetically identical HPeV3 in infected patients and in asymptomatic children in their families have suggested that family members may be a source of infection in neonates and young infants.[74]

The finding of HPeV in the stool of asymptomatic infants, along with the high seroprevalence among young infants and children in the general population, provides evidence that the majority of HPeV infections are subclinical.[37,42,54] Given the high prevalence of HPeV infections, it is likely that the reported cases of severe HPeV infections represent infrequent events.

UNDIFFERENTIATED FEBRILE ILLNESS/SEPSIS SYNDROME

HPeVs have been isolated from the stool, nasopharyngeal swabs, urine, and blood of neonates and infants with an undifferentiated febrile illness (UFI).[40,54,71,75–82] The overwhelming majority of reported cases have occurred in male infants younger than 2 months. Seven percent of infants younger than 36 months evaluated for fever without a source in the emergency department of a children's hospital had a HPeV infection.[83] In some cases, cardiovascular and respiratory dysfunction is severe enough to warrant a clinical diagnosis of sepsis. Although multiple HPeV types have been associated with UFI, cases of sepsis have been most frequently associated with infection due to HPeV3.[40,76,80–82] HPeV4 infection may also be associated with serious, sepsis-like disease.[81,84,85]

In addition to fever, irritability, and poor feeding, tachycardia and tachypnea are typically present.[c] A rash may be present on the trunk

[a]References 8, 12, 14, 20, 23, 25.
[b]References 38, 39, 43, 47, 48, 53, 54, 56, 57.

and extremities, including the palms and soles, which has been described as macular, maculopapular, or erythematous.[76,79,80,86–88] Mild respiratory (rhinorrhea, cough) and gastrointestinal symptoms (diarrhea, abdominal distention) may be present. Some may present with or develop a sepsis-like syndrome with severe tachycardia, retractions, apnea, oxygen desaturation, poor capillary filling, mottling, or shock.[80–83] In these patients, hepatitis may be present.[80,83]

Admission to the intensive care unit may be necessary for monitoring and cardiovascular or respiratory support and may be more common for infants younger than 1 to 2 months.[80–83] In a review of three recent series involving very young infants, totaling over 200 cases, only a single death was reported.[80,81,82] A recent study raised concerns regarding normal neurodevelopment among infants hospitalized with HPeV sepsis-like presentation.[89] With use of a standard questionnaire, follow-up was performed 1 year after hospitalization in 42 infants who had been hospitalized with HPeV sepsis-like syndrome, 12% of whom had encephalitis. The investigators found that half of the infants may have had some abnormal neurodevelopment, including possible significant abnormalities in one-fifth of the infants, most prominently in the gross motor and problem solving domains. These findings suggest that careful follow-up of young infants hospitalized with HPeV disease may be warranted.

CENTRAL NERVOUS SYSTEM INFECTIONS

HPeVs have been conclusively documented to cause various central nervous system (CNS) syndromes, including acute flaccid paralysis (AFP),[23,26,90,91] encephalitis,[44,48,75,80–82,92] and meningitis.[d]

Meningitis

HPeVs have been detected from the cerebrospinal fluid (CSF) or blood of individuals with meningitis.[51] Retrospective analysis of archival CSF specimens negative for enterovirus and a prospective report detected HPeV in approximately 1% to 8.8% of individuals undergoing evaluation for possible infection.[e] The annual prevalence varies widely and ranges from 0% to 17% (mean, 5.3%). With the exception of one report, the overwhelming cause of HPeV meningitis is HPeV3.[75] More than 80% of cases occur in infants younger than 3 months. Males account for the majority of cases.

Clinical findings consist of fever and irritability in nearly all cases.[f] Poor feeding and decreased activity are frequent manifestations. An exanthem may be present and has been described as maculopapular or erythematous, affecting primarily the extremities and involving the palms and soles as noted earlier.[48,80,88] Gastrointestinal signs, such as emesis, diarrhea, and distention, and respiratory symptoms such as rhinorrhea, cough, tachypnea, apnea, and wheezing, may also be present. Conspicuously absent are clinical findings indicative of increased intracranial pressure (bulging fontanel) or meningeal irritation (nuchal stiffness, Kernig, or Brudzinski signs).

Minimal or no abnormalities are found at CSF evaluation in the overwhelming majority of patients.[g] Pleocytosis is absent in the overwhelming majority of cases and, if present, is generally minimal (<25 cells/mm^3). Hypoglycorrhachia and increased CSF protein are uncommon.[46,48,94]

The majority of infants with HPeV meningitis are hospitalized as a result of the need to exclude bacterial infection.[44,46,48,94] The median duration of hospitalization varies from 3 to 7 days.[g] Death is extremely rare.[g]

Encephalitis

HPeV encephalitis occurs almost exclusively in neonates and very young infants.[h] Although HPeV3 is the dominant cause, cases due to HPeV1 have been reported.[92,96] Seizures, which may be intractable, constitute the predominant presenting clinical finding, followed by fever and irritability. An exanthem is seen in the majority of patients. Apnea and hypotension, requiring inotropic support, may occur. Additional findings include stupor, hypertonia, decreased appetite, and diarrhea.

The CSF is normal in nearly all cases. Cerebral magnetic resonance imaging (MRI) or ultrasonography reveals abnormalities in nearly all cases.[75,86,92,95,97] White matter changes consisting of high signal intensity and punctate lesions are seen at MRI in a majority of cases. These changes occur even in children without seizures.[75,86] Ultrasound usually reveals severe periventricular echogenicity. Electroencephalography findings are abnormal and document epileptic discharges and background slowing.[95]

Fatalities rarely occur.[76,91,97] Limited information on long-term neurologic outcome is available; however, in more than half of reported cases, patients were neurologically normal at the time of the clinical report. A recent report documented neurodevelopment sequelae in the majority of infants with confirmed HPeV encephalitis. At discharge, one-third were noted to have sequelae. However, at the 12-month follow-up, 62.5% were assessed to have sequelae: cerebral palsy, visual impairment, and concern regarding gross motor development.[95] Other neurologic sequelae include epilepsy, learning disability, and mild distal hypotonia.[43,86] Repetitive seizures and a discontinuous background on electroencephalogram were found to correlate with poor neurodevelopmental outcome.[86,95] Striking similarities between the clinical features and neurologic sequelae caused by HPeV3 encephalitis and hypoxic ischemic encephalopathy of the newborn have been noted.[98]

Acute Flaccid Paralysis

HPeV types 1, 3, 5, 6, 7, and 12 have been identified from stools and CSF in patients with AFP.[i] With the exception of a single report of an outbreak of HPeV1-associated AFP,[87] all cases have been sporadic occurrences. The outbreak occurred in Jamaica and involved five individuals—one adult and four children.[90] Individuals with AFP are older than those with other HPeV-related CNS diseases, ranging in age from 1 month to 27 years.[23,90,91,96,99] Detailed clinical data are limited.[23,90,96] The onset is acute and in the majority of cases involves the lower extremities. Loss of deep tendon reflexes occurs in the involved extremities. Respiratory failure due to involvement of the muscles of respiration may occur. Fever and diarrhea may be present at the onset. Other findings include paresthesias, cranial nerve involvement, and signs of meningeal irritation. CSF examination generally reveals increased protein concentration and absence of pleocytosis. Although occasional cases of transient paralysis have been reported,[23] the majority of reports have indicated residual weakness or palsy of the involved extremity.

GASTROINTESTINAL DISEASE

Hemorrhage-Hepatitis Syndrome

Neonates and very young infants may develop a syndrome characterized by hepatitis, thrombocytopenia, and coagulopathy reminiscent of hemorrhage-hepatitis syndrome observed with enteroviruses (see Chapter 172) after HPeV infection.[43,75,87,100] All infants have been younger than 2 months, and HPeV3 has been isolated in all cases. Infants present with fever, fussiness, and poor feeding. As the illness progresses, ecchymosis and bleeding from puncture sites occur. Marked elevation of aspartate and alanine aminotransferases levels, prolongation of prothrombin, and partial thromboplastin times attest to hepatitis and liver failure. Thrombocytopenia is universally present. Deaths have occurred, and liver transplantation as a result of severe liver necrosis has been necessary.[43,100]

Necrotizing Enterocolitis

Infection with HPeV1 and HPeV3 has been associated with necrotizing enterocolitis in neonates and very young infants.[36,100] Clinical findings include feeding intolerance, abdominal distention, and bloody stools. Nonspecific symptoms such as apnea, rhinitis, tachypnea, and lethargy may also occur. Radiograph findings include dilated loops of bowel and pneumatosis intestinalis.

Acute Gastroenteritis

Multiple studies that vary in stringency, controls, number of specimens, and clinical venues have examined the role of HPeV in the etiology of

[c]References 40, 43, 71, 75, 76, 79.
[d]References 44, 46, 48, 51, 73, 75, 81–82, 87, 93.
[e]References 44, 46, 48, 51, 73, 87, 93.
[f]References 46, 48, 73, 80–82, 87, 93.
[g]References 44, 46, 48, 80, 81, 82, 94.
[h]References 43, 44, 48, 58, 75, 80, 81, 82, 86, 95, 96, 97.
[i]References 23, 26, 90, 91, 96, 99.

acute gastroenteritis (AGE) in children[j] and adults.[42] Further befuddling the analysis of the role of HPeV in AGE is that gastrointestinal signs and symptoms accompany almost every syndrome caused by these agents.

An analysis of reports of stool specimens shown to be negative for known viral cases of AGE, and screened for HPeV with NAATs, indicates that prevalence of HPeV among hospitalized children ranges from 0% to 2.7%.[42,47,102] No difference in prevalence was observed among children from developing versus industrialized nations.

The prevalence of HPeV in cases of AGE has been examined in the outpatient setting in different countries by using large numbers of stool samples negative for other viral causes of AGE. A report from Germany found that only 1.8% of all samples contained HPeV. No difference was found in HPeV-AGE patients compared with a control non-AGE group.[42] All positive specimens were found in children younger than 6 years. The prevalence in children younger than 2 years was 11.6%. Among nonhospitalized Japanese children with AGE, 8.3% had detectable HPeV.[57] Lastly, a report from the CDC found HPeV present in only 4.4% of 782 stool specimens submitted from children younger than 5 years with AGE.[103] These researchers concluded that there was no causal role for HPeV in AGE. On the basis of the available information, HPeV appears to have at best a small role as a cause of AGE.

Respiratory Disease

Respiratory symptoms, either alone or in combination with other HPeV syndromes, are common.[k] Several recent large studies cast doubt on the role of HPeV as a significant cause of respiratory disease. In a report from Norway, 161 asymptomatic children attending daycare were studied, and 9% were found to be positive for HPeV.[104] A study conducted in Northern Italy found that only 0.4% (14) of 3525 patients with respiratory syndromes had HPeV isolated. Of those, all but 4 had a coinfection with other respiratory viruses.[78] In a report from Scotland, the prevalence of HPeV was 1.2% among individuals with respiratory tract illness.[42] HPeV accounted for 2.9% of cases of those in whom any respiratory virus was identified. Of significance, coinfection with one or more respiratory viral pathogens was found in 76% of HPeV-positive children. All PeVs were isolated from children 0.5 to 5 years of age, and 89% were younger than 3 years. The observed incidence of HPeV infections in children younger than 5 years was within the expected frequency of the general population. Comparison of the clinical profile of those in whom HPeV was detected coincided more closely with those with negative samples.[41] Lastly, a report from a Midwest children's hospital in the United States found a 3% prevalence rate for HPeV infection among 637 children for whom respiratory specimens were submitted.[105] All children were younger than 3 years, and 79% were younger than 3 months. Only nonspecific respiratory symptoms were present. Taken together, these findings fail to support a significant etiologic role for HPeV in respiratory disease.

[j]References 38, 42, 47, 49, 54, 56, 57.
[k]References 42, 44, 47, 57, 59, 76, 103.

MISCELLANEOUS CLINICAL ASSOCIATIONS

HPeVs have been isolated from patients with a wide array of syndromes, including sudden infant death,[70] Reye syndrome,[25,26,106] Guillain-Barré syndrome,[90,107] myocarditis,[43,108,109] myositis and epidemic myalgia,[26,110,111] hemolytic uremic syndrome,[112] hand-foot-mouth disease,[26,54] herpangina,[52] otitis media,[113] hemophagocytic lymphohistiocytosis,[114,115] and hyperferritinemia.[116,117] In the majority of reports, HPeVs were detected from sites where prolonged shedding is known to occur and not from the involved organ system or tissue. Some reports fail to indicate whether other infectious agents known to be associated with the syndrome were excluded. Further adding to the complexity of ascribing a HPeV etiology to an illness is the codetection of a second agent known to be linked to the disease in question.[41,54,57]

DIAGNOSIS

Samples for detection of HPeV include throat swabs, stool specimens, CSF, or blood. The HPeVs produce an enterovirus-like cytopathic effect on appropriate cell lines.[35,118] However, optimal recovery of the HPeV requires the use of multiple cell lines because not all types replicate equally on the same primary cells or cell lines. Many of these are not routinely found in diagnostic laboratories.

Reverse-transcriptase polymerase chain reaction (RT-PCR) is the methodology of choice for detection of HPeV because of its sensitivity and ability to detect all known HPeV types in a clinically meaningful time frame.[28] Speciation of HPeV is accomplished by amplification and sequencing of the VP1 coding region of the viral genome.[28,29]

THERAPY AND PREVENTION

No specific antiviral therapy has been described for HPeVs, although it is an area of active research.[119,120,121] Intravenous immunoglobulin has been used to treat serious HPeV infections, but its efficacy is not established.[122] Monoclonal antibodies with neutralizing activity against HPeV have been reported and may offer an approach to specific therapy.[123] Because their major mode of transmission is the fecal-oral route, hand hygiene prevents their spread. For hospitalized patients with HPeV-related syndromes, infection control measures similar to those for the enteroviruses, consisting of use of standard precautions, are sufficient.[124] No vaccine is currently available for the prevention of HPeV-associated disease.

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174 Hepatitis A Virus

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SHORT VIEW SUMMARY

Definition
- Hepatitis A is an acute inflammatory condition of the liver caused by hepatitis A virus (HAV) and characterized by constitutional symptoms including anorexia, fatigue and weight loss, and jaundice.

Microbiology
- HAV is a nonenveloped RNA virus member of the picornavirus family. Three genotypes infect humans (I, II, and III).
- HAV is relatively resistant to heat but can be inactivated at higher temperatures.
- HAV is resistant to organic solvents and detergents and can survive in acidic environments to a pH of 3.
- HAV can be inactivated by hypochlorite (bleach) and quaternary ammonium formulations containing hydrochloric acid (HCl) (found in many toilet cleaners).

Epidemiology
- Transmission is via the fecal-oral route, with peak viral shedding before onset of symptoms.
- Humans are the only important reservoir for HAV.
- Spread is most commonly person to person (including sexual contact) but can occur through foodborne and waterborne transmission, among injection drug users and men who have sex with men, and rarely through use of infected blood products.
- Risk factors in the United States include common-source (foodborne) outbreaks, and close contact (sexual, household or other) with a hepatitis A–infected person in the context of a community outbreak.
- In the United States, the incidence of hepatitis A has decreased dramatically over the past 20 years as a result of implementation of routine vaccination of children. Large community-wide epidemics previously common are now rare. However, high-profile foodborne outbreaks and multistate outbreaks among persons who experience homelessness, persons who use injection and noninjection drugs, and their close direct contacts have emerged; such outbreaks are now more apparent with the overall decreases in background incidence.
- Globally, hepatitis A is a common cause of jaundice and kills an estimated 11,000 persons annually. Disease incidence paradoxically may increase as improvement in living conditions delays infection to older ages, when symptoms are likely to be more severe.

Diagnosis
- Hepatitis A is clinically indistinguishable from other forms of viral hepatitis.
- Laboratory diagnosis is made through detection of immunoglobulin M (IgM) antibodies to HAV in a single, acute-phase serum sample.

Therapy
- Most illnesses are self-limited and can be managed with supportive and symptomatic measures.
- Fulminant cases are rare but need to be managed appropriately and may necessitate transplantation in extremely ill patients.
- Chronic hepatitis A does not occur, although relapse can occur.

Prevention
- In the United States, vaccination with hepatitis A vaccine is recommended for all children at age 12 to 23 months. Globally, few countries recommend hepatitis A vaccination for children. Vaccination of older children may be warranted, and vaccination of international travelers and persons in selected high-risk groups is recommended, including patients with chronic hepatitis B and C (see Table 174.4).
- Administer postexposure prophylaxis with hepatitis A vaccine (preferred) or immune globulin (IG) if exposure occurred within the previous 2 weeks. IG is recommended over vaccination if the patient is younger than 12 months, is immunocompromised, has chronic liver disease, or is a person with a contraindication to vaccine (see Table 174.2).

Hepatitis A is generally an acute, self-limited infection of the liver by an enterically transmitted picornavirus, hepatitis A virus (HAV). Infection may be asymptomatic or result in acute hepatitis. Rarely, fulminant hepatitis can ensue. Although the duration and severity of symptoms vary widely, HAV infections never cause chronic liver disease. The availability of effective vaccines against hepatitis A has markedly affected the epidemiology of hepatitis A in places where the vaccines are widely used, as in the United States (see later).

HISTORY

The earliest accounts of contagious jaundice are from ancient China.[1] Although the signs and symptoms that were described are similar to those currently found in people with hepatitis A, it should be remembered that a number of other infections produce a similar clinical picture. The earliest outbreaks of hepatitis that were almost certainly hepatitis A were documented in Europe in the 17th and 18th centuries, especially during periods of war. From 1855, the disease became known as "catarrhal jaundice" because the pathologists Bamberger and Virchow believed that the disease was caused by blockage of the common bile duct by a plug of inspissated mucus.[2] The first suggestion that the disease was caused by an infectious agent was made by McDonald,[3] who, unable to demonstrate the involvement of enteric bacteria, suggested that the infection might be caused by a virus. Shortly thereafter, Cockayne[4] proposed that the sporadic and epidemic forms of jaundice were manifestations of the same disease. In 1923, Blumer analyzed a large number of epidemics of hepatitis in the United States and identified its predilection for young adults and children and peak incidence in winter and fall.[5] Hepatitis A had an incubation period of between 15 and 49 days and was transmitted by the fecal-oral route.[6–8] Later studies demonstrated that the virus could be detected in feces or blood during the acute infection, that infection could be transmitted experimentally by both the oral and parenteral routes, and that infection was followed by long-term immunity and could be prevented by previous administration of normal human immune globulin (IG).[5,9] In addition, the disease was shown to be associated with a filterable agent resistant to heating at 56°C for 30 minutes and resistant to diethyl ether. In the 1950s and 1960s, Krugman[10] expanded these observations with a series of studies in human volunteers that further defined the incubation period, period of infectivity, and period of viremia, and developed standardized reagents representing hepatitis A. In 1973, Feinstone and colleagues[11] detected

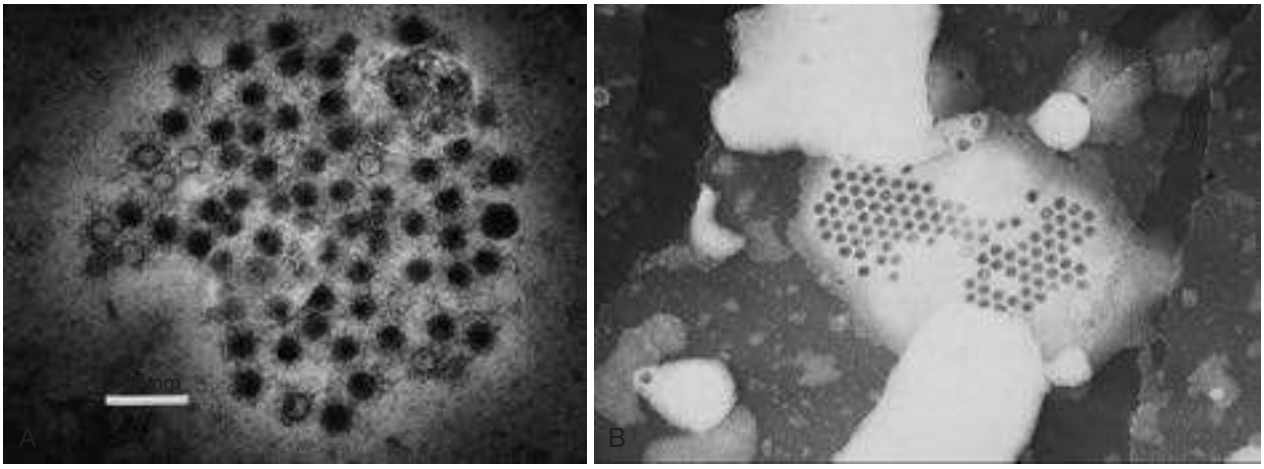

FIG. 174.1 Electron micrographs of hepatitis A virus particles (A) aggregated by antibody, 27 to 28 nm in diameter and highly concentrated, purified hepatitis A virus from human feces (B).

27-nm virus-like particles in the stools of volunteers infected with hepatitis A and demonstrated that they were aggregated by convalescent but not by preinfection serum, thus indicating that the particles represented the etiologic agent of the disease. The identification of HAV, transmission of the disease to marmosets and chimpanzees, propagation of HAV in cell culture, and molecular cloning of the viral genome ushered in a new era of research that culminated almost 2 decades later in the development and licensing of effective vaccines.[12–18]

CLASSIFICATION AND PHYSICOCHEMICAL AND BIOLOGIC PROPERTIES OF HEPATITIS A VIRUS

HAV is a nonenveloped, positive-strand RNA virus member of the Picornaviridae family. This family contains 35 genera such as *Aphthovirus* (foot-and-mouth disease virus), *Enterovirus, Parechovirus,* and others. Although HAV shares general structure and genomic organization with the other picornaviruses, it has limited nucleotide sequence homology and certain distinguishing characteristics that have resulted in its being classified in its own genus, *Hepatovirus.*[19–21] Novel *Hepatovirus* species related to HAV have been found in seals and small mammals.[22,23] Human isolates of HAV are relatively closely related on the basis of partial genomic sequences. However, human HAV strains have been divided into three genotypes—I, II, and III—on the basis of phylogenetic analysis of sequences of the complete VP1 region.[24] The other three genotypes—genotypes IV, V, and VI—are all single simian HAV isolates.[25,26] Phenotypic differences such as disease severity among the genotypes have not been well recognized. Recently, however, some association of fulminant hepatitis with infections with certain genotype IB strains in the United States was suggested.[27] In Korea, patients infected with genotype IIIA were reported to have significantly higher aminotransferase levels, prothrombin time and leukocyte count, and more severe symptoms than patients infected with IA.[28] In addition, mutations in the 2B coding region were shown to be associated with severe and fulminant disease.[29,30] Although antigenic variants exist among all genotypes, there is only one recognized serotype of HAV on the basis of cross-neutralization studies.

Structure

HAV virions recovered from feces of infected individuals are 27- to 28-nm spherical, nonenveloped particles,[11] (Fig. 174.1) with a surface structure suggesting icosahedral symmetry.[31] Good-quality cryoelectron microscopy has confirmed the icosahedral symmetry of the virion with typical picornavirus threefold and fivefold axes and 60 repeated pentamers comprising the capsid (Fig. 174.2). The cryoelectron microscopic views of HAV reveal the plateau surrounding the fivefold axis that is typical for picornaviruses, but not the pit or canyon believed to be the receptor-binding site of enteroviruses and rhinoviruses (see Fig. 174.2). The prominence at the threefold axis of symmetry is distinct from other picornaviruses and is believed to be the major antigenic site at which neutralizing antibodies are targeted. High-resolution x-ray crystallography has confirmed the icosahedral symmetry and the lack of a canyon.[32] It has also revealed that the structure of HAV is notably different from that of typical picornaviruses and bears similarities to insect picorna-like viruses, suggesting that HAV may be a link between modern and primordial picornaviruses.

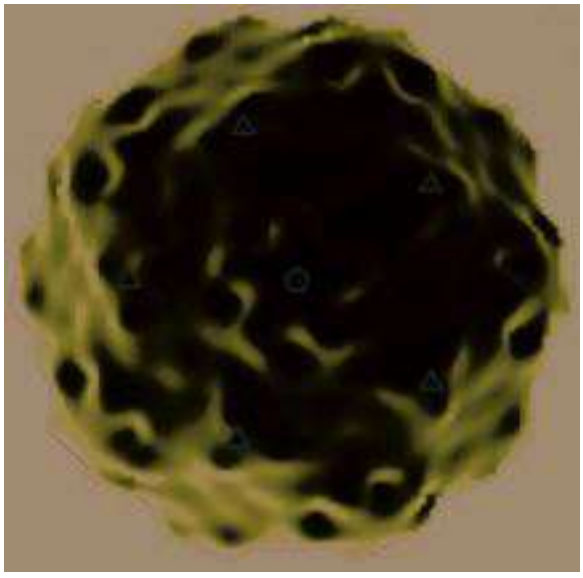

FIG. 174.2 Cryoelectron micrograph showing the surface structure of hepatitis A virus (HAV). The pentagon designates the fivefold axis of symmetry plateau typical of picornaviruses. The triangles designate the prominence of the threefold axis that is unique among picornaviruses and represents the major antigenic site of HAV. *(Courtesy Dr. Holland Cheng, University of California at Davis.)*

Purification of virus from clinical samples or tissue culture yields three distinct populations of particles[33]: (1) mature hepatitis A virions that band at 1.32 to 1.34 g/cm^3 in cesium chloride and sediment at approximately 160 S (similar to enteroviruses and cardioviruses), (2) a lower-density fraction that bands at approximately 1.27 g/cm^3 in cesium chloride, and (3) sediments at 70 to 80 S that may represent empty capsids or particles with incomplete genomes and a high-density fraction (1.4 g/cm^3) that may represent particles with a more open virion structure that allows increased penetration and binding of cesium chloride to

the viral particle. These high-density particles have been shown to contain RNA but tend to be less stable than mature virions.[34,35]

HAV released from cells was shown to be enveloped in host-derived membranes that protect the virion from antibody-mediated neutralization. These quasienveloped virions are fully infectious and circulate in the blood of infected humans. They are secreted nonlytically from infected cells in the form of small extracellular vesicles approximately 50 to 110 nm in diameter. Antibodies to capsid restrict HAV replication after infection with the enveloped virus, suggesting a possible mechanism for postexposure prophylaxis (PEP).[36]

Resistance to Physical and Chemical Agents

HAV is a relatively stable virus under a variety of environmental conditions. HAV is more resistant to heat than other picornaviruses and may not be completely inactivated (depending on the conditions) by exposure to 60°C for 10 to 12 hours.[37–39] Complete inactivation in food requires heating to higher than 85°C for at least 1 minute.[39] Outbreaks of hepatitis A have been reported after ingestion of steamed shellfish, suggesting that the internal temperature achieved by steaming sometimes may be insufficient to destroy the virus.[40] However, HAV can be reliably inactivated by autoclaving (121°C for 30 minutes).[41] Studies have also shown that HAV can be inactivated in shellfish by a nonthermal method using high hydrostatic pressure.[42] The virus is resistant to most organic solvents and detergents and to a pH as low as 3.[41,43] HAV can be inactivated by many common disinfecting chemicals including hypochlorite (bleach) and quaternary ammonium formulations containing 23% hydrochloric acid (HCl) (found in many toilet bowl cleaners).[41] Currently licensed vaccines are inactivated by 1:4000 formalin at room temperature for at least 15 days to exceed complete inactivation by at least threefold. As a result of several outbreaks of hepatitis A in hemophiliacs who received factor VIII concentrates that had been treated by means of a solvent detergent method for inactivation of lipid-enveloped viruses, interest has focused on techniques capable of inactivating nonenveloped viruses without compromising the biologic activity of the product.[44] Various manufacturers use different techniques to inactivate or remove HAV and human parvovirus B19. These include nanofiltration and other purification techniques, sensitive nucleic acid testing methods such as polymerase chain reaction (PCR) on minipools of source plasma, pasteurization at 60°C for 10 hours, and dry heat on lyophilized products.

Genome and Proteins

Although HAV had the characteristics of a picornavirus and indirect tests suggested an RNA genome, molecular cloning and sequence analysis demonstrated that the HAV genome is composed of single-stranded, positive-sense linear RNA of 7478 nucleotides (strain HM175) and a molecular weight of approximately 2.25×10^6 Da, with an overall structure and gene order typical of picornaviruses.[17,19,45]

The 5′ end of the genome does not have a cap structure, but instead, as is typical for picornaviruses, has a small, covalently bound protein termed VPg.[42] The genome itself has a long 5′ untranslated region beginning with UU as found in all picornaviruses. This 5′ untranslated region folds to form a highly ordered secondary structure that is important for both replication and translation. These structured regions include an internal ribosome entry site (IRES) that directs the initiation of translation at either of two AUG codons at nucleotide positions 735–737 and 741–743 (Fig. 174.3).[45] The AUG codon initiates translation of a single long open reading frame of 6681 nucleotides that encodes a polyprotein that is 2227 amino-acid residues in length. The coding region of picornaviruses has been arbitrarily divided into three parts, termed P1, P2, and P3, and the peptides that are ultimately cleaved from the translation products of these regions are referred to as 1A, 1B, 1C, 2A, 2B, 2C, and so forth, in order of translation from the 5′ to the 3′ end of the genome.[46] The HAV genome ends with a 3′ noncoding region of 63 nucleotides that is followed by a virus-encoded poly (A) tail.

A peculiar property of the HAV coding region is a highly deoptimized codon usage. The HAV genome is significantly biased against codons used abundantly by human cells. The accumulation of rare codons was hypothesized to result in the reduced translation rate and modulation of translation kinetics of HAV RNA, thus playing an important role in HAV replication and evolution.[47,48]

The four capsid proteins of mature virus particles are coded by the 5′-end 2373 nucleotides of the long open reading frame (P1 part) and the nonstructural proteins by the remainder (P2 and P3 parts). The four HAV capsid polypeptides named by analogy with other picornaviruses are referred to in descending order of size as virion proteins (VP): VP1 = peptide 1D (molecular weight, 32,800 Da), VP2 = 1B (24,800 Da), VP3 = 1C (27,300 Da), and VP4 = 1A (2500 Da).[49,50] A VP0 protein (1AB) that is the precursor to VP4 and VP2 can also be detected, especially from cell cultures where immature virions (provirions) may accumulate in large amounts.[51] The VP4 molecule is believed to be liberated during the maturation cleavage of VP0, which converts

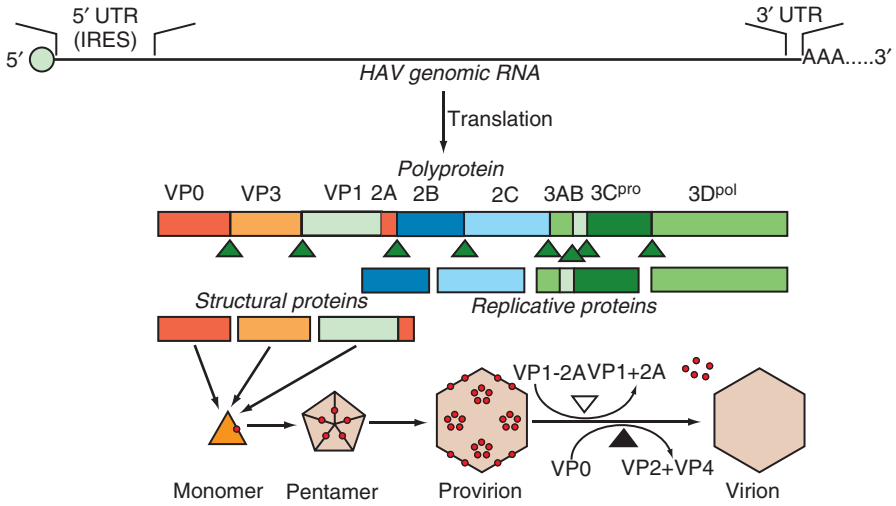

FIG. 174.3 Organization of the RNA genome of hepatitis A virus (HAV), polyprotein cleavage, and viral assembly. The 7.5-kb positive-strand RNA is covalently attached to VPg (5′ end) and has a poly (A) tail. The 5′ untranslated region *(UTR)* of 734 nucleotides functions as an internal ribosome entry site *(IRES)* to initiate translation *(vertical arrow)* of the precursor polyprotein of 2227 amino acids. Regions of the polyprotein are indicated according to standard nomenclature. The single viral protease, $3C^{pro}$, cleaves itself from the polyprotein and subsequently cleaves elsewhere in the polyprotein to yield the structural protein precursors VP0, VP3, and VP1-2A (PX) and replicative proteins 2B, 2C, 3A, 3B, and 3D (RNA-dependent RNA polymerase). VP0, VP3, and VP1-2A probably remain associated as a monomer and then form pentamers that are a stable precursor in capsid formation. Assembly of 12 pentamers together with RNA forms the provirion, after which 2A is susceptible to cleavage by host cell protease(s) *(open arrowhead)*. The final maturation cleavage of VP0 to VP2 and VP4 *(solid arrowhead)* is dependent on the encapsidated viral RNA.

provirions to virions (see later), but VP4 has never been experimentally determined to be within the virion particle and, at just 23 amino acids, is approximately one-third the size of the VP4 proteins of other picornaviruses.

Assembly of HAV particles proceeds through several steps (see Fig. 174.3). Cleavage of the polyprotein by the 3C protease yields three capsid-related proteins—VP0, VP3, and VP1-2A (also known as PX)—which constitute a monomer and subsequently assemble into pentameric subunits. Twelve copies of the pentamer then associate with viral RNA to form provirions or to form empty capsids (procapsids) without viral RNA. The involvement of the VP1-2A precursor in assembly is unique to HAV, and it has been shown that the 2A extension is essential for proper processing and assembly of the pentameric subunit.[52–54] After assembly, 2A is removed from VP1 by cellular proteases,[55,56] and in the final maturation step, VP0 is cleaved to yield VP2 and VP4. The VP0 cleavage is dependent on the presence of viral RNA in the particle, and procapsids therefore fail to cleave VP0, but HAV procapsids are quite stable and seem to have the same antigenic structure as mature virions.

Antigenic Composition and Viral Diversity

Although a variety of genotypes of HAV have been identified through analysis of genome sequences (Fig. 174.4), there seems to be only one serotype.[57–59] This view is supported by the observation that IG prepared in developed countries and monovalent vaccines prepared from strains originating in Australia, Central America, or Europe protect travelers from disease equally well, irrespective of their destination.[60,61]

Neutralization sites for HAV are located primarily on the structural proteins VP1 and VP3, with possibly a minor contribution from VP2.[57] Monoclonal antibodies in competition binding assays and the generation of neutralization escape mutants suggest that the dominant neutralization site is composed of overlapping epitopes on VP1 and VP3 that combine to form a conformational antigenic site at which neutralizing antibodies are targeted.[57,59,62]

Classification of HAV into genotypes and subtypes through use of a 168-nucleotide fragment containing the VP1/P2A junction was originally suggested. Genotypes differ by at least 15% and subtype by 7% to 7.5% from each other in this region.[26] Seven genotypes, I to VII, identified with this approach were recently reclassified into six genotypes, I to VI, using the complete VP1 region. Genotypes I, II, and III are divided into subtypes A and B. The former genotype VII is classified as subtype IIB (2). These three genotypes infect humans, whereas genotypes IV to VI have a simian origin.[26,63,64] Genotypes have a particular geographic distribution.[26,65,66] Genotype I is most prevalent worldwide, with subtype IA being more common than IB, and is the most common in the United States.[26,65] Although IB usually constitutes only a small fraction of genotype I strains circulating in South and North America, Europe, Asia, and Africa,[64] it was reported among 35% to 70% of patients infected with HAV genotype I in Brazil and was identified in an outbreak in Thailand.[67–69] Genotype IB is predominant in the Middle East[70,71] and South Africa.[72] Genotype II isolates originally identified in France in 1979 and Sierra Leone in 1988[26] are not frequently reported. Subtype IIA was suggested to have West African origin.[73] Genotype III can be identified globally,[64] but it is endemic in Southeast and Central Asia.[26] An increase in genotype IIIA infections was reported in Korea and Russia.[28,74] In India, reported hepatitis A outbreaks have all been caused by genotype IIIA infections.[75–77] Genomic heterogeneity of HAV is used to track transmissions during outbreak investigations,[66] with the VP1/P2A region being most frequently applied for genetic identification of HAV strains.[66,78] Although individual strains of HAV have differences at the molecular level that may be useful for epidemiologic studies, a high degree of identity in nucleic acid (up to 90%) and amino-acid sequence (up to 98%) is generally seen between strains.[66,79]

Biology of Hepatitis A Virus in Cell Culture

HAV was first propagated in marmoset liver explant cultures and a cloned line of fetal rhesus monkey kidney cells (FRhK-6) with a strain of virus (CR326) that had been adapted by multiple passages in *Saguinus mystax* and *Saguinus labiatus* marmosets.[14] Many HAV strains have subsequently been isolated from clinical material, although the procedure may take several weeks. Until recently, only epithelial or fibroblast cells of primate origin had been shown to support growth of the virus.[15,16,80] However, in a systematic search for cells that would support HAV replication, growth was detected in cells of guinea pig, dolphin, and porcine origin.[81]

The major characteristics of HAV in cell culture are slow growth and low yields relative to other picornaviruses, which is likely associated with the inefficient IRES,[82–84] inability to induce the cellular protein synthesis shutoff,[85] and highly deoptimized codon use.[47,48] In addition, the virus remains largely cell associated, does not usually produce a cytopathic effect, and readily leads to persistently infected cell lines.[86] With adaptation, more rapid replication and higher yields can be obtained and cytopathic variants have been selected. These cell culture–adapted viruses are useful for virus titrations, neutralization, inactivation kinetics, and virus replication and have made the production of inactivated vaccines practical.[14,16,87–89]

In one study, direct isolation of a wild-type strain of HAV was achieved through use of a modified cell line.[90] Detection of HAV in either patients' or environmental samples is now done primarily using PCR or other nucleic acid–based technology. The kinetics of viral replication and biosynthetic events has been studied in cells infected with cell culture–adapted strains of HAV, and a number of differences from most other picornaviruses have been revealed. After attachment to cells, the uncoating of virus is delayed for more than 8 hours[91,92]; this exceeds the duration of an entire growth cycle for many picornaviruses. The delayed uncoating seems to be related to the protracted maturation cleavage of VP0 to VP2 and VP4 because virions are uncoated more rapidly than provirions.[93] Accumulation of new viral RNA can be detected as early as 12 hours after infection of BS-C-1 cells with a fast-growing, cytopathic variant of strain HM175, but levels of viral replicative intermediates (double-stranded RNAs) remain much lower than in cells infected with other picornaviruses.[91] As outlined previously, translation of the viral polyprotein is directed by the IRES within the 5′ untranslated region, but the IRES of HAV is relatively inefficient, with initiation approximately 1% of that seen for the IRES of encephalomyocarditis virus.[82–84]

The initial proteolytic processing of HAV polyprotein is accomplished by the viral 3C protease, and assembly of viral particles proceeds via monomers and pentamers (see Fig. 174.3).[52] After infection of cell cultures with rapidly replicating and cytopathic variants of HAV, pentamers are first detected at 9 hours after infection and reach peak levels after approximately 18 hours even though the amount of viral RNA (and presumably viral translation) increases beyond this time.[94] These cells continue to produce virus for 2 to 3 days before cell death, whereas most HAV variants progress to a persistent infection with reduced levels of virus production over many weeks and subsequent cell passages.

Repeated passage in cell culture has been used to apply mutational pressure to HAV to alter the phenotype. For example, HAV variants that grow more rapidly or are resistant to neutralization by monoclonal antibodies have been selected.[89,95,96] Attenuated strains of HAV have been selected by means of multiple tissue culture passages, and cold adaptation has been achieved by means of passage at reduced temperature.[31,97] Some of the mutations responsible for these altered phenotypes have been identified through molecular cloning and sequencing of the mutant. Mutations within the 5′ untranslated region and mutations within the 2B and 2C coding regions of HAV RNA have been shown to enhance virus replication in vitro.[96,98,99] However, mutations within the VP1-2A and 2C proteins seem to be most important for attenuation of virulence.[96]

Most viruses initiate infection by first binding to a specific cell surface receptor molecule or, in many cases, may require binding to both receptors and coreceptors to facilitate virus entry and uncoating. Identification of a specific receptor for HAV remained elusive for many years. However, Kaplan and colleagues[100] succeeded in the isolation of one specific receptor molecule, havcr-1, first in cells of simian origin and later in human cells.[101] This molecule is a novel mucin-like class I integral membrane glycoprotein of 451 amino acids, known as T-cell immunoglobulin and mucin domain1 (TIM-1), with the amino-terminal, cysteine-rich domain responsible for binding to HAV.[101] Because this

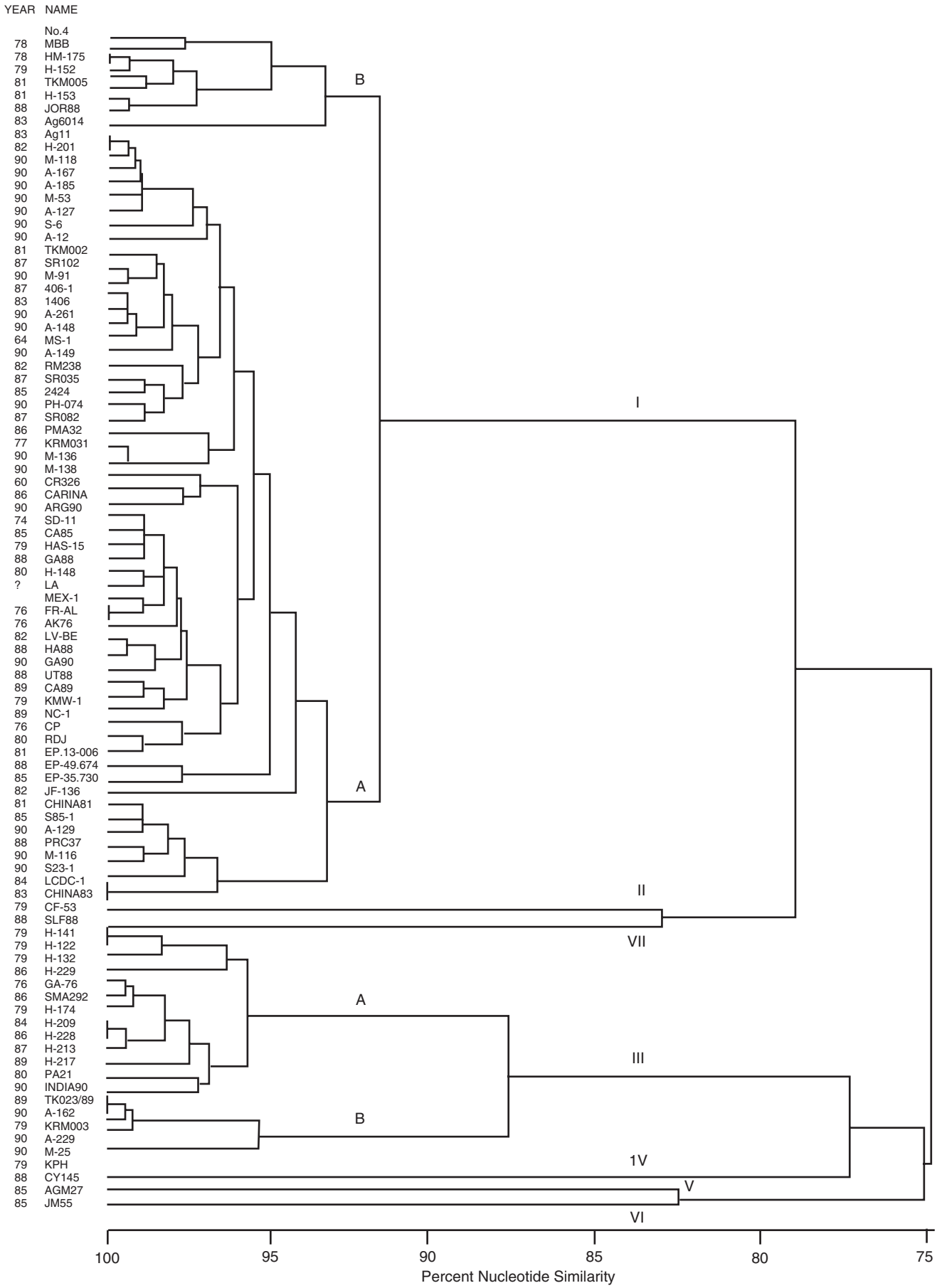

FIG. 174.4 Relationship among hepatitis A virus strains determined by the sequence of the VP1-2A region. Nucleotide sequence alignments of 92 isolates were compared. The genotypes were divided into groups I to VII, and groups I and III were divided into subgroups A and B. *(Courtesy S.M. Lemon, Galveston, TX.)*

molecule is expressed on cells from many tissues that are not susceptible to HAV infection, it is likely that specific coreceptors contribute to the organ tropism of HAV. However, a soluble form of havcr-1 has some ability to neutralize particles of HAV directly, which is consistent with roles in both attachment and uncoating of virus.[102]

It remains possible that HAV may use other pathways for cell entry in addition to havcr-1. Studies have demonstrated that the asialoglycoprotein receptor can also mediate infection of cells with HAV when the virus is first complexed with specific immunoglobulin A (IgA), leading to the interesting hypothesis that IgA may play a role as both carrier and targeting molecule during infection and transmission, particularly in relapsing cases of HAV.[103]

Two forms of HAV virions, naked and quasienveloped, appear to use different mechanisms for cell entry. The quasienveloped HAV enters cells via an endocytic pathway. Slow removal of the membrane in endosomal compartment exposes the capsid to neutralizing antibody for 6 to 8 hours but allows for interaction with the cell receptor. The naked HAV uncoats rapidly without endosomal acidifications and is not affected by postendocytic neutralization. Both forms likely bind to the same cell receptor, TIM-1 (HAVCR1).[104] However, recent studies have shown that TIM-1 does not seem to be essential for cell entry of either HAV form.[104]

Host Range

Humans are considered to be the only important reservoir of HAV. However, the existence of extrahuman reservoirs of infection remains possible. In 1961, Hillis[105] described an outbreak of hepatitis A among chimpanzee handlers who apparently contracted the infection from the chimpanzees. Epidemiologic data suggested that the animals had become infected during captivity but before their importation into the United States. Interesting to note, although epidemics of hepatitis were recognized in American primate handlers, the disease was rarely seen in Africa, presumably because most handlers were already immune. Widespread screening of nonhuman primates revealed antibodies to HAV in chimpanzees, gorillas, orangutans, gibbons, macaques, owl monkeys, pigtail monkeys, rhesus monkeys, and several species of South American tamarin monkeys.[106,107] It is unclear whether such primates may serve as reservoirs of infection or as transient hosts after exposure to HAV from human sources. However, both the PA21 and AGM-27 strains of HAV seem to be true simian viruses.[108,109] Interesting to note, the AGM-27 virus produces attenuated disease in chimpanzees and was the subject of some study as a potential live-attenuated vaccine.[110]

Deinhardt[111] pioneered the use of nonhuman primates for hepatitis A studies in both chimpanzees and tamarins (*Saguinus* species). He showed that in chimpanzees, liver function test abnormalities developed on inoculation with known infectious material of human origin. Since that time, the chimpanzee, several species of tamarins, and the owl monkey have all been shown to be susceptible to HAV infection and to develop hepatitis.[111] These animals have been valuable tools in the study of hepatitis A pathogenesis and for the development of vaccines. Recently, it was shown that guinea pigs express a receptor similar to the human HAV receptor and that both guinea pig cells and guinea pigs can be infected with HAV. However, the animals did not have evidence of hepatitis.[81,112]

Molecular mechanisms of the HAV host specificity are not well understood. Recent research using a murine model of HAV infection suggested a role of the intrahepatic immune responses in defining the host range, with the HAV evasion of MAVS-mediated type I interferon responses being essential for restricting the infection to humans and nonhuman primates.[113] Although restricted in its natural infection of host species, adaptation of HAV to growth in murine and guinea pig cells suggests a certain capability to replicate in other hosts.[112,114]

EPIDEMIOLOGY

Modes of Transmission

HAV is primarily transmitted via the fecal-oral route. After replication in the liver, the virus is excreted in bile and is found in highest concentrations in stool. In experimental studies, infectivity of stools was demonstrated from 21 days before to 8 days after onset of jaundice, but the highest concentrations occur during the 2-week period before jaundice

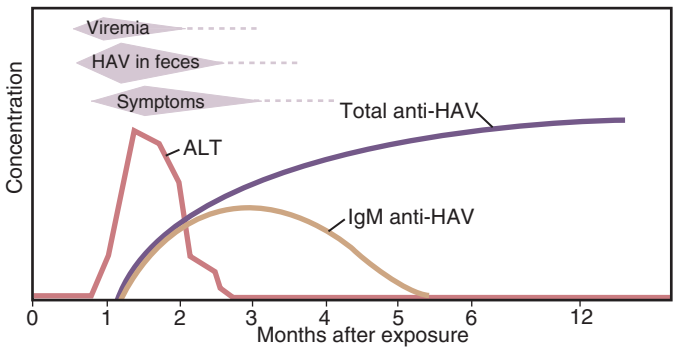

FIG. 174.5 Clinical, virologic, and serologic events associated with hepatitis A virus *(HAV)* infection. *ALT,* Alanine aminotransferase.

develops or liver enzymes increase, followed by a rapid decrease after the appearance of jaundice (Fig. 174.5).[11,115,116] Data from epidemiologic studies also suggest that peak infectivity (risk of transmission) occurs during the 2 weeks before the onset of symptoms.[117] Shedding of HAV in stool may continue for longer periods in infected infants and children than in adults. HAV RNA has been detected in stool of infected newborns for as long as 6 months after infection.[118] Excretion in older children and adults was demonstrated 1 to 3 months after clinical illness.[61,118] Although chronic shedding of HAV does not occur, the virus has been detected in stool during relapsing illness.[119]

During the period of viremia, which begins during the prodrome and extends through the period of increased liver enzymes (see Fig. 174.5), HAV concentrations in serum are several orders of magnitude lower than in stool.[120–122] However, in experiments conducted in nonhuman primates, HAV was several orders of magnitude more infectious when administered via the intravenous compared with the oral route, and animals were successfully infected with low concentrations of HAV administered via the intravenous route.[123] Although HAV may occasionally be detected in saliva in experimentally infected animals and may be present in human saliva,[124,125] transmission by saliva has not been demonstrated.

The infectious dose of HAV in humans is unknown. Enzyme immunoassays and PCR may detect defective (noninfectious) and infectious viral particles. Thus, the detection of HAV antigen in the stool with enzyme immunoassays or HAV RNA in the serum, stool, or saliva with PCR does not mean that an infected person is necessarily infectious, and it is likely that the period of infectivity is shorter than the period during which HAV RNA is detectable. For practical purposes, both children and adults with hepatitis A can be assumed to be noninfectious 1 week after jaundice appears.

Person to Person

Person-to-person transmission via the fecal-oral route remains the primary means of HAV transmission throughout most of the world.[126,127] Transmission can occur among close contacts, particularly in households and extended family settings.[128] In the United States prior to the introduction of hepatitis A vaccine, young children had the highest rates of infection, and children often served as a source of HAV infection for others because HAV infection is often asymptomatic and standards of hygiene are generally lower in young children compared with adults, as in daycare facilities.[128–130] Since the introduction of vaccine, person-to-person transmission in the United States is now more commonly seen among special populations including people experiencing homelessness, people who use injection and noninjection drugs, and men who have sex with men (MSM).[131,131a]

Similar patterns are emerging in Western Europe.[132–134]

Foodborne and Waterborne

HAV can remain infectious in the environment for long periods of time,[135] allowing for common source outbreaks and sporadic cases to occur from exposure to fecally contaminated food or water. Many uncooked foods have been recognized as the source of outbreaks. Cooked

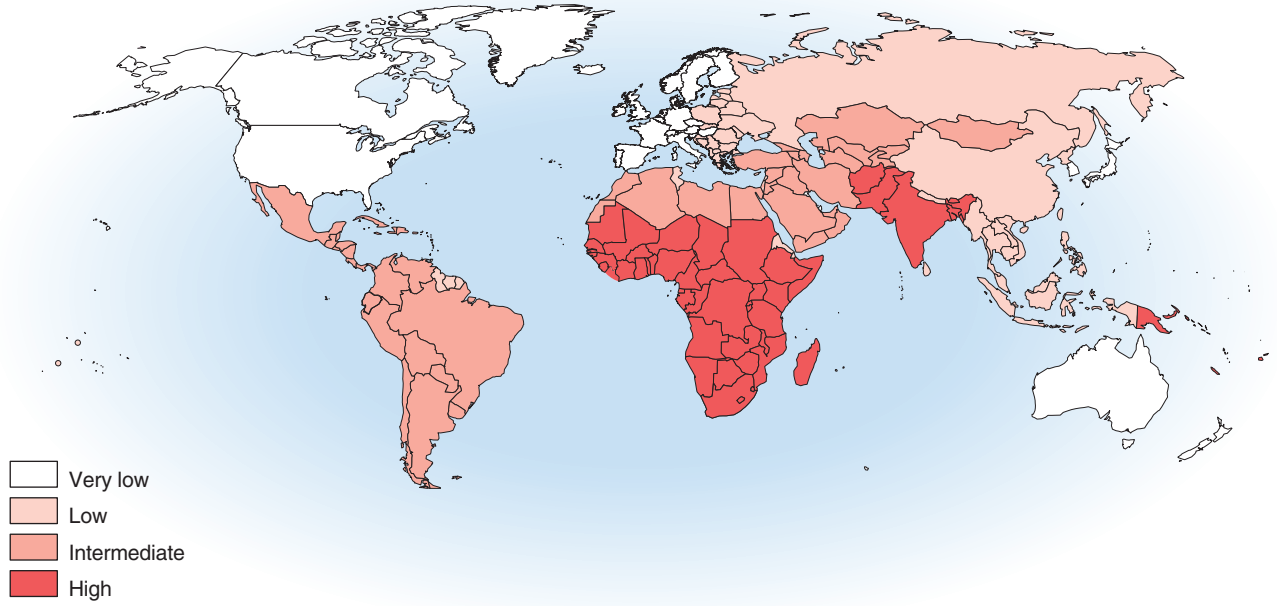

FIG. 174.6 World map indicating patterns of endemicity of hepatitis A virus infection, 2005. The patterns of high, transitional, intermediate, low, and very low endemicity are shown. *(Modified from Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005.* Vaccine. *2010;28:6653–6657.)*

foods also can transmit HAV if the cooking is inadequate to kill the virus or if the food is contaminated after cooking, as commonly occurs in outbreaks associated with infected food handlers.[136,138,139] Contaminated shellfish were responsible for a large outbreak in Shanghai, China, in 1988.[140,141] Although such outbreaks continue to be reported,[142–144,145,146] shellfish-associated HAV outbreaks in the United States and other developed countries have become increasingly uncommon. In Italy, detection of HAV in samples of shellfish has decreased, believed to be the result of hepatitis A vaccination programs in that country.[147] Several large outbreaks traced to produce contaminated before distribution have been reported in recent years from the United States, Europe, and Australia.[148–150,151–157] Waterborne outbreaks of hepatitis A are also uncommon in developed countries, and are difficult to differentiate from other forms of transmission in the absence of well-designed studies in less developed countries. Molecular techniques now allow for identification of common source foodborne hepatitis A outbreaks around the globe.[153–155,158]

Bloodborne

Transfusion-related hepatitis A is rare because HAV does not result in chronic infection, and, in the developed world, blood donors have been screened for many years for elevated aminotransferase levels, further decreasing the risk of transmission during the viremic prejaundice phase of infection. However, transmission through transfusion of blood or blood derivatives collected from donors during the viremic phase of their infection has been reported, including outbreaks in Europe and the United States among patients who received factor VIII and IX concentrates prepared with solvent-detergent treatment to inactivate lipid-containing viruses.[44,122,159,160–162] HAV is resistant to solvent-detergent treatment, and contamination presumably occurred from plasma donors with hepatitis A who donated during the incubation period. The degree to which transfusion-related transmission occurs in countries where screening for blood and blood products may be limited is unknown.

Vertical

Two published case reports describe intrauterine transmission of HAV during the first trimester, resulting in fetal meconium peritonitis.[163,164] After delivery, both infants were found to have a perforated ileum. The risk of transmission from pregnant women who develop hepatitis A in the third trimester of pregnancy to newborns appears to be low.[165] However, newborns who acquire infection in this manner are usually asymptomatic, and an outbreak among hospital staff related to exposure to such an infant has been reported.[166]

Worldwide Disease Patterns

Globally, tens of millions of hepatitis A infections occur annually,[167–169] resulting in an estimated 11,000 deaths annually from hepatitis A.[170] Although hepatitis A occurs worldwide, major geographic differences exist in endemicity and resulting varying epidemiologic patterns (Fig. 174.6).[167–169] The degree of endemicity is closely related to hygienic and sanitary conditions and other indicators of the level of development. In less developed areas, especially when there is limited access to clean water and inadequate disposal of human feces, HAV infects most people early in life, when infection is rarely clinically apparent When high standards of hygiene and sanitation apply, the majority of adults remain susceptible. Distinct patterns of HAV infection can be described, each characterized by particular age-specific anti-HAV prevalence (Fig. 174.7) and hepatitis A incidence; these patterns are a result of prevailing environmental (hygienic and sanitary) and socioeconomic conditions.[126,167–169]

In areas of high endemicity, represented by the least developed countries (i.e., parts of Africa and South Asia), poor hygienic and sanitary conditions allow HAV to spread readily (see Figs. 174.6 and 174.7). Infection is nearly universal in early childhood, when asymptomatic infection predominates, and most of the population is infected before reaching adolescence, as demonstrated by the age-specific prevalence of anti-HAV (see Fig. 174.7).[167,171,172] Because most adults are immune, reported disease rates in this population are low and few outbreaks occur. However, susceptible adolescents and adults in these areas are at high risk for hepatitis A. Recent surveillance data have demonstrated increasing numbers of cases among adults and frequent outbreaks, and small seroprevalence studies have identified greater susceptibility of some children into adolescence, in some highly endemic countries.[173–176] Differences in susceptibility to hepatitis A in low- and middle-income countries can be defined by socioeconomic status, wherein more affluent children living under more hygienic conditions are more likely to escape HAV infection as infants and young children, leaving them susceptible to HAV infection as adolescents and adults.[177] In past years, some ethnic

or geographically defined groups within highly developed countries also experienced high endemicity, including Native Americans, aboriginal populations in Australia, and Roma populations in Slovakia.[178–180]

In areas of intermediate endemicity, HAV is not transmitted as readily because of better sanitary and living conditions, and the predominant age at infection is older than in areas of high endemicity (see Figs. 174.6 and 174.7).[168,181] Paradoxically, the overall incidence and average age in reported cases are often higher than in highly endemic areas because high levels of virus circulate in a population that includes many susceptible older children, adolescents, and young adults who are more likely to develop symptoms with HAV infection.[182] Large common source food- and water-associated outbreaks can occur because of the relatively high rate of virus transmission and large number of susceptible persons, especially among those of higher socioeconomic level. The largest such outbreak was reported from Shanghai in 1988, with more than 300,000 cases associated with consumption of clams harvested from water contaminated with human sewage.[140] Nevertheless, person-to-person transmission in community-wide epidemics continues to account for much of the disease in these countries.

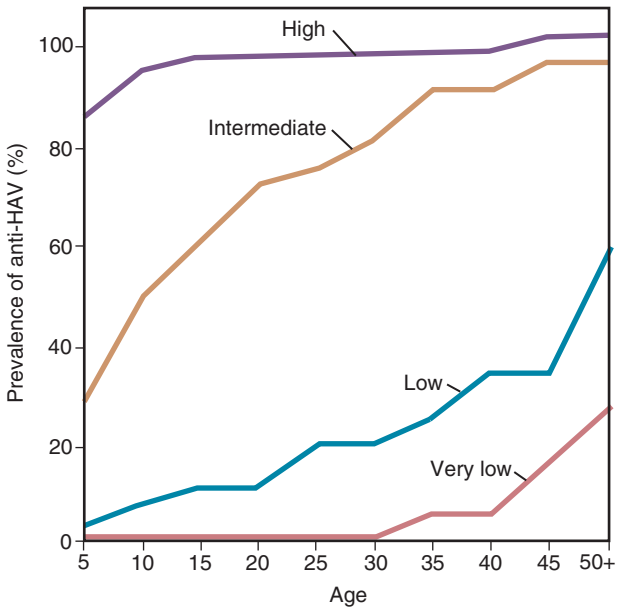

FIG. 174.7 Patterns of hepatitis A virus *(HAV)* infection worldwide. Age-specific prevalence of anti-HAV in areas of high, intermediate, low, and very low endemicity is shown.

Shifts in age-specific prevalence patterns that reflect a transition from high to intermediate endemicity are occurring in many parts of the world (see Fig. 174.6).[183–188] A feature of this transitional pattern is striking variations in hepatitis A epidemiology among countries and within countries and cities, with some areas displaying a pattern typical of high endemicity and others of intermediate endemicity.[126,183,189–194,195–200] Considerable hepatitis A–related morbidity and associated costs occur with this transition, even in developing countries.[201,202] For example, hepatitis A was the cause of fulminant hepatitis in two-thirds of children presenting to two hospitals in Argentina during a 15-year period, and, in one of these hospitals in which liver transplantations were performed, one-third of liver transplantations among children were performed for fulminant hepatitis A.[201]

In high-income, developed countries, including the United States, Canada, and much of western Europe, the endemicity of HAV infection is low or very low (see Fig. 174.6). Few children are infected, the incidence of disease is generally low, and disease is usually sporadic. Community-wide outbreaks are becoming increasingly rare; in the United States this may be because of routine hepatitis A vaccination of children.[126,162,179,203–207] Population-based seroprevalence surveys show a gradual increase in the prevalence of anti-HAV with increasing age, primarily reflecting declining incidence, changing endemicity, and resultant lower childhood infection rates over time. In countries where hepatitis A vaccine has been introduced, such as the United States, anti-HAV prevalence is increasing among children as a result of vaccination.[208] In low-and very-low-endemicity countries, most cases occur in defined risk groups such as travelers returning from endemic areas, MSM, and users of injection drugs.[162,207,209–214] The United States has recently experienced outbreaks among people experiencing homelessness and people who use injection and noninjection drugs.[131a] There is documented risk of hepatitis A among children and other contacts of family members who are residing in low endemic countries and travel to their high or intermediate endemic country of origin.[215–218] An emerging risk for hepatitis A transmission in low endemic countries stems from asylum seekers or other persons displaced from their home country owing to conflict.[219–221]

Epidemiology in the United States

Although large national outbreaks were recognized during the 1950s, data collection for hepatitis A in the United States started in 1966. Surveillance data demonstrates that periodic outbreaks, approximately every decade, occurred through the 1990s, before hepatitis A vaccine was widely available (Fig. 174.8).[222,223] The highest rates and the majority of cases occurred in the western and southwestern states.[224–226] With the advent of routine vaccination of children, large community-wide outbreaks and cyclic peaks have ceased. The geographic variations that characterized hepatitis A incidence in the past have also disappeared.[225]

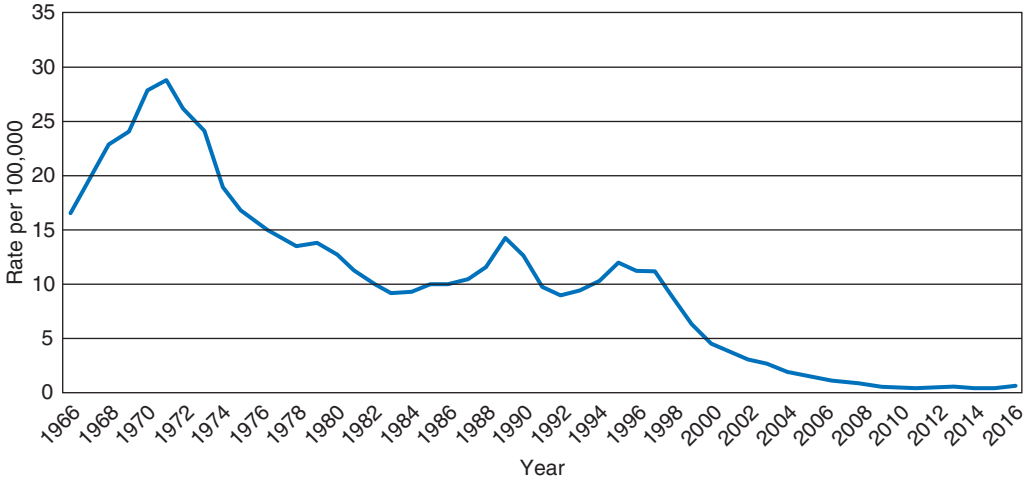

FIG. 174.8 Hepatitis A incidence, United States, 1966–2016. *(From Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, GA.)*

In 1995, when hepatitis A vaccine was licensed in the United States, more than 31,000 cases of hepatitis A were reported (incidence, 12 cases per 100,000 persons), making hepatitis A one of the most frequently reported vaccine-preventable diseases.[227] In 1996–99 the Advisory Committee on Immunization Practices (ACIP) recommended targeted vaccination for children at 2 years of age in states with high hepatitis A rates.[224,228] Incidence rates declined sharply after implementation of these recommendations (see Fig. 174.8).[223,229] In 2006, universal vaccination was recommended in all states for all US infants starting at 1 year of age.[226] The number of cases reported annually in the United States dropped steadily from the late 1990s through 2010, plateauing at between 1500 and 2000 cases reported per year (i.e., <1 case per 100,000 population) through 2016.[223] Since 2012, high-profile multistate outbreaks of hepatitis A and increased reporting associated with heightened awareness around the outbreaks may have accounted for the plateauing of the number of reports.[155] Nevertheless, it is clear that hepatitis A rates have declined since vaccine introduction from 11.7 cases per 100,000 population in 1996 to less than 1 per 100,000 population in 2016, a greater than 10-fold decline.[229a]

Young children typically have asymptomatic or unrecognized infections, and have historically, before vaccine introduction, played an important role in the epidemiology of hepatitis A in the United States, serving as a major reservoir for HAV transmission.[230] Before vaccine introduction in the United States, the highest hepatitis A rates were reported among children 5 to 14 years old, with approximately one-third of cases occurring among children younger than 15 years;[224] the majority of infections were among asymptomatic children <5 years of age.[130] Hepatitis A rates among children have declined more sharply than among adults since vaccine introduction and, through 2016, rates among both adults and children have been around 1 case per 100,000, or less, with children <10 years of age having the lowest rates of any age group (Fig. 174.9).[223] Historically, rates of hepatitis A among Hispanics and Native Americans/Alaska Natives have been higher than those of other racial or ethnic populations, but currently these differences have largely disappeared.[223] Mortality associated with hepatitis A in the United States is uncommon; 60 to 80 cases were reported annually during 2012 to 2016, with highest rates among persons aged 55 years and older.[223]

In the United States, before the introduction of vaccine when community outbreaks and outbreaks in child care centers were common, studies of outbreaks wherein serologic testing of household contacts was performed found that 25% to 40% of contacts <6 years of age had serologic evidence of acute HAV infection,[128,231] resulting in some immunity among children as they entered adolescence and adulthood.[232,233] A nationally representative serosurvey conducted during 2007–2010 found that among persons ages 6 to 19, the prevalence of anti-HAV was 37.6%, compared with an anti-HAV prevalence of 13.1% conducted during 1988–94, before the availability of hepatitis A vaccine, reflecting increasing immunity among children due to vaccination. However, results from serial national serosurveys among the entire population conducted for the periods 1988–94 and 2003–06 indicate an overall decline in the age-adjusted seroprevalence of anti-HAV in the US population from 32.5% in 1988–94 to 26.7% in 2003–06, reflecting the decreased incidence in the country over time.[208] Substantial declines in HAV incidence, likely fueled in part by the childhood vaccination program, have resulted in an overall decrease in seroprevalence rates among US adults and therefore an increase in susceptibility to HAV among older adults.[208,234] A 2013 multistate outbreak of hepatitis A occurred largely in adults, with few cases among vaccinated and unvaccinated children.[155] In summary, the epidemiology of hepatitis A in the United States has fundamentally changed as endemic transmission, outbreaks in child care centers, and large community outbreaks have been virtually eliminated, in part through improvements in hygiene, but largely through the childhood vaccination program. We are likely to continue to see a greater proportion of cases among adults due to international travel, outbreaks associated with MSM, outbreaks associated with imported food contaminated with HAV, and transmission among marginalized populations including people experiencing homelessness and people who use illicit drugs.

Potential Sources of Infection

Risk factor and exposure data for hepatitis A cases are often lacking in the United States. Before 2010, on the basis of the data collected from enhanced disease surveillance, the most commonly reported exposure to HAV in the United States was international travel (accounting for 46% of cases), followed by household or sexual contact with individuals with hepatitis A.[235] Although data are limited by underreporting, approximately three-quarters of hepatitis A cases reported through routine surveillance in the United States do not have a recognized source of infection.[223] Surveillance data from 2016 identified food (and waterborne) outbreaks as the most common source of infection (75.8%) among case reports with an identified risk factor or exposure.[223] Cases associated with child care centers or preschools, which previously accounted for a

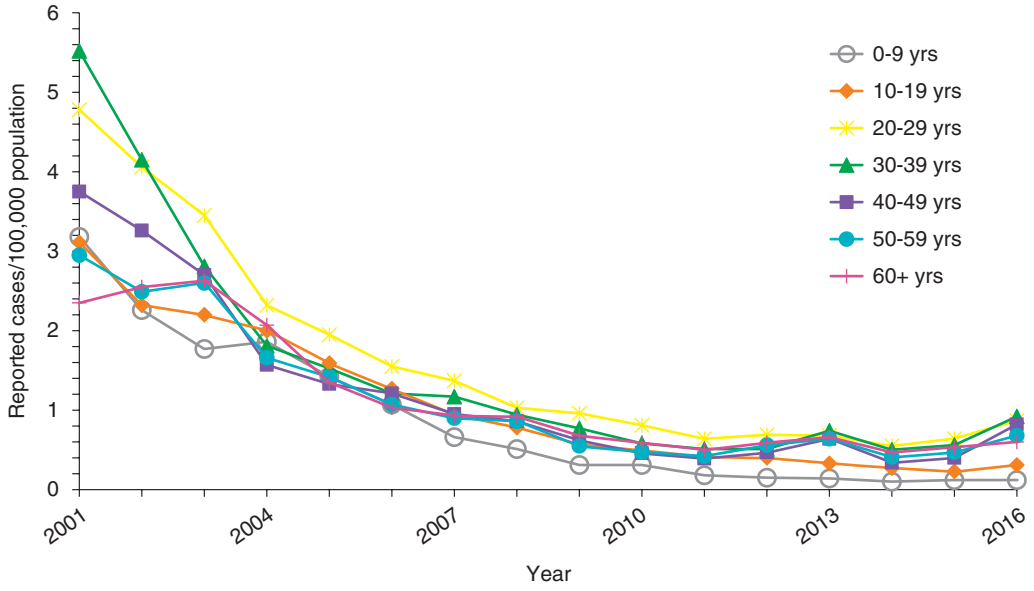

FIG. 174.9 Incidence of hepatitis A by age group—United States, 2001–2016. Rates of reported hepatitis A reached a low point in 2014 for all age groups except 10 to 19 years; for that group the low point occurred in 2015. Rates increased for all age groups from 2015 and 2016, except for those aged 0 to 9, whose rates remained stable. When the 2016 hepatitis A rates of all age groups are compared, persons aged 20 to 29 years and 30 to 39 years had the highest rate (0.9 cases per 100,000 population) and persons aged 0 to 9 years had the lowest rate (0.1 cases per 100,000 population). *(From Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System [NNDSS].)*

substantial proportion of reported exposures, were responsible for only 8.6% of all reported exposures in 2016.[223] Other reported exposures include international travel (6.5%), household or sexual contact (3.8%), illicit drug use (3.6%), and MSM (1.5%).[223] Increasingly in the United States, outbreaks among people experiencing homelessness are being recognized[131a]; however, data regarding homelessness as an exposure are not currently collected with routine surveillance.[223]

Specific Groups and Settings of Infection

International Travel

Hepatitis A is a risk for unvaccinated persons from developed countries who travel to regions with high, transitional, or intermediate endemicity (see Fig. 174.6).[236–241] A study of Swiss travelers that was published in 2006 estimated the risk to be 6 to 30 cases per 100,000 months of stay in developing countries among persons who did not receive IG or vaccine before departure.[242] A more recent study from the Netherlands found rates of 3.5 to 7.5 per 100,000 Dutch travelers.[240] The risk may be higher among travelers staying in areas with poor hygienic conditions[243] and varies according to the region and the length of stay. Declines in the number of hepatitis A cases attributed to improved hygiene and infrastructure in endemic countries have been reported for travelers from some European countries.[240,244,245] In the United States, international travel is a rarely reported risk factor.[223] In the United States and Europe, the risk of hepatitis A for travelers, especially children, traveling to endemic countries to visit relatives and friends poses a particular risk.[218] Compared with persons traveling for work or recreation, these persons generally have trips of longer duration and, by staying in family settings rather than in hotels, may have greater exposure to HAV circulating in the community. These persons also are less likely to seek or to adhere to pretravel advice.[215,246–252] Travelers who acquire hepatitis A infection during their trip may also transmit the virus to others on their return.[253] Vaccination of international travelers, including those visiting friends and family in endemic regions, remains an important consideration for pretravel preparations.[254]

International Adoption and Migrant and Refugee Populations

International adoptees with asymptomatic hepatitis A may transmit the virus to family members and close contacts. Most adoptee-associated cases of hepatitis A are among unvaccinated nontraveling contacts of the adoptee exposed during the first 60 days after arrival in the United States; secondary and tertiary transmission has been described.[255–258] Serologic results from 270 adoptees who received testing within 4 months of arrival in the United States showed 1% with evidence of acute hepatitis A; rates varied by country of origin. All acutely infected adoptees were asymptomatic.[258] A retrospective review from Minnesota, United States, from 2007 to 2009 identified 21 hepatitis A cases of among international adoptees within ≤60 months of age.[259] Another study found the prevalence of HAV infection to be 4.6% among international adoptees based on screening of adoptees from 2006 to 2010; the study noted the substantial interruption of care and social support associated with secondary caretaker infection.[260] Screening of international adoptees from endemic areas was suggested as a preventive measure; however, there is a need for a better understanding of the impact and public health importance of international adoptees introducing hepatitis A into the community.

In recent years, there has been an increase in persons displaced by conflict, resulting in refugees and migrants and particularly affecting Europe and the Mediterranean. Increased transmission of hepatitis A has reemerged as a public health problem associated with conflict and war. Conflict can lead to increases in refugees, migrants, and internally displaced people (IDPs). In recent years, conflict has directly resulted in increases in cases and outbreaks of hepatitis A being recognized in Sri Lanka among IDPs in the wake of a war[261] and among refugees and asylum seekers in Greece, Turkey, and Germany.[219–221]

Users of Injection and Noninjection Drugs and People Experiencing Homelessness

Outbreaks of hepatitis A have been reported among people who use injection and noninjection drugs in North America, Australia, and Europe.[131a,262–272] In the United States, these outbreaks have involved users of injection and noninjection drugs.[127,131a,263,267,273] Cross-sectional serologic surveys have demonstrated that injection drug users have a higher prevalence of anti-HAV than the general US population,[274–279] although a small study from Switzerland found a decrease in the seroprevalence in this population compared with previous years.[280] A randomized intervention trial of 469 injection (70%) and noninjection (30%) drug users in methadone maintenance found 56% were anti-HAV positive.[281] Transmission among injection drug users probably occurs predominantly through fecal-oral routes, but percutaneous transmission may occur.[267] People experiencing homelessness in the United States and Europe may be at increased risk of hepatitis A infection, may have a higher seroprevalence of anti-HAV, and may be associated with outbreaks.[131a,282] The risk of hepatitis A among people experiencing homelessness may be related to lack of access to water and sanitation, sexual and illicit drug use practices, years experiencing homelessness, and country of origin.[269,282–284] It is anticipated that the childhood vaccination program that began in the United States in the late 1990s will offer protection to future generations of people experiencing homelessness who were vaccinated as children.

Men Who Have Sex With Men

Hepatitis A outbreaks among MSM have been reported in urban areas in the United States, Canada, Europe, and Australia and may occur in the context of an outbreak in the larger community or a mass gathering such as a Pride Festival.[a] Seroprevalence surveys have not consistently demonstrated an elevated prevalence of anti-HAV among MSM compared with a general population of similar age.[274,294] Some studies conducted during outbreaks and seroprevalence surveys have identified specific sex practices associated with illness, whereas others have not demonstrated such associations.[275,286,289] A large cross-sectional prevalence study among MSM conducted from 1994 to 2000 in the United States found a similar prevalence of anti-HAV to the general population of similar age.[295] The study also found that factors associated with HAV infection among MSM in nonoutbreak settings are likely similar to those among non-MSM.

Transfusion and Health Care Settings

Transfusion-related hepatitis A is rare.[296] The risk of infection in patients with hemophilia is unknown, but results of one serologic survey of persons with hemophilia before the licensure of hepatitis A vaccine suggested they might have been at increased risk.[297] Outbreaks have been reported in Europe and the United States among patients who received factor VIII and factor IX concentrates.[160,298] However, no HAV infections attributed to blood products were identified in an analysis of serosurveillance data collected from 140 hemophilia treatment centers in the United States between 1998 and 2002, suggesting that improved viral inactivation procedures, donor screening, and increased hepatitis A vaccination coverage among clotting factor recipients has reduced the risk of HAV transmission.[299]

Outbreaks of hepatitis A have been reported in neonatal intensive care units after transmission to hospital staff from a neonate with asymptomatic HAV infection acquired from a blood transfusion.[118,300–302] Nosocomial transmission of hepatitis A from adult patients to health care workers also is rare because most patients with hepatitis A are hospitalized after the onset of jaundice, when infectivity is low, but it has been reported in association with fecal incontinence of patie nts.[115,303,304] Health care workers were not found to have an increased prevalence of anti-HAV compared with control populations in serologic surveys conducted in the United States before vaccine introduction.[305]

Foodborne and Waterborne Hepatitis A

Foodborne hepatitis A outbreaks are of increasing concern globally, particularly in Europe and the United States. Historically, in the United States foodborne outbreaks were most commonly recognized in association with contamination of food during preparation by a food handler with HAV infection.[136–138,306–309] Food contaminated before retail distribution, such as lettuce or fruit contaminated at the growing or processing

[a]References **127**, 131, 132, 134, 212, 272, 285–293.

stage, can be the source of hepatitis A outbreaks, some of which can be large or spread over a wide geographic area.[148–151,310–313] Since 2000, common source food exposures, such as the 2003 outbreak associated with contaminated green onions[150,308] and more recently contaminated berries imported from countries with endemic transmission of hepatitis A, have been increasingly recognized in hepatitis A low and very low endemic regions.[b] According to the Rapid Alert System for Food and Feed database in Europe, 35 notifications of HAV in food were reported between 1999 and 2013 in eight EU countries from multiple food sources.

Mixed frozen berries were implicated in a hepatitis A genotype IA outbreak in Italy in 2013.[314,316–318] An outbreak likely associated with berry mix buttermilk cake imported frozen from Germany[319] occurred in 2013–14. Other recent outbreaks of HAV genotype IB illness associated with food from the Middle East were also reported. A Nordic outbreak (Denmark, Finland, Sweden, and Norway) occurred in 2012–13 from contaminated strawberries, suspected to have originated from Egypt and Morocco.[320] Frozen pomegranate arils from Egypt were implicated in an outbreak in Canada in 2012, and imported pomegranate arils from Turkey were associated with a multistate outbreak in the United States in 2013.[155] Semidried tomatoes from Turkey were associated with cases in Europe and Australia in 2009–11.[153,154,321] Foodborne outbreaks can be resource intensive to investigate and control, and it may be challenging to identify the point source or source country of the contaminated produce.[156,157]

In low and very low endemic countries, outbreaks related to contaminated shellfish[78] have become less common but are still occasionally identified.[143–147] Waterborne hepatitis A outbreaks are rare and generally are related to sewage contamination or inadequate treatment of water.[322–324]

Although results of some serologic surveys conducted among sewage workers in Europe indicated a possible elevated risk of HAV infection, findings have not been consistent.[325–327] In published reports of three serologic surveys conducted among US sewage workers and appropriate comparison populations, no substantial or consistent increase in prevalence of anti-HAV was found among sewage workers.[328–330] No work-related instances of HAV transmission have been reported among sewage workers in the United States.

Institutions Including Daycare for Children and Facilities for Persons With Developmental Disabilities

Historically, HAV infection was commonly reported from daycare centers and endemic in institutions for developmentally disabled people in the United States. With the adoption of universal hepatitis A vaccination, transmission in daycare settings is now rarely reported.[223] For persons with disabilities, with deinstitutionalization and the shift to smaller facilities, some with improved conditions and increased availability of vaccine, the incidence and prevalence of infection have decreased and outbreaks rarely are reported in the United States.[226,227,331] However, unvaccinated disabled adults cared for in group homes are at risk because they typically live in close quarters and often are incontinent and nonverbal.[332] During 2013, an outbreak in Michigan occurred in which eight unvaccinated residents of five group homes for adults with disabilities were diagnosed with hepatitis A, and one died.[332] Unvaccinated staff working in group homes may also be at risk during such outbreaks.

PATHOGENESIS

Although HAV shares many virologic characteristics with enteroviruses, it has several differentiating features that influence the pathogenesis and clinical expression of the disease. HAV is resistant to heat, solvents, and acid and grows slowly in living cells, where it has been shown to be relatively noncytolytic and to have little effect on the rate of host protein synthesis.

Incubation Period

Determination of the incubation period of hepatitis A is imprecise because the early signs and symptoms are often vague and nonspecific. Jaundice may not be noticed by the patient, so a change in urine color to very dark urine, which is almost always recognized by the patient, is the most common reason for seeking medical attention for patients with hepatitis A. The range of incubation is between 15 and 50 days, with a mean of approximately 28 days (see Fig. 174.5). Although HAV can be transmitted orally or parenterally, the incubation period is independent of the route of inoculation.[333] Experiments in primates and observations in humans suggest that the incubation period is dependent on the infectious dose.[334]

Site of Viral Replication

HAV is generally transmitted by the fecal-oral route. Because the virus is acid resistant, it passes through the stomach, replicates lower in the intestine,[335–337] and is then transported to the liver, which is the major site of replication.[335,338,339] Evidence of replication in the oropharynx has been obtained in chimpanzees, and HAV has been identified in human saliva.[124,125] HAV, like many other picornaviruses, is highly organ specific with little evidence of significant replication outside the liver. Virus is shed from infected liver cells into the hepatic sinusoids and canaliculi, passes into the intestine, and is excreted in feces. In humans and nonhuman primates, HAV has been detected in the liver, bile, and feces.[340,341] The first indirect evidence that virus may replicate in the gut was the detection of co-proantibodies in the feces,[342,343] followed by the demonstration of hepatitis A antigen in duodenal lining cells.[335] Nonetheless, the major pathology is restricted to the liver.

Pathology

HAV is generally not cytopathic in cell culture, and histopathologic findings in experimental animals and humans do not show widespread hepatocyte necrosis, although the vast majority of hepatocytes at the peak of viral replication appear to be infected by immunohistochemical staining. Therefore, immune mechanisms have been invoked to explain the pathogenesis of the disease.[344] It has been postulated that liver cell damage occurs through a cell-mediated immune response, whereas circulating antibodies are probably more important in limiting the spread of virus to uninfected liver cells and other organs. This hypothesis is consistent with observations in animal models and humans. For example, intravenous inoculation of marmosets with a large dose of HAV resulted in mildly abnormal liver function test results and detectable hepatitis A antigen in hepatocytes within 1 week. Enzyme levels stabilized or even decreased until the third week after inoculation, when a second, higher peak was observed coincident with the appearance of serum antibodies.[345] One explanation is that the early mild hepatitis was due to a direct viral effect, but the second, more severe episode was due to an immune response. The presence of large quantities of virus in hepatocytes before the onset of hepatitis also argues against a major direct cytopathic effect of HAV.[336] It has been suggested that virally elicited T cells target infected liver cells and induce immunopathology. In human studies, Vallbracht and colleagues[344,346] found that lymphocytes from convalescing patients produced cytotoxic effects against autologous epidermal cell lines infected with HAV and that $CD8^+$ T-cell clones demonstrated cytotoxic activity against autologous fibroblasts infected with hepatitis A. These findings are consistent with the hypothesis that $CD8^+$ T lymphocytes mediate liver cell damage. Furthermore, natural killer cells have been demonstrated to be capable of lysing HAV-infected tissue culture cells.[347]

Although liver damage occurs at the time that circulating antibodies become detectable, it has not been proved that the pathology is antibody dependent.[347] Circulating immune complexes containing HAV and specific immunoglobulin M (IgM) antibodies have been found during infection. However, IG and complement deposits were not found at the sites of liver cell damage, and resolution of disease occurred even when antibody levels were increasing and hepatitis A antigen could still be detected in the liver.[348,349,350]

MANIFESTATIONS

Hepatitis A is an acute infection of the liver and is indistinguishable from other forms of acute viral hepatitis on clinical grounds, although the diagnosis may be suspected in a patient with typical symptoms during an outbreak. Although the clinical expression of infection varies widely, the disease is usually self-limited, sometimes subclinical, but

[b]References 154, **155**, 156, 157, 314, 315.

typically is symptomatic with jaundice. The most important determinant of the likelihood of clinical expression is the age at which infection occurs.[351] The vast majority of infections in children younger than 5 years are silent, and the proportion of symptomatic infections increases with age. The proportion of icteric cases (compared with anicteric cases) varies by age at time of infection and ranges from <10% among children younger than 5 years to over 70% among adolescents and adults.[232,352,353] In an epidemic in Greenland during the 1970s, the frequency of clinically recognizable hepatitis increased from 1% in children younger than 1 year to 24% in 15-year-olds.[354,355] Similar low rates of clinical symptoms have been noted in children involved in outbreaks in daycare centers in the United States[356]; however, adults infected in these outbreaks usually became jaundiced.

Signs and Symptoms

Patients with hepatitis A often describe a mild illness, the prodrome (see Fig. 174.5), that appears 1 to 7 days before the onset of dark urine (bilirubinuria) and jaundice, although longer periods have been recorded.[236,351] These symptoms may not be severe enough to cause the patient to seek medical attention or to stay home from work. In the early stages, flulike symptoms are common; fever (as high as 40°C) may be accompanied by chills, mild headache, malaise, and fatigue. Loss of appetite, nausea, vomiting, and weight loss are common. Occasionally, children may experience atypical symptoms such as diarrhea, cough, coryza, and arthralgia.

A common early sign of disease and the one that causes most patients to seek medical attention is dark urine (bilirubinuria), which is usually followed within a few days by pale or clay-colored feces (lacking bilirubin pigment), jaundice (yellow discoloration of the skin and mucous membranes), and scleral icterus (yellow discoloration of the sclera). The return of color to the stool occurs 2 or 3 weeks after the onset of illness and is an indication of resolution of disease. Pruritus, a sign of cholestasis, occurs in less than 50% of patients but may be severe enough to require antipruritics or other medications.

On physical examination, hepatomegaly is common and usually associated with tenderness to palpation. Palpable splenomegaly may be found in 5% to 15% of patients. Other extrahepatic manifestations such as skin rashes and arthralgias may be seen in up to 15% of patients, and immune complex–associated disorders and vasculitis occur rarely.

The duration of illness varies, but by the third week most patients feel better, have lost their hepatomegaly, and have normal or nearly normal levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In many patients, the appearance of jaundice is associated with rapid resolution of symptoms. In a study of 59 patients in the United States, approximately two-thirds recovered within 2 months, 85% within 3 months, and nearly all by 6 months.[357]

The clinical course and histologic findings do not differ in pregnancy.[358,359] Intrauterine transmission of HAV during the first trimester that resulted in fetal meconium peritonitis has been described in two case reports.[163,164] At delivery, both infants had a perforated ileum. No evidence has suggested that more severe infection or subsequent loss of immunity occurs in the presence of human immunodeficiency virus infection.[360] Although not demonstrated in all published studies, on balance, it seems that HAV infection in persons with chronic liver disease is more severe and more likely to result in fulminant hepatitis A.[361–363]

Complications

The clinical course of hepatitis A is usually benign, and long-term sequelae in recovered patients have not been observed. However, complications can occur, including cholestasis and acalculous cholecystitis, hemolysis, prolonged and relapsing disease, fulminant hepatitis, autoimmune hepatitis, pancreatitis, and autoimmune extrahepatic disease. During a large 1995 community-wide hepatitis A outbreak in Memphis, Tennessee, a total of 14% of hospitalized patients experienced serious complications. The most common complications were acalculous cholecystitis and hemolysis. Consistent with other series, complications were more common among older adults (25% of patients 40 years of age or older) but did occur among younger adults and children (11% of younger patients).[363,364,365,366] Hepatitis A may be a common cause of acute hepatic failure and hospitalization of children with jaundice in endemic countries.[367,368] Although unusual in low endemic countries, fulminant hepatic failure requiring transplantation and death from hepatitis A can occur.

Cholestatic hepatitis, characterized by fever, pruritus, and prolonged jaundice, has been reported as an occasional complication. Cholestasis developed in 4 of 59 (7%) patients in a hospital-based study.[357] In a detailed description of 6 patients, peak serum bilirubin levels of 12 to 29 mg/dL were recorded and jaundice lasted for 12 to 18 weeks. In each case, peak ALT levels were less than 500 IU/L.[369] Liver biopsy specimens revealed centrilobular cholestasis and portal inflammation.

Relapsing disease has been reported as an occasional complication in both adults and children.[119,357,370] It has been reported that a relapse occurs after a typical initial course in 3% to 20% of cases. Typically, symptoms decrease but may not completely resolve during the recovery phase, and the relapse disease is usually milder than the first. In one clinical series,[357] the mean ALT level was 3500 mIU/mL and the mean bilirubin level was 4.9 mg/dL during the first peak and 1554 mIU/mL and 2.5 mg/dL, respectively, during the second. Viral excretion during the relapse has been detected. Although the pathogenesis of relapses has not been elucidated, these cases resolve without sequelae.[370,371] Extrahepatic manifestations of hepatitis A rarely include cardiac involvement, although patients with acute hepatitis may have bradycardia and electrocardiograms may show prolongation of the PR interval and some mild T-wave depression. These changes resolve rapidly during convalescence.[372] Less common extrahepatic complications include postviral encephalitis, Guillain-Barré syndrome, cholecystitis, acute pancreatitis, acute renal failure secondary to interstitial nephritis, aplastic or hemolytic anemia, agranulocytosis, thrombocytopenic purpura, and pancytopenia. Several cases of arthritis, vasculitis, and cryoglobulinemia have also been reported.[349,373,374] Some patients may become depressed, and occasionally the depression may be severe enough to require treatment, but it is usually mild and self-limited.

The most serious complication of hepatitis A is fulminant hepatic failure, defined by the appearance of severe acute liver disease with hepatic encephalopathy in a previously healthy person.[375,376] Signs may include excitability, irritability, insomnia, confusion, and severe vomiting. Laboratory and clinical evidence of deteriorating liver function, especially prolonged prothrombin times or abnormal international normalized ratio (INR), correlates with the histologic picture of almost complete destruction of the hepatic parenchyma, with only a reticulin framework and portal tracts remaining. Occasionally, small groups of surviving hepatocytes can be seen close to the portal tracts, which may represent foci of regeneration. Surprisingly, little indication of a vigorous inflammatory response has been noted. Fulminant hepatitis A is now rarely seen in the United States. However, hepatitis A can be more severe in older patients and in patients with other comorbidities or hepatitis B or C, resulting in a greater proportion of patients requiring hospital care,[376,377] although overall hospitalization rates for hepatitis A have been decreasing in the United States.[378] Spontaneous survival from fulminant hepatitis A occurs more commonly than from fulminant hepatitis of other causes.[376]

Recent hepatitis A outbreaks in the United States among people who use illicit substances and people experiencing homelessness have resulted in a large proportion of patients with acute hepatitis A requiring hospitalization (71%), and a high proportion of deaths (3%).[131a] This is likely associated with high rates of comorbid conditions, including chronic hepatitis B and hepatitis C, in this population. Another reason for this may include a demographic shift among hepatitis A outbreaks of the past, with more recent outbreaks having a greater proportion of affected individuals who are older and/or have more comorbid conditions.[377]

THERAPY AND GENERAL MANAGEMENT

There is no specific therapy available for hepatitis A, and management is supportive. In the rare event of fulminant hepatitis, identification of patients requiring liver transplantation is difficult because up to 60% of patients, especially children, with fulminant hepatic failure caused

by hepatitis A survive.[376,379] Transplantation is used for the management of carefully selected patients who have a poor prognosis with medical management alone. The survival rate is reported to be 80%, although reinfection has been reported.[380,381]

For most patients with hepatitis A, admission to the hospital is not indicated, provided that patients have access to supportive care. If hospitalized, fecally incontinent patients, patients with diarrhea, and small children should be given a separate room and toilet, and contact precautions should be implemented. There is no objective evidence that bed rest or restriction of physical activity affects the outcome of the disease. There also are few data indicating that dietary restrictions, including prohibition of even modest amounts of alcohol, affect outcome. Nevertheless, abstaining from alcohol is usually recommended because alcohol has been linked with relapse of jaundice.[382]

LABORATORY DIAGNOSIS

Hepatitis A is not clinically distinguishable from other forms of viral hepatitis, although the diagnosis may be suspected in a patient with typical symptoms during an outbreak. Liver enzyme tests (see Fig. 174.5), especially serum levels of ALT and AST, are sensitive measures of parenchymal liver damage but are not specific for hepatitis A. In one case series[357] the peak mean ALT level was 1952 mIU/mL and the mean peak AST level was 1442 mIU/mL, with the highest ALT level being 9711 mIU/mL, although values greater than 20,000 mIU/mL have been observed. The ALT levels returned to normal by a mean of 7.4 weeks (range, 1–29 weeks). Although elevated ALT levels are detected in patients with severe hepatitis, high levels are not necessarily correlated with an adverse outcome. Alkaline phosphatase levels are usually only mildly elevated, and persisting elevated levels suggest hepatitis-associated cholestasis.[369] Peak bilirubin levels can exceed 30 mg/dL and tend to be higher with increasing age.[357] The presence of mild lymphocytosis and occasional atypical mononuclear cells is a common laboratory finding among patients with acute hepatitis A.[357,383]

The diagnosis of acute hepatitis A is most commonly confirmed by detection of specific IgM in a single acute-phase serum sample.[384] The hepatitis A–specific IgM antibody is usually present at the initial evaluation and may be detectable at the time of the first increase in ALT (see Fig. 174.5). However, serologic assays can be initially negative in 10% of patients who are tested soon after the onset of symptoms; testing should be repeated for persons testing negative for IgM anti-HAV if hepatitis A is clinically suspected.[385,386] IgM anti-HAV can be detected in nearly 100% of patients with acute hepatitis A at least 2 days after their peak ALT and remains positive in most for 3 to 6 months; it is rarely detected more than 2 years after initial exposure. False-positive test results occur particularly among persons who have no other evidence of recent infection, suggesting a low positive predictive value when testing is performed in asymptomatic persons with no known recent HAV exposures.[387] Assays for total antibody to the virus are of little diagnostic value because immunoglobulin G (IgG) persists for many years and may be related to a past infection or vaccination. Antibodies to naturally acquired HAV infection are primarily directed against the virion and do not react well with the individual peptides that make up the virion capsid. Low levels of antibodies to nonstructural proteins are found in the serum of convalescing patients, but attempts to use this phenomenon to distinguish the antibody response to natural infection from the response to vaccination have not yielded consistent results.[388,389]

HAV or viral antigen can be detected in the stools of patients 1 to 2 weeks before symptoms develop, but such detection has little place in routine clinical diagnosis because the tests are not widely available and shedding may have ceased before the patient seeks medical attention.[390,391] Nucleic acid–based diagnostic techniques, primarily PCR or other nucleic acid amplification assays, have been used in research laboratories when a highly sensitive test for the presence of HAV is required. PCR has been useful in the study of environmental samples.[392,393] In response to several outbreaks of hepatitis A associated with pooled plasma products, screening by nucleic acid testing of plasma pools intended for manufacture into various plasma components has been instituted by most plasma fractionators for process testing. Although these nucleic acid tests are now commercially available for testing plasma, they are not recommended for use as diagnostics for patients with acute hepatitis. The performance of these assays in the diagnostic setting has not been evaluated. The use of PCR for identifying HAV in stool samples also has not been validated as a diagnostic assay. Many stools have inhibitors of PCR, which could result in false-negative results. The use of these types of assays for diagnosis is currently not recommended outside the research setting.

Liver biopsy is rarely indicated to establish a diagnosis in acute hepatitis because this procedure is associated with a small, but measurable, risk and the histopathologic findings are not usually diagnostic. In one study done in Japan, where biopsy for acute hepatitis was routine, 86 patients with serologically established acute hepatitis A were evaluated for quantitative and qualitative light microscopic features, together with biopsy samples from 78 patients with acute hepatitis B and from 76 patients with acute non-A, non-B hepatitis. Hepatitis A was characterized by more pronounced portal inflammation than was non-A, non-B hepatitis, but less conspicuous parenchymal changes such as focal necrosis, Kupffer cell proliferation, acidophil bodies, and ballooning. Nonspecific reactive hepatitis with slightly increased serum transaminase levels was often seen during recovery from hepatitis A and needs to be distinguished from the longer-lasting cases of acute hepatitis B and C.[394,395] Hepatitis A antigen and HAV particles can be detected in the cytoplasm of infected cells with immunostaining techniques or thin-section electron microscopy.[340,396]

IMMUNITY

The high prevalence of antibody in older individuals in countries that now have a low incidence of hepatitis A suggests that anti-HAV IgG usually persists for life. After a natural infection, second attacks of hepatitis A have not been documented in the field and have not been induced experimentally. In two sets of experiments involving a total of 19 volunteers, reinoculation with HAV 6 to 9 months after the initial illness failed to induce disease.[5,6]

Past experience with IG has shown that it provides protection against hepatitis A, suggesting that serum antibody alone is sufficient to prevent infection. It has been difficult to judge the effect of mucosal immunity because IgA antibodies in saliva or feces are not detected or are present at very low levels.[397] The antibody response to HAV infection is generally brisk and high titered. Both IgG and IgM can usually be detected at the time of the first expression of clinical illness (see Fig. 174.5). Neutralizing antibody as measured with in vitro tissue culture assays can also be detected early in disease. Because HAV is not usually cytopathic, a radioimmunofocus reduction test that is equivalent to a plaque reduction assay was devised.[398] With this assay, both IgM and IgG have been shown to possess neutralizing activity. It has also been demonstrated that patients convalescing from hepatitis A may have high titers of in vitro neutralizing antibody. Serum dilutions of 1:100,000 to 1:500,000 or more are not uncommon.

The role of T lymphocytes in protection from HAV infection has not been fully elucidated. Undoubtedly, T-cell responses do occur and $CD8^+$ cytotoxic T lymphocytes and possibly natural killer cells are important in pathogenesis.[399–401]

PREVENTION

Infection Prevention and Control

Hygiene and sanitation are an effective methods to control hepatitis A and other enteric infections. Good hygienic practices with particular emphasis on hand washing and restriction of activities of workers who are ill are of primary importance in the food preparation industry. Travelers to hepatitis A–endemic countries should be advised to eat only properly cooked food, avoid uncooked vegetables and shellfish, and consume only treated or purified water and ice. Nosocomial infections have been reported but are not common, and transmission is usually from a patient who is not suspected of having hepatitis A.[402,403] Hence, hospitalized patients with confirmed or suspected hepatitis A need routine precautions. Private rooms, gowns, and masks are not necessary unless the patient is incontinent, in which case contact precautions should be instituted. Gloves should be worn when handling any material potentially contaminated with feces. Frequent hand washing, whether gloves are worn or not, should be emphasized.

Passive Immunization

Before the licensing of hepatitis A vaccines, the mainstay of hepatitis A immunoprophylaxis was passive immunization with pooled serum IG, which has been referred to as gamma globulin or immune serum globulin. IG has proved useful for the prevention of hepatitis A in travelers, Peace Corps volunteers, and military personnel and for PEP. Although IG has proven effective for prevention of hepatitis A in individuals, it has not been successful in altering the epidemiology of hepatitis A in communities; this can be attributed to the transient nature of the protection and the high population coverage rates necessary to alter the epidemiology and induce herd immunity.

IG is manufactured by means of cold ethanol precipitation from large pools of plasma collected from tens of thousands of donors.[404] At present, the individual plasma units used in these pools are screened for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by the appropriate serologic and other assays. Minipools of plasma are tested with nucleic acid testing for hepatitis C and human immunodeficiency virus, and the product itself undergoes at least one specific viral inactivation step in its manufacturing process. In the United States, the prevalence of antibody to HAV in the population has been decreasing, raising concern that antibody levels against HAV in IG preparations might drop below effective levels. Although no standard for anti-HAV levels exists in IG preparations in the United States, reduced anti-HAV antibody titers have been documented in pooled plasma in the United States and Europe.[405] Studies have not demonstrated reduced efficacy in the prevention of hepatitis A at differing doses of IG.[226,406] However, in July 2017, the prescribing information for GamaSTAN S/D, the only IG product approved by the US Food and Drug Administration for HAV prophylaxis, was updated owing to concerns around decreasing HAV IgG antibody levels. Changes were made in 2017 to the dosing instructions, increasing the dose, for both preexposure and postexposure HAV prophylaxis (Table 174.1).[407] Eventually the manufacture of IG from selected antibody-positive donors may need to be considered to develop a hyperimmune globulin for hepatitis A prevention, analogous to other agent-specific hyperimmune globulins.[408]

IG is effective for preexposure prophylaxis (PrEP) and PEP of hepatitis A.[409] The efficacy of IG was first demonstrated in an outbreak at a summer camp in 1944 and has been confirmed many times since.[410,411] Several studies have demonstrated the effectiveness of IG in preexposure settings, such as among travelers, military personnel,[412] and Peace Corps workers.[413] The rate of HAV infections in Peace Corps volunteers dropped from 1.6 to 2.1 cases per 100 per year before mandatory administration of IG every 4 months, to 0.1 to 0.3 case per 100 per year after the institution of a mandatory program.[413] When administered before exposure or within 2 weeks after exposure, IG is more than 85% effective in preventing hepatitis A.[410,414,415] Whether IG completely prevents infection or leads to asymptomatic infection and the development of persistent anti-HAV (passive-active immunity) is probably related to the amount of time that has elapsed between exposure and IG administration.[410,416]

The use of IG for PrEP and PEP has become limited with the licensure of inactivated hepatitis A vaccines, but it remains indicated for some rare situations both for PEP (Table 174.2).[407,417,417a] A randomized trial comparing hepatitis A vaccine versus IG for PEP found them to have equivalent efficacy among persons ≤40 years of age.[418] After exposure, IG should be used to protect children younger than 12 months, immunocompromised persons, persons with chronic liver disease, and persons for whom the vaccine is contraindicated.[417] Previously the preference was also to use IG for persons older than 40 years; however, although limited effectiveness data were available, recommendations in the United States changed in 2018 to provide hepatitis A vaccination for all individuals aged ≥12 months and to consider additional use of IG for persons aged ≥40 years based on clinical assessment (Table 174.4).[417a] These changes were driven by the increase in the IG dosing instructions (due to concerns of decreased IG potency) and by the decreased availability of IG during outbreaks.[417a] Similarly, for PrEP such as is needed for travel to endemic areas, recommendations have changed to provide hepatitis A vaccine to children 6 to 11 months of age who also require measles, mumps, and rubella (MMR) vaccine because IG cannot be given with MMR vaccine, which is also recommended for PrEP for this age group prior to travel to countries with endemic measles (see Table 174.3). Children should then receive the recommended two-dose series of HAV beginning at age 12 months or older, and their recommended MMR vaccine series as indicated.[417a] IG should be used instead of vaccine for persons who have a contraindication to the vaccine.

The current recommended dose of IG is a single IM injection of 0.1 mL/kg for postexposure and preexposure travel of up to 1 month; 0.2 mL/kg should be given before exposure for travel up to 2 months and repeated every 2 months for extended travel (see Table 174.1).[407] Hepatitis A vaccine, if not contraindicated, is a better choice for PrEP and PEP (see "Active Immunization" later).

Intramuscular preparations of IG should never be given intravenously, and the intravenous preparations of IG are not intended for hepatitis A prevention and are formulated at a lower globulin concentration.

IG does not interfere with the immune response to oral poliovirus or yellow fever vaccine or, in general, to inactivated vaccines. However, IG can interfere with the immune response to some live-attenuated vaccines (e.g., MMR vaccine; varicella vaccine). Administration of the

TABLE 174.1 Indications and Updated Dosage Recommendations for GamaSTAN S/D Human Immune Globulin for Preexposure and Postexposure Prophylaxis Against Hepatitis A Infection

INDICATION	DOSAGE RECOMMENDATION
Preexposure prophylaxis	
Up to 1 month of travel	0.1 mL/kg
Up to 2 months of travel	0.2 mL/kg
2 months of travel or longer	0.2 mL/kg (repeat every 2 months)
Postexposure prophylaxis	0.1 mL/kg

Modified from Nelson NP. Updated Dosing Instructions for Immune Globulin (Human) GamaSTAN S/D for Hepatitis AVirus Prophylaxis. MMWR Morb Mortal Wkly Rep. *2017;66:959–960.*

TABLE 174.2 Recommendations for Postexposure Prophylaxis, by Age Group and Risk Category

INDICATION/ AGE GROUP	RISK CATEGORY/ HEALTH STATUS	HAV	IG
Postexposure Prophylaxis			
<12 mo	Healthy	No	0.1 mL/kg[a]
12 mo–40 yr	Healthy	1 dose[b]	None
>40 yr	Healthy	1 dose[b]	0.1 mL/kg[c]
≥12 mo	Immunocompromised or chronic liver disease	1 dose[b]	0.1 mL/kg[d]
≥12 mo	Persons who elect not to receive vaccine or for whom vaccine is contraindicated[e]	No	0.1 mL/kg

HAV, Hepatitis A vaccine; *IG,* immune globulin.
[a]Measles, mumps, and rubella vaccine should not be administered for at least 3 months after receipt of IG.
[b]A second dose is not required for postexposure prophylaxis; however, for long-term immunity, the HAV series should be completed with a second dose at least 6 months after the first dose.
[c]The provider's risk assessment should determine the need for IG administration. If the provider's risk assessment determines that both vaccine and IG are warranted, HAV and IG should be administered simultaneously at different anatomic sites.
[d]Vaccine and IG should be administered simultaneously at different anatomic sites.
[e]Life-threatening allergic reaction to a previous dose of HAV, or allergy to any vaccine component.
Data from Nelson NP, Link-Gelles R, Hofmeister MG, et al. Update: Recommendations of the Advisory Committee on Immunization Practices for Use of Hepatitis A Vaccine for Postexposure Prophylaxis and for Preexposure Prophylaxis for International Travel. MMWR Morb Mortal Wkly Rep. *2018;67:1216–1220.*

TABLE 174.3 Recommendations for Preexposure Protection, by Age Group and Risk Category

INDICATION/ AGE GROUP	RISK CATEGORY/ HEALTH STATUS	HAV	IG
<6 mo	Healthy	No	0.1–0.2 mL/kg[b]
6–11 mo	Healthy	1 dose[c]	None
12 mo–40 yr	Healthy	1 dose[d]	None
>40 yr	Healthy	1 dose[d]	0.1–0.2 mL/kg[b,e]
All ages	Immunocompromised or chronic liver disease	1 dose[d]	0.1–0.2 mL/kg[b,e]
>6 mo	Persons who elect not to receive vaccine or for whom vaccine is contraindicated[f]	No	0.1–0.2 mL/kg[b]

HAV, Hepatitis A vaccine; *IG,* immune globulin.
[a]IG should be considered before travel for persons with special risk factors for either HAV infection or increased risk for complications in the event of exposure to HAV.
[b]0.1 mL/kg for travel up to 1 month; 0.2 mL/kg for travel up to 2 months, 0.2 mL/kg every 2 months for travel of ≥2 months' duration.
[c]This dose should not be counted toward the routine 2-dose series, which should be initiated at age 12 months.
[d]For persons not previously vaccinated with HAV, administer dose as soon as travel is considered, and complete series according to routine schedule.
[e]May be administered, based on providers' risk assessment.
[f]Life-threatening allergic reaction to a previous dose of HAV, or allergy to any vaccine component.
Data from Nelson NP, Link-Gelles R, Hofmeister MG, et al. Update: Recommendations of the Advisory Committee on Immunization Practices for Use of Hepatitis A Vaccine for Postexposure Prophylaxis and for Preexposure Prophylaxis for International Travel. MMWR Morb Mortal Wkly Rep. *2018;67:1216–1220.*

TABLE 174.4 ACIP Recommendations for Routine Preexposure Use of Hepatitis A Vaccine

GROUP	COMMENTS
All children at age 12–23 mo	2 doses separated by 6–18 months, between the first and second birthdays; a series begun before the second birthday should be completed even if the child turns 2 before the second dose is administered.
Children aged 2–18 years	Catch-up vaccination; anyone 2 years of age or older may receive hepatitis A vaccine if desired; minimum interval between doses is 6 months
International travelers or workers	Travelers to or workers in high- or intermediate-endemicity countries (see Fig. 174.6)
Persons who anticipate close contact with an international adoptee	Includes unvaccinated household or other close contacts (e.g., regular babysitting) 12 mo of age or older of international adoptees from a country of high or intermediate hepatitis A endemicity during the first 60 days after arrival of the adoptee in the United States; first dose of vaccine should be administered at least 2 weeks before the arrival of the adoptee
Men who have sex with men	Includes adolescents
People who use injection and noninjection drugs	Includes adolescents
Persons with chronic liver disease	Increased risk of fulminant hepatitis A with HAV infection
Persons receiving clotting factor concentrates	
Persons who work with HAV in research settings or work with nonhuman primates	
Anyone who wants to obtain immunity	

ACIP, Advisory Committee on Immunization Practices; *HAV,* hepatitis A virus.
Data from Centers for Disease Control and Prevention (CDC). Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. *2006;55(RR-7):1–23; ACIP CDC. Update: Prevention of hepatitis A after exposure to hepatitis A virus and in international travelers: updated recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Morb Mortal Wkly Rep. *2007;56:1080–1084; CDC. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees.* MMWR Morb Mortal Wkly Rep. *2009;58:1006–1007; ACIP, Fiore AE, Wasley A, Bell BP. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Recomm Rep. *2006;55(RR-7):1–24.*

MMR vaccine and of varicella-containing vaccines should be delayed for at least 3 months after administration of IG, and IG should not be given within 2 weeks after the administration of the MMR or varicella-containing vaccines, unless the benefits of IG administration are greater than the benefits of vaccination.[419]

Serious adverse events from IG are rare. Because anaphylaxis has been reported after repeated administration to persons with IgA deficiency, these persons should not receive IG.[420] Pregnancy or lactation is not a contraindication to IG administration. For infants and pregnant women, a preparation that does not include thimerosal is preferable; in the United States, the only available preparation, GamaSTAN S/D, does not contain thimerosal.

Active Immunization

Active immunization with hepatitis A vaccines has developed along classic lines similar to the path followed for polio vaccines. As with poliovirus, the initial breakthrough came with the in vitro cultivation of HAV in cell lines suitable for vaccine production.[14] Formalin-inactivated, cell culture–produced, whole-virus vaccines have now been approved in much of the world, and a live-attenuated vaccine is available in China.

Two inactivated hepatitis A vaccines are approved for use in the United States and widely throughout the world. Other inactivated hepatitis A vaccines are available in Europe and other parts of the world.[421,422] Both US-licensed vaccines are produced from highly cell culture–adapted virus strains that have also been shown to be highly attenuated in humans, which gives them an extra measure of safety.[423] The US-licensed vaccines—Havrix (GlaxoSmithKline Biologicals, Philadelphia, PA) and VAQTA (Merck & Co., Whitehouse Station, NJ)—are both grown in MRC-5 cells, purified, inactivated by formalin, and formulated with alum as an adjuvant. Both vaccines are licensed in a two-dose series, with the second dose given 6 to 18 months after the first, depending on the vaccine (Table 174.5). Inactivated hepatitis A vaccines are also manufactured in Europe and China and are available in many countries of the world. Currently there are two hepatitis A vaccines that have

TABLE 174.5 Recommended Doses and Schedules for Inactivated Hepatitis A Vaccines Licensed in the United States

AGE (yr)	VACCINE	DOSE	VOLUME (mL)	NO. OF DOSES	SCHEDULE (mo)[a]
1–18	Havrix VAQTA	720 ELU[b] 25 U[c]	0.5 0.5	2 2	0, 6–12 0, 6–18
≥19	Havrix VAQTA	1440 ELU 50 U	1.0 1.0	2 2	0, 6–12 0, 6–18
≥18	Twinrix[d]	720 ELU	1.0	3	0, 1, 6

[a]0 months represents timing of initial dose; subsequent numbers represent months after the initial dose.
[b]Enzyme-linked immunosorbent assay units.
[c]Hepatitis A virus antigen unit.
[d]Hepatitis A (720 ELU) and hepatitis B (recombinant; 20 μg) vaccine. Standard dosing: a series of 3 doses (1 mL each) given on a 0-, 1-, and 6-month schedule. Accelerated dosing: a series of 4 doses (1 mL each) given on days 0, 7,and 21 to 30 followed by a booster dose at month 12.

been prequalified by the World Health Organization (WHO): Havrix and Sinovac (Biotech).[424] In the United States, a combination hepatitis A and hepatitis B vaccine, Twinrix (GlaxoSmithKline), is also available for persons aged 18 and older.

Clinical trials have indicated that inactivated hepatitis A vaccines are safe, highly immunogenic, and efficacious.[60,61,421,425] In one US study, 1037 healthy seronegative children 2 to 16 years of age in a community experiencing yearly outbreaks of hepatitis A received either a single dose of formalin-inactivated vaccine (n = 519) or placebo (n = 518). No cases of hepatitis occurred in the vaccinated group, except for a few that appeared within 3 weeks of vaccination. These cases represented patients who were already incubating the infection at the time of vaccination. In the period from 21 days to 103 days, 34 cases of hepatitis A were observed, all in the placebo group, indicating a 100% vaccine protective efficacy during that period of observation.[425,426] In a large field trial of an inactivated vaccine involving more than 40,000 children in Thailand, the vaccine was found to be at least 80% effective compared with placebo and was without serious adverse reactions.[61]

Although the absolute level of antibody required to protect against infection has not been rigorously established, it is accepted on the basis of comparisons with protective antibody levels associated with passive immunization with IG that antibody concentrations of 10 to 20 mIU/mL (depending on the assay used) are protective.[60,427,428] The licensed inactivated hepatitis A vaccines have all been shown to be highly and rapidly immunogenic. They induce seroconversions to protective levels of antibody in as little as 2 weeks after the initial dose.[429,430] The level of antibody after vaccination varies with the dose and schedule of the vaccine. However, after a single dose of vaccine, antibody titers are higher than titers produced by known protective levels of IG but are generally lower than titers measured after natural infection.[431–433] The quality of the antibody response after vaccination has also been studied by comparing antibodies detected with radioimmunoassay, radioimmunoprecipitation, and in vitro neutralization in sera from persons passively immunized with IG and in persons immunized by vaccine. With the antibody normalized between the two groups by radioimmunoassay, the IG recipients had higher neutralization titers but negligible radioimmunoprecipitation titers compared with the group that was vaccinated.[433] However, it has also been shown that IG prepared from the serum of vaccinees could protect a chimpanzee from HAV challenge when the titer of antibody achieved by passive immunization in the chimpanzee was similar to that found in humans receiving IG prophylaxis.[434] Nevertheless, there are no direct correlations between in vitro neutralization assays and seroprotection. Regardless of the results of antibody measurements after vaccination, clinical trials have demonstrated that the vaccine is highly effective within 1 month of the first dose.[418]

Certain factors may reduce the response to the vaccine. For instance, only 50% to 75% of HIV-positive vaccinees develop protective levels of antibody, and those who respond have lower antibody titers than vaccinees without HIV infection.[435–438] However, response improves with higher $CD4^+$ counts and suppression of HIV.[437,438] The final antibody concentrations achieved in patients with chronic liver disease are also lower than in normal subjects, but the seroprotection rates were approximately the same; vaccination before development of cirrhosis is advisable.[439] The common recommended schedule of a single dose followed by a booster dose 6 to 18 months later produces high levels of antibody; the high levels of antibody produced by vaccination are well in excess of the levels achieved after passive immunization with IG, which are known to be effective in preventing hepatitis A. After the completed series, it is estimated that protective levels of antibody will persist for at least 20 to 30 years.[440,441,442,443] Because the incubation period for hepatitis A averages 28 to 30 days, and the anamnestic responses observed after the second dose are rapid and robust, it has been suggested that vaccinees who have seroconverted will be protected even if their antibody levels have fallen below protective levels.[60] Long-term follow-up studies are being performed to confirm the duration of protection.

A freeze-dried live-attenuated vaccine based on the H2 strain, a derivative of the cell culture–adapted, attenuated HM175 strain, has been used in China.[31,423,444–447] The vaccine is administered as a single subcutaneous dose. Antibody has been detected for at least 8 years after vaccination, and postlicensure community-based studies have demonstrated protection against hepatitis A in vaccinated populations.[441,448]

RECOMMENDATIONS FOR PREVENTION

Postexposure Prophylaxis

For decades, IG has been recommended for PEP to prevent infection after known exposure to HAV. However, with the demonstration of postexposure efficacy from hepatitis A vaccine,[418] and the relative public health advantages of vaccine compared with IG including the induction of active immunity and longer protection, greater ease of administration, and greater acceptability and availability, in the United States hepatitis A vaccines are now recommended in most circumstances for PEP (see Table 174.2).[417,417a] Hepatitis A vaccines should be administered for PEP for most persons aged ≥12 months; however, in addition to hepatitis A vaccine, IG may be administered to persons aged >40 years depending on the providers' risk assessment.[417a]

When using hepatitis A vaccine alone for PEP, single-antigen vaccine should be used; no data exist regarding the performance of the combination hepatitis A and hepatitis B vaccine for prophylaxis after exposure to HAV, and the concentration of HAV antigen in the currently available combination vaccine is half that included in the single-antigen vaccine available from the same manufacturer. IG may still be used for PEP in certain cases at a dose of 0.01 mL/kg, such as children younger than 12 months, immunocompromised persons, persons who have had chronic liver disease diagnosed, and persons for whom vaccine is contraindicated (see Table 174.2).

PEP, with vaccine, is recommended to prevent hepatitis A after exposure in certain settings.[226,417] Household and sexual contacts of patients with hepatitis A should receive appropriate PEP as soon as possible. Casual contacts such as school classmates or coworkers who have not had close physical contact usually do not require PEP. PEP may be indicated to control hepatitis A outbreaks in homeless shelters, substance abuse treatment centers, or correctional facilities when a resident, client, or inmate, respectively, or an employee is diagnosed with hepatitis A.[131a] Outbreaks in other settings (e.g., hospitals, facilities for developmentally disabled persons) are rare but can occur. When a food handler is identified with hepatitis A, PEP with vaccine or IG should be administered to other food handlers at the food establishment and can be considered for patrons if certain other conditions exist.[136,138,226,417] Once cases that are associated with a food service establishment are identified, it is generally too late to administer PEP to patrons because the 2-week postexposure period during which IG or vaccine is known to be effective will have passed.

Preexposure Prophylaxis

Hepatitis A vaccine is preferred over IG for PrEP. Hepatitis A vaccine is indicated for preexposure protection of susceptible persons 12 months of age or older, persons at increased risk of hepatitis A, and for any person wishing to obtain immunity. In addition, hepatitis A vaccine should also be administered to children 6 to 11 months of age traveling outside the United States when protection against hepatitis A is recommended (see Table 174.3) (MMWR, ACIP 2018, pending publication).[255,406] IG should be used for children younger than 6 months or other persons who cannot or choose not to receive hepatitis A vaccine but who require protection against hepatitis A when protection against hepatitis A is recommended (see Table 174.3).

Prevaccination serologic testing may be considered to reduce costs by eliminating vaccination of persons with previous immunity, such as older adolescents and adults in certain population groups with a high prevalence of infection (e.g., persons born in areas of high hepatitis A endemicity). However, prevaccination testing should take into account the cost of testing, vaccine cost, and the likelihood that the person will return for vaccination.[449] Vaccination of immune individuals is not harmful, and therefore, for most situations, vaccinating individuals without prevaccination testing is preferred. Postvaccination testing is not indicated because of the high rate of vaccine response and because some commercially available assays may not detect lower anti-HAV concentrations generated by immunization.

Disease-Control Strategies

Routine Vaccination of Children

Hepatitis A vaccine was licensed and became available in the United States in 1995. It was soon recognized that a strategy of widespread routine vaccination of children had the potential to achieve a sustained reduction in the overall incidence of hepatitis A in the community.[450] The initial recommendations, published in 1996, called for routine vaccination of children living in communities with the highest hepatitis A rates (e.g., Native American and Alaska Native communities).[228] Although effective in reducing disease rates in communities covered by these recommendations, implementation of these recommendations had little impact on overall disease incidence nationwide because only a small proportion of nationally reported cases occurred among persons in such communities. Recommendations for use of hepatitis A vaccine were updated and incrementally expanded in 1999, 2006, and 2009.[224,226,228,255,451] In 1999, the recommendation for routine vaccination of children was extended to include those living in states, counties, and communities with consistently elevated hepatitis A rates.[224] Hepatitis A incidence decreased sharply in the states included in the 1999 recommendations for routine vaccination of children, and by 2001, rates were similar across all regions of the country, and the majority of cases were occurring in states that historically had low rates and where hepatitis A vaccination of children had not been widely implemented.[225] In 2006, after hepatitis A vaccines were licensed for children beginning at age 12 months (vs. 24 months previously), the recommendations for routine vaccination were expanded to include all children 12 to 23 months of age nationwide (see Table 174.4).[226] This strategy has resulted in a historic low of reported hepatitis A cases and rates among all age groups in the United States (see Figs. 174.8 and 174.9).[223] Similar decreases in hepatitis A have been documented throughout the world in countries implementing vaccination strategies that target children.[144,452–457] By 2017, 18 countries had introduced hepatitis A vaccine into their routine childhood vaccination programs, including some countries that have a single-dose schedule.[458] In 2012, the WHO Strategic Advisory Group of Experts (SAGE) on Immunization reviewed evidence of efficacy after a single dose of inactivated hepatitis A vaccine in a randomized controlled trial in Nicaragua and from national use of a single-dose schedule of hepatitis A vaccine in Argentina. The SAGE concluded that national programs may consider a single-dose schedule, although additional evidence of long-term immunogenicity is needed, and for certain high-risk and immunocompromised individuals a two-dose schedule is recommended.[459,460]

In the United States, it is recommended that children who did not receive two-dose hepatitis A vaccine by 2 years of age should be offered catch-up vaccination through age 18 years (see Table 174.4).[226] The first dose does not need to be repeated if the first dose was given.[385]

Vaccination of Persons at Increased Risk of Hepatitis A Infection or Severe Consequences

In addition to routine vaccination of children, hepatitis A vaccine is also indicated for persons in the following groups that have an increased risk of HAV infection or of severe consequences if infected (see Table 174.4).[226]

Men Who Have Sex With Men

Adolescent and adult MSM should be vaccinated, regardless of reported level of sexual activity.[226] Prevaccination serologic testing is not necessary for vaccination of adolescents and young adults, but it could be considered for older adults (see Table 174.4).[226]

Users of Illicit Drugs

Vaccination is recommended for users of injection and noninjection illegal drugs (see Table 174.4).[226] Prevaccination testing could be considered for adults; the need might depend on the particular characteristics of the population of drug users, including the type and duration of drug use.[272,274,275]

International Travelers

Susceptible persons ≥6 months of age who travel to or work in countries where hepatitis A is endemic should be vaccinated to prevent infection and illness. Although IG is still an option, hepatitis A vaccination is preferred for most travelers (see Tables 174.3 and 174.4). The first dose should be administered as soon as travel is considered. For most healthy persons, one dose of single-antigen hepatitis A vaccine given any time before departure can provide adequate protection. For travel that will begin within 2 weeks to countries with high or intermediate endemicity of hepatitis A, older adults, immunocompromised persons, and persons with chronic liver disease or other chronic medical conditions should receive the initial dose of the vaccine and may also be simultaneously administered IG (0.01–0.02 mL/kg), depending on providers risk assessment, at a different anatomic injection site (see Tables 174.1 and 174.3). Travelers who elect not to receive hepatitis A vaccine, are aged <6 months, or are allergic to a component of hepatitis A vaccine should receive IG at the appropriate dose (see Table 174.1).

Completion of the vaccine series according to the licensed schedule is recommended for long-term protection. Prevaccination serologic testing could be considered for older travelers or younger travelers who were born in a country in which hepatitis A is endemic.

Close Contacts of Newly Arriving International Adoptees

Susceptible household members and other close personal contacts (e.g., regular babysitters) who anticipate close personal contact during the first 60 days after arrival of adopted children from countries with high or intermediate levels of hepatitis should receive hepatitis A vaccination (see Table 174.4).[255] The first dose should be administered as soon as adoption is planned, ideally 2 or more weeks before the arrival of the adoptee. Parents traveling to high or intermediate endemic countries (see Fig. 174.6) to bring adoptees home should be vaccinated as per the recommendations for international travelers.[417]

Recipients of Blood or Plasma-Derived Products

The risk of hepatitis A from a blood transfusion or from plasma derivatives is extremely low, but both have been reported.[44,122] Individuals who receive these products regularly should receive hepatitis A vaccine (see Table 174.4).

Persons With Chronic Liver Disease

Although individuals with chronic liver disease are not at increased risk of acquiring hepatitis A, acute hepatitis A in such patients can have serious or fatal consequences.[461] Therefore, it is recommended that all such persons, no matter the cause of their liver disease, be vaccinated against hepatitis A (see Table 174.4). Vaccination should be performed early after the diagnosis of chronic liver disease in order to obtain optimal response to the vaccine.

Other Groups and Settings

Persons who work with HAV in research settings or have exposure to nonhuman primates should be vaccinated (see Table 174.4).[226]

Hepatitis A Vaccination During Outbreaks

Large community-wide outbreaks have become rare in the United States since the implementation of routine childhood immunization, and ongoing vaccination of children should reduce the occurrence of these outbreaks further. The United States and Europe have experienced large outbreaks associated with contaminated shellfish, produce, and frozen berries.[146,155–157,319] Beginning in 2017, there have been multiple outbreaks of hepatitis A among substance users and people experiencing homelessness in the United States.[131a] Outbreaks can be very disruptive, resource intensive, and logistically challenging to respond to with accelerated vaccination. Furthermore, such efforts may have limited impact on transmission.[226] During outbreaks, persons who work as food handlers, who are not at increased risk of hepatitis A because of their occupation, may also transmit HAV to others when they contract hepatitis A.[138,307] To reduce the frequency of evaluations of food handlers with hepatitis A and the need for PEP of patrons, public health officials in some jurisdictions have instituted measures to promote hepatitis A vaccination of food handlers.[462] Although outbreaks associated with eating establishments continue to garner media attention, often during an ongoing

community outbreak, transmission from infected food handlers generally accounts for a small proportion of incident cases. Therefore, vaccination of food handlers is not likely to affect overall disease incidence and has not been found to be cost-effective.[463]

DIRECTIONS FOR THE FUTURE

In the United States, Western Europe, and many other high-income countries, low levels of endemic transmission and outbreaks among specific population may occur, whereas international travelers to high- and intermediate-endemicity countries remain at risk. In these countries, hepatitis A is relatively well controlled, and as more children are vaccinated, in countries with childhood vaccination programs the population immunity will continue to increase, further reducing the risk of hepatitis A. However, in much of Europe, where routine vaccination of children has mostly not been implemented, population susceptibility continues to grow.[464] Globally, hepatitis A continues to be responsible for significant morbidity and mortality.[170] As of 2017, only 18 countries had introduced hepatitis A vaccine into their routine vaccination program.[458] In many countries previously thought to be highly endemic and transitioning into intermediate hepatitis A endemicity, a sizable proportion of adolescents and adults are increasingly susceptible to hepatitis A, particularly in urban areas with improving water quality and sanitation. Therefore the relative cost-effectiveness of hepatitis A vaccination strategies compared with other major public health priorities deserves greater scrutiny.[465–467] Establishment of surveillance systems and use of surveillance data can shed light on the burden of hepatitis A and provide important inputs for cost-effectiveness analyses.[173,176] Cost-effectiveness analyses of various vaccination strategies have been conducted and are being used for reevaluating hepatitis A vaccine policy in countries with transitional or intermediate endemicity.[460,465,467–472] The global disease burden associated with hepatitis A is likely to continue to increase in the coming years in the absence of vaccination programs.[126,167,169] If vaccine was available at a low cost and vaccination was shown to be cost-effective, more countries in which a significant susceptible adolescent and adult population has developed might find it useful to include hepatitis A in their vaccination programs. One strategy that has been considered as a potential way to reduce the costs is vaccinating with only a single dose. Experiences in Nicaragua, where a single dose was evaluated for efficacy,[473] and Argentina, where a single-dose childhood vaccination strategy was implemented in response to a large nationwide outbreak, were effective in controlling the outbreak.[456,467,474] This led WHO to issue a permissive recommendation allowing for the use of a single-dose vaccination strategy.[460] However, long-term protection remains an important and unanswered question for countries choosing to implement this policy.[460]

International support from the Gavi Alliance for hepatitis A vaccination for eligible countries could catalyze implementation and accelerate control efforts globally.

In the United States, vaccination of successive cohorts of children has resulted in a sustained reduction in disease incidence nationwide, providing the opportunity to eliminate HAV transmission, although recent outbreaks among people experiencing homelessness and people who use injection and noninjection drugs highlight that significant challenges remain.[131a] HAV has been considered a target for eradication, but international bodies have not made this recommendation, primarily because of considerations of cost and feasibility.[475] At present, the disease can best be controlled by improving living conditions in the developing world and prudent application of vaccination strategies.

ACKNOWLEDGMENT

The authors gratefully acknowledge Anna Koscelski, BS, for her numerous contributions to this chapter.

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The complete reference list is available online at Expert Consult.

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175 Rhinovirus

Raphael Dolin

SHORT VIEW SUMMARY

Definition
- Rhinoviruses are the most frequent causes of the common cold.
- Rhinoviruses cause combinations of sneezing, sore throat, rhinorrhea, nasal congestion, and coughing.

Epidemiology
- Rhinoviruses are among the most common human pathogens.
- Rhinoviruses cause 0.75 infections per year in adults and 1.2 to 6 infections per year in children.
- Infections occur year-round with seasonal peaks in spring and fall in temperate climates.
- Spread occurs by hand-to-nose or hand-to-eye contact and by aerosol.

Microbiology
- Rhinoviruses are single-stranded, nonenveloped RNA viruses in the family Picornaviridae.
- There are three species, RV-A, RV-B, and RV-C, which have at least 156 serotypes and genotypes.

Clinical Manifestations
- The common cold is the most frequent illness.
- Infection exacerbates asthma and chronic pulmonary diseases.
- Infection can be associated with otitis media and sinusitis.
- Lower respiratory tract illness including pneumonia can occur.
- Infection is more severe in immunosuppressed patients.

Diagnosis
- Specific virologic diagnosis is made through detection of virus in nasal secretions (aspirates, swabs, washings) or pulmonary samples.
- Reverse-transcriptase polymerase chain reaction is the most sensitive and efficient laboratory technique for virus detection.

Therapy
- Common colds are self-limited, but symptomatic therapy can be used to provide relief.
- Antiviral therapy is not available.

Prevention
- No established method of prevention exists.
- Vaccines are under active investigation.

Rhinoviruses are among the most frequent causes of viral infections in humans. They are the major cause of the common cold and are significant contributors to other upper respiratory syndromes as well as certain lower respiratory illnesses. The term *common cold* is believed to derive from ancient descriptions of illness in traditional Chinese and Roman sources, in which the illness was believed to be caused by cold temperatures and being chilled.[1] Scientific investigations into the cause of the common cold began in the early 20th century in studies by Kruse,[2] in which filtrates of nasal secretions from individuals with colds were administered to volunteers and subsequently induced colds. These studies, along with similar investigations conducted later by Dochez and colleagues[3] indicated that colds were caused by filterable agents (i.e., presumed to be viruses).

The first isolation of rhinoviruses was achieved by Price[4] and Pelon and coworkers,[5] who inoculated nasopharyngeal washings from individuals with colds into rhesus monkey kidney tissue cultures. Andrewes and colleagues[6] suggested the name "rhinovirus," and a group designation was proposed by Tyrrell and Chanock in 1963.[7] Once in vitro isolation techniques were established, the filtrates used in transmission experiments were demonstrated to contain rhinoviruses. Subsequently the application of sensitive molecular techniques led to characterization of rhinovirus strains that did not grow in tissue culture and to extensive new knowledge of the epidemiology and pathogenesis of rhinovirus infection.

VIROLOGY

Classification

Rhinoviruses are members of the *Enterovirus* genus of the Piconoviridae family and comprise the RV-A, RV-B, and RV-C species. Classification is based on genome organization, capsid properties, and sequence conservation.[8,9] Within species, rhinoviruses are subdivided into numerical genotypes. Rhinoviruses have also historically been characterized by immunologic serotype, as defined by neutralization with antiserum. A total of 101 serotypes, numbered 1A, 1B, and 2 to 100, were identified. Classification into new serotypes ended in 1987 and has been supplanted by genomic characterization, which includes newly discovered strains that do not grow in tissue cultures.[10] The recognized serotypes are divided into two species, RV-A and RV-B (except for RV-A87, which is actually an enterovirus, EVD68). The third species, RV-C, consists of previously unrecognized strains of rhinoviruses detected by genomic sequencing and currently has 55 genotypes.[11]

Receptor specificity for rhinovirus has also been used for group designations. The major receptor group consists of 88 serotypes that use the intercellular adhesion molecule 1 (ICAM-1) to infect cells.[12,13] The minor receptor group consists of 11 serotypes that use the low-density lipoprotein and related proteins as receptors. The major receptor group comprises both RV-A and RV-B species, whereas the minor receptor group comprises RV-A species only. The receptor for the RV-C species is human cadherin-related family member 3.[14] Susceptibility to antiviral compounds has also been used to divide rhinoviruses into two groups (A and B) by Andries and coworkers,[15] who suggested that those groups reflect sequence and possibly pathogenic differences.

Structure

Rhinoviruses are single-stranded RNA viruses of approximately 30 nm in diameter that have a rigid protein shell (capsid) composed of four viral proteins, VP1, VP2, VP3, and VP4. The protein subunits are called protomers; consist of one copy of each of the viral proteins; and are organized into 12 pentamers, each of which contains 5 protomers (Fig. 175.1). In each of the pentamers, VP1 has a prominent symmetrical depression or "canyon," which extends around the fivefold axis of symmetry and contains the binding site for the ICAM-1 receptor. This canyon also provides immunogenic surfaces. Antibodies with neutralizing activities against rhinovirus bind to the receptor site in the canyon. A hydrophobic "pocket" is present at the base of the canyon, which is the site of binding for antiviral drugs such as pleconaril or WIN 52084.[15]

The overall structure of the rhinovirus capsid is similar to that of enteroviruses in general; however, in contrast to enteroviruses, rhinoviruses are unstable at acid pH (<5 or 6) and are completely inactivated

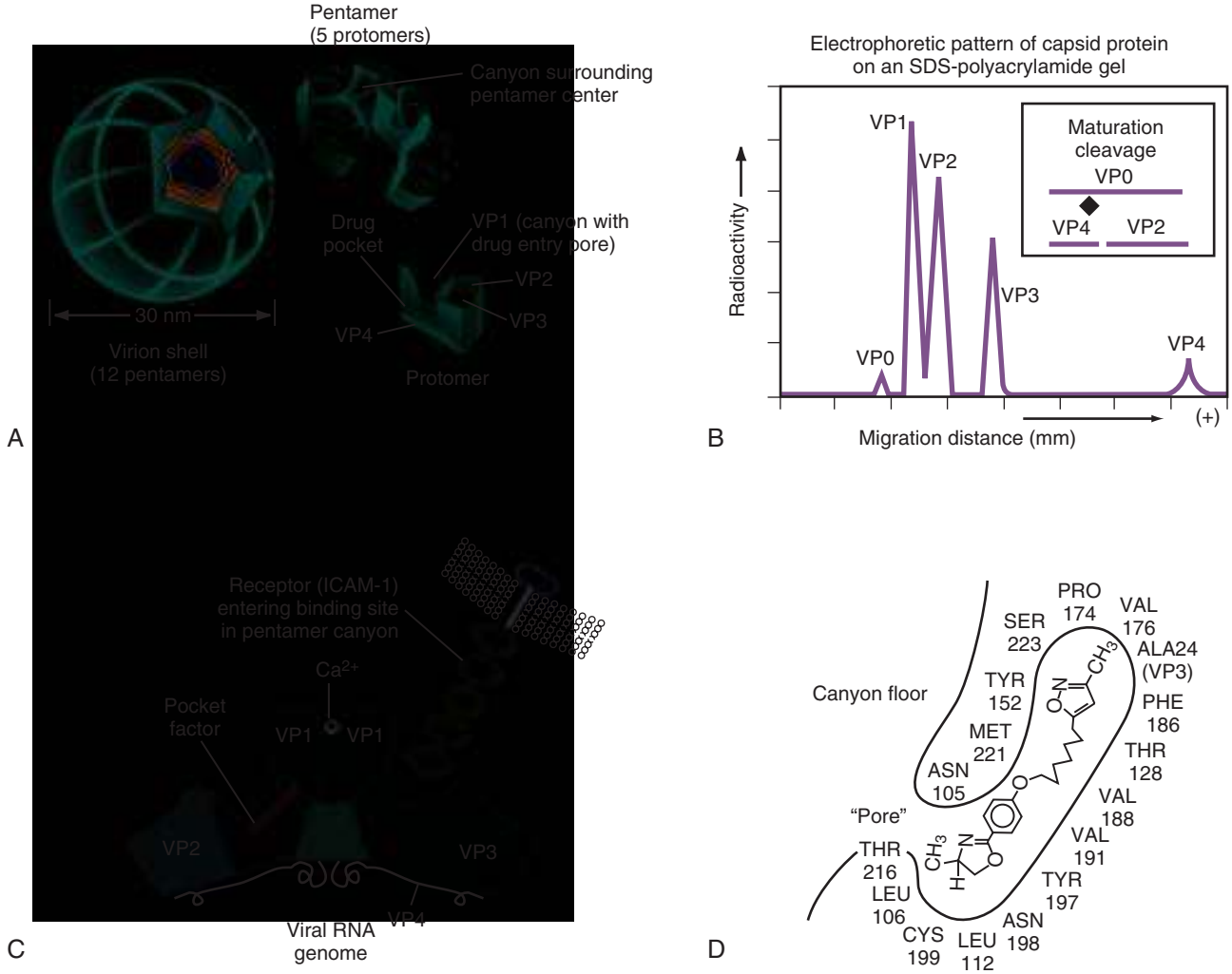

FIG. 175.1 Key features of a human rhinovirus (RV). (A) The virion shell consists of 12 pentamers, one of which has been removed to show the approximate location of the RNA packed tightly into a central cavity. Each pentamer, in turn, consists of five wedge-shaped subunits, called protomers. The canyon *(stippled)* is shown in only 1 of the 12 pentamers. (B) The virion contains four major proteins (VP1, VP2, VP3, and VP4) plus traces of another, VP0, representing residual precursor after the maturation cleavage *(inset)* required for acquisition of infectivity. (C) Transverse section through the center of a pentamer depicting entry of its cellular receptor (intercellular adhesion molecule 1 [ICAM-1]) and the location of the drug-binding pocket just beneath the canyon floor. An ion, located at each pentamer center in RV-1A, RV-14, and RV-16, is tentatively identified as calcium, which is necessary for attachment of some rhinoviruses. (D) Detail showing orientation of a capsid binder (WIN 52084) and identity of amino-acid residues lining the canyon floor and drug-binding pocket in a single protomer. In RV-14, the drug prevents attachment of its receptor, ICAM-1. *SDS,* Sodium dodecyl sulfate.

at pH <3.[16] Rhinoviruses retain stability for hours to days at 24°C to 34°C on environmental surfaces and have viability for years at freezer temperatures of −70°C.[17]

Rhinoviruses lack envelopes and thus are relatively resistant to lipid solvents such as chloroform and ether. Polar organic solvents decrease infectivity of rhinoviruses, perhaps because of partial denaturation of their protein shell or because of inactivation of the hydrophobic pocket. Rhinoviruses are resistant to nonionic detergents but are sensitive to commonly used disinfectants such as chlorine, iodine, hydrogen peroxide, and ozone.[18] Physical treatments such as gentle heating, desiccation, or ultraviolet light also decrease infectivity of rhinoviruses.

Genomic Organization

The rhinovirus genome consists of a single strand of positive sense RNA of approximately 7200 bases, which codes for a single open reading frame for a large polyprotein with almost 2200 amino acids. The RNA genome also contains multiple important RNA motifs.[15] The 5′ end has a cloverleaf structure that is believed to serve as a regulatory element for translation and replication. Distinct secondary and tertiary structures are present that represent internal ribosomal entry sites that enable ribosomes to initiate translation without the necessary "caps" required by most cellular messenger RNAs.[17] A shorter nontranslated region is present at the 3′ end, which is believed to play a role in termination of translation (Fig. 175.2).[19]

EPIDEMIOLOGY

Age

Rhinoviruses are worldwide in distribution. Infections occur most commonly in infants and young children. Infections appear to be less frequent in first the 6 months of life and then have high rates in infancy and early childhood.[9,18,20–22] Among children in the United States, the rate is 1.2 to 6 infections per person-year, and among adults, it is 0.75 infections per person-year.[22–26] Infection rates appear to be higher in young women with children than in men of similar age; however, rates are higher in older men than in older women.[23]

Seasonality

Rhinovirus infection occurs throughout the year, with increased rates in the spring and fall in the temperate climates.[22,23,27,28] The fall peak usually occurs in late August or early September in the Northern Hemisphere, and then the rates are usually lower until the peak in early April and May.[26] Overall, rhinovirus infections may account for up to

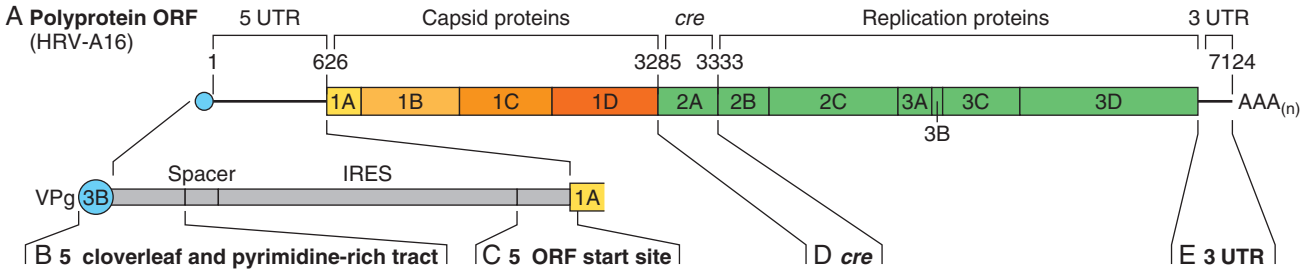

FIG. 175.2 (A) The genome of a human rhinovirus encodes a single polyprotein open reading form *(ORF)*. Important RNA structural motifs include a (B) 5′ cloverleaf, (C) ORF stat-site stem, (D) a *cre* element, and (E) 3′ stem motif. *(Modified from Gern J, Palmenberg AC. Rhinoviruses. In: Knipe DM, Howley PM, eds.* Fields Virology. *Philadelphia: Lippincott, Williams & Wilkins; 2013:533.)*

80% of upper respiratory illnesses during peak seasons.[29] Even during the low periods in the summer months, rhinoviruses may still be responsible for up to 50% of upper respiratory illnesses.[23,26]

The reasons for the seasonality of rhinovirus infections are not fully understood. Environmental high humidity improves rhinovirus survival, and high indoor humidity has been associated with an increased incidence of rhinovirus infections.[30] An increase in transmission has also been attributed to periods of time when schools are in session.[30,31] In tropical climates, rhinovirus infections appear to occur with little relationship to climatic factors.

PATHOGENESIS

The pathogenesis of rhinovirus infections has been studied in experimental infection of volunteers and in naturally occurring infection in patients with various rhinovirus-associated illnesses. Initial infection occurs in the nasal mucus or the eye. Experimental studies have shown that extremely low quantities of rhinovirus (1 median tissue culture infective dose) can induce infection when administered via the conjunctiva or nasal mucosa.[32]

The primary site of infection is the epithelium of the upper respiratory tract via receptors described earlier. Inoculation of rhinoviruses into the nasal cavity is followed by spread to the posterior nasopharynx.[33–36] Infection of the upper respiratory tract includes involvement of the paranasal sinuses, and rhinovirus can be detected in sinus secretions by polymerase chain reaction (PCR).[37] Sinus abnormalities are frequently noted on computed tomography or magnetic resonance imaging in both experimentally induced and naturally occurring colds.[38] Rhinoviruses grow preferentially at temperatures of 33°C to 35°C, rather than at higher temperatures of 37°C or greater. Studies indicate that rhinovirus replication at higher temperatures results in more expression of interferon types 1 and 3 and of interferon-stimulated genes, and this may account for the preferential growth at lower temperatures.[39] These observations led to the early concept that rhinoviruses may not be able to infect lower airways that have higher core temperatures. More recent studies indicate that medium and lower airways have core temperatures lower than 37°C and that they support rhinovirus replication efficiently.[9] After experimental infection of the upper airway, rhinoviruses are found in the lower airway by bronchoscopy or bronchoalveolar lavage in 50% of specimens.[40,41] It has also been noted that RV-C species grow well at 37°C in human sinus epithelial cells in contrast to RV-A and RV-B species.[42] Thus it is clear that rhinoviruses can infect the lower respiratory tract, but how frequently this occurs remains undetermined.[43]

Once symptoms of a cold are already present, biopsy specimens of the nasal mucosa and nasopharynx surprisingly show only small foci of infection, along with large areas of apparently uninfected cells.[36,44] Examination of specimens of nasal epithelium during rhinovirus infection by light or electron microscopy do not show consistent histopathology.[45]

Rhinovirus infection involves primarily epithelial ciliated cells, although nonciliated cells are also infected. It has been suggested that most of the rhinovirus-infected cells are likely shed into nasal secretions.[46]

Inflammatory Responses

Although histopathologic changes in the nasal mucosa are not prominent in rhinovirus infection, even when symptoms are clearly present, a variety of inflammatory and cellular immune responses can be seen. Polymorphonuclear leukocytes are present in both nasal mucosa and secretions.[47,48] A modest increase in peripheral polymorphonuclear leukocytes is seen during the first 2 to 3 days after experimental virus infection, which is not observed in volunteers who did not become ill.[49] Modest increases in T-cell lymphocyte counts in the nasal mucosa and nasal secretions also occur during rhinovirus infection, but data are conflicting on the effect of rhinovirus infection on peripheral lymphocyte counts.[50,51]

Rhinovirus infections stimulate a variety of innate immune responses including proinflammatory cytokines and mediators of inflammation. The signaling pathways that induce these effects are not fully determined, but Toll-like receptor 3, phosphatidylinositol 3-kinase, virus-induced oxidant stress, and mitogen protein kinases all have been implicated.[17,52,53] Ceramide-enriched cell membrane platforms may also play a role in stimulation of the signaling pathways.[17,54,55] Chemokines and cytokines that are present in increased concentrations in nasal secretions include interleukin-1β, interleukin-6, interleukin-8, and interferon-γ-induced protein 10.[17] Concentrations of interleukin-6, interleukin-8, and interferon-γ-induced protein 10 in nasal secretions are increased during colds, correlate with symptom severity, and decrease as symptoms abate.

Kinins, including bradykinin and lysyl-bradykinin are found in nasal mucosa during colds, and their concentrations correlate with the severity and time course of illness.[48,56] However, administration of a bradykinin antagonist did not reduce cold symptoms in one study[57]; reduction of the concentration of kinins by administration of steroids also did not reduce symptoms.[58] Histamine is present in nasal mucosa, and although antihistamines are commonly used for symptomatic treatment of colds, a trial of a second-generation antihistamine did not show an effect on rhinovirus-induced colds.[59]

Nasal Fluid Production

Parasympathetic innervation controls secretory function of nasal seromucous glands. Most of the nasal fluid produced by colds is derived from these glands, along with fluid that emerges by transudation.[60] Intranasal administration of drugs with anticholinergic activity such as atropine or ipratropium and oral administration of first-generation antihistamines reduce nasal fluid by one-third in experimentally induced colds.

The inflammatory processes that result in increased passage of serum into the nasal mucosa and nasal secretions are particularly prominent early in the course of illness and are major components of nasal obstruction. Increased secretion from nasal glands becomes more prominent later during the course of a cold.[16,17]

TRANSMISSION

Person-to-person transmission of rhinovirus infection occurs by direct contact or by aerosol. Direct contact appears to be the more efficient route of transmission. Rhinovirus survives for several hours on the skin of infected volunteers and can be recovered from 65% of fingers of infected subjects after finger-to-nose contact.[61,62] Self-inoculation into the eye or nasal mucosa can result via rhinovirus-contaminated fingers. Larger particle aerosols such as those produced by sneezing or coughing transmit infection in experimental studies.[63,64] Small particle

aerosols appear to have a lesser role in transmission, although rhinovirus RNA has been detected in such aerosols.[17] In experimentally induced infection, rhinovirus transmission was most efficient when there were large concentrations of virus in the nose (>1000 median tissue culture infective dose), when virus was present on the hands and nasal mucosa, and when symptoms of a cold were most severe.[65] These findings occurred most commonly during the second or third day after virus inoculation.[17]

The efficiency of transmission of rhinovirus infection is increased by time and closeness of contact between infected and susceptible individuals. Children not only have the highest rates of infection but also have the highest rates of transmission to other children and to adults.[66,67] In experimental studies, the highest transmission rates occurred among married couples, under crowded living conditions, and in association with severity of illness.[68–70]

In studies of naturally occurring rhinovirus infection, the importance of the direct contact route of transmission is less clear cut. Rhinovirus can be isolated from approximately 40% of individuals with colds and from 6% to 15% of objects found in their environment.[17,61,62] Individuals routinely make finger-to-nose or finger-to-eye contact that can self-inoculate infection. Application of 2% aqueous iodine to fingers of mothers of children with colds appeared to prevent infection in the mothers in one study.[61] However, a study of the use of a virucidal hand disinfectant in young adults did not reduce acquisition of rhinovirus infections.[71]

The role of fomites in transmission of rhinovirus infection remains uncertain. Although objects in the environment can be readily contaminated by contact with hands that contain virus, attempts to demonstrate experimental transmission from such fomites have not succeeded.[72]

CLINICAL MANIFESTATIONS OF COMMON COLD

The most frequent illness associated with rhinovirus infection is the common cold, an upper respiratory syndrome (Fig. 175.3). After experimental infection, there is a 1- to 2-day incubation period followed by the onset of throat discomfort, usually described as "scratchy" or sore. Rhinorrhea and nasal obstruction develop shortly thereafter, along with various combinations of hoarseness, sneezing, headache, malaise, and feverishness. Cough occurs in approximately 30% of cases, usually begins after the appearance of nasal signs and symptoms, and may persist after those subside.[17] Illness is similar in adults and children except that fever occurs in approximately one-third of children but is usually absent in adults.[73]

The duration and severity of rhinovirus-induced colds are quite variable in severity and duration. Naturally occurring illness has been reported to last 1 to 33 days, with a median duration of 7 days, and approximately one-fourth last for 2 weeks.[17,74] Peak signs and symptoms of illness generally occur 2 to 4 days after onset, and illness usually subsides or ends by 1 week. Some minor symptoms may linger longer and likely account for the prolonged illness reported in some studies.[9,68,69]

Computed tomography performed by Gwaltney and colleagues[38] showed that paranasal involvement, consisting of mucosal thickening, swelling, or fluid accumulation, occurred in one or more sinuses in 87% of subjects with clinically typical colds. Rhinovirus RNA has also

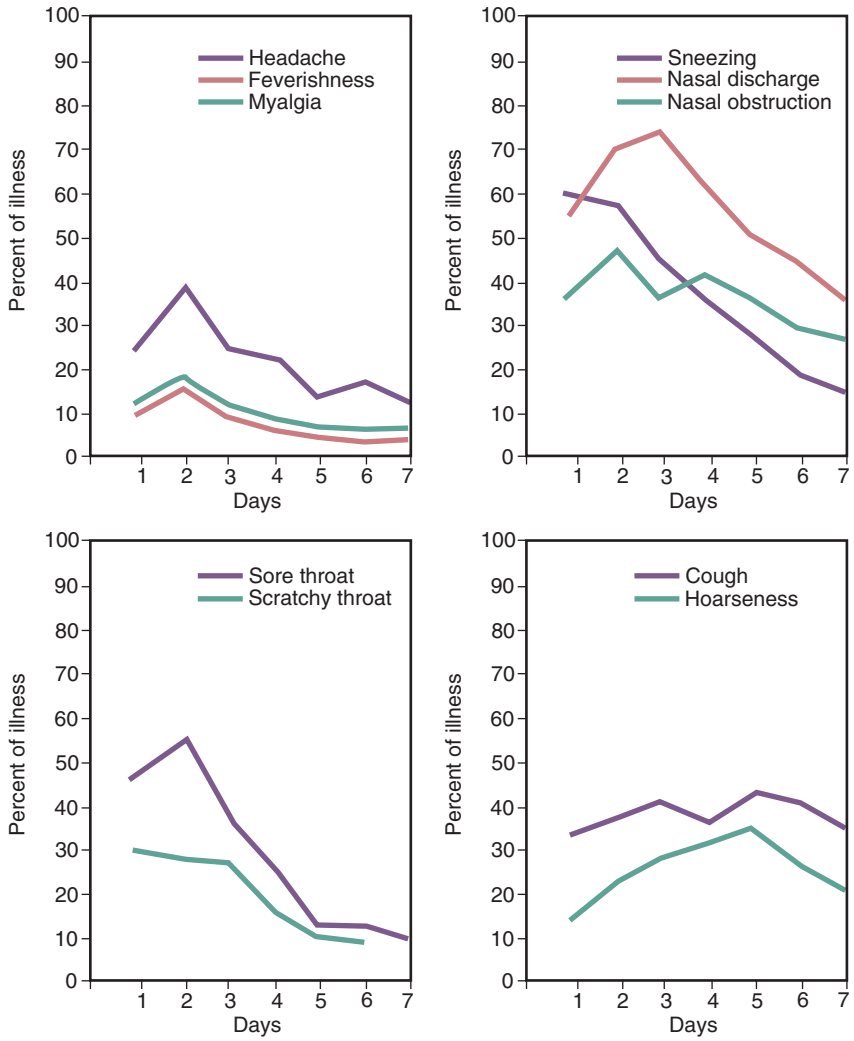

FIG. 175.3 **Rhinovirus cold symptoms (139 adults with natural infection).**

been found in brushings from sinus cavities,[75] and rhinoviruses have been isolated from sinus aspirates. On this basis, it has been suggested that colds might be appropriately characterized as a viral rhinosinusitis.[46] This common rhinovirus involvement of sinuses resolves without specific therapy,[75,76] in contrast to the complication of bacterial sinusitis described next.

COMPLICATIONS

Acute Bacterial Sinusitis

The frequency of acute bacterial sinusitis as a complication of a cold is difficult to ascertain, in part because of the frequent involvement of sinuses in uncomplicated colds. Various studies have reported that 0.5% to 8% of colds have been complicated by bacterial sinusitis.[77] The precipitating factors for development of acute bacterial sinusitis in association with colds have not been fully elucidated. Infection may result from retrograde propulsion of bacteria into sinuses by nose blowing, by occlusion of drainage of paranasal ostia, and by obstruction of the ethmoid and infundibulum.[17] Facial swelling, pressure, pain, and tenderness accompanied by fever should increase the suspicion that bacterial sinusitis may be present. The persistence of a respiratory illness beyond the typical 2-week duration of a cold should also increase that suspicion. (Also see Chapter 62.)

Acute Bacterial Otitis Media

Rhinovirus infections frequently result in abnormalities and dysfunction in the eustachian tube and middle ear.[78,79] Increased middle ear pressures occur in three-fourths of patients with rhinovirus colds, sometimes in association with middle ear effusions.[17] Rhinovirus has been found alone or in combination with bacteria in middle ear fluids of 24% of patients with otitis media.[80] It has been estimated that otitis media is associated with 30% of colds in children and with 2% of colds in adults.[77,81] (Also see Chapter 61.)

Asthma

Rhinovirus infections are major causes of wheezing syndromes and of exacerbations of asthma.[82,83] They are associated with 60% to 70% of asthma exacerbations in school-aged children and have been implicated in precipitation of asthma attacks in older children and adults. The basis for this association is not well understood. Exacerbation appears to occur most often when rhinovirus infection is superimposed on airway inflammation caused by allergens, as reflected by serum immunoglobulin E (IgE) concentrations or by airway eosinophilia.[17,84–87] Precipitation of asthma episodes by rhinovirus infection in association with allergen-specific IgE appears to be particularly important in children older than 2 years of age. Studies suggest that rhinovirus infection may interact with a genetic risk for development of childhood asthma.[88]

Exacerbation of Chronic Bronchitis

Rhinoviruses have also been implicated in up to 40% of exacerbations of chronic bronchitis, as characterized by fever, increased production of purulent sputum, and deterioration of ventilation.[17] Exacerbation of bronchiectasis, chronic obstructive pulmonary disease, and cystic fibrosis and induction of bronchiolitis have also been described.

Lower Respiratory Tract Infections

The precise role of rhinovirus as pathogens in infections of the lower respiratory tract is also difficult to ascertain. This is due in part to the frequency of rhinovirus infection and shedding in the upper respiratory tract but also to other microbial pathogens that are often isolated concomitantly with rhinovirus from the lower respiratory tract.

Using reverse-transcriptase (RT)–PCR techniques, rhinoviruses have been detected as the sole pathogen in approximately 10% of children hospitalized with bronchiolitis.[89,90] Another study found rhinovirus in 21% of children with bronchiolitis, but it was the only pathogen in 2% of patients.[91,92]

Rhinovirus can be frequently detected in the upper respiratory tract of patients with pneumonia.[93] Using RT-PCR, one study reported that 24% of children with pneumonia had rhinovirus detected in the upper respiratory tract, but more than half of these had evidence of a concomitant bacterial pathogen.[94] Broadly based surveillance studies of community-acquired pneumonia have described detection of multiple viral and bacterial potential pathogens. In specimens from these studies, rhinoviruses have been frequently detected in the United States (9%),[95] Norway (12%),[96] and New Zealand (13%).[97] The rate of detection of rhinoviruses in subjects without pneumonia was significantly less.[95,97] Rhinoviruses were also frequently detected in patients with community-acquired pneumonia who required mechanically assisted ventilation.[98] Based on these and other studies, investigators have suggested that rhinoviruses should be considered as potential etiologic agents of pneumonia.[99,100]

Infections in Immunocompromised Patients

Assessment of the impact of rhinovirus infections in immunocompromised patients is challenging because of the clinical complexity of immunocompromised patients, the frequency of rhinovirus infections throughout the year, and the concomitant detection of other potential pathogens along with rhinoviruses. It is well documented that rhinovirus infection is frequent and can be prolonged in immunocompromised patients. Prolonged shedding is seen, ranging from 30 to 455 days (means of 61 and 92 days), and has been described in patients with hematologic malignancies, hematopoietic stem cell transplants, and other immunosuppressed states.[101–104]

Upper respiratory illnesses were most frequently encountered in immunocompromised patients, but lower respiratory tract illnesses were also seen. Most of the lower respiratory illnesses had concomitant bacterial, fungal, or other viral pathogens noted, but there were instances in which rhinoviruses were the only pathogens detected in the lower respiratory tract.

The overall impact of rhinovirus infection is difficult to ascertain. In one study, the mortality of patients with bronchoalveolar lavage confirmed rhinovirus infection at 1 year was 55% compared with 41% in the total hematopoietic stem cell transplant cohort. Another study reported a similar severity of disease in immunocompromised patients with influenza A/H1N1 infections in 2009 and immunocompromised patients with rhinovirus infections.[105] A recent study reported higher morbidity and mortality in hospitalized elderly patients with rhinovirus infections compared with hospitalized elderly patients with influenza, most of whom had infections with A/H3N2.[106]

DIAGNOSIS

The clinical characteristics of infections caused by rhinoviruses are not sufficiently distinctive either clinically or epidemiologically to make a specific etiologic diagnosis without laboratory techniques. Historically, detection of rhinoviruses has been carried out by isolation of virus in tissue cultures, and much of our understanding of rhinovirus infection has been derived on that basis. Culture has now been largely supplanted by molecular techniques such as RT-PCR, which are considerably more sensitive and efficient

Rhinovirus can be detected in nasal aspirates, swabs, or washings. After experimental infection, virus shedding in the upper airways peaks at 2 to 4 days after inoculation and generally lasts 1 to 2 weeks and occasionally up to 3 weeks.[9,35]

Virus Isolation in Tissue Culture

Detection of rhinoviruses by growth in tissue cultures is carried out using human diploid embryonic lung fibroblasts (WI-38, MRC-5) or HeLa cells. Growth of rhinoviruses is most efficient at 33°C or 34°C and when cultures are incubated in rolling drum conditions. Cultures are maintained for 10 to 14 days after incubation, and cytopathic effect can be readily observed. Tissue cultures may vary in sensitivity for rhinovirus growth, and lots should be monitored for this characteristic. Blind passage in HeLa cells may increase sensitivity for growth. As discussed earlier, rhinovirus species A and B can be grown in tissue culture, but species C does not grow in standard cultures and requires molecular techniques for detection.

Molecular Techniques

RT-PCR has become the standard technique for detection of rhinovirus. Primers for this technique take advantage of the fact that the 5′

untranslated region contains subregions that are highly conserved among all types of rhinoviruses.[107–109] Additional regions such as those on VP2-4 or VP1 can be used for genotyping. The use of multiple different primers increases sensitivity.[110]

Antigen Detection

Serotype-specific antibodies conjugated with immunofluorescence or immunoperoxidase techniques can be used to detect individual serotypes but lack the sensitivity and breadth for use as diagnostic aids.[17]

Neutralizing Antibody Assays

Development of serotype-specific neutralizing antibodies occurs within 7 to 14 days after infection and consists mostly of the IgG1 subclass in serum and of IgA in nasal secretions.[22,111,112] Because the assays are serotype specific, they are diagnostically useful only when the serotype is known and as in experimentally induced infection or in outbreaks in families or in closed environments.

THERAPY

Symptomatic Therapy

A wide variety of symptomatic therapies have been proposed and advocated for common colds regardless of their etiology including colds caused by rhinoviruses. Studies of several of these have shown varying degrees of effectiveness of treatments for nasal congestion, rhinorrhea, and sore throat. These are discussed in detail in Chapter 58. It should be remembered that the common cold, although very frequent, is overwhelmingly a self-limited illness that resolves spontaneously; hence any potential benefit of treatment should be weighed carefully against risks and costs.

Antivirals

Antivirals with in vitro activity against rhinoviruses by a variety of mechanisms have been described. The most intensively studied of these have been agents that bind to the rhinovirus capsid at the hydrophobic pocket formed by VP1.These agents block attachment by rhinovirus to the cell receptor, inhibit ingress of virus into the cell, and interfere with release of viral RNA intracellularly. The most extensively studied of these in clinical trials is pleconaril, which is active against 90% of rhinovirus clinical isolates in vitro (see Chapter 48).[113] Pleconaril administered at 400 mg three times a day for 5 days reduced the duration of naturally occurring uncomplicated colds by approximately 1 day in two studies.[114,115] An intranasal formulation of pleconaril was found to be ineffective in prevention of naturally occurring colds.[116] The US Food and Drug Administration has not approved pleconaril for these indications. The drug was also noted to induce CYP3A enzymes and consequently has the potential for multiple drug interactions.

Vapendavir (BTA798) is another capsid-binding agent that binds to VP1 of RV-A and RV-B. The drug was well tolerated after oral administration, and given 2 days before a rhinovirus challenge, it reduced the frequency of rhinovirus infection and peak virus load.[117] A phase IIb study in patients with asthma failed to show a beneficial effect in naturally occurring rhinovirus infection, although an antiviral effect was noted in subjects who received drug during the first 24 hours of symptoms.

The 3C protease of rhinoviruses is also an attractive target for antiviral inhibition. It cleaves the rhinovirus polyprotein that provides essential structural and enzymatic proteins for rhinovirus replication and is highly conserved among rhinovirus serotypes. Rupintrivir, a 3C protease inhibitor, was studied in clinical trials of experimental rhinovirus infection and was shown to have a modest effect on infection rate and severity of illness.[118]

Interferon

Intranasally administered leukocyte and recombinant interferon have been studied extensively in experimentally induced and naturally occurring colds in both prophylaxis and treatment of rhinovirus infections.[119] Intranasally administered interferon-α was effective in prophylaxis of naturally occurring rhinovirus-induced colds but was ineffective in treatment. Intranasal application of interferons for 1 to 2 weeks was associated with nasal side effects and local toxicity, which halted further development of this approach.

PREVENTION

The economic impact of the common cold in the industrialized world based on added costs and loss of productivity is enormous. In the United States, it has been estimated to exceed $40 billion per year. Because of this, there has been great interest in development of preventive measures, but few have been demonstrated to have any effects.

Virus Inactivation on Skin

As direct contact via hand to nose or hand to eye is a major route of transmission of rhinovirus, it appears reasonable that measures to remove or inactivate virus on hands may prevent infections that occur through self-inoculation. However, the effectiveness of such measures has been difficult to demonstrate. Hand sanitizers that contain 62% ethanol clearly inactivate virus on skin, but use in the setting of naturally occurring infection did not prevent colds.[120–122] Hand treatment with 2% aqueous iodine solution resulted in virus inactivation and did provide prevention against rhinovirus infection. However, the iodine stains on fingers made its use unacceptable.[61]

Acid Inactivation of Virus

The acid lability of rhinoviruses has led to the addition of organic acids to nasal tissues, hand treatments, and sprays in attempts to prevent infection. Studies of nasal tissues that contain citric acid, malic acid, and sodium laurel sulfate showed little effect on the frequency of colds under natural conditions.[123] Organic acids incorporated into hand treatments have also been ineffective in prevention of colds.[71] A study of a nasal spray containing low pH saline found a very modest reduction in virus titer and had no effect on symptoms.[124]

Intercellular Adhesion Molecule 1 Receptor Blockade

Monoclonal antibody directed at the major cellular receptor of rhinovirus, ICAM-1, has been demonstrated to block rhinovirus infection in vitro. Studies of this antibody in prophylaxis of experimental infection did not show an overall consistent benefit on virus shedding or on symptoms of colds.

An additional approach used generation of truncated forms of ICAM-1 through deletion of transmembrane and intracellular domains of the protein, referred to as soluble ICAMs, which inhibited rhinovirus infection in vitro. However, in a clinical trial of experimental infections, soluble ICAMs administered 7 hours before or 12 hours after challenge did not affect the rate of infection, although symptoms were reduced.[125]

VACCINES

The discovery of rhinovirus more than 60 years ago was associated with great interest in the development of vaccines. It was recognized early that the presence of rhinovirus serotype-specific neutralizing antibodies was associated with some protection against infection with homologous, but not with heterologous, serotypes.[126] Early trials with formalin-inactivated rhinoviruses as vaccine candidates, given intramuscularly or intranasally, showed protection against homologous challenge in terms of reduction of symptoms but not in reduction of rates of infection.[127,128] However, the multiplicity of rhinovirus antigenic types has presented a major impediment to the development of vaccines since those early studies. Molecular studies have identified a region of the N-terminus that can raise type cross-reactive neutralizing antibodies, and this may be useful as a vaccine candidate.[129] Use of modern techniques has led to generation of polyvalent rhinovirus vaccines with up to a 50-valent composition, which is broadly immunogenic in nonhuman primates, that are undergoing further development.[130]

ACKNOWLEDGMENT

Sections of this chapter are based in part on previous chapters in *Principles and Practice of Infectious Diseases* authored by Jack Gwaltney and Ron Turner.

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176 Noroviruses and Sapoviruses (Caliciviruses)

Raphael Dolin and John J. Treanor

SHORT VIEW SUMMARY

Definition
- The genera *Norovirus* and *Sapovirus,* of the family Caliciviridae, cause acute gastroenteritis.

Epidemiology
- Noroviruses are the major worldwide cause of viral gastroenteritis, cause both foodborne and person-to-person outbreaks, and can be spread via fomites.
- Noroviruses account for 699 million illnesses worldwide annually, 21 million cases of gastroenteritis in the United States, and 219,000 deaths annually in the developing world, mostly among children younger than 5 years.
- Sapoviruses cause gastroenteritis less frequently than noroviruses.

Microbiology
- Noroviruses and sapoviruses are single-stranded, positive-sense RNA viruses, which are divided into 7 genogroups (noroviruses) and 14 genogroups (sapoviruses). Each genogroup has multiple genotypes.
- Human noroviruses have been cultured in vitro for the first time recently.

Clinical Manifestations
- Illness caused by noroviruses and sapoviruses consists of combinations of vomiting and diarrhea, often accompanied by abdominal cramps, nausea, and low-grade fever.
- Illness usually lasts 12 to 60 hours and remits spontaneously.
- More severe disease and even fatalities can occur in young children and in the elderly. Immunocompromised patients may have prolonged shedding of virus, along with severe and persistent gastrointestinal illness.

Diagnosis
- Noroviruses may be suspected as a cause of outbreaks of gastrointestinal illness if the aforementioned clinical features are present, and particularly if a large number of individuals are involved. However, the illness is not sufficiently distinctive to permit a diagnosis to be made on clinical grounds alone.
- Specific diagnosis is made by detection of virus in stool specimens with reverse-transcriptase polymerase chain reaction assay. Enzyme immunoassays are also available, but they are generally of lower sensitivity and specificity.

Therapy
- Only supportive therapy is available, and maintenance of hydration is particularly important.

Prevention
- The main control measures are to prevent contamination of food supplies and water through good hygiene and restriction of food handling by ill individuals until at least 2 to 3 days after gastrointestinal illness has resolved.
- Vigorous hand washing by ill individuals should be maintained.
- Decontamination of exposed surfaces should be carried out with Environmental Protection Agency–recommended disinfectants; for example, household bleach should be used at 1:10 to 1:50 dilutions.
- Vaccines against norovirus are under active development, including virus-like particles, which are undergoing clinical trial.

Acute infectious gastroenteritis is an exceedingly common and widespread illness throughout the world. Noroviruses are major causes of this illness,[1,2] which is generally self-limited but can result in hospitalization,[3] mortality in young children in the developing world,[4] and severe illness in elderly[5] and immunocompromised patients.[6] *Sapovirus,* another calicivirus, and *Astrovirus* (see Chapter 177) also cause gastroenteritis but are much less common than noroviruses.

HISTORY

The failure to isolate causative agents, bacterial or viral, from apparently infectious outbreaks of diarrhea or vomiting, or both, led to the widely held assumption that undetected viruses were responsible for such disease. In 1945, Reimann and coworkers[7] transmitted disease to volunteers by administering bacteria-free filtrates of throat washings, stool filtrates, or both from naturally occurring cases. Gordon[8] and Jordan[9] and their associates also induced disease in volunteers with bacteria-free material. These studies described two transmissible agents of subbacterial size, the Marcy and FS agents, which appeared to be antigenically distinct. However, these workers were unable to detect viral agents in vitro with techniques available at that time. Despite extensive virologic investigations in laboratories throughout the world, relatively little progress was made in this area until 1972, when the Norwalk virus, the prototype of this group, was described and partially characterized.[10,11] This virus was initially detected in diarrheal stools obtained during an outbreak of gastroenteritis in Norwalk, Ohio, that involved students in an elementary school and family contacts. Subsequently, additional viruses with similar properties were described, including the Hawaii,[12] Montgomery County (MC),[13] Taunton,[14] and Snow Mountain[15] viruses, also named for the geographic regions in which they were first recognized. All these viruses had a similar small, round, structured morphologic appearance with electron microscopy; were of a similar size and density; did not grow in any in vitro propagation system; and were responsible for acute gastroenteritis, commonly in epidemic form with high secondary attack rates.[16–18] At the same time, viruses with more readily identifiable morphologic features with electron microscopy, referred to as *human caliciviruses,*[19] were observed in the stools of individuals, primarily children, with gastroenteritis.

A major advance in this field occurred when polymerase chain reaction (PCR) techniques were applied to amplify the genome of the Norwalk virus from virion-containing stool samples.[20,21] These

studies identified the Norwalk virus as a member of the Caliciviridae family and allowed determination of the complete nucleotide sequence of this virus.[22] Subsequent molecular studies have clearly identified all these viruses as caliciviruses and established them as major causes of gastrointestinal disease in both adults and children worldwide.

VIROLOGY

Taxonomy

The name *calicivirus,* and hence the virus family Caliciviridae, is derived from the characteristic appearance of the viral particles on electron micrographs, which consists of a scalloped border with cuplike indentations on its surface (Fig. 176.1), from which the Latin name *chalice* or *calyx* is derived.[23,24] Caliciviruses have been detected in a variety of animal species, including marine mammals, swine, felines, and rabbits, in addition to humans. Five genera have been described: *Norovirus, Sapovirus, Vesivirus, Lagovirus,* and *Nebovirus.* Human infections are caused by *Norovirus*[25,26] and *Sapovirus.*[27] *Vesivirus* causes vesicular diseases in swine, cats, and marine mammals; *Lagovirus* causes hemorrhagic diseases in rabbits; and *Nebovirus* causes enteric disease in calves. The prototypical *Norovirus* is the Norwalk virus, and the prototypical *Sapovirus* is the Sapporo virus. In addition to sequence differences, the genera differ in minor details of genome organization. Whereas many animal caliciviruses replicate efficiently in cell culture, the propagation of human noroviruses or sapoviruses in vitro has been difficult to achieve, although transfection of viral RNA from norovirus in human embryonic cells[28,29] and infection of human intestinal organ cultures[30] have been reported. Thus, much of the initial microbiologic information on noroviruses was based on physical properties determined with electron microscopy or by means of physicochemical manipulation of infectious inocula. Because of the small numbers of virions characteristically found in stool samples, it is sometimes necessary to enhance electron microscopic visualization of the particles through the addition of immune serum, which obscures the typical morphologic features (Fig. 176.2). Recently a major breakthrough was reported, in which efficient culture of human norovirus was achieved in enterocytes obtained from stem cells in human intestinal epithelium.[31] The cultivation of noroviruses in vitro in a B-cell line has also been reported.[32,33] These advances should enable efficient, detailed molecular characterization of noroviruses to be carried out, along with facilitation of efforts to develop effective vaccines and therapeutics (see later).

Genome Organization

Characteristics of the noroviruses include a single-stranded, positive-sense RNA genome with a polyadenylated 3′ tail[22,34] and a single capsid polypeptide of 59- to 62-kDa molecular mass.[35] The virions are 26 to 34 nm in diameter, have cubic symmetry with a buoyant density in cesium chloride of 1.34 to 1.41 g/mL, and are relatively heat and acid stable and ether resistant.[11]

The genomic organization of noroviruses is shown in Fig. 176.3.[36] Noroviruses have a positive-sense, single-stranded RNA genome of approximately 7700 nucleotides, excluding the polyadenylated tail. Three long open reading frames (ORFs) are present. The first ORF encodes a polyprotein of about 57-kDa molecular weight, which includes and codes for seven nonstructural proteins, including the viral RNA polymerase, helicase, and protease functions.[37] The second ORF encodes the viral capsid protein (VP1) of 58-kDa molecular weight, which determines the antigenic phenotype and interacts with host cell receptors.[38] When expressed in eukaryotic cells, the capsid protein spontaneously assembles into virus-like particles (VLPs), which are immunogenic and react specifically with convalescent human sera.[38] The three-dimensional structure of these empty capsids has been studied with electron cryomicroscopy, which suggests that the capsid has icosahedral symmetry with T = 3.[39] The x-ray crystallographic structure of these VLPs shows that the capsid contains two domains, a shell (S) domain and a protruding (P) domain that may be involved in binding to susceptible cells.[36,40] Finally, the third ORF encodes a minor structural protein (VP2) of 12- to 29-kDa molecular weight,[22,41] which may add to particle stability and may also be involved in capsid assembly and genome encapsidation.[42] The overall genetic organization of sapoviruses is similar to that of noroviruses, except that sapoviruses contain two ORFs. ORF-1 encodes both nonstructural and the major structural protein (VP1), whereas ORF-2 encodes the minor structural protein.[27,43] A third ORF has been predicted in several sapoviruses, but its function is unknown.

Based on phylogenetic analyses, noroviruses have been subdivided into seven genogroups designated GI to GVII,[26,44,45] and the genogroups have been further divided into at least 34 genotypes. Individual genotypes are designated by Arabic numerals after the genogroup designation—for

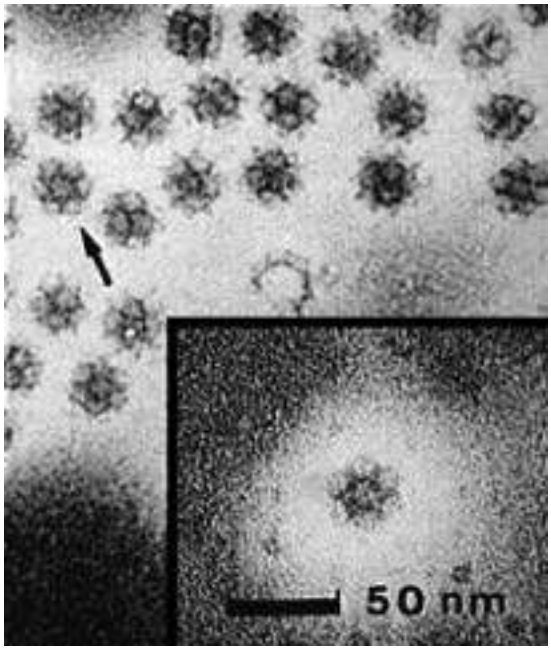

FIG. 176.1 Calicivirus particles *(arrow)* in a fecal extract from a child with gastroenteritis. *Inset* is higher magnification of particle indicated by *arrow. (From Chiba S, Sakuma Y, Kogasaka R, et al. An outbreak of gastroenteritis associated with calicivirus in an infant home.* J Med Virol. *1979;4:249–254.)*

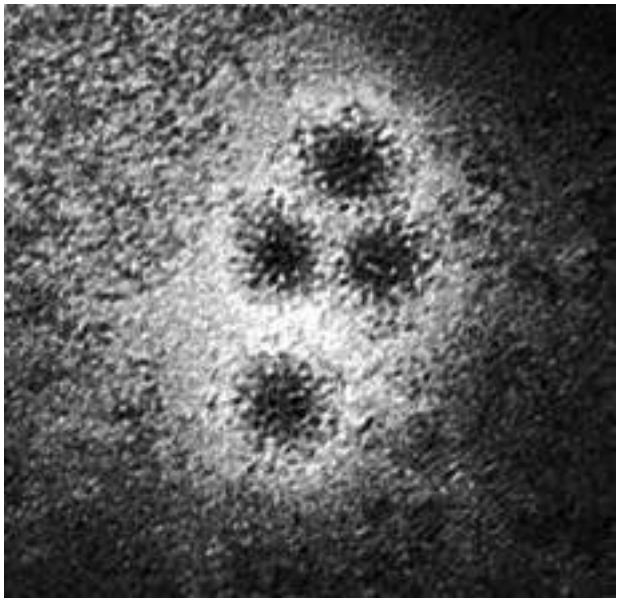

FIG. 176.2 Snow mountain virus in stool filtrate from a volunteer with experimentally induced disease as visualized with immune electron microscopy. Particles are 27 nm in diameter and are stained with 2% phosphotungstic acid.

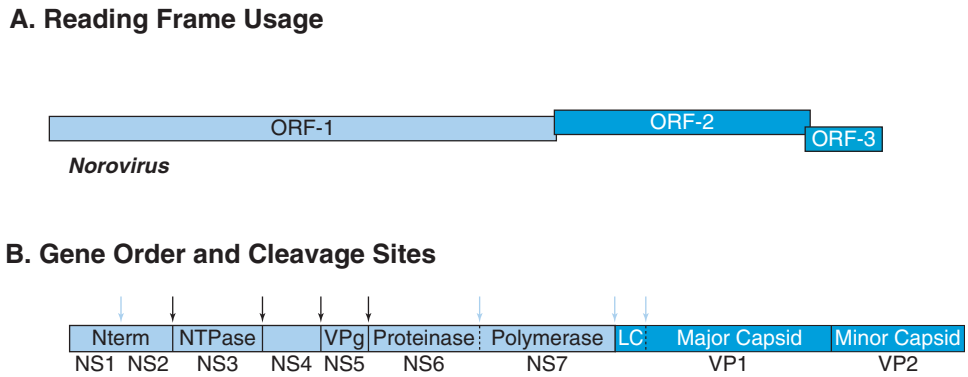

FIG. 176.3 Genome organization of norovirus. (A) Reading frames: Open reading frame 1 (ORF-1) codes for nonstructural proteins: ORF-2 codes for the major capsid VP1; ORF-3 codes for the minor capsid VP2. (B) Gene order and cleavage sites: ORF-1 codes a polyprotein that has seven distinct proteins. ORF-2 codes for a leader protein *(LC)* and for VP1. ORF-3 codes for VP2. *(Modified from Green KY. Caliciviridae: the noroviruses. In:* Fields Virology. *6th ed. Philadelphia: Lippincott; 2013.)*

example GI.1 or GII.1. Most of the strains implicated in human disease fall into genogroups GI and GII, but GIV strains also infect humans.[26] The GI genogroup includes the Norwalk virus, whereas the Snow Mountain and Hawaii viruses belong to genogroup GII.

Sapoviruses have also been further subdivided into 14 genogroups (GI to GXIV), of which GI, GII, GIV, and GV are known to infect humans.[46] Multiple genotypes within the genogroups have also been noted.[26]

Antigenic Characterization

Because of the lack of a convenient in vitro propagation system, antigenic characterization of these viruses has not been straightforward until now. Not unexpectedly, predicted amino-acid homology within the capsid region is less than that within the polymerase region.[44] Thus, phylogenetic trees based on capsid sequence have a slightly different structure than those based on polymerase structure.[47] VLPs have been generated by expression of the capsid regions of many of the noroviruses, including Norwalk[38] and Desert Shield[48] viruses (genogroup GI) and MX,[49] Lordsdale,[50] Snow Mountain,[47] Hawaii,[51] and Toronto[52] viruses (genogroup GII), in addition to the prototype sapovirus, Sapporo.[53] In general, hyperimmune animal sera raised against capsids are very specific. However, tests of postinfection human sera have suggested a significant degree of cross-reactivity among viruses within a genogroup.

The most clear-cut antigenic distinction among noroviruses is between the Norwalk and Hawaii viruses because these agents have been compared through cross-challenge experiments in human subjects.[54] In these studies, infection with the Norwalk virus provided short-term protection against rechallenge with the Norwalk virus but not against the Hawaii virus, and vice versa. Because this type of experiment is the closest analogue to virus neutralization that has been previously available, this is direct evidence that there are at least two distinct norovirus serotypes, roughly corresponding to the GI and GII genogroups described earlier.

EPIDEMIOLOGY

With the advent of efficient means for their detection (see later), noroviruses have emerged as major, worldwide causes of gastroenteritis in diverse populations and in both children and adults. Noroviruses are the most common cause of epidemic gastroenteritis and account for more than 90% of outbreaks of viral gastroenteritis and for about 50% of all-cause outbreaks worldwide.[18,26,55–60] Noroviruses are also important causes of sporadic cases of gastroenteritis and account for 4.4% to 30.7% of children younger than 5 years who are hospitalized with gastroenteritis throughout the world.[26,61] Overall, it has been estimated that noroviruses cause 699 million illnesses and 219,000 deaths annually worldwide. Mortality is believed to occur primarily in children younger than 5 years.[4,62] The Centers for Disease Control and Prevention (CDC) estimates that noroviruses cause 40% to 50% of foodborne outbreaks of gastroenteritis in the United States and overall account for 21 million cases of gastroenteritis, 2 million office visits, 70,000 hospitalizations, and up to 800 deaths annually in the United States.[26,63,64]

Antigenic Variation

The role of antigenic variation in the epidemiology of noroviruses remains an area of continued investigation. In general, antibody to noroviruses within genogroup II appears to be more common than to viruses within genogroup I. However, a number of diverse genotypes often cocirculate, including recombinants among different strains,[65–67] and there may be year-to-year variation in the predominant viruses associated with illnesses.[68,69] Despite this, viruses from a single genotype, GII.4, have been responsible for the majority of outbreaks worldwide since 1995.[26,70–72] Novel pandemic GII.4 variants have evolved every 2 to 3 years since the 1990s and replaced previously predominant GII.4 strains. GII.4 strains were the predominant strains associated worldwide with outbreaks from 2001 to 2012.[73,74] In 2014, GII.17 virus emerged and spread worldwide in 2014–2015.[75–79]

The epidemiology of sapoviruses has been less extensively studied than that of noroviruses, but sapoviruses appear to be substantially less frequent causes of outbreaks of gastroenteritis than noroviruses.[27] The original Sapporo virus was reported in Japan and appeared to affect young children primarily.[80] Subsequently, sapoviruses have been reported in outbreaks in adults in various settings, including long-term care facilities.[80–84] Reports have indicated that sapoviruses caused 1.8% to 8.0% of outbreaks that were studied in diverse geographic locales.[27]

Seroprevalence

In developed countries, serum antibody to the noroviruses is first noted at ages 3 to 4 years, with antibody prevalence gradually rising to greater than 50% by the fifth decade of life.[85–88] Studies using recombinant Norwalk antigen have suggested that significant increases in antibody prevalence occur in infancy, at entry into primary schools, and in young adulthood.[89] Seroepidemiologic studies of the sapoviruses carried out in Japan, Southeast Asia, and the United Kingdom have indicated that antibody is acquired in early childhood and can be detected in up to 90% of older children and adults.[90–92] Antibody appears to be acquired more rapidly in developing countries[85] and may be rare or nonexistent in some isolated populations.[85,93] Transmission of noroviruses occurs year round but with a higher incidence of disease in the winter months in temperate climates.[94]

Seroprevalence studies of sapovirus antibodies indicated that infection also occurs early in childhood and reaches >90% in school age children. Seroprevalence remained high (80%–100%) in pooled immunoglobulins obtained from adults.[27]

Transmission of Infection

The experimental induction of illness in volunteers suggests that the major route of person-to-person transmission is fecal-oral. Epidemiologic reports have also implicated vomitus as a vehicle of transmission,[95,96]

and virus has been detected in vomitus with electron microscopy[97] and PCR.[98] Airborne transmission has also occasionally been implicated,[99,100] but limited attempts to experimentally transmit virus with nasopharyngeal washings from an ill volunteer were unsuccessful.[11] It has been estimated that fewer than 100 viral particles are required for infection of a susceptible individual,[101] although studies have suggested that 1300 to 2800 particles may be necessary.[102]

Incubation periods are generally 24 to 48 hours, although ranges from 18 to 72 hours have been observed. Virus shedding in stools is maximal over the first 24 to 48 hours after illness.[13,15] In volunteer studies, virus has been rarely detected beyond 72 hours after the onset of vomiting or diarrhea[13,15] with immune electron microscopy. However, virus can be detected for 3 to 4 weeks after illness with use of sensitive enzyme-linked immunosorbent assay techniques[103] or PCR.[104–106] Prolonged shedding of norovirus for months may occur in immunocompromised indivduals.[107] The clinical significance of the prolonged detection of virus in stools is unclear, but epidemiologic data have implicated individuals who are not symptomatic in the transmission of illness.[108]

Noroviruses were first recognized in association with point-source outbreaks of gastroenteritis, and such outbreaks are a common situation in which noroviruses have been implicated as etiologic agents. Several features are characteristic of such outbreaks that may be useful in suspecting norovirus as a cause of the illness. These include a short-lived illness of 2 to 3 days' duration, with vomiting as a prominent symptom in most affected individuals; an incubation period of 24 to 48 hours; high secondary attack rates; and lack of other identifiable pathogens on routine examinations of stool samples.[109] The application of modern diagnostic techniques has shown that the frequency of norovirus infection in such outbreaks is extremely high.[65]

Outbreaks of norovirus gastroenteritis are particularly common in closed settings, such as in hospitals, nursing homes, ships, schools, and the military.[110–113] Secondary transmission is a prominent feature of such outbreaks. Although most of these outbreaks will terminate spontaneously after 1 to 2 weeks, some may be quite prolonged. For example, up to 12 recurrent outbreaks of norovirus gastroenteritis have been reported on cruise ships despite stringent attempts to determine the source and disinfect the ship between cruises.[114]

Environmental Contamination

Contamination of objects can result in transmission of infection via fomites, and such surfaces should be decontaminated using Environmental Protection Agency (EPA)–recommended disinfectants (see "Prevention").

Almost any type of food that has contact with contaminated water or is contaminated by food handlers may serve as a vehicle for outbreaks of norovirus gastroenteritis.[115] The most common foods implicated in norovirus outbreaks are sandwiches and salads, particularly those that require handling but not subsequent cooking. Contamination of lettuce and salad greens with noroviruses accounts for nearly 25% of all produce-associated outbreaks.[26,116] Outbreaks have also been associated with drinking contaminated water or even swimming in pools or lakes in which ill individuals have also been swimming,[117,118] which indicates the highly infectious nature of these viruses. Of note, these viruses appear to be relatively resistant to inactivation by chlorine.[96] Because such products as shellfish or contaminated commercial ice[119] can be distributed to multiple sites, these outbreaks can encompass a wide geographic area.[120] Contamination of foodstuffs has been traced to both presymptomatic[121] and postsymptomatic[108] food handlers, complicating infection-control recommendations.

Shellfish, such as clams and oysters, are filter feeders and efficiently concentrate microorganisms from contaminated water. When consumed, these foods are frequently implicated in the transmission of enteric viruses in general and of norovirus gastroenteritis in particular.[122] Because noroviruses are relatively resistant to heat inactivation, steaming of shellfish does not entirely eliminate the risk for transmission.[123,124]

Recommendations for evaluation and control of nosocomial outbreaks[101] include identification and elimination of common sources and the use of hand washing and barrier methods to prevent secondary transmission. Exclusion of ill employees may be important in limiting the spread of nosocomial outbreaks.[125] Health departments have recommended that individuals with norovirus-associated illness not be involved in food handling or preparation until 2 to 3 days after gastrointestinal signs and symptoms have resolved. These methods have generally been found to be more effective in limiting the spread of outbreaks from unit to unit within an institution than in terminating an outbreak in an individual unit once it has begun.[126,127] The Viral Gastroenteritis Section of the CDC is available for advice regarding such outbreaks.

Although noroviruses were initially recognized primarily in association with outbreaks of acute gastroenteritis mostly involving adults, there has been an increasing recognition of the role of these viruses as causes of sporadic gastroenteritis in children in various parts of the world.[19,23,128–132] Toronto virus has been reported to be the second most common virus detected in the stools of young children with gastroenteritis.[133] The frequency of norovirus gastroenteritis has been estimated at between 10% and 100% of that of rotavirus in children, when direct comparisons have been made.[134–136] In one study, 49% of prospectively followed Finnish infants and children seroresponded to norovirus over a 2-year period.[137] Sapoviruses and noroviruses have also been detected in community-wide, daycare,[138] and nosocomial outbreaks of gastroenteritis in children.[139] After the introduction of the rotavirus vaccines, norovirus has become the leading cause of medically attended acute gastroenteritis in US children, with nearly 1 million health care visits annually.[140]

Norovirus outbreaks occur throughout the year but tend to peak during cold weather months in temperate climates.[141,142] Frequency, seasonality, and geographic location of outbreaks may vary substantially from year to year, and the factors related to this variation are unclear. The markedly increased number of outbreaks reported in a number of years since 1995 have been associated with the emergence of novel GII.4 strains,[18,142] including the emergence of a novel strain in 2012, GII.4 Sydney.[72–74] In 2014, novel GII.17 viruses emerged and spread worldwide.[75,76,78,79]

PATHOGENESIS

Although norovirus infection of nonhuman primates and gnotobiotic piglets has been accomplished,[18] convenient animal models for gastroenteritis induced by the Norwalk viruses are not available, and therefore information about the pathogenesis of this illness is based largely on studies of experimentally induced disease in healthy volunteers. Acute infection with Norwalk and Hawaii viruses results in a reversible histopathologic lesion in the jejunum,[12,143–145] with apparent sparing of the stomach[146] and rectum (Fig. 176.4). The villi are blunted, but the mucosa is otherwise intact. Round cells and polymorphonuclear leukocytic infiltration are seen in the lamina propria. With electron microscopy, the epithelial cells are similarly intact, microvilli are shortened, and widened intercellular spaces are noted. These histopathologic changes

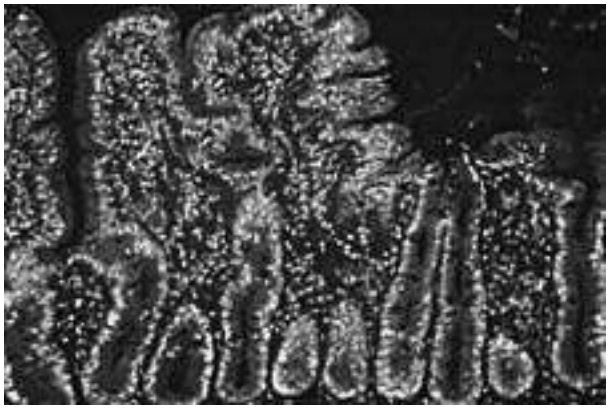

FIG. 176.4 Hawaii virus–induced disease. Light micrograph of a jejunal mucosal biopsy specimen from a volunteer with Hawaii virus–induced disease 48 hours after challenge. Blunted villi and inflammatory cell infiltrate in the lamina propria are present. (Hematoxylin-eosin stain, ×140.)

appear within 24 hours after virus challenge, are present at the height of illness, and persist for a variable period of time after the illness. The histopathologic changes have generally cleared within 2 weeks after the onset of illness, although some jejunal changes have been noted as late as 6 weeks after challenge. Histopathologic changes have been described in both clinical and subclinical cases of infection[144,145] and appear to be indistinguishable between Norwalk virus– and Hawaii virus–induced disease.

Diarrhea induced by the Norwalk virus is associated with a transient malabsorption of D-xylose and fat[147] and with decreased activity of brush-border enzymes, including alkaline phosphatase and trehalase.[143] Absorption and brush-border enzyme levels return to normal values within 2 weeks after challenge. During acute illness, a variable amount of intestinal fluid is produced, but infection with Norwalk and Hawaii agents has not been associated with detectable enterotoxin production. Adenylate cyclase levels in jejunal biopsy specimens appear to be normal during infection.[148] Thus, the precise mechanisms of virus-induced diarrhea, vomiting, or both remain unknown at the present. Calicivirus infections of animals have been associated with atrophy of the small intestinal mucosa, along with a mild inflammatory infiltrate in the lamina propria.[149,150]

The cellular receptor for infection with norovirus has not been characterized. In human challenge studies, susceptibility to infection with certain norovirus strains has been associated with the presence of H blood group carbohydrate antigens (HBGAs), which are also expressed on gastrointestinal epithelial cells.[151–156] These observations, along with in vitro studies demonstrating binding of noroviruses to these carbohydrate antigens within a pocket of the P2 domain of the viral capsid,[157] have suggested that these carbohydrates may represent or be related to cellular receptors for noroviruses. It is postulated that allelic variation in expression of HBGAs affects susceptibility to norovirus infection.[158] These findings may explain, at least in part, the observations of a poorly defined long-term resistance to norovirus infection seen in previous challenge studies, in which some individuals consistently remained well despite repeated challenge with virus.[159] Epidemiologic studies have supported the concept that expression of HBGAs increases susceptibility to norovirus infection, but they suggested that the effects may be strain specific.[106,160] Sapoviruses do not bind to HBGAs, and susceptibility to infection with sapoviruses does not appear related to HBGA phenotypes.[27]

IMMUNE RESPONSE

Infection with the Norwalk virus results in the induction of virus-specific serum immunoglobulin G (IgG), IgA, and IgM antibody,[161–164] even in the presence of previous exposure. IgA and IgM responses appear to be relatively short lived, whereas elevations in Norwalk-specific serum IgG persist for months.[163,164] In addition to recognizing the infecting strain of norovirus, serum antibody responses to infection may recognize other variants within the same genogroup, although generally to lower titer.[165] Such heterologous antibody responses are more common within a genogroup than between genogroups.[162,166] Serum IgM and IgA antibody may be more specific for the infecting strain of virus.[162,167] With use of baculovirus-expressed capsid proteins, it has been demonstrated that responses to viruses within genogroup GI may be more specific than responses to infection with viruses within genogroup GII.[168] Heterologous responses have also been seen in individuals infected with the sapoviruses.[91] These broad responses are in contrast to the extremely specific antibody response of animals hyperimmunized with capsid antigen[169] and may in part reflect the extensive prior exposure of most adults to related viruses. The significance of such heterologous responses with respect to protection against reinfection is not clear.

Mucosal immune responses have not been studied extensively, but jejunal IgA synthesis has been shown to be elevated in biopsy specimens obtained 2 weeks after challenge with the Norwalk agent,[170] and fecal IgA responses after Norwalk infection have also been reported.[171] Limited studies of cell-mediated immune responses in these infections have indicated that acute illness is associated with a transient lymphopenia that involves thymus-derived, bone marrow–derived, and null cell subpopulations.[172] Antigen-specific cellular responses to the capsid have been demonstrated in peripheral blood after experimental infection and are predominantly of the Th1 type.[173] Such responses are also cross-reactive within a genogroup.

Parameters defining protective immunity to noroviruses are poorly understood. After infection with Norwalk virus, most individuals manifest resistance to reinfection that persists for at least 4 to 6 months.[159,174] Multiple exposure appears to increase this resistance.[174] This short-term resistance does not appear to extend to other antigenically distinct viruses.[54] Infection-induced resistance eventually wanes, and after 2 to 3 years, such individuals are susceptible to reinfection with the same virus.[159] As noted earlier, the absence of secretion of HBGAs is associated with resistance to infection with the Norwalk virus, and these antigens may represent or be related to cellular receptors for noroviruses.[18] Norwalk virus infections can induce serum antibodies that block binding of virus to HBGAs, and this may serve as a surrogate for neutralizing activity.[175]

Studies of the role of serum antibody in mediating protection have yielded conflicting results. In most studies in adults, infection and illness induced by Norwalk-like agents occur in the presence of a wide range of preexisting serum antibody levels, which thus correlate poorly with protection.[162,174] However, after repeated experimental exposure of adults[174] and in epidemiologic studies of noroviruses and sapoviruses in children,[176–178] there has been a better correlation between the presence of serum antibody and protection from illness. Studies in children have shown that high serum levels of norovirus genotype–specific blocking antibodies correlate with protection against infection with genotype-identified noroviruses.[179] Antibody activity has been assessed with a surrogate neutralizing assay using histo–blood group antigens.[180] Protection may also be related to other host defense factors such as local mucosal antibody. However, direct measurements of intestinal antibody have failed to show a correlation with protection from Norwalk-induced illness,[181] and the presence of prechallenge Norwalk-specific fecal IgA was also not protective against challenge.[104] Studies of related animal caliciviruses viruses have also suggested a role for innate immunity in resistance to norovirus infection.[182] The role of T cells in immunity to noroviruses remains poorly understood. Prolonged norovirus-associated diarrhea in an immunosuppressed patient was reported to improve when T-cell counts increased.[183]

CLINICAL MANIFESTATIONS

Clinical characteristics of illness induced by the noroviruses appear to be similar in both naturally occurring and experimentally induced disease (Fig. 176.5), and there are no apparent differences in clinical findings between genogroups. Illness induced by sapovirus infections appears to be similar to that induced by noroviruses, although earlier reports suggested that illness may be milder than that seen with norovirus infection.[136] The onset of symptoms can be either gradual or abrupt, and most persons complain first of abdominal cramps or nausea. In general, both vomiting and diarrhea occur, although either can be present alone. Myalgias, malaise, and occasional headaches are also seen. Low-grade fever, with temperatures of 101°F to 102°F, occurs in about half of cases. Diarrheal stool is generally moderate in amounts, with four to eight nonbloody stools usually being produced over a period of 24 hours. Disease manifestations generally last 48 to 72 hours and usually remit without sequelae.

More severe disease has been reported in elderly patients, including deaths that have been observed in outbreaks in long-term care facilities.[84,184–187] Cases in such outbreaks have shed high titers of virus in stools for up to 3 weeks.[188] These outbreaks often involve person-to-person transmission, although foodborne spread may also occur. Prolonged symptomatic infection and virus shedding of noroviruses have been reported in pediatric oncology patients[189] and in transplant recipients, both hematopoietic stem cell and solid organ transplants.[6,190–193] Prolonged diarrhea for months may be present, along with associated malnutrition, dehydration, graft failure, and kidney injury Chronic norovirus infection for periods as long as 8 years has also been described in patients with enteropathy and common variable immunodeficiency.[194]

DIAGNOSIS

Clinical Diagnosis

A clinical diagnosis of norovirus-related illness can be suspected on the basis of epidemiologic information and on the absence of other documented pathogens. Outbreaks of illness have a high likelihood of

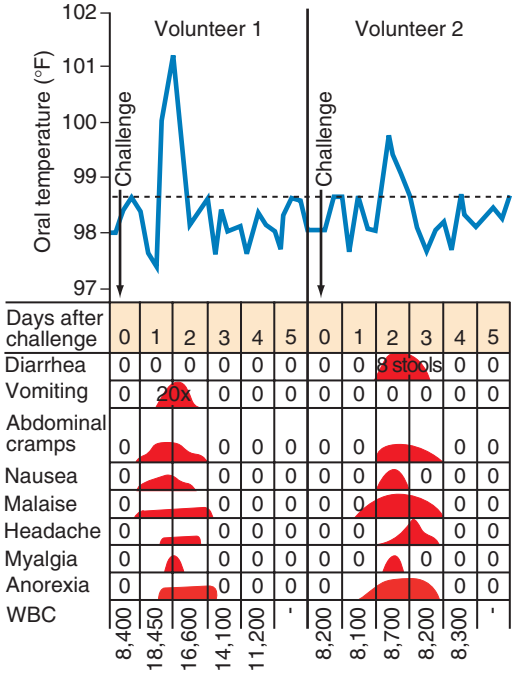

FIG. 176.5 Clinical response of two volunteers after the oral administration of the norwalk virus. The height of the shaded curve is proportional to the severity of the sign or symptom. *WBC,* White blood count. *(From Blacklow NR, Dolin R, Fedson DS, et al. Acute infectious non-bacterial gastroenteritis: etiology and pathogenesis.* Ann Intern Med. *1972;76:993–1008.)*

being caused by noroviruses if vomiting occurs in more than 50% of persons, if the incubation period is 24 to 48 hours, if illness lasts 12 to 60 hours, and if bacterial pathogens are not identified.[195] However, the signs and symptoms of illness are not sufficiently characteristic to enable a diagnosis in an individual case to be made on clinical grounds alone. Routine laboratory tests are also generally not helpful in making a specific diagnosis of norovirus infection. Peripheral white blood cell counts are normal or slightly elevated, with a relative polymorphonuclear leukocytosis and lymphopenia, but with otherwise unremarkable white cell morphology. The results of liver function tests, blood urea nitrogen and creatinine determinations, and urinalysis are generally within normal limits. The absence of fecal leukocytes, as determined by means of microscopic examination of stools stained with methylene blue,[196] is a useful tool with which to exclude infection with enteroinvasive pathogens such as *Shigella* spp.

Laboratory Diagnosis

Specific diagnosis requires laboratory confirmation. Because these agents currently cannot be cultivated in vitro in clinical diagnostic laboratories, a variety of methods have been developed to detect virus directly in stool samples.[197] Immune electron microscopy, in which immune sera are used to aggregate and highlight virions in stool suspensions (see Fig. 176.2), was the method used originally to identify these viruses[15,198] but is no longer commonly used.

The diagnostic tests of choice are highly sensitive and specific reverse-transcriptase polymerase chain reaction (RT-PCR) techniques that have been developed for detection of noroviruses and sapoviruses and are now widely available.[199–204] The success of this strategy depends on the ability to remove inhibitors of reverse transcription from the samples and on the choice of primers in relatively conserved regions of the genome, to widen the scope of viruses detected.[205,206] Conversely, carefully selected primers within relatively more divergent regions can be used to genotypically differentiate virus strains.[207] RT-PCR may be particularly useful for detecting contamination of food and environmental samples.[98,208–212]

Enzyme immunoassays (EIAs) to detect norovirus antigens in stool are commercially available, but are generally of lower sensitivity and specificity than PCR assays.[26,213–215] EIAs may be useful to assess specimens from outbreaks when multiple samples are available.

Techniques have also been adapted for the detection of antibody to noroviruses and may be useful for serologic diagnosis of infection, particularly when appropriate stool samples are not available. Serum antibody titer rises can be detected within 10 to 14 days after the onset of illness.[216–218]

THERAPY

Specific antiviral therapy is not available, and therefore treatment consists entirely of supportive measures. Oral fluid replacement with isotonic liquids is generally adequate to replace fluid losses. Rarely, parenteral intravenous therapy may be required if severe vomiting and diarrhea develop. Symptomatic treatment of headache, myalgias, and nausea with analgesics and antiemetics may provide relief. In one study, the administration of bismuth subsalicylate reduced gastrointestinal symptoms in Norwalk virus–induced disease in volunteers, but had no effect on the number or character of stools or on virus shedding.[219] Although antiperistaltic agents are frequently prescribed to control diarrhea, their effect on the disease course and on excretion of virus has not been rigorously evaluated.

The recent success in cultivation of human norovirus in vitro should facilitate the search for effective antivirals.[31] Previously, the cultivation of a murine norovirus in tissue culture had identified antiviral activities of nucleoside analogues.[220] Recently, 2′-C-methylcytidine has been reported to reduce shedding of murine norovirus in a mouse model and in vitro.[221–225] Rupintrivir, an enterovirus protease inhibitor, has been reported to have anti–human norovirus activity in vitro.[226] A small clinical study has suggested that nitazoxanide may result in modestly reduced symptoms in norovirus-associated illness.[227]

For norovirus infections in immunosuppressed patients, the most important therapeutic measure is reduction of immunosuppression when possible.[193] Therapies with nitazoxanide, ribavirin, or pooled immunoglobulins have been attempted, with uncertain results.[193,228]

PREVENTION

The primary control measures for norovirus outbreaks are prevention of contamination of water and food supplies by means of proper hygiene procedures, including restriction of the activity of symptomatic food handlers. Many health departments recommend that individuals with norovirus illness refrain from being involved in food handling or preparation until 2 to 3 days after illness has resolved.[229] Vigorous hand washing should be used during and after illness to reduce spread. The use of soap and water appears to be more effective than alcohol-based hand sanitizers.[229] Potential sources of contamination of fomites should be treated with appropriate cleaning solutions. Because of the resistance of noroviruses to freezing, heating, and standard cleaning solutions, decontamination procedures should include EPA-recommended disinfectants, such as chlorine bleach with a concentration of 1000 to 5000 ppm (1:50 to 1:10 dilution of household bleach).[229]

Vaccines

The availability of an effective vaccine against norovirus-associated illness would have significant economic impact. In the United States, it has been estimated that it could prevent more than 2 million episodes of gastroenteritis annually, and reduce health expenditures by $2.1 billion per year.[230] However, such vaccines face multiple challenges.[230–232] As described earlier, infection may not induce long-term protective immunity, although short-term protection has been demonstrated. The likely presence of multiple antigenic types is also a challenge. However, progress toward a candidate norovirus vaccine has been made using VLPs.[233,234] Norwalk VLPs have been shown to be immunogenic when administered orally to human volunteers, inducing serum IgG, mucosal IgA, and cellular responses that resemble those seen after infection, although of substantially lower magnitude.[235,236] Intranasally administered norovirus VLPs, administered in two doses 3 weeks apart, provided protection of 47% against gastroenteritis induced by challenge with a Norwalk virus.[237] Somewhat less protection (26%) was provided against infection with norovirus. Additional studies of intramuscularly administered VLPs have been carried out, which showed that such

administration was also well tolerated and immunogenic.[238] A vaccine regimen of two intramuscular injections of VLPs followed by an oral challenge of norovirus did not meet the predefined end point of reduction of cases of illness or infection, but it did result in reduced severity of illness.[239] A large-scale field trial of this VLP vaccine, sponsored by Takeda vaccines, has been undertaken. Additional candidate vaccines based on adenovirus serotype 5 vectors are also under development. In addition, Norwalk virus has proved to be an excellent model to evaluate expression of vaccine antigens in plants as a method for oral immunization.[240]

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The complete reference list is available online at Expert Consult.

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177 Astroviruses and Picobirnaviruses

Raphael Dolin and John J. Treanor

SHORT VIEW SUMMARY

Definition
- Astroviruses cause acute gastroenteritis. Picobirnaviruses have been detected in stools but have not been established as causes of illness in humans.

Epidemiology
- Astroviruses are worldwide in distribution; they cause disease most frequently in young children, but can also cause gastroenteritis in adults and in immunosuppressed patients. Transmission is likely by the fecal-oral route.

Microbiology
- Astroviruses are single-stranded RNA viruses composed of multiple genogroups and genotypes, with a characteristic star-shaped morphologic appearance on electron micrographs. Picobirnaviruses are double-stranded RNA viruses with a bisegmented genome.

Clinical Manifestations
- Illness induced by astroviruses consists of diarrhea, headache, malaise, nausea, low-grade fever, and, less commonly, vomiting. Illness may be somewhat less severe than that seen with noroviruses and rotaviruses.

Diagnosis
- Astroviruses in stools can be detected with electron microscopy, enzyme immunoassay, and reverse-transcriptase polymerase chain reaction assay, which is the most sensitive technique.

Therapy
- Treatment is supportive. Intravenous immune globulin has been used in immunosuppressed patients, but its efficacy is not established.

Prevention
- Although virus-like particles from astroviruses have been produced, vaccines have not been developed.

Viral gastroenteritis is caused largely by noroviruses and sapoviruses (see Chapter 176) and by rotaviruses (see Chapter 150). Astroviruses are also established as important causes of gastroenteritis, albeit substantially less frequently than the aforementioned viruses. Picobirnaviruses are more recently described viruses that have a possible, although not yet established, role as agents of gastroenteritis.

ASTROVIRUSES

Virology

Classification

Astroviruses are members of the Astroviridae family, which consists of two genera: *Mamastrovirus*, which infects mammals and includes the human astroviruses, and *Avastrovirus*, which includes avian viruses. Astroviruses were first detected by their characteristic starlike appearance on electron micrographs (Fig. 177.1) and have subsequently undergone extensive molecular virologic characterization.[1–3] The ability to grow astroviruses in cell culture, including primary HEK cells and a variety of monkey cell lines, has facilitated their study.[4–6]

Mamastroviruses are subdivided into two genogroups (GI and GII), which have at least 19 genotypes. The first clade of these includes eight human astroviruses (HAstV1 to HAstV8) that correspond to the eight serotypes first noted in human astrovirus infections.[1–3,7–11] Genotypes in two additional clades have been subsequently identified: HAstV-MLB and HAstV-VA/HMO.[3,12] HAstV serotypes can be distinguished with immunofluorescence or plaque neutralization techniques,[13,14] whereas considerable cross-reactivity exists with enzyme immunoassays (EIAs).[4]

Structure

Astroviruses are positive-stranded RNA viruses that are found in a wide variety of animal species, in addition to humans. The virions are nonenveloped, display icosahedral symmetry, and are about 28 to 30 nm in diameter. Under the electron microscope, the particles in stool samples have a characteristic morphologic appearance that consists of round smooth edges with multiple triangular electron-lucent areas and an electron-dense center that results in the appearance of a five- or six-pointed star from which the virus derives its name (see Fig. 177.1).[1–3,12] Analysis of virus grown in cell culture has shown the virus particles to exhibit a layer of 41-nm spikelike projections on the surface.[15] The human astroviruses have a density of 1.35 to 1.37 g/mL in cesium chloride and contain a positive-sense, single-stranded 35S RNA genome with a 3′ polyadenylated tail.

Genomic Organization

The genomic organization includes three open reading frames (ORFs) (Fig. 177.2). ORF1a encodes the protease region, ORF1b encodes the RNA-dependent RNA polymerase, and ORF2 encodes a polyprotein that is cleaved into the core capsid and virion spike proteins. A possible additional ORF has been detected (ORF-X) whose function is uncertain.[1–3,12,16–19] From the perspective of genetic sequences, ORF1b is the least divergent, and ORF2 is the most divergent.[20] The capsid is translated from a subgenomic polyadenylated RNA in infected cells.[3,21,22] The number of viral structural proteins appears to vary with the serotype (see later in this section), with one to three reported for the human viruses,[23,24] possibly reflecting differences in the processing of the capsid precursor.[25]

Epidemiology

Astrovirus infection is widely geographically[26] distributed, and HAstV[1–3,12,15–18] serotypes or genotypes have been detected throughout the world.[8–11] HAstV-1 appears to be the most common, based on both serotypic[27–29] and genotypic surveys.[11,30–33]

Astroviruses are a recognized cause of gastroenteritis worldwide, most commonly in children, usually younger than 2 years. They have been estimated to cause 2% to 9% of cases of nonbacterial gastroenteritis.[2,3,34] Astroviruses have been detected in the stools of children with diarrhea brought to medical attention in a variety of settings.[35] Rates of 2% to 16% in children with diarrhea have been found in hospital-based studies and 5% to 17% in community-based studies.[36–38] Outbreaks have been described in schools, daycare settings, and pediatric wards.[39–42] Gastroenteritis associated with astroviruses has also been reported in adults in a variety of settings.[2,43–46] These include outbreaks in healthy adults[44,45]; elderly populations[46]; immunosuppressed patients

with diarrhea, including human immunodeficiency virus (HIV)–infected patients[47]; patients who have undergone hematopoietic cell transplantation[48]; and children with primary immunodeficiencies[49] or hematologic malignancies.[50] Astrovirus infections in immunosuppressed patients were associated with prolonged shedding of virus and persistent diarrhea, but often other pathogens were also present in stool specimens. The severity of illness in HIV-infected children with astroviruses, however, was no greater than that seen in healthy children.[51,52] Astroviruses, which are phylogenetically divergent from HAstV-1 through HAstV-8 and are found in mink and cattle, have been associated with encephalitis in immunosuppressed children.[53,54]

A winter predilection has been noted in temperate climates in some studies,[24,55] whereas infection occurs throughout the year in tropical climates,[56] similar to the pattern reported for rotaviruses. Transmission is presumably by the fecal-oral route, and astrovirus infection has been induced by oral administration of stool filtrates to normal volunteers.[23] HAstV can be shed in high amounts in the stools of infected subjects, with up to 10^{23} genome copies/g, contributing to the spread of infection.[57]

Contamination of foods and water supplies appears to be important in astrovirus-associated outbreaks.[2]

Seroprevalence studies in worldwide sites, including in the United States, indicate that HAstV infections occur early in life, such that by age 5 more than 90% of children have antibody to at least one HAstV serotype.[12,27] Seroprevalence in healthy adults appears to be somewhat lower.[12]

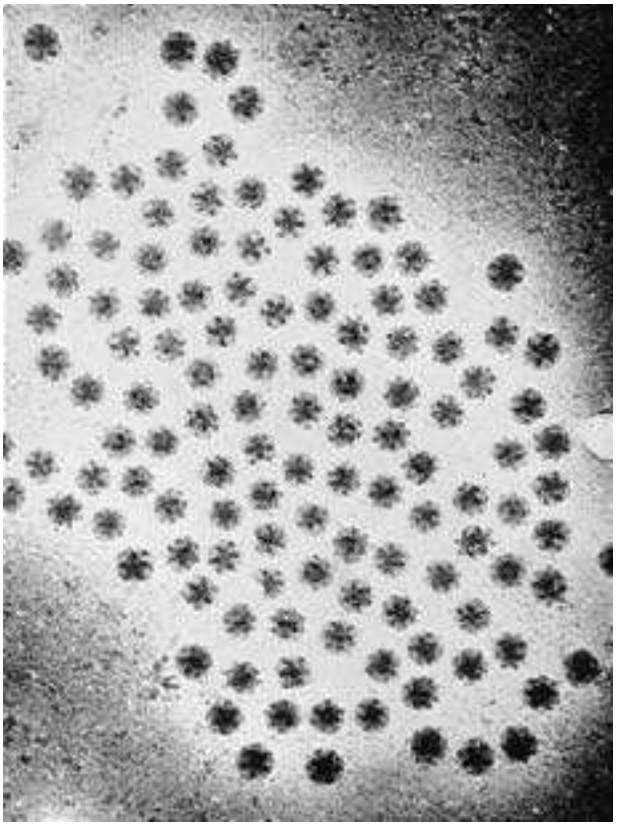

FIG. 177.1 Astrovirus in the intestinal contents of gnotobiotic lambs. Particles are 30 nm in diameter. *(From Snodgrass DR, Gray W. Detection and transmission of 30 nm virus particles [astroviruses] in the faeces of lambs with diarrhoea.* Arch Virol. *1977;55:287–291.)*

Pathogenesis

The pathogenesis of astrovirus-induced illness is not well understood, and studies have been limited by the lack of a convenient animal model for HAstV infection. Astrovirus infections in animals have been associated with small intestinal villus shortening and with mild inflammatory infiltrates in the lamina propria.[58,59] Astrovirus infection may result in decreased intestinal disaccharidase activity and subsequent osmotic diarrhea,[60] similar to the mechanism postulated for rotavirus. As noted previously, stool filtrates that contain astroviruses can infect volunteers after oral administration, but in comparison to noroviruses, astroviruses containing filtrates appear to induce illness less frequently when administered to healthy adults.[23,61]

Clinical Manifestations

Illness attributed to astroviruses consists primarily of diarrhea, headache, malaise, abdominal pain, and nausea; vomiting appears to be less common.[2,3] In general, the symptoms are similar to those seen in norovirus and rotavirus infection in children but are milder, and children with astrovirus gastroenteritis are less likely to become dehydrated.[2,43,62] Low-grade fever is frequently present. The incubation period of illness has been estimated to be 3 to 4 days, and in the absence of coexisting pathogens, disease manifestations usually last 5 days or less, with occasionally longer duration. The duration of virus shedding as assessed with EIA is generally short (mean of 1.5 days; range, 1–9 days).[63] Detection of shedding with polymerase chain reaction (PCR) is longer (mean of 4 days), with a prolonged range reported in one outbreak (1–35 days)[63]

Astrovirus infection may also occur in immunocompromised patients, in whom it can be more severe and prolonged, including multiorgan dissemination in severely immunocompromised children.[2,12,64,65]

Immune Responses

Immune responses to astroviruses and their role in protection from infection and illness are not well understood. Epidemiologic observations suggest that infection results in at least short-term protection against reinfection with the same serotype.[66]

In volunteer experiments, the ability to infect participants with astrovirus and the severity of subsequent illness were inversely correlated with the presence and magnitude of serum anti-HAstV.[23,61] Mucosal immunoglobulin A (IgA) antibodies are also induced by HAstV infection, but their role in protection is unclear.[67] T-cell responses to inactivated HAstV have been demonstrated in mucosal biopsy specimens from healthy adults.

Diagnosis

In contrast to caliciviruses, astroviruses are often shed in large amounts in stool and can be readily detected with electron microscopy even without immune aggregation. Detection of astroviruses has been carried out with immune electron microscopy (IEM) or with immunofluorescence, and an EIA technique[68] that detects the astrovirus group antigen has been used widely in epidemiologic studies. EIA is of comparable sensitivity (91%) and specificity (98%) to IEM.[2,3] Reverse-transcriptase polymerase chain reaction (RT-PCR) techniques have been developed and are the most widely used techniques to detect astroviruses, and they are significantly more sensitive than EIA or IEM.[2,63,69,70]

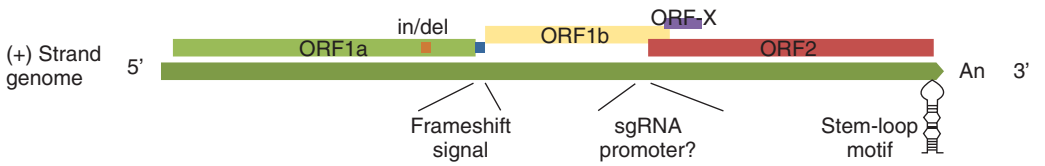

FIG. 177.2 Genome organization of human astrovirus. The genomic RNA contains three open reading frames *(ORF1a, ORF1b, ORF2),* and possibly a fourth *(ORF-X).* See "Genomic Organization" in text. *(Modified from Mendez E, Arias CF. Astroviruses. In:* Fields Virology. *6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013:611.)*

Therapy

Illness associated with astroviruses is generally self-limited, and treatment, if required at all, is supportive and directed at maintaining hydration and electrolyte balance. Intravenous immune globulin has been used in immunosuppressed patients with astrovirus gastroenteritis, but its efficacy, if any, is not established by clinical trials.[71] Antivirals against astroviruses are not available, but in vitro activity of flavonoids has been reported.[72]

Prevention

The major approaches to prevention of infection with HAstVs are avoidance of contamination of water and food supplies. Measures to avoid person-to-person transmission from active cases also appear prudent. Virus-like particles of astroviruses have been generated,[73] raising the possibility of vaccine approaches at some point in the future.

PICOBIRNAVIRUSES

Picobirnavirus is the only genus within the virus family Picobirnaviridae.[74,75] Picobirnaviruses are small, nonenveloped icosahedral viruses with a segmented double-stranded RNA genome consisting of two segments. The genomes have been characterized into two types or profiles[76,77]—a large profile (2.3–2.6 kbp for the large segment) and a small profile (1.75–1.55 kbp)—which are now being used for molecular epidemiologic studies.[78] Their exact taxonomic position is unclear, and they derive their name from the observation that they (usually) have a *bi*segmented double-stranded RNA genome, similar to the *Bi*rnaviridae, but are smaller *(pico),* with a virion size of 30 to 40 nm.[79] They were first observed in the stools of humans[80] in the course of studies using polyacrylamide gel electrophoresis to detect rotavirus. Picobirnavirus genomes have been found in the stools of a variety of animals[75] and in children and adults with diarrhea, including HIV-infected adults,[47,78,81–84] and in patients with hematopoietic stem cell transplants.[85] Two genogroups and a potential third one have been identified among human viruses.[86] However, the role of these viruses as causative agents of gastroenteritis remains unclear because the prevalence of picobirnaviruses in stools of individuals with and without diarrhea is similar.[87]

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The complete reference list is available online at Expert Consult.

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178 Hepatitis E Virus

Hirsh D. Trivedi and Sanjiv Chopra

SHORT VIEW SUMMARY

Definition
- Hepatitis E virus (HEV) is an RNA virus which is a member of the Hepeviridae family, genus *Hepevirus,* composed of five genotypes, of which four are associated with human infection.

Epidemiology
- HEV was first recognized in the 1980s and has emerged as the leading cause of acute viral hepatitis worldwide. It can occur as outbreaks or in sporadic forms.
- Initially, HEV was thought to be present only in underdeveloped nations, but it has been noted in developed countries, where it is likely underdiagnosed.
- One-third of the world's population has positive seroprevalence for IgG antibodies against HEV, suggesting prior exposure.
- The most common forms of transmission are through fecal-oral contamination of drinking water supplies (genotypes 1 and 2) and through zoonoses (genotypes 3 and 4). (See Table 178.1.)
- Acute HEV infection is most often self-limiting. However, in women in the third trimester of pregnancy, it has a striking mortality of 20% to 25% (genotype 1). It can also be seen in immunocompromised patients.
- Chronic HEV has been described in organ-transplant recipients (genotype 2).

Microbiology
- HEV is a nonenveloped, icosahedral virus that measures 32 nm in diameter.
- It has a positive-sense, single-stranded RNA genome with three open reading frames.
- There are four genotypes that cause infection in humans. (See Table 178.1.)

Diagnosis
- Clinical characteristics are similar to those of other forms of acute viral hepatitis.
- Diagnosis with immunoglobulin M (IgM) antibodies to HEV is used in endemic areas.
- Reverse-transcriptase polymerase chain reaction (RT-PCR) is required for diagnosis in nonendemic areas. It is more sensitive and specific than serologic diagnosis.

Therapy
- Supportive care is the mainstay of therapy because most cases are self-limiting.
- Ribavirin treatment and interferon treatment have been used in severe acute cases and in immunosuppressed patients with encouraging results.

Prevention
- Maintaining clean water supplies is the cornerstone of prevention of HEV.
- A vaccine that is safe and effective has been approved in China but not yet in other countries.

Hepatitis E virus (HEV) is a ribonucleic acid (RNA) virus that causes a viral hepatitis that is indistinguishable from other forms of viral hepatitis. The World Health Organization (WHO) estimates a global incidence of 20 million cases of HEV infection annually, accounting for 3.3% of mortality from viral hepatitis.[1] HEV infection most often results in an acute, self-limited disease that does not necessitate hospitalization. Rarely, it can be a fatal cause of viral hepatitis. Certain individuals, such as those who are pregnant, are immunocompromised, or have coinfections, are at higher risk of significant hepatitis and death. The virus was first recognized in 1980 after several outbreaks of infection.[2–5] It has since emerged as an important and often missed cause of viral hepatitis. Initially thought to be limited only to underserved, underdeveloped nations, HEV has been increasingly documented in developed countries and is now the most common cause of acute viral hepatitis worldwide.

A BRIEF HISTORY

The disease was first recognized in the 1980s when sera collected during a large epidemic of viral hepatitis in New Delhi, India in 1955[6] was found to lack the typical serologic markers of hepatitis A or B virus.[5,7] This virus was therefore referred to as enterically transmitted, water-borne hepatitis non-A, non-B type virus.[5] Similar observations in serologic studies were made after an epidemic of viral hepatitis in 1978, in Kashmir, India.[5,8] During the Soviet occupation of Afghanistan in the 1980s, there was another unexplained outbreak of viral hepatitis at a military camp.[2] A member of the research team ingested a pool of fecal extract from the soldiers and became ill. The new virus was detected in the researcher's stool with electron microscopy. Electron microscopic diagnosis was also used in other epidemics to identify the virus (Fig. 178.1).[9] In the 18th and 19th centuries, several hepatitis outbreaks around the world had features that were similar to those of the HEV epidemics.[10] Endemic HEV infection was subsequently discovered in many underdeveloped countries, where it was thought to be more prevalent. More recently, HEV infection has been observed even in developed nations.

GENETICS

HEV is a small, icosahedral, nonenveloped single-stranded RNA virus with a positive-sense genome.[1] It measures 27 to 34 nm in diameter and is a member of the Hepeviridae family and *Hepevirus* genus. Large open reading frames (ORFs) of the RNA virus have been described; the largest one consists of 1693 codons.[11]

There are four genotypes of the virus that can infect humans (Table 178.1).[1] Genotypes 1 and 2 cause acute disease and are found only in humans, whereas genotypes 3 and 4 are found in animals (including pigs, deer wild boars, and others). Genotypes 3 and 4 can occasionally infect humans but do not cause acute disease.

NATURAL HISTORY OF HEPATITIS E

Burden of Disease

Hepatitis E is more common than previously realized. An estimated 20 million HEV infections annually occur worldwide.[1,12] About 3.3 million of these cases are symptomatic HEV infection.[1] WHO estimated

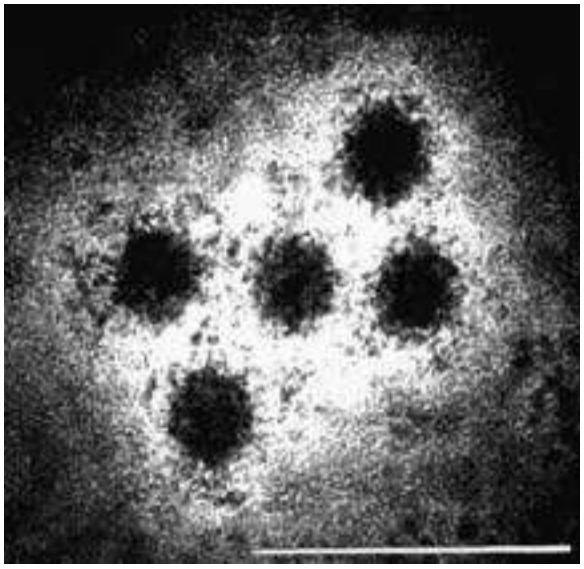

FIG. 178.1 Electron microscopy of antibody-coated hepatitis E virus particles in feces. *(Modified from Ticehurst J, Popkin TJ, Bryan JP, et al. Association of hepatitis E virus with an outbreak of hepatitis in Pakistan: serologic responses and pattern of virus excretion.* J Med Virol. *1992;36:84–92.)*

44,000 deaths due to HEV infection in 2015, accounting for 3.3% of the mortality cases of viral hepatitis.[1] Large outbreaks and sporadic cases occur frequently in endemic areas compared with nonendemic countries. Case-fatality rates in endemic areas range from 0.5% to 4% in hospital-based data and 0.07% to 0.6% in population surveys during outbreaks.[3,7]

Seroprevalence is characterized as positivity for immunoglobulin G (IgG) antibody against HEV without active infection. Data on the seroprevalence of HEV infection suggest a lifetime exposure risk in one-third of the world's population.[12] The prevalence of HEV antibody is 10% to 70% in developing countries, compared with 1% to 21% in developed countries.[13–15]

Geographic Distribution

HEV infection is a global health concern (Fig. 178.2). It is the most common cause of acute viral hepatitis worldwide. HEV was originally thought to occur only in underdeveloped nations with frequent water contamination but is now an emerging infectious disease in developed countries with safe drinking water supplies.[16] The highest prevalence of HEV infection is in East and South Asia.[1] It is the most common cause of acute hepatitis in highly endemic areas such as India, Egypt, and parts of China.[17] Sporadic cases of infection have been reported in South Africa,[18] Tunisia, Chad, and Morocco.[19] An epidemic of HEV has been noted in Mexico.[20] Cuba has been reported to have a high prevalence of clinically apparent HEV infection,[21,22] whereas only isolated cases of infection have been observed in South America.[23–25] Areas that

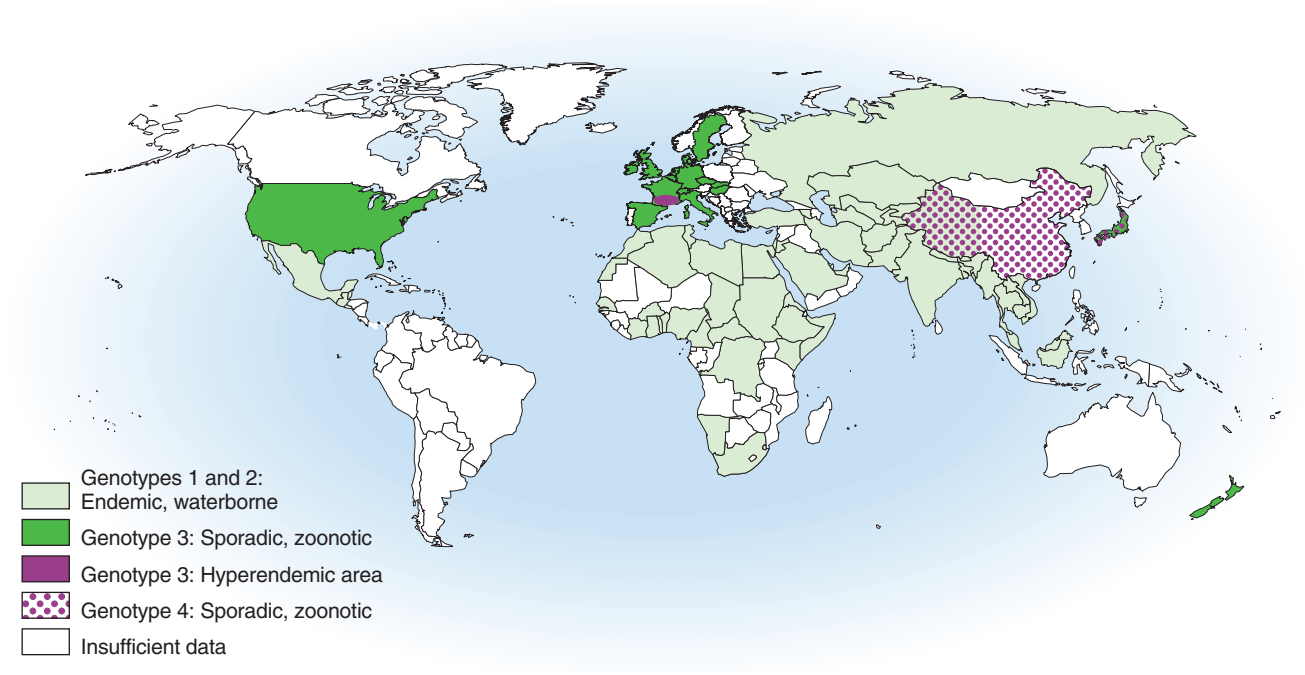

FIG. 178.2 Geographic distribution of clinical cases of hepatitis E virus infection. *(Modified from Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E.* Lancet. *2012;379:2477–2488.)*

TABLE 178.1 Genotype of Hepatitis E Virus

	GENOTYPE 1	GENOTYPE 2	GENOTYPE 3	GENOTYPE 4
Presentation	Acute	Acute	Chronic	Chronic
Source	Humans	Humans	Animals	Animals
Transmission	Contaminated water	Contaminated water	Undercooked meat	Undercooked meat
Geographic Distribution	Asia, India, North Africa	Asia, India, North and West Africa, Mexico	Western countries, Asia, North America	Asia, Europe

are commonly affected include hospitals,[26] war zones, and camps for refugees or displaced populations, where the water supply is often contaminated.[1] HEV has led to major epidemics in refugee settings such as Darfur, Sudan.[27,28] Developed countries do not experience such outbreaks but can encounter sporadic cases of HEV infection. The true incidence and prevalence of HEV infection in developed countries has not yet been systematically investigated.

Recent outbreaks of HEV infection have been reported. An outbreak in Am Timan, Chad involved 693 cases of HEV infection and led to 11 deaths from acute jaundice syndrome. In April 2017, WHO was informed of another outbreak of HEV infection in the Diffa region of Niger. Of the 282 cases reported, 27 resulted in death. All cases resulting in death, except 1, occurred in pregnant women.

Transmission

Fecal-oral spread is the most common route of transmission for HEV infection (Fig. 178.3). Infected individuals excrete the virus in their stool. Fecal contamination of drinking water supplies leads to widespread outbreaks of HEV in underdeveloped countries. The infected person excretes the virus in the stool starting a few days before to 3 to 4 weeks after the development of clinical symptoms. In developed nations, however, fecal contamination of food products and undercooked meats is more common, leading to sporadic cases of HEV infection.

Transmission through other routes does occur but is far less common.[1] These include vertical transmission from mother to child and transfusion of infected blood products. In addition, the ingestion of uncooked or raw shellfish can also transmit infection. Individuals with immunosuppression or chronic liver disease are susceptible to infection from the consumption of these foods.

CLINICAL PRESENTATION

Acute Hepatitis E

The more common form of HEV infection is acute viral hepatitis. It is usually caused by genotypes 1 and 2. Acute HEV clinically resembles other forms of viral hepatitis infection. Most cases of HEV infection are self-limiting. The average incubation period after exposure to HEV is 15 to 70 days. Early reports suggested an incubation period of 28 to 40 days between exposure and clinical onset of disease (Fig. 178.4).[29] Symptomatic infection within endemic areas is most common in young adults between 15 and 40 years of age; symptoms in children are less prevalent, often leading to the lack of diagnosis.

The clinical presentation of HEV infection is similar to that of other forms of acute viral hepatitis and often indistinguishable. HEV and hepatitis A virus share similar characteristics, making them difficult to differentiate (Table 178.2). The clinical presentation usually starts with a prodromal phase. The clinical symptoms include, but are not limited to, fever, anorexia, nausea, vomiting, abdominal pain, skin rash, and arthralgia. The prodromal phase usually lasts from 1 to 7 days and is followed by pruritus and jaundice, lasting up to a few weeks. Dark urine and pale-colored stools often accompany jaundice. Organomegaly,

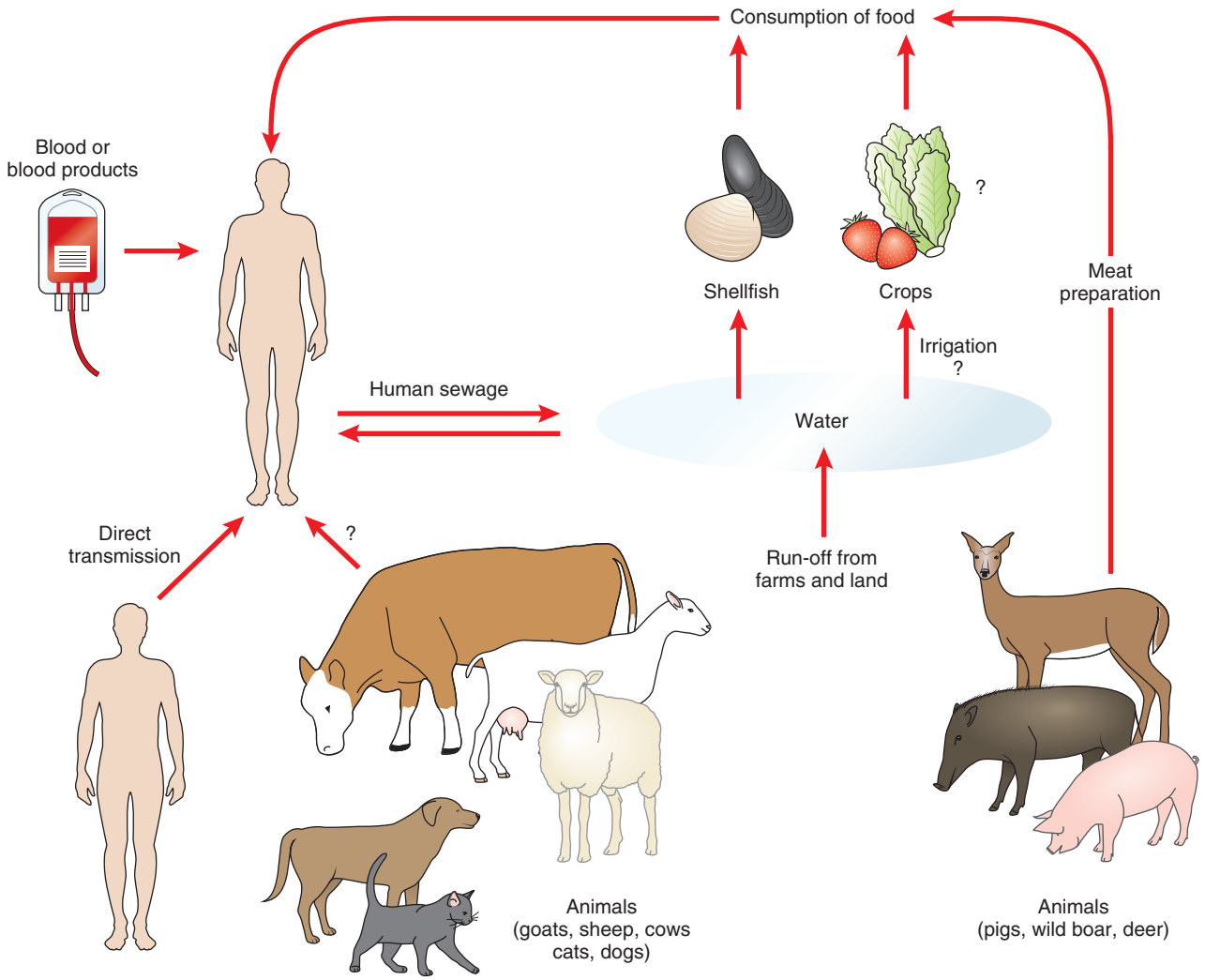

FIG. 178.3 Sources and routes of transmission of hepatitis E virus. *(Modified from Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E.* Lancet. *2012;379:2477–2488.)*

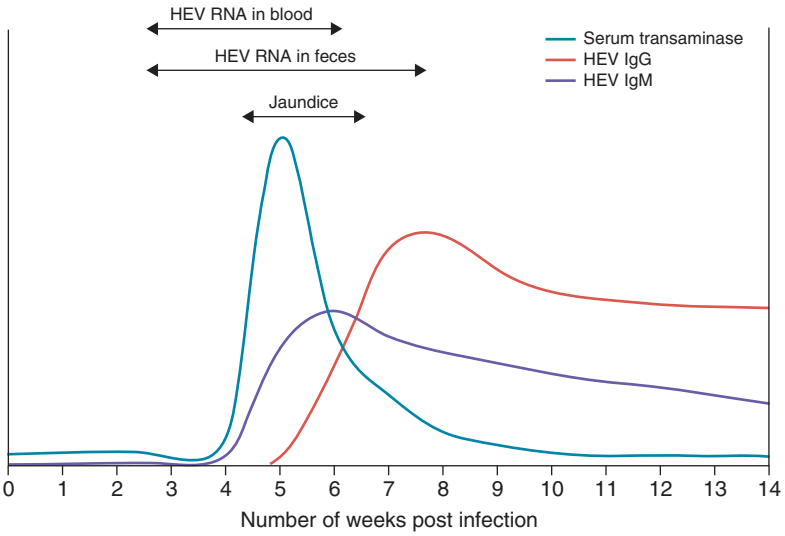

FIG. 178.4 Time course of hepatitis E virus infection including viral detection and shedding, clinical manifestations, and serologic response. HEV, Hepatitis E virus; IgG, immunoglobulin G; IgM, immunoglobulin M. *(Modified from Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries.* Lancet Infect Dis. *2008;8:698–709.)*

TABLE 178.2 Comparison Between Hepatitis A Virus and Hepatitis E Virus

CHARACTERISTICS	HEPATITIS A	HEPATITIS E
Genus and family	*Hepatovirus,* Picornaviridae	*Hepevirus,* Hepeviridae
Common mode of transmission	Fecal-oral route	Fecal-oral route
Incidence	1.4 million cases per year	20 million cases per year
Mortality rate	0.2%	1.0%
Clinical presentation	Self-limiting Fulminant hepatitis rare Cholestatic injury in <5%	Self-limiting Fulminant hepatitis is 20%–25% in third trimester of pregnancy Cholestatic liver injury in 60%
Geographic distribution	Developed and underdeveloped nations	Developed and underdeveloped nations
Diagnosis	HAV-IgM RT-PCR	Exclude HAV Endemic: HEV-IgM Nonendemic: RT-PCR
Treatment	Supportive care	Supportive care RBV or IFN in selected cases
Vaccination	Available	Available only in China

HAV, Hepatitis A virus; *HEV,* hepatitis E virus; *IFN,* interferon; *IgM,* immunoglobulin M; *RBV,* ribavirin; *RT-PCR,* reverse-transcriptase polymerase chain reaction.

abdominal tenderness, jaundice, and skin excoriations from itching can be found at clinical examination.

Acute hepatitis E can rarely result in severe, fulminant hepatitis and death. The results of laboratory and investigative tests are often abnormal and can help aid in the diagnosis. The abnormalities include elevations in alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), and direct bilirubin from a conjugated hyperbilirubinemia. In experimental studies, liver enzyme elevations peaked at 42 to 46 days after ingestion of the virus.[9] In animal studies of macaques administered intravenous challenge with a high dose inoculum of HEV, viremia was detected as early as 9 days after exposure, and disease became apparent as early as 2 weeks after exposure.[30–32] The serum ALT elevation may precede symptoms but does not correlate with severity of disease. Mild leukopenia and relative lymphocytosis can also be seen. An abdominal ultrasound examination is important in order to rule out biliary obstruction. Ultrasound findings in HEV infection can include an enlarged liver with increased echogenicity, enlarged spleen, edematous gallbladder wall, and prominence of portal venules. Most often, resolution of HEV infection occurs with normalization of serum ALT and bilirubin levels within 6 weeks. Histologic resolution lags behind and may take up to 6 months.

Chronic Hepatitis E

Hepatitis E was initially thought to cause only acute infection. However, chronic hepatitis E has been identified in areas with low endemicity and with genotype 3 and genotype 4 infection.[7] Hepatitis E viremia and fecal excretion can last for several months to years in patients who are immunosuppressed, such as organ-transplant recipients,[33–36] those receiving chemotherapy,[37–39] and those with human immunodeficiency virus (HIV) coinfection.[40–42] Individuals with chronic HEV infection can have a variable clinical presentation ranging from unremarkable symptoms to signs of liver disease with elevated serum laboratory parameters and progressive inflammation that can culminate in fibrosis and cirrhosis.[35] However, it is unclear how often this occurs.

Extrahepatic Manifestations

Extrahepatic complications of HEV infection are seemingly rare, but do occur. Neurologic manifestations have particularly been observed in several case reports and case series. Genotypes 1 and 3 of HEV infection have been implicated in causing neurologic consequences in case studies. Several cases were identified in India and in parts of Europe, such as France.[43] The observed neurologic manifestations include, but are not limited to, acute meningoencephalitis,[44] acute transverse myelitis,[45] brachial plexus neuritis,[46] Bell palsy,[47] and Guillain-Barré syndrome.[48,49] A case series of genotype 3 infections also included neurologic sequela with ataxia, proximal myopathy, bilateral brachial neuritis, encephalitis, Guillain-Barré syndrome, and inflammatory polyradiculopathy.[50] Some patients had detectable HEV RNA in the cerebrospinal fluid (CSF).[50,51] In general, the patients who cleared their HEV infection had resolution of their neurologic complaints.[50]

Other extrahepatic systemic involvement has been noted with HEV infection. Hematologic manifestations including severe thrombocytopenia[52] and hemolytic anemia in patients with glucose phosphate dehydrogenase deficiency have been reported during acute cases of infection.[53] Acute pancreatitis is rare but has also been found in acute HEV infection.[54] Chronic HEV genotype 3 infections have been associated with renal manifestations such as membranous glomerulonephritis and

TABLE 178.3 Extrahepatic Manifestations of Hepatitis E Virus

SYSTEM OR TYPE	MANIFESTATION
Central nervous system and peripheral nervous system	Meningoencephalitis Ataxia Transverse myelitis Pseudotumor cerebri Pyramidal syndrome Bell palsy Oculomotor nerve palsy Guillain-Barré syndrome Neuralgic amyotrophy
Gastroenterologic	Acute pancreatitis
Hematologic	Anemia (hemolytic, aplastic) G6PD deficiency Thrombocytopenia Pure red cell aplasia Hemophagocytic syndrome Monoclonal gammopathy of undetermined significance
Immune-mediated	Autoimmune disease Thyroiditis Myocarditis Henoch-Schönlein purpura Myasthenia gravis
Renal	IgA nephropathy Membranous glomerulonephritis Membranoproliferative glomerulonephritis Nephroangiosclerosis

G6PD, Glucose-6-phosphate dehydrogenase; *IgA,* immunoglobulin A.

membranoproliferative glomerulonephritis.[55] Table 178.3 lists the full spectrum of possible extrahepatic manifestations of HEV infection, with the majority being noted in isolated cases.

Special Considerations

Certain health states predispose to more aggressive forms of HEV infection. A heightened awareness of these conditions is imperative because their presence can result in significantly worse outcomes with significant morbidity and mortality.

Pregnancy

Fulminant hepatitis occurs more frequently with HEV infection in pregnancy and leads to an increased mortality.[1] In particular, those in the second and third trimesters are at an increased risk of developing acute liver failure, fetal loss, and death. Mortality rates as high as 20% to 25% have been reported in women in the third trimester of pregnancy.[56–58] HEV viral loads with genotype 1 infection in pregnant patients are higher compared with nonpregnant women.[59] The mechanism by which HEV infection increases mortality in pregnancy is not clearly understood.[19]

In the Kashmir, India epidemic of 1978 involving around 270 patients, HEV infection developed in 17.3% of pregnant women, compared with 2.1% in nonpregnant women and 2.8% in men of similar age.[8] Of the pregnant patients who were infected, 19.4% and 18.6% were in the second and third trimesters, respectively. Fulminant hepatic failure developed in 22% of the infected pregnant women, leading to abortions, stillbirths, and neonatal deaths. Vertical transmission of HEV with severe hepatitis in neonates has been reported.[60,61] It has not been associated with congenital abnormalities.

Solid Organ Transplantation

Immunocompromised individuals, particularly those with solid organ transplantation, are susceptible to HEV infection. The incidence of genotype 3 infections in France has been reported to be 3.2 cases per 100 person-years.[62] Transmission occurs with consumption of wild meat, pork, and mussels after transplantation.[63] Transmission of HEV infection via the transplanted organ is possible, but fortunately rare.[62,64] In a single patient in Germany, hepatic failure and death were reported in the transplant recipient.[64] HEV reactivation after organ transplantation in individuals who are seropositive for the virus is usually not a concern[62] but has been reported in a patient after allogeneic hematopoietic stem cell transplantation for acute lymphoblastic leukemia.[65]

The clinical presentation of organ transplant recipients with chronic HEV infection is variable. Most patients do not have clinical symptoms, whereas others can develop signs and symptoms of progressive liver disease and jaundice.[34] Laboratory parameters show mild-to-moderate elevations compared with acute hepatitis, with the ALT being approximately 300 IU/L or six times the upper limit of normal.[34] Diagnosis is challenging because their immunocompromised state prevents them from developing HEV IgG or IgM antibodies and seroconversion may never occur.[34] Genomic techniques to confirm diagnosis and measure therapeutic response to treatment are critical.[19]

Around 60% of solid organ transplant recipients with acute HEV infection fail to clear the virus and go on to develop chronic hepatitis.[34] Several factors have been associated with reduced clearance of the virus after acute infection. These factors include the degree of immunosuppression, time since transplantation, presence of leukopenia, and total and T-cell lymphopenia.[33,34] However, a study using multivariate analysis identified the use of tacrolimus and the presence of thrombocytopenia as the only two predictive factors for the development of chronic HEV infection in patients after solid organ transplantation.[34] Individuals with chronic hepatitis may develop fibrosis,[66] with 10% of cases progressing to cirrhosis.[34] Liver transplantation may be necessary to prevent death from decompensated liver failure.[35] Recurrence of chronic HEV infection is fortunately rare, having been reported in a single patient after a second liver transplantation.[67]

Coinfections

HEV infection can occur in patients who are HIV positive. Fortunately, coinfection is extremely rare; the estimated incidence is 0% to 0.9%.[41,68–70] All identified coinfections with HIV virus are genotype 3 HEV infections, whereas other genotypes have not been recognized.[40–42,68–72] Patients with coinfection usually have acute hepatitis,[40–42] but one patient with cirrhosis has been reported.[40] Although acute hepatitis is more common, the development of chronic HEV infection is associated with low CD4 counts.[2]

DIAGNOSTIC EVALUATION

General Principles

Timely identification and accurate diagnosis of HEV are essential. The implications are important for patient management, clinical outcomes, reduction of transmission, and optimization of disease control. The clinical manifestations of HEV infection are similar to those of other causes of viral hepatitis, making its diagnosis difficult.[73–75] Ruling out hepatitis A virus infection is important in the initial diagnostic work up of HEV infection because hepatitis A and HEV infections share many similarities in their characteristics (see Table 178.2). Hepatitis B and C should also be ruled out, particularly in HEV-nonendemic areas.

HEV should be suspected in the appropriate epidemiologic and clinical setting. However, the increasing detection of HEV in nonendemic areas suggests that it should be an important consideration in dealing with all cases of acute hepatitis regardless of location. Knowledge of HEV among clinicians is lacking compared with other forms of viral hepatitis. The diagnosis of HEV infection is often missed, and other causes, such as drug-induced liver injury, may be suspected. One analysis found that 2.2% of cases that expert hepatologists designated as drug-induced liver injury were actually acute HEV infection.[76] As a result, it is absolutely imperative that treating clinicians have knowledge about HEV infection.

Serologic Evaluation

The diagnosis of acute HEV infection is usually made serologically. However, comparison studies have shown significant differences in the sensitivities and specificities of the different serologic tests,[77,78] suggesting the need for improved diagnostic options. The detection of IgM antibodies to HEV is often adequate in endemic areas to make the diagnosis of acute infection.[1,7] The anti-HEV IgM antibodies occur in early phases of infection and usually last 4 to 5 months (see Fig. 178.4). These

antibodies can be detected in the majority of HEV outbreaks. The sensitivities of anti-HEV IgM antibody assays range from 72% to 98%; the specificities range from 78% to 96%.[78] The presence of IgG antibodies against HEV suggests a convalescent phase or past or chronic infection.[7] IgG antibodies develop a few days after IgM antibodies and can remain positive up to several years, but the exact duration is unknown. IgA antibodies to HEV infection have also been identified in 50% of infected patients.[79] These antibodies typically have a rapid decline to undetectable levels but can occasionally persist longer than IgM antibodies.[80,81] The role of IgA in HEV immunity is unknown, but it may help in establishing the diagnosis.

Molecular Diagnosis

Although serologic diagnosis is widely available, it is not currently licensed for use within the United States. Detecting HEV RNA in the blood or stool with reverse-transcriptase polymerase chain reaction (RT-PCR) has higher specificity and is used in areas where HEV is less common or in order to diagnose chronic HEV infection. It is an essential confirmatory test for IgM-positive individuals in nonendemic areas[82] and can be used to monitor infection.[2] RT-PCR is also popularly used for research purposes,[83–87] but is often impractical in the field.[19] It requires specialized laboratory facilities, which often leads to a longer time to diagnosis. It has poor sensitivity in diagnosing acute infection[88] and has interlaboratory variability,[89] further limiting its use. A viral antigen detection test also exists and is currently under investigation for its diagnostic usefulness in HEV infection. Genomic-based methods are important for the diagnosis of chronic HEV infection because many patients never have seroconversion.[40] Use of molecular methods with HEV viremia is vital to monitor response to antiviral treatment inpatients with chronic HEV infection.[90,91]

Histopathology

Liver biopsy for histopathologic diagnosis of HEV infection can be considered when dealing with fulminant hepatitis or when serologic and molecular tests are not ideal. The histologic changes in HEV infection can reveal a pattern of cholestatic injury or a typical acute hepatitis picture.[2,92] The characteristic findings in HEV infection (Fig. 178.5) include a lobular disarray with portal tract enlargement, focal hepatocyte and bridging necrosis, hepatocyte ballooning and acidophilic degeneration, Kupffer cell proliferation, and mononuclear cell infiltration.[19] There is also intrahepatocytic dilation of endoplasmic reticulum cisternae and an increase of cytoplasmic lysosomes. The mitochondrial matrix may appear condensed with dilation of the outer mitochondrial membrane. Prominent features of the cholestatic injury associated with HEV infection include bile stasis and glandular transformation of liver cell plates. Cholestatic changes persist until clinical recovery, whereas the other histopathologic features generally resolve over a 3- to 6-month period.[19] These histologic changes are not distinctive to HEV and should be used in conjunction with clinical parameters for appropriate and timely diagnosis.

In order to optimize outcomes of fulminant HEV infection, accurate diagnosis in a timely fashion is imperative. The histologic features of fulminant hepatitis include parenchymal necrosis with lobular collapse, foamy hepatocyte swelling, acinar pattern of hepatocyte arrangement, small bile ductular proliferation, portal and central hepatic vein phlebitis, and portal inflammation with neutrophilic and lymphocytic proliferation.[19,56,92,93] Involvement of other sites, such as biliary epithelial cells and extrahepatic sites, has been discovered in animal models[94,95] but has not been noted in humans.



FIG. 178.5 Histologic appearance of liver of a patient with acute hepatitis E virus infection. In the hematoxylin and eosin–stained section, there is portal tract expansion with inflammatory infiltrate, occasional lymphoid aggregates, and bile ductile proliferation *(white arrow).* The inflammatory infiltrate consists of lymphocytes, including plasma cells *(white arrowhead),* neutrophils, and rare eosinophils *(black arrow).* The periportal hepatocytes show ballooning degeneration *(black arrowheads). (Modified from Dalton HR, Bendall R, Ijaz S, et al. Hepatitis E: an emerging infection in developed countries.* Lancet Infect Dis. *2008;8:698–709.)*

THERAPEUTIC STRATEGIES

Acute Hepatitis E

Acute HEV infection most commonly has a self-limiting course. Hospitalization is generally not required. Treatment revolves around supportive care, and specific therapy is rarely warranted. Patients with hepatic failure require admission to the intensive care unit, where measures to monitor for signs of cerebral edema can be used and evaluation for transplantation can be performed.[7] Pregnant patients, who are at higher risk of morbidity and mortality, also require hospitalization with close monitoring, although adequate therapeutic interventions for pregnant patients have yet to be established. The benefit of early termination of pregnancy has not been proven.[7] Patients with HEV genotype 3 infections have been noted to have a favorable response to ribavirin therapy, leading to reduced viral loads and improved clinical outcomes.[96,97] Ribavirin's teratogenicity contradicts its use in pregnancy, and alternative antiviral measures require investigation.[2]

Chronic Hepatitis E

The treatment of chronic HEV infection revolves around minimizing the use of immunosuppressive agents in organ transplant recipients. The reduction in immunosuppression can lead to clearance of HEV in about 30% of patients[66,90] and potentially decrease the risk of subsequent cirrhosis. Antiviral therapy should be considered for patients in whom a reduction of immunosuppression is not possible or in those who have not achieved viral clearance after decreased doses. Interferon-α and ribavirin either in combination or as monotherapy have been tried[90,91,98,99] and have been successful in certain cases.[39,99–102] Monotherapy with ribavirin can lead to viral clearance within a few weeks.[2] In a retrospective case series with a total of 59 solid-organ transplant recipients who received ribavirin monotherapy, 95% of patients were observed to have viral clearance with a median dose of 600 mg/day for a median duration of 3 months.[102] In addition, sofosbuvir, an RNA-dependent polymerase inhibitor used to treat patients with chronic hepatitis C, has been shown to inhibit replication of HEV and may provide additional antiviral effects.[103] Randomized trials have not been conducted, and treatments in other immunocompromised populations are less well studied.

PREVENTION

General Measures

Prevention of HEV infection is key in reducing the burden of disease. General measures of prevention should be based on the endemicity of the geographic area. In highly endemic areas, focus should be directed on maintaining clean drinking water supplies, protecting water from fecal contamination, and paying strict attention to sewage disposal.[7,19] In fact, many epidemics in underdeveloped countries occurred as a result of sewage pipes leaking into drinking water supplies, and hence maintaining a barrier between these two conduits is essential to prevent transmission of HEV infection.[19] Chlorination and filtration are usually

inadequate for cleaning a contaminated water supply.[28] Simple measures to improve the quality of water, such as boiling water, have led to a decline in new cases during epidemics.[7]

The history of previous outbreaks of HEV infection has led to the development of important preventive strategies. Often, extreme weather conditions can lead to massive contamination of water supplies in developing countries.[19] Certain environments such as refugee camps or other emergency settings are particularly susceptible to this type of contamination. In 2004, a significant outbreak of HEV in Darfur had a short 6-week interval from the first case of infection to rapidly reaching the peak of the epidemic.[27,28] Attempts at chlorination to sanitize water supplies were unsuccessful.[28] Timely detection of acute HEV infection in these settings is imperative.

In developed countries, strong public sanitation systems and low secondary HEV transmission rates lead to a low incidence of HEV infection. A study done in the United States in 1997 found no association between background HEV seroprevalence and risk factors for enterically transmitted viruses,[104] suggesting alternative routes of HEV transmission. In geographical locations with low endemicity of HEV infection, emphasis should be placed on avoiding undercooked meats and thoroughly cooking pork products to prevent zoonotic transmission.[7,19] These measures are particularly important in patients who are immunocompromised or have chronic liver disease

Travelers should take certain precautions when traveling to endemic areas of HEV infection. Measures should be geared toward avoiding the consumption of contaminated water, ice, and foods. Pregnant women should try to avoid travel to endemic areas altogether, and immunocompromised patients should pay attention to their food consumption.

Vaccination

Experimental studies in animal models have tested various vaccines containing recombinant HEV capsid proteins. Preclinical studies have demonstrated protection against hepatitis and viremia, but fecal excretion of virus was not prevented.[105] Cross-challenge studies in monkeys indicated that vaccines derived from one genotype could provide cross-protection against all four genotypes.[106]

Two vaccines have undergone trials in humans.[107] The first vaccine was with a recombinant truncated HEV capsid protein produced in insect cells as a virus-like particle (VLP) with aluminum hydroxide as an adjuvant.[107] In a phase II, double-blind, placebo-controlled safety and efficacy clinical trial in Nepal, nearly 2000 participants were randomly assigned to receive three doses of vaccine or a matched placebo at 0, 1, and 6 months.[107] Participants were followed for 2 years. There was a 95.5% protective effect against acute HEV infection after three doses of the vaccine.[107] The vaccinated individuals had high anti-HEV IgG levels at 1 month after the third dose of vaccine, but this effect had declined to 56% at the end of follow-up.[107]

A second vaccine with 239–amino acid truncated ORF2 protein is expressed in *Escherichia coli*.[108] This vaccine was studied in a large population-based trial in southern China with more than 110,000 subjects.[108] There was a robust protective effect against acute HEV infection at a rate of 100% during a 13-month follow-up period after three administered doses of the vaccine. The genotypes that were detected were primarily genotype 4 (92%) and only one case of genotype 1 (8%). Thus, although the vaccine is anticipated to provide pangenotypic protection, this has not yet been demonstrated. This vaccine is currently approved for use in China. Vaccination against HEV infection has not yet been approved for use in other countries.

Although passive immunization has shown beneficial results in preclinical studies,[109] the administration of immune globulin for pre-exposure or postexposure prophylaxis does not affect the incidence of HEV infection.[7] The administration of immune globulin in endemic and nonendemic regions has been ineffective and offers little to no protective function.[20,110] However, the identification of neutralizing monoclonal antibodies and their specific target epitopes provides a possible means for passive immunization in the future.[111–114]

The future of vaccination against HEV infection remains hopeful. Vaccines will eventually be available in countries where the infection is endemic. Vaccination is imperative to reduce disease burden and decrease morbidity and mortality in pregnant patients.[115] An ongoing uncertainty that requires further investigation is the duration of the protective role of the current investigational vaccines and whether booster immunization will be required. WHO identified the HEV-239 vaccine as a promising vaccine that has shown efficacy in 16- to 65-year-old healthy individuals in China,[116] but has not yet gathered enough information to recommend its use. DNA-based vaccines also show potential for the prevention of HEV infection.[117]

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179 Prions and Prion Disease of the Central Nervous System (Transmissible Neurodegenerative Diseases)

Patrick J. Bosque and Kenneth L. Tyler

SHORT VIEW SUMMARY

Definition
- Prion diseases are transmissible neurodegenerative conditions of mammals characterized by a rapidly progressive dementia, usually with ataxia and other motor signs.
- Human prion diseases include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, variably protease-sensitive prionopathy, and kuru.
- Important prion diseases of animals are scrapie of sheep and goats, chronic wasting disease of deer and related species, and bovine spongiform encephalopathy.

Epidemiology
- Annual incidence of prion disease is 1 to 2 per million annually.
- About 90% of cases are sporadic.
- About 10% of cases are genetic, caused by a mutation in the prion protein gene (*PRNP*).
- Infectious transmission accounts for less than 1% of human cases.

Microbiology
- The prion is composed primarily of an aggregate of misfolded forms of a normally produced protein, the prion protein (PrP).
- The aggregate serves as a catalytic template for the further aggregation of PrP, and thus the prion replicates.
- The prion contains no information-bearing nucleic acid.

Diagnosis
- Treatable mimics of prion disease should be excluded before making the diagnosis.
- A highly sensitive and specific test, real-time quaking-induced conversion (RT-QuIC), is now available to diagnose the most common form of prion disease from a sample of cerebrospinal fluid (CSF).

Therapy
- No effective therapy exists for prion infection.

Prevention
- Only standard precautions are needed for most procedures to prevent iatrogenic transmission of prion disease.
- Additional precautions are needed for neurosurgical cases and handling of CSF.
- Transfusion of red blood cells and plasma protein products can transmit one form of prion disease, variant CJD.
- Tissue from persons with prion disease must not be transplanted.
- Dietary exposure to ruminant animal prions should be avoided.

Prions are the transmissible agents of a class of neurodegenerative diseases of humans and other mammals. Prions differ from other infectious agents in that they contain no information-bearing nucleic acid. Rather, they are composed mainly, perhaps only, of abnormal conformations of a normally produced cell surface glycoprotein called the *prion protein* (PrP). These abnormal conformations of PrP are able to trigger the abnormal folding of previously normal conformers of PrP. It is the abnormal conformation of PrP, rather than the polypeptide itself, that propagates in prion diseases. The human forms of prion disease are most commonly referred to as Creutzfeldt-Jakob disease (CJD), although some specific clinical forms carry other names, such as kuru, Gerstmann-Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI). The most important prion diseases of animals are scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle, and chronic wasting disease (CWD) of deer and related species.

BRIEF HISTORY OF PRION DISEASE RESEARCH

Scrapie, a disease of sheep and goats, is the prototypical prion disease. References to it are found at least as early as 1750 in Germany.[1] Studies on scrapie in the late 19th through mid-20th century[2] established that the disease was transmissible by a small filterable agent after incubation periods of up to 22 months. It was thus thought to be a virus or, because of the long latency between infection and the emergence of symptoms, a "slow virus." However, studies in the mid-20th century revealed the scrapie agent to be resistant to ionizing radiation and other physical and chemical treatments that ordinarily inactivate viruses. This led Alper and coworkers[3] to suggest that the agent might replicate despite lacking nucleic acid. The link between the agent of scrapie and human disease was established by Gajdusek and Zigas.[4] Gajdusek and coworkers then identified kuru, a neurodegenerative condition endemic in certain cannibalistic tribes in the highlands of New Guinea, as a human prion disease when they transmitted the condition to primates.[5] They were aided in this discovery by the insights of the veterinary neuropathologist Hadlow, who remarked on the histopathologic similarity of kuru to scrapie, triggering the search for the potential transmissibility of kuru.[6] The similarities between kuru and scrapie that first drew Hadlow's attention included a "soap bubble"–like vacuolation of the neuropil, profound neuronal loss, and intense reactive astrogliosis in the absence of an associated inflammatory response. Modern neuropathologists would add only the immunohistochemical detection of abnormal forms of PrP to this list of key pathologic features of prion diseases. Prusiner and coworkers[7,8] first purified the infectious agent. They found that a previously unidentified protein was the chief component of a highly infectious fraction isolated from brains of hamsters with scrapie.[8] In 1982, Prusiner proposed the name *prion* (pronounced PREE-on) for the agent responsible for scrapie and related neurodegenerative diseases.[9] The term *prion* initially was chosen to emphasize the hypothesis that the causative agents in these diseases were *p*roteinaceous *i*nfectious particles that could be distinguished from viruses and viroids by their apparent lack of nucleic acid. The protein that comprised the dominant component of the infection fraction was thus named the "prion protein."

MOLECULAR BIOLOGY AND PATHOPHYSIOLOGY OF PRION DISEASES

The Prion Protein

The process of prion propagation involves the conversion of a normally produced form of the prion protein (Fig. 179.1), usually designated PrP^{C} ("C" for cellular), to an abnormal disease-causing form, often designated PrP^{Sc} ("Sc" for scrapie). PrP^{C} is a cell surface glycoprotein, transcribed off a chromosomal gene, *PRNP,* located at 20p13 in humans.[10] The protein is modified during biosynthesis by the addition of a glycosylphosphatidylinositol moiety that anchors the carboxyl terminus of the mature protein to the external surface of the cell membrane.[11] It may be further modified by oligosaccharides (glycans) linked to asparagine at positions 181 and 197 in the polypeptide.[12] PrP^{C} is constitutively expressed at high levels in the normal brain.[13,14] The protein is also expressed at high levels in certain cells of the reticuloendothelial system, and this distribution contributes to the pathogenesis of epidemic prion disease (see "Transmission by Oral Exposure" later). The normal function of PrP^{C} remains obscure. Mice in which production of PrP is abolished by disruption of the *PRNP* gene survive to adulthood, and show no gross anatomic or behavioral abnormalities. The PrP sequence is highly conserved in evolution, so it presumably confers an as-yet unrecognized fitness benefit on the host. Candidate functions for PrP^{C} include cell adhesion, synaptic function, signal transduction, metabolism of amyloid β (the protein involved in Alzheimer disease pathogenesis), and a role in copper metabolism.[15,16] High-resolution structures for PrP^{C} have been obtained by nuclear magnetic resonance spectroscopy.[17] This shows PrP^{C} to be a globular protein composed of three α-helical segments and two short β strands that form a small antiparallel β sheet.

Infectious Prions

Prion diseases are marked by the appearance of a pathologic form of PrP, designated PrP^{Sc}. PrP^{Sc} has biochemical properties different from those of PrP^{C} (Table 179.1). In particular, the C-terminal portion of PrP^{Sc} is resistant to digestion by proteases, so that after treatment with the serine endopeptidase proteinase K, only the N-terminal portion of PrP^{Sc} is digested, whereas PrP^{C} is completely hydrolyzed. The generation of protease-resistant C-terminal fragments of characteristic size is a widely used assay for PrP^{Sc}. Spectroscopic studies indicate the PrP^{Sc} polypeptide has acquired extensive β sheet structure that is not present in PrP^{C}.[18] These and other data indicate that PrP^{Sc} is composed of aggregates of PrP tightly bound by interpeptide β sheets. In contrast, PrP^{C} exists as monomers or perhaps low-order multimers. The tertiary structure of PrP^{Sc} (i.e., the conformation of the polypeptide chain in aggregates) has proved difficult to determine, in part because available methods to determine high-resolution protein structure require that the protein be soluble, but the aggregates of which PrP^{Sc} is composed are insoluble. The conformation of PrP^{Sc} appears to be central to the process of prion propagation, so efforts to decipher this structure using advanced techniques are underway.[19,20]

Propagation of Prions

The precise mechanism of prion propagation is not completely understood. A popular model views prion propagation as similar to the "seeded polymerization" mechanism of amyloid formation. "Amyloid" is defined as a fibrillary protein aggregate, in which the protein monomers are bound together in β sheets oriented perpendicularly to the long axis of the fibril. Such aggregates may form the large "plaques" that are histologically identifiable by apple green birefringence under polarized light when stained with the dye Congo red (the classical definition of amyloid), but need not do so. The kinetics of amyloid fibril formation in vitro suggests a mechanism for prion propagation. Typically, a pure solution of monomers of an amyloidogenic peptide will exhibit a lag phase, often days in duration, before the rapid conversion of monomers into amyloid fibrils. If a small "seed" fibril is introduced into a solution of monomeric peptides, the lag phase is eliminated and the monomers rapidly polymerize. By analogy, an inoculum of PrP^{Sc} acts as seed to trigger aggregation of PrP^{C}. PrP amyloid of the classical type is seen in some forms of human and animal prion disease. Uncertainty persists over whether amyloid fibrils or a protofibrillary state may be the actual infectious moiety of prion diseases.[20,21] A conceptual model of prion propagation is presented in Fig. 179.2.

In vitro Synthesis of Prions

The seeded aggregation approach has been used to synthesize infectious prions in vitro. In the simplest application of this in vitro method, termed *prion misfolding cyclic amplification,* a small inoculum of infectious prions is added to a larger volume of brain homogenate.[22] This mixture is incubated at a warm temperature and subject to cycles of sonication. In this process, PrP^{C} in the brain homogenate is converted to PrP^{Sc}. A small amount of this treated mixture is then inoculated into another volume of brain homogenate. The process is repeated through many cycles. Infectious prions and PrP^{Sc} are present in the final mixture, yet the original inoculum has been diluted to nothingness. This method has been further refined to produce infectious prions using only defined components, confirming the prion hypothesis.[23,24] Further modifications of this method have been exploited to develop sensitive and specific minimally invasive (typically lumbar puncture) tests for prion disease (see "Laboratory Diagnosis of Prion Disease" later).

Other Macromolecules Contributing to Prion Propagation

Whether proteins or macromolecules other than PrP play a role in prion propagation is uncertain. In the yeast prion state [PSI+] (see

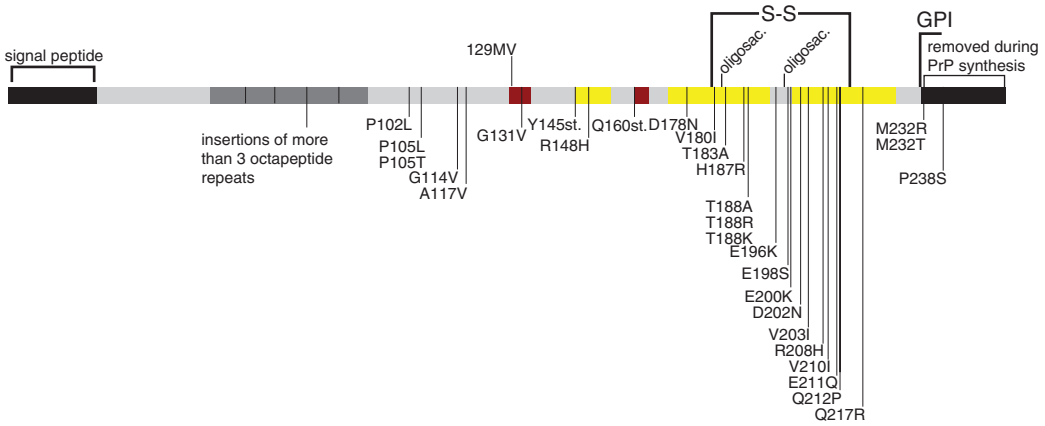

FIG. 179.1 Structural elements of prion protein (PrP) and mutations associated with genetic human prion disease. The PrP polypeptide is depicted as a horizontal bar running from the amino terminus on the left to the carboxyl terminus on the right. Amino and carboxyl termini removed in posttranslational processing are shown in black, and the locations of the five octapeptide repeats are shown in dark gray. The elements of regular secondary structure in recombinant cellular PrP (PrP^{C}) are the α-helix *(yellow)* and two short β strands *(red).* The sites of covalent modifications are shown above the bar, as is the location of the common codon 129MV polymorphism. PrP mutations associated with human prion disease are shown below the bar. Not shown are rare, apparently nonpathogenic polymorphisms. *GPI,* Glycosylphosphatidylinositol.

TABLE 179.1 The Properties of the Normal and Scrapie-Associated PrP Isoforms

FEATURE	PrP^{C} (NORMAL ISOFORM)	PrP^{Sc} (SCRAPIE ISOFORM)
Present in normal brain	Yes	No
Present in scrapie-infected brain	Yes	Yes
Covalent modifications	GPI anchor, Asn-linked polysaccharides, single intramolecular disulfide bond	Probably identical to PrP^{C}
Soluble in mild detergent	Yes	No
Effect of protease	Hydrolyzed to small peptides	Protease resistant C-terminal portion of variable length
Conformation		
Secondary structure: Local conformation of the polypeptide chain	About 40% α-helical, little β sheet	30% β sheet, 20% α-helix
Tertiary structure: Overall fold of the polypeptide	3 α-helical regions, unstructured N-terminus	Not determined
Quaternary structure: Association with other polypeptides	Monomeric or few-subunit oligomer	Aggregated

Asn, Asparagine; *GPI,* glycosylphosphatidylinositol.
Modified from Reisner D. Biochemistry and structure of PrP(C) and PrP(Sc). Br Med Bull. *2003;66:21–33.*



FIG. 179.2 A conceptual model of prion replication. (A) PrP^{C} *(green)* is formed of a nonaggregated polypeptide that contains α-helical domains and little β sheet, whereas PrP^{Sc} *(blue)* exists in aggregates of PrP polypeptides joined by intramolecular β sheet. (B) PrP^{Sc} and PrP^{C} interact and in the process a PrP^{C} adopts the conformation of PrP^{Sc} in the aggregate and joins the aggregate. (C) This process repeats and the aggregate grows. (D) At some point the aggregate fractures, creating two (or more) aggregates, each of which can serve as the nidus for further recruitment of PrP^{C} into PrP^{Sc} aggregates; the prions thus replicate. The process requires a source of PrP^{C} to continue, because it is the abnormal conformation of PrP that is propagating, not the PrP polypeptide itself.

"Yeast Prions" later), protein folding chaperones such as heat shock proteins can influence the rate of prion propagation.[25,26] The evidence of similar effects for mammalian prion disease is less clear.[27,28] To date, no protein other than PrP has been unequivocally demonstrated to participate in mammalian prion propagation. Genome-wide surveys of human populations have suggested that genes other than *PRNP* may contribute to a risk for prion disease, but conclusive evidence is lacking.[29–31] In vitro studies of prion replication indicate that phosphatidylethanolamine and RNA (of any sequence) may be important cofactors to produce infectious prions.[32]

Species Barrier to Transmission of Prion Diseases

Prion diseases are enzootic in several species of ruminants to which humans are exposed through food consumption and other routes, raising concerns of transmission of these conditions to humans (see "Variant Creutzfeldt-Jakob Disease" later). The amino-acid sequence of PrP can have a dramatic influence on prion propagation. The transmission of prion diseases across species is generally less efficient than transmission within the species. This phenomenon is known as the "species barrier." A crucial determinant of the species barrier is differences in the amino-acid sequence of PrP between species. Studies in transgenic mice demonstrate that the species barrier can be abrogated by the introduction of a gene directing expression of PrP of the prion donor species into the host.[33] Thus mice, which are usually not susceptible to human sporadic CJD (sCJD), are readily infected if the mouse *Prnp* gene has been replaced by a transgene coding for the human PrP sequence. This phenomenon has been exploited to generate transgenic mice that can be used to model prion diseases of several species.[34] The efficiency of cross-species transmission of prions is also determined by the strain of prion involved, as discussed in the next section.

Prion Strains

One of the most enigmatic features of prion diseases is the existence of what are termed *prion strains* (Table 179.2).[35] In experimental animals, prion strains can be distinguished by clinical features such as disease incubation time in animals or the pattern of clinical signs at disease onset.[36] Pathologically, strains can be characterized by the region of the brain in which PrP^{Sc} accumulates, by the degree of brain vacuolization, and by the morphology and distribution of PrP amyloid accumulation. Of particular importance to human health in the presence of animal prion disease epidemics, the species barrier to prion disease transmission may vary considerably between prion strains.[35] Thus the resistance of humans to prions of other species cannot be predicted simply from a comparison of *PRNP* sequences.

Because the prion consists of host-derived PrP, the means by which the properties of individual strains are maintained is puzzling. The biochemical basis of prion strain variety appears to reflect differences in PrP^{Sc} conformation between various strains (Fig. 179.2). Evidence for this includes differences in size of the protease-resistant carboxyl-terminal fragment between strains, different stabilities of the PrP aggregate to denaturation with chaotropic salts, and differential binding of antibodies to PrP^{Sc} of different strains.[37,38] Taken together, these and other observations suggest that subtle conformational differences between the PrP^{Sc} associated with the various strains somehow dictate the clinical and pathologic differences.

It appears that differences in the clinical manifestation of certain human prion diseases (e.g., sCJD, sporadic fatal insomnia, variant CJD [vCJD], and so-called variable protease-sensitive prionopathy) may relate to differences in the strain of the prion involved.

Transmission by Oral Exposure

Only a small proportion of cases of human prion disease are infectiously transmitted, but intraspecies transmission through the oral route is the major cause of epidemic prion disease in animals. After dietary exposure to prions, the reticuloendothelial system plays a major role in the initial propagation of prions and in carrying infection to the central nervous

TABLE 179.2 Comparison of Two Hamster Prion Strains

	STRAIN	
PROPERTY	**HYPER**	**DROWSY**
Incubation time[212]	65 days	165 days
Clinical signs[212]	Hyperexcitability and ataxia	Lethargy
Size of protease-resistant fragment (nonglycosylated band)[213]	21 kDa	20 kDa
Sensitivity of resistant carboxyl-terminal fragment to prolonged exposure to protease[213]	Present after 24 h of digestion	Hydrolyzed after 12 h
Resistance to denaturation (concentration of guanidine HCl that denatures 50% of PrP^{Sc})[214]	1.5 M	1.1 M
Distribution of PrP^{Sc} in the brains of clinically affected hamsters[215]	Most in medial geniculate nucleus and deep cerebellar nuclei	Most in regions of hippocampus, cerebellar granular layer, and occipital cortex
Distribution of PrP^{Sc} outside the CNS[216]	In spleen and other lymphoreticular organs	Not found in lymphoreticular system
Species barrier[212]	Nonpathogenic in mink	Pathogenic in mink

[a]Perhaps the two most well-studied prion strains are "hyper" and "drowsy," which are adapted to hamsters from transmissible mink encephalopathy, a prion disease of mink. The two strains are typically propagated in Syrian golden hamsters. Some characteristic properties are compared in the table. The number of potential strains that can exist on a single genetic background is not known, but evidence indicates that it is more than two.[213]
CNS, Central nervous system; *PrP^{Sc},* scrapie prion protein.

system (CNS). Prion titers rise first in gut-associated lymphoid tissue. Mice deficient in the number of functional Peyer patches show increased resistance to oral prion challenge.[39] Similarly, a number of studies have demonstrated that follicular dendritic cells are necessary for mice inoculated intraperitoneally with mouse-adapted scrapie to propagate prions to the brain.[40] Prion infection is carried to the brain from lymphoid tissue by axoplasmic transport in neurons of the sympathetic nervous system.[41–44]

Neurodegeneration in Prion Disease

How propagation of PrP^{Sc} leads to neurodegeneration remains unknown. Although the phenotypic expression of yeast prions, as discussed in the next section, is in several instances due to the loss of the protein's normal function, this does not appear to be the case with mammalian prions. Rather, the conversion of PrP^{C} to PrP^{Sc} causes a toxic "gain of function."[45] The nature of this toxic function remains poorly understood. Toxicity requires expression of PrP, and this PrP must be glycosylphosphatidylinositol anchored.[45,46] Substantial evidence suggests that the toxic species of PrP associated with prion infection may be different from the infectious species.[47] As with other neurodegenerative diseases associated with protein aggregates, dysfunction of the ubiquitin-proteasome system has been invoked as a cause of neurodegeneration in prion disease.[48]

Yeast Prions

Wickner first proposed that certain epigenetic traits of yeast could be manifestations of a process analogous to that which occurs in mammalian prion diseases.[49] To date, at least 10 yeast prions and 1 prion of the filamentous fungus *Podospora anserina* ([Het-s]) have been identified.[50] These traits are transmitted through exchange of cytoplasm but are not linked to the mitochondrial genome. Each particular prion trait is linked to a different protein, encoded by the nuclear genome, that is found to be in an aggregated state when the prion trait is expressed. Unlike the mammalian PrP prion, the phenotype of at least some yeast prions is equivalent to an inactivating mutation in the cognate protein. The [Het-s] prion is particularly interesting in that it appears to convey a useful property, mating incompatibility, upon its host. Yeast prions are intensively studied as models of mammalian prions and the processes of protein folding and aggregation in general. Yeast prions do not play any role in the initiation or transmission of mammalian prion diseases.

Prion-Like Behavior of Other Neurodegenerative Diseases

Prions formed of PrP remain the only proven mammalian prions, but there is some evidence that prion-like behavior of other proteins may play a role in neurodegenerative diseases such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis.[51] Limited evidence suggests that under unusual circumstances these conditions might be iatrogenically transmitted.[52,53]

HUMAN PRION DISEASES

Classification and Epidemiology of Human Prion Disease

Traditionally, human prion diseases have been classified by a combination of epidemiology, clinical features, histopathology, and family history. This has led to a proliferation of named syndromes that fundamentally share a similar pathophysiology. A more useful and consistent classification considers the various manifestations of human prion disease in terms of origin: sporadic, genetic, or infectiously transmitted. Most human prion disease is sporadic, that is, there is no determined infectious or genetic cause. In general, the term *Creutzfeldt-Jakob disease* refers to human prion disease and includes sporadic, genetic, and infectiously acquired forms that have not been given another name. The substantial clinical and pathologic diversity of human prion disease may be the manifestations of different strains of prions. However, typing of human prion strains is an emerging technology, and the basis of prion strain differences is incompletely understood. It is not clear whether the diverse manifestations of human prion disease can be attributed to differences in the properties of the initiating prion strains or result from other as-yet unidentified factors.

Epidemiology of Creutzfeldt-Jakob Disease

The term *Creutzfeldt-Jakob disease* was first used by Spielmeyer in 1922 to refer to a puzzling, rapidly progressive neurodegenerative syndrome initially described separately by the German neurologists Creutzfeldt and Jakob.[54–56] CJD is rare, with an annual prevalence and incidence typically said to be 1 case per 1 million population worldwide. In several countries, the rate of CJD has increased since the mid-1990s.[57] This most likely is the result of improved surveillance in response to the outbreak of BSE and vCJD. Based on these studies, the actual incidence of human prion disease may approach 2 cases per million annually.[58] In the United States, studies find a substantially lower rate of CJD among African Americans and other nonwhites than among whites.[59] Whether this reflects reduced ascertainment in these groups or a truly reduced incidence is not certain.

Sporadic Creutzfeldt-Jakob Disease Epidemiology

sCJD comprises approximately 85% to 94% of all cases of human prion disease.[60,61] It shows no gender predilection. Mean age at onset is 57 to 66 years, although patients as young as 17 years and older than 80 years

with sCJD have been reported.[61] Several studies found a peak incidence, approaching 6 per 1 million population, in the eighth decade and then a distinct decline in incidence in those older than 80 years.[59–61]

Clinical Features

The most distinctive clinical feature of sCJD is the pace of its progression, typically described as "rapid" or "subacute." In the context of neurodegenerative conditions, these terms refer to perceptible declines in cognitive and motor function that are obvious over a period of a few weeks. In contrast, in more common neurodegenerative conditions, such as Alzheimer or Parkinson disease, decline is typically only apparent over periods ranging from months to years. Some observers have noted that the pace of decline in sCJD accelerates until the later stages of akinetic mutism, when neurologic dysfunction is so severe that it is difficult to appreciate further decline. A second distinctive feature of sCJD is the prominent involvement of multiple brain systems in which motor signs, such as ataxia, bradykinesia, or spasticity, are combined with memory and other cognitive deficits. There is a great deal of variability in the clinical manifestations of sCJD, and this has led to attempts to describe a variety of clinical subtypes, including those with predominance of visual,[62] cerebellar,[63] thalamic,[64] and striatal[65,66] features. The existence of these syndromes indicates that sCJD may affect particular brain regions disproportionately. In some cases, the particular regional predominance of pathology may reflect the strain of prion involved.

In many patients with sCJD, there is a prodromal phase of psychiatric disturbance before the onset of neurologic signs. Of 126 mostly sporadic cases of CJD, 26% of patients had psychiatric signs in the prodromal or presenting phase.[67] Most common were sleep disturbance, depression, and anxiety. In approximately one-third of patients, initially prominent visual or cerebellar symptoms may overshadow dementia. Mental deterioration typically is rapidly progressive, and the average duration of illness from onset of symptoms to death is 7 to 9 months. In addition to profound and rapidly progressive mental deterioration, another very common feature is involuntary twitches or jerks of muscles, known as myoclonus. However, myoclonus in demented patients is not pathognomonic of sCJD. It can occasionally occur in Alzheimer disease and is common in Lewy body dementia. Extrapyramidal and cerebellar signs, including bradykinesia, rigidity, ataxia, nystagmus, and tremor, ultimately develop in approximately two-thirds of patients. Approximately 40% to 80% of patients have signs of corticospinal tract dysfunction, including hyperreflexia, spasticity, and extensor plantar responses. Prominent visual disturbances, which can include visual field cuts, cortical blindness, and visual agnosia, occur in 50% of sCJD patients.

Some patients have vague sensory complaints, including pruritus and aching limbs. It is unclear if these sensations are of peripheral or central origin.[68] Signs of a motor or sensory peripheral neuropathy are occasionally found in sCJD, although these signs are almost always overshadowed by the dramatic signs of CNS dysfunction. One study found clinical evidence of peripheral neuropathy in approximately 20% of cases examined and electrophysiologic abnormalities in 14 of 16 surveyed cases of sCJD.[69] On occasion, signs of sensory or motor neuropathy may dominate the early disease course. Rarely, fasciculations and muscle wasting will be so prominent as to suggest amyotrophic lateral sclerosis.[70,71] Some cases of sCJD also have been reported in which the clinical features indicated prominent autonomic nervous system involvement. These features included hypohidrosis, bowel dysfunction, abnormal pupillary responses to autonomic drugs, abnormal diurnal blood pressure variation, and electrocardiogram abnormalities. Such findings are cardinal features of FFI.

Certain neurologic disturbances occur only rarely as prominent features in sCJD, and their presence should prompt clinicians to consider other diagnostic possibilities. Although seizures occur in 10% to 20% of cases, they are rarely a dominant feature and typically are amenable to therapy. Cranial nerve involvement is never prominent, although isolated cases have been reported with involvement of the pupils,[72] extraocular movements,[73] and involvement of the auditory[74] and vestibular systems.[75]

Strains

At least two distinctive sporadic forms of sCJD bear the hallmarks of discrete prion strains. First, very rare sporadic cases of sporadic fatal insomnia, a clinical and pathologic syndrome indistinguishable from FFI (see "Familial Prion Disease" next), will be encountered in patients without *PRNP* mutations.[76,77] This form of sporadic prion disease maintains a biochemical signature identical to that of FFI upon serial transmission in transgenic mice expressing human PrP. It thus behaves as a distinct human prion strain.

Second, variably protease-sensitive prionopathy, a recently described condition, is a form of sCJD with fewer and less prominent motor and sensory signs and a pattern of cognitive impairment said to resemble frontotemporal dementia.[78,79] Progression is slower than typical sCJD, with a mean duration of 30 months. PrP^{Sc} from these patients is more sensitive to protease digestion than in typical sCJD. The predominant protease-resistant fragment is 8 kDa and cleaved at both the amino and carboxyl termini, whereas in sCJD the predominant fragment is cleaved only at the amino terminus and is 19 or 21 kDa in size. This 8-kDa fragment is also observed in the genetic prion disease GSS (see "Gerstmann-Sträussler-Scheinker Syndrome" later) leading some to speculate that variably protease-sensitive prionopathy is a sporadic form of GSS. The disease is quite rare; 13 cases were identified by the main US prion disease pathology center over an 8-year period, and 5 cases were identified in Britain in a retrospective review of 20 years.[79,80] Ignoring ascertainment problems, which may be significant, these translate into an annual incidence of approximately 0.5 per 100 million in either population. Attempts to transmit the condition to rodents, including transgenic mice expressing only human PrP, have been minimally successful, with some evidence of prion replication on first passage, but no propagation on later passages.[81,82]

Familial Prion Disease

Overview and Epidemiology

Approximately 10% of cases of human prion disease are caused by some amino-acid sequence–changing mutation in *PRNP*, although the precise proportion varies among countries.[60,83] At least 30 distinct mutations in the *PRNP* gene have been associated with the inherited prion diseases (see Fig. 179.1).[84] *PRNP* mutations are found in all cases from families with a history of inherited prion disease, but not all patients with genetic forms of prion disease present with a family history of dementia. In a large European survey of prion disease, a family history of a CJD-like dementia was elicited in only half of genetic cases.[83] Familial prion diseases are transmitted in an autosomal-dominant pattern, usually with high penetrance. Recent analysis of large data sets of the expressed genome in humans has discovered that some *PRNP* mutations previously reported only in association with prion disease or atypical dementia are found in persons in the general population without symptoms or a family history of prion disease.[85] This indicates that for these mutations penetrance is low, and in some cases that the association with prion disease may be coincidental. For example, the frequency of the V180I mutation in control populations without a history of prion disease suggests that the lifetime risk of prion disease in a carrier is around 1%.[85]

Specific mutations in the *PRNP* gene tend to be associated with particular clinical and pathologic disease phenotypes.[83] However, significant variability is seen both within and between kindreds harboring identical mutations. The disease associated with many mutations is essentially indistinguishable from sCJD. Among these is the most common mutation worldwide, a lysine-for–glutamic acid substitution in codon 200 (E200K).[86] This mutation has been found in geographic clusters of familial CJD in Slovakia and Chile and among Sephardic Jews in Greece, Libya, Tunisia, and Israel. Before the mutation was discovered to be prevalent in Libyan Jews, the high rate of CJD in this group was misattributed to the consumption of sheep brains or eyes.[87] The median age of onset is 58 years. The mutation is highly but incompletely penetrant so that approximately 80% of carriers will develop CJD by the eighth decade of life.[88] Because the mutation is found in inbred populations, rare carriers are homozygous for the mutation. Surprisingly, these people seem to develop illness only slightly earlier than heterozygotes (50 vs. 59 years).[89]

Some *PRNP* mutations are associated with syndromes markedly different from sCJD.

Gerstmann-Sträussler-Scheinker Syndrome

In some families with inherited prion disease, most victims develop prominent early ataxia and signs of corticospinal tract degeneration.

This picture is often associated with accumulations of PrP in large amyloid plaques in the CNS.[90,91] This clinicopathologic picture is known as *Gerstmann-Sträussler-Scheinker syndrome.* GSS is linked to several different mutations in *PRNP,* including P102L, P105L, A117V, Q187H, F198S, D202N, Q212P, Q217R, Y145STOP, and an insertion of eight or nine octapeptide repeats.[92] The duration of illness ranges from 3 months to 13 years, with a mean of 5 or 6 years.[92] Overt dementia occurs late in the disease.

The characteristic neuropathologic finding of GSS is widespread amyloid plaques composed of PrP. Within the cerebellum, where the concentration is typically most dense, plaques are found in the molecular layer and are often multicentric. The multicentric morphology of GSS plaques distinguishes them from plaques seen in kuru, which are typically unicentric.[93] The degree of spongiform change is variable, ranging from substantial and severe to completely absent. Western blot analysis of protease-resistant PrP finds mainly an 8-kDa fragment, as opposed to the 21-kDa or 19-kDa fragments associated with most forms of human prion disease. GSS is inconsistently transmitted to mice expressing human PrP, but transmission to another rodent, the bank vole, appears to be more consistent.[94]

Fatal Familial Insomnia

FFI first was reported as a human disease in 1986.[95] Subsequently, the immunohistochemical detection of abnormal PrP followed by the discovery of a D178N mutation in *PRNP* marked FFI as a prion disease with unusual features.[96] The D178N mutation can occur in two different *PRNP* allele forms, with either a methionine or a valine at the polymorphic codon 129. The D178N/129M allele associates with FFI, whereas a syndrome more closely resembling typical sCJD occurs in patients carrying the D178N/129V allele.[97] In the typical form of FFI, patients develop insomnia as a prominent and early complaint, along with signs of autonomic hyperactivity (increased sweating, tearing, salivation, mild nocturnal hyperthermia, tachycardia, and hypertension). Motor disturbances develop later and can include ataxia, myoclonus, spasticity, hyperreflexia, and dysarthria.[98,99] Marked memory impairment is not prominent early in the disease, although a delirium-like hallucinosis may occur. The mean age of onset in FFI is 50 to 56 years, somewhat younger than in sCJD, but cases in patients as young as 19 years and as old as 83 years of age have been reported.[83,100,101] Series of patients with the FFI genotype display substantial clinical heterogeneity. Insomnia is not always noted by the patient, family, or clinicians.[100,102] Polysomnography is a sensitive test for FFI, being abnormal in almost all cases, but investigations have shown this is true for sCJD as well.[103] Neuropathologic changes, including neuronal loss and reactive gliosis, are found consistently in the anterior ventral and mediodorsal nuclei of the thalamus and the inferior olives.[98] Immunostaining of brain material for PrP^{Sc} is positive, although the concentration of protein is 5 to 10 times less than that seen in sCJD.

As mentioned earlier, a rare sporadic form of human prion disease—sporadic fatal insomnia—presents with clinical and histopathologic features identical to those of FFI.[76,77]

Long-Duration Genetic Prion Disease

Certain *PRNP* mutations frequently cause disease with exceptionally slow progression. These include large expansions in the octapeptide repeat region and the missense mutations T183A and H187R.[104–107] Some mutations may be associated with lifelong psychiatric disturbances that precede the onset of progressive dementia by decades.[108]

Polymorphisms in *PRNP*

Polymorphisms at codon 129 of *PRNP* play a role in CJD expression and susceptibility. In European populations, approximately 60% to 70% of alleles have methionine at this position, and the rest have valine. The alleles are in Hardy-Weinberg equilibrium, so genotypes in the general population are approximately 42% MM, 46% MV, and 12% VV at codon 129.[109] In contrast, 95% of patients who develop sCJD exhibit homozygosity (either MM or VV) at this locus.[110–112] Codon 129 allele distributions vary across ethnic groups, but the tendency for overrepresentation of homozygotes in CJD cases holds.[113] Genome-wide association surveys have demonstrated that the codon 129 polymorphism genotype is a significant risk factor for both sCJD and acquired CJD.[84] A polymorphism at codon 219 is found mostly in Asian populations. Persons heterozygous at this codon appear to be resistant to sCJD, but may have increased susceptibility to vCJD.[113a]

Infectiously Acquired Human Prion Disease

Infectiously acquired prion disease is quite rare in humans, constituting less than 1% of cases in most countries. Nonetheless, the risk of transmission of these incurable conditions is a significant concern. Humans have acquired prion disease both through dietary exposure and iatrogenic routes.

Kuru

Kuru is a neurodegenerative disease that was endemic within the Fore linguistic tribal group of the Eastern Highlands of Papua New Guinea.[114] It arose in the early 20th century and was transmitted through the practice of ritual cannibalism at funeral feasts.[115] No one born since the cessation of this practice has developed kuru. The mean incubation period was 10 to 13 years, with 90% of cases occurring within 21 to 27 years of exposure. The incubation period was likely related to exposure dose and was shorter in women than in men and shorter in older women than in younger women, reflecting the likelihood of participation in cannibalistic practices.[116] The last known case of kuru occurred in 2009, likely as a result of exposure before cannibalism ceased in 1960. Thus in some cases the incubation period for kuru approached 50 years.[117]

Although it has disappeared as a clinical entity, kuru remains important conceptually as the largest outbreak of human-to-human transmission of a prion disease and as an example of human prion disease transmitted via the alimentary route. In its clinical and pathologic manifestations, it is distinct from most types of human prion disease. Kuru typically begins insidiously with a prodrome of headaches and aching limbs that may last for several months.[118] This prodrome is followed by the development of an inexorably progressive neurologic disease resulting in death within 3 to 24 months of onset, usually from intercurrent pneumonia and malnutrition. Typical disease duration is 12 months. Prominent clinical features include cerebellar ataxia, action tremor, and involuntary movements (choreoathetosis, myoclonic jerks, and coarse fasciculations). Dementia is usually not noted until late in the illness, 8 or more months after the onset of ataxia.

Neuropathologic examination of kuru brains shows neuronal loss, astrogliosis, and the accumulation of PrP^{Sc}, all findings typical of prion disease.[119,120] The pathologic hallmark of kuru is the presence of PrP amyloid plaques, predominantly in cerebellar tissue. These plaques are usually unicentric (in contrast to the multicentric plaques of GSS), located in the granular layer of the cerebellum, and often associated with microglial cells. The genetics of the *PRNP* gene in the kuru epidemic region show the powerful selection pressure that the disease exerted. In the kuru epidemic region, older women who potentially were exposed to infected brain material during the era of cannibalism, but who survived without developing kuru, show a higher-than-expected frequency of heterozygosity at codon 129.[121] Thus this heterozygosity may have played a protective role against the transmission of prion diseases among the Fore. More strikingly, a *PRNP* G127V mutation is found only among survivors of the kuru epidemic and their descendants, and in no other population in the world.[122] This mutation appears to have conferred resistance to kuru and to have been strongly selected for during the epidemic.

The properties of kuru prions on transmission to transgenic mice expressing human PrP suggest that it is the same strain as that most commonly found in sCJD.[123] This finding is consistent with the hypothesis that the kuru epidemic arose out of a naturally occurring case of sCJD that was then spread by cannibalism. It is puzzling that the clinical and pathologic features of kuru are quite distinct from those of typical sCJD. It is possible that the oral route of exposure alters the clinical and pathologic presentation, or that current methods of typing strains are insensitive to differences between kuru and sCJD.

Variant Creutzfeldt-Jakob Disease

Epidemiology

Beginning in 1995, cases of a new variant of CJD were reported from the United Kingdom.[124–126] As of April 2018, a total of 178 cases have

been reported to the UK National CJD Research & Surveillance Unit (see http://www.cjd.ed.ac.uk/surveillance for latest case totals). This includes three symptomatic "secondary" cases transmitted through non–leukocyte-depleted red blood cell transfusion and two asymptomatic cases—one from red blood cell transfusion and the other probably transmitted through pooled plasma-derived factor VIII.[127–129] An additional 53 cases have occurred outside the United Kingdom, with 27 of these in France and the remainder in the Republic of Ireland, Italy, the Netherlands, Portugal, Spain, Japan, Saudi Arabia, the United States, and Canada. Of the five North American cases, four appear to be due to exposure of the victims to infected beef products in the United Kingdom or Saudi Arabia.[130] Retrospective review of available autopsy material suggests that vCJD did emerge as a new disease entity in 1995; no current evidence suggests that earlier cases occurred.[131,132] It is likely that millions of people were exposed to BSE-infected food.[133] However, fears of a large epidemic of vCJD have not been realized. The largest number of annual cases (28) was reported in 2000.[134] The incidence of vCJD is declining; since 2012 there have been only two deaths from vCJD in Great Britain.[134]

Clinical Features

The epidemiologic, clinical, and pathologic features of the vCJD cases set them apart from typical sCJD.[126,135,136] Patients with vCJD have been considerably younger than patients with sCJD, with a mean age at onset of 26 years (range, 12–74 years) compared with 65 years for sCJD. Ninety percent of vCJD patients are younger than 40 years at the onset of their disease. The reason for this striking age dependence is not clear, but it appears to be due to greater susceptibility in the young rather than greater exposure to BSE-contaminated foodstuffs.[137] The duration of illness in vCJD is longer (average, 14 months) than in sCJD (average, 4.5 months). This longer duration may be a reflection of the younger age of the patients because younger patients with sCJD have a similar duration of illness.[138,139]

Patients with vCJD frequently present with sensory disturbances and psychiatric manifestations. Such symptoms are not unusual in young-onset sCJD, but in vCJD they are more prominent, perhaps because dementia and more obvious neurologic signs supervene later than in sCJD.[138] Among the sensory symptoms are vague pain, cold sensation, or paresthesias involving the face and limbs or in a hemisensory distribution. Most studied patients have had normal electromyography or mild abnormalities that do not point to a peripheral origin for the sensory disturbance.[68] Psychiatric manifestations frequently include dysphoria, withdrawal, anxiety, irritability, insomnia, and loss of interest in usual activities.[140,141] These symptoms often prompt an initial diagnosis of psychiatric illness. Neurologic signs and symptoms are uncommon within 4 months of onset. As disease progresses, the most frequent neurologic signs include dysarthria and gait disturbance. In the later stages of disease (>6 months after onset), prominent neurologic signs include hyperreflexia, myoclonus, incoordination, and other cerebellar signs.

Transmission by Blood Products

Blood products have very likely transmitted vCJD on at least five occasions. Among a group of 66 individuals identified as having received blood components from donors who later developed vCJD, only 33 of the transfusion recipients survived more than 5 years after transfusion, with the rest dying of conditions other than CJD or dementia.[142] Three of the remaining 33 recipients developed vCJD 6 to 8 years after transfusion. The donors had given blood 16 months to 3.5 years before the onset of vCJD. One donor was linked to vCJD cases in two recipients. All cases received non–leukocyte-depleted red blood cells. Remarkably, in an autopsied case, PrP^{Sc} was found in the tonsils, suggesting that the accumulation of PrP^{Sc} within tonsils that is found in most vCJD cases is not due to the alimentary route of exposure but, rather, is a tropism inherent in the vCJD prion strain.[127] Another of these 33 recipients died of causes unrelated to vCJD but was found at autopsy to have PrP^{Sc} in the spleen.[143] The rate of vCJD in transfusion recipients from donors who later developed vCJD (4 per 66, or 6%) is about 10,000-fold greater than the rate of vCJD in the British population, conclusively implicating the transfusion as the cause of disease. Finally, a survey of tissue obtained at autopsy from 11 persons with hemophilia, known to have received UK-sourced, pooled, plasma-derived clotting factor concentrates, found PrP^{Sc} in a single spleen.[144] Both cases of asymptomatic infection occurred in blood product recipients who were heterozygous (M/V) at *PRNP* codon 129, whereas that codon was homozygous (M/M) in the three symptomatic cases.

Neuropathology

The neuropathologic features of vCJD are strikingly different than those of sCJD.[126] The neuropathology of vCJD includes prominent involvement of the cerebellum in almost all cases, whereas only in a subset of sCJD brains, the Brownell-Oppenheimer variant and GSS cases, is the cerebellum prominently affected. vCJD cases have prominent amyloid plaques distributed throughout the cerebrum and cerebellum and, to a lesser extent, the basal ganglia and thalamus. These plaques have a dense eosinophilic center and pale periphery and are surrounded by vacuoles in the neuropil that are arranged around the plaque like petals on a flower (hence, the name "florid plaques"). This flower-like morphology is common in plaques from vCJD but is infrequently seen in other plaque-forming types of human prion disease. vCJD cases show typical spongiform change, neuronal loss, and astrogliosis in the cortex, basal ganglia, and thalamus, but these do not distinguish vCJD from sCJD. Patients with vCJD also have a consistent pattern of electrophoretic mobility of PrP^{Sc} protein (type 4 isoform) that is distinct from the mobility patterns encountered in sCJD and is similar to the pattern seen in BSE PrP^{Sc}.[145]

vCJD Caused by Exposure to BSE

Compelling evidence indicates that vCJD is the result of bovine-to-human transmission of BSE.[146–150] From an epidemiologic standpoint, cases of vCJD followed a massive epidemic of BSE in the United Kingdom, with a lag period that is consistent with the known incubation period of prions. During the BSE epidemic, it was estimated that several hundred thousand BSE-infected cattle might have entered the human food chain.[133] The number of BSE-infected cattle peaked during 1992 and 1993 and subsequently declined steadily. This decline has been attributed to bans on using ruminant protein for ruminant feeds (July 1988) and on using bovine brain, spinal cord, and other specified offal as feed for nonruminant animals and poultry (September 1990). Another ban prohibited use of certain bovine tissues for human consumption (November 1989). It has been suggested that the BSE epidemic was triggered by changes in the rendering process, particularly the abandonment of the use of organic solvents.[151]

Additional evidence for a BSE-vCJD link comes from the close neuropathologic similarities between the two diseases. Transgenic mice expressing bovine PrP develop indistinguishable neurologic illness and neuropathologic changes after a similar incubation period when injected with brain material from either cattle with BSE or humans with vCJD, and this differs from the pattern and incubation time seen after inoculation with scrapie.[150] In addition, the PrP^{Sc} protein isoforms isolated from the brains of the BSE-inoculated or vCJD-inoculated mice show an identical fragment size and glycosylation pattern, which differs from that seen after scrapie inoculation.[148] Furthermore, macaques inoculated with BSE prions develop florid plaques histologically indistinguishable from those seen in vCJD, whereas those inoculated with sporadic CJD prions do not develop plaques.[149] Finally, the susceptibility of mice expressing murine PrP, or transgenic mice expressing PrP of humans, to vCJD more closely resembles the susceptibility of these same mice to BSE than to other forms of human prion disease.[152]

Genetics of vCJD Susceptibility

With two possible exceptions, all vCJD patients tested to date are homozygous for methionine at polymorphic codon 129 (129M). One young patient in Great Britain with the 129VV genotype developed CJD with clinical and pathologic features not typical of sCJD or vCJD. Protease-resistant PrP^{Sc} from this patient showed a size and glycoform pattern identical to that found in vCJD.[153] Whether this was a sporadic case or, like vCJD, was caused by exposure to BSE prions is undetermined. Recently, a 36-year-old man died of prion disease in Great Britain. The molecular, but not the clinical, features of the disease were

consistent with vCJD.[154] It is fortunate, though puzzling, that despite the widespread exposure of populations to BSE, comparatively few persons have developed vCJD. As mentioned earlier, genome-wide association studies have sought loci that might confer exceptional susceptibility to vCJD upon some people. No convincing evidence for genetic risk factors, except for homozygosity for methionine at *PRNP* codon 129, has been found.[30,31]

Subclinical vCJD

The wide exposure of the British population to BSE-contaminated food products and the sometimes very long incubation period of prion diseases have raised concerns that human infection with BSE prions may be more prevalent than is apparent from the number of clinical cases. In this regard, in certain animal models of transmission of prions across species, animals can be efficiently infected, as indicated by demonstrable prion replication in the spleen and other organs of the reticuloendothelial system, but never show clinical signs of disease. Immunohistochemical surveys of large numbers of archived tonsils and appendices have been undertaken in Great Britain to look for subclinical prion infection. A survey of approximately 15,000 appendices collected between 1995 and 1999 found 3 with immunohistochemically detected PrP^{Sc}.[155] The study was repeated on approximately 32,000 appendices collected between 2000 and 2012, and 16 were found to harbor PrP^{Sc}.[156] Together, these studies suggest that about 1 in 2000 Britons may be subclinically infected with prions. However, as yet, no study has been performed on a control group, for instance, a population not known to have exposure to BSE. Therefore, it is uncertain whether these findings are related to BSE exposure, represent some other aspect of prion biology, or are artifactual. Interestingly, similar investigations of more than 60,000 tonsillectomy specimens failed to find any conclusive evidence of PrP^{Sc}.[155,157] If the PrP^{Sc} detected in appendices is the result of BSE prions propagating in human lymphoid tissue (i.e., subclinical vCJD), then it is possible that a large number of symptomatic cases could eventually occur and that donated blood products, organs, or tissues from subclinically infected Britons might transmit prion disease to recipients.

Diagnosis of vCJD should be considered when patients with a history of residence in a BSE-endemic area, such as the United Kingdom, develop a progressive neurodegenerative disease. Table 179.3 lists the World Health Organization criteria for vCJD. These criteria identified vCJD cases with 77% sensitivity and 100% specificity.[136] The newly developed real-time quaking-induced conversion (RT-QuIC) assays for prions in CSF (see "Specific Testing for Prion Disease [RT-QuIC]" later) appear to have limited sensitivity to detect vCJD prions.[158]

TABLE 179.3 World Health Organization Case Definitions for Sporadic and Variant Creutzfeldt-Jakob Disease (CJD)

Sporadic CJD

Possible CJD

Progressive dementia *and*
EEG atypical or not known *and*
Duration <2 years *and*
At least two of the following clinical features:
- Myoclonus
- Visual or cerebellar disturbance
- Pyramidal/extrapyramidal dysfunction
- Akinetic mutism

Probable CJD (in the Absence of an Alternative Diagnosis From Routine Investigation)

Progressive dementia *and*
At least two of the following four clinical features:
- Myoclonus
- [a]A typical EEG, whatever the clinical duration of the disease, *and/or*
- [a]A positive 14-3-3 assay for CSF, *and*
- A clinical duration to death <2 years

Definite CJD

Neuropathologic confirmation *and/or*
Confirmation of protease-resistant prion protein (immunocytochemistry or Western blot) *and/or*
The presence of scrapie-associated fibrils

Variant CJD

I. A. Progressive neuropsychiatric disorder
B. Duration of illness >6 months
C. Routine investigations do not suggest an alternative diagnosis
D. No history of potential iatrogenic exposure
E. No evidence of a familial form of TSE
II. A. Early psychiatric symptoms
B. Persistent painful sensory symptoms
C. Ataxia
D. Myoclonus or chorea or dystonia
E. Dementia
III. A. EEG does not show the typical appearance of sporadic CJD (or no EEG performed)
B. MRI brain scan shows bilateral symmetrical pulvinar high signal
IV. A. Positive tonsil biopsy

Definite CJD: I A *and* neuropathologic confirmation of variant CJD
Probable CJD: I *and* 4/5 of II *and* III A *and* III B *OR* I *and* IV A
Possible CJD: I and 4/5 of II and III A

[a]Note: These criteria have not been revised since the development of minimally invasive assays such as real-time quaking-induced conversion (RT-QuIC) of CSF. In cases of sporadic CJD, the RT-QuIC assay is superior to EEG or a 14-3-3 assay, and should be taken as evidence of probable or definite CJD.
CSF, Cerebrospinal fluid; *EEG,* electroencephalography; *MRI,* magnetic resonance imaging; *TSE,* transmissible spongiform encephalopathy.
From World Health Organization. *WHO Manual for Surveillance of Human Transmissible Spongiform Encephalopathies Including Variant Creutzfeldt-Jakob Disease.* Geneva: World Health Organization; 2003.

Iatrogenic Creutzfeldt-Jakob Disease

Examples of iatrogenic prion disease are exceedingly rare, but cases have occurred after transplantation of dura mater grafts,[159] corneal transplantation,[159] liver transplantation, use of dura mater material in radiographic embolization procedures,[160,161] use of contaminated neurosurgical instruments or stereotactic depth electrodes,[159] and, in cases of vCJD, blood and plasma concentrate transfusions.[142] Although the numbers are evolving constantly as new cases are identified, a review in 2012 identified 230 cases of iatrogenic CJD (iCJD) associated with human pituitary hormone administration (226, growth hormone; 4, gonadotropin), 228 cases associated with cadaver lyophilized dura mater grafts, 6 cases associated with contaminated neurosurgical instruments or intracortically implanted electroencephalography (EEG) depth electrodes, and 2 cases associated with cadaver corneal transplantation.[159]

Transmission Through Dura Mater Grafts

In the case of dura mater grafts, most implicated grafts have been Lyodura, the product of a single German commercial producer, manufactured before 1987. More cases (at least 154 by 2018) have been reported from Japan than from all other countries worldwide. [159,162] Cases have occurred in at least 17 additional countries, including at least 8 cases from Canada and the United States. The incubation period in these cases has ranged from 16 months to 30 years (median, approximately 12 years). In Japan, two distinct clinicopathologic forms of dura mater graft–associated CJD have been reported.[163] Approximately 75% of cases present very similarly to typical sCJD, with rapid progression, myoclonus, periodic sharp waves on the EEG, and a diffuse cortical distribution of PrP^{Sc}. About one-third of cases present with a picture reminiscent of GSS, with slower progression, absent myoclonus, absent or late appearance of sharp waves on the EEG, and deposition of PrP in amyloid plaques, some with a "florid" pattern similar to that seen in vCJD cases.

Transmission Through Cadaver-Derived Hormones

At least 226 cases of iCJD have been reported in young patients who received cadaver-derived human growth hormone for the treatment of endocrine disorders, with at least 4 additional cases in women receiving cadaver pituitary gonadotropin for infertility, practices that were discontinued when recombinant forms of these hormones became available.[159] Patients with human growth hormone–associated CJD typically received injections of growth hormone prepared from pools of 15,000 pituitary glands, several times weekly for several years. The risk of developing CJD differs among countries and cannot be absolutely determined because new cases continue to occur. As with kuru, incubation times can be extraordinarily long, with cases documented up to

40 years after exposure.[164] In 1977 a column chromatography step was introduced into the purification process for growth hormone produced under the National Hormone and Pituitary Program in the United States, and all but 1 of the US cases of growth hormone–associated CJD have occurred in the approximately 2700 patients who received treatment before that year.[159] The exception is a person who received commercially produced growth hormone from 1983 to 1985, and who developed prion disease in 2010.[165] France has an unusually high rate of iCJD among growth hormone recipients, in part because patients continued to receive the pituitary-derived product for several years after other countries abandoned the practice.[166]

Transmission Through Corneal Transplants

iCJD has been reported in 10 recipients of corneal transplants from 1974 to 2006 worldwide. However, in only two of those cases was the donor known to have had CJD. When the large number of corneal transplants performed annually is taken into account, it has been estimated that sCJD might coincidentally occur in a corneal graft recipient every 1.5 years in the United States alone.[167] Thus most of the 10 reported cases are likely coincidental.

Transmission Through Surgical Instruments

Five cases of iCJD have followed surgery with contaminated neurosurgical instruments, four from the United Kingdom and one from France.[168,169] The incubation period after surgery ranged from 12 to 28 months (median, 17 months). In two cases from Switzerland, intracortically implanted stereotactic depth electrodes for EEG monitoring were implicated.[170] The incubation period was 16 and 20 months in the two affected patients. Documented cases of neurosurgical transmission are remarkably rare, despite the large number of neurosurgical procedures performed. Epidemiologic studies have been mixed, but several show an increased risk of iCJD in those patients who have undergone *any* major surgical procedure.[171] Many of these studies may have been contaminated by recall and other biases, but a case-control study based on national hospital discharge registries in Sweden and Denmark also found an approximately twofold risk of CJD in those who had undergone major surgery, again of various sorts.[172] Odds ratios were mostly in the range of 3 or less. A modestly increased risk for a rare disease such as CJD translates into only a small increase in absolute risk, and the increased risk observed after surgery, even if real, does not necessarily implicate an infectious etiology.

Inadvertent Surgical Exposure

Several hospitals in the United States, Canada, and the United Kingdom have reported incidents in which neurosurgical instruments were inadvertently reused in other patients after having been used initially for diagnostic brain biopsies in patients who subsequently were found to have CJD. Despite these medical mishaps, no patient so exposed is known to have developed iCJD, although the time since surgery in most of these instances is insufficient to exclude absolutely the possibility that this may occur in the future. The Joint Commission on Accreditation of Healthcare Organizations has issued a sentinel event alert concerning this risk.[173] Hospitals should be aware of this potential and should establish guidelines for the handling, quarantine, and tracking of neurosurgical instruments in patients undergoing brain biopsy for dementia or other unknown neurodegenerative illnesses. In such cases, disposable instruments can be used if practicable, reducing the need for quarantine and tracking.

Blood Products and Sporadic CJD

As discussed previously, five cases of human-to-human transmission of vCJD prions through blood transfusion have occurred. In contrast, many epidemiologic studies have failed to find a convincing link between blood transfusions and any other form of human prion disease,[142,174] and a history of preceding transfusion does not seem to increase the risk for CJD in epidemiologic studies.[175] The reasons for this remarkable divergence are unknown. Animal transmission studies suggest that whole blood, serum, or buffy coat derived from patients with vCJD, cattle with BSE, deer with CWD, or animals experimentally inoculated with prions can transmit prion disease to at least some intravenously inoculated animals.[175] Recently, two cases of CJD occurred in Great Britain in patients with hemophilia. It is uncertain whether there is a causal connection between the lifelong receipt of clotting factors in these patients, or whether the occurrence of CJD was coincidental.[176]

CJD in Health Professionals

Cases of CJD occasionally occur in physicians, nurses, and other health care professionals. Despite the natural concern these reports produce, the incidence of CJD in this group does not seem to exceed what would be expected by chance alone.[177] There have been no documented reports of clear-cut transmission of disease from patients to hospital or mortuary staff. Similarly, although isolated cases of conjugal CJD have been reported, there does not seem to be any increased risk to spouses or other family members from exposure to CJD. As noted previously, the presence of familial cases of CJD seems invariably to result from genetic factors, rather than from person-to-person spread of illness.

Nosocomial and Iatrogenic Infection Risk

No special precautions need be taken in the routine care of patients with prion disease. Most bodily fluids, including blood, can be safely handled using standard precautions.[178] WHO recommends that clinical laboratories employ special precautions in the handling of CSF. Neurosurgical instruments utilized on patients with possible CJD should be sterilized by prolonged autoclaving (steam sterilization) or by using a combination of sodium hydroxide and autoclaving. (See Chapter 299 for further discussion.)

Prion Disease in Ruminants

Overwhelming evidence implicates the transmission of BSE from cattle to humans as the cause of vCJD. Prion disease is also endemic or epidemic in other ruminant species consumed by humans, including sheep and goats (caprinae), and deer, elk, and related species (cervidae). Recently, prion disease has been discovered in camels. The risk that prions in these species pose to humans appears low but is under active study. Intensive surveillance for prion disease in cattle and sheep has uncovered what appear to be strains of prion disease other than classic BSE and scrapie, and the risk of transmission of these forms to humans is especially uncertain.

Atypical Forms of Bovine Spongiform Encephalopathy

Two atypical forms of bovine prion disease, known as BSE-L (sometimes called BASE) and BSE-H, were first described in 2004.[179] They are uncommon, the rate being less than 0.5 per million adult cattle in France.[179,180] These forms are histopathologically distinct from classic BSE, and the protease-resistant fragments migrate differently from classic BSE on Western immunoblots. This, and data from transmission studies in various lines of transgenic and nontransgenic mice, supports the notion that these forms are strains of prions different from that which causes classic BSE.[180] These conditions are diagnosed mostly in aged cattle and seem to be found at a similar rate wherever cattle are intensively surveyed for prion disease. It is therefore suspected that these represent spontaneous cattle prion disease, rather than an exogenously acquired condition. A concern with these recently discovered strains of BSE prions is that they may be more pathogenic for humans than the classic BSE strain. Indeed, BSE-L has been transmitted both to transgenic mice expressing human PrP and to macaques, with incubation times suggesting the condition might more readily transmit to humans than does classic BSE.[181,182]

Atypical Forms of Scrapie

Scrapie has long been endemic in sheep, and epidemiologic evidence indicates that the disease either is not transmitted to humans or transmits at a very low rate.[183] As with BSE, intensive surveillance in recent years has revealed previously unrecognized strains of scrapie in domesticated sheep. Nor98 is the most distinctive of these strains, with an unusual 12-kDa protease-resistant core and a characteristic neuropathologic profile in sheep and in transgenic mice expressing ovine PrP.[184] Unlike classic scrapie, it is not clear if Nor98 is horizontally transmitted within flocks. Inoculation of transgenic mice expressing human PrP with either

classic scrapie or atypical forms such as Nor98 failed to cause disease, suggesting a substantial transmission barrier of sheep scrapie to humans.[185]

Chronic Wasting Disease

CWD was first discovered in captive mule deer in Colorado,[186] but now appears to be spreading worldwide.[187] It is found in free ranging and captive populations of deer, elk, and moose in North America. Recently it has been discovered in reindeer and moose in Norway. CWD is remarkable for the high rate of horizontal transmission. In northern Colorado, as many as 5% of all mule deer are infected, and, in captive populations, infection rates have exceeded 30%.[188] Human hunters and others have certainly consumed infected animals, but fortunately it appears that CWD is not readily transmissible to humans.[189] Nonetheless, the dynamics of interspecies transmission of prion diseases are poorly understood, so humans should avoid consuming CWD-contaminated deer and elk.

Camel Prion Disease

A prion disease has recently been discovered in more than 3% of dromedary camels sent to an abattoir in Algeria.[190] There is evidence that camels in this region may have been affected since the 1980s. Camels are a source of milk and meat for humans in northern and eastern Africa, the Middle East, and part of Asia. No information is currently available on the potential transmissibility of camel prion disease to humans.

LABORATORY DIAGNOSIS OF PRION DISEASE

In most cases, diagnosing human prion disease is approached by first excluding some more common conditions that might mimic prion disease, and then, if prion disease remains a diagnostic possibility, pursuing specific testing for these conditions. Typically, when prion disease is considered, the patient presents with a rapidly progressive dementia often with additional signs such as ataxia, parkinsonism, myoclonus, and spasticity. (See descriptions of the clinical presentations of these signs in the previous section, "Human Prion Diseases".) This is a relatively rare presentation, and certain conditions figure prominently in the differential diagnosis (Table 179.4). Many of these conditions are less common than prion disease, but some are treatable, so effort should be devoted to diagnosing them promptly.

Prion Disease Mimics

Suggested initial laboratory tests for a patient with rapidly progressive dementia are listed in Table 179.5. Common blood tests are typically normal in prion disease, so abnormalities might suggest other diagnoses. In prion disease, the CSF protein is usually normal or mildly elevated while cell counts and glucose concentration are usually normal. Rare reports exist of an inflammatory CSF profile in prion disease, but the presence of a significant pleocytosis or hypoglycorrhachia should prompt a search for other diagnostic possibilities.[191] These include infections, CNS neoplasms, and autoimmune encephalitis. In regard to this last possibility, an increasing number of autoantibodies against neural antigens are found to be associated with subacutely progressive neurologic illnesses, many of which share clinical features with prion diseases. A more detailed description of these syndromes is beyond the scope of this chapter, but they have been recently reviewed.[192,193] Typically signs of inflammation (elevated white blood cell counts and protein) are present in CSF, and areas of increased T2 signal may be seen in magnetic resonance imaging (MRI) sequences, but this is not always the case. Medication intoxications and nutritional deficiencies, particularly Wernicke encephalopathy, are encountered surprisingly often as prion disease mimics.

Brain MRI is useful both to suggest prion disease and to look for alternative causes of rapidly progressive dementia. Enhancing lesions and patterns of diffusion-weighted imaging (DWI) abnormality indicating infarction or white matter involvement suggest a diagnosis other than prion disease. The MRI findings in prion disease are discussed under "Brain Imaging" later.

Specific Testing for Prion Disease (RT-QuIC)

If the initial evaluation fails to suggest an alternative diagnosis, specific testing for prion disease should be pursued. Until very recently, the only highly reliable diagnosis of sporadic human prion disease was the demonstration of PrP^{Sc} in brain tissue obtained by biopsy or autopsy. Now, however, very specific and, as the technology develops, increasingly sensitive tests can be performed on CSF or other fairly easily obtained specimens to identify prions. These assays look for the ability of the specimen to cause the in vitro aggregation of recombinant PrP.[194] The precise techniques differ and are evolving, but the RT-QuIC method currently available in the United States through the National Prion Disease Pathology Surveillance Center has a 92% sensitivity and about a 99% specificity when performed on CSF.[195] Some forms of prion disease

TABLE 179.4 Differential Diagnosis of Prion Disease

CLASS	EXAMPLES[a]	COMMENT
Neurodegenerative	Alzheimer disease Diffuse Lewy body disease Frontotemporal dementia	The slower pace of progression usually distinguishes these from prion disease, but atypical rapidly progressive forms may occur.
Autoimmune encephalitis	SREAT Anti-NMDA Lupus cerebritis	Many treatable mimics of prion disease fall in this category.
Vascular	Primary angiitis of CNS Lupus-associated vasculitis CADASIL	
Nutritional/Metabolic	Thiamine deficiency Vitamin B_{12} deficiency	
Toxins	Serotonin syndrome Bismuth intoxication	
Neoplasms	Intravascular lymphoma Meningeal carcinomatosis	
Infections	Syphilis Lyme disease HIV	
Psychiatric	Factitious Catatonia	
Endocrine	Hyper- and hypothyroidism	

[a]Lists are not complete.
CADASIL, Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; *CNS,* central nervous system; *HIV,* human immunodeficiency virus; *NMDA, N*-methyl-D-aspartate; *SREAT,* steroid-responsive encephalopathy with antithyroid antibodies.

may not be reliably detected by this method.[158] Fig. 179.3 depicts the RT-QuIC methodology. With the availability of these sensitive and specific minimally invasive tests, older diagnostic criteria for sporadic CJD are obsolete (see Table 179.3).

Supportive Testing for Prion Disease

CSF Proteins

Until recently, measuring elevated levels of certain proteins in CSF was important in diagnosis of sCJD. Such tests still may be useful in suggesting the diagnosis when RT-QuIC assay is negative. The intracellular neuronal protein 14-3-3 is found at elevated levels in the CSF of many patients with CJD. Testing for CSF 14-3-3 protein is available through commercial laboratories, but interpretation of 14-3-3 results is not standardized, so more reliable results may be available through the large central prion disease laboratories of the United States (National Prion Disease Pathology Surveillance Center, at https://case.edu/medicine/pathology/divisions/prion-center/), the United Kingdom, and the European Union. Although early studies reported a very high sensitivity and specificity for an elevated CSF 14-3-3 level, in subsequent practice the specificity has proved considerably lower. The American Academy of Neurology recently issued a guideline, based on a literature review, on the use of 14-3-3 CSF testing in the diagnosis of human prion disease. They reported a sensitivity of 92% and a specificity of 80% for the diagnosis of CJD. The guideline concluded that a negative 14-3-3 may reduce the suspicion of CJD but that a positive test can be found in potentially treatable conditions that mimic CJD.[196]

TABLE 179.5 Initial Evaluation of Rapidly Progressive Dementia

Blood
Complete blood count
Complete metabolic panel
Vitamin B12
Thyroid-stimulating hormone
Erythrocyte sedimentation rate
C-reactive protein
Antinuclear antibody
Syphilis and HIV serology
CSF[a]
Glucose
Protein
immunoglobulin G index
Oligoclonal bands
Cytology
Herpes simplex virus type 1
Imaging
Brain magnetic resonance imaging with and without contrast

[a]If prion disease is considered, instruct the laboratory to hold 2–5 mL frozen at –80°C. If initial evaluation fails to find an alternative diagnosis, this sample should be sent for real-time quaking-induced conversion testing.

Brain Imaging

Brain MRI is the most useful imaging technique in the diagnosis of CJD (Fig. 179.4). DWI is probably the most sensitive MRI sequence for detecting characteristic abnormalities associated with sporadic CJD.[197] In sporadic CJD the most common abnormality seen on standard MRI sequences is increased T2, DWI, or fluid-attenuated inversion recovery (FLAIR) signal in the striatum. Ribbon-like increased signal intensity in the cerebral cortex is also common. Increased signal intensity in the basal ganglia has been reported to have 67% sensitivity and 93% specificity for diagnosis of sCJD. In a study of 193 consecutive cases of suspected CJD, sensitivity of MRI changes ranged from 50% to 70%, depending on the evaluator, whereas specificity was approximately 80%.[198] The neuropathologic substrate for DWI abnormalities has not been characterized fully, although these abnormalities correlate with areas of severe neuropathologic change, including spongiform degeneration.[199] MRI is particularly useful in the diagnosis of vCJD.[200] In a prospective study of 368 MRI scans of patients with clinical histories suspicious for CJD, the presence of the "pulvinar sign" identified 74 of 82 patients eventually proven by neuropathologic studies to have vCJD. The pulvinar sign consists of increased signal in the posterior thalamus, usually seen on

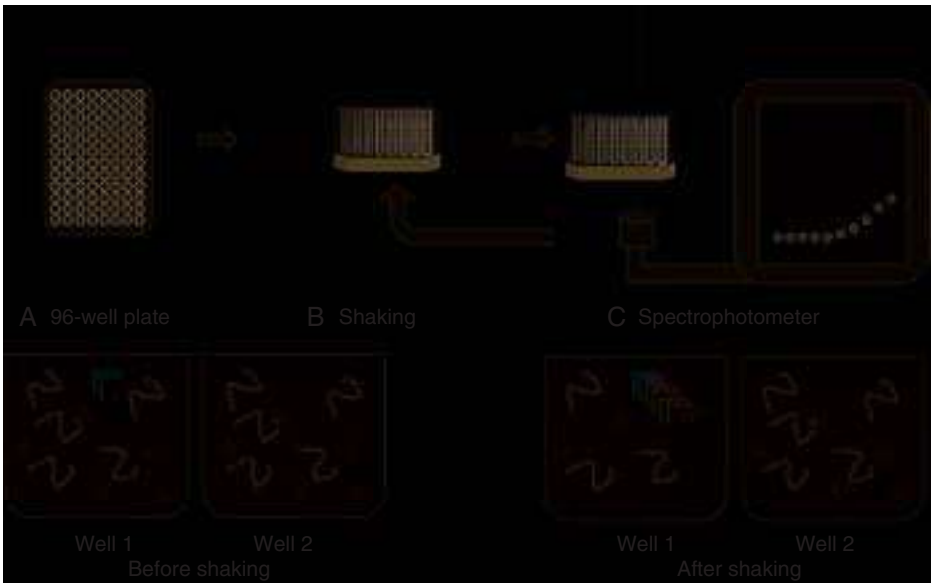

FIG. 179.3 The real-time quaking-induced conversion (RT-QuIC) method. RT-QuIC is an automated technique for amplifying and detecting seeded aggregation of PrP in vitro. The process is performed in 96-well plates, which allow for efficient processing of many samples and the inclusion of controls. The figure depicts the overall process above, and the contents of two hypothetical 96-well plates below. (A) The substrate consists of recombinant PrP *(blue lines)* in a solution containing thioflavin T *(stars)*. This compound undergoes a shift in fluorescence when it binds to amyloid fibrils. A sample to be tested, typically cerebrospinal fluid, is added to the substrate solution. (B) The plate is then shaken at a warm temperature. If PrP^{Sc} *(red)* is in the sample (well 1), recombinant PrP is incorporated to the fibril, and thioflavin T binds, undergoing a shift in fluorescence (depicted as a change to *green stars*). If there is no PrP^{Sc} in the sample (well 2), no fibrils are formed. (C) After shaking, the amount of bound thioflavin T is determined by spectrophotometry. The cycles of shaking and spectrophotometry are repeated, and samples containing PrP^{Sc} show a steady increase in the amount of bound thioflavin T, reflecting the increase in amyloid fibrils. This method has approximately 99% specificity and greater than 90% sensitivity for the diagnosis of sporadic Creutzfeldt-Jakob disease.

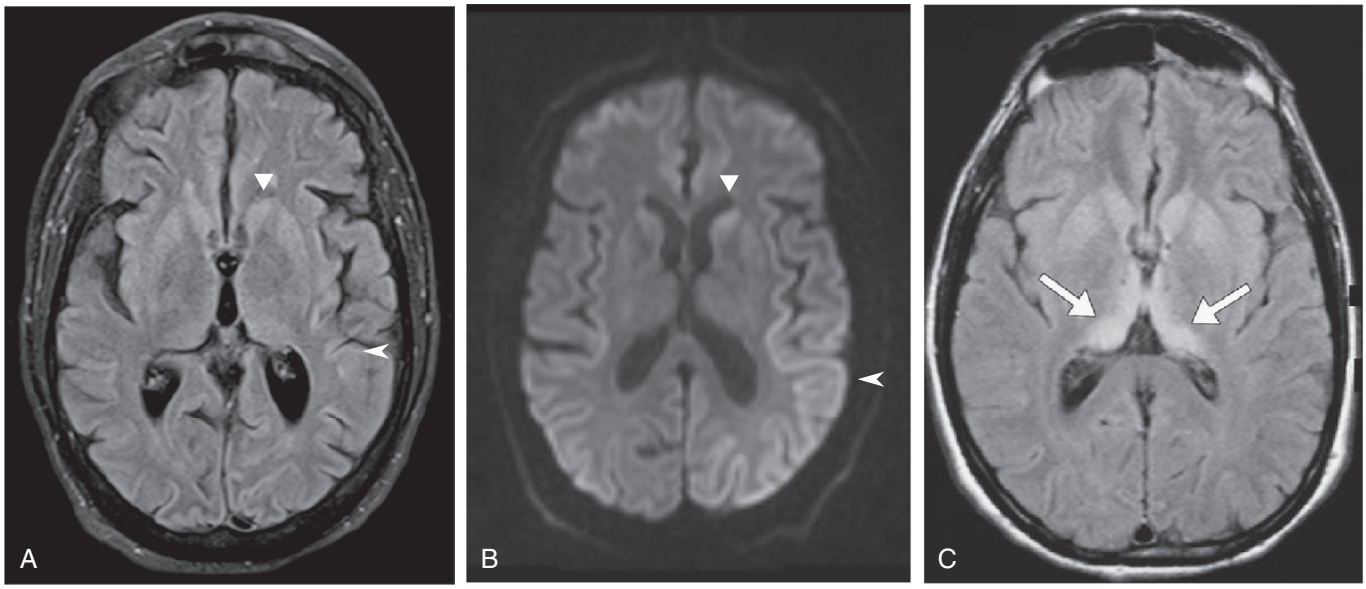

FIG. 179.4 The magnetic resonance imaging appearance of prion disease. (A and B) Fluid-attenuated inversion recovery (FLAIR) image (A) and diffusion-weighted image (DWI) (B) from a 63-year-old man with sporadic Creutzfeldt-Jakob disease (CJD). There is subtle hyperintensity in the cortical ribbon *(arrowheads)* and head of the caudate *(triangles),* seen more clearly on DWI than on FLAIR. (C) FLAIR image from a patient with variant CJD displays hyperintensity in the posterior thalamus *(arrows),* the so-called pulvinar sign that is characteristic of this form of CJD. *(C, From Tschampa HJ, Zerr I, Urbach H. Radiological assessment of Creutzfeldt-Jakob disease.* Eur Radiol. *2007;17:1200–1211.)*

image sequences sensitive to T2 effects (T2, FLAIR, DWI, etc.). Rarely, T1 hyperintensity is recognized in the pulvinar as well. When strictly defined as greater hyperintensity in the posterior thalamus relative to other deep brain nuclei, the pulvinar sign is specific for vCJD. Increased pulvinar and other deep gray matter hyperintensity occurs in some cases of sCJD; however, in these cases the signal in other nuclei, especially the anterior putamen, is greater than in the pulvinar.

Computed tomography is usually not helpful in the diagnosis of CJD, except that it may exclude some other conditions. Studies evaluating other neuroimaging techniques, such as positron emission tomography and single-photon emission computed tomography, are too few to determine the sensitivity or specificity of these tests.

Electroencephalography in Creutzfeldt-Jakob Disease

The EEG formerly played an important role in the diagnosis of CJD. The classic EEG pattern, which ultimately appears in 67% to 95% of patients, consists of a slow background interrupted by generalized, bilaterally synchronous biphasic or triphasic periodic sharp wave complexes (PSWCs).[201,202] These occur at intervals of 0.5 to 2.5 seconds and have a duration of 100 to 600 msec. PSWCs may be absent early in disease and may disappear in the terminal stages. They are more dramatic during periods of alertness, and may disappear during sleep or under the influence of certain drugs. Given the availability now of more specific and sensitive tests, the role of the EEG in the diagnosis of prion disease is uncertain.

Histology of Creutzfeldt-Jakob Disease

Brain histology remains the gold standard for diagnosis of prion diseases. The classic neuropathologic features of neuronal loss, reactive gliosis, and vacuolation of the neuropil (spongiform change), with an absence of inflammatory changes, typically are present in such cases and are consistent with the diagnosis. However, the modern diagnosis of prion disease rests on the immunohistochemical demonstration of abnormal forms of PrP. A variety of immunohistochemical techniques have been developed to detect PrP^{Sc} in paraffin-embedded brain tissue. The general strategy is to hydrolyze PrP^{C} while enhancing the antigenicity of PrP^{Sc} by partially denaturing the aggregates. A widely used protocol includes autoclaving tissue sections in water (hydrolytic autoclaving), followed by treatment with formic acid and then a chaotropic salt (guanidine thiocyanate).[203] With these techniques, a variety of PrP staining patterns have been identified in CJD brain tissue.[204] This tissue may show positive PrP staining limited to plaques or a more diffuse staining pattern that colocalizes with synaptic markers throughout the gray matter, or a combination of both patterns. In the United States, the National Prion Disease Pathology Surveillance Center at Case Western Reserve University in Cleveland, Ohio, assists clinicians and pathologists in analyzing fixed brain material for characteristic histopathology and the presence of PrP^{Sc}, and performs PrP^{Sc} immunohistochemistry and prion isoform analysis on frozen brain tissue from patients with suspected prion diseases. Instructions for shipping material are available at the center's website (https://case.edu/medicine/pathology/divisions/prion-center/).

Tissue Biochemical Tests

If frozen tissue is available, analysis of protease-resistant forms of PrP by Western immunoblot assay is generally performed. Two similar classification schemes correlate the clinical features of sporadic human prion disease with the biochemical properties of the PrP^{Sc} that accumulate in these cases. In these schemes, patients are classified by (1) the size of the protease-resistant fragment of PrP^{Sc} isolated from the brain and (2) the genotype at the polymorphic codon 129 of PrP (MM, MV, or VV). These "molecular" types are then correlated to the patients' clinical syndromes. In the scheme of Parchi and coworkers the protease-resistant fragment is either 21 kDa (type 1) or 19 kDa (type 2) in size.[205] Thus there are six possible molecular types of human prion disease under this scheme (MM1, MM2, MV1, etc.). In the scheme originally proposed by Collinge and coworkers,[146] three PrP^{Sc} size variants are recognized (with type 1 and type 2 being close in size to Parchi and coworkers' type 1). The subtle differences between the schemes may be an artifact.[206] At any rate, the molecular types do correlate to clinical syndromes, with distinct differences in age of onset, duration, and dominant clinical symptoms between the types. For example, under the Parchi scheme, patients with the MM1 molecular type have a mean age of onset of 66 years and a duration of 4 months, whereas those with the rare VV1 type have a mean onset of 40 years and a duration of 15 months. These molecular types likely reflect, in part, the same sorts of biochemical differences in the PrP^{Sc} seen between well-characterized animal prion strains, with an additional, incompletely understood influence from the PrP genotype.

Genetic Testing and Creutzfeldt-Jakob Disease

In a symptomatic patient with a family history of prion disease, finding a *PRNP* gene mutation can be taken as definitive evidence of CJD. A

family history of unusual dementia is absent in as many as 46% of patients with a genetic prion disease.[83] Such cases can be due to new mutations, incomplete family history, incomplete penetrance, or misidentified paternity. Thus it may be worth obtaining the sequence of the *PRNP* coding region in patients with undiagnosed subacute or otherwise unusual dementias. Genetic techniques are highly specific but insensitive when used for diagnosis of isolated cases of prion disease because approximately 90% of prion disease patients will have no *PRNP* mutation.

WHO has published criteria for the clinical diagnosis of CJD (see Table 179.3). The definition of "probable CJD" is obsolete, and needs to be revised to account for the availability now of highly specific and sensitive tests such as RT-QuIC.

PRION DISEASE THERAPY

All prion diseases remain incurable and uniformly fatal; however, the availability of excellent animal models of prion disease has encouraged investigations of a large number of different therapeutic strategies. These strategies include immunotherapies, such as antibodies and immunization against PrP, various small molecule inhibitors of PrP^{Sc} propagation identified by in vitro screening methods, polyanionic and polycationic compounds, and small interfering RNA and other inhibitors of PrP expression.[207,208] Several of these treatments are able to abolish scrapie infection in cultured cells. A number can delay the onset of clinical illness in rodents when administered before inoculation with prions and, in some cases, after inoculation but before the emergence of clinical signs. However, no treatment significantly alters the course of disease after the appearance of obvious clinical signs—the situation that would be most useful for treatment of sporadic human prion disease. Five compounds have been tried in series of humans symptomatic with prion disease.[209,210] Pentosan polysulfate by intraventricular infusion may have slowed disease progression in some patients, but the studies are uncontrolled. Studies of orally administered quinacrine treatment generally suggest no substantial benefit. Amantadine may have transiently improved symptoms in some patients, but it probably provides no clinically important benefit. In a placebo-controlled trial of flupirtine, the drug may have slowed cognitive decline.[211] A mostly observational trial of tetracycline suggested that it might slightly increase survival time.[210]

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The complete reference list is available online at Expert Consult.

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C Chlamydial Diseases

180 *Chlamydia trachomatis* (Trachoma and Urogenital Infections)

Byron E. Batteiger and Ming Tan

SHORT VIEW SUMMARY

Definition

- *Chlamydia trachomatis* is an obligate intracellular bacterium that mainly infects ocular, genitourinary, and rectal epithelium.
- The ocular disease trachoma is the leading infectious cause of blindness worldwide.
- Chlamydial genital infections are the most common bacterial sexually transmitted infections in the world.
- Lymphogranuloma venereum (LGV), caused by distinct serovars of *C. trachomatis,* is a less common disease characterized by enlarged inguinal lymph nodes or severe proctocolitis.

Epidemiology

- Trachoma is common in poor, rural areas of developing countries, with an estimated 21.4 million active cases worldwide in 2011.
- Active infection in trachoma largely affects young children, often those younger than 1 year of age, whereas the scarring sequelae that produce blindness occur later in life.
- The sexually transmitted urogenital infections are common worldwide, with an estimated 131 million new cases in 2012.
- Chlamydial genital infections are most prevalent among adolescent women and men, in whom repeated infections are common.
- Chlamydial genital infections are often asymptomatic but can cause reproductive sequelae in women, including pelvic inflammatory disease (PID), ectopic pregnancy, and involuntary infertility.
- Transmission during vaginal delivery can lead to neonatal conjunctivitis and pneumonia.
- *C. trachomatis* infection is a notifiable disease that is reportable to the Centers for Disease Control and Prevention and all state health departments in the United States.

Pathophysiology

- Disease manifestations are largely mediated by the host response to the infection, which is initiated and sustained by actively infected nonimmune host epithelial cells.
- In a substantial minority of persons with trachoma and genital infections, the host inflammatory response leads to tissue scarring, resulting in end-organ dysfunction and sequelae.

Microbiology

- *C. trachomatis* is a gram-negative bacterium, but Gram stain is not used for identification.
- The organism is cultivated in cell culture because it does not divide and grow axenically.
- This obligate intracellular bacterium replicates within a cytoplasmic inclusion in a host cell.
- The two morphologic forms are the infectious but nondividing elementary body and the reticulate body, which is an intracellular, noninfectious, replicating form.

Clinical Manifestations

- Trachoma is a chronic keratoconjunctivitis that may progress over years in a minority of cases to conjunctival scarring and corneal opacification.
- Active trachoma is frequently asymptomatic but may present as chronic conjunctivitis with redness, discomfort, photophobia, and mucopurulent discharge.
- *C. trachomatis* urogenital infection in men manifests as nongonococcal urethritis with scant, nonpurulent urethral discharge and dysuria.
- *C. trachomatis* urogenital infection in women is mostly asymptomatic, but may present as mild and nonspecific symptoms, such as vaginal discharge and bleeding, mild abdominal pain, or dysuria.
- Classic LGV is a chronic, systemic illness that progresses from a genital lesion to prominent inguinal lymphadenopathy.
- LGV proctocolitis presents with mucopurulent anal discharge and rectal pain, ulceration, and bleeding.

Diagnosis

- Diagnosis of trachoma in endemic areas is typically by physical examination of the eye according to criteria established by the World Health Organization.
- Diagnosis of genital infections requires microbiologic testing, ideally by nucleic acid amplification tests of first-catch urine in men and vaginal swabs in women.

Therapy (See Table 180-3)

- Therapy of trachoma is generally provided by repeated mass treatment of hyperendemic communities with single-dose azithromycin.
- Recommended treatment of uncomplicated urogenital infection is either azithromycin 1 g orally as a single dose or doxycycline 100 mg orally twice daily for 7 days. Treatment of sex partners is crucial to prevent repeated infection.
- Treatment of complicated infections requires longer durations of therapy: for salpingitis, 14 days; for epididymitis, 10 days; and for mild proctitis, 7 days.
- Classic LGV, and severe proctocolitis caused by LGV serovars, are treated with doxycycline for 21 days.

Prevention

- Trachoma can be prevented by face washing, access to clean water, and improvements in sanitation.
- In the United States and other developed countries, prevention of sexually transmitted genital infections and complications is largely focused on screening and treating nonpregnant, sexually active women under 25 years of age on an annual basis. Uptake of screening in the United States is relatively low.
- Screening of all pregnant women is recommended.
- Screening and treatment of women older than 25 years of age is recommended if risk factors are identified, such as new or multiple sexual partners.
- One-time screening and treatment in women decreases risk of symptomatic PID in the following year. Data are currently lacking as to whether screening programs reduce population prevalence of chlamydial genital infection.
- Screening of young men in high-risk settings (sexually transmitted infection and adolescent clinics, correctional facilities) should be considered if resources allow.
- No vaccine is currently available for either trachoma or chlamydial genital infections. Induction of sterilizing immunity appears unlikely, and future vaccine efficacy evaluation may be based on demonstrating reduced risk of inflammatory sequelae in the eye and symptomatic PID in women.

Chlamydia trachomatis imposes a tremendous burden on human health because it is one of the most common causes of bacterial infection. Worldwide in 2011, an estimated 21 million people had active trachoma, which is an ocular *C. trachomatis* infection that is the leading cause of infectious blindness.[1] The World Health Organization (WHO) estimated that in 2016, 1.9 million individuals were blind or had severe visual impairment as a long-term consequence of trachoma.[2] This disease has disappeared from the developed world coincident with improved sanitation and access to clean water. However, an estimated 190 million people live in endemic areas, mainly in poor, rural regions of 41 low-income countries, and require interventions to prevent blinding disease.[2]

C. trachomatis genital tract infection is the most common bacterial sexually transmitted infection (STI) in the world, with an estimated 131 million new cases in 2012.[3,4] Women disproportionately suffer the major complications, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility, and can transmit the bacterium to their newborns, who develop conjunctivitis and pneumonia. In 2016, 1.59 million new cases of *C. trachomatis* infection were reported to the Centers for Disease Control and Prevention (CDC),[5] making it the most commonly reported infectious disease in the United States.[6] Not all *C. trachomatis* infections are diagnosed, however, and the actual incidence is higher. For example, in 2008 the annual incidence of *C. trachomatis* genital infections in the United States was estimated to be 2.86 million cases, which was more than twice the number that were reported.[7] In a population-based assessment, chlamydial prevalence in the United States in the 14- to 39-year-old age group was estimated to be 2.0% for women and 1.4% for men for 2007 to 2012, with stable overall prevalence during this period.[8] Women have had consistently higher rates, which may be due to increased screening and diagnosis compared to men. Higher chlamydial prevalence is reported when screening targeted populations, who tend to be higher-risk groups. For example, in women 15 to 24 years old, chlamydial prevalence ranging from 6.6% to 15.6% has been reported, with the highest prevalence in STI clinics and juvenile detention centers.[5] In the United States, reported case rates continue to rise, largely as a result of increased screening and improved diagnostic tests (Fig. 180.1A), but population prevalence appears stable, with notable disparities by age and race/ethnicity (Fig. 180.1B). Data from 2016 demonstrate that the disease is most common in adolescents and young adults ages 15 to 24 years, with higher rates in women and among African Americans compared with Hispanics/Latinos and non-Hispanic whites.[5] *C. trachomatis* infections thus continue to be a major public health problem.

Human *C. trachomatis* infections are associated with ocular, genitourinary, and respiratory disease syndromes, and although clinically distinct, they share common characteristics. These include the propensity to cause (1) infections that are often long-lasting in the absence of treatment; (2) repeated infections after natural clearance or antibiotic treatment of an initial infection; (3) infections that are often asymptomatic or minimally symptomatic; and (4) infections that produce inflammatory and scarring sequelae in the absence of treatment, often with few or no symptoms until end-organ dysfunction is manifest.[9]

CHLAMYDIAL BIOLOGY

Chlamydiae are obligate intracellular bacteria that replicate within eukaryotic cells.[10] *C. trachomatis,* like all other *Chlamydia* spp., has one of the smallest bacterial genomes. Its 1.04-Mb genome encodes approximately 900 genes, which is less than a fifth of the genes in *Escherichia coli.*[11] The large majority of these genes (668) are shared among all

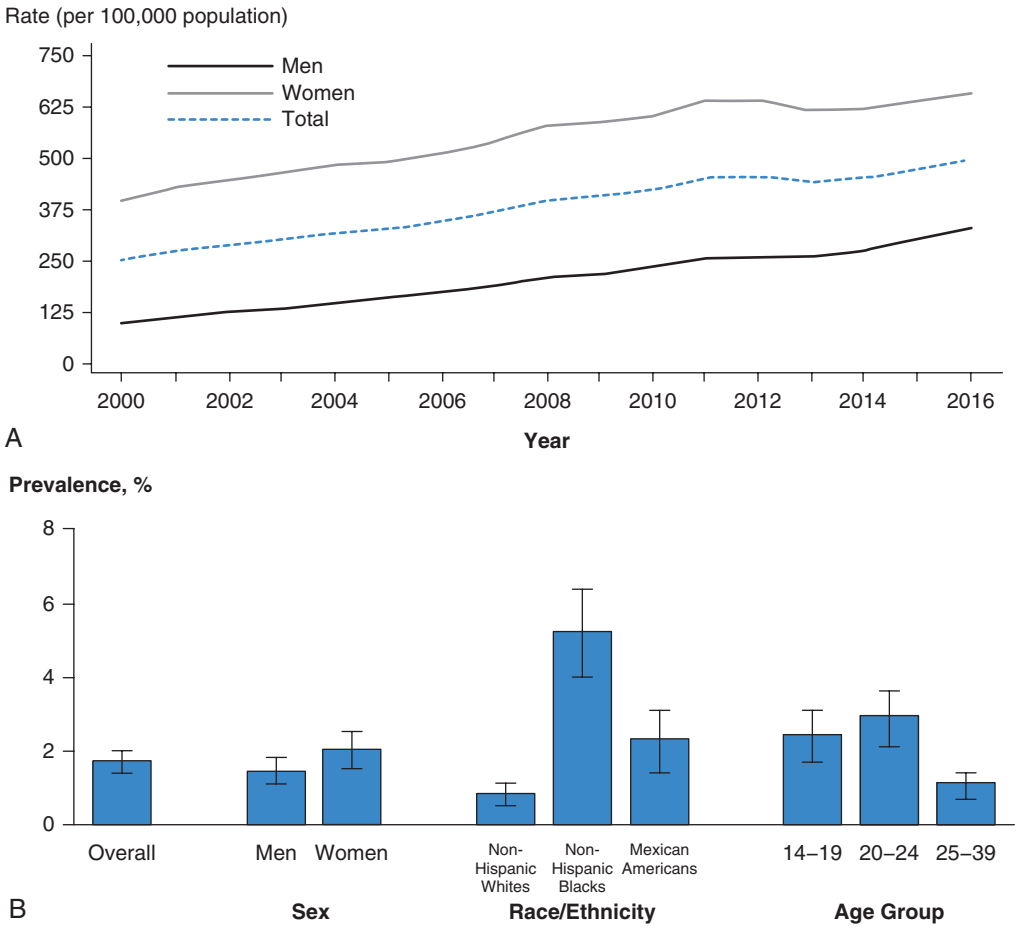

FIG. 180.1 Prevalence of *Chlamydia trachomatis*. (A) Reported case rates of *Chlamydia trachomatis* in the United States. (B) Population prevalences of *Chlamydia trachomatis* in the United States, 2007–2012. Note: Since 2000, all 50 states and the District of Columbia require the reporting of *Chlamydia* cases. *(From Centers for Disease Control and Prevention.* Sexually Transmitted Disease Surveillance, 2016. *Atlanta: US Department of Health and Human Services; 2017; and data from Torrone E, Papp J, Weinstock H; Centers for Disease Control and Prevention. Prevalence of* Chlamydia trachomatis *genital infection among persons aged 14–39 years—United States, 2007–2012.* MMWR Morb Mortal Wkly Rep. *2014;63:834–838.)*

Chlamydia, representing a core set of genes necessary for the intracellular chlamydial lifestyle.[12] The small genome size is due to reductive evolution in which *C. trachomatis* has lost enzymes and metabolic pathways for nutrients that are readily available from the host cell.[13] As a consequence, however, *Chlamydia* is dependent on its host cell to complete its developmental cycle. Other common obligate intracellular bacteria include *Rickettsia,* which also has a small genome, and *Mycobacterium leprae,* which is undergoing active genome reduction.

Chlamydia is unusual among bacteria in having two developmental forms, which are morphologically different and functionally specialized.[10] The reticulate body (RB) is the vegetative form that grows and divides by binary fission.[14] Chlamydiae lack many enzymes and pathways for biosynthesis of amino acids and nucleotides[15] and thus are auxotrophic for many essential metabolites. The RB compensates by having numerous membrane transport systems to acquire nutrients—including amino acids, oligopeptides, nucleotides, ATP, carbohydrates, and metal ions—from the host cell.[15,16] The RB thus resembles a typical bacterium, but it occupies an intracellular niche in which it can obtain many nutrients from its host cell.

The spore-like elementary body (EB) is a unique chlamydial adaptation that is the transmission vehicle to infect another host cell.[10,17] It is environmentally stable, with a rigid envelope of cross-linked membrane proteins,[18–20] and DNA condensed into chromatin by two *Chlamydia*-specific histone-like proteins, Hc1 and Hc2.[21–23] The most abundant of its membrane proteins is the major outer membrane protein (MOMP), which is also the immunodominant antigen.[18] Extracellular EBs have limited metabolic and biosynthetic activity.[24,25] However, EBs contain the machinery for central metabolism and glucose catabolism,[15] and large stores of ATP,[26] which prime it for a burst of metabolic activity upon entry into a host cell.

For many years, it was not clear if chlamydiae have peptidoglycan, which is a major structural component of the bacterial cell wall.[27] It has now been shown that *C. trachomatis* RBs form a narrow peptidoglycan ring at the septum during chlamydial cell division.[28] However, EBs lack peptidoglycan, and the classical mesh-like exoskeleton of peptidoglycan found in most other bacteria is not present in pathogenic *Chlamydia* spp.[28]

All *Chlamydia* spp. share an unusual biphasic developmental cycle in which there is conversion between RBs and EBs inside an infected host cell (Fig. 180.2).[10,14,29] An EB binds and enters a susceptible epithelial cell, within a membrane-bound vacuole called the chlamydial inclusion, and converts into an RB within 2 to 8 hours. RBs divide repeatedly by binary fission and then begin converting into EBs at about 24 hours after entry. The delayed and asynchronous timing of RB-to-EB conversion has been proposed to be controlled by RB size, which progressively decreases by sixfold as the RB population in an infected cell expands via successive rounds of replication.[14] By 48 hours, several hundred to 1000 EBs are released by lysis of the host cell, or by extrusion of the inclusion from an intact host cell.[30]

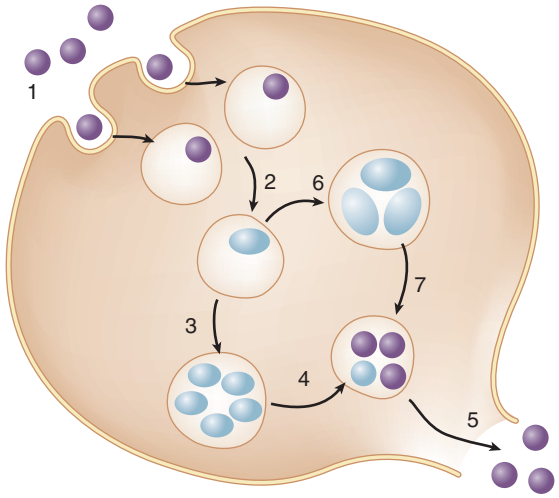

FIG. 180.2 Developmental cycle of *Chlamydia trachomatis* in cell culture. *1,* Infectious elementary bodies (EBs) attach to and are taken up by epithelial cells. *2,* Inclusions fuse and EBs differentiate into reticulate bodies (RBs). *3,* RBs divide by binary fission. *4,* Delayed and asynchronous conversion of RBs into EBs. *5,* EBs release, often with cell death, to infect other cells. *6,* Altered growth state under stressful conditions (e.g., interferon-γ exposure) leading to enlarged aberrant RBs but no EBs. *7,* With removal of stress, reactivation back to normal developmental cycle and production of EBs.

EB attachment to an epithelial host cell occurs through a two-step process.[31] The first step is a low-affinity interaction between an EB surface protein OmcB,[32] and heparan sulfate proteoglycan associated with the host cell surface.[33] The second step is an irreversible, high-affinity interaction between chlamydial ligands, including MOMP and the Pmp family of polymorphic membrane proteins,[34] and specific host receptors.[31] *C. trachomatis* has been reported to bind a number of host receptors, which may contribute to strain-specific differences in tissue tropism, but could also represent a strategy to increase the chance of binding to a host cell.[17]

This bacterium plays an active role in promoting its own entry into a host cell, which allows it to infect nonphagocytic cells.[35–37] Upon contact, the EB induces actin cytoskeletal rearrangements in the host cell by secreting preformed protein effectors via a polarized, type III secretion (T3S) mechanism.[38,39] For example, a chlamydial effector called Tarp (translocated actin-recruiting phosphoprotein) nucleates actin polymerization directly, while also activating the host actin-nucleating machinery,[40–42] and another effector (CT694) binds the actin-binding protein AHNAK.[43] In addition, binding of the EB to receptor tyrosine kinases, such as epidermal growth factor receptor, fibroblast growth factor receptor, and platelet-derived growth factor receptor, activates the MEK-ERK and phosphoinositide 3-kinase signaling pathways, promoting chlamydial entry and host cell survival.[31,44,45]

The inclusion provides a protected intracellular niche within which chlamydiae grow and replicate. Maintenance of this intracellular compartment depends on inhibition of phagolysosomal fusion and selective recruitment of vesicles carrying nutrients such as lipids and iron.[31] *Chlamydia* inserts inclusion membrane proteins into the inclusion membrane, where they interact with host regulators of vesicular trafficking and fusion, including Rab guanosine triphosphatases and SNARE proteins.[36,46,47] There may initially be multiple small inclusions in a host cell, from infection by more than one EB, but they coalesce into a single inclusion through fusion mediated by an inclusion membrane protein called IncA (see Fig. 180.2).[48]

The inclusion has interactions with a number of organelles in the host cell that help promote the intracellular infection.[31] Early in the infection, the inclusion migrates along microtubules from the cell periphery to the microtubule organizing center in the perinuclear region of the host cell.[49] Chlamydiae acquire host lipids from the Golgi apparatus via exocytic vesicles, from the endoplasmic reticulum by recruiting the host lipid biosynthetic machinery to the inclusion, and via lipid-rich organelles called lipid droplets.[31,50] These host lipids are necessary for the enlarging inclusion membrane and may contribute to the chlamydial cell membrane.[50–52] The inclusion also recruits recycling endosomes and mitochondria to facilitate acquisition of iron and ATP, respectively.[31,53]

Chlamydial genes are expressed as three major temporal groups that correspond to three main stages of the developmental cycle.[54–56] Early genes are first transcribed during EB-to-RB conversion and have a likely role in establishing the chlamydial inclusion. The largest temporal group consists of midcycle genes, which include most housekeeping genes as well as virulence genes that are expressed during RB replication. Data indicate that midcycle genes and a subset of early genes are activated by higher levels of negative DNA supercoiling in midcycle.[57,58] This novel use of its DNA topology to regulate developmental gene expression makes *Chlamydia* dependent on enzymes such as DNA gyrase that modulate supercoiling levels.[59] It also provides a *Chlamydia*-specific target for the fluoroquinolones, including ofloxacin and levofloxacin, which are DNA gyrase inhibitors used to treat chlamydial infections. Late genes, which are the third and last temporal group, have roles in RB-to-EB conversion and EB function. They are negatively regulated by the transcription factor EUO, providing a mechanism to prevent their premature expression.[60,61]

Chlamydia actively promotes the survival of its host cell so that it can complete its developmental cycle.[31,62] At the time of entry, binding of the EB to receptor tyrosine kinases and ephrin receptors activates prosurvival pathways involving MEK-ERK and phosphoinositide 3-kinase.[31] In addition, chlamydiae block intrinsic apoptosis by downregulating and sequestering proapoptotic factors, and by upregulating and stabilizing antiapoptotic factors.[62] *Chlamydia* also inhibits extrinsic apoptosis by blocking caspase activation.[63]

Chlamydiae grown in cell culture can enter an altered growth state of viable but noncultivable bacteria that is called in vitro persistence.[64,65] Infectious progeny cannot be recovered because there is no RB-to-EB conversion. Persistence can be induced by exposing *Chlamydia*-infected cells to interferon (IFN)-γ, which induces host indoleamine 2,3,-dioxygenase to deplete tryptophan.[66] It can also be induced by penicillin treatment, iron or nutrient starvation, herpesvirus coinfection, or host cell differentiation.[65] In vitro persistence is reversible because removal of the inducing agent causes reactivation back to the normal developmental cycle and production of EBs.[67] The relevance of in vitro persistence to clinical infection and disease is not clear.[65]

Chlamydia causes centrosomal and DNA defects that may explain its association with cervical cancer.[68] *Chlamydia* alters centrosomal function by inducing the formation of extra centrosomes, centrosome clustering, and mitotic spindle defects, each of which may lead to chromosome segregation errors.[69–71] *Chlamydia* causes DNA damage in an infected cell by inducing DNA double-strand breaks and impairing the DNA damage response.[72] In addition, *Chlamydia* can transform infected cells in cell culture and cause cervical dysplasia in mice.[73]

ANTIGENIC AND GENETIC DIVERSITY

C. trachomatis has been historically classified into 15 serovars on the basis of antigenic cross-reactivity in the microimmunofluorescence test of Wang and Grayston.[74] The serovars can be grouped into two biovariants that are associated with different clinical presentations. The trachoma biovar consists of serovars A, B, Ba, and C, which cause trachoma, and serovars D through K, which cause urogenital disease and inclusion conjunctivitis. The lymphogranuloma venereum (LGV) biovar includes serovars L1, L2, and L3, which cause LGV and proctocolitis in men who have sex with men (MSM). Occasionally, serovars B and Ba have been isolated from the genital tract, but A and C have not. Alternatively, serovars can be divided into two subgroups on the basis of cross-reactivity patterns: the B complex consists of serovars B, Ba, D, E, L1, and L2, and the C complex is composed of serovars A, C, H, I, J, K, and L3. Serovars F and G bridge the two complexes, although they are more closely related to the B complex.[74] Additional serovars have been proposed (Da, Ia, and L2a),[75] as well as serovars such as L2b that have caused a recent outbreak of LGV proctitis.[76] When full-length sequencing of *ompA* is used for strain identification, additional stable variants have been described.[77]

Species-, subspecies-, and serovar-specific antibodies recognize epitopes located in four variable sequence regions of MOMP.[78] Serovar-specific epitopes are mostly found in variable sequence regions 1 and 2, whereas the more broadly shared epitopes cluster in variable region 4.[78] However, some serovars contain more than one serovar-specific epitope, and serovar-specific epitopes are found in variable region 4 as well.[79] The immunodominant genus-reactive epitope is in lipopolysaccharide (LPS), which is closely related to LPS of other gram-negative bacteria, particularly the deep rough (Re) mutants of enterobacteria.[80] Chlamydial LPS is technically a lipooligosaccharide composed of a 3-deoxy-D-manno-octulosonic acid trisaccharide in a 2,4 linkage and a *Chlamydia*-specific 2,8 linkage.[81] Additional genus-reactive epitopes have been identified in MOMP,[82] the 60-kDa cysteine-rich protein (OmcB),[82] and the 60-kDa heat shock protein..[83]

With the advent of affordable whole-genome sequencing, chlamydial strains are increasingly being typed by genetic differences on a genomic scale.[84,85] Genomic comparison has confirmed that *C. trachomatis* can be divided into three lineages, with the urogenital and ocular clades more closely related to each other than to the LGV clade.[85] There is strong conservation of gene content and order between *C. trachomatis* strains, with only 4860 single nucleotide polymorphisms, out of just over 1 million bases, between the trachoma and LGV biovars.[86] This overall genetic similarity has led to a search for specific genes that contribute to differences in tropism and clinical presentation. A hot spot of genetic diversity called the plasticity zone is located at the replication terminus of the chlamydial genome, which is a site of large-scale rearrangements in many bacterial genomes.[12,87] Between *Chlamydia* spp., there is considerable variation in the size of the plasticity zone, from 18 kb to 81 kb, and the presence or absence of genes encoding virulence factors, such as membrane attack complex/perforin,[88] phospholipase D,[89] cytotoxin,[90] and tryptophan synthetase.[91,92] Notably, only *C. trachomatis* serovars that cause urogenital disease, and not trachoma strains, encode a functional tryptophan synthase.[91,92] Urogenital strains may have retained this enzyme to convert indole, produced by vaginal microbiota, into tryptophan as a defense mechanism to counter IFN-γ–mediated tryptophan depletion.[93,94] Another difference is that the enzymatic portion of the cytotoxin gene is only present in urogenital, but not ocular or LGV, strains.[95] A family of autotransporters, called the polymorphic membrane proteins, has a high concentration of single nucleotide polymorphisms, which appear to correlate with tissue tropism.[96,97] Genetic differences have also been identified between urogenital and rectal clinical isolates of *C. trachomatis*.[98] This identification of strain-specific differences will be made easier by new methods for whole-genome sequencing of chlamydial clinical isolates without cultivation.[99,100]

Recombination between *C. trachomatis* strains is another source of genetic diversity.[86,101] It occurs in nature, most commonly between strains that infect the same tissue site, providing evidence for mixed infections with more than one *C. trachomatis* strain.[102] In addition recombination has been documented between urogenital and ocular strains, and even between trachoma and LGV biovars.[102] As a dramatic example, a hypervirulent *C. trachomatis* LGV strain isolated from a patient with severe hemorrhagic proctitis was found to be a recombinant of an invasive L2 strain and a noninvasive urogenital D strain.[103] Nevertheless, the high degree of genomic conservation among *C. trachomatis* strains suggests that most recombination events are not fixed as gene gain or loss. Recombination events have been fixed at a higher rate near the MOMP gene (*ompA*), most likely because of selective pressure from the host immune system. *ompA* has transferred between phylogenetically unrelated strains on multiple occasions, leading to serovar switching and chimerism.[86,102] These findings explain why MOMP is not a good phylogenetic marker[104] and why MOMP strain typing does not correlate well with clinical manifestations.[105–107]

Experimental methods to genetically manipulate chlamydiae have recently become available.[108,109] These methods include transformation of chlamydiae,[110–112] generation of mutants by chemical mutagenesis,[113] and, in a few instances, targeted gene disruption and replacement.[114,115] Chlamydiae appear to be naturally competent because recombinant progeny can be readily obtained by coinfecting the host cell with two chlamydial strains.[116] The phenotypes of mutants can be studied with linkage analysis, conditional mutants, and complementation.[117,118] These approaches have been aided by the use of whole-genome sequencing to identify genetic alterations that can then be related to phenotypes in cell culture and infection models.[119] These methodologic breakthroughs provide the potential for forward and reverse genetic approaches to study chlamydial gene function. However, their widespread use has been limited by the low efficiency of EB transformation and the time (in months) to produce and isolate mutants. The downsized *C. trachomatis* genome is expected to contain a high proportion of essential genes that cannot be deleted.

PATHOGENESIS

The target cells of the trachoma biovar of *C. trachomatis* are the squamocolumnar epithelial cells of the endocervix and upper genital tract in women, the epididymis and perhaps the prostate in men, and the conjunctiva, urethra, rectum, and to a lesser extent the pharynx in both women and men.[120–123] In infants, the columnar epithelial cells of the respiratory tract are also commonly infected.[120] *C. trachomatis* strains exhibit strong tissue tropism during natural infection, thereby producing very different clinical presentations; genital serovars preferentially infect genital tract epithelial cells, ocular serovars favor conjunctival epithelial cells, and LGV serovars infect macrophages and spread to lymph nodes.[124]

Regardless of the site, the initial response to infection appears to be primarily a polymorphonuclear leukocyte response.[125–127] Infected epithelial cells produce proinflammatory cytokines, such as the chemokine interleukin (IL)-8, that direct the initial innate and acquired responses to chlamydial infection and drive inflammatory responses that both produce disease and resolve the infection.[128–131] LPS may be the predominant chlamydial antigen capable of inducing proinflammatory cytokines.[132] In immortalized human cells, IL-8 induction depends on host cell signaling,[133] in part via the MEK-ERK pathway.[134] Independently, IL-8 is also induced via the pattern response receptor nucleotide-binding oligomerization domain 1, which is a peptidoglycan sensor.[135] *C. trachomatis* pathogenesis is often studied with the related species *Chlamydia muridarum,* which offers a convenient mouse model of human genital infection.[136,137] *C. muridarum* infection of mouse oviduct epithelial cell lines induces a variety of cytokines, including IL-1α, IL-6, tumor necrosis factor-α, and cytokines that augment IFN-γ production, including type I interferons IFN-α/β and IL-12p70.[131,138] In this mouse model of chlamydial genital infection, Toll-like receptor 2 (TLR2) is the principal pattern response receptor responsible for induction of acute-phase mediators and chronic inflammatory pathology.[139,140] Chlamydiae can also be detected by the intracellular nucleotide sensors cyclic GMP-AMP synthase (cGAS) and STING (stimulator of interferon genes), which induce expression of type I interferons.[141,142] Mice deficient in receptors for type I interferons (α and β) have reduced duration and shedding of organisms during infection, less oviduct pathology, and enhanced CD4 recruitment to cervical tissue compared with wild-type mice, suggesting that induction of type I interferons exacerbates infection by inhibiting the specific CD4 response to chlamydiae.[143]

The initial neutrophilic infiltration is followed by tissue infiltration with lymphocytes, macrophages, plasma cells, and eosinophils.[120] In ocular and genital infections, plasma cells are generally present in large numbers,[144,145] whereas in infant pneumonia, eosinophils and neutrophils predominate.[126] In ocular and genital disease with the trachoma biovar, lymphoid follicles (aggregates of lymphocytes and macrophages in the submucosa) form as the acute inflammation begins to subside. There is thinning or loss of epithelium overlying the follicles, and they may become necrotic as the disease progresses.[120] In the conjunctiva, these follicles are clinically apparent as raised avascular lesions. Epithelial proliferation leads to formation of papillae and papillary hypertrophy. As the infection then begins to resolve, fibrosis and scarring occur.

Initial infection of the eye in humans[120] and of the eye[145] and genital tract in monkeys[146] resolve with little or no residual tissue damage. However, in the human eye[147] and the monkey genital tract,[148] as few as two to three repeated infections produce an accelerated and more intense inflammatory response with scarring and tissue damage; multiple rechallenges amplify the effect in the primate eye.[149] The potentially important role of reinfection in chlamydial disease was first recognized during human trachoma vaccine trials in which volunteers were immunized and then subsequently challenged with live organisms.[150] In these and other studies in humans and monkeys, limited serovar-specific protection against infection could be induced. However, when infection did occur after immunization in humans, it was more severe than in unvaccinated people and the increased severity was not serovar specific.

Studies have raised questions about the relative contributions of conjunctival infection by *C. trachomatis* and other bacterial pathogens to trachoma development.[151] It was previously thought that a chlamydial component, the chlamydial 60-kDa heat shock protein (cHSP60), was driving disease expression by hypersensitivity mechanisms. Although serologic data show an association between antibody response to cHSP60 and risk of PID[152] and scarring trachoma,[153] these responses are likely due to complicated or severe disease and are not reflective of a causal link. In early follicular and intense trachoma, the inflammatory response and early disease expression are driven and sustained by *Chlamydia*-infected nonimmune epithelial cells. However, trachomatous scarring in adults shows a greater association with other bacteria than with *C. trachomatis* infection.[151] It has therefore been proposed that *C. trachomatis* plays an initiating role but that chronic conjunctival inflammation and tissue damage may be due to the host immune response to other bacterial pathogens after the *C. trachomatis* infection.

Chlamydial infection induces production of IFN-γ,[154] which inhibits chlamydial replication[155] and in animal models shortens the duration of infection.[156] In cell culture, it induces a dose-related persistent infection as described in "Chlamydial Biology" earlier. However, subsequent removal of IFN-γ allows the organisms to be rescued and to resume replication and conversion into infectious EBs. Long-lasting infections are known to occur relatively frequently in the absence of treatment in women[157,158] and in men.[159] The in vivo bacterial and inclusion morphology of these long-lasting infections in people is unknown. However, it is possible that cyclic changes in inhibitory cytokines, chlamydial replication, and antigen production and the resulting continued inflammatory signaling from nonimmune epithelial cells could explain the chronic inflammation and scarring often associated with chlamydial infections.[160]

The LGV biovar of *C. trachomatis* is distinctive in being able to cause a systemic infection. This organism gains entrance through breaks in the skin or infects epithelial cells of the mucous membranes of the genital tract or rectum. It has tropism for the lymphatic system and is carried by lymphatic drainage to the regional lymph nodes, where it multiplies inside mononuclear phagocytes.[120] Bacteremic spread may also occur, and the central nervous system may be infected. The characteristic histopathology is that of granuloma formation with development of small abscesses that may become necrotic or coalesce into suppurative foci.[161,162]

Almost all strains of *C. trachomatis* carry a conserved 7.5-kb plasmid that has a role in pathogenesis.[163] It encodes eight proteins, including Pgp3, which is an effector that is secreted into the host cytosol, and an immunodominant antigen that is being used as a serologic marker.[164,165] Another plasmid gene, *pgp4,* regulates transcription of chromosomal genes involved in virulence and glycogen synthesis.[166] Rare *C. trachomatis* clinical isolates lacking this plasmid have been described.[167] Studies with chlamydial strains experimentally cured of the plasmid show that the plasmid modulates chlamydial exit from host cells, infectivity, and virulence.[163,168,169] For example, a plasmid-cured *C. muridarum* strain was able to infect the lower genital tract but did not produce upper tract disease in the mouse oviduct because it failed to activate TLR2-dependent cytokine production.[170] These results suggest that the plasmid controls expression of the chlamydial TLR2 ligand, which has not yet been identified. Plasmid exchange between different *C. trachomatis* strains only occurs infrequently.[102]

There is evidence that chlamydial infections may involve a mixed population of genetic variants. These variants may differ in pathogenicity, such as their ability to cause lower versus upper genital tract disease.[171] Experimental work with animal models indicates that these variants can compete or cooperate, and that mixed infections may differ from monoinfection with a single variant.[171]

IMMUNITY

Natural infection with *C. trachomatis* appears to confer little protection against reinfection, and this limited protection is short-lived.[172] Multiple or long-lasting infections are an essential factor in the pathogenesis of trachoma.[173] Rates of repeated infection are also high in young, sexually active individuals with genital tract infections: 29% of men and women attending an STI clinic during a 3½ -year period,[174] as well as 38.4% of adolescent women observed prospectively for up to 2 years, had repeated infections.[175] However, other data suggest that genital tract infections confer at least partial immunity against reinfection. In women with endocervical infection, the presence of secretory immunoglobulin (Ig) A correlated inversely with the numbers of organisms shed.[176] Men experiencing their first episode of nongonococcal urethritis (NGU) are more likely to have *C. trachomatis* recovered from their urethra than men with a prior history of NGU.[177] A study of commercial sex workers demonstrated reduced chlamydial detection with increasing duration of sex work.[178] In another study of female sex workers in Nairobi, protective immunity against incident infection was observed and correlated with cHSP60-specific IFN-γ production in peripheral blood mononuclear cells.[179] In the trachoma vaccine trials mentioned previously, partial serovar-specific immunity could be elicited, but protection lasted for only 1 or 2 years.[173] Repeated genital infections in adolescent women are common after effective treatment,[77] and it has even been suggested

that treatment may inhibit the development of protective immunity.[180] In support of this intriguing hypothesis, women who spontaneously resolved an uncomplicated chlamydial infection before they could be treated were less likely to have reinfection at 1 to 12 months than women whose infections were ended by treatment.[181]

In mouse models of *C. muridarum* infection, CD4 lymphocytes of the Th1 type that traffic to the genital mucosa are crucial for restriction of intracellular growth and resolution of infection.[182] Antibodies directed at epitopes on MOMP are neutralizing in cell culture and may play a role in reducing acquisition of infection.[183] In the mouse genital infection model, antibodies against MOMP or LPS partially protect against reinfections but not primary infections, indicating that the protective effect is dependent on CD4-mediated adaptive immunity acquired at the initial infection.[184] Antibodies may also influence the severity of upper genital tract pathology in the mouse.[185] IgA antibodies are not absolutely required for protective immunity in this model.[186] Both antibody and cell-mediated mechanisms are important in protective immunity in the guinea pig model.[187] It is possible that antigen presentation in natural mucosal infection may be relatively ineffective in producing strong protective immunity, because dendritic cells pulsed in vitro with inactivated chlamydiae are capable of conferring protective immunity in the mouse model.[188] The current consensus is that $CD4^+$ T cells and B cells are most critical in mediating recall immunity to *C. trachomatis* infection and $CD8^+$ T cells are less important.[189] The latter may exert antichlamydial activity by production of IFN-γ rather than by cytotoxic activity. In *C. muridarum* genital tract infections in the mouse, two independent subsets of *Chlamydia*-specific CD4 T cells exhibit different effector functions, one dependent on inducible nitric oxide synthase (iNOS) and the other requiring expression of *Plac8.* If *Plac8*-deficient mice are treated with an iNOS inhibitor, the mice are unable to resolve genital tract infections.[190]

The natural history of human infection is not well defined, but we know that many infections resolve without treatment, while many are long-lasting. In one of the few studies of its kind, Molano and colleagues[158] found that 46% of untreated women had persistent infection with identical strains at 1 year. These infections were likely to represent long-lasting infection rather than reinfection because most of these women were older than 30 years of age, and 83% reported a single lifetime sexual partner. It is not clear how *C. trachomatis* is able to avoid immune clearance for prolonged intervals or why an effective response is so slow to develop.[191] One hypothesis is that *C. trachomatis* may utilize nutrients produced by the genitourinary microbiota to evade inhibitory effects of IFN-γ, which restricts chlamydial replication by inducing tryptophan starvation. *C. trachomatis* is auxotrophic for tryptophan, but urogenital serovars can use indole to synthesize tryptophan. The microbiomes of the vagina in women and the urethra in men sometimes contain *Prevotella* species and other bacteria that produce indole.[192,193] In vitro studies indicate that vaginal secretions, and bacterial supernatants containing indole, mitigate the inhibitory effect of IFN-γ on chlamydial growth.[93,94] Additional chlamydial genes not involved in the tryptophan biosynthetic pathway appear to contribute to IFN-γ resistance.[194]

Vaccine development efforts over the past 20 years have been directed at defining relevant epitopes for use as components in a synthetic or genetically engineered vaccine.[195,196] This subunit approach is important because of the combination of protective and deleterious effects produced by infection or vaccination with the whole organism.[129] Studies of DNA vaccines utilizing the *ompA* gene of *C. muridarum* showed reduced organism burden and mortality in a mouse pneumonia model,[197] but similar studies have not demonstrated an influence on the course of experimental genital infection in mice.[198] Vaccines composed of native conformations of trimeric MOMP of *C. muridarum* compounded with human vaccine adjuvants have been shown to induce partial protection in genital tract challenge in mice.[199] Chlamydial protease–like activity factor (CPAF) is a possible vaccine candidate because it is an immunodominant antigen, and mice immunized with recombinant CPAF, together with IL-12 or CpG, are protected against genital challenge.[200] Promising recent data have established that strains of *C. trachomatis* and *C. muridarum* lacking the 7.5-kb plasmid are attenuated for pathology in the primate eye[201] and in the mouse oviduct[202] even in the absence of sterilizing immunity. Such plasmidless strains hold promise as live-attenuated vaccines against both trachoma and complications of genital infection, including PID.[201,203]

Efforts are also underway to improve vaccine response by developing mucosal vaccines. For example, a vaccine composed of ultraviolet light–inactivated *C. trachomatis* conjugated to synthetic adjuvant nanoparticles induced a robust systemic memory T-cell response and long-lived protection from genital infection in mice immunized via the intrauterine or intranasal route.[204]

LABORATORY DIAGNOSIS

Among *C. trachomatis* infections, only trachoma can be diagnosed on clinical grounds alone, given the proper epidemiologic setting in a high-prevalence area.[205] Other chlamydial infections are often associated with specific clinical syndromes but require laboratory confirmation for definitive diagnosis.[206,207] Detection of *C. trachomatis* indicates the presence of an infection because the organism is intrinsically invasive as an intracellular pathogen and there is no extracellular colonization state. The mainstay of modern diagnostic testing of *C. trachomatis* is the nucleic acid amplification test (NAAT). Other laboratory methods for detection of chlamydial antigens and nucleic acids have been largely superseded as routine diagnostic tests. Microbiologic identification by isolation of *C. trachomatis* in cell culture remains important but in a specialized role.

Nucleic Acid Amplification Tests

NAATs are the preferred diagnostic and screening test for *C. trachomatis* genital infection in the United States because they are sensitive and can be used for noninvasive testing without the need for a pelvic examination or a urethral swab.[208–210] The optimal specimens are a vaginal swab in women,[211,212] which can be obtained by either a clinician or the patient,[213] and first-catch urine in men.[214] Some NAATs have been cleared by the US Food and Drug Administration (FDA) for use on liquid-based cytology specimens collected for Papanicolaou smears.[215] NAATs target and amplify conserved nucleic acid sequences that are present in almost all clinical strains of *C. trachomatis,* including the urogenital, LGV, and ocular serovars.[210] However, NAATs are only approved by the FDA for diagnosis of *C. trachomatis* urogenital infections and have not been cleared to detect the organism in extragenital sites, such as the rectum, oropharynx, and eye.[209] Some laboratories have performed in-house validations for NAAT testing of extragenital specimens in order to conform to Clinical Laboratory Improvement Amendments (CLIA) regulations. Some NAATs can also detect *Neisseria gonorrhoeae,* providing a convenient means to test for chlamydial infection and gonorrhea in the same clinical specimen.[210]

NAATs are considerably more sensitive and nearly as specific as culture, which was the historical gold standard for identifying *C. trachomatis.*[206,207] The high sensitivity is due to the amplification step, which allows detection of chlamydial nucleic acids from a single infected host cell. NAATs are 15% to 20% more sensitive than culture performed in an experienced laboratory and may be up to 40% to 50% more sensitive than culture or enzyme immunoassay tests in other settings (Table 180.1). NAATs can be used to detect *C. trachomatis* in traditional urogenital specimens, including endocervical swabs in women and urethral swabs in men, which require an invasive procedure for collection. However, a major advantage is that NAATs have a comparable high sensitivity for noninvasive specimens, such as a vaginal swab or first-catch urine.[211,212,214] Thus noninvasive testing of chlamydial infections is now possible, allowing innovations such as self-collected vaginal swabs that make widespread screening and prevention programs feasible.[213] NAATs have also been shown to be sensitive and specific for detection of *C. trachomatis* infection of the eyes in patients with trachoma[216] and in the rectum and oropharynx in MSM.[217,218]

Although NAATs are sensitive, a number of disadvantages have been discussed.[219] They are expensive and thus may not be affordable by health departments for comprehensive screening. In addition, they may be technically demanding for some routine laboratory settings, resulting in reproducibility issues and false-positive and false-negative rates that are higher than the reported rates. Another disadvantage is that the current FDA-approved NAATs do not distinguish between LGV and

TABLE 180.1 Comparative Performances of Selected Diagnostic Tests in the Detection of *Chlamydia trachomatis*

TEST	SENSITIVITY RELATIVE TO EXPANDED GOLD STANDARD (%)[a]	SPECIFICITY (%)	DETECTABILITY LEVEL (ELEMENTARY BODIES)
Enzyme immunoassay	40–60	99.5[b]	1000–10,000
Nonamplified genetic probe	40–65	99.0	1000–10,000
Direct fluorescent antibody	50–80	99.8	50–1000
Cell culture	50–90	99.9	10–100
Nucleic acid amplification tests	Cervix: 81–100 Vagina: 91–98 Male urine: 90–96	99.7	1–10

[a]Defined using a combination of different test methodologies, including culture, direct fluorescent antibody, and polymerase chain reaction (PCR) or ligase chain reaction (LCR) directed against a target sequence distinct from that used in the routine PCR or LCR assays.
[b]Specificity using confirmatory assays.

non-LGV strains, which is important because the duration of treatment is longer for LGV infections. Nonapproved NAATs that can distinguish between LGV and non-LGV strains have been described but are not widely available.[220] Another issue is that NAATs detect chlamydial DNA or RNA rather than live organisms, and positive NAATs are not uncommon 3 weeks after completion of antibiotic therapy.[221] Thus the NAAT should not be used as a test-of-cure assay, except in pregnant women in whom it is justified to document cure at 3 to 4 weeks after completion of therapy in efforts to prevent infection in the infant.[209]

Commercially available NAATs differ in the method used to amplify chlamydial nucleic acids from a clinical sample.[210] Although the tests also amplify different DNA or RNA sequences, these targets are not intrinsic to the amplification method and can be altered if necessary. Transcription-mediated amplification is sensitive because it amplifies *C. trachomatis*–specific 23S ribosomal RNA, which is present in high copy number. The DNA strand displacement assay amplifies sequences on the chlamydial plasmid. It has excellent sensitivity because the chlamydial plasmid is present in all clinical isolates, with rare exceptions, and there are approximately four to eight copies of the plasmid in each organism.[222] Polymerase chain reaction (PCR) is another commonly used amplification method that amplifies different sequences on the chlamydial plasmid or chromosome. Ligase chain reaction was a chlamydial NAAT method that was used for many of the initial validation studies, but it is no longer available commercially.[223]

In 2006, a new variant strain of *C. trachomatis* that was not detectable with a PCR-based NAAT was discovered in Sweden.[224] This strain has a 377-bp deletion in the region of the chlamydial plasmid that was amplified by two different PCR-based NAATs (AMPLICOR [CT/NG] Test [Roche Diagnostics, Indianapolis, IN] and *m*2000 RealTi*m*e [Abbott Laboratories, Abbott Park, IL]), resulting in false-negative tests. Its discovery was prompted by an unexpected decrease in chlamydial prevalence in Sweden due to underreporting from counties that used these two NAATs compared with other counties that used a strand displacement NAAT assay that targeted a different region on the plasmid.[225] Once recognized, the strain was found to be highly prevalent in counties where it was not being detected, demonstrating that missed diagnosis and treatment of infected individuals can confer a diagnostic selective advantage that promotes the spread of a particular strain. It also provides an important lesson about the limitation of detecting a pathogen by only targeting a single small region of DNA or RNA. In addition, the plasmid may not be an ideal NAAT target because other deletion and recombination events within the plasmid have been reported.[102] Improved versions of the Roche and Abbott PCR-based NAATs targeting two regions of the *C. trachomatis* genome are now available. However, there are still other FDA-approved *Chlamydia* NAATs that only target a single sequence on the *C. trachomatis* plasmid or chromosome.[210] The Swedish new variant strain of *C. trachomatis* has been largely restricted to the Nordic countries, where its prevalence has decreased with the use of NAATs that detect both wild-type and variant strains.[226] So far, this clonal strain has not been detected in the United States.[227]

Antigen Detection and Nucleic Acid Hybridization

Before the introduction of NAATs, a number of molecular tests were developed to detect chlamydial antigens or nucleic acids in clinical samples without the need to culture the organism.[207] The three main nonculture tests are direct fluorescent antibody (DFA) staining and enzyme-linked immunoassay (EIA), which both detect chlamydial antigen, and nucleic acid hybridization, which uses a DNA probe to detect chlamydial ribosomal RNA without an amplification step. These tests have sensitivities of 60% to 80% and specificities of 99% at best, which provide acceptable positive and negative predictive values, except in low-prevalence populations (<5% infected) where the false-positive rate is high. However, this level of sensitivity can only be achieved with invasive specimens, such as a cervical swab in women or a urethral swab in men. In general, the sensitivity is higher in patients who are symptomatic and shedding large numbers of chlamydiae compared with asymptomatic patients shedding fewer organisms.[228,229] The *C. trachomatis* nonculture tests have been largely replaced because of the superior performance of NAATs even when performed on noninvasive specimens.

Isolation in Cell Culture

Culture has been supplanted by NAATs as the routine method for detecting *C. trachomatis* in clinical samples because it has lower sensitivity and is more difficult to perform. Even under optimal conditions, the sensitivity of culture is estimated at between 70% and 80% on the basis of the comparison to the more sensitive NAAT and may be as low as 40% to 50% outside experienced laboratories.[206,207] Many of the relative disadvantages of culture stem from the need to grow the organism in cell culture, which requires expertise, proper handling and storage of specimens, and a long turnaround time.[230] *C. trachomatis* is most commonly cultivated in HeLa or McCoy cells, but the bacterium grows well in a variety of cell lines.[231] The most sensitive and specific way to identify intracytoplasmic inclusions is by direct immunofluorescent staining with antichlamydial monoclonal antibodies, which is usually performed after 36 to 72 hours of incubation. The chlamydial inclusion can also be detected by staining with Giemsa stain.[230] *C. trachomatis* differs from other *Chlamydia* spp. in containing glycogen, which can be stained with iodine.[206]

The isolation rate of cell culture depends on the number of viable organisms in the clinical specimen.[228] For example, the highest concentrations of chlamydiae, and thus the highest isolation rates, are with ocular infections such as active trachoma and neonatal or adult inclusion conjunctivitis. In urogenital infections, many more organisms are recovered from the endocervix compared with the male urethra, and even fewer from the female urethra.[232] Infections associated with symptoms and signs, as well as infections in younger individuals, generally have higher isolation rates.[233]

Chlamydial culture is still used as a specialized diagnostic method and for research purposes.[230] One important advantage is that it allows recovery of the clinical isolate, which molecular methods such as NAATs

do not. Culture can be important for public health issues, including antibiotic susceptibility testing and identification of outbreak or mutant strains. The high specificity of culture, which approaches 100%, makes it the recommended test for medicolegal evaluation of child abuse, although the NAAT can be used for *C. trachomatis* detection from the urine or vagina in girls.[209] In sexual assault in adults and adolescents, NAATs are recommended because they are more sensitive and widely available.[209]

Cytologic Diagnosis

Typical intracytoplasmic inclusions and free chlamydiae can be identified in Giemsa-stained cell scrapings from the eye. Stained conjunctival scrapings are positive in 90% of infants with neonatal conjunctivitis and 50% of adults with inclusion conjunctivitis. It is less sensitive for active trachoma, in which only 10% to 30% of conjunctival scrapings are positive.[234] Cytology has also been used to evaluate endocervical scrapings, including those obtained for Papanicolaou smears, but the sensitivity and specificity are low.[235]

Rapid Tests

There has been great interest in the development of rapid tests for *C. trachomatis* infection, to shorten the time between sample collection and test results. Currently, the best rapid test for *C. trachomatis* is the Xpert CT/NG (Cepheid, Sunnyvale, CA) rapid PCR test, which can produce results in approximately 90 minutes.[236] This test is a rapid NAAT for *C. trachomatis* and *N. gonorrhoeae,* and it has performance in both low- and high-prevalence populations similar to existing NAATs, which typically have a processing time of 1 to 2 days. The CT/NG GeneXpert has been approved by the FDA as a "moderate complexity" test, which means that it can be performed in many more clinic and hospital laboratories than other NAATs, which are classified as "high complexity" tests. It has been approved for use on endocervical and vaginal swabs and for urine.

The ideal rapid *Chlamydia* test is a point-of-care (POC) test that is performed while the patient is being evaluated, producing immediate, yet sensitive and specific, results. POC *Chlamydia* tests have been developed that use a rapid immunoassay to detect chlamydial antigens in a patient specimen. These tests are similar in principle to a rapid group A streptococcus or pregnancy test and have the potential to be simple to perform and to interpret. However, current non-NAAT *C. trachomatis* POC tests have low sensitivities compared with NAATs, and reported performances have been highly variable from study to study.[237–239] These *Chlamydia* POC tests are commercially available in some countries but have not been approved for use in the United States. A portable NAAT that can produce results in 1 hour has been developed and evaluated as a POC test in a hospital emergency department setting.[240] It utilizes a microfluidic cartridge to capture and process chlamydial nucleic acids, and a mobile phone to operate the cartridge and to collect the optical signal with its built-in camera.

Serology

Serologic tests are not recommended for diagnosis of most common oculogenital *C. trachomatis* infections.[206] Anti-*Chlamydia* IgM is infrequent in adults with genital tract infection, whereas anti-*Chlamydia* IgG is long lived and cannot distinguish past from current infection. Although a rise in convalescent titers can be used to diagnose an acute infection, it is not a practical option given the wide availability of *Chlamydia* NAATs. Serology can be used to support a diagnosis of LGV infection because commercial NAATs do not distinguish between LGV and non-LGV serovars of *C. trachomatis,* but this role is limited because reliable serologic testing for LGV is not widely available.[210]

The complement fixation test has been used to diagnose LGV, but it is technically demanding and performed in few clinical laboratories.[206] It measures complement-fixing antibodies against the genus-specific LPS antigen, which is present in *C. trachomatis* as well as *Chlamydia pneumoniae* and *Chlamydia psittaci.* Thus it lacks specificity because it can also be positive in patients with recently acquired *C. pneumoniae* infections and some patients with oculogenital infections, although titers tend to be higher with LGV because it is a more invasive disease. A confirmed result requires a fourfold or greater rise in titer between acute and convalescent sera, but patients with LGV often present 3 or 4 weeks into their illness when their antibody titers are stable. Complement fixation serology has no diagnostic utility for *C. trachomatis* urethritis, cervicitis, or conjunctivitis because most patients with these infections have low or nonexistent titers.

The microimmunofluorescence (MIF) test is the historical gold standard for detection of chlamydial antibodies because of its sensitivity, and species and serovar specificity. It is not widely used because it is primarily available in research laboratories,[206] although less well-characterized immunofluorescence serologies are commercially available. In its most common format, elementary bodies from each of the 15 *C. trachomatis* serovars are used as antigens to detect antibodies against chlamydial cell wall components.[74] This test reliably differentiates among *C. trachomatis, C. pneumoniae,* and *C. psittaci,* and occasionally, but not reliably, among *C. trachomatis* serovars. In an experienced laboratory it is more sensitive than the complement fixation test. Many adult patients with symptomatic eye or genital infection have a fourfold rise in antibody titers between acute and convalescent sera. Titers are especially high in women with PID or perihepatitis, in whom its diagnostic sensitivity may be highest. However, among *C. trachomatis* infections, the MIF test is most useful for diagnosing neonatal chlamydial pneumonia because almost all affected infants test positive, with an IgM titer of 1:32 or greater.[241] Anti-*Chlamydia* IgM is also present in approximately 30% of infants with neonatal inclusion conjunctivitis.[241]

The limited availability of the MIF test and the interlaboratory variability in its interpretation emphasize the need for more clinically practical serologic tests. As an example of an assay in development, an enzyme-linked immunosorbent assay test using whole EBs as antigens has been reported to be more sensitive than a commercially available MOMP peptide-based assay and able to distinguish between *C. trachomatis* and *C. pneumoniae* infection.[242] This and other assays may prove useful in defining seroprevalence and duration of antibody positivity after infection in advance of future vaccine trials. In addition, reproducible assays will be necessary to document preexisting antibodies and the response after vaccination.

CLINICAL MANIFESTATIONS

C. trachomatis infections can be divided into four clinical categories: (1) trachoma, (2) oculogenital diseases in adults, (3) perinatal infections, and (4) LGV.

Trachoma

Trachoma is a chronic keratoconjunctivitis caused by repeated infections with serovars A, B, Ba, and C of *C. trachomatis.*[205,243] The disease has been recognized as a cause of blindness since antiquity, with therapy for trachoma and its complications described in China in the 27th century BC and in Egypt in the 19th century BC.[205] The countries with the highest prevalence are in sub-Saharan Africa, but about half of all active trachoma cases are located in just five countries: Ethiopia, India, Nigeria, Sudan, and Guinea.[244,245] In high-prevalence areas, active trachoma is present in more than 50% of children younger than 10 years of age.[245] The economic burden from blindness and lost productivity has been estimated to be $5.3 billion annually.[246]

Active *C. trachomatis* infection mostly occurs in children, but a small proportion of individuals go on to have complications of impaired vision and blindness in adulthood.[205] In areas where trachoma is endemic, the first infection usually occurs in the first 2 years of life, and active infection and inflammation persist for several months. Although initial infections may resolve spontaneously, they are frequently followed by reinfection or by superimposed bacterial conjunctivitis. This active stage of trachoma manifests as a chronic follicular conjunctivitis with papillary hypertrophy and inflammatory infiltration. Individuals are frequently asymptomatic, but can have mild symptoms of chronic conjunctivitis with redness, discomfort, photophobia, and mucopurulent discharge.[205] Many individuals will clear the infection and inflammation without sight-threatening consequences. However, in a significant minority there is scarring of the tarsal conjunctiva lining the eyelid. Over years, this distortion of the eyelid causes the eyelashes to turn in and abrade the cornea (trichiasis), resulting in ulceration, scarring, corneal opacification, and loss of vision as an adult, typically at 30 to 40 years of age. Trachoma

is a clinical diagnosis, and laboratory detection of *C. trachomatis* is not used for diagnosis.[1] The WHO simplified grading scheme for evaluating trachoma by direct examination of the conjunctiva is presented in Table 180.2. The assessment can be easily performed in the field, and the only equipment necessary is a pair of binocular loupes, which are simple magnifying glasses.

In endemic areas, children with ocular infection are the primary reservoir.[205] Transmission occurs by hand-to-eye contact between children and their caregivers, by contact with fomites contaminated with ocular or nasal secretions, and via the feet of flies. Flies feed on the exudate from children with active conjunctivitis and act as a mechanical vector. Hygienic factors important for control of disease include face washing, access to clean water, improvements in sanitation, and reduction of household fly density. Although global estimates of trachoma prevalence have limitations, a downward trend has been noted in both active trachoma and trachoma-related blindness since 1981.[245]

Treatment

Individuals can be successfully treated with systemic antibiotics, but in high-incidence areas they will be easily reinfected, and treatment of the entire community is necessary to have an impact.[205,243] WHO recommends mass drug administration in communities in which more than 10% of children have follicular trachoma. More than 85 million people worldwide received single-dose azithromycin for trachoma in 2016.[2] Trials of mass treatment with azithromycin at the village level indicate that both infection and clinical disease are markedly decreased at 6 and 12 months, but multiple rounds of treatment over 3 to 5 years are necessary in hyperendemic areas.[247] Of the regimens recommended by WHO, single-dose oral azithromycin is the preferred treatment.[205] Topical application of tetracycline ointment to the eyes is an alternative, but adherence is difficult because of the 6-week course. In addition, topical treatment does not clear the bacterium from extraocular sites, such as the nasopharynx and rectum, that are also infected in children with trachoma.[205] Surgery for trichiasis is performed to correct in-turned eyelashes and has some value in preventing progression to corneal opacity and blindness, but recurrence of trichiasis after surgery is common, occurring in about 20% of persons at 1 year and in up to 60% at 3 years.[205] An added benefit of trachoma treatment programs is an associated reduction in childhood mortality, diarrhea, and respiratory infections.[243]

WHO has initiated a program to eliminate blinding trachoma by 2020.[205] The objective is not necessarily to eliminate the trachoma serovars of *C. trachomatis,* but to eliminate or at least markedly reduce clinically active disease. The key elements of the S-A-F-E strategy are *S*urgery for deformed eyelids, *A*ntibiotics administered as periodic mass treatment of villages with single-dose azithromycin, *F*ace washing and hygiene, and *E*nvironmental improvements to control flies, using approaches such as building latrines outside villages. In some areas, trachoma appears to be disappearing coincident with economic gains and without the introduction of specific control programs, illustrating the strong historic link between poor socioeconomic conditions and endemic trachoma.

TABLE 180.2 World Health Organization Simplified Grading Scheme for Trachoma

Trachomatous inflammation—follicular (TF): ≥5 follicles in the upper tarsal conjunctiva (follicles must be at least 0.5 mm in diameter).
Trachomatous inflammation—intense (TI): Pronounced inflammatory thickening of the tarsal conjunctiva, which obscures half of the normal deep tarsal vessels.
Trachomatous conjunctival scarring (TS): The presence of easily visible scars in the tarsal conjunctiva.
Trachomatous trichiasis (TT): At least one eyelash rubs on the eyeball. Evidence of recent removal of in-turned lashes was also graded as trichiasis.
Corneal opacity (CO): Easily visible corneal opacity present over the pupil, which was so dense that at least part of the pupil margin was blurred when seen through the opacity.

Modified from Thylefors B, Dawson CR, Jones BR, et al. A simple system of the assessment of trachoma and its complications. Bull World Health Organ. *1987;65:477–483.*

Oculogenital Disease in Adults

C. trachomatis serovars D through K, and occasionally B and Ba, produce a wide variety of oculogenital infections (Fig. 180.3).

Inclusion Conjunctivitis

C. trachomatis ocular infection is called inclusion conjunctivitis because of the pathognomonic presence of the prominent chlamydial inclusion inside infected epithelial cells. In adults, chlamydial eye infection manifests as an acute follicular conjunctivitis, often with a foreign body sensation in the eye. Symptoms are usually unilateral. The clinical picture in the first 2 weeks is dominated by hyperemia and a mucoid discharge that becomes purulent.[248] This is followed by lymphoid follicle formation (frequently with corneal lesions and epithelial keratitis[249,250]) and infiltration of the upper margin of the cornea by small blood vessels (pannus), which is indistinguishable from early trachoma. Preauricular lymphadenopathy and otitis media may be present.[249] The condition may persist for many months if untreated but usually resolves without complications.[251] Scarring similar to that seen in mild trachoma may occur in occasional cases. A common scenario for chlamydial ocular infection is conjunctivitis for weeks to months that has not responded to topical antibiotics. The differential diagnosis is primarily conjunctivitis caused by adenovirus or other viruses.[250]

Slightly more than half of adults with inclusion conjunctivitis have a concurrent *C. trachomatis* genital tract infection, although they may not have genital symptoms.[251,252] In such individuals, the presumed mode of transmission is autoinoculation of the eye with infected genital secretions or, in some cases, direct inoculation from an infected partner. Eye-to-eye spread between individuals by transfer of infected secretions without sexual contact may also occur.[252] In adolescents and adults, the peak frequency of *C. trachomatis* as a cause of keratoconjunctivitis is in 16- to 20-year-olds, accounting for 9% of cases and most likely reflecting high sexual activity and risk of chlamydial genital infection.[251] However, for perspective it is important to remember that chlamydial ocular infection is an uncommon complication that is seen in less than 1% of individuals with proven genital tract infection.[251,252]

A definitive diagnosis can be made by demonstration of the organism by culture if it is available. Of the nonculture tests, DFA has been approved for detection of chlamydiae in conjunctival swabs. NAATs can also be used, but are not FDA approved. The condition responds promptly to the administration of appropriate systemic antibiotics such as azithromycin or doxycycline with decreased discharge, hyperemia, and keratitis symptoms within 48 hours.[248] The patient and partners should be evaluated and treated for genital tract infection.

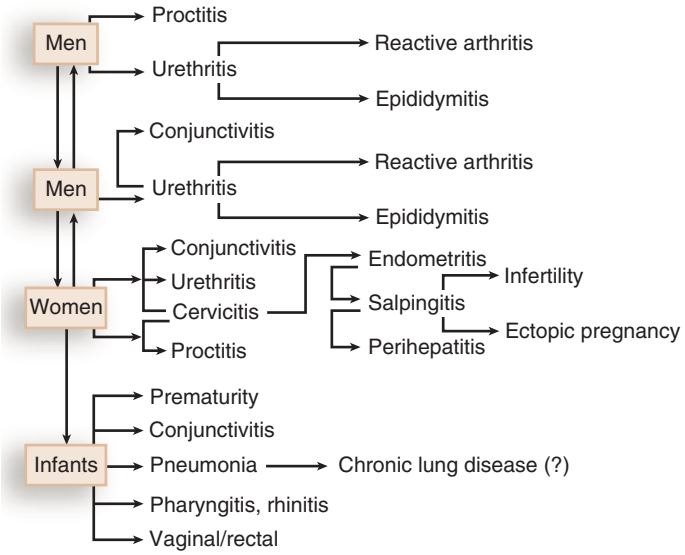

FIG. 180.3 Clinical manifestations and sequelae of *Chlamydia trachomatis* urogenital infections and patterns of transmission.

Urogenital Infections

C. trachomatis urogenital infections are extremely prevalent, accounting for 60% of infections reported to the CDC each year.[253] *C. trachomatis* is the most common cause of bacterial STI in the United States and the world.[4,6] Direct medical costs of chlamydial genital infections in the United States are estimated to be more than $500 million each year (in 2010 dollars).[254]

The risk of acquisition of *C. trachomatis* from a single episode of sexual intercourse with an infected partner has been estimated to be substantially less than that of *N. gonorrhoeae.*[255] By extrapolating data from partner notification programs, and from couples with discordant cell culture–proven infection, the transmission probability within a heterosexual partnership has been estimated to be 0.39 from men to women and 0.32 from women to men.[256] Another heterosexual partnership study that used a NAAT to establish infection found that 72.9% of male partners of positive women, and 68.6% of female partners of positive men, were themselves infected.[257] A modeling study used similar concordance data from an earlier partnership study[258] to estimate transmission probabilities of 0.40 to 0.68.[259] However, these estimates are based on the average frequency of intercourse within a partnership and do not represent the transmission probability of a single encounter. The transmission probability from a single act of unprotected coitus has been estimated to be 0.13 from a stochastic model[260] that used data from a longitudinal study of young women[77] and 0.10 from a partnership study.[259]

In contrast to gonococcal infections, *C. trachomatis* genital infections have characteristics of a prevalent infection rather than an incident infection. Recent exposure to a new partner was much more strongly associated with gonorrhea than with chlamydial infection.[261] In adolescents, chlamydial infection was strongly associated with frequency of intercourse, both in those reporting one lifetime partner and in those reporting more than one partner.[262] These differences may be explained by the less overt presentation of chlamydial infection. Most individuals infected with *N. gonorrhoeae* develop symptoms and seek care quickly, but many men and most women with *C. trachomatis* are either asymptomatic or minimally symptomatic and are only diagnosed as a result of screening or because a contact is symptomatic.[261] Women were also less likely to be infected with *C. trachomatis* if their male partners were identified through a screening program rather than a symptomatic infection.[263]

C. trachomatis is recovered more often from women who acquire gonorrhea than from similarly exposed women who do not have gonorrhea.[264] In individuals with gonorrhea, the recurrence of *C. trachomatis* infection with the same serovar is significantly greater than can be explained by variables related to likely exposure.[265] Furthermore, individuals infected with both *C. trachomatis* and *N. gonorrhoeae* shed larger numbers of *C. trachomatis* than those infected with *C. trachomatis* alone.[228] These data, based on cell culture diagnosis, suggest that acquisition of a gonococcal infection increases the likelihood of acquiring or detecting a chlamydial infection.

Urogenital Infections in Men

Urethritis

C. trachomatis is well known for being a major cause of symptomatic NGU.[266] There are differences in the clinical presentation of chlamydial and gonococcal urethritis, but a reliable distinction cannot be made on clinical grounds without testing.[267] The incubation period for symptomatic chlamydial urethritis is longer, taking 7 to 14 days compared with 4 days for gonococcal urethritis. Both infections present with dysuria, but the urethral discharge with chlamydial urethritis is usually white, gray, or sometimes clear, in contrast to the more purulent discharge observed with gonococcal urethritis.[268] The discharge of NGU may be so slight as to be demonstrable only after penile "stripping" or "milking" and only in the morning. Some patients may deny the presence of discharge but may note stained underwear in the morning. The preferred rapid diagnostic test for urethritis is a Gram stain of the urethral discharge. Previously, the definition of NGU for treatment purposes was 5 or more white blood cells per oil immersion field (1000× magnification), without organisms that looked like *N. gonorrhoeae.* The most recent 2015 STI treatment guidelines have reduced this threshold to 2 or more white blood cells per oil immersion field,[209] based on the finding that *C. trachomatis* is detected by a NAAT with appreciable frequency at this lower cutoff.[269]

In the United States, the incidence of NGU exceeds that of gonococcal urethritis by more than 2:1 in most STI clinics, with a higher ratio in many private practice settings.[270] *C. trachomatis* is a major cause of symptomatic NGU, outnumbering cases caused by *Ureaplasma urealyticum, Trichomonas vaginalis, Mycoplasma genitalium,* or herpes simplex virus.[271,272] The proportion of NGU due to *C. trachomatis* was 23%,[273] 43%,[274] and 42%[262] in three recent treatment trials. In addition, *C. trachomatis* can be recovered from approximately 20% of men with gonococcal urethritis.[275] Risk factors for chlamydial urethritis in men include age younger than 20 years, black race, and heterosexual orientation.[268]

There is limited information about the natural history of untreated urethral infection.[191] Several small studies suggest that untreated chlamydial infection of the urethra may persist in men for up to 6 months.[159,276,277] Asymptomatic infections are common in men, as shown by a study in which only one of eight infected men who were observed without treatment for a minimum of 21 days developed symptomatic urethritis.[278] The primary complications of chlamydial urethritis in men are (1) epididymitis, (2) sexually reactive arthritis, and (3) transmission to women.

Epididymitis and Prostatitis

C. trachomatis[279] and *N. gonorrhoeae* are the most frequent causes of epididymitis in men younger than 35 years, whereas Enterobacteriaceae (primarily *E. coli*) are the usual pathogens in men older than 35 years.[280] In younger men, urethritis is usually also present but may be asymptomatic and only noted on examination. The absence of urethritis does not exclude chlamydial infection or gonorrhea as the cause of epididymitis. Most men with *E. coli* epididymitis have other risk factors for urinary tract infection, including recent catheterization, urologic surgery, or rectal insertive intercourse. Chlamydial epididymitis is often associated with oligospermia during the acute phase,[281] but there are no data indicating that future fertility is impaired. In addition, epididymitis is usually unilateral, and attempts to correlate chlamydial infections with male factor infertility have been unsuccessful.[282] A presumptive diagnosis of epididymitis in the setting of NGU can be confirmed as chlamydial in etiology by a NAAT on first-void urine sample (Table 180.3).

Typically, acute epididymitis presents with unilateral testicular pain and tenderness, hydrocele, and palpable swelling of the epididymis.[209,280] Patients also have dysuria, fever, and, in some cases, shaking chills. Many patients can be managed in the outpatient setting, but others require hospitalization for parenteral antibiotics, scrotal elevation, analgesia, and observation. An alternative diagnosis of testicular torsion should always be considered in a young man with acute onset of severe unilateral scrotal pain and should be ruled out with ultrasound.

The role of *Chlamydia* in prostatic infection remains controversial. From available data, this bacterium does not appear to play a role in acute prostatitis, which is mainly caused by *E. coli,* other gram-negative rods, or enterococci. Its role in chronic nonbacterial prostatitis remains more controversial. Although some investigators have recovered *C. trachomatis* from prostatic expressate or biopsies, convincing evidence that *Chlamydia* plays an etiologic role in chronic nonbacterial prostatitis has yet to be developed and antibiotic therapy is not recommended.[283]

Sexually Reactive Arthritis

Reactive arthritis is defined as an immune-mediated inflammatory response in the joints due to a primary infection at a distant mucosal site.[284] Approximately 10% to 15% of adults report rheumatologic symptoms following a bacterial enteric infection caused by *Salmonella, Shigella, Campylobacter, Yersinia* spp., or *E. coli* O157; two-thirds of these cases are confirmed as reactive arthritis by a rheumatologist.[285] Reactive arthritis can also be triggered by chlamydial and other STIs, but its frequency is not well defined because only the more severe cases are identified. A commonly cited estimate is that 0.8% of men with NGU develop sexually reactive arthritis. This estimate is based on hospitalized arthritis cases ($n = 182$) from 1942 to 1956 normalized to

TABLE 180.3 Clinical Characteristics of Common *Chlamydia trachomatis* Infections

INFECTION	SYMPTOMS AND SIGNS	PRESUMPTIVE DIAGNOSIS	DEFINITIVE DIAGNOSIS	TREATMENT
Men				
Nongonococcal urethritis	Urethral discharge, dysuria	Urethral leukocytosis; no gonococci seen	Urine or urethral NAAT	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
Epididymitis	Unilateral epididymal tenderness, swelling; pain; fever; presence of NGU	Urine or urethral NAAT	Urethral leukocytosis; pyuria on urinalysis	*STI likely:* Ceftriaxone, 250 mg IM, plus doxycycline, 100 mg PO bid, for 10 days *History of insertive anal intercourse:* Ceftriaxone, 250 mg IM, plus levofloxacin, 500 mg once daily, for 10 days
Women				
Cervicitis	Frequently asymptomatic or mild symptoms of vaginal discharge and bleeding, mild abdominal pain, dysuria; a small minority have mucopurulent cervical discharge; ectopy, easily induced bleeding	≥20 PMN/OIF on cervical Gram stain	Urine, vaginal, or cervical NAAT	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
Urethritis	Dysuria, frequency; no hematuria	Pyuria on UA; negative urine Gram stain and culture	Urine, cervical, or urethral NAAT	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
PID	Lower abdominal pain, adnexal pain, cervical motion tenderness	Evidence of mucopurulent cervicitis	Urine or cervical NAAT	*Inpatient:* Cefoxitin 2 g IV q6h or cefotetan 2 g IV q12h, plus doxycycline 100 mg PO bid; transition after clinical improvement to doxycycline alone to complete 14-day course *Outpatient:* Ceftriaxone, 250 mg IM (single dose), plus doxycycline, 100 mg PO bid, for 14 days, with or without metronidazole, 500 mg PO bid, for 14 days
Men and Women				
Conjunctivitis	Ocular pain, redness, discharge; simultaneous genital infection	Gram stain of conjunctival swab negative for bacterial pathogens; PMNs on smear	DFA or NAAT on conjunctival swab	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
Proctitis (non-LGV)	Rectal pain, discharge and bleeding; history of receptive anal intercourse	≥1 PMN/OIF on rectal Gram stain; no gonococci seen	Urine or urethral NAAT; rectal culture or NAAT	Doxycycline, 100 mg PO bid, for 7 days
LGV	Painful, tender inguinal lymphadenopathy; women more likely to have backache and pelvic pain	"Groove sign"	Urine, urethral, lymph node, or rectal NAAT; rectal or lymph node culture; LGV-specific testing if available	Doxycycline, 100 mg PO bid, for 21 days
LGV proctitis and proctocolitis	Rectal pain, discharge, and bleeding in MSM; absence of inguinal lymphadenopathy	≥1 PMN/OIF on rectal Gram stain; no gonococci seen	Urine, urethral, or rectal NAAT; rectal culture; LGV-specific testing if available	Doxycycline, 100 mg PO bid, for 21 days
Newborns				
Conjunctivitis	Ocular pain, redness, discharge; simultaneous genital infection	Gram stain of conjunctival swab negative for bacterial pathogens; PMNs on smear	DFA or NAAT of conjunctival swab; vagina, rectum, pharynx also often positive	Erythromycin base, 50 mg/kg/d PO, divided into four doses for 14 days; evaluate and treat parents as well
Pneumonia	Staccato cough, tachypnea, hyperinflation	Diffuse interstitial infiltrate, eosinophilia	Nasopharyngeal NAATs or culture; MIF serology (IgM)	Erythromycin base, 50 mg/kg/d PO, divided into four doses for 14 days; evaluate and treat parents as well
Children				
Trachoma	Frequently asymptomatic or mild eye symptoms of redness, discomfort, photophobia, mucopurulent discharge; a small minority have progression to trichiasis, corneal opacification, and blindness	Residence in endemic area; direct examination of conjunctiva	DFA or NAAT of conjunctival swab (not usually performed)	Community-wide mass treatment with azithromycin, 1 g PO (single dose)

DFA, Direct fluorescent antibody; *IgM,* immunoglobulin M; *LGV,* lymphogranuloma venereum; *MIF,* microimmunofluorescence; *MSM,* men who have sex with men; *NAAT,* nucleic acid amplification test; *NGU,* nongonococcal urethritis; *OIF,* oil immersion field; *PID,* pelvic inflammatory disease; *PMN,* polymorphonuclear neutrophil; *STI,* sexually transmitted infection; *UA,* urinalysis.

22,010 outpatient cases of urethritis of any etiology at the same institution over this time period.[286] Better data come from a study in an STI clinic in which 271 consecutive patients at risk and treated for chlamydial infection were carefully screened for rheumatologic symptoms; 4.1% had features of reactive arthritis, which was relatively mild and mostly short-lived.[287] Retrospective data from a sexual health clinic in Sydney, Australia, found that reactive arthritis diagnoses declined from 1992 through 2012 despite increasing rates of *Chlamydia* detection.[288]

The full complex of sexually reactive arthritis consists of arthritis, conjunctivitis, urethritis, and skin lesions. However, limited data suggest that NGU-associated cases of reactive arthritis predominantly present with enthesitis and oligoarthritis,[287] similar to patients with reactive

arthritis following enteric infection.[285] In men with untreated reactive arthritis who have urethritis, *C. trachomatis* can be recovered from the urethra in up to 69% at the onset of the acute arthritis.[289] Reactive arthritis patients also have elevated synovial and serum antibody levels to *C. trachomatis*.[289] In older studies, approximately 80% of reactive arthritis patients had the histocompatibility marker human leukocyte antigen (HLA)-B27,[290] but this percentage is much lower in more recent population-based studies.[285,287] In addition to reactive arthritis, there appears to be an association between chlamydial infection, HLA-B27, and undifferentiated oligoarthritis.[291]

There is some evidence that *C. trachomatis* may be present in the joints of reactive arthritis patients in a form that either cannot be cultured or is difficult to culture.[292] Synovial lymphocytes from reactive arthritis patients show higher proliferative responses in vitro to chlamydial antigens than do peripheral blood lymphocytes from the same patients or synovial fluid lymphocytes from control patients.[284] Early reports of *C. trachomatis* recovery by culture from the synovial membranes of at least some patients with reactive arthritis have not been replicated even with improvements in isolation technique.[284] However, organisms with morphology consistent with *Chlamydia* have been identified in the synovium of reactive arthritis patients by electron microscopy, immunocytochemical staining,[284,289] and molecular hybridization and amplification techniques,[284] suggesting that *Chlamydia* may persist in some form in the synovial membranes of patients with reactive arthritis. Chlamydial DNA was found in synovial biopsies together with messenger RNA detected by reverse-transcriptase PCR, suggesting that the detectable forms and DNA represent viable, persistent organisms.[293] A double-blind study comparing lymecycline (tetracycline-L-methylene lysine) and placebo in patients with reactive arthritis suggested that 3 months of treatment was efficacious in arthritis associated with chlamydial infection but not in reactive arthritis associated with other causes.[292] Furthermore, when patients with reactive arthritis were treated for genitourinary infections with antichlamydial agents or broad-spectrum antibiotics, the incidence of arthritic relapses was significantly reduced compared with patients left untreated or treated with penicillin.[294] However, several other human treatment studies have not shown antibiotics to be of benefit.[295] A more recent prospective, placebo-controlled trial compared a 6-month treatment course of either doxycycline or azithromycin plus rifampin versus placebo in patients with chronic *Chlamydia*-associated reactive arthritis and suggested improvement in clinical end points.[296] The PCR assays used in this study to establish the association with chlamydiae are specialized research assays and not standard commercially available amplification assays used for screening purposes.

Urogenital Infections in Women

Most women with *C. trachomatis* genital infection are asymptomatic, and yet most of the serious consequences occur in women. Risk factors vary in different population groups, but in general the risk of *C. trachomatis* infection is in excess of 30% for the female sexual partner of a man with either gonococcal urethritis or NGU.[270] In the United States, higher prevalence rates in sexually active individuals have been associated with younger age (15 to 24 years old), African-American ethnicity, unmarried status, new or multiple sexual partners, and oral contraceptive use.[270,297] Higher rates are also seen in the southeastern part of the country. Young age is the single factor most strongly associated with increased risk of chlamydial infection among sexually active females. For example, chlamydial prevalence in sexually active individuals is nearly three times greater among 14- to 24-year-olds compared with 25- to 39-year-olds.[298] In addition, young age is associated with an increased risk of repeated infection[299] and with an associated increased risk of PID, ectopic pregnancy, and infertility.[300] Oral contraceptives may increase susceptibility or enhance detection because of increased cervical ectopy, resulting in more exposure of susceptible endocervical cells. Alternatively, oral contraceptive use may be a surrogate marker for increased sexual activity.[297] In some studies,[301] but not others,[261] a recent change of sexual partners and increased numbers of partners have also been associated with increased prevalence of chlamydial genital infection.

The natural history of endocervical infection with *C. trachomatis* in women is not fully known.[191] In most animal models, including primates, an immune response is mounted after infection or reinfection, and the organism can no longer be detected.[302] It is likely that a similar response occurs in a substantial proportion of infected women, but other data suggest that chlamydiae can cause chronic asymptomatic infections in the female genital tract. For example, 68 of 85 (80%) infected but asymptomatic adolescent women were still infected when retested 2 months or more after their initial evaluation.[303] Two epidemiologic studies indicate that untreated genital infection persists for 1 year in 45% to 50% of women.[157,158] Further evidence of a prolonged infection comes from reports of young women who repeatedly tested positive for up to 5 years with a genetically identical strain of *C. trachomatis*.[304,305]

The epidemiology and natural history of anorectal infections in women is another area of uncertainty. In two large studies, the prevalence of anorectal chlamydial infection among women giving a history of anorectal intercourse was 14.6%[306] and 16%,[307] respectively; all women were asymptomatic. A recent Canadian study carried out at two different clinic sites (Calgary and Edmonton) assessed genital and anorectal chlamydial infection by sampling female STI clinic attendees who received a pelvic examination as routine clinic procedure (Calgary) or were screened for either symptoms, contact with STI, anorectal sex, sex work, or sexual assault (Edmonton).[308] The prevalence of rectal infection was 11.7% at Calgary and 13.5% at Edmonton. A substantial proportion of chlamydial infections were detected only at the rectal site (44.6% at Calgary, 17.8% at Edmonton). Reported anorectal sex among women with rectal-only infection was only 15.9% at Calgary and 22.6% at Edmonton. The reason(s) for the disparity between anorectal infection and anorectal intercourse could relate to underreporting of anorectal sex, autoinoculation from the genital site, difference in spontaneous clearance rates at the genital and rectal sites, or false-positive NAATs at the rectal site.[308] Alternatively, Rank and Yeruva[309,310] hypothesized an asymptomatic reservoir in the lower gastrointestinal tract established via the fecal-oral route, which is a well-documented and common characteristic of chlamydial infections in birds and mammals. More research is needed to confirm the frequency and mechanism of rectal carriage in women, to determine whether routine screening at the anorectal site is warranted to increase case finding, and to evaluate the response of anorectal infection to the currently recommended treatment regimens.

Cervicitis and Urethritis

The cervix is the most common site of *C. trachomatis* infection in women. Indeed, mucopurulent cervicitis caused by *C. trachomatis* has been called the female counterpart of male NGU.[125] However, only a minority of patients have the classic presentation of mucopurulent cervicitis with discharge of mucus and pus from the cervical os and easily induced endocervical bleeding by gentle passage of a cotton swab through the os.[125,311] Instead, approximately 70% of women with endocervical infection are asymptomatic or have mild symptoms, such as vaginal discharge and bleeding, mild abdominal pain, or dysuria.[270] Vaginal discharge is likely due to endocervical rather than vaginal infection because *C. trachomatis* cannot infect the squamous epithelium of the adult vagina. However, vaginitis can be present in girls because the vagina is lined with transitional cell epithelium before puberty. Dysuria may reflect concurrent urethral infection. On examination, the cervix may appear normal or may exhibit edema, erythema, and hypertrophy. Diagnostic testing with a NAAT is necessary to distinguish between *C. trachomatis* and *N. gonorrhoeae* as the two common causes of cervicitis.

Acute urethral syndrome is defined as dysuria and urinary frequency with fewer than 10^5 organisms per milliliter of urine.[312] In one study of 59 women with this syndrome, 42 also had pyuria and 11 of the 42 were infected with *C. trachomatis,* as were 3 of 66 women without symptoms and 1 of 35 women with cystitis related to *E. coli.* Most of the remainder of the women with pyuria and acute urethral syndrome had low urine concentrations of *E. coli* or *Staphylococcus saprophyticus* demonstrated by culture of urine obtained by suprapubic aspiration.[312] Young, sexually active women with this clinical syndrome should be evaluated for possible *C. trachomatis* infection, and they respond to appropriate antibiotics such as doxycycline. *C. trachomatis* has also been isolated from the Bartholin glands in women with bartholinitis.

C. trachomatis has been associated with cervical cancer in seroepidemiologic studies and proposed as a cofactor for human papillomavirus (HPV) in the development of this common cancer in women. In a meta-analysis of 19 retrospective studies and 3 prospective studies, *C. trachomatis* infection in women was significantly associated with increased risk of cervical cancer (odds ratio [OR], 2.21; 95% confidence interval [CI], 1.88–2.61).[68] The risk was present with *C. trachomatis* as an independent factor (OR, 1.76; 95% CI, 1.03–3.01), and increased when there was also HPV infection (OR, 4.03; 95% CI, 3.15–5.16).[68] Case-control studies have found an association between cervical dysplasia or neoplasia and *C. trachomatis* infection.[313–317] This association has been noted for both cervical squamous cell carcinoma and cervical adenocarcinoma,[68] and is more pronounced for specific strains of *C. trachomatis*.[318,319] Coinfection with *C. trachomatis* was associated with more prolonged shedding of HPV in a longitudinal study of adolescents.[320] Biologic support for a cofactor role comes from studies indicating that *C. trachomatis* infection may cause chromosomal and genetic instability and induce cellular transformation, as described earlier in the "Chlamydial Biology" section. Chronic inflammation induced by *Chlamydia* could also potentially contribute to cancer development.

Also of concern is the possible association between chlamydial cervicitis and acquisition of human immunodeficiency virus (HIV) infection by women.[321] In a case-control study of female prostitutes in Zaire, the adjusted OR for HIV seroconversion with *C. trachomatis* infection was 3.6, with a 95% CI of 1.4 to 9.1.[321] Other studies support the idea that *C. trachomatis* and other sexually transmitted agents that produce genital mucosal inflammation cause increased shedding of HIV in genital secretions.[322]

Pelvic Inflammatory Disease

PID is an upper genital tract disease that encompasses some combination of endometritis, salpingitis, and peritonitis. For example, in a study of women with suspected PID, 70% had histologic evidence of plasma cell endometritis, 67% had laparoscopically verified salpingitis, and 67% had peritonitis.[323] Endometritis is present in 40% of women with mucopurulent cervicitis, suggesting early spread of infection from the cervix to the endometrium.[144] In clinical practice, PID is rarely confirmed pathologically or by direct visual inspection of the fallopian tubes by laparoscopy. Instead the diagnosis is usually made on a clinical basis in a sexually active woman with lower abdominal pain and cervical, uterine, and adnexal tenderness on pelvic examination.[324] Approximately 20% of women with acute PID have *C. trachomatis* detected in urogenital samples, although the percentage ranges from 5% to 51%, depending on the population studied and the techniques used.[270] A more recent estimate of acute PID cases attributable to *C. trachomatis* was derived by examining multiple data sources from the United Kingdom; the overall rate was estimated at 20%, but was 35% in younger women (ages 16–24).[325]

The proportion of women with endocervical *C. trachomatis* infections who develop acute symptomatic PID is difficult to determine accurately. Estimates vary widely, which may be due to differences in the patient populations, with clinic-based studies having more cases of recently acquired incident infections. For example, 18 of 109 (16.5%) infected but asymptomatic adolescent women who were observed for 2 months or more became symptomatic, but only 2 (1.8%) developed clinical PID.[303] In contrast, 6 of 20 (30%) women infected with both *C. trachomatis* and *N. gonorrhoeae,* but only treated for gonorrhea, developed acute salpingitis during a 7-day follow-up interval.[326] More consistent estimates can be obtained from randomized controlled trials that screen asymptomatic women who are followed longitudinally to detect clinical PID. For example, the Prevention of Pelvic Infection (POPI) trial of *Chlamydia* screening and treatment in London suggested that the rate of clinical PID observed in the year after a positive *Chlamydia* screening test is about 10%.[327] More recently, data from the POPI trial[327] and two other randomized controlled trials[328,329] were reanalyzed using Markov modeling.[325] The estimated risk of PID following chlamydial infection was 14.8% (95% CI, 4.8–24.8%) for symptomatic PID and 17.1% (95% CI, 5.6–28.9%) if asymptomatic PID was also included.[330] The timing of progression to PID after acquisition of a chlamydial infection is not established. Although it may occur early, modeling of data from the POPI trial suggests that clinical PID attributed to *Chlamydia* may occur at a constant rate over the course of a year after infection.[331] These findings support a role for screening and treatment to prevent PID even after acquisition of *C. trachomatis* infection.

The spectrum of PID associated with *C. trachomatis* infection ranges from acute, severe disease resembling gonococcal salpingitis, with associated perihepatitis and ascites, to asymptomatic or "silent" PID.[332] Undiagnosed PID appears to be more common than diagnosed acute disease,[330] while completely asymptomatic disease may only account for about 11% of cases.[330] Compared with women with gonococcal or nongonococcal, nonchlamydial salpingitis, women with chlamydial salpingitis are more likely to experience a chronic, subacute course with a longer duration of abdominal pain before seeking medical care. Yet they have as much or more tubal inflammation at laparoscopy.[333] In a prospective study, one-time screening of asymptomatic women for chlamydial infection, followed by treatment if infected, reduced the incidence of PID in a health maintenance organization setting.[328]

Infertility and Ectopic Pregnancy

The long-term consequences of both acute PID and silent, subclinical disease are tubal infertility, ectopic pregnancy, and chronic pelvic pain syndrome.[330,334] In developed countries, infertility affects approximately one in six couples and occlusion of the fallopian tubes is a factor in 10% to 30% of these cases.[324] The general mechanisms responsible for tubal occlusion are not understood, but *Chlamydia* is believed to induce chronic inflammation, scarring, and eventual blockage of the fallopian tubes from repeated or prolonged infection. In a nonhuman primate model, repeated endocervical infections followed by direct tubal inoculation of *C. trachomatis* produced peritubular adhesions and plasma cell endometritis.[335] Consistent with the greater inflammation seen with chlamydial disease,[333] women with nongonococcal salpingitis are more likely to have an adverse reproductive outcome than women with gonococcal salpingitis.[336]

The relationship between PID and infertility was examined in a prospective study of women with laparoscopy-verified salpingitis.[337] Over a mean period of 94 months, 16% of the patients and 2.7% of the control subjects failed to conceive. Ten percent of patients and none of the control subjects had confirmed tubal infertility. Tubal infertility increased with severity of infection from 0.6% after a case of mild PID to 21.4% after severe PID. It also increased with the number of episodes of PID, from 8% after one episode to 19% after two and 40% after three. The ectopic pregnancy rate was 9.1% among patients versus 1.4% among control subjects.[337]

Tubal infertility and ectopic pregnancy have a strong association with serologic evidence of prior chlamydial infection, even though most women with these reproductive health issues do not have a known history of an STI or PID.[270] *Chlamydia* has been recovered from fallopian tube biopsies in women undergoing microtuboplasty for surgical correction of damaged tubes.[338,339] In one study, 22% of women experiencing ectopic pregnancy had histologic evidence of plasma cell salpingitis and antichlamydial antibodies.[340] Repeated *Chlamydia* diagnosis is associated with ectopic pregnancy risk[341]; however, further studies are necessary to define the relative role of prolonged versus repeated infection in increasing the risk of ectopic pregnancy. Recent analyses have led to contemporary estimates that *C. trachomatis* infections are complicated by PID in 17% of cases, by tubal factor infertility in 0.5%, and by *Chlamydia*-attributable ectopic pregnancy in 0.2%.[334]

Pregnancy Complications

Some data indicate that *C. trachomatis* infections affect pregnancy outcome. In one study, women experiencing recurrent spontaneous abortions had high titers of antichlamydial IgG but negative endocervical cultures for *C. trachomatis,*[342] suggesting an association between prior or chronic *C. trachomatis* infection and spontaneous abortion. Some women with recent *C. trachomatis* infection had infants with lower birth weight compared with women lacking *Chlamydia*-specific IgM.[343,344] An association between chlamydial infection and prematurity and premature rupture of membranes has been reported.[345,346] In a large study, treatment of *C. trachomatis* infection was significantly associated with decreased premature rupture of membranes, and there was a trend

toward increased perinatal survival.[347] Pregnancy outcomes have also been compared in 244 treated women who were cured of a *C. trachomatis* infection and 79 treated women who had a treatment failure or recurrent infection.[348] Successful treatment significantly decreased the frequency of premature delivery (OR, 0.16; 95% CI, 0.06–0.47), premature rupture of membranes (OR, 0.31; 95% CI, 0.14–0.69), and small-for-gestational-age infants (OR, 0.45; 95% CI, 0.23–0.88).

These studies suggest that identification and treatment of *C. trachomatis* infection in pregnancy is likely to improve pregnancy outcome. A major difficulty in these studies is the potential interaction of other infections that may influence pregnancy outcomes, including genital infections caused by *Mycoplasma, Ureaplasma,* herpesviruses such as cytomegalovirus, and *Trichomonas,* as well as bacterial vaginosis, urinary tract infections, and vaginal colonization with gram-negative rods. Studies need to evaluate and analyze all of these factors comprehensively, making such studies both large and complex.[349]

Extragenital Infections

Proctitis (Rectal Infections)

C. trachomatis genital serovars D through K can cause proctitis, but this rectal infection is not as severe as LGV proctitis.[350] It results from direct inoculation of the rectum in either men or women through receptive anal intercourse but can also be caused by secondary spread of secretions from the cervix in women. The primary manifestations are anal pruritus and pain and a mucous rectal discharge that may become mucopurulent. However, many patients are asymptomatic. The infection remains superficial, is limited to the rectum, and closely resembles gonococcal proctitis. Leukocytes are usually present on rectal Gram stain even if the infection is asymptomatic.[351,352] Proctitis with concomitant infection by LGV and non-LGV strains of *C. trachomatis* has been reported in MSM.[353] Non-LGV serovars of *C. trachomatis* can also cause asymptomatic rectal carriage in infants[354] and adults.[355] Proctitis caused by LGV serovars of *C. trachomatis* is discussed in "Lymphogranuloma Venereum" later in this chapter.

Oropharyngeal Infections

Oropharyngeal infections have been documented by cell culture in heterosexual men (3.7%) and women (3.2%),[121] and more recently in MSM, by NAATs (2.9%).[122] Oral sex is a risk factor, and virtually all of the infections are asymptomatic. However, transmission to genital sites likely occurs,[356,357] and treatment is recommended if detected. Routine screening for oropharyngeal infection is not recommended, although screening is suggested for MSM who engage in oral sex.[209]

Other Infections

Pneumonia caused by *C. trachomatis* is primarily a disease of infants, although there have been isolated reports of *C. trachomatis* pneumonia in immunocompromised patients.[358] There are rare reports of pulmonary infection in laboratory workers exposed to high concentrations of LGV serovars.[359] The route of transmission is believed to have been by inhalation of aerosolized organisms, and the individuals responded promptly to treatment with tetracycline or erythromycin. In retrospect, an older report of serologic association of *C. trachomatis* with community-acquired pneumonia[360] was likely due to cross-reacting antibody with then-unrecognized *C. pneumoniae*. Case reports and case-control studies have reported serologic association of *C. trachomatis* with meningoencephalitis,[361] myocarditis,[362] and endocarditis.[363]

Treatment of Genital and Ocular Infections in Adults

A number of antibiotics have excellent activity against *Chlamydia* in cell culture, including the tetracyclines, macrolides and related compounds, rifamycins (including rifampin), and some of the fluoroquinolones.[364] The fluoroquinolones block bacterial DNA replication and generate double-strand DNA breaks by targeting DNA gyrase and topoisomerase IV, which are enzymes that regulate DNA supercoiling levels. *C. trachomatis* DNA gyrase and topoisomerase IV have been confirmed to be susceptible to ciprofloxacin,[59] and mutations in these enzymes confer fluoroquinolone resistance.[365] Because DNA supercoiling levels control the temporal expression of chlamydial genes, the fluoroquinolones are also likely to have a *Chlamydia*-specific activity in blocking progression of the chlamydial developmental cycle.[56,59] Chlamydiae are susceptible to ampicillin and penicillin because they contain peptidoglycan, which has been shown to be present in RBs during chlamydial cell division, but not in EBs.[28] Cephalosporins lack activity due to poor affinity for chlamydial penicillin-binding proteins.[366] Aminoglycosides are not effective because they do not penetrate the eukaryotic host cell.

Considerable clinical data are available on the treatment of uncomplicated urogenital tract infections in both men and women.[367] For many years, standard therapy for uncomplicated genital tract infection has been doxycycline, 100 mg orally twice daily for 7 days. However, azithromycin given as a single 1-g dose is as effective as a 7-day course of doxycycline[368] and is a recommended regimen in the 2015 CDC guidelines for the treatment of STIs.[209] Single-dose therapy is attractive because it minimizes treatment failure from lack of medication adherence, but so far azithromycin is the only approved single-dose option for treating *C. trachomatis* infection. Rifalazil, which is highly active against *C. trachomatis* and has a long half-life, had shown promise as a single-dose treatment for chlamydial NGU.[369] However, a randomized trial did not establish its noninferiority with azithromycin in treatment of uncomplicated chlamydial genital infections in women,[370] and it is no longer in development because of severe adverse effects.

Questions have been raised about the efficacy of azithromycin for treating chlamydial genital infections. Early repeated chlamydial infection has been reported in up to 5% to 13% of adolescents treated with azithromycin, which could represent reinfection, but treatment failure could not be excluded in some cases.[371,372] Although a meta-analysis found an azithromycin cure rate of 97%, it was based on trials that used cell culture for diagnosis.[373] In a recent randomized controlled trial of NGU in men that used NAATs as a more sensitive test, there was an unexpectedly low chlamydial cure rate of 77.4% for azithromycin versus 94.8% for doxycycline.[274] In contrast, another NAAT-based study showed a cure rate for azithromycin of 86% versus 90% for doxycycline,[273] and a third study found an azithromycin cure rate of 94%.[369] The authors of these three trials harmonized their data and calculated an overall azithromycin treatment failure rate of 6.2% to 12.8% for symptomatic urethral chlamydial infection in men, although acquisition of new infection could not be completely excluded.[374] In women with uncomplicated infection, two observational studies using NAATs documented use effectiveness of azithromycin therapy of 92%.[77,375] A recent randomized trial, with observed treatment, in a youth correctional setting found that largely asymptomatic men and women with uncomplicated urogenital chlamydial infection had cure rates of 97% for azithromycin and 100% for doxycycline, although the noninferiority of azithromycin was not established.[376] The high cure rates in this institutionalized group suggest that lower cure rates in the wider population could be due to acquisition of new infection, although it is also possible that asymptomatic infections have a higher cure rate than symptomatic ones. In addition, a meta-analysis of randomized controlled trials suggested a degree of increased efficacy for doxycycline, particularly among symptomatic urethral infection in men.[377]

Despite these efficacy questions, azithromycin continues to be recommended for treatment of uncomplicated chlamydial infections in the 2015 Sexually Transmitted Diseases Treatment Guidelines.[209] This recommendation takes into consideration the major advantage of azithromycin as a single-dose regimen, which enhances adherence and makes directly observed therapy feasible. Azithromycin is now available as a generic drug, and its cost to our STI clinic in Indianapolis of about 60 cents per 1-g dose is about the same as a 7-day course of doxycycline (J. Arno, personal communication). The FDA released a warning on March 12, 2013 that azithromycin can cause potentially life-threatening arrhythmias. Patients with known QT interval abnormalities, or who take drugs to treat arrhythmias, should receive doxycycline instead.

For *C. trachomatis*, ofloxacin, 300 mg twice daily for 7 days, and levofloxacin, 500 mg once daily for 7 days, are included as alternative agents in the 2015 CDC treatment guidelines.[209] However, neither should be used in adolescents younger than 18 years or in pregnant women.[209] Delayed-release doxycycline (Doryx) 200 mg daily for 7 days has been approved by the FDA for treatment of uncomplicated chlamydial genital infection in men and women.[209] This once-daily preparation could

improve treatment adherence and was shown to be noninferior to the standard twice-daily regimen in both men and women.[378]

To date, antimicrobial resistance among wild-type chlamydiae has not been demonstrated to be a clinically important problem.[379] Reports of tetracycline resistance in *C. trachomatis* clinical isolates have not been substantiated.[380] However, resistance can be induced in the laboratory to fluoroquinolones, rifampin, and other drugs.[365,381,382] There have been isolated reports of patients with persistently symptomatic genital infection with repeatedly positive *C. trachomatis* NAATs despite antibiotic treatment, suggestive of treatment failure.[383] Currently there is no active surveillance program in the United States to detect emergence of resistance in *C. trachomatis.*

Guidelines from the CDC in 2015 recommend azithromycin, 1 g orally, as the treatment for chlamydial infections in pregnancy.[209] Amoxicillin, 500 mg orally three times a day, and erythromycin preparations are listed as alternatives.[384–386] Doxycycline, ofloxacin, and levofloxacin are contraindicated in pregnancy. Clindamycin is only partially effective in eradicating *C. trachomatis* in men with NGU,[387] but it appears to be as efficacious as erythromycin in pregnant[388] and nonpregnant[389] women with *C. trachomatis* infection.

Test of cure after treatment is not necessary in most patients because of high cure rates with first-line antibiotics. It is reserved for specific circumstances, including pregnancy, persistent symptoms, and suboptimal antibiotics (erythromycin or amoxicillin). However, retesting 3 months after therapy is recommended because of the high risk of reinfection in women and men (see "Prevention Strategies" later).[209]

Presumptive treatment of *C. trachomatis* for both patient and sexual partners is warranted for clinical conditions with a high likelihood of a chlamydial infection, such as NGU in heterosexual men, epididymitis in men younger than 35 years, PID, and gonococcal infection in either men or women.[209] Presumptive treatment of mucopurulent cervicitis is more controversial, but current guidelines accept it as a reasonable approach in women at high risk for chlamydial infection (≤25 years old, with new or multiple sex partners, partners who themselves have concurrent partners, or partners with known STI). Otherwise, diagnostic testing with NAATs and follow-up for treatment is the recommended approach.[209]

Proctitis in homosexual men and the acute urethral syndrome in women may be managed either by presumptive therapy or by therapy based on test results. Empirical therapy of both proctitis and epididymitis should include treatment for gonorrhea—for example, ceftriaxone, 250 mg IM in a single dose followed by 7 days of doxycycline, 100 mg orally twice daily.[209] Azithromycin should probably be avoided for treatment of symptomatic rectal infections due to reports of treatment failure.[390] Doxycycline treatment should be extended to 21 days when an LGV serovar is known or suspected to be the causative agent in a patient with more severe symptoms.[209,391] Treatment of asymptomatic rectal infection in MSM is with either single-dose azithromycin or 7 days of doxycycline according to the 2015 CDC guidelines,[209] but doxycycline is preferred in the 2015 European guidelines.[392] A survey of observational studies suggests that azithromycin may be less effective for treatment of rectal chlamydial infection in MSM,[393] and a randomized trial comparing azithromycin with doxycycline is underway in Australia.[394]

The management of PID, even when gonorrhea is present, should always include therapy directed against *C. trachomatis,* as well as *N. gonorrhoeae* and anaerobic bacteria. Randomized trials have shown that parenteral and oral regimens have similar clinical efficacy for mild-to-moderate PID, although doxycycline is given orally if possible because intravenous infusion is painful.[209] Recommended parenteral regimens include cefoxitin or cefotetan along with a 14-day course of doxycycline or, alternatively, clindamycin plus gentamicin, or ampicillin-sulbactam plus doxycycline.[209] Outpatient regimens include initial, single-dose, intramuscular therapy with a second- or third-generation cephalosporin plus 14 days of doxycycline, with or without metronidazole, 500 mg twice daily for 14 days. Due to the emergence of quinolone-resistant *N. gonorrhoeae,* regimens that include a quinolone are no longer recommended for PID treatment.

Few comparative data exist on the treatment of adult inclusion conjunctivitis, but doxycycline is effective when given for a 2- or 3-week period of time.[395] Erythromycin is an alternative.

Perinatal Infections

Neonatal Inclusion Conjunctivitis

C. trachomatis was recognized as a cause of ophthalmia neonatorum when the introduction of perinatal ocular prophylaxis for gonorrhea did not eliminate all cases of neonatal conjunctivitis. However, even before the modern antibiotic era, cytoplasmic inclusions, which are identical to those seen in patients with trachoma, had been observed in conjunctival scrapings from neonates with conjunctivitis and subsequently in cells from the cervix of mothers and the urethra of fathers of infected infants.[396] Infant infection is usually acquired during passage through an infected birth canal. Exceptions include occasional infants who appear to have acquired infection perinatally despite birth by cesarean section, or postnatally from an infected caregiver by hand-to-eye contact.[397] Between 30% and 50% of infants born to *C. trachomatis*–infected women develop neonatal conjunctivitis.[398] The usual incubation period is 5 to 12 days from birth, although onset may be as late as 6 weeks of age.[399] Typically, a watery ocular discharge appears, which becomes progressively more purulent. The eyelids swell, and the conjunctivae become erythematous and thickened. At birth, the conjunctiva lacks a lymphoid layer, so follicles do not develop initially but may become apparent after 3 to 6 weeks.

The progression of the disease is similar to inclusion conjunctivitis in adults, with spontaneous resolution occurring in most untreated infants after 3 to 12 months.[161] However, mild or subclinical infection may persist for several years,[400] and late sequelae such as scars and corneal lesions occur in a small proportion of cases.[401] A mucopurulent rhinitis and vulvovaginitis in female infants are often associated with the conjunctivitis. The primary differential diagnosis in a newborn is gonococcal ophthalmia, which is uncommon in children who receive ocular prophylaxis at birth, but can still occur.[402] Topical erythromycin ointment, which is the standard prophylaxis for gonococcal ophthalmia in neonates, does not prevent perinatal transmission of *C. trachomatis* infection.[209]

Infant Pneumonia

Between 11% and 20% of infants born by vaginal delivery to mothers with *C. trachomatis* genital infection develop chlamydial pneumonia.[403] Infected infants usually become symptomatic before 8 weeks of age with nasal obstruction or discharge, tachypnea, and cough, or a combination of these.[404] Typically, infants have been symptomatic for 3 or more weeks when they present for care between 4 and 11 weeks of age. Most are afebrile and only moderately ill,[405] but half have a history of conjunctivitis and a majority have middle ear abnormalities.[404] Paroxysms of staccato coughing that interfere with sleeping and eating are sometimes present. Auscultation may reveal scattered crackles, but breath sounds are usually good and wheezing is usually absent. Chest radiographs show bilateral interstitial infiltrates with hyperinflation (Fig. 180.4).[404] Peripheral eosinophilia, arterial hypoxemia, and elevated serum immunoglobulins are characteristic.[404–406] *C. trachomatis* can usually be recovered from nasopharyngeal swab specimens, and antichlamydial IgM titers are elevated.[403]

Untreated, the course is protracted, often lasting weeks to months.[405] Especially in very young infants, the initial respiratory manifestations of *C. trachomatis* infection may be more severe and include prolonged spells of apnea or respiratory failure.[407,408] Although published reports emphasize more serious disease, it is likely that most patients with *C. trachomatis* infant pneumonia are treated as outpatients, often without laboratory confirmation of diagnosis. Long-term respiratory sequelae may be significant in more severe cases, as shown by a higher frequency of obstructive airway disease and physician-diagnosed asthma in children who were diagnosed with chlamydial pneumonia in the first 6 months of life.[409,410]

Diagnosis of Neonatal Inclusion Conjunctivitis and Infant Pneumonia

Sensitive and specific methods for diagnosis of ophthalmia neonatorum and infant pneumonia include cell culture and nonculture tests, such as DFA, EIA, and NAAT. DFA is the only nonculture test cleared by the FDA for conjunctival and nasopharyngeal samples.[209] NAATs have not been cleared by the FDA for conjunctival and nasopharyngeal

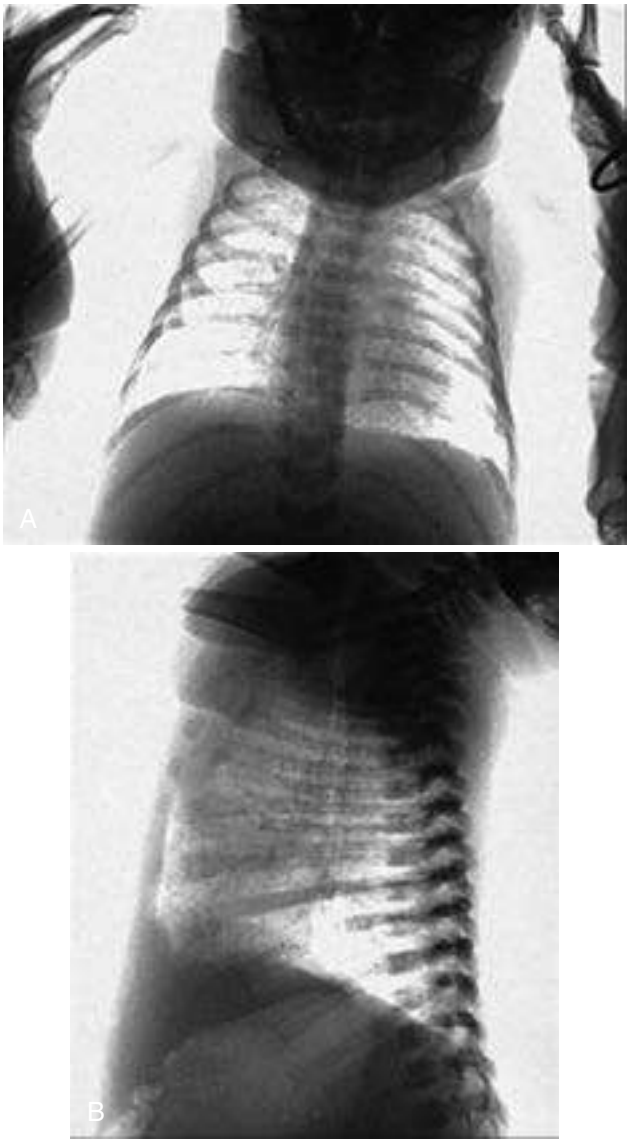

FIG. 180.4 ***Chlamydia trachomatis*** **infant pneumonia.** (A and B) Chest radiographs of a 2-month-old infant with *Chlamydia trachomatis* pneumonia demonstrate typical patchy interstitial infiltrates and flattened diaphragms. *(Courtesy Dr. John Gaebler, Indianapolis.)*

samples, but they can be used for testing these sites if the laboratory has performed in-house validations to conform to CLIA regulations.

Prevention and Treatment of Infant Infections

Oral erythromycin is the treatment for *C. trachomatis* conjunctivitis or pneumonia in infants. The recommended dose is 50 mg/kg of body weight per day, divided into four doses, for 14 days, and the efficacy of therapy is approximately 80%, although a second course may be required.[398] Topical treatment of inclusion conjunctivitis is not recommended because of difficulty in application and failure to eliminate concurrent nasopharyngeal carriage, which can result in recurrent conjunctivitis, pneumonia, or both.[411] Mothers of infants with *C. trachomatis* should be evaluated and treated, if necessary, as should their sexual partners.[209]

Prenatal screening and treatment of *C. trachomatis* infection is the best strategy for preventing neonatal chlamydial infections because it is approximately 90% effective in preventing transmission during birth.[412,413] However, in populations at high risk for reinfection, particularly adolescents, reacquisition of infection after the first trimester is frequent,[414] and repeated prenatal screening is warranted. Current recommendations are to obtain a test of cure at 3 to 4 weeks after treatment, preferably by a NAAT. Such a test of cure is not routinely done for chlamydial genital infections but is recommended for pregnant women to ensure therapeutic cure in light of the serious sequelae of neonatal infection. Women younger than 25 years of age should be retested during the third trimester. Thus a woman who is positive in the first trimester should receive a test of cure, a follow-up test at 3 months, and a retest during her third trimester.[209] The incidence of neonatal chlamydial infections has greatly decreased in the United States because of widespread screening and treatment of pregnant women, but *C. trachomatis* remains a common cause of neonatal conjunctivitis in countries that do not perform prenatal screening.[398]

Chlamydial Infections in Prepubertal Children

Perinatally acquired *C. trachomatis* infection may persist in the nasopharynx, urogenital tract, or rectum for 2 to 3 years.[209,400] Consequently, differentiating infection acquired at birth from infection related to sexual abuse may be particularly difficult in children younger than 3 years of age. However, chlamydial infection among prepubertal children 3 years and older should be considered indicative of sexual abuse. NAATs can be used for vaginal and urine specimens from girls, but the CDC does not recommend NAATs for boys due to insufficient data.[209] In the setting of possible sexual abuse, data are insufficient to support use of NAATs at extragenital sites. The CDC continues to recommend *Chlamydia* culture for detection of urogenital *C. trachomatis* in boys and at extragenital sites in both boys and girls.[209]

Lymphogranuloma Venereum

LGV is an STI caused by serovars L1, L2, and L3 of *C. trachomatis*.[415] These serovars produce a more invasive disease than the genital infections caused by serovars D to K.[416] In endemic areas in Africa, India, Southeast Asia, South America, and the Caribbean, LGV commonly presents in its classic or bubonic form as a chronic, systemic illness with a genital lesion, and prominent regional lymphadenopathy. LGV can also cause a severe proctitis and proctocolitis, sometimes with prominent systemic symptoms, that occurs as clusters of cases outside endemic areas, primarily in MSM.

Classic LGV

There are three distinct stages in classic LGV.[416] The first stage is formation of a primary lesion, usually on the genital mucosa or adjacent skin. *C. trachomatis* cannot infect squamous epithelial cells, and when the primary lesion occurs on the external genitalia or in the vagina, the organism probably gains entry through minute lacerations or abrasions.[417] The primary lesion is usually a small papule or herpetiform ulcer that produces few or no symptoms and is generally not noticed. It appears between 3 and 30 days after acquisition of infection[161] and heals rapidly without leaving a scar. Depending on the site of inoculation, the initial infection can present as symptomatic urethritis, cervicitis, or proctitis.

Days to weeks after the primary lesion has resolved, there is a secondary stage with lymphadenopathy and systemic symptoms.[416] Lymphadenopathy is unilateral in two-thirds of patients and involves lymph nodes that drain the area of the primary lesion.[161,417] Thus in men, inguinal lymphadenopathy is most common because the primary lesion is usually on the penis or in the urethra, which drain to the inguinal nodes. In contrast, inguinal lymphadenopathy is the main manifestation in only 20% to 30% of women, occurring when the primary infection is in the vulva or lower vagina, which drain into the inguinal and femoral nodes. In contrast, inguinal lymphadenopathy is not prominent with an upper vaginal or cervical infection because these sites drain to the iliac and obturator nodes in the pelvis. The internal iliac nodes are involved when there is a rectal infection. Initially, the lymph nodes are discrete and tender with overlying erythema, but because of extensive periadenitis, the inflammatory process spreads from the lymph nodes into the surrounding tissue, forming an inflammatory mass. Abscesses within the mass coalesce, forming a bubo that may rupture spontaneously with development of loculated abscesses, fistulas, or sinus tracts.[418] Mild leukocytosis, with an increase in monocytes and eosinophils, is frequent.[417]

The secondary stage is marked by systemic manifestations, including fever, headache, and myalgias.[416] Women often have backache and pelvic pain from enlarged, inflamed pelvic lymph nodes. Meningitis may occur, and in some cases the organism has been recovered from blood or cerebrospinal fluid.[417] Rupture of the fluctuant inflammatory mass (bubo) relieves pain and fever,[417] although sinus tracts may continue to drain thick, yellowish pus for several weeks or months before fully resolving.[419] Excised inguinal nodes often have a characteristic inflammatory response, with central stellate coalescing abscesses that contain neutrophils and necrotic debris, surrounded by a zone of palisaded epithelioid cells, macrophages, and occasional multinucleated giant cells, and an outer layer of lymphocytes and plasma cells. With time, the nodal architecture is effaced and replaced by progressive fibrosis. This histopathologic presentation is suggestive of the diagnosis of LGV or cat-scratch disease but is not unique to these entities.

Healing leaves some scarring in the inguinal region but does not result in significant sequelae in most cases. Relapse occurs in approximately 20% of untreated cases.[417] Only approximately one-third of buboes become fluctuant and rupture. The others harden and form inguinal masses, which gradually involute over time.[419] The separation of enlarged femoral and inguinal nodes by the inguinal ligament produces the "groove sign," which is characteristic of LGV.[417,420] Inguinal or femoral lymphadenopathy may be misdiagnosed as inguinal hernia, whereas internal iliac node involvement may raise a question of appendicitis.

If untreated, some patients progress to a chronic tertiary stage of the infection.[416] Complications include elephantiasis of the male or female genitalia from lymphatic obstruction and esthiomene (Gr., "eating away"), which refers to hypertrophic chronic granulomatous enlargement with ulceration of the external genitalia (vulva or scrotum and penis).[419]

The differential diagnosis of inguinal lymphadenopathy in the age group likely to have LGV includes genital herpes, syphilis, chancroid, and, occasionally, lymphoma.[418] LGV manifesting as inguinal lymphadenopathy can be distinguished from genital herpes by the presence of multiple painful ulcers at the site of the primary herpes infection, in contrast to the painless primary lesion of LGV, and by matting of the lymph nodes in LGV. Also, lymphadenopathy is frequently bilateral in herpes but not in LGV. A diagnosis of syphilis is suggested by a primary lesion with indurated margins (chancre) and bilateral and nontender inguinal lymphadenopathy. Large ulcers that are multiple and extremely tender in association with lymphadenopathy suggest chancroid. The pseudobuboes that occur in granuloma inguinale are nodules in the skin and subcutaneous tissue, with lymph node involvement being the result of secondary infection.[161] However, the clinical presentations of sexually transmitted agents causing inguinal lymphadenopathy clearly overlap, and appropriate laboratory tests are usually required to distinguish among them. When an ulcer is present, a darkfield examination would be helpful to identify *Treponema pallidum,* but it is not usually available, and serologic tests for syphilis should be obtained.[161,418]

LGV Proctitis and Proctocolitis

LGV proctitis or proctocolitis is a severe rectal infection, caused by *C. trachomatis* LGV serovars, that primarily occurs in MSM.[421,422] This infection is called *proctitis* if it is restricted to the distal 15 cm of the rectum, or *proctocolitis* if there is more extensive involvement into the colon.[207] Infection results from direct inoculation of the rectum via receptive anal intercourse. In women, there can also be lymphatic spread to the rectum from the posterior vaginal wall or cervix, or spread of infected secretions from the cervix.[417,423] The disease can extend into the colon, and a granulomatous inflammatory process is present in the bowel wall, with both noncaseating granulomas and crypt abscesses. As the disease progresses, muscle layers are replaced by fibrous tissue, which contracts to form rectal strictures. Sinus tract formation can lead to rectovaginal fistulas in women.[419,423] Involvement of the distal rectal mucosa can lead to perirectal abscesses and anal fissures. Lymphatic drainage from the rectum leads to lymphadenopathy of the internal iliac nodes, which may cause lower abdominal and back pain.[419] Lymphatic obstruction can lead to outgrowths of lymphatic tissue resembling hemorrhoids.

Since 2003, there has been an increase in reports of chlamydial proctitis and proctocolitis caused by LGV strains.[76,415] This outbreak has occurred as clusters of cases in large cities in Europe, North America, and Australia, outside regions where LGV is endemic. Almost all the cases have been in MSM. The majority of these patients have been infected with HIV, and they have had high rates of other STIs. However, the patients were typically not immunosuppressed, and LGV proctitis does not appear to be an opportunistic infection. Rectal ulcers commonly present in LGV proctitis may facilitate transmission and acquisition of HIV and other infections.

Patients with LGV proctitis and proctocolitis often have mucopurulent anal discharge, as well as rectal pain, ulceration, and bleeding.[76] Tenesmus and constipation are reported less consistently. A minority of patients have systemic symptoms, such as fever, malaise, and weight loss.[76] LGV proctitis may also be asymptomatic, as shown by a UK screening study that found an absence of rectal symptoms in 15 of 55 (27%) MSM with LGV detected by rectal swab PCR.[424] One unusual feature of the current outbreak of LGV proctitis is that most patients have not had genital lesions or inguinal lymphadenopathy typical of classic LGV.[76,415] These cases have been caused by a clonal strain of *C. trachomatis* serovar L, designated L2b.[102] By retrospective testing, this serovar was already present in San Francisco in the early 1980s, leading to questions about how new the outbreak is.[422,425] Endoscopic findings include mucopurulent exudate with hyperemic and friable mucosa, easy bleeding, multiple ulcers, erosions, and granulomatous tissue, but are not specific for LGV proctitis.[76] The differential diagnosis of severe infectious proctitis in a high-risk population includes gonorrhea, genital herpes, and syphilis.[350,415] Proctocolitis caused by LGV strains has clinical and histologic similarities to other inflammatory bowel diseases, such as Crohn disease, which can lead to misdiagnosis and inappropriate therapy.[350,351]

Diagnosis of Lymphogranuloma Venereum

The diagnosis of LGV has been hampered by the lack of a good, widely available test.[209,415,426] Standard NAATs will identify *C. trachomatis* but not the serovar; additional PCR-based testing is required to identify LGV serovars but is not available outside specialized laboratories.[209,426] Specimens that can be tested include a swab of the primary genital ulcer, if present; lymph node aspirate or biopsy; rectal swab or biopsy; urine; and urethral swab. NAATs are not FDA approved for rectal specimens, but some laboratories can perform the test because they have done validation studies.[209] In 30% of cases, *C. trachomatis* can be recovered by culture from bubo aspirates, genital tissue, or rectal tissue, but *Chlamydia* culture is no longer routinely performed in clinical microbiology laboratories.[417] A complement fixation titer greater than 1:64 supports a diagnosis of LGV in the appropriate clinical setting but is not widely available.[209] Histopathologic changes in LGV are not specific. A skin test (Frei test) has been used in the past but is no longer available.[417,427] With this lack of widely available LGV-specific diagnostic tests, it is recommended that patients with a clinical presentation of LGV or LGV proctitis be treated presumptively.

Treatment of Lymphogranuloma Venereum

For classic LGV, the CDC recommends 21 days of doxycycline, 100 mg twice daily, for treatment of LGV, with erythromycin as an alternative.[209] Data are lacking, but if doxycycline cannot be used because of allergy or intolerance, azithromycin, 1 g orally once weekly for 3 weeks, is probably effective, and levofloxacin for extended intervals might be effective as well.[209,391] In addition, fluctuant buboes should be aspirated to prevent rupture and sinus tract formation.[417] Antibiotic therapy results in rapid abatement of constitutional symptoms but has only a limited effect on bubo resolution.[417] Effects on late complications such as strictures are variable.[161,417] Sexual partners should be examined and treated. Pregnant women with LGV should be treated with erythromycin because doxycycline should be avoided.[209]

With the overall lack of access to specific testing for LGV serovars, all cases of severe chlamydial proctitis and proctocolitis should be assumed to be due to LGV strains and treated with the 21-day regimen of doxycycline.[209] However, this regimen from the CDC is based on the experience with classic LGV, and concern about the deep-seated nature of LGV buboes, and is not based on comparative studies of treatment duration for LGV proctitis.[391] The optimal duration of treatment for asymptomatic or minimally symptomatic LGV proctitis is not known.

A recent observational study of MSM from the United Kingdom showed that most asymptomatic or mildly symptomatic rectal infections due to an LGV serovar resolved microbiologically with 7 days of doxycycline.[428] However, clinical trials are needed to confirm that a shorter 7-day doxycycline course would suffice.

PREVENTION STRATEGIES

Primary prevention strategies are centered on (1) inducing behavioral changes that reduce the risk of acquisition of chlamydial infections, as well as other STIs; and (2) identification and treatment of people with genital infection before they can transmit the infection or develop complications. Behaviors that reduce risk of infection include delaying age of first intercourse, decreasing numbers of partners, and promoting use of condoms. Vaginal microbicides such as vaginal sponges containing the spermicide nonoxynol-9 were previously thought to be of value, but a meta-analysis of multiple trials concluded that nonoxynol-9 was ineffective in preventing cervical gonorrhea, chlamydial infection, or HIV infection.[429] Other microbicides that may be more effective and less toxic than nonoxynol-9 are under development, but none is yet available.

Many developed countries have initiated programs with the stated goal of reducing transmission and sequelae of *C. trachomatis* by screening high-risk populations for asymptomatic infections and by aggressive partner notification and treatment programs. These efforts are based on the previous success of such measures with gonococcal infections and on a few controlled clinical trials that sought to determine the effect of screening on development of symptomatic PID.[430] Most clear-cut is a randomized trial that used selective screening criteria in a health maintenance organization and demonstrated a 60% reduction in PID among screened women during 12 months of follow-up.[328] Largely on the basis of these data, the US Preventive Services Task Force recommendations for screening women have been published and are summarized in Table 180.4.[431,432] However, adoption of such opportunistic screening in the United States has been relatively low,[433] and the effects on population prevalence are less clear. A small longitudinal study of repeated infections in adolescent women showed that screening and treatment as frequently as every 3 months did not reduce point prevalence over time in a high-risk population.[77] On a larger scale, a recent controlled trial of registry-based screening did not demonstrate a reduction in prevalence of *C. trachomatis* infection.[434] At present there is insufficient evidence to recommend routine *C. trachomatis* screening in men.

Patients with *C. trachomatis* urogenital infection should be retested 3 months after treatment because of the high frequency of repeated chlamydial infections within the first several months after treatment.[209,372,435] This retesting is to identify any new chlamydial infection, which is usually from an untreated sex partner or a new partner, and is not to test for cure of the initial infection. However, despite these recommendations, a recent study found that only 22.3% of men and 38% of nonpregnant women were retested.[436] Interventions under evaluation to improve these rates include home-obtained vaginal swabs or urine collection using mailed kits.[437,438]

TABLE 180.4 Screening for Chlamydial Infection, US Preventive Services Task Force, 2014

Screen all sexually active women ≤24 years old
Screen older, high-risk women[a]
Screen pregnant women ≤24 years old
Screen older, high-risk pregnant women[a]
Insufficient evidence to recommend male screening at present

[a]High risk for chlamydial infection as defined in various populations can include women ≤24 years old; new or multiple sexual partners, a sex partner with concurrent partners, or a sex partner with a sexually transmitted infection; previous or concurrent sexually transmitted infection; inconsistent condom use among persons who are not in mutually monogamous relationships; and exchanging sex for money or drugs.

Noninvasive testing of urine and self-collected vaginal swabs with NAATs has facilitated screening efforts in sexually active young men and women. Target groups include nontraditional settings such as high schools, military intake centers, juvenile detention centers, and other nonclinic sites in the community.[439–441] The benefits of screening women have been assessed primarily in ecologic studies in which there have been declining case rates of infection and PID in the screened population during the first several years of screening implementation. However, some programs have noted an unexpected increase in chlamydial case rates and repeated infections as screening continued,[180] while the PID rate has continued to decline.[442] The "arrested immunity" hypothesis attempts to account for these observations by proposing that early identification and treatment of *C. trachomatis* infections interferes with the development of protective immune responses, leaving the individual more susceptible to reinfection,[443] while also interfering with deleterious immune responses that lead to PID.[442]

Notification and treatment of sexual partners are also effective in reducing infection and can be cost-effective despite the resources required.[444,445] The effectiveness of sex partner management has been shown to be improved by expedited partner-delivered therapy.[446] In this innovative approach, the sex partner does not have direct medical contact or evaluation, but instead receives empirical single-dose antibiotic therapy through the index case, often with solicitation of mailed-in urine or vaginal swabs for NAAT testing. Collectively, these prevention strategies have the goal of reducing the high prevalence of *C. trachomatis* genital infections.

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181 Psittacosis (Due to *Chlamydia psittaci*)

David Schlossberg

SHORT VIEW SUMMARY

Definition
- Psittacosis is a systemic infection that frequently causes pneumonia.

Epidemiology
- It is acquired by inhalation after exposure to infected birds, or less commonly from domesticated animals. Occasional patients provide no history of relevant exposure.

Microbiology
- The etiologic agent is *Chlamydia psittaci* of the family Chlamydiaceae.

Diagnosis
- Diagnosis is made serologically in most cases; when available, polymerase chain reaction may be positive. Because diagnostic methods are imperfect, treatment should not await definitive diagnosis.

Therapy
- Doxycycline or tetracycline is the treatment of choice. Macrolides and fluoroquinolones have good in vitro activity, but there is limited clinical experience.

Prevention
- Treat imported birds and birds that have suspected infection.

Psittacosis is a systemic infection that frequently causes pneumonia. Its relationship to bird exposure has been known for more than 100 years. In 1879, Ritter[1] studied an outbreak in Switzerland and called it pneumotyphus. Morange[2] applied the term *psittacosis* (from the Greek word for parrot) in 1892 after studying cases associated with sick parrots. In 1930, the organism was identified in several laboratories, by Bedson in the United Kingdom, Kromwede in the United States, and Levinthal in Germany.[3]

The name *psittacosis* has persisted, even though the term *ornithosis* more accurately depicts the potential for all birds to spread this infection. In fact, even mammals, including humans, are rare sources of psittacosis.

The causative agent of psittacosis is *Chlamydia psittaci.* A proposed reclassification grouped *C. psittaci* with *Chlamydia pneumoniae, Chlamydia pecorum, Chlamydia abortus, Chlamydia caviae,* and *Chlamydia felis* in a new genus, *Chlamydophila*, but use of the latter has declined.[4]

EPIDEMIOLOGY

Chlamydia psittaci is common in birds (often as asymptomatic carriers) and domestic animals. Infection is therefore a hazard to pet owners, pet shop employees, poultry farmers (turkey-associated psittacosis has the highest attack rate in psittacosis epidemics), workers in abattoirs and processing plants (psittacosis is the most common abattoir-associated pneumonia), and veterinarians. However, anyone in contact with an infected bird or animal is at risk. Human cases occur both sporadically and in outbreaks.[5,6]

Most patients with psittacosis have had some contact with a bird, usually as a pet. In fact, the importation of exotic birds (sometimes illegally) has been correlated with an increase in human psittacosis in the United States, Sweden, England, and Wales. Often, the bird was recently acquired or was ill. Bird contact may achieve surprising levels of intimacy. Patients have acquired psittacosis by kissing their parrot or by performing mouth-to-mouth resuscitation on a dying bird. Other patients have had more trivial or transient exposure, such as visits to public bird parks, transporting pigeons by car, passing through a room in which infected birds were sitting, sharing a stage with a parrot, or guarding crates of pigeons at a railroad depot. Still, some patients (25%) have had no avian exposure,[7] and other animal sources may rarely be implicated, as in a recent outbreak in a veterinary school following exposure to equine fetal membranes.[8]

Birds transmit the infection to their nestlings, which in turn shed the organism during periods of both illness and good health. In bird populations studied, there is a baseline prevalence of 5% to 8% of *C. psittaci* carriage. This may increase to 100% when birds are subjected to the stress of shipping, crowding, and breeding.[3,7]

It is likely that all birds are susceptible. More than 130 avian species have been documented as hosts of *C. psittaci.*[3] These include members of the parrot family (macaws, cockatoos, parakeets, budgerigars), finches (canaries, bullfinches, goldfinches, sparrows), poultry (hens, ducks, geese, turkeys), pigeons, pheasants, egrets, seagulls, and puffins.

Infection may appear in birds years after exposure. Infected birds may be asymptomatic or obviously sick. In the latter case, birds may exhibit shivering, depression, anorexia, emaciation, dyspnea, and diarrhea, frequently with closed eyes and ruffled feathers. Spontaneous relapse and remittance of the illness may occur, although it is during periods of illness that infected birds excrete the largest numbers of organisms. Discharge from their beaks, eyes, feces, and urine is infective; their feathers and the dust around their cage become contaminated.

The infection is generally spread by the respiratory route, by direct contact or aerosolization of infective discharges or dust. Rarely, the bird may spread the infection by a bite. If untreated, 10% of infected birds become chronic asymptomatic carriers.[7]

Strains from turkeys and psittacine birds are the most virulent for humans. Although most human exposure comes from avian strains of *C. psittaci,* disease has occurred in ranchers after exposure to infected tissues from parturient cows, goats, and sheep. Endocarditis has been attributed to avian and nonavian strains, and cats have spread feline pneumonitis to humans and other mammals. The growing practice of pet-associated therapy in nursing homes has produced a new epidemiologic risk for psittacosis.[9]

Human-to-human[10,11] and nosocomial[12] transmissions are rare, and it is therefore thought unnecessary to isolate patients in the hospital or to give antibiotic prophylaxis to contacts. However, cases acquired from humans tend to be more severe than avian-acquired disease. Environmental sanitation is important because the organism is resistant to drying and can remain viable for months at room temperature.[3,7]

CLINICAL MANIFESTATIONS AND DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS

The disease begins after an incubation period of 5 to 15 days. Onset may be insidious or abrupt, and the clinical manifestations tend to be nonspecific. Several syndromes may result. The infection may be

subclinical, or it may resemble a nonspecific viral illness with fever and malaise or a mononucleosis-like syndrome with fever, pharyngitis, hepatosplenomegaly, and adenopathy. A typhoidal form manifests as fever, bradycardia, malaise, and splenomegaly. However, the presentation most suggestive of the cause is atypical pneumonia, with nonproductive cough, fever, headache, and chest film abnormalities more dramatic than would be suggested by the physical findings. The illness ranges in severity from an inapparent or mild disease to a fatal systemic illness with prominent respiratory symptoms.

Because many patients have an illness with nonspecific findings, the list of initial diagnoses for which patients have been referred to hospitals is extensive. This list reflects the various organ systems that may be involved in *C. psittaci* infection and includes the diagnoses of meningitis, tonsillitis, pneumonia, pulmonary embolism, myocardial infarction, gastroenteritis, hepatitis, peritonitis, pancreatic carcinoma, urinary tract infection, endocarditis, vasculitis, septicemia, malaria, brucellosis, fever of unknown origin, and polymyositis.[9,13]

The list of considerations in the differential diagnosis is extensive, and the diagnostic possibilities depend on the presentation. A typhoidal picture suggests the mononucleosis syndrome, typhoid fever, brucellosis, tularemia, influenza, malaria, or subacute bacterial endocarditis. Respiratory signs and symptoms plus headache and myalgias should orient the clinician to causes of atypical pneumonia, such as viral pneumonia, Q fever, legionellosis, and infection with mycoplasma and *C. pneumoniae.* Helpful clues to a diagnosis of psittacosis, when present, are relative bradycardia, rash, hemoptysis, epistaxis, and splenomegaly.

The most common sign is fever, occurring in 50% to 100% of patients. Cough has been reported in 50% to 100%, but often it appears late in the illness and is not present initially. Headache, myalgias, and chills are reported in 30% to 70% of patients. The nonspecificity of these signs and symptoms may be puzzling until cough supervenes. Even then, the long list of other signs and symptoms that occur in fewer than half the patients may be particularly confusing due to the lack of specificity: diaphoresis, photophobia, tinnitus, ataxia, deafness, anorexia, nausea and vomiting, abdominal pain, diarrhea, constipation, sore throat, dyspnea, hemoptysis, epistaxis, arthralgia, and rash. Chest soreness is reported, but true pleuritic pain is rare.[5,7,13,14]

The signs most frequently reported are fever, pharyngeal erythema, rales or other abnormalities on chest auscultation, and hepatomegaly. These occur in more than half of cases. Fewer than 50% of patients show the signs of somnolence, confusion, tachycardia, relative bradycardia, pleural rub, splenomegaly (this occurs toward the end of the first week and is helpful diagnostically), adenopathy, palatal petechiae, herpes labialis, Horder spots (see later), and muscle tenderness.[5,7,13]

Specific end-organ involvement reflects the systemic nature of psittacosis. The organ most commonly involved in humans is the lung. This is manifested clinically by cough, dyspnea, and a variety of nonspecific auscultatory findings on physical examination. On occasion, the pneumonitis may progress to acute respiratory distress syndrome. Cardiac manifestations include pericarditis (rarely with effusion and tamponade), myocarditis, idiopathic dilated cardiomyopathy,[15] and "culture-negative" endocarditis. *Chlamydia psittaci* endocarditis is associated with preexisting heart disease and may cause valvular destruction. Arterial embolism to major vessels occurs rarely. The source of these emboli and the mechanism are unknown; some are attributed to endocarditis or mural thrombi.[16]

Hepatitis may develop, sometimes with jaundice. Anemia may result from hemolysis (both Coombs test positivity and cold agglutinins are reported) and from a reactive hemophagocytosis, in which case pancytopenia may be present. Disseminated intravascular coagulation (DIC) also complicates psittacosis.[17,18] Reactive arthritis occurs 1 to 4 weeks after the initial illness; although most of the described cases are polyarticular, monarticular arthritis has also been described.

Neurologic abnormalities include cranial nerve palsy (including sensorineural hearing loss), cerebellar involvement, transverse myelitis, confusion, meningitis, encephalitis, transient focal neurologic signs, and seizures. Results of cerebrospinal fluid examination on lumbar puncture are usually normal; a small number of white cells (predominantly lymphocytes) may be seen, and the protein level on occasion is greatly elevated.[19–24]

Dermatologic phenomena include Horder spots, which are a pink, blanching, maculopapular eruption on the face or trunk resembling the rose spots of typhoid fever. Also described are erythema multiforme, erythema marginatum, erythema nodosum, and urticaria, as well as acrocyanosis, subungual splinter hemorrhages, and superficial venous thromboses. Acute glomerulonephritis, acute tubulointerstitial nephritis, and acute tubular necrosis have been reported. Psittacosis has severe consequences in pregnancy and often causes DIC, hepatic dysfunction, and placentitis, with fetal compromise.[25,26] Additional clinical complications of psittacosis include phlebitis, pancreatitis, and thyroiditis. Bacteremia has been demonstrated in a patient with a sarcoid-like illness.

Recent observations have suggested that *C. psittaci* is associated with ocular adnexal lymphomas involving orbital soft tissue, lacrimal glands, and conjunctiva. *Chlamydia psittaci* has been detected in lymphoma biopsies by polymerase chain reaction (PCR) assay, and tumors in some patients have regressed after treatment with doxycycline. However, the prevalence of *C. psittaci* in these lymphomas varies, particularly geographically,[27–29] and some tumors with no evidence of *C. psittaci* have also responded to doxycycline therapy, suggesting that *C. psittaci* detection methods are inadequate or that other doxycycline-responsive organisms may cause this malignancy, or that the presumed lymphoma may not be a true malignancy but a hyperproliferative response to an infecting agent.[30,31]

In addition to ocular adnexal lymphomas, *C. psittaci* has recently been associated with nongastrointestinal mucosa–associated lymphoid tissue lymphomas and autoimmune precursor lesions (Hashimoto thyroiditis and Sjögren syndrome).[32]

There is no documented protection after infection, and second infections have been seen in spite of elevated levels of complement-fixing (CF) antibodies.[3] Treated birds can also be reinfected.

LABORATORY FINDINGS

The total white blood cell count is usually normal or slightly elevated. Two-thirds of patients have a leftward shift. Eosinophilia has been seen in convalescence. Results on liver function testing are mildly abnormal in 50% of cases and may suggest cholestasis. Culture of the organism is possible from blood in the first 4 days of illness and from sputum in the first 2 weeks. However, although the organism can be isolated in cell culture and by animal inoculation, these methods are dangerous, and serologic diagnosis is preferred (see "Diagnosis" later).

Appearance on chest radiography is abnormal in approximately 75% of patients (range, 50%–90%) and is usually more abnormal than auscultation would predict. The most frequent finding is consolidation in a single lower lobe, seen in 90% of the abnormal chest radiographs. However, a variety of patterns have been reported, including a homogeneous ground-glass appearance, a patchy reticular pattern radiating from the hila, segmental or lobar consolidation with or without atelectasis, a miliary pattern, unilateral or bilateral hilar enlargement, and the halo sign. These chest radiograph findings may take as long as 20 weeks to resolve, with resolution occurring by 6 weeks on average. Pleural effusions are seen in up to 50% of cases but are usually small and asymptomatic.[5] As noted, hilar enlargement may be present but never as the sole manifestation of disease.

PATHOLOGIC FINDINGS

Birds show involvement predominantly in the liver, spleen, and pericardium, but in humans the lung is most frequently and characteristically involved. The trachea and bronchi become inflamed, with widespread mucous plugging. The inflammation spreads from respiratory bronchioles to the alveoli in a lobular pattern. Alveolar and then interstitial exudates accumulate; these are composed of mononuclear cells with a few polymorphonuclear leukocytes, red blood cells, epithelial cells, and fibrin. There is hyperplasia, proliferation, and desquamation of alveolar lining cells, which contain basophilic intracytoplasmic inclusions. Hilar lymph nodes swell, and the lungs become rubbery and solid. The classic sequence of congestion, edema, and red and then gray hepatization is seen.

The brain is congested and edematous, with diffuse arachnoiditis. Meningeal exudate contains macrophages with intracytoplasmic inclusions. The heart shows monocytic infiltration, edema, fatty degeneration,

and subendocardial hemorrhage. The pathologic findings in acute glomerulonephritis include hyaline glomerular occlusion, with subepithelial electron-dense deposits on electron microscopy. The liver may show nonspecific hepatitis or granulomas. Infected placental tissue shows intervillositis with trophoblastic cytoplasmic inclusions.[25] In emboli, polymorphonuclear leukocytes, platelets, and fibrin are seen but not organisms or chlamydial antigen.[18]

DIAGNOSIS

Culture from sputum, pleural fluid, and clotted blood is possible but dangerous because of the risk to technicians of laboratory-acquired infection, and direct identification in tissue specimens is not standardized, so diagnosis depends on serology. The Centers for Disease Control and Prevention considers a *confirmed* case as one with a compatible clinical illness plus either laboratory confirmation by culture from respiratory secretions, or fourfold or greater rise in CF or microimmunofluorescence (MIF) antibody (immunoglobulin G) in specimens drawn at least 2 weeks apart.[32a] A *probable* case is one associated with a compatible illness plus either an antibody (immunoglobulin M) titer of at least 1:32 in a single specimen or detection of *C. psittaci* DNA in a respiratory specimen by PCR. There are both false-positive and false-negative PCR results. Also, the complement fixation test is only genus-specific and does not distinguish *C. psittaci* from *Chlamydia trachomatis* or *C. pneumoniae,* both of which are common pathogens. MIF testing is species-specific but is not generally available, and cross-reactions still occur. Thus serologic testing remains imperfect. In addition, antibiotic therapy can delay or diminish the antibody response. PCR assay can detect *C. psittaci* in avian and human tissues[33–36] and has been used for real-time diagnosis in both birds and humans.[33,37] A recent study in the Netherlands utilized PCR successfully to diagnose psittacosis in patients with community-acquired pneumonia.[38] However, PCR assay is not routinely available and may yield false-negative results. A recent report described rapid identification of *C. psittaci* inclusion bodies in mucosal lesions stained by the Diff-Quik cytologic stain.[39] Thus, since none of the diagnostic methods combines the features of sensitivity, specificity, speed, and availability, therapy for psittacosis should be initiated on the basis of clinical suspicion, usually in the setting of a relevant exposure history.

THERAPY

The treatment of choice is doxycycline, 100 mg PO twice daily, or tetracycline hydrochloride, 500 mg PO four times daily for 10 to 21 days. Some observers recommend the longer course to prevent relapse, but this is controversial. Minocycline is probably equivalent to doxycycline, but clinical data are limited. Macrolides have good in vitro activity, and azithromycin has been effective in birds; thus some have recommended azithromycin as alternative therapy in children and pregnant patients. However, macrolides may be less efficacious in severe cases and might not protect the fetus when administered during pregnancy. Fluoroquinolones, particularly levofloxacin and moxifloxacin, demonstrate good in vitro activity, but clinical experience with these agents is also lacking.[40]

Most patients respond within 24 hours subjectively. Without treatment, the fatality rate is approximately 20%; with treatment, it drops to 1%. The best therapy for endocarditis is valve replacement and prolonged antimicrobial therapy with doxycycline, possibly with macrolides or fluoroquinolones.[7,16] A theoretical advantage of fluoroquinolone chlamydiacidal activity in intravascular infection awaits further clinical experience.

PREVENTION

Infected birds should be treated with tetracycline, chlortetracycline, or doxycycline for at least 45 consecutive days. The US Department of Agriculture (USDA) requires that imported birds be quarantined for 30 days to prevent introduction of Newcastle disease. During this period, birds are treated with chlortetracycline. The USDA recommends that importers continue treatment for an additional 15 days, but this is not always done, and, if treated for fewer than 45 days, some infected birds will continue to shed the organism.[7]

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182 Chlamydia pneumoniae

Margaret R. Hammerschlag, Stephan A. Kohlhoff, and Charlotte A. Gaydos

SHORT VIEW SUMMARY

Microbiology and Epidemiology
- Obligate intracellular bacterium, must be grown in tissue culture.
- Capable of causing persistent infection, often subclinical.
- Worldwide distribution, infects many animals as well as humans.
- Primarily a respiratory pathogen in humans, causing community-acquired pneumonia.
- Can cause epidemics in enclosed populations: military bases, schools, nursing homes.

Diagnosis
- *Chlamydia pneumoniae* causes pneumonia; clinically it cannot be differentiated from other causes of atypical pneumonia, especially *Mycoplasma pneumoniae*.
- The most accurate method of diagnosis is identification of the organism in respiratory samples by culture or nucleic acid amplification test (NAAT).
- Serology is of limited value and requires paired sera, and many patients who are positive by culture or NAAT will be seronegative.

Therapy
- *Chlamydia pneumoniae* is susceptible to macrolides, quinolones, and tetracyclines. Data on efficacy, including optimal dose and duration of therapy, are limited.
- Ten- to 14-day courses of erythromycin, clarithromycin, doxycycline, levofloxacin, or moxifloxacin or 5 days of azithromycin are clinically effective and result in approximately 80% microbiologic eradication.

Chlamydiae are obligate intracellular bacterial pathogens whose entry into mucosal epithelial cells is necessary for intracellular survival and subsequent growth. Chlamydiae cause a variety of diseases in animal species at virtually all phylogenic levels, from amphibians and reptiles to birds and mammals. In 1999, Everett and coworkers[1] reported a taxonomic analysis involving the 16S and 23S ribosomal RNA (rRNA) genes and found that the order Chlamydiales contained at least four distinct groups at the family level. Moreover, within the family Chlamydiaceae there were two distinct lineages, which suggested splitting the genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila*. This classification was not universally accepted by the *Chlamydia* scientific community, and recently it was agreed that the family Chlamydiaceae contains a single genus, *Chlamydia*.[2] This position has been supported by additional data on chlamydial genome sequences. The genus *Chlamydia* contains nine recognized species: *Chlamydia trachomatis, Chlamydia psittaci* (agent of psittacosis; many species of birds), *Chlamydia pneumoniae, Chlamydia pecorum* (ruminants and koalas), *Chlamydia muridarum* (formerly the agent of mouse pneumonitis), *Chlamydia suis* (an important pathogen of swine), *Chlamydia abortus* (causes abortion in cattle and sheep; rarely causes abortion in humans), *Chlamydia caviae* (formerly *C. psittaci,* the guinea pig inclusion conjunctivitis strain), and *Chlamydia felis* (causes epidemic keratoconjunctivitis in cats).[1–4] *Chlamydia trachomatis* and *C. pneumoniae* are the most significant human pathogens, and *C. psittaci* is an important zoonosis.

Recently, several chlamydiae-like organisms that are endosymbionts of free-living amebas have been identified.[3,5] These organisms, which include *Parachlamydia acanthamoebae, Simkania negevensis,* and *Neochlamydia hartmannellae,* have been termed *environmental chlamydiae.* Analyses of nearly full-length 16S rRNA gene sequences of these isolates showed that they clustered with other members of the order Chlamydiales but in a lineage separate from those of *Chlamydia* (16S rRNA sequence similarities >88%). This bacteria-protozoa interaction might have been a driving force for the development of effective mechanisms by bacteria to survive phagocytosis by unicellular eukaryotes, which in turn may have been a first step in the evolution of intracellular bacterial pathogens of higher organisms.[5]

Although *C. pneumoniae* is a human respiratory pathogen, the organism has also been isolated from nonhuman species, including horses, cats, koalas, bandicoots, and amphibians.[4,6,7] Molecular data suggest that the animal strains are ancestral to human strains and that the organism may have crossed from animals to humans as the result of a relatively recent zoonotic event.[4] Whole-genome sequencing has found that human isolates are highly conserved, whereas a *C. pneumoniae* strain from a koala was 12 kb larger, with several interesting differences, including a plasmid that has not been described in human isolates.[4]

HISTORY

The first isolates of *C. pneumoniae* were serendipitously obtained during trachoma studies in the 1960s. After the recovery of a similar isolate from the respiratory tract of a college student with pneumonia in Seattle, Grayston and colleagues[8] applied the designation TWAR after their first two isolates, TW-183 and AR-39. Only one serotype of *C. pneumoniae* has been identified so far. Studies have found a high degree of genetic relatedness (>98%) among human *C. pneumoniae* isolates tested.[4,9]

MICROBIOLOGY

Chlamydiae have a gram-negative envelope without detectable peptidoglycan; however, recent genomic analysis has revealed that both *C. trachomatis* and *C. pneumoniae* encode for proteins that form a nearly complete pathway for synthesis of peptidoglycan, including penicillin-binding proteins.[10] Chlamydiae also share a group-specific lipopolysaccharide antigen and use host ATP for the synthesis of chlamydial protein.[10] Although chlamydiae are auxotrophic for three of four nucleoside triphosphates, they do encode functional glucose-catabolizing enzymes, which can be used for generating ATP.[10] As with peptidoglycan synthesis, for some reason these genes are turned off, which may be related to their adaptation to the intracellular environment. All chlamydiae also encode an abundant protein called the major outer membrane protein (or outer membrane protein A) that is surface exposed in *C. trachomatis* and *C. psittaci* but apparently not in *C. pneumoniae.*[10] The major outer membrane protein is the major determinant of the serologic classification of *C. trachomatis* and *C. psittaci* isolates. Chlamydiae are susceptible to antibiotics that interfere with DNA and protein synthesis, including tetracyclines, macrolides, and quinolones. *Chlamydia pneumoniae* lacks a tryptophan recovery or biosynthesis pathway and is resistant to sulfonamides and trimethoprim.[4]

Chlamydiae have a unique developmental cycle with morphologically distinct infectious and reproductive forms: the *elementary body* (EB) and the *reticulate body* (RB; Fig. 182.1). After infection, the infectious EBs, which are 200 to 400 nm in diameter, attach to the host cell by a

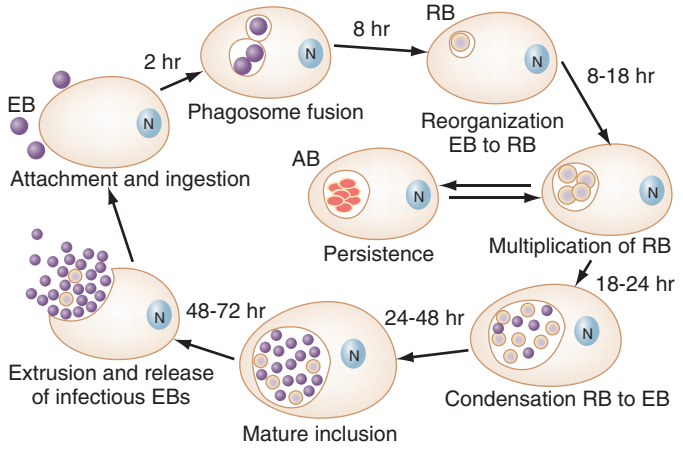

FIG. 182.1 Life cycle of chlamydiae in epithelial cells. *AB,* Aberrant body; *EB,* elementary body; *N,* nucleus; *RB,* reticulate body. *(Modified from Hammerschlag MR, Kohlhoff SA, Darville T:* Chlamydia pneumoniae *and* Chlamydia trachomatis. *In: Fratamico PM, Smith JL, Brogden KA, eds.* Post-infectious Sequelae and Long-term Consequences of Infectious Diseases. *Washington, DC: American Society for Microbiology; 2008.)*

process of electrostatic binding and are taken into the cell by endocytosis that does not depend on the microtubule system. EBs are sporelike; they are metabolically inactive but stable in the extracellular environment. Within the host cell, the EB remains within a membrane-lined phagosome, with inhibition of phagosomal-lysosomal fusion. The inclusion membrane is devoid of host cell markers, but lipid markers traffic to the inclusion, which suggests a functional interaction with the Golgi apparatus. Chlamydiae appear to circumvent the host endocytic pathway, inhabiting a nonacidic vacuole that is dissociated from late endosomes and lysosomes. EBs then differentiate into RBs that undergo binary fission. After approximately 36 hours, the RBs differentiate back into EBs. Despite the accumulation of 500 to 1000 infectious EBs in the inclusion, host cell function is minimally disrupted. At about 48 hours, release may occur via cytolysis or a process of exocytosis or extrusion of the whole inclusion, leaving the host cell intact. This strategy is very successful and enables the organism to cause essentially silent chronic infection.

A number of in vitro studies have challenged this biphasic paradigm. Chlamydiae may enter a persistent state in vitro after treatment with certain cytokines, such as interferon-γ (IFN-γ); treatment with antibiotics, specifically penicillin; restriction of certain nutrients, including iron, glucose, and amino acids; infection in monocytes; and heat shock.[4,11] While in the persistent state, metabolic activity is reduced and the organism is often refractory to antibiotic treatment. These different systems produce similar growth characteristics, including loss of infectivity and development of small inclusions that contain fewer EBs and RBs, and ultrastructural findings, specifically morphologically abnormal RBs, which suggests that they are somehow altered during their otherwise normal development. These abnormal RBs are often called aberrant bodies (ABs). Restriction of certain nutrients has also been shown to induce persistence in chlamydiae. Ultrastructural analysis of IFN-γ–treated *C. pneumoniae* also reveals atypical inclusions that contain large reticulate-like ABs with no evidence of redifferentiation into EBs.

Another model of persistent *C. pneumoniae* infection is long-term continuous infection. In contrast to the previously described models, continuous cultures become spontaneously persistent when both chlamydiae and host cells multiply freely in the absence of stress. *Chlamydia pneumoniae* infection was maintained in human epithelial type 2 (HEp-2) and A549 cells for more than 4 years without centrifugation, addition of cycloheximide, or IFN-γ treatment.[12] Infection levels in these infected cells were high (70%–80%). Ultrastructural studies revealed three types of inclusions in these cells. Approximately 90% were typical large inclusions that ranged approximately from 5 to 12 μm in diameter. The second type (altered inclusions) contained both normal EBs and RBs, but in considerably lower numbers than typical inclusions, and pleomorphic ABs, which were up to four to five times the size of normal RBs (2.5 μm in diameter); their cytoplasm was homogeneous. The third type of inclusion was small aberrant inclusions, on average 4 μm in diameter, containing about 60 ABs that were similar in size to normal RBs but appeared electron dense and no longer retained a smooth spherical shape. These dense ABs retained the characteristic chlamydial outer membrane structure, with very little periplasmic space, and the membranes were more tightly bound to the chlamydial body, similar to normal RBs. No EBs were observed in these inclusions. These findings show that the developmental cycle of *C. pneumoniae* can combine the typical development forms with the persistent phase in tissue culture.

Another possible mechanism of chlamydial persistence could be through a direct effect on the host cell, possibly through an effect on apoptosis, which is an important regulator of cell growth and tissue development. Apoptosis is a genetically programmed, tightly controlled process, unlike necrosis, which involves nonspecific inflammation and tissue damage and intracellular enzymes, condensation of nucleus, and cytoplasm and fragmentation. Many microbial pathogens, including chlamydiae, have been found to modulate cellular apoptosis to survive and multiply. *Chlamydia* spp. have been shown to both induce and inhibit host cell apoptosis, depending on the stage of the chlamydial developmental cycle.[13] Chlamydiae protect infected cells against apoptosis as a result of external stimuli during early stages of infection and may induce apoptosis of the host cell during later stages of the life cycle. Thus chlamydiae may protect infected cells against cytotoxic mechanisms of the immune system, and the apoptosis observed at the end of the infection cycle may contribute to the inflammatory response because apoptotic cells secrete proinflammatory cytokines and facilitate the release of the organism from the infected cells. Studies with IFN-γ–treated cultures have reported that cells infected with *C. trachomatis* and *C. pneumoniae* resist apoptosis as the result of external ligands, via inhibition of caspase activation. Data from studies with the long-term continuously infected cell model showed marked differences in the effect of *C. pneumoniae* on apoptosis in acute and chronically infected A549 cells.[13] Acute *C. pneumoniae* infection *induced* apoptotic changes in A549 cells within the first 24 to 48 hours after infection. Induction of apoptosis in acute infection may facilitate release of *C. pneumoniae* from the host cell. Chronic *C. pneumoniae* infection *inhibited* apoptotic changes within the first 24 hours and up to 7 days. These results suggest that inhibition of apoptosis may help to protect the organism when it is in the intracellular, persistent state.

LABORATORY TESTING FOR *CHLAMYDIA PNEUMONIAE*

Although numerous methods can be used to detect *C. pneumoniae* in clinical samples, in practice detection is very difficult, primarily because of the lack of standardized well-validated methods. Determination of whether *C. pneumoniae* infection is an acute primary infection or reinfection, a chronic persistent stage, or a past infection is also very difficult. Cell culture, immunohistochemistry (IHC), and nucleic acid amplification tests (NAATs) detect living bacteria, antigen, and nucleic acid, respectively. These techniques are primarily used in research settings or require experienced specialized laboratories. In clinical settings, routine diagnosis of *C. pneumoniae* infection has been based on results of serologic testing to identify anti–*C. pneumoniae* immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) antibodies. This approach is problematic for a number of reasons subsequently outlined in detail.

Two new commercially available multiplex nucleic acid amplification assays for the detection of *C. pneumoniae* in conjunction with other respiratory pathogens were cleared by the US Food and Drug Administration (FDA) in 2012 and 2017 (discussed later under "Single and Multiplex Nucleic Acid Amplification Tests").[14,15]

Cell Culture

Chlamydia pneumoniae, as an obligate intracellular parasite, can be isolated by means of cell culture, but the organism is fastidious and slow growing. *Chlamydia pneumoniae* will grow, although not as readily, in cell lines that are usually susceptible to *C. trachomatis,* such as McCoy and HeLa cells. Growth has been observed to be somewhat easier in HL (human line) and HEp-2 cells.[16,17]

Chlamydia pneumoniae has been isolated from the respiratory tract (nasopharyngeal and throat cultures, bronchoalveolar lavage fluids) and tissue biopsies, including lung and adenoids. The organism can also be isolated from sputum, but sputum can be toxic to cell culture and often is contaminated by overgrowing fungi or bacteria. If nasopharyngeal or pharyngeal swab specimens are collected, use of aluminum- or plastic-shafted Dacron-tipped swabs is mandatory because calcium alginate on cotton-tipped swabs and those with wooden shafts may inhibit the growth of the organism in tissue culture and may be toxic to cells. Specimens for culture must be stored in a suitable transport medium optimized for chlamydiae. A suitable medium is sucrose-phosphate-glutamate buffer with antibiotics and fetal calf serum, but ready-to-use media are also commercially available.

Specimens that can be processed within 24 hours should be kept refrigerated at 4°C and shipped on wet ice. Samples that cannot be processed within 24 hours should be held at 4°C before freezing at –70°C because more rapid freezing decreases the titer of viable organisms. Specimens need to be treated with sterile glass beads or sonication to disrupt cells and then centrifuged onto the cell monolayers to facilitate absorption. Cell cultures are incubated at 37°C with 5% carbon dioxide for at least 72 hours per passage. Culture confirmation is assessed by staining inclusion bodies, using a *Chlamydia* genus–specific fluorescent antibody and epifluorescence microscopy (Fig. 182.2). More than one subculture may be necessary for isolation; thus culture is not a straightforward attempt to diagnose the microorganism in a timely fashion. Because the organism has been difficult to grow and because of the lack of any other commercially available diagnostic assay, most original associations with respiratory diseases have been via use of serology with the microimmunofluorescence (MIF) test. Patients who have IgG autoantibodies against IgM may cross react with anti–*C. pneumoniae* IgM antibody.[18]

Antigen Detection

Chlamydia pneumoniae has also been detected in tissue sections or cells with monoclonal antibodies labeled with a peroxidase (IHC) or fluorescent (immunofluorescent) marker. Antigen detection testing, in general, allows preservation of tissue morphology. On the other hand, interpretation of the staining pattern to distinguish the organism from background or nonspecific staining is subjective and influenced by a number of technical issues.[19] In complex biologic samples, IHC gives rise to cross-reactions between antitarget antibodies and nontarget proteins that produce nonspecific signals (e.g., immunoreactivity for *C. pneumoniae* was frequently present in atheroma and nonatheroma sections of vessel walls). The sites with positive results with *C. pneumoniae* IHC assays precisely matched the sites with autofluorescent ceroid deposits.[20] The interpretation of IHC staining must be performed with the utmost caution.

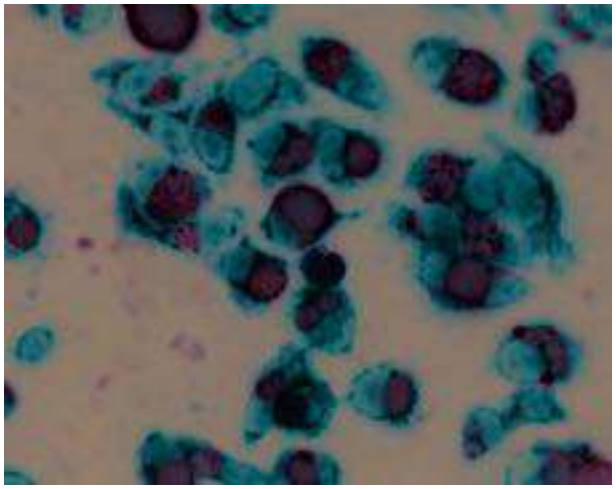

FIG. 182.2 Direct immunofluorescence staining of cell culture 72 hours after infection. The apple-green *C. pneumoniae* inclusion bodies are seen in red counterstained HEp-2 cells (magnification, ×600). *(Courtesy P. Apfalter.)*

Single and Multiplex Nucleic Acid Amplification Tests

Multiple in-house NAATs (such as polymerase chain reaction [PCR]) methodologies have been published, but the older literature has been confounded by lack of standardization and validation. In 2000, a Centers for Disease Control and Prevention (CDC) workshop suggested a few assays that were considered to be "validated" enough to be used for research.[19] Some of these included early developed and validated ones.[21–25] These have been improved, and others have been developed since then.[26–29] The advantages of these NAAT or PCR assays are their sensitivity, decreased possibility of contamination, and ability to quantify DNA. Nearly a decade later, however, many of these tests turned out to be highly prone to false-positive results.[30,31] Until recently, not a single NAAT for the detection of *C. pneumoniae* was commercially available or listed in the in vitro diagnostic database of the FDA (Devices @ FDA; http://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm). Numerous in-house PCR-based tests still are performed, but these assays range from those that are well validated to those that are not validated at all. Although NAATs offer the promise of exquisite sensitivity, theoretically allowing detection of a single organism in a clinical sample, both false-negative and false-positive results can and do occur because of a large number of technical issues that were summarized recently.[32] Currently, real-time PCR technology for the detection of *C. pneumoniae* should be used.[33,34] Real-time PCR offers significant advantages over conventional PCR in its rapidity, the ease with which it can be automated, the potential decreased risk of carryover contamination, and the potential provision of a quantitative result.

Until recently, there have been no commercially available NAAT assays. Abbott Laboratories (Abbott Park, IL) developed a research-use-only PCR assay that was used in a multicenter study comparing PCR results by using in-house PCRs from five different laboratories. The assay performed very well but it was never taken to a clinical trial.[35] Becton Dickinson (Franklin Lakes, NJ) performed a clinical trial for a strand displacement assay, but it was not cleared by the FDA. BioFire Technologies (formerly Idaho Technologies; Salt Lake City, UT) developed a FilmArray assay for the detection of 17 viruses, which is FDA cleared.[36,37] The FilmArray system now includes assays on the same platform for some of the atypical agents of pneumonia, including *C. pneumoniae, Mycoplasma pneumoniae,* and *Bordetella pertussis.* This assay received FDA clearance in July 2012. The BioFire FilmArray system combines nucleic acid extraction, nested PCR, detection, and data analysis in a single-use pouch.[14]

The BioFire FilmArray automated system is simple to perform, and results are ready in 1 hour. This single-test platform enables the detection of numerous viral and bacterial respiratory pathogens in a single test. The pouch is loaded into the FilmArray instrument, and the remainder of the test is completely automated. The system is very robust, detecting a low concentration of pathogen in the presence of a high concentration of a second pathogen. The respiratory panel detects adenoviruses, bocaviruses, coronaviruses, influenza A and B, influenza A subtypes (novel H1-2009, H1, H3), metapneumovirus, parainfluenza viruses types 1 to 4, respiratory syncytial virus, and rhinoviruses, as well as *B. pertussis, C. pneumoniae,* and *M. pneumoniae.*[14,36] However, data on the clinical performance of this assay for detection of *C. pneumoniae* from clinical specimens from patients with community-acquired pneumonia (CAP) are very limited. Most of the data on sensitivity and specificity were derived from contrived specimens.

The GenMark Respiratory Pathogen (RP) Panel (GenMark Diagnostics, Carlsbad, CA), another new multiplex panel for molecular amplification identification of respiratory viruses and bacteria, including *C. pneumoniae* and *M. pneumoniae,* was recently cleared by the FDA. Results were reported from a large multicenter evaluation that compared this assay to the BioFire FilmArray. However, *C. pneumoniae* was detected in only 6 of 2908 (0.3%) patients tested, and the data on performance parameters were derived from contrived specimens.[15,38] The GenMark RP Panel is a qualitative nucleic acid multiplex test for use on GenMark's new ePlex instrument. The ePlex RP Panel is a sample-to-answer multiplex assay that runs on a single-use cartridge that automates all aspects of nucleic acid testing, including extraction, amplification, and detection.[39] The ePlex RP Panel detects a similar range of viral and bacterial targets

as the Biofire FilmArray assay: viral targets include adenovirus, coronavirus (subtypes 229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A subtypes (H1, H1-2009, H3), influenza B, parainfluenza viruses types 1 to 4, and respiratory syncytial virus types A and B.[40] From specimen receipt to result, time to identification is 1 hour 45 minutes with a hands-on time per sample of 1 minute 35 seconds. Overall agreement between the ePlex RP Panel and BioFire FilmArray in this clinical trial was greater than 95% for all targets. Having new commercially available assays that can detect copathogens, as well as single organisms, will improve and better define the role of *C. pneumoniae* in diseases processes. However, we need more data on the performance of both assays for detection of *C. pneumoniae* in clinical specimens.

Specimens for research PCR testing have previously included nasopharyngeal swabs; secretions from the respiratory tract, including sputum and bronchoalveolar lavage fluid; tissue; and peripheral blood mononuclear cells (PBMCs). Swabs should be sent in tubes without transport medium. Sputum, bronchoalveolar lavage fluid, and tissue should also be collected in a sterile device without transport medium. Of note, currently FDA-cleared multiplex NAATs are only approved for use with nasopharyngeal swab specimens.

Serologic Testing

Several types of serologic assays are currently commercially available for the detection of antibodies to *C. pneumoniae.* However, none is currently approved by the FDA for this indication. The test used most frequently and recommended by the CDC remains the MIF assay.[19] Tests based on an enzyme-linked immunosorbent assay (ELISA) format are particularly easy to perform and do not need sophisticated laboratory equipment, which makes them the preferentially offered diagnostic chlamydial tool for laboratories. *Chlamydia pneumoniae,* however, is an intracellular pathogen, and the poor correlation between direct detection (e.g., with culture or NAAT) and serologic results is not surprising. Besides specificity issues, it is not at all clear which classes and titers of antibodies might represent acute first infection or reinfection, or chronic, persistent, or past *C. pneumoniae* infection.[19] This is true for complement fixation tests (measurement of antibodies against chlamydial lipopolysaccharide, therefore not specific for *C. pneumoniae*), ELISA-based tests (purified *C. pneumoniae* EBs or recombinant antigens detected; specificity unclear), and also the gold standard MIF test (formalinized *C. pneumoniae* EBs fixed onto glass slides). A serologic test can only be as specific as the antigen used. Cross-reactivity between *C. pneumoniae* and other *Chlamydia* species has been shown with the MIF test. Factors such as strain type, purity, and concentration of antigen used, and the assay procedure itself, might contribute to the fact that the MIF is less specific for *C. pneumoniae* than was thought 20 years ago. Data also show significant problems with subjective interpretation and intralaboratory and interlaboratory reproducibility.[41] The problems in context with *C. pneumoniae* serology have been discussed in detail in two review articles.[42,43] For an example of the complexity of this issue, consider that two multicenter pneumonia treatment studies in children showed that although 7% to 13% of the patients in the study had positive culture results and 7% to 18% met the serologic criteria with the MIF test for acute infection, they were not the same patients. Only 1% to 3% of the patients with positive culture results met the serologic criteria, and approximately 70% with positive culture results for *C. pneumoniae* were seronegative.[44]

Benitez and coworkers[34] reported similar data in adults from an investigation of a *C. pneumoniae* outbreak in a prison. MIF serology (IgG and IgM) had a positive predictive value of only 30% compared with real-time PCR. Another problem with serologic diagnosis of *C. pneumoniae* infection is that the MIF method used to detect serum antibodies is not standardized; studies have shown substantial interlaboratory variation in the performance of these tests.[41] In summary, serology seems not only to be insufficient for diagnosis of *C. pneumoniae* respiratory tract infection but also to be an inadequate methodology to study associations between *C. pneumoniae* and other diseases. *C. pneumoniae* serology is most problematic in terms of defining specificity, reproducibility, and titer in a given clinical picture or disease, even if prospectively defined.

It is important to know that new environmental *Chlamydia* spp. are being steadily described. Ample evidence exists for a huge diversity and wide distribution of chlamydiae in nature, and humans are exposed to that diversity of species. As an example, the recovery of a novel environmental *Chlamydia* strain from activated sludge with cocultivation with an *Acanthamoeba* sp. was reported; it was shown to also invade mammalian cells.[45] These new environmental chlamydiae (i.e., *Simkania, Waddlia,* and *Parachlamydia*) may interfere with serologic testing for traditional Chlamydiaceae (*Chlamydia*).[46,47]

EPIDEMIOLOGY

The mode of transmission of *C. pneumoniae* remains uncertain but probably is through infected respiratory secretions. Acquisition of infection via droplet aerosol was described during a laboratory accident.[48] *Chlamydia pneumoniae* can remain viable on Formica countertops for 30 hours and can survive small particle aerosolization.[49] Spread within families and enclosed populations, including military recruits, prisons, and nursing homes, has been described.[34,50–52] A review of the role of *C. pneumoniae* in respiratory illness in US Army training centers from January 2013 through December 2016 found that the organism was responsible for approximately 10% of illness, following rhinovirus and *M. pneumoniae* (J. Gaydos, personal communication).

Several serologic surveys have documented rising chlamydial antibody prevalence rates, beginning in school-age children and reaching 30% to 45% by adolescence.[8] Seroprevalence antibody, as determined with the MIF method, can exceed 80% in some adult populations.[53,54] The proportion of CAP in children and adults associated with *C. pneumoniae* infection has ranged from 0% to more than 44%, varying with geographic location, the age group examined, and the diagnostic methods used (Table 182.1).[55] The proportion of CAP attributable to *C. pneumoniae* appears to be significantly lower in studies published after 2000. Whether this is secondary to the methods used (most of the more recent studies have used real-time PCR) or possible cycling, as is seen with *M. pneumoniae,* is unknown. Four studies published after 2010, from diverse geographic areas (Europe, Africa, and Thailand), that used real-time PCR found prevalences of *C. pneumoniae* infection ranging from 0% to 3.8%.[56–59] This was compared with 11.4% in a Chinese study that used MIF serology; 30% of the patients only had a single serum sample (see Table 182.1).[60] In Germany from 2011 to 2012, CAP as assessed by molecular methods was more often attributable to *M. pneumoniae* (12.3%) and *C. psittaci* (2.1%) than *C. pneumoniae* (1.4%).[61] Early studies that relied on serology suggested that infection in children younger than 5 years was rare; however, subsequent studies with culture or PCR assay have found the prevalence rate of infection in children beyond early infancy to be similar to that found in adults.[55] Approximately 50% or more of children with culture-documented *C. pneumoniae* respiratory infection (pneumonia and asthma) show seronegativity with the MIF.[55] Prolonged respiratory infection, documented with culture, that lasts from several weeks to several years after acute infection has been reported.[62]

Coinfections with other organisms, specifically *Streptococcus pneumoniae* and *M. pneumoniae,* may occur frequently.[55,63] Clinically, these patients cannot be differentiated from those infected with a single organism. In these cases, *C. pneumoniae* may not be the primary cause of the pneumonia but might disrupt the normal clearance mechanisms and enable other pathogens to invade. This may have been the case in an outbreak of pneumonia and fatal pneumococcal meningitis among US Army trainees.[52] Six of 12 trainees with pneumonia were infected with *C. pneumoniae,* which suggested a simultaneous outbreak of both infections. Asymptomatic respiratory infection may occur in 2% to 5% of adults and children.[53,63] The role asymptomatic carriage plays in the epidemiology of *C. pneumoniae* is not known. Acute respiratory infection with *C. pneumoniae* does not appear to vary by season, but no systematic surveillance for *C. pneumoniae* infection exists in the United States.

Prior to the availability of research and commercial multiplex NAAT assays for viruses and atypical pathogens, few studies had focused on the role of *C. pneumoniae* as a pathogen in CAP with multiple viral etiologies and as a copathogen. A recent study reported the presence of 14 respiratory viruses and atypical bacteria (*M. pneumoniae, C. pneumoniae*), via multiple PCR assays in patients under 18 years old

TABLE 182.1 Summary of Selected Studies of Respiratory Infection From *Chlamydia pneumoniae* in Adults and Children Published Since 2001

LOCATION	AGE (NO. TESTED)	DIAGNOSTIC METHODS	NO. POSITIVE	COMMENTS
United Kingdom	>16 yr (316)	Serology,[a] gene amplification	55 (17%)	All positive with serology
Netherlands	1–88 yr (159)	EIA, Pst1-based PCR	5 (3.1%)	All positive with serology alone
Netherlands	≥18 yr (107)	Conventional test or PCR[b]	0	Test not described
Taiwan	17–99 yr (168)	MIF	12 (7.1%)	—
Japan	17–99 yr (232)	MIF, PCR, culture	15 (6.5%)	Proportion of results positive with each test not stated
Germany	≥18 yr (546)	MIF, PCR	5 (0.9%)	All 5 positive with 2 different PCR assays, negative with MIF
Thailand	1 mo–15 yr (333)	MIF	149 (44.7%)	14 of 149 positive with single titers
United States	6 wk–18 yr (154)	MIF, EIA	14 (9%)	Proportion positive with MIF and EIA not stated
Switzerland	Ages not stated (1583)	RT-PCR	2 (0.013%)	
Kenya	>2 mo (2158)	RT-PCR	81 (3.8%)	
China	≥18 yr (507)	MIF	60 (11.4%)	Paired sera were obtained from 320 (63%) patients
Thailand	All ages, range not specified (3417)	RT-PCR, MIF	92 (2.7)	Proportion diagnosed by PCR or serology not specified
Sweden	18–93 yr (184)	PCR, EIA	0	

[a]PCR or serology method not described.
[b]Study enrolled both patients and age-matched control subjects.
EIA, Enzyme immunoassay; *MIF,* microimmunofluorescence; *PCR,* polymerase chain reaction, *Pst1,* a restriction enzyme isolated from *Providencia stuartii*; *RT-PCR,* real-time PCR.
Modified from references 55–60.

hospitalized due to CAP.[64] Atypical pathogens were detected in 40% (58/146), viral etiologies in 36% (52/146), and coinfections in 19% (27/146). The most common etiologic agent was *M. pneumoniae* (n = 47), followed by *C. pneumoniae* (n = 11). The most frequent respiratory viruses detected were respiratory syncytial virus subtype A (n = 35), influenza virus (n = 21), and parainfluenza virus (n = 10). Virus-bacterium and bacterium-bacterium coinfections were found in 27 cases. *Mycoplasma pneumoniae* and *C. pneumoniae* were detected as frequently as respiratory viruses (36%) in this population. However, the prevalence of *C. pneumoniae* was significantly higher in this pediatric population compared to what has recently been reported in similar populations in the United States. The prevalence of *C. pneumoniae* was only 0.3% in the multicenter study evaluating the GenMark RP Panel; 58% (1222/2098) were ≤21 years of age and 842 (40%) were <5 years of age.[15]

Until more prospective studies are conducted with commercially available multiplexed NAAT assays for viruses and atypical bacteria, which also include diagnoses of typical bacteria such as *S. pneumoniae,* the role of *C. pneumoniae* in CAP remains less clear than when only serology was used for diagnosis of *C. pneumoniae.*[65]

CLINICAL MANIFESTATIONS

Most respiratory infections from *C. pneumoniae* are probably mild or asymptomatic. Initial reports emphasized mild atypical pneumonia clinically resembling that associated with *M. pneumoniae.*[8] Subsequent studies have found that pneumonia associated with *C. pneumoniae* has been clinically indistinguishable from other pneumonias.[55,66] The spectrum of disease in a recent outbreak in which molecular diagnosis with multiplex PCR was used is illustrative. *Chlamydia pneumoniae* was found in 73% of samples from patients with pneumonia but also in 36% of samples from patients with upper respiratory disease without pneumonia, all of whom recovered without therapy.[38] *Chlamydia pneumoniae* has been associated with severe illness and even death, although the role of preexisting chronic conditions as contributing factors in many of these patients is difficult to assess. *Chlamydia pneumoniae* can be a serious pathogen even in the absence of underlying disease; it was isolated from the respiratory tract and the pleural fluid of a previously healthy adolescent boy with severe pneumonia complicated by respiratory failure and pleural effusions.[67]

The role of host factors remains to be determined. Although *C. pneumoniae* has been detected in bronchoalveolar lavage fluid from 10% of a group of patients with acquired immunodeficiency syndrome and pneumonia, its clinical role in these patients is uncertain because most were coinfected with other well-recognized pathogens such as *Pneumocystis jirovecii* and *Mycobacterium tuberculosis.*[68] Gaydos and colleagues[23] identified *C. pneumoniae* infection with PCR assay in 11% of a group of immunocompromised adults with human immunodeficiency infection, malignant neoplasms, and other immune disorders, including systemic lupus erythematosus, sarcoidosis, and common variable immunodeficiency. *Chlamydia pneumoniae* was responsible for 6 of 31 episodes (19%) of acute chest syndrome in children with sickle cell disease in New York.[70] *Chlamydia pneumoniae* infection in these patients appeared to be associated with more severe hypoxia than was infection with *M. pneumoniae.*

The relationship of *C. pneumoniae* and upper respiratory infections, including pharyngitis, sinusitis, and otitis media, is less clear.

THERAPY

Data on treatment of *C. pneumoniae* respiratory infection are limited. *Chlamydia pneumoniae* is susceptible to antibiotics that affect DNA and protein synthesis, including macrolides; azalides, specifically azithromycin; tetracyclines; and quinolones (Table 182.2). However, in vitro activity may not always predict in vivo efficacy. Most published pneumonia treatment studies, including all recent studies, have used serology alone for diagnosis of *C. pneumoniae* infection, which is at best a clinical end point. Results of several multicenter treatment studies that used culture showed 70% to 86% efficacy of treatment with erythromycin, clarithromycin, azithromycin, levofloxacin, and moxifloxacin in eradicating *C. pneumoniae* from the nasopharynx of children and adults with CAP.[44] Most patients had clinical improvement despite persistence of the organism. Persistence did not appear to be the result of the development of antibiotic resistance because the minimal inhibitory concentrations of the isolates obtained after treatment did not change. Antibiotic resistance is unusual in chlamydiae.[44,71] Investigators were unable to select for macrolide resistance after passage of *C. pneumoniae* in subinhibitory concentrations of azithromycin.[72] In contrast, resistance to quinolones has been selected in vitro after passage of *C. pneumoniae* in subinhibitory concentrations of moxifloxacin.[73] These isolates were found to have a point mutation in the *gyrA* gene. Studies with long-term continuously infected cells suggest that *C. pneumoniae* may be refractory to antibiotics when in the persistent state.[74]

On the basis of these limited data, the following regimens can be used for respiratory infection from *C. pneumoniae* in adults: doxycycline, 100 mg orally twice daily for 14 to 21 days; tetracycline, 250 mg orally

TABLE 182.2 Comparative in vitro Activities of Currently Available Antimicrobials Against *Chlamydia pneumoniae*

ANTIMICROBIAL AGENT	MIC RANGE (μg/mL)
Doxycycline	0.015–0.5
Tigecycline	0.125–0.25
Erythromycin	0.015–0.25
Azithromycin	0.05–0.25
Clarithromycin	0.004–0.03
Ciprofloxacin	1–4
Levofloxacin	0.25–1
Moxifloxacin	0.125–1
Rifampin	0.0075–0.03
Trimethoprim	≥128
Sulfamethoxazole	≥500

MIC, Minimal inhibitory concentration.
Modified from Hammerschlag MR, Kohlhoff SA. Treatment of chlamydial infections. Expert Opin Pharmacother. *2012;13:542–552.*

four times daily for 14 to 21 days; azithromycin, 500 mg orally once daily followed by 250 mg/day for 4 days; clarithromycin, 500 mg orally twice daily for 10 days; levofloxacin, 500 mg intravenously or orally once daily for 7 to 14 days; or moxifloxacin, 400 mg orally once daily for 10 days. For children, the following regimens can be used: erythromycin suspension, 50 mg/kg/day for 10 to 14 days; clarithromycin suspension, 15 mg/kg/day for 10 days; or azithromycin suspension, 10 mg/kg once on the first day, followed by 5 mg/kg once daily for 4 days. Some patients may need re-treatment.

CHLAMYDIA PNEUMONIAE AND CHRONIC DISEASE IN HUMANS

One of the distinguishing characteristics of chlamydiae is their ability to cause persistent, often subclinical, infections. From a clinical standpoint, chlamydiae may be the persistent infection par excellence, capable of persisting in the host for months to years, often without causing obvious illness. From a microbiologic standpoint, persistence also refers to long-term intracellular infection that can be detected with antigen, microscopy, or nucleic acid–based amplification methods. Chronic persistent infection with *C. pneumoniae* has been implicated in the pathogenesis of several chronic diseases initially not thought to be infectious, including asthma, arthritis, and atherosclerosis. However, studies of the association of *C. pneumoniae* and these disorders have been hampered with difficulty in diagnosis of chronic persistent infection with the organism, which, in turn, makes determination of the efficacy of interventions difficult, especially with antibiotics.

Chlamydia pneumoniae and Asthma

Infection with *C. pneumoniae* has been linked to asthma by a large number of epidemiologic and clinical studies. The controversy about definition of infection and diagnostic tests contributes to the difficulty in interpretation and comparison of studies. The field is further complicated by differences in study populations in regard to asthma phenotype and the presence of acute symptoms. Table 182.3 summarizes selected studies that have examined the association of *C. pneumoniae* infection and asthma. The wide range of positivity illustrates the sometimes contradictory findings regarding an association between *C. pneumoniae* and asthma, some of which may be explained by differences in populations and diagnostic methods.

In 1991, Hahn and colleagues[75] reported an association between serologic evidence of acute *C. pneumoniae* infection and adult-onset asthma and asthmatic bronchitis in the United States. Studies that have shown an association or lack of association between the presence of antibodies (IgG, IgM, and IgA) and higher antibody titers (IgG) against *C. pneumoniae* with asthma have been reported since then in a variety of populations.[76] Studies that used direct detection methods (culture or PCR) were more consistent in establishing a role of *C. pneumoniae* in exacerbations of asthma (see Table 182.2). In patients with stable asthma symptoms, evidence for infection with *C. pneumoniae* of up to 22% with PCR, alone or in combination with *M. pneumoniae,* may suggest chronic infection.[76] The clinical implications of *C. pneumoniae* infection in patients with asthma who have no acute symptoms are not clear; the obvious concern is that the persistent presence of the pathogen may lead to ongoing inflammation and thus contribute to severity and progression of asthma.[77]

Currently, minimal data exist to examine the immunologic basis for the association between *C. pneumoniae* and asthma pathology. Persistent infection with *C. pneumoniae,* which has been shown in patients with asthma, might be the result of an insufficient Th1 response in these patients, which is critical for clearance of the intracellular bacterium.[66,78] In analogy to the correlation of abnormal host immune response to *C. trachomatis* infection and tissue sequelae, a similar relationship may conceivably exist between respiratory infection with *C. pneumoniae* and asthma pathology.[79] Abnormal cellular immune responses to respiratory infections with *C. pneumoniae* in patients with asthma may in part be related to genetic variation in immune mediator genes.[80] Genetic variation of Toll-like receptor 2 is under investigation as a major factor in the development of asthma and may be related to susceptibility to *C. pneumoniae.* In Toll-like receptor $2^{-/-}$ mice, decreased IFN-γ and adaptive cell responses led to poor control of respiratory infection with *C. muridarum* and prolonged inflammation.[81] Differences in *C. pneumoniae* IgG antibody responses were seen in children with asthma, depending on variant mannose-binding lectin alleles.[82] An association between wheezing and anti–*C. pneumoniae* IgE in children infected with *C. pneumoniae* was shown, which suggests a Th2 response to the bacterium in patients with asthma.[83] The role of stress and host genetics in delayed or suboptimal Th1 response to chlamydial infection and development of complications in certain individuals, and the role of specific *C. pneumoniae* antigens in eliciting harmful immune responses in patients with asthma, are currently unclear.

Therapy

If infection with *C. pneumoniae* contributes to inflammation in patients with allergic asthma, diagnosis and treatment of these infections is important. Interactions may also exist between *C. pneumoniae* infection and asthma drugs. Treatment of asthma exacerbations frequently includes systemic steroids, which have been shown to enhance the in vitro infectivity of *C. pneumoniae*[84]; this was reflected in significant increases of inclusions but did not affect the in vitro activities of azithromycin, erythromycin, and doxycycline against *C. pneumoniae.*[84]

Several studies have addressed the question of whether antibiotic treatment of *C. pneumoniae* infection in patients with asthma leads to improvement in disease activity. Study design has been complicated by the fact that macrolides, quinolones, and tetracyclines all have immunomodulatory activity independent of their antimicrobial activity.[85,86] Any positive treatment outcomes may therefore be the result of antichlamydial or immunomodulatory effects, or a combination of the two. Several uncontrolled studies showed beneficial effects of antibiotics on patients with asthma with proven or presumed *C. pneumoniae* infection.[66,87] Subsequent placebo-controlled trials attempted to confirm the benefits suggested by these preliminary studies. A placebo-controlled 6-week trial of roxithromycin in patients with asthma who were seropositive for *C. pneumoniae* showed significantly higher morning peak expiratory flow in the treatment group at the end of treatment but not at subsequent time points.[88] In the absence of clear evidence that patients with asthma in this study had persistent *C. pneumoniae* infection, one could conclude that the treatment effect was the result of the antiinflammatory action of roxithromycin, which disappeared after stopping the drug. A double-blind, randomized, placebo-controlled study of telithromycin in patients with acute exacerbations of asthma found reduction of asthma symptoms among those treated with the active drug; however, the study could not adequately assess the effect of infection because only 1 of 278 enrolled patients was positive for *C. pneumoniae* with PCR assay of upper airway samples.[89] Similarly, in a randomized, controlled study of clarithromycin, the number of asthma patients who were PCR-positive for either *C. pneumoniae* or *M. pneumoniae* was only 12 and therefore the

TABLE 182.3 Summary of Clinical Studies of the Role of *C. pneumoniae* in Asthma

POPULATION (yr)	NO. WITH ASTHMA/CONTROL	CULTURE+ ASTHMA/ CONTROL (%)	PCR+ ASTHMA/ CONTROL (%)	SEROLOGY: MIF ASTHMA/ CONTROL	COMMENTS
United States, adults (1991)	365	—	—	Positive correlation between IgG titers (MIF) and wheezing	
Italy, adults (1994)	74/—	—	—	IgG seroconversion (10%/—)	Asthmatics with acute exacerbation
United States, children (1994)	118/41	11/4.9	—	No significant difference in IgG titers between groups; 58% of culture-positive asthmatics without IgG/IgM response	Asthmatics with acute exacerbation
Japan, adults (1998)	168/108	1.2/0	5.4/0.9	Higher prevalence rate of IgG and IgA in asthmatics (85%/68% and 48%/17%); mean IgG titers: 39/18	Asthmatics with acute exacerbation
Great Britain, adults (1998)	123/1518	—	—	No difference in prevalence rate of IgG titers (≥512 and ≥64–256) between groups (5.7%/5.7% and 15%/13%, respectively)	IgG ≥64–256 more common in subgroup of severe asthmatics (34.8%)
New Zealand, children and adults (2000)	96/102	—	—	No positive correlation between diagnosis of asthma and IgG titer at 11 yr and 21 yr of age	Asthma-enriched birth cohort; self-reported asthma
Italy, children (2000)	71/80	—	8/2.5	Serologic response consistent with acute infection: 13%/0%	Asthmatics with acute exacerbation
United States, adults (2001)	55/11	0/0	12.7[a]/0	Serologic response consistent with acute infection in 42% of PCR-positive asthmatics	Stable asthmatics
Great Britain, adults (2004)	74/74	—	22[b]/9[b]	—	Cases: stable atopic asthmatics; Controls: nonatopic spouses
Finland, adults (2005)	83/162	—	—	No difference in titers or conversion rates	Population-based cohort
Finland, adults (2006)	103/30	—	21[c]/37	—	Stable asthmatics

[a]Respiratory specimens obtained from lower airway (bronchoalveolar lavage, biopsy, or airway brushing).
[b]Positivity rate during 3-month (October to December) longitudinal study (at least one positive sample obtained on repeat sampling).
[c]Positivity rate in mild asthmatics: 20.8%; in moderate asthmatics: 22%.
Ig, Immunoglobulin; *MIF,* microimmunofluorescence; *PCR,* polymerase chain reaction.
Modified from Hammerschlag MR, Kohlhoff SA, Darville T. Chlamydia pneumoniae *and* Chlamydia trachomatis. *In: Fratamico PM, Smith JL, Brogden KA, eds.* Post-infectious Sequelae and Long-term Consequences of Infectious Diseases. *Washington, DC: American Society for Microbiology; 2008.*

study was underpowered to test the effect of clarithromycin on asthma outcome variables.[90] A randomized controlled trial of minocycline in patients with allergic asthma showed improved asthma symptoms and reduced total serum IgE, a beneficial effect that did not appear to be the result of a respiratory infection with *C. pneumoniae;* seropositivity for *C. pneumoniae* was not significantly different between patients and control subjects, and no patient had positive nasopharyngeal cultures for *C. pneumoniae.*[91]

Comparing studies of antibiotic treatment of patients with asthma is complicated by the use of different criteria of *C. pneumoniae* infection status (culture, PCR, serology, or a combination of these tests), use of nonstandard methods, and the unclear definition of chronic infection. Most studies have been underpowered to show effects of infections status. In conclusion, although diagnosis and treatment of *C. pneumoniae* infections in patients with asthma with signs and symptoms of an airway infection are recommended, the benefit of using antibiotics with activity against atypical bacteria in patients with asthma without laboratory evidence of infection remains controversial.

Chlamydia pneumoniae and Other Chronic Diseases

Persistent *C. pneumoniae* infection has also been implicated in the pathogenesis of several chronic diseases initially not thought to be infectious, including atherosclerosis, multiple sclerosis (MS), temporal arteritis, stroke, Alzheimer disease, lung cancer, and macular degeneration.[76] Studies in mice have shown that *C. pneumoniae* disseminates to the spleen and other organs after respiratory infection via macrophages.[92] However, this effect has not been conclusively shown to occur in humans. In addition, studies of the association of *C. pneumoniae* and these disorders have been hampered by difficulty in diagnosis of chronic persistent infection with the organism; no validated serologic or other surrogate markers exist for chronic *C. pneumoniae* infection.[19] The high prevalence of chlamydial infections and transient immunity after infection makes differentiation of persistent infection from reinfection or even past infection difficult. This, in turn, makes determination of the efficacy of any therapeutic intervention difficult.

Chlamydia pneumoniae and Atherosclerosis

Conventional risk factors, including cigarette smoking, hypertension, and high serum lipid levels, do not fully explain the incidence, prevalence, and distribution of coronary artery disease (CAD). Inflammation of the vessel wall plays an essential role in the initiation and progression of atherosclerosis, erosion, fissure, and eventual rupture of the atheromatous plaques.[93] Various markers of systemic inflammation, including C-reactive protein, have been found to predict future cardiovascular events, including nonfatal and fatal myocardial infarction and stroke. Although inflammation is present, the exact cause is still not known. Infectious agents, including cytomegalovirus, human herpesviruses, enteroviruses, *Helicobacter pylori,* bacteria involved with periodontal disease, and *C. pneumoniae,* have also been investigated as possible causes for this inflammation.

The first report that suggested a possible association between *C. pneumoniae* infection and CAD came from a case-control study from Finland published in 1988, showing that patients with proven CAD were significantly more likely to have antibodies to *C. pneumoniae* than control subjects selected at random.[94] This report was quickly followed by additional seroepidemiologic studies and studies that identified *C. pneumoniae* in atheroma with various methods, including culture, immunohistochemical staining (IHS), and PCR.[42,43] Animal studies have shown that *C. pneumoniae* can either induce or enhance the development

of atherosclerosis in mice.[95] In vitro studies have shown that *C. pneumoniae* can infect and replicate within monocytes, macrophages, and vascular endothelial and smooth muscle cells and that all are important components of atherosclerotic plaque.[93,96] In vitro infection also results in oxidation of cellular low-density lipoprotein; the production of proinflammatory cytokines involved in atherogenesis, including tumor necrosis factor-α, interleukin-6 and interleukin-1β, and interferon-α; and the transendothelial migration of neutrophils and monocytes.[93,96] *Chlamydia pneumoniae* can induce human macrophage foam cell formation in vitro, a key event in early atheroma development.[97] However, this may not be specific because the key component appears to be the chlamydial lipopolysaccharide, which is conserved in all chlamydial species, including *C. trachomatis*.

However, no single serologic, PCR, or IHS assay has been used consistently across all studies, and these assays are not standardized. In 2002, Boman and Hammerschlag[42] reviewed 14 seroepidemiologic studies published from 1992 to 2000 and found a great deal of heterogeneity among these studies in terms of the serologic tests used and the criteria for seropositivity. In some studies, an IgG or IgA titer of 1:64 or more was used as an indicator of chronic infection; in others, the same criteria were used as indicators of *past* infection. Nine of these studies used the MIF assay; all were in-house tests. The antigen used was only specified in four of the MIF assays. The remaining studies used a variety of other methods, including genus-specific enzyme immunoassays and whole-cell immunofluorescence. As stated previously, MIF assays are not standardized and are subject to significant operator variation.[41] Background seropositivity rates in the general population often exceed 70%, which can also make demonstration of an association between the presence of *C. pneumoniae* antibodies and CAD difficult. Earlier case-control studies that showed an association were generally small and based on single serum samples, which does not take into account that antibody titers fluctuate over time. A meta-analysis of 12 studies only found combined odds ratios of 1.15 and 1.13 for IgG and IgA antibodies, respectively.[98]

Boman and Hammerschlag[42] also analyzed 43 studies, published from 1992 through 2000, that examined 2679 samples of atheromatous tissue for the presence of *C. pneumoniae* with culture, electron microscopy, PCR, and IHS. The overall rates of detection of *C. pneumoniae* ranged from 0% to 100%, with 49.7% being positive by at least one method. However, when specimens were analyzed with more than one method, the prevalence rate of specimens positive by at least two methods (usually IHS and PCR) was only 15.14%. As with the serologic studies, major variation was found in the methods, including the antibodies and techniques for IHS and PCR. IHS has also been found to have problems with interpretation and reproducibility. Studies from Hoymans and colleagues[20] reported that ceroid, an insoluble lipid present in plaque, could cause nonspecific reactions with IHS.

The extent of interlaboratory variation with performance of PCR was shown by Apfalter and colleagues,[30] who sent a panel of 15 homogenized clinical atheroma specimens (carotid and coronary) and control specimens to nine laboratories in Europe and the United States for detection with PCR. The positivity rate in the clinical specimens ranged from 0% to 60%, and three laboratories identified *C. pneumoniae* in negative control specimens. The concordance between the assays was only 25% for one specimen. Subsequently, Apfalter and colleagues[32] showed that contamination was practically impossible to avoid with nested PCR assays. Ieven and Hoymans[43] published an analysis of studies reported through 2003, many of which used real-time PCR and were found to be predominantly negative. Real-time PCR is much less subject to contamination from amplicon carryover. Ieven and Hoymans[43] also noted that in studies where serology was done in addition to PCR, no correlation was found between presence of *C. pneumoniae* in the atheroma tissue and presence of anti–*C. pneumoniae* antibodies in individual patients.

With extrapolation from the observation that *C. pneumoniae* can disseminate systemically in mice after intranasal inoculation,[92] the presence of *C. pneumoniae* in PBMCs has been suggested to act as a surrogate marker for infection with *C. pneumoniae* in individuals with cardiovascular and other diseases.[42,43] More than 20 studies that examined the presence of *C. pneumoniae* DNA in PBMCs have been published, and as seen with studies of vascular tissue, the reported prevalence rate of *C. pneumoniae* DNA in PBMCs has also varied significantly, from 0% to 59% of patients with CAD and 0% to 46% of healthy blood donors.[99] Kohlhepp and others[100] examined PBMCs from more than 300 blood donors, either younger than 20 years or older than 60 years. The samples were divided and sent to two different laboratories, where they were tested for *C. pneumoniae* DNA with real-time, touchdown, and nested PCR assays. Only two samples from the younger–than–20-year-old group were positive in one of the laboratories but negative in the second. None of the samples for the more–than–60-year-old group was positive in either laboratory. This study showed that two different laboratories, with different extraction methods and real-time PCR targets, did not detect *C. pneumoniae* DNA in both cohorts of patients, but evidence of interlaboratory discrepancy was found with two specimens. More recently, West and coworkers[101] examined PBMCs from 86 patients with angiogram-documented CAD and 91 age and gender control subjects for the presence of *C. pneumoniae* DNA by using two different real-time PCR assays that used different genetic targets. No *C. pneumoniae* DNA was detected in any of the specimens, including serial specimens from a subset of patients followed for 8 months. The background prevalence of anti–*C. pneumoniae* IgG of greater than or equal to 1:16 was 74% in case and control subjects.

Results of the initial seroepidemiologic and organism detection studies led to several preliminary studies that investigated the efficacy of antibiotic treatment directed at *C. pneumoniae* for the prevention of secondary cardiac events. The results of these preliminary studies suggested an effect but the studies were underpowered and raised questions about the antibiotic regimens used and methods of identification of patients with *C. pneumoniae* infection. The major assumption of many of the seroepidemiologic studies of the association of *C. pneumoniae* and atherosclerosis and other chronic conditions is that the presence of anti–*C. pneumoniae* antibody implies the presence of the organism somewhere in the body. However, earlier studies of patients with respiratory infection often found a poor correlation between serology and isolation of the organism from the respiratory tract.[55]

Gupta and others[102] randomized 60 men with prior myocardial infarction and who were seropositive with MIF (IgG ≥8) to receive either azithromycin 500 mg/day for 3 or 6 days or placebo. They found that the patients who received azithromycin showed a decrease in MIF IgG titers and had a lower risk of a secondary adverse cardiac event than the patients who received placebo. The antibiotic regimen used by Gupta and colleagues[102] was never studied for treatment of *C. pneumoniae* infections. A meta-analysis of 11 randomized trials, which enrolled a total of 19,217 patients, was published in 2005.[103] Seven of these trials used azithromycin; length of treatment ranged from 500 mg/day for 3 to 6 days to 500 to 600 mg/wk for 6 weeks to 1 year. Three studies used roxithromycin for 30 days to 6 weeks; one used clarithromycin, 500 mg/day for 85 days; and one used gatifloxacin 400 mg/day for 10 days/month for 2 years. The duration of follow-up ranged from 3 months to 2 years. The results of two of six of the earlier small studies (≤150 patients in each arm) favored antibiotic treatment, but all of the remaining five large studies favored placebo for all end points, including total mortality; subsequent myocardial events, including infarction; and unstable angina. Also, no relationship was found between outcome and *C. pneumoniae* serologic status. A similar analysis with similar results was published by Baker and Couch in 2007.[104]

A number of possible reasons have been proposed for the failure to show a positive effect of antibiotic treatment, including populations studied, trial design, and duration of treatment. Given lack of a reliable marker for endovascular *C. pneumoniae* infection and the largely negative results in recent organism detection studies, additional studies are unlikely to show any benefit of long-term antibiotic treatment in reducing mortality or cardiovascular events in patients with CAD.

Chlamydia pneumoniae and Multiple Sclerosis

During the past 60 years, 20 different bacteria and viruses have been proposed to be associated with MS. The results were often inconsistent. The possible association of *C. pneumoniae* and MS was first described in

a case study from researchers at Vanderbilt University Medical Center (VUMC); this study was then followed by a series of patients from VUMC in which the researchers claimed that they identified the organism with culture and PCR.[105] The hypothesis of how *C. pneumoniae* might cause MS was not clear. The results of subsequent studies from a number of other groups were conflicting, some finding *C. pneumoniae* DNA in approximately 30% to more than 80% of cerebrospinal fluid (CSF) specimens from patients with MS and in approximately 20% of CSF specimens from patients with other neurologic diseases, and others finding none in CSF and brain tissue with culture and PCR.[106,107]

In an effort to deal with the issue of laboratory-to-laboratory differences in methods used to detect *C. pneumoniae* in studies of MS, prospectively collected CSF specimens from patients with MS and other neurologic diseases were sent to laboratories at VUMC, Johns Hopkins University (JHU), and the University of Umea (UU) in Sweden and subsequently also to the CDC.[108] Thirty specimens from patients with MS and 22 control specimens were tested; none was positive with PCR at JHU, UU, and the CDC, but 73% of the CSF specimens from patients with MS and 23% of the control specimens were positive with PCR at VUMC. Reasons for these discrepant results were discussed and included poor sensitivities of the assays used by JHU, UU, and the CDC or specificity problems with the assays used by VUMC. The primer sets used by VUMC in the multicenter study were analyzed at the CDC and were found to have high sequence similarity to human DNA, as determined with a BLAST (basic local alignment search tool), suggesting that they were not specific for *C. pneumoniae*.[109]

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The complete reference list is available online at Expert Consult.

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183 *Mycoplasma pneumoniae* and Atypical Pneumonia

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SHORT VIEW SUMMARY

Definition
- Atypical pneumonia caused by *Mycoplasma pneumoniae* is a syndrome that in contrast to lobar pneumonia is:
  - Slower in onset
  - Generally milder
  - Not associated with lobar consolidation
  - Not associated with resolution by crisis
- Many other viral and bacterial agents can also produce an atypical pneumonia syndrome.

Etiologic Agent
- *M. pneumoniae*
  - Bacterium lacking cell wall
  - Adhesins and adhesion organelle important in pathogenesis
  - Fastidious
  - May be cultivable from sputum after symptoms of illness resolve

Extrapulmonary Sites and Manifestations of Infection
- Skin (maculopapular or vesicular rashes, Stevens-Johnson syndrome)
- Cardiac (pericarditis, myocarditis)
- Central nervous system (encephalitis or meningitis, myelitis, radiculopathy)
- Musculoskeletal (myalgia/arthralgia, rhabdomyolysis, arthritis)
- Raynaud phenomenon
- Renal (glomerulonephritis, nephrotic syndrome)
- Hematologic

Diagnosis
- Clinical suspicion by syndrome
- Presence of cold agglutinins in the presence of the syndrome and absence of another explanation
- Detection of *M. pneumoniae*–specific genetic components
- Detection of rising titers of antibodies to *M. pneumoniae*
- Detection of *M. pneumoniae*–specific antigens

Treatment
- Macrolides (e.g., azithromycin, 500 mg day 1, 250 mg day 2 to 5)
  - Macrolide resistance is increasing and may limit usefulness
- Tetracyclines (e.g., doxycycline, 100 mg twice daily for 7–14 days)
- Fluoroquinolones (e.g., moxifloxacin, 400 mg daily for 7–14 days)

The concept of atypical pneumonia antedated the start of the antibiotic era. At least as early as World War I it was recognized that "…in the larger number of cases observed in the [military] camps the pneumonia was of an atypical nature. The onset tended to be slower than that of the lobar pneumonia of civil life; the course more prolonged. Crisis was relatively rare; physical signs were slow of development and of patchy distribution and scattered in several lobes."[1]

With the introduction of sulfonamides in the 1930s and penicillins in the 1940s, it was recognized that some cases of pneumonia did not respond to these antibiotics and that many of these could not be attributed by Gram stain or culture to a known bacterial cause. The term "primary atypical pneumonia" was given to these cases; the prefix "primary" indicated that no causative agent could be determined.

Since then, as diagnostic microbiology and virology advanced, it was recognized that, in addition to *Mycoplasma pneumoniae*, multiple etiologic agents can produce the atypical pneumonia syndrome, including influenza virus, adenovirus, respiratory syncytial virus, cytomegalovirus, *Chlamydia* spp., *Legionella* spp., *Pneumocystis jirovecii*, and metapneumovirus. Additional agents will surely be recognized in the course of time, and the prefix "primary" is now mostly of historical interest. Atypical pneumonia is best regarded as a syndrome to be contrasted with the classic symptom complex of "typical" or lobar pneumonia, as exemplified by pneumococcal pneumonia.[2]

HISTORY

In 1938 Reimann described seven patients with similar clinical characteristics that he termed *atypical pneumonia*.[3] During World War II the syndrome assumed special importance because the majority of pneumonias encountered in the military were atypical.[4]

In 1944 *M. pneumoniae* was first identified as a transmissible cause of atypical pneumonia by Monroe Eaton and coworkers[5,6] and became known as the Eaton agent. In these original experiments sputum and homogenized lung tissue from autopsied patients with primary atypical pneumonia were inoculated into cotton rats, where they produced pneumonitis. The agent was then serially passed and titered in cotton rats and in hamsters, where it also produced pneumonitis. The agent produced no lesions when inoculated and passed in chick embryos. However, when transferred from chick embryo into cotton rats or hamsters, it produced pneumonia. The agent could be passed through a Millipore filter, was neutralized by sera from patients convalescing from atypical pneumonia, and could not be grown on standard bacteriologic media. Thus it was originally thought to be a virus. Demonstration that the agent could be inactivated by certain antibiotics cast doubt on this hypothesis, and the organism was subsequently grown on artificial media and shown to share properties with the group of infectious agents known as the pleuropneumonia-like organisms, or PPLOs.[7]

In 1943 Maxwell Finland and coworkers identified "cold agglutinins," isohemagglutinins that were active at 4°C, in the blood of some patients with atypical pneumonia, and in subsequent work they showed that the cold agglutinins were present in cases associated with the Eaton agent but not in cases associated with influenza virus.[8,9]

The relation of the Eaton agent to the atypical pneumonia syndrome was established by the observations that human serum from some patients recovering from atypical pneumonia neutralized the agent.[10,11] The causal link with atypical pneumonia was strengthened with the demonstration that neutralizing activity was present in convalescent serum from volunteers who were infected by inoculation of ultrafiltrates from those with naturally occurring disease.[12] Subsequently, the agent was grown in tissue culture[13] and cell-free media,[14] and its morphology was described.[15] The Koch postulates were completely fulfilled when the cultivated organism produced the disease in volunteers.[16] Although the similarity of the Eaton agent with organisms that caused pneumonia

in cattle led to its initial description as a pleuropneumonia-like organism, they were rapidly identified as mycoplasmas and named *Mycoplasma pneumoniae*.[6,17]

MICROBIOLOGY

The genus *Mycoplasma* falls within the class Mollicutes, prokaryote microorganisms that lack cell walls. *M. pneumoniae* is one of several members of this genus that cause disease in humans. It is among the smallest of free-living organisms that cause disease in man (≈10 × 200 nm) and has a relatively small genome of 816,394 base pairs of DNA encoding 679 genes.[18] Because it lacks a cell wall, *M. pneumoniae* is insensitive to penicillin and other β-lactam antibiotics and does not take up Gram stain.

M. pneumoniae can be grown in cell-free artificial media but has complex growth requirements and grows slowly. On horse serum and yeast extract–enriched agar medium, it requires 6 to 7 days to form granular or characteristically "fried egg"–appearing colonies. The fried egg appearance results because the centers of the colonies are dense and embedded in the agar medium, whereas the less dense periphery is spread on the agar surface. At maturity colonies may range in size from 50 to 100 microns in diameter. When stained with Dienes stain, colonies contain densely stained small granules. *M. pneumoniae* ferments glucose and other sugars, including xylose, mannose, maltose, dextrin, and starch. It produces a hemolysin that will lyse human, guinea pig, or horse erythrocytes within 24 to 48 hours in artificial media.[19]

Lacking a cell wall, *M. pneumoniae* is bounded by a trilaminar cell membrane that is rich in sterols. It divides by binary fission and is pleomorphic when grown on an inert surface.

M. pneumoniae adheres to respiratory epithelial cells and to red blood cells via sialic acid receptors.[20,21] Adherence is mediated by a complex set of adhesion proteins, including P1, P30, proteins B and C, P116, and HMW1-3. These form an organelle at one end of the rodlike structure of the organism.[22,23] The adhesion organelle also allows the organism to have gliding motility.[24,25] Types 1 and 2 *M. pneumoniae* strains differ because of major variations of the P1 adhesion protein.[26] Distinguishing among these types is important for surveillance, epidemiologic, and clinical purposes.[24,27]

M. pneumoniae also elaborates a cytotoxin that has been called the community-acquired respiratory distress syndrome (CARDS) toxin. This is a cell-associated adenosine diphosphate ribososylating and vacuolating cytotoxin that is present in the inflamed airways of infected experimental animals and elicits antibodies that appear as the infection wanes.[28]

M. pneumoniae forms biofilms.[29] These are volcano-like structures composed of polysaccharide, protein, and lipid. The organisms become encased in the biofilm. It has been shown that types 1 and 2 *M. pneumoniae* strains form very different biofilms and that these confer differential resistance to antibiotics and may alter the ability of antibodies, complement, and white blood cells to penetrate to and attack the organisms.[30]

As macrolide resistance has become more prevalent, case reports have suggested that resistant isolates might be more virulent than sensitive ones[31,32]; however, in one family, a wide spectrum of severities were found.[33] Also, in a single case report severe disease was seen in a 14-year-old adolescent with infectious mononucleosis. At present, any association between macrolide resistance and disease severity must be judged speculative.

EPIDEMIOLOGY

M. pneumoniae are distributed worldwide with minor, if any, effects of climate on the incidence of disease. Long-term studies have shown a pleomorphic annual cycle: In some years there are seasonal peaks in fall and winter, whereas in other years there is little evidence of seasonality.[34–38]

Infection has been described at all ages; however, it is primarily a disease of childhood and adolescence, with the peak incidence of infection between 5 and 15 years of age. It has been reported that children younger than 3 years develop primarily upper respiratory tract infection, whereas those 5 to 20 years of age tend to develop bronchitis and pneumonia.[39] In older infected adults pneumonia predominates.[40] Because asymptomatic infection is common, descriptions of the disease's epidemiology must be interpreted in light of whether the data was collected from cases of symptomatic disease or from systematic survey of defined populations.

Seroepidemiologic studies in the United States showed an incidence of mycoplasmal pneumonia of 5 per 1000 per year among those 10 years of age and 1 per 1000 per year among those 25 to 50 years of age. Annual incidence fell as age increased further. There were no major sex differences.[34] It was estimated that, overall, at least one case of mycoplasmal pneumonia occurs annually for each 1000 persons, or more than 2 million cases annually. The total incidence of mycoplasmal infection at all sites in the respiratory tract may be 10 to 20 times higher.[3]

A prospective study of all adults from two counties in Ohio, who required admission for community-acquired pneumonia during 1991, demonstrated similar rates of hospitalization for *M. pneumoniae* and *Streptococcus pneumoniae* for those between 15 to 34, 34 to 65, and 66 to 79 years of age, with a continued increase in the incidence of disease from each pathogen in those older than 80 years, but in this oldest group there was a divergence, with the rate of *S. pneumoniae* disease rising above that resulting from *M. pneumoniae*. *M. pneumoniae* was not seasonal in this study, whereas *S. pneumoniae* had a clearly defined seasonality.[41]

M. pneumoniae is spread from person to person. Spread of infection appears to be by droplet infection. The infection is transmitted by coughing and spread of droplets, and patients may remain infectious for prolonged periods after many of the symptoms other than cough have disappeared. The organism and disease associated with it can relapse or be transmitted even after treatment of patients with effective antibiotics.[42]

When assessed within families, the rate of spread is slow, but, because of prolonged carriage, it is extensive. In one study, spread occurred in 23 of 36 families, and among the 23, 84% of children and 41% of the adults became infected.[43] In another study the total infection rate was 58% (81% in children).[44]

In closed populations, such as military recruit camps and boarding schools, *M. pneumoniae* disease can be epidemic.[1,45,46] Outbreaks have been described in schools,[47] hospital workers,[48] and even the confined spaces of nuclear submarines.[49] *M. pneumoniae* also can cause nosocomial infections in long-term care (LTC) facilities. A report from the Centers for Disease Control and Prevention (CDC) described an outbreak that occurred in an LTC facility in Nebraska.[50] The respiratory illness, characterized by cough and fever, occurred in 55 residents of the LTC and resulted in 12 hospitalizations and 7 deaths. *M. pneumoniae* DNA was detected by polymerase chain reaction (PCR) in 40% of the specimens collected. The outbreak was terminated by strict observance of good infection control practices. This report points out that serious *M. pneumoniae* infections are not confined to children and young adults, can cause disease in the elderly, and should be considered in clusters of nosocomial pneumonia.

Reports of two outbreak scenarios illustrate how transmission occurs and the disease spreads. One occurred among 26 of 55 members of a college fraternity who attended a pledging banquet.[51] Symptoms occurred in the first of the infected fraternity members 1 week after the banquet, and the epidemic reached its peak 6 days later. In this instance multiple members of the fraternity were simultaneously exposed to a high concentration of infectious *M. pneumoniae* and developed disease synchronously. In a second outbreak employees of a large hospital in New York City developed illness over a 6-month period (July–December), but a single source for the outbreak could not be identified.[52]

Because infection is often asymptomatic or mild in children, and shedding persists long after illness, even after treatment children are often the reservoir from which spread can occur.[53,54]

IMMUNOLOGY AND RESISTANCE

Innate Immunity

Initial host-parasite interactions with *M. pneumoniae* are likely to involve the innate immune system; however, the details are just beginning to be elucidated. Cathelin-related antimicrobial peptide, a molecule that protects the host from other infectious agents, was shown to inhibit the growth of *M. pneumoniae*, and its presence was increased in the cells of *M. pneumoniae*–infected mice.[55] Mice lacking the gene for heat

shock factor 1, a transcription-controlling molecule that is active in the response to a wide variety of stresses, had a higher concentration of *M. pneumoniae* in their lungs than did those with the gene.[56] Mice that lacked the gene for human SPLUNC1 (short palate, lung, nasal, and epithelial clone 1) protein had lower concentrations of *M. pneumoniae* than did those in which it had been inserted.[57]

Infection of cell cultures of A549 lung cancer cells with *M. pneumoniae* has been shown to increase the level of proinflammatory cytokines (interleukin [IL]-8, IL-1) in the cultures.[58]

In a guinea pig model peritoneal and alveolar macrophages ingested and killed opsonized *M. pneumoniae* at a rate slightly slower than other bacteria; however, the authors, Erb and Bredt,[59] concluded that the delay was not clinically significant. Of note, the cell-adherent but unopsonized organisms were relatively resistant to killing mediated by complement.

Adaptive Immunity

M. pneumoniae induces a rich immune response. The humoral response includes protective immunoglobulin G (IgG) and IgA antibodies. Mice born to immune mothers were protected from infectious challenge, whereas those born to nonimmune mothers were not. The protection was shown to be due to IgG antibody in maternal colostrum.[60] Similar protective effects were shown in a hamster model in which passive transfer of serum conferred humoral but not cellular immunity.[61] In human studies the level of IgA antibody in nasal secretions correlated with protection after experimental challenge.[62] Protection is probably mediated by antibody against *M. pneumoniae* polysaccharide rather than against its proteins, and the antibody may act by blocking the binding of the pathogen to epithelial surfaces.[63–65]

One of the striking aspects of the immune response to *M. pneumoniae* is the production of isohemagglutinins directed against the I antigen expressed on the surface of adult erythrocytes.[66] As discussed above, the presence of "cold agglutinins" was described in 1942 by Finland and coworkers.[67] They showed that such antibodies were present in 50% to 70% of patients with Eaton agent pneumonia and that the agglutination, produced by holding erythrocytes at 4°C, was reversible by warming them to 37°C.[8,68]

Cold Agglutinins

Cold agglutinins are IgM antibodies and are often present at the start of or in the first week of clinical illness. They may persist for several months after illness. Several theories have been suggested to account for their appearance. One is that the I antigen, present on erythrocytes, is also present in respiratory epithelium and is part of the receptor through which the organism attaches to the cell. Thus the antibody is actually directed at the binding complex or a part of it.[69] Another theory suggests that the antibody is actually directed against a polysaccharide component of *M. pneumoniae*. This is based on the observation that immunization of rabbits with either *M. pneumoniae*, *Streptococcus* MG (a group F α-hemolytic streptococcus), or *Listeria monocytogenes* produced cold agglutinins that were inactivated by incubation with *M. pneumoniae* membrane-associated lipopolysaccharide.[70,71]

Cold agglutinins have no known role in the development of pneumonitis, but they may occasionally be directly pathogenic, particularly when the antibody titers are high, producing hemolysis, capillary obstruction with the Raynaud phenomenon, renal failure, and, rarely, gangrene (Fig. 183.1B and C).[72–74]

Complement-fixing *M. pneumoniae*–specific antibodies also appear early in the course of illness and high levels persist for several months after infection. Seroprevalence surveys suggest that these antibodies persist at a low level for much more prolonged periods.[75–77]

Resistance and Susceptibility to Infection

There are few or no data relating specific conditions to the infectious inoculum of *M. pneumoniae*. Knowledge about which factors affect severity and duration of *M. pneumoniae* infection rests primarily on case reports or small series of patients with severe disease. Because severe disease is also seen in those without obvious predisposition, such associations must be considered tentative.[78–80]

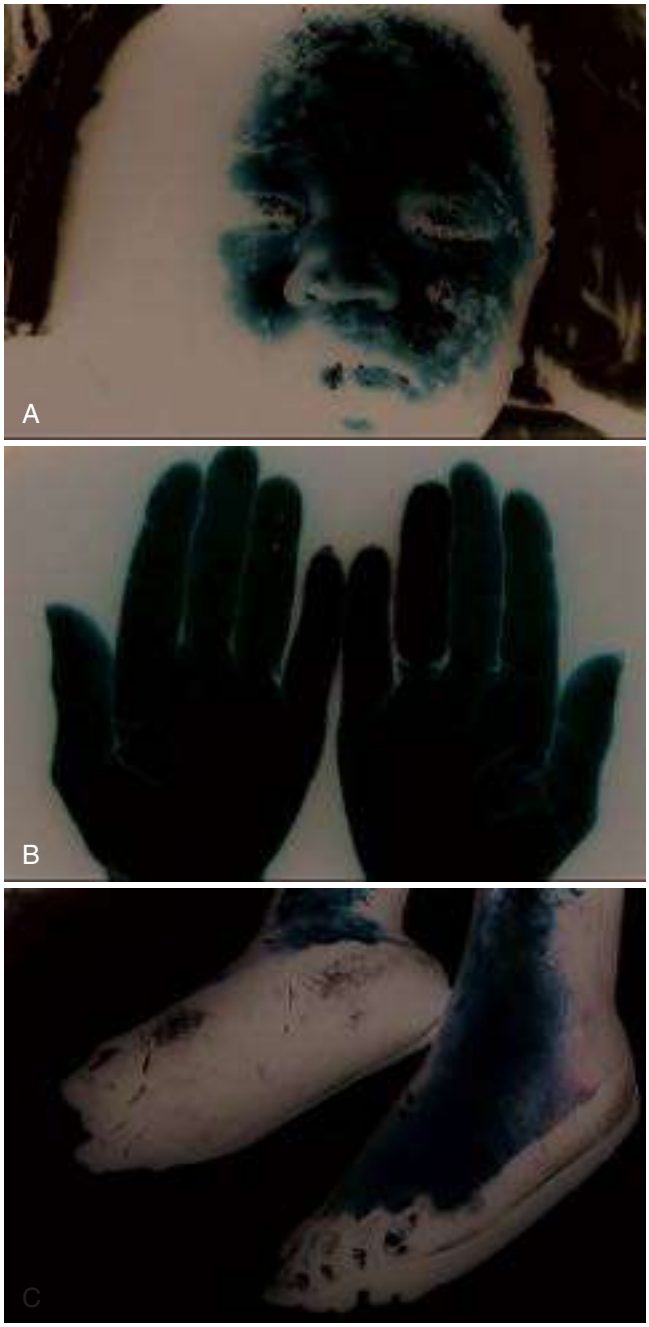

FIG. 183.1 Skin conditions associated with *Mycoplasma pneumoniae* infection. (A) Stevens-Johnson syndrome in a child with *M. pneumoniae* infection. (B) Raynaud phenomenon in a young woman with *M. pneumoniae* infection and high titers of cold agglutinins. (C) Necrosis of distal extremities in a patient with sickle cell disease who contracted *M. pneumoniae* infection accompanied by very high cold agglutinin titers.

Although the immune systems may play a role in pathogenesis of clinical disease, the humoral response to infection provides immunity to reinfection for some period of time. Reinfections with *M. pneumoniae* are generally milder than initial infections,[81–83] and both antibody (human and murine) and colostrum (murine) were protective against *M. pneumoniae* in murine models.[60,84] Intensity of antibody response was inversely correlated with severity of *M. pulmonis* infections in rats.[85] Additional studies in mice showed that in comparison with immunocompetent mice, those with X-linked immunodeficiency failed to show severe lung lesions despite *Mycoplasma pulmonis* in their lungs. Mice with severe combined immunodeficiency also had milder lung lesions but allowed the mycoplasma infection to spread to the joints, where they produced severe pathology.[86]

Several reports have suggested that children with sickle cell SS or SC hemoglobinopathy are predisposed to severe disease,[87,88] but severe disease has not been reported in association with sickle trait (hemoglobin SA). *M. pneumoniae* has been found to be responsible for 16% to 20% of cases of sickle cell anemia–associated "chest syndrome."[89,90] Children with Down syndrome have also been reported to be disposed to severe disease.[91,92] Neither adults with chronic obstructive lung disease nor cigarette smokers are predisposed to *M. pneumoniae* infection.[3,93,94]

M. pneumoniae is not generally considered an opportunistic pathogen in immunocompromised patients; however, a few reports suggest that cytotoxic chemotherapy and neutropenia may be associated with severe and prolonged disease.[95,96] A 7-year-old boy with hypogammaglobulinemia was reported to recover completely from mycoplasmal pneumonia but had continued carriage over a 2-year period despite additional courses of antibiotics.[97]

Many studies suggest that genetic factors, as yet undefined, may play a role. In the mouse there are marked strain differences in disease severity, and male sex is associated with severe disease.[98,99] Reviews of severe mycoplasmal pneumonia in humans have also shown a predominance of males.[100] One case report documents markedly different disease severity in identical twins, demonstrating that severity is not solely conditioned by genetic factors.[101]

Suprainfection during the course of mycoplasmal pneumonia is unusual; however, there are suggestions that when there is concomitant pulmonary inflammation or coinfection the clinical severity is worsened.[102] Thus, in a study of Kenyan refugee camps, persons coinfected with "atypical pathogens" and respiratory viruses were significantly more likely to have severe respiratory illness.[103] Acute exposure of mice to nitrogen dioxide worsened the pathologic findings when the exposed mice were subsequently infected with *Mycoplasma pulmonis*.[104]

PATHOLOGY

M. pneumoniae infections are common in children and young adults but are very uncommonly fatal. Postmortem examination in one fulminant case showed extensive exudates in the lungs, with abscess formation, pericarditis, and disseminated intravascular coagulation with involvement of skin, lungs, and adrenals. A review of 11 other autopsied cases showed similar findings.[105]

There are several mechanisms by which *M. pneumoniae* can cause disease. First, attachment of the organism to sialic acid receptors on respiratory epithelium, via its attachment organelle, may directly damage the respiratory epithelium and its ciliary activity.[20,23] Second, *M. pneumoniae* elaborates a cytotoxin, the CARDS toxin, that may also directly damage the respiratory tract.[28] Third, the organism may alter antigens on the surface of cells to which it is attached to the extent that autoantibody formation is elicited. This is presumed to be the mechanism by which the anti-I cold hemagglutinin antibody is formed.[66] Finally, *M. pneumoniae* and/or its CARDS cytotoxin may elicit the influx of inflammatory cells, which then produce proinflammatory cytokines that may result in damage to the host.[106,107]

DISEASE SYNDROMES

Respiratory Illnesses

Most *M. pneumoniae* infections are symptomatic, and most of these occur in children and adolescents. Although disease in children younger than 5 years does occur, it predominantly manifests itself as coryza, but *M. pneumoniae* may also be responsible for as much as 5% of episodes of bronchiolitis. In older children and adolescents the most common syndrome is a combination of tracheobronchitis and upper respiratory tract infection, such as coryza, pharyngitis, and otitis media.[39]

Atypical pneumonia is the predominant syndrome seen in young adults and adults (Fig. 183.2). The incubation period was 9 to 13 days in volunteer experiments, but estimates from clinical studies suggest that it may be as long as 3 weeks. After the incubation period, low-grade (<102°F) fever, malaise, headache, myalgia, and cough develop over a few days. The gradual onset of atypical pneumonia is in contrast to the sudden onset of classic lobar pneumonia, with its high fever, shaking chill, and pleurisy, as well as to classic influenza, with its high fever and rapid progression of symptoms from the nose to the throat and chest.

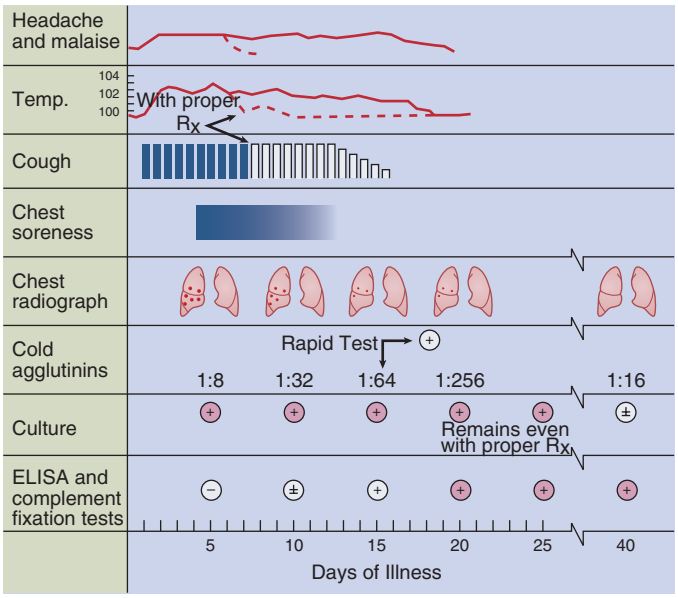

FIG. 183.2 Major clinical and laboratory manifestations of mycoplasmal pneumonia. *ELISA,* Enzyme-linked immunosorbent assay; *Rx,* treatment; *Temp.,* temperature. *(Modified from Baum SG. Mycoplasmal infections. In: Wyngaarden JB, Smith LH Jr, eds.* Cecil Textbook of Medicine. *17th ed. Philadelphia: Saunders; 1985:1506.)*

Coughing or tracheitis may result in substernal soreness, but pleuritic chest pain is rare. Gastrointestinal symptoms are unusual.

Physical examination of a patient with mycoplasmal pneumonia shows relatively few findings for observed degree of malaise. Cough is persistent, frequent, and debilitating. Nonexudative pharyngitis may be seen, but cervical lymphadenopathy is unusual. Examination of the chest by palpation and percussion is generally normal. Auscultation may indicate patches of rales at one or more sites, but they are often absent or heard only inconsistently. Wheezes and ronchi are not characteristic. The remainder of the examination is usually unrevealing unless there is a rash or extrarespiratory infection, as discussed in the following section.

Otitis and bullous myringitis were commonly seen in experimentally induced disease in adults[16] and in case series,[108] but other studies suggest that in clinical practice *M. pneumoniae* is only an occasional cause of otitis and that bullous myringitis is due to the same spectrum of pathogens as are other ear infections.[109] No useful diagnostic information is provided by the absence of bullous myringitis and its presence is of uncertain sensitivity and specificity.

Routine laboratory data are generally normal. There may be a mild leucocytosis in as many as 25% of cases, but the total white blood cell count rarely exceeds 15,000 per mL. Expectorated sputum is generally scanty and not viscous. Gram stain of sputum, if of sufficient quality for interpretation, shows a mixture of polymorphonuclear and mononuclear cells. Chest radiographs or other imaging may show one or more patches of alveolar fluid or bronchopneumonia, and some consolidation may be evident in approximately a quarter of the cases. Severe cases may show a bronchopneumonia extending from the hilum and involving the entire lung. Pleural effusions, generally small and transudative, may be seen in 5% to 20% of cases.[108] Typically, imaging shows more disease than was anticipated from the physical signs. Lobar consolidation is unusual but has been reported.[110]

The natural history of such a case is one of extended illness, with fever and cough lasting well into the third week of illness, and then followed by gradual recovery. There is no "crisis" as there is in untreated pneumococcal pneumonia. Appropriate antibiotics shorten the duration of illness but do not reliably eradicate the organism from the respiratory tract, and convalescent cases can serve as sources of infection to others.

That *M. pneumoniae* infection might precipitate exacerbations of childhood asthma was suggested in 1970.[111] Although such a role seems

well established, it is not unique to this organism.[112,113] Whether *M. pneumoniae* has any role as an initiator of childhood asthma remains unclear and controversial.[114] In infants without asthma, *M. pneumoniae* may be an etiologic agent for acute bronchiolitis.[115]

Other Illnesses

Although respiratory illness is the most common syndrome in *M. pneumoniae* infections, other organs of the host can be involved, and extrapulmonary symptoms may be present or predominant. The extrapulmonary sites that are most commonly involved are the skin and mucous membranes, central nervous system (CNS), heart, hematopoietic system, kidneys, and musculoskeletal systems.

DERMATOLOGIC SYNDROMES

Skin disorders are common in *M. pneumoniae* infection, occurring in up to 25% of cases.[116] Signs may range from relatively transient exanthems[43] to severe Stevens-Johnson syndrome (see Fig. 183.1A).[117] Among 29 patients with *M. pneumoniae* infection, cutaneous lesions were macular in 4 patients, maculopapular in 14, vesicular in 14, bullous in 6, urticarial in 2, and petechial in 1 patient. The rash involved the trunk and extremities in most of the patients. It generally began with the onset of fever and lasted for a week or more in most. Twenty-one of the patients had oral lesions, 14 had ulcerative stomatitis, and 7 had tonsillitis or pharyngitis. Eleven patients had conjunctivitis, 8 of them deemed severe.[118]

In a review of reports of dermatologic complications of *M. pneumoniae* infection, exanthematous rashes were noted to be reported in 8% to 33% of patients, urticaria in 7%, and Stevens-Johnson syndrome in 1% to 5%.[119] Because respiratory symptoms do not always predominate, *M. pneumoniae* infection should be considered in the differential diagnosis of patients who present with Stevens-Johnson syndrome. In a recent series from the Mayo Clinic, *M. pneumoniae* infection was present in 6 (22%) of such patients.[120]

Bullous erythema multiforme has also been reported in association with *M. pneumoniae* infections. The bullous lesions seen in this condition are commonly caused by herpes simplex virus, and an early review concluded that bullous erythema multiforme was not caused by *M. pneumonia*,[121] but a more recent review identified 33 such cases in association with *M. pneumoniae* infection and concluded that this infection could also be a cause.[119]

Raynaud Phenomenon

The Raynaud phenomenon is a vasospastic disorder characterized by blanching of the fingers and/or toes after exposure to cold temperatures, followed by extreme cyanosis, and then flushing when the extremities are rewarmed. During this process the digits go from white to blue to red in color. The Raynaud phenomenon occurs more commonly in women than in men, and it is often seen in patients with rheumatologic disorders.

The Raynaud phenomenon has been reported very rarely in association with *M. pneumoniae* infection (see Fig. 183.1B). A 3-year-old child developed the Raynaud phenomenon in association with respiratory symptoms, a patchy pulmonary infiltrate, elevated titers of cold hemagglutinins, and an elevated titer of complement-fixing antibody to *M. pneumoniae*.[73] In another series of cases, "cold hands" but none of the other features of the Raynaud phenomenon, were observed in 1 of 40 patients with proven *M. pneumoniae* infection seen during an outbreak in Scotland.[66]

CARDIAC SYNDROMES

M. pneumoniae infection can affect the heart. Among 560 patients with serologically confirmed *M. pneumoniae* infection seen in hospitals in Helsinki, Finland, 25 (4.5%) had evidence of carditis. Patients with carditis ranged in age from 1 to 68 (mean, 38) years. Thirteen were female and 12 were male. Six had pericarditis (2 without and 4 with pericardial effusion). Electrocardiographs (ECGs) were abnormal in 19 patients. In 5 patients there were ST-T wave changes only, and in 4 patients there were ST-T wave changes with atrioventricular block. Two patients had atrial fibrillation, 1 had atrial flutter with variable conduction, and 1 had supraventricular tachycardia. Two patients had ventricular ectopic beats, 1 patient had ventricular fibrillation, and 2 had ventricular bigeminy. Nineteen patients had myopericarditis: 2 with ECG changes only; 5 with ECG changes and cardiac enlargement; 8 with ECG changes and a transient murmur; 4 with ECG changes, a transient murmur, and enlargement of the heart. Fourteen of 23 patients developed long-term sequelae of carditis (2 were not followed). Four of the patients had persistent heart failure, and 9 had persistent rhythm disturbance. One individual, who was 25 years of age at his initial presentation with *M. pneumoniae* and carditis, had acute myocardial infarctions 3 and 5 months later.[122]

In another series from Pennsylvania, 13 (9.2%) cases of pericarditis and myopericarditis occurred among 141 patients with proven *M. pneumoniae* infection. Five patients had myopericarditis, and 8 had pericarditis alone. Six were deemed critically ill, and 1 was moderately ill. Three of the 5 patients with myopericarditis developed heart failure, and 1 developed a hemopericardium. All recovered from the infection and were discharged from the hospital, but 2 died during the follow-up period.[123]

Individual case reports confirm the presence of *M. pneumoniae* in cardiac sites. In two cases *M. pneumoniae* was cultured from pericardial fluid or tissue.[124] A marrow transplant recipient developed pericarditis, and the organism was demonstrated by electron microscopy and PCR.[125]

NEUROLOGIC SYNDROMES

Neurologic complications are a well-described complication of *M. pneumoniae* infection, and CNS symptoms occur in up to 7% of patients hospitalized with this infection. A cluster of encephalitis cases among schoolchildren led to the discovery of a much more widespread outbreak of *M. pneumoniae* respiratory infections in the community.[47]

Manifestations include encephalitis, aseptic meningitis, transverse myelitis, stroke, and radiculopathy.[126–129] In one series of 38 cases of atypical pneumonia in which neurologic complications occurred, 24 patients had meningoencephalitis, 5 patients had transverse myelitis, 4 had stroke, 4 patients had ascending paralysis, and 1 patient had cranial nerve palsy.[130] Three recently published articles illustrate the severe neurologic complications of *M. pneumoniae* infection that can occur in children. The first described 7 children who developed severe Guillain-Barré syndrome (GBS) in association with IgM antibody to *M. pneumoniae*.[131] Five of the children required mechanical ventilation, 1 died, and only 2 recovered completely. A second series from France reported on 9 children ranging in age from 4 to 14 years of age with neurologic problems complicating *M. pneumoniae* infection, including meningoencephalitis in 6 children, ophthalmoplegia in 2 children, myositis in 1 child, and GBS in 1 child.[132] The third series involved 42 children who developed neurologic diseases of 365 children with proven *M. pneumoniae* infection seen at the Hospital for Sick Children in Toronto, Canada over a 16-year period from 1996–2013. Of these children with neurologic disease, 22 had encephalitis, 5 had acute disseminated encephalomyelitis, 5 had transverse myelitis, and 7 had other conditions, including 2 with GBS.[133] The authors of this series noted two patterns of onset of neurologic disease. First were the children who developed with little (<7 days) or no prodromal respiratory disease, and the second group had respiratory symptoms (usually dry cough) for 7 days or more. The former were predominantly PCR positive for *M. pneumoniae* in cerebrospinal fluid (CSF) but not in the respiratory tract, whereas the latter were predominantly PCR positive in the respiratory tract but not in CSF. The authors concluded that the first group had direct invasion of the CNS by the infection, whereas that mechanism in the latter CNS involvement was indirect and most likely immunologically mediated.

Brainstem involvement and dysfunction may be seen.[134] Bickerstaff encephalitis and Miller Fisher syndrome, two syndromes associated with ataxia and ophthalmoplegia, have been reported to occur.[135,136] Both syndromes are also associated with increased CSF protein concentrations without pleocytosis and with increased concentrations of anti-CQ1b antibody. Both may respond to corticosteroids, plasmapheresis, or immunoglobulin therapy.

MUSCULOSKELETAL, RENAL, AND HEMATOLOGIC SYNDROMES

Myalgia and arthralgia are common symptoms in *M. pneumoniae* infections, occurring in up to 45% of patients.[137,138] Rhabdomyolysis has been reported.[139–141] *M. pneumoniae* osteomyelitis is very rare but

has been reported in immunocompromised individuals.[142,143] A case report of periprosthetic joint infection caused by *M. pneumoniae* has been published.[144]

M. pneumoniae infection has been associated with acute glomerulonephritis and the nephrotic syndrome.[145–149]

Hemolytic anemia is an uncommon, but reported, complication of *M. pneumoniae* infection.[118,137,150,151] Other hematologic complications include thrombocytopenia, pancytopenia, and disseminated intravascular coagulation. These complications are generally seen in the most fulminant cases[105,138,152–154] and are associated with the presence of cold agglutinins (see "Immunology and Resistance").

DIAGNOSIS

As a common cause of illness in children and young adults, *M. pneumoniae* should be regularly considered as a possible etiology in any upper respiratory infection in a child or young adult, as well as in older adults who have not responded to β-lactam antibiotics. In adults of any age with community-acquired pneumonia of mild-to-moderate severity, the present of persistent intractable cough is an important clinical clue that should suggest the possibility of mycoplasmal infection, as well as adult pertussis, especially in older adults. In the immunocompromised patient *M. pneumoniae* may cause severe pneumonia that is typically not lobar or consolidating in its radiologic appearance.

Multiple laboratory tests are available for diagnosis of *M. pneumoniae* infection. It appears that the best of these tests are those based on the detection of *Mycoplasma*-specific nucleic acids, although no single test is so good as to make the others obsolete.

Cold Agglutinins

The use of cold agglutinins as a diagnostic test is mainly of historical interest. A 1944 survey of the prevalence of cold agglutinins in various conditions in adults indicated that titers greater than 1:40 could be found in 137 of 200 patients with the atypical pneumonia syndrome (sensitivity, 68.5%) but only in 5 of 200 patients with acute bacterial pneumonia (specificity, 97.5%) and 9 of 658 patients with other conditions (specificity, 98.6%).[67] Similar results (sensitivity, 70%; specificity 97%) were found in a 1990 survey of 130 children with pneumonia by using a rapid cold agglutinin test with a diagnostic cutoff at a titer of greater than 1:32.[155] The description of a bedside test for cold agglutinins and evidence of a strong correlation with a titer of 1:64 by a more quantitative test[156] promoted widespread use; however, it is not clear if the performance characteristics of the bedside test, which may be performed by inexperienced physicians, match those of the laboratory test (Fig. 183.3).



FIG. 183.3 A positive bedside test for cold agglutinins. *(Modified from Callahan CW. Pneumonia and bacterial pulmonary infections. In: Panitch H, ed.* Pediatric Pulmonology. *Philadelphia: Mosby; 2005.)*

A test with a sensitivity of 70% and a specificity of 98% can provide useful diagnostic information. Applied to a person with pneumonia in whom the pretest probability of *M. pneumoniae* infection is low, for instance, 20%, a positive test would yield a posttest probability of 90%. Unfortunately, a negative test provides relatively little information. Applied to a person with pneumonia in whom the pretest probability is 80%, a negative test would only reduce the posttest probability to 55%.

Culture

Most clinical microbiology laboratories do not perform routine *Mycoplasma* cultures on respiratory secretions. Recovery and speciation of *M. pneumoniae* by routine methods takes too long to be useful in management; however, a rapid (24 hours to a presumptive positive) culture system has been developed.[157] Unfortunately, even when recovered, an isolate of *M. pneumoniae* cannot be regarded as diagnostic of illness etiology. As previously noted, asymptomatic carriage may be prolonged after infection, and many from whom the organism is recovered lack evidence of current infection.[158]

Detection of *Mycoplasma pneumoniae*–Specific Antibodies

A fourfold rise in the titer of antibodies to an uncultured organism is generally deemed indicative of concomitant infection with that organism. Because determination of a rising titer requires two separate samples of blood, spaced in time, saved, and then tested simultaneously in the same assay, the method is onerous and does not return results soon enough to be useful for decisions regarding management. To avoid delay, methods have been explored to identify single high titers of IgM antibody, which would presumably reflect current initial infection, and rising titers of IgG, which would be present on either initial or reinfection. Multiple commercial tests are available based on enzyme-linked immunosorbent assay, complement fixation, and particle agglutination.[159–161] A comparison of 12 such tests in 31 patients with positive and 96 with negative PCR tests for *M. pneumoniae* showed widely different sensitivities (35%–77%) and specificities (49%–100%) when using a diagnostic criterion of a single titer of IgM greater than or equal to 1:64. For the combined criterion of either seroconversion or a threefold rise in titer of IgG, the sensitivities ranged from 26% to 68%.[161] Using the specific PCR as the gold standard, another study demonstrated a sensitivity of 62.2% for a commercial IgM assay. Of note, many discordant results for PCR and IgM tests were observed, and IgM^+/PCR^- patients had frequent coinfections documented, including adenoviral and pneumococcal infections.[162]

Detection of *Mycoplasma pneumoniae*–Specific Antigens

Antigen capture immunoassays have been developed for the detection of *M. pneumoniae* in respiratory secretions. The sensitivity of one early test was 91.3% and the specificity was 100% when compared with seropositivity as the gold standard.[163] More recently, a commercial rapid antigen kit in Japan for the detection of the *Mycoplasma pneumoniae* ribosomal protein L7/L12 was found to have an approximately 60% sensitivity using PCR as the standard.[164] Although antigen detection remains an area of research interest, it has largely been supplanted by PCR-based diagnostic methods.

Detection of *Mycoplasma pneumoniae*–Specific Nucleic Acids

The detection of bacterial or viral nucleic acids for diagnostic purposes has been an active area of transitional research in the past 2 decades, and detection of *M. pneumoniae* DNA is now the diagnostic method of choice where available. PCR was applied to throat swabs from sputum to permit the detection of *M. pneumoniae* DNA encoding the *P1* adhesin gene as early as 1989 and to the detection of bacterial 16S RNA by 1991.[165,166] Early evaluation suggested that, compared with culture, sensitivity was 73% to 92%[167–169] and specificity was 96% to 100%, although at least one study subsequently showed lower sensitivity (57%).[170] Another study evaluated PCR and serologic testing in clinically diagnosed cases in outbreak settings and noted a 48% sensitivity of PCR if testing was performed during the first 3 weeks of illness.[171] PCR has been found

to be superior to IgM testing in acute infection.[172] In a comparison of 12 commercially available serologic tests with PCR, none of the serologic tests appeared to have superior performance.[161]

Differences in sensitivity between individual reports are likely due in part to differences in case definitions and timing of the tests over the course of illness, as well as in differences in the concentration of organisms or nucleic acids in clinical specimens. Sputum samples are more likely to yield positives than are nasopharyngeal aspirates or throat swabs.[173] Occasional detection of *M. pneumoniae* nucleic acids in clinical specimens from asymptomatic individuals suggests that a positive result should be deemed to have the same significance as would a positive culture. It does not prove that *M. pneumoniae* is the cause of the symptoms that generated the test. Currently, two US Food and Drug Administration–cleared or –approved multiplex PCR panels include testing for *M. pneumoniae*.

TREATMENT

Many *M. pneumoniae* infections in children produce few or no symptoms and resolve spontaneously without treatment. The more severe infections are brought to medical attention and, if recognized, can be treated with antibiotics; however, the carriage of *M. pneumoniae* is not always eradicated. In a classic study Smith and coworkers[42] treated 35 Eaton agent–infected volunteers with either tetracycline or erythromycin. They demonstrated that treatment shortened the duration of symptoms, with improvement in symptoms generally within 24 hours. They noted that 8 of 25 tetracycline-treated and 5 of 10 erythromycin-treated continued to shed *M. pneumoniae* after completion of therapy.[42]

M. pneumoniae lacks a cell wall. Hence it is insensitive to the penicillins and other β-lactam antibiotics. The organism has generally been susceptible to fluoroquinolones, both older and newer macrolide antibiotics, and tetracyclines. Azithromycin has been favored due to its lower minimum inhibitory concentration (MIC) in *M. pneumoniae* and fewer contraindications in children than quinolones and tetracyclines. However, macrolide-resistant *M. pneumoniae* strains have become increasingly prevalent, particularly in Asia, and resistance is primarily due to point mutations in the peptidyl-transferase loop of the 23S rRNA, most commonly the A2063G mutation.[174,175] A 2010 report from China documented a 69% prevalence of resistance and treatment failure when the MIC was 2 μg/mL or greater,[176] A survey of Chinese isolates from 2008–12 found a rate of macrolide resistance of 90.6%,[177] and a nationwide survey of pediatric isolates in Japan over the same 5-year period found macrolide-resistance mutations in 70.3% of isolates.[178] Rates of resistance in the United States appear lower. In a study of 91 *M. pneumoniae* specimens collected from six locations in the United States from 2012–14, 13.2% of isolates were found on genotypic and phenotypic analyses to be macrolide resistant.[179] Tetracycline antibiotics remain effective against macrolide-resistant strains.[180] In a retrospective review of outcomes in hospitalized patients with *M. pneumoniae* pneumonia in Japan from 2010–13, during which macrolide-resistance rates were high, fewer patients begun on tetracyclines or fluoroquinolones required switches to other agents than those begun on macrolides.[181] Although a newer fluoroquinolone antibiotic has been approved for use in the pediatric population in Japan, it is not clear which agents might be acceptable for use in the pediatric populations of Europe and the United States if these macrolide-resistant organisms become more prevalent as a cause of symptomatic disease.[182]

The optimum duration of therapy is uncertain, although reports of success with 1, 3, and 5 days of azithromycin therapy have been reported.[183,184] Some authors suggest that shorter durations of treatment may lead to relapse.[185] Selection of the optimum antibiotic in a particular case involves considerations of local prevalence of macrolide resistance, patient age, potential drug interactions, risk of photosensitivity, presence of liver or renal disease, and pregnancy. For otherwise healthy adults in areas with a low prevalence of macrolide resistance, treatment options include a second-generation macrolide (e.g., azithromycin, 500 mg orally on day 1 and 250 mg daily on days 2–5), a tetracycline (e.g., doxycycline, 100 mg orally twice daily for 7–14 days, or a fluoroquinolone (e.g., moxifloxacin, 400 mg orally once daily for 7–14 days).

PREVENTION

Infection Control

M. pneumoniae infection is believed to spread by droplet transmission, and CDC guidelines for hospital infection control recommend droplet precautions for the duration of illness. As shedding continues to occur for prolonged periods, convalescent cases are a reservoir from which new infections arise in small groups such as families or larger ones such as military recruits.[43,82]

In an observational, nonrandomized study of an outbreak in a facility for the developmentally disabled, the investigators assessed effect of "standard" epidemic control measures (i.e., active surveillance, use of standard and droplet precautions for cases, cohorting of residents and staff, and promoting hand washing) and standard measures plus preventive use of azithromycin. The adjusted secondary attack rate in the absence of any measures was 17.6%; for standard measures alone, it was 7.5%; and for standard measures plus azithromycin, it was 0.9%. The observational nature of this study prevents the drawing of any definitive conclusion, but the results are consistent with the efficacy of both standard control measures and preventive antibiotic therapy.[186]

Vaccination

The occurrence of epidemic *M. pneumoniae* in military camps and the importance of mycoplasmas as veterinary pathogens have maintained interest in the creation of vaccines for human and animal diseases. Several live-attenuated or inactivated vaccines are in veterinary use in cattle, goats, sheep, and poultry; however, a review in 2009 concluded that evidence for efficacy was weak, and they "provide only transient or partial immunity and often induce unpleasant side effects."[187]

Human vaccine development has not yet yielded a vaccine suitable for general use. Formalin-inactivated vaccines were developed for human use in the 1960s and 1970s and have been tested on relatively large groups of people. In a meta-analysis of six such studies, four of them involving 66,458 military recruits and two of them involving 810 pediatric patients, there was an overall efficacy for preventing pneumonia of 41% in the military studies but only 14% among the children. This difference, although large, was not statistically significant. Overall efficacy, considering all six studies, was 30%.[188]

Live-attenuated *M. pneumoniae* vaccines have been developed and tested using a hamster model, and these vaccines were also shown to have low efficacy: 50% for one strain and 10% for a second.[189–191]

Cell-free protein-based vaccines have not been superior to the whole-cell preparations. Immunization of guinea pigs with the adhesion protein, which binds the organism to respiratory epithelium, was not protective. In immunized animals severe pneumonia developed with infectious challenge, despite the fact that the animals had enhanced levels of *M. pneumoniae*–specific IgG, IgA, and adherence-neutralizing antibody.[192]

DNA vaccination of mice against *M. pulmonis* was described as protective in 1997.[193] DNA vaccine candidates are currently being evaluated for the prevention of porcine enzootic pneumonia caused by *M. hyopneumoniae*.[194,195]

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The complete reference list is available online at Expert Consult.

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184 Genital Mycoplasmas: *Mycoplasma genitalium*, *Mycoplasma hominis*, and *Ureaplasma* Species

David H. Martin

SHORT VIEW SUMMARY

Microbiology and Taxonomy

- The genital mycoplasmas include *Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum,* and *Ureaplasma parvum.* The two *Ureaplasma* spp. were assigned to separate species only more recently.
- The genital mycoplasmas lack cell walls and have the smallest genomes of known free-living microorganisms.

Epidemiology

- All of these organisms are primarily sexually transmitted but also may be transmitted to infants at birth. There is evidence that they may persist in children into adulthood.
- The epidemiology of infection with *M. genitalium* closely parallels that of *Chlamydia trachomatis* and *Neisseria gonorrhoeae.*

Clinical Manifestations

- *U. urealyticum* causes urethritis, whereas *U. parvum* appears to be a colonizer. Only relatively sexually inexperienced men are susceptible to *U. urealyticum* disease, which is likely due to the development of immunity after multiple exposures to this common urethral organism.
- Although the pathogenesis is unclear, the ureaplasmas are strongly associated with bronchopulmonary dysplasia in very-low-birth-weight infants.
- Both *Ureaplasma* spp. may cause hyperammonemia syndrome in immunocompromised patients, especially patients receiving lung transplants.
- *M. hominis* plays a minor role in genital tract disease but is implicated in postpartum fever, bacteremia, and postoperative mediastinal and wound infections.
- *M. genitalium* is a cause of urethritis in men and endocervicitis in women. It also is associated with pelvic inflammatory disease, infertility, ectopic pregnancy, and preterm birth.

Diagnosis

- Most clinical laboratories are not equipped to diagnose infections caused by these organisms. Only *M. hominis* is detectable using methods routinely employed in clinical laboratories, but sensitivity is poor. The ureaplasmas require special media for growth, and *M. genitalium* cannot be cultured at all outside research laboratories. Nucleic acid amplification assays for this organism are increasingly becoming available for clinical use.

Therapy

- Tetracyclines are active against most ureaplasmas and *M. hominis* but not *M. genitalium.* Macrolides are effective for the ureaplasmas but not for *M. hominis* and are only partially effective for *M. genitalium.* Quinolones, especially moxifloxacin, are active against all of the genital mycoplasmas.

TAXONOMY AND MICROBIOLOGY

The clinically important genital mycoplasmas—*Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum,* and *Ureaplasma parvum*—belong to the Mollicutes class and Mycoplasmataceae family of bacteria. *Mycoplasma* spp. and *Ureaplasma* spp. are the only two genera in this family. In this chapter the trivial term *genital mycoplasmas* is used to refer to these genera collectively. *Ureaplasma* spp. are most prominently distinguished from *Mycoplasma* spp. by virtue of their ability to hydrolyze urea for energy production. The two *Ureaplasma* spp. differ phenotypically and genotypically and were officially designated as separate species in 2002.[1] Originally they were believed to be biovariants of a single species and were first known as T-mycoplasmas and later renamed *Ureaplasma urealyticum.* This background information is important because textbooks and research papers published before 2002 did not distinguish the two species. The trivial term *ureaplasmas* is used in this chapter in reference to published research that did not differentiate the two species or in reference to conditions that are associated with both species.

Mollicutes in general have the distinction within the bacterial kingdom for having the smallest genomes of any known self-replicating organisms. The smallest of these is the genome of *M. genitalium,* which is only 580,000 base pairs in length and therefore is believed to be close to the minimal set of genes necessary for independent life. For this reason, its genome was among the first to be completely sequenced as well as the first to be completely reconstructed in vitro.[2,3] Mollicutes evolved from *Clostridia*-like gram-positive bacteria by virtue of extensive gene deletion. *M. genitalium* is very closely related genetically to *Mycoplasma pneumoniae,* having evolved away from this organism by additional gene deletions.[4] As with all members of the Mollicutes class of bacteria, the genital mycoplasmas lack a cell wall and thus are osmotically fragile. Reflective of their small genomes, they have limited biosynthetic capacity, which is why complex growth media containing sterols are required for their cultivation in vitro.

The natural habitat of the genital mycoplasmas is the human genitourinary tract, where they adhere to mucosal surface epithelial lining cells, although *M. genitalium* also can be found intracellularly.[5,6] They are found outside this environment less frequently, and, when they are, it is often in the setting of immune deficiency such as that found in very-low-birth-weight premature infants. Although the mechanisms differ among species, the genital mycoplasmas all have the ability to vary immunogenic proteins on their cell surface.[7] This appears to be the major mechanism by which they avoid the host immune response and is the reason they are able to persist for months to years in the same host.[7,8] *Mycoplasma fermentans* and *Mycoplasma penetrans* are two other mycoplasmas that have been identified in the human genitourinary tract, but they have not been consistently associated with human disease.[9] These are not discussed further in this chapter.

EPIDEMIOLOGY

All of the genital mycoplasmas are sexually transmitted, although their roles in genital tract disease vary significantly, as discussed in detail in "Clinical Manifestations."[10–13] The ureaplasmas are more commonly found in both the male and female genital tracts than either of the two *Mycoplasma* spp. In a study of healthy college men, ureaplasmas were present in the urethras of 29%, whereas *M. hominis* was present in 7%. For both organisms, prevalence correlated strongly with the number of sex partners.[14] Among sexually active female nursing students, the same group of investigators found that the prevalence of ureaplasmas varied from 38% to 75% and the prevalence of *M. hominis* varied from 9% to 17%, depending on the number of lifetime sex partners.[11] In a nationally representative sample of young adults in the United States, the prevalence of *M. genitalium* was 1.0%, whereas in the same population

the prevalence of *Chlamydia trachomatis* was 4.2%.[15] In this and another survey study,[16] prevalence of *M. genitalium* was related to the number of reported sex partners. Evidence for sexual transmission of *M. genitalium* was further strengthened by studies demonstrating high rates of genotype concordance within couples.[17,18] The epidemiology of *M. genitalium* closely parallels that of *C. trachomatis* and *Neisseria gonorrhoeae.* All are found more frequently in younger sexually active men and women and are more common in African Americans than in other racial groups.[15,19] Young age is also a risk factor for infection by the ureaplasmas and *M. hominis,* but racial associations have been less well studied.[14,20]

Neonates born vaginally are frequently colonized in both the genital and the respiratory tracts by ureaplasmas and less frequently by *M. hominis*.[21,22] Colonization after cesarean section occurs less commonly, supporting the hypothesis that exposure to vaginal secretions at birth is the primary risk factor for acquisition in neonates. Boys in general are less likely to be colonized than girls.[22,23] Colonization of the genital tract in girls persists in some cases and is likely to be a reservoir for infection of boys when sexual intercourse is first initiated.[24,25] Much less is known about transmission of *M. genitalium* to neonates at delivery. However, it was reported that 9% of 319 children undergoing bronchoscopy for medical indications at a mean age of 8 years had *M. genitalium* detected in lavage fluid by polymerase chain reaction (PCR) assay. This was compared with positivity rates of 10% for *M. pneumoniae,* 5% for *M. hominis,* and 9% for *U. parvum*.[26] If these data can be confirmed, it may be that all the genital mycoplasmas have the ability to colonize the respiratory tract and possibly may be present for long periods of time.

CLINICAL MANIFESTATIONS

See Table 184.1.

Ureaplasmas

The role of ureaplasmas in nongonococcal urethritis (NGU) has long been controversial in that numerous earlier studies failed to show that the organisms were more common in men with disease compared with control subjects without disease.[27] The best evidence for a pathogenic role for ureaplasmas in NGU comes from studies of men who are relatively inexperienced sexually and are having their first episodes of urethritis. In these men, the rate of isolation and the concentration of ureaplasmas in first-voided urine specimens are significantly greater in those with *C. trachomatis*–negative NGU than in those with *C. trachomatis*–positive NGU and in men without urethritis.[28] Data compiled from three Seattle studies showed that ureaplasmas were isolated significantly more often from men with a first episode of urethritis than from those who had had previous episodes or from men without urethritis.[28–30] The results of studies of selective antibiotic eradication of ureaplasmas also support their role in NGU.[28,30,31] Further evidence that the ureaplasmas cause NGU comes from self-inoculation studies performed by two investigators, both of whom developed evidence for urethral inflammation that responded to treatment.[32] Studies over the 15 years subsequent to differentiation of the two *Ureaplasma* spp. have further enhanced understanding of the role of these organisms in NGU. The evidence indicates that *U. urealyticum* is a urethral pathogen, whereas *U. parvum* does not appear to induce inflammation at this site.[33,34,35] The study by Wetmore and coworkers[35] reproduced the results of the previous studies by Bowie and colleagues[28,29,30] in that the strongest association of *U. urealyticum* with NGU was among men with the fewest lifetime sex partners. *U. parvum* was not associated with NGU at all in this study.[35] Thus, although it is reasonably clear now that *U. urealyticum* is a cause of symptomatic urethritis in men, this occurs in a subpopulation of men with relatively little sexual experience, so overall the proportion of NGU cases caused by this organism is small.

Ureaplasmas are present in the amniotic fluid of some women experiencing preterm labor and delivery and in cases of preterm premature rupture of membranes, leading to the hypothesis that the organism causes these conditions.[36] Supporting this hypothesis is a study of amniotic fluid obtained from normal pregnant women between 15 and 17 weeks of gestation, which showed that 11% were positive by PCR assay for ureaplasmas and that these women were significantly more likely to have preterm labor and delivery than women who were uninfected.[37] Additionally, the presence of ureaplasmas in the placenta at the time of delivery is strongly associated with chorioamnionitis.[38] However, a placebo-controlled treatment trial in pregnant women vaginally colonized with ureaplasmas did not show any benefit of erythromycin treatment.[39] These seemingly disparate outcomes may be explained by a molecular analysis of amniotic fluid from women in preterm labor with intact membranes. In addition to ureaplasmas, a number of fastidious bacteria were found that would have been missed by older culture-based studies, suggesting the possibility that the effect previously attributed to ureaplasmas could have been caused by other organisms.[40]

TABLE 184.1 Relationship Between *M. genitalium* and Disease Compared With *M. hominis* and *Ureaplasma* Species

	M. GENITALIUM		*M. HOMINIS*		*UREAPLASMA* SPP.	
CONDITION	**A**	**C**	**A**	**C**	**A**	**C**
Nongonococcal urethritis						
Acute	++++	++++	–		+++[b]	+++
Persistent	++++	++++	–		++	++
Balanoposthitis	++	+	–		–	
Chronic prostatitis	+	+	–		–	
Epididymitis	+	+	+	–	++	++
Bacterial vaginosis	++	–	++++	–	++	–
Cervicitis	+++	+++	–		–	
Infertility	+++	++	–		+	–
Ectopic pregnancy	+	?	+	–	+	–
Pelvic inflammatory disease	+++	+++	++	++	+	–
Postpartum fever	NS		+++	+++	+	–
Preterm delivery	+++	+++	+	–	+++	+
Neonatal lung disease	NS		+	?	+++	+

[a]Shown are the relative probabilities of the indicated mycoplasma being associated with (A) or causing (C) the conditions shown in the first column. ++++, very high; +++, high; ++, moderate; +, low; –, zero; ?, not certain; NS, not studied.
[b]The values shown for the nongonococcal urethritis categories are based on studies of *U. urealyticum.*
Modified from Taylor-Robinson D, Jensen J. Mycoplasma genitalium: *from chrysalis to multicolored butterfly.* Clin Microbiol Rev. *2011;24:498–514.*

As noted earlier in "Epidemiology," ureaplasmas are frequently transmitted to neonates at the time of delivery. Especially among very-low-birth-weight infants, ureaplasmas are associated with bronchopulmonary dysplasia.[7,36,41] However, antibiotic treatment trials in colonized preterm infants have not clearly demonstrated efficacy for the prevention of bronchopulmonary dysplasia.[42] Ureaplasmas may be isolated from the cerebrospinal fluid of preterm infants, but the significance is unknown.[43] In a prospective study, cerebrospinal fluid white blood cell counts and protein and glucose levels did not differ between *Ureaplasma* PCR-positive samples and culture-negative samples. Moreover, the infections appeared to resolve without therapy.[44] Similarly, cord blood and venous blood specimens are positive in 15% to 20% of very-low-birth-weight infants, but the clinical significance is unclear.[7,36] There appears to be no difference between the two *Ureaplasma* spp. in their potential to cause neonatal disease, but further studies are required.

Ureaplasmas can cause systemic infections in adults. Postpartum fever occasionally is a consequence of *Ureaplasma* bacteremia or endometritis.[45] The ureaplasmas are urease positive and have been implicated rarely as a cause of urinary infection stones.[46] There is evidence from two studies using suprapubic urine aspirations that ureaplasmas cause some cases of cystitis and urethral syndrome in women,[47,48] although there is no evidence that they are a cause of pyelonephritis.[49] Pericarditis and mediastinitis following open heart surgical procedures have been reported, especially in heart and lung transplant cases.[50] Postoperative sternal wound infections rarely occur in immunocompetent patients.[51] Hypogammaglobulinemic individuals appear to be at higher risk for systemic ureaplasma infections, especially septic arthritis.[52,53] Single cases of ambulatory peritoneal dialysis–associated peritonitis, meningitis, and brain abscess have been reported.[53–55] Because appropriate culture methods for ureaplasmas are not available in most clinical microbiology laboratories, the reported cases may represent the "tip of the iceberg." Ureaplasmas should be considered in culture-negative cases of any of these conditions.

Hyperammonemia is an uncommon but usually fatal syndrome in immunocompromised patients, particularly among recipients of lung transplants. A recent study has shown that this syndrome is caused by ureaplasmas, which is a major advance in that these infections readily respond to appropriate antibiotics.[56] Both *Ureaplasma* spp. have been implicated.

Mycoplasma hominis

Although a common inhabitant of the male genital tract, *M. hominis* is not a cause of NGU. Numerous studies have shown no difference in the prevalence of this organism in urethral specimens of symptomatic and asymptomatic men.[49] *M. hominis* has been implicated in a few cases of pyelonephritis.[57] *M. hominis* is frequently present in vaginal fluid, and in this setting it is strongly associated with bacterial vaginosis.[58] Moreover, quantitative PCR studies have demonstrated that significantly higher concentrations of the organism are present in women with bacterial vaginosis than in women whose microbiota is dominated by *Lactobacillus* spp.[59,60] Thus, although *M. hominis* has been associated with genitourinary tract syndromes such as vaginal discharge and pelvic inflammatory disease, it is not clear whether it is a true pathogen or is simply one of the many organisms comprising the bacterial communities associated with bacterial vaginosis, itself a well-documented risk factor for multiple genitourinary tract morbidities in women.[61] However, Sha and associates[59] reported that after controlling for bacterial vaginosis as defined by the Nugent score, high levels of *M. hominis* correlated strongly with shedding of human immunodeficiency virus (HIV) in the vagina. Interestingly, *M. hominis* has a symbiotic relationship with certain strains of *Trichomonas vaginalis*. In fact, the organism can survive intracellularly in the parasite.[62] There appears to be a subset of women with trichomoniasis with high levels of *M. hominis* in vaginal fluid in the absence of bacterial vaginosis who have clinical evidence of marked cervical and vaginal inflammation compared with other women with trichomoniasis.[61] Taken together, these data suggest that high levels of *M. hominis,* perhaps as the result of *T. vaginalis* coinfection, may be a risk factor for HIV transmission independent of bacterial vaginosis.[63]

M. hominis colonizes neonates and has been isolated from the chorioamnion of premature infants along with the ureaplasmas.[64] However, several recent studies using molecular methods for bacterial detection in amniotic fluid and placentas obtained from women delivering premature infants only rarely found *M. hominis* even in the presence of chorioamnionitis.[38,40,65]

These data argue against a significant causative role for *M. hominis* in prematurity. Moreover, the organism does not appear to be associated with chronic lung disease in very-low-birth-weight infants, in contrast to the ureaplasmas.[7] However, *M. hominis* has been isolated from blood in 13% of women with postpartum fever,[49,66] as it has from women with postabortion fever.[67]

Among the genital mycoplasmas, *M. hominis* is most commonly reported to play a role in systemic infections. At least in part, this may be because *M. hominis* is the only one that can be cultivated, albeit with some difficulty, using the standard media used by clinical laboratories (see "Laboratory Diagnosis"). Particularly well documented are postoperative wound infections, especially after open heart procedures. These include sternal wound infections, mediastinitis, and pericarditis.[68] In 1997, Garcia-Porrúa and associates[69] reviewed 17 cases of septic arthritis caused by *M. hominis*. The organism has been reported uncommonly as the cause of pneumonia in immunocompromised patients, patients in intensive care, and healthy people.[70,71] Often these infections are manifested by the sepsis syndrome.[72] Occasional cases of endocarditis have been documented,[73] and the organism is capable of forming abscesses, although this is relatively rare.[74–76] The key clinical point to remember is that *M. hominis* should be considered in all well-documented systemic or local infections in which properly collected samples do not reveal an etiologic agent in the absence of prior antibiotic treatment.

Mycoplasma genitalium

Of all the genital mycoplasmas, the evidence for *M. genitalium* as a genitourinary tract pathogen is strongest. Its role in systemic infections has not been investigated because it is the most difficult of all of the genital mycoplasmas to cultivate in vitro. *M. genitalium* is the second most common cause of urethritis in men after *C. trachomatis*.[77] The clinical manifestations are very similar to those associated with *C. trachomatis,* and both include milder symptoms and signs of inflammation than observed with gonococcal urethritis. Because *M. genitalium* is relatively resistant to the antibiotics recommended for NGU treatment, it is the most common cause of persistent or recurrent symptomatic NGU.[77] The role of *M. genitalium* in epididymitis has not been studied, but given the similarity between diseases caused by this organism and *C. trachomatis,* a well-documented cause of epididymitis, it seems likely that at least a few cases are caused by this organism.

Endocervicitis, evidenced by mucopurulent discharge, cervical friability, and erythema, is associated with *M. genitalium,* although the relationship does not appear to be as strong as it is with *C. trachomatis*.[78] Several studies of clinically diagnosed pelvic inflammatory disease have found an association with *M. genitalium*.[78] Meta-analyses indicate that the organism also is associated with abnormal pregnancy outcomes including spontaneous abortion and preterm delivery.[78] The evidence suggesting that *M. genitalium* also is associated with infertility is less strong than for the above-mentioned complications, but this issue needs further study.[78] Despite evidence that *M. genitalium* infection is associated with morbid events in women, the case cannot yet be made for a national prevention program for this relatively common sexually transmitted infection. Clear evidence is lacking that a population-based *M. genitalium* screening and treatment program can prevent outcomes such as infertility, PID, HIV infection, or abnormal pregnancy outcomes and that doing this is not harmful and is cost-effective.[79]

LABORATORY DIAGNOSIS

Most clinical microbiology laboratories do not routinely test for the genital mycoplasmas. Optimal in vitro growth of both *M. hominis* and the ureaplasmas requires specialized liquid and solid media containing amino acids, nucleic acid precursors, and horse or fetal calf serum. Additionally, urea is required by the ureaplasmas and arginine is required by *M. hominis* as sources of energy.[7,80] Cultures for these organisms from clinical specimens can be done at selected reference laboratories. Only *M. hominis* is sometimes detectable using methods routinely employed in clinical laboratories, although sensitivity is poor. Growth

of *M. hominis* in the commonly used blood culture systems is inhibited by the anticoagulant polyanethole sulfonate. This can be neutralized by the addition of gelatin to a 1% concentration in the blood culture medium, but this is not done routinely.[81] Automated blood culture systems rely on production of gas, primarily carbon dioxide, for the detection of growing bacteria including *M. hominis* followed by Gram stain of a sample of the culture medium to preliminarily identify the organism. A clue to the presence of *M. hominis* is the fact that the Gram stain of the medium is negative. Acridine orange DNA staining will reveal the organism, but subculture on blood agar is required to confirm the diagnosis. *M. hominis* grows slowly on blood agar, producing tiny translucent nonhemolytic colonies after 2 to 7 days of incubation.[72] *M. hominis* will grow directly on blood agar from specimens obtained from abscesses and wound infections if not overgrown by other organisms and if incubated long enough. Nucleic acid amplification assays are becoming the standard for diagnosing infections caused by *M. hominis* and the ureaplasmas. These tests are increasingly becoming available from reference laboratories. Antibiotic susceptibility determination is dependent on cultured isolates and is rarely available outside of research laboratories.

In vitro growth of *M. genitalium* requires cocultivation with mammalian cell cultures. Vero cells are used most commonly, but these systems are available only in a few research laboratories. Moreover, the organism grows very slowly and may not be detectable for weeks. Even Vero cell cocultivation cultures are negative in many cases of documented infection.[82] A number of different nucleic acid amplification tests have been developed for *M. genitalium* detection.[82,83] In the United States, most of these tests are available only for research, although a growing number of reference laboratories are providing Clinical Laboratory Improvement Act–approved *M. genitalium* tests. In some cases these tests include detection of the common gene mutations associated with macrolide resistance.[83]

THERAPY

A number of in vitro antibiotic susceptibility studies have been done with *M. hominis* and the ureaplasmas, but there have been no comparative clinical trials to establish optimal therapeutic approaches. The opposite is true of *M. genitalium*. Because of the difficulty in culturing the organism, only a few in vitro studies have been done, whereas there have been several well-designed clinical treatment trials using nucleic acid amplification testing to assess clinical efficacy. The mycoplasmas do not have cell walls and therefore are not susceptible to β-lactam and glycopeptide antibiotics. In addition, mycoplasmas do not synthesize folic acid, and thus sulfonamides are not active against them. Of ureaplasma strains, 85% to 90% are susceptible in vitro to tetracyclines.[84] Tetracycline resistance is due to the *tetM* determinant, and it appears that this has become more common in recent years.[85] Men with NGU who fail treatment with a tetracycline class drug may be infected with one of these strains and should be re-treated with a 1-g dose of azithromycin in addition to metronidazole, which is recommended in this circumstance for the possibility of a *Trichomonas* infection.[88] Ureaplasmas are generally susceptible to the macrolide and quinolone classes of antibiotics, although resistance to the latter may occur. For patients with systemic ureaplasma infection, azithromycin should be the antibiotic of choice. A recent in vitro study of 250 ureaplasma clinical isolates in the United States showed that 95% of strains were susceptible to levofloxacin, whereas ciprofloxacin was less active,[86] so levofloxacin is an alternative drug for infections caused by these organisms. Use of both azithromycin and levofloxacin for patients with the life-threatening hyperammonemia syndrome is advisable at least initially.[56] *M. hominis* is uniformly resistant to the macrolide drugs but is generally susceptible to tetracyclines, although the *tetM* determinant also has been found occasionally in this organism. Levofloxacin also has good activity against *M. hominis*.[82,85]

Clinical studies of *M. genitalium* infections have shown that doxycycline has relatively little activity against the organism because microbiologic treatment failure rates are 55% to 70%.[87–89] Azithromycin is more active but results in microbiologic treatment failure rates of 13% to 32% in most studies,[87,88,90] although in one study it was 60%.[89] Thus it is now clear why *M. genitalium* is a major cause of recurrent and persistent NGU in men. Molecular studies have shown that organisms in patients failing azithromycin treatment have mutations in the V region of the 23S rRNA gene.[91] An azithromycin 1.5-g dosage given over 5 days only modestly improves the cure rate and does not decrease the rate of resistance emergence significantly.[92] Thus in the United States the 1-g single dose of azithromycin is still recommended for initial treatment of NGU.[77] Moxifloxacin 400 mg daily for 7 days is very effective in curing men with NGU who have clinically failed azithromycin treatment. For such patients this drug in combination with a single 2-g dose of metronidazole for possible *Trichomonas* is the most reasonable approach.[77] The problem is that moxifloxacin is relatively expensive and is not provided by public health departments, so some men will be unable to access treatment. Further complicating the cost issue is the fact that sexual partners should be treated similarly as their male partners. Quinolone resistance genes are increasingly being reported in many parts of the world, and so even this class of drug may be effective for only a limited time.[92] Investigations into new drugs for treating *M. genitalium* are underway, so there is hope that new options for *M. genitalium* infections will become available in the future.[92]

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The complete reference list is available online at Expert Consult.

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E Rickettsioses, Ehrlichioses, and Anaplasmoses

185 Introduction to Rickettsioses, Ehrlichioses, and Anaplasmoses

Didier Raoult

BACTERIOLOGY

Originally, all small gram-negative bacteria, associated (or not) with arthropods and strictly or facultatively intracellular, were considered Rickettsiaceae. The advent of 16S ribosomal RNA gene sequencing and phylogeny has deeply challenged this classification. The controversy has centered on how much difference between strains should constitute a subspecies.[1–5] Among the agreed-upon changes, *Orientia* was created from an independent branch of its phylum. The *Ehrlichia* group has been reclassified[6] into four genera, with *Ehrlichia* and *Anaplasma* being associated with ticks, *Neorickettsia* with helminths, and *Wolbachia* with both arthropods and helminths. This chapter is limited to the Rickettsiales. All are intracellular Alphaproteobacteria associated with eukaryotic hosts (arthropods or helminths). Based on antigenic and genetic data, pathogenic rickettsiae are traditionally divided into three groups—the spotted fever group, the typhus group, and the scrub typhus group (Table 185.1). The spotted fever group accounts for most tick-borne rickettsioses. The typhus group comprises *Rickettsia prowazekii,* the agent of epidemic typhus that is transmitted by the human body louse, and *Rickettsia typhi,* causing murine typhus, which is transmitted by rat and cat fleas. The scrub typhus group comprises *Orientia tsutsugamushi* only, which is transmitted by chiggers (Trombiculidae).

HISTORY AND EMERGING DISEASES

The development of polymerase chain reaction (PCR) and DNA sequencing, as well as the use of cell culture assays, has allowed the description of many new rickettsioses and ehrlichioses during the past 30 years (Table 185.2).[7] Seven ehrlichioses and 14 rickettsioses have been described since 1980. Three major conditions determined the description and separation of these species. Some were discovered after clinical description in countries where spotted fever had been unknown (*Rickettsia japonica* in Japan, *Rickettsia honei* on Flinder's Island, and Astrakhan fever in Russia). Some were recognized by bacterial identification based on culture and PCR in places where the new pathogen was confounded with another known rickettsial pathogen (*Rickettsia africae* with *Rickettsia conorii, Rickettsia heilongjiangensis* with *Rickettsia sibirica, R. sibirica* subsp. *mongolitimonae* and *Rickettsia aeschlimannii* with *R. conorii, Rickettsia felis* with *R. typhi,* and *Anaplasma phagocytophilum* and *Ehrlichia ewingii* with *Ehrlichia chaffeensis*). Some were identified through association by physicians and microbiologists when an atypical unknown disease (*E. chaffeensis, Rickettsia slovaca, Rickettsia raoultii,* and *Rickettsia helvetica*) was being explored.[7]

In addition to the description of new species, old rickettsioses, such as epidemic typhus or scrub typhus, reemerged apparently because of lack of social control or ecologic changes. These diseases, which were the more deadly rickettsioses for the human species, remain a threat. Studies in Asia identified rickettsioses such as murine typhus and scrub typhus among the most common causes of fever.[8]

Wolbachia, an essential symbiont of human filarial worms, has been shown to play a major role in the pathology and clinical manifestations of filariasis. It introduces a completely new concept in infectious diseases.[9] It appears that inflammatory reactions of patients during the disease and during the treatment of filariasis are caused by the release of lipopolysaccharide-like molecules from the symbiotic *Wolbachia.*

Many rickettsiae were found in their vectors long before a particular disease could be associated with them. However, the denomination *nonpathogenic rickettsiae,* which is used for bacteria found only in ticks, is misleading.[7] Among famous pathogens first classified as nonpathogenic rickettsiae are *Legionella pneumophila; Coxiella burnetii,* the agent of Q fever; *Rickettsia parkeri*[10]; and *R. africae.* Several rickettsiae have been found in ticks throughout the world, the pathogenic potential of which remains unknown. A rarely recognized febrile disease caused by *Neorickettsia sennetsu,* acquired by eating raw infected fish, has been described in Laos and may be present throughout Southeast Asia.[11]

PATHOPHYSIOLOGY

Rickettsia, Ehrlichia, and *Anaplasma* species are host-associated pathogens. These pathogens depend on their environment for the supply of many nutriments. *Rickettsia* species escape rapidly from the phagosome to multiply within the cytoplasm. Spotted fever rickettsiae, which are motile in the cytoplasm through actin polymerization,[12] invade neighboring cells. *Rickettsia prowazekii* is devoid of such motility and is released only by destruction of the host cell. Phospholipase D may play a key role in cellular invasion.[13]

The target cell of *Rickettsia* is the vascular endothelial cell, except for *Rickettsia akari* and *O. tsutsugamushi,* which multiply in monocytic cells. *Ehrlichia chaffeensis* and *Ehrlichia muris* subsp. *eauclairensis* multiply in monocytic cells; *A. phagocytophilum* and *E. ewingii* multiply in polymorphonuclear cells. Some animal ehrlichiae multiply in blood platelets.

GENETICS

The complete genome sequences of most *Rickettsia, Orientia, Ehrlichia, Anaplasma, Neorickettsia,* and *Wolbachia* species have been published. These bacteria have small genomes (0.7–2.1 Mb) with or without plasmids and are undergoing genomic reduction through progressive gene degradation and loss.[14] This phenomenon has been suggested to be associated with increasing virulence.[15]

EPIDEMIOLOGY

The geographic and temporal distribution of rickettsioses and ehrlichioses is mainly determined by their vectors (see Table 185.1). Louse-transmitted diseases occur worldwide, and the human louse is distributed worldwide. Lice often parasitize poor people, preferentially in cold places and during wars. Common fleas, such as cat and dog fleas (*Ctenocephalides felis*

TABLE 185.1 Rickettsioses, Ehrlichioses, and Anaplasmoses of Humans and Their Vectors

	TICK-BORNE	FLEA-BORNE	LOUSE-BORNE	MITE-BORNE	OTHER
Rickettsiae					
Spotted fever group	*R. rickettsii* *R. conorii* *R. japonica* *R. sibirica* *R. australis* *R. slovaca* *R. africae* *R. honei* *R. aeschlimanii* *R. helvetica* *R. parkeri* *R. heilongjiangensis* *R. raoultii* *R. massiliae* *R. monacensis* *R. philipii* strain 364D	*R. felis*		*R. akari*	
Typhus group		*R. typhi*	*R. prowazekii*		
Scrub typhus group *(Orientia)*				*O. tsutsugamushi*	
Anaplasma	*A. phagocytophilum* *A. capra*				
Ehrlichia	*E. chaffeensis* *E. ewingii* *E. muris* subsp. *eauclairensis*				
Candidatus Neoehrlichia	*Candidatus* N. mikurensis				
Neorickettsia sennetsu					Raw fish
Wolbachia					Helminths

TABLE 185.2 Historical Data on Diseases Caused by *Rickettsia* Species (First and Senior Authors)

YEAR	DISCOVERY	AUTHORS
1760	Description of exanthematic typhus	Boissier de Sauvage
1879	First report of scrub typhus	Nagayo
1899	Description of Rocky Mountain spotted fever	Maxcy
1906	Isolation of *Rickettsia rickettsii*	Ricketts
1909	Role of body lice in typhus	Nicolle [Nobel Prize]
1909	Description of Mediterranean spotted fever	Conor et al.
1910	Serology test based on *Proteus*	Wilson
1911	Isolation of *Rickettsia prowazekii*	Nicolle
1914	Tick role in Mediterranean spotted fever	Wilson
1916	Weil-Felix test	Weil and Felix
1921	Identification of *Rickettsia typhi*	Mooser
1925	Description of the tâche noire in Mediterranean spotted fever	Pieri
1930	First isolation of *Orientia tsutsugamushi* (*Rickettsia orientalis*)	Nagayo
1930	Role of chiggers in scrub typhus	Kawarimura
1930	Role of fleas in murine typhus	Dyer
1932	Isolation of *Rickettsia conorii*	Brumpt
1935	Description of Siberian tick typhus	Shmatikov et al.
1938	Isolation of *Rickettsia sibirica*	Krontovuka et al.
1940	*Rickettsia phagocytophila*	Gordon
1946	Description of rickettsialpox	Huebner
1946	Isolation of *Rickettsia akari*	Huebner
1946	Isolation of *Rickettsia australis*	Plotz and Smadel
1946	Queensland tick typhus	Plotz and Smadel
1956	*Ehrlichia sennetsu*	Kobayashi
1968	Isolation of *Rickettsia slovaca*	Brezina et al.
1974	Culture of *R. conorii*	Goldwasser

Continued

TABLE 185.2 Historical Data on Diseases Caused by *Rickettsia* Species (First and Senior Authors)—cont'd

YEAR	DISCOVERY	AUTHORS
1979	Isolation of *Rickettsia helvetica*	Burgdorfer and Peter
1981	*Ehrlichia chaffeensis*	Anderson
1984	Japanese spotted fever	Mahara
1985	Culture of *Rickettsia heilongjiangensis*	Udida and Walker
1987	First case of human ehrlichiosis in United States	Maeda and McDade
1989	Culture of *Rickettsia japonica*	Lov
1990	First human cases of granulocytic ehrlichiosis	Bakken
1990	Isolation of *Rickettsia africae*	Kelly
1991	Flinder's Island spotted fever	Stewart
1992	Molecular identification of *Ehrlichia ewingii*	Anderson
1992	First case of infection by *R. africae*	Kelly and Raoult
1992	Culture and identification of *R. conorii*	Tarasevitch and Raoult
1992	Culture of *Rickettsia honei*	Baird et al.
1993	Culture and identification of *R. sibirica* subsp. *mongolitimonae*	Yu and Raoult
1994	First case of flea-borne spotted fever	Schriefer and Azad
1996	Infection by *R. sibirica* subsp. *mongolitimonae*	Raoult et al.
1997	First infection by *R. slovaca*	Raoult et al.
1997	Culture of *Rickettsia aeschlimanii*	Beati and Raoult
1999	Description of Astrakhan fever	Tarasevitch and Raoult
1999	First human cases of infection with *E. ewingii*	Buller
2000	Role of *Wolbachia* in filariasis	Taylor
2000	First case of acute infection by *R. helvetica*	Fournier and Raoult
2000	Culture of *Rickettsia felis*	Raoult et al.
2002	First case of infection by *R. aeschlimanii*	Raoult et al.
2004	First case of infection by *Rickettsia parkeri*	Paddock et al.
2006	Description of infection by *R. heilongjiangensis* (Far Eastern spotted fever)	Mediannikov et al.[35]
2007	First case of *Rickettsia monacensis* infection	Jado et al.[36]
2008	First case of *Rickettsia massiliae* infection	Parola et al.[37]
2008	First case of infection with *Rickettsia raoultii*	Parola et al.[38]
2009	First infection with *Ehrlichia muris* subsp. *eauclairensis*	Pritt et al.[39]
2010	First case of infection with *Rickettsia philipii* strain 364D	Shapiro et al.[40]
2010	First infection with *Candidatus* Neoehrlichia mikurensis	Welinder-Olsson et al.[41]
2015	First case of *Anaplasma capra* infection	Li et al.[42]

Data from Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev. *1997;10(4):694–719; and Shapiro MR, Fritz CL, Tait K, et al. Rickettsia 364D: a newly recognized cause of eschar-associated illness in California.* Clin Infect Dis. *2010;50:541–548.*

and *Ctenocephalides canis,* respectively) and rat fleas (*Xenopsylla cheopis* and *Pulex irritans*) are also present worldwide, as are their transmitted diseases—murine typhus *(R. typhi)* and flea-borne spotted fever (caused by *R. felis*). Lice, fleas, or exposure to ectoparasite excreta may account for the continued appearance of clusters of flying squirrel *(Glaucomys volans)*–associated sylvatic typhus.[16] Tick species are highly dependent on their environment; very few are found worldwide, with the exception of *Rhipicephalus sanguineus*—the brown dog tick, vector of *R. conorii* in the Old World and *R. rickettsii* in the United States and of *Rickettsia massiliae* and *Ehrlichia canis* worldwide. Tick-transmitted diseases are usually restricted to areas of the world where they can be transmitted by the local fauna. Among rickettsioses and ehrlichioses transmitted by ticks, only *A. phagocytophilum* is currently found worldwide.

Tick behavior may determine the targeted human population and the seasonality. It may also influence the clinical presentation. For example, *Amblyomma* ticks are aggressive hunting ticks. They frequently attack in groups, a behavior that explains grouped cases and several inoculation eschars per patient. *Dermacentor* species wait for their host in an ambush strategy, falling onto a hairy host from a height of 1 m.[17] Therefore they bite frequently in the hair, and children are a primary target. As a consequence, *Dermacentor*-transmitted rickettsioses, such as Rocky Mountain spotted fever (RMSF), and infections by *R. slovaca* more frequently involve children than do other rickettsial diseases.[18] Wide variations in the annual incidence of tick-transmitted diseases, such as RMSF and Mediterranean spotted fever, have been observed. The incidence from 2008 to 2012 in the United States has been reported and suggests significant differences in the risk of death among different ethnic groups (relative risk, 5.2 for American Indians/Alaska Natives compared with white race and 5.7 for Asians/Pacific Islanders) and children (case-fatality rate, 1.6%).[19]

Finally, rickettsialpox, caused by *R. akari* and transmitted to humans by *Liponyssoides sanguineus* (the mouse mite), is diagnosed in the United States, Europe, Ukraine, Korea, and South Africa (see Chapter 187).

TABLE 185.3 Clinical Findings and Target Cells for Rickettsioses, Ehrlichioses, and Anaplasmoses

DISEASE	RASH	RASH SPECIFICITY	ESCHAR	ENLARGED LYMPH NODES
Rocky Mountain spotted fever *(Rickettsia rickettsii)*	90%	45% purpuric	No	No
Mediterranean spotted fever *(Rickettsia conorii)*	97%	10% purpuric	72%	Rare
Siberian tick typhus *(Rickettsia sibirica* subsp. *sibirica)*	100%	Macular	77%	Yes
Queensland tick typhus *(Rickettsia australis)*	100%	Vesicular	65%	Yes
Israeli spotted fever *(R. conorii* subsp. *israelensis)*	100%	Macular	Rare	No
Flinder's Island spotted fever *(Rickettsia honei)*	85%	8% purpuric	28%	Yes
Astrakhan fever *(R. conorii* subsp. *caspiensis)*	100%	Macular	23%	No
African tick-bite fever *(Rickettsia africae)*	30%	Vesicular	100% multiple	Yes
Japanese spotted fever *(Rickettsia japonica)*	100%	Macular	90%	No
Lymphangitis-associated rickettsiosis *(R. sibirica* subsp. *mongolitimonae)*	Yes	Macular	Yes (could be multiple)	Yes
Tick-borne lymphadenopathy *(Rickettsia slovaca, Rickettsia raoultii)*	No	Macular	Yes	Yes
Rickettsia helvetica	No	No	No	No
Far Eastern spotted fever *(Rickettsia heilongjiangensis)*	Yes	Macular	Yes	Yes
Rickettsia aeschlimanii	Yes	Macular	Yes	No
Rickettsia parkeri	Yes	Macular	No	Yes
Pacific coast tick fever (*Rickettsia philipii* strain 364D)	Yes	Yes	Yes	No
Rickettsia monacensis	50%	Macular	30%	No
Flea-borne spotted fever *(Rickettsia felis)*	Yes	Macular	Yes	?
Rickettsialpox *(Rickettsia akari)*	100%	Vesicular	100%	Yes
Epidemic typhus *(Rickettsia prowazekii)*	50%	Macular	No	No
Murine typhus *(Rickettsia typhus)*	50%	Macular	No	No
Scrub typhus *(Orientia tsutsugamushi)*	30%	Macular	50% (could be multiple)	Yes
Ehrlichiosis *(Ehrlichia chaffeensis)*	36%	Macular	No	25%
Anaplasmosis *(Anaplasma phagocytophilum)*	<10%	Macular	No	No
Infection by *Anaplasma capra*	36%	Macular	Yes	29%
Infection by *Ehrlichia ewingii*	No	No	No	No
Infection by *Ehrlichia muris* subsp. *eauclairensis*	No	No	No	No
Neorickettsia sennetsu	No	No	No	Yes
Infection by *Candidatus* Neoehrlichia mikurensis	No	No	No	No
Filariasis *(Wolbachia)*	No	No	No	No

CLINICAL FINDINGS

Fever, rash, and headache were considered for years the diagnostic clue for rickettsial diseases. Indeed, this remains a major triad, but spotless RMSF has been reported, and many of the newly described rickettsial diseases have no rash.[20] Major findings in rickettsioses and ehrlichioses include fever in a patient with exposure to a potential vector that may be associated with rash, inoculation eschar, and/or localized lymphadenopathy (Table 185.3). Biologically, neutropenia, thrombocytopenia, and moderate increases in transaminases are common. This may prompt a diagnostic test and eventually treatment with doxycycline.

The severity of these diseases varies with the causative agent and the host. Some *Rickettsia* species, such as *R. rickettsii* and *R. prowazekii,* and *O. tsutsugamushi* are often more severe. Some variations in the same disease are seen between regions for scrub typhus and RMSF. Currently, the RMSF fatality rate reported by the Centers for Disease Control and Prevention is very low, possibly suggesting misdiagnosis.[5] Host factors also play a role in severity. Old age, alcoholism, and deficit in glucose-6-phosphate dehydrogenase have been associated with more severe disease.[21] In such patients, a multiple-organ dysfunction syndrome that usually leads to a fatal outcome can be observed. Purpuric rash and gangrene of the extremities can also be observed in severe cases. The year-round occurrence in Arizona in the United States, in combination with a lack of specificity of clinical and laboratory abnormalities, has highlighted the need for broad awareness by treating physicians in that context, with a low threshold for early treatment with doxycycline.[22,23]

DIAGNOSIS

Culture remains extremely difficult for these organisms and is usually restricted to laboratories equipped for cell culture, and diagnosis mainly relies on serology and PCR. Swabbing vesicular or escharotic lesions has been proven very efficient for molecular detection. The reference technique for serology is immunofluorescence. Many cross-reactions among species are observed, and precise determination of the infecting agent may be difficult. Testing of several antigens on the same slide, to compare reactivity, may help in discriminating among cross-reacting agents. Western blotting may be more specific in early sera. Cross-absorption, coupled with immunofluorescence or Western blot, may help to resolve these problems, but it is technically demanding and expensive.[7]

PCR has been effectively used for the diagnosis of ehrlichiosis and anaplasmosis from blood samples. Observation of morulae in monocytes or neutrophils in blood smears has also been helpful in the diagnosis of those infections. With rickettsiosis, biopsies of skin lesions are preferable

because biopsies can also be used for immunohistochemistry.[24,25] PCR from early sera and swabs of eschars may also be of value for patients with rickettsial disease.[26]

TREATMENT

The most useful drug in children and adults for the treatment of rickettsiosis, ehrlichiosis, and anaplasmosis is doxycycline. It can be prescribed in short courses or even single doses (for typhus, scrub typhus, and Mediterranean spotted fever). Dental problems should not be a problem for children if fewer than three courses of several days of doxycycline are prescribed during childhood. Chloramphenicol should not be prescribed as first-line therapy because it is not active for ehrlichiosis[27] and it is less active than doxycycline for RMSF. However, it remains widely used in Asia, where rickettsiosis and typhoid are common. Quinolones, which have been disappointingly inactive in cases of typhus and scrub typhus despite good in vitro efficacy (see Chapters 189 and 191), are not effective for ehrlichiosis.

REMAINING QUESTIONS AND PERSPECTIVES

In the United States in recent years, increased reporting of cases of RMSF with a low fatality rate is evidenced by the fact that several rickettsial diseases prevalent in the United States were circulating under this name,[5] including those associated with the serologically indistinguishable *R. parkeri,*[10] *Rickettsia amblyommatis*, *R. massiliae*, and *Rickettsia philipii* strain 364D.[28,29] Changes in the range of the tick host are also consistent with a broader distribution of less pathogenic species and stability of *R. rickettsii* incidence.[30]

The high incidence of *R. felis* in febrile patients in sub-Saharan Africa,[31,32] paralleling that of malaria, has questioned the possibility that this rickettsia might be transmitted by mosquitoes.[33] DNA of *R. felis* has been detected in *Aedes* and *Anopheles* mosquitoes, the vectors of malaria,[31,34] and it was recently demonstrated that *Anopheles gambiae* has the potential to be a vector of *R. felis* infection.

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186 *Rickettsia rickettsii* and Other Spotted Fever Group Rickettsiae (Rocky Mountain Spotted Fever and Other Spotted Fevers)

Lucas S. Blanton and David H. Walker

SHORT VIEW SUMMARY

Etiology
- Spotted fever group (SFG) rickettsiae are small, gram-negative, obligately intracellular bacteria that cause tick-, mite-, and flea-borne human infections.
- In humans, rickettsiae infect endothelial cells and exert their pathophysiologic effects through endothelial injury, with a resultant increase in vascular permeability.

Epidemiology
- *Rickettsia rickettsii* is the agent that causes Rocky Mountain spotted fever (RMSF). *Dermacentor variabilis, Dermacentor andersoni,* and *Rhipicephalus sanguineus* ticks transmit the infection in the eastern two-thirds, western, and southwestern United States, respectively. RMSF also occurs in Central and South America.
- In the United States, RMSF is most prevalent in the South Atlantic and South Central regions. Infections usually occur during the late spring and summer, when ticks are most active.
- Other tick-borne SFG rickettsiae with a broad range of distribution include *Rickettsia conorii* (Europe, Africa, and South Asia), *Rickettsia sibirica* (eastern Russia and Asia), *Rickettsia africae* (sub-Saharan Africa and West Indies), *Rickettsia parkeri* (North and South America), and *Rickettsia slovaca* (Europe).

Clinical Manifestations
- RMSF typically manifests with fever early in the course. Other manifestations include headache, myalgias, nausea, vomiting, and abdominal pain.
- Rash is common but may not occur in the first few days of illness. Rash typically starts on the wrists and ankles before spreading proximally. Involvement of the palms and soles occurs in 36% to 82% of cases but is often a late sign.
- Skin necrosis, gangrenous digits, neurologic complications, azotemia, pulmonary edema, and acute respiratory distress syndrome are manifestations of severe infection.
- The case-fatality rate of RMSF is 23% without appropriate antimicrobial treatment and up to 4% despite appropriate antimicrobials.
- Other SFG rickettsioses manifest with a wide spectrum of disease severity but are generally less severe than RMSF and often have an associated eschar at the tick bite site.

Diagnosis
- The indirect immunofluorescence assay is the serologic method of choice. A fourfold rise in immunoglobulin G (IgG) titer from acute illness to convalescence retrospectively confirms the diagnosis.
- Immunohistochemical detection of SFG rickettsiae in skin biopsy specimens can establish the diagnosis during acute illness.
- Polymerase chain reaction amplification of rickettsial nucleic acids in blood, skin or eschar biopsy specimen, or eschar swab is a useful tool for diagnosis and species identification.
- Treatment should not be withheld pending laboratory confirmation.

Treatment and Prevention
- Doxycycline, 100 mg twice daily for 7 to 10 days, is the treatment of choice for RMSF and other SFG rickettsioses.
- Where available, chloramphenicol is an alternative, but its use is associated with a higher case-fatality rate in those with RMSF.
- Azithromycin and clarithromycin are alternatives for less severe SFG rickettsioses.
- Prevention is aimed toward the avoidance of contact with vectors through repellents and protective clothing.

The spotted fevers comprise a large group of tick-, mite-, and flea-borne zoonotic infections that are caused by closely related rickettsiae. These include Rocky Mountain spotted fever (RMSF), boutonneuse fever, African tick bite fever, North Asian tick typhus, lymphangitis-associated rickettsiosis, Queensland tick typhus, Flinders Island spotted fever, Japanese spotted fever, tick-borne lymphadenopathy, Far Eastern spotted fever, flea-borne spotted fever, and rickettsialpox. Rickettsiae are emerging or reemerging pathogens in many parts of the world.[1–29] Associated diseases have a broad spectrum of severity; the most virulent, RMSF (historically in Montana), exhibited a case-fatality rate of 66%.[30] Even young and previously healthy people may die of RMSF. In recent years, the wide distribution and potential severity of the other spotted fevers have been recognized, especially in Europe, Africa, Australia, Asia, and Japan. Early diagnosis remains deceptively difficult.

ROCKY MOUNTAIN SPOTTED FEVER

The Pathogen

RMSF was first described in Idaho in the late 19th century.[31] Ricketts established the infectious nature of the illness and demonstrated the role of ticks as the vector in western Montana in 1906.[31,32] Wolbach, in 1919, clearly identified the causative rickettsiae in endothelial cells.[33,34] The causative agent was designated *Rickettsia rickettsii.* Geographic origin, clinicoepidemiologic observations, and serotyping were the historical basis for species designation of subsequent rickettsial isolates. Phylogenetic analyses have more accurately revealed the evolutionary relationships among the rapidly increasing number of clinical and environmental isolates. It is likely that virulence was a relatively late mechanism of evolutionary survival for *Rickettsia*, which arose as vertically transmitted symbiotes of insects, arachnids, leeches, and amebas,[35] with gain of virulence associated with genome reduction.[36,37] Contemporary phylogenomics based on whole-genome sequences has defined the ancestral, typhus, transitional, and spotted fever groups.[38] *Rickettsia australis, Rickettsia felis*, and *Rickettsia akari* are members of the transitional group. Criteria for species designation of *Rickettsia* based on historically named species, an approach considered inappropriate by some respected taxonomists,[39] have been proposed to be determined by the divergence of 16S ribosomal RNA (rRNA), citrate synthase, outer membrane proteins A and B, and Sca4 of the most closely related, previously named species.[40] These criteria have resulted in proposals for species designation of rickettsial strains that are much more closely

related than other bacterial species. For example, a proposed criterion of 0.2% divergence of the 16S rRNA gene has created many more species names than the usual 1% to 1.3% divergence of other bacterial taxa.[41,42] An attractive approach, concatenated phylogeny based on multilocus sequence typing using eight loci of *Rickettsia* genes has revealed that species designation of *Rickettsia sibirica, Rickettsia africae*, and *Rickettsia parkeri* should be subject to further discussion.[43] The spotted fever group (SFG) rickettsial strains that have been strongly or weakly associated with human infections (*R. rickettsii, R. conorii, R. africae, R. sibirica* [including the *mongolitimonae* strain], *R. honei, R. japonica, R. slovaca, R. parkeri, R. massiliae, R. monacensis, R. aeschlimannii, R. heilongjiangensis, R. amblyommatis*, and *R. helvetica*) definitely merit identification that is useful for clinical and epidemiologic purposes. It is controversial whether they and a rapidly growing number of candidates merit separate designations as different at the species level. An excellent guide for diagnosis and management of spotted fever rickettsioses has been published by the Centers for Disease Control and Prevention (CDC).[44]

Virulence Factors

SFG rickettsiae are obligately intracellular bacteria that reside in the cytosol and less often in the nuclei of host cells. These rickettsiae are small, measuring approximately 0.3 by 1.0 µm. They have one of the smallest bacterial genomes, ranging between 1.1 and 1.6 Mb. The cell wall, which has the ultrastructural appearance of a gram-negative bacterium, contains peptidoglycan and lipopolysaccharide (LPS). Rickettsiae are difficult to stain with ordinary bacterial stains but are conveniently stained with the Gimenez method or with acridine orange. They have not been cultivated in cell-free medium. Growth requires living host cells, such as the yolk sac of embryonated eggs, experimental animals, or cell culture (e.g., Vero, HEL, and L-929 cells). Rickettsiae have undergone remarkable genome reduction with exploitation of their cytosolic environment by being highly adapted for intracellular survival with effective transport systems for adenosine triphosphate, amino acids, and phosphorylated sugars, in addition to their own independent metabolic enzymes. Rickettsiae exhibit a large family of surface proteins, (autotransporters) that are a major source of antigenic differences.[45] Among these, OmpA (190 kDa) and OmpB (135 kDa) contain conformational epitopes that are targets of humoral immunity and were the antigenic basis for serotyping; other antigens are also shared among the SFG.[46,47] The LPS of SFG rickettsiae contains highly immunogenic antigens that are strongly cross-reactive among all members of the group. However, antibodies to LPS do not provide protection against infection.[48]

Epidemiology

The role of a tick bite in the transmission of RMSF was demonstrated by McCalla and Brereton and reported in 1908[33]; a tick obtained from a patient with RMSF transmitted the disease to two volunteers. The seasonal distribution of RMSF parallels tick activity. The tick is both the vector and the main reservoir.[49] *Dermacentor variabilis,* the American dog tick, is the prevalent vector in the eastern two-thirds of the United States and the Far West; *Dermacentor andersoni,* the Rocky Mountain wood tick, in the western states; *Rhipicephalus sanguineus,* in Mexico and Arizona[50]; and *Amblyomma cajennense, Amblyomma sculptum, Amblyomma mixtum, Amblyomma patinoi, Amblyomma tonelliae,* and *Amblyomma aureolatum* in Central and South America.[51-55] *Amblyomma tenellum* is a potential vector for *R. rickettsii* in Mexico and is likely involved in its maintenance in nature.[56] Causes for the variation in infection rates among populations of ticks are not clear, although in *Dermacentor* only a small portion of ticks (generally 4%) carry any rickettsiae, and fewer than 1 in 1000 ticks carry virulent *R. rickettsii.* One limiting factor is the deleterious effect that *R. rickettsii* has on ticks; another is the inhibition of establishment of transovarial transmission of *R. rickettsii* by the presence of another *Rickettsia* species in the tick.[57–60] Humidity, climatic variations, human activities altering the vegetation and fauna, and the use of insecticides have been suspected to play a role in the fluctuation of tick populations and the prevalence of human rickettsiosis.

R. rickettsii is transmitted transstadially (stage to stage) and transovarially in ticks, thus maintaining the agent in nature. Horizontal transmission through vertebrate hosts would also appear to occur to a small degree and to be a necessary factor for the maintenance of *R. rickettsii* in nature.[49] In most mammals, rickettsemia is of very short duration and low titer, allowing infection of only a small proportion of feeding ticks. Of the three tick stages—larva, nymph, and adult—only adult *Dermacentor* ticks feed on humans. The prevalence of pathogenic rickettsiae in various populations of ticks, their efficiency at transstadial and transovarial transmission, and their ability to infect uninfected ticks while cofeeding on the same host are variable.[61–63] Many rickettsiae of unknown pathogenicity have been isolated and characterized in the United States, including *Rickettsia bellii, Rickettsia montanensis, Rickettsia rhipicephali*, and *Rickettsia peacockii.*[58,64]

The tick transmits the disease to humans during a prolonged period of feeding that may last for 1 to 2 weeks. The blood meal and host temperature associated with tick feeding modulate the expression of genes that may upregulate virulence factors of rickettsiae.[65] Furthermore, salivary products of the tick may enhance establishment of rickettsial infection.[66] The bite is painless and frequently goes unnoticed. After the attached tick has fed for 6 to 10 hours, rickettsiae begin to be injected from the salivary glands. An even longer period may be required for reactivation of rickettsial virulence in unfed ticks. Humans can also be infected by exposure to infective tick hemolymph during the removal of ticks from persons or domestic animals, especially when the tick is crushed between the fingers.

The mean infectious dose of *R. rickettsii* is 23 organisms, but as few as one bacillus can cause infection.[67] Laboratory-acquired infection[68] transmitted by infectious aerosols or parenteral inoculation of *R. rickettsii* may be prevented with careful technique and the use of biohazard containment hoods, masks, and gloves.[69]

From the 1870s until 1931, RMSF was recognized as existing only in the western United States. At present, the prevalence of the disease is higher in the South Atlantic states and in the South Central regions than in the Rocky Mountain states (Fig. 186.1).[70–73] Local prevalence in highly endemic areas, such as North Carolina, has been as great as 14.59 per 100,000.[71] The incidence reported among American Indians is as high as 16.8 per 100,000 according to passive national surveillance data and 94.6 per 100,000 with use of International Classification of Diseases, Ninth Revision (ICD-9) codes from Indian Health Service records.[74,75] Tribal lands in Arizona have been found to be highly endemic areas for RMSF. These populations are affected economically through medical costs, loss of productivity, and loss of life related to the disease.[76] Indeed, the 10% case-fatality rate in Arizona is currently higher than in other areas of the United States.[77] The report of a focus in the South Bronx emphasizes that the ecologic conditions that permit the establishment of RMSF are widely distributed.[78] Most cases are diagnosed during late spring and summer. However, especially in the southern states, a few cases occur during the winter.

In the southern states, incidence is highest in children, adults 60 to 69 years old, and patients who are known to be exposed more often to ticks than are matched controls.[73,79] In the western mountainous states, because of transmission by the wood tick *D. andersoni,* a higher proportion of men contract the disease because of occupational exposure. On tribal lands of Arizona, incidence is highest in children younger than 10 years, and cases are associated with exposure to ticks and contact with dogs.[80] The case-fatality rates reported from 1999 to 2012 are highest for children younger than 10 years old and those older than 70.[77,81]

Consistent with unexplained 30- to 40-year cycles of waxing and waning incidence of RMSF, the number of cases reported to the CDC has skyrocketed (13,561 cases from 2008 to 2012); Fig. 186.2).[77] Problematic issues are the low proportion of laboratory-confirmed cases (15%, with only 5% with specific evidence for *R. rickettsii*); a significant population of healthy persons with serum antibodies reactive with *R. rickettsii,* which shares antigens with other SFG rickettsiae; discovery that *R. parkeri* transmitted by *Amblyomma maculatum* causes human infections; evidence that highly prevalent *R. amblyommatis* carried by *Amblyomma americanum* ticks causes mild or subclinical infections; and a reported RMSF case-fatality rate of 0.4% compared with 23% in the preantibiotic era and 5% in recent years.[82–88,89,90,91–93] It is likely that many of these patients had human monocytotropic ehrlichiosis and were misdiagnosed based on the presence of antibodies stimulated at

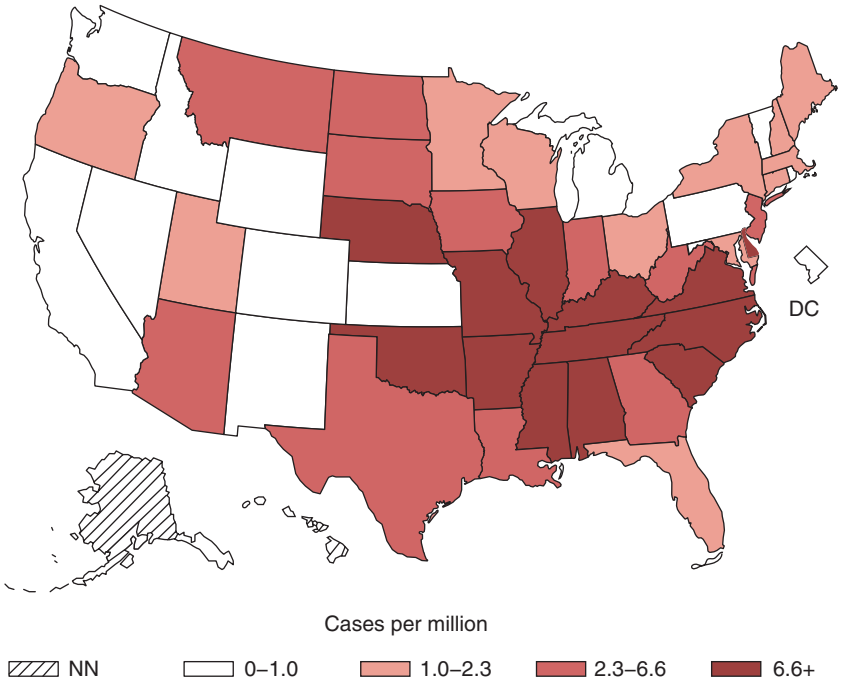

FIG. 186.1 US incidence of Rocky Mountain spotted fever, 2014. *NN,* Not notifiable.

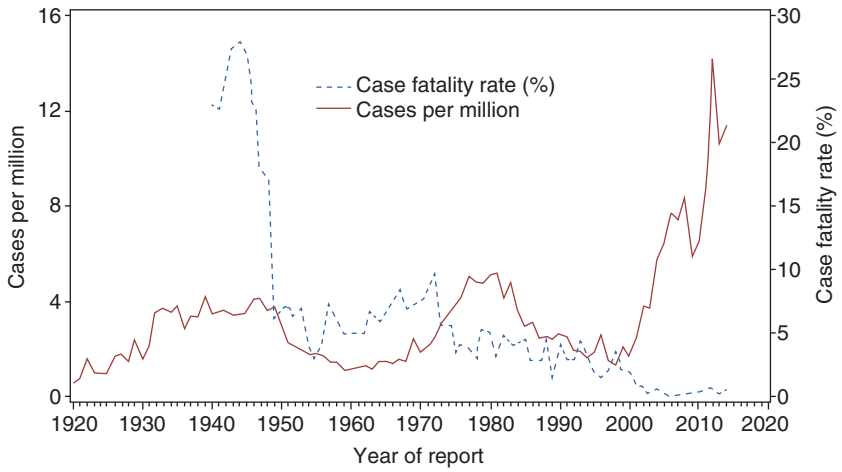

FIG. 186.2 Reported Rocky Mountain spotted fever (RMSF) incidence and case-fatality rate in the United States, 1920 to 2014.

a previous time by *R. amblyommatis.* Some of them likely were infected with *R. parkeri.*[2]

Phylogenetic analysis of isolates of *R. rickettsii* has demonstrated that the organism exists in several clades, likely originated in North America, and subsequently drifted into Central and South America,[94,95] where it is currently emerging in Argentina and reemerging in Brazil, Colombia, Panama, Costa Rica, and Mexico, largely unrecognized, and misdiagnosed as dengue or other febrile exanthems.[10,11,15,16] *R. rickettsii* infections appear to be more severe in these regions, with case-fatality rates of up to 40% in Brazil and Mexico.[96,97,98] The alarming situation in northern Mexico has prompted the Mexican Ministry of Health to declare an epidemiologic emergency.[98,99] Cases originating from northern Mexico have spilled over into the United States with fatal outcomes due to delay in early treatment.[100] Cases have also occurred with community and familial clustering likely associated with large populations of *R. sanguineus.*[101,102]

Pathogenesis

Rickettsiae introduced into the skin apparently spread via lymphatics and small blood vessels to the systemic and pulmonary circulation where, by means of OmpA, OmpB, Sca1, Sca2, and fibroblast growth factor receptor-1, they attach to and induce phagocytosis by their target cells—the vascular endothelium—to establish numerous disseminated foci of infection.[103–113,114] Comparison of the genomes of highly passaged isolates of *R. rickettsii* originating from the western and eastern United States, three virulent and one attenuated, has revealed numerous deletions and amino-acid substitutions between western and eastern isolates.[115,116] A notable mutation in the avirulent stain was disruption of the *ompA* gene leading to absence of its expression. Targeted knockout of *ompA* in the virulent Sheila Smith strain failed to attenuate disease in an animal model, indicating alternative mechanisms contributing to pathogenesis.[117] Other mutations in the eastern isolates include four amino-acid substitutions in OmpB,[116] which in the case of the attenuated Iowa strain does not undergo normal posttranslational processing.[118] After entry, the rickettsiae escape rapidly from the phagosome into the cytosol in association with expression of membranolytic phospholipase D and hemolysin C,[119] and less frequently invade the nucleus. Rickettsiae proliferate intracellularly by means of binary fission. The movement of spotted fever rickettsiae within the cytoplasm, into projections invaginating into the nucleus and into cell projections from which they are released

extracellularly or spread into the adjacent cell, is caused in part by propulsion by the host cell's actin filaments.[120] SFG rickettsial protein Sca2 is associated with activation of Arp2/3 and polymerization of actin at one pole of the rickettsiae, and RickA also plays a role during the early stage of infection in actin-based mobility.[121–125,126] Evolutionary and experimental disruption of actin-based motility seems to attenuate SFG rickettsial virulence.[127,128]

The consequence of cell-to-cell spread in the body is a focal network of hundreds of contiguous infected endothelial cells corresponding to the lesions (e.g., maculopapular rash). The presence of greater quantities of rickettsiae in damaged cells supports the concept of direct cell injury.[121] No convincing data support endotoxin or exotoxin as a pathogenic mechanism. Rickettsial injury to the host cell is caused, at least in part, by free radical–induced damage to host cell membranes and rickettsial phospholipase and protease activities.[129–134] More pathogenic rickettsiae are associated with increased endothelial injury and cell death.[135] The major pathophysiologic effect of endothelial cell injury is increased vascular permeability, which in turn results in edema, hypovolemia, hypotension, and hypoalbuminemia.[136] Endothelial cell induction of heme oxidase and cyclooxygenase, production of prostaglandins, and phosphorylation-induced destabilization of vascular endothelial cadherin may contribute to vasodilatation and increased vascular permeability.[127–141] Hyponatremia is the result of secretion of antidiuretic hormone as an appropriate response to hypovolemia.[142] High quantities of rickettsiae infecting the pulmonary microcirculation are associated with increased vascular permeability and cause noncardiogenic pulmonary edema.[143,144] Vascular injury and the subsequent host lymphohistiocytic response correspond to the distribution of rickettsiae and include interstitial pneumonia, interstitial myocarditis, encephalitis, and similar vascular lesions in the rash, gastrointestinal tract, pancreas, liver, skeletal muscles, and kidneys. However, even severe vascular injury rarely leads to clinically significant hemorrhage. Platelets are consumed locally in numerous foci of infection; consequently, thrombocytopenia is observed in 32% to 52% of patients.[145,146] A procoagulant state ensues, including endothelial injury, release of procoagulant components, activation of the coagulation cascade with thrombin generation, platelet activation, increased antifibrinolytic factors, consumption of natural anticoagulants, activation of the kallikrein-kinin system, and secretion of coagulation-promoting cytokines.[147–150,151–154] These observations are supported by numerous studies of endothelial cells in culture, such as the demonstration that tissue factor is secreted by *Rickettsia*-infected endothelial cells,[155–157] but true disseminated intravascular coagulation occurs only rarely, and occlusive vascular thrombosis is not the basic pathophysiologic event.[150,158]

The host immune and inflammatory responses are critical for clearance of infection, but cytokines may contribute also to increased vascular permeability, and T-regulatory cells may contribute to suppression of immunity in overwhelming fatal illness.[159,160,161] T lymphocytes (particularly CD8 cells) are important effectors of immune clearance of rickettsiae, and interferon-γ (IFN-γ) and tumor necrosis factor-α activate infected endothelial cells to kill intracellular rickettsiae.[103,162–165,166] Dendritic cells stimulated via Toll-like receptor 4 activate natural killer cells in the early innate immune response and secrete IFN-γ, which dampens the rickettsial burden.[159,167–169] The adaptor molecule MyD88 is a key component of Toll-like receptor–mediated recognition of *Rickettsia*.[170] In macrophages, activation of the inflammasome contributes to host control of the bacterium.[171] The ability to invade and proliferate in macrophage-like cells has been noted in pathogenic but not in nonpathogenic rickettsiae.[172] Cytotoxic T lymphocytes are crucial to the clearance of rickettsial infection.[173] OmpA and OmpB cell wall proteins are important immunogens.[174–179]

Clinical Manifestations

The incubation period of RMSF ranges from 2 to 14 days, with a median of 7 days. Variation in incubation time may be related in part to inoculum size. The disease usually begins with fever, myalgia, and headache, most likely the effects of proinflammatory cytokines (Table 186.1). The temperature is higher than 102°F (38.9°C) in 63% of patients during the first 3 days and in 90% later.[145] The variable incidences of reported headache and myalgia in different series are likely related to the proportion of young children who may not articulate the concept of pain.

TABLE 186.1 Features of Rocky Mountain Spotted Fever (RMSF), Boutonneuse Fever (BF), and African Tick Bite Fever (ATBF)

FEATURE	RMSF (%)	BF (%)	ATBF (%)
Fever	99–100	100	81–88
Headache	79–91	56	83
Rash	88–90	97	26–46
Vesicular rash			16–45
Tache noire	<1	72	32–95
Multiple eschars	0	0	21–54
Myalgia	72–83	36	63–87
Nausea, vomiting	56–60		
Abdominal pain	35–52		
Petechial rash	45–49	10	
Conjunctivitis	30	9	
Lymphadenopathy	27		43–49
Stupor	21–26	10	
Diarrhea	19–20		
Edema	18–20		
Ataxia	5–18		
Meningismus	18	11	
Splenomegaly	14–16	6	
Hepatomegaly	12–15	13	
Jaundice	8–9	2	
Pneumonitis	12–17		
Cough	33	10	
Dyspnea		21	
Coma	9–10		
Seizures	8		
Shock, hypotension	7–17		
Decreased hearing	7		
Arrhythmia	7–16		
Myocarditis	5–26	11	
Death	4–8	2.5	
Increased aspartate transaminase level	36–62	39	
Thrombocytopenia	32–52	35	
Anemia	5–24		
Hyponatremia	19–56	25	
Azotemia	12–14	6	

Other signs and symptoms are frequently prominent early in the course before the onset of rash, at which time gastrointestinal involvement with nausea, vomiting, abdominal pain, diarrhea, and abdominal tenderness occurs in substantial numbers of patients; this may suggest gastroenteritis or an acute abdominal condition requiring surgery.

The rash, the major diagnostic sign, appears in a small fraction of patients on the first day of the disease and in only 49% during the first 3 days, usually appearing 3 to 5 days after the onset of fever and occurring in 88% to 90% of patients overall. Rocky Mountain "spotless" fever occurs more often in older patients and in black patients.[145,180] A delay in diagnosis is occasionally associated with the absence or late onset of rash. The rash typically begins around the wrists and ankles but may start on the trunk or be diffuse at the onset. Involvement of the palms and soles is considered characteristic, but it occurs in only 36% to 82%

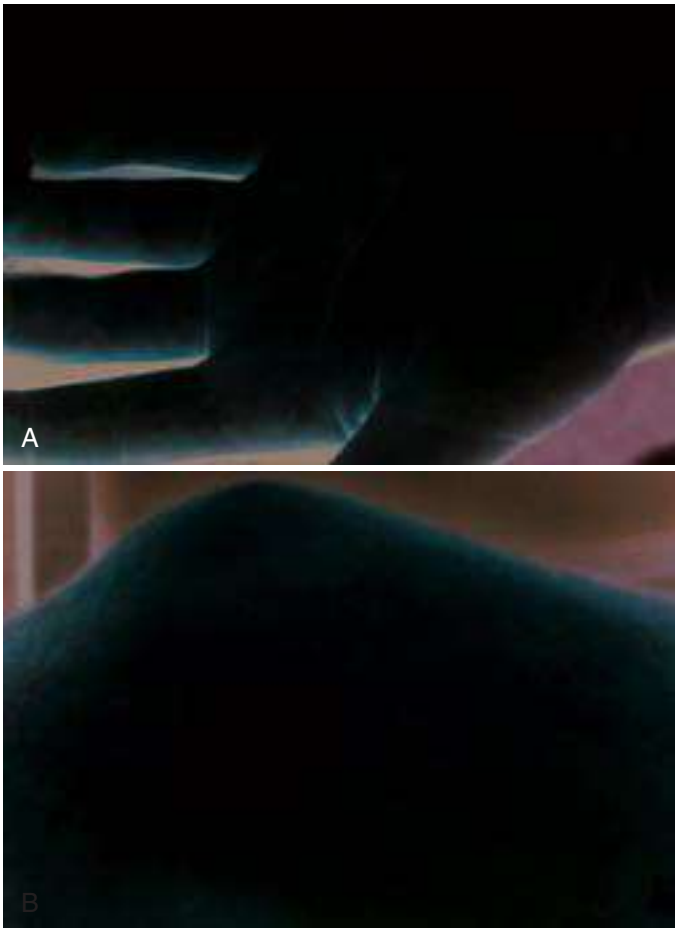

FIG. 186.3 Rocky Mountain spotted fever rash. (A) The wrist and palm manifest the rash of Rocky Mountain spotted fever with central petechiae in some of the maculopapules. (B) Early petechial rash on arm.



FIG. 186.4 Late acute stage of Rocky Mountain spotted fever. The lower portion of the arm shows a florid petechial rash.

of patients who have a rash; it often appears late in the course (Figs. 186.3 and 186.4). Skin necrosis or gangrene develops in only 4% of cases as a result of rickettsial damage to the microcirculation.[146] Gangrene involves the digits or limbs and occasionally necessitates amputation. Careful examination seldom reveals an eschar at the site of the tick bite in RMSF.[181,182] Headache is usually quite severe. Focal neurologic deficits, transient deafness, meningismus, and photophobia may suggest meningitis or meningoencephalitis. The cerebrospinal fluid (CSF) contains increased leukocytes in one-third of patients, with either lymphocytic or polymorphonuclear predominance[146]; CSF protein concentration is increased in one-third of patients. However, glucose concentration is low in the CSF of only 8% of patients.

The electroencephalogram may show diffuse cortical dysfunction. In general, neurologic involvement portends a bad prognosis. Among 37 patients followed for 1 to 8 years after acute RMSF, including some in the preantibiotic era, 21 had residual neurologic abnormalities.[183] These sequelae were headache and other subjective findings, but 12 cases involved electroencephalographic abnormalities. In a more recent series of cases of children with RMSF, 15% of survivors had neurologic deficits at discharge, including global encephalopathy, ataxia, and blindness.[184] Sequelae occur less often in patients with early antibiotic treatment. On funduscopic examination, retinal vein engorgement, arterial occlusion, flame hemorrhage, and papilledema without increased CSF pressure have been noted. These changes may reflect retinal vasculitis with increased permeability and focal thrombosis. Renal failure is an important problem in severe RMSF.[185] Prerenal azotemia related to hypovolemia responds to intravenous hydration; however, in patients with acute tubular necrosis, hemodialysis may be required. Pulmonary involvement is suggested by cough and radiologic changes, such as alveolar infiltrates, interstitial pneumonia, and pleural effusion.[186] Patients with pulmonary edema with impairment of pulmonary function or acute respiratory distress syndrome may require oxygen therapy and ventilatory assistance. Echocardiographic studies reveal minimal myocardial dysfunction,[187] and normal pulmonary capillary wedge pressure measurements document the noncardiogenic nature of the pulmonary edema.

In classic RMSF, death occurs 7 to 15 days after the onset of symptoms when appropriate therapy is not given in a timely manner. In fulminant RMSF, death occurs within the first 5 days. Several features account for the extreme difficulty associated with the diagnosis of fulminant RMSF—the course is rapid, the rash develops shortly before death if at all, antibodies to *R. rickettsii* do not have time to develop, and the pathologic lesions even appear different, containing more thrombi and lacking the characteristic lymphohistiocytic component.[188] Fulminant RMSF is more often observed in black males with glucose-6-phosphate dehydrogenase (G6PD) deficiency, apparently because of an unidentified secondary effect of the usually moderate degree of hemolysis. After hemolysis has depleted the older red blood cells (RBCs) with low G6PD content, evaluation of hemolysis through plasma haptoglobin may be more revealing than assay of G6PD activity in the remaining RBCs. Hemolysis may be the pathogenic factor, rather than the reduced G6PD enzyme activity itself. Other risk factors for a lethal outcome in classic RMSF include older age and possibly alcoholism.

Characteristic laboratory data may support the clinical diagnosis of classic RMSF but are relatively nonspecific.[145,146] The white blood cell count is generally normal, but increased quantities of immature myeloid cells occur frequently. Anemia is observed in 5% to 30% of cases. Thrombocytopenia occurs in more severe cases but also in some patients with mild disease. Coagulopathy with prolonged coagulation times and decreased concentrations of fibrinogen and other clotting factors occurs infrequently because the hemostatic system usually functions effectively to prevent severe bleeding from vascular lesions and generally does not contribute significantly to the pathologic state.[150] Disseminated intravascular coagulation is rarely documented. Hyponatremia is observed in half of patients with RMSF. Increased concentrations of serum lactate dehydrogenase, creatine kinase, and other enzymes are related to diffuse tissue injury, such as multifocal rhabdomyonecrosis.

The prognosis in RMSF is largely related to the timeliness of initiation of appropriate therapy.[189,190] The intervals between onset of disease and appearance of the rash, clinical diagnosis, and effective antibiotic treatment are significantly longer in patients who die than in those who survive.[30,145,189] Patients with fatal cases more frequently have hepatomegaly, jaundice, stupor, and renal insufficiency and report a history of tick exposure less often.[191] Patients who survive RMSF have solid immunity to *R. rickettsii.* Long-term sequelae are mainly neurologic or result from amputation of gangrenous limbs.[192,193]

Diagnosis

The diagnosis of RMSF before the onset of rash is clinical and epidemiologic. Early clinical diagnosis remains essential for this life-threatening disease. The differential diagnosis at the first consultation includes typhoid fever, measles, rubella, respiratory tract infection, gastroenteritis, acute abdominal condition requiring surgery, enteroviral infection, meningococcemia, disseminated gonococcal infection, secondary syphilis, leptospirosis, immune complex vasculitis, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, infectious mononucleosis, drug reaction, ehrlichiosis, anaplasmosis, and other rickettsial diseases. Laboratory diagnosis of RMSF may be achieved through isolation of *R. rickettsii* from the blood. Because *R. rickettsii* is a Biosafety Level 3 organism, few laboratories undertake its isolation, which can be performed by means of inoculation of guinea pigs, embryonated hen's eggs, or cell culture. A centrifugation-enhanced cell culture system—the shell vial assay—has been successfully applied to rickettsial isolation[194] and has been successfully modified to be carried out on a 24-well plate.[195]

Some hospitals and public health laboratories can demonstrate *R. rickettsii* in cutaneous biopsy specimens with immunohistochemistry, a timely diagnostic method used during the acute phase for patients with a rash.[196–198] Serology, the usual method for confirmation of the diagnosis, is retrospective; serum antibodies usually become detectable during convalescence. Therefore, serologic confirmation of diagnosis requires seroconversion or demonstration of a fourfold rise in titers from acute- and convalescent-phase sera.[44] Serology does not allow discrimination of the particular causative SFG *Rickettsia* species unless Western immunoblotting and cross-absorption with appropriately selected antigens are performed.[199] The Weil-Felix test, using *Proteus* OX-19 and OX-2 agglutination, is not to be relied on. This method lacks sensitivity and specificity. Antibodies to rickettsial antigens are detected with indirect immunofluorescence and enzyme immunoassay. The indirect immunofluorescence assay for immunoglobulin G (IgG) reactive against SFG antigens is the most sensitive and specific test. Immunoglobulin M (IgM) lacks specificity and, compared with IgG, does not appear much earlier in illness to make it of great usefulness.[44,200] Indirect immunofluorescence and dot enzyme immunoassay have commercially available reagents.

Polymerase chain reaction (PCR) amplification of the 17-kDa protein, citrate synthase, and *ompA* and *ompB* genes has been employed to identify rickettsiae from a variety of specimens.[201] Unfortunately, amplification of *R. rickettsii* DNA has not proved to be a sensitive diagnostic method for blood samples, except late in the course, particularly for fatal cases.[202–204] Although real-time PCR offers improved analytic sensitivity, the few circulating organisms within the blood limit its clinical sensitivity.[205] When used on banked clinical specimens, real-time PCR revealed rickettsiae in 18% compared with 16% for nested PCR.[206,207] PCR has been successfully applied to other SFG rickettsioses, such as specimens from biopsies of cutaneous rash and eschars during Mediterranean spotted fever (MSF) and other SFG rickettsioses.[199] A multiplexed real-time PCR assay, used by the CDC on formalin-fixed, paraffin-embedded skin biopsy specimens, has the ability to differentiate RMSF from other SFG rickettsioses of concern in the United States.[208] Although not necessary for clinical care, nor available in clinical laboratories, sequencing of PCR products or use of restriction fragment length polymorphism analysis can confirm *R. rickettsii* as the etiologic agent when a species-specific diagnosis is sought.[209] Loop-mediated isothermal amplification assays for the detection of rickettsiae have been developed.[210] Although this platform offers a simple and potentially field-applicable platform, it will likely have the same limitations as PCR when used on blood in the clinical setting.

Treatment

Doxycycline is the treatment of choice. Since the introduction of chloramphenicol and the tetracyclines, including doxycycline, the lethality of the disease has decreased dramatically but remains significant at 3%.[70] In vitro and in ovo, *R. rickettsii* is susceptible not only to chloramphenicol and tetracycline but also to rifampin, some quinolone compounds, including ciprofloxacin and levofloxacin, and the macrolides clarithromycin and azithromycin. Clinical experience with these agents has been insufficient to enable recommendation of their use for RMSF.[211,212] The organism is resistant to β-lactam antibiotics, aminoglycosides, erythromycin, and trimethoprim-sulfamethoxazole.

Tetracyclines are the drug class of choice to treat RMSF, and doxycycline 100 mg every 12 hours is the preferred agent. Doxycycline is effective when given orally but should be given intravenously to patients with nausea and vomiting and to those who are seriously ill. Treatment is usually administered for 7 days or is continued for 3 days after the patient has become afebrile. Single-day treatment with 200 mg of doxycycline has proved to be safe and efficient in adults and children with MSF but has not been tested and is not recommended for RMSF. Tetracyclines have been avoided in young children because of concerns for staining the teeth, but it is recommended that doxycycline be used for suspected RMSF in children of all ages because of the life-threatening nature of RMSF and the demonstration that a single course of doxycycline does not stain the teeth.[213–215] Although the issue of doxycycline in children with RMSF is addressed in clinical guidelines, health care workers are often unaware of this important recommendation.[216–218]

Chloramphenicol, 50 to 75 mg/kg/day given in four divided doses, is also effective for the treatment of RMSF, but it is no longer available in the United States. It is also associated with a higher case-fatality rate when compared with those receiving tetracyclines such as doxycycline.[219] The effects of tetracycline on fetal bones and teeth and fatal hepatotoxicity during pregnancy are rarely seen with doxycycline.[220] When not treated appropriately, RMSF during pregnancy can have devastating consequences for both the mother and the fetus.[221] In addition, chloramphenicol is also not effective for ehrlichiosis, an infection that closely resembles RMSF. For these reasons, doxycycline should be used during pregnancy. Hypersensitivity to doxycycline is a rare event, but desensitization protocols are available when this scenario arises.[222,223]

Avoidance of therapeutic delay is critical for a favorable prognosis.[30,189,190] Severely ill patients require intensive supportive care. Fluid management is critical for maintenance of organ perfusion. Because of increased vascular permeability and risk of extravasation of fluid into pulmonary alveoli, a Swan-Ganz catheter may be needed to monitor hemodynamics in some patients. Glucocorticoids are sometimes given to severely ill patients, but there is no documentation of efficacy and they are not recommended.

Prevention

Although no vaccine is available currently, immunodominant surface proteins have been identified, and the major surface protein antigens (OmpA and OmpB) of *R. rickettsii* and *R. conorii* are candidate vaccine antigens. Immunization of guinea pigs with OmpA provided protective immunity against a virulent challenge, and protective fragments of OmpB have been identified.[174–177] The presence of antibodies to OmpA or OmpB is associated with protection.[48] The surface exposed protein YbgF is also protective.[224,225] A vaccine may be developed that would protect against all SFG rickettsiae, or even typhus group and SFG rickettsiae, but currently no vaccine is available for any of the SFG rickettsiae.[226–228]

Currently, the best means of prevention remains the avoidance of contact with ticks through the use of repellents and protective clothing. Permethrin-treated clothing is effective at reducing the number of tick bites[229] and is effective through at least a year of regular wear.[230] In experimental conditions, infected unfed ticks may take up to 12 hours to transmit *R. rickettsii,* but partially fed ticks may transmit the bacterium in as few as 10 minutes.[231] Therefore, frequent checks of the body, including scalp, pubic, and axillary hair, should be performed to allow removal of ticks before rickettsial transmission. To remove an attached tick, one should use forceps to detach the intact tick without leaving mouth parts in the skin. The tick bite wound should be cleansed. In foci with high rates of RMSF, methods to treat the environment or infested animals with acaricides have been shown to reduce tick populations,[232,233] but such efforts are expensive and laborious.[98]

OTHER SPOTTED FEVER GROUP RICKETTSIOSES

Other SFG rickettsioses have been given a variety of names, including geographic names, that often do not indicate their full distribution (e.g.,

Flinders Island spotted fever, which also occurs in mainland Australia and Southeast and South Asia),[12,234,235] and clinically descriptive names (e.g., tick-borne lymphadenopathy).[3] Aside from the latter highly distinctive disease, the other SFG rickettsioses form a spectrum of similar clinical illnesses with indistinguishable symptoms at onset and subsequent courses with overlapping clinical manifestations, such as varying in severity from occasionally life-threatening to nonfatal; in incidence of the rash from almost all to only half; in characteristics of the rash (usually maculopapular; in some illnesses, occasionally vesicular-pustular, frequently petechial to never petechial); in incidence of eschars; in incidence of regional lymphadenopathy that may be painful (from frequently to never); and in incidence of lymphangitis (from almost half to never). The severity of illness is associated with both the particular *Rickettsia* species or strain and host factors. Among the 15 other SFG *Rickettsia* species reported with evidence to have caused infection of humans (*R. conorii, R. sibirica, R. japonica, R. australis, R. honei, R. africae, R. parkeri, R. slovaca, R. aeschlimannii, R. massiliae, Candidatus* R. philipii*, R. heilongjiangensis, R. helvetica, R. felis,* and *R. akari*), the first 13 are transmitted by tick bite. SFG cross-reactive protein and LPS antigens are detected serologically, and cross-protection is shared among SFG rickettsiae.[226,236] *R. conorii,* a typical SFG rickettsia with high genetic homology with *R. rickettsii,* appears to have greater intraspecies antigenic and genetic diversity than *R. rickettsii* and consists of four strains.[237,238] *R. sibirica* has two distinct strains.[239] The proposal to designate the strains as subspecies is controversial because several already named species are so closely related that some taxonomists would combine them into one. This situation could create the rationale to convert some former species names into subspecies.

Boutonneuse Fever (Mediterranean Spotted Fever)

The most severe SFG rickettsiosis other than RMSF is boutonneuse fever, caused by *R. conorii.* Infection has been designated by many geographic names: Marseilles fever, MSF, Kenya tick typhus, Israeli tick typhus, Astrakhan spotted fever, and Indian tick typhus. Historically, boutonneuse fever was first described by Conor and Bruch in 1909 in Tunisia, although the *tache noire* (black spot)—that is, the eschar at the site of the tick bite—was not described until 1923 by Pieri in Marseilles.[241] *R. conorii* has been identified in India, Pakistan, Israel, Russia, Georgia, Bulgaria, Turkey, Ukraine, Ethiopia, Kenya, South Africa, Morocco, and southern Europe.[241–243] The epidemiology of boutonneuse fever and ecology of *R. conorii* are closely related to *R. sanguineus* ticks. *R. conorii* is maintained transovarially in ticks and is transmitted to humans by tick bite. Dogs have been experimentally shown to be a competent reservoir for *R. conorii,* serving as a reservoir for acquisition and transmission of the agent to *R. sanguineus* ticks.[244,245] Experiments in immune dogs have also demonstrated horizontal transmission through cofeeding (acquisition of *Rickettsia* by naïve ticks during simultaneous feeding adjacent to *Rickettsia*-infected ticks).[246] The frequent absence of a history of tick bite is likely because of transmission by immature larvae and nymphs, which are often not noticed. Cases occur mainly in the warm months, with peak incidences in July, August, and September in many Mediterranean locations. Imported cases occur in travelers returning to the United States and northern Europe from southern Europe. Necropsies of fatal cases of boutonneuse fever reveal disseminated vascular infection and injury by *R. conorii,* including meningoencephalitis and vascular lesions in kidneys, lungs, gastrointestinal tract, liver, pancreas, heart, spleen, and skin.[247,248] Hepatic biopsy specimens reveal multifocal hepatocellular necrosis and granuloma-like lesions.[249] In a large series of well-documented cases, patients infected with the *R. conorii* Israeli spotted fever (ISF) strain were more severely ill than those infected with the Malish strain. Although eschars were present in many patients with both strains, tick bite inoculation lesions occurred more often with Malish strain. Severity and fatality were manifested by more frequent petechial rash, gastrointestinal symptoms, obtundation, dehydration, tachypnea, hepatomegaly, leukocytosis, coagulopathy, acute renal failure, hyperbilirubinemia, and elevated serum transaminase and creatine kinase levels.[250] Host factors that are risks for severity include diabetes mellitus, cardiac insufficiency, alcoholism, old age, and G6PD deficiency.[250,251,252] The disease is milder in children. In France, Israel, and Spain, the death rate among hospitalized patients ranges from 1.4% to 5.6%, similar to that of RMSF.[252] In contrast, SFG rickettsiosis in northeastern Spain documented with cross-reactive serology is a milder illness, suggesting that another strain might be the actual agent.[253] Other rickettsiae that share antigens and cause similar disease in Europe include *R. massiliae, R. monacensis, R. sibirica mongolitimonae* strain, and *R. akari.*[18,20,23,254,255] Boutonneuse fever is associated with a procoagulant state,[148–150,151,256] and 9.6% of cases are complicated by deep venous thrombosis late in the course.[257] Plasma levels of tumor necrosis factor rise during infection.[154] After a mean incubation period of 7 days, fever, myalgias, and headache characterize the onset (see Table 186.1). Careful clinical examination may reveal an eschar, which facilitates the clinical diagnosis.

Spotted Fevers: Moderate Severity

R. sibirica was discovered in the 1930s in eastern Russia and subsequently in China, Mongolia, and Pakistan. A genomic variant considered a strain or a subspecies, named *mongolitimonae,* has been found in Europe, Asia, and Africa.[20,258–260] *R. sibirica* causes North Asian or Siberian tick typhus, which closely resembles RMSF. Nearly half of patients infected with the *R. sibirica mongolitimonae* strain manifest a very unusual ropelike lymphangitis between an inoculation eschar and the draining lymph node.[20,261] This clinical feature led to the term *lymphangitis-associated rickettsiosis* and sometimes also is present in infections with other rickettsial species. Severe complications, including retinal vasculitis and septic shock, have been reported.[262,263] *R. australis* is limited to eastern Australia, and *R. honei*, the causative agent of Flinders Island spotted fever, also causes human infections on mainland Australia and in Asia.[12,24,234,235]

Although Japanese spotted fever has been described as a typically moderate SFG rickettsial illness (fever 100%, rash 100%, and eschar 90%), more severe illness, including meningoencephalitis, respiratory failure, shock, and even death associated with elevated proinflammatory cytokines, does occur.[264–268] *R. heilongjiangensis*, the agent of Far Eastern spotted fever, is present in eastern Russia, Thailand, and China.[25] It causes typical non–life-threatening SFG rickettsiosis, with some patients exhibiting ropelike lymphangitis.

R. africae is prevalent throughout sub-Saharan Africa, where the vector ticks—*Amblyomma hebraeum* and *Amblyomma variegatum*—are present.[24] The identification of *R. africae* infections in the West Indies apparently conforms to the distribution of *A. variegatum.*[26] African tick bite fever is the only tick-transmitted rickettsiosis in which several inoculation eschars are observed in a high proportion of cases.[26] *R. africae,* the most frequently imported rickettsiosis, is often observed in patients who have hunted or traveled in the bush in southern Africa.[26,269,270–273] The attack rate in an exposed group can be rather high. In a cohort of 940 Norwegian travelers to South Africa, a 4% attack rate was observed in all travelers and in 25% of those traveling to hunt game.[274] Patients with African tick bite fever typically have headache, fever, and myalgia, and acute-stage elevations of serum tumor necrosis factor-α, interleukin (IL)-6, IL-8, IL-13, IFN-γ, RANTES (*r*egulated on *a*ctivation, *n*ormal *T*-cell *e*xpressed and *s*ecreted), and MIP-1α (macrophage inflammatory protein-1α).[275] Rash may be vesicular, maculopapular, sparse, or even absent in more than half of patients. Distinctive features include frequent regional lymphadenitis that drains the region of the eschars. The infection is common among native Africans, in whom it is frequently suspected to be malaria or typhoid fever.[21] Local seroprevalence rates are highest in habitats that favor abundant tick populations.[276]

R. parkeri infection has been documented in about 40 patients in the United States,[277] transmitted largely by the Gulf Coast tick *(A. maculatum).* The agent has been detected in 20% to 40% of *A. maculatum* ticks collected in several states.[278–280] *R. parkeri* is also associated with human infections in Uruguay, Brazil, Colombia, and Argentina,[2,13,14,85,281–283] where it is apparently transmitted by *Amblyomma triste, Amblyomma tigrinum,* and *Amblyomma ovale.*[284–288] Transmission by *A. triste* was also documented in Arizona.[289] It causes a spotted fever very similar to African tick bite fever, the agent to which *R. parkeri* is closely related. It is likely one of the causes of cases reported as RMSF based on serology, although *R. parkeri* infection is less severe than RMSF. As another

possible difference from RMSF, more than 90% of patients develop an eschar, which provides useful tissue for PCR diagnosis.[277,281,282,289,290] Other eschar-associated infections in North and South America have been described. In California, the agent designated *Candidatus* R. philipii strain 364D, likely transmitted by *Dermacentor occidentalis,* causes an eschar-associated spotted fever rickettsiosis.[291,292] Cases of eschar-related disease in Brazil have been reported and attributed to a strain of *R. parkeri.*[293,294]

Spotted Fevers: Mild Severity

In Europe, human infections with *R. slovaca*, *Rickettsia raoultii,* and sometimes other species (e.g., *Candidatus* Rickettsia rioja and *R. massiliae*) are called TIBOLA (*ti*ck-*b*orne *l*ymph*a*denopathy) or SENLAT (*s*calp *e*schar and *n*eck *l*ymphadenopathy *a*fter *t*ick bite).[3,19,27,295–301] *R. slovaca* is transmitted to humans most often by the bite of adult *Dermacentor marginatus* ticks during winter and early spring. The illness is characterized by an eschar, typically on the scalp, and enlarged, often tender, draining cervical lymph nodes. Fewer than half of patients manifest a fever, and rash occurs rarely. The eschar site may have prolonged alopecia. There is persistent asthenia in a small fraction of patients, despite response to antirickettsial treatment. It should be noted that other bacteria have been documented in those with clinical presentations as similar to the aforementioned syndrome (i.e., *Francisella tularensis*, *Bartonella henselae*, *Borrelia burgdorferi*, and *Coxiella burnetii*).[299] *R. raoultii* has been found to cause illness in China, but, unlike in Europe, fever is a more prominent finding, and eschar and lymphadenopathy are less frequently reported.[302,303] The isolation of an *R. slovaca*–like agent from *D. variabilis* originating from the southeastern United States raises the possibility of another pathogenic rickettsial species in the United States.[304]

The possibility of chronic symptoms following an SFG rickettsial infection has also been suggested in patients in Australia.[305] The fact that SFG rickettsiae can cause an eschar or papule with surrounding erythema—the mildest manifestation of illness, short of asymptomatic seroconversion—and did so in two otherwise healthy persons[306,307] was demonstrated for *R aeschlimannii.*[4] Another case of *R. aeschlimannii* infection manifesting as typical SFG rickettsiosis has also been reported.[17] *R. massiliae* infects *R. sanguineus* ticks in the United States and worldwide.[308,309] It has been isolated from a patient in Sicily[23]; identified molecularly in patients from France, Argentina, Tunisa, and Sicily[310–313]; and implicated through serologic cross-absorption techniques in Romania.[301] A patient with acute febrile illness in Sweden was reported with molecular and serologic evidence of *R. helvetica* infection.[28] Another patient from Sweden with mild meningeal symptoms accompanying fever had *R. helvetica* isolated from CSF.[314] In addition, numerous distinct SFG rickettsiae (e.g., *R. amblyommatis, R. montanensis, R. peacockii, R. rhipicephali,* and *R. andeanae*) have been identified only in ticks.[1,240,315] Some of these may prove to be pathogenic for humans, even if they cause only a short, nonexanthematous, febrile illness or asymptomatic seroconversion.[87]

Flea-borne Spotted Fever

R. felis, the agent of flea-borne spotted fever, is maintained transovarially in fleas, largely cat fleas, and is presumably transmitted by them.[6] It was the first *Rickettsia* shown to contain a plasmid.[316] The cat flea host, *Ctenocephalides felis,* is ubiquitously distributed throughout the world, and infections have been documented molecularly or serologically in North and South America, Europe, Africa, Asia, Australia, and Oceania. A review of 34 documented cases in the literature describe the following: fever (32 cases), rash (usually maculopapular; 24 cases), eschar (4 cases), gastrointestinal symptoms (3 cases), and pulmonary involvement (4 cases).[317] Although infections tend to be mild, severe manifestations (e.g., hepatitis, meningitis, and a splenic infarct) have been reported.[318–320] *R. felis* DNA has been detected from febrile persons in Africa but has also been detected in afebrile control subjects,[321,322] from skin swabs of ulcerative skin lesions and from the skin of healthy volunteers,[323] and from a variety of hematophagous and nonhematophagous arthropods.[324] These findings have brought scrutiny to the significance of *R. felis* as a pathogen.[325,326]

Diagnosis

The diagnosis of SFG rickettsiosis may be established by immunohistologic demonstration of organisms in skin biopsy; PCR assay has been used with blood, skin biopsy, or swabs of eschar bases for *R. conorii, R. africae, R. slovaca, R. felis*, *R. japonica, R. sibirica, R. australis, R. honei, R. monacensis,* and *R. massiliae.*[a] Pitfalls associated with ascribing an illness to a causative agent by PCR are emphasized in the dubious association of *R. helvetica* with sarcoidosis and chronic myopericarditis.[334–337] A novel approach that can be used to diagnose boutonneuse fever before the onset of rash is immunofluorescent detection of *R. conorii* in circulating endothelial cells captured by immunomagnetic beads coated with a monoclonal antibody to the human endothelial cell surface.[196,202,338] Timely diagnosis can also be established by isolating SFG rickettsiae in a shell vial cell culture system.[194,339] As with other infectious agents, the sensitivity of isolation is decreased with prior effective antimicrobial therapy.[340] Serology is the mainstay of diagnosis, but reactive antibodies are usually absent during early illness. Thus, convalescent-phase serum demonstrating seroconversion or a fourfold rise in titer is often necessary to establish a diagnosis. Production of antibodies to SFG rickettsiae can be demonstrated with the use of microimmunofluorescence, latex agglutination, enzyme immunoassay, Western blot, or complement fixation. Commercially available multiplexed slides with the ability to use automated techniques have been developed.[341] Antibodies to *R. africae* appear late in convalescence in African tick bite fever.[342] The detection of rickettsial nucleic acid from ticks removed from humans has been proposed as a tool to assess the risk of infection.[343]

Treatment

Tetracyclines such as doxycycline and minocycline are the drugs of choice for SFG rickettsioses. Unlike RMSF, wherein recommendations for tetracyclines are based on extensive clinical experience, there have been prospective studies of tetracyclines and various other drugs for the treatment of MSF.[344] For MSF and other SFG rickettsioses, successful treatment is achieved with doxycycline, 100 mg twice daily for 7 days. Shorter regimens have been studied. In adults with MSF, a single day of doxycycline (taken in two 100-mg doses) was as effective as a 10-day course of tetracycline hydrochloride.[345] Effectiveness was demonstrated with ciprofloxacin, 750 mg twice daily for 7 days, in those with mild-to-moderate MSF,[346,347] but the findings of a subsequent study suggest that fluoroquinolone use may be associated with severe courses.[348,349] The newer macrolides clarithromycin and azithromycin have been shown to be effective in those with mild disease[350,351,352] and can be considered during pregnancy. Chloramphenicol has long been considered an effective antibiotic, but with effective and safe alternatives, the potential for serious adverse events should raise caution regarding its use.

[a]References 5, 6, 199, **241**, 247, 254, 327–333.

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187 *Rickettsia akari* (Rickettsialpox)

Pierre-Edouard Fournier and Didier Raoult

SHORT VIEW SUMMARY

Definition
- Vesicular fever with eschar caused by mite bites; may be confused with anthrax.

Epidemiology
- Found worldwide but is particularly common in New York City.

Microbiology
- Infection caused by the intracellular bacterium *Rickettsia akari.*

Diagnosis
- Based on serology and polymerase chain reaction assay of swab from a vesicle or eschar.

Therapy
- Doxycycline, 100 mg twice daily for 7 days.

Prevention
- Mouse eradication from buildings will prevent transmission to humans.

Rickettsialpox is a worldwide mite-borne rickettsiosis presenting as a febrile and vesicular eruption. It is caused by *Rickettsia akari,* associated with mice, and transmitted by its ectoparasite, the mite *Liponyssoides sanguineus.*

ETIOLOGY

Rickettsia akari is classified among spotted fever group rickettsiae based on antigenic and genetic data. Its genome has a size (1.23 Mb) similar to those of other *Rickettsia* species but its plasmid content varies, possibly according to the strain and/or culture passage, as described for other closely related species such as *Rickettsia felis.*[1,2]

It differs from other *Rickettsia* species in that its target cell in humans is the macrophage and not the endothelial cell,[3] and it is transmitted by the bite of *Liponyssoides sanguineus,* the mouse mite.

EPIDEMIOLOGY

The epidemiology of rickettsialpox is linked to house mice. Another vector (perhaps the tick or flea) is suspected in that the seroprevalence of *R. akari* is high in New York City dogs.[4] In Korea, a sylvatic cycle of *R. akari* in voles is suspected.[5] Rickettsialpox was initially described in New York City in 1946[6] and has since been reported in Europe, Ukraine, Korea, and South Africa.[7,8] The 2001 bioterrorist attack with anthrax directed the attention of physicians to skin eschars and rash and allowed the identification of 34 cases of rickettsialpox in New York City[7] from February 2001 to August 2002. The patients were suspected of suffering cutaneous anthrax or smallpox. The usual yearly incidence in New York City is 5 cases. A surprisingly high seroprevalence of *R. akari* has been reported in intravenous drug users from Baltimore[9] and in patients using a free clinic in Los Angeles County.[10] Because this disease is not actively tracked, its overall prevalence is completely unknown.

CLINICAL MANIFESTATIONS

The typical triad of the disease, which includes fever, vesicular rash, and eschar,[11] was found in 92% of patients investigated in New York City (Table 187.1). Indeed, the disease is recognized by only a few physicians; in the New York City cases, half of patients were identified by a single physician and 75% by three. The incubation period is approximately 7 to 14 days. Eschar is the clinical hallmark of the disease.[12] It starts as a primary papule; a vesicle then appears in the center and, when it dries, it leaves a brown or dark eschar (Fig. 187.1). Palpable regional lymph nodes draining this eschar are common and are usually tender.[11] The rash usually appears on the third or fourth day. It is papular at the beginning and becomes vesicular in many patients. The vesicles dry, and each leaves a black crust. Patients typically have 20 to 40 skin lesions. In contrast with other spotted fever group rickettsioses, palms and soles are not involved. The disease is benign, and patients usually recover. A transient leukopenia can be documented, as can thrombocytopenia and elevated aminotransferases.[13] A case was described of a human immunodeficiency virus–positive patient who recovered.[14]

DIAGNOSIS

Serology has been the easiest tool that can be used for diagnosis. Cross-reactions have been noted between *R. akari* and *Rickettsia rickettsii.* Assay of homologous antigens is more sensitive and is preferable. Immunoglobulin M and immunoglobulin G are detected 7 to 15 days after onset. Immunohistochemistry is of value with skin biopsies and was considered the most efficient tool in the New York City series. A polymerase chain reaction (PCR) assay targeting DNA sequences coding for a 17-kDa antigen was used on fresh tissues.[7] Recently, it was found that swabbing eschar or vesicles of patients with rickettsioses allows DNA detection by PCR[15,16] and should be the preferred sample. Isolation from skin biopsy may be performed on Vero cells in specialized laboratories. Consideration of *R. akari* in the differential diagnosis is critical in that its eschar can be misdiagnosed as inoculation anthrax. Moreover, it is one of the few infections that causes vesicular rashes, along with smallpox, varicella, herpes zoster, herpes simplex, and some other rickettsioses (e.g., Queensland tick typhus and African tick-bite fever).

THERAPY

Treatment[17] for rickettsialpox is doxycycline, 200 mg per day, given as 100 mg twice daily, for 7 days. The alternative treatment is chloramphenicol. Although clinical efficacy is unknown, *R. akari* is susceptible to many antibiotics, including azithromycin.



FIG. 187.1 Patient with eschar of rickettsialpox in 2002. *(Courtesy C. Paddock.)*

TABLE 187.1 Clinical and Epidemiologic Findings in Rickettsialpox

SERIES	PADDOCK ET AL.[7]	GREENBERG ET AL.[6]	KASS ET AL.[11]
Number of cases	34	144	13
Year of study	2003	1947	1994
Fever	97%	100%	100%
Mice at residence or work	67%	—	—
Eschars	90%	99.8%	100%
Any rash	100%	100%	100%
Vesicular rash	—	—	92%
Fever plus eschars plus rash	92%	—	100%
Hospitalization	32%	—	—
Headache	NA	90%	100%

NA, Not available.

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188 *Coxiella burnetii* (Q Fever)

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SHORT VIEW SUMMARY

Definition
- Q fever manifests as a self-limited febrile illness, pneumonia, endocarditis, or hepatitis. There are many less common manifestations such as meningitis, encephalitis, and osteomyelitis. Infections are divided into acute and chronic (persistent) focalized forms.

Epidemiology
- Q fever is a zoonosis that is acquired by inhalation or, much less commonly, by ingesting unpasteurized milk.
- Cattle, sheep, and goats are the most common reservoirs.
- Infected parturient cats are important in the spread of *Coxiella* in some areas.
- There is an extensive wildlife reservoir.
- The environment is contaminated at the time of parturition by an infected animal.
- Windborne spread from the contaminated environment can occur over a distance of at least 10 km.
- Distribution is worldwide except for New Zealand, Polynesia, Antarctica, and the Arctic.

Microbiology
- *Coxiella burnetii* is a pleomorphic gram-negative (structurally—does not stain with Gram stain) coccobacillus.
- *C. burnetii* is an intracellular pathogen.
- The organism has a spore stage.
- *C. burnetii* undergoes phase variation.
  - Acute infection leads to antibody predominantly against phase II antigens, whereas persistent infections such as endocarditis are characterized by immunoglobulin G (IgG) antibody predominantly against phase I antigens.

Diagnosis
- Indirect immunofluorescent serologic tests are most commonly used.
- A fourfold rise in titer between acute and convalescent samples or presence of IgM or positive polymerase chain reaction (PCR) is diagnostic of acute disease.
- Persistent focalized infection is diagnosed on the basis of evidence of a lesion (e.g., endocarditis, osteomyelitis, vascular infection) in conjunction with an IgG phase I titer at least 1:800, positive PCR of the blood, or biopsy.
- In persistent Q fever, the IgG phase I titer is usually much higher than the phase II IgG titer, but rare cases occur in which the phase II titer is higher.
- Isolation of *C. burnetii* by a shell vial technique can be done.
- PCR assay can be used to amplify *C. burnetii* DNA from a variety of clinical specimens such as blood, heart valves, and joint fluid. Fluorescent in situ hybridization can identify organisms in tissue, although standard histopathologic stains are negative.

Therapy
- Acute Q fever: 14 days of doxycycline or minocycline
- Q fever endocarditis: doxycycline plus hydroxychloroquine for 1½ years for patients with native valve endocarditis and 2 years for patients with prosthetic valve infections

Prevention
- Good animal husbandry practices should be in place.
- Seronegative sheep or goats should be used in research facilities.
- Vaccination should be offered to high-risk groups where available.
- Pregnant women and patients with valve lesions or prosthetic valves should avoid contact with reservoir.

Q fever remains an intriguing disease despite being studied for more than 80 years. Molecular diagnostics and genomic analysis are adding to our understanding of the disease. The large outbreak in the Netherlands,[1] other outbreaks, and longitudinal studies from France are changing diagnostic and treatment approaches.[2] Although progress has been made, the name *Q fever* ("Q" for query) remains appropriate, as many questions remain unanswered.

Q fever is an acute febrile illness that occurs worldwide.[3] The most common animal reservoirs for this zoonosis are cattle, sheep, and goats, but a large wildlife reservoir exists, and soft ticks may be infected. Domestic ungulates, when infected, shed the desiccation-resistant organisms in urine, feces, milk, and especially birth products. The placenta of an infected sheep contains up to 10^9 organisms/g of tissue. Humans are infected by inhalation of contaminated aerosols and after an incubation period of 20 days (range, 1–39 days) become ill with severe headache, fever, chills, fatigue, and myalgia. There is a dose-response effect on the incubation period, with incubation periods of only 2 days occurring on occasion. Other signs and symptoms depend on the organs involved.

PATHOGEN

Coxiella burnetii is the causative agent of Q fever. *C. burnetii* is a highly pleomorphic coccobacillus with a gram-negative cell wall. It measures 0.3 to 0.7 µm long[4] and enters the cell by a passive mechanism. It survives within the phagolysosome of host cells; the low pH of this environment is necessary for the metabolic functioning. Large and small variants exist, and a spore stage has been described, which explains the ability of *C. burnetii* to withstand harsh environmental conditions.[5,6] It survives for 7 to 10 months on wool at 15°C to 20°C, for more than 1 month on fresh meat in cold storage, and for more than 40 months in skim milk at room temperature.[7] Although it is destroyed by 2% formaldehyde, the organism has been isolated from infected tissues stored in formaldehyde for 4 to 5 months. It has also been isolated from fixed paraffinized tissues. Either 1% Lysol or 5% hydrogen peroxide kills *C. burnetii*.

C. burnetii undergoes phase variation.[7] In nature and in laboratory animals, it exists in the phase I state, to which the immunoglobulin G (IgG) response, but not the IgM response, is delayed compared with phase II. Repeated passage of phase I virulent organisms in embryonated chicken eggs leads to gradual conversion to phase II avirulent forms by chromosomal deletions.[8] There is no morphologic difference between the two phases, although they differ in the sugar composition of their lipopolysaccharides,[9] in their buoyant density in cesium chloride, and in their affinity for hematoxylin and basic fuchsin dyes. *C. burnetii* lipopolysaccharide is nontoxic to chicken embryos at doses greater than 80 mg/embryo, in contrast to *Salmonella typhimurium* smooth-type and rough-type lipopolysaccharide, which is toxic in nanogram amounts.[10] Plasmids have been found in both phase I and phase II cells.[11] Some

strains are without plasmids. There are variations in virulence depending on the strain.[3]

The *C. burnetii* genome contains genes with potential roles in adhesion, invasion, intracellular trafficking, and host cell modulation.[12] A difference from other intracellular bacteria is the observation that the genome of *C. burnetii* contains 32 insertion sequences dispersed in the chromosome.[12] The genomes of many[13] strains of *C. burnetii* have been sequenced.[14] The ability to grow *C. burnetii* in an axenic medium will enhance studies of the molecular biology of this microorganism.[15]

Epidemiology

History

In 1935, Derrick, a medical officer in Queensland, Australia, investigated a febrile illness that affected 20 of 800 employees of a Brisbane meatworks.[16] He coined the term *Q* (or *query*) *fever* for this illness, for which he had no diagnosis but suspected was a new disease. Burnet and Freeman[17] showed that the microorganism isolated from the blood and urine of Derrick's patients was a rickettsia. At about the same time, Davis and Cox[18] isolated a microorganism from ticks *(Dermacentor andersoni)* collected near Nine Mile Creek, Montana. Later, Dyer[19,20] showed that *Rickettsia burnetii* (Burnet and Freeman's organism) was the same as *Rickettsia diaporica* (Cox's organism); it is now known as *Coxiella burnetii.*

Zoonotic Sources

C. burnetii has been identified in arthropods (specifically soft ticks), fish, birds, rodents, marsupials, and livestock.[4] The most common animal reservoirs are cattle, sheep, and goats, although a variety of animals (horses, dogs, swine, camels, water buffalo, pigeons, ducks, geese, turkeys, several species of wild birds, squirrels, deer mice, harvest mice, rats, cats, and rabbits) may be infected.[21] Collared doves have been suspected of carrying *C. burnetii* from Western Europe to Ireland. In Nova Scotia, exposure to infected parturient cats resulted in several outbreaks of Q fever.[22,23] A recent outbreak in Guyana was linked to three-toed sloths.[3,24] Animal reservoirs are key to infection, as they lead to high levels of environmental contamination. One study in the United States reported 24% of 1600 samples including nonagricultural sites being positive via quantitative polymerase chain reaction (PCR).[25]

Q fever has a worldwide distribution except for French Polynesia, New Zealand, the Arctic, and Antarctic.[26,27,28] Growing evidence suggests that Q fever is underreported in areas of Africa.[29] It is usually an occupational disease affecting people with direct contact with infected animals such as farmers, veterinarians, and abattoir workers, although indirect contact with infected animals has resulted in outbreaks of Q fever, as in Switzerland, where more than 350 people who lived along a road over which sheep traveled from mountain pastures developed Q fever.[30] Exposure to contaminated straw, manure, or dust from farm vehicles resulted in Q fever in British residents who lived along a road traveled by these vehicles.[31] Exposure may be even more indirect, as in the case of laundry workers who developed Q fever after handling contaminated laundry.[32] Ingestion of contaminated raw milk,[33,34] exposure to infected parturient cats,[22] and skinning of infected wild rabbits are also ways in which Q fever may be acquired. *C. burnetii* has also been isolated from human milk[35] and human placentas.[36] *C. burnetii* is known to undergo reactivation during pregnancy in animals other than humans. It is likely that this happens in humans as well, and Q fever complicating human pregnancy is probably underdiagnosed.[37] Laboratory exposure to *C. burnetii*[38] and transport of infected sheep through hospitals to research laboratories have resulted in large outbreaks of Q fever.[39,40] Nearly 250 travel-acquired cases of acute Q fever have been reported.[41] Q fever is associated with military operations with more than 150 cases occurring in US soldiers deployed to Iraq.[42] Live-cell therapy, an injection of processed fetal cells from animals, was associated with Q fever in travelers to Germany from the United States and Canada, where the procedure is not approved. Patients presented with symptoms compatible with Q fever and seroconversion to *C. burnetii*.[43]

Transmission Between Humans

Q fever has been transmitted by blood transfusion,[44] during autopsies,[45–47] and rarely during clinical care. It appears that delivery of pregnant women infected with Q fever or procedures leading to potential aerosolization present the highest risk. An obstetrician developed Q fever after delivery of a woman infected with *C. burnetii*.[48] A female patient who shared a room and toilet with another patient with acute Q fever who had delivered during their time in the same room together subsequently developed Q fever.[49] Following removal of an infected breast implant, three health care providers (one of three had taken prophylactic doxycycline) seroconverted without clinical disease.[50] There was apparent human-to-human transmission among members of a household.[51] *C. burnetii* may also be transmitted sexually.[49,52]

Incidence

Q fever became a notifiable disease in the United States in 1999 because of its potential as a biological warfare agent.[53,54] From 2000 to 2012, 1366 cases were reported.[55] The male-to-female ratio was 3:1. There were no reported cases of Q fever among pregnant women during this time period in the United States. Despite agriculture being listed as the most common occupation (36%), 61% of patients denied direct contact with livestock. Among patients, 8.4% reported drinking unpasteurized milk, which is double the national prevalence of this practice.[55]

Karakousis and colleagues[56] reviewed the published cases of Q fever endocarditis from the United States. They found seven such cases, likely a gross underestimate, from 1976 to 2004. From 2008 to 2012, 110 cases were reported, which again suggests the disease is underreported.[55] This suggestion is supported by a study by Dahlgren and coworkers,[57] who compared two different reporting systems within the United States. They compared case report forms submitted to the US Centers for Disease Control and Prevention (CDC) and National Death Certificate data and estimated that Q fever endocarditis was underreported by a factor of 14 for case report forms and a factor of 5 for death certificates.

Seroprevalence data in the United States show that 3.1% of Americans test positive for *C. burnetii*.[58] Seropositivity was more common in males (3.8% vs. 2.5%), in Mexican Americans (7%), in older individuals, in foreign-born individuals, and in individuals living in poverty. Data suggest that Q fever remains underrecognized and underreported in the United States.

Outbreaks

Q fever has historically been a disease that causes intermittent outbreaks, which often provide important learning about the disease. A superspreading infected ewe at a farmer's market resulted in hundreds of cases of Q fever in Germany.[59] Urbanization of sheep farming is a major factor in the spread of Q fever in Germany; in one outbreak, people who lived within 50 m of a sheep meadow had an attack rate of 11.8%.[60]

One of the most remarkable events in the history of Q fever has been the outbreak in the Netherlands from 2007 to 2010 resulting in more than 4000 cases[1] from multiple genotypes from likely one strain previously found in Germany and France.[61] Although high-density goat farming in proximity to urban populations was the source and explanation for ongoing transmission of infection, environmental factors also played a role.[62] Farms without transmission had higher vegetation densities and shallower ground water conditions than farms where transmission did occur.[63] The ability to detect organisms in ambient air in the year following the outbreak revealed an association between the intensity of detection and goat kidding as well as the distance to and size of the farm.[64]

PATHOGENESIS

In the most likely sequence of events in the cycle of transmission of *C. burnetii* to humans, the organism is maintained in ticks or other arthropods. These ectoparasites infect domestic and other animals including a variety of small mammals by bite or through contamination of the skin by infected feces. Infected domestic ungulates are usually asymptomatic, although abortion or stillbirth may result. The placenta contaminates the environment at the time of parturition. Air samples are positive for up to 2 weeks after parturition, and viable organisms are present in the soil for up to 150 days.[65–67] Humans are infected by the inhalation of contaminated aerosols. Clinical severity of infection depends on the strain—the QPH_1 plasmid-containing strain being more

virulent than the QPRS-containing plasmid both in humans and experimental models. The microorganisms proliferate in the lungs, and bloodstream invasion follows. This invasion results in the onset of systemic symptoms and a variety of clinical manifestations depending on the dose of the microorganism inhaled and probably on the characteristics of the infecting strain.[68] Depending on the host, primary infection may be symptomatic (acute Q fever) or asymptomatic. Children and women are less likely to be symptomatic, which may partly be dependent on steroid hormones.[69] Pregnant women are even more commonly asymptomatic after primary infection.[70] The evolution to endocarditis does not appear to be related to specific strains[71] but mainly to host factors, and it can follow symptomatic or asymptomatic primary infection. Multiplication of *C. burnetii* is controlled by macrophages, and granulomas are formed. In some people, the macrophages cannot kill *C. burnetii,* seemingly because of the secretion of interleukin-10 (IL-10).[72] Patients with endocarditis have increased levels of IL-10 secreted by stimulated blood monocytes. Patients with cancer, valve lesions, or arterial aneurysms or who are pregnant are at increased risk for endocarditis if infected with *C. burnetii.*[73] High levels of IgG antibodies to cardiolipin have been reported to be associated with development of acute or chronic endocarditis.[74] The early role of antiphospholipid (aPL) antibodies in the generation of aseptic vegetations is suspected and is associated with increased risk of thrombosis.[74]

CLINICAL MANIFESTATIONS

Humans are the only animals known to develop illness regularly as a result of *C. burnetii* infection.[75] In one large series of 207 patients, the mortality rate was 2.4%, which is consistent with US fatality data (2%).[55,76] There are several clinical syndromes, as follows:

1. Self-limited febrile illness (2–14 days)
2. Pneumonia
3. Endocarditis and vascular infections
4. Hepatitis
5. Osteomyelitis
6. Q fever in the immunocompromised host
7. Q fever in infancy
8. Neurologic manifestations: encephalitis, aseptic meningitis, extrapyramidal disease
9. Q fever in pregnancy
10. Post–Q fever fatigue syndrome

SELF-LIMITED FEBRILE ILLNESS

Self-limited febrile illness is probably the most common form of Q fever. In many areas, 11% to 12% of individuals have antibodies to *C. burnetii;* most do not recall pneumonia or other severe illness.[77] The age at which infection occurs, the sex, and the dose of the agent likely determine clinical severity.[78–80]

PNEUMONIA

Pneumonia is the most common presentation of Q fever and occurs as rapidly progressive pneumonia, atypical pneumonia, or pneumonia as an incidental finding in a febrile patient.[81,82] The rapid form mimics legionnaires' disease and the pneumonic form of tularemia.[83] Cough is present in only 28% of patients with radiographically confirmed Q fever pneumonia. Fever occurs in all patients. Severe headache is present in about 75% of patients and is a useful clue to the diagnosis. Other symptoms and the frequency with which they occur are fatigue (98%), chills (88%), sweats (84%), myalgia (68%), nausea (49%), vomiting (25%), pleuritic chest pain (28%), and diarrhea (including as presenting symptom; 21%).[84]

Physical examination of the chest is often unremarkable. The most common physical finding is inspiratory crackles.[81]

Radiologic Findings

The radiographic picture of Q fever pneumonia is variable. Nonsegmental and segmental pleural-based opacities are common, and atelectasis and hilar adenopathy may occur.[85,86] Multiple rounded opacities are very suggestive of Q fever that follows exposure to infected parturient cats.[85] Pleural effusion has been found and is usually small.[86] The resolution time ranged from 10 to 70 days.[86]

Outcome

C. burnetii pneumonia is rarely fatal, and in such instances there is usually a coexisting condition.[87] Information regarding the histology of this form of pneumonia in humans is limited. Pierce and coworkers[88] found small coccobacilli in alveolar macrophages on a transbronchial biopsy specimen in a patient with Q fever. A fatal case of pneumonia in a 43-year-old man was characterized by severe intraalveolar hemorrhagic and focal necrotizing pneumonia, with associated necrotizing bronchitis. Histiocytes, lymphocytes, and plasma cells were in the alveoli. This was thought to be Q fever pneumonia on the basis of organisms seen with a modified Giemsa stain.[89] A resolving, *C. burnetii* pneumonia lesion was characterized by an inflammatory pseudotumor (subsequently described in additional patients),[90] a lung mass composed of mixtures of macrophages, giant cells, plasma cells, and lymphocytes. The bronchiolar epithelium was focally absent, regenerated, or hyperplastic.[91] The changes that result from the inoculation of the lungs of rhesus monkeys resemble changes reported in humans. The resulting consolidation is peribronchial or peribronchiolar.[92] The interstitial infiltrate had more lymphocytes than monocytes (Fig. 188.1).

Laboratory Findings

The white blood cell count is usually normal, but one-third of patients have an increased count. Moderate thrombocytopenia is very common, but thrombocytosis (platelet counts >1 million/mm^3) may occur. Elevation (two to three times normal) of the hepatic transaminase level occurs in many patients. The serum bilirubin level is usually normal; however, jaundice may occur.[82] Rarely, the syndrome of inappropriate secretion of antidiuretic hormone occurs.[93]

Diagnosis of Acute Q Fever

The initial diagnosis of acute Q fever cannot be made on clinical grounds but may be suspected, and treatment should not be delayed while awaiting results of diagnostic testing.[94] The diagnosis of Q fever pneumonia is confirmed serologically or by PCR. *C. burnetii* is highly infectious, and tissues from patients with Q fever should be processed under Biosafety Level 3 conditions. PCR assays are an option if available but are most useful early in illness.[94,95–97]

Microagglutination,[98] complement fixation,[99] and immunofluorescent tests[100,101] as well as enzyme-linked immunosorbent assay[102] have been used in the serologic diagnosis of this illness. The indirect immunofluorescent test is best for the diagnosis of acute and chronic Q fever. Cutoff titers for a positive test may vary by population and laboratory methods.[103,104] The source of antigen can also influence the results. When treating patients with endocarditis, it is critical that laboratories store serum samples so that the previous sample can be tested concurrently with the current sample. This is the only way that a change in titer can be definitively determined.

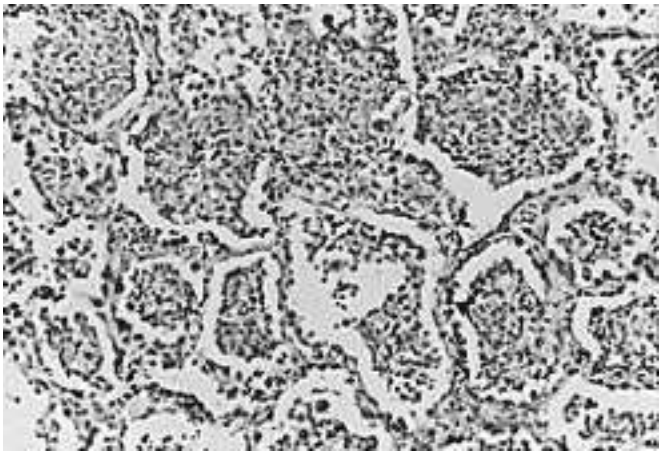

FIG. 188.1 Photomicrograph of an open lung biopsy specimen from a patient with Q fever pneumonia. The alveolar spaces are filled with an inflammatory exudate consisting of lymphocytes and macrophages. Note the hyperplasia of the alveolar lining cells. (Hematoxylin and eosin stain, ×500.)

A fourfold rise in titer between acute and convalescent samples is diagnostic of Q fever. Most patients seroconvert within 2 weeks (90% by week 3).[94] Cross-reactions have been reported between antibodies to *Bartonella* and *Legionella micdadei* and antibodies to *C. burnetii*.[3] Some authors have advocated using the indirect immunofluorescent test to detect antibodies to IgM so that a single serum specimen may be used for the diagnosis of acute Q fever.[73] A titer of 1:50 or higher has a high positive predictive value.[105] However, IgM antibodies may persist for 678 days[106]; in one study,[107] 3% of 162 patients still had a significant IgM antibody level 1 year after the infection. The CDC considers a phase II IgG ≥1:128 consistent with probable infection.[94]

Treatment of Acute Q Fever

The treatment of choice for acute infection with *C. burnetii* including pneumonia is doxycycline, 100 mg twice a day for 14 days.[94] Sobradillo and colleagues[108] performed a prospective, randomized, double-blind study of doxycycline and erythromycin in the treatment of pneumonia presumed to be caused by Q fever. Of 48 patients with Q fever proven by serologic studies, 23 received doxycycline, 100 mg twice daily, and 25 received erythromycin, 500 mg four times daily for 10 days. Fever resolution was faster in the doxycycline-treated group (3 ± 1.6 days vs. 4.3 ± 2 days for erythromycin-treated patients; $P = .05$). The authors concluded that doxycycline is more effective than erythromycin but recognized the self-limiting nature of most cases of Q fever pneumonia.

A retrospective review of 113 patients with acute Q fever pneumonia requiring hospitalization treated between 1989 and 1996 was performed by Gikas and coworkers.[109] This was a retrospective study with patients divided into three treatment groups: doxycycline, macrolide, and β-lactam. Patients treated with doxycycline had a quicker resolution of fever (2.39 days) than patients treated with macrolides (clarithromycin [3.3 days], roxithromycin [3.89 days], and erythromycin [3.93 days]) or β-lactams (6.4 days).

Morovic[110] reported on the effectiveness of doxycycline, clarithromycin, and moxifloxacin for treatment of 77 patients with Q fever pneumonia. Time to defervescence was similar: 2.4 days for doxycycline ($n = 20$), 1.9 days for clarithromycin ($n = 32$), and 2.2 days for moxifloxacin ($n = 25$). The regimens were well tolerated, and there were no documented relapses. The outbreak in the Netherlands provided the largest cohort to date looking at antibiotic regimens for acute Q fever. Dijkstra and colleagues[111] reported on 438 patients with acute Q fever. Patients treated with doxycycline and moxifloxacin had lower risk of hospitalization compared with azithromycin and β-lactams. Doxycycline was the most efficacious. Doxycycline is usually effective as outlined, but rarely a strain may be resistant, or a patient may be intolerant of doxycycline. In such situations, trimethoprim-sulfamethoxazole (TMP-SMZ), chloramphenicol, and rifampin are efficacious.

Monitoring Patients With Acute Q Fever

There is debate about the best way to follow patients with a diagnosis of acute Q fever, although there is agreement that patients should be monitored for development of cardiovascular infection.[26,42,94,112–114] This debate as well as evolving evidence has led one of the authors (D.R.) to propose that the term *chronic Q fever* be abandoned and replaced with *persistent focalized infection,* as chronic infection without focalization does not exist.[26,115] The hope is that this change in nomenclature and the evolving evidence will help provide clarity in the approach to persistent infection. This is necessary to clarify that patients with high antibody titers (phase 1 IgG >1:800) but no lesions or fever do not have "chronic" Q fever.

Monitoring serology every 3 months for up to 2 years after an episode of acute Q fever has been advocated for early detection of endocarditis.[26,94] This is based on the observation of endocarditis or other forms of persistent infection following acute Q fever, especially in patients with unrecognized or mild valvular heart lesions.[116] The risk of endocarditis in patients with acute Q fever and valvular heart disease was 39% in one study.[117] This increased risk in patients with underlying valvulopathies has led some to the recommendation that all patients with acute Q fever undergo screening transthoracic echocardiography (TTE).[26,117] TTE may be performed as soon as possible to detect acute endocarditis.[118] This is important because studies in France and the Netherlands found that prolonged treatment of acute Q fever with doxycycline and hydroxychloroquine prevented development of endocarditis in patients with preexisting valve lesions (bicuspid aortic valve or other significant valvulopathy).[119] We recommend that these patients be treated with hydroxychloroquine and doxycycline for 1 year and followed closely.

If the initial TTE is negative, patients should continue serologic follow-up. For patients who have rising serologic titers (phase I IgG >1:6400 [France] and phase I IgG >1:1024 [United States and Netherlands]) following treatment of acute infection, additional evaluation is warranted to ensure there is not a persistent infection (e.g., endocarditis, vascular infection, osteomyelitis). Evaluative approaches vary based on clinical guidelines.[26,94,120] If there are concerns for infection based on clinical status and not serology alone, repeat TTE or transesophageal echocardiography or both can be pursued. PCR of the blood can be done looking for *Coxiella*. Positron emission tomography (PET) has demonstrated promise when looking for evidence of persistent infection and resulted in adjustments to treatment in 20% of patients in one study.[121] PET is particularly efficient in detecting mycotic aneurysm and osteoarticular infection.[122]

Risk factors to consider during follow-up are rapid rise in antibody to phase I antigen, age older than 50 years, male sex, and appearance of aPL antibody. A newly described syndrome in France associates aPL antibodies with valvular vegetations in patients without prior valvular heart disease. The intensity of the aPL antibody (>100 IgG phospholipid units) and immunosuppression were independently associated with acute Q fever endocarditis, which was present in 1.2% of Q fever cases followed at the National Reference Center from 2007 to 2014.[118] A more recent study demonstrated that aPL antibody levels were significantly higher among patients proven to have endocarditis offering a potential marker of infection.[123]

ENDOCARDITIS

It is now recognized that chronic Q fever has a variety of persistent manifestations including endocarditis, infection of a vascular prosthesis, infection of aneurysms, osteomyelitis, hepatitis, interstitial pulmonary fibrosis, and pseudotumor of the lung.[124] Endocarditis is the prime manifestation of chronic Q fever.[125–147,148] The incidence of Q fever endocarditis is increasing, but this may reflect increased recognition of this entity.[2,112,125,138,146,149] This is supported by the decrease of endocarditis in France following prophylaxis.[114] Usually, abnormal native or prosthetic cardiac valves are affected[147]; however, any part of the vascular tree may become infected,[145] including clot in a left ventricular aneurysm. Such patients have a defective cell-mediated immune response to *C. burnetii*. Q fever endocarditis is rare in children.[143,150]

Clinical Manifestations of Endocarditis

The clinical presentation is consistent with culture-negative endocarditis, and the presence of fever is variable. Marked clubbing of the fingers, hypergammaglobulinemia, splenomegaly, and hepatomegaly may occur depending on diagnostic delay. A purpuric rash related to leukocytoclastic vasculitis may occur in about 20%. The erythrocyte sedimentation rate is usually increased; anemia and microscopic hematuria are also found. Arterial emboli complicate the course of some patients.

The vegetations in chronic Q fever may be absent in half of cases. The gross (Fig. 188.2) and microscopic appearance differ from vegetations seen in pyogenic bacterial endocarditis. Microscopically, there is a subacute and chronic inflammatory infiltrate. Many large foamy macrophages are present. Characteristic microorganisms are seen within these cells on electron microscopy.

Diagnosis of Endocarditis

Confirmation of the diagnosis in most cases is serologic (Table 188.1). A complement fixation titer of 1:200 or higher to phase I antigen has been said to be diagnostic of chronic Q fever, although not all patients in the series of Turck and coworkers[125] had this titer. In acute Q fever, complement fixation antibody titers to phase I antigen do not typically reach this level but can be observed in convalescent sera.

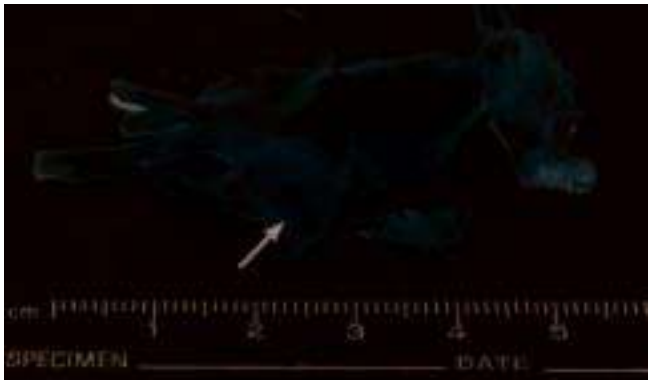

FIG. 188.2 Mitral valve of a patient with Q fever endocarditis. The nodule *(arrow)* was full of *Coxiella burnetii* organisms within foamy macrophages. *(From Raoult D, Raza A, Marrie TJ. Q fever endocarditis and other forms of chronic Q fever. In: Marrie TJ, ed.* Q Fever: The Disease. *Boca Raton, FL: CRC Press; 1990:179–199.)*

TABLE 188.1 Definition of Q Fever Endocarditis

A. Definite
Positive culture, PCR, or immunochemistry for *Coxiella burnetii* of a cardiac valve
B. Major Criteria
Microbiology
Positive culture or PCR of blood or an embolus for *C. burnetii* or serology with IgG phase I antibodies ≥1 : 6400
Evidence of Endocardial Involvement
Echocardiogram positive for IE: oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of alternative anatomic explanation; or abscess; or new partial dehiscence of prosthetic valve; or new valvular regurgitation (worsening or changing of preexisting murmur not sufficient)
PET scan showing a specific valve fixation and mycotic aneurysm
C. Minor Criteria
Predisposing heart condition (known or found on echography)
Fever, temperature >38°C
Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm (see at PET scan), intracranial hemorrhage, conjunctival hemorrhages, and Janeway lesions
Immunologic phenomena: glomerulonephritis, Osler nodes, Roth spots, or rheumatoid factor
Serologic evidence: IgG phase I antibodies ≥ 1 : 800 < 1 : 6400
Diagnosis Definite
1 A criterion 2 B criteria 1 B and 3 C criteria (including microbiologic evidence and cardiac predisposition)
Diagnosis Possible
1 B criterion, 2 C criteria (including 1 microbiologic evidence and cardiac predisposition) 3 C criteria (including positive serology and cardiac predisposition)

IE, Infective endocarditis; *IgG,* immunoglobulin G; *PCR,* polymerase chain reaction; *PET,* positron emission tomography.
Modified from Raoult D. Chronic Q fever: expert opinion versus literature analysis and consensus. J Infect. *2012;65:102–108.*

Studies have reported high titers of IgA antibodies to phase I antigen in endocarditis and granulomatous hepatitis,[106,151] whereas another study found that patients with acute Q fever also produced IgA antibodies to phase I antigen, albeit in low titer.[106] Fournier and colleagues[152] used a phase I IgG antibody titer of 1 : 800 or greater by immunofluorescence as diagnostic of Q fever endocarditis, which was added as a major criterion to the Modified Duke Criteria for diagnosis of endocarditis.[153] Updated criteria for the diagnosis of Q fever endocarditis were proposed by Raoult in 2012 (Table 188.2).[26,114] Reliance on serology only to make

TABLE 188.2 Criteria for Diagnosis of Q Fever Vascular Infection

A. Definite
Positive culture, PCR or immunochemistry for *Coxiella burnetii* of arterial samples (prosthesis or aneurysm) or periarterial abscess or spondylodiskitis linked to aorta
B. Major Criteria
Microbiology
Positive culture, PCR of blood or embolic material for *C. burnetii,* or serology with IgG phase I antibodies ≥6400
Evidence of Vascular Involvement
CT scan: aneurysm or vascular prosthesis plus periarterial abscess, fistula, or spondylodiskitis
PET scan: specific localization to aneurysm or vascular prosthesis
C. Minor Criteria
Serologic IgG phase I antibodies ≥ 1 : 800 < 1 : 6400
Fever, temperature ≥38°C
Emboli
Underlying vascular predisposition (aneurysm or vascular prosthesis)
Diagnosis Definite
1 A criterion 2 B criteria 1 B criterion and 2 C criteria (including microbiologic evidence and vascular predisposition)
Diagnosis Possible
Vascular predisposition, serologic evidence, and fever or emboli

CT, Computed tomography; *IgG,* immunoglobulin G; *PCR,* polymerase chain reaction; *PET,* positron emission tomography.
Modified from Raoult D. Chronic Q fever: expert opinion versus literature analysis and consensus J Infect. *2012;65:102–108.*

the diagnosis of Q fever endocarditis is to be discouraged.[94] Instead, clinical judgment and using all the information available, as listed in Table 188.1, is recommended. Wegdam-Blans and associates[113] reviewed the literature on chronic Q fever and found 607 reports; after applying a number of criteria, they were left with 17 articles that they analyzed. Eight reports dealt with echocardiographic criteria, 6 of which described valvular vegetations that were present in 21% to 50% of cases; abscesses were described in 4 reports and were present in 7% to 20% of patients (Fig. 188.3).[149] PET can be useful in the diagnosis of Q fever endocarditis, osteomyelitis, and endovascular infection.[133]

Treatment of Q Fever Endocarditis

There is a growing body of evidence guiding the treatment of Q fever endocarditis and other persistent forms of Q fever.[2,26] An algorithmic approach used by one of the authors (DR) has demonstrated good clinical outcomes and is available online for use by health care providers.[153a] For cases that do not fit into these algorithms, health care providers should contact an expert.[120]

The preferred treatment regimen for Q fever endocarditis is doxycycline, 100 mg twice daily, plus hydroxychloroquine, 200 mg three times per day, for 1½ years for patients with Q fever native valve endocarditis and 2 years for patients with prosthetic valve infection.[3,94,154] Maurin and colleagues[155,156] reported that the bactericidal effect of doxycycline is enhanced when the phagolysosome is alkalinized with chloroquine or amantadine. Some experts have recommended hydroxychloroquine and doxycycline serum levels be monitored, as they may vary among patients, and adjusted to maintain a serum concentration between 0.8 µg/mL and 1.2 µg/mL for hydroxychloroquine and >5 µg/mL for doxycycline, whereas others have recommended checking levels if a patient fails to respond clinically and serologically.[94,157–159] Resistance to doxycycline appears to be rare, but in one patient who died, the minimal inhibitory concentration of his *C. burnetii* to doxycycline was 8 µg/mL.[158]

The combination of hydroxychloroquine plus doxycycline has been compared with other regimens, and there are options if they are not

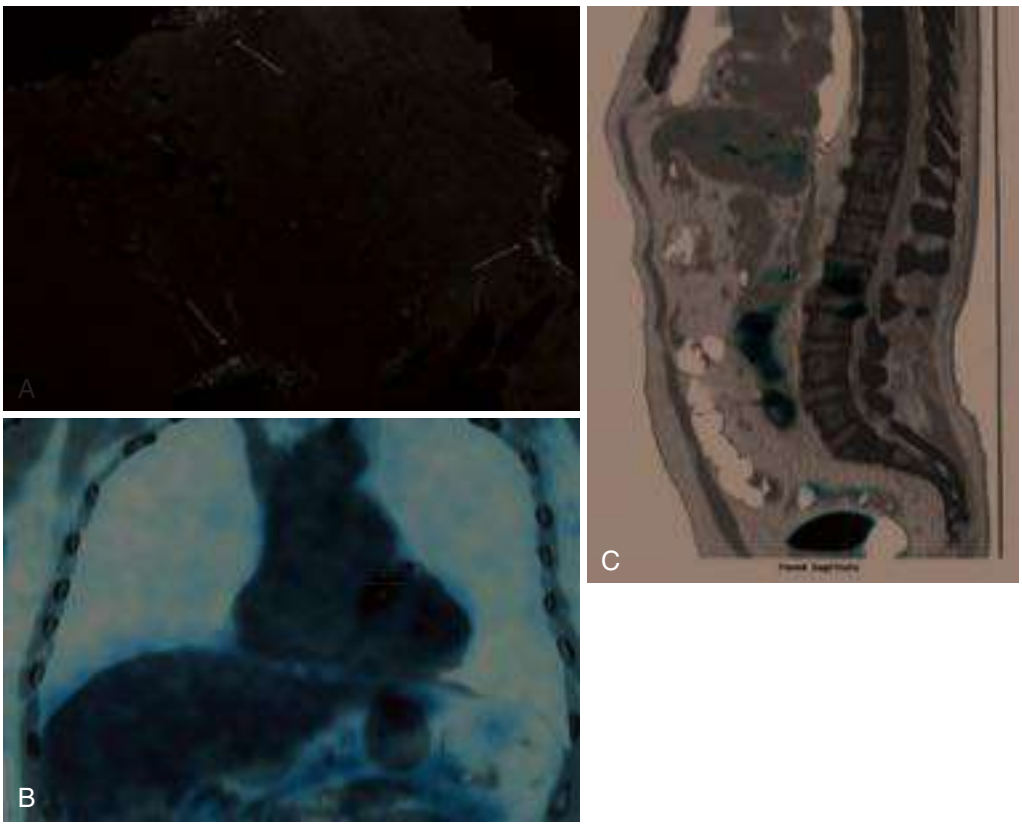

FIG. 188.3 (A) Analysis of an excised cardiac valve from a patient with Q fever endocarditis. Focal and small inflammatory infiltrates with infected macrophages *(arrows)* show that Q fever endocarditis is an insidious latent infection that can evolve over years without any symptoms. (B) ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) scan showing hypermetabolism of the aortic valve in an asymptomatic patient 3 months after febrile hepatitis was diagnosed as acute Q fever. Although the patient was asymptomatic, PET scan led to a diagnosis of Q fever endocarditis, together with serology (phase I immunoglobulin G [IgG] was 52,000). Transthoracic echocardiography was negative, but vegetation was later demonstrated by transesophageal echocardiography. After 5 months of treatment with doxycycline and hydroxychloroquine and surgery of an infected abdominal aortic aneurysm, hypermetabolism completely disappeared. A good outcome was confirmed by serology (phase I IgG was 12,800 at 1 year). (C) ^{18}F-FDG-PET scan led to identification of a Q fever vascular infection with contiguous spondylodiskitis.

tolerated or unable to be used. Hydroxychloroquine is contraindicated in patients with glucose-6-phosphate dehydrogenase deficiency, but the evidence of the risk of hemolysis is weak. Preexisting retinal or visual field deficits is a contraindication according to some authors,[94] although the French group does not exclude these patients.[159a] A Dutch group led by van Roeden[160] compared tetracyclines plus hydroxychloroquine versus tetracyclines plus quinolones and found no difference in outcomes, but a longer follow-up may be necessary to identify relapses.[2] The study by van Roeden and associates[160] showed high rates of clinical failure if monotherapy was used, which supports previous findings. A prior study using doxycycline in combination with ciprofloxacin or rifampin for 2 years had lower success rates.[154] Patients with aneurysms or vascular prosthetic infection have poor outcomes and high mortality (24% in Dutch outbreak).[161] Treatment requires prolonged doxycycline and hydroxychloroquine, and surgery has been found to be critical for the cure of infected prostheses.[162]

Follow-Up

Patients being treated with doxycycline and hydroxychloroquine need to be monitored for clinical and serologic response monthly as well as for side effects of these medications. Hydroxychloroquine can cause retinal toxicity, so a baseline ophthalmic examination as well as examinations every 6 months should be conducted to monitor for visual deficits. Recommendations for serologic monitoring during treatment vary from monthly[94] to every 3 to 6 months during therapy and every 3 months for the first 2 years after the cessation of therapy. Successful therapy is accompanied by a declining erythrocyte sedimentation rate, correction of anemia, and resolution of hyperglobulinemia. Valve replacement is frequently necessary but should be dictated by the patient's clinical status. Empirically, in France, we recommend a duration of 2 years for prosthetic valve infection and 1½ years for native valve and stop the treatment if IgG antibody levels have decreased by fourfold.[163]

HEPATITIS

Hepatitis or elevation of the aminotransferases is the most common manifestation of acute Q fever infection in France.[76,164,165] In the United States, hepatic involvement is present in most cases of Q fever. Hepatitis is more frequent in sheep-breeding and goat-breeding areas.

There are three presentations of Q fever hepatitis, as follows:[166–171]

1. An infectious hepatitis–like picture
2. Fever of unknown origin with increased transaminases
3. An incidental finding in a patient with acute Q fever pneumonia

In patients with fever of unknown origin related to Q fever, the typical doughnut granuloma is seen on liver biopsy, but liver biopsy is rarely performed now.[167,168] This granuloma with a dense fibrin ring surrounded by a central lipid vacuole is suggestive of Q fever but may be seen in other diseases (Hodgkin disease and infectious mononucleosis). *C. burnetii* has been isolated from the liver of patients with Q fever hepatitis, but the organism has not been visualized within the hepatic parenchyma.[125] Antibiotic treatment for 2 weeks is probably sufficient. The rare cases of Q fever hepatitis with high antibodies should be investigated, as isolated chronic hepatitis is mainly a consequence of endocarditis. These patients should be observed with serial antibody titers until the liver enzymes are normal and the titers decrease.

Some patients with hepatitis continue to have fever despite appropriate antibiotic therapy. These patients frequently exhibit anti–smooth muscle and aPL antibodies,[126] and a rapid defervescence of fever occurs with short-term corticosteroid therapy.[127,128] The autoimmune antibodies that are seen in Q fever hepatitis have prompted some authors to suggest that this infection may trigger autoimmune liver disease such as primary

biliary cirrhosis.[129] Our recommendation at the present time is to treat these patients with hydroxychloroquine until the fever resolves. Rarely, cholestatic jaundice and acalculous cholecystitis may complicate acute Q fever.[82,125]

NEUROLOGIC MANIFESTATIONS

Severe headache (often resulting in lumbar puncture) is the most common manifestation and probably represents central nervous system infection, although there is little evidence of serious brain involvement in Q fever.[172,173,174,175] Aseptic meningitis, encephalitis, or both complicate 0.2% to 1.3% of cases of Q fever.[173] A review of 16 cases of Q fever meningoencephalitis revealed that 8 patients had an elevated cerebrospinal fluid white blood cell level (predominantly mononuclear cells), ranging from 18 to 1392 cells/mm^3.[176] The protein level was usually increased, and the glucose level was normal.[176] The electroencephalogram was abnormal in 5 of 6 patients.

In a study from Plymouth, England, Reilly and coworkers[177] reported an astoundingly high 22% incidence of neurologic complications in 103 patients, of whom 46 had acute Q fever, 5 had chronic Q fever, and 52 had past infections. Of the 45 patients with acute Q fever, 6 had residual neurologic impairment including weakness, recurrent meningismus, blurred vision, residual paresthesias, and sensory loss involving the left leg. The meningoencephalitis of Q fever may be accompanied by seizures and coma.[178] Behavioral disturbance, cerebellar symptoms and signs, cranial nerve palsies, extrapyramidal disease, and the Miller-Fisher variant of Guillain-Barré syndrome (areflexia and ophthalmoparesis) have been reported to complicate acute Q fever. Demyelinating polyradiculoneuritis developed in a 71-year-old man 10 weeks after the onset of *C. burnetii* pneumonia.[179]

Q FEVER IN THE IMMUNOCOMPROMISED HOST

Q fever has been reported infrequently in the immunocompromised host[180–184]; however, this may reflect a lack of consideration. When Raoult and colleagues[184] examined serum samples from 500 human immunodeficiency virus (HIV)–positive individuals, they found that 10.4% of the 500 individuals had IgG antibodies to *C. burnetii* at a titer of 1:25 or greater compared with 4.1% of 925 apparently healthy blood donors ($P < .001$). They also found that from 1987 to 1989, 5 of 68 patients (7.3%) hospitalized with Q fever were positive for HIV. They estimated that in HIV-positive individuals, the number of cases of Q fever was 13 times higher, and these patients were symptomatic more frequently than the general population.

In a review of all cases of chronic Q fever in France from 1982 to 1990, the investigators noted that 20% of 84 patients were immunocompromised (cancer, chronic myeloid leukemia, acquired immunodeficiency syndrome, renal transplantation, corticosteroid therapy, renal dialysis, postpartum state, and chronic alcoholism).[124] *C. burnetii* infection resulted in a fatal interstitial pneumonia in an 11-year-old boy with chronic granulomatous disease.[185] In several cases, we have observed the concurrence of lymphoma and Q fever endocarditis. In a review from the French National Referral Center for Q fever, an association with non-Hodgkin lymphoma has been described. An excess risk of follicular lymphoma and diffuse large B-cell lymphoma was detected in patients with Q fever from 2004 to 2014. Although not a causative association, the complex and multifactorial risk for lymphoma may now include Q fever with other infectious diseases in some settings.[186] Patients with history of Q fever and adenopathy should be followed and evaluated for lymphoma.

OTHER MANIFESTATIONS OF Q FEVER

Osteomyelitis is an uncommon manifestation of *C. burnetii* infection, although there are increased reports in recent years possibly related to improved diagnostics including PET.[187–189] Q fever may also occur in infancy; it has caused pneumonia, febrile seizures, pyrexia of unknown origin, malaise, and meningeal irritation in infants.[190] Hematologic manifestations include bone marrow necrosis,[191] histiocytic hemophagocytosis,[192] and hemolytic anemia[193]; occasionally this disease may have simulated lymphoma.[194] Other hematologic manifestations include transient hypoplastic anemia[195] and splenic rupture.[196] Optic neuritis[197] and erythema nodosum[198] have also rarely been reported.

Q Fever in Pregnancy

C. burnetii replicates within trophoblasts.[199] In a trophoblast cell culture, by day 3 there is a 90-fold increase in the inoculum, and by day 9, 2.6×10^6 colony-forming units. Most data suggest that Q fever infection during pregnancy leads to worse fetal outcomes including abortion if infection occurs during the first trimester.[37,200,201,202,203,204] A study from Spain showed that women who had spontaneous abortions were more frequently seropositive than women who gave birth.[203] The study also found that phase II IgG antibody titers ≥1:160 were present in 9.6% of the abortion case group versus 3.1% of the group that delivered, and overall Q fever accounted for 12% of the spontaneous abortions.[203]

Data suggest that outcomes in pregnancy may vary depending on geographic region.[201,205,206] During the outbreak in the Netherlands, pregnancy outcomes were no different for seropositive women compared with seronegative women, although there was a small association with infants being small for gestational age.[201,205,206] However, these patients did not have acute Q fever, and there may be strain differences possibly related to specific plasmids (QpVD) between isolates from France and the Netherlands.[201] The combined data leave it unclear whether or not to screen pregnant women for *C. burnetii* infection during an outbreak.

Q fever during pregnancy should be treated with TMP-SMZ for the duration of the pregnancy.[94,204] In one retrospective study, this approach reduced obstetric complications from 81% to 44%. There were no intrauterine fetal deaths in the TMP-SMZ–treated group.[207] Patients with a chronic Q fever serologic profile should be treated with doxycycline and hydroxychloroquine for 1 year after delivery.

Post–Q Fever Fatigue Syndrome

The term *Q fever fatigue syndrome* (QFFS) is used to describe the protracted state of fatigue that can develop in up to 20% of patients who develop acute infection with *C. burnetii.*[208,209] This syndrome consists of a constellation of symptoms including fatigue, headaches, sweats, arthralgia, myalgias, blurred vision, muscle fasciculations, and enlarged and painful lymph nodes.[75] In a case-control study performed 5 years after a large outbreak of Q fever in individuals in a community in the West Midlands, England, Ayres and coworkers[210] found that participants in whom acute Q fever was diagnosed during the initial outbreak had more complaints of fatigue, sweats, blurring of vision, and dyspnea than age-matched, sex-matched, and geographically matched control subjects and that 42.3% of the infected individuals fulfilled the CDC criteria for chronic fatigue state.[211,212] In a study conducted in Newfoundland, in which 49 patients with goat-related Q fever were followed and the Short Form–36 health survey was used to assess quality of life, 38% (19 of 49) who had acute Q fever during the outbreak had persistent symptoms 3 months later and had significantly lower scores on all components of the Short Form–36 health survey than patients who did not have persistent symptoms.[213] During the outbreak in the Netherlands approximately half of infected patients had fatigue and reduced quality of life up to 4 years following the outbreak,[214] and one study suggested symptoms may last 7 years.[215]

Hickie and coworkers[216] enrolled 253 patients with Epstein-Barr virus infection ($n = 68$), Q fever ($n = 43$), Ross River virus infection ($n = 60$), and an unconfirmed group ($n = 82$) in a prospective longitudinal study to determine the frequency of chronic fatigue after each of these acute infections and the factors that predict the fatigue state. There was no difference in incidence among the infecting agents, and the authors concluded that aspects of the host response were the likely determinants of the postinfective fatigue syndrome. Other evidence suggests "cytokine dysregulation and immunomodulation from persistence of *C. burnetii*" in the host may be responsible for QFFS.[211,212]

Few studies have examined the effects of treatment for QFFS. A small study compared 4 patients with postinfective fatigue treated with 3 months of doxycycline with control subjects and demonstrated a decrease in headaches and mean body temperature.[217] Another study of 20 patients with QFFS reported similar decreases in fatigue and

headaches.[218] A randomized controlled trial in patients with QFFS comparing doxycycline, cognitive-behavioral therapy, and placebo showed a decrease in fatigue-related symptoms when comparing cognitive-behavioral therapy with placebo. The doxycycline arm showed no difference compared with placebo.[219] Consequently, patients with QFFS should not be given doxycycline. Additionally, some patients with QFFS have high levels of antibodies without evidence of ongoing infection, which prompted the new classification of Q fever to avoid treating patients with fatigue and high antibody titers by antibiotics.[220]

PREVENTION

Vaccination

Vaccination of people at risk for infection (e.g., abattoir workers, veterinarians) is an effective strategy to prevent infection.[221,222] At the present time, the vaccine, Q-VAX (Commonwealth Serum Laboratories, Victoria, Australia), is licensed and available only in Australia. It is a formalin-killed, whole-cell vaccine and has an estimated effectiveness for preventing clinical disease of 92% to 98%.[223,224] Skin testing is required before vaccination because individuals with prior exposure are at risk for adverse site reactions. Vaccination has also been used in animals as a potential means of reducing human exposure.[225]

Risk Reduction

The CDC has outlined steps to prevent infection in research facilities and hospitals.[94] Because of the lack of person-to-person spread, there is no need to isolate patients hospitalized with Q fever, unless the patient is undergoing a procedure that causes aerosolization or has recently given birth.[94] Simple measures such as the consumption of only pasteurized milk serve to eliminate cases of Q fever transmitted in this manner. In Cyprus, the incidence of *C. burnetii* infection among sheep and goats was reduced by a program in which aborted material was destroyed, affected dams were isolated, and the premises were disinfected.[226] Control of ectoparasites on cattle, sheep, and goats is also important in the control of Q fever. It seems prudent not to accept blood donors from a region undergoing an outbreak of Q fever, both during the outbreak and for up to 4 weeks after cessation of the outbreak. Ensuring veterinarians engage in practices that could prevent zoonotic transmission is important and may be overlooked.[227]

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The complete reference list is available online at Expert Consult.

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189 *Rickettsia prowazekii* (Epidemic or Louse-Borne Typhus)

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SHORT VIEW SUMMARY

Microbiology
- *Rickettsia prowazekii* is a small, obligately intracellular gram-negative coccobacillus.
- Its 1.1-Mb genome has undergone evolutionary reduction, resulting in the reliance on the host cell cytosol for many biosynthetic functions.
- An extracellular dormant form remains infectious in louse feces for months or longer.

Epidemiology
- The agent is transmitted between patients by the human body louse *(Pediculus humanus corporis).*
- Lice become infected while feeding on rickettsemic patients. The organism is inoculated into a new host by scratching of rickettsiae-laden louse feces into louse-bitten skin or by being rubbed into mucous membranes.
- Recovered patients remain latently infected, are susceptible to reactivation with rickettsemia, and can serve as a source for an epidemic. Brill-Zinsser disease is recurrence of typhus, often in milder form, even decades after the initial infection.
- Epidemics are associated with conditions that promote louse infestations through poor hygiene—poverty, cold climate, jails, and displacement of populations by war and other calamities.
- In eastern North America, an extrahuman reservoir of *R. prowazekii* exists in flying squirrels *(Glaucomys volans),* with infections transmitted to humans through mucous membrane or inhalational exposure to the squirrel's flea or louse feces.

Clinical Manifestations
- Frequent symptoms include fever, headache, chills, myalgia, and rash (see Table 189.1).
- Untreated illness may progress to cause pulmonary edema, encephalitis, and death.
- Recrudescent typhus and flying squirrel–associated typhus manifest similarly but are less severe.

Diagnosis
- The indirect immunofluorescence assay is the mainstay of serologic diagnosis. A fourfold rise in immunoglobulin G (IgG) titer from acute illness to convalescence confirms the diagnosis.
- Polymerase chain reaction assay and immunohistochemical detection of *R. prowazekii* in blood or tissue, respectively, can establish the diagnosis during acute illness.
- Treatment should not be withheld while one awaits laboratory confirmation.

Treatment and Prevention
- Doxycycline, 100 mg twice daily for 7 days, is the treatment of choice.
- Control of body lice by changing and washing garments in hot water is essential for prevention and outbreak control.

Rickettsia prowazekii is the only rickettsial species that can cause devastating, naturally occurring epidemics capable of killing a substantial proportion of human populations infested with body lice. Epidemics are associated with conditions that prevent bathing and washing of clothes in hot water, such as war and poverty, natural disasters such as earthquakes and floods, displacement of populations, jails, and lack of hygiene. Historical accounts of typhus epidemics in Mexico, associated with times of extreme poverty and hunger, have been correlated with drought through the study of tree-ring data to infer annual precipitation.[1] A continued problem in impoverished, louse-infested populations, epidemic typhus threatens to reemerge as it did during the Civil War in Burundi, where an estimated 100,000 persons developed typhus in 1997.[2]

Based on his observations of an Italian epidemic in 1528, epidemic typhus was described vividly by Hieronymus Fracastorius as a previously unknown, life-threatening disease in which a petechial rash appeared 4 to 7 days after the onset of fever and was accompanied by stupor and delirium. The word *typhus* is derived from the Greek *typhos,* meaning "smoky" or "hazy," which describes the state of confusion accompanied by stupor. Luis de Toro described the same course and signs of an illness that occurred in soldiers on the Iberian Peninsula in 1557, also noting winter seasonality and association with contact with clothing of the ill. The term *exanthematic typhus* was proposed by Boissier de Sauvages in 1760 and differentiated the disease from typhoid.[3] During an epidemic of typhoid fever in Philadelphia in 1836, Gerhard distinguished these diseases by the presence of intestinal lesions in typhoid patients. In 1909, Charles Nicolle experimentally established the fact that typhus was a transmissible infection with the human body louse as its vector. Investigations from 1910 to 1922 by Ricketts, von Prowazek, da Rocha-Lima, and Wolbach used microscopy, xenodiagnosis in lice, and histochemistry to establish that the agent was a bacterium that proliferated in human endothelial cells and louse gut epithelium but could not be cultured axenically.[4]

In 1896 and 1910, Nathan Brill described a series of patients in New York with a febrile illness that was shown by Hans Zinsser in 1934 to be a rickettsial infection.[5,6] Zinsser hypothesized correctly that the illness is a recrudescence of long-latent *R. prowazekii,* now called Brill-Zinsser disease. The distinction of *Rickettsia typhi* as a separate agent that causes an endemic, clinically similar disease transmitted to humans by fleas from a zoonotic cycle involving rats was established by the work of Neill (1917),[7] Mooser (1928),[8] Maxcy (1929),[9] and Dyer (1931).[10] The feared specter of epidemic louse-borne typhus receded with the following: (1) the success of a killed *Rickettsia* vaccine in preventing the deaths of allied soldiers during World War II; (2) the effectiveness of insecticides in curtailing epidemics by louse control; and (3) the effective treatment of illness with tetracyclines and chloramphenicol. Currently, epidemics of typhus are increasingly recognized, lice resistant to various insecticides have been detected, antibiotic-resistant rickettsiae have been developed, and aerosol-transmitted, weaponized *R. prowazekii* has emerged as a biothreat. Lack of direct human-to-human transmission is a limitation of use as a bioterror weapon. *R. prowazekii* has been isolated from *Amblyomma* and *Hyalomma* ticks in Ethiopia and in *Amblyomma tenellum* ticks in Mexico.[11,12] The presence of *R. prowazekii* DNA has also been found within *Ixodes ricinus* ticks in the Netherlands.[13] Moreover, in 1975, *R. prowazekii* was identified in a highly prevalent zoonotic cycle in flying squirrels in the eastern United States.[14]

MICROBIOLOGY

The causative agent of louse-borne typhus, *R. prowazekii*, is an obligately intracellular, small (1×0.3 µm) coccobacillus. Its 1.1-Mb genome has undergone considerable reduction, contains a subset of the genes of

Rickettsia conorii,[15] and exhibits little variation among isolates.[16] *R. prowazekii's* conserved structure necessitates its ability to manipulate host cell processes, as demonstrated for small GTPases.[17] Many biosynthetic functions are provided by the organism's milieu in the resource-rich host cell cytosol, to which it has adapted by means of evolutionary selection for transport mechanisms for adenosine triphosphate (ATP), amino acids, phosphorylated sugars, and triose phosphate.[15,18] Its gram-negative cell wall contains an abundant 135-kDa, immunodominant, tetragonally arranged, S-layer surface protein; lipopolysaccharide; and peptidoglycan.[19] Differential expression of genes occurs with its infection of mammalian or arthropod host cells.[20] It possesses an extracellular dormant form that remains infectious in louse feces for months or more. Technical barriers to the genetic manipulation of *R. prowazekii* have limited extensive study of its virulence mechanisms, but development of insertional mutants,[21,22] site-directed knockouts,[23] and maintenance of inserted plasmids offer tools for more extensive study.[24] To date, only phospholipase D and phospholipase A have been identified as virulence factors of *R. prowazekii.*[23,25]

EPIDEMIOLOGY

Louse-Borne Epidemic Typhus

Typhus affected the outcomes of wars from the 1500s until the end of the 19th century.[26] During the Russian campaign of 1812, typhus was responsible for the deaths of many of the 700,000 troops of Napoleon.[27] During World War I, the Bolshevik revolution, and its aftermath, an estimated 30 million cases of typhus occurred in the Soviet Union alone, with 3 million deaths.[28] During World War II, epidemics of typhus occurred in eastern Europe, North Africa, concentration camps, and southern Italy, where dichlorodiphenyltrichloroethane (DDT) was used against lice to abort an epidemic in Naples in 1944.

R. prowazekii is transmitted between patients by the human body louse *(Pediculus humanus corporis)*, which is strictly adapted to humans, lives in the clothes, and takes a blood meal five times daily.[29] Lice become infected while feeding on the blood of rickettsemic patients. *R. prowazekii* enters the louse gut epithelial cells and replicates by means of binary fission until the massively infected cells burst[30] and are released into the louse feces 5 to 7 days after ingestion. Lice are not adapted to an elevated body temperature and leave febrile patients for a new host. *Rickettsia*-laden louse feces are deposited on the skin and clothes and are introduced into the new host by being scratched into the louse-bitten skin or being rubbed into mucous membranes such as the conjunctiva; they are also transmitted through inhalation.

Persons who recover from typhus fever remain latently infected and are susceptible to reactivation of the infection and rickettsemia, which can infect body lice and ignite an epidemic under circumstances of crowding, extreme poverty, cold climate, and poor hygiene, which can lead to a high prevalence of louse infestation (Fig. 189.1). The louse population can expand rapidly (11% daily).[30,31] Lice are currently prevalent in poor countries and found on homeless persons in developed countries. In France, 35% of members of the homeless population have lice. Risk factors for reactivation of latent infection in humans have not been determined, but waning immunity, poor nutrition, alcoholism, and stress have been hypothesized as potentially important.

Typhus is endemic in the Peruvian Andes, Burundi, and Rwanda. The most recently documented outbreak occurred in a youth rehabilitation center, where the recorded attack and case-fatality rates were 10% and 3.5%, respectively.[32] Cases have been diagnosed in Russia, in patients from Morocco, Algeria, and Senegal, and in a homeless person in France.[2,33–37] Given the geographic distribution of latent typhus infection from known historic epidemics, the neglect with which this disease has been handled, and the limited availability of laboratory diagnostic methods, the current epidemiology of louse-borne typhus, particularly in marginal populations of mountainous areas in Asia, South and Central America, and Africa, is unknown.

Sylvatic Typhus

An extrahuman reservoir of *R. prowazekii* exists in a large portion of southern flying squirrels *(Glaucomys volans),* which are distributed from Florida to Maine and westward to Minnesota and eastern Texas.[14] In stark contrast with the louse-human cycle, in which 15% of humans

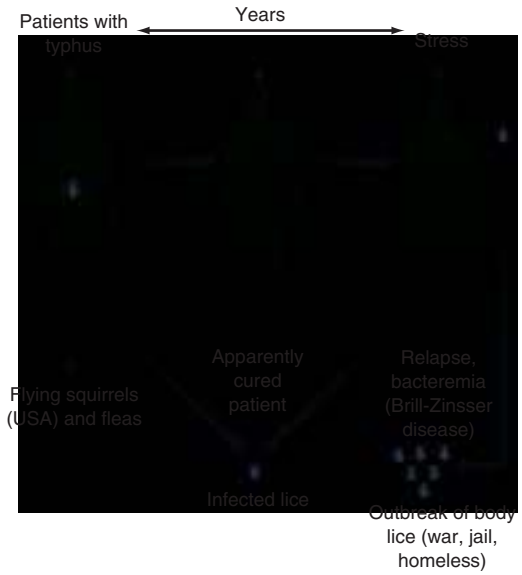

FIG. 189.1 Transmission of typhus.

die and 100% of lice develop rickettsial destruction of the intestine with extravasation of the red blood meal into the hemocele and death, *R. prowazekii* causes subclinical rickettsemia in flying squirrels and no ill effects on the squirrel's own species of lice and fleas.[38] Infections are apparently transmitted to humans by flying squirrel fleas or mucous membrane or inhalation exposure to the feces of the flea or louse. These illnesses occur mainly during winter, when flying squirrels enter buildings.[39] Clusters of cases have occurred in association with flying squirrel–infested attics and walls of dwellings.[40,41] A case of recrudescent typhus has been described in a patient 11 years after *R. prowazekii* infection related to close contact with flying squirrels.[42] The role of ticks in transmitting *R. prowazekii* is currently unknown, although this source would appear to be a potential explanation for a case of *R. prowazekii* originating in southern Texas.[43]

PATHOGENESIS

After inoculation into the skin, *R. prowazekii* spreads throughout the body via the bloodstream. Rickettsiae enter mainly endothelial cells and, to a lesser extent, macrophages by induced phagocytosis; they escape from the phagosome into the cytosol, where they proliferate until the cell bursts.[44] Phospholipase D has been found to play a significant role in pathogenesis, with evidence for mediating rickettsial phagosomal escape in concert with rickettsial hemolysin C.[45,46] The posttranslational methylation of *R. prowazekii* outer membrane protein B appears to play a role in virulence.[47] Point mutation–derived inactivation of a methyltransferase is responsible for the attenuation of the Madrid E strain (the historical vaccine strain).[48] *R. prowazekii* lacks actin-based mobility that mediates the cell-to-cell spread of spotted fever group rickettsiae. Rickettsial phospholipase A_2 activity has been hypothesized to play a role in lysis of infected cells. A gene for a patatin-like protein has been identified in *Rickettsia,* with sequence similarity to phospholipase A.[49,50] The *R. prowazekii* genome encodes two of these proteins.[51] One is similar in sequence to the *Pseudomonas aeruginosa* cytotoxin ExoU phospholipase A and similarly exhibits superoxide dismutase–dependent activation without experimental evidence for pathologic effect.[25] The principal pathophysiologic effects of rickettsial infection include increased vascular permeability and petechial hemorrhage. Thrombosis occurs in only a tiny minority of foci of vascular infection and injury, and ischemic necrosis is a rare consequence.[4] The most important vital target organs are the brain and the lung. The petechial maculopapular rash is a manifestation of cutaneous vascular infection by *R. prowazekii.* Classic pathologic lesions are composed of swollen, infected endothelial cells in the microcirculation, with adjacent perivascular infiltration by lymphocytes and macrophages, which represents the host effector cellular immune response.[4,52] The primary animal model for epidemic

typhus is guinea pigs infected intraperitoneally with *R. prowazekii.* Lesions typical of typhus encephalitis are produced.[53] Balb/C mice have been reported to become infected by a narrow dose range of *R. prowazekii,* develop illness associated with growth of *R. prowazekii,* mount an effective immune response, and develop recrudescence after immunosuppression with corticosteroids.[54,55] Nonhuman primate models have been described that reproduce the pathologic lesions of human typhus.[56,57]

CLINICAL MANIFESTATIONS

Louse-Borne Epidemic Typhus

Experiments in human volunteers during the preantibiotic era demonstrated that after an incubation period of 8 to 16 days (mean, 11.1 days) and a prodrome of 2 days, rash developed in 79% of subjects an average of 4 days after onset of illness, and the fever lasted for 12 days. In an epidemic in Poland after World War I, a prodrome of 1 day or longer occurred in 88%, followed by fever (100%), headache (89%), chills (74%), myalgias (54%), rash (an entry criterion of the study), conjunctival injection (87%), and rales (74%).[4] Erythematous 2- to 6-mm macules appeared most often on the trunk on day 5 and later on the extremities. Without the availability of antibiotics, the course was characterized by marked delirium (48%), severe cough (38%), hemorrhagic rash (34%), gangrene (4%), coma (6%), death (13%) at a median of 12.5 days after onset, or defervescence at a median of 14 days after onset. Studies from Ethiopia and Burundi have demonstrated a lower incidence of visible rash in darkly pigmented skin, marked myalgia, variable incidence of stupor, cough, and conjunctivitis (Table 189.1), and a lower case-fatality rate resulting from effective antimicrobial treatment in many patients.[2,58]

Onset is usually abrupt, with rigors, malaise, and severe headache. The tongue is dry and the patient is constipated. The rash progresses through macules that disappear on pressure to maculopapules with petechiae. The face, palms, and soles are usually spared. In darkly pigmented patients, cutaneous lesions are more easily visible in the axilla. Chest radiographs frequently reveal interstitial pneumonia. Cases have not occurred in settings in which clinical laboratory abnormalities have been studied extensively, except for the Ethiopian series, which demonstrated values characteristic of disseminated rickettsial vascular injury and increased vascular permeability (Table 189.2).[58]

TABLE 189.1 Clinical Manifestations of Epidemic Typhus

	PLACE	
MANIFESTATION	**BURUNDI**	**ETHIOPIA**
Number of cases	102	60
Fever >39°C	100%	100%
Headaches	100%	100%
Any rash	25%	38%
Purpuric rash	11%	33%
Stupor	81%	35%
Coma	4%	—
Cough	70%	38%
Nausea, vomiting	57%	43%
Conjunctivitis	15%	53%
Diarrhea	13%	—
Splenomegaly	8%	13%
Photophobia	—	33%
Myalgias	100%	70%

Data for Burundi from Fournier PE, Ndihokubwayo JB, Guidran J, et al. Human pathogens in body and head lice. Emerg Infect Dis. *2002;8:1515–1518; data for Ethiopia from Perine PL, Chandler BP, Krause DK, et al. A clinico-epidemiological study of epidemic typhus in Africa.* Clin Infect Dis. *1992;14:1149–1158.*

Sylvatic Typhus

Flying squirrel–associated typhus cases have generally been less severe, with no fatalities recorded. Patients have developed fever (100%), headache (81%), maculopapular rash (66%), confusion (44%), and myalgia (42%).[39,59–61]

Recrudescent Typhus

In 1910, Brill's clinical description of recrudescent typhus was that of an illness resembling nonfatal typhus, with onset characterized by chills, intense headache, fever, myalgias, nausea, and sometimes vomiting. Patients are prostrate with apathy, dulled sensorium, and persistent headache as an overriding symptom. Between the fifth and seventh days of illness, a maculopapular rash appears on the back and abdomen and spreads rapidly. Congested conjunctivae and constipation are prominent features in many cases. The untreated illness lasts about 2 weeks. Brill stated, "In the case of an epidemic of typhus fever, in my opinion, it would be simply impossible to say that these cases which I have described were not mild typhus fever."[5] Twenty-four years later, Hans Zinsser isolated rickettsiae from the blood of similar patients—louse-free immigrants from typhus-endemic regions of Europe. Thus, the eponymous designation, Brill-Zinsser disease, is used to refer to recrudescent typhus.[6]

DIAGNOSIS

In the midst of a recognized epidemic, the patient with a late-stage florid rash or even one with fever, severe headache, and myalgia without a rash would likely be diagnosed with louse-borne typhus. The diagnosis of louse-borne typhus early in an outbreak, when only a few cases have been reported, is more challenging. The most important differential diagnoses are typhoid fever and malaria in tropical countries.[33] The prominent cough and crackles often suggest a diagnosis of pneumonia. Neurologic signs and cerebrospinal fluid pleocytosis may lead to consideration of viral or bacterial meningoencephalitis. Nausea, vomiting, and abdominal tenderness raise the diagnostic possibilities of viral or bacterial enterocolitis or even an acute abdominal condition requiring surgery. Jaundice and elevated hepatic enzyme levels suggest viral hepatitis. The hemorrhagic rash may lead to diagnostic consideration of arenaviral and filoviral hemorrhagic fevers.[62] Other differential diagnoses include leptospirosis, arboviral and enteroviral infections, meningococcemia, trench fever, relapsing fever, and other rickettsioses.

Other circumstances under which *R. prowazekii* infection should be considered involve immigrants from regions where epidemic typhus has been prevalent, persons who have been exposed to flying squirrels, and homeless persons.[34,39,59] It is unlikely that cases of aerosol-transmitted typhus in a bioterrorist attack would be diagnosed clinically before the onset of rash, if even then.[63]

The laboratory diagnosis of louse-borne typhus generally relies on the detection of antibodies with a fourfold rise in titer in convalescence.

TABLE 189.2 Clinical Laboratory Findings in Patients With Epidemic Typhus

FINDING	**INCIDENCE (%)**
White blood cell count	Low, 3; elevated, 14
Thrombocytopenia	43
Increased serum AST	63
Increased serum ALT	35
Increased serum LDH	82
Increased serum CPK	31
Increased BUN	31
Decreased serum protein	38

ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *BUN,* blood urea nitrogen; *CPK,* creatine phosphokinase; *LDH,* lactate dehydrogenase.
Modified from Perine PL, Chandler BP, Krause DK, et al. A clinico-epidemiological study of epidemic typhus in Africa. Clin Infect Dis. *1992;14:1149–1158.*

Usually, a diagnostic titer is detected during the second week of illness. The standard serologic method is indirect immunofluorescence assay, and an immunoglobulin G (IgG) titer of 1:128 or an IgM titer of 1:32 confirms the diagnosis. Enzyme immunoassays that yield reliable results have also been developed. Although the *Proteus vulgaris* OX-19 agglutination (Weil-Felix reaction) has been demonstrated to be poorly sensitive and nonspecific, it may be useful when it is the only method available in a developing country. Antibodies stimulated by *R. prowazekii* react with shared antigens of *R. typhi,* allowing diagnostic detection of cross-reactive antibodies. A fourfold higher titer against *R. prowazekii* than *R. typhi* distinguishes epidemic typhus from murine typhus in fewer than half of cases.[64] The predominance of IgG antibodies and the absence of IgM and Weil-Felix antibodies in recrudescent typhus is controversial.[65]

Although serodiagnosis is retrospective, methods that can establish a diagnosis during the acute stage of infection—namely, the polymerase chain reaction (PCR) assay and immunohistochemical detection of *R. prowazekii* in blood or tissue, respectively—are seldom available in regions where typhus epidemics occur.[66–68] Although loop-mediated isothermal amplification offers a relatively rapid and field-applicable molecular diagnostic tool,[69] the presence of few circulating rickettsial organisms within the bloodstream limits the utility of even the most analytically sensitive molecular assays for clinical use.[70] Feasibility has been demonstrated for serodiagnosis on blood spotted onto filter paper and for rickettsial isolation or PCR detection in lice removed from the patient, either of which can be sent to a referral laboratory by mail.[71,72] Strain typing with PCR to amplify a minimal gene set may be useful to study the origin of strains during an epidemic or to identify strains used in bioterrorism.[73] Rickettsiae may also be isolated most effectively from blood, buffy coat, plasma, or tissue in shell vial cell culture.[74]

TREATMENT

The treatment of choice for all patients who are not allergic to tetracyclines and who are not pregnant is doxycycline, 100 mg twice daily for 7 days.[75] Under chaotic epidemic conditions and in other situations in which doxycycline availability is limited, a single dose of 200 mg of doxycycline is effective, although in a small portion of patients relapse may occur.[76] Where available, chloramphenicol, 60 to 75 mg/kg/day in four divided doses (not to exceed 4 g total), and tetracycline, 25 to 50 mg/kg/day in four divided doses, are also effective. Other antibiotics, including β-lactams, aminoglycosides, and sulfonamides, are ineffective. Although fluoroquinolones, rifampin, and some of the newer macrolides show inhibition of growth of *R. prowazekii* in cell culture, none has proven to be efficacious clinically.

PREVENTION

Control of body lice is the mainstay in the prevention of epidemic typhus. When an outbreak of lice appears, the first step is to change all garments and wash them in hot water. Introducing regular washing of clothes will stop outbreaks. Only when this is impossible is delousing with insecticides, such as Lindane in powder form, useful. Application of 30 to 50 g of 1% permethrin dusting powder per adult both inside and outside of clothing and on bedding may be repeated every 6 weeks to kill lice.[77] Mass treatment of clothed individuals with permethrin can be achieved by use of a compressed air duster.[78]

No vaccine is currently available for the prevention of typhus. However, the identification of the attenuating point mutation of a previously successful live vaccine (E strain) that was prone to reversion offers the opportunity to develop a permanently attenuated protective vaccine against *R. prowazekii.*[48,79,80] The discovery of protective T-cell antigens via the in silico analysis of 834 *R. prowazekii* proteins shows promise for further vaccine development.[81]

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190 *Rickettsia typhi* (Murine Typhus)

Lucas S. Blanton, J. Stephen Dumler, and David H. Walker

SHORT VIEW SUMMARY

Microbiology
- Murine typhus is caused by *Rickettsia typhi,* an obligately intracellular gram-negative bacterium.
- The organism infects endothelial cells in mammalian hosts and midgut epithelial cells in flea hosts.

Epidemiology
- Murine typhus is distributed worldwide and is prevalent in tropical and subtropical seaboard regions where rats (*Rattus* spp.) and their fleas (*Xenopsylla cheopis*) serve as reservoirs and vectors, respectively.
- In the United States, most cases are reported in southern Texas and southern California where the cat flea (*Ctenocephalides felis*) is the predominant vector and opossums are the suspected reservoir.
- The disease is transmitted by the inoculation of infected flea feces into a flea bite wound. The incubation period is 1 to 2 weeks.

Clinical Manifestations
- Frequent symptoms in early illness include fever, headache, nausea, and vomiting.
- Rash occurs in about one-half of patients. It is usually described as macular or maculopapular, and is most often observed on the trunk.
- The clinical course is usually uncomplicated, but occasionally central nervous system abnormalities, renal insufficiency, respiratory failure, and death occur.
- Elevations in serum hepatic aminotransferase levels are frequent laboratory findings. Vascular injury often leads to hypoproteinemia, hypoalbuminemia, and electrolyte abnormalities (e.g., hyponatremia and hypocalcemia).

Diagnosis
- Early diagnosis of murine typhus is based on clinical suspicion and epidemiology.
- Immunofluorescence assay is the mainstay of serologic diagnosis, which is generally retrospective. A fourfold rise in immunoglobulin G titer from acute illness to convalescence confirms the diagnosis.
- Immunohistochemical detection of *R. typhi* in a skin biopsy specimen can establish the diagnosis during acute infection. Polymerase chain reaction assay amplification of rickettsial nucleic acids in blood or skin biopsy samples can also establish the diagnosis, but sensitivity is poor on blood.
- To avoid prolonged illness and severe or potentially fatal infection, treatment should not be withheld while awaiting laboratory confirmation.

Treatment and Prevention
- Doxycycline, 100 mg twice daily for 7 to 10 days, is the treatment of choice.
- Prevention is directed toward the control of flea vectors and potential flea hosts.

In 1922, Hone first described human infections "closely resembling typhus fever."[1] Since 1926, when Maxcy successfully identified murine typhus as a distinct clinical and epidemiologic entity, and 1931, when Dyer isolated a new typhus group named *Rickettsia* from rats and fleas, murine typhus has been recognized as a worldwide zoonotic problem.[2] Often underrecognized and believed to be clinically mild, murine typhus may occur in epidemics or with high prevalence in certain geographic regions.[3–9] Illness may be severe, particularly in the elderly, with death occurring in a small proportion of individuals.[10,11] The association with both rat and cat fleas is now well established.[12,13]

MICROBIOLOGY

Rickettsia typhi, the causative agent of murine typhus, is an obligately intracellular bacterium that infects endothelial cells in mammalian hosts and midgut epithelial cells in the flea hosts.[12] A new rickettsial agent, *Rickettsia felis,* has been recognized as sharing some antigenic and genetic components with *R. typhi* but is best phylogenomically characterized as a transitional group bacterium between typhus and spotted fever group rickettsiae.[14,15] As typical for the genus *Rickettsia, R. typhi* contains rickettsial outer membrane protein B (OmpB) and the autotransporter Sca4, but its lack of rickettsial outer membrane protein A (OmpA), a characteristic of spotted fever group rickettsiae, resembles the typhus group.[16] In addition to *ompB,* three other autotransporters—*sca1, sca2,* and *sca3*—are expressed.[17] OmpB mediates adhesion and entry events for other *Rickettsia* spp., and Sca2 also functions as an adhesin in spotted fever rickettsiae.[16,18] In addition, the *R. typhi* genome encodes genes for several potentially membranolytic proteins (*tlyA, tlyC, pldA,* and two phospholipase A2 genes) and RalF, a type IV secretion system effector that could facilitate cell invasion or endosomal escape.[15,19–22] These organisms are well adapted for intracellular life because they lack enzymes for carbohydrate metabolism, lipid biosynthesis, nucleotide synthesis, and amino acid metabolism and a complete tricarboxylic acid cycle but possess several ATP-ADP translocase genes that likely mediate host energy parasitism.[15] The genome encodes Sec, TolC, and type IV secretion system components.[15,23–25] As in the case of TolC-dependent secretion of an ankyrin repeat–containing protein, Rickettsia ankyrin repeat protein 1 (RARP-1), these secretion system components suggest delivery of effector proteins into the host cell to influence cell function and fate.[25] Unlike the situation for spotted fever group rickettsiae, *R. typhi* lacks *rickA,* which encodes a protein promoting intracellular motility via actin polymerization.[16]

EPIDEMIOLOGY

Murine typhus is found worldwide and is especially prevalent in tropical and subtropical seaboard regions, where the most important rat reservoirs (*Rattus* spp.) and flea vectors (*Xenopsylla cheopis*) are found (Fig. 190.1).[2,12,26] Urban ports, often rife with rats, have been associated with a higher prevalence of murine typhus.[27] An important vector in some areas (southern Texas and southern California) is the cat flea (*Ctenocephalides felis*), and opossums have been implicated as a potential reservoir in these areas.[8,12,26,28–30] Thus residents and visitors to these urban and suburban regions are at risk when flea-bearing animals bring infected fleas into close proximity to humans. The demonstrable seroprevalence to typhus group antibodies in areas where the disease is endemic, and in areas where the disease is not well recognized, indicates that murine typhus is likely vastly overlooked.[31–34]

Murine typhus was once more prevalent in the United States, especially in the warm humid South. In 1944, at the peak of reporting, there were 5401 cases.[35] Efforts to control rat populations, and more likely the strategic use of DDT on rat runs and harborages, made dramatic strides

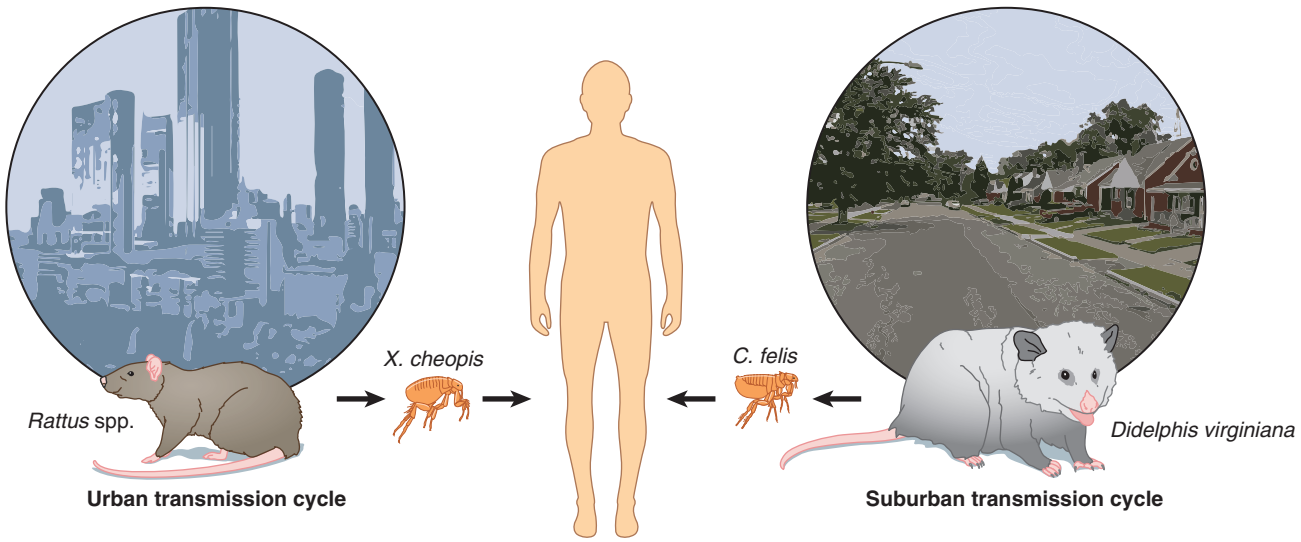

FIG. 190.1 Transmission of murine typhus. In the urban transmission cycle, rats (*Rattus* spp.) serve as reservoirs and their fleas (*Xenopsylla cheopis*) serve as vectors. In the suburban transmission cycle, the cat flea (*Ctenocephalides felis*) is the predominant vector and opossums (*Didelphis virginiana*) are the suspected reservoir.

to break the rat-flea transmission cycle and decrease the prevalence of murine typhus.[36,37] By 1956 fewer than 100 cases were reported in the United States.[35] In the decades following this dramatic decline of murine typhus, there persists a low but increasing level of reported infections, with most cases seen in south Texas and southern California.[29] In Texas, the last several decades have witnessed the majority of cases occurring in the southernmost counties along the Mexican border, but in the last 10 years there have been increasing reports of murine typhus occurring in more northern municipalities.[38,39] Outbreaks in an urban area of central Texas, the northern Texas coast, and in Orange County, California, have been associated with opossums and *R. typhi*–infected *Ct. felis.*[30,32,40,41] Epidemiologic observations have led to the hypothesis that cats, hosts for *Ct. felis,* play a role in the transmission of murine typhus.[28,42] In addition, *R. typhi* has been isolated from *Rhipicephalus sanguineus* ticks collected from dogs.[43] More studies are needed to elucidate the role domestic animals play in the transmission and maintenance of *R. typhi.*

Outbreaks throughout the world occur when there is inadequate vector and reservoir control.[2,44–48] Among displaced Khmers at the Thailand-Cambodia border with unexplained fever, 70% were cases of murine typhus, with an attack rate of 172 per 100,000 adults.[9] Murine typhus has been implicated in as many as 4% to 26% of cases of undifferentiated febrile illness in Nepal,[49–52] 0.4% to 7% in countries of Southeast Asia,[53–55] and as few as 0.5% in northern Tanzania.[56] In northern Vietnam, when malaria, dengue, typhoid fever, and leptospirosis are excluded, *R. typhi* has been attributed to 33% of undifferentiated febrile illness.[57] Most patients are adults, although persons of all ages may become ill.[58] Cases are recognized year-round, with peak prevalence from April through June in Texas[8] and during the warm months of summer and early fall elsewhere.[2,46,59]

Murine typhus has been increasingly recognized as a febrile illness in travelers returning from endemic regions throughout the world. In an analysis of 3655 travelers considered to have acute and potentially life-threatening illnesses, murine typhus was the seventh most prevalent cause (identified in 16), and when excluding the diagnosis of *Plasmodium falciparum* malaria, occurred in 1.9% of febrile patients.[60] Africa, Southeast Asia, South Asia, and the Americas have been reported as sites of disease acquisition in returning travelers.[60–62]

The disease is transmitted after the inoculation of infected flea feces into a flea bite wound. The incubation period is 1 to 2 weeks, commonly 12 days. Because predominantly gut epithelial cells are infected in the flea vector, a reservoir of infected fleas is maintained mostly by horizontal transmission from flea to vertebrate host to uninfected flea.[63] Once infected, the flea maintains the rickettsial infection for the duration of its life. *Rickettsia typhi* may also infect the flea reproductive organs, which explains the low levels of transovarial (vertical) transmission.[64] The longevity of fleas is unaffected by gut epithelial cell or disseminated *R. typhi* infection.[12]

PATHOLOGY AND PATHOGENESIS

Few accurate descriptions of the histopathology of murine typhus are available, despite the fact that the case-fatality rate ranges between 1% and 4%.[8] Pathologic findings indicate systemic endothelial infection similar to epidemic typhus and Rocky Mountain spotted fever.[65,66] Lymphohistiocytic vasculitis may affect any organ, and in fatal cases interstitial pneumonitis, interstitial nephritis, interstitial myocarditis, meningoencephalitis, and portal triaditis may be present. Rickettsiae may be demonstrated in many organs and are especially numerous in foci of vasculitis.[65] This underlying vasculitic lesion and the rickettsia-induced vascular injury account for most of the clinicopathologic abnormalities. Evaluation of serum markers reveals endothelial damage and activation of the coagulation and fibrinolytic pathways.[67] As vascular injury accumulates, a substantial loss of intravascular volume, albumin, and electrolytes occurs, and platelets are consumed at foci of infection. With multifocal heavy infection and attendant inflammation, vascular and parenchymal injury may yield localized symptoms, signs, or laboratory findings related to the sites of infection and injury. The induction of hypovolemia insufficiently corrected by normal homeostatic mechanisms further exacerbates tissue perfusion compromise and may lead to renal insufficiency. Mild-to-moderate hepatic injury is a frequent finding in murine typhus and probably results from multifocal infection of hepatic sinusoidal and portal endothelium, with bystander hepatocyte damage.[8,68] Ocular manifestations, which are often subclinical, are detected by careful ophthalmologic examination, and reflect retinal choroidal vascular injury.[69] With extensive rickettsial vascular injury and hypoperfusion secondary to volume loss, the result may be renal failure, respiratory failure, central nervous system abnormalities, or multiorgan failure.[8,65]

Immunity to *R. typhi* is mediated mainly by early interactions with and maturation of dendritic cells, and early control is provided by natural killer cells. This is followed by adaptive responses via CD4 and CD8 T lymphocytes, their cytokine products interferon-γ and tumor necrosis factor-α, and antibody, which likely plays an adjunctive role.[70–75]

CLINICAL MANIFESTATIONS

Signs and Symptoms

Only a small proportion of patients (median, 4%) with murine typhus recall a flea bite or exposure, and an incubation period of 1 to 2 weeks may transpire before abrupt onset of illness occurs. The presentation



FIG. 190.2 **Truncal rash of murine typhus.**



FIG. 190.3 **Petechial rash of murine typhus.**

is often nonspecific, and fever (93%–100%), headache (10%–91%), myalgia (10%–78%), and nausea or vomiting (14%–59%) are the most frequently reported early clinical manifestations.[8,46,76–80] Rash is noted in up to 18% of patients at presentation, but over the course of illness as few as 3% and as many as 80% will develop this sign, which is more often detected in lightly pigmented than darkly pigmented skin (Fig. 190.2).[81] As the illness progresses, most patients continue with fever and can have gastrointestinal (nausea, 48%; vomiting, 40%; and anorexia, 35%) or respiratory tract (cough in 14%–44%) involvement. Some studies record the presence of hepatomegaly and splenomegaly in up to 22% and 17% of patients, respectively.[82–85] Severe neurologic complications occur in as many as 17% of patients, usually manifesting as photophobia, confusion, stupor, seizures, or localized findings such as ataxia.[86,87]

The absence of rash or lack of petechiae should not dissuade one from a diagnosis of murine typhus. In fact, when rash is identified, it is described as macular or maculopapular in 75% to 80% of cases and petechiae are noted in less than 13% (Fig. 190.3).[8,58,81,83] The rash is most often distributed on the trunk (88% of cases), but involvement of the extremities is not infrequent (>45%). The initial rash distribution is equally frequent on the extremities and on the trunk and is present on the palms and soles in less than 3% of patients.[46]

The clinical course of murine typhus is usually uncomplicated, and childhood murine typhus is often mild,[45,83,88] with one series reporting only nighttime fever with normal daytime activities.[77] The case-fatality rate in the postantibiotic era is 0.4%.[10] However, occasional patients develop central nervous system abnormalities,[69,89–94] renal insufficiency,[78] or respiratory failure requiring intubation.[95] Patients are ill enough that 10% require admission to an intensive care unit, and up to 4% of hospitalized adult patients die of the infection.[8]

Once the diagnosis has been considered and appropriate therapy begun, most patients defervesce rapidly (median, 3 days). Findings associated with severe illness include leukocytosis, elevated blood urea nitrogen or creatinine levels, and a high blood urea nitrogen–to-creatinine ratio. Advanced age and a prolonged interval before the administration of specific antirickettsial therapy are also significantly correlated with severity.[8,11] One report has suggested a link between hemolytic disorders such as glucose-6-phosphate dehydrogenase deficiency, hemoglobinopathy, and thalassemia and more severe illness, including jaundice.[68] A trend toward more severe infection is also noted in patients treated with trimethoprim-sulfamethoxazole.

Laboratory Features

Early mild leukopenia (which coincides with thrombocytopenia) is present in 25% to 50% of patients during the first 7 days of illness. Subsequently, leukocytosis develops in less than one-third. Prothrombin times are occasionally prolonged, but true disseminated intravascular coagulation with hypofibrinogenemia is infrequently documented. The most frequent laboratory abnormality in murine typhus, a mild-to-moderate elevation in serum aspartate aminotransferase levels, is present in most patients (67%–92%), and related indices of hepatic and cellular injury (alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase levels) are often elevated in parallel.* Rickettsia-induced vascular damage frequently leads to hypoproteinemia (45%) and hypoalbuminemia (89%) and is probably responsible in large part for multiple serum electrolyte abnormalities, especially hyponatremia (60%) and hypocalcemia (79%). Even in the presence of symptomatic central nervous system abnormalities, cerebrospinal fluid examination can be normal or reveal findings resembling viral or leptospiral meningoencephalitis. The fluid is usually clear with a normal glucose concentration; abnormalities include pleocytosis, usually lymphocytic, with an increased protein concentration.[90,96]

DIAGNOSIS

Early diagnosis of murine typhus is still based mostly on clinical suspicion. Because timely specific antirickettsial therapy is indicated to avoid severe or potentially fatal infection, treatment should not be withheld while laboratory confirmation is awaited.[8,58] When early suspicion is followed by prompt empirical treatment, length of hospitalization is decreased.[97] Although it affords a definitive diagnosis, culture is rarely attempted because of a reputation of biohazard and difficulty, reducing the use of this potentially valuable adjunct to diagnosis. The shell vial culture technique—or a modification using a 24-well plate—has been successfully used to obtain isolates.[32,98,99]

*References 8, 29, 38, 52, 65, 67.

The predominant method of laboratory confirmation is serologic. Because antibodies are infrequently detected during acute illness, serologic diagnosis is retrospective.[83] Obsolete Weil-Felix agglutination reactions have proved insensitive, are intrinsically nonspecific, and, as such, should not be used to establish a definitive diagnosis.[100] Instead, sensitive serologic tests that use specific *R. typhi* antigens, such as indirect fluorescent antibody (IFA) assay, are preferable. With the use of a sensitive and specific test such as the IFA assay, diagnostic titers are present in approximately 50% of murine typhus patients within 1 week and in almost all patients within 15 days after the onset of illness.[8,83] Although a single reactive immunoglobulin G titer during a clinically compatible illness is suggestive of the diagnosis, confirmation generally requires seroconversion or a fourfold rise in immunoglobulin G titer between acute- and convalescent-phase sera. Even in areas where the disease is recognized to be endemic, confirmatory testing is often not performed or reported.[101,102] The accuracy of IFA end point titers are subject to microscopist experience and often exhibit interobserver variability.[103] Sera from blood-spotted filter paper may be an inexpensive yet effective method for sample collection and storage prior to serologic testing.[104] Because typhus group rickettsiae share antigens, routine serologic evaluation does not distinguish between epidemic and murine typhus.[105] In occasional sera, reactions against both typhus and spotted fever groups are observed, creating further diagnostic difficulties.

Other methods for laboratory confirmation of rickettsiosis include the immunohistologic demonstration of *R. typhi* in tissues, but the sensitivity, specificity, and predictive value are unknown.[65,106,107] Polymerase chain reaction (PCR) assay amplification of rickettsial nucleic acids in peripheral blood or skin biopsy samples has been achieved. Unfortunately, despite the ability of quantitative real-time PCR to detect very low copy numbers of target genes, the low numbers of circulating rickettsiae within the bloodstream limits its clinical sensitivity. A meta-analysis reveals that PCR for the detection of typhus group rickettsiae has a median clinical sensitivity of 3% and 6% from blood and tissue specimens, respectively.[108] Considering this limitation, it is doubtful that development of more field-applicable, rapid turnaround assays (e.g., loop-mediated isothermal amplification and recombinase polymerase amplification) will have much clinical utility in their current forms.[109,110] Neither immunohistochemistry nor PCR is generally available in clinical laboratories.

Most patients are initially investigated for fever of undetermined origin, and less often patients are investigated for upper or lower respiratory tract infection, urinary tract infection, cerebrovascular accident, gastroenteritis, or neoplasm, among other diagnoses.[8,111] Initial diagnostic workup may be extensive and costly prior to the consideration of murine typhus.[112] Despite the occasional presence of findings that suggest alternative diagnoses because of attention to isolated organ system involvement, an early clue toward the successful diagnosis of murine typhus is recognition of the systemic manifestations associated with fever. Other rickettsioses may cause considerable difficulty in the differential diagnosis. In the Western Hemisphere, Rocky Mountain spotted fever is the most frequent, whereas in Europe, Africa, and Asia, other spotted fever rickettsioses or scrub typhus should be considered. Murine typhus and spotted fever rickettsiosis may be distinguished on the basis of the history, clinical presentation, and fourfold differences in titers in specific serologic tests.[113] Many patients with murine typhus are exposed to animals or flea vectors in urban or suburban regions,[3,13,59,114] and a small proportion (1%–40%) report a flea bite or exposure, whereas patients with Rocky Mountain spotted fever often acquire illness after outdoors exposure or documented tick bites and more often develop rash and petechiae. An inoculation lesion or eschar is exceedingly rare in both murine typhus and Rocky Mountain spotted fever but is more often present in other spotted fever group rickettsioses and scrub typhus.[107] In areas where both murine typhus and scrub typhus are common, those living in more densely populated areas, such as urban centers, are more likely to be exposed to the vectors of *R. typhi* than those of *Orientia tsutsugamushi*.[115] The distribution of rash is of little help in individual cases.[8,58,59,78] The likelihood of monocytic ehrlichiosis or human granulocytic anaplasmosis is diminished if leukopenia and thrombocytopenia are minimal or absent, although serum hepatic aminotransferase levels may be elevated in both murine typhus and ehrlichiosis. Murine typhus, spotted fever rickettsiosis, and ehrlichiosis or anaplasmosis occur during warm seasons, when the arthropod vectors are most active. In contrast, the louse vector of epidemic typhus is most active and likely to spread *Rickettsia prowazekii* in cool seasons when layers of clothing are worn, persons are crowded indoors, and personal hygiene of extremely poor persons diminishes. Differentiation of sporadic cases of sylvatic typhus caused by *R. prowazekii* from murine typhus may be difficult, but the former illness is suggested when exposure to the flying squirrel reservoir is elicited.

There are many differential diagnoses of murine typhus because of its usually nonspecific presentation. Aside from the rickettsioses and ehrlichiosis, alternative diagnoses include meningococcemia, measles, typhoid fever, bacterial and viral meningitis, secondary syphilis, leptospirosis, toxic shock syndrome, and Kawasaki disease.

TREATMENT AND PREVENTION

The preferred drug for treatment of *R. typhi* infection is a tetracycline, such as doxycycline. In vitro, doxycycline, rifampin, chloramphenicol, and the fluoroquinolones (e.g., ciprofloxacin and levofloxacin), as well as azithromycin, inhibit rickettsial growth and have ratios of maximal serum concentration to minimal inhibitory concentration achievable in human therapy.[116–118] No prospective clinical trials have been conducted regarding treatment of murine typhus; thus all current recommendations are based on retrospectively analyzed series and cases. The current recommendation is for administration of twice-daily doxycycline, 100 mg orally or, in severely ill patients, intravenously. The use of doxycycline and tetracycline is supported by extensive experience as detailed in a monograph that analyzed over 600 patients from numerous case series and reports.[119] Although chloramphenicol, 50 to 75 mg/kg/day in four divided doses, has been long advocated as the best alternative treatment for murine typhus, the evidence for its use is derived from case series comprising treatment in only 97 patients. Oral chloramphenicol is not currently available in the United States, and the parenteral formulation is exceedingly difficult to procure. Clinical trials of fluoroquinolones for treatment of spotted fever rickettsioses have shown their effectiveness as alternatives to tetracyclines, and for murine typhus, reports of successful treatment[92,120–125] with ciprofloxacin outnumber reports of poor responses,[126] although these data only involved treatment of 37 patients. Corticosteroids are occasionally used for severe central nervous system disease, but no controlled study to evaluate their efficacy has been performed. Infected pregnant patients must be evaluated individually, and when possible, doxycycline is preferred. Although tetracycline has been associated with serious adverse events in pregnant women (hepatotoxicity) and in the developing fetus (teratogenicity and tooth discoloration), these tetracycline class effects appear less prevalent with doxycycline.[127] Poor outcomes associated with a third of neonates in a cohort of pregnant women with suboptimal treatment for murine typhus must be considered when weighing the risks and benefits of doxycycline in this population.[128] Alternately, and where available, chloramphenicol can be used (early trimester). Case reports touting good outcomes with azithromycin during pregnancy[129] are tempered by the lack of benefit in neonatal outcomes in the aforementioned cohort.[128] Antimicrobial therapy should be continued until 3 days after defervescence. After initiation of therapy, patients become afebrile at a median interval of 3 days. Single-dose doxycycline therapy was effective in almost 80% of patients in one study[82] but is not routinely advocated because relapse may occur.

Prevention is directed primarily toward the control of flea vectors and potential flea hosts.[12] Success of this approach is exemplified by the control efforts using dichlorodiphenyltrichloroethane (DDT) in the United States during the 1940s.[35,36] Because of the potential for epidemic spread associated with foci of infected flea infestations, all suspected cases of murine typhus should be reported promptly to local public health authorities. Although it is usually considered a mild illness, murine typhus may be fatal or severe if misdiagnosed or inadequately treated. Unfortunately, no vaccine of proven effectiveness exists for murine typhus. Recovery from natural infection confers solid, long-lasting immunity to reinfection.

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The complete reference list is available online at Expert Consult.

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191 *Orientia tsutsugamushi* (Scrub Typhus)

Daniel H. Paris

SHORT VIEW SUMMARY

Definition
- Scrub typhus is a serious rickettsial illness characterized by an eschar at the mite bite site, possible skin rash, and an initial flulike febrile illness with complications such as respiratory and renal failure, meningoencephalitis, and severe multiorgan failure.

Epidemiology
- *Orientia* spp. are predominantly spread across the Asia-Pacific region, but emerging evidence suggests a worldwide distribution in tropical and subtropical zones. Scrub typhus is the leading cause of treatable undifferentiated febrile illness in many parts of Southeast Asia.

Microbiology
- Scrub typhus is caused by *Orientia* spp.—most commonly *Orientia tsutsugamushi*—an obligate intracellular pathogen that is transmitted to humans by trombiculid mites.

Diagnosis
- Historically the gold standard diagnosis of scrub typhus was based on serology, but the added value of polymerase chain reaction (PCR) in the early bacterial dissemination phase of disease has led to a combination of "PCR plus serology" for most rickettsial diseases.

Therapy
- Scrub typhus is easily treated with appropriate antibiotics (doxycycline, azithromycin, and chloramphenicol), and upon clinical suspicion early consideration of empirical treatment is justified.

Prevention
- The many heterogeneous strains of *O. tsutsugamushi* and the lack of long-term protective natural immunity pose difficulties in vaccine development. Currently, no licensed vaccines or vector-control efforts are available for scrub typhus.

HISTORY

The earliest clinical reports of possible scrub typhus date back to the Chinese manual *Zhouhofang* in 313 BC. In 1810 the association of a febrile illness with mite transmission was made in the Niigata Prefecture in Japan, which led to the first clinical definition of tsutsugamushi fever. Historically, the occurrence of scrub typhus was associated with its dominant presence in Asia, in an area defined as the "Tsutsugamushi Triangle," but emerging reports of *Orientia* spp. and clinical cases reported from regions in Africa, Europe, and South America suggest a wider global distribution in tropical to subtropical regions (Fig. 191.1). The recent description of confirmed human cases in Chile has led to a paradigm shift in the epidemiology of scrub typhus.[1] Today, scrub typhus is probably the world's most relevant rickettsial disease based on its disease burden, and although the understanding of its global distribution is expanding, the available literature remains limited.[2,3]

DEFINITION

Scrub typhus is a vector-borne infectious disease caused by the obligate intracellular bacterium *Orientia tsutsugamushi*. The initial clinical presentation includes nonspecific "flulike" symptoms such as fever, fatigue, frontal headaches, myalgia, cough, restlessness, and insomnia. In many cases an inoculation lesion may appear, termed eschar or "tache noire" (Fig. 191.2A–B). However, in areas where scrub typhus is common and the population is regularly exposed, the presence of an eschar may occur less frequently; this is likely due to preexisting cellular immunity against *Orientia* spp.[4,5] Similarly, a diffuse, macular or maculopapular skin rash can develop within 3 to 8 days after the onset of fever. Confusion and gastrointestinal symptoms are common, and complications include respiratory and renal failure, meningitis or meningoencephalitis, and, very rarely, disseminated intravascular coagulation. Severe scrub typhus manifested with severe multiorgan failure in up to a third of hospitalized patients in India, with an average 24% mortality rate.[2,6] In Lao patients with central nervous system (CNS)–related complications, a mortality rate of 14% was observed,[2,7] and scrub typhus during pregnancy was associated with poor maternal and fetal outcomes (Laos and Thai-Myanmar border), with approximately a third of cases resulting in either abortion or stillbirth.[2,8,9] However, to what extent such outcomes depend on the strain of *Orientia,* immune competence, and genetics of the patient remains to be defined.

In most endemic areas of scrub typhus, the following differential diagnosis for "typhus-like illnesses" should be considered: malaria, murine typhus, dengue, leptospirosis, Q fever, typhoid, melioidosis, and chikungunya fever; malaria and meningitis should always be ruled out first.

ECOLOGY AND EPIDEMIOLOGY

Scrub typhus is a zoonotic disease, and *Orientia* spp. are transmitted to humans by trombiculid mites (phylum Arthropoda, family Trombiculidae, genus *Leptotrombidium*) via the bite of the larval stage, termed *chiggers* (see Fig. 191.2C–D). Mites represent both vectors and major reservoirs of *Orientia* spp. in nature; bacteria can be passed on transstadially through all stages in the life cycle and maintained transovarially over multiple generations. The life cycle of trombiculid mites takes place in the soil, ranging from mountainous heights (i.e., Himalayas), semiarid regions, jungle, and shrubby fringes to river banks and beaches at sea level. Humans play no role in the *Orientia* mite life cycle and are dead-end hosts; this is relevant because any human-based preventive interventions such as treatment or vaccines will not affect disease incidence rates. Scrub typhus can exhibit a pronounced seasonality, with high transmission peaks before and after the rainy season in regions of Southeast Asia, with a regular year-round transmission common in tropical and subtropical regions.[7]

Scrub typhus is a truly neglected tropical disease. Prospective causes-of-fever studies have shown it to be a leading cause of treatable nonmalarial febrile illness in many regions of Asia, affecting mainly rural populations but occurring increasingly also in urban settings, especially in fast-growing metropolitan areas in China and Korea.[2,7,10,11] Given the median seroprevalence of 22%, approximately every fifth person carries antibodies against *Orientia* spp. in Asia. All five countries with a passive national surveillance system in place have reported an apparent rise in

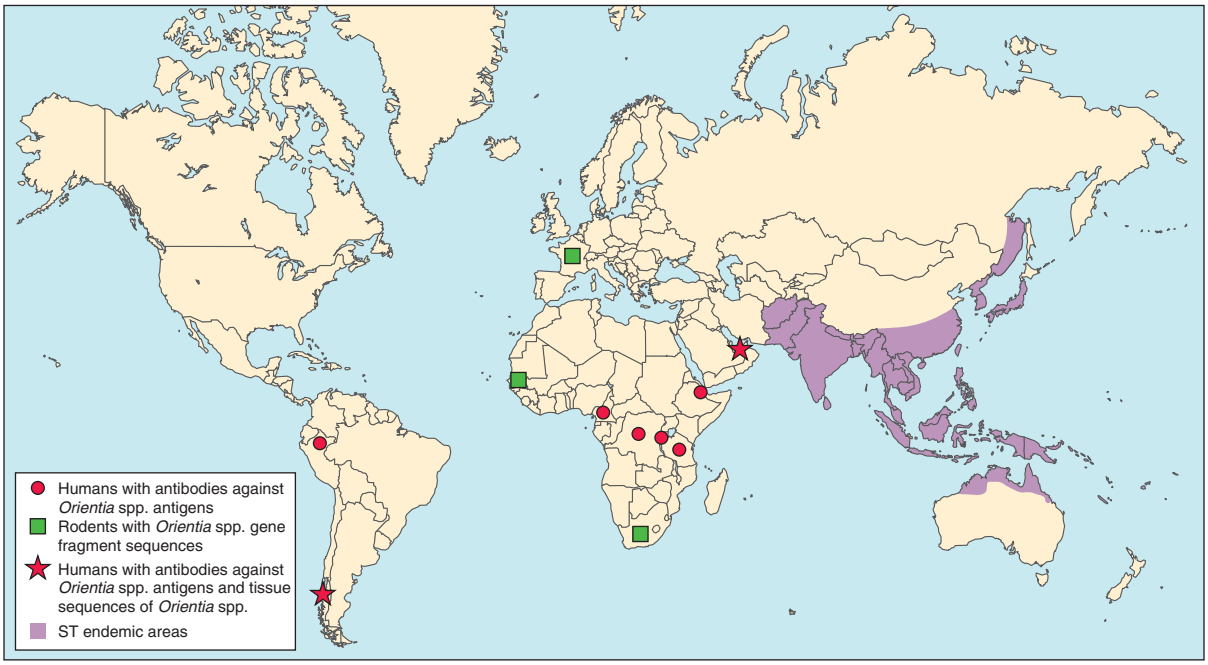

FIG. 191.1 World distribution of scrub typhus cases and *Orientia* spp. Originally scrub typhus was believed to be restricted to the "Tsutsugamushi Triangle," with most cases occurring in the Asia-Pacific region *(shaded in purple).* However, serologic evidence from Kenya, Congo, and Cameroon, recent reports of *Orientia* spp. in febrile patients from the Arabian Peninsula and Chile (polymerase chain reaction [PCR], culture and sequencing), and reports in rodents from southern France, South Africa, and Senegal (PCR) are suggestive of a more worldwide distribution along the tropical/subtropical belt. *(Modified from Jiang J, Richards AL. Scrub typhus: no longer restricted to the tsutsugamushi triangle.* Trop Med Infect Dis. *2018;3:E11.)*

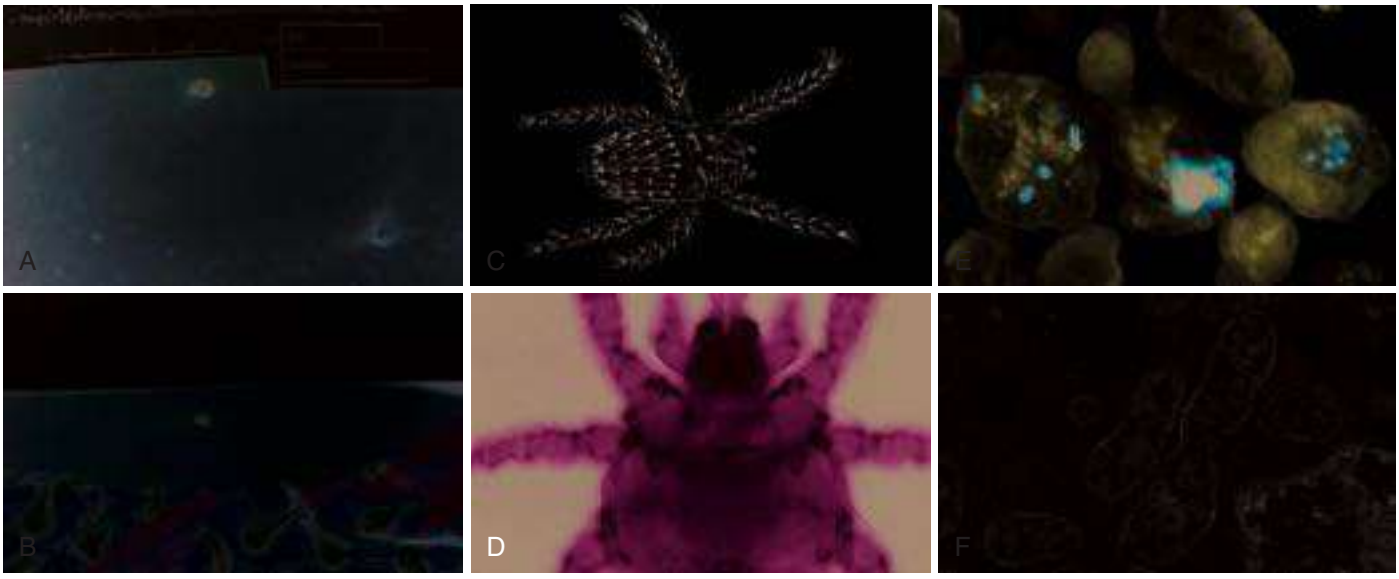

FIG. 191.2 Images of scrub typhus eschars, trombiculid mites, and *Orientia tsutsugamushi* bacteria. (A, B) Scrub typhus eschars typically occur along areas of restrictive clothing, (e.g., belts, collars, sarongs, or elastic bands of bras) or in axillae, in groin areas, or under the breasts. Detection requires careful examination and often special counseling by physicians owing to the cultural sensitivities of certain body areas involved. Chiggers—the larval stages of trombiculid mites—transmit *Orientia tsutsugamushi* to humans via their saliva during feeding (C). These mites are very small (larvae, approximately 0.2–0.3 mm; adults, approximately 2–3 mm) and have autofluorescent properties under ultraviolet light (D). (E) *O. tsutsugamushi* are obligate intracellular bacteria requiring in vitro cell culture; here the pathogens colored *brown* multiply in the perinuclear region of COS-1 cells. The bacterial double-membrane has a thicker outer leaflet in *Orientia* spp. than the inner one, as seen in (F), where *O. tsutsugamushi* is undergoing mitosis in L929 cells. *(Images A and B were generously provided by Dr. Rattanaphone Phetsouvane from Laos, and image D by Dr. Rawadee Kumlert from Thailand.)*

minimum disease incidence of scrub typhus over the past 8 to 10 years, with the highest being in China.[2] The mortality of untreated cases varies widely around a median of 6% to 8%, while current reports place mortality for treated scrub typhus at 1.4%.[2,10] However, much higher overall mortality is associated with complications such as CNS involvement (14% mortality)[12] and multiorgan dysfunction (24%)[6] in adults, while the high number of pregnancy miscarriage rates associate with poor maternal and neonatal outcomes.[8]

MICROBIOLOGY

Orientia spp. are obligate intracellular gram-negative coccobacillary bacteria that acquire their nutrition (adenosine triphosphate [ATP] and glycogen) primarily from the cytoplasm and thus locate to the microtubule organization center (MTOC) of the host cell. The *Orientia* bacterial membrane differs from that of its closest relatives, the rickettsia, by its outer membrane, which is considerably thicker than its inner one (see Fig. 191.2E–F). The typical constituents of gram-negative

cell walls—peptidoglycan and lipopolysaccharide—are not evident in *Orientia*,[13] and a major outer surface protein, the 56-kDa type-specific antigen (TSA), which contains four hypervariable regions, is mainly responsible for the organism's antigenic heterogeneity.[14]

To date two human-pathogenic *Orientia* spp. are known: the widely recognized and dominant *O. tsutsugamushi* and the recently described *Orientia chuto,* originating from the United Arabic Emirates.[15] In the past, serology-based strain characterization revealed the dramatic antigenic diversity of *O. tsutsugamushi.*[14,16] Genotyping efforts based on the immunodominant TSA 56-kDa gene demonstrate even greater diversity, but the combined data suggest that the clinically most relevant and common strains found in scrub typhus endemic areas are Karp, Gilliam, Kato, and TA716.[14] These data and new approaches involving whole-genome sequencing or antigenic cartography will provide insight into relevant antigens and cross-reactivity of antibodies, to inform diagnostic and vaccine development efforts in the future.[17]

The size of the *O. tsutsugamushi* genome is approximately 2 Mb, and among all sequenced bacterial genomes it contains the highest number of repetitive elements; an impressive 47% of the genome consists of repeats derived from integrative, conjugative, and transposable elements, generated by extensive gene degradation and amplification.[18–21] The underlying reasons for the pronounced proliferation of mobile elements and the selective pressures driving the process remain to be investigated. It is hoped that population genetics of genome sequences derived from mites, rodents, and humans will shed light on this intriguing conundrum.[19,20]

DIAGNOSIS

Historically, the gold standard diagnosis of scrub typhus was based on serology, but the added value of DNA-based pathogen detection in the early bacterial dissemination phase of disease has resulted in a combination of "polymerase chain reaction (PCR) plus serology" for most rickettsial diseases. The notoriously unreliable discontinued Weil-Felix OX-K agglutination test—discovered in 1916 and based on the cross-reactivity of anti-*Orientia* antibodies to *Proteus mirabilis,* specifically the OX-K (Kingsbury) strain—was replaced by the indirect immunofluorescence assay (IFA) in the 1960s.[22–24] The IFA was improved with new diagnostic positivity criteria defined in the 1970 and 1980s, and represented the gold standard for many years.[25,26] Only recently has the apparent lack of consensus for standardization of antigens and diagnostic positivity criteria led to the requirement of a dynamic titer rise (paired fourfold rising immunoglobulin M [IgM] or IgG titers) as a positivity criterion.[27] The development of enzyme-linked immunosorbent assays (ELISAs) using either culture-derived *O. tsutsugamushi* antigens or recombinant proteins to detect *Orientia*-specific antibodies has contributed to improved diagnostic accuracy and enabled higher throughput, over the often cumbersome IFAs and indirect immunoperoxidase test.[28,29] Point-of-care rapid diagnostic tests (RDTs) targeting anti-*Orientia* IgM and IgG are under validation, and it is hoped that future development will include antigen capture–based RDTs to enable early diagnosis at the patient bedside. Of note, the preexisting IgM and IgG in human populations of disease-endemic regions may interfere with the serological diagnosis during the acute phase. The presence of background immunity due to repeated exposure can lead to false positivity, especially in the absence of a convalescent serum sample, which would enable detection of a dynamic rise in titer (greater or equal to a fourfold rise).

The onset of the use of PCR has triggered a paradigm shift in rickettsial diagnostics; the long-term reliance on IFAs, which notably led to underdiagnosis and inappropriate therapies, is being replaced by the more objective ELISAs coupled with PCR assays to expand the diagnostic detection window toward early stages of infection. With their high diagnostic accuracies, PCR assays now represent a central pillar in scrub typhus diagnosis. Common target genes are the 56-kDa, 47-kDa, 16S rRNA, and *groEL* genes. This composite diagnostic approach has led to improved use of adequate antirickettsial therapy and reduction of morbidity and mortality, while providing more robust evidence in sample characterization for diagnostic and vaccine development.

The time from onset of symptoms to health care presentation is documented as "days of fever prior to admission"; this is of high value, because this number informs on the state of disease progression at admission and can guide the optimal use of diagnostics. Usually the bacteremia window can last up to 10 days from fever onset, and the antibody response in immune-naïve individuals is not generated until after approximately 7 to 10 days of fever.[30,31] Ideally, both a PCR assay and a serology test (i.e., RDT, ELISA) should be performed for optimal diagnosis (Fig. 191.3).

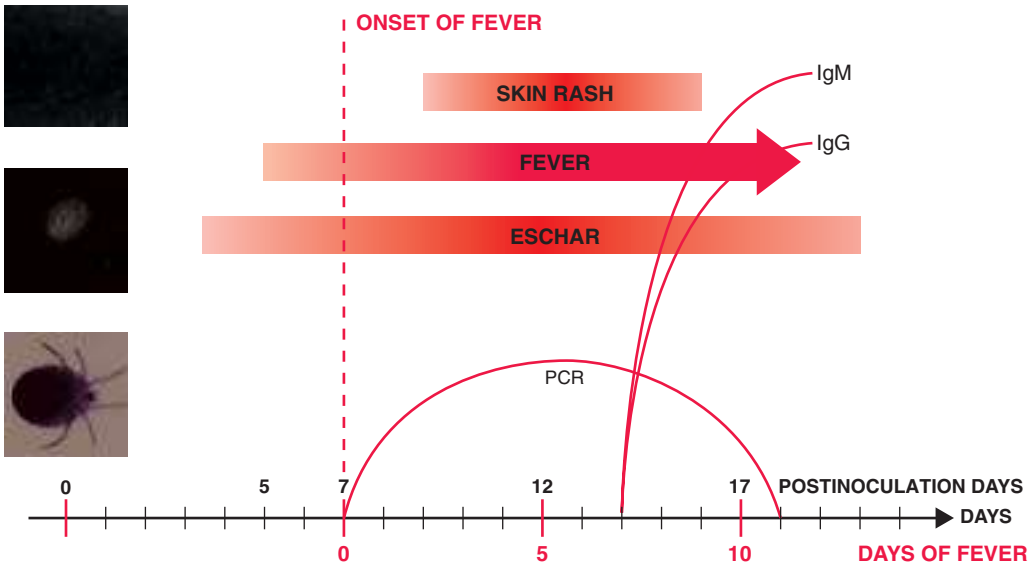

FIG. 191.3 Disease dynamics of scrub typhus clinical features, bacteremia, and anti–*Orientia tsutsugamushi* antibodies. Scrub typhus time course dynamics in relation to post–mite inoculation days *(black numbers)* and post–fever onset days *(red numbers)* are illustrated along the time axis (days). The day of the mite bite is shown as day 0 *(black).* The mite is shown in the lime green figure in the *bottom inset* as photographed under ultraviolet light. A skin rash can appear after 2 to 3 days of fever but is not always present *(upper inset).* After the mite bite, an eschar *(middle inset)* starts to form 2 to 3 days before fever onset and remains until approximately 14 days later. The bacteremia phase persists for about 10 days after onset of fever; during this time, polymerase chain reaction (PCR) assay and culture success is likely. Although anti-*Orientia* antibodies can appear as early as after 3 to 5 days of fever—and IgM can rise earlier, but often together with IgG in endemic areas—serology is likely to be the better modality to diagnose scrub typhus after approximately 7 days of fever. Patients with a history of 5 to 7 days of fever can be PCR, rapid diagnostic test, and serology positive. Because these numbers vary according to the inoculation dose (higher inoculation dose = faster dynamics), it is wise to always perform both PCR assay and serology for diagnosis of scrub typhus. *IgG,* Immunoglobulin G; *IgM,* immunoglobulin M.

If available, eschar swab or crust samples are excellent noninvasive diagnostic specimens; their high bacterial loads and separation from the circulation support early accurate molecular diagnosis until late in the disease course, even after initiation of treatment.[32] Culture of *Orientia* spp. from blood is difficult and can take several weeks owing to the fastidious nature of the bacteria, necessitating cell culture and Biosafety Level 3 facilities.

IMMUNE RESPONSE

Scrub typhus is a systemic vasculopathy with prominent infiltrates of lymphocytes, monocytes, and macrophages leading to prominent perivascular cuff formation (Fig. 191.4). *Orientia*-infected target cells include the endothelium and antigen-presenting cells, of which a subpopulation of infected monocytes has the propensity to enter the circulation and disseminate the pathogens in a "Trojan horse" phenomenon.[33] This bacteremia phase coincides with the onset of fever and lasts for approximately 10 days (see Fig. 191.3). Unfortunately, the natural immune response to scrub typhus is short-lived and is poorly cross-protective among different strains; the acquired immune protection can wane within months, resulting in symptomatic illness on reexposure to heterologous (different) strains, but could last for over a year for a homologous (same strain) reexposure.[34] The natural immune response against *Orientia* is a combination of synergistic humoral and cell-mediated immune responses; whereas the humoral component is characterized by a predominantly homologous protection with poor cross-protection to other strains, the cell-mediated immune response supports predominantly homologous protection, with initial short-lived heterotypic protection, that wanes over time. Investigations of the balance between these two arms in the context of bacterial dissemination dynamics will be crucial for the future success of vaccine design.

With appropriate treatment, patients with scrub typhus usually clear their fever within 48 hours; however, in northern Thailand and southern India, delayed fever clearance times and high case-fatality rates of 12% to 13% have been described.[35,36] Currently, ongoing investigations regarding the possible association of poor clinical responses with potential antibiotic resistance of *Orientia* spp. in northern Thailand and southern India are underway.

THERAPY

Scrub typhus is easily treated with appropriate antibiotics; these are doxycycline (preferred), azithromycin, chloramphenicol, or a combination of two. Early empirical treatment is justified upon clinical suspicion, and a response with reduction of fever within 48 hours of administration of doxycycline is often observed in scrub typhus.

The standard regimen is doxycycline 100 mg given orally twice daily for 7 days. Tetracycline is not commonly used, but 500 mg q6h for 7 days is an alternative regimen. Azithromycin has comparable efficacy to doxycycline and is usually administered as a 3-day regimen with a loading dose of 1000 mg or 500 mg on the first day, followed by 500 or 250 mg daily for another 2 days, respectively. Azithromycin remains the preferred drug during pregnancy or for children, owing to the fear of tetracycline side effects. Chloramphenicol is an excellent drug and a good alternative to doxycycline; it is administered as 500 mg q6h in adults or 50 to 75 mg/kg/day in children for 7 days. Unfortunately, the hematologic side effects (1:21,600) and the very rare occurrence of gray baby syndrome in premature infants (circulatory collapse) has led to significant reduction in its use.

Drug combinations of doxycycline plus either azithromycin or chloramphenicol are beneficial in cases with a delayed treatment response; reduction of fever clearance time also reduces adverse outcomes, especially in pregnancy, and no negative interactions have been reported to date. Decisions regarding combinations including cotreatment with rifampicin should consider the potential reduction of drug levels, and adequate dosage adjustments should be considered.

When clinical suspicion of typhoid or typhus arises, either a high-dose regimen of azithromycin (typhoid dosage) or chloramphenicol can be considered, because this would cover both infections. For uncomplicated scrub typhus, shorter regimens have been evaluated; 3 days of doxycycline performed equally as well as 7-day regimens, with comparable fever clearance times and no documented treatment failures.[37] Azithromycin is an effective alternative for potentially doxycycline-resistant scrub typhus with prolonged fever clearance times, as reported from northern Thailand.[38]

PREVENTION

Scrub typhus is among the top 10 causes of acute and potentially life-threatening diseases among ill returning western travelers from the tropics.[39] With improving awareness of medical staff and the availability of better diagnostics, increasing numbers of returning travelers with scrub typhus are being reported, including severe and fatal cases.[40,41]

Most reported cases are acquired after travel to Southeast Asia, as the disease-transmitting trombiculid chigger mites are most prevalent in scrub vegetation and tall grass of rural areas where tourists, local residents, and military personnel are exposed. Prevention is limited to the rigorous use of insect repellents, such as *N,N*-diethyl-meta-toluamide (DEET), which provides effective but short-lasting protection against ticks and mites during travel in rural areas of endemic countries. No licensed vaccines are available.

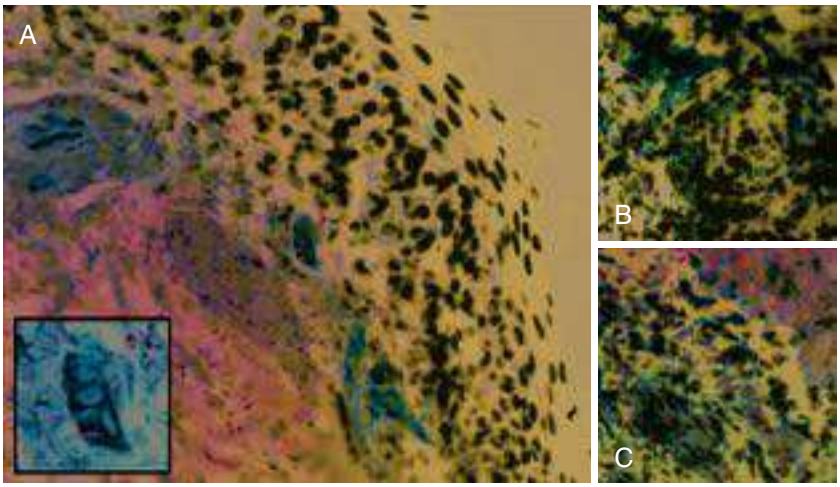

FIG. 191.4 *Orientia tsutsugamushi (green)* in skin and perivascular infiltrates. *O. tsutsugamushi (green)* in perivascular infiltrates at the superficial dermal-epidermal junction of the skin. The red-stained cells are endothelial cells of the vasculature; the inset represents a laser scanning micrograph (LSM) localizing the bacteria to the vasculature (A, with *inset*). (B and C) Two cross-sectional images of the typical cuff-forming infiltrates around smaller blood vessels consisting of large numbers of red-stained mononuclear cells (CD14-positive monocytes/macrophages); B and T cells (CD3 positivity) are shown. (TM2193, magnification ×400, LSM inset ×1000. Double-immunolabeling: CD31 in red [A], CD14 in red [B] and CD3 in red [C]; *O. tsutsugamushi* in green; DAPI nuclear counterstain in blue.)

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The complete reference list is available online at Expert Consult.

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192 *Ehrlichia chaffeensis* (Human Monocytotropic Ehrlichiosis), *Anaplasma phagocytophilum* (Human Granulocytotropic Anaplasmosis), and Other Anaplasmataceae

J. Stephen Dumler and David H. Walker

SHORT VIEW SUMMARY

Definition
- Ehrlichiosis and anaplasmosis are infections caused by intracellular bacteria in the Anaplasmataceae family.

Microbiology
- Organisms of the family Anaplasmataceae of the order Rickettsiales are obligately intracellular gram-negative bacteria.
- All members of the Anaplasmataceae survive within vacuoles of host cells generally derived from the bone marrow, but also occasionally endothelial cells.
- *Ehrlichia chaffeensis, Ehrlichia canis, Ehrlichia muris,* and *Neorickettsia helminthoeca* infect mostly monocytes and macrophages in blood and tissues of mammalian hosts.
- *Anaplasma phagocytophilum* and *Ehrlichia ewingii* infect mostly neutrophils and other granulocytes in the blood of mammalian hosts.
- The mammalian target cell of *Candidatus* Neoehrlichia mikurensis is not known.

Epidemiology
- Human monocytotropic ehrlichiosis (HME) and human *E. ewingii* infection are distributed predominantly in south-central and eastern North America, where *Amblyomma americanum* ticks and white-tailed deer *(Odocoileus virginianus),* respectively, serve as vectors and reservoirs.
- Human granulocytic anaplasmosis (HGA) is distributed worldwide, especially in northern latitudes of North America, Europe, and Asia, where *Ixodes persulcatus* clade ticks or *Haemaphysalis longicornis* ticks (China) and multiple small mammals serve as vectors and reservoirs.
- Human *E. muris* subsp. *eauclairensis* infection is distributed in the upper Midwest of the United States (primarily Wisconsin and Minnesota), where *Ixodes scapularis* ticks and white-footed mice and other small mammals serve as vectors and reservoirs.
- Human *Anaplasma capra* infections are documented only in the northeast parts of China, and transmission is likely by the bites of *I. persulcatus* ticks.
- Human *Candidatus* N. mikurensis infection occurs in Europe and northern China, where *I. persulcatus* clade ticks and small mammals serve as vectors and reservoirs. The presentation of disease may differ (thrombotic and embolic events) in patients with B-cell malignancies and autoimmune disease, or as subclinical to moderately severe febrile disease in immunocompetent patients and subjects.
- Sennetsu neorickettsiosis occurs in eastern and southeastern Asia, where digenean trematodes serve as vectors and aquatic animals, mostly fish, serve as reservoirs through which infection is acquired by means of oral transmission.

Clinical Manifestations
- Frequent early manifestations include fever, headache, myalgia, nausea, and vomiting.
- Rash is infrequent or rare.
- The clinical course is usually uncomplicated, but severe complications can include a septic shock–like syndrome, respiratory distress, meningoencephalitis, renal failure, and death, particularly with HME.
- Leukopenia, thrombocytopenia, and elevations in serum hepatic aminotransferase and C-reactive protein levels are frequent findings.

Diagnosis
- Early diagnosis of HME, HGA, and other human infections caused by bacteria in the Anaplasmataceae family is based on clinical suspicion and epidemiologic clues.
- Rapid laboratory confirmation can sometimes be achieved through blood smear examination for morulae (inclusions) in circulating leukocytes or detection of bacterial DNA in blood by means of polymerase chain reaction assay.
- Seroconversion or fourfold increase in antibody titer from the acute to the convalescent phase confirms the diagnosis retrospectively when the specific tests are available.
- Early therapy improves outcomes and prevents severe complications or sequelae.

Therapy
- Doxycycline, 100 mg twice daily for 5 to 10 days until fever is resolved, is the treatment of choice.
- Chloramphenicol should not be used.
- Rifampin has been successfully used in children.

Prevention
- Prevention is directed toward avoidance of tick exposures and bites and early removal of attached ticks. Neorickettsiosis could be prevented by avoidance of uncooked or fermented fish food products in regions where the disease occurs.

Until 1987, infections by members of the family Anaplasmataceae, including the genera *Ehrlichia, Anaplasma,* and *Neorickettsia,* were known mainly as veterinary diseases (Table 192.1). Canine ehrlichiosis was first described in 1935 by Donatien and Lestoquard in Algeria. This disease is produced by *Ehrlichia canis,* which is transmitted to dogs by *Rhipicephalus sanguineus* ticks. The disease is characterized by fever associated with the presence of clusters of small Giemsa-stained organisms in circulating monocytes. *Ehrlichia* spp. generally have a tick vector and tropism for macrophages, granulocytes, or sometimes endothelial cells, where they grow within cytoplasmic membrane–bound vacuoles. Consequently, *Ehrlichia* was recognized as distinct from other genera of obligately intracellular bacteria of medical importance

TABLE 192.1 Anaplasmataceae Causing Medical and Veterinary Diseases

CAUSATIVE AGENT	MAMMALIAN HOST	MAJOR TARGET CELL	VECTOR, TRANSMISSION
Ehrlichia chaffeensis	Humans, deer, dogs, coyotes, marsh deer	Monocytes and macrophages	Ticks *(Amblyomma americanum, Dermacentor variabilis, Ixodes pacificus)*
Ehrlichia ewingii	Dogs, humans, deer	Granulocytes	Ticks *(A. americanum, D. variabilis)*
Ehrlichia muris subsp. *muris* and subsp. *eauclairensis*	Humans, *Apodemus* mice, voles, white-footed mice	Macrophages, endothelial cells?	Ticks *(Ixodes persulcatus, Ixodes scapularis, Haemaphysalis flava)*
Ehrlichia canis	Dogs, humans	Macrophages	Ticks *(Rhipicephalus sanguineus)*
Ehrlichia ruminantium	Cattle, sheep, wild ruminants	Endothelial cells	Ticks *(Amblyomma variegatum)*
Anaplasma phagocytophilum	Humans, white-footed mice, wood rats, bank voles, wood mice, yellow-necked mice, horses, dogs, cats, sheep, cattle, white-tailed deer, roe deer, red deer, fallow deer	Granulocytes	Ticks *(I. scapularis, I. pacificus, Ixodes ricinus, I. persulcatus), Haemaphysalis longicornis*
Anaplasma platys	Dogs	Platelets, macrophages	*Rhipicephalus* ticks
Anaplasma marginale	Cattle, wild ruminants	Erythrocytes	Ticks (e.g. *Rhipicephalus*)
Anaplasma centrale	Cattle, wild ruminants	Erythrocytes	Ticks (e.g. *Rhipicephalus*)
Anaplasma capra	Humans, goats	Unknown	Ticks (*I. persulcatus*)
Candidatus Neoehrlichia mikurensis	Wild rodents, dogs, humans	Endothelial cells, neutrophils?	Ticks *(Ixodes ovatus, I. ricinus, I. persulcatus, Haemaphysalis concinna)*
Neorickettsia sennetsu	Humans	Macrophages	Possibly ingestion of raw fish infested by digenean trematodes
Neorickettsia risticii	Horses	Macrophages, enterocytes, mast cells	Ingestion of aquatic insects (e.g., mayflies) infested by digenean trematodes
Neorickettsia helminthoeca	Dogs	Macrophages	Ingestion of trematode-infested salmon

(*Rickettsia, Coxiella,* and *Chlamydia*). In 1937 the genus name *Ehrlichia* was suggested in honor of the German bacteriologist Paul Ehrlich.[1] Subsequent phylogenetic studies have shown that two other economically important veterinary pathogens, *Anaplasma marginale* (described in 1910) and *Ehrlichia* (formerly *Cowdria*) *ruminantium* (described in 1925), are also ehrlichiae.[2] The first human disease demonstrated to have an ehrlichial cause was sennetsu neorickettsiosis, an infectious mononucleosis–like illness recognized to have occurred only in western Japan, Malaysia, and Laos. Although human infections caused by all members of the reorganized family Anaplasmataceae have been generically referred to as "ehrlichiosis," and the causative agents are referred to as "ehrlichiae," it is increasingly apparent that the clinical manifestations and causative agents are distinct. Because of the confusion arising from omitting species-specific diagnosis, more clarity in reporting the results of diagnosis is needed in order to eliminate a substantial burden of "undetermined" cases.[3]

The first diagnosed case of human ehrlichiosis in the United States occurred in a 51-year-old man who became ill in April 1986, 12 to 14 days after tick bites in rural Arkansas.[4] Although the disease was initially thought to be caused by the canine pathogen *E. canis*, the main causative agent of human monocytotropic ehrlichiosis (HME) is *Ehrlichia chaffeensis,* which was finally described in 1990.[5] In 1994, *Anaplasma phagocytophilum* was identified as the causative agent of a distinctly different infection, now called human granulocytotropic anaplasmosis (HGA).[6,7] Subsequently, human infections have been documented as caused by *E. canis,*[8] *Ehrlichia ewingii,*[9] and *Ehrlichia muris* subspecies *eauclairensis*[10,11]; by a bacterium related to *E. ruminantium* that is called Panola Mountain *Ehrlichia*[12]; by a bacterium recently identified and assigned to a new genus, *Candidatus* Neoehrlichia mikurensis[13]; by the newly recognized *Anaplasma capra*[14]; and, in a single case of infection, by *Anaplasma ovis* in a patient from Cyprus.[15]

ETIOLOGY

Members of the family Anaplasmataceae are defined predominantly by their genetic similarities and differences but also by phenotypic characteristics and host affinities (see Table 192.1). These are small (0.5 µm) gram-negative bacteria. Their clustered inclusion-like appearance of a microcolony in the host cell vacuole is called a morula, from the Latin word for mulberry (Fig. 192.1).



FIG. 192.1 Human monocytotropic ehrlichiosis. Peripheral blood smear (buffy coat preparation) showing intracellular inclusions *(arrows)* in mononuclear cells of a patient with human monocytotropic ehrlichiosis (Wright stain, ×400).

The taxonomic relationships of *Ehrlichia, Anaplasma, Neorickettsia, Neoehrlichia, Wolbachia, Orientia, Rickettsia, Coxiella,* and *Chlamydia* have been clarified through molecular and metabolic studies. The evolutionary relationships determined by comparison of whole genomes and the genes *rrs* (16S ribosomal RNA gene) and *groESL* indicate that *Ehrlichia, Anaplasma, Neorickettsia, Neoehrlichia, Wolbachia, Orientia,* and *Rickettsia* evolved from a common ancestor[2]; in contrast, *Coxiella* and *Chlamydia* are phylogenetically unrelated to ehrlichiae. Ehrlichiae and chlamydiae superficially resemble one another in that both reside within cytoplasmic vacuoles. Unlike chlamydiae, however, ehrlichiae are able to synthesize adenosine triphosphate through metabolism of glutamine, a metabolic characteristic shared with members of the genus *Rickettsia,* and lack the pathogen-associated molecular patterns

lipopolysaccharide and peptidoglycan. Because of the increasing use of molecular methods for genetic identification, the diversity and number of potential clades within Anaplasmataceae exceeds 323 entries within the NCBI Taxonomy database (see https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi), spanning established genera described here, but also including the genera *Candidatus* Xenohaliotis, *Candidatus* Xenolissoclinum, and *Candidatus* Cryptoplasma. Whether any of these molecular "isolates" are bacteria with the capacity to cause human disease is unknown. The family Anaplasmataceae contains at least five genera that are actually very different from one another. *E. chaffeensis* shares many antigens and genetic sequences with the predominantly canine pathogens *E. canis* and *E. ewingii*; *E. muris* and closely related species found in Japanese wild mice, voles, and ticks; and more recently in humans, a bacterium with a close genetic relationship to the ruminant pathogen *E. ruminantium*.[2,5,16–18] A second genus includes a granulocytotropic bacterium, *A. phagocytophilum*; an organism of uncertain tropism, *A. capra*; and a suspected erythrocytic bacterium, *A. ovis,* which can infect humans. All of these are related genetically to the veterinary pathogens *Anaplasma platys, Anaplasma bovis,* and *A. marginale*.[2,7,14,15] The third contains *Neorickettsia sennetsu*, which is closely related to *Neorickettsia risticii, Neorickettsia helminthoeca,* and an unnamed organism found in Japanese fish flukes.[2] The fourth genus, *Neoehrlichia,* contains two species: *Candidatus* N. mikurensis and *Candidatus* Neoehrlichia lotoris.[19,20] The former was first identified in *Ixodes ricinus* ticks in Europe[21] and then in *Ixodes ovatus* ticks and wild rats from Japan,[19] and it is the only species identified as a human pathogen in the genus. The final genus, *Wolbachia*, contains bacterial endosymbionts of invertebrates, including insects and helminths, some of which may contribute to disease in human filariasis.[22]

Ehrlichia and *Anaplasma* have two ultrastructural forms, a larger reticulate cell and a smaller, dense core cell, and the cell wall differs from that of *Rickettsia* spp., with thinner outer and inner leaflets reflecting the absence of lipopolysaccharide and lipooligosaccharide.[23] Genes coding for the enzymes required for the biosynthesis of peptidoglycan and lipopolysaccharide are not present in the *E. chaffeensis* or *A. phagocytophilum* genome.[24] The genomes of these obligately intracellular bacteria are small, ranging from 1.2 to 1.6 Mb to as low as 900 kb for *Neorickettsia*. Bacteria in the Anaplasmataceae family possess multiple genes that are members of the pfam01617.8, Surface_Ag_2 gene family, encoding major surface porin proteins responsible for antigenic variation and host cell adhesion in genera as diverse as *Neisseria, Brucella,* and *Pseudomonas. E. chaffeensis, E. canis, E. ewingii,* and *A. phagocytophilum* have gene families that encode more than 19 paralogous, surface-exposed pfam01617.8 proteins of 22 to 30 kDa (p28/Omp-1 family) for *Ehrlichia* and approximately 105 paralogous genes encoding 41 to 49 kDa (major surface protein-2 [Msp2]) for *A. phagocytophilum*.[25–27] In accordance with the pfam01617.8 predictions, roles have been shown for *A. phagocytophilum* Msp2 in antigenic variation,[28,29] and Msp2/p44 and p28/Omp-1 could function as porins in both *A. phagocytophilum* and *E. chaffeensis*.[30,31] Antigenic diversity within a single strain is based on the presence of hypervariable regions in the *Ehrlichia p28/omp1* family, where each gene is transcribed independently yet is dominated by the expression of a single protein in mammalian infection, and a different protein is expressed in ticks.[31,32] Reinfection with different *E. chaffeensis* and *A. phagocytophilum* strains has been reported, underscoring the role of antigenic diversity in immune protection.[33,34] Similarly, Msp2/p44 of *A. phagocytophilum* is characterized by conserved domains that flank a hypervariable region, but expression requires gene conversion into a single genomic site. This condition may be further complicated by segmental conversion that generates even greater antigenic complexity.[35–37] Infection in vivo yields a large number of transcriptional and antigenic variants that presumably contribute to persistence in reservoir hosts.[38]

E. chaffeensis undergoes a 72-hour developmental cycle in which the infectious dense core stage attaches, enters, and converts into the reticulate stage, which replicates numerous times by binary fission in an acidified early endosome and converts into the dense core stage, which is released from the host macrophage. The *Ehrlichia* genome encodes more than 20 outer membrane proteins (OMPs) that are porins, only one of which is expressed in humans and another in ticks, and several proteins that contain a series of tandem repeat units (TRPs). TRP120 and TRP47 are expressed exclusively on dense core ehrlichiae.[39,40] These play a role in adhesion to the target cell, in part regulated by bacterial response regulator CtrA.[41,42] Attachment of TRPs is most likely to host Wnt receptors and of EtpE to DNAX, triggering internalization via caveolae. Ehrlichiae residing in early endosomes acquire nutrients by induction of autophagy by Etp-1 and block lysosomal fusion via protein expression controlled by a two-component regulatory system. TRPs translocate to the nucleus of the infected cell, where they bind DNA at sites that are differentially transcribed with infection to reprogram host cell gene expression, downregulating interleukin (IL)-12 and IL-18 and upregulating apoptosis and cyclin inhibitors that favor ehrlichial growth. TRP120 also interacts with multiple host proteins involved in cell signaling, protein trafficking, and actin cytoskeleton organization, providing multiple opportunities to reprogram the host cell's functions.[43,44] Ehrlichiae also modulate host Wnt, Notch, and Jak/Stat signaling and SUMOylation pathways, resulting in downregulated pattern recognition receptor expression and innate proinflammatory immune responses, further creating an environment for ehrlichial survival.[45]

Both *E. chaffeensis* and *A. phagocytophilum* express functional type IV secretion systems, and at least one *A. phagocytophilum* substrate, AnkA, is translocated into the infected host cell, where it regulates bacterial entry by interacting with Abl-interactor 1 (ABI1), which influences epidermal growth factor receptor signal transduction and cytoskeletal changes.[46] AnkA eventually translocates to complex with host nuclear heterochromatin, where it binds widely throughout the genome to intergenic regions sequestered into the nuclear lamina and to gene promoters and by recruiting histone deacetylase alters histone acetylation, chromatin structure, and ultimately transcriptional activity.[47–50] Both bacteria also express two-component histidine kinase regulatory systems that influence trafficking of the parasitophorous vacuole after bacterial entry.[51]

Epidemiology and Epizootiology of Human Monocytotropic Ehrlichiosis

Human ehrlichioses are tick-borne zoonoses. Most patients give a history of tick exposure during the month before the onset of illness. The seasonality of HME, with peak incidence in May to August, reflects the tick-transmitted epidemiology.[52] Exposures are predominantly rural and suburban and involve recreational, peridomestic, occupational, and military activities. More than 60% of patients are male. Documented cases of HME have been reported in 47 states, particularly in the south-central and southeastern United States. This region conforms to the distribution of the Lone Star tick, *Amblyomma americanum,* the range of which is expanding northward and westward; along with white-tailed deer, this maintains the ehrlichiae in nature through acquisition of *E. chaffeensis* during feeding as a larva or nymph on persistently infected deer or by cofeeding with infected ticks and subsequently transmitting ehrlichiae to nonimmune deer. The pathogen is transmitted transstadially from larvae to nymphs and from nymphs to adults, but not transovarially.[53] Organisms closely related to *E. chaffeensis* and evidence of human ehrlichial infections have also been reported in South America, Africa, and eastern Asia.[54–58]

Between 1987 and 2017, 15,527 cases of HME were reported in *Morbidity and Mortality Weekly Reports* (*MMWR;* Centers for Disease Control and Prevention [CDC]). From 2008 to 2012, the incidence of HME was 3.2 cases per million population in the United States, a fourfold increase from 2000, with hospitalization in 57%, a life-threatening complication in 11%, and a case-fatality rate of 1% (4% in children <5 years and 3% in those ≥70 years of age).[52] An active, prospective, 3-year study in Cape Girardeau, Missouri, revealed 29 cases in a family practice of 7000 patients, an average annual incidence of 138 cases per 100,000 population.[59] Similarly, selected communities have prevalence rates as high as 330 cases per 100,000 population under permissive ecologic circumstances.[60]

It is presumed that most infections are not diagnosed, because cross-sectional seroprevalence in endemic regions ranges from 1.3% to 12.5% for HME.[60] Thus, subclinical seroconversion could reflect exposure to other *Ehrlichia* or *Anaplasma* species or other antigenic stimuli. *E. canis,* which induces cross-reactive serologic responses, also infects patients in South and Central America. *E. muris* subsp. *eauclairensis* and

E. ewingii are known to cause milder infections that result in serologic cross-reactions with *E. chaffeensis.*[8,9,11] A single case of infection by an *E. ruminantium*–like bacterium, called the Panola Mountain *Ehrlichia*, has been identified in a 31-year-old man in Georgia.[12] Human infection by *E. muris* subsp. *eauclairensis* has, to date, occurred only in Wisconsin and Minnesota.[11] Transfusion-transmitted HME is possible,[61] and transfusion-related *E. ewingii* infection has been reported,[62] but both are probably rare. *Candidatus* N. mikurensis is found in *Ixodes* ticks throughout Europe, Asia, and Africa. Surveillance with polymerase chain reaction (PCR) of asymptomatic forest workers in Central Europe found an infection rate of 1.6%.[63] Life-threatening transplant-associated disease occurred in both recipients of kidneys from the same deceased donor.[64]

Epidemiology and Epizootiology of Human Granulocytic Anaplasmosis

Human granulocytic anaplasmosis (HGA) also has a seasonal occurrence, peaking in June but continuing through November, in accordance with the activity of nymphal and adult stages of *Ixodes scapularis* ticks.[65] Although risk for HGA is associated with outdoor activity, a substantial proportion of cases occurs in suburban areas of northeastern and upper Midwestern cities.[65–67] HGA occurs in specific geographic locations; 92% of US cases are reported from New England and the Upper Midwest, with the highest rates in Wisconsin, Minnesota, and Rhode Island.[65] Infection rates increased from 2008 to 2012 and increased with age, and the geographic range expanded. The majority of diagnoses were made by means of PCR. The distribution is almost identical to that of Lyme disease because of the shared *Ixodes* spp. tick vectors. HGA is documented throughout Europe, particularly in Slovenia, Sweden, and Norway, and in China, Korea, Japan, and other parts of Asia.[55,68–71] Serologic studies suggest a global distribution in the Northern Hemisphere for HGA, *A. phagocytophilum,* and its tick vectors.

Between 1995 and 2017, a total of 30,759 cases of HGA were reported by the CDC in *MMWR.* The incidence of HGA nationally was 8.0 cases per million person-years in 2012, but above 50 cases per million person-years in Minnesota, Wisconsin, and Rhode Island[65]; however, active case collection has yielded an incidence of 14 to 16 cases per 100,000 population in the upper Midwest between 1990 and 1995, with rates as high as 24 to 58 cases per 100,000 population in some northwest Wisconsin counties in 1994–1995 and in Connecticut in 1997–1999.[72,73] Cross-sectional seroprevalence studies have shown that up to 15% of the population in northwestern Wisconsin, 1% of Connecticut residents and US. military personnel, 17% of Slovenians, and 12% of the population of Sweden's Koster Islands have antibodies reactive with *A. phagocytophilum* in the absence of antecedent clinical evidence for HGA.[74–78] Alternatively, in some centers in Sweden and Finland, the rate of asymptomatic seroconversion from bites of infected ticks is probably rare.[79] The demonstration of mildly affected patients who recover spontaneously, even in the absence of specific therapy, suggests that HGA could frequently be subclinical.[80] Transfusion-transmitted HGA has become an increasing threat in spite of leukoreduced blood products in the United States and in Europe.[81–88]

From 4% to 36% of patients with serologic evidence of *A. phagocytophilum* infection also have serologic evidence of *Borrelia burgdorferi* or *Babesia microti* infection, possibly representing serial infections with each agent; both agents are also transmitted by *Ixodes* spp. tick bites.[89–91] Concurrent HGA and Lyme disease, documented by isolation of both agents, has been reported,[92] although a prospective study has shown only a 2% incidence of coinfection in patients with erythema migrans and Lyme disease.[93] Coinfection with tick-borne encephalitis virus has been demonstrated in Europe and is likely in the United States with the increasing incidence of Powassan and deer tick virus infections.[94] Whether concurrent infection with these agents results in increased severity, prolonged duration of illness, or more frequent and severe sequelae has yet to be definitively determined.[91]

A. phagocytophilum is transmitted to humans by the bites of nymphal and adult *I. scapularis* ticks in the eastern United States, *Ixodes pacificus* in California, *I. ricinus* in Europe, and presumably *Ixodes persulcatus* in parts of Asia. However, *A. phagocytophilum* DNA was also found in *Haemaphysalis longicornis* ticks examined in conjunction with 62 cases of HGA in Shandong Province in China.[69] Although transstadial transmission of the infectious agent occurs, *A. phagocytophilum* is not maintained transovarially, and thus natural maintenance requires horizontal (tick-mammal-tick) transmission.[95] A major proven reservoir host is the white-footed mouse, *Peromyscus leucopus*; however, other small mammals, such as sciurids (squirrels) in the western United States, and ruminants have been found naturally infected or have serologic evidence of infection, including voles, wood rats, white-tailed deer, red deer, and roe deer.[96–98] Current serologic evidence suggests that larval ticks acquire *A. phagocytophilum* after feeding on small mammals infected earlier in the season by nymphal ticks. White-footed mice develop immunity to *A. phagocytophilum* after a period of bacteremia that may last from several days to weeks; prior immunity reduces small mammal reservoir competence and transmission.[99] Small mammals are not adversely affected by the infection, and some may become persistently infected. The contribution of persistently infected ruminants and cervids as reservoir hosts for *A. phagocytophilum* requires further investigation. In northern China, where *A. capra* infects goats, 28 of 477 (6%) persons with a history of tick bite had PCR evidence of infection, and all had a history of a nonspecific febrile illness, including 10 with rash or eschar.[14]

PATHOGENESIS AND PATHOLOGY

Human Monocytotropic Ehrlichiosis

After entering the skin by tick bite inoculation and being spread presumably through lymphatic and blood vessels, ehrlichiae invade target cells of the hematopoietic and lymphoreticular systems. Morulae of *E. chaffeensis* are observed mainly in macrophages and monocytes, less frequently in lymphocytes, and rarely in polymorphonuclear leukocytes.[100–103] Ehrlichial morulae have been identified in peripheral blood, bone marrow, hepatic sinusoids, lymph nodes, splenic cords, splenic sinusoids, splenic periarteriolar lymphoid sheaths, cerebrospinal fluid (CSF) macrophages, and macrophages in perivascular lymphohistiocytic infiltrates in organs such as the kidney, appendix, and heart.

The best studied tissue in HME is bone marrow, largely because of the frequency of leukopenia, thrombocytopenia, and anemia. Frequent findings include granulomas, myeloid hyperplasia, and megakaryocytosis.[100] Erythrophagocytosis and plasmacytosis occur in smaller proportions of patients with HME. Focal hepatocellular necrosis; hepatic granulomas, including ring granulomas; cholestasis; splenic and lymph node necrosis; diffuse mononuclear phagocyte hyperplasia in the spleen, liver, lymph nodes, and bone marrow; perivascular lymphohistiocytic infiltrates of various organs, including the kidney, heart, liver, meninges, and brain; and interstitial mononuclear cell pneumonitis are also observed.[104,105] It is worthy of emphasis that direct endothelial injury and thrombosis have not been described. The observation of erythrophagocytosis, myeloid hyperplasia, and megakaryocytosis in the bone marrow of patients with HME suggests peripheral consumption of blood elements and a compensatory response. In contrast, evidence in murine models exists for myelosuppression, which suggests either direct bacterium-related or indirect chemokine effects on bone marrow.[106]

Although *E. chaffeensis* causes a direct cytopathic effect when grown in cell culture, it appears that the host responses account for most of the clinical manifestations. The toxic shock manifestations of HME are likely to be the systemic effects of increased levels of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), IL-1α and IL-1β, and IL-6; defective production of Th1 cytokines, including interferon-γ (IFN-γ) and IL-2; the increased production of antiinflammatory IL-10; and increased levels of chemokines.[107] TNF-α is produced by natural killer T cells and CD8 T lymphocytes in murine models and possibly in humans and, together with perforin expressed by these cells, results in killing of CD4 T lymphocytes, significant reductions in IFN-γ expression, and apoptotic tissue injury.[108,109] IFN-γ stimulates macrophage killing of *E. chaffeensis* through the sequestration of iron, and opsonization with immune serum enhances the destruction of ehrlichiae by macrophages.[110,111] *E. chaffeensis* circumvents host defenses by inhibiting the fusion of infected phagosomes with lysosomes and inhibiting the signal transduction pathway of IFN-γ–mediated anti-ehrlichial activity.[112] There is evidence that *E. chaffeensis* also evades immunity through downregulation of other host defense genes of the infected macrophage and manipulation of the host cell as a niche favorable to its survival and growth.[43,44,113,114]

Human Granulocytotropic Anaplasmosis

A. phagocytophilum is observed predominantly in neutrophils in peripheral blood and tissues from infected individuals.[67,72] Dramatic histopathologic findings involve the presence of opportunistic pathogens, especially severe fungal and viral infections, which account for most fatalities. Pathologic findings in humans and animal models include normocellular or hypercellular bone marrow, erythrophagocytosis in mononuclear phagocytic organs, hepatocellular apoptoses and periportal lymphohistiocytic infiltrates, focal splenic necrosis, mild interstitial pneumonitis, and pulmonary hemorrhage.[115] Vasculitis, endothelial injury, granulomas, and meningeal inflammation have not been described.

A. phagocytophilum disseminates to blood, bone marrow, and spleen after a tick bite, likely through local infection of neutrophils attracted to the tick bite wound in the dermis.[116] In the bone marrow, progenitors of myeloid and monocytic lineages can be infected.[117,118] Endothelial cells can be infected in vitro,[119] but evidence for in vivo infection is lacking.[115,116] With neutrophil infection, the bacteria attach to the cell surface P-selectin glycoprotein ligand-1 (CD162) and perhaps other ligands,[120] enter a vacuole that is altered by secreted *A. phagocytophilum* effectors to avoid autophagy, mimic recycling endosomes by accumulating an array of Rab guanosine triphosphatases (GTPases) to the membrane, and thereby preclude lysosome fusion and replicate.[121,122]

In vitro, *A. phagocytophilum* survives by deactivation of the neutrophil antimicrobial response through AnkA-mediated silencing of granulocyte *RAC2* and *CYBB* ($gp91^{phox}$) transcription and prolonged downregulation of phagocyte oxidase activity.[47,49,123,124] Additional pathogenetic processes modified in infected granulocytes include delayed apoptosis, ineffective binding to and transmigration of activated endothelium, and inhibition of phagocytic activity.[125–131] However, infection also paradoxically stimulates an inflammatory response with neutrophil activation, chemokine secretion, and degranulation.[132–134] Increased proinflammatory activity, in part mediated by TLR2 and NALP4 inflammasome activation of macrophages[135,136] and by transcriptional upregulation of chemokine genes,[133,137,138] allows the recruitment of new neutrophil host cells and localized tissue injury that further exacerbates inflammatory stimulation when neutrophils cannot generate effective antimicrobial responses. These findings are consistent with the dissociation of bacterial burden and histopathologic evidence of tissue injury in mouse and horse models, suggesting a role for host immunity in disease.[139–142] *A. phagocytophilum* infection is initially controlled by IFN-γ, which results in macrophage activation and marked increases in inflammatory tissue injury in mouse models, paralleling evidence of macrophage activation as a mechanism of increased severity in humans[143,144]; downregulation of IFN-γ expression in animal models results in a higher bacterial burden but less inflammatory tissue injury and lessened clinical disease.[139–141] Paradoxically, infection of Stat1-deficient animals results in ample IFN-γ production with massive inflammatory consequences and marked increases in bacterial burden.[134] Although involved in inflammatory injury with infection, host innate and adaptive immune mechanisms contribute little to control of *A. phagocytophilum* infection except for CD4 T lymphocytes.[145]

CLINICAL MANIFESTATIONS

Human Monocytotropic Ehrlichiosis

Signs and Symptoms

The clinical picture of HME in immunocompetent patients is of a mild-to-severe multisystem illness, with a median duration of 23 days (Table 192.2).[52,59,146–148] Approximately 12% to 30% of infections have been reported in immunocompromised patients in whom *E. chaffeensis* acts as an opportunistic pathogen, wherein a 3.0 relative risk for life-threatening complications is documented and fatal overwhelming infections can occur.[149–151] The median incubation period is 9 days. Symptoms at onset of illness include fever, chills, headache, myalgia, and malaise. Later in the course, patients often develop nausea, anorexia, vomiting, and weight loss. Physical signs are not striking. Fewer than half of patients have a rash, which is maculopapular and can be petechial. Rash is observed more frequently in children. Adult patients with severe illness are more likely to have a cough, diarrhea, confusion, and lymphadenopathy, whereas pediatric patients may develop edema of the hands or feet. Severe complications occur in 9% to 17% of patients and include pneumonia and adult respiratory distress syndrome (18% require mechanical ventilation); disseminated intravascular coagulation (DIC)– and sepsis-like syndromes; acute renal insufficiency; central nervous system (CNS) abnormalities such as meningoencephalitis; and death.[52,107,152–154] *E. chaffeensis* infection can also lead to hemophagocytic lymphohistiocytosis or macrophage activation syndrome.[155–159] CSF pleocytosis usually shows a predominance of lymphocytes and increased protein concentration and can demonstrate the presence of infected cells.[103] Nearly half of patients with chest radiographic evaluation have infiltrates. Failure to recognize the frequency of pulmonary and CNS involvement is associated with delayed treatment and greater likelihood of intensive care unit (ICU) admission and severe complications.[149]

TABLE 192.2 Clinical and Laboratory Abnormalities in Human Monocytotropic Ehrlichiosis (HME) and Human Granulocytotropic Anaplasmosis (HGA)

SIGN, SYMPTOM, OR LABORATORY FINDING	HME MEDIAN % WITH ABNORMAL FINDING (IQR)[a]	HGA MEDIAN % WITH ABNORMAL FINDING (IQR)[b]
Fever	96 (95–99)	100 (94–100)
Headache	72 (69–72)	82 (60–96)
Myalgia	73 (63–75)	73 (61–82)
Malaise	77 (73–80)	97 (92–99)
Nausea	57 (56–59)	40 (35–52)
Vomiting	47 (37–56)	22 (17–35)
Diarrhea	25 (20–31)	20 (13–25)
Rash	26 (21–34)	5 (3–10)
Cough	28 (26–31)	27 (21–33)
Confusion/mental status changes	20 (19–22)	17 (17–18)
Leukopenia	60 (60–71)	63 (53–76)
Thrombocytopenia	79 (68–88)	80 (61–90)
Anemia	50 (38–54)	40 (14–48)
Elevated AST/ALT	88 (86–91)	80 (69–98)
Elevated creatinine	29	49 (25–71)
Elevated C-reactive protein	data not available	96 (83–100)

[a]HME meta-analysis data from references 59, 149, 151, 153, 154, 164, 217, 218.
[b]HGA meta-analysis data from references 66, 72, 68, 219, 220, 69, 177–186, 221–228.
ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *IQR,* interquartile range.

Important laboratory features are thrombocytopenia, mild-to-moderate leukopenia, and elevations of serum hepatic aminotransferase levels (see Table 192.2). The nadir of leukopenia is usually between 1300 and 4000 cells/μL. Neutropenia, lymphopenia, or both account for the leukopenia. Thrombocytopenia occurs concurrently with leukopenia and is usually between 50,000 and 140,000 platelets/μL, although it is occasionally profound (less than 20,000 platelets/μL).

Course

The clinical course of illness ranges from mild illness to a fatal outcome. The higher incidence and risk for life-threatening complications in older patients suggests that host factors are important in disease severity. A virulent form of HME occurs in human immunodeficiency virus–infected individuals and is often associated with overwhelming infection, a toxic shock– or sepsis-like syndrome, and fatality.[107,150] Immune compromise related to corticosteroid therapy, etanercept, or immunosuppression with organ transplantation is also associated with increased severity, although prompt treatment abrogates this increased risk.[151,160,161]

The median duration of hospitalization is about 1 week. Convalescence is often prolonged. Persistent infection has been documented in only one patient with HME.[162] Fatalities occur in approximately 1% to 3%.[52] Many patients treated with doxycycline or tetracycline recover rapidly. On the other hand, most patients who receive no effective anti-ehrlichial treatment also have uncomplicated complete recovery.

Diagnosis

A diagnosis based on epidemiologic and clinical factors, including a high index of suspicion, allows prompt empirical anti-ehrlichial treatment.[149] Patients with fever, leukopenia, thrombocytopenia, elevated serum aminotransferase levels, and a history of a recent tick bite in endemic regions from May through July should be considered as possibly having HME. No absolute clinical criteria distinguish HME from Rocky Mountain spotted fever,[163] although patients with ehrlichiosis are less likely to have a rash and more likely to have leukopenia (median white blood cell count, 3500/μL). Morulae are observed in less than 7% of patients with HME, most often in immunocompromised patients with overwhelming infection.[150]

Although effectively used in one large series of cases of HME,[164] culture is only a research tool that uses special methods and unique cell lines.

Because of the presence of *E. chaffeensis* in peripheral blood or CSF mononuclear cells during active infection and before the presence of diagnostic levels of serum antibodies, methods to detect bacterial DNA, such as PCR assay, are highly sensitive and useful diagnostic tools. There are a large number of potential gene targets, with sensitivity ranging from 60% to 100%.[59,165,166]

At present, the major diagnostic confirmatory criterion for human ehrlichiosis is serologic, as determined by means of indirect immunofluorescence assay (IFA) with *E. chaffeensis*–infected cells. To be considered positive, the patient's sera must show a fourfold or greater rise in immunoglobulin G (IgG) antibody titer during the course of the disease, with a minimal peak titer of 128. IFA shows a peak geometric mean titer of 1280 at 6 weeks after onset.[167] Only 22% to 44% of the sera tested in the first week of illness have a titer of 80 or higher. Among sera from patients tested in the second week, 68% are diagnostic. Sera tested 4 or more weeks after the onset of illness should demonstrate seroconversion.

Differential Diagnosis

Early in the course of the disease, when the patient presents with fever, headache, myalgia, and malaise, the differential diagnoses may include various viral syndromes, Rocky Mountain spotted fever, upper respiratory illness, sepsis, and urinary tract infection. If nausea, vomiting, and anorexia are prominent symptoms, gastroenteritis is often suspected. With prominent cough, pneumonia is often considered. CNS signs and symptoms with CSF pleocytosis suggest viral or bacterial meningoencephalitis. On obtaining a history of recent tick bite, the physician should consider a tick-borne febrile illness, such as Rocky Mountain spotted fever, relapsing fever, tularemia, Lyme borreliosis, Colorado tick fever, babesiosis, or Powassan, Heartland, or Bourbon virus infections. Other diagnostic considerations include meningococcemia, toxic shock syndrome, leptospirosis, hepatitis, enteroviral infection, influenza, murine typhus, Q fever, typhoid fever, bacterial sepsis, endocarditis, Kawasaki disease, collagen-vascular diseases, and leukemia. *E. chaffeensis* is associated with the development of hemophagocytic lymphohistiocytosis syndrome. A comparison of the ehrlichioses and Rocky Mountain spotted fever is presented in Table 192.3.

No prospective randomized, controlled clinical studies to establish efficacy of antimicrobials for HME have been conducted. Based on retrospective clinical studies and in vitro investigations, doxycycline is the drug of choice, even in pregnant patients and children, and is administered to adults at a dose of 100 mg twice daily until the patient has become afebrile and clinically improved.[149] Courses of treatment of 5 to 10 days have yielded a favorable outcome in many patients. *E. chaffeensis* has been demonstrated to be susceptible to rifampin and resistant to fluoroquinolones in cell culture.[168,169] Patients benefit from doxycycline administration at hospital admission with significantly decreased rates of ICU transfer and mechanical ventilation, shorter hospital stays, and shorter length of illnesses.[149]

TABLE 192.3 Comparison of Human Monocytotropic Ehrlichiosis (HME) and Human Granulocytic Anaplasmosis (HGA) in the United States With Rocky Mountain Spotted Fever (Rmsf)

Similarities
History of tick attachment
Incubation period of about 1 week between tick bite and onset of symptoms
Peak incidence in late spring and summer
Acute onset with headache, fever, myalgia, and malaise
Severe cases may include coagulopathy, azotemia, and encephalopathy
White blood cell count usually not elevated, platelet count often low, serum aspartate aminotransferase level often increased
Diagnosis through acute and convalescent serology
Treatment—a tetracycline is effective
Differences
Cough, dyspnea, and vomiting present less commonly in ehrlichioses and HGA
RMSF—rash is present in 90% of patients and is petechial in about half the cases
Ehrlichiosis and anaplasmosis—rash is present in less than half of adult patients with HME and infrequently in HGA, is maculopapular and rarely petechial
Leukopenia with absolute lymphopenia and/or neutropenia common in hospitalized patients with HME and neutropenia in those with HGA, but less frequent in RMSF
Inclusions (morulae) seen infrequently in monocytes and macrophages of HME patients, occasionally in neutrophils of HGA patients but not in RMSF patients
Chloramphenicol is not likely effective for HME or HGA
Histopathologic vasculitis, hallmark of RMSF, not observed in ehrlichioses or anaplasmosis

Ehrlichiosis Caused by *Ehrlichia ewingii* and *Ehrlichia muris*

E. ewingii ehrlichiosis has been diagnosed in Missouri, Tennessee, Delaware, Maryland, Virginia, Georgia, Illinois, Kansas, Louisiana, Ohio, Oklahoma, and South Carolina and in *A. americanum* or other ticks across the same geographic regions.[170,171] *E. ewingii* has also been detected in Korea and Cameroon.[172,173] One-quarter of infections are reported in immunocompromised patients, including those with organ transplantation or history of cancer. Clinical manifestations are similar to those of HME, and despite a 77% hospitalization rate, overall the illness is not as severe, with fewer complications and no deaths reported.[52,150,151] Diagnosis requires PCR with species-specific primers or probes or with DNA sequencing of amplicons of broad-range PCR. Sera of most patients with *E. ewingii* ehrlichiosis react with *E. chaffeensis* IFA antigens, and peptides or recombinant *E. ewingii* 28-kDa proteins distinct from those of *E. chaffeensis* could allow creation of a specific serodiagnostic test.[174]

E. muris was first detected in Japan, where it was found in *Apodemus* mice and *Haemaphysalis flava* ticks; antibodies to *E. muris* were present in 1% of humans in Japan in a large serosurvey,[175] and *E. muris* or a closely related organism was also detected in *I. persulcatus* ticks in the Perm region of Russia, where 86 patients with an acute febrile illness had antibody titers of 80 to 1200 against the antigenically related *E. chaffeensis*.[176] In 2009, infection with the recently reclassified *E. muris* subsp. *eauclairensis* was identified among patients with suspected ehrlichiosis or anaplasmosis in Wisconsin and Minnesota and in *I. scapularis* ticks from the Upper Midwest United States.[10,11] Some patients had strong serologic reactivity in convalescence to *E. chaffeensis* but not *A. phagocytophilum* antigens. Among 69 human infections (including samples from Michigan, North Dakota, and Indiana), all had tick bites or exposures in Wisconsin or Minnesota during 2007 to 2013. The mean age was 63 years, and 27% were immunocompromised. The clinical features were similar to both HME and HGA, including fever (87%), headache (67%), myalgias (60%), lymphopenia (53%), and thrombocytopenia (67%). Nearly 23% of patients were hospitalized, but no deaths occurred.[11]

Human Granulocytotropic Anaplasmosis

Signs, Symptoms, and Course

Infection with *A. phagocytophilum* generally manifests with acute undifferentiated fever. Male patients comprise 60% of infections, and

the incidence rate is at least 10 times higher in patients aged 50 years or older compared with children younger than 10 years.[65] After an incubation period of approximately 1 to 2 weeks, the illness can be mild to severe, with fever, headache, malaise, myalgias and arthralgias in most patients.[66,69,72,177–186] Nausea, vomiting, diarrhea, cough, stiff neck, and confusion are present in less than half of patients, less than 10% have rash, and most of these reflect erythema migrans with concurrent Lyme disease. Most doxycycline-treated patients are well within 7 days; if individuals are untreated, the median duration of illness is 9 days (range, 1–60 days).[178,187] Approximately 31% of infections are severe enough to warrant hospitalization, and 3% to 7% of patients have life-threatening complications or require ICU admission.[65,67] Severe manifestations include respiratory insufficiency, a septic shock– or DIC-like illness, renal failure, and pneumonia or acute respiratory distress syndrome.[65] Although reported in 0.4% of cases,[65] meningoencephalitis and CSF pleocytosis are rare in documented cases of HGA; however, other neurologic sequelae can include facial diplegia, brachial plexopathy, and demyelinating polyneuropathy.[188–190] At least 18 patients died after HGA in 2008 to 2012 alone.[65] Among 62 patients with HGA in Shandong Province in China, a subset of 16 patients from one region had significantly greater disease severity, including multiorgan dysfunction; gastrointestinal, renal, and hemorrhagic manifestations; worse laboratory features; and a case-fatality rate of 16% versus 2.6% in other regions.[69] Similar but less severe findings, including hemorrhagic manifestations (ecchymosis, epistaxis) in another study of HGA in China,[185] suggest the potential for strain-related severity differences or the potential for coinfection with tick-borne hemorrhagic fever bunyaviruses known to be present in those regions.[191–193]

Laboratory features observed in a substantial portion of cases include thrombocytopenia, leukopenia, mild anemia, and increases in serum hepatic aminotransferase activities within the first 7 days of illness.[66,72,186,194] Neutropenia with a left shift and relative lymphocytosis can occur. Leukocyte, erythrocyte, and platelet counts return to normal by 14 days, but the left shift can persist.[195] Doxycycline therapy reverses the decline in leukocyte and platelet counts and blunts the degree of left shift, usually within 5 to 7 days; anemia and normalization of serum hepatic aminotransferase activities respond more slowly. Hyperferritinemia and proinflammatory cytokine response have been identified as risk factors for severity in several studies of HGA.[144,185,186]

Diagnosis

Unlike the rarity of morulae in circulating mononuclear cells in HME, 20% to 80% of patients with HGA have morulae identified in peripheral blood neutrophils (Fig. 192.2).[66,67] Culture of *A. phagocytophilum* requires 1 week or longer and is not routinely available, whereas PCR amplification of *A. phagocytophilum* nucleic acids from blood is 54% to 100% sensitive and highly specific and can be performed in a timely manner.[166,187]

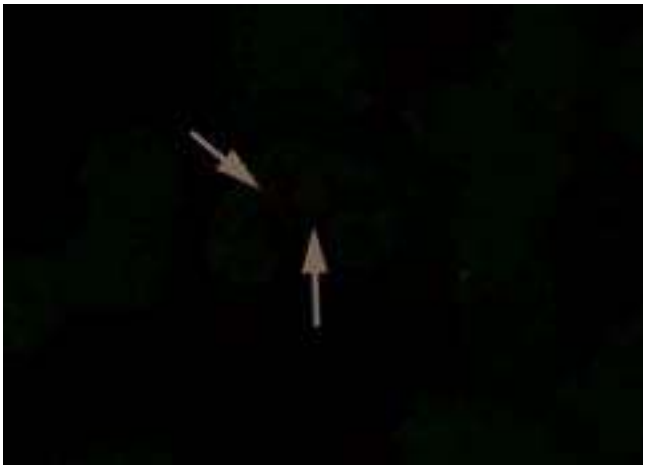

FIG. 192.2 Human granulocytic anaplasmosis. Peripheral blood smear showing intracellular inclusion within a neutrophil of a patient with human granulocytic anaplasmosis *(arrows)* (Wright stain, ×1000).

Serologic diagnosis is most often achieved retrospectively by detection of IgG antibodies reactive with *A. phagocytophilum* in infected tissue culture cells.[196,197] By current criteria, an IgG titer of at least 64 is considered supportive diagnostic information; however, because 15% to 16% of the population in the upper Midwest and New York State have preexisting serologic reactions, a fourfold rise to at least a titer of 128 provides more definitive evidence for infection.[76,78] IgM testing could be useful because reactions are demonstrated only during the first 45 to 60 days, but this test lacks sensitivity and is not generally advocated.[196] A role for Western immunoblot confirmation or use of recombinant antigens for serodiagnosis is not currently defined for humans.

TREATMENT AND PREVENTION

No prospective controlled treatment trials to determine antimicrobial efficacy for HGA have been conducted. In retrospective treatment studies, doxycycline, 100 mg twice daily, or tetracycline, 500 mg four times a day, has been used successfully. Based on *A. phagocytophilum* cell culture, doxycycline and rifamycins are bactericidal and chloramphenicol is not effective.[168,198,199] The clinical efficacy of rifampin has not yet been evaluated, but it has been used in children and during pregnancy.[200,201] Although fluoroquinolones have in vitro minimal inhibitory concentration values that predict in vivo effectiveness, relapse after use of levofloxacin unrelated to mutations in the bacterial *gyrA* gene has been documented in HGA.[202]

CANDIDATUS NEOEHRLICHIA MIKURENSIS HUMAN INFECTIONS

Documented human infections with *Candidatus* N. mikurensis are few, but increasing in number and geographic distribution. Infection has been described in humans in multiple recent cases in Europe, in 7 of 622 febrile Chinese patients, and in 5 of 316 asymptomatic foresters in Poland.[13,63,203–210] Infection was identified within peripheral blood granulocytes in 1 patient, and no isolate has been made from any patient. European patients were from Czech Republic, Germany, Sweden, and Switzerland. Eight patients had preexisting immunocompromise (chronic lymphocytic leukemia; follicular and mantle cell lymphomas; chronic inflammatory demyelinating polyneuropathy; rituximab, cyclophosphamide, or prednisolone treatment; and orthotopic liver transplantation), and several developed severe sepsis-like syndromes, sometimes protracted over weeks. Among these patients there is a high prevalence of thromboembolic complications, with an initial clinical presentation including fever, muscle and joint pain, and vascular events including deep vein thrombosis and transient ischemic attacks. The diagnosis is often delayed secondary to the similarity of the presentation to nonspecific inflammatory complications. All patients receiving doxycycline recovered. In contrast, none of the Chinese patients,[206] 2 patients from Sweden,[210] and 5 asymptomatic Polish subjects[63] were known to be immunocompromised, and all experienced only mild disease, including nausea, vomiting, myalgia, and stiff neck, or were asymptomatic. Cases were predominantly diagnosed with broad-range amplification of eubacterial *rrs* genes or with newly developed microbe-specific PCR assays. Doxycycline treatment was associated with clinical resolution in most cases; 1 patient died, and in 2 patients the infection resolved after phenoxymethylpenicillin treatment.

PREVENTION OF EHRLICHIOSIS AND ANAPLASMOSIS

At present, prevention of human ehrlichioses and anaplasmosis must rely on avoidance of tick exposure, regular careful search of the body for ticks after exposure, and prompt removal of ticks from the body. Although *A. phagocytophilum* may be transmitted within 4 hours of a tick bite, no analysis of prophylactic antibiotic therapy has been conducted.[211]

SENNETSU NEORICKETTSIOSIS

Physicians outside of Asia are unlikely to see a patient with sennetsu neorickettsiosis, which has been documented in Japan, Malaysia, and recently in Laos and is associated with consumption of raw fish and not with arthropod vectors.[212–214] Despite high seroprevalence in some

areas, disease of sufficient clinical severity to necessitate hospitalization remains rare.[215] *N. sennetsu* was isolated in 1953 from the blood, bone marrow, and lymph node of a 25-year-old man who had fever, severe headaches, myalgia, anorexia, lymphadenopathy, and an increased quantity of atypical lymphocytes in his peripheral blood.[216] Organisms isolated in mice were inoculated into human volunteers, who developed a syndrome resembling infectious mononucleosis. Neorickettsiae were recovered from their blood samples.

The average incubation period of 14 days is followed by sudden onset of chills and a fever that lasts for 2 weeks unless treated effectively. Patients also complain of headache and myalgia. Postauricular and posterior cervical lymphadenopathy appears 5 to 7 days after onset. Hepatosplenomegaly occurs in one-third to one-half of patients. Aseptic meningitis is observed only occasionally, and rash very rarely. Early in the illness leukopenia occurs; in the late febrile and convalescent phases, absolute lymphocytosis is observed, with 10% or greater atypical lymphocytes. Mild-to-moderate elevations occur in serum aminotransferase levels. Laboratory diagnosis can be made with PCR on buffy coat blood or through demonstration of specific serum antibody by IFA. Treatment with one of the tetracycline antimicrobials, including doxycycline or minocycline, results in defervescence after 1 to 2 days.

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The complete reference list is available online at Expert Consult.

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F Bacterial Diseases

193 Introduction to Bacteria and Bacterial Diseases

Jennifer A. Philips and Martin J. Blaser

Bacteria, the oldest forms of life on earth, are remarkably diverse and exist in astounding numbers. Diseases caused by bacteria include some of the most common infections in the world and some of the most important human scourges, past, present, and probably future. At the same time, each of us is colonized by as many bacterial cells as we have human cells in our bodies. In general, this is a peaceful and even productive (symbiotic) relationship, but occasionally even these well-tolerated residents of the human biosphere cause disease.[1–3]

We are surrounded by and always exposed to bacteria, including those that evolved to live well with us (e.g., *Bacteroides* species) and those whose evolution has promoted the tendency to cause disease (e.g., *Mycobacterium tuberculosis*) and death (e.g., *Bacillus anthracis*). In consequence, and not surprisingly, many of the presently recognized infectious diseases are caused by bacteria. It also may be safely predicted that many important illnesses not yet recognized or widespread ("emerging" infectious diseases) will be found to be caused by bacteria,[4] as will some chronic inflammatory diseases of unknown cause and malignancies (see later). Therefore knowledge of pathogenic bacteria, the diseases to which they lead, and current preventive and therapeutic strategies are critical for all health care providers, especially specialists in infectious diseases. An emerging concept is that our residential bacteria, part of human physiology and protective against introduced pathogens, are changing, with important health consequences.[4–8]

CLASSIFICATION OF BACTERIA

Bacteria have been classified according to phenotype, including size, shape, staining properties, and biochemical properties, since the beginning of microbiology. In recent years classification has been dominated by genotype, especially relying on conserved molecules, such as 16S ribosomal RNA.[9] Although there is a considerable degree of overlap between phenotype and genotype, as would be expected, dichotomies occur. In the future, taxonomy, understanding of pathogenesis, and diagnostics will be increasingly based on genotype and gene expression. Even in resource-limited settings, rapid genotypic identification of drug-resistant *M. tuberculosis* is close at hand.[9] Proteins expressed under a given condition can be determined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which generates proteomic mass patterns or fingerprints. As a method for identification of microorganisms, this is rapidly being integrated into the routine analytic pipeline of clinical microbiology laboratories. More advanced "proteotyping" will provide strain-level characterization, including virulence and antibiotic resistance factor expression.[10] Thus physicians and other students of infectious diseases must broaden their knowledge of molecular biology and taxonomy. As bacteriology advances in its differentiation of genera and species, as subspecies diversity is increasingly appreciated,[11] as variation within individual hosts is better understood, and as the evolution of pathogens is better outlined,[12,13] a grounding in evolutionary biology and ecology also will become more critical. The recent National Institutes of Health Human Microbiome Project already has provided a new scientific basis for the biomedical community toward understanding strain and species variation among the indigenous organisms in human hosts.[14] Over time, this information will be translated into clinical advances in prevention, diagnosis, and treatment of infectious diseases; studies of the human microbiome are already suggesting bacterial roles in diseases that were not previously suspected to have a microbial cause.[2–4]

VARIATION IN BACTERIAL INFECTIONS

Because all organs of the body are subject to bacterial infection, a recitation of these sites would be exhaustive and thus not useful. However, at the least, bacterial infections may be considered as varying in cause, mechanisms, and time frame. Infections may be caused by gram-positive or gram-negative bacilli or cocci; these were the first recognized bacterial agents of disease. However, this simple taxonomy does not fully account for other causative bacterial agents, including *Mycobacterium* species, treponemes, mycoplasmas, rickettsiae, chlamydiae, and actinomyces, all of which are Eubacteria. Each of these types of organisms has particular stereotypic features that characterize its interactions with hosts, but exceptions and variations abound. In recent years Archaea, a widespread and ancient group of prokaryotes, most closely resembling bacteria, has been isolated from human specimens[15]; the extent to which they play roles in human diseases is not yet known.

The mechanisms whereby bacteria cause disease are quite varied (Table 193.1). There is no universal mechanism or principle; the causative organism need not even be present in the human body. For example, food poisoning is commonly caused by the ingestion of preformed toxin produced by *Clostridium botulinum* or *Staphylococcus aureus* when they are growing in food, not in the host. The scope of bacterial infections includes interactions across time frames that vary from minutes to decades, or longer (Table 193.2). The descendants of the organisms that we each acquire from our mother[16] as a newborn can be the cause of our death in old age (e.g., caused by a perforated diverticulum) to carry the argument to the farthest extreme. Each bacterial infection is unique and reflects the underlying virulence properties of the bacteria and the predisposition of the host. For example, commensals or generally nonpathogenic environmental organisms can cause devastating disease in certain hosts because of genetic inborn errors[17,18]; acquired immunodeficiencies from diverse causes, such as human immunodeficiency virus, autoantibodies,[19] or immunomodulatory drugs; or from anatomic defects, such as perforated viscera, indwelling lines, or traumatic injuries. This complexity increases the difficulty in grasping the underlying concepts but also makes the practice of infectious diseases so intellectually satisfying.

POLYMORPHISM AND BACTERIAL INFECTION

From the earliest days of microbiology, when it was recognized that some organisms of the same species were encapsulated and many were not, it has been clear that pathogenic bacteria are polymorphic. With the development of antisera came the recognition that apparently identical organisms showed variation; this information became the basis for typing

schemes based on capsular, somatic O-antigen, or flagellar antigenic differences. Such polymorphisms have enabled better classification of virulent (and avirulent) meningococci, pneumococci, and *Escherichia coli*, for example, and have led to diagnostics, therapeutic antisera, and vaccines. However, in recent years has come the understanding that once bacteria begin to multiply in a host, their own populations become polymorphic.[20–21] Antigenic variation is one subtheme of that phenomenon and has been known since the studies of *Borrelia recurrentis*, the cause of relapsing fever. Increasingly, with the tools provided by the sequencing of whole bacterial genomes and single cell transcriptomics, we are learning just how polymorphic are bacteria, often considered as clonal organisms, and how dynamic their changes in relation to individual hosts. Even the most highly clonal bacteria, such as *M. tuberculosis*, exhibit extensive phenotypic variation, as well as genetic variation at particular loci, often those involving interaction with hosts.[21–22]

In parallel, we have been learning more about human genetic polymorphisms and their relationship to bacterial diseases. Medical science is rapidly advancing from phenotypes (e.g., blood groups) to genotypes (e.g., alleles of the interleukin-1β promoter). The genetic composition of each individual helps determine its response to bacterial infections and thus the outcome. Increasingly, these host characteristics will become the focus of the information that clinicians will need when considering prevention, differential diagnosis, and therapy of bacterial infections.[18,23]

TABLE 193.1 Disease Mechanisms Involved in Bacterial Infections

MECHANISM	EXAMPLES
Pyogenic infection	Pneumococcal pneumonia, staphylococcal abscess
Granulomatous infection	Pulmonary tuberculosis, brucellosis, syphilis
Intoxication (augmentation of host physiology)	Cholera *(Vibrio cholerae)*
Intoxication (tissue destruction)	Gas gangrene *(Clostridium perfringens)*, diphtheria *(Corynebacterium diphtheriae)*
Immunologic mediation	Guillain-Barré syndrome after *Campylobacter jejuni* infection; reactive arthritis after shigellosis; acute rheumatic fever after pharyngitis due to *Streptococcus pyogenes*
Neoplasia	Adenocarcinoma of the stomach as a consequence of *Helicobacter pylori* persistence; adenocarcinoma of the esophagus as a consequence of physiologic and microbiologic changes induced by absence of *Helicobacter pylori*

BACTERIA AS "NEW" CAUSES FOR "OLD" DISEASES

The finding that an indigenous bacterium, *Helicobacter pylori*, plays pathogenetic roles in two important illnesses, peptic ulcer disease and gastric cancer,[24] has advanced a new paradigm—that many of the diseases that we consider diseases of unknown cause may in fact be infectious diseases.[4] This idea, which gained prominence with the knowledge about the relationship of streptococcal pharyngitis to rheumatic fever, has been growing since the 1930s, and other examples have been recognized. For example, after an episode of enteritis caused by *Campylobacter jejuni*, the Guillain-Barré syndrome may develop.[25] Similarly, more than 30 years ago it was recognized that acute infection with enterohemorrhagic *E. coli* may lead to the hemolytic uremic syndrome.[26] Thus acute, transient, and self-limited infections of the respiratory or gastrointestinal tracts may trigger cardiac, neurologic, or systemic diseases, with consequences lasting months or permanently. Of importance, the disease locus (e.g., kidney, peripheral nervous system, heart) may be distant from the original infection at a mucosal surface. How many other examples of parallel phenomena may be present? The uncovering of clinical and epidemiologic associations has led to research identifying pathogenetic mechanisms that provide new paradigms for autoimmunity.[27] Could a more persistent bacterial pathogen trigger multiple sclerosis or Graves disease? Conversely, does exposure to diverse environmental microbes modulate development of the immune system and protect against atopic conditions?[28]

Diseases such as sarcoidosis, ulcerative colitis, Crohn disease, Wegener granulomatosis, and thyroiditis are chronic inflammatory diseases for which a bacterial role in causation is not improbable. In addition, there is a growing appreciation for the role of our microbiome and its metabolites in modulating human development, physiology, and immunity.[2] Our metagenome and metatranscriptome may become useful indices for interpreting human physiology.[29] Thus unnecessary use of antibiotics may have significant unforeseen immediate health consequences, including enhanced susceptibility to viral infections[30] and long-term sequelae. For example, antibiotic use in early life in both farm and experimental animals produces major shifts in microbiota characteristics, host developmental phenotypes, and adiposity.[31–33] Whether such precedents are applicable to human children is unknown, but it seems likely. New studies suggest connections between the bacteria we carry and seemingly unrelated diseases, such as diabetes and hypertension.[34–35]

BACTERIAL EVOLUTION

The study of infectious diseases is a dynamic field, at least in part because of the evolving nature of the pathogens we consider. An obvious and absolutely critical aspect of bacterial evolution is the acquisition of resistance to antimicrobial agents. As the prescribing of antimicrobial therapies flourishes—whether they address important, controversial,

TABLE 193.2 Variation in Time Courses for Representative Bacterial Infections

TIME FRAME	DISEASE	REPRESENTATIVE CAUSATIVE ORGANISM	CLINICAL MANIFESTATIONS	MECHANISMS
Minutes	Food poisoning	*Clostridium perfringens*	Vomiting, diarrhea	Preformed enterotoxin
Hours	Necrotizing fasciitis	*Streptococcus pyogenes*	Devitalization of muscle, sepsis	Bacterial spread across tissue planes
Days	Anthrax	*Bacillus anthracis*	Cough, chest pain, fever, dyspnea	Resistance to macrophage killing
Weeks	Lung abscess	Oral anaerobes	Cough, fever, chest pain	Necrotizing pyogenic process
Months	Subacute bacterial endocarditis	α-Hemolytic streptococci	Fever, anemia, stroke, heart failure, uremia	Infection of immunologically privileged site
Years	Whipple disease	*Tropheryma whipplei*	Fever, diarrhea, weight loss	Resistance to macrophage killing
Decades (persistence)	Osteomyelitis	*Staphylococcus aureus*	Fever, wound discharge, pain	Pyogenic infection of devitalized tissue (±foreign body)
Decades (latency)	Pulmonary tuberculosis	*Mycobacterium tuberculosis*	Cough, fever, weight loss	Reactivation of latent focus into active granulomatous process
Decades (oncogenesis)	Gastric cancer	*Helicobacter pylori*	Cachexia, abdominal pain	Persistent inflammation leading to progressive metaplastic and dysplastic conditions

or even trivial indications—antimicrobial resistance by pathogens and indigenous organisms continues to grow.[36–38] Understanding resistance patterns is pivotal to understanding proper therapeutic approaches.[36] Also important is that understanding the biology, epidemiology, and mechanisms for resistance will lead to the methods needed to prevent and curtail resistance in the populations of microbes that infect and colonize humans, which may provide reservoirs for resistance.[38,39] Physicians, especially those who are specialists in infectious diseases, must be at the forefront of efforts to reduce the development of resistance.

Of interest, considerations of resistance are useful for understanding other important aspects in the evolution of infectious diseases; we live in a world of natural selection. Resistance is among the easiest phenotypes to detect and understand, but bacteria continue to be selected on the basis of differences in the soaps used (also a function of resistance), sizes of families and other social groups, presence of daycare centers and jet planes, and changing dietary habits (functions of transmission).[40] As the connectivity of human populations increases, there may be greater selection for virulence.[13] We may already be witnessing progressively increasing virulence of *M. tuberculosis*,[22] in addition to its progressively increasing antibiotic resistance.[41] The spontaneous and widespread emergence of hypervirulent *Clostridioides difficile* (formerly *Clostridium difficile*) strains of different genotypes but similar phenotypes (hypertoxigenic because of deletions involving regulatory elements) indicates the power of microbes to adapt to strong selective forces.[42,43] Study of bacterial infections provides a rich school, not only for the health care provider but also for the student of human evolutionary biology.[22,44,45] The lessons learned will also be critical in clinical medicine and epidemiology.

BACTERIA AS THERAPEUTICS

Since Metchnikoff's time and earlier, physicians have sought ways to harness bacteria to fight disease. At present, the highly lethal exotoxin of *C. botulinum* is being used as a therapeutic agent for medical and cosmetic purposes. The bacillus Calmette-Guérin vaccine, an attenuated form of *Mycobacterium bovis*, is used as adjuvant therapy for bladder cancer. Although at first glance such harnessing of bacteria for useful purposes seems extraordinary, it actually makes great sense. The bacteria that live with us, or that attack us, often know us well; their evolution has selected for organisms that exploit chinks in our armor.[13,46–48] They are skilled cell biologists, immunologists, and physiologists and are great competitors with one another. Bacteria have potential usefulness as probiotics to treat disease.[49] Perhaps the best example is the least defined—the utility of fecal transplantation to cure recurrent *C. difficile* infection, which has become the standard of care.[50] This therapy is a proof of principle that using a bacterial mixture that normalizes the gut microbiome can cure an infectious disease. Defined probiotic strains and prebiotics, nutrients that provide a substrate for particular bacteria or biochemical processes, extend the concept of harnessing bacteria for treatment by another step.[51] Engineering bacteria to do our work will be a further development.[52] Understanding the clinical manifestations and pathogenesis of bacterial infections will lead to new approaches to medicine—new therapeutics[53] and new preventives.[7] Predictably, each of the new treatments will lead to new complications. A thorough grounding in knowledge about bacterial infections will enable physicians and researchers to develop these new therapeutic modalities and to predict and treat the expected complications.

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194 *Staphylococcus aureus* (Including Staphylococcal Toxic Shock Syndrome)

Yok-Ai Que and Philippe Moreillon

SHORT VIEW SUMMARY

Definition
- *Staphylococcus aureus* is a gram-positive pathogen that is responsible for superficial and deep-seated infections.
- It is a frequent colonizer of asymptomatic carriers.
- The organism is responsible for both pyogenic and toxin-related diseases.
- It is the primary cause of community- and hospital-acquired bloodstream infections and the first cause of invasive infections including infective endocarditis and osteomyelitis.
- Frequently, *S. aureus* is resistant to methicillin (methicillin-resistant *S. aureus* [MRSA]) and almost all β-lactam drugs (in up to 50% of hospital isolates).
- Often it is coresistant to many clinically available antibiotics.

Epidemiology
- *S. aureus* is a colonizer of the anterior nostrils in 20% to 40% of the normal population.
- With regard to drug-resistant nosocomial infections, it is the number one public health problem.
- Clonal spread of MRSA occurs from health care settings (health care–associated [HCA] MRSA) and other permissive environments (see Fig. 194.2).
- Polyclonal dispersion of susceptible strains occurs in the community, but successful clones of community-acquired (CA) MRSA may spread worldwide (e.g., USA300).
- The organism is a superantigen producer responsible for toxic shock syndrome and food poisoning.

Microbiology
- *S. aureus* is the most virulent species of the more than 40 *Staphylococcus* spp. taxa.
- Its conserved core genome consists of approximately 2.8 million base pairs.
- It has multiple mobile genetic elements (MGEs): pathogenic and genomic islands, transposons, and prophages encoding virulence and antibiotic-resistance genes (see Tables 194.1 and 194.3).
- Multiple immune-evasion strategies are used by the organism, which impedes vaccine development (see Table 194.4).
- Evolution of successful clones occurs by means of mutations and acquisition of MGEs.
- Methicillin resistance is conferred by a polymorph family of SCC*mec* cassettes.

Diagnosis
- Conventional cultures are mandatory and critical for the detection of new resistance phenotypes.
- Molecular tests are useful for rapid identification of known drug-resistance genes.
- Molecular typing is critical for management of MRSA epidemics and infection control.

Therapy (See Tables 194.9 and 194.10)
- The first choice for methicillin-susceptible *S. aureus* is a penicillinase-resistant β-lactam or first-generation cephalosporin—for instance, cephalexin or dicloxacillin orally; nafcillin or oxacillin intravenously; or, outside the United States, flucloxacillin. Alternatives include clindamycin or trimethoprim-sulfamethoxazole (TMP-SMX) orally or intravenously.
- The first choice for MRSA would be vancomycin or daptomycin intravenously, or, if susceptible, TMP-SMX or clindamycin given orally or intravenously. Alternatives include ceftaroline, linezolid, or telavancin or, for acute bacterial skin and skin structure infections, dalbavancin, oritavancin, or tedizolid.
- A partner drug for combinations is rifampin.
- The benefit of aminoglycosides not well demonstrated and no longer recommended for native valve endocarditis.

Prevention
- Prevention measures include the following:
  - Decolonization of staphylococcal carriers (see Table 194.8)
  - Detection of HCA-MRSA and epidemic CA-MRSA
  - Hand hygiene measures
  - Vaccines in development

Staphylococcus aureus is a highly successful opportunistic pathogen. It is a frequent colonizer of the skin and mucosa of humans and animals (it is present in the anterior nares of up to 30% of the healthy human population) and can produce a wide variety of diseases. These diseases encompass relatively benign skin infections, such as folliculitis and furunculosis, and life-threatening conditions, including erysipelas, deep-seated abscesses, osteomyelitis, pneumonia, sepsis, and endocarditis.[1] In addition to infections in which the organism is physically present at the infected site, *S. aureus* is also capable of producing "distant" diseases, which are mediated by the secretion of toxins.[2] The toxins can be produced *directly* by bacteria that colonize the skin or mucosa or *indirectly* by microorganisms that colonize food or beverages. The former is exemplified by staphylococcal scalded skin syndrome (SSSS),[3,4] which is the result of skin, mucosal, or wound colonization by *S. aureus*–producing exfoliative toxin A or B (ETA or ETB) and by staphylococcal toxic shock syndrome (TSS),[2,5] which is the result of the production of toxic shock syndrome toxin 1 (TSST-1) or exotoxins B or C. The latter is exemplified by *S. aureus* food intoxication, in which the toxin is ingested with the contaminated dish, and disease follows shortly thereafter in the form of vomiting and diarrhea. Food intoxication is the result of staphylococcal toxins called *enterotoxins*.[2,6] These toxins are heat stable. Cooking may kill the contaminants but does not denature the toxins. Hence, subsequent culture of the dish may fail to grow the culprit bacterium.

S. aureus has an extraordinary capacity to adapt and survive in a great variety of environments. During the past decades, molecular and genetic dissection of *S. aureus* has revealed a great number of surface adhesins, which mediate adherence to and colonization of target tissues, and secreted enzymes, toxins, superantigens (SAgs) and immune evasion determinants that are responsible for invasion and distant disease

(Table 194.1).[1,7–9] The availability of now several thousands of *S. aureus* genome assemblies and annotation reports (www.ncbi.nlm.nih.gov/genome/genomes/154) has helped complete this portrait. *S. aureus* is part of the Firmicutes phylum and shares approximately 50% of orthologue genes with notoriously nonpathogenic *Bacillus subtilis,* which indicates that the two organisms have evolved from a common ancestor.[10–12] Homology searches on the chromosome revealed numerous new surface-attached and secreted factors that represent additional pathogenic factors. *S. aureus* harbors a large number of mobile genetic elements (MGEs) from exogenous origin, including insertion sequences, transposons, bacteriophages, pathogenicity islands, and genomic islands, which contain specific determinants responsible for disease and antibiotic resistance.[7,8,10,13,14] The presence of these exogenous elements attests to the high capacity of *S. aureus* to undergo horizontal gene transfer and exchange genetic elements with other organisms, including staphylococcal and nonstaphylococcal genera. Because gene exchange is a key player of evolution, this peculiar genetic plasticity is a likely explanation for the success of *S. aureus,* both as a colonizer and a disease-producing microbe. In the case of SAgs (see later discussion), one of the trading partner is suspected to be *Streptococcus pyogenes*.[2]

THE MICROORGANISM

Members of the *Staphylococcus* genus are gram-positive cocci (0.5–1.5 µm in diameter) that occur singly and in pairs, tetrads, short chains, and irregular grapelike clusters. Ogston[15] introduced the name *Staphylococcus* (Greek *staphylé,* "a bunch of grapes") to describe micrococci responsible for inflammation and suppuration. Staphylococci are nonmotile, non–spore forming, and usually catalase positive, and they are often unencapsulated or have a limited capsule (Fig. 194.1). Most species are facultative anaerobes.[11,12]

The genus *Staphylococcus* contains up to 40 taxa, 16 of which are commonly found in humans (Table 194.2). Only a few are pathogenic in the absence of predisposing immunosuppression or implanted foreign material. The most virulent ones include *S. aureus, Staphylococcus lugdunensis,* and *Staphylococcus schleiferi* in humans, and *S. aureus* and *Staphylococcus intermedius* in animals. Although *Staphylococcus epidermidis, Staphylococcus haemolyticus,* and *Staphylococcus saprophyticus* are commonly responsible for device-related and urinary tract infections, they produce substantially less devastating disease syndromes than *S. aureus.*

S. aureus harbors some unique features when compared with its less–disease-producing congeners. These include coagulase and clumping factors (or fibrinogen-binding proteins), which have laboratory diagnostic value because they help rapidly discriminate between coagulase-positive (i.e., *S. aureus*) and coagulase-negative staphylococci (CoNS; see Table 194.2). Moreover, *S. aureus* carries between more than 20 and more than 30 adhesin and toxin genes, respectively, as compared with 10 or fewer adhesin genes and virtually no toxin genes for the CoNS mentioned previously.[7,16–18] Thus, *S. aureus* is a distinct pathogen within the *Staphylococcus* genus.

Habitat

Staphylococci are ubiquitous colonizers of the skin and mucosa of virtually all animals, including mammals and birds.[11,12] Some species have preferential niches as indicated by their names (see Table 194.2). *S. epidermidis* and *Staphylococcus capitis* are constant colonizers of the skin and scalp, respectively. *Staphylococcus pseudintermedius* is a colonizer of cats and dogs and may be misidentified as *S. aureus* (tube coagulase positive, slide coagulase negative) when transmitted by animal bites.[19]

In animals, *S. aureus* is a major cause of livestock infection, including mastitis in bovine and ovine herds. In humans, *S. aureus* has a niche

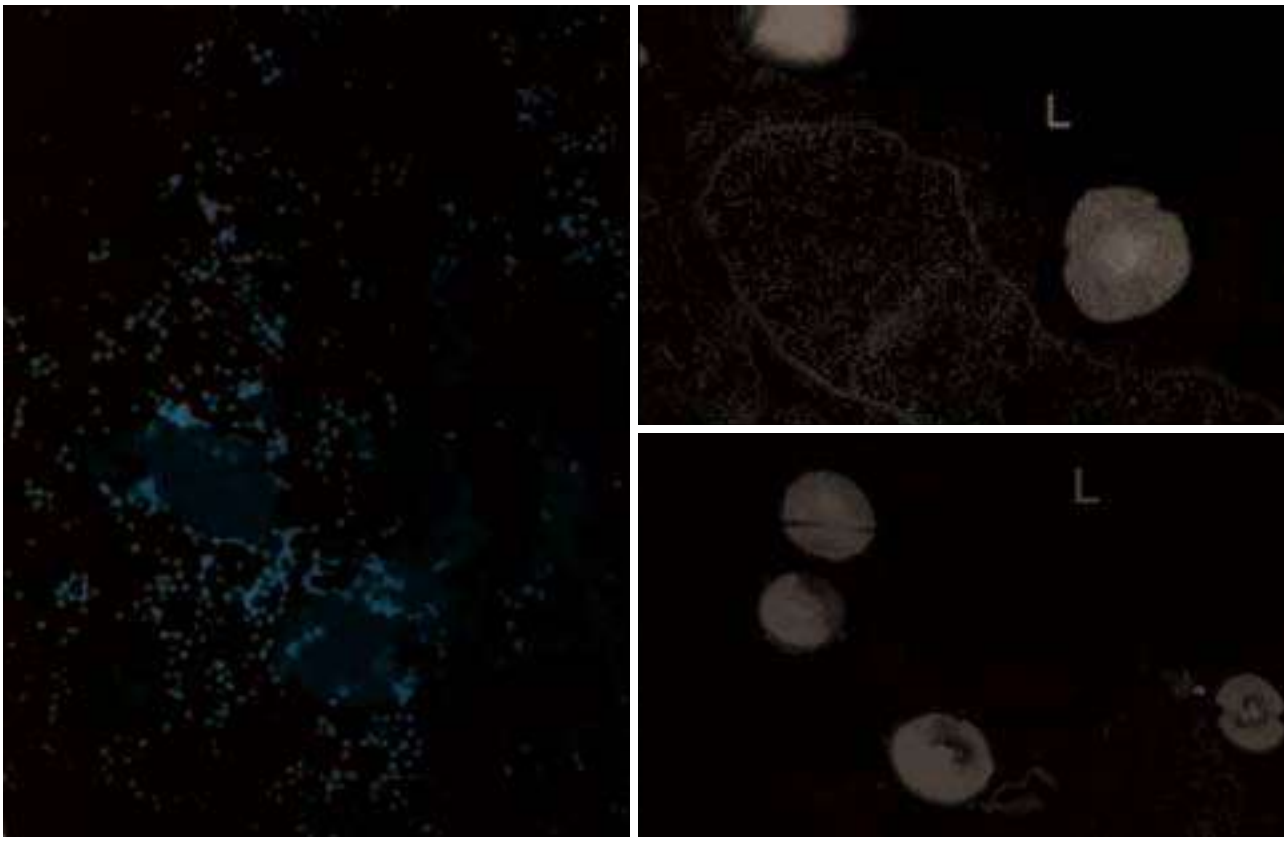

FIG. 194.1 Gram staining and transmission electron microscopy of clinical samples of *Staphylococcus aureus.* *Left,* Gram-stained sputum specimen from 20-year-old patient with fulminant hemorrhagic *S. aureus* pneumonia (see also Fig. 194.8). Grapelike clusters of bacteria and eukaryotic cells are visible. *Right,* Electron microscopy images from rat with experimental aortic endocarditis caused by *S. aureus. Upper part* depicts *S. aureus* in process of invading valve endothelial cell. *Lower part* depicts endothelial cell that has been lysed by invading bacteria, probably as a result of *S. aureus* hemolysin secretion. *L,* Lumen side of endothelium.

TABLE 194.1 Some of the Major *Staphylococcus aureus* Extracellular Factors Involved in Pathogenesis and Response to Global Regulatory Elements During Bacterial Growth

GENE	LOCATION	PRODUCT	ACTIVITY OR FUNCTION	TIMING[a]	ACTION OF REGULATORY GENES[b]			
					agr	*saeRS*	*rot*	*sarA*
Surface Proteins								
spa	Chromosome	Protein A	Blocks IgGs, binds von Willebrand factor	Exp	–	See footnote[c]	+	
cna	Chromosome	Collagen BP	Collagen binding	Exp	–			
fnbA	Chromosome	Fibronectin BPA	Fibronectin binding	Exp	–			-
fnbB	Chromosome	Fibronectin BPB	Fibronectin binding	Exp	–			+
clfA	Chromosome	Clumping factor A	Fibrinogen binding	Exp	0			
clfB	Chromosome	Clumping factor B	Fibrinogen binding	Exp	0		+	0
sdrC	Chromosome	Serine-aspartate repeat protein	Fibrinogen binding Cytokeratin binding	Exp				+
Capsular Polysaccharides								
cap5	Chromosome	Polysaccharide capsule type 5	Antiphagocytosis	Pxp	+			+
cap8	Chromosome	Polysaccharide capsule type 8	Antiphagocytosis	Pxp	+			
Cytotoxins								
hla	Chromosome	α-Hemolysin	Hemolysin, cytotoxin	Pxp	+	+	–	See footnote[c]
hlb	Chromosome	β-Hemolysin	Hemolysin, cytotoxin	Pxp	+	+	–	See footnote[c]
hld	Chromosome	δ-Hemolysin	Hemolysin, cytotoxin	Pxp	+	0		+
hlg	Chromosome	γ-Hemolysin	Hemolysin, cytotoxin	Pxp	+		–	See footnote[c]
lukS/F	PVL phage	PVL	Leucocidin	Pxp	+		–	
Superantigens								
sea	Bacteriophage	Enterotoxin A	Food poisoning, TSS	Xp	0			
seb	SaPI3[d]	Enterotoxin B	Food poisoning, TSS	Pxp	+			See footnote[c]
sec	SaPI4[d]	Enterotoxin C	Food poisoning, TSS	Pxp	+			
sed	Plasmid	Enterotoxin D	Food poisoning, TSS	Pxp	+			
eta	ETA phage	Exfoliatin A	Scalded skin syndrome	Pxp	+			
etb	Plasmid	Exfoliatin B	Scalded skin syndrome	Pxp	+			
tst	SaPI1,2, bov1[d]	Toxic shock toxin 1	TSS	Pxp	+			See footnote[c]
Enzymes								
SplA-F	Chromosome	Serine protease-like	Putative protease		+		–	
ssp	Chromosome	V8 protease	Spreading factor	Pxp	+	0		–
aur	Chromosome	Metalloprotease (aureolysin)	Processing enzyme?	Pxp	+			–
sspB	Chromosome	Cysteine protease	Processing enzyme?	?			–	
scp	Chromosome	Staphopain (protease II)	Spreading, nutrition	Pxp	+			–
geh	Chromosome	Glycerol ester hydrolase	Spreading, nutrition	Pxp	+	0	–	See footnote[c]
lip	Chromosome	Lipase (butyryl esterase)	Spreading, nutrition	Pxp	+	0		See footnote[c]
fme	Chromosome	FAME	Fatty acid esterification	Pxp	+			See footnote[c]
plc	Chromosome	PI-phospholipase C		Pxp	+			
nuc	Chromosome	Nuclease	Nutrition	Pxp	+	+		
has	Chromosome	Hyaluronidase	Spreading factor	Xp	See footnote[c]			
coa	Chromosome	Coagulase	Clotting, clot digestion	Exp		+	+	+
sak	Bacteriophage	Staphylokinase	Plasminogen activator	Pxp	+	0		

[a]Timing: Xp, throughout exponential phase; Exp, early exponential phase only; Pxp, postexponential phase; 0, no effect of gene on. Expression: +, upregulated; –, downregulated.
[b]*agr,* Accessory gene regulator; PVL, Panton-Valentine leukocidin; *saeRS, S. aureus* exoproteins; *rot,* repressor of toxins; *sarA, Staphylococcus* accessory regulator.
[c]Controversial.
[d]*SaPI, S. aureus* pathogenic island.
BP, Binding protein; *FAME,* fatty acid modifying enzyme; *TSS,* toxic shock syndrome.
Modified from Cheung AL, Projan SJ, Gresham H. The genomic aspect of virulence, sepsis, and resistance to killing mechanisms in Staphylococcus aureus. Curr Infect Dis Rep. *2002;4:400–410; and Novick RP, Geisinger E. Quorum sensing in staphylococci.* Ann Rev Genet. *2008;42:541–564.*

TABLE 194.2 Some Staphylococcal Species From Mammals and Relationship Between Production of Coagulase and Clumping Factor (Fibrinogen-Binding Protein A) and Potential Virulence

HOST	SPECIES	COAGULASE[a]	CLUMPING FACTOR[a]	VIRULENCE[a]
Human and other primates	*S. aureus*	++	++	+++
	S. epidermidis	–	–	+
	S. capitis	–	–	±
	S. caprae	–	–	±
	S. saccharolyticus	±	–	–
	S. warneri	–	–	–
	S. pasteuri	–	–	–
	S. haemolyticus	–	–	+
	S. hominis	–	–	±
	S. lugdunensis	–	±	+
	S. auricularis	–	–	±
	S. saprophyticus	–	–	+
	S. cohnii	–	–	–
	S. xilosus	–	–	–
	S. simulans	–	–	–
	S. schleiferi	±	+	+
Carnivores	*S. intermedius*	+	–	++
	S. felis	–	–	++

[a]Semiquantitative estimate of production of coagulase and clumping factor and relation to virulence.
Modified from Kloos WE, Schleifer KH, Goetz F. The genus Staphylococcus. *In: Balows A, Trüper HG, Dworkin M, et al, eds.* The Prokaryotes. *2nd ed. New York: Springer-Verlag; 1992:1369–1420; and Kloos WE, Bannerman TL.* Staphylococcus *and* Micrococcus. *In: Murray PR, Baron EJ, Pfaller MA, et al, eds.* Manual of Clinical Microbiology. *6th ed. Washington, DC: ASM Press; 1995:282–298.*

preference for the anterior nares, especially in adults,[20–22] and is shed onto healthy skin, including axilla and perineum. However, certain clones may have preferences for more hidden niches, as was shown in the case of a peculiar epidemic hospital methicillin-resistant *S. aureus* (MRSA) clone that colonized the groin and rectum.[23] *S. aureus* can exist as a resident or a transient member of the normal flora. Nasal carrier rate varies from 10% to 40% in both the community and the hospital environment. *S. aureus* carriage in various anatomic sites may put certain populations at an increased risk for infection, such as patients with recurring furunculosis and patients who are subject to medical procedures, including hemodialysis, peritoneal dialysis, and surgery (see later section "Carriage of *Staphylococcus aureus*").[24–26]

S. aureus carriage has also become a way of persistence and spread of multiresistant staphylococci, especially MRSA.[20,21,23,26] Because MRSA can resist many of the antibiotics in common use, it has risen to the level of a public health threat in the hospital for 3 decades and in the community since the beginning of this century.[25,27]

Culture and Identification

Live organisms obtained by means of culture are critical for phenotypic diagnosis and revealing emerging antibiotic resistant phenotypes from as yet unknown mechanisms. In addition, molecular diagnosis helps speed up the results, which take a few hours instead of 1 to 3 days with bacterial subculturing. Molecular methods also help detect the presence of nonculturable microbes, mostly when patients have taken antibiotics before sample collection.

Techniques for culture and identification of staphylococci have been described.[11] Specimens should be inoculated both on blood agar and into rich liquid media such as Mueller-Hinton broth. With *S. aureus,* abundant growth occurs normally within 18 to 24 hours. However, morphologic variants (see subsequent discussion) may require prolonged growth periods, and plates should be kept 2 to 3 days in order to detect them. Colonies should be Gram stained, subcultured, and tested for genus, species, and antibiotic susceptibility when appropriate. Phenotypic tests for species identification include coagulase tests and agglutination tests, which detect the presence of surface determinants, including clumping factor, protein A, and polysaccharides.[28] Phenotypic antibiotic susceptibility tests vary from agar-diffusion methods (e.g., Kirby-Bauer and Etests) to automated measurement of metabolic activity or growth rates. Macro broth or agar dilution methods are precise but are not routinely performed in the laboratory.[29]

Molecular specification may be necessary in case of unclear phenotype, such as, for instance, in the case of morphologic variants (see next section).

Morphologic Variants

Prolonged incubation is particularly important for the detection of morphology variants such as *small colony variants* (SCVs). SCVs grow into tiny colonies that are difficult to distinguish and may be mistakenly disregarded as contaminants.[30] They are usually recovered from protracted, difficult-to-treat infections such as chronic osteomyelitis and infected osteosynthetic prostheses, and have also been described in patients with cystic fibrosis.[31]

The most classic types of SCVs are selected during aminoglycoside therapy and result from alterations in the respiratory chain. Such SCVs have a lower transmembrane potential, which impedes the intake of the drug.[32] Interesting to note, switching from normal colonies to SCVs occurs naturally in the absence of antibiotic at a high rate (about 10^{-6}), and switching back from SCVs to normal colonies also occurs.[33] Hence, SCVs are proposed to result from an intrinsic capacity of the bacterium to survive in unfavorable conditions rather than fortuitous mutations.

SCVs are also selected by other antimicrobial agents, including triclosan.[34] They were recovered from the sputa of up to 25% of children with cystic fibrosis and were statistically significantly associated with previous trimethoprim-sulfamethoxazole (TMP-SMX) therapy.[31] Such SCVs carry mutations in the thymidylate synthase gene *(thyA)* and are dependent on exogenous thymidine to grow.[35] *S. aureus* synthesizes thymidine by using *thyA* plus tetrahydrofolate to convert uridine monophosphate into thymidine monophosphate. TMP inhibits the synthesis of tetrahydrofolate, thus making *thyA* useless. By mutating the *thyA* gene, *S. aureus* forces itself to rely on exogenous vital thymidine by importing it. This makes the bacterium resistant to TMP. Thymidine is available in DNA-rich lung secretions of patients with cystic fibrosis and in abscesses. However, the rate of thymidine import is limiting, which results in slow growth and SCV phenotype.

In spite of slow growth, SCVs are equally as or more infective than their fast-growing parents in experimental infections such as osteoarthritis[36] and endocarditis.[37] Moreover, SCVs are particularly prone to invade eukaryotic cells and persist in them,[30,38] which may explain their occurrence in latent infections. SCVs are cross-resistant to drug-induced killing by most antibiotics,[32] and their eradication necessitates prolonged antibiotic therapy including drug combinations with rifampin.

MOLECULAR DIAGNOSIS

Molecular diagnosis plays an increasing role in rapid detection of microbial pathogens and identification of drug-resistance determinants. Techniques based on molecular probing have been reviewed.[39] One of

these techniques relies on fluorescent detection of 16S rRNA with a peptide nucleic acid probe (peptide nucleic acid fluorescence in situ hybridization [PNA-FISH]). Such a technique has been shown to be highly specific and to help in discriminating *S. aureus* from CoNS in blood culture within 4 hours.[40] In addition, its usefulness in clinical therapeutic decision making has also been demonstrated.[41]

Multiplex real-time polymerase chain reaction (PCR) assays are being developed to quantify organisms directly in clinical samples. Genes representative of both species and resistance mechanisms are amplified simultaneously. For MRSA, the resistance gene sought is *mecA,* which encodes low-affinity penicillin-binding protein A (PBP2A).

However, *mecA* is also present in methicillin-resistant CoNS and thus detects simultaneously both MRSA and commensal methicillin-resistant CoNS, which may result in false diagnosis. One way to bypass this limit is to extend the *mecA* amplification product to *orfX*. *orfX* is an open reading frame that is specific for *S. aureus,* and its amplification ensures the correct diagnosis.[42] Another way is to choose additional *S. aureus* or CoNS specific genes. These include typical *S. aureus* gene versions such as *femA,*[42] protein A *(spa)*, coagulase *(coa)*, and nuclease *(nuc)*.[43] Other genes or gene combinations were also successfully used to discriminate between *S. aureus* and CoNS in clinical samples.[42,44,45]

Limits may occur with PCR amplification techniques. In some cases, proprietary DNA targets, known only to the manufacturer, can make it difficult to assess the vulnerability of commercial molecular assays to changes in the DNA sequence of isolates being tested. In a large multicenter US study of one molecular platform for identifying MRSA, 3 of 93 MRSA isolates were called methicillin-susceptible *S. aureus* (MSSA), and 8 of 102 MSSA isolates were called MRSA, indicating that although molecular typing may be useful for rapid screening of carriers, it may carry the risk of misdiagnosis in clinical care.[46] Likewise, PCR amplification using standard primers failed to amplify a new version of the *mecA* gene, renamed *mecC,* that emerged in livestock MRSA.[47] In this very case, methicillin resistance was detected with phenotypic tests.[48] Moreover, MSSA isolates have been found that had PCR-detected *mecA* elements but later reverted to methicillin resistance under therapy. The loss of a transposon that interrupted *mecA* and replication errors accounted for this conversion.[49]

Currently, more rapid identification by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is being developed, allowing MRSA identification from a colony within 5 minutes as compared with several hours with PCR.[50]

However, although more rapid than phenotypic tests, these molecular techniques still require prior growth of the organisms, which may take 12 to 24 hours, and do not test all possible antibiotic resistance genes. Whole-genome sequencing may become an option to screen for species and resistance genes, but the bioinformatic workload remains a limiting factor. One emerging technique relies on metagenomic analysis of DNA amplified directly from clinical samples, without prior culturing, through use of next-generation sequencing (NGS). Here, identification of genus, species, and known resistance genes will rely on comparison with large databases,[51] but unknown resistance genes will be missed. Thus, phenotypic testing must always be kept in mind.

Molecular Typing

There is a dual interest in studying the genealogy of life. One is academic and aims at solving the evolutionary journey of peculiar organisms. The other is epidemiologic and aims at tracing a peculiar pathogen responsible for clinical problems. *S. aureus* is a common pathogen both in the hospital and in the community.[27,52] Although the prevalence of MRSA has been slowly decreasing over the last decade in the United States and Europe,[53] this trend is not global and the proportion of MRSA in health care–related infections remains over 50% in other geographic locations.[54]

MRSA is highly clonal, and a few highly successful clones, named according to the place where they were described, can be recovered at multiple locations both nationwide and worldwide (i.e., the Iberian, Brazilian, Hungarian, New York/Japan, Pediatric, and EMRSA-16 pandemic clones).[55,56] The main molecular typing methods underlying this comprehension are briefly presented later. More complete total genome sequencing may not be required for routine tracing of epidemic strains.

Pulsed-Field Gel Electrophoresis

The seminal method is a restriction-fragment length technique based on large chromosomal fragments generated by digestion with the low-frequency cutting enzyme *Sma*I. The fragments are separated with pulsed-field gel electrophoresis (PFGE) and yield banding patterns specific for particular clones. Banding comparison allowed identification of the major epidemic clones listed earlier, which represented 70% of more than 3000 MRSA isolates recovered worldwide.[56]

One limitation of PFGE is that it does not provide accurate information on the genealogy of the organism. Indeed, the length of chromosomal fragments, and thus the clone-specific banding, may be modified with acquisition or loss of mobile DNA (MGEs) such as transposons, prophages, or pathogenicity islands. The new banding pattern may identify a different clone, which is in fact the same bacterium that has gained or lost MGEs. This is exemplified by the fact that several PFGE major MRSA epidemic clones belonged to the same multilocus sequence typing (MLST; see later) group.[55] Thus, PFGE is useful to follow epidemic clones, but not to build the parental staphylococcal genealogy.

Multilocus Sequence Typing

In contrast to PFGE, MLST is a sequence-based method that allows the unambiguous assignment of the ancestral phylogeny of the staphylococcal population.[57] It consists of sequencing seven housekeeping genes (i.e., *arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) and comparing them with the sequences of other isolates collected in a central database (www.mlst.net). It compares allelic diversity based on approximately 500-bp internal gene fragments. Thousands of sequences have been submitted, generating numerous sequence types (STs). Organisms that share all seven alleles are defined as *clones,* those that share five of seven identical alleles are defined as *clonal complexes* (CC), and those that share less than five alleles are defined as *unrelated*. Within such arborescence, STs can be considered as founders of further evolutionary groups such as CCs.

Because housekeeping genes are independent of acquired MGEs, MLST traces staphylococci back to their latest common ancestor. Of the seven pandemic clones mentioned previously, six could be traced back to three ancestral MSSA types (i.e., CC5, CC8, and CC30; Fig. 194.2).[58] Thus, a few ancestral clones of MSSA took the lead and successfully colonized humans and animals before antibiotic resistance developed. Later acquisition of MGEs carrying drug-resistance or virulence genes helped further adaptation to new conditions (e.g., antibiotic use in hospitals), generating a new PFGE makeup on similar ancestral parents (see "Comparative Genomics and Evolution"). Moreover, genomics now shows that acquisition of antibiotic resistance genes is reversible and that the contemporary decrease in MRSA prevalence is associated with the loss of the methicillin-resistance determinants.[53]

Spa Typing and Double-Locus *Spa-ClfB* Typing

Spa typing and double-locus *spa-clfB* typing rely on PCR amplification of strain-specific regions within hypervariable segments of the *spa* (protein A) or *clfB* (clumping factor B) genes.[59] The variable regions are made of 24 nucleotide repeats in *spa* and serine-aspartate repeats in *clfB,* the length of which may vary from duplication or accidental loss of DNA material. Single PFGE or MLST types can evolve into different *spa* or *clfB* sublineages. Hence, combining these techniques generates unambiguous data sets that can be compared in multicenter studies.

Typing is critical in order to understand the *S. aureus* epidemiology. On the other hand, although a handful of founding ST types appear to be prevalent in MRSA[55] and PVL-positive strains,[60] no specific types could be attributed to disease-producing versus mere colonizing strains.[10]

PATHOGENESIS

Regulation and Virulence Determinants

S. aureus is extremely well equipped in surface factors and secreted proteins that mediate host colonization and disease (see Table 194.1).[1]

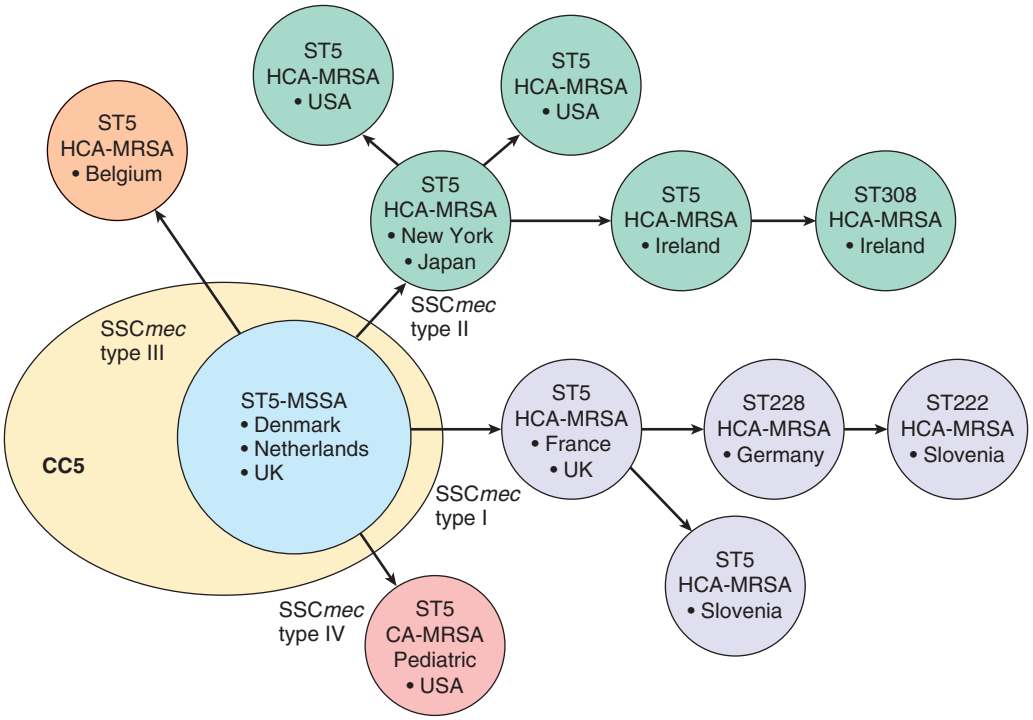

FIG. 194.2 Evolution of methicillin-susceptible *Staphylococcus aureus (MSSA)* into methicillin-resistant *S. aureus (MRSA)* as exemplified by sequence type 5 *(ST5).* ST5 belongs to clonal cluster 5 *(CC5),* which gathers *S. aureus* isolates sharing homologies in five of the seven genes (*arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) compared with method of multilocus sequence typing (MLST). Parental ST5 is an MSSA that has been isolated in several countries, including Denmark, the Netherlands, and the United Kingdom. It acquired various types of SCC*mec* at several independent occasions, probably from coagulase-negative staphylococci (CoNS) donor strains. After SCC*mec* acquisition, new MRSA clones followed their own geographic and genetic evolution, spreading either as HCA-MRSA (SCC*mec* I, II, or III) or CA-MRSA clones (SCC*mec* IV) and sometimes evolving into new ST types (e.g., ST222, ST228, and ST308). Three clonal clusters (CC5, CC8, and CC30) generated six of the seven major pandemic MRSA clones described over the past 3 decades. *(Modified from Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant* Staphylococcus aureus. Antimicrob Agents Chemother. *2003;47:3926–3934.)*

In addition to these features, *S. aureus* is equipped with regulatory systems that sense environmental conditions and respond by fine-tuning the expression of given metabolic and virulence determinants (for review, see Novick and Geisinger,[61] Pragman and Schlievert,[62] and Balasubramanian and colleagues[63]). Some aspects of this subtle adaptation machinery are described subsequently.

Regulation

At least three families of regulatory elements intertwine to adjust gene expression to specific environmental conditions: first, two-component regulatory systems, of which *agr* (for accessory gene regulator) is a paradigm; second, DNA-binding proteins, largely represented by the Sar (for staphylococcal accessory regulator) family of proteins; and third, small regulatory RNAs.

agr and Other Two-Component Regulatory Systems

The paradigm of two-component regulatory systems virulence gene regulation is *agr,* which is schematized in Fig. 194.3.[64] *agr* functions as a quorum sensing control that reacts to bacterial density, allowing the preferential expression of surface adhesins during the exponential phase of growth (low cell density) and switching to the expression of exoproteins during the postexponential and stationary growth phases (high cell density).[61,64,65] The switch is composed of two divergent operons (see Fig. 194.3). On the left hand, promoter P2 drives the transcription of a series of components that comprises (1) a transmembrane protein (AgrB); (2) an autoinducing peptide precursor (AgrD), which is processed and exported by membrane-spanning AgrB; (3) a transmembrane sensor (AgrC), which is the cognate receptor of the AgrD-derived autoinducing peptide; and (4) a transcription regulator (AgrA) that can be activated by AgrC. At low cell density (exponential growth phase), the P2 promoter is off and the operon is transcribed at a low level. As cell growth proceeds, the concentrations of both bacteria and extracellular autoinducing peptide increase in the milieu, thereby augmenting the chance of the autoinducing peptide to make contact with its cognate AgrC receptor. On contact, AgrC activates the response regulator AgrA, a process that may involve AgrA dephosphorylation.[61,64]

Activated AgrA is a DNA-binding protein that turns on the transcription from both promoter P2, generating a positive feedback on the system, and promoter P3, which drives the transcription of δ-hemolysin and of a peculiar effector called RNAIII. RNAIII has a reciprocal effect and activates the expression of several secreted proteins while downregulating the expression of surface-bound factors (see Table 194.1). RNAIII has a complex three-dimensional structure and a long half-life (up to 15 minutes). It regulates gene expression in several ways, including at the translational level by blocking the messenger RNA (mRNA) ribosome-binding site (RBS) of the target genes, or by prolonging the half-life of mRNA of downstream pleiotropic transcriptional regulators such as MgrA.[66]

The *S. aureus* chromosome encodes for up to 16 two-component regulatory systems involved in both metabolic environmental control and virulence gene regulation.[61,64,65] Important two-component regulatory systems regarding virulence genes include *saeR/S* (for *S. aureus* exoproteins),[67] *srrAB* (for staphylococcal respiratory response),[68] and *arlS* (for autolysis-related locus sensor).[69] *saeR/S* was identified with transposon mutation in a pleiotropic mutant defective in exoprotein synthesis other than that regulated by *agr* (e.g., coagulase and nuclease; see Table 194.1).[70] *saeR/S* acts independently of *agr* and responds to environmental stimuli such as high salt, low pH, glucose, and subinhibitory antibiotic concentrations. *srrAB* and *arlS* interfere with growth in microaerobic conditions and autolysis, respectively. *srrAB* represses the expression of TSST-1 and protein A in microaerobic conditions,[68] an observation that may be relevant for the pathogenesis of tampon-related

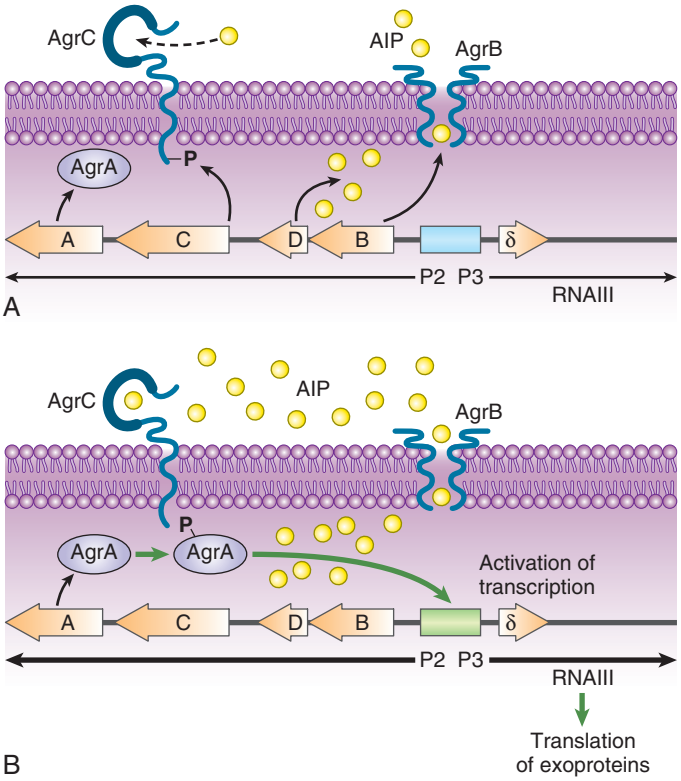

FIG. 194.3 Schematic representation of *Staphylococcus aureus* global regulatory system *agr* (accessory gene regulator). (A) System at rest. It consists of two divergent operons, transcribed from promoters P2 and P3. Promoter P2 encodes putative membrane protein AgrB, precursor of autoinducing peptide *(AIP)* AgrD, which is processed by AgrB; transmembrane receptor AgrC; and response regulator AgrA. Promoter P3 encodes δ-hemolysin and RNAIII. At low bacterial density, P2 and P3 are off and only a small amount of AIP is secreted because of promoter leakiness *(A).* As bacterial growth proceeds and bacterial density increases *(B),* chance of AIP encountering its cognate receptor AgrC increases. On contact, AgrC undergoes conformational change and phosphorylates (or dephosphorylates) response regulator AgrA. (B) Activated AgrA activates transcription from both P2 and P3, resulting in positive feedback. δ-Hemolysin is membrane-active protein toxic for eukaryotic cells. RNAIII is an intracellular regulator that acts in *trans* and regulates expression of many virulence genes, including numerous toxins (see Table 194.1). Although *agr* is pivotal in quorum-sensing regulation of gene expression, it is not the only regulator of pathogenic determinants in *S. aureus. sar, saeRS, rot,* and other systems may affect the expression of *agr* itself or affect virulence genes directly (e.g., *sar*) or both (see Table 194.1).

TSS (see later discussion).[5] Both *srrAB* and *arlS* interact reciprocally with *agr.*[62,65]

DNA-Binding Proteins

sar is an important locus that encodes the DNA-binding protein SarA, which positively controls *agr* (Fig. 194.4), and maybe also *sae* and *arlS.*[61,65] In addition, *sar* directly regulates adhesin genes (see Table 194.1). The *sarA* transcripts peak at the end of the logarithmic phase of growth, thus promoting *agr* expression. Moreover, *sarA* itself is transcribed downstream of three alternate promoters, which can themselves be regulated by as yet incompletely solved factors.

SarA is the prototype of a growing family of DNA-binding proteins that may drive a number of transcriptional activities, including the expression of housekeeping genes and phage-related genes. *sarA* homologues include *sarR, sarS, sarT, sarV, sarU, sarY, rot,* and *mgrA.*[62,65,71] *rot* stands for "repressor of toxins" and counters toxin expression by repressing *agr.*[72] Inactivation of *rot* partially restored the *agr* phenotype in *agr*-negative mutants, probably by alleviating a repressing effect on the downstream P3 cascade of the *agr.* This downstream cascade might be the target of several additional regulators that also affect the *agr* phenotype (see Fig. 194.4). *mgrA* stands for multiple gene regulator.[71]

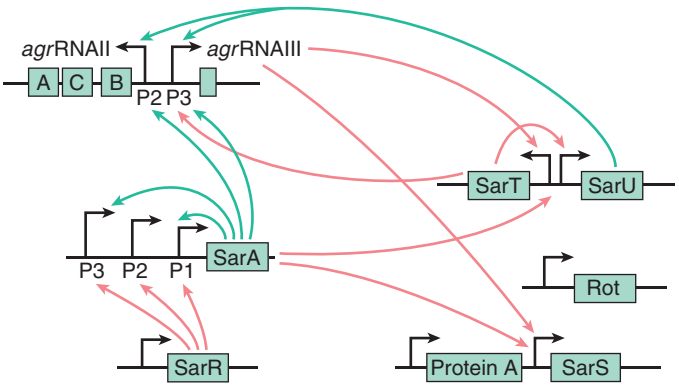

FIG. 194.4 Regulatory network of *agr* and Sar family of DNA-binding proteins. Intertwining of activation *(green arrows)* and repression *(red arrows)* underlines complexity of system. Gene expression is further modulated by additional factors, including alternative σ^B, *arlS, sae,* and *srrAB,* which can act on *agr* promoters or directly on specific genes. Gene promoters are denominated P1, P2, and P3 and represented by *black lines. (Modified from Pragman AA, Schlievert PM. Virulence regulation in* Staphylococcus aureus*: the need for in vivo analysis of virulence factor regulation.* FEMS Immunol Med Microbiol. *2004;42:147–154.)*

It controls the transcription of up to 355 genes (175 upregulated and 180 downregulated), including capsule, and protein A and α-hemolysin genes in an *agr*-dependent way.[66,71]

Sigma factors (σ) are another major mechanism of response to environmental stimuli. In bacteria, σ factors combine with and activate RNA polymerase to transcribe specific sets of genes. *S. aureus* contains one σ^A and two alternative σ^B and σ^C. Alternative σ^B is important for the microbial response to a variety of stresses, including temperature, energy depletion, and chemical stimuli.[73] It acts mostly via the global regulatory network and affects the expression of up to 251 genes (198 positively, 53 negatively),[74] but also has some direct effect by activating the expression of coagulase and fibronectin-binding proteins at the early growth phase, and downregulating certain secreted proteins in the stationary phase. Mutants overexpressing σ^B were more virulent in experimental endocarditis, probably by increasing the expression of surface adhesins.[75] Conversely σ^B defective mutants were less infective in a model of catheter-related systemic infection.[76]

Small RNAs and Endoribonuclease III

Small RNAs (sRNAs) are increasingly recognized as major players in regulation of gene expression. They act mainly at the translational level via antisense hybridization with mRNA, where they can alter mRNA stability, hide RBSs from ribosome recognition, or conversely reveal RBSs that are hidden in secondary mRNA structures by unfolding these very structures. Alternatively, sRNA can also bind regulatory DNA-binding proteins, thus sequestering them from their original gene regulatory function. A genome-wide analysis generated a "Staphylococcal Regulatory RNA Database" (SRD; http://srd.genouest.org/) and identified at least 550 potential regulatory sRNAs. The best functionally characterized of them are RNAIII, which orchestrates the *agr* response, and RNAI, which regulates the replication of multiresistance plasmid pSK41.

In symmetry, posttranscriptional expression is also modulated by direct RNA alteration via endoribonuclease III (RNase III). This RNA double-stranded endonuclease plays a critical role in RNA processing and decay. It has been shown to modulate posttranscriptional expression through various mechanisms, including turnover of transcribed and nontranscribed RNAs, and by maturating the 5′ untranslated region (5′UTR) of the mRNAs of the cold-shock protein *cpsA* and maybe the protein A *spa* genes, to increase their stability and translation.[77]

The regulatory network must be considered as a metabolic hub that integrates both external and internal information and responds in the most appropriate way. The observed phenotypes result from complex interplays among sometimes contradicting signals of sensors and transcriptional and posttranscriptional regulators, the understanding of which will require a systems biology approach.[78] Moreover,

experimentally interrupting one of these circuitries may cause compensation by others, thus introducing biases in the observed phenotype. In this complex system, *agr* appears to be a central switch toward which many other regulators converge (see Fig. 194.4).

Role in Pathogenesis

The intuitive *agr*-based model suggests that scattered growing bacteria produce primarily adhesins, promoting tissue colonization, whereas installed organisms that form dense populations switch to the production of hydrolytic enzymes and toxins for the purpose of feeding and escaping host defenses.[61,65] Accordingly, inactivation of the function of *agr* alone decreased pathogenicity in experimental models of tissue destruction (e.g., subcutaneous abscesses), where exoprotein production is likely to be important.[79] On the other hand, *agr* inactivation did not much influence the course of experimental endocarditis, where bacterial surface adhesins are critical for valve colonization.[80] Indeed, although *agr*-negative mutants are hampered in exoprotein production, they are still fully equipped with surface-bound colonizing determinants (see Table 194.1). In contrast, inactivation of *sar* decreased infectivity in experimental endocarditis[80] because in addition to its effect on *agr* expression (see Table 194.1 and Fig. 194.4), *sar* also acts directly on expression of surface-bound fibronectin-binding protein A (FnBPA), which promotes experimental endocarditis.[81,82]

In addition, in vivo gene expression revealed a further level of complexity.[62] For instance, although *sar* transcripts were detected in infected vegetations during experimental endocarditis, they were expressed from both P1 and P2 promoters, rather than only from the P1 promoter as observed in vitro.[83] Likewise, in vivo expression of several genes appeared dissociated from their control regulator as described in vitro. Although *agr* positively regulates TSST-1 in vitro (see Table 194.1), the toxin was still expressed by an *agr*-negative mutant in a rabbit model of TSS in vivo.[84] This may result from alternative regulation by other regulators that act either downstream of the *agr* locus or directly on the *tss* gene promoter. Eventually, *agr*-negative mutants can be recovered from clinical samples as in cystic fibrosis[85] and in carrier and bacteremic patients.[86] Such *agr*-negative clinical isolates, and *agr*-negative laboratory mutants, have increased surface adhesins and an increased ability to form biofilms, and are found in chronic infections such as osteomyelitis and device infections.[87]

Hence, the pathogenic implication of regulatory circuitries cannot be drawn merely from in vitro observations. In vivo experimentation reveals the plurality of *S. aureus* infection forms, which may be variously altered by novel antivirulence therapies. For instance, inhibition of the *agr* loop by action on the autoinducing peptide impedes acute tissue destruction[79] but might promote biofilm formation and chronic infection.[87,88]

Ecologic and Epidemiologic Implication of *agr*

Genetic and functional experiments revealed the existence of at least four *agr* groups in *S. aureus,* which were characterized by specific variations in all three AgrB, AgrD, and AgrC proteins (see Fig. 194.3).[89] Whereas the autoinducing peptide of a given *agr* group stimulated signaling in other strains sharing the same *agr* group, it either cross-inhibited (e.g., group I and group IV) or cross-activated (e.g., group I and group II) members of other groups. This suggests that certain antagonistic *agr* groups could be mutually exclusive with attempts to simultaneously colonize the same niche. However, studies regarding this hypothesis gave conflicting results. In particular, patients with cystic fibrosis colonized with *S. aureus* can successfully harbor organisms from two antagonistic *agr* groups.[90]

Although *agr* and other global regulators control the timely expression of pathogenic genes, they are not bona fide pathogenic factors themselves. The *agr* locus has homologues in numerous nonpathogenic staphylococci. A phylogenic study of nonpathogenic CoNS indicated that variations in *agr* genes followed parallel variations in species-specific rRNA genes.[91] In fact, *agr* groups diverged very early during the evolution of staphylococci (see "Comparative Genomics and Evolution") and represent a lineage marker of strains that evolve in distinct environments rather than a strategy to exclude potential competitors. Thus, global regulators were originally meant to control the expression of useful metabolic genes. How exogenous virulent genes, which were acquired later, succeeded in taking advantage of such systems remains a fascinating question of evolutionary genetics.

The Journey to Invasive Disease

Although *S. aureus* is an innocuous resident of the skin and mucosal flora in up to 30% of the human population,[20–22] healthy carriers are notoriously more prone than noncarriers to develop invasive *S. aureus* infections. This is exemplified by recurrent skin and wound or bloodstream infections (BSIs), which are due to the patient's own carriage strain in up to 80% of the cases.[21,24]

Colonization of the anterior nares is ideal for microbial dissemination. Outward dissemination is illustrated in Fig. 194.5. A few drops of fluorescein were instilled intranasally in a volunteer, followed by ultraviolet imaging. Two hours after instillation, fluorescein was all over the hands and clothes, ensuring both sneezing-induced and contact dissemination.

Inward dissemination by host invasion is an opportunity for ample bacterial proliferation. Host invasion is often considered a bacterial dead end, because invading microorganisms may be destroyed by the immune system. However, this only holds true if the immune system can eliminate the invading organisms, which is mostly not the case with *S. aureus* (see "Immune Evasion" later). Alternatively, invading microbes can kill the host, but then incur the risk of disappearing with the decaying corpse. Nevertheless, although this is expected in humans wherein dead bodies are eliminated through burial or cremation, it is different in the wild, where scavengers eat corpses, thus contributing to further dissemination. As a result, *S. aureus* has little evolutionary pressure to dampen its invasive lifestyle—which comes in addition to commensalism—whereas it has ample reasons to withstand host defenses, including resistance to antibiotics.

Mucosal and Skin Colonization

Persistent mucosal and skin colonization is critical. Factors involved in attachment to nasal epithelia involve teichoic acids,[92] which may attach



FIG. 194.5 Example of environmental spread of bacteria colonizing the nose. A few drops of fluorescein were instilled in the anterior nares of a volunteer, who was left to go about his routine activities and was photographed under ultraviolet light 2 hours later. Fluorescein is found all over face, hands, and clothes, exemplifying the appropriateness of colonizing the nose to ensure rapid microbial spread.

to lectin glycoproteins on the surface of mucosal cells, fibrinogen-binding protein B (clumping factor B or ClfB), serine-aspartate rich proteins C and D (SdrC and SdrD), SasG, and IsdA (Table 194.3).[93–95] ClfB, SdrC, SdrD, SasG, and IsdA are members of a family of *S. aureus* surface-bound proteins referred to as MSCRAMMs (for matrix surface components recognizing matrix molecules).[96] MSCRAMMs are covalently attached to the *S. aureus* peptidoglycan via the membrane-bound transpeptidase sortase A (SortA) (Fig. 194.6). SortA-attached proteins include at least 21 members,[97] some of them having functions different than host-matrix adherence (see Table 194.3). However, all to them are recognized by SortA at an LPXTG signature motif, cleaved by SortA between LPXT and G and covalently attached to the last G (glycine) residue of the peptidoglycan pentaglycine side chain (see Fig. 194.6).[98,99]

ClfB, SdrC, and SdrD are able to bind fibrinogen and keratin in vitro. SdrD was also shown to bind desmoglein 1, a desmosomal transmembrane protein that binds epidermal cells to keratin,[100] which is likely to facilitate *S. aureus* persistence in deeper layer of keratinized epithelia. The epithelial ligand of SasG is not known, but SasG is involved

TABLE 194.3 *Staphylococcus aureus* MSCRAMMs Belonging to Sortase-Mediated Cell Wall–Associated Proteins

GENE	PROTEIN	AA	SORTASE	MOTIF	LIGAND SPECIFICITY	POTENTIAL IMPLICATION IN DISEASE
Spa	Protein A	508	SrtA	LPETG	Antibody Fc fragment (IgG, IgM) von Willebrand factor, TNFR1, platelets	Experimental sepsis, experimental osteoarthritis
clfA	Clumping factor A	933	SrtA	LPDTG	Fibrinogen, platelets	Experimental endocarditis
clfB	Clumping factor B	913	SrtA	LPETG	Fibrinogen, cytokeratin 10, platelets	Colonization of nasal mucosa
cna	Collagen-binding protein	1183	SrtA	LPKTG	Collagen	Experimental osteomyelitis, septic arthritis
fnA	Fibronectin-binding protein A	1018	SrtA	LPETG	Fibronectin, fibrinogen, elastin Platelets	Experimental endocarditis Cell invasion, experimental mastitis
fnB	Fibronectin-binding protein B	914	SrtA	LPETG	Fibronectin, fibrinogen, elastin, platelets	Experimental mastitis
sdrC	Serine-aspartate repeat protein	947	SrtA	LPETG	Fibrinogen, cytokeratin of nasal epithelia	Nasal colonization
sdrD	Serine-aspartate repeat protein	1315	SrtA	LPETG	Fibrinogen, desmosomal desmoglein	Nasal, deep skin colonization, biofilm
sdrE	Serine-aspartate repeat protein	1166	SrtA	LPETG	Bridges fibrinogen and complement factor H on the *S. aureus* surface	Immune evasion
pls	Plasmin-sensitive protein	1637	SrtA	LPDTG	Cellular lipids, ganglioside M3; nasal epithelial cells	Colonization of nasal mucosa
sraP (or sasA)	Serine-rich adhesin for platelets	2261	SrtA	LPDTG	Platelets	Experimental endocarditis
IsdA	Iron-regulated surface determinant A	354	SrtA	LPKTG	Fibrinogen, fibronectin	Nasal colonization
(sasE)					Hemoglobin/transferrin	
IsdB	Iron-regulated surface determinant B	645	SrtA	LPQTG	Hemoglobin/hemin	Experimental bacteremia and renal abscesses
(sasJ)						
isdC	Iron-regulated surface determinant C	227	SrtB	NPQTN	Hemin	Experimental bacteremia and renal abscesses
isdH (or haR)	Iron-regulated surface determinant H	895	SrtA	LPKTG	Haptoglobin/hemoglobin complex	Nasal colonization
sasI	Putative *S. aureus* surface protein I				Undetermined	Associated with bovine gangrenous mastitis strains
sasB	*S. aureus* surface protein B	937	SrtA	LPDTG	Undetermined	—
sasC	*S. aureus* surface protein C	2186	SrtA	LPNTG	Intercellular adhesion	Involved in biofilm
sasD	*S. aureus* surface protein D	241	SrtA	LPAAG	Undetermined	Involved in biofilm
sasF	*S. aureus* surface protein F	637	SrtA	LPKAG	Undetermined	—
sasG	*S. aureus* surface protein G	1117	SrtA	LPKTG	Nasal epithelial cells	Associated to invasive disease
Sas (or adsA)	*S. aureus* surface protein H	308	SrtA	LPKTG	Cell wall associated adenosine synthase	Escape phagocyte-induced killing
sasK	*S. aureus* surface protein K	211	SrtA	LPKTG	Undetermined	—
fmtB	Formyl transferase B		SrtA	LPXTG	Cell wall synthesis, β-lactam resistance	Antibiotic resistance

AA, Protein length in amino acids; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *MSCRAMMs,* microbial surface components recognizing adhesive matrix molecules; *Srt,* sortase; *TNFR1,* tumor necrosis factor receptor 1.
Modified from Roche FM, Massey R, Peacock SJ, et al. Characterization of novel LPXTG-containing proteins of Staphylococcus aureus *identified from genome sequences.* Microbiology. *2003;149:643–654; Clarke S, Foster S. Surface adhesins of* Staphylococcus aureus. Adv Microb Physiol. *2006;51:187–224; and Dedent A, Marraffini L, Schneewind O. Staphylococcal sortases and surface proteins. In: Fischetti V, Novick RP, Ferretti J, et al, eds.* Gram-Positive Pathogens. *2nd ed. Washington, DC: ASM Press; 2006:486–495.*

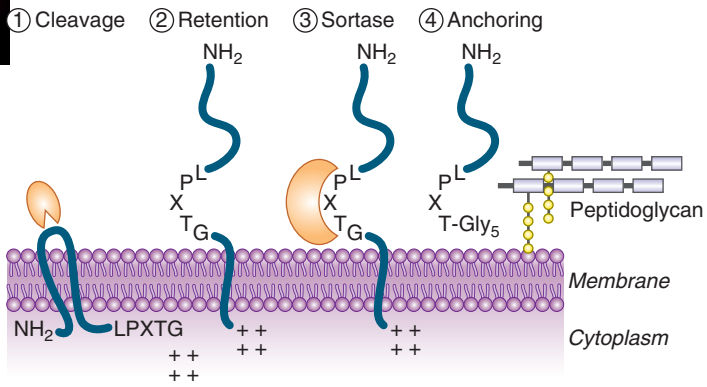

FIG. 194.6 Anchoring of gram-positive surface proteins to peptidoglycan through sortase-mediated processing of LPXTG consensus motif. During membrane translocation, amino-terminal leader sequence is clipped off. Protein is then transiently retained on cell surface through membrane-anchor domain, rich in positively charged amino acids at its intracellular carboxyl-terminal portion. LPXTG consensus region is then processed by sortase that clips between threonine and glycine (T-G) and transfers covalent bond to glycine acceptor in peptidoglycan meshwork. *(Modified from Fischetti VA, Pancholi V, Schneewind O. Conservation of a hexapeptide sequence in the anchor region of surface proteins from gram-positive cocci.* Mol Microbiol. *1990;4:1603–1605; Mazmanian SK, Ton-That H, Schneewind O. Sortase-catalysed anchoring of surface proteins to the cell wall of* Staphylococcus aureus. Mol Microbiol. *2001;40:1049–1057; and Bradshaw WJ et al. Molecular features of the sortase enzyme family.* FEBS J. *2015;282:2097–2114.)*

in biofilm formation, which is an ingredient of local persistence.[101] Likewise, the exact role of IsdA in nasal colonization is unclear. However, because IsdA is a heme-binding protein,[102] it might help acquire essential iron for the colonizing bacteria.[95] Finally, *S. aureus* can also survive in a dormant state inside nasal epithelial cells.[103] This is one reason, in addition to biofilm, that explains why it is difficult to eradicate chronic carriage, especially with antimicrobials that do not penetrate inside eukaryotes cells—for instance, β-lactams.

Whether the fibrinogen-binding capacity of ClfB, SdrC, and SdrD may facilitate further invasion in case of mucosal or skin breaches is not known. On the other hand, SasG (among few others) elicited an antibody response in patients with invasive *S. aureus* diseases,[97] a finding that supports its involvement in deep-seated infections.

Most interestingly, there is an as yet unexplained privileged liaison between *S. aureus* and chronic nasal carriers. In one study, *S. aureus* carrier and noncarrier volunteers had the nose disinfected and reinoculated with a mixture of four *S. aureus* strains, including the carriage strain in case of chronic carriers. Over a few days, noncarriers tended to eliminate all inoculated strains, whereas chronic carriers eliminated all foreign strains and reselected their own.[104]

In certain occurrences, mucosal or wound colonization with *S. aureus* may produce distant diseases such as SSSS or TSS. These issues are discussed in dedicated sections later.

Taken together, although the subtle relation between *S. aureus* and its host remains incompletely solved, it is critical to detect and eliminate *S. aureus* carriage in groups at risk of severe infection, such as patients undergoing operation,[26] dialysis patients,[105] and possibly patients with prosthetic heart valves, in whom the 1-year mortality of *S. aureus* valve infection is up to 50%.[106]

Host Invasion

Because *S. aureus* is nonmotile, invasion takes advantage of mucosal or skin breaches, where the microorganism engages with constituents of deeper tissue and blood compartments. The first encounters are constituents of microthrombi, which occur as a normal healing process of tissue breaches. *S. aureus* avidly binds to soluble fibrinogen and clotted fibrin via clumping factor A (ClfA)[107] and the fibrinogen-binding domain of fibronectin-binding protein A (FnBPA).[108] ClfA and FnBPA are SortA-LPXTG wall-associated MSCRAMMs (see Fig. 194.4 and Table 194.3), that serve at least two purposes. On one hand, they encourage *S. aureus* attachment at the place of preexisting lesions.[81] On the other hand, they also act as immune camouflage factors against complement-induced phagocytosis by surrounding the bacterium with a shield of soluble fibrinogen and fibronectin (see Table 194.3).[109]

Once attached to microthrombi, *S. aureus* may encourage further thrombus formation via the action of two secreted coagulases—coagulase (Coa) and von Willebrand–binding protein (vWbp). Coa and vWbp bind to prothrombin and induce a conformational change that converts it into active staphylothrombin.[110] Staphylothrombin is unique in that it polymerizes fibrinogen into fibrin and activates platelets even in blood anticoagulated via coumarin therapy, or exposed to heparin or calcium chelators.

Then, in order to avoid local trapping and hamper further spread, *S. aureus* needs to control local coagulation. To this end, coagulase is only transiently produced in the early exponential phase of growth. Moreover, *S. aureus* can escape clots by secreting staphylokinase (Sak), a protease that activates host plasminogen into active plasmin, which in turn disintegrates fibrin clots[111] and promotes extension of local infections.[112] Sak also cleaves complement opsonin C3b and preformed antibodies, contributing to the antiphagocytic properties of *S. aureus* (see "Immune Evasion" later). Sak is produced in both the early exponential and the late stationary growth phases. Its gene *(sak)* is located, together with SCIN (for staphylococcal complement inhibiting protein) and CHIPS (for chemotaxis inhibitory protein of *Staphylococcus*), on a so-called immune escape cluster (IEC) as part of a ϕSa3 β-hemolysin–converting prophage, which is present in >90% of human *S. aureus* isolates, but usually not in animal isolates.[113,114] Thus, the ϕSa3 prophage and its IEC cargo are believed to participate to the *S. aureus* specificity for human hosts.

Contribution of Coagulation

It was originally thought that *S. aureus* surface MSCRAMMs would mediate direct binding to ligands present in target organs, such as binding to collagen via collagen-binding protein (Cna) in osteoarthritis.[115,116] However, in the case of hematogenous dissemination, reaching the target organ requires prior *S. aureus* extravasation from the microcirculation. It was shown that ClfA and FnBPA are critical to colonize and invade damaged or inflamed endothelia, and this presumably occurred by direct attachment to the injured tissues.[81] However, in the bloodstream, ClfA and FnBPA become rapidly saturated with soluble fibrinogen and fibronectin, which interfere with direct binding to injured vessels.[109] Thus there is a missing link, which *S. aureus* circumvent by hijacking the coagulation system.[117]

While circulating *S. aureus* organisms become saturated with soluble fibrinogen and fibronectin, ClfA and FnBPA induce a fibrinogen conformational change that triggers its docking to the platelet GPIIb/IIIa receptor and activates platelets.[118] In addition, preexisting anti-ClfA or anti-FnBPA antibodies, if present, activate platelets by docking to the immunoglobulin G (IgG) platelet receptor FcγRIIa. These microaggregates are then conveyed through the blood to inflamed endothelia or to nascent platelet-fibrin clots present on injured tissues, to which activated platelets attach.

The convergence of these *S. aureus*–platelet microaggregates to inflamed endothelia is further strengthened by the second staphylocoagulase vWbp.[110] vWbp has the dual capacity to activate blood coagulation and to bind endothelial-attached von Willebrand factor (vWF). vWF is secreted as monomer by inflamed endothelia or activated platelets. vWF monomers attach to injured tissues and polymerize into discrete strings floating in the vascular flow, which bind activated platelets and microaggregates in a sheer-dependent manner.[119] *S. aureus*–secreted vWbp binds on one hand to the floating WWF strings, and on the other hand to *S. aureus*–attached ClfA,[120] thus also favoring the halt of circulating *S. aureus* onto inflamed endothelia.

This platelet-staphylothrombin scenario was validated in experimental models of endovascular colonization and endocarditis, in which prophylaxis with antiplatelet (acetylsalicylic acid and ticlopidine) or antithrombin (dabigatran) agents successfully prevented *S. aureus* endovascular infections.[121,122] In contrast, coumarin anticoagulation,

which relies on a different mechanism, did not prevent experimental endovascular infection.

Of note, platelet activation may be a double-edged sword in that platelet degranulation produces platelet-microbicidal peptides (PMP) that destabilize bacterial membranes and can kill bacteria.[123] However, *S. aureus* strains that produce successful endovascular infection are known to resist PMP-induced killing via plasma membrane modification.[124,125]

Eventually, inflamed endothelial cells also express α5β1 integrins, which bind soluble fibronectin and normally act as a landing runway for neutrophils. However, α5β1-bound fibronectin also promotes *S. aureus* attachment via FnBPA, which triggers active bacterial internalization by endothelial cells.[81] Local tissue destruction ensues, and more specific molecules can enter into action, including specific MSCRAMMs (e.g., Cna),[115,116] biofilm facilitating factors,[126] hemolysins, and immune evasion molecules.

Immune Evasion

Along with invasion and tissue colonization, *S. aureus* has to confront several layers of host defenses against which it applies an extremely sophisticated immune evasion armamentarium, which is briefly described in the following sections (Table 194.4) (for review, see Foster,[9] Kim and colleagues,[127] and Guerra and colleagues[128]).

Escaping Phagocytosis

The first line of anti–*S. aureus* host defense is phagocyte engulfment, primarily by neutrophils, either by direct recognition of pathogen-associated molecular patterns (PAMPs),[129] or via complement-mediated opsonization. Direct PAMP recognition is hampered by the production of polysaccharidic capsules (mostly type 5 or 8 in human *S. aureus* isolates), which are not recognized by professional phagocytes and physically block their access to underlying PAMPs, such as teichoic acids, lipoteichoic acids, peptidoglycan, and even C3b complement opsonins attached to these PAMP structures.

In addition, *S. aureus* uses several secreted and SortA-LPXTG anchored surface factors to counters phagocytosis. Secreted factors include the chemotaxis inhibitory protein CHIPS[111] and the extracellular adherence protein Eap (or Map).[130] CHIPS belongs to the ϕSa3 prophage IEC and blocks the neutrophil receptor for formyl-peptides, a universal signature of bacterial protein synthesis, and the C5a receptor for chemotaxis.[111] Eap binds intercellular adhesion molecule 1 (ICAM-1) and fibrinogen and vitronectin, and blocks leukocyte adhesion and neutrophil recruitment mediated by β2-integrin and urokinase receptors in vitro and in vivo.[130]

SortA-LPXTG anchored molecules include protein A (Spa), ClfA, and adenosine synthase A (AdsA, also called SasA) (see Tables 194.3 and 194.4). Spa blocks antibody-mediated phagocytosis by binding IgGs by their Fc fragments and exposing the Fab fragments instead, which are not recognized by complement.[127] ClfA interferes with phagocytosis in a fibrinogen-dependent manner (probably by bacterial shielding) and an as yet unclear fibrinogen-independent manner.[131] AdsA converts adenosine monophosphate into adenosine, a dual immuno-modulator compound that has proinflammatory antiinflammatory properties.[132] *S. aureus*–generated adenosine was shown to impede neutrophil-mediated bacterial clearance and to promote abscess formation in a mouse model of sepsis and kidney abscess.[133]

Luring Complement

If not directly triggered by PAMPs, phagocytosis may be promoted by complement-mediated opsonization. The lectin and the alternative complement pathways are mainly involved against *S. aureus*. The classical pathway, which requires prior antibodies, is largely hampered protein A (Spa) and Sak, as mentioned earlier,[9] and by secreted staphylococcal binder of immunoglobulin (Sbi).[134] Sbi is both secreted and loosely attached to the bacterial envelope. Its envelope-attached form binds immunoglobulin Fc fragments similarly to Spa, and its secreted form binds complement factor H and C3d, which accelerate the decay of preopsonin C3. Soluble Sbi-C3d-factor H complexes also bind the complement receptor CR2 of B lymphocytes, promoting their apoptosis and impeding antibody production.[127] In addition, direct complement-induced bacterial killing via the C8-C9 polymerization membrane attack complex (MAC) is not effective against gram-positive bacteria, because their plasma membrane is physically protected from MAC by the thick peptidoglycan cell wall (for review see Zipfel[135]).

The lectin and alternative complement pathways are triggered by PAMPs, which activate the lectin or alternative pathway-dependent convertases C4b2a and C3bBb. The convertases cleave C3 into C3a, which amplifies the chemoattractant loop, and C3b, which binds to staphylococcal teichoic acids and attracts phagocytes.[135] *S. aureus* counteracts complement-mediated opsonization by means of several mechanisms. First, as mentioned earlier, it can produce polysaccharidic capsules, hindering phagocyte access to teichoic acid–attached C3b. Second, it secretes Sak, which cleaves C3 and C3b. Third, it produces staphylococcal complement inhibitory protein SCIN, which binds to and inhibits the C4b2a and C3bBb convertases, thus blocking the production of C3a and C3b.[111] Like Sak, SCIN and CHIPS are located on the ϕSa3 prophage EIC.[113] They are expressed in the exponential phase of growth, whereas Sak is also expressed later in the late stationary growth phase.[111]

A fourth mechanism involves secreted extracellular fibrinogen binding protein (Efb), a dual adhesin capable of binding bacterial-attached C3b proximally, and soluble fibrinogen distally. As a result, Efb contributes an additional external fibrinogen shield, preventing the contact of neutrophils with C3b.[136]

Finally, a most astounding host-hijacking mechanism is conferred by SortA-LPXTG anchored SdrE. In addition to binding fibrinogen in vitro, SdrE binds to and attracts complement factor H on the *S. aureus* surface.[137] Factor H is a complement regulatory protein that normally binds to host cells and accelerates the decay of C3b in order to protect them from nonspecific assaults from self-host defenses. By attracting factor H on the *S. aureus* surface, SdrE usurps the complement host control system to its advantage.

Resisting Oxidative Burst

Activated neutrophils trigger oxidative burst and bacterial killing via NADPH oxidase and myeloperoxidase (MPO), or via nitric inducible oxide synthase (iNOS).[138] The cascade uses superoxide (O_2^-) to produce highly oxidative molecules such as H_2O_2 or hypochlorous acid (HOCl). Oxidation results in protein, lipid, and nucleic acid damage that can kill pathogens either extracellularly or inside phagolysosomes. Extracellular oxidative burst is exemplified by the neutrophil extracellular traps (NETs), which are constituted of neutrophil granules and chromatin proteins and contain up to 80% of neutrophil-released MPO.[139] *S. aureus* can disable these mechanisms by reducing enzymes such as catalase, which converts H_2O_2 to water and O_2, or direct rescue of oxidized molecules by means of several reducing agents listed in Table 194.4.

Resisting Antimicrobial Peptides

Insects and animals produce an array of antimicrobial peptides (AMPs) consisting most often of 20– to 100–amino acid pore-forming β-sheet structures.[140] Human produces various AMPs in skin and mucosal tissues, and large quantities that are stored in granules of neutrophils and platelets (see "Contribution of Coagulation" earlier). A hallmark of these AMPs is that they are positively charged and are attracted by the negatively charged wall teichoic acids (Fig. 194.7) and membrane phospholipids of the gram-positive bacterial envelope. *S. aureus* modulates its susceptibility to AMPs by modulating its surface charge, either by means of the D-alanine lipoteichoic acid ligase *(dlt)* operon, which decorates teichoic acids with alanine residues, or by means of a lysyltransferase that transfers lysine residues to membrane phospholipids.[141,142] Both mechanisms result in a more positively charged bacterial envelope and thus in AMP repulsion. In particular, successful endocarditis *S. aureus* strains were shown to be consistently resistant to platelet-secreted PMPs,[125] a property that may discourage the development of AMPs for therapeutic purposes.

Killing Leukocytes

S. aureus kills eukaryotic cells via secreted hemolysins, leukocidins, and phenol-soluble modulins (PSMs).[11,143,144] There are four types of hemolysins, referred to as α-hemolysin (Hla), β-hemolysin (Hlb), δ-hemolysin (Hld), and γ-hemolysin (Hlg).

TABLE 194.4 Main Immune Evasion Determinants

INTERFERENCE WITH	DETERMINANT	LOCATION	FREQUENCY IN *STAPHYLOCOCCUS AUREUS* ISOLATES	ACTION
Neutrophil chemotaxis, migration and phagocytosis	Polysaccharidic capsule (mainly types 5 and 8)	Core genome	20%–60%	Not recognized as PAMPs Steric blockage of neutrophil access to deeper cells wall structures including wall attached C3 and C3b
	CHIPS (chemotaxis inhibitory protein of *Staphylococcus*)	ϕSa3 IEC	>60%	Blocks neutrophil C5a receptor Blocks neutrophil formyl-peptide receptor
	Eap (or MAP) (extracellular adherence protein)	Core genome	>95%	Interferes with neutrophil migration and extravasation by blocking the docking of neutrophil LFA-1 to endothelial ICAM-1 (See anticomplement activity below)
	AdsA (or SasH) (adenosine synthase A)	Core genome	70%–80%	Converts adenosine monophosphate to adenosine Interferes with inflammation and phagocytosis
Complement	SdrE (serine-aspartate repeat protein)	Core genome	40%–60%	Antiopsonic Binds factor H on the *S. aureus* surface
	SCIN	ϕSa3 IEC	>60%	Antiopsonic Inhibits lectin and alternative complement pathways Binds to and inhibits the C3 convertases C4b2 and C3bBb, thus blocking the generation of the C3b opsonin
	Sak (staphylokinase)	ϕSa3 IEC	>60%	Antiopsonic Converts plasminogen into plasmin which cleaves fibrin (solubilizing clots), IgGs, and bacterial attached C3b (See anti-AMP activity below)
	Eap (or MAP) (extracellular adherence protein)	Core genome	>95%	Antiopsonic Inhibits classical and lectin complement pathways Binds to and inhibits C3 convertase C4b2 and further C3b-mediated opsonization
	Efb (extracellular fibrinogen-binding protein)	IEC2	60%–70%	Antiopsonic Binds C3 components and fibrinogen Binds to staphylococcal wall-attached C3b and attracts plasma fibrinogen over it, thus shielding it from recognition by neutrophils
	Ecb (Extracellular complement binding protein)	IEC2	>95%	Antiopsonic Blocks binding of the neutrophil complement receptor CR1 to bacterial-attached C3b, thus preventing phagocytosis
Oxidative burst	SodA and SodM (super oxide dismutases)	Core genome		Convert superoxide radicals to H_2O_2 and O_2 (using manganese as a co-factor)
	KatA (catalase)	Core genome		Converts H_2O_2 into H_2O and O_2
	AhpC and AhpF (alkyl hydroperoxide reductases)	Core genome		Convert H_2O_2 to H_2O and O_2 Convert alkyl hydroperoxides to alcohol and water
	bNOS (bacterial nitric oxide synthase)	Core genome		Scavenges HOCl (hypochlorous acid).
	Bacillithiol Coenzyme A Staphyloxanthin	Core genome		Protection by *S*-thiolation of oxidants Antioxidant mechanism unclear
Antimicrobial peptides (AMPs)	Dlt (D-alanine (lipo)teichoic acid ligase)	Core genome	>95%	Neutralizes negatively charged wall surface by alanine substitution of ribitol teichoic and lipoteichoic acids Decreases surface affinity for positively charged AMPs
	MprF (muropetide resistance factor)	Core genome	>95%	Idem by adding L-lysine residues to phosphatidylglycerol at the extracellular side of the plasma membrane
	Sak (staphylokinase)	ϕSa3 IEC		Proteolytic degradation of fibrin (via plasmin activation), C3 components, IgGs, and AMPs
	Aur (aureolysin)	Core genome		Proteolytic cleavage of cathelicidin AMPs
Leukocyte lysis	Hla (α-hemolysin)	Core genome		Forms heptamer barrels in the plasma membrane of target cells
	Hlg (γ-hemolysin)	Core genome	>95%	Bicomponent leukocidin Lyses both erythrocytes and leukocytes
	Luk E/D (leukocidin E/D)	Genomic island beta	30%–40%	Synergohymenotropic bicomponent leukodicin
	Luk F/M (leukocidin F/M)	ϕSa1		Synergohymenotropic bicomponent leukocidin
	Panton-Valentine leukocidin	ϕSa2	2%–4%	Synergohymenotropic bicomponent leukocidin
	Hld (delta-hemolysin)	Core genome	>95%	Idem phenol-soluble modulins (PSMs) below
	PSM alpha 1–4	Core genome	100%	Phagocyte lysis by membrane destabilization
	PSM beta-1 and 2 (phenol-soluble modulins)			Mechanism analogous to the delta-hemolysin mechanism of membrane damage

AMPs, Antimicrobial peptides; *ICAM-1,* intercellular adhesion molecule 1 (ligand of LFA-1); *IEC,* immune escape cluster; IgG, immunoglobulin G; *LFA-1,* lymphocyte function-associated antigen h1; *MAP,* major histocompatibility complex class II analogous protein; *PAMPs,* pathogen associated molecular patterns; *ϕSa1* to *ϕSa3,* *Stapylococcus* prophages 1 to 3; *SasH, Staphylococcus* surface protein H; *SCIN,* staphylococcal complement inhibitory protein.

Modified from McCarthy AJ and Lindsay JA. S. aureus *innate immune evasion is lineage-specific: a bioinfomatics study. Infect Genet Evol. 2013;19:7–14; Lindsay JA.* S. aureus *evolution: lineages and mobile genetic elements (MGEs). In: Lindsay J, ed.* Staphylococcus aureus *Molecular Genetics. Norfolk, UK: Casiter Academic Press; 2008:45–69; and Peakock SJ, et al. Virulent combinations of adhesin and toxin genes in natural populations of* Staphylococcus aureus. *Infect Immun. 2002;70:4987–4996.*

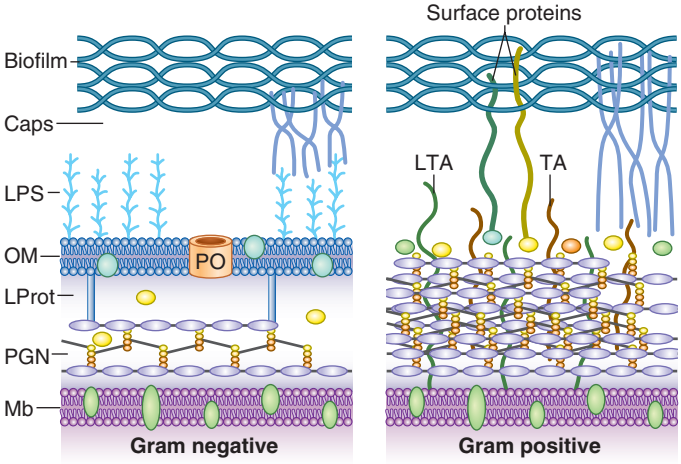

FIG. 194.7 Schematic representation of gram-negative *(left)* and gram-positive *(right)* bacterial envelopes. Gram-negative bacteria have very thin peptidoglycan *(PGN)* and an outer membrane *(OM),* made of lipopolysaccharide *(LPS),* which are not present in gram-positive bacteria. Peptidoglycan is very conserved and constituted of glycan chains made of *N*-acetylglucosamine and *N*-acetylmuramic acid disaccharide subunits, in which the *N*-acetylmuramate moiety is linked to highly conserved pentapeptide or tetrapeptide stems (L-alanine–D-isoglutamine–L-lysine–D-alanine–D-alanine). The chains of disaccharide peptide are cross-linked via peptide bridges between the penultimate D-alanine and the diamino acid L-lysine located in position 3 of a neighboring stem peptide. In *S. aureus,* the interpeptide bridge typically contains a polyglycine linking piece that comprises one to five glycine residues. The addition of glycines to the wall precursors is driven by the *femABC* and *fmhB* genes (see Fig. 194.13). Teichoic acids *(TA)* represent up to 50% of the dry weight of purified staphylococcal walls and are made of polyribitol-phosphate polymers cross-linked to *N*-acetylmuramic acid residues of the peptidoglycan and decorated with D-alanine and *N*-acetylglucosamine residues. Lipoteichoic acids *(LTA)* are the plasma membrane–bound counterparts of teichoic acids. They are made of polyglycerol phosphates chains and are linked to a diacylglycerol moiety that serves as a plasma membrane anchor. TA and LTA are not present in gram-negative bacteria. *Caps,* Capsule; *Lprot,* lipoprotein; *Mb,* plasma membrane; *PO,* porin. *(Modified from Gotz F, Bannerman T, Schleifer K-H. The genera* Staphylococcus *and* Macrococcus. *In: Balows A, Truper AG, Dworkin M, et al, eds.* The Prokaryotes. *Vol. 4. New York: Springer Science+Business Media; 2006:41–75.)*

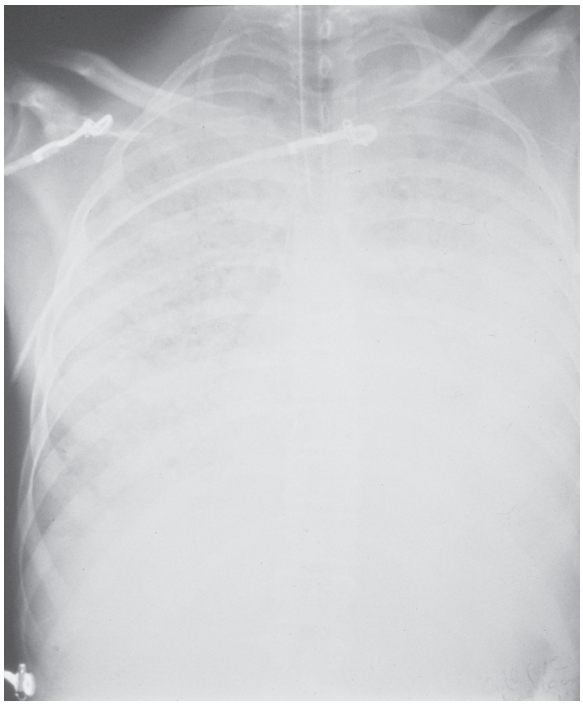

FIG. 194.8 Fulminant hemorrhagic pneumonia in 20-year-old patient infected with Panton-Valentine leukocidin–producing *Staphylococcus aureus*.

Hla and Hld are secreted in nontoxic soluble forms and multimerize on eukaryotic membranes to form lytic pores. α-Hemolysin (or α-toxin) is involved in a great variety disease.[145,146] It multimerizes as heptamers on phosphocholine-containing membranes, a process which depends on the presence of host cell transmembrane protein ADAM10, which reunifies both metalloprotease and disintigrin (integrin-binding) properties.[147] Moreover, α-hemolysin interferes with adherens junction proteins to induce cell killing, most notably plekstrin-homology domain-containing protein 7 (PLEKHA7). Indeed, PLEKHA7-deficient cells can readily recover from Hla cytotoxicity.[148] Hence, polymorphism in this determinant could influence individual susceptibility to infection.

Hld acts in a similar way and belongs to the same family as PSMs, which were described more recently.[144] There are four PSMα types (PSMα_{1-4}), consisting of approximately 20 amino acids, and two PSMβ types (PSMβ_{1-2}), consisting of approximately 40 amino acids, the genes for which are located on the staphylococcal chromosome. Hld is located upstream of *agr* RNAIII regulatory RNA (see "Regulation" section). The structure of Hld and PSMs consists of amphipathic α-helices, which confer several properties aside from membrane cell damage, including biofilm turnover and inflammatory responses.[149]

Hlb is distinctive because it is a sphingomyelinase that damages membranes by means of enzymatic alteration of their lipid content.

Hlg is also peculiar in that it is composed of two types of proteins called S and F, for slow and fast elution at chromatography. It promptly lyses white blood cells in addition to other cells and is sometimes referred to as leukocidin. It is encoded by two distinct operons, one that encodes a unique HlgA (S protein) and another that encodes for one S protein (HlgC) and one F protein (HlgB). S and F proteins must assemble to form membrane-perforating complexes. Therefore, this class of hemolysins is also referred to as synergohymenotropic toxins. Active α-Hemolysin exists in two bioactive forms: HlgA-HlgB and HlgA-HlgC.

Panton-valentine leukocidin. PVL is a peculiar homologue of Hlg, which was originally reported in 1932 by Panton and Valentine.[150,151] PVL is encoded by two genes, *lukS* and *lukF,* which can assemble either between themselves or with the components of Hlg, thus producing chimera complexes. Like the other hemolysins, PVL is regulated by *agr* (see Table 194.1). Unlike the other hemolysins, PVL is encoded by mobile phages, including ϕSLT, ϕSa2958, ϕSa2MW, ϕPVL, ϕ108PVL, ϕ7247PVL, ϕSa119, ϕTCH60, and ϕSa2USA, which can transfer PVL to other strains.[151,152] Also unlike the other hemolysins, the prevalence rate of PVL is usually low (≤2%) in MSSA and health care–associated MRSA (HCA-MRSA),[150] whereas it is present in almost 100% of isolates of the community-acquired MRSA (CA-MRSA) USA300 cluster, which is peculiarly prevalent in North America.[151,153]

PVL-producing *S. aureus* is associated with skin and soft tissue infection (SSTI) and severe hemorrhagic pneumonia in children and young adults.[154] In contrast, it is rarely responsible for other infections, such as osteomyelitis, septicemia, and endocarditis. The reason for clustering in young patients is unclear. The clustering could be linked to an age-related permissive milieu or permissive immunologic window. Nevertheless, the connection is important; a young adult with recurrent boils and pneumonia should receive particular attention because the mortality rate of hemorrhagic lung disease is high (Fig. 194.8).

Escaping Cell-Mediated Immunity

Among the first lines of skin and mucosal innate defenses are γ/δ T cells and antigen-presenting Langerhans cells. γ/δ T cells are not major histocompatibility complex (MHC) restricted and respond to epithelial stress and injury. They promote healing via the production of growth factors and attract neutrophils and T cells via the production of IL-17A,[155] which also upregulates the production of AMPs.[156] Stimulation of

neutrophils by IL-17A decreased the severity of experimental *S. aureus* SSTIs and facilitated *S. aureus* nasal eradication.[156,157] Thus, γ/δ T cells and the production of IL-17A comprise an important nonspecific first-line defense against invading microbes. Besides, Langerhans cells should phagocytose invading organisms and present surface antigens to boost humoral immunity, thus completing the continuum from innate to acquired host immunity.

However, *S. aureus* is well equipped to counter recognition by phagocytes and migration of neutrophil and lymphocytes, and impede cytokine-mediated cell recruitment and antibody production, including Eap (or Map), which interferes with lymphocyte migration,[130] Spa, Sbi,[134] and Sak (see "Escaping Phagocytosis" earlier). In addition, the most impressive interference of *S. aureus* with cell immunity is the ubiquitous production of SAgs, which trigger massive and nonspecific activation of the T-lymphocyte compartment, resulting in TSS.[2] One consequence of this T-cell distraction is immune paralysis and anergy. SAgs also aggravate atopic dermatitis and psoriasis by promoting local inflammation, serum suffusion, and access to nutrients.[158] SAgs, of which TSST-1 is a paradigm, are discussed in the "Superantigens" section later.

Producing Biofilm

Biofilm is an ultimate way to settle and escape host defenses. It consists in an extracellular polysaccharidic and proteinaceous meshwork that gathers bacterial communities within a mechanically cohesive scaffold. Biofilm-trapped bacteria cannot be physically phagocytized, a phenomenon referred to as frustrated phagocytosis,[159] and are dormant.[88] As result, they are phenotypically tolerant to antibiotic-induced killing.

Biofilm formation is a major therapeutic problem.[160] It was widely described in CoNS but is also formed by *S. aureus,* especially in the settings of colonization of catheters and biomaterials. Biofilm-producing staphylococci were associated with persistence and virulence in various experimental models, including *Caenorhabditis elegans* and mice with foreign-body infection.[161]

Biofilm formation evolves in three steps, starting with nonspecific adherence of individual cells to the materials, followed by growth and biofilm formation, and ending with detachment of surface bacteria. In CoNS, it is associated with the production of polysaccharide intercellular adhesion (PIA), which consists of β-1,6-glucosamine chains that are *N*-substituted with succinate residues.[162] PIA is synthesized by an operon called *ica* composed of a regulator *(icaR)* and biosynthetic *(icaADBC)* genes.[163]

An *ica* homologue has also been described in *S. aureus.* Its role in colonizing amorphous surfaces might be identical to that shown in CoNS. However, its role in disease initiation is debated.[164] In *S. aureus,* biofilm production relates to a large network of genes including surface-attached and secreted proteins in addition to complex regulatory circuitries.[126] For instance, although biofilm deep-seated bacteria must express adherence molecules, surface bacteria must be prone to detach in order to colonize additional organs. Detachment depends on, among other factors, *agr* expression, which represses expression of adhesins and promotes that of secreted factors including PSMs.[87] In turn, PSMs are involved in remodeling biofilm surfaces and creating channels to feed inner parts of the structure.[165] Thus, *ica* could be a relatively ancestral colonization mechanism that is still present in *S. aureus* but is surpassed by more effective means.

Taken together, the myriad immune evasion strategies collected by *S. aureus* highlight its remarkable adaptation to the animal world and make it a major challenge for host defense-mediated elimination. This explains the as yet unsuccessful attempts to develop an antibacterial vaccine against it, leaving only hope for antitoxin neutralizing vaccines, which will not eradicate the bacterium but might help reduce tissue destruction and disease symptoms.

Exfoliative Toxins and Staphylococcal Scalded Skin Syndrome

SSSS is a superficial skin disorder that varies from local blistering to impressive generalized scalding (Fig. 194.9). It was originally described by the German physician Baron Gotfried Ritter von Rittershain, who published a series of 297 cases in young children in 1878.[166] Hence, the syndrome is sometimes referred to as Ritter disease. SSSS clusters in neonates and infants younger than 1 year and rarely in adults. It is typically the result of mucosal or skin colonization (e.g., umbilical cord) with a toxigenic *S. aureus* strain that produces either ETA or ETB, encoded by the *eta* and *etb* genes, respectively. The toxin genes are located either on a phage *(eta)*[8] or on a plasmid *(etb).* Two additional isoforms of SSSS toxins (exfoliative toxins C [ETC] and D [ETD]) were isolated through pathologic observations in animals and with genome screen, but seem not to be involved in humans.[167,168]



FIG. 194.9 Staphylococcal scalded skin syndrome. Blisters are expression of toxin-related (exfoliative toxin A or B) distant disease and usually do not contain microorganisms.

A US study estimated the annual incidence of SSSS to be 8 cases per million US children, increasing to 45 cases per million in children younger than 2 years.[169] The crude inpatient mortality was low (0.33%) and similar to that in children without SSSS sharing comparable clinical conditions.[169] Similar figures were reported in France.[4] SSSS is often related to *S. aureus* infections or carriage in close contacts, and may evolve as small epidemics that result from clonally related strains, usually in nurseries. Nasal carriage of the organism may be found among the medical staff, and all caretakers should be screened for this possibility. The proportion of *S. aureus* carrying *eta* or *etb* in overall staphylococcal nasal carriers or clinical isolates is low (≤2% of isolates),[170] which explains the rarity of the disease and its clustering in favorable milieus.

The toxins act by a direct effect on the stratum granulosum of the epidermis. Mucosa are never involved. This consideration is important for differential diagnosis with more severe Lyell syndrome, which usually involves mucosa.[3] Lyell syndrome, or toxic epidermal necrolysis, results from cleavage below the dermoepidermal junction. It is associated with a reaction to more than 100 drugs and sometimes vaccination and has a high fatality rate.

Molecular Pathogenesis of Staphylococcal Scalded Skin Syndrome

The toxin is released by staphylococci locally, passes through the body, and localizes at the level of the stratum granulosum. The toxin is a glutamate-specific serine protease whose molecular target is desmoglein-1 (Dsg1). Dsg1 is a transmembrane desmosomal glycoprotein that is important to maintenance of interkeratinocyte adhesion.[171] The human skin harbors four Dsg isoforms (Dsg1 to Dsg4) that are localized in various layers of the epidermis, but only Dsg1 is present at the level of the stratum granulosum and is the target of SSSS toxins, which remove its amino-terminal extracellular domain.[172]

An incompletely solved question is why the disease primarily affects children and adults with peculiar skin diseases. One hypothesis is that the toxin targets Dsg1 in the vicinity of the cell membrane ganglioside (GM4), which is present only in the skin of young children or in adults with peculiar skin diseases. This could explain the clustering of SSSS in these particular populations. GM4-like gangliosides are present in the skin of suckling mice and can inhibit the effect of the toxin when coincubated before injection to susceptible animals.[3] The toxin has a serine protease activity, but only after it has reached the skin, which

suggests that a locally induced conformational change is needed for activity.

Clinical Aspects

The two forms of SSSS are a generalized form and a localized form. In the generalized form, the toxin spreads throughout the body and localizes at the level of the skin, where it produces generalized scalding (see Fig. 194.9). The skin easily detaches by mere rubbing (Nikolsky sign). The blister liquid is clear. Because scalding is the expression of a distantly secreted toxin, the responsible staphylococci are usually not found in the lesions. The disease is self-limited and wanes within 4 to 5 days, which probably parallels the appearance of specific antitoxin immunoglobulins. Indeed, in addition to age-related expression of GM4 or other specific factors in the skin, the presence of antitoxin antibodies in older children and adults also explains the restriction of SSSS to the younger age groups.

The localized form of SSSS is sometimes referred to as bullous impetigo (Fig. 194.10). It results from the local spread of the toxin around a colonized wound in individuals who already bear some immunity against the toxin, as is the case in neonates still benefiting from passive maternal immunity (often around the umbilicus), or in older individuals who are already immunized. The presence of antibodies hinders distant dissemination of the toxin but not local spread around the colonized area. Unlike the generalized form, scalding is localized and the blister liquid often contains bacteria and sometimes white blood cells.

Patients may have general symptoms that include fever and lethargy, especially in the generalized form. Treatment includes general measures such as antiseptic wound dressing and fluid support, specific antibiotic therapy to eradicate the causative agent, and screening and decontamination of caretakers, especially in nurseries. If appropriately handled, the prognosis of SSSS in children is usually good and, as mentioned, the mortality rate far less than 5%.[169] In contrast, the mortality rate can be very high in adults (>50%) and is usually associated with an underlying condition.

As mentioned, the differential diagnosis with Lyell syndrome (toxic epidermal necrolysis) is critical because the etiology, treatment, and prognosis of the diseases are different. In doubtful cases, skin biopsy is useful to provide the definitive answer.

Superantigens

TSST-1 and staphylococcal enterotoxins (SEs) are the paradigm of a large family of pyrogenic exotoxins called *superantigens*.[2,5,173] SAgs are proteins that do not activate the immune system by means of normal contact between antigen-presenting cells and T lymphocytes. Normally, antigens are taken up by antigen-presenting cells, hydrolyzed, and presented as restricted peptides to cognate T lymphocytes. The peptides are expressed within a groove on the MHC class II receptor on the surface of the antigen-presenting cell. Cognate T cells recognize the peptide–MHC class II complex by specific contacts with the five variable domains of the α and β chain of the T-cell receptor (Vβ, Dβ, Jβ, Vα, Jα).

SAgs can bypass this highly specific interaction. They attach to an external portion of the Vβ domain from large quantities of lymphocytes and directly wedge them to the MHC class II receptors of antigen-presenting cells. This nonspecific contact activates up to 20% of the total pool of T cells, instead of approximately 1 per 10,000 during normal antigen presentation. The consequence is a massive burst in cytokine release, which drives an overwhelming inflammatory response that results in endotoxin-like shock, including endothelial leakage, hemodynamic shock, multiorgan failure, and possibly death.

S. aureus can produce a large number of SAgs. Aside from TSST-1, it can produce at least 15 different enterotoxins (SEs A, B, C_n, D, E, G, H, I, J, K, L, M, N, O), which by definition are emetic when administered to rodents. However, the nomenclature has become more complicated with the discovery of enterotoxin homologues that did not have emetic properties, and which are now called SEl, for "staphylococcal enterotoxin-like," followed by specific letterings.[173] Moreover, additional screens have revealed a family of at least 14 proteins based on homologies in the conserved C- and N-terminal domains of SAgs.[174] These proteins do not bind MHC class II molecules but can variously interact with immunoglobulins and complement. They are referred to as SSL for "staphylococcal superantigen-like" and tend to cluster together on staphylococcal pathogenicity islands (discussed in the "Genomics and Mobile Genetic Elements" section later).

Although quite some variation exists in the primary structure of many SAgs, they all share a common architecture, as shown with crystallography. They consist of A and B globular domains, which are made of β-sheet barrels and α-helices and rejoined by a discrete linking piece. In TSST-1, the region binding to the Vβ chain of the T-cell receptor has been mapped at the A-B hinge region.

A genealogy of SAgs was built on the base of their sequence homologies. The SAgs studied were segregated into five groups.[5] Group I was represented only by TSST-1. Group III contained only staphylococcal SAgs (SEs H, I, K, L, and P), and group IV contained only streptococcal SAgs (SPs E, C, G, and SME Z). On the other hand, groups II and V contained both staphylococcal and streptococcal SAgs. Group II contained staphylococcal SEs B, C, and G and streptococcal SSA and SPE A, and group V contained staphylococcal SEs I, K, L, and P and streptococcal SPs E and H. This underlines the likelihood of horizontal gene transfer between these two genera, a fact that is becoming increasingly apparent with genome comparisons.[2]

Toxic Shock Syndrome

TSS has been sporadically reported as staphylococcal scarlet fever since 1927.[175] Interest in TSS dramatically increased in the early 1980s, when a number of staphylococcal TSS cases occurred in young women who used high-absorbency tampons during menses.[176] The disease was associated with a toxin called TSST-1 that was secreted locally by toxigenic strains. TSST-1 and other SAgs can cross the mucosal membrane by several means. At the level of mechanical barriers, tight junctions are not uniformly present on mucosal surfaces, and staphylococcal Hla may help further disrupt their surface. At the level of SAgs, a conserved dodecapeptide (YNKKKATVQELD) was shown to mediate transcytosis of the toxin to deeper mucosal layers, promoting contact with immune cells and triggering inflammation—a phenomenon referred to as outside-in signaling mechanism.[5,177] There are two clinical forms of TSS: menstrual TSS and nonmenstrual TSS.

Menstrual Toxic Shock Syndrome

Menstrual TSS starts within 2 days of the beginning or the end of menses and is primarily associated with the use of high-absorbency tampons. Clinical signs include high fever, capillary leak syndrome with hypotension and hypoalbuminemia, generalized nonpitting edema, and a morbilliform rash, followed by desquamation after a few days. The toxin is produced locally, and blood culture results are typically negative. The organisms responsible were represented by a single clone in most reported cases.

The disease proceeds by SAg-induced hyperactivation of the immune system (see previous discussion). TSST-1 production is regulated by



FIG. 194.10 Localized staphylococcal scalded skin syndrome, also called bullous impetigo. Disease is caused by local production of exfoliative toxins, and bacteria may be found in blister liquid.

agr (see Table 194.1). However, its expression requires specific conditions that include (1) an elevated protein level; (2) a relatively neutral pH (6.5–8); (3) an elevated pCO_2; and (4) an elevated pO_2.[68] All four conditions are met when menstruation is combined with the use of high-absorbency tampons. The high protein concentration and neutral pH are provided by blood proteins and their buffering capacity. The high PCO_2 is ensured by the higher than atmospheric CO_2 content of venous blood. Eventually, the high concentration in O_2 is introduced into the vaginal anaerobic flora by the high-absorbency tampon. Thus, the O_2 brought in by the tampon might be the trigger that modifies an otherwise equilibrated ecosystem and stimulates the production of TSST-1 by colonizing staphylococci.

TSST-1–producing *S. aureus* may be found in up to 20% of isolates from both carrier and clinical specimens, and higher in MSSA than in MRSA.[27,170] The fact that TSST-1 expression has special requirements may partially explain the comparatively low prevalence rate of the disease (approximately 1–3 cases per 100,000 patient-years).[2]

Nonmenstrual Toxic Shock Syndrome

Nonmenstrual TSS has attracted less attention than menstrual TSS, yet it can occur in any patient. In addition to TSST-1, nonmenstrual TSS can be the result of enterotoxins SEB and SEC, which are *agr* regulated (see Table 194.1). Responsible organisms may colonize virtually any site of the body, including surgical wounds (surgical TSS), lung (influenza-associated TSS), mucosa or skin (recalcitrant desquamative syndrome in patients with acquired immunodeficiency syndrome [AIDS]), contraceptive diaphragms, and dialysis catheters in patients undergoing chronic peritoneal dialysis. The development of general symptoms with high fever and cutaneous rash should suggest the possibility of nonmenstrual TSS in such patients.

A special feature of wound colonization is that the affected tissues often do not appear inflamed. This is believed to result from the toxin itself, which is able to prevent the influx of professional macrophages.

Predisposing Factors

In addition to the use of high-absorbency tampons or colonization with a toxigenic strain, most patients who are TSS susceptible also lack specific antibodies that block the responsible SAg. In one study, antibody titers considered protective against TSST-1 (≥1:100) were detected in 30% of 2-year-old children and in more than 90% of women and men 25 years of age. However, low or negative titers of anti–TSST-1 antibodies (<5) were found in acute-phase sera from 90.5% of patients with menstrual TSS, and less than 50% had positive titers of anti–TSST-1 antibody that developed during convalescence.[2,178] Hence, some patients remain susceptible to recurrent TSS.

An interesting feature of SAgs is that they primarily trigger a CD_4^+ T-cell response, which privileges a helper T-cell type 1 (Th1) cytokine release response without a significant type 2 (Th2) response. A consequence of the dominant Th1 response is a decreased antibody expression, which could explain the relative lack of antibody response in patients with TSS. An additional explanation for the anergy could be SAg-induced apoptosis of responsive T cells, which could account for the prolonged anergy toward the deleterious toxin.

Diagnosis

The diagnosis of TSS is based on a constellation of clinical and laboratory signs as proposed by the Centers for Disease Control and Prevention.[179] Table 194.5 also proposes additional laboratory features, such as isolation of a toxin-producing organism to broaden the diagnostic tools.[180] The criteria of streptococcal TSS, from toxigenic *S. pyogenes* isolates, are presented for comparison. Although both syndromes are the results of similar kinds of SAgs, they differ in two important aspects. First, in contrast to staphylococcal TSS, streptococcal TSS is almost always associated with the presence of streptococci in deep-seated infections, such as erysipelas or necrotizing fasciitis, which has been referred to as flesh-eating disease. Second, mortality rates are very different in staphylococcal and streptococcal TSS. Mortality rates of menstrual and nonmenstrual (in children) staphylococcal TSS were reportedly <1%.[181,182] In contrast, mortality of streptococcal TSS in children was 28%[182] and up to 45% in adults,[183] especially in cases of necrotizing fasciitis, which necessitates aggressive treatment with generous surgical débridement of infected tissues, and sometimes amputation.

TABLE 194.5 Diagnostic Criteria for Staphylococcal and Streptococcal Toxic Shock Syndrome

STAPHYLOCOCCAL TOXIC SHOCK SYNDROME[a]	STREPTOCOCCAL TOXIC SHOCK SYNDROME
Fever	Isolation of group A streptococci from:
Hypotension	Sterile site for definite case
Diffuse macular rash with subsequent desquamation	Nonsterile site for probable case
Three of following organ systems involved:	Hypotension
Liver	Two of the following symptoms:
Blood	Renal dysfunction
Renal	Liver involvement
Mucous membranes	Erythematous macular rash
Gastrointestinal	Coagulopathy
Muscular	Soft tissue necrosis
Central nervous system	Adult respiratory distress syndrome
Negative serologic studies for measles, leptospirosis, and Rocky Mountain spotted fever and negative blood or cerebrospinal fluid cultures for organisms other than *Staphylococcus aureus*	

[a]Proposed revision of diagnostic criteria for staphylococcal toxic shock syndrome (TSS) includes (1) isolation of *S. aureus* from mucosal or normally sterile site; (2) production of TSS-associated superantigen by isolate; (3) lack of antibody to implicated toxin at time of acute illness; and (4) development of antibody to toxin during convalescence.

Modified from McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol. *2001;55:77–104.*

Therapy and Prevention

Treatment of staphylococcal TSS consists of elimination of the causative agent with antibiotic treatment and appropriate drainage of affected tissues if necessary. Antibiotic regimens should include active drugs such as β-lactams or vancomycin (in case of MRSA) plus protein inhibitors such as clindamycin or linezolid, which block the production of toxins.[184,185] Supportive care that includes intravenous fluid and vasopressors might be necessary. The immunologic gap that allows the toxin to be active in susceptible patients suggests that passive immunotherapy such as intravenous immune globulin (IVIG) could be effective. However, the success of IVIG therapy has been disputed in several recent analyses.[186,187] Because the mortality of menstrual staphylococcal TSS is low, immunotherapy should be considered only for life-threatening cases of streptococcal TSS.

Prevention is aimed at avoiding the use of hyperabsorbent tampons and preventing staphylococcal colonization of wounds and mucosa. In the case of nasal carriage, this is achieved with topical application of antibacterial agents such as mupirocin. In the case of extranasal colonization, additional complete body washing with antiseptics such as chlorhexidine is recommended for at least 1 week (see Table 194.8 later). Control cultures should be taken thereafter.

Active immunization with a TSST-1 vaccine could be a potential alternative. A phase I trial with recombinant TSST-1 demonstrated good tolerance and immunogenicity.[188] Further evaluation is awaited.

Enterotoxins and Food Poisoning

S. aureus harbors up to 15 enterotoxins, which are defined as SAgs able to produce gastrointestinal symptoms that include vomiting and diarrhea in primate models.[2,5] Although many of these toxins have potential SAg activity, not all of them have a clear role in human disease. As mentioned, SEB and SEC are associated with nonmenstrual TSS. Likewise, SEA is by far the most frequent culprit of food poisoning, whereas SED, SEB, and SEC are less frequently involved.[189]

Foodborne disease is a major public health problem that may account for 6 to 8 million cases per year in the United States. *S. aureus* food poisoning follows ingestion of toxins that have been released into

contaminated food stocks or beverages. The toxins are heat stable and thus are not denatured by cooking. The disease typically starts 2 to 6 hours after ingestion with general malaise, nausea, vomiting, abdominal pain, and diarrhea. No fever occurs, but the symptoms may be distressing enough to justify hospital consultation in approximately 10% of patients. The symptoms spontaneously resolve within 6 to 12 hours, and the prognosis is excellent, except in the case of severe dehydration in young children and elderly patients.

Although the mode of action of SAgs at the level of T lymphocytes is known, their mechanism at the surface of the intestinal mucosa is less clear. They might share transcytosis mechanisms with TSST-1.[5,177]

Other Implications of Superantigens

Although SAgs can result in dramatic subversion of the host immune system, they are not ultimate bacterial weapons because they affect only a restricted subgroup of patients who do not mount an appropriate blocking antibody response. Many of these staphylococcal genes are physically contiguous, which suggests that they may have arisen by duplication, maybe for the purpose of diversity.[8,190] The versatility of SAgs is further supported by the discovery that one of them (i.e., SHE) develops its SAg activity by binding to the Vα rather than the Vβ domains of the T-cell receptor, thus expanding different sets of T-cell lineages than classic SAgs.[191]

The clinical relevance of this multiplicity of toxins is not entirely understood. Toxin genes are dispensable elements that are not needed for growth in rich media and in the absence of competition. Some SAgs (e.g., TSST-1 and SEA, SEB, and SEC) obviously provide a way for the bacterium to escape host immunity. For instance, SAgs have been involved in the etiology of psoriasis and atopic dermatitis,[192] where SAg-induced skin modification could promote bacterial survival. On the other hand, the survival advantage of provoking allergic diseases including rhinitis and asthma[193,194] is less intuitive, except maybe to promote airborne dispersal (see Fig. 194.5). Altogether, the multiplicity of SAgs could enable *S. aureus* to interfere with the immune response of various animal species, thus broadening its host spectrum.

Genomics and Mobile Genetic Elements

At the time of writing, several thousands of *S. aureus* genome assemblies and annotation reports are available in public databases (www.ncbi.nlm.nih.gov/genome/genomes/154). *S. aureus* genomes are circular and contain approximately 2.8 million base pairs that represent up to 2700 coding sequences, plus structural and regulatory RNAs. They are divided into (1) a core genome, which contains mostly housekeeping genes, is quite conserved along various staphylococcal species, and accounts for about 80% of the whole DNA, and (2) an accessory genome, which carries mobile DNA (MGEs), contains most *S. aureus* pathogenic and drug-resistance features, and may vary among different species and strains.[7,8,10,16–18] In addition, certain elements of the core genome can vary according to lineages—for example, by the presence or absence of core genes that are specific of given clades. Therefore the core genome is sometimes subdivided into the core-stable and core-variable genome.[7,10]

Comparative Genomics and Evolution

Genome evolution is driven by random point mutations that lead to single nucleotide polymorphism (SNP), larger variations in core genes (e.g., deletions or duplication of repeat regions) that may differ between lineages, and MGEs that include insertion sequences, transposons, viruses, and pathogenicity and genomic islands.[7,8,10,16–18]

Beyond academic interest, understanding the evolution of *S. aureus* may help understand the fundamentals of successful clones and eventually help design strategies to block their spread. Based on 7- and 14-gene MLST analyses,[195,196] an evolutionary scenario was proposed in which a common ancestor of *Staphylococcus* spp. first segregated into non–*S. aureus* and *S. aureus* species (Fig. 194.11). The *S. aureus* branch acquired the genomic islands νSaα and νSaβ (see later), which are absent from other staphylococci and encode for type I restriction modification systems, and further evolved into two subbranches that gave rise to different STs and CCs. Although still discussed,[196,197] it seems that the global regulator *agr* groups I to IV differentiated early in these two

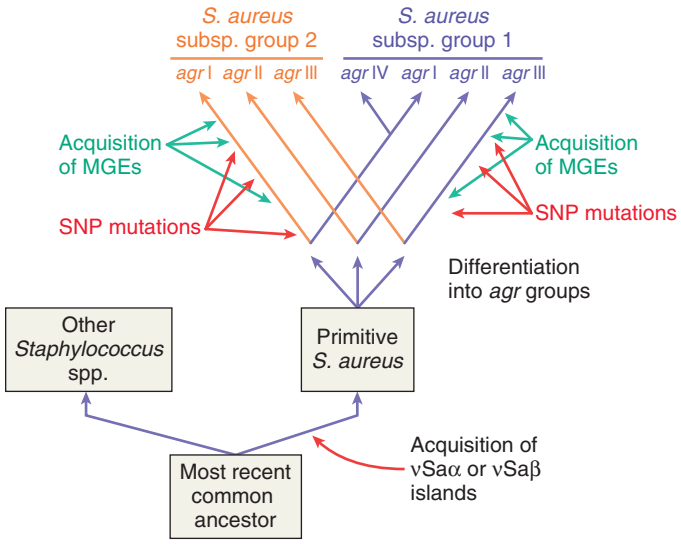

FIG. 194.11 Molecular evolution of *Staphylococcus* spp. into species and subspecies. *Staphylococcus* spp. evolved from a common ancestor into non–*S. aureus* and *S. aureus* species. The *S. aureus* branch distinguishes itself by the early acquisition of the νSaα or νSaβ genomic islands, which carry the genes for type I restriction modification systems. *agr*-interference groups differentiated shortly after, primarily by mutations. However, the *agr* two-component regulatory system is likely to be very old, because it is also present in non–*S. aureus* species. Sequence types (STs) and clonal clusters (CCs) evolved later, mostly via mutations, and determined two *S. aureus* subspecies (subsp. 1 and subsp. 2). Accordingly, the two subspecies contain different CCs, such as notoriously successful CC30 in subsp. 1 and CC5 in subsp. 2. On the other hand, they may contain similar *agr* interference groups, because these interference groups differentiated earlier, with the exception of group IV. *(Modified from Robinson DA, Monk AB, Cooper JE, et al. Evolutionary genetics of the accessory gene regulator* (agr) *locus in* Staphylococcus aureus. J Bacteriol. *2005;187:8312–8321; and Feng Y, Chen CJ, Su LH, et al. Evolution and pathogenesis of* Staphylococcus aureus*: lessons learned from genotyping and comparative genomics.* FEMS Microbiol Rev. *2008;32:23–37.)*

branches, which explains why the four *agr* groups may be found in different downstream CCs (see Fig. 194.11). Eventually, MGEs were acquired later and are dispersed in almost any of the STs or CCs. Yet, a few exceptions to this rule exist. Indeed, the TSST-1 gene and the PVL gene locus are classically associated with *agr* group III, the exfoliatin genes with *agr* group IV, and the vancomycin intermediate-resistance phenotypes with *agr* groups I or II.[7] Whether this is due to peculiarly favorable *agr*-related genetic backgrounds or to favorable contemporary conditions for the extension of specific clones is unclear. Moreover, a functional expression of *agr* is dispensable in certain circumstances, as for instance in biofilms.[87,88,165] Indeed, the association of clinical outcome with *agr* dysfunction and SCC*mec* type was observed in a study from South Korea, in which MRSA bacteremia-associated mortality was highest with types II/III, in which *agr* dysfunction is significantly increased.[198]

Using whole-genome sequencing, McAdam and colleagues[199] traced back the evolution of notoriously virulent CC30 to over 100 years ago. In the late 1800s, CC30 first segregated into phage type 80/81 and Southwest Pacific clades, which encoded PVL and produced severe community- and hospital-acquired infections due to penicillin-resistant (but methicillin-susceptible) strains in the mid 1900s. In the mid 1950s, a third hospital-related clade emerged (EMRSA-16), which was devoid of PVL but had acquired a new methicillin-resistance MGE named staphylococcal cassette chromosome *mec* (SCC*mec;* see later discussion). All three clades gave downstream variants related to further SNPs or MGE acquisitions. Moreover, the CC30 evolution demonstrates acquisition, loss, and reacquisition of PVL and other MGEs, underlying the virulence plasticity of this particular organism. It will be important to understand whether the success of CC30 is due to a peculiar ability to capture useful

MGEs or rather to contemporary environmental conditions that favored clonal expansion (e.g., in the hospital), or perhaps both.

Staphylococcus aureus Cross-Species Jump Between Humans and Animals

S. aureus is also an animal pathogen that raises particular concerns in livestock and cattle. Human and animal *S. aureus* strains tend to segregate in different lineages.[200] However, cross-species jumps exist and need to be considered. Companion animals and veterinary providers have been shown to share strains.[201] Two outbreaks have been described in Israel involving horses and veterinary personnel.[202,203]

Another example is the swine-related MRSA CC398, which appeared to colonize swine husbandries since the early 2000s.[204] Price and colleagues[205] proposed that CC398 was first transferred from human to swine. Indeed, CC398 is also present in humans and had been devoid of the SCC*mec* cassette (and thus was methicillin susceptible).[206] The swine CC398 has lost the ϕSa3 β-hemolysin–disrupting prophage, which is present in the human CC398. As mentioned, in human strains prophage ϕSa3 disrupts the β-hemolysin gene and simultaneously imports virulence factors, including the IEC carrying Sak, CHIPS, and SCIN (discussed under "Immune Evasion" earlier).[207] In swine CC398, the loss of prophage ϕSa3 restores the β-hemolysin gene, which may be important for skin or mucosal colonization in animals.[114,208] The swine CC398 also acquired a new SCC*mec* cassette that may carry useful determinants for survival in swine husbandries, including antibiotic use. Thus, CC398 was first a human MSSA. It was transferred to swine with the parallel loss of ϕSa3 and acquisition of a new SCC*mec*. The loss of prophage ϕSa3 seems critical in this evolution. This raises the question as to whether reacquisition of prophage ϕSa3 by swine CC398 could promote its reestablishment in humans, carrying along a new SCC*mec* cassette. Indeed, cases of invasive human infection with swine-CC398 MRSA are increasingly reported.[209] Likewise, a linear increase of 66% of human CC398 MRSA cases occurred in Denmark from 2004 to 2011; one-third of these patients reported no livestock exposure.[210] Most important, an epidemiologic evaluation in the Republic of Ireland has revealed an elaborate pattern of cases mixing human and animal disease, wherein a few human CC398 MRSAs did not carry the ϕSa3 prophage IEC cluster, thus resembling typical animal strains, whereas some animal CC398 MRSAs did carry the ϕSa3 prophage IEC cluster, thus resembling human strains.[211] In this line, a large study in Iowa found higher rates of *S. aureus* carriage in swine workers, with more than a third carrying livestock-associated strains that did cause infection in humans.[212] This strongly supports the likelihood constant of interspecies passage.

Similar scenarios were described with the ST5 *S. aureus* strain that invades poultry[213] and with the notoriously virulent human CC8 *S. aureus* strain that infects cows.[114,214] As in swine CC398, the bovine version of CC8 first lost the ϕSa3 prophage and then acquired a new composite SCC element, which is as yet devoid of the methicillin resistance *mecA* gene but carries a new LPXTG surface protein that might be responsible for colonization of the bovine mammary gland. Thus, gene trafficking and genome evolution should be apprehended globally.

Pathogenicity and Genomic Islands

S. aureus pathogenicity (SaPI) and genomic islands (νSa) are continuous structures that vary in size from approximately 15 kb to 70 kb and can harbor many virulence or resistance genes. They mostly contain heterologous DNA that indicates exogenous acquisition. A common feature of these elements is that they are bracketed by direct or inverted repeats and carry recombinase genes. The repeats serve as an attachment site *(att)* for integration into homologous regions of the bacterial chromosome. The recombinase, which is often an integrase, catalyzes integration into the chromosome.[215] At least seven SaPIs have been described and are reunified in four groups based on the homology of their integrase genes.[10] SaPI1 and SaPI2 harbor the gene for TSST-1 and are responsible for most cases of TSS. SaPI3 and SaPI4 contain numerous enterotoxin genes. SaPIbov encodes for a bovine version of TSST, and SaPIbap encodes a bovine adherence protein that might play a role in bovine mastitis.

The *S. aureus* chromosome also carries two larger elements called genomic islands (νSaα and νSaβ), which were acquired early during staphylococcal speciation (see Fig. 194.11).[8,17,58,190] νSaα and νSaβ carry not only restriction modification systems, but also a variety of SEs and other virulence genes and thus have been variously referred to as an enterotoxin gene cluster *(egc)* or virulence gene nursery.[6,173]

Pathogenicity and genomic islands are terminology variations of mobile elements deriving from ancestral prophages, and their mobilization is still dependent on so-called helper prophages.[13] A seminal study showed that *S. aureus* SaPI1 could be mobilized from the bacterial chromosome by ϕ80α and ϕ11 and transferred into naïve recipients thereafter.[216] SaPIs often carry remnants of integrase/excisase *(int/xis)* genes, and λ phage *cI*-like repressor genes that repress the expression of *int/xis* and forbid spontaneous excision of their cognate SaPIs. When induced, helper prophages produce a nonessential protein that blocks the SaPI *cI*-like repressor, thus freeing the expression of *int/xis* genes and promoting SaPI excision. Excised SaPI can replicate and then undergo illegitimate packaging into nascent phage capsids of the helper prophage via *pac* sites. Following phage-induced bacterial lysis, capsids that are illegitimately hijacked by SaPIs can bind to new *S. aureus* recipients and inject their SaPI-DNA cargo, a process called generalized transduction. The delivered SaPI DNA then undergoes Campbell-like site-specific integration into the bacterial chromosome using its *att* site and its own integrase.[13,217] Such phage mobilization was observed with other pathogenicity islands (e.g., SaPI2) and is likely to be a general mechanism of gene transfer for these large elements. Excision and transfer of such elements are triggered by stresses such as exposure to ultraviolet light and certain antibiotics in vitro.[216–218] They are also likely to be promoted by antibiotics in the clinical setting as well.

Resistance Island Staphylococcal Cassette Chromosome *mec*

MRSA contains one resistance island called *staphylococcal cassette chromosome* (SCC) *mec,* wherein *mec* is the genetic element that confers resistance to methicillin.[219] SCC*mec* is an exogenous piece of DNA that may vary between 15 and 60 kb. It inserts at the 5′ end of the *orfX* gene at a conserved 15–base-pair chromosomal attachment site *(attB)* that recombines with an homologous site on the cassette *(attS),* generating two flanking direct repeats (*attL* and *attR*) after insertion. The SCC*mec* critical genes are the recombinases *ccrA*/*ccrB* and *ccrC,* which can mediate mobilization of the whole element, and the *mecA* gene, which mediates β-lactam resistance. The rest of SCC*mec* contains various additional determinants and is referred to as "J" for junkyard.[220]

mecA encodes for penicillin-binding protein 2A (PBP2A), which has a low affinity for methicillin and most other β-lactam drugs and confers intrinsic resistance of MRSA to almost all β-lactams (see "Mechanism of Methicillin Resistance").[221] The *mecA* gene is preceded or not by the *mecRI* and *mecI* regulator determinants, which are homologues of the *blaRI* and *blaI* regulators of penicillinase *(bla)* genes. *mecRI* (and *blaRI*) encodes for a membrane receptor, and *mecI* (and *blaI*) encodes for a gene repressor. In the presence of penicillin, the extracellular portion of the membrane *mecRI (blaRI)* receptor triggers an autocatalytic cleavage of its intracytoplasmic portion. The liberated intracytoplasmic peptide acts as a metalloprotease, which further cleaves the *mecI (blaI)* repressor, thus derepressing gene expression.[222] The *mecA* gene is bracketed by one or two copies of IS431, which is believed to serve as a gene collector and might promote the local insertion of additional determinants, such as antibiotic resistance genes.[220]

Several types of SCC*mec* have evolved from more generalist SCC cassettes, which are structures able to integrate a variety of genes and are functional equivalents to integrons in gram-negative bacteria. Up to 12 SCC*mec* types have been described according to a consensus classification system.[223,224] The classification is based on the *mecA* complex, which encompasses the *mecA* gene and its surrounding regulatory genes, and the *ccr* complex, which includes the *ccrA*/*ccrB* and *ccrC* genes.[223] The *ccrA*/*ccrB* and *ccrC* genes do not come together on the same SCC*mec*. *ccrA*/*ccrB* are cotranscribed and come in four different alleles, whereas *ccrC* has only one allele. Up to now there are nine types of SCC*mec* that carry various combinations of *ccrA* and *ccrB* and three types that carry one of two different *ccrC* alleles, including one in type V and VIII and a newer one in type XII.[224]

SCC*mec* types mirror major original MRSA clones. Types I, II, and III were shown to belong to HCA-MRSA. They harbor multiple resistance determinants, they have relatively large sizes (35–60 kb), and they are therefore difficult to mobilize. Types IV, V, and VI were associated with CA-MRSA.[225,226] They are much smaller (about 15 kb) than their hospital congeners and do not carry multiple antibiotic-resistance genes. However, they appear to be associated with other elements in the same bacterium, including prophage-related PVL and multiple SE genes.[8] One particularly successful clone of CA-MRSA (clone USA300 of CC8 lineage) has also acquired a so-called arginine catabolic mobile element (ACME) inserted downstream of the SCC*mec* cassette.[227] ACME was acquired from *S. epidermidis* and confers survival advantages in acidic and maybe other environments. It improved survival and fitness of USA300 in a rabbit model of bacteremia.[228] Together these elements may render the organism particularly fit and virulent.

Whereas HCA-MRSA types are clonal and carry large and difficult-to-mobilize SCC*mec* cassettes, CA-MRSA types carry small SCC*mec* cassettes, which are more prone to mobilization[220] and less clonal than HCA-MRSA, at least in Europe, where the USA300 clone is rather sporadic.[229,230] CA-MRSA did not arise from HCA-MRSA that permeated the community, but emerged independently by acquiring SCC*mec* most likely from CoNS donors.

Recently, a new SCC*mec* carrying a *mecA* variant gene ($mecA_{LGA251}$) with only 70% homology to *mecA* was missed by molecular probing and incidentally identified through methicillin-resistance phenotyping.[48] This variant, renamed *mecC*,[47] was mainly found in livestock-associated MRSA (e.g., CC130, CC1943, and ST425). Although few *mecC*-positive MRSA strains could be traced back to the mid-1970s,[231] they have clearly been increasing since 2003, representing almost 3% of all MRSA isolates recovered in Denmark in 2011.[231] This adds to the need to closely follow staphylococcal cross-species jumps between humans and animals.

Beyond epidemiologic implications, the use of molecular diagnostics insensitive to non-*mecA* methicillin resistance should be considered, and a newer generation of the Cepheid Xpert (new Xpert MRSA Gen 3; Cepheid, Sunnyvale, CA) PCR assay includes *mecC* as a target.[212] The therapeutic implications of *mecC* have not been reported, but this variant PBP has a higher affinity for penicillins than for cephalosporins, and in vitro, *mecC* isolates are susceptible to the addition of clavulanate to penicillin.[232] This is reminiscent of the greater affinity of old penicillin G and amoxicillin for PBP2A, and the fact that amoxicillin-clavulanate combinations successfully cured experimental infections due to MRSA, with clavulanate being required to block secreted penicillinase.[233] Moreover, expression of methicillin-resistance by *mecC*-positive MRSA appears to be temperature dependent. MICs of flucloxacillin (an analogue of methicillin) were much lower when tested at 37°C than at 30°C. As a result, flucloxacillin successfully cured experimental endocarditis due to *mecC*-positive MRSA, whereas it is known to fail against *mecA*-positive MRSA.[234]

ANTIBIOTIC RESISTANCE

S. aureus has developed resistance to virtually all antibiotic classes available for clinical use. These encompass cell wall inhibitors such as β-lactams and glycopeptides; ribosomal inhibitors that include macrolide-lincosamide-streptogramin B (MLS_B), pleuromutilins, aminoglycosides, tetracyclines, fusidic acid, and the new oxazolidinones; the RNA polymerase inhibitor rifampin; the DNA gyrase–blocking quinolones; the antimetabolite TMP-SMX and iclaprim; and the newer lipopeptides and lipoglycopeptides.[235–238] FDA-approved versions of such drugs and the main resistance mechanisms of *S. aureus* are summarized in Table 194.6. Some are discussed subsequently.

β-Lactams

β-Lactams inhibit bacterial growth by interfering with cell wall assembly. They bind to the active site of a series of membrane-bound enzymes responsible for inserting the peptidoglycan precursors into the nascent wall (Fig. 194.12).[239] Certain enzymes are bifunctional and carry both transglycosidase and transpeptidase activity. Transpeptidation takes place at the D-ala-D-ala terminal of the precursor. It hydrolyzes the covalent bond between the penultimate and the terminal D-ala and then transfers the penultimate D-ala to a free NH_2 terminal (the ε—NH_2 of lysine) of neighboring peptidoglycan stem peptides. The terminal D-ala is released, and a new stem peptide cross-link is created (see Fig. 194.12A).

Penicillin and other β-lactams are steric analogues of the cell wall D-ala-D-ala terminal of the precursors. They are mechanism-based inhibitors that compete with D-ala-D-ala binding to the active site of the membrane-bound transpeptidase, which they block irreversibly—hence the term *penicillin-binding protein* (PBP) coined for these enzymes.

Resistance to Penicillin

The most common resistance mechanism of *S. aureus* to β-lactams is penicillinase, which is encoded by the *bla* gene usually carried on a plasmid. The gene is inducible and preceded by the *blaRI* and *blaI* regulatory determinants (see "Resistance Island Staphylococcal Cassette Chromosome *mec*"). Penicillinase is a secreted enzyme that hydrolyzes penicillin and other penicillinase-susceptible compounds into inactive penicilloic acid. Penicillinase-producing *S. aureus* emerged rapidly after penicillin was introduced as a therapeutic agent in the mid 1940s. It is now prevalent both in the hospital and in the community, where it represents close to 80% of the isolates.[240,241]

The minimal inhibitory concentration (MIC) of penicillin G for fully susceptible *S. aureus* is approximately 0.01 mg/L. In contrast, the MIC of penicillinase-stable drugs such as nafcillin, oxacillin, flucloxacillin, or cephalosporins is 10-fold greater. Thus, penicillin G remains the best choice against penicillin-susceptible staphylococci. However, major concerns remain in the United States about recommended detection of penicillin susceptibility and resistance, because currently recommended susceptibility assays may miss more than 35% of β-lactamase–producing *S. aureus*.[242,243] Moreover, the use of a single β-lactam, such as oxacillin or cefoxitin, to predict susceptibility of *S. aureus* to other β-lactams may have limitations. For example, an analysis of *S. aureus* MICs found that oxacillin was a better predictor than cefoxitin for susceptibility to ceftriaxone, ceftaroline, and cefazolin.[244]

Methicillin-Resistant *Staphylococcus aureus*

The first penicillinase-stable β-lactams such as cephalosporins and semisynthetic methicillin and nafcillin became available in the late 1950s. Ironically, MRSA was first described at about the same time.[245] The prevalence of MRSA progressively increased thereafter. One survey of the National Nosocomial Infections Surveillance System (NNIS) found that the hospital prevalence of MRSA increased from 2.1% in 1975 to 35% in 1991.[246] It went up to 60% in certain centers in the United States,[52] and more than 70% in a report from Shanghai,[247] but great geographic variations exist worldwide. Several reports have indicated a declining trend in the prevalence of MRSA over the last decade, both in Europe and in North America.[53,248–250] Although this decline most probably results from hospital hygiene measures, the exact reason may be more intricate, such as a better fitness of MSSA replacing MRSA in the absence of antibiotic pressure.[251] This was elegantly demonstrated in competition experiments showing that antibiotic concentrations that were several orders of magnitude below the MIC of susceptible strains favored the growth of MRSA over MSSA, whereas MSSA overgrew MRSA in the total absence of antibiotics.[252] Such low antibiotic concentrations are found in sewages of urban environments[253] and are likely to be decreasing with antibiotic consumption control, thus favoring MSSA over MRSA resurgence.

Health Care–Associated Versus Community-Acquired Methicillin-Resistant *Staphylococcus aureus*

Although originally confined to the hospital environment, MRSA has emerged as a community-associated infection over the past 2 decades.[25,153,254] CA-MRSA is different from HCA-MRSA from both epidemiologic and molecular points of view. Case-definition studies showed that HCA-MRSA and CA-MRSA represented different organisms that produced different clinical syndromes.[255,256] HCA-MRSA was associated with risk factors that included recent hospitalization or surgery, residence in a nursing home, or presence of an indwelling catheter or device. It produced mostly hospital-related pneumonia and

TABLE 194.6 *Staphylococcus aureus* Resistance Mechanisms to Major Classes of Antibiotics

ANTIMICROBIAL AGENTS	RESISTANCE MECHANISMS: TARGET MODIFICATION	RESISTANCE MECHANISMS: DRUG INACTIVATION	RESISTANCE MECHANISMS: DECREASED ACCUMULATION	RESISTANCE GENE: NATURE	RESISTANCE GENE: ORIGIN	RESISTANCE GENE: LOCATION[a]
β-Lactams						
Penicillinase-S	Yes	Yes	No	Penicillinase	Acquired	Plasmid
				PBP2A[b]	Acquired	SCC*mec* (chromosome)
				(PBP2C in some livestock animals)		
Penicillinase-R	Yes	No	No	PBP2A[b]	Acquired	SCC*mec* (chromosome)
				(PBP2C in some livestock animals)		
Glycopeptides						
Intermediate resistance	Yes	No	No	Mutations in wall-building genes	Intrinsic	Chromosome
Full resistance	Yes	No	No	*vanA* and *vanH*	Acquired	SCC*mec* (chromosome)
Lipoglycopeptides						
Daptomycin	Yes	No	No	Mutations in genes involved in wall-building and membrane charges *(mprF)*	Intrinsic	Chromosome
Macrolide-lincosamide-streptogramin B						
Macrolides	Yes	No	Yes	*erm*	Acquired	Plasmid or chromosome
				msrA	Acquired	Plasmid
Lincosamide[c]	Yes	Yes	No	*erm*	Acquired	Plasmid or chromosome
				linA	Acquired	Plasmid or chromosome
Streptogramin B[c]	Yes	Yes	Yes	*erm*	Acquired	Plasmid or chromosome
				vgb (rare)	Acquired	Plasmid or chromosome
				msrA (rare)	Acquired	Plasmid or chromosome
Streptogramin A	No	Yes	Yes	*vat, vatA* (rare)	Acquired	Plasmid or chromosome
Quinupristin-dalfopristin	Yes	Yes	Yes	*vga, vgaB* (rare); combinations of above (rare)	Acquired	Plasmid or chromosome
Linezolid	Yes	No	No	Mutation in 23S rRNA gene	Intrinsic	Chromosome
				cfr	Acquired	Plasmid
Tetracyclines	Yes	No	Yes	*tet*(M), tet(O)	Acquired	Plasmid or chromosome
				tet(K), *tet*(L)	Acquired	Plasmid or chromosome
Gentamicin	No	Yes	Yes	*aac*(6′)-*aph*(2′)	Acquired	Plasmid or chromosome
				Respiratory chain mutants		Chromosome
Chloramphenicol	No	Yes	No	*cat*	Acquired	Plasmid or chromosome
Fusidic acid	Yes	No	Yes	*fusA* mutation	Intrinsic	Chromosome
				pUB101	Acquired	Plasmid
Rifampin	Yes	No	No	*rpoB* mutation	Intrinsic	Chromosome
Fluoroquinolones	Yes	No	Yes	*grlA* and *gyrA*	Intrinsic	Chromosome
				norA	Intrinsic	Chromosome
Trimethoprim	Yes	No	No	*dfrA* mutation	Intrinsic	Chromosome
				dfrA	Acquired	Plasmid or chromosome (acts by mutation or overproduction)
Sulfamethoxazole	Yes	No	No	*dpsA*	Intrinsic	Chromosome
					Acquired	Plasmid (probable) (acts by mutation or overproduction)

[a]*SCC*mec, Staphylococcal chromosomal cassette *mec* (see text for details).
[b]*PBP2A,* Penicillin-binding protein 2A; *PBP2C,* penicillin-binding protein 2C.
[c]*erm* gene must be induced or constitutively expressed to confer resistance to lincosamides and streptogramins B. Only macrolides are good inducers. Lincosamides and streptogramins do not induce resistance but are inactive against constitutively MLS_B-resistant strains.
aac(6′)-*aph*(2′), Bifunctional aminoglycoside acetyl-transferase and phosphor-transferase determinant, present on transposons Tn*4001*; *cat,* chloramphenicol acetyl-transferase; *cfr (chloramphenicol-florfenicol resistance),* 23S rRNA methyltransferase; *dfrA,* dihydrofolate reductase gene; *dpsA,* dihydropteroate synthase; *erm,* erythromycin-resistance methylase, mainly *ermA* (chromosome, transposons Tn*554*) and *ermC* (plasmid); *fusA,* gene encoding elongation factor G (EF-G); *grlA* and *gyrA,* genes encoding for the DNA topoisomerase and gyrase, respectively; *linA,* lincosamide nucleotidyl transferase; *mprF* (muropeptide resistance factor), lysylphosphatidylglycerol synthase; *msrA,* macrolide-streptogramin resistance, ABC-transporter; *norA,* gene encoding for staphylococcal efflux pump; *pUB101,* plasmid encoding penicillin-resistance (penicillinase), cadmium-resistance, and a protein (Far1) conferring impermeability to fusidic acid; *rpoB,* gene encoding for β subunit of RNA polymerase; *tet(M) and tet(O),* responsible for ribosomal-modification and protection; *tet(K) and tet(L),* responsible for active efflux of tetracyclines; *vanA* and *vanH,* vancomycin resistance A and H genes (see text for details); *vat* and *vatA,* acetyl transferase genes; *vga* and *vgaB,* streptogramin A efflux gene, ABC-transporter; *vgb,* virginiamycin hydrolysis.

bacteremia. In contrast, CA-MRSA was not associated with any risk factors and produced primarily SSTIs (often furunculosis), and sometimes rapidly fatal necrotizing pneumonia. It was also described as being responsible for necrotizing fasciitis and bone and joint infections.[257,258] In addition, whereas HCA-MRSA was multiresistant and highly clonal, CA-MRSA was pauciresistant[259,260] and seemingly more polyclonal, at least in Europe and Australia.[229,260] Indeed, CA-MRSA has been highly clonal in North America, with a first clone (USA400 and ST1) prevailing until approximately 2000. Since then, it has been replaced with the very successful and highly prevalent clone USA300 (ST8),[153] accounting for 61% of all MRSA isolates in the United States in one recent surveillance survey.[261] Most interesting, Strauss and colleagues[262] traced USA300 back

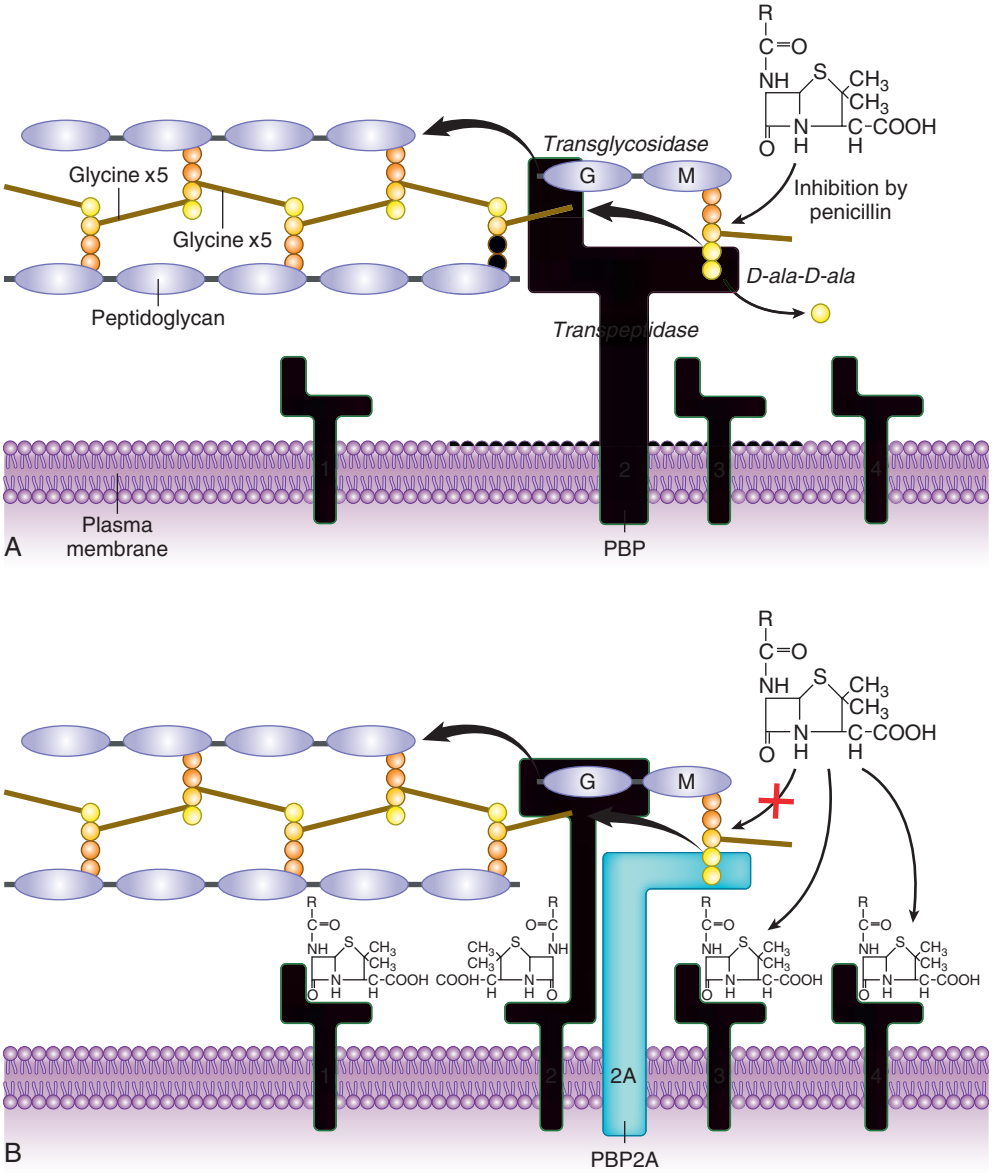

FIG. 194.12 Peptidoglycan assembly in wild-type *Staphylococcus aureus* and in methicillin-resistant *S. aureus* (MRSA). (A) Cell wall precursors consist of units of disaccharides-peptides where disaccharides (*N*-acetylglucosamine–*N*-acetylmuramic acid) are linked to pentapeptides (L-ala–D-glu–L-lys–D-ala–D-ala) at the *N*-acetylmuramate residue. After membrane translocation, precursors are handled by membrane penicillin-binding proteins *(PBPs).* High-molecular-weight PBPs are bifunctional enzymes that perform both a transglycosidase step, linking incoming *N*-acetylglucosamine *(G)* to muramic acid *(M)* in nascent wall, and a transpeptidase step, linking penultimate D-ala to glycine acceptor in nascent wall. In *S. aureus,* lysine in position 3 of stem peptide is almost always decorated with pentaglycine side chain *(orange bars).* Penicillin is a mechanism-based inhibitor of the transpeptidase domain of PBPs. (B) MRSA carries an additional PBP called PBP2A, which has very low affinity for most available β-lactam drugs. Therefore, when β-lactams are present, they block normal PBPs but not PBP2A. PBP2A has only a transpeptidase domain and must "hijack" the transglycosidase domain of normal PBP2 to be active. *(From de Lencastre H, Wu SW, Pinho MG, et al. Antibiotic resistance as a stress response: complete sequencing of a larger number of chromosomal loci in* Staphylococcus aureus *strain COL that impact on the expression of resistance to methicillin.* Microb Drug Resist. *1999;5:163–175.)*

to Europe—not to Australia, as formerly thought—where it became prevalent as PVL-negative MSSA in the mid-1800s. It then spread through Europe and invaded the United States in the early 1900s, where it acquired PVL-containing prophage ϕSA2 in the late 1930s, followed by the ACME locus and then several introductions of SCC*mec* after the 1960s. Since then, it has spread transcontinentally and intercontinentally to South America and Africa but as yet has not become prevalent in Europe.

As mentioned, CA-MRSA is associated with SCC*mec* type IV (and type V and VI in a few cases) and almost always carries PVL.[153,259,263] PVL is epidemiologically associated with SSTI and necrotizing pneumonia, but its specific role in disease has been controversial because, among other considerations, experimental results were opposite in rabbit versus mouse experimental models.[228,264,265] This issue was solved by two more recent studies. The first one demonstrated that PVL toxicity was host specific.[266] PVL appeared to bind to and lyse neutrophils via the C5a receptor and was effective against human and rabbit versions of C5a, but not against mouse and dog C5a, thus explaining the rabbit and mouse differences. The second demonstrated that PVL was toxic in humanized SCID mice, but not in wild-type mice.[267] These are important findings because they provide a rationale for treating PVL-producing staphylococcal infection with toxin-blocking antimicrobial combinations containing, for instance, clindamycin or linezolid,[268] or maybe passive immunotherapy.[269]

Thus, HCA-MRSA and CA-MRSA are not alike. Practically, MRSA in patients at risk is likely to be of the multiresistant hospital type,

whereas MRSA in patients without risk factors is likely to more susceptible to antibiotics but more invasive.

Mechanism of Methicillin Resistance

The main mechanism of methicillin resistance is not mediated by penicillinase but by the newly acquired PBP2A, encoded by *mecA*.[221] The few staphylococci that express borderline methicillin resistance from the overexpression of penicillinase are usually not considered as clinically relevant, although awareness of their possible implication in poor therapeutic outcome is resurging.[270] Because of its low β-lactam affinity, PBP2A can take over the cell wall assembly when normal staphylococcal PBPs are blocked by β-lactams (see Fig. 194.12B).[271] However, although this confers high intrinsic resistance to virtually all β-lactams, PBP2A has a special requirement for particular cell wall precursors. These must contain a pentaglycine decorating side chain attached to the position 3 L-lysine of their stem peptide and other specificities, such as an amidated D-glutamine in position 2 of the peptide (Fig. 194.13).

Providing this adequate substrate to PBP2A requires the functionality of several additional genes implicated in the normal wall building machinery, including 14 or more accessory determinants.[272] Some of them (*femABC* and *fmhB*) are responsible for adding the glycine side-chain residues critical for the PBP2A function.[273] Any alteration in these elements decreases the expression of methicillin resistance in spite of the fact that PBP2A is present.

Another fragility of PBP2A is that it carries only a transpeptidase domain and misses a transglycosidase activity (see Fig. 194.12B). Thus, for successful assembly of the peptidoglycan, PBP2A needs the assistance of the transglycosidase domain of normal staphylococcal PBP, namely PBP2.[274] This is a salient example of protein cooperation in antibiotic resistance but also represents the Achilles' heel of the system. Because most β-lactams can readily block the normal staphylococcal PBPs, further drug development needs only to target additional PBP2A to be effective. Both experimental work and recent crystallographic evidence indicate that such an approach is feasible.[275] Successful treatment of experimental endocarditis from MRSA was achieved with an array of older and newer β-lactams with good PBP2A affinity.[233,276] This approach is driving the development of new anti-MRSA compounds,[277] which recently generated some novel molecules of the cephalosporin (e.g., ceftobiprole and ceftaroline) and carbapenem classes.[278,279]

Glycopeptides

As a general rule, current *glycopeptides* (e.g., vancomycin) are less bactericidal than β-lactams against MSSA. Therefore they should not be used as first-line treatment against β-lactam–susceptible organisms.[280] However, vancomycin is still a gold standard against severe MRSA infections, recently enriched by daptomycin, novel anti-MRSA β-lactams (which are discussed later), and combinations of vancomycin or daptomycin plus β-lactams to take advantage of the so-called "seesaw effect" (also discussed in "Daptomycin" and "Alternatives Treatments" later).

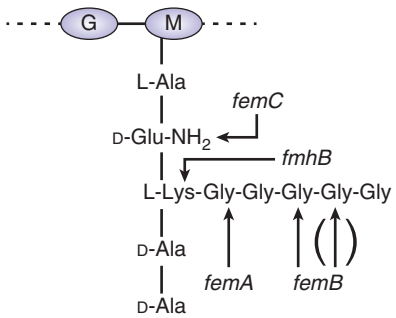

FIG. 194.13 Peptidoglycan precursor required for wall assembly by PBP2A. To be functional, penicillin-binding protein 2A (PBP2A) requires that the cell provide fully decorated precursors, containing both pentaglycine side chain and amidated glutamine. Inactivation of *femB, femA,* and *fhmB* genes blocks addition of pentaglycines and thus decreases expression of methicillin resistance even though PBP2A is present in bacterial membrane. Inactivation of *femC* has a similar effect. *(Modified from Berger-Bächi B. Expression of resistance to methicillin.* Trends Microbiol. *1994;2:389–393.)*

Two types of resistance to glycopeptides were reported in clinical isolates of *S. aureus,* namely "intermediate" and "high-level" resistance. According to the 2012 guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS])[281] the vancomycin MIC breakpoints for *S. aureus* are as follows: ≤2 mg/L for susceptible isolates; 4 to 8 mg/L for intermediate-resistant isolates; and ≥16 mg/L for high-level–resistant isolates. Both resistance phenotypes result from different mechanisms and may be of different clinical and epidemiologic relevance.

Therapeutic Monitoring of Vancomycin in Adult Patients

Although CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/clinical_breakpoints/) agree on the ≤2 mg/L susceptibility breakpoint, several reports and meta-analyses have indicated that infections due to *S. aureus* with vancomycin MICs >1 mg/L but ≤2 mg/L (referred to as high MICs) result in a greater mortality than infections due to *S. aureus* with vancomycin MICs ≤1 mg/L (referred to as low MICs).[282,283] Therefore, special attention was given to pharmacokinetic and pharmacodynamic parameters that could help predict treatment efficacy.[284] A consensus review proposed the following guidelines for vancomycin treatment and monitoring in severe infection, based on twice-daily administration of the drug:

From the therapeutic point of view:

1. The trough concentration of vancomycin in the serum is the best indicator of efficacy and prevention of resistance selection.
2. Trough levels of vancomycin should be between 15 and 20 mg/L.
3. Obtaining peak levels is not indicated.
4. Drug dosages should be adapted to the patient's body weight and renal function. A loading dose of 25 to 30 mg/kg IV must be considered, followed by 15 to 20 mg/kg IV q12h depending on the renal function and drug dosage monitoring.

From the toxicity point of view:

1. Unlike original preparations of vancomycin (in the late 1950s), ototoxicity is no longer an issue if vancomycin is given in monotherapy.
2. Nephrotoxicity at high doses in monotherapy has been reported.
3. Vancomycin-induced nephrotoxicity should be considered if two to three consecutive measurements of renal function are abnormal during therapy.
4. Peak concentration of vancomycin does not seem to be a good correlate of toxicity.

Applying appropriate vancomycin dosage might explain the results of a more recent study reporting no difference in the mortality rate of patients infected with low or high vancomycin MIC *S. aureus.*[285]

Intermediate Resistance to Glycopeptides

Intermediate-resistant *S. aureus* isolates were originally described in Japan and in the United States[286,287] but are ubiquitous. The first isolate,[286] called Mu50, was recovered from a 4-month-old child with MRSA sternal wound infection after cardiac surgery. The infection did not respond to vancomycin treatment. The organism had an MIC of vancomycin of 8 mg/L, as detected with standard broth dilution methods. At that time, the CLSI defined staphylococci for which the MIC of vancomycin was 4 mg/L or less as susceptible, for which the MIC was 8 to 16 mg/L as intermediate, and for which the MIC was 32 mg/L or more as resistant.[29] Therefore, the Mu50 isolate was defined as a vancomycin (or glycopeptide)-intermediate *S. aureus* (GISA).

The same author reported that a second *S. aureus* isolate, called Mu3, was responsible for vancomycin treatment failure in an adult patient with pneumonia.[288] Although the vancomycin MIC for this isolate was 4 mg/L, formally considered as susceptible at the time, Mu3 contained GISA subpopulations ($\leq 10^{-6}$ colony-forming units) that grew in the presence of 5 to 9 mg/L of vancomycin and were not detected with standard drug-susceptibility testing. The term *heteroresistant GISA* (hGISA) was coined to define the Mu3 phenotype. Since then, a number of cases of GISA and hGISA have been described worldwide and were associated with vancomycin treatment failures both in animal experiments and in human cases.[289] As mentioned earlier, CLSI and EUCAST breakpoints now classify isolates with a vancomycin MIC of

4 and 8 mg/L as GISA. Currently the term "heteroresistant" is applied to isolates with a vancomcyin MIC of 2 μg/mL or less but which harbor resistant colonies (see Chapter 30).

Intermediate glycopeptide resistance arises from pleiotropic chromosomal mutations that affect the structure of the wall peptidoglycan. In susceptible strains, glycopeptides inhibit cell wall assembly by binding to the D-ala-D-ala terminal of cell wall precursors and block both transpeptidation and transglycosylation. GISA harbors a thickened cell wall that contains an increased number of free non–cross-linked D-ala-D-ala terminals. This increased amount of free D-ala-D-ala is believed to act as a lure that traps glycopeptide molecules before they reach their target.[290]

Genomic analyses indicate that mutations in two-component sensing systems are involved, including *vraSR* (for vancomycin resistance–associated sensor/regulator),[291] *graSR* (for glycopeptide resistance–associated regulator),[292,293] and *walKR*.[294] *vraSR* is a cell wall stress response regulator that affects the expression of up to 100 genes, including cell-wall building enzymes such as PBP2 and MurZ.[291] *vraSR* dysfunction affects peptidoglycan sturdiness, which is compensated for by wall thickening. *graSR* affects the expression of up to 200 genes including the *dlt* operon and muropeptide resistance factor *mprF.* As mentioned in the "Immune Evasion" section, the *dlt* operon and *mprF* are involved in cell surface charge modulation by means of alanylation of teichoic acids and lysine decoration of membrane phospholipids, respectively.[142] This explains the phenomenon of GISA cross-resistance with AMPs and daptomycin, the antibacterial activities of which depend on membrane charges (see also discussion later).[295] *walKR* is a wall metabolism-associated regulon that is highly conserved in low GC gram-positive bacteria and senses bacterial wall changes—for instance, in response to antibiotic exposure—in order to adapt cell wall metabolism.[296] *walKR* mutations affect both vancomycin and daptomycin resistance by means of *mprF* deregulation, among other processes.[294,296]

Experimental work indicates that like SCVs, GISA strains preexist and are selected during therapy.[297] However, their low level of resistance and sometimes-heterogeneous phenotype make them hard to detect in the laboratory.[29] Pending the development of efficient automated systems, a convenient low-technology dual-antibiotic Etest strip assay containing vancomycin and teicoplanin performed remarkably well. It detected GISA and hGISA with a high sensitivity (95%) and specificity (94%), which was almost identical to much more cumbersome population analysis, which is not performed in routine laboratory testing.[298]

Full Resistance to Glycopeptides

Full vancomycin resistance (MIC ≥16 mg/L for *S. aureus*) has been known for more than two decades in *Enterococcus* spp.[299] In these organisms, glycopeptide resistance results from the acquisition of either Tn*1546* or Tn*1547,* two transposons that encode for a series of genes that modify the D-ala-D-ala terminal of the bacterial peptidoglycan precursor, the very target of glycopeptide compounds, to D-ala-D-lactate. The modified D-ala-D-lactate–containing precursor has a low affinity for glycopeptides and therefore confers resistance. Tn*1546,* which encodes the so-called VanA resistance phenotype, could be transferred to *S. aureus* experimentally.[300] Thus, the recent emergence of fully vancomycin-resistant *S. aureus* (VRSA) that expresses the VanA phenotype among human clinical isolates is not astonishing. Only 14 cases were described in the United States until 2015,[301] but these organisms must be taken seriously. First, most patients had evidence of previous MRSA and enterococcal infection, but not all had received vancomycin. Thus, transfer of the transposon may occur by more generalized triggering effects, perhaps involving unrelated antibiotics. Second, the VRSA phenotype may be missed with routine automated antibiotic susceptibility testing[302] and could be more prevalent than observed. Third, new cases were described in hospitals in the Middle East.[303] Fourth, bloodstream isolates of both methicillin-susceptible and methicillin-resistant, but fully vancomycin-resistant, have been described in Brazil. The conjugative plasmid carrying the *vanA* cluster has been identified, and in vitro transfer demonstrated.[304,305] Finally, a few isolates of VanA-positive MRSA were recovered in a screen of river surface water in Turkey.[306] Taken together, this information indicates that VRSA is pending and warrants constant attention in the diagnostic laboratory.

Daptomycin

Daptomycin is a relatively new lipopeptide that was approved in the United States and elsewhere for *S. aureus*–complicated SSTI, bacteremia, and right-sided infective endocarditis.[236] It is increasingly used as a replacement for vancomycin against MRSA. Daptomycin is an amphiphilic molecule that requires calcium to solubilize as octamer-micelles in liquid phases.[307] Because of its large size, it cannot traverse the outer membrane of gram-negative bacteria, which are naturally resistant to the drug. In gram-positive organisms, it diffuses through the peptidoglycan toward the plasma membrane, where the calcium ions disperse and leave the lipid moiety of daptomycin to interact with the plasma membrane and destabilize its electric potential.[308] Daptomycin is highly bactericidal, but its activity is dose dependent and the dosage of the drug must be large enough to ensure supra-MIC tissue levels. Standard recommendations are 4 to 6 mg/kg IV q24h, but 8 or even 10 mg/kg q24h have been used in severe infections without notable side effects.[309–311] Prospective observational studies and large retrospective series indicate that daptomycin may also be considered for use against left-sided infective endocarditis due to MRSA,[310,312] but approval by official agencies is still pending. Daptomycin should not be used against airway-acquired pneumonia because it is inactivated by alveolar surfactant.[313]

Mutants with decreased daptomycin susceptibility (MIC >4 mg/L) were recovered both in the laboratory and in clinical samples.[236,314] As mentioned in the "Intermediate Resistance to Glycopeptides" section earlier, daptomycin resistance is mediated by mutations in or deregulation of *mprF* (via *vraSR* or *graSR*), which increases the charge of staphylococcal plasma membrane, and is often associated with intermediate vancomycin resistance.[315,316] This cross-resistance raises caution in antibiotic use.

Interesting to note, however, is the fact that daptomycin-resistant mutants have a decreased fitness that may hamper their virulence and dissemination.[317] Moreover, like intermediate vancomycin resistance, daptomycin resistance is associated with the seesaw effect, characterized by a resensitization to β-lactams related to a general perturbation of the cell-call building machinery and an alteration of PBP expression.[318,319] Taking advantage of the seesaw effect opens ways to alternative antibiotic combination strategies.

Protein Synthesis Inhibitors

The MLS_B antibiotics and the oxazolidinone linezolid are discussed in this section. The tetracycline derivatives glycylcycline tigecycline and omadacycline are addressed in the subsequent section "Alternative Treatments."

MLS_B Antibiotics

MLS_B antibiotics comprise separate classes of molecules (i.e., macrolides, ketolides, lincosamides, and streptogramin B) that all bind to the bacterial 50S ribosomal subunit and block protein synthesis. Resistance proceeds by any of the three classic mechanisms: modification of the bacterial drug target, modification-inactivation of the drug itself, and decreasing intracellular accumulation of the drug.

Ribosome modification and drug efflux are the most frequent resistance mechanisms in *S. aureus*.[320] Ribosome modification is mediated by the *erm* gene (for erythromycin methylase), which encodes a methylase that adds one or two methyl groups to the 23S rRNA of the 50S ribosomal subunit. This inflicts a steric alteration that greatly decreases the affinity of the drug for its target. The *erm* determinants belong to a family of methylase genes preferentially located on mobile elements such as transposons (e.g., Tn*554* and *ermA*) or plasmids (e.g., pE194 and *ermC*). An additional sophistication in *S. aureus* is that the expression of *erm* may be inducible or constitutive.[320] In the case of the inducible form, the *erm* product is synthesized only in the presence of inducing drugs. Thus, the bacterium does not spend worthless metabolic energy in the absence of antibiotic pressure. Among MLS_B drugs, only macrolides are good *erm* inducers. However, once induced, the gene product confers cross-resistance to the other members of the group, including the newer ketolides, lincosamides, and streptogramin B, but not streptogramin A. Moreover, mutations that result in constitutive *erm* expression, and hence, global MLS_B resistance, occur at high frequency (10^{-7} to 10^{-8}). Therefore, lincosamides (e.g., clindamycin) should be used with great caution against *erm*-inducible isolates (i.e., resistant to erythromycin

but susceptible to lincosamides and streptogramin B) because the drug might select for constitutive MLS_B mutants, which are resistant to the whole group of compounds.[321]

Newer drugs of the ketolide subfamily, including telithromycin and solithromycin, have a greater ribosomal affinity, are poor *erm* inducers, and have lower MICs than erythromycin for *S. aureus.*[322] However, they are still affected by constitutive expression of *erm* and thus are not useful against these types of organisms, which include the majority of HCA-MRSA isolates.[322–324]

In the laboratory, the MLS_B resistance phenotype is detected with the disk diffusion D-test in which erythromycin and clindamycin disks are placed at a distance on a plate inoculated with bacteria and the diffusion of erythromycin toward the clindamycin disk induces clindamycin resistance. As a result, the zone of inhibition around the clindamycin disk takes a D shape.[321] In contrast, constitutive MLS_B resistance yields no inhibition zone at all around the clindamycin.

Drug Efflux

Active macrolide efflux has been reported in both streptococci and staphylococci.[325,326] In *S. pyogenes* and *Streptococcus pneumoniae,* efflux is mediated by the *mefA* and *mefE* genes, respectively, which are members of the major facilitator transporter and export only macrolides (M-resistance phenotype). *S. aureus* and CoNS may contain *msrA,* which belongs to the complex ABC-transporter (adenosine triphosphate [ATP]–binding cassette) set of genes[325] and confers resistance to both macrolides and streptogramin B (MS-resistance phenotype). In contrast to major facilitators, ABC transporters use ATP hydrolysis as a source of energy for active efflux. The *msrA* complex is located on a plasmid and is frequently observed in MLS_B-resistant CoNS. It can be transferred into *S. aureus,*[325] but its clinical relevance for MLS_B resistance is unclear because it is rarely detected in clinical isolates.[327] Of note, lincosamides (e.g., clindamycin) are not subject to efflux by these pumps.

Constitutive MLS_B resistance, associated or not with drug efflux, is extremely frequent (>90%) in HCA-MRSA.[324] Therefore, use of MLS_B drugs should never be considered against such organisms. The only exception is the quinupristin-dalfopristin combination (streptogramin B and A; see "Alternative Treatments"). In contrast, only 5% of CA-MRSA isolates are reported as clindamycin resistant and mostly are of the inducible phenotype.[153,328] Thus, clindamycin remains a therapeutic option against CA-MRSA.

Oxazolidinones

The oxazolidinone linezolid prevents initiation of protein synthesis by binding to the 23S rRNA of the 50S ribosomal subunit, near its interface with the 30S subunit. It is active only against gram-positive bacteria and is essentially bacteriostatic. It is approved in the United States for complicated SSTI and nosocomial pneumonia from susceptible organisms, including MRSA. Although originally controversial, linezolid was shown to be superior to vancomycin against MRSA nosocomial pneumonia (daptomycin cannot be used in pulmonary infections) in a randomized double-blind study.[329] A systematic review on infective endocarditis from multiresistant bacteria, including 18 MRSA and vancomycin-intermediate *S. aureus* (VISA) isolates, reported a success rate of about 60%, which suggests that compassionate use of linezolid might be an option in such complicated situations.[330] Moreover, evidence-based reviews indicate that it is equal to vancomycin in a number of clinical situations[331] including osteomyelitis in children.[332]

One asset of linezolid is that it can be administered orally and thus is useful for outpatient therapy. Another is that, like clindamycin, it inhibits the secretion of TSST-1 and other toxins and should be considered against toxin-associated infections, including CA-MRSA hemorrhagic pneumonia.[268,333] On the other hand, linezolid is not suitable for long-term (>28 days) therapy because prolonged treatment may be associated with thrombocytopenia, sometimes peripheral or optic neuropathy, and lactic acidosis.[334]

Linezolid resistance has been reported episodically in clinical settings. It is primarily the result of mutations in the 23S rRNA gene.[335] Because staphylococci harbor six to seven copies of rRNA genes, mutation in only one of them does not yield high-level resistance at once. MIC increments are progressive, and MICs of such mutants are usually 4 to 8 mg/L compared with a baseline of 2 mg/L. However, plasmid-mediated high-level resistance was also detected in clinical isolates of *S. aureus* and of *S. epidermidis* (MIC, 8 and >257 mg/L, respectively).[336] The resistance gene *(cfr)* encodes a 23S rRNA methylase that confers cross-resistance to other drugs that bind at the same site, including chloramphenicol, lincosamides (i.e., clindamycin), and streptogramin A.[337] Plasmid-born *cfr* was recently reported in several isolates from livestock in Europe and from human patients in the United States and Ireland.[338,339] Nevertheless, the overall rate of linezolid resistance in *S. aureus* clinical isolates remains very low (<1%) even after more than 15 years of use.[340,341]

The newer semisynthetic tedizolid derivative, approved both in the United States and in Europe for SSTI, has 2× to 8× lower MICs than parent linezolid for MRSA and is not affected by *cfr*-mediated resistance.[342] It is noteworthy, however, that the improved in vitro efficacy is partially offset by a greater binding to plasma proteins—that is, 85% as compared with 30% for linezolid.[343] In two phase III trials of acute skin and skin structure bacterial infections, tedizolid 200 mg once daily for 6 days compared favorably with linezolid 600 mg twice daily for 10 days.[344]

Quinolones

Quinolones are an important class in the antiinfective armamentarium. They originated in the 1960s as a byproduct from the synthesis of antimalarial quinines. Fluorinated derivatives such as ciprofloxacin, norfloxacin, and ofloxacin appeared in the 1980s. They had low MICs (on the order of 0.01 mg/L) for most gram-negative pathogens. However, the MIC for gram-positive bacteria was relatively high (0.25–2 mg/L for *Staphylococcus* spp. and *Streptococcus* spp.)[345] and close to therapeutic concentrations in the serum of humans (2 mg/L for peak concentration of ciprofloxacin). Use of these borderline active drugs against MRSA facilitated the selection for resistant derivatives. The prevalence of quinolone resistance in HCA-MRSA has been around 90% for a long time and is close to 40% in CA-MRSA,[229,346] which makes older and newer quinolones mostly inappropriate against MRSA.

Mechanisms of Resistance

Known quinolone-resistance mechanisms result from chromosomal mutations (see Table 194.6). Plasmid-mediated resistance has been described in gram-negative pathogens and is associated with the *qnr* gene, which protects the quinolone targets.[347] A *qnr*-like gene has been described in *Enterococcus faecalis* and could confer resistance to *S. aureus.*[348] However, such a mechanism was not yet described in clinical isolates.

Quinolone resistance proceeds by two types of mechanisms, including overexpression of the efflux pump NorA[349] and structural mutations in the quinolone targets topoisomerase IV (*grlA* and *grlB*) and gyrase *(gyrA, gyrB)* genes.[350] Resistance is acquired stepwise. A first *grlA* mutation, which occurs at frequencies of 10^{-7} to 10^{-8}, produces a moderate increase in MIC (e.g., 0.5–2 mg/L of ciprofloxacin) that is still considered susceptible (<4 mg/L). However, this first mutation paves the way to a second mutation in the *gyrA* gene, which, combined with the *grlA* mutation, results in high-level resistance. Because the initial *grlA* mutation jeopardizes the efficacy of quinolones, it is critical to avoid selecting it at first, by ensuring appropriate drug levels in the blood and tissues.

Older quinolones readily select for such alterations, yielding highly resistant organisms after only a few serial exposures to the drug.[351] Quinolones with improved anti–gram-positive activity (levofloxacin, moxifloxacin, gatifloxacin, garenoxacin) are less selective. However, they may still select for higher resistance levels in bacteria that already acquired a first degree (*grlA* mutants) of ciprofloxacin resistance (MIC, 2–8 mg/L).

The newer agent delafloxacin might be more promising. Its modified chemical structure ensures dual binding to gyrase and topoisomerase IV, thus resulting in 10 to 100 times lower MICs then previous quinolones, including against *grlA* mutants, in addition to insensitivity to NorA-mediated efflux.[352] At the time of writing, delafloxacin is still investigational.[237]

Pharmacokinetic and pharmacodynamic criteria help predict quinolone efficacy and risk for resistance. Efficacy was predicted by peak drug-level/MIC ratios of 8 or more and ratios of area under the concentration-time curve/MIC of 100 or more.[353] With regard to resistance prevention, in vitro and in vivo experiments have suggested

that drug dosage might be adjusted on so-called mutation prevention concentration, which is two to four times higher than the MIC, rather than on MIC.[354] However, the clinical relevance of mutation prevention concentrations has not yet been assessed.

Alternative Treatments

Treatment of infections from multiresistant HCA-MRSA may be problematic. The activity of all available drugs must be tested against the isolate to establish whether some could still be used. Evaluation of the severity of the disease is also important because not all drugs are equally appropriate in serious conditions.

Alternatives Against Non–Life-Threatening Infections

Superficial and non–life-threatening infections may respond to a variety of drugs, including TMP-SMX, to which HCA-MRSA and CA-MRSA are often susceptible, combined or not with other substances such as rifampin. Other alternatives include the agents discussed in the following sections.

Tigecycline

A modified version of minocycline from the tetracycline family of molecules, tigecycline is almost universally active against gram-negative and gram-positive pathogens, with the notorious exception of *Pseudomonas aeruginosa* and a few other gram-negative organisms that can extrude the drug via efflux pumps.[355] Tigecycline overcomes current *S. aureus* tetracycline-resistance mechanisms, including ribosome protection and active efflux, and thus is effective against all tetracycline-resistant isolates. It is approved in the United States and Europe for the treatment of complicated SSTI. However, it is strictly bacteriostatic, and no trials on *S. aureus*–specific severe infections have been reported. It is still uniformly efficacious against MRSA in recent antimicrobial surveys.[356] The drug must be administered intravenously. Increased mortality in the aggregated clinical trials is also a concern.[357] The drug should not be used in severe situations.

Aminomethylcyclines

This new class of minocycline derivatives, structurally related to tigecycline, includes two recently approved drugs, omadacycline (see Chapter 26) and eravacycline. Both have promising in vitro activity against *S. aureus*, both methicillin-susceptible and methicillin-resistant isolates, but insufficient clinical data to estimate their use.

Quinupristin-Dalfopristin

Quinupristin-dalfopristin combines a streptogramin B (quinupristin) and a streptogramin A (dalfopristin).[358] It is active against both MLS_B-susceptible and MLS_B-resistant staphylococci. It is highly bactericidal against MLS_B-susceptible isolates, but tends to be less bactericidal in the case of constitutive MLS_B resistance, which is practically always the case with HCA-MRSA. Standard dosage is 7.5 mg/kg IV q12h, but larger doses (7.5 mg/kg q8h) have been suggested against constitutive MLSB resistant strains. Experimental data indicate that combining quinupristin-dalfopristin with a β-lactam increases its activity against MRSA, even though the β-lactam is inactive on its own.[359] One limitation of quinupristin-dalfopristin is its venotoxicity, which makes it necessary to administer the drug through a central catheter. Arthralgia and myalgia are significant side effects.

Lipoglycopeptides

Semisynthetic derivatives of glycopeptides, lipoglycopeptides carry modifications of specific functional groups. Three lipoglycopeptides are currently approved for SSTIs in the United States: telavancin, dalbavancin, and oritavancin.[237,360,361] Like vancomycin, they bind to the D-ala-D-ala terminal of peptidoglycan precursors, thus inhibiting both transpeptidation and transglycosylation. In addition, the presence of lipophilic pharmacophores allows the molecules to interact with the plasma membrane, which leads to dispersion of the membrane potential and rapid bacterial killing. Their lipophilic nature confers high binding (≥90%) to plasma proteins and thus prolongs their plasma half-life.[362] For instance, dalbavancin can be given only once a week.

All three compounds are active against antibiotic-susceptible and antibiotic-resistant (including vancomycin) gram-positive pathogens and showed efficacy in various animal infection models. In a double-blind complicated SSTI trial, dalbavancin given once weekly for 2 weeks (1 g at day 1 and 0.5 g at day 8, IV) was highly effective (success rate, >90%) and comparable to linezolid given twice a day (600 mg IV q12h; may be later switched to oral therapy) for the same length of time.[363] Oritavancin given 1200 mg IV once for acute SSTI, including MRSA infections, gave comparable results to vancomycin 1 g (or 15 to 20 mg/kg) IV q12h for 7 to 10 days.[364] The high cost of dalbavancin and oritavancin appears to factor in the cost of intravenous vancomycin administration.

Of note, however, high binding to plasma protein may hamper diffusion of the drug inside therapeutic sanctuaries such as abscesses or infected vegetations. This limitation was demonstrated years ago with teicoplanin[365] and might have been responsible for recently described treatment failure of dalbavancin in right-sided *S. aureus* endocarditis.[366] Data are lacking regarding use of this drug family against severe *S. aureus* infections.

Alternatives Against Severe Infections

There is a notorious lack of good alternatives for use in deep-seated or life-threatening infection. Vancomycin is one of the first choices in such situations. However, poor response to vancomycin may occur in a substantial proportion of patients because the drug is poorly bactericidal and selects for resistant mutants.[282,283,297]

Combination Therapy

Combining vancomycin with an aminoglycoside increases in vitro bactericidal activity. However, the clinical benefit of adding aminoglycosides to vancomycin is not demonstrated, and both kidney and ototoxicity are a matter of concern.[367]

An interesting alternative is to take advantage of the vancomycin–β-lactam or vancomycin-daptomycin seesaw effects,[318] as discussed in the "Daptomycin" section earlier. The fact that resistance to methicillin or daptomycin and resistance to vancomycin are mutually exclusive has yielded several successful therapeutic attempts.[368] An open-label study in MRSA bacteremia demonstrated that combining vancomycin with flucloxacillin shortened the duration of bacteremia to 1.94 days, as compared with 3 days with vancomycin.[369] Moreover, the so-called CAMERA2 trial is now studying vancomycin or daptomycin combinations with antistaphylococcal β-lactams (flucloxacillin, cloxacillin, or cefazolin) on a larger scale.[370]

In addition, there is a growing interest in combining fosfomycin plus β-lactams (e.g., imipenem) as a rescue therapy for complicated MRSA bacteremia or endocarditis.[371] This combination is still being evaluated.

β-Lactams With Improved Penicillin-Binding Protein 2A Affinity

Improving the affinity of β-lactams for MRSA-specific PBP2A has been the purpose of intensive research.[278] Work on the structure of PBP2A and potential blocking of PBP2A–β-lactams indicates that the active site of the enzyme is closed in the resting state and thus difficult to reach with the drug. However, when the enzyme is exposed to cell wall precursors or β-lactams with appropriate pharmacophores, allosteric interactions at other portions of PBP2A trigger opening of the active site, providing access to the precursors or to the blocking drug.[372] The success of this interaction probably depends on the hydrophobic pentaglycine side chain of the precursor (see Figs. 194.12 and 194.13) and thus on the presence of hydrophobic pharmacophores on the β-lactam molecule.[278] This poses solubility problems, which were solved by delivering the compound as a prodrug, as in ceftobiprole medocaril.

Ceftaroline is the first of these agents approved for use in the United States for complicated skin and skin structure infections and nonstaphylococcal community-acquired pneumonia (CAP). It has activity against MSSA and MRSA, with MICs for MRSA of 0.5 to 2 mg/L, and 0.12 to 0.25 mg/L for MSSA. The "AWARE" susceptibility survey, which gathered 21,046 *S. aureus* isolates from 42 US centers from 2010 to 2016, showed persistent (>97%) susceptibility to ceftaroline.[341] Ceftaroline was equivalent to ceftriaxone in MSSA pneumonia; MRSA pneumonia was not

systematically studied. Ceftaroline has been examined in parallel studies of complicated skin and skin structure *S. aureus* infections in comparison with vancomycin-aztreonam. The ceftaroline-vancomycin population microbiologic profiles consisted of 73.4% and 84% *S. aureus* infection (34.3% and 32% MRSA; 39.9% and 50.9% MSSA), respectively. Clinical cure rates were 95.1% and 91.4%, respectively, with microbiologic and adverse event outcomes that were similar to those in the comparator.[373,374] The clinical use of the drug is compromised by the lack of a comparative study in major MRSA diseases, such as bacteremia, endocarditis, and osteomyelitis. Multiple reports have focused on its activity in vitro against isolates nonsusceptible to vancomycin and daptomycin, and its clinical use in combination with other agents for the treatment of severe MRSA infections such as bacteremia and endocarditis, which does not answer the question of whether ceftaroline alone would be an effective treatment.[341,374–376] Case series of salvage treatment in refractory MRSA bacteremia, pneumonia, and endocarditis (including prosthetic valve infection) have been reported and suggest that the drug may be an effective option, alone or in combination.[319,377–380]

Development of Nonantibiotic and Vaccine Strategies

A number of nonantibiotic strategies are being investigated to circumvent *S. aureus* antibiotic resistance. These encompass novel AMPs, virulence modulators, antibiotic resensitization, immunomodulation, bacteriophages (phages), phage endolysins (lysins), and vaccines.

Antimicrobial Peptides

AMPs include primarily cathelicidin-like peptides purified from various animal or insect venoms, and synthetic derivatives of them.[381,382] AMPs are positively charged amphiphilic peptides. It was recently shown that artificially increasing their charges increased their bactericidal activity as well.[383] On the other hand, *S. aureus* resists the detrimental effect of AMPs by increasing the charge of its own cell wall or plasma membrane via the *dlt* operon and *mprF,* which act as repelling factors (discussed in "Immune Evasion" earlier).[142] Whether increasing the AMP charges will upset the bacterial resistance mechanism is not clear. Moreover, AMPs still have relatively high MICs for *S. aureus*.[383]

Virulence Modulation

Virulence modulators include primarily inhibitors of SortA, which block the anchoring of *S. aureus* surface adhesins[96,98]; iron-capturing determinants, which also promote infection[97,99,102,384]; and inhibitors of *agr,* which block the secretion of toxins.[61,64] Although the blocking of surface protein and toxin secretion is conceptually sound, none of these methods reached total protection in animal models.[385,386]

Because *S. aureus* virulence results from the intertwining of both surface-attached and secreted factors, one should ideally administer the two kinds of inhibitors simultaneously to prevent or damper infection severity. However, although promising, this method will probably not offset the need to eradicate the microorganisms from the infection site.

Immunomodulation

Reports on the production of IL-17A by γ/δ T cells and the benefit of IL-17A in stimulating innate immunity and impeding skin and nasal colonization by *S. aureus* are quite promising[156,157] (see also "Escaping Cell-Mediated Immunity" earlier). Another strategy consists of sensitizing *S. aureus* cells to complement and phagocytes, referred to as "lysibodies"[387]; this is described in the "Phage Lysins" section later. Whether prescribing IL-17A or lysibodies will be beneficial in humans remains to be determined.

Antibiotic Resensitization

Statins have pleiotropic effects that alter membrane compartmentalization in both eukaryotes and prokaryotes[388] and were associated with improved clinical outcomes in infected patients.[389,390] In the case of MRSA, it was recently shown that antibiotic resensitization was due to altered PBP2A positioning.[391] The authors demonstrated that PBP2A positioning in the cell membrane depended on the dynamics of "functional membrane micro-domains" (FMMs), which are the bacterial equivalent of membrane rafts in eukaryotic cells. FMMs depend on the interaction of membrane lipids with the scaffold protein "flotillin," with which statins interfere. Thus, statins alter both eukaryotes and prokaryotes,[388] in which they may restore susceptibility to antibiotics—that is, to methicillin and its derivatives in the case of MRSA. This remarkable antibacterial benefit of statins, associated with additional in vitro[392] and clinical observations,[389,390] warrants further scrutiny.

Another antibiotic sensitization study addressed the eradication of intracellular *S. aureus* persistence. It showed that conjugating antibody and antibiotics in a system in which antibiotics became activated only in the phagolysosomes was superior to vancomycin in eliminating intracellular *S. aureus* and treating bacteremia in mice.[393] However, once again, the limitation may be the antiphagocytic strategies of *S. aureus.*

Phage Lysins

Phage lysins and phage therapy are coming close to clinical applicability.[394,395] Phages produce peptidoglycan hydrolases (lysins) to burst the host bacteria and release their progeny at the end of their replication cycle. Lysins can be purified and used to lyse gram-positive bacteria very effectively from the outside. Gram-negative bacteria are protected from outside-in lysin diffusion by their outer membrane and are thus naturally resistant to lysin therapy. Several anti–*S. aureus* phage lysins were produced, and demonstrated potent in vitro and in vivo activity in animal models[396–398]; however, few successful cases of treatment of refractory *S. aureus* skin infections in humans have been reported.[399] Lysins are not yet available for routine clinical use, but two prospective clinical trials are underway in *S. aureus* dermatitis[400] and BSIs (https://clinicaltrials.gov/ct2/show/NCT03163446?term=cf-301&rank=1).

An interesting development of lysins in the field of immunomodulation is via so-called lysibodies.[387] It consists of fusing a lysin peptidoglycan-binding domain to an IgG Fc fragment. The lysin peptidoglycan-binding domain of the construct binds to the conserved peptidoglycan and exposes its Fc portion at the bacterial surface. The Fc portion attracts complement and professional phagocytes and promoted bacterial clearance both in vitro and in a mouse sepsis model.[387] It remains to be determined whether *S. aureus* will be able to circumvent lysibodies via their immune escape armamentarium (discussed in "Immune Evasion" earlier).

Phage Therapy

The strategy of phage therapy is generating growing interest.[395] Phages have been used against bacteria since the early 1920s, but were abandoned in Western countries after the introduction of antibiotics. In contrast, phages were continuously developed in the countries of the former Soviet Union, particularly at the Eliava Institute in Tbilisi, Georgia.[401] Phages are very rapidly bactericidal and can synergize with antibiotics.[402] Aside from ample experience from the Eastern countries, no reported phase III comparative clinical trials on phage therapy have been performed, with the exception of the multicenter European Phagoburn study on burn wounds infected with *P. aeruginosa* (http://www.phagoburn.eu), the results of which are still preliminary. On the other hand, there are recent case reports on successful phage therapy of *S. aureus* osteomyelitis and diabetic ulcers,[401,403] and at least one ongoing phase I study using anti–*S. aureus* phages in chronic rhinosinusitis is planned (https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=368275). Phage therapy is promising, but it still requires careful comparative assessment.

Vaccines

Vaccination is a very important area of research both in human and in veterinary medicine. However, considering the arsenal of immune evasion strategies of *S. aureus* (discussed in "Immune Evasion" earlier), this issue is an immense challenge. Vaccines pursue one of three aims: blocking the effect of toxins, blocking the functional surface adhesins or other relevant proteins, or stimulating phagocytosis. Experimental vaccines have been developed by a variety of means, including DNA vaccines, and against constituents as diverse as the capsule or specific surface determinants, including PBP2A and adhesins. Most of these vaccines conferred some protection in experimental models, and in one trial a conjugated capsular vaccine conferred promising but transient

protection in patients on hemodialysis.[404] A trial using a vaccine targeted on the IsdB iron-binding LPXTG-bound protein to prevent deep sternal wound infections did not provide protection and paradoxically increased the mortality rate in *S. aureus*–infected patients.[405]

One critical point regarding extrapolation of results in mouse models to human is that mouse thrombocytes do not carry a platelet immunoglobulin Fc receptor similar to FcγRIIA in human thrombocytes.[406] As discussed in the "Contribution of Coagulation" section, *S. aureus* activates platelets and hijacks them to colonize tissues with inflamed or injured endothelia. Hence, antibodies directed to the *S. aureus* surface expose Fc fragments that in turn activate platelets via FcγRIIA (in humans) instead of binding complement and attracting leukocytes, and help channel staphylococcal aggregates to injured tissues. In contrast, this is not expected to occur in the case of neutralizing antibodies that are directed against soluble toxins, which might be a better option. These opposed effects of surface-directed versus toxin-directed antibodies are convincingly supported by studies examining vaccination against experimental PVL-related pneumonia.[407]

As yet, no approved antistaphylococcal vaccine is available for clinical use, but there is one pending phase I/II study targeting leukocidins (https://clinicaltrials.gov/ct2/show/results/NCT01011335?term=staphylococcus+aureus&draw=5&rank=43), and hope still exists regarding the targeting of mixtures of *S. aureus* surface-bound and secreted determinants.[408]

CLINICAL ASPECTS AND EPIDEMIOLOGY

Over the last 2 decades, the increase in medical procedures such as major operations, chronic dialysis, organ transplantation, and cancer chemotherapy has profoundly modified the epidemiology of *S. aureus* infections worldwide. With the recent rise in invasive procedures, *S. aureus* has become a leading cause of nosocomial and health care–related infections.[409,410] Along with the pressure toward shorter lengths of stay in acute-care settings, increasing numbers of such health care–related *S. aureus* infections are now diagnosed in subacute or long-term care facilities.[411,412]

S. aureus invasive infections consistently result in mortality rates as high as 20% to 30%,[413–415] especially when associated with antibiotic resistance (i.e., methicillin resistance)[411] or when occurring in acutely critically ill patients[416] or in patients with chronic conditions such as hemodialysis.[417] This translates to an important social and economic burden: Inpatients with *S. aureus* infection had a hospital stay that was three times longer (14.3 vs. 4.5 days), charges that were three times higher (US $48,824 vs. $14,141), and a risk of in-hospital death that was five times greater (11.2% vs. 2.3%).[418] Additional costs incurred because of methicillin resistance were estimated in 2015 to be approximately $3.3 billion yearly for US intensive care units (ICUs).[419]

Clinical Spectrum

S. aureus is responsible for an array of infections wherein it is either present at the infection site or acts at a distance by secreting toxins (see "Regulation and Virulence Determinants" section). Incidence rates range between 28.4 and 35.4 per 100,000 inhabitants per year[413,420]; it causes mainly skin and soft tissue infection (40%), lower respiratory tract infection (20%), and BSI (20%).[421]

A rising incidence of *S. aureus* health care–related infections has been observed and can be attributed to medical progress.[422] The increasing number of invasive procedures and the use of inserted or implanted foreign bodies has indeed created a unique niche for this versatile bacterium, which is very well equipped with numerous virulence factors (see "The Journey to Invasive Disease" earlier). Accordingly, *S. aureus* is a leading cause of surgical site infections (SSIs; 20%–45% of cases),[409,423] catheter-related bloodstream infections (CRBSIs; 13%–40% of cases),[409,424] and ventilator-associated pneumonia (25%–28% of cases).[409,425]

In the community, it is also one of the primary causes of native and prosthetic valve endocarditis (23%–30% of cases)[422,426–428] and the leading organism responsible for osteoarticular infections (40%–70% of cases).[429–431] Finally, *S. aureus* is the second most frequent cause of community-onset bacteremia after *Escherichia coli* (15%–23.5%).[432]

Risk Factors for *Staphylococcus aureus* Infection

Population-based studies have consistently identified male and very young and elderly individuals as being at increased risk for *S. aureus* infections. Moreover, two studies showed that the most important risk factor is necessity for dialysis, either peritoneal (relative risk [RR], 150–204) or hemodialysis (RR, 257–291). Other conditions that increase the risk of invasive *S. aureus* infections include diabetes (RR, 7), cancer (RR, 7.1–12.9), rheumatoid arthritis (RR, 2.2–9.2), human immunodeficiency virus (HIV) infection (RR, 23.7), intravenous drug use (RR, 10.1), and alcohol abuse (RR, 8.2; Table 194.7).[420,433]

Rare but classic predisposing factors also encompass chemotactic and phagocytosis defects. Inheritable chemotactic defects include Job syndrome, Chédiak-Higashi syndrome, Wiskott-Aldrich syndrome, and Down syndrome. *Job syndrome* is a condition that involves recurrent eczema with repeated skin infections and cold abscesses. *Chédiak-Higashi syndrome* is defined clinically by albinism and recurrent *S. aureus* infections and cytologically by giant granules in phagocytic and other cells. Acquired chemotactic defects are also relatively rare and include rheumatoid arthritis and decompensated acidotic diabetes mellitus. Opsonic defects, whether inherited or acquired, are predisposing factors for all kinds of pyogenic infections and are not specific for *S. aureus*. They are exemplified by selective or combined hypogammaglobulinemias and various kinds of complement defects.

However, one of the most important factors that independently adds to all these predisposing conditions is chronic *S. aureus* nasal carriage (see later discussion). Whether they are in the hospital or in the community, patients mostly become infected with their own carriage strain.[24] Therefore it has been proposed that patients at high risk for *S. aureus* nasal or cutaneous carriage should be screened, and in positive cases should be decontaminated with mupirocin ointments or other means (Table 194.8).[434] Implementation of this policy for selected patients admitted to the hospital should be considered.[26]

The Burden of Antibiotic Resistance

At the beginning of last decade, MRSA was one of the most commonly identified antibiotic-resistant pathogens in many parts of the world.[247,421] During the period from 2000 to 2005, the proportion of hospital-onset *S. aureus* infections that were methicillin resistant peaked to 56.2% in US hospitals,[435] a proportion that was even higher (64.4%) in US ICUs. For some years now, surveillance programs from various areas have reported a steady decrease in the incidence of MRSA infections in the community[436] and in health care[437] or military settings,[438] the largest decrease being observed among hospital-onset MRSA infections[411] (see "Methicillin-Resistant *Staphylococcus aureus*" earlier).

In contrast to the diversity of strains observed in diseases caused by MSSA, MRSA outbreaks are caused by a limited number of successful clones.[439,440] In the health care setting, HCA-MRSA infections are associated with greater lengths of stay,[441] higher mortality,[442] and increased costs.[419] Whether MRSA is more virulent than MSSA is still a matter of debate. The molecular typing of thousands of carriage- and disease-associated *S. aureus* strains revealed that MRSA did not represent specific lineages and that all types of *S. aureus* can become invasive given the appropriate circumstances.[443] On the other hand, most MRSA infections are mainly of nosocomial origin and manifest as complications of health care procedures or underlying disorders. In this specific context, patient differences could account for the variation in mortality because a greater number of older patients with severe underlying disease contract MRSA infections.[442] In addition, ineffective or delay in effective antibiotic therapy could also play a large role in suboptimal response to therapy.[444]

CA-MRSA has now become the most frequent cause of SSTIs acquired in the community.[445] Groups with high-intensity physical contact are particularly affected. This includes competitive athletes, children in daycare centers, military recruits, injections drug users, jailed inmates, and men who have sex with men.[254] Five percent to 10% of CA-MRSA infections are invasive and can cause severe, sometimes fatal disease, such as necrotizing pneumonia, bacteremia, or necrotizing fasciitis[446] (see "Methicillin-Resistant *Staphylococcus aureus*" earlier). Successful CA-MRSA clones have established themselves as nosocomial pathogens and are now a common cause of health care–associated

TABLE 194.7 Risk of Invasive *Staphylococcus aureus* Infection Associated With Selected Underlying Conditions in Adults 20 Years Old or Older

UNDERLYING CONDITION	NO. OF PATIENTS WITH INVASIVE *S. AUREUS* INFECTION (*N* = 226)	ANNUAL INCIDENCE, PER 100,000	RR (95% CI)	*P* VALUE
Hemodialysis	24	7692	257.2 (161.0–393.6)	<.001
Peritoneal dialysis	3	4918	150.0 (30.5–44.1)	<.001
HIV infection	4	778	23.7 (6.4–61.4)	<.001
Solid-organ transplantation	3	683	20.7 (4.2–61.3)	<.001
Heart disease	114	362	20.6 (15.8–27.0)	<.001
Cancer	47	348	12.9 (9.1–17.8)	<.001
Illicit intravenous drug use	13	321	10.1 (5.3–17.7)	<.001
Alcohol abuse	31	241	8.2 (5.4–12.0)	<.001
Diabetes mellitus	48	192	7.0 (5.0–9.7)	<.001
Stroke	16	200	6.4 (3.6–10.6)	<.001
Chronic obstructive pulmonary disease	26	120	3.9 (2.5–5.9)	<.001
Systemic lupus erythematosus	2	80	2.4 (0.3–8.7)	.3
Rheumatoid arthritis	5	74	2.2 (0.7–5.3)	.1

CI, Confidence interval; *HIV,* human immunodeficiency virus; *RR,* relative risk.
Modified from Laupland KB, Church DL, Mucenski M, et al. Population-based study of the epidemiology of and the risk factors for invasive Staphylococcus aureus *infections.* J Infect Dis. *2003;187:1452–1459.*

TABLE 194.8 Example of Decontaminating Scheme for Patients Colonized or Infected With Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Protective Measures

Put patient in contact isolation (one or several contaminated patients in single room with restricted access)
Use protective gown and gloves
Use protective mask and glasses if risk for splashing of contaminated liquids
Clean hands with alcoholic solution at glove removal and between caregiving procedures
Leave any disposable item in room and discard for sterilization in special containers

Decontamination Measures

Apply nasal mupirocin (2%) every 8 hours for 5–7 days
Apply chlorhexidine-based oral spray three to four times a day for 5–7 days
Take daily shower or clean body thoroughly with chlorhexidine-based soap for 5–7 days
In the case of dental prostheses, clean and soak the prosthesis daily in chlorhexidine-based solution for 5–7 days

Control Cultures and Decision

Take control swabs of any contaminated sites 48 and 96 hours after the end of treatment
Keep isolation measures in force until laboratory results are available
If no MRSA is present in control cultures, consider patient decontaminated; discontinue isolation, and swab weekly for follow-up cultures.
If MRSA is present in control cultures, pursue isolation measures and repeat whole decontamination scheme

Modified from Current Recommendations at the University Hospital of Lausanne, Switzerland.

hospital-onset infections, especially BSIs.[447,448] Compared with HCA-MRSA, CA-MRSA retained susceptibility to many non–β-lactam antibiotics. If hospital-associated MRSA infections appear to be on the decline, only modest changes have been reported for CA-MRSA so far.[449]

Carriage of *Staphylococcus aureus*

The primary reservoirs of *S. aureus* are the anterior nares, but the organism can be isolated from other sites, especially the throat.[450] Traditionally, three patterns of carriage have been distinguished: persistent carriers (20% of healthy people, range 12%–30%), intermittent carriers (30% of healthy people, 16%–70%), and noncarriers (50% of healthy people, 16%–69%).[20] Persistent carriers differ markedly from others: They carry the same strain for extended periods of time, have higher *S. aureus* loads, and are at higher risk of acquiring *S. aureus* infections.[22,451] Because intermittent carriers and noncarriers share similar characteristics, such as a low risk of infection, identical *S. aureus* nasal elimination kinetics, and comparable antistaphylococcal antibody profiles, it has been proposed that *S. aureus* carriage be reclassified such that only persistent carriers are distinguished from others.[451]

Bacterial and host determinants for *S. aureus* carriage have been thoroughly investigated.[452] In contrast to toxins, staphylococcal cell surface–associated and immune evasion molecules seem to be important for colonization efficacy[453] (see "The Journey to Invasive Disease" earlier). If some results suggest a role for cell wall teichoic acid, SdrC, SdrD, and SasG in binding to squamous cells in vitro, only ClfB and IsdA have been shown to be associated with *S. aureus* carriage in humans.[454,455] Patients exposed to skin lesions have a greater risk of *S. aureus* nasal carriage. They include patients with insulin-dependent diabetes, patients undergoing hemodialysis or peritoneal dialysis, intravenous drug users, patients with recurrent *S. aureus* skin infections or atopic dermatitis, patients with HIV, and healthy patients receiving repeated injections for allergies.[452]

Carriage rates vary with geographic location, age, sex, and ethnicity. Most infants become colonized shortly after birth, usually with the same strain as their mother. Carriage then decreases with age (40%–60% at 2 months, and 21%–28% at 6 months), reflecting both the development of an immune response to *S. aureus* and competition between microorganisms in the nasopharynx. This is particularly true for *S. aureus* and *S. pneumoniae* because being a *S. pneumoniae* carrier is inversely associated with *S. aureus* carriage and vice versa.[456,457] Children and adolescents younger than 20 years seem to have higher persistent carriage rates than adults. Since 2000, the reported prevalence rate of *S. aureus* nasal colonization has decreased to 20% to 30%.[458] Explanations for this decline might include improved personal hygiene, changes in socioeconomic class, and smaller families.[22]

Traditionally, control of *S. aureus* has been focused on preventing cross-infection between patients. However, at least three sets of observations indicate that nasal carriage of *S. aureus* is an important risk factor for sepsis: persistent carriers have higher rates of infections than others[22]; a large proportion of nosocomial *S. aureus* infections originate from patients' own flora[24]; and eradication of carriage reduces nosocomial infections, especially after orthopedic and cardiosurgery-related infections.[26,459]

Carriage of Methicillin-Resistant *Staphylococcus aureus*

The prevalence of MRSA colonization increased from 0.8% in 2001 to 2002 to 3% in 2011 to 2012.[460,461] MRSA colonization is particularly important in the hospital environment because colonized and infected patients represent the most important reservoir of MRSA in health care facilities. Factors associated with MRSA carriage at time of hospital admission include prior health care exposure (i.e., nursing home resident or hospitalization in past 12 months), prior contact with nosocomial pathogens (i.e., history of MRSA and/or vancomycin-resistant enterococci [VRE] carriage, history of *Clostridioides difficile* [formerly *Clostridium difficile*] infection), or selected comorbid conditions such as diabetes, chronic obstructive pulmonary disease (COPD), and congestive heart failure, probably because those conditions lead to repeated hospital exposure.[462]

Despite the increasing rate of CA-MRSA infections, the prevalence rate of MRSA among persons without typical risk factors remains relatively low, and most MRSA colonization and infection still develop among those who have health care–associated risk factors or contact with other persons who have such risks. When patients known to be colonized with nosocomial MRSA are discharged from the hospital or nursing home into the community, spread to family members or close contacts can occur. Rates of transmission between positive case patients and household members range from less than 10% to 43%. About one-half of MRSA carriers are colonized for 2 months or less, but an estimated 20% are persistent MRSA carriers who remain positive for months or years.[463]

Preventive Measures to Limit Health Care–Associated Infections Methicillin-Resistant *Staphylococcus aureus* Infections

Health care–associated infections is a leading cause of preventable illness. As a major cause of such infections, MRSA has been given priority in infection-control strategies; the United Kingdom and several US states have even mandated anti-MRSA strategies. Typical methods to limit infections by MRSA include a comprehensive set of interventions. Infection-control measures should always involve the laboratory. Determining the clonality of MRSA recovered from several patients is important in order to differentiate sporadic cases of MRSA from more problematic epidemic situations (see "Molecular Typing" earlier).

Improving the rational use of antibiotics and the implementation of standard precaution and hand hygiene are clearly cornerstones of MRSA prevention and control. Other anti-MRSA bundle measures (e.g., systematic active screening using either rapid or conventional testing, isolation and decolonization of carriers; see Table 194.8) when implemented routinely in settings with endemic MRSA are effective, but their use is a matter of debate because they are economically not cost-effective.[464–466]

Screening and isolation of MRSA carriers at admission is a resource-consuming protective measure still widely used worldwide. Both rapid PCR tests and standard culture methods can help decrease MRSA infection rates in hospitals, particularly in settings with hyperendemic MRSA cross-infections.[465] Decolonization of MRSA carriage has been an important part of the control of MRSA dissemination so far. Most decontamination regimens recommend a 1-week daily total body washing with a chlorhexidine-based soap, plus nasal mupirocin application (see Table 194.8). Low-level mupirocin resistance (MIC, 8–256 mg/L) and high-level resistance (MIC, >512 mg/L) exist but are uncommon and usually follow prolonged administration.[467] Monitoring susceptibility is essential to detect appearance of resistance to the decolonizing agent.

Recent Dutch guidelines, which use an aggressive "search and destroy" strategy, achieved a rate of up to 80% of eradication.[468] They differentiate between uncomplicated carriers, who have no associated MRSA infections, and complicated carriers, who may have skin or deeper infection. Uncomplicated carriers are treated only with topical measures (see Table 194.8), whereas complicated carriers receive concomitant systemic antibiotic such as TMP-SMX (160/800 mg, orally q12h) plus rifampin (600 mg orally q12h) for 1 week.[468] Nevertheless, eradication of MRSA carriage is often difficult, and the role of MRSA decolonization in the infection-control measure remains uncertain.[459]

About 5% of health care workers become colonized with MRSA, and it has long been recommended to decolonized them.[469] However, health care workers most frequently act as vectors of transmission, not as main sources of MRSA. A study reported in 2017 demonstrated the limited role of the environment and health care workers in transmission of *S. aureus* to patients.[458] The worrying trend of increasing rates of MRSA has now been reversed in many high-income countries. In this context, should we still target high-risk pathogens, or should we instead protect patient populations that are susceptible to infection from many health care–associated pathogens? Indeed, universal practices such as hand hygiene and chlorhexidine body washings[470] or specific measures to reduce the rate of CRBSIs including use of chlorhexidine sponges[471,472] helped decrease the rates of infection not only with MRSA but also with other pathogens.[473] Infection-control measures should therefore be regarded as a whole and should not target only a specific pathogen.[474]

CLINICAL SYNDROMES

Infection begins with the colonization of target tissues by the microbes. Further spread results from more specific invasion processes, during which bacteria interact directly or indirectly (e.g., via toxins) with the host. Thus, any localized infection has the potential to become the seeding site of a more severe infection by means of contiguous extension, distant spread through the blood circulation, or production of toxins including TSST-1 and PVL (see "The Journey to Invasive Disease" earlier). Pyogenic infections are described subsequently

Skin and Soft Tissues Infections

Classification

S. aureus SSTIs include primary pyoderma (such as folliculitis, furuncles, carbuncles, and impetigo) and soft tissue infections (i.e., cellulitis, erysipelas, and pyomyositis). They are commonly classified according to the anatomic structure involved (Fig. 194.14): (1) infection of the epidermis—impetigo; (2) infection of the superficial dermis—folliculitis; (3) infection of deep dermis—furuncles, carbuncles, and hidradenitis suppurativa; and (4) infection of subcutaneous cellular tissues—erysipelas, cellulitis, fasciitis, and pyomyositis.

The diagnosis of an *S. aureus* SSTI is most frequently made clinically. The basic anatomic lesion induced is a pyogenic exudate or an abscess. Superficial infections can often be treated with local care, surgical drainage, and rarely, systemic antibiotics. When the infection penetrates to the deeper subcutaneous tissue and/or surgery is required, it is considered complicated; erysipelas, lymphangitis, lymphadenitis, cellulitis, and necrotizing fasciitis are severe diseases that may be life-threatening. They require hospitalization, systemic antibiotic therapy, and prompt surgical drainage and débridement[184] (Tables 194.9 and 194.10).

Impetigo

Impetigo is a superficial infection of the skin that involves only the epidermis. It affects mostly children, usually on exposed areas of the

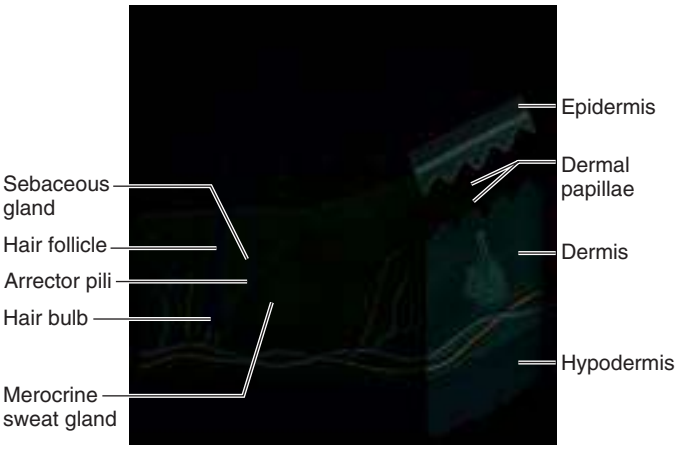

FIG. 194.14 Skin anatomy delineating various levels at which *Staphylococcus aureus* infection can occur (see text for details).

TABLE 194.9 Initial Management of Adults With Community-Acquired Purulent Skin and Soft Tissue Infections Presumed to Be Staphylococcal (Dosages Given for Patients With Normal Renal Function)

1. Incise and drain purulent foci. Consider heat, elevation, and immobilization of site.
2. Obtain wound Gram stain, culture, and two blood cultures with susceptibility testing.
3. Consider admitting for observation patients who require extensive débridement, have large areas of erythema, are elderly, are immunosuppressed, have important comorbidities (such as chronic renal failure, morbid obesity, diabetes mellitus), are unable to take medication reliably, or who are clinically unstable.
4. Administer intravenous therapy for hospitalized patients
   - A. Initially cover MRSA with
     - a. Vancomycin: 15 mg/kg IV q12h
     - b. Alternatives include:
       - i. Daptomycin: 6 mg/kg IV q24h
       - ii. Ceftaroline: 600 mg IV q12h
   - B. If cultures grow MSSA, can switch to:
     - a. Oxacillin, nafcillin, or flucloxacillin (not in United States): 2 g IV q4h, or
     - b. Cefazolin: 2 g IV q8h
5. Patients appropriate for outpatient therapy (with follow-up scheduled for 48–72 h): trimethoprim-sulfamethoxazole—2 double-strength tablets (320/1600 mg orally q12h)
   Alternatives include:
   - A. Clindamycin: 300–450 mg orally q8h
   - B. Doxycycline or minocycline: 100 mg orally q12h
   - C. Linezolid: 600 mg orally q12h
   - D. Tedizolid: 200 mg orally q24h
   - E. Oritavancin: 1.2 g IV over 3 h once, or dalbavancin 1 g IV over 30 min, followed by 500 mg IV 1 week later
   - F. If cultures grow MSSA can switch to:
     - a. Dicloxacillin: 500 mg orally q6h
     - b. Cephalexin: 500 mg orally q6h

MRSA, Methicillin-resistant *Staphylococcus aureus*; *MSSA,* methicillin-susceptible *S. aureus.*



FIG. 194.15 Infective skin lesions in staphylococcal impetigo.

body (e.g., the face and the legs). Primary impetigo occurs via direct bacterial invasion of intact healthy skin, whereas secondary impetigo occurs via infection at sites of minor skin trauma such as abrasions, insect bites, or eczema. Most cases of impetigo are caused by *S. aureus, S. pyogenes* or mixed infections of staphylococci and streptococci.[475]

The disease usually starts as a red macule that evolves into vesicles that contain cloudy fluid based on the area of erythema (Fig. 194.15). Bullous impetigo is mostly exclusively due to *S. aureus* strains producing exfoliative toxins (see "Exfoliative Toxins and Staphylococcal Scalded Skin Syndrome" earlier). The bullae are the result of epidermolytic toxins of the serine protease family. The vesicles rapidly rupture and leave a yellowish, thick, wet crust with a diameter exceeding 1 cm that is surrounded by erythema. Most affected children present with multiple lesions of various ages. General symptoms are absent, but a local inflammatory lymph node reaction is a rule. At the beginning, the differential diagnosis includes other vesicular eruptions, such as herpes simplex and varicella. However, the evolution is typical and rapidly differentiates the diseases. Although of mild severity, the disease is extremely contagious and the affected child should be kept apart from other children until an effective treatment has been applied. A basic treatment that combines disinfection with a chlorhexidine-based or povidone-iodine–based soap and additional bacitracin zinc ointment in patients with limited lesions, or fusidic acid cream (not available in the United States) in cases of more extensive lesions, is generally sufficient. Oral antibiotics are rarely needed. Mupirocin should be reserved for the treatment of *S. aureus* nasal carriage.

Folliculitis

Folliculitis is defined as a pyoderma that involves the hair follicle and its immediate surroundings. It manifests as a series of raised painful reddish lesions with indurated bases, each of them centered on a hair follicle. Extensive folliculitis of the bearded area of the face is called *sycosis barbae.* General symptoms are usually absent, and local antiseptic measures are the treatment of choice.

Furuncles and Carbuncles

Furuncles (boils) represent extension of the infectious process involving the hair follicle and are located, by definition, on the hairy areas of the body, with a predilection for the face, neck, axilla, and buttocks. The disease starts as a painful red nodule and rapidly evolves into a hot, painful, raised, and indurated lesion with a diameter of 1 to 2 cm. Its evolution is characterized by the appearance of a yellowish area in its center. On rupture (either spontaneous or surgical), it liberates a small amount of yellowish creamy discharge of purulent and necrotic material. Secondary foci from autoinoculation are frequent. General symptoms are normally absent. Local treatment is usually sufficient. In case of recurrent episodes, testing for nasal carriage and appropriate eradication may be necessary, although its effectiveness in reducing the risk of recurrence is not clearly shown.

CA-MRSA is a particular issue in furuncles.[254,445] They are often centered by a necrotic spot, are multiple, and occur in outbreaks. Lesions can progress to abscesses and cellulitis. In young patients with boils and systemic signs of infection, one must remember the risk of severe hemorrhagic pneumonia (see Fig. 194.8) or necrotizing fasciitis[446] (discussed in "The Journey to Invasive Disease" earlier).

Another remarkable situation is when furuncles are located around the nares or upper lip. Such lesions may lead to life-threatening septic thrombophlebitis of the cavernous sinus. Therefore, furuncles in this location should be treated with high-dose parenteral antibiotics.

Carbuncles are deep-seated infections that involve several hair follicles and result from the coalescence and spreading of the infectious process into the depths of subcutaneous tissue. They are usually localized at the base of the neck. The disease leads to the development of a central necrotic crater, which heals with the development of a hard hypertrophic violaceous scar. Fever and malaise are generally present. Carbuncles may be the source of bacteremia and require parenteral antibiotic therapy.

Hidradenitis Suppurativa

Hidradenitis suppurativa is a pyogenic infection of the apocrine sweat glands that manifests as crops of furuncles that develop in the axillary, perineal, and genital areas. After spontaneous drainage, hypertrophic scarring may occur. As in furunculosis, treatment is primarily limited to local care and topical disinfectants. Administration of oral antimicrobials is indicated only in the case of systemic symptoms.

Mastitis

Symptoms of *mastitis* occur in up to 25% of mothers in the United States who are breastfeeding, but the incidence of staphylococcal mastitis necessitating therapy is approximately 2.5%.[476] The infection develops most commonly during the second or third week of the puerperium. The diagnosis of mastitis is usually clinical, with patients presenting with focal tenderness in one breast accompanied by fever and malaise.

TABLE 194.10 Suggested Therapy for Native Valve and Prosthetic Valve Endocarditis Caused by Staphylococci

ANTIBIOTIC	FREQUENCY, DOSAGE, AND ROUTE	DURATION	COMMENTS
Native Valves ***Methicillin-Susceptible Staphylococci***			
Flucloxacillin (not in United States) or oxacillin or nafcillin	2 g IV q4h	4–6 wk	Addition of gentamicin no longer recommended for native valve endocarditis
Cefazolin (or other first-generation cephalosporins)[a]	2 g IV q8h	4–6 wk	Alternative for patients allergic to penicillins (not in case of immediate-type penicillin hypersensitivity)
Methicillin-Resistant Staphylococci			
Vancomycin	15–20 mg/kg IV q12h	4–6 wk	
Daptomycin	8–10 mg/kg IV q24h	4–6 wk	
Prosthetic Valves ***Methicillin-Susceptible Staphylococci***[b]			
Flucloxacillin (not in United States) or oxacillin or nafcillin	2 g IV q4h	≥6 wk	
with rifampin	900 mg IV q24h or 300 mg orally q8h	≥6 wk	Rifampin increases hepatic metabolism of numerous drugs, including warfarin
and gentamicin	3 mg/kg IV q24h or 1 mg/kg IM q8h	2 wk	
Methicillin-Resistant Staphylococci			
Vancomycin	15–20 mg/kg IV q12h	≥6 wk	
plus rifampin	900 mg IV q24h or 300 mg orally q8h	≥6 wk	
and gentamicin	3 mg/kg IV q24h or 1 mg/kg IM q8h	2 wk	

[a]American Heart Association.
[b]Rifampin plays a special role in prosthetic device infection because it helps kill bacteria attached to foreign material. Rifampin should never be used alone because it selects for resistance at a high frequency (about 10^{-6}).
Modified from Que YA, Moreillon P. Infective endocarditis. Nat Rev Cardiol. *2011;6:322–336; and Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America.* Circulation. *2015;132:1435–1486.*

Treatment includes analgesics, changing breastfeeding technique, and reversing milk stasis, often with the assistance of a lactation consultant. Continued breastfeeding should be encouraged in the presence of mastitis and generally does not pose a risk to the infant. Evidence has suggest that mastitis can be either prevented[477] or treated[478] with probiotics such as lactobacilli. When antibiotics are needed (i.e., in the presence of acute pain, systemic symptoms, or fever), those effective against *S. aureus* are preferred. Breast abscess is the most common complication of mastitis. It can be prevented with early treatment of mastitis and continued breastfeeding. Once an abscess occurs, surgical drainage or needle aspiration is needed.

Surgical Site Infection

SSIs are infections that occur at or near the incision site within 30 days of the procedure, or within 90 days if prosthetic material is implanted at surgery; they account for 20% to 30% of all hospital-acquired infections.[410] The commonly reported incidence rate of 2% to 5% of patients undergoing inpatient surgery is probably underestimated because of inadequate postdischarge surveillance.[479] *S. aureus* is the most prevalent pathogen that causes SSI for most types of surgery (15%–45% of cases),[409,410,423] especially after clean procedures. Gram-negative rods and enterococci are, however, the most prevalent pathogens that cause SSI after abdominal surgery.[435] Most infections are caused by *S. aureus* strains that are carried by the patient at admission to the hospital.[24] Hence, nasal carriage of *S. aureus* is a risk factor for subsequent infection in patients undergoing surgery, and decolonization has been recommended, especially for patients undergoing orthopedic or cardiothoracic procedures.[480]

SSIs are characterized by progressive edema, erythema, and pain around the surgical incision. General symptoms are frequently associated. Careful inspection of the wound, ulcer, or lesion is essential. If deeper structures are not involved, release of the stitches, repeated cleansing, and antibiotic coverage for 7 to 10 days is usually curative. If the infection involves deeper structures (e.g., bone) or foreign material (e.g., prosthetic devices), prolonged parenteral antibiotic therapy (4–6 weeks) may be necessary, and removal of the foreign material is warranted.[184] The evolution of wound infection is highly dependent on the patient's comorbidities. Healing may be delayed, particularly in vascular insufficiency and diabetes.

Erysipelas, Cellulitis, and Fasciitis

Superficial or deep extension of infection may result in erysipelas, cellulitis, and fasciitis. Erysipelas and fasciitis are commonly the result of *S. pyogenes*, but not exclusively. Fasciitis can arise from hematogenous seeding. An important common feature of all three entities is severe pain. In the case of erysipelas, soreness is associated with typical skin lesions. In the case of cellulitis or fasciitis, on the other hand, severe pain symptoms are disproportional with regard to the visible anatomic lesions (Fig. 194.16). Hence, high fever, severe local pain, and relatively meager clinical findings at visible examination are highly suggestive of one of these entities. The consideration is important because emergency surgical drainage is indicated in the case of fasciitis, and prompt intervention may be delayed because of the confounding picture.

Erysipelas is a superficial cellulitis with prominent lymphatic involvement, with an indurate, "peau d'orange" appearance with a raised border that is demarcated from normal skin. It often complicates edematous extremities and skin ulcers such as in varicose limbs. As in impetigo, it may be the result of mixed *S. pyogenes* and *S. aureus* infection. Clinical signs of sepsis with high fever are present. In most cases, an etiologic diagnosis is not established. Local sampling with puncture may be attempted, and blood cultures must be drawn. Prompt empirical treatment



FIG. 194.16 ***Staphylococcus aureus*** **cellulitis of the elbow in cancer patient with low neutrophil counts.** Pain was disproportional to visual appearance of the lesion. Patient was bacteremic.

should be started with parenteral antibiotics covering at least both staphylococci and streptococci.

In patients with underlying conditions, such as diabetic foot, mixed pictures of erysipelas and cellulitis may occur and may also be the result of gram-negative bacteria, including *P. aeruginosa.* Therefore the spectrum of empirical treatment should be broadened to cover these agents until the microbiologic results are available. If gram-negative bacteria or MRSA is suspected, the addition of aminoglycoside or vancomycin to a broad-spectrum β-lactam may be warranted.

Cellulitis involves deeper anatomic structures and does not produce the typical geographic skin lesion of erysipelas (see Fig. 194.16). Therefore it is more confusing and may be mistaken for nonspecific lesions such as trauma. Associated pain and fever are important signs. Cellulitis may be from multiple other organisms, including gram-negative bacteria, especially in patients with immunocompromise. Therefore, microbiologic sampling (including blood cultures) should be promptly followed by broad-spectrum antibiotic therapy with both anti–gram-positive and anti–gram-negative coverage. Radiologic examination is unnecessary in most cases of cellulitis. Computed tomography (CT) is useful when subjacent osteomyelitis is suspected, whereas magnetic resonance imaging (MRI) helps to differentiate cellulitis from necrotizing fasciitis and osteomyelitis from adjacent soft tissue infection.

Necrotizing fasciitis is the most severe condition and paradoxically causes the least superficial signs at visual observation of the skin and soft tissues. The pain may be so intense that opiate administration is required for relief. The condition is often the result of *S. pyogenes,* but *S. aureus* may be involved, especially in the presence of CA-MRSA.[254] Gram-negative bacteria, including *P. aeruginosa,* may be responsible, especially in patients with immunocompromise, and must be considered in the choice of initial empirical treatment.

Whatever its cause, fasciitis is an absolute emergency that necessitates immediate and generous surgical débridement and drainage. The evolution is a matter of minutes rather than hours and may rapidly result in amputation or death. Prompt clinical diagnosis and multidisciplinary evaluation are warranted. Imaging may help delineate the lesions, but urgent surgical exploration and fasciotomy should not be delayed. High-dose and broad-spectrum antibiotic therapy is necessary and can be readjusted after the bacterial pathogen has been isolated. In the case of *S. pyogenes* and severe refractory shock, compassionate use of intravenous immunoglobulins has been proposed[186] (see also "Nonmenstrual Toxic Shock Syndrome" earlier).

Management of Skin and Soft Tissue Infection

Localized lesions can be handled with disinfectant or topical antibiotics such as fusidic acid. However, increasing rates of resistance to topical agents have been reported. Mupirocin should be reserved for the treatment of *S. aureus* nasal carriage. Moderate and severe SSI should be treated with systemic antibiotic. A decision to hospitalize a patient is made based on clinical judgment (large abscesses, signs of systemic infection) and the at-risk characteristics of the patients (age <6 months, diabetes, or immunodeficiency). Surgical drainage is a major part of treatment, and most benign SSTIs are cured with drainage alone.[184]

Empirical therapy of health care–associated SSTI should cover multiresistant HCA-MRSA and thus include vancomycin or maybe linezolid. Empirical therapy of community-acquired SSTI is complicated by the occurrence of CA-MRSA. β-Lactams should be used with caution and according to the local epidemiology. Unlike HCA-MRSA, CA-MRSA is often susceptible to clindamycin and TMP-SMX, sometimes to tetracyclines, and mostly to linezolid. However, if coinfection with *S. pyogenes* is found and antibiotics are required, linezolid, vancomycin, or maybe tigecyclin should be considered for first-line treatment. If toxin secretion is an issue, linezolid might be preferred.[481]

Bloodstream Infection

BSI is defined as one or several positive blood cultures associated with general symptoms such as fever or hypotension.[482] Its incidence rate increased from 7.4 episodes per 1000 admissions in the 1950s[483] to 31.2 episodes per 1000 admissions in 2006.[484] Population-based studies have estimated the rate of BSI to be around 140 to 160 BSIs per 100,000 inhabitants/year in high-income countries.[432] The most common isolates are *E. coli* and *S. aureus,* with incidence rates estimated at 30 to 50 per 100,000 inhabitants/year and 20 to 35 per 100,000 inhabitants/year, respectively.[432,485] Higher incidence rates of *S. aureus* BSI are observed in countries with a greater burden of MRSA, in men, or at either extreme of life (<1 year and >75 years). Mortality of *S. aureus* BSI (15%–25%) has stabilized since the 1990s and varies as a function of age and underlying conditions.[414] Despite the increased prevalence of SSTI from CA-MRSA in outpatients in the United States, a parallel increase in CA-MRSA bacteremia or endocarditis has not been reported.[485] Studies have suggested that PVL-positive strains are more likely to infect skin and soft tissues (see previous section).

BSI is usually divided into two categories: nosocomial BSI, wherein positive blood cultures occur 2 days or more after hospital entry; and community-acquired BSI, which occurs in the community or before 2 days of hospitalization. However, the increasing number of individuals treated in outpatient programs makes these two categories progressively overlapping. Hence, community-acquired BSI may be more appropriately referred to as *community-onset BSI,* which is further subdivided into *health care–associated BSI* (HCA-BSI) and *community-associated BSI* (CA-BSI). The subdivision is similar to that made between HCA-MRSA and CA-MRSA.

Community-Onset Bacteremia

Community-onset HCA-BSI is comparable with nosocomial BSI in terms of risk factors of multiresistant organisms. These include intravenous devices, a history of surgical treatment, and dialysis. In contrast, community-onset CA-BSI, which occurs in patients without underlying conditions, is mostly from antibiotic-susceptible organisms and always associated with a detectable infected focus, including SSTI, deep-seated abscesses, pneumonia, osteoarticular infections, or infective endocarditis.[486] Of note, patients on dialysis are at particularly high risk for staphylococcal endocarditis and represent a distinct at-risk group for this disease.[426,487]

Nosocomial and Health Care–Associated Bloodstream Infection

S. aureus is the leading cause of nosocomial BSI and HCA-BSI, together with CoNS.[409,410] These are mostly associated with the presence of intravascular catheters or devices, procedures in contaminated sites, SSI, and sometimes *S. aureus* pneumonia.[409] Complications involve peripheral metastatic foci, which can reveal themselves later. Nosocomial *S. aureus* bacteremia enters in the differential diagnosis of any hospital-related febrile or septic episodes. The risk for patients with catheter-induced *S. aureus* bacteremia to develop infective endocarditis is about 10%.[280,488] Thus, catheter-related *S. aureus* bacteremia must be taken very seriously and consideration given to excluding infective endocarditis with transesophageal echocardiography.[489–491]

Management of *Staphylococcus aureus* Bloodstream Infection

With *S. aureus,* even a single positive blood culture should prompt initiation of antibiotic therapy, sampling of blood for follow-up cultures, determination of the source and extent of infection, and finally search for endocarditis with transesophageal echocardiography.[490] Approximately one-third of patients with *S. aureus* BSI develop metastatic complications, especially in cases involving prosthetic material. The strongest indicators of clinical complication are a positive result of follow-up blood culture after 48 to 96 hours of treatment and persistent fever at 72 hours.[492]

Removal of the original focus is a golden rule, especially in the case of infected intravascular material or prosthetic devices. Failure to do so is strongly associated with recurrence. In the case of difficult-to-remove implanted catheters, the use of antibiotic locks between infusions may be attempted, but success is variable.[493] In case of a removable infection source (e.g., a catheter), a 10-day to 14-day post–catheter removal antibiotic treatment may be appropriate: (1) after the removal of all prosthetic material and endovascular catheter; (2) after the exclusion of endocarditis; (3) as long as the follow-up blood cultures drawn 2 to 4 days after initial positive cultures are negative for *S. aureus*; (4) if the fever has vanished within 72 hours after the initiation of antistaphylococcal therapy; and (5) when the absence of metastatic foci has been confirmed.[493,494]

Although no consensus recommendations exist regarding therapy, most authorities advocate a 14-day course of antibiotic treatment in case of BSI related to a removable catheter or drainable localized infection.[495] Deeper infections, such as arthritis, osteomyelitis, and endocarditis, must be treated with antibiotics for 4 to 6 weeks, with or without surgery depending on individual circumstances (see Chapters 80, 81, 103, 104, and 105 for more detailed recommendations). Empirical antibiotic treatment must take into account the probability of methicillin-resistant staphylococci, which may represent more than 50% of staphylococcal infections in the hospital milieu.[490]

Infective Endocarditis

Infective endocarditis is one the most severe complications of *S. aureus* bacteremia. The disease is uniformly lethal if not treated with antibiotics with or without surgery. *S. aureus* endocarditis typically follows an acute course with multiple peripheral septic emboli, valve destruction, myocarditis, and mixed cardiogenic and septic shock. Appropriate care involves a multidisciplinary evaluation, including infectious disease and microbiology experts, cardiologists, intensive care specialists, cardiac surgeons, and sometimes neurologists.[489,491]

Epidemiology

In spite of improved health care, the overall incidence rate of infective endocarditis has remained at 2 to 8 cases per 100,000 population per year over the last 40 years.[426–428,496] Risk factors have, however, been changing. Chronic rheumatic heart disease, which was a prime risk factor in the preantibiotic era, is being replaced by other at-risk groups, including patients with age-related degenerative valve lesions, prosthetic valves,[497] intracardiac devices, or intravascular prostheses. Coexisting conditions such as hemodialysis,[498] diabetes, intravenous drug use, or HIV infection increase the risk for infective endocarditis.[487]

As a consequence, the epidemiology of infective endocarditis has changed; the mean age of patients with infective endocarditis has increased from 30 years in the 1950s to older than 60 years since the 1990s. The incidence of infective endocarditis is now highest in men aged 75 to 79 years.[427] Oral streptococci, which are still a leading cause in developing countries, have been supplanted by *S. aureus* and CoNS, especially in industrialized countries.[422,427,499,500] This finding also correlates with the fact that the portal of entry has become more often cutaneous or procedure related than dental. Accordingly, patients with health care–associated infective endocarditis may represent up to one-fourth to one-third of all cases of infective endocarditis in recent studies.[427,499]

Pathogenesis

Role of Bacterial Adhesins

The pathogenesis of *S. aureus* endocarditis implicates a close relationship between certain *S. aureus* surface adhesins (MSCRAMMs) and host proteins present on the surface of damaged or inflamed valves. Physically damaged endothelia are covered by a meshwork of fibrin, platelets, and numerous host matrix proteins.[501]

S. aureus is extremely well equipped with both surface-bound and secreted factors that mediate tissue colonization and invasion (see Table 194.3 and "The Journey to Invasive Disease" earlier). With a system developed in *Lactococcus lactis,*[502] Que and colleagues[81,503] showed that *S. aureus* ClfA was necessary and sufficient for early valve colonization and infection in rats with experimental infective endocarditis but not sufficient for invasive and persistent disease. The same authors showed that fibronectin-binding protein A (FnBPA) promoted both early valve colonization and persistent infection. FnBPA is a peculiar MSCRAMM that carries at least three binding specificities, to fibronectin, to fibrinogen, and to elastin.[504] Construction of truncated and chimera proteins indicated that although fibrinogen binding was necessary and sufficient for early valve colonization, as observed with ClfA, fibronectin binding was necessary for further invasion and persistence. This invasive phenotype was associated with the capacity of fibronectin binding to trigger active internalization of staphylococci into eukaryotic cells, both in vitro and in vivo. Thus, valve infection proceeds through consecutive binding to fibrinogen, for early colonization, and fibronectin, for invasion and persistence.[81] Such interadhesin cooperation could also occur between other MSCRAMMs, adding even more flexibility to the already wealthy set of *S. aureus* surface determinants.[81,82]

In the case of physically intact but inflamed endothelia, *S. aureus* fibronectin-binding proteins might be of primary importance. During inflammation, endothelial cells express integrins of the β_1 family, which can bind plasma fibronectin at the luminal pole of the cell. The resulting fibronectin coat functions as a ligand surface for circulating *S. aureus* that expresses fibronectin-binding proteins. The contact between the adhesin and its ligand triggers the active internalization of *S. aureus* by endothelial cells and by other cells.[505] Once internalized, *S. aureus* may either persist locally, protected from host defenses and antimicrobial therapy, or multiply and secrete hemolysins (see Tables 194.1 and 194.3), which lyse the host cell and allow bacteria to spread both locally and to distant organs. This second scenario probably explains many cases of infective endocarditis on anatomically normal valves.

Role of Platelets

The role of coagulation is critical in funneling circulating *S. aureus* clumps toward damaged endothelia (see "Contribution of Coagulation" earlier). However, bacteria-induced platelet activation is a double-edged sword. On the one hand, platelets contribute to the formation of the vegetation. On the other hand, platelets also contribute to antiinfective host defenses by releasing AMPs and inflammatory mediators. These cationic AMPs are contained in the α-granules of thrombocytes and are released into the surroundings by activated platelets. They kill numerous gram-positive organisms by perturbing their membrane potential.[506,507]

Experimental evidence indicates that platelet-resistant mutants of staphylococci or streptococci have an increased ability to produce endocarditis in animals. In addition, clinical studies have indicated that isolates of *S. aureus* recovered from patients with endocarditis are more often resistant to platelet-induced killing than *S. aureus* isolated from other infected sites.[508] Resistance of *S. aureus* to AMPs is mediated by the *dlt* and *mrp* operons (see "Immune Evasion" section earlier). Additional resistance strategies include binding and inactivation of AMPs by staphylococcal proteases such as Sak and Aur (see Table 194.1).[507,509]

Experiments have confirmed that fibrinogen adherence and platelet aggregation cooperated to induce experimental endocarditis in rats.[510] In addition, interaction with vWF is also involved.[120] Finally, retrospective studies suggested that patients on prior aspirin therapy were significantly less likely to have vegetations on cardiovascular implantable electronic device leads or heart valves than those who had not received it,[511] yet without any impact on survival[511] or embolism.[512,513]

This raises the question regarding whether antiaggregant therapy might be useful in the prevention or treatment of infective endocarditis. Experimental antiaggregant prophylaxis is therefore being revisited in experimental models and has generated promising results.[121] Likewise, antiaggregant therapy with acetosalicylic acid decreases the severity of experimental endocarditis from *S. aureus.*[514]

One randomized, double-blind, placebo-controlled trial showed that a daily dose of aspirin (325 mg orally q24h) in addition to the conventional antibiotic therapy did not reduce the risk of embolic events in patients with infective endocarditis and was even associated with an increased risk of bleeding.[515] Similarly, anticoagulants increased the risk of secondary bleeding at the site of septic emboli, including hemorrhagic stroke, and are not recommended.[516,517] However, these studies did not look at endocarditis prevention sensu stricto, because the studied patients had already developed valve infection and thus were more prone to have secondary bleeding. A more realistic protocol should include patients who are at risk of endocarditis and are taking or not taking prophylactic antiaggregants, and test whether or not they have a modified risk of contracting endocarditis.

Host Defenses and Prevention

The role of host defenses is marginal in infective endocarditis. Once staphylococci have colonized the valves, their intrinsic procoagulant activities (e.g., fibrinogen polymerization by coagulase and platelet activation by fibrinogen-binding protein) trigger further deposition of platelets and fibrin on top of the microorganisms, thus providing a protective niche inside the vegetation. Moreover, *S. aureus* can be internalized into endothelial cells via bridging with fibronectin (see previous discussion). Both cases result in a failure of professional phagocytes to eradicate the organisms (see also "Immune Evasion" earlier).

Killing by T-cell–mediated effectors is not operative in endocarditis. Therefore, the only alternative is antibody-mediated protection, which could act before colonization by blocking *S. aureus* surface adhesins or by increasing the speed of blood clearance via opsonization. Active research is dedicated to such an approach, but few promising results are available yet. The limitation of preventive vaccines in endocarditis might be the short (1-minute to 2-minute) delay between blood invasion and valve colonization, which leaves a small window for antibodies to be active.

Other preventive measures are scarce. Because *S. aureus* is a ubiquitous skin colonizer, prevention starts with hygiene and disinfection. Decontamination of carriers and proper antisepsis at the site of injection and catheter placement is mandatory. Antibiotic prophylaxis may be useful but is limited to cases of medicosurgical procedures in the area of well-defined infective foci (see Chapter 83 for details). Thus, detection of patients with risk factors, and respect for proper hygiene and antisepsis measures, remain the cornerstones of *S. aureus* endocarditis prevention.

Clinical Spectrum

S. aureus infective endocarditis often manifests as an acute septic syndrome with fever, tachycardia, and often hypotension. Dyspnea may be present from congestive heart and from septic pulmonary emboli in the case of right-sided endocarditis. General signs such as arthralgia or myalgia, back pain, and pleuritic pain are present in 10% to 50% of cases. Specific signs include a new cardiac murmur, usually of valve regurgitation, in about 90% of cases; septic emboli in the form of petechiae and Janeway lesions; and central nervous system manifestations in up to one-third of patients (Figs. 194.17, 194.18, and 194.19).

Cardiac failure is a major indication for emergency valve replacement. A defect in atrioventricular conduction may represent a mycotic aneurysm of the sinus of Valsalva, usually the noncoronary cusp. Transesophageal echocardiography is useful in detecting this complication. Large vegetations (≥1 cm) are relatively frequent in *S. aureus* endocarditis and have been associated with an increased risk of embolization. However, the risk of embolization rapidly decreases within the first days of efficacious therapy.[518]

Vascular Complications

Embolic skin lesions encompass Janeway spots, occurring in acute endocarditis and often containing bacteria (see Fig. 194.17), or more delayed immune-related vasculitis (Roth spots) that are a sign of relatively chronic infection and are not a usual feature of acute *S. aureus* endocarditis. Larger septic emboli from broken-off vegetations can occlude the coronary or peripheral arteries, especially in the brain. Mycotic aneurysms are found in up to 15% of patients with bacterial endocarditis, and probably more frequently in *S. aureus* endocarditis. They may arise either from direct invasion of the arterial wall by the infecting organisms, from septic embolization of the vasa vasorum, or from the deposition of immune complexes that trigger local inflammation and weakening of the arterial wall.



FIG. 194.17 Embolic skin lesions (Janeway spots) in framework of acute mitral valve endocarditis caused by *Staphylococcus aureus*.



FIG. 194.18 *Staphylococcus aureus* endocarditis of the mitral valve. Note large ulcerovegetative lesion on anterior leaflet. *(Courtesy Drs. A. Lobrinus and I. Letovanec, Pathology Institute, Lausanne University.)*

Neurologic Complications

Cerebral complications are frequent, occurring in 15% to 25% of patients[517,519] and are associated with poor outcome.[520] Systematic brain imaging with MRI may reveal cerebral events in up to 80% of patients; among them, 50% are asymptomatic.[521] Lesions include ischemic and hemorrhagic stroke, transient ischemic attacks, silent cerebral embolism,

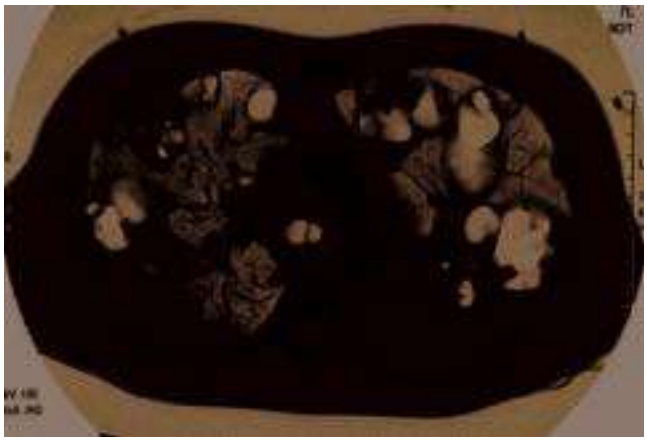

FIG. 194.19 Chest computed tomography scan of 30-year-old intravenous drug user with tricuspid valve *Staphylococcus aureus* endocarditis and bilateral lung abscesses and empyema.

abscesses, mycotic aneurysms, encephalopathy, and meningitis.[517,519,521] Control of the infection is essential because embolization sharply decreases thereafter.[518] Patients with large mobile mitral vegetations are at higher risk of embolism.[517] Recurrent embolization after the onset of efficacious therapy may be an indication for urgent valve replacement. This decision is difficult because anticoagulation therapy during extracorporeal circulation and after valve replacement puts the patients at increased risk of secondary intracerebral hemorrhage.[489,517] However, recent studies have indicated that early surgery (i.e., within the hospital stay) is usually beneficial.[522]

Diagnosis

Criteria for the diagnosis of infective endocarditis changed with the use of echocardiography and are discussed in detail in Chapter 80. In *S. aureus* endocarditis, the first two blood cultures are positive in more than 90% of cases. The volume of blood cultures is critical because persistent bacteremia in infective endocarditis is often of low level, representing 1 to 100 bacteria per milliliter of blood. For each culture, 8 to 12 mL of blood should be drawn, with careful antiseptic precautions.

Management of *Staphylococcus aureus* Endocarditis

Treatment options are listed in Table 194.10[489,491] (see Chapters 80 and 81).

Management of Right-Sided Endocarditis in Injection Drug Users

S. aureus is the most common, yet not unique, pathogen in right-sided endocarditis in intravenous drug users.[523] Although standard treatment of left-sided *S. aureus* endocarditis requires 4 to 6 weeks of antibiotic therapy (see Table 194.10 and Chapter 80), the duration of treatment for right-sided endocarditis can be considerably shortened in injection drug users[489,491,524,525] because right-sided endocarditis has somewhat different physiopathologic features and is easier to cure or can heal spontaneously in experimental models. Moreover, the prognosis is less severe than in left-sided valve infection. Agents proven appropriate for 2-week treatment of right-sided endocarditis are nafcillin and oxacillin. Combination with an aminoglycoside was shown to be not necessary.[524,525] However, short-course therapy is limited to favorable conditions, including infection with an MSSA, prompt response to treatment, infection limited to the tricuspid valve, <20-mm vegetations, absence of extracardiac infected sites such as empyema, and absence of severe immunodepression (>200 CD4 cells/mL) in HIV-infected patients.[489,491,524]

Another proposed regimen that may allow intravenous-oral switch therapy is ciprofloxacin plus rifampin.[526,527] Today newer anti–gram-positive quinolones (e.g., levofloxacin or moxifloxacin) should be preferred to ciprofloxacin, because they are less prone to select for resistance. Some authors do not start rifampin until 3 days of effective therapy have been given, hoping to reduce the chance of secondary rifampin resistance. Daptomycin is an alternative for *S. aureus* right-sided infective endocarditis therapy (see Chapter 31).[236] However high doses (10 mg/kg/24 h) should be used, and combination with oxacillin or fosfomycin to avoid the development of resistance has been suggested (see "Daptomycin" section earlier). On the other hand, glycopeptides (vancomycin or teicoplanin) should not be used for short-course treatment because of their lack of bactericidal activity and poor vegetation penetration.

Infections of the Central Nervous System

Meningitis

S. aureus meningitis is an uncommon disease that accounts for about 5% of cases of bacterial meningitis.[528] Two different modes of pathogenesis have been described: (1) postoperative meningitis, associated with neurosurgical procedures, shunt devices, or head trauma; and (2) hematogenous meningitis, sometimes in the context of infective endocarditis.[529]

Postoperative and spontaneous *S. aureus* meningitis are two different clinical syndromes. *Postoperative meningitis* usually appears as hospital-acquired infection in young people, and most cases are associated with ventricular shunt devices,[530] recent neurosurgery, or cerebrospinal fluid leak. On the other hand, *spontaneous meningitis* is a community-acquired infection associated with a variety of clinical sources (primary bacteremia, infective endocarditis, osteomyelitis) that mainly affects older patients with severe underlying conditions.[529] The mortality rate associated with *S. aureus* meningitis is high, around 50%; the mortality rate of spontaneous meningitis is usually higher than that of postoperative meningitis.[530–532]

Spinal Epidural Abscess

Spinal epidural abscess is a rare infection of the central nervous system, usually occurring after operation or trauma, that can be associated with damage to the spinal cord and nerve roots. *S. aureus* is the leading bacterial pathogen causing spinal epidural abscess, accounting for about two-thirds of cases. Classically, patients have back pain, fever, and neurologic signs.[533] In general, surgical decompression and a long course of intravenous antibiotics are required in order to achieve a successful outcome.

Pulmonary Infections

Epidemiology

S. aureus is responsible for 2% to 5% of CAP for which hospitalization is required,[534,535] but for 20% to 30% of hospital-acquired pneumonia (HAP).[409] The prevalence of *S. aureus* CAP varies widely by country and continent.[536] Although *S. aureus* CAP cases may cluster with influenza cases, *S. aureus* CAP occurs year round. In recent studies, patients with *S. aureus* CAP had similar demographic characteristics, comorbidities, and presenting signs and symptoms compared with those with non–*S. aureus* CAP.[534] Mortality is high (10%–15%) compared with pneumococcal CAP (about 5%),[534] and is often associated with a more severe course.[535]

In the hospital, *S. aureus* is becoming the pathogen most frequently responsible for nosocomial pneumonia. Its frequency increased from 13% of the cases between 1981 and 1986 to 25% between 2011 and 2014,[409] with a proportion of MRSA that peaked at 55% during the last decade.

Clinical Spectrum

The clinical manifestations of *S. aureus* pneumonia are frequently indistinguishable from those of pneumonia caused by other pathogens,[534] although the pneumonia caused by *S. aureus* is typically a necrotizing infection with rapid progression to tissue destruction and cavitation. The infection may result either from airborne contamination or aspiration or from hematogenous seeding during bacteremia or right-sided endocarditis. In both cases, the pulmonary infection can lead to local complications, such as abscesses and pleural empyema.

Pneumonia in young, previously healthy adults with a preceding influenza-like illness characterized by severe respiratory symptoms, hemoptysis, high fever, leukopenia, very high C-reactive protein level (>400 mg/L), hypotension, and a chest radiograph that shows multilobar cavitating alveolar infiltrates should lead one to suspect CA-MRSA infection.[411] The fatality rate of CA-MRSA necrotizing pneumonia can be as high as 60%.[537] Airway bleeding, erythroderma, and leukopenia are strong predictors of mortality.[537] Fig. 194.8 illustrates a case of acute

hemorrhagic pneumonia from PVL toxin–secreting *S. aureus* associated with influenza A infection in a 20-year-old patient (see "Killing Leukocytes" section earlier).

S. aureus remains a common cause of pleural empyema and accounts for about one-fifth of the cases.[538] Acute empyema usually arises by means of direct extension from *S. aureus* pneumonia or lung abscess. It is also often seen as a complication of thoracic surgery. Radiologic findings on CT scan or ultrasound scan confirm the clinical suspicion. Demonstration of a pleural air-fluid level in the absence of a previous thoracocentesis suggests a bronchopleural fistula, another feared complication of *S. aureus* infection. Fig. 194.19 illustrates the case of multiple lung abscesses and pleural effusion in a young patient with right-sided endocarditis.

Therapy

Rapid institution of appropriate antibiotic therapy is essential. Delay is associated with poor outcome. Because the rapid determination of the etiology of severe pneumonia is possible only in a limited number of cases, initial broad-spectrum antibiotic therapy that treats MRSA and other nosocomial pathogens should be instituted early in the cases of health care–associated pneumonia. However, deescalation therapy should occur whenever possible; in particular, a switch to a more rapidly bactericidal β-lactam agent should be done whenever possible according to susceptibility testing. Because of the low prevalence of MRSA in CAP,[534,536] routine empirical anti-MRSA antibiotics are not recommended in the initial treatment of CAP.[539]

For years, vancomycin was the only treatment for MRSA pneumonia, but the cure rate was disappointing. This led to the recommendation to increase trough concentrations of vancomycin in the serum to 15–20 μg/mL for severe infections. Linezolid is an alternative to vancomycin for the treatment of MRSA pneumonia. Clinical outcomes of *S. aureus* HAP and VAP treated with linezolid therapy were found to be significantly better than in those treated with vancomycin in retrospective subgroups analysis. However, these results were not confirmed in a randomized trial. Thus it is still unclear whether one drug is better than the other. For patients with pneumonia caused by MRSA strains with vancomycin MICs of 1.0 mg/mL or more who require concomitant nephrotoxic therapy or who have preexisting renal failure, linezolid (600 mg IV, q12h, with possible later switch to oral therapy) is advised.[540]

The duration of treatment of *S. aureus* pneumonia is determined by the general picture of the disease. The less complicated cases of CAP without overt tissue destruction and without associated deep-seated infections respond to appropriate antibiotic therapy in 10 to 15 days. With the exception of MRSA pneumonia, 7 to 8 days of treatment is sufficient for most patients with HAP or VAP without bacteremia. In the case of surgical drainage of empyema, the treatment duration is adjusted according to cultures and persistence of the pleural effusion. In the case of right-sided endocarditis, therapy is prolonged to 4 weeks according to standard recommendations for endocarditis treatment (see previous discussion).

Osteoarticular Infections

S. aureus is the leading cause of bone and joint infections in both children and adults.[541] The prevalence of those infections is increasing, mainly because of a longer life expectancy, the rise in chronic diseases such as diabetes mellitus, and the increasing use of bone fixation devices and prosthetic joints in the elderly.[542]

Osteomyelitis

Epidemiology

Osteomyelitis has been described since antiquity. Its epidemiology has, however, dramatically changed over the last decades, in parallel with the increased use of medical devices in orthopedic surgery; the higher incidence of chronic disease, especially diabetes mellitus; and the emergence of resistant bacterial strains (e.g., MRSA and CA-MRSA).[543] In parallel, immunization programs led to the disappearance of causative organisms such as *Haemophilus influenzae,* and improved identification methods revealed new causative microorganisms (i.e., *Kingella kingae*). Nevertheless, *S. aureus* continues to be the leading organism, isolated in 30% to 60% of cases (Table 194.11).[486,544]

TABLE 194.11 Frequency of Osteomyelitis Caused by Various Microorganisms

	FREQUENCY OF OSTEOMYELITIS (%)			
	BLYTH ET AL.[547]	TICE ET AL.[557]	KREMERS ET AL.[542]	MURILLO ET AL.[543]
Microorganisms				
Staphylococcus aureus	65	ND	44	59
Coagulase-negative staphylococci	5	13.9	17	3
Non–group D streptococci	30	13.7	16	17
Pseudomonas aeruginosa	ND	4.4	7	2
Other	0	13.8	16	19
Total	100	100	100	100
Demographics				
No. of patients or episodes	20	454	760	618
Age (yr; median)	0.1–12 (5.4)	6–92 (51)	52 +/- 25	53–77 (65)

ND, Not determined.

The mortality of osteomyelitis has markedly decreased since the introduction of antibacterial therapy. In one pediatric case review, the mortality rate decreased from more than 30% before the introduction of sulfa derivatives (years 1936 through 1940) to approximately 13% afterward (from 1941 to 1945).[545] The mortality rate from osteomyelitis continued to decline with modern antibiotics and is now stabilizing at around 5%.[546]

The reported incidence of osteomyelitis has risen from around 3 cases per 100,000 population per year in 1997[547] to 24.4 cases per 100,000 population per year in recent population-based studies.[542] Whereas the incidence of osteomyelitis is increasing in adults, it has been declining in children younger than 13 years; in the area of the Greater Glasgow Board Health Center (United Kingdom), the incidence rate of osteomyelitis declined by about three times over the last 30 years and by two times during the 1990s.[547] Some studies reported, however, an increased incidence in children related to CA-MRSA.[548]

The incidence of osteomyelitis varies with age and the presence of underlying risk factors such as male sex (men are affected twice as often as women) or diabetes. Other risk factors include individuals with an increased risk of bacteremia, such as patients undergoing hemodialysis or intravenous drug users, and immunosuppression.

Pathogenesis

Lew and Waldvogel differentiated three different groups of osteomyelitis etiologies: (1) hematogenous osteomyelitis occurring mostly in children; (2) osteomyelitis due to local spread from a contiguous source after trauma or surgery; and (3) secondary osteomyelitis in patients with vascular insufficiency or concomitant neuropathy, with most of the cases following a foot soft tissue infection.[549] Some authors propose the addition of a fourth etiologic group of osteomyelitis occurring in special hosts with an increased risk for bone infection, including intravenous drug users, individuals with sickle cell anemia, and patients with Gaucher disease.

Healthy bone is generally highly resistant to infection. The two ways by which bacteria can infect the bones are: 1, hematogenous seeding; and 2, contiguous contamination. Both have epidemiologic and pathogenic correlates. Bone infection requires certain predisposing circumstances. In children with hematogenous osteomyelitis, the disease is usually located at the distal end of the long bone metaphysis, including the humerus, femur, and tibia.[548] The nature of the blood flow close to the growing plate may be responsible. Terminal arterioles followed by stagnant blood in the venous sinusoids may facilitate the settlement of

blood-borne staphylococci. A similar model may apply for vertebral osteomyelitis (Fig. 194.20A), wherein blood flow at the vertebral interface is somewhat similar. In addition, microtrauma (or macrotrauma) to bone may facilitate infection by affecting the local blood supply or exposing host matrix proteins to which staphylococci can adhere.

On the bacterial side, *S. aureus* is equipped with several surface adhesins or MSCRAMMs (see Tables 194.1 and 194.3, and "The Journey to Invasive Disease" earlier), including collagen-binding protein and sialoprotein-binding protein, which were shown to promote experimental osteoarticular infection. After local settlement, secreted proteases, such as aureolysin, and toxins, such as alpha-type PSMs,[550] promote profound alteration in bone remodeling, tissue destruction, and invasion, as indicated by a decreased virulence of *sae, sar,* and *agr* mutants that are affected in secretion of virulence factors.[551,552] The combined effect of both *S. aureus* factors and immune cell-mediated production of oxygen radicals and cytokines results in local necrosis and abscess formation. If adequate antibacterial therapy is given, the nascent abscess can heal totally. Alternatively, bone remodeling and necrosis can extend and circumscribe devitalized bone fragments, or sequestra, floating in the abscess cavity (see Fig. 194.20B). The formation of necrosis and sequestra exemplifies the evolution of acute osteomyelitis to the chronic form (see subsequent discussion) and requires the combination of antibiotics and surgical débridement and sequestrectomy for successful treatment.

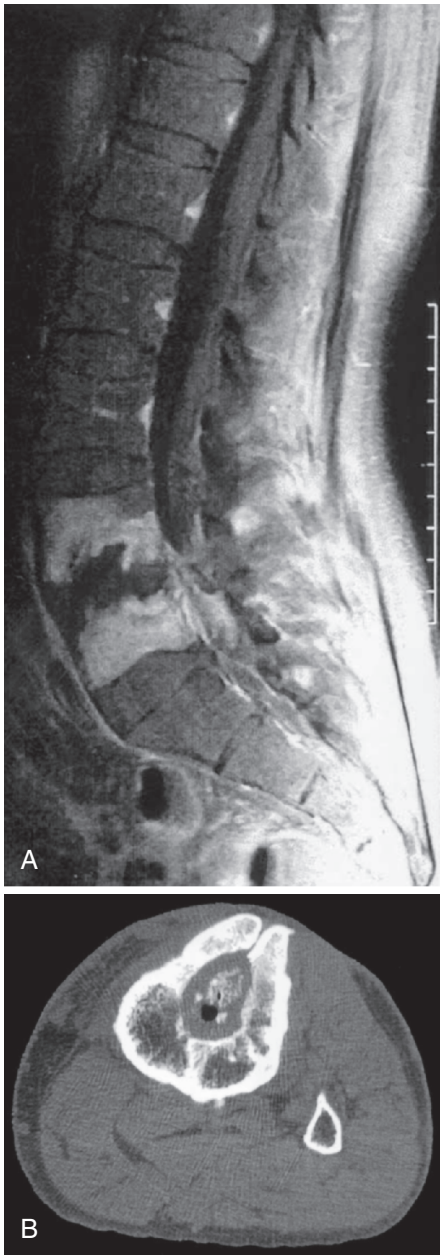

FIG. 194.20 Osteomyelitis. (A) T1-weighted magnetic resonance image of L4-L5 lumbar osteomyelitis after injection of gadolinium contrast medium. (B) Chronic osteomyelitis of left tibia. A vast necrotic cavern containing sequestrum surrounded by air can be seen. A fistula with drainage to the skin is also present. *(Courtesy Dr. U. Flückiger, Hirslanden Klinik, Aarau, Switzerland.)*

Clinical Features

Osteomyelitis can be classified according to pathogenesis, to localization, to the presence of an implant, or to anatomy and comorbidity. It is conventionally divided into acute and chronic disease. Acute osteomyelitis is defined as a first episode that responds to medical treatment within 6 weeks. It is usually hematogenous and predominant in children and elderly patients. Symptoms are those of an acute septic syndrome with chills, high fever, malaise, and local pain and swelling. Blood cultures are positive in about 50% of cases, and blood or tissue cultures in 65% of cases.

Chronic osteomyelitis is considered in all other situations, including the relapse of previously treated or untreated disease and infection arising by contiguity. The process can evolve over months or even years and is characterized by low-grade inflammation, the presence of necrosis, sequestra, pus, fistula, and recurrences.[549]

Aside from open-wound fractures, contiguous osteomyelitis involves diabetes-related and unrelated vascular diseases and prosthesis-related osteomyelitis. Diabetes-related osteomyelitis and vascular-related osteomyelitis principally involve the feet. They complicate chronic ulcers, which may be paradoxically painless because of associated neuritis. The ulcerative lesion should be explored gently, but in depth, with a surgical probe. If the probe encounters the bone surface, osteomyelitis is present.[553] Other investigations involve radiology and surgical biopsy. Cultures of deep tissues and bone biopsy are mandatory for microbiologic diagnosis. Cultures of surface swabs and fistula fluids mostly yield skin contaminants but not the responsible pathogen.

Diagnosis

Diagnosis of osteomyelitis integrates clinical signs, radiology, and microbiology. However, clinical signs may be scarce in chronic infection. Imaging techniques are of primary importance, and dominated now by MRI because it is highly sensitive and provides information on both solid and soft tissues. Nuclear studies (bone scans) are performed only if metal hardware precludes MRI or CT.

Microbiologic cultures are indispensable to guide therapy. Whenever possible, both blood cultures and tissue biopsies should be performed. Important to note, chronic infection may be associated with persistent forms of *S. aureus,* such as SCVs (small colony variants), especially if aminoglycosides have been used in conjunction with osteosynthetic material (see earlier "Culture and Identification" section).[554] The diagnostic laboratory should be made aware of this possibility so appropriate measures may be taken.

Therapy

Rapid institution of antibiotic therapy is mandatory to prevent bone necrosis and the passage of acute osteomyelitis to more problematic chronic disease. The duration of drug treatment in most studies is 4 and 6 weeks but may vary up to 10 weeks or more in complicated situations. A trial suggested that 6 weeks of treatment is noninferior to 12 weeks.[430] Empirical treatment of osteomyelitis should include adequate coverage of MRSA in areas where CA-MRSA is endemic. Adequate representative cultures should be obtained to ensure the early identification of MRSA.

The classic regimens are as follows: (1) for penicillin-susceptible *S. aureus,* intravenous penicillin G, 4 millions units IV q6h; (2) for penicillin-resistant *S. aureus,* intravenous nafcillin, oxacillin, or, outside the United States, flucloxacillin, 2 g IV q6h; and (3) for MRSA, intravenous vancomycin, 1 g IV q12h (followed a loading dose as proposed in "Therapeutic Monitoring of Vancomycin in Adult Patients"). Clindamycin might be a good alternative to vancomycin in the presence of susceptible CA-MRSA (see Chapter 104).

In children with hematogenous *S. aureus* osteomyelitis, a relatively short course (4–7 days) of intravenous treatment is often followed by oral therapy. A series comparing 131 children showed equivalence

between short (total of 20 days) and long (total of 30 days) treatment durations.[555] Intravenous treatment (median 3.7 days) was followed by oral amoxicillin, first-generation cephalosporin, or clindamycin for the remaining period. Oral treatment is currently not recommended in adults, although newer quinolones may be appropriate because of their high bioavailability. However, outpatient therapy is also increasingly used. Although controversial, some workers use ceftriaxone (2 g once a day), which was shown to be effective against *S. aureus* osteomyelitis in two studies.[556,557]

Finally, osteomyelitis from multiresistant MRSA remains difficult to treat. Although vancomycin is still recommended as first-line therapy, daptomycin and linezolid are good alternatives.[558]

Native Joint Septic Arthritis

Septic arthritis is a disease that usually arises in elderly people and very young children.[546,559] *S. aureus* remains the most frequent cause of septic arthritis in both children and adults.[429,543] Previous joint pathology (e.g., rheumatoid arthritis, osteoarthritis, crystal arthropathy) and diabetes are risk factors. The disease may follow both hematogenous seeding and local trauma and may be of iatrogenic nature in the case of joint puncture or arthroscopy. Postarthroscopic septic arthritis has a prevalence of around 14 cases per 10,000 procedures.[560] Symptoms associated are acute pain and joint swelling. Joint destruction occurs within a few days from both bacterial and host inflammatory factors and probably also ischemic lesions from the increased intraarticular hydrostatic pressure. Therefore, patients with underlying arthritis with acute pain in a single joint should undergo aspiration immediately; the fluid should be examined for cell count, chemistry, and gout crystals (if appropriate), and culture should be performed.

The prognosis of hematogenous arthritis in children is good. In adults, the mortality rate is around 11%[429] and the prognosis is mostly associated with that of the underlying disease (i.e., rheumatoid arthritis or endocarditis or other deep-seated condition responsible for the initial bacteremia). Medical treatment is identical to that of osteomyelitis. Open joint drainage is usually unnecessary, except for hip infections in children, where it may help prevent necrosis of the femoral head.

Septic Bursitis

Septic bursitis is an acute infection that involves the periarticular bursa. It is most often located in pressure areas such as the olecranon and the patella. It manifests as an acute juxtaarticular inflammation. The overlying skin is usually inflamed. Unlike arthritis and osteomyelitis, the underlying bone and joint are usually painless at pressure or mobilization. The portal of entry is likely to be local, such as spread from cellulitis. More than 80% of bursitis is the result of *S. aureus*.[561] Diagnosis is made with puncture and examination of the bursa fluid. Important to note, septic bursitis may be at the origin of both local and distant septic complication. Thus, careful clinical evaluation is mandatory. The prognosis is good but requires 2 to 3 weeks of appropriate antibiotic therapy. Repeated aspiration of the bursa is preferable to incision and drainage for patient with persistent swelling and pain. Patients with complicated cases may require hospitalization and intravenous treatment. Surgical excision of the bursa may be considered in the case of recurrences, once inflammation has subsided.

Prosthetic Joint Infections

With the use of perioperative antimicrobial prophylaxis and laminar airflow operating rooms, prosthetic joint infections are fairly rare, with an estimated incidence rate of 1.5 infections per 1000 joint-years.[431] The infection rate after primary joint replacement is usually less than 1% in hip and shoulder prostheses, less than 2% in knee prostheses, and less than 9% in elbow prostheses.[562] Sixty percent to 70% of infections occur during the first 2 years after surgery. Infection rates after surgical revision are usually considerably higher.

Implant-associated infections are typically caused by microorganisms that grow in structures known as biofilms. *S. aureus* and CoNS are the main causative organisms.[431,562] Infections within the 12 first weeks after implantation are considered early, or acute; infections that occur from 12 weeks to 24 months after implantation are considered delayed, or low-grade, infections; and those that occur after 24 months are considered late, or chronic.[562]

S. aureus is mostly responsible for early infection.[431] As for prosthetic heart valves, the organisms usually originate from the skin and are likely to be introduced at the time of operation. Although early symptoms may be acute, patients with low-grade or chronic infection may have few general symptoms, and the clinical signs may become centered around local pain and loosening of the prosthesis. Prosthetic joints remain susceptible to hematogenous seeding during their entire lifetime. The overall risk for prosthetic joint infection after *S. aureus* bacteremia is high, ranging from 30% to 40%.[563,564]

Blood culture results are often negative. Cultures of the fluid from the artificial joint are critical, preferably with broth culture, but can have negative results as well. Molecular diagnosis with PCR should be considered (see "Culture and Identification" earlier). If the prosthesis is surgically removed, multiple microbiologic samples should be taken at various sites of the prosthesis and bone cavity because bacteria may remain clustered in circumscribed areas.

Successful treatment combines surgical intervention and antibiotics. The suggested antibiotic duration is 3 months for hip prostheses and 6 months for knee prostheses. Intravenous therapy should be administrated for the first 2 to 4 weeks.[431] The addition of rifampin to a conventional antistaphylococcal regimen appeared clearly useful in patients with prosthetic joint infections. Rifampin has an excellent activity on slow-growing and adherent staphylococci. In general, retention of the implant should not be attempted. Débridement with retention is an option only for patients with early postoperative or acute hematogenous infection, if duration of clinical signs and symptoms is less than 3 weeks, if the implant is stable, and if effective therapy against biofilm microorganisms is available. The prerequisites for one-stage exchange are satisfactory condition of soft tissue, the absence of severe coexisting illnesses, and the absence of difficult-to-treat bacteria. In all other cases, a two-stage exchange is preferred, with a 2-week to 4-week interval between procedures (see Chapter 105).[431,565]

Pyomyositis

Primary pyomyositis, also called tropical myositis, infective myositis, pyogenic myositis, and myositis purulenta tropica, is a rare subacute purulent infection of skeletal muscles. It does not follow contiguous contamination and is most probably of hematogenous origin. The rarity of the disease is attributed to the resistance of muscles to infection. A history of muscle trauma is often reported.

Pyomyositis is frequently seen in Africa and the South Pacific, but it is rare in the Northern Hemisphere. Hence, it could be related to particular local conditions or bacterial properties. In a review of 676 cases, the disease occurred in all age groups. However, it was about twice as frequent in children and adults younger than 30 years than in older adults, and males were predominantly affected.[566] Any muscle may be involved, but the quadriceps and iliopsoas muscles were most often implicated, in 26% and 14%, respectively. *S. aureus* was the etiologic agent in about 80% of cases. In a series from the Amazon Basin of Peru, 11 of 12 patients carried the Panton-Valentine toxin suggesting that PVL might be involved (see "Killing Leukocytes" section earlier).[567]

Clinical symptoms evolve in three stages. They first start with the insidious onset of dull cramping and low-grade fever, general malaise, and muscle aches. Because only the aponeurosis is innervated, overt muscle pain may be delayed for 1 or 2 weeks, before frank abscess formation. Second, the formation of muscle abscess becomes symptomatic, with pain, muscle swelling, tenderness, and sepsis. Most patients are seen at this stage. If left untreated, the disease evolves into the third stage, with muscle destruction, local extension with osteomyelitis, or osteoarthritis, septicemia, and distant dissemination. Diagnosis involves radiographic imaging (CT and MRI) and bacteriologic diagnosis with blood cultures and muscle puncture. Treatment is essentially based on antibiotic therapy. Treatment duration is a matter of debate. Parenteral treatment is often recommended for 7 to 14 days, followed by oral treatment for up to 6 weeks. The prognosis before stage 3 is usually excellent.

ACKNOWLEDGMENT

This work was supported by Swiss National Science Foundation grants CR31I3_166124 and 320030_176216 (to YA Que) and 310030_143650 (to P. Moreillon) on staphylococcal disease and new phage therapy.

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195 *Staphylococcus epidermidis* and Other Coagulase-Negative Staphylococci

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SHORT VIEW SUMMARY

Microbiology

- A heterogeneous group of more than 40 species, coagulase-negative staphylococci are gram-positive cocci that are closely related to the intrinsically more virulent *Staphylococcus aureus* and differentiated from *S. aureus* by their inability to produce coagulase.
- *Staphylococcus epidermidis,* the most common clinically encountered species of coagulase-negative staphylococci, is a prominent part of the normal commensal flora of human skin. *S. epidermidis* owes its pathogenic success to three factors: (1) its normal niche on human skin, giving it access to any device inserted or passed through the skin; (2) its ability to adhere to biomaterials and elaborate biofilm; and (3) changes in the human host population resulting in greater numbers of immunosuppressed patients and greater use of bioprosthetic devices.[1–3]
- Other noteworthy species of coagulase-negative staphylococci include *Staphylococcus saprophyticus,* a common cause of uncomplicated urinary tract infection in sexually active women; *Staphylococcus haemolyticus,* often resistant to glycopeptides; and *Staphylococcus lugdunensis,* a more virulent coagulase-negative *Staphylococcus* that mimics infections due to *S. aureus.*

Epidemiology

- *S. epidermidis* is a prominent cause of intravascular catheter–related infection and infection of a variety of medical devices, such as prosthetic joints, artificial heart valves, and cerebrospinal fluid shunts.
- Strains of *S. epidermidis* can establish predominance in hospital environments and spread from unit to unit, hospital to hospital, and country to country.

Diagnosis

- Differentiating "true" infection-causing coagulase-negative staphylococci from contaminants can, at times, be a diagnostic challenge. Finding coagulase-negative staphylococci at high numbers or repetitively in situations clinically consistent with infection is indicative of a true infection. Unfortunately, in some situations, infections due to coagulase-negative staphylococci can be indolent and diagnosis is difficult.

Therapy

- Most nosocomial *S. epidermidis* strains are multidrug resistant, and glycopeptide or alternative antistaphylococcal antibiotics directed at methicillin-resistant strains are used in treatment. Isolates of *S. epidermidis* with an oxacillin minimal inhibitory concentration (MIC) of 0.25 μg/mL or less may be treated with oxacillin or nafcillin. Vancomycin is the drug with which there is the most clinical experience in coagulase-negative staphylococcal infections, although case reports support use of daptomycin and linezolid. Most species are susceptible in vitro to the newer agents telavancin, dalbavancin, oritavancin, ceftaroline, and tedizolid, and to the older agents quinupristin-dalfopristin and tigecycline, but their clinical utility for coagulase-negative staphylococcal infections remains to be defined.
- In some situations, rifampin is added to the regimen for better activity against biofilm-associated organisms.
- Information is accruing with regard to treatment of biomaterial-based infections with the device in situ, but most of these data are anecdotal. In general, infected devices should be removed whenever possible. A number of biofilm-directed therapeutic modalities appear to hold promise.

Prevention

- Biomedical devices must be inserted or implanted with scrupulous attention to aseptic practices. There is great interest in developing biomedical devices that are less prone to bacterial adherence and infection.

The genus *Staphylococcus,* with more than 80 recognized species and subspecies (https://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date/prokaryotic-nomenclature-up-to-date.html) is one of the most abundant microbes inhabiting normal human skin and mucous membranes.[1,4] They infrequently cause primary invasive disease and are most commonly encountered by clinicians as contaminants of microbiologic cultures. However, because of relatively recent changes in the practice of medicine and changes in underlying host populations, coagulase-negative staphylococci, most notably *Staphylococcus epidermidis,* have arisen to become formidable pathogens.[2] *S. epidermidis* is a very common cause of primary bacteremia and is frequently encountered in infections of indwelling medical devices.[3–5] *S. epidermidis* owes its pathogenic success to two major features: its natural niche on human skin, thus resulting in ready access to any device inserted or implanted across the skin, and its ability to adhere to biomaterials and form a biofilm.[3–7] Infections caused by *S. epidermidis* are often indolent and may be clinically difficult to diagnose. Differentiation of culture contamination from true infection can be challenging. Treatment is made more difficult by increasing rates of antibiotic resistance in coagulase-negative staphylococci and by the effect of biofilms on host defense and antimicrobial susceptibility. Unfortunately, infected prosthetic devices must often be removed to exact cure. Because the use of indwelling medical devices will most likely continue to increase, it is anticipated that the clinical significance of coagulase-negative staphylococci will similarly increase.

MICROBIOLOGY AND ECOLOGY

The staphylococci are members of the family Micrococcaceae, which also includes *Micrococcus, Stomatococcus,* and *Planococcus.* These bacteria are catalase-positive, gram-positive cocci that divide in irregular clusters, producing a "grapelike cluster" appearance when viewed under the microscope. *Staphylococcus* comprises at least 80 defined species and subspecies (Table 195.1).[7,8] In the clinical microbiology laboratory, staphylococci are typically categorized as those that have the ability to coagulate rabbit plasma (i.e., coagulase-positive staphylococci or *Staphylococcus aureus*) and those that do not (i.e., coagulase-negative staphylococci). The most common coagulase-negative staphylococci associated with human disease include *S. epidermidis, Staphylococcus saprophyticus, Staphylococcus lugdunensis,* and *Staphylococcus haemolyticus.* However, numerous other species have less commonly been associated with disease

TABLE 195.1 Taxonomy of Coagulase-Negative Staphylococci

Human

Species Frequently Associated With Disease

Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus lugdunensis
Staphylococcus saprophyticus

Species Rarely Associated With Human Disease

Staphylococcus auricularis
Staphylococcus capitis
Staphylococcus caprae
Staphylococcus carnosus
Staphylococcus cohnii
Staphylococcus hominis
Staphylococcus pasteuri
Staphylococcus petrasii
Staphylococcus pettenkoferi
Staphylococcus pulvereri
Staphylococcus saccharolyticus
Staphylococcus schleiferi
Staphylococcus simulans
Staphylococcus warneri
Staphylococcus xylosus

Animal

Staphylococcus agnetis
Staphylococcus arlettae
Staphylococcus caseolyticus
Staphylococcus chromogenes
Staphylococcus condimenti
Staphylococcus delphini
Staphylococcus devriesei
Staphylococcus equorum
Staphylococcus felis
Staphylococcus fleurettii
Staphylococcus gallinarum
Staphylococcus hyicus
Staphylococcus intermedius
Staphylococcus kloosii
Staphylococcus lentus
Staphylococcus lutrae
Staphylococcus muscae
Staphylococcus nepalensis
Staphylococcus piscifermentans
Staphylococcus pseudintermedius
Staphylococcus rostri
Staphylococcus sciuri
Staphylococcus simiae
Staphylococcus succinus
Staphylococcus vitulinus

(see Table 195.1). An alternative to the coagulase test commonly used in clinical microbiology laboratories includes rapid agglutination kits (containing antibody bound to beads) that target specific *S. aureus* antigens. The ability to identify coagulase-negative staphylococci to the species level correctly is difficult because of the number of biochemical tests required to yield accurate results.[8,9] However, most phenotypic systems used in clinical laboratories today can accurately identify those species most commonly isolated from human disease, including *S. aureus, S. epidermidis, S. haemolyticus,* and *S. saprophyticus.*[9,10] A simplified scheme used to identify *S. lugdunensis* from other coagulase-negative staphylococci includes a positive pyrrolidonyl aminopeptidase (PYR) and ornithine decarboxylase test.[10,11] Owing to complexities of identifying bacteria through phenotypic means, clinical laboratories are increasingly using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to identify bacterial pathogens, including staphylococci.[12–14,27,28] In addition, US Food and Drug Administration (FDA)–approved polymerase chain reaction (PCR) platforms are commonly used to rapidly detect staphylococci (both *S. aureus* and coagulase-negative *Staphylococcus*) from positive blood culture bottles.[15,16] Indeed, the use of MALDI and rapid PCR identification testing systems will most certainly replace phenotypic identification of *Staphylococcus* species in the near future. Lastly, research-based PCR assays that identify coagulase-negative staphylococci to the species level primarily target 16S-23S rRNA regions,[17] *hsp60,*[10,18] *rpoB,*[10] *sodA,*[10] *tuf,*[10] and transfer RNA intergenic spacer length polymorphisms.[19]

Coagulase-negative staphylococci are normal commensal skin and mucous membrane microbes and are indigenous to a variety of mammalian hosts. Depending on the anatomic site, healthy human skin or mucous membranes support from 10^1 to 10^6 colony-forming units (CFUs) per square centimeter of coagulase-negative staphylococci. We are just now beginning to investigate the fundamental biologic significance of staphylococcal skin colonization. For example, recent data demonstrate that *S. epidermidis* colonization augments the local T-cell response, providing protection against *Leishmania major* infection in mice.[20] Furthermore, Nakatsuji and colleagues found evidence of bacterial communities extending beneath the dermal layer to the basement membrane, indicating interactions with various cell types.[21] Indeed, future studies to document the biologic function of coagulase-negative staphylococci and other skin commensals may be of utmost importance in the understanding of normal skin development and immunity. Of interest, the *S. epidermidis* protease Esp has the ability to inhibit biofilm formation of *S. aureus,* suggesting that some strains of *S. epidermidis* may be important in the inhibition of *S. aureus* colonization in humans.[22,23] More recent studies have found that *S. lugdunensis, Staphylococcus hominis,* and *S. epidermidis* produce antimicrobial peptides (AMPs) that, in some cases, synergize with the human AMP LL-37, to inhibit *S. aureus* growth on skin.[24,25] These interactions between coagulase negative staphylococci and *S. aureus* appear to be vital in the pathogenesis of staphylococcal infections in atopic dermatitis.[25] In addition, Horswill and colleagues found that a strain of *Staphylococcus caprae* was able to inhibit *S. aureus* dermonecrosis in a mouse model by inhibition of the Agr quorum sensing system via synthesis of its own autoinducing peptide.[26] Thus, the study of coagulase-negative staphylococci and their interactions with other commensal flora (e.g., *Cutibacterium acnes*) and potential pathogens, including *S. aureus*, is a fertile area of investigation that may lead to novel prevention strategies.

Aptly named, *S. epidermidis* is one of the most prevalent species found on human skin, with the average person consistently carrying 10 to 24 different strains.[1,8,9] Because of varying characteristics of human skin, including varying moisture content, nutrient substances, pH range, and temperature, *S. epidermidis* must adapt to a variety of environmental conditions. Certain species of coagulase-negative staphylococci are well adapted to exist in specialized niches, such as *Staphylococcus capitis* (human scalp), *Staphylococcus auricularis* (human ear canal), and *S. saprophyticus* (human alimentary and genitourinary tracts); however, the loci that function in staphylococcal skin colonization are not well characterized.

ANTIBIOTIC RESISTANCE

Coagulase-negative staphylococci isolated from nosocomial environments are almost always resistant to multiple antimicrobial agents. In two large surveillance studies from the United States and North America, 73% to 88% of isolates were resistant to oxacillin, 55% to 66% were resistant to levofloxacin, 70% to 73% were resistant to erythromycin, 35% to 52% were resistant to clindamycin, and 35% to 48% were resistant to trimethoprim-sulfamethoxazole.[29,30] Similar results were obtained from the United Kingdom.[31] Although resistance determinants have been defined in some cases, little resistance is observed clinically to agents such as linezolid, tedizolid, daptomycin, quinupristin-dalfopristin, tigecycline, ceftaroline, dalbavancin, telavancin, and oritavancin, or to the investigational agents ceftobiprole and iclaprim.[30,32–45] A few coagulase-negative staphylococci with *van* genes have been isolated from nonclinical sources.[50] In addition, isolates with elevated minimal inhibitory concentrations (MICs) to glycopeptide antibiotics, especially within *S. haemolyticus,* have been reported.[46–49] To date, "vancomycin creep" as found in *S. aureus* has not been observed in coagulase-negative staphylococci.[50] The ability to isolate subpopulations of *S. epidermidis* clinical isolates with markedly elevated vancomycin MICs (>32 g/mL) has not been associated with a clinical consequence to date.[51–53]

Phenotypic expression of methicillin (oxacillin) resistance in coagulase-negative staphylococci is much more heterotypic than that observed in *S. aureus,* meaning that the percentage of the population

that expresses high-level oxacillin resistance is smaller. To address this expression difference, the MIC breakpoint to detect oxacillin resistance is lower for coagulase-negative staphylococci (except *S. lugdunensis*) than *S. aureus* (≥0.5 μg/mL vs. >4 μg/mL, respectively).[59] Regardless of the degree of heterotypy observed, all isolates containing *mecA* (the gene conferring oxacillin resistance) are clinically resistant to all β-lactam antibiotics.[55] Alternative methods to detect oxacillin resistance include a cefoxitin disk test, which is used as a surrogate to detect *mecA*-mediated oxacillin resistance,[54] PCR assay for *mecA* detection,[56] and commercial assays to detect PBP2A production (gene product of *mecA*).[57] However, in some coagulase-negative staphylococci, PBP2A is detected only after oxacillin induction. Note that *mecA*, and thus oxacillin resistance, is rapidly detected with PCR-based blood culture identification systems.[15,58,59]

A particularly onerous aspect of treatment of most coagulase-negative staphylococcal infections is their ability to form biofilms on biomaterials (e.g., catheters, prostheses; see later). Tolerance to antibiotics and persister cells is a common theme with staphylococci and other bacteria growing within a biofilm; these facts need to be taken into consideration during treatment.[60,61] Studies testing the effectiveness of antibiotics against staphylococci growing in a biofilm have demonstrated that they are significantly less effective than when treating planktonic cells.[62] However, antibiotic combinations containing daptomycin alone or in combination with rifampin seem promising in treating or at least reducing the bacterial burden of staphylococcal biofilm infections.[63,64] Interesting to note, studies have shown that staphylococci growing in a biofilm lead to the establishment of an antiinflammatory environment.[65,66] Redirecting this response with proinflammatory macrophages can lead to partial clearance of *S. aureus* biofilm, suggesting that the combination of antibiotics plus a cell-based therapy could be a promising approach in the future.[67]

MOLECULAR EPIDEMIOLOGY

Pulsed-field gel electrophoresis (PFGE) is the gold standard method for addressing the short-term molecular epidemiology of *S. epidermidis* and other coagulase-negative staphylococci. There is extreme diversity in pulsed-field patterns when *S. epidermidis* epidemiology is studied.[68,69] Therefore the finding of indistinguishable PFGE patterns within the context of an outbreak assessment is highly relevant. Longer-term, population-based relationships and trends are better addressed with multilocus sequence typing (MLST) analysis, which suggests that the population structure of *S. epidermidis* is epidemic and that nine clonal lineages are disseminated worldwide.[70] One major clone, CC2, represented 74% of isolates worldwide in one study; similar results were found in other MLST studies.[70–74]

Interesting to note, population structure and the presence or absence of five genetic markers (*icaA, IS256, sesD, mecA,* and the ACME pathogenicity island) have the ability to discriminate between hospital and nonhospital sources of *S. epidermidis*.[75] However, rapid evolution (and thus PFGE patterns) occurs through frequent transfer of mobile genetic elements and recombination, possibly through insertion sequence elements. Other molecular typing methods, including sequence analysis of repeat regions of *sdrG/aap* genes and MLVA (multiple-locus variable-number tandem repeat analysis), have been developed and have yielded similar discriminatory power as MLST or PFGE.[76,77]

PATHOGENESIS

In contrast to *S. aureus,* which produces an array of toxins and adherence factors, there are few defined virulence factors in *S. epidermidis* (Table 195.2) and other coagulase-negative staphylococci. However, significant advances made in the past 20 years have helped define the pathogenesis of infections caused by *S. epidermidis*.[78] The ability of *S. epidermidis* to adhere and form biofilm on the surface of biomaterials is thought to be the most significant virulence factor associated with this bacterium.[79–81] However, other factors, such as the secretion of poly-gamma-DL-glutamic acid (PGA)[82] and phenol-soluble modulins (PSMs),[83,84] appear to complement and increase virulence.

Virulence Factors

Biofilm

Staphylococcal biofilm formation is thought to occur in multiple stages, including adherence to a surface, multiplication, maturation and tower development, and subsequent dispersal (Fig. 195.1).[85] It is well established that bacteria growing within a biofilm are unique compared with those growing exponentially in the planktonic phase. Microarray studies have demonstrated that both *S. epidermidis* and *S. aureus* growing in a biofilm state have unique transcriptional responses compared with cells growing exponentially.[86–88] For example, these experiments have shown that staphylococci growing in a biofilm shift their physiology toward anaerobic or microaerobic metabolism and downregulate protein, cell wall, and DNA synthesis. Although these experiments have been extremely helpful in defining the "average" transcriptional response of biofilm growth (as all cells growing in a biofilm were examined), it is also well established

TABLE 195.2 Defined and Proposed Virulence Factors of *Staphylococcus epidermidis*

DEFINED AND PUTATIVE VIRULENCE FACTORS	PROPOSED MECHANISM
Biofilm	**Immune System Avoidance, Antimicrobial Tolerance**
Polysaccharide intercellular adhesin (PIA)	Polysaccharide component of biofilm
Accumulation-associated protein (Aap)	Accumulation of biofilm
Bap homologue protein (Bhp)	Accumulation of biofilm
Extracellular DNA	Structure of biofilm
Adhesin Molecules	**Adherence to Host Proteins or Plastic**
Autolysin, adhesin (Aae)	Binds fibrinogen, vitronectin, fibronectin
Autolysin (AtlE)	Binds vitronectin
Bap homologue protein (Bhp)	Binds polystyrene
Elastin-binding protein (Ebp)	Binds elastin
Extracellular matrix binding protein (EmbP)	Binds fibronectin
Fibrinogen-binding protein (Fbe)	Binds fibrinogen
Glycerol ester hydrolase (GehD)	Binds collagen
Staphylococcal conserved antigen (ScaA)	Binds fibrinogen, vitronectin, fibronectin
Staphylococcal conserved antigen (ScaB)	Binds undefined ligand
Serine aspartate repeat protein F (SdrF)	Binds collagen
Serine aspartate repeat protein G (SdrG)	Binds fibrinogen
Staphylococcal surface protein 1 (Ssp-1)	Binds polystyrene
Staphylococcal surface protein 2 (Ssp-2)	Binds polystyrene
Teichoic acid	Binds fibronectin
Other Putative Virulence Factors	**Mechanisms**
Peptidoglycan, lipoteichoic acid	Stimulates cytokine production
Phenol-soluble modulins	Immune system modulation, biofilm dispersion
Poly-D-glutamic acid	Immune system avoidance, resistance to antimicrobial peptides
Delta toxin	Immune system avoidance
Exoenzymes	
Fatty acid–modifying enzyme (FAME)	Inactivates fatty acids on skin, skin colonization
Lipases	Skin and wound colonization
Proteases	Destruction of host tissue
Elastase	Immune modulation, skin colonization
Lantibiotics	
Epidermin, epilancin, epicidin, Pep5, K7	Bacterial interference and skin colonization

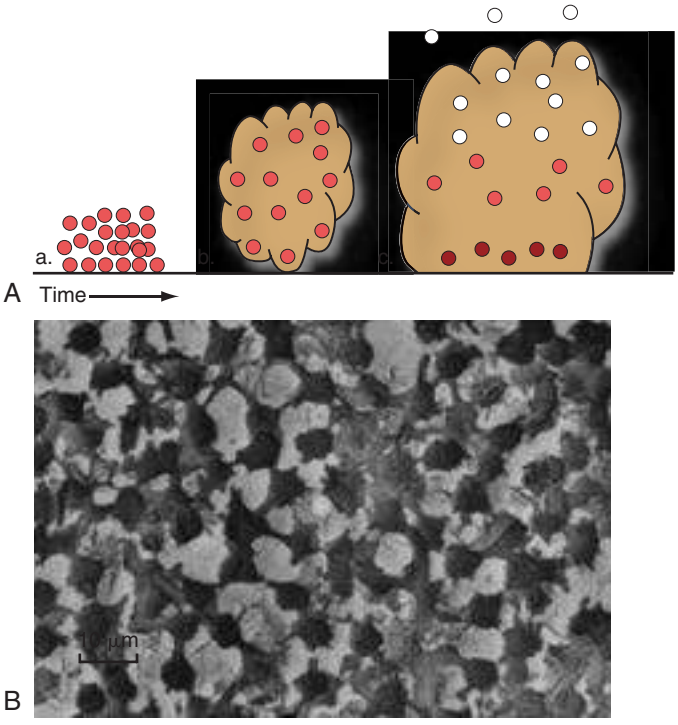

FIG. 195.1 Biofilm formation in *Staphylococcus epidermidis.* (A) Model of biofilm formation in *S. epidermidis.* Staphylococci initially adhere to biomaterial by binding to serum matrix proteins coated on the biomaterial and through direct biomaterial interactions *(a).* Once bound, *S. epidermidis* produces extracellular polymeric substances (polysaccharide intercellular adhesin, environmental DNA) and other proteinaceous factors (Aap, Embp, Bap), which facilitate intercellular adherence *(b).* A mature biofilm has multiple metabolic states, including cells growing under aerobic conditions (white cells), microaerobic or anaerobic conditions (bright red cells), and dormant cells (dark red cells) *(c).* Through the action of phenol-soluble modulins, cells disperse from the biofilm, which facilitates colonization at other sites. (B) Scanning electron micrograph of *S. epidermidis* biofilm adhering to a tissue cage in a guinea pig model (×1360).

that cells growing within a biofilm have spatial and temporal responses to their immediate environment (e.g., nutrient and oxygen availability and interactions with metabolic waste).[89] It is hypothesized that these unique physiologic states found within a biofilm allow for tolerance to antibiotics and development of persister and/or dormant cells.[60]

Adherence

Biomaterials placed within a human host are rapidly coated with serum matrix proteins, including fibrinogen and fibronectin. Genome and functional analyses have suggested that *S. epidermidis* possesses at least the ability to bind fibrinogen, fibronectin, collagen, vitronectin, and elastin.[90–95] Furthermore, there is evidence that *S. epidermidis* autolysins have the ability to bind directly to plastic and contain matrix protein binding sites.[96,97] Lipase (GehD), in addition to its enzymatic function, has been shown to bind collagen.[94] Mutants that do not have the ability to bind fibrinogen or produce autolysin are less virulent in pertinent in vivo models, suggesting that initial adherence to serum matrix proteins is critical.[98,99] Lastly, the bifunctional protein accumulation-associated protein (Aap) has been shown to bind to catheters in a rat catheter model, but the binding partner within serum or host cells is currently unknown.[100–102]

Maturation

After adherence to the biomaterial, intercellular adherence of the bacteria is primarily mediated by polymeric molecules. It has been shown that extracellular DNA is a major component of both *S. aureus* and *S. epidermidis* biofilms, and mutants defective in DNA release produce deficient biofilms.[103] Some clinical strains of *S. epidermidis* produce an abundance of polysaccharide intercellular adhesin (PIA), but not all strains produce PIA and it is a minor component of an *S. aureus* biofilm.[104,105] PIA (or poly-*N*-acetylglucosamine [PNAG]) is a β-1,6-linked *N*-acetylglucosamine[78] synthesized by the *ica* operon gene products.[105,106] The *ica* operon is composed of four genes: *icaA, icaD, icaB,* and *icaC.*[81] A divergently transcribed repressor, *icaR,* is found just upstream of *ica.*[107] PIA appears to be important in *S. epidermidis* surface colonization, biofilm formation, and immune system evasion.[108,109] Regulation of *icaADBC* transcription has been an intense area of study and involves SarA, SarZ, σB, IcaR, and the TCA cycle, among others.[110–116] In *S. aureus,* PIA enhances virulence in murine systemic disease models and is a vaccine candidate for both *S. aureus* and *S. epidermidis.*[117–119] It is important to reiterate that approximately 50% of clinically relevant strains of *S. epidermidis* do not contain the *icaADBC* operon, and other alternative, proteinaceous biofilm maturation strategies exist (e.g., AAP, Embp, Bap).[92–95,104,120–124] PIA-positive strains of *S. epidermidis* may be selected for in niches of high shear stress such as the catheter lumen; indeed, isolates obtained from high-shear environments are more likely to produce PIA-mediated biofilms than those isolates obtained from other sources, suggesting that an additional function of PIA is related to stability of the biofilm structure under high shear.[104] Furthermore, allelic replacement of *icaADBC* confers increased fitness during colonization of human skin as compared with the isogenic wild-type isolate.[125] Therefore, although production of PIA is highly advantageous to the organism during the infection process, PIA production may be deleterious to the organism during colonization of human skin. An additional well-studied protein that functions in biofilm maturation and accumulation is Aap. Once Aap binds to its target, the N-terminal portion of the protein is cleaved via an *S. epidermidis* protease, SepA, and intracellular accumulation occurs via Aap proteins on neighboring cells.[102]

Dispersal

The last stage of biofilm development is dispersal and subsequent spread to other potential sites. The production of PSMs by *S. epidermidis* has been shown in flow cell biofilm experiments to mediate the detachment of the upper layers of the biofilm, although other mechanisms may exist, including nuclease-mediated dispersal.[85,87] PSMs, which are regulated by the quorum-sensing global regulator *agr,* act as surfactants, leading to loss of cellular clusters. In addition, *S. epidermidis* PSMs are proinflammatory and have been shown to recruit, activate, and lyse human neutrophils during infection with *S. aureus.*[126,127]

Other Virulence Factors

Phenol Soluble Modulins

S. epidermidis typically produces five PSMs, a family of amphipathic α-helical peptides that have many functions, including spread on epithelial surfaces.[128] However, some PSMs, including those produced by *S. aureus,* have cytolytic activities. A study by Otto and coworkers found that PSM-mec, which is encoded within SCC*mec,* the genomic island conferring methicillin resistance within the staphylococci, contributes significantly in a mouse model of sepsis.[129] These data may be particularly relevant to neonatal sepsis, which is typically mediated by methicillin-resistant *S. epidermidis* strains encoding *psm-mec.*

Poly-Gamma-DL-Glutamic Acid

A somewhat surprising finding is that in contrast to *S. aureus,* multiple species of coagulase-negative staphylococci, including *S. epidermidis,* produce PGA.[82] PGA is a cell surface–associated, antiphagocytic polymer first described as a virulence factor in *Bacillus anthracis.*[130,131] PGA appears to have a bifunctional role in *S. epidermidis* and functions to inhibit innate host defense and facilitate colonization of human skin.

Antimicrobial Peptide Resistance

Although *S. epidermidis* does not produce multiple toxins to protect against professional phagocytes, several other mechanisms are used to defend against phagocytosis. First, as previously mentioned, biofilm production is itself antiinflammatory, and production of PIA is antiphagocytic.[132] Second, the Aps system can sense the presence of AMPs and upregulate mechanisms to protect the cell against this injury; this includes

increased D-alanylation of teichoic acids and lysylation of phospholipids, thus increasing the overall positive charge of the bacterium.[133]

Lantibiotics

S. epidermidis produces several lantibiotics (e.g., epidermin, Pep5, epilancin, epicidin), which are bacteriocins. These thioether amino acid–containing AMPs are active against a variety of bacteria and may function in bacterial interference and successful colonization and persistence on human skin.[134]

Other Coagulase-Negative Species

Because of the lack of well-developed genetic systems within other coagulase-negative staphylococci, investigation of virulence mechanisms in other staphylococcal species is limited. However, significant progress has been made with *S. saprophyticus* and *S. lugdunensis*. *S. saprophyticus* shows strong adherence to uroepithelial cells and erythrocytes and produces urease, facilitating the growth of this pathogen in urine.[135–138] In addition, genome sequencing has revealed that *S. saprophyticus* ATCC15305 is particularly adapted to growth in urine because of expansion of systems involved in osmotolerance and inorganic ion transport.[136] *S. lugdunensis* does not produce toxins and adherence factors similar to *S. aureus* despite its apparently increased virulence.[10,11] However, several studies have found that *S. lugdunensis* binds human host matrix proteins, including collagen, immunoglobulin G (IgG), fibrinogen, laminin, vitronectin, fibronectin, thrombospondin, von Willebrand factor, and plasminogen.[91,136,139,140] In addition, although it contains the *icaADBC* locus, *S. lugdunensis* appears to produce protein-dependent biofilms that are not PIA dependent, similar to *S. aureus*.

EPIDEMIOLOGY AND CLINICAL SYNDROMES

Previously disregarded as nonvirulent contaminants, coagulase-negative staphylococci have more recently been increasingly recognized as true pathogens. Coagulase-negative staphylococci cause a wide variety of clinical infections, many related to foreign bodies and prosthetic medical devices.

Colonization and Transmission

Certain species of coagulase-negative staphylococci have a predilection for specific niches on the human body that may predispose to certain types of infections. At times, the species names reflect the anatomic site where the particular species is most frequently encountered. For example, *S. auricularis* colonizes the external ear and *S. capitis* is frequently recovered from the scalp. *S. haemolyticus* and *S. hominis* prefer apocrine glands found in the axilla and pubis. Similarly, *S. lugdunensis* is frequently found to colonize the perineum and groin region. Consequently, it is not surprising that *S. capitis* has been associated with cerebrospinal fluid (CSF) shunt infections nor *S. auricularis* with middle ear infections, and *S. lugdunensis* has caused bacteremia and endocarditis after surgical procedures involving the groin and scrotum. As previously mentioned, *S. epidermidis* is well suited to colonize human skin. It possesses a number of osmoprotective systems to cope with the harsh environmental conditions and high salt concentrations encountered on the dermal surface.[141] Most strains of *S. epidermidis* (and some strains of *S. haemolyticus* and *S. capitis*) contain the arginine catabolic mobile element (ACME), which is thought to improve the organism's ability to colonize the skin and mucosal membranes.[142] In general, body sites with greater moisture are favored, and thus coagulase-negative staphylococci are more readily recovered from the axilla, groin, gluteal, umbilical, antecubital, popliteal, and plantar foot and toe web areas, in addition to mucosal surfaces such as the nares, conjunctiva, and vagina.[50] *S. epidermidis* is also well suited to survive in hospital environments. It can survive on fabrics and inanimate surfaces for weeks to months[143] and is often resistant to multiple antibiotics.[3] *S. epidermidis* often possesses efflux pumps, making it less susceptible to certain antiseptics and other biocides.[144] *S. epidermidis* produces PSMs that are proinflammatory cytolysins and appear to exact selective antimicrobial activity against other organisms, including *S. aureus,* thus helping *S. epidermidis* to outcompete other microbes for dominion of the skin niche.[145,146] Similarly, *S. epidermidis* serine protease Esp appears to inhibit nasal colonization by *S. aureus*.[19,23] These findings hold promise that certain strains of *S. epidermidis* may someday be used as probiotics to exclude the more pathogenic *S. aureus* from skin and nasal colonization.

Investigators have documented in various patient groups that shortly after admission to the hospital, a patient's endogenous, usually antibiotic-susceptible, *S. epidermidis* flora is replaced by nosocomial, usually antibiotic-resistant, strains of *S. epidermidis*.[147–149] Predominant clones of *S. epidermidis* often emerge and can persist in hospitals for decades.[149,150] Health care providers may be carriers of these endemic *S. epidermidis* strains and spread them from patient to patient.[151,152] Furthermore, under the selective pressure of antimicrobial use, these highly successful strains of *S. epidermidis* have been shown to spread from unit to unit, hospital to hospital, and even country to country.[47,72,153–156] Although nosocomial spread of coagulase-negative staphylococci primarily occurs via contact with health care providers' hands, fomites, or contaminated environmental surfaces, nosocomial strains of *S. epidermidis* have been recovered from hospital air.[157] In addition, coagulase-negative staphylococci appear to serve as a pool for the horizontal transfer of antibiotic-resistant genes to *S. aureus*.[158,159] Interesting to note, at least part of the ability of community-associated methicillin-resistant *S. aureus* (MRSA) (strain USA300) to rapidly spread in the human population is thought to be due to the transfer of ACME from coagulase-negative staphylococci to *S. aureus*. Some authors have suggested that in order to better control the emergence and spread of multidrug-resistant *S. aureus,* infection-control measures should be introduced to detect and prevent the spread of multidrug-resistant *S. epidermidis*.[160] However, practical and feasible methods to control the spread of multidrug-resistant *S. epidermidis* are completely undefined.

Bacteremia

Coagulase-negative staphylococci are the most common cause and account for approximately 30% of health care–associated bloodstream infections. Most of these infections are caused by involvement of intravascular catheters or other prosthetic medical devices. Immunosuppressed patients, particularly neonates or those with severe neutropenia, are at increased risk of bloodstream infection with coagulase-negative staphylococci. In addition to intravascular catheters, mucosal breakdown caused by cytotoxic chemotherapy may precipitate infection in oncology patients.

Because coagulase-negative staphylococci, particularly *S. epidermidis,* occupy such a prominent position in the commensal flora of human skin and mucous membranes, they are frequently encountered as culture contaminants. Approximately 1% to 6% of blood cultures are contaminated, and coagulase-negative staphylococci are responsible for 70% to 80% of cases.[161–163] Typically, rates of true bacteremia range from 10% to 35% when coagulase-negative staphylococci are isolated from blood cultures.[163–167]

Determining the clinical significance of coagulase-negative staphylococci isolated from blood cultures is difficult, and a variety of clinical and laboratory parameters should be examined when making this determination. This is not a trivial issue because contaminants treated as true pathogens result in unnecessary antibiotic treatment, emergence of antibiotic resistance, excessive use of laboratory resources, antibiotic-associated side effects and toxicity, and greater expense.[168] Many clinicians use fever or other signs of infection (e.g., leukocytosis or leukopenia, hypotension) to assist with the interpretation of blood cultures that yield coagulase-negative staphylococci, and a number of studies have offered support.[161,162,165,169] However, most blood cultures are obtained because of a clinical suspicion of bacteremia, and other investigators have not found clinical signs and symptoms helpful in differentiating contamination from infection.[166,170,171] Another parameter often used is the number or proportion of cultures that yield coagulase-negative staphylococci. Most studies relate that the likelihood of true bacteremia increases when multiple cultures are obtained and yield coagulase-negative staphylococci,[172] but this has not been universally observed.[172,173] The problem is compounded in neonates, from whom it is common practice to obtain only one blood culture when entertaining the possible diagnosis of bacteremia. A study examining the usefulness of a single-sample strategy for blood culture found that the positive predictive values for a "true" bloodstream infection were 3.5%, 61.1%, 78.9%, and 100% when one, two, three, and four or more blood culture

bottles yielded coagulase-negative staphylococci.[174] However, Seybold and colleagues noted through use of molecular typing methods that contamination may be frequent even when two or more blood cultures reveal coagulase-negative staphylococci in a 24-hour period.[175] A shorter time to growth or a greater burden of bacteria in quantitative blood cultures has also been used to differentiate true bacteremia from contamination, with mixed success.[176] In general, a time to positivity of less than 24 hours is considered consistent with true bacteremia.[167] Savithri and colleagues noted that a time to positivity of less than 24 hours for blood cultures yielding coagulase-negative staphylococci was associated with a higher organ failure score and a trend toward increased 28-day mortality in a group of critical care unit patients.[177] Schnell and colleagues formulated an algorithm to assess the clinical significance of coagulase-negative staphylococcal bacteremia, taking into account the number of positive cultures, antibiogram, clinical signs, and source of infectious foci, and they related positive and negative predictive values of 78% and 68%, respectively.[178] Similarly, Garcia-Vasquez and colleagues formulated an algorithm to predict clinically significant coagulase-negative staphylococcal bacteremia with a 62% sensitivity and 93% specificity; this algorithm included Charlson score ≥3, Pitt score ≥1, neutropenia, presence of a central venous catheter (CVC), time to culture positivity of <16 hours, and *S. epidermidis* speciation.[167]

In differentiating intravascular catheter–associated coagulase-negative staphylococci bloodstream infection from bacteremia from other sources, the differential time for a blood culture to become positive between peripheral blood and catheter blood can now be readily determined with most automated blood culture methods. When the catheter blood is positive 2 or more hours sooner than the peripheral blood, this can be taken as an indicator that the catheter is the source of bacteremia.[145,146,179,180] Other laboratory parameters that may have some usefulness for differentiating contaminants from pathogens in certain settings include C-reactive protein (CRP)[181] and serum procalcitonin levels,[182] molecular typing,[183,184] antibiogram,[185] biofilm production,[186] presence of adhesins, and absence of *bap* gene.[181–187] Kitao and colleagues noted that more than half of *S. epidermidis* isolates obtained from blood cultures possessed genes for biofilm production *(icaAB)* and methicillin resistance *(mecA),* whereas only 1.5% of isolates from the skin were positive by PCR for these potential markers for clinical infection.[188]

Methods that have been used to prevent blood culture contamination include use of effective skin antiseptics, phlebotomy teams, culture bottle preparation, blood culture kits, and double-needle bottle inoculation.[161,162,189] More recently, a device that diverts and sequesters the initial 1.0 mL to 1.5 mL of blood has proven very effective in preventing blood culture contamination thought to stem from dislodged skin cells.[190] In general, blood cultures should be obtained with careful aseptic technique in persons in whom a clinical suspicion for bacteremia exists. Paired cultures should be drawn, and if a CVC source is being considered, one of the blood samples should be obtained from the catheter. The site (catheter or periphery) and time of obtainment should be recorded. Patients with clinical signs of sepsis, with multiple positive cultures for coagulase-negative staphylococci that reveal growth in less than 24 hours, are much more likely to have true bacteremia. Although the low virulence of coagulase-negative staphylococci is well known, coagulase-negative staphylococcal infections can at times be severe, even in the absence of a biomaterial-based process. Chu and coworkers noted that 8% of all cases of native valve endocarditis were caused by coagulase-negative staphylococci, and these patients experienced a mortality rate that is similar to that associated with *S. aureus* endocarditis (approximately 25%).[191] Among 87 patients with clinically significant bacteremia, Pien and colleagues noted an attributable mortality of approximately 2%.[163]

Intravascular Catheter Infections

Intravascular catheter infections are discussed more fully in Chapter 300. More than 150 million intravascular catheters are used in the United States annually. Although the incidence of central line–associated bloodstream infections (CLA-BSIs) decreased by 50% in US acute-care hospitals between 2008 and 2014, tens of thousands of patients continued to experience CLA-BSI each year, resulting in substantial morbidity, mortality, and increased cost.[192,193] The mean rate of CLA-BSIs in US hospitals ranges from 0 to 2.9 per 1000 CVC days depending on the type of unit.[192,194] The rate of CLA-BSI in Western Europe is generally comparable to rates reported in the United States.[195] Unfortunately, the incidence of CLA-BSI in limited-resource countries is substantially higher (1.6–44.6 per 1000 CVC days).[195a] Coagulase-negative staphylococci are the most common cause of these infections (Fig. 195.2). For the years 2009 and 2010, 27,766 CLA-BSIs were defined in the Centers for Disease Control and Prevention's National Healthcare Safety Network, of which 20.5% were caused by coagulase-negative staphylococci. Over a 6-year observation period in Quebec, from 2003 to 2009, coagulase-negative staphylococci were responsible for 53% of CLA-BSIs.[196] A detailed discussion of the pathogenesis of CVC-associated bloodstream infection is beyond the scope of this chapter. Briefly, for peripheral intravascular catheters and nontunneled CVCs, infection most commonly results from coagulase-negative staphylococci entering via the cutaneous surface of the catheter to the bloodstream. The tissue around the intravascular catheter appears to be an important niche for microbes, and large numbers of coagulase-negative staphylococci can be cultured from pericatheter tissue.[197] For tunneled CVCs, hub colonization and passage of organisms along the lumen appear to be an important route of infection. In a cohort of hemodialysis patients with tunneled CVCs, endoluminal colonization by *S. epidermidis* was detected a median of 31.5 days before a clinically evident catheter-related bloodstream infection was defined.[198] The ability of coagulase-negative staphylococci to adhere to the catheter and elaborate biofilm are crucial traits that allow them to cause infection of vascular catheters.[199]

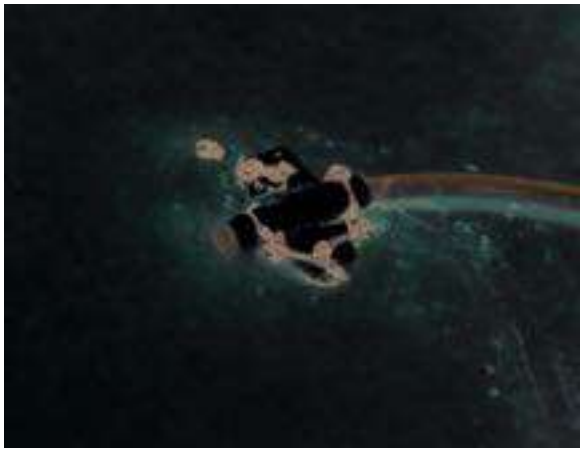

FIG. 195.2 Infected central venous catheter. Although many infected central venous catheters appear innocuous, this *Staphylococcus epidermidis*–infected implanted catheter site exhibits erythema, tenderness, and purulent drainage at the skin exit site.

The diagnosis of coagulase-negative staphylococci CVC bloodstream infection can be difficult because most infected CVCs have no obvious local evidence of inflammation or infection[199] and, as already discussed, coagulase-negative staphylococci are most often clinically encountered as blood culture contaminants.[199] In the past, diagnosis of CVC-associated bloodstream infection was considered to require removal of the catheter and semiquantitative or quantitative culture of the catheter tip.[200,201] Many clinicians take advantage of automated, continuously monitored blood culture systems and use a 2-hour cutoff differential time to positivity assessment on blood cultures drawn from the periphery and CVC.[180] The limitations of this technique are discussed in greater detail in Chapter 300.

In general, short-term, nontunneled CVCs infected with coagulase-negative staphylococci should be removed because these infections can be associated with sepsis and increased morbidity.[202,203] In patients with coagulase-negative staphylococci–infected tunneled CVCs who do not exhibit signs of severe sepsis, it is permissible to attempt catheter salvage. If the CVC is retained, it is advisable to use systemic antimicrobials, based on susceptibility testing, through all the lumens of the catheter for 7 to 14 days.[179] Antimicrobial lock therapy, discussed in greater detail in Chapter 300, is increasingly employed in attempt to treat CVC-related infection with the catheter in situ. Various antimicrobial

agents have been used, either alone or in combination, in lock solutions used to treat coagulase-negative staphylococcal CVC-related bloodstream infection, including vancomycin, daptomycin, minocycline, cephalosporins, aminoglycosides, fluoroquinolones, hydrochloric acid, alcohol, and various chelators.[204] In general, success rates of 80% to 90% are noted, with a relapse of 20% to 30%. Similarly, lock therapy can be used to prevent CVC-associated bloodstream infection.[205,206] If fever or bacteremia persists for more than 3 days after initiating therapy, the CVC should usually be removed. In a group of 23 patients with coagulase-negative staphylococcal bacteremia lasting for longer than 48 hours despite appropriate antibiotics, antibiotic lock therapy was successful in only 30% of patients.[207] Most health care–associated coagulase-negative staphylococci are methicillin resistant, and vancomycin is most frequently used to treat these infections. If once-daily daptomycin is used, each dose should be distributed among all the lumens of a multiport catheter. As mentioned, if the catheter is retained, the clinician should be alert for relapse because this occurs in a substantial minority of patients.[204,208] Tunnel track or port pocket infections necessitate catheter removal, whereas exit site infections can usually be successfully treated with the CVC in place.

Endocarditis

Prosthetic valve endocarditis (PVE), although uncommon, is caused by coagulase-negative staphylococci in 15% to 40% of cases.[209–213] Both bioprosthetic and mechanical valves can be infected, as can valves replaced via a transcatheter approach.[213,214] The infection is usually health care related (resulting from inoculation at the time of surgery) and manifests within 12 months of valve placement.

Coagulase-negative staphylococci cause approximately one-third of early-onset PVE (onset <2 months after valve insertion) and 40% of intermediate-onset disease (onset 2–12 months after valve insertion).[212] These isolates are likely to be methicillin resistant. The cases of PVE that present after this time period are less commonly caused by the coagulase-negative staphylococci, usually associated with trauma to mucosal surfaces or incidental infection and, if caused by coagulase-negative staphylococci, are more apt to be methicillin susceptible.[215] Coagulase-negative staphylococci cause 10% to 20% of late-onset PVE (>12 months after valve insertion).[211,212] The aortic valve is most frequently involved, and patients may present in an acute or more indolent fashion. An acute presentation is characterized by fever and physical evidence of valve dysfunction, whereas peripheral stigmata of endocarditis are more commonly observed in patients exhibiting a more indolent course. The diagnosis is usually confirmed by documenting repeatedly positive blood cultures and vegetations with transesophageal echocardiography. Positron emission tomography (PET)/computed tomography (CT) is being increasingly used in ambiguous cases and should be interpreted by a specialist with expertise in cardiac imaging and the findings integrated into the clinical context.[216] Heart failure occurs in 54% of cases, and more than 80% of patients have complications, including prosthetic valve dysfunction and intracardiac abscesses. Typically, antibiotic therapy consists of vancomycin and rifampin for at least 6 weeks combined with gentamicin for the first 2 weeks (see Chapter 81).[217,218] Isolates susceptible to penicillinase-stable penicillins should be treated with oxacillin or nafcillin instead of vancomycin. There is increasing concern regarding vancomycin "MIC creep," and clinicians are sometimes turning to daptomycin or other vancomycin alternatives. Valve replacement surgery is usually necessary. Despite aggressive therapy, the mortality caused by coagulase-negative staphylococci PVE remains high, at approximately 25%.[211,213]

Unlike PVE, native valve endocarditis caused by coagulase-negative staphylococci is relatively rare, occurring in only 5% to 12% of endocarditis cases.[191,209,213,219,220] This infection is the result of hematogenous seeding of previously damaged heart valves and endocardium. The incidence of endocarditis caused by coagulase-negative staphylococci appears to be increasing and is probably driven by an increase in health care–associated interventions (e.g., intravenous catheters, hemodialysis), and the causative isolates are usually methicillin resistant.[221] In the rare cases of polymicrobic endocarditis, coagulase-negative staphylococci are involved in approximately 50% of cases.[219] Prolonged symptoms and physical signs (fever, vascular or immunologic findings) before diagnosis are relatively common,[156] and patients often have a complicated clinical course because of embolic events, rhythm conduction abnormalities, and congestive heart failure.[191,222] At times, PCR may be helpful in defining the causative role of coagulase-negative staphylococci when cultures of blood and tissue are sterile.[223] The aortic valve is most frequently involved (70%), and more than half of these cases require valve replacement. Despite aggressive combined medical-surgical treatment, mortality continues to be approximately 25%.

Cardiovascular Implantable Electronic Devices

Cardiovascular implantable electronic device (CIED) infection (pacemakers, defibrillators) occurs in 1% to 2% of device placement procedures (see Chapter 82); coagulase-negative staphylococci (predominantly *S. epidermidis*) account for approximately 50% to 60% of these infections.[224–230] Unfortunately, the incidence of CIED infection has increased and has paralleled the increased use of implantable cardiac devices.[231] A particularly challenging subset of patients includes those with a CIED infection who also have a left ventricular assist device in place.[232,233] Infection can be limited to the pocket or can spread via intravascular leads to involve endocardial tissue. One-fourth of pacemaker infections manifest acutely within 1 month of insertion, but delays of up to 1 to 2 years are commonly observed.[231] Factors associated with increased risk of CIED infection include older age, diabetes, end-stage renal disease, chronic obstructive pulmonary disease, corticosteroid use, history of previous device infection, malignancy, heart failure, preprocedural fever, anticoagulant drug use, and skin disorders.[233] Clinically, most patients present with inflammation at the pocket site, whereas systemic symptoms are less frequently observed. Diagnosis is generally made through culture of the generator pocket, culture of the device itself, or multiple positive sequential blood cultures with the same strain of coagulase-negative staphylococci. However, only about one-third of patients with implantable cardiac device infection are bacteremic.[226,228] Transesophageal echocardiography is recommended for all patients and is much more sensitive than transthoracic echocardiography.[225,234,235] Interesting to note, coagulase-negative staphylococci can be cultured from a substantial number of explanted devices from asymptomatic patients, particularly if sonication is used.[236,237] Some of these patients go on to develop device infection with the same species, leading to the thought that the devices can be colonized without overt infection.[236] Successful treatment of implanted electrical cardiac devices generally requires complete removal of the device. Although some clinicians are reluctant to remove leads for fear of associated complications, these concerns have been minimized with use of laser extraction. Complete lead extraction can be safely achieved in more than 90% of patients with minimal risk of significant morbidity.[238] Relapse rates and mortality are substantially increased if complete removal of the device is not accomplished.[225–227, 229,235,239] A multivariate analysis demonstrated a sevenfold increase in 30-day mortality if the CIED was not removed.[234] Antibiotic therapy typically consists of vancomycin or daptomycin, with or without rifampin, continued for 14 days after device removal for patients with infection limited to the pocket and for 6 weeks for patients with bacteremia, lead involvement, or endocarditis.[234] Device reimplantation, if necessary, should be at a new site when the patient is no longer bacteremic.

Vascular Grafts

Although infection is a relatively rare complication of arterial reconstruction (<1%–6%, depending primarily on location of the graft), coagulase-negative staphylococci are one of the most common causes (20%–30%) of this feared entity.[240–242] The coagulase-negative staphylococci, usually *S. epidermidis,* causing these infections are thought to be inoculated at the time of surgery from the patient's skin. Major risk factors include a groin incision, diabetes, emergency aortic aneurysm repair, steroid therapy, and remote infections. Most cases of vascular graft infection caused by coagulase-negative staphylococci present in an indolent fashion, months to years after surgery, and manifest as a false aneurysm, fistula or sinus tract formation, or hemorrhage at the anastomotic site. Diagnosis is usually entertained on the basis of local physical findings and supported by radiographic modalities, such as CT, magnetic resonance imaging

(MRI), or ultrasonography. Blood cultures are often negative because infection may not extend to the graft lumen. Radiographic-guided aspiration of perigraft fluid can be helpful in establishing the diagnosis.[242]

Confirmation of the causative role of coagulase-negative staphylococci is maximized by sonication of the explanted graft at the time of surgery to recover biofilm-associated organisms.[243] Optimum treatment requires a combined medical and surgical approach. Intensive and prolonged antibiotic therapy is important, but surgery is required for cure. "Conservative" therapy with antibiotics and without surgery is associated with a high mortality rate and should be avoided if possible.[244] Treatment failure has been associated with antibiotic regimens that did not include rifampin.[245] Surgical strategy can be summarized as graft excision with extraanatomic bypass or graft excision with in situ reconstruction using a prosthetic conduit, allograft, or autogenous tissue. These techniques have been summarized elsewhere but are all associated with mortality of approximately 10% to 25%. As expected, in situ reconstruction is associated with less risk of amputation but an increased risk (15%–20%) of recurrent infection. There is some support for the use of antibiotic-treated or silver-coated grafts in the treatment of vascular graft infection or, similar to their application in orthopedic surgery, the placement of antibiotic-impregnated beads into the infected tissue bed.[246] A systematic review and meta-analysis of perioperative strategies to prevent infection in patients undergoing peripheral arterial reconstruction has confirmed the beneficial role of prophylactic antibiotics but did not find benefit associated with rifampin-bonded Dacron grafts, suction groin wound drainage, or perioperative bathing with antiseptic agents.[241]

Orthopedic Prosthetic Device Infections

Coagulase-negative staphylococci, usually *S. epidermidis,* are responsible for 30% to 43% of cases and are the most common cause of infection of prosthetic orthopedic devices (see Chapter 105).[247–249] These organisms are generally inoculated at the time of the arthroplasty and, because of their relatively avirulent nature, may be quite indolent in their clinical presentation. Risk factors consistently observed include previous joint surgery, perioperative wound complications, and rheumatoid arthritis.[250] Risk factors observed in some studies but not others include history of malignancy, diabetes, corticosteroid use, obesity, age, nutritional status, infection at remote site at time of surgery, psoriasis, hemophilia, sickle cell anemia, dialysis, acquired immunodeficiency syndrome (AIDS), and solid-organ transplantation. Infections can be classified as early (within 3 months of surgery and are often caused by *S. aureus*); delayed (3 months to 2 years postoperatively; most frequently caused by coagulase-negative staphylococci); and late (longer than 2 years after surgery; usually caused by hematogenous inoculation of organisms from some other source). Delayed infections caused by coagulase-negative staphylococci are usually indolent and manifest as pain at the affected joint, without fever or other systemic manifestations. The diagnosis is supported by the presence of an elevated erythrocyte sedimentation rate or CRP level.[251] Radiographic imaging studies may be helpful but are often limited by poor sensitivity and specificity or imaging artifact caused by metallic components of the implant. Labeled leukocyte scans combined with either technetium or gallium bone scans may be helpful.[251] PET scanning has been reported to offer an 82% sensitivity and 87% specificity in defining prosthetic joint infection.[252] Aspiration and synovial fluid analysis or tissue biopsy may also be helpful but can be limited by the localized nature of inflammation or infection and potential contamination. Diagnostic yield is improved by obtaining five or six periprosthetic tissue specimens for bacterial culture.[253] The recovery of organisms can be optimized by sonication of the prosthesis at the time of removal or by prolonging incubation time to 15 days.[247,254,255] In addition, PCR has proven helpful in some circumstances, but is also associated with "false-positive" results.[256] Coagulase-negative staphylococci may also be a prominent cause of culture-negative prosthetic joint infection manifesting as aseptic loosening of the joint.[257] It is thought that prior antibiotic therapy and the presence of a metabolically quiescent population of coagulase-negative staphylococci encased in biofilm may explain this condition. In addition, small colony variants are noted in approximately one-third of prosthetic joint infections caused by *S. epidermidis.*[258] Prosthetic joint infection caused by *S. epidermidis* small colony variants appear to have a more indolent presentation, but are not associated with excess treatment failure compared with infections caused by strains with a normal phenotype.[258] Finally, unsuspected prosthetic joint infection, defined by positive cultures at the time of revision surgery, is noted in a small proportion of patients undergoing repeat joint replacement surgery.[259] Such patients, undergoing knee revision, had inferior infection-free survival compared with patients with sterile cultures.[259]

Surgical management of prosthetic joint infections consists of débridement and resection of the prostheses or replacement of the prostheses in one-stage or two-stage procedures.[260] Depending on the type of surgical procedure, antibiotic therapy, usually using vancomycin directed at the methicillin-resistant strains of coagulase-negative staphylococci, lasts from 4 to 6 weeks to up to 6 months.[251,260] In two-stage procedures, the use of antibiotic spacers may improve outcome. Rifampin is recommended in combination therapy in cases of rifampin-susceptible coagulase-negative staphylococcal infection.[248,260] Success rates of 90% to 95% are noted.[261] Decreased susceptibility to vancomycin in strains of coagulase-negative staphylococci isolated from prosthetic joint infections has been noted.[262] Coagulase-negative staphylococci are also occasionally noted to cause osteomyelitis or septic joints in the absence of orthopedic devices.[263,264] There are limited data regarding treatment with vancomycin alternatives, such as linezolid, daptomycin, telavancin, ceftobiprole, or ceftaroline.[265–267] Prevention strategies include the use of laminar flow operating suites, antimicrobial prophylaxis, and antibiotic-impregnated bone cement.[268–271]

Cerebrospinal Fluid Shunt Infections

Infection is one of the most significant complications associated with CSF shunt implantation, ranging in incidence from 1.5% to 38%, but more recently occurring in approximately 5% of patients (see Chapter 92). Coagulase-negative staphylococci, most predominantly *S. epidermidis,* are the most common cause and are responsible for approximately one-third to one-half of cases.[272] Risk factors predisposing to infection include age younger than 6 months, shunt revision surgery, scalp dermatitis, long duration of procedure, proficiency of the surgeon, and intraoperative use of a neuroendoscope. One study found that in 80% of cerebral spinal fluid shunt implant procedures, the gloves of neurosurgeons and other members of the surgical team quickly became contaminated with coagulase-negative staphylococci, and the authors hypothesized that this may be the route of inoculation and infection.[273] Patients who receive a short-term ventriculostomy (external ventricular drain) are at substantial risk (approximately 10%) of developing ventriculitis or meningitis, and coagulase-negative staphylococci are the predominant pathogens,[274–276] although some reports have indicated a shift toward gram-negative pathogens.[277] The long duration of catheterization appears to be the major risk factor for infection that is not substantially modified by catheter exchange or prophylactic antibiotics. Other risk factors are intraventricular blood and previous trauma.[274] Signs and symptoms of shunt infection typically develop within 2 months of shunt insertion and should be suspected in patients with local signs of inflammation; nausea or vomiting; signs of increased intracranial pressure; or shunt malfunction. The diagnosis is confirmed by means of isolation of coagulase-negative staphylococci from CSF obtained from the shunt. PCR may prove helpful in timely identification of coagulase-negative staphylococci or other pathogens.[278] A modest pleocytosis is usually evident, accompanied by an elevated protein level. Positive blood cultures are observed in patients with infected ventriculoatrial shunts.

Most infections are caused by methicillin-resistant strains of coagulase-negative staphylococci, and combination therapy with vancomycin, gentamicin, and rifampin is a traditional regimen. Vancomycin and gentamicin are often delivered intraventricularly. Rifampin achieves excellent CSF concentration with systemic administration. Experience with newer antistaphylococcal agents, such as linezolid or daptomycin, is limited. Successful treatment usually requires shunt removal,[227] but some selected patients may be treated successfully with the shunt in situ.[272,279] In patients with coagulase-negative staphylococcal infections and normal CSF findings, reshunting can be performed on the third day after shunt removal. If CSF abnormalities are present,

7 days of therapy are generally recommended before reshunting as long as repeat CSF cultures are sterile and the CSF protein level is lower than 200 mg/dL. In a systematic review and meta-analysis of 17 trials involving 2134 patients, the administration of perioperative (24 hours) prophylactic antibiotics in CSF shunt surgery significantly decreased the risk of infection.[280] Additional reduction in infection was observed through application of strict operative aseptic technique.[281] Although most studies examining the use of antimicrobial-impregnated catheters for prevention of CSF shunt infection are not randomized trials, meta-analysis indicates a reduction in the risk of infection, but use of antibiotic-impregnated shunt catheters has been associated with inconsistent results.[282–286] There is some concern that antibiotic-impregnated extraventricular drains may result in false-negative surveillance cultures and an increased risk of infection with implantation of a permanent shunt.[287]

Ommaya reservoirs are subcutaneous devices used to deliver chemotherapy into the CSF of patients with cancer. Approximately 5% of patients with Ommaya reservoirs develop infection, and approximately 50% are caused by coagulase-negative staphylococci.[288] Treatment generally requires device removal along with systemic and intra-Ommaya administration of vancomycin. In one series, approximately 15% of patients were successfully treated with retention of the Ommaya reservoir.[288]

Surgical Site Infections

Surgical site infections caused by the coagulase-negative staphylococci occur frequently; these organisms are third only to *S. aureus* and *E. coli* as a causative agent.[289] Coagulase-negative staphylococci are more often causative of superficial incisional infections than of deep incisional infections and rarely cause organ or space infections. A notable exception is mediastinitis after median sternotomy for cardiac surgery (see Chapter 85). In addition, coagulase-negative staphylococci are more likely to cause infections involving clean procedures than those classified as contaminated (e.g., bowel, genitourinary). Superficial incisional infections generally manifest within 5 to 10 days after the procedure and usually result from inoculation of organisms from the patient's endogenous flora or, less frequently, from the operating personnel or environment. Risk factors include long duration of the surgical procedure; host factors (e.g., extremities of age, obesity, poor nutritional status); and experience of the surgeon and surgical staff. Signs and symptoms of a surgical site infection include pain, tenderness, swelling, warmth, erythema, drainage at the incisional site, leukocytosis, and fever. The causative pathogen is confirmed by recovery of coagulase-negative staphylococci from wound cultures. Culture results require careful interpretation because coagulase-negative staphylococci are commonly regarded as contaminants or colonizers. In general, coagulase-negative staphylococci are interpreted to be the causative agent of the infection if they are the predominant or only isolate from purulent drainage and/or are repeatedly cultured from the same source. Treatment depends on the severity of the infection and ranges from topical wound care alone to surgical débridement and parenteral antibiotics. Preventive measures should be emphasized, and detailed guidelines are available (see Chapter 313 and Berrios-Torres and colleagues[290]). Because many coagulase-negative staphylococcal surgical site infections are caused by strains that are resistant to traditional prophylactic antibiotics, some interest has been expressed in broader use of glycopeptide-containing regimens.[290] Other preventive strategies include use of perioperative mupirocin and antiseptic body washes.[291] Triclosan-coated sutures did not prevent coagulase-negative staphylococcal sternal wound infections.[292]

Peritoneal Dialysis Catheter–Associated Infections and Peritoneal Dialysis–Associated Peritonitis

Coagulase-negative staphylococci, accounting for 25% to 50% of cases, are the most frequent cause of peritonitis in patients undergoing peritoneal dialysis.[293] *S. epidermidis* is responsible for 50% to 80% of infections caused by coagulase-negative staphylococci, with a wide variety of other coagulase-negative staphylococcal species less frequently observed[294]; clonal spread of coagulase-negative staphylococci has been documented at some centers.[293–296] Coagulase-negative staphylococci gain access to the peritoneum from the patient's skin via the intraluminal route or from the exit site via the periluminal route. Factors for the development of peritoneal dialysis-associated peritonitis include age, diabetes, heart failure, anemia, and low serum albumin.[297] Obese patients appear to be at particular risk of peritonitis due to coagulase-negative staphylococci.[298]

Clinically, compared with infection with S. *aureus,* peritonitis caused by coagulase-negative staphylococci is relatively benign and infrequently leads to catheter removal.[299] Peritonitis caused by biofilm-producing strains of coagulase-negative staphylococci is associated with worse outcome compared with biofilm-negative strains.[299] Signs and symptoms of infection include abdominal pain and tenderness, fever, nausea, and vomiting. Diagnosis is confirmed by documentation of more than 100 white blood cells per milliliter in dialysate fluid and recovery of coagulase-negative staphylococci in cultures of the fluid. When culturing the dialysate, it is necessary to culture large volumes of fluid (>100 mL) or to use a filter technique or broth enrichment to detect small numbers of organisms. However, care must be taken because it may be difficult to differentiate contaminants from causative pathogens.[293] Although historically most of these infections were caused by methicillin-susceptible strains, the antimicrobial susceptibility pattern has recently shifted, and most causative strains are now methicillin resistant.[299,300] Treatment with vancomycin via the dialysate fluid is a relatively convenient administration method and is often successful. Recalcitrant or recurrent peritonitis is often an indication for catheter removal.[301]

Prevention of infection depends on proper catheter placement, exit site care, infusion with Y sets and twin bag systems, disconnect systems, mupirocin application at the exit site, and careful training of patients regarding aseptic practices.[302] Perioperative antibiotic prophylaxis significantly reduces the risk of early peritonitis but not exit site or tunnel infections.[303,304]

Endophthalmitis

Coagulase-negative staphylococci are readily recovered from conjunctival cultures of preophthalmologic surgery patients, and thus it is not surprising that they are the most frequent cause of postoperative endophthalmitis or infection following intravitreal injection, responsible for 33% to 70% of cases (see Chapter 114).[305–309] Symptoms typically develop within 1 week of surgery and usually consist of pain, redness, and decreased visual acuity.[310] Fever is generally absent and leukocyte count is normal. The physical examination reveals conjunctival injection and a hypopyon. Optimal treatment consists of vitrectomy and intravitreal administration of antibiotics.[311] A large proportion of coagulase-negative staphylococci causing eye infections are multidrug resistant.[312] Vancomycin is usually administered at an intravitreal dose of 1 mg, and bactericidal concentrations usually persist up to 2 to 3 days.[313] Intraocular lens removal is generally not required. Although prognosis largely depends on presenting visual acuity, residual visual impairment is frequently observed. Postinfection visual acuity is generally better preserved when the infection is due to coagulase-negative staphylococci compared with other pathogens.[310]

Urinary Tract Infection

Urinary tract infections (UTIs) caused by the coagulase-negative staphylococci fall into two major groups. The first is caused by *S. saprophyticus,* which is covered more fully in a separate section of this chapter (also see Chapter 72). The second group is uncommon and occurs almost exclusively in hospitalized patients with underlying urinary tract complications. Most of these patients have a urinary catheter in place and have recently undergone urinary tract surgery or a kidney transplantation, or they have experienced kidney stone disease or have a neurogenic bladder or obstructive uropathy. Coagulase-negative staphylococci cause approximately 3% of nosocomial UTIs, with *S. epidermidis* responsible for 90% of these isolates.[289] Additional risk factors include advanced age and extended length of hospital stay. Approximately 1% of UTIs in outpatients are caused by coagulase-negative staphylococci.[314] Infection with coagulase-negative staphylococci is associated with a lesser degree of pyuria than infection with gram-negative bacilli (mean urine leukocyte count, of 39 vs. 121 white blood cells per milliliter), and most of these patients are asymptomatic.[315] Coagulase-negative staphylococci causing

nosocomial UTIs are usually methicillin resistant, and treatment, if required, should be based on the susceptibility profile of the organism. Coagulase-negative staphylococci have also been noted as a cause of chronic prostatitis, and strains causing this condition are more frequently noted to inhibit lysozyme and platelet microbicidal protein than strains isolated from the semen of healthy men.[316]

Infections of Genitourinary Prostheses

S. epidermidis is responsible for 35% to 60% of infections of synthetic urinary sphincters and penile prostheses, in which an overall infection rate of 2% to 4% is observed.[317] Coagulase-negative staphylococcal infections of penile prostheses are often indolent and may take up to a year from the date of implantation to manifest clinically. Those with infected prostheses exhibit local pain, swelling, induration, and erythema of the penis. Occasionally fistula formation is observed, and malfunction or impairment of the device is frequent. Rarely do systemic signs of infection occur. Diagnosis is made clinically and by culture of any drainage or of the device itself. Surgical removal of the device is usually required, accompanied by 10 to 14 days of systemic antibiotics for uncomplicated infection.[318] Treatment with retention of the prosthesis has been successful in selected patients.[319] In prevention of infection, alcoholic chlorhexidine appears to be superior to povidone iodine in reduction of skin flora at the surgical site in patients undergoing genitourinary prosthetic surgery.[320] In addition to fastidious surgical technique and antibiotic prophylaxis, the use of antibiotic-coated prostheses appears promising as a means to prevent infection.[321,322]

Mastitis and Infections of Breast Implants

Although *S. aureus* is the major etiologic agent of mastitis in lactating women, coagulase-negative staphylococci may also play a role.[323] Causative strains usually produce biofilm and are antibiotic resistant. Infection associated with breast implant surgery occurs in approximately 1% to 2% of patients and is most often caused by coagulase-negative staphylococci (27%).[324–326] The rate of infection is highest in patients undergoing mastectomy and immediate tissue expander reconstruction (6%).[326] *S. epidermidis* inhabits the glandular tissue ducts of the breast and from there may gain access to the space surrounding the implant.[327] Infection may manifest acutely or may be indolent. Signs and symptoms are predominantly localized and include erythema, tenderness, pain, swelling, induration, and drainage. Acute infections are often associated with systemic findings, such as fever and leukocytosis. Diagnosis is confirmed by culture of the drainage or fluid surrounding the implant or of the implant itself. Recovery of coagulase-negative staphylococci is increased by sonication of the implant.[328] Treatment consists of antibiotic therapy and a two-stage replacement procedure.[327] Capsular contracture remains the most common complication after breast augmentation. Increasing evidence points to chronic low-grade or subclinical infection with coagulase-negative staphylococci as a cause for capsular contracture.[329] *S. epidermidis* is also seen as a cause of infection after nipple piercing and insertion of nipple rings or studs.

Miscellaneous Prosthetic Device Infections, Implant Infections, and Other Infections

Almost any biomaterial or device that is inserted or implanted across the skin or mucous membranes can become colonized or infected by coagulase-negative staphylococci. Miscellaneous devices that have been associated with infections caused by coagulase-negative staphylococci include ventricular assist devices, coronary stents, hemodialysis shunts and catheters, implantable neurologic stimulators and peripheral nerve catheters, cochlear implants, fracture fixation devices and other orthopedic implants, ureteral or urethral stents, and surgical mesh. It can be anticipated that infection caused by coagulase-negative staphylococci will parallel the increasing use of such devices.[318,330]

In recent years, a large number of infections have been characterized as biofilm associated. It is therefore not surprising that coagulase-negative staphylococci, whose chief virulence determinant is the ability to elaborate a biofilm, have been incriminated as a cause of a variety of biofilm-associated infections such as otitis, osteomyelitis, endocarditis, and chronic cystitis.

PATIENT POPULATIONS AT INCREASED RISK OF INFECTION WITH COAGULASE-NEGATIVE STAPHYLOCOCCI

Transplant Patients and Neutropenic Hosts

Solid-organ or hematopoietic stem cell transplant patients are susceptible to coagulase-negative staphylococcal infections owing to immunosuppression, intravascular catheterization, and mucosal or skin breakdown.[331,332] These infections most often manifest as an *S. epidermidis* bloodstream infection and are due to an infected intravenous catheter. However, empirical therapy directed toward coagulase-negative staphylococci is discouraged in patients with febrile neutropenia.[333,334] Mucositis and the breakdown of gastrointestinal mucosal integrity, related to cytotoxic chemotherapy or radiation therapy, may be an alternate source for coagulase-negative staphylococcal bacteremia.[335,336] The Centers for Disease Control and Prevention surveillance definition for CLA-BSI takes into account mucosal breakdown as a source for bacteremia due to coagulase-negative staphylococci and other organisms that frequently colonize mucosal membranes.[335] Cardiac transplant patients are at increased risk of sternal wound infections and mediastinitis caused by coagulase-negative staphylococci (see Chapter 85).

Neonates

Early-onset neonatal sepsis (occurring at less than 72 hours of life) is rarely due to coagulase-negative staphylococci. In contrast, late-onset neonatal sepsis is frequently caused by coagulase-negative staphylococci, and the incidence varies, depending on the degree of maturity and birth weight, from 0.6% to 14.2%.[337] Over one-half of these infections are caused by coagulase-negative staphylococci.[337–339] *S. epidermidis* accounts for 60% to 93% of the infections caused by coagulase-negative staphylococci, with lesser contributions by *S. haemolyticus, S. hominis, Staphylococcus warneri, S. saprophyticus, Staphylococcus cohnii, S. capitis,* and other species. After implementation of infection-prevention measures directed primarily at reducing intravascular catheter–associated infection, some centers have noted a dramatic fall in the incidence of neonatal sepsis due to coagulase negative staphylococci.[53]

Neonates become colonized with coagulase-negative staphylococci on their skin and in their nares, umbilicus, pharynx, and gastrointestinal tract within days of their admission to the neonatal intensive care unit (NICU), and in most cases these organisms do not originate from the mother but are acquired from the hospital environment and health care workers.[340] The neonatal gut, in particular, appears to be a niche quickly colonized by antibiotic-resistant strains of coagulase-negative staphylococci.[340–342] In one study using multiple molecular epidemiologic typing methods, 62% of NICU nurses harbored methicillin-resistant strains of coagulase-negative staphylococci that were identical to bacteremic strains.[343] Risk factors for developing coagulase-negative staphylococcal bacteremia include low birth weight, the presence and duration of use of CVCs and umbilical catheters, mechanical ventilation, and total parenteral nutrition, especially with intravenous lipid emulsions.[338,344–346]

Coagulase-negative staphylococcal bacteremia in neonates is often indolent, and signs of infection may include abdominal distention, apnea, bradycardia, inability to maintain body temperature, feeding difficulties, lethargy, neutropenia, thrombocytopenia, hyperglycemia, and metabolic acidosis.[344,345] Procalcitonin may be a more useful test in diagnosis of infection due to coagulase-negative staphylococci than CRP, white blood cell count, or immature-to-total neutrophil ratio.[339,347] Persistent bacteremia was associated with the ability to produce biofilm in one study.[348] Although most bloodstream infections occur in infants with indwelling catheters, some cases are also found when there are no intravenous catheters present. These may be the result of skin lesions or respiratory or gastrointestinal colonization by these organisms.[341] Differentiating true bacteremia from contamination is made even more difficult in neonates for several reasons, including the difficulty in obtaining blood from low-birth-weight infants, the small volume of

blood generally obtained (0.1–1 mL), and the common practice of obtaining a single sample of blood for culture to preserve blood volume.[338,349] It is usually necessary to correlate other laboratory findings along with the clinical presentation and the blood culture results to arrive at the correct diagnosis.[338] In addition to bacteremia, coagulase-negative staphylococci can also cause skin infections, pneumonia, UTIs, and meningitis in the newborn. A particularly common problem, conjunctivitis, is reported to occur in 4% to 22% of neonates.[350] Coagulase-negative staphylococci are the most frequently isolated pathogen. Erythema toxicum neonatorum, a benign self-limited eruption, may be caused by an innate immune response to coagulase-negative staphylococci that have colonized the hair follicles. Similarly, there are data associating *S. epidermidis* and neonatal necrotizing enterocolitis.[337,351] Coagulase-negative staphylococcal infections in the neonate infrequently result in mortality but are often associated with morbidity necessitating many additional days of care in the hospital while the infant receives antimicrobial therapy. Prevention of coagulase-negative staphylococcal infection in neonates has largely concentrated on prevention of intravascular catheter–associated infection. Catheters should be inserted with meticulous attention to aseptic practices. Staff should adhere to appropriate protocol in caring for the catheter site and in accessing the catheter, including thorough disinfection of the catheter connector valve or hub.[205] With some restrictions in place, usually based on gestational age, most NICUs in the United States report the use of chlorhexidine as a skin disinfectant to prevent intravascular catheter infection.[352–354] Other preventive measures have included use of prophylactic antibiotics or antimicrobial flush solutions. A meta-analysis of five randomized controlled trials that evaluated the safety and efficacy of prophylactic vancomycin in preventing late-onset sepsis in neonates has confirmed a beneficial effect.[355] However, mortality and length of stay were not significantly different between the two groups. There were insufficient data to evaluate the risk of development of vancomycin-resistant organisms. Similarly, a trial of a vancomycin-heparin catheter lock solution proved beneficial in preventing bloodstream infections.[356]

Burn Patients

Not surprisingly, burn patients have an exceptionally high rate of bloodstream infection. The rate of CLA-BSI is higher in burn units (2.9 per 1000 CVC days) than in any other surveyed unit, and methicillin-resistant coagulase-negative staphylococci are among the most frequently isolated pathogens.[194,357] These infections are associated with increased morbidity and length of hospital stay,[357–359] which emphasizes the need for scrupulous attention to CVC insertion and care guidelines.

NON–*STAPHYLOCOCCUS EPIDERMIDIS* SPECIES OF COAGULASE-NEGATIVE STAPHYLOCOCCI

In addition to *S. epidermidis,* several other species of coagulase-negative staphylococci should be specifically discussed because of their pathogenic potential and other unique features.

Staphylococcus haemolyticus

S. haemolyticus is typically the second or third most common species of coagulase-negative staphylococci to be incriminated as a cause of infection.[360,361] These infections are usually nosocomial bloodstream infections related to intravascular catheters, but skin and soft tissue infection, UTI, meningitis, endocarditis, and a variety of device-associated infections have been described.[362–365] *S. haemolyticus* has been implicated in outbreaks, most often in NICUs. Companion animals have been noted to be colonized with antibiotic-resistant strains of *S. haemolyticus.*[366] *S. haemolyticus* contains several putative virulence factors, including phenol soluble modulins.[367] Approximately 75% of clinical isolates of *S. haemolyticus* produce biofilm. The most noteworthy feature of *S. haemolyticus* is that it is often resistant to multiple antibiotics, including glycopeptides.[368,369] The glycopeptide-resistant strains possess highly cross-linked peptidoglycans with serine instead of glycine in their cross bridges. It has been suggested that *S. haemolyticus* serves as a reservoir of resistance genes for other species of staphylococci.[360] Outbreaks of infection due to linezolid-resistant strains of *S. haemolyticus* have been described.[370]

Staphylococcus lugdunensis

S. lugdunensis, first described in 1988, is a constituent of the normal human skin flora and an increasingly described human pathogen. *S. lugdunensis* behaves clinically in a manner similar to *S. aureus* and has been described to cause fulminant native valve endocarditis, endocarditis associated with implanted cardiac electrical devices, PVE, skin and soft tissue infection, adenitis, bacteremia, ocular infection, UTI, peritoneal catheter infection, central nervous system infection, bone and joint infection, and peritonitis.[10,11,371–378] When MALDI-TOF-MS is used to associate coagulase-negative species with clinical significance, 40% of *S. lugdunensis* isolates are associated with infection risk.[379] *S. lugdunensis* appears to colonize various skin and mucous membrane sites much more frequently than *S. aureus*, which may explain a propensity for *S. lugdunensis* to cause infectious complications, including endocarditis, after vasectomy.[380,381] It is thought that *S. lugdunensis* is more virulent than other coagulase-negative staphylococci because of the production of several virulence factors, including a delta toxin–like hemolytic peptide; a variety of adhesins promoting adherence to collagen, fibronectin, fibrinogen, laminin, and vitronectin; a variety of enzymes, including DNase and lipase; lysozyme resistance; and biofilm formation.[10,11,382–384] Its prominent role in native valve endocarditis may be the result of the production of a von Willebrand factor–binding protein that allows it to bind to endothelial lesions.[91] In addition, it possesses an accessory gene regulator system (*agr* locus), similar to *S. aureus.*

Identification of this species in the clinical laboratory can be difficult. *S. lugdunensis* can be easily confused with *S. aureus* if identification is based only on the latex agglutination test because the production of clumping factor by *S. lugdunensis* will yield a positive test result.[10,11] Detection of genes encoding tannase production can be used to quickly identify *S. lugdunensis.*[385] It is commonly susceptible to most antistaphylococcal antibiotics because β-lactamase production is found in only about 25% of strains, but methicillin resistance is now reported in populations (dialysis patients) commonly colonized with multidrug-resistant pathogens.[386] Infections due to *S. lugdunensis* are treated similarly to those caused by *S. aureus.* The interested reader is referred to reviews on *S. lugdunensis.*[10,11,387] Although similar putative virulence determinants that have been noted in *S. lugdunensis* have also been described in *Staphylococcus schleiferi,* this species is only rarely associated with disease in humans.[6,7] However, it appears to be a significant cause of skin disease and otitis in companion animals.[388]

Staphylococcus saprophyticus

S. saprophyticus colonizes the rectum or urogenital tracts of approximately 5% to 10% of women[314] and is a frequent causative agent of uncomplicated UTIs in young, sexually active women.[389–392] *S. saprophyticus* is only rarely implicated as a cause of UTI in men. *S. saprophyticus* UTIs in women have a seasonal predilection (late summer and fall), often follow sexual intercourse or menstruation, and may occur concomitantly with vaginal candidiasis.[393] Of patients with *S. saprophyticus* UTIs, 90% are symptomatic with dysuria, frequency, or urgency, and 80% have pyuria or hematuria. Whole-genome sequence of *S. saprophyticus* has been defined, which has allowed for greater insight into the pathogenesis of UTI.[136] *S. saprophyticus* possesses a unique adhesion protein, UafA, which allows it to adhere to human uroepithelial cells and mediates hemagglutination.[135] In addition, this bacterium encodes several transport proteins, enabling it to adjust rapidly to osmotic and pH changes, and it produces abundant urease, allowing it to proliferate in urine.[137] Data regarding the molecular epidemiology of *S. saprophyticus* remain relatively scarce, and it is unclear whether specific strains or clones are preferentially responsible for UTI.[394] Timely identification of *S. saprophyticus* can be achieved through PCR testing.[395] *S. saprophyticus* can also be differentiated from other coagulase-negative staphylococci because of its resistance to novobiocin. However, it remains susceptible to other antimicrobial agents, and *S. saprophyticus* UTIs are usually successfully treated with urinary tract antimicrobials, with only rarely reported sequelae. Rare cases of native valve endocarditis, endophthalmitis, and septicemia have been cited in the literature.

OTHER SPECIES OF COAGULASE-NEGATIVE STAPHYLOCOCCI

A large number of other species of coagulase-negative staphylococci are increasingly described as infrequent causes of human infection. Some of the more noteworthy reports include linezolid-resistant *S. cohnii, Staphylococcus kloosii,* and *Staphylococcus pettenkoferi*[396,397]; native valve endocarditis due to *Staphylococcus simulans*[398]; brain abscess due to *Staphylococcus massiliensis*[399]; a multicenter outbreak of bloodstream infections in neonates due to methicillin-resistant *S. capitis* with reduced susceptibility to vancomycin[400]; a series of orthopedic infections due to *S. warneri*[401]; meningitis caused by *S. capitis* without underlying trauma or implant[402]; and finally, an infection due to a relatively newly defined species,[396–402] *Staphylococcus petrasii.*[403] *Staphylococcus pseudintermedius* is a cause of infections in dogs but may occur in humans, usually as a skin and soft tissue infection in a dog owner.[404] However, in general, coagulase-negative staphylococci do not appear to be shared between dogs and their owners.[404] Methicillin resistance is common, and susceptibility testing using cefoxitin as a surrogate for methicillin resistance is not reliable.[405,406]

NOVEL THERAPEUTIC OPTIONS FOR INFECTIONS DUE TO COAGULASE-NEGATIVE STAPHYLOCOCCI

The increasing prevalence of multidrug-resistant *S. epidermidis* and a greater understanding of the pathogenesis of *S. epidermidis* infections and the role of biofilm have prompted investigators to explore novel therapeutic modalities.[407] Developmental agents, many of which are thought to interfere with *S. epidermidis* biofilm, include cinnamon oil,[408] ultrashort cinnamic acid peptide derivatives, cathelicidin[409] berberine,[410] furanones,[411] cationic lipopeptides, flavonoids (quercetin), pleuromutilins,[412] and various other AMPs such as short salt-resistant synthetic peptides.[408–416] Biomedical engineers continue to work on prosthetic devices that are coated or impregnated with constituents that inhibit staphylococcal adherence or proliferation.[417] Antibiofilm approaches and other innovative technologies include immunoprophylaxis (*S. epidermidis* vaccine),[418] quorum sensing interference,[417] impairment of *S. epidermidis* adhesion or biofilm accumulation,[419] immunotherapy (bacteriophage)[420] enzymatic disruption or removal of biofilm,[421] immunomodulation, and use of nanoparticles to deliver antibiofilm agents.[417–422] Some evidence indicates that other microbes produce substances that inhibit *S. epidermidis* biofilm,[423] and an extract from medicinal maggots *(Lucilia sericata)* has a strong impact on biofilm.[423,424] Similarly, a defensin derived from bedbugs appears to have antimicrobial activity against *S. epidermidis.*[425] Although some of these developments appear promising, at the present, coagulase-negative staphylococci continue to offer vexing challenges to patients, clinicians, and medical microbiologists. Unfortunately, with the increased use of medical devices, the challenge of coagulase-negative staphylococcal infections is not likely to diminish.

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The complete reference list is available online at Expert Consult.

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196 Classification of Streptococci

John P. Dekker and Kathryn L. Ruoff

Members of the genus *Streptococcus* are catalase-negative, gram-positive bacteria that form oval or coccoid cells arranged in pairs and chains. Streptococci are nutritionally fastidious and require complex media, preferably supplemented with blood, for optimal growth. They are homofermentative lactic acid bacteria, producing lactic acid without gas as the major end product of glucose metabolism. Although streptococci are referred to as facultative anaerobes, growing both aerobically and anaerobically, streptococci do not use oxygen metabolically. In addition, some strains are capnophilic, whereas others grow better under anaerobic conditions. This large and heterogeneous group of commensals of humans and animals harbors relatively avirulent normal microbiota organisms as well as some of the most impressive human pathogens.[1]

Early attempts to classify streptococci of clinical importance centered around their action on blood-containing agars[2] and antigens contained in their cell walls.[3] Some streptococci (β-hemolytic) can lyse blood cells and cause complete clearing of blood agar in the vicinity of their growth (Fig. 196.1). Other strains cause no change in blood agar (γ-hemolytic or nonhemolytic), whereas the remainder of the streptococci (α-hemolytic) reduce hemoglobin and cause a greenish discoloration of the agar (Fig. 196.2). Lancefield,[3] concentrating initially on virulent, β-hemolytic streptococci, found that they could be subdivided based on cell wall antigens. It was thought that β-hemolytic organisms with the same Lancefield antigen were closely related, but this correlation was not always valid for non–β-hemolytic strains. Other phenotypic traits of streptococci were also examined and catalogued throughout the 20th century, giving rise to various classification schemes. In 1937 Sherman[4] classified the streptococci into the pyogenic, viridans, enterococcal, and lactic divisions, based on phenotypic traits. Subsequent molecular studies generally upheld these basic divisions,[5] but revealed multiple genera among organisms traditionally thought to be streptococci.

By the mid-1980s, the enterococcal streptococci (Lancefield group D, bile esculin positive, and salt tolerant) had taken up residence in their own newly created *Enterococcus* genus, and the "dairy" or "lactic" streptococci (Lancefield group N, occasionally documented in human infection) were moved to the new *Lactococcus* genus. Ensuing studies of streptococci isolated from human and animal infections gave rise to updated classification schemes based on 16S ribosomal RNA sequences and other molecular information. These investigations also aided accurate differentiation of genera of streptococcal-like, catalase-negative, gram-positive cocci (e.g., *Leuconostoc, Pediococcus*, and numerous others) that had previously been unrecognized in clinical specimens. Many, but not all, β-hemolytic streptococci were found to be members of the pyogenic group, whereas the viridans streptococci were divided into groups of closely related species, one of which, the *Streptococcus mitis* species group, includes *Streptococcus pneumoniae*. Although the viridans strains, normal microbiota of the oral cavity and gastrointestinal tract, were traditionally characterized as α-hemolytic, it was realized that some members of this group also displayed β-hemolysis. Organisms considered to be nutritionally variant streptococci (also referred to as pyridoxal-dependent or satelliting streptococci) were reclassified in two new genera, *Abiotrophia* and *Granulicatella*.[1,6] It became apparent that the hemolytic reactions, Lancefield antigens, and other phenotypic characteristics relied on in the past were not always accurate predictors of genetic relatedness among strains. These characteristics are, however, still useful to clinical laboratorians for the presumptive identification of many commonly encountered streptococci. Table 196.1 summarizes currently described streptococcal species and species groups that are frequently isolated from humans. Streptococci normally associated with animals but infrequently isolated from human infection are presented in Table 196.2.

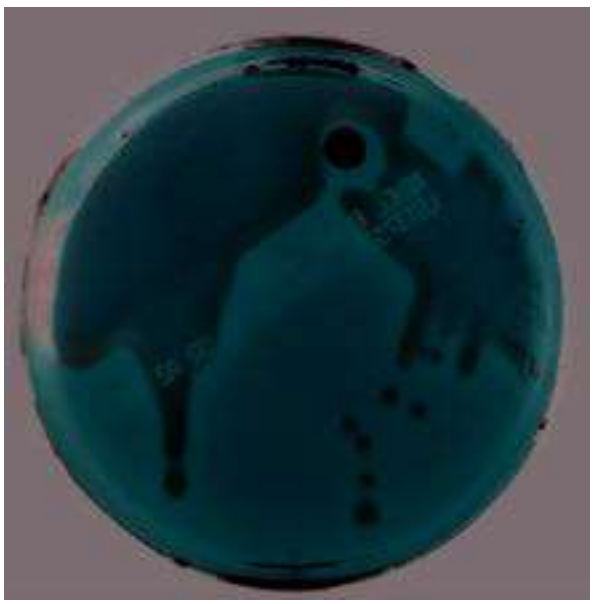

FIG. 196.1 Group A streptococci growing in pure culture on a sheep blood agar plate. Individual colonies are surrounded by zones of complete β-hemolysis. Subsurface hemolysis (agar stab) is caused in part by the action of streptolysin O, which is oxygen labile. The zone of inhibition around a low-potency bacitracin disk is a presumptive test for group A organisms.



FIG. 196.2 Sheep blood agar plate with α-, β-, and γ-hemolytic streptococci.

TABLE 196.1 Classification of Streptococci Commonly Isolated From Humans

SPECIES	LANCEFIELD ANTIGEN(S)	HEMOLYTIC REACTION(S)	COMMENTS
S. pyogenes	A	β	Pyogenic; can be differentiated from β-hemolytic anginosus group strains with the group A antigen by the formation of relatively large colonies and other phenotypic traits; agent of pharyngitis and respiratory, skin, and other infections; can cause nonsuppurative sequelae (acute rheumatic fever, acute glomerulonephritis)
S. agalactiae	B	β, γ	Pyogenic; hemolytic reaction is weak; agent of chorioamnionitis, puerperal sepsis, neonatal sepsis and meningitis, and infections in nonpregnant adults
S. dysgalactiae subsp. *equisimilis*	C, G[b]	β	Pyogenic; formerly named *S. equisimilis;* can be differentiated from β-hemolytic *S. anginosus* group strains with the C or G antigen by the formation of relatively large colonies and other phenotypic traits; agent of respiratory and deep tissue infections, cellulitis, and septicemia
S. pneumoniae and the *S. mitis* spp. group	Not detected or not useful for differentiation	α	*S. pneumoniae,* an agent of respiratory infections, otitis media and meningitis, is a close relative of members of the *S. mitis* spp. group, a viridans streptococcal group. The nonpneumococcal *S. mitis* spp. group members include *S. mitis, S. oralis, S. sanguinis, S. gordonii,* and *S. pseudopneumoniae.* Some nonpneumococcal species produce extracellular polysaccharides[c] and play roles in subacute bacterial endocarditis and infections in neutropenic hosts
S. anginosus spp. group	A, C, F, G, or no detectable antigen	α, β, γ	Viridans streptococcal group composed of three species: *S. anginosus, S. constellatus,* and *S. intermedius;* two subspecies of *S. constellatus: S. constellatus* subsp. *constellatus* and *S. constellatus* subsp. *pharyngis,* have been described[7]; formerly known as *S. milleri;* β-hemolytic strains form small colonies compared with those of pyogenic β-hemolytic group A, C, and G streptococci and also differ in other phenotypic traits; agents of purulent infections
S. bovis spp. group	D	α, γ	Viridans streptococcal group formerly known as group D nonenterococcal streptococci; strains commonly isolated from humans are currently classified as *S. gallolyticus* subsp. *gallolyticus* (formerly *S. bovis* biotype I), *S. gallolyticus* subsp. *pasteurianus* (formerly *S. bovis* biotype II/2), *S. infantarius* subsp. *infantarius,* and *S. infantarius* subsp. *coli* (formerly *S. bovis* biotype II/1)[8]; some strains produce extracellular polysaccharides[c]; agents of endocarditis, meningitis; isolated from blood in patients with colonic cancer
S. mutans spp. group	Not useful for differentiation	α, γ, occasionally β	Viridans streptococcal group; *S. mutans* and *S. sobrinus* spp. are commonly isolated from humans; produce extracellular polysaccharides[c]; agents of dental caries and endocarditis
S. salivarius spp. group	Not useful for differentiation	α, γ	Viridans streptococcal group; *S. salivarius* and *S. vestibularis* spp. commonly isolated from humans; strains in the *S. salivarius* group may react with Lancefield group K antiserum and may produce extracellular polysaccharides[c]; infrequent opportunists in compromised hosts

[a]See reference 1 for additional information on streptococci mentioned in this table.
[b]Isolates with the group A antigen have also been described.
[c]Extracellular polysaccharides (dextran, levan) are thought to aid colonization and may play a role in virulence.

TABLE 196.2 Streptococci Primarily Isolated From Animals That May Occasionally Cause Human Infection

SPECIES	LANCEFIELD ANTIGEN(S)	HEMOLYTIC REACTION	COMMENTS
S. dysgalactiae subsp. *dysgalactiae*	C, L	α, β, γ	Pathogen of domesticated animals; participation in human infections not well documented
S. equi subsp. *equi*	C	β	Agent of equine strangles; participation in human infections not well documented
S. equi subsp. *zooepidemicus*	C	β	Agent of bovine mastitis and infection in other domesticated animals; implicated in outbreaks of nephritis in humans
S. porcinus	E, P, U, V	β	Swine are usual hosts; *S. porcinus*-like strains isolated from the human female genital tract are currently classified as *S. pseudoporcinus*[1]; may cross react with commercially available group B streptococcal grouping reagents
S. canis	G	β	Dogs and other animals are usual hosts; documented as an infrequent human pathogen
S. suis	R, S, T	α, β[b]	Swine are usual hosts; isolated infrequently from cases of human meningitis
S. iniae	No detectable antigen	β	Fish are usual hosts; isolated infrequently from cutaneous and systemic infection in humans

[a]See reference 1 for additional information on streptococci mentioned in this table.
[b]α-Hemolytic on sheep blood agar, but some strains may be β-hemolytic on horse blood agar.

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197 *Streptococcus pyogenes*

Amy E. Bryant and Dennis L. Stevens

SHORT VIEW SUMMARY

Definition

- *Streptococcus pyogenes* is an important global human pathogen that causes a wide variety of acute infections, such as soft tissue infections and pharyngitis; severe life-threatening infections, such as streptococcal toxic shock syndrome (strep TSS); and devastating postinfectious sequelae, such as rheumatic fever and glomerulonephritis.

Epidemiology

- The most common infection is streptococcal pharyngitis.
- Superficial skin and soft tissue infections include impetigo, erysipelas, and cellulitis.
- Severe life-threatening infections include scarlet fever, bacteremia, pneumonia, necrotizing fasciitis, myonecrosis, and streptococcal toxic shock syndrome.
- Postinfectious sequelae include acute rheumatic fever and poststreptococcal glomerulonephritis.

Microbiology

- *S. pyogenes* is a gram-positive, β-hemolytic *Streptococcus* that is catalase negative. More than 150 different strains have been identified based on different M-protein types. It is a group A *Streptococcus* based on its carbohydrate structure, according to Lancefield typing of β-hemolytic strains. Mucoid strains are rich in hyaluronic acid capsules, and numerous extracellular toxins are produced by most strains, which include streptolysin O (SLO; a cholesterol-dependent cytolysin), streptolysin S (SLS; a cell-associated hemolysin), nicotine adenine dinucleotidase (NADase; a metabolic toxin), fibrinogen-binding proteins, streptokinase (a fibrinolysin), numerous pyrogenic exotoxins that act as superantigens (e.g., streptococcal pyrogenic exotoxin A, [SpeA]), and a cysteine protease called streptococcal pyrogenic exotoxin B (SpeB).

Diagnosis

- Diagnosis of *S. pyogenes* pharyngitis is suspected based on specific clinical criteria and can be substantiated by rapid antigen tests or by bacteriologic culture.
- Similarly, impetigo, cellulitis, and erysipelas are suspected based on their clinical presentations. Cultures of impetiginous lesions will distinguish *Streptococcus* from *Staphylococcus aureus* as the cause. Cultures of lesions associated with cellulitis and erysipelas are useful only 20% of the time, and blood cultures are rarely positive.
- Invasive *S. pyogenes* is more difficult to diagnose early in the course, although blood cultures are positive in greater than 50% of cases. In the 50% of patients with necrotizing fasciitis associated with a portal of entry, such as surgical incision, postpartum sepsis, or insect bites, cultures of these sites are positive in most cases. In the 50% of patients with no portal of entry, infection begins deep in the fascia and muscle, and these patients often present with a history of previous nonpenetrating trauma, severe pain, and systemic toxicity. Classic signs of necrotizing infections are not apparent until late in the course, at a time when the patient has systemic shock and organ failure.
- Rheumatic fever is diagnosed based on clinical suspicion of patients with an antecedent pharyngitis who present with carditis, Sydenham chorea, migratory arthritis, and evidence that the pharyngitis was due to *S. pyogenes* by positive culture or rising anti-SLO titers and using the Jones criteria, which are reviewed elsewhere.
- Poststreptococcal glomerulonephritis is diagnosed based on evidence of renal failure with glomerular damage, as indicated by red blood cell casts and an antecedent streptococcal infection, which can be either pharyngitis or impetigo (see Chapter 200).

Therapy

- Penicillin remains the drug of choice for treatment of most streptococcal infections.
- Most cases of impetigo can be treated with topical bacitracin, mupirocin, or retapamulin, and only in severe cases or in epidemic situations is oral or parenteral administration of penicillin necessary.
- For severe cases of *S. pyogenes* infections, such as necrotizing fasciitis, myonecrosis, and strep TSS, the current recommendation includes the addition of clindamycin, which suppresses toxin production and in animal studies and limited human studies is superior to penicillin. Intensive care support, aggressive fluid resuscitation, ventilator support, and surgical intervention are commonly required.

Prevention

- Epidemics of streptococcal pharyngitis have been prevented in military recruits by the administration of monthly benzathine penicillin given intramuscularly.
- Rheumatic fever can be prevented by administration of penicillin within 9 days of the onset of streptococcal pharyngitis. Secondary prophylaxis should be considered in patients with rheumatic heart disease based on age, small children in the household, and exposure to cases of streptococcal infection.
- Improved living conditions, improved personal hygiene, and topical treatment of impetiginous lesions can prevent spread to susceptible individuals.
- Prevention of secondary cases should be considered in health care workers and family members with prolonged, intimate contact with patients with necrotizing fasciitis/strep TSS, particularly those who are immunocompromised, have varicella, have had recent surgery, or have recently given birth. The risk for secondary severe infection is low, but colonization and streptococcal pharyngitis can occur commonly. Oral penicillin for 7 to 10 days is reasonable, although no definitive studies have been done.

Streptococcus pyogenes (group A *Streptococcus;* [GAS]) is one of the most important bacterial pathogens of humans. This ubiquitous organism is the most frequent bacterial cause of acute pharyngitis, and it also gives rise to a variety of cutaneous and systemic infections. Its unique place in medical microbiology stems from its propensity to initiate two nonsuppurative sequelae: acute rheumatic fever and poststreptococcal glomerulonephritis. The former malady has been responsible for suffering, disability, and mortality in all parts of the world.

HISTORY

Streptococci were demonstrated in cases of erysipelas and wound infections by Billroth in 1874 and in the blood of a patient with puerperal sepsis by Pasteur in 1879. Fehleisen, in 1883, isolated chain-forming organisms in pure culture from erysipelas lesions and then demonstrated that these organisms could induce typical erysipelas in humans. Rosenbach applied the designation *Streptococcus pyogenes* to these organisms in 1884.

Initial progress toward a rational classification of streptococci dates from the description by Schötmuller in 1903 of the blood agar technique for differentiating hemolytic from nonhemolytic streptococci. In 1919 Brown[1] made a systematic study of patterns of hemolysis and introduced the terms α-, β-, and γ-hemolysis (see Chapter 196).

Rebecca Lancefield's classification of β-hemolytic streptococci into distinct serogroups in 1933 was a major turning point in our understanding of the epidemiology of streptococcal infections.[2] Most strains pathogenic for humans were found to belong to serogroup A *(S. pyogenes).* Systems of serotyping GAS were later developed on the basis of M-protein precipitin reactions (Lancefield) or T-protein agglutination reactions (Griffith). In addition, Lancefield established the critical role of M protein in streptococcal virulence and the type-specific nature of protective immunity to group A streptococcal infection. Studies by Dochez and collaborators and by George and Gladys Dick in the 1920s established the relationship of scarlet fever to hemolytic streptococcal infection. A few years later, Todd's description of the method for titration of anti–streptolysin O (ASO) in serum added still another important tool to the armamentarium available for study of the immunology and epidemiology of streptococcal disease. Such tools were used by a number of investigators, including Coburn, Collis, Rammelkamp, Stollerman, and Wannamaker, to establish the relationship of group A streptococcal infection to acute rheumatic fever and acute glomerulonephritis. Much of our knowledge of the detailed epidemiology of streptococcal infections and of acute rheumatic fever derives from the pioneering studies performed at Warren Air Force Base, Wyoming during 1949–51 by Rammelkamp, Wannamaker, and Denny.[3–5]

In 1981 the first superantigen was described from *Staphylococcus aureus* as a cause of staphylococcal toxic shock syndrome toxin 1 (TSST-1).[6] Later, pyrogenic exotoxins from GAS were shown to be superantigens as well (reviewed in Spaulding and colleagues[7]). These extracellular protein toxins have the ability to create a cytokine storm by simultaneously stimulating both T lymphocytes and macrophages (see discussion of virulence factors later).

In 1989 Stevens and colleagues[8] described 20 patients with streptococcal toxic shock syndrome (strep TSS) and implicated production of specific pyrogenic exotoxins associated with this condition.

DESCRIPTION OF THE PATHOGEN

GAS grow as spherical or ovoid cells 0.6 to 1.0 μm in diameter and occur as pairs or as short to moderate-length chains in clinical specimens. When growing in broth media enriched with serum or blood, long chains are frequently formed, and many strains produce capsules of hyaluronic acid. The organisms are gram positive, nonmotile, non–spore forming, catalase negative, and facultatively anaerobic. GAS are nutritionally fastidious and are usually cultivated in complex media, often supplemented with blood or serum.

When cultured on blood agar plates, *S. pyogenes* appears as white-to-gray colonies, 1 to 2 mm in diameter and surrounded by zones of complete (β) hemolysis. (Strains that fail to produce such hemolysis occur but are rare.) Strains that produce copious amounts of the hyaluronate capsular material appear mucoid, at times resembling a water drop on the plate. Less mucoid strains assume a crinkled, so-called matte appearance. Small opaque colonies of organisms that lack capsules and detectable M protein are termed *glossy.*

The complete genome sequences from several *S. pyogenes* serotypes have been reported, and this information is providing insight into the subtle genetic differences among streptococcal types that arm them to produce specific syndromes. Recent whole-genome studies have also revealed the phylogenic relatedness of GAS, including globally disseminated hypervirulent M-1 and M-3 strains.[9] A large number of somatic constituents and extracellular products of GAS have been identified. The most important of these are indicated in the following sections.

Somatic Constituents

The organism is enveloped in a hyaluronic acid capsule that serves as an accessory virulence factor in retarding phagocytosis by polymorphonuclear neutrophils (PMNs) and macrophages of the host.[10,11] Streptococcal strains vary greatly in their degree of encapsulation, and those with the most exuberant capsule production have a mucoid appearance when cultivated on blood agar plates. In certain heavily encapsulated group A streptococcal strains, the capsule may take precedence over M protein in mediating resistance to phagocytosis.[11] Group A streptococcal capsular hyaluronate is chemically similar to that found in human connective tissue. For this reason, it is a poor immunogen, and antibodies to group A streptococcal hyaluronic acid have not been demonstrated in humans.

The cell wall is a complex structure containing many different antigenic substances. The group-specific carbohydrate of group A strains is a dimer of rhamnose and *N*-acetylglucosamine in a ratio of approximately 2 : 1. The mucopeptide (peptidoglycan) layer provides rigidity to the cell wall; it is composed of polymers of repeating subunits of *N*-acetylglucosamine and *N*-acetylmuramic acid connected by amino-acid side chains.

M protein is the major somatic virulence factor of GAS. Strains rich in this protein are resistant to phagocytosis by PMNs, multiply rapidly in fresh human blood, and are capable of initiating disease. Strains that do not express M protein are avirulent.[12] GAS may be divided into serotypes on the basis of antigenic differences in M-protein molecules and more recently into genotypes on the basis of nucleotide differences in the *emm* gene encoding M protein. More than 150 such serotypes and more than 220 genotypes are currently recognized.[13] Acquired human immunity to streptococcal infection is based on the development of opsonic antibodies directed against the antiphagocytic moiety of M protein. Such immunity is type specific and quite durable, lasting for many years and perhaps indefinitely. Various vaccine strategies have targeted multiple individual M proteins with some success. Recently, however, convalescent serum samples from patients with skin and soft tissue infection demonstrated cross-reactive immune responses that align with M-protein clusters, suggesting that fewer individual M-types may be required for an effective vaccine against GAS.[14]

The M protein itself is a filamentous macromolecule that exists as a stable dimer with an α-helical coiled coil structure.[15] The molecule, which is anchored to the cell membrane, traverses and penetrates the cell wall. The more proximal portion of the molecule contains epitopes widely conserved among GAS, whereas the more distal portion contains type-specific epitopes.[16] This configuration localizes the type-specific moiety on the tips of fibrils protruding from the cell surface (Fig. 197.1). In the nonimmune host, M protein exerts its antiphagocytic effect by inhibiting activation of the alternative complement pathway on the cell surface.[17,18] Such inhibition appears to be mediated by the binding to the M-protein molecule of host proteins, among which are complement control proteins (factor H, a factor H–like protein, and human C4b-binding protein)[19–21] and fibrinogen.[22–24] The antiphagocytic effect is nullified in the presence of adequate concentrations of type-specific antibody. There is evidence that immunity caused by opsonic anti–M-type antibody may be strain and not type specific.[25,26] M proteins analogous to those of GAS are present in many strains of groups C[27] and G[28] streptococci.

Additional surface proteins related to M protein have now been identified. Although their structure is overall similar to that of M protein, they differ in the types of repeats and in their ability to interact with different human proteins. Genes encoding these proteins (e.g., *enn,*

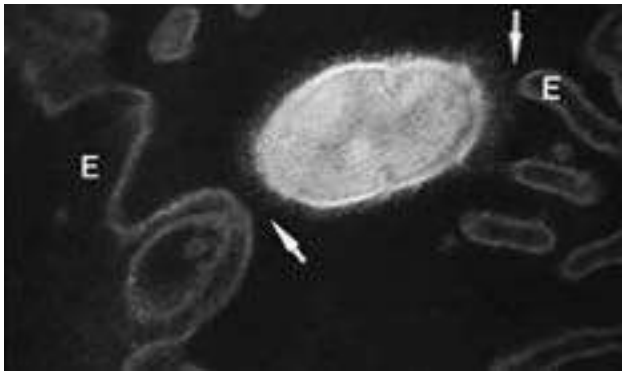

FIG. 197.1 Electron micrograph of group A streptococci. Surface fibrils contain type-specific, antiphagocytic epitopes of M protein. Lipoteichoic acid and fibronectin binding proteins facilitate adherence of streptococci to the membrane *(arrows)* of a human oral epithelial cell *(E)* (×67,500). *(From Beachey EH, Ofek I. Epithelial cell binding of group A streptococci by lipoteichoic acid on fimbriae denuded of M protein.* J Exp Med. *1976;143:759–771.)*

mrp, fcrA, arp, protH) have been designated as members of the *emm* gene superfamily. A number of the M-like proteins bind immunoglobulin (Ig)G or IgA at the non–antigen-binding site and appear to be cooperative with M protein in antiphagocytic effect.[29,30] Indeed, a notable function of the M-protein family is its ability to bind to a wide range of host proteins, including, among others, albumin, fibrinogen, and plasminogen. Still other antiopsonic surface proteins continue to be described.[31] For example, Mac, a secreted group A streptococcal protein with homology to a human β_2-integrin, binds to CD16 on the surface of human PMNs and inhibits phagocytosis and bacterial killing.[32] An additional surface protein, streptococcal heme-associated protein (Shp), has been found in M1 strains of GAS and likely has a role in transport of iron intracellularly. Antibody against Shp has been found in convalescent sera and has opsonic capability.[33] These observations underscore the extreme virtuosity with which the bacterium develops multiple mechanisms to evade phagocytic killing.

A protein antigen very closely associated with the M-protein molecule of GAS is the so-called serum opacity factor (OF). This factor is an α-lipoproteinase that is detected by its ability to opacify horse serum and that also has fibronectin-binding properties.[34] Strains of a minority of the currently identified M types elaborate this antigen.[35] OF itself is antigenic and type specific, that is, its ability to opacify serum can be specifically inhibited by antiserum raised against homologous but not heterologous M types. Type-specific and non–type-specific immune responses to streptococcal M protein are generally weaker after pharyngeal infection with OF-positive than with OF-negative types.[36] The former importance of this substance as an ancillary typing system for strains that could not be M serotyped has been obviated by the advent of *emm* genotyping. Of interest, antibody against OF has opsonic activity and has been shown to synergize with anti–M protein antibody in protecting mice against challenge with OF-positive strains.[37]

A number of somatic streptococcal constituents play critical roles in the first step of colonization, namely, adherence to the surface of human epithelial cells. At least 17 adhesin candidates have been described,[38] but the most extensively studied have been lipoteichoic acid (LTA), M protein, and fibronectin-binding proteins. Through hydrophobic interactions, LTA serves as a "first-step" adhesin, bringing the organisms into close contact with host cells and then allowing other adhesins to promote high-affinity binding.[39] Although M protein does not appear to promote adhesion to human buccal or tonsillar epithelial cells,[40,41] it does mediate adherence to skin keratinocytes via the attachment of the C repeat region to keratinocyte membrane cofactor CD46.[42,43] Group A streptococcal surface proteins that bind fibronectin have been studied extensively and are important in adherence to both throat and skin. These include protein F1 (PrtF1),[44] also known as SfbI (streptococcal fibronectin-binding protein I),[45] and related proteins known as SbfII,[46] FBP54,[47] protein F2,[48] and PFBB.[49]

Moreover, the expression of these adhesins has been reported to be environmentally regulated.[50] Expression of protein F1 is enhanced in an oxygen-rich environment, whereas that of M protein is greater at higher partial pressures of carbon dioxide.[51] Thus, teleologically, it might be postulated that the organism displays protein F1 on its surface when it seeks to adhere to the cutaneous surface but expresses M protein in the deeper tissues, where it is more likely to encounter phagocytic cells.

Extracellular Products

During the course of growth in vitro or in vivo, GAS elaborates numerous extracellular products, only a limited number of which have been well characterized. Two distinct hemolysins are recognized. SLO derives its name from its oxygen lability. It is reversibly inhibited by oxygen and irreversibly inhibited by cholesterol. In addition to its effect on erythrocytes, in high concentrations it is toxic to a variety of cells and cell fractions, including PMNs, platelets, endothelial cells, lysosomes, and isolated mammalian cardiomyocytes.[9,52] In subcytotoxic doses SLO stimulates hyperresponsiveness in these same cell types, including enhanced neutrophil degranulation, increased platelet activation and adhesion, induced synthesis of lipid mediators by endothelial cells and spontaneous, nonpaced and hyperaugmented contractions in cardiomyocytes.[53] SLO is produced by almost all strains of *S. pyogenes*, as well as many group C and G organisms, and is antigenic. Measurement of ASO antibodies in human sera is useful as an indicator of recent streptococcal infection.

Streptolysin S (SLS) is a hemolysin produced by streptococci growing in the presence of serum (hence the "S") or in the presence of a variety of other substances, such as serum albumin, α-lipoprotein, ribonucleic acid, or detergents such as Tween. SLS is nonantigenic, or at least no antibody to it has been detected that neutralizes its hemolytic activity. Like SLO, SLS can damage the membranes of PMNs, platelets, and subcellular organelles. Unlike SLO, it is not inactivated by oxygen, but it is thermolabile.[54,55] Most strains of *S. pyogenes* produce both hemolysins. Hemolysis on the surface of blood agar plates is primarily caused by SLS, whereas SLO exerts its hemolytic effect best in subsurface colonies, in pour plates, or in anaerobic cultures. An occasional strain may produce only one of the two hemolysins. Rarely, strains are encountered that lack both hemolysins.

Recently emerged, globally disseminated strains of hypervirulent GAS have been associated with production of nicotine adenine dinucleotidase (NADase), whereas older strains had the gene for this toxin but did not produce a functional enzyme.[56] Recent isolates have mutations in the SLO/NADase coregulator that result in enhanced coproduction of both virulence factors. It has been demonstrated that the binding of NADase to SLO stabilizes both toxins and enhances group A streptococcal virulence.[57]

Several extracellular products may theoretically serve to facilitate the liquefaction of pus and the spreading of streptococci through tissue planes characteristic of streptococcal cellulitis and necrotizing fasciitis. These include the following: (1) four antigenically distinct enzymes that participate in the degradation of deoxyribonucleic acid (DNases A, B, C, and D); (2) hyaluronidase, which enzymatically degrades hyaluronic acid found in the ground substance of connective tissue; (3) streptokinase, which promotes the dissolution of clots by catalyzing the conversion of plasminogen to plasmin; (4) streptococcal pyrogenic exotoxin B (SpeB), which is a potent protease; and (5) C5a peptidase, which specifically cleaves the human chemotaxis factor C5a at the PMN binding site.[58,59] SpeB also cleaves IgG bound to GAS, thus interfering with ingestion and killing by phagocytes.[60] The streptococcal pyrogenic exotoxins are a family of bacterial superantigens believed to be associated with strep TSS, necrotizing fasciitis, and other severe infections. This family includes the bacteriophage-encoded SpeA[61] and SpeC, historically known as the scarlatinal toxins because of their association with scarlet fever, as well as the cysteine protease SpeB; a number of additional pyrogenic exotoxins (e.g., mitogenic factor [MF, SpeF] and streptococcal superantigen [SSA]) have more recently been identified.[62] SSA and SpeC have been implicated in a recent upsurge of scarlet fever in China.[63] SpeB has also been implicated as a causative antigen in poststreptococcal glomerulonephritis, having been found in glomerular membranes in such patients.[64,65] Despite its many functions and its widespread distribution among clinical isolates of GAS[66] the role of SpeB in pathogenesis

remains controversial. One view is that SpeB is a key contributor to pathogenesis; alternatively, GAS may be under in vivo pressure to downregulate this toxin.

Superantigens are potent immunostimulators able to bind simultaneously to the class II major histocompatibility complex (MHC) and specific V-β regions of the T-cell receptor.[67] This binding results in clonal proliferation of these T cells. Superantigen activation of T cells leads to increased secretion of proinflammatory cytokines produced by both antigen-presenting cells and T lymphocytes. This issue is discussed in more detail later in the section "Streptococcal Toxic Shock Syndrome."

Emerging concepts regarding the molecular biology of streptococcal virulence, colonization, and tissue invasion have been reviewed.[62,68] Control of the expression of the heretofore-mentioned virulence factors over time and under diverse environmental circumstances depends on a complex system of genetic modulation. Of the known transcriptional regulators in *S. pyogenes,* the two most intensively studied are Mga[69] (multiple gene regulator) or Mry (regulator of M-protein expression) and a two-component regulatory system known as CsrRS[70] (capsule synthesis regulator) or, alternatively, CovRS[71] (control of virulence genes), which represses the synthesis of the capsule and several exotoxins.[72] The regulator of proteinase B (RopB) has been shown to have various polymorphisms that regulate the virulence of *S. pyogenes.*[73]

STREPTOCOCCAL PHARYNGITIS

Epidemiology

Streptococcal sore throat is among the most common bacterial infections of childhood. It is estimated that more than 600 million cases of streptococcal pharyngitis occur annually worldwide.[74] GAS are responsible for the great majority of such infections, but strains of other serogroups, especially groups C and G,[75] are occasionally involved. The disease occurs primarily among children 5 to 15 years of age, with the peak incidence occurring during the first few years of school. All age groups are susceptible, however, and severe epidemics are common in military training facilities. There is no gender predilection. The disease is ordinarily spread by direct person-to-person contact, most likely via droplets of saliva or nasal secretions. Crowding such as occurs in schools or barracks favors interpersonal spread of the organism (Fig. 197.2) and may also enhance its virulence by processes of natural selection analogous to those that occur during mouse passage in the laboratory. The effect of crowding in facilitating transmission may account in part for the increased incidence of streptococcal pharyngitis in northern latitudes during the colder months of the year. Explosive foodborne or waterborne outbreaks are also well documented. Contamination of dust, clothing, blankets, or other fomites does not appear to play a significant role in contagion.

GAS frequently colonize the throats of asymptomatic persons. Pharyngeal carriage rates among normal schoolchildren vary with geographic location and season of the year. Carriage rates as high as 15% to 20% have been noted in several studies. The carriage rate among adults is considerably lower.

Studies of experimentally induced human infections and of transmission in military barracks have shed considerable light on the variables involved in interpersonal spread. During the acute phase of tonsillopharyngeal infection, M-typeable GAS are frequently present in large numbers in the nose and throat. In untreated infections organisms may persist for many weeks, although the signs and symptoms of illness abate within a few days. During convalescence the organisms decrease in numbers and tend to disappear from the anterior nares sooner than from the throat. In addition, the M-protein content and virulence of persisting organisms gradually decline. The result of these qualitative and quantitative changes is that convalescent carriers are much less likely to transmit the organism to close contacts than acutely infected persons.

In patients who do not receive effective antibiotic therapy for acute streptococcal pharyngitis, type-specific antibodies are frequently detectable in the serum between 4 and 8 weeks after the infection. These opsonic antibodies protect against subsequent infection with organisms of the same M type, but the person remains susceptible to infection by heterologous types. Prompt and effective antibiotic therapy may ablate the type-specific immune response.

Clinical Manifestations

The usual incubation period of streptococcal pharyngitis is 2 to 4 days. The onset of illness is heralded by the rather abrupt onset of sore throat accompanied by malaise, feverishness, and headache. Nausea, vomiting, and abdominal pain are common in children. Prominent physical findings include redness, edema, and lymphoid hyperplasia of the posterior portion of the pharynx; enlarged, hyperemic tonsils; patchy discrete tonsillopharyngeal exudates (Fig. 197.3); enlarged, tender lymph nodes at the angles of the mandibles; and a temperature of 38.3°C (101°F) or higher. In the absence of these symptoms and signs, simple coryza, hoarseness, cough, or conjunctivitis does not suggest the presence of streptococcal infection. Laboratory findings include a positive throat culture for β-hemolytic streptococci and a total white blood cell (WBC) count usually exceeding 12,000/mm,[3] with increased numbers of PMNs. The test for C-reactive protein is usually positive.[76]

Not all patients with streptococcal pharyngitis have the full-blown syndrome just described. Endemically occurring infections in open populations manifest a wide spectrum of clinical severity. For example, only approximately 50% of such patients with sore throats and positive throat cultures have tonsillar or pharyngeal exudates. Patients who have undergone tonsillectomy tend to experience a milder clinical syndrome. In infants the response to streptococcal infection is much less sharply focalized to the lymphoid tissue of the faucial and posterior pharyngeal

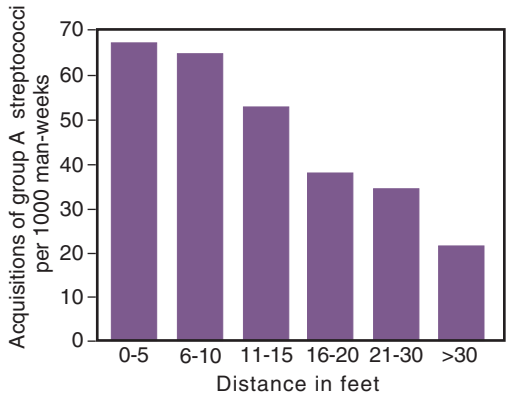

FIG. 197.2 Transmission of group A streptococci in a military barracks according to bed distance from the nearest carrier. *(From Wannamaker LW. The epidemiology of streptococcal infection. In: McCarty M, ed.* Streptococcal Infections. *New York: Columbia University Press; 1953:157–175.)*

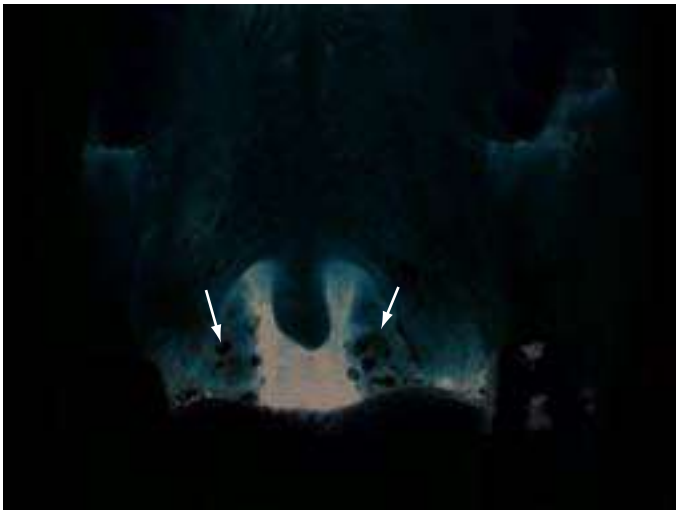

FIG. 197.3 Streptococcal tonsillopharyngitis. Exudates *(arrows)* are present on the enlarged erythematous tonsils. *(From Nimishikavi S, Stead I. Images in clinical medicine: streptococcal pharyngitis.* N Engl J Med. *2005:352:e10.)*

area. Rhinorrhea, suppurative complications, low-grade fever, and a more protracted course tend to characterize infections at this age. Exudative pharyngitis in children younger than 3 years is rarely streptococcal in cause.

In the absence of suppurative complications the disease is self-limited. Fever abates within 3 to 5 days. Almost all acute signs and symptoms subside within 1 week, although several additional weeks may be required for tonsils and lymph nodes to return to their usual size. Penicillin shortens the period of fever, toxicity, and infectivity.[77–79] Given the rather brief time course of untreated disease, however, such shortening of the clinical syndrome may not be striking unless therapy is initiated within the first 24 hours of illness. Antibiotic treatment shortens the clinical symptoms by 24 hours, and the main reason for treatment is the prevention of rheumatic fever.[80]

Scarlet Fever

Scarlet fever results from infection with a streptococcal strain that elaborates streptococcal pyrogenic exotoxins (erythrogenic toxins). Although this disease is usually associated with pharyngeal infections, it may follow streptococcal infections at other sites, such as wound infections or puerperal sepsis. The clinical syndrome is similar in most respects to that associated with nontoxigenic strains, save for the scarlatinal rash. The latter must be differentiated from those of viral exanthems, drug eruptions, staphylococcal TSS, and Kawasaki disease.

The rash usually appears on the second day of clinical illness as a diffuse red blush, with many points of deeper red that blanch on pressure. It is often first noted over the upper part of the chest and then spreads to the remainder of the trunk, neck, and extremities. The palms, soles, and usually the face are spared. Skin folds in the neck, axillae, groin, elbows, and knees appear as lines of deeper red (Pastia lines). There are scattered petechiae, and the Rumpel-Leeds test of capillary fragility is positive. Occlusion of sweat glands imparts a sandpaper texture to the skin, which is a particularly helpful finding in dark-skinned patients.

The face appears flushed, except for marked circumoral pallor. In addition to findings of exudative pharyngitis and tonsillitis, patients display an enanthem characterized by small, red, hemorrhagic spots on the hard and soft palates. The tongue is initially covered with a yellowish white coat through which may be seen the red papillae (white strawberry tongue). Later, the coating disappears, and the tongue is beefy red in appearance (red strawberry tongue). The skin rash fades over the course of 1 week and is followed by extensive desquamation lasting for several weeks. A modest eosinophilia may be present early in the course of the illness.

Severe forms of scarlet fever, either associated with local and hematogenous spread of the organism (septic scarlet fever) or with profound toxemia (toxic scarlet fever), are characterized by high fever and marked systemic toxicity. The course may be complicated by arthritis, jaundice, and, very rarely, hydrops of the gallbladder. Such severe forms of the disease are infrequent in the antibiotic era. In the late 1800s scarlet fever was associated with mortalities of 20% in Chicago, New York, and Scandinavia. Recently, an epidemic of 900 cases of scarlet fever occurred in Hong Kong between January and July 2011 associated with *emm12 S. pyogenes* strains.[81] Similarly, in Great Britain, the scarlet fever incidence increased from 8.2 per 100,000 population in 2013 to 33.1 per 100,000 in 2016, and 1 in 40 cases required hospitalization, suggesting increased virulence among prevalent strains.[74] In both studies the *emm12* type was predominant, and those from China were 100% resistant to erythromycin and clindamycin[82] and harbored the genes for superantigens SSA and SpeC.[63]

Suppurative Complications

Inflammation in the facial area induced by acute streptococcal infection may affect structures that are directly contiguous to the pharynx or that drain that site. Such relatively rare complications include peritonsillar cellulitis, peritonsillar abscess, retropharyngeal abscess, suppurative cervical lymphadenitis, mastoiditis, acute sinusitis, and otitis media.[83] Peritonsillar or retropharyngeal abscesses, however, frequently contain a variety of other oral flora, including anaerobes, with or without GAS.[84] GAS are responsible for only a small minority of cases of otitis media or sinusitis.

Extension up the cribriform plate of the ethmoid or via the mastoid bone may cause meningitis, brain abscess, or thrombosis of the intracranial venous sinuses. Streptococcal pneumonia, another potential suppurative complication, is discussed later. Finally, bacteremic spread of the streptococci may result in a variety of metastatic foci of infection, such as suppurative arthritis, endocarditis, meningitis, brain abscess, osteomyelitis, or liver abscess. Such complications of streptococcal pharyngitis are extremely rare since the advent of effective chemotherapy.

Nonsuppurative Complications

The nonsuppurative complications of streptococcal pharyngitis, acute rheumatic fever and acute poststreptococcal glomerulonephritis, are discussed in Chapter 198. The role of streptococci vis-à-vis other infectious and noninfectious agents in initiating certain other acute inflammatory disorders, such as erythema nodosum and anaphylactoid purpura, remains unresolved.

Diagnosis

Pharyngitis and tonsillitis may be caused by infectious agents other than *S. pyogenes.*[75,85] Among these are streptococci of groups C[86,87] and G.[88–90] *Corynebacterium diphtheriae,* the other major bacterial pathogen associated with exudative pharyngitis, is now extremely rare in the United States[91] and, when it occurs in the classic form, is differentiated by the appearance of the diphtheritic membrane, respiratory embarrassment, severe systemic toxicity, and myocardial and neurologic manifestations. Other bacterial agents such as *Neisseria gonorrhoeae* and perhaps *Neisseria meningitidis* occasionally cause pharyngitis, as does *Mycoplasma pneumoniae.*

Pharyngitis due to *Arcanobacterium* (formerly *Corynebacterium*) *hemolyticum,* although rare, may closely mimic that caused by *S. pyogenes.*[92,93] *A. hemolyticum* affects primarily teenagers and young adults, and the patients may exhibit both an exudative pharyngitis and a scarlatiniform rash. The organism is more readily identified on rabbit or human blood agar than on sheep blood agar. Another rare cause of acute pharyngitis is *Yersinia enterocolitica.*[94] Patients infected with this organism may appear quite ill and may or may not have associated enteric symptoms. When *Y. enterocolitica* pharyngitis is associated with disseminated yersiniosis, the mortality rate may be appreciable. Diagnosis depends on clinical clues because the organism is unlikely to be detected on routine throat cultures and antistreptococcal therapy is unavailing (see Chapter 229B). The oropharyngeal form of tularemia is characterized by severe sore throat, exudative and ulcerative tonsillopharyngitis, and cervical adenopathy (see Chapter 227).

Acute pharyngitis is more frequently caused by viruses than by bacteria. Infectious mononucleosis and adenovirus infections frequently give rise to exudative pharyngitis and thus may closely mimic streptococcal sore throat. Herpes simplex viruses 1 and 2,[95–97] influenza,[98] and parainfluenza viruses may also simulate streptococcal pharyngitis, as may initially the acute retroviral syndrome in human immunodeficiency virus (HIV) infection. Pharyngitis associated with the acute retroviral syndrome is, however, not exudative.[99] Even when careful microbiologic techniques are used to detect bacteria, mycoplasmas, and viruses, no causative agent can be detected in a substantial proportion of all cases of acute sore throat.[100] A more complete discussion of the differential diagnosis of acute pharyngitis may be found in Chapter 59.

Approximately one-fourth to one-third of all children complaining of sore throat have a positive throat culture for GAS. Of these, about half can be demonstrated to have immunologically significant infection, as judged by a significant rise in serum titer of one or more antistreptococcal antibodies. Many of the remainder are likely to be asymptomatic carriers because the average carriage rate among school-age children in temperate climates during the winter months may approximate 15% to 20%. Such asymptomatic carriers are at no risk for developing suppurative and nonsuppurative complications and do not require antibiotic therapy. Although acutely infected individuals tend to have more strongly positive throat cultures, this distinction cannot be made with confidence in patients whose signs and symptoms are compatible with those of streptococcal pharyngitis.

Numerous studies have tested the precision with which physicians may differentiate between streptococcal and nonstreptococcal sore throat

by clinical criteria alone. In the presence of a classic scarlatinal rash or during a documented epidemic of streptococcal infections, such differentiation is usually easy. On the other hand, in the case of endemically occurring infections, the problem is much more complex. Certain clinical findings, particularly fever, sore throat, tonsillopharyngeal exudate, and tender, enlarged lymph nodes at the angles of the jaws, have a statistically significant correlation (≈85%) with the presence of positive throat cultures for GAS.[101] Such findings are not diagnostic, however. Although only approximately 50% of patients with immunologically proven streptococcal sore throat have tonsillar exudate, a substantial proportion of cases of exudative pharyngitis are nonstreptococcal in cause.

It is possible to identify individual patients in which "strep throat" can be effectively excluded on a combination of epidemiologic (see earlier) and clinical grounds. For example, symptoms of the common cold are not caused by *S. pyogenes*. Similarly, the presence of hoarseness and conjunctivitis and the absence of fever or pharyngeal erythema make streptococcal pharyngitis very unlikely. A number of investigators have developed clinical algorithms in children and adults to assist in determining the likelihood that a particular patient has group A streptococcal pharyngitis.[101–107] These algorithms are useful and accurate in identifying patients whose risk for streptococcal infection is so low as to obviate the need for further microbiologic testing. Otherwise, such testing should be performed.

One published practice guideline[108,109] has suggested that in adults with features strongly suggestive of streptococcal pharyngitis, empirical antimicrobial therapy without microbiologic confirmation is an acceptable alternative. That guideline uses an algorithm, developed by Centor and coworkers,[107] using four clinical criteria—presence of tonsillar exudates, presence of swollen tender anterior cervical nodes (i.e., cervical lymphadenitis), lack of cough, and history of fever—that have been reported to be independently associated with the likelihood of a positive throat culture for GAS.[110] A subsequent cost-effectiveness analysis[111] and two prospective clinical studies[112,113] have concluded that such empirical therapy is neither the most effective nor least expensive strategy for diagnosis of strep throat in adults. Furthermore, empirical therapy in adults leads to considerable overuse of antibiotics.[114] It is important to realize that the most common age group of streptococcal pharyngitis is in the 5- to 15-year-old group. This is of particular concern because annually 73% of the 6.7 million adults who visit primary care providers in the United Sates with the complaint of sore throat receive a prescription for antibiotics.[115]

Thus, for adults and children, expert panels of the Infectious Diseases Society of America,[80] American Heart Association (AHA),[116] and American Academy of Pediatrics (AAP)[117] recommend that the presence of GAS in the pharynx should be documented by a throat culture or rapid antigen detection test (RADT).[79,116,117] It should be noted that a positive test does not discriminate between active streptococcal infection versus colonization and a concomitant viral infection. Clinical criteria for streptococcal pharyngitis should be present before antibiotic treatment is considered.

Throat Culture

Throat culture remains the gold standard for diagnosing streptococcal pharyngitis. Failure to isolate β-hemolytic streptococci in a carefully obtained and accurately interpreted throat culture rules out the diagnosis of streptococcal sore throat for practical purposes. In cases in which doubt exists as to the validity of a negative culture, it may be preferable to repeat the culture rather than to treat empirically with antimicrobial agents.

Although a negative culture eliminates the necessity for therapy, a positive culture does not differentiate between acute infection and asymptomatic carriage. Serum antibody titers do not rise until convalescence and thus are of no help in short-term management. Although the degree of positivity of the throat culture may assist in making this differentiation, it is best to assume that all positive cultures in patients with acute pharyngitis are significant and to treat accordingly while recognizing that, even with the use of the throat culture, some degree of overtreatment is inevitable.

Detailed instructions for obtaining and processing a throat culture have been published by the AHA.[118] Sheep blood agar is preferred because clear-cut patterns of hemolysis are obtained using this medium. In regard to isolation of GAS, there is controversy in the literature as to the relative merits of plain sheep blood agar plates versus plates to which trimethoprim-sulfamethoxazole has been added to suppress competing normal pharyngeal flora. Similar controversy exists about the optimal atmosphere for incubation—aerobic, aerobic in the presence of 5% to 10% carbon dioxide, or anaerobic. Detailed analyses of these issues have been published.[119,120] If blood agar plates are not immediately available, the swab may be placed in a dry sterile tube for transportation to the laboratory. After overnight incubation at 35°C to 37°C, culture plates from patients with streptococcal pharyngitis show colonies surrounded by clear zones of hemolysis and β-hemolysis around the agar stab. Plates that are negative on first reading should be reexamined after an additional 24 hours of incubation. Serologic grouping of β-hemolytic streptococcal isolates may now be readily performed by using commercially available kits. Fluorescent antibody techniques provide excellent results and specifically identify group A organisms. No quantitative information is gained about the degree of positivity of the culture. A less expensive screening procedure, the bacitracin sensitivity test, is best performed once the organism has been isolated in pure culture. This susceptibility procedure is based on the observation that greater than 95% of all group A streptococcal strains are inhibited by low-potency (0.04 unit) bacitracin disks, whereas 80% to 90% of non–group A strains are resistant.

Because no GAS resistant to penicillin or cephalosporins have yet been detected, antibiotic testing is unnecessary if these drugs are to be used. The same holds true in general for macrolides because GAS resistant to this drug are rare in the United States at this time[121] (see later section "Treatment" for caveats).

Rapid Antigen Detection Tests

RADTs allow detection of the presence of the group A carbohydrate antigen directly from throat swabs. Unlike the throat culture, which requires overnight or longer to yield a definitive result, RADTs can be completed in a matter of minutes. By facilitating early diagnosis and therapy, a RADT may possibly shorten the duration of illness, decrease secondary spread of the organism, and allow earlier return of patients and parents to school and work. Earlier tests based on latex agglutination methodology have been largely replaced by enzyme immunoassays that are easier to interpret and more sensitive. More recently, tests using optical immunoassay (OIA) and chemiluminescent DNA probes have become available.

Most currently commercially available RADTs are highly specific (95% or higher), so a positive result obviates the need for a throat culture. Unfortunately, the sensitivity of these tests is lower than that of the conventional throat culture, and therefore they may be negative in patients in whom conventional culture proves to be positive. Some investigators[122,123] have found newer tests such as OIA to have a sensitivity equivalent to that of culture, but others have reached opposite conclusions.[124–126] At present the AAP recommends that a negative RADT be confirmed with a throat culture. In view of conflicting data about sensitivity of commercially available RADTs, as well as the paucity of studies directly comparing the various tests with each other, physicians who elect to use any RADT in children and adolescents without culture backup of negative results should do so only after confirming in their own practice that the rapid test is comparable in sensitivity to the throat culture.[117]

In considering appropriate laboratory diagnostic testing for adults, certain epidemiologic distinctions from pediatric disease deserve consideration. The GAS cause 15% to 30% of cases of acute pharyngitis in pediatric patients but only approximately 10% of such illnesses in adults.[106,127,128] However, the risk for streptococcal pharyngitis may be higher in parents of school-age children and adults whose occupation brings them into close association with children. Moreover, the risk for a first attack of acute rheumatic fever is extremely low in adults in the United States and most other developed countries, even if they experience an undiagnosed and untreated episode of streptococcal pharyngitis. These facts make performance of RADT without culture backup of negative results an acceptable alternative to throat culture.[79,116] The generally high specificity of RADTs should minimize overprescribing

of antimicrobial agents for adults. This latter point is of particular importance in view of national data, indicating that antibiotics are prescribed for approximately 75% of adults consulting community primary care physicians for the complaint of sore throat and that the prescription of more expensive, broader-spectrum antibiotics is frequent.[115] Physicians who wish to ensure they are achieving maximal sensitivity in diagnosis may continue to use the conventional throat culture or to back up a negative RADT with a culture. It should be clear that demonstration of GAS in the throat is not sufficient to initiate treatment, the exception being in those with a history of acute rheumatic fever. However, in one recent adult study,[113] OIA without culture backup was performed in adults exhibiting two or more of Centor and coworkers' criteria.[107] When compared with throat culture, this strategy led to nearly optimal treatment (94%) and antibiotic prescription (37%), with minimal antibiotic overuse (3%) and underuse (3%).

Therapy

Antimicrobial therapy is indicated for children and adults with symptomatic pharyngitis (see Centor criteria earlier) after the presence of the organism in the throat is confirmed by culture or RADT. The goals of antimicrobial therapy are (1) prevention of acute rheumatic fever, (2) prevention of suppurative complications, (3) improvement in clinical symptoms and signs, and (4) rapid decrease in infectivity so as to reduce transmission of group A β-hemolytic streptococci to family members, classmates, and other close contacts and to allow the rapid resumption of usual activities. There is no firm evidence that poststreptococcal acute glomerulonephritis is preventable by treatment of the antecedent streptococcal infection.[129]

Treatment of group A streptococcal sore throat as long as 9 days after onset is still effective for the prevention of rheumatic fever.[130] Thus, if the patient is seen early in the course of the illness, the delay in initiation of therapy occasioned by obtaining a positive throat culture is not ordinarily a matter of concern in this regard. As noted, patients with signs and symptoms of acute pharyngitis and a positive rapid test (properly performed and interpreted) for group A carbohydrate antigen should receive appropriate antimicrobial therapy.

In the minority of patients who are severely ill or toxic at presentation and in whom there is clinical and epidemiologic evidence resulting in a high index of suspicion, oral antimicrobial therapy can be initiated while awaiting the results of the throat culture (either as a primary diagnostic tool or in confirmation of a negative RADT). If oral therapy is prescribed, a positive throat culture serves as a guide to the necessity of completion of a full antimicrobial course or, alternatively, of recalling the patient for an injection of penicillin G benzathine. Early initiation of antimicrobial therapy may result in faster resolution of the signs and symptoms in children and adults,[77–79] but group A streptococcal pharyngitis is usually a self-limited disease; fever and constitutional symptoms are markedly diminished within 3 or 4 days of onset, even without antimicrobial therapy.[131] Thus antimicrobial therapy initiated within the first 48 hours of onset hastens symptomatic improvement by only 1 to 2 days.

The drug of choice in the treatment of streptococcal infection is penicillin because of its efficacy in the prevention of rheumatic fever, safety, narrow spectrum, and low cost (Table 197.1).[79,116,117] Prevention of acute rheumatic fever is believed to require eradication of the infecting streptococcus from the pharynx, an effect that depends on prolonged rather than high-dose penicillin therapy. This objective may be accomplished by the administration of a single injection of 1.2 million units of penicillin G benzathine. For children weighing 60 pounds (27 kg) or less, the dose is reduced to 600,000 units. Most physicians in the United States, however, elect to administer oral therapy. In this case penicillin V, in one of the regimens listed in Table 197.1, must be continued for a full 10 days. Amoxicillin is often prescribed in preference to penicillin V in children requiring liquid medication because of poor palatability of oral suspensions of penicillin V. Once-daily amoxicillin therapy is effective for the treatment of group A streptococcal pharyngitis[132–134] in children. An oral time-release formulation of amoxicillin has recently been approved by the US Food and Drug Administration (FDA) for once-daily treatment of group A streptococcal pharyngitis in adolescents and adults. Because of its convenience, low cost, and relatively narrow spectrum, once-daily amoxicillin is an acceptable alternative regimen for the treatment of group A β-hemolytic streptococcal pharyngitis.[116]

TABLE 197.1 Primary Prevention of Rheumatic Fever (Treatment of Streptococcal Tonsillopharyngitis)

AGENT	DOSAGE	ROUTE	DURATION (DAYS)
Penicillins			
Penicillin V (phenoxymethyl penicillin)	Children ≤27 kg (60 lb): 250 mg two to three times daily Children >27 kg, adolescents, and adults: 500 mg two to three times daily	Oral	10
or			
Amoxicillin	50 mg/kg once daily (maximum, 1 g)	Oral	10
or			
Benzathine penicillin G	600,000 U for patients ≤27 kg; 1.2 million U for patients >27 kg	Intramuscular	Once
For Individuals Allergic to Penicillin			
Narrow-spectrum cephalosporin[b] (cephalexin, cefadroxil)	Variable	Oral	10
or			
Clindamycin	20 mg/kg/d divided in three doses (maximum, 1.8 g/d)	Oral	10
or			
Azithromycin	12 mg/kg once daily (maximum, 500 mg)	Oral	5
or			
Clarithromycin	15 mg/kg/d divided twice daily (maximum, 250 mg twice daily)	Oral	10

[a]The following are not acceptable: sulfonamides, trimethoprim, tetracyclines, and fluoroquinolones.
[b]To be avoided in those with immediate (type I) hypersensitivity to a penicillin.
From Gerber M, Baltimore R, Eaton C, et al. Prevention of rheumatic fever and diagnosis of acute streptococcal pharyngitis. A scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and Quality of Care and Outcomes Research Interdisciplinary Working Group. Circulation. *2009;119:1541–1551.*

A variable percentage of patients continue to harbor GAS of the originating serotype in their pharynx after completion of a course of oral penicillin.[135] Such bacteriologic treatment failures are sometimes associated with symptomatic relapse. Unless such children have symptomatic sore throat as clinically defined earlier, they should not be re-treated unless there is a history of rheumatic fever. Because penicillin is ineffective in eradicating asymptomatic streptococcal pharyngeal carriage, apparent treatment failures may actually represent persistence of such carriage in patients with superimposed viral pharyngitis.[136]

Oral cephalosporins are highly effective in the treatment of streptococcal pharyngitis, and meta-analyses have suggested that streptococcal eradication rates and clinical cure rates attained with these agents are slightly higher in children and adults than those achieved with penicillin.[135,137,138] However, these analyses have been strongly challenged on methodologic grounds.[139] This is related to high efficacy in treatment in patients with no prior antibiotic treatment and in vitro data that has never identified penicillin resistance in GAS. In penicillin-allergic patients, macrolide (erythromycin or clarithromycin) or azalide (azithromycin)

antimicrobial agents, clindamycin, or first-generation cephalosporins are the agents of choice.[79] The latter should be avoided in those with immediate (type I) penicillin hypersensitivity (see Table 197.1). Cephalosporins should not be administered to patients with a history of immediate (anaphylactic-type) hypersensitivity to penicillin. The physician should bear in mind the possibility of an increased risk for allergic reactions to cephalosporins when treating penicillin-allergic patients.

Erythromycin is less expensive than clarithromycin or azithromycin but may be associated with more gastrointestinal side effects. Although there have been reports of relatively high levels of resistance to macrolide antimicrobial agents from several countries[140–142] (see section on invasive infection later, where resistance to these agents is high in China) and reports of increased rates of macrolide resistance in certain localized areas of the United States,[143,144] three multistate surveillance studies conducted during 2002 and 2003 detected overall macrolide resistance rates of 3.8%,[145] 5.2%,[146] and 6.8%, respectively.[147] However, given the increasing use of azalides for upper and lower respiratory tract infections, the situation may change. Physicians should therefore be cognizant of local patterns of antimicrobial resistance. In areas in which macrolide resistance is known to be prevalent, antimicrobial susceptibility testing should be performed if these agents are used to treat group A streptococcal infections. Furthermore, continued surveillance of national trends in macrolide susceptibility is warranted.

There has been considerable recent interest in abbreviated courses of antimicrobial therapy. It has been reported that second- and third-generation cephalosporins, such as cefuroxime,[148,149] cefixime,[150] ceftibuten,[151] cefdinir,[152] cefpodoxime,[153] and cefditoren,[154] are effective in eradication of GAS from the pharynx when administered for 5 days, although not all of these are approved by the FDA for a 5-day course of therapy for acute streptococcal pharyngitis at the time of this writing. Although such shortened courses might theoretically enhance patient compliance, the potential ecologic effects of using these broad-spectrum agents to treat such a common bacterial infection are of great concern. This is particularly true should these agents be used as first-line therapy for strep throat. Moreover, even when administered for short courses, they are considerably more expensive than penicillin. Clearly, penicillin treatment is also associated with potential adverse effects.

Similar favorable results of short-course therapy have also been reported for the newer macrolides or azalides, clarithromycin,[155] and azithromycin.[156–159] Because of its long intracellular half-life and slow release from tissue sites, a 5-day course of azithromycin is approved by the AHA [116] for use in penicillin-allergic patients. As noted, promiscuous use of macrolides has been associated with development of resistance by GAS.[140,141]

Because tetracycline-resistant GAS are prevalent in many areas, this drug is not recommended. Sulfonamides, which are effective for the secondary prophylaxis of rheumatic fever (see Chapter 198), are ineffective for the eradication of pharyngeal organisms or the prevention of rheumatic fever when used as therapy for acute pharyngeal infections.

Patients with more severe suppurative infections, such as those involving the mastoid or ethmoid, may require higher doses of penicillin or other β-lactam antibiotics administered parenterally. When streptococcal upper respiratory tract infection is complicated by the development of abscesses associated with suppurative cervical adenitis or in the peritonsillar or retropharyngeal soft tissues, aspiration or incision and drainage is usually required.

Because prevention of rheumatic fever appears to require eradication of the streptococcal organism from the pharynx, treatment failures are of concern. In addition to true treatment failure (i.e., reisolation of the original infecting streptococcal serotype shortly after completion of a full course of antibiotic therapy), causes of posttreatment culture positivity include failure of compliance with oral medication schedules and reinfection with the same or different streptococcal types in the home or school environment. Apparent failure may also occur when the patient is in reality an asymptomatic postconvalescent streptococcal carrier suffering from an acute viral pharyngitis. In everyday practice it is often impossible to differentiate among these alternatives.

Nevertheless, routine reculture of the throat after a course of antistreptococcal therapy in an asymptomatic patient is not advised[79] because the cost-benefit ratio of such cultures continues to decline in parallel with the incidence of acute rheumatic fever in developed countries. Such cultures should be undertaken in high-risk circumstances (e.g., if the patient or a family member has a history of rheumatic fever) or when symptoms compatible with streptococcal infection persist or recur. When an increased incidence of acute rheumatic fever is detected in a community, as happened in a number of US cities during the 1980s, the approach to streptococcal infection must be particularly rigorous, and serious consideration should be given to routine performance of posttreatment cultures. If reculture is undertaken in patients with a history of rheumatic fever, re-treatment with an oral cephalosporin might be considered in view of the slightly increased eradication rates observed with these agents; this may be due to β-lactamase production by the oral microbiota.

The presence of persistently but weakly positive throat cultures after repeated courses of antibiotic therapy in an otherwise asymptomatic patient is not a cause for alarm. Such persons are asymptomatic postconvalescent streptococcal carriers[136] who are neither at risk for developing rheumatic fever nor likely to spread their infection to others. Their most frequent problem is anxiety produced by multiple medical consultations associated with the streptococcal colonization. In the event in which, for medical or psychological reasons, eradication of chronic streptococcal carriage becomes highly desirable, clindamycin,[160] amoxicillin-clavulanate,[161] or azithromycin may be efficacious.[79,162] Any of these antibiotic treatments can have adverse events, such as *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.

Streptococcal acquisition rates of 25% or higher have been recorded in family contacts. Certainly, family contacts with symptoms of upper respiratory tract infection should be cultured and treated appropriately if positive. Asymptomatic family contacts should also be cultured in high-risk circumstances, such as a family member who has had rheumatic fever or known cases of rheumatic fever or poststreptococcal glomerulonephritis occurring in the general area. In situations of lesser risk, routine culture of asymptomatic family contacts is not recommended.[79,116] The advisability of culture and/or prophylaxis of household contacts of patients with invasive group A streptococcal infection is discussed later.[162]

There is no firm evidence to suggest that tonsillectomy reduces the incidence of rheumatic fever, either in healthy persons or in persons who have had rheumatic fever and faithfully maintained continuous antibiotic prophylaxis. In certain patients with recurrent bouts of tonsillopharyngitis, however, tonsillectomy may decrease the frequency of incapacitating acute infections.[163,164] Tonsillectomy should be considered only for the most severely affected patients.[165]

STREPTOCOCCAL PYODERMA

Pyoderma, impetigo, and *impetigo contagiosa* are terms used synonymously to describe discrete purulent lesions that are primary infections of the skin and that are extremely prevalent in many parts of the world. In the great majority of cases pyoderma is caused by β-hemolytic streptococci and/or *S. aureus.*

Epidemiology

Pyoderma occurs most frequently in economically disadvantaged children dwelling in tropical or subtropical climates. It is also prevalent in northern climates during the summer.[166] The peak incidence of impetigo is in children age 2 to 5 years. This disorder also occurs among older children and adults whose recreational activities or occupation results in cutaneous cuts or abrasions.[167–169] There is no gender predilection, and all races appear to be susceptible. The prevalence of streptococcal pyoderma is markedly influenced by several factors, the most important of which appear to be climate and level of hygiene.

Meticulous prospective studies of streptococcal impetigo have demonstrated that the responsible microorganisms initially colonize the unbroken skin,[166] an observation that probably explains the influence of personal hygiene on disease incidence. Development of skin colonization with a given streptococcal strain precedes the development of impetiginous lesions by an average interval of 10 days. The mechanism of production of skin lesions is unproved, but it is most likely caused by intradermal inoculation of surface organisms by abrasions, minor trauma, or insect bites. Frequently, there is a transfer of the streptococcal strains from the skin and/or pyoderma lesions to the upper respiratory

tract. The interval between colonization of the skin and colonization of nose or throat or both averages 2 to 3 weeks.

Bacteriology and Immunology

Streptococci isolated from pyodermal lesions are primarily group A, but on occasion, representatives of other serogroups, such as C and G, are responsible. GAS that cause impetigo differ in several respects from those usually associated with tonsillitis and pharyngitis. Skin strains belong to different M serotypes or genotypes from the classic throat strains; because most have been identified more recently, they tend to comprise the higher-numbered M types. Throat and skin strains can also be differentiated by genetic markers.[170] A relatively small number of serotypes seem capable of regularly initiating both pharyngitis and pyoderma.[171]

Assays of streptococcal antibodies are of no value in the diagnosis and management of impetigo, but they provide helpful supporting evidence of recent streptococcal infection in patients suspected of having poststreptococcal glomerulonephritis. The ASO response is weak in patients with streptococcal impetigo,[172,173] presumably because the activity of SLO response is inhibited by skin lipids (cholesterol),[174] whereas anti–DNase B levels are elevated.[172,173]

Clinical Manifestations

The lesion of streptococcal pyoderma begins as a papule that rapidly evolves into a vesicle surrounded by an area of erythema. The vesicular lesions are evanescent and rarely recognized clinically; they give rise to pustules that gradually enlarge and then break down over a period of 4 to 6 days to form characteristic thick crusts (Fig. 197.4). The lesions heal slowly and leave depigmented areas. A deeply ulcerated form of impetigo is known as *ecthyma*.

Streptococcal impetigo occurs on exposed areas of the body, most frequently on the lower extremities or face. The lesions remain well localized but are frequently multiple. Although regional lymphadenitis may occur, systemic symptoms are not ordinarily present.

In the past the lesions just described could be rather confidently diagnosed as streptococcal. This was the predominant form of impetigo and could be distinguished from bullous impetigo caused by phage group II *S. aureus*. Although bullous impetigo remains almost exclusively staphylococcal in cause, the bacteriology of nonbullous impetigo has changed.[175] *S. aureus,* either alone or in combination with *S. pyogenes,* is now the predominant causative agent.[176–178] Almost all such staphylococci are penicillinase producers. Therefore treatment with penicillin, which in the past had been highly effective against nonbullous impetigo, even when both streptococci and staphylococci were isolated from the lesions, now frequently fails.[177]

Therapy and Prevention

Because of the current frequency of isolation of *S. aureus* from nonbullous impetigo lesions and concomitant reports of penicillin failures,[177,179,180] penicillinase-resistant penicillins or first-generation cephalosporins are preferred.[177] Erythromycin has long been a mainstay of pyoderma therapy, but its use may be lessened in areas in which erythromycin-resistant strains of *S. aureus* or, more recently, *S. pyogenes* are prevalent. Topical therapy with mupirocin is equivalent to oral systemic antimicrobial therapy[181,182] and may be used when lesions are limited in number. It is expensive, however, and some strains of staphylococci may be resistant.[183] Retapamulin, a novel pleuromutilin antibacterial agent, has recently been approved by the FDA for treatment of bullous and nonbullous impetigo caused by GAS and methicillin-susceptible strains of *S. aureus* in children 9 months of age or older.[184–186] In vitro data have suggested that it may be more effective than mupirocin against methicillin-resistant *S. aureus*.

Adherence to good regimens of personal hygiene is the most effective measure currently available for prevention of impetigo.



FIG. 197.4 Multiple pyoderma lesions on the lower extremities of a child in rural Mississippi. *(Courtesy Dr. K. Nelson, Baltimore.)*

Complications

Suppurative complications are uncommon. For as yet unexplained reasons, rheumatic fever has never been shown to occur after streptococcal pyoderma. On the other hand, cutaneous infections with nephritogenic strains of GAS are the major antecedent of poststreptococcal glomerulonephritis in many areas of the world. There are as yet no conclusive data to indicate that treatment of an individual case of pyoderma prevents the subsequent occurrence of nephritis in these patients. Such therapy is nevertheless important as an epidemiologic measure in eradicating nephritogenic strains from the environment.

INVASIVE STREPTOCOCCAL INFECTIONS OF SKIN AND SOFT TISSUES

In the mid-1980s outbreaks of acute rheumatic fever began to occur throughout the United States, concomitant with the reappearance of certain streptococcal strains exhibiting characteristics known to be associated with rheumatogenicity (see Chapter 198). Shortly thereafter, invasive streptococcal infections, of a frequency and severity not seen in the preceding decades, began to be reported both in the United States and abroad.[187–190] Although strains of a number of group A streptococcal M types have been isolated from invasive infections, there has been a definite and consistent tendency for M types 1 and 3 to be associated with life-threatening infections.[8,188–191] A high proportion of these cases has occurred in adults, and the portal of entry is frequently the skin or soft tissues. In some cases the infections give rise to shock and multiorgan failure, features that simulate, in certain respects, staphylococcal TSS.[192] Thus these life-threatening group A streptococcal infections have been termed strep TSS. Clinical features of serious streptococcal skin and soft tissue infections and TSS are described later.

Erysipelas

Erysipelas is a superficial cutaneous process, usually restricted to the dermis but with prominent lymphatic involvement. It is distinguished clinically from other forms of cutaneous infection by three features: The lesions are raised above the level of the surrounding skin; there is a clear line of demarcation between involved and uninvolved tissue; and the lesions are a brilliant salmon red. This disorder is more common in infants, young children, and older adults. It is almost always caused by β-hemolytic streptococci. Using immunofluorescence techniques combined with conventional culture on skin biopsies from patients with lower limb infections, GAS were found responsible for 26 of 27 cases of erysipelas and 11 of 15 cases of acute cellulitis.[193] Uncommonly, group B streptococci or *S. aureus* may be the culprit. In older reports erysipelas was described as characteristically involving the butterfly area of the face (Fig. 197.5), but at present the lower extremities are more frequently involved (Fig. 197.6). In patients with facial erysipelas there is frequently a history of preceding streptococcal sore throat, although the exact mode of spread to the skin is unknown. When erysipelas involves the extremities, breaks in the cutaneous barrier serve as portals of entry; these include surgical incisions, trauma or abrasions, dermatologic diseases such as psoriasis, or local fungal infections.

The cutaneous lesion begins as a localized area of erythema and swelling and then spreads rapidly with advancing red margins, which



FIG. 197.5 Facial erysipelas. The lesion is well demarcated from surrounding skin and illustrates the typical butterfly distribution. *(From Bisno AL. Cutaneous infections: microbiologic and epidemiologic considerations.* Am J Med. *1984;76:172–179.)*



FIG. 197.6 Erysipelas in saphenous venectomy limb. This patient had undergone coronary artery bypass grafting.

are raised and well demarcated from adjacent normal tissue. There is marked edema, often with bleb formation, and in facial erysipelas the eyes are frequently swollen shut. The lesion may demonstrate central resolution while continuing to extend on the periphery. The cutaneous inflammation is accompanied by chills, fever, and toxicity.

The differential diagnosis is limited. Early on the lesions of facial herpes zoster, contact dermatitis, or giant urticaria may be confused with erysipelas. Lesions resembling erysipelas may occur in patients with familial Mediterranean fever. Cutaneous lesions similar in appearance to those of erysipelas may occur on the hands of patients who sustain cuts or abrasions while handling fish or meats. This entity, known as erysipeloid of Rosenbach and caused by *Erysipelothrix rhusiopathiae,* is usually unaccompanied by fever or systemic symptoms.

With early diagnosis and treatment, the prognosis is excellent. Rarely, however, the process may spread to deeper levels of the skin and soft tissues. Blood cultures are positive for β-hemolytic streptococci in 4.6% to 9% of patients.[194] Penicillin, either parenterally or orally, depending on clinical severity, is the treatment of choice. If staphylococcal infection is suspected, a penicillinase-resistant semisynthetic penicillin or cephalosporin should be selected. In a randomized, prospective multicenter trial,[195] roxithromycin, a macrolide antimicrobial agent, was equivalent to penicillin. Increased levels of macrolide resistance among GAS, however, have been detected in certain areas of the United States.[143,144]

Streptococcal Cellulitis

Streptococcal cellulitis, an acute spreading inflammation of the skin and subcutaneous tissues, results from infection of burns, wounds, or surgical incisions but may also follow mild trauma. Clinical findings include local pain, tenderness, swelling, and erythema. The process may extend rapidly to involve large areas of skin. Systemic manifestations include fever, chills, and malaise, and there may be associated lymphangitis, bacteremia, or both. In contrast to erysipelas, the lesion is not raised, the demarcation between involved and uninvolved skin is indistinct, and lesions are more pink than salmon red. Often, however, the clinical differentiation between these entities is not clear cut.

Two predisposing causes of streptococcal cellulitis deserve special mention. One is the parenteral injection of illicit drugs.[196–198] These cases are often associated with bacteremia and deep tissue infections, such as septic thrombophlebitis, suppurative arthritis, osteomyelitis, and, occasionally, infective endocarditis. Second, patients who have impaired lymphatic drainage from upper or lower extremities are prone to recurrent episodes of streptococcal cellulitis. Examples include individuals with filariasis and women who have undergone radical mastectomy with axillary node dissection.[199] It is speculated that repetitive infection further damages local lymphatics and worsens lymphatic stasis.[200]

Recurrent episodes of severe cellulitis have also been reported in certain patients who have undergone coronary artery bypass grafting.[201] The lesion invariably occurs in the extremity from which the saphenous vein was removed, and at times it may exhibit features of erysipelas (see Fig. 197.6). Patients with tinea pedis of the venectomy limb appear to be particularly at risk.[202–204] As with other forms of cellulitis, pathogenic bacteria are difficult to recover during these episodes. The appearance of the lesions and the response to penicillin therapy suggest, however, a streptococcal cause. The few β-hemolytic streptococci that have been recovered and characterized often belong to serogroups other than A.[205]

Disruption of the cutaneous barrier (leg ulcers, wounds, dermatophytosis) is a risk factor for the development of cutaneous streptococcal infection.[206] There is suggestive evidence that local dermatophyte infection (i.e., athlete's foot) may serve as a reservoir for β-hemolytic streptococci that initiate episodes of erysipelas or cellulitis of the lower extremities.[202,207] Thus care should be taken to eradicate such fungal infections in patients who experience recurrent bouts of erysipelas or cellulitis. Another potential reservoir is intestinal streptococcal colonization.[208] Other risk factors include venous insufficiency, edema, and obesity.[206] Not surprising, an increased risk for recurrent cellulitis has also been reported in homeless persons.[209]

Cellulitis may be caused by infection with a variety of bacterial pathogens (see Chapter 93), but most cases are caused by *S. pyogenes* (or, on occasion, streptococci of groups B, C, or G) or by *S. aureus. S. aureus* infection of the skin is usually associated with a pyogenic, fluctuant

focus with surrounding erythema and is referred to as "purulent cellulitis." In the absence of positive blood cultures, which are present in only 5% of cases of nonpurulent cellulitis, a specific microbiologic diagnosis is often not possible. Aspirate or biopsy samples from sites of active cellulitis are helpful when positive on smear or culture, but unfortunately such specimens are usually negative in adult patients.[210–212]

It is often impossible to differentiate streptococcal from staphylococcal cellulitis on initial presentation confidently. In this case a semisynthetic penicillinase-resistant penicillin should be used. In penicillin-allergic patients a first-generation cephalosporin may be used if the hypersensitivity is not of the immediate type. Clindamycin or vancomycin may be used in patients who manifest anaphylactic hypersensitivity to β-lactam antibiotics, and the latter should be administered if there is reason to suspect infection with methicillin-resistant *S. aureus*. Patients with milder cases of streptococcal cellulitis may be switched to oral medications after an initial favorable response to parenteral therapy.

The role of continuous antimicrobial prophylaxis[213–215] in patients prone to frequent recurrences remains unsettled. At present such prophylaxis seems justified only for patients with very frequent or severe episodes, and the optimal regimen has not been established.

Elevation of the affected extremity and evaluation of blood flow are important adjunctive measures to improve circulation and to detect potential compartment syndrome, which reduces distal blood flow.

Necrotizing Fasciitis (Streptococcal Gangrene)

Necrotizing fasciitis is an infection of the deeper subcutaneous tissues and fascia, characterized by extensive and rapidly spreading necrosis and by gangrene of the skin and underlying structures. As detailed in Chapter 198, this entity may arise in several distinct epidemiologic settings, may be caused by multiple aerobic and anaerobic microorganisms, and may vary in clinical manifestations. The present discussion is limited to necrotizing fasciitis caused by GAS[216] and described by Meleney[217] in 1924 as hemolytic streptococcal gangrene. Characteristically, streptococcal gangrene begins at a site of trivial or even inapparent trauma or in an operative incision. The initial lesion may appear only as an area of mild erythema but over the next 24 to 72 hours undergoes a rapid evolution. The inflammation becomes more pronounced and extensive, the skin becomes dusky and then purplish, and bullae containing yellow or hemorrhagic fluid appear. Bacteremia is frequently present, and metastatic abscesses may occur. By the fourth to fifth day, frank gangrenous changes are evident in the affected skin,[218] followed by extensive sloughing. The process may march inexorably over large areas of the body unless measures are taken to contain it. The patient with streptococcal gangrene appears perilously ill, with high fever and extreme prostration. Mortality rates are high, even with appropriate treatment.[218] Fournier gangrene, a form of necrotizing fasciitis involving the male genital area, may rarely be caused by GAS.

The course of necrotizing fasciitis today appears to be much more fulminant than that described by Meleney.[217] Specifically, ecchymoses and bullae may appear within 2 to 3 days, and associated myonecrosis is more common. In addition, the mortality rate in 1924 was 20%, whereas mortality rates of 20% to 70% have been reported in the current era. This difference is even more remarkable because antibiotics, intravenous (IV) fluids, ventilators, and dialysis were not available in 1924.

Diagnosis and Differential Diagnosis

Successful management of necrotizing fasciitis is dependent on early recognition, yet early in their course, patients may present with fever and toxicity when the cutaneous lesion may appear relatively benign.[219] Fever and severe pain are the first manifestations of disease. In those with a defined portal of entry, such as a surgical incision, burn, insect bite, or varicella lesion, there is redness of the skin, pain, and swelling. In the 50% of patients who develop necrotizing fasciitis without a defined portal of entry, the infection begins deep to the skin, frequently at the site of a hematoma, muscle strain, or traumatic joint injury. In these patients crescendo pain is the most reliable clinical clue and is likely related to tissue ischemia arising from toxin-induced vascular occlusion.[220]

Routine radiographs, computed tomography (CT), and magnetic resonance imaging (MRI) may show localized swelling of the deep structures but characteristically do not show frank abscess formation or gas in the tissue and *thus are not definitive procedures*. This is particularly problematic in those patients without a portal of entry who have deep infection at the site of recent trauma, such as muscle tear, hematoma, or prior surgery, in whom the clinician cannot distinguish the cause of the deep swelling. Unfortunately, imaging studies often serve to delay rather than facilitate a diagnosis. Clinical judgment is crucial, and initiation of aggressive medical and surgical treatment may be caused by the time it takes for imaging studies. Fever and increasingly severe pain are the best and earliest signs of infection. Some patients do not present with fever,[219] and others may have taken nonsteroidal antiinflammatory drugs (NSAIDs) that mask fever and reduce pain. Unexplained tachycardia, marked left-sided shift, and an elevated creatine phosphokinase level are also important clues to the diagnosis of necrotizing fasciitis, and their presence should prompt surgical inspection of the deep tissues. Gram stains of aspirated fluid reveal chains of gram-positive cocci that contain few, if any, WBCs. Similarly, a biopsy with frozen section may aid in the diagnosis of necrotizing fasciitis.[221,222]

Myositis and Myonecrosis

Most cases of purulent muscle infection occur in the tropics, and *S. aureus* is the predominant causative agent. Myositis caused by GAS has been rare but occurs in many patients with necrotizing fasciitis and strep TSS. Most of these cases occur after blunt nonpenetrating trauma or occur spontaneously. Most likely, bacteria are translocated to the deep tissue hematogenously from the throat. Systemic toxicity is common, and mortality as high as 80% has been reported.[223] Destruction of tissue is poorly understood, but infection and inflammation within the confined muscle compartment space may result in pressures exceeding arterial pressure, necessitating emergent fasciotomy and débridement (Fig. 197.7). There is much overlap in the clinical features of necrotizing fasciitis and myonecrosis,[218,223] and the differentiation must be made by surgical inspection or biopsy.

Streptococcal Toxic Shock Syndrome

Strep TSS is defined as described in Table 197.2 but, simply put, it is any streptococcal infection associated with the sudden onset of shock and organ failure. Such cases were first described in the mid to late 1980s, and reports of strep TSS have subsequently emanated from North America, Europe, Australia, and Asia.[188,192,195,224–232] Most cases have occurred sporadically. The highest incidence of invasive streptococcal disease occurred in a small Minnesota community, where 26 cases per 100,000 population were recorded.[231] In addition, outbreaks of invasive group A streptococcal infections have occurred in closed environments, such as nursing homes[233–237] and hospitals.[238] Secondary cases of strep TSS are unusual, but transmission to family members[238,239] or health

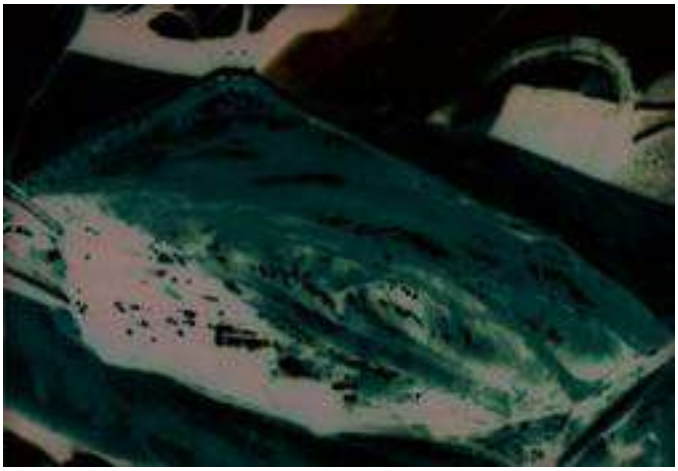

FIG. 197.7 Surgical exploration of patient with streptococcal toxic shock syndrome. This patient had necrotizing fasciitis and myositis that occurred spontaneously, with no prior injury to the site. *(From Stevens D. Streptococcal toxic shock syndrome.* Infect Med. *1992;9:33–39.)*

TABLE 197.2 Case Definition for the Streptococcal Toxic Shock Syndrome

I. Isolation of group A streptococci *(Streptococcus pyogenes)*
   A. From a normally sterile site (e.g., blood, cerebrospinal, pleural, or peritoneal fluid, tissue biopsy, surgical wound)
   B. From a nonsterile site (e.g., throat, sputum, vagina, superficial skin lesion)

II. Clinical signs of severity
   A. Hypotension: systolic blood pressure ≤90 mm Hg in adults or below fifth percentile for age in children
   and
   B. Two or more of the following signs:
      1. Renal impairment: creatinine ≥177 μmol/L (≥2 mg/dL) for adults or ≥2 times the upper limit of normal (ULN) for age; in patients with preexisting renal disease, a twofold or greater elevation over the baseline level
      2. Coagulopathy: platelets ≤100 × 10^{11}/L (≤100,000/mm^3) or disseminated intravascular coagulation defined by prolonged clotting times, low fibrinogen level, and the presence of fibrin degradation products
      3. Liver involvement: serum aspartate aminotransferase, alanine aminotransferase, or total bilirubin levels greater than or equal to two times the ULN for age; in patients with preexisting liver disease, a twofold or greater elevation over the baseline level
      4. Adult respiratory distress syndrome defined by acute onset of diffuse pulmonary infiltrates and hypoxemia in the absence of cardiac failure, evidence of diffuse capillary leak manifested by acute onset of generalized edema, or pleural or peritoneal effusions with hypoalbuminemia
      5. A generalized erythematous macular rash that may desquamate
      6. Soft tissue necrosis, including necrotizing fasciitis or myositis, or gangrene

[a]An illness fulfilling criteria IA and II (A and B) can be defined as a definite case. An illness fulfilling criteria IB and II (A and B) can be defined as a probable case if no other cause for the illness is identified.
From Defining the group A streptococcal toxic shock syndrome: rationale and consensus definition. The Working Group on Severe Streptococcal Infections. JAMA. *1993;269:390–391.*

TABLE 197.3 Factors That Increase the Likelihood of Developing Streptococcal Toxic Shock Syndrome

Age (neonates and older adults)
Diabetes
Alcoholism
Surgical procedures
Trauma
  Penetrating (insect bites, lacerations, slivers, abrasions, burns)
  Nonpenetrating (hematoma, bruise, muscle strain, hemarthrosis)
Varicella
  Contact with a case
  High prevalence of invasive strains in the community
Nonsteroidal antiinflammatory drugs[a]

[a]Based on limited evidence.

care workers[238,240] has been well documented by demonstrating identical pulsed-field gel electrophoresis patterns from cross-infecting strains. Although many of the studies cited earlier described strep TSS in adults, several reports have documented that this disorder also occurs in children.[225,230,231,241,242] Thus persons of all ages can be afflicted and, although some have underlying medical conditions such as diabetes and alcoholism,[225,227,243–247] many have no predisposing medical condition and are not immunocompromised. This contrasts sharply to reviews of group A streptococcal bacteremia from several decades ago[243–245] that found the disease to occur primarily among the very young, the very old, or patients with predisposing conditions, such as cancer, renal failure, leukemia, severe burns, or iatrogenic immunosuppression.

The portals of entry for streptococci are the vagina, pharynx, mucosa, and skin in 50% of cases.[8] Surgical procedures such as suction lipectomy, hysterectomy, vaginal delivery, bunionectomy, reduction mammoplasty, hernia repair, bone pinning, and vasectomy have provided portals in other cases (Table 197.3). Rarely, infection occurs secondary to streptococcal pharyngitis.[248–250] Virus infections such as varicella and influenza have provided portals of entry in other cases.[8,230,248]

Additional factors that increase the risk for invasive group A streptococcal infection, including bacteremia, strep TSS, and necrotizing fasciitis, are listed in Table 197.3. Three studies have demonstrated that a high or increasing prevalence of M-1 or M-3 strains among throat isolates may signal an increased incidence of strep TSS in a community.[230,246,251] NSAIDs taken for problems such as muscle strain, trauma, or postpartum pain may mask the early signs and symptoms of streptococcal infection or possibly predispose to more severe infection, such as necrotizing fasciitis or strep TSS.[8,252,253]

Pathogenesis

Entry of GAS into the deeper tissues and bloodstream may occur as a result of breach of a barrier, or the organism itself may penetrate intact mucous membranes, such as the pharyngeal mucosa. Although bacteremia is a very uncommon phenomenon in streptococcal pharyngitis, transient bacteremia must occur in those 50% of patients who develop invasive infections without a portal of entry. In either case, GAS avoid phagocytosis largely because of the antiphagocytic properties of M protein.[12] Adherence of GAS to pharyngeal mucosal cells is a prerequisite to colonization or infection and has been related to surface structures, such as lipoteichoic acid and fibronectin-binding proteins. Penetration or translocation of GAS through respiratory epithelial cells has been demonstrated for M-1 types of GAS. Some have suggested that those M-1 strains possessing an invasin *(inv+)* gene penetrate more efficiently.[254] If penetration of mucosal barriers occurs commonly, it does not result in clinically detectable bacteremia in the vast majority of patients because the incidence of invasive infection is very low, that is, 3.5 cases per 100,000 population.[255] Thus clearance of GAS must be highly efficient in the vast majority of humans either because of preexisting type-specific immunity or nonspecific clearance mechanisms in the reticuloendothelial system. Recent studies have suggested that after colonization of mucosa or skin, SpeB may play a role in attenuation of the local host response because of its proteolytic activity. Later in the growth cycle, group A streptococcal production of SpeB is curtailed through alteration of the control of virulence regulator (CovR), allowing these particular strains to bind plasminogen, evade the immune system, and switch to an invasive phenotype.[256]

How and why GAS cause deep infection of traumatized muscle and fascia in the absence of penetrating injury remains an enigma. Recent studies have demonstrated that injured muscle cells express vimentin on their surface during the healing and repair process and that GAS bind vimentin.[257] In addition, intravenously injected GAS traffic to the site of muscle injury, and this process is augmented in the presence of NSAIDs.[258] Although controversy remains regarding a link between NSAID use and increased risk and severity of group A streptococcal infections (for review see reference 259), experimental evidence from three independent laboratories supports this notion.[258,260,261]

Mechanisms of Shock and Organ Failure

Within the deeper tissues and bloodstream, the induction of cytokine synthesis plays a critically important role in the production of shock and organ failure. Pyrogenic exotoxins (scarlatina toxins, erythrotoxins) have the ability to cause fever, enhance susceptibility to endotoxin, suppress IgM antibody synthesis, and act as superantigens. These toxins, such as the staphylococcal enterotoxins (A, B, C) and TSST-1, can stimulate T-cell responses through their ability to bind to both the class II MHC antigen-presenting cells and the V-β region of the T-cell receptor.[262] The net effect is the induction of monocyte cytokines (tumor necrosis factor-α [TNF-α], interleukin [IL]-1β, and IL-6) as well as the lymphokines (TNF-β, IL-2, and interferon-γ).[263–269] There is evidence that M-protein fragments may also act as superantigens.[267] Pyrogenic exotoxins C and MF, as well as SSA and several new streptococcal pyrogenic exotoxins, are also capable of inducing massive quantities of proinflammatory cytokines and lymphokines,[268] which contribute to shock and organ failure. Some clinical studies have suggested that variation in human leukocyte antigen haplotype may predispose worse outcomes in some patients with strep TSS.[269]

Other streptococcal virulence factors are also capable of inducing proinflammatory cytokines, such as TNF-α and IL-1β. Specifically,

SpeB, a potent cysteine protease, causes release of IL-1β from preformed intracellular pools.[270] SLO also stimulates mononuclear cells to produce TNF-α and IL-1β and, in the presence of SpeA, has synergistic effects on IL-1β production.[271] Heat-killed GAS, as well as peptidoglycan and lipoteichoic acid, are also potent inducers of TNF-α and IL-1β.[272,273] Noncytokine mechanisms of shock may also play a role. A cysteine protease produced by GAS was shown to release bradykinin from a high-molecular-weight kininogen.[274] Bradykinin is a potent vasodilator of systemic and pulmonary vasculature and could be responsible, at least in part, for the early hypotension observed in strep TSS.[274] Recent studies demonstrated that SLO causes early, direct cardiomyocyte contractile dysfunction.[53] These effects were mediated by an influx of calcium through SLO-induced membrane pores. Upon removal of SLO, normal electrical pacing resumed, suggesting that membrane lesions were repaired and normal intracellular calcium levels were restored. These observations are consistent with the clinical observation that cardiomyopathy is a reversible condition in patients who survive strep TSS.[275]

Thus there are likely many streptococcal and host factors that contribute to the shock and organ failure characteristic of strep TSS. That TNF plays a central role is supported by two observations. First, high levels of TNF were observed in a baboon model of group A streptococcal bacteremia at a point in time when profound hypotension was manifest.[276] Second, in that model a neutralizing monoclonal antibody against TNF restored normal blood pressure and reduced mortality by 50%.[276] Diffuse capillary leak contributes to volume depletion and hypotension and is likely related to cytokine release but may also be related to circulating M-protein–fibrinogen complexes.[277] Recent evidence suggests that cardiomyocyte-derived cytokines are produced after direct *S. pyogenes* stimulation and after exposure to *S. pyogenes*–activated inflammatory cells.[278] In addition, viable *S. pyogenes* induced production of cardiomyocyte-derived stimulator(s) that boost(s) macrophage production of matrix metalloproteinase-9, proinflammatory cytokines (IL-1β, IL-6), and cardiodepressant factors (inducible nitric oxide synthase).[279] These locally produced, cardiomyocyte-derived cytokines, termed *cardiokines,* may further mediate cardiac contractile dysfunction in some patients with strep TSS.[275]

Clinical Manifestations

The first phase of strep TSS begins with an influenza-like prodrome characterized by fever, chills, myalgias, nausea, vomiting, and diarrhea that precedes hypotension by 24 to 48 hours.[8] Confusion and/or combativeness is present in 55% of patients. Where there is a defined or superficial portal of entry such as a laceration, suspicion of streptococcal infection or frank evidence of infection may be present at this phase of infection. In contrast, in patients without a portal of entry (50% of cases) and who subsequently develop necrotizing fasciitis or frank myonecrosis, postpartum infection, peritonitis, or joint space infection, pain that progressively increases in severity is the most common initial symptom that prompts patients to seek medical care and, of interest, precedes clinical evidence of localized infection by 12 to 24 hours.[8] In both children[230] and adults[8] the soft tissues are the most common primary site of infection. In the remaining cases, pneumonia, meningitis, endophthalmitis, peritonitis, myocarditis, joint infection, and intrauterine infection have been described.[8,230]

The second phase of strep TSS is characterized by tachycardia, tachypnea, persistent fever, and, in patients who subsequently have necrotizing fasciitis or myonecrosis, increasingly severe pain at the site of infection. In others, fever and severe pain are the best early clinical clues.[280] In children, toxicity during varicella or persistence of fever longer than 4 days should also prompt careful evaluation. Many patients are seen in emergency departments at this stage and frequently sent home, on one or two occasions with mistaken diagnoses, such as deep vein thrombophlebitis, muscle strain, viral gastroenteritis, dehydration, and sprained ankle.[219] High fever and excruciating pain, particularly in individuals with no risk factors for deep vein thrombosis, should arouse suspicion of a deep-seated infection. The laboratory tests described later are helpful, and CT and MRI may be useful to define the level of tissue injury but are not specific (see earlier discussion of necrotizing fasciitis).

The third phase of strep TSS is characterized by the symptoms and signs mentioned but with the sudden onset of shock and organ failure. Many patients are in florid shock at the time of admission, but in almost 50% of patients, hypotension is apparent during the first 4 to 8 hours after admission. Clinical evidence of necrotizing fasciitis is frequently a late finding, often occurring after hypotension is present. The appearance of purple bullae and dusky-appearing skin is a bad prognostic sign and should prompt emergent surgical exploration (see earlier discussion of necrotizing fasciitis). It should be noted that currently the progression of necrotizing fasciitis from red skin to purple bullae may take place within a 24-hour period, whereas that described by Meleney[217] in 1924 took 7 to 10 days. In addition, the rapidity with which shock and multiorgan failure can progress is impressive, and many patients die within 24 to 48 hours of hospitalization.[8]

Laboratory tests should be performed in patients with aggressive soft tissue infections or in patients with severe pain and fever who appear toxic. The serum creatinine measurement is particularly useful because renal impairment (creatinine level more than twice normal) is apparent even during the second phase, before hypotension is apparent. In addition, creatine phosphokinase levels in serum are markedly elevated in those with necrotizing fasciitis and myonecrosis. The WBC count is usually normal or elevated at admission but with a profound left shift that includes myelocytes and metamyelocytes. Finally, serum albumin and calcium levels are usually low on admission and drop precipitously as a diffuse capillary leak syndrome develops. Thrombocytopenia does not develop until later in the course but is the earliest sign of disseminated coagulopathy.[8] Profound metabolic acidosis develops early in the third phase, and serum bicarbonate, lactate, and blood gas pH determinations are crucial tests to follow therapeutic progress. Because the acute respiratory distress syndrome develops in 55% of patients with strep TSS, pulse oximetry and, later, blood gas levels are necessary to evaluate the need for intubation and ventilation.

Management

Source Control

Prompt and aggressive surgical exploration and débridement of suspected deep-seated streptococcal infection are mandatory. It is as important to establish the cause of the infection as it is to determine the extent of necrosis. CT and MRI are helpful to locate the primary site of infection, but because the GAS do not form gas or frank abscess, radiologic interpretations are frequently not definitive. Such findings in a patient with extreme pain and fever or who is toxic should prompt surgical consultation. Once necrosis is established, extensive débridement is necessary because shock and organ failure continue to progress if devitalized tissue remains. Although necrosis of the fascia may be present, it is important to know that necrosis of muscle, skin, and subcutaneous tissue also is commonly present.

Fluid Resuscitation

If several liters of crystalloid IV fluid challenge do not rapidly improve blood pressure (mean arterial pressure > 60 mm Hg) or tissue perfusion, then invasive monitoring is indicated. The goal should be to maintain a pulmonary artery occlusion pressure of 12 to 16 mm Hg.[281] If this goal is reached but hypotension persists, the serum albumin concentration and hematocrit should be checked because profoundly low albumin levels are common and because hemolysins produced by GAS can cause dramatic drops in circulating red cell mass. Thus transfusion with packed red blood cells, with or without albumin, may be useful to improve blood pressure and preserve tissue perfusion.

Because of intractable hypotension and diffuse capillary leak, massive amounts of IV fluids (10–20 L/day) may be required in an adult. Albumin replacement may be necessary because many patients' serum albumin levels decrease to lower than 2.0 g/dL.

Antimicrobial Therapy

Prompt antimicrobial therapy is mandatory, and empirical broad-spectrum coverage for septic shock should be instituted initially. Once the streptococcal cause is confirmed, high-dose penicillin and clindamycin should be given.[282] This recommendation is based on the following information: (1) all strains of GAS remain sensitive to penicillin; (2)

resistance to erythromycin is currently found in less than 5% of GAS in the United States, but the rate is higher in certain locales, and there have been rare reports of resistance to clindamycin; (3) clindamycin and erythromycin are more active in experimental models of necrotizing fasciitis and myonecrosis; (4) penicillin-binding proteins are not expressed during stationary-phase growth of GAS, and thus penicillin is ineffective in severe deep infections in which large numbers of bacteria are present; (5) clindamycin suppresses exotoxin and M-protein production by GAS; (6) clindamycin has a much longer half-life; (7) combinations of penicillin and clindamycin have indifferent interaction against GAS in vitro at clinically relevant concentrations of antibiotics (no antagonistic effects were found)[283]; and (8) clindamycin and azithromycin suppress cytokine production by human mononuclear cells.[284,285] Recent reports from China concerning scarlet fever after strep throat have documented azithromycin and clindamycin resistance in 100% of strains[63,82] suggesting that susceptibility testing should be performed in such patients, particularly those with severe symptoms and signs of pharyngeal or invasive infection.

Management in the Intensive Care Unit

In patients with persistent hypotension, monitoring of cardiac outputs, pulmonary artery occlusion pressure, and mean arterial pressure is important. Intubation and ventilator support are usually required because of the high incidence of acute respiratory distress syndrome (55%) in patients with strep TSS. Pressors such as dopamine are used frequently, although no controlled trials have been performed in strep TSS. In patients with intractable hypotension, high doses of dopamine, epinephrine, or phenylephrine have been used, but caution should be exercised in those with evidence of disseminated intravascular coagulation and in particular in those with cold, cyanotic digits. Symmetrical gangrene involving all 20 digits, the tip of the nose, ear lobes, and the breast areola have been described. In addition, we have observed amputation of one, two, three, and even four extremities. In these cases, both excessive pressors and disseminated intravascular coagulation likely contributed to symmetrical gangrene.

Dialysis and Hemoperfusion

Either of these methodologies may be necessary because greater than 50% of patients develop acute renal failure. Both dialysis and hemoperfusion may also nonspecifically reduce the concentrations of circulating toxins. It is interesting that a study from Sweden that used hemofiltration achieved the lowest mortality rate ever recorded in patients with strep TSS.[247] Finally, a polystyrene superantigen absorbing device was developed in Japan and shown to be highly efficacious in absorbing pyrogenic exotoxin A or TSST-1 from plasma and, when used extracorporeally in animals infused with TSST-1 and lipopolysaccharide, mortality improved from 100% to 50%.[286]

Intravenous Immune Globulin

The rationale for the use of intravenous immune globulin (IVIG) in the treatment of strep TSS is based on the data implicating extracellular exotoxins as mediators of shock and organ failure. George and Gladys Dick, in 1924, demonstrated that convalescent sera from patients with scarlet fever neutralized scarlatina toxins and, when passively administered, attenuated the course of severe scarlet fever.[287] Just as penicillin was becoming available, anti–scarlatina toxin horse serum became commercially available in the United States; however, because of the availability of penicillin and the decline in the severity of scarlet fever, it was never used. Several reports have described the successful use of IVIG in patients with strep TSS.[288–290] One large treatment group (15 patients) showed a significant reduction in mortality compared with matched historical controls.[291] The mortality rate of 70% in the control group was among the highest ever reported, whereas the rate was 30% in the IVIG group. This rate is similar to that of some series that did not use IVIG.[8] A recent comparative observational (retrospective) study in Sweden analyzed the outcomes of 67 patients with streptococcal TSS. Twenty-three patients were treated with IVIG and had a 28-day survival of 87%, whereas the non–IVIG-treated group had a 28-day survival of 50%.[292] A double-blind clinical trial was undertaken in northern Europe comparing IVIG with albumin in patients with strep TSS. All patients received clindamycin. The mortality rate in the IVIG group was 16%, whereas that in the albumin group was 32%.[293] Unfortunately, the study was stopped because of low enrollment, and only 7 or 8 patients with proven group A streptococcal infections were in each group. Thus the differences were not significant. A retrospective study in patients with strep TSS has also shown no benefit of IVIG on mortality.[294] It is hoped that further double-blind studies with sufficient numbers of cases will resolve the continuing dilemma regarding the potential efficacy of IVIG.[295] Until then, IVIG could be given early, and more than one dose should be used because batches of IVIG have variable neutralizing activity against streptococcal exotoxins.[268,296]

There have been no comparative trials describing the efficacy of hyperbaric oxygen treatment in strep TSS, although some state that such treatment reduces mortality and the need for further débridements.[297] Certainly, use of this modality should not delay or be used in preference to surgical débridement when the latter is indicated.

BACTEREMIA

Group A streptococcal bacteremia has been relatively uncommon in the antibiotic era.[298] Before the mid-1980s, bacteremia occurred predominantly at the extremes of life and was usually community acquired. Occasional cases were seen in young and middle-age adults associated with surgical wound infections and endometritis.

During the past decade, however, there has been an increase in the number of reported cases of group A streptococcal bacteremia, reflecting the changing epidemiology and clinical patterns of invasive streptococcal infection as noted earlier. Many of the patients were previously healthy adults between the ages of 20 and 50 years. There has been an apparent increase in cases associated with parenteral injection of illicit drugs,[8,98,245] as well as nosocomial outbreaks in nursing homes.[233–237]

Bacteremia in children may emanate from an upper respiratory tract infection, but it is more commonly associated with cutaneous foci, including burns and varicella.[240] Older patients with streptococcal bacteremia present with a variety of chronic illnesses; their relation to the bacteremia is often unclear. However, diabetes mellitus, cirrhosis, and peripheral vascular disease, and possibly fatty liver, appear to be predisposing factors in older adults,[299] and, as in children, the portal of entry is usually the skin. Malignancy and immunosuppression are risk factors in both age groups.[218,300] Although group A streptococcal bacteremia may at times be transient and relatively benign,[301] it is more often fulminant. The onset is abrupt, with chills, high fever, and prostration. Rarely, patients may present with acute abdominal pain.[301,302] Mortality in five modern series[243,301–304] has ranged from 27% to 38%.

OTHER STREPTOCOCCAL INFECTIONS

Lymphangitis may accompany cellulitis or may occur after clinically minor or inapparent skin infection. Lymphangitis is readily recognized by the presence of red, tender, linear streaks directed toward enlarged, tender, regional lymph nodes. It is accompanied by systemic symptoms, such as chills, fever, malaise, and headache. Puerperal sepsis follows abortion or delivery when streptococci colonizing the patient herself or transmitted from medical personnel invade the endometrium and surrounding structures, lymphatics, and bloodstream. The resulting endometritis and septicemia may be complicated by pelvic cellulitis, septic pelvic thrombophlebitis, peritonitis, or pelvic abscess. This disease was associated with high mortality in the preantibiotic era. Although endocarditis caused by *S. pyogenes* was relatively common in the preantibiotic era, it is now rarely reported.[305,306] Meningitis caused by *S. pyogenes* usually follows upper respiratory tract infection, including sinusitis or otitis,[307] or neurosurgical conditions.[308] It is indistinguishable clinically from other forms of acute pyogenic meningeal infection.[309]

Pneumonia caused by *S. pyogenes* is frequently associated with preceding viral infections such as influenza, measles, or varicella or with chronic pulmonary disease. Numerous epidemics have been described in military recruit populations.[310,311] An increased number of cases has been reported over the past few years in association with the resurgence of invasive streptococcal infections. In one-third or fewer of the cases, there was a history of preceding streptococcal upper respiratory tract infection. The onset is typically abrupt, and the disease

is characterized by chills, fever, dyspnea, cough productive of blood-streaked sputum, pleuritic chest pain, and, in more severe cases, cyanosis. The pulmonary picture is that of bronchopneumonia, with consolidation being uncommon. Empyema develops in 30% to 40% of cases, tends to appear early in the disease, and typically consists of copious amounts of thin serosanguineous fluid. Bacteremia occurs in 10% to 15% of cases. Complications include mediastinitis, pericarditis, pneumothorax, and bronchiectasis; and the clinical course of the disease is often prolonged. Recent case reports describe a rapidly fulminant hemorrhagic pneumonia associated with hypervirulent serotype M1 strains.[312] Outside of these cases, mortality has generally been low with penicillin therapy and adequate drainage of empyema, perhaps reflecting its occurrence in healthy military recruits. However, in a recent Canadian report of 222 cases of community-acquired pneumonia among adults (median age, 56 years), the case-fatality rate was 38%.[313] Of interest, a recent review of deaths in the 1918 pandemic of influenza has demonstrated that the major cause of death was secondary bacterial pneumonia.[314] Whereas *S. pneumoniae* was the most common cause, GAS was the second, and *S. aureus* was the third. Among patients with empyema complicating pneumonia, GAS was the most common cause. Investigators have demonstrated in a mouse model that a nonlethal influenza infection greatly enhanced the severity and mortality of secondary respiratory tract infection after challenge with GAS.[315]

Group A streptococcal perianal cellulitis and vulvovaginitis are symptomatic but benign disorders primarily affecting children.[316,317] Asymptomatic carriage of GAS in the vagina, anus, scalp, or, rarely, upper respiratory tract of adults has, however, been the source of outbreaks of nosocomial streptococcal infection.[318]

Prophylaxis and Risk for Secondary Cases of Streptococcal Toxic Shock Syndrome

Strep TSS is most commonly community acquired and sporadic, yet clusters of invasive cases have been described in nursing homes,[233–237] families,[238,239] and hospital workers.[240,319] In San Francisco 23 hospital workers became colonized or infected with GAS as a result of contact from a single case of strep TSS.[319] This example, as well as many historical studies in schools, military posts, and nursing homes, shows that GAS are highly contagious. Luckily, mere contact or colonization is usually not sufficient to cause a secondary case of invasive group A streptococcal infection. Epidemiologic studies by the Centers for Disease Control and Prevention found one secondary case of invasive infection among more than 1500 contacts.[162] This would extrapolate to 66 per 100,000 population per year for secondary cases.[320] As noted, the current incidence of primary cases of invasive group A streptococcal infections in the United States is 3.5 per 100,000 population per year. Thus the risk to contacts is roughly 20 times greater than that for the general population but is still very low. Given the relative infrequency of these infections and the lack of a clearly effective chemoprophylactic regimen, routine screening for and prophylaxis against streptococcal infection are not recommended for household contacts of index patients. In deciding who should receive prophylaxis, the clinician needs to factor in the duration of contact, intimacy of contact, and underlying host factors of individual contacts. Specifically, contacts with open wounds, recent surgery, recent childbirth, concurrent viral infections such as varicella or influenza, or immunodeficiency diseases should receive prophylaxis. In a multicenter study of adults age 18 to 45 years, HIV infection and injecting drug use were independently associated with an increased risk for invasive group A streptococcal disease. In those 45 years of age or older, diabetes, cardiac disease, cancer, and corticosteroid use were significant risk factors.[299] Moreover, adults 65 years or older are at increased risk for mortality should they contract invasive disease. Thus it may be prudent to initiate prophylaxis in households with older adults or those with the just-mentioned risk factors.

Lacking firm data on which to base antimicrobial prophylaxis, it seems reasonable to choose those agents that have achieved highest rates of pharyngeal eradication in asymptomatic individuals; among these are clindamycin and azithromycin. Specific regimens have been published elsewhere.[162]

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The complete reference list is available online at Expert Consult.

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198 Nonsuppurative Poststreptococcal Sequelae: Rheumatic Fever and Glomerulonephritis

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SHORT VIEW SUMMARY

Definitions
- Acute rheumatic fever (ARF) and acute poststreptococcal glomerulonephritis (APSGN) are immune-mediated illnesses that develop after some group A streptococcal infections.
- ARF predominantly affects the heart and joints and can lead to chronic rheumatic heart disease, whereas APSGN is an immune complex nephritis.

Epidemiology
- ARF can follow an untreated group A streptococcal infection of the pharynx in individuals who appear to be genetically susceptible.
- ARF is most frequent in children 5 to 15 years of age and occurs most commonly in winter or spring.
- ARF now is much less common in developed areas of the world, compared with several decades ago. Most cases now occur in developing countries or in minority populations within Australia and New Zealand. In North America, there appears to be increased incidence in Mormon populations and Orthodox Jews in New York, perhaps related to large family size.
- APSGN can follow group A streptococcal infections of the skin or throat.
- Postpharyngeal APSGN occurs mainly in school-age children in winter or spring, whereas postpyoderma APSGN is most common in pre–school-age children in late summer or fall.

Microbiology
- Certain M types of group A streptococci (GAS; M-1, M-3, M-5, M-6, M-14, M-18, M-19) are considered rheumatogenic, that is, they have much greater ability to trigger the immune events that result in ARF when compared to nonrheumatogenic types. The molecular basis of rheumatogenicity is unknown.
- Nephritogenic M types of GAS are those with great propensity to lead to APSGN, specifically M-1, M-4, M-12, and M-25 after pharyngitis and M-2, M-9, M-55, M-57, M-59, M-60, and M-61 after pyoderma. The specific antigen(s) involved in this immune complex nephritis is still somewhat unclear.

Diagnosis
- Diagnosis of ARF is based upon the Jones criteria, comprising five major criteria, four minor criteria, and a requirement for evidence of antecedent group A streptococcal infection. The Jones criteria revision of 2015 expanded the definition of carditis to include echocardiographic evidence without a murmur and established slightly different minor criteria for patients from low-risk geographic areas and moderately high-risk areas.
- The five major Jones criteria are carditis, arthritis, chorea, erythema marginatum, and subcutaneous nodules. The four minor criteria are fever, arthralgia, elevated acute-phase reactants (erythrocyte sedimentation rate, C-reactive protein), and prolonged PR interval. Diagnosis of ARF requires one major plus at least two minor criteria or two major criteria plus evidence of antecedent group A streptococcal infection.
- The diagnosis of APSGN is generally based upon acute onset of hematuria with hypertension, azotemia, and hypocomplementemia, and with evidence of recent streptococcal pharyngitis or pyoderma.

Therapy
- Treatment of ARF includes an antiinflammatory agent (aspirin for those with arthritis, mild carditis, or both; corticosteroids for those with moderate or severe carditis [see Table 198.3]); an agent such as phenobarbital, diazepam, haloperidol, valproate, or risperidone for those with chorea; and a prophylactic antibiotic (see "Prevention" later). A single dose of intramuscular (IM) benzathine penicillin (or an alternative agent for penicillin-allergic individuals) is indicated.
- Treatment of APSGN includes correction of circulatory overload and hypertension by salt and fluid restriction, diuretics as needed, and antihypertensives only if severe hypertension or hypertensive encephalopathy is present. Immunosuppressives are not beneficial.

Prevention
- Because ARF frequently recurs with subsequent group A streptococcal pharyngitis episodes, long-term prophylaxis is indicated with IM benzathine penicillin every 4 weeks or oral penicillin V twice daily, or with a daily sulfadiazine, macrolide, or azalide for penicillin-allergic individuals (see Table 198.4), usually at least until age 21.
- Because APSGN only very rarely recurs, no preventative antibiotic therapy is indicated.

RHEUMATIC FEVER

Acute rheumatic fever (ARF) is a disease characterized by nonsuppurative inflammatory lesions involving primarily the heart, joints, subcutaneous tissues, and central nervous system. In its classic form, ARF is acute, febrile, and largely self-limited. However, damage to heart valves may occur, and such damage may be chronic and progressive and lead to severe cardiac failure, total disability, and death many years after the acute attack. The manifestations of ARF are extremely variable; the disorder remains for the most part a clinical syndrome for which no specific diagnostic test exists. All cases of ARF follow group A streptococcal upper respiratory tract infection, although the exact mechanisms mediating development of the disease remain speculative. Persons who have suffered an attack of ARF are predisposed to recurrent episodes after subsequent group A streptococcal infections.

History

Guillaume de Baillou (1538–1616), also known as Ballonius, first clearly distinguished acute arthritis from gout. Thomas Sydenham (1624–1689) described chorea but failed to associate this entity with other manifestations of ARF. Raymond Vieussens (1641–1715) published pathologic descriptions of mitral stenosis and aortic insufficiency. It remained, however, for William Charles Wells, in 1812, to emphasize the association of rheumatism and carditis and to provide the first clear description of subcutaneous nodules. Jean-Baptiste Bouillard, in 1836, and Walter B. Cheadle, in 1889, published extensive studies of rheumatic arthritis and carditis that synthesized the syndrome of rheumatic fever and have come to be regarded as classic works in this field and that form the basis for modern clinical concepts of ARF. In 1904, Ludwig Aschoff described the specific rheumatic lesion in the myocardium.[1]

In 1880, J. K. Fowler pointed out the association between sore throat and rheumatic fever from his personal experience and, shortly after the dawn of the 20th century, Bela Schick identified ARF as one of the "nachkrankheiten" (sequelae) of scarlet fever. The introduction of Rebecca Lancefield's grouping system for β-hemolytic streptococci allowed clarification of the epidemiology of the disease by a number of investigators in the United States and the United Kingdom, including Coburn, Collis, Rammelkamp, Denny, Wannamaker, Massell, and Stollerman. Finally, the widespread introduction of antibiotic agents after World War II resulted in the development of strategies for primary and secondary prevention of rheumatic fever.[1]

Etiology and Pathogenesis

ARF is a delayed nonsuppurative sequela of upper respiratory infection caused by group A streptococci (GAS), a conclusion firmly supported by several lines of evidence. There is a close temporal relationship between epidemics of streptococcal sore throat and scarlet fever and epidemics of ARF. About two-thirds of patients with ARF relate a history of preceding pharyngitis; even in the absence of such clear-cut evidence, elevated serum levels of antistreptococcal antibodies almost always document recent streptococcal infection. Prospective studies of primary and recurrent ARF have shown that this disease occurs only after an immunologically significant streptococcal infection. Finally, continuous antimicrobial prophylaxis, when successful in preventing intercurrent group A streptococcal infections, also effectively prevents ARF recurrences in rheumatic persons.

An intriguing and as-yet unexplained aspect of the host-parasite relationship is the fact that, as far as is known, cutaneous streptococcal infections do not initiate ARF. This may indicate a requirement for the pharyngeal site, with its rich endowment of lymphoid tissue, for initiation of the disease process, or it may result from a lack of rheumatogenicity among the so-called pyoderma strains of GAS. However, some suggest that cutaneous infections might play a role in the etiology of ARF, based on epidemiologic observations and circulating GAS strains in hyperendemic areas, but this remains unclear.[2,3]

Host genetic factors appear to influence the susceptibility to ARF. Observational studies in the 19th century recognized familial tendencies to develop ARF, and in the early 1940s, studies showed familial tendencies to develop the disease, with greater risk occurring in children if both parents had rheumatic heart disease.[4] Considerable efforts examining human leukocyte antigen type predisposition to ARF have been undertaken, and more recently, an allele in an immunoglobulin heavy-chain locus was shown to be associated with rheumatic heart disease in Micronesian populations.[5] Alternatively, clustering by family also might reflect differences in the microbiome.

A substantial body of evidence indicates that GAS do vary in their rheumatogenic potential. Studies of outbreaks of streptococcal pharyngitis show that strains of certain M serotypes or genotypes are strongly and repeatedly associated with ARF (Table 198.1),[6] whereas strains of other equally prevalent types fail to initiate the disease or even to reactivate it in exquisitely susceptible hosts.[7] Investigations of endemic ARF cases in Trinidad[8] and Chile[9] indicated that streptococci causing ARF belong to different serotypes than those causing acute glomerulonephritis (AGN) when the conditions occur simultaneously in the same population. Strains of GAS isolated from ARF patients may, however, differ widely in different geographic locales.[10] Although the association of pyoderma strains of GAS (see Chapter 197) with ARF has been postulated by Australian investigators,[10,11] such strains have never been definitively associated with ARF[12,13] even when, as sometimes occurs, they colonize the throat. A study comparing group A streptococcal pharyngitis serotypes in Chicago in the 1960s, when ARF was common, to serotypes causing pharyngitis from 2001 to 2007, when ARF was very uncommon, showed marked reduction in circulating rheumatogenic types in the latter period.[14]

Rheumatogenic streptococcal strains exhibit distinct biologic characteristics. Their M protein molecules share a particular surface-exposed antigenic domain against which ARF patients mount a strong immunoglobulin G (IgG) response.[15] These strains fail to elaborate α(l)-lipoproteinase (so-called streptococcal opacity factor), and they are frequently heavily encapsulated.[16,17] The latter feature is manifested by the formation of mucoid colonies on blood-agar plates. Whether such strains express a unique rheumatogenic antigen, however, remains unknown.

TABLE 198.1 M Serotypes of Group A Streptococci Associated with Nonsuppurative Sequelae in the Western Hemisphere

ACUTE RHEUMATIC FEVER	PHARYNGITIS-ASSOCIATED AGN	PYODERMA-ASSOCIATED AGN
1	1	2
3	4	49[b]
5	12	55[b]
6	25	57
14		59
18		60
19		61
24		

[a]This list represents the major serotypes known to be associated with acute rheumatic fever and AGN in the Western Hemisphere, but it is not all-inclusive. M types of streptococcal strains isolated from various geographic areas vary widely.[10,184]
[b]M types 49 and 55 have also been reported on occasion to cause pharyngitis-associated AGN.
AGN, Acute glomerulonephritis.

It is probable that not all strains of rheumatogenic serotypes are equally dangerous. The propensity of a given strain to cause ARF likely depends on its degree of virulence, a reflection of quantitative factors such as expression of M protein, hyaluronate, or other less well-defined biologic properties. Virulence is likely to be enhanced in epidemiologic settings that favor rapid person-to-person transmission.

Although GAS is the causative agent of rheumatic fever, the exact mechanism whereby this microorganism induces the disease remains unexplained. Several theories have been advanced. These include the following: (1) toxic effects of streptococcal products, particularly streptolysins S or O, which are known to be capable of inducing tissue injury; (2) serum sickness–like reaction mediated by antigen-antibody complexes, perhaps localized to sites of tissue injury; (3) autoimmune phenomena induced by similarity or identity of certain streptococcal antigens to a wide variety of human tissue antigens[18]; and (4) binding of streptococcal M protein to the CB3 region of collagen type IV, which may trigger antibody to collagen, leading to ground substance inflammation in the subendothelial matrix of endothelium and perivascular connective tissue.[19]

Although none of these theories has been unequivocally proved or refuted, most attention has been focused on the concept of autoimmunity related to molecular mimicry.[20] Interest in this mechanism has been spurred by the identification of antibodies in the sera of patients with ARF or rheumatic heart disease (RHD) that react with the human heart in a variety of test systems. These so-called heart-reactive antibodies (HRAs) are also present at a much lower titer in sera of patients with uncomplicated streptococcal pharyngitis. The presence of bound immunoglobulin and complement in the myocardia of children dying of rheumatic carditis suggests that circulating HRAs may have pathogenetic significance.

Molecular techniques have been used to study the relationship between specific peptides of the M protein molecule and human tissues. Epitopes of streptococcal M proteins have been identified that share antigenic determinants with cardiac myosin,[21,22] sarcolemmal membrane proteins,[23] synovium, and articular cartilage.[24]

Goldstein and colleagues[25] described a cross-reaction between group A streptococcal polysaccharide and a structural glycoprotein of human and bovine heart valves. Such a cross-reaction might explain the observation that serum levels of antibody to group A streptococcal carbohydrate appear to remain elevated for many years in patients with rheumatic valvulitis but not in rheumatic patients without valvulitis,[26] and the levels decline remarkably after valve resection.

There is evidence that antibody to cardiac myosin in rheumatic carditis targets the S2 region and is similar among diverse populations worldwide.[27] Many children with Sydenham chorea have circulating antibodies that react both with neurons of the caudate and subthalamic nuclei and with group A streptococcal cell wall carbohydrate.[28] Taken together, these cross-reactive and toxic phenomena could explain most of the individual manifestations of ARF. On the other hand, it should be emphasized that no direct proof exists that these systems play any role in the pathogenesis of rheumatic fever.

Much of the work reviewed in the preceding paragraphs, particularly that related to HRAs and group A streptococcal carbohydrate, focused on humoral immune responses to streptococci. Indeed, serum antibody responses to streptolysin O, non–type-specific M-related antigens, and almost every other streptococcal antigen are, on average, more vigorous in patients with ARF than in those with uncomplicated streptococcal infections. However, it is likely that cellular immune responses to streptococcal antigens also play a critical role in the etiology of ARF.[29,30] Preparations of streptolysin S contain a nonspecific mitogen that is closely related but separable from the hemolytic activity. In rheumatic persons, lymphocyte reactivity to streptococcal cell walls and membranes is heightened, but the reactivity to membranes is more striking and persists for several years after an acute attack.[31] During active rheumatic carditis, both the number of helper (CD4) lymphocytes and the ratio of CD4 to CD8 cells are increased in heart valves as well as in peripheral blood.[32,33] Production of interleukin-1[34] and interleukin-2[34,35] is enhanced. That both M protein[36] and streptococcal pyrogenic exotoxins[37] function as superantigens suggests a potential mechanism mediating the unrestrained immunologic assault postulated to cause ARF.

Pathologic Findings

Rheumatic fever is characterized pathologically by the presence of exudative and proliferative inflammatory lesions of connective tissue, most notably in the heart, joints, blood vessels, and subcutaneous tissue.[38] In the early stages of the disease, there is fragmentation of collagen fibers, cellular infiltration that is predominantly lymphocytic, and fibrinoid deposition. This is followed shortly by the appearance of the myocardial Aschoff nodule (Fig. 198.1). The Aschoff nodule is a perivascular focus of inflammation that consists of an area of central necrosis surrounded by a rosette of large mononuclear and giant multinuclear cells. The nuclei of these cells may contain a clear area just within the nuclear membrane (owl-eyed nucleus) or present a serrated (caterpillar) appearance, depending on their orientation in microscopic cross section. Such cells are known as Anichkov myocytes, although immunohistochemical studies demonstrated that they are of macrophage-histiocyte origin.[39,40] Cardiac findings may include pericarditis, myocarditis, endocarditis, or combinations of these. Endocarditis involves the left side of the heart in almost all instances. A thickened and roughened area is frequently seen in the left atrium above the base of the posterior leaflet of the mitral valve (MacCallum patch). Valvular lesions begin as edema and cellular infiltration of the leaflets and chordae, with small verrucae along the line of closure. As healing progresses, the valves may become thickened and deformed, the chordae shortened, and the valve commissures fused, thereby resulting in valvular insufficiency with stenosis manifesting years later.



FIG. 198.1 **Aschoff nodule.**

The joint lesions are characterized by fibrinous exudate over the synovial membrane and serous effusion without joint destruction. Histologic findings include cellular infiltration and fibrinoid degeneration. Subcutaneous nodules resemble Aschoff bodies in many features. They consist of a central zone of fibrinoid necrosis surrounded by histiocytes and fibroblasts; perivascular accumulations of lymphocytes and polymorphonuclear leukocytes are also apparent. Although scattered areas of arteritis and petechial hemorrhages have been found in the brain, their relationship to Sydenham chorea remains uncertain.

Epidemiology

ARF is most frequent in children ages 5 to 15 years. Only approximately 5% of cases occur in children younger than 5 years.[41] Indeed, its relative rarity in infants and pre–school-aged children has led to the idea that repeated "primary" infections might be a precondition for the development of this disease. Both initial and recurrent episodes also occur in adults uncommonly.[42–44] There is no clear-cut gender predilection, although a female preponderance exists in certain clinical manifestations, notably mitral stenosis and Sydenham chorea when the latter occurs after puberty. In temperate climates, rheumatic fever tends to occur more during winter or spring and less frequently during the summer.

It is difficult for North American physicians to comprehend the magnitude of the problem of ARF in developing countries. The disease is highly endemic in the Middle East, the Indian subcontinent, and selected areas of Africa and South America.[45,46] The World Health Organization has estimated that approximately a half-million individuals worldwide acquire rheumatic fever annually, of whom 300,000 develop RHD. More than 15 million persons have been estimated to be living with this disease, and 319,000 die each year from RHD or its complications.[47,48] Extraordinarily high rates of ARF and RHD are seen in Aboriginal populations, such as those in New Zealand and Australia, and in Pacific Islanders. The annual incidence of ARF in Aboriginal children ages 5 to 14 years in Australia's Northern Territory was 150 to 380 per 100,000, and the point prevalence of RHD among the Aboriginal population has approached 2%.[2] In addition, the recognition that silent mitral regurgitation is common in school-age populations in many parts of the developing world may lead to a large increase in the estimated number of individuals affected by ARF/RHD.[49–52]

The overall incidence of ARF in the United States cannot be ascertained precisely because of inherent difficulties in diagnosing the disease and because most states no longer maintain rheumatic fever registries. There is general agreement, however, that the incidence of ARF and RHD declined markedly during the 20th century in the United States and Western Europe. The rate of decline appears to have been particularly steep during the 1960s and 1970s. A survey in Memphis, Tennessee,[53] indicated that during 1977 through 1981, the incidence of ARF in white suburban schoolchildren was only 0.5 per 100,000 annually. Similar rates were reported from many geographic areas of the United States. Traditionally, ARF in the United States was largely a disease of lower socioeconomic groups. The incidence was much higher in blacks than whites,[53,54] a fact that appears to relate to basic environmental conditions rather than to any genetic predisposition of blacks for the development of ARF. The major identified predisposing environmental condition is household crowding. The degree of crowding markedly influences the acquisition rate of GAS (see Chapter 197) and hence the risk of development of ARF.[55]

In the mid-1980s, a resurgence of ARF occurred in many communities in the United States.[56] Beginning in early 1985, an epidemic occurred in Salt Lake City, Utah, and the surrounding intermountain area.[55] By 2000, more than 500 cases had been diagnosed at the Primary Children's Medical Center in Salt Lake City. Smaller clusters of ARF, ranging from 15 to 40 cases, were reported during approximately the same time period from the following regions: Columbus and Akron, Ohio; Pittsburgh,

Pennsylvania; Nashville and Memphis, Tennessee; Kansas City, Missouri; Morgantown and Charleston, West Virginia; Dallas, Texas; and New York City, New York. Moreover, for the first time in many years, outbreaks occurred in army and navy training camps.[56–59]

Quite surprisingly, several of the 1980s civilian outbreaks[55,60] involved children of middle-class families residing in suburban or rural settings. The group A streptococcal strains most strongly associated epidemiologically with these ARF outbreaks belong to the well-recognized rheumatogenic serotypes (e.g., types M-1, M-3, M-5, M-6, and M-18); particularly prominent in this regard were highly mucoid strains of M-18.[17,61] Since the 1980s ARF has been very uncommon in most areas of the mainland United States, though outbreaks of rheumatic fever have been reported in the United States, such as that which occurred in Utah in the 1980s.[62]

Persons who have suffered an initial attack of ARF have a marked predilection to develop recurrences after subsequent episodes of streptococcal pharyngitis. The risk of recurrence after streptococcal infection is highest within the first few years after the initial attack and then declines. It is unclear whether the reason for this decline is the length of time since the preceding attack or the older age of the patient. Nevertheless, rheumatic patients remain at an increased risk of recurrence well into adult life. Two other factors that positively correlated with a risk of rheumatic recurrences after streptococcal infection are the magnitude of the anti–streptolysin O (ASO) response and the presence of preexisting heart disease. In the classic studies conducted at Irvington House, New York,[63] for example, 56% of streptococcal infections occurring in persons with RHD and accompanied by fourfold or higher ASO titer rises induced an ARF recurrence.

Clinical Manifestations

Rheumatic fever manifests itself as a variety of signs and symptoms that may occur singly or in combination. The most important of these, in terms of diagnosis, have been termed the *major manifestations* and include carditis, polyarthritis, chorea, subcutaneous nodules, and erythema marginatum. Certain additional findings frequently present in ARF, but those nonspecific in nature constitute the so-called *minor manifestations:* fever, arthralgia, first-degree heart block, and acute-phase reactants in the blood (C-reactive protein [CRP], leukocytosis, and erythrocyte sedimentation rate [ESR]).

The latent period between the onset of preceding streptococcal sore throat and the onset of ARF averages 19 days.[64] The range is 10 days to 5 weeks. The average latent period is the same for recurrent attacks as for initial episodes.

The mode of onset is variable. If acute polyarthritis is the initial complaint, the disease may have a rather abrupt onset and may be marked by fever and toxicity. On the other hand, when isolated mild carditis is the initial manifestation, the onset of ARF may be insidious or even subclinical.

Most attacks begin with polyarthritis. Carditis, if it appears, usually does so early in the course of the disease. Overall, arthritis occurs in approximately 75% of first attacks of ARF, clinically evident carditis in 40% to 50%, chorea in 15%, and subcutaneous nodules and erythema marginatum in less than 2%.[65,66] These incidences vary with age; carditis occurs most frequently when ARF strikes younger children, whereas the proportion of cases with arthritis increases with the age of the patient.

Carditis is the only manifestation of ARF that has the potential to cause long-term disability or death. Heart involvement in ARF may be pancarditis involving the endocardium, myocardium, and pericardium. While myocarditis and pericarditis may occur in ARF, the predominant manifestation of carditis is involvement of the endocardium presenting as valvulitis. Thus myocarditis or pericarditis without evidence of valvulitis is inconsistent with ARF. Nevertheless, in the absence of high fever or symptoms of acute pericarditis or congestive heart failure, it may be asymptomatic. Carditis almost always manifests itself within the first 3 weeks of an attack of ARF, if it appears at all. The clinical signs of carditis include the development of organic heart murmur(s) not previously present, cardiac enlargement, congestive heart failure, pericardial friction rubs, or signs of effusion. Because echocardiography is superior to auscultation for detection of valvular pathology,[67] the 2015 Jones criteria were revised to include mitral regurgitation demonstrated using



FIG. 198.2 Chronic rheumatic valvular heart disease. The mitral valve leaflets and chordae are thickened, fibrotic, and distorted; intercommissural adhesions are present. *(Courtesy Dr. L. Alvarez, Veterans Administration Medical Center, Miami, FL.)*

echocardiography. Thus now both pathologic and physiologic criteria that meet World Heart Federation criteria (rather than only pathologic) valvular involvement fulfills the criterion for carditis.[68] Intractable heart failure with pancarditis may cause death in the acute phase of ARF, but this is rare. Echocardiographic studies show that patients with ARF and congestive heart failure have preserved left ventricular systolic function and severe mitral or aortic regurgitation or both.[69–72] Serum levels of cardiac troponin I are not elevated in ARF patients with congestive heart failure.[69–73] Thus the cause of heart failure appears to be acute valvular dilatation rather than myocarditis.[74]

Chronic inflammatory changes involving the myocardium and endocardium may lead to the delayed development of chronic RHD (Fig. 198.2). Endocarditis involves the mitral valve more frequently than the aortic valve. There are three characteristic murmurs of acute rheumatic carditis: a high-pitched blowing holosystolic apical murmur of mitral regurgitation, a low-pitched apical mid-diastolic flow murmur (Carey Coombs murmur), and a high-pitched decrescendo diastolic murmur of aortic regurgitation at the secondary and primary aortic areas. Murmurs of mitral and aortic stenosis are associated with chronic but not with acute rheumatic valvular disease. The tricuspid valve is involved much less frequently and the pulmonic valve very rarely. Delayed atrioventricular conduction, as manifested by first-degree or greater degrees of heart block,[75] is a toxic phenomenon associated with ARF but is not in itself diagnostic of rheumatic carditis.

Joint involvement in ARF ranges from arthralgia without objective findings to frank arthritis characterized by heat, swelling, redness, and exquisite tenderness. There is an inverse relationship between the severity of joint involvement and the risk of development of carditis.[76] The most frequently involved joints are the knees, ankles, elbows, and wrists. The small joints of the hands are less frequently affected, and the spine is only rarely involved. When the course of the illness has not been suppressed by antiinflammatory drugs, classically, multiple joints are usually involved; approximately 50% of patients develop arthritis in more than

six joints. Arthritis in ARF is typically migratory in nature, that is, the inflammation travels from joint to joint. Once a joint becomes involved, inflammation begins to subside within a few days to a week. The evolution of arthritis in individual joints tends to overlap, so multiple joints may be inflamed at the same time. The typical migratory polyarthritis pattern may not be present, however, if effective antiinflammatory therapy is administered early in the course of the disease. In ARF, arthritis responds dramatically to salicylates or nonsteroidal antiinflammatory drugs (NSAIDs), and the absence of such a response should call the diagnosis into question. Moreover, the classic migratory pattern is not invariable. In some cases, the pattern may initially be additive, persisting in several joints simultaneously or even, rarely, monoarthritic.[77] It should be noted that the 2015 revision of the Jones criteria introduces less stringent arthritis/arthralgia criteria only for those individuals in moderate- and high-risk populations[78] (Table 198.2).

In most cases, the entire bout of polyarthritis subsides within 4 weeks, leaving no residual articular damage or deformity. One possible exception to this is the very rare occurrence of the so-called Jaccoud form of periarticular fibrosis after rheumatic arthritis.

The existence of poststreptococcal reactive arthritis distinct from ARF has been postulated to occur in some patients whose arthritis is atypical in time of onset or duration,[44] is nonmigratory, is unaccompanied by other major manifestations of rheumatic fever, and fails to respond promptly to salicylate therapy.[79–81] The ultimate prognosis of such cases is unknown, but, in a very few cases, mitral valve disease has apparently ensued.[82–84] Although the issue remains controversial,[85] it seems prudent to consider all cases of poststreptococcal polyarthritis that fulfill the Jones criteria as representing ARF,[79] provided that other common causes of polyarthritis have been excluded.[86–88]

Subcutaneous nodules are rare but are almost always associated with severe carditis and tend to occur several weeks after its onset. They are firm and painless and vary in size from a few millimeters to 2.0 cm. Such nodules are usually found over extensor surfaces or prominences and over tendons. Common sites are adjacent to elbows, knees, wrists, or ankles and over Achilles tendons, the occiput, or spinous processes of the vertebrae. Their number varies from one, requiring a thorough physical examination to detect, to a few dozen, which can be quite prominent as described in 1889 by Cheadle (Fig. 198.3). They usually persist for a few weeks. Somewhat similar but more persistent lesions are seen in rheumatoid arthritis. Pathology of the lesions is variable depending on the time since onset, but usually includes fibrous tissue (increasing in older lesions) and polymorphonuclear cells, as described by Jones in 1937 (Fig. 198.4).

Erythema marginatum is a rare nonpruritic, nonpainful erythematous eruption usually seen on the trunk or proximal aspects of the extremities but rarely, if ever, on the face. The individual lesions are evanescent, moving over the skin in serpiginous patterns that can change before the observer's eyes and are often likened to smoke rings, with a tendency

TABLE 198.2 2015 Revision of the Jones Criteria for the Diagnosis of Acute Rheumatic Fever in the Era of Doppler Echocardiography

CRITERIA	LOW-RISK POPULATIONS[a]	MODERATE-HIGH-RISK POPULATIONS
Criteria required	Initial diagnosis: 2 major manifestations or 1 major plus 2 minor manifestations Recurrent ARF: 2 major or 1 major and 2 minor or 3 minor	
Major Criteria		
Carditis	Carditis: clinical and/or subclinical[b]	
Arthritis	Polyarthritis only	Monoarthritis or polyarthritis OR polyarthralgia[c]
Erythema marginatum Subcutaneous nodules Chorea		
Minor Criteria		
Arthralgia[d]	Polyarthralgia	Monoarthralgia
Fever	≥38.5°C	≥38.0°C
Inflammatory markers	ESR[e] ≥60 or CRP[e] ≥3.0 mg/dL	ESR[e] ≥30 or CRP[e] ≥3.0 mg/dL
Prolonged PR interval	After accounting for age variability (unless carditis is a major criterion)	

[a]Low risk is defined as ≤2 per 100,000 school-age children per year or all-age rheumatic heart disease prevalence of ≤1 per 1000; moderate-high risk is defined as >2 per 100,000 school-age children per year or all-age rheumatic heart disease prevalence of >1 per 1000.
[b]Subclinical carditis indicates echocardiographic valvulitis.
[c]See section in Gewitz and colleagues[78] on polyarthralgia, which should only be considered as a major manifestation in moderate- to high-risk populations after exclusion of other causes. As in past versions of the criteria, erythema marginatum and subcutaneous nodules are rarely "stand-alone" major criteria. In addition, joint manifestations can only be considered in either the major or minor categories but not both in the same patient.
[d]Joint manifestations can only be considered in either the major or minor categories but not both in the same patient.
[e]CRP value must be greater than upper limit of normal for laboratory. Also, because ESR may evolve during the course of ARF, peak ESR values should be used.
ARF, Acute rheumatic fever; *CRP,* C-reactive protein; *ESR,* erythrocyte sedimentation rate.
From Gewitz MH, Baltimore RS, Tani LY, et al. Revision of the Jones criteria for the diagnosis of acute rheumatic fever in the era of Doppler echocardiography: a scientific statement from the American Heart Association. Circulation. *2015;131:1806-1818.*



FIG. 198.3 Subcutaneous nodules.

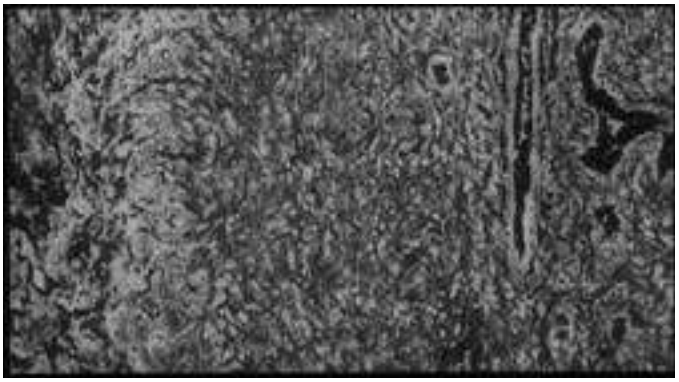

FIG. 198.4 Pathology of subcutaneous nodules. *Right,* Perivascular edema with lymphocytic infiltration from a subcutaneous nodule resected 7 days after detection. *Left,* Necrotic edematous collagen.



FIG. 198.5 Erythema marginatum: annular plaques (complete rings) of varying sizes, with macular centers and erythematous raised margins. Note the serpiginous borders formed by coalescence of several partial rings. *(Courtesy Dr. M. Tyrell, Saskatoon, Saskatchewan, Canada; and from Bisno AL. Noncardiac manifestations of rheumatic fever. In: Narula J, Virmani R, Reddy KS, et al, eds.* Rheumatic Fever. *Washington, DC: Armed Forces Institute of Pathology; 1999;245-256.)*

to advance at the margins while clearing in the center (Fig. 198.5). The lesions are usually macular with slightly raised margins and appear to be more a vasomotor phenomenon than a manifestation of cutaneous pathologic changes. Individual lesions may come and go in minutes to hours, but the process may go on intermittently for weeks to months and can be exacerbated by a warm cloth. Histopathologic examination may show perivascular infiltration of lymphocytes and neutrophils in the dermis.[89]

Sydenham chorea (St. Vitus dance) is a neurologic disorder characterized by emotional lability, muscular weakness, and rapid, uncoordinated, involuntary purposeless movements. The choreiform movements disappear during sleep and may be partially suppressed by sedation. The nonrhythmic movements are most notable in the face, hands, and feet. Sensation remains intact. Detailed descriptions of the nature of the choreiform movements can be found elsewhere.[1,90] Individual attacks in hospitalized patients usually last 2 to 4 months.

Chorea may occur in close temporal association with other major rheumatic manifestations or in isolated form (pure chorea). In cases of pure chorea, laboratory evidence of acute inflammation (elevated CRP, elevated ESR) or recent streptococcal infection (elevated levels of antistreptococcal antibodies) may be lacking. This observation, which led investigators in the past to question the relationship of ARF to pure chorea, is now known to result from the fact that Sydenham chorea often occurs with a substantially longer latent period than the other manifestations of ARF. Relapses of pure chorea may occur in some patients despite faithful adherence to prophylaxis with intramuscular benzathine penicillin.[91] Some patients with pure chorea are found on follow-up to have RHD manifested by mitral regurgitation or even mitral stenosis.[92]

Interest has focused on the possibility that certain other neurobehavioral conditions, including tics, obsessive-compulsive disorder, and Tourette syndrome, may be poststreptococcal sequelae.[93,94] This hypothesized entity has been termed *p*oststreptococcal *a*utoimmune *n*europsychiatric *d*isorders *a*ssociated with *s*treptococci (PANDAS). A prospective blinded cohort study of patients meeting suggested diagnostic criteria for PANDAS found that, after group A streptococcal infection, exacerbations of childhood tics and obsessive-compulsive disorders were more frequent in such patients than in control subjects. However, there has been no consistent relationship demonstrated between exacerbations and GAS infections. Streptococcal infection was not the only or even the most common antecedent of such exacerbations.[95] The relationship of streptococci to this proposed entity is unproven at this time.[93]

Several clinical manifestations of ARF occur with some frequency but are not in themselves specific enough to be considered major criteria. These minor criteria include fever, which accompanies almost all ARF attacks at their onset, and arthralgia. Slight differences in these minor criteria have been incorporated into the revised Jones criteria between low- and moderate- to high-risk populations. The pulmonary parenchyma in ARF rarely may be involved by a variety of pathologic processes, including pulmonary edema, atelectasis, pulmonary embolism, or thromboses.[96] Some believe that, in addition, a specific rheumatic pneumonia may occur in rare cases. Abdominal pain or epistaxis can occur.

The average duration of an attack, in the absence of antiinflammatory therapy, is approximately 3 months. Less than 5% of cases persist for longer than 6 months, justifying the designation of "chronic" rheumatic fever. Stollerman[1] listed the criteria for continuing clinical activity as follows: joint symptoms, new organic murmurs, changing heart size, congestive heart failure in the absence of long-standing valvular disease, subcutaneous nodules, sleeping pulse rate higher than 100 beats/min, erythema marginatum, chorea, elevated CRP levels, and a rectal temperature of 100.4°F or higher for 3 or more consecutive days.

Diagnosis

Because ARF can have such diverse manifestations (acute polyarthritis, congestive heart failure, chorea, or combinations of these) and because there is no specific diagnostic test for the disease, the differential diagnostic possibilities in an individual case may be extensive. Among the diseases that most frequently need to be differentiated are rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, serum sickness, sickle cell crisis or cardiopathy, septic arthritis (especially gonococcal arthritis in adolescent patients), Lyme disease,[97] infective endocarditis, viral myocarditis, and Henoch-Schönlein purpura. Choreiform movements have been described in patients with systemic lupus erythematosus,[98] neoplasms involving the basal ganglia,[99] legionnaires' disease,[100] hypoparathyroidism,[101] antiphospholipid syndrome,[102] Wilson disease, and Huntington disease. Chorea is also seen occasionally in women taking oral contraceptives[103] and during pregnancy (chorea gravidarum).[104]

Arriving at the correct diagnosis is particularly important in ARF, not only in terms of treatment for the acute attack and formulating an accurate prognosis but also because of the necessity for prescribing continuous antistreptococcal prophylaxis. To minimize overdiagnosis and underdiagnosis, the criteria originally formulated in 1944 by Jones[105] and updated and modified by a committee of the American Heart Association in 1992[87] and again in 2015[78] are generally accepted as the basis for reaching a diagnosis of ARF (see Table 198.2). The criteria are to be applied most stringently to the diagnosis of an initial ARF attack. Although most patients with recurrences fulfill the criteria, the diagnosis of recurrent ARF may be less apparent. In a patient with established RHD, for example, it may be difficult to diagnose recurrent carditis confidently, unless a previously normal valve is affected. The criteria therefore allow a presumptive diagnosis of recurrent ARF to be made if clinical findings are suggestive and there is supporting evidence of recent streptococcal infection.

Patients with ARF may have echocardiographic evidence of valvular regurgitation in the absence of an audible murmur. Although valvular regurgitation can also be detected in normal individuals by two-dimensional Doppler echocardiography and color flow Doppler imaging techniques, criteria for discriminating physiologic from pathologic regurgitation have been proposed by experienced investigators and endorsed by the World Heart Federation.[68,106–108] In a recent study from India, 333 patients suspected of having ARF were investigated using stringent echocardiographic criteria, and 15% were judged to have evidence of subclinical carditis.[68] A number of echocardiographic surveys of schoolchildren in several developing countries have identified up to 5% or more with subclinical (silent) mitral regurgitation.[49–52] The 2015 revision to the Jones criteria added so-called echocarditis (silent

regurgitation) as part of the major criteria.[78] The issue had been controversial,[74,109] but in some areas of the world with a high incidence of ARF (Australia, New Zealand), positive echocardiographic evidence had already been used to establish the diagnosis of carditis.[110,111] Handheld echocardiography has been utilized as an inexpensive tool in resource-limited settings to screen for RHD,[112] even when used by nonexperts. Using echocardiography, pathologic mitral regurgitation to fulfill the carditis criterion requires visualization in at least two views, a jet length ≥2 cm in at least one view, a peak velocity of >3 m/s, and a pansystolic jet in ≥1 envelope. Pathologic aortic regurgitation requires visualization in at least two views, a jet length ≥1 cm in at least one view, a peak velocity of >3 m/s, and a pandiastolic jet in ≥1 envelope.[78]

The Jones criteria are not infallible, particularly when the diagnosis rests on acute polyarthritis as the sole major criterion, with supporting evidence of fever plus an elevated ESR or CRP level. For this reason, evidence of recent streptococcal infection must be obtained to satisfy the Jones criteria. Such evidence might include a recent microbiologically documented episode of streptococcal pharyngitis; a positive throat culture or rapid streptococcal antigen test for GAS, although differentiation of infection from colonization presents a problem; or an elevated serum titer of antistreptococcal antibodies. In most cases, physicians rely on the latter criterion.

If a serum sample is obtained within 2 months of onset, approximately 80% of patients with ARF have an elevated ASO titer. If a second streptococcal antibody test is performed on the same serum specimen, the proportion of patients with ARF with at least one elevated titer will rise to 90%.[113] Although an elevated antistreptococcal antibody titer is certainly not diagnostic of ARF, failure to demonstrate evidence of recent immunologically significant streptococcal infection using ASO and anti–DNase B tests makes the diagnosis of ARF doubtful. An exception to this statement is the patient with pure chorea whose antibody titers may have declined to the normal range because of the long latent period between the antecedent streptococcal infection and the onset of this manifestation. Similarly, the onset of isolated carditis may be difficult to date; if recognition of isolated carditis is delayed, immunologic evidence of recent streptococcal infection may have disappeared.

Therapy and Prognosis

The objectives of therapy in ARF are to relieve inflammation, decrease fever and toxicity, and control cardiac failure. The mainstays of treatment are salicylates and corticosteroids. Neither of these agents prevents or modifies the development of chronic RHD.[114] A suggested treatment schedule is outlined in Table 198.3. Analgesics without antiinflammatory properties are recommended for patients with mild disease. This allows complete expression of the clinical manifestations to aid in diagnosis and also avoids posttherapeutic rebounds. Most patients require salicylates (50–70 mg/kg/day). If the high doses of salicylates required cannot be tolerated because of gastric irritation or if symptoms of salicylism develop, a reduction in the aspirin dosage or a change to corticosteroids is necessary. The more potent antiinflammatory action of corticosteroids should be used whenever salicylates fail to control the inflammatory process or whenever carditis with congestive heart failure is present. Although NSAIDs appear a reasonable alternative for patients who do not tolerate salicylates but do not require corticosteroids, there is a paucity of data on the use of these agents in ARF. Studies using either naproxen[115,116] or tolmetin[117] reported NSAIDs to be equivalent to aspirin in efficacy, with fewer side effects, though the numbers of patients in these studies was limited.

TABLE 198.3 Suggested Schedule of Antiinflammatory Therapy in Rheumatic Fever

CLINICAL SEVERITY	TREATMENT
Arthralgia or mild arthritis; no carditis	Analgesics only, such as codeine or acetaminophen
Moderate or severe arthritis; no carditis, or carditis *with or without* cardiomegaly, but without failure	Aspirin, 50–70 mg/kg/d for 3 weeks, increased if necessary; 25–35 mg/kg/d for the subsequent 6 weeks
Carditis with congestive failure, with or without joint manifestations	Prednisone, 2 mg/kg, max 60 mg/d; methylprednisolone sodium succinate intravenous in fulminating cases; after 2–3 weeks, slow withdrawal to be completed in 3 more weeks. Aspirin started when steroid withdrawal begins and continued for 1 month after discontinuation of prednisone.

Modified from Stollerman GH. Rheumatic Fever and Streptococcal Infection. *New York: Grune & Stratton; 1975.*

Reactivation of clinical or laboratory manifestations of rheumatic inflammation, or both, may occur after cessation of antiinflammatory therapy. This "rebound" phenomenon is more frequent after corticosteroids than aspirin. For this reason, therapy should be tapered rather than discontinued abruptly. Aspirin should be instituted as the steroid is being tapered and then should be continued for 1 month after treatment with corticosteroids is discontinued.

Heart failure should be treated by conventional measures. The potential risk of digitalis-induced arrhythmias in the patient with active myocarditis must be kept in mind. As noted, in the absence of preexisting valvular disease, congestive heart failure in ARF patients is usually attributable primarily to valvular dilatation and not to myocardial failure. Patients with chorea require a quiet, nonstimulatory environment and sedation. An agent such as phenobarbital or diazepam may be used. In patients with severe and debilitating hyperkinesis, haloperidol has been used. A review has suggested valproic acid as first-line treatment and risperidone for nonresponders.[118] Evidence suggests that corticosteroids may be beneficial in decreasing both the intensity and duration of chorea.[119] A prospective randomized trial of intravenous immunoglobulin in ARF in New Zealand failed to show that it altered the natural history of the disease.[120]

The only long-term sequela of ARF is RHD. The prognosis in rheumatic fever patients is greatly improved by prevention of recurrent attacks, with their concomitant threat of additional valvular damage. The ultimate prognosis of an individual attack is related to the severity of cardiac involvement during the acute phase. This was best studied in a joint UK-US study.[121] In that study, only 6% of patients with no carditis or with only questionable carditis during their attack of ARF had heart murmurs when reexamined 10 years later. Heart disease was present at follow-up in 30% of the patients initially found to have only apical systolic murmurs, in 40% of those with basal diastolic murmurs during the acute phase, and in 68% of those who initially suffered from congestive heart failure, pericarditis, or both. Patients with pure chorea appear to have a relatively high incidence of late development of RHD, even if carditis is not recognized at the time of the initial attack. These findings need to be reevaluated in the echocardiographic era.

RHD can be detected in asymptomatic individuals by auscultation of a murmur characteristic for mitral stenosis, as documented by Paul Dudley White in the 1950s.[122] Our concepts of the prevalence of RHD in developing countries requires revision in view of the results of echocardiographic screening of some 5800 randomly selected schoolchildren in Colombia and Mozambique and other studies in underdeveloped areas.[49–52] These studies found the prevalence of RHD to be approximately 10-fold greater than recognized by conventional clinical auscultation by ordinary observers alone. Prospective follow-up of these children is required to determine the functional implications of these imaging studies.

Prevention

Prevention of ARF in persons without a prior history of this disease depends on accurate diagnosis and appropriate treatment of the antecedent streptococcal infection. This approach (primary prevention) is outlined in Chapter 197. It is effective[123] but suffers from the limitation that one-third or more of ARF cases follow streptococcal infections that are entirely subclinical or too mild to bring them to medical attention. Guidelines for treatment of streptococcal pharyngitis were published in 2012 by the Infectious Diseases Society of America.[124]

Rheumatic patients are at high risk of developing recurrent ARF after immunologically significant streptococcal upper respiratory infections and require continuous prophylaxis to prevent intercurrent streptococcal

TABLE 198.4 Secondary Prevention of Rheumatic Fever (Prevention of Recurrent Attacks)

AGENT	DOSE	MODE
Benzathine penicillin G	600,000 U for children ≤27 kg (60 lb), 1.2 million U for those >27 kg (60 lb) every 4 weeks[a]	Intramuscular
Penicillin V	250 mg twice daily	Oral
Sulfadiazine	0.5 g once daily for patients ≤27 kg (60 lb) 1.0 g once daily for patients >27 kg (60 lb)	Oral
For Individuals Allergic to Penicillin and Sulfadiazine		
Macrolide or azalide	Variable	Oral

[a]In high-risk situations, administration every 3 weeks is justified and recommended.
From Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. Circulation. *2009;191:1541-1551.*

TABLE 198.5 Duration of Secondary Rheumatic Fever Prophylaxis

CATEGORY	DURATION AFTER LAST ATTACK
Rheumatic fever with carditis and residual heart disease (persistent valvular disease[a])	10 yr or until age 40 yr, whichever is longer; sometimes lifelong prophylaxis (see text)
Rheumatic fever with carditis but no residual heart disease (no valvular disease[a])	10 yr or until age 21 yr, whichever is longer
Rheumatic fever without carditis	5 yr or until age 21 yr, whichever is longer

[a]Clinical or echocardiographic evidence.
From Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. Circulation. *2009;191:1541-1551.*

infections. The recommended regimen[125] for most patients in the United States and other countries in which ARF incidence is low consists of a single injection of penicillin G benzathine administered every 4 weeks (Table 198.4). In the most comprehensive study reported,[126] children following this regimen experienced a rheumatic fever recurrence rate of only 0.4 per 100 patient-years of observation. In areas of the world in which ARF and RHD remain very highly prevalent, ARF recurrence rates have been found to be even lower when injections of penicillin G benzathine are administered every 3 weeks rather than every 4 weeks.[127]

Oral sulfadiazine and penicillin V are also acceptable prophylactic agents but are less effective than penicillin G benzathine (see Table 198.4). The lesser efficacy of oral regimens is at least in part the result of the extreme difficulty of enforcing adherence. Patients allergic to penicillin and sulfadiazine may be given a macrolide or an azalide. Patients requiring protection for many years are often begun on a regimen of penicillin G benzathine, which is changed to oral prophylaxis later when the risk of recurrence is deemed to be lower.

The optimal duration of continuous antimicrobial prophylaxis remains controversial. The risk of ARF recurrence is neither continuous nor uniform. It declines with the age of the patient and the number of years since the most recent attack. It is positively correlated with the number of previous attacks and with the presence and severity of RHD. Thus the risk of recurrence becomes low in older adults without heart disease who are not in intimate contact with school-age children. The physician must decide whether and when to discontinue prophylaxis after careful assessment of the patient's risk of acquiring a streptococcal infection, the anticipated recurrence rate per infection, and the likely consequences of such recurrence. Even when all these factors are favorable, prophylaxis should not be discontinued generally until the patient has reached his or her early 20s and at least 5 years have elapsed since the most recent rheumatic attack.[128] Recommendations of the American Heart Association are given in Table 198.5.

Investigative efforts are currently being directed toward the development of a safe, effective vaccine for the prevention of streptococcal infection and ARF. Such a vaccine would have to provide protection against the major serotypes associated with ARF and highly invasive infections.[129,130] Finally, a school-based intervention to screen and treat cases of GAS sore throat in New Zealand was shown to decrease rates of ARF in a hyperendemic area.[131] The recent observation that 65% of new GAS acquisitions caused no symptoms yet were associated with an antibody response in children may suggest that primary prevention in high-prevalence areas of ARF/RHD may need to focus on both symptomatic and asymptomatic children,[132] but this requires further study.

GLOMERULONEPHRITIS

Poststreptococcal AGN is an acute inflammatory disorder of the renal glomerulus, characterized pathologically by diffuse proliferative glomerular lesions and clinically by edema, hypertension, hematuria, and proteinuria. The disease is a delayed nonsuppurative sequela of pharyngeal or cutaneous infection with certain nephritogenic group A streptococcal strains belonging to a limited number of serotypes.

History

Richard Bright (1789–1858) clearly differentiated cardiac from renal dropsy in 1836.[133] He also noted the association between acute scarlet fever and AGN.[133] Subsequently, many investigators confirmed the relationship between β-hemolytic streptococcal infections and subsequent AGN. Schick,[134] in 1907, commented on the similarity of the latent period in serum sickness to that in AGN, thus suggesting the possibility of an immunologic basis for the latter disease. Longcope[135] noted the association of streptococcal infections and AGN. Rammelkamp and Weaver[136] explained the puzzling variations in attack rate of AGN after group A streptococcal infection by proposing that only certain serotypes of *Streptococcus pyogenes* were nephritogenic. Classic detailed prospective studies of the epidemiology, bacteriology, immunology, and natural history of pyoderma-associated nephritis by Wannamaker[137] in Minnesota, Potter and colleagues[138] in southern Trinidad, and Dillon and coworkers[139] in Alabama have added greatly to our understanding of this disease.

Etiology and Pathogenesis

Poststreptococcal AGN follows infection with a limited number of group A streptococcal serotypes (see Table 198.1). Type 12 is the most frequent M serotype causing AGN after pharyngitis or tonsillitis, whereas M-49 is the type most frequently related to pyoderma-associated nephritis. Not all streptococcal strains belonging to these serotypes are nephritogenic, however. As yet, there are no reliable biologic markers to differentiate nephritogenic from nonnephritogenic streptococci. Poststreptococcal AGN is almost always caused by strains of serogroup A. Well-documented outbreaks caused by group C organisms (*Streptococcus equi* subsp. *zooepidemicus*) have been reported, however.[140,141]

The precise mechanism whereby streptococcal infection gives rise to AGN has not been delineated, although evidence strongly favors the view that the renal injury is immunologically mediated. This includes the approximately 7-day latent period between infection and the development of AGN, the associated hypocomplementemia, and the fact that IgG, complement components, and antigens that react with streptococcal antisera are present in the renal glomerulus early in the course of the disease.[142,143] It is possible that antibodies elicited by nephritogenic streptococci react with renal tissues in such a way as to produce glomerular injury, because antigenic similarities between constituents of the streptococcus and human kidney are described.[144,145] However, the electron microscopic finding of nodular subepithelial "humps" in renal biopsy specimens in AGN is compatible with renal injury caused by deposition of preformed complexes consisting of streptococcal antigen and host antibody within the glomerulus. These deposits are characteristic of experimentally induced disease caused by circulating immune complexes and of other immune complex–mediated

disorders. Several groups have detected circulating immune complexes in AGN.[146]

The identity of the streptococcal constituent(s) involved in the pathogenesis of AGN remains unknown. M protein is an obvious candidate because of the close association of nephritogenicity and certain M serotypes. Monoclonal antibodies raised against human glomeruli have been found to cross react with streptococcal M protein.[145] Moreover, in an animal model of nephritis induced by nephritogenic type 12 streptococci, eluted bound glomerular antibodies were found to be directed against type 12 M protein but not against other streptococcal and renal antigens. Others, however, have described cross-reactions between fragments of streptococcal cell membrane and human glomerular basement membrane.[142]

Several antigens isolated from nephritogenic streptococci have been investigated regarding their role in the pathogenesis of AGN, including streptococcal pyrogenic exotoxin B (SpeB)[147,148] and a nephritis-associated plasmin receptor (NAPlr) and nephritis-strain–associated protein (NSAP).[149–151] Streptokinase production has been postulated to play a role in the pathogenesis of AGN and has been found to be essential for development of the disease in a mouse model.[151] However, there is no unique reactivity to group A streptococcal streptokinase in sera of AGN patients, nor has streptokinase deposition been demonstrated in biopsy specimens obtained early in the disease.[152]

Pathologic Characteristics

In the acute phase of AGN, light microscopic examination of renal biopsy specimens demonstrates a marked increase in glomerular intracapillary cellularity caused by endothelial and mesangial cell proliferation. These changes involve almost all the glomeruli, which appear enlarged and bloodless, tending to fill the Bowman space.[153] In addition to this diffuse proliferative endocapillary process, a variable degree of polymorphonuclear leukocytic exudation is observed. Proliferation of parietal and visceral epithelial cells occurs only to a modest degree and is rarely extensive enough to give rise to well-developed crescent formation. Thin sectioning and special strains may reveal discrete deposits on the epithelial side of the basement membrane that correspond to the humps visible on electron microscopy. Focal degeneration, interstitial edema, and cellular infiltration also occur in the renal tubular cells, but these tubular changes are far less prominent than is the glomerulitis. Arterioles are normal or almost normal in most cases of AGN.

Immunofluorescence studies demonstrate considerable variability in the pattern of deposition of immunoglobulin and complement components. C3 is almost always present in the glomeruli, and granular deposits of IgG are also frequently demonstrable as discrete deposits similar in size and location to the subepithelial humps visualized by electron microscope,[143,154] although deposits of C3 may also occur in an interrupted linear pattern along the basement membrane or in the mesangium. Deposits of immunoglobulin M, C1q, C4, and fibrin are found less commonly. The rather weak and inconsistent deposition of early complement components suggests that activation of the alternate complement pathway plays a role in the immunopathology of AGN.

Epidemiology

It has been estimated that more than 470,000 cases of AGN occur annually worldwide, with approximately 5000 deaths.[155] The great majority of these occur in less-developed countries. The epidemiologic characteristics of AGN largely reflect those of the antecedent group A streptococcal infection, that is, pharyngitis or pyoderma (Table 198.6). Thus the classic streptococcal sore throat occurs primarily among school-age children during the cooler months of the year. Pyoderma is largely a disease of children ages 2 to 6 years and, in temperate climates, occurs during the summer and early fall. Given a skin infection with a nephritogenic strain, the attack rate of AGN is higher in children 6 years of age or younger than in older children.[156] AGN can also follow cutaneous infections other than pyoderma.[157,158] The latent period of AGN is variable but averaged 10 days after pharyngeal infection in the studies of Stetson and colleagues[159]; prospective studies at the Red Lake Indian Reservation in Minnesota have indicated the usual latent period of pyoderma-associated AGN to be 3 weeks or longer.[156]

TABLE 198.6 Epidemiologic Characteristics of Pharyngitis-Associated and Pyoderma-Associated Acute Glomerulonephritis (AGN)

FEATURE	PHARYNGITIS-ASSOCIATED AGN	PYODERMA-ASSOCIATED AGN
Age	Early school age	Pre–school-age
Gender	Male-to-female ratio ≅2:1	Equally distributed
Season	Winter and spring	Late summer and early fall
Geographic distribution	North and south	Predominantly south
Familial occurrence	Variable	Variable
Latent period	10 d	3 wk
Attack rate[a]	10%–15%	10%–15%
Serologic types	Limited types	Also limited but different types
Recurrences	Rare	Rare

[a]After infection with known nephritogenic strain.
Modified from Wannamaker LW. Differences between streptococcal infections of the throat and of the skin. N Engl J Med. *1970;282:23-31.*

Although the attack rate of AGN after throat or skin infection with a nephritogenic strain is substantial (i.e., 10%–15%),[156] the disease differs dramatically from ARF in that recurrences are rare. This is attributable at least in part to the relatively limited number of streptococcal strains that are nephritogenic and presumably also to the acquisition of type-specific protective immunity to the serotype that elicited the initial attack. When second attacks of AGN occur, they are clinically and histologically indistinguishable from the initial attack.[160] More common than recurrent AGN attacks is the propensity for streptococcal infections to precipitate exacerbations of chronic glomerulonephritis.[161] Such exacerbations often occur after a relatively brief latent period of 1 to 4 days. The coexistence of ARF and AGN in the same patient after pharyngeal infection is rare, but a few such cases have been reported.[162]

The introduction of a highly nephritogenic strain into a family unit may result in multiple cases. When systematic screening of sibling contacts for hypertension, urinary abnormalities, and serum complement levels has been performed, the incidence of proven and suspected cases of AGN in sibling contacts has been extremely variable,[163–165] with estimates ranging as high as 20%.

Clinical and Laboratory Features

The typical clinical features of AGN in children include edema, hypertension, and smoke- or rust-colored urine. Patients also exhibit pallor and may complain of lethargy, malaise, weakness, anorexia, headache, and dull back pain. Fever is not a prominent finding.

Facial and periorbital edema are usually present, especially in the morning, but edema also involves dependent areas, such as the feet and legs, scrotum, and sacrum. In severe cases, ascites or pleural effusions may occur. Another manifestation of fluid overload is circulatory congestion, which may give rise to dyspnea, orthopnea, rales at the lung bases, distended neck veins, and even frank pulmonary edema. Manifestations of circulatory overload tend to be particularly prominent in the occasional cases of AGN occurring in older adults and, in these patients, may obscure the correct diagnosis if urinary findings are not properly interpreted.

Hypertension occurs in most patients but is usually of modest degree. Hypertensive retinopathy or heart failure does not ordinarily complicate the clinical picture. On the other hand, up to 5% to 10% of AGN patients develop severe hypertension complicated by signs and symptoms of encephalopathy. These range from headache and vomiting to confusion, somnolence, and convulsions.

Although these clinical features are typical of hospitalized patients, many cases of AGN are so mild as to escape detection unless those at risk are tested for urinary sediment abnormalities and serum complement levels. Two studies that included renal biopsy data concluded

that in epidemic situations, as many as 50% of cases of AGN may be subclinical.[165,166]

Laboratory findings include a mild normocytic normochromic anemia, elevated ESR, slight hypoproteinemia, and elevations of the blood urea nitrogen and serum creatinine concentrations. Hypercholesterolemia and hyperlipemia may also be present. Serum levels of total hemolytic complement and C3 complement are markedly reduced in the great majority of patients with clinically apparent AGN. Urine volume may be significantly diminished, and the urine itself is smoky, rusty, or brownish, with a high specific gravity and positive test findings for protein and hemoglobin. Total urinary protein excretion is usually less than 3 g/day.[167] Microscopic examination of the urine reveals erythrocytes, leukocytes, and hyaline, granular, and red blood cell casts.

The urinary abnormalities in AGN must be distinguished from the transient mild hematuria and proteinuria that may be seen during the acute phase of acute streptococcal infection and other febrile illnesses. The relationship, if any, of these early urinary findings to the development of AGN is unknown.[167] Finally, diagnostic confusion is almost inevitable in the rare cases in which pronounced clinical manifestations of AGN occur in patients with minimal or no urinary sediment abnormalities.[168]

Diagnosis

The diagnosis of AGN is based on the clinical history, physical findings, and confirmatory evidence of antecedent streptococcal infection. The latter may include a recent history of scarlet fever, isolation of group A streptococci from throat or skin lesions, or demonstration of elevated serum titers of streptococcal antibodies. Even in the absence of bacteriologic isolation of streptococci, the presence of skin lesions compatible with streptococcal impetigo is highly suggestive.

It is almost always possible to demonstrate an elevated level of streptococcal antibodies in AGN, although, in cases with relatively short latent periods, serial bleedings may be necessary. In pyoderma-associated nephritis ASO responses are weak, and it is frequently necessary to assess levels of anti–DNase B. Although antistreptozyme titers rise in pyoderma nephritis, technical problems limit the reliability of the test. Finally, if renal biopsy is performed, the demonstration of diffuse proliferative glomerulonephritis with subepithelial electron-dense deposits is a very helpful confirmatory finding.

Poststreptococcal acute glomerulonephritis must be differentiated from other infectious processes involving the kidney and from other primary renal diseases. For example, often it may be extremely difficult to differentiate an acute exacerbation of chronic glomerulonephritis, such as may be precipitated by streptococci or by other intercurrent infections, from a true attack of AGN. A short latent period of 1 to 4 days suggests that the episode is an exacerbation of preexisting renal disease. Patients with infective endocarditis tend to develop high serum levels of circulating immune complexes and may develop diffuse proliferative or focal glomerulonephritis, which may be confused clinically with poststreptococcal nephritis. Other bacterial and protozoan illnesses, such as pneumococcal pneumonia, typhoid fever, leptospirosis, syphilis, toxoplasmosis, and *Plasmodium falciparum* malaria, on occasion are associated with nephritis. Viral infections, such as hepatitis B and C, infectious mononucleosis, measles, mumps, and enteroviral disease, have similarly been implicated as causes of viruria, transient renal dysfunction, or actual glomerulonephritis.[169] In addition to the development of focal and segmental glomerulosclerosis, patients infected with human immunodeficiency virus may rarely develop an immune complex glomerulonephritis.[170] Other entities that may at times mimic AGN are Henoch-Schönlein purpura, systemic lupus erythematosus, polyarteritis nodosa, acute tubular necrosis, focal glomerulonephritis with hematuria, hereditary nephritis, rapidly progressive glomerulonephritis, idiopathic nephrotic syndrome, and malignant hypertension.

Therapy

Because no form of treatment is known to alter the long-term prognosis of AGN, therapy is directed toward management of the acute issues. Attention is directed to the most immediate problem—circulatory overload—and to hypertension. In most cases, this is handled adequately by salt and fluid restriction alone, but diuretics may be required. Digitalis is not indicated because the risk of toxicity is substantial, and usually myocardial function is intact.[171] Specific antihypertensive therapy is usually unnecessary, except in cases of severe hypertension and hypertensive encephalopathy. Patients developing acute pulmonary edema or severe and prolonged oliguria require measures conventionally used for these conditions. Immunosuppressive therapy is not of benefit.

All nonallergic patients should receive penicillin, preferably intramuscular penicillin G benzathine (see Chapter 197 for dosage schedule), to eradicate the nephritogenic streptococcal strain. Penicillin-allergic patients should receive one of the alternative regimens listed in Chapter 196.

With skillful use of the supportive measures outlined here, mortality during the acute phase of AGN is now rare. Perhaps 1% or less of patients develop severe and irreversible renal failure. In the remainder, signs and symptoms often begin to abate within a few days after admission. Serum complement levels return to normal within a month, but microscopic hematuria and cylindruria frequently persist for months, despite the patient's general feeling of well-being.

Prevention

Although penicillin treatment of the antecedent streptococcal infection is highly efficacious in preventing ARF, the same does not appear to be the case in AGN. Stetson and colleagues,[159] in a controlled study of an epidemic of pharyngitis-associated (serotype M-12) AGN in a military population, found a small but not statistically significant preventive effect of penicillin. Uncontrolled observations during an epidemic of nephritis in Israel (both throat and skin infections caused by serotype M-55) documented the occurrence of AGN in a number of subjects who had received prior antibiotic therapy according to various dosage regimens.[172] Moreover, there was no difference in the clinical severity of AGN between subjects who had and those who had not received antibiotic therapy.

As noted, penicillin is nevertheless effective in epidemiologic attempts to eradicate nephritogenic strains by treatment of AGN patients and their colonized family contacts. In appropriate high-risk settings during epidemics of AGN, universal penicillin prophylaxis of selected populations might be considered in a manner somewhat analogous to that used in US military recruit camps for rheumatic fever control. Such universal prophylaxis is rarely indicated and should be used only after careful consideration of the specific epidemiologic parameters involved. Because recurrent episodes of AGN are so rare, continuous antistreptococcal prophylaxis, such as that used in the secondary prevention of rheumatic fever, is unnecessary.

Prognosis

One of the most important issues relating to poststreptococcal glomerulonephritis is the frequency with which patients afflicted with the disease eventually develop chronic glomerulonephritis. In a very small percentage of AGN patients, the acute attack is never resolved and the disease enters a subacute phase, leading to an almost complete loss of renal function within 6 months to 2 years. It is the ultimate fate of the remainder of the patients in whom the illness appears to have resolved clinically that remains controversial. Most observers agree that the long-term prognosis in children is excellent. A 10-year follow-up of 61 patients involved in an epidemic at Red Lake, Minnesota,[173] revealed no cases of chronic glomerulonephritis. Moreover, in a 12- to 17-year follow-up of 534 Trinidadians convalescent from AGN,[174] only 3.5% of the subjects had persistent urine abnormalities, 3.7% were hypertensive, and none had serum creatinine values higher than 1.25 mg/dL. These figures are not in excess of what would be expected in surveys of normal populations. Almost all the Trinidadian patients had been children at the time of their attack of AGN. There was no difference in outcome of sporadic AGN cases as opposed to those associated with epidemics. A report from Australia, however, noted increased prevalences of albuminuria and hematuria in Aboriginal children several years convalescent from two epidemics of AGN. There were no significant differences between patients and control subjects in blood pressure, serum creatinine level, or calculated glomerular filtration rate.[175] However, a recent report indicated that in the aboriginal Australians, chronic nephritis occurred later in life in many who had poststreptococcal AGN in childhood.[176]

Similarly, Baldwin[177] followed 168 subjects for periods up to 18 years and concluded that irreversible renal damage ensued in 50% of these patients, as evidenced by the presence of proteinuria, hypertension, or both, although clinical uremia occurred in only 6 patients. Renal biopsy specimens from the subjects in this series showed that proliferative changes had decreased, whereas glomerulosclerosis of marked degree was present in more than half of the specimens. Their study population contained a high proportion of adults, who are generally agreed to have a worse prognosis than children.[178] Moreover, the results presented have been challenged because of the difficulty of sorting out exacerbations of chronic nephritis from true de novo attacks of AGN in studies of sporadically occurring disease[179] and because of the paucity of published data documenting the poststreptococcal cause of the cases studied. More recently, however, a follow-up of Brazilian adults who contracted AGN from consumption of cheese contaminated with *S. equi* subsp. *zooepidemicus* revealed a high rate of hypertension and renal abnormalities, with some patients having reached end-stage renal disease at 5-year follow-up, though some improvement in glomerular filtration rate was noted by 10 years after the outbreak.[141,180,181]

Based on the bulk of currently available data, it seems likely that greater than 90% of children with AGN make an uneventful recovery and that this disease in children is not an important precursor of chronic glomerulonephritis or hypertension. The prognosis appears more guarded in adult patients,[141,182,183] but the proportion who might be left with residual renal function impairment is at present unknown.

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The complete reference list is available online at Expert Consult.

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199 *Streptococcus pneumoniae*

Edward N. Janoff and Daniel M. Musher

SHORT VIEW SUMMARY

Epidemiology

- *Streptococcus pneumoniae* is the leading bacterial cause of pneumonia and meningitis in children younger than 5 years and older adults worldwide.
- The incidence is highest in children younger than 2 years and adults older than 65 years; mortality is highest in older adults.
- Asymptomatic colonization is common and precedes almost all symptomatic clinical infections.
- Large outbreaks are uncommon, but smaller outbreaks occur under crowded conditions (prisons, nursing homes, military training).
- Groups at increased risk for serious pneumococcal disease include individuals at the extremes of age (particularly <2 years and >65 years of age), those with underlying organ dysfunction (asplenia and splenic dysfunction; chronic heart, lung, liver, and kidney disease), and immunocompromising conditions (particularly antibody defects, complement deficiencies, neutropenia, and malignancies).

Microbiology

- *S. pneumoniae* is a gram-positive, α-hemolytic, lancet-shaped diplococcus. Growth is inhibited by optochin, and colonies are soluble in bile.
- The 97 or more distinct capsular polysaccharides are identifiable by serotyping. The capsules are the principal source of resistance to phagocytosis.
- Effective phagocytosis and killing in vivo typically require antibodies (most often to capsular polysaccharide), complement, and phagocytes (neutrophils and macrophages).
- Pneumolysin is the cholesterol-binding, pore-forming primary toxin that causes both epithelial and endothelial damage and perturbs complement activity.

Diagnosis

- Gram stain and culture of good-quality sputum (>10 neutrophils/epithelial cell) from patients with pneumonia support a presumptive diagnosis of pneumococcal pneumonia. Blood cultures are positive in about 20% of patients with pneumococcal pneumonia, establishing a diagnosis of proven pneumococcal pneumonia.
- Detection of pneumococci by Gram stain and culture of cerebrospinal fluid establishes the diagnosis of pneumococcal meningitis.
- Detection of pneumococcal cell wall polysaccharide in urine (approximately 70% sensitive in adults with bacteremia; not specific in children) or of capsular polysaccharide in urine (sensitive, but limited to a small number of serotypes) is diagnostic of pneumococcal infection.

Clinical Manifestations

- The spectrum of pneumococcal infection ranges from asymptomatic pharyngeal colonization to mucosal disease (otitis media, sinusitis, pneumonia) to invasive disease (bacteria in a normally sterile site: bacteremia, meningitis, empyema, endocarditis, arthritis).
- Otitis media is the most common clinical syndrome in children; acute purulent sinusitis is the most common syndrome in adults, followed by pneumonia.
- Most invasive disease results from bacteremic seeding, but meningitis and empyema may also result from extension of local infection.

Therapy

- β-Lactam antibiotics are the mainstay of therapy for pneumococcal infection. Decreased susceptibility to penicillin, derived from evolved modifications of penicillin-binding proteins, compromises the efficacy of β-lactams in treatment of meningitis, but typically not pneumonia.
- Intravenous therapy, particularly ceftriaxone, is recommended for the initial treatment of serious pneumococcal infection; vancomycin is added initially with meningitis.
- Combined therapy with a β-lactam and macrolide (and addition of corticosteroids) may improve outcomes in severe pneumococcal pneumonia.
- Carbapenems, quinolones, linezolid, and vancomycin also show clinical activity against *S. pneumoniae*.

Prevention

- Two vaccines provide protection against invasive pneumococcal disease. The 13-valent pneumococcal polysaccharide-protein conjugate vaccine (PCV13), recommended for all children, provides them with >90% protection against bacteremia, up to 30% against pneumonia, and some protection against otitis media and meningitis. It also provides adults with 75% protection against bacteremia and 45% against pneumonia caused by vaccine-specific serotypes.
- The 23-valent pneumococcal polysaccharide vaccine for adults (PPSV23) provides 54% to 81% protection against bacteremia, but efficacy is limited for pneumonia. PPSV23 alone is recommended for persons under 65 years with underlying disease. Serial immunization with PCV13, then PPSV23, is approved for adults ≥65 years (given 1 year apart) and immunocompromised adults (given ≥8 weeks apart).
- Widespread pneumococcal vaccination of children has reduced the incidence of invasive disease and hospitalization for pneumonia in all age groups in the United States.

Long recognized for causing asymptomatic colonization and as a prominent cause of pneumonia, bacteremia, meningitis, sinusitis, and otitis media, *Streptococcus pneumoniae* is likely the most common cause of serious bacterial respiratory infection in both children and adults worldwide. Antimicrobials and vaccines have substantially reduced the incidence of, and morbid outcomes from, pneumococcal infection. However, acquisition of antibiotic resistance, the more limited impact of vaccines on mucosal disease (e.g., pneumonia, otitis media), the emergence of nonvaccine serotypes, and a growing immunocompromised population provide challenges for ongoing control of this prevalent and invasive pathogen.

HISTORY

S. pneumoniae has played a prominent role in the history of microbiology. Identified concurrently in 1881 in France by Pasteur and in the United States by Sternberg, this bacterium was soon recognized as the most common cause of lobar pneumonia and became known as the pneumococcus. Based on its appearance in Gram-stained sputum, the name *Diplococcus pneumoniae* was assigned in 1926 and changed to *Streptococcus pneumoniae* in 1974, based on its morphology during growth in liquid medium.

S. pneumoniae was the first organism to be recognized as showing characteristics of a prototypic extracellular bacterial pathogen by replicating extracellularly in mammalian tissues. Resistance to uptake by

phagocytic cells is largely related to the polysaccharide capsule. In the early 1890s, Felix and Georg Klemperer showed that immunization with killed pneumococci protected rabbits against subsequent pneumococcal challenge and that protection could be transferred by infusing serum ("humoral" substance) from immunized rabbits into naïve recipients. The basis for this immunity was shown by Neufeld and Rimpau to be the presence of factor(s) in serum that facilitate ingestion by white blood cells (WBCs), a process they called *opsonization,* derived from the Greek word for buying provisions.[1] These observations provided the basis for what we now call humoral immunity. Individual capsular serotypes were recognized when injection of killed organisms into a rabbit stimulated the production of serum antibody that agglutinated and caused capsular swelling ("Quellung") of the immunizing strain, as well as some, but not all, other pneumococcal isolates. Early in the 20th century, serotypes 1, 2, and 3 were distinguished, and all other pneumococci were called group 4.

In the first decade of the 20th century, the concept of humoral immunity was used to address the problem of epidemic lobar pneumonia that each year affected as many as 1 in 10 African miners.[1,2] A program of immunization with whole killed pneumococci substantially reduced the incidence of pneumonia. In the 1920s, Heidelberger and Avery[3] demonstrated that the protective antibody reacted with surface capsular polysaccharides, the first nonprotein antigen identified. Felton[4] prepared the first purified pneumococcal capsular polysaccharides (PPSs) for immunization of human subjects, and mass vaccination with Felton's type 1 polysaccharide aborted an epidemic of pneumonia at a state hospital in Massachusetts in the winter of 1937 to 1938.[5] Thus specific bacterial polysaccharide antigens could be used to stimulate antibodies that conferred protection against epidemic human infection. These observations were confirmed during World War II when MacLeod and coworkers[6] showed that vaccinating military recruits with capsular material from four serotypes of *S. pneumoniae* greatly reduced the incidence of pneumonia caused by serotypes in the vaccine but not by other serotypes.

S. pneumoniae also played a central role in the discovery of DNA. Experiments by Griffith[7] in the 1920s revealed that intraperitoneal injection into mice of live, unencapsulated (and therefore avirulent) pneumococci, together with heat-killed encapsulated pneumococci, led to the emergence of encapsulated virulent bacteria, a process he called *transformation.* This observation remained unexplained until the 1940s, when Avery and coworkers[8] provided conclusive evidence that these mutants had recovered the capacity to produce a capsule by taking up nucleic acids from killed, virulent organisms. Therefore this discovery was the first to identify a functional transferable material (DNA) that encoded for phenotype.

Finally, pneumococcal infections were among the first to be treated with an antimicrobial agent, in this case optochin (ethylhydrocupreine), a quinine derivative. The organism was also among the first to develop resistance to such therapy, resulting in failure of treatment both in experimental animals and in humans.

MICROBIOLOGY

S. pneumoniae is a gram-positive coccus that replicates in chains in liquid medium but appears as lancet-shaped diplococci in clinical specimens. The organism is catalase negative, but generates hydrogen peroxide (H_2O_2) via a flavoenzyme system and therefore grows better in the presence of a source of catalase, such as red blood cells. Pneumococci produce pneumolysin (formerly called α-hemolysin), which breaks down hemoglobin into a green pigment that surrounds the colonies during growth on blood and chocolate agar plates, a phenomenon still termed *α-hemolysis.* Pneumococci may be identified in the microbiology laboratory by three reactions: (1) α-hemolysis of blood agar, (2) susceptibility to optochin, and (3) solubility of colonies in bile salts (sodium deoxycholate). Some pneumococci are optochin resistant. A related species, *Streptococcus pseudopneumoniae,* which is associated with exacerbation of chronic obstructive pulmonary disease or pneumonia, is optochin-susceptible during growth in room air at 37°C but optochin-resistant when grown in the presence of increased carbon dioxide. These factors have led to greater reliance on the use of bile solubility and commercial DNA probes for the ribosomal RNA (rRNA) gene for definitive identification.

Peptidoglycan and teichoic acid are the principal constituents of the pneumococcal cell wall[9] (Fig. 199.1). Peptidoglycan consists of long chains of alternating *N*-acetyl-D-glucosamine and *N*-acetylmuramic acid, from which extend chains of four to six amino acids called stem peptides. Stem peptides are cross-linked by pentaglycine bridges, which provide substantial strength to the cell wall. Teichoic acid, a carbohydrate polymer that contains phosphorylcholine, is covalently linked to the peptidoglycan on the outer surface of the bacterial wall and protrudes into the capsule. Teichoic acid and tightly adherent fragments of peptidoglycan make up C-polysaccharide, a substance present in all pneumococci but only in a few species of viridans-group streptococci. C-polysaccharide reacts with acute-phase reactants in the blood during inflammation, including C-reactive proteins. Many proteins are expressed on the pneumococcal cell surface. Of particular importance in the pathogenesis of pneumococcal disease are those that bind to choline, including pneumococcal surface proteins A and C (PspA, PspC); surface adhesins choline-binding protein A (CbpA) and choline-binding protein C; and proteins involved in receptivity to DNA acquisition (or natural competence) (Table 199.1).

Nearly every clinical isolate of *S. pneumoniae* contains an external polysaccharide capsule, but unencapsulated isolates have been implicated in outbreaks of conjunctivitis.[10] Capsules (see Fig. 199.1) of repeating oligosaccharides are synthesized in the cytoplasm, polymerized, and transported to the bacterial surface by cell membrane transferases.[11] These polysaccharides are covalently bound to peptidoglycan and C-polysaccharide, which explains the difficulty of separating capsular from cell wall polysaccharide in vaccine preparations. Genetic control of this complex set of events has been elucidated for some serotypes; for example, a cassette of 15 genes that function as a single transcriptional unit is responsible for encapsulation in serogroup 19.[12] At least 97 serotypes of *S. pneumoniae* have been identified on the basis of antigenic differences in their capsular polysaccharides. Among the multiple genes that encode production of individual capsules, some are specific for individual polysaccharides, whereas others are conserved among nearly all pneumococci and even some other streptococci.[13]

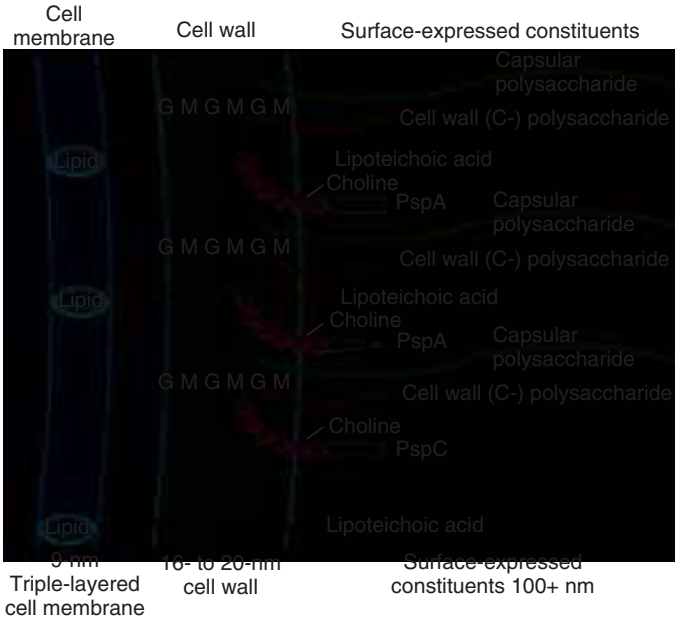

FIG. 199.1 Anatomy of the pneumococcus. A representation of the cell membrane, cell wall, and capsule of *Streptococcus pneumoniae.* Within the cell wall, M = *N*-acetylmuramic acid and G = *N*-acetyl-D-glucosamine. The stem peptides and the cross-linked pentaglycine bridges that extend from the long M-G-M-G chains are not shown. Cell wall (C-) polysaccharide consists of teichoic acid with peptidoglycan and phosphorylcholine (not shown). F antigen is the lipid/teichoic acid moiety in the cell membrane that extends into the cell wall. *PspA,* Pneumococcal surface protein A; *PspC,* pneumococcal surface protein C.

TABLE 199.1 Role of Pneumococcal Constituents as Virulence Factors

PNEUMOCOCCAL CONSTITUENT	MECHANISM	STRENGTH OF EVIDENCE AS A VIRULENCE FACTOR	
		Antibody Prevents Disease[b]	Mutants Lack Virulence
Capsular polysaccharide	Prevents phagocytosis; activates complement	4+	4+
Cell wall polysaccharide	Stimulates inflammation by strongly activating complement and stimulating release of cytokines	0	ND
Pneumolysin	Cytotoxic; activates complement, cytokines	2–3+	2–3+
PspA	Inhibits phagocytosis by blocking activation and deposition of complement on bacterial surface	2+	2+
PspC	Inhibits phagocytosis by binding complement factor H	1–2+	1–2+
PsaA	Mediates adherence	1–2+	1–2+
Autolysin	Causes bacterial disintegration, releases components	1+	2+
Neuraminidase	Possibly supports adherence	0–1+	0–1+

[a]The grading system is subjective and indicates (on a scale of 1+ to 4+) the stringency and importance of the demonstrated effect. For discussion and references, see the text and Weiser et al.[69]
[b]Animal models only, except capsular polysaccharides.
ND, Not done; *Psa,* pneumococcal surface adhesin; *Psp,* pneumococcal surface protein.

Antibodies induced in rabbits immunized with specific capsular types cause agglutination and create a hydrophobic border around the capsule. This latter reaction, called the Quellung reaction, renders the capsule refractile and therefore more readily visible under the microscope. Because serum antibody is the basis for identifying these types of pneumococcus, they are called serotypes. The American system numbers the serotypes sequentially in the order in which they were identified historically. The more widely accepted Danish numbering system distinguishes 46 serogroups, with groups containing antigenically related serotypes. For example, Danish serogroup 19 includes serotypes 19F, 19A, 19B, and 19C (the letter F indicates the first member of the group to be identified, followed by A, B, C, etc.), which in the American system would be serotypes 19, 57, 58, and 59, respectively. The serotypes that most frequently caused human disease were the earliest to be identified and the first to be assigned numbers, which explains why the lower-numbered serotypes are generally more likely to be implicated in human infection. In the 1930s, serotyping was used to direct therapy with capsule-specific horse antisera. Today, serotypes are important to define epidemiologic and public health surveillance, as targets for vaccines, and for understanding pathogenesis, but not for therapy.

Pneumococci express a competence-sensing protein and internalize DNA from other pneumococci or from other bacterial species.[14] This horizontal transfer of genetic information to pneumococci, called transformation, enables pneumococci to acquire new traits. Of note, a pneumococcus of one serotype can acquire DNA that encodes a different capsular polysaccharide, thereby changing its serotype. This exchange of genetic information occurs under experimental conditions as well as in nature.[15] Thus in addition to a highly conserved genetic core, *S. pneumoniae* supports a large number of noncore genes that provide remarkable diversity of genetic loci between isolates, particularly related to antimicrobial targets and targets of immune recognition (capsular polysaccharides and adhesins).[11,16,17]

EPIDEMIOLOGY

The spectrum of pneumococcal infections can range from asymptomatic colonization to mucosal disease (otitis media, sinusitis, pneumonia) to invasive infections (infection of previously sterile sites). Although otitis media may be the most common clinical manifestation, pneumococcal pneumonia has the greatest impact on morbidity and mortality. Indeed, pneumonia is the leading cause of death of children from infection worldwide, accounting for 1 in 5 deaths, and *S. pneumoniae* is the leading cause of bacterial childhood pneumonia, particularly severe pneumonia.[18] Pneumococcal pneumonia, sepsis, and meningitis cause more deaths in children younger than 5 years than acquired immunodeficiency syndrome [AIDS], malaria, and measles combined,[19] particularly in resource-limited countries. *S. pneumoniae* is the leading

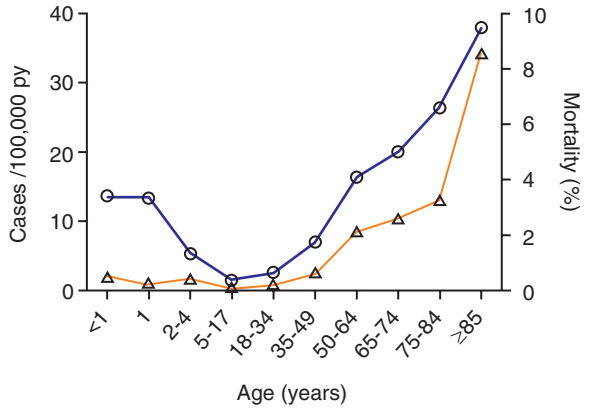

FIG. 199.2 Age-specific incidence and mortality with invasive *S. pneumoniae*, United States, 2016. The incidence of invasive pneumococcal infection has declined substantially since the introduction of pneumococcal conjugate vaccine in 2000, particularly in young children, such that the incidence is now highest in adults older than 65 years, exceeding that in children younger than 2 years.[3,12] However, the bimodal incidence pattern at the extremes of age is consistent with results since the 1920s.[1,18] Mortality is low in young children in the United States but highest in older adults, particularly those with underlying diseases. Blue line with circle represents cases per 100,000 person-years, and orange line with triangle represents mortality (%). *py,* Patient-years. *(Modified from Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Network,* Streptococcus pneumoniae*; 2016.* http://www.cdc.gov/abcs/reportsfindings/survreports/spneu16.pdf.*)*

identified cause of bacterial pneumonia in adults in Kenya[20] and in adults in the United States.[21]

Invasive pneumococcal infections are most prominent at the extremes of life (Fig. 199.2).[22] Consistent results in multiple ethnic and geographic groups highlight the tremendous impact of age on the incidence of bacteremia.[1,18,23] In the pre–conjugate vaccine era (before 2000), pneumococcal bacteremia occurred at an approximately 10-fold higher rate among children younger than 2 years than among adults in the general population and in all populations studied, independent of ethnicity (e.g., White Mountain Apaches) or underlying disease (sickle cell disease, splenectomy, human immunodeficiency virus [HIV] infection).[24] These results are likely due to the limited ability of children under 2 years of age to generate protective antibodies to capsular polysaccharides. Infants in the first few months of life tend to be relatively spared in association with the passive transfer of capsule-specific mucosal immunoglobulin A (IgA) and innate factors to the upper respiratory tract by breast milk and specific immunoglobulin G (IgG) to serum transplacentally through

cord blood, levels of which decline by 6 months of age. Implementation of pneumococcal vaccination at 2, 4, and 6 months of age for infants in the United States (7-valent pneumococcal polysaccharide-protein conjugate vaccine [PCV7], then 13-valent pneumococcal polysaccharide-protein conjugate vaccine [PCV13] in 2010) has reduced invasive pneumococcal disease by over 90% in young children and by half in older adults.

Among children 6 months to 2 years of age, invasive pneumococcal disease is diagnosed primarily when blood cultures are obtained to evaluate for fever. Many of the affected children with "primary bacteremia" have no apparent focus of infection and are not hospitalized, and one-third resolve spontaneously. Hospitalization is more common with associated underlying cardiac, respiratory, and neurologic disease. Unlike in adults, among whom bacteremia is most often a complication of pneumonia (>80%), pneumonia in young children accounts for 28% to 77% of pneumococcal bacteremias in developing countries and 13% to 60% in more industrialized countries.[25] Primary bacteremia accounts for 61% to 70% of invasive pneumococcal disease in infants in the United States but is uncommonly diagnosed in developing countries. Whether the relatively low levels of serum antibodies to capsular polysaccharides or other protein antigens, or innate factors, in healthy adults underlie their relatively low incidence of pneumococcal disease is not well understood.[26,27]

Adults older than 65 years comprise about 15% of the population but experience one-third of all cases of invasive pneumococcal disease (approximately 15,000 episodes/yr, with ≥15% mortality). Hospitalization for pneumonia increases from 1.5% to 3.9% per year from age 65 to over 85, particularly among those with diabetes and organ dysfunction.[28] Most invasive cases result from complications of pneumonia (70% to >80%), but 5 to 10 times as many older adults experience pneumococcal pneumonia without bacteremia. In a recent prospective observational study, 8.8% of confirmed cases among adults over 65 years of age hospitalized with serotype-defined pneumococcal pneumonia were complicated by bacteremia,[29] as were 14% to 30% of such cases in a large prospective vaccine trial.[30] Thus the overall mortality associated with *S. pneumoniae* is likely much greater in the population than the numbers for invasive disease predict.[31] Mortality also increases substantially with age (see Fig. 199.2) and is more than two- to fivefold greater among adults with underlying disease (advanced lung, heart, kidney, or liver disease; diabetes; asplenia; solid and hematologic malignancies; immunosuppression) than in healthier older adults.[32]

Deaths from pneumococcal bacteremia tend to occur quickly, often in the first day to week[31,33,34] of hospitalization. Despite advances in antimicrobial therapy, the 5% to 10% early mortality with pneumococcal bacteremia has remained constant over the last century (Fig. 199.3). Although chronologic age itself is a factor, most pneumococcal disease and mortality in older adults occur in subjects with diabetes, underlying organ dysfunction (e.g., liver, kidney, heart, lung), humoral immune defects (hypogammaglobulinemia, chronic lymphocytic leukemia, multiple myeloma), and use of immunosuppressive medication (Fig. 199.4).[35–37] These data suggest an independent or additive contribution of underlying disease to the increased risk of serious infection with age.

Pneumococcal disease is not generally highly contagious. Pneumococci are transmitted from one person to another as a result of close contact,[38] such as among toddlers in daycare centers,[39,40] but many steps intervene between spread of organisms, colonization, and development of disease.[38] However, community-wide epidemics are well recognized in the meningitis belt in West Africa, where capsular serotype 1 predominates,[41] and smaller outbreaks of disease, often caused by capsular serotypes 1, 5, and 12F,[42,43] occur among adults in crowded living conditions, such as in military camps,[44] prisons,[45] shelters for the homeless,[46] and nursing homes.[47] In contrast, close contact in schools or the workplace is generally not associated with outbreaks of pneumococcal disease.[38,48] A very high incidence of invasive infections occurs at all ages among African Americans,[49] Native Americans,[50,51] and Alaska Natives[52] and

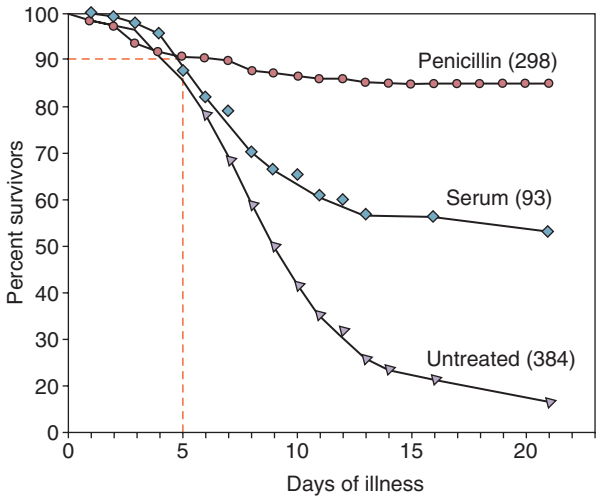

FIG. 199.3 Effect of therapy on survival with pneumococcal bacteremia in adults. Despite dramatic improvement in survival with immune horse serum against pneumococcal serotypes 1 and 2 in the 1920s, and then by penicillin and now multiple antibiotics and advanced supportive care, the 5% to 10% mortality of patients with pneumococcal bacteremia at 72 hours and 10% to 15% at 7 days has remained relatively constant.[33,362]

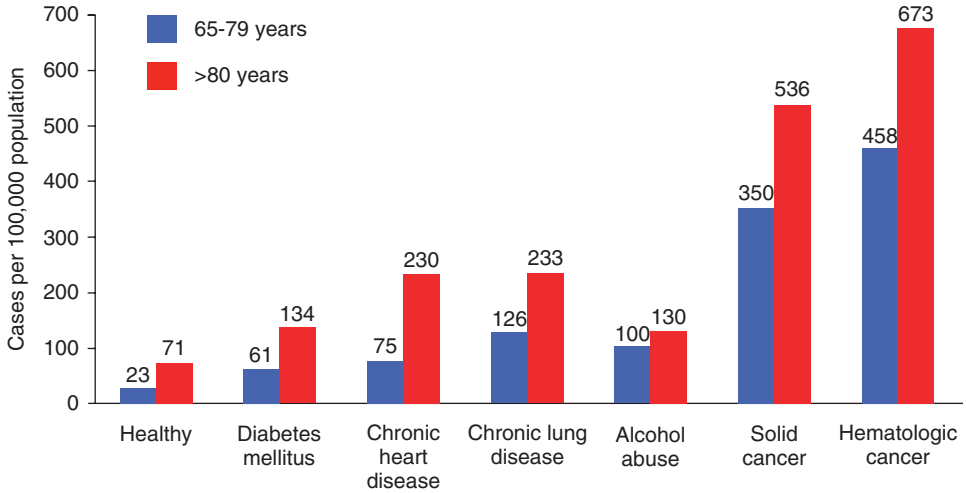

FIG. 199.4 Incidence of invasive pneumococcal disease in older adults by age group and illness. *(Modified from Kyaw MH, Rose CE Jr, Fry AM, et al. The influence of chronic illnesses on the incidence of invasive pneumococcal disease in adults.* J Infect Dis. *2005;192:377–386.)*

Australian Aboriginals,[53] presumably related to both socioeconomic genetic factors. Infants from these populations and other disadvantaged members of developed societies[54] are more likely to be colonized with high numbers of pneumococci, even within the first few weeks of life, adding exposure to their risk for disease.

Pneumococcal colonization and disease occur with a seasonal pattern, with a midwinter increase, although pneumococci can be recovered from healthy children and adults throughout the year. The incidence of pneumococcal otitis media clusters largely from November to April.[55,56] Bacteremia[57,58] shows a clear midwinter peak in temperate climates (Fig. 199.5). The association may relate in part to the colder temperatures and lower humidity, which appear to predispose to transmission of respiratory viruses such as influenza,[57–59] and perhaps closer contact indoors. However, in Houston, Texas, invasive disease in children coincides with the school year, from September through May, sparing the summer months and with no clear midwinter peak.[58] The seasonal distribution is less prominent in tropical climates.

PATHOGENETIC MECHANISMS

Colonization

The prevalence of pneumococcal colonization attests to the success of this organism in adapting to adherence and survival in the nasopharynx. The vast majority of colonizing episodes remain asymptomatic, but most symptomatic infections are likely initiated after asymptomatic colonization.[56,60] Nasopharyngeal colonization with pneumococci begins in the first weeks of life. The prevalence of colonization increases from less than 10% over the first several months of life to a peak at 70% to 100% at age 1 year, persists through the second and third year of life, and decreases thereafter to adult rates under 5%. Defining such rates depends on the sampling location, methods, and culture,[61] and, more recently, high-throughput sequencing of the 16S rRNA gene[62] and detection of the *lytA* gene by polymerase chain reaction (PCR). Duration of carriage can range from 1 week to 6 months. In adults, an individual serotype persists for shorter periods, usually 2 to 4 weeks,[63] but sometimes for several months.[1]

Living in resource-limited countries and the presence of siblings in the home are consistent risks for colonization.[54,64] Crowding (e.g., in home, daycare, barracks),[65] lower socioeconomic status, and ethnic background, as mentioned earlier, have been associated with high carriage rates, as have smoke exposure (passive or active smoking[45] and cooking fires in the home), antibiotic use (with associated risk of carriage of resistant organisms), and respiratory viral infections. Colonization is seasonal, peaking in the winter months but present in children year-round. Colonizing organisms can be transmitted from person to person by an aerosol route from coughing, with particles in the 1- to 5-μm size range depositing primarily in the upper respiratory tract[66] or acquired from saliva and shared drinking vessels.[67]

The frequency of pneumococcal colonization can be influenced by mucosal viral infections and the resident polymicrobial microbiome of co-colonizing organisms, which include other streptococci and *Neisseria* species, as well as *Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus*, and other organisms. These bacteria interact and can compete for nutrients and binding sites, produce inhibitory molecules, and modify both local innate and specific immune responses.[64,68] Bacterial components can have specific interactions with host mucosal constituents.[64,69] A more diverse nasopharyngeal microbiome was associated with an increased likelihood of colonization in subjects experimentally challenged nasopharyngeally with pneumococcus.[70] Pneumococcal neuraminidases A and B may modify neighboring bacteria and cleave sialic acid on host mucins and glycopeptides to facilitate exposure of *N*-acetylglucosamine receptors on resting epithelial cells that bind pneumococcal surface-associated proteins, such as pneumococcal surface adhesion protein A. Local inflammation induced by pneumolysin, rhinovirus or influenza infections, and conditions that elicit proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1, facilitate pneumococcal binding, uptake, and likely migration of the bacteria across the epithelium and endothelium.[68] Such inflammation elicits upregulation of epithelial platelet-activating factor receptor that binds bacterial cell wall phosphorylcholine (ChoP), which intercalates through the capsule. Moreover, sialic acid, lacto-*N*-neotetraose, and polymeric immunoglobulin receptors on activated epithelial cells bind surface-expressed CbpA to advance transcytosis.[69]

Other innate host macrophage receptors, including scavenger receptor A, mannose receptor, and particularly, macrophage receptor with collagenous structure (MARCO), have been proposed in murine models to enhance mucosal clearance of *S. pneumoniae*. The role of MARCO may be to enhance interactions between the organisms and other innate receptors, such as Toll-like receptor 2 and nucleotide oligomerization domain protein 2, to promote cytokine and chemokine production.[71] In addition to its cytotoxic and complement-modifying activities, pneumolysin, largely conserved among strains, appears to engage Toll-like receptor 4, and, in conjunction with the cell wall, to limit mortality after mucosal challenge.[72] Finally, specific host IgA bound to the pneumococcal capsule, and potentially to other surface proteins, can be cleaved by bacterial IgA1 protease. The bound but cleaved IgA modifies the surface charge and can enhance binding of encapsulated pneumococci that otherwise bind less well to the epithelium.[73] Pneumococci themselves may also respond to local environmental conditions by increasing expression of ChoP and CbpA to enhance adherence to mammalian cells.[69] Lower expression of capsule may expose epithelial-binding cell

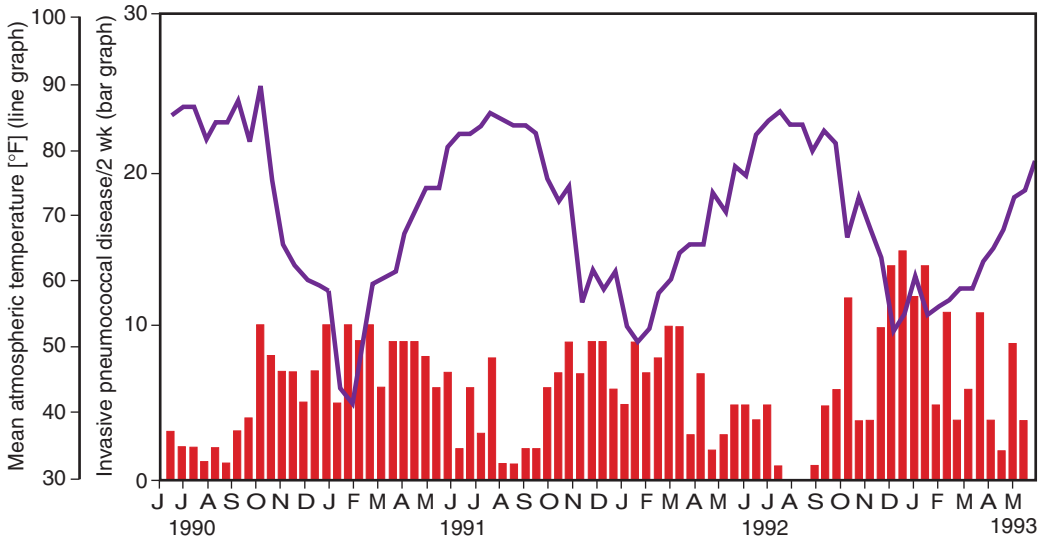

FIG. 199.5 Seasonal distribution of invasive pneumococcal disease. The bar graph indicates the total cases of bacteremic pneumococcal pneumonia and pneumococcal meningitis during each 2-week period over a period of 3 years at four tertiary care hospitals (adult and pediatric) in Houston, Texas. The line graph shows mean ambient temperature, indicating its association with the prevalence of invasive pneumococcal disease.[363]

wall–bound ligands, but higher capsule expression may facilitate bacterial escape from mucin binding and shedding.[21] Thus *S. pneumoniae* adapt to and interact with their mucosal milieu to effect colonization, but the presence of inflammation may promote the conversion from asymptomatic colonization to local and systemic disease. When symptomatic infections do accompany colonization, they typically occur within a few weeks of acquiring a new strain rather than from chronic carriage, because antibody production generally follows soon after colonization in children[56] or in adults.[74]

Pneumococcal Capsular Serotypes and Progression From Colonization to Disease

The serotypes of colonizing pneumococci can vary by age and geography and are predictive of the incidence, syndrome, and outcome of pneumococcal infections. Only a relatively small number of the more than 92 capsular serotypes typically cause serious disease, particularly among unvaccinated children. Differences among these capsular types account for approximately 60-fold differences in the invasiveness of the organism.[75–78] Indeed, capsular serotype appears to be a primary determinant of which strains are likely to remain as colonizers (e.g., 3, 6A, 6B, 9N, 19F, and 23F), which are more likely to progress from carriage to cause more invasive disease (e.g., 1, 5, 7F, 8, 14, 18C, 33F, and 38), and, among those causing invasive disease, which are more likely to cause fatal infections.[64,76,79–82] Based on a process called phase variation, organisms that express less capsule in vitro are more likely to colonize, whereas those producing more capsule show greater resistance to phagocytosis.[83] The clinical distinctions among organisms are associated with their interaction with the host. Colonizing strains are proposed to resist nonopsonic phagocytosis but to be cleared by alveolar macrophages and by complement in blood and, perhaps, lung, thereby resulting in a lower incidence of invasive disease.[84] In contrast, invasive serotypes are less common colonizers that, if not cleared by neutrophils, show increased adhesion-mediated binding to and translocation across the epithelium and are more resistant to killing by alveolar macrophages and complement. However, when colonizing strains do cause invasive disease (e.g., serotypes 3, 6A, 6B, 9N, 19F, and 23F), mortality is higher than with infection with the usual invasive strains, perhaps related to decreased host resistance.

Although the serotype distribution varies by age, these differences are likely not sufficient to explain the increases in mortality (1) from infancy to adulthood, (2) from age 65 to older than 80 years, or (3) in the presence of underlying disease among adults older than 65 years.[35] Disease in immunocompromised adults is more often caused by pediatric serotypes.[85] Thus the interaction between microbial factors, particularly expression of capsular serotype,[81] and the integrity of anatomic and immune host defense[35,82] determine the development of disease and mortality, but serotype appears to be the primary determinant of outcome in young healthy adults.

These morbid outcomes highlight the importance of understanding, potentially preventing, or at least successfully managing pneumococcal colonization and thereby its consequences. In this context, use of the polysaccharide-protein conjugate vaccines over the past 18 years has been associated with decreased rates of colonization in immunized children.[86,87] This decrement in childhood colonization and disease has been accompanied by a progressive reduction in adult invasive disease with the pediatric vaccine serotypes.[88] Colonization itself may serve as an immunizing event, and antibodies to pneumococcal neuraminidase A, PspA, and capsular polysaccharides have been detected in serum after carriage.[64,69,74,89] These antibodies, as well as antibody-independent $CD4^+$ T-cell–mediated responses,[90] perhaps involving Th17 $CD4^+$ T cells,[84,91,92] may also mediate protection against subsequent colonization and the development of disease and provide alternative approaches to immunization.

Immunologic Mechanisms of Defense Against and Susceptibility to *S. pneumoniae* Infection

After successful initial colonization, a range of immunologic mechanisms in the host contribute to protection against disease, and a panoply of bacterial factors conspires to evade these defenses. Critical to the process of defense against disease are the interplay among innate immune factors, including complement, antibodies to the bacteria, and the activity of phagocytes. Each is required and none is sufficient alone to clear this invasive mucosal pathogen. The development of pneumococcal disease among patients with specific congenital or acquired immune defects reveals the role of these factors in defense against serious pneumococcal infections.

Antibodies are essential for defense against pneumococcal disease. Antibodies to capsular polysaccharides, the primary virulence factor,[75–78] are implicated in protection against disease. In support of their protective role, (1) anticapsular antibodies appear in the bloodstream 5 to 8 days after the onset of infection, when fever spontaneously resolves in the absence of treatment; (2) administration of immune horse serum with capsule type-specific antibody in the preantibiotic era was moderately effective in treating pneumococcal pneumonia (see Fig. 199.3)[33]; (3) capsular polysaccharide vaccines elicit both specific antibody and protection against invasive infection; and (4) capsule-specific antibodies support dose-dependent uptake and killing of pneumococci in vitro[93–96] and protection of experimental animals after pneumococcal challenge in vivo.[97] Failure to produce capsule-specific antibodies in young children and compromised adults results in increased rates of invasive disease.[37,98–101] Similarly, Bruton (X-linked) agammaglobulinemia in children and common variable immunodeficiency in adults (all deficient in immunoglobulin M [IgM], IgG, and IgA)[21,102] predispose to serious pneumococcal infection, as may deficiencies of IgG2 subclass with selective IgA deficiency[103] and selective defects in responses to polysaccharide antigens.[104,105]

Other than indirect evidence of an association between antibody to pneumolysin[106] or PspA,[107] few data in humans support a protective role for antibody to antigens other than capsular polysaccharides. Maternally derived antibodies to a range of pneumococcal proteins were associated with either an increase or a decrease in infant colonization.[108] Epidemiologic evidence suggests that antibody-independent immune mechanisms provide protection in the population.[109] Indeed, in the preantibiotic era, a proportion of patients recovered from pneumococcal pneumonia without producing measurable amounts of anticapsular antibody. Moreover, young children with documented primary bacteremia and only mild symptoms can clear the infection at a time when their ability to make anticapsular antibodies is very limited. Thus antibody-independent mechanisms, including the effects of $CD4^+$ Th17 T cells during colonization, may also contribute to defense against this pathogen.

Antibodies defend against infection by binding through their variable Fab regions to bacterial surface components, including polysaccharide capsules and proteins. Upon binding, the effector constant Fc region of the antibody engages Fc receptors on phagocytic cells, such as neutrophils and macrophages, thereby providing a bridge between the phagocyte and the organism. Supporting antibody-dependent phagocytosis and killing of the organism, complement also binds to the surface of *S. pneumoniae* and to complement receptors on the phagocyte. Complement activation by each of the three activation pathways (classical, lectin, and alternative) and deposition on the surface of pneumococci (particularly complement protein C3b) occurs by both antibody-dependent and antibody-independent mechanisms and is largely required for effective uptake and killing of the organism.[110] In general, all three elements are required for clearance of pneumococci: complement, phagocytes, and antibodies; no two alone are sufficient.

Capsule-specific antibodies are proposed to derive from selected subsets of B cells.[111] The ability of polysaccharides to bind and cross link surface antibodies may directly activate naïve and IgM memory B cells, initiating an IgM response. Thus pneumococcal polysaccharides are considered "T-independent" antigens. Decreases in these B-cell subsets are associated with advanced HIV disease[112] and limited IgM responses to pneumococcal vaccine. Impaired vaccine responses and a high incidence of invasive pneumococcal disease characterize patients with common variable immunodeficiency.[113] A decreased frequency of response to pneumococcal vaccine among patients with splenectomy may be related to immunosuppression and underlying malignancy.[114] Very limited IgM responses to pneumococcal vaccine are common with HIV infection[115] and in elderly and, particularly, frail adults.[116]

Although considered T-independent antigens, pneumococcal polysaccharides may also engage and depend upon $CD4^+$ T cells to provide help. Activated T cells can engage B cells directly as well as secrete cytokines to enhance these early responses and to support the switch from production of IgM to IgG and IgA. The mechanisms by which polysaccharides stimulate T cells are controversial. The presence of proteins, particularly those linked directly and covalently to the polysaccharide on the organism or with conjugate vaccines, appears to be required to help B cells produce antibodies in sufficient amounts and of sufficient quality to control these infections. Such enhancement may derive from binding of protein-linked polysaccharides to polysaccharide-specific B cells by their surface antibody. Uptake and processing of the linked protein by these B cells provides protein-derived peptides bound to major histocompatibility complex (MHC) class II molecules on the B-cell surface. Activation of $CD4^+$ helper T cells by these MHC-bound peptides on the B cell may then support the development of these polysaccharide-specific B cells to produce antibodies that bind avidly to the capsular polysaccharides.[117,118] In this scenario, the processed protein stimulates the T cells. Alternatively, the linked protein may serve to anchor the polysaccharide to MHC class II molecules on the B-cell surface, allowing the polysaccharide itself to stimulate the T cell and enhance antibody production by that B cell.[119] The key to understanding the limitations of antibody responses to capsular polysaccharides and to enhancing these responses with vaccines lies in characterizing and exploiting these protein-polysaccharide interactions. Indeed, the introduction of vaccines with proteins covalently conjugated to polysaccharides has revolutionized vaccine development and efficacy against *S. pneumoniae* and other encapsulated pathogens by generating capsule-specific antibodies to opsonize and kill the organisms.[120,121]

The spleen is the principal reticuloendothelial organ that clears unopsonized pneumococci from the bloodstream.[122,123] In humans, highly opsonized particles are removed from the circulation in part by the liver. However, particularly with limited opsonization, the spleen assumes the most prominent role.[124] Presumably, the slow passage of blood through the spleen and prolonged contact time with reticuloendothelial cells in the cords of Billroth and the splenic sinuses allow the relatively less efficient removal of nonopsonized particles through natural immune mechanisms.[125] Overwhelming pneumococcal infection occurs in children and adults from whom the spleen has been removed or in whom it does not function normally, with an incidence 5- to 15-fold greater than that in other adults and greater yet in children.[37,122,126] The herald event in an outbreak of pneumococcal pneumonia in a metropolitan prison was the rapid, septic death of two prisoners who had undergone splenectomy.[45] Pneumococcal disease progressed so rapidly in these cases that pneumonia was not initially detectable clinically or even with certainty by chest radiographs before autopsy. The 35- to 100-fold increase in the incidence of pneumococcal bacteremia or meningitis in children with sickle cell disease is probably due to splenic dysfunction, although other factors, such as antibody and complement abnormalities, may also contribute.[123,127] Complications of these high-grade infections can include adrenal insufficiency with Waterhouse-Friderichsen syndrome and peripheral symmetrical gangrene with necrosis of multiple digits and limbs.

FACTORS THAT PREDISPOSE TO PNEUMOCOCCAL INFECTION

S. pneumoniae is a prototypic extracellular bacterial pathogen. Host defenses against infection rely, as noted earlier, on the interaction between antibody, complement, and phagocytic cells, specifically neutrophils. Both primary (or congenital) and secondary clinical conditions and underlying mechanisms may hamper the immunologic capacity of the host and predispose to pneumococcal infection (Table 199.2). Although these risks include defects in anatomy, antibody production, complement, and phagocytes (typically low neutrophil number rather than impaired function), cell-mediated abnormalities in T and natural killer cells do not figure prominently among them. These predisposing conditions do, however, include underlying liver, kidney, heart, and lung dysfunction; diabetes; alcoholism; and malignancies, particularly in older adults, which may invoke a more subtle constellation of predisposing risks.

TABLE 199.2 Conditions That Predispose to an Increased Incidence and/or Severity of Pneumococcal Infection

ABNORMALITY	PRIMARY	SECONDARY
Anatomic	Congenital CSF leak	Poor eustachian tube drainage Traumatic CSF leak Cochlear implants COPD Asthma Preceding viral/influenza infection
Antibody defects	Congenital agammaglobulinemia Common variable immunodeficiency IgG2 subclass deficiency (± selective low IgA) Selective hyporesponsiveness to polysaccharides Hyper-IgM syndrome Hyper-IgE syndrome	CLL Multiple myeloma HIV infection
Low complement	Classical pathway (low C2, C1, C4) Alternative pathway (low factors I, H, and B) Low C3 (all pathways) MBL deficiency and polymorphisms	Nephrotic syndrome Complement consumption
Neutropenia	Cyclic neutropenia	Drug-induced neutropenia Aplastic anemia
Neutrophil dysfunction	Fcγ receptor IIa (*R131* allele) (low avidity for IgG2) Chédiak-Higashi syndrome	Diabetes
Reticuloendothelial cell defects	Congenital asplenia Hyposplenia	Splenectomy Sickle cell disease Portal hypertension
Combined/other	IRAK4 deficiency (decreased cytokines)	Extremes of age HIV/AIDS Sickle cell disease (spleen, antibody, ± complement) Chronic organ dysfunction (lung, liver, kidney, heart) Chronic alcohol use Solid-organ and bone marrow transplantation Lymphoma
Environmental		Environmental smoke Smoking (tobacco) Crowding (daycare, homeless shelters, prison, military training) Stress (military training) Cold season

C, Complement component; *CLL,* chronic lymphocytic leukemia; *COPD,* chronic obstructive pulmonary disease; *CSF,* cerebrospinal fluid; *HIV/AIDS,* human immunodeficiency virus/acquired immunodeficiency syndrome; *Ig,* immunoglobulin; *IRAK4,* interleukin-1 receptor-associated kinase 4; *MBL,* mannose-binding lectin.

In addition to the potential defects in antibody production considered previously, the heat-labile complement components of humoral immunity are essential for defense. Of the many possible defects in complement, only those factors required to generate C3b for binding to the bacterial surface and inactivated C3b (iC3b) for phagocytosis and killing by phagocytes are associated with pneumococcal infection.[110] Because the pore-like membrane attack complex (MAC; complement component C6, C7, C8, or C9) that lyses gram-negative organisms cannot pierce the thick pneumococcal cell wall, pneumococci are not killed by serum alone, and defects in these components do not predispose to these infections. In contrast, genetic deficiencies in complement component C3, essential for the activity of each of the three complement activation

pathways (classical, lectin, and alternative) are associated with recurrent pneumococcal infection.[110,128] Deficiencies in complement components C1, C4, and most commonly C2, whether congenital or acquired, are expected to increase susceptibility to pneumococcal infection,[129] although they do so only rarely. The absence of mannose-binding protein in serum, which triggers the lectin complement pathway, may be associated with susceptibility to pneumococcal bacteremia.[130,131] However, in addition to host defects in complement production, *S. pneumoniae* expresses a range of proteins that promote degradation of C3 (PhpA) and interfere with deposition of opsonins (PspA, pneumolysin), with activation and generation of convertase (pneumolysin, factor H–binding inhibitor of complement [Hic], and PspC) and with ligands for complement receptors on phagocytes (Hic, PspA/PspC, CbpA, and pneumolysin). These data highlight the importance of the complement system in defense of the host and survival of the bacteria.[110]

Neutropenia of whatever cause is associated with *S. pneumoniae* infection, although functional leukocyte defects, such as leukocyte adhesion deficiency syndrome (MAC-1 deficiency) generally are not,[101,132] due to the redundancy of killing pathways in neutrophils. Similarly, defective bacterial killing by polymorphonuclear leukocytes (PMNs), as seen in chronic granulomatous disease, does not predispose to infection with *S. pneumoniae*; the absence of catalase renders this organism susceptible to the interaction between its endogenous H_2O_2 and myeloperoxidase and the halide present in PMNs. At the time of initial hospitalization for acute leukemia, patients are more likely to have infection caused by other gram-positive pathogenic bacteria,[133] akin to the pretreatment situation in multiple myeloma.[134] Limitations in antibody binding to Fc receptors, particularly among subjects with homozygous expression of the *R131* allele of the neutrophil Fcγ receptor II that binds the Fc region of IgG2 only poorly,[135] can predispose to these infections.

The susceptibility of elderly persons to pneumococcal pneumonia is multifactorial; the bacteria can exploit the defects described earlier as well as those accompanying impaired functional status, such as weakening of the gag and cough reflexes, malnutrition, and organ dysfunction. The effect of alcoholism is also multifactorial and involves lifestyle (such as cold exposure and malnutrition), suppression of the gag reflex, and possibly deleterious effects on PMN function, although in most instances these alterations have been difficult to attribute to the effect of alcohol alone and may well involve liver disease as well.[136,137] Heffron[1] cited studies from the preantibiotic era showing a 30% to 50% incidence of alcohol abuse in patients with pneumococcal pneumonia, results confirmed in more recent studies (about one-third of such patients).[23,34,138] A disproportionately high number of patients with pneumococcal infection have diabetes mellitus,[23,34,138–140] a condition in which PMN chemotaxis is reduced[141] and phagocytic function is defective,[142] as well as dysfunction of other organ systems and underlying hematologic malignancies that often accompany aging (see Fig. 199.4).

Regarding antibody production in older adults, both the differentiation potential of hematopoietic stem cells and the numbers of naïve T cells and B cells decrease with advancing age,[143–145] as may the subsets of IgM memory B cells proposed to respond preferentially to polysaccharide antigens.[111,146–149] These perturbations and decreased responses to capsular polysaccharides have been most closely related to weight loss and frailty rather than to age alone.[150,151] Studies have been begun to distinguish between innate age-dependent "immunosenescence" and the effects of accumulated underlying disease and frailty on age-related immune dysfunction, particularly decreased responses to vaccination.

As mentioned earlier, prior respiratory viral infection, especially that caused by influenza virus, plays a prominent role in predisposing to pneumococcal infection.[57,58,152,153] Upregulation of surface receptors during viral infection enhances pneumococcal adherence[154,155] and invasion. Bacteria show greater epithelial adherence and impaired clearance from the airways because of virus-induced damage. Pneumococcal disease is greatly increased in people with altered pulmonary clearance, such as those who have chronic bronchitis, asthma, or chronic obstructive pulmonary disease, and cigarette smoking, including passive exposure,[48] particularly among otherwise noncompromised nonelderly adults. In the United States, socioeconomic factors may contribute substantially to the increased risk for these infections among persons of African-American descent, whereas the very high incidence among certain Native American populations, such as the Navajo, likely reflects genetic and environmental factors.[50,51,156]

HIV Infection

Pneumococcal pneumonia occurs 10 to 25 times more commonly with untreated HIV infection than in the general age-matched population (approximately 90 cases/1000 person-years).[157,158] Rates of invasive pneumococcal disease (e.g., bacteremia) were increased by approximately 80- to 100-fold with advanced untreated HIV disease (overall 176 vs. 3.8/100,000 patient-years for HIV vs. general population, respectively),[159] with recurrence in approximately 20% of cases.[160,161] HIV-associated risk factors for pneumococcal disease include stage of HIV disease, $CD4^+$ T-cell number,[37] drug and alcohol use, smoking,[158] organ dysfunction, previous pneumonia, and African-American ethnicity,[162,163] often with impaired antibody responses. Despite a greater than 90% decline in other opportunistic infections, effective antiretroviral treatment has limited the incidence of bacterial pneumonia and pneumococcal disease by only half,[158,164–168] or even shown no benefit.[169–171]

HIV infection is complicated by impaired IgG antibody responses, especially primary responses, to immunization[172–174] with pneumococcal and other vaccines.[175] That preimmunization levels may be lower, for instance, with influenza or measles[175,176] suggests a paradoxical loss of specific antibody despite hypergammaglobulinemia. Indeed, selective antigen-specific deficits after immunization were proposed to underlie the increased incidence of pneumonia and, relatively, pneumococcal infection after administration of 23-valent polysaccharide vaccine among HIV-infected adults in Uganda.[177] Pneumococcal vaccine failures were associated with low vaccine responses[178] and opsonic activity.[179] As discussed later, current pneumococcal vaccines show limited or transient protection against invasive disease, highlighting the need to limit HIV-associated immunodeficiency and circumvent related defects with more effective vaccine platforms.

CLINICAL SYNDROMES

S. pneumoniae causes infection of the middle ear, sinuses, trachea, bronchi, and lungs by direct spread of organisms from a nasopharyngeal site of colonization and causes empyema by direct extension to the pleural space from the lungs. Infections of the heart valves, bones, and joints are initiated by hematogenous spread, and those of the central nervous system (CNS) and peritoneal cavity by either route. The spectrum of invasive disease[180] includes primary bacteremia without an apparent source or focus of infection. In a population-based study of pneumococcal bacteremia in Israeli adults,[181] 71% of patients had recognizable pneumonia by plain chest radiograph, 8% had meningitis, and 4% had otitis media or sinusitis; 18% had primary bacteremia without an obvious source, a finding that is more common in children than adults.

Otitis Media

Diagnosis of otitis media depends on visualizing the tympanic membrane and testing its function.[182] Virtually every study of culture-proven acute otitis media has shown *S. pneumoniae* to be the most common isolate or second only to nontypeable *H. influenzae*, although nearly all of these studies were carried out in populations that had not received conjugate polysaccharide vaccine (see below). Among children ages 6 months to 4 years before widespread use of pneumococcal conjugate vaccine, *S. pneumoniae* was implicated in 40% to 50% of cases in which an etiologic agent was isolated or in 30% to 40% of all cases. PCR of middle ear fluid from children with otitis media suggests that 50% of specimens that used to be regarded as sterile may be caused by *S. pneumoniae* or *H. influenzae*.[183] Pneumococci have historically been the most prevalent pathogens in otitis media in adults as well.[184] Prior infection by a respiratory virus may play a major contributory role by causing congestion of the opening to the eustachian tube, which prevents expulsion of bacteria by normal clearance mechanisms. Prospective longitudinal studies[56,60,185] have shown that, when otitis media occurs, it follows within a few weeks after colonization by a new pneumococcal serotype and before the appearance of circulating anticapsular antibody.

Sinusitis

Acute bacterial rhinosinusitis has the same pathogenesis as, and is initially caused by, the same organisms as acute otitis media, with

S. pneumoniae and *H. influenzae* predominating.[186] Obstruction of orifices by viral infection, atmospheric pollutants, or allergens, together with accumulation of fluid in the paranasal sinus cavities, even during simple colds,[187] provides a medium for bacterial proliferation and subsequent acute sinus infection. Acute bacterial sinusitis is most consistently differentiated from viral causes by the persistence of symptoms (≥10 days), severity of symptoms and signs (temperature ≥39°C, purulent discharge or pain for greater than 3 days), and worsening of symptoms (fever, headache, increased nasal discharge) after initial improvement following a simple upper respiratory tract infection.[188] Evolution from acute to chronic sinusitis is associated with more complex bacteriologic findings.

Meningitis

Except during an outbreak of meningococcal infection, *S. pneumoniae* is the most common cause of bacterial meningitis in adults.[189–191] In countries that have implemented effective vaccination programs for *H. influenzae* type b, pneumococci have become the most common sporadic cause of meningitis in children older than 6 months, as well. Even after the widespread use of pneumococcal conjugate vaccine in children, *S. pneumoniae* remains the most common cause of bacterial meningitis in the United States, but the burden of disease has shifted from young children to older adults.[191]

Meningitis may result from hematogenous spread or by direct extension of bacteria from the sinuses or the middle ear.[192,193] Favoring the role of direct extension are the association between acute otitis media or sinusitis and infection of the CNS and the well-documented role of *S. pneumoniae* as the most common cause of recurrent bacterial meningitis associated with head trauma, cerebrospinal fluid (CSF) leak, cochlear implants,[194] or any other break in the integrity of the dura.[195] Favoring hematogenous spread is the association between pneumococcal pneumonia or bacteremia without a known focus and meningitis. In addition, an autopsy study of the temporal bones of children who died of bacterial meningitis[196] showed no evidence for extension from the middle ear, supporting the possibility that even after otitis media, meningitis may develop as a result of bacteremia. Although hematogenous spread to the choroid plexus was originally thought to be responsible for most cases of pneumococcal meningitis, it is now proposed that infection upregulates platelet-activating factor on vascular endothelial surfaces in the meninges and that pneumococci adhere and are internalized by this mechanism.[197] Direct spread to the CNS via lymphatics may also be responsible.[198] Communication through the cochlear aqueduct between the inner ear and the subarachnoid space[198,199] may explain deafness, a common complication in patients with hematogenous bacterial meningitis.

Once pneumococci appear in the meninges or subarachnoid space, the capacities to escape phagocytosis and produce inflammation are central to the disease process. Intracisternal injection of pneumococcal cell wall constituents in rabbits (principally peptidoglycan, also teichoic acid) causes the CSF abnormalities of bacterial meningitis, presumably through a variety of inflammatory mediators (e.g., complement protein C5a, tumor necrosis factor, interleukin-1, interleukin-6).[189,193,200] Interactions with Toll-like receptors 2 and 4 may initiate some protective response, but also stimulate further inflammation.[198]

No distinctive clinical features distinguish meningitis due to *S. pneumoniae* from that due to other bacteria. Examination of a Gram stain from a centrifuged specimen of CSF provides the correct diagnosis in a large majority of cases[201] with confirmation by appropriate culture. However, bacterial numbers and diagnostic sensitivity are substantially decreased if antibiotics have been given more than 4 hours in advance of CSF sampling. Immunologic detection of pneumococcal capsular material ("bacterial antigen") generally does not add information beyond what is determined by Gram staining[202]; the value of available single and multiplex PCR methods is currently under study.

Acute Exacerbation of Chronic Bronchitis

S. pneumoniae is the third most common bacterial cause of exacerbation in patients who have chronic bronchitis,[203,204] following *H. influenzae* and *M. catarrhalis.* Exacerbations are highly associated with acquisition of a new pneumococcal strain.[205]

Pneumonia

Overview

In the preantibiotic era, pneumococcus was identified as the cause in up to 90% to 95% of cases of community-acquired pneumonia (CAP).[1] More recent studies from Europe have attributed 25% to 40% of pneumonia cases to *S. pneumoniae,* results based, in part, on techniques that have not been validated.[206,207] Recent studies from the United States implicate pneumococcus as a cause of about 5% to 10% of CAP cases, even when an intensive effort has been made to identify the causative organism,[208] consistent with incidence of for rigorously identified cases in the Netherlands (5.4%).[30] The earlier widespread use of conjugate pneumococcal vaccine in children and a lower rate of cigarette smoking in U.S. compared with European adults may contribute to these geographic differences.

Pathogenesis

Unrecognized aspiration of oropharyngeal bacteria likely initiates most cases of lower respiratory infections. That many organisms are present in the nasopharynx but *S. pneumoniae* most often causes clinical pneumonia speaks to the relative virulence of this organism. Pneumonia develops when potentially protective mechanisms fail to prevent both the access of pneumococci to the alveoli and their subsequent replication. Bacteria proliferate in alveolar spaces and spread via the alveolar septa. In these sites, pneumococci activate complement, generate cytokine production, and upregulate receptors on vascular endothelial surfaces. Exudative fluid and WBCs accumulate in the septa and alveoli and extend to uninvolved areas through the interalveolar pores of Kohn. This filling of alveoli with microorganisms and inflammatory exudate defines the presence of pneumonia, and a clinical diagnosis is made when fluid accumulation is great enough to be seen radiographically as a nonlucent region of "infiltration" or "consolidation" (Fig. 199.6).

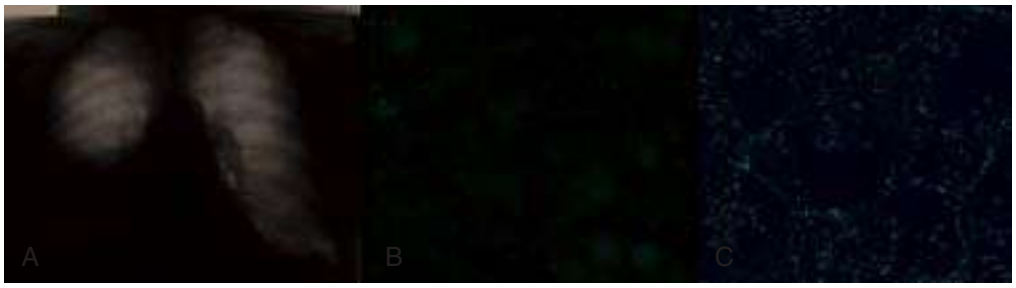

FIG. 199.6 Radiographic, microbiologic, and pathologic features of pneumococcal pneumonia in a 62-year-old man. A 62-year-old man described sudden-onset chills, fever, cough, and purulent sputum for 2 days. He smoked two packs of cigarettes per day, had alcohol binges, and had congestive heart failure. He was acutely ill, had a temperature of 103.4°F (39.7°C), respiratory rate of 32 breaths/min and labored breathing, blood pressure of 106/62 mm Hg, and pulse of 112 beats/min. He had decreased breath sounds and density to percussion in the right posterior chest. (A) Chest radiograph. (B) Gram stain of sputum with many neutrophils and gram-positive diplococci (note that bacteria are not within the phagocytes). (C) Histology of lung tissue from this man's autopsy when he died on day 2 of hospitalization while treated with ceftriaxone and azithromycin with supplemental oxygen in the intensive care unit.

Predisposing Factors

In a recent prospective study of pneumococcal pneumonia in a veteran population,[34] nearly all patients had two or more predisposing conditions, such as cigarette smoking, chronic obstructive pulmonary disease (both affect pulmonary clearance), alcohol abuse, liver disease (hepatitis, cirrhosis, or both), diabetes mellitus (these three latter conditions adversely affect PMN function), recent intravenous drug use, congestive heart failure, malignancy, HIV infection (greatly suppressed ability to produce IgG), or neurologic disease (decreased ability to swallow, decreased gag reflex, and increased aspiration). One-third of patients had been discharged from a hospital within the preceding 6 months. The increase in adult pneumococcal pneumonia during winter[58] and the striking association with viral infections in children and adults has long been noted.[58,152,153,209] Among patients hospitalized for influenza and found to have pneumonia, 25% were coinfected with *S. pneumoniae.*[210]

Symptoms and Physical Findings

The acute onset of cough (92%), fatigue (63%), shortness of breath (47%), and dyspnea (23%) with documented or subjective fever (92%), chills (77%), sweats, purulent sputum, and pleuritic chest pain (79%), as described by Heffron in 1938,[1] remain the most frequent symptoms of pneumococcal pneumonia.[31,211,212] These are all more prominent in younger than in older patients.[213,214] Patients with pneumococcal pneumonia usually appear ill and have a grayish, anxious appearance that may differ from that of persons with viral or mycoplasmal pneumonia. The temperature may be elevated to 102°F to 103°F, the pulse to 90 to 110 beats/min, and the respiratory rate to greater than 20 breaths/min and labored, with use of accessory muscles. Elderly patients may have a less well-defined clinical syndrome and only a slight or no temperature elevation, which may delay or obscure the diagnosis, but they are more likely to have an increased respiratory rate.[213,214] Fully one-half of patients admitted to a Department of Veterans Affairs hospital with CAP did not have a temperature >99.4°F in the first 48 hours of admission.[208]

Physical examination may reveal diminished respiratory excursion (splinting) on the affected side because of pain. Dullness to percussion is present in about half of cases. Crackles or abnormal lung sounds are heard on careful auscultation in nearly all cases, but in patients who have chronic lung disease, it is often difficult to be certain that such sounds signify the presence of pneumonia. Increased tactile fremitus, often overlooked, is very useful in detecting consolidation. Bronchial or tubular breath sounds may be heard if dense consolidation is present. Flatness to percussion at the lung base, with an inability to detect the expected degree of diaphragmatic motion based on the patient's respiratory excursion, and decreased tactile fremitus strongly suggest the presence of pleural fluid. Normal vital signs substantially reduce the likelihood of pneumonia, but no set of physical findings can reliably replace the chest radiograph in diagnosing the presence or absence of pneumonia.[215] Accordingly, the presence of cough, fever, increased respiratory rate, and abnormal lung sounds indicates the need for a chest radiograph. Indeed, patient management depends upon the results of chest imaging, limiting both overdiagnosis of pneumonia and antibiotic use.[216] The finding of a new heart murmur, particularly a diastolic murmur, raises concern about endocarditis, a rare but serious complication. The presence of fever with headache, confusion, obtundation, or neck stiffness suggests the presence of meningitis and is a compelling indication for a lumbar puncture.

Radiographic Findings

In most cases of pneumococcal pneumonia, chest radiography reveals an area of infiltration involving one or more segments within a single lobe (see Fig. 199.6A).[34] Consolidation, defined as a dense infiltrate (e.g., showing the presence of air bronchograms), is more frequent in bacteremic cases.[34,217] Necrotizing changes can be seen on chest radiographs in about 2% of patients and by computed tomography in 10%.[34,218] Only rarely does *S. pneumoniae* infection cause a thick-walled lung abscess.[219] Although pleural effusion may be detected by careful search in up to 57% of patients with pneumococcal pneumonia, only 10% have sufficient amounts of fluid to aspirate. Empyema is present in only 2% to 8% of patients.[31,220]

General Laboratory Findings

Twenty-five percent of patients with pneumococcal pneumonia have hemoglobin at 10 mg/dL or less.[34] The WBC count is elevated in the majority of patients (>12,000/mm^3) but normal in one-quarter; a WBC count less than 6000/mm^3, occurring in 5% to 10% of adults hospitalized for pneumococcal pneumonia, indicates a very poor prognosis.[221] Low serum albumin may result from predisposing malnutrition or from sepsis-related catabolism and fluid shifts.[34] Elevations of serum bilirubin to 3 to 4 mg/dL may result from hypoxia, hepatic inflammation with elevated transaminases, and breakdown of red blood cells in the lung. Levels of lactate dehydrogenase may be elevated. The likelihood that underlying disease is present must always be considered when evaluating abnormal laboratory findings. Laboratory abnormalities in empyema are reviewed in Chapter 68.

Diagnostic Microbiology

Microscopic examination of Gram-stained sputum is both sensitive and specific if the sample is available and contains large numbers of PMNs (>25/high-power field) and very few epithelial cells (<1 per 10–20 PMN), and if the patient has not received more than 6 to 12 hours of antibiotics (see Fig. 199.6B).[222,223] Sensitivity declines after 6 hours of antibiotic therapy, so every effort should be made to obtain a sample before that time. Although often unavailable, with adequate sputum specimens and in the absence of prior antibiotic treatment, Gram staining and culture both reveal pneumococci in expectorated sputum from about 60% of patients with pneumococcal pneumonia, and 30% to 40% of patients with CAP.[31,222] When a patient who has pneumonia cannot initially provide an expectorated specimen, inhalation of humidified air or hypertonic saline may be used to obtain one, but these should not delay therapy. Antibiotics should be given promptly and at the point where the diagnosis of pneumonia is made. An earlier requirement to administer antibiotics within 4 hours of a patient's arrival at an emergency room has recently been rejected.[224] We suggest that reasonable attempts to determine an etiologic agent should be made for all adults sufficiently ill to be hospitalized; upcoming guidelines for management of CAP recommend such efforts only for those with severe pneumonia.

If accepted terminology is strictly followed, a presumptive diagnosis of pneumococcal pneumonia is then made if *S. pneumoniae* is identified by sputum culture, whereas the diagnosis is regarded as proven if pneumococci are isolated from blood or another normally sterile body site. About 20% to 25% of patients hospitalized for pneumococcal pneumonia have detectable bacteremia.[1] A guide to interpreting results of blood and sputum culture is shown in Table 199.3.

In addition to analysis of sputum and blood cultures, other diagnostic techniques focus on the detection of pneumococcal antigen. Most commonly used is the rapid point-of-care urine immunochromatographic membrane test (BinaxNow *S. pneumoniae* urinary antigen test; Alere, Waltham, MA) for C-polysaccharide approved by the US Food and Drug Administration (FDA). In 22 studies of more than 4600 patients with pneumococcal pneumonia, the test has a sensitivity of 75% (range, 58%–93%), specificity of 95% (range, 58%–93%), a positive predictive value of 79% (range, 25%–100%), and a negative predictive value of 93% (range, 74%–100%), showing greater sensitivity than other methods,[31] particularly in those with bacteremia. The test can remain positive for weeks and is not useful in children because of positive results with heavy pharyngeal colonization.[226] False-positive results have rarely been reported.[227,228] A technique to detect PPSs from the 13 serotypes present in the 13-valent conjugate vaccine in the urine is more sensitive than BinaxNow,[229] but these serotypes have become much less common causes of pneumonia because of the use of the 13-valent conjugate vaccine, so this test is not likely to be useful diagnostically. Other techniques include the following:

(1) Quantitative PCR for DNA that encodes *lyt*A of *S. pneumoniae* in sputum or nasal secretions[230,231] modestly increases the diagnostic yield compared with standard bacteriologic techniques, as may multiplex PCR assays,[232] the predictive values of which are being determined in community settings. This test also was useful in HIV-infected subjects in South Africa but has yet to be validated in non-AIDS patients in a developed country.

TABLE 199.3 Microscopic Examination and Culture of Sputum and Blood Diagnosis of Pneumococcal Pneumonia

GRAM STAIN	SPUTUM CULTURE	BLOOD CULTURE	COMMENT
+	+	+	Generally regarded as confirmed diagnosis of invasive pneumococcal pneumonia but does not exclude contribution by another etiology, such as influenza virus infection or lung cancer
+	+	–	Good evidence for presumptive (nonbacteremic) pneumococcal pneumonia if a clinical syndrome suggesting pneumonia is present, microscopic examination of Gram-stained sputum is characteristic (see Fig. 199.5), and culture shows strongly predominant growth of pneumococci with no other likely pathogenic bacteria
±	–	+	With symptoms and signs of pneumonia and an infiltrate on the chest radiograph, findings are taken to indicate invasive pneumococcal pneumonia even if organisms are not found in sputum
+	–	–	In the presence of the appropriate clinical syndrome, still remains suggestive of pneumococcal pneumonia because organisms can be missed on culture as a result of sampling error and overgrowth of streptococci from saliva; however, gram-positive cocci may be streptococci of the mouth
–	+	–	In the presence of the appropriate clinical syndrome, this yields a diagnosis of presumptive (nonbacteremic) pneumococcal pneumonia
–	–	–	Does not support a diagnosis of pneumococcal pneumonia but does not exclude it because of numerous difficulties in obtaining a valid sputum sample

(2) A quantitative assay to detect pneumococcal capsular polysaccharide directly in nasopharyngeal and blood specimens.[233]

(3) Quantitative PCR that has been studied on sputum in CAP patients who can provide a sputum specimen. Although they used excellent negative and positive controls to standardize the assay, the authors found multiple isolates of common bacterial pathogens that are readily identifiable by traditional techniques, so the assay is evolving for clinical application.[234]

Levels of C-reactive protein and procalcitonin are often elevated in patients with bacterial pneumonia but do not discriminate *S. pneumoniae* from other bacteria.

Complications

Pleural fluid appears in a substantial proportion of cases of pneumococcal pneumonia but is usually reactive. The fluid is tapped when of sufficient amount to cause respiratory distress or when response to treatment is delayed, suggesting the presence of an empyema. Some authorities suggest that, in every case of pneumonia, if sufficient fluid is present a pleural tap should always be done. When bacteria reach the pleural space, either hematogenously or as a result of extension to the visceral pleura, empyema results; this is seen in 2% to 8% of cases.[220] Persistence of fever, even if "low grade," and leukocytosis after 4 to 5 days of appropriate antibiotic treatment for pneumococcal pneumonia is suggestive of empyema, and this diagnosis is even more likely if the radiograph shows persistence of pleural fluid. The presence of frank pus in the pleural space, a positive Gram stain, or fluid with pH of 7.1 or less are each indications for aggressive and complete drainage with prompt insertion of a chest tube, followed by immediate removal of infected material by pleuroscopy or open thoracotomy if drainage is not complete, if the patient remains febrile or with leukocytosis, or both.[235]

Other Infectious Syndromes

S. pneumoniae is implicated in a wide variety of infectious states. Isolated or epidemic *conjunctivitis* is essentially the primary condition in which unencapsulated pneumococci play a role.[10] A case of pneumococcal *endocarditis*[236] is seen once or twice per decade at a large tertiary care hospital in the United States; 14 cases were identified in Denmark (population of 5 million) in a 10-year period.[237] Alcoholic dependence is common in these cases, most infections involve previously normal heart valves, and the disease tends to be fulminating.[236–238] Purulent *pericarditis*[239] caused by pneumococci has also become exceedingly rare, whether it occurs as a separate entity or together with endocarditis. Pneumococci cause a small proportion of *peritonitis,* occurring by hematogenous or local inoculation of the peritoneal cavity.[240] Preexisting ascites can serve as a site of diminished resistance, with pneumococci reaching the peritoneum by hematogenous spread, even in the absence of a documented source of infection elsewhere. Local inoculation occurs either when pneumococci are carried to the peritoneal cavity via the female reproductive tract, with or without clinically recognizable infection (e.g., salpingitis), or as a result of bowel perforation. Pneumococcal infections of the female reproductive organs[241–243] occur with or without peritonitis. *Septic arthritis* develops spontaneously in a natural or prosthetic joint[244] or as a complication of rheumatoid arthritis.[245] Multiple joints are involved in less than 25% of cases,[246] and the functional outcome is often bad. *Osteomyelitis* in adults tends to involve the vertebral bones.[247] *Epidural and brain abscesses* are rarely described.[248] *Soft tissue* infections[249,250] are found in persons who have connective tissue diseases or HIV infection. Bacteremic pneumococcal *cellulitis* generally occurs in patients who have severe underlying diseases, and a respiratory focus is often apparent.[251] Finally, the development of pneumococcal pneumonia or bacteremia or the appearance of *unusual infections* in a young adult should prompt testing for HIV infection.[252,253]

Noninfectious Syndromes

Noninfectious sequelae may complicate infections,[254,255] including pneumococcal pneumonia.[222] Consistent with earlier reports of cardiovascular complications of CAP,[254,255] among 170 US veterans hospitalized for pneumococcal pneumonia, 33 (19.4%) had at least one major cardiac event, including acute myocardial infarction, arrhythmia, or worsening congestive heart failure.[222] Possible mechanisms for these cardiac events include (1) increased local inflammatory response in a vulnerable coronary artery atherosclerotic plaque, with plaque rupture and subsequent infarction[256]; (2) sepsis causing increased demand ischemia in myocardial muscle exacerbated by ventilation-perfusion mismatch and hypoxemia; or (3) actual invasion of or damage to myocardium by pneumococci.[257] Pneumococcal bacteremia may also be complicated by peripheral symmetrical gangrene with necrosis of digits and limbs, as well as hemolytic-uremic syndrome.[258]

ANTIBIOTIC SUSCEPTIBILITY AND RESISTANCE

Definitions of Antibiotic Susceptibility

Before 1977, with isolated exceptions, virtually all *S. pneumoniae* were inhibited by penicillin at a concentration of 0.06 μg/mL or less. In 1977–1998, pneumococcal antimicrobial resistance was described in South Africa and was associated with clinical failures of treatment, particularly in the cases of meningitis. Isolates with a minimal inhibitory concentration (MIC) of 0.06 μg/mL or less were called penicillin susceptible, or resistant if the MIC was 0.12 μg/mL or greater.[259] Definitions of susceptibility to penicillin were changed in 2008 to reflect the site of infection, particularly for meningitis, and the route of therapy.[260–263] Current accepted definitions of susceptibility and resistance to penicillins

TABLE 199.4 Current Definitions of Susceptibility or Resistance of Pneumococci to Penicillins or Cephalosporins

	PENICILLIN[a]	CEFTRIAXONE[b]
Non-CNS, Oral		
Susceptible	≤0.06 μg/mL	—
Intermediate	>0.06 and ≤1 μg/mL	—
Resistant	>1 μg/mL	—
Non-CNS Parenteral		
Susceptible	≤2 μg/mL	≤1 μg/mL
Intermediate	>2 and ≤4 μg/mL	>1 and ≤2 μg/mL
Resistant	>4 μg/mL	>2 μg/mL
CNS		
Susceptible	≤0.06 μg/m	≤0.5 μg/mL
Intermediate	—	>0.5 and ≤1 μg/mL
Resistant	>0.06 μg/mL	>1 μg/mL

[a]Also applies to amoxicillin.
[b]Also applies to cefotaxime.
CNS, Central nervous system.

and cephalosporins, summarized in Table 199.4, may be overly conservative for penicillin and ceftriaxone for CNS infections.

Mechanisms of Antibiotic Susceptibility and Resistance

Penicillin inhibits the replication of *S. pneumoniae* by binding one or more enzymes needed to synthesize peptidoglycan, including higher-molecular-weight transpeptidases and a lower-molecular-weight carboxypeptidase, resulting in bacterial autolysis. The binding is covalent, and a serine ester–linked, enzymatically inactive penicilloyl complex is formed. Six peptidoglycan synthesis enzymes, which are also called penicillin-binding proteins (PBPs), have been identified (1a, 1b, 2a, 2b, 2x, and 3). Resistant isolates are characterized when penicillin shows decreased affinity for one or more of these enzymes due to mutations in the genes that encode them.[264] Alterations in PBP2b are more likely to account for low-level resistance, whereas mutations in PBP2x have been associated with high-level resistance.[265] Alterations in PBP2x and 1a also render pneumococci more resistant to third-generation cephalosporins, such as cefotaxime or ceftriaxone.[266]

Pneumococci develop antibiotic resistance by transformation, the unique capacity to acquire genetic material from other bacteria with which they coexist in close proximity. In fact, the altered sequence in the gene for PBP2b in many penicillin-resistant isolates appears to have been acquired from *Streptococcus mitis.*[267] Extensive diversity among isolates[268] or within the transpeptide-encoding region of the pneumococcal genome[265,266] indicates that many discrete mutational events have occurred, some of which reflect acquisition and others, rearrangement of DNA. Alterations in several PBPs within an individual isolate result in a mosaic array of PBPs. Nevertheless, the major source of resistance worldwide has been the geographic spread of a few clones that seem to have special capacity to spread and to colonize.[269] In this context, a strain that was prevalent in Spain during the 1992 Olympics was imported into and rapidly spread throughout Iceland.[270] Similarly, the dominant factor in the emergence of antibiotic-resistant pneumococci in the United States has been human-to-human spread of relatively few clonal groups that harbor resistance determinants to multiple classes of antibiotics[271] and have spread worldwide.

Geographic spread is greatly facilitated by antibiotic pressure, which explains why many of the widespread colonizing clones exhibit antibiotic resistance, including the clonal spread of a penicillin-resistant *S. pneumoniae* serotype 19A.[272] A prominent site for this selection in the United States is daycare centers, in which a remarkably high proportion of children are receiving antibiotics. Conditions in these centers (1) suppress susceptible microbiota, thereby creating a niche for resistant organisms; (2) spare antibiotic-resistant pneumococci; (3) increase the prevalence of antibiotic-resistant viridans-group streptococci, thus setting the stage for further transformation of pneumococci to antibiotic resistance; and (4) provide close contact among small children, which allows the spread of organisms. Similar conditions for emerging resistance are present in nursing homes.

Decreased susceptibility to penicillin is associated with resistance to other antimicrobial agents, because resistance usually results from acquisition of a cassette of genetic elements. Even low-level increases in MICs for penicillin are associated with resistance to other widely used antibiotics, including macrolides, trimethoprim-sulfamethoxazole, tetracyclines, and, to a lesser extent, quinolones.

Understanding the complexity of macrolide resistance is important clinically. Macrolides insert into a pocket of the 23S subunit of the 50S ribosome, specifically by attaching at domain V of the peptidyl transferase loop, thereby blocking protein assembly. In doing so, these drugs—generally regarded as bacteriostatic drugs against gram-positive pathogens, such as *S. aureus*—are bactericidal against *S. pneumoniae.* Acquisition of genetic material, designated *ermB* or *mefA,* often together with genes that encode penicillin resistance, may lead to resistance. The gene *ermB* encodes an enzyme that methylates a base in domain V of the 23S rRNA (nucleotide A2058), thereby altering the ribosomal pocket. Because the macrolide no longer fits into the pocket, increasing its concentration has little effect, resulting in high-level resistance (≥64 μg/mL). The gene *mefA* encodes an efflux pump that extrudes macrolides. High antibiotic concentrations might be expected to overcome the pump, forcing enough antibiotic into the bacterium to exert an antibacterial effect. This resistance is at a lower level (usually ≤16 μg/mL) and, at a sufficient dose, a macrolide might be expected to be effective. The debate about whether such resistance is clinically meaningful[273] is based on the fact that the majority of macrolide-resistant isolates in the United States have *mefA,* and that present doses of macrolides may be effective despite the in vitro finding that an isolate is resistant. Other mutations are responsible for resistance in a small percentage of isolates.[274]

Prevalence of Resistance

Despite earlier overestimates of the frequency of pneumococcal resistance to β-lactam antibiotics, currently, about 65% of non-CNS isolates in the United States are susceptible to penicillin or amoxicillin given orally, 17% are intermediately resistant, and 17% are resistant (Tables 199.4 and 199.5).[275] About 96% of all pneumococci are susceptible to penicillin given parenterally, 4% are intermediate, and <1% are resistant.[276] In cases of meningitis, 65% of organisms are susceptible to penicillin and 35% are resistant (no intermediate resistance is defined). For ceftriaxone, in non-CNS infections, 96% of organisms are susceptible, 4% are intermediate, and <1% are resistant; in CNS infections, these percentages are 88%, 7%, and 5%, respectively.[277] With the notable exception of the Netherlands and Germany, where accepted standards of practice strictly limit antibiotic usage,[278] rates of resistance are higher in most European countries than in the United States, and greater yet where antibiotic usage is uncontrolled.

Pneumococci with low MICs for penicillin remain susceptible to most other antibiotics. As resistance to penicillin increases, resistance to other commonly used antibiotics also increases. In the United States, about 35% of all pneumococci are resistant to macrolides, 10% to clindamycin, 30% to trimethoprim-sulfamethoxazole, 18% to doxycycline, and 2% to the newer quinolones.[279] *ermB* and *mefA* strains are present in equal proportions, but *ermB* gene-related macrolide resistance is higher in Europe. In general, greater than 98% of isolates remain susceptible to fluoroquinolones, probably because these drugs are not used to treat children. In Canada, an increase in resistance has paralleled increased quinolone use,[280] and in high-usage locales, such as chest clinics[281] or nursing homes,[282] the rate of resistance may exceed 5%. A remarkable incidence of colonization by a single clone of *S. pneumoniae* (sequence type 156; serotype 19A or 23) was documented in a long-term care facility in Israel; many of the colonized patients had not, themselves, previously received a quinolone.[283] Pneumococcal resistance to vancomycin, the oxazolidinones (linezolid), ceftaroline, the ketolides (such as telithromycin and cethromycin), the glycylcyclines (such as tigecycline), or daptomycin has not yet been documented.

TABLE 199.5 Estimated Likelihood of in vivo Bacteriologic Response of *S. pneumoniae* to Antibiotics in Patients With Non-CNS Infections Using Standard Therapy in the United States

ANTIBIOTIC	LIKELIHOOD (%)
Penicillin (parenteral), ampicillin, piperacillin	95[b]
Amoxicillin	95[b]
Cefuroxime, cefpodoxime, cefdinir	85[b]
Cefotaxime, ceftriaxone, cefepime	97[b]
Imipenem, meropenem, ertapenem	97
Azithromycin, clarithromycin	80[c]
Clindamycin	93
Telithromycin	100
Trimethoprim-sulfamethoxazole	65
Doxycycline	84
Vancomycin	100
Quinolones[d]	98

[a]See text discussion and Musher and coworkers.[260] These estimates of bacteriologic coverage are not equivalent to National Committee for Clinical and Laboratory Standards–defined in vitro susceptibility nor the likelihood of cure in vivo, which depend on both microbiologic and host clinical factors.
[b]With high doses of these drugs, almost all pneumococci are expected to be susceptible.
[c]This estimate balances the 25% rate of resistance in vitro and a generally high rate of clinical response.
[d]Quinolones = levofloxacin and moxifloxacin.

THERAPY

The basic principles of treating pneumococcal infection are similar to those for treating other bacterial infections: (1) prompt diagnosis and administration of an antibiotic that provides a level sufficient to inhibit or kill the infecting organism, (2) continuation of treatment at least until the host is able to complete the curing and healing processes, (3) drainage of infections of closed spaces if necessary, (4) knowing what response to expect, and (5) being prepared to reevaluate if this response is not observed. Application of these principles is by no means simple. Obstacles include (1) for most pneumococcal diseases, therapy is begun before the etiologic agent is known or no microbiologic studies are done; (2) even if *S. pneumoniae* is known to be causative, antibiotic susceptibility is not known when treatment is begun; (3) the appropriate duration of therapy is not evidence based; (4) in otitis media and sinusitis, the most common infections caused by *S. pneumoniae,* drainage is not usually done; and (5) many physicians do not clearly understand the natural history of disease or what response to expect after treatment has begun.

Otitis Media

Treatment of otitis media is the leading indication for antibiotic use in children. For children under 2 years of age with first-episode otitis media requiring antibiotics (bulging of the tympanic membrane, other signs of acute infection, *and* middle ear effusion), the American Academy of Pediatrics and the American Academy of Family Physicians recommend amoxicillin, 90 mg/kg in two daily doses for 10 days (<2 years old) or 5 to 7 days (>2 years old)[284] for *S. pneumoniae,* the most common identifiable cause of this infection and the one associated with the greatest morbidity. Treatment of recurrent or recently treated disease with amoxicillin-clavulanate is directed to β-lactamase–producing *H. influenzae,* because this medication has no additional effect on penicillin-resistant *S. pneumoniae,* for which cephalosporins may be more effective.

Sinusitis

Because the pathogenesis and causative organisms of acute bacterial rhinosinusitis are comparable to those of otitis media, the same therapeutic considerations apply. Treatment guidelines are largely empirical, without knowing whether *S. pneumoniae* is present or, if present, its antibiotic susceptibility.[92,285] First-line therapy with amoxicillin is likely beneficial in 80% to 90% of cases. However, amoxicillin-clavulanate is increasingly recommended as first-line therapy in children and adults because of a high frequency of β-lactam resistance among isolates of *H. influenzae.*[188] High-dose amoxicillin-clavulanate (180 mg/kg/day orally in two or three divided doses, not to exceed a total of 4 g/day) is considered for severe infections, immunocompromised hosts, and extremes of age, and when the frequency of penicillin nonsusceptibility is high (>10%). Because clavulanic acid causes diarrhea, a proposed option is to mix 500 mg of amoxicillin together with another 500 mg of amoxicillin plus 125 mg clavulanic acid three to four times daily. Quinolones may be used in adults but are not approved for children. If subsequent therapy with ceftriaxone fails, referral to an otolaryngologist is appropriate.

Pneumonia

Outpatient Therapy

When a microbiologic diagnosis is sought, *S. pneumoniae* has been the most common organism isolated.[21] The response to therapy generally appears to be excellent irrespective of the drug selected; specifically, penicillins with or without β-lactamase inhibitors, macrolides, doxycycline, or a newer fluoroquinolone (as opposed to ciprofloxacin) all seem to be equally effective,[224] although clinical failures may complicate macrolide treatment of outpatient pneumonia caused by macrolide-resistant pneumococci.[286]

Inpatient Therapy

Guided but not bound by published guidelines,[224] the importance of the decision to hospitalize a patient with pneumonia, even directly to intensive care, cannot be overemphasized. The severity index established by the Pneumonia Outcomes Research Team (PORT)[287] is preferred over the more accessible but less reliable CURB-65 (*c*onfusion, blood *u*rea nitrogen, *r*espiratory rate, systolic *b*lood pressure)[288] criteria. However, clinical judgment should outweigh the results of a scoring system in considerations of hospitalizing the patient, at least for the initiation of therapy. Because the PORT score is so highly dependent on age of the patient, clinical judgment becomes more important in younger adults.

Pneumonia caused by pneumococci that are susceptible or intermediately resistant to penicillin responds to treatment with penicillin, 1 million units intravenously every 4 hours; ampicillin, 1 g every 6 hours; or ceftriaxone, 1 g every 24 hours. A principal concern is whether pneumonia caused by a relatively uncommon pneumococcus judged to be resistant by present definitions will respond to such therapy, whether higher doses of β-lactam antibiotics are needed, or whether treatment should be changed to another class of antibiotic, such as a quinolone. Patients who are treated for pneumococcal pneumonia with an effective antibiotic generally have substantially reduced fever and feel much better within 48 hours. If a patient has responded to treatment with a β-lactam antibiotic, this therapy should be continued independent of susceptibility results. However, if a clear response is not observed and the organism is resistant, therapy should be changed in accordance with susceptibility testing results. Several retrospective case-control studies and prospective trials suggest that patients with pneumococcal infection have better outcomes if a macrolide is added, especially if they are critically ill.[289–291] Proposed mechanisms for these observations include immunomodulatory or antiinflammatory effects of the macrolide,[292] treatment of coinfections (e.g., *Mycoplasma, Chlamydia, Legionella*), increased bacterial kill rates, or decreased adherence of organisms.

The optimal duration of therapy for pneumococcal pneumonia is uncertain. Pneumococci are not readily detected in sputum microscopically or by culture more than 24 hours after the administration of an effective antibiotic.[293] Experience obtained early in the antibiotic era showed that 5 to 7 days of therapy sufficed, even when blood cultures were positive. Antibiotic therapy for pneumococcal pneumonia should be continued for 2 days after achieving clinical stability (temperature <99°F, respiratory rate ≤24 breaths/min, oxygen saturation >90%, blood pressure >90 mm Hg), generally for a total of 5 to 7 days.[294] Failure of a patient to defervesce within 3 to 5 days should stimulate a review of the organism's antibiotic susceptibility, search for another organism,

attempts to exclude a loculated infection such as empyema, or search for a noninfectious source. Once clinically stable, patients have a less than 1% chance of failure, defined by death or immediate readmission, but if discharged with two or more abnormal signs, they have a 46% risk of death or readmission.[31,294] The time to achieve clinical stability is no different among bacteremic and nonbacteremic patients with pneumococcal pneumonia,[295] so treatment duration should be comparable in both settings.

Meningitis

Pneumococcal meningitis is the syndrome in which antimicrobial resistance is most likely to have adverse clinical outcomes. Pneumococcal meningitis has been treated with 12 to 24 million units of penicillin every 24 hours or 2 g ceftriaxone every 12 hours. Either regimen is effective against antibiotic-susceptible *S. pneumoniae* and may be effective against intermediately resistant ones; pharmacokinetic considerations and achievable CSF levels favor the use of ceftriaxone or cefotaxime. During treatment of resistant strains, β-lactam antibiotics are likely not to achieve therapeutic levels in CSF, justifying the recommendation that vancomycin be given together with ceftriaxone or cefotaxime until susceptibility results are reported. In patients who have documented penicillin and cephalosporin allergies, unless the history suggests a class 1, life-threatening reaction, ceftriaxone or cefotaxime should be used. Meropenem is an alternative for the β-lactams; imipenem is not typically recommended because of the risk of seizures.

An important randomized clinical trial in adults with severe meningitis showed that the addition of dexamethasone, 10 mg four times daily for 4 days, decreased morbidity and mortality from pneumococcal meningitis,[296] although benefits had not been consistently demonstrated previously in adults or children.[297] If administered, steroids should be given before or with the first dose of antibiotics and should not be continued beyond the recommended 4 days. Repeat spinal taps may be needed if there is any suggestion of a delayed clinical response.

Endocarditis

Pneumococcal endocarditis is associated with rapid destruction of heart valves. All patients with this disease should be seen by a cardiologist and a cardiovascular surgeon as soon as the diagnosis is suspected. Because of the (albeit unlikely) possibility of β-lactam resistance, initial therapy should include vancomycin and ceftriaxone until the results of minimal bactericidal concentration testing are known.

PREVENTION OF PNEUMOCOCCAL INFECTIONS

Despite the availability of effective vaccines in children against invasive disease caused by *S. pneumoniae* and *H. influenzae* type b, these encapsulated pathogens remain leading causes of bacterial death worldwide in young children.[19] Interventions to prevent pneumococcal disease and death in children include minimizing crowding, limiting indoor air pollution from fires for cooking and heating (and avoiding chronic secondary smoke exposure from cigarettes[48]), and promoting maternal health, sanitation, and access to care.[80] Optimizing nutrition by reducing prematurity and low birth weights, exclusive breastfeeding, and micronutrient supplementation (with zinc and, likely, vitamin A) are also beneficial. Case management includes early diagnosis, assessment of severity, early therapy, and the use of oxygen in severe cases. Immunization for measles and perhaps influenza also should reduce the incidence of pneumococcal disease.[298]

Vaccination with capsular polysaccharides is the mainstay of control of pneumococcal disease. Two pneumococcal polysaccharide vaccines are approved for use in the United States: (1) a pneumococcal polysaccharide vaccine, PPSV23, marketed as Pneumovax (Merck, Whitehouse Station, NJ), or Pnu-Immune (Wyeth, Collegeville, PA), consisting of capsular material from 23 pneumococcal types that historically caused about 75% to 85% of pneumococcal disease in children or adults, and (2) a protein-conjugate pneumococcal vaccine, PCV, initially marketed as Prevnar or Prevnar 7 (PCV7; Wyeth) and now replaced by Prevnar13 (PCV13; Pfizer, Philadelphia, PA), containing capsular polysaccharide from the 13 most common types that caused disease in children, each covalently linked to a nontoxic protein congener of diphtheria toxin (cross-reacting material 197 [CRM197]).[299] PPSV23 and PCV13 share serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23, whereas PPSV23 also contains serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F, but serotype 6A is unique to PCV13. The more limited immunogenicity and efficacy of the polysaccharide vaccine in very young children is overcome by the ability of the conjugate to enhance levels, generation of IgG1 rather than IgG2 antibodies, avidity, and memory for pneumococcal capsular polysaccharide antibodies in children by engagement of dendritic cell–dependent and T-cell–dependent mechanisms,[118,119] with greater than 90% protection against invasive disease.[300,301]

Antibody Responses to Pneumococcal Capsular Polysaccharides

The primary goal of pneumococcal vaccination is to elicit antibodies to the primary virulence factor of *S. pneumoniae,* its polysaccharide capsules. Both younger and older adults often show evidence of prior exposure to a range of pneumococcal serotypes. Among persons older than 65 years, each serotype was recognized by IgG (>1 μg/mL) from 5% to 35% of subjects.[27,96] After vaccination of adults, capsule-specific IgM and IgG, as well as IgA, can be detected after about 7 days and IgG peaks at 1 to 2 months.[27,94,302–304] About 80% of older adults showed appreciable responses to 18 or more of 23 serotypes and 50% to 15 or more serotypes after receiving PPSV23,[27] with 65% to 95% showing greater than 1 μg/mL of specific IgG to each serotype. Levels of specific IgG then wane to near baseline levels over the next 5 to 7 years.[305] However, no reliable cutoff for a protective level has been established in adults. Levels of capsule-specific IgG show some correlation with the ability of these sera to support opsonophagocytosis and killing of the organism. Multiple factors contribute to these functional outcomes, including antibody isotype, subclass and avidity, and phagocyte function, each of which may be compromised in older adults.[116,306]

That IgG appears as soon as IgM after primary vaccination and that these capsule-specific IgG cells are class-switched and show substantial somatic hypermutation of the antigen-binding immunoglobulin genes may suggest that the vaccine is stimulating memory cells in these adults.[307,308] However, whether pure polysaccharides generate memory responses is controversial. The substantial response to the polysaccharide PPSV23 in adults, compared with low levels in young infants, may be due to memory or to greater immune maturation. With classic memory responses to proteins (e.g., tetanus toxoids), booster responses are of greater magnitude than those to primary immunization. In contrast, responses to revaccination with PPSV23, which has been best studied among the elderly, are of similar or even lower magnitude than those to the original challenge.[302,305] Whether the latter responses to revaccination with PPSV23 represent blunting of secondary responses ("hyporesponsiveness"), as has been described with meningococcal polysaccharides, in older adults is debated.[305,309,310] However, recent data suggest that revaccination within 1 year, rather than many years, after primary immunization and giving the conjugate vaccine as the first dose, independent of which vaccine is given second, is associated with enhanced antibody levels among adults older than 70 years, compared with giving PPSV23 first.[311] These data and recent efficacy results[30] underlie the recent CDC Advisory Committee on Immunization Practices (ACIP) recommendation to use PCV13 for primary immunization in older adults followed by PPSV23; the former may provide a more immunogenic base for revaccination and the latter elicits broader serotype coverage. Nevertheless, because of the effectiveness of PCV7 and now PCV13 in reducing transmissible pneumococcal carriage and disease in children, herd protection results in an ever-decreasing proportion of clinical disease in adults caused by PCV13 serotypes.[312]

Pneumococcal Conjugate Vaccine Efficacy in Children

Despite earlier evidence of protection against death from pneumonia in children in Papua, New Guinea,[313] young children, particularly those younger than 2 years, do not respond adequately to the PPSV23 vaccine used in adults. The defect may relate in part to engagement of T cells and impaired ability of children to produce the IgG2 subclass predominantly elicited by polysaccharides. Their ability to now recognize and respond to the capsular polysaccharides within the conjugate vaccine

elicits robust antibody responses, predominantly of the IgG1 subclass that supports opsonophagocytosis of *S. pneumoniae* with complement. After three to four doses of 7- or 9-valent vaccines delivered early in life (ages 2, 4, 6, and 12 months in the United States), protection against invasive pneumococcal disease caused by vaccine serotypes ranged from 77% to 97% in the United States, South Africa, and the Gambia.[300,314–316] Such protection against bacteremia in children younger than 2 years[300] includes low-birth-weight and preterm infants[317] and persists over time.[318] Across countries, overall protection against vaccine serotypes ranges from 39.9% in Spain to 99.1% in the United States.[319] For all vaccine-related invasive serotypes, protection had a median of 65.5% for all international sites and 94% in the United States,[312] and 45% for invasive pneumococcal disease of all serotypes in the United States.

Conjugate vaccine protection extended to other syndromes in young children. Protection was significant but more modest against otitis media in children: 6% (0.4%–16%) against all-cause otitis, 34% (21%–45%) against confirmed pneumococcal otitis, and 57% (44%–67%) against otitis caused by vaccine serotypes.[301,320,321] Of greatest importance, the conjugate vaccine provides substantial protection against pneumonia in children,[322] both clinically diagnosed and, particularly, radiologically confirmed pneumonia, which is the leading cause of death from pneumococcal disease in children.[323] All-cause pneumonia was reduced by 4.3% (−3.5% to 11.5%), but 30% (4.4%–34%) with disease confirmed by chest radiographs. These results were confirmed in the Gambia in West Africa, where, among children younger than 1 year, a 9-valent conjugate supported 7% (1%–12%) protection against clinical pneumonia but 37% against radiographically confirmed pneumonia.[314] Consistent with these results, use of the protein-polysaccharide conjugate vaccine (PCV7 and PCV13) in young children has also yielded dramatic and sustained decreases in hospitalizations for pneumonia.[324,325] Indeed, in the period after the introduction of PCV7 and its broad uptake in the population (1997–2009), the annual incidence of invasive pneumococcal disease fell most dramatically in children under 2 and adults 65 years and older (Fig. 199.7),[312] as did all-cause hospitalization for pneumonia in the United States,[324] as well as specifically for pneumococcal pneumonia in children under 18 years (Fig. 199.8).[325] Based on these remarkable findings, an advancing goal is to introduce effective, accessible, and affordable vaccines against the pneumococcus in resource-limited settings (Fig. 199.9).

Finally, meningitis, which has the highest case fatality and long-lasting sequelae, has also decreased by 26% to 64% in children younger than 2 years, in association with use of the pediatric vaccine, as well as by 54% among adults older than 65 years by herd protection. Some of the concomitant increase in nonvaccine serotypes with PCV7 (especially 19A, 22F, and 35B)[277] are included in PCV13 (e.g., 19A). Results of testing the use of an additional dose of PPSV23 after PCV13 in children have shown mixed results, some of which are promising.[309]

Efficacy of 23-Valent Pneumococcal Polysaccharide and 13-Valent Polysaccharide Conjugate Vaccine in Adults

Protection against pneumococcal disease in adults, particularly older adults, needs to be considered in the context of bacteremia and pneumonia. In two field trials in South Africa in the 1970s, pneumococcal polysaccharide vaccines showed significant efficacy against pneumococcal pneumonia among otherwise healthy, young gold miners with a high incidence of disease.[326,327] Based on these results, the 14-valent, then the 23-valent polysaccharide vaccines were licensed. Currently, in the United States, one-half to two-thirds of persons older than 65 years and with relevant underlying illnesses have received PPSV23 (and one-third have received PCV13).[328] Case-control and indirect cohort

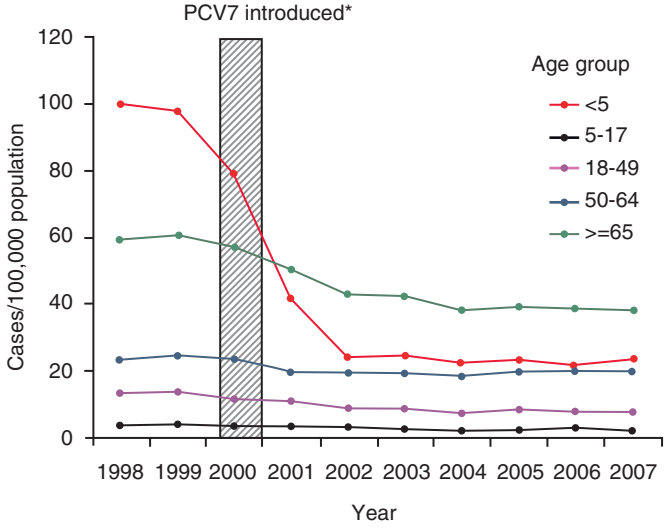

FIG. 199.7 Broad protection of children and adults by immunization of children with pneumococcal conjugate vaccine. An active vaccination program in infants and toddlers by using 7-valent pneumococcal conjugate vaccine (PCV7) beginning in 2000 greatly reduced the incidence of invasive pneumococcal disease in persons of all age groups (45%) from 1998 to 2007, including in adults who did not receive this vaccine.[312] The incidence of all invasive pneumococcal disease and of that caused by the seven vaccine serotypes declined by 76% and almost 100% in children younger than 5 years, respectively, and by 37% and 92%, respectively, in adults age 65 years or older. *(Modified from Pilishvili T, Lexau C, Farley MM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine.* J Infect Dis. *2010;201:32–41.)*

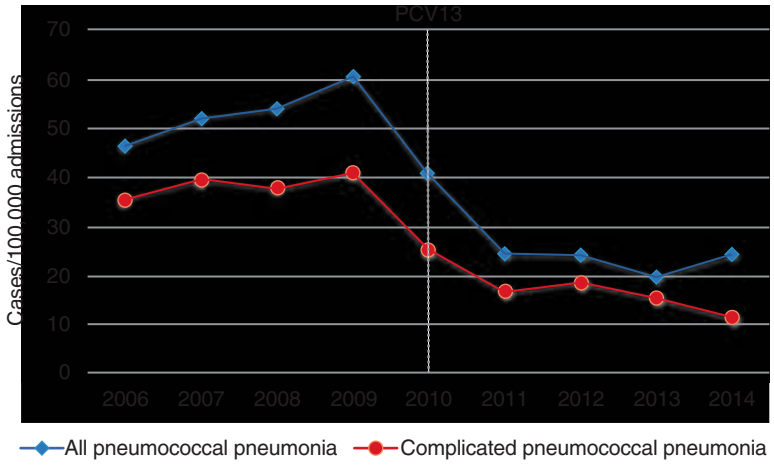

FIG. 199.8 Hospitalization rate for pneumococcal pneumonia in children <18 years old in eight US cities, 2006–2014. Children's hospitals in eight cities were prospectively monitored for microbiologically confirmed cases of pneumococcal pneumonia in the period surrounding the introduction of PCV13 in 2010. *(From Olarte L, Barson WJ, Barson RM, et al. Pneumococcal pneumonia requiring hospitalization in US children in the 13-valent pneumococcal conjugate vaccine era.* Clin Infect Dis. *2017;64:1699–1704.)*

Indications for Adult Pneumococcal Vaccination

Indications:

PPSV-23 Alone	Both PCV-13 and PPSV-23
Patients 19-64 years with ≥1 choronic condition below:	**All patients ≥65 years**
Cigarette smoking	**Patients 19-64 years with ≥1 immunocompromising condition below:**
Chronic heart disease (CHF, cardiomyopathy)	Cerebrospinal fluid leak
Chronic lung disease (asthma, COPD)	Cochlear implant
Diabetes mellitus	Congenital or acquired immunodeficiency
Alcoholism	HIV infection
Chronic liver disease (cirrhosis)	Functional or anatomic asplenia
Reside in nursing home or long-term care facility	Chronic renal failure or nephronic syndrome
	Malignancy
	Solid organ transplant
	Immunosuppression (glucocorticoids, radiation)

Vaccination Timing:

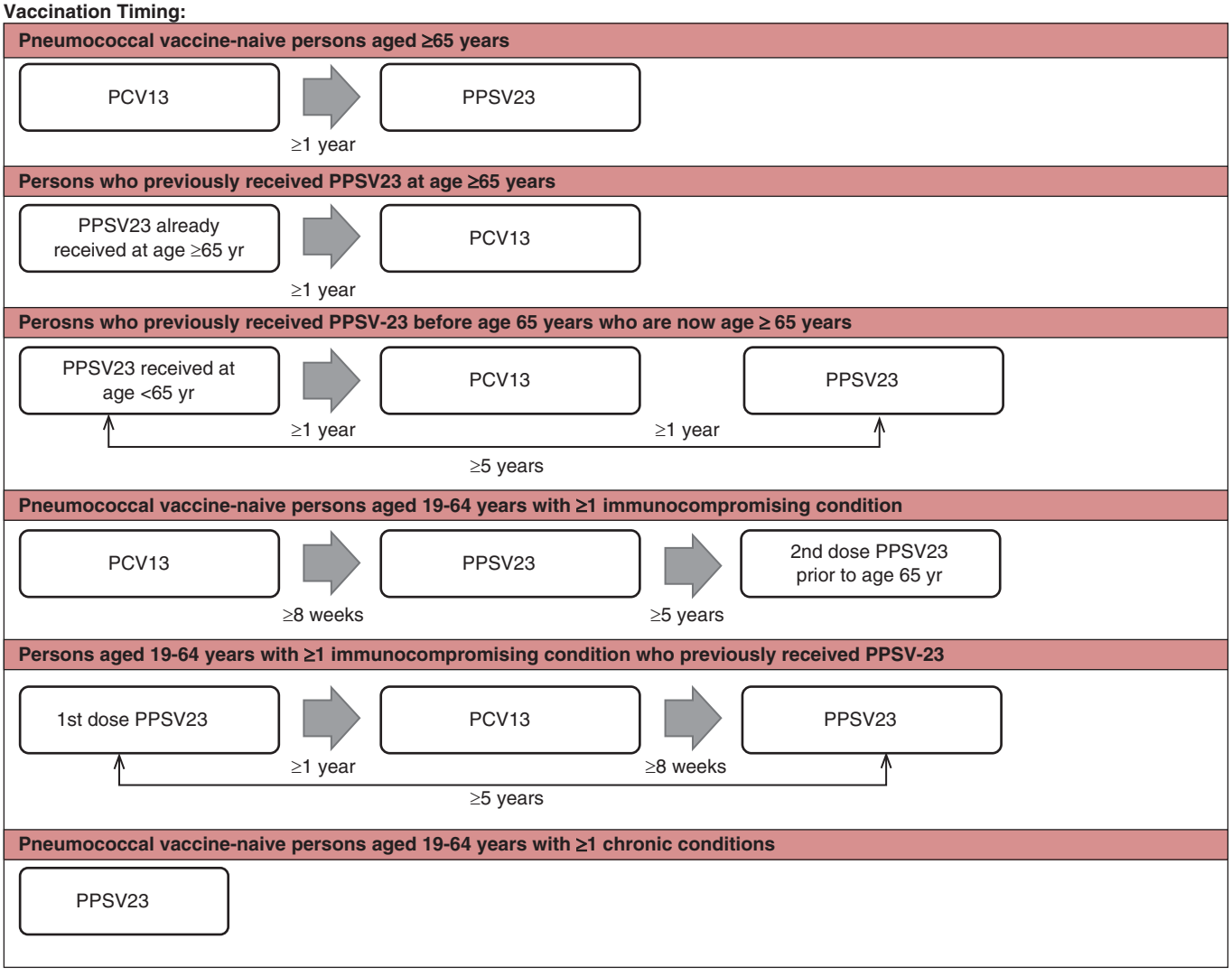

Refer to http://www.cdc.gov/vaccines/acip/index.html

FIG. 199.9 Recommendations for pneumococcal vaccination (PCV13 and PPSV23) in adults age 19 years and older. *Schedule is integrated from references* **359**–361 and 364–366. CHF, *Congestive heart failure;* COPD, *chronic obstructive pulmonary disease;* HIV, *human immunodeficiency virus;* PCV13, *pneumococcal conjugate vaccine (Prevnar);* PPSV23, *pneumococcal polysaccharide vaccine (Pneumovax).*

studies performed postlicensure revealed that the vaccine is protective against invasive disease, particularly bacteremia, in immunocompetent older adults. In the largest study, Shapiro and coworkers[329] showed that invasive pneumococcal disease caused by vaccine serotypes was reduced by 56%, consistent with other studies of invasive disease (54%–81%).[36,330–333] Shapiro and coworkers highlighted clinical protection, a decreasing effect with advancing age, and suggested a waning protective effect over time from initial immunization, consistent with waning levels of specific antibody. However, such protection was not afforded to older adults with immunocompromising conditions, among whom vaccine efficacy has not been confirmed.

Pneumococcal pneumonia comprises the major burden of pneumococcal disease, and most pneumococcal pneumonia and deaths are not associated with bacteremia. Evidence in support of a beneficial

effect of PPSV23 in prevention of pneumococcal pneumonia in older adults is limited,[331,334,335] although diagnosing *S. pneumoniae* as the specific cause of CAP in the absence of bacteremia can be problematic. Among nine clinical trials of PPSV23 against all-cause pneumonia,[336–340] three have shown benefit.[341–343] Among the latter, 1006 nursing home residents in Japan were randomized to receive PPSV23 or placebo.[341] All-cause pneumonia and pneumococcal pneumonia were significantly lower in the vaccine group compared with the control group (cases/1000 person-years: 55 vs. 91 and 12 vs. 32, respectively; both $P < .001$). Moreover, death rates from pneumococcal pneumonia, but not all-cause pneumonia, were lower in the vaccine group. Thus although there is no consensus as to whether PPSV23 reliably prevents all-cause CAP, only a minority of cases are due to *S. pneumoniae.* Use of more sensitive and specific tests to diagnose pneumococcal pneumonia with current methods—PCR assay of respiratory samples and serotype-specific urine antigen detection—may help to resolve this question.

Perhaps the most striking recent advance in pneumococcal vaccine is a prospective, randomized, placebo-controlled trial of PCV13 among almost 85,000 adults age 65 years or older in a community in the Netherlands.[30] Using culture and vaccine serotype–specific urine antigen detection, the study is the first to show efficacy by PCV13 against bacteremia in adults (75%), and, of note, against CAP as well (45.6%), both limited to vaccine serotypes (Table 199.6), with this robust trial design. The 139 vaccine serotype–related cases represented 9% of all CAP in the population, for which no overall efficacy was detected. The study was done prior to the introduction of conjugate vaccine in children in the Netherlands, so the vaccine serotypes likely represented a higher proportion of adult disease than is now present in the United States. Nevertheless, the demonstration of protection against vaccine serotypes with a conjugate vaccine provides proof of concept that pneumococcal pneumonia may be a vaccine-preventable disease in older adults at risk. These data led to time-limited approval of PCV13 for older adults, and recent observational results consider these promising findings in the US community setting.[29] Less clear is whether the broad introduction of conjugate vaccine in adults has led to an additional decrement in adult cases of pneumococcal pneumonia and bacteremia independent of the community protection effects of childhood immunization. Additional strategies are in development to address prevention of the additional, and now majority, of serotypes causing pneumonia and bacteremia in adults that are not currently included in the pediatric-directed PCV13 now used in adults.

Impact of Infant Immunization on Adult Disease

In addition to the direct effects of pneumococcal vaccination of adults, a beneficial indirect effect of vaccination of children has been a progressive and significant decline in both invasive disease and pneumonia[312] in adults, particularly older adults. These effects appear to be a consequence of the decreased frequencies of invasive disease, and, particularly, decreased colonization in vaccinated children,[88,312,344] because the decrement in adults is largely limited to PCV7 and now PCV13 vaccine and vaccine-related serotypes and does not extend substantially to nonvaccine serotypes. This protective effect beyond the vaccinated group is called "herd protection," in which adults experience a lower incidence of disease based on decreased exposure from fewer infected infants, rather than because these adults have increased vaccine-associated immune protection themselves.

Consistent with these results, use of the protein-polysaccharide conjugate vaccine in young children has also yielded dramatic and sustained decreases in hospitalizations for pneumonia in adults as well as children.[324] Hospitalizations for pneumonia declined by 22% in adults age 85 years or older and 51% in adults of all ages, despite no appreciable changes in immunization of the adults themselves, accounting for an estimated 168,000 fewer hospitalizations for pneumonia annually in the United States overall. In 2007, PCV13 serotypes accounted for about one-third of invasive disease in adults age 65 years or older, and the 11 serotypes unique to PPSV23 accounted for 25%.[345] These proportions have continued to decline in children and older adults since PCV13 was introduced in 2010,[346] highlighting the need to extend the vaccine-associated serotype coverage in adults.

Vaccine Protection During HIV Infection

With almost 35 million people living with HIV/AIDS worldwide and their very high incidence of pneumonia and bacteremia, this population comprises perhaps the largest acquired risk group for pneumococcal disease. Antibody responses to PPSV23 are impaired in untreated adults.[347,348] A large prospective, randomized, placebo-controlled trial of 23-valent polysaccharide vaccine among HIV-infected adults in Uganda showed no protection against invasive disease.[177] In fact, the rate of all-cause pneumonia was significantly increased after immunization compared with that in placebo recipients. Pneumococcal vaccine failures were associated with an impaired IgG response to vaccine capsular serotypes.[178,179] However, among HIV-infected children in South Africa, a similarly designed trial of 9-valent conjugate vaccine revealed 53% vaccine efficacy (95% confidence interval, 21%–73%) against invasive disease caused by vaccine serotypes.[315] This efficacy waned rapidly within the first year after vaccination.

Compared with the 23-valent polysaccharide vaccine, the immunogenicity of the 7-valent conjugate vaccine may be somewhat higher among HIV-infected adults with initial immunization,[349] as has been described in patients with splenectomy,[350] but the magnitude of the differences is limited.[351] Alternating two sequential doses of the 23-valent polysaccharide and the 7-valent conjugate vaccine showed higher levels of capsule-specific antibody for three of four serotypes with the conjugate, but the second dose of 7-valent vaccine was beneficial for only one serotype (type 4).[352] In no case was an initial dose of PCV7 detrimental to subsequent responses to either vaccine.

Ultimately, the most powerful data in support of the conjugate vaccine in adults derive from a trial among HIV-infected adults in Malawi who had a previous episode of invasive pneumococcal disease[353] and among whom 20% or greater experienced a second episode.[177,354] This randomized, placebo-controlled, double-blind clinical trial with two doses of PCV7 showed a relative risk of 0.25 (range, 0.08–0.71) against vaccine serotypes in the first year compared with placebo. However, the risk against any invasive pneumococcal disease was not significant because

TABLE 199.6 Effect of PCV13 on Community-Acquired Pneumococcal Pneumonia and Invasive Disease Caused by Vaccine Serotypes Among Adults 65 Years and Older in the Netherlands (2008–2013)

	PCV13 (n = 42,237)	PLACEBO (n = 42,255)	PROTECTION	P
	Cases			
Pneumonia	49	90	45.6%	0.006
Invasive disease	7	28	75.0%	0.0067
	Incidence (100,000/yr)		**No. Needed to Vaccinate**[a]	
Pneumonia	57.6	105.8		656
Invasive disease	8.2	32.9		20,400

Currently, PCV13 is used in approximately 35% of cases and PPSV23 is used in approximately 65%.
No effect on all community-acquired pneumonia cases or mortality among 13 total deaths.
[a]To prevent a single case of the indicated illness.
Data from Bonten MJ, Huijts SM, Bolkenbaas M, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. N Engl J Med. *2015;372:1114–1125.*

TABLE 199.7 Recommendations for Pneumococcal Vaccines in Adults >19 Years of Age (Advisory Committee on Immunization Practices, 2014)

		PCV13	PPSV23	
RISK GROUP	**UNDERLYING MEDICAL CONDITION**	**Recommended**	**Recommended**	**Revaccination 5 Years After First Dose**
Immunocompetent persons	Chronic heart disease[b]		×	
	Chronic lung disease[c]		×	
	Diabetes mellitus		×	
	Cerebrospinal fluid leak	×	×	
	Cochlear implant	×	×	
	Alcoholism		×	
	Chronic liver disease, cirrhosis		×	
	Cigarette smoking		×	
Persons with functional or anatomic asplenia	Sickle cell disease/other hemoglobinopathy	×	×	×
	Congenital or acquired asplenia	×	×	×
Immunocompromised persons	Congenital or acquired immunodeficiency[d]	×	×	×
	Human immunodeficiency virus infection	×	×	×
	Chronic renal failure	×	×	×
	Nephrotic syndrome	×	×	×
	Leukemia	×	×	×
	Lymphoma	×	×	×
	Hodgkin disease	×	×	×
	Generalized malignancy	×	×	×
	Iatrogenic immunosuppression[e]	×	×	×
	Multiple myeloma	×	×	×

[a]All adults age 65 years or older should receive a dose of PCV13 if not previously vaccinated with PPSV23, followed by PPSV23 after 12 months. Adults 65 years or older who have been vaccinated with PPSV23 after age 65 should receive a dose of PCV13 at least 12 months from the initial vaccination. Adults vaccinated with PPSV23 before age 65 should receive a dose of PCV13 at age 65 at least 12 months after initial vaccination and then receive PPSV23 in 12 months.
[b]Including congestive heart failure and cardiomyopathies, excluding hypertension.
[c]Including chronic obstructive pulmonary disease, emphysema, and asthma.
[d]Includes B- (humoral) or T-lymphocyte deficiency, complement deficiencies (particularly C1, C2, C3, and C4), and phagocytic disorders (excluding chronic granulomatous disease).
[e]Diseases requiring treatment with immunosuppressive drugs, including long-term systemic corticosteroids and radiation therapy.

most cases were due to nonvaccine serotypes, and there was no protection against death or all-cause pneumonia. Counterbalancing the results of this efficacy study with the broader prevalence of serotypes in the community, the ACIP recommended the use of a two-dose schedule with a first dose of 13-valent conjugate vaccine, followed 8 weeks or more later with the 23-valent vaccine for patients with HIV infection and adults with immunocompromising conditions.[15] Ongoing studies will reveal whether this rapid succession of doses enhances both the magnitude and persistence of capsule-specific antibody. Current evidence is mixed on the immunogenicity of reimmunization with either PPSV23 or PCV7 (now PCV13) among HIV-infected patients who received a PPSV23 dose 3 to 7 years earlier, even with effective antiretroviral therapy.[355–357]

Vaccine Recommendations

Children are recommended to receive PCV13 at 2, 4, and 6 months of age with a booster dose at 12 to 15 months. Current recommendations for pneumococcal vaccination in adults have evolved over the last several years (Table 199.7).[358] On August 13, 2014, the ACIP recommended routine use of PCV13 for adults age 65 years or older.[359] Rather than receiving a single dose of PPSV23, pneumococcal vaccine–naïve persons or those with unknown age status or those age 65 years or older are recommended to receive PCV13 first, followed by PPSV23 6 to 12 months later. Adults age 19 to 64 years with chronic conditions should receive a dose of PPSV23. Those in this age category with more severe immunocompromising conditions, including HIV infection, should be given PCV13 followed by PPSV23 at least 8 weeks later. In all cases, revaccination with PPSV23 should be delayed until 5 years after the initial PPSV23 dose. Among adults older than 50 years, both PPSV23 and PCV13 are now licensed by the FDA as options.[345] Persons previously vaccinated with PPSV23 are recommended to be vaccinated with PCV13 1 year or more after the most recent PPSV23 vaccination. Persons due for a second PPSV23 injection should have it administered at least 12 months after PCV13 and 5 years or more after the initial PPSV23 dose.[360] Of note, for all immunocompromised adults older than 19 years, those with functional or anatomic asplenia, or immunocompetent adults with CSF leak or cochlear implants, a sequential two-dose regimen is recommended, with a dose of PCV13 first, followed by a dose of PPSV23 at least 8 weeks later (see Table 199.7).[361] Primary immunization of children should include three doses of PCV13 between 2 and 6 months of age or two doses between 7 and 11 months of age, with each receiving a booster dose at 12 to 15 months of age. Children 12 to 23 months of age are given two doses; those 24 to 59 months of age, one dose; and those with chronic or immunocompromising conditions, two doses between 24 and 71 months of age, each without a booster dose.[358]

Revaccination with PPSV23 is indicated for children age 2 years or older and adults with functional or anatomic asplenia and immunocompromising conditions, if given 8 weeks or more after the last dose of PCV13. Of note, for those with chronic conditions, revaccination with PPSV23 is not recommended in either age group because of lack of data supporting the clinical benefit of revaccination. All of these recommendations will likely continue to change as diagnostic methods improve, serotype prevalence changes within groups, and results of clinical trials are revealed. Enhancing our understanding of the epidemiology, microbiology, pathogenesis, and immunology of *S. pneumoniae* will drive the development of more efficient and effective vaccines and approaches to prevention and management of pneumococcal colonization and disease.

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200 *Enterococcus* Species, *Streptococcus gallolyticus* Group, and *Leuconostoc* Species

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SHORT VIEW SUMMARY

Microbiology and Taxonomy

- Enterococci are gram-positive facultatively anaerobic bacteria that usually appear oval in shape and can be seen as single cells, pairs, short chains, or very long chains.
- Enterococci are capable of growing in media containing 6.5% sodium chloride and at temperatures between 10°C and 45°C and are able to hydrolyze esculin in the presence of 40% bile salts.
- *Enterococcus faecalis* and *Enterococcus faecium* are the most clinically relevant species.
- The former *Streptococcus bovis* group is now divided into three main species: *S. gallolyticus*, *S. pasteurianus,* and *S. infantarius*.
- The *Leuconostoc* genus comprises catalase-negative, gram-positive cocci that are usually arranged in pairs or chains and are intrinsically resistant to vancomycin.

Colonization, Virulence, and Genomics

- Enterococci are normal commensals of the gastrointestinal tract.
- Enterococci are capable of dominating the gut microbiota of hospitalized patients who receive broad-spectrum antibiotics.
- Specific hospital-associated genetic lineages of *E. faecium* and *E. faecalis* have evolved to become successful in the nosocomial environment.
- The *S. gallolyticus* group of organisms carries genes encoding potential cell surface determinants that interact with host proteins, which may help explain the association with endocarditis and colonic malignancy.

Epidemiology

- Enterococci are known to cause hospital-associated infections, particularly in critically ill or immunosuppressed patients.
- Enterococci are among the most common organisms causing both hospital-associated and community-associated infective endocarditis (IE).
- Enterococci are able to spread in the hospital via the hands of health care workers and via the environment.
- Infection control measures are critical to prevent acquisition of these microorganisms.
- IE and bacteremia caused by *S. gallolyticus* are highly associated with gastrointestinal malignancies.

Clinical Presentations

- Enterococci are capable of causing bloodstream infections in community-associated and hospital-associated clinical settings.
- IE is one of the most serious and life-threatening infections caused by enterococci.
- Enterococci are one of the leading causes of nosocomial urinary tract infections.
- Enterococci have been described in soft tissue infections, intraabdominal infections, and meningitis.
- The *S. gallolyticus* group of organisms can cause bacteremia and IE.
- *Leuconostoc* causes opportunistic infections mainly in immunocompromised patients, although cases in immunocompetent patients have been reported.

Therapy and Antimicrobial Resistance

- Although most *E. faecalis* isolates are susceptible to ampicillin and vancomycin, *E. faecium* isolates typically exhibit high minimal inhibitory concentrations of ampicillin, and most clinical isolates in the United States are vancomycin-resistant.
- Bactericidal therapy for *E. faecalis* often requires the use of a cell wall agent plus an aminoglycoside or ceftriaxone.
- The therapy of choice for severe infections caused by *E. faecalis* is the combination of ampicillin plus either an aminoglycoside (gentamicin or streptomycin) or ceftriaxone.
- Ampicillin plus ceftriaxone is considered an equivalent alternative to ampicillin plus an aminoglycoside as therapy for *E. faecalis* endocarditis.
- Therapy of severe infections caused by vancomycin-resistant *E. faecium* is challenging, and no consistently reliable therapy has been established.
- Linezolid is the only US Food and Drug Administration–approved drug for multidrug-resistant *E. faecium,* but this compound has important limitations because of the lack of bactericidal effect, toxicity, side effects, and emergence of resistance.
- Daptomycin is a lipopeptide antibiotic with in vitro bactericidal activity against enterococci, but development of resistance during therapy seems to be a limitation for the use of this antibiotic.
- Daptomycin combinations (e.g., with β-lactams, aminoglycosides, tigecycline) may offer some promise in the future for treatment of multidrug-resistant enterococcal infections.
- The therapy of choice for endocarditis caused by the *S. gallolyticus* group of organisms is typically a β-lactam, but therapy varies according to the penicillin minimal inhibitory concentration of the organism.
- Ampicillin or penicillin is the drug of choice for treatment of *Leuconostoc* infections.

HISTORICAL BACKGROUND

The first time that the term *entérocoque* was used appears to have been in an article in the French literature in 1899.[1] The article was referring to a diplococcus found in the gastrointestinal (GI) tract that had the potential to become pathogenic for humans. The first clinical and pathologic description of an enterococcal infection was published the same year (1899)[2] and concerned a patient admitted to "Dr. Osler's Service" with a clinical picture of endocarditis who succumbed to the infection. The authors isolated gram-positive cocci in "pairs and short chains" from the patient's blood and several organs (postmortem). The virulence properties of the organism were confirmed after inoculating it into several animal models and reproducing the pathologic findings observed in the patient. This organism was initially designated *Micrococcus zymogenes* because of its fermentative properties. In 1906 Andrews and Horder[3] described in detail a study of streptococci pathogenic for humans and used the name *Streptococcus faecalis* for the first time to denote the most common species of streptococci present in the intestine of humans and other vertebrates. They referred to previous environmental experiments performed by themselves and Houston in London,[3] indicating that the most common microorganisms collected from London's

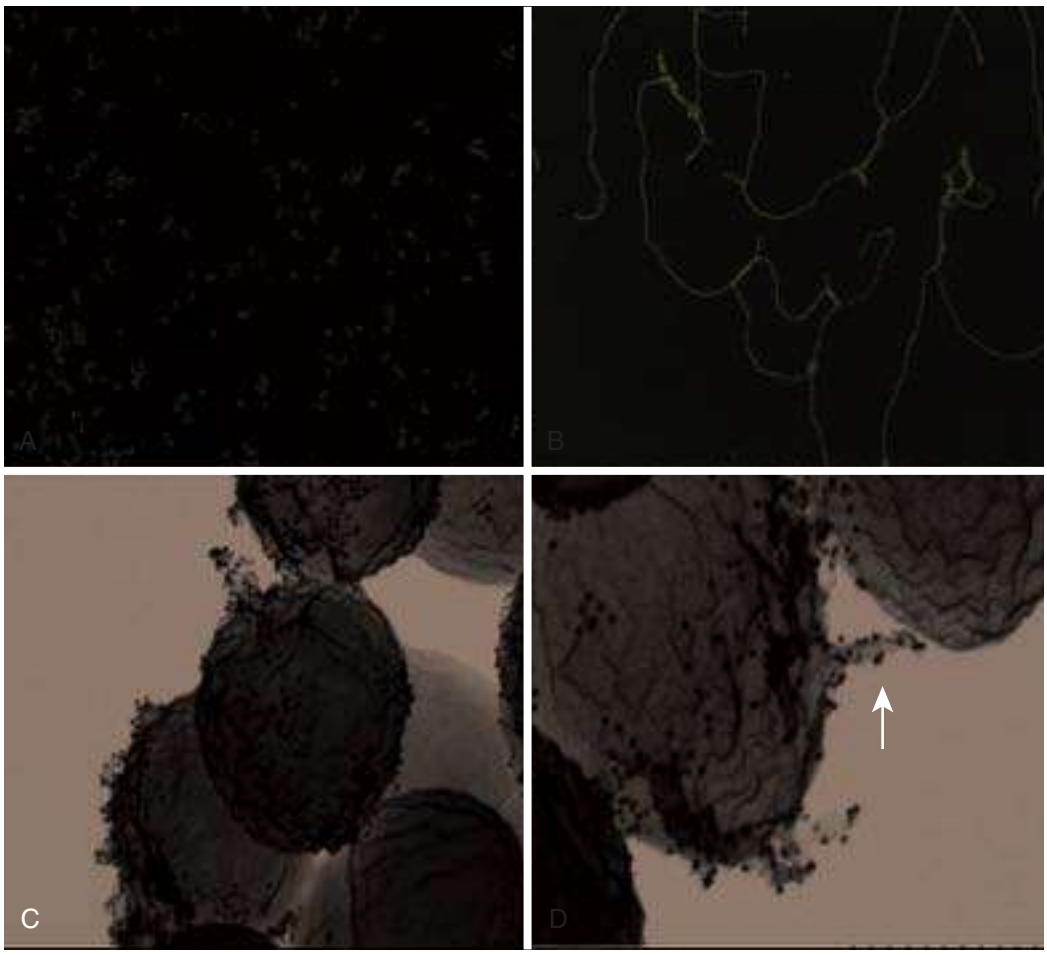

FIG. 200.1 Gram stain and scanning electron microscopy (SEM) of *enterococcus faecalis* isolates. (A) Gram stain of *E. faecalis* V583 after growth in brain heart infusion broth for 5 hours, exhibiting pairs, single cells, and short chains (×100 magnification). (B) *E. faecalis* TX0623, an isolate that produces β-lactamase, showing very long chains after growth using the same conditions indicated in (A) (×50 magnification). (C–D) Immunogold labeling and SEM of *E. faecalis* pili stained with anti-Ebp antibodies (one of the pilus proteins). Pili are seen as projections from the cell surface (*arrow* in D) (C, ×90,000; D, ×150,000 magnification).

air were "intestinal streptococci," which probably originated from horse dung, "which forms so large of a part of the organic contamination of London's air."

In December 1937 Sherman,[4] addressing the Society of American Bacteriologists, indicated that "the enterococcus" was a nonspecific term used for streptococci isolated from the gut, which was "a screen behind which the investigator could hide his ignorance of the organisms with which he worked." Sherman[4] proposed to group the enterococci as *S. faecalis, S. faecalis* var. *hemolyticus, S. faecalis* var. *zymogenes, S. faecalis* var. *liquefaciens,* and *S. durans,* all of which displayed common phenotypic characteristics that included growth in the presence of 6.5% sodium chloride, pH 9.6, and high temperature. Studies in the 1940s and 1950s showed that an organism initially identified in 1919 as *S. faecium*[5] had distinct characteristics that differentiated it from *S. faecalis,*[6,7] and in 1970 a formal proposal that the enterococcal streptococci be considered a new genus based on their distinct phenotypic characteristics was put forward.[8] However, it was not until 1984 that *Enterococcus* was widely confirmed as a separate genus from *Streptococcus* after nucleic acid hybridization experiments were performed, and genetic tools were subsequently applied to differentiate the varied species of enterococci.[9] More recently, using next-generation sequencing technology and molecular clocks to analyze the genomes of an array of representatives of the genus, the emergence of the ancestors of the modern enterococci was estimated to coincide with the advent of terrestrial land animals some 400 million years ago, speaking to the long and successful history of this durable commensal and opportunistic pathogen.[10]

MICROBIOLOGY AND TAXONOMY

Organisms belonging to the genus *Enterococcus* are gram-positive facultatively anaerobic bacteria that usually appear oval in shape and can be seen as single cells, pairs, short chains, or very long chains (Fig. 200.1). They are capable of growing in media containing 6.5% sodium chloride and at temperatures between 10°C and 45°C, and they are able to hydrolyze esculin in the presence of 40% bile salts and produce a leucine aminopeptidase and pyrrolidonyl arylamidase (PYR) (except for *E. cecorum, E. columbae, E. pallens,* and *E. saccharolyticus*). Enterococci are usually α-hemolytic or γ-hemolytic on trypticase soy, 5% sheep blood agar (Table 200.1), whereas some are β-hemolytic (due to the acquisition of a hemolysis/cytolysin gene) on horse, rabbit, or human blood. Most enterococci react with Lancefield group D antisera and some react with group Q antisera, and some of them are motile (e.g., *E. casseliflavus* and *E. gallinarum*). Table 200.2 lists some enterococci spp. isolated from human infections; the vast majority of clinical infections are produced by two species (*E. faecalis* and *E. faecium*), and clinical laboratories usually do not identify enterococci to the species level. However, in certain clinical scenarios or in epidemiologic studies, it may be important to differentiate between these two species because they appear to differ in their virulence and antibiotic resistance profiles (see later).

Conventional methods to identify enterococci at the species level include manual biochemical differentiation based on several tests (e.g., acid formation and hydrolysis of arginine); nonetheless, because of the laboriousness of this approach, laboratories usually rely on automated

TABLE 200.1 Phenotypic Differentiation Among Enterococci, *Leuconostoc,* and *Streptococcus Gallolyticus* Group

	PHENOTYPIC CHARACTERISTICS									
GENUS	**VAN**	**Gas**	**BE**	**PYR**	**LAP**	**NaCl**	**10° C**	**45° C**	**HEM**	**Lancefield Group**
Enterococcus spp.	S/R	—	++	++	++	++	++	++	α/γ	D (+)
Leuconostoc spp.	R	++	+	—	—	+	+/–	+/–	α/γ	D(+/–)
Streptococcus gallolyticus group[a]	S[b]	—	++	—	++	—	+/–	++	α/γ	D (+)

[a]Includes *Streptococcus gallolyticus* subsp. *gallolyticus (S. gallolyticus), S. gallolyticus* subsp. *pasteurianus (S. pasteurianus),* and *S. infantarius* subsp. *infantarium (S. infantarium).*
[b]Vancomycin-resistant isolates carrying the vanB gene cluster have been described.
++, Positive in ≥85% of isolates; *+,* positive in 50%–84% of isolates; *+/–,* positive in 16%–49% of isolates; *—,* positive in ≤15% of isolates; *10°C,* growth at 10°C; *45°C,* growth at 45°C; *BE,* hydrolysis of esculin in the presence of bile; *Gas,* production of gas in de Man, Rogosa, and Sharpe broth; *HEM,* hemolysis in sheep blood agar; *LAP,* production of leucine aminopeptidase; *NaCl,* growth in medium containing 6.5% sodium chloride; *PYR,* production of pyrrolidonyl arylamidase; *R,* resistant; *S,* susceptible; *VAN,* vancomycin susceptibility.
Modified from Teixeira LM, Carvalho MGS, Facklam RR, et al. Enterococcus. In: Murray PR, Baron EJ, Jorgen JH, et al, eds. Manual of Clinical Microbiology. *11th ed. Washington, DC: American Society for Microbiology Press; 2015:403–421.*

TABLE 200.2 Enterococcal Species Isolated From Human Infections

E. faecalis
E. faecium
E. gallinarum
E. durans
E. avium
E. raffinosus
E. pallens
E. gilvus
E. cecorum
E. malodoratus
E. italicus
E. sanguinicola
E. mundtii
E. casseliflavus/flavescens
E. dispar
E. hirae
E. pseudoavium

methods or rapid biochemical methods such as the analytical profile index system, which appear to be accurate for *E. faecalis,* but not for other enterococcal species. Because *E. faecium* is capable of fermenting arabinose, some selective arabinose-containing agars have been used to differentiate *E. faecium* from other clinically relevant enterococcal species.[11] In addition, several molecular techniques have been developed for species differentiation, although they are not used routinely by clinical laboratories; among the most popular, amplification of *ddl* genes,[12] amplification of or a probe for the *ace* gene,[13] and sequencing of the 16S ribosomal RNA (rRNA) gene appear to reliably differentiate the relevant enterococcal species. Automated nucleic acid hybridization–based rapid diagnostic technologies and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry are now being used more commonly in the clinical microbiology laboratory; these techniques use species-specific sequence variation or protein fragment profiles to quickly identify *E. faecalis* and *E. faecium*. Furthermore, resistance to ampicillin usually indicates *E. faecium,* whereas nonsusceptibility to quinupristin-dalfopristin (Q-D) is usually seen in *E. faecalis.*

COLONIZATION, VIRULENCE, AND GENOMICS

Enterococci have well-adapted mechanisms to survive in the GI tract of humans. The human colonic microbiota comprises approximately 10^{13} commensal bacteria per gram of contents, encompassing more than 100 culturable bacterial species and many more nonculturable species, with a predominance of anaerobes.[14] Enterococci are clearly a minor population in relation to the anaerobic commensals in a normal host; they also appear to have a symbiotic relationship with the immune system and the other bacteria. However, one of the main effects that antibiotics have in the human gut is to alter the dynamics of colonization in favor of enterococci, which are naturally tolerant to a number of antimicrobial compounds (see "Therapy and Antimicrobial Resistance" later). Antibiotics that are excreted in the bile or have substantial antianaerobic activity without inhibiting enterococci (e.g., certain cephalosporins) have been shown to dramatically increase colonization of the GI tract by enterococci (e.g., vancomycin-resistant enterococci [VRE]).[15,16,17] Moreover, administration of broad-spectrum antibiotics favors VRE colonization of the murine GI tract in part by downregulating the intestinal expression of the antimicrobial peptide RegIIIγ (a bactericidal lectin produced by intestinal epithelial and Paneth cells), which has activity against gram-positive intestinal organisms. This effect appears to be due to the fact that broad-spectrum antibiotics suppress gram-negative organisms (including anaerobes) in the gut that are responsible for the activation of the signals necessary for the production of the RegIIIγ peptide through the lipopolysaccharide present in their outer membranes.[18] Moreover, the dominance of enterococci (particularly VRE) in the GI tract of hospitalized neutropenic patients after the administration of antibiotics has been shown to be a predictor of subsequent bloodstream infection in these patients.[19] Intestinal colonization of VRE also seems to be influenced by the presence of specific bacterial species of the gut anaerobic microbiota. Microbiota containing *Barnesiella* species conferred resistance to the domination by VRE in mice[20]; the presence of this species also correlated with decreased GI VRE colonization of patients undergoing allogeneic hematopoietic stem cell transplantation and receiving antibiotics.[20] A consortium of bacteria isolated from ampicillin-treated mice resistant to VRE colonization highlighted the complexity of unraveling the contributions of individual species within the microbiome. *Blautia producta,* a commensal anaerobe, directly inhibited VRE growth in the mouse GI tract after oral challenge; however, this inhibitory action depended on the presence of *Clostridium bolteae*. Furthermore, the β-lactamase–producing anaerobes *Bacteroides sartorii* and *Parabacteroides distasonis* were also required, presumably to clear inhibitory concentrations of ampicillin from the local environment and allow *B. producta* to proliferate.[21] Greater understanding of the interplay between the organisms in the microbiome and the host may allow for the therapeutic use of beneficial microbial cocktails to prevent VRE colonization in high-risk patients. Another factor that may also play a role in colonization and overgrowth of enterococci in the gut is increased stomach pH, usually secondary to the administration of proton pump inhibitors, a strategy commonly used in critically ill patients to reduce the incidence of aspiration pneumonitis.[22]

Considerable research has been performed in the investigation of pathogenic determinants that increase the ability of enterococci to cause disease by enhancing their virulence, survival, or colonizing capacity in human hosts. The cytolysin hemolysin is a bacterial toxin, often encoded by pheromone-responsive plasmids, that is capable of lysing eukaryotic (and prokaryotic) cells and has been shown to contribute to *E. faecalis* virulence.[23] Gelatinase and serine protease are bacterial enzymes that contribute to virulence in *E. faecalis*[24] by several potential mechanisms that include, among others, (1) facilitation of microbial invasion by altering immunoglobulins or complement molecules; (2) processing of virulence factors to regulate autolysis and release of high-molecular-weight extracellular DNA, a critical component for the development of *E. faecalis* biofilms[25]; and (3) degradation of host connective tissues, exposing ligands for bacterial attachment and possibly providing nutrients for the cell. The expression of gelatinase and serine protease genes is regulated by *fsr,* a two-component quorum-sensing global regulatory system that is similar to the *agr* system of *Staphylococcus*

aureus.[26,27] Gls24 of *E. faecalis* and Gls20/Gls33 of *E. faecium* are thought to be general stress proteins that have been shown to be important in virulence in both mouse peritonitis[28,29] and rat endocarditis[30] models. Although their function has not been fully elucidated, they are associated with resistance of enterococci to bile salts, and their homologues in *S. aureus* have been linked to upregulation of VraSR and PrsA, important members of the cell wall stress response.[31] Using comparative genome analysis, a locus encoding a putative phosphotransferase system was shown to increase the ability of *E. faecium* to colonize the murine intestinal tract during antibiotic treatment, and this system has been implicated in both biofilm formation and pathogenesis in a rat model of endocarditis.[32,33]

Cell surface components are important factors in bacterial virulence because they are usually the first molecules to interact with the host tissue or immune system or both. Aggregation substance of *E. faecalis* is encoded by pheromone-responsive plasmids and assists in a particular type of conjugative plasmid transfer as well as contributing to virulence (i.e., endocarditis). Aggregation substance proteins increase the adherence and internalization of enterococci into several eukaryotic cells (phagocytes, renal cells, intestinal cells, and epithelial cells) and enhance adherence of producing bacteria to serum and extracellular matrix proteins such as fibrin, fibronectin, thrombospondin, vitronectin, and collagen type I.[34] The *E. faecalis* surface protein (Esp) is another property found in some strains (and its homologue in *E. faecium*); it is a protein that appears to function as an adhesin involved in the formation of biofilms in a glucose-dependent manner.[35] Ace (adhesin to collagen of *E. faecalis*) belongs to the family designated as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) and is considered part of the *E. faecalis* core genome. The Ace protein binds to collagen via what has been called the collagen hug model, in which the protein embraces the collagen molecule after initial docking.[36] A similar protein, Acm, identified in *E. faecium,* is important in the pathogenesis of endocarditis and implicated in the emergence of *E. faecium* as an important nosocomial pathogen.[37]

Another surface protein found to be important in enterococcal pathogenesis is ElrA (enterococcal leucine-rich repeat-containing protein). This polypeptide is a member of the WxL family of surface proteins, and deletion of the encoding gene attenuated *E. faecalis* virulence in a mouse peritonitis model.[38] The characterization of pili on the surface of gram-positive bacteria has been a major step in the understanding of bacterial virulence. In *E. faecalis,* the presence of pili (Ebp) was demonstrated (see Fig. 200.1), and the characterization of *ebp* genes encoding the pilus subunits led to establishing that these structures play a major role in biofilm formation and fibrinogen adhesion and are important in the pathogenesis of experimental endocarditis and urinary tract infections (UTIs).[39,40] Genes encoding homologous pilus subunits have also been identified in *E. faecium,*[41] indicating that the pili are ubiquitous structures of enterococci.

Polysaccharides on bacterial surfaces may be important pathogenic determinants and may affect leukocyte-mediated killing of bacteria. Certain *E. faecium* strains are resistant to polymorphonuclear cell killing, a characteristic that might be due to a carbohydrate-containing surface moiety.[42] In addition, antibody to a capsular polysaccharide component purified from an *E. faecalis* strain enhanced phagocytosis and killing of some strains of both *E. faecalis* and *E. faecium;* the capsular material may have vaccine potential because a reduction in bacterial numbers recovered from different organs of immunized mice was obtained compared with nonimmunized control subjects.[43,44] Using rabbit antisera for the typing of *E. faecalis,* four capsular serotypes (1, 2, 4, and 7) were found to be present in most clinical isolates,[45] and at least two types of gene clusters for the production of polysaccharide have been characterized (designated *epa*[46] and *cps* loci[47]). Passive immunization with antibodies against *E. faecalis* lipoteichoic acid promoted the clearance of *E. faecalis* bacteremia in mice. The antibodies bound lipoteichoic acid from other gram-positive organisms and opsonized *Staphylocccus epidermidis, S. aureus,* and group B streptococci,[48] raising the possibility of using these antibodies for possible vaccine development.

In *E. faecium,* acquisition of a very large plasmid (approximately 250 kb) by a commensal strain increased the virulence of the organism in a mouse peritonitis model.[49] These virulence plasmids have been highly associated with clinical strains versus commensal isolates.[50] More recently, genes associated with the regulation of oxidative stress have been identified as important in the virulence of *E. faecium*. One of these regulators is AsrR, which uses cysteine oxidation to sense hydrogen peroxide mediating the activation of many genes potentially involved in pathogenesis (e.g., *acm*), antibiotic and antimicrobial peptide resistance, oxidative stress, and adaptive responses. Among the genes regulated by AsrR was *pbp5*, encoding a low-affinity penicillin-binding protein 5 (PBP5); deletion of *asrR* markedly decreased the bactericidal activity of ampicillin and vancomycin and increased the ability of the mutant to form biofilm and persistence in *Galleria mellonella* and mouse systemic infection models.[51]

Sequencing of enterococcal genomes has been another useful tool to facilitate the understanding of the complicated pathway by which enterococci evolved from commensals to pathogens. Sequencing of the genome of the first vancomycin-resistant *E. faecalis* strain isolated in the United States (designated V583) indicated that more than one-quarter of its genome is mobile DNA. A pathogenicity island, which is a large genetic element carrying a set of putative virulence-associated genes,[52] a transposon carrying the *vanB* gene cluster, three plasmids with antibiotic-resistance determinants, and insertion sequences were the most prominent, potentially mobile elements of V583.[53] The pathogenicity island encodes factors that may enable enterococci to gain an advantage in the gut, such as the cytolysin that has antibacterial properties, several surface adhesins, several carbohydrate utilization pathways, and enzymes that may permit colonization of certain areas of the intestine. It has been postulated that this pathogenicity island was acquired by an ancestral *E. faecalis* clonal strain that evolved to acquire antibiotic resistance determinants, thus becoming equipped to cause problematic infections in humans. Importantly, V583 and other multidrug-resistant strains lack an active CRISPR (clustered regularly interspaced short palindromic repeats)/Cas system, which acts as an immune system protecting the bacterial cell from incoming foreign DNA (i.e., phages). This deficiency has been associated with an increased frequency of mobile DNA elements (including plasmids and transposons), which can carry resistance determinants and may explain their nosocomial predominance.[54] Similarly, sequencing of the entire genome of another *E. faecalis* strain (*E. faecalis* OG1RF) revealed considerable variation in gene content in this species. As opposed to V583, *E. faecalis* OG1RF lacks plasmids and the pathogenicity island but is still able to cause infection in animal models. This strain has an intact CRISPR/Cas system and lacks the mobile elements typical of V583 but harbors genes predicted to encode proteins involved in adherence, defense against bacteriophages, metabolism of *myo*-inositol, and novel surface proteins.[55] Recent genome comparisons indicate that there are two ancestrally distinct clades of *E. faecium* (animal and human commensal)[56,57] that diverged from each other at least thousands of years ago; a third subclade responsible for most human, health care–associated *E. faecium* infections is estimated to have evolved from the animal clade approximately 75 years ago, coinciding with the time that antibiotics were introduced in clinical medicine to become successful in the nosocomial environment.[56–58] This hospital-associated *E. faecium* clade, which mostly contains clinical and outbreak-associated strains, characteristically contains *pbp5-R,* the allele encoding the ampicillin-resistant version of PBP5, and insertion sequences, such as IS*16*.

EPIDEMIOLOGY OF ENTEROCOCCAL INFECTIONS

The two most common species responsible for the vast majority of enterococcal infections are *E. faecalis* and *E. faecium.* The only other species of enterococci known to be responsible for outbreaks and nosocomial spread, albeit rare, is *E. gallinarum.*[59] Taken together, enterococci were the third leading cause of nosocomial infections in the United States from 2011 to 2014, with VRE now accounting for approximately 30% of enterococcal infections with most VRE isolates being *E. faecium* (>90%).[60] The first step in the infectious process appears to involve colonization of the GI tract by hospital-associated strains, which may persist for months or years, although direct inoculation onto intravenous or urinary catheters, onto intravenous stop-cock sets, or via thermometers has been reported. The hospital environment can be heavily colonized

with VRE, including bed rails, linen, doorknobs, bedpans, urinals, blood pressure cuffs, stethoscopes, and monitoring equipment, among others.[61] Risk factors associated with increased VRE colonization include the presence of immunosuppression or serious comorbid conditions (e.g., diabetes, renal failure, high Acute Physiology and Chronic Health Evaluation [APACHE] score); increased length of hospital stay; residence in a long-term care facility; proximity to another colonized or infected patient, including sharing a room or hospitalization in a room previously occupied by a patient colonized with VRE; and invasive procedures and administration of broad-spectrum antibiotics (e.g., cephalosporins), antianaerobic drugs (e.g., metronidazole), or vancomycin.[62,63] The hands of health care workers seem to be the most common source of transmission of VRE, and the Society for Health Epidemiology of America has published specific guidelines to curtail this transmission.[64] The organisms are capable of surviving on the hands, gloves, and gowns of health care workers for prolonged periods, and independent risk factors for glove and gown contamination include contact with a colonized patient's catheter or drain, trunk, or lower extremity.[65]

Once a patient becomes colonized with VRE, the risk of developing a subsequent bloodstream infection with the same VRE-colonizing strain appears to increase,[66] although some studies have not found an association. Rates of bloodstream infections in patients colonized with VRE range from 0% to 34% and seem to be higher among patients with cancer and solid-organ and bone marrow transplant recipients. Risk factors associated with developing a VRE bloodstream infection in a patient already colonized with VRE include cancer or diabetes (relative risk [RR], 3.91), GI procedures (RR, 4.56), acute renal failure (RR, 3.1), exposure to vancomycin (RR, 1.95), infection of a site other than blood (odds ratio, 3.9),[67] and dominance of VRE in the GI tract following use of broad-spectrum antibiotics.[19] Among patients with leukemia, concurrent *Clostridioides difficile* (formerly *Clostridium difficile*) infection was associated with increased risk of developing a VRE bloodstream infection.[68] In addition, two meta-analyses evaluated the mortality of patients with VRE bacteremia compared with patients with vancomycin-susceptible enterococci (VSE).[69,70] Both studies concluded that patients with bacteremia due to VRE were approximately 2.5 times more likely to die than patients with VSE bacteremia, indicating that the development of vancomycin resistance is a poor prognostic sign in critically ill patients. Despite the introduction of antibiotics with in vitro bactericidal activity against VRE, such as daptomycin, this effect persists, with data showing VRE bacteremia associated with an increase in mortality (odds ratio, 1.8) and longer hospital stays (mean 5.01 days) compared with VSE bacteremia.[71]

CLINICAL MANIFESTATIONS OF ENTEROCOCCAL DISEASE

Bacteremia and Endocarditis

Bacteremia and infective endocarditis (IE) are common presentations of enterococcal disease. Bacteremia without endocarditis is by far the more frequent presentation, and enterococci are one of the leading causes of nosocomial bacteremias.[60] Frequent sources of the bacteremia are the genitourinary and GI tracts in patients with infections originating outside the hospital (endocarditis should always be ruled out). Intravascular or urinary catheters are the most common sources of nosocomial bacteremia, and intraabdominal, pelvic, biliary tract, wound (including in burned patients), and bone infections have also been documented as sources of bacteremia. Enterococcal bacteremia often occurs in debilitated patients who have received antibiotics and have serious underlying conditions, and polymicrobial bacteremia can be seen in approximately 50% of cases; however, polymicrobial bacteremia has not been independently associated with mortality.[72,73] Data suggest that *E. faecium* bloodstream infections may have a worse prognosis than *E. faecalis,* probably because these organisms are much more resistant to antibiotics and are increasingly difficult to treat.[74] Enterococcal bacteremia and meningitis (see "Meningitis") have also been associated with the *Strongyloides* hyperinfection syndrome (see Chapter 286).[75]

The percentage of patients who have endocarditis as the cause of detectable enterococcal bacteremia varies according to the study and population studied, ranging from about 1% to 32%. Enterococci are the second (after staphylococci) most common cause of health care–associated endocarditis and third (after staphylococci and streptococci) most common cause of endocarditis in the community setting depending on the series and patients examined, accounting for 5% to 20% of cases of endocarditis. In a worldwide observational cohort study that included 2781 patients with endocarditis, enterococci were the third most common etiologic agents after *S. aureus* and streptococci, with a higher incidence in North America compared with other regions of the world.[76] Factors independently associated with IE in 647 patients presenting with *E. faecalis* bacteremia included monomicrobial cultures with *E. faecalis* (hazard ratio [HR], 3.6), prosthetic heart valve (HR, 6.2), male sex (HR, 2.0), and community acquisition (HR, 1.8).[77] The NOVA score, a scoring system used to identify patients with predominately *E. faecalis* bacteremia at low risk of IE in whom transesophageal echocardiography could be deferred, has been validated using an external cohort.[77,78] The criteria include three of three positive blood cultures (or the majority if more than three blood cultures obtained; 5 points), unknown origin of bacteremia (4 points), prior valvular disease (2 points), and presence of a heart murmur (1 point), with patients receiving a score <4 at low risk of IE. Although the majority of the validation cohort with IE had a score ≥4 (97%), clinical judgment and an index of suspicion should still be used to guide performing transesophageal echocardiography in select patients.

Enterococci can affect both native and prosthetic valves and can cause both community-acquired and nosocomial endocarditis, with *E. faecalis* being recovered much more frequently than *E. faecium* or other enterococcal species. The disease usually occurs in the setting of damaged heart valves, and the mitral and aortic valves are usually involved, although endocarditis of apparently intact valves also occurs.[79,80] Most patients tend to be male and elderly with comorbidities, although enterococcal endocarditis in women of childbearing age has been well documented. The infection usually originates from the genitourinary or GI tract. Procedures associated with the development of enterococcal endocarditis include cystoscopy, cesarean section, prostatectomy, transrectal prostatic biopsy, transjugular intrahepatic portosystemic shunt, extracorporeal shock wave lithotripsy, colonoscopy, fiberoptic sigmoidoscopy, and liver biopsy.[7,80] Malignant and inflammatory lesions of the gut and biliary tract may also be the source of endocarditis.[81]

Most patients with enterococcal endocarditis display a subacute course, and the most common clinical manifestations include fever; presence of a murmur; and constitutional symptoms such as weight loss, generalized aches, and malaise. Peripheral signs of endocarditis such as petechiae, Osler nodes, and Roth spots have been found less frequently (approximately 15%) than in endocarditis caused by other organisms.[80] Atypical manifestations include polyarthritis,[82] spondylodiskitis,[83] metastatic abscesses in the spleen,[84] and empyema.[85] The most common complication of enterococcal endocarditis is heart failure, which occurs in about half of patients, with a significant percentage requiring valve replacement. Embolization occurs in 27% to 43% of patients,[80,86] and the brain appears to be the most common end organ. Mortality ranges from 11% to 35%; in a cohort of 500 patients with confirmed enterococcal endocarditis, increasing age (HR, 1.02 per 1-year increment), heart failure (HR, 2.43), and stroke (HR, 1.9) were all associated with an increase in 1-year mortality.[87]

Urinary Tract Infections

Enterococcal UTI in young healthy women without a history of urinary tract instrumentation or anatomic abnormalities is infrequent (<5% of all UTIs) and was first reported in 1906.[3] Conversely, enterococcal UTIs are well documented in the hospital and usually associated with indwelling catheters, instrumentation, and abnormalities of the genitourinary tract. Data from the Centers for Disease Control and Prevention National Healthcare Safety Network from 463 hospitals across the United States indicate that enterococci are the fifth most common organism isolated from catheter-associated UTIs, with *E. faecium* and *E. faecalis* accounting for the majority of organisms isolated.[60] It is sometimes difficult to differentiate between infection and colonization in the hospital setting; therefore the isolation of greater than 10^5 colony-forming units of *Enterococus* spp. from urine may represent colonization, and removal of the catheter may suffice to eradicate the presence of the organism. Recurrent UTIs and previous antibiotic treatment have also

been associated with enterococcal UTIs. The infection appears to be more common in older men, and associated prostatitis or epididymitis have been documented. Enterococci can also cause complicated UTIs (although less frequently than *Escherichia coli*), with the development of pyelonephritis and perinephric abscesses that can lead to bacteremic episodes.[7]

Meningitis

Enterococci are uncommon causes of meningitis, accounting for approximately 0.3% to 4% of meningitis cases according to different series.[88,89] Two presentations are usually described: spontaneous and postoperative meningitis. *E. faecalis* is the most common species isolated, followed by *E. faecium, E. gallinarum, E. avium,* and *E. casseliflavus*. Spontaneous meningitis is a community-associated infection that often manifests in patients with severe comorbidities such as diabetes, chronic renal failure, pulmonary or cardiovascular disease, immunosuppression (including steroid use and human immunodeficiency virus), malignancies, transplantation, and splenectomy. In approximately 15% of cases, meningitis can manifest in apparently healthy individuals with no clear focus of infection.[89,90] In children, spontaneous meningitis has been reported in association with central nervous system (CNS) pathology (neural tube defects and hydrocephalus), prematurity, recent surgery, or congenital heart disease. Meningitis in the setting of disseminated strongyloidiasis has also been well characterized. Postoperative meningitis is a hospital-associated infection, and the presence of shunt devices appears to be the most important predisposing factor. Enterococcal meningitis has rarely been reported as a complication of lumbar or ventricular tap, placement of CNS electrodes, and epidural anesthesia.[89]

The clinical characteristics of meningitis are similar in patients with spontaneous and postoperative presentations. Patients have an acute course with fever, altered mental status, and signs of meningeal irritation; in some cases, coma, petechial rash, shock, and focal CNS deficits may develop. Cerebrospinal fluid (CSF) findings include pleocytosis, increased protein levels, and low glucose. In a series of 140 patients with enterococcal meningitis, the median CSF leukocyte count was 533 mm^3, and only 35% of patients had a leukocyte count of less than 200 mm^3, with a positive Gram stain seen in up to 40% of patients.[89] Accompanying bacteremia can be found in more than half of cases of spontaneous meningitis. Complications of enterococcal meningitis include hydrocephalus, stroke, and brain abscesses. The overall mortality approaches 20%, and poor prognostic factors include seizures, altered consciousness, advanced age, respiratory failure, septic shock, and the presence of hypoglycorrhachia or decreased white blood cell count in the CSF[88]; residual sequelae in approximately 17% of patients[89] were reported in one study.

Intraabdominal and Pelvic Infections

Enterococci are commensals of the GI and genitourinary tracts and are commonly isolated from abdominal and pelvic infections, usually with gram-negative and anaerobic organisms; the role of enterococci in these infections is controversial.[91] An analysis of six clinical trials with the objective of examining the use of antibiotics without enterococcal activity in the treatment of intraabdominal infections did not find any case of treatment failure, despite evidence of the presence of enterococci in 20% to 30% of initial cultures.[92] Similarly, several studies have shown that community-associated complicated intraabdominal infections with mixed microbiota that include enterococci can be treated with surgery and antibiotics that do not exhibit in vitro activity against enterococci. Moreover, data from animal experiments indicate that enterococci alone do not cause intraabdominal sepsis when injected intraperitoneally, unless other substances or organisms that promote abscess formation are added.[93] Nonetheless, several well-conducted studies have demonstrated that enterococci can cause treatment failures and adverse outcomes; a randomized, prospective, double-blind trial comprising 330 patients concluded that the isolation of enterococci from intraabdominal collections was a predictor of treatment failure.[94] Several other series confirmed these observations, concluding that the presence of enterococci increases postoperative infectious complications and mortality in these patients.

The bulk of evidence indicates that the use of antienterococcal antibiotics may not be necessary for initial treatment of acute intraabdominal infections in most cases. However, the apparent increased frequency of isolation of nosocomial multidrug-resistant enterococci indicates that antienterococcal therapy should be considered for immunocompromised and severely ill patients with nosocomial peritonitis and abdominal sepsis or persistent collections who have received broad-spectrum antibiotics that do not have activity against enterococci (e.g., cephalosporins) as well as patients with peritonitis and damaged or prosthetic heart valves (to prevent endocarditis).[91] Enterococci are also capable of producing spontaneous peritonitis and empyema in cirrhotic patients and patients with chronic renal failure and have been reported as etiologic agents in peritonitis associated with chronic ambulatory peritoneal dialysis.[7]

Neonatal Infections

Enterococci are part of the normal adult vaginal microbiota and can be acquired by neonates during delivery. Although carriage of enterococci is common, colonization with VRE is rare; it was found in about 1% in a multicenter study of 1320 infants.[95] The organisms have been implicated in approximately 6% of late-onset sepsis cases, 5% of pneumonias, 9% of surgical site infections, 10% of bacteremias, 17% of UTIs, and 6% of meningitis cases in neonatal units.[96,97] In cases of late-onset sepsis, enterococcal infections are usually nosocomial and may be polymicrobial. Affected patients usually have had a prolonged hospital stay, low birth weight, prior antibiotic therapy, and several invasive procedures. The clinical presentation of sepsis is usually associated with localized sites of infection or necrotizing enterocolitis. Endocarditis is rare in the neonatal period but can be seen in infants with prolonged enterococcal bacteremia.[96]

Several outbreaks of neonatal sepsis have been documented in the literature. In 1982, an outbreak of *E. faecium* was documented in Virginia[98]; the infants had severe underlying conditions, with the presence of endovascular devices and nasogastric tubes, and were premature. Similarly, a 6-month outbreak of *E. faecalis* neonatal sepsis was documented in Colorado in 1987; most of the infants were also premature, with low birth weight, and had intravascular devices and had undergone bowel surgery.[99] Outbreaks of VRE causing sepsis in neonates have also been well characterized in different parts of the world.

Skin, Soft Tissue, and Other Infections

Enterococci have been associated with skin and soft tissue (including wound) infections. When found in clinical samples from soft tissues, they are usually accompanied by other microorganisms; therefore their pathogenic role in these infections is unknown. Decubitus and diabetic foot ulcers are the usual lesions associated with the presence of enterococci; in some cases the organisms have been isolated from bone affected by osteomyelitis.[100] The simultaneous presence of vancomycin-resistant *E. faecalis* and *S. aureus* in diabetic wound infections has been associated with the transfer of the *vanA* gene cluster (encoding proteins necessary for vancomycin resistance) from enterococci to *S. aureus*.[101] Enterococci are rare causes of soft tissue abscesses; however, liver, lung, and brain abscesses have been reported.[102] The authors have seen a breast abscess caused by *E. faecium* in a patient hospitalized in the critical care unit. Enterococcal pneumonia and spontaneous empyema are also uncommon but have been occasionally described.

THERAPY AND ANTIMICROBIAL RESISTANCE

The main hurdle the clinician faces in the treatment of enterococcal infections is that these organisms are intrinsically resistant to a number of compounds and have an ability to acquire antibiotic-resistance genes. These therapeutic problems have long been recognized; in the past, approximately 60% of failures in the treatment of enterococcal endocarditis occurred when penicillin was used as monotherapy,[7] as opposed to endocarditis caused by streptococci. Bactericidal therapy is needed for optimal cure rates in endocarditis and other endovascular infections, which is usually not achieved with available single agents; therefore combination therapy should usually be given (Figs. 200.2 and 200.3; Table 200.3). The emergence of resistance to various antimicrobial agents seen commonly in *E. faecium* poses enormous clinical problems because the goal of bactericidal therapy often cannot be achieved.

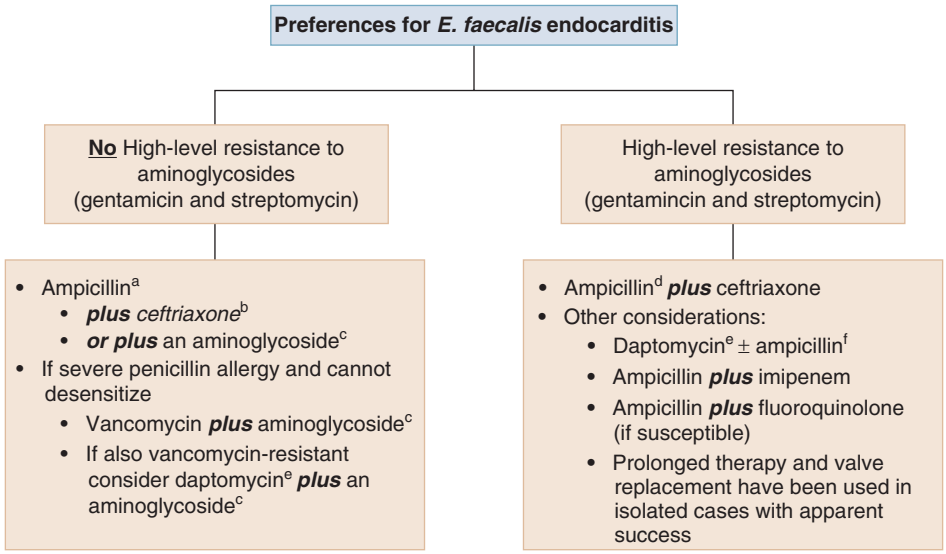

FIG. 200.2 Possible therapeutic alternatives for the treatment of endocarditis caused by *enterococcus faecalis*. [a]In rare cases of β-lactamase–producing isolates, the American Heart Association (AHA) recommends ampicillin-sulbactam 12 g/day. [b]The AHA recommends ampicillin plus ceftriaxone for patients whose creatinine clearance is <50 mL/min. [c]Gentamicin or streptomycin (see text); the AHA recommends gentamicin administered at 3 mg/kg/day in two or three divided doses. Data are insufficient to recommend once-daily dosing regimens for enterococcal endocarditis in patients with normal renal function. [d]Continuous infusion of ampicillin or ampicillin-sulbactam as monotherapy is preferred by some. [e]High-dose daptomycin (8–12 mg/kg) is preferred. [f]Addition of another active agent (e.g., ampicillin, ceftaroline) might be considered. None of these uses are approved by the US Food and Drug Administration, and prospective, randomized trials have not been conducted. Recommendations are based on literature review and personal opinion. Therapy duration is generally 6 weeks for both native and prosthetic valve endocarditis; 4 weeks of ampicillin plus an aminoglycoside appears to suffice for native valve endocarditis of less than 3 months' duration. Longer durations of therapy should be considered when neither a β-lactam nor vancomycin can be used. *(Modified from Arias CA, Contreras GA, Murray BE. Management of multidrug-resistant enterococcal infections.* Clin Microbiol Infect. *2010;16:555–562.)*

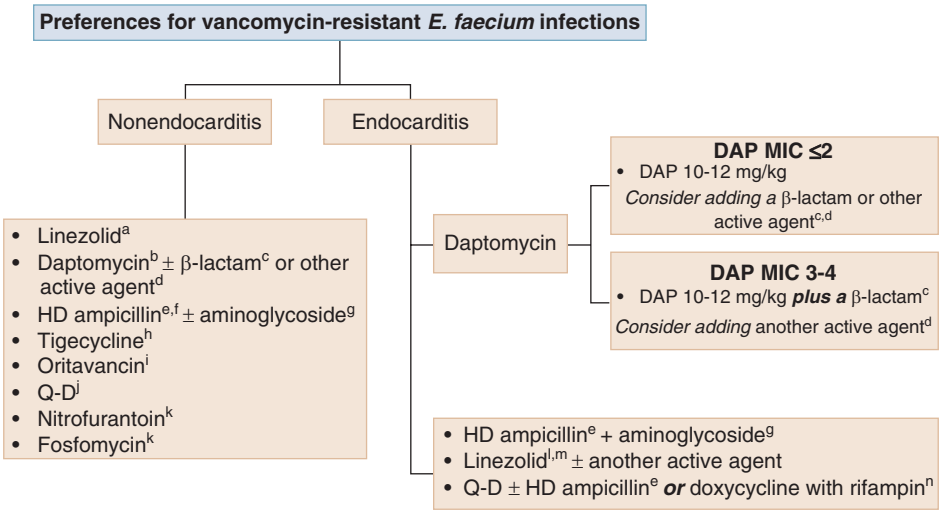

FIG. 200.3 Possible therapeutic alternatives for treatment of infections caused by vancomycin-resistant *enterococcus faecium*. [a]Linezolid is approved by the Food and Drug Administration (FDA) for nonendocarditis vancomycin-resistant *E. faecium* infections. Linezolid, daptomycin, and quinupristin-dalfopristin *(Q-D)* (the latter two also used intrathecally) have been successfully used against meningitis caused by vancomycin-resistant isolates of *E. faecium*. [b]Use of high-dose *(HD)* daptomycin (8–12 mg/kg) is preferable to 6 mg/kg. [c]In vitro data suggest that ampicillin, ceftaroline, and ertapenem are reasonable choices as additive or synergistic β-lactam therapy. [d]Other potentially active agents include (there is need to confirm with in vitro susceptibilities) tigecycline, fluoroquinolones, Q-D, or an aminoglycoside (gentamicin or streptomycin). [e]If minimal inhibitory concentration *(MIC)* is ≤64 μg/mL, HD ampicillin up to 30 g/day could be considered. However, toxicity at high ampicillin doses has not been systematically assessed. HD ampicillin *plus* imipenem (for imipenem MIC ≤32 μg/mL) is a consideration if high-level resistance to aminoglycosides is present. [f]Because of high urine concentrations obtained with ampicillin therapy, ampicillin is likely to be effective in lower urinary tract infections, even when caused by organisms with MICs ≥128 μg/mL (*plus* catheter removal). [g]If the organism lacks high-level resistance to gentamicin or streptomycin. [h]Not approved for any *E. faecium* infection but active in vitro; concerns exist in treatment of bacteremia because of low tigecycline serum levels, although it may have a role in combination therapies (e.g., with daptomycin) against multidrug-resistant *E. faecium*. It has favorable pharmacokinetics in intraabdominal tissues. [i]Case report of prosthetic valve endocarditis, initial dosing was 1200 mg every other day for three doses, then once weekly for 6 weeks. The dosing interval was increased to twice weekly after the patient experienced a relapse. Mild transaminitis and elevation of alkaline phosphatase was reported at 10 weeks on a twice-weekly dosing regimen. [j]Q-D was the first agent approved by the FDA for vancomycin-resistant enterococci (VRE), but this indication has since been withdrawn. Q-D has been used both systemically and as intrathecal therapy for VRE meningitis. [k]Urinary tract infections only. [l]Linezolid and daptomycin are listed in the American Heart Association/Infectious Diseases Society of America recommendations for the treatment of vancomycin and ampicillin-resistant *E. faecium*. [m]Concerns over reports of lack of efficacy or development of resistance with monotherapy. [n]If susceptible to each agent. The recommendations for compounds that are not FDA-approved are based on literature review and personal opinion. No prospective, randomized trials have been conducted. *(Modified from Arias CA, Contreras GA, Murray BE. Management of multidrug-resistant enterococcal infections.* Clin Microbiol Infect. *2010;16:555–562.)*

TABLE 200.3 Treatment of Nonendovascular Infections Caused by β-Lactam and Glycopeptide-Susceptible Enterococcal Species

DISEASE	SUGGESTED REGIMEN	DOSAGE AND DURATION (NORMAL RENAL AND LIVER FUNCTIONS)
Bacteremia[a]	Ampicillin[b] *or* Penicillin G *or* Vancomycin	9–12 g daily IV in divided doses q4–6h for 14 days[c] 18-30 million U IV daily divided q4–6h for 14 days[c] 30 mg/kg daily IV in divided doses for 14 days[c]
Meningitis[d]	Ampicillin[e] *or* Penicillin G[e] *plus* Ceftriaxone[f] *plus* Gentamicin[g] *or* Streptomycin	2 g q4h IV for 14 days 4 million U q4h IV for 14 days 2 g IV q12h 2 mg/kg loading dose, followed by 1.7 mg/kg IV q8h *or* 3 mg/kg IV q24h 7.5 mg/kg body weight q12h (evaluation of drug levels is strongly recommended) for 14 days
Urinary tract infections	Nitrofurantoin Fosfomycin Amoxicillin[h]	100 mg PO q6h for 5 days 3 g PO (single dose) 875 mg to 1 g PO q12h for 5 days

[a]Adding an aminoglycoside for synergistic therapy (gentamicin, 1 mg/kg q8h, or streptomycin, 7.5 mg/kg q12h) with a cell wall agent should be considered in seriously ill patients or patients with high risk of developing endocarditis, as indicated in Fig. 200.2. Adding ceftriaxone to ampicillin is an alternative to aminoglycosides.
[b]In rare β-lactamase–producing isolates, ampicillin-sulbactam (12 g daily in divided doses) should be considered.
[c]In catheter-associated bacteremias, a shorter duration of therapy (5–7 days) may be sufficient after removal of the catheter.
[d]In refractory cases, linezolid has been reported to be successful; intrathecal vancomycin, daptomycin, or gentamicin may be considered in cases of postoperative meningitis or with failure to respond to systemic antibiotics.
[e]For patients with penicillin allergy, intravenous vancomycin, 15–20 mg/kg q8–12h (adjust dosage to achieve vancomycin serum trough concentrations of 15–20 μg/mL), is recommended.
[f]Ampicillin plus ceftriaxone, along with the addition of an aminoglycoside (if no high-level resistance to aminoglycosides is present), may be considered for treatment of meningitis.
[g]Once-daily dosing of gentamicin may be considered in this context. Clinical data are not available, but the higher peak level of the extended interval dosing regimen may facilitate drug penetration to the cerebrospinal fluid.
[h]For bacteria that produce β-lactamase, amoxicillin-clavulanate can be used.
IV, Intravenously; *PO,* per os (orally).

Combination of β-Lactam and Aminoglycosides or Cephalosporins

β-Lactam antibiotics inhibit the penicillin-binding proteins of susceptible bacteria, thus interfering with cell wall synthesis, and this class of antibiotics should be the first choice for the treatment of susceptible enterococcal isolates (see Table 200.3). Relative resistance to β-lactams with minimal inhibitory concentrations (MICs) of penicillin 10 to ≥100 times those of streptococci is a well-described characteristic of enterococci. Many strains are also tolerant to β-lactams, that is, not killed with concentrations of antibiotics ≥16 times higher than the MIC.[103] The most potent activity is observed with the aminopenicillins (e.g., ampicillin) and ureidopenicillins, followed by penicillin G and imipenem. Although the MIC breakpoint defined by the Clinical and Laboratory Standards Institute for ampicillin resistance is ≥16 mg/L, high doses of ampicillin can achieve plasma concentrations ≥150 mg/L, which has led to the suggestion that isolates with ampicillin MICs ≤64 mg/L might be successfully treated with doses of 18 to 30 g/day of ampicillin or ampicillin-sulbactam[104] usually combined with an aminoglycoside (see later), although there are few safety data for use of this high-dose β-lactam regimen.

Resistance to penicillins and carbapenems is usually found in clinical isolates of *E. faecium* and rarely in *E. faecalis*. The mechanisms of resistance in *E. faecium* appear to involve the expression of a resistant *pbp5* allele (*pbp5-R*) (whose DNA sequence differs from that of susceptible *pbp5* by approximately 5%)[105] with decreased affinity for ampicillin.[106] Differences in the genetic environment of the *pbp5* gene correlated with increased expression of PBP5 in strains belonging to the ampicillin-resistant clinical clade (also known as health care–associated clade), suggesting factors other than amino acid sequence also play a role in PBP5-mediated resistance.[107] A laboratory strain of *E. faecium* with a penicillin-binding protein–independent mechanism of β-lactam resistance involving a novel transpeptidation pathway of peptidoglycan synthesis has also been reported,[108] although no clinical isolates have so far been found to exhibit this mechanism. β-Lactam resistance in *E. faecalis* can be mediated by the production of a β-lactamase enzyme[109]; although rare, occasional outbreak strains harboring this enzyme have been reported, mainly in the United States and Argentina. The presence of this enzyme is not detected by routine susceptibility testing, and therefore testing specifically for β-lactamase in endocarditis or serious enterococcal infections should be considered.[110] Some strains of *E. faecalis* have been found to be susceptible to ampicillin but resistant to penicillin and imipenem[111]; although the mechanism of this discrepancy on β-lactam susceptibility has not been demonstrated experimentally, decreased susceptibility correlates with specific amino acid changes in PBP4.

A bactericidal regimen should be used for the treatment of enterococcal endocarditis (see Figs. 200.2 and 200.3) and is recommended for any other endovascular infection. However, as mentioned before, β-lactams are not often bactericidal for enterococci, but a synergistic and bactericidal effect is usually achieved with the addition of an aminoglycoside. In vitro, synergism in enterococci is defined as a ≥2 $\log_{10}$ increase in killing at 24 hours by the combination compared with the β-lactam (or glycopeptides; see later) alone when the concentration of the aminoglycoside does not have any effect on the growth curve of the microorganism, with a 99.9% decrease from the starting bacterial inoculum resulting from the antibiotic combination. Gentamicin and streptomycin are the only two aminoglycosides recommended for achieving this synergistic effect in clinical practice. The use of other aminoglycosides for this purpose is discouraged (see later).

High-level resistance (HLR) to aminoglycosides is defined by growth at concentrations of 2000 mg/L and 500 mg/L of streptomycin and gentamicin, respectively, on brain heart infusion agar or 1000 mg/L of streptomycin when using brain heart infusion broth. The presence of HLR to both gentamicin and streptomycin abolishes the synergistic effect of these compounds in clinical practice. The emergence of enterococci with HLR to both aminoglycosides was reported in 1983[112] and has increased since then in both *E. faecalis* and *E. faecium*. HLR to gentamicin is mostly due to the presence of a bifunctional aminoglycoside-modifying enzyme, AAC(6′)-IE-APH(2″)-Ia, that confers high-level resistance to gentamicin (as well as HLR or resistance to

synergism with tobramycin, netilmicin, sisomicin, kanamycin, and amikacin, but not streptomycin). HLR to streptomycin can be due to mutations in the 30S ribosomal subunit[113] and to the presence of a streptomycin adenylyltransferase.[112] Evaluation for the presence of HLR has been the standard of care for treatment of all enterococcal isolates causing endovascular or severe infections. As a caveat, rare isolates of both *E. faecalis* and *E. faecium* (and *E. gallinarum*) whose MICs of gentamicin are lower than 500 mg/L (i.e., reported as not HLR to gentamicin) may be resistant to the synergistic effect of the combination with a cell wall agent because of the presence of the APH(2″)-Ic enzyme or other, yet unidentified mechanisms. Hence this situation must be considered in patients who do not respond appropriately to combination therapy with aminoglycosides with isolates reported as not having HLR to aminoglycosides.

Aminoglycosides other than gentamicin and streptomycin are not recommended for the treatment of enterococcal infections (except possibly arbekacin and tobramycin in *E. faecalis,* with no HLR to gentamicin; see later) because (1) as stated earlier, the common mechanism of resistance to gentamicin in clinical isolates is mediated by the AAC(6′)-Ie-APH(2″)-Ia enzyme, which confers resistance to synergism with all aminoglycosides commonly available in the United States except streptomycin; (2) *E. faecium* (as a species characteristic) produces an aminoglycoside modifying enzyme, 6′-acetyltransferase (6′-AAC), that results in higher MICs of tobramycin as well as kanamycin, netilmicin, and sisomicin, resulting in loss of the synergistic effect with cell wall agents; and (3) the *aph(3′)-IIIa* gene (encoding a kanamycin/neomycin phosphotransferase) is commonly found in enterococci and confers HLR or resistance to synergism with amikacin and kanamycin. In Japan, the aminoglycoside arbekacin is approved for clinical use, and this compound appears to be more stable to the action of the AAC(6′)-Ie-APH(2″)-Ia enzyme. It has also been shown that arbekacin exhibited synergism in vitro when combined with ampicillin against 40% of enterococci possessing the bifunctional enzyme.[114] Therefore this compound may be useful for certain isolates with HLR to aminoglycosides.

Typically the cephalosporins have weak activity against all species of enterococci with two exceptions. First is ceftriaxone (or cefotaxime) in combination with ampicillin; this regimen was initially used for the treatment of endocarditis caused by isolates of *E. faecalis* exhibiting HLR to all aminoglycosides (see Fig. 200.2).[115,116] The rationale for this approach is based on the observation that low concentrations of an aminopenicillin may be capable of partially saturating low-molecular-weight PBP4 and PBP5, but not PBP2 and PBP3, which then could actively participate in the synthesis of the bacterial cell wall. The addition of cefotaxime (or ceftriaxone) could produce total saturation of PBP2 and PBP3 and result in a bactericidal synergistic effect[117] against *E. faecalis.* (This effect is not observed in clinical isolates of *E. faecium.*) In the initial open-label and nonrandomized trial in Spain, 43 patients with *E. faecalis* endocarditis were treated successfully (clinical cure rate at 3 months was 67.4%) with the combination of ceftriaxone (2 g every 12 hours) and ampicillin (2 g every 4 hours) given for 6 weeks.[115] A subsequent observational, nonrandomized, comparative multicenter cohort study at 17 Spanish hospitals and 1 Italian hospital compared ampicillin-gentamicin versus ampicillin-ceftriaxone combinations for *E. faecalis* endocarditis. There were no differences in mortality while on antimicrobial treatment (22% vs. 21%, $P = .81$) or at 3-month follow-up (8% vs. 7%, $P = .72$), in treatment failure requiring a change in antimicrobials (1% vs. 2%, $P = .54$), or in relapses (3% vs. 4%, $P = .67$). Interruption of antibiotic treatment because of adverse events (mainly because of increases in creatinine values) was much more frequent in the gentamicin arm than in patients receiving ampicillin plus ceftriaxone (25% vs. 1%, $P < .001$) (≥25% increase in baseline creatinine concentration; 23% vs. 0%, $P < .001$), indicating that ceftriaxone-ampicillin is a good alternative in patients at risk of aminoglycoside toxicity, if not the therapy of choice.[116]

Ceftobiprole and ceftaroline are examples of a new generation of cephalosporins with increased affinity for penicillin-binding proteins of many resistant species, mainly PBP2a of methicillin-resistant *S. aureus*, that have relatively good activity against clinical isolates of *E. faecalis,* but not ampicillin-resistant *E. faecium*.[118] Ceftobiprole has potent activity against β-lactamase–producing and vancomycin-resistant strains of *E. faecalis,* it has exhibited synergism with aminoglycosides against selected isolates of *E. faecalis,* and its activity was comparable to ampicillin in an in vivo mouse peritonitis model.[118]

Glycopeptides and Lipoglycopeptides

The two glycopeptides currently used in clinical practice to treat enterococcal infections are vancomycin and teicoplanin. The mechanism of action of these compounds includes the inhibition of the last steps of peptidoglycan synthesis, which involves transglycosylation and transpeptidation of the pentapeptide units. Glycopeptides have been mainly used in the past in the treatment of β-lactam–resistant *E. faecium.* However, the increased prevalence of vancomycin resistance (e.g., approximately 80% of *E. faecium* isolates in the United States are resistant to vancomycin) has reduced the clinical use of these compounds against enterococci. Oritavancin is a semisynthetic glycopeptide antibiotic derived from chloroeremomycin and approved for acute bacterial skin and skin structure infections including vancomycin-susceptible isolates of *E. faecalis*, but not VRE. In vitro, oritavancin has potent dose-dependent bactericidal activity against enterococci including VRE with MICs ranging from 1 to 2 mg/L.[119,120] This antibiotic binds to peptidoglycan precursors in a similar manner as the glycopeptides, and it appears to have an extra binding site to peptidoglycan. Furthermore, oritavancin inserts into the cell membrane, producing depolarization that is thought to increase its bactericidal activity.[121] Clinical trials testing oritavancin in skin and soft tissue infections indicate that the antibiotic is safe and fulfilled the noninferiority criteria against the comparators[122]; however, data for the use of oritavancin in other human infections and against VRE are limited to case reports. Telavancin appears to have limited activity against glycopeptide-resistant enterococci with minimal inhibitory concentration in 90% of isolates (MIC_{90}) for vancomycin-resistant *E. faecalis* and *E. faecium* between 4 mg/L and 16 mg/L and 2 mg/L and 16 mg/L, respectively (although the MICs are severalfold lower than MICs of vancomycin). Similarly, dalbavancin does not bind peptidoglycan precursors terminating with D-Ala-D-Lac (produced by enterococci bearing VanA) and has little activity against VRE (MIC 16–32 mg/L). These compounds may be useful in the treatment of skin and soft tissue infections in which vancomycin-susceptible enterococci play a role.

Daptomycin

Daptomycin is a lipopeptide antibiotic that was approved by the US Food and Drug Administration (FDA) in 2003 for the treatment of complicated skin and soft tissue infections including infections with vancomycin-susceptible *E. faecalis,* and it was granted an additional indication in 2006 for *S. aureus* (including methicillin-resistant *S. aureus*) bacteremia and right-sided endocarditis. Daptomycin is not approved by the FDA for the treatment of *E. faecium* (regardless of susceptibility) or for VRE infections. The molecular events involved in its antibacterial action have not yet been completely elucidated, and it is thought that daptomycin inserts into the bacterial cell membrane in a calcium-dependent manner with preferential binding to septal areas, producing alterations of membrane homeostasis and cell division. These effects eventually lead to bacterial cell death by an unknown mechanism. In vitro, daptomycin exhibits rapid concentration-dependent bactericidal killing of enterococci; in vivo, the area under the curve (free drug)/MIC ratio appears to be the best parameter that correlates with clinical success.[123] The activity of daptomycin in vivo appears to be affected by its high binding affinity to albumin (90%–94%), and the unbound fraction might be crucial in the treatment of endovascular enterococcal infections when bactericidal therapy is a requirement. This observation, plus higher MICs and breakpoints than for staphylococci, has led to the notion that higher doses (10–12 mg/kg) may be of clinical benefit (see Figs. 200.2 and 200.3) and is supported by in vitro studies showing that higher doses of daptomycin may decrease the emergence of resistance on therapy.[124] Two nonrandomized cohort studies, one a retrospective study of enterococcal bacteremia in patients in a Veterans Affairs (VA) hospital in the United States and the other a prospective observational cohort in Taiwan, assessed the clinical impact of high-dose daptomycin (defined as ≥10 mg/kg/day and ≥9 mg/kg/day, respectively) on VRE bloodstream infections.[125,126] Both studies favored the use of high-dose daptomycin, and in the VA study high-dose daptomycin was significantly

associated with both lower 30-day mortality (RR, 0.83) and microbiologic clearance (HR, 0.70).[125]

Combination therapies that include daptomycin have been used in recalcitrant enterococcal infections. One of the most promising strategies involves the use of daptomycin plus a β-lactam to take advantage of an in vitro phenomenon known as the seesaw effect. This refers to the observation that resistance to cationic antimicrobial peptides (such as daptomycin or human cathelicidin LL-37) results in increased sensitivity to β-lactam antibiotics (such as ampicillin, ceftaroline, or ertapenem). Although robust clinical data are lacking, combinations of daptomycin plus ampicillin or ceftaroline have been successfully used to treat recurrent deep-seated enterococcal infections that have failed daptomycin monotherapy.[127,128] A variety of other combination regimens have been reported for difficult VRE infections. For example, failure of daptomycin monotherapy in the treatment of *E. faecium* endocarditis was overcome by the use of a combination of daptomycin (8 mg/kg/day), high-dose ampicillin, and gentamicin.[129] Similarly, the combination of daptomycin, gentamicin, and rifampin successfully treated a case of prosthetic valve endocarditis caused by a vancomycin-resistant isolate of *E. faecium* that failed with linezolid,[130] and in vivo experiments have demonstrated that the renal toxicity of gentamicin appears to be attenuated by the concomitant use of daptomycin. An additional anecdotal successful combination used in the treatment of endocarditis caused by a multidrug-resistant (including HLR to aminoglycosides) *E. faecium* included the combination of daptomycin (6 mg/kg every 48 hours) and tigecycline.[131]

Enterococci resistant to daptomycin have been well documented (daptomycin MICs range from 6 to >32 mg/L), although they remain relatively rare. Daptomycin resistance emerging during prolonged therapy (average exposure to daptomycin of 18 days) has been documented in *E. faecalis, E. faecium,* and *E. durans* isolates, and a retrospective study at a large cancer hospital found that exposure to ≥13 days of daptomycin in the preceding 3 months most significantly correlated with subsequent isolation of daptomycin-resistant *E. faecium* from the blood (60% vs. 11%).[132] De novo resistance has also been observed in isolates without previous exposure to the antibiotic.[133] Data suggest that a pivotal event in the mechanism of daptomycin resistance in enterococci involves changes in genes predicted to orchestrate the bacterial cell envelope stress response (designated *liaFSR*) and encoding enzymes of phospholipid metabolism (e.g., cardiolipin synthase).[134] Moreover, mutations in *liaFSR* in daptomycin-susceptible enterococci have been shown to lead to in vitro daptomycin tolerance (loss of the bactericidal activity of the antibiotic), and these mutations are common in isolates with MICs near the breakpoint (3–4 mg/L).[135,136] A retrospective cohort of 62 patients with VRE bacteremia found that MICs of 3–4 mg/L were associated with microbiologic failure, although MICs were not predictive of microbiologic failure or all-cause mortality in other studies.[126,137,138] Thus daptomycin dose and combination therapy should be carefully considered in patients with isolates near the breakpoint.

Linezolid

The efficacy of linezolid against enterococci (including VRE) has been evaluated in several clinical trials and in a meta-analysis.[139] In the case of endocarditis, controversial reports can be found in the literature regarding the outcomes of these patients because the antibiotic has been used when other recommended options have failed or when resistance or allergies to approved agents have been documented. In an intention-to-treat analysis of 22 patients with VRE endocarditis treated with linezolid on a compassionate use basis, cure was achieved in only 10 (45%) patients. Conversely, linezolid was used as a single agent in the treatment of 5 patients with endocarditis caused by *E. faecalis* (3 were vancomycin resistant) and 4 patients with *E. faecium* (all vancomycin resistant). The overall cure or improvement rate was 78% (7 of 9 patients), with development of thrombocytopenia in 33% of patients.

The American Heart Association (AHA)/Infectious Diseases Society of America (IDSA) lists linezolid as one of the two drugs (the other being daptomycin) that can be used as first-line therapy for endocarditis caused by *E. faecium* resistant to β-lactams, glycopeptides, and aminoglycosides[140] (evidence is "expert opinion"). The question of which antibiotic, linezolid or daptomycin, should be the preferred agent for treatment of VRE bloodstream infection remains undecided. There was no difference between the two drugs in one meta-analysis,[141] whereas in two others linezolid had lower all-cause[142] and infection-related[143] mortality. Each of the analyses had substantial limitations including small sample sizes, heterogeneous outcomes, and a lack of patient-specific data. A subsequent study using a large retrospective cohort of 644 patients admitted to a VA hospital with VRE bacteremia and treated with either daptomycin or linezolid found that treatment with linezolid was associated with higher 30-day all-cause mortality and microbiologic failure rates.[144] Furthermore, analysis of a propensity-matched cohort of VA patients with endocarditis due to VRE showed that daptomycin was associated with lower mortality than linezolid.[145] We suggest that linezolid should be used with caution in the treatment of VRE enterococcal endocarditis and only when combinations of β-lactams and aminoglycosides, high-dose daptomycin, or daptomycin plus other agents cannot be used because of resistance, toxicity, or therapeutic failure. In patients allergic to β-lactams, desensitization should be considered in severe infections caused by *E. faecalis* (see Fig. 200.2). Linezolid has also been successfully used in the treatment of enterococcal meningitis caused by different species, but no clinical trials have been conducted because of the paucity of cases.

Linezolid resistance appears to be increasing, and horizontal spread of specific linezolid-resistant outbreak strains has been reported[146] including in patients without previous exposure to the antibiotic. Risk factors for the acquisition of nosocomial linezolid-resistant strains included peripheral vascular disease; receipt of a solid-organ transplant; total parenteral nutrition; and administration of piperacillin-tazobactam, or cefepime, or both. The common mechanism of resistance involves mutations in the central loop of domain V of the 23S rRNA. The mutation G2576T (*E. coli* 23S rRNA gene numbering) is commonly found in resistant strains, and other mutations (G2505A, G2512T, G2513T, C2610G) have been found in enterococci, mainly in vitro. The rRNA mutations appear to interfere with the oxazolidinone interaction with its target at the core of the ribosomal peptidyltransferase center. *E. faecalis* and *E. faecium* contain four and six copies of the rRNA operons, respectively, and the increase in MIC of linezolid has been associated with the number of mutated rRNA genes[147]; intracellular homologous recombination between mutated and wild-type alleles may play a role in increasing the number of copies with the mutation. Studies in the GI tract of gnotobiotic mice also indicate that the dose and duration of linezolid exposure directly influence the number of mutated rRNA genes. Nonmutational resistance to linezolid is mediated by the presence of a gene designated *cfr* (chloramphenicol-florfenicol resistance), which was originally described on a plasmid in an animal isolate of *Staphylococcus sciuri,* and encodes an enzyme capable of methylating position A2503 of the 23S rRNA of bacterial ribosomes. The presence of *cfr* conferring linezolid resistance in a clinical isolate of *E. faecalis* indicated some evidence of animal-to-human transmission.[148]

The new oxazolidinone tedizolid has better in vitro activity against *cfr*-positive isolates. A second gene conferring resistance to linezolid is *optrA,* which encodes a protein similar to the adenosine triphosphate (ATP)–binding subunit of ATP-binding cassette transporters and mediates resistance to oxazolidinones and phenicols through ribosomal protection (displacement of the antibiotic from the site of action).[149] In contrast to *cfr,* the presence of *optrA* was associated with a fourfold to eightfold increase in the tedizolid MIC, which could have a clinically significant impact on the use of this agent.[150]

Tigecycline

Tigecycline is approved by the FDA for the treatment of complicated skin and soft tissue infections and abdominal infections caused by susceptible organisms including vancomycin-susceptible *E. faecalis*. Animal models of peritonitis and endocarditis have documented the efficacy of tigecycline against enterococci, regardless of the presence of vancomycin or tetracycline resistance. It is thought that its prolonged half-life, postantibiotic effect, and homogeneous diffusion into the cardiac vegetation could enhance the in vivo activity of this compound against enterococci in valvular vegetations. However, no clinical data are available to support the use of tigecycline monotherapy in enterococcal endocarditis or any other endovascular infections, and concerns over the

use of tigecycline for the treatment of bloodstream infections have been raised because only low serum concentrations are achieved at the recommended dose. Tigecycline may have a role as a part of combination therapy, especially for *E. faecium,* as the MIC_{90} is 0.12 to 0.25 μg/mL and tends to be slightly lower than for *S. aureus* or *E. faecalis* as determined in broad antimicrobial susceptibility surveys.[151,152]

Emergence of resistance to tigecycline during therapy has been documented in an enterococcal isolate (*E. faecalis*) from the urine of a patient who was receiving treatment for a respiratory infection caused by *Stenotrophomonas maltophilia*.[153] Resistance to tigecycline has been associated with changes in the S10 ribosomal protein in the 30S ribosomal subunit that lies near the binding site of the drug; it is likely that mutations interfere with the ability of tigecycline to effectively bind the 16S rRNA target.[154] Nonetheless, in phase III trials of complicated skin and soft tissue and intraabdominal infections, tigecycline was noninferior to the comparators (vancomycin-aztreonam and imipenem-cilastatin, respectively) against vancomycin-susceptible *E. faecalis,* supporting the potential clinical usefulness of tigecycline against enterococci.[155] As mentioned earlier, tigecycline has been successfully used in combination with daptomycin in the treatment of at least three patients with endocarditis and one patient with meningitis unresponsive to other regimens.

Quinupristin-Dalfopristin

Q-D is a parenteral, semisynthetic antibiotic combination of streptogramin type A (dalfopristin) and type B (quinupristin) that was the first FDA-approved antibiotic for the treatment of VRE infections (this indication was subsequently withdrawn). Q-D is not generally active against *E. faecalis* because of the presence of an ATP-binding cassette (ABC) protein designated Lsa (similar to *optrA* [see "Linezolid"]).[156] Q-D has been evaluated in patients with severe vancomycin-resistant *E. faecium* infections in two prospective noncomparative, emergency use studies, with overall success rates (clinical and bacteriologic) of approximately 65%.[157] Q-D side effects such as phlebitis, myalgias/arthralgias, and metabolic abnormalities appear to be the most relevant problems with this antibiotic, which may lead to treatment interruptions. Successful treatment of patients with endocarditis caused by a vancomycin-resistant *E. faecium* with several combination therapies, in which one of the components was Q-D, has been documented and includes Q-D plus doxycycline and rifampin and Q-D plus high-dose ampicillin (32 g/day).[158] Q-D has also been used successfully in the treatment of enterococcal meningitis (using intravenous and intrathecal administration).

Nonsusceptibility to Q-D in enterococci is due to several mechanisms including (1) the macrolide-lincosamide-streptogramin B (MLS_B) type of resistance mediated by the *erm* genes (encoding a 23S rRNA methyltransferase) that appears to decrease the bactericidal activity of Q-D in vitro and in vivo and (2) the presence of the *vatD* and *vatE* genes, which encode acetyltransferases that inactivate streptogramin A. These genes are usually carried on plasmids and confer resistance to the related streptogramin, virginiamycin, which is an antibiotic previously used as a growth promoter in the veterinary industry.

Other Antienterococcal Antimicrobials

Quinolones (mainly ciprofloxacin and moxifloxacin) have been reported as part of combination therapy for treatment of enterococcal infections (see Fig. 200.2). However, the increased rates of resistance observed, the selection of resistant mutants during therapy, and the lack of effect in certain animal models make the quinolones less appealing for enterococcal infections, particularly as monotherapy. A potential role of quinolones may be as long-term suppressive therapy against fluoroquinolone-susceptible enterococci in endovascular infections in combination with amoxicillin. Tetracyclines (doxycycline and minocycline) also have been sporadically used in enterococcal infections either as monotherapy or in combination with other compounds when isolates are susceptible to these antibiotics.

Although resistance to chloramphenicol in enterococci mediated by the chloramphenicol acetyltransferase enzyme has been long documented, the prevalence among VRE isolates appears to be low, and hence chloramphenicol was used early on in the treatment of resistant enterococcal infections. In a case series of 51 patients with VRE bloodstream infections treated with chloramphenicol, 61.1% demonstrated a clinical response, and 79.1% exhibited microbiologic eradication with no serious side effects that could be definitely attributed to chloramphenicol.[159] Similarly, successful treatment of prosthetic valve endocarditis and meningitis with chloramphenicol plus minocycline and chloramphenicol monotherapy, respectively, has been reported.[160,161] However, blood levels of this antibiotic at doses generally used (100 mg/kg/day divided every 6 hours) may not be adequate, and treatment failures have occurred; hence chloramphenicol should be considered only as an alternative in certain clinical settings (when available), and close monitoring for hematologic toxicity is strongly recommended.

Antibiotics that concentrate in the urine such as β-lactams (very high concentrations of ampicillin may be obtained in urine) could be potentially useful for the treatment of enterococcal UTIs, even in cases of isolates with increased ampicillin MICs. Nitrofurantoin also achieves good levels in urine, and a randomized open-label trial comparing nitrofurantoin for 5 days versus trimethoprim-sulfamethoxazole for 3 days in the treatment of UTIs including those caused by enterococci indicated that nitrofurantoin was equivalent to the comparator drug.[162] Successful treatment of UTIs caused by vancomycin-resistant and ampicillin-resistant *E. faecium* with nitrofurantoin in an outbreak setting (100 mg every 6 hours) has also been documented.[163] Similarly, fosfomycin tromethamine has activity against enterococci and has an FDA indication for treatment of UTI caused by *E. faecalis*. Although clinical trials are lacking, in vitro activity against *E. faecium* isolates from UTIs (MIC_{90}, 64 mg/L) indicates that it would seem a reasonable option to consider in UTIs caused by *E. faecium* that are susceptible using the *E. faecalis* criteria.[110]

Intrathecal antibiotics have been used in the treatment of enterococcal meningitis, mostly combined with systemic therapy. The antibiotics used include vancomycin, teicoplanin, gentamicin, daptomycin, and Q-D. However, there are no clear clinical data that favor the use of intrathecal antibiotics over systemic compounds alone in the management of enterococcal meningitis, and this approach may be considered in cases with multiresistant organisms in which systemic therapy fails and removal of intrathecal catheters or shunts cannot be achieved (see Table 200.3).

STREPTOCOCCUS GALLOLYTICUS (BOVIS) GROUP

Streptococci belonging to the previously designated *Streptococcus bovis* group are organisms that have been considered opportunistic pathogens in humans. These organisms also react with Lancefield group D antisera but can be differentiated from the enterococci, as they are PYR negative and do not grow in the presence of 6.5% sodium chloride. Growth on trypticase soy plus 5% sheep's blood agar typically produces γ-hemolytic or α-hemolytic colonies (see Table 200.1). Genetic data have led to a taxonomic reclassification within the *S. bovis* group, which traditionally had been divided into three different biotypes based on the fermentation of mannitol and the presence of β-glucuronidase activity. The former *S. bovis* biotype I (the most commonly associated with endocarditis and bacteremia) has been designated *S. gallolyticus* subsp. *gallolyticus (S. gallolyticus)*, and biotype II/2 has been designated *S. gallolyticus* subsp. *pasteurianus (S. pasteurianus)*. The former biotype II/1 has been divided into two species, *S. infantarius* subsp. *infantarius (S. infantarius)* and *S. infantarius* subsp. *coli (S. lutiensis)*.[164,165] This change in nomenclature has generated some confusion among clinicians because the important association between bacteremia/endocarditis caused by the *S. gallolyticus* group of organisms and colonic malignancies may have been missed because of the lack of recognition of the new species names. The differentiation between these species can be difficult at times. Nucleic acid tests including sequencing of 16S ribosomal DNA or whole-genome sequencing will allow accurate identification to the subspecies level. MALDI-TOF mass spectrometry often provides rapid and reliable identification to the species level, which is sufficient to lead to further pursuit of endocarditis or colorectal malignancy, if clinically warranted.[166] Although a degree of strain variability exists, the *S. gallolyticus* group of organisms (particularly *S. gallolyticus* subsp. *gallolyticus*, formerly *S. bovis* biotype I) has been found to adhere in vitro to individual proteins

of the extracellular matrix such as collagen, fibronectin, and fibrin, which is thought to be important in the pathogenesis of endocarditis.[167]

The *S. gallolyticus* group of organisms is estimated to cause approximately 7% of all cases of endocarditis and approximately 20% of streptococcal endocarditis. Bacteremia and endocarditis caused by the *S. gallolyticus* group of organisms have been highly associated with the presence of colonic malignancy (particularly *S. gallolyticus* subsp. *gallolyticus,* although *S. gallolyticus* subsp. *pasteurianus* has been linked to malignancy in other locations in the GI tract)[168,169] and hepatobiliary disease, although the reason for this association remains obscure. In vitro models of *S. gallolyticus* subsp. *gallolyticus* infection have suggested that the ability to form biofilm on collagen-rich surfaces and translocate at the site of premalignant colonic lesions while evading immune activation may underlie the association with endocarditis and colonic malignancy.[170] Further studies of host-pathogen interaction suggest that *S. gallolyticus* induces expression of β-catenin, promoting cell proliferation and tumorigenesis in cultured human colon cancer cell lines.[171] This suggests that not only is *S. gallolyticus* associated with GI malignancy, but that it also may contribute to pathogenesis. In a study analyzing the clinical characteristics of 20 cases of endocarditis (excluding drug addicts) caused by the *S. gallolyticus* group over 13 years, the aortic valve was the most frequent valve affected. Simultaneous involvement of two cardiac valves, moderate-to-severe regurgitation, and embolic events were also commonly found. Colonic neoplasms were documented in 77% of patients.[172]

The AHA/IDSA guidelines for infectious endocarditis recommend the use of a β-lactam (ceftriaxone or penicillin G) plus an aminoglycoside (gentamicin) for 2 weeks or a β-lactam alone (in patients older than 65 years of age with concomitant impairment of renal function or eighth cranial nerve function) for 4 weeks for the treatment of native valve endocarditis caused by the *S. gallolyticus* group when MICs to penicillin are ≤0.12 mg/L. For isolates with MICs greater than 0.12 but less than or equal to 0.5 mg/L, the combination of the β-lactam (4 weeks) and aminoglycoside (2 weeks) should always be considered. Similar recommendations are given for the treatment of prosthetic valve endocarditis, although the duration of β-lactam therapy should be extended to 6 weeks.[140] For isolates with MIC greater than 0.5 mg/L, 4 to 6 weeks of therapy with ampicillin or penicillin G plus gentamicin is recommended. Vancomycin should be used in patients unable to tolerate the β-lactams, and a minimum of 4 or 6 weeks of therapy is recommended for native or prosthetic valves, respectively. The *vanB* gene cluster conferring high-level vancomycin resistance has been identified in members of the *S. gallolyticus* group. Shorter regimens should be considered for bacteremia without endocarditis, and patients should undergo evaluation of the GI tract to rule out malignancies, particularly if *S. gallolyticus* is involved.

LEUCONOSTOC SPECIES

Members of the *Leuconostoc* genus are catalase-negative, gram-positive cocci usually arranged in pairs or chains and often found in plants, dairy products, and foods. A minority (up to 31%) of *Leuconostoc* spp. react with group D antisera, and they do not produce either PYR or leucine aminopeptidase.[173] Also, in contrast to the enterococci and *S. gallolyticus* group, *Leuconostoc* spp. are capable of producing gas on fermentation of glucose. Strains exhibit variable growth in broth containing 6.5% sodium chloride, at 10°C, and at 45°C (see Table 200.1). Although these organisms were previously considered of low pathogenic potential for humans, they have emerged as sporadic causes of infection. Because *Leuconostoc* spp. can phenotypically resemble *Streptococcus, Enterococcus, Pediococcus,* and *Lactococcus,* they may sometimes be misidentified; their distinguishing characteristics include vancomycin resistance, negative reaction for PYR and leucine aminopeptidase (see Table 200.1), and lack of gas production from glucose. The most common *Leuconostoc* spp. isolated from human infections are *L. mesenteroides, L. pseudomesenteroides, L. paramesenteroides, L. citreum,* and *L. lactis.* A taxonomic study using 16S rRNA sequences indicated that members of the *Leuconostoc* genus could be grouped into three subclusters: *L. mesenteroides* subcluster, which contains most of the human pathogenic species; *L. fructosum* subcluster, which was proposed to be renamed as *Fructobacillus;* and *L. fallax* subcluster.[174]

Leuconostoc spp. appear to be normal colonizers of the GI tract, which could be the initial portal of entry in human infections. The first report of human infections with *Leuconostoc* spp. was in 1985 in two immunocompromised patients with bloodstream infections,[175] and since then they have been implicated in a variety of infection such as bacteremia (including catheter-related infections), endocarditis, pulmonary infections, meningitis, brain and liver abscesses, and osteomyelitis, among others, affecting both immunocompetent and immunocompromised patients (majority) including children and newborns. It has been postulated that previous antibiotic therapy (e.g., vancomycin), presence of intravascular devices, and underlying GI disease may be risk factors for *Leuconostoc* infections. Nosocomial outbreaks of *Leuconostoc* spp. have also been described, suggesting that they have the potential to disseminate in the hospital environment.

Antimicrobial susceptibility testing is crucial for treatment of *Leuconostoc* infections because these bacteria are naturally resistant to glycopeptides owing to the production of peptidoglycan precursors ending in D-alanine-D-lactate. The organisms are usually susceptible to penicillin G and ampicillin (although MICs tend to be higher than streptococci), which are generally more active than the cephalosporins and should be considered as the treatment of choice. The carbapenems (i.e., imipenem) have good activity, but a carbapenem-resistant isolate from a patient with postoperative meningitis has been documented. Resistance to trimethoprim, sulfonamide, and fosfomycin appears to be common among the members of the *Leuconostoc* genus. Erythromycin, clindamycin, tetracycline, minocycline, and chloramphenicol appear to remain active against the majority of the clinical isolates. Linezolid MICs are higher for *Leuconostoc* spp. compared with streptococci (MIC_{90} of 4 mg/L), whereas daptomycin is highly active in vitro and has been used successfully in the treatment of catheter-related bacteremia caused by *Leuconostoc* spp. for two patients who underwent bone marrow transplantation.[176]

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The complete reference list is available online at Expert Consult.

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201 *Streptococcus agalactiae* (Group B Streptococci)

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SHORT VIEW SUMMARY

Definition
- Group B streptococci (GBS) are gram-positive bacteria that cause invasive hematogenous infection in at-risk individuals.

Epidemiology
- GBS asymptomatically colonize the lower gastrointestinal or genital tract in approximately one-fourth to one-third of men and women.
- Distribution is worldwide, but rates vary geographically.
- Disease burden from GBS disease is increasing, especially in adults with underlying conditions including diabetes mellitus and persons with chronic liver or kidney disease; cancer, heart, or cardiovascular conditions; or neurologic impairment.
- Nursing home residents have a markedly higher incidence of invasive GBS disease than do community-dwelling adults.

Microbiology
- β-Hemolytic streptococci exhibit a narrow zone of hemolysis on blood agar.
- GBS have a polysaccharide capsule with pilus-like structures that enhance adherence and invasion.
- GBS grow readily in blood culture media and specimens from cerebrospinal fluid and other sites of infection.

Diagnosis
- In adults, onset is acute, with chills and fever, or acute-on-chronic, with indolent symptoms, especially in association with skin and soft tissue manifestations.
- In infants younger than 3 months, bacteremia without a focus; meningitis; or, less often, other focal infections, such as pneumonia or soft tissue infection, occur.
- Growth of GBS from blood or another normally sterile site is diagnostic.

Treatment
- Penicillin is the drug of choice; isolates are uniformly susceptible to β-lactams and meropenem. Isolates are generally susceptible to vancomycin; there have been only a few case reports of resistance.
- The duration of treatment ranges from 10 days for uncomplicated bacteremia to a 4-week minimum treatment course for endocarditis.

Prevention
- Intrapartum antibiotic prophylaxis given to colonized parturients during labor prevents early-onset infections in neonates, thus reducing the overall rate of neonatal sepsis (see Table 201.3).
- Both glycoconjugate and protein-based vaccines are in clinical trials and offer the potential for prevention of infections in pregnant women, neonates, and young infants, and invasive infections in nonpregnant adults.

HISTORICAL PERSPECTIVE

Group B streptococci (GBS; *Streptococcus agalactiae*) were reported as human pathogens in 1938 by Fry, who described three cases of fatal puerperal sepsis.[1] Infections in humans were reported infrequently until the 1960s, when it became evident that disease was occurring more commonly.[2] By the 1970s, GBS emerged as the predominant pathogens causing septicemia and meningitis in neonates and young infants and as a cause of substantial pregnancy-related morbidity. Implementation of maternal intrapartum chemoprophylaxis in the mid-1990s resulted in a dramatic decrease in early-onset neonatal disease incidence and in a decline in invasive disease during pregnancy.[3]

In the past nearly 3 decades, twofold to fourfold increases in the incidence of group B streptococcal disease have occurred in nonpregnant adults; most of these patients have had underlying medical conditions or are 65 years of age or older.[4,5] Active, population-based surveillance indicates that more than 90% of invasive group B streptococcal disease cases now occur in nonpregnant adults.[3,6] The highest case-fatality rate as a consequence of group B streptococcal infection is among nonpregnant adults older than 65 years.[7] These shifts in incidence and outcome suggest that at-risk adults might benefit from immunization with a group B streptococcal glycoconjugate vaccine, should this vaccine become available commercially. Publication of the complete genome sequences of major GBS capsular types opened new avenues for the identification of novel vaccine targets and for further elucidating the molecular basis for virulence of the organism.[8,9]

DESCRIPTION

Classification and Morphologic Characteristics

S. agalactiae is the species designation for streptococci belonging to Lancefield group B. The serologic differentiation of β-hemolytic streptococci by groups is based on capillary precipitin reactions between the group-specific carbohydrate cell wall antigen and hyperimmune rabbit antisera. GBS are facultative, gram-positive diplococci that grow on a variety of culture media. Isolated colonies on sheep blood agar are 3 to 4 mm in diameter and grayish white. The flat, somewhat mucoid colonies are surrounded by a narrow zone of β-hemolysis. One percent to 2% of strains are nonhemolytic. Selective media have been used to enhance the accurate detection of low numbers of GBS from sites such as the genital or gastrointestinal (GI) tract. These usually contain Todd-Hewitt broth with or without sheep red blood cells and antimicrobial agents, such as nalidixic acid and gentamicin or colistin, or chromogenic agar.

Identification

Definitive identification of GBS is based on detection of the group B–specific cell wall antigen common to all strains. A number of serologic methods using hyperimmune group B–specific antisera are used for detection of the group B antigen. Latex agglutination is the most widely used. When the manufacturer's instructions are followed, these products are comparable to the Lancefield capillary precipitin method.

Biochemical methods that permit the presumptive identification of GBS include resistance to bacitracin or trimethoprim-sulfamethoxazole, positive sodium hippurate hydrolysis, and the production of an orange pigment during anaerobic growth on certain media. Production of CAMP (Christie, Atkinson, Munch, Peterson) factor, a thermostable extracellular protein that results in synergistic hemolysis on sheep blood agar with the β-lysin of *Staphylococcus aureus,* is observed in 98% to 100% of GBS. The combination of the CAMP test with bacitracin susceptibility testing and the bile esculin reaction is adequate for presumptive differentiation of GBS from other serogroups of β-hemolytic streptococci.

Serologic Typing

GBS are differentiated serologically by capsular polysaccharide type and by cell surface–expressed proteins. Lancefield defined two cell wall carbohydrate antigens, the group B–specific or C substance, common to all strains, and the S substance, which allowed classification into types I, II, and III.[10] Later, she reported differences in type I strains and designated these Ia and Ib.[11] These strains possessed capsular polysaccharide Ia or Ib, and some strains also had a surface protein designated C protein, subsequently found to have α and β components. The nomenclature was revised in 1984 to designate the capsular polysaccharides as type antigens, with surface proteins as additional markers.[12] Additional capsular types, IV through IX, have been defined, and additional candidates are being evaluated.

The α C protein is the prototype for a family of proteins (α-like proteins) that includes Rib and Alp1 to Alp4 and is found in most GBS. R proteins (R1 to R4) are found on some strains. Each of the common polysaccharides has a characteristic protein expression pattern, but exceptions occur. For example, Alp1 is found frequently in type Ia strains, Rib is most often associated with type II and III strains, and Alp3 is associated with type V strains.[13] The β C protein binds to human immunoglobulin A (IgA) and is found mainly in type Ib strains.

Antibodies directed against polysaccharide antigens were shown by Lancefield to provide passive protection in mice challenged with homologous, but not heterologous, antigen-containing strains.[14] The α and β C proteins and Rib also elicit protective antibodies in animals,[15,16] but their role in human infections is not known. Antibodies directed at the group B antigen are not protective.

GBS consist of a large and diverse core population from which recombinant clones with limited diversity have emerged with properties that have allowed them to successfully disseminate and adapt to a special habitat, such as that of the human neonate.[17] The classic method for capsular typing of GBS is by capillary precipitins or immunodiffusion in agarose between acid extracts of the organism and hyperimmune rabbit antisera. Molecular assays for capsule type identification include multilocus sequencing typing, pulsed gel electrophoresis, and polymerase chain reaction (PCR). PCR-based and whole-genome sequencing methods offer powerful tools to document the epidemiologic relatedness of strains and to distinguish among the known types.[18–20] Such methods can determine whether recurrent infections are caused by separate or identical strains and can identify virulent clone families that may be disproportionate causes of invasive disease.[20] Molecular methods do not permit an assessment of polysaccharide or protein expression. However, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has allowed rapid detection of proteins specific for highly virulent strains.[21]

EPIDEMIOLOGY AND TRANSMISSION

Asymptomatic Colonization

GBS colonize the genital area or lower GI tract of women at rates ranging from 10% to 40%; in men, GI tract carriage is estimated to be 10% to 25%. Variations in prevalence of asymptomatic colonization relate to sites sampled, methods of detection, and demographic differences in populations (Table 201.1). When multiple appropriate sites, such as the lower vagina or the periurethral area *and* the rectum, are sampled and when the selective broth media are used, colonization rates typically exceed 20%. The association of GBS with asymptomatic bacteriuria during pregnancy is a marker for a high density of organisms or heavy genital colonization.

Colonization with GBS occurs more frequently among black women than in other racial or ethnic groups. Diabetes mellitus also is independently associated with higher rates of group B streptococcal colonization during pregnancy. Multiple partners and frequent or recent sexual intercourse are associated with increased risk for vaginal acquisition over time, possibly because sexual activity alters the vaginal microenvironment to make it more permissive for colonization. Sexual activity and particularly male-to-female oral sex increase the risk for cocolonization with identical group B streptococcal strains in heterosexual college couples.[22] Sexually inexperienced persons have low genital colonization rates. Pregnancy does not influence colonization prevalence.

TABLE 201.1 Factors Influencing Detection of Group B Streptococcal Colonization

	EFFECT ON ISOLATION RATE		
	INCREASED	**DECREASED**	**NONE**
Method Used			
Culture medium	Broth media Antibiotic-containing media	Agar media Nonselective broth media	— —
Sites in women	Lower vagina and rectum	Cervical os	—
Sites in infants and adults	Multiple sites	Single site	—
Interval	Two or more cultures in interval of 6–8 wk	Single sampling time	—
Genital Carriage in Women			
Pregnancy	—	—	+
Time during pregnancy	—	—	+
Day of menstrual cycle	First half	—	—
Age	≤20 yr	—	—
Sexual activity	Active	No sexual debut	—
Frequency of sexual intercourse or total number of partners	+	—	—
Vaginal discharge	—	—	+
Birth control method	Intrauterine device	—	Oral contraceptives
Parity	Primigravida	More than three pregnancies	—
Ethnic origin	Black	Asian	—
Marital status	—	—	+
Socioeconomic group	Lower income	—	—

+, Documented to have effect indicated on isolation rate.

The principal reservoir for GBS is the lower GI tract. The presence of GBS in the GI tract is a risk factor for vaginal colonization.[23] Changes in capsule expression and recolonization with antigenically distinct group B streptococcal clones can occur over time, and specific sequence types associated with loss or persistence of colonization have been defined.[24] The prevalence of oropharyngeal colonization in adults is about 5% but can approach 20% in men who have sex with men. The colonization rate in healthy older adults is about 20%.[25]

Transmission

Maternal-to-infant transmission occurs in utero by the ascending route or at the time of delivery. Vaginally colonized pregnant women are at an increased risk for premature labor. The rate of vertical transmission among neonates born to women colonized with GBS who do not receive intrapartum antibiotic prophylaxis before delivery is about 50%. A high maternal genital inoculum at delivery significantly increases the likelihood of vertical transmission. Transmission also can occur within the hospital setting, albeit uncommonly, likely through hand-to-hand contact, and in closed populations such as nursing homes.

Incidence and Serotype Distribution of Isolates

The mean incidence of invasive group B streptococcal disease globally in infants younger than 3 months is estimated to be approximately 0.5 cases per 1000 live births.[26] There is marked variation by geographic

region, with higher rates in Africa, followed by the Americas and Europe, and the lowest rates in Southeast Asia.[27,28,29] The incidence of early-onset (0 through age 6 days) neonatal group B streptococcal infection in the United States, formerly 1 to 3 per 1000 live births, declined by 2016 to 0.21 per 1000 live births in association with widespread use of antenatal culture screening and intrapartum antibiotic prophylaxis for colonized women.[30,31] Use of this prevention strategy has had no impact on late-onset (age 7 through 89 days) disease incidence, which still occurs at a rate of approximately 0.3 per 1000 live births.

The incidence of invasive disease in pregnancy declined significantly in association with the use of intrapartum antibiotic prophylaxis, but pregnancy-associated group B streptococcal disease is responsible for peripartum febrile morbidity. Intrapartum vaginal colonization with GBS also is an independent risk factor for intraamniotic infection. Women with "heavy" (≥50 colonies on the primary blood agar plate) or high-inoculum GBS colonization are at significantly greater risk for intraamniotic fluid infection than those with "light" (1–10 colonies) vaginal colonization. Among women with invasive group B streptococcal disease for whom pregnancy outcome was known, 61% had a spontaneous abortion or a stillborn infant, 30% had infants without apparent illness, 5% had live-born infants who developed clinical infections, and 4% had induced abortions.[30]

Invasive group B streptococcal disease among nonpregnant adults has been increasing for decades.[6] Adults now account for in excess of 90% of all invasive group B streptococcal disease cases in the United States.[31] Most adults have at least one predisposing medical condition, such as diabetes mellitus, chronic liver or renal disease, human immunodeficiency virus infection, malignancy, or stroke. The incidence of adult disease increased from 3.6 cases per 100,000 population in 1990 to 10.9 cases per 100,000 population in 2016.[32,32a] An estimated 27,700 cases of invasive disease and 1500 deaths occurred in the US population in 2016. A multinational population-based assessment found that bloodstream infection rates increased among older adults across all study regions, especially in association with diabetes.[33] In one population-based surveillance report, adults 65 years of age and older represented one-third of cases of invasive group B streptococcal disease. Nursing home residents have a markedly higher incidence (72 per 100,000 population) than community residents 65 years of age and older.[7]

Group B streptococcal types Ia, III, and V represent more than two-thirds of colonizing isolates in pregnancy and the same proportion of isolates in early-onset neonatal disease. Late-onset infant disease is caused predominantly by type III strains.[30] Among nonpregnant adults, type Ia strains account for 23% of almost 2000 cases of invasive disease in 2016, followed by types II and V (18% each), Ib (15%), III (12%) and IV (11%).[32a] Serotypes Ia, Ib, II, III, and V accounted for 86% and 94%, respectively, of invasive strains in nonpregnant adults in contemporary surveillance studies in the United States and France.[33,34] In Brazil, a surveillance study confirmed the high prevalence of the same serotypes but showed regional variability in the distribution of serotypes.[35] Types IV, VI through VIII, and nontypable isolates are uncommonly associated with invasive infection, but in Japan serotypes VI and VIII are frequently isolated from pregnant women.[36] Type IV strains are increasing as a cause of invasive disease in adults globally[37,38]

PATHOGENETIC MECHANISMS

Adherence

To cause disease, GBS must colonize mucosal surfaces and then breach these surfaces to enter normally sterile sites such as the bloodstream. GBS have surface-exposed protective antigens that are components of high-molecular-weight, covalently linked, pilus-like structures, and these structures contribute both to adherence and to invasion of host cells.[39]

GBS adhere to several human cells, including vaginal and intestinal epithelium, placental membranes, respiratory tract epithelium, and the blood-brain barrier endothelium. A role for the α protein is proposed in the interaction between the bacterium and the host cell.[40] The α protein is a prototype for a family of long, tandem repeat–containing surface proteins that are common to GBS types causing most invasive disease. The pilus operon codes for three proteins that contain the conserved amino-acid motif LPXTG (leucine, proline, any amino acid, threonine, glycine), associated with cell wall–anchored proteins, together with two genes coding for sortase enzymes.[39] Genes encoding the surface-associated pili have a role in adhering to cell surfaces such as brain endothelium.[41] The widely conserved, surface-exposed fibrinogen receptor FbsA also promotes adherence of GBS to human epithelial cells.[42]

Invasion

GBS can cross the epithelial barrier by a paracellular route, and translocation of epithelial barriers is proposed as the predominant route for dissemination of the organism in the human host.[43,44] In support of this mechanism, pili can locate in the intercellular space ahead of translocating bacteria. That pilus backbone also contributes to paracellular translocation of GBS is supported by experiments in which deletion of the pilus backbone protein reduces the capacity of the organism to transcytose through differentiated human epithelial cells.[45]

GBS can invade respiratory epithelial and endothelial cells and brain microvascular endothelial cells in vitro.[46] It is proposed that α C protein mediates translocation across epithelial barriers by facilitating intracellular invasiveness through binding to glycosaminoglycan on the surface of the host cell.[47] Proper anchoring of lipoteichoic acid on the group B streptococcal surface facilitates invasion,[48] and the group B streptococcal pore-forming β-hemolysin/cytolysin also promotes invasion of lung epithelial cells.[49] β-Hemolysin/cytolysin expression also correlates with lung epithelial cell injury and promotes injury of lung microvascular endothelial cells, increasing cell permeability in vitro. This increased permeability contributes to alveolar edema and hemorrhage that could be a feature of group B streptococcal pneumonia.[50]

Bacterial Virulence Factors

GBS produce a capsule that inhibits complement deposition and phagocytosis. Strains that effectively evade host phagocytes can damage tissues by inducing the release of inflammatory modulators or by production of substances that directly damage cells. A high quantity of cell-associated sialic acid and its elaboration in supernatant fluid are associated with virulence of type III strains.[51] Population studies reveal a strong correlation of capsular polysaccharide type III with CC17 hypervirulent strains that are documented to cause invasive neonatal disease worldwide.[52] Changes in capsular expression are associated with a loss of virulence in experimental models of lethal infection. The unique structures of GBS capsules also may enhance the invasiveness of one capsular type over another. A novel insertion sequence in the hyaluronidase gene has been identified predominantly in group B streptococcal strains causing endocarditis.[53] The mechanism by which this genetic alteration and the lack of hyaluronidase increase disease potential is speculative.

The pore-forming β-hemolysin/cytolysin enhances virulence by promoting invasion and cell injury and by inducing cytolysis and apoptosis of phagocytes in concert with shielding by carotenoid of GBS from oxidative damage.[54] Hyperhemolytic variants promoted joint injury compared with a nonhemolytic group B streptococcal variant in a murine model of infection. Group B streptococcal β-hemolysin/cytolysin also directly impairs cardiomyocyte viability and function,[55] and hyperhemolytic strains can disrupt the amniotic barrier and penetrate placental membranes.[56] The surface-localized β protein binds human complement factor H, and the complexed factor H retains its ability to downregulate complement activation.[57] In addition, *cspA,* a novel gene that encodes a surface-localized, serine protease–like protein, has been identified. It promotes group B streptococcal survival by evasion of opsonophagocytosis and is required for group B streptococcal cleavage of fibrinogen.[58]

Host Factors

Baker and Kasper[59] reported in 1976 that neonates and young infants at risk for invasive type III group B streptococcal infection were those with low concentrations of passively acquired maternal antibodies to the type III capsular polysaccharide. Women colonized with type III GBS who delivered healthy neonates had higher concentrations of type III–specific IgG than women whose infants developed early-onset type III disease. A low concentration of maternal antibodies to other capsular polysaccharides also is a determinant of neonatal susceptibility to infection. Colonization is a dynamic process, however. In longitudinal studies

during pregnancy, the average duration of colonization is about 6 weeks, so duration of colonization per se does not predict maternal immunity.

Little is known regarding host factors that predispose adults to develop group B streptococcal infection. A broad range in concentration of antibodies to the capsular polysaccharide of the infecting strain is found in acute-phase sera from adults with invasive infection. Some adults already have relatively high concentrations of antibodies to the infecting capsular polysaccharide when illness occurs. As a group, nonpregnant adults demonstrate an immune response to capsular polysaccharides and to pilus proteins during recovery from invasive infection, but whether these function to protect against infection is unknown.[60] Although anticapsular antibody may be protective in many cases, the susceptibility of some adult patients to group B streptococcal infection may be the result of defects in other aspects of the host defense, such as phagocytosis.

CLINICAL MANIFESTATIONS

Invasive group B streptococcal infection causes substantial morbidity and mortality, and the disease burden is increasing, especially among older persons, blacks, and adults with diabetes.[30] In a prospective, population-based assessment of invasive group B streptococcal disease, adults accounted for 92% of cases.[30] Adults with bacteremia unrelated to pregnancy are often elderly, but ages range from 18 to 103 years.[61–67,32a] The mean age in one report was 63 years, and 59% were men. The incidence increases with age, is higher in blacks than in other races or ethnicities, and is increased among elderly adults in long-term care facilities compared with community-dwelling older adults.[68]

One or more medical conditions predisposing to infection are present almost uniformly in adults with invasive disease (Table 201.2). In a multistate, population-based survey, 95% of cases occurred in persons with at least one underlying condition, most often obesity (54%), diabetes (53%), or neurologic disease (26%). Smoking, cardiovascular disease, renal disease, immunosuppressive conditions, liver disease, and lung disease also were underlying conditions.[32a] Among patients with soft tissue infections, ~40% report diabetes mellitus as a comorbid condition.[67] Neurologic illnesses associated with invasive infections include dementia, cerebrovascular disease causing altered mental status, and paraplegia or quadriplegia. Young adults (20–40 years of age) with diabetes, cancer, or human immunodeficiency virus (HIV) infection are at significantly increased risk (28- to 30-fold) for invasive disease.[6] Hospital-associated infection was independently associated with placement of a central venous line, diabetes, congestive heart failure, and seizure disorder.[68] Age 65 years or older is a risk factor for invasive group B streptococcal infection. The incidence doubles when 50- to 64-year-olds are compared with those 65 years of age and older.[67] Among a group of community-dwelling older adults, the age-specific incidence increased steadily and was about threefold higher in those older than 85 years than in a 65- to 74-year-old group.

Mortality is increased in older patients, in those with polymicrobial infection, and in patients with diabetes mellitus, liver disease, or malignancy. Contemporary case-fatality rates in nonpregnant adults range from 5% to 25%. Shock at diagnosis, alcoholism, and cancer are associated independently with an increased risk for death.[69] Nursing home residents are more likely to have a fatal outcome than are community-dwelling older adults. Health care–associated infection can occur, but case clustering has not been observed. The latter suggests that endogenous respiratory, genitourinary, or GI colonization, rather than the acquisition of GBS in the hospital, is the source of infection. About one-fourth of patients with GBS isolated from the bloodstream have polymicrobial bacteremia. *S. aureus* is a frequently observed second isolate. The age distribution, mortality rate, and proportion of health care–associated cases do not differ between patients with bacteremia caused only by GBS and those with polymicrobial bacteremia.[68]

Most group B streptococcal infections manifest with one of several clinical expressions of infection.

Primary Bacteremia

The most common presenting manifestations of nonfocal infections in adults are fever, chills, and change in mental status.[70] When no evident site of infection can be established, patients from whom GBS have been isolated from blood are considered to have primary bacteremia. Primary bacteremia can account for 20% to 50% of cases, and this manifestation carries a high fatality rate. Among survivors, recurrence of infection can occur in which a focus of infection is identified, such as endocarditis or osteomyelitis.[71]

Infections of the Female Genital Tract

In adults, a substantial number of infections caused by GBS are associated with pregnancy. The female genital tract is the source of these infections. GBS alone or as a component of a polymicrobial infection is among the most commonly isolated facultative aerobes from women with early postpartum endometritis. One-half of cases of pregnancy-associated bacteremia are associated with infection of the upper genital tract, placenta, or amniotic fluid, resulting in fetal death. Other manifestations include bacteremia without a focus in one-third of women, endometritis without fetal death, chorioamnionitis without fetal death, pneumonia, and puerperal sepsis.[30] Overall, GBS account for an estimated 15% of cases of peripartum endometritis, 15% of pregnancy-associated bacteremias, and up to 15% of wound infections after cesarean section.[72]

Focal signs and symptoms of infection usually develop within 48 hours after delivery. Among patients who deliver abdominally, women colonized with GBS have a significantly increased frequency of premature rupture of membranes, postpartum fever, and endometritis when compared with noncolonized women. The clinical findings of endometritis are nonspecific and include fever greater than or equal to 100.4°F with or without chills, malaise, moderate uterine tenderness, and normal lochia. Life-threatening sequelae of endometritis, such as pelvic abscess, septic shock, or septic thrombophlebitis, can occur, albeit rarely. Another manifestation of morbidity from group B streptococcal infection in pregnant women is urinary tract infection. Bacteriuria typically is asymptomatic or, less often, can manifest as cystitis or, rarely, pyelonephritis.

The role of GBS as an etiologic agent causing vaginitis has not been established. It is regarded as a commensal organism in the vagina and does not elicit a vaginal inflammatory response. Anecdotal reports suggest the pathogenic potential of GBS in vaginitis and resolution of symptoms of vaginitis in association with a short course of antibiotic administration.[73]

There is growing evidence to suggest that preterm birth is associated with maternal group B streptococcal colonization, especially when there

TABLE 201.2 Underlying Conditions in Invasive Group B Streptococcal Infections

CONDITION	*n* (%)
Diabetes mellitus	82 (30)
Liver disease or history of alcohol abuse	66 (24)
Neurologic impairment	58 (21)
Malignancy	52 (19)
Renal insufficiency or failure	37 (14)
Cardiovascular disease or heart failure	37 (14)
Pulmonary disease	21 (8)
Urologic disease	11 (4)
Peripheral vascular disease	9 (3)
Human immunodeficiency virus infection	7 (3)
Intravenous catheter-related infection	5 (2)
Gastrointestinal disease	5 (2)
Steroid administration	5 (2)
Hypertension	4 (1)
Functional or surgical splenectomy	3 (1)
Other	7 (3)
None	5 (2)

[a]*N* = 271 adults. Streptococcal infections listed were not related to pregnancy. Some patients had more than one underlying condition.
From references 5 and 61–65.

is evidence of ascending infection. In cohort studies, preterm birth is associated with maternal bacteriuria.[74] Among the estimated 2.6 million stillbirths globally each year, GBS is associated with approximately 1% of all stillbirths in developed countries and 4% of those in South Africa.[75]

Infections in Infants

Early-onset infection, occurring in neonates younger than 7 days, has a mean onset at 12 hours of age. Maternal obstetric complications are common, and infants born at less than 37 weeks' gestation have significantly higher attack rates than those born at term. Bacteremia or septicemia, pneumonia, and meningitis are the common clinical expressions of infection, and they occur at frequencies of about 85%, 10%, and 5%, respectively.[30] The presenting signs are nonspecific and are indistinguishable from those in neonates with bacterial infections of other causes. Signs of respiratory distress are observed in most infants. In the era of maternal screening, virtually all cases of early-onset disease occur in infants whose mothers have silent or clinically overt intraamniotic infection and in those who either did not receive intrapartum antibiotic prophylaxis during preterm labor or were negative for group B streptococcal colonization at screening culture at 35 to 37 weeks of gestation.[76]

Late-onset infection has an onset between 7 and 89 days of age, with a median of 36 days.[77] Currently, about 30% of the infants with late-onset disease in the United States are born prematurely. Other maternal obstetric complications are uncommon, and the case-fatality ratio is 3% to 5%.[30] Bacteremia and meningitis are common manifestations of infection, but focal infections, including osteomyelitis, septic arthritis, or cellulitis, can occur. Infants can present with fulminant infection characterized by rapid progression to a moribund state with septic shock and seizures. Among survivors of group B streptococcal meningitis, whether onset is early or late, approximately 50% have permanent neurologic sequelae.[78]

Pneumonia

Patients with group B streptococcal pneumonia have in common the apparent inability to limit the spread of the organism from colonizing mucous membrane sites to the bloodstream. The most common underlying medical conditions among these adult patients are diabetes mellitus and neurologic disease. These patients are often debilitated and bedridden. Fever, leukocytosis, and hypoxia are common presenting features. Chest radiographs can show bilateral or lobar infiltrates. Pleural empyema can occur. Fatality rates in patients with pneumonia range from 30% to 85%.

Endocarditis

A shift in the clinical expression of group B streptococcal endocarditis was first documented by Lerner and colleagues.[64] In contrast to the predominance of acute endocarditis in pregnant women during the preantibiotic era, cases reported since 1945 have had no gender predilection, have been both acute and subacute in onset, and have occurred in older patients (mean age, about 50 years). The mitral valve most often is affected (48%); infections involving the aortic (29%), mitral and aortic (10%), and tricuspid valves (5%) also have been described. Tricuspid valve involvement typically occurs in injection drug users. Prosthetic valve endocarditis has been noted.[79] Underlying heart disease exists in most cases, and valvular disease, atherosclerotic or rheumatic heart disease, and mitral valve prolapse each can be predisposing conditions.[80] An aggressive course with large friable vegetations is a frequent feature of group B streptococcal endocarditis.[81] Vegetations can resemble atrial myxomas, and embolization can occur early in the clinical course. Rapid valve destruction can necessitate early valve replacement in some patients.[82] The mortality rate is about 35% to 50%.

Arthritis

Group B streptococcal arthritis is monoarticular in two-thirds of patients and involves more than one joint in the remainder. In a review of 75 adults with group B streptococcal arthritis in which patients with prosthetic joint infection were excluded, the mean age was 58 years, and 45% were men.[83] The most commonly affected joints, in descending order of frequency, were the knee, shoulder, hip, and sternoclavicular and sacroiliac joints. One or more underlying medical conditions were present in three-fourths of patients, most commonly diabetes mellitus, malignancy, or chronic liver disease. One-third had a nonjoint focus of group B streptococcal infection, such as vertebral osteomyelitis or urinary tract infection. The most common presenting features were fever and joint pain, but about 14% of patients were afebrile. The erythrocyte sedimentation rate, when performed, was more than 30 mm/h in 95% of patients, and GBS were isolated from blood cultures in two-thirds. Surgical drainage was performed in 36% of cases.

Group B streptococcal prosthetic joint infections (≈75% of cases) usually occur 3 or more months after implantation and are usually (>90%) associated with comorbidities. In one review of 34 patients with prosthetic hip or knee infections, all patients underwent surgery.[84] Fifteen patients were treated with débridement with retention of the implant (relapse occurred in 2), 3 with a one-stage exchange arthroplasty, and 12 with a two-stage exchange, and 4 required removal of the implant. With appropriate antimicrobial therapy, the infection was cured in most patients, and functional mobility was preserved in more than 80%.

Osteomyelitis

Osteomyelitis occurs as a consequence of adjacent arthritis, peripheral vascular disease, orthopedic surgery, or an adjacent focus of infection. In a review of 39 cases of group B streptococcal osteomyelitis, one-half of the episodes were diagnosed as acute and one-half as chronic.[85] The mean age was 56 years, one-third of patients were older than 65 years, and two-thirds were men. The most commonly affected bones were the vertebrae, followed in order of frequency by the foot, bones around the hip, tibia, and toes. Underlying medical conditions were almost invariably present, most commonly diabetes mellitus, previous bone surgery, prosthetic bone or joint, or peripheral vascular disease. Foot bone involvement occurred predominantly in diabetic patients. Bones around the hip were involved in association with prior surgical procedures or trauma to the hip joint.

No specific clinical signs were associated with group B streptococcal osteomyelitis. The majority of patients were afebrile and had a normal erythrocyte sedimentation rate at the time of presentation. Cultures of bone or blood yielded GBS in about 90% and 40% of patients, respectively. Approximately one-fourth of infections are polymicrobial, with *S. aureus* or Enterobacteriaceae the most commonly associated microorganisms.[86] Most patients require a combined medical and surgical approach. The mean duration of antibiotic therapy is 10 weeks. Fatal infections are uncommon, but amputation may be required for resolution of infection in some patients with vascular insufficiency.

Skin and Soft Tissue Infections

Skin and soft tissue are the most common focal group B streptococcal infections in adults, accounting for more than one-third of infections. In these patients the diagnosis is most often made through isolation of the organism from one or more blood cultures and/or a surgical site tissue specimen. Cellulitis, foot ulcers, abscess, and infection of decubitus ulcers are frequent manifestations (Fig. 201.1). Cellulitis has occurred in association with foreign bodies, such as breast or penile implants. Relapsing cellulitis occurs in individuals with skin affected by chronic lymphedema.[87] Less common manifestations of skin and soft tissue infections are pyomyositis, blistering dactylitis, and necrotizing fasciitis, on occasion associated with toxic shock–like syndrome.[88,89] There are no features of these infections unique to GBS except that underlying conditions, such as diabetes mellitus and obesity, generally exist.

Meningitis

Meningitis caused by GBS has been reported in adults, most of whom have predisposing comorbid conditions.[90,91] The mean age of nonpregnant adults is 52 years; almost one-half are men. Several have had proven or possible disruption of the anatomic barrier protecting the brain as a consequence of surgery for tumors or chronic sinusitis. The overall case-fatality rate is 34%. Advanced age and overwhelming illness with presenting features such as coma or septic shock are associated with a poor outcome. Deafness, reported in 7% of survivors, is the most common neurologic sequela.



FIG. 201.1 Recurrent lower extremity cellulitis in a 70-year-old man who had undergone a saphenous venectomy.

Urinary Tract Infections

The spectrum of group B streptococcal urinary tract infection includes asymptomatic bacteriuria, cystitis, urethritis, pyelonephritis, and urosepsis. Asymptomatic bacteriuria during pregnancy is a surrogate for heavy maternal vaginal colonization. Clinically, urinary tract infections caused by GBS are not distinctive. Symptoms are consistent cystitis in approximately 80% and pyelonephritis in approximately 20% of patients.[92] Most infections are community acquired, occurring in middle-aged women, most of whom have an underlying condition such as alteration of urinary flow or stones. Symptomatic infection is common among residents of long-term care facilities. Treatment failure or relapse can occur, most likely as a consequence of persistent vaginal or enteric colonization.

Uncommon Manifestations of Infection

GBS, alone or as a component of mixed infection, have been isolated from a number of patients with keratitis or endophthalmitis. These infections occurred in eyes with severely damaged surfaces; the outcome was poor, with light perception being lost in one-half of the affected eyes.[93] Other unusual infections caused by GBS include breast abscess in a nonlactating woman,[94] ventriculoperitoneal shunt infection,[95] spinal epidural abscess,[96] mycotic aneurysm,[97] liver abscess,[61] intraabdominal abscess,[98] peritonitis,[4,64] and infection of a pacemaker wire after sigmoidoscopy.[99] GBS also have been reported to cause a toxic shock–like syndrome.[100]

Recurrent Invasive Group B Streptococcal Infection

Four percent to 7% of nonpregnant adults who survived an episode of group B streptococcal bacteremia had a recurrence.[32a,71] The mean interval between episodes of bacteremia is 24 weeks, but the interval is shorter when the recurrent episode is caused by the same capsular type (mean, 14 weeks) than when it is caused by another serotype (mean, 43 weeks between episodes). Several patients in whom primary bacteremia was the diagnosis of the first episode presented with focal infection, such as endocarditis or osteomyelitis, during the second episode.

DIAGNOSIS

Isolation of GBS from blood, cerebrospinal fluid, another usually sterile site, or a site of focal suppuration is the only means by which the diagnosis of invasive infection can be documented. Recovery of the organism from mucous membrane sites is of no diagnostic significance.

Intrapartum detection of colonization with GBS in women presenting for delivery can assist in identifying at-risk patients who will benefit from intrapartum chemoprophylaxis or empirical treatment. One fluorogenic real-time PCR technique for rapid detection of GBS in pregnant women at delivery had a sensitivity of 97% and a specificity of 100% when compared with cultures of vaginal and rectal swabs inoculated into selective broth medium.[101] Results were available in 45 minutes, compared with 100 minutes for conventional PCR and 36 hours or longer for the conventional culture technique. Field testing of commercially manufactured assays such as the Xpert GBS Assay (Cepheid, Sunnyvale, CA), which uses automated real-time PCR technology, and IDI-Strep B (Infectio Diagnostic, Quebec, Canada), which uses a PCR assay to amplify GBS-specific DNA and a fluorogenic probe to detect the amplified group B streptococcal target, has been performed.[102,103] When compared with conventional culture methods, these assays are sufficiently robust to be performed for intrapartum patients in point-of-care settings but have not replaced the culture-based method used for antenatal identification of women who are colonized with GBS as candidates for intrapartum antibiotic prophylaxis to prevent early-onset neonatal infection.

TREATMENT

GBS remain susceptible to penicillins and cephalosporins in vitro, although several strains with somewhat reduced susceptibility to penicillin have been identified in Japan.[104] Penicillin G is the drug of choice. The organism also is susceptible to vancomycin, meropenem, and imipenem. There have been recent reports of vancomycin-resistant strains associated with invasive disease in the United States.[105] Rates of fluoroquinolone resistance are variable and are associated with mutations in the gyrase and topoisomerase IV genes.[106] Increasing resistance to erythromycin and clindamycin now precludes their use as empirical treatment for invasive infection or for intrapartum prophylaxis unless susceptibility is established. Among isolates from patients with invasive disease, resistance rates are approximately 40% for erythromycin and 20% for clindamycin. Most macrolide-resistant strains present a macrolide, lincosamide, and streptogramin B (MLS_B)–resistant phenotype, mainly constitutive, because of *erm* genes.[107] A chromosomally integrated, composite transposon has been defined for *ermB*-type macrolide resistance.[108] Tetracycline resistance has increased to nearly 90%. GBS are uniformly resistant to nalidixic acid, trimethoprim-sulfamethoxazole, metronidazole, and aminoglycosides.

Therapy of 10 days' duration is recommended for the treatment of bacteremia, pneumonia, and pyelonephritis, whereas a 14-day minimal duration is recommended for the treatment of soft tissue infections or meningitis, and a 4-week minimum for the treatment of osteomyelitis, endocarditis, or ventriculitis. In infants, oral therapy is never appropriate. Relapses of infection have occurred in association with both an inadequate dosage and an inadequate duration of therapy. High-dose penicillin therapy does not reliably eliminate mucous membrane infection with GBS, a source that explains some recurrences.

PREVENTION

The impact of maternal intrapartum antibiotic prophylaxis on the incidence of early-onset group B streptococcal disease was first assessed through active, population-based surveillance in selected counties of eight states and reported in 2000.[3] The incidence of early-onset neonatal disease decreased by 65% from 1993 to 1998 and from 1.7 to 0.6 per 1000 live births, and the incidence of invasive disease in pregnant women declined by 21%. A multistate retrospective cohort comparison found that the culture-based approach was 50% more effective than a risk-based approach in preventing early-onset group B streptococcal infection.[109] Guidelines for prevention of perinatal group B streptococcal infection issued by the Centers for Disease Control and Prevention in 2010 specify that lower vaginal and rectal swab screening cultures be performed at 35 to 37 weeks of gestation for all pregnant women, with the exception of patients with documented group B streptococcal bacteriuria during the current pregnancy or with a previous infant with invasive group B streptococcal disease. The indications for intrapartum prophylaxis are shown in Table 201.3. Revision of the guidelines expanded recommendations on laboratory methods for the identification of GBS, clarified that

TABLE 201.3 Indications and Nonindications for Intrapartum Antibiotic Prophylaxis to Prevent Early-Onset Group B Streptococcal Disease

INTRAPARTUM GBS PROPHYLAXIS INDICATED	INTRAPARTUM GBS PROPHYLAXIS NOT INDICATED
Previous infant with invasive GBS disease	Colonization with GBS during a previous pregnancy (unless an indication for GBS prophylaxis is present for current pregnancy)
GBS bacteriuria during any trimester of the current pregnancy[a]	GBS bacteriuria during previous pregnancy (unless an indication for GBS prophylaxis is present for current pregnancy)
Positive GBS vaginal-rectal screening culture in late gestation[b] during current pregnancy[a]	Negative vaginal and rectal GBS screening culture in late gestation[b] during the current pregnancy, regardless of intrapartum risk factors
Unknown GBS status at the onset of labor (culture not done, incomplete, or results unknown) and any of the following: • Delivery at <37 wk gestation • Amniotic membrane rupture ≥18 h • Intrapartum temperature ≥100.4°F (≥38.0°C)[c] • Intrapartum NAAT[d] positive for GBS	Cesarean delivery performed before onset of labor on a woman with intact amniotic membranes, regardless of GBS colonization status or gestational age

[a]Intrapartum antibiotic prophylaxis is not indicated in this circumstance if a cesarean delivery is performed before onset of labor on a woman with intact amniotic membranes.
[b]Optimal timing for prenatal GBS screening is at 35 to 37 weeks' gestation.
[c]If amnionitis is suspected, broad-spectrum antibiotic therapy that includes an agent known to be active against GBS should replace GBS prophylaxis.
[d]NAAT for GBS is optional and might not be available in all settings. If intrapartum NAAT is negative for GBS but any other intrapartum risk factor (delivery at <37 weeks' gestation, amniotic rupture at ≥18 hours, or temperature ≥100.4°F [≥38.0°C]) is present, then intrapartum antibiotic prophylaxis is indicated.
GBS, Group B streptococci; *NAAT,* nucleic acid amplification test.
From Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. MMWR Recomm Rep. *2010;59(RR-10):1–32.*

a threshold of 10^4 colony-forming units/mL should be used to screen urine for the presence of GBS in women with asymptomatic bacteriuria, and updated algorithms for screening and prophylaxis for women with preterm labor or preterm premature rupture of membranes.[110]

Penicillin G remains the preferred agent for intrapartum prophylaxis because of its relatively narrow spectrum of antimicrobial activity and documented efficacy. The recommendation is to administer 5 million units of penicillin G initially and then 2.5 to 3.0 million units every 4 hours until delivery. Ampicillin (2 g initially, then 1 g every 4 hours) is an acceptable alternative agent, but it has a broader antimicrobial spectrum and can potentially affect the incidence of ampicillin-resistant *Escherichia coli* or other forms of non–group B streptococcal sepsis.[111] Cefazolin (2 g initially, then 1 g every 8 hours until delivery) is recommended for use in penicillin-allergic women who do not have a history of anaphylaxis, angioedema, respiratory distress, or urticaria after administration of a penicillin or cephalosporin. Because of increasing resistance rates of GBS to clindamycin in the United States, susceptibility testing should be performed for antenatal group B streptococcal isolates from penicillin-allergic women at high risk for anaphylaxis. Clindamycin (900 mg intravenously every 8 hours) can be used for prophylaxis only *if* the isolate is susceptible and the result of testing for inducible clindamycin resistance is negative. Vancomycin (1 g every 12 hours until delivery) is the suggested alternative for penicillin-allergic women at high risk for anaphylaxis, but its ability to penetrate into amniotic fluid or to be effective in preventing early-onset disease has not been established.[112]

Several studies have evaluated the potential association between intrapartum antibiotic prophylaxis and the rates of neonatal sepsis caused by other pathogens. The incidence of early-onset *E. coli* cases is slightly decreased, and the proportion of isolates from term infants that are susceptible to ampicillin has not increased. However, for infants born prematurely, especially those weighing less than 1500 g at birth, there has been a significant decrease of *E. coli* isolates causing sepsis that are susceptible to ampicillin. This most likely results from the large proportion of women with preterm labor being treated with antimicrobial agents before sustained labor, but this is an area that requires further studies.

The 2010 guidelines provide a detailed algorithm for management of infants born to mothers who have received intrapartum antibiotic prophylaxis.[110] These advocate a full diagnostic evaluation and empirical treatment for infants with signs of neonatal sepsis and a limited diagnostic evaluation and empirical treatment if intrapartum antibiotics were administered for suspected chorioamnionitis. The guidelines recommend a limited sepsis evaluation with observation for at least 48 hours for infants of less than 37 weeks of gestation or with membrane rupture of at least 18 hours in mothers who did not receive intrapartum prophylaxis with penicillin, ampicillin, or cefazolin for at least 4 hours before delivery. A healthy-appearing infant at term gestation whose mother received 4 or more hours of intrapartum prophylaxis can be discharged home after 24 hours of observation if other discharge criteria have been met and a person able to comply fully with instructions for home observation is available.

Group B Streptococcal Vaccines

Because the risk for invasive group B streptococcal disease in pregnant women and neonates is associated with low concentrations of maternal antibodies to the type-specific capsular polysaccharides of these organisms at delivery, immunization to prevent these infections is desirable. Uncoupled group B streptococcal capsular polysaccharides were poorly immunogenic in healthy adults with low concentrations of capsular-specific antibodies. Capsular polysaccharide-protein conjugate vaccines subsequently were developed. The first, composed of type III polysaccharide covalently linked to tetanus toxoid, was well tolerated and significantly more immunogenic than uncoupled capsular polysaccharide.[113] Additional conjugate vaccines were developed for capsular types Ia, Ib, II, and V polysaccharides. Each of these monovalent conjugate vaccines was well tolerated and immunogenic when administered as a single dose in healthy nonpregnant adults. A type III polysaccharide–tetanus toxoid conjugate vaccine has been administered to healthy women in the third trimester (mean gestation, 31 weeks) of pregnancy.[114] The concentration of capsular polysaccharide–specific IgG was sufficient to provide transplacental antibodies that were functional in vitro through the first 2 months of life for infants born to these women. The concept that two monovalent conjugate vaccines combined can elicit immune responses comparable to those of the monovalent vaccines alone has been proved by the administration to healthy adults of bivalent type II and III group B streptococcal conjugate vaccines.[115]

Administration of a multivalent group B streptococcal polysaccharide–tetanus toxoid conjugate vaccine to women during the last third of pregnancy theoretically could provide capsular type–specific antibodies in sufficient concentrations to passively protect neonates from early- and late-onset disease. Such a multivalent conjugate vaccine tested in a murine model provided protection of pups against lethal infection with multiple group B streptococcal types.[116] This approach to prevention, in contrast to maternal intrapartum antibiotic prophylaxis, offers a method that is simple, cost-effective, and durable and would not promote antimicrobial resistance. Another attribute of maternal GBS immunization is that it would reduce the proportion of US women, currently estimated to be 30%, receiving antibiotics during labor that may be adversely affecting the infant microbiome. The demonstration that type V group B streptococcal polysaccharide–tetanus toxoid conjugate vaccine was well tolerated and immunogenic in healthy older adults also offers promise that immunization could confer protection from invasive infection in at-risk nonpregnant adults, but further studies are needed.[117]

The discovery that surface-associated pili are widely distributed among GBS and that vaccines based on combinations of recombinant pilus components protect mice against lethal challenge with a wide variety of group B streptococcal strains paves the way for design of pilus-based, multivalent vaccines against GBS.[118] Furthermore, pilus islands 1, 2a, and 2b, alone or in combination, were uniformly identified

on more than 200 isolates from infants and adults with invasive group B streptococcal disease, and most were highly surface expressed.[119] Other group B streptococcal surface proteins, including Rib and components of C protein, also offer potential as carriers in conjugate vaccines.[120,121]

Development of multivalent vaccines to prevent group B streptococcal infection has been undertaken by the pharmaceutical industry. In a phase I study that enrolled healthy, nonpregnant women, a single dose of unadjuvanted, preservative-free vaccine consisting of purified capsular polysaccharides of group B streptococcal types Ia, Ib, and III conjugated to cross-reacting material 197 (CRM197) was well tolerated and immunogenic.[122] This trivalent vaccine, when administered during pregnancy in an open-label, phase II, multicenter trial, was less immunogenic in women with HIV infection than it was in those not HIV infected.[123] However, a multivalent conjugate vaccine incorporating six capsular types is needed to prevent most group B streptococcal–associated mortality and morbidity in pregnancy and in fetuses and young infants globally, and possibly to reduce the burden of group B streptococcal disease in adults.

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The complete reference list is available online at Expert Consult.

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202 Viridans Streptococci, Nutritionally Variant Streptococci, and Groups C and G Streptococci

Cheston B. Cunha

SHORT VIEW SUMMARY

Overview
- The habitat, epidemiology, microbiology, clinical manifestations, and therapy of the viridans streptococci, including the *Streptococcus anginosus* group, nutritionally variant streptococci (NVS), and groups C and G streptococci, are discussed in this chapter.

Habitat
- The viridans streptococci are part of the normal microbiota of the oropharynx, skin, and gastrointestinal (GI) tract. These organisms lack the virulence factors of β-hemolytic streptococci and are opportunistic pathogens in specific clinical settings, for instance, damaged heart valves (subacute endocarditis [SBE]) and severe neutropenia (bacteremia).

Microbiology
- Most viridans streptococci demonstrate α- or γ-hemolysis and are not typeable by Lancefield antisera. These organisms are gram-positive cocci arranged in chains and are facultatively anaerobic. Viridans streptococci are catalase and coagulase negative. The NVS require thiol compounds for growth. Groups C and G streptococci may demonstrate β-hemolysis resembling that of group A streptococci (GAS).

Virulence
- Viridans streptococci are primarily colonizers of the oral and GI mucosa. Strains produce varying amounts of capsular polysaccharide (dextran) essential for adherence and colonization. Strains with abundant capsule production are the most likely strains to cause SBE. Capsular polysaccharide also inhibits phagocytosis. Most viridans streptococci are not organisms that induce a pyogenic response. Abscesses formation is a characteristic of the *S. anginosus* group of viridans streptococci.

Clinical Manifestations
- The most frequent infection caused by the viridans streptococci, including NVS, is native valve SBE. Abscesses of teeth, brain, liver, and abdomen are caused by the *S. anginosus* group of viridans staphylococci, but these organisms rarely cause SBE. Groups C and G streptococci most often cause pharyngitis or cellulitis in the elderly, diabetic persons, cirrhotic persons, or those with malignancy.

Diagnosis
- The diagnosis of viridans streptococcal SBE is based on demonstrating a high-grade continuous bacteremia and a valvular vegetation. With suspected SBE due to NVS, blood media are supplemented with vitamin B_6 and subcultured on chocolate agar. In *S. anginosus* group abscesses, diagnosis is by culture of the organism. Acute pharyngitis due to groups C and G streptococci resembles GAS pharyngitis and is diagnosed by throat culture. Cellulitis due to group C or G streptococci is indistinguishable from GAS or group B streptococci cellulitis unless the organism is identified in positive blood cultures.

Antimicrobial Therapy
- The viridans streptococci remain highly susceptible to penicillin and β-lactams. Resistance is minimal and, if present, is a "relative resistance" responsive to high-dose penicillin and β-lactams. The NVS are relatively more penicillin resistant than other viridans streptococci. Like staphylococci, viridans streptococci may demonstrate antibiotic "tolerance." The main therapeutic challenge in treating viridans streptococcal SBE is not antibiotic resistance to penicillin or β-lactams, but it is related to antibiotic penetration of valvular vegetations.

OVERVIEW

The viridans group streptococci (VGS) are a diverse group of organisms and are the predominant microbiota in the oropharynx and gastrointestinal (GI) tract. The viridans streptococci cause a significant percentage of all cases of infective endocarditis (IE). *Streptococcus mutans* is responsible for dental caries. Several typing schemes for the VGS have been proposed. There are at least 30 recognized species of VGS, which are classified into six major groups: *S. mutans* group, *Streptococcus salivarius* group, *Streptococcus mitis* group, *Streptococcus sanguinis* group, *Streptococcus anginosus* group, and *Streptococcus bovis* group.[1] The *S. anginosus* group has been the one that is most difficult to classify because all isolates are not uniformly α-hemolytic; some are γ-hemolytic or β-hemolytic. VGS usually do not react with Lancefield grouping antisera, exceptions being some strains of *S. anginosus*, which may react with Lancefield A, C, F, or G antisera. *S. bovis*, a nonenterococcal group D streptococcus, has some characteristics of the enterococci and reacts with Lancefield D antisera.

VGS are catalase-negative, gram-positive cocci in chains, which are leucine aminopeptidase positive, pyrrolidonyl arylamidase (PYR) negative, and do not grow in bile esculin agar or 6.5% sodium chloride (NaCl) broth. The differentiation of *Streptococcus pneumoniae* from VGS is by optochin (ethylhydrocupreine hydrochloride) susceptibility and bile solubility.[1] Because of its major role in disease, Chapter 199 is devoted to *S. pneumoniae*.

Viridans streptococci are so named for their green discoloration surrounding colonies, (α-hemolysis) on blood agar, but some species produce no greenish discoloration (γ-hemolysis). Viridans streptococci are common colonizers of the oropharynx, GI tract, and skin. Colonizing strains of viridans streptococci produce abundant capsular material that facilitates adherence to epithelial cells. Colonization of mucosal surfaces, for instance, the oropharynx by viridans streptococci, is protective against colonization by other organisms, such as aerobic gram-negative bacilli.

With the exception of *S. pneumoniae*, *Streptococcus mitis*, and the *S. anginosus* group, viridans streptococci are relatively avirulent and are opportunistic pathogens usually requiring damaged tissue, such as heart valves, or impaired neutrophil function, such as chemotherapy-induced neutropenia. Unlike the other viridans streptococci, the *S. anginosus* group is associated with abscess formation. Streptococci are gram-positive cocci and are facultatively anaerobic or microaerophilic, catalase negative, and arranged in pairs or chains. With the exception of group A streptococci (GAS), streptococcal virulence is inversely related to chain length, for instance, *S. pneumoniae* (diplococci) are more virulent than viridans streptococci (long chains). Most streptococci may be classified on the basis of their pattern of hemolysis on blood agar, Lancefield antigens, and biochemical reactions.

Streptococci are nutritionally fastidious, grow best in blood-supplemented media, and produce lactic acid without gas from glucose.

Lancefield groups A, B, C, and G streptococci are β-hemolytic. α-Hemolytic streptococci include numerous species, but the most important are *S. pneumoniae* and *S. mitis*. Viridans streptococci are a diverse group of streptococci usually demonstrating α-hemolysis and generally possessing no Lancefield cell wall antigens. They are collectively referred to as VGS.

Viridans streptococci consists of groups of organisms, for instance, the *S. mitis* group, *S. mutans* group, *S. salivarius* group, and *S. sanguinis* group (Tables 202.1 and 202.2). Each viridans group is associated with particular infections of oral origin. *S. mutans* is associated with dental caries and pleuropulmonary infection. *S. mutans* and *S. sanguinis* are frequent causes of subacute endocarditis (SBE).

The *S. anginosus* group differs from other viridans streptococci in its ability to cause invasive pyogenic infections, such as abscesses (brain, dental, lung, liver, abdomen). Unlike the other viridans streptococci, the *S. sanguinis* group rarely causes SBE.[2]

Another distinctive group of viridans streptococci are the nutritionally variant (deficient) streptococci (NVS), also referred to as pyridoxal (vitamin B_6)–dependent or "satelliting streptococci." The NVS consist of two genera: *Abiotrophia* sp. and *Granulicatella* spp. SBE is the classic infection caused by these NVS (*Abiotrophia* sp. and *Granulicatella* spp.) and is often clinically more severe than infection caused by other viridans streptococci.[3] Although consisting of four species in two genera, the term *NVS* will be used in this chapter for historical reasons, and this term is a reminder of their fastidious nutritional requirements.

MICROBIOLOGY

Viridans Streptococci

Viridans streptococci are gram-positive cocci arranged in pairs and chains and are facultative anaerobes, but some strains are microaerophilic. As a group viridans streptococci are fastidious organisms and grow best on blood agar but form small, raised colonies. They are nonmotile and nonspore formers. The viridans streptococci produce lactic acid, but not gas, from glucose. Colony size and characteristics vary by species.[4]

Greenish discoloration surrounding viridans streptococcal colonies ("viridans" from the Latin *virdis,* meaning "green") results in a greenish discoloration on blood agar that is due to partial red blood cell destruction. In general, the type of hemolysis distinguishes the viridans streptococci, but some strains may demonstrate γ-hemolysis or β-hemolysis. The intensity of α-hemolysis, that is, the intensity of greenish discoloration varies with the medium and viridans streptococcal species. Streptococcal colonies often glisten. After 24 hours of incubation, β-hemolytic streptococci (GAS, group C streptococci [GCS], and group G streptococci [GGS]) form large colonies (>0.5 mm in diameter); β-hemolytic small colonies suggest the *S. anginosus* group. These small *S. anginosus* (<0.5 mm in diameter) "pinpoint colonies" have honey or caramel odor. Group B β-hemolytic streptococcal colonies are larger and demonstrate less intense β-hemolysis (smaller zone) than other β-hemolytic streptococci. Colonies of viridans streptococci demonstrate α-hemolysis and have a "domed" appearance, in contrast to the flat colonies of other streptococci. The α-hemolytic colonies of *S. pneumoniae*

TABLE 202.1 Viridans Streptococci Group

	MOST COMMON HUMAN ISOLATES	OTHER GROUP SPECIES
***S. mitis* Group**		
S. cristatus		*S. cristatus*
S. infantis		*S. infantis*
S. mitis	*S. mitis*	
S. oralis	*S. oralis*	
S. peroris		*S. peroris*
S. tigurinus		*S. tigurinus*
***S. mutans* Group**		
S. cricetus		*S. cricetus*
S. downei		*S. downei*
S. ferus		*S. ferus*
S. hyobvaginalis		*S. hyobvaginalis*
S. macacae		*S. macacae*
S. mutans	*S. mutans*	
S. ratti		*S. ratti*
***S. salivarius* Group**		
S. alactolyticus		*S. alactolyticus*
S. hyointestinalis		*S. hyointestinalis*
S. infantarius		*S. infantarius*
S. salivarius	*S. salivarius*	
S. thermophilus		*S. thermophilus*
S. vestibularis	*S. vestibularis*	
***S. sanguinis* Group**		
S. gordonii	*S. gordonii*	
S. parasanguinis		*S. parasanguinis*
S. sanguinis	*S. sanguinis*	
***S. anginosus* Group**[a]		
S. anginosus	*S. anginosus*	
S. intermedius		*S. intermedius*
S. constellatus	*S. constellatus*	
Nutritionally Variant Streptococci		
Abiotrophia defectiva *Granulicatella adiacens* *Granulicatella elegans*	*Abiotrophia defectiva* *Granulicatella adiacens* *Granulicatella elegans*	
Granulicatella para-adiacens		*Granulicatella para-adiacens*

[a]Formerly *S. milleri* group.

TABLE 202.2 Some Biochemical Characteristics of Viridans Streptococci

SPECIES	VOGES-PROSKAUER	ARGININE	ESCULIN	MANNITOL	SORBITOL
S. mutans	+	–	+	+	+
S. salivarius	+	–	+	–	–
S. anginosus	+	+	+	–	–
S. sanguinis	–	+	+	–	V
S. gordonii	–	+	+	–	V
S. mitis	–	–	–	–	V
S. oralis	–	–	V	–	–

V, Variable.

typically have a depressed center. In general, viridans streptococci are not groupable by Lancefield antisera, but important exceptions include the Lancefield group F streptococci of the *S. anginosus* group.

Microbiologically, it is important to differentiate viridans streptococci from those in groups A, B, C, and G streptococci; group D enterococci; and nonenterococcal group D streptococci. *S. pneumoniae* is a lancet-shaped diplococcus and does not form long chains typical of the viridans streptococci. *S. pneumoniae* is α-hemolytic and demonstrates optochin inhibition.[1]

Group D enterococci are bile soluble, grow in 6.5% NaCl broth, and are penicillin resistant. In contrast, *S. bovis, (S. gallolyticus),* a nonenterococcal group D streptococcus, is penicillin susceptible.

Streptococcus anginosus Group

The *S. anginosus* group differs from other viridans streptococci in its ability to cause invasive pyogenic infections, that is, particularly abscesses (brain, dental, lung, heart, liver, abdomen).[5,6] Clinically, the *S. anginosus* group behaves more like *Staphylococcus aureus* than the other viridans streptococci, which are relatively avirulent. Viridans "small colony" streptococci (originally known as "minute hemolytic streptococci"), the *S. anginosus* group, may be rapidly differentiated from other viridans streptococci by three key tests: a positive Voges-Proskauer (VP) test, arginine hydrolysis, and inability to ferment sorbitol (Table 202.3). If present, a deacetyl metabolite is responsible for the "caramel odor" and is a distinctive feature of *S. anginosus* colonies on solid media. Some isolates of the *S. anginosus* group may react with Lancefield A, C, F, or G group antisera, but if the isolate is Lancefield group F positive, then the organism is one of the *S. anginosus* group.

Nutritionally Variant Streptococci: *Abiotrophia* sp. and *Granulicatella* spp.

The NVS have aspects in common with the viridans streptococci but have distinctive features. Like the viridans streptococci, they are commensals of the oropharynx and GI tract. *Abiotrophia* sp. and *Granulicatella* spp. differ from other viridans streptococci in their growth requirements. For NVS culture, media must contain thiol compounds (L-cysteine or vitamin B_6 or pyridoxamine). There is no growth on solid subculture media unless supplemented with pyridoxal (vitamin B_6). Alternatively, using a *S. aureus* streak to provide thiol compounds, subculture produces satellite colonies ("satelliting") surrounding the *S. aureus* streak. NVS colonies closest to the streak have uniform coccal morphology on Gram stain but show increasing pleomorphism the further from the *S. aureus* cross-streak. Aside from their vitamin B_6 growth requirement, they are PYR positive, whereas the viridans streptococci are PYR negative. In blood cultures NVS appear as oval to bulbous bacilliform cocci in short chains resembling *Streptobacillus moniliformis,* and some have long filaments. Microbiologic clues to NVS on blood culture are gram-variable pleomorphic streptococci that fail to grow on unsupplemented solid media subculture.

Currently, NVS have been placed in two distinct genera consisting of four species: one *Abiotrophia* sp., that is, *Abiotrophia defectiva,* and three *Granulicatella* spp., that is, *Granulicatella adiacens*, *Granulicatella elegans*, and *Granulicatella para-adiacens.* Species are differentiated by hydrolysis of hippurate, arginine, galactosidase, glucuronidase, and carbohydrate fermentation.[1]

EPIDEMIOLOGY

Viridans Streptococci and the *Streptococcus anginosus* Group

The viridans streptococci are part of the normal microbiota of the mouth and GI tract. The concentration of viridans streptococci in the GI tract is greatest in the oropharynx. Colonization of the oral cavity varies by species, for instance, *S. mitis* and *S. anginosus* (buccal mucosa); *S. oralis*, *S. mitis*, and *S. sanguinis* (early dental plaque); *Streptococcus gordonii* (supragingival plaque); and *S. anginosus* (subgingival plaque).[7,8] The adherence of the oral viridans streptococci is directly proportional to capsule production, which is related to fibronectin adherence to oral epithelial cells, that is, fibronectin selectively promotes adherence of oral streptococci, such as *S. mutans*.[9,10] In hospitalized patients with impaired fibronectin function, decreased adherence of oral streptococci predisposes to colonization by other organisms, such as nosocomial gram-negative bacilli. The frequency of viridans streptococci causing SBE is directly related to their relative capsule production. During bacteremia originating in the oral cavity, viridans streptococci with polysaccharide capsules are able to adhere to damaged heart valves and initiate the development of valvular vegetations. After bacteremia adherence precedes colonization, and colonization precedes infection on damaged cardiac valves.[9,11] Viridans streptococcal strains, most numerous in the oropharynx, with the most abundant capsule production are the most frequent species to cause SBE on damaged heart valves. Of interest, although the *S. anginosus* group is an oral commensal and associated with dental plaque as are the other oral viridans streptococci,

TABLE 202.3 Viridans Streptococci: Clinical Infectious Disease Spectrum

	COMMON COLONIZATION SITES	NONPATHOGENIC AT SITE	FREQUENT INFECTIOUS MANIFESTATIONS
Viridans streptococci[a]	Oropharynx (not a cause of pharyngitis) Skin (not a cause of cellulitis or abscess) GI tract (not a cause of cholecystitis, appendicitis, diverticulitis) Female GU tract	Lung[b,c] Bone[c] (not a cause of osteomyelitis) Sacral decubitus ulcers[c] Pelvis Skin Urinary tract (kidney, bladder, prostate)	Dental caries SBE[d] Late PVE Bacteremia (in neutropenic hosts)
S. anginosus (*S. milleri*) group	Oropharynx Upper respiratory tract	Bone Skin Urinary tract (kidney, bladder, prostate)	Brain abscesses Dental abscesses Lung abscesses Empyema Hepatic abscesses Myocardial abscesses Abdominal abscesses Pelvic abscesses
NVS (*Granulicatella* spp., *Abiotrophia* sp.)	Oropharynx Upper respiratory tract	Lung Skin GI tract GU tract	SBE Bacteremia (in neutropenic hosts)

[a]*S. mitis* group, *S. mutans* group, *S. salivarius* group, *S. sanguinis* group, and NVS.
[b]May be isolated from abscesses.
[c]As a commensal/colonizer cultured from tissues commonly.
[d]CNS involvement (aseptic meningitis) emboli, mycotic aneurysm.
CNS, Central nervous system; *GI,* gastrointestinal; *GU,* genitourinary; *NVS,* nutritionally variant streptococci; *PVE,* prosthetic valve endocarditis; *SBE,* subacute endocarditis.

the *S. anginosus* group is a rare cause of SBE.[12] In addition, in the setting of decreased neutrophil numbers, such as neutropenia, in severely leukopenic oncology patients, viridans streptococci may cause primary bacteremia.[13,14,15]

Nutritionally Variant Streptococci: *Abiotrophia* sp. and *Granulicatella* spp.

The NVS were first described as a species closely related to *S. mitior* (*S. mitis*) but were found to be genetically unrelated. *Abiotrophia* sp. and *Granulicatella* spp., like viridans streptococci, are colonizers of the oropharynx, GI tract. As with other streptococci and staphylococci, some NVS strains may demonstrate "tolerance," that is, minimal bactericidal concentration (MBC) ≥ 32 × minimal inhibitory concentration (MIC). If antibiotic "tolerance" is present, it has important therapeutic implications.[16]

VIRULENCE

Viridans Streptococci

Except for *S. mitis* and *S. pneumoniae*, viridans streptococci lack the virulence factors of β-hemolytic streptococci, are relatively avirulent, and are not pathogenic for laboratory animals. To cause infection, viridans streptococci require impaired host defenses, for instance, severe leukopenia in oncology patients. Viridans streptococci are the most frequent pathogens in native valve SBE and late prosthetic valve endocarditis (PVE) pathogens. Viridans streptococci are not primary pathogens in meningitis, bone, skin, or urine infections. However, viridans streptococci are commonly cultured alone or with other colonizers and are often present in dental, hepatic, or GI abscesses. Before ascribing an infection to viridans streptococci and considering the causative organism a pathogen, the organism should be repeatedly isolated (in pure culture) from sterile body sites.

In viridans streptococcal SBE virulence is determined by lipoteichoic acid (fibronectin adhesin). After adherence to damaged cardiac valves, viridans streptococci promote platelet aggregation via tissue factor, which is the initial step in the formation of cardiac vegetations.[11] Viridans streptococci are the most frequent pathogens causing native valve SBE in individuals with previous heart valve damage due to rheumatic heart disease, mitral valve prolapse, or degenerative valvular disease.[17,18] Viridans streptococci are important pathogens in late prosthetic valve endocarditis (not early PVE). With normal heart valves, repeated bacteremias from the oral cavity are common but do not result in SBE. The ability of viridans streptococci to cause SBE is directly related to the amount of polysaccharide (dextran) capsule produced and is species dependent. Strains with more abundant capsules are the most frequent SBE pathogens; conversely, strains with little or no capsule, such as *S. mitis,* are infrequent causes of SBE.

S. mutans is particularly associated with dental caries. Adherence to dental enamel is the initial step in caries development and is mediated by glucan. *S. mutans* glucan-mediated colonization occurs and in the presence of fermented sucrose produces acid that damages the enamel.

S. anginosus group, unlike other viridans streptococci, produces a variety of hydrolytic enzymes, such as hyaluronidase and DNase. Some species are able to use sialic acid as a sole carbon source and may be a growth factor for these organisms. Some species, for instance, *S. mitis,* produce superantigens that stimulate nonspecific T-lymphocyte proliferation.[19] Superantigens are potent inducers of cytokine release in systemic infections.

Streptococcus anginosus Group

Strains of *S. anginosus* group blunt chemotaxis and are relatively resistant to neutrophil killing.[20] The mechanism of abscess formation by the *S. anginosus* remains unknown. The *S. anginosus* group rarely causes SBE, but when it does, infection is often complicated by myocardial abscess with heart block. Species of the *S. anginosus* group may be cultured from abscesses alone or with other viridans streptococci.

Nutritionally Variant Streptococci: *Abiotrophia* sp. and *Granulicatella* spp.

Like viridans streptococci, NVS cause SBE on previously damaged heart valves. NVS causes SBE, which is often more severe than that caused by other viridans streptococci.[21] Among NVS organisms, *A. defectiva* seems especially suited to cause endovascular infection because of its ability to adhere to extracellular fibronectin.[22] Like other viridans streptococci, NVS may cause bacteremia in severely neutropenic oncology patients.

CLINICAL MANIFESTATIONS

Abscesses: *Streptococcus anginosus* Group

S. anginosus group organisms are frequent isolates in head and neck abscesses in pure culture or together with what used to be called the "oral pigmented *Bacteroides,*" for instance, *Prevotella melaninogenica*. Organisms of the *S. anginosus* group are often cultured from brain abscesses with or without other viridans streptococci. Brain abscesses do not result from viridans streptococcal SBE but result from bacteremia with central nervous system (CNS) seeding occurring in patients with suppurative lung disease, such as bronchiectasis, lung abscess, or cyanotic heart disease (R→L shunts). Alternatively, brain abscesses may result from spread from a contiguous focus.

Aseptic Meningitis: Viridans Streptococci

Not uncommonly, SBE due to viridans streptococci may be accompanied by seeding of the cerebrospinal fluid (CSF), clinically manifested as "aseptic meningitis." In this form there is an aseptic/viral CSF profile, with Gram stain/culture negativity with normal glucose and mild lymphocytic pleocytosis. This is in contrast to *S. aureus* acute bacterial endocarditis (ABE), in which the CSF seeding results in acute bacterial meningitis (ABM), showing a CSF "septic profile" with CSF Gram stain/culture positivity for *S. aureus*.

CNS emboli from viridans streptococcal SBE usually occurs in the terminal branches of the middle cerebral artery. The most common embolic CNS manifestation of viridans streptococci is an embolic stroke or mycotic aneurysm. Most mycotic aneurysms are asymptomatic, but rupture results in intracranial hemorrhage.

Subacute Bacterial Endocarditis: Viridans streptococci, *Streptococcus anginosus,* and Nutritionally Variant Streptococci

In the preantibiotic era, viridans streptococci accounted for approximately 75% of cases of IE. At the present time their relative frequency in endocarditis has declined to as low as 20%.[23] This change in epidemiology largely reflects an increase in the number of patients acquiring staphylococcal endocarditis in association with injection drug use, intravenous (IV) catheters, or prosthetic valves, and a decrease in the overall incidence of streptococcal endocarditis in industrialized nations.[24] In patients with SBE due to viridans streptococci, fevers usually are low grade (<102°F) and without rigors. Essentially all adults with native valve SBE have a cardiac murmur secondary to prior cardiac valvular damage. In viridans streptococcal SBE, splenomegaly and peripheral manifestations are a function of time. Of importance, peripheral adenopathy or hepatomegaly are not features of viridans streptococcal SBE and should suggest an alternative diagnosis. Viridans streptococci are also important pathogens in late-onset PVE. With the exception of the *S. anginosus* group, intracardiac abscesses or heart block are not clinical features of viridans streptococcal SBE. The diagnosis of SBE is based on the presence of fever, cardiac murmur, valvular vegetation, and continuous high-grade bacteremia due to an SBE pathogen, such as viridans streptococci. As the name suggests, SBE onset is subacute with low-grade fevers without chills. The fever in viridans streptococcal SBE has no particular pattern or periodicity, but it usually is prolonged and low grade. Night sweats and weight loss without anorexia may occur. Eye involvement is common, with conjunctival hemorrhage and/or Roth spots. The longer that SBE has been present, the more common are the peripheral manifestations.[25] Mild splenomegaly (nontender) is a common finding. Because hepatomegaly is not a feature of SBE, if present alone or with splenomegaly, an alternative explanation for liver enlargement should be sought. Alternatively, acute onset of otherwise unexplained hemorrhagic stroke, blindness, or abdominal pain due to a splenic infarct may suggest the diagnosis of SBE. A cardiac murmur with SBE is nearly always present, but there will not be a valvular murmur if related to a ventricular septal

defect. In contrast to new or changing murmurs, ABE murmurs are unchanging. Native valve viridans streptococcal SBE is diagnosed based on the presence of fever, cardiac murmur, continuous high-grade bacteremia, and a valvular vegetation by cardiac echocardiography.

In an adult with a cardiac murmur, even with low-grade fevers, a cardiac vegetation, and peripheral manifestation of SBE, the growth of viridans streptococci in one or two blood cultures is not diagnostic of SBE. With only one or two blood cultures positive for viridans streptococci, the clinician should consider the blood culture isolates a skin bacterial contaminant. Aside from the clinical findings described, the diagnosis of viridans streptococcal SBE requires the presence of high-grade continuous bacteremia. Streptococcal bacteremia, fever, murmur, cardiac vegetation, and peripheral manifestations without high-grade continuous viridans cultures should suggest the possibility of a SBE mimic, for instance, marantic endocarditis and Libman-Sacks endocarditis. The systemic embolic potential of viridans streptococcal SBE is directly related to vegetation size.

Typical nonspecific laboratory test abnormalities associated with viridans streptococcal SBE include an otherwise unexplained highly elevated erythrocyte sedimentation rate, mild leukocytosis, mild anemia (of chronic disease), and occasionally thrombocytosis. Unless there is associated heart failure, serum aminotransferases are not elevated. Microscopic hematuria is common and due to the focal glomerulonephritis that is the most common renal manifestation of SBE. *S. anginosus* group bacteria clinically behave like *S. aureus* with ABE and myocardial abscesses.

The NVS are a rare cause of SBE. NVS should be suspected as likely pathogens in SBE with positive blood cultures for streptococci that fail to grow on subculture. Cardiac vegetations are larger with NVS than viridans streptococcal SBE, but clinical manifestations of NVS SBE are often more subtle than viridans streptococcal SBE. Nevertheless, mortality of NVS SBE is higher than viridans streptococci SBE (Table 202.4).

Positive Blood Cultures and Nonsterile Body Sites: Viridans Streptococci

Viridans streptococci that are not invasive and relatively avirulent are commonly cultured from skin, sacral decubitus ulcers, and are common blood culture contaminants.[26] Unlike group D enterococci, viridans streptococci do not cause urinary tract infections. They are predominately colonizers of the oropharynx, GI tract, and skin. Until proven otherwise, culture of viridans streptococci from nonsterile sites alone or with other organisms should be considered as colonizers and not pathogens. Repeated isolation in pure culture from sterile body sites may indicate a pathogenic role, but microbiologic results should always be interpreted in the appropriate clinical context. Having blood cultures in which only one or two are positive for viridans streptococci argues against a diagnosis of SBE. High-grade (3/4–4) continuous viridans streptococcal bacteremia suggests SBE until proven otherwise (see Table 202.3).

Primary viridans streptococcal bacteremia may occur in patients with profound/prolonged neutropenia secondary to chemotherapy.[27,28] Viridans streptococci may gain bloodstream access from colonized intestinal mucosa damaged by chemotherapy. Fatality rates among neutropenic patients with bacteremia caused by penicillin-resistant viridans streptococci have been higher than for penicillin-susceptible strains.[29,30]

TABLE 202.4 Clinical Spectrum of Group C and G Streptococci

	GROUP C STREPTOCOCCI	GROUP G STREPTOCOCCI
Habitat	*Normal flora* Oropharynx Skin Female genital tract	*Normal flora* Oropharynx Skin Female genital tract
Predisposing factors	Animal or bird contact Ingestion of unpasteurized dairy products (milk, cheese) Malignancy Alcohol abuse DM	Malignancy Alcohol abuse DM Lymphedema Elderly IVDA
Colonization common	Oropharynx Skin	Oropharynx Skin
Rare causes of infections	1° bacteremia UTIs Osteomyelitis Septic arthritis CAP ABM (secondary to bacteremia)	1° bacteremia UTIs Osteomyelitis Septic arthritis ABM (2° to bacteremia)
Uncommon infections	ABM (2° to ABE) Brain abscess (2° to ABE) Endophthalmitis CAP Puerperal sepsis[a] Endometritis ABE[b,c]	ABM (2° to ABE) Brain abscess (2° to ABE) CAP Endophthalmitis Puerperal sepsis[a] Endometritis ABE[b,c]
Common infectious manifestations	Cellulitis (often recurrent) Pharyngitis[d] Septic arthritis[e,f]	Cellulitis (often recurrent) Pharyngitis Wound infections[a] Septic arthritis 2° bacteremia (from any of the above)
Postinfectious and immune manifestations	AGN Reactive arthritis	

[a]Nosocomial infections.
[b]Myocardial abscess, rapid valvular destruction, paravalvular abscess, congestive heart failure, and septic emboli.
[c]Skin usual source.
[d]May be severe.
[e]With normal and preexisting joint damage. Native joint septic arthritis usually polyarticular.
[f]2° bacteremia from cellulitis or endocarditis.
1°, Primary; *2°,* secondary; *ABE,* acute bacterial endocarditis; *ABM,* acute bacterial meningitis; *AGN,* acute glomerulonephritis; *CAP,* community-acquired pneumonia; *DM,* diabetes mellitus; *GCS,* group C streptococci; *GGS,* group G streptococci; *IVDA,* intravenous drug abuser; *UTI,* urinary tract infection.

THERAPY: VIRIDANS STREPTOCOCCI

Subacute Bacterial Endocarditis

Recommended regimens for treatment of streptococcal endocarditis are based largely on clinical observations and experimental animal models of endocarditis.[31] Optimal antibiotic selection for the therapy of viridans streptococcal endocarditis is based on two primary therapeutic considerations. First, the antibiotic selected should have a high degree of activity against the pathogen. Second, as important a consideration is achieving therapeutically effective concentrations at the site of infection, for instance, with infected cardiac valvular vegetation. Using pharmacokinetically based dosing, dosing interval, and duration, eradication of the bacteremic component of SBE is relatively straightforward. However, eradicating the source infection is critical to therapeutic success with SBE, that is, the ability of the selected antibiotic to sterilize the infected heart valve vegetation. With appropriate antibiotic therapy for viridans streptococcal SBE, elimination of the organism from the blood is rapid and easily achieved. Viridans streptococcal SBE is treated for weeks (see Chapter 80) so as to penetrate and sterilize the cardiac vegetation. With appropriate antimicrobial therapy, the continuous bacteremia of viridans streptococcal SBE rapidly resolves. Viridans streptococcal SBE relapses are rare with adequate duration of therapy. Cardiac vegetations decrease in size during effective antimicrobial therapy and usually completely resolve with therapy.

Meningitis: Viridans Streptococci

Despite the frequency with which viridans streptococci cause bacteremia, they are an uncommon cause of meningitis, accounting for only 0.3% to 5% of culture-proven cases.[32,33] Bacteremic meningitis in the absence of cardiac involvement has been described primarily in severely immunocompromised patients.[34] Viridans streptococcal SBE is often accompanied by systemic emboli. Clinically, CNS embolization may manifest as "aseptic meningitis." Typically, the CSF shows a mild lymphocytic pleocytosis, but viridans streptococci are not usually cultured from the CSF. Culture of viridans streptococci from CSF in a patient with viridans streptococcal SBE usually represents skin contamination

of the specimen. In such situations Gram staining of the CSF will be negative due to the few skin organisms contaminating the CSF culture medium. In a series of 43 patients from whom various species of α-hemolytic streptococci were isolated from CSF culture, only eight isolates (19%) were determined to be clinically relevant.[35]

Because the treatment of viridans streptococcal SBE is with high-dose penicillin or a β-lactam, additional CSF penetration is unnecessary. However, therapeutic CSF levels are an unintended effect that occurs as a result of high-dose endocarditis regimens, which are the same as for meningeal dosing, for instance, penicillin G 3 million units, IV, every 4 hours, or ceftriaxone 2 g, IV, every 24 hours. Although primary meningitis due to viridans streptococci remains rare, it can occur after medical procedures. In a review of bacterial meningitis occurring after dural puncture, streptococci were responsible for greater than 50% of the 179 cases.[36] Spinal and epidural anesthesia, myelography, and diagnostic lumbar puncture were the most common predisposing events. Iatrogenic viridans streptococcal meningitis has been reported in association with failure to adhere to infection control measures while performing lumbar puncture, especially regarding the use of face masks.[37] The same infection control measure may be important in preventing viridans streptococcal infection during joint injection.[38]

Endophthalmitis: Viridans Streptococci

With the increased use of intravitreal injections for macular degeneration and other conditions, viridans streptococci are an emerging pathogen in patients with endophthalmitis. A retrospective study of more than 33,000 injections at one large center showed that, although the overall rate of endophthalmitis was very low (13 cases over 10 years, for a rate of 0.04%), postinjection viridans streptococcal endophthalmitis occurred much more rapidly and had far worse final visual outcomes than cases caused by other organisms.[39] An outbreak of 12 patients with postinjection endophthalmitis, most caused by *S. mitis*/*S. oralis*, was linked to preparation of syringes of bevacizumab at a compounding pharmacy.[40]

Reactive and Septic Arthritis: Viridans Streptococci, Group C *Streptococcus*, and Group G *Streptococcus*

Reactive arthritis is the most common manifestation of viridans streptococcal SBE. As with CNS involvement, viridans streptococci are not culturable from synovial fluid in reactive arthritis. However, with some viridans streptococci, for instance, GCS or GGS, septic arthritis may occur from a nonendocarditis-related bacteremia. NVS septic arthritis remains rare, and specific ribosomal RNA polymerase chain reaction assays have been developed to aid in the identification of NVS.[41] With septic arthritis, antibiotic selection is based on the activity of the antibiotic against GCS or GGS and the ability of the antibiotic to penetrate synovial fluid in therapeutically effective concentrations. This is readily accomplished with β-lactam therapy, but synovial fluid penetration is suboptimal with vancomycin.

Abscesses: *Streptococcus anginosus* Group

Most viridans streptococci do not form abscesses, but abscess formation is particularly associated with the *S. anginosus* group. Abscesses may occur in many organs. Large abscesses, such as in the liver and abdomen, usually require drainage for source control. To be effective, abscess drainage must be adequate, and partially or completely drained abscesses usually require additional drainage and are unlikely to resolve with antibiotic therapy alone. Antibiotic penetration into abscesses is suboptimal with antibiotics, and antibiotic therapy alone should not be expected to effectively eliminate large abscesses.

Resistance: Viridans Streptococci and Nutritionally Variant Streptococci—*Abiotrophia* sp. and *Granulicatella* spp.

Viridans streptococci remain highly susceptible (MIC ≤ 0.12 μg/mL for penicillin) to penicillin and β-lactams.[42] Resistance, if present, is "relative resistance" (MIC = 0.25–2 μg/mL for penicillin). MICs are easily exceeded in blood and cardiac vegetations with the high-dose β-lactam therapies used for SBE. Even highly resistant strains (MIC ≥ 4 μg/mL for penicillin) are susceptible to high-dose penicillin or β-lactam therapy.[43] S. *mitis* and *S. oralis* are relatively more resistant than other viridans streptococci.[44] Among the viridans streptococci resistance is mediated by alterations in penicillin-binding proteins and not via β-lactamases. Trimethoprim-sulfamethoxazole (TMP-SMX) and ciprofloxacin have little activity against the viridans streptococci and should not be used, and resistance to tetracyclines and macrolides is common.[45,46,47] Decreasing susceptibility to other agents, including the third-generation cephalosporins and clindamycin, has been reported.[48] Effective alternatives to β-lactams include linezolid, levofloxacin, dalbavancin, and tigecycline.[49–51] High-level resistance to daptomycin monotherapy may occur rapidly. Resistance to daptomycin may be prevented or overcome with concomitant treatment with gentamicin.[52,53,54]

Some viridans streptococci demonstrate "tolerance," that is, MBC ≥ 32× the MIC. Clinically, tolerance should be suspected when the organism appears susceptible in vitro but the infection fails to clear even with appropriate antibiotic doses.[55] Gentamicin may be added to β-lactam therapy in the treatment of viridans streptococcal SBE to overcome such tolerance.[56] With SBE due to viridans streptococci, resistance is, in part, related to the abundance of capsular polysaccharide, and gentamicin added to a β-lactam decreases the relapse rate.[57] With uncomplicated viridans streptococcal SBE, duration of therapy can be as short as 2 weeks. If treated for 4 weeks, gentamicin may be given during the initial 2 weeks of therapy and may enhance outcomes.[57] Therapeutically achievable antibiotic concentrations in cardiac vegetations exceed the MIC of even "relatively resistant" strains of viridans streptococcal SBE. The prolonged duration of therapy of viridans streptococcal SBE is necessary for eradication of the organisms in the privileged site of infected vegetations.

Because NVS have unique growth requirements, uncommon recovery from clinical specimens, and difficulties in standardizing and interpreting testing methodologies, limited antimicrobial susceptibility data for these organisms are available. Approximately 33% to 67% of strains are relatively resistant (MIC, 0.25–2.0 μg/mL), and some isolates are highly resistant to penicillin (MIC ≥ 4 μg/mL).[58] Treatment of NVS, which are usually relatively resistant to penicillin, is usually 4 to 6 weeks with penicillin or β-lactam plus gentamicin for the initial 2 weeks.[56,57]

GROUPS C AND G STREPTOCOCCI

Group C Streptococci

Habitat

GCS are primarily animal pathogens, that is, of horses, cows, and swine. *S. equi* subsp. *zooepidemicus* causes significant, often epidemic, infections in domestic animals (horses, cattle, sheep, and pigs). It is not considered part of the normal human microbiota. Human infection is uncommon and is often reported in those with immune compromise; cases have been associated with consumption of homemade cheese and unpasteurized cow's milk and in humans who have had direct contact with animals.[59,60] However, colonization of GCS in the upper respiratory tract is relatively common.[61] In total, human infections are generally regarded as zoonotic and transmitted by ingestion of contaminated milk or dairy products.

Microbiology

Lancefield GCS have a polysaccharide capsule and may be α-, β-, or γ-hemolytic on sheep blood agar. GCS resemble GAS in some respects, but the terminal antigenic carbohydrate determinant is *N*-acetyl-galantamine in GCS, rather than *N*-acetylglucosamine as in GAS. GCS may resemble GAS on sheep blood agar. The appearance of large and small colony variants of groups C and G streptococci can make identification challenging. They are differentiated on the basis of biochemical tests, that is, VP negative and β-glucosidase positive, but some variants are VP positive and β-glucosidase negative. Because of variability in bacitracin testing with GCS, bacitracin testing cannot reliably differentiate GCS from GAS, and identification depends on group-specific antisera.[1] Unlike GAS, GCS are usually susceptible to TMP-SMX, whereas clindamycin resistance is uncommon among GCS but has been reported.[62]

Virulence
As with GAS, the primary virulence factors for GCS are the hyaluronic acid capsule and an M protein. GCS virulence is related to the abundance of its mucoid capsule, and some strains produce hemolysins.[1,63]

Clinical Manifestations
Human GCS infections may manifest clinically as pharyngitis, cellulitis, and only rarely as pneumonia, SBE, or meningitis.[60,64–67]

After colonization of the oropharynx, epidemics of GCS pharyngitis have occurred among college students.[68] Clinically, GCS pharyngitis resembles GAS pharyngitis, with fever, exudative pharyngitis, and anterior cervical adenopathy.[69–72] Of importance, like GAS pharyngitis, GCS pharyngitis may also elevate the antistreptolysin titer. However, acute rheumatic fever has not been linked to GCS pharyngitis.[73] GCS pharyngitis is usually less severe than GAS pharyngitis.[74]

In addition to the oropharynx, GCS are common colonizers of skin and female genitourinary (GU) tract. Like GAS, GCS may cause cellulitis or erysipelas. In parturient women GCS may cause puerperal sepsis or toxic shock syndrome. Nosocomial outbreaks have occurred in maternity units and burn wards.[75–77] Community-acquired pneumonia (CAP) rarely may be due to GCS and may be complicated by empyema.

Infections are most common in patients with cirrhosis, diabetes, and malignancy. Because skin colonization is common, positive blood cultures due to contamination should be differentiated from true bacteremia. Because continuous bacteremia is a feature of SBE, clinically the diagnosis of SBE should be made only with otherwise unexplained high-grade/sustained bacteremia. In patients with prolonged GCS bacteremia and clinical findings of SBE, a presumptive diagnosis may be made. GCS meningitis is usually due to CNS seeding secondary to endocarditis and may be complicated by brain abscess or subdural empyema.[66,78,79] Meningitis cases have been reported in association with ingestion of unpasteurized goat's milk[80] and from frequent contact with horses.[81]

Treatment
The antimicrobial agent of choice for group C β-hemolytic streptococci remains penicillin.[82] Other agents with good in vitro activity include amoxicillin, ureidopenicillins, cefazolin, cefotaxime, vancomycin, linezolid, and dalbavancin. Oxacillin and nafcillin are not effective. However, aside from vancomycin, the clinical experience with antimicrobial agents other than penicillin is limited. Resistance of GCS is about 30% to tetracycline, 25% to erythromycin, and 10% to ciprofloxacin. Two isolates had intermediate susceptibility to clindamycin.[83] Macrolide resistance is widespread in many countries.[63] Tolerance to penicillin has been reported in GCS, with MBCs ranging from 32- to 512-fold higher than the MICs.[84,85]

The addition of gentamicin or rifampin to a β-lactam or vancomycin results in bactericidal activity against GCS.[84,85] A marked synergy for killing of GCS by penicillin plus gentamicin has also been demonstrated, independent of penicillin tolerance. Although the clinical relevance of these findings is uncertain, combination therapy for patients with severe infections, such as endocarditis, meningitis, septic arthritis, or bacteremia in neutropenic hosts, caused by GCS should be considered.

GROUP G STREPTOCOCCI

GCS are rare causes of CAP. The clinical presentation is similar to GAS CAP, that is, segmental/lobar infiltrates with a moderate/large serosanguinous pleural effusion. [86]

Habitat
GGS are normal commensals of the oropharynx, skin, GI tract, and female GU tract.

Microbiology
GGS are characterized by two group-specific capsular polysaccharides—galactosamine and rhamnose—and GGS are usually β-hemolytic, resembling GAS. Animal, but not human, strains ferment trehalose. As with GCS, GGS produce large and small colonies, which can lead to misidentification. In some cases GGS are susceptible to bacitracin, as are GAS.

Virulence
As with GAS, M protein is the major virulence factor of GGS. M protein is protective against phagocytosis. GGS also produce C5a peptidase, which inactivates C5a chemotactic factor and fibronectin-binding protein.[1]

Clinical Manifestations
Infections due to GCS are often more severe than with other streptococci, particularly in patients with malignancy. GGS occurs in patients with cirrhosis, diabetes, and those on steroids or immunosuppressive therapy, and in IV drug abusers. GGS frequency colonizes the oropharynx but may cause acute pharyngitis, often in outbreaks.[87,88] Clinically, GGS pharyngitis resembles GAS pharyngitis but is often more severe. In contrast to GAS pharyngitis, which occurs in those younger than 30 years, GGS pharyngitis is most frequent in the elderly. As with GAS, GCS or GGS may be cultured from the throat in those with an underlying viral pharyngitis, such as due to Epstein-Barr virus. As with GCS, infection with GGS is not a cause of acute rheumatic fever.[73] Culture from the throat indicates presence (colonization) and is not diagnostic of a pathogenic role (see Table 202.4).

GGS are common skin colonizers, and skin infections also are common.[89] Most frequently, GGS occur in cellulitis and may be accompanied by bacteremia. Septic arthritis after bacteremia may result in patients with underlying joint disease and those with prosthetic joints.[90] Septic arthritis due to GCS is often polyarticular, and osteomyelitis may occur with septic arthritis. Osteomyelitis due to GGS occurs primarily in the elderly with malignancies and those with cirrhosis, osteoarthritis, or prosthetic joint materials, or with impaired host defenses.[91] GGS rarely causes CAP or SBE.[86,92] GGS SBE is often complicated by embolic complications, congestive heart failure, and acute bacterial meningitis.[78,93–95] Uncommonly, GGS infections may be the causes of puerperal sepsis.[67] Secondary bacteremia may follow skin or joint infections, but primary bacteremia also may occur, but rarely.[96,97] Although GCS are associated with malignancy, the association is even stronger with GGS.[65,67,98] Of importance, otherwise unexplained GGS bacteremia should prompt a search for an underlying malignancy or other structural lesions in the GI tract.

Treatment
GGS are also susceptible in vitro to multiple antimicrobial agents, including penicillin G, ureidopenicillins, most cephalosporins, vancomycin, linezolid, and dalbavancin.[50,83,99] The most active drugs are penicillin, ampicillin, and cefotaxime; however, oxacillin and nafcillin are not effective therapeutic agents. Clindamycin, erythromycin, and tetracycline have relatively poor activity against GGS. Resistance to tetracyclines, clindamycin, and macrolides is increasing.[100] Penicillin tolerance is not a major feature of GGS, and it has been demonstrated only in the presence of a high inoculum.

Streptococcus suis
S. suis is a major pathogen in swine populations worldwide, and human infection can result in persons who have had close contact with sick animals or contaminated pork products.[59] Most human cases have arisen in Southeast Asia due to farm contacts between humans and pigs. Although most infections have been sporadic, there was a large outbreak in Southwest China in 2005.[101]

Sepsis and meningitis are major manifestations of *S. suis* infection, and hearing loss is a frequent complication. In contrast to the auditory nerve damage, which can occur as a complication of *S. pneumoniae* and *Haemophilus influenzae* meningitis, the cause of deafness in patients with *S. suis* infection is labyrinthitis. This is an important distinction because *S. suis* meningitis may be associated with more profound hearing loss compared with other causes of acute bacterial meningitis.[102] Despite *S. suis* generally being very susceptible in vitro to penicillin, relapses have been seen in patients with meningitis that has been appropriately treated. Relapse should prompt an assessment for occult foci of infection

and decreased antimicrobial susceptibility, but, in the absence of those two factors, longer courses of therapy may be required.[103]

Viridans-Like Organisms

Rothia

Rothia mucilaginosa (formerly *Stomatococcus mucilaginosus*) is a gram-positive aerobic coccus that was traditionally found as a cause of oral, cutaneous, and CNS infections in impaired hosts. Infections in immunocompetent hosts, including meningitis, necrotizing fasciitis, and prosthetic joint infection, have also been reported.[104–106] Patients with hematologic malignancies and profound neutropenia with central-line catheters have been found to be at higher risk of developing *Rothia* infection.[107] Most *Rothia* strains are susceptible to ceftriaxone, vancomycin, carbapenems, and levofloxacin.[108]

Pediococcus

Pediococcus spp. are gram-positive aerobic cocci that have caused nosocomial infections, particularly bacteremia and intraabdominal infections. They are intrinsically resistant to vancomycin; therapy has traditionally been with β-lactams, but the successful use of daptomycin has also been reported.[109]

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The complete reference list is available online at Expert Consult.

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203 *Streptococcus anginosus* Group

Cathy A. Petti and Charles W. Stratton IV

SHORT VIEW SUMMARY

Definition
- The *Streptococcus anginosus (milleri)* group comprises three distinct species: *S. anginosus, S. constellatus,* and *S. intermedius.*

Epidemiology
- Members of the *S. anginosus* group are found as part of the oropharyngeal, urogenital, and gastrointestinal microbiota. When pathogenic, these streptococci are often associated with polymicrobial abscess formation, bacteremia, and endocarditis.

Microbiology
- These microorganisms are microaerophilic, catalase-negative, gram-positive cocci and are easily identified with conventional methods. They are generally susceptible to β-lactam antibiotics but often cause polymicrobial infections with copathogens.

Diagnosis
- Depending on the clinical presentation, infections are usually diagnosed by culturing a specimen from the source of infection.

Therapy
- β-Lactam antibiotics are generally used; abscesses may require incision and drainage and antimicrobial therapy with broader coverage, such as that provided by ampicillin-sulbactam or piperacillin-tazobactam, in order to cover copathogens.

The *Streptococcus anginosus (milleri)* group is a subgroup within the viridans-group streptococci that includes three separate streptococcal species: *S. anginosus, S. constellatus,* and *S. intermedius.*[1–5] These viridans-group streptococci are part of the normal flora of the human oropharynx[6] and urogenital and gastrointestinal tracts,[7] but tend to be more virulent than other viridans streptococci and are known to cause serious infections.[1–9] Microbiologically, members of this group are recognized by their microaerobic or anaerobic growth requirements, their formation of minute colonies, and the frequent presence of a characteristic caramel-like odor.[a] This chapter defines the three species[1,2,5] and subspecies[6,16–19] currently making up the *S. anginosus* group and discusses their role in clinical infections.[2–4,7–9,12,20–24]

EPIDEMIOLOGY

Clinically, this group causes invasive pyogenic infections in both adults[b] and children,[2–4,22–24] which usually differentiates them from the other viridans-group streptococci.[2–4,19,25] Members of the *S. anginosus* group have been considered normal commensals of the human microbiota.[6,7] *S. constellatus* is usually found in the pharynx. *S. intermedius* is more commonly found in dental plaque, whereas *S. anginosus* is more frequently found in the gastrointestinal tract.[7] Spread from the gastrointestinal tract to the vagina with subsequent vaginal colonization is common.[26] Owing to a number of virulence factors, these streptococci are associated with a wide variety of infections.[2–4,7–9,12,20–24] When pathogenic, the members of the *S. anginosus (milleri)* group are characterized by their proclivity to cause abscesses and should be considered true pathogens when isolated from blood cultures or other clinically relevant cultures.

MICROBIOLOGY

S. intermedius, S. constellatus, and *S. anginosus* are three distinct species[1,2] that constitute the *S. anginosus* group.[1–5] This group is also commonly referred to as the *S. milleri* group.[3,4] Genetic and phenotypic studies[1–9,16,17,20] have clearly demonstrated that the *S. anginosus* group consists of these three distinct streptococcal species, and further subspecies of *S. constellatus* and *S. anginosus* have been identified.[6,9,16–18,20] The clinical relevance of these subspecies remains to be determined.

Molecular Subspecies

Within these three distinct streptococcal species, a number of subspecies have been recognized or proposed based primarily on molecular testing rather than phenotypic methods, the latter being highly variable.[2,6,9,16–18,20] *S. constellatus* has three subspecies: *S. constellatus* subsp. *constellatus, S. constellatus* subsp. *pharyngis,* and *S. constellatus* subsp. *viborgensis. S. anginosus* has two subspecies: *S. anginosus* subsp. *anginosus* and *S. anginosus* subsp. *whileyi.* A third genomosubspecies has tentatively been proposed for *S. anginosus*: *S. anginosus* subsp. *vellorensis.*[18] Of note is that *S. anginosus* subsp. *vellorensis* harbors superantigen and extracellular DNase coding genes identical to genes found in *Streptococcus pyogenes.*[18] The various species and subspecies appear to be associated with a number of different body habitats and sites and types of clinical infection. Microbiologically, members of this group are recognized by their microaerobic or anaerobic growth requirements, their formation of minute colonies, and the frequent presence of a characteristic caramel-like odor.[2–5,7–11,20] This chapter defines the three species and subspecies currently making up the *S. anginosus* group and discusses their role in clinical infections.

Phenotypic Characteristics

Members of the *S. anginosus* group share the phenotypic characteristics of the members of the genus *Streptococcus,* whose classification in general is based on patterns of hemolysis, Lancefield serologic antigenic reactions, growth properties, and biochemical reactions (Fig. 203.1). Like other streptococci, these organisms may be β-hemolytic, α-hemolytic, or γ-hemolytic on sheep blood agar. Members of the *S. anginosus* group often exhibit Lancefield antigens A, C, F, or G[2,5,6,11] but can be differentiated from other Lancefield-grouped streptococci by the small size (less than 0.5 mm) of their colonies. In general, β-hemolytic strains of *S. constellatus* react with Lancefield serologic group F antibody.[5,6,12,13] Strains containing the group F antigen may cross-react with the other grouping sera (Lancefield groupings) and therefore are of little value in identifying these organisms.[2,5,6,11]

Gram staining of *S. intermedius* strains reveals gram-positive spherical or ovoid cells that form short chains or pairs. *S. anginosus* group can be differentiated from other streptococci by a combination of three

[a]References 1, 2, 5, 6, 8, 10–15.
[b]References 2–4, 7–9, 16, 17, 20, 21.

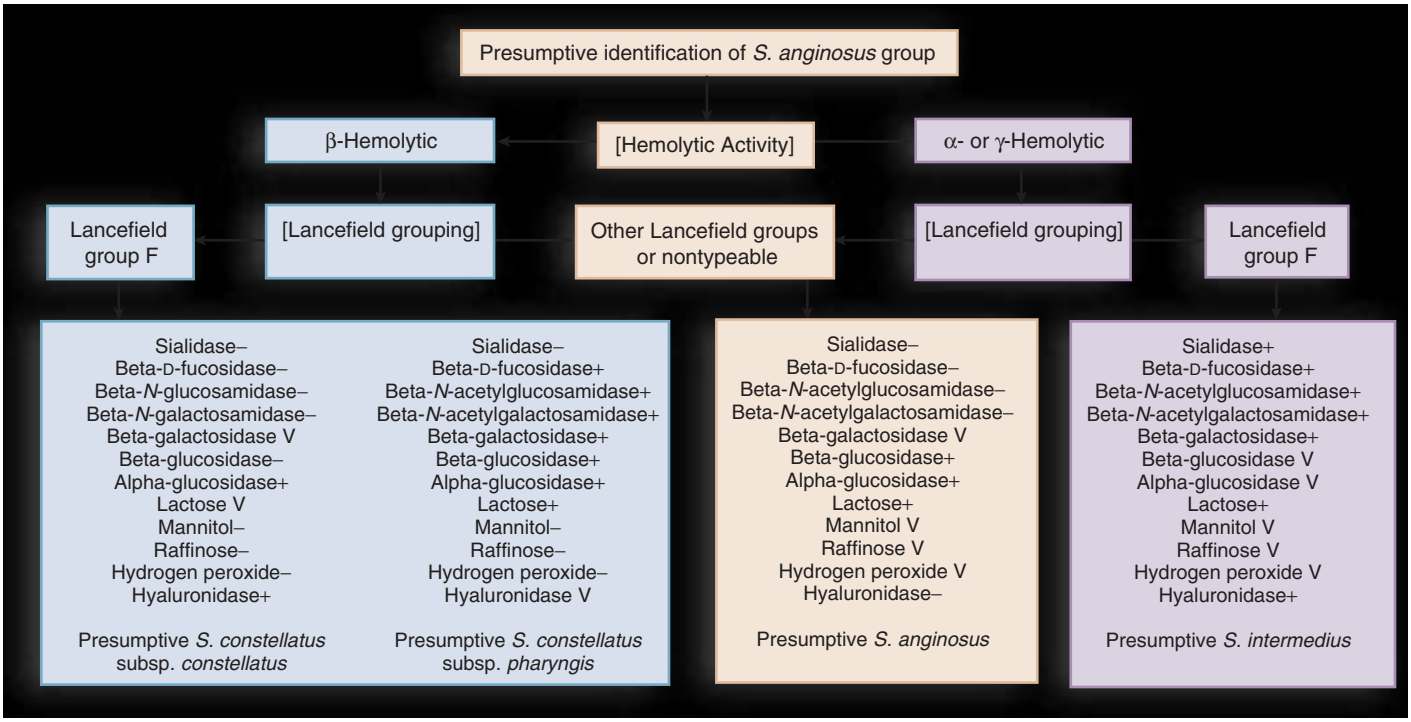

FIG. 203.1 Phenotypic differentiation of the members of the *Streptococcus anginosus* group. +, At least 90% of strains have a positive reaction; –, at least 90% of strains have a negative reaction; *V,* variable. *(Modified from Whiley RA, Fraser HY, Hardie JM, et al. Phenotypic differentiation of* Streptococcus constellatus, Streptococcus intermedius, *and* Streptococcus anginosus *[the* Streptococcus milleri *group]: association with different body sites and clinical infections.* J Clin Microbiol. *1990;28:1497–1501; and Whiley RA, Hall LMC, Hardie JM, Beighton D. A study of small colony, beta-haemolytic, Lancefield group C streptococci within the anginosus group: description of* Streptococcus constellatus *subsp.* pharyngis *subsp. nov., associated with the human throat and pharyngitis.* Int J Syst Bacteriol. *1999;49:1443–1449.)*

TABLE 203.1 Presumptive Identification of Members of the *Streptococcus anginosus* Group

Growth of Minute Streptococcal Colonies Under Microaerobic-Anaerobic Conditions
Acid from:
Insulin –
Sorbitol –
Salicin +
Hydrolysis of:
Hippurate –
Esculin +
Deoxyribonuclease –
Arginine dihydrolysis +
Voges-Proskauer test[a] +
Caramel-like odor V
↓
Presumptive *S. anginosus* group

+, ≥90% of strains have a positive reaction; –, ≥90% of strains have a negative reaction; *V,* variable.
[a]Rare exceptions.
Modified from Spellerberg B, Brandt C. Streptococcus. *In: Murray PR, Baron EJ, Jorgenson JH, et al, eds.* Manual of Clinical Microbiology. *9th ed. Washington DC: American Society for Microbiology Press; 2007:412–429; Whiley RA, Fraser HY, Hardie JM, et al. Phenotypic differentiation of* Streptococcus constellatus, Streptococcus intermedius, *and* Streptococcus anginosus *(the* Streptococcus milleri *group): association with different body sites and clinical infections.* J Clin Microbiol. *1990;28:1497–1501; and Hinnebusch CJ, Nikolai DM, Bruckner DA. Comparison of API Rapid Strep, Baxter Microscan Rapid Pos ID panel, BBL Minitek Differential Identification System, IDS RapID STR System, and Vitek GPI to conventional biochemical tests for identification of viridans streptococci.* Am J Clin Pathol. *1991;96:459–463.*

rapid tests: a positive Voges-Proskauer test for acetoin production, hydrolysis of arginine, and failure to ferment sorbitol.[2–4] In addition, the presence of the caramel-like odor, often attributed to the production of a diacetyl metabolite, can be helpful.[10,11] These characteristics are summarized in Table 203.1.

Taxonomy

The diversity of hemolytic and Lancefield groupings has made identification of these pathogens difficult in many laboratories.[2–5] Whiley and coworkers[5] have noted that almost all *S. intermedius* strains (93%) are not β-hemolytic, whereas 38% of *S. constellatus* and 12% of *S. anginosus* are β-hemolytic. Laboratories can readily differentiate the three members of the *S. anginosus* group by using phenotypic characteristics that correlate well with molecular taxonomic techniques.[2–9,16,17,20] A number of commercial systems are available for the identification of viridans streptococci, and studies have shown similar performance to manual biochemical testing.[14,15,26,27]

Molecular Diagnostic Methods

Nucleic acid amplification assays have been developed for the identification of clinically relevant viridans-group streptococci to the species and group level. Targets for these assays have included the 16S rRNA gene, the 16S-23S rRNA intergenic spacer region, *tuf* gene, *rpoB* gene, or *groEL* gene. Most assays require both amplification and sequencing of the targeted region, making the method impractical for routine use. In addition, viridans streptococci are competent—that is, they freely exchange genetic material within and between species. This phenomenon makes the taxonomic classification of viridans streptococci with DNA target sequencing and their identification to species level much more challenging. Non–sequence-based methods for identification, such as matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry, are fast, reliable and cost-effective identification methods for *S. anginosus* group, but identification to the species level is challenging.[28,29]

PATHOGENESIS

The ability of the *S. anginosus* group to cause invasive infections and the tendency to form abscesses have long been recognized.[2–4] The pyogenic potential among the members of the *S. anginosus* group does vary considerably.[7,8,20,21,30] Although the reasons for these pathogenic

characteristics are not yet completely understood, a number of factors contribute to invasion and abscess formation.

Mixed Infections Contribute to Abscess Formation

Mixed infections involving members of the *S. anginosus* group and other microbes (e.g., *Eikenella corrodens,* Enterobacteriaceae, and anaerobes) contribute to abscess formation by allowing more rapid replication of the streptococci.[18,22,24,31–33] Murine models of pneumonia have been used to demonstrate synergy between members of the *S. anginosus* group and oral anaerobes.[31,32] These studies have found that mortality was higher, abscesses or empyema were more frequently noted on histopathologic examination, and viable bacteria were more numerous in the lungs of mice with mixed infections caused by members of the *S. intermedius* group and oral anaerobes than in the lungs of mice with monomicrobial infection. In vitro studies by these and other investigators have confirmed that anaerobes enhance the growth of *S. anginosus* group organisms.[31–33] In clinical cases in which *S. anginosus* group members cause acute pneumonia, pulmonary abscess, and/or thoracic empyema in humans, the predominant species recovered along with *S. anginosus* group isolates were anaerobic bacteria,[32,34–38] confirming the clinical importance of anaerobic copathogens in pulmonary and thoracic infections.

Virulence Factors

Anginosus group streptococci also possess virulence factors that are likely to be involved in their ability to cause serious invasive infections. For example, members of the *S. anginosus* group may express a number of different adhesins on their cell surfaces that facilitate adherence to substrates found in their natural environment.[39–46] All members of the *S. anginosus* group are able to bind fibronectin via a cell surface protein,[41] and some strains are able to bind to platelets, fibrin, fibrin clots, and fibrinogen.[43] This property is thought to be a factor in the ability of these pathogens to cause endocarditis. Fibrinogen binding may, in turn, aid in platelet aggregation, which would also facilitate the development of endocarditis.[44,45] In an experimental rat endocarditis model in which all three species of the *S. anginosus* group were studied, *S. anginosus* strains produced infective vegetations and bacteremia in almost all catheterized rats, *S. constellatus* strains did so less frequently, and *S. intermedius* strains did so only occasionally.[46] Moreover, the vegetations infected with *S. anginosus* strains harbored significantly higher numbers of microorganisms than those infected by other strains.

Cytotoxins

The production of pyrogenic exotoxins by other *Streptococcus* species is well known.[47,48] A unique cytolytic toxin specific for human cells, intermedilysin, has been described from a strain of *S. intermedius* isolated from a liver abscess.[49] In particular, intermedilysin has been noted to have a potent hemolytic effect on human erythrocytes, suggesting that this or similar exotoxins may be responsible for β-hemolysis on blood agar plates. Moreover, intermedilysin is essential for the invasion of human hepatic cells and is thus an important factor in the pathogenesis of liver abscesses.[50] The intermedilysin gene has been found only in *S. intermedius*.[51] Production of intermedilysin in *S. intermedius* isolates from deep-seated abscesses is higher than that in strains from normal habitats, suggesting that this cytolysin is a virulence factor.[51]

Hydrolytic Enzymes

Members of the *S. anginosus* group produce a wide variety of hydrolytic enzymes, including hyaluronidase, deoxyribonuclease, and chondroitin sulfatase.[18,52] These enzymes may facilitate the spread of these pathogens through tissues, play a role in microbial nutrition, and assist in liquefaction of pus. One of the most prevalent hydrolytic enzymes is hyaluronidase,[53] which has been found in pus[54] and shown to be a growth factor.[55] Another hydrolytic enzyme, chondroitin sulfatase, is produced by *S. intermedius*.[56] In addition, a novel glycosaminoglycan depolymerase isolated from *S. intermedius* acts on both chondroitin sulfate and hyaluronic acid.[57] Yet another enzyme that may play a role in pathogenesis is sialidase (neuraminidase), which is produced by *S. intermedius*.[58] Sialidase production by other bacteria is an important pathogenic feature because sialic acid is known to be a nutrient source for these microorganisms; members of the *S. anginosus* group also use sialic acid efficiently as a sole carbon source. Sialidase, therefore, may be a growth factor and may play a role in the ability of these microorganisms to proliferate in humans.

Immune Factors

A number of other possible virulence factors related to the host immune response have been identified. One such factor is an immunosuppressive and B-cell mitogenic protein (P90) that is produced by *S. intermedius*.[59] Mice treated with this protein were 50 times more susceptible to infection by *S. intermedius*. This virulence effect is thought to be mediated by stimulation of suppressor lymphocytes.

Superantigens

Another important virulence factor for members of the *S. anginosus* group in relation to the host immune response appears to be superantigens.[18,60] Superantigens are bacterial proteins that bind to major histocompatibility complex class II and T-cell receptors and cause stimulation of large numbers of T cells.[61] *Streptococcus dysgalactiae* subspecies *equisimilis* has been found to possess the superantigen genes *speM, ssa,* and *smeZ,* thought to be the result of the transfer of these genes from group A streptococci (GAS). In addition, the authors demonstrated the *smeZ* allele in *Streptococcus canis,* demonstrating the wide dissemination of GAS *smeZ* in another streptococcal species. Multilocus sequence analysis[18] and case reports of cervical necrotizing fasciitis involving *S. anginosus* group isolates[60] have suggested that it is very possible that these superantigen genes identical to those of *S. pyogenes* have also been transferred from GAS to new genomosubspecies of the *S. anginosus* group.

Resistance to Phagocytosis

The interaction of the *S. anginosus* group and human neutrophils also has been examined because of the striking propensity of these organisms to cause abscesses. A potential virulence factor is the frequent presence in members of the *S. anginosus* group of a polysaccharide capsule[11,62] that hinders phagocytosis. The ability to escape phagocytosis would allow these pathogens to replicate after arriving at and adhering to a site of tissue damage. A murine model used to investigate the pathogenicity of *S. constellatus* in pulmonary infections has demonstrated that virulent strains are less likely to be phagocytized and killed than avirulent strains, presumably because of capsular variation.[62] This study demonstrated that a virulent strain of *S. constellatus* is less likely to be killed by human neutrophils than the avirulent strains.[62] Another study has shown that members of the *S. anginosus* group stimulate less chemotaxis than *Staphylococcus aureus,* which may provide an advantage for proliferating bacteria.[63] In the latter study, members of the *S. anginosus* group survived ingestion by neutrophils better than strains of *S. aureus*.[62] These characteristics help explain the ability of members of the *S. anginosus* group to cause abscesses.

CLINICAL PRESENTATIONS

Head and Neck Infections

Members of the *S. anginosus* group were initially recovered from dental abscesses and continue to be recovered from endodontic and periapical dental abscesses, although their role, if any, in these processes is unclear.[64] The presence of members of the *S. anginosus* group in the oral cavity clearly predisposes to oral and maxillofacial infection[65] and to head and neck infection.[66] A transient bacteremia may occur with dental abscesses (or with dental procedures) and has been associated with intracranial metastatic abscesses.[67–72] Moreover, the anginosus group streptococci represented the most common microorganisms isolated in sinus-induced intracranial sepsis; *S. anginosus* group isolates have been recovered from intracranial and orbital empyemas in up to 50% of cases.[68–72] Because of the potential for metastatic spread, head and neck infections and sinusitis caused by *S. anginosus* group isolates require aggressive management.[70]

Intracranial Complications of Head and Neck Infections[67–72]

Bacteremia

Both bacteremia[73–80] and endocarditis[81–86] caused by *S. anginosus* group isolates have been well documented.[73–80] Many of these bacteremic

episodes are associated with an identifiable focus of infection—usually a deep-seated abscess in a visceral organ, implicating the gastrointestinal tract as the source.[77] Bacteremia also raises the possibility of endocarditis[81]; an echocardiogram may be indicated. Finally, viridans streptococci, including members of the *S. anginosus* group, have been recognized as an increasingly important cause of bacteremia in neutropenic cancer patients undergoing chemotherapy.[73] The primary species associated with bacteremia in one recent bacteremia study was *S. anginosus*.[79]

Consider the source and consequences of bacteremia. Bacteremia caused by members of the *S. anginosus* group should alert the clinician to initiate an appropriate investigation for the detection of a possible source and a possible suppurative metastatic focus of infection. The primary species associated with bacteremia in one recent bacteremia study was *S. anginosus*.[79]

Endocarditis

Endocarditis caused by *S. anginosus* group isolates also has been well documented.[81–86] Bacteremia by *S. anginosus* group isolates may be caused by, or may cause, bacterial endocarditis.[81,82] It is estimated that members of this group represent between 3% and 15% of streptococcal isolates from patients with endocarditis.[7,86] The propensity for suppuration of these pathogens contributes to their propensity to cause the complication of myocardial abscess or metastatic abscess,[74,75] although these complications do not occur in all patients. Endocarditis results either from an abnormal heart valve or a prosthetic heart valve, although the exact attachment mechanism is unknown. *S. anginosus* group strains have been shown to adhere to buccal epithelial cells and also to bind to fibronectin, which may contribute to their pathogenicity in endocarditis.[41,43–46]

Central Nervous System Infections

S. anginosus group organisms have a strikingly prominent association with brain abscesses and have been isolated in approximately 50% to 80% of these infections.[69,74,87] These organisms may be isolated in pure or mixed culture. In addition, these organisms have been found in culture-negative intracerebral abscesses by gene sequencing from direct specimens.[88] Factors associated with brain abscesses caused by these isolates include congenital heart defects, oral infections, sinusitis, otitis media, liver disease, and direct trauma. Although *S. intermedius* can be found in the mouth, it has been suggested that most brain abscesses caused by this pathogen originate from the intestine.[74] On rare occasions, *S. anginosus* group organisms cause meningitis; this often is preceded by trauma or purulent infection at another site.[89] Transient or persistent bacteremia often results in epidural abscesses of the spine. Rapid surgical drainage is a critical prognostic factor for effective management of these spinal cord abscesses. Of the three members, *S. intermedius* appears to be the one most commonly isolated from brain abscesses. For brain abscesses, the source of the infection should always be considered.[74,89]

Intraabdominal Infections

Given that members of the *S. anginosus* group are considered commensal organisms of the intestinal tract, it is not surprising to find these pathogens causing infections within the abdominal cavity. Such infections include liver abscesses, peritonitis, pelvic abscesses, subphrenic abscesses, appendicitis, abdominal wound infections, and cholangitis.[c] The use of antimicrobial drugs with minimal or no activity against the *S. anginosus* group for prophylaxis or therapy involving the abdominal cavity has been associated with the development of infections by these organisms.[90] Specifically, metronidazole alone or in combination with gentamicin appears to allow these bacteria to become pathogens, possibly through the suppression of competing organisms, such as *Bacteroides* species or *Fusobacterium* species. Infections caused by members of the *S. anginosus* group can be seen after abdominal surgery, particularly if prophylactic antibiotics do not cover these pathogens. The proclivity for liver abscess and bacteremia[91] and cholangitis[92] must be appreciated. Finally, *S. anginosus*[7] and *S. constellatus*[8] are the species in the *S. anginosus* group that are most frequently recovered from infections in the abdominal cavity.

If bacteremia is present, surgical sepsis should always be considered.[c]

[c]References 7, 8, 9, 20, 21, 90–93.

Thoracic Infections

The presence of *S. anginosus* group organisms in the oropharynx can lead to aspiration pneumonia followed by pulmonary complications, such as lung abscess, pleural empyema, or both.[31–38,62,94] Pulmonary complications may be related, in part, to the ability of *S. anginosus* group organisms to cross tissue planes and extend into adjacent tissues.[95] Moreover, pulmonary complications are particularly likely to occur with mixed pulmonary infections.[31–38] Predisposing factors to *S. anginosus* group pulmonary infections include male gender, previous pneumonia, alcoholism, cancer, and possibly cystic fibrosis and/or chronic obstructive pulmonary disease.[96–98] Significant morbidity and mortality (death rates of 15%–30%) have been associated with these pulmonary infections. Management of pulmonary infections caused by the *S. anginosus* group must be aggressive and often requires operative intervention.[34,35,99,100] Empyema necessitans and mediastinitis have also been reported.[101,102] *S. constellatus* and *S. anginosus* are the species of the group most frequently identified from respiratory tract infections.[7,8]

Pulmonary Complications

Pulmonary infections[31–38,95] and/or exacerbations of chronic obstructive pulmonary disease,[34,35,96–100] empyema necessitans, and mediastinitis[101,102] also may be due to these organisms. A number of other infections caused by members of the *S. anginosus* group have been reported. These include osteomyelitis,[103–105] septic arthritis,[105,106] and subcutaneous abscess or cellulitis.[107,108] Drug addicts appear to be at risk for subcutaneous abscesses caused by members of the anginosus group.[107] This risk may be related to blowing through needles or having other oral contacts with injection sites. Finally, peritonsillar abscesses involving anaerobic microorganisms mixed with *S. anginosus* group members have been described.[66]

THERAPY

Clinically, infections caused by members of the *S. anginosus* group continue to respond well to penicillin G and cephalosporins. Most of these streptococci have minimal inhibitory concentrations (MICs) to penicillin G of less than 0.125 μg/mL, with only occasional strains having MICs greater than 1.0 μg/mL.[79,109–114] As noted, streptococci are naturally competent, and the potential for penicillin and cephalosporin resistance clearly exists because of the horizontal transfer of genes among streptococci.[115] In one study, 29% of viridans streptococci overall, including members of the *S. anginosus* group, were tolerant to intermediate concentrations of penicillin G (MIC = 0.25 to 2 μg/mL), and 9% of all strains had an MIC to penicillin G greater than 4 μg/mL.[114] However, other studies report high MICs to penicillin in fewer than 2% of *S. anginosus* group isolates.[79,110–114,116,117] When penicillin resistance is seen, it is more common among *S. anginosus* and *S. intermedius* isolates. Although most strains of the *S. anginosus* group are relatively resistant to aminoglycosides, synergy with a β-lactam agent usually can be demonstrated. Therefore, the addition of an aminoglycoside to a β-lactam agent for treatment of endocarditis caused by members of the *S. anginosus* group is a reasonable practice, particularly for strains with intermediate MICs. This occasional increase in MICs to penicillin G does suggest that penicillin G combined with gentamicin may be prudent for treating endocarditis, although higher doses of penicillin or vancomycin could be used. Of the cephalosporins that are clinically available, cefepime, cefotaxime, and ceftriaxone have been noted to be superior in potency and spectrum for empirical coverage of patients at risk for streptococcal bacteremias.[110] Vancomycin and clindamycin have been useful for patients with β-lactam allergies. Moreover, resistance to erythromycin and clindamycin has been described.[112,113,116,117] Therefore, macrolides do not appear to be potent enough for empirical therapy.[116,117] Finally, abscesses often involve mixed infections, which may require anaerobic coverage, and surgical drainage.

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The complete reference list is available online at Expert Consult.

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204 *Corynebacterium diphtheriae* (Diphtheria)

Paul G. Saleeb

SHORT VIEW SUMMARY

Definition

- *Corynebacterium diphtheriae* is a gram-positive bacillus that causes toxic upper respiratory disease or cutaneous lesions in susceptible hosts.

Epidemiology

- *C. diphtheriae* is transmitted via respiratory or cutaneous secretions. Asymptomatic carriers and, less commonly, contaminated fomites serve as reservoirs for infection. The incidence of diphtheria has precipitously declined with widespread use of the toxoid vaccine. Persons at risk for disease are those with a history of inadequate vaccination who have been exposed to an endemic or epidemic setting.

Microbiology

- *C. diphtheriae* is a gram-positive, unencapsulated, club-shaped, aerobic bacillus that is subdivided into four biovars. The organisms display "Chinese character" arrangement on Gram stain and form black colonies with brown halos on tellurite-containing culture media. The diphtheria exotoxin is responsible for the disease's cardiac and neurologic manifestations.

Clinical Manifestations

- Pharyngeal diphtheria presents with fever, sore throat with an associated pseudomembrane, submandibular edema, and cervical lymphadenopathy, resulting in a "bull neck" appearance. Pseudomembranous extension and obstruction of the airways may lead to respiratory failure. Cardiac and neurologic toxicities are complications of severe disease. Cutaneous diphtheria classically presents as an ulcerative lesion and is not typically associated with toxicity.

Diagnosis

- A clinical diagnosis can be made in an individual with appropriate epidemiologic risk factors presenting with pharyngitis complicated by pseudomembrane formation and cardiac or neurologic toxicity. A definitive diagnosis is made by culturing respiratory or cutaneous specimens on tellurite-containing media and performing additional biochemical testing. Polymerase chain reaction or enzyme immunoassay can be used to detect the diphtheria toxin.

Therapy

- Equine diphtheria antitoxin should be administered to all persons with suspected diphtheria. A 14-day course of antibiotic therapy with erythromycin or procaine penicillin G prevents further elaboration of toxin and transmission to susceptible hosts. All individuals should be vaccinated during convalescence, as infection may not induce a protective level of immunity.

Prevention

- A primary series of vaccination with diphtheria toxoid affords protective immunity in children and adults. To counter the effect of waning immunity, booster doses should be administered every 10 years to older children and adults who have completed the primary vaccination series. Close contacts and *C. diphtheriae* carriers should be treated with a course of antibiotics until microbiologic clearance is documented and should also undergo primary or booster vaccination as necessary.

A primary series of vaccination with diphtheria toxoid affords protective immunity in children and adults. To counter the effect of waning immunity, booster doses should be administered every 10 years to older children and adults who have completed the primary vaccination series. Close contacts and *Corynebacterium diphtheriae* carriers should be treated with a course of antibiotics until microbiologic clearance is documented and should also undergo primary or booster vaccination as necessary.

Diphtheria was one of the most lethal diseases of childhood until the middle of the 20th century. Scientific study of the disease and its causative pathogen, *C. diphtheriae*, led to seminal discoveries and significant advancement in the fields of bacteriology, immunology, and vaccine science. A precipitous decline in the incidence of disease has occurred worldwide since the introduction of the toxoid vaccine into routine immunization schedules. However, the 1990s epidemic in the former Soviet Union republics reinforces the crucial need to maintain robust immunization programs.

HISTORY

The earliest written records of diphtheria, some of which are attributed to Hippocrates, can be traced back to the Hellenic period during the 5th or 4th century BC. Vivid depictions of diphtheria were also documented during the Renaissance: Bartholin described the disease as "angina puerorum" and "morbus strangulatorius," evoking its propensity to cause disproportionate morbidity in young children.[1] Outbreaks occurred sporadically in Spain during the 1600s, and a major epidemic broke out in New England from 1735–40, decimating the population of children in several towns. Belief that divine retribution was the cause of the outbreak may have contributed to the Great Awakening of the mid-1700s.[2] During an 1818–20 epidemic in Tours, Bretonneau described diphtheria's salient clinical findings and differentiated it from other causes of "throat distemper." He named the disease "diphtheria" (from the Greek "diphthera," meaning "leather hide"), aptly depicting its characteristic pseudomembrane. He may have also been the first clinician to perform a tracheostomy successfully for pharyngeal diphtheria in 1825.[3]

Understanding of the etiology, pathogenesis, and treatment of diphtheria advanced significantly as discoveries were made in the late 1800s and early 1900s. Klebs isolated *C. diphtheriae* from a pseudomembrane in 1884, and Loeffler proved it to be the etiologic agent of diphtheria. In 1888 Roux and Yersin discovered the exotoxin and described its clinical effects.[3] The treatment of diphtheria advanced significantly in 1890 when von Behring and Kitasato developed antitoxin in guinea pigs, demonstrating the concept of passive immunity. For this seminal discovery von Behring was awarded the inaugural Nobel Prize for Physiology and Medicine in 1901.[4] A combination toxin-antitoxin vaccine was initially used for prevention of diphtheria, until

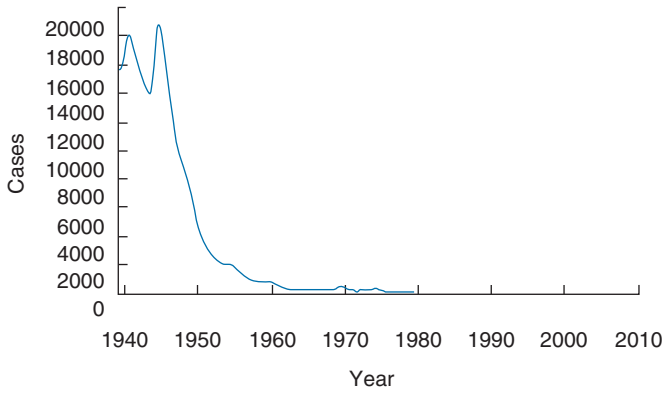

FIG. 204.1 Diphtheria: United States, 1940–2011. *(From Centers for Disease Control and Prevention. Diphtheria. In: Hamborsky J, Kroger A, Wolfe S, eds.* Epidemiology and Prevention of Vaccine-Preventable Diseases. *13th ed. Washington DC: Public Health Foundation; 2015:107–118.* https://www.cdc.gov/vaccines/pubs/pinkbook/dip.html.*)*

Ramon developed a safe and immunogenic heat- and formalin-inactivated toxoid vaccine in 1923. Aluminum salt adjuvants were incorporated into the toxoid vaccine to increase its immunogenicity, and by the 1940s an effective vaccine had been developed. Cases of diphtheria in the United States decreased by over 99% from approximately 206,000 cases in 1921 to 5 reported cases since 2000 (Fig. 204.1).[5,6] In the 1990s, however, there was a resurgence of diphtheria in the former Soviet Union republics, as their health care system was disrupted. At the peak of the epidemic there were more than 140,000 cases and approximately 4000 deaths.[7] Targeted vaccination campaigns and a coordinated international effort halted the outbreak.

THE PATHOGEN

The genus *Corynebacterium* is grouped within the order Actinomycetales and consists of more than 80 species, several of which are medically important. *C. diphtheriae* (from "korune" and "diphthera," Greek for "club" and "leather," respectively) is named for its characteristic clubbed-shape appearance on Gram stain and its propensity to form a leather-like pseudomembrane. The organism is a gram-positive, unencapsulated, nonmotile, nonsporulating, aerobic rod. On Gram stain the bacilli display a characteristic "Chinese characters" arrangement and may even appear gram variable, due to thinning of the cell wall leading to decolorization of stain.[8] Metachromatic granules containing polyphosphate are found in the polar regions of the bacterium and appear bluish-purple to red when stained with methylene blue. Loeffler developed his eponymous culture medium containing dextrose, horse serum, and beef heart infusion for isolation of *C. diphtheriae*. Due to the tendency for overgrowth of commensals on Loeffler medium, more selective media containing telluric acid (e.g., Mueller-Miller, Tinsdale) were later developed.[9] On Tinsdale medium potassium tellurite inhibits gram-negative organisms and most upper respiratory flora; *C. diphtheriae* and *C. ulcerans,* another medically important *Corynebacterium* sp., appear as grayish-black colonies with a surrounding brown halo. Urease testing can distinguish between the two organisms, as *C. diphtheriae* is urease negative, whereas *C. ulcerans* is urease positive.

C. diphtheriae is subdivided into four biovars (biotypes): *belfanti*, *gravis*, *intermedius*, and *mitis*. An individual may harbor more than one biovar concurrently. Although clinically similar, biovars may be distinguished on the basis of colony morphology, hemolysis, biochemical reactions (e.g., API Coryne:; bioMérieux, La Balme Les Grottes, France), and, more recently, 16S ribosomal RNA sequencing.[10,11] Ribotyping and pulsed-field gel electrophoresis have been used to type *C. diphtheriae* strains during outbreaks and for surveillance purposes; these methods have been supplanted by multilocus sequence typing due to its improved reproducibility.[12,13]

In the late 19th century Loeffler discovered the presence of avirulent, nontoxigenic strains of *C. diphtheriae* in healthy carriers and noted that these strains are morphologically indistinguishable from toxigenic strains. Corynebacteriophages carry *tox*, the gene for exotoxin production, and convert strains of *C. diphtheriae* from nontoxigenic to toxigenic via a lysogenic cycle. Expression of *tox* is regulated by DtxR, an iron-activated repressor that is derepressed in low iron states. The potent diphtheria toxin is composed of three domains: a cell receptor binding domain, a transmembrane domain, and a catalytic N-terminal adenosine diphosphate (ADP)-ribosyltransferase domain.[8] *C. pseudotuberculosis* and *C. ulcerans* also elaborate the diphtheria toxin; both species may be differentiated from *C. diphtheriae* by means of biochemical testing.[10,14] Laboratory methods for detection of toxin include polymerase chain reaction (PCR), enzyme immunoassay (EIA), and immunochromatography.[14,15]

EPIDEMIOLOGY

The role of the asymptomatic carrier as a reservoir for infection was first recognized in the late 1800s.[16] Humans were originally thought to be the only reservoir, but *C. diphtheriae* has now been isolated from horses, cattle, and domestic cats.[17–19] Although diphtheria has been classically understood as an upper respiratory disease acquired via inhalation of infected droplets, cutaneous lesions may provide a more efficient means of transmission, resulting in both respiratory and cutaneous diphtheria. Cutaneous lesions are probably the major reservoir for infection in resource-limited environments, serving as a source of natural immunity in these settings.[20] Transmission of infection between skin lesions and between the respiratory tract and skin lesions (bidirectional) has been documented.[21] Cases of reinfection, probably due to transmission via contaminated fomites, have also been described.[22]

Young children suffered disproportionately from diphtheria during the prevaccine era; up to 70% of cases occurred in children younger than 15 years.[20] In the New England epidemic of 1735–40, 40% of all children below 10 years of age died in a single year in Hampton Falls, New Hampshire. In 1881 greater than 1% of deaths in children younger than 10 years in New York were caused by diphtheria.[23] Children ages 5 to 14 experienced high attack rates of up to 683 per 100,000 population from 1921–24 in Baltimore; the case fatality rate in that city ranged from 5% to 8%.[24] Epidemics tended to peak in the fall at the beginning of the school year and affected those at the age of school entry. In England in the late 1930s diphtheria was the second most common cause of mortality in children, after pneumonia, causing 32 deaths per 100,000 population in those younger than 15 years.[25]

Although introduction of antitoxin treatment in the early 1900s had a beneficial effect on mortality, incidence rates of disease remained elevated. In the immediate prevaccine era the incidence rate in the United States was 237 per 100,000 population per year.[26] Incidence rates decreased worldwide from the 1930s onward, coincident with the introduction of routine toxoid vaccination in children. During World War II, however, outbreaks occurred in European countries that had experienced decreasing rates of infection in the previous years. German soldiers infected with *C. diphtheriae* biovar *gravis* reintroduced the disease in several occupied territories.[25] Unlike earlier epidemics in which younger children were disproportionately affected, a relatively high percentage of older children and adults contracted disease, likely due to wartime displacement of large populations and improved living conditions over the preceding decades, leading to decreased exposure of younger children to infection.[20] After World War II incidence rates in industrialized countries declined precipitously as childhood immunization programs were strengthened. By 1965 the attack rate in the United States had declined to 0.08 per 100,000 population.[27] Isolated outbreaks did occur among minority and indigent groups; unimmunized individuals constituted the majority of these cases. One such example was the 1970s outbreak in the Skid Road neighborhood of Seattle, which was concentrated in Native American men with high rates of alcohol dependence and was characterized by a predominance of cutaneous diphtheria.[22,28]

A major epidemic of diphtheria occurred in the former Union of Soviet Socialist Republics (USSR) in the early 1990s. The incidence rate in the USSR was as low as 0.04 per 100,000 population in the mid 1970s. By the 1980s, however, a combination of factors contributed to the 1990s epidemic: decreased rates of immunization in children due to vaccine shortages and antivaccine propaganda, vaccination of children with the reduced-dose adult formulation of diphtheria toxoid, waning adult immunity, and transmission of infection from unvaccinated

members of the military returning from Afghanistan.[23] After the breakup of the USSR in 1991 the health care system collapsed, and the incidence of diphtheria spiked to as much as 50,000 cases in 1995. The predominant strain in Russia, Ukraine, Belarus, the Baltic republics, and northern Kazakhstan was *gravis*, whereas *mitis* was predominant in southern Kazakhstan, Tajikistan, Uzbekistan, and Kyrgyzstan. In Russia, where the majority of cases occurred, incidence rates in adolescents and adults were more than 20 per 100,000 population.[25] Mobilization of an intensive vaccination campaigns in coordination with World Health Organization (WHO) and the United Nations Children's Fund brought about an end to the epidemic in the late 1990s.

Global incidence rates of diphtheria have declined steadily as a result of widespread implementation of the WHO's Expanded Programme on Immunization (EPI). From 1980 to 2000 there was a greater than 90% decrease in global incidence rates. Vaccination has prevented an estimated 40 million cases of diphtheria.[26,29] Despite these impressive figures, outbreaks continue to occur in resource-limited settings, highlighting gaps in vaccination coverage. India is currently the country with the greatest number of cases worldwide. In northern Kerala there were 533 cases in 2016, the majority of which occurred in individuals older than 10 years.[30,31]

In the prevaccine era placental passage of maternal antibodies afforded passive immunity to neonates. As passive immunity waned at approximately 6 months of age, active immunity developed naturally by means of disease or through clinically silent infection; the majority of children had developed immunity to diphtheria by age 15 years.[32] After the introduction of childhood vaccination, however, the burden of disease shifted to susceptible older children and adults. Examples were clearly seen in Jordan and Sudan, where younger children were mostly affected during epidemics before the implementation of effective childhood vaccination programs, but adults and older children were disproportionately affected during outbreaks occurring after the institution of these programs.[33,34] Waning immunity to diphtheria underpins the rationale for booster vaccination of older children and adults.

With the decline of disease due to toxigenic *C. diphtheriae*, the pathogenicity of nontoxigenic strains is becoming increasingly recognized. The *tox* gene is not necessary for the life cycle or metabolism of *C. diphtheriae*; immunized individuals are more likely to harbor nontoxigenic strains, which are consequently more likely to circulate in the community.[28] Nontoxigenic *C. diphtheriae* has been associated with blood stream infections and endocarditis in individuals with comorbid conditions, such as alcoholism, dental disease, and intravenous (IV) drug use.[35–37] The majority of nontoxigenic strains do not carry the gene *tox*; however, nontoxigenic strains that harbor the gene without expressing the protein exotoxin have recently been detected.[38]

PATHOGENESIS

Although the diphtheria exotoxin is responsible for much of the disease manifestations, other virulence factors also play a role in pathogenesis. Neuraminidase and *trans*-sialidase facilitate binding to the host cell and allow the organism to scavenge sialic acid for nutrition and metabolism.[8,39] SpaA, SpaD, and SpaH pili mediate adherence to pharyngeal, laryngeal, and respiratory epithelial cells.[40,41] DIP0733 and DIP2093 adhesins facilitate binding to the extracellular matrix, adhesion and invasion of epithelial cells, biofilm formation, and survival in macrophages.[42–45] Both toxigenic and nontoxigenic strains are capable of converting fibrinogen to fibrin, a finding that may explain the rare occurrence of pseudomembranes in the setting of nontoxigenic *C. diphtheriae* infection.[46]

The diphtheria exotoxin, containing 535 amino acids, is composed of two covalently bonded fragments, fragment A and fragment B. Fragment B contains the receptor binding and transmembrane domains, which allow cell surface binding and transport into the cytosol, respectively. Fragment A, containing the catalytic domain, facilitates ADP-ribosylation and consequent irreversible inhibition of elongation factor 2, a eukaryotic protein necessary for the coordinated movement of transfer RNA, messenger RNA, and the ribosome during the elongation cycle of protein synthesis.[8,47] One molecule of the exotoxin is sufficient to kill a cell; the lethal dose in humans may be as little as 100 ng/kg.[48,49]

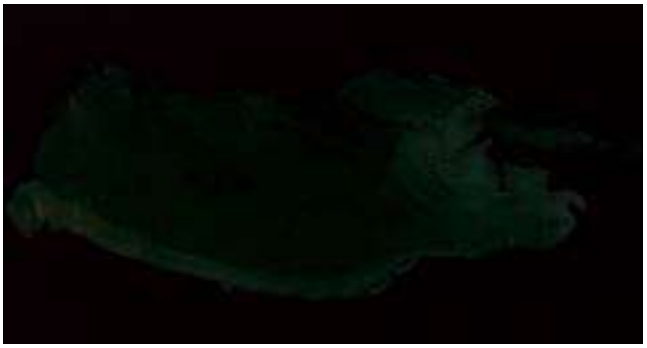

FIG. 204.2 Pharyngeal pseudomembrane. Epithelium is absent; at one side, inflammatory exudate extends to underlying muscle. Hematoxylin and eosin staining. Original magnification ×2.5. *(From Hadfield TL, McEvoy P, Polotsky Y, et al. The pathology of diphtheria.* J Infect Dis. *2000;181(suppl 1):S116–S120; with permission of Oxford University Press.)*

C. diphtheriae infection leads to mucosal edema with subsequent necrosis and ulceration. A fibrinous exudate overlying the desquamated mucosae forms the adherent pseudomembrane. On histopathology the pseudomembrane consists of fibrin and denuded epithelial cells with an associated neutrophilic infiltrate and clusters of *C. diphtheriae* organisms (Fig. 204.2). The pseudomembrane may extend to form a cast of the upper airways. Forcible removal of the pseudomembrane may cause bleeding; dislodgement may lead to aspiration and asphyxiation.[50] Edematous cervical, parabronchial and mediastinal lymph nodes often hemorrhage or necrose.[51]

CLINICAL MANIFESTATIONS

C. diphtheriae usually causes upper respiratory tract or cutaneous disease. Cardiac and neurologic complications are the most frequent toxin-mediated manifestations. Both toxigenic and nontoxigenic strains may rarely disseminate to distant sites.

Respiratory Tract Diphtheria

Clinical signs and symptoms of respiratory tract disease become apparent after an incubation period of 2 to 5 days. The fauces are most commonly involved; however, disease may also occur at other sites, including the anterior nares, larynx, and tracheobronchial tree.

Anterior Nasal

Anterior nasal diphtheria is characterized by a mucopurulent discharge that may be slightly bloody. In more severe cases a white membrane develops on the anterior nasal mucosae and septum. Rarely, the membrane erodes through the nares and upper lip.[50] Systemic toxicity in anterior nasal diphtheria is uncommon, even in the presence of a pseudomembrane.[6]

Faucial

Early symptoms of infection of the tonsillar pillars and pharynx include sore throat, malaise, and low-grade fever (usually less than 39°C). Approximately 3 days later a pseudomembrane forms on the tonsils or proximal pharynx in 50% to 80% of individuals (Fig. 204.3).[52,53] The membrane is initially white, then becomes grayish-green or black and may extend to the soft palate, nasopharynx, laryngopharynx, or bronchi. Forceful removal of the membrane causes bleeding of the underlying mucosae. Approximately one-third of affected individuals develop a "bull neck" appearance as a result of cervical lymph node enlargement and submandibular edema.[54] Local complications of pharyngeal diphtheria include stridor, airway obstruction, and subsequent respiratory failure. The case fatality rate of pharyngeal diphtheria is approximately 10%. In the absence of a pseudomembrane, disease is less severe and associated with improved outcomes.[52,53]

Laryngeal and Tracheobronchial

Although primary infection of the larynx, trachea, and bronchial tree may occur, these sites are more often secondarily involved as a result



FIG. 204.3 Pharynx of a 39-year-old woman with bacteriologically confirmed diphtheria. Photograph taken 4 days after the onset of fever, malaise, and sore throat. Hemorrhage due to removal of the membrane by swabbing appears as dark area on the left.



FIG. 204.4 A diphtheria skin lesion on the leg. *(From Centers for Disease Control.* Public Health Image Library. Diphtheria Photos: Photo ID #1941. https://www.cdc.gov/diphtheria/about/photos.html.*)*

of pseudomembranous extension from the pharynx. Prominent symptoms include stridor, hoarseness, and a barking cough. Airway edema or membrane dislodgement leads to eventual respiratory embarrassment and asphyxiation.[6,50]

Cardiac Toxicity

Studies from Vietnam and former Soviet Union republics indicate that up to 25% of individuals with pharyngeal diphtheria develop cardiac toxicity.[53–56] Fever, tonsillar pseudomembrane, and "bull neck" appearance are predictive of cardiac involvement, which may occur acutely or approximately 10 days after the initial onset of symptoms. Electrocardiographic abnormalities in diphtheritic cardiomyopathy consist of ST-segment and T-wave changes and QT interval prolongation. Severe complications of cardiac involvement include cardiac dilatation, arrhythmias, and heart block. Approximately one-third of patients with diphtheritic cardiomyopathy suffer a fatal outcome; in Vietnam, third-degree atrioventricular block and ST-segment depressions/T-wave inversions were associated with worse outcomes. Resolution of electrocardiographic abnormalities occurred in all survivors.[56]

Neurologic Toxicity

Although less common than cardiac toxicity, neurologic complications of pharyngeal diphtheria are a cause of significant morbidity.[50,52–54] A local motor neuropathy, manifesting as paralysis of the soft palate and posterior pharyngeal wall, occurs initially. Afterward, bulbar and oculomotor neuropathies may develop, leading to further paralysis of the pharynx and involvement of the extraocular and ciliary muscles. Peripheral neuritis, occurring early in the disease or up to 3 months after respiratory symptoms have abated, is characterized by a descending motor neuropathy involving the diaphragm and limbs.[57–59] Cerebrospinal fluid analysis usually reveals a cytoalbuminologic dissociation, similar to Guillain-Barré syndrome; the latter may be distinguished from diphtheritic polyneuropathy by its characteristic ascending paralysis. Sensory involvement occurs in a stocking-and-glove distribution. Signs of autonomic dysfunction, such as hypotension and urinary retention, may also develop. Cranial nerve neuropathies tend to improve at around the same time during the disease course as peripheral nerve function worsens.[59] After several weeks of neurologic involvement, complete recovery of peripheral motor and sensory nerve function is the norm.

Other Complications

Acute kidney injury, due to direct activity of the exotoxin, may occur. The exotoxin has been shown to induce necrosis of the kidneys, liver, and adrenal glands in animal models.[60,61]

Cutaneous Diphtheria

Although widespread vaccination has led to a decline in the incidence of respiratory tract disease, cutaneous diphtheria has become increasingly recognized. Cutaneous infection due to toxigenic *C. diphtheriae* may occur in unimmunized individuals, but the majority of cases are caused by nontoxigenic strains. Outbreaks have been described in impoverished communities, among racial minorities, and in settings with high rates of alcoholism and IV drug use.[21,22,62] Cutaneous infection is well described in resource-limited settings, where asymptomatic skin carriage induces natural immunity in the host and also serves as an important reservoir for transmission to susceptible individuals in the community. Cutaneous transmission may be more efficient than the respiratory route and may lead to contamination of fomites, thereby facilitating reinfections during outbreaks. Cutaneous diphtheria classically manifests as an ulcerative lesion that may be associated with a pseudomembrane (Fig. 204.4). Skin involvement may present uncharacteristically, however, as a scaly, impetiginous, or erythematous lesion.[21] Coinfections with other organisms, including *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Arcanobacterium haemolyticum* have been described.[62]

Invasive Disease

Nontoxigenic *C. diphtheriae* strains are known to cause bacteremia with metastatic complications, including endocarditis, septic arthritis, pseudoaneurysms, and mycotic cerebral aneurysms.[37,63,64] Most cases of invasive disease have occurred in impoverished communities in individuals with comorbid conditions, such as diabetes mellitus, alcoholism, and IV drug use. The clinical presentation is often fulminant and may be associated with a fatal outcome.

Other Sites

Localized infection may occur at other sites. Cases of diphtheritic conjunctivitis, corneal ulcers, otitis media, mastoiditis, and vulvovaginitis have been described in the literature.[65,66]

DIAGNOSIS

Respiratory diphtheria should be considered in the differential diagnosis whenever an individual with appropriate epidemiologic risk factors (i.e., travel or residence within an epidemic or endemic setting; history of inadequate vaccination) presents with sore throat, low-grade fever, and cervical adenopathy. Other conditions that may have similar findings include: Streptococcal pharyngitis, infectious mononucleosis, epiglottitis, Vincent angina, retropharyngeal space infection, oropharyngeal candidiasis, and acute retroviral syndrome after human immunodeficiency virus (HIV) infection. Although streptococcal pharyngitis may present with tonsillar exudates, the infection is more acute in onset than diphtheria and is characterized by high fever and an exquisitely sore throat. Infectious mononucleosis tends to occur in adolescents and young adults, does not evolve into a toxic illness, and may be differentiated on the basis of positive serum heterophile antibodies or Epstein-Barr virus serologies. Epiglottitis is characterized by stridor and a barking cough; however, it is not associated with pharyngitis or a pseudomembrane. Vincent angina, or acute necrotizing ulcerative gingivitis, will often present as a gingival exudate that may extend to the pharynx in

an individual with fetid breath, poor dentition, and underlying gingival disease. The neck swelling associated with a retropharyngeal space infection may resemble the "bull neck" appearance of pharyngeal diphtheria. Retropharyngeal space infections do not have an associated pseudomembrane, though, and are readily diagnosed with an imaging study of the neck. Oropharyngeal candidiasis may present with tonsillar exudates and odynophagia if the esophagus is involved, but the infection is not characterized by fever and occurs in individuals with appropriate risk factors (e.g., cellular deficiency; use of inhaled corticosteroids). The acute retroviral syndrome seen in early HIV infection may be associated with pharyngitis, cervical adenopathy, and low-grade fever. Acute HIV infection does not evolve into a toxic illness characterized by stridor and impending respiratory failure, and it may be distinguished from diphtheria on the basis of epidemiologic risk factors (e.g., sexual history) and appropriate diagnostic testing.

Droplet or contact precautions should be instituted once respiratory or cutaneous diphtheria is suspected, respectively. Cultures should be performed on swabs of the throat, tonsils, nasopharynx, and any skin lesions. A carefully removed portion of the pseudomembrane and a swab of the underlying mucosae should also be cultured, if feasible. It is necessary to inform the microbiology laboratory of the suspicion of diphtheria such that selective media are used; *C. diphtheriae* may be discounted as a contaminant if specimens are only plated on blood agar. Specimens should be plated on both sheep blood agar to detect *Streptococcus* spp. and on selective tellurite-containing media, such as modified Loeffler or Tinsdale.[8,14] Potassium tellurite inhibits the growth of upper respiratory flora, and *Corynebacterium* spp. appear as black colonies with a brown halo. The organisms are club-shaped, gram-positive bacilli and may demonstrate "Chinese character" arrangement. Biochemical assays (e.g., API Coryne) differentiate the four biovars and *C. ulcerans*. *C. ulcerans* may cause an illness similar to diphtheria and may also elaborate diphtheria toxin; however, it is usually associated with zoonotic transmission and is urease positive, unlike *C. diphtheriae*.[67,68]

Guinea pigs were used for the detection of diphtheria toxin in clinical specimens until the middle of the 20th century, at which time the Elek immunodiffusion test supplanted in vivo testing. Due to inconsistencies and laboratory variation in interpretation of the Elek test, PCR of the *tox* gene was developed. An important limitation of *tox* PCR testing is that some *C. diphtheriae* strains may harbor the gene without expressing it. EIA, using monoclonal antibody to fragment A of the exotoxin, is highly accurate and able to overcome the drawbacks of both the Elek test and PCR.[69]

THERAPY

The mainstay of treatment of respiratory diphtheria is antitoxin therapy. Equine serum containing diphtheria antitoxin (DAT) was shown in 1898 to reduce mortality from 12% to 3% in what may have been the first randomized clinical trial.[70] DAT neutralizes circulating unbound toxin and is of greatest benefit in preventing disease progression when administered within the first 48 hours of symptoms.[6] To minimize the risk of serum sickness or anaphylaxis, the scratch test should be performed with a 1:1000 dilution of DAT in patients without a history of hypersensitivity to equine serum and, if negative, followed by an intradermal test with the same dilution. If there is a history of hypersensitivity or if either test is positive, desensitization to antitoxin should be performed.[71] DAT is administered intravenously at a dose of 20,000 to 40,000 U for pharyngeal or laryngeal disease of 2 days' duration or less; 40,000 to 60,000 U for nasopharyngeal disease; and 80,000 to 120,000 U for extensive disease with neck swelling or for disease greater than 3 days' duration.[71,72] Although the intravenous route is preferred for administration of DAT, intramuscular injection may be considered for less severe cases. There is currently a worldwide shortage of antitoxin due to decreased production over the past several years. In the United States DAT is only available under an Investigational New Drug (IND) protocol and can be obtained by contacting the Centers for Disease Control and Prevention (CDC) at 770-488-7100. Much higher antitoxin titers are needed for treatment compared with prevention of infection. Human donor serum does not contain a sufficient concentration of antitoxin for treatment; even with enrichment techniques, prohibitively high volumes of donor serum would be necessary to achieve such a concentration.[73] Efforts are currently underway to develop recombinant antibodies to exotoxin for treatment.[74]

Antibiotic therapy prevents the propagation of organisms and further elaboration of toxin while decreasing transmission. *C. diphtheriae* is susceptible to a wide range of antimicrobials, including β-lactams, erythromycin, ciprofloxacin, tetracycline, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole, and rifampin. Penicillin and erythromycin have traditionally been used for therapy, although there have been concerns with erythromycin due to its arrhythmogenic potential and gastrointestinal side effects. Moreover, despite erythromycin's greater in vitro activity than penicillin, there have been reports of strains that are macrolide resistant.[75–77] A randomized trial comparing the two antibiotics in 44 Vietnamese children showed that defervescence occurred more quickly with penicillin; however, there was no difference between the two arms in time to bacteriologic clearance or resolution of pseudomembrane.[75] CDC guidelines recommend that treatment consist of 14 days of oral or parenteral erythromycin at 40 mg/kg/day (maximum 2 g/day) or intramuscular procaine penicillin G at a dose of 300,000 U twice daily for those weighing 10 kg or less or 600,000 U twice daily for those weighing more than 10 kg.[6] Procaine penicillin G may be switched to penicillin VK once oral intake is feasible.

Airway management is critical during treatment of respiratory diphtheria, as respiratory failure may occur as a result of extension of the pseudomembrane, leading to obstruction or aspiration. Preemptive endotracheal intubation is recommended in most instances, and severe cases may require tracheotomy.[52] Patients should be hospitalized in units with cardiac monitoring capabilities for early diagnosis and management of myocardial toxicity. Droplet precautions should be maintained until the 14-day course of antibiotic treatment has been completed and two cultures taken 24 hours apart after the discontinuation of antibiotics are negative. Vaccination with diphtheria toxoid after convalescence is necessary, as natural infection may not induce an adequate level of protective immunity.

Cutaneous diphtheria is treated with the same antibiotic regimen as that for respiratory disease. DAT has not been shown to be beneficial in the treatment of cutaneous diphtheria, as the majority of cases are caused by nontoxigenic strains. There is limited data on treatment of invasive or systemic nontoxigenic infection. A case series on the treatment of *C. diphtheriae* endocarditis reported similar outcomes with combination therapy consisting of a β-lactam or vancomycin in conjunction with an aminoglycoside as with single-drug therapy.[78] A literature review of 29 cases of endocarditis by the same authors found no significant difference in mortality or in need for surgical treatment with combination therapy. The review was limited by its small sample size and heterogeneity, especially relating to the duration of aminoglycoside therapy. Given the paucity of data, complicated systemic infection, such as endocarditis or septic arthritis, should be treated for a prolonged course; combination therapy with an aminoglycoside for at least a short duration is not unreasonable if there are no contraindications precluding such therapy.

PREVENTION

Incorporation of the diphtheria toxoid vaccine into routine immunization schedules has resulted in a dramatic decrease in the global disease incidence. In adequately immunized individuals the vaccine has a clinical efficacy of approximately 97% in preventing the development of toxigenic disease.[6] The minimum concentration of antitoxin needed for protection is 0.01 IU antitoxin/mL. A level of 0.1 IU antitoxin/mL or greater, affording full protection against disease, is achieved after administration of a series of four primary doses in children or three in adults.[6,79,80] Waning immunity is of significant concern in regions with high rates of childhood vaccination but lack of booster coverage in older children and adults. A British study demonstrated that, in the absence of boosting, only 50% of adults older than 60 years were immune to diphtheria.[81]

Pediatric formulations of the vaccine contain 15 to 25 Lf ("limes flocculationis" [limit of flocculation]) of diphtheria toxoid. Local and systemic (e.g., anaphylaxis, urticaria, angioedema) reactions are common in adults and older children who are vaccinated with this dose.[80,82] Adult formulations of toxoid vaccine, administered to those age 7 years and older, contain less toxoid (2–2.5 Lf), are less likely to cause reactions,

and are as equally immunogenic as the children's dose. Diphtheria toxoid is combined with tetanus toxoid in both pediatric and reduced-dose adult formulations of the vaccine (DT and Td, respectively); both formulations are available as combined vaccines with acellular pertussis components (DTaP and Tdap, respectively). DTaP vaccines are also available in combination with vaccines against hepatitis B virus, poliovirus (inactivated vaccine), and *Haemophilus influenzae* serotype b.

The following is a summary of the schedule for vaccination against diphtheria, as recommended by the Advisory Committee on Immunization Practices and the CDC[6,83,84]:

From 6 weeks to 7 years of age: A series of three doses of DTaP spaced 4 to 8 weeks apart should be started at age 2 months and no earlier than 6 weeks of age. After a minimum interval of 6 months after the third dose, a fourth primary dose is given at age 15 to 18 months. A fifth dose is administered at age 4 to 6 years (around the time of school entry) if the fourth dose was administered before the fourth birthday. The series does not need to be restarted if a dose is missed; in this case the next dose should be administered and the series resumed.

For unvaccinated individuals 7 years of age and older: A primary series of three doses of Td is administered with an interval of at least 4 weeks between the first two doses and 6 to 12 months between the second and third doses. The first vaccination in the series should ideally be with Tdap.

For individuals 10 years of age and older who have completed the primary vaccination series: A booster dose of Td should be given every 10 years. The first booster should be with Tdap if it was never administered previously. Pregnant women should receive a dose of Tdap at 27 to 36 weeks' gestation of each pregnancy, preferably during the earlier weeks of this interval, regardless of their vaccination history.

Vaccination does not prevent infection, transmission, or asymptomatic carriage of *C. diphtheriae*. Close contacts should be vaccinated with a booster dose if more than 5 years have elapsed since their most recent vaccination. If their vaccination history is unknown or incomplete, a primary series should be initiated or completed. All contacts should also be treated with a single dose of intramuscular benzathine penicillin G (600,000 U if <6 years old; 1.2 million U if 6 years or older) or 7 to 10 days of erythromycin (40 mg/kg/d for children; 1 g/d for adults).[6] Although increasingly rare, any identified carriers of toxigenic strains should be treated with a similar course of benzathine penicillin G or erythromycin. Droplet precautions should be maintained until two cultures obtained at least 24 hours apart and no earlier than 14 days after the completion of antibiotic therapy are negative. If cultures remain positive, erythromycin should be administered for another 10 days, due to its greater effectiveness in eradicating the carrier state, and follow-up cultures obtained after the completion of therapy.[85,86]

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The complete reference list is available online at Expert Consult.

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205 Other Coryneform Bacteria, *Arcanobacterium haemolyticum*, and Rhodococci

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SHORT VIEW SUMMARY

Definition
- Coryneform bacteria encompass several genera, of which *Corynebacterium* is the most frequently encountered in clinical infections.
- Coryneform bacteria are characterized as irregularly shaped, non–spore-forming, aerobic, gram-positive rods.

Epidemiology
- Coryneform bacteria are ubiquitous in the environment (soil and water), commensal colonizers of skin and mucous membranes in humans, and commensals in animals.
- Infections caused by coryneform bacteria are broadly categorized as community acquired or nosocomial; sporadic cases of zoonoses have been reported.
- *Rhodococcus equi* usually occurs in individuals with defective cell-mediated immunity, particularly with human immunodeficiency virus infection, with or without a history of animal exposure.

Microbiology
- Coryneform bacteria readily grow on standard culture media. For lipophilic strains, growth is enhanced with addition of Tween 80.
- Species identification and antimicrobial testing of coryneform bacteria are recommended when specimens are collected from normally sterile sites, high colony counts are present with a strong leukocyte reaction, or high colony counts of *Corynebacterium urealyticum* are recovered from urine culture.
- Molecular tests, such as 16s ribosomal RNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry are better than biochemical tests for species identification of coryneforms and *Rhodococcus*.

Diagnosis
- Coryneform bacteria are considered clinically significant when patients present with symptoms consistent with infection, along with recovery of bacteria as aforementioned (see "Microbiology").

Therapy
- Coryneform bacteria are uniformly susceptible to glycopeptides, such as vancomycin, and most strains are susceptible to daptomycin and linezolid.
- *R. equi* is usually susceptible to vancomycin, teicoplanin, erythromycin, fluoroquinolones, rifampin, carbapenems, aminoglycosides, and linezolid.

Prevention
- Prevention of infections caused by coryneform bacteria includes proper skin antisepsis before invasive procedures and caution when handling animals.

CORYNEFORM BACTERIA OTHER THAN *CORYNEBACTERIAE DIPHTHERIAE*

Corynebacterium was proposed as a genus by Lehmann and Neumann in 1896, who derived the name from the Greek *koryne,* which means "club," and *bacterion,* meaning "little rod."[1] The coryneforms are a diverse group of organisms. *Corynebacterium diphtheriae* serves as the type species, leading to the term *diphtheroids* to describe other bacteria sharing similar morphologic features. Also known as *coryneform bacteria,* bacteria demonstrating morphologic characteristics similar to those of corynebacteria include the genera *Corynebacterium, Arcanobacterium, Trueperella, Brevibacterium, Dermabacter, Microbacterium, Rothia, Turicella, Arthrobacter, Oerskovia, Leifsonia, Helcobacillus, Exiguobacterium, Cellulomonas, Cellulosimicrobium, Curtobacterium, Auritidibacter, Janibacter, Pseudoclavibacter, Brachybacterium,* and *Knoellia*.[2,3] The 16S ribosomal RNA (rRNA) sequencing data show that the genera *Corynebacterium* and *Turicella* are more related to the partially acid-fast bacteria and to the genus *Mycobacterium* than to the other coryneforms discussed in this chapter.[3]

Coryneform bacteria are widely distributed in the environment as normal inhabitants of soil and water. They are commensals colonizing the skin and mucous membranes of humans and other animals.[4,5] In the hospital setting, coryneform bacteria may be cultured from the hospital environment, including surfaces and medical equipment; corynebacteria are able to produce biofilm.[6] Coryneform bacteria other than *C. diphtheriae* have been isolated frequently in clinical specimens and were commonly considered contaminants without clinical significance. There is an increasing body of evidence of the pathogenicity of the coryneform bacteria, particularly as a cause of nosocomial infection in hospitalized and immunocompromised patients.[7,8] Several members of the genus *Corynebacterium* are better known as pathogens in animals and only incidentally cause infection in humans as zoonoses.

The coryneform bacteria are pleomorphic, demonstrating different forms at various stages of the life cycle, irregularly shaped gram-positive rods that are aerobically cultured, not spore forming, and not partially acid fast.[2,3] A history of misidentification of coryneform bacteria has made interpretation of the medical literature difficult. Initial identification is aided by observation of colony size and appearance, and the presence or absence of hemolysis on sheep blood agar. Odor production by colonies assists in identification, particularly of *Brevibacterium casei* and *Corynebacterium urealyticum.* Several of the medically relevant coryneform bacteria are lipophilic, demonstrating enhanced growth with the addition of Tween 80 to the culture medium.

True corynebacteria demonstrate club-shaped gram-positive rods on Gram staining, whereas other coryneform bacteria may not appear distinctly club shaped. Cells demonstrate variable sizes and appearance, from coccoid to bacillary forms, depending on the stage of the life cycle, and Gram-stain results may be uneven. Coryneform bacteria typically form arrangements such as "Chinese letters" or picket-fence configurations as a result of "snapping" after the cells divide. Lack of spore formation helps distinguish them from *Bacillus* species.[2]

The spectrum of human infections attributed to the coryneform bacteria is broad but can be understood in two general categories: community-acquired infections and nosocomial infections. Community-acquired infections include pharyngitis, skin and soft tissue infections, native valve endocarditis, genitourinary tract infections, acute and chronic prostatitis, and periodontal infections (Table 205.1).[9,10] Many case series of nosocomial infections attributed to coryneform bacteria are in the medical literature and include intravascular catheter–associated septicemia, native and prosthetic valve endocarditis, device-related infections,

TABLE 205.1 Community-Acquired Coryneform Infections

Conjunctivitis or keratitis	*Corynebacterium macginleyi* *Corynebacterium propinquum* *Corynebacterium pseudodiphtheriticum*
Pharyngitis	*Arcanobacterium haemolyticum* *Corynebacterium ulcerans* *C. pseudodiphtheriticum*
Peritonsillar and pharyngeal abscess	*A. haemolyticum*
Odontogenic infections	*A. haemolyticum* *Rothia dentocariosa*
Lymphadenitis	*Corynebacterium pseudotuberculosis*
Genitourinary tract infection	*Corynebacterium glucuronolyticum* *Corynebacterium riegelii*
Chronic prostatitis	*C. glucuronolyticum*
Skin and soft tissue infections	*A. haemolyticum* *Trueperella pyogenes* *Corynebacterium minutissimum* *C. pseudotuberculosis* *Corynebacterium confusum* *C. ulcerans*
Breast abscess	*Corynebacterium kroppenstedtii* *Corynebacterium tuberculostearicum* *C. minutissimum*
Native valve endocarditis	*A. haemolyticum* *R. dentocariosa* *C. pseudodiphtheriticum* *C. propinquum*
Native joint infection	*Corynebacterium striatum*

TABLE 205.2 Nosocomial Infections Caused by Coryneform Bacteria

Cerebrospinal fluid shunt infections	*Corynebacterium jeikeium*
Meningitis	*C. jeikeium* *Brevibacterium* spp.
Pneumonia	*Corynebacterium amycolatum (Corynebacterium xerosis)* *Corynebacterium striatum* *Corynebacterium urealyticum* *Corynebacterium pseudodiphtheriticum*
Intravenous catheter–related bloodstream infection	*C. jeikeium* *C. amycolatum* *C. striatum* *C. urealyticum* *Brevibacterium casei* *Corynebacterium macginleyi* *Corynebacterium minutissimum* *Corynebacterium afermentans* subsp. *afermentans* *Trueperella bernardiae* *Trueperella pyogenes* *Oerskovia* spp. *Microbacterium* spp.
Native valve endocarditis	*C. amycolatum* *C. jeikeium* *C. striatum* *C. urealyticum*
Prosthetic valve endocarditis	*C. jeikeium* *C. amycolatum* *C. striatum* *B. casei*
Skin and soft tissue infection	*C. amycolatum* *C. minutissimum* *C. urealyticum*
Postsurgical infections	*C. jeikeium* *C. urealyticum* *C. striatum* *C. minutissimum* *C. amycolatum*
Prosthetic joint infections	*C. jeikeium*
Urinary tract infections and encrusted cystitis	*C. urealyticum*
Continuous ambulatory peritoneal dialysis–related peritonitis	*C. jeikeium* *Brevibacterium* spp. *C. urealyticum* *Dermabacter* *Rothia dentocariosa*

peritonitis in peritoneal dialysis patients, and postoperative surgical site infections.[9,11,12] Common nosocomial pathogens include *Corynebacterium jeikeium, C. urealyticum, Corynebacterium amycolatum*, and *Corynebacterium striatum* (Table 205.2).[13] Nosocomial infections with the coryneform bacteria will continue to increase, reflecting the increased numbers of severely ill patients with extended stays in intensive care units and multiple antibiotic exposures.

Taxonomy

The taxonomy of the coryneform bacteria has evolved extensively over the past 30 years and continues to be refined. Hollis and Weaver,[14] at the Special Bacteriology Laboratory, Centers for Disease Control and Prevention (CDC) in Atlanta, completed the first extensive compilation of coryneform bacteria isolated from clinical specimens. Coryneform bacteria were grouped based on colony and biochemical characteristics. Since then, further work has been done to analyze these groups and define species. Table 205.3 lists the significant coryneform bacteria and the CDC group to which they previously belonged.

To date, more than 90 species of *Corynebacterium* have been identified; more than 50 species have been associated with disease in humans.[3,15,16] The use of molecular genetics has resulted in continued revision of the taxonomy of the coryneform bacteria and provides useful information on the epidemiology and pathogenicity of the genera. Molecular genetic studies, such as 16S rRNA and *rpoB* gene sequencing, are used in reference laboratories to confirm identification at the species level; 16S rRNA gene sequencing has become the standard by which new species are identified.[2,3,16–18] Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is another analytical test that is being used for identification of *Corynebacterium* spp.[19] With the MALDI-TOF technique, a mass spectrometer is used to analyze proteins that are extracted from the bacteria, and they are compared with a database.

Microbiology

Because the coryneforms are frequently cultured in polymicrobial infections and may be contaminants in cultures collected with poor sterile technique, clinician communication with the microbiology laboratory is essential in order to determine when species identification is appropriate. The decision to identify the coryneform bacteria to the species level is recommended when (1) the bacteria are cultured from normally sterile sites, such as blood (two or more positive blood cultures, except when recovered from the same set) or cerebrospinal fluid (CSF), (2) if the bacteria appear in adequately collected clinical material as the predominant organism on Gram staining and have a strong inflammatory reaction, or (3) are from urine specimens in which the bacterium (e.g., *C. urealyticum*) is the only organism recovered with a colony count greater than 10^4/mL *or* if it is the predominant bacterium cultured and the total bacterial count is greater than 10^5/mL.[3,16]

Media used for initial specimen processing are standard blood agar plates for most specimens, thioglycolate broth for wound cultures, and standard blood culturing systems using continuous monitoring for carbon dioxide (CO_2) production. Special media used for species identification include sheep blood agar supplemented with Tween 80, to assess lipid-enhanced growth.[3]

Identification to the species level in the microbiology laboratory is confirmed by biochemical testing. Initial testing includes the catalase test with 3% hydrogen peroxide. Additional tests include oxidation or fermentation; nitrate reduction; urea hydrolysis; esculin hydrolysis; and acid production from glucose, maltose, sucrose, mannitol, and xylose. A frequently used system of biochemical testing for medically relevant coryneform bacteria is the API Coryne system (API-bioMérieux, Marcy

TABLE 205.3 Medically Relevant Coryneform Bacteria

CLASSIFICATION	CDC CORYNEFORM GROUP
Nonlipophilic, fermentative corynebacteria	
Corynebacterium ulcerans	C. diphtheriae group
C. pseudotuberculosis	C. diphtheriae group
C. xerosis	F-2, I-2
C. striatum	I-1
C. minutissimum	
C. amycolatum	F-2, I-2
C. glucuronolyticum	
Others: *C. argentoratense, C. matruchotii, C. riegelii, C. confusum, C. simulans, C. sundsvallense, C. thomssenii, C. freneyi, C. aurimucosum, C. tuscaniae, C. coyleae, C. canis, C. falsenii, C. freiburgense, C. massiliense, C. pilbarense, C. stationis,* and *C. timonense*	
Nonlipophilic, nonfermentative corynebacteria	
C. afermentans subsp. *afermentans*	ANF-1
C. auris	
C. pseudodiphtheriticum	D-1
C. propinquum	ANF-3
Lipophilic corynebacteria	
C. jeikeium	JK
C. urealyticum	D-2
Others: *C. afermentans* subsp. *lipophilum, C. accolens, C. macginleyi, C. tuberculostearicum, C. kroppenstedtii, C. bovis,* CDC coryneform group F-1, *C. lipophiloflavum*	6 *(C. accolens)* G-1 *(C. macginleyi)* G-2 *(C. tuberculostearicum)*
Arcanobacteria	
Arcanobacterium haemolyticum	
Trueperella pyogenes (Arcanobacterium pyogenes)	
Trueperella bernardiae (Arcanobacterium bernardiae)	2
Other coryneform bacterial genera: *Turicella, Arthrobacter, Brevibacterium, Dermabacter, Rothia, Oerskovia, Microbacterium, Leifsonia aquatica*	A-1, A-2 (*Oerskovia* spp.) A-4, A-5 (*Microbacterium* spp.) B-1, B-3 *(Brevibacterium casei)* 3, 5 *(Dermabacter hominis)*
Rhodococci	

CDC, Centers for Disease Control and Prevention.

l'Étoile, France), which includes 20 biochemical tests and enables identification of many of the important corynebacteria and other coryneform bacteria, including *Arcanobacterium* spp. and *Brevibacterium* spp., and *Rhodococcus equi.*[20] An evaluation of the API Coryne database 2.0 found correct identification of 90.5% of the coryneforms tested.[21] Another identification system, RapID CB Plus (Thermo Fisher Scientific, Waltham, MA), correctly identifies 80.9% of strains to the species level and an additional 12.2% to the genus level. It has the advantage of requiring only 4 hours to perform, compared with 24 hours for the API Coryne system.[22] In a few cases, the Christie-Atkins-Munch-Petersen (CAMP) test helps to identify the organism to the species level.[2] MALDI-TOF has been used in comparison with biochemical identification for *Corynebacterium* species and coryneform bacteria. Comparison with sequencing of 16S ribosomal RNA genes demonstrated higher rates of identification with MALDI-TOF at the genus and species level for both *Corynebacteria* spp. and coryneform-like bacteria.[23]

The Clinical and Laboratory Standards Institute (CLSI) released standards for susceptibility testing of coryneform bacteria in 2016.[24] Isolates generally show susceptibility to vancomycin, daptomycin, and linezolid. Susceptibility to newer agents such as oritavancin, telavancin, and tedizolid has also been demonstrated.[25,26] Species of *Corynebacterium* are capable of expressing the *ermX* methylase gene, which is linked to the resistance phenotype macrolide, lincosamide and streptogramin B (MLS_B); this phenotype confers resistance to erythromycin and clindamycin and is associated with cross-resistance to other antimicrobial agents.[27] The *vanA* gene has been identified in *Oerskovia turbata* and *Arcanobacterium haemolyticum,* but no documented infections with vancomycin-resistant coryneforms have appeared in the literature.[28] When a clinically important isolate is obtained, susceptibility testing is recommended in order to ensure antimicrobial activity.

For consistency, the coryneform bacteria are reviewed here within groups identified by the presence or absence of lipid-enhanced culture (lipophilic or nonlipophilic) and fermentation activity.

Nonlipophilic, Fermentative Corynebacteria

Corynebacteria have been divided into lipophilic and nonlipophilic, fermentative and nonfermentative. Lipophilic species have enhanced growth in the presence of certain lipids, such as Tween 80. Fermentative strains produce acid from certain sugars. Advances made in the identification of species in the nonlipophilic fermentative group have resulted in a revision of thinking regarding the pathogenic role of several species, particularly *Corynebacterium xerosis* and *C. amycolatum.*[29] Interpretation of the literature that does not include detailed information on laboratory identification is difficult because of historical misidentification of species in the nonlipophilic fermentative group.

Corynebacterium ulcerans and *Corynebacterium pseudotuberculosis*

C. ulcerans and *C. pseudotuberculosis* are members of the *C. diphtheriae* group and are known primarily as animal pathogens, although disease in humans has been reported as zoonotic infections. Both *C. ulcerans* and *C. pseudotuberculosis* may elaborate diphtheria-like toxin.

Increasingly, *C. ulcerans* has been implicated in causing diseases such as exudative pharyngitis and cutaneous ulcers in humans indistinguishable from *C. diphtheriae.*[30,31] In the United Kingdom, *C. ulcerans* exceeded *C. diphtheriae* as the causative agent in diphtheria infection; in total, 59 cases of toxigenic *C. ulcerans* infection were reported from 1986 to 2008, and 12 cases from 2007 to 2013.[32,33] The European-based Diphtheria Surveillance Network reported an increase in diphtheria cases attributable to *C. ulcerans* during a 9-year surveillance period.[34] *C. ulcerans* has been implicated in human infection because of contact with domesticated animals; the use of multilocus sequence typing has allowed for rapid confirmation of zoonotic transmission.[35–37] This has made the identification of the causative organism important for epidemiology, and guidelines for laboratory diagnosis of diphtheria cases have been published; molecular testing with techniques such as real-time polymerase chain reaction (PCR) facilitates identification of toxigenic corynebacteria.[38,39] The spectrum of illness with *C. ulcerans* is similar to that with *C. diphtheriae.*[30,31] Fatalities have been reported, including sudden death from toxin-induced cardiac injury and a case of fatal necrotizing sinusitis.[40] Skin infection with *C. ulcerans* mimics that with *C. diphtheriae.*[41] Infection of the lower respiratory tract may occur, causing pneumonia and pulmonary nodules.[42,43] One case of possible human-to human transmission of *C. ulcerans* has been reported.[44] Treatment of pharyngitis caused by *C. ulcerans* is similar to treatment of diphtheria, including the use of antibiotics such as erythromycin and diphtheria antitoxin when appropriate.

C. pseudotuberculosis is a significant pathogen in animals, particularly sheep, in which it causes caseous lymphadenitis. Human disease is rare, manifesting as granulomatous lymphadenitis, found mainly in farm workers and veterinarians who have had exposure to infected animals.[45] It has been reported to cause a diphtheria-like illness and pneumonia, and has also been isolated from soft tissue abscesses in a young butcher.[32,46,47] Management of *C. pseudotuberculosis* infection includes excision of affected lymph nodes and treatment with β-lactam antibiotics, macrolides, or tetracyclines.

Corynebacterium xerosis

C. xerosis is a colonizer of the human nasopharynx, conjunctiva, and skin.[48] Historically, *C. xerosis* has been described in the literature as a pathogen that causes serious human disease, especially in immunocompromised hosts, including sepsis, endocarditis, pneumonia, peritonitis, ventriculoperitoneal shunt infection, and postoperative sternal wound infection. Subsequent investigations have questioned the reliability of identification of *C. xerosis* in the microbiology laboratory.[49,50] In one study, all isolates originally identified as *C. xerosis* were in actuality *C.*

amycolatum.[50] This calls into question preceding case reports attributing disease to *C. xerosis* because true *C. xerosis* isolates apparently are quite rare. *C. xerosis* infections in humans have included blepharitis, a brain abscess, and a case of sepsis in a pediatric patient with sickle cell disease.[51–53] True *C. xerosis* strains are susceptible to most antibiotics, which helps to distinguish them from *C. amycolatum,* which demonstrates multiple antibiotic resistances.

Corynebacterium striatum

C. striatum has been one of the more commonly isolated coryneform bacteria in the clinical microbiology laboratory.[2,3] As with other nonlipophilic fermentative corynebacteria, a high degree of misidentification of *C. striatum* has occurred in the past in microbiology laboratories, and investigators have found many isolates to be *C. amycolatum* on detailed retesting.[49,54]

C. striatum is ubiquitous and colonizes the skin and mucous membranes of normal hosts and hospitalized patients. Historically, *C. striatum* was not routinely identified to the species level and was often considered a contaminant. With the use of analytic tests such as MALDI-TOF, *C. striatum* is increasingly being recognized as an emerging pathogen.[55] Reports of true infection confirmed with isolation of *C. striatum* have increased in frequency and have been described in patients with indwelling devices, chronic pulmonary disease, and immunosuppression.[56–58] In addition, significant *C. striatum* infection has been reported in immunocompetent hosts. Case reports in the literature include native and prosthetic valve endocarditis, meningitis, pulmonary nodules, necrotizing fasciitis, septic arthritis, tubo-ovarian abscess, empyema, and osteomyelitis.[59–67] There is evidence for patient-to-patient transmission of *C. striatum* in hospital settings, which may account for the frequency with which it is isolated in hospitalized patients.[68,69] *C. striatum* has the ability to produce biofilm and has been implicated in a nosocomial outbreak of a multidrug-resistant strain.[70] Nosocomial outbreaks of *C. striatum* have been reported in patients with chronic obstructive pulmonary disease.[71]

Historically, *C. striatum* has been shown to be uniformly susceptible to vancomycin and other antimicrobials with broad gram-positive activity.[72] Resistance has been demonstrated to penicillin, ciprofloxacin, erythromycin, clindamycin, and tetracyclines, limiting potential oral antimicrobial options for therapy.[73,74] Increasingly, resistance to daptomycin has been reported; specifically in the setting of prior daptomycin therapy and during therapy in patients with infections of left ventricular assist devices.[75–77]

Corynebacterium minutissimum

Defined in 1983 by Collins, *C. minutissimum* is a colonizer of human skin, particularly moist intertriginous areas.[78] As with other members of this group, *C. amycolatum* has been misidentified as *C. minutissimum* in the past.[79] Although *C. minutissimum* historically has been considered the causative agent in erythrasma, that association has been questioned because cultures tend to show polymicrobial infection.[2] Erythrasma is a superficial skin infection that occurs in intertriginous areas between skin folds, axillae, groin, and fingers and toes.[10] It causes reddened scaling patches that may be accompanied by pruritus. Skin patches glow coral-red under a Wood lamp. Diagnosis is made by means of clinical appearance and symptoms and by culture of skin scrapings. Colonies also appear coral-red under ultraviolet light. Treatment includes topical and oral antibiotics. Recurrences are frequent.

Other rare infections attributed to *C. minutissimum* include septicemia and endocarditis in immunocompromised patients and patients with indwelling central venous catheters, peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD), pyelonephritis, costochondral abscess, breast abscess, postoperative abdominal infection, and vascular graft infection.[80–82] One case of bacteremia and meningitis has been reported, in addition a case of an infected pseudomeningocele.[83,84]

Corynebacterium amycolatum

Defined as a new species in 1988 by Collins, *C. amycolatum* was first isolated from the skin of healthy humans.[85] Noted for its lack of mycolic acids, the species corresponds to the CDC coryneform groups F-2 and I-2. It is the nonlipophilic coryneform bacterial species most frequently isolated from clinical specimens.[7,8] *C. amycolatum* forms small dry nonhemolytic colonies of 1 to 2 mm in diameter when cultured at 37°C.[3] The organisms are pleomorphic and vary from single organisms to an array of Chinese letters. Because of variability in biochemical reactions, *C. amycolatum* had been misidentified previously as *C. minutissimum, C. xerosis,* and *C. striatum.* Currently, the API Coryne system can correctly identify *C. amycolatum,* but confirmatory tests should be performed.[3]

Although case reports of infections attributed to *C. amycolatum* are rare, many previously reported infections by other members of the nonlipophilic fermentative group were most likely caused by *C. amycolatum.* Reports with reliable information on organism identification include nosocomial endocarditis after intravenous catheter–related infection, septic arthritis, a case of native valve endocarditis with aorta–left atrial fistula, and sepsis in pediatric oncology patients.[86–88] *C. amycolatum* has been implicated in ear infections and orbital implant infections.[89,90] Susceptibility testing has shown resistance to penicillins, cephalosporins, macrolides, and fluoroquinolones, and susceptibility to vancomycin, daptomycin, and linezolid.[91] There is variable resistance to aminoglycosides and tetracyclines.[92,93] Reports of successful treatment of endovascular infection include the use of vancomycin and daptomycin in combination with rifampin.[94]

Corynebacterium glucuronolyticum

C. glucuronolyticum was defined in 1995; since 2000, the species has included isolates previously identified as *Corynebacterium seminale* that had been defined by Riegel and coworkers.[95,96] Although it has been primarily isolated from the genitourinary tract of animals, in humans it may be included in the normal flora of the genitourinary tract. It is commonly isolated from males with genitourinary tract infections and is associated with chronic prostatitis; a case of encrusted cystitis due to *C. glucuronolyticum* has been reported.[97–99] *C. glucuronolyticum* strains are susceptible to β-lactam antibiotics, gentamicin, and vancomycin but demonstrate variable resistance to fluoroquinolones, macrolides, and tetracyclines.[92]

Other Nonlipophilic, Fermentative Corynebacteria

Corynebacterium argentoratense has been isolated from the throats of healthy volunteers and from mucosal biofilms on adenoid tissue from children with chronic or recurrent otitis media. The clinical significance of this finding is unclear.[100] *Corynebacterium matruchotii* is identified by its characteristic "whip handle" appearance on Gram staining.[2,3] It was previously identified as *Bacterionema matruchotii* until 1983, when it was reclassified as a *Corynebacterium* species by Collins. Mainly an inhabitant of the oral cavity of humans and animals, *C. matruchotii* has been rarely associated with human disease.

In 1998, Funke and colleagues[101] identified a new species of *Corynebacterium* isolated from female patients with symptomatic urinary tract infections. Given the name *Corynebacterium riegelii,* it is nonlipophilic, weakly fermentative, and facultatively anaerobic. Similar to the lipophilic *C. urealyticum,* it demonstrates strong urease activity. It is susceptible to penicillins, cephalosporins, gentamicin, fluoroquinolones, and tetracyclines.

Corynebacterium confusum was defined in 1998 by Funke and colleagues[29]; it is nonlipophilic and very slowly fermentative.[102] *C. confusum* has been isolated from a blood culture, foot infections, and a breast abscess.[102] Additional nonlipophilic fermentative *Corynebacterium* spp. identified from human clinical specimens include *C. simulans, C. sundsvallense, C. thomssenii, C. freneyi, C. aurimucosum, C. tuscaniae, C. coyleae, C. canis, C. falsenii, C. freiburgense, C. massiliense, C. pilbarense, C. stationis,* and *C. timonense.*[103–114]

Nonlipophilic, Nonfermentative Corynebacteria

The nonlipophilic, nonfermentative corynebacteria do not produce acid from any sugars and were designated as absolute nonfermenters (ANFs) by Hollis and Weaver.[14] They are colonizers of the human respiratory tract and ear canal and are infrequent pathogens.

Corynebacterium afermentans subsp. *afermentans*

C. afermentans subsp. *afermentans* was included in the CDC coryneform group ANF-1 until 1993, when Riegel and coworkers[115] defined the species as *C. afermentans* with two subspecies: *C. afermentans* subsp. *afermentans* and *C. afermentans* subsp. *lipophilum. C. afermentans* subsp. *afermentans* is a rare human pathogen but has been reported to cause septicemia in immunocompromised patients.[116]

Corynebacterium auris

As in the case of *Turicella otitidis, C. auris* was initially isolated from middle ear fluid of pediatric patients with otitis media and was presumed to be among the pathogens that cause otitis media. Subsequent studies have cultured *C. auris* from the external ear canal and cerumen of healthy subjects, both children and adults, and its role as a pathogen has been discounted.[5,117] *C. auris* is resistant to penicillins, clindamycin, and erythromycin and susceptible to fluoroquinolones, gentamicin, tetracyclines, and vancomycin.[92]

Corynebacterium pseudodiphtheriticum

C. pseudodiphtheriticum is included in the normal bacterial flora of the human upper respiratory tract. Lehmann and Neumann[1] described the organism in 1896, giving it the name *Bacillus pseudodiphtheriticum.* Since 1925, it has been known as *C. pseudodiphtheriticum.* Historically, *C. pseudodiphtheriticum* was associated with endocarditis of native and prosthetic valves.[118] The first cases of infections at other sites attributable to *C. pseudodiphtheriticum* became known in 1982, and since then, *C. pseudodiphtheriticum* has been associated primarily with respiratory infections, particularly in immunocompromised hosts and in patients with chronic lung disease.[119,120] It has been isolated from patients with pneumonia and advanced acquired immunodeficiency syndrome (AIDS), and from children with cystic fibrosis and respiratory infections.[121,122] Other sites of infections have been the eye, intervertebral disks, joints, lymph nodes, urine, peritoneal fluid, intravenous catheters, and surgical wounds.[123] Although *C. pseudodiphtheriticum* does not elaborate toxins, it has been isolated from patients with exudative pharyngitis with pseudomembrane formation, not unlike *C. diphtheriae.*[124] Corneal scrapings from patients with bacterial keratitis due to *C. pseudodiphtheriticum* were evaluated for host immune response to infection; elevated expression of Toll-like receptors and proinflammatory cytokines interleukin (IL)-6 and Il-1β were noted.[125] Isolates of *C. pseudodiphtheriticum* have demonstrated resistance to macrolides and lincosamides but have maintained susceptibility to penicillins, cephalosporins, doxycycline, and glycopeptides. One large case series of 113 *C. pseudodiphtheriticum* strains from a single institution showed moderate levels of resistance to β-lactams, imipenem, tetracycline, erythromycin, ciprofloxacin, aminoglycosides, and clindamycin; all strains were susceptible to vancomycin.[123]

Corynebacterium propinquum

Before 1994, *C. propinquum* was known as CDC coryneform group ANF-3; it is primarily isolated from the human respiratory tract.[120,126] *C. propinquum* has been implicated in native and prosthetic valve endocarditis; species identification was confirmed with 16s rRNA gene sequencing and MALDI-TOF.[127,128] *C. propinquum* has also been isolated from a pulmonary pleural effusion, an infected orthopedic device, and a plaque associated with keratitis in a diabetic patient who used a therapeutic contact lens; a case of nongonococcal urethritis due to *C. propinquum* has been reported.[129,130]

Lipophilic Corynebacteria

Lipophilic corynebacteria are fastidious, slow-growing bacteria that form tiny nonhemolytic colonies on standard media but demonstrate enhanced growth with the addition of lipids to the culture medium.[2] The group includes the significant human pathogens *C. jeikeium* and *C. urealyticum.*

Corynebacterium jeikeium

C. jeikeium was initially described in 1976 as a highly resistant coryneform bacteria that caused severe sepsis in patients with hematologic malignancies and profound neutropenia and in one patient with a ventricular CSF shunt.[131] In 1979, it was designated as CDC group JK, and in 1988, the designation was revised to *C. jeikeium.*[132] Whole-genome sequencing has revealed that *C. jeikeium* is actually a group of four genomospecies.[133] *C. jeikeium* colonizes the skin of hospitalized patients, especially those treated with multiple antibiotics, and can also be isolated from the hospital environment.[134] There is some evidence that patient-to-patient transmission occurs in the hospital. It is the most frequently isolated *Corynebacterium* in the acute-care setting and is the most important pathogen of the lipophilic corynebacteria.[7]

Microbiology

C. jeikeium is a pleomorphic gram-positive rod that varies in form from coccobacillary to bacillary; some appear club shaped. It is nonhemolytic on standard media and forms small gray-white colonies on routine culture.[2] It is lipophilic and forms large colonies on sheep blood agar supplemented with Tween 80. *C. jeikeium* does not produce urease or reduce nitrate, and it ferments glucose.

Pathogenicity

C. jeikeium is a cause of severe infections in the hospitalized patient.[135] Predisposing factors for infection include immunocompromised states such as malignancy, neutropenia, and AIDS.[135,136] Other risk factors include the presence of indwelling medical devices such as central venous catheters, peritoneal dialysis catheters, prosthetic valves, and CSF shunts. Prolonged hospital stay, treatment with broad-spectrum antibiotics, and impaired skin integrity are well-described risk factors for development of infection with *C. jeikeium.*

Infectious processes include septicemia from infected intravascular devices, native and prosthetic valve endocarditis, CSF shunt infections, meningitis and transverse myelitis, and prosthetic joint infections.[137,138] It has been reported to cause postsurgical infections, peritonitis in patients who undergo CAPD, liver abscess, malignant otitis externa, and osteomyelitis; glomerulonephritis attributable to left ventricular assist device infection has been reported.[139,140] Skin findings with *C. jeikeium* infection are common: neutropenic patients with *C. jeikeium* septicemia commonly have reported skin findings including rash and subcutaneous nodules.[141,142]

Treatment

C. jeikeium is resistant to many antibiotics, including penicillins, cephalosporins, and aminoglycosides; there is inducible resistance to macrolides.[143,144] It remains susceptible to vancomycin, which is the recommended treatment. Although catheter removal has been routinely recommended in the setting of intravascular catheter–related infection, experience has shown a high success rate in catheter salvage with appropriate antimicrobial therapy.[145] Successful treatment with daptomycin and tigecycline have been reported; one case of a daptomycin-resistant strain of *C. jeikeium* in a previously treated neutropenic patient has been reported.[146–148]

Corynebacterium urealyticum

First described in 1974, this bacterium was designated as CDC group D2 until 1992, when the name *C. urealyticum* was proposed.[149] *C. urealyticum* colonizes the skin of 25% to 37% of hospitalized patients. Because of its ability to adhere to uroepithelial cells, it is most commonly associated with urinary tract infections, especially in cases of abnormal anatomy, and has been implicated as the cause of encrusted cystitis and encrusted pyelitis.[150]

Microbiology

Colonies of *C. urealyticum* are slow growing and lipophilic and appear nonhemolytic and pinpoint when cultured on sheep blood agar under CO_2 enrichment for 48 hours.[2] It is a strict aerobe, with no growth under anaerobic conditions. On Gram staining, organisms are palisading, non–spore-forming coccobacilli. They are catalase positive and oxidase negative, with a rapid production of urease. Laboratories should be made aware of the need for further investigation of diphtheroid bacilli from urinary tract specimens in the proper clinical setting because *C. urealyticum* may not grow in standard urine culture.[2]

Pathogenicity

C. urealyticum is primarily a cause of chronic and recurrent urinary tract infections, occurring mainly in elderly people and those with debilitation or immunosuppression. Additional risk factors include prolonged hospitalization, the use of percutaneous and bladder drainage catheters, and urinary tract procedures.[151,152] It has been reported to cause infections in renal transplant recipients.[153] Clues to diagnosis of *C. urealyticum* infection include sterile pyuria, alkaline urine, and the presence of white blood cells and struvite crystals.[154] *C. urealyticum* causes encrusted cystitis, which appears as chronic inflammation of the bladder mucosa with crystal deposits on the bladder mucosa, surrounded by erythema. Encrusted pyelitis may occur if there are abnormalities of the upper urinary tract. In rare cases, *C. urealyticum* has been reported as a causative agent in peritonitis, endocarditis, pneumonia, septicemia, osteomyelitis, soft tissue infections, and superinfection of wounds.[151,152,155]

Treatment

In general, *C. urealyticum* is resistant to β-lactams, aminoglycosides, and trimethoprim-sulfamethoxazole. There is variable susceptibility to fluoroquinolones, macrolides, and tetracycline.[7,151,155] The treatment of choice is vancomycin, to which it remains susceptible. For urinary tract infections, in addition to vancomycin, endoscopic removal of bladder mucosa encrustations or acidification of urine by instillation of acid into the bladder in cases of encrusted cystitis may be required, and urologic consultation is recommended.[156] The use of percutaneous nephrostomy tube placement and irrigation of the upper urinary tract with Thomas acid solution, in cases of upper tract disease, has been described.[157]

Other Lipophilic Corynebacteria

C. afermentans subsp. *lipophilum* is a rarely reported human pathogen.[115] It has been reported to cause intravascular catheter–related septicemia, prosthetic valve endocarditis, lung abscess, empyema, and brain abscess. *Corynebacterium accolens* was previously known as CDC coryneform group 6. There were discrepancies in the definition until 1991, when it was defined further by Neubauer and associates[158] and given the name *Corynebacterium accolens.* Known to colonize the human upper respiratory tract, *C. accolens* is a rarely reported human pathogen but has been reported to cause septicemia, endocarditis, breast abscess, and pelvic osteomyelitis.[159–161] As compared with controls, infants with cystic fibrosis had higher levels of *C. accolens* in the nasopharyngeal microbiota than of other *Corynebacterium* spp.[162] *Corynebacterium macginleyi* (formally CDC coryneform group G-1) was initially isolated solely from the human eye as a cause of conjunctivitis.[163] In an 8-year survey at a single institution, *C. macginleyi* was identified as the causative agent of microbial keratitis in 20% of patients with this condition.[164] Case reports of *C. macginleyi* infection include intravascular catheter–associated bloodstream infection, urinary tract infection associated with a bladder drainage catheter, ventilator-associated pneumonia in a lung cancer patient, and septicemia in immunocompromised patients.[165,166] Other lipophilic corynebacteria, including *Corynebacterium tuberculostearicum* (formally CDC coryneform group G-2) and *Corynebacterium kroppenstedtii,* have been cultured from inflammatory breast tissue in cases of granulomatous mastitis; in particular, *C. kroppenstedtii* has been reported in multiple case series in association with inflammatory breast disease.[167–170] *C. kroppenstedtii* was identified as a cause of cystic neutrophilic granulomatous mastitis, a condition that is characterized by lipogranulomas with cystic spaces surrounded by neutrophils.[171] The first case positing a causal role for *C. kroppenstedtii* in granulomatous mastitis occurred in a patient with abnormal neutrophil responses to a NOD2 agonist.[172]

A case series from a single center reviewed 16 patients with granulomatous mastitis due to *Corynebacterium* spp.; *C. kroppenstedtii* was the most frequently identified organism, followed by *C. tuberculostearicum.* Susceptibility testing showed that *C. kroppenstedtii* was resistant to β-lactam antibiotics, whereas *C. tuberculostearicum* was multidrug resistant.[168] A strain of multidrug-resistant *C. kroppenstedtii*–carrying multiple resistance genes has been described in a patient with granulomatous mastitis.[173] *C. kroppenstedtii* has been identified as the causative agent in a case of prosthetic valve endocarditis.[174] *C. tuberculostearicum* has also been associated with postsurgical deep wound infections and osteomyelitis.[175] CDC *Corynebacterium* group G has caused native and recurrent prosthetic valve endocarditis.[176] *Corynebacterium bovis* is a cause of bovine mastitis, but in humans has been described as a cause of endocarditis, chronic otitis media, central nervous system (CNS) infection, line-related septicemia, and joint infection.[177–179] CDC coryneform group F-1 may be a cause of urinary tract infection; it is similar to *C. urealyticum* in its very rapid urease reaction and differs from the latter in its very high susceptibility on antimicrobial testing.[180] *Corynebacterium lipophiloflavum* has been isolated from a patient with bacterial vaginosis. *Corynebacterium resistens* is a multidrug-resistant coryneform bacteria isolated from blood, bronchial aspirate, and abscess specimens.[181] *Corynebacterium ureicelerivorans* has been implicated in bacteremia and peritonitis.[182,183] New species of lipophilic coryneform bacteria found in human specimens continue to be defined; these include *Corynebacterium aquatimens, Corynebacterium sputi,* and *Corynebacterium pyruviciproducens.*

Arcanobacteria

Collins defined the genus *Arcanobacterium* in 1982, from *arcane,* meaning "mysterious or secret," and *bacterium.*[184] For many years, *A. haemolyticum* was the only species in this genus. However, in 1997, further investigation of several *Actinomyces* spp. resulted in the reclassification of *Actinomyces pyogenes* and *Actinomyces bernardiae* as *Arcanobacterium* spp. and defined two additional new species of arcanobacteria.[185] In 2011, *Arcanobacterium pyogenes* and *Arcanobacterium bernardiae* were reclassified to a new genus, *Trueperella,* and are currently identified as *Trueperella pyogenes* and *Trueperella bernardiae.*[186]

Arcanobacterium haemolyticum

A. haemolyticum was first isolated by MacLean and coworkers[187] in 1946 from American soldiers and Pacific Islanders with pharyngeal and skin infections in the South Pacific. The initial classification as *Corynebacterium haemolyticum* endured until 1982, when the genus *Arcanobacterium* was defined by Collins. *Corynbacterium haemolyticum* is no longer used.

Microbiology

A. haemolyticum is a catalase-negative, gram-positive to gram-variable rod that does not form spores and is nonmotile.[2] It is β-hemolytic, but expression can vary with culture media and conditions; hemolysis is best observed on human blood agar.[188] Growth is enhanced in the presence of CO_2. It is known for forming dark pits under the colonies. Poor growth on tellurite helps to differentiate it from *C. diphtheriae.*

Colony morphology has been described as either rough or smooth type.[189] Rough-type colonies are most frequently associated with respiratory isolates; smooth biotypes are most frequently associated with wound isolates. *A. haemolyticum* does not ferment xylose, which differentiates it from *T. pyogenes.* A positive α-mannosidase test can be used to identify *A. haemolyticum* and differentiate it from *T. pyogenes* and other coryneform-like bacteria, including *R. equi* and *Erysipelothrix rhusiopathiae.* Because of the presence of phospholipase D activity similar to *C. ulcerans* and *C. pseudotuberculosis,* the reverse CAMP test result will be positive, with inhibition of the hemolytic zone of a β-lysin–producing strain of *Staphylococcus aureus.* Other virulence factors include neuraminidase and hemolysin production.[190]

Infections in Humans

A. haemolyticum is a well-recognized cause of pharyngitis in humans, with a spectrum of illness from mild to diphtheria-like.[191–193] It accounts for about 0.5% of pharyngeal infections overall and 2.0% in individuals in the 15- to 25-year-old age range. In studies, *A. haemolyticum* has not been isolated from healthy control populations but has been isolated from 2.5% of a symptomatic young adult population.[193–195] It is indistinguishable from streptococcal pharyngitis in clinical appearance, and about 50% of cases of pharyngitis are exudative. Cervical adenopathy is usually present. *A. haemolyticum* pharyngitis is accompanied by an exanthem in about 50% of cases. The rash generally appears after the onset of the pharyngitis and has a variable appearance, often described as an erythematous morbilliform or scarlatiniform rash, appearing on



FIG. 205.1 Skin rash in a patient with *Arcanobacterium haemolyticum* pharyngitis.

the trunk, neck, and extremities (Fig. 205.1). It may also manifest as an erythematous urticarial rash with an appearance similar to that of erythema multiforme. Complications of *A. haemolyticum* pharyngitis include peritonsillar and pharyngeal abscesses, with *A. haemolyticum* being the sole pathogen in 50% of cases in adolescents and young adults, and the remaining 50% coinfected with β-hemolytic streptococci.[194]

A. haemolyticum has been isolated from soft tissue infections, including chronic ulcers, wound infections, cellulitis, and paronychia.[196] It is frequently a component of polymicrobial infection in this setting but has also been isolated as the sole pathogen.[197] Underlying conditions in polymicrobial chronic ulcers include diabetes and peripheral vascular disease. Posttraumatic wound infections have been reported, as has coinfection or superinfection with leprosy ulcers.[198]

Lemierre disease with *Fusobacterium necrophorum* and *A. haemolyticum* has been reported, accompanied by a skin rash typical for *A. haemolyticum* infection; Lemierre syndrome and septicemia caused by *A. haemolyticum* have also been reported.[199,200] Sepsis syndrome from *A. haemolyticum* has been described, occurring in all age groups and without predisposing factors.[201] Other infections reported include sinusitis, orbital cellulitis, orbital necrotizing fasciitis and osteomyelitis, brain abscess, endocarditis, cavitary pneumonia, and osteomyelitis.[202,203]

Treatment

Susceptibility information for *A. haemolyticum* has been reviewed extensively.[204] Although in vitro studies show most strains to be penicillin susceptible, treatment failures may occur because of tolerance and poor penetration into the intracellular space. Other β-lactams have also shown in vitro activity. Susceptibility data showed low minimal inhibitory concentrations to erythromycin and azithromycin.[204] Clindamycin and doxycycline are also efficacious, as are ciprofloxacin and vancomycin. Resistance to trimethoprim-sulfamethoxazole and tetracycline is well documented.[205] Surgical management of wound infections and drainage of soft tissue abscesses are recommended.

Trueperella (Arcanobacterium) pyogenes

Initially described by Glage in 1903, this organism was initially named *Bacillus pyogenes.* It was known as *Corynebacterium pyogenes* until 1982, when it was reassigned to the genus *Actinomyces.* In 1997, *Actinomyces pyogenes* was transferred to the genus *Arcanobacterium* and was renamed *Arcanobacterium pyogenes.*[185] In 2011, *Arcanobacterium pyogenes* was further reclassified as *Trueperella pyogenes.*[186] *T. pyogenes* is primarily an animal pathogen that causes pyogenic infections in cattle, including pneumonia, endometritis, endocarditis, wound infections, and mastitis. Abscess formation is aided by neuraminidases, which facilitate adhesion to host epithelial cells.[206] Transmission of *T. pyogenes* by flies has been proposed. *T. pyogenes* has not been isolated as normal human flora. Most human cases are acquired in rural settings and include outbreaks of leg ulcers in Thai children, septicemia in a patient with colon carcinoma, polymicrobial-infected diabetic foot ulcers, spondylodiskitis and psoas abscess, subcutaneous abscesses, and intraabdominal infections.[207,208] Cases of fatal endocarditis in patients with no animal contact have been reported.[209,210] *T. pyogenes* is cultured on sheep blood agar under CO_2 enrichment. Colonies are weakly hemolytic at 24 hours and become more strongly hemolytic at 48 hours.[2] Differentiation from *A. haemolyticum* is made with observation of the CAMP reaction, by fermentation of xylose, and by the α-mannosidase test. *T. pyogenes* is susceptible to most antibiotics, including penicillins, cephalosporins, macrolides, tetracyclines, and aminoglycosides.

Trueperella (Arcanobacterium) bernardiae

Originally described as CDC coryneform group 2 in 1987, this organism was assigned the species name *Actinomyces bernardiae* in 1995. In 1997, *Actinomyces bernardiae* was transferred to the genus *Arcanobacterium* as *Arcanobacterium bernardiae*; in 2011, *Arcanobacterium bernardiae* was further reclassified as *Trueperella bernardiae.*[185,186] On Gram staining, it appears as short gram-positive rods without branching. It is identified through its ability to ferment maltose more rapidly than glucose, which separates it from other coryneform bacteria. It is distinguished from *T. pyogenes* by the inability to ferment sucrose, mannitol, and xylose.[2] *T. bernardiae* is a rare human pathogen, with recovery of the organism from the bloodstream, abscesses, the urinary tract, joints, the eye, and wounds; it has also been implicated as a cause of necrotizing fasciitis and prosthetic joint infection.[211–214]

Miscellaneous Coryneform Bacteria

Turicella otitidis

Initially isolated from patients with otitis media, *T. otitidis* is believed to be a colonizer of the human auditory canal and not a true pathogen in this setting because it has been isolated in the same frequency from an asymptomatic control population.[5,117,215] It has been reported as a cause of mastoiditis and posterior auricular abscess in immunocompetent children and septicemia in a neutropenic child. *T. otitidis* is resistant to clindamycin and erythromycin but susceptible to penicillins, cephalosporins, tetracyclines, fluoroquinolones, linezolid, and vancomycin.[216]

Arthrobacter Species

An environmental coryneform found in animal sheds, schools, and daycare centers, *Arthrobacter* has rarely been isolated from human clinical specimens. Commonly identified species include *Arthrobacter cumminsi* and *Arthrobacter oxydans.*[217,218] There are reports of septicemia in immunocompromised patients and isolation of *Arthrobacter* from human urine specimens. Disseminated intravascular coagulation due to *Arthrobacter* septicemia in a pregnant patient was implicated as contributing to intrauterine fetal demise.[219]

Brevibacterium Species

Brevibacterium spp. are short coryneforms isolated from milk and dairy products and are known colonizers of human skin.[8] They have been identified in environmental dust in schools, daycare centers, and animal sheds. Brevibacteria have a biphasic morphologic appearance on culture, with young colonies demonstrating typical coryneform features. As colonies age, the organisms mature into cocci or a coccobacillary appearance.[2] Brevibacteria have been implicated in causing human foot odor when confining footwear results in a moist environment. Only a few species of *Brevibacterium* have been noted to cause infection.

B. casei is the species of this genus that is most frequently isolated from human clinical specimens.[220] On culture, it forms white-gray colonies with a distinctive cheese odor. On Gram staining, it is a short, club-shaped rod that is catalase positive and non–spore forming.[2,221] Human infections with brevibacteria have most frequently been

intravascular catheter–related bloodstream infections, particularly in immunocompromised patients and patients with AIDS. In one case series, six of 11 patients with pulmonary hypertension receiving continuous iloprost via a central venous catheter developed catheter-related infection due to *Brevibacterium* spp.[222] There have been additional reports of meningitis, brain abscess, cholangitis, salpingitis, and peritonitis in patients undergoing CAPD.[223,224] Susceptibility testing shows some resistance to β-lactam antibiotics, fluoroquinolones, clindamycin, and macrolides.[216,225] Vancomycin is the treatment of choice for serious infections.[225] Other *Brevibacterium* spp. that have been reported to cause invasive disease include *Brevibacterium sanguinis, Brevibacterium epidermidis,* and *Brevibacterium otitidis.*[226–228]

Dermabacter hominis

Dermabacter spp. were previously identified as CDC group 3 and group 5 coryneform bacteria and are skin colonizers of humans.[229] They have been a cause of bacteremia in patients with prolonged hospitalizations and peritonitis in immunocompromised persons who undergo CAPD. *Dermabacter* has been isolated from a cerebral abscess in a renal transplant recipient and from a patient with chronic osteomyelitis with *Actinomyces neuii* as copathogen.[230] *D. hominis* exhibits variable resistance to many antibiotics, including penicillins, fluoroquinolones, macrolides, chloramphenicol, and tetracyclines, and susceptibility to vancomycin and linezolid; high rates of resistance to daptomycin have been reported.[216,231]

Rothia dentocariosa and *Rothia mucilaginosa*

Rothia are found as colonizers of the human oral cavity and have been isolated from dental plaque and in cases of periodontal disease.[232] *R. dentocariosa* has the potential for misidentification as a *Dermabacter* or *Actinomyces* species in the microbiology laboratory.[2] Case reports with reliable information on identification of the organisms have found it to be a pathogen in several cases of native and prosthetic valve endocarditis, including presentations with abscesses, mycotic aneurysms, and vertebral osteomyelitis.[233,234] It has also been a cause of bacteremia without endocarditis.[234,235]

R. mucilaginosa, formerly *Stomatococcus mucilaginosus,* is a normal resident of the human mouth and nasopharynx. On culture, it usually appears as gram-positive cocci in clusters—hence, the previous classification as a *Stomatococcus. R. mucilaginosa* is a rare cause of true bacteremia and sepsis; two case series from large academic institutions each identified more than 20 patients with true *Rothia* bacteremia. *R. mucilaginosa* was identified as the predominant species recovered; the majority of patients had neutropenia and hematologic malignancy.[235,236] *R. mucilaginosa* has been found in cases of pneumonia in patients with leukemia and lung cancer and peritonitis in patients undergoing CAPD.[237,238] A case of granulomatous dermatitis attributable to *R. mucilaginosa* bacteremia has been reported.[239]

Oerskovia and *Cellulosimicrobium* Species

Included in CDC group A-1 and A-2, *Oerskovia* spp. are rare human pathogens but have been reported to cause infection in immunocompromised hosts, patients with implanted devices, and those with indwelling central venous catheters.[2] The spectrum of infections has ranged from bacteremia, endocarditis, meningitis associated with CSF shunt infection, soft tissue infection, prosthetic joint infection, and peritonitis in a patient undergoing CAPD.[240–242] Two species, *O. turbata* and *Oerskovia xanthineolytica,* have been reclassified as *Cellulosimicrobium funkei* and *Cellulosimicrobium cellulans,* respectively, based on 16S rRNA sequencing.[243] Infections due to *C. cellulans* include peritonitis in a patient who was undergoing CAPD, and pyogenic flexor tenosynovitis in a patient with traumatic injury due to introduction of a foreign body (wooden splinters) in one finger.[244,245]

Microbacterium Species

CDC coryneform group A-4 and A-5 bacteria were defined as *Microbacterium* spp., and in 1998 the genus *Aureobacterium* was reclassified and renamed within the genus *Microbacterium.*[246,247] *Microbacterium* spp. have been found as a cause of bacteremia, peritonitis in patients who undergo CAPD, and endophthalmitis.[248–250] Most commonly, it has been a nosocomial pathogen in debilitated and immunocompromised patients. In a study of 50 human isolates, the most common species recovered were *Microbacterium oxydans, Microbacterium paraoxydans,* and *Microbacterium foliorum.*[251]

Leifsonia aquatica

Corynebacterium aquaticum was reclassified in 2000 as *Leifsonia aquatica.*[252] Because of inconsistencies of identification and confusion with *Aureobacterium* in previous reports, it has been difficult to determine the pathogenicity of this species. Case reports for *L. aquatica* are rare; *L. aquatica* had been reported to cause septicemia in immunocompromised hosts, peritonitis in patients on CAPD, and bacteremia in a hemodialysis patient.[253] One case of septicemia due to *L. aquatica* after retinal detachment surgery has been reported.[254]

Other medically relevant coryneform bacteria

Other medically relevant coryneform bacteria include the genera Auritidibacter, Exiguobacterium, Cellulomonas, Helcobacillus, Curtobacterium, Janibacter, Pseudoclavibacter, Brachybacterium, and Knoellia.[3] Isolation of these rare organisms has typically been in the context of clinical material submitted to a microbiologic research laboratory for further characterization.

RHODOCOCCI

Taxonomy

Rhodococcus ("red coccus") belongs to the family Nocardiaceae, order Actinomycetes, which includes *Nocardia, Corynebacterium, Mycobacterium,* and *Gordonia* spp. This genus is made up of genetically and physiologically diverse bacteria that have environmental, clinical, and industrial significance. *R. equi* is the most commonly isolated species causing human infection, especially among immunocompromised hosts with defective cell-mediated immunity. Recent debate over taxonomy and nomenclature has led to a proposed reclassification of *R. equi* as *Rhodococcus hoagii* or *Prescottella equi.*[255] Other members of this genus that are human pathogens include *Rhodococcus rhodochrous, Rhodococcus fascians (Rhodococcus luteus),* and *Rhodococcus erythropolis.*

Rhodococcus equi

Epidemiology

R. equi (formerly *Corynebacterium equi*) was first identified as a pathogen in 1923, when it was isolated from the lungs of foals with pyogranulomatous pneumonia. It has subsequently been identified in a variety of animals, including cattle, swine, sheep, goats, deer, bears, wild birds, seals, dogs, and cats.[256] The first case of human infection was reported in 1967, when *R. equi* was cultured from lung specimens of a young man who worked in a stockyard, was being treated with corticosteroids and 6-mercaptopurine for autoimmune hepatitis, and presented with fever and cavitary pneumonia. During the next decade, sporadic cases of infection in humans were reported. Beginning in the early 1980s, the incidence of *R. equi* infection increased markedly. This increase has been attributed to the human immunodeficiency virus (HIV) infection epidemic, advances in chemotherapy for malignancies, and organ transplantation.[257,258] In addition, improvements in microbiology laboratory identification techniques and increasing recognition of *R. equi* as a pathogen may also explain part of the increase in incidence.[259] The frequency of *R. equi* infections in HIV-infected patients seems to have decreased in recent years, largely related to highly active antiretroviral therapy and possibly to prophylaxis with azithromycin. More than 200 cases of infection caused by *R. equi* have been published. *R. equi* has been isolated from water and soil worldwide and from the manure of herbivores.[256] Infection in both animals and humans is thought to be acquired through inhalation or ingestion of the organism. Inoculation into a wound can also lead to infection. Exposure to farm soil, animals, or manure has been reported in many human cases, although it is less common in HIV-positive patients.[260,261] *R. equi* has been rarely isolated from healthy persons without an identified immunosuppressive condition.[262,263] An environmental or occupational exposure (farmer, horse breeder) was identified in 50% of cases.[263] Most infected individuals have had defective cell-mediated immunity, including HIV infection, with or without a history of animal exposure. Health care–associated

cases of *R. equi* have been reported.[264] Human-to-human transmission has been suspected in cases of *R. equi* pneumonia acquired by HIV-infected patients who were roommates of patients infected with *R. equi.*[265] Occupational acquisition of *R. equi* by a healthy laboratory worker who developed pneumonia has been reported. *Rhodococcus* spp. with properties very similar to those of *R. equi* have been isolated as nasal flora in adults.[266]

Microbiology

R. equi is a gram-positive obligate aerobe that is asporogenous and nonmotile. It may appear coccoid or bacillary, depending on growth conditions. Its bacillary appearance varies from long, curved, clubbed forms to short filaments with branching. *R. equi* can grow at a variety of temperatures but grows optimally at 30°C. Colonies on solid media appear large, irregular, smooth, and mucoid. They are pale salmon-pink in color; however, this characteristic color may not appear until days 4 to 7 of incubation. Although it grows well on ordinary media, if cultured in this manner the organism may be overlooked or discarded as a nonpathogenic coryneform or misidentified as *Nocardia* or *Micrococcus.* Isolation of *R. equi* from contaminated specimens is facilitated by the use of selective media, such as colistin-nalidixic agar, phenylethyl alcohol agar, or ceftazidime-novobiocin agar. *R. equi* is catalase, lipase, urease, and phosphatase positive. It is oxidase, elastase, deoxyribonuclease, and protease negative. Differentiation from other pathogenic coryneforms has been historically based on a lack of ability to ferment carbohydrates or liquefy gelatin.[2] Because it is sometimes acid fast, it may be mistaken for a *Mycobacterium.*[256] It can be distinguished from some mycobacterial species by the 14-day arylsulfatase test because *Rhodococcus* is negative for this reaction. Two special features of *R. equi* help distinguish it from other similar organisms: (1) When *R. equi* is cultured on sheep blood agar that is cross-streaked with other bacteria, such as *S. aureus, C. pseudotuberculosis,* or *Listeria monocytogenes,* synergistic hemolysis occurs (the CAMP test); and (2) in vitro antagonism between imipenem and other β-lactams is widespread among *R. equi* isolates.[267] In general, the identification of *Rhodococcus* spp. with traditional phenotypic and biochemical tests may be difficult and is unreliable. Molecular techniques, especially gene sequencing, are currently the only methods that provide definitive identification of most aerobic actinomyces, such as *Rhodococcus, Gordonia,* and *Tsukamurella.* The 16S rRNA sequencing method provides a rapid and accurate identification of *Rhodococcus* spp. and other aerobic actinomycetes, including *Gordonia* and *Tsukamurella.*[268,269] MALDI-TOF offers promise for the rapid identification of *Rhodococcus.*[270]

Pathogenicity

R. equi is a facultative, intracellular pathogen. It infects macrophages and survives inside the lysosomes. After phagocytosis by macrophages, *R. equi* arrests phagosomal maturation.[271] Its ability to cause chronic infection may be based on its complex cell wall, which is thought to prevent phagosome-lysosome fusion, resists the oxidative burst, and causes a nonspecific degranulation of lysosomes, which permits intrahistiocytic survival.[272] *R. equi* can also survive within human alveolar epithelial cells.[273] Virulence factors associated with *R. equi* infections in animals and in humans have been defined and include the plasmid-encoded antigens VapA and VapB, although they do not always appear to be necessary for infection in humans.[274,275] Histopathologic evaluation usually reveals a necrotizing granulomatous reaction. Endobronchial granulomas have been reported.[276] Multiple microabscesses may be seen. Malakoplakia is a rare, chronic, granulomatous inflammatory process that is associated with an impaired ability to process microorganisms within histiocytes. It is characterized by accumulations of benign macrophages associated with intracellular and extracellular aggregates of periodic acid–Schiff stain–positive histiocytes that contain lamellated iron and calcium inclusions and are termed *Michaelis-Guttman bodies.*[272] Lung malakoplakia is a rare condition; most of the reported patients had *R. equi* pneumonia.[277]

Clinical Manifestations

R. equi has been cultured from a variety of human tissues and fluids, including sputum, bronchial washings, lung tissue, pleural fluid, blood, heart valves, CSF, brain, skin, lymph nodes, peritoneal fluid, bone, stool, pharyngeal exudates, and wounds.[257,260,278–291] It has been recovered from peritoneal dialysate, intravenous catheters, and CSF after ventriculoperitoneal shunt insertion.[260,292,293] It produces a biofilm on intravascular catheters.[294] Pneumonia accounts for about 80% of human cases of infection reported in the literature.[259,295] Most published cases of pulmonary infection have occurred in immunocompromised hosts.[295] The lung was the only site of infection in more than 80% of cases; a concurrent extrapulmonary site was reported in about 20% of cases of pulmonary infection.[260] Typically, the presentation is subacute in onset. Common symptoms include fever, productive or nonproductive cough, and fatigue.[260] Pleuritic chest pain is also common. Hemoptysis has been reported in about 15% of patients. *R. equi* bacteremia frequently complicates pneumonia. Other complications include the development of lung abscess, pleural effusion, empyema, pneumothorax, endobronchial lesions, pericarditis, cardiac tamponade, and mediastinitis.[260,289,295,296] Chest radiographs reveal nodules, cavities (single or multiple), infiltrates, and pleural effusions.[265,289] More than one type of lesion may be present. In a case series of pulmonary cavitary lesions in HIV-infected persons, *R. equi* was the fifth most common microbiologically proven cause, accounting for about 9% of cases.[297] It followed *Mycobacterium tuberculosis, Pneumocystis jirovecii, Pseudomonas aeruginosa*, and *S. aureus* in frequency. The cavities have been described as thick walled and sometimes have an air-fluid level.[260] Necrotizing pneumonia caused by *R. equi* closely resembles tuberculosis or nocardiosis.[298] Nodules or cavities of the upper lung lobes, or both, may be seen. Air-fluid levels are seen in cavitary lesions caused by *R. equi* but not in those seen with tuberculosis. Mediastinal enlargement has been noted. The most common computed tomographic finding is consolidation with cavitation.[299] Although a good-quality sputum specimen can yield a microbiologic diagnosis, in many instances invasive techniques such as bronchoscopy, thoracentesis, or surgical resection are required in order to make a microbiologic diagnosis. Blood cultures are positive in about 50% of HIV-infected individuals and in 25% of solid-organ transplant recipients who are infected with *R. equi.* Up to 30% of immunocompetent hosts are bacteremic.[262,278,280]

Extrapulmonary infection with *R. equi* occurs in about 20% of cases with pulmonary infection; infection of extrapulmonary sites occurs in about 25% of cases without evidence of pulmonary involvement. The most common extrapulmonary sites reported were brain and subcutaneous abscesses.[260,300,301] Extrapulmonary infection is frequently a late manifestation of the initial pulmonary infection. Abscesses in the liver, spleen, thyroid, kidney, psoas muscle, bone, prostate, intraabdominal cavity, and paraspinous tissue have occurred.[295,300,302] Extrapulmonary infections not associated with pulmonary disease have been noted to manifest in three distinct patterns.[260] The first pattern includes wound infections, traumatic septic arthritis, and endophthalmitis after ocular injury. In these cases, infection remains localized at the primary site, and drainage procedures appear to hasten recovery. The second group consists of cases of isolated bacteremia that manifested with fever. Most of these patients had malignancies and were neutropenic or had recently received chemotherapy. Central venous catheters were present in most of these cases. The third pattern may have resulted from inoculation of the gastrointestinal tract with dissemination to regional lymph nodes. Conditions in this group include peritonitis, pelvic masses, and mesenteric adenitis. Other reported types of infection include otitis media with mastoiditis; colonic polyps infiltrated with *R. equi*; and osteomyelitis of the vertebrae, long bones, and mandible.[260,302]

More than 85% of cases of *R. equi* infection described in the literature have occurred in immunocompromised hosts, particularly those with HIV infection. HIV-infected patients account for two-thirds of cases.[259] Other immunocompromised hosts reported to be infected with *R. equi* include recipients of solid organ and hematopoietic stem cell transplants; diabetics; alcoholics; those with chronic renal failure, leukemia, lymphomas, lung cancer, or sarcoidosis; and preterm infants. Infection has occurred as a complication of chemotherapy, corticosteroid use, and treatment with monoclonal antibodies.[303,304] Immune reconstitution syndrome (IRIS) secondary to *R. equi* has been reported in a patient with HIV and Burkitt lymphoma.[305] Infection of immunocompetent persons with *R. equi,* however, may be more common than previously assumed because in a recent series, immunocompetent hosts accounted

for 42% of cases.[262] Clearance of *R. equi* is impaired in the immunocompromised host, and relapses are common despite maintenance antibiotic therapy.[306] In the pre–antiretroviral therapy era, relapses of pneumonia were described in up to 80% of HIV-infected patients. Infection occurs primarily in patients with CD4 counts of less than 100 cells/μL.[259] About 10% of *R. equi* infections occur in transplant recipients receiving immunosuppressive therapy and are generally a late complication.[259,282,288,306–309] Most of these patients were solid-organ transplant recipients. The primary site of infection was the lung. Findings included both nodular lesions and infiltrates. Cavitary lesions were frequent. Pseudotumor has been reported.[308] In about half of transplant recipients, extrapulmonary infection was present and included brain abscesses, paravertebral abscess, purulent pericarditis, subcutaneous nodules, and osteomyelitis of the femur. Among immunocompetent hosts, localized infections account for nearly 50%.[262] Pulmonary infection was present in more than 40%. Disseminated infection also occurred.[286,287,300] Recurrent infection has been reported.[310] The mortality rate is greatest among patients with AIDS and has been reported to be as high as 58%.[260,278,280] In a study from Thailand, HIV-infected persons with community-acquired pneumonia caused by *R. equi* were more likely to die than those infected with other organisms.[311] The use of highly active antiretroviral therapy has greatly improved the survival rates to 90% to 100% for those with HIV infection.[312,313] Mortality in immunocompetent hosts has been reported to be 11%; it is about 20% for non–HIV-infected immunocompromised hosts.[259,282]

Treatment

Susceptibility testing should be performed, based on CLSI recommendations, with a regular gram-positive panel. *R. equi* is usually susceptible in vitro to vancomycin, macrolides, fluoroquinolones, rifampin, teicoplanin, carbapenems, aminoglycosides, and linezolid.[259,260,314–316] Of the quinolones, moxifloxacin and gatifloxacin are the most active in vitro.[317,318] Gatifloxacin is no longer available in the United States. Susceptibility to clindamycin, tetracycline, chloramphenicol, and cephalosporins is variable; *R. equi* is usually resistant to penicillins, and even if susceptible in vitro, the use of penicillins and other β-lactams (except carbapenems) is not recommended because resistance can develop.[260,262] Mechanisms of resistance include altered penicillin-binding proteins and β-lactamase production.[262,298] In an animal model, the most effective agents were vancomycin, imipenem, and rifampin.[319] Rifampin, erythromycin, clarithromycin, vancomycin, and doxycycline exhibit a relatively long postantibiotic effect.[320] Rifampin-resistant isolates have been reported.[321] The incidence of resistance to macrolides and rifampin in animal isolates has been increasing.[322] Monotherapy has been ineffective in a number of cases and is not recommended. Monotherapy can lead to ciprofloxacin-resistant mutants.[323] Combinations of two or three antimicrobial agents have generally yielded partial or complete therapeutic responses. Localized, non-CNS infections in immunocompetent hosts can usually be treated with oral agents.[262] Two-drug regimens that include a macrolide, rifampin, fluoroquinolone, or a combination can be started empirically and should be adjusted based on the results of susceptibility testing.[259] Immunocompromised hosts and those with serious infections should be treated with two- or three-drug regimens that include vancomycin or a carbapenem (imipenem, ertapenem, or meropenem), rifampin, a fluoroquinolone, an aminoglycoside, or a macrolide.[309,324] Linezolid has been used successfully to treat relapsing infection and in cases of osteomyelitis.[306] It has been suggested that intravenous antibiotics be continued until clinical improvement occurs or for a minimum of 2 to 3 weeks.[259] Oral agents should then be given until cultures are negative and signs and symptoms have resolved. A 2- to 8-week course of two drugs might be sufficient for mild-to-moderate pneumonia, although optimal treatment of this infection is still unclear.[298] A minimum of 2 to 6 months of antimicrobial therapy is advised for immunocompromised hosts and those with pulmonary, bone, joint, or CNS infections. Brain abscess has been successfully treated with 8 weeks of intravenous therapy.[300] Because the CNS is a frequent secondary site of infection, agents that penetrate this site should be administered.[260] Drainage or débridement of localized abscesses, empyema, infected pericardial effusion, and large cavities may be beneficial.[325,326] Lung lobectomy has been performed when poor clinical response was noted with antimicrobial therapy.[327] It is generally recommended that after the treatment course is completed, HIV-infected individuals and persons with ongoing immunosuppression receive long-term suppressive therapy with a macrolide plus rifampin or a quinolone or doxycycline with rifampin. For HIV-positive patients, oral suppressive therapy should be continued until immune reconstitution occurs. Reducing immunosuppressive therapies may improve eradication of *Rhodococcus* spp.[308] Infection may develop or may manifest at other sites during therapy. Relapses are common. They can occur at the initial site of infection or at other sites.

Other *Rhodococcus* Species and Related Genera

Infections caused by other *Rhodococcus* spp. and related genera, such as *Gordonia* and *Tsukamurella,* have generally been associated with medical procedures or devices. *Gordonia* spp., previously classified as *Rhodococcus* spp., have caused pulmonary infections, bacteremia, endocarditis, septic arthritis, and CNS infections in both immunocompromised and immunocompetent adults and children.[328–338] Major pathogens include *Gordonia bronchialis, Gordonia sputi,* and *Gordonia terrae. G. terrae* has caused CNS infections, central venous catheter–associated bacteremia and endocarditis in children, and infections of medical devices including peritoneal dialysis catheters.[329, 330, 333,336] *G. sputi* has caused skin infections, mediastinitis after coronary bypass surgery, and bacteremia.[335, 337] *G. bronchialis* (formerly *Rhodococcus bronchialis*) has been reported to cause a cluster of sternal wound infections after coronary artery bypass surgery, bacteremia, osteomyelitis, pleural infection, and recurrent breast abscess.[339–345] Pulmonary infection resembling tuberculosis has been reported to be caused by *Gordonia rubropertincta* (formerly *Rhodococcus rubropertinctus*) in a patient who was not immunosuppressed.[346] Other human pathogens include *Gordonia polyisoprenivorans, Gordonia amicalis, Gordonia araii, Gordonia effusa, Gordonia otitidis,* and *Gordonia aichiensis.* They cause predominately skin infection, pulmonary infection, and bacteremia.[337,338] Speciation is best accomplished by 16S rRNA sequencing.[333] MALDI-TOF mass spectrometry has been used successfully to identify *G. bronchialis,* but other systems have been ineffective because of current database limitations.[270,342] *Gordonia* isolates are most predictably susceptible to imipenem, ciprofloxacin, amikacin, linezolid, gatifloxacin, and gentamicin. Eighty-nine percent of isolates were susceptible to vancomycin.[333] Initial treatment may consist of a carbapenem or a fluoroquinolone with or without an aminoglycoside.

Tsukamurella spp., including *Tsukamurella paurometabola* (formerly *Rhodococcus aurantiacus* and *Corynebacterium paurometabolum*), have caused bacteremia associated with central venous catheters in patients with malignancies and patients who were receiving parenteral nutrition, and in those with infection of an implantable cardioverter defibrillator, pneumonia (including tuberculosis-like pneumonia), meningitis, conjunctivitis, keratitis, otitis media, skin and soft tissue abscesses, brain abscess, peritonitis resulting from CAPD, and necrotizing tenosynovitis.[298,346–356] Underreporting of cases has probably occurred as a result of misidentification as atypical *Mycobacterium* spp. or as *Rhodococcus* spp.[298,356,357] Susceptibility varies with species. Most isolates have been susceptible to fluoroquinolones, macrolides, imipenem, vancomycin, trimethoprim-sulfamethoxazole, and aminoglycosides and resistant to penicillins and cephalosporins. Topical tetracycline or levofloxacin has been used to treat ocular and ear infections.

R. fascians (R. luteus) and *R. erythropolis* have been associated with chronic endophthalmitis after lens implantation.[358] *R. erythropolis* has been isolated from patients with peritonitis who were undergoing ambulatory peritoneal dialysis, from subcutaneous nodules in a patient with AIDS, from blood, and from sputum in a patient with pneumonia.[359–361] *R. rhodochrous* has caused pneumonia, bacteremia, pericarditis, skin lesions, meningoencephalitis, ventriculoperitoneal shunt infection, and chronic corneal ulceration.[362,363] *Rhodococcus globerulus* bacteremia was associated with a subacute illness with severe hepatitis in a hematopoietic stem cell transplant recipient. It also was associated with a corneal abscess after LASIK (laser-assisted in situ keratomileusis) therapy.[364,365] Meningitis caused by non-*equi Rhodococcus* has been reported in an immunocompetent host.[366] Antimicrobial therapy for these infections should be based on susceptibility testing. When *Rhodococcus* spp. infection occurs in association with a medical device, the device should be removed.

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206 *Listeria monocytogenes*

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SHORT VIEW SUMMARY

Diagnosis
- Culture of blood, cerebrospinal fluid, or other normally sterile body fluid or, in the case of gastroenteritis, from stool.
- Serology not useful for invasive disease.

Microbiology
- Short, gram-positive rod; grows readily on blood agar; tumbling motility.
- May be mistaken for diphtheroid contaminant.
- Will grow in refrigerated food.

Epidemiology
- Zoonosis, particularly herd animals.
- Human transmission from contaminated food or from pregnant woman to fetus or newborn.
- Highest food risks from delicatessen-style meats and unpasteurized cheeses.
- Most cases in neonates, pregnant women, adults 60 years old or older, and individuals with impaired cell-mediated immunity resulting from underlying condition (hematologic malignancy, organ or bone marrow transplantation, acquired immunodeficiency syndrome) or therapy (corticosteroids, anti–tumor necrosis factor agents). Notify laboratory for special stool cultures if outbreak of febrile gastroenteritis.

Clinical Settings
- Neonatal sepsis or meningitis.
- Meningitis or focal central nervous system (CNS) lesions in immunosuppressed patients or adults older than 50 years.
- Rhombencephalitis occurs in previously healthy patients who develop fever; cranial nerve palsies; cerebellar signs; and hemiparesis, hemisensory deficits, or both.
- Fever in pregnancy, especially third trimester.
- Outbreak of foodborne febrile gastroenteritis.

Treatment
- Ampicillin (2 g IV every 4 hours); consider adding gentamicin (5 mg/kg/day) for CNS infection or endocarditis.
- Trimethoprim-sulfamethoxazole (5/25 mg/kg IV every 8 hours) for penicillin-allergic patients.

Prevention
- *Pneumocystis* prophylaxis with trimethoprim-sulfamethoxazole likely prevents listeriosis.

DEFINITION

Listeria monocytogenes is a gram-positive bacillus and zoonotic and foodborne pathogen found worldwide that causes listeriosis. Listeriosis spans the clinical spectrum from self-limited febrile gastroenteritis in immunocompetent people to more severe and invasive disease that mostly affects pregnant women, newborn infants, older adults, and individuals with cell-mediated immunodeficiencies. It is a rare but important pathogen because of the populations it infects and its high rates of mortality.

EPIDEMIOLOGY

L. monocytogenes was first described in 1926 after it was identified as the causative pathogen responsible for an outbreak and sudden death of a number of laboratory animals at Cambridge University. Initially named *Bacterium monocytogenes,* it was isolated from infected laboratory animals, a rabbit and a guinea pig, that were also noted to have a peripheral monocytosis.[1] Already known to be an important zoonotic disease infecting more than 40 mammalian and avian species, *L. monocytogenes* was first recognized as a human pathogen in 1929.[2] Additionally, *L. monocytogenes* was known to be transmissible by direct inoculation from an infected animal to a human causing cutaneous infection as well as vertically from mother to fetus causing abortion, stillbirth, or neonatal infection. However, the route of transmission causing bacteremia and meningoencephalitis eluded scientists until 1983, when Schlech and colleagues[3] traced an outbreak of *L. monocytogenes* serotype 4b in Nova Scotia, Canada, to contaminated coleslaw and cabbage. *L. monocytogenes* is now widely recognized as a serious but rare cause of foodborne infections and has the highest case-fatality rate of any foodborne pathogen in the Western Hemisphere.[4–6]

Although most cases of listeriosis are sporadic, *L. monocytogenes* is a cause of major foodborne outbreaks globally.[7,8] Longer incubation periods for listeriosis can affect recall and can make trace-back investigations and identification of the causative foods difficult.[9] Foods that have been linked to outbreaks are those that are at risk for contamination; support the growth of *L. monocytogenes;* may be stored for long periods of time allowing for a higher inoculum of bacteria on ingestion; and are refrigerated, as it can replicate at lower temperatures.[10] *L. monocytogenes* can also withstand high-salt and lower pH environments.[9] Specific foods implicated in outbreaks include soft cheeses; cheeses made from raw milk; raw produce including packaged salads; cantaloupe and sprouts; caramel apples; frozen vegetables; smoked seafood; ice cream; and ready-to-eat meats such as delicatessen meats, hot dogs, and pâté.[11–13] Food contamination can occur at any level of production, from preharvest to food processing. *L. monocytogenes* is a ubiquitous organism found worldwide in water, soil, animal feces, and vegetation. It forms biofilms and can persist in food-processing environments for years.[14] Pasteurization and antimicrobial agents used in foods before and after packaging effectively neutralize *Listeria.* Careful cleaning of food-processing equipment and clean postprocessing handling practices are ways of reducing contamination.[10]

In 2010 listeriosis caused an estimated 23,150 illnesses, 54,463 deaths, and 172,823 disability-adjusted life-years worldwide.[7] Much higher rates in industrialized countries are likely due to more standardized reporting, although other potential factors such as dietary habits, food processing, testing, and host factors may contribute.[15] Seasonal trends have been observed in Europe and the United States with peak incidence of invasive listeriosis cases in the summer months.[16–18]

MICROBIOLOGY

The genus *Listeria* contains 17 species of small, gram-positive, rod-shaped bacteria, of which three, *L. monocytogenes, Listeria ivanovii,* and *Listeria grayi,* are opportunistic pathogens in humans.[19–22] *L. monocytogenes* was the first *Listeria* spp. identified and is responsible for nearly all cases of listeriosis in humans. *L. monocytogenes* is a nonsporulating, catalase-positive, oxidase-negative, flagellated facultative anaerobe that grows optimally at 30°C to 37°C.[23] It demonstrates characteristic tumbling motility at 20°C to 28°C, is able to grow at temperatures as low as 4°C, and is tolerant of high-salt environments.[23,24] Morphologically, *L. monocytogenes* can be decolorized by alcohol during the Gram staining process and appear as gram-variable or gram-negative.[25] Different growth

media may produce short rods, longer rods, or elliptical cocci.[25] For these reasons, it can be mistaken for diphtheroids, *Enterococcus* spp., *Streptococcus pneumoniae, Haemophilus influenzae,* or enteric bacteria on Gram stain.[25] Some commercially available polymerase chain reaction (PCR) film arrays for cerebrospinal fluid (CSF) include *L. monocytogenes* and should facilitate rapid diagnosis.

L. monocytogenes grows well on most routine culture media and usually forms a narrow zone of β-hemolysis on blood agar, although nonhemolytic strains have been described.[19,23] Selective media used to prevent overgrowth from other bacteria present can be used to culture *L. monocytogenes* from nonsterile sources such as the vaginal canal, stool, and food.[26,27]

There are more than 14 serotypes, which are differentiated based on their somatic (O) and flagellar (H) antigens.[28] Serotypes 1/2a, 1/2b, and 4b are implicated in more than 95% of cases of human listeriosis.[11] Various PCR methods can be used to quickly identify the different high-risk serotypes.[29] Whole-genome sequencing has been a valuable tool in outbreak tracing.

PATHOGENESIS

L. monocytogenes is a hardy organism that is adapted to live as a saprophyte in external environments or as an intracellular pathogen in a number of animal hosts.[30] Within its animal hosts, it maintains an arsenal of defensive mechanisms to survive in, respond to, and proliferate in the diverse environments it encounters.[31] *L. monocytogenes* can employ a number of virulence factors such as Internalin A (InlA) encoded by *inlA* and listeriolysin O (LLO) encoded by *hly* to help facilitate entry into and movement through the host cells, to escape phagosomes, and to circumvent the host immune response.[32] Virulence genes are often used as targets for PCR detection and subtyping.[33]

In nearly all cases of infection, *L. monocytogenes* enters into human hosts via the gastrointestinal tract after ingestion of contaminated food. In the stomach, gastric pH of less than 3 is informally bactericidal; however bacterial survival increases at a pH of 3.5 and even more at a pH of 4.[34] This is perhaps why the widespread use of proton pump inhibitors and thus elevated gastric pH has been shown to increase the risk of nonperinatal invasive listeriosis after adjusting for confounding factors.[35] *L. monocytogenes* then moves into the duodenum where the high concentration of bile creates a hostile environment for most microbes.[36] The expression of bile salt hydrolases and the ability to tolerate high-salt conditions allow *L. monocytogenes* not only to survive transit through the duodenum but also to colonize the gallbladder.[37,38] Luminal antibodies have not been shown to be protective against *L. monocytogenes,* whereas innate immune mechanisms such as bactericidal peptides produced by Paneth and epithelial cells are successful host defenses in the small intestine.[37]

The intestinal microbiota also provides an independent and important first line of defense against *L. monocytogenes* infection and indirectly augments host defenses.[37,39] Potential mechanisms of protective properties of commensal bacteria are nutrient competition, contact-dependent inhibition and production of bacterial soluble mediators, and bacteriocins, which are toxic molecules created by certain common gut bacteria.[37] Conversely, dysbiosis, from exposure to antibacterial agents or other causes, can allow for the expansion of *L. monocytogenes* within the intestine. Listeriolysin S is a virulence factor found in epidemic strains of *L. monocytogenes* and the only known bacteriocin produced in the *Listeria* genus that disrupts the host gut microbiota creating a beneficial environment for *L. monocytogenes* during infection.[40]

Once at the intestinal wall, *L. monocytogenes* is able to invade both phagocytic and nonphagocytic cells by employing a number of different strategies. Bacterial virulence factors and surface proteins InlA and InlB bind to the surface receptors E-cadherin and Met on nonphagocytic host cells such as epithelial cells and facilitate entry by receptor-mediated endocytosis.[41] After internalization, the low pH of the phagosome activates LLO, which, along with phospholipases PlcA and PlcB, causes pore formation of the organelle wall and inhibits lysosome fusion, allowing the bacterium to escape.[32,42] Within the cytosol, *L. monocytogenes* is able to replicate with a doubling time of about 1 hour.[42] Bacterial surface protein ActA recruits and binds actin from the host cell to form an actin comet tail at one pole enabling the bacterium to propel itself through the cytoplasm.[43] The bacterium then finds the plasma membrane of the infected host cell. It becomes enveloped in the membrane, extruded from the infected cell, and then phagocytosed by the neighboring cell.[43] It again uses LLO to escape the phagosome, infecting the new host cell and thereby enabling cell-to-cell spread while avoiding the humoral immune system (Fig. 206.1A).[43]

L. monocytogenes is also able to invade goblet cells expressing E-cadherin by InlA-mediated transcytosis and is rapidly transported through the cell, independent of InlB, LLO, and ActA.[44] In some macrophages, *L. monocytogenes* is able to replicate within vacuoles called spacious *Listeria*-containing phagosomes before phagosome escape (Fig. 206.1B).[41]

Having traversed the gastrointestinal border, *L. monocytogenes* then disseminates from lymph nodes into the bloodstream to the spleen and liver and across the blood-brain and placental barriers in the appropriate hosts.[41] Invasion into the central nervous system (CNS) is accomplished by a number of different mechanisms including Internalin receptor-mediated endocytosis through endothelial cells; the neural route, where *L. monocytogenes* migrates from peripheral neurons to central neurons by cell-to-cell spread; and transport inside infected monocytes, the so-called Trojan horse.[45] Transplacental infection likely occurs predominantly by Internalin receptor-mediated endocytosis and cell-to-cell spread but may also occur via inflammation-mediated and primary hematogenous spread.[46,47] Newly identified virulence factors may better explain organ-specific tropism in invasive listeriosis such as InlP and placental tissue.[48]

Despite the ubiquitous and robust nature of *L. monocytogenes,* actual infection is relatively rare owing to active host defenses. Innate and adaptive host defenses against *L. monocytogenes* have been extensively studied in in vivo murine models and in vitro human cells, so much is extrapolated as to how these systems work in humans.[49] The innate immune response is rapid and crucial for host survival.[50] On escape from the phagosome within an infected macrophage, LLO activates at least three separate pathways that produce distinct host responses.[51] One pathway promotes pyroptotic host cell death.[51] Microbial products released from infected macrophages recruit macrophages and promote their differentiation into dendritic cells.[50] The dendritic cells produce inducible nitric oxide synthase and tumor necrosis factor (TNF) to induce microbial killing.[50] Natural killer cells produce interferon-γ that, along with TNF, further activate macrophages, which are, along with neutrophils, directly listeriacidal.[50,52]

Cell-mediated immunity, as opposed to humoral immunity, plays the predominant role in the adaptive response and is necessary for clearance of infection.[53] The innate immune response to *L. monocytogenes* infection is essential for the development of the host adaptive response.[51]

CLINICAL MANIFESTATIONS

As noted earlier, *L. monocytogenes* can cause a broad spectrum of disease ranging from self-limiting febrile gastroenteritis to invasive disease such as bacteremia and meningoencephalitis. Development of invasive disease usually depends on host factors, with higher incidence in pregnant women, neonates, elderly adults, and individuals with cell-mediated immunodeficiencies. Localized and focal infections can be caused by either direct inoculation of *Listeria* into a site or via hematogenous spread.

Acute Febrile Gastroenteritis

L. monocytogenes been recognized as a cause of outbreaks of febrile gastroenteritis only in the past few decades. The first fully evaluated outbreak happened in 1994, when 45 people developed febrile gastroenteritis after drinking pasteurized chocolate milk contaminated after pasteurization and stored in conditions that allowed for bacterial proliferation.[54] *Listeria* strains from stool cultures, the chocolate milk, and the milk storage tank were indistinguishable, providing for the first time the most compelling evidence for *L. monocytogenes* as an etiologic agent of gastroenteritis.[54] Multiple other outbreaks have since been reported including one directly linking the epidemic strain to subclinical mastitis in a goat whose milk was used to produce fresh cheese.[55] Serotypes 1/2a and 1/2b are responsible for most cases of febrile gastroenteritis, whereas serotype 4b more commonly causes invasive diseases. High

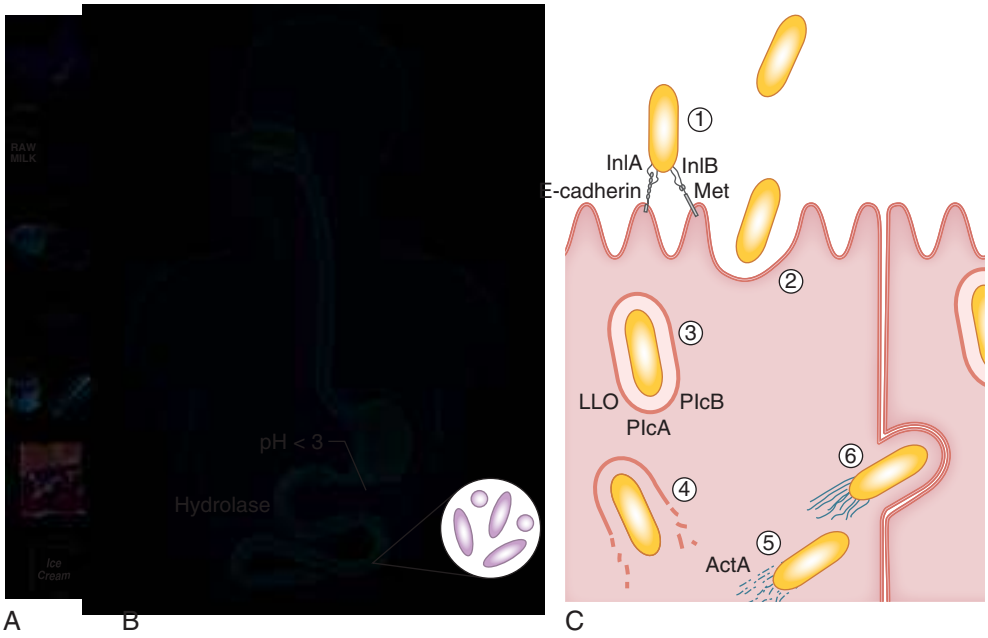

FIG. 206.1 (A) Foods associated with listeriosis outbreaks—cantaloupe, raw dairy products, ready-to-eat meats such as deli meat, sprouts, caramel apples, hot dogs, prepackaged salads, and ice cream. (B) Host defenses and transit through the gastrointestinal tract. In the stomach, gastric pH of less than 3 is informally bactericidal. Bacterial survival increases above a pH of 3.5. *Listeria monocytogenes* expresses bile salt hydrolases and is able to tolerate high-salt conditions, enabling it to survive transit through the duodenum. Paneth and epithelial cells in the small intestine produce bactericidal peptides. Intestinal microbiota provides an independent and important first line of defense against *L. monocytogenes* infection as well as indirectly augmenting host defenses. (C) Intracellular invasion. Bacterial virulence factors and surface proteins Internalin A (InlA) and Internalin B (InlB) bind to the surface receptors E-cadherin and Met *(1)* on nonphagocytic host cells and facilitate entry by receptor-mediated endocytosis *(2)*. After internalization, the low pH of the phagosome activates listeriolysin O (LLO), which, along with phospholipases PlcA and PlcB *(3)*, causes pore formation of the organelle wall and inhibits lysosome fusion, allowing the bacterium to escape *(4)*. Bacterial surface protein ActA recruits and binds actin from the host cell to form an actin comet tail at one pole enabling the bacterium to propel itself through the cytoplasm *(5)*. The bacterium then finds the plasma membrane of the infected host cell, in which it is enveloped, extruded from the infected cell, and phagocytosed by the neighboring cell *(6)*.

organism density in the source food was found in many of the reported outbreaks, suggesting that high inoculum is probably required to produce infection. Virulence and host factors also likely play a role but have yet to be elucidated. Attack rates during outbreaks have ranged from 52% to 100%.[56]

In contrast to invasive listeriosis, gastrointestinal listeriosis occurs most frequently in healthy children and adults. Infection usually has a much less severe clinical course than invasive infection and is generally self-limiting. Symptoms last an average of 1 to 3 days but can persist for a week.[56] Fever, headache, fatigue, arthralgias, nausea, and nonbloody diarrhea are common symptoms. The incubation period is short, with symptoms often occurring within 24 hours after ingestion of contaminated food.[56,57] However, there was one case in an outbreak in Sweden in which a person developed symptoms 10 days after ingestion of contaminated fresh cheese.[58] A small number of invasive infections were reported during febrile gastroenteritis outbreaks, but these were usually in people with underlying risk factors for invasive disease such as older age and chemical immunosuppression.[54,58]

Sporadic cases of *Listeria* gastroenteritis are likely uncommon. A 2-year prospective study in Nova Scotia looked at 6785 stool samples submitted for bacterial culture and found only 13 grew *L. monocytogenes,* whereas there were 124 isolates of *Salmonella* spp. and 128 isolates of *Campylobacter* spp.[59] Study participants, when matched with control cases from the cohort that grew either *Salmonella* spp. or *Campylobacter* spp., had a longer duration of diarrhea and were more likely to have underlying gastrointestinal problems such as inflammatory bowel disease, irritable bowel syndrome, rectal carcinoma, and *Clostridioides difficile* (formerly *Clostridium difficile*) colitis in one case.[59] Participants with stool cultures positive for *L. monocytogenes* were less likely to report fever than persons in the control group (17% vs. 54%).

Additionally, stool carriage of *L. monocytogenes* in the general human population is approximately 1% to 5%, with a higher percentage in individuals in households with members who develop invasive listeriosis,[60–62] presenting a potential challenge in interpreting positive stool cultures. However, *L. monocytogenes* should be considered in patients with febrile gastroenteritis and negative testing for other common pathogens.[56]

Cutaneous Listeriosis

Primary cutaneous listeriosis is rare and has been described in otherwise healthy individuals predominantly with occupational or recreational exposures such as cattle farming, large animal veterinary medicine, and, in one reported case, gardening.[63] Infection occurs via direct inoculation and in many documented cases from an infected source, most commonly aborted bovine fetuses.[63,64] A papulopustular rash develops at the site of inoculation 1 to 7 days after exposure. Nearly all rashes are accompanied by fever. Given the mode of transmission, arms and hands are the most frequent site of infection. In nearly all documented cases, patients made a full recovery with only a quarter receiving treatment with antibiotics, suggesting infection is usually self-limited. Secondary cutaneous listeriosis due to disseminated disease has been reported only in neonates (discussed in "Neonatal Infection") and immunosuppressed adults.[65,66]

Invasive Listeriosis

Pregnancy

Women who are pregnant are at a 17-fold greater risk of listeriosis (12 cases/100,000) than the general population (0.7 cases/100,000) and almost 6-fold greater risk than individuals older than 70 years of age (2.1 cases/100,000).[42] Pregnant women represent about one-sixth of invasive listeriosis cases.[17] Although maternal infection is usually mild, vertical transmission to the fetus can cause fetal loss, preterm labor, and neonatal listeriosis, a condition with high morbidity and mortality. In one study, about 20% of pregnancy-related listeriosis cases led to spontaneous abortion or stillbirth.[67] Among pregnancies that did not end in spontaneous abortion or stillbirth, about 68% of neonates became

infected, and the remaining 32% did not become infected. A large prospective study in France found that major fetal or neonatal complications occurred in up to 83% of maternal cases, which is higher than previous retrospective analyses.[4]

Development of listeriosis earlier in pregnancy correlates with poorer fetal outcomes.[4,68] Women are more susceptible to listeriosis in the third trimester, probably due to a decline in cell-mediated immunity during that period.[67,69,70] Additionally, most pregnant women who develop listeriosis have no other underlying risk factors usually associated with listeriosis and are generally healthy.[67] Certain subpopulations, such as Hispanic women in the United States, women of Asian or Afro-Caribbean descent in the United Kingdom, women from the Maghreb, women from sub-Saharan Africa in France, and non–English-speaking women in Australia, are disproportionally represented among cases of maternal listeriosis.[4,17,68,71]

Clinical manifestations occur an average of 23 days (range, 0–67 days) after exposure and are often subtle and nonspecific, with fever being the most common sign in a number of studies.[4,67,72] Other manifestations may be flulike symptoms or obstetric signs and symptoms such as contractions, labor, abnormal fetal heart rate, abdominal pain, diarrhea, vomiting, headache, amnionitis, or septic abortion.[4,67,68] Blood cultures are positive in 43% to 58% of women, and vaginal or cervical cultures are positive in 26% to 34%.[4,67,71] For reasons not yet elucidated, cases of severe disseminated listeriosis and meningitis are rare in pregnant women, even in women with bacteremia.[67]

Neonatal Infection

From the infected mother, *L. monocytogenes* can invade through the placenta leading to severe complications such as chorioamnionitis, spontaneous abortion, stillbirth, preterm labor, and neonatal infection. Congenital listeriosis, which is rare outside of the neonatal period in children, is a cause of meningitis and disseminated bacterial infections in infants 31 days old or less, with *Listeria,* group B *Streptococcus,* and *Escherichia coli* accounting for more than 70% of total cases.[73,74] The incidence and epidemiology of neonatal listeriosis vary geographically. Historically, *L. monocytogenes* has been one of the top causes of severe bacterial infection in the neonatal period. However, there has been an epidemiologic shift in recent decades with listeriosis becoming less common among other bacterial pathogens affecting neonates, especially in the United States.[75–77] Case rates have also significantly decreased from 4.78 cases for the years 1992–95 to 1.31 cases for 2003–13 per 10,000 admissions in the United States.[75] This shift and decrease in incidence may be explained by a number of factors including improved food safety and implementation of antenatal group B *Streptococcus* screening and prophylaxis with penicillin or amoxicillin.[75]

In the past 50 years, the United Kingdom has seen a decrease in mortality from neonatal listeriosis from 35%[5] to 21% to 24%.[71] A 17-year study from Taiwan found a 29% mortality rate.[78] Neonatal listeriosis produces severe disease with a high mortality rate of 20% to 30%, whereas preceding maternal infection may be absent or produce self-limiting mild flulike symptoms or fever with a sepsis syndrome.[67,79,80] Neonatal listeriosis can manifest in three forms: early-onset sepsis syndrome, late-onset meningitis, and, much less commonly, granulomatosis infantiseptica.[69]

Granulomatosis infantiseptica and probably early-onset listeriosis are acquired by transplacental infection in utero. Granulomatosis infantiseptica is a rapidly fatal disseminated infection present at birth with widespread microabscesses and granulomas within the liver and spleen and sometimes on the skin and bacteria seen on Gram stain of the meconium.[67,69]

Early-onset neonatal listeriosis occurs within the first few days of life. It is associated with preterm birth; chorioamnionitis; and diagnosis of one or a combination of bacteremia, pneumonia, or meningitis.[67,79] In one large case series including 6 cases from 3 large hospitals and 94 cases from the literature, 20.2% of neonates had pneumonia; 25.5% had bacteremia; 29.8% had bacteremia and pneumonia; 9.6% had bacteremia and meningitis; 5.3% had meningitis; and 5.3% had bacteremia, meningitis, and pneumonia.[67] Clinical features, which may be present at birth, include respiratory distress, meconium staining, fever, jaundice, lethargy, and maculopapular or papulovesicular rash.[67,71,78]

Late-onset neonatal listeriosis occurs an average of 5 to 14 days after birth. Neonates are usually full term, are healthy at birth, and are born to asymptomatic mothers who have had uncomplicated pregnancies.[67,79] Although mode of transmission remains unclear, inoculation from the vaginal tract or maternal gastrointestinal tract and nosocomial infection[67,79,81–83] are thought to be potential sources. Clinical features are nonspecific and similar to other bacterial pathogens causing bacteremia and meningitis during this period. CNS involvement is more common in this disease than in early-onset listeriosis and is the predominant clinical syndrome.[5,67] Sequelae of neonatal listeriosis can be significant, with 40% of surviving infants having neurologic or neurodevelopmental impairment on follow-up in one case series.[71]

Bacteremia

Bacteremia is the most common cause of invasive listeriosis and can lead to neurolisteriosis as well as other localized infections such as endocarditis and septic arthritis. Most cases are sporadic.[9] Of nonpregnant individuals who develop *L. monocytogenes* bacteremia, 97% have at least one underlying immunodeficiency, either due to one or more medical conditions or due to immunomodulating medications.[4] Specific risk factors include solid-organ and hematologic malignancy, kidney disease, cirrhosis, diabetes mellitus, giant cell arteritis, solid-organ transplantation, acquired immunodeficiency syndrome (AIDS), and older age (generally 65 years old and older).[4,69,84–86] Infection occurs in older adults even without other risk factors, with the incidence in individuals age 75 years and older nearly 20 times greater (0.98 cases/100,000) than in individuals younger than age 65 years (0.05 cases/100,000).[84] Current or recent use of a proton pump inhibitor also increases the risk of developing invasive infection.[35] Three-month mortality is 46% and is associated with older age, female sex, ongoing malignancy, multiple organ failure, worsening of prior organ disease, weight loss, monocytopenia <200 cells/μL, and elevated neutrophil count.[4]

Clinical manifestations occur an average of 5 days after exposure (range, 0–29 days).[72] In a large prospective study of 427 cases of bacteremia, 94% of patients presented with fever, tachycardia, or both, and C-reactive protein was elevated in 96%.[4] Signs and symptoms are general, nonspecific, and similar to other pathogens that cause bacteremia. Patients may report an antecedent diarrheal illness or nausea.

Neurolisteriosis

Neurolisteriosis is the second most common manifestation of invasive listeriosis after bacteremia. In the past 2 decades the overall incidence of bacterial meningitis including *L. monocytogenes* has declined to 0.7 to 0.9/100,000 cases in the Western world due to vaccines for *Streptococcus pneumoniae, Neisseria meningitidis,* and *H. influenzae* as well as improved food-processing techniques, safer food practices, and education.[87] Incidence in the developing world remains high.[87,88] In the United States the incidence of *L. monocytogenes* meningitis has declined by 46% over 2 decades from 0.10 cases/100,000 in 1998–99 to 0.05 cases/100,000 in 2006–07.[89] However, *L. monocytogenes* remains one of the top five most common pathogens of CNS infections in the Western world; is third worldwide; and is second among patients with diabetes, with alcohol dependence, and on immunosuppressive therapies.[87,89,90] Despite this overall decline, case mortality rates of neurolisteriosis remain the same, and compared with other pathogens causing CNS infections, *L. monocytogenes* mortality continues to be among the highest.[89]

In contrast to other common pathogens that cause bacterial meningitis, *L. monocytogenes* has a proclivity for the brain itself.[69] Isolated meningitis (13%) is less common than meningoencephalitis (84%).[4] *L. monocytogenes* can also cause localized infections such as cerebritis; abscess; and rhombencephalitis, a rare form of encephalitis involving the cerebellum and brainstem. The presence of encephalitis is associated with older age and increased number of comorbidities and a threefold greater mortality compared with neurolisteriosis without encephalitis.[4]

Although the same risk factors exist for individuals who develop *Listeria* bacteremia as individuals who develop CNS disease, 14% to 37% of persons who develop neurolisteriosis have no identifiable risk factors.[4,91,92] A large prospective study in France found 4% of patients with neurolisteriosis were younger than 40 years old, excluding neonates,

and had no identifiable risk factors including pregnancy.[4] This suggests a possible genetic susceptibility in this small subset of patients or perhaps exposure to a higher bacterial inoculum.[4] Individuals without underlying disease or other risk factors have a lower mortality rate than individuals with existing risk factors. Mortality rates range from 9% to 38% in this previously healthy group and higher depending on geographic location.[4,69,91,93]

The average incubation period is 10 days (range, 0–21 days).[72] Fever, headache, myalgia, chills, gastroenteritis, and other systemic symptoms may occur an average of 3 to 4 days before presentation.[91] Fever is the most common presenting symptom (>90%),[4,94] followed by altered mental status (66%) and headache (46%).[4,91] Seizures, cranial neuropathies, and other focal neuropathies may also be present. One-half to two-thirds of patients have nuchal rigidity.[4,91]

Gram stain of CSF reveals gram-positive rods in only about one-third of cases.[4,91,94] CSF analysis usually reveals a pleocytosis with neutrophil predominance. Blood cultures are positive in more than half of patients. In one small study, hyponatremia was present in 22 of 30 (73%) cases.[94] In a large prospective study, in a subanalysis of neuroradiographic findings in 71 patients with neurolisteriosis, magnetic resonance imaging was more sensitive than computed tomography for showing meningeal and parenchymal involvement of the brain.[4]

Three-month mortality is 30% and similar to patients with bacteremia without CNS involvement. Factors associated with mortality include older age, female sex, ongoing malignancy, multiple organ failure, worsening of prior organ disease, weight loss, monocytopenia <200 cells/μL, elevated neutrophil count, bacteremia, and dexamethasone administration.[4]

Of patients who survive, about 40% recover fully, and about 45% have persistent neurologic impairment such as limb motor deficiencies, cerebellar symptoms, and eighth nerve palsy.[4] Encephalitis is the strongest predictor of persistent neurologic impairment.[4]

Focal Invasive Infection

L. monocytogenes can cause endocarditis,[95] endovascular infections,[96] septic arthritis,[97,98] osteomyelitis,[98] peritonitis,[99] adenitis,[4] urinary tract infections,[100] pneumonia,[101] and other focal infections via hematogenous spread. Focal infections can also precede systemic infection.[102] Ocular infections can occur as a result of either direct inoculation or hematogenous spread.[103–105]

DIAGNOSIS

Listeriosis should be particularly suspected in the following clinical scenarios: respiratory distress, sepsis, or meningitis in neonates; meningitis or parenchymal brain infection in persons ≥50 years of age with subacute presentation of meningitis, hematologic malignancy, solid-organ malignancy, organ transplant, or AIDS or on immunosuppressive therapies (e.g., chemotherapy for cancer, corticosteroids, anti-TNF agents); concomitant infection of the meninges and brain parenchyma; subcortical brain abscess; fever during pregnancy, especially in the third trimester; and foodborne outbreak of febrile gastroenteritis with negative stool cultures or standard gastrointestinal panel multiplex PCR.[106]

Diagnosis of invasive listeriosis is usually made by isolation of *L. monocytogenes* by culture from sterile sources such as blood, CSF, amniotic fluid, placental tissue, aqueous humor, or vitreous fluid. Culture from peripheral sites such as the vagina, cervix, and stool in adults and gastric aspirate, ear, anus, and pharynx in neonates can aid in diagnosis.[4] Neurolisteriosis may be diagnosed in patients with positive blood cultures and neurologic symptoms consistent with meningitis, encephalitis, or cerebritis without positive CSF cultures. Cultures may be negative if patients received empirical antimicrobial treatment before sample collection. PCR is more sensitive and more specific than culture, offers rapid diagnosis, and can be used in cases to confirm diagnoses when cultures are negative but clinical suspicion is high.[107] Multiplex PCR testing for CSF for an array of viruses, bacteria including *L. monocytogenes,* and fungi is now commercially available.[108]

Patients usually have fever and a leukocytosis with predominance of polymorphonuclear cells.[4] In nonperinatal cases, 79% to 84% of patients have lymphopenia, but this is less common in maternal cases.[4] Despite its name, *L. monocytogenes* infections rarely produce a monocytosis in humans.[4,69]

Isolation of *L. monocytogenes* from wound and urine cultures can be due to contamination, colonization, or true infection. Results should be interpreted alongside other tests such as urinalysis as well as within the clinical context from which the culture was obtained.[100] Selective media are required for isolation from stool, and the receiving microbiology laboratory should be notified if *Listeria* gastroenteritis is suspected.[56] Although not useful in invasive disease, elevated levels of LLO antibodies may be helpful in the diagnosis of febrile gastroenteritis.[54,106]

TREATMENT

Although there are no randomized controlled trials to establish the most effective antimicrobial treatment of listeriosis, benzylpenicillin (penicillin G) and aminopenicillin (ampicillin) are considered the mainstay of treatment either alone or in combination with an aminoglycoside for synergy in severe infections such as CNS infections or endocarditis.[109] Second-line therapies include trimethoprim-sulfamethoxazole, meropenem, and piperacillin-tazobactam, although the latter should not be used in CNS infections. Cephalosporins should not be used.[69,110]

In vitro and extracellular susceptibilities of clinical isolates of *L. monocytogenes* demonstrate sensitivity to many antibiotics including penicillin, ampicillin, fluoroquinolones, vancomycin, tetracycline, rifampin, and cefazolin with high resistance rates to clindamycin and third-generation cephalosporins.[111] Intracellular activity may vary,[112] and there have been clinical failures with vancomycin and development of listeriosis while on ciprofloxacin.[113,114] In rare instances, plasmid-mediated resistance to macrolides, gentamicin, and trimethoprim occurs.[115]

In in vitro studies, β-lactams are bacteriostatic for *L. monocytogenes,* and the addition of an aminoglycoside has been shown to synergistically enhance killing.[110] However, there are mixed data with regard to benefit of aminoglycosides in both animal and clinical studies given their toxicity. A number of retrospective studies did not show improved outcomes with aminoglycoside synergy, and one showed higher mortality with aminoglycoside use.[116–118] Other large studies including a more recent prospective study showed statistically significant increased survival.[4,91] Consequently, both US and European guidelines for the treatment of *L. monocytogenes* meningitis recommend considering the addition of an aminoglycoside rather than a formal recommendation for combination therapy.[119,120]

Local infections such as cutaneous and febrile gastroenteritis, which occur mainly in immunocompetent hosts, are often self-limited and resolve without antibiotics.[63,121] Pregnant women with febrile gastroenteritis and presumptive exposure to *L. monocytogenes* should be treated with ampicillin or amoxicillin.[122,123] Otherwise, antibiotics may be considered in protracted cases or cases with underlying risk factors for severe infection.

The optimal duration of treatment for invasive listeriosis is unknown. Patients with pregnancy-associated listeriosis and bacteremia should receive at least 2 weeks of antimicrobial treatment.[124] Trimethoprim-sulfamethoxazole should be avoided in women in the first trimester of pregnancy, as it can cause neural tube and cardiovascular defects.[123]

In neonates, individuals with predisposing risk factors, and adults older than 50 years of age, empirical treatment for bacterial meningitis should include an anti-*Listeria* agent.[119,120] *Listeria* meningitis should be treated for at least 21 days.[119,120] Treatment of focal infections such as brain abscess may require treatment for 6 to 8 weeks.[69,91] Adjunctive treatment for bacterial meningitis with steroids, which have been shown to improve mortality in cases of *S. pneumoniae* meningitis,[125] should be stopped once *L. monocytogenes* is identified as the causative pathogen because steroid therapy is associated with increased mortality in this subset of patients.[4]

PREVENTION

Pneumocystis prophylaxis with trimethoprim-sulfamethoxazole likely also protects against listeriosis, but the incidence of listeriosis in the same populations is too low to evaluate efficacy. Prevention of listeriosis requires a multipronged approach including regulation and testing at the food-processing level as well as education of high-risk populations on safe food consumption and handling practices.

Since 1987, the US Department of Agriculture Food Safety and Inspection Service has implemented on-site testing for *L. monocytogenes* at meat and poultry plants and random sampling programs and prohibited

TABLE 206.1 Dietary Recommendations for Preventing Foodborne Listeriosis

General Recommendations
Washing and Handling Food

- Rinse raw produce thoroughly under running tap water before eating, cutting, or cooking. Even if produce will be peeled, it should still be washed first.
- Scrub firm produce, such as melons and cucumbers, with a clean produce brush.
- Dry produce with a clean cloth or paper towel.

Keep the Kitchen Cleaner and Safer

- Wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods.
- Be aware that *Listeria monocytogenes* can grow in foods in the refrigerator. The refrigerator should be 40°F or lower, and the freezer should be 0°F or lower.
- Clean up all spills in the refrigerator right away, especially juices from hot dog and lunchmeat packages, raw meat, and raw poultry.

Cook Meat and Poultry Thoroughly

- Thoroughly cook raw food from animal sources such as beef, pork, or poultry to a safe internal temperature.
- Use precooked or ready-to-eat food as soon as you can. Do not store the product in the refrigerator beyond the use-by date.
- Use leftovers within 3–4 days.

Choose Safer Foods

- Do not drink raw (unpasteurized) milk, and do not eat foods that have unpasteurized milk in them.

Recommendations for Persons at Higher Risk[a]
Meats

- Do not eat hot dogs, luncheon meats, cold cuts, other delicatessen meats (e.g., bologna), or fermented or dry sausages unless they are heated to an internal temperature of 165°F or until steaming hot just before serving.
- Avoid getting fluid from hot dog and lunchmeat packages on other foods, utensils, and food preparation surfaces, and wash hands after handling hot dogs, luncheon meats, and delicatessen meats.
- Do not eat refrigerated pâté or meat spreads from a delicatessen or meat counter or from the refrigerated section of a store. Foods that do not need refrigeration, such as canned or shelf-stable pâté and meat spreads, are safe to eat. Refrigerate after opening.

Cheeses

- Do not eat soft cheese such as feta, queso blanco, queso fresco, brie, Camembert, blue-veined, or panela (queso panela) unless it is labeled as "*Made With Pasteurized Milk*."

Seafood

- Do not eat refrigerated smoked seafood unless it is contained in a cooked dish, such as a casserole, or unless it is a canned or shelf-stable product.
- Canned and shelf-stable tuna, salmon, and other fish products are safe to eat.

Melons

- Wash hands with warm water and soap for at least 20 seconds before and after handling any whole melon.
- Scrub surface of melons with a clean produce brush under running water and dry them with a clean cloth or paper towel before cutting. Be sure that your scrub brush is sanitized after each use.
- Promptly consume cut melon or refrigerate promptly. Keep cut melon refrigerated for no more than 7 days.
- Discard cut melons left at room temperature for more than 4 hours.

Raw Sprouts

- Do not eat any kind of raw or lightly cooked sprouts and ask that raw or lightly cooked sprouts not be added to food when eating out.
- Cooking sprouts thoroughly can reduce the risk of getting sick.

[a]Recommendations for persons at higher risk such as pregnant women, persons with weakened immune systems, and older adults are in addition to the recommendations listed under General Recommendations.
Modified from Lorber B. Listeria monocytogenes. In: Bennett JE, Dolin R, Blaser MJ, eds. Principles and Practice of Infectious Diseases. *8th ed. Philadelphia: Saunders; 2015.*

the selling of contaminated foods.[9,16] These policies, guidelines, and regulations, along with better treatments for human immunodeficiency virus and education of high-risk groups, have contributed to a decrease in incidence of sporadic cases of listeriosis as well as a decrease in mortality annually in the United States of 10.74% for the years 1990–96 to 4.29% for the years 1996–2005.[16] The development of surveillance and reporting systems in the United States in the late 1990s and early 2000s has improved and expedited detection of outbreaks resulting in smaller and shorter outbreaks.[9] Additionally, these systems have resulted in a decrease in incidence overall since implementation, although for the years 2001–09 the number of cases of invasive disease plateaued with the exception of a peak in 2011 that was due to a large cantaloupe-associated outbreak.[9,13,17,126] In Europe a decrease in incidence was seen for the years 1999–2005, but overall incidence increased in 2006 due to an increase in sporadic cases of bacteremia in individuals older than 60 years of age and individuals younger than 60 with underlying risk factors such as leukemia.[18]

High-risk populations often report consumption of foods associated with listeriosis and should be educated on the risks of listeriosis and advised to avoid foods implicated in previous outbreaks.[17] Recognition and education of subset populations at even higher risk such as pregnant Hispanic women in the United States should be prioritized.[9,17] Recommendations for safer food handling practices at home are summarized in Table 206.1.

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207 *Bacillus anthracis* (Anthrax)

*Gregory J. Martin and Arthur M. Friedlander**

SHORT VIEW SUMMARY

Epidemiology and Microbiology

- Sporadic worldwide, anthrax is most common in Africa, the Middle East, India, Southeast Asia, and Latin America.
- Naturally acquired human cases are usually associated with animal products.
- Spore-forming gram-positive bacillus grows readily in the laboratory.
- When protected from ultraviolet light, spores remain viable for decades or longer.
- Pathogenicity is associated with edema and lethal toxins and a capsule.

Clinical Manifestations and Diagnosis

- *Cutaneous anthrax:* accounts for 95% of naturally acquired cases. After skin inoculation, a pruritic papule forms in 2 to 5 days. Vesicles rupture, leading to formation of a black eschar at the base of a shallow ulcer. An injectional form has more recently been described in people who use injection heroin and is associated with a more aggressive course. Surgical débridement may be required. Gram stain of vesicle fluid, scraping of base of ulcer, or punch biopsy may show gram-positive bacilli and a paucity of polymorphonuclear neutrophils. Culture of material is frequently positive. Direct fluorescent antibody (DFA) test and polymerase chain reaction (PCR) assay also may be used.
- *Inhalational anthrax:* results from handling of animal products such as wool, hides, or bones or after intentional spore release in bioterrorism. This form has the most dangerous presentation, with near 100% mortality without early antibiotics. It is primarily a mediastinal (not an airspace) process. Blood and pleural fluid cultures are positive. Pleural fluid Gram stain may be positive. DFA test or PCR assay may give the most rapid results.
- *Gastrointestinal anthrax:* responsible for approximately 1% of human cases, typically occurring 1 to 5 days after ingestion of contaminated meat. Blood, stool, and ascites all should be obtained for culture, DFA test, and PCR assay. Gram staining of ascitic fluid may reveal gram-positive bacilli.
- *Anthrax meningitis:* secondary seeding of the meninges occurs during bacteremia in fulminant disease. Death occurs within 24 hours in 75% of cases. Cerebrospinal fluid reveals gram-positive bacilli, and cultures are positive. DFA test and PCR assay may provide the most rapid diagnosis.

Therapy

- Rapid initiation of antibiotics for all stages is crucial (see Table 207.2).
- For cutaneous anthrax, ciprofloxacin or doxycycline alone is used.
- Cutaneous anthrax with systemic symptoms, inhalational anthrax, gastrointestinal anthrax, injectional anthrax, and anthrax meningitis should be treated with two bactericidal agents, preferably a quinolone such as ciprofloxacin and a β-lactam such as meropenem, combined with a protein synthesis inhibitor such as linezolid, clindamycin, or chloramphenicol (although not a protein synthesis inhibitor, rifampin may be included as an option). Consider central nervous system penetration of antibiotics for treatment of potential meningitis.
- Anthrax antitoxins such as anthrax immune globulin and monoclonal anti–protective antigen antibodies should be considered in conjunction with antibiotics in severe cases and in some spore exposures.

Prevention

- Current vaccines (anthrax vaccine absorbed [AVA] in the United States and anthrax vaccine precipitated in the United Kingdom) are cell-free supernatants containing protective antigen adsorbed to aluminum hydroxide (AVA) or precipitated with aluminum potassium sulfate (anthrax vaccine precipitated).
- Postexposure vaccination with AVA should be administered at 0, 2, and 4 weeks and administered subcutaneously in conjunction with antibiotics.

Anthrax as an Agent of Bioterrorism

- Anthrax is generally considered the most likely agent for bioterrorism via an aerosol route.
- Gram quantities of stable spores are easy to transport and could cause thousands of cases.
- Early identification of the first cases is difficult owing to the presentation with nonspecific flulike symptoms.
- Nasal swabs are used to identify exposure areas, *not* to determine individual exposures.
- Exposed patients should be given antibiotic prophylaxis with 60 days of ciprofloxacin or doxycycline and anthrax immunization at 0, 2, and 4 weeks.
- Exposed patients should be decontaminated with soap and water. Surfaces may be remediated with a number of different chloride-containing compounds including household bleach.
- Anthrax is *not* transmissible from patients after they have been decontaminated, and isolation is *not* required.

Anthrax has never been a cause of the massive loss of life associated with cholera, plague, or smallpox, but it has played a prominent role in the history of infectious diseases. While much of the industrialized world is focused on anthrax as an agent of bioterrorism, anthrax remains a significant cause of animal deaths as well as more limited numbers of human cases in much of the developing world.

References to a disease that likely was anthrax appear in the Bible, and descriptions of inflamed papules from exposure to tainted wool occur in Virgil's writings.[1] Anthrax was the first disease definitively attributed to a bacterium, which was discovered by Robert Koch in 1877 and was used to first demonstrate Koch's postulates. Louis Pasteur established the concept of attenuating a bacterial pathogen by serial passage of *Bacillus anthracis* and used this approach in 1881 to develop an anthrax vaccine shown to be protective in a field trial in domesticated animals.[2] With the initiation of the factory processing of hides and wool in the industrial age, deaths from inhalational anthrax among 19th-century British and German woolsorters and ragpickers introduced the concept of occupational risks for infectious disease and the need to protect workers from these risks.[3,4] In 1979 an accidental release of anthrax spores from a Soviet military microbiology facility in Sverdlovsk, Russia, was responsible for approximately 70 cases of inhalational anthrax

*The opinions and assertions herein are those of the authors and should not be construed as official or representing the views of the Department of State, Bureau of Medical Services, the Department of Defense, or the US government.

that were originally reported to be gastrointestinal anthrax until details were finally published years later.[5] This outbreak and the revelations that Iraq had produced anthrax spores in 1991[6] raised the possibility of anthrax being used as a weapon. This possibility was realized with the dissemination of anthrax spores from letters sent through the US Postal Service in 2001 that led to 22 cases of human anthrax and 5 deaths and made what had been nearly a forgotten disease in Europe and North America the subject of intense public attention and renewed scientific and medical interest. In the years since the US anthrax attacks there have been significant advances in the understanding of the biology of *B. anthracis,* the pathophysiology of the disease manifestations, and improvements in diagnostic and therapeutic options.

EPIDEMIOLOGY

Anthrax is a worldwide disease of domesticated and wild animals that may secondarily infect humans. Estimates of worldwide cases vary widely, but it is estimated by the World Health Organization (WHO) that there are 2000 to 20,000 human cases per year (Fig. 207.1).[7] Although cases occur worldwide, there is little genetic diversity among isolates. Examination of variable number tandem repeats loci identifies six major clones among two branches. Based on identification of variable number tandem repeats in different geographic areas, it appears that southern Africa has the greatest diversity of strains and is believed to be the geographic origin of *B. anthracis.*[8,9] The actual number of anthrax cases worldwide has been difficult to ascertain owing to poor reporting, but anthrax in animals has been reported from 82 nations. It is significantly more common among grazing herbivores in some areas of the Middle East, Africa, and Latin America than in more developed countries. The enormous areas of savanna and large populations of ungulate herbivores in southern Africa provide an ideal environment for the development of anthrax. In 1923 in South Africa, it was estimated that 30,000 to 60,000 animals died of anthrax.[10] In the last decade, isolates of *Bacillus cereus* strains possessing the virulence plasmids of *B. anthracis* and expressing the anthrax toxins and capsule have been obtained from diverse species of animals in tropical rainforests in sub-Saharan Africa and may threaten the survival of chimpanzees in that area.[11] No human cases with these strains have been reported to date.

The largest human outbreak of anthrax occurred in Zimbabwe during the years 1979–85 with approximately 10,000 reported cases and 182 deaths. These cases, almost all of which were cutaneous, were associated with cattle ranching and lapsed veterinary control practices during the civil war that established the country.[12,13] Anthrax remains enzootic in much of sub-Saharan Africa with continued cases in wildlife and livestock and more cases in humans than in most of the rest of the world combined.[14,15]

In most of Europe and North America, human cases are rare, and animal cases are sporadic and uncommon. A single animal death usually is met with an intense veterinary public health response that mandates proper disposal of carcasses; decontamination of fields; and immunization of surviving, potentially exposed animals. A single human case in a nonagricultural, nonrural setting appropriately raises concern for an act of bioterrorism.

The natural cycle of anthrax infection in humans and animals is illustrated in Fig. 207.2.[16] Many animals that die of anthrax have a characteristic terminal hemorrhage from the nose, mouth, and anus that contains large amounts of anthrax bacilli. Animals are then infected when they graze on fields or grain contaminated with spores or through the bites of flies that have fed on infected carcasses. Seasonal variations in anthrax cases have been noted for decades. Heavy spring rains may serve to concentrate spores into low-lying areas, and if this is followed by a hot, dry period, animals grazing on these areas with high spore burdens may become infected. River beds that dry out after flooding and serve as pasture for animals have been repeatedly implicated.[14] Additionally, in periods of drought, animals grazing on dried grasses close to the soil surface have increased oral abrasions from the dry vegetation. These abrasions provide areas for spore entry and germination and a subsequent increase in animal cases of anthrax.[17] In 2016 anthrax reappeared in the remote Yamal Peninsula in Siberia for the first time in 75 years. As global climate change has melted permafrost, the carcasses of reindeer that had been frozen for decades have thawed and infected reindeer herds and local people with deaths of thousands of reindeer and one human.[18] The Siberian experience demonstrates the resilience of anthrax spores in the right environmental settings. Naturally acquired human cases are usually associated with exposure to infected animals or contaminated animal products. Numerous products have been implicated in transmission to humans including wool, hair, bone and bone meal, meat, horns, and hides. The source may not be readily evident because the animal product may have been processed (e.g., shaving brushes, goat-skin drums, wool-based tapestries, and bone meal–based fertilizers).[19,20,21,22] In 2013 an inhalational anthrax case occurred in an American who had traveled through four US states with endemic anthrax. Despite an extensive investigation, it appears his only risk factor was driving through herds of bison and burros a few days before developing symptoms.[23] Transmission from flies has also been documented; biting flies may carry spores or vegetative forms from a carcass to another animal or human, and even nonbiting

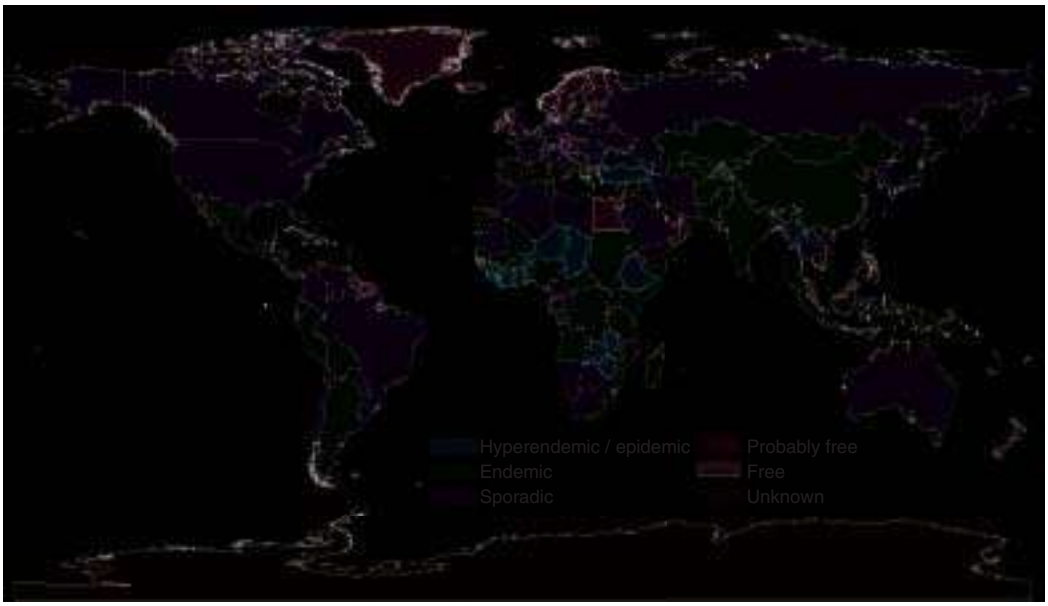

FIG. 207.1 World distribution of anthrax cases as compiled by the World Health Organization. *(From Louisiana State University School of Veterinary Medicine. Welcome to the World Anthrax Data Site.* http://www.vetmed.lsu.edu/whocc/mp_world.htm. *Accessed March 26, 2014.)*

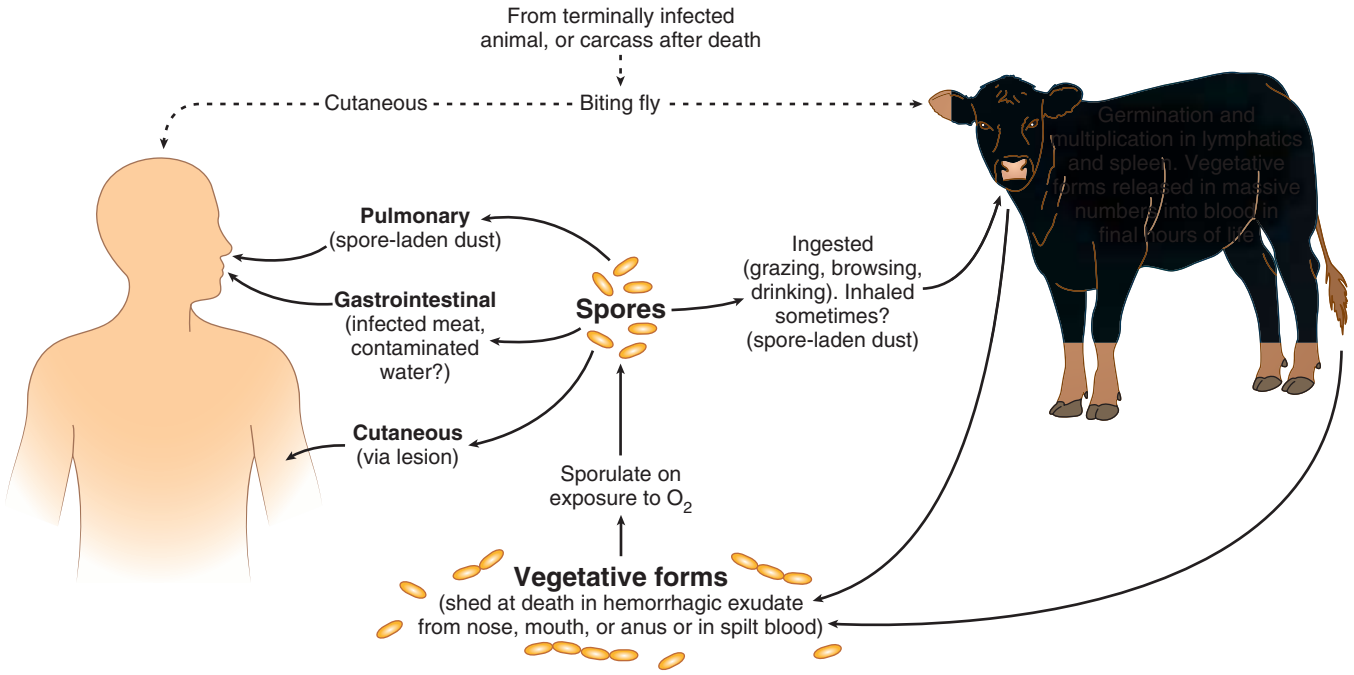

FIG. 207.2 Cycle of infection in anthrax. The spore is central to the cycle, although vegetative forms may also play a role in establishing infection when, for example, humans or carnivores eat meat from an animal that died of anthrax or when biting flies transmit the disease. *(From World Health Organization.* Anthrax in Humans and Animals. *4th ed. Geneva: WHO Press; 2008.)*

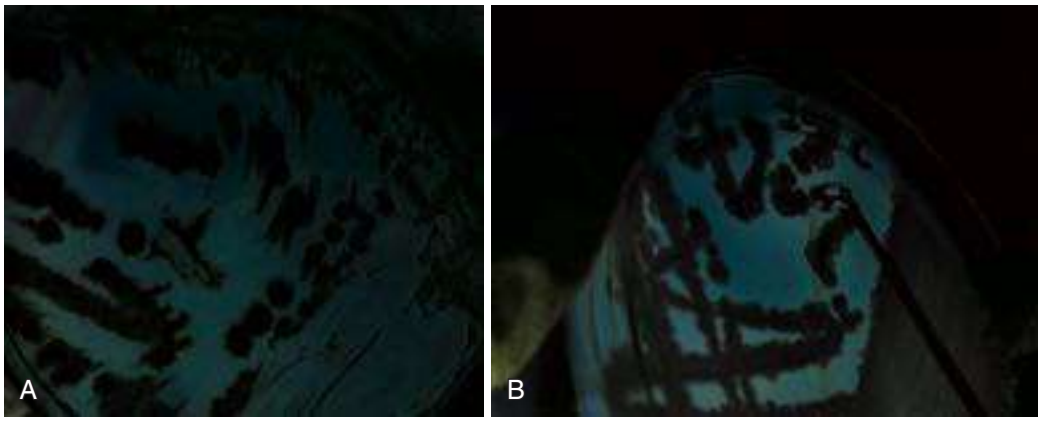

FIG. 207.3 Appearance of *Bacillus anthracis* colonies. (A) Colonies of *B. anthracis* on sheep blood agar demonstrating white-gray colonies and "comet trail" or "Medusa's head" outgrowths from colony margins. (B) "Whipped egg white" appearance of tenacious *B. anthracis* colonies while being removed from sheep blood agar. *(Courtesy Robert Paolucci, National Naval Medical Center, Bethesda, MD.)*

flies have been shown to carry *B. anthracis* in feces or vomit that they deposit onto vegetation. Birds such as vultures shed anthrax spores in their feces for up to 2 weeks after they ingest infected meat.[24] In Europe, people who used injection drugs developed anthrax infections from injecting heroin contaminated with spores possibly acquired from the goat skin containers used in the transport of the drug from Turkey.[25-23]

Spores are the usual infecting form of anthrax. However, ingestion of either spore or vegetative forms of *B. anthracis* in contaminated meat may lead to gastrointestinal infection.

The world distribution of anthrax cases in humans and animals is tracked via a geographic information system and remote sensing by WHO as part of the World Anthrax Data Site. This includes an updated nation-by-nation breakdown of cases by species and year.[7]

MICROBIOLOGY

B. anthracis, the causative agent of anthrax, is a large (1–1.5 μm × 3–8 μm), gram-positive bacillus with rapid, nonhemolytic growth on blood agar that readily forms spores in the presence of oxygen. Colonies have a characteristic "Medusa's head appearance," sometimes also referred to as a "comet tail," appearing slightly curled at the periphery (Fig. 207.3A). The white or gray-white colonies are tenacious when attempts are made to remove them from agar, and this is often described as "a whipped egg white appearance" when a loop is passed through a colony (Fig. 207.3B). In culture the bacilli may form long chains with prominent central or paracentral oval spores that do not cause swelling of the bacilli (Fig. 207.4). In infected tissue, bacteria occur singly or in short chains of two to three bacilli without spores. In the presence of carbon dioxide in the laboratory, or of bicarbonate in tissue, *B. anthracis* forms a prominent poly-D-γ-glutamic acid capsule important in the inhibition of phagocytosis of the vegetative bacilli. Catalase positivity and nonmotility of organisms are further characteristics that differentiate *B. anthracis* from other *Bacillus* spp. These basic identification techniques can typically be performed in nearly all microbiology laboratories, but definitive identification of *B. anthracis* requires further demonstration of lysis by γ phage, detection of the capsule

by fluorescent antibody, and identification of toxin genes by polymerase chain reaction (PCR) assay, usually best performed at a reference laboratory.[26,27]

In contrast to growth during in vitro cultivation, *B. anthracis* sporulation does not occur in viable tissues until they are exposed to atmospheric levels of oxygen, typically after an infected animal has died and the carcass is opened. The spores, although sensitive to prolonged ultraviolet radiation, are extremely hardy and may survive in certain soil conditions for decades. In the interior of buildings, typically shielded from ultraviolet light, spores may also remain viable for years. Although anthrax spores have demonstrated viability in soil and carcasses for decades, and even longer in bones from an archeological site,[28] in most environments where the organism must compete with other soil-dwelling bacteria they typically survive only for months and rarely more than 4 years.

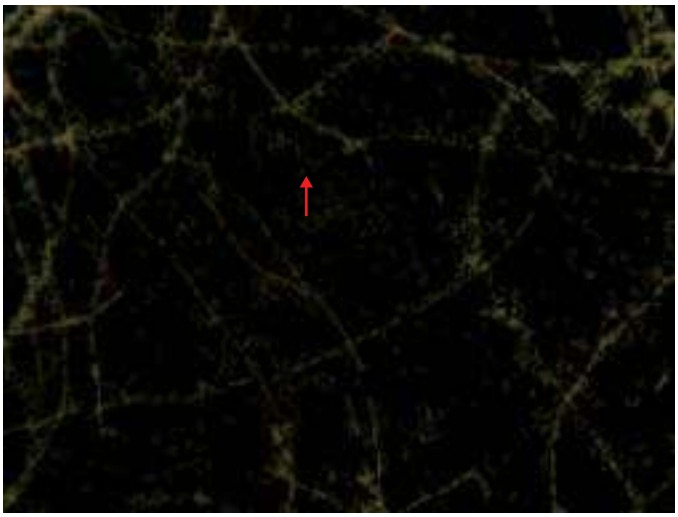

FIG. 207.4 Gram stain of *Bacillus anthracis* demonstrating long chains of bacilli that form when grown in culture. The prominent central or paracentral spores do not stain with Gram staining and appear as clear areas in many of the bacilli in chains (example shown at *red arrow*). *(Courtesy Robert Paolucci, National Naval Medical Center, Bethesda, MD.)*

Spores may also remain dormant, but viable, in living animals for a period of at least 100 days, as demonstrated by primate studies in which viable organisms were recovered at necropsy from the lungs of apparently healthy animals,[29–31] a finding that has important therapeutic implications, as discussed later.

The two major virulence factors are the antiphagocytic poly-D-γ-glutamic acid capsule, encoded on the pX02 plasmid, and two exotoxins, encoded on a separate plasmid, pX01. The anthrax toxins have been intensively studied, and components of the toxins have important use in vaccines, in diagnostics, and as targets for new adjunctive therapeutics. A schematic representation of the main anthrax toxins in shown in Fig. 207.5.[32]

The pX01 plasmid encodes three toxin components, known as protective antigen (PA), edema factor (EF), and lethal factor (LF), each of which individually is biologically inactive. Studies in the 1950s and 1960s established that edema toxin, composed of PA combined with EF, produced local skin edema and that lethal toxin, composed of the same PA combined with LF, was highly lethal for experimental animals. The combination of all three components was the most lethal and produced many characteristics of an actual anthrax infection.[33]

PA, which was originally identified as an antigen that induced protective immunity in experimental animal models, attaches to the anthrax toxin receptors—tumor endothelial marker 8 (TEM8) and capillary morphogenesis protein 2 (CMG2)—on the cell surface and is cleaved by a cell surface protease into PA_{63}, which forms a heptamer to which up to three EF and/or LF molecules may attach.[34] CMG2 appears to be the major toxin receptor in the mouse model in which neutrophils contribute to resistance to infection.[35] PA may also be cleaved by a serum protease with formation of toxins in the circulation. When the PA heptamer complexes with EF, it forms edema toxin, and with LF it forms lethal toxin. The toxins are then taken into the cytosol, where they mediate cellular damage. LF is a calmodulin-dependent zinc metalloprotease that cleaves and inactivates multiple mitogen-activated protein kinases and interferes with signal transduction, whereas EF is an adenylate cyclase that increases intracellular cyclic adenosine monophosphate concentrations and interferes with cell function. The toxins have been shown in vitro to impair cell functions associated with innate immunity including neutrophil chemotaxis; phagocytosis; superoxide production; and macrophage, T and B lymphocyte, and dendritic cell function[36] and likely affect many other cells possessing toxin receptors including endothelial cells resulting in increased vascular

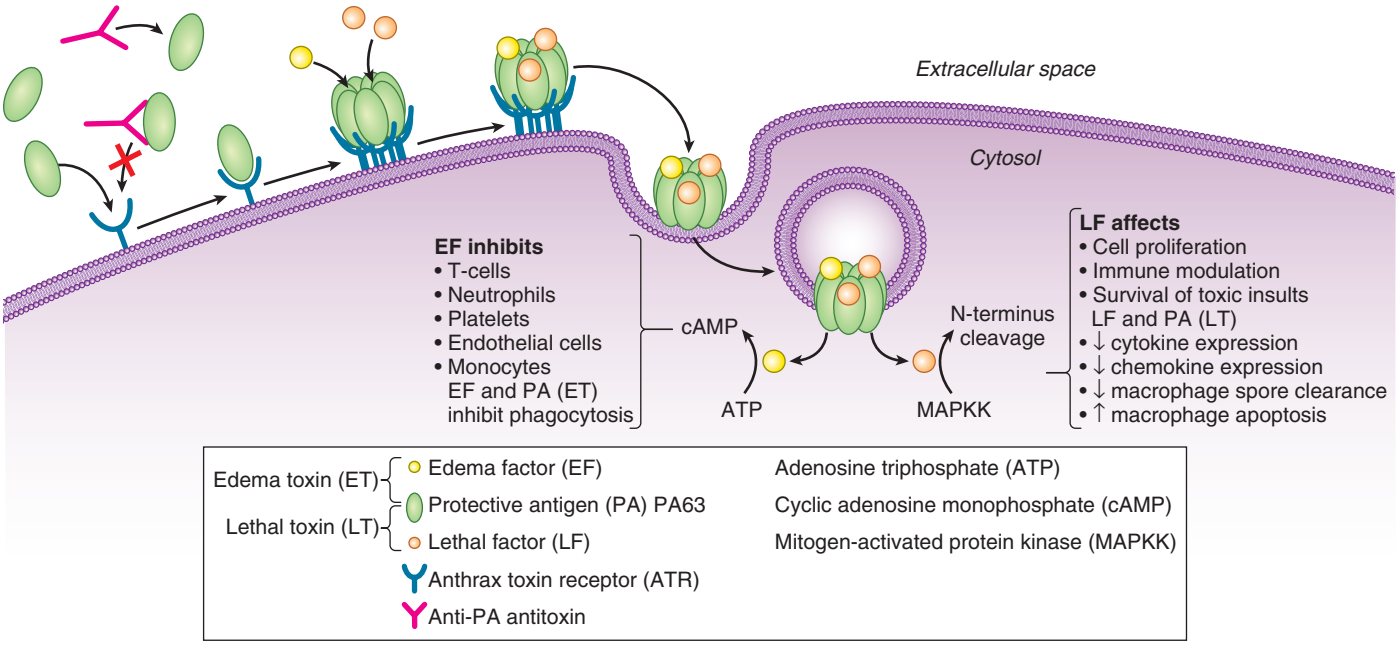

FIG. 207.5 Formation and activity of main anthrax toxins. *(From Bower W, Hendricks K, Pillai S, et al. Clinical framework and medical countermeasure use during an anthrax mass-casualty incident.* MMWR Morb Mortal Wkly Rep. *2015;64:1–23.)*

permeability[37] and cells of the cardiovascular system and liver.[35] Studies with isolated toxins suggest they both cause hypotension in experimental animals and are additive, but shock has not been a prominent finding in patients presenting with inhalational anthrax. Edema toxin was so named because of its ability to cause edema in experimental animals, and lethal toxin has also been shown to increase vascular permeability. Lethal toxin demonstrates effects on the intestinal epithelium that may allow for secondary infections with enteric pathogens.[38] However, there are discrepancies between effects of toxins observed in cells in vitro and in vivo and findings observed in experimental infections. The cell targets and exact role and mechanisms of toxin-induced host dysfunction during infection remain under investigation, and numerous other bacterial factors contribute to virulence, although they are of less importance than the capsule and toxins.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

The clinical manifestations of anthrax in animals and humans have been well described since the 1800s, when cases were relatively common in many areas of the world. The three primary forms of anthrax are dependent on the route of exposure: cutaneous, gastrointestinal, and inhalational. In the past decade an additional form of cutaneous anthrax has been described among people who inject heroin. Heroin contaminated by *B. anthracis* spores may cause severe systemic infection similar to that seen in advanced inhalational anthrax.[39,40] Bacteremia secondary to any of the primary forms of anthrax may lead to seeding of any site including the central nervous system (CNS), with the resulting hemorrhagic meningoencephalitis being nearly always fatal.

Online resources for clinicians considering a diagnosis of anthrax are readily available. Frequently updated and extensively referenced anthrax websites for both naturally occurring and bioterrorism-associated disease are provided by the Centers for Disease Control and Prevention (CDC) (www.cdc.gov/anthrax/), the European Centre for Disease Prevention and Control (https://ecdc.europa.eu/en/anthrax), and the Center for Infectious Disease Research and Policy (http://www.cidrap.umn.edu/infectious-disease-topics/anthrax). Additional resources are provided by WHO at www.who.int/csr/disease/Anthrax/resources/en/. The WHO site features a comprehensive handbook of WHO guidelines for anthrax in animals and humans that includes information on handling of carcasses, disinfection, and control of infections.[16] Laboratory guidelines[26] and guidelines for shipping and handling of clinical specimens[41] are continually revised by the American Society for Microbiology and are available at https://www.asm.org/index.php/guidelines/sentinel-guidelines.

Cutaneous Anthrax

Naturally occurring anthrax infections in humans cause cutaneous disease in more than 95% of cases. Series of cutaneous anthrax cases from the 19th century and early 20th century that were untreated demonstrated 16% to 39% mortality. As anthrax immune serum was used for treatment in cutaneous case series in the years 1903–41, mortality decreased to 0% to 28%, although no controlled studies were reported. With penicillin treatment available, mortality rates have generally decreased to less than 5%.[42] In a series from 1955, Gold[43] carefully reported the findings of 117 cases of anthrax he observed near a Philadelphia goat-hair mill over 20 years. All but one case was cutaneous anthrax, and the only fatalities were the single pulmonary case and one cutaneous case. The Chinese Center for Disease Control and Prevention reported 120,111 human cases of anthrax for the years 1955–2014 with 4341 fatalities (3.6%). Of 258 confirmed Chinese anthrax cases for the years 2005–14, 98% were cutaneous anthrax, and only 2% of all anthrax cases were fatal.[44]

As described earlier, a multitude of contaminated animal sources have been implicated in naturally acquired cutaneous anthrax in humans. After the introduction of anthrax spores into the skin, often with just trivial trauma, there is an incubation period of 1 to 19 days (more commonly 2–5 days), leading to the development of a small, pruritic papule at the inoculation site.[45] Most lesions are on exposed areas of the head, neck, and extremities. Owing to the associated pruritus, patients (and clinicians) often attribute these painless lesions to an insect or spider bite and ignore them.

A day or two after the formation of the papule, vesicles form around the lesion and may become 1 to 2 cm in diameter. The vesicles contain a clear to serosanguineous fluid, and Gram stain reveals numerous bacilli but a paucity of leukocytes. Culture of vesicular fluid will readily yield *B. anthracis* in most cases in which antibiotics have not been administered. There is no purulence, and the lesions remain painless unless secondarily infected. The vesicles are thin roofed and easily rupture, leading to formation of a dark brown eschar that turns black at the base of a shallow ulcer. The ulcer is typically surrounded by an area of induration, and in some cases nonpitting edema may be marked (Fig. 207.6).[46] Most other organisms causing skin infections are not typically associated with extensive induration around a skin lesion or with frank edema, so these findings may be the first clue to the diagnosis of anthrax. As the ulcer matures, its base becomes characteristically black and is the source of the name anthrax, which derives from the Greek word *anthrakis,* meaning "coal."

In uncomplicated cases (i.e., without secondary spread), lesions slowly heal over a period of 1 to 3 weeks, and the eschar loosens and falls off, typically without leaving a scar. Antibiotics do not affect the evolution of the skin lesions. In most cases, patients report associated headache, malaise, and low-grade fever even if the infection does not progress to bacteremia.

Multiple lesions may occur in some cases, probably representing multiple inoculation sites, but at other times small satellite lesions may appear around an initial isolated lesion. Serious cutaneous disease may be marked by extensive edema that involves an entire extremity or the trunk from neck to groin. This has been described as "malignant edema" and may be associated with inflammation of the overlying skin with pain, signs of toxemia, and subsequent secondary seeding of other sites as bacteremia develops; cutaneous anthrax with severe edema has been more commonly seen in injectional anthrax (see later discussion).

Untreated cutaneous disease in humans has been associated with a fatality rate of 10% to 20%, whereas treated cutaneous disease (before the onset of secondary bacteremic spread) is rarely fatal. Repeat infections are rarely reported and tend to be milder, implying some degree of acquired immunity.[43,45]

Clinical characteristics that should place cutaneous anthrax high in the differential diagnosis include a painless lesion (during initial stages), the presence of edema out of proportion to the size of the lesion, and a Gram stain of vesicular fluid or ulcer swab with gram-positive rods but rare white blood cells.

Differential Diagnosis of Cutaneous Anthrax

The differential diagnosis of the unusual skin lesions associated with cutaneous anthrax includes some diagnoses uncommonly encountered by most clinicians. Brown recluse spider bites, which may also cause a black eschar and some associated edema, may be confused with cutaneous anthrax lesions. The key difference is the significant pain associated with a recluse spider bite and the absence of pain in anthrax lesions (although there may be tender adenopathy in association with an anthrax skin lesion). The differential diagnosis of cutaneous anthrax also includes tularemia, scrub typhus, rat-bite fever, blastomycosis, cutaneous fungus acquired from animals, and mycobacterial infection with *Mycobacterium marinum.*

Laboratory Diagnosis of Cutaneous Anthrax

Diagnostic procedures for cutaneous anthrax should preferably be performed before initiation of antibiotics because vesicular fluid and biopsy material are quickly rendered noninfectious after initiation of antibiotics. Appropriate samples for Gram stain and culture include vesicle fluid, either in a syringe or on a swab; a specimen from swabbing the edge of the base of an eschar; and material from a full-thickness punch biopsy of the edge of a vesicle and/or the center of an eschar (Table 207.1). A *Bacillus* species in a culture specimen can be confirmed as *B. anthracis* by demonstration of bacterial lysis in the presence of the γ phage, detection of the capsule by direct fluorescent antibody (DFA), and identification of toxin genes by PCR assay as described later (all generally available in reference laboratories).[26]

Since the anthrax attacks in the United States in 2001, a two-component DFA assay that uses fluorescein-labeled monoclonal

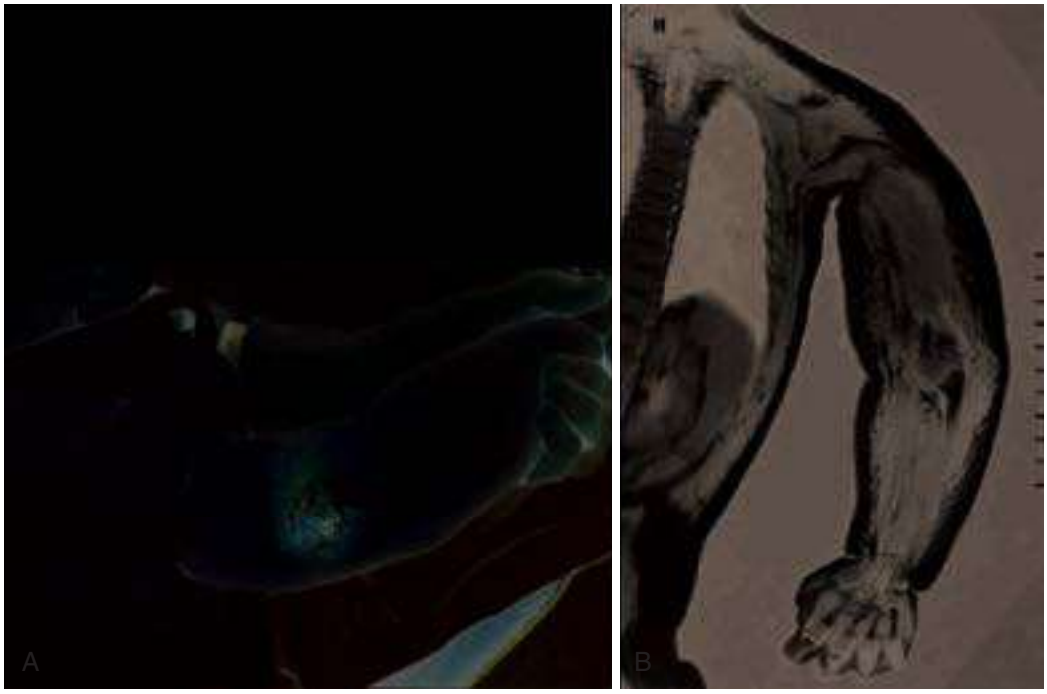

FIG. 207.6 Clinical and magnetic resonance imaging appearance of cutaneous anthrax. (A) Cutaneous anthrax with extensive nontender swelling and erythema in a 7-month-old child in New York in 2001. (B) Magnetic resonance imaging demonstrates extensive subcutaneous edema from shoulder to hand. *(From Roche KJ, Chang MW, Lazarus H. Cutaneous anthrax infection: images in clinical medicine.* N Engl J Med. *2001;345:1611. Copyright © 2001 Massachusetts Medical Society. All rights reserved.)*

antibodies (MAbs) specific to the *B. anthracis* cell wall and capsule has been developed.[47,48] Rapid PCR assays are also now available.[49,50] Testing may be obtained through the CDC Laboratory Response Network (local Sentinel laboratories, city or state Reference laboratories, or CDC and military National laboratories) and some hazardous material teams. DFA and real-time PCR can be used for rapid and definitive identification of culture isolates and for presumptive identification of *B. anthracis* directly from clinical specimens and, in some cases, environmental samples. Caution must be used in interpreting these results because false-positive and false-negative findings may occur. In even the most experienced laboratories, cross-contamination is always a risk with PCR, and false-positive results can lead to considerable confusion.[51]

Because blood cultures are frequently positive in cases that have progressed to sepsis, consideration should be given to obtaining blood cultures early in the evaluation, especially if there are any systemic symptoms. Automated blood culture systems commonly used in hospitals readily support growth of *B. anthracis*.

Culture of *B. anthracis* remains the gold standard for diagnosis of anthrax infections. Table 207.1 outlines diagnostic specimen preparation, handling, and testing. Despite development of molecular diagnostics for anthrax, there is still a role for serology. Three of the cutaneous cases in 2001 had no culture or PCR evidence of disease, but acute and convalescent serology demonstrated an anti-PA antibody response that confirmed the diagnosis. Acute and convalescent serum samples should be obtained for serology at 0 to 7 days of illness and at 14 to 28 days. A rapid enzyme-linked immunosorbent assay (ELISA) that measures total antibody to PA has been approved. A number of anthrax-PA ELISA kits have been approved by the US Food and Drug Administration (FDA) and can be used on serum to diagnose all types of anthrax or to demonstrate seroconversion after immunization. Retrospectively, anthrax PA antibody was detected by ELISA in 100% of both cutaneous and inhalation cases from 2001. However, ELISA is not positive early in disease; PA antibody is not detected until approximately 1 week after symptoms begin.[27,52]

Anthrax diagnostics for both environmental and clinical samples continue to be developed. A number of lateral flow devices (handheld assays) designed for environmental samples are available. Although easy to perform and more rapid than other diagnostics, they are typically not as sensitive or specific as more traditional methods and should not be used on clinical specimens.

Injectional Anthrax

Injectional anthrax is an uncommon form of cutaneous anthrax that has been described in people who use injection drugs who introduced contaminated heroin either into the skin or intravenously. Both *Clostridium* and *Bacillus* spp. (usually *B. cereus*) are spore-forming organisms that have been previously associated with infections in people who use injection drugs. In 2009 a few sporadic injectional anthrax cases occurred in Europe; in 2009–10 there were 119 cases among people who injected heroin in the United Kingdom, mainly in Scotland. Injectional anthrax is difficult to diagnose because skin infections are common among people who use injection drugs, but the clinical presentations of injectional anthrax cases were atypical. Skin lesions were not typical cutaneous anthrax lesions; rather, patients presented with advanced localized soft tissue infections accompanied by disproportionate edema, often with less pain than is typically associated with other serious soft tissue infections such as necrotizing fasciitis. Fever was not a prominent feature. Some patients had no localized injection-related lesions but presented with features of systemic anthrax infection; deteriorated rapidly; and died with meningitis, sepsis, and multiorgan failure. Patients were treated with conventional antibiotics, and some required extensive surgical débridement for necrosis associated with deep infections and subsequent reconstructive surgery; this is in contrast to typical cutaneous anthrax, for which surgical débridement is not recommended. Fourteen of the patients also received intravenous (IV) therapy with anthrax immune globulin (AIG).[25,53]

Inhalational or Pulmonary Anthrax

Even a single case of inhalational anthrax should raise the possibility of a deliberate spread of spores because naturally occurring inhalational disease is currently extraordinarily rare. Inhalational anthrax is an exceptionally dangerous type of *B. anthracis* infection that in the preantibiotic era was nearly uniformly fatal. In a review of 82 reported cases of inhalational anthrax in the years 1900–2005, there was an

TABLE 207.1 Collection and Transport of Laboratory Specimens for Diagnosis of Anthrax

TYPE OF ILLNESS	SPECIMEN COLLECTION AND TRANSPORT	COMMENTS
Cutaneous anthrax	*All stages:* Collect two swabs, one for Gram stain and culture and one for PCR assay. *Vesicular stage:* Perform Gram stain, culture, and PCR assay of fluids from unroofed vesicle (soak two dry sterile swabs in vesicular fluid). *Note:* Gram stain is most sensitive during vesicular stage. *Eschar stage:* Perform Gram stain, culture, and PCR assay of ulcer base or edge of eschar without removing it. *Ulcer (no vesicle or eschar present):* Swab base of ulcer with premoistened sterile saline. A punch biopsy for IHC testing and a second biopsy for culture, Gram stain, PCR assay, and frozen tissue IHC if patient has not received antibiotics should be obtained on all patients with suspected cutaneous anthrax. Include skin adjacent to papule or vesicle. If vesicles and eschars are both present, separate biopsy specimens should be obtained. *Serum:* Collect acute serum within first 7 days of symptom onset and convalescent serum 14–35 days after symptom onset. Collect blood for culture and PCR assay and serum for LF detection with evidence of systemic involvement.	*Swabs:* Moisten with sterile saline or water; transport in sterile tube at 2°C–8°C. Transport swabs for PCR assay only at –70°C. Do not use transport medium. *Tissue, fresh:* ≥5 mm^3; store and transport at 2°C–8°C (≤2 h) or frozen at –70°C (>2 h). *Tissue, preserved in 10% buffered formalin:* 1.0 cm^3; store and transport at room temperature. Obtain biopsy specimen of lesions for histopathology, preserved in 10% buffered formalin: 0.3 mm diameter; store and transport at room temperature. Freeze serum after separation at –20°C or colder, ship on dry ice. Ship part of sample (>1.0 mL) and retain part in case of shipping problems. Obtain blood for culture per local protocol. Collect blood for PCR assay in EDTA (purple top) tube. Ship at room temperature (≤2 h transport) or 2°C–8°C (>2 h transport). Assay for serum LF toxin and presence of capsule available at CDC.
Inhalational anthrax	If sputum is being produced, collect sputum specimen for Gram stain and culture (*note*: inhalational anthrax does not usually result in sputum production). Obtain blood for smear, culture, and PCR assay and serum for LF detection. If a pleural effusion is present, collect a specimen for culture, Gram stain, PCR assay, and LF detection. Collect CSF if meningeal signs are present or meningitis is suspected for culture, Gram stain, PCR assay, and LF detection. *Serum:* Collect acute serum within first 7 days of symptom onset and convalescent serum 14–35 days after symptom onset. *Biopsy material:* Bronchial or pleural biopsy material can be evaluated if available.	*Sputum:* Transport at room temperature in sterile, screw-capped container (<1 h transport time) or at 2°C–8°C (>1 h transport time). *Blood cultures:* Obtain appropriate blood volume, number, and timing of sets per laboratory protocol; transport at room temperature. *Blood for PCR assay:* 10 mL in EDTA (for pediatric patients collect volumes allowable). Transport directly to laboratory at room temperature (2°C–8°C if transport ≥2 h). *Pleural fluid:* Collect >1 mL in sterile container. Store and transport at 2°C–8°C. *CSF:* Transport directly to laboratory at room temperature, or 2°C–8°C if transport ≥2 h. Transport serum or citrated plasma (separated and removed from clot) at 2°C–8°C (transport <2 h) or freeze at –20°C or colder (transport ≥2 h); ship on dry ice. Ship part of sample (>1.0 mL) and retain part in case of shipping problems. Preserve biopsy specimens in 10% buffered formalin, and transport at room temperature.
Gastrointestinal anthrax	Obtain stool specimen for culture (>5 g). Obtain rectal swab from patients unable to produce stool (insert swab 1 inch beyond anal sphincter). Obtain blood for smear and culture (and possibly PCR testing and LF detection). Blood cultures most likely to yield *Bacillus anthracis* if taken 2–8 days postexposure and before administration of antibiotics. If ascites is present, obtain a specimen for Gram stain and culture (and possibly PCR testing and LF detection).	*Stool:* Transport in sterile container unpreserved (≤1 hr transport time) or at 2°–8°C in Cary-Blair medium or equivalent (>1 hr transport time); specimen >5.0 g. *Blood:* Transport at room temperature.
Anthrax meningitis	Obtain CSF specimen for Gram stain, culture, PCR assay, and LF detection. Obtain blood for Gram stain, culture, and PCR assay, and serum for LF detection.	See comments above for collection and transport of blood and CSF for Gram stain, culture, PCR assay, and LF detection.

CDC, Centers for Disease Control and Prevention; *CSF,* cerebrospinal fluid; *EDTA,* ethylenediaminetetraacetic acid; *IHC,* immunohistochemistry; *LF,* lethal factor; *PCR,* polymerase chain reaction.
Modified from Center for Infectious Disease Research and Policy. Anthrax: Clinical Laboratory Testing. http://www.cidrap.umn.edu/infectious-disease-topics/anthrax#overview&1-5.

overall 92% mortality rate despite treatment with anthrax antiserum or antibiotics or both in the majority of cases.[54] During the 2001 anthrax attacks in the United States, 5 of the 11 (45%) patients with inhalational anthrax died despite aggressive intensive care unit (ICU) management and appropriate antibiotics. Early diagnosis, initiation of antibiotics, and aggressive management of inhalational anthrax are crucial to survival.

During the 19th century, inhalational anthrax (woolsorter's disease) occurred among factory workers handling hair, wool, or hides contaminated with anthrax spores, with studies demonstrating that as many as 50% of samples of raw materials were contaminated with spores. In the Bradford district of England, 23 cases of inhalational anthrax were reported during the year 1880. Much of our experience with naturally acquired inhalational anthrax was gained in the preantibiotic era.[55,56] Studies in the 1950s revealed that during an 8-hour period mill workers inhaled hundreds of spores smaller than 5 µm, and some had positive nasal or pharyngeal cultures, and yet inhalational anthrax remained extraordinarily uncommon.[56] In a serologic study of unvaccinated mill employees, nearly 15% had antibodies to anthrax.[57] It is evident that there is some threshold number of spores that can be destroyed through the innate immune response even in the absence of prior immunization.[58,59] As safeguards were built into the process so that wool was decontaminated before handling by workers and ventilation was improved in factories, the number of annual cases in developed countries in the second half of the 19th century decreased significantly; with the addition of vaccines and respirators in the 1950s and 1960s, cases dropped nearly to zero.

In 2005 Lucey[60] proposed a modified three-level staging system for inhalational anthrax characterized by an early prodromal stage leading to the intermediate progressive stage followed by the late fulminant

stage that has generally become accepted as reflecting the course of both terrorist-associated and recent naturally occurring inhalational anthrax and is used here. As spores are inhaled, those larger than 5 μm are captured in the upper airways and transported out of the airways via the mucociliary elevator to the mouth. Spores in particles smaller than 5 μm may reach the terminal bronchioles and alveoli, where they are quickly phagocytized by alveolar phagocytic cells and transported to draining lymph nodes and then to mediastinal lymph nodes. More recent studies have suggested that spores may be transported to lymphatics through alveolar epithelial cells more commonly than phagocytes.[61] This early prodromal stage is a clinically silent incubation period and is the presymptomatic stage of inhalational anthrax occurring 1 to 6 days after initial exposure. Although it has been extremely rare to see inhalational cases develop more than 1 week after natural exposure, significant controversy exists about potential incubation periods of 60 days or longer after very-low-dose exposure.[62,63]

The first symptoms occur in the early prodromal stage with a flulike illness characterized by low-grade fever, malaise, fatigue, and myalgias usually without upper respiratory tract symptoms. Headache may be prominent, fatigue may be profound, and blurred vision and photophobia occur in some cases. Dry cough and mild precordial discomfort are also seen in some patients. Patients may experience a biphasic illness during which they feel somewhat improved after the 2 to 3 days of the prodromal illness, whereas others progress directly to the intermediate progressive stage associated with high fever, declining pulmonary status, respiratory distress, dyspnea, marked diaphoresis, pleuritic chest pain, and confusion or syncope. Blood cultures are typically positive in this stage, and mediastinal widening and pleural effusions are noted radiographically. Diagnosis during this stage and treatment with appropriate multiple antibiotics as well as antitoxin therapies (AIG and/or MAbs) coupled with intensive supportive care are associated with survival in most cases.

With or without therapy patients may progress to the late fulminant stage (often referred to in older literature as the fulminant acute phase). These patients have some combination of respiratory failure requiring intubation, sepsis, meningitis, and multiorgan failure associated with overwhelming bacteremia/toxemia. Death frequently occurs within 24 hours. In addition to aggressive antibiotics and intensive care management, these patients should be considered for treatment with antitoxin therapy such as AIG and anthrax MAbs.[59–64]

Inhalational anthrax is a mediastinal process and not a primary airspace disease. Although the majority of inhaled spores are believed to germinate into vegetative organisms while being carried to (or after arrival in) the mediastinal lymph nodes, studies in nonhuman primates have demonstrated that some spores remain dormant for weeks to months.[31] As the vegetative bacilli destroy and burst out of the cell that transported them across the alveoli, they become encapsulated and are released into the systemic circulation, leading to seeding of multiple organs including the meninges. Vegetative bacteria reach high levels in the blood and may be visible on staining of the buffy coat. Levels of the lethal toxin may become high enough terminally that a bacteria-free serum sample may contain enough toxin to kill another animal. The initial signs and symptoms of inhalational anthrax are not very specific, and discriminating between early inhalational anthrax and influenza can be quite difficult, although the characteristic upper respiratory tract symptoms found with influenza are usually absent in anthrax.[54,65]

Chest radiography, or more commonly computed tomography (CT), reveals a widened mediastinum and often bilateral pleural effusions. The progression of inhalational anthrax with chest radiographs and CT from a 2001 bioterrorism case is seen in Fig. 207.7.[66] Before the bioterrorist-associated anthrax cases in 2001, it was generally accepted that pulmonary infiltrates or consolidation were not typically prominent in inhalational anthrax (because it is not a primary parenchymal lung disease), but 7 of 10 inhalational cases in the 2001 attacks were noted to have pulmonary infiltrates. However, it was found that areas of pulmonary infiltrate on chest radiography actually corresponded to pulmonary edema and hyaline membrane formation at necropsy, not pneumonia with bacterial infiltration of the alveoli.[67,68] Pleural effusions are seen in most cases and are typically serosanguineous or hemorrhagic. They may rapidly reaccumulate after thoracentesis, requiring drainage with tube thoracostomy. Adequate pleural fluid drainage is important to achieve because it was associated with a significant survival advantage in the meta-analysis of 82 inhalational cases.[54]

Diagnosis of Inhalational Anthrax

Table 207.1 outlines guidelines for diagnostic specimen preparation, handling, and testing, which are generally identical to guidelines described for cutaneous anthrax earlier. Although inhalational anthrax is typically not associated with a cough productive of sputum, if sputum is produced, it should be sent for Gram stain, culture, and PCR analysis. Pleural fluid is more frequently present and should be sent for diagnostic testing because it is much more likely to yield bacilli on staining, culture, or PCR assay. Much more commonly than in cutaneous anthrax, the diagnosis of inhalational anthrax is made by finding positive blood cultures, and these should be obtained before any antibiotics are administered. Especially in patients who have received antibiotics, blood samples should be sent for PCR assay and antigen detection. Buffy coat smears can also be examined for the presence of bacilli, an ominous sign that the patient has entered the late fulminant stage of anthrax. Immunohistologic studies of tissue specimens for the presence of bacillus cell wall and capsule antigens may be of particular value in treated patients because results may be positive when culture, Gram stain, and PCR are negative.[52]

Gastrointestinal Anthrax

Oropharyngeal or intestinal infections with *B. anthracis* are indicative of gastrointestinal anthrax. This form of anthrax is quite common in the grazing herbivores that are the usual hosts for anthrax infections but is uncommon in humans, responsible for only approximately 1% of cases, almost exclusively in rural areas of the developing world. It is generally believed to be underreported.[69] Similar to inhalational anthrax, gastrointestinal anthrax is more likely to be associated with bacteremia, sepsis, and seeding of other sites and, without antibiotics, is associated with a mortality rate of approximately 40%. Recognition and early treatment are crucial to survival, but because many victims are impoverished inhabitants in remote regions, antibiotics are often delayed until the disease has progressed to later stages.

Most human cases are associated with the ingestion of undercooked meat (or uncooked dried meat) from an animal infected with anthrax, but a case in the United States was associated with a contaminated drum and probably demonstrates that spores deposited in the upper airways frequently are trapped in secretions and are eventually swallowed.[21] This is the only such case reported, and the patient had an associated *Enterobius vermicularis* infection of the small intestine and appendix. Large outbreaks in communities that have shared meat from dead animals have occurred, especially in Africa and Asia. In these settings, gastrointestinal anthrax cases may exceed the number of cutaneous cases.[69,70] Anthrax typically halts milk production in infected cows, but cow still producing have not been demonstrated to shed bacilli or spores in their milk. Furthermore, pasteurization kills vegetative *B. anthracis,* but not spores.[70] There have been no documented cases of natural infection from milk.

Typically symptoms develop 1 to 5 days after exposure for either oropharyngeal or intestinal disease, which may also be present concomitantly. In oropharyngeal anthrax, symptoms and signs of swelling, severe pharyngitis, dysphagia, and odynophagia at the site of inoculation in the mouth or pharynx; fever; and, in some cases, respiratory distress due to marked edema and lymphadenitis develop. An ulcer may be observed in the mouth or pharynx, and in one Turkish series it was localized to the tonsil in five of six patients and to the tongue in the sixth patient.[71] Pseudomembranes often form over the ulcer after the first week, bringing diphtheria into the differential diagnosis. Although significant neck swelling is seen in all oropharyngeal cases, massive facial and neck edema is occasionally seen. A peritonsillar abscess often is considered, but incision never yields purulent drainage. A swab of the base of oropharyngeal lesions typically reveals gram-positive bacilli and yields a positive culture.[72]

Intestinal disease occurs with infection of the stomach or bowel wall. The patient presents with nausea, vomiting, and fever, followed by severe abdominal pain that often manifests as a surgical abdomen.

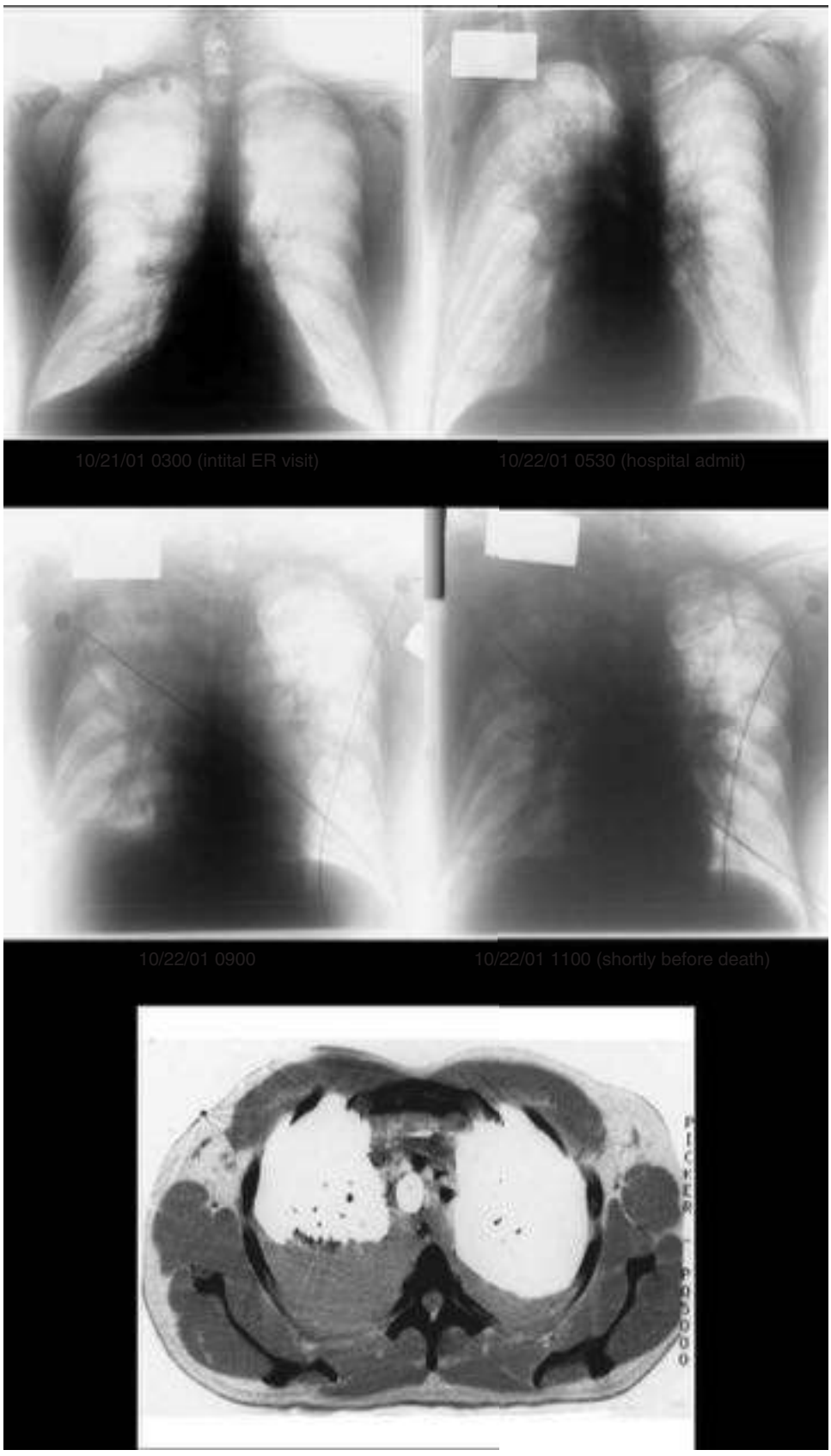

FIG. 207.7 Chest radiographs and computed tomography (CT) scan from a 47-year-old postal worker who had been ill for 5 days when he presented to the hospital with inhalational anthrax. Note progressive bilateral perihilar and infrahilar infiltrates, widened mediastinum, and rapid evolution. CT scan demonstrates mediastinitis and large right and smaller left pleural effusions. *ER,* Emergency room (department). *(Modified from Borio L, Frank D, Mani V, et al. Death due to bioterrorism-related inhalational anthrax.* JAMA. *2001;286:2554–2559.)*

Many cases are associated with hematemesis, massive ascites, and bloody diarrhea. Secondary meningitis is also common.

Table 207.1 outlines guidelines for diagnostic specimen preparation, handling, and testing. Blood, stool, and ascites samples all should be considered for culture and PCR testing.

Anthrax Meningitis

The frequency of anthrax meningitis is difficult to ascertain from published reports. Meningitis is an uncommon sequela of cutaneous anthrax but a frequent complication due to the bacteremia in inhalational or gastrointestinal disease, occurring in up to 50% of cases of the inhalational form. Although meningitis may rarely be the presenting symptom in some anthrax cases, it does not represent the initial site of infection (with the exception of a few case reports) and thus is not considered one of the primary forms of the disease.[73] In a review of reported anthrax cases from 1880–2013, 132 of 363 cases with systemic anthrax met criteria for anthrax meningitis. Severe headache, altered mental status, meningeal signs, and other neurologic signs at presentation independently predicted meningitis. Presence of even one of these factors had an 89% sensitivity for finding meningitis.[74]

As anthrax bacilli are released from macrophages into the bloodstream, the ensuing high-level bacteremia leads to seeding of other sites. The lethal and edema toxins have also been demonstrated to inhibit the innate immune responses of the blood-brain barrier in experimental infection, which may allow for easier access of the vegetative bacilli to the CNS.[75,76] Spread to the CNS may result from a focus of hemorrhagic necrosis that permits bacilli to pass to the meninges, cerebrospinal fluid (CSF), and brain parenchyma. The hallmark of anthrax meningitis is its hemorrhagic component associated with large gram-positive bacilli. CNS involvement may also include parenchymal brain hemorrhage and subarachnoid hemorrhage, possibly owing to a diffuse cerebral arteritis or necrotizing vasculitis. As might be expected from anthrax infections at other sites, cerebral edema may also be prominent.

Symptoms of meningitis usually occur in the presence of fulminant disease and are followed by death within 24 hours in 75% of cases. Initial symptoms include abrupt onset of severe headache, malaise, fever, chills, nausea, and vomiting. Meningeal signs such as nuchal rigidity may be absent early in the course but develop as the patient's condition deteriorates. Seizures, delirium, and coma usually follow within hours. Death was inevitable in the preantibiotic era but is currently estimated at approximately 95% of cases.[77] Early initiation of three or more antibiotics (focused on CNS penetration) has been associated with a greater potential for survival. Table 207.1 outlines guidelines for diagnostic specimen preparation, handling, and testing for anthrax meningitis. Blood and CSF should be obtained for stains, culture, and PCR testing.

THERAPY

Rapid initiation of appropriate antibiotic therapy is crucial in the treatment of anthrax, especially in the more severe noncutaneous cases. Table 207.2 outlines the oral treatment for cutaneous anthrax without evidence of systemic involvement. Table 207.3 outlines initial consideration for treatment of severe anthrax including anthrax meningitis.

Penicillin had been the drug of choice for all types of anthrax since the 1940s, but naturally occurring strains are increasingly reported to be resistant. However, *B. anthracis* is sensitive to a broad range of antibiotics including tetracyclines, macrolides, aminoglycosides, fluoroquinolones, carbapenems, linezolid, clindamycin, rifampin, quinupristin-dalfopristin, daptomycin, and first-generation (but not second- or third-generation) cephalosporins; chloramphenicol has demonstrated variable in vitro effectiveness in some studies.[78,79] In a study of 96 French anthrax isolates collected from the environment, animals, and one human case between 1994 and 2000, there was uniform resistance to third-generation cephalosporins, aztreonam, and trimethoprim-sulfamethoxazole. In 11.5% of the strains, penicillin and amoxicillin resistance and decreased sensitivity to second-generation cephalosporins were demonstrated, but there was nearly 100% sensitivity to the other 16 antibiotics tested.[80] Agents under study for their use in prophylaxis after spore exposure or treatment in clinical disease include oritavancin, cethromycin, daptomycin, and the novel inhibitor of the bacterial stringent response, Relacin, among others.[81–83] Several studies have demonstrated the combination of a bactericidal agent such as a penicillin, quinolones, or carbapenems with a protein synthesis inhibitor to be crucial in improving survival in animal models and the limited number of human cases reviewed.[84] An additional study also showed the combination of a bactericidal agent with a protein synthesis inhibitor was effective against a ciprofloxacin-resistant strain in an animal model.[85]

Most strains of naturally occurring *B. anthracis* have a chromosomally mediated, weak, inducible β-lactamase and cephalosporinase, and there have been rare reports of the development of resistance during therapy with penicillin, especially if subtherapeutic doses may have been administered. However, it is relatively easy to select for antibiotic-resistant strains of *B. anthracis* in the laboratory, and the resistance pattern of bioterrorist strains must be carefully assessed and therapy modified accordingly.[86,87] The guidelines for bioterrorism-associated anthrax recommend use of fluoroquinolones, carbapenems, and doxycycline until resistance testing is available, as β-lactam resistance in such strains is presumed to be likely.[88] Guidelines for treatment of severe anthrax, especially with meningitis, recommend use of three antibiotics—two bactericidal agents and a third protein synthesis inhibitor. All systemic anthrax infections should be treated with combination intravenous antibiotics for at least 2 weeks or until the patient is clinically stable, whichever is longer.[42,89]

TABLE 207.2 Oral Treatment for Cutaneous Anthrax Without Systemic Involvement

For all strains, regardless of penicillin susceptibility or if susceptibility is unknown
- **Ciprofloxacin, 500 mg every 12 hours**
  or
- **Doxycycline, 100 mg every 12 hours**
  or
- **Levofloxacin, 750 mg every 24 hours**
  or
- **Moxifloxacin, 400 mg every 24 hours**
  or
- Clindamycin, 600 mg every 8 hours[b]
  or

Alternatives for penicillin-susceptible strains
- Amoxicillin, 1 g every 8 hours
  or
- Penicillin VK, 500 mg every 6 hours

[a]Preferred drugs are indicated in **boldface**. Alternative drugs are listed in order of preference for treatment for patients who cannot take first-line treatment or if first-line treatment is unavailable. Duration of treatment is 60 days for bioterrorism-related cases and 7–10 days for naturally acquired cases.
[b]Based on in vitro susceptibility data, rather than studies of clinical efficacy.
From Hendricks KA, Wright ME, Shadomy SV, et al; Workgroup on Anthrax Clinical Guidelines. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. Emerg Infect Dis. *2014;20. doi:10.3201/eid2002.130687.*

Cutaneous Anthrax Without Systemic Manifestations

Naturally acquired (i.e., not occurring after an intentional release of spores) cutaneous anthrax with no evidence of systemic symptoms has been traditionally treated with oral penicillin for 7 to 10 days. As penicillin resistance appears in approximately 10% of naturally occurring strains of *B. anthracis* and older recommendations may lead to subtherapeutic dosing, the consensus of authorities convened by the CDC was that ciprofloxacin, doxycycline, levofloxacin, moxifloxacin, and clindamycin were the preferred first-line agents with amoxicillin or penicillin as alternatives once the sensitivity profile was determined (see Table 207.2). The duration of therapy for naturally acquired cases is 7 to 10 days. Unless there is clear evidence that an infection was naturally acquired, it should be considered to result from an intentional release with presumed concomitant inhalation of spores, and 60 days of treatment[42,90] with a fluoroquinolone or doxycycline is recommended as first-line therapy (ciprofloxacin, 500 mg orally twice daily, or levofloxacin, 750 mg daily, or doxycycline, 100 mg orally twice daily). If cultures were obtained from the lesions, modifications of the regimen may be made in response to the resistance profile seen. If systemic symptoms have developed,

TABLE 207.3 Intravenous Triple Therapy for Severe Anthrax With Possible or Confirmed Meningitis

1. A Bactericidal Agent (Fluoroquinolone)
Ciprofloxacin 400 mg q8h
or
Levofloxacin 750 mg q24h
or
Moxifloxacin 400 mg q24h
plus
2. A Bactericidal Agent (β-Lactam)
a. For All Strains, Regardless of Penicillin Susceptibility or if Susceptibility Is Unknown
Meropenem 2 g q8h
or
Imipenem[c] 1 g q6h
or
Doripenem 500 mg q8h
or
b. Alternatives for Penicillin-Susceptible Strains
Penicillin G 4 million units q4h
or
Ampicillin 3 g q6h
plus
3. A Protein Synthesis Inhibitor
Linezolid[d] 600 mg q12h
or
Clindamycin 900 mg q8h
or
Rifampin[e] 600 mg q12h
or
Chloramphenicol[f] 1 g q6–8h
Duration of Therapy
For 2–3 weeks or longer, until clinically stable. Will require prophylaxis to complete an antibiotic course of up to 60 days from onset of illness.

[a]Drug names in **boldface** are preferred agents. Alternative selections are listed in order of preference for therapy for patients who cannot tolerate first-line therapy or if first-line therapy is unavailable.
[b]Severe anthrax includes meningitis; inhalational, injectional, and gastrointestinal; and cutaneous with systemic involvement, extensive edema, or lesions of the head or neck.
[c]Increased risk for seizures associated with imipenem/cilastatin therapy.
[d]Linezolid may exacerbate thrombocytopenia; use for >14 days carries additional hematopoietic toxicity.
[e]Rifampin is not a protein synthesis inhibitor but may be used based on in vitro synergy.
[f]Should be used only if other options are not available, owing to toxicity concerns.
From Hendricks KA, Wright ME, Shadomy SV, et al; Workgroup on Anthrax Clinical Guidelines. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. Emerg Infect Dis. *2014;20. doi:10.3201/eid2002.130687.*

the patient should be treated with IV antibiotics as described for inhalational anthrax (see Table 207.3).

Cutaneous Anthrax (With Systemic Manifestations), Injectional Anthrax, Inhalational Anthrax, Gastrointestinal Anthrax, and Meningeal Anthrax

These frequently lethal forms of anthrax require aggressive management with multiple IV antibiotics, a vasopressor, a ventilator, and ICU support.[42] The use of antitoxin therapies such as AIG (Anthrasil; Cangene Corporation, Winnipeg, Canada) or one of the anti-PA MAbs raxibacumab (ABthrax; GlaxoSmithKline, Philadelphia, PA),[64] obiltoxaximab (Anthim; Elusys Therapeutics, Pine Brook, NJ), and Valortim (MDX-1303; PharmAthene/Medarex/Bristol-Myers Squibb, New York, NY) as well as investigational drugs should be considered in consultation with the CDC and other experts.[91,92] Patients frequently have an acute, rapid deterioration as anthrax infection progresses to the fulminant stages of bacteremia and, when possible, should be closely monitored in the ICU even if they initially appear clinically stable. Meningitis and brain parenchymal infection should be considered in all severe cases of anthrax because hemorrhagic seeding of the CNS occurs in approximately half of cases.[5,54]

Initial therapy should include two bactericidal agents and a protein synthesis inhibitor. If meningitis is suspected or confirmed, agents that have demonstrated adequate CNS penetration should be included in the regimen. CDC guidelines recommend an IV fluoroquinolone such as ciprofloxacin plus a carbapenem such as meropenem as the bactericidal agents. Protein synthesis inhibitors are added with the goal of diminishing bacterial toxin synthesis. Owing to its likely improved CNS penetration, the preferred protein synthesis inhibitor is linezolid, but clindamycin, rifampin, and chloramphenicol are alternative or additional recommended agents. Once antibiotic sensitivities of the *B. anthracis* isolate are known, penicillin may replace the carbapenem in the regimen. These guidelines with recommendations for all preferred and alternative agents are presented in Table 207.3.[42,88,89,90]

Owing to the critical need to use the most effective antimicrobial agents, the usual avoidance of quinolones and tetracyclines in children and pregnancy is superseded. As the resistance profile of the anthrax strain is determined and clinical improvement has been demonstrated, modifications of regimens can be made to diminish possible toxicity. Patients may also be transitioned to oral therapy as improvement occurs. Since the 2001 anthrax attacks, the recommendations for duration of therapy have been for a total of 60 days out of concern for delayed germination of inhaled spores. In most cases, a minimum of 10 to 14 days of IV therapy is required, followed by oral therapy. Data from studies in nonhuman primates with inhalational anthrax demonstrated that after a 10-day course of antibiotic therapy, begun after animals were bacteremic, the surviving animals developed an immune response and were protected against death from the delayed germination of retained spores even after discontinuance of antibiotic therapy.[63]

Management of Pleural Effusion and Ascites

Inhalational anthrax is associated with significant lymphatic obstruction leading to pulmonary edema and rapid accumulation of pleural fluid. Similarly, gastrointestinal anthrax may be associated with massive ascites. The 2001 anthrax cases demonstrated that the pleural fluid had the highest levels of anthrax bacilli as well as bacterial cell wall and capsular antigens.[67] PCR analysis for *B. anthracis* was most often positive from the pleural fluid.[50] After review of the cases of inhalational anthrax in 2001 and 2006 as well as the statistically significant decrease in mortality seen in the series of cases from 1990–2005, the consensus of experts is that early and aggressive management of pleural effusions with repeated thoracentesis or thoracostomy drainage is associated with increased survival.[19,54,67,68] In addition to the improved oxygenation afforded by minimizing loss of lung volume, study of toxin levels in the 2006 case demonstrated that pleural fluid has very high levels of LF.[93] This is essentially subjecting inhalational anthrax to the same standard of care as for an empyema or a complicated parapneumonic effusion. Ascites should also be continually monitored and drained because ascitic fluid can serve as a toxin reservoir, and significant fluid accumulations may further compromise pulmonary function.[94]

Role of Corticosteroids and Management of Severe Edema

The addition of corticosteroids has been advocated for treatment of cerebral edema and increased intracranial pressure in anthrax meningitis, based on its effect in improving outcomes in pneumococcal meningitis, although no controlled studies have been reported for anthrax. In addition, most recommendations for treatment of increased intracranial pressure include the use of hyperventilation and mannitol. Corticosteroid treatment has also been considered for the severe edema often associated with cutaneous, inhalational, and gastrointestinal cases resulting in life-threatening obstruction, massive pleural effusions, and ascites despite the lack of any controlled studies demonstrating efficacy.[95] Animal studies with corticosteroids and elucidation of the pathogenesis of fluid accumulation and meningitis are expected to give more objective evidence to support the use of corticosteroids in human anthrax.

Anthrax Antitoxin Therapies (Immunotherapeutics)

In the preantibiotic era, treatment of anthrax included incision, cautery, and application of acid. In the late 1890s French and Italian researchers developed animal-derived hyperimmune serum that eventually became the standard of care in North America by the 1920s and was used well into the 1950s. Antiserum was used to treat all forms of anthrax and was reported to have decreased the overall mortality rate of cutaneous cases from 24% to 6% in uncontrolled studies.[96] Serum preparations that were used and the quantity and routes of administration were almost arbitrarily determined, but case reports of survival of even bacteremic patients and lack of other effective treatments established antiserum as the treatment of choice. With the development of effective antibiotics, the use of antiserum gradually fell out of favor in most of the world, and it is no longer available in most countries. Anthrax antiserum is still available in some countries of the former Soviet Union and China, although it is seldom used.

Since the 2001 anthrax attacks, because of the high mortality associated with inhalational anthrax and the renewed appreciation of the role of the toxins in pathogenesis, there has been interest in the development of adjunctive therapies including antibodies. AIG is produced from plasmapheresis of individuals recently immunized with the licensed vaccine that consists mainly of PA. AIG was approved by the FDA in 2015 and is produced and marketed as Anthrasil under contract to the US government by Cangene Corporation. AIG was used in conjunction with antibiotics and ICU management for 19 patients: 3 with inhalational anthrax, 15 with injection anthrax, and 1 with gastrointestinal anthrax. All patients appeared to tolerate the antitoxin, and 2 of the patients with inhalational anthrax, 10 of the patients with injectional anthrax, and the 1 patient with gastrointestinal anthrax survived (Bower W, CDC, personal communication).[93,97]

Three human MAbs with high affinity for PA—raxibacumab,[64] obiltoxaximab, and Valortim (MDX-1303)—are currently approved by the FDA and are stockpiled by the US Department of Homeland Security. Other MAbs are also under development. One of the potential advantages of using MAbs, demonstrated in limited animal studies, is that their administration may not interfere with production of natural antibodies to anthrax.[98] Their half-life in humans after a single IV or intramuscular (IM) dose is approximately 4 weeks, with measurable antibodies for up to 2 months. The relative advantages of MAbs versus polyclonal antibodies remain to be determined in clinical studies, and polyclonal antibodies may be more effective for strains with PA resistant to a single MAb. Both are most effective in animal studies when given before or shortly after spore exposure but also have demonstrated efficacy in more advanced disease, especially where initiation of antibiotics may have been delayed.[98,99,100] In animal studies where antibiotics and antitoxin therapy were initiated at the onset of symptoms there was no advantage of combination therapy over antibiotics alone. However, when there was a delay in initiation of antibiotics after symptom onset, there was a survival advantage with combination therapy, but it did not reach statistical significance.[101]

The most experience with anthrax antitoxins in humans occurred with the recognition of injectional anthrax in people who used intravenous drugs in Scotland in 2009–10 where 15 recipients of AIG were compared retrospectively with 28 individuals who did not receive AIG. Death rates did not differ significantly between recipients of AIG and individuals who did not receive it, but the results were confounded due to the AIG recipients being sicker and at higher risk for death.[102]

In three human cases of inhalational anthrax treated with both antibiotics and antitoxin therapy, two patients survived and one died. Experts at the CDC who reviewed the three cases were unable to determine which of the intensive treatment measures was associated with survival. Antitoxin therapy blocks toxin translocation and does not affect toxin that has already entered cells. The patient who died developed respiratory symptoms earlier in the course of illness, and the CDC reviewers hypothesized that there may be a time point in disease progression after which intracellular damage is irreversible.[101] The CDC currently recommends use of anthrax antitoxin therapy in patients with all forms of anthrax who present with systemic symptoms. Although approved by the FDA, none of the antitoxin therapies is readily available without consultation with public health authorities at the CDC, Department of Defense, or state level. Experience in using the different antitoxin therapies is insufficient to recommend one as superior; expert consultation would help determine which product should be used.[42] The role of antitoxin therapy has not yet been defined in postexposure prophylaxis (PEP) but may be useful for strains of unknown antibiotic sensitivity in conjunction with antibiotics and immunization as soon after exposure as possible.[91,100,101,103]

PREVENTION

Vaccines against anthrax for both animals and humans have been used for more than 100 years, and effective use of live-attenuated veterinary vaccines has been associated with the decrease in animal and human cases in many regions of the world. Although there is some anecdotal evidence that immunity develops after infection based on the observation that human anthrax reinfections are rare and less severe, the best data are from studies in nonhuman primates demonstrating resistance to reinfection after recovery from inhalational anthrax.[104] Humoral immunity plays a critical role in the response to anthrax. Anti-PA is the most important antibody, and PA is the protective immunogen and the basis of the protection afforded by the human vaccines currently available. The role of the immune response to the other toxin components, EF and LF, in protection from anthrax infection is controversial, with conflicting studies demonstrating either additional protection or no added protection benefit when used to vaccinate animals despite adequate antibody responses.[105–107]

The currently approved US human vaccine, anthrax vaccine adsorbed (AVA) (BioThrax; Emergent BioSolutions Inc., Gaithersburg, MD), is a cell-free culture supernatant containing PA, derived from an unencapsulated, toxin-producing strain of *B. anthracis* that has been adsorbed to an aluminum hydroxide gel. The vaccine, developed by Wright and coworkers in the early 1950s, was licensed in 1970 and has been used for preexposure prophylaxis by veterinarians; laboratory, textile, and other workers who may be occupationally exposed; and the US military. PEP with AVA was approved by the FDA in 2015 and has been recommended by the US Advisory Committee on Immunization Practices to be used in conjunction with antibiotics for optimal management after exposure to aerosolized spores.[108]

In 2008 the FDA approved a modified five-dose regimen and administration route for preexposure AVA. The change from subcutaneous to IM administration decreases local side effects, and dropping the 2-week dose simplifies the regimen with no significant decrease in immunogenicity at 7 months, although antibody levels were significantly lower from 4 weeks to 6 months.[109] At 0 and 4 weeks, 0.5 mL of AVA is administered by IM injection, followed by subsequent injections at 6, 12, and 18 months as well as yearly boosters. For postexposure vaccination, a three-dose AVA regimen should be administered subcutaneously (in contrast to preexposure IM dosing) on a 0-, 2-, and 4-week schedule.

The vaccine approved in the United Kingdom, anthrax vaccine precipitated, is similar to AVA but is given as a four-dose series at 0, 3, and 6 weeks and 6 months, followed by annual boosters. Mild local side effects are as common with AVA as with other common adult vaccines, as are rare, idiosyncratic, serious side effects.[110] In a US Army study of 601 AVA recipients, 20% reported symptoms that they personally judged as mild enough to be ignored, 15% reported symptoms that affected their activity for a short time but did not limit their work duties, 8% reported short-term symptoms that were relieved with nonprescription medication, and only 2% reported symptoms unrelieved with medication and with short-term limitation of their work duties. Itching, subcutaneous nodules, and erythema were the most commonly reported symptoms, and reported symptoms were more common in women than in men.[111]

The safety of AVA has been the source of considerable controversy in relation to its mandatory use in the US military and objections to its side effects and purported long-term effects. However, the safety and efficacy of AVA were confirmed in an extensive review by the Institute of Medicine of the National Academy of Sciences in 2002[112] as well as additional safety studies since then.[113] No serious adverse events were noted in a review by the CDC of individuals given the vaccine as PEP

after the 2001 anthrax events.[114] As a cell-free filtrate, AVA cannot cause anthrax. Its use has not been associated with birth defects, and a possible association with optic neuritis and multiple sclerosis was shown not to be statistically significant.[112,115]

A live-attenuated vaccine consisting of spores from an unencapsulated toxigenic strain of *B. anthracis* has been used in the former Soviet Union since 1953. The vaccine, given via scarification or subcutaneously, is said to be reasonably well tolerated and is reported to afford some degree of protection against cutaneous anthrax.[111] The Chinese developed a similar live spore vaccine in the 1960s.

Since the 2001 anthrax attacks there has been an intense effort to produce newer anthrax vaccines with a less cumbersome dosing schedule and less local reactogenicity. Several highly purified, recombinant PA-based vaccines adjuvanted with aluminum hydroxide (and other novel adjuvants) have been shown to protect nonhuman primates against inhalational anthrax with only one or two doses and are undergoing clinical trials.[116–118] Additional efforts to improve PA-based vaccines include using PA with other adjuvants and delivery systems including DNA vaccines and given by different routes. Research has also identified additional antigens as potential future vaccine candidates such as the anthrax capsule,[119,120] spore proteins,[121] and others that have been shown to be protective in experimental animals.[111,122]

ANTHRAX AS AN AGENT OF BIOTERRORISM

When bioterrorism became a reality in the autumn of 2001, worldwide interest became focused on *B. anthracis,* a bacterium associated with what had become a relatively obscure disease of the developing world. With a few grams of anthrax spores dispersed in letters, the recognition of the threat of bioterrorism prompted a dramatic increase in research, training, public health preparedness, countermeasures, and response infrastructure. The general level of understanding of all the agents of bioterrorism was raised not only in the medical community but also among first responders, legislators, and the general public.

Anthrax remains the agent of greatest concern for future use as a bioterrorist's weapon. With naturally occurring cases not uncommon in much of the world, *B. anthracis* is readily accessible to terrorists; easy to grow in even a rudimentary laboratory; and, in the spore form, stable, easily stored, and portable in small quantities that can wreak havoc when dispersed.

History of *Bacillus anthracis* as a Bioterrorist Agent

The history of anthrax being spread intentionally to infect others is relatively recent (compared with plague and smallpox), extending only as far back as World War I, when Germans were reported to have shipped infected horses and cattle with *B. anthracis* to be used by the Allies.[123] A more bizarre story is that of Finnish independence activist Baron Otto Karl von Rosen, who was apprehended by Norwegian police in 1917 with 19 sugar cubes, each with an embedded glass capillary tube supposedly filled with anthrax. Apparently, the plan was to infect reindeer and horses used to haul British arms through Norway. The sugar cubes would be fed to the animals, whose teeth would break the glass tubes inside, thereby lacerating their gums and allowing oropharyngeal or gastrointestinal anthrax to ensue.

During World War II, both the Axis and the Allies had biological warfare programs that involved anthrax, including the British, whose spore bomb experiments on the Scottish Gruinard Island rendered areas of the island heavily contaminated for decades.[124] The Japanese carried out extensive research on biological weapons. They used anthrax-infected animals to spread the disease in Russia and intentionally infected Chinese residents of Manchuria and China by various means including an unsuccessful attempt to infect children with anthrax-laden chocolate.[125]

Beginning in the 1940s the United States maintained an offensive biological warfare program that performed numerous studies on anthrax weaponization and defense that remain the basis of much of our understanding of bioterrorism today. The offensive program at Fort Detrick, Maryland, was disestablished by President Nixon in 1969 and replaced by the US Army Medical Research Institute of Infectious Diseases, which has been on the forefront of biodefense for more than 40 years.

The Soviet Union maintained an active anthrax program well into the 1990s. The widely studied accidental release of anthrax spores in 1979 from a Soviet military microbiology facility in Sverdlovsk, Russia, was responsible for approximately 70 human cases of inhalational anthrax.[5,126] It remains the largest outbreak of inhalational anthrax known.

During the reign of Saddam Hussein, Iraq was known to have an active biological warfare program, and 16 other nations were suspected of having biological warfare programs, but it is unknown which may have been working with anthrax.[127] At the close of the first Gulf War, Iraqi authorities admitted to having produced 8500 L of anthrax and placed 6500 L into munitions but denied ever having used them.[6,128]

Far more widely known is the use of the US Postal Service in 2001 to mail anthrax spore–laden letters, after which 22 people developed anthrax, 11 with inhalational infections and 11 with cutaneous infections. There were five deaths among the inhalational cases. An astute infectious diseases physician first considered the diagnosis when a Florida man was noted to have gram-positive rods in his CSF. Subsequent letters were sent to recipients in New York City and Washington, DC, including media outlets and offices of Senators Thomas Daschle and Patrick Leahy in the US Capitol.

The epidemiology of the 2001 anthrax cases completely changed understanding of the dispersal of anthrax spores. The ease with which spores were released from sealed envelopes during mail handling was a startling development that was not recognized until hospitalization of postal employees with inhalational anthrax occurred in individuals never suspected to have been exposed. Approximately 10,000 Americans subsequently received anthrax PEP with antibiotics because of possible exposure. The extensive cross-contamination of mail that led to additional cases geographically distant from the source was a completely unpredicted finding.[129,130] Finally, the substantial reaerosolization of spores from surfaces was not anticipated from studies performed decades previously and contributed to the $100 million spent on anthrax remediation (decontamination) in the United States, with $23 million spent at the US Capitol complex alone.[125–131]

The anthrax events of 2001 fueled a massive investment by the US government in broad areas of research on the agents of bioterrorism.[132] Since these events, new rapid, sensitive, and specific diagnostic tests have become available and are dispersed regionally as part of the Laboratory Response Network system of laboratories with varying biosafety levels (BSLs): sentinel (BSL-2), reference (BSL-3), and national (BSL-4) laboratories. Extensive bioterrorism educational efforts were made with physicians, nurses, infection control practitioners, and first responders. The intensive search for the source of the anthrax spores drove rapid improvements in microbial forensics, and the decontamination efforts in postal facilities, the US Capitol, and multiple media outlets advanced the science behind environmental remediation.[133–135] The anthrax events, occurring soon after the terrorist attacks of September 11, 2001, provoked a sense of urgency in both legislators and the scientific and medical communities that has led to considerable improvements in countermeasures such as antibiotics, therapeutic antitoxin antibodies, and vaccines. Project BioShield was created in 2004 in the United States to provide the Strategic National Stockpile funds to maintain a supply of antibiotics and other therapeutics, vaccines, and diagnostics in the event of a national emergency. Furthermore, BioShield not only provides funding for phase III clinical trials to obtain approval by the FDA but also gives the option of using supplies that may not have been fully approved by the FDA if the CDC declares an emergency.[136] The Biomedical Advanced Research and Development Authority was subsequently created by the US Congress to fund advanced development of countermeasures and oversee BioShield contracts.

Dissemination of Anthrax as a Bioterrorist Agent

History has already presented a number of methods in which anthrax can be weaponized. Anthrax has proven itself to be a versatile agent for a terrorist to use. Spores can be dispersed or sprayed as a powder or liquid, or animals can be infected and released with the intent to spread infection among others. Anthrax can be delivered by an aerosol in bombs, sprayed from a plane or backpack sprayer, or sent in the mail. It is generally believed that an intentional release of anthrax would

most likely be associated with aerosols and subsequent inhalational infections. Although contamination of human food or water could cause gastrointestinal anthrax, evidence from experimental animals suggests that it is not an efficient route and requires much higher doses of spores to lead to infection.[137] Anthrax is also an efficient agent; the 2 g of spores that were in the letter sent to Senator Thomas Daschle in 2001 were estimated to contain more than 10 million human LD_{50} (lethal dose for 50% of individuals).[138]

In a report to the US Congress, it was estimated that 100 kg of anthrax spores released from a plane over Washington, DC, would kill 1 to 3 million people under ideal meteorological conditions.[137] Other studies indicated there could be 50% fatalities as far as 160 km downwind from an aerosol spore release.[139]

A 1999 Canadian study that investigated the effect of aerial spraying of *Bacillus thuringiensis* for control of gypsy moths in British Columbia was indicative of why anthrax is considered such a high-threat agent. *B. thuringiensis* is a spore-forming species closely related to *B. anthracis* but a nonpathogen in animals. A plane sprayed a slurry of spores at a droplet size of 110 to 130 µm, and samples were taken from the environment, inside homes, and from nasal swabs of asthmatic children. Thirty minutes after release, spore concentrations were highest in the outdoor environment (mean, 739 spores/m^3 of air), but 5 or 6 hours later spore concentration inside homes exceeded outdoor concentrations (mean, 245 spores/m^3). A significant amount of small droplet aerosolization occurred with droplets of 2 to 7 µm formed in sufficient quantities to penetrate houses, yielding positive nasal swabs in 76%. (Droplets <5 µm can reach the alveoli.) If this had been *B. anthracis,* some estimates are that potentially 15% of the population sprayed may have been exposed to a lethal dose of anthrax.[140] Furthermore, the spray formulations and equipment used are readily available to the public through agricultural supply stores, and these formulations do not clog spray nozzles.[141]

Determinations of the number of inhaled spores per hour raise questions about the infective dose (ID) and lethal dose (LD) of anthrax spores. Obviously, controlled studies cannot be performed on humans; therefore we must rely on extrapolation of data from estimates of known inhalational cases and from studies in nonhuman primates. Even this is difficult because different animals have markedly different sensitivities to anthrax spores: some are very sensitive and others are quite resistant.[59,142] Despite the uncertainty inherent in extrapolation, the LD_{50} for humans is generally considered to range from approximately 4000 to 55,000 spores based on studies in nonhuman primates.[31,59,104,143] Data from nonhuman primates suggest that inhaled doses of 1000 to 5500 spores results in mortalities from 10% to 25%.[144] The determination of the LD_1 (or ID_1) is even more difficult to ascertain, and it may be significantly lower, but there are no data on this point. From a public health perspective, even an LD_1 or LD_{10} release may be significant because a city of 1 million might experience tens of thousands of cases of anthrax.[140]

Studies from mill workers in the 1950s and 1960s revealed that many employees were inhaling hundreds of anthrax spores less than 5 µm on a daily basis, and yet inhalational anthrax was extraordinarily rare.[29,145,146] Furthermore, 15% of workers developed measurable anti-anthrax antibodies, demonstrating some degree of exposure by an unknown route, suggesting some degree of innate immunity in avoiding clinically evident infections.[57] Even in the absence of measurable antibodies, exposures in the US Capitol appear to have been associated with evidence of a cellular immune response in a few cases.[147] Historically, some individuals with inhalational anthrax had evidence of preexisting pulmonary disease, which may have made them more susceptible.[144,148] What may be a "safe" number of spores for a healthy 20-year-old may be a lethal dose for an immunocompromised individual.

Outbreak Characteristics After Use of Anthrax as a Bioterrorist Agent

A terrorist considering using anthrax may contemplate which route to deliver spores to reach his or her objective. Although spores could be introduced into food supplies or water, the cutaneous, oropharyngeal, and gastrointestinal disease that would result is far less dramatic and far less fatal than inhalational anthrax.[138] Knowledge of the number of spores needed to infect humans via ingestion is not available. It is generally understood that significantly more are needed than via inhalation, and experiments in nonhuman primates suggest it is very difficult to deliberately cause infection by the oral route. In addition, the majority of ingestions are not associated with full-blown gastrointestinal anthrax, which is frequently fatal, but rather with gastroenteritis, a far less dramatic presentation than inhalational anthrax.[69]

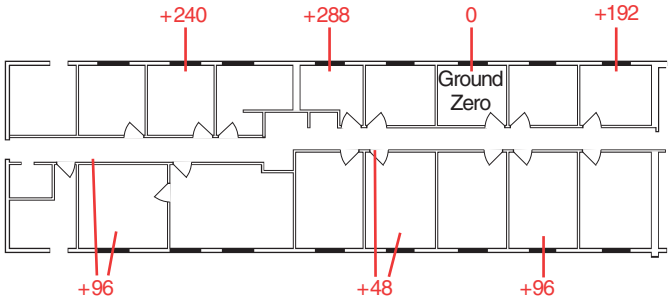

FIG. 207.8 Spores released in an office spread very rapidly through doors and in hallways. Time in seconds for peak spore deposition at a site after release of spores from an envelope at "ground zero." *(From Kournikakis B, Ho J. Objective assessment of the hazard from anthrax terrorist attacks in an office environment. Presented at the Anthrax Incident Management Workshop, Medicine Hat, Alberta, Canada. April 30, 2002.)*

An aerosol release, whether from an envelope, a sprayer on the ground, or on a larger scale from a plane, is considered the most likely terrorist scenario. Studies done in Canada with envelopes containing *Bacillus globigii (Bacillus atrophaeus)* spores demonstrated how spores spread like a gas in an office after opening an envelope containing 1 g of spores (Fig. 207.8). The estimates were that with 10 minutes of exposure, hundreds to thousands of times a human LD_{50} would be inhaled by people in the room.[149,150]

Individual *B. anthracis* spores are 1.5 to 3 µm; in nature they are most likely clustered together to form aggregates that are 10 to 100 µm in diameter or greater. As Fig. 207.9 demonstrates, particles larger than 5 µm typically cannot reach the terminal bronchioles and alveoli; they are captured in the respiratory tract mucus, removed by the mucociliary elevator to the mouth, and swallowed.[151] This is likely why inhalational anthrax has been uncommon in natural settings but was more common in factories where wool, hair, or hides were dried and manipulated by machinery, resulting in particle sizes of 1 to 5 µm. When spores are grown and engineered in a laboratory with the intent of preventing clumping by coating with silica or other substances, they may not cluster at all or may form small spore aggregates that may be efficiently delivered to the alveoli. It can be assumed that a sophisticated terrorist using anthrax spores would consider the following:

1. Engineering of spores
   a Enhancement of stability and infectivity
   b Neutralization of electrostatic charges, thereby reducing clusters and maintaining spores in small respirable aggregates less than 5 µm
2. Use of high concentrations of spores to overcome any degree of innate immunity
3. Selection of a strain demonstrating antimicrobial resistance
4. Genetic modifications to decrease protection from vaccination or increase toxin production

When anthrax spores pass the terminal bronchioles into the alveoli, they are rapidly scavenged by alveolar phagocytic cells or are translocated across alveolar epithelial cells and move through the lymphatics to the tracheobronchial lymph nodes and then mediastinal lymph nodes. Some spores rapidly transform to the vegetative state within the macrophages, whereas others are thought to remain quiescent potentially for months.

The incubation period in natural inhalational anthrax is generally considered to be 2 to 10 days, but with large inocula it may be as short as 1 day. In the 2001 cases for which the date of exposure was known, the incubation period was 4 to 6 days.[68] There is some controversy regarding how long the incubation can potentially be and whether small spore inocula may be associated with extended incubation periods.[29,129,144] The often-cited last case of anthrax in Sverdlovsk occurred 43 days after

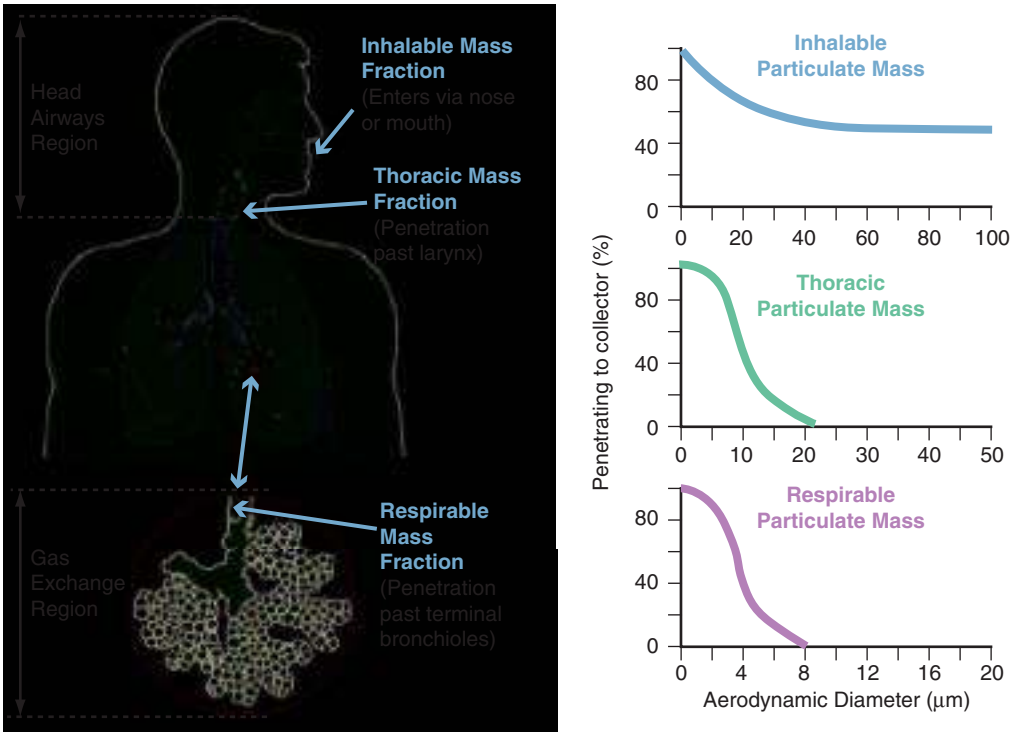

FIG. 207.9 Deposition of anthrax spores in the human respiratory tract depends on the size of spore aggregates. Individual spores are 1.0 to 1.5 μm but form aggregates 10 to 100 μm or larger that are deposited in the upper airways and cleared; weaponized spores may not cluster and are efficiently delivered to the alveoli, where they may lead to inhalational anthrax. *(Modified from Hoover MD. Uncertainty and probability distribution analyses for anthrax dispersion and human exposure. Presented at the Anthrax Incident Management Workshop, Medicine Hat, Alberta, Canada. April 30, 2002.)*

the release of spores, but median incubation for those closest to the release was 10 days and for those farthest away was 21 days.[126] However, these data are of dubious value because crucial details are not available, and patients may have received antibiotics, which are known to extend the incubation period. The case of the 94-year-old woman in Connecticut who developed anthrax in 2001, presumably related to the terrorism cases that year, occurred 35 days after the last letter was mailed and 56 days after the first, suggesting she may have inhaled a small dose. However, the possibility of exposure resulting from a different unrecognized intentional release of spores cannot be excluded.[129] In a series of 58 nonhuman primates, one of us (A.M.F.) noticed the time to death after exposure to spores varied from 2 to 9 days. However, there are reports of three infected and untreated animals with possible times to death of 20, 28, and 98 days after exposure.[29,31] Animals sacrificed 42 days after inhalation had a large number of spores in the lungs; after 100 days, small numbers of viable anthrax spores remained.[31]

The ability to detect anthrax spores soon after an aerosol release or to diagnose the first case of anthrax has improved significantly in the past decade. In a large spore release over a city, every day earlier that the exposure is recognized will result in earlier initiation of PEP and thousands fewer cases and millions of dollars in saved resources.[152,153] Even with an extensive system of detection in place, it is likely that the first evidence of anthrax bioterrorism, as in 2001, will be a critically ill patient discovered to have *B. anthracis* in a blood culture. Again, as in 2001, it can be expected that some patients will present with cutaneous disease (because aerosolized spores deposited on the body can be introduced into the skin) or gastrointestinal disease (as demonstrated in the American woman at an animal hide drumming event),[21] and others will present with inhalational disease or meningitis. If an extensive outbreak is recognized, local medical resources will be severely taxed attempting to initiate PEP, evaluating individuals potentially developing symptoms, and caring for individuals already confirmed infected and potentially critically ill. The past decade has been marked by numerous studies modeling the effects of an anthrax spore release and the subsequent response by government, health care institutions and facilities, and private individuals.[154,155]

Although there are numerous rapid assays in development, there is currently no rapid method approved to diagnose anthrax early in disease. The early symptoms of inhalational anthrax are nonspecific and similar to those of influenza. It is important to rapidly determine who has inhalational anthrax, influenza or influenza-like illness, or community-acquired pneumonia so that appropriate therapy can be initiated.[65,156,157] Kuehnert and associates[157] combined data from patients presenting with each of these diagnoses and developed a scoring system. They found that compared with patients who had influenza-like illness, patients who had inhalational anthrax were more likely to have tachycardia, high hematocrit, low albumin, and low sodium levels and were less likely to have myalgias, headache, sore throat, and nasal symptoms. Compared with patients who had community-acquired pneumonia, patients with inhalational anthrax were more likely to have nausea or vomiting, tachycardia, high aminotransferase levels, low sodium levels, and normal white blood cell counts (Tables 207.4 and 207.5).[157] The use of rapid influenza and respiratory syncytial virus diagnostics in the clinic or emergency department is also indicated. Patients with fever and spore exposure should also have blood cultures obtained; all seven patients with inhalational anthrax in 2001 who were not taking antibiotics had positive blood cultures. Kyriacou and coworkers[158] compared 47 inhalational anthrax cases with 376 community-acquired pneumonia or influenza-like illness cases and found the most accurate predictor of anthrax was a chest radiograph demonstrating mediastinal widening or pleural effusion. The epidemiologic as well as diagnostic significance of mediastinal widening needs to be emphasized in recognizing aerosol route of exposure to anthrax. It has been erroneously stated[159] and repeated in the literature[70] that mediastinal widening was reported to occur in a case of gastrointestinal anthrax. However, a careful reading of the original article reveals no mention of mediastinal widening, but rather that pneumonia was present on chest radiography, which the authors believed to be secondary to bacteremia. This is consistent with our ideas of pathogenesis in that the lymph nodes draining the site where the spores are introduced are those anticipated to become infected and enlarged. Thus mediastinal widening on a radiograph should alert the physician to suspect inhalational anthrax from an aerosol exposure

TABLE 207.4 Signs and Symptoms of Patients Presenting With Inhalational Anthrax, Influenza or Influenza-Like Illness, and Community-Acquired Pneumonia

SIGN OR SYMPTOM	INHALATIONAL ANTHRAX (*N* = 11), %	INFLUENZA OR INFLUENZA-LIKE ILLNESS (*N* = 684), %	COMMUNITY-ACQUIRED PNEUMONIA (*N* = 650), %
Tachycardia	82	14 (*P* = .0001)	49 (*P* = .04)
Sore throat	18	76 (*P* = .0001)	25 (*P* = 1.0)
Nasal symptoms	27	81 (*P* = .0002)	34 (*P* = .76)
Headache	45	86 (*P* = .002)	38 (*P* = .76)
Myalgias	64	91 (*P* = .01)	41 (*P* = .21)
Fever >37.8°C (100°F)	73	57 (*P* = .37; with flu *P* < .05)	53 (*P* = .23)
Cough	91	89 (*P* = 1.0)	79 (*P* = .47)
Fatigue	100	98 (*P* = 1.0)	NA
Abdominal pain	27	NA	21 (*P* = .71)
Diarrhea	9	NA	20 (*P* = .70)
Nausea or vomiting	82	NA	35 (*P* = .002)
Chest pain	64	NA	31 (*P* = .04; if pneumococcal bacteremia *P* > .05)
Dyspnea	82	NA	80 (*P* = 1.0)
Chills	82	NA	59 (*P* = .21)

NA, Not applicable.
Modified from Kuehnert M, Doyle T, Hill H, et al. Clinical features that discriminate inhalational anthrax from other acute respiratory illnesses. Clin Infect Dis. *2003;36:328–336.*

TABLE 207.5 Laboratory Findings in Patients Presenting With Inhalational Anthrax, Influenza or Influenza-Like Illness, and Community-Acquired Pneumonia

LABORATORY PARAMETER	INHALATIONAL ANTHRAX (*N* = 8–11), %	INFLUENZA OR INFLUENZA-LIKE ILLNESS (*N* = 630–687), %	COMMUNITY-ACQUIRED PNEUMONIA (*N* = 185–645)
Leukocytosis	27	7 (*P* = .04; without confirmed flu, *P* > .05)	61 (*P* = .03)
Neutrophilia	72	43 (*P* = .06; without confirmed flu, *P* < .05)	78 (*P* = .71)
High hematocrit	36	6 (*P* = .004)	NA
Low platelets	22	4 (*P* = .05)	NA
High bilirubin	38	6 (*P* = .009)	NA
High AST	89	18 (*P* < .0001)	29 (*P* = .0004)
High ALT	89	32 (*P* = .0008)	29 (*P* = .0005)
Low albumin level	67	2 (*P* < .0001)	NA
Low sodium	80	9 (*P* < .0001)	35 (*P* = .0005)
High BUN	50	4 (*P* < .0002)	23 (*P* = .10)
High creatinine	0	1 (*P* = 1.0)	NA
Low potassium	10	2 (*P* = .21)	NA
Low calcium	100	44 (*P* = .002)	74 (*P* = .21)

ALT, Alanine aminotransferase; *AST,* aspartate aminotransferase; *BUN,* blood urea nitrogen; *NA,* not applicable.
Modified from Kuehnert M, Doyle T, Hill H, et al. Clinical features that discriminate inhalational anthrax from other acute respiratory illnesses. Clin Infect Dis. *2003;36:328–336.*

and a bioterrorist event until proved otherwise. The evolution of chest radiographs and CT in inhalational anthrax is demonstrated in US cases from 2001 as shown in Fig. 207.7.[160] The Center for Infectious Disease Research and Policy has developed a helpful clinical pathway to guide clinicians assessing the probability of anthrax exposure and evaluating patients with possible inhalational anthrax (Fig. 207.10 and Table 207.6).[27]

ANTHRAX COUNTERMEASURES

Diagnostics

It is important to notify the laboratory that anthrax is in the differential diagnosis because the typical clinical laboratory may discard gram-positive rods as probable contaminants or not work them up beyond *Bacillus* spp. Furthermore, if *B. anthracis* is isolated, it should be handled in at least a BSL-2 laboratory (i.e., under a hood) because secondary cases have occurred among laboratory workers.

The frequency of encountering other *Bacillus* spp. has been part of the problem in developing sensitive and specific rapid tests for anthrax from both environmental and clinical sources. When Dahlgren and coworkers[146] reported on *B. anthracis* aerosols in goat hair mills, they noted the difficulty in finding the organism because it was obscured by other spore-forming bacteria in a ratio of 115:1 to 700:1. Recently developed, more specific PCR assays have become available that should aid in rapid diagnosis and minimize the number of false-positive samples.[49]

The role of nasal swabs in the "diagnosis" of anthrax must also be clarified. In the 2001 outbreak, patients considered nasal swabs as a determination of whether they had been exposed or not. The reality is that although a positive nasal culture for anthrax clearly indicates an exposure, a negative culture does not rule out an exposure. In Senator Thomas Daschle's suite in the Hart Building of the US Capitol, all 13 staff members in the room where the spore-laden letter was opened had heavy growth on blood agar plates from their nasal swabs. Many of these individuals had a separate swab from each nostril, and in at least two cases, one of us (G.J.M.) noted that one nostril of the individual yielded heavy growth and the other nostril yielded no *B. anthracis* growth. Nasal swabs essentially use the nose to sample whether the individual has filtered anthrax spores in the recently (nasally) inhaled air. They are therefore helpful as a public health tool in determining the zone of exposure. Demonstrating that one individual in a space has a positive nasal swab requires that everyone in that space receive PEP regardless of negative nasal swabs for the others. The optimal timing of obtaining nasal swabs after exposure has not been determined, but clearly the sooner the better, and it is likely that the yield 24 hours later (e.g., after showering) is much lower. Thus obtaining nasal swabs more than 24 hours after exposure should be discouraged. All 28 positive cultures in the US Capitol were obtained within hours of exposure; the remaining 6000 cultures done during the subsequent days were negative despite environmental samples demonstrating varying levels of contamination from multiple other US Capitol sites. One of us (G.J.M.) observed that repeat nasal swabs of all the culture-positive individuals in the US Capitol 1 week after exposure were negative. In evaluation of future exposures, efforts to determine if an individual was exposed might also include culturing pharyngeal washings because a study of wool mill workers revealed that addition of such cultures doubled the number of individuals with positive cultures compared with culturing only nasal swabs.[145] Gram stain of cutaneous or oral lesions, pleural fluid, CSF, or even buffy coats of blood may be positive for gram-positive rods indicative (in the right setting) of anthrax, and culture will confirm the diagnosis. Nasal swab, pharyngeal washes, and stool samples should be cultured for anthrax, but Gram staining of these samples is not helpful.

In the 2001 outbreak, there were no serologic tests readily available for anthrax. In the aftermath, numerous serologic assays have been in development and a number of rapid ELISAs that measure total antibody

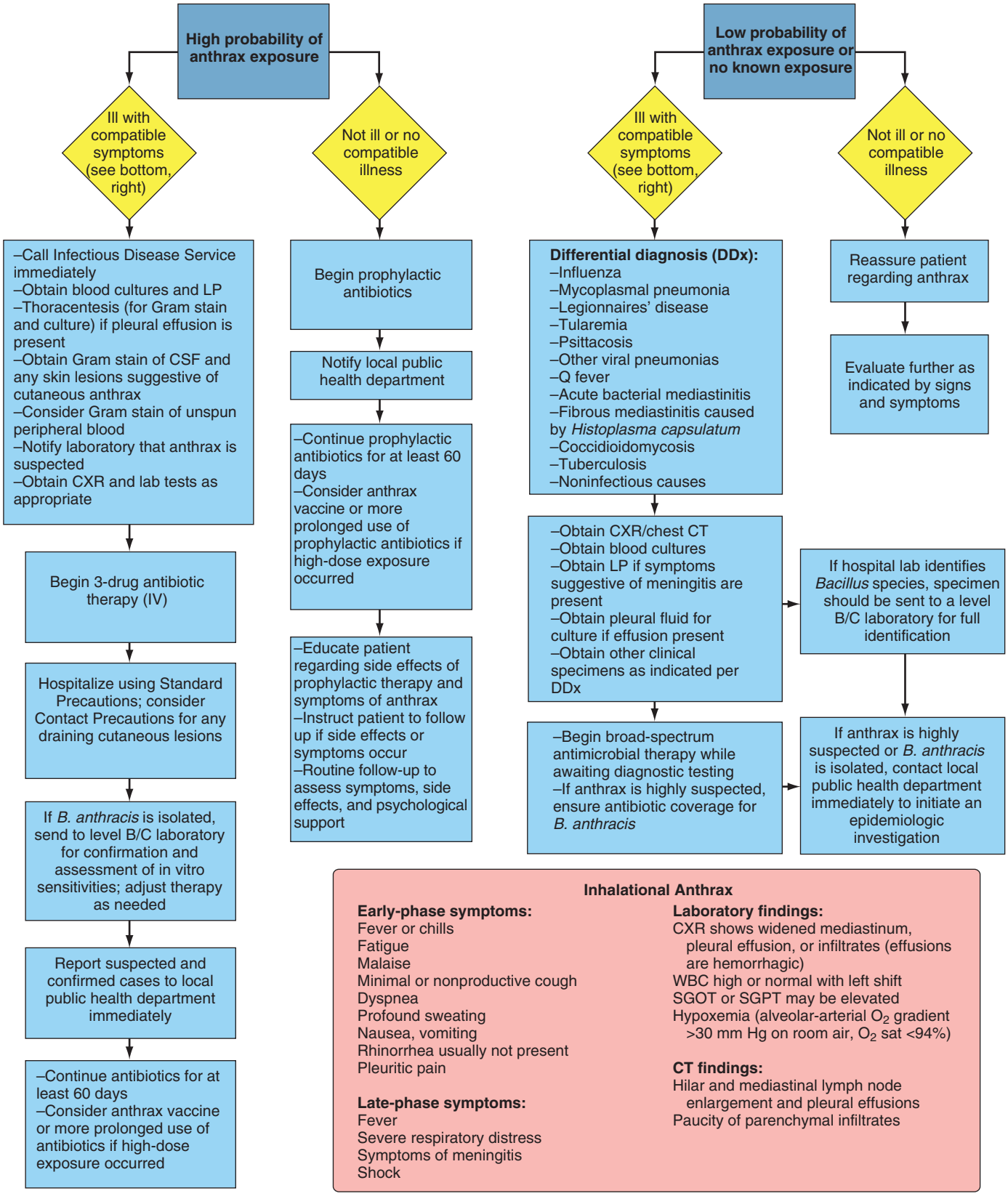

FIG. 207.10 Clinical pathway: anthrax inhalational exposure. *CSF,* Cerebrospinal fluid; *CT,* computed tomography; *CXR,* chest radiograph; *IV,* intravenous; *LP,* lumbar puncture; *SGOT,* serum glutamic oxaloacetic transaminase; *SGPT,* serum glutamic pyruvate transaminase; *WBC,* white blood cell count. *(Modified from the Center for Infectious Disease Research and Policy.)*

TABLE 207.6 Assessing the Probability of Anthrax Exposure

High Probability
During a Known Anthrax Event

Persons exposed to an airspace where a suspicious material may have been aerosolized (e.g., near a suspicious letter containing powder during opening)
Persons who shared an airspace likely to be the source of an inhalational anthrax case (e.g., being exposed to a shared ventilation system)
Persons who may have been exposed to an item contaminated with *Bacillus anthracis* (e.g., an envelope or other vehicle) along the transit path of the item (e.g., a postal sorting facility in which an envelope containing *B. anthracis* was processed)

In Situations in Which Anthrax Has Not Previously Been Identified[a]

Persons who opened a suspicious letter or package that was found to contain a white powder suspected to be a source of *B. anthracis*
Persons exposed to an airspace where suspicious material may have been aerosolized (e.g., near a suspicious letter containing powder during opening)
Sudden appearance of multiple patients with acute onset of characteristic illness (suggests common source exposure such as would be seen with a bioterrorist attack)

Low Probability

No history of exposure to an item (e.g., an envelope or other vehicle) or powder confirmed or suspected to harbor *B. anthracis* spores
No history of exposure to an airspace where a suspicious material could have been aerosolized (e.g., being present at the time a letter containing powder was opened)
No history of exposure to an airspace likely to have been the source for a confirmed case of inhalational anthrax

[a]In situations in which anthrax exposure is suspected but no prior cases of anthrax have been confirmed, a risk assessment should be conducted by local public health and law enforcement officials. If the probability of anthrax exposure is considered high on the basis of the risk assessment, prophylactic antimicrobial therapy should be initiated for asymptomatic exposed persons while the suspect material is being tested for *B. anthracis*. Any persons who have symptoms compatible with anthrax should be treated with appropriate antibiotics until anthrax can be confirmed or ruled out.
From the Center for Infectious Disease Research and Policy (www.cidrap.umn.edu).

TABLE 207.7 Centers for Disease Control and Prevention Recommendations for Postexposure Prophylaxis After Exposure to *Bacillus Anthracis* Spores

Recommended Initial Antibiotic

Ciprofloxacin 500 mg orally bid[b]
or
Doxycycline 100 mg orally bid[b]
or
Levofloxacin 750 mg orally once daily
or
Moxifloxacin 400 mg orally once daily
or
Clindamycin 600 mg orally q8h

Alternatives: Penicillin-Sensitive Strains

Amoxicillin 1 g orally q8h
or
Penicillin VK 500 mg orally q6h

[a]Duration 60 days (or with anthrax vaccine administered continue for 14 days after third dose of vaccine).
[b]Ciprofloxacin and doxycycline are considered equal preferred initial agents.[42]
From Hendricks KA, Wright ME, Shadomy SV, et al; Workgroup on Anthrax Clinical Guidelines. Centers for Disease Control and Prevention expert panel meetings on prevention and treatment of anthrax in adults. Emerg Infect Dis. *2014;20. doi:10.3201/eid2002.130687.*

TABLE 207.8 Comparison of Anthrax Vaccine Adsorbed for Preexposure and Postexposure Use

CLINICAL SETTING	NO. AND ROUTE OF DOSES	SCHEDULE
Preexposure	5, intramuscular	Weeks 0, 4 Months 6, 12, 18
Postexposure	3, subcutaneous	Weeks 0, 2, 4

to PA have been approved by the FDA. These assays can be used on serum to diagnose all types of anthrax or demonstrate seroconversion after immunization. Retrospectively, it was positive in 100% of both cutaneous and inhalation cases from 2001. However, it becomes positive only after approximately 1 week of symptoms. This was demonstrated in the 2006 inhalational anthrax case.[19,52] Numerous other assays are in the development stages including assays for anti-LF antibodies.[161] Further research on new rapid diagnostic tests yielding results in a few hours, based on detection of toxin proteins[48,162] and capsule in the serum,[163] are in development and anticipated to be licensed in the near future.

To ensure they are obtaining all the appropriate specimens[26] and preparing and shipping[41] them correctly, clinicians considering anthrax in the differential diagnosis should refer to Table 207.1 or the CDC website at https://www.cdc.gov/anthrax/specificgroups/lab-professionals/cdcspecimens.html.

Antibiotics

The role of antibiotics in preventing the development of anthrax after exposure to inhaled spores has been well documented in animal studies since the 1940s and in humans after the 2001 anthrax attacks. Antibiotic PEP and therapy are the most important countermeasures currently available for bioterrorism-associated anthrax. The antibiotic regimens recommended by the CDC were reviewed in previous sections. The focus of the discussion here is on PEP and newer agents approaching approval for anthrax.

Current recommendations for PEP have been repeatedly updated and are for initiation of 60 days of antibiotic prophylaxis with appropriate drugs and use of anthrax vaccine. These are summarized in Tables 207.7 and 207.8.[42,90,164] In the event of a release of anthrax spores, the determination of which antibiotic should be used will be guided by the sensitivity profile of the *B. anthracis* isolated. Although ciprofloxacin 500 mg and doxycycline 100 mg administered orally twice daily have been recommended by the CDC as first-line agents to be used as PEP, it is relatively easy to develop resistant strains of anthrax through serial passages in low concentrations of antibiotics, and a terrorist may develop and use a highly resistant strain.[86] Furthermore, in an unrecognized outbreak of inhalation disease many patients may have had treatment initiated for community-acquired pneumonia with third-generation cephalosporins, to which even most natural-occurring strains of *B. anthracis* are resistant.[87,165]

Initiation of antibiotics as soon as possible after (or before) exposure ensures the presence of antibiotics as spores germinate. Ungerminated spores, sequestered in the lung or macrophages, do not appear to be affected by antibiotics, which are active only on germinated spores or vegetative bacilli. The question of how long antibiotics should be maintained remains unanswered. A competing-risks model to determine the optimal duration of PEP suggests that this is dependent on the size of the inhaled inoculum, with small exposures requiring shorter courses and large exposures requiring courses of 4 months or more.[62]

The concern about prolonged PEP is that adherence to a 60-day course of antibiotics is quite poor. In the 2001 anthrax attacks, the overall adherence with 60 days of antibiotics was 44%. Adverse events were common, reported in 57% of patients during the first 60 days of PEP, and occurred nearly equally with either ciprofloxacin or doxycycline. These data are deceptive in that many of the approximately 10,000 individuals placed on PEP did not perceive themselves to have been at high risk and so were less likely to continue PEP if they were experiencing even a mild adverse event. Conversely, individuals who enrolled in the investigational new drug (IND) application, involving the use of anthrax vaccine and prolonged antibiotics, actually had the best adherence.[166] This was reported and personally observed by one of us (G.J.M.) to be a surrogate marker of individuals' perceived risk for exposure but also

reflected the recommendations of clinicians who strongly advised individuals who had been most highly exposed in US Senate offices to maintain strict adherence with PEP as well as enroll in the IND anthrax vaccine protocol.

There were similar findings reported in 2014 when the CDC experienced inadvertent potential aerosolization of anthrax spores in two laboratories. PEP was recommended for 42 individuals. Of individuals who initiated PEP, 74% did not complete either 60 days of antibiotics or postexposure anthrax immunization. Of individuals who discontinued PEP, 38% did so due to their perceived low risk of infections, whereas 31% discontinued due to minor PEP-related adverse events, and 10% cited both reasons.[167]

A number of new or recently developed antibiotics are being tested for their efficacy in the prophylaxis and treatment of anthrax. Oritavancin, a novel lipoglycopeptide in late development for skin and soft tissue infections, was very effective in the mouse model for preexposure and postexposure use. In addition, development of resistance to oritavancin in the laboratory has been much more difficult than with current drugs used for anthrax.[168] Cethromycin, a novel ketolide in development for pneumonia and sinusitis, has also demonstrated promise for *B. anthracis* infection. It can be administered orally once daily, and it showed efficacy equal to that of ciprofloxacin in the mouse model.[81]

Vaccines

Current CDC guidelines are for 60 days of PEP with either doxycycline or ciprofloxacin and initiation of postexposure anthrax immunization. AVA, the currently approved vaccine in the United States, is now approved for use in preventing anthrax preexposure as well as for PEP. The recommendation to add postexposure anthrax immunization to antibiotic PEP is due to the concern that retained spores may still be present at the end of 60 days and could germinate and lead to inhalational anthrax after discontinuance of antibiotics. Even after heavy spore exposures, animals taking antibiotics do not usually develop a significant humoral immune response. By initiating anthrax immunization, antibodies develop while the individual is protected by antibiotics. After the 60-day course of antibiotics is completed, there should be an adequate immune response to immunization to prevent any remaining spores that might germinate from causing anthrax.

For PEP, AVA given as three doses subcutaneously at 0, 2, and 4 weeks results in excellent antibody responses in nearly 100% of recipients.[169] Although the FDA approved eliminating the week 2 dose in the preexposure regimen, it is maintained in the postexposure IND regimen, and doses are given subcutaneously.[108] The preexposure and postexposure schedules are outlined in Table 207.8.

Animal studies have demonstrated the prospect of shorter courses of antibiotics for individuals who are immunized. Among rhesus macaques given approximately 1600 LD_{50} of inhaled spores and begun on PEP with oral ciprofloxacin for 14 days, 44% survived, whereas 100% of those administered three doses of anthrax vaccine in conjunction with the same course of 14 days of ciprofloxacin survived.[170]

AVA is a PA vaccine produced from filtration of the supernatant of *B. anthracis* and has been the source of much controversy even before the anthrax attacks of 2001. The frequency of side effects, requirement for numerous doses, and stability of the vaccine while stockpiled led the US government to fund development of a second-generation recombinant PA vaccine. As some products moved into human trials, stability of the vaccine became a problem, and the candidate vaccine stalled in development. This remains an active area of research, and a new recombinant PA vaccine is likely to be approved in the United States in the near future. Candidate products have been injectable or nasally or orally administered.[44,101,110,171–173] In addition, a new formulation of AVA combined with the synthetic immunostimulatory oligonucleotide adjuvant CpG 7909 has shown a more rapid and enhanced immune response in human clinical trials and is being developed for PEP.[174]

Antitoxin Immunotherapy

The role of anthrax hyperimmune sera in the treatment of anthrax in the preantibiotic era is well documented,[96] and the role of immunotherapy in potential future bioterrorist attacks has not been defined but is almost certain. Through Project BioShield, the US government has purchased supplies of AIG and the approved anti-PA MAb products raxibacumab, Valortim (MDX-1303), and obiltoxaximab for the Strategic National Stockpile.

The protective effect of anthrax antitoxin therapy in animals has been dramatic when given early after exposure and after detection of antigen in serum or onset of fever.[100] Expert opinion is mixed about the appropriate timing and which antitoxin immunotherapeutic to use in a postexposure but presymptomatic scenario. PEP antibiotics are considered most important but whether PEP antitoxin therapy should be added to antibiotics would probably be determined by the number of individuals exposed, the intensity of the exposure, and the availability of antitoxin products. Considering the low toxicity the antitoxin therapies have demonstrated and the high mortality with systemic anthrax infections, adding antitoxin to antibiotic therapy should be considered with expert consultation. Additionally, the prospect of a highly antibiotic-resistant strain of anthrax being used by a terrorist may make the role of antitoxin therapy critical to prevent infection or to treat established disease.[99,101]

Infection Control

B. anthracis has not been demonstrated to spread in household or health care settings from individuals who are infected with anthrax, with the exception of two cases associated with direct contact with cutaneous lesions.[175] Inhalational anthrax is not an airspace disease and is usually not associated with sputum production; when it is, sputa usually do not contain *B. anthracis*. Furthermore, vegetative anthrax bacteria, in contrast to the spores, are not hardy in the environment.

Anthrax-infected patients may be managed in a standard hospital room with standard universal precautions. Cutaneous lesions should be covered and dressings from lesions, chest tube drainage, blood, and so on, should be considered potentially infectious and incinerated or autoclaved. Hand washing with soap and water, 2% chlorhexidine, or chlorine-containing towels decreased spores of other *Bacillus* spp. in testing, but the use of waterless ethyl alcohol–containing hand sanitizers was not effective in removing or destroying spores.[176]

The main concern for health care workers, especially in emergency departments, is from exposed individuals who have not been decontaminated and may pass spores from clothing, skin, or hair to people caring for them. Similarly, hazardous materials and remediation teams may inadvertently carry spores outside the contaminated areas and expose other individuals. These teams often wear personal protective equipment in buildings with the heating, ventilation, and air conditioning systems shut down, so they are prone to heat-associated injuries and emergency department visits. Potentially exposed individuals should be decontaminated outside the emergency department if possible by individuals wearing personal protective equipment. Patients' clothes should be removed and bagged, they should shower, and skin and hair should be washed thoroughly with soap and water and thoroughly rinsed. Surfaces they contact (e.g., ambulances, benches) should be wiped down with a solution of 1:10 household bleach. PEP antibiotic prophylaxis should be considered for people who have cared for patients before their decontamination. Further consideration for postexposure anthrax immunization and antitoxin administration should be done with public health authorities.

Inadvertent contamination of laboratory workers may also occur because *B. anthracis* sporulates on agar while being cultured, and, if not handled carefully under BSL-2 or higher conditions, it may be associated with secondary infections. In the 2001 Capitol Hill anthrax events all the anthrax-positive nasal swabs produced extensive *B. anthracis* growth on culture plates within 12 hours of being streaked.[177]

Remediation (Decontamination)

One of the more controversial topics regarding bioterrorist-associated use of anthrax is the method and cost associated with making spore-contaminated areas safe. This is an additional advantage to the terrorist of using an agent that has the demonstrated persistence of the anthrax spore. As a result of the massive cleanup effort in the wake of the 2001 attacks, there has been increased understanding of decontamination of spores. The Environmental Protection Agency outlined eight steps in the remediation process of contaminated sites, and these are presented in Table 207.9.[133]

TABLE 207.9 Recommended Steps in Remediation of Sites Contaminated With Anthrax Spores

1. Perform a site assessment to include environmental sampling to assess contamination.
2. Isolate contaminated areas.
3. Remove any critical items, artifacts, antiques, etc.
4. Perform source reduction if ongoing contamination.
5. Remediate contaminated areas and contaminated articles removed from areas.
6. Perform postremediation environmental sampling.
7. Perform repeat remediation of persistently contaminated areas; consider using a different remediation method.
8. Safely dispose of all contaminated waste.

Modified from Canter D. Remediating sites with anthrax contamination: building on experience. Presented at the AWMA/EPA Indoor Air Quality Problems and Engineering Solutions Specialty Conference and Exhibition, Research Triangle Park, NC. 2003.

One of the least expensive and most commonly available compounds used to destroy anthrax spores is household bleach, and many of the high-tech remediation methods use bleach in some manner. Bleach, chlorine dioxide, ethylene oxide, hydrogen peroxide, peroxyacetic acid, methyl bromide, paraformaldehyde, and vaporized hydrogen peroxide all were used to some degree in the federal decontamination process in 2001 and 2002.[176] As might be expected, one agent is not suitable for all applications. Chlorine dioxide gas and liquid were used extensively in the US Capitol Hart Senate Office Building but were not found to be very effective for porous surfaces such as carpeting, chairs, and fabric surfaces, which were subsequently decontaminated with other agents.

In the event of widespread contamination of individuals and households where the public will be expected to be performing much of the decontamination efforts, it is likely that household bleach in 1 : 10 dilution will be recommended because it is readily available. Contaminated individuals should be advised to remove clothing and place it in a bag either before entering their home or immediately after entering (to minimize spores coming off clothes into the home). They should shower using soap and water and shampoo their hair. Clothes can be decontaminated by washing in hot water with bleach and machine drying. Dry cleaning will also destroy spores.

How extensively remediation must be performed remains controversial. Because it is well known from studies of wool mill workers and nonhuman primates that the innate immune system can eradicate an as yet undefined number of spores, preventing the development of inhalational anthrax, must every spore be removed from every surface? There may be an acceptable level of contamination that will allow for a timelier and cost-effective remediation effort after a city-wide exposure without serious compromise to the public health of the community. The National Academy of Sciences reviewed remediation of buildings after anthrax contamination and addressed many of these controversial areas but concluded that it cannot be determined what lowest level of spore contamination is acceptably safe for exposure.[178]

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The complete reference list is available online at Expert Consult.

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208 *Bacillus* Species and Related Genera Other Than *Bacillus anthracis*

Thomas Fekete

SHORT VIEW SUMMARY

Definition
- Gram-positive spore-forming bacilli

Epidemiology
- Worldwide distribution
- Commonly found in soil and water
- Major pathogens of insects
- Infrequent cause of infection in mammals (including humans)
- Normal flora in children and adults

Microbiology
- Aerobic or facultatively anaerobic
- Easily cultivated with standard culture technique
- Numerous genera (>56) and species (>545)
- Frequently toxin producing, and toxin contributes to many clinical manifestations of disease
- Susceptibility tests not standardized but can be helpful

Diagnosis
- Culture on routine media at standard temperatures (25°–37°C)
- Can be challenging to distinguish true pathogen from contaminant
- Characteristic Gram stain properties but can be confused with *Clostridium*
- For food poisoning, can be isolated from food products
- Should be suspected when sterilization of equipment is inadequate
- Infection may follow traumatic injury

Therapy
- Food poisoning is normally self-limited and requires only supportive treatment
- Deep infection requires antibiotics
- Vancomycin and fluoroquinolones usually active
- May require removal of foreign body such as vascular catheter

Prevention
- Optimal management and storage of food
- Careful attention to sterilization techniques
- Early removal of unneeded devices

MICROBIOLOGY

Bacteria of the genus *Bacillus* are well adapted to their normal environment of soil. This includes *Bacillus anthracis,* discussed elsewhere in this text (see Chapter 207). These gram-positive or gram-variable, aerobic or facultatively anaerobic, rod-shaped bacilli have rounded or squared-off ends, form endospores, tolerate extremes of temperature and moisture, and are ubiquitous. They are found in superficial lake and ocean sediment, even in deep water. Their hardiness under conditions of desiccation and heat has been used to determine the efficacy of heat sterilization *(Bacillus stearothermophilus)* and fumigation procedures *(Bacillus subtilis).* For many members of the genus *Bacillus,* an association with animals (either saprophytic or pathogenic) has also been noted. These animals range from small insects to large mammals, including humans.

Genomic tools have reshaped the taxonomy of *Bacillus* spp. Earlier changes included the movement of *Bacillus alvei* into the genus *Paenibacillus* and placement of both *Bacillus brevis* and *Bacillus laterosporus* into the genus *Brevibacillus.*[1] Newer changes indicate upwards of 56 genera and 545 total species of aerobic, gram-positive, spore-forming bacteria, although few of them can cause human disease.[2] Recent analyses of *Bacillus cereus* (sensu lato) have shown it to be essentially a pangenome with clonality throughout *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. weihenstephanensis*, *B. pseudomycoides,* and *B. anthracis.* Environmental strains resemble those found in cultures of human clinical material.[3] For practical purposes, we retain the current species because they are fairly easy to distinguish, and because it is useful to make distinctions around the public health threat of *B. anthracis* and to recognize the commercial role of *B. thuringiensis.*[3] Another organism in the *B. cereus* group, *Bacillus cytotoxicus,* has been associated with foodborne illness.[4] This species can produce a number of toxins and has unusual thermotolerance, being able to grow up to 50°C. Less-related species of *Bacillus* that may occasionally be encountered in the human clinical microbiology laboratory are *B. subtilis, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus*, and *Bacillus sphaericus. Bacillus* spp. grow quickly at 25° to 37°C on the usual culture media of the clinical laboratory. By definition, all have the capacity to form spores, but they vary widely in motility, colony morphology, and nutritional requirements. They are fairly large bacteria, with dimensions ranging from 3×0.4 to 9×2 µm. Although they are usually gram positive in early growth, old cultures can be gram variable or even gram negative.[5] In most clinical laboratories, the most urgent task is to distinguish *B. anthracis* from other *Bacillus* spp. *B. anthracis* is nonhemolytic on sheep or horse blood agar and nonmotile, whereas most other clinical isolates are motile and β-hemolytic. Strains of *B. anthracis* that are slightly hemolytic have been reported, and some of the less frequently isolated non-*anthracis* strains are nonmotile and nonhemolytic. For the latter strains, detailed biochemical analysis and toxin testing may be required. It is perhaps surprising, yet fortunate, that the commercial tests to distinguish *B. anthracis* from its near relatives generally are easily carried out on widely available commercial kits. Some species, such as *B. sphaericus* and *Bacillus badius,* are biochemically unreactive and difficult to identify using commercial biochemical kits. Fortunately, newer technologies, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, have been shown to be reliable for species determination.[5]

EPIDEMIOLOGY

The widespread distribution in nature of *Bacillus* spp. explains its frequent isolation in the laboratory. In many cases the isolation of *Bacillus* spp. from a clinical specimen raises the possibility of contamination because environmental spores can germinate quickly on various laboratory media. *Bacillus* spp. are infrequently cultured but commonly present in human intestinal contents when a genomic analysis is performed.[6] They represent the most common members of Actinobacteria, the phylum that also includes mycobacteria, corynebacteria, nocardia, and actinomyces. Using more conventional culture techniques, children and adults were tested for the presence of *B. cereus* in the stool, and rates of recovery from 0% to 43% were found in the absence of diarrhea. The density of *B. cereus* in stool is usually low (≈100 viable organisms/g) but can be considerably higher. Strains of *B. cereus* in the stool are the same as

those found in the food supply, and the ubiquity of *B. cereus* is reflected in a large number of different strains in fecal cultures of healthy people. However, during outbreaks, it can be shown that the strain of *B. cereus* causing food poisoning is consistent by biotype, serotype, toxin production, and phage type among patients.[7]

Hospital outbreaks of *Bacillus* spp. infection include one in which *B. cereus* was an ongoing cause of positive respiratory cultures and morbidity, including two cases of true bacteremia and one fatal pneumonia, in an intensive care unit.[8] This epidemic was the consequence of inadequate sterilization of respiratory circuits. No other bacterial infections occurred at higher than usual rates during the epidemic period because the degree of sterilization was sufficient to eradicate non–spore-forming bacteria but not *B. cereus.* Failure to sterilize surgical instruments has been associated with a number of postoperative infections, including those caused by *Bacillus* spp. In a Scottish outbreak, *Bacillus* spp. and coagulase-negative staphylococci were isolated from a variety of autoclaved instruments, and a number of patients had infections in operative sites caused by various bacteria, including coliforms, staphylococci, and *Bacillus* spp.[9] A more dramatic example of a large-scale outbreak occurred at a hospital in Singapore during a period of major construction.[10] A 10-fold increase in isolates of *Bacillus* spp. (including true infections) was noted in association with drilling and excavation. Linens were heavily contaminated by *Bacillus,* and more meticulous handling of hospital linens reduced the rate of infections, but a relaxation of linen cleaning standards allowed a resurgence of *Bacillus* infections.

Other species of *Bacillus* can persist for a long period and then cause intermittent medical problems, such as 12 cases in 10 years of *B. sphaericus* bacteremia in a children's cancer hospital in Italy.[11]

PSEUDOINFECTION AND CONTAMINATION

More common than true outbreaks are pseudoepidemics, in which a strain or strains of *Bacillus* spp. are recovered from patients with a common source of contamination.[12,13] In these settings biochemical and molecular studies can show that a single strain is found, even though it was not actually causing disease. Conversely, clusters of *Bacillus* spp. infection may look like point source outbreaks when they represent a higher than expected rate of infection by environmental organisms. One small cluster of serious *Bacillus* spp. infections, all of which were accompanied by bacteremia, occurring over 10 days in a children's cancer ward showed that the strains recovered were different from one another and from other isolates submitted for analysis.[14] Because *Bacillus* spp. is such a common contaminant and such a rare cause of disease, many laboratories do not identify *Bacillus* to the species level, except to exclude the possibility of *B. anthracis. Bacillus* spp. can survive in high concentrations of ethyl alcohol, up to 95%, including the sprays of 70% ethanol that are sometimes used for hand hygiene.[12] The failure of alcohol to kill *Bacillus* spp. led to a serious problem when a manufacturing defect in alcohol prep pads allowed for an outbreak of some serious infections.[15] Fresh prep pads tested positive for *Bacillus* spp., whereas other sterilizing liquids were, in fact, sterile.

Bacillus spp. contamination has resulted in false-positive rates of up to 0.1% to 0.9% of all blood cultures submitted.[16] Construction on a hospital driveway resulted in a 13-fold increase in the number of blood cultures that tested positive for *Bacillus* spp., although no true infections were identified.[13] The problem was related to direct contamination of stored blood culture bottles and inadequate cleaning of the bottles before introduction of the specimen. Even in the absence of a pseudoepidemic, it can be difficult to separate true *Bacillus* spp. infection from contamination. The best indicator of true bacteremia is the presence of multiple positive cultures or recurrent bacteremia. In one study that compared patients for whom both bottles were positive in a set with *Bacillus* spp. against patients with only a single bottle positive, 29% (5/17) of episodes with both bottles positive were associated with a subsequent positive blood culture, as opposed to 3% (2/59) in patients with only a single bottle positive.[17] This suggests that skin preparation may be less important than specimen handling in false-positive blood cultures for *Bacillus* spp. In a Japanese hospital 29 patients were noted to have *Bacillus* spp. Bacteremia; more than half of these were *B. cereus*.[18] However, these patients were not treated for *Bacillus* spp. and did well clinically. Review of infection control policies showed suboptimal approaches to handling the catheters—wrong disinfectant, pauses during infusion, and reuse of caps on stopcocks. When these shortcomings were corrected, the *Bacillus* spp. bacteremia pseudoepidemic ceased. False-positive cultures of cerebrospinal fluid (CSF) for *Bacillus* spp. have also been reported.[19] A Polish study of microbial contamination of postcollection and postprocessing peripheral blood and bone marrow products found a contamination rate of 0.87% in the postprocessing products, of which 67% were *Bacillus* spp.[20]

COMMERCIAL USES OF *BACILLUS* SPECIES

The toxins of the insect pathogen *B. thuringiensis* (Bt) have been purified and are among the most widely used "natural" control agents in agriculture. Bt organisms or their purified toxins can be applied to commercially important plants to reduce damage from insect pests, and these can be easily purchased in garden centers to spray or dust in areas of insect activity. Despite its close genetic relatedness to potential pathogens, Bt used for farming and horticulture is very safe for humans.[21] Genetic engineering has allowed the insertion of the toxin gene from Bt into other bacteria that can live closely with plants (e.g., among their roots or even between their cells) and protect them. Bt toxin genes have been inserted into commercially farmed plants, such as tobacco, tomato, and cotton, making them naturally resistant to insects. *Bacillus* spp. spores have been marketed in nonchemical drain cleaners that work when the spores germinate and enzymatically digest part of the clog.[22] There is a growing practice of including *Bacillus* spp. in commercial probiotic formulations.[23] In one case a *B. subtilis* probiotic resulted in infection of an immunocompromised patient.[24]

ADHERENCE PROPERTIES

Adherence of some *Bacillus* spp. to plastic intravascular catheters may help account for the frequency with which *Bacillus* spp. infection presents as bacteremia, accounting for 26 of 38 patients in one series.[25] Scanning electron microscopy of a Hickman catheter removed from a cancer patient with persistent *Bacillus* spp. bacteremia showed vegetative organisms embedded in a layer of glycocalyx.[26] The spores of *B. cereus* are also sticky and have tendrils that stick out from the dense spore and provide a mode of attachment to various surfaces.[27] *B. licheniformis* is often mucoid in colonial morphology, which may account in part for its ability to cause somewhat indolent but difficult-to-treat infections in patients with long-term indwelling vascular catheters.[28]

CLINICAL MANIFESTATIONS

Food Poisoning

Intoxication from the ingestion of *Bacillus* species–derived toxins is an uncommon but well described form of food poisoning. A report from England and Wales in the mid-1980s showed that there was 1 food poisoning from *Bacillus* spp. for every 129 of *Campylobacter,* 95 of other bacteria (e.g., *Salmonella, Shigella*), and 5.6 of *Clostridium perfringens.*[29] Like other toxin-mediated food poisonings, *Bacillus* spp. food poisoning occurs within 24 hours of eating, often within a few hours of the offending meal. *Bacillus* spp. toxins can produce one of two distinct syndromes: diarrheal and emetic. The diarrheal syndrome is characterized by profuse diarrhea and cramping but rarely vomiting or fever. The onset is about 8 to 16 hours after the ingestion of contaminated food, and the illness is brief (median duration, 24 hours). The emetic form (similar to *Staphylococcus aureus* food enterotoxin) has an even faster onset (1–5 hours) and is characterized by nausea, vomiting, and cramps, although diarrhea can occur in about one-third of cases. It also resolves within 24 hours. The toxins responsible for these two clinical syndromes are quite different. The diarrheal toxin is a mixture of two or more proteins with molecular weights of 36 to 45 kilodaltons (kDa). The precise mode of action is unknown, although in animal models the toxins disrupt cell membranes and may have sphingomyelinase activity. The diarrheal toxin is heat labile and can be reduced or eliminated by heating food to a high enough temperature to kill the vegetative phase of the organism. This is important because it is believed that the ingestion of toxin-producing *Bacillus* spp. can lead to diarrheal food poisoning by elaboration of toxin in the gastrointestinal (GI) tract. Foods most commonly

associated with *Bacillus* spp. diarrheal food poisoning include meats, vegetables, and sauces.[29] Because diarrheagenic toxin genes are found in most strains of *B. cereus,* vegetative cells are adherent to mucosal surfaces, and the organism is ubiquitous in the environment, it is not known why food poisoning is so infrequent.[27,30] Although most isolates of the diarrheal form of *Bacillus* spp. food poisoning are *B. cereus,* there have been outbreaks related to *B. licheniformis* and *B. pumilus.*[31]

The emetic toxin is a small peptide of about 10 kDa. It is heat stable and associated with starchy foods, such as rice. This problem is worsened when rice is kept at room temperature overnight (to prevent clumping during refrigeration) and reheated the next day (e.g., fried rice). Heating or reheating food may eliminate viable *Bacillus* spp. organisms and the diarrheal toxin but not the emetic toxin. Strains of *Bacillus* may produce one toxin or the other but rarely both. Genetic studies of strains that produce emetic toxin show that they belong to a single *B. cereus* genetic group (III) and subgroup (BC05), whereas strains producing diarrheal toxin may belong to any one of five of the seven *B. cereus* genetic groups.[32] More recent scholarship has shown that affiliation with group rather than with species is helpful in understanding the enterotoxigenic properties within the *B. cereus* group.[33] At least one outbreak of *B. licheniformis* food poisoning was clinically comparable with the emetic syndrome of *B. cereus,* but the polypeptide toxin of *B. licheniformis* differs from that found in *B. cereus.*[34] On rare occasions emetic toxin can lead to significant liver disease, including fulminant hepatic failure.[35] This is thought to be the result of inhibition of mitochondrial fatty acid oxidation.

Both types of food poisoning can be diagnosed by culturing food, feces, or vomitus. Although cultivation of the *Bacillus* spp. organisms is easily done, it is not routine in the evaluation of diarrhea when stool cultures are submitted. Testing for the toxins themselves is difficult because commercial assays are not widely available. Sometimes, the cause of food poisoning is inferred rather than documented. Large studies of restaurant-associated outbreaks have allowed a probabilistic determination of the cause of foodborne illness based on the relative contributions of poor hygiene, poor cooking, poor holding, or contaminated equipment.[36] Improper food holding is the most likely culprit for *B. cereus*–associated disease, as is the case for *C. perfringens* and *S. aureus* toxin–related vomiting and diarrhea. Although *Bacillus* spp. food poisoning often occurs in point source epidemics, the exact infective or toxic dose of *Bacillus* spp. in food is not known. Food screening that finds concentrations of *B. cereus* higher than 10^3/g of food is worrisome and should lead to a careful assessment of food handling and storage, even in the absence of known food poisoning.[37] In one outbreak in which *B. cereus* food poisoning was associated with mayonnaise in potato salad, only 10^3 bacteria were recovered per gram of mayonnaise.[38] Diners may have been exposed to a higher concentration of *B. cereus* because the potato salad was left at room temperature. In London diarrheal *B. cereus* food poisoning involved at least 139 out of almost 1000 people who ate a barbeque meal together.[39] Of the responders with food poisoning, one-fifth had fever (low grade) and one-third developed symptoms outside the usual 6- to 24-hour window (mostly 6 hours) after exposure. Some people were ill for up to 20 days after the start of symptoms, although the median duration was 2 days. Leftover pork had more than 10^3/g *B. cereus*. In an outbreak from a hospital cafeteria, 160 of 249 (64%) employees reported an illness compatible with the diarrheal form of *Bacillus* spp. food poisoning related to rice or chicken, both of which cultured positive for *B. cereus.*[40]

A distinct form of food poisoning has been associated with *B. subtilis.* This syndrome is characterized by a short incubation period (median, 2.5 hours); vomiting; diarrhea (in about 50% of cases); and various other manifestations, such as flushing, sweating, and headaches, in about 10% of patients.[29] Large amounts of *B. subtilis* are required to cause this syndrome; cultures of vomitus and food show 10^6 to 10^9 organisms/g.

Systemic Infections

The rare but definite association between *Bacillus* spp. and deep infection has been recognized for more than 4 decades (Table 208.1). Bacteremia has been the most common presentation, but distinguishing infection from contamination may be difficult.[8,18,41] In a review of positive blood cultures for *Bacillus* spp. in a North Carolina hospital in the 1980s, 5 of 78 isolates were thought to represent true infection.[42] All the definite infections were caused by *B. cereus,* whereas 70% of the possible and only 45% of the nonsignificant isolates were *B. cereus.* A series from an academic hospital in France of cases collected from 2008–12 found that *B. cereus* infection was associated with bacteremia in 24 of 57 cases. Skin infections were 28.1%, and bone and joint infections were 17.5%. Mortality was 11.8% and was significantly associated with β-lactam therapy.[43] The most common feature in true *Bacillus* spp. bacteremia is the presence of an intravascular catheter, particularly a surgically implanted catheter.[28] Catheter removal is often required for cure. The largest number of the bloodstream isolates of *Bacillus* are *B. cereus,* but other species, such as *B. licheniformis,* have also been reported.[28]

A case-control study from South Korea and correspondence related to it illustrate important points about *Bacillus* bacteremia in patients with cancer. In South Korea risk factors for bacteremia included the presence of a central venous catheter, consistent with many reports as noted earlier, and antecedent use of an extended-spectrum cephalosporin within the preceding month, consistent with poor susceptibility to this class of β-lactam antimicrobial drug. However, hematopoietic stem cell transplantation had a negative association.[44] A potential explanation was provided in a response reporting results from M.D. Anderson Cancer Center, where surveillance cultures are performed weekly, and quantitative cultures found that 97% of *Bacillus* spp. bacteremias had less than 10 colony-forming units/mL, which was interpreted as not representing a "true" bacteremia, but rather catheter colonization. The author suggests that therapy initiated to treat a catheter colonized with other low-grade pathogens, such as coagulase-negative staphylococci, as detected by routine surveillance, might lessen the number of *Bacillus* bacteremias, consistent with the Korean experience.[45]

Disseminated *Bacillus* spp. infections in neonates and young children have been described. These infections can cause multisystem involvement, and in neonates they seem to be acquired perinatally.[46] A case of probable maternal-fetal infection has been reported in an injection drug user.[47] An analysis of two preterm infants who died of *B. cereus* sepsis showed that the strains were different and indistinguishable from environmental strains making it likely that their risk factor was immature immunity.[48]

Bacillus spp. infections can be serious and even fatal, especially when the patient has major host defense compromise, such as neutropenia.[41,49] Injection drug users are at higher risk of serious although not usually

TABLE 208.1 *Bacillus* Species and Related Genera With Their Reported Clinical Syndromes, Other Than Anthrax

SPECIES	CLINICAL SYNDROMES
B. cereus	Bacteremia, pneumonia, ophthalmitis, keratitis, osteomyelitis, endocarditis, soft tissue infections, nosocomial infections, meningoencephalitis, fulminant hepatitis, diarrheal food poisoning, emetic food poisoning
B. circulans	Meningitis, cerebrospinal fluid shunt infection, endocarditis, wound infection, endophthalmitis
B. licheniformis	Bacteremia, catheter-related sepsis, food poisoning, central nervous system infections after surgery or trauma
B. megaterium	Meningitis, bacteremia
B. pumilus	Meningitis, bacteremia, soft tissue infection
B. sphaericus	Peritonitis, pleuritis, pericarditis, pseudotumor of the lung, meningitis, bacteremia
B. subtilis	Meningitis after lumbar puncture or head trauma, otitis, mastoiditis, wound infection, bacteremia, pneumonia, endocarditis, shunt infection, emetic food poisoning
Brevibacillus brevis	Keratitis, food poisoning
Brevibacillus laterosporus	Bacteremia
Paenibacillus alvei	Sepsis, meningitis, prosthetic joint infection, wound infection

fatal *Bacillus* spp. infection from injection of the organism from the drugs or injection paraphernalia.[50] *Bacillus* spp. can be disseminated via bacteremia to other body sites, such as the bones or eyes. *Bacillus* spp. are rarely the cause of native valve endocarditis, and when this occurs it is usually in injection drug users, although serious infections have occurred in other settings.[50,51,52] Although a central catheter was present in a case report of *B. cereus* endocarditis, a review showed that the major risk factors for valve infection were presence of a prosthetic valve and injection drug use.[53]

Central Nervous System Infections

Bacillus spp. usually enters the neuraxis through trauma or surgery, particularly implantation of a CSF shunt.[54] Removal of the hardware is usually required to achieve a cure. Lumbar puncture for diagnostic or therapeutic purposes can also lead to *Bacillus* spp. meningitis.[55] Brain abscess or encephalitis can be found alone or in combination with meningitis.[56] Five cases from a single institution presented with protean manifestations, including meningoencephalitis, brain abscess, ventriculitis, and catastrophic hemorrhage. All patients were in the neutropenic phase of therapy for acute leukemia, and mortality was 80%. Invasion of cerebral blood vessel walls by *B. cereus* with thrombosis and hemorrhage has been reported in a neutropenic leukemic patient with bacteremia and a rapidly fatal course.[57] Concomitant pathology in the gut of some cases with ulcerations colonized or infected by *B. cereus* and an extensive source investigation suggested that there could have been an association with a food source, perhaps bananas.[58,59] There were genetic differences between the strains in that report, arguing against a single source, and GI lesions have been uncommonly reported in *Bacillus* bacteremia. A retrospective review of *B. cereus* bacteremias in a single large center in Japan noted an association between brain abscess as a sequela and neutropenia in five cases over 9 years, in which GI complaints were present in four. Only one patient died, and empirical therapy was with a carbapenem and vancomycin.[60] A *B. cereus* brain abscess in a 5-year-old boy undergoing induction therapy for acute leukemia manifested only as fever and resolved after prolonged therapy with meropenem.[61]

Respiratory Infections

Bacillus spp. are rarely the cause of pneumonia, but there have been sporadic cases of severe pneumonia associated with *B. cereus*.[62–64] Detailed evaluation of these strains has shown that they differ from typical *B. cereus* strains by virtue of capsule production and close genetic relatedness to *B. anthracis*. An example of such a case was a welder who presented with positive blood cultures and a rapidly progressive pneumonia with a striking preponderance of gram-positive bacilli in his respiratory secretions.[65] Ultimately, the patient died, and numerous cultures confirmed an organism that typed out as *B. cereus* but had some hallmarks of *B. anthracis*. Capsule, plasmid carriage, and animal virulence studies of *B. cereus* strains (*B. cereus* biovar *anthracis*) that caused an anthrax-like disease in great apes in Central Africa suggest that these lineages provide further evidence on the evolution and evolutionary advantages of *B. cereus* with increased pathogenic potential.[66]

Eye Infections

Bacillus spp., usually *B. cereus,* can cause a rapidly destructive endophthalmitis, resulting from ocular trauma, therapeutic injection or surgery, or hematogenous dissemination.[67–70] The latter route is usually in an injection drug user. A large case series of *Bacillus* spp. endophthalmitis from India, mostly but not entirely the result of trauma, has shown that aggressive therapy combining vitrectomy, topical and systemic antibiotics, and occasionally steroids could result in a surprisingly good outcome.[71] A similar series from the United States reported 22 patients with *Bacillus* spp. endophthalmitis who were treated with vitrectomy and antibiotics (intraocular vancomycin plus a cephalosporin or aminoglycoside), and 18% retained a visual acuity of 20/60 or better.[72]

Bacillus spp. keratitis is an uncommon sequela to eye trauma or other conditions that affect the cornea. Corneal scrapings reveal gram-positive or gram-variable rods that grow easily in culture.[73] The eye complaints usually begin soon after injury but may be delayed for weeks or even months. Topical ophthalmic treatment is often successful in curing the infection with reasonable visual acuity. There have also been reports of *B. cereus* keratitis as a result of contact lens wear.[74] In this study normal disinfection methods for the contact lens case were insufficient to eliminate *B. cereus.* One of the rare infectious complications of refractive surgery is keratitis from *Bacillus* sp. This was seen in a case of delayed lamellar keratitis caused by *B. megaterium* in a healthy adult despite aseptic surgical technique and perioperative antibiotics.[75]

Soft Tissue, Skin, and Muscle Infection

Bacillus spp. soft tissue and bone infection has been associated with injuries and wounds, notably including those received in motor vehicle accidents.[76] In one series of Swedish orthopedic patients with postoperative or posttraumatic wounds, about one patient per month had *Bacillus* spp. obtained from wounds, of which half were considered to represent moderate-to-severe infections.[77] *Bacillus* spp. were isolated from 25% of patients with wound complications after total hip arthroplasty, and these patients had a longer hospital stay than other patients. Ninety-four scalp infections occurred among 660 university military cadets in the summer of 2004.[78] Three cadets had scalp cultures with indistinguishable strains of *B. cereus,* whereas numerous environmental *B. cereus* strains (including from barber clippers) were distinct from one another and from the clinical isolates. The timing of the lesions suggested a point source outbreak abetted by microabrasions caused by the recent haircut. In a case series from Costa Rica, *B. cereus* was found in 14 of 18 patients with traumatic wounds acquired in the rain forest.[79] These isolates were toxin producers, and in most cases wound cultures showed *B. cereus* to be present in pure cultures and in large numbers. A drug abuser with *Bacillus* spp. crepitant cellulitis had the same isolate obtained from his heroin.[80]

PREVENTION

Guidelines for the safe preparation and handling of food are available at www.cdc.gov/foodsafety. Education of commercial food vendors is of obvious importance.[37] The best way to avoid both forms of food poisoning is to cook foods adequately and eat them immediately. Cooking will kill vegetative *Bacillus* spp. and destroy preformed diarrheal toxin, although not emetic toxin. If food cannot be consumed immediately, it should be refrigerated as soon as possible because *Bacillus* spp. metabolism and toxin production are inhibited by cold temperature. Cooked rice should not be held at room temperature for prolonged periods before preparation of fried rice. Education of contact lens wearers about proper decontamination is important in preventing keratitis.[74]

THERAPY

There is no specific treatment for the food poisoning syndromes other than supportive measures. For deep tissue infections, removal of prosthetic material, including infected intravascular catheters, is vital to achieve cure.[17] Most *Bacillus* spp. isolates are susceptible to vancomycin, clindamycin, fluoroquinolones, aminoglycosides, carbapenems, and, variably, penicillins and cephalosporins.[81] *B. cereus* is often resistant to all β-lactams, other than carbapenems, and serious infections are best treated with vancomycin, fluoroquinolones, or clindamycin, with or without an aminoglycoside.[27]

B. cereus, the most frequent species causing bacteremia, is uniformly susceptible to vancomycin, ciprofloxacin, levofloxacin, linezolid, and gentamicin.[82–84] *B. cereus* is usually, but not always, susceptible to carbapenems or clindamycin, in part depending on the testing method.[83] Resistance is usual in trimethoprim-sulfamethoxazole, tetracycline, macrolides, penicillins, amoxicillin-clavulanate, and cephalosporins.[84] There is little clinical outcome data on *B. cereus* bacteremia, but a Japanese group showed, in a case series, that treatment with "appropriate" antibiotics (based on in vitro susceptibility data) had a better 2-day defervescence rate than treatment with inappropriate therapy.[85] The study was underpowered to show other benefits. The β-lactamase of *B. cereus* and several other *Bacillus* spp. is a zinc-based enzyme that is evolutionarily different from β-lactamases in other gram-positive bacteria. For strains other than *B. cereus,* various β-lactams are active in vitro,[19] but clindamycin is less reliably active. Vancomycin appears to be active in vitro

against most *Bacillus* strains, but resistance via the *vanA* gene cluster has been reported.[86]

For native valve endocarditis a long course of therapy has been reported to be successful,[49] and clindamycin has been surprisingly effective in a few cases, despite its variable susceptibility and bacteriostatic activity. For prosthetic valve disease, valve replacement is usually performed.[50] In patients with positive cultures of blood or other body fluid, the challenge is to be certain the isolate is not a contaminant.[19] Surgical drainage and removal of necrotic debris or implanted devices, including central venous catheters, are vital. In endophthalmitis, pars plana vitrectomy and intravitreal antibiotics have been advocated, but prognosis is poor. *Bacillus* spp. keratitis is treated topically, for example, with a fluoroquinolone. A good visual outcome is most likely when the lesion is treated early and does not involve the central part of the cornea.[69]

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209 *Erysipelothrix rhusiopathiae*

Annette C. Reboli

SHORT VIEW SUMMARY

Definition
- *Erysipelothrix rhusiopathiae* is a pleomorphic, nonsporulating gram-positive bacillus.
- It causes three major forms of disease in humans: erysipeloid (localized cutaneous infection), diffuse cutaneous infection, and systemic or invasive infection (bacteremia with or without endocarditis or vocal organ or deep tissue infection).

Epidemiology
- A zoonosis, it is widespread in nature and infects wild and domestic animals, including swine, poultry, sheep, and fish.
- Infection in humans is usually due to occupational exposure.
- Portal of entry is usually through abrasions or puncture wounds of the skin, but infection may also follow ingestion of undercooked pork or seafood.

Microbiology
- It is an aerobe or facultative anaerobe.
- Most strains produce hydrogen sulfide, a diagnostically important reaction.
- It is sometimes confused with other gram-positive bacilli.
- Vitek 2, the API Coryne system, the API ID 32 Strep system, and matrix-assisted laser desorption/time-of-flight (MALDI-TOF) mass spectroscopy are reliable for identification.

Diagnosis
- A provisional diagnosis can often be made based on a history of appropriate epidemiologic exposure and, in the case of erysipeloid, characteristic physical findings.
- Definitive diagnosis requires isolation of the organism from blood, other sterile body fluid, or a biopsy specimen.
- Standard methods for culturing blood or biopsy tissue suffice.
- The polymerase chain reaction assay has been used for rapid diagnosis in swine and has been applied successfully to human and environmental samples.

Therapy
- *E. rhusiopathiae* is highly susceptible to penicillins, cephalosporins, clindamycin, imipenem, linezolid, daptomycin, and ciprofloxacin.
- Most strains are resistant to vancomycin, sulfa drugs, and aminoglycosides.
- Erysipeloid may resolve in the absence of therapy; however, appropriate therapy shortens the illness and reduces the risk for relapse.
- Penicillin is the drug of choice for all forms of infection. When β-lactams are contraindicated, use of fluoroquinolones, daptomycin, linezolid, or clindamycin may be considered.

Prevention
- Hand hygiene, use of protective attire such as gloves, and disinfection of contaminated surfaces are essential.
- Commercial vaccines are available for veterinary use.

Erysipelothrix rhusiopathiae is a thin, pleomorphic, nonsporulating gram-positive rod. First isolated from mice by Robert Koch in 1878 and from swine by Louis Pasteur in 1882, it was established as the etiologic agent of swine erysipelas in 1886 by Löffler and as a human pathogen in 1909 when Rosenbach isolated it from a patient with localized cutaneous lesions.[1] Rosenbach coined the term *erysipeloid* to avoid confusion with *erysipelas,* a superficial cellulitis with prominent lymphatic involvement that is almost always caused by group A streptococci.[2]

MICROBIOLOGY

E. rhusiopathiae is a straight or slightly curved aerobic or facultatively anaerobic bacillary organism; it is 0.2 to 0.4 μm in diameter and 0.8 to 2.5 μm in length. It is gram positive but may appear gram negative because it decolorizes readily. Organisms may be arranged singly, in short chains, in pairs in a V configuration, or grouped randomly. Nonbranching filaments, which can be longer than 60 μm, are sometimes seen. Colonial and microscopic appearance varies with the medium, pH, and temperature of incubation.[1] After growing for 24 hours at 37°C, colonies are small and transparent, with a smooth, glistening surface. On blood agar, the organism may be α-hemolytic. *E. rhusiopathiae* is negative for catalase, oxidase, indole, Voges-Proskauer, and methyl red.[2] Acid without gas is produced from the fermentation of glucose, fructose, lactose, and galactose. Most strains produce hydrogen sulfide, a diagnostically important reaction. On triple sugar iron (TSI) agar slants, hydrogen sulfide causes a blackened butt. In gelatin stab cultures, *E. rhusiopathiae* produces a distinctive "pipe cleaner" pattern of growth. *E. rhusiopathiae* is sometimes confused with other gram-positive bacilli, in particular, *Listeria monocytogenes, Trueperella (Arcanobacterium) pyogenes,* and *Arcanobacterium haemolyticum,* but these three species are β-hemolytic on blood agar and do not produce hydrogen sulfide in the butt on TSI agar slants. Furthermore, *L. monocytogenes* is catalase positive and motile.[1] The Vitek 2 and Phoenix automated systems and the API Coryne and API ID 32 Strep systems are reliable for the identification of *E. rhusiopathiae.*[3] Matrix-assisted laser desorption/time-of-flight (MALDI-TOF) mass spectroscopy has been shown to accurately and rapidly identify *Erysipelothrix.*[4–6]

EPIDEMIOLOGY

E. rhusiopathiae is found worldwide. It has been reported as a commensal or a pathogen in a wide variety of vertebrate and invertebrate species, but the major reservoir is believed to be domestic swine.[2] Mites may serve as a vector of the organism, allowing it to persist in coops and pens.[7] It does not appear to cause disease in fish but can persist for long periods in the mucoid exterior slime of these animals. It may live long enough in soil to cause infection weeks or months after initial contamination. The greatest commercial impact of *E. rhusiopathiae* infection is the result of disease in swine, but infection of poultry and sheep is also important. Human infection is a zoonosis. The organism is communicable from animals to humans by direct cutaneous contact. Abrasions or puncture wounds of the skin probably serve as the portal of entry of *Erysipelothrix* organisms in most cases of infection in humans and animals. There have been reports of bacteremia, one with endocarditis, which occurred after ingestion of undercooked pork. There was an outbreak of *E. rhusiopathiae* in racing pigeons after ingestion of compost.[8]

The risk for human infection with *Erysipelothrix* is closely related to the opportunity for exposure to the organism; accordingly, most human cases are related to occupational exposure. Although infection with *Erysipelothrix* has been associated with many occupations, people at greatest risk include fishermen, fish handlers, butchers, farmers, slaughterhouse workers, veterinarians, and homemakers.[2] Infection is especially common among people who handle fish.[9] "Whale finger" is erysipeloid seen in people who sustain cuts to the fingers and hands while engaged in whaling. Human-to-human transmission of infection has not been reported. Cases of infection that do not have an occupational link have occurred mainly in immunocompromised hosts, including children, and suggest that colonization of the oropharynx or gastrointestinal tract may occur.[10,11] Chronic alcoholism is a common underlying condition. There have been a few reports of erysipeloid after cat and dog bites.[12] Most human infections are caused by serovars 1 and 2.[3] Ribotyping, randomly amplified polymorphic DNA, pulsed-field gel electrophoresis, and nucleotide sequence analysis of the *spaA* gene are useful for typing isolates.[3]

PATHOGENESIS

Progress has been made in the understanding of the pathogenesis of *E. rhusiopathiae.* Virulence factors include a capsule, enzymes (neuraminidase and hyaluronidase), and cell wall–associated proteins such as transporter and adhesion proteins.[13,14,15,16] The capsule is composed of polysaccharide antigen and confers resistance to phagocytosis. Neuraminidase is important in attachment and entry into host cells. The surface protective antigen proteins, SpaA, SpaB, and SpaC, are major protective antigens. SpaA and SpaC elicit a protective immune response in swine and murine animal models.[17] In the absence of specific antibodies, *E. rhusiopathiae* evades phagocytosis, but even if phagocytized it is capable of intracellular replication.[13]

CLINICAL MANIFESTATIONS

Three well-defined clinical categories of human disease have been described: (1) erysipeloid, a localized skin lesion; (2) a diffuse cutaneous eruption with systemic symptoms; and (3) systemic infection, predominately bacteremia, which is often associated with endocarditis.

The localized cutaneous form—the "erysipeloid" of Rosenbach—is a subacute cellulitis and is the most common type of *Erysipelothrix* infection seen in humans. Because the organism is acquired through contact with infected animals or fish, or with products made from them, gaining entrance via cuts or abrasions on the skin, most lesions are on the fingers. After an incubation period of 2 to 7 days, pain, which is often severe and described as burning, itching, or throbbing, and swelling of the involved digit or part of the hand develop. The lesion is well defined, slightly raised, and violaceous (Fig. 209.1).[2,18] As it spreads peripherally, the central area fades. Vesiculation may occur. Regional lymphadenopathy and lymphangitis occur in approximately one-third of cases.[18] There may be inflammation of an adjacent joint. Systemic symptoms are uncommon. Approximately 10% of the patients have low-grade fever and arthralgias.[18] Clinically, erysipeloid resembles staphylococcal or streptococcal cellulitis, but a history of occupational exposure, lesions on the hands, subacute course, absence of suppuration, lack of pitting edema, violaceous color, and the disproportionate pain should suggest the possibility of erysipeloid.[19] Because organisms are located only in deeper parts of the skin in erysipeloid, aspirates or biopsy specimens should incorporate the entire thickness of the dermis, and tissue from the periphery of the lesion, to maximize the chance of recovery of the organism. Erysipeloid usually resolves without treatment within 3 or 4 weeks.



FIG. 209.1 Lesion of erysipeloid.

The diffuse cutaneous form, which is rare, occurs when the violaceous cutaneous lesion progresses proximally from the site of inoculation or appears at remote areas.[1,2] Most cases arise from percutaneous exposure. Cases have also occurred after ingestion of contaminated seafood or undercooked pork and are presumed to occur after occult bacteremia. Lesions may appear urticarial, with the rhomboid pattern characteristic of swine erysipelas.[2] Fever and arthralgias are common. Blood cultures are negative. The course is more protracted than in the localized form, and recurrence is common.

Systemic infection with *E. rhusiopathiae* is unusual. More than 100 cases of bacteremia have been reported, most complicated by endocarditis.[20,21,22–28,29,30,31] This number may be falsely elevated because of reporting bias in case reports.[32,33] Although this organism has caused prosthetic valve endocarditis, most reported cases of endocarditis have involved native valves. There was a history of an antecedent or concurrent skin lesion of erysipeloid in 36% of patients.[21] When clinical features of *E. rhusiopathiae* endocarditis were compared with those of endocarditis caused by other bacteria, there was a higher male-to-female ratio (which probably reflects occupational exposure), a greater propensity for involvement of the aortic valve, and a much higher mortality rate among patients with *E. rhusiopathiae* endocarditis (38% vs. 20% in endocarditis caused by other organisms).[27,29] In approximately 60% of patients, *E. rhusiopathiae* endocarditis developed in previously normal heart valves. In patients with bacteremia, endocarditis, or both, routine blood culture techniques are adequate for recovery of the organism.[1,3] Complications of *Erysipelothrix* endocarditis include congestive heart failure, myocardial abscess, aortic valve perforation, meningitis, brain infarctions, glomerulonephritis, septic arthritis, and osteomyelitis.[20,21,22–25,27,28] More than one-third of the patients required valve replacement.[21]

Bacteremia without endocarditis has been reported with increasing frequency.[32,33,34,35] It has occurred primarily in immunocompromised hosts.[36–38] Brain abscess, endophthalmitis, pneumonia, necrotizing fasciitis, meningitis, epidural and paravertebral abscesses, peritonitis (including peritoneal dialysis–related peritonitis), intraabdominal abscesses, psoas abscess, tenosynovitis, osteomyelitis, and septic arthritis (including infection of native joints, prosthetic joints, and a reconstructed ligament) have also been reported.[a]

DIAGNOSIS

Definitive diagnosis of infection with *Erysipelothrix* requires isolation of the organism from a biopsy specimen, blood, or other sterile body fluid. Standard methods for culturing blood or biopsy tissue should suffice if incubation is continued up to 7 days. *Erysipelothrix* grows best at 35°C in 5% carbon dioxide. Culture-based methods can be problematic and may lead to an incorrect diagnosis.[52] Despite the use of selective media to improve isolation from contaminated specimens, it is difficult to isolate *E. rhusiopathiae* from heavily contaminated specimens because of its small colony size, its slow growth rate, and the inability to inhibit all contaminants. Recommended culture media for isolation include blood or chocolate blood agar, tryptic soy, Schaedler, or thioglycolate broth.[3] There are no reliable serologic tests for the diagnosis of infection in humans. The polymerase chain reaction assay has been used for rapid diagnosis in swine and has been applied successfully to human and environmental samples.[53]

THERAPY AND PREVENTION

Susceptibility data for *E. rhusiopathiae* are limited. Most strains are highly susceptible to penicillins, cephalosporins, clindamycin, imipenem, linezolid, ciprofloxacin, and daptomycin.[48,54,55] Penicillin and imipenem are the most active agents in vitro. Susceptibility to chloramphenicol, erythromycin, and tetracycline is variable. Resistance to macrolides has been reported to be plasmid mediated.[56] Most strains are resistant to vancomycin, sulfonamides, trimethoprim-sulfamethoxazole, novobiocin,

[a]References 2, 3, 35, 39–49, 50, 51.

teicoplanin, and aminoglycosides.[57] Resistance to vancomycin is important because this agent is often used empirically to treat bacteremia caused by gram-positive organisms. A Clinical and Laboratory Standards Institute guideline for broth microdilution susceptibility testing has been published.[58] Because minimal inhibitory concentrations (MICs) of penicillin range from 0.0025 to 0.06 μg/mL and minimal bactericidal concentrations (MBCs) have been reported in the range of 0.0025 to 0.75 μg/mL, penicillin G (12–20 million units/day) is the drug of choice for serious infections caused by *E. rhusiopathiae*. Ampicillin and ceftriaxone have also been used successfully. Ciprofloxacin has MIC and MBC values similar to those obtained with β-lactam antibiotics.[54,55] Daptomycin has demonstrated in vitro activity against clinical isolates of *E. rhusiopathiae*.[59] Use of fluoroquinolones, daptomycin, clindamycin, or linezolid may be considered in *Erysipelothrix* infections when β-lactams are contraindicated. In cases of endocarditis, the duration of intravenous antibiotic therapy should be 4 to 6 weeks, although shorter courses (2 weeks of intravenous therapy followed by 2–4 weeks of oral therapy) have been successful.[2] Although erysipeloid usually resolves spontaneously, healing is hastened by antibiotic therapy. Oral therapy with amoxicillin or a quinolone can be used.

Prevention of infection in people in high-risk occupations depends on adequate hand hygiene, the use of protective attire such as gloves, and disinfection of contaminated surfaces.[2,14] Unprotected direct contact with animal body tissues and secretions should be avoided. Although commercial vaccines are available for veterinary use, research to develop more immunogenic and safer vaccines continues.[17] Use of vaccination along with other measures, such as improved waste disposal, have helped control swine erysipelas.

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The complete reference list is available online at Expert Consult.

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210 Whipple Disease

Thomas Marth and Thomas Schneider

SHORT VIEW SUMMARY

Definition
- Whipple disease (WD) is a rare systemic infectious disorder caused by the actinomycete *Tropheryma whipplei.*

Epidemiology
- Classic WD is rare and is found in middle-aged individuals, approximately three times more often in men than in women.
- *T. whipplei* is found frequently in the stools of children with acute diarrhea.
- *T. whipplei* can cause culture-negative endocarditis.

Microbiology and Pathogenesis
- *T. whipplei* is a gram-positive bacterium with high guanine and cytosine content.
- *T. whipplei* is resistant to glutaraldehyde.
- The hallmark of WD is the invasion of the intestinal mucosa with macrophages incompetent to degrade *T. whipplei.*
- An immunologic defect in the pathogenesis of WD is evident and includes macrophages, T cells, and an impaired humoral immune response.

Clinical Features and Diagnosis
- WD occurs as an acute transient disease (presenting, e.g., with fever and diarrhea in children).
- WD occurs in a localized form (e.g., endocarditis or central nervous system disease).
- WD occurs in various clinical manifestations in association with immunosuppression.
- WD occurs as a classic systemic disease with weight loss, arthralgia, diarrhea, and a possible broad spectrum of clinical signs and symptoms.
- Diagnosis is established usually by duodenal biopsy showing typical periodic acid–Schiff–positive cells in the lamina propria.
- Diagnosis should be confirmed by polymerase chain reaction assay or immunohistochemistry, which can be performed alternatively from various organ samples or body fluids.

Therapy
- Intravenous induction therapy with ceftriaxone for 2 to 4 weeks
- Followed by long-term therapy with oral trimethoprim-sulfamethoxazole for 1 year
- Close follow-up of treatment success over several years
- Complications: relapses, neurologic defects, heart valve destruction, immune reconstitution inflammatory syndrome

Prevention
- Ubiquitous bacterial agent, prevention not yet possible

Whipple disease (WD) is a rare systemic infectious disorder caused by the actinomycete *Tropheryma whipplei*. This chronic disease, first described by Whipple as "intestinal lipodystrophy," preferentially affects middle-aged white men, who may present with weight loss, arthralgia, diarrhea, and abdominal pain. Various other clinical patterns, such as involvement of the heart, lung, or central nervous system (CNS), are frequent. In addition, individuals with isolated heart valve involvement or asymptomatic carriers may be observed. The diagnosis often is established by small bowel biopsy, which is characterized by periodic acid–Schiff (PAS)–positive inclusions representing the causative bacteria. *T. whipplei* can be detected by a specific polymerase chain reaction (PCR) assay, immunohistochemistry, or electron microscopy, and it has been cultured. Several studies show that subtle defects of cell-mediated immunity exist in active and inactive WD, which may predispose individuals with a certain human leukocyte antigen (HLA) type to a clinical manifestation of *T. whipplei* infection. As confirmed in a more recent controlled trial, most patients respond well to prolonged antibiotic treatment, although some may develop an immune reconstitution inflammatory syndrome (IRIS) that may be successfully treated by corticosteroids. However, for a few patients with relapsing disease or with CNS manifestations, the prognosis may be poor.

ETIOLOGY

In 1907 Whipple found rod-shaped structures with silver stain in his original case but noted the following: "Whether this is the active agent in this peculiar pathological complex cannot be determined from the study of this single case but its distribution in the glands is very suggestive."[1] A characteristic, rod-shaped (0.25 × 1.5–2.5 µm) organism was observed by electron microscopy within cells in various stages of degradation and in the extracellular space (Fig. 210.1A). Morphologically, the organism uniformly possesses a trilaminar plasma membrane and a surrounding homogeneous cell wall of 20-nm thickness with two inner layers and an outer trilaminar membrane-like structure. The bacillus can be found typically in macrophages of the lamina propria of the small intestine and its lymphatic drainage but also has been observed in endothelial and epithelial cells, muscle cells, and various cells of the immune system, including polymorphonuclear leukocytes, plasma cells, mast cells, and intraepithelial lymphocytes.[2] The organisms disappear from the lamina propria on antibiotic-induced clinical improvement, which argues for their etiologic significance.[3]

Specific segments of bacterial 16S ribosomal RNA from duodenal lesions of patients were amplified genomically using PCR assay.[4] The sequence analysis enabled the classification of the causative organism phylogenetically as a new genus and species, and the name *Tropheryma whippelii* (*trophe*, "nourishment," and *eryma*, "barrier") was proposed.[4]

After several unsuccessful attempts at culture, the Whipple bacillus isolated from heart valve tissue tentatively had been propagated for a short time in peripheral blood mononuclear cells deactivated by interleukin (IL)-4 and IL-10.[5] In 1999 the growth of the organism was stably established in human fibroblast (HEL) cells in minimal essential medium with 10% calf serum.[6] Because the bacterial strains have been deposited in bacterial collections, the name of the Whipple bacillus officially was corrected to *T. whipplei.*[7] The bacterium is slow growing, with an estimated doubling time of 17 days; under certain in vitro conditions (e.g., axenic medium), it may be shorter.[6] The site of multiplication of *T. whipplei* in vivo remains controversial. It has been suggested that bacteria multiply in the digestive lumen, become phagocytized, and then are degraded in macrophages.[2] *T. whipplei* replicates within peripheral blood mononuclear cells (which release the bacteria), and within macrophages of WD patients; it also replicates within HeLa cells, where the bacillus actively multiplies in acidic vacuoles at pH 5.[8] This high acidity, which may impair antibiotic activity, is a probable cause

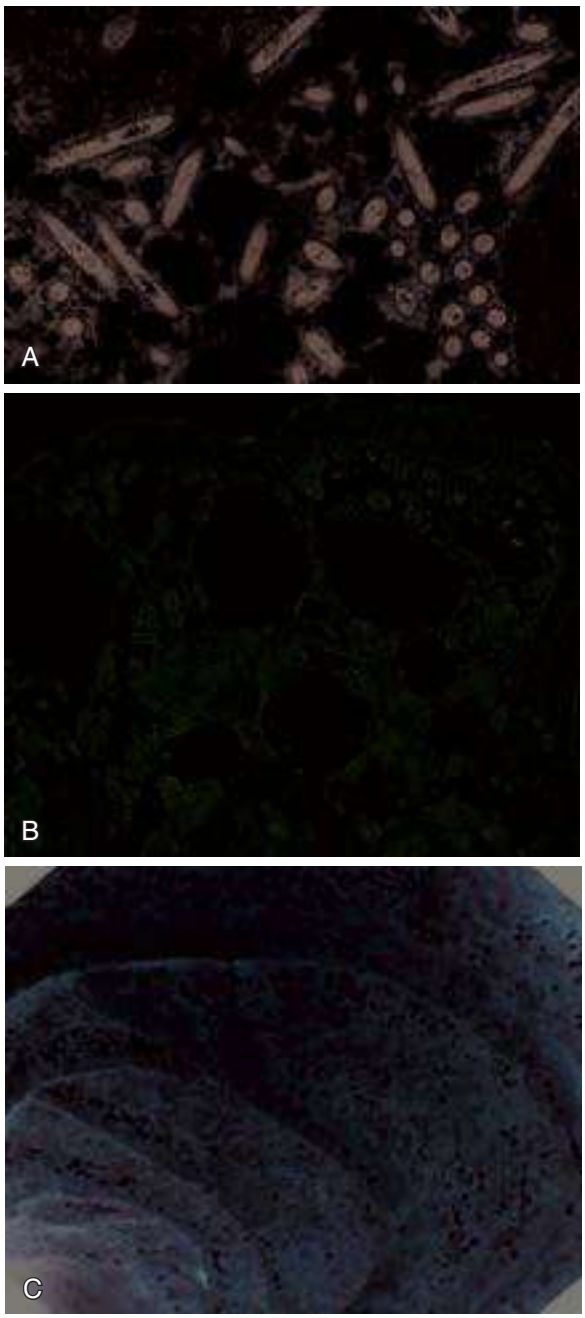

FIG. 210.1 Whipple disease. (A) Electron microscopic view of the Whipple bacillus, *Tropheryma whipplei.* The characteristic, rod-shaped (0.25 × to 2.5 μm) organism can be observed in the extracellular space in florid disease or within cells in various stages of degradation. The bacillus is found typically in macrophages of the lamina propria of the small intestine. *T. whipplei* is characterized by a trilaminar plasma membrane, a surrounding homogeneous cell wall of 20-nm thickness, and an outer trilaminar membrane-like structure (×20,000). (B) Periodic acid–Schiff stain of duodenal biopsy specimen in a patient with Whipple disease. Large numbers of purple-stained macrophages in the lamina propria and lymphectasia can be seen. (C) Endoscopic picture of distal duodenum with whitish yellow plaques representing engorged lymphatic vessels sometimes found in classic Whipple disease. *(B courtesy C. Loddenkemper, Berlin; C from Schneider T, Moos V, Loddenkemper C, et al. Whipple's disease: new aspects of pathogenesis and treatment.* Lancet Infect Dis. *2008;8:79–90.)*

for the lack of efficacy of some antibiotics. *T. whipplei* is resistant to glutaraldehyde.[8]

The genome of *T. whipplei* has been sequenced and deposited in Genbank.[9,10] *T. whipplei* has a single circular chromosome and small genome size (925 kilobases), and it seems to possess characteristics that suggest immune evasion and host interaction play an important role in its replication. In particular, *T. whipplei* seems to have a high degree of sequence variation and lacks several important biochemical pathways, including unique deficiencies in amino-acid biosynthesis (e.g., no thioredoxin) and carbohydrate metabolism, suggesting a host-dependent lifestyle.[9,10]

The organism has been placed phylogenetically within the subdivision of gram-positive bacteria with high guanine and cytosine content, the actinomycetes, and is closely related to several actinobacteria, nocardioforms, and cellulomonads. Genomic variants are associated with neither the geographic residence of the patients nor the organotropism of the agent.[11]

Similar to some of its phylogenetic relatives, *T. whipplei* occurs in the environment; it has been found by PCR assay in sewage water, in sewage plant workers, in saliva, and in human feces.[8,12,13] It is speculated that *T. whipplei* may contaminate humans through drinking water. Humans are the only known host in which *T. whipplei* can replicate, and all environmental sources are in relation to humans and their wastes; therefore it may be speculated that the agent has a sporelike formation that facilitates survival in the environment and may explain its high tenacity, even against glutaraldehyde.

EPIDEMIOLOGY AND PATHOGENESIS

The spectrum of infections resulting from *T. whipplei* has been increasingly recognized by the availability of molecular detection methods and new culture techniques. The bacterium was found in the stool of young children with gastroenteritis; in a study including 241 samples from children aged 2 to 4 years, *T. whipplei* was found in 15% of cases; one-third were coinfected with other diarrhea pathogens.[14] In Europe the prevalence of the bacterium in fecal samples from healthy adults ranges from 1% to 11%. In addition, the bacterium has been detected in sewage and is more prevalent in the fecal samples of healthy sewage workers (12%–26%) compared with the general population.[12,13] *T. whipplei* has also been associated with fever, cough, and sleep disturbances in rural West Africa.[15] These conditions, including isolated Whipple endocarditis, should be separated from the classic form of WD, which was described initially and is characterized by certain immune dysfunctions.

Tropheryma whipplei was also found more frequently in the bronchioalveolar lavage from human immunodeficiency virus (HIV)-positive individuals, compared with HIV-negative ones.[16]

Classic Whipple disease (CWD) is rare, and no valid estimation on the incidence is available. The prevalence recently has been estimated at approximately 10 per 1 million people.[16a] The disorder has been described most frequently in whites. Only rare occurrences have been reported in Hispanics, African Americans, Native Americans, and Asians.[2] It has been presumed that the disease may occur in local clusters, that many patients reside in rural areas, and that farming often is found among the documented occupations.[2,8] Specific environmental factors or habits have not yet been associated with WD.

WD occurs primarily in middle-aged individuals, with a mean age at diagnosis of about 50 years. In the published literature the disease is approximately eight times more common in men than in women[2,8]; newer series find a relatively higher incidence in women (male-to-female ratio 3:1).[17] There are only several case reports of familial clusters (brother pairs, father/daughter), and most of the analyzed cases do not exhibit familial components. A polygenic susceptibility was suggested, and in a larger European cohort, an HLA association (to DRB1*13 and DQB1*06, but not B27) has been observed. The cumulative odds ratios for disease were 2.23 for the DRB1*13 allele ($P < .0001$) and 2.25 for the DQB1*06 allele ($P < .0001$).[18] Recently, a gene defect of a transcription factor involved in immunity has been detected in some patients with familial WD.[18a] The interferon regulating factor 4 (IRF4) haploinsufficiency may be a rare but important factor to predispose for *T. whipplei* infection in certain individuals and in an age-dependent manner.

An immunologic defect in the pathogenesis of WD is probably based on the specific features of the *T. whipplei* genome, suggesting an immune evasion and host-adapted lifestyle, and the very low disease incidence despite the ubiquitously present bacteria. The protean clinical picture, years ago, led to immunologic studies. The immunologic defect in WD

is subtle and specific for the WD organism because patients with the disease usually are not predisposed to infections with other organisms. As an exception, a few case reports have pointed out that WD also may occur in immunodeficient states, in immunosuppression, or concomitant with other infections (e.g., in patients with acquired immunodeficiency syndrome [AIDS] or in patients with *Nocardia* or *Giardia* infections).[8] Newer data point to the possibility that intestinal manifestations (i.e., diarrhea) in WD are triggered by medical immunosuppression, which is given for the treatment of unclear arthropathy.[19]

The immunohistologic features of the lamina propria in WD are unique because the intestinal tissue shows a relative paucity of lymphocytic infiltration, including plasma cells, despite the massive and unusual influx of macrophages, which persist even after completion of treatment.[20] *T. whipplei* is found and replicates mostly intracellularly but also may be metabolically active extracellularly. Although the lack of lymphocytic infiltrate and disturbed immunity in WD could be attributed partially to an intestinal lymphangiectasia, many studies have found a more profound phenotypic and functional alteration of immunologic features in patients with WD.

It has been shown that lamina propria and circulating populations *on the T-cell level* in active WD are characterized by a reduced $CD4^+/CD8^+$ T-cell ratio, a shift toward mature T-cell subpopulations (e.g., CD45RO expression increased, CD45RA expression decreased), and increased cell activation markers.[21] In addition, disturbed function of peripheral T cells, that is, by reduced T-cell proliferative responses to various stimuli and impaired delayed-type hypersensitivity reaction to skin antigens (mostly recall antigens), has been found even in patients with long-standing remission.[21] Newer studies show that *T. whipplei*–specific $CD4^+$ cells are reduced in the periphery and on the mucosal level,[22] and patients exhibit diminished production of Th1 cytokines in response to *T. whipplei* and other antigens.[22,23] In contrast, there is an increase in functional Th2 responses in peripheral T cells,[22,23] lending support to the observation that *T. whipplei* replicates in cytokine (IL-4 and IL-10)-deactivated macrophages,[5] and that the cytokine milieu might inhibit dendritic cell maturation. In the lamina propria of untreated CWD patients, the number of $CD4^+$ T-regulatory (Treg) cells is increased, whereas the percentages in the peripheral blood were similar in CWD patients and healthy control subjects.[24] However, peripheral Treg cells of CWD patients were more activated than those of control subjects. Elevated secretion of IL-10 and transforming growth factor-β in the duodenal mucosa of CWD patients indicated locally enhanced Treg activity. Thus the increased numbers of Treg cells in the duodenal mucosa in untreated CWD might contribute to the deactivation of macrophages and to the chronic infection and systemic spread of *T. whipplei* in CWD. On the other hand, it may prevent a mucosal barrier defect by reducing local inflammation.[24] As a further indication for the pathogenetic relevance of the impaired cellular immune responses, it was reported that in one patient with WD refractory to antibiotic regimens and with reduced interferon-γ (IFN-γ) levels in vitro, treatment with antimicrobials and supplemental recombinant IFN-γ led to the rapid clearance of the infection.[25]

Studies *on the B-cell level* have found reduced numbers of immunoglobulin A (IgA)-positive cells but increased numbers of surface IgM-positive B cells in the lamina propria.[26] Recent studies on a greater number of Whipple patients revealed that all immunoglobulin-producing cells are reduced in Whipple patients compared with control subjects. Secretory IgA production is especially impaired in WD patients. However, this deficiency does not completely abolish *T. whipplei*–specific secretory IgA production.[27] Humoral immune responses to infectious agents in the periphery and total serum IgG levels usually are normal, whereas IgM and IgG2 subclass levels may be decreased, and IgA is increased in acute stages of the disease.[2,8,27,28]

Studies *on the macrophage level* in WD have been sparse for some years. Macrophages from patients with WD manifested decreased intracellular degradation of several organisms, and intestinal macrophages showed some decrease of phagocytosis. In addition, patients with WD have reduced numbers of circulating cells expressing CD11b, a molecule that serves as a facilitator of microbial phagocytosis/antigen processing and mediates IFN-γ–induced intracellular killing of ingested bacteria.[21,28] Impaired functions of antigen-presenting cells in WD may be related to reduced monocyte and macrophage IL-12 production.[20,28] IL-12 is a cytokine that has important functions in regulating cell-mediated immune responses. More recent investigations have shown that intestinal macrophages show an alternatively activated phenotype and express (the Th2-type cytokine) IL-10.[29] *T. whipplei* is able to replicate in monocytes and macrophages from normal individuals if deactivated with IL-16, and neutralizing antibodies to this cytokine inhibit the growth of the bacterium.[29] In addition, because IL-16 is a stimulator of macrophage apoptosis, high levels of IL-16 might promote the dissemination of *T. whipplei* and correlate with the activity of the disease. Recent studies on a greater number of WD patients confirmed the alternative activated phenotype of the macrophages in the lamina propria, which correlated with a lack of excessive local inflammation and may explain in part the hallmark of WD—invasion of the intestinal mucosa with macrophages incompetent to degrade *T. whipplei*.[20]

Together, the local immune response on the mucosal level is apparently insufficient to kill ingested bacteria in patients with WD. Despite the more recent findings describing the subtle and persistent immune disturbance causing a disturbed phagocytosis and intracellular degradation of *T. whipplei*, it remains unclear whether the immune defect is primarily macrophage or T-cell related.

PATHOLOGY

On gross inspection the duodenum and jejunum, which are the sites most frequently affected, often appear thickened and edematous.[2] The infiltration of the bowel wall is associated with a widening and flattening of the villi, with dilatated lacteals containing yellow lipid deposits that are the result of a blockade of villous lymphatics. Based on these and similar observations in the draining mesenteric lymph nodes, G. H. Whipple assumed, in 1907, a disorder of fat metabolism and suggested the name *intestinal lipodystrophy*.[1] Pathophysiologically, the disturbance of the villous architecture is presumably the cause of the steatorrhea and the subsequent malabsorption syndrome that accompany the disease. In addition, the intestinal lymphangiectasia and protein-losing enteropathy seem to be mainly secondary to the lymphatic blockage.[2]

Light microscopic examination of duodenal biopsy specimens in WD usually reveals the typical infiltration of the lamina propria with large macrophages (also called *sickle-form particle-containing cells*) containing granular-foamy, purple-stained, PAS-positive inclusions that are diastase resistant, silver positive, and often gram positive; they represent more or less intact remnants of ingested bacteria (see Fig. 210.1B).[2,3,8] The PAS positivity is believed to be a reaction with bacterial capsular mucopolysaccharides located in the cell wall, as noted first in 1949. In florid disease undigested extracellular bacteria also are seen.[2] Although it seems reasonable to assume that the route of infection occurs through the intestinal lumen, which results in a secondary accumulation of *T. whipplei* in the cells of the lamina propria, another possibility is the penetration of the bacillus through the intestinal lymphatics because the most viable organisms were seen at the base and not the apex of the epithelial cells.[2,8]

Because WD is a systemic disease, PAS-positive macrophages, positive reactions to *T. whipplei* with PCR or immunohistochemistry, and electron microscopically detectable bacilli have been shown in many cell types, as noted earlier, and in almost all organs and in body fluids, including the heart, lung, CNS, cerebrospinal fluid (CSF), eye, vitreous humor, liver, spleen, ascites, lymph nodes, endocrine glands, joints, synovium, and bone marrow.[2] The involvement of heart valves in histopathologic investigations, mostly the aortic and mitral valves, warrants special mention because it may lead frequently to clinical symptoms or isolated valve manifestations and is present in more than one-third of autopsy cases. Other frequent pathologic features include pericarditis or myocarditis, pleuritis, hepatosplenomegaly, ascites or polyserositis, uveitis and ependymitis, cortical atrophy, and demyelination of the CNS.[2]

CLINICAL FEATURES AND DIAGNOSIS

The molecular characterization of *T. whipplei* and new data based on its presence in acute disease, in localized body compartments, and in healthy people has led to a much better understanding of the nature of the infection. This allows definition of the following clinical categories (Table 210.1):

TABLE 210.1 Clinical Features and Spectrum of *Tropheryma Whipplei* Infection

	TRANSIENT AND ACUTE	ASYMPTOMATIC	LOCALIZED	CLASSIC	ASSOCIATED WITH IMMUNOSUPPRESSION
Acronym	TWD	AWD	LWD	CWD	IS→WD
Description	Occurs mostly in children with diarrhea and fever (in Africa)	Asymptomatic carriers. More frequent, e.g., in sewage workers	Localized infection, e.g., endocarditis or CNS disease without systemic symptoms	Classic form with weight loss, arthralgia, diarrhea, and a broad spectrum of other (systemic) symptoms	Occurrence of diarrhea or localized disease after immunosuppression. Coinfection in HIV patients, IRIS
Remarks	Newly recognized. Probably frequently occurring (up to 75%). Frequency in western countries yet unknown. Points to fecal-oral route of infection	Frequency varies from 1%–26%. Development to CWD unproven, presumably rare	LWD is most frequent form of culture-negative endocarditis. Severe CNS damage possible. LWD is very difficult to diagnose	CWD is rare. Published cases exemplify the immense spectrum of clinical symptoms	Protean clinical features hinder early diagnosis. IS→WD may be explained by parasitic features of *T. whipplei* and immune defects

AWD, Asymptomatic Whipple disease; *CNS,* central nervous system; *CWD,* classic Whipple disease; *HIV,* human immunodeficiency virus; *IRIS,* immune reconstitution inflammatory syndrome; *IS,* immunosuppression; *LWD,* localized Whipple disease; *TWD,* transient Whipple disease; *WD,* Whipple disease.

1. *Transient and acute infection with T. whipplei/transient and acute Whipple disease (TWD).* This has been shown so far most impressively in French and African children. In the course of short-term and self-limiting diarrheal disease, up to 75% of patients may excrete *T. whipplei* in their stools, have *T. whipplei* bacteremia, or cough.[14,15,30] TWD argues, along with a high intrafamilial *T. whipplei* occurrence,[31] that the bacterium is a transmissible agent, for instance, via the fecal-oral route.
2. *Asymptomatic carriers of T. whipplei/asymptomatic Whipple disease (AWD).* In these cases most of the individuals pass the agent into their stool. *T. whipplei* is present in saliva, or individuals may carry serum antibodies. Presumably, most have acquired *T. whipplei* via a subclinical or short-term acute infection.[14,15,30] Very few will develop systemic WD later in their lives. An increased proportion (≈12%–26%) of asymptomatic carriers were found in workers who have close contact with sewage.[12,13] The bacterial load in the stool of these patients is lower than in untreated patients with CWD.
3. *Localized T. whipplei infection/localized Whipple disease (LWD)* without systemic or CWD. This manifestation occurs most frequently as culture-negative bacterial endocarditis, for instance, on degenerative valve lesions, leading to a slowly progressive valve damage.[32] In addition, LWD can be found in the eye, CNS, lymph nodes, and bone.
4. *Classic and systemic Whipple disease (CWD),* with the leading symptoms of weight loss, diarrhea, and arthropathies. In 75% of cases these symptoms are found together by the time of diagnosis.[8,33] The clinical presentation of patients may vary to a great extent, however, because of the differential organ involvement and the stage of the disease. In cases of CWD a reduced $CD4^+$ T-cell response against *T. whipplei* is present.[22]
5. *Whipple disease in association with immunosuppression (IS→WD).* In some instances immunosuppressive therapy, such as tumor necrosis factor blocker therapy, is initiated in patients with unclear arthritis. Some of these patients may be diagnosed with WD at a later time point and may present with a heterogeneous clinical picture.[34–36]

Immunosuppressive treatment may favor the occurrence of diarrheal disease, leading to the diagnosis of systemic WD.[19] Alternatively, medical immunosuppression therapy may lead to isolated endocarditis, spondylitis, or CNS manifestations.[32,35,37,38] In many of these cases the clinical picture before WD diagnosis is considered to reflect the symptoms of the underlying autoimmune disorders.[39] This form of WD, underscoring the role of *T. whipplei* as opportunistic invader,[9,10,19,22,23] is of major importance because the diagnosis probably often could be established earlier. In some cases WD is diagnosed in association with malignancies (e.g., lymphomas) that could be due to prior immunomodulation or chemotherapy.[8,38] In addition, after the use of immunosuppressive therapy, WD has a more complicated course after the initiation of therapy (see discussion of IRIS later) (see Table 210.1).

The clinical features of CWD are described in more detail, in many instances (63% in our large series)[33] with CWD beginning insidiously with arthropathy. This symptom may precede the diagnosis by a considerable length of time (mean, 8 years in one series, up to 30 years),[33] and consists usually of chronic migratory, nondestructive, and seronegative joint disease involving predominantly the peripheral joints; in addition, it often is accompanied by myalgias.[2,8] Because new diagnostic tools enable detection of *T. whipplei* in the synovial fluid, these patients may now be diagnosed earlier[40,40a] (Table 210.2).

Weight loss is found nearly invariably in all patients with CWD at the time of diagnosis. We found that weight loss was present in two-thirds of patients more than 4 years before diagnosis and was clinically relevant (often loss of ≥20% of initial weight).[17,33]

Gastrointestinal (GI) symptoms, which usually begin later and ultimately often lead to diagnosis, consist of episodic and watery diarrhea or steatorrhea, in many cases accompanied by colicky abdominal pain and occult blood in the stool.[41,42] These symptoms and concomitant anorexia may lead to the full picture of a malabsorption syndrome with severe weight loss, weakness, and general cachexia, and it may be associated with ascites.

Systemic symptoms occur frequently (i.e., in about half of patients with WD). These symptoms consist of intermittent, mostly low-grade fever and night sweats. Other frequent features of WD are peripheral and abdominal lymphadenopathy; mesenteric lymphadenopathy is found often in radiologic investigations but also may manifest as an abdominal mass. Skin hyperpigmentation, particularly affecting light-exposed skin, has been observed. Chronic, nonproductive cough, chest pain, and reversible pulmonary hypertension have been described.[43] In 3% of bronchioalveolar lavages of intensive care unit patients and in 13% of HIV patients, *T. whipplei* has been found by PCR assay.[16,44] Pleuritis, polyserositis, ascites, hypotension, and edema are among other frequently found signs and symptoms. Hepatomegaly or splenomegaly may be present in some patients with this disorder. Less frequently, involvement of the genitourinary system and the endocrine system have been reported (see Table 210.2).[2,8]

Cardiac involvement in CWD may occur. In recent years endocarditis with *T. whipplei* (LWD) is reported more frequently without CWD (no other evidence of clinical WD and duodenal biopsy may be negative).[32] These cases seem to be of increasing clinical relevance. Clinical symptoms depend on the stage of the disease and may include cardiac murmur and valve (often aortic or mitral) insufficiency, leading finally to valve replacement. Patients are typically diagnosed by histology and PCR assay on the explanted valve.[32,40] Patients often do not fulfill Duke criteria for infective endocarditis and progress more slowly than patients with typical streptococcal or staphylococcal endocarditis. In one large retrospective series, greater than 6% of all infective endocarditis cases were due to *T. whipplei* infection. Thus it was the most frequent cause of culture-negative endocarditis.[32]

A major and frequently overlooked area of involvement in CWD is the CNS. This involvement manifests most often as memory disorder, personality change, and dementia. Other frequent clinical signs include ophthalmoplegia, nystagmus, or myoclonia. These often may be found in combination with a disturbed sleep pattern, ataxia, seizure, or

TABLE 210.2 Signs and Symptoms in Classic Whipple Disease

	INCIDENCE RANGE (%)[a]
Major Clinical Features	
Weight loss	75–100
Arthropathy	70–100
Diarrhea	70–85
Abdominal pain	30–90
Frequent Signs and Symptoms	
Fever	35–60
Lymphadenopathy	35–60
Cardiac murmurs	30–40
Hyperpigmentation	25–60
Occult bleeding	20–30
Hypotension	15–60
Chronic cough	10–20
Splenomegaly	10–20
Hepatomegaly	5–15
Ascites	5–10
Other Clinical Features	
Pleuritis, pleural effusion, endocarditis, muscle wasting, glossitis, peripheral neuropathy, eye involvement (e.g., visual loss, uveitis, retinitis)	
Involvement of central nervous system (e.g., dementia, ophthalmoplegia, myoclonus, ataxia, nystagmus)	
Organ-Specific Involvement	
Gastrointestinal tract	95–100
Cardiac involvement	55
Pulmonary involvement	50
Central nervous system	20–30
Ocular involvement	10

[a]Incidence ranges reported in the literature: references 2, 8, 33, 38, and 45.

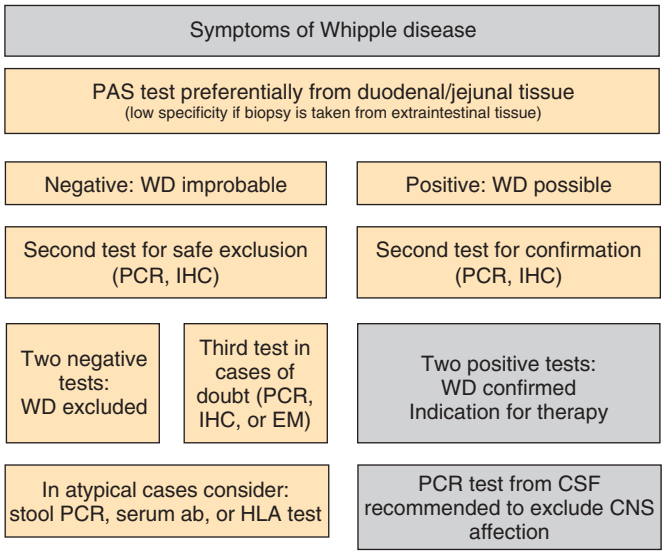

FIG. 210.2 Diagnostic algorithm for detection of Whipple disease *(WD)* based on classic histology, polymerase chain reaction, and immunohistology. Tissues for diagnostic sampling should be prepared in accordance with the symptoms of the patient. If only one diagnostic tool results in a positive finding, it should be confirmed by a second method. If alternative tests are not practicable with the samples available, additional tissue samples should be analyzed to avoid false-positive results. *ab,* Antibody; *CNS,* central nervous system; *CSF,* cerebrospinal fluid; *EM,* electron microscopy; *HLA,* human leukocyte antigen; *IHC,* immunohistochemistry; *PAS,* periodic acid–Schiff; *PCR,* polymerase chain reaction.

symptoms of cerebral compression (because of hydrocephalus). Various cranial nerve symptoms, such as hearing loss and blurred vision, have been reported. In some patients a specific, if not pathognomonic, oculomasticatory myorhythmia or myoclonus with ophthalmoplegia has been described.[38,45] There seem to exist some characteristic radiologic signs of CNS WD by using specific sequences ("flair" and "T1" sequences) of magnetic resonance imaging.[46] CNS symptoms have a frequency of about 15%. CNS and ocular symptoms, such as blurred vision or ophthalmoplegia, may occur with minimal or absent GI involvement (i.e., localized WD).[45]

The diagnosis of CWD usually is made by upper endoscopy. Endoscopically, WD findings often are described as a pale yellow shaggy mucosa alternating with an erythematous, erosive, or mildly friable mucosa in the postbulbar region of the duodenum or the jejunum; alternatively, whitish yellow plaques may be seen (see Fig. 210.1C). Magnification endoscopy may reveal edematous mucosa with blunted microvilli and yellow spots representing engorged lymphatic vessels. WD has been diagnosed recently with newer endoscopic techniques (e.g., i-scan, confocal laser, capsule endoscopy).[47,48] More than three sufficiently sized and deep specimens should be taken from different parts, such as from the proximal and the distal duodenum or the jejunum, because involvement can be patchy or submucosal.[8] The diagnosis usually can be established if the characteristic PAS-positive material is present in the lesions (see Fig. 210.1B). Endoscopy is also an important part of patient follow-up. The duodenal mucosa recovers during the first weeks to months of antibiotic therapy, whereas the PAS-positive material in the macrophages may persist in single cases for several years; an increase of PAS-positive material may be the first indicator of a relapse.[33] Based on clinical manifestations, other samples may be tested, such as CSF, cardiac valve tissue, lymph nodes, or synovial tissue (Fig. 210.2).[40]

There are several histopathologic pitfalls in the diagnosis of WD. Involvement of the GI tract or lymphatic tissue may be accompanied by noncaseating, epithelioid cell (sarcoid-like) granulomas. Sarcoid-like granulomas have been found in patients with CWD in other organ systems and the skin. Infections with *Rhodococcus equi* and *Mycobacterium tuberculosis* complex in patients with AIDS and infections with fungi, *Histoplasma* spp., and others, may be histologically similar to WD, some of which may be ruled out by a Ziehl-Neelsen stain. Lipid deposits and lymphangiectasia have to be differentiated. Biopsy specimens taken from the colon or the rectum can be misleading because of other conditions that are accompanied by PAS-positive cells (e.g., melanosis coli or histiocytosis).[2,40]

Although the clinical picture, together with a pathognomonic PAS-positive histology from the duodenum, usually may be sufficient to establish the diagnosis, a specific diagnostic test, such as PCR and/or immunohistochemistry, is recommended in every newly identified patient; it is mandatory in cases of doubt or if the diagnosis is based on extraduodenal tissue (see Fig. 210.2). This recommendation reflects the fact that PAS staining is of limited value in extraintestinal tissue and for monitoring the effect of therapy.

Electron microscopy now is used only infrequently for diagnosis.

A large amount of experience has been gained with PCR-based detection of *T. whipplei* DNA. PCR and quantitative PCR of *T. whipplei* in stool or saliva in individuals with TWD, AWD, LWD, or CWD differ to a great extent (e.g., in stool approximately 1%–75%).[12–15,30,48a] Quantitative PCR of saliva and stool are not helpful in establishing diagnosis of WD requiring therapy but could be used for noninvasive screening or follow-up purposes in the future. Newer studies show that PCR-based *T. whipplei* diagnosis by urine samples in untreated patients is interesting.[48b] The diagnosis of CWD is highly probable if the bacterial load is high in these specimens. In these cases the diagnosis should be confirmed by upper endoscopy and PAS staining of small intestinal biopsy specimens.

TABLE 210.3 Comparison of Clinical Data and Immunological Studies Versus Predictions From Newly Described Gene Defect (IRF4 Haploinsufficiency) in Patients with WD

	PREVIOUS DATA	PREDICTIONS FROM IRF4 GENE DEFECT
Age	Mean age at diagnosis approximately 50 years	Disease onset in older patients due to age-dependent incomplete penetrance
Sex	Male predominance (male:female ratio from 3:1–8:1)	Autosomal dominant; male preference possible
Organ involvement	Endoscopy and histopathologic studies: *Tropheryma whipplei* is almost invariably found in duodenum and jejunum	IRF4 gene and protein is expressed in a high level in the small bowel
Bacterial distribution	Although *T. whipplei* is ubiquitously present and high numbers of asymptomatic persons (carriers) have been found, only a small number of patients develop WD	Infection with *T. whipplei,* which is found in water and soil, develops to WD only in predisposed individuals. Heterozygous IRF4 mutation probably favors the development of chronic *T. whipplei* carriage.
Immunologic features	Defects in Th1 pathways, reduction of IFN-γ and IL-12, increase in Th2/Th17 cytokine and pathways, particularly on the mucosal level	IRF4 is a transcription factor involved in immunity. Affected patients have reduced Th1 and IFN signaling. Disease mechanism is subtle and specifically affects protective immunity to *T. whipplei.*
Disease course and relapses	Several relapses may occur in the same patient and even with different *T. whipplei* strains. Familial WD cases has been described.	Inheritance of a gene defect: autosomal-dominant genetic disease predisposing for infection
Open questions	—	IRF4 protein levels are higher in the cytoplasmic compartment (not in the nucleus) in patients with the mutation. It is therefore possible that a modifier allele at another locus contributes to the development of WD.

IFN, Interferon; *IL-12,* interleukin-12; *WD,* Whipple disease.

Before definitive diagnosis, and particularly when atypical cases are reported, the use of at least two PCR tests, based on primers obtained from two different genes to avoid a false-positive result caused by contamination, or the use of immunohistochemistry is recommended. Sample specimens usable for PCR-based diagnosis of WD are duodenal biopsy, synovial fluid, lymph node, cardiac valve, vitreous humor, and CSF.[8,13,40] Culture of *T. whipplei* is performed only in highly specialized laboratories and requires several weeks.[6,7]

The clinician always has to interpret the histopathologic and laboratory findings, in diagnostic and monitoring situations, in the context of the clinical features of the patient (i.e., a positive PCR test without clinical correlation should not result in the initiation of treatment). In unclear situations it is advisable that specialists usually should be consulted. Fig. 210.2 is an algorithm for the suggested diagnostic approach.

Laboratory testing can reveal evidence of malabsorption and protein-losing enteropathy: reduced serum levels of β-carotene, various vitamins (B_{12}, D, K, and folic acid), albumin, cholesterol, and electrolytes; lymphocytopenia; elevated stool fat excretion; and reduced D-xylose absorption.[2,19,33] Some patients with WD have eosinophilia and abnormalities of serum immunoglobulins, such as low IgM, IgG2, or high IgA.[2,27,28,38] Finally, other, less specific laboratory abnormalities in WD include elevated erythrocyte sedimentation rate and elevated acute-phase proteins, such as C-reactive protein, thrombocytosis, and hypochromic anemia.[2,19,38]

Finally, it is interesting to note that many data and observations from previous clinical and immunological studies concur well with predictions from the newly described *IRF4* gene defect.[18a] This is depicted in Table 210.3.

THERAPY AND PROGNOSIS

WD was considered to be a fatal disorder before empirical establishment of antibiotic therapy in the 1950s. Antibiotic therapy leads to a rapid improvement of the clinical status in most patients with WD and to a lasting remission. Diarrhea and fever may disappear within 1 week of therapy, whereas arthropathy and other symptoms often are improved after 2 to 4 weeks. The laboratory findings normalize often over several weeks. Clinical improvement usually is accompanied by a gradual reconstitution of the villous architecture of the small intestine and by a disappearance of the bacteria over several weeks.[2,3,33] Finally, immunologic parameters, such as increased IgA or shifts in T-cell subpopulations, return to normal within several months. In contrast, the subtle defect in cell-mediated immunity persists for years, if not indefinitely.[21,22]

Current treatment recommendations are based not only on retrospective analysis of small patient cohorts but, since 2010, also on a prospective randomized controlled trial.[17] Recommendations include an induction treatment for 2 weeks with intravenous antibiotics that achieve high CSF levels, such as ceftriaxone or meropenem (Table 210.4). This is followed by 1-year (continuous) treatment with oral cotrimoxazole (trimethoprim-sulfamethoxazole) twice daily. The results of the therapy trial show a very high rate of clinical remission, which might be due to better adherence of medication intake during controlled studies.[17] Because new study data argue for a short treatment duration of, for instance, 3 months,[49] an issue that is currently still controversial, and others argue for a lifelong treatment, further treatment trials are awaited. So far, short-term treatment should be reserved for clinical studies.

TABLE 210.4 Recommended Treatment in Whipple Disease

Initial Parenteral Therapy (Intravenous)

General therapy: 2 weeks, ceftriaxone, 2 g daily
Central nervous system disease: 4 weeks, ceftriaxone, 2 g daily
Endocarditis: 4 weeks, ceftriaxone, 2 g daily; or penicillin G, 10 million units daily
Alternatives in cases of allergy or relapse: meropenem or penicillin

Long-Term Therapy (Oral)

At least 1 year of trimethoprim-sulfamethoxazole, 160/800 mg twice daily
Alternatives in cases of allergy or relapse: doxycycline, 100 mg twice daily plus hydroxychloroquine, 200 mg three times daily

Although most patients recover completely and long-lastingly after antibiotic therapy, on occasion, inflammation reappears (mostly as high and recurrent fevers) after initial improvement; this is often interpreted as refractory or recurrent disease. However, PCR assay for *T. whipplei* in tissue of these patients is frequently negative, indicating absence of vital bacteria, and this reinflammation does not respond to antimicrobials but does respond to corticosteroids. This pathologic condition was first described in HIV patients with low CD4 T cells after initiation of highly active antiretroviral therapy and was named immune reconstitution inflammatory syndrome (IRIS). In a recent study IRIS was found in approximately 10% of patients with WD, with an outcome that varied from mild to fatal. The great majority of WD patients who developed IRIS had previous immunosuppressive therapy, which has been given under the suspected diagnosis of rheumatic disease. IRIS should be considered in patients with WD in whom inflammatory symptoms recur after effective treatment. Early diagnosis and treatment with corticosteroids may be beneficial.[50]

In patients with CNS symptoms, neurologic defects are difficult to reverse. Although nonspecific focal lesions of the brain and

ophthalmoplegia and other movement disorders respond better to antibiotic treatment, other more structural changes, such as granulomas, infarcts, abscesses, or atrophic changes, may lead to persistent symptoms or even to fatal courses.[38,40,45] Additional treatment with corticosteroids has been beneficial in cases of severe structural CNS manifestations (and in patients with IRIS; see earlier), a therapy that may reduce local inflammation, edema, and endothelial damage.[40]

In cases of allergic reaction to ceftriaxone, an alternative induction therapy may consist of meropenem or penicillin. A possible alternative to long-term oral cotrimoxazole may be doxycycline (2 × 100 mg/day; in some cases minocycline has been used) in combination with hydroxychloroquine (600 mg/day), which enhances the in vitro activity of doxycycline by increasing the pH in the phagolysosomes of the macrophages.[51,52] This treatment is based on molecular data of resistance against sulfamethoxazole,[53] a finding that does not yet reflect broad clinical experience.

Relapsing disease may occur in some patients despite an adequate and prolonged antibiotic treatment, still after many years of remission, more than once, and remarkably even with different strains of *T. whipplei*.[8,54] Relapses of CNS disease, particularly, may have a detrimental course. Additional supportive therapy with Th1 cytokines (IFN-γ) has been described as beneficial.[25] As mentioned, the immunologic defect seems to be specific for *T. whipplei* because a correlation with other infectious diseases was not found.

All patients should be followed with duodenal biopsies and, in cases of cerebral involvement, with CSF analysis at 6 months and 1 year after diagnosis. If PAS-positive material is absent after 1 year and no bacteria are detected by PCR assay, antibiotic therapy can be stopped. We recommend, even in successfully treated patients in whom therapy has been discontinued, performing follow-up biopsies at increasingly longer intervals for at least 5 years after the establishment of diagnosis. If bacterial material persists after 1 year of treatment, therapy should be continued. Therapy probably can be stopped safely if the histology has been stationary for more than 2 years. Monitoring of therapy in the future may be improved with the use of PCR assay (e.g., from the stool), immunohistology, or serology.

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The complete reference list is available online at Expert Consult.

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211 *Neisseria meningitidis*

David S. Stephens

SHORT VIEW SUMMARY

Definition

- *Neisseria meningitidis,* the cause of epidemic cerebrospinal fever and septicemia, is an obligate human bacterial pathogen most often presenting as acute bacterial meningitis and/or mild bacteremia to devastating septicemia.

Microbiology

- *N. meningitidis* is a gram-negative diplococcus (0.6 × 0.8 μm) and member of the bacterial family Neisseriaceae, which includes the human pathogen *Neisseria gonorrhoeae.*

Biology and Pathogenesis

- Meningococcal biology and pathogenesis are defined by (1) *N. meningitidis* human-to-human transmission, acquisition, and colonization; (2) virulence factors that facilitate these events and invasive meningococcal disease; and (3) human "host" susceptibility to invasive meningococcal disease. Each of these components influences disease incidence, disease severity, and prevention strategies.

Epidemiology

- Meningococcal disease occurs worldwide, but overall incidence is declining. Capsular polysaccharides A, B, C, W, X, and Y expressed by specific meningococcal genetic clonal complexes (CCs) designated ST-5 and ST-7 (capsular serogroup A); ST-41/44, ST-32, ST-18, ST-269, ST-8, and ST-35 (usually serogroup B); ST-11 (usually serogroups C or W); ST-23 and ST-167 (serogroup Y); and ST-181 (serogroup X) meningococci cause almost all invasive meningococcal disease (see Fig. 211.4), but new virulent genotypes continue to emerge.

Clinical Manifestations and Pathophysiology

- The common presentations of invasive meningococcal disease are meningococcemia and acute meningitis. Less common presentations are primary pneumonia (up to 10%, especially with serogroups Y and W), septic arthritis (2%), purulent pericarditis, chronic meningococcemia, conjunctivitis, epiglottitis, sinusitis, otitis, urethritis, and proctitis.
- *Meningococcemia:* In 20% to 30% of cases, septicemia with shock is the dominant clinical finding, with sudden onset of fever, gastrointestinal symptoms, generalized malaise, weakness, cold extremities and skin pallor, leukocytosis or leukopenia, erythematous blanching, petechial or purpuric rash, headache and/or drowsiness, and hypotension.
- *Meningitis:* Meningitis is the most common presentation of invasive meningococcal disease and occurs in 40% to 65% of cases, reflecting the meningeal tropism of *N. meningitidis.* Findings include sudden-onset headache, fever, vomiting, myalgias, photophobia, irritability, decreased ability to concentrate, agitation, drowsiness and meningeal signs (neck stiffness, Kernig or Brudzinski sign), and cloudy cerebrospinal fluid. An erythematous, petechial, or purpuric rash may or may not be present.
- *Rash:* Skin lesions are present in 28% to 77% of patients with invasive meningococcal disease on admission.
- Complement system deficiencies increase the risk for meningococcal disease—for example, C5-C9 terminal pathway deficiencies, properdin deficiency, use of the complement inhibitor eculizumab, and C3 deficiency.
- Chronic meningococcemia manifests as intermittent fever (often low grade), migratory arthralgias or arthritis, and a nonspecific, often maculopapular rash.

Complications

- Immune complex–mediated complications, such as pericarditis or arthritis, may follow meningococcal disease.
- Overall mortality is 10% to 20% of cases. Long-term sequelae including learning disabilities or hearing or digit loss occur in 11% to 19% of cases.
- Family and community impact is very significant.

Diagnosis

- The definitive diagnosis of invasive meningococcal disease is based on bacteriologic isolation or antigen or DNA identification (by polymerase chain reaction assay) of *N. meningitidis* in a usually sterile body fluid, such as blood, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, or other tissue.

Treatment

- Invasive meningococcal disease is a medical emergency, and early parenteral antibiotic treatment with ceftriaxone, cefotaxime, or meropenem should be the primary goal (see Table 211.1).
- Adjuvant therapy and supportive care should be provided.

Prevention

- Chemoprophylaxis eliminates meningococci from close contacts of confirmed or presumptive cases, protects susceptible individuals, and disrupts the further spread of meningococci. Recommended antibiotics for chemoprophylaxis are rifampin, ceftriaxone, ciprofloxacin, or azithromycin (see Table 211.2).
- Meningococcal capsular polysaccharide-protein conjugate vaccines are licensed for prevention of meningococcal disease due to serogroups A, C, Y, and W (see Table 211.3) and are recommended for children, adolescents, and others at risk for meningococcal disease.
- Two meningococcal outer membrane protein–based vaccines targeting serogroup B (MenB) are now available and currently licensed in the United States for use in children ≥10 years and adolescents and adults at risk for MenB meningococcal disease (see Table 211.3). They have efficacy against most currently circulating serogroup B strains, unlike previous MenB meningococcal vaccines.

DEFINITION AND HISTORY

Neisseria meningitidis (the meningococcus) is the cause of epidemic cerebrospinal fever, clusters and sporadic cases of acute bacterial meningitis,[1,2,3,4,5] mild bacteremia to devastating septicemia, pneumonia, and, less commonly, septic arthritis, pericarditis, chronic bacteremia, conjunctivitis, epiglottitis, otitis, sinusitis, urethritis, and proctitis. Historically, half a million cases of invasive meningococcal disease occur worldwide each year, but incidence is declining owing to the widespread use of conjugate vaccines that provide herd protection,[6] new vaccines for serogroup B,[7] targeted and widespread antibiotic use that eliminates carriage, and other factors including reductions in population risk factors for disease such as smoking and crowding. Mortality remains at approximately 10% to 15% in developed countries and is higher, approximately 20%, in the developing world.[4,8] Morbidity occurs in an additional 11% to 19% of systemically infected individuals and includes digit and limb loss, scarring, hearing loss, cognitive dysfunction, visual impairment, educational difficulties and developmental delays, motor nerve deficits, seizures, and behavioral problems.

Meningococcal disease was first clearly described in the late winter and spring in Geneva, Switzerland, in 1805, as "an epidemic outbreak of rapid onset, hemorrhagic eruption, febrile course, and high mortality with gross inflammation of the central nervous system; 33 deaths in three months were recorded."[9] In 1806, a similar epidemic outbreak was reported in New Bedford, Massachusetts.[10] Subsequently, large outbreaks were reported across Europe and in the United States from 1805 through World War II. Curiously, no description of this singular epidemic disease and its distinct presentation was reported before 1805, nor were there reports of epidemics in the meningitis belt of sub-Saharan Africa before the late 1800s.[11] This and meningococcal molecular phylogeny studies[12] have given rise to speculation that *N. meningitidis* epidemics were, at the time, a new emerging disease.

Marchiafava and Celli[13] (1884) first described intracellular oval micrococci in cerebrospinal fluid (CSF), and Anton Weichselbaum[14] (1887) identified and cultured the organism, which he named *Diplococcus intracellularis meningitidis* because of the organism's presence inside neutrophils from CSF, thus establishing the etiologic relationship between *N. meningitidis* and epidemic meningitis. Kiefer[15] in 1896 and Albrecht and Ghon[16] in 1901 found that healthy persons were nasopharyngeal carriers of the meningococcus. Outbreaks of meningococcal meningitis in New York City in the early 1900s led in 1907 to the introduction of intrathecal equine serum therapy for the treatment of meningococcal meningitis by Flexner.[17] Mortality was reduced to 40% from 75% to 85%. In 1908, Bruns and Hohn[18] noted a close relationship between the carrier rate in a population and the onset, rise, and decline of an epidemic. Serologically different meningococci were first recognized by Dopter[19] in 1909, and *N. meningitidis* was found to be distinct from the commensal *Neisseria lactamica.* Before and during World War I, the organism caused significant outbreaks. Glover[20] (1917) was the first to note that carrier rates in military recruit camps increased with periods of crowding, and Gordon and Murray[21] in 1915 developed the first meningococcal classification system (I, II, III, and IV). Later (1918), a similar system (A, B, C, D) was developed by Nicolle, Debains, and Jouan.[22] In 1928 to 1930 and in 1941, significant US and worldwide epidemics (serogroup A) occurred.[23–25] Rake[26] (1934) further defined the epidemiology of the meningococcal carrier state. In 1937, sulfonamide therapy radically altered the outcome of meningococcal infection and replaced serum as the initial treatment.[27] Beeson and Westerman[28] reported on 3575 cases in England and Wales in 1939 to 1941 managed with sulfonamides; mortality was 16%. In the 1940s and 1950s, Branhan further defined meningococcal serogroups based on differences in capsular polysaccharide and developed the internationally standardized nomenclature A, B, and C,[29,30] which was later expanded in the 1960s by the work of Slaterus and by the Walter Reed Institute of Medical Research to include serogroups E, X, Y, Z, and W.[31,32] Later work identified serogroups H, I, K, and L.[33,34] Meningococci that do not have the capacity to express capsules are commonly found as commensals in the pharynx.

Although *N. meningitidis* remains a devastating and worldwide cause of sepsis and meningitis, significant advances in control have been made in the past 2 decades, led by the introduction of new effective vaccines to prevent meningococcal disease.[6,7,35–37] Study of the meningococcus has also led to the discovery of important insights into bacterial pathogenesis and of pathogen evolution in humans.[38] The new meningococcal polysaccharide (MPS) conjugate vaccines against serogroups A, C, Y, X, and W (X in development), and new serogroup B vaccines, hold great promise for worldwide prevention in expanded human populations (e.g., infants and young children) and can (at least for the protein-polysaccharide conjugate vaccines) induce significant indirect effects and herd protection.[4,35,36,39] Major challenges are the global implementation of meningococcal conjugate vaccines (MCVs), the full introduction and breadth of coverage of vaccines against serogroup B, the duration of protection of meningococcal vaccines, increasing antibiotic resistance in meningococci, and the emergence or reemergence in the past 2 decades of serogroups (e.g., Y, W, X) and new invasive genotypes that now cause significant endemic and epidemic meningococcal disease.

MICROBIOLOGY

The meningococcus is a gram-negative diplococcus (0.6 × 0.8 μm), a β-proteobacterium, and a member of the bacterial family Neisseriaceae, which includes many commensal species (e.g., *N. lactamica*, *Neisseria subflava*, *Neisseria polysaccharea*) and also the human pathogen *Neisseria gonorrhoeae.*[40,41] The adjacent sides of the diplococcus are flattened to produce the typical biscuit or coffee bean shape. The meningococcus has a rapid autolytic rate. Lysis appears to be mediated by expression of lytic transglycosylase and cytoplasmic *N*-acetylmuramyl-L-alanine amidase genes and an outer membrane phospholipase,[42] which can result in considerable size and shape variation in older cultures. The organism may produce a polysaccharide capsule; structural differences in the capsular polysaccharide are the basis of the serogroup typing system.

Because the meningococcus is considered fastidious, appropriate media and growth conditions are necessary. On solid media, the meningococcus grows as a round, colorless-to-gray, nonpigmented, nonhemolytic colony that is 1 to 5 mm in diameter. Colonies are convex and, if large amounts of polysaccharide are present, can appear to be mucoid rather than smooth. Optimal growth conditions are achieved in a moist environment at 35°C to 37°C under an atmosphere of 5% to 10% carbon dioxide. The meningococcus will grow well on a number of medium bases, including blood agar base, trypticase soy agar, supplemented chocolate agar, and Mueller-Hinton agar. Classic confirmation of this organism in clinical specimens has depended on a positive oxidase test (the meningococcus contains cytochrome oxidase in its cell wall) and a series of carbohydrate fermentations. The meningococcus will metabolize glucose and maltose to acid without gas formation and fails to metabolize sucrose or lactose. The expanded use of molecular methods based on a variety of polymerase chain reaction (PCR) techniques[43,44] has confirmed and supplemented cultures in the diagnosis of patients infected with the meningococcus. This is particularly true for clinical specimens from patients treated with antibiotics before cultures are obtained.

BIOLOGY AND PATHOGENESIS

The dynamic pathobiology of *N. meningitidis* resembles the story of "Dr. Jekyll and Mr. Hyde." As an obligate human pathogen with no other reservoir, the organism is an ancient common human commensal that can be carried for months, most often in the human nasopharynx. Cross-sectional studies in multiple populations project an estimated 230 million to more than 1 billion meningococcal carriers worldwide (3%–25% of populations).[45,46] Carriage is an immunizing process, often resulting in natural protective immunity. In contrast, the meningococcus is a devastating human pathogen, historically causing approximately 500,000 cases of invasive meningococcal disease worldwide annually,[8] with high mortality and morbidity and with increased incidence in often otherwise healthy children and adolescents.

Meningococcal biology and pathogenesis can be defined by three interrelated components: (1) *N. meningitidis* human-to-human transmission, acquisition, and colonization; (2) virulence factors that facilitate these events and invasive meningococcal disease; and (3) human "host" susceptibility to invasive meningococcal disease. Each of these areas has implications for prevention strategies.

The potential *virulence* of *N. meningitidis* (defined as the ability to cause invasive disease) differs extensively among meningococcal strains. Unencapsulated strains rarely cause invasive disease, but even among

encapsulated strains there is considerable variability in virulence. Organism characteristics that facilitate survival during invasive disease and/or that also promote transmission and acquisition will increase disease incidence. Meningococcal disease patterns and incidence vary dramatically, both geographically and over time in populations. This is related to the appearance and disappearance in populations of invasive meningococci of specific genotypes designated as clonal complexes (CCs),[47] often but not exclusively associated with a specific capsular polysaccharide. Currently, 12 genomic CC types (see "Epidemiology") cause most endemic and epidemic invasive meningococcal disease worldwide.[47,48] The genomic population structure of colonizing meningococci is considerably more diverse.[49] One approach to the assessment of meningococcal virulence relates the number of cases in a population due to a specific clade or CC to the number of carriers of that clade or CC. This can range from much less than 1 case per 10,000 carriers to more than 1 case per 10 to 20 carriers for highly virulent CCs.[50,51] For example, the case-to-carrier ratio is much higher for serogroup A CCs or serogroup C, CC ST-11, than for serogroup X or serogroup Y meningococci, presumably reflecting a marked difference in meningococcal virulence.[52]

The meningococcus has a single chromosome of 2.1 to 2.3 megabases. The first whole-genomic sequences were from serogroup B and serogroup A strains and were reported in 2000.[53,54] With high-throughput whole-genome sequencing (WGS), the number of genomes as gap-closed, as finished genomes, or now more commonly as incomplete or draft genomes is rapidly increasing in available databases (http://neisseria.org/nm/genomes; http://patricbrc.org/portal/portal/patric/Home; http://PuBMLST.org). The *Neisseria* PubMLST database contains genetic data for a collection of over 50,000 isolates and over 19,000 genomes that represent the total known diversity of *Neisseria* species (http://PuBMLST.org). The core genome consists of 1300 to 1600 genes and differs by 3% to 5% from other sequenced strains, with the meningococcal "pan genome" estimated at more than 2500 genes.[55–58] The overall G and C content is 51%, but low G and C regions suggest horizontal transfer and contain genes that are important in pathogenesis. Work has identified a transcriptome of approximately 1100 transcribed open reading frames per strain with over 300 operons. The genome encodes core metabolic, fitness, and key virulence factors, including capsular polysaccharide, a requirement for virtually all invasive meningococcal disease and the basis for serogrouping; lipooligosaccharide (LOS), a lipopolysaccharide molecule without repeating *O* side chains and the basis for immunotyping; the pilus organelle complex that facilitates motility and initiates attachment; and other virulence-associated outer membrane proteins (PorA, PorB, Opc, Opa, NadA, FetA, FHbp), which are the basis for serotyping and serosubtyping (Fig. 211.1). Repetitive nucleotide sequences and polymorphic regions are present, usually in large heterogeneous arrays, suggesting active areas of genetic recombination. Transformation is the major means of horizontal gene transfer and a source of strain diversity. Over 1900 copies of the *Neisseria* uptake sequence (5′-GCCGTCTGAA-3′) ease *Neisseria* spp. DNA exchange by transformation and homologous recombination. Recombination events are recognized, including transfer of DNA including complete genes between meningococci, gonococci, and commensal *Neisseria* spp. and other bacteria.[57,58] The genome is modified and regulated at multiple levels. Genetic switches involved in gene regulation include small noncoding regulatory RNAs, CRISPR (clustered regularly interspaced short palindromic repeats), slipped-strand mispairing of repetitive nucleotides, global protein regulators of promoters (e.g., *fur*), intergenic recombination, IS (insertion sequence) element movement, methylation, hypermutator phenotypes, and two-component systems regulation. There are large genetic islands (e.g., IHT-A1 [8.5 kb], IHT-A2 [5.4 kb], IHT-B [17.1 kb], IHT-C [32.6 kb], IHT-E) in different meningococcal strains[55,58,59] containing bacteriophages, phage elements and remnants, and many restriction enzymes related to CC genomic structure virulence factors and other genes to encoding surface proteins. For example, the IHT-A2 locus encodes an ABC transporter homologue and a secreted protein and the IHT-C locus encodes 30 open reading frames, including toxin homologues, a bacteriophage, and potential virulence proteins.[50] Although one bacteriophage has been proposed as a marker of virulence,[59] the current evidence is that no specific virulence gene pool is exclusively present in all *N. meningitidis* strains from hyperinvasive lineages.[55,58]

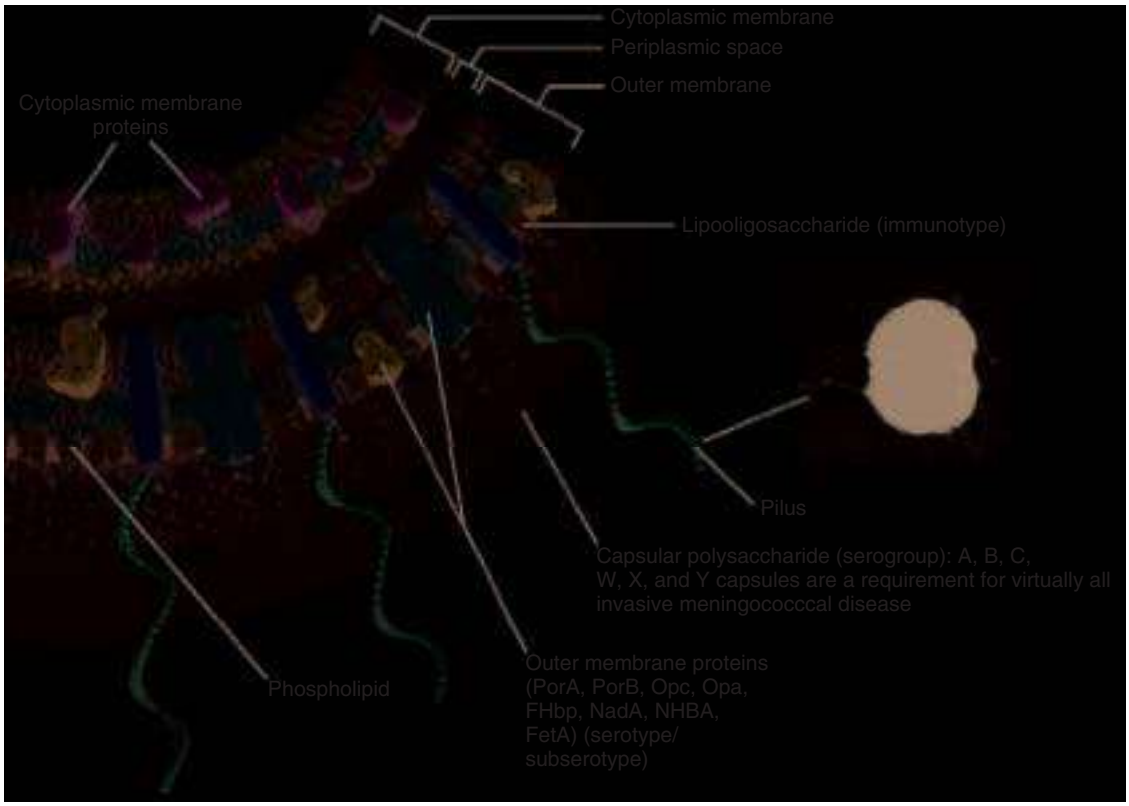

FIG. 211.1 Cross-sectional and scanning electron microscopic views of the meningococcus. *(Modified from Rosenstein NE, Perkins BA, Stephens DS, et al. Meningococcal disease.* N Engl J Med. *2001;344:1378–1388.)*

The major virulence factor for invasive disease produced by the meningococcal genome is capsular polysaccharide. Serologic typing and the biochemical composition of capsular polysaccharides have classified *N. meningitidis* capsular polysaccharides into a total of 13 serogroups (A, B, C, D, E, H, I, K, L, W, X, Y, and Z),[3] with A, B, C, E, H, I/K, L, W, X, Y, and Z now confirmed genetically.[60–62] However, six capsular serogroups (A, B, C, W, X, and Y) cause almost all invasive meningococcal disease. Serogroups B and C capsular polysaccharides are sialic acid homopolymers of (α2→8) and (α2→9) linkages, respectively, whereas serogroups Y and W are alternating units of D-glucose or D-galactose and sialic acid, respectively. Serogroup A *N. meningitidis* expresses a homopolymeric (α1→6) *N*-acetyl mannosamine-1-phosphate capsule, whereas serogroup X expresses (α1→4) linked *N*-acetyl-D-glucosamine-1-phosphate. Capsular polysaccharides provide antiadherent properties, thereby promoting meningococcal spread from mucosal surfaces, and provide protection in intracellular environments and against complement-mediated killing.

Capsule is produced by a 30-kb region containing the IHT-A1 genetic island with genes for capsule biosynthesis, assembly, and transport.[62–65] The island has been lost or never acquired in commensal *Neisseria* species, such as *N. lactamica,* the pathogen *N. gonorrhoeae,* and many commensal *N. meningitidis*[64]; but recent work shows evidence of a *cps* locus in some commensals such as *N. subflava*. The acquisition or reacquisition and evolution of this genetic island was a key to the emergence of invasive meningococcal disease. Capsule subunit and polymer biosynthesis, acetylation, assembly, protection from degradation, and transport to the cell surface are encoded by genes of the region. The region is related to capsule encoding islands in *Pasteurella* and other bacterial species. The different capsule structures are the result of evolutionary divergent biosynthesis, polymerization, or acetylation genes found in the capsule locus.

The serogroup B polysaccharide capsule ([α2→8]-linked polysialic acid) is similar to the capsular polysaccharides of *Escherichia coli* K1, *Pasteurella haemolytica* A2, *Moraxella nonliquefaciens,* and human polysialic acid structures, such as the neural cell adhesion molecule (NCAM).[66,67] Due to NCAM similarity, the serogroup B capsule is poorly immunogenic as an antigen in humans and animals.

Capsule, as with many other meningococcal virulence factors, is subject to genetic regulation. On↔off phase variation, regulation of amount of capsule expressed, and modifications to structure (e.g., capsule acetylation) are well described.[63–65,68] Thermoregulation of capsule expression (increased capsule expression at 37°C due to RNA thermosensors in the *cps* operon) is reported.[69] Meningococci "capsule switching" resulting in structural change also occurs, providing a mechanism of immune escape.[63,70–72] Gene conversion by transformation and homologous recombination of the capsule locus was first noted in a serogroup B outbreak in the United States in the 1990s[63]; otherwise identical serogroup C expressing strains appeared during the outbreak. Similarly, CC, ST-11 serogroup W *N. meningitidis* outbreaks associated first with the Hajj in 2000 and 2001 and more recently with other CC, ST-11 W clades were likely the result of distant capsule switching events from ST-11 serogroup C ancestors. In large meningococcal isolate collections, capsule switching events are detected in approximately 3% of isolates.[70]

Type IV pili (see Fig. 211.1) are complex surface appendages requiring at least 23 proteins (e.g., PilE, PilC, secretin, PilT, PilQ), either as structural components of the organelle or required for biogenesis. Pili are anchored in the outer membrane and radiate, through an oligomeric ring, several thousand nanometers from the meningococcal surface (see Fig. 211.1).[73–76] The major subunit may undergo *O*-linked glycosylation. Pili and accessory proteins (e.g., PilX) facilitate meningococcal aggregation and initial attachment and anchoring to human epithelial or endothelial cells.[77] Through cycles of polymerization and depolymerization that produce retraction and extension, pili are responsible for "twitching motility" (1–2 μm/sec). Pilus attachment to human cells initiates localized remolding of the human cell cytoskeleton. Pili also facilitate aggregation and microcolony formation but, when glycosylated, promote meningococcal disaggregation and dissemination. Pili are also necessary for DNA transformation.

The endotoxin of *N. meningitidis,* an LOS, lacks *O*-side chains and has a lipid A structure distinct from gram-negative enterics.[78,79] This LOS can mimic the i and I human blood group antigens.[80] LOS also is an important virulence factor, influencing cell damage, meningococcal attachment and invasion of host cells, and complement-mediated killing. Mass spectrometry analysis of the structure of LOS shows highly phosphorylated lipid A forms that influence innate immune responses and clinical outcomes.[81,82] The release of LOS during bloodstream or CSF invasion is a key factor in meningococcal sepsis and meningitis.[83] Serum or CSF levels of LOS are directly correlated with the severity of meningococcal sepsis and meningitis. For example, when LOS levels exceed 100 ng/L, septic shock and/or death are highly correlated.[84,85] LOS stimulates chemokine and cytokine release systemically by binding Toll-like receptor 4 (TLR4) on macrophages and other cells and stimulating MyD88-independent and MyD88-dependent pathways, resulting in tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein-3α (MIP-3α), IL-6, IL-8, IL-10, MCP-5, and release of other chemokines and cytokines.[79,83,85] LOS also downregulates genes involved in oxidative phosphorylation and mitochondrial function in human cells.[86] Non-LPS molecules can contribute to inflammation, but 10-fold to 100-fold higher concentrations are required to reach the same responses as those induced by LOS.[83]

Outer membrane porins are involved in host-cell interactions and are targets for bactericidal antibodies. PorB, which is the major outer membrane porin, inserts in membranes, induces Ca^{2+} influx, and activates Toll-like receptor 2 (TLR2) and cell apoptosis.[87] PorA is a second important porin and a major target of meningococcal outer membrane vesicle (OMV) vaccines including OMV in one of the new MenB vaccines. Meningococci also express variable proteins such as Opa and Opc, which are important in adherence and host cell interactions.[74] Another important outer membrane protein, a principal target for the new MenB meningococcal vaccines, is the factor H binding protein (FHbp), a lipoprotein involved in meningococcal resistance to complement-mediated killing. Other outer membrane vaccine-targeted proteins include neisserial adhesin A (NadA), a mediator of adhesion and cell invasion; and neisserial heparin binding antigen (NHBA), also involved in adhesion and in protection against complement-mediated killing.[37,88]

Iron is necessary for meningococcal survival, colonization, and infection. The meningococcus scavenges iron from the human proteins transferrin, lactoferrin, and hemoglobin through a series of highly evolved, surface-exposed receptors and TonB-derived energy.[1,61,75,89] More than 80 genes are regulated by the iron-responsive repressor Fur.[89] Meningococcal iron-acquiring proteins include HmbR (hemoglobin), TbpA and TbpB (transferrin), HbpA and HbpB (lactoferrin), and HpnA and HpnB (hemoglobin-haptoglobin complex) and FetA (ferric enterobactin receptor). Iron-loaded animals are more susceptible to fatal meningococcal infection.[90] New putative iron acquisition proteins, adhesion or invasion proteins, and toxin proteins have been identified in meningococcal genome searches.

Meningococcal Colonization and Transmission

The dynamic life cycle of *N. meningitidis* is shown in Fig. 211.2. The human nasopharynx is the most frequent site of meningococcal colonization and carriage and the major source of transmission to other humans.[46,51,74,76,91–95] The human nasopharynx, optimal for *N. meningitidis* growth, has a CO_2 content of 3% to 4%; high, 75% to 80%, humidity; and a temperature of 34°C. Colonization is a complex process of meningococcal interaction with upper respiratory mucosa. The initial adherence of meningococci is facilitated by type IV pili, which may recognize integrin α-chains or other receptors. Surface movement, proliferation, aggregation, and microcolony formation are facilitated on mucosal surfaces by type IV pili–induced twitching motility involving pilus retraction, and the phase variation of glycan decorations on pili. Meningococci attach to nonciliated nasopharyngeal epithelial cells and induce apical cortical plaques (see Figs. 211.2 and 211.3A), which anchor the organisms against loss by mucus and ciliary action and promote the formation of meningococcal microcolonies and biofilms (see Fig. 211.3A).[74–76] This process is mediated by close adherence involving the meningococcal outer membrane ligands (Opc, Opa, LOS, NadA, and others) and the human host receptors such as carcinoembryonic antigen–related cell adhesion molecules (CEACAMs) and integrins,

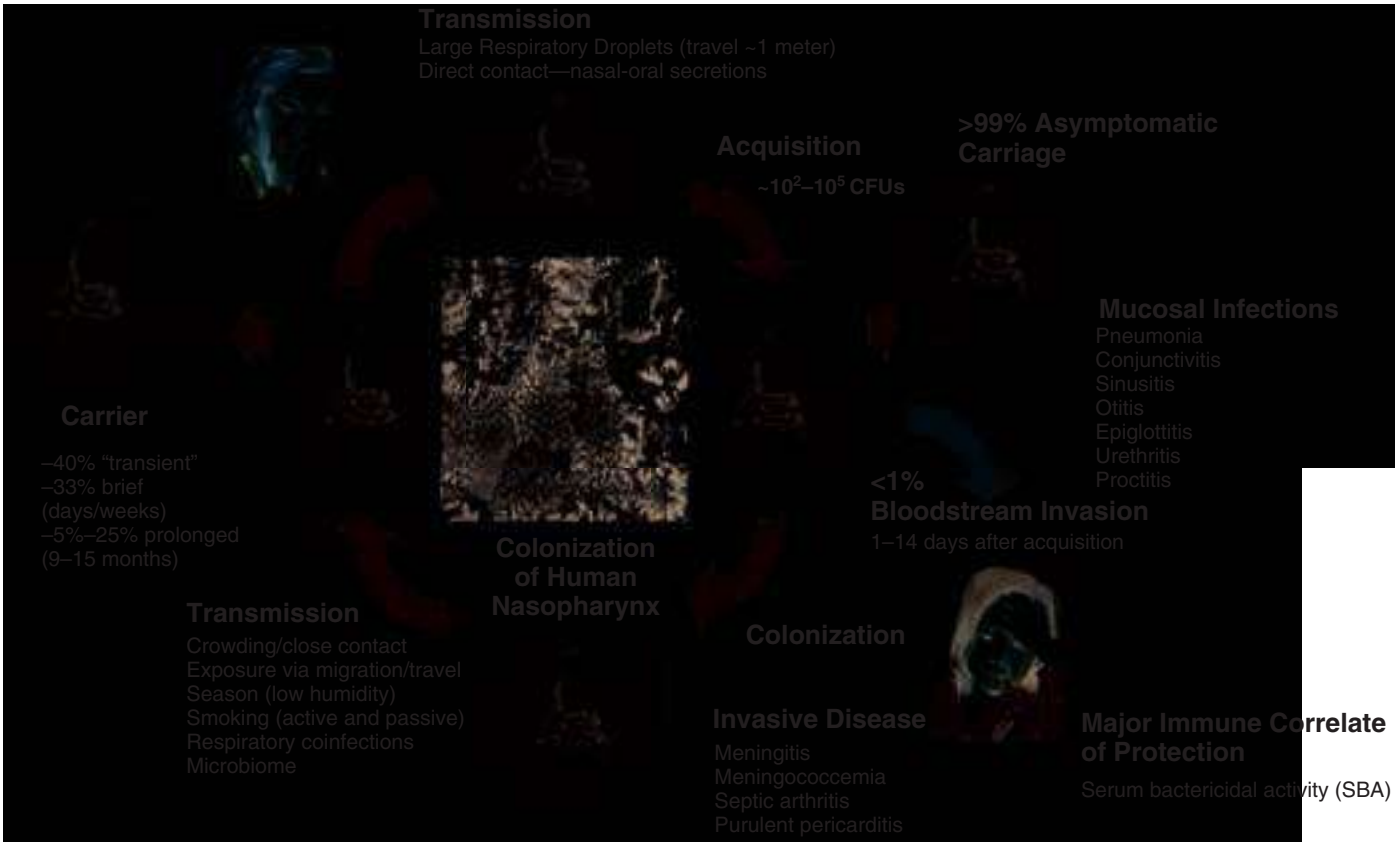

FIG. 211.2 **The life cycle of *Neisseria meningitidis.*** *CFUs,* Colony-forming units.

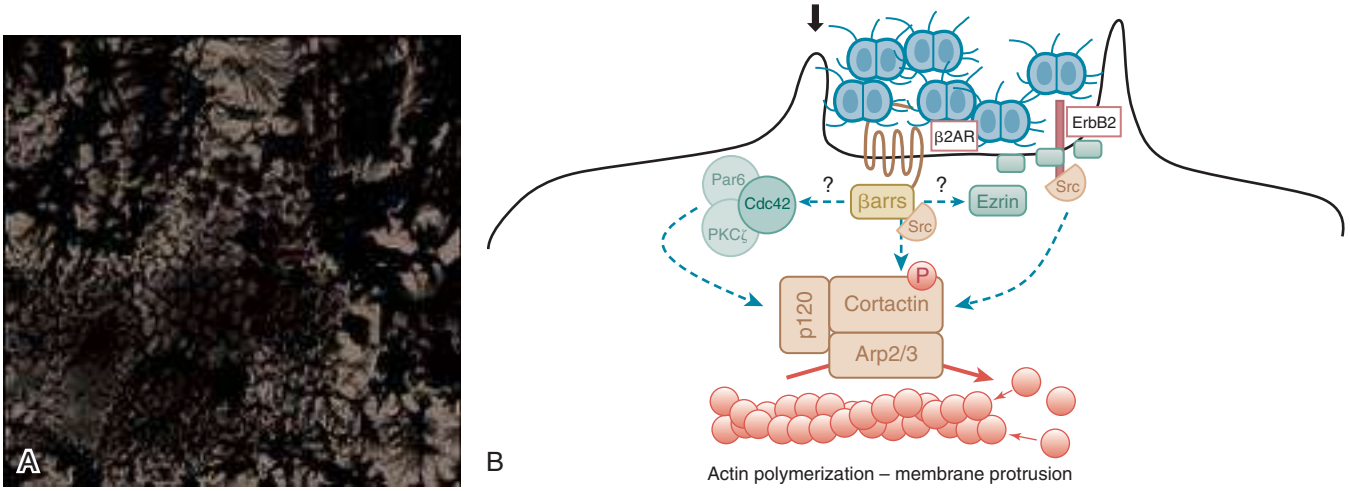

FIG. 211.3 **Dynamics of meningococcal attachment to human nasopharynx epithelial cells and human vascular endothelium.** (A) Electron micrograph of *Neisseria meningitidis (green arrow)* attaching to human nasopharyngeal mucosa. (B) Schematic of the molecular events occurring in (A). *(Modified from Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium* Neisseria meningitidis. Vaccine. *2009;27(suppl 2):B71–B77; and Coureuil M, Join-Lambert O, Lécuyer H, et al. Mechanism of meningeal invasion by* Neisseria meningitidis. Virulence. *2012;3:164–172.)*

leading to cortical actin polymerization and plaque formation (see later). The induction of cortical plaques also leads to internalization of meningococci within epithelial cells, a site where capsule is advantageous for survival. Cell entry is a potential pathway to mucosal invasion and access to the bloodstream. Capsule expression and glycan expression on pili result in meningococcal disaggregation and spread along and from mucosal surfaces.

As previously noted, meningococci are common commensals, colonizing 3% to 25% of human populations worldwide.[50,51,92–94] Carriage rates are low in infants and young children and highest in adolescents and in closed populations, but there is considerable global diversity. Carriage may be transient or prolonged (e.g., months). Carriage does not directly predict an outbreak or the course of the outbreak, but "if there are no carriers, there are no cases."[96] Although usually less than 1% of persons exposed develop invasive disease, expanded transmission of meningococci with the capacity to cause disease will result in increased incidence. Transmission is by direct contact with respiratory secretions or by inhalation of large respiratory droplet nuclei that travel approximately 1 meter (3 feet); respiratory droplets may increase in low-humidity environments.[97] Meningococci are fastidious but can survive for hours

or more on innate surfaces.[96,98] Factors that influence meningococcal transmission and carriage include crowding or close contact, exposure through migration or travel (e.g., the Hajj), season, active and passive smoking, party or club attendance, male sex, and respiratory coinfections.[94,97,99–102] The human upper respiratory microbiome also influences meningococcal colonization (e.g., the presence of *N. lactamica* is negatively correlated with meningococcal carriage). A challenge in understanding the dynamics of meningococcal carriage has been the variable expression of capsule or other virulence factors detected with serologic technologies in colonizing isolates. WGS is a more accurate approach to characterizing meningococcal carriage isolates.[103]

The meningococcal inoculum required for nasopharyngeal colonization or needed to cause invasive disease is not known. However, data from experimental *N. gonorrhoeae* urethral infections in male subjects revealed a median infective dose (ID_{50}) of approximately 10^5 colony-forming units of gonococci, which could be further reduced to approximately 3×10^2 if gonococci obtained from the initial passage after infection were used.[104] In settings of very high exposure (e.g., military recruits), the acquisition of meningococci in the nasopharynx is transient (or not detected) in about 40% of individuals; in approximately 33%, the period of nasopharyngeal colonization and carriage is brief (i.e., days to weeks); and in approximately 25%, meningococcal carriage becomes fully established and can last 9 to 15 months or longer.[50,105] Meningococci that gain access to the bloodstream may cause a transient or mild bacteremia but also the clinical syndromes of septicemia and meningitis described later. Invasive meningococcal disease usually occurs 1 to 14 days after mucosal acquisition.[1,3] Cofactors associated with invasive meningococcal disease are respiratory tract infections (e.g., from mycoplasma, or influenza and other viral agents), active and passive smoking, and environmental damage to the upper respiratory tract (e.g., from low humidity, dusty winds of the Harmattan in Africa).[1,3,4,99,100] *N. meningitidis* also has the capacity to damage respiratory epithelial cells, can cause a pharyngitis, and can spread to adjacent mucosal surfaces of the upper respiratory tract to produce focal respiratory infections (e.g., pneumonia in up to 10% of presentations) but is an uncommon cause of otitis media or sinusitis. Meningococcal pneumonia and conjunctivitis are also recognized as portals of entry for systemic disease.

Once in the bloodstream, type IV pili facilitate meningococcal attachment to peripheral vascular endothelial cells. This results in the formation of microcolonies on vascular endothelium, the activation of signaling pathways in endothelial cells and formation of cortical plaques, and meningococcal entry into endothelial cells. A humanized mouse model shows meningococcal adherence only to human vessels.[101] Adherence results in extensive damage, inflammation, and development of a purpura at these sites. The specific steps in brain and other microvascular endothelial cortical plaque formation have been dissected[106–109] and involve the recruitment and activation of the β_2-adrenoreceptor and CD147 in hetero-oligomeric complexes, the accumulation of ezrin and ezrin-binding receptors, β-arrestins and β-arrestin–binding molecules such as Src and VE-cadherin, and the activation of the cortactin/Arp2/3 complex, leading to actin polymerization, membrane protrusions, and cortical plaque formation (see Fig. 211.3B). In brain microvascular endothelium there is also breakdown of tight junctions between endothelial cells, which also may allow meningococci to cross the blood-brain barrier and gain access to the subarachnoid space.[109]

Host Susceptibility

The absence of protective bactericidal activity (deficiency in bactericidal antibody or complement) is the most important single predisposing human factor for susceptibility to systemic meningococcal disease, but other genetic polymorphisms and other host cofactors contribute to invasive disease potential or severity.[110–112] Disappearance of maternal antibodies increases the risk for infants and young children. Congenital and acquired antibody deficiencies also increase risk.[110,111,113,114] Rapidly progressive, fatal meningococcemia can arise in patients deficient in properdin, and there is an increased risk for recurrent meningococcal infections for those with C3 deficiency and congenital or acquired defects (e.g., eculizumab therapy) in the terminal complement pathway (C5-C9).[115] Studies also have linked polymorphisms in complement factor H *(CFH)* and complement factor H related 3 *(CFHR3)* as contributing to meningococcal disease susceptibility.[116] However, opsonization and phagocytic function can also contribute to meningococcal host defense mechanisms, as suggested by disease reduction after meningococcal vaccination in individuals with congenital terminal complement deficiencies.[115] As detailed later, the risk of meningococcal disease in individuals receiving eculizumab, a potent inhibitor of C5, is >300 per 100,000 and opsonization and phagocyte function are also impaired in these patients.

Polymorphisms in genes coding for the Fcγ-receptor II *(CD32),* Fcγ-receptor III *(CD16),* mannose-binding lectin (MBL), TLR4, TNF, the collectin SP-A2, and the β_2-adrenocepter gene *(ADRB2)* have been associated with increased risk for meningococcal disease.[117–127] MBL is a plasma opsonin that initiates complement activation; specific polymorphisms in this gene have been identified more frequently in children with meningococcal disease than in controls[118] in some studies but not others.[119,120] Plasminogen activator inhibitor-1 (PAI-1) concentrations affect risk for severity and mortality of meningococcal sepsis, suggesting that impaired fibrinolysis is an important factor in the pathophysiology of meningococcal sepsis.[124] Genetic variation in the β_2-adrenoceptor has also been associated with susceptibility to meningococcal meningitis.[125]

Meningococcal disease is also linked to immunosuppressive disorders such as glomerulonephritis, hypogammaglobulinemia, splenectomy, and other chronic illnesses including human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) (i.e., an approximate 10-fold increased risk for sporadic meningococcal disease[126] versus about a 100-fold increased risk for infection with the pneumococcus in HIV/AIDS). In an outbreak in New York City of serogroup C meningococcal disease, the risk for disease was 12.6 per 100,000 in men who have sex with men (MSM), most of whom were infected with HIV, compared with 0.16 per 100,000 in non-MSM.[128] Serogroup C meningococcal outbreaks in MSM have also occurred in Chicago and Los Angeles.[129] However, there have been no well-documented increases in epidemic outbreaks of meningococcal disease in developing countries with high rates of HIV infection. Other chronic illnesses (e.g., chronic dialysis, diabetes mellitus, transplantation) in children and adults have been associated in case reports with susceptibility to meningococcal infections, due either to underlying disease or immunosuppressive therapy. Many of the meningococcal isolates from these patients are of low virulence, including capsule null *N. meningitidis.*[130]

EPIDEMIOLOGY

The epidemiology of meningococcal disease is dynamic. The rates of disease are influenced by the virulence of circulating strains, environmental and host factors influencing transmission, carriage and disease, population immunization, and other prevention strategies.[1,4,131,132] Meningococcal isolates have been historically classified by serologic typing based on the biochemical composition of the capsular polysaccharide. Serogroups A, B, C, E, H, I/K, L, W, X, Y, and Z are confirmed genetically[60]; however, six serogroups (A, B, C, W, X, and Y) currently cause almost all worldwide life-threatening disease.[1,4,48,60]

Outer membrane protein serotyping and serosubtyping and LOS immunotyping have also been used, especially for serogroup B meningococcal strains. Genomic typing (e.g., multilocus sequence typing [MLST]) and whole-genome comparisons have unlocked a broader understanding of the global epidemiology of meningococcal disease. With MLST,[133] meningococcal isolates are classified into different sequence types (STs) and CCs based on polymorphisms in seven housekeeping genes considered not to be under selective pressure. CCs ST-5 and ST-7 (serogroup A); ST-41/44, ST-32, ST-18, ST-269, ST-8, and ST-35 (serogroup B); ST-11 (serogroups C, W, or B); ST-23 and ST-167 (serogroup Y); and ST-181 (serogroup X) meningococci cause almost all invasive meningococcal disease[48,70,134]; but other new genotypes associated with disease continued to emerge (e.g., ST-10217, serogroup C in Africa[135,136]; ST-4821, serogroups C and B in China[136]). The emergence of rapid WGS as an epidemiologic technique and tool for better understanding pathogenesis has further defined meningococcal strain relatedness, diversity, and virulence.

Meningococcal disease is worldwide (Fig. 211.4), but with significant differences in serogroup/CC predominance. Incidence also varies in populations over time. Disease can be epidemic, endemic (sporadic),



FIG. 211.4 Global epidemiology of meningococcal disease by serogroup, 2018. Serogroups are linked to 12 major clonal complexes: ST-5 and ST-7 (serogroup A); ST-41/44, ST-32, ST-18, ST-269, ST-8, and ST-35 (serogroup B); ST-11 (serogroups C or W); ST-23 and ST-167 (serogroup Y); and ST-181 (serogroup X) meningococci currently cause almost all invasive meningococcal disease. *(Modified from Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and* Neisseria meningitidis. Lancet. *2007;369:2196–2210.)*

or hyperendemic. Meningococcal epidemiology is affected by the sequence types and serogroups circulating, by age and other host susceptibility (e.g., the frequency in the population of complement deficiencies), by the widespread use of antibiotics that eradicate the meningococcal carrier state, and by vaccines. The introduction of polysaccharide vaccines in the 1970s decreased the incidence of meningococcal disease through widespread use in the military, in sub-Saharan Africa, in China, and in countries associated with the Hajj and Umrah pilgrimages. More recently, the A, C, W, Y MCVs that both induce individual protection and interfere with transmission resulting in herd protection have had greater impact.[70] Other changes in populations (e.g., in strain- or clonal group–specific immunity) and other environmental factors (e.g., crowding, smoking, humidity, dust, viral, or mycoplasmal coinfections) also influence the incidence of meningococcal transmission and disease.[1,4,131,132]

Meningococcal disease epidemiology dramatically changed in industrialized nations after World War II.[a] Before and during this war, large serogroup A outbreaks occurred in the United States, Europe, Asia, South America, and Africa that were cyclical, having peaks and troughs every 5 to 12 years. These large serogroup A outbreaks dominated meningococcal disease throughout the world in the 19th and first half of the 20th centuries; disappeared from the United States, Western Europe, and Japan after World War II; but continued in parts of Asia, South America, and sub-Saharan Africa. For example, before and during World War II the incidence of meningococcal disease in the United States was 3 to 4 per 100,000 population, with episodic serogroup A outbreaks raising the incidence to 8 to 17 per 100,000.[137] With the disappearance of serogroup A outbreaks and serogroup A carriage for reasons that are not well understood, overall attack rates in the United States declined to 1.7 per 100,000 population by the mid-1990s, but with periodic "hyperendemic" fluctuations in the incidence of B, C, or Y meningococcal disease. In the United Kingdom, incidence decreased from more than 5 per 100,000 population in the 1990s to less than 3 per 100,000 population after the virtual elimination of serogroup C disease as a result of the introduction of C specific conjugate vaccines (see later).[138] However, in 2010 the rates of serogroup W ST-11 disease began to increase in the United Kingdom.[139] The increase is associated with the emergence of new ST-11 serogroup W sublineages with severe disease, often in adolescents and older adults, and clinical presentations that include pneumonia, gastroenteritis (nausea, vomiting, diarrhea), septic arthritis, and epiglottitis or supraglottitis. The W:CC 11 lineages have appeared globally, causing increased disease in South America, Canada, Europe, China, Australia, and sub-Saharan Africa.[139,140] These events have led to the increasing use of A, C, W, Y conjugate vaccines in many countries.

Although increases in W:CC 11 disease have occurred, the overall meningococcal disease incidence and carriage rates in many industrialized countries' populations have continue to decline[b] (in the United States in 2015 the invasive meningococcal disease incidence was 0.12 per 100,000). In Japan, rates of disease are now <0.04 per 100,000 (and carriage rates are 0.4%), with a high percentage of the remaining meningococcal disease occurring in complement-deficient individuals.[115,141,143]

In the United States, serogroups B (ST-41/44, ST-32), Y (ST-23), and C (ST-11), although declining in incidence, continue to cause most (87%) of the meningococcal disease.[4] The United States saw the emergence in the early 1970s and again in the mid-1990s into the 2000s of serogroup Y (ST-23) disease. Serogroup Y disease incidence peaked in 1997, accounting for approximately 50% of reported cases, in contrast to approximately 2% in the early 1990s.[2–4] Almost all of the serogroup Y meningococcal disease in the United States in the 1990s was caused by ST-23 CC strains.[48,70] In 1998, a carriage study of high school students from counties in the metropolitan area of Atlanta, Georgia, found the rate of meningococcal carriage to be 7.7%; and of these isolates, 48% were serogroup Y.[146] However, in 2006–07, a similar carriage study in high school students found a much lower carriage rate of less than 3% and a much lower proportion of serogroup Y carriage.[48,147] The lower frequency of serogroup Y and overall meningococcal carriage correlated with the decline in invasive serogroup Y cases after 1998. However, serogroup Y continues to cause disease in the US population and is seen globally.

The overall declines in disease also continue to be interrupted by localized serogroup C outbreaks in the United States and Europe, such as in MSM[142,148,149]; or by serogroup B outbreaks in communities and institutions such as on college campuses.[150] The increased serogroup C rates in MSM compared with non-MSM populations led to specific targeting of educational and vaccination efforts in New York City and in other locations.[129]

In South and Latin America (in particular, Brazil and Chile), serogroup W:CC 11 is currently a major cause of disease, followed by serogroups B and C.[151] In Europe, serogroups B (ST-41/44, ST-32, ST-18, ST-269, ST-8), W (ST-11), C (ST-11),[144] and Y (ST-23) currently predominate; in Russia, serogroups B, C, A, W, and Y; in China and Mongolia, serogroups C, W, B, and A; in India and Nepal, serogroups A, C, and B[152,153]; in Oceania, serogroups W, B, Y, and C; in the Middle East and North Africa,[154] serogroups W, B, A, and Y; and in sub-Saharan Africa, serogroups C (ST-10217), W (ST-11), and X (ST-181).[52] Serogroup Y CC ST-11 and ST-167 have been increasingly reported in South Africa, Israel, parts of Europe, and Japan.

Serogroup A is historically associated with the highest incidence and largest epidemics of meningococcal disease,[1,11,131,155,156] especially meningitis. Serogroup A global pandemics in the 20th century were caused by five major ST CCs (e.g., ST-5, ST-7). Three pandemic waves arising in China and spreading to Russia, the Middle East, Africa, and globally were recorded in the 1960s to 1970s, 1980s, and into the 1990s. Serogroup A strains are genetically distinct from other meningococci and appear to have evolved from a common ancestor in the 19th century.[12] In sub-Saharan countries of Africa, extending from Senegal and The Gambia in the west to Ethiopia, Eritrea, and northern Kenya in the east, a region known as the African meningitis belt, large periodic epidemics of serogroup A meningococcal disease occurred every 5 to 12 years from the late 1800s to 2010.[11,157] Virulent meningococci may have been first introduced into the region by returning Hajj pilgrims. The size of these epidemics was enormous. In major African epidemics, the attack rate ranged from 100 to 800 per 100,000 population during epidemics, and individual communities have reported rates as high as 1 per 100.[c] Between 1988 and 1997, 704,000 cases and more than 100,000 deaths were reported; from 1996 to 1997, more than 300,000 cases and 30,000 deaths, the largest serogroup A epidemic year ever recorded, with spread to countries south of the meningitis belt (e.g., Rwanda, Burundi, Tanzania, Zambia, and the Central African Republic); between

[a]References 1, 3, 4, 131, 137, 539.

[b]References 1, 4, 131, 132, 138, 141–145.

[c]References 11, 17, 155, 156, 158, 159.

1998 and 2002, 224,000 cases; and, in 2009, 88,199 meningococcal meningitis cases occurred in the belt.[158]

The CCs, ST-5 and ST-7, were responsible for these global serogroup A pandemics, the African meningitis belt outbreaks, endemic serogroup A meningococcal disease in the belt, and the worldwide cases of serogroup A disease.[157] The epidemics in the meningitis belt have been linked to environmental factors, such as climatic changes (dry season, "dust" and winds of the Harmattan), coinfections, poor living conditions, overcrowded housing, travel and population displacements, and specific population immunologic susceptibility.[d] Disease in this region predominantly occurs in the dry season (December to June), ending during the intervening rainy season. Respiratory droplets that facilitate meningococcal transmission have higher density in the dry season.[97] Although serogroup W, X, and rarely C had had caused epidemic outbreaks in sub-Saharan populations, serogroup A epidemic meningococcal disease was a major public health threat in the meningitis belt and other areas of the developing world. Over the past 2 decades, great strides have been made in development and implementation of serogroup A–directed MCVs. The successful introduction beginning in 2010 of a new serogroup A MCV, MenAfriVac (Serum Institute of India, Pune, India), in sub-Saharan Africa[160] (see "Meningococcal Conjugate Vaccines") has radically altered meningococcal disease in this region. The serogroup A conjugate vaccine has been introduced into the 26 countries in the African meningitis belt, resulting in a remarkable decline in the burden of disease. However, the region does continue to see serogroup C meningococcal disease, with two large outbreaks in Nigeria and Niger,[161] serogroup W in Ghana[162] and Burkina Faso, and serogroup X in Burkina Faso, Chad and Togo. This is a consequence of bacterial evolution rather than serogroup replacement.[162] The cost of vaccination remains a primary obstacle to further progress against other serogroups in the African belt, but a low-cost pentavalent meningococcal conjugate A, C, W, X,Y is in development, projected for implementation in 2020 or 2021.

The epidemiology of meningococcal disease in parts of Africa outside the meningitis belt is not as well defined. In a report from rural Mozambique, the average incidence of endemic meningococcal disease was 11.6 per 100,000, with both sepsis and meningitis identified and with W (ST-11) as a major cause.[163] It is important to note that epidemics of *S. pneumoniae* meningitis (serotype 1, ST 217 CC) are also reported in the meningitis belt,[164,165] and febrile encephalopathy due to malaria can mimic meningococcal meningitis.[166] More longitudinal and laboratory-based surveillance is needed in Africa.

As noted, serogroup W has emerged in the past 2 decades as a cause of global epidemic outbreaks of considerable size.[139,167–169] In 2000 and 2001 several hundred pilgrims attending the Hajj in Saudi Arabia were infected with *N. meningitidis* serogroup W. Then in 2002, serogroup W emerged in Burkina Faso, striking 13,000 people and killing 1500. Global spread with secondary cases from these epidemics was also observed. The outbreaks were caused by W (ST-11) strains closely related to ST-11 serogroup C strains. Serogroup X (ST-181) has caused localized outbreaks in certain African countries, including Kenya, Niger, and Ghana, but has rarely been a cause of meningococcal disease outside Africa.[52,170] Curiously, serogroup B disease is quite rare in sub-Saharan Africa but does cause significant disease in South Africa.

Serogroup B is the major cause of prolonged outbreaks, hyperendemic disease, and endemic (sporadic) meningococcal disease, especially in infants and young children in developed countries.[1,4,131,171–174] Serogroup B epidemic outbreaks have also occurred worldwide and are usually of lower overall incidence compared with serogroup A outbreaks, but can reach levels of 20 to 45 per 100,000 in highly affected populations, as was seen in Pacific Islanders and Maoris in New Zealand and in infants. Serogroup B outbreaks can persist over many years. The serogroup B (ST-32) outbreaks of meningococcal disease in the US Pacific Northwest[171] from 1988 to approximately 2007 and in New Zealand[173] in 1992 to 2003 are examples. Recently in the United States, college campuses have been sites of a series of serogroup B meningococcal outbreaks (13 from 2008 to 2017).

There is greater genetic diversity, and thus antigenic diversity, in serogroup B strains that cause meningococcal disease. Most serogroup B disease is caused by seven major ST CCs: ST-41/44, ST-32 (members of these first two complexes account for over two-thirds of serogroup B disease), ST-18, ST-35, ST-269, ST-8, and ST-11.[48] However, several other STs are found in collections of serogroup B isolates, and novel serogroup B strains can cause disease worldwide. The diversity of CCs causing serogroup B disease has presented a challenge to control through vaccination, because the B capsule structure has identity to human polysialic acid determinants.[67] Also, ST-11 isolates, a CC usually associated with serogroup C, can express the serogroup B capsule as a result of "capsule switching,"[63,70,71] allowing escape from vaccine-induced or natural protective immunity. This escape mechanism has raised concerns about serogroup B replacement as a threat to the effectiveness of meningococcal A, C, Y, and W conjugate vaccines.

Vaccines have only recently become available for the routine prevention of serogroup B disease. OMV vaccines were initially developed for control of serogroup B clonal outbreaks (see "Serogroup B Vaccines") such as in 2004 in the New Zealand serogroup B outbreak. A serogroup B outer membrane protein–containing vaccine, VA-MenGOC-BC (Finlay Institute, Havana, Cuba), was also extensively used in Cuba and Latin America.[175–178] New approaches to serogroup B vaccines based on conserved outer membrane proteins are now approved and in use in Europe, Canada, Australia, the United States, and other countries (see "Serogroup B Vaccines").[37,179]

Serogroup C (predominantly CC ST-11) continues to account for significant meningococcal disease, especially in older children and adolescents and young adults throughout the world.[48,153,180–182] Increases in serogroup C meningococcal disease were seen in the United States and Europe in the 1980s and 1990s. In response, the meningococcal serogroup C conjugate vaccines were developed and first introduced in the United Kingdom in 1999. After serogroup C conjugate vaccine introduction, serogroup C invasive disease rapidly declined in the United Kingdom[35,36,138,183] and in other European countries,[184] but it has not been eliminated.

In the United States, surveillance including Active Bacterial Core surveillance (ABCs), a prospective laboratory- and population-based surveillance system, has tracked invasive bacterial pathogens, including *N. meningitidis*.[4,132] This surveillance has documented the contribution of the *Haemophilus influenzae* type b capsular conjugate vaccines, the pneumococcal conjugate vaccines, and the MCVs to the marked decline in bacterial meningitis in the United States. However, *N. meningitidis* remains an important cause of bacterial meningitis and septicemia in infants, children, and adults. In the United States, it has been the second most common cause of community-acquired adult bacterial meningitis.[185]

Based on this recent detailed surveillance, the incidence of meningococcal disease in the United States in the last quarter-century peaked at 1.7 per 100,000 population in the mid-1990s and since has continually declined; it was 0.35 per 100,000 in 2007,[3] and in 2015 it was at a historic low of 0.12 per 100,000 population,[4] a decrease of greater than 92%. From 2006 to 2015,[4] a total of 7924 cases of meningococcal disease were reported in the United States; 14.9% of these cases were fatal. "Sporadic" cases accounted for 98% of cases, but molecular typing is better defining invasive isolate relatedness in communities.[186] The incidence of meningococcal disease varies by season, with more cases occurring during January through March (34.7 % of cases) and the fewest cases occurring during August and September (11.2% of cases). The incidence of meningococcal disease in 2006 through 2015 by state ranged from 0.13 cases per 100,000 population to 0.79 cases per 100,000 population (median, 0.25 per 100,000). The incidence was highest in Oregon (0.79 cases per 100,000 population), reflecting the persistence of the longstanding serogroup B ST-32 strains in the region. The incidence of serogroup B–specific meningococcal disease was 0.31 cases per 100,000 population in Oregon, compared with 0.07 cases per 100,000 population in the other states.

Although the highest attack rates occur in the very young (<1 year old, 2.45 per 100,000), 73% of cases of invasive meningococcal disease in the United States occur in adolescents and adults. The rates of meningococcal disease have declined in all age groups in the United States since the mid-to-late 1990s.[3,4] Male patients accounted for 50.6% of cases. Female patients were significantly older than male patients (median age, 35 years in female patients compared with 22 years in

[d]References 1, 11, 99, 100, 102, 155, 159.

male patients). The incidence of meningococcal disease among black persons was 0.27 cases per 100,000 population, compared with 0.20 cases per 100,000 population among white persons and 0.20 cases per 100,000 population among other race categories combined. The distribution of serogroups causing disease has also shifted in the United States.[3,4,187] From 2006 to 2015, serogroup B caused 35.8% of all meningococcal disease, serogroup C was responsible for approximately 22.8% of endemic disease, and case clusters and local outbreaks and serogroup Y caused 28.5% of cases. The case-fatality rates of serogroups C (20.2%) and W (20.9%) were higher than those of serogroup B (11.5%) or serogroup Y (13.7%).

CLINICAL MANIFESTATIONS AND PATHOPHYSIOLOGY

The common presentations of invasive meningococcal disease are *meningococcemia* and acute *meningitis*. In several large series of cases, predominantly of serogroup B or C disease[4,83,188–192] in industrialized settings, 40% to 65% of patients presented with meningitis, 10% to 20% had fulminant meningococcemia with shock but without meningitis, 7% to 12% had both meningitis and fulminant meningococcemia, and 18% to 33% had bacteremia without shock or meningitis. Less common presentations are *primary pneumonia* (up to 10%, especially with serogroup Y), *septic arthritis* (2%), *purulent pericarditis, chronic meningococcemia, gastroenteritis, conjunctivitis, epiglottitis, sinusitis, otitis, urethritis,* and *proctitis*.[4,83,188–191] Rare presentations include necrotizing fasciitis.[193] Invasive disease often occurs in otherwise healthy individuals and is difficult to identify early. In part, this is because sporadic meningococcal disease is not common and the classic clinical features of disease (e.g., petechial or purpuric rash, meningismus, and impaired consciousness) often appear late in the illness.

In fulminant meningococcemia, the time window between the progression from initial symptoms to death can be narrow (hours). In a study by Thompson and colleagues,[191] nonspecific symptoms, such as fever, loss of appetite, nausea and vomiting, and sometimes diarrhea (which may have been preceded by a coryza and pharyngitis), occur in the first 4 to 6 hours, with more severe symptoms developing by 8 hours, such as leg pains, cold hands and feet, labored breathing, abnormal skin color, and rash; the median time to hospital admission was 19 hours, and by 24 hours, individuals can be dead or moribund.[188] Physicians and health care providers should be alert to the concern of parents or relatives about the abrupt or rapid deterioration of a patient.[194,195] Major complications of meningococcemia and/or meningococcal meningitis are necrosis and limb or digit loss, scarring, cranial nerve palsies (mostly commonly eighth nerve loss resulting in deafness), postinfectious immune complex–mediated polyarticular arthritis or pericarditis, long-term learning and cognitive disabilities, seizures, motor deficits, and chronic renal dysfunction. The overall case-fatality rate, despite antibiotics and aggressive support, remains at approximately 10% to 15% in developed countries.

Although the clinical features of meningococcal disease are similar in all epidemiologic situations, the number of patients who have a specific clinical presentation can vary from outbreak to outbreak for reasons that are not well understood. For example, meningitis is the major presentation, and septicemia is recorded less frequently during serogroup A epidemics in the African meningitis belt in comparison with patients in industrialized countries.

Meningococcemia

In 10% to 20% of cases, septicemia with shock is the dominant clinical picture and presentation is acute, with sudden-onset fever, generalized malaise, weakness, cold extremities and skin pallor, leukocytosis or leukopenia, rash, headache and/or drowsiness, and hypotension.[5,196] A petechial or purpuric rash, a classic sign of meningococcal septicemia, is seen in 40% to 80% of cases of meningococcemia but may be difficult to detect initially. A maculopapular blanching rash can also be an early sign in the disease, can progress to a petechial or purpuric rash, or can persist in 13%.[197] Median time from onset to admission for meningococcemia in two studies was 13 hours and 12 hours, which is less than half the time recorded for patients with meningitis.[85,198] Generalized muscle tenderness may also be an important differential sign. Occasionally the pain from these myalgias is quite intense and causes the patient considerable discomfort. Clinical signs of meningeal irritation are usually absent, few meningococci are present in CSF, and CSF pleocytosis is negligible.[1]

A rapid proliferation of meningococci in the circulation characterizes meningococcal septicemia (including the classic Waterhouse-Friderichsen syndrome[199] with adrenal hemorrhage) (Figs. 211.5 to 211.7), resulting in very high concentrations of bacteria (10^5–10^8/mL) and meningococcal endotoxin (10^1–10^3 endotoxin units [EU]/mL).[1] Cases with fatal outcome have higher concentrations of meningococcal endotoxin in plasma than mild cases, and these patients are sicker than survivors.[1] The adherence of intact meningococci and/or OMVs to the microvasculature and association with macrophages and mononuclear cells in fulminant meningococcemia is striking.[200] Expression of human CD46 by macrophages may accelerate inflammatory responses on meningococcal infection or LOS stimulation.[201] The rapid bacterial growth in the bloodstream causes an exaggerated and destructive intravascular inflammatory response, leading to progressive circulatory collapse and severe coagulopathy.[1] There is also evidence of meningococcal proliferation and massive local inflammatory response in specific organs.[202,203] Shock all too frequently dominates the clinical picture. Patients can present with severe, persistent shock that lasts more than 24 hours or until death. The patient is poorly responsive, and peripheral vasoconstriction is present, with cyanotic, poorly perfused extremities. Arterial blood gas analysis demonstrates evidence of acidosis in the range of pH 7.25 to 7.3, and, depending on the degree of shock, anoxia may manifest with an arterial oxygen pressure below 70 mm Hg.

Patients develop impaired renal, adrenal, and pulmonary function and disseminated intravascular coagulation (DIC), with thrombotic lesions in the skin, limbs, kidneys, adrenals, choroid plexus, heart, and occasionally the lungs. Clinical evidence of DIC includes increasing petechiae within prescribed areas, gastric or gingival bleeding, or oozing at sites of venipuncture or intravenous infusions. Myocardial dysfunction is also well described in adults and children with meningococcemia.[204–206] Postmortem studies by Hardman[207] and by Gore and Saphir[208] indicated that myocarditis of varying degrees of severity is present in more than half the patients who die of meningococcal disease. The hypotension and vascular complications can lead to extensive scarring and loss of digits or limbs, and survivors can be severely handicapped.[196]

Mild or transient bacteremia without sepsis is a presentation in less than 5% of cases. Admission or emergency evaluation is often for an upper respiratory tract illness, fever, or presumed viral exanthema. After recovery in 2 to 5 days and frequently after release without specific antimicrobial therapy, the unexpected results of blood cultures are reported as positive for *N. meningitidis*. In one study, Sullivan and LaScolea[209] reported that the levels of bacteremia in this presentation were low, from 22 to 325 organisms per milliliter of blood. Meningococcal disease in terminal complement-deficient patients is also often milder and associated with a lower mortality for reasons that are not well understood (see "Complement Deficiency and Meningococcal Disease").[115] Also, patients with isolates with LOS lipid A mutations have less rash and coagulopathy.[210]

Meningitis

Meningitis is the most common presentation of invasive meningococcal disease and is seen in 40% to 65% of cases,[e] reflecting the meningeal tropism of *N. meningitidis*. In older children, adolescents, and adults, sudden-onset headache, fever, vomiting, myalgias, photophobia, irritability, decreased ability to concentrate, agitation, drowsiness, and meningeal signs (neck stiffness, Kernig or Brudzinski sign), and cloudy CSF, with or without a rash, are seen.[211–224] Uncommonly, the conus medullaris syndrome,[216] or more frequently cranial nerve dysfunction (particularly of the sixth, seventh, and eighth cranial nerves), can complicate meningococcal meningitis.[217] Although focal neurologic signs and seizures may be less common in meningococcal meningitis than in pneumococcal meningitis or in meningitis caused by *H. influenzae* type b,[217] van de Beek and associates[224] reported that 33% of 257 adult patients with meningococcal meningitis had focal neurologic deficits and 5% had

[e]References 1, 4, 5, 188–192, 194, 196.

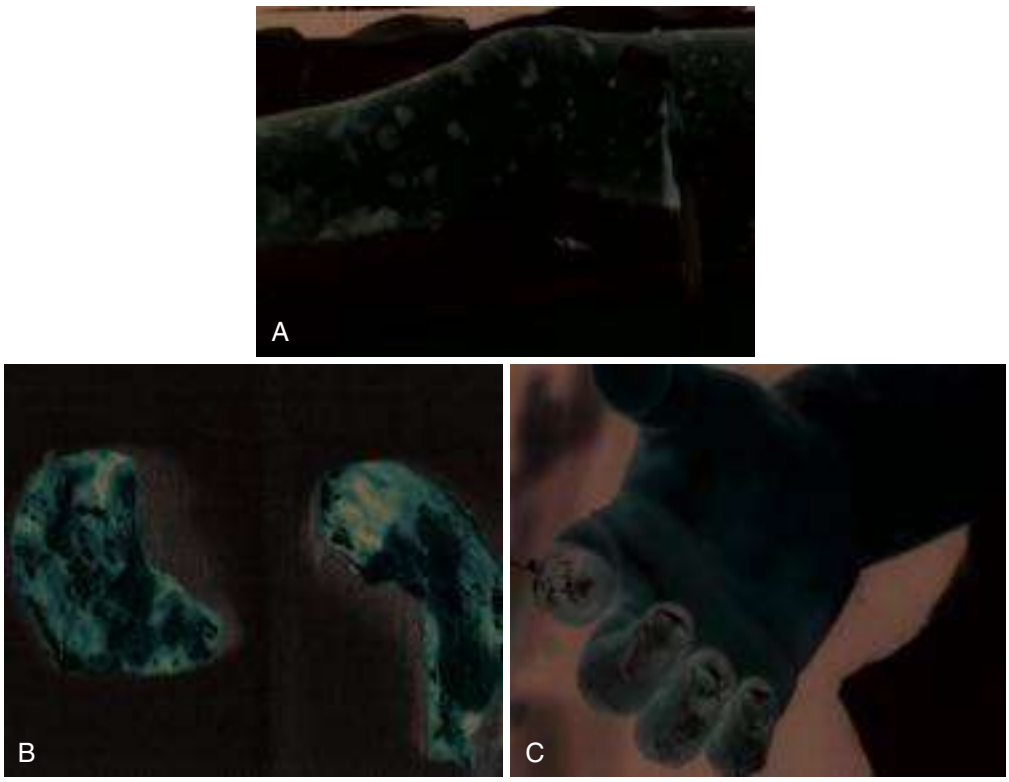

FIG. 211.5 Fulminant meningococcal septicemia. (A) Ecchymoses. (B) Hemorrhagic adrenals. (C) Thrombosis and gangrene of the fingers of a child. *(From Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and* Neisseria meningitidis*.* Lancet*. 2007;369:2196–2210.)*

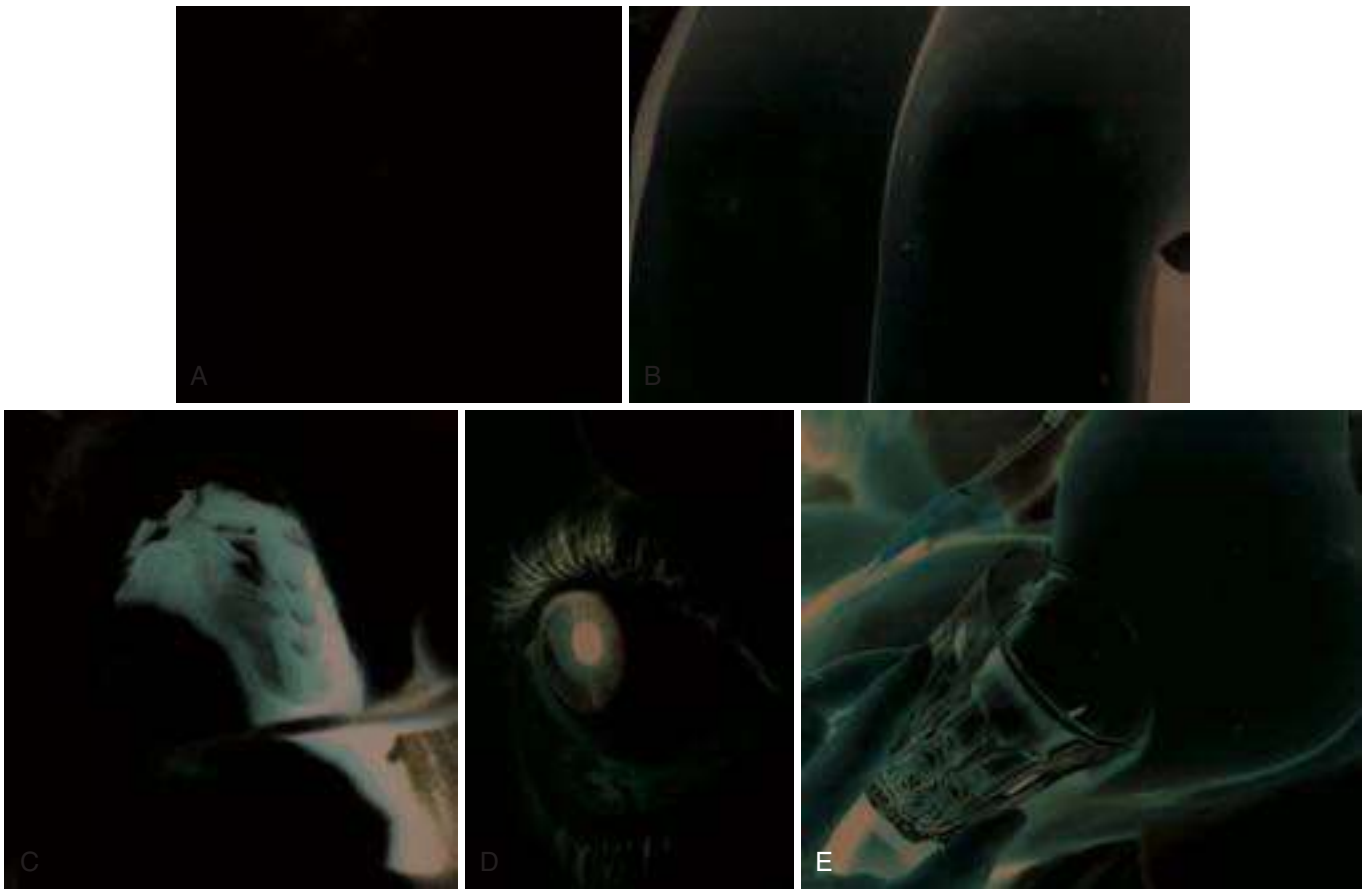

FIG. 211.6 Rashes of meningococcal disease. (A) Rubella-like rash seen early in meningococcal sepsis. Macular (B) and nonblanching petechial (C–E) rashes in meningococcal disease. *(From Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and* Neisseria meningitidis*.* Lancet*. 2007;369:2196–2210.)*



FIG. 211.7 Subcutaneous ecchymoses, embolic lesions, and petechiae of meningococcal disease. (A) Subcutaneous ecchymoses on a sole resulting from meningococcal sepsis. (B) Subcutaneous ecchymoses on the back resulting from meningococcal sepsis. (C) Embolic lesions on a palm secondary to meningococcal sepsis. (D) Petechial lesions on legs in meningococcal disease. (E) Petechiae on the dorsum of a hand resulting from meningococcal sepsis. *(Courtesy Dr. Peter Densen.)*

seizures during their clinical course and that seizures may increase to one-third in children.[5,218] Subdural empyema, in contrast to *Streptococcus pneumoniae* meningitis, is a rare complication of meningococcal meningitis. Some patients with meningococcal meningitis are comatose with an encephalitic picture and pathologic reflexes.

The inflammatory response triggered within the subarachnoid space by replicating meningococci is responsible for the symptoms and signs of meningitis.[219] A rash may be present in 26% to 62% of patients with meningococcal meningitis[220,221] and is commonly petechial but may have fewer lesions than seen in meningococcal septicemia, and/or atypical lesions. In a prospective observational cohort study, the classic meningitis triad of fever, neck stiffness, and altered mental status was present in 70 of the 258 patients (27%) with meningococcal meningitis; when rash was added, 89% of patients had at least two of these four signs.[223] The classic triad is much more common in pneumococcal meningitis (58% in the same cohort study).[224] As reviewed by Pace and Pollard,[5] children younger than 5 years with meningococcal meningitis may present with irritability or lethargy, fever, vomiting, impairment in consciousness, or seizures[211,225] as predominant features without signs of meningeal irritation.[211,225,226] Infants may have inconsolable crying, poor feeding, and a bulging fontanelle.[225,227]

Patients with meningococcal meningitis usually have a low concentration of meningococci ($<10^3$/mL) and endotoxin (<3 EU/mL) in plasma but high concentrations in CSF,[85,198,228–232] leading to a large compartmentalized inflammatory response in the subarachnoid space, with pronounced increase in concentrations of TNF-α, interleukins (IL-1β, IL-6, IL-8, and IL-10), different chemokines, and other inflammatory mediators. The inflammatory response in the systemic vasculature, as shown by cytokines and complement activation, is modest.[85,198] Raised intracranial pressure, caused by cerebral inflammation and edema, in meningococcal meningitis may lead to cerebral herniation and death. Thomas[233] found that focal cerebral involvement in meningococcal meningitis was rare. The cause of death in meningococcal meningitis was related to cerebral edema and to secondary effects on the vital centers in the midbrain region. Meningococcal meningitis has a mortality of 5% to 18%.[4,189,224] Although either meningococcemia or meningitis usually predominates, about 12% of patients have features of both.[188]

Brandtzaeg and coworkers have made major contributions to our understanding of the physiologic effects of LOS during sepsis and meningitis caused by *N. meningitidis*.[f] These studies have placed a pathogenetic rationale for the clinical states of infection described by Wolfe and Birbara[192] and others, have demonstrated the ability to measure LOS in the plasma and CSF of infected patients, and have shown a

[f]References 83, 85, 198, 219, 228, 230, 232.

close correlation between plasma LOS levels and prognosis. The compartmentalization of LOS production correlates with the clinical findings in meningococcal infections.[230] LOS levels in patients defined as having septicemia were high in plasma (median, 3500 ng) and low in CSF, whereas in patients with meningitis, LOS was detectable in the plasma of 3 of 19 patients and in the CSF of 18 of 19 patients with median levels of 2500 ng. The release of LOS from the surface of the meningococcus in the form of membrane blebs[231] is now considered to be the principal factor associated with the high endotoxin levels in meningococcal sepsis or meningitis. Meningococci covered with multiple, long membrane protrusions, thus indicating surplus outer membrane (blebbing), occurs in both the blood and CSF in vivo.[228,229] Mass spectrometric analysis of the endotoxin from patients with meningococcal sepsis indicated that the endotoxin was of meningococcal origin rather than arising from the gastrointestinal tract as a result of increased permeability during infection.[234]

Rash of Meningococcal Disease

In endemic and epidemic disease outbreaks in industrialized countries, skin lesions are present in 28% to 77% of patients with invasive meningococcal disease on admission[235–237] but can be more difficult to see in patients with dark skin.[238] The classic petechial rash manifests as discrete lesions 1 to 2 mm in diameter, most frequently on the trunk and lower portions of the body (see Fig. 211.6) but also on mucosal membranes and sclera. Lesions are commonly seen in clusters in areas where pressure may be applied to the skin by elastic in underwear or stockings. Petechiae of meningococcemia are usually larger and bluer than pinpoint petechiae caused by thrombocytopenia, leukocytoclastic vasculitis induced by other infections, or those induced by vomiting or coughing. The petechial lesions can coalesce and form larger lesions that appear ecchymotic. Ecchymoses (diameter >10 mm) or purpura are mainly noted in patients with severe DIC.[198,235] Ecchymosis are secondary to subcutaneous hemorrhage, can occasionally be vesicular, and can desquamate as the patients recover. Petechiae correlate with the degree of thrombocytopenia and are clinically important as an indicator in the evolution of bleeding complications secondary to the DIC. Early and aggressive intervention with antimicrobial agents and support of vascular perfusion are keys. At times, surgical débridement of lesions and skin grafting may be necessary. Deep necrosis of limbs or digits may call for amputation. Biopsy samples from dermis show that meningococci are present in and around the microvascular endothelial cells.[200,239,240] Meningococci in these lesions express the polysaccharide capsule, pili, and PorA and can be cultured up to 12 hours after effective antibiotic treatment is started.[200,239,240]

Petechial lesions are a common harbinger of systemic meningococcal infections, but occasionally if the patient is not completely undressed when examined or if examination of mucous surfaces such as the palpebral conjunctiva is omitted, these important telltale lesions or other rash can be missed (see Fig. 211.6C–D). Differential diagnosis of the rash of meningococcal disease includes Henoch-Schönlein purpura (leukocytoclastic vasculitis), Rocky Mountain spotted fever, typhus, viral infections, idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura; a petechial purpuric rash can also be seen in sepsis due to *S. pneumoniae, H. influenzae, Staphylococcus aureus,* and other bacterial pathogens. In two studies of children with a nonblanching rash,[221,222] 11% and 15% were caused by *N. meningitidis*. Clinical signs such as fever greater than 38.5°C (101.3°F), purpura, slow capillary refill time, circulatory collapse, and signs of meningitis increase the likelihood of meningococcal disease for patients with a nonblanching rash. Children with a nonblanching rash confined to the distribution of the superior vena cava are very unlikely to have meningococcal infection.[221]

A number of authors have described a maculopapular blanching eruption in invasive meningococcal disease that can vary somewhat in hue and can be mistaken for a wide variety of viral exanthems, particularly rubella (see Fig. 211.6).[192,241,242] This eruption is not initially purpuric or pruritic but may progress to petechiae or purpura or be transient. In some patients, red maculae with a diameter greater than 1 cm are the only signs of meningococcemia (see Figs. 211.6 and 211.7).[198]

COMPLEMENT DEFICIENCY AND MENINGOCOCCAL DISEASE (See also Chapter 9)

The importance of the human complement system in protection against meningococcal infections is well recognized, in particular the risk for C5 to C9 deficiencies of the terminal pathway complement pathway, properdin (factor P, a promoter of the alternative pathway of complement and link to innate NK cell activity) deficiency, and C3 deficiency.[243–253] Up to 39% of individuals with late complement deficiency and 6% with properdin deficiency develop systemic meningococcal infections. Terminal complement deficiency is associated with a 1400- to 10,000-fold increase in development of meningococcal disease, and 40% to 50% of patients have recurrent infections.[243,244] Vaccinating individuals deficient in late complement components may shift the burden of host defense from serum bactericidal activity (SBA) to opsonophagocytosis and confer protection.[251,252]

Studies by Lim and coworkers first demonstrated an absence of the sixth complement component as a risk factor for meningococcal disease,[246] and Alper and associates noted that a patient with recurrent meningococcal disease lacked C3.[247] Human deficiency of C8 was found in persons with meningococcal infections, disseminated gonococcal infection,[248] and gonococcal meningitis.[249] Ellison and coworkers[250] evaluated the complement system in 20 patients with first episodes of serious systemic meningococcal infection. Six of 20 (30%) had a complement deficiency. Three had deficiencies in a terminal complement protein or proteins, and three had deficiencies of multiple factors associated with underlying disease states. Densen and coworkers studied a family with properdin deficiency whose members had a high rate of fatal meningococcal disease[251]; about 50% of properdin-deficient individuals develop invasive meningococcal disease.[244]

Complement deficiency is also a factor in the risk for meningococcal disease associated with systemic lupus erythematous and C3 and C4 nephritic factor (autoantibody) states,[245] such as in glomerulonephritis and in patients in whom the complement inhibitor eculizumab is used.[254] The role of complement in meningococcal infections has been reviewed by Densen[243] and by Ram and colleagues.[244] The role of the lectin complement activation pathway in meningococcal disease is controversial. Data have supported the hypothesis that MBL polymorphisms may play a role in susceptibility to meningococcal disease in early childhood[2] or even colonization, but data from large case-control studies revealed that MBL2 structural polymorphisms do not predispose children or adults to invasive meningococcal disease.[120,255]

Chronic Meningococcemia

Chronic meningococcemia is an uncommon meningococcal infection that can last from weeks to months.[256–260] The symptoms are intermittent fever (often low grade), migratory arthralgias or arthritis, and a nonspecific, often maculopapular rash; however, petechial, purpuric, and vesicular rashes are also reported. Symptoms might completely disappear for days before they return with fever, joint pain, and signs of vasculitis. The distribution and appearance of the cutaneous lesions can resemble those seen in disseminated gonococcal infection, for which it can be mistaken.[261] Systemic meningococcal infection should be in the differential diagnosis of the acute arthritis-dermatitis syndrome. Chronic meningococcemia can progress to meningitis and death. Recently, meningococcal isolates with a mutation in the *lpx*L1 gene that results in an underacylated LOS, a less potent endotoxin inducer of proinflammatory cytokines and tissue factor,[210,262] have been associated with chronic meningococcemia.[263]

Primary (Purulent) Pericarditis

Meningococcal bacteremia can result in pericardial infection, sometimes presenting as a massive pericardial effusion and tamponade.[264–270] Primary meningococcal pericarditis is described more in adolescents and adults and has been predominantly caused by serogroup C and W, especially CC ST-11 strains.[267,268] With appropriate antibiotic treatment and drainage of pericardial fluid, patients can do well. Sterile pericarditis is also a postinfectious complication of meningococcal disease.

Septic Arthritis

Primary (purulent) meningococcal arthritis[5,261,268,271–273] is often monoarticular, with the knee and ankle being the joints most frequently affected,[5,272] often occurs in adolescents and young adults (mean age of 21 years), and has been associated with hyperinvasive strains (serogroup C or W) of the ST-11 CC.[5,268] Meningococcal septic arthritis can also resemble the arthritis-dermatitis syndrome caused also by *N. gonorrhoeae.*[3] Response to antibiotics is excellent, and a prolonged course or drainage is usually not required. Septic arthritis should be distinguished from postinfectious immune complex–mediated arthritis,[273] which occurs during convalescence after meningococcal disease (see "Complications").

Respiratory Tract Infections

Meningococcal pneumonia has been recognized as a clinical syndrome for more than 80 years.[4,268,274–276] Of all meningococcal cases, 5% to 10% manifest as pneumonia, and approximately 10% of patients with invasive meningococcal disease have an infiltrate evident on a chest radiograph. Primary meningococcal pneumonia is a more common presentation in adults and especially older adults (>50 years). In patients from whom *N. meningitidis* was isolated, serogroup Y has been the most prevalent single serogroup causing pneumonia (44%), followed by W (16%), B (17%), and C (15%).[4,268,274] In a study of the etiology of community-acquired pneumonia in Finland, *N. meningitidis* was implicated as the etiologic agent in 6 of 162 cases.[277] In the US surveillance of meningococcal cases in patients older than age 65 years, approximately 35% manifested as pneumonia.[4] Because of the nasopharyngeal carriage of the meningococcus, establishing the diagnosis by sputum culture or stain alone is difficult. Koppes and associates used transtracheal cultures to establish the diagnosis in Air Force recruits with group Y meningococcal pneumonia.[275] In that series, a history of cough, chest pain, chills, and previous upper respiratory tract infection occurred in more than half the patients. Rales and fever occurred in almost all patients, and evidence of pharyngitis was present in more than 80%. The disease involved more than one lobe in 40%, with the right lower and middle lobes involved most frequently. The prognosis was good, with no deaths occurring in the 68 recruits with pneumonia. In the elderly, however, the picture is different, with significant mortality of 15.9% with meningococcal pneumonia.[4] One difficulty in the interpretation of infiltrates is the association of invasive meningococcal infections with preceding viral respiratory tract infections. Young and coworkers investigated an outbreak of meningococcal infection in an aged population, most of whom had serologic evidence of influenza.[276]

Meningococcal sinusitis and otitis are described,[238] but in contrast to *S. pneumoniae, H. influenzae, or Moraxella catarrhalis,* the meningococcus is an uncommon cause of these focal respiratory tract infections. However, in one study, *N. meningitidis* was found in 4.7% of persistent middle ear effusions.[278] Meningococcal upper respiratory tract infection (pharyngitis) associated with contacts of cases and as a prior manifestation in cases of serious meningococcal disease has also been described.[279] Meningococcal supraglottitis is a rare presentation of meningococcal infection. Supraglottitis was first reported as a syndrome in 1995; since then, multiple case reports have been published including recent presentations of serogroup W (CC:ST-11). The diagnosis should be considered with the patient's clinical picture of sore throat, dysphagia, fever, muffled voice, and swollen supraglottic tissues, as seen on plain radiography, fiberoptic laryngoscopy, and cervical computed tomography.[280]

Conjunctivitis

Meningococcal conjunctivitis, typically manifesting with a unilateral hyperacute purulent exudate with hyperemia and edema, has been reported in all age groups, including neonates, but is more common in childhood (mean age, approximately 8 years).[5,281,282] *N. meningitidis* accounts for approximately 2% of cases of bacterial conjunctivitis reported.[282] Direct inoculations of secretions or airborne droplet spread is the presumed mode of transmission. Meningococcal conjunctivitis may progress to panophthalmitis or to invasive disease (10%–40% of patients) or both; thus, systemic therapy with intravenous antibiotics and contact chemoprophylaxis is the preferred method of treatment.[282] Conjunctivitis and endophthalmitis are also reported as complications of meningococcal bacteremia.

Meningococcal Urethritis and Proctitis

Meningococci can be isolated from the urethra and can be the etiologic agent of urethritis. Recently, increased cases of meningococcal urethritis have been reported from sexually transmitted infection clinics in the United States.[283] An association between orogenital sex and acquisition of the organism has been suggested.[284,285] The US urethritis outbreak is due to a specific CC ST-11.2 clade.[286] The clade has acquired gonococcal genes important for anaerobic growth. In MSM, the meningococcus can be frequently isolated from the oropharynx, rectum, and urethra.[284] The meningococcus can be carried asymptomatically in the rectum of both men and women and has been linked to approximately 3% of symptomatic proctitis in homosexual men.[287–289]

Complications

Immune complex–mediated complications such as arthritis, cutaneous vasculitis, iritis, episcleritis, pleuritis, and pericarditis can first appear several days to 2 to 3 weeks after onset of invasive meningococcal disease, when the patient is otherwise improving. These complications are due to the deposition of antigen–antibody complexes composed of meningococcal capsular polysaccharide or other antigens, meningococcal-specific immunoglobulins and C3; they complicate 6% to 15% of meningococcal meningitis or septicemia.[5,290,291] The subject has been reviewed by Pace and Pollard.[5] These inflammatory complications may be associated with reemergence of fever and an increase in the number of leukocytes and in C-reactive protein (CRP) levels; and it is more commonly seen after severe disease, following serogroup C infections, and in adolescents and adults.[5] The most common manifestation, an aseptic monoarticular arthritis in 7% to 14% of patients, tends to affect the knees, ankles, or elbows, although polyarthritis may also occur.[291] Cutaneous vasculitis in 2% to 8% has a diverse presentation varying from a maculopapular rash, which may be mistaken as a drug-induced rash, to vesicular-bullous lesions, ulcers, and nodules.[291,292] Pleuritis and pericarditis may be associated with an effusion, which rarely requires drainage.[291,292] Treatment is with aspirin or nonsteroidal antiinflammatory drugs, and resolution is complete, usually within 14 days from the onset and usually without residual sequelae.[5] A case report noted excellent response with intravenous immunoglobulin to an immune complex reaction after meningococcal sepsis.[293]

Long-term sequelae and morbidity after meningococcal disease are significant. Neurologic impairment occurs in 7% to 10% of patients with meningococcal meningitis.[294] Palsies of cranial nerves VI, VII, and VIII and hemiparesis and quadriparesis occur. Unilateral or bilateral sensorineural hearing loss occurs in 2% to 9% of patients,[292,294,295] which is profound in 2% of affected persons[8] and necessitates cochlear implantation in 0.4%.[295] A formal audiologic assessment should be performed as soon as possible after discharge. Neurodevelopmental impairment, including behavioral and psychological problems, learning difficulties, memory deficits, executive function problems, decreased academic performance, spasticity, seizures, and focal neurologic signs, is also seen in approximately 10% of patients.[292–294,296–300] Visual difficulties, seizures, and motor deficits are reported in 2% to 3%, with multiple neurologic disabilities occurring in 1% to 2% of affected individuals; reported incidence of the latter is twice as high in Africans and Southeast Asians.[294,301] Survivors of meningococcal sepsis and meningitis in childhood have, in 5% to 37% as young adults, long-term behavioral and emotional problems, problems with intellectual functioning, and illness-related physical or social consequences.[302,303]

Headache and lower limb pain are also frequent chronic symptoms observed in children after meningococcal septicemia, although data on the incidence of chronic pain are limited. Scarring of the skin, secondary to necrotic purpura, may vary from unnoticeable to requiring skin grafting.[304] Multiple areas may be involved, with the lower limbs being most frequently affected, followed by the arms, chest, and face.[305] Amputations of the digits or limbs are frequently multiple, result from necrosis of the skin,[306] muscle, and bone of the affected parts (see Fig. 211.5), and, depending on the site and extent, prostheses may be required in order to improve function or appearance.[307] Bone growth disturbances, stump overgrowth, scar contractures, and soft tissue and bone infections may complicate amputations.[308] Limb-length discrepancies, which may result from the growth plate infarction, often necessitate further surgical

intervention.[305] After acute renal failure at presentation, renal function recovers in the majority of individuals; however, evidence of renal dysfunction may persist for more than 4 years in both children[309] and adults,[310] with the risk being higher in those who required renal replacement therapy.

Family and Community Impact

Meningococcal disease and its complications, often occurring rapidly in otherwise healthy individuals, have significant individual, family, community, health care, and public health impact.[311–320] The physical and emotional toll on individuals who survive[312] and of the families of those with meningococcal disease in intensive care units, of those who survive with complications, and of those who die is considerable and a global phenomenon.[311–315] In communities, meningococcal disease may also create considerable fear and anxiety.[316] Posttraumatic stress disorder (PTSD) occurs at a higher frequency in both patients and families, often months after the illness.[314–316] In one study, PTSD was present in 15% of children, in half of the mothers, and in 19% of fathers[314] at 3 months. Meningococcal disease and its complications also result in substantial hospital and long-term health care costs.[318,319] Furthermore, delay in the diagnosis of meningococcal sepsis and meningitis and septicemia is a common reason for successful litigation.[320,321]

DIAGNOSIS

Confirming the cause as *N. meningitidis* is important in order to initiate prevention of secondary cases, anticipate complications, and have cultures or DNA for antibiotic susceptibility testing and epidemiology. Meningococci with increasing resistance to the penicillins, and rarely resistance to chloramphenicol or quinolones, have been reported (see "Treatment").

The definitive diagnosis of invasive meningococcal disease is based on bacteriologic isolation of *N. meningitidis* antigen or DNA identification of *N. meningitidis* in a usually sterile body fluid such as blood, CSF, synovial fluid, pleural fluid, urine, or pericardial fluid; or detection of *N. meningitidis* in formalin-fixed tissue by immunohistochemistry.[1,3,322–324] Blood and CSF are the most fruitful sources of positive cultures and for DNA identification via the PCR assay, but urine and skin lesions can also yield results in systemic meningococcal disease. The diagnosis of meningococcal meningitis is confirmed through CSF pleocytosis and Gram stain showing gram-negative diplococci (often inside neutrophils) and/or CSF culture, latex agglutination (LA) tests detecting meningococcal capsular polysaccharide in CSF, or PCR assay identifying *N. meningitidis* in CSF. In patients considered to have meningococcemia without clinical evidence of meningitis, CSF cultures are also often positive.[325]

In an analysis of 727 cases of meningococcal disease, Hoyne and Brown described the results of 400 blood cultures, of which 51.4% were positive for meningococci.[326] CSF examination of 423 patients from the same series found 94% positive for gram-negative diplococci by either CSF Gram stain or culture for the meningococci from CSF. Carpenter and Petersdorf indicated that 46% of their cases of meningococcal meningitis were positive by CSF culture.[327] In an additional 12% of the cases, the diagnosis was aided by Gram stain of CSF. Levin and Painter studied 28 patients with culture-proven meningococcal disease; and in 22 of 27 patients tested, the CSF was positive, whereas 15 of 28 had positive blood cultures.[325] In 8 of 12 patients considered to have meningococcemia without clinical evidence of meningitis, the CSF cultures were positive. Feldman has quantitated the bacterial counts of meningococcal meningitis in CSF and reported a mean of 1.27×10^5 (1.5×10^2 to 6×10^7) organisms per milliliter.[328] In cases of meningococcal and pneumococcal meningitis in adults, the Gram stain of CSF has a sensitivity of 40% to 80% and a specificity of over 95%.

Carpenter and Petersdorf[327] examined the CSF of 58 patients with meningococcal meningitis. The median leukocyte count was about 1200, with a range of less than 10 to 65,000/mm.[3] About 75% had CSF glucose levels below 40 mg/dL. CSF protein levels ranged from 25 to more than 800 mg/dL, with a median value of about 150 mg/dL. Although studies have shown higher CSF pleocytosis and protein levels and a lower glucose concentration in the CSF of patients with bacterial meningitis compared with those with nonbacterial, aseptic, or viral meningitis,[329] no clinically reliable threshold can discriminate between bacterial and other forms of meningitis.[330] Early in the course of meningococcal meningitis, CSF findings may be minimal although cultures are positive. Appropriate antibiotics should be given if there is clinical suspicion of the infection and if the cell count is abnormal (>20 cells/μL in neonates and >5 cells/μL in older children).[330]

The ability to see or to culture meningococci in petechial skin and mucosal lesions varies. Hoyne and Brown[326] reported identification in 69.8% of the petechial smears examined. In a prospective study of the use of Gram stain and culture from biopsy specimens from skin lesions in patients with suspected meningococcal infection and controls,[331,332] the sensitivities of culture of the aspiration and skin specimens were 44% and 36%, respectively, and with the combination of Gram stain and culture the sensitivities were 62% and 56%, respectively. Patients with meningococcal disease at presentation can have peripheral leukocytosis and high CRP levels.[329,330,333] However, there is no useful white blood cell threshold; and although in one study[330] a CRP level greater than 6 was 100% sensitive in detecting children with meningococcal disease, it had very poor specificity.

LA tests for meningococcal capsular polysaccharides have been used for detection of *N. meningitidis* in CSF, serum, and also urine samples, especially in patients who received prior antibiotics. LA is less sensitive than a PCR assay, especially for serogroup B. LA assays can cross-react with K1 *E. coli* capsular polysaccharide, but LA can be used in laboratories or epidemiologic settings (e.g., sub-Saharan Africa)[334,335] where a PCR assay is not readily available.

PCR assays are rapid, sensitive, and specific and are increasingly used as the major test for the diagnosis of meningococcal disease. The assay can also determine serogroup and MLST and has the potential for detecting antibiotic resistance determinants.[44,323,324] PCR techniques include real-time PCR testing of CSF, blood, and other sterile specimens, and multiplex assays for other meningitis pathogens. Urine is a less sensitive fluid for PCR assay. An increasing number of patients are now diagnosed by means of PCR testing without culture.[336] The sensitivity and specificity of the PCR assay for the diagnosis of meningococcal meningitis are greater than 90% to 95%; in contrast, the sensitivity of CSF or blood culture is less than 65%.

The PCR assay is particularly useful in diagnosis in situations in which culture has little value because of prior antibiotic administration.[336] Sensitivity is not affected by prior antibiotic administration, which can sterilize the CSF within 4 hours. Multiplex PCR assays permit simultaneous testing for meningococci, pneumococci, and *H. influenzae* type b in establishing the diagnosis of disease.[32,337] PCR assay also has the capability of rapidly genetically typing strains, which is a useful adjunct in situations that appear to be an evolving epidemic.[44] Although the PCR assay has not replaced traditional culture methods, it is more sensitive than culture or antigen detection and is established as a very important tool in the rapid diagnosis of invasive meningococcal infections. However, a negative PCR test result does not rule out meningococcal disease.

TREATMENT

Before passive immune or antibiotic treatment was available, the mortality of invasive meningococcal disease was 70% to 90%.[17] Although the introduction of antibiotics dramatically improved the prognosis of meningococcal disease, current mortality remains 10% to 15% in developed countries (this has not changed significantly in the last 50 years[338]), and is higher in the developing world.[1,4,8,297,339] The value of early diagnosis in lowering the mortality rate is exemplified by the historical results at Fort Dix, New Jersey,[340] where an intense surveillance program was established between 1968 and 1969 and the mortality was less than 5%. An analysis of 7924 cases of meningococcal disease in the United States from 2006 to 2015[4] found that the overall mortality was 14.9%; case-fatality ratios were highest among adults 65 years or older (22%) and decreased with lower age (8.6% in infants younger than 1 year). Isolated bacteremia and meningococcal pneumonia have higher case-fatality ratios (13.2% and 15.9%, respectively) than meningococcal meningitis (9.0%).[4] The case-fatality ratio is highest among cases caused by serogroup W (20.9%) and serogroup C (20.2%) and lower among cases caused by serogroup B strains (11.5%) and serogroup Y (13.7%).[4]

Early recognition by parents and health care professionals of the importance of fever and headache with a rash, prehospital antibiotic treatment, rapid transportation to a local hospital, early effective antibiotics, and stabilization in an intensive care unit can reduce the case-fatality rate. In addition to the early use of antibiotics, the application of supportive care to treat the problems of DIC, shock, heart failure, prolonged mental obtundation, pericarditis, and pneumonia, which complicate this infection, can affect individual cases. For patients in intensive care, recognition of the different pathophysiologic processes associated with meningococcal meningitis (which causes death predominantly by cerebral edema) and meningococcal septic shock (which causes death predominantly through hypovolemia, capillary leak, myocardial dysfunction, and multiorgan failure) results in distinct treatment and management strategies for these two different forms of the disease. Management of shock using volume expansion, intensive care unit monitoring, inotropic support, management of raised intracranial pressure, and the correction of hemostatic metabolic abnormalities can reduce rates of mortality in fulminant meningococcemia.[1]

Antibiotic Therapy

Invasive meningococcal disease is a medical emergency, and early antibiotic treatment should be the primary goal,[341] because effective antibiotics immediately stop the proliferation of *N. meningitidis*.[228,342] Meningococci in CSF are killed within 3 to 4 hours after intravenous treatment with an adequate dose,[343] and concentrations of endotoxin in plasma fall by 50% within 2 hours.[228,342] The concentrations of key cytokines and chemokines fall in parallel.[344,345] Antibiotic treatment does not induce a large release of meningococcal endotoxin or lead to an increased inflammatory response (Herxheimer reaction).[228,346–348] Traditionally, patients have been treated for 7 to 14 days,[349] depending on the clinical syndrome and response of the patient, but 3 or 4 days of intravenous treatment has been shown to cure patients with meningococcal meningitis without relapse.[350–352] However, the duration of antibiotic therapy will vary with the manifestation of the disease and with the response of the patient.

The recommendations for antibiotic treatment of meningococcal meningitis and meningococcemia are summarized in Table 211.1. A goal of antibiotic therapy for meningitis is to establish concentrations of antibiotics in the CSF that approximate 10 times the minimal inhibitory concentration (MIC) of the organism for that agent.[353] Patients with suspected bacterial meningitis of unknown etiology are usually given ceftriaxone or cefotaxime initially, often combined with vancomycin (and sometimes ampicillin in adults older than 50 years) until the causative agent has been identified.[354–356] When *N. meningitidis* is identified, antibiotic treatment should be continued with a third-generation cephalosporin (e.g., ceftriaxone or cefotaxime). Meropenem is also highly active clinically and bacteriologically in the treatment of meningococcal meningitis. If patients are allergic to penicillin and cephalosporin, moxifloxacin and chloramphenicol are potential choices. Fluoroquinolones such as moxifloxacin (used in suspected pneumococcal-resistant meningitis) have excellent in vitro activity against *N. meningitidis,* but clinical data are limited and fluoroquinolone resistance (mutations in *gyr*A) of meningococcal isolates (e.g., CC ST-4821 in China, Japan, and Canada), although rare, has emerged.[356–359]

TABLE 211.1 Antibiotic Treatment of Meningococcal Meningitis and Meningococcemia in Adults With Normal Renal Function

Ceftriaxone, 2 g IV every 12 hours
or
Cefotaxime, 2 g IV every 4–6 hours
or
Meropenem, 2 g IV every 8 hours, 6 g/day
or
If patient has severe allergy to β-lactams (e.g., anaphylaxis), moxifloxacin 400 mg IV once daily; or chloramphenicol, 25 mg/kg IV every 6 hours up to 1 g every 6 hours

Originally modified from Shin SH, Kwang Kim SK. Treatment of bacterial meningitis: an update. Exp Opin Pharmacother. *2012;13:2189–2206.*

The era of chemotherapy for meningococcal infection began with a report of Schwentker and associates in 1937 that demonstrated that sulfonamides could be successfully used in the treatment of meningococcal meningitis and meningococcemia,[360] resulting in a dramatic change in the prognosis. However, studies by Schoenback and Phair[361] and by Love and Finland[362] revealed small populations of sulfonamide-resistant meningococci; and in 1963, an epidemic of group B meningococcal infection occurred at Ford Ord, California, in which the infecting strain was resistant to sulfonamides.[363,364] The majority of *N. meningitidis* isolates are now resistant to sulfadiazine.[365] Thus, sulfonamides now are no longer used in the treatment of meningococcal infections.

Early studies of penicillin given in relatively low doses (120,000 units/day IM) indicated that it was not as effective as sulfonamides.[366] Using larger amounts of the drug, Kinsman and D'Alonzo demonstrated that treatment results with penicillin were identical to those with sulfonamides.[367] Historically, high-dose penicillin therapy, using an intravenous dose of 300,000 units/kg/day with an upper limit of 24 million units/day administered as 2 million units every 2 hours, was effective for meningococcal infections. Although the majority of *N. meningitidis* isolates have remained susceptible to penicillin, 3% to 26% of isolates (approximately 10% in the United States) now have MICs in the intermediate susceptibility range (0.12–1 μg/mL), and sensitivity of meningococci to penicillin has been decreasing worldwide.[368–376] Relative resistance to penicillin is due to a reduced affinity of penicillin-binding proteins 2 and *penA* gene polymorphisms. CSF levels of penicillin averaged 0.8 μg/mL on the first day of therapy. This concentration approximates the MIC for penicillin G for the most intermediate-resistance isolates. Intermediate-resistance strains are also relatively resistant to cefuroxime, but cefotaxime, ceftriaxone, and meropenem generally remain very active against these strains in vitro. The MICs of cefotaxime, ceftriaxone, and meropenem are generally not affected by the presence of most *penA* polymorphisms (see later). Penicillin-insensitive *N. meningitidis* strains, with MICs greater than 1.0 μg/mL, have been reported from Great Britain, Spain, and, less commonly, the United States and other countries.[372] High-level resistance due to β-lactamase–producing strains of *N. meningitidis* was reported in the 1980s[377] but has not been subsequently confirmed.

An important issue is that antimicrobial susceptibility testing is not routinely performed in many practice settings, and rapid assays used for other organisms (e.g., Etests, disk diffusion, and automated systems) are not standardized or approved for *N. meningitidis*.[378] Also, broth microdilution and agar diffusion reference methods are impractical for clinical laboratories that do not regularly isolate *N. meningitidis*.[378] Cost and episodic limited availability of aqueous penicillin G have also lessened enthusiasm for this agent as the first-line drug in therapy for meningococcal disease.

The therapeutic efficacy of first-generation cephalosporins was studied in the 1960s and produced variable results[379]; and first generation cephalosporin use is contraindicated in treating meningococcal infections. In contrast, third-generation cephalosporins (e.g., ceftriaxone, cefotaxime) have demonstrated excellent in vitro and in vivo effectiveness against the meningococcus and achieve excellent central nervous system concentrations to treat meningococcal infections.[380,381] With the recommended doses of third-generation cephalosporins, CSF concentrations of these antibiotics obtained at a second lumbar puncture are 45-fold to 8750-fold higher than are the MICs (up to .012 μg/mL) for most isolated meningococci.[382] The ability to administer ceftriaxone twice daily makes this drug particularly attractive as the most cost-effective regimen, and ceftriaxone is the first-line recommendation in infants older than 3 months, children, and adults (see Table 211.1).[383–385] In younger infants, cefotaxime is preferred.

Of concern is a recent report from France indicating that 2% of invasive meningococcal isolates from 2012 to 2015 demonstrated reduced susceptibility (0.125 μg/mL, a 10-fold increase in MICs) to third-generation cephalosporins.[386] These isolates harbored a novel *penA327* allele also detected in meningococcal isolates from urethritis and in gonococci. However, the MICs of these isolates remained below the breakpoint (0.250 μg/mL) for resistance, and third-generation cephalosporins remain effective.

The efficacy of chloramphenicol as a therapeutic agent was demonstrated by McCrumb and coworkers.[387] Chloramphenicol is bactericidal for *N. meningitidis* and penetrates the blood-brain barrier effectively. Chloramphenicol has been an effective substitute in patients with immediate reactions (e.g., anaphylaxis) or other severely penicillin-allergic patients[388] but potential toxicity has limited its use. Chloramphenicol-resistant strains have been reported but remain rare in the United States and in most countries.[1,389,390]

Prehospital antibiotic treatment is now advocated[348,391–393] as soon as the diagnosis is suspected and has led to a significantly more favorable outcome. In one study, a single death occurred in 119 patients treated for meningococcal disease with antibiotics before admission, compared with 15 deaths in 329 similar patients ($P = .04$) who were not given prehospitalization therapy. The primary goal is to reduce the case-fatality rate for patients with fulminant meningococcal septicemia with rapidly increasing concentrations of meningococci and endotoxin in the circulation.[85,394] In view of the unimpeded growth of meningococci in the vasculature of some patients with an estimated doubling time of bacteria of 30 to 45 minutes, the window of opportunity closes rapidly. In one study of 51 patients, the chance of surviving the shock was 75% with admission plasma concentrations of endotoxin in the range of 10 to 50 EU/mL, 15% for those with concentrations of 50 to 250 EU/mL, and zero for those with concentrations above 250 EU/mL; 50% of nonsurviving patients with shock die within the first 12 hours of hospital admission.[85,198,345]

If antibiotic treatment is initiated before admission, ceftriaxone or another effective antibiotic should be injected intravenously or intramuscularly in adults and intramuscularly in children. The recommended site for intramuscular injection is the quadriceps muscle. During epidemics in developing countries, a single injection of ceftriaxone injected intramuscularly is as effective as a single injection of long-acting chloramphenicol injected intramuscularly; ceftriaxone has become the preferred treatment for epidemic meningitis in developing countries.[395]

Adjunctive Therapy and Supportive Care

Common complications of meningococcemia are vascular collapse and shock, primarily caused by the effects of meningococcal LOS, a potent toxin. There is also evidence of organ-specific inflammation including the heart, leading to acute myocarditis. The levels of meningococcal LOS in blood from patients with meningococcal disease are closely associated with the clinical presentation and outcome. The cytokine and chemokine cascade induced by LOS and other meningococcal components from endothelial and mononuclear cells leads to vascular collapse and shock. TNF-α released in large quantities by LOS is a mediator of endotoxic shock.[396,397] Girardin and coworkers demonstrated that serum levels of TNF-α, IL-1, and interferon-γ correlated with the severity of meningococcemia in children.[398] Brandtzaeg and coworkers have extensively studied the host responses in meningococcemia and meningococcal meningitis in patients in Norway.[g] These investigators found that IL-6, IL-1, and the antiinflammatory cytokine IL-10 are released into the serum and coexist with TNF-α and LOS in the systemic circulation during the initial phases of meningococcal septic shock. High levels of IL-6, IL-1, and notably IL-10 are associated with a fatal outcome.[399] LOS was compartmentalized in the plasma and in tissues such as heart and lungs in patients[202] with meningococcemia, whereas patients with meningitis had high levels in CSF and low or undetectable levels in plasma. LOS also is a potent inducer of tissue factor mRNA in human monocytes.[85] Tissue factor is the main initiator of blood coagulation and is known to be essential in the development of coagulopathy in meningococcal disease. This group has also shown that extensive complement activation due at least in part to LOS occurs in fulminant cases of meningococcemia, suggesting that complement-activating products in concert with other mediators may contribute to the multiple-organ failure and death occurring in the most severe cases.[400] Based on the pathophysiology, many adjuvant therapies have been tried in efforts to affect meningococcal sepsis and meningitis,[1,341,401–414] with no or marginal success.

[g]References 85, 198, 228, 230, 234, 349, 396, 400, 541-543.

Although initial clinical studies in patients with septic shock treated with recombinant activated protein C substitution (drotrecogin alfa [Xigris]) indicated efficacy, the incidence of serious bleeding was increased.[402–405] In 2011, recombinant activated protein C was withdrawn from the market after the failure of a worldwide trial that did not demonstrate improved outcomes in sepsis.[406] Randomized clinical controlled trials using hyperimmune serum, antibodies, or recombinant bactericidal or permeability-increasing protein ($rBPI_{21}$) designed to inactivate *N. meningitidis* endotoxin have been done, without beneficial effect on survival.[407–409]

Plasmapheresis, blood exchange, extracorporeal membrane oxygenation, and intraaortic balloon counter pulsation have been used in meningococcal purpura fulminans and shock.[411–414] However, no controlled trials have been done. Plasmapheresis and blood exchange have little additive effect to the endogenous clearance of LOS and cytokines from the circulation,[1,411,412] and low concentrations of protein C and antithrombin are not corrected. Extracorporeal membrane oxygenation has been used in several centers,[413,414] with better results for children with acute pulmonary failure than for those with refractory septic shock.[414]

The management of shock and its therapy are best accomplished in an intensive care unit. Circulatory collapse, which is the primary cause of death from meningococcemia in industrialized countries, is a result of an altered endothelial barrier function leading to the capillary leak syndrome, vasodilation, and a pronounced reduction in myocardial function.[1,85,198,204–206] The rapidly rising levels of meningococci in the circulation trigger exaggerated release of cytokine (IL-6, for example, is linked to myocardial dysfunction[206]), bradykinin, and presumably nitric oxide.[1,85] At a later stage, very substantial complement activation contributes to the altered endothelial barrier function and relaxation of the smooth muscles in the vessel wall through large generation of anaphylatoxins (C3a and C5a). The capillary leak syndrome results in increased flux of albumin and water across the altered capillary wall to the extravascular space. The magnitude of this flux can be judged by the volumes of fluids needed to combat insufficient tissue perfusion and hypotension. The patient accumulates a large amount of fluid in the extravascular tissue. Plasma concentration of albumin falls and rises only temporarily after intravenous infusion of albumin. The concentration of albumin in the urine increases and equals the concentration recorded in patients with nephrotic syndrome.[83]

The primary goal in fluid management is to increase the circulating blood volume by means of aggressive fluid treatment.[1,5,198] Both colloids and crystalloids (saline 0.9%) are used,[1] without a demonstrated difference in effectiveness. The volume infused seems to be more important than the type of fluid. Patients with persistent shock need large volumes of fluid. The total fluid volume needed per 24 hours is decided by the response to the treatment (e.g., tissue perfusion, blood pressure, urine output, and evidence of intravascular volume overload). Detailed treatment recommendations for the administration of fluids in fulminant meningococcemia have been reported.[198,349,384,415–419] The volume treatment is usually combined with vasopressor therapy, including dopamine, norepinephrine, epinephrine, or dobutamine.[420,421] Fluid treatment in patients with shock may be complicated by reduced renal function. Patients may need dialysis or hemofiltration to compensate for the failing kidney and to reduce the substantial edema that accumulates. Hyperglycemia is also seen in patients admitted with meningococcal sepsis and septic shock but usually resolves without insulin.[422] Myocarditis will resolve if effective antibiotic therapy is administered early enough.

Patients with meningitis or mild meningococcemia should be given the normal daily requirement of fluid intravenously supplemented with the volume lost before admission unless there was evidence of the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). Excessive volume treatment in patients with meningitis can contribute to fatal brain edema and herniation, but fluid restriction is not of value in meningitis unless there is evidence of raised intracranial pressure or SIADH.[384]

The use of corticosteroids in meningococcal sepsis, particularly in patients with evidence of purpura fulminans and concomitant adrenal hemorrhage (Waterhouse-Friderichsen syndrome), remains controversial.

In the 1950s, several investigators recommended the use of corticosteroid replacement therapy. However, the studies of Belsey and colleagues were inconclusive in demonstrating a beneficial effect of the application of low-dose corticosteroid in meningococcal infections.[423] Patients with fulminant meningococcal septicemia often develop adrenal hemorrhage (see Fig. 211.5). The corticotropin concentration is higher, the cortisol concentration is lower, and the corticotropin-to-cortisol ratio is higher in patients with fatal meningococcal shock than in survivors.[1,424,425] Dexamethasone along with appropriate antibiotic therapy has a beneficial effect on meningococcal sepsis in transgenic mice expressing human transferrin.[426] Adults with septic shock and indications of inadequate adrenal function are often given low doses of corticosteroids.[1,427] A benefit has not been documented in children, although intensive care specialists use low doses of corticosteroids in children with shock caused by *N. meningitidis*.[1]

Dexamethasone has been shown to reduce morbidity (e.g., especially hearing loss) in pneumococcal and *H. influenzae* type b meningitis in industrialized countries[354,355,428,429] and Vietnam,[430] but a benefit has not been established in Africa.[431,432] The evidence for reduction in morbidity in *N. meningitidis* meningitis is also less clear. Randomized controlled clinical trials have not demonstrated that dexamethasone significantly reduces death or sequelae from meningococcal meningitis. However, the numbers of patients in these studies were limited. In one study done from 1998 to 2011 from The Netherlands of adults with meningococcal meningitis, unfavorable outcome was similar in those who received or did not receive dexamethasone.[223] The rate of hearing loss was 3 of 96 (3%) and of death was 4 of 100 (4%) in those who received dexamethasone versus 30 of 258 (12%) and 19 of 256 (7%) in those who did not ($P = .67$ and $P = .24$, respectively).[223] There was no evidence of harm, and the rate of arthritis was significantly decreased in the group receiving dexamethasone.[433] Many now recommend that dexamethasone (10 mg every 6 hours for the first 4 days, and in children at a dose of 0.15 mg/kg every 6 hours for 4 days beginning before or with the first dose of antibiotics) be given early in suspected or confirmed bacterial meningitis.[223,384,433–438] Glycerol has been used to reduce intracranial pressure in bacterial meningitis,[439–441] but its value is not proven.

As pointed out earlier, the problem of DIC is ominous. Petechiae are frequent accompaniments of meningococcal sepsis. The development of increasing petechial lesions, confluent ecchymoses, persistently bleeding venipuncture sites, and bleeding gums despite adequate antimicrobial therapy and supportive care is indicative of DIC. Heparin treatment of this complication of meningococcal disease[1,442] is probably not indicated because as many patients can be harmed by the inappropriate treatment of DIC, as by DIC itself. To complicate matters, in severe DIC the problem of plasmin activation with fibrinolysis becomes a clinical reality. It appears that impairment of the protein C anticoagulation pathway is critical to the thrombosis associated with sepsis and to the development of purpura fulminans in meningococcemia. This occurs because protein C activation is impaired, a finding consistent with the downregulation of the thrombomodulin–endothelial protein C receptor pathway.[443]

Anticoagulant treatment has frequently been given to patients with invasive meningococcal disease and DIC but has not been documented to improve outcome.[198,349,444] Patients with fulminant meningococcal septicemia have retained the capacity of the endothelium to convert protein C to activated protein C,[445] and infusion of protein C concentrate restored the plasma concentration in patients with meningococcemia.[446] As noted earlier, initial reports[403,404,446] with concentrated protein C suggested a beneficial effect with bleeding risks,[405] but there were no adequate control groups, and a phase III study of recombinant activated protein C in children with severe sepsis of all causes (26% meningococcal sepsis) was stopped because no benefit was noted.[406,447] The use of recombinant human tissue plasminogen activator to ease thrombolysis is not lifesaving, increases the risk for cerebral hemorrhage, and is not recommended.[448] Other major life-threatening complications necessitating therapy include adult respiratory distress syndrome, neurologic sequelae ranging from coma to diabetes insipidus, pneumonia that is not necessarily meningococcal but may be secondary to aspiration during the obtunded state, and pericarditis.

PREVENTION

Chemoprophylaxis

Chemoprophylaxis eliminates meningococci from close contacts of confirmed or presumptive cases, protects susceptible individuals, and disrupts the further transmission of meningococci. The occurrence of meningococcal disease in individuals in the same household, especially children and individuals in other close contact settings (e.g., daycare or preschool centers, playmates, teammates, military recruits, closed environments such as college dormitories, residential schools, prisons, other institutions), or anyone else directly exposed to an infected patient's oral or nasal secretions (e.g., via kissing, close social contact, sharing of eating or drinking containers, mouth-to-mouth resuscitation) is 100-fold or greater (e.g., 500- to 800-fold in households) than in the normal population.[3,449,450] The recommended therapy for meningococcal prophylaxis is rifampin, ceftriaxone, ciprofloxacin, or azithromycin (Table 211.2), all of which have activity against meningococci in the nasopharynx, but each agent has caveats regarding use. Chemoprophylaxis with the agents recommended is 90% to 95% effective.[322]

Shortly after the clinical use of sulfonamides for the treatment of meningococcal disease in the 1940s, it became apparent that short courses of the sulfadiazine resulted in the disappearance of meningococcal carriage for prolonged periods.[451,452] With the recognition of widespread sulfonamide-resistant meningococci and the failure of sulfadiazine to have an impact on an epidemic in 1963 at Fort Ord, California,[453–455] these agents were abandoned for meningococcal chemoprophylaxis except in instances in which the meningococcal case strains were known to be sulfa sensitive. Over half of meningococcal strains remain sulfonamide resistant (owing to mutations in *fol*P, which encodes dihydropteroate synthase[365,456,457]), and thus sulfonamides, once highly effective, are rarely used now for chemoprophylaxis.

The search for new agents for chemoprophylaxis has been extensive. Penicillin has proved ineffective: penicillins may suppress but do not eradicate nasopharyngeal carriage.[458] Minocycline and rifampin were found to eradicate the carrier state rapidly, and this eradication persists for up to 6 to 10 weeks after treatment[459]; however, the use of minocycline is not recommended because of vertigo, and the effect on the vestibular system. Rifampin treatment can result in the rapid emergence of rifampin-resistant meningococci in 10% to 27% of patients treated.[460] In addition, rifampin causes red urine and other secretions, which can stain contact lenses and be quite disconcerting unless the patient is forewarned. Rifampin should be avoided in pregnant women and may reduce the efficacy of oral contraceptives. Studies by Pugsley and coworkers[461] demonstrated that ciprofloxacin, 500 mg every 12 hours for 5 days, eradicated the meningococcus from the nasopharynx in 100% of individuals for up to 13 days after the completion of therapy. Gilja and coworkers[462] used a single dose of 400 mg of ofloxacin in a controlled study and showed that it can eradicate nasopharyngeal carriage for up to 33 days in 97% of subjects. A single dose of ceftriaxone (250 mg IM for adults and 125 mg IM for children <15 years) has also been shown to eradicate nasopharyngeal carriage for 14 days.[463] Furthermore, a single 500-mg dose of azithromycin has been found to be as effective as rifampin in eradicating meningococci from the nasopharynx of asymptomatic carriers.[464]

A number of other agents active against meningococci in vitro have been tested but have failed to provide prophylaxis. These agents include erythromycin, trimethoprim, cephalexin, oxytetracycline, and nalidixic acid. Hoeprich[465] studied a number of these drugs and found that the primary factor determining effectiveness as a meningococcal prophylactic agent is the ability to achieve bactericidal levels in tears and saliva.

The question of who should receive prophylaxis has concerned public health officials since the advent of effective chemoprophylaxis. Initially sulfonamides were used, little discrimination between high-risk and low-risk populations was attempted, and the drugs were administered very widely to people without an increased risk for disease. With the clinical emergence of sulfa resistance and the problem of finding agents that are safe and effective, attention focused on the populations at greatest risk. The definition of "close contact" includes persons who have had prolonged contact while in close proximity (3 feet is the general limit for large-droplet spread) to the patient or who have been directly exposed to the patient's oral or nasal secretions.[322] As noted earlier, during

TABLE 211.2 Antibiotic Chemoprophylaxis for Household or Intimate Contacts

ANTIBIOTIC	DOSAGE	COMMENT
Rifampin	Adults: 600 mg q12h orally for 2 days Children <1 mo: 5 mg/kg q12h orally for 2 days Children >1 mo: 10 mg/kg q12h (maximum, 600 mg) orally for 2 days	Rifampin can interfere with efficacy of oral contraceptives and some seizure prevention and anticoagulant medications; may stain soft contact lenses. Not recommended for pregnant women.
Ceftriaxone	Children <15 yr: 125 mg, single IM dose Children >15 yr and adults: 250 mg, single IM dose	Ceftriaxone is recommended for prophylaxis in pregnant women.
Ciprofloxacin	Adults: 500 mg, single oral dose	Not recommended routinely for persons <18 yr, but use in infants and children (20 mg/kg) may be justified after careful assessment of the risks and benefits. Not recommended for pregnant or lactating women. Cases of ciprofloxacin resistance have been reported,[538] but it remains rare.
Azithromycin	10 mg/kg (maximum, 500 mg) single oral dose	Equivalent to rifampin for eradication of meningococci from nasopharynx, but data are limited.

[a]Recommended groups for chemoprophylaxis,[322,469,470] based on exposure to the index patient in the week before onset of illness:

- Household contacts and persons sharing the same living quarters, particularly young children; the attack rate for household contacts exposed to patients who have meningococcal disease is estimated to be four cases per 1000 persons exposed, which is 500–800 times greater than the rate for the total population.
- Daycare center, nursery school or child care contacts, frequent playmates of young children.
- Close social contacts who were exposed to oral secretions in week before onset, such as by kissing or sharing eating or drinking utensils or toothbrushes.
- For airline travel lasting more than 8 hours, passengers who are seated near an infected person should receive prophylaxis.
- Prophylaxis is recommended for health care professionals who have had intimate exposure (e.g., management of airway or contact with respiratory secretions) to an infected person. Vaccination is recommended for microbiologists who may handle *Neisseria meningitidis* isolates.
- Because the risk for secondary cases is highest during the first few days after exposure, chemoprophylaxis should be initiated as soon as possible, ideally <24 hours after identification of the index patient.
- If more than 14 days have passed since the last contact with the index patient, chemoprophylaxis is not likely to be of benefit.
- Pharyngeal cultures are not helpful in determining the need for chemoprophylaxis and may unnecessarily delay the use of effective chemoprophylaxis.
- Chemoprophylaxis has also been recommended for patients given penicillin or chloramphenicol for treatment, because pharyngeal carriage may not be eliminated with these antibiotics and the patient could remain colonized with a virulent strain.
- Ceftriaxone is recommended for pregnant women.
- May want to avoid ciprofloxacin or azithromycin in individuals at risk of QT prolongation.

epidemics and in endemic situations in civilian populations, household contacts have been shown to be at increased risk for infection. Analysis by the meningococcal surveillance group of the Centers for Disease Control and Prevention (CDC) showed that the attack rate in this group was 500 to 800 times greater than that determined for the general population studied.[466] Similar high-risk situations exist in closed populations such as in some college dormitories, long-term care hospitals, nursery schools, and military barracks.[467,468] Recently, close contact of individuals during air travel was identified as a risk factor. In the community, chemoprophylaxis is recommended for household contacts, daycare center members, and anyone exposed to oral secretions but not usually school, transportation, or office contacts unless they meet the criteria for close contact.[322,469,470]

Secondary cases usually occur within 1 to 14 days of the primary case. Close surveillance of this group for at least 14 days would ensure prompt treatment of secondary cases that might arise in the absence of effective chemoprophylaxis. Beginning chemoprophylaxis more than 2 weeks after exposure to the index case is likely too late to prevent secondary cases. Most hospital personnel are not at increased risk and in general should not receive chemoprophylaxis; however, medical staff who have intimate exposures such as mouth-to-mouth resuscitation or endotracheal intubation should receive prophylaxis.[449,469–471] Nosocomial transmission of meningococcal disease between patients has been reported.[472,473] For hospitalized patients, in addition to standard precautions, droplet precautions are recommended until 24 hours after initiation of effective antimicrobial therapy. Laboratory personnel handling cultures also should be vaccinated.[471] The index patient should also be treated before leaving the hospital if penicillin, ampicillin, or chloramphenicol was used because these antibiotics do not reliably eradicate the carrier state.

Immunoprophylaxis

Immune Correlates of Protection and Meningococcal Polysaccharide Vaccines

Simon Flexner, in 1907, demonstrated that a passive immune-based approach using meningococcal antiserum raised in horses and given intrathecally reduced the death rate (>75%) of meningococcal meningitis by one-half.[17] The first meningococcal vaccine candidates based on heat-killed bacterial cultures were developed in 1912 but were ineffective and highly reactogenic. George D. Heist first demonstrated differences in bactericidal activity of human blood against the meningococcus.[474] Unfortunately, Dr. Heist's own sera was a negative control and he died at age 36 (1920) of meningococcal meningitis. In 1935, Henry Scherp and Geoffrey Rake first identified meningococcal capsular material of serogroup A meningococci as a polysaccharide.[475] The introduction of effective therapy and chemoprophylaxis in the 1940s with sulfonamides, penicillin, and other antibiotics put meningococcal vaccine approaches on a slower trajectory. However, with outbreaks of meningococcal disease in military recruits and the appearance of sulfonamide resistance in the meningococcus, renewed interest in meningococcal vaccines began in earnest in the 1960s.

The importance of human SBA as the correlate of protection against meningococcal disease was further defined by Goldschneider, Gotschlich, and Artenstein of Walter Reed Army Institute of Research in a 1969 study of military recruits.[110] Depending on the assay, SBA titers greater than or equal to 4, or greater than or equal to 8, indicate protection against meningococcal disease when human or baby rabbit complement sources, respectively, are used. A greater than or equal to fourfold rise in the SBA titer also indicates protection. Strain selection, complement source, and assay standardization are critical for the interpretation of the SBA. As the validated correlate of protection in efficacy trials of MPS vaccines, SBA has become an accepted basis of new meningococcal vaccine (conjugate, MenB) licensure without efficacy trials.

Work at the Walter Reed Army Institute of Research in the 1960s identified high-molecular-weight meningococcal capsular polysaccharide of serogroups A and C as vaccine antigens inducing protective SBA responses. Artenstein and coworkers[476] then demonstrated the effectiveness of a capsular polysaccharide group C vaccine in studies of US Army recruits; and Makela and associates[477] showed that administration of group A polysaccharide to Finnish military recruits significantly lowered the incidence of meningococcal disease caused by that serogroup when compared with an unvaccinated control population. The work led to the introduction of MPS vaccines for serogroups A, C, and later Y and W in the 1970s and 1980s in military and civilian populations and for control of disease outbreaks.[478] The MPS vaccines were safe (reactions usually limited to local erythema of the vaccine recipients). Millions of doses have been used worldwide; MPS vaccines have excellent efficacy (>85%) in older children and adults. But these vaccines are poorly immunogenic (C>A) in children younger than 18 to 24 months; induce protective SBA titers of limited duration (around 3–5 years of protection); create at best a transient herd protection effect; do not generate immunologic memory responses or antibody affinity maturation; and demonstrate immunologic hyporesponsiveness to repeat vaccinations of the polysaccharides.

Meningococcal Conjugate Vaccines

The successful introduction of capsular polysaccharide-protein conjugate vaccines for *H. influenzae* type b in 1990, led by efforts of David Smith, Porter Anderson, John Robbins, and Rachel Schneerson,[479] fueled the development of the MCVs in the 1990s. Covalent linkage of a saccharide to immunogenic carrier protein to create a glycoconjugate had been first investigated in 1929 by Avery and Goebel.[480] In contrast to a T-cell–independent antigen of the saccharide alone, conjugation leads to a T-cell–dependent antigen recruitment of T-cell help by the carrier protein. The two MCVs licensed in the United States use diphtheria proteins. Menveo uses CRM197, a nontoxic variant of diphtheria toxin, and Menactra uses diphtheria toxoid.

MCVs[35,183] are safe, have excellent immunogenicity, induce immune memory responses and amnestic antibody levels with repeated doses in contrast to the hyporesponsiveness seen with MPS vaccines, have equal efficacy to the polysaccharides, and provide prevention in expanded human populations (infants, young children). They have now largely replaced MPS vaccines in the vaccine prevention of meningococcal disease. Unlike the MPS vaccines, MCVs have long-term effects in reducing transmission, resulting in significant herd protection at modest levels of vaccine coverage (due to a low R_0 of *N. meningitidis*; see "Herd Protection and Vaccine Strategies").[481] Special populations, such as solid-organ transplant recipients, still represent a vulnerable population with poor responses to both polysaccharide nonconjugate and conjugate vaccines.[482]

In response to a significant burden of serogroup C meningococcal disease, the first MCVs used were serogroup C conjugates and were introduced in the United Kingdom in 1999 to 2000 as a broad catch-up campaign for those younger than 19 years of age; the highest meningococcal carriage rates are in adolescents. These vaccines were then incorporated in the routine vaccination program for infants and young children.[483,484] Serogroup C disease decreased dramatically in the first 2 years after vaccine introduction and virtually disappeared by 2005.[138] A major contributor (approximately 50% of effectiveness) to this rapid decline was herd protection created by the generation of mucosal immunity by these vaccines and the interference with transmission of serogroup C expressing meningococci.[485–487] Vaccine effectiveness for reducing nasopharyngeal carriage of serogroup C in adolescents was more than 75% (serogroup C specific carriage declined from 0.42% to 0.09%),[487] and reduction in serogroup C meningococcal disease due to herd protection has persisted now for almost 2 decades. No significant change in serogroup B disease (serogroup replacement) was observed. The introduction of serogroup C conjugate vaccines in other countries in Europe has been the driving force behind the reductions in serogroup C disease across the continent.[184]

This success led to the rapid development of MCVs by major vaccine manufacturers, a public-private partnership (Meningitis Vaccine Program for the African meningitis belt), and multiple formulations and conjugation approaches for C, A, A/Y, X, and A/C/Y/W, sometimes in combination with other antigens (e.g., *H. influenzae* type b conjugate). A tetravalent conjugate vaccine (MCV4, Menactra; A, C, Y, W) was first approved for use in the United States in 2005 in adolescents, and now two MCV4s are available (Table 211.3).[488,489] Recent data suggest an impact of the US MVC4 program in reducing incidence of A, C, Y, and W disease in this age group.[490]

An important example of a cost-effective approach to conjugate and other vaccine development was the Meningitis Vaccine Program, a partnership among PATH, the World Health Organization, and the GAVI Alliance, for the development of a group A MCV for Africa,[160,491] designated MenAfriVac. At less than 50 cents a dose, MenAfriVac is produced at the Serum Institute of India, Ltd. Because of the huge impact of herd protection of the serogroup C conjugate vaccine introduction in the United Kingdom, MenAfriVac was introduced as a mass vaccination strategy for those aged 1 to 29 years. The MenAfriVac vaccination campaign began in Burkina Faso in December 2010[160,492] and was extended to Mali, Chad, Niger, Nigeria, Benin, Ghana, Senegal, Cameroon, Sudan, and other regions of the African meningitis belt. Over 280 million doses in 26 African countries have been administered. Vaccine effectiveness through the elimination of serogroup A carriage has been demonstrated through the African Meningococcal Carriage Consortium (MenAfriCar) and other surveillance efforts (MenAfriNet).[159,493,494] Results show the virtual elimination of serogroup A meningococcal disease in the countries in which vaccination was performed in the first 7 years after the mass campaigns began.

TABLE 211.3 Vaccines for *Neisseria meningitidis*: United States, 2018

Menveo (GlaxoSmithKline)	Meningococcal (groups A, C, Y, and W) Oligosaccharide Diphtheria CRM197 Conjugate
Menactra (Sanofi Pasteur)	Meningococcal (groups A, C, Y, and W) Polysaccharide Diphtheria Toxoid Conjugate Vaccine
Bexsero (GlaxoSmithKline)	Meningococcal group B directed Recombinant Protein Vaccine
Trumenba (Pfizer)	Meningococcal group B directed Recombinant Protein Vaccine

Serogroup B Vaccines

The successful development and use of the effective polysaccharide-protein glycoconjugate vaccines for serogroups A, C, Y, and W focused attention on serogroup B *N. meningitidis* (expressing an [α2→8]-linked polysialic acid capsule), a leading cause of meningococcal disease, especially in infants, young children, and adolescents, in many countries. Because the serogroup B polysaccharide resembles the human NCAM and other human antigens, the molecule is poorly immunogenic and carries a theoretical risk for autoimmunity. Serogroup B vaccines based on OMV proteins (especially the PorA protein) were successful in the control of clonal outbreaks of serogroup B disease in Norway, New Zealand, Cuba, France, Brazil, and Chile.[175,495–497] However, due to meningococcal surface structure heterogeneity, difficulties in the consistency of manufacture and composition, limited duration (approximately 2 years) of protection, and lack of evidence of herd protection, these vaccines have not been candidates for routine prevention of endemic serogroup B disease. The use of meningococcal OMV vaccines in infants and young children is also limited, requiring multiple doses, and is associated with higher reactogenicity.

New serogroup B vaccines based on semiconserved surface-protein antigens identified by "reverse vaccinology" have now been introduced. The first of these, MenB-4C (Bexsero), licensed in Europe in 2013 and in the United States in 2015, contains semiconserved surface-protein antigens—(1) a member of the FHbp family, (2) NadA, and (3) NHBA—and a meningococcal serogroup B PorA-containing OMV preparation previously used to control the serogroup B clonal outbreak in New Zealand.[498,499] Alum is the adjuvant. The second serogroup B vaccine licensed in the United States in 2014 and in Europe in 2017, MenB-FHbp (Trumenba), is based on two distinct members of the FHbp family. MenB-FHbp has been used in a college outbreak in several states.[500] Initial evaluations of the immunogenicity and safety of these vaccines have been excellent.[498,501–504] Both vaccines have a higher incidence of pain at the injection site. Both have been approved for the prevention of invasive meningococcal disease in persons aged 10 through 25 years and have been used in response to control of college campus outbreaks in the United States. In the United Kingdom, MenB-4C has been introduced in infants and young children as part of the routine immunization program and has been used to control a serogroup B outbreak in Quebec.[503,504]

In the United States, the Advisory Committee on Immunization Practices (ACIP) recommends that adolescents and young adults aged 16 to 23 years (the age of greatest risk) may be vaccinated with a serogroup B vaccine.[502] Important evaluation continues regarding the use of these vaccines, such as effectiveness in different countries and regions, reactogenicity in infants and children, whether herd protection will be generated, and the length of persistence of the protective immune responses. However, the prevention of serogroup B disease is a significant step closer with these vaccines.[179] There is also evidence of cross-protection against other meningococcal serogroups that share related outer membrane protein epitopes. For example, serum from MenB-vaccinated children demonstrate SBA against the hypervirulent W CC:11 strain circulating in England (see "Epidemiology" earlier).[139,492,503–505]

Whether MenB vaccines have a herd protection effect remains controversial.[506–508] There is limited evidence to support an effect,[506] and in other studies MenB-FHbp did not rapidly reduce or prevent serogroup B carriage.[508] Persistence after the initial priming series for both MenB vaccines is also an important issue. Data in several vaccinated populations show an approximately 50% decline in hSBAs 12 to 18 months after the priming series.[509–512] A booster is amnestic, indicating immunologic memory with hSBA titers rising quickly to high levels and possibly persisting for longer, although the duration of this booster response is not known. If the risk of MenB disease persists or recurs (e.g., college outbreak) a booster is needed.

Of interest, retrospective case-control and cohort studies of the OMV MenB vaccine used in New Zealand (MeNZB) and of Bexsero used in an outbreak in Canada have suggested that these vaccines may have activity against *N. gonorrheae*.[513] Estimated vaccine effectiveness of MeNZB against gonorrhea in 15- to 30-year-olds was 31%.[513] The advances in meningococcal vaccinology now suggest the possibility of worldwide control and even the potential eradication of *N. meningitidis* as a human pathogen.[514,515] However, important issues remain to be addressed with the new meningococcal vaccines.

Duration of Protection

The older MPS vaccines in adults induced 3 to 5 years of protection. It was initially anticipated that MCVs that induce memory responses and higher antibody levels would provide protection of more than 8 years. However, data suggest that the protection provided after a single dose of MCVs may not be much longer than with the polysaccharide vaccines.[4] The ACIP recommends that persons aged 2 to 55 years who remain at increased risk for meningococcal disease 5 years after vaccination with an MCV should be revaccinated and that a booster dose should be given to adolescents (routine vaccination at age 11, with a booster at age 16).[488] The duration of protection after the new serogroup B vaccines after the initial series is also a concern, and boosters may be required.[509]

Immunologic Memory and Interference

In the absence of protective anticapsular antibody concentrations (human SBT ≥1 : 4), immunologic memory alone is insufficient for protection against *H. influenzae* type b or meningococcal disease.[488,516–518] Four to 7 days are required for an immunologic memory response to develop, and the time from exposure to disease is often less than 7 days. Immune interference and concurrent administration with other vaccines is another important consideration with conjugate vaccines.[519] For example, although immune enhancement can be seen with the coadministration of two tetanus toxoid (TT) vaccines, CRM197 and TT together induce bystander interference, and CRM197 and TT produce carrier-induced epitopic suppression.[520] One example of interference is the coadministration of MenACWY-D and PCV7, which results in lower geometric mean concentrations of immunoglobulin G (IgG) antibodies to some pneumococcal serotypes when compared with corresponding IgG geometric mean concentrations at PCV7 administered alone.[488]

Vaccine Population Coverage and Effectiveness

Given the ever-changing epidemiology of meningococcal disease, effective coverage of the population against circulating strains is an essential determinant in decisions about new meningococcal vaccines. Thus, accurate and ongoing laboratory-based surveillance and assessment of the epidemiology and of the strains causing meningococcal disease remain essential. For example, the coverage of serogroup B isolates by new serogroup B vaccines is an important consideration in decisions about the introduction and use of these vaccines.[179] The population effectiveness over individual efficacy (e.g., through herd protection) is also an increasingly important factor in meningococcal vaccine strategies.

Herd Protection and Vaccine Strategies

Protection against the bacterial meningitis pathogens through herd protection is remarkable and powerful, and was an unanticipated, effect of bacterial polysaccharide-protein conjugate vaccines.[39] Herd protection can account for approximately one-half of the MCV effectiveness at preventing meningococcal disease. Herd protection is an important strategy in planning for meningococcal vaccine introduction, implementation, and evaluation. As noted, in 1999 to 2000 the meningococcal serogroup C conjugate vaccines were introduced in the United Kingdom as a broad catch-up campaign for those younger than 19 years. This approach reduced nasopharyngeal carriage of serogroup C in adolescents by more than 75%, and created herd protection that has persisted for almost 2 decades.[483,487] The remarkable herd protection effect is related to the low R_0 (basic reproduction number, or the average number of secondary infectious cases that are produced by a single index case in a completely susceptible population), estimated at 1.3, for meningococcal disease. Thus, herd protection is achieved with 17% to 26% MCV coverage of a population.[39,521] In contrast, measles, mumps, pertussis, polio, and rubella have an R_0 greater than 5 and require much higher thresholds (i.e., >80% vaccine coverage for herd protection).[481,514] The immunologic basis of mucosal (herd) protection with conjugate vaccines, however, remains poorly defined. Generation of capsule-specific mucosal immunoglobulins, transudation of high-avidity serum IgG to mucosal surfaces, and Th17-induced immunity via macrophage clearance are proposed as mechanisms.[39] Cost-effectiveness, measured as a quality-adjusted life-year (QALY) score, is an important consideration of vaccine recommendations in public health, and herd protection significantly enhances benefit-cost analyses.[489]

Meningococcal Vaccine Recommendations

Two meningococcal quadrivalent polysaccharide-protein conjugate vaccines (MCV4s) that provide protection against meningococcal serogroups A, C, W, and Y (MenACWY-D [Menactra; Sanofi Pasteur, Swiftwater, PA] and MenACWY-CRM [Menveo, Novartis Vaccines, Cambridge, MA]) are licensed in the United States and other countries. MenACWY-CRM is licensed in the United States for infants ages 2 through 23 months. MenACWY-D is licensed as a two-dose series for infants and toddlers aged 9 through 23 months. MenACWY-D and MenACWY-CRM are licensed for persons aged 2 through 55 years as a single dose. Two serogroup B vaccines (MenB-4C [Bexsero; GlaxoSmithKline, Research Triangle Park, NC] and MenBFHbp [Trumenba; Pfizer, Philadelphia, PA]) have been licensed in the United States for persons aged 10 through 25 years, Bexsero as a two-dose series and Trumenba as a two-dose or three-dose series.[502,522]

Adolescents and Adults

The ACIP recommends routine vaccination with a quadrivalent (MenACWY, MCV4) MCV (MenACWY-D or MenACWY-CRM) for adolescents aged 11 or 12 years, with a booster dose at age 16 years as an effective strategy to reduce meningococcal disease among adolescents and young adults.[488,523] Vaccination is also recommended for adults at risk for meningococcal disease. The following adult populations are at risk for meningococcal disease: college freshmen living in dormitories who were not previously immunized; microbiologists who are routinely exposed to *N. meningitidis*; military recruits; persons who travel to or reside in countries in which *N. meningitidis* is hyperendemic or epidemic; persons who have complement component deficiencies (e.g., C3, C5-9, properdin, factor H, factor D) or those taking eculizumab (Soliris); persons who have functional or anatomic asplenia; and persons at risk because of an A, C, W, or Y outbreak. Adults who have HIV type 1 infection are at increased risk for meningococcal infection, and vaccination is now also recommended for this group. Use of an MCV4 is preferred for persons aged 11 to 55 years and may be administered to older individuals. A two-dose primary series with an MCV4 is recommended for adults with asplenia, for those with persistent complement component deficiency, and for persons with HIV infection; otherwise, an initial single dose is indicated. Booster doses of an MCV4 administered every 5 years are recommended for persons who remain at increased risk for meningococcal disease. Individuals at risk who previously received an MCV4 conjugate vaccine also should get an MCV after they reach 55 years of age.

MenACWY-D or MenACWY-CRM is administered as a single 0.5-mL intramuscular dose. Protective levels of antibodies are usually achieved

within 7 to 10 days after vaccination. In persons aged 2 through 55 years, MCV4 vaccines can be administered concomitantly with other vaccines but at a different anatomic site. Providers administering vaccinations should be aware of the potential for syncope after vaccination, especially among adolescents, and should take appropriate measures to prevent potential injuries. Two studies have provided evidence that the risk for Guillain-Barré syndrome (GBS) is not increased over the background risk in adolescents after MCVs.[488] The ACIP has recommended removing the precaution against use of MCVs for persons with a history of GBS, although it remains a listed precaution in the US Food and Drug Administration (FDA) package insert for MenACWY-D.

MenB vaccines, either as a two- or a three-dose series (Trumenba, MenB-FHbp) or as a two-dose series (Bexsero, MenB-4C), may be administered to adolescents and young adults aged 16-23[502,522,524–526] to provide short-term protection against serogroup B disease. The preferred age for MenB vaccination is 16 to 18 years of age. The two available MenB vaccines are not interchangeable. Trumenba or Bexsero may be administered concurrently with other vaccines indicated for patients of this age but at a different anatomic site, if feasible. The choice of the dosing series for MenB-FHbp is based on the patient's risk for exposure and susceptibility to serogroup B meningococcal disease.[526]

MenB vaccines are also recommended for persons 10 years of age or older who are at increased risk for serogroup B meningococcal disease.[524] This includes persons with persistent complement component deficiencies including those taking eculizumab, persons with anatomic or functional asplenia, microbiologists exposed to isolates of *N. meningitidis,* and persons identified as at increased risk because of a serogroup B meningococcal disease outbreak such as on college campuses. Adults who have HIV type 1 infection are also at increased risk for serogroup B meningococcal infection, and vaccination should also be considered for this group.

Infants and Young Children

In the United States, routine meningococcal vaccination of infants and children 2 months to 10 years of age is not currently recommended. The current low burden of disease limits the potential impact of a routine meningococcal vaccination program for infants and children. A targeted approach is recommended to protect infants and children who are at increased risk for meningococcal disease. This contrasts with the routine vaccination program for infants and children in the United Kingdom, where the incidence of meningococcal disease is higher. In the United States the approach includes MenACWY-CRM vaccination for children aged 2 months to 10 years or MenACWY-D for children aged 9 months to 10 years who are at increased risk for meningococcal disease—for example, children who have complement deficiencies (C3, C5-9, factor P or properdin, factor H, or those taking eculizumab), those with anatomic or functional asplenia including sickle cell anemia, and those with HIV infection.[524] Certain infants with congenital heart disease have asplenia, and infants with sickle cell disease may initially have functional spleens but develop functional asplenia during childhood. So as not to interfere with the immune response to the pneumococcal conjugate vaccine (PCV) series, MenACWY-D should not be used until age 2 in children with asplenia (including those with sickle cell or HIV, and not until at least 2 weeks after completion of all PCV 13 doses). MenACWY-CRM is preferred. The MCV4 vaccines are also used in children in communities with a meningococcal disease outbreak for which vaccination is recommended, and travelers to or residents of countries in which meningococcal disease is hyperendemic or epidemic. Infants at risk are recommended to receive a four-dose vaccination series with MenACWY-CRM with doses at ages 2, 4, 6, and 12 months. A two-dose primary series is recommended for children aged 7 to 23 months, with the second dose at ≥12 months of age and ≥3 months after the first dose, and a two-dose primary series also for all persons with asplenia, persistent complement component deficiency, or HIV infection of any age who are at increased risk for meningococcal disease; otherwise, an initial single dose is indicated. Booster doses of MCV4 administered every 5 years are recommended for persons who remain at increased risk for meningococcal disease except for children who received their previous dose before their sixth birthday; these children should receive a booster dose 3 years after their previous dose.

Hib-MenCY-TT (MenHibrix), previously available for children aged 2 through 18 months for prevention of *H. influenzae* type b infection and serogroups C and Y meningococcal disease, has been withdrawn from the US market. MenB vaccines are recommended in the United States for children ≥10 years of age[522] at high risk for serogroup B disease (e.g., those with complement deficiencies including those on eculizumab, asplenia, HIV infection).

Outbreak Immunoprophylaxis

Cases of invasive meningococcal disease can occur in communities or organizations (e.g., college campuses) several days, weeks, or months after onset of the disease in an index cases. Thus, meningococcal vaccination is an adjunct to expanded chemoprophylaxis when an outbreak is caused by a vaccine-preventable serogroup. Guidelines for evaluation, management, and reevaluation of outbreaks of meningococcal disease, either organizational or community based, recently revised by the CDC, are available[525] and include decision making about vaccination, chemoprophylaxis, the population at risk, and the importance of molecular typing including WGS of isolates. Both MCV4 or MenB vaccines are used in outbreak control.

Hajj and Umrah Pilgrimages

The occurrence of meningococcal outbreaks during the Hajj and Umrah pilgrimages has been related to high meningococcal transmission and carriage in pilgrims.[527] Three million participate in the annual Hajj pilgrimage. In one historic study from Mecca, meningococcal carriage rates were as high as 80%.[528] Owing to the previous serogroup A and C meningococcal outbreaks associated with the Hajj, the A and C MPS vaccine became a requirement for the attendance of the Hajj in 1986. In 2000 and 2001, two large outbreaks caused by meningococcal serogroup W[529,530] resulted in the inclusion of a quadrivalent (A, C, Y, W) vaccine, either polysaccharide within 3 years or conjugate vaccine within 5 years, as a requirement for a Hajj or Umrah visa.[527,531,532] In addition, visitors arriving from countries in the African meningitis belt except pregnant women receive chemoprophylaxis with ciprofloxacin at entry to lower the rates of meningococcal carriage.[532] It is estimated that 400,000 to 460,000 pilgrims receive the recommended doses at the port of entry in Saudi Arabia.[528] Compliance with meningococcal vaccination among arriving international pilgrims is 97% to 100%.[533] Carriage rates in pilgrims are now <5%, and 0% to 0.6% in returning pilgrims.

Eculizumab (Solaris)

Prevention of meningococcal disease in those receiving eculizumab is a special concern. Eculizumab is a humanized monoclonal antibody that binds C5 and inhibits the terminal complement pathway. Eculizumab is approved for use in complement-mediated thrombotic microangiopathies such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic-uremic syndrome, and its use is now extending to a variety of other conditions.[534] The risk of meningococcal disease in individuals receiving eculizumab is >300 per 100,000. Both ACWY and MenB vaccines are recommended before individuals receive eculizumab, but vaccine-induced protection is incomplete,[534–536] possibly because of eculizumab's inhibition of complement-mediated opsonic and phagocytic functions.[537] Over half of cases in those receiving eculizumab are due to nongroupable strains. Antibiotic chemoprophylaxis is recommended, but development of resistance to oral penicillin regimens has been reported.[536] Heightened awareness, early care seeking, vaccination of close contacts, and administration of ceftriaxone or other effective antibiotic agents at first signs of symptoms are recommended.

Future Meningococcal Disease Vaccine Prevention Strategies

Vaccines that provide long-term protection early in life, through adolescence, and into adulthood against the major meningococcal serogroups and CCs that cause meningococcal disease are now available and have the potential to greatly reduce the global burden of

meningococcal disease. Challenges are the costs and other barriers to worldwide implementation of the meningococcal conjugate and MenB vaccines, gaps in vaccine coverage (e.g., certain serogroup B serotypes, serogroup X), the need for periodic boosters for meningococcal vaccines, and the introduction of meningococcal vaccines in conjunction with routine vaccination regimens. In addition, the continuing evolution and adaptability of *N. meningitidis* result in a dynamic disease epidemiology, and the emergence of new CCs and serogroups that can result in vaccine escape. Of note, meningococcal vaccine work may provide potential new insights into gonococcal vaccine strategies.

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The complete reference list is available online at Expert Consult.

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212 *Neisseria gonorrhoeae* (Gonorrhea)

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SHORT VIEW SUMMARY

Definition
- Gonorrhea is a sexually transmitted infection caused by the organism *Neisseria gonorrhoeae.*

Epidemiology
- Gonorrhea occurs most commonly in adolescents and young adults worldwide.
- Overall, incidence has been declining; however, an exception is among men who have sex with men.
- *N. gonorrhoeae* should be considered in the evaluation of genital inflammatory syndromes, including cervicitis, urethritis, and pelvic inflammatory disease.
- Most infections, however, involve neither signs nor symptoms; thus, routine screening of young women (≤25 years) or older women with key risk factors is recommended.
- Untreated maternal infection may result in ophthalmia neonatorum, which may be avoided with neonatal prophylaxis.

Microbiology
- This gram-negative, intracellular diplococcus is a fastidious microorganism that grows only in vitro in a narrow temperature range (35°C–38°C) on complex media.
- It has a marked ability to develop resistance to antibiotics; recently there has been the global appearance of strains with increasing resistance to third-generation cephalosporins.
- The gonococcus is naturally competent. This is a factor that has led to the rapid acquisition of antimicrobial resistance.
- *N. gonorrhoeae* is an exclusively human pathogen. It uses different pathogenic mechanisms to infect the epithelium of men and women. In men, the asialoglycoprotein receptor present on the urethral epithelia interacts with the terminal galactose of the lipooligosaccharide (LOS) to enter human cells. In women, a cooperative interaction occurs among C3b covalently linked to the LOS, gonococcal porin and pilus, and complement receptor 3 (CR3) on the surface of the cervical epithelial cell.

Diagnosis
- Culture and nucleic acid amplification testing are sensitive and specific and can be performed at all potentially infected anatomic sites.

Therapy
- Options are limited, given widespread resistance to numerous antibiotic classes.
- Parenteral therapy with a third-generation cephalosporin is currently recommended and should be accompanied by treatment with azithromycin for additional coverage (see Table 212.2).
- Sex partners of infected people should be treated presumptively, regardless of diagnostic test results.
- Patients in whom treatment with standard regimens fails should undergo assessment for reinfection; if reinfection is unlikely, antibiotic resistance should be considered.

Prevention
- Because of the asymptomatic nature of most infections, routine screening of young women (≤25 years) and pregnant women is recommended.
- Condoms are very effective in preventing transmission.

Gonorrhea is a common bacterial infection that is transmitted almost exclusively by sexual contact or perinatally and primarily affects the mucous membranes of the urethra and cervix and, less frequently, those of the rectum, oropharynx, and conjunctivae. Ascending genital infection in women leads to endometritis and salpingitis—collectively called pelvic inflammatory disease (PID)—the predominant complication and one of the most common causes of female infertility. Other complications include acute epididymitis; ophthalmitis; disseminated infection with arthritis, dermatitis, and sometimes endocarditis; and transmission to the neonate with attendant conjunctivitis (ophthalmia neonatorum).

Gonorrhea is one of the oldest known human illnesses, and references to sexually acquired urethritis can be found in ancient Chinese writings, the biblical Old Testament (Leviticus), and other works of antiquity. Galen (AD 130) introduced the term *gonorrhea* ("flow of seed"), implying interpretation of urethral exudate as semen. The causative organism was described by Neisser in 1879 and was first cultivated in 1882 by Leistikow and Löffler. Untreated infections were understood to resolve spontaneously over several weeks or months, but reinfection was recognized. Many therapies were tried, but not until the advent of the sulfonamides in the 1930s and penicillin in 1943 was truly effective treatment available. Since that time, therapy has changed radically in the face of antimicrobial resistance. We are now facing a major crisis as resistance to the last of the "single-dose" third-generation cephalosporin therapies is increasing. Growth of fundamental knowledge about the organism and the host response to infection was slow for 80 years, but a surge of new information began in the 1970s, and the molecular biology of the gonococcus and the pathogenesis of gonorrhea have been well elucidated. Public health control efforts have met with variable success, and gonorrhea remains the second most common reportable disease in the United States (following sexually transmitted chlamydial infection), a prime example of the influence that social, behavioral, and demographic factors can have on the epidemiology of an infectious disease despite highly effective antimicrobial therapy.

THE ORGANISM

Description

Neisseria gonorrhoeae is a non–spore-forming, gram-negative coccus that characteristically grows in pairs (diplococci) with adjacent sides flattened. It does not have flagella but can move based on extension and retraction of its type IV pilus.[1] This process is known as "twitching" and is the basis of the colony types seen in piliated and nonpiliated gonococcal strains. The gonococcus closely resembles the related pathogen *Neisseria meningitidis* and several species of nonpathogenic *Neisseria*. All *Neisseria* spp. rapidly oxidize dimethyl-*p*-phenylenediamine or tetramethyl-*p*-phenylenediamine, the basis of the oxidase test. Traditionally, gonococci are differentiated from other *Neisseria* by their ability to grow on selective media; to use glucose but not maltose, sucrose, or lactose; and to reduce nitrites, and also by their inability to grow well at reduced temperature or on simple nutrient agar.[2]

Growth and Cultivation

Gonococci do not tolerate drying, and patient samples to be used for cultivation should be inoculated immediately onto an appropriate growth or transport medium. Growth is best for most strains at 35°C to 37°C,

and many freshly isolated strains have a relative or absolute requirement for atmospheric carbon dioxide in concentrations of approximately 5%. All strains are strictly aerobic under usual growth conditions, but the organism grows anaerobically when nitrite is provided as an electron acceptor. Colonies appear in 24 to 48 hours, but on most media viability is rapidly lost after 48 hours because of autolysis.[2]

Gonococci are inhibited by many fatty acids, and it is necessary to incorporate starch or other substances that absorb fatty acids into most growth media. All strains have complex growth requirements, including requirements for several vitamins, amino acids, iron, and other factors. For clinical purposes, a satisfactory medium is chocolate agar enriched with glucose and other defined supplements. Isolation of gonococci from sites that normally contain high concentrations of the normal microbiota, especially the pharynx, rectum, and cervix, may be difficult because of overgrowth of the hardier normal microbiota—a problem that is largely overcome with use of media containing antimicrobial agents that inhibit most nonpathogenic *Neisseria* and other species but permit growth of most strains of *N. gonorrhoeae* and *N. meningitidis*. Chocolate agar that contains vancomycin, colistin, nystatin, and trimethoprim (modified Thayer-Martin medium) is widely used for this purpose in the United States; a similarly constituted translucent selective medium (New York City medium) is also commonly used. Specimens from sites that usually do not harbor indigenous microbiota (e.g., blood, synovial fluid, and cerebrospinal fluid) should be cultured on antibiotic-free media.

Surface Structures

The envelope of *N. gonorrhoeae* is similar in basic structure to that of other gram-negative bacteria. As the interface between the gonococcus and host, the cell surface has been intensively studied (Fig. 212.1), and specific surface components have been related to adherence, tissue and cellular penetration, cytotoxicity, and evasion of host defenses both systemically and at the mucosal level.

Type IV Pili

Type IV pili (fimbria) are strong flexible filaments extending from the gonococcal cell surface. They can be several microns in length and 50 to 80 angstroms in width. Pili traverse the outer membrane of the gonococcus through an integral outer membrane protein known as PilQ.[3] Mature pili are composed of repeating protein subunits (pilin) with a molecular weight of 19 ± 2.5 kDa.[4] Through the activity of the proteins PilT and possibly PilB, gonococci can depolymerize and repolymerize the pilus strand, causing the bacteria to "twitch." The pilin subunit has regions of considerable interstrain antigenic similarity, especially near the amino terminus, but areas of extreme antigenic variability are also present.[4,5] A single strain of *N. gonorrhoeae* is capable of producing pili with differing antigenic compositions. This has compromised the usefulness of pilus-based vaccines against gonorrhea. The presence or absence of pili result in varied colonial forms, which can be distinguished when *N. gonorrhoeae* is grown on translucent agar.[6] Fresh clinical isolates initially form colony types P^{+} and P^{++} (formerly called T1 and T2), and the organisms have numerous pili extending from the cell surface (Fig. 212.2); P^{-} colonies (formerly T3 and T4) lack pili. Piliated gonococci are better able than organisms from P^{-} colonies to attach to human mucosal surfaces and are more virulent in animal and organ culture models and in human inoculation experiments than nonpiliated variants. Expression of pili is a function of the *pil* gene complex. A spontaneous shift between P^{+} or P^{++} colonies to P^{-} colony types, known as phase variation, occurs after 20 to 24 hours of growth in vitro. This is caused by errors in DNA replication, resulting in *pilE* genes that are out of frame and fail to produce functional protein.[5]

In addition to mediating attachment, pili contribute to resistance to killing by neutrophils. In the fallopian tube mucosa model (Fig. 212.3), pili facilitate attachment to nonciliated epithelial cells, which

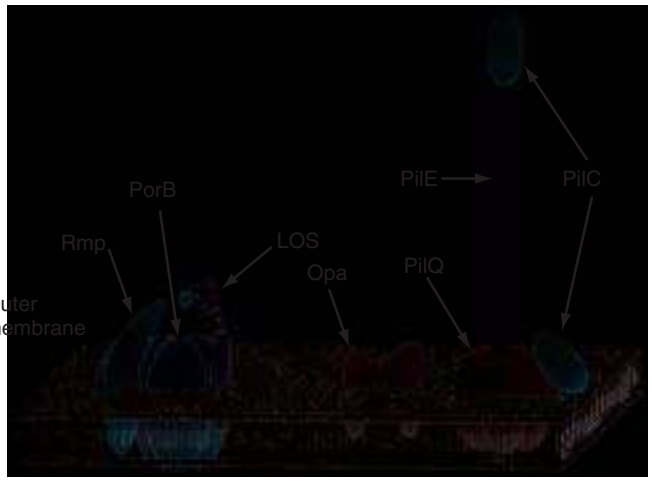

FIG. 212.1 Illustration of gonococcal outer membrane depicting many of the antigens described in the text. *LOS,* Lipooligosaccharide: the branched and phase variable LOS polysaccharide chain is shown as being bound by sialic acid, designated by a red hexagon; *Opa,* opacity protein; *PilC,* the outer membrane protein PilC, which is proposed to be presented by the pilus fibril as a tip adhesin; *PilE,* pilin, the subunits that are assembled into the α-helical pilus fibril; *PilQ,* pilin accessory protein Q, a secretin through which the assembled pilus extrudes; *PorB,* porin protein; *Rmp,* reduction modifiable protein. *(Courtesy C.E. Thomas, University of North Carolina School of Medicine, Chapel Hill, NC.)*

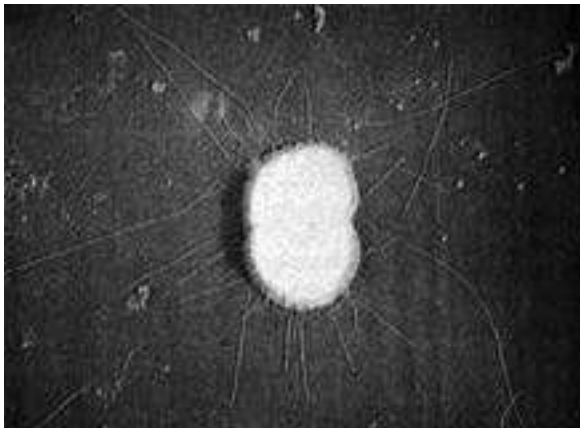

FIG. 212.2 *Neisseria gonorrhoeae* with numerous pili extending from the cell surface. *(Courtesy Dr. Gour Biswas, University of North Carolina School of Medicine, Chapel Hill, NC.)*

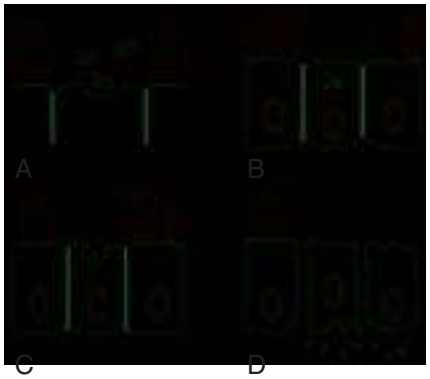

FIG. 212.3 Schematic representation of the interaction between fallopian tube explant epithelial cells and *Neisseria gonorrhoeae.* (A) Attachment of the piliated gonococci to the surface of a nonciliated host cell. (B) Endocytosis of gonococci and loss of cilia on adjacent cells, mediated by lipooligosaccharide (LOS). (C) Transport of gonococci through an epithelial cell in an endocytotic vacuole, in which the organism may replicate; progression of LOS-associated cytotoxicity. (D) Release of organisms into subepithelial space. *(From Dallabetta G, Hook EW III. Gonococcal infections.* Infect Dis Clin North Am. *1987;1:25–54.)*

initiates a process of entry and transport through these cells into intercellular spaces near the basement membrane or directly into the subepithelial space while concurrently nearby ciliated mucosal cells lose their cilia and are sloughed.[7] CD46 was considered to be the main pilin receptor, but the issue is currently uncertain and the identity of the pilus receptor is an area of active study. Other pilus-associated proteins are likely to be important to adhesion to host cells, particularly PilC.[8,9] Other factors also mediate attachment, notably opacity (Opa) proteins, lipooligosaccharide (LOS), and porins (Por).

Outer Membrane

Like all gram-negative bacteria, the gonococcus possesses a cell envelope composed of three distinct layers: an inner cytoplasmic membrane, a middle peptidoglycan cell wall, and an outer membrane. The outer membrane contains LOS, phospholipid, and a variety of proteins (see Fig. 212.1). Porin, formerly designated protein I, has a molecular weight of 32 to 36 kDa and is closely associated in the membrane with LOS. Porin provides channels that allow aqueous solutes to pass through the otherwise hydrophobic outer membrane and is believed to play an important role in pathogenesis. Porin is the product of a gene designated *porB.* Porin proteins occur in two major antigenic classes, designated PorB1A and PorB1B, each of which is composed of many distinct genetic variants. Variations in Por sequence or antigenic types form the basis for the most commonly used gonococcal typing systems.[10] Strains expressing PorB1A and occasionally PorB1B are associated with genotypic resistance of *N. gonorrhoeae* to the bactericidal effect of normal (nonimmune) human serum and, perhaps as a direct result, with an enhanced propensity to cause bacteremia. Porin-related serum resistance is due to binding to loops on the porin protein of the complement downregulatory components C4bp or factor H (fH).[11,12] PorB1A also appears to directly promote invasion of epithelial cells, which also helps explain the propensity for bacteremic dissemination.

Opa proteins are outer membrane proteins with molecular weights of 20 to 28 kDa. They are members of a family of proteins, each produced from its own *opa* gene. The amino-acid sequence of the Opa proteins varies somewhat, primarily because of differences in two hypervariable regions in each protein.[13] Expression of Opa varies because of high-frequency variations in *opa* DNA that result in translational frame shifting. An individual strain of *N. gonorrhoeae* can express none or up to 11 Opa variants but usually not more than 3 at a time. Gonococci isolated from mucosal sites usually express Opa and their colonies are opaque, but most isolates obtained from the cervix during menstruation and isolates from normally sterile sites, such as fallopian tubes, blood, and synovial fluid, generally lack Opa and form translucent colonies. Many Opa proteins increase adherence between gonococci and to a variety of eukaryotic cells, including phagocytes.[14] Certain Opa variants appear to promote invasion of epithelial cells. Two classes of Opa receptor on eukaryotic cells have been identified: heparin-related compounds and CD66, or carcinoembryonic antigen–related cell adhesion molecules (CEACAMs).[15,16] Certain Opa proteins are able to bind to CEACAM receptors on B and T cells, resulting in downregulation of immune responses.[17] This may help account for the poor immune response to natural infection.

Reduction-modifiable protein (Rmp) has a molecular weight of 30 to 31 kDa; is present in all gonococci in close association with porin and LOS; and shows little, if any, interstrain antigenic variation. Rmp can stimulate blocking antibodies that reduce serum bactericidal activity against *N. gonorrhoeae,* which may potentiate infection after sexual exposure to an infected partner.[18] Several other outer membrane proteins have been identified, including multiple iron-repressible proteins, some of which are shared with *N. meningitidis.* Two of the iron-repressible proteins (85 and 110 kDa) constitute a specific receptor for human transferrin,[19] and two others form a receptor for human lactoferrin.[20] The transferrin receptor is required for successful experimental urethral infection, but the role of the lactoferrin receptor is unclear; it does not influence infectivity. Two additional proteins constitute a receptor for hemoglobin.[21] Other proteins are expressed only during anaerobic growth.[22] The ability of *N. gonorrhoeae* to grow anaerobically after removing available oxygen from the microenvironment contributes to its ability to survive in the cervical and vaginal microaerobic environment.[23] Immunoglobulin A1 (IgA1) proteases, present in *N. gonorrhoeae* and *N. meningitidis* but not in nonpathogenic *Neisseria,* are assumed to protect the organism from secretory IgA antibody at mucosal surfaces, but this role has not been proven.

Gonococcal LOS is composed of lipid A and a core oligosaccharide that, in contrast to the polysaccharide of most gram-negative bacteria, lacks O-antigen side chains. Sialylation of LOS core sugars in vitro or in vivo masks epitopes on both LOS and porin and contributes to resistance to bactericidal antibodies.[24] LOS possesses endotoxic activity and contributes to ciliary loss and the death of mucosal cells in the fallopian tube explant model (see Fig. 212.3). LOS core sugars undergo high-frequency phase and antigenic variation in vitro and in vivo, which may contribute to the pathogenesis of infection, including resistance to bacterial anti-LOS antibodies present in normal serum and invasion of epithelial cells.

The peptidoglycan layer of *N. gonorrhoeae* may also contribute to the inflammatory response. The gonococcus has lytic transglycosylases, which produce and release highly inflammatory peptidoglycan monomers.[25] These peptidoglycan fragments are toxic in the fallopian tube explant system and cause complement consumption in vitro. In addition, peptidoglycan fragments have been found in the apparently sterile synovial fluid of patients with gonococcal arthritis-dermatitis syndrome.[26] Gonococci produce a surface polyphosphate that may have capsule-like functions, such as creating a hydrophilic, negatively charged cell surface. However, a carbohydrate capsule analogous to that of *N. meningitidis* or *Streptococcus pneumoniae* is not produced.

Strain Typing

Studies of the clinical manifestations and epidemiology of gonorrhea have been greatly enhanced by the development of reproducible methods for typing *N. gonorrhoeae.* These methods are not available in clinical laboratories, however. They consist of characterization of gonococcal strains based on two primary methods: auxotyping and serotyping using monoclonal antibodies to variable epitopes on the porin protein. In the future, with the introduction of rapid and inexpensive whole-genome sequencing, strains will be compared through use of these methods.

Genetics

Plasmids

Many gonococci possess a 24.5-mDa conjugative plasmid and can thereby conjugally transfer other non–self-transferable plasmids with high efficiency; chromosomal genes are not mobilized. Many gonococci carry a plasmid (Pc^r) that specifies production of a TEM-1 type of β-lactamase (penicillinase). The two most common Pc^r plasmids have molecular weights of 3.2 and 4.4 mDa and are closely related to each other and to similar plasmids found in certain *Haemophilus* spp., including *Haemophilus ducreyi.* In fact, it is suspected that gonococci first acquired Pc^r plasmids from *H. ducreyi.*[27] Pc^r plasmids are commonly mobilized to other gonococci by the conjugative plasmid.

Gonococci with plasmid-mediated high-level resistance to tetracycline, with minimum inhibitory concentrations (MICs) of 16 mg/L or greater, carry the 24.5-mDa conjugative plasmid into which the *tetM* transposon has been inserted.[28] The *tetM* determinant also confers tetracycline resistance to a variety of other bacteria, including some *Streptococcus* and *Mycoplasma* spp. and various genital organisms such as *Gardnerella vaginalis* and *Ureaplasma urealyticum.* Because of its location on the conjugative plasmid, high-level tetracycline resistance is readily transferred among gonococci. The *tetM* determinant functions by encoding a protein that protects ribosomes from the effect of tetracycline. Finally, all gonococci contain a small (2.6 mDa) cryptic plasmid of unknown function.

Chromosomal Mutations and Transformation

Mutations in biosynthetic pathways are common, presumably reflecting the ready availability in vivo of essential nutrients such as amino acids, purines, and pyrimidines at infected mucosal sites. Nevertheless, *N. gonorrhoeae* is not highly mutable in that it lacks error-prone repair systems and is relatively resistant to external mutagenic stimuli such as ultraviolet light. Instead, gonococci have evolved efficient systems

for phase and antigenic variation of surface components (pili, Opa, and LOS) that do not depend on such mutagenic pathways.

Gonococci also use transfer of naked DNA between cells (transformation) to promote genetic variability. The piliated variants of virtually all clinical isolates of *N. gonorrhoeae* are highly competent in transformation, but loss of the ability to express pili is always accompanied by a dramatic reduction in transformation competence. Uptake of transforming DNA is limited to homologous (i.e., gonococcal) DNA, which reflects recognition of a unique nucleotide sequence by a surface receptor.[29] No bacteriophages have been found in *N. gonorrhoeae.*

Chromosomal resistance of *N. gonorrhoeae* to β-lactam antibiotics and the tetracyclines results from interactions among a series of individual mutations, some of which (e.g., the *mtr* determinant) alter the net accumulation of antimicrobial agents inside the cell. The *mtr* locus has been shown to be an efflux pump similar to other membrane transporters.[30] The *penA* locus alters penicillin-binding protein 2 to reduce its affinity for penicillin.[31] For epidemiologic purposes, chromosomal resistance is defined when the MIC is such that clinical failures are common with the maximum practical therapeutic dose, which corresponds to MICs of 2 mg/L or greater for both tetracycline and penicillin G. Clinically significant resistance to the fluoroquinolones, indicated by MICs of ciprofloxacin of 1 mg/L or higher (up to 16 mg/L), result from the additive effects of multiple chromosomal mutations involving the genes *gyrA* and *gyrB,* which code for DNA gyrases, and *parC* and *parE,* which code for topoisomerases.[32]

PATHOBIOLOGY OF GONOCOCCAL INFECTION

An unusual characteristic of gonococcal infection is that the pathobiologic events that result in infection in men and women are different, leading to different clinical expression of the infection.[33]

Infection of the Male Urethra

N. gonorrhoeae infection in men occurs as an acute urethritis, which develops from the concomitant inflammatory response directed at infecting gonococci. One hallmark of gonococcal disease in men is the frequent presence of a purulent urethral discharge, which is associated with polymorphonuclear leukocyte (PMN) influx and shedding of urethral epithelial cells. Human volunteer studies indicate that there is an incubation period from the time of infection to the onset of clinical symptoms of disease; during this time, gonococci cannot be cultured from the urethra for up to 40 hours after the initiation of infection.[34] In vitro infection assays and microscopic analysis of patient exudates indicate that gonococci can enter urethral epithelial cells and are released, and then infected epithelial cells are shed from the mucosal surface to the urethral lumen.[35] Experimental infection in men has also demonstrated that high concentrations of the chemokine interleukin (IL)-8 and cytokines IL-6 and tumor necrosis factor-α (TNF-α) are present within the urethral lumen with progressive gonococcal infection.[36] Recent evidence demonstrates that LOS elicits TNF-α, IL-1β, IL-6, and IL-8 secretion from urethral epithelial cells.[37] Release of cytokines and chemokines from the urethral epithelium may therefore initiate the inflammatory response associated with gonococcal urethritis by triggering PMN influx. PMN influx in conjunction with cytokine release from the urethral epithelium subsequently potentiates the clinical symptoms associated with disease. Unless intercepted by effective antimicrobial therapy, this process is cyclic during the course of infection, with extension into the upper male genital tract. The acquired immune response in humans is ineffective in slowing disease progression or preventing reinfection.

Microscopic examination of urethral exudates from men documented to have culture-proven gonorrhea indicates that gonococci are found within PMNs and urethral epithelial cells.[38] The interaction of gonococci with PMNs is dependent on the presence of Opa, but it does not require pili. Opa proteins can be divided into two broad classes represented by Opa_{50} (i.e., proteins that recognize host cell heparin sulfate proteoglycans [HSPGs]) and Opa_{52} (i.e., proteins that recognize CEACAMs[39]). Experimental infection of men with Opa^- gonococci results in a shift to an Opa^+ phenotype.[36] An Opa^+ phenotype is also prevalent in clinical isolates obtained from men with naturally acquired gonococcal infection. Primary male urethral epithelial cells do not express CEACAMs, and an *N. gonorrhoeae* strain FA1090Δ Opa mutant is not impaired in its ability to cause infection in a human experimental model. This would suggest that the role of Opa proteins in gonococcal urethritis in men resides in their ability to facilitate a gonococcal-PMN interaction. (Accordingly, generating such an inflammatory response may give a survival advantage to the gonococcus because it can replicate within the PMN.[40])

CEACAMs can serve as coreceptors for other cell surface receptors (e. g., integrins) present on professional phagocytic cells. Engagement of CEACAMs sends a priming signal within PMNs that activates adhesion receptors without triggering a respiratory burst or the release of inflammatory mediators. An Opa-CEACAM interaction may therefore enhance gonococcal survival within these cells.

Porin also contributes to the intracellular survival of gonococci within PMNs. Gonococcal porin is unique among gram-negative bacterial porins in its ability to translocate to and insert into a targeted host cell membrane. Within a host cell membrane, porin forms an anion-selective, voltage-gated channel that is modulated through its interaction with adenosine triphosphate or guanosine triphosphate.[41] Insertion of porin into the PMN membrane inhibits degranulation by causing a change in membrane potential without triggering the respiratory burst within these cells.[42] Further modulation of host cell function attributed to porin involves its ability to inhibit phagosome maturation[43] and to downregulate cell surface receptors important to immune function (e.g., FcγRII, FcγRIII, and complement receptors 1 [CR1] and 3 [CR3]).[44] A single primary receptor has not been identified that uniquely modulates the interaction of the gonococcus with PMNs; however, this association occurs independently of CR3.[45] Fcγ-, CEACAMs, HSPGs, and integrin receptors present on the PMN cell surface may all play a role in gonococcal adherence and/or its internalization.

The initial site of gonococcal disease in men is the urethral epithelial cell (Fig. 212.4A). Disease occurs as a sequential process in which an initial interaction occurs between gonococcal pilus and the urethral epithelium. Pilus expression is required for efficient infection of the urethral epithelium. An intimate association between the urethral epithelium and the gonococcus (see Fig. 212.4B) is achieved through the interaction of the asialoglycoprotein receptor (ASGP-R) and gonococcal LOS,[46] a major constituent of the gonococcus cell membrane. Engagement of the ASGP-R by the gonococcus results in pedestal formation beneath the bacterium. Endocytosis ensues primarily because of actin-[47] and clathrin-dependent[35] processes. Endocytosis mediated by the ASGP-R results in endosomal fusion and acidification, which results in clathrin-coat disassembly and uncoupling of the ASGP-R–ligand complex. After gonococcal internalization, ASGP-R is recycled to the urethral cell surface, where it is available to bind more gonococci. Small proportions of infecting gonococci enter urethral epithelial cells by a macropinocytic mechanism, although membrane ruffling is not observed.[48] The intracellular fate of the gonococcus is unclear. The gonococcus does induce antiapoptotic events that prolong the life of the epithelial cell.[49,50]

Adherence of the gonococcus to the ASGP-R is dependent on the presence of an exposed galactose on the terminal lacto-*N*-neotetraose (LNnT) moiety on LOS. This moiety mimics human paragloboside and provides one means by which the gonococcus escapes immune recognition. In addition, the LNnT epitope can serve as a sialic acid acceptor. The presence of sialic acid on gonococcal LOS confers (unstable) resistance to the bactericidal action of normal human serum (i.e., serum resistance). The importance of the LNnT moiety to gonococcal pathogenesis in men can be inferred from human experimental infection studies and from clinical data obtained from men with naturally acquired gonorrhea, which demonstrate that LNnT is selected for in vivo.[51,52] Gonococci bearing the LNnT moiety on their LOS exhibit enhanced infectivity in human volunteer studies.[53] It is thought that serum resistance conferred by LOS sialylation allows a greater proportion of gonococci to survive the innate immune factors in the urethral lumen during disease. Consequently, a lower infectious dose is required to establish disease because a greater proportion of the infection inoculum survives and proliferates.

Sialylation of gonococcal LOS occurs when it is intracellular. LOS sialylation is mediated by gonococcus-encoded sialyltransferase[54–59]

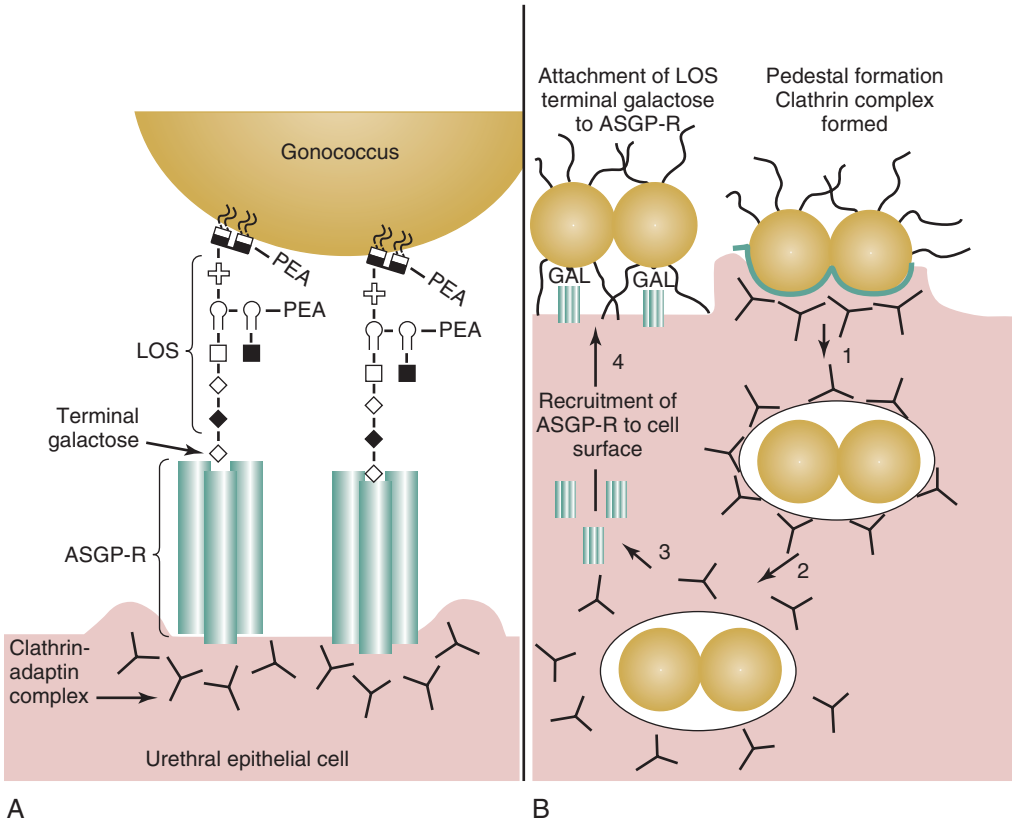

FIG. 212.4 Infection of the urethra by the gonococcus is accomplished by receptor-mediated phagocytosis of the organism by the urethral epithelial cell. (A) Interaction between the asialoglycoprotein receptor *(ASGP-R)* and the terminal galactose of the lipooligosaccharide *(LOS)* initiates a signaling cascade, which results in recruitment of clathrin to the site of ASGP-R, and gonococcus–ASGP-R complexes are internalized in clathrin-coated pits in an actin-dependent process. (B) The bacterium is engulfed by and in close interaction with the epithelial cell membrane and is taken into the cell. The enzyme dynamin cleaves the now-engulfed bacterium from the cell plasma membrane. The bacterium is within a tightly formed endosome, and within this endosome a drop in pH is proposed to release the ASGP-R from the gonococcus surface and clathrin molecules also are released. The receptor clathrin complexes begin to disperse from the bacteria-containing endosome and are available for recruitment back to the cell membrane. This results in further uptake of bacteria at the epithelial cell surface. Sialylated gonococci are eventually released from the urethral epithelium, where they can then be transmitted to a female partner. *GAL,* Galactose; *PEA,* phosphoethanolamine.

present in the gonococcal outer membrane. The gonococcus lacks the ability to synthesize cytidine 5′-monophosphate *N*-acetylneuraminic acid (CMP-NANA) and must parasitize this substrate from its human host.[60,61] Sialylation of the LNnT epitope impairs the ability of gonococci to invade primary urethral epithelial cells and epithelial cell lines, to cause disease in human volunteers, and to be phagocytized by neutrophils. Gonococcal infectivity is restored with sialic acid removal by neuraminidase or by the replication of gonococci within the lumen of the urethra in the absence of host-derived CMP-NANA. Within the lower female genital tract, sialylated gonococci may become modified to enhance disease transmission to men—that is, neuraminidases produced by the vaginal microbiota (e.g., *G. vaginalis*[62]) may remove sialic acid from sialylated gonococci. Cervical epithelia also produce neuraminidase[63]; however, the specificity of this enzyme to cleave endogenous or exogenous substrates exhibits cyclic variability. The level of sialic acid found within the microenvironment of the cervix also exhibits cyclic variation. Neuraminidase and the ASGP-R are also present on human sperm.[64] Sialylated gonococci in proximity to sperm cells may become desialylated through the action of neuraminidase present on these cells. Subsequent gonococcal adherence to the ASGP-R on sperm can then, in turn, facilitate disease transmission.[64]

Infection of the Lower Female Genital Tract

The majority of gonococci transmitted from men to their partners have sialylated LOS. However, the presence or absence of sialic acid on LOS does not influence the interaction of the gonococcus with the cervical epithelium.

In contrast to the overt inflammatory response generated with gonococcal infection of the male urethra, 50% to 80% of women with lower genital tract *N. gonorrhoeae* infection are asymptomatic and 70% to 90% of women with disseminated infection lack signs of genital tract involvement. Analysis of cervical secretions obtained from uninfected women and from women infected with the gonococcus reveal that an antibody response is not generated with uncomplicated infection. These findings are consistent with the ability of the gonococcus to evade and to subvert host immune function.[65]

Within the lower female genital tract, the cervical epithelium provides a source of alternative pathway (AP) complement (C′) activity, albeit at a level only comparable to approximately 10% of that observed for human serum. These components are produced by the cervical epithelia. Within minutes of infection of cervical epithelia, C′ protein C3b is deposited on the lipid A portion of gonococcal LOS[66] and is rapidly inactivated to iC3b (Fig. 212.5A).[67] These data are supported by the predominance of iC3b (in comparison to C3b) on the surface of clinically isolated gonococci.[68,69] The affinity of C′ fH for sialylated LOS[12] and for porin of a PI.A isotype[12] may augment C3b inactivation. However, C3b inactivation occurs in a kinetically similar manner on gonococci of either a PI.A or a PI.B isotype and on sialylated gonococci or on gonococci that are not sialylated.[67]

CR3 serves as the primary receptor for *N. gonorrhoeae* adherence to and invasion of the ectocervix and endocervix (see Fig. 212.5A).[67] Binding of gonococcal pilus to the I-domain of CR3 probably allows the gonococcus to overcome the electrostatic repulsion between its own cell surface and that of the cervical cell. The twitching action of the gonococcal pilus with reduction in pilus length may act to juxtapose the gonococcus

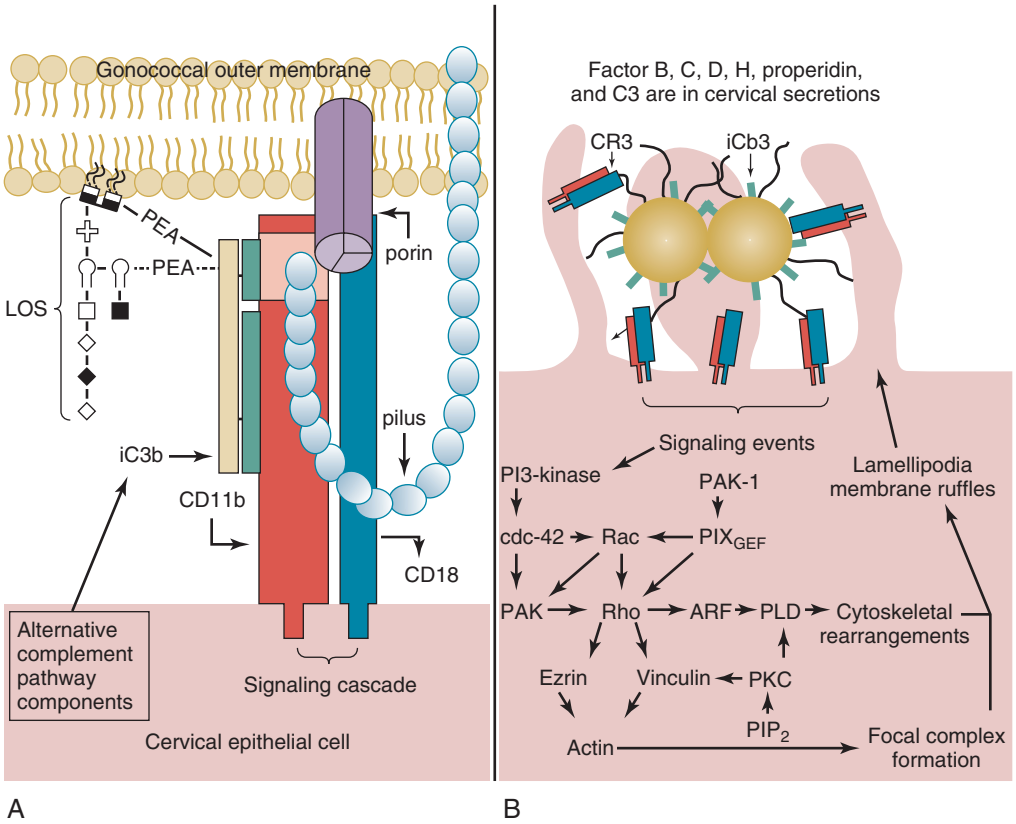

FIG. 212.5 Infection of cervical epithelia by the gonococcus. (A) Alternative complement pathway components are produced and released by the cervical epithelia. On *Neisseria gonorrhoeae* infection, complement is activated. Gonococcal pilus binds to the I-domain (red region on CD11b) of CR3 receptor, which comprises the integrins CD11b and CD14, allowing the bacterium to overcome the electrostatic repulsion between its own cell surface and that of the host cell. As the pilus retracts, this places the gonococcus within proximity to the cervical cell surface where complement components would be sufficient to allow deposition upon the bacterial cell surface. C3b forms a covalent association with amine groups on the deep core region of gonococcal lipooligosaccharide (LOS) and is rapidly inactivated to iC3b. The affinity of gonococcal porin for factor H may augment iC3b formation. The proximity of porin to LOS in the outer membrane may spatially favor the intimate association between iC3b and porin with the CR3 I-domain. (B) Engagement of CR3 triggers a complex signal transduction cascade mediated by the Src-family tyrosine kinases and Rho GTPases. These processes result in vinculin- and ezrin-enriched focal complex formation and membrane ruffling, that is, the trigger mechanism of invasion. Secreted *N. gonorrhoeae* phospholipase D modulates cervical cell signal transduction by playing a role in CR3 recruitment to the cervical cell surface and by modulating cytoskeletal reorganization. Additionally, this protein augments intracellular survival of gonococci after their internalization within macropinosomes. Sialylated gonococci are eventually released from the cervical cell, where they are free to invade more of the cervical epithelia or where they become primed for transmission to the male urethra on sialic acid removal by sialidases present within the female genital tract. However, sialic acid on the gonococcus surface does not influence the ability of these organisms to infect the human cervical epithelial cell.

at the cervical cell surface, where C′ concentrations would be expected to allow efficient opsonization for the subsequent intimate adherence of iC3b on the organism surface and gonococcal porin to the I-domain. Thus, binding of the gonococcus requires the cooperative action of iC3b bound to the gonococcal surface in conjunction with gonococcal porin and pilus.[67] Engagement of CR3 results in a complex signaling cascade in which a vinculin- and ezrin-enriched focal complex formation occurs before membrane ruffle formation.[70] A signal transduction cascade that is dependent on the activation of wortmannin-sensitive kinases (i.e., phosphatidylinositol 3-kinase or mitogen-activated protein kinases)[70] and Rho GTPases[67] initiates ruffling (see Fig. 212.5B). Gonococci are then internalized within macropinosomes.[70]

On infection of cervical epithelia, gonococci release a phospholipase D homologue that gains access to the cervical intracellular environment nonspecifically through macropinocytosis of gonococci.[71] Gonococcal phospholipase D (NgPLD) appears to promote infection of the cervical epithelium in several ways. Data indicate that this secreted gonococcal protein augments signaling events that trigger CR3 mobilization to the cervical cell surface.[71] This ensures gonococcal receptor availability and, consequently, efficient targeting to and association with the cervical cell surface. NgPLD also modulates cervical cell signal transduction events, leading to membrane ruffling. Mutant gonococci that lack functional NgPLD activity do not elicit membrane ruffling, and they are impaired in their ability to associate with and to invade primary human cervical cells.[71]

As with invasion of male urethral epithelial cells, the intracellular fate of gonococci within the cervical epithelium is unclear. Ligand binding to the I-domain of CR3 does not invoke a proinflammatory response in professional phagocytic cells. Consequently, the ability of gonococci to subvert cervical cell signal transduction cascades and the complement system in such a manner to allow a cooperative mechanism of CR3-mediated adherence to and invasion of the cervical epithelium may also enhance its survival within the lower female genital tract. Gonococcal invasion in the absence of a respiratory burst increases the number of gonococci that survive intracellularly, whereas inactivation of the C′ system enhances gonococcal survival extracellularly. Consequently, subversion of host cell signal transduction and the C′ system by the gonococcus within the lower female genital tract allows this bacterium to obtain a carrier-like state. Ascending infection of the uterus and fallopian tubes may occur as a consequence of hormonal changes that alter the mucosal epithelia, molecules available for gonococcal use, and/or virulence factors expressed by the gonococcus. In this regard, menses is associated with an increased risk to women for gonococcal PID and disseminated infection. C3 production by the cervical epithelium exhibits cyclic variability, and the highest levels of C3 are detected during menses. A correlation can also be made between the presence or absence of

Opa and the site of gonococcal isolation. Opa⁻ (or transparent) gonococci predominate in the fallopian tubes and in the cervix at the time of menses. Conversely, Opa⁺ gonococci predominate in the male urethra and the cervix at the time of ovulation.

Infection of the Upper Female Genital Tract

Approximately 45% of women with gonococcal cervical infection will develop an ascending infection,[72] the prerequisite to PID. Ascent to the upper female genital tract may be facilitated by the ability of gonococci to exhibit twitching motility in conjunction with hormonal changes, which influence the expression of C′ and molecules serving as gonococcal receptors within the female genital tract. Human expression of CR3 progressively decreases in an ascending manner from the ectocervix to the fallopian tubes.[67] Conversely, expression of the lutropin receptor (LHr) increases in an ascending manner from the endometrium to the fallopian tubes, and expression is upregulated during menses.[72,73] The LHr serves as a receptor for gonococcal invasion of fallopian tube epithelia.[74] The interaction of the gonococcus with the LHr increases the invasive character of the gonococcus for the fallopian tube epithelia and therefore is said to occur in a contact-inducible manner.[74,75] Human chorionic gonadotropin (hCG), a ligand for LHr, can competitively inhibit the LHr-gonococcus interaction. These data suggest that gonococci possess a surface molecule that mimics hCG. Recently, Spence and coworkers[75] reported that this hCG-like molecule is the ribosomal protein L12. L12 is shown to facilitate gonococcal transcytosis through the fallopian tube epithelia. The LHr is also present on the human uterus, placenta, decidua, and fetal membranes.[76] Hypothetically, a gonococcus-LHr interaction occurring on decidua and placental membranes could result in severe complications of disease and may in part contribute to the increased risk for spontaneous abortion associated with *N. gonorrhoeae* infection.

Gonococcal adherence via the LHr occurs selectively on nonciliated cells[7,74]; however, it is the ciliated cells of the fallopian tube epithelia that are subsequently shed.[77] If the infection is left untreated, complete loss of ciliary action can occur. Cytotoxicity of ciliated cells is attributed to gonococcal peptidoglycan[78] and LOS[79,80] either directly or indirectly through the induction of increased production of the inflammatory cytokine TNF.[81] The loss of ciliated cells within the fallopian tube provides the gonococcus access to subepithelial tissues. Access to subepithelial tissue is also obtained with invasion of nonciliated cells, after which gonococci are transcytosed to the basal lateral surface of these cells and released. In Hec1B cells, the gonococcal protein L12 mediates transcytosis to the basal lateral surface[75]; however, this has yet to be demonstrated in a fallopian tube organ model. Sialylation of intracellular gonococci (before their exocytosis) might prime these organisms for disseminated infection because of the increased serum resistance.

THE HUMAN IMMUNE RESPONSE TO *NEISSERIA GONORRHOEAE*

Adaptive Immune Response

Studies of human immune responses to *N. gonorrhoeae* have yielded little information about the factors necessary for development of a protective response. It has been recognized for years that recurrent infection by the gonococcus after successful treatment is a common event. The diary of Boswell describing his frequent recurrences of infection are testimony to this fact.[82] Some of his infections persisted for months before clearing. Modern studies have shown that the gonococcus can persist in asymptomatic women and men for over 6 months, and probably considerably longer.[1,2] Using a human experimental model of infection, by challenging subjects with the homologous organism[3] after clearing the first infection with antibiotics, investigators showed that recurrent infection can occur. The inability of the human to readily clear the infection and the reports of long-term carriage and reinfection by the same organism all are indicative of very poorly protective immune response to the gonococcus. Studies of the human immune response focused on the evolution of serum antibodies to pilus and porin after infection but yielded little evidence that a protective response could be developed or sustained.[4,5] Despite these observations, a study was undertaken to protect individuals from gonorrhea through use of a pilus-based vaccine. Analysis of the results of this study indicated that the vaccination was completely ineffective.[6] Since the application of molecular techniques to the gonococcus and the elucidation of the genome, a number of organism factors have been found that allow the organism to evade the host immune response. These include the ability of a large number of surface structures to undergo phase and antigenic variation,[7–9] the molecular mimicry by surface glycolipids of human antigens,[10–12] and horizontal gene exchange.[13] In addition, studies in mice suggest that the gonococcus may directly inhibit the adaptive and innate immune responses.[14] The mechanisms for these effects are not known. Analysis of individuals receiving the four-component meningococcal B vaccine suggested that limited protection against gonococcal infection occurred.[15] These results need to be confirmed in future studies.

Innate Immune Response

More recent interest in the innate immune response has stimulated similar studies on *N. gonorrhoeae*. The hallmark of symptomatic gonococcal infection in humans is a robust exudative PMN response. In spite of this, the gonococcus persists and the inflammatory response can continue untreated for prolonged periods.[82] Studies have shown that gonococci can delay fusion with primary granules in the neutrophil[16] and that lytic enzymes involved in peptidoglycan biosynthesis impart resistance to killing of the gonococcus by lysozyme and human neutrophils.[17] The gonococcus appears to be immune to the effects of antimicrobial peptides as a result of a variety of mechanisms from novel pumps to modifications of lipid A structures by phosphoethanolamine.[18–22] Studies have shown that in vivo the sialylated LOS enhances deposition of fH, an inhibitor of the alternative pathway of complement to the bacterial surface. This results in the degradation of C3 to inactive C3b and loss of complement lytic activity.[23–25] Taken together, the findings of previous studies of the immune response, both adaptive and innate, reveal an organism that is remarkably adapted to the human host.

EPIDEMIOLOGY

Incidence

Many industrialized countries but few developing ones possess reporting systems that permit reliable estimates of the incidence of gonorrhea. The number of reported cases in the United States increased from approximately 250,000 cases in the early 1960s to a high of 1.01 million cases in 1978. The peak incidence of reported infection in modern times, 468 cases per 100,000 population, occurred in 1975 (Fig. 212.6). The incidence then declined rapidly, largely the result of systematic public health prevention measures implemented in the 1970s. The decline resulted in a historical low of 98.1 cases per 100,000 in 2009. After slight increases each subsequent year, rates increased markedly during 2015 and 2016: the overall rate increased by 18.5% (22% among men and 13.8% among women) to a total of 468,514 cases for an overall rate of 145.8 cases per 100,000.[83] These statistics likely represent a significant underestimate of the true burden of disease.

The incidence of gonorrhea is substantially lower in all countries of western Europe than in the United States, but high and rising rates have been documented in eastern Europe. In the past, the highest incidences of gonorrhea and its complications occurred in developing countries, and this likely remains true in some areas of the world, with particularly devastating consequences for women and their reproductive health. However, extensive use of antibiotic regimens for syndromic management of genital complaints, including urethral and vaginal discharge and PID, has effected a decline in gonorrhea prevalence in many countries.[84]

In the United States, the highest rates occur in 15- to 24-year-old females and males, but after adjustment for sexual experience, the highest rates are seen in sexually active 15- to 19-year-old females (Fig. 212.7).[85] According to the population-based National Health and Nutrition Examination Survey, from 1999 to 2008 the prevalence remained higher among non-Hispanic blacks relative to whites.[86] The Add Health study of young adults showed similar results. Among 12,548 adults aged 18 to 26 years, the prevalence of gonorrhea was 0.43% (95% confidence interval [CI], 0.29%–0.63%), and it was strikingly higher in blacks than in whites (2.13%; 95% CI, 1.46%–3.10%).[87]

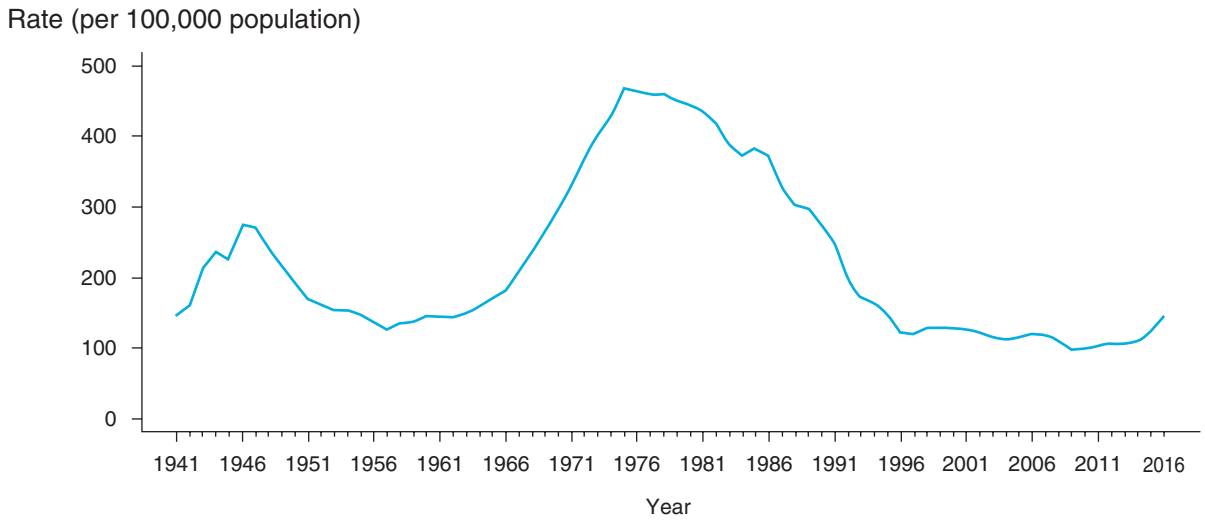

Note: Data collection for gonorrhea began in 1941; however, gonorrhea became nationally notifiable in 1944. Refer to National Notifiable Disease Surveillance System (NNDSS) website for more information: https://wwwn.cdc.gov/nndss/conditions/gonorrhea/

FIG. 212.6 Incidence of reported gonorrhea per 100,000 residents, United States, 1941 to 2016. *(From Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2016. Atlanta: US Department of Health and Human Services; 2017.)*

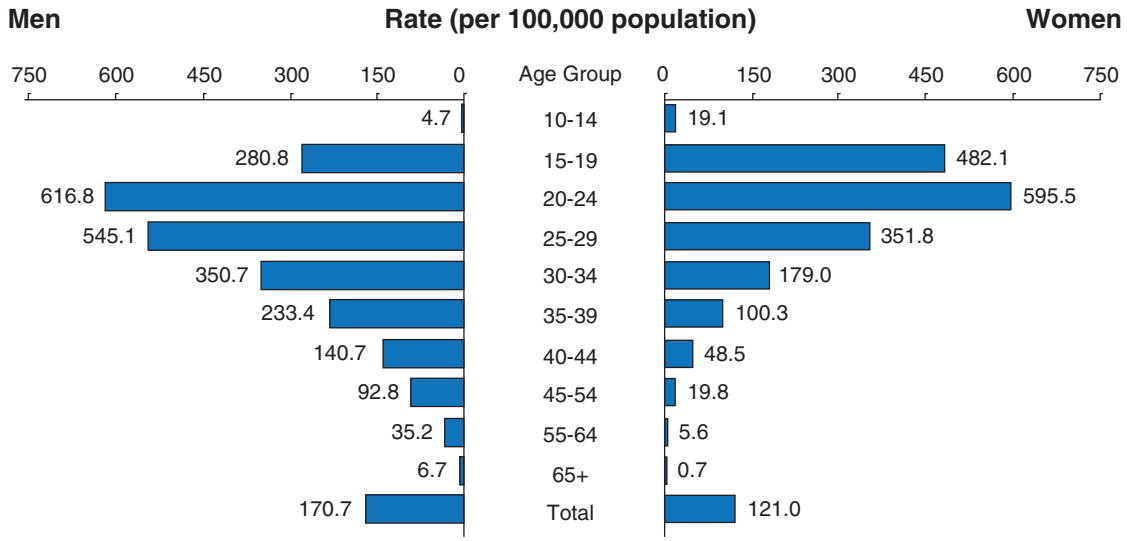

FIG. 212.7 Distribution of reported gonorrhea incidence according to age and sex, United States, 2016. *(From Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2016. Atlanta: US Department of Health and Human Services; 2017.)*

Overall, more cases of gonorrhea are reported in men than in women, likely reflecting both a greater ease of diagnosis in men and a substantially higher rate of infection in men who have sex with men (MSM) than in heterosexual men and women. The incidence of gonorrhea among MSM in industrialized countries has been relentlessly increasing in the past several years, presumably a result of behavioral disinhibition in response to improved therapy and survival of people with human immunodeficiency virus (HIV) infection and, more recently, the increasing use of HIV preexposure prophylaxis.[88,89] These trends are evident in data from the Centers for Disease Control and Prevention (CDC) STD Surveillance Network (SSuN), a representative sample of gonorrhea case reports from across the United States. In 2016 the proportion of MSM with rectal or urethral gonorrhea and chlamydia at sexually transmitted disease (STD) clinics varied by site (Figs. 212.8). Across all SSuN sites in 2016, 44.7% of gonorrhea cases were estimated to be among MSM or men who reported having sex with both men and women. Among six sites that continuously participated from 2010 through 2015, estimated rates among MSM increased from 1369 cases per 100,000 to 3435 cases per 100,000 in that timeframe, suggesting that population-based estimates align with those from clinic-based sites.[90]

The rate of gonorrhea in African-American populations in the United States is almost 25 times higher than that in whites or people of Asian ancestry; Hispanics and Native Americans experience intermediate rates (Table 212.1). Only a small portion of these differences can be explained by greater attendance of nonwhite populations at public clinics, where case reporting is more complete than in private health facilities. Race and ethnicity are demographic markers of increased risk, not factors that directly denote a high risk for gonorrhea or other STDs. Other markers of gonorrhea risk in the United States include lower socioeconomic attainment, lesser education, residence in the southeastern part of the country, unmarried status, and illicit drug use. Contrary to popular perceptions, the population-based incidence of gonorrhea is as high in many rural settings in the United States as in urban ones. Differing incidence rates among population subgroups are related less to variations in numbers of sex partners than to complex and poorly understood differences in sex partner networks, access to health care, and related

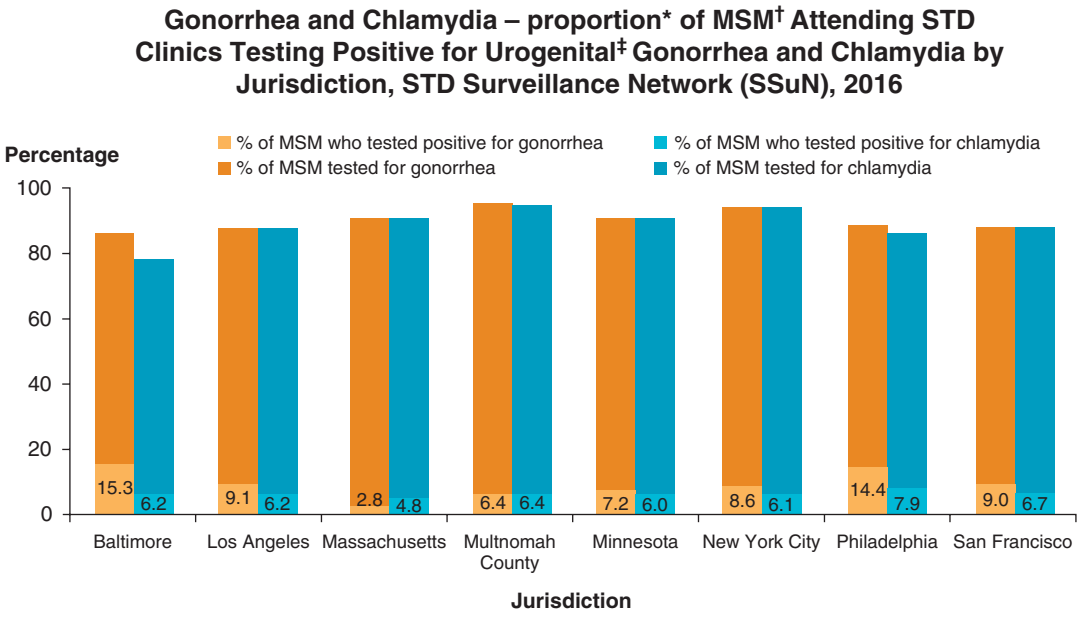

* Results based on data obtained from 22,404 patients tested for urogenital gonorrhea and 22,152 patients tested for urogenital chlamydia attending SSuN STD clinics in 2016; data from Florida and Seattle were not available.

† MSM = Gay bisexual, and other men who have sex with men (collectively referred to as MSM).

‡ Urogenital includes results from both urethral and urine specimens.

A

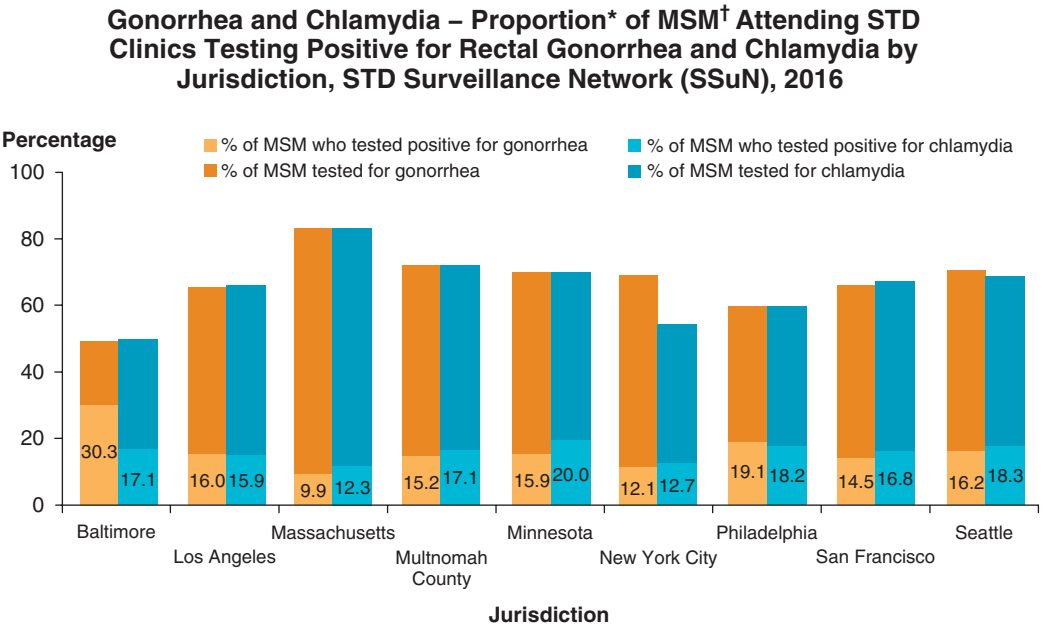

* Results based on data obtained from 17,467 tested patients tested for rectal gonorrhea and 17,445 patients tested for rectal chlamydia attending SSuN STD clinics in 2016; data from Florida were not available.

† MSM = Gay, bisexual, and other men who have sex with men (collectively referred to as MSM).

B

FIG. 212.8 (A) Proportion of men who have sex with men *(MSM)* with urethral gonorrhea or chlamydia in the Sexually Transmitted Disease Surveillance Network *(SSuN),* 2016. (B) Proportion of MSM with rectal gonorrhea or chlamydia in the SSuN, 2016. *(From Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2016. Atlanta: US Department of Health and Human Services; 2017.)*

societal factors. For example, a detailed analysis of increasing gonorrhea incidence in California from 2003 to 2005 raised the importance of contact with a recently incarcerated partner as a major risk and highlighted the relatively understudied contribution of this infection in correctional facilities settings.[91] Moreover, in a large prospective study of 4843 women tested annually for gonorrhea with incidence of 3.5% person-years at risk, number of reported recent sex partners was not independently significantly associated with acquisition.[92] In an elegant analysis, Gomez-Gardenes and colleagues demonstrated that failure to account for these differences in the structure of male-male, female-male, and male-female transmission models can have marked consequences for estimation of transmission dynamics.[93] The demographic predictors of gonorrhea throughout the world are qualitatively similar to those in the United States.

Transmission

The main risk factor for acquiring gonorrhea is sexual intercourse with an infected partner. The risk for transmission of *N. gonorrhoeae* from an infected woman to the urethra of her male partner is approximately 20% per episode of unprotected vaginal intercourse and increases to

TABLE 212.1 Reported Cases and Rates of Gonorrhea According to Race and Ethnicity, United States, 2016

RACIAL OR ETHNIC GROUP	REPORTED CASES	CASES PER 100,000 POPULATION	RATE RATIO
White, non-Hispanic	110,311	55.7	Referent
Black, non-Hispanic	192,114	481.2	8.6
Hispanic	54,299	95.9	1.7
American Indians/Alaska Natives	5757	242.9	4.3
Asians	4934	28.3	0.5
Native Hawaiians/Other Pacific Islanders	928	165.8	3.0
TOTAL	468,514	145.8	

From Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2016. Atlanta: US Department of Health and Human Services, 2017.

60% to 80% after four or more exposures.[94] The risk for male-to-female transmission has been less well studied but probably approximates 50% to 70% per contact.[95] Transmission by anal intercourse is efficient, but the risk per episode has not been quantified. Transmission occurs less readily by fellatio, especially from the oropharynx to the urethra, and transmission in either direction by cunnilingus is believed to be rare. Whether women using hormonal contraception are at increased risk for gonorrhea is not known; if so, it is likely that the magnitude of the effect is small.[96]

The incubation period is brief, and prominent genital symptoms often promptly bring infected people to treatment so that the mean duration of gonorrhea is short, typically several days in men and often less than 2 weeks in women. The approximate 50% transmission efficiency of uncomplicated gonorrhea through heterosexual intercourse dictates that especially high rates of sex partner change—an average of two new partners within the 1- or 2-week interval between acquisition of infection and its resolution, equivalent to 50 or more partners per year—are required to sustain transmission in a population. People who have unprotected intercourse with new partners with sufficient frequency to maintain a stable prevalence in the community are defined as core transmitters. Originally developed as a mathematical model, the core transmission hypothesis has been empirically confirmed by several studies,[97] and a central focus of gonorrhea control is to identify the core group and to target members for case finding, treatment, and other prevention strategies. Demographic and social characteristics that directly or indirectly influence the frequency with which new partners are acquired include young age, low educational and socioeconomic levels, commercial sex, illicit drug use, and similar factors. Other characteristics of core transmitters include poorly understood psychosocial determinants of partner selection, cultural factors that affect the response to symptoms, and reduced access to health care (whether real or perceived). A recent variation on the core group concept is the "risk space" hypothesis—namely, that the locations where risk populations live and the sites where sexual exposures occur may be equally or more important determinants of STD incidence than other demographic predictors. Analysis of sexual networks in clusters of gonorrhea is increasingly important in understanding these processes and indicates, for example, that the likelihood that an urban African-American adolescent residing in a high–STD prevalence community will be exposed to an STD is related to the presence of sexual links between his or her recent sex partners and the community.[98,99]

Gonorrhea and other STDs are usually transmitted by people with asymptomatic infections or by those who have symptoms that they ignore or discount.[100] Nevertheless, most people with new genital symptoms cease sexual activity and seek care. It follows that many transmitters belong to a subset of infected people who lack or ignore symptoms. This concept underlies the importance of taking active steps to ensure treatment of the sex partners of infected people, who often will not spontaneously seek health care.

Antimicrobial Resistance

As for most bacterial pathogens, the antimicrobial susceptibility of *N. gonorrhoeae* has evolved under the influence of antimicrobial therapy, and we are now facing a critical juncture as the emergence of resistance to cephalosporins evolves. Unfortunately, this is a clear model for how resistance emerges in the modern age. Initially, gonococci were almost uniformly susceptible to sulfonamides, penicillin, tetracyclines, macrolides, and fluoroquinolones, but none of these is now suitable for routine therapy for uncomplicated gonorrhea in most of the world. Declining susceptibility to penicillin, now attributed to chromosomal mutations, was documented almost immediately after the drug was introduced in the 1940s, but for almost 3 decades penicillin remained useful despite gradually rising relative resistance that necessitated incrementally higher doses and cotreatment with probenecid to enhance and prolong blood levels.

Two nearly simultaneous developments rendered the penicillins unsuitable for routine gonorrhea therapy worldwide. Most dramatic was the appearance in the 1970s of β-lactamase–producing strains of *N. gonorrhoeae* bearing plasmids with the Pc^r determinant, followed by worldwide dissemination within a decade. Several plasmid variants now carry the Pc^r determinant, including the still dominant 4.4- and 3.2-mDa plasmids originally associated with Asia and Africa, respectively.[101] Second, in the 1970s and 1980s, chromosomal resistance progressed to the point that treatment failures became common with the maximum practical single-dose regimens of procaine penicillin, ampicillin, or amoxicillin.

Strains of *N. gonorrhoeae* with plasmid-mediated high-level tetracycline resistance were first documented in the United States in 1985. The location of the responsible *tetM* gene on the conjugative plasmid probably contributed to especially rapid worldwide spread of such strains. The prevalence of tetracycline-resistant *N. gonorrhoeae* (TRNG) peaked in 1997 at 7.3% and decreased for several years. Fortunately, the tetracyclines are no longer recommended and are little used as sole therapy for gonorrhea.

The mutations responsible for chromosomal resistance include *mtr,* which results in increased efflux of several antibiotics and other toxic compounds, such as fatty acids and bile salts; *penA,* which modifies the affinity of penicillin-binding proteins to β-lactam antibiotics; and *penB,* which alters the ability of antibiotics to transit the cell membrane through the porin protein.[30] Simultaneous mutations are common, with additive effects that result in resistance to several antibiotics and to all or most members of each affected drug class. Thus, simultaneous chromosomal resistance to penicillin, the tetracyclines, and macrolides is common. Infection with chromosomally resistant *N. gonorrhoeae* is especially common in MSM. This likely occurs because rectal infection is required for propagation of gonorrhea in MSM and because fecal bile salts and fatty acids confer selection pressure for *mtr.*[102]

As for penicillin and other drugs, low-level resistance to the fluoroquinolones began to appear in *N. gonorrhoeae* almost immediately after they were introduced and soon was observed sporadically throughout the world. Clinically significant resistance, with ciprofloxacin MICs of 1 to 16 mg/L, began to evolve soon thereafter owing to the additive effects of multiple chromosomal mutations, particularly in the DNA gyrase complex (*gyrA* and *gyrB*) and topoisomerases (*parC* and *parE*).[32] Ciprofloxacin MICs of 4 mg/L or greater are associated with at least 50% rates of treatment failure with the recommended regimens of ciprofloxacin or other fluoroquinolones.[103] In the Philippines, the prevalence of high-level resistance to the fluoroquinolones among people with gonorrhea increased from 12% in 1994 to more than 70% in 1996 and 1997,[104] and similar prevalences are now the norm throughout Asia and the Pacific. The proportion of isolates derived from the US Gonococcal Isolate Surveillance Project (GISP) identified as quinolone resistant was 19.2% in 2014 and is notably higher among MSM (30.0% compared with 12.7% in men who reported sex only with women).[105] Such increases prompted the CDC to recommend in 2007 that fluoroquinolones no longer be used to treat gonorrhea in the United States.

Most recently, isolates with resistance to both azithromycin and, most alarmingly, third-generation cephalosporins have emerged.[106] Starting in 2009, the percentage of GISP isolates with cefixime MICs of 0.125 μg/mL increased each year from 1.4% to 1.7% in 2011, decreased to 0.4% in 2013, and increased again to 0.8% in 2014[105] (Fig. 212.9). As was the case with fluoroquinolones, isolates obtained from MSM demonstrate this trend more dramatically. These trends prompted the CDC in August 2012 to modify its STD treatment guidelines for gonorrhea to recommend treatment with parenteral ceftriaxone and to discourage the use of oral cefixime.[107] Moreover, the evolving resistance to cephalosporins detected through routine monitoring has been accompanied by increasing numbers of reports of frank treatment failure, including an increasing number of cases in which parenteral ceftriaxone failed.[108] Emerging data suggest that outbreaks linked to cephalosporin-resistant gonococci may be clonal in origin, which may offer some slim hope for bringing these trends under control in the future.[109–112] Identified mechanisms of cephalosporin resistance are chromosomally mediated, most commonly in the *penA* region, and affect binding of the drug at the β-lactam pocket. However, other mutations are likely needed to effect significant resistance, and the genetics of this process are under intense study.

Finally, the emergence of cephalosporin resistance has been paralleled by a similar trend for azithromycin, with attendant reports of treatment failure and documentation of resistant isolates[113,114] (Fig. 212.10). In 2013, this trend as measured by GISP was stable, with the proportion

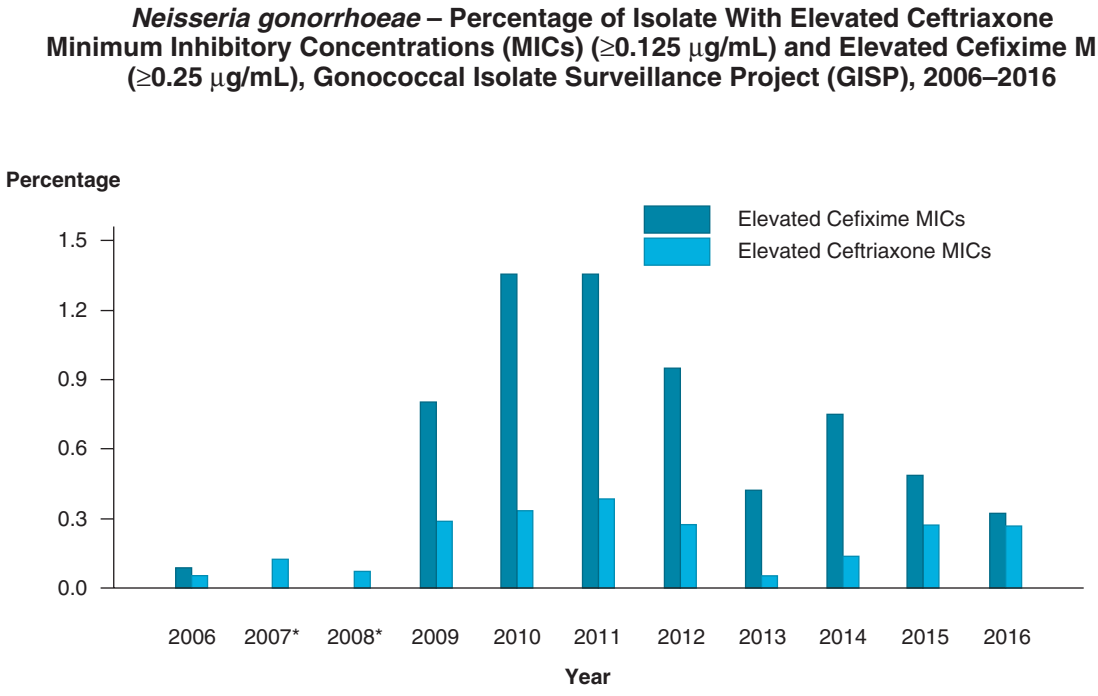

FIG. 212.9 Distribution of minimum inhibitory concentrations *(MICs)* to ceftriaxone in the Gonococcal Isolate Surveillance Project, 2006 to 2016. *(From Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2016. Atlanta: US Department of Health and Human Services; 2017.)*

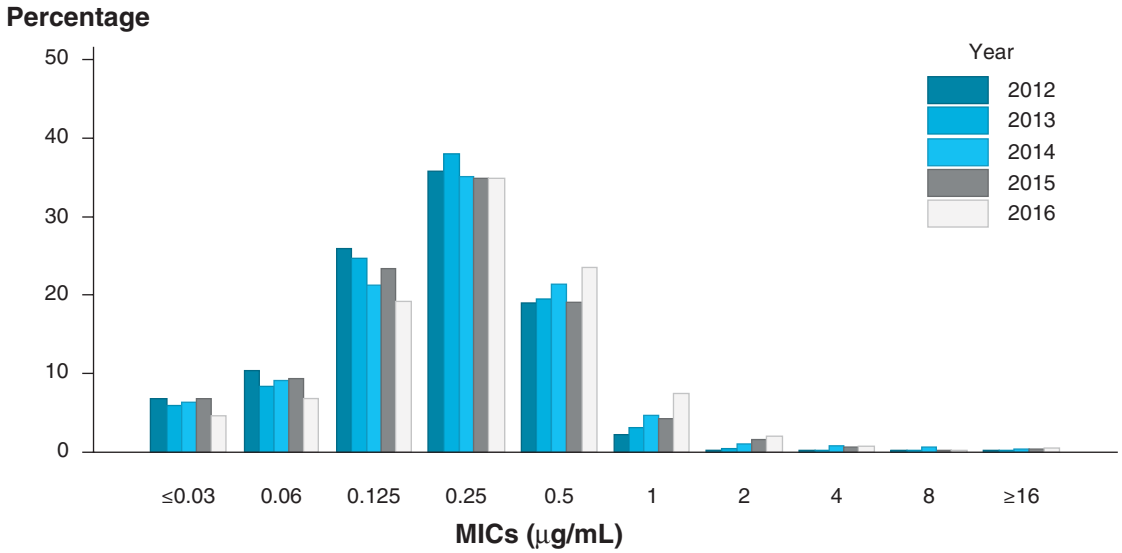

FIG. 212.10 Distribution of minimum inhibitory concentrations *(MICs)* to azithromycin in the Gonococcal Isolate Surveillance Project, 2012 to 2016. *(From Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance, 2016. Atlanta: US Department of Health and Human Services; 2017.)*

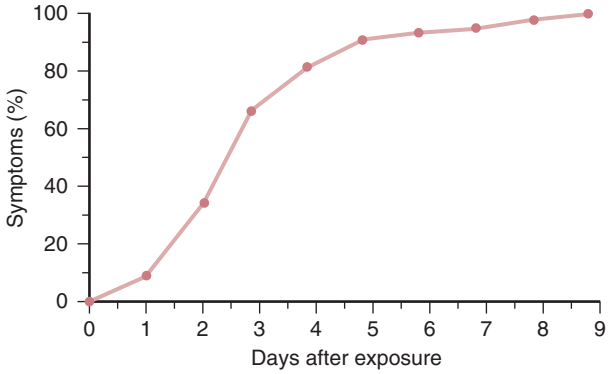

FIG. 212.11 Incubation period in 44 men with gonococcal urethritis. *(From Harrison WO, Hooper RR, Weisner PJ, et al. A trial of minocycline given after exposure to prevent gonorrhea.* N Engl J Med. *1979;300:1074–1078.)*

of isolates with azithromycin MICs greater than 2.0 μg/mL at 0.6%, relatively unchanged from 0.5% in 2010.[115] Unfortunately, this trend has progressed to the point at which some have proposed entirely eliminating this antibiotic as treatment, including in combination with ceftriaxone. In Seattle, Washington, from 2014 to 2016, 5% of gonorrhea cases among MSM had reduced azithromycin susceptibility, defined as MIC ≤1 μg/mL,[116] a trend also reported in Europe.[117]

CLINICAL MANIFESTATIONS

Genital Infection in Men

Uncomplicated Infection

Acute urethritis is the predominant manifestation of gonorrhea in men (see Chapter 109). The incubation period is typically 2 to 5 days but ranges from 1 to 10 days or longer (Fig. 212.11). Urethral discharge and dysuria, usually without urinary frequency or urgency, are the major symptoms. The discharge may initially be scant and mucoid, but within 1 or 2 days it becomes overtly purulent. These observations have been confirmed in studies of experimental gonococcal urethritis in humans.[118] Compared with nongonococcal urethritis, the incubation period of gonorrhea is shorter, dysuria is usually more prominent, and the discharge is generally more profuse and purulent (Fig. 212.12). However, exceptions are common, and a small proportion of men with urethral gonorrhea remain asymptomatic and lack signs of urethritis.[100] The symptoms of urethral gonorrhea depend in part on the infecting organism. Some Por IA serovars and the AHU$^-$ and related auxotypes of *N. gonorrhoeae* are more frequently associated with asymptomatic urethral infection in men than are other gonococcal types.[119] The decline in the prevalence of such strains in North America probably has been accompanied by a decreasing frequency of asymptomatic gonorrhea in men. Most cases of untreated gonococcal urethritis resolve spontaneously over several weeks.

Localized Complications

Acute epididymitis is the most common complication of urethral gonorrhea but now is uncommon in industrialized countries; most cases of epididymitis in young men are due to *Chlamydia trachomatis* (see Chapter 112).[120] Penile edema without other overt inflammatory signs ("bull-headed clap") is occasionally seen in gonococcal or nongonococcal urethritis. Penile lymphangitis, periurethral abscess, acute prostatitis, seminal vesiculitis, and infections of Tyson and Cowper glands are uncommon complications. Urethral stricture as a result of gonorrhea is also uncommon. Although once considered a common consequence of gonococcal urethritis, many strictures in the preantibiotic era might have resulted primarily from treatment with urethral irrigation with caustic solutions, such as silver nitrate or potassium permanganate, rather than from gonorrhea.

Uncomplicated Urogenital Infection in Women

The primary loci of genital infection in women are the columnar epithelial cells that line the endocervix (see Chapter 110). *N. gonorrhoeae* can often



FIG. 212.12 Gonococcal urethritis. (A) Purulent exudates due to gonorrhea. (B) Mucopurulent discharge mimicking the usual appearance of nongonococcal urethritis due to *Chlamydia trachomatis* and other pathogens.

be recovered from the urethra or rectum and occasionally from the periurethral (Skene) glands and the ducts of Bartholin glands, but these are rarely the sole infected sites except in women who have undergone hysterectomy. The vagina per se is not infected in sexually mature women because under the influence of estrogen the squamous epithelium of the vaginal mucosa is not susceptible to gonococcal infection.

The natural course of gonorrhea is less well understood in women than in men. Symptoms probably develop in most infected women,[121] but many remain asymptomatic or have only minor symptoms and do not seek medical care.[122] Thus, women with subclinical infection accumulate in the population, and in settings in which sexually active women are routinely screened for subclinical infection up to 80% of women with gonorrhea are likely asymptomatic. Women infected with AHU$^-$ and related auxotypes of *N. gonorrhoeae,* like men, are more likely to have asymptomatic infection and more subtle inflammatory signs.[119]

Most infected women who develop symptoms do so within 10 days. The dominant symptoms are increased vaginal discharge, dysuria (often without urgency or frequency), and intermenstrual bleeding, sometimes triggered by coitus. Abdominal or pelvic pain usually denotes ascending infection, but some women with these symptoms lack evidence of salpingitis at laparoscopy. Physical examination may show purulent or mucopurulent cervical exudate (Fig. 212.13) and other signs of mucopurulent cervicitis, such as edema in a zone of cervical ectopy or endocervical bleeding induced by gentle swabbing[123]; in most infected women, these cervical signs are absent. Purulent discharge can sometimes be expressed from the urethra or the ducts of Bartholin glands.

Rectal Gonococcal Infection

N. gonorrhoeae can be isolated from the rectum in up to 40% of women and a similar proportion of MSM with uncomplicated gonorrhea. The rectum is often the only infected site in MSM. Isolated rectal infection in women is probably more common than traditionally thought; in a large study in Baltimore, 2.75% of 4402 women had gonorrhea detected at the rectum; routine screening of women at this site is, however, not routinely recommended.[124] Rectal infection is acquired both through



FIG. 212.13 Purulent endocervical exudate in gonococcal cervicitis. *(From Handsfield HH.* Color Atlas and Synopsis of Sexually Transmitted Diseases. *2nd ed. New York; McGraw-Hill; 2001; courtesy King K. Holmes.)*



FIG. 212.14 Acute gonococcal conjunctivitis in an adult. *(From Handsfield HH.* Color Atlas and Synopsis of Sexually Transmitted Diseases. *2nd ed. New York; McGraw-Hill; 2001.)*

receptive anal intercourse, which accounts for virtually all cases in men, and perineal contamination with cervicovaginal secretions, which is believed to be responsible for some infections in women. Rectal gonococcal infection is usually asymptomatic, but some patients have acute proctitis manifesting with anal pruritus, tenesmus, purulent discharge, or rectal bleeding. The apparently higher rate of clinically apparent proctitis in men than in women suggests that inoculum size or trauma during anal intercourse may influence the development of symptoms. Anoscopy sometimes reveals mucopurulent exudate and inflammatory changes in the rectal mucosa, but infection with *C. trachomatis,* herpes simplex virus, or other sexually transmitted pathogens can produce the same findings.

Pharyngeal Infection

Pharyngeal gonococcal infection is acquired through receptive oral sex but probably rarely, if ever, by kissing. Acquired more efficiently by fellatio than by cunnilingus, pharyngeal infection can be found in 10% to 20% of heterosexual women with gonorrhea and 10% to 25% of infected MSM, but it is present in only 3% to 7% of heterosexual men with gonorrhea. Almost all pharyngeal infections are asymptomatic, but rare cases may cause overt pharyngitis.

The importance of documenting pharyngeal gonorrhea is debated, and several factors argue against routine screening or diagnostic testing in the general sexually active adult population. Most cases are asymptomatic and resolve spontaneously, and pharyngeal infection is probably less transmissible than rectal or genital gonorrhea. Furthermore, pharyngeal non–culture-based diagnostic assays for identification of *N. gonorrhoeae* have not been cleared by the US Food and Drug Administration (FDA), although they have been validated by numerous health department and several commercial laboratories. On the other hand, pharyngeal infection can be symptomatic and is sometimes the source of transmission to sex partners, especially among MSM,[125] or of systemic dissemination of *N. gonorrhoeae.* Current recommendations are to routinely test for pharyngeal infection in HIV-infected people who report receptive oral sex,[127] and many experts recommend routine pharyngeal screening in MSM given the continued high incidence of disease in this group.[127]

Other Local Manifestations

Gonococcal conjunctivitis in adults is usually seen in people with genital gonorrhea, and most cases probably result from autoinoculation, but some cases may be acquired by other routes, such as orogenital exposure. Gonococcal conjunctivitis is usually painful, with prominent photophobia and copious, purulent exudate (Fig. 212.14), and corneal ulceration can supervene rapidly in the absence of prompt antibiotic therapy. However, some infections are mild, perhaps related to specific gonococcal strains. *N. gonorrhoeae* has been isolated in cases of acute gingivitis, otherwise unexplained oral ulcerations, and intraoral abscesses. Cutaneous abscesses have been described, typically involving the finger or the penile shaft, and probably result from inoculation of preexisting lesions. For example, gonococcal abscess is sometimes the first clinical presentation of a congenitally patent median raphe duct of the penis. Finally, in the absence of neonatal prophylaxis, maternal infection with *N. gonorrhoeae* may result in ophthalmia neonatorum, typically manifesting as purulent, edematous conjunctivitis. In areas without routine neonatal prophylaxis, blindness can result. Infection of other superficial mucosal surfaces can also occur.

Pelvic Inflammatory Disease

PID (see Chapter 111) refers to a spectrum of upper genital tract infections and may occur with or without overt symptoms. When symptomatic, PID manifests with various combinations of endometritis, salpingitis, tubo-ovarian abscess, pelvic peritonitis, and perihepatitis. The immediate and long-term sequelae are a primary impetus for prevention strategies against gonorrhea and chlamydial infection. Infection with *N. gonorrhoeae, C. trachomatis,* perhaps *Mycoplasma genitalium,* and vaginal anaerobes associated with bacterial vaginosis may be involved, often in combination. Adolescent girls are at higher risk than older women, probably because of both innate susceptibility, perhaps due to a high prevalence of cervical ectopy that may facilitate ascending infection, and sexual network factors that confer a higher likelihood of exposure to infected sex partners. Women using anovulatory hormones have been shown to have a lower risk for chlamydial salpingitis but not of gonococcal PID in some studies[128]; however, other analyses have not reported this association.[129]

PID is estimated to occur in 10% to 20% of women with gonorrhea detected at the cervix. In 57 women with gonorrhea but with no clinical signs or symptoms of PID, endometrial biopsies revealed histologic endometritis (termed *subclinical PID* by the authors) in 15 (26%); this was a significantly higher percentage than that seen in women without gonorrhea (11%; $P < .001$).[130]

The most consistent symptom of PID is low abdominal pain, and most women also have symptoms of lower genital tract infection. Gonococcal PID often follows the onset of menses by a few days.[122] Fever, chills, nausea, and vomiting may occur, but most patients lack these manifestations. The primary finding at physical examination is pelvic adnexal tenderness, usually bilateral. Other common findings are uterine fundal tenderness, pain elicited on moving the cervix, and one or more tender adnexal masses. Abdominal examination usually elicits tenderness over the lower quadrants, and signs of peritoneal inflammation are common in severe cases. Many women with PID have bacterial vaginosis, and many have signs of mucopurulent cervicitis. Fever, leukocytosis, and an elevated erythrocyte sedimentation rate or C-reactive protein level are common, but they are absent in approximately one-third of patients with laparoscopically documented PID. In practice, the clinical diagnosis of PID is imprecise.

The proportion of PID cases associated with gonorrhea varies greatly across population groups and with the background rates of gonorrhea and chlamydial infection. In the 1980s, 20% to 40% of cases of PID in most urban areas of the United States were associated with gonorrhea. The presence of cervical gonococcal or chlamydial infection does not exclude fallopian tube infection with other organisms, nor does failure to isolate *N. gonorrhoeae* or *C. trachomatis* definitively exclude their contribution to salpingitis. Along with *M. genitalium,* facultative and anaerobic bacterial microbiota of the vagina may also contribute to acute PID, especially in patients with pelvic abscess or otherwise severe infections.[131]

Infertility resulting from fallopian tube obstruction is the most common serious consequence of PID and occurs in 15% to 20% of women after a single episode and 50% to 80% of those who experience three or more episodes.[132] Infertility may be more common after chlamydial than after gonococcal PID, perhaps because the more acute inflammatory signs associated with gonorrhea bring women to diagnosis and treatment sooner. This pattern was evident in the PID Evaluation and Clinical Health study, which enrolled more than 800 female patients aged 14 to 37 years with symptomatic PID.[133] Despite clinical cure and apparent microbiologic eradication of gonorrhea, as evidenced by lower tract cultures, infertility rates were 13% for participants with *N. gonorrhoeae* identified, 19% for those with *C. trachomatis,* and 22% for those with anaerobic bacteria during a median follow-up period of 35 months.[134] The rate of chronic pelvic pain was 27% among women with gonococcal infection.[135]

Perihepatitis

Acute perihepatitis, or Fitz-Hugh–Curtis syndrome, occurs primarily by direct extension of *N. gonorrhoeae* or *C. trachomatis* from the fallopian tube to the liver capsule and overlying peritoneum. Some cases may result from lymphangitic spread or bacteremic dissemination, which may explain rare cases of apparent perihepatitis in men. Perihepatitis results in abdominal pain, hepatic tenderness, and right upper quadrant peritoneal inflammatory signs. Most cases occur in association with overt PID, but many women lack pelvic symptoms or signs. Perihepatitis should be considered in the differential diagnosis of right upper quadrant pain in young, sexually active women; it is commonly mistaken for acute cholecystitis or viral hepatitis. Laparoscopy may show the characteristic "violin string" adhesions between the liver capsule and the parietal peritoneum.

Gonorrhea in Pregnancy

Gonorrhea during pregnancy is associated with spontaneous abortion, premature labor, early rupture of fetal membranes, and perinatal infant mortality.[136] The clinical manifestations of gonorrhea are unchanged in pregnant women, except that PID and perihepatitis are uncommon after the first trimester, when the products of conception obliterate the uterine cavity. Reports are conflicting as to whether pregnancy is a risk factor for gonococcal bacteremia.

Disseminated Gonococcal Infection

Disseminated gonococcal infection (DGI) results from bacteremic dissemination of *N. gonorrhoeae,* although immune complexes or other indirect immunologic mechanisms may contribute to pathogenesis and symptoms in some cases. Although once estimated to occur in 0.5% to 3% of infected patients, the rate undoubtedly is lower at present because of declining prevalences of gonococcal strains prone to disseminate.[137] However, discrete outbreaks of DGI have recently been documented.[138] Septic arthritis and a characteristic syndrome of polyarthritis and dermatitis are the predominant manifestations, and DGI is a cause of infective arthritis in young adults. Gonococcal endocarditis was common in the preantibiotic era but now is rare. Meningitis, osteomyelitis, septic shock, and acute respiratory distress syndrome are rare manifestations.

Properties of *N. gonorrhoeae* classically associated with dissemination include resistance to the bactericidal action of nonimmune human serum, the AHU$^-$ auxotype, specific Por IA serovars, and marked susceptibility to penicillin—characteristics also associated with asymptomatic genital gonorrhea. However, recent DGI cases have been associated with other auxotype/serovar classes as the prevalence of Por IA/AHU$^-$ gonococci has declined and antibiotic-resistant strains have caused DGI.

Complement deficiency predisposes to gonococcal and meningococcal bacteremia.[139] Up to 13% of patients with DGI have been reported to have complement deficiencies, and patients with repeated episodes of neisserial bacteremia should be tested with an assay for total hemolytic complement activity. Other host factors associated with dissemination include female sex, menstruation, and perhaps pharyngeal gonococcal infection and pregnancy. In approximately half of affected women, symptoms of DGI begin within 7 days of the onset of menses.

The most common presentation of DGI is the arthritis-dermatitis syndrome.[140] During the first few days, most patients experience polyarthralgias that primarily involve the knees, elbows, and more distal joints; the axial skeleton is usually not involved. Physical examination usually shows objective signs of arthritis or tenosynovitis in at least two joints. Asymmetrical involvement of only a few joints helps distinguish DGI from polyarthritis caused by most immune complex–mediated disorders, which typically manifest with symmetrical involvement of many joints. A characteristic dermatitis (Fig. 212.15) is present in approximately 75% of patients and consists of discrete papules and pustules, often with a hemorrhagic component. Hemorrhagic bullae or overtly necrotic lesions that mimic ecthyma gangrenosum are sometimes seen. The lesions usually number 5 to 40 and occur predominantly on the extremities. Fever, systemic toxicity, and polymorphonuclear leukocytosis are common, but they are usually mild and are often absent.

If the infection is not treated, inflammation regresses in most joints and the dermatitis resolves, but overt septic arthritis supervenes in one or two joints, most commonly the knee, ankle, elbow, or wrist, although any joint may be involved. Septic gonococcal arthritis develops in some patients without prior polyarthritis or dermatitis; in the absence of the characteristic dermatitis or overt genital infection, it is clinically indistinguishable from septic arthritis of any etiology.

During the arthritis-dermatitis stage, gonococci often can be recovered through blood culture, but synovial fluid, if it can be obtained, usually contains fewer than 20,000 leukocytes/mm^2 and is sterile. Gonococci can often be seen by means of immunochemical methods in biopsy specimens of skin lesions, but cultures are generally sterile. In septic gonococcal arthritis, synovial fluid usually contains more than 50,000 leukocytes/mm^2 and culture is often positive, but at this stage blood cultures are usually negative.

Gonococcal bacteremia is often intermittent, so a minimum of three blood cultures should be obtained when DGI is suspected. Synovial fluid should be cultured if a specimen can be obtained. However, only approximately half of patients with DGI have positive cultures of blood or synovial fluid. Although uncommonly positive, Gram-stained smears and cultures of pustular skin lesions are simple to perform and should be obtained. *N. gonorrhoeae* can be recovered from a mucosal site in at least 80% of patients, so the urethra or endocervix, the rectum, and the pharynx should be tested regardless of symptoms or exposure history, and patients' sex partners should be examined and tested. Nucleic acid amplification tests (NAATs), including polymerase chain reaction assay, are more sensitive than culture in detecting gonococci in synovial fluid and have been applied to skin lesions of DGI with success.[141] The diagnosis of DGI is confirmed if *N. gonorrhoeae* is identified in a nonmucosal specimen such as blood, synovial fluid, or a skin lesion, and the diagnosis is probable if infection is documented at a mucosal site or in a sex partner of a patient with a typical clinical syndrome.

The differential diagnosis of the gonococcal arthritis-dermatitis syndrome includes meningococcemia, septic arthritis due to other pyogenic bacteria, and the entire range of inflammatory arthritis. Reactive arthritis is easily confused with DGI because it is common in sexually active young adults and is associated with urethritis, cervicitis, and skin lesions that sometimes have a pustular component. Usually, careful clinical and microbiologic assessment readily differentiates these disorders, but a trial of antibiotic therapy may be required.

Gonococcal endocarditis, which usually involves the aortic valve, is a rare but serious manifestation that occurs in an estimated 1% or 2% of patients with DGI.[142] Although often associated with the arthritis-dermatitis syndrome, endocarditis may be the sole manifestation of DGI. In the preantibiotic era, median survival was 6 to 8 weeks, reflecting



FIG. 212.15 Cutaneous lesions in disseminated gonococcal infection. (A) Early papular lesions. (B) Pustular lesion associated with subungual hemorrhage. (C) Ulcerated pustular lesion.

a typical rate of valve destruction between that of acute staphylococcal or pneumococcal endocarditis and subacute endocarditis caused by viridans streptococci.

Neonatal and Pediatric Infections

Gonococcal conjunctivitis of the newborn (ophthalmia neonatorum) is the most common clinically recognized manifestation of neonatal infection[143]; it was once a common cause of blindness in the United States and may remain so in some developing countries. Prophylaxis by means of instillation of antibiotics into the conjunctivae soon after delivery is highly effective, although occasional failures occur. The most important preventive measure is routine screening and treatment of pregnant women for gonorrhea before term. The diagnosis of gonococcal ophthalmia should be suspected clinically when acute conjunctivitis develops within a few days of delivery and is confirmed by identification of gonococci in conjunctival secretions. Systemic illness with septicemia and arthritis can also develop in neonates exposed to gonorrhea, but these are rare. *N. gonorrhoeae* can often be recovered from the gastric aspirates of infants born to infected mothers, but many cases probably reflect transient colonization rather than clinically important infection.

Rectal gonococcal infection is sometimes seen in neonates, but vaginal infection is uncommon because the neonatal vaginal mucosa is well estrogenized by circulating maternal hormone. Purulent vaginitis is the primary manifestation of gonorrhea in prepubertal girls after the neonatal period, but otherwise the clinical manifestations of gonorrhea in children are not materially different from those in adults. After the neonatal period until 1 year of age, most cases in children appear to be acquired nonsexually from an infected parent, usually in a setting of poor hygiene. After 1 year of age, most cases are acquired by sexual abuse. Nonsexual transmission of ocular infection occasionally occurs in young children in tropical settings.

DIAGNOSIS

Isolation of *N. gonorrhoeae* is the historical standard for diagnosis. Culture is inexpensive and reasonably sensitive and preserves an isolate for antimicrobial susceptibility testing when clinically indicated or for surveillance. Culture is widely considered the only appropriate test in forensic settings—for example, in testing people who have been sexually assaulted or children with suspected gonorrhea. Nevertheless, NAATs have supplanted culture in most settings, owing to convenient specimen management and their usefulness in testing urine and self-obtained vaginal swabs. Nonamplified DNA probe tests remain in use despite their lower sensitivity compared with culture or NAATs. Microscopy of gram-stained smears is effective in the diagnosis of symptomatic urethritis in men but has marginal usefulness in other settings. There is no clinically useful serologic test.

Culture

A single culture on antibiotic-containing selective medium, such as modified Thayer-Martin agar, has a sensitivity of 95% or more for urethral specimens from men with symptomatic urethritis and 80% to 90% for endocervical infection in women. Results may vary depending on the quality of the medium and the adequacy of the clinical specimen. Simultaneous inoculation of both selective and nonselective media maximizes sensitivity for cervical infection but is impractical in most settings. Normally, sterile clinical specimens, such as blood, synovial fluid, and cerebrospinal fluid, should be inoculated onto enriched chocolate agar or another nonselective medium in addition to broth medium and should also be tested with NAATs, but no published studies have systematically analyzed the detection of *N. gonorrhoeae* in broth cultures with the automated techniques now used by most clinical laboratories.

Up to 5% of gram-negative, oxidase-positive diplococci isolated from the genital tract and substantially greater proportions of those from the rectum and pharynx are in fact *N. meningitidis*.[144] Some of these cases are associated with urethritis, cervicitis, or proctitis that is clinically indistinguishable from gonorrhea.[145,146] Therefore, speciation of *Neisseria* isolates is desirable for positive genital cultures and should be routine for pharyngeal and rectal specimens. Available NAATs for *N. gonorrhoeae* reliably exclude meningococcal infection. Antimicrobial susceptibility testing of *N. gonorrhoeae* is not generally recommended except when one is conducting surveillance for antimicrobial resistance, but it should be routine after treatment failure and in cases of disseminated infection.

Nucleic Acid Amplification Tests

Currently available NAATs for *N. gonorrhoeae* use several related technologies, including transcription-mediated amplification, polymerase chain reaction assay, and the DNA strand displacement assay. The NAATs have largely replaced culture in most settings in which people are screened for asymptomatic genital infection and, in particular, in which assays with combined targets are used for gonorrhea and chlamydial infection (e.g., the APTIMA Combo 2 assay [Hologic]). These tests are not significantly more sensitive than culture for detecting *N. gonorrhoeae* in cervical or urethral specimens (unlike the diagnosis of *C. trachomatis,* for which the NAATs are more sensitive than all other tests). However,

the NAATs for *N. gonorrhoeae* have specificities greater than 99%, offer important advantages in specimen management, and retain sensitivity when used to test voided urine or self-collected vaginal swabs, an important advantage for laboratory screening in many settings. The transcription-mediated amplification test has a positive predictive value of at least 95% in women in settings in which gonorrhea prevalence is as low as 0.5%, indicating that false-positive results are rare even when low-prevalence populations are screened.[147] Concern has been expressed that persisting DNA may cause false-positive results when NAATs are used to test patients soon after treatment, but NAAT results for *N. gonorrhoeae* become negative within 1 to 2 weeks of successful treatment.[148] In fact, the recommendation to obtain a test of cure at 1 week if indicated includes NAAT testing and assumes that the results of these assays will rapidly become negative for *N. gonorrhoeae.*[107] The APTIMA test (and possibly others, although fewer data exist at this point) for *N. gonorrhoeae* is also reliable for diagnosis of rectal and pharyngeal infection.[149,150] Although these assays have not been cleared by the FDA for routine use, the CDC recommends their use as long as laboratories are Clinical Laboratory Improvement Amendments (CLIA) compliant with regard to test modifications, before reporting the results for patient management.[151] These observations may not extend to other NAATs; the Probe-Tec assay demonstrated low positive predictive value when applied to pharyngeal and rectal specimens in a community-based sample of MSM.[152]

Gram-Stained Smears

The Gram stain has been used for more than a century to identify gonococci in clinical specimens; methylene blue and other dyes have also been used. Microscopy findings of Gram-stained smears are positive if gram-negative diplococci of typical morphology are observed in association with neutrophils (Fig. 212.16), negative if no such organisms are seen, and equivocal if typical morphotypes are not associated with neutrophils or if morphologically atypical organisms are seen. Nonpathogenic members of the Neisseriaceae are not usually associated with leukocytes. In men with symptomatic urethritis, microscopy is at least 95% sensitive and is highly specific for diagnosis of gonorrhea, but microscopy is less useful for other anatomic sites. The sensitivity approximates 50% for asymptomatic urethral infection in men and for cervical or rectal infection; the specificity is said to be high, but this likely is true only for highly experienced observers. Microscopy is both insensitive and nonspecific for detection of pharyngeal gonococcal infection and is not recommended.

Other Diagnostic Methods

Nonamplified DNA probe tests are less sensitive than culture or NAATs, and they are not useful in the diagnosis of rectal or pharyngeal infection or for testing urine. However, these assays are inexpensive and are offered by many laboratories in combination with assays for *C. trachomatis.* Immunochemical detection of gonococcal antigens, such as with enzyme immunoassays with polyclonal antigonococcal antibodies and fluorescein-conjugated monoclonal antibodies for direct fluorescence microscopy, now is little used.



FIG. 212.16 Gram-stained smear of urethral exudates showing intracellular gram-negative diplococci that are characteristic of gonorrhea.

THERAPY

The antimicrobial susceptibility of *N. gonorrhoeae* is labile, varies greatly across geographic areas and populations, and fluctuates over time. Clinical treatment decisions almost invariably are made without knowledge of the antimicrobial susceptibility of the infecting strain. Therefore, when possible, the regimens for routine treatment of gonorrhea should have efficacies that approach 100%, regardless of the distribution of sensitive and resistant strains of *N. gonorrhoeae* in the community, and treatments with efficacies less than 95% should be avoided.[153] Other factors that influence therapeutic decisions for gonorrhea are the pharmacokinetic characteristics of the agent, its efficacy in complicated versus uncomplicated infection, differential efficacy at various anatomic sites of infection, toxicity, availability, convenience of administration, and cost.[154] An additional consideration is the potential efficacy of an agent for concurrent infection. The most common coexisting pathogen in people with gonorrhea is *C. trachomatis,* and many studies during 3 decades have demonstrated consistent results: 15% to 25% of heterosexual men, 10% to 15% of MSM, and 35% to 50% of women with gonorrhea have concurrent chlamydial infections.[155] The treatment of gonorrhea has been greatly facilitated by the excellent efficacy of single-dose regimens; however, with increasing resistance to several classes of antibiotics (detailed later), this approach has been reevaluated. Gonorrhea in pregnant women responds to the recommended regimens.

Uncomplicated Gonorrhea in Adults
Initial Single-Dose Treatment

Recommendations for the treatment of uncomplicated gonorrhea, modified from those published in the 2015 Sexually Transmitted Diseases Treatment Guidelines from the CDC, are summarized in Table 212.2.[153,154]

TABLE 212.2 Options for the Treatment of Gonorrhea

Uncomplicated Infection of the Cervix, Urethra, and Rectum
Ceftriaxone, 250 mg IM single dose *and* Azithromycin, 1 g PO single dose
Infection of the Pharynx
Ceftriaxone, 250 mg IM single dose *and* Azithromycin, 1 g PO single dose
Conjunctivitis (Not Ophthalmia Neonatorum)
Ceftriaxone, 1 g IM single dose
Disseminated Gonococcal Infection
Ceftriaxone, 1 g IM or IV every 24 hours for 24–48 hours[b] after improvement, with switch to oral therapy for completion of 1 week total antibiotic therapy, including cefixime, 400 mg PO twice daily
Meningitis and Endocarditis
Ceftriaxone, 1–2 g IV every 12 hours for 10–14 days (meningitis) or ≥4 weeks (endocarditis)
Ophthalmia Neonatorum
Ceftriaxone, 25–50 mg/kg IV or IM in a single dose, not to exceed 125 mg[c]

[a]Patients should abstain from sex for 1 week after single-dose treatment. Test of cure for pharyngeal infection is recommended at 14 days if the ceftriaxone regimen is not used.
[b]Ceftriaxone administered IM may be reconstituted in 1% lidocaine solution to minimize injection pain. Alternative parenteral regimens include cefotaxime, ceftizoxime, and spectinomycin. See www.cdc.gov/std/treatment for specific regimens.
[c]Topical antibiotic therapy alone is inadequate for treatment of ophthalmia neonatorum.
Modified from Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Morb Mortal Wkly Rep. *2015;64:60–68; and www.cdc.gov/std/treatment.*

A single 250-mg intramuscular dose of ceftriaxone is recommended for infection of all mucosal sites, with cure rates that exceed 98% for urethral, cervical, and rectal gonorrhea and 90% for pharyngeal infection; this should be combined with azithromycin given as a single dose of 1 g orally. The rationale for giving a second drug with a different mechanism of action rather than the primary treatment is that it may reduce selection pressure for antimicrobial resistance in *N. gonorrhoeae*. However, as noted earlier, increasing rates of resistance to azithromycin may soon compromise this approach.[116,117] Hypothetically, the worldwide spread of fluoroquinolone-resistant gonococci may have been slower than the dissemination of penicillinase-producing strains 25 years earlier in part because penicillin monotherapy was the rule in the 1970s, whereas antichlamydial cotherapy was the norm in the 1990s. Because of increasing resistance to the cephalosporins, neither of the widely available oral third-generation cephalosporins—cefixime and cefpodoxime—is recommended for primary therapy. Of note, allergic cross-reactions between penicillin and third-generation cephalosporins appear to be uncommon, and penicillin-allergic people can often be treated for gonorrhea with a cephalosporin.[156] Both ceftriaxone and azithromycin are safe and effective in pregnant women.

Unfortunately, owing to the relentless worldwide increase in the resistance of *N. gonorrhoeae* strains to fluoroquinolones, these agents now have limited clinical usefulness. Increasingly widespread fluoroquinolone resistance in the United States led the CDC to recommend against the use of these agents to treat gonorrhea in MSM in 2004 and, in August 2007, against routine use for all cases of gonorrhea. Nonetheless, fluoroquinolones are highly effective against infection due to susceptible gonococci, with cure rates of at least 98% for all anatomic sites. Although their general use should be discouraged, these drugs retain a useful role in patients in whom no other option is feasible, such as those with absolute contraindications to cephalosporin; however, as noted previously, allergic cross-reactions between penicillin and ceftriaxone appear to be uncommon, and penicillin-allergic people often can be treated for gonorrhea with ceftriaxone. When a fluoroquinolone is the only feasible option for gonorrhea treatment, a culture isolate should be obtained so that susceptibility testing can be performed and for reference in the event of treatment failure.

In one clinical trial, treatment of uncomplicated urogenital gonorrhea with dual therapy consisting of oral azithromycin (2 g) and either a single oral dose of gemifloxacin (320 mg) or a single intramuscular dose of gentamicin (240 mg) was associated with cure rates of 99.5%, and 100%, respectively.[157] Few infections in this trial occurred at rectal and pharyngeal sites, limiting the reliability of cure estimates at these sites; regardless, if available, either of these regimens may be considered alternative therapies, although gastrointestinal side effects limited tolerability.

Spectinomycin, 2 g intramuscularly, is effective for genital and rectal gonorrhea, but it is ineffective for pharyngeal infection and is currently not available in the United States; its eventual availability is uncertain. Its sole indication is the treatment of pregnant women with a history of rapid-onset allergic reactions to penicillin or documented cephalosporin allergy—a rare confluence of events. Other options to treat such women are limited and have not been studied; these include desensitization to recommended agents or use of alternative drugs with test of cure.

Follow-Up of Patients Treated for Uncomplicated Gonorrhea

Failure of treatment of infections with organisms that are susceptible to recommended regimens is uncommon. Retesting should be performed to evaluate persistence of symptoms at any infected site; ideally, this should be performed with culture to facilitate assessment for antimicrobial resistance. Documentation of cure should be performed with retesting for pharyngeal infections treated with a regimen other than ceftriaxone. In this case, test of cure using NAATs or culture should be obtained 14 days after treatment was provided. It is important to realize, however, that most patients with gonorrhea remain at risk for repeat infection even if initial treatment was successful; studies have found recurrent gonorrhea in 10% to 15% of people retested 1 to 4 months after treatment.[158–160] Accordingly, retesting for recurrent or persistent infection, or rescreening, should be done routinely 3 or 4 months after treatment.

TABLE 212.3 Recommended Treatment of Acute Pelvic Inflammatory Disease

Hospitalized Patients

Regimen A

Cefotetan, 2 g IV q12h, or cefoxitin, 2 g IV q6h
plus
Doxycycline, 100 mg IV or PO q12h
Continue both drugs IV for 24 hours after the patient substantially improves, then continue doxycycline, 100 mg PO bid, to complete 14 days total therapy. Either clindamycin or metronidazole may be added to the oral regimen if tubo-ovarian abscess is suspected.
or

Regimen B

Clindamycin, 900 mg IV q8h
plus
Gentamicin, 2 mg/kg IV once, followed by 1.5 mg/kg q8h[a]
Continue both drugs IV for 24 hours after the patient substantially improves, then continue doxycycline, 100 mg PO twice daily, or clindamycin, 450 mg PO four times daily, to complete 14 days total therapy. Clindamycin may be preferable when tubo-ovarian abscess is suspected.

Outpatients

Single-dose cefoxitin, 2 g IM, plus probenecid, 1 g PO; or ceftriaxone, 250 mg IM; or other parenteral third-generation cephalosporin (e.g., ceftizoxime or cefotaxime)
plus
Doxycycline, 100 mg PO bid for 14 days
with or without
Metronidazole, 500 mg PO bid for 14 days

[a]Single daily dosing may be substituted.
Modified from Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. MMWR Morb Mortal Wkly Rep. *2015;64:60–68; and www.cdc.gov/std/treatment.*

Rescreening is also indicated for men and women with chlamydial infection. Testing urine or self-collected vaginal specimens with NAATs can be accomplished in asymptomatic patients without a clinical examination or, if using mailed specimens, without a clinic visit.

Pelvic Inflammatory Disease

The treatment of PID is addressed in detail in Chapter 111. In individual patients, the specific pathogens responsible for ascending genital infection usually are not known and the recommendations for initial treatment of acute PID are similar regardless of whether the initial infection is due to *N. gonorrhoeae, C. trachomatis,* or other pathogens. Guidelines for the treatment of PID are summarized in Table 212.3. The main regimen for outpatient therapy is single-dose treatment with ceftriaxone or with cefoxitin plus probenecid, followed by oral therapy with doxycycline, 100 mg twice a day orally, and metronidazole, 500 mg twice a day orally for 14 days. In a well-designed study, this regimen (without metronidazole) was equally as effective as the equivalent inpatient parenteral regimen both in short-term resolution and in preservation of fertility after a 3-year follow-up period.[134] The use of azithromycin for treatment of PID has been controversial and is not currently recommended. The CDC guidelines stipulate that fluoroquinolone may be considered as the antibiotic for gonorrhea coverage in PID if the community prevalence and individual risk for fluoroquinolone-resistant gonorrhea are low, and the guidelines recommend performing diagnostic testing with appropriate management according to the results. In addition, in many communities gonorrhea is now a relatively uncommon cause of PID, reducing concern about antibiotic resistance.

Regardless of the initial treatment, close follow-up is indicated. Clinical progression or absence of improvement within 3 days is an indication for diagnostic imaging and hospitalization of the patient for parenteral therapy and possible laparoscopy to confirm the diagnosis and obtain intraabdominal culture specimens to facilitate selection of improved, parenteral antimicrobial therapy. The patient's sex partner(s) should be tested and treated for chlamydial infection and gonorrhea, unless both infections can be reliably excluded in the index patient.

Acute Epididymitis

The treatment of acute epididymitis is addressed in Chapter 112. Most cases in young adults are due to *C. trachomatis* or *N. gonorrhoeae,* but coliforms and other urinary tract pathogens are common causes in older men or after urinary tract instrumentation and perhaps in men who participate in unprotected insertive anal intercourse. Most cases can be managed on an outpatient basis with ceftriaxone, 250 mg intramuscularly, plus 10 days of treatment with doxycycline, 100 mg twice daily. Ofloxacin, 400 mg orally twice daily, or levofloxacin, 500 mg daily, for 10 days, provides better coverage for coliforms and nonsexually transmitted pathogens and is effective against chlamydial infection but should be avoided if gonorrhea is suspected.

Disseminated Gonococcal Infection

Few data are available on the treatment of patients with DGI since the evolution and spread of gonococci with high levels of antibiotic resistance, and all recommendations are empirical. Antimicrobial susceptibility testing should be routinely used to guide therapy in case the initial response to empirical treatment is suboptimal. Patients with the gonococcal arthritis-dermatitis syndrome should be treated initially with ceftriaxone, 1 g once daily, given either intramuscularly or intravenously.[153] Most patients without complications can be treated as outpatients. Equivalent doses of other third-generation cephalosporins undoubtedly would be effective and are recommended as options. No reported studies have systematically evaluated the fluoroquinolones, but these drugs undoubtedly are effective if the organism is susceptible. After clinical improvement begins, treatment of patients without septic arthritis or other complications may be switched to an oral cephalosporin (e.g., cefixime 400 mg twice daily) or a fluoroquinolone (e.g., levofloxacin 500 mg daily) to complete 7 to 10 days of total therapy. Patients with gonococcal endocarditis should receive 4 weeks of parenteral therapy, usually starting with ceftriaxone or an equivalent cephalosporin and later modifying the regimen if dictated by the results of antimicrobial susceptibility testing. Meningitis should be treated with a 10- to 14-day course of ceftriaxone.

Gonorrhea in Children

Relatively few cases of gonorrhea are seen in children, and treatment has not been well studied; most treatment recommendations are extrapolated from those for adults. Uncomplicated infections in neonates and older children should normally be treated with ceftriaxone in a single intramuscular dose of 25 to 50 mg/kg, up to 125 mg.[153] Little is known about the prevalence of chlamydial infection in pediatric patients with gonorrhea, and the usual practice is to perform a test for *C. trachomatis* and withhold specific treatment unless infection is diagnosed. DGI and gonococcal conjunctivitis in children are treated with 7 to 10 days of ceftriaxone, 25 to 50 mg/kg/day intramuscularly or intravenously, or with an equivalent regimen of another third-generation cephalosporin. Continuous irrigation of the conjunctivae with physiologic saline solution is often used in gonococcal conjunctivitis, but topical antibiotics probably offer no additional benefit.

Management of Sex Partners

Management of sex partners is an integral part of treating patients with gonorrhea and other STDs because failure to ensure that the partner is treated risks reinfection of the patient and fosters continued transmission. Few state or local health departments in the United States provide direct assistance in contacting or managing the partners of people with gonorrhea or chlamydial infection diagnosed outside public clinics (and often not there); the physician and patient should work together to this end.

Ideally, the sex partners of people with gonorrhea or chlamydial infection should be examined, tested for both infections and other common STDs, and counseled on prevention. Unfortunately, success in bringing partners to clinical care is low, and in most settings treatment can be documented for less than half the partners of infected people.[161] Studies suggest that this proportion can be increased and the rate of reinfection in index patients reduced by arranging for treatment without examination—that is, by giving the index patient an antibiotic for the partner or a prescription in the partner's name or by calling in a prescription directly for the partner. Collectively, these strategies have been termed *expedited partner therapy* (EPT).[162] Several states with formerly restrictive laws or regulations have modified them to permit EPT, and others are considering such steps. The CDC provides up-to-date reports of the legal status of EPT throughout the United States at www.cdc.gov/std/ept. Clinicians should routinely use EPT for the partners of people with gonorrhea or chlamydial infection, to the extent permitted by regional laws and regulations, whenever success is not ensured in personal evaluation of the partners. EPT for gonorrhea can be accomplished with single-dose oral therapy active against both *N. gonorrhoeae* (e.g., cefixime) and *C. trachomatis* (azithromycin); for the partners of patients with chlamydial infection alone, azithromycin is appropriate.

PREVENTION AND CONTROL

Public Health Strategies

Screening of sexually active people is a mainstay of public health strategies to prevent gonorrhea and the other treatable bacterial STDs, and it is largely responsible for the dramatic declines observed in the incidence of gonorrhea nationwide after the mid-1970s. Women at risk who undergo routine pelvic examinations should be tested for *N. gonorrhoeae* and *C. trachomatis* according to published guidelines where available. The US Preventive Services Task Force recommends routine annual testing for gonorrhea in sexually active women younger than 25 years and for older women with specific risks.[163] Some authors have used more refined epidemiologic analyses to help narrow these relatively broad recommendations for screening and have suggested that local gonorrhea prevalence rates may be especially useful.[164] When pelvic examination is otherwise not indicated, urine or a self-collected vaginal swab can be tested with NAATs. Universal screening of all heterosexual men and women usually is not cost-effective except in certain settings, such as public STD clinics. In screening women or heterosexual men, a pharyngeal culture should be obtained if symptoms of pharyngitis are present, and it may be warranted if the patient has performed oral sex on a person known to have genital gonorrhea.

Although asymptomatic cervical gonorrhea has been recognized for some time, of increasing concern is the underappreciated prevalence of asymptomatic gonorrhea in some populations, particularly rectal and pharyngeal infection in MSM. Sexually active MSM should be tested routinely for both gonococcal and chlamydial infection, depending on the anatomic sites exposed. Furthermore, it was shown more than a decade ago that urethritis increases HIV viral load in semen, and treatment concomitantly lowers levels of HIV RNA.[165] Thus, prompt diagnosis and treatment of urethritis along with routine periodic screening at all exposed anatomic sites is recommended for all HIV-infected people at initial evaluation and thereafter at least every 12 months and more frequently for those at higher risk.[127] Among asymptomatic MSM, the highest yield will be achieved by testing the rectum if receptive anal intercourse is reported, urethra if insertive anal sex is reported, and pharynx if receptive oral sex is reported.

Public health control measures for gonorrhea and chlamydial infection also include routine rescreening and treatment of patients' sex partners (see previous discussion). Other elements include appropriate diagnostic testing in people with compatible clinical syndromes, adherence to recommended treatment regimens, and periodic testing of gonococcal isolates to monitor trends in antimicrobial resistance. Reporting of cases by health care providers, preferably supplemented by direct reporting by laboratories of people with positive test results, is important for epidemiologic monitoring and to facilitate targeted control efforts. Public education and personal counseling, in an effort to encourage healthy sexual behavior and the use of barrier contraceptives, is central to the control of gonorrhea and all STDs. Finally, all people with a newly acquired STD should routinely be tested for other common infections. For example, screening tests for gonorrhea, chlamydial infection, syphilis, and HIV should be undertaken in people diagnosed with any STD; women with STDs should undergo cervical cytology and diagnostic testing for vaginal infections.

Condoms and Microbicides

Properly used male condoms provide a high degree of protection against transmission or acquisition of gonorrhea, chlamydial infection, HIV, and other STDs transmitted by infected secretions.[153] Methodologic

limitations of most studies that have attempted to estimate the magnitude of condom effectiveness tend to result in an underestimate of protection. Nevertheless, a well-designed systematic review reported that most studies found condom use to be associated with reduced risk for both gonorrhea and chlamydia.[166] The female condom likely provides comparable protection against gonococcal infection.[167] Logically, diaphragms and cervical caps might be expected to offer some protection against gonorrhea. Although a randomized controlled cohort study in South Africa failed to show a protective effect against HIV, a subset analysis revealed that women who reported consistent diaphragm use experienced a significant reduction in incident gonococcal infection (relative hazard, 0.61; 95% CI, 0.41–0.91).[168] The spermicide nonoxynol-9 provides no significant protection against gonorrhea or chlamydial infection and is associated with increased risk for vulvovaginal candidiasis and bacterial urinary tract infection and increased risk for HIV acquisition in a large trial.[169] Although spermicidal preparations with nonoxynol-9 enhance the contraceptive efficacy of barrier methods, they should be avoided by people at high risk for STDs, as should condoms packaged with lubricant containing nonoxynol-9. Research is underway to identify alternative microbicides for vaginal or rectal use. No evidence has been presented that such time-honored measures as washing, urinating, or douching after exposure materially reduce the risk for gonorrhea or any other STD.

Other Prevention Strategies

The development of a vaccine to prevent gonorrhea is a high research priority, but an experimental vaccine containing purified gonococcal pili conferred only partial protection against experimental infection with the homologous strain of *N. gonorrhoeae* and no protection from heterologous challenge.[170] The extraordinary degree of antigenic variability in pili, Opa proteins, and LOS, both at the community level and during the course of each infection, presents formidable barriers to developing a gonorrhea vaccine based on these antigens. More antigenically stable proteins, including porin, are under investigation as possible vaccine candidates, but success probably lies in the distant future. Most intriguing is a recent study that used a retrospective case-control approach of patients at sexual health clinics in New Zealand who were eligible to have received an outer membrane vesicle meningococcal B vaccine (MeNZB) in the 1980s. Vaccinated individuals were 31% significantly less likely to have gonorrhea diagnosed subsequently ($P < .001$), suggesting a protective effect and providing a potential pathway for effective vaccine development.[171]

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The complete reference list is available online at Expert Consult.

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213 *Moraxella catarrhalis, Kingella*, and Other Gram-Negative Cocci

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SHORT VIEW SUMMARY

Definition
- *Moraxella catarrhalis* is a gram-negative diplococcus that colonizes the upper respiratory tract of children.
- It is a common cause of otitis media in children and exacerbations of chronic obstructive pulmonary disease (COPD) in adults.
- *Kingella kingae* is an increasingly recognized pathogen that causes osteoarticular infections and bacteremia in children 6 months to 4 years of age and endocarditis in all ages.

Epidemiology
- *M. catarrhalis* is recovered exclusively from humans.
- Nasopharyngeal colonization is common in infancy and childhood and decreases with age.
- *K. kingae* colonizes the oropharynx of children and is more often isolated from those who attend daycare centers. Infections occur sporadically, but occasional clusters are seen among children in close contact.

Microbiology
- *M. catarrhalis* is easily overlooked in culture because it is phenotypically identical to *Neisseria* in Gram stain and on culture plates.
- *M. catarrhalis* produces oxidase, catalase, and DNase, which are used for speciation.
- *K. kingae* is difficult to isolate and to identify, growing more readily in liquid culture than standard solid agar media.

Diagnosis
- An etiologic diagnosis of the most common clinical manifestations of *M. catarrhalis* (otitis media in children and exacerbations of COPD in adults) is not made routinely in clinical practice. Rather, these infections are usually treated empirically, based on a presumptive diagnosis using clinical manifestations.
- A diagnosis of *K. kingae* infections is made by recovering the organism in culture from normally sterile body fluid, including blood and joint fluid aspirate. Difficulties in isolation can lead to an incorrect diagnosis of culture-negative bone or joint infection. Polymerase chain reaction–based assays are more sensitive than culture in detecting the organism in joint fluid.

Therapy
- Most isolates of *M. catarrhalis* produce β-lactamase and are thus resistant to ampicillin. Isolates are susceptible to amoxicillin/clavulanic acid, macrolides, fluoroquinolones, and extended-spectrum cephalosporins.
- Otitis media and exacerbations of COPD are treated with oral antimicrobial agents, with the choice generally guided by expert guidelines.
- *K. kingae* isolates are usually susceptible to penicillins, cephalosporins, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, and macrolides, although a plasmid-encoded β-lactamase has been found in a few strains.

Prevention
- No vaccines are available currently, but research is in progress to develop vaccines to prevent *M. catarrhalis* infections, including otitis media and exacerbations of COPD.
- Interrupting outbreaks of *K. kingae* osteoarticular infections in daycare centers by prophylactic antibiotics has met with mixed success.

Over the past 3 decades *Moraxella (Branhamella) catarrhalis* has emerged as an important and common human respiratory tract pathogen. In this chapter *M. catarrhalis* is discussed. In addition, *Kingella* and other gram-negative cocci, including *Neisseria* other than *N. meningitidis* and *N. gonorrhoeae*, and other *Moraxella*, which are less common causes of human infection, are considered. *Acinetobacter* is discussed in Chapter 222, and *Oligella* is discussed in Chapter 236. *N. meningitidis* and *N. gonorrhoeae* are discussed in Chapters 211 and 212, respectively.

MORAXELLA CATARRHALIS

History

M. catarrhalis has an interesting and checkered taxonomic history. The bacterium was first described a century ago and was suspected by Sir William Osler to be the cause of his own terminal pneumonia.[1] After having been initially named *Micrococcus catarrhalis*, the organism's name was subsequently changed to *Neisseria catarrhalis* because of its similarities in phenotype and ecologic niche to *Neisseria* spp. In 1970 it was transferred to the new genus *Branhamella* on the basis of differences in fatty-acid content and DNA hybridization studies, compared with other Neisseriaceae.[2] The name *Moraxella catarrhalis* was subsequently proposed, and this is the most widely accepted name at this time.

For most of the last century *M. catarrhalis* was regarded as an upper respiratory tract commensal. However, since the late 1970s, investigators from many centers have accumulated compelling evidence that *M. catarrhalis* is an important and common respiratory tract pathogen in humans.[3–9]

Microbiology

Current taxonomic classification schemes include three genera in the family Moraxellaceae—*Moraxella, Acinetobacter*, and *Psychrobacter. M. catarrhalis* is a gram-negative diplococcus that is indistinguishable from *Neisseria* by Gram stain. The organism grows well on blood agar, chocolate agar, and a variety of media. *M. catarrhalis* are difficult to distinguish from *Neisseria* by colony morphology, particularly after overnight growth on agar plates. After 48 hours of growth, *M. catarrhalis* colonies tend to be larger than *Neisseria* and take on a pink color. In addition, colonies display the hockey puck sign by sliding along the surface of the agar when pushed. Because samples from the respiratory tract frequently contain *Neisseria*, suspicious colonies should be tested for the possibility that they are *M. catarrhalis*. The similarity in colony morphology between commensal *Neisseria* and *M. catarrhalis* results in the underestimation of *M. catarrhalis* in cultures of human respiratory tract samples.

M. catarrhalis produces oxidase, catalase, and DNase. Several kits to speciate *M. catarrhalis* are commercially available.[10,11]

Epidemiology and Respiratory Tract Colonization

M. catarrhalis has been recovered exclusively from humans. The prevalence of colonization is highly dependent on age. The upper respiratory tract of approximately 1% to 5% of healthy adults is colonized by *M. catarrhalis*.[12,13] By contrast, nasopharyngeal colonization with *M. catarrhalis* is common throughout infancy. Some published studies have shown a higher rate of colonization during winter months; this higher rate may be a result of the appearance of respiratory viral illnesses during colder months. Substantial regional differences in colonization rates are observed. For example, 66% of infants in a study in Buffalo, New York, were colonized during the first year of life,[14] whereas a similar study in Göteborg, Sweden, showed a colonization rate of approximately half of that level.[15] A study of rural Aboriginal infants near Darwin, Australia revealed that 100% of infants were colonized by *M. catarrhalis* by the age of 3 months.[16] The explanation for the marked differences in rates of colonization is not yet known. Several factors, including living conditions, daycare, crowding, hygiene, environmental factors (e.g., household smoking), genetic characteristics of the populations, and host factors, may play a role.[17,18] Microbial interactions in the nasopharynx among cocolonizing respiratory pathogens, including viruses and bacteria, and normal flora contribute to the complexity and dynamics of nasopharyngeal colonization.[19–25] The widespread use of pneumococcal conjugate vaccines has caused changes in patterns of nasopharyngeal colonization by reducing colonization with vaccine serotypes of *Streptococcus pneumoniae*. This effect has resulted in "replacement" of vaccine serotypes of *S. pneumoniae* by nonvaccine pneumococcal serotypes. Whether replacement by nontypeable *Haemophilus influenzae* and *M. catarrhalis* also occurs will require careful monitoring.[26–28]

Nasopharyngeal colonization with middle ear pathogens, including *M. catarrhalis*, is associated with otitis media. Early colonization is a risk factor for recurrent otitis media.[14,29,30] Otitis-prone children are colonized with *M. catarrhalis* at a higher rate compared with healthy children.[14,31,32]

M. catarrhalis is isolated from the sputum of adults with chronic obstructive pulmonary disease (COPD). Approximately 50% of episodes of acquisition of *M. catarrhalis* are associated with acute exacerbations of COPD.[33] Adults with COPD develop systemic and mucosal immune responses and clear *M. catarrhalis* from the respiratory tract efficiently after a relatively short duration (<1 month).[34,35] A smaller proportion of episodes of colonization may persist for up to 2 years.[33] Acquisition and clearance of the organism results in strain-specific protection.

Pathogenesis

M. catarrhalis causes mucosal infections in children and adults. The pathogenesis of infection appears to involve contiguous spread of the bacterium from its colonizing position in the respiratory tract to cause clinical signs of infection. In the case of otitis media the isolates recovered from the middle ear are present in the nasopharynx, indicating that the middle ear isolate came from the nasopharynx via the eustachian tube. Colonization of the upper respiratory tract with middle ear pathogens, including *M. catarrhalis*, is a necessary first step in the pathogenesis of otitis media. However, colonization alone is not sufficient to cause disease. An inciting event, such as a viral infection, in a child colonized with a middle ear pathogen is probably necessary for bacteria to move to the middle ear and cause otitis media. In the case of infection in adults with COPD the acquisition of a new strain is critical in the pathogenesis of infection.[33,36]

A key step in the initiation of infection is adherence of *M. catarrhalis* to the human respiratory epithelium. Several adhesins with varying specificities for host cells have been identified (Table 213.1).[3,7,37] Some of these surface antigens and others are being evaluated as potential vaccine antigens.[6,38–44] In addition to adherence to the epithelial surface, *M. catarrhalis* also resides within and beneath the epithelium and invades host cells.[45,46] Indeed, colonization of the nasopharynx by *M. catarrhalis* is more frequent than is revealed by surface culture.

TABLE 213.1 Adhesins and Putative Adhesins of *Moraxella catarrhalis*

ADHESIN	MOLECULAR MASS (kDa)	OBSERVATION
UspA1	88 (oligomer)	Adhesin for respiratory epithelial cells; binds laminin
MID/Hag	200	Hemagglutinin; binds IgD
OMP CD	45	Adhesin for respiratory epithelial cells; binds mucin; OMP A–like protein
McmA	110	Metallopeptidase-like adhesin
MchA1 (MhaB1)	184	Homology with filamentous hemagglutinin of *Bordetella pertussis*
MchA2 (MhaB2)	201	
McaP	66	Adhesin and phospholipase B
OlpA	24	Putative adhesin based on homology with Opa proteins
OMP J	19 16	Exists in two forms; putative adhesins based on homology with Opa proteins
Type 4 pili	16	Also essential for transformation; involved in biofilm formation

Hag, Hemagglutinin; *IgD,* immunoglobulin D; *McaP, M. catarrhalis* adherence protein; *MchA, M. catarrhalis* hemagglutinin-like protein A; *McmA, M. catarrhalis* metallopeptidase-like adhesin A; *MhaB2, M. catarrhalis* (filamentous) hemagglutinin adhesin-like protein B2; *MID, Moraxella* IgD-binding protein; *OMP,* outer membrane protein; *Opa,* opacity associated protein; *Olp,* Opa-like protein; *UspA1,* ubiquitous surface protein A1.

The outer membrane of *M. catarrhalis* contains lipooligosaccharide (LOS). LOS consists of a lipid A core coupled to oligosaccharides. The structure of the LOS resembles that of other nonenteric gram-negative bacteria in that the molecule lacks the long polysaccharide side chains observed in enteric gram-negative bacteria. Three major antigenic types of LOS can be distinguished, accounting for 95% of all strains.[47] The different serotypes are based on differences in terminal sugars in the LOS molecule. A detoxified LOS molecule is a potential vaccine antigen.[48,49]

The interaction of selected surface antigens with receptors of various host cells in the respiratory tract has important effects in mediating host responses to *M. catarrhalis*. For example *M. catarrhalis* is mitogenic for B lymphocytes through interaction with the MID/Hag protein.[50,51] The surface protein UspA2 regulates nuclear factor kappa B and subsequent interleukin-8 release by human respiratory epithelial cells.[52] LOS stimulates human monocytes to produce proinflammatory cytokines in both Toll-like receptor 4– and CD14-dependent pathways.[53] Like many gram-negative bacteria, *M. catarrhalis* sheds vesicles from its surface; these vesicles are internalized by respiratory epithelial cells and mediate several virulence mechanisms, including B-cell activation, induction of inflammation, and delivery of β-lactamase, which may promote survival of copathogens.[54–56] The availability of the genome sequence of clinical isolates is facilitating studies of pathogenesis.[57,58] *M. catarrhalis* evades host immunity through specific binding of several human extracellular matrix molecules in connective tissue, including vitronectin, fibronectin, collagen, plasminogen, and cartilage oligomeric matrix protein.[58–64] These host molecular patterns become exposed when epithelial integrity is interrupted, for example, by chronic inflammation or viral infection.

A reliable animal model that parallels human infection has not yet been developed for *M. catarrhalis*. The specificity of *M. catarrhalis* for humans creates challenges for the development of a useful model to study pathogenesis. The chinchilla model of otitis media is used widely to study otitis media caused by other bacteria, but chinchillas readily clear *M. catarrhalis* when it is instilled into the middle ear. *M. catarrhalis* colonizes the nasopharynx of the chinchilla, so this model may prove useful.[65,66] The most widely used model is a mouse pulmonary clearance model that measures the rate of clearance of *M. catarrhalis* from the lungs after intratracheal challenge. This model does not parallel human infection but has been used as a guide to identify and study potential

vaccine antigens. Models that involve coinfection of *M. catarrhalis* with other viral and respiratory tract pathogens may prove to be useful in understanding the role of *M. catarrhalis* as a copathogen.[67–69]

Clinical Manifestations

Otitis Media

Approximately 80% of children experience at least one episode of acute otitis media by the age of 3 years. A subset of children experiences recurrent otitis media, which is associated with a delay in speech and language development. Careful studies from many centers have defined the cause of acute otitis media by culturing middle ear fluid obtained by tympanocentesis, which has been considered the gold standard for determining the etiology of otitis media. Although some differences among studies are observed, the results from centers in the United States and Europe are remarkably consistent in showing that *S. pneumoniae*, nontypeable *H. influenzae*, and *M. catarrhalis* are the predominant bacterial causes of acute otitis media. Overall, based on cultures of middle ear fluid, approximately 5% to 20% of cases of acute otitis media are caused by *M. catarrhalis*.[70–78] However, culture detects pathogens in only a subset of otitis media.[79] Using more sensitive molecular analysis, *M. catarrhalis* is detected alone or with other pathogens in 30% to 50% of middle ear fluid samples from children with otitis media (Fig. 213.1).[80–86] Analysis of middle ear fluid by PCR increases the frequency of detection of *S. pneumoniae* and nontypeable *H. influenzae* 3.2-fold compared with culture, whereas *M. catarrhalis* is 4.5 times more likely to be identified by PCR.[87] Several lines of evidence indicate that the presence of bacterial DNA in clinical samples indicates active infection, including (1) detection of messenger RNA, (2) viable bacteria in biofilms, and (3) studies in the chinchilla model.[88–90] When *M. catarrhalis* is isolated from middle ear fluid in acute otitis media, it is present as a sole pathogen in approximately 75% of cases and as a copathogen in the remaining 25%. The widespread use of pneumococcal conjugate vaccines is altering the distribution of pathogens in otitis media, emphasizing the importance of continuous monitoring.[91,92]

Lower Respiratory Tract Infections in Chronic Obstructive Pulmonary Disease

M. catarrhalis causes lower respiratory tract infections in adults, particularly in the setting of COPD. The recognition of *M. catarrhalis* as a pathogen in this setting was delayed until the past 20 years because *M. catarrhalis* is indistinguishable from commensal *Neisseria* by Gram stain and difficult to distinguish by colony morphology. Therefore, unless clinical microbiology laboratories specifically test colonies that appear to be *Neisseria, M. catarrhalis* will be missed as a potential pathogen in sputum.

Several lines of evidence have established that *M. catarrhalis* causes exacerbations of COPD[93,94]:

- Analysis of sputum samples of a subset of patients with exacerbations of COPD demonstrates a predominance of gram-negative diplococci on Gram stain and almost pure cultures of *M. catarrhalis.*
- Studies using transtracheal aspiration and bronchoscopy with the protected specimen brush to sample the lower airways have revealed pure cultures of *M. catarrhalis* in some patients with exacerbations of COPD.
- The levels of inflammatory markers in the sputum of patients with exacerbations with positive cultures for *M. catarrhalis* are higher than the level in sputum of culture-negative exacerbations.[95]
- A specific immune response has been observed after exacerbations of COPD associated with *M. catarrhalis* in the sputum.[33–35]
- Acquisition of a new strain of *M. catarrhalis* is associated with clinical exacerbation.[33,36]
- Exacerbations resulting from acquisition of a new strain of *M. catarrhalis* are associated with protease-antiprotease imbalance in the airways.[96,97]

M. catarrhalis causes approximately 10% of exacerbations of COPD, making the bacterium the second most common bacterial cause of exacerbations after nontypeable *H. influenzae*.[3,33,98]

The clinical manifestations of exacerbations of COPD caused by *M. catarrhalis* are similar to those of exacerbations caused by other bacteria, such as nontypeable *H. influenzae.* Patients experience increased cough and sputum production, increased sputum purulence, and increased dyspnea compared with baseline symptoms. Sputum Gram staining shows intracellular and extracellular gram-negative diplococci as the exclusive or predominant bacterial form (Fig. 213.2), and cultures grow predominantly *M. catarrhalis.*

Pneumonia in Older Adults

M. catarrhalis causes pneumonia in older adults, but it is difficult to state the precise proportion. Most older patients who experience pneumonia caused by *M. catarrhalis* have underlying illnesses, including COPD, congestive heart failure, and diabetes. Although *M. catarrhalis* causes a significant illness in older adults, fulminant pneumonia is uncommon.

Nosocomial Respiratory Tract Infections

Nosocomial lower respiratory tract infections caused by *M. catarrhalis* have been observed in respiratory units in health care facilities. The

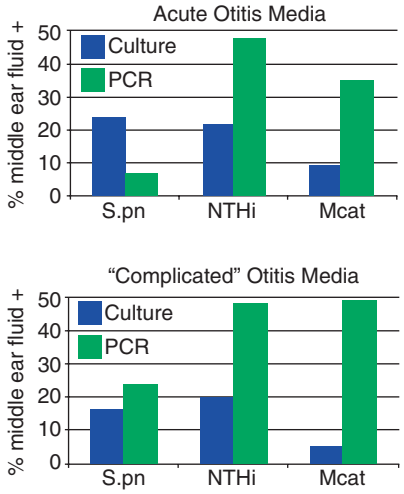

FIG. 213.1 Causes of otitis media. These are the results of bacterial culture *(blue)* and polymerase chain reaction *(PCR) (green)* of middle ear fluid from children with acute otitis media *(top)* and complicated otitis media defined as recurrent, refractory, or chronic otitis media and otitis media with effusion *(bottom).* Studies included 1565 (acute) and 894 (complicated) samples from seven studies.[80–86] *Mcat, Moraxella catarrhalis; NTHi,* Nontypeable *Haemophilus influenzae; S.pn, Streptococcus pneumoniae.*

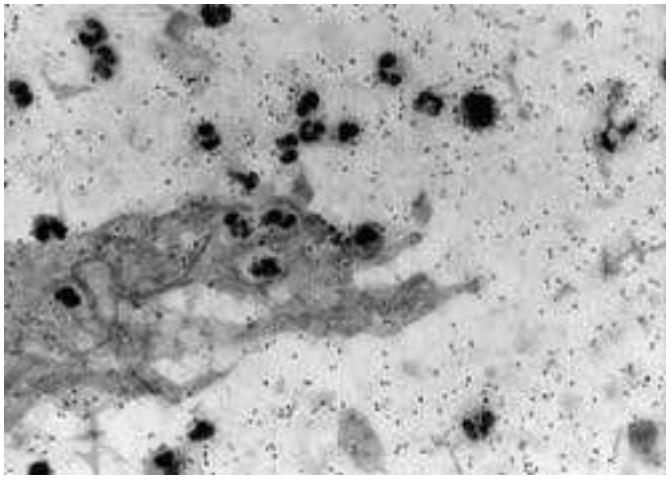

FIG. 213.2 Sputum sample from a patient with chronic obstructive pulmonary disease experiencing an exacerbation caused by *Moraxella catarrhalis*. Note the abundance of leukocytes, the presence of large numbers of gram-negative diplococci as the exclusive bacterial form and the presence of intracellular bacteria in leukocytes (Gram stain; ×1000).

presence of a susceptible population of adults with underlying cardiopulmonary disease may be important in these apparent outbreaks. Analysis of isolates by various typing methods has indicated that some of these clusters involved multiple strains of *M. catarrhalis,* and some were caused by a single strain, indicating person-to-person spread of the organism.[99]

Sinusitis

The cause of sinusitis is determined by culture of sinus aspirates, a relatively invasive procedure that is not performed routinely. Studies that have used sinus aspiration to determine the cause of sinusitis have shown that *M. catarrhalis* is the third most common cause of sinusitis in adults and children after nontypeable *H. influenzae* and *S. pneumoniae.*

Bacteremia

Published reports have documented the occurrence of bacteremia caused by *M. catarrhalis.*[100–102] Bacteremia is an infrequent manifestation of *M. catarrhalis* infection. The severity of clinical manifestations ranges from mild to life threatening. Bacteremia has been reported in those of all ages, from neonates to older adults. Most patients have clinical evidence of respiratory tract infection. Whereas many infants with bacteremia caused by *M. catarrhalis* are immunocompetent, most adults have underlying illnesses, including cardiopulmonary disease, malignancy, immunodeficiency, and chronic debilitation. A review of *M. catarrhalis* bacteremia has noted a mortality of 21%.[102] The underlying illness is an important determinant of outcome.

Therapy

A rapid increase in the proportion of strains that produce β-lactamase occurred simultaneously in the United States and Europe, beginning in the late 1970s.[103] This is one of the most dramatic examples of a rapid increase in antimicrobial resistance by a bacterial species. Currently, almost all strains of *M. catarrhalis* produce β-lactamase. Three different β-lactamases (BRO-1, BRO-2, and BRO-3) have been identified and characterized.[104,105] The β-lactamase of *M. catarrhalis* is inducible and cell associated. Because an inoculum-dependent susceptibility to ampicillin is observed, ampicillin should not be used for β-lactamase–producing strains, regardless of the results of susceptibility testing. Antimicrobial susceptibility patterns for *M. catarrhalis* have remained relatively stable. However, the detection of macrolide- and tetracycline-resistant isolates, thus far confined to China and the Asia Pacific region, demands continued vigilance.[106–111]

Most infections caused by *M. catarrhalis* can be treated with oral antibiotics. The organism is generally susceptible to amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tetracyclines, oral cephalosporins (e.g., cefixime, cefpodoxime, cefprozil, cefaclor, cefuroxime, and others), macrolides (e.g., azithromycin, clarithromycin), and fluoroquinolones. *M. catarrhalis* is also uniformly susceptible to ticarcillin, piperacillin, second- and third-generation cephalosporins, and aminoglycosides. *M. catarrhalis* is resistant to penicillin, ampicillin, vancomycin, and clindamycin.

Vaccines to prevent *M. catarrhalis* infections are not yet available, but several candidate vaccine antigens are under active investigation.[112–114]

OTHER *NEISSERIA*

N. meningitidis and *N. gonorrhoeae* have long been recognized as the pathogenic *Neisseria.* Other *Neisseria* spp. are common components of the normal flora of the upper respiratory tract of humans and are often called commensal *Neisseria.* Table 213.2 lists several biochemical and growth characteristics used to distinguish among various *Neisseria* spp. and *M. catarrhalis.* Commensal *Neisseria* spp. lack several virulence factors, including pili, opacity-associated (Opa) proteins, and the H8 antigen, that are expressed by meningococci and gonococci and have an altered lipid A structure compared with that in the LOS of pathogenic *Neisseria.*[115,116] However, commensal *Neisseria* express a variety of surface antigens that share homology with antigens of *N. meningitidis* and *N. gonorrhoeae.*[117,118] Immune responses to cross-reactive antigens on commensal *Neisseria*, particularly *N. lactamica*, may contribute to acquisition of natural immunity to *N. meningitidis,* and this characteristic is under evaluation as a potential approach to vaccine development.[119–121]

Neisseria spp. such as *N. sicca, N. subflava, N. cinerea, N. lactamica,* and others occasionally cause invasive infections in humans. These infections, documented primarily by individual case reports, include meningitis, endocarditis, bacteremia, ocular infections, pericarditis, empyema, peritonitis, septic arthritis, bursitis, and osteomyelitis. *N. cinerea* appears to have a propensity to cause ocular infections in young children.

N. weaveri (formerly Centers for Disease Control and Prevention group M5) is a component of the normal oropharyngeal flora of dogs and is an important cause of infection in dog bite wounds in humans. These infections are occasionally associated with bacteremia.

Many of these infections have been treated successfully with penicillin and ampicillin. However, isolates of *Neisseria* spp. have shown increased resistance to penicillin, so susceptibility testing should be performed on isolates that cause invasive infections, and the results should be used to guide antimicrobial therapy.

Neisseria are naturally competent for uptake of DNA. Genetic recombination occurs among bacteria that make up the complex flora of the upper respiratory tract. Virulence determinants are exchanged with pathogenic *Neisseria*, genes encoding altered penicillin-binding proteins are passed between species, and extensive interspecies recombination of a variety of genes occurs in vivo.[122–126] These observations have important implications in the role of acquisition of antibiotic resistance and evolution of pathogens in the human respiratory tract. Indeed, interspecies transfer of *penA* genes from commensal *Neisseria* is an important mechanism of acquisition of penicillin resistance of *N. gonorrhoeae* and *N. meningitidis.*[122]

OTHER *MORAXELLA*

Bacteria of the genus *Moraxella* are normal commensals of the human upper respiratory tract and are occasionally recovered from the skin and urogenital tract. Species are generally differentiated biochemically. Table 213.3 shows biochemical reactions and growth characteristics used to distinguish among several *Moraxella* spp.

Moraxella spp. other than *M. catarrhalis* are unusual pathogens in humans. Several reports have emphasized the role of other *Moraxella* as ocular pathogens. These bacteria cause conjunctivitis, keratitis and, rarely, endophthalmitis.[127–129] Most episodes of *Moraxella* keratitis have predisposing local ocular pathology. Healing may take weeks to months. *Moraxella* spp. are susceptible to all conventional topical ocular antibiotics.

Case reports have established *Moraxella* spp. as unusual causes of invasive infections in humans, including endocarditis, bacteremia, septic arthritis, purulent pericarditis, cellulitis, and meningitis. Patients who experience meningitis caused by *Moraxella* spp. have a high frequency of inherited and acquired complement deficiencies, and these should be investigated after recovery.[130]

Antimicrobial susceptibility should be performed on isolates recovered from normally sterile sites, but *Moraxella* spp. are generally susceptible to penicillins and cephalosporins.

KINGELLA

History and Microbiology

Current taxonomic classification schemes place *Kingella* in the family Neisseriaceae. *Kingella* are short gram-negative cocci to medium-sized rods with tapered ends. Four species have been identified: *K. kingae, K. indologenes, K. denitrificans*, and *K. oralis. Kingella* spp. are recovered from the human respiratory tract and have previously been recognized as rare causes of human disease. However, in the past 2 decades infections caused by *Kingella* have been recognized with increasing frequency.[131]

The increase in the recognition of *Kingella* infections is a result of several factors. Because the bacterium is slow growing and fastidious, special attention by the microbiology laboratory is often required to isolate the organism. Another factor is that the bacterium has likely been misidentified as *Moraxella* and other *Neisseria* by many laboratories. Finally, the development and application of PCR-based diagnostic testing, along with an elevated index of suspicion, has been important in recognizing this emerging pathogen in children.[131–135] *K. kingae*, the most common human pathogen of the *Kingella* spp., grows on blood and chocolate agar but fails to grow on MacConkey agar. The bacterium has a tendency

TABLE 213.2 Biochemical and Growth Characteristics of *Neisseria* and *Moraxella catarrhalis*

	PRODUCTION OF ACID FROM										
SPECIES	**GLUCOSE**	**MALTOSE**	**SUCROSE**	**LACTOSE (ONPG)**	**FRUCTOSE**	**H_2S[a]**	**OXIDASE**	**EXTRA CO_2**	**GROWTH AT 22°C**	**POLYSACCHARIDE[b]**	**PIGMENT[c]**
N. gonorrhoeae	+	—	—	—	—	—	+	VI	—	NG	—
N. meningitidis	+	+	—	—	—	—	+	I	—	NG	—
N. lactamica	+	+	—	+	—	+	+	v	v	—	+ Y
N. sicca	+	+	+	—	+	+	+	—	+	+	— (slY)
N. subflava	+	+	v	—	v	+	+	—	+	v	+ Y
N. mucosa	+	+	+	—	+	+	+	—	+	+	– (slY)
N. flavescens	—	—	—	—	—	+	+	—	+	+	+ Y
N. cinerea	—	—	—	—	—		+	—	v	—	Grayish
N. polysaccharea	+	+	—	—	—		+		—	+	— (slY)
N. elongata	—	—	—	—	—		+		+	—	Grayish/slY
N. weaveri	—	—	—	—	—	+	+		+	—	— (slY)
M. catarrhalis	—	—	+	—	—	+	+	—	v	—	Grayish

[a]With lead acetate paper.
[b]Synthesis of polysaccharide from 5% sucrose.
[c]On Loeffler slant.
CO_2, Carbon dioxide; *H_2S,* hydrogen sulfide; *I,* important for growth; *NG,* no growth; *ONPG,* *o*-nitrophenol-β-D-galactopyranoside; *sl,* slightly; *VI,* very important for growth; *v,* variable; *Y,* yellow.
From Gröschel DM. Moraxella catarrhalis and other gram-negative cocci. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. *4th ed. New York: Churchill Livingstone; 1995:1926.*

TABLE 213.3 Laboratory Procedures Useful for Identification of *Moraxella, Oligella, Moraxella*-like Organisms, and *Kingella*

SPECIES	MOTILITY	OXIDASE	CATALASE	OXIDATION/ FERMENTATION GLUCOSE	SERUM REQUIRED	UREASE	INDOLE	NITRATE	PHENYLALANINE	GELATIN	ASSIMILATION OF ACETATE	GROWTH ON MACCONKEY AGAR
M. catarrhalis	—	+	+	—	—	—	—	—	—	—	v	v
M. lacunata	—	+	+	—	+	—	—	+	v	v	—	—
M. nonliquefaciens	—	+	+	—	v	—	—	+	—	—	—	—
M. osloensis	—	+	+	—	—	—	—	v	v	—	+	v
M. phenylpyruvica	—	+	+	—	—	+	—	v	+	—	v	v
M. atlantae	—	+	+	—	+	—	—	—	—	—	v	+
O. urethralis	—	+	+	—	—	—	—	—	+	—	v	+
Neisseria weaveri (M5)	—	+	+	—	—	—	—	—	+	—	—	—
N. elongata (M6)	—	+	—	—	—	—	—	+	—	—	v	v
K. kingae[a]	—	+	—	F[b]	—	—	—	—	—	—		v
K. indologenes	—	+	—	F	—	—	+	—	—	—		
K. denitrificans	—	+	—	F	—	—	—	+	—	—		—

[a]Most strains hemolytic on blood agar.
[b]May take 3 or more days; some strains require serum supplement.
F, Fermentation; *v,* variable.
From Gröschel DM. Moraxella catarrhalis and other gram-negative cocci. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. *4th ed. New York: Churchill Livingstone; 1995:1926.*

to resist decolorization and may therefore sometimes be mistaken for a gram-positive organism. It is oxidase positive, produces acid from glucose and maltose, and lacks catalase, urease, and indole. Table 213.3 lists several characteristics that distinguish *Kingella* from related bacteria. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and 16S ribosomal DNA sequencing are also accurate means of identifying *Kingella* spp.

With the recognition of *K. kingae* as a human pathogen, recent work is beginning to shed light on the genetic population structure of the species and on mechanisms of pathogenesis. Genotyping of clinical isolates suggests the presence of substantial genetic heterogeneity with a subset of clones that appear to be associated with invasive disease.[136–139] Key virulence factors include type IV pili, a polysaccharide capsule, and a "repeat-in-toxin" toxin with wide cellular specificity has been identified in *K. kingae*.[140–146]

Epidemiology and Respiratory Tract Colonization

K. kingae frequently colonizes the throats of young children. Blood agar medium with vancomycin 2 μg/mL is used to improve isolation from the oropharynx. The organism has not been recovered from cultures of the nasopharynx. The highest rate of colonization is observed in children ages 6 months to 4 years, which corresponds to the peak age incidence of invasive disease. Infants younger than 6 months are not colonized. This pattern of colonization parallels that of other respiratory tract pathogens, such as *M. catarrhalis* and *S. pneumoniae*, which show low rates of colonization in the neonatal period (presumably as a result of maternal antibodies), followed by higher rates of colonization and infection in infancy and childhood, with a declining incidence of infection in adulthood. *K. kingae* is transmitted person to person, and outbreaks of invasive infection have been observed in daycare centers.[139,142–153]

In studies of the microbiology of the human oral cavity a bacterium that resembled *Eikenella corrodens* was recovered frequently from dental plaque.[154] On the basis of 16S ribosomal sequences, these organisms were designated as the new species *K. oralis*.[155] *K. oralis* is present in plaque or on the tooth surface in most people with or without periodontal disease.[156] The role of *K. oralis* in periodontal disease is not known at this time.

Clinical Manifestations

K. kingae is the most frequent human pathogen of the *Kingella* spp. Approximately 90% of invasive disease caused by *K. kingae* occurs in previously healthy children younger than 4 years. Underlying medical conditions are generally present in older children who experience infections caused by *K. kingae*.[157] Invasive infections have not been reported in infants younger than 6 months. Infection shows a seasonal distribution, with the rate of cases being higher in the autumn and winter months.[158] Outbreaks in daycare centers and closed communities are well documented.[143] The most common clinical manifestations of *K. kingae* disease are septic arthritis, osteomyelitis, tenosynovitis, spondylodiskitis, endocarditis, and bacteremia.[159] The clinical presentation of *K. kingae* infections is often subtle, with low-grade fever and normal levels of acute-phase reactants, emphasizing the importance of a high index of suspicion.[131,160–162]

Skeletal Infections

K. kingae shows a propensity to cause osteoarticular infections in young children and is now recognized as a common cause of such infections.[132,163–167] The disease most frequently presents as septic arthritis of large weight-bearing joints, especially the knee and ankle. Gram staining of the joint fluid is usually negative. The diagnosis is made by recovering the organism from culture of joint fluid. Inoculating blood culture bottles with joint fluid substantially enhances the likelihood of recovering the organism, compared with direct inoculation of agar plates, and should be done routinely with joint fluid samples from young children.[168,169] PCR-based assays have revealed that *K. kingae* is a more common cause of osteoarticular infection than is revealed by culture.[131,132,150,170,171] Osteomyelitis caused by *K. kingae* most frequently involves the bones of the lower extremity. The onset is insidious, and the diagnosis is often delayed. Hematogenous invasion of the intervertebral disk by *K. kingae* is observed most commonly in the lumbar intervertebral spaces but can occur at any level.[150,166,172]

Endocarditis

In contrast to other clinical manifestations of *Kingella* infections, endocarditis can be seen at all ages, including school-age children and adults. Endocarditis has involved native and prosthetic valves. Although many cases of endocarditis occur in those who have preexisting valvular disease, *Kingella* can cause endocarditis on normal valves as well. Cases of endocarditis can be caused by *K. denitrificans* and *K. indologenes* as well as *K. kingae*. *Kingella* spp. are part of the so-called HACEK group of organisms, which include fastidious bacteria capable of causing endocarditis. The HACEK group consists of the following: *Haemophilus parainfluenzae*; three *Aggregatibacter* spp.—*A. (Haemophilus) aphrophilus, A. (Haemophilus) paraphrophilus*, and *A. (Actinobacillus) actinomycetemcomitans; Cardiobacterium hominis; Eikenella corrodens*; and *K. kingae.* The difficulty in recovering and identifying *K. kingae* in blood cultures frequently results in a delay in the diagnosis, which may account for the relatively high rate of morbidity seen with *Kingella* endocarditis. Because of the serious nature of *Kingella* endocarditis, all patients with *Kingella* bacteremia should be carefully evaluated for the presence of endocarditis.

Bacteremia

Approximately 50% of children with *K. kingae* bacteremia have a concomitant focal source, such as the skeletal system. The remainder have occult bacteremia. The presumed source of the bacteremia is the respiratory tract.

Other Infections

Kingella spp. have been documented by case reports to cause infections in a variety of sites, including pneumonia, epiglottitis, meningitis, soft tissue infections, and ocular infections.

Therapy

Kingella spp. are susceptible to a wide variety of penicillins and cephalosporins, although rare isolates of β-lactamase–producing *K. kingae* have been identified.[173–175] Disease-associated isolates should be tested for antimicrobial susceptibility; if the isolate is susceptible, a penicillin or cephalosporin should be used. Other agents with in vitro activity include aminoglycosides, trimethoprim-sulfamethoxazole, tetracycline, erythromycin, and fluoroquinolones. Although specific guidelines for treatment are not yet established, a rational approach is to administer antibiotic therapy for 2 to 3 weeks for *K. kingae* septic arthritis, for 3 to 6 weeks for osteomyelitis, and 3 to 12 weeks for spondylodiskitis. Children are often treated initially with a β-lactam antibiotic intravenously, followed by oral administration once the clinical condition has improved.[131] Attempts to stop outbreaks in daycare centers with prophylactic antimicrobial drugs, such as rifampin, have met with mixed success.[143]

The drugs of choice for the treatment of endocarditis caused by *Kingella* spp. (and other HACEK organisms) are the third-generation cephalosporins cefotaxime or ceftriaxone.[176] The duration of treatment for native valve endocarditis should be 3 to 4 weeks, and the duration of treatment for prosthetic valve endocarditis should be 6 weeks.

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The complete reference list is available online at Expert Consult.

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SHORT VIEW SUMMARY

Microbiology and Epidemiology

- *Vibrio cholerae* is a curved motile gram-negative bacillus.
- *V. cholerae* is a noninvasive intestinal pathogen.
- *V. cholerae* O1 and O139 serogroup organisms are the causes of epidemic cholera.
- Non-O1 and non-O139 *V. cholerae* can cause isolated cases of usually mild gastroenteritis.
- Cholera results from secretory diarrhea caused by the actions of cholera toxin (CT) in the intestine.
- CT is an adenosine diphosphate–ribosylating enzyme that leads to chloride, sodium, and water loss from intestinal epithelial cells.
- *V. cholerae* has an aquatic reservoir, particularly in brackish estuarine water.
- Fecal-oral transmission is associated with unsafe water and inadequate sanitation.
- There are an estimated 2 to 3 million cholera cases, resulting in approximately 100,000 deaths each year.
- The current cholera global pandemic began in 1961 and represents the seventh in the historical record.
- Cholera is now endemic in more than 50 countries.
- Cholera can lead to explosive epidemics and outbreaks.
- The current pandemic is caused by *V. cholerae* O1 El Tor organisms, with the largest burdens in South Asia, sub-Saharan Africa, Haiti, and Yemen.

Clinical Manifestations

- Acute severe watery diarrhea can result in death from dehydration within 6 to 12 hours of onset of clinical symptoms.
- Bowel movements during cholera can become progressively more watery, eventually resembling rice water with a fishy odor.
- Vomiting, ileus, and muscle cramps are common.
- The presence of fever should prompt consideration of additional diagnoses.
- Complications of cholera largely are those resulting from hypotension and hypoperfusion and may include acute renal tubular necrosis and stroke, usually in older individuals. Aspiration pneumonia from vomiting may also occur. Death from cholera almost always results from dehydration.

Diagnosis

- Cholera should be considered when an adult or child 5 years of age or older develops severe dehydration or dies of acute watery diarrhea in any area or when an individual 2 years of age or older develops acute watery diarrhea in an area known to be endemic for cholera.
- Rapid antigen tests are available, including dipstick stool assays.
- Confirmatory microbiologic culturing permits definitive identification and assessment of antimicrobial resistance profiles.

Management

- The cornerstone of management is rapid assessment of the degree of dehydration, followed by prompt fluid restoration (see Table 214.2) and vigorous monitoring and matching of ongoing fluid losses.
- Severe dehydration (>10% fluid loss) may require greater than 100 mL/kg of fluid replacement to restore euvolemia, ideally administered within the first 3 to 4 hours of clinical presentation (in 6 hours for children younger than 1 year).
- Additional stool losses should be matched at approximately 10 to 20 mL/kg per diarrheal stool or vomiting episode.
- Moderate dehydration (5%–10% fluid loss) may require 75 to 100 mL/kg of fluid resuscitation within the first 3 to 4 hours of clinical presentation. Patients with no or mild dehydration may be treated with oral rehydration solution (ORS) alone or liquid ad libitum.
- Intravenous fluids are indicated in patients with severe dehydration and in patients unable to ingest adequate ORS. Lactated Ringer solution supplemented with 5% dextrose (ideally supplemented with additional potassium) is an optimal choice for patients with cholera, although intravenous normal saline or normal saline supplemented with dextrose (and potassium) can also be used.
- Oral rehydration treatment should be encouraged in all patients able to ingest, regardless of degree of dehydration and regardless of potential ongoing administration of intravenous fluid.
- ORS can be made in resource-limited settings by adding ½ tsp of table salt to 6 tsp of table sugar in 1 L of safe water, ideally supplemented with locally available potassium sources such as coconut milk, bananas, or orange juice.
- Antibiotics play a secondary role in treatment of patients with cholera; usually a macrolide or fluoroquinolone antibiotic is administered. Tetracyclines can be used in nonpregnant patients older than 7 years in areas with confirmed susceptibility (see Table 214.3).
- Antibiotics decrease the duration of diarrhea and may limit secondary transmission.
- Infants younger than 6 months should also receive 10 mg of zinc each day for 10 days; children 6 months to 5 years of age should receive 20 mg of zinc each day for 10 days.

Prevention

- Control primarily focuses on surveillance, case detection, fluid resuscitation and management, vaccination, and provision of safe water and adequate sanitation.
- A number of oral killed cholera vaccines are currently commercially available and approved by the World Health Organization for global use (see Table 214.4).
- A live attenuated oral cholera vaccine is approved by the US Food and Drug Administration.
- Additional cholera vaccines are under development.

Cholera is an acute and often severe watery diarrheal disease that is among the most rapidly fatal infectious diseases of humans. Previously well individuals infected with *Vibrio cholerae,* the comma-shaped gram-negative rod that causes this disease, can die from dehydration in less than a day. Two epidemiologic features of cholera also distinguish it from other diarrheal diseases. First, cholera can appear in explosive epidemics, particularly among populations that lack access to clean water and adequate sanitation and who are immunologically naïve to the organism. Second, cholera occurs in pandemics, where one strain of *V. cholerae* spreads around the world in essentially clonal fashion. The ongoing seventh pandemic of cholera began in 1961 on the Indonesian island of Sulawesi. Cholera is thought to have afflicted human populations for centuries, if not longer, particularly on the Indian subcontinent.[1] Throughout the centuries, cholera epidemics have influenced human history and killed millions. Studies of cholera and *V. cholerae* have had a broad impact in several scientific fields, and rehydration strategies developed for the treatment of cholera have been extremely useful for treatment of other diarrheal diseases.[2] Snow's studies in London in the 1840–50s linking the spread of cholera to the water supply are considered central to the establishment of the field of epidemiology. Koch isolated *V. cholerae* from patients with cholera in Calcutta (Kolkata) in 1883; however, Pacini likely detected the organism in 1854 in the intestines of cholera victims in Florence.[3]

CLASSIFICATION AND GENOMICS

V. cholerae organisms are oxidase-positive facultative anaerobes that can be identified using a variety of biochemical tests and selective media.[4] The organisms are characteristically highly motile and bear a unipolar sheathed flagellum. *V. cholerae* is classified into more than 200 serogroups; differences in the composition of the O-specific polysaccharide (OSP) chains of lipopolysaccharide (LPS) molecules establish the chemical basis for the distinct antigenicity of the serogroups. The O1 serogroup of *V. cholerae* was the only serogroup associated with epidemic cholera until 1992, when *V. cholerae* O139 emerged. However, some non-O1, non-O139 *V. cholerae* serogroups have given rise to gastroenteritis outbreaks, but not to large epidemics. In general, the majority of *V. cholerae* isolates from the environment including those of *V. cholerae* O1 do not produce the virulence factors, such as cholera toxin (CT), that are required to cause cholera. The O1 serogroup is divided into two principal serotypes, Inaba and Ogawa, which differ by the presence of a 2-*O*-methyl group in the nonreducing terminal sugar of the Ogawa OSP, which is absent from Inaba OSP.[5] Switching of serotypes during epidemics is well described; the driving forces underlying switching are not known, but they do not appear to be random.[6] CT-producing (toxigenic) O1 serogroup strains are also divided into two biotypes based on a number of microbiologic and biochemical differences.[4] The classic biotype of *V. cholerae* O1 caused the second[7] and sixth cholera pandemics and probably the earlier and intervening pandemics as well, whereas the El Tor biotype of *V. cholerae* O1 is the cause of the ongoing seventh cholera pandemic. The classic biotype of *V. cholerae* is now likely extinct.

V. cholerae, similar to many other bacterial enteric pathogens, is classified as a Gammaproteobacteria. In contrast to most Gammaproteobacteria such as *Escherichia coli,* whose genomes consist of single circular chromosomes, the genome of *V. cholerae* is multipartite. The *V. cholerae* genome (and that of all *Vibrio* spp.) is unequally divided between two circular chromosomes.[8] Its large chromosome (≈3 kb) contains the vast majority of the essential genes as well as genes implicated in pathogenicity, whereas the small chromosome (≈1 kb) is enriched for genes of unknown function. A plausible explanation for the evolution of the bipartite *V. cholerae* genome is that a remote ancestor of *V. cholerae* (and all vibrios) acquired a large plasmid that subsequently obtained essential genes and other chromosome-like features.[9] Acquisition of virulence-linked genes from other (unknown) donor organisms via lateral gene transfer has played a central role in the evolution of pathogenic *V. cholerae*.[10] For example, the genes encoding CT are encoded within a bacteriophage that infected and integrated its DNA into the genome of a nontoxigenic precursor of toxigenic *V. cholerae* O1.

PATHOGENICITY

V. cholerae has no known natural vertebrate host besides humans. However, the pathogen is not dependent on humans for its propagation; *V. cholerae* grows in brackish estuaries and coastal seawaters, often in close association with copepods or other zooplankton. *V. cholerae* can also grow in water of lower salinity when it is warm and adequate organic material is available.[11] Because patients with severe cholera can excrete up to 20 L of "rice water" stool laden with ≈10^9 *V. cholerae* cells/mL per day, humans greatly facilitate the propagation and dissemination of the pathogen. Humans usually become infected with *V. cholerae* after ingestion of contaminated water or food, although there may be direct fecal-oral spread between people that does not involve water or food. The infectious dose in volunteer studies carried out in the United States is relatively high.[12] Low stomach pH is known to lower the infectious dose in volunteers; additional factors likely impact the infectious dose as well.[12] Because the frequency of hypochlorhydria (caused by chronic *Helicobacter pylori* infection) in cholera endemic regions is often high, the *V. cholerae* infectious dose may be considerably lower in these regions.

After passage through the stomach, *V. cholerae* has the unusual capacity to survive and multiply in the small intestine. Although many processes and gene products contribute to the organism's ability to colonize the small intestine,[13] toxin coregulated pili (TCP) are the most critical identified factors primarily dedicated to mediating colonization.[14] The major subunit of these pili is encoded by *tcpA,* the first gene of the *tcp* operon. Most of the other genes of the operon encode the machinery for the biogenesis of TCP,[15] but *tcpE* encodes a protein found at the tip of the pilus, and *tcpF* encodes a protein secreted by the TCP assembly apparatus that is also required for colonization.[16] TCP are thought to promote *V. cholerae* intestinal colonization in at least three ways: TCP (1) mediate bacterium-bacterium interactions, enabling microcolony formation within the small intestine; (2) confer protection against toxic factors produced in the intestine; and (3) promote attachment to the intestinal epithelium.[15] The *tcp* operon is found within the TCP pathogenicity island, a chromosome segment ≈41 kb that also encodes additional virulence-associated factors,[17,18] which appears to have been acquired via horizontal gene transfer by a nonpathogenic ancestor of contemporary *V. cholerae*. In addition, the type VI secretion system may be induced in the intestine, promoting colonization potentially by enabling interactions with the microbiota.[19]

While colonizing the small intestine, *V. cholerae* secretes CT, the AB_5 subunit–type protein toxin that causes the secretory diarrhea that is characteristic of cholera. The sufficiency of CT to elicit cholera-like diarrhea was demonstrated experimentally; volunteers fed as little as 5 µg of purified CT developed severe diarrhea indistinguishable from cholera.[20] Thus cholera is a toxin-mediated disease, and *V. cholerae* is a noninvasive mucosal pathogen. The pentameric B subunit of CT binds to GM1, a glycosphingolipid on the surface of epithelial cells. The CT-GM1 interaction targets the toxin through a retrograde trafficking pathway from the plasma membrane to the Golgi apparatus and endoplasmic reticulum and then to the cytoplasm.[21] In the cytoplasm, the enzymatic A subunit of CT mediates the transfer of adenosine diphosphate ribose from nicotinamide adenine dinucleotide to the G protein that regulates adenylate cyclase activity, leading to elevation in the intracellular cyclic adenosine monophosphate (cAMP) concentration.[22] Increased cAMP levels promote chloride ion (Cl^-) secretion by intestinal crypt cells and decreased absorption by villous cells. Water moves from epithelial cells into the bowel lumen to maintain osmolality, and diarrhea results when the resorptive capacity of the remainder of the gut is exceeded. Additional actions of CT (besides dysregulation of adenylate cyclase) likely contribute to the secretory response underlying cholera.[22] Also, other factors besides CT contribute to the diarrheal response because volunteers who ingest *V. cholerae* deficient for *ctxA* often develop mild diarrhea.[23]

The genes encoding CT, *ctxA* and *ctxB (ctxAB),* are not part of the ancestral *V. cholerae* genome; instead, these genes are embedded in the genome of CTXΦ, a filamentous bacteriophage that infected *V. cholerae,* rendering it toxigenic.[24] Of note, the receptor for this phage is TCP, suggesting that a TCP+, *ctxAB*– strain was the precursor of contemporary pathogenic *V. cholerae*. The intricate interdependence of these two mobile elements—the TCP pathogenicity island and the CTX prophage—extends

even further; ToxT, a key transcriptional activator of *ctxAB* expression, similar to the *tcp* operon, is encoded within the TCP island. Mobile genetic elements such as phages, plasmids, integrative conjugative elements, and pathogenicity islands have played critical roles in the evolution of most bacterial pathogens.

Many of the key virulence factors of *V. cholerae* such as CT and TCP are not constitutively expressed. Instead, fairly shortly after *V. cholerae* is ingested, as yet undefined host signals trigger the coordinated expression of the principal virulence factors of *V. cholerae*.[14] A membrane-embedded transcription activator, ToxR, sits at the top of a signaling cascade that promotes expression of many of the pathogen's virulence-associated genes.[25] There may be a specific temporal order of *V. cholerae* gene expression during expression.[26] Furthermore, as the pathogen passes to more distal parts of the intestine, expression of late genes promotes *V. cholerae* survival outside the human host and engenders a transient hyperinfectious state, which promotes transmission of the organism to new hosts.[27,28] Additional regulatory pathways such as quorum sensing systems, which modify gene expression according to bacterial density and the concentrations of autoinducer molecules, modulate expression of *V. cholerae* virulence. Notably, it has been suggested that crosstalk between the quorum sensing molecules of the microbiota and quorum regulation of *V. cholerae* impact the pathogen's capacity to colonize the intestine.[29]

EPIDEMIOLOGY

Clinical descriptions of cholera-like illness exist in ancient Sanskrit texts, and cholera has likely been endemic to the Ganges delta regions for centuries, if not longer. Seven cholera pandemics have been recorded since 1817. All the pandemics are thought to have originated in Asia, particularly from the Indian subcontinent. The El Tor O1 *V. cholerae* strain causing the ongoing seventh pandemic is evolving. Polymorphisms in the genome of the pandemic strain have been reported to have arisen in the Bay of Bengal and then to have independently spread to other continents.[30] All seven pandemics were likely caused by *V. cholerae* O1, but in 1992 *V. cholerae* O139 emerged in India and Bangladesh to become the first non-O1 serogroup to give rise to epidemic cholera. Although *V. cholerae* O139 initially caused large cholera outbreaks, this serogroup is not a significant cause of disease anywhere at the present time; additional O139 cases were never reported outside of Asia. Horizontal gene exchange appears to explain the molecular origins of *V. cholerae* O139; the genome of this novel *V. cholerae* serogroup is essentially identical to that of El Tor O1 *V. cholerae* except that the genes encoding O1 antigen biosynthesis have been replaced by genes encoding the O139 O-antigen.[31]

Cholera had been absent from the Americas for nearly a century until 1991, when a large outbreak began in Peru and rapidly spread throughout much of South and Central America. During the ensuing decade, more than 1 million cases were reported. Whole-genome–based epidemiologic analyses suggest that there were two separate introductions of El Tor O1 strains, from Africa and Asia, that gave rise to this epidemic.[32] Cholera did not reach the Caribbean during this outbreak. However, in October 2010, a severe cholera epidemic began in Haiti on the Caribbean island of Hispaniola. Since the onset of the epidemic, more than 800,000 cases and 9500 deaths have been recorded in Haiti, and focal related outbreaks and cases have occurred throughout the Caribbean basin.[33] Several lines of evidence indicate that cholera may have been introduced to Haiti by United Nations security forces from Nepal,[34,35,36] suggesting that asymptomatic carriers of *V. cholerae* can play an important role in transmitting cholera over long distances. In October 2016 a large cholera outbreak began in the capital city of Yemen, and by September 2017 almost the entire country was affected, resulting in more than 700,000 cases and 2000 deaths.[37]

The number of cholera cases reported to the World Health Organization (WHO) varies by year depending on the size and extent of outbreaks and endemic disease. In 2011 there were approximately 600,000 cases of cholera and nearly 8000 cholera-related deaths reported to the World Health Organization from 58 countries (Fig. 214.1).[38] In 2015 there were approximately 170,000 cases and 1300 deaths reported from 42 countries.[39] Due to large outbreaks, including in Yemen, these numbers increased dramatically to 1,227,391 cases and 5654 deaths reported from 34 countries in 2017.[39a] These estimates of cholera morbidity and mortality significantly underrepresent the true burden of this disease because many cases are not diagnosed or reported. More recent estimates suggest that approximately 2 to 3 million cholera cases occur annually worldwide with 100,000 deaths.[40] In the decade preceding the outbreaks in Haiti and Yemen, more than 90% of reported cholera cases were from Africa, although the burden of disease in Asia is significantly underreported. More recent analysis suggests that cholera outbreaks in Africa represent repetitive reintroductions of *V. cholerae* from Asia.[41]

CLINICAL MANIFESTATIONS

Cholera manifests as an acute, often severe watery diarrhea that can lead to death from dehydration within hours of onset. The spectrum of disease in individuals infected with *V. cholerae* includes asymptomatic intestinal colonization of *V. cholerae,* observed in individuals with preexisting immunity and mild, moderate, and severe diarrhea. In severe cholera the volume of watery diarrhea can exceed 1 L/h. Vomiting is common. In the initial stages, diarrhea may contain intestinal contents; however, as diarrhea progresses, it becomes more watery, eventually becoming clear, with flecks of white mucus (rice water stool) with a fishy odor. Individuals with cholera are usually afebrile and may be hypothermic because of severe dehydration. The extensive fluid secretion into the intestinal lumen can lead to abdominal cramping and discomfort, and the severe dehydration and perturbations in calcium and potassium levels can lead to ileus, muscle pain and spasm, and tetany. In severely dehydrated patients the skin can become mottled or darkened, reflecting the hyperviscosity and poor circulation of blood.

The clinical manifestations of severe cholera reflect the degree of dehydration. Individuals may be lethargic or unresponsive, have sunken eyes, and have markedly decreased skin turgor often leading to skin tenting (i.e., when skin is pulled back, it takes a long time for it to fall back into place). Individuals may have cold and clammy skin, have dry mucous membranes, and be anuric. Extensive loss of bicarbonate in stool and lactic acidosis secondary to compromised circulation and perfusion can lead to Kussmaul breathing.[42] The peripheral pulse is often rapid in the initial stages of dehydration; however, it can become progressively fainter, eventually becoming nonpalpable as dehydration progresses. Hypoglycemia can be severe, especially in children with limited glycogen stores, and can contribute to lethargy, seizures, and coma. Paradoxically, stool volume can decrease as the degree of dehydration increases, giving a false impression of improvement before total circulatory collapse. Cholera is an acute illness, and the complications of cholera reflect severe hypoperfusion and can include stroke, acute tubular necrosis with renal dysfunction, and aspiration pneumonia from the extensive vomiting.[43] Diarrhea is usually most prominent for 1 to 3 days.

The clinical manifestations of cholera caused by *V. cholerae* O1 and O139 are indistinguishable. Similarly the disease caused by the Ogawa and Inaba serotypes of *V. cholerae* O1 is indistinguishable. At the population level, disease caused by classic biotype *V. cholerae* O1 is more severe than that caused by O1 El Tor biotype strains. Differences in the amino-acid sequences of the CT produced by the two biotypes may account for the heightened severity of diarrhea associated with the classic biotype. So-called variant (or hybrid) strains of *V. cholerae* O1 El Tor that express classic biotype CT may be associated with more severe clinical manifestations than traditional El Tor strains, although the reason for this increased severity is unclear.[44]

Host factors likely also contribute to the clinical manifestations of cholera. For example, when *V. cholerae* is first introduced into an immunologically naïve population (e.g., into Haiti in 2010), individuals of all ages can be equally affected, and disease is often severe. In comparison, in regions where cholera is endemic, many older children and adults have preexisting immunity, and these individuals can be asymptomatically colonized or present with mild-to-moderate diarrhea.[45] Individuals with blood group O are more likely to present with severe cholera than individuals with non-O blood groups.[46] Hypochlorhydria increases the likelihood and severity of disease.[47,48] Retinol deficiency is associated with an increased risk for cholera,[49,50] and concomitant intestinal parasitic and bacterial infection can modulate anti–*V. cholerae* immune responses after cholera.[51,52] First-degree relatives of patients with cholera also have greater odds of becoming infected than



FIG. 214.1 Map of global distribution of annual incidence of cholera. Results represent mean annual incidence for the years 2014–2016. Data do not include large 2017 outbreak in Yemen *(asterisk). (Figure created by Martine Piarroux using Philcarto, http://philcarto.free.fr.)*

non–first-degree relatives in the same household, independent of blood group,[49] suggesting that other genetic factors may contribute to susceptibility, and in a candidate gene study a variant in the promoter region of *BPIFB1* (also known as *LPLUNC1*) involved in innate immune responses was associated with cholera.[53,54] In a genome-wide study, host susceptibility to cholera was also associated with potassium channel genes involved in cAMP-mediated Cl^- secretion and for components of the innate immune system involved in nuclear factor kappa B signaling.[55]

DIAGNOSIS

Cholera should be considered in any area of the world when an individual 5 years of age or older develops severe dehydration or dies as a result of acute watery diarrhea and when an individual 2 years of age or older develops acute watery diarrhea in an area of the world known to be endemic for cholera.[56] Microbiologic analyses of stool samples permit confirmation of *V. cholerae* and determination of antimicrobial susceptibility profiles; however, once cholera is known to be endemic in an area and the antimicrobial susceptibility profile of the outbreak strain is known, routine stool culture from each individual is not indicated. *V. cholerae* can be isolated from stool by using selective media such as taurocholate tellurite gelatin agar or thiosulfate citrate bile sucrose agar, and recovery of organisms can be enhanced by enrichment of stool in alkaline peptone water.[57] Serogroup and serotype identification are determined with specific antibodies.[58] Darkfield microscopy can be used to rapidly evaluate whether watery stools contain the characteristic "shooting star" motility of vibrios, which can be inhibited by the action of specific antibodies.[59] A number of immunoassays that can detect *V. cholerae* LPS or CT or both directly in stool have been developed.[60] These assays are particularly helpful when included in surveillance systems in areas of the world at risk for cholera. During laboratory evaluation and under field conditions, the sensitivity and specificity of these assays have ranged from 60% to 100%.[60] A commercially available PCR panel for detection of gastrointestinal pathogens in stool includes *Vibrio cholerae* (Biofire FilmArray).

TREATMENT

The primary focus of treating patients with cholera is fluid management. Patients with suspected cholera should be rapidly restored to euvolemia, and ongoing fluid losses need to be matched. Algorithms for rapid assessment of fluid status in any setting, including in sites lacking electricity or laboratory support, are shown in Table 214.1. Although patients with severe cholera or who are unresponsive require administration of intravenous fluids in the initial stages of treatment, many patients with less severe cholera and who are alert can be managed with oral rehydration therapy alone.

Management of patients with cholera does not usually require any laboratory analysis, although focused laboratory analysis can assist clinical decisions in complicated cases including in patients who do not respond to fluid replacement therapy or have ongoing coma, seizures, or severe ileus. In these cases, focused testing for persistent hypoglycemia, hyponatremia, hypernatremia, hypokalemia, hypocalcemia, renal dysfunction, and degree of hemoconcentration can assist in management.[61]

Patients with severe dehydration from cholera have greater than a 10% deficit of their total fluid volume and often require greater than 100 mL/kg of fluid via the intravenous route within 3 hours of presentation (6 hours in children younger than 1 year) to restore euvolemia, in addition to matching ongoing losses. Once patients are mentally alert, oral rehydration fluid should supplement administration of intravenous fluids. Intravenous therapy should be discontinued once the patient can maintain euvolemia through oral ingestion alone, usually within 3

TABLE 214.1 How to Approach Rehydration in Patients With Suspected Cholera

		DEGREE OF DEHYDRATION		
		None (<5%)	**Some (5%–10%)**	**Severe (>10%)**
Assess degree of dehydration	Mentation	Alert	Restless, irritable	Lethargic or unconscious
	Eyes	Normal	Sunken	Sunken
	Skin turgor	Normal recoil	Slow recoil	Very slow recoil (>2 s)
	Pulse	Normal	Rapid, low volume	Weak or absent
	Thirst	Drinks normally	Thirsty, drinks eagerly	Drinks poorly or unable to drink
Approach to rehydration[a]	Fluid replacement	Ongoing losses only	75 mL/kg in addition to ongoing losses	>100 mL/kg in addition to ongoing losses
	Preferred route of administration	Oral[b]	Oral or intravenous	Intravenous
	Timing	Usually guided by thirst	Replace fluids over 3–4 h	As rapidly as possible until circulation is restored; complete the remainder of fluids within 3 h
	Monitoring	Observe until assured ongoing losses can be adequately replaced by ORS	Observe every 1–2 h until all signs of dehydration resolve and patient urinates	Once circulation is established, monitor every 1–2 h

[a]Patients with comorbid conditions including severe malnutrition and significant complications, infants, and elderly patients may require adjustments from these standard guidelines for the inpatient treatment of severely malnourished children. See references 56, 63, and 77.
[b]If losses are greater than 10 mL/kg/h, it may not be possible to successfully initially use oral therapy.
The Cholera Outbreak Training and Shigellosis Program (www.cotsprogram.com) is an excellent resource that provides free online information regarding the management of patients with cholera based on World Health Organization standards.
ORS, Oral rehydration solution.
Modified from Harris JB, Larocque RC, Qadri F, et al. Cholera. Lancet. *2012;379:2466–2476.*

to 6 hours of presentation, and subsequent fluid support should be delivered solely through the oral route. If intravenous fluids are not available, an orogastric or nasogastric tube may be inserted in unresponsive patients to permit administration of appropriate oral fluid containing both glucose and sodium in equimolar amounts.

Patients with the most severe form of the disease, cholera gravis, may require on average 200 mL/kg of fluid replacement within the first 24 hours and may require as much as 350 mL/kg in this period.[56,61,62,63] Ongoing fluid losses may exceed 10 to 20 mL/kg/h, and output should be matched by fluid replacement. A cholera cot (Fig. 214.2) is a simple and inexpensive device that allows care workers to monitor fluid losses, enabling accurate matching fluid replacement. If a cholera cot is not available, fluid replacement should be guided by estimating 10 to 20 mL/kg of body weight for each diarrheal stool or episode of severe vomiting during cholera. Severely malnourished children with cholera require special clinical care.[64]

Patients with some dehydration often have a fluid deficit of 5% to 10% and may be irritable and restless but are not lethargic or unconscious. Euvolemia should be reestablished within 3 to 4 hours of presentation and often requires at least 75 mL/kg of fluid in addition to matching ongoing losses. Individuals with less than 5% dehydration will usually be mentally alert and able to drink normally and should largely be treated with oral fluid management.

When intravenously administered fluids are indicated, lactated Ringer solution (LRS), ideally supplemented with 5% dextrose (D5LR), is considered optimal among the most commonly available fluids.[61] D5LR contains not only sodium chloride and glucose but also potassium and bicarbonate to partially match ongoing losses, although the amount of potassium present in LRS is insufficient to match the ongoing losses associated with cholera (Table 214.2). Although normal saline can also be administered with the primary goal of maintaining circulatory support, the absence of potassium, bicarbonate, and glucose (unless supplemented) make it a less ideal agent. Normal saline with 5% dextrose should be used only if D5LR is not available, and D5LR should ideally be supplemented with additional potassium if available. In highly endemic areas, local manufacturers will sometimes produce cholera saline, often known as Dhaka solution, which contains increased potassium and bicarbonate and glucose compared with LRS and which was developed to optimally match fluid losses during cholera.[61]

Oral rehydration solutions (ORSs) are based on the delivery of equimolar concentrations of sodium and glucose in the small intestine to maximize absorption by relying on a different absorption pump than that affected by CT.[65] WHO initially recommended that patients with cholera be treated with an ORS with an electrolyte profile that closely matched that of cholera stools; however, with the broader use of ORS to prevent dehydration-associated deaths and morbidity from all causes of gastroenteritis, that formula has been modified to include less salt. This lower-osmolarity formula more closely reflects the electrolyte profile of stool associated with all-cause gastroenteritis globally. Lower osmolarity ORS limits the risk of hypernatremia in young children with non–cholera-associated dehydration, and although it increases the likelihood of subclinical hyponatremia in treated patients with cholera, this degree of hyponatremia does not appear to be clinically significant at a population level.[66] If available, rice-based ORS formulas can decrease stool losses in severe cholera and duration of diarrhea.[67] During emergency situations, ORS can be made by mixing ½ tsp of table salt with 6 tsp of table sugar in 1 L of safe water.[68] Potassium replacement can occur through ingesting locally available potassium sources such as coconut milk, bananas, or orange juice.

Unresponsive patients or patients with persistent hypoglycemia should receive 0.25 to 0.50 g/kg of intravenous glucose, and glucose should be continued until the patient is able to drink and eat to replenish glucose stores.[69] Potassium and bicarbonate levels should be evaluated in patients with severe or persistent ileus because these individuals may require supplemental potassium replacements. Rapid administration of intravenous fluids, especially fluids containing bicarbonate, can lead to calcium shifts that can precipitate tetany, which can be treated with intravenously administered calcium gluconate.

Despite the role that antibiotics play in many infectious diseases, their role in the treatment of a patient with cholera is secondary. Antibiotics should be administered to patients with moderate or severe dehydration from cholera.[70] The administration of effective antibiotics reduces the volume of diarrhea by approximately 50% and shortens the duration of diarrhea.[71,72] Because patients with moderate or severe dehydration from cholera consume the largest amounts of fluid in resource-limited settings, targeting antibiotic treatment to these individuals can maximize use of limited supplies. Appropriate antimicrobial therapy can also limit the shedding of organisms and may decrease the risk of ongoing transmission.[71,72] Increasing antimicrobial resistance in *V. cholerae* is occurring globally, and the seventh pandemic strains currently circulating contain an integrative conjugative element that mediates resistance to multiple antibiotics. Individuals with cholera who are treated with an antimicrobial agent should receive a macrolide such as azithromycin or a fluoroquinolone antibiotic based on local resistance profiles (Table 214.3). Resistance to tetracyclines is common, so these agents (including doxycycline) should be used only during outbreaks when antimicrobial susceptibility has been confirmed, and tetracyclines should, in general, not be used in pregnant women and children younger than 8 years.[73] Macrolide antibiotics are of particular use in the treatment of women of childbearing age and in young children.

Breastfeeding should be continued for infants with cholera in addition to other avenues of fluid replacement. To limit the impact of malnutrition



FIG. 214.2 (A) Buckets of rice water stool from a patient with cholera. (B) Cholera cots; note central hole and sleeve leading to buckets to assist with monitoring ongoing fluid losses. (C) Obtunded, severely dehydrated patient with cholera; note sunken eyes. (D) Same patient after rehydration therapy. *(From Chowdhury F, Khan AI, Faruque AS, et al. Severe, acute watery diarrhea in an adult.* PLoS Negl Trop Dis. *2010;4:e898; and Harris JB, Larocque RC, Qadri F, et al. Cholera.* Lancet. *2012;379:2466–2476.)*

TABLE 214.2 Composition of Cholera Stools and Therapeutic Fluids for Cholera

		CONCENTRATION (mmol/L)					
		Na^+	K^+	Cl^-	HCO_3^-	Carbohydrate	COMMENTS
Intravenous fluid	LRS	130	4	109	28	— (278 if D5LR available)	LRS contains potassium and bicarbonate and is preferred over NS. LRS and NS can be supplemented with dextrose. Dhaka solution more closely approximates losses during cholera, but is not readily available.
	NS	154	0	154	0	—	
	Cholera saline (Dhaka solution)	133	13	154	48	140	
Oral rehydration therapy	ORS (WHO, 2002)[a]	75	20	65	10 (citrate)	75 (glucose)	WHO ORS uses glucose as a carbohydrate source. Rice-based ORS formulations have been found in randomized trials to reduce duration of diarrhea and stool losses in severe cholera.[67] A homemade preparation of ORS could be used in an emergency situation.
	Rice-based ORS (e.g., CeraORS 75)	75	20	65	10 (citrate)	27 g rice syrup solids	
	Homemade ORS[68]: ½ tsp table salt and 6 tsp table sugar in 1 L of safe water	≈75	0	≈75	0	≈75	
Electrolyte losses in stools (composite estimates)	Cholera stool, adult	130	20	100	45	—	Mean maximal rate of purging in severe cholera may exceed 10 mL/kg/h. Sodium losses in cholera stools exceed those seen in other causes of diarrheal illness.
	Cholera stool, child	100	30	90	30	—	
	Noncholera stool, child (ETEC)	50	35	25	20	—	

[a]In 2002 WHO replaced its previous formulation of ORS with the current lower osmolarity formulation to reflect the broad use of ORS for treating dehydration from all-cause gastroenteritis.

Cl^-, Chloride ion; *D5LR,* 5% dextrose in lactated Ringer solution; *ETEC,* enterotoxigenic *Escherichia coli;* HCO_3^-, bicarbonate ion; K^+, potassium ion; *LRS,* lactated Ringer solution; Na^+, sodium ion; *NS,* normal saline; *ORS,* oral rehydration solution; *tsp,* teaspoon; *WHO,* World Health Organization.

See references 56, 77, and 143.

Modified from Harris JB, Larocque RC, Qadri F, et al. Cholera. Lancet. *2012;379:2466–2476.*

TABLE 214.3 Antimicrobial Options for Treating Patients With Cholera

CLASS	ANTIBIOTIC	PEDIATRIC DOSE[a]	ADULT DOSE	COMMENTS
Macrolides	Erythromycin Azithromycin	12.5 mg/kg/dose qid × 3 days 20 mg/kg × single dose	250 mg qid × 3 days 1 g × single dose	Single-dose azithromycin is often preferred therapy, especially in children, and has been shown to be more effective than ciprofloxacin in randomized trials in regions where reduced susceptibility to fluoroquinolones is common.[144,145] There are rare reports of macrolide resistance.
Fluoroquinolones	Ciprofloxacin	15 mg/kg/dose bid × 3 days	500 mg bid × 3 days	In highly susceptible strains, single-dose ciprofloxacin compares favorably against erythromycin[146] and doxycycline[147] in randomized trials. Reduced susceptibility to fluoroquinolones has become common in endemic areas and is associated with treatment failure.[144,148]
Tetracyclines	Tetracycline Doxycycline	12.5 mg/kg/dose qid × 3 days 4–6 mg/kg × single dose	500 mg qid × 3 days 300 mg × single dose	Antibiotic resistance to all tetracyclines is common.[73] Empirical use is often reserved for outbreaks caused by documented susceptible isolates. In general, tetracyclines are not recommended for pregnant women or children less than 8 years old.

[a]Pediatric doses are based on weight; should not exceed maximum adult dose.
bid, Twice a day; *qid,* four times a day.
Modified from Harris JB, Larocque RC, Qadri F, et al. Cholera. Lancet. *2012;379:2466–2476.*

and energy deprivation, a high-energy diet should be encouraged as soon as fluid status is recovered for all individuals with cholera. WHO recommends zinc for children with diarrhea who are younger than 5 years, regardless of cause; zinc supplementation has been associated with a decreased volume and duration of diarrhea in children with cholera.[74] Zinc supplementation is usually given as 20 mg/day for 10 days for children 6 months of age to 5 years and 10 mg/day for 10 days for children younger than 6 months.[56] Vomiting of the supplement by young children is common during the initial stages of fluid resuscitation, but ingestion should be encouraged. Although not specifically studied with cholera, vitamin A supplementation is recommended for children with diarrhea in resource-limited settings. Although development of an antisecretory drug could theoretically limit fluid losses during cholera, no such agent has yet been identified and developed for widespread use. Antimotility agents should be avoided during cholera because they could lead to intestinal pooling of fluid (but not decreased production of fluid), yielding a false sense of improvement, and they can impede the ability to monitor ongoing fluid losses. Individuals caring for patients with cholera, who often include family members in resource-limited settings, should be carefully instructed that diarrhea will persist and may even worsen once an affected individual becomes rehydrated. Diarrhea usually resolves over 1 to 3 days.

Most patients who die of cholera die as a result of severe dehydration. The most common clinical mistakes in caring for patients with cholera are (1) inadequate initial fluid resuscitation; (2) inadequate matching of ongoing fluid losses, especially in young children; and (3) use of suboptimal or incorrect fluids.[75] With proper fluid management, mortality from cholera in patients who are able to reach clinical care should be less than 1%. However, many individuals die of cholera before they reach medical care, often within 6 to 12 hours of onset of symptoms, and case-fatality rates of 2% to 10% in the initial stages of complex emergencies are not infrequent.[76] Establishment of cholera treatment centers within communities with cholera outbreaks facilitates rapid access to care. Resources are available to assist in handling cholera outbreaks including the Cholera Outbreak Training and Shigellosis Program, an online resource that provides information, tools, and algorithms for the treatment of individuals and populations with cholera (www.cotsprogram.com).[77]

The treatment of a population with cholera, as opposed to an individual patient, can significantly strain limited resources, staff, and infrastructure. Cholera epidemics can cause panic in a population and in care providers who are unfamiliar with the disease. At-risk populations should be informed through community announcements, text messaging, the Internet, radio announcements, placards, posters, and other outreach programs as well as by local community leaders and personalities to recognize the initial symptoms of cholera, the importance of fluid replacement, and the need to seek medical care early in the illness. Simultaneous outreach programs should focus on the importance of safe water and adequate sanitation, and delivery efforts should focus on the provision of safe water and adequate sanitation including distribution of chlorine tablets for point-of-use treatment of unsafe water, if indicated. Integration of cholera vaccination programs should also be considered.

A community response to a cholera outbreak requires closely coordinated efforts that should involve community leaders as well as local, regional, national, and international health authorities. The unacceptably high case-fatality rates that are common in the initial phases of cholera outbreaks underscore the difficulty of establishing such coordinated efforts in the displaced, disenfranchised, and impoverished populations that are frequently most at risk for cholera as well as the often very complex nature of the emergencies that can afflict such populations.

IMMUNE RESPONSES

Although the genomes of *V. cholerae* O139 and El Tor O1 are extremely similar, including containing identical CT genes,[31,78] infection with *V. cholerae* O1 provides little to no protection against *V. cholerae* O139 and vice versa.[58] Similarly, although immune responses targeting CT are common after cholera, they do not mediate long-lived protective immunity.[79] As such, protective immunity against cholera appears to be largely serogroup specific and toxin independent, and serogroup specificity is determined by the OSP of LPS.[80]

Historically the vibriocidal response has been commonly used as a gauge of protective immunity against cholera.[81] This assay measures a complement-dependent bactericidal antibody response in blood that presumably is a surrogate marker for as yet poorly understood mucosal responses.[82] Every twofold increase in the vibriocidal response is associated with an approximately 40% reduction in the risk of cholera in Bangladesh.[83] The vibriocidal response is largely composed of immunoglobulin M antibodies targeting LPS, and anti-LPS responses (and anti-LPS memory B cells) have been associated with protection against cholera in household contacts of index cholera patients in Bangladesh.[49,84] OSP and LPS responses highly correlate, and vibriocidal responses are adsorbed away by OSP, suggesting the importance of OSP in mediating protective immunity to cholera.[80,85] Indeed, OSP-specific plasma and memory B-cell responses are associated with protection against cholera in household contacts of cholera index patients, and OSP-specific antibodies are associated with protection against cholera in North American volunteers experimentally infected with virulent *V. cholerae*.[85a,85b] T-cell activation during cholera is also associated with induction of memory B-cell responses,[86] and activation of innate immune responses during acute cholera correlates with subsequent immune responses.[87,88]

Analysis of the humoral response against *V. cholerae* suggests that the vast majority of the responses target a relatively small number of immunodominant antigens including OSP, CT, and sialidase, a bacterial enzyme that potentiates toxin activity.[89,90] Young children develop immune responses after cholera that are comparable to responses that develop in older children and adults including to antigens considered T-cell independent such as LPS and OSP.[91,92] The mechanism by which young

children surviving cholera develop such responses is unclear, but the potent adjuvant activity of CT may contribute.[91,92]

PREVENTION AND VACCINES

Prevention or eradication of cholera from the world is extremely laudable and a potentially achievable goal. However, because *V. cholerae* has an environmental reservoir, complete eradication of cholera from the world will be a very difficult task. Control of epidemic cholera has already been achieved in many countries. In the United States and Northern Europe, epidemic cholera was eliminated before the wide use of antibiotics and vaccines through the provision of safe water and adequate sanitation. When such basic public health measures are available globally, epidemic cholera will likely be eliminated.[93] This is a daunting goal; WHO currently estimates that more than 800 million individuals currently lack safe water and that more than 2 billion individuals currently lack adequate sanitation.[94] In light of these realities and the projected population growth over the next few decades, especially in densely populated regions with limited infrastructure, it is likely that human populations will be at risk for cholera for decades.[95] Indeed, the number of countries endemic for cholera and the number of outbreaks, their duration, their rate of recurrence, and their associated case-fatality rates all have increased during the current pandemic that began in 1961.[76,95]

Because the global provision of safe water and adequate sanitation will be extremely costly and take many years to achieve, integrated control programs should in many situations include use of cholera vaccines. The development of both oral and parenteral cholera vaccines has a long history, dating back to the late 1800s. A killed whole-cell parenteral cholera vaccine was often required for international travel for much of the mid-1900s. This vaccine had only modest short-term protective efficacy, required two to three immunizations as a primary series, required booster immunizations every 6 months, and had a high frequency of adverse events.[96] The vaccine eventually fell out of favor, in part because of the perceived decreased risk for routine international travelers of acquiring cholera and the impracticality of its ongoing use among populations in areas endemic for cholera. The shortcomings of this vaccine were part of the calculus that led to the removal of cholera vaccines from public health responses to control cholera in the latter part of the 1900s.

This calculus changed in light of the prolonged and worsening ongoing pandemic and the development of safe, better tolerated, and more effective cholera vaccines that induce longer-term immunity.[82] A number of oral killed cholera vaccines approved by WHO are currently commercially available internationally (Table 214.4).[39] One, Dukoral (Valneva, Lyon, France), a whole-cell/recombinant–B subunit oral cholera vaccine, contains approximately 10^{13} killed *V. cholerae* O1 classic and El Tor organisms and is supplemented by 1 mg of the nontoxic B subunit of cholera toxin (CtxB). Another vaccine, Shanchol (Shantha Biotechnics–Sanofi Pasteur, Hyderabad, India), is bivalent and includes a number of *V. cholerae* O1 strains as well as *V. cholerae* O139; it is not supplemented by CtxB. A related vaccine is the oral cholera prevention vaccine Euvichol (Eubiologics, Republic of Korea); Euvichol is now the primary vaccine in the WHO cholera vaccine global stockpile.[97] A locally produced Vietnamese version of the bivalent vaccine that is not internationally available is mORCVAX (VaBiotech, Hanoi, Vietnam).

The three prequalified oral cholera vaccines (Euvichol, Shanchol, Dukoral) are safe and well tolerated.[98–112] Dukoral is approved for use in children 2 years of age and older; Shanchol and Euvichol are approved for use in children 1 year of age and older. The absence of supplemental CtxB from Shanchol and Euvichol removes the requirement for buffer and decreases the amount of fluid that must be administered at the time of immunization. The vaccines are administered as two or three oral immunizations and provide approximately 60% to 80% protective efficacy for 6 to 60 months, depending on the vaccine used and age of the recipient. Booster immunizations are currently recommended with Dukoral every 6 months for children younger than 5 years of age and after 2 years for all other recipients. Shanchol may be associated with longer-term protection, although its protective efficacy in children younger than 5 years is also of lower magnitude and shorter duration than that afforded to older recipients.[113]

The manufacturers of Shanchol and Euvichol do not currently have recommendations regarding revaccination.[39] At the present time, the manufacturers do not recommend administration of either vaccine to pregnant women, although the scientific basis for this recommendation is unclear because the vaccines contain only killed organisms; the vaccines are not systemically administered or absorbed; and pregnant women who develop cholera are at significant risk of severe dehydration, clinical complications, and loss of the pregnancy. Administration of Dukoral was not associated with an increased risk of adverse birth defects or sequelae among pregnant women who inadvertently received the vaccine.[114] Shanchol has also been safely administered in populations with a high prevalence rate of human immunodeficiency virus.[111,115] Oral killed cholera vaccines may not require a cold chain and have shown some, albeit reduced, efficacy after a single dose.[116,117,118]

Modeling suggests that vaccine coverage rates of 50% or higher can potentially interrupt transmission secondary to vaccine herd affect[119,120] and that the earlier a cholera vaccine rollout program occurs during an outbreak, the more significant the impact of immunization.[121–123,124,125] Some studies have found cholera vaccine use to be cost-effective, whereas others have not.[126,127]

In light of the safety, immunogenicity, protective efficacy, and potential impact of cholera vaccines, the WHO Cholera Control Task Force now recommends that cholera vaccines be considered as part of an integrated cholera control program in areas endemic for cholera, in humanitarian crises with high risk of cholera, and during cholera outbreaks.[39] In

TABLE 214.4 Internationally Available and WHO Prequalified Oral Killed Cholera Vaccines

VACCINE	DOSES[a]	DOSING INTERVAL[a]	DOSING VOLUME[a]	BOOSTERS[a]	PROTECTIVE EFFICACY	COMMENTS
Shanchol (Shantha Biotechnics–Sanofi Pasteur); Euvichol (Eubiologics)						
≥1 year of age	2	14 days (7–42 days probably permissible)	1.5 mL	No recommendations from manufacturer	60%–70% protective efficacy decreasing to baseline over 36–60 months in older children and adults; 40% protective efficacy and shorter duration in younger children	Appears to provide longer-term protection than that provided by Dukoral. Does not require buffer to administer vaccine. Maintained in WHO Global Cholera Vaccine Stockpile
Dukoral[b] (Valneva)						
Children 2 to <6 years of age	3	14 days (7–42 days permissible)	3 mL vaccine and 75 mL buffer	Every 6 months	60%–85% protective efficacy within 6 months of vaccination, decreasing to baseline over 24–36 months	Licensed in many countries. Provides short-term protection against diarrhea caused by heat-labile toxin expressing strains of ETEC.
≥6 years of age	2	14 days (7–42 days permissible)	3 mL vaccine and 150 mL buffer	Every 2 years		

[a]Per manufacturer.
[b]Field studies have involved both the current preparation of whole-cell vaccine supplemented with recombinant cholera toxin B subunit and an initial preparation of whole-cell vaccine containing nonrecombinant B subunit.
ETEC, Enterotoxigenic *Escherichia coli; WHO,* World Health Organization.

support of this recommendation, the WHO and international health authorities established an international cholera vaccine stockpile in 2013.[128] By 2017, more than 12 million doses of oral cholera vaccine had been distributed from the stockpile as part of 46 deployments. The number of cholera vaccines shipped each year has roughly doubled since inception of the stockpile.[129] In 2017, a global strategy on cholera control with a target to reduce cholera deaths by 90% by 2030 was launched by the Global Task Force on Cholera Control.[129a]

In 2016 a live attenuated oral cholera vaccine was approved by the US Food and Drug Administration for use in adults in the United States (Vaxchora [CVD 103 HgR]; PaxVax, San Diego, CA).[130] This vaccine provided 90% protective efficacy against experimental challenge in North American adult volunteers within 10 days of vaccination and 80% protective efficacy against challenge at 90 days.[131] The duration of protection and vaccine efficacy in endemic zone populations is currently unknown. Additional live attenuated oral cholera vaccines are in various stages of development and analysis including Peru-15 (Haikou VTI Biological Institute, China), HaitiV,[131a] and several others.[132–138,139,140,141] A number of other cholera vaccines including subunit and conjugate vaccines are in various stages of development.[142]

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The complete reference list is available online at Expert Consult.

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215 Other Pathogenic Vibrios

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SHORT VIEW SUMMARY

Definition
- Vibrios are gram-negative rods whose environmental niche is primarily marine and estuarine waters.
- They may also be found in freshwater sources.
- The halophilic vibrios require higher concentrations of salt for growth.

Epidemiology
- The most common *Vibrio* species causing human illness are *Vibrio parahemolyticus* and *Vibrio vulnificus.*
- The incidence of vibriosis in the United States has been increasing, indicating that current prevention efforts are not effective.
- Illness is more common in the warmer summer months when *Vibrio* populations are higher and there is increased human exposure from shellfish consumption and recreational water exposure.
- *Vibrio* species primarily cause diarrhea of varying severity, soft tissue infection, and/or bacteremia.
- *V. vulnificus* causes a distinct soft tissue infection with rapidly developing hemorrhagic bullae.
- *Vibrio* infections are more severe and more often fatal in people with cirrhosis, other underlying liver disease, or immune compromise.

Microbiology
- The major pathogenic vibrios grow well in blood culture media and common nonselective media used for wound cultures.
- Culture-independent diagnostic testing by DNA amplification panels is increasing the frequency of detecting *Vibrio* infections in the United States.

Diagnosis
- *Vibrio* infection should be suspected in people with diarrhea, sepsis, or wound infection after seafood consumption or marine water exposure.

Therapy
- Rapid recognition of possible *V. vulnificus* infection is key to improving survival and requires antimicrobial treatment as well as surgical débridement.
- Survival is improved in *V. vulnificus* infection when a third-generation cephalosporin is added to doxycycline or a quinolone, provided débridement of necrotic tissue is done.
- Treatment with doxycycline or a quinolone can decrease symptoms and fecal shedding in people with diarrhea continuing for 5 days.

Prevention
- No vaccine is available for the noncholera vibrios.
- Thorough cooking of seafood with prompt consumption or refrigeration is needed to decrease the risk for *Vibrio* infection.

Vibrio species are ubiquitous in estuarine waters in the temperate zones. Plankton blooms and temperature upshifting in the spring are followed by rapid outgrowth of most vibrios. Molluscan shellfish, which are filter feeders, acquire vibrios as part of their normal microbiota during the warmer months. Shellfish contamination by these vibrios thus occurs as a consequence of the normal climate-associated changes in *Vibrio* prevalence in coastal waters. In addition to this mechanism, sewage contamination of shellfish beds in epidemic cholera can contribute significantly to disease burden.

In the United States, illnesses caused by the commonly isolated pathogenic vibrios have a marked seasonal peak, with more than 90% of cases occurring between April and October. This presumably reflects seasonal changes in shellfish consumption, recreational water exposure, and the increase in densities of vibrios in marine waters during the warmer months. The incidence of vibriosis in the United States has been increasing despite prevention efforts.[1] This may be reflective of both host and exposure factors, such as increases in water-related outdoor activities (swimming, boating), natural disasters (hurricanes, flooding), increased consumption of seafood, and more persons living with chronic liver disease and immunocompromising conditions. Recently, more widespread use of DNA amplification panels for detection of diarrheal pathogens has led to a 54% increase in the number of *Vibrio* infections reported in 2017 compared with the prior 2 years.[2]

In addition to *Vibrio cholerae* serotype O1 and *Campylobacter fetus* (formerly known as *Vibrio fetus*), other vibrios have been clearly associated with human disease. The best known among the halophilic (salt-requiring) vibrios are *V. parahaemolyticus* and *V. vulnificus,* both of which can cause serious illness and death. Three other halophilic vibrios, *V. alginolyticus, V. fluvialis,* and *V. furnissii,* cause infections of appreciable frequency but lesser clinical severity. The former *V. hollisae* and *V. damselae* have been reclassified and are now *Grimontia hollisae* and *Photobacterium damselae,* respectively. *V. metschnikovii, V. cincinnatiensis,* and *V. harveyi* (*V. carchariae*) rarely cause human illness. The nonhalophilic vibrios include non-O1/O139 *V. cholerae* and *V. mimicus;* like *V. cholerae,* they cannot grow at higher salt concentrations. They are worldwide in distribution and have frequently been incriminated in human illness.

VIBRIO PARAHAEMOLYTICUS

V. parahaemolyticus, a halophilic vibrio, is a major cause of acute diarrheal disease in Japan.[3] In the United States, *V. parahaemolyticus* was the most commonly isolated *Vibrio* species and the most common of the speciated vibrios reported.[1] This pathogen was the most common bacterial cause of foodborne disease in Taiwan, accounting for 35% of all outbreaks[4]; in less-developed countries, it has been incriminated in up to 20% of acute diarrheal illnesses. A specific serotype, *V. parahaemolyticus* O3:K6, emerged as an important cause of human illness in Southeast Asia[5] and, since 1996, became established globally by a pandemic clone.[6] In 1998, this serotype first appeared in the United States,[7] causing a large multistate outbreak that prompted regulatory changes in programs for bacteriologic monitoring of shellfish.[8] In 2004, an outbreak of gastroenteritis caused by serotype O6:K18 was traced to raw oysters from the Gulf of Alaska, harvested when the water temperature was higher than 15°C.[9] Whether global warming trends portend locally acquired *V. parahaemolyticus* infection even at high northern latitudes is a concern and emphasizes the need for surveillance and appropriate diagnostics, a recurring theme in emerging infectious diseases.

The genome of *V. parahaemolyticus* has been sequenced.[10] Like *V. cholerae, V. parahaemolyticus* has two circular chromosomes, but unlike *V. cholerae,* a type III secretion system has been found on both of the two chromosomes of *V. parahaemolyticus.*[11] This finding likely underlies the inflammatory diarrhea seen in *V. parahaemolyticus,* but

not *V. cholerae,* infection, and suggests distinctly different mechanisms for diarrhea from these two pathogenic vibrios.

As suggested both by clinical manifestations and by animal studies, *V. parahaemolyticus* has the genetic endowment to produce an enterotoxin and to cause an inflammatory reaction in the small bowel mucosa. Major degrees of intestinal fluid loss are seldom seen, and the tissue damage that this vibrio causes is generally less extensive than that observed in shigellosis. Two hemolysins, thermostable direct hemolysin (TDH) and TDH-related hemolysin, have enterotoxin activity; strains negative for TDH and TDH-related hemolysin are usually avirulent.

Epidemiology

Because of lack of specificity of the clinical features of the illness, the epidemiologic history usually provides the most important clue to diagnosis. *V. parahaemolyticus* is ubiquitous in coastal waters,[3] although it typically is not recovered from estuarine waters during winter months in temperate zones. During periods of low temperature or nutrient deprivation, it enters a viable but nonculturable state.[12]

Consumption of raw or undercooked shellfish is the most common means of acquiring *V. parahaemolyticus* infection. In the United States, raw oysters are the most common vehicle.[13] In a microbiologic survey of oysters harvested from US waters, the frequency of *V. parahaemolyticus* contamination was consistently greater than that for *V. vulnificus.*[14,15] Inadequately cooked seafood can harbor small numbers of surviving vibrios, as can food contaminated by seawater on ships. *V. parahaemolyticus* can proliferate rapidly to reach high colony counts in contaminated foods held at ambient temperature for a few hours. This presumably contributes to the high attack rates seen in common source outbreaks.

Person-to-person transmission has not been documented, suggesting that the infective dose for normal persons is relatively high. *V. parahaemolyticus* has rarely been cultured from asymptomatic people, and no carrier state has been identified.

Clinical Manifestations

Gastroenteritis is the most common clinical illness associated with *V. parahaemolyticus* infection; wound infections and septicemia may be seen, but much less frequently. Enteric illness ranges from mild watery diarrhea to a frank dysentery-like syndrome. Illness commonly begins with the acute onset of explosive watery diarrhea, generally within 24 to 72 hours of ingestion of the contaminated seafood. Often, the diarrhea is accompanied by mild to moderately severe cramping and abdominal pain; low-grade fever, mild chills, and headache occur in fewer than half of infected people. The fluid loss is rarely severe, and death caused by *V. parahaemolyticus* is rare, usually occurring in very young children, older adults, or people with underlying disease.

Laboratory Findings

The diarrheal fluid is characteristically watery, sometimes mucoid, and occasionally bloody (<15% of cases). Fecal leukocytes are often present. *V. parahaemolyticus* is a pleomorphic gram-negative rod that is a facultative anaerobe. It is readily identified on the selective thiosulfate citrate–bile salts–sucrose (TCBS) agar, on which it appears as distinct opaque green colonies. While culture-independent diagnostic testing has greater sensitivity (lower limit of detection) compared with stool cultures, it does not provide antibiotic susceptibility or molecular subtyping results to compare isolates. Reflex cultures from fecal specimens positive for *Vibrio* species by DNA amplification panels were only positive in 38% of cultures.[2]

Nearly all clinical isolates of *V. parahaemolyticus* cause β-hemolysis of human erythrocytes (the Kanagawa reaction), which is caused by the production of TDH. Interestingly, growth in bile salt–containing environments enhances the expression of several virulence traits in *V. parahaemolyticus.*[16]

In patients with *V. parahaemolyticus* enteric infection, both serum and coproantibody responses to lipopolysaccharide and to TDH were detected.[17] Mucosal biopsies from the duodenum and rectum showed inflammatory changes, suggesting that the small and large intestines are affected. Tumor necrosis factor-α (TNF-α) levels were noted to be elevated acutely in *V. parahaemolyticus* infection, similar to observations in shigellosis but in contrast to cholera infections, in which TNF-α levels are not elevated.

Differential Diagnosis

Because *V. parahaemolyticus* is ubiquitous in coastal waters throughout the temperate and tropical zones of the world, this pathogen must be considered in the differential diagnosis of all acute diarrheal illnesses that follow the ingestion of seafood. There are no clinical features that, in the individual case, reliably distinguish diarrhea caused by *V. parahaemolyticus* from that caused by enterotoxigenic *Escherichia coli* or from milder cases of shigellosis or salmonellosis. Vomiting is characteristically less prominent than in disease caused by staphylococcal enterotoxin, and the cramping abdominal pain is generally less severe than that typical of food poisoning caused by *Clostridium perfringens.*

Therapy

No treatment is required by most patients because the gastroenteritis is usually self-limited. However, antimicrobial therapy could be considered for those patients with diarrhea lasting longer than 5 days.[18] Therapy with doxycycline or a quinolone would be expected to shorten the clinical course and duration of pathogen excretion. In the United States, 16% to 20% of patients with *V. parahemolyticus* infection were hospitalized; the overall case fatality rate was <1%.[1] Occasionally patients, usually at the extremes of age, may become sufficiently dehydrated that oral or intravenous rehydration is needed. Wound infections due to *V. parahaemolyticus* are managed similarly among all the vibrios causing soft tissue infection, including antibiotic treatment and débridement as needed.

Prevention

V. parahaemolyticus can remain viable in shrimp or crab meat for several minutes at temperatures as high as 80°C, and it is especially important when cooking large quantities of such foods to ensure that all portions of the seafood are heated to cooking temperatures adequate to kill *Vibrio* species. Of only slightly less importance is the necessity of refrigerating cooked seafood if it is not to be eaten immediately after cooking. Shipboard outbreaks can be prevented by avoiding the use of untreated seawater in galleys. Irradiation at a dose of 3.0 kGy can reduce *V. parahaemolyticus* levels by 6 logs without killing the oysters or adversely affecting their organoleptic qualities[19]; however, lower doses may not inactivate viruses.

It is not known whether protective immunity is conferred by clinical infection. No effective vaccine is currently available.

VIBRIO VULNIFICUS

Like other pathogenic halophilic vibrios, *V. vulnificus* is part of the normal marine microbiota and, in the temperate zones, reaches sufficient concentrations to cause clinical illness only in the warmer months of the year. Almost all oysters harvested in the summer from the Chesapeake Bay contain this pathogen, as do 10% of crabs. In the United States, 25% of all *Vibrio* infections were from a non-foodborne source and of these, *V. vulnificus* was the most common species isolated. Almost half of all *V. vulnificus* infections were non-foodborne.[20] After Hurricane Katrina in 2005, 18 cases of wound infection caused by vibrios were reported; 14 (82%) were caused by *V. vulnificus,* resulting in three deaths.[21] Infections from *V. vulnificus* may be increasing in the United States, particularly in association with Gulf coast oyster consumption,[22] and warmer water temperatures (>22°C) in the Gulf of Mexico may be contributing in part to the increase.

Over 80% of reported *V. vulnificus* cases were hospitalized in the United States; the case-fatality rate of 30% is the highest among infections caused by any *Vibrio* species.[1] *V. vulnificus* is estimated to account for 90% of all seafood-related deaths in the United States. Hippocrates may have provided the first description of a *V. vulnificus* infection in the 5th century BC when he described the rapid progression of a severe foot cellulitis with black blisters in Criton of Thasos, which was fatal in 48 hours.[23]

Clinical Manifestations

V. vulnificus is primarily associated with a severe, distinctive soft tissue infection or septicemia or both, rather than diarrheal illness.[24] In

compromised hosts, especially patients with cirrhosis, *V. vulnificus* can invade the bloodstream without causing gastrointestinal symptoms. The clinical picture is one of abrupt onset of chills and fever, often (in 33% of cases) followed by hypotension, usually (in 75%) followed by the development of metastatic cutaneous lesions within 36 hours after onset. These begin as erythematous lesions and rapidly evolve to hemorrhagic bullae or vesicles and then to necrotic ulcers (Fig. 215.1). *V. vulnificus* bacteremia has been fatal in over 50% of cases in which it occurred. More than 90% of such patients had a history of having consumed raw oysters in the 7 days before illness onset; concentrations of *V. vulnificus* of 10^3 colony-forming units per gram of oysters have produced illness. Although oysters harbor genetically heterogeneous populations of *V. vulnificus,* only one strain type has been recovered from human tissues in invasive infections.[25] In addition to cirrhosis, other risk factors for the septicemic form of *V. vulnificus* infection include other liver diseases; iron overload states such as hemochromatosis, hemolytic anemia, or chronic renal failure; malignancy; human immunodeficiency virus infection; and immunosuppressive medications.

Posttraumatic wound infections with *V. vulnificus* usually occur on the legs and arms; the diagnosis can be suspected from the distinct clinical picture (see Fig. 215.1).[21] After contamination of a superficial wound by warm seawater, *V. vulnificus* can cause a rapidly developing, intense cellulitis, necrotizing vasculitis, and ulcer formation in both healthy people and immunocompromised hosts, and bacteremia is frequent. This was graphically illustrated in a report of a surgeon with a minor cut to his finger while saltwater fishing, with severe advancing cellulitis developing <12 hours later. The rapidity and severity (arm amputation despite appropriate antibiotics and emergency débridements) may have been related to adalimumab (a TNF-α inhibitor), which he was receiving for psoriatic arthritis.[27] In another case, *V. vulnificus* apparently survived on intact skin for more than 24 hours after handling tilapia fish, with the patient subsequently developing a necrotic cellulitis after traumatic injury to the hand while working on a motor vehicle engine.[28,29]

V. vulnificus is an infrequent cause of acute, self-limited diarrheal illness in individuals receiving gastric acid suppression therapy,[30] ocular infections usually occurring after an injury from molluscan shell fragments,[31] and, rarely, septic arthritis.[32]

The major determinant of virulence in *V. vulnificus* is its polysaccharide capsule, which renders the bacterium resistant to phagocytosis and directly stimulates release of inflammatory cytokines such as TNF-α.[33] Other contributors to pathogenicity include a variety of extracellular proteins and cell wall lipopolysaccharide. Host-derived factors that contribute to the pathogen's virulence include the availability of iron and at least one inflammatory mediator. *V. vulnificus* can alter expression of several genes differentially in response to low-iron and iron-rich conditions and, in the latter, can multiply rapidly.[34] The predilection of this *Vibrio* species to cause disease in patients with iron overload states can be explained by its ability to sequester iron from hemoglobin and 100% saturated (but not 30%, or normally saturated) transferrin.

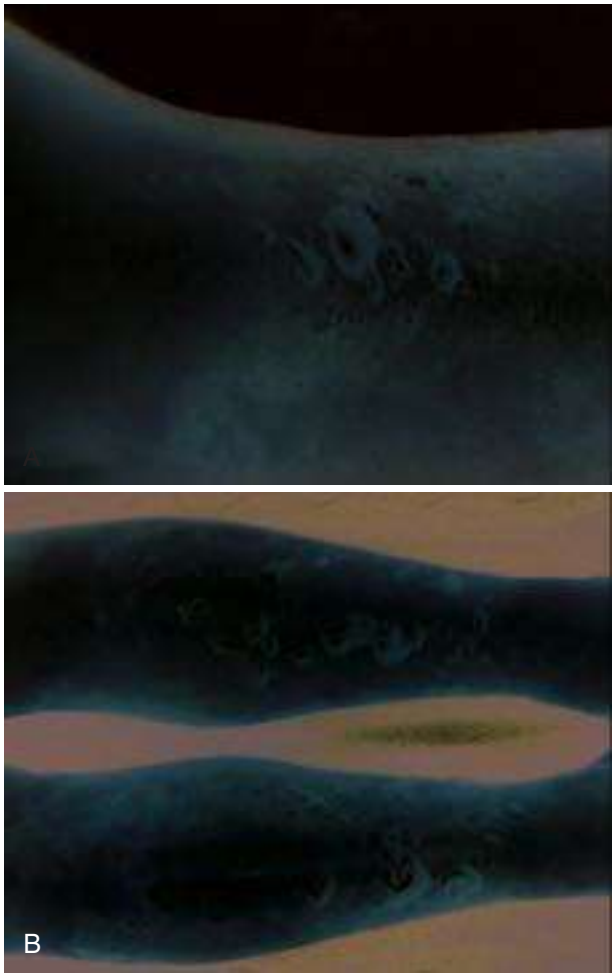

FIG. 215.1 Cellulitis (A) and hemorrhagic bullae (B) in *Vibrio vulnificus* infection. *(From Centers for Disease Control and Prevention. Vibrio illnesses after Hurricane Katrina—multiple states, August-September, 2005.* MMWR Morb Mortal Wkly Rep. *2005;54:928–931.)*

Differential Diagnosis

V. vulnificus should be suspected in any immunocompromised host (but especially with cirrhosis or underlying liver disease) who develops a septicemic illness associated with necrotizing cutaneous lesions within 1 to 3 days after the ingestion of oysters. Although rare, the clinical syndrome is distinct and should suggest this diagnosis.

Similarly, the development of cellulitis in humans occupationally or recreationally exposed to seawater should suggest infection with *V. vulnificus* (especially in the presence of severe, necrotizing cellulitis). Other *Vibrio* species may cause soft tissue infections, but these are usually less severe.

V. vulnificus grows readily in blood culture media, on blood or other nonselective agar for wound cultures, and on MacConkey and TCBS media; final identification is made by standard biochemical tests. Because *V. vulnificus* ferments lactose, it can be overlooked in cultures grown on MacConkey agar unless the laboratory is advised to specifically look for it; thus communication between the clinician and microbiology laboratory personnel is key. *V. vulnificus* can also be identified from stool specimens by DNA amplification panels that increasingly are being adopted by clinical laboratories for syndromic testing (e.g., diarrhea).[35]

A real-time quantitative polymerase chain reaction (PCR) assay to detect the *toxR* gene of *V. vulnificus* was capable of detecting as few as five copies per microliter of serum; positive PCR results were obtained in 5 of 22 cases with negative blood cultures but in which *V. vulnificus* was isolated from soft tissue.[36] In a retrospective study using real-time PCR for *V. vulnificus toxR,* DNA copy numbers in tissue were considerably higher than in blood. Such testing could add diagnostic speed as well as specificity, especially in persons who had received antibiotics prior to obtaining cultures.[37]

Therapy

Early administration of antibiotics in *V. vulnificus* infection is critical because the cellulitis can advance very rapidly. Immediate surgical consultation should be obtained because débridement of all devitalized tissue or even amputation may be lifesaving.[27] In one retrospective case series, temporizing surgical management for those patients with shock at admission was associated with lower mortality versus aggressive débridement (26% vs. 60%), but choice and timing of antimicrobial therapy were not detailed.[38]

Fluoroquinolones, third-generation cephalosporins, and doxycycline are highly active against *V. vulnificus,* and the combination of ciprofloxacin and cefotaxime exhibited synergy.[39] Survival in *V. vulnificus* necrotizing fasciitis was higher in those treated with minocycline or ciprofloxacin added to a third-generation cephalosporin, provided that prompt surgical intervention was obtained.[40,41]

Prevention

Although patients with underlying liver disease and other chronic illnesses should be warned of the hazards of eating raw oysters, this has not been accomplished effectively in the United States, even when required by law.[1] A capsular polysaccharide conjugate vaccine has been developed, but studies indicate that polyclonal immunoglobulin, actively or passively derived, was necessary for cross-protection among capsular types of *V. vulnificus*.[42] At present, thorough cooking of seafood remains the only effective means of prevention.

VIBRIO ALGINOLYTICUS

V. alginolyticus has been predominantly associated with cellulitis and acute otitis media or externa rather than gastroenteritis.[20] These infections generally occurred after local trauma in otherwise healthy seawater from swimmers or fishermen and responded well to appropriate antibiotics.[43,44] In a review of *V. alginolyticus* infection in the United States from 1988 to 2012, there was a 12-fold increase during this period, such that it is now the second most common *Vibrio* infection. Most infections were reported from coastal states, with the highest rates from the Gulf coast.[45]

Necrotizing fasciitis has developed after a soft tissue injury from a coral reef to the leg of an apparently immunocompetent individual.[46] *V. alginolyticus* caused a pacemaker pocket infection in a man who swam off the Atlantic coast of France,[47] showing that *Vibrio* infections also can be diseases of medical progress. Isolation is similar to that for *V. vulnificus;* however, *V. alginolyticus* does not ferment lactose.

HALOPHILIC VIBRIOS

Several other *Vibrio* species are recognized as causative agents of human disease acquired through ingestion of contaminated seafood or contact of traumatized skin with seawater or brackish water. *Grimontia hollisae* (formerly *V. hollisae*) primarily causes a moderate to severe diarrheal illness, often requiring hospital admission.[1,48,49] *Photobacterium damselae* (formerly *V. damselae*) causes serious wound infections, with findings reminiscent of the clinical picture of *V. vulnificus*.[50,51] Such cases were previously reported with exposures in warmer southern waters; a fatal case with an extremely aggressive necrotizing fasciitis due to *P. damselae* was reported in New England.[52] *V. fluvialis* previously was primarily associated with sporadic gastroenteritis; more recently, it has caused peritonitis (one case of which was in association with continuous ambulatory peritoneal dialysis)[53,54] and severe hemorrhagic cellulitis.[55] Of potentially greater significance is the finding of carbapenem resistance in clinical isolates of *V. fluvialis* from persons with diarrhea in India. These isolates produced New Delhi metallo-β-lactamase 1 (NDM-1) and were resistant to nearly all antibiotics tested except azithromycin and doxycycline.[56]

V. furnissii has been rarely isolated in sporadic cases of diarrhea.[14] However, a nonfatal case in a patient with poorly controlled diabetes described *V. furnissii* bacteremia and skin lesions similar to those in *V. vulnificus* infection.[57] *V. metschnikovii* has been a rare cause of severe infections, including pneumonia, cellulitis, and bacteremia[58–60]; *V. cincinnatiensis* has caused bacteremia and meningitis.[61] *V. harveyi* (formerly *Vibrio carchariae*) has caused cellulitis after a shark bite[62] and sepsis in a pediatric patient with a central venous catheter.[63]

NONHALOPHILIC VIBRIOS: NON-O1/O139 *VIBRIO CHOLERAE* AND *VIBRIO MIMICUS*

Vibrios that are biochemically similar to *V. cholerae* but that do not agglutinate in *V. cholerae* O1 or O139 antiserum are taxonomically included in the species *V. cholerae* and are referred to as non-O1/O139 *V. cholerae*. *V. mimicus* is closely related to non-O1 *V. cholerae* but differs biochemically in being sucrose negative and Voges-Proskauer reaction negative. These nonhalophilic vibrios require only trace amounts of sodium chloride for growth in culture medium; this characteristic distinguishes them from the true halophilic vibrios *V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus,* which require higher concentrations of sodium chloride in culture media and have the remarkable ability to grow in 10% sodium chloride.

Epidemiology

V. cholerae organisms are worldwide in distribution and ubiquitous in seawater and estuarine water sources, but they also have been isolated from fresh water. The burden of disease from these strains has been in the severity of illness in individual patients. They have not been observed to cause sweeping epidemics, as have *V. cholerae* O1 and O139, although a few non-O1 strains have caused outbreaks. The molecular basis for this epidemiologic behavioral difference resides in a large (39.5-kb) *Vibrio* pathogenicity island (VPI) that contains gene clusters responsible for cholera toxin acquisition and expression as well as sequences involved in colonization.[64] The group of virulence genes within the VPI is strongly tied to epidemic ability. The VPI is present in epidemic and pandemic *V. cholerae* O1 strains, in Bengal O139, and in two non-O1 *V. cholerae* strains that caused outbreaks. The VPI was absent in sporadic diarrheal and nontoxigenic environmental isolates of non-O1 *V. cholerae*.[64] Horizontal gene transfer of this pathogenicity island may be an initial step for the acquisition of epidemic capability by non-O1 *V. cholerae* strains.

In temperate regions, vibrio infections are usually seasonal; the higher number of cases in summer months is associated with warmer sea surface temperatures (>15°C) that promote growth of vibrio species. Northern Europe has seen an increase in vibrio infections (despite being nonreportable), particularly in years with heat waves.[65] There was a substantial increase in non-O1/O139 *V. cholerae* infections in Sweden and Finland in 2014, a year that had the highest (Sweden) and second highest (Finland) recorded temperatures in >150 years. The cases, negative for cholera toxin, were reported from within 100 miles of the Arctic Circle.[66]

Clinical Manifestations

Non-O1 *V. cholerae* organisms produce a wide spectrum of diarrheal illness, ranging from severe watery diarrhea indistinguishable from that with cholera to the milder traveler's diarrhea of the type commonly associated with enterotoxigenic *E. coli.* Some clinical isolates of non-O1 *V. cholerae* produce cholera toxin, but most are nontoxigenic. No clinical features distinguish the severe diarrheal illnesses caused by enterotoxin-producing non-O1 *V. cholerae* from those caused by classic *V. cholerae.* This was aptly illustrated in a report of eight sporadic cases of severe diarrhea with dehydration in the southeastern United States; *V. cholerae* serotype O75 was isolated from the patients and environmental samples, and all isolates produced cholera toxin.[67] Non-O1 *V. cholerae* strains can rarely cause bacteremia, almost invariably in patients with liver disease[68,69] but occasionally also in healthy people.

V. mimicus has caused sporadic cases of acute diarrheal illness in the United States and the tropics. Illness was associated with ingestion of raw seafood, including turtle eggs[70] and crayfish.[71]

Laboratory Findings

With intestinal infections caused by both non-O1 *V. cholerae* and *V. mimicus,* the diarrheal fluid varies from the watery isotonic fluid characteristic of cholera gravis to loose stools in which small numbers of leukocytes and erythrocytes may be seen. The organisms are readily identified on TCBS agar, on which *V. cholerae* appears as opaque yellow colonies and *V. mimicus* shows as green colonies; final speciation is made by biochemical tests and lack of agglutination in O1 antisera.

Therapy

Other than oral fluid and electrolyte repletion, no treatment is required by the large majority of patients with diarrheal disease, and antimicrobial agents have not been shown to shorten the clinical course. In occasional patients, especially those in the developing world, the intestinal fluid loss is sufficient to require intravenous electrolyte therapy. In this situation, therapy is guided by the same principles used for the treatment of cholera.

Prevention

Because non-O1/O139 *V. cholerae* organisms exist in a variety of water sources, ranging from freshwater rivers to the oceans, purification of water sources and adequate cooking of fish and other seafood provide the only certain protection against these occasional pathogens.

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The complete reference list is available online at Expert Consult.

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216 *Campylobacter jejuni* and Related Species

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SHORT VIEW SUMMARY

Definition

- *Campylobacter* spp. are small, curved, gram-negative rods that cause acute gastrointestinal illness that typically lasts from 1 to 7 days. The diarrhea is frequently bloody and is associated with abdominal pain. Postinfectious sequelae occur infrequently. *C. jejuni* is the most common human clinical isolate.

Epidemiology

- *Campylobacter* spp. are found in a variety of animals and are a common cause of human diarrheal disease worldwide. In developed nations, diarrhea occurs in all ages, whereas in low- and middle-income countries, symptomatic illness occurs mostly in young children. Consumption or handling of poultry is a common mode of transmission.

Microbiology

- Many, but not all, *Campylobacter* spp. thrive at 42°C. The gram-negative bacilli are small (0.3–0.6 μm in diameter) and motile. They are microaerophilic and grow best at an oxygen concentration of 5% to 10%.

Diagnosis

- The gold standard for diagnosis is isolation of the organism from a stool or occasionally a blood culture. However, many highly sensitive, non–culture based techniques are now used for diagnosis by clinical microbiology laboratories.

Therapy

- The most important tenet of treatment is to replace fluid and electrolytes to prevent dehydration. Antibiotics may shorten the duration of symptoms. Azithromycin 500 mg orally daily for 3 days will treat most infections.

Prevention

- The best way to prevent infection is by thoroughly cooking poultry and other foods of animal origin and taking other measures to ensure food and water are not contaminated with animal feces. No effective vaccine is currently available.

Campylobacteriosis refers to the group of infections caused by gram-negative bacteria of the genus *Campylobacter.* Among the most common bacterial infections of humans in all areas of the world, *Campylobacter* spp. cause both diarrheal and systemic illnesses and may be associated with long-term sequelae. Infection of domesticated animals with *Campylobacter* is widespread. The name *Campylobacter* is derived from the Greek *campylos,* meaning "curved", and *baktron,* meaning "rod". After the recognition of *Campylobacter jejuni* as a major human pathogen, numerous related *Campylobacter, Arcobacter,* and *Helicobacter* species have been identified. The solving of the first *Campylobacter jejuni* genomic sequence in 2000 opened new doors in our understanding of these organisms.[1] Whole-genome sequences for multiple *Campylobacter* spp. have been determined[2] and are available on various websites.

MICROBIOLOGY

Campylobacter organisms are motile, non–spore-forming, comma-shaped, gram-negative rods.[3] Originally isolated from aborted sheep fetuses in 1909, these and similar organisms were considered subspecies of *Vibrio fetus.* However, because these organisms did not ferment carbohydrates and differed in their guanine plus cytosine (G + C) DNA content from true members of the genus *Vibrio,* a new genus, *Campylobacter,* was created. Fourteen species have been recognized within the genus; however, in recent years, taxonomic studies have indicated that splitting the genus is more appropriate.[4] The genus *Arcobacter* has been created, which now includes *Arcobacter butzleri* and *Arcobacter skirrowi.*[5] *Helicobacter cinaedi* and *Helicobacter fennelliae* had been named *Campylobacter cinaedi* and *Campylobacter fennelliae* when first discovered.[6] Although transfer to the genus *Helicobacter* is more appropriate on taxonomic grounds, because these two species cause intestinal rather than gastric illnesses they are discussed in this chapter. *Helicobacter pylori,* previously named *Campylobacter pylori,* is discussed in Chapter 217. It is clear that new members of *Campylobacter* and related genera are being identified with regularity[7–9] and that many of these will be found to be human pathogens.

Table 216.1 lists the *Campylobacter* and related species most commonly associated with human disease and indicates the differentiating characteristics. Certain species, such as *Campylobacter nitrofigilis* and *Arcobacter cryaerophilus,* have not yet been associated with human illness. In contrast, the "nitrate-negative" campylobacters are associated with diarrheal illnesses, but the appropriate nomenclature for the organisms has not been determined. Two types of illnesses are directly associated with *Campylobacter* spp.: enteric and extraintestinal. For each of these illnesses, one *Campylobacter* species predominates and other species are less commonly present. The prototype for enteric infection is *C. jejuni;* for extraintestinal infection it is *Campylobacter fetus* (Table 216.2). Because the organisms causing enteric and extraintestinal illnesses are generally the same, they are considered together in the following discussion.

Campylobacters and related organisms grow best in an atmosphere containing 5% to 10% oxygen and are thus considered microaerophilic.[3,4] Although most of these organisms will not grow under aerobic or anaerobic conditions, *C. jejuni* can grow in candle jars, which permits isolation when the optimal atmosphere cannot be achieved. All campylobacters grow at 37°C; however, *C. jejuni* grows best at 42°C. Because *C. jejuni* is the most common enteric pathogen of humans, many laboratories have used incubation at 42°C for optimal isolation; however, use of this temperature will not permit detection of infections by many of the related species. In particular, *Campylobacter upsaliensis* may be missed.

Campylobacter spp. multiply more slowly than do the usual bacteria of the enteric flora and therefore cannot be isolated from fecal specimens unless selective techniques are used. The most common isolation methods use blood-based, antibiotic-containing media. Three such media—Skirrow medium, Butzler agar, and Campy-BAP medium—or variations of these have been in wide use.[4] The last two media contain cephalothin, which inhibits *C. fetus* and several other *Campylobacter* subspecies, but are best suited for isolating *C. jejuni.* Several enrichment broths have been developed, but because ill humans usually excrete 10^6 to 10^9 *C. jejuni* colony-forming units per gram of stool, enrichment usually is not necessary. Blood-free media can also be used.[10] Owing to their small

TABLE 216.1 Differential Characteristics of *Campylobacter* and Related Species Most Commonly Associated With Pathogenicity in Humans

	GROWTH				H_2S PRODUCTION			SUSCEPTIBILITY TO 30-μg DISK		
SPECIES	25°C	37°C	42°C	NITRATE REDUCTION	On TSI	On Lead Acetate Paper	HIPPURATE HYDROLYSIS	Cephalothin	Nalidixic Acid	C-19 FATTY ACID REDUCTION
Campylobacter jejuni	–	+	+	+	–	+	+[a]	R	S	+
Campylobacter coli	–	+	+	+	v	+	–	R	S	+
Campylobacter lari	–	+	+	+	–	+	–	R	R	+
Campylobacter fetus subsp. *fetus*	+	+	v	+	–	v	–	S	R	–
Campylobacter hyointestinalis	v	+	v	+	+	+	–	S	R	+
Helicobacter cinaedi	–	+	–	+	–	+	–	S	S	–
Campylobacter upsaliensis[b]	–	+	+[c]	+	–	+	–	S	S	–
Helicobacter fennelliae	–	+	–	–	–	+	–	S	S	–

[a]Approximately 5% to 10% of *C. jejuni* strains are hippurate negative.
[b]Catalase negative or weak.
[c]Occasional isolates fail to grow at 42°C.
–, Does not have the characteristic; +, has the characteristic; *R,* resistant; *S,* susceptible; *TSI,* triple sugar iron agar slant; *v,* variable (some strains show the characteristic).

TABLE 216.2 *Campylobacter, Helicobacter,* and *Arcobacter* Species Associated With Different Clinical Manifestations of Infection

ENTERIC DISEASE	EXTRAINTESTINAL DISEASE
Major Pathogen	**Major Pathogen**
Campylobacter jejuni	*Campylobacter fetus*
Minor Pathogens	**Minor Pathogens**
Campylobacter coli *Campylobacter lari* *Campylobacter fetus* *Helicobacter fennelliae* *Helicobacter cinaedi* *Campylobacter upsaliensis* *Arcobacter butzleri* *Arcobacter skirrowi* *Arcobacter cryaerophilus*	*Campylobacter jejuni* *Campylobacter coli* *Campylobacter lari* *Helicobacter fennelliae* *Helicobacter cinaedi* *Campylobacter sputorum* *Campylobacter hyointestinalis* *Helicobacter rappini*



FIG. 216.1 Fine-curved, S-shaped, or spiral, lightly staining gram-negative appearance of *Campylobacter jejuni* in pure culture (×1000).

size (0.3–0.6 μm in diameter) and motility, *Campylobacter* spp. and related organisms pass through 0.45- or 0.65-μm filters that retard the usual enteric flora. Filtration methods permit isolation without use of antibiotic-containing media. It is now clear that use of filtration techniques and nonselective rich media such as chocolate agar, with incubation of plates at 37°C, improves stool culture yields of both *C. jejuni* and the "atypical" enteric *Campylobacter* spp.[11,12] The development of filtration techniques represents a significant advance over the use of selective media, and such techniques are now recommended for primary isolation of *Campylobacter* spp. from fecal specimens or swabs.

Visible colonies usually appear on the plating media within 24 to 48 hours, and usually longer for the "atypical" species. *Campylobacter* spp. can be distinguished from other microorganisms on the basis of several standard criteria and can be distinguished from one another on the basis of biochemical testing.[4,13] Organisms from young cultures have a typical vibrioid appearance (Fig. 216.1), but after 48 hours of incubation, organisms appear coccoid. The ability to hydrolyze hippurate distinguishes *C. jejuni* from most other members of the genus, but hippurate-negative *C. jejuni* isolates also occur. Isolation of organisms from sites without a normal microbiota, such as the bloodstream, is not difficult, although when *Campylobacter* is the suspected pathogen, incubation of cultures should be extended to 2 weeks. With radiometric detection systems, turbidity of the medium may not be present, and the increase in released radiolabeled substance may be less than usually specified thresholds, reflecting suboptimal conditions for certain of these organisms.[14] State-of-the-art identification to the species level should include polymerase chain reaction studies of 16S recombinant RNA (rRNA) or other targets for comparison with known species.[15–17] As discussed later (see "Diagnosis"), these techniques have been developed for culture confirmation and for typing of strains and may be more sensitive than traditional culture methods.[18–20]

As with other bacteria whose ecologic niche is the gastrointestinal tract of mammals and avian species, the serotypical diversity of *C. jejuni* is enormous. More than 90 different serotypes based on somatic (O) antigens and 50 different serotypes based on heat-labile (capsular and flagellar) antigens have been identified[4]; phase variation of flagellar

antigens occurs. O-antigen variation reflects the presence of differing genetic cassettes that contain the enzymes for O-antigen formation. No group somatic or flagellar antigen has been identified; however, several superficial proteins appear to have broad serotypical specificity, a factor that may aid in the development of a broadly specific vaccine.

C. jejuni cannot long withstand drying or freezing temperatures, which are characteristics that limit its transmission.[21] However, *C. jejuni* survives in milk or other foods or in water kept at 4°C for several weeks. Pasteurization effectively destroys the organism, as does chlorine at concentrations in standard use for water disinfection.

EPIDEMIOLOGY

Campylobacteriosis is a worldwide zoonosis. *Campylobacter* spp. are commonly found as commensals of the gastrointestinal tract in wild or domesticated cattle, sheep, swine, goats, dogs, cats, rodents, and all varieties of fowl.[3,21] *C. jejuni* has a very varied reservoir, but *Campylobacter coli* and *Campylobacter hyointestinalis* are most commonly isolated from swine, and *C. upsaliensis* is most commonly isolated from dogs.[22,23] *C. fetus* subsp. *fetus* has been isolated from sheep, cattle, poultry, reptiles, and swine.[3] Primary acquisition of *Campylobacter* spp. by animals often occurs early in life and may lead to morbidity or mortality, but in most colonized animals a lifelong carrier state develops. The vast reservoir in animals is probably the ultimate source for most enteric *Campylobacter* infections in humans. Meats originating from infected animals frequently become contaminated with intestinal contents during the slaughtering process.[21] In particular, commercially raised poultry is nearly always colonized with *C. jejuni,* slaughterhouse procedures amplify contamination, and chicken and turkey in supermarkets, ready for consumers to take home, frequently are contaminated.[21,24] Excreta from infected animals may contaminate soil or water. Most infections in humans probably result from consumption of contaminated food and water. Investigations of more than 50 outbreaks indicate that unpasteurized (raw) milk is such a vehicle.[21,25] Similarly, untreated surface water has been responsible for both endemic and epidemic campylobacteriosis. Backpackers in Wyoming who drank untreated water and developed acute diarrheal illnesses had three times more *Campylobacter* infections than *Giardia* infections.[26] Several large outbreaks have been traced to defects in municipal water systems.[27,28] Undercooked meats, especially poultry, have been associated with infection.[29–33] Other vehicles include raw clams, raw or undercooked beef, and unpasteurized cheeses and goat's milk. In one US study, children riding in grocery store shopping carts next to raw meat or poultry had higher rates of *Campylobacter* infections.[34,35] Nevertheless, consumption of undercooked poultry is estimated to be responsible for 50% to 70% of sporadic *Campylobacter* infections in developed countries. Increases in the isolation of *Campylobacter* spp. reflect both improved recognition and increased consumption of poultry in recent years.

Direct contact with infected animals may result in transmission. Household pets, especially young dogs and cats with diarrhea, have been implicated as vectors for campylobacteriosis.[19,22] In 2017, a large multistate US outbreak of multidrug-resistant *C. jejuni* infections occurred in puppies and was transmitted to more than 100 people.[36] Because healthy dogs, cats, rodents, and birds may excrete *Campylobacter* and related organisms, it is not surprising that human infections associated with these animals also have been reported. People with occupational exposure to cattle, sheep, and other farm animals are at increased risk for infection, and laboratory-acquired infections have been reported. *C. fetus* strains in reptiles and mammals probably diverged 200 million years ago[37]; however, humans may become infected with reptile strains, possibly due to consuming a food of reptile origin. Most reported strains in the United States and elsewhere have been from peoples of Asian origin,[38] suggesting that some particular contaminated food is involved. The reptile isolates are sufficiently distinct that a new subspecies has been proposed: *C. fetus* subsp. *testudinum.*

As with other enteric pathogens, fecal-oral person-to-person transmission of *C. jejuni* has been reported. People in contact with the excreta of infected individuals who are not feces continent (e.g., infants) are at risk for infection. Infected school-age children rarely may transmit *Campylobacter* infection. Transmission from infected food handlers who are asymptomatic is at best uncommon. Perinatal transmission from a mother who may not have been symptomatic may be due to exposure in utero, during passage through the birth canal, or during the first days of life.[39] Infection has been associated with blood transfusion from an infected patient.[40] Because of a variety of sexual practices, homosexual men appear to be at increased risk for infection caused by *H. cinaedi, H. fennelliae,* and other "atypical" *Campylobacter* spp.[41] Human immunodeficiency virus (HIV)–infected patients are at substantially increased risk for infection.[42] The standardization of serotyping methods[43] and the development of molecular methods for identification and typing of *C. jejuni* and related organisms[15–20,44,45] should improve our understanding of transmission.

C. jejuni infections occur year-round in the United States and other developed countries but with a sharp peak in summer and early fall. *C. fetus* infections show the same seasonal variation, but the peak is less marked. The reason for this seasonal variation is unclear. In developed countries, the incidence of infection is higher when air temperatures rise.[46] Flies also have been suggested as a potential source of transmission to humans.[47] In tropical countries, the seasonal variation of *C. jejuni* infection appears to be influenced by rainfall.

For many years, the incidence of *C. jejuni* infections continued to rise in the United States and Europe and exceeded rates of *Salmonella* and *Shigella* infections combined.[48,49] Beginning in the mid-1990s, the incidence has waxed and waned but *C. jejuni* remained one of the most frequently reported pathogens that cause foodborne illness. Improved hygienic practices on farms and especially in poultry slaughterhouses may have contributed to stabilization in disease frequency. Beginning in 2014, the incidence began to increase again.[50,51] Indeed, in 2017, the most recent year reported by the Centers for Disease Control and Prevention's Foodborne Diseases Active Surveillance Network (FoodNet), the incidence of *Campylobacter* rose by 10%, climbing to 19.1 per 100,000 population.[52] Over the past decade, the incidence of *Campylobacter* infections has also increased in Europe and Israel.[53,54] The reasons for these increases are not obvious, but increasing reliance on culture-independent diagnostic tests (CIDTs) could be playing a role. In the United States, the number of *Campylobacter* cases diagnosed by CIDTs alone increased by 114% in 2016 when compared with 2013 to 2015.[55] Because CIDT methods are quicker and more readily available, clinicians may order these tests more frequently, making year-to-year comparisons difficult. As a result, changes in *Campylobacter* incidence over time and determinations of the effectiveness of prevention strategies are becoming increasingly challenging. Nevertheless, *Campylobacter* infections have always been underdiagnosed vis-à-vis infections with Enterobacteriaceae, due to their slow and fastidious nature in culture, the need for specialized culture conditions, and the importance of laboratory experience in isolating and identifying these organisms. The increasing use of CIDTs is permitting a better assessment of *C. jejuni* infections, but unless the constituents of the panels are broadened in the future, will fail to identify infections due to the less common species, further underdiagnosing these infections. Other technologic advancements, such as whole-genome sequencing of *Campylobacter* isolates, may be available in the future for use in outbreak investigations and in further characterizing the epidemiology of these infections.[56]

Based on current estimates, there are probably more than 2 million *Campylobacter* infections annually in the United States, although there is great geographic variability in incidence rates, even within the United States.[57] Population-based studies show peak incidence in children younger than age 1 year and in people 15 to 29 years old[58]; however, cases have been reported in patients of all ages. The incidence in males may be higher. The prevalence of infection in healthy people is very low (<1%).

The epidemiology of infection in developing countries is markedly different. *C. jejuni* is often isolated from healthy people, and the infection is especially common during the first 5 years of life.[59–61] During the first 2 years of life, most children have numerous *Campylobacter* infections, but those occurring early in life frequently are symptomatic, whereas later infections are mostly asymptomatic.[60] The source of these frequent infections has not been defined, but preliminary evidence suggests that human-to-human transmission may be more common than in developed countries. The substantial age-related difference in the infection-to-illness ratios in developed and developing countries

appears primarily to be due to differences in age- or exposure-related immunity of the populations rather than to differences in the isolates.[62,63] Areas where *Campylobacter* infection is most prevalent are associated with growth shortfalls among children.[64] Interestingly, even in developed nations, the incidence of infection in rural areas is higher, similar to patterns observed in developing nations, and possibly associated with more direct contact with animal vectors of the infection.[65] *C. jejuni* and other *Campylobacter* spp. are important causes for the acute diarrheal illnesses suffered by travelers.[66]

PATHOGENESIS AND PATHOLOGIC CHARACTERISTICS

The absence of a nonprimate animal model that is closely analogous to human infection makes understanding *Campylobacter* pathogenesis more difficult. However, it is clear from outbreak investigations and from volunteer studies that not all *Campylobacter* infections produce illness. Although all factors responsible for this phenomenon are not known, three of the most important appear to be the dose of organisms reaching the small intestine, the virulence of the infecting strain, and the specific immunity of the host to the pathogen ingested. Among exposed people who become ill, the incubation period varies from 1 to 7 days, a characteristic that is probably inversely related to the dose ingested. Although there are reports of illness occurring within 24 hours of exposure,[67,68] most infections occur 2 to 4 days after exposure. In one study, volunteers became ill after ingesting as few as 500 organisms, but with a dose of less than 10^4 organisms, illness was infrequent.[69,70] *C. jejuni,* like *Salmonella typhimurium,* is susceptible to hydrochloric acid.[70] Taken together, these data suggest that the infectious dose for *C. jejuni* is similar to that for *Salmonella.* The acidic milieu of the stomach provides an effective barrier against *Campylobacter* infection. However, vehicles such as milk, fatty foods, and water that favor passage through the gastric acid barrier may permit some infections to occur at relatively low doses. Similarly, patients who use proton pump inhibitors or histamine type 2 blockers are more susceptible to infection.[71,72] Also, some *Campylobacter* spp. appear to be well adapted to survival outside animal hosts and are more resilient to physical stresses[73]; these strains may be more available to infect humans.

C. jejuni multiplies in human bile[70] a characteristic that aids colonization of the bile-rich upper small intestine early in infection. The sites of tissue injury include the jejunum, ileum, and colon, with similar pathologic features in each. Inspection of affected tissues may reveal a diffuse, bloody, edematous, and exudative enteritis,[74] but pathologic examinations are generally only performed on specimens from patients with the most severe cases. Microscopic examination of rectal biopsy specimens has shown a nonspecific colitis with an inflammatory infiltrate of neutrophils, mononuclear cells, and eosinophils in the lamina propria; degeneration, atrophy, loss of mucus, and crypt abscesses in the epithelial glands; and ulceration of the mucosal epithelium.[75,76] Rectal biopsy samples with these nonspecific features have been interpreted as showing acute ulcerative colitis or Crohn disease. In other cases, the appearance of the rectal biopsy sample has been similar to that of specimens obtained in *Salmonella* or *Shigella* infections. In a series of 124 patients with *C. jejuni* infection, 18 of the most severely ill patients underwent sigmoidoscopic examination or rectal biopsy; 17 of these procedures showed colonic involvement.[77] Some patients have terminal ileitis as well as colitis. Unspecified host factors are also clearly important; in volunteers, a single strain produced a wide spectrum of clinical manifestations.[69]

The presence of bacteremia in some patients, the finding of cellular infiltration in biopsy specimens, and the presence of blood in stools from patients with *Campylobacter* colitis also suggest that tissue invasion occurs. The process of *C. jejuni* invasion is multifactorial. Some evidence suggests *C. jejuni* breaches epithelial cell barriers via a paracellular route by disruption of tight junctions.[78] Other evidence exists for basolateral and apical transcellular routes of invasion.[79] Invasion requires microtubule-dependent mechanisms,[80,81] and subsequently *Campylobacter* organisms are contained within a compartment that does not fuse with lysosomes but allows release of the organisms to the basolateral side of the epithelial cell and underlying lamina propria.[82] Subsequent detection of the organisms by the host immune system leads to an influx of inflammatory cells and cytokines/chemokines and to tissue destruction, which ultimately produce the disease signs and symptoms. The bacteria's flagellae are also important virulence factors because they promote the motility and chemotaxis needed for *C. jejuni* to colonize the intestinal tract.[83,84] The bacterial flagellar export apparatus is involved in the secretion of a number of proteins that affect invasion.[85–87] Some secreted proteins produce rapid apoptotic death of cell cultures[88]; others may modulate the immune response to favor bacterial survival.[89] However, the role of these proteins in causing diarrhea is not known. Unlike other enteric pathogens such as *Salmonella, Campylobacter* flagellins are not recognized by the host pattern recognition receptor Toll-like receptor (TLR) 5 due to differences in specific regions of flagellin genes, and do not elicit production of the proinflammatory cytokine interleukin-8, suggesting that flagellins are involved in evasion of innate immune responses in their reservoir hosts.[90,91]

Unlike other pathogens such as *Escherichia coli*, the *C. jejuni* genome does not encode any conventional known enterotoxins.[1] Some strains express the cytolethal distending toxin (Cdt), named for its effect on mammalian cell lines,[91] but its role in pathogenesis is unclear because it is not always present in strains isolated from patients with diarrhea.[92] However, this toxin may affect cell cycle kinetics[93,94] and may play a role in suppressing innate immunity by inducing death of macrophages.[95,96]

A high-molecular-weight plasmid (pVir) encodes proteins involved in secretion and also enhances the invasive capabilities of *C. jejuni* virulence.[97,98] It has been identified in some clinical *Campylobacter* isolates and has been significantly associated with bloody stools, although strains lacking pVir may remain virulent.[99,100] Several epithelial cell adhesins have been identified in *C. jejuni.* The superficial antigen (PEB1) that appears to be the major adhesin[101] and is conserved among *C. jejuni* strains also is a target of the immune response,[102] and may represent a vaccine candidate.[103,104] Other important adhesins include JIpA, a surface-exposed lipoprotein,[105] and CadF, which mediates adhesion by binding to fibronectin.[106] Acquisition of ferrous and ferric iron in the gut is critical for colonization by *C. jejuni,* and the molecules involved in the process may be considered as virulence factors and targets for interventions as well.[107]

Campylobacter outer membranes contain lipopolysaccharides (LPSs) with typical endotoxic activity.[108–110] The structure of the LPS O antigen is highly variable.[109,110] Many *C. jejuni* O antigens possess sialic acid–containing structures that are recognized by host immune cells bearing sialoadhesins.[111–113] The close resemblance of these sialylated structures to those seen in human gangliosides such as GM1, GD1a, GD3, and GT1a and their presence in strains isolated from patients who developed the Guillain-Barré syndrome (GBS) support a role in the pathogenesis of this disorder, via molecular mimicry.[111,112,114] Certain capsular genotypes are also associated with the sialylated lipooligosaccharide structures implicated in GBS, raising the possibility that capsule also may contribute to GBS pathogenesis.[115] Sialylated lipooligosaccharides confer resistance to host cationic antimicrobial peptides and proteins[116] and may also be associated with more severe gastrointestinal disease.[117]

Bacteremia can sometimes be detected in patients with *Campylobacter* infections, whether or not they show signs of systemic illness. Most bacteremias reported to the Centers for Disease Control and Prevention have been due to *C. fetus* subsp. *fetus,* whereas *C. jejuni* is by far the more common pathogen overall. One explanation for the apparently greater tendency of *C. fetus* to cause bacteremia is that it is usually resistant to the bactericidal activity present in normal human serum.[118] Important risk factors for *C. fetus* bacteremia and meningitis include immunosuppression and occupational exposure to infant animals that are the reservoir hosts for *C. fetus.*[119,120] *C. fetus* is covered with a surface (S)–layer protein that functions as a capsule.[121,122] Virtually all human isolates of *C. fetus* possess an S-layer protein that completely disrupts C3b binding to these organisms.[123] Lack of C3b binding explains both serum and phagocytosis resistance.[120] *C. fetus* also has the ability to change the major S-layer protein expressed. This results in antigenic variation[124] and is facilitated by recombination among several highly homologous genes encoding full-length proteins.[125,126] The S-layer protein of *C. fetus* is the major virulence factor explaining its extraintestinal spread (Fig. 216.2).

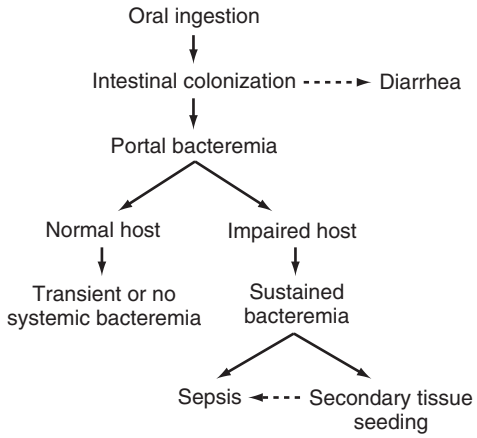

FIG. 216.2 Pathogenesis of *Campylobacter fetus* infections. *(From Blaser MJ. Campylobacter fetus: emerging infection and model system for bacterial pathogenesis at mucosal surfaces.* Clin Infect Dis. *1998;27:256–258.)*

IMMUNOLOGY

The adaptive human immune responses responsible for protection and recovery from *Campylobacter* infection are not completely understood. Immunocompetent individuals who develop *C. jejuni* diarrheal disease usually resolve the diarrhea and become asymptomatic without antibiotic treatment. Protection from recurrent disease may not develop following a single infection, as has been shown in human experimental infection and reinfection models of *C.jejuni*[127]; early antibiotic treatment might thwart the development of a full immune response to the infection. Patients infected with *Campylobacter* spp. develop specific immunoglobulin (Ig) G, IgM, and IgA antibodies in serum[128,129] and IgA antibodies in intestinal secretions.[129,130]

In low-income countries, where *C. jejuni* infection is hyperendemic, serum IgA levels rise progressively with age, and the decreasing case-to-infection ratio with age suggests acquisition of protective immunity following multiple exposures. Further supporting the importance of humoral immunity are multiple reports of severe and recurrent *C. jejuni* infection in patients with congenital or acquired hypogammaglobulinemia.[131,132] In HIV-infected patients as well, failure of *C. jejuni* infection to respond to antimicrobial therapy has been correlated with failure to produce a humoral response to infection.[131] Overall, patients with immunodeficiency from HIV have a markedly increased incidence of *C. jejuni,*[42,133] although the role of cellular immunity is not well understood. The heightened incidence is consistent with an epidemiologic model of wide circulation of the organism in human foods, but most often at low doses to which immunocompetent hosts are not susceptible, but to which immunocompromised hosts are.

Components of the innate immune response have been demonstrated to both limit infection and trigger inflammation. *C. jejuni* is serum sensitive, allowing for complement-mediated killing as well as opsonization followed by phagocytosis.[134] Phagocytes, intestinal epithelial cells, and many other cells express the key pattern recognition receptors TLRs 2 and 4, which recognize cell wall components and LPS/lipooligosaccharide. Engagement of these receptors triggers proinflammatory signaling. This leads to the production of inflammatory cytokines (e.g., interleukin-6, tumor necrosis factor-α) and chemokines (e.g., interleukin-8) that assist in the recruitment and activation of host cells that kill *C. jejuni* directly via neutrophilic phagocytosis, for example.[135,136] Unlike most other enteric pathogens, the flagellin of *C. jejuni* is not recognized by TLR5, and TLR9 is poorly activated by *C. jejuni* as well, suggesting these are means of immune evasion.[137,138] Nonetheless, *C. jejuni* is susceptible to killing by cationic antimicrobial agents, including α- and β-defensins produced by intestinal epithelial cells and phagocytes.[139]

As discussed earlier, one important virulence factor in *C. fetus* that is not found in *C. jejuni* is a proteinaceous paracrystalline array attached to the O antigen of LPS known as an S layer. This renders *C. fetus* serum resistant, owing to the inability of the complement component C3b to opsonize its surface.[140] Patients with *C. fetus* infections much more frequently have evidence of impaired immunity, including conditions such as chronic alcoholism, liver disease, old age, diabetes mellitus, and malignancies,[141,142] permitting an organism that a priori can resist innate immunity to take hold.

CLINICAL MANIFESTATIONS

Campylobacter jejuni Infections

The clinical manifestations of infections caused by all of the *Campylobacter* spp. that cause enteric illnesses appear identical; *C. jejuni* infection may be regarded as the prototype. Acute enteritis is the most common presentation of *C. jejuni* infection. Symptoms may last from 1 day to 1 week or longer. Often, there is a prodrome with fever, headache, myalgia, and malaise 12 to 24 hours before the onset of intestinal symptoms.[143] In some patients, the constitutional symptoms may coincide with the intestinal phase or, less often, may follow it. The most common symptoms are diarrhea, malaise, fever, and abdominal pain.[143–145] Diarrhea may range in severity from loose stools to massive watery or grossly bloody stools. In any patient, the entire spectrum of diarrhea may be seen. For most patients, there are 10 or more bowel movements on the worst day of the illness. Abdominal pain is usually cramping and is relieved by defecation; it may be the predominant manifestation of illness. *Campylobacter* enteritis is frequently self-limiting, with a gradual resolution of symptoms over several days; however, illness lasting longer than 1 week occurs in 10% to 20% of patients seeking medical attention, and relapse may be seen in another 5% to 10% of patients who do not receive treatment.[44,143–146]

Infection also may be manifested as an acute colitis, with symptoms of fever, abdominal cramps, and bloody diarrhea persisting for 1 week or longer.[75,145] Fever may be low grade or consist of daily peaks above 40°C (104°F). Initially, stools may be watery, but as the illness progresses they may become frankly bloody; tenesmus is a common symptom. In the most severe forms, patients appear very ill, and toxic megacolon has been reported.[147] Because of the propensity of *Campylobacter* infection to affect young adults and the characteristic clinical presentation, it may be readily confused with ulcerative colitis or Crohn disease.[75,143] The pathologic findings on rectal biopsy are nonspecific, and the clinical features and radiographic findings are also nondiagnostic. Therefore the clinician should have a high index of suspicion for *Campylobacter* infection in a patient who presents with this symptom complex. Because of the often fastidious nature of these organisms,[148,149] a single negative culture does not rule out infection, especially if optimal filtration methods are not used for primary isolation of a pathogen.

Occasionally, acute abdominal pain may be the major or only symptom of infection.[150] Although any quadrant of the abdomen may be affected, patients most often complain of pain in the right lower quadrant. As with *Yersinia enterocolitica* and *Salmonella enteritidis, C. jejuni* may cause pseudoappendicitis.[44,143] In most cases, the removed appendix has shown minimal or no inflammation. Enlarged mesenteric nodes (mesenteric adenitis) and terminal ileitis[44] also may be responsible for symptoms. Diagnosis is often made during the postoperative period, when diarrhea ensues. *Campylobacter* infection occasionally may present solely as a gastrointestinal hemorrhage.[151] Among neonates, *C. jejuni* infection may be manifested as one or more grossly bloody stools and no other symptoms, with findings suggesting intussusception,[152] or with extraintestinal foci.[153] Fever also may be the sole manifestation of *C. jejuni* infection. Temperature elevation may be so severe and persistent that typhoid fever is the initial diagnosis until *C. jejuni* is isolated from stools. Febrile convulsions in young children before the onset of the enteric phase of illness also may occur.[154]

Bacteremia has been noted in less than 1% of patients with *C. jejuni* infection. In part, this low frequency reflects the fact that physicians rarely perceive diarrheal illness as an indication for blood culture, even when fever is present. Nevertheless, bacteremia appears to be more common in infections in people at the extremes of age.[58,155,156] Meningitis and endocarditis are rare manifestations of *C. jejuni* infection. In general, three patterns of extraintestinal *C. jejuni* infection have been noted.[157] First, there may be a transient bacteremia in a normal host with acute *Campylobacter* enteritis. The bacteremia may be discovered several days after blood cultures are obtained, by which time the patient usually has completely recovered. The course is benign, and no specific treatment

based on the positive blood culture result is usually indicated. Second, there may be a sustained bacteremia or deep focus of infection in a previously normal host; usually the patient has an acute enteritis as well. The *C. jejuni* isolates are generally relatively or absolutely serum resistant.[157] Bacteremia usually has its origin in the intestinal tract inflammation and responds to antimicrobial therapy. Third, sustained bacteremia or deep infection may occur in an immunocompromised host; many such patients do not have an acute enteritis. *C. jejuni* isolates are usually serum sensitive.[157] However, as with other gram-negative bacteria, *C. jejuni* bacteremia may produce fever and shock.[158] Antimicrobial therapy, which may need to be prolonged, is required for elimination or suppression of this infection.

C. jejuni may cause septic abortion,[159] but sustained bacteremia in a pregnant patient does not necessarily imply fetal infection or a bad outcome.[157] There have been infrequent reports of *C. jejuni* infections manifesting as acute cholecystitis,[160] pancreatitis,[161] and cystitis.[162] People with immunoglobulin deficiencies often develop prolonged, severe, and recurrent *C. jejuni* infections,[131,132] often with bacteremia and other extraintestinal manifestations such as erysipelas-like skin lesions or osteomyelitis.[163]

Long-term colonization by immunocompromised hosts, such as common variable immunodeficiency, has been recognized and may last for years.[164] Symptomatic and asymptomatic recrudescence of *C. jejuni* infection following appropriate antibiotics in healthy adults is also a newly recognized phenomenon, and suggests that *C. jejuni* both may be shed in the feces for longer than recognized postinfection and may persist in the host in a protected site such as intestinal biofilms.[165]

C. jejuni infections have been associated with multiple postinfectious sequelae, especially GBS but also reactive arthritis, irritable bowel syndrome, and immunoproliferative disorders of the small intestine. GBS is a strongly associated but uncommon consequence of *C. jejuni* infection (estimated at 1 case per 2000 infections) that usually occurs 2 or 3 weeks after the diarrheal illness.[166,167] From 20% to 50% of GBS cases follow *C. jejuni* infections, reflecting in part the high incidence of these infections.[166–170] A particular group of *C. jejuni* organisms marked by LPS serotype O:19 is overrepresented among people who develop GBS.[170–172] A recent study demonstrated that *C. jejuni* O:19 chaperone proteins share high primary sequence homology with heat shock proteins found on human peripheral nerves; these structures as well as capsular and LPS glycolipids may be involved in the molecular mimicry triggering GBS.[173] Serotype O:41 has also been implicated, and other sporadic cases may be due to specific *C. jejuni* strains with sialylation of their LPS molecules.[174–176] Postinfectious reactive arthritis may occur up to several weeks after infection, and prolonged rheumatic symptoms have also been reported. The relation of this phenomenon to the presence of HLA-B27 histocompatibility antigens is not clear.[177–179] The organism was detected by polymerase chain reaction assay in small intestinal biopsy specimens from patients with mucosa-associated lymphoid tissue (MALT),[180] analogous to the role of *H. pylori* in gastric MALT lymphomas. Myopericarditis,[181,182] hepatitis,[183] cellulitis,[184] interstitial nephritis, the hemolytic-uremic syndrome, and IgA nephropathy[185] are other reported complications.

Campylobacter fetus Infections

Although *C. fetus* may occasionally cause diarrheal disease, it has the propensity to cause dissemination, including bacteremia, endovascular infections, and cellulitis, and may do so in the absence of intestinal symptoms.[141,186] As summarized in Table 216.3, the clinical, laboratory, and epidemiologic characteristics of *C. jejuni* infections differ significantly from those of *C. fetus* subsp. *fetus,* which often produce systemic manifestations. *C. fetus* infections may cause intermittent diarrhea or nonspecific abdominal pain without localizing signs. The diarrheal illness may manifest exactly like *C. jejuni* infection and is more common than was suspected several years ago. Clinical manifestations are similar and sequelae uncommon. Nearly all affected patients survive the infections when appropriate antibiotic treatment is given and usually do well without antibiotic treatment. *C. fetus* also may cause a prolonged relapsing illness characterized by fever, chills, and myalgias, in which a source of infection cannot be demonstrated.[142,187] Occasionally, secondary seeding of an organ will occur, leading to a more complicated infection[187,188] and sometimes to a fulminant, fatal course.

TABLE 216.3 Biologic and Clinical Characteristics of *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus*

FEATURE	*CAMPYLOBACTER JEJUNI*	*CAMPYLOBACTER FETUS* SUBSP. *FETUS*
Epidemiologic Characteristics		
Major reservoir	Avian species, food animals	Cattle and sheep (reptiles)
Affected hosts	Normal hosts; all ages affected; often in clusters of cases	Opportunistic agent in debilitated hosts; clustering rare; healthy hosts may be affected
Laboratory Characteristics		
Range of growth temperatures	32°–42°C	25°–37°C[a]
Usual source of isolation	Feces	Bloodstream
Clinical Characteristics		
As a cause for diarrheal illness	Common	Uncommon
Clinical manifestations	Acute gastroenteritis, colitis	Systemic illness with bacteremia, meningitis, vascular infections, abscesses; gastroenteritis
Outcome of infection	Usually self-limited	May be fatal in debilitated hosts

[a]Occasionally grows at 42°C.

C. fetus infections appear to have a predilection for vascular sites; vascular necrosis occurs in patients with endocarditis and pericarditis resulting from this organism.[189–191] Mycotic aneurysms of the abdominal aorta and, rarely, peripheral arteries also occur.[192] Thrombophlebitis may be associated with *C. fetus* bacteremia, but whether it is the primary event or a secondary manifestation of the infection is uncertain. Patients with a bacteremic illness without localization should be carefully evaluated for the presence of septic thrombophlebitis, because the response is good when this condition is treated with appropriate antibiotics. Infections during pregnancy primarily have been manifested as upper respiratory tract symptoms, pneumonitis, fever, and bacteremia. However, four of five *C. fetus*–infected second-trimester patients were delivered of dead infants despite antibiotic therapy. One patient received antibiotic therapy and had a normal term infant. All the mothers survived their infection.[193]

Central nervous system (CNS) infections with *C. fetus* occur in neonates and adults. The prognosis is poor for premature infants, but five of six full-term neonates in one series survived infection. Infection is manifested as a meningoencephalitis with a cerebrospinal fluid polymorphonuclear pleocytosis. Subdural effusion may complicate infection. Meningoencephalitis is also the most common CNS manifestation of *C. fetus* infection in adults.[194] Cerebrovascular accidents, subarachnoid hemorrhages, and brain abscesses also occur. The prognosis is better in adults than in neonates, with a survival rate of approximately 67%, although neurologic sequelae are frequent. *C. fetus* has been shown to cause a variety of other types of localized infections, including septic arthritis, spontaneous bacterial peritonitis, salpingitis, lung abscess, empyema, cellulitis, urinary tract infection, vertebral osteomyelitis, and cholecystitis.[195,196] Although most patients with these illnesses recovered with appropriate antibiotics and drainage procedures, the clinical course was frequently prolonged and relapsing. Antibiotic resistance to fluoroquinolones may develop in immunocompromised patients who receive monotherapy regimens.[197] Nevertheless, in other patients, self-limiting bacteremia without any sequelae has been observed. Hypogammaglobulinemic patients may have persistent bacteremia and local symptoms unless given chronic suppressive therapy with antibiotics.

C. fetus is found in multiple animals and animal products, mainly cattle and sheep, which are probably the main source of human infection.[198]

Foodborne sources of infection likely include raw milk products, raw liver, and raw meat of such animals.[199] Similarly, reptiles may carry a different group of *C. fetus* strains,[200,201] and human infections with these organisms have been reported as well.[198,202] Reports of a cluster of *C. fetus* cases among men who have sex with men suggest the possibility of person-to-person transmission.[203]

Infection Caused by Other Enteric *Campylobacter* Species

The clinical manifestations of infection caused by other enteric *Campylobacter* spp. overlap substantially with those of *C. jejuni* infection.[149,204,205] On average, *C. coli* may produce milder disease.[62] In one series of homosexual men, *H. cinaedi* and *H. fennelliae* infections were more often asymptomatic than were those caused by *C. jejuni.*[206] Among immunocompromised patients, especially those with acquired immunodeficiency syndrome or hematologic malignancies, these "atypical" *Helicobacter* spp. have caused cellulitis, osteomyelitis, and bacteremia.[207–209] As with *C. fetus, C. upsaliensis* mostly causes diarrheal diseases in previously normal people[22,23,210] and bacteremia in immunocompromised hosts[211]; most strains of the latter species are serum resistant. Other extraintestinal manifestations, such as breast abscess, have been observed.[212] Although dogs have long been considered an important source of human *C. upsaliensis* infections, genetics studies have shown that human and canine strains are distinct.[213] Cellulitis may occur in immunocompromised hosts infected with any of a variety of these "atypical" species.[209] *C. hyointestinalis,* which resembles *C. fetus* in its biochemical characteristics,[214] also may cause bacteremia in immunocompromised hosts. *A. butzleri* may cause abdominal cramps without diarrheal illness.[215] *Helicobacter (Flexispira) rappini* has been reported to cause bacteremia in immunocompromised hosts.[216]

C. fetus subsp. *venerealis,* which had never been considered a human pathogen, was reported to have been isolated from stools from two homosexual men in Australia and from two women with bacterial vaginosis. *C. fetus* subsp. *fetus* has been isolated from two other patients with vaginosis. *Campylobacter curvus* has caused septicemia, liver abscess, and possibly chronic (Brainerd) diarrhea.[217,218] *Campylobacter sputorum* subsp. *sputorum,* which is indigenous to the human mouth and intestine, has been isolated from perianal boils and lung abscesses. *C. sputorum* subsp. *bubulus,* a commensal of sheep and cattle, has been isolated from boils and skin abscesses from humans. *Campylobacter insulaenigrae,* a recently identified species seen primarily in marine mammals, has been isolated from the stool and blood of a patient with end-stage renal and hepatic disease.[219] *Campylobacter concisus,* long believed to be part of the microbiota of healthy people, is now considered a possible cause of human gastrointestinal illness.[220–222] An increasing body of evidence also has linked *C. concisus* infection to childhood Crohn disease.[223–227] Other recently identified *Campylobacter* spp. that may be clinically relevant include *C. urealyticus,*[228–231] *C. troglodytis,*[232] *C. lari* subsp. *concheus, C. peloridis,*[233] *C. gracilis,*[234] and *C. fetus* subsp. *testudinum.*[235] Since these organisms are fastidious and can only be diagnosed by culture, it is likely that they are not as rare as it presently appears.

DIAGNOSIS

The gold standard for diagnosis of *Campylobacter* infection remains isolation by culture; however, multiple new non–culture-based techniques are now in use.

Bacteriologic Studies

Confirmation of the diagnosis of *C. jejuni* infection is based on a positive result on stool culture or, occasionally, blood culture, as described in the "Microbiology" section earlier. Because blood cultures are not often performed in the evaluation of patients presenting with diarrheal symptoms, the frequency of bacteremia is not known. *Campylobacter* spp. cannot be isolated from fecal specimens unless microaerobic incubation conditions and selective techniques that reduce the growth of competing microorganisms are used.[4,43] *C. fetus* is usually isolated from blood cultures 4 to 14 days after the specimen has been obtained.[142] Occasionally, *C. fetus* may be isolated from feces of patients with either diarrheal or systemic infections.[236] If *C. fetus* or another of the atypical species is suspected, incubation at 37°C and use of media without cephalosporins are necessary. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry can also provide rapid identification of clinical *Campylobacter* species (e.g., VITEK 2 system; bioMerieux USA, Durham, NC).

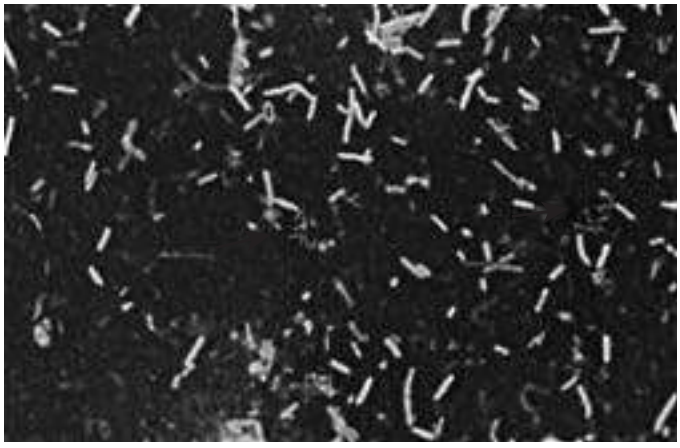

FIG. 216.3 Gram stain of fecal specimen from a patient with *Campylobacter* enteritis. Arrows point to typical gram-negative fine, small, spiral, and *Vibrio*-like organisms (×1024).

Molecular Techniques and Immunoassays

Use of enzyme immunoassays and nucleic acid amplification tests (NAATs, such as polymerase chain reaction) for *Campylobacter* diagnosis are also rapidly expanding in the clinical microbiology laboratory. Although no standardized guidelines exist for use, these techniques are significantly more rapid and sensitive than culture-based methods and often include at least *C. jejuni* and *C. coli.*[237] NAATs for *Campylobacter* diagnosis are usually part of a multiple enteric pathogen panel, including *Salmonella, Shigella*, and others.[237] However, when antibiotic resistance is a concern, the bacterial isolate is still needed and culture should be concurrently performed, especially in outbreak settings. In addition, NAATs detect bacterial DNA, not viable organisms, and should be interpreted with caution.

Direct Examination of Feces

Examination of diarrheal fecal specimens by darkfield or phase-contrast microscopy within 2 hours of passage can permit a rapid presumptive diagnosis of *Campylobacter* enteritis if the characteristic darting motility of the *Campylobacter* organism is seen.[145,238] This test is particularly useful in the acute phase of the illness. Similarly, the presence of vibrioid forms in Gram-stained stool specimens is a very specific diagnostic feature, although the sensitivity of this finding is 50% to 75% (Fig. 216.3).[239] Although infrequently performed, these techniques could be used by clinicians and laboratorians who want to make a rapid diagnosis in cases in which the index of suspicion for *Campylobacter* infections is high.

THERAPY

Fluid and electrolyte replacement constitutes the cornerstone of treatment of diarrheal illnesses. Patients with *Campylobacter* infections who are severely dehydrated should undergo rapid volume expansion using intravenous solutions of electrolytes in water. For patients with less serious volume depletion, oral rehydration using glucose and electrolyte solutions is indicated. People infected with *C. jejuni* who are ill enough to seek medical attention and from whom a fecal culture is obtained represent only a subset of all those infected. Nevertheless, even among these patients, fewer than half are candidates for specific antimicrobial therapy.[143] In a meta-analysis of 11 small randomized trials, antimicrobial agents reduced the duration of intestinal symptoms by approximately 1.3 days.[240] Because the greatest therapeutic benefit occurs when antimicrobial therapy is started early, the rapid presumptive diagnosis of *Campylobacter* infection by means of direct visualization of the organisms in stool is clinically relevant. Earlier diagnosis also has been

facilitated by the development of rapid CIDTs. Treatment with antibiotics seems prudent in those patients with high fever, bloody diarrhea, or more than eight stools per day; in patients whose symptoms have not lessened or are worsening at the time the diagnosis is made; or in those in whom symptoms have persisted for more than 1 week.[241]

In vitro, *C. jejuni* is susceptible to a wide variety of antimicrobial agents, including erythromycin, macrolides, the tetracyclines, the aminoglycosides, chloramphenicol, the quinolones, the nitrofurans, and clindamycin.[242,243] Erythromycin was once considered the agent of choice; however, because erythromycin, is primarily metabolized by cytochrome P-450 isoenzyme 3A4, there is a slight risk for sudden cardiac death. Therefore this agent is no longer recommended. We agree with the recommendation made in the 2017 Infectious Diseases Society of America guidelines that first-line therapy is either a macrolide or a fluoroquinolone.[244] Therapy with extended-spectrum macrolides such as clarithromycin or azithromycin should be equally effective.[245] The recommended dosage of azithromycin is 500 mg orally daily for 3 days; a single dose of 1000 mg may be equally effective, although this dosage is associated with more nausea. In children, the recommended dosage of azithromycin is 10 mg/kg per day for 3 days.

The dosage of ciprofloxacin is 500 mg orally twice daily for 5 to 7 days,[246] although as with azithromycin, a single dose (750 mg) may be just as effective.[247] Fluoroquinolones have the advantage of possessing activity across a broad spectrum of bacteria causing diarrheal illness, including *Campylobacter* spp. However, fluoroquinolones should be used with caution because rising rates of resistance to these agents have limited their usefulness in treating *Campylobacter* infections (see later section on "Resistance to Fluoroquinolones"). Another alternative agent is tetracycline, except in children younger than age 9 years; in such patients, clindamycin may be used. Most *C. jejuni* and *C. coli* isolates are not susceptible to most cephalosporins or penicillin, and these agents should not be used. However, amoxicillin or ticarcillin plus clavulanic acid appears to be universally effective.[248] Some macrolide- and fluoroquinolone-resistant *C. coli* strains may be susceptible to fosfomycin.[249] Susceptibility to sulfonamides and metronidazole is variable. Unlike in *Salmonella* infections, treatment with antimicrobial agents does not prolong carriage of *C. jejuni;* on the contrary, erythromycin eliminates carriage within 72 hours in most patients.[242]

H. cinaedi and those *Campylobacter* strains acquired in developing countries, especially *C. coli,* are more likely to be resistant to macrolides and tetracycline.[250] In such cases, when treatment is indicated, alternative agents should be used until susceptibility is known. Use of an antimotility agent appears to prolong duration of symptoms and has been associated with fatalities.[251] The necessity for treating septic or bacteremic episodes with agents other than macrolides has not been established. For those patients who appear very ill, treatment with gentamicin, imipenem, or chloramphenicol (where available) is indicated, but susceptibility tests should be performed. In hypogammaglobulinemic patients with recurrent *C. jejuni* bacteremias, fresh-frozen plasma with appropriate antibiotics may eradicate the infection[163]; oral immune globulin therapy may have some value as well for recurrent diarrheal illness.[252] Systemic *C. fetus* infections should be treated parenterally, but erythromycin should not be used.[253,254] Occasionally, systemic infections diagnosed only retrospectively by positive results on blood culture resolve after empirical oral therapy. In these cases, follow-up cultures are recommended; if results are no longer positive, further treatment is not required. If necessary, ampicillin treatment has been associated with good results. Patients with endovascular infections caused by *C. fetus* require at least 4 weeks of therapy, and gentamicin or ampicillin is probably the agent of choice.[254] Treatment with imipenem or meropenem constitutes another alternative. Infections of the CNS should be treated with ampicillin, imipenem, or chloramphenicol (where available) for 2 or 3 weeks. Patients with other serious infections should also receive parenteral gentamicin or another aminoglycoside, ampicillin, or imipenem for at least 2 weeks. Because antibodies to *C. fetus* are not usually present in serum from normal people, intravenous immune globulin is not helpful for this infection in immunodeficient patients.[188] For *C. fetus*–infected patients with diarrheal illness or other less severe infections, treatment need not be as intense or as prolonged.

TABLE 216.4 Resistance Mechanisms in *Campylobacter*

RESISTANCE MECHANISM	EXAMPLES
Modification of an antibiotic's target or alteration in the expression level of the target	Modification of the DNA gyrase target of quinolones/fluoroquinolones[258] Modification of the ribosomal A site target of tetracyclines via binding of bacterial TetO[259] Mutations in 23S ribosomal RNA target of macrolides[260,261] Variant forms of the dihydrofolate reductase target of trimethoprim[262]
Inability of the antibiotic to reach its target	Major outer membrane protein[263,264] Lipopolysaccharide and capsule-mediated exclusion[265]
Efflux of the antibiotic	Efflux of macrolides, fluoroquinolones, β-lactams, and tetracyclines via CmeABC[257]
Modification or inactivation of the antibiotic	Aminoglycoside-modifying enzymes[266] β-Lactamases[267–269]

Antibiotic Resistance

Campylobacter species are often naturally transformable organisms, allowing for the acquisition of resistance genes from other *Campylobacter* spp. as well as other gram-positive and gram-negative organisms.[255,256] These genetic elements represent a combination of endogenous and acquired genes that may be chromosomal-, transposon-, or plasmid-borne and include the resistance mechanisms shown in Table 216.4.[257–269]

High-level antibiotic resistance often occurs due to the combined effects of antibiotic efflux plus a second mechanism. The most well-studied efflux mechanism is a tripartite multidrug-resistance pump that also confers resistance to bile acids, known as CmeABC.[257,270] This pump is encoded by three genes: *cmeC,* corresponding to an outer membrane protein; *cmeA,* corresponding to the periplasmic portion, and *cmeB,* encoding the inner membrane drug transporter.[257,271] Additional potential efflux pumps that may contribute to antibiotic resistance (CmeDEF and CmeG) have also been reported, but their clinical significance is unclear.[272,273] Although there are numerous examples of resistance to specific antibiotics, the following discussion will be restricted to those antibiotics most commonly used to treat acute diarrhea: fluoroquinolones and macrolides.

Resistance to Fluoroquinolones

The fluoroquinolones (e.g., ciprofloxacin) are the most commonly used class of antibiotics used to treat acute bacterial diarrhea.[274] Because diarrhea due to *Campylobacter* is generally clinically indistinguishable from other causes of acute diarrhea, many cases are treated empirically with fluoroquinolones. Therefore fluoroquinolone resistance is of concern.

Through the late 1980s into the early 1990s, fluoroquinolone resistance rates were generally low in the United States and worldwide.[275,276] In 2013, the Centers for Disease Control and Prevention designated *Campylobacter* as a microorganism with a serious antibiotic resistance threat.[277] This was driven principally by the increase in fluoroquinolone resistance observed in *C. jejuni* from 15% in 1998 to 25% in 2015.[278] Resistance rates in certain parts of the world are much higher than in the United States—as high as 75% in travelers to Asia[279]—highlighting the need for an accurate travel history when assessing the traveler with diarrhea. Inappropriate and overprescription of fluoroquinolones in humans, combined with increased fluoroquinolone use in the poultry industry in particular, have contributed to the increased prevalence of fluoroquinolone resistance. In 2004, the US Food and Drug Administration reversed its prior approval for the therapeutic use of the veterinary fluoroquinolone enrofloxacin because of the concern that the observed increase in fluoroquinolone resistance in human isolates was due to an increase in fluoroquinolone-resistant poultry isolates.[276,280] Only approximately 2% to 3% of US isolates are resistant to azithromycin, and so macrolides are the first-line therapies.[244] Unfortunately, macrolide resistance rates are much higher in parts of Asia and Africa; for example,

in studies in South Africa and Nigeria, 80% or more of isolates were resistant to macrolides.[281,282]

The target of fluoroquinolones is bacterial DNA gyrase, which unwinds double-stranded DNA to allow for DNA replication and transcription. The two main subunits of DNA gyrase are encoded by *gyrA* and *gyrB*.[283] Fluoroquinolones form a stable complex with these enzymes on DNA, thereby inhibiting DNA replication and transcription, leading to bacterial cell death.[284] Resistance in *Campylobacter* occurs most commonly via a Thr-86-Ile substitution in the quinolone resistance-determining region (QRDR) of *gyrA*. Unlike fluoroquinolone resistance in *Salmonella* and *E. coli,* this single mutation confers high-level resistance to nalidixic acid and fluoroquinolones.[258] Because only the single Thr-86-Ile mutation is needed to mediate high-level resistance and does so without an apparent (or large) fitness cost, fluoroquinolone resistance appears quickly in animals and humans.[285–288] In contrast, less common mutations in *gyrA* (i.e., Asp-90-Asn and Ala-70-Thr) confer only intermediate-level resistance.[289] However, if the efflux pump CmeABC is present also, synergy between these two mechanisms produces high-level fluoroquinolone resistance.[290–292] Finally, although mutations in *gyrB* occur, none confers resistance to fluoroquinolones.[293]

Resistance to Macrolides

Macrolides, specifically azithromycin, are the treatments of choice for diarrheal illnesses due to *Campylobacter,* owing to the unacceptably high rates of fluoroquinolone resistance.[274] These antibiotics are large molecules (>700 Da) that inhibit protein synthesis by reversibly binding to the P site on the 50S subunit of bacterial ribosomes, leading to bactericidal or bacteriostatic effects.

The two principal mechanisms that mediate macrolide resistance in *Campylobacter* are (1) modification of the target site on bacterial ribosomes and (2) efflux via the multidrug-resistance efflux pump CmeABC. These mechanisms can work synergistically to produce high-level macrolide resistance in *Campylobacter.*[294,295] Specific point mutations at positions 2074 or 2075 (positions 2058 and 2059 in *E. coli* numbering) of the peptidyl encoding region in domain V of the 23S rRNA gene leads to high-level resistance when all three copies of the 23S rRNA genes are mutated.[261,296–298] There appears to be a gene dosage effect because strains harboring mutations on two 23S rRNA genes exhibit only intermediate resistance[299,300]; mutation in a single rRNA gene has not been reported. The low spontaneous mutation rate of the 23S rRNA genes (approximately 10^{-10} per cell per generation) contributes to the apparent higher barrier to the emergence of resistance compared with fluoroquinolones.[295] In another contrast to fluoroquinolone resistance, competition experiments suggest that macrolide resistance imparts a fitness cost.[301–305]

The second major mechanism of macrolide resistance is via the multidrug-resistance efflux pump CmeABC.[257,261] High-level resistance can occur in concert with ribosomal mutations as well as by mutation in *cmeB* alone.[306] For example, in macrolide-resistant strains that express wild-type 23S rRNA, gene disruption or silencing of *cmeB* or *cmeA* causes reversion to a macrolide-susceptible phenotype.[294,307] In other instances, the CmeABC efflux pump and rRNA mutations function synergistically to effect high-level macrolide resistance.[294,308,309]

PROGNOSIS

The vast majority of patients recover fully after *C. jejuni* infections, either spontaneously or after appropriate antimicrobial therapy. The "reactive arthritis," formerly called Reiter syndrome, occurring in human leukocyte antigen HLA-B27–positive people closely resembles that seen after *Yersinia, Salmonella,* or *Shigella* infections and is not specific to *C. jejuni* infection. However, rheumatologic symptoms may persist for several months or possibly for years in a few affected people. GBS is an uncommon sequela of *Campylobacter* enteritis, but because of their high prevalence, *Campylobacter* infections are the most important recognized antecedent of this clinically devastating disorder. Occasional deaths after *C. jejuni* infections have been reported in developed countries[251]; in most cases, the victim was an elderly person or an immunocompromised host. However, fatalities in previously healthy young adults may occur, probably as a result of volume depletion. Some of the deaths that occur in GBS patients can be attributed to the consequences of *C. jejuni* infection. Because in developing countries most symptomatic *Campylobacter* infections occur in children younger than 2 years of age[60] and frequently produce a dysenteric picture, it is reasonable to conclude that *C. jejuni* infection may play a role in the dehydration and malnutrition that often accompany infantile diarrhea in these geographic areas. The outcome of infections caused by newly discovered *Campylobacter*-like organisms remains to be determined.

C. fetus infection may be lethal to patients with chronic compensated diseases such as cirrhosis or diabetes mellitus or may hasten the demise of seriously immunocompromised patients. For immunocompromised hosts with systemic *C. fetus* infections, prognosis is most dependent on the rapidity with which appropriate antimicrobial therapy is begun. Previously healthy people infected with *C. fetus* usually survive the illness without permanent sequelae.

PREVENTION

Because most human *Campylobacter* infections are acquired from consumption or handling of poultry, thorough cooking of chicken and other poultry products is an important step in preventing transmission of infection to people. Furthermore, meticulous attention to avoiding cross-contamination of utensils and cutting boards during food preparation also may reduce the risk of infection. Elimination or reduction of use of antibiotics in animals used for food production may reduce the rate of antibiotic resistance among *Campylobacter* species. Finally, because *C. jejuni* infections are "accidentally" acquired by humans, and because there is evidence for the natural development of immunity among people in developing countries, the goal of producing a vaccine is probably achievable.

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The complete reference list is available online at Expert Consult.

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217 *Helicobacter pylori* and Other Gastric *Helicobacter* Species

Timothy L. Cover and Martin J. Blaser

SHORT VIEW SUMMARY

Definition
- *Helicobacter pylori* is a gram-negative bacterium that persistently colonizes the human stomach.
- Most *H. pylori*–colonized persons remain asymptomatic, but the presence of *H. pylori* is associated with an increased risk of peptic ulceration and gastric cancer.
- Increasing evidence shows benefits associated with gastric *H. pylori* colonization in addition to the known disease risks.

Epidemiology
- *H. pylori* is present in humans throughout the world, but the prevalence is variable and depends on age and geography.
- The prevalence of *H. pylori* in developed countries has been declining over the past several decades.

Microbiology
- *H. pylori* is a urease-positive, gram-negative, spiral-shaped bacterium.

Diagnosis
- Noninvasive diagnostic approaches include serology, urea breath test, and stool antigen test.
- Invasive diagnostic approaches are endoscopy and analysis of gastric tissue.

Therapy
- Antimicrobial treatment is indicated in patients with peptic ulceration or patients considered to have a high risk of gastric cancer.
- Treatment consists of a combination of antimicrobials and often includes an agent to diminish gastric acidity.
- An increasing proportion of strains show resistance to antibiotics. Knowledge of antimicrobial susceptibility allows optimization of treatment regimens.

Prevention
- Prevention of *H. pylori* acquisition by immunization is not routinely practiced. An important focus is on the prevention of *H. pylori*–associated illnesses, including gastric adenocarcinoma.

Helicobacter pylori (formerly known as *Campylobacter pylori* or *pyloridis*) was first isolated from humans in 1982.[1,2] This highly motile, curved, gram-negative rod lives within the mucus layer overlying the gastric mucosal epithelium.[3] *H. pylori* is commonly found in the human stomach, and when present, it is usually the single dominant species. Essentially all persons colonized with *H. pylori* have a cellular infiltrate in the lamina propria of the gastric antrum and fundus.[4,5] The first isolation of *H. pylori* in pure culture and recognition of its association with gastritis and peptic ulcer disease led to the awarding of the Nobel Prize in Medicine in 2005 to Barry Marshall and Robin Warren, two physicians in Australia. The presence of *H. pylori* increases the risk of peptic ulcer disease[6–8] and gastric cancer,[9–13] but also may be associated with health benefits, including a decreased risk of esophageal reflux and its consequences and protection against childhood asthma and related disorders.[9,14–18] With the development of effective therapies to eradicate *H. pylori,* physicians are faced with the challenge of determining which patients will benefit from therapy and which may be harmed.

MICROBIOLOGY

H. pylori organisms are small (0.5–1.0 μm in width and 2.5–4.0 μm in length), curved, microaerophilic, gram-negative rods.[2,19] Because they closely resemble members of the genus *Campylobacter,* they were initially considered to belong to that genus. However, multiple genotypic and phenotypic characteristics are different from those of campylobacters, and a new genus, *Helicobacter,* was established.[19] Most mammals studied to date are colonized with one or more distinct *Helicobacter* species. Examples include *Helicobacter mustelae* in ferrets,[19,20] *Helicobacter felis* in dogs and cats,[20] *Helicobacter muridarum* in mice, *Helicobacter nemestrinae* in nonhuman primates, and *Helicobacter acinonychis* in cheetahs.[21] Individual *Helicobacter* species preferentially colonize either the stomach or the intestinal tract, and typically are adapted for colonization of specific mammalian hosts.

H. pylori is the most important human-associated species from a medical perspective and may be considered the prototype for this group of gastric organisms. Related gastric bacteria that colonize the human stomach are considered in a separate section of this chapter. *Helicobacter fennelliae* and *Helicobacter cinaedi* are intestinal organisms that cause diarrheal illnesses, and *H. cinaedi* also can cause bacteremia and cellulitis, especially in immunocompromised patients.[22] Because the clinical features of these infections resemble those of *Campylobacter* spp., they are discussed in Chapter 216. *Helicobacter* spp., such as *Helicobacter hepaticus, Helicobacter bilis,* and *Helicobacter rappini,* have been identified in the colon and biliary tract of rodents,[23] and there is human carriage as well. Preliminary evidence suggests that these species might colonize the diseased human biliary tract,[24,25] but whether they contribute to biliary pathology is uncertain.[26] Thus there are both gastric and intestinal residential *Helicobacter* species in humans. In contrast to *H. pylori,* the residential intestinal species are not known to cause disease in nonimmunocompromised humans.

H. pylori cells are highly motile, with a rapid corkscrew motion, and have multiple, polar, sheathed flagella.[19] Although these cells are classically curved or spiral in fresh cultures, spherical (coccoid) forms are present in older cultures. The major biochemical properties of *H. pylori* and several related bacteria are shown in Table 217.1. A prominent biochemical characteristic of gastric helicobacters is production of urease. *H. pylori* urease is a hexadimer consisting of 61- and 28-kDa subunits, both of which are essential for activity.[27]

The complete genome sequences of hundreds of different *H. pylori* strains have been determined,[28–30] and the transcriptome of one strain has been analyzed in detail.[31] Comparison of these genome sequences has permitted the definition of a core genome,[32–34] and numerous strain-specific genes have been identified, including genes that encode restriction-modification enzymes[35] and others that encode cell surface components.[36,37] In comparison to many other bacterial species, *H. pylori*

TABLE 217.1 Biochemical Characteristics of *Helicobacter pylori* and Related Bacteria

CHARACTERISTIC	*H. PYLORI*	*HELICOBACTER MUSTELAE*	*HELICOBACTER FELIS*	*CAMPYLOBACTER JEJUNI*
Urease	+	+	+	–
Catalase	+	+	+	+
Oxidase	+	+	+	+
H_2S production	–	–	–	+
Guanosine plus cytosine content (%)	35–38	36	42.5	33–36
Hippurate hydrolysis	–	–	–	+
Nitrate reduction	–	+	+	+
Resistance to nalidixic acid (30-µg disk)	+	–	+	–
Cephalothin (30-µg disk)	–	+	–	+
Growth at 42°C	–	+	+	+
Growth at 37°C	+	+	+	+
Growth at 25°C	–	–	–	–

Data from Goodwin et al.,[19] Paster et al.,[20] Eaton et al.,[21] and Kiehlbauch et al.[22]

has few two-component regulatory systems. Frameshift mutations occur commonly within open reading frames encoding certain *H. pylori* proteins, which suggests that *H. pylori* may use mutation to control phenotype, with the host selecting for the "most fit" organism within a particular environmental niche.[38,39] Plasmids are present in most *H. pylori* isolates; they vary in size, and their functional properties are mostly unknown at present. Bacteriophages also have been detected in *H. pylori*.[40]

Although *H. pylori* is highly homogeneous in the biochemical characteristics analyzed in clinical microbiology, including urease, oxidase, and catalase positivity, there is substantial genetic variation among strains, both in gene content and in nucleotide sequences of individual genes.[41,42] Humans may be simultaneously colonized with more than one strain of *H. pylori,* and as many as five different strains have been identified in an individual subject.[43–45] *H. pylori* strains are naturally competent (i.e., able to take up heterologous DNA) and frequently undergo intergenomic and intragenomic recombination.[42,45,46] The strain-specific restriction-modification systems diminish recombination and may facilitate colonization of a host by several different strains simultaneously.[35,47,48] Because of point mutations and recombination, *H. pylori* are among the most varied of all species in the human biosphere.[49–52]

Distinct clonal lineages of *H. pylori* are not readily identifiable when comparing strains from unrelated humans,[45] but multilocus sequence typing has revealed the existence of multiple *H. pylori* populations with distinct geographic distributions.[53] Classification of strains by this approach is useful for anthropologic studies, and may be relevant to differences in pathogenicity of strains in hosts of different ethnicities, as described in recent studies from South America.[54] The most important dichotomy among *H. pylori* strains is the presence or absence of the cytotoxin-associated genes (*cag*) pathogenicity island, a 35- to 40-kilobase chromosomal region encoding the secreted protein CagA and genes encoding components of a type IV secretion system that mediates delivery of CagA into host cells.[55–59] Both $cagA^+$ and $cagA^-$ strains are present in *H. pylori* populations in most parts of the world,[58] which suggests that this chromosomal region was acquired in the distant past. The *cag* status of an *H. pylori* strain is relevant to the risk of a number of clinical outcomes (as discussed later). Another heterogeneous locus that provides a basis for typing strains is *vacA,* a conserved gene that encodes a secreted protein (vacuolating cytotoxin) that interacts with epithelial cells.[60] Three regions of *vacA* have major polymorphisms: the *s* region (with allelic types s1 and s2), the *m* region (with allelic types m1 and m2), and the *i* region (with allelic types i1 and i2).[61–63] Type s1 *vacA* genotypes are strongly linked to the presence of *cagA,* and therefore $cagA^+$ and *vacA* s1–positive genotypes are each associated with similar clinical outcomes.[64]

EPIDEMIOLOGY

H. pylori has been isolated from persons in all parts of the world.[53,65,66] Similar organisms have been isolated from primates, but other animal sources for *H. pylori* have not been identified, nor have reservoirs been found in food, soil, or water. It now appears likely that humans are the major, if not sole, reservoir for *H. pylori.* Analyses of genetic heterogeneity among strains indicate that *H. pylori* has been present in humans for at least 100,000 years, if not longer, and the current geographic distribution of *H. pylori* alleles reflects ancient migrations of human populations.[53,66–68] The recent finding of *H. pylori* DNA in a 5300-year-old corpse, frozen in ice, provides further evidence for the long-standing association of *H. pylori* with humans.[69] These data support the notion that *H. pylori* is indigenous to humans, as its relatives are to other mammals.[70] Prolonged coevolution of *H. pylori* with human populations may be linked to reduced pathogenicity. For example, a study from Colombia found that African-ancestry *H. pylori* was relatively benign in humans of African ancestry but was associated with higher risk of gastric cancer in individuals of Amerindian ancestry.[54]

The high prevalence of colonization among persons in settings where sanitary conditions are suboptimal, including institutions for individuals with intellectual disabilities and orphanages, suggests that horizontal fecal-oral transmission or oral-oral transmission occurs.[65,71] *H. pylori* has occasionally been isolated from feces, especially from children.[72] *H. pylori* has also been isolated from dental plaque,[43] and *H. pylori* DNA may be detected in saliva by the polymerase chain reaction assay,[73] which raises the possibility of oral-oral transmission as well. Studies of persons attending clinics for either sexually transmitted diseases or infertility indicate that sexual transmission does not occur very frequently, if at all.[74] The relative contribution of fecal-oral, oral-oral, or vomitus-oral[75] transmission of *H. pylori* is not known. *H. pylori* infection clusters in families,[76] and the presence of the organism in a child is highly associated with large family size and older siblings.[77,78] On occasion, transmission occurs from person to person via improperly cleaned endoscopes.[79]

In most populations, *H. pylori* appears to be almost universally acquired during childhood,[65,80] but not in the first year of life.[81] Once acquired, *H. pylori* can persistently colonize the stomach for decades or for an entire lifetime. *H. pylori* has also been found to transiently colonize children and nonhuman primates, a phenomenon that may be associated with less pathogenic strains,[82] or the selective bottleneck of transmission to a new host, with failure to sufficiently adapt to sustain colonization. The prevalence of *H. pylori* colonization varies considerably among different populations around the world[83] and is dependent on age[5,71] and socioeconomic development (Fig. 217.1). In developing countries, by age 10 years, more than 70% of persons carry *H. pylori.* Among non-Hispanic whites in the United States, little colonization is

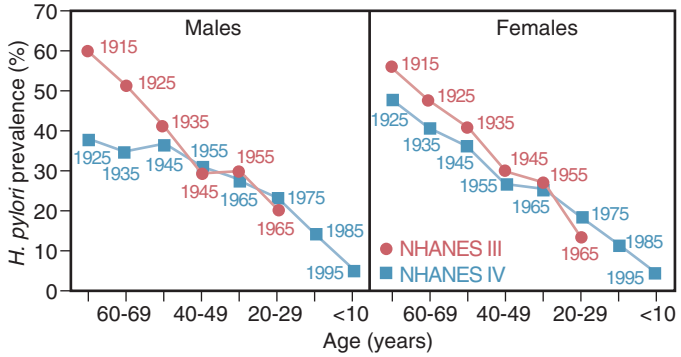

FIG. 217.1 Age-specific prevalence of *Helicobacter pylori* positivity among men and women in the National Health and Nutrition Examination Survey (NHANES) III, phase I (1988–1991; *red circles*) and NHANES IV, phase I (1999–2000; *blue squares*), by age and year of birth. Year of birth is shown at the midpoint of decade birth categories. Prevalence data for participants 19 years of age or younger were not available in NHANES III. *(From Chen Y, Blaser MJ.* Helicobacter pylori *colonization is inversely associated with childhood asthma.* J Infect Dis. *2008;198:553–560.)*

currently occurring during childhood, and the relatively high prevalence rates in adults reflect acquisition many decades ago. Among blacks and Hispanics, a high prevalence is seen at all ages.[84–86] The annual incidence of acquisition has ranged from 0.5% among epidemiologists in the United States to 7.4% among persons at an institution for individuals with intellectual disabilities in Australia.[87] The incidence of *H. pylori* has been progressively declining in the United States and other developed countries,[87–89] probably as a result of smaller family sizes, decreased crowding, improved sanitation, and more than 70 years of widespread antibiotic use.[70,90,91] Thus the age-related increase in prevalence reflects a birth cohort phenomenon (with persons born earlier having higher acquisition rates in childhood), continuing exposure and low-level new colonization into young adulthood, and continuing loss of *H. pylori* because of collateral effects from antibiotic use. The birth cohort effect predominates. The prevalence of *H. pylori* is increased among immigrants and persons of lower socioeconomic status.[5,84,86] In total, in less than 1 century in the United States, colonization has gone from being ubiquitous to being present in 5% of children[16]; this is a change in human microecology of major proportion.

PATHOLOGY AND PATHOGENESIS

H. pylori is able to survive and multiply in the acidic gastric environment, which is hostile to the growth of most bacteria. When intraluminal acidity diminishes as a result of gastric atrophy, *H. pylori* is less able to colonize the stomach, possibly because of competing organisms. *H. pylori* characteristics that permit gastric colonization include microaerophilism for survival within the mucous gel, spiral shape, flagella for motility within this viscous layer, and urease activity, which generates ammonium ions that buffer gastric acidity.[92,93] Although most organisms appear to be free-living in the mucous layer, smaller numbers appear to be adherent to the mucosal epithelial cells. Organisms may be found at the luminal surface of the gastric mucosa and also deeper within gastric glands.[94] *H. pylori* localizes almost exclusively in association with gastric-type epithelium. Affected gastric epithelium may be in the gastric antrum or fundus[95] or may be ectopic in the duodenum or in the esophagus.[4,96–98] In contrast, *H. pylori* does not colonize intestinal epithelium, even when present in the stomach.[4] Several important *H. pylori* adhesins have been identified, including the outer membrane proteins BabA (which binds to fucosylated Lewis b receptor on gastric epithelial cells), SabA (which binds to sialyl Lewis X receptors), HopQ (which binds to carcinoembryonic antigen-related cell adhesion molecules), AlpA, and AlpB.[99–104]

The gastric tissue colonized by *H. pylori* almost always exhibits an inflammatory infiltrate.[5] The lamina propria most commonly contains mononuclear cells, including lymphocytes, monocytes, and plasma cells. Neutrophils and, to a lesser extent, eosinophils may be present in the lamina propria and epithelium. The epithelial glands have a more complex architecture and less mucus when *H. pylori* is present than when it is absent.[4] In children, a follicular lymphoid pattern is common. The presence of *H. pylori* induces these changes, and the bacterium is not just a secondary colonizer.

Persons colonized with *H. pylori* have different gastric secretory physiology than do those who are not colonized. On average, colonized persons have higher gastrin levels, which are reduced by eradication of the organism.[105,106] The mechanism for increased gastrin production appears to be related to low gastric somatostatin levels,[107,108] which may reflect cytokine production in the colonized antrum.[109,110] Increased gastrin may contribute to the increase in parietal cell mass observed in many patients with duodenal ulceration. *H. pylori* products may directly affect parietal cells,[111,112] which may diminish acid production. Effects of *H. pylori* on both the fundus (the site of acid production) and the antrum (which produces factors regulating acid production) may in part be responsible for the multiplicity of outcomes of colonization.[97,109,113]

H. pylori does not appear to invade tissues, except as an incidental finding. Thus *H. pylori*–induced alterations in host tissue are likely a response to extracellular bacterial products or to cellular alterations induced by contact with the organism. Many of the alterations in gastric epithelial cells caused by *H. pylori* are attributable to CagA, which is secreted and translocated into cells through a type IV secretion system.[59,114–118] The 3′ region of *cagA* contains DNA repeats flanking sites that encode tyrosine phosphorylation motifs.[114] *H. pylori* populations include individual cells with zero, one, two, or more tyrosine phosphorylation motifs in CagA, which arise through intragenomic recombination.[119] Once injected into the epithelium by the type IV secretion system, Src and Abl kinases phosphorylate these tyrosine residues,[120,121] and phospho-CagA interacts with multiple intracellular regulatory molecules, including SHP-2[122,123] and PAR1b/MARK2,[124,125] that affect mitogen-activated protein kinases and the actin cytoskeleton; these pathways affect cell shape, cell cycle events, and cytokine production.[126–128] Nonphosphorylated CagA also can interact with cellular proteins, resulting in altered signaling events.[129] Experiments in animal models indicate that CagA and the type IV secretion system contribute to gastric inflammation and, in some cases, can promote histologic changes that are linked to gastric cancer.[118,130–132] The *cag* type IV secretion system contributes to the entry of heptose-1,7-bisphosphate (an intermediary metabolite of *H. pylori* lipopolysaccharide biosynthesis) into epithelial cells, which stimulates proinflammatory signaling.[133,134] The *cag* island is metastable, and isolates in individual patients may vary in the presence of the island, specific genes or regions, or subgenic sequences, as in the case of the 3′ region of *cagA*.[119] This instability creates a population of variants that can interact with the host in myriad ways.

Besides CagA, several other secreted or released *H. pylori* proteins are relevant. The secreted pore-forming protein VacA can cause several alterations in gastric epithelial cells (including alterations in endosomal compartments and cell death), and can also target several types of immune cells (T cells, B cells, mast cells, and macrophages).[60,135–138] Certain forms of VacA (type s1) are active in cellular assays in vitro, whereas other forms of VacA (type s2) are inactive in these assays.[61,139,140] T-cell activity is downregulated by both VacA and *H. pylori* γ-glutamyl transpeptidase; this downregulation may contribute to *H. pylori* persistence, as well as protection against allergic and asthmatic conditions.[141–144] HtrA, a secreted protease, facilitates access of *H. pylori* to the basolateral surface of gastric epithelial cells.[145] Urease may be shed by *H. pylori* cells, has been observed in affected tissues, and is a chemoattractant and activator of host phagocytic cells.[146,147]

The presence of *H. pylori* overlying the gastric mucosa activates epithelial cells to produce proinflammatory cytokines[148–151] and activates mononuclear and polymorphonuclear cells to produce cytokines, superoxide, and other proinflammatory molecules.[147,152,153] Although *H. pylori* colonization of the stomach is consistently accompanied by an inflammatory gastric host response, several factors serve to downregulate host responses.[38,109] Bacterial lipopolysaccharide usually has proinflammatory activities, but *H. pylori* lipopolysaccharide has remarkably little.[154] Similarly, *H. pylori* flagellin is modified so that it is poorly recognized by Toll-like receptor 5 (TLR5).[155] *H. pylori* lipopolysaccharide may express type II Lewis antigens (Le^x, Le^y, neither, or both of these antigens),[36,37,156] as well as type I antigens (Le^a, Le^b).[37] This observation

is significant because these antigens are present on gastric epithelial cells, and there is evidence that the host Lewis phenotype selects for the particular Lewis expression of the *H. pylori* population.[157,158] This may represent a form of molecular mimicry that promotes *H. pylori* persistence. T-regulatory cells in the gastric mucosa may downregulate local inflammatory responses.[159,160] Finally, *H. pylori* glucosylation of host-derived cholesterol contributes to immune evasion.[161]

Both bacterial and host factors may be determinants of outcome.[97,162] Virtually all patients with duodenal ulceration are colonized by strains possessing *cagA* (and thus the *cag* pathogenicity island).[6,163,164] Similarly, *cagA*$^+$ strains have been associated with a higher risk of stomach cancer than *cagA*$^-$ strains.[10,97,165,166] In East Asia, most *H. pylori* strains are *cagA*$^+$, which may account for the relatively high incidence of gastric cancer in this part of the world. Strains from patients with ulcers or stomach cancer more commonly contain type s1 *vacA* and more frequently express the BabA adhesin compared with strains from patients without these diseases.[60,61,167,168] Differences among colonized hosts in cell-mediated immunity and cytokine responses to *H. pylori* are other possible determinants of outcome variability.[169,170] Humans are polymorphic in the genetic loci involved in regulating proinflammatory cytokine production, and allelic variation in genetic determinants of interleukin-1β and interleukin-10 production affects risk of gastric cancer in *H. pylori*–positive persons.[168,171,172]

Findings similar to those observed in humans develop in several animal models of infection, including nonhuman primates and Mongolian gerbils.[173–180] The development of experimental *H. pylori* infections in these models and in human volunteers has allowed new avenues for exploring host-microbe interactions.[181,182]

CLINICAL CONSEQUENCES ASSOCIATED WITH *H. PYLORI* COLONIZATION

In the majority of *H. pylori*–colonized persons, the presence of this organism is not associated with any readily identifiable clinical consequences. Nevertheless, colonization is associated with certain types of upper gastrointestinal pathology, and appears to protect against other diseases (Table 217.2). From a clinical standpoint, the major consequences of *H. pylori* colonization are as follows.

Gastrointestinal Diseases

Acute Acquisition

Natural, voluntary, or accidental *H. pylori* acquisition may cause an acute upper gastrointestinal illness with nausea and upper abdominal pain.[1,79,183–185] Vomiting, burping, and fever may also be present. Symptoms last from 3 to 14 days, with most illnesses persisting less than 1 week. A diagnosis of food poisoning may be made in persons seeking medical attention. For many individuals, the acquisition of *H. pylori* is clinically silent.[184] Most data suggesting symptomatic acquisition relate to adults, but worldwide, most acquisition actually occurs in children[81]; the relative proportion of symptomatic and asymptomatic acute acquisition at any age is not known. In the weeks after acquisition, intense gastritis develops; hypochlorhydria ensues and may persist for as long as 1 year. In children, there is a transient increase in serum pepsinogen I levels.[81] One adult volunteer who ingested *H. pylori* seemed to have had an acute self-limited infection[185]; the frequency of this phenomenon is not known.

Persistent Colonization

It now is clear that, after acquisition, *H. pylori* persists for years, if not decades, in most persons (Fig. 217.2).[184,186] Not every exposure to *H. pylori* leads to persistent colonization, because of either lack of adaptation to the particular host[187] or coincident or proximate use of antibiotics. Tissue and serologic responses to colonization develop in essentially all persistently colonized persons.[5] The acute *H. pylori*–induced upper gastrointestinal symptoms resolve and do not return in most persons; most with persistent *H. pylori* colonization are asymptomatic. However, studies of patients with nonulcer dyspepsia indicate that *H. pylori* may be slightly more common in such patients than in age-matched control subjects[188] and that *H. pylori* colonization may be one of the causes of this common but poorly defined and heterogeneous group of disorders. Supporting this hypothesis are the results of some studies indicating that some patients with nonulcer dyspepsia who are colonized with *H. pylori* show better responses to antimicrobial therapy than treatment with placebo,[188] an effect not seen in patients with nonulcer dyspepsia who do not have *H. pylori* colonization.[189] However, in other studies, no difference between *H. pylori* treatment and placebo was found.[190,191] In total, *H. pylori* is probably responsible for fewer than 10% of cases of nonulcer dyspepsia.[192,193] Even if such an association exists, no markers are available to identify those patients with nonulcer dyspepsia in whom antibiotics would have a beneficial effect. Better definition of nonulcer dyspepsia and ascertainment of both *H. pylori* and host genotypes in individual patients should lead to a better understanding of whether *H. pylori* persistence is associated with symptoms in particular patients in the absence of ulceration or neoplasia.

TABLE 217.2 Association of *Helicobacter pylori* with Common Pathologic Lesions of the Upper Gastrointestinal Tract and with Nongastrointestinal Diseases

LESION	ASSOCIATION WITH *H. PYLORI*
Chronic diffuse superficial gastritis	Nearly always associated[4,5]
Type A (pernicious anemia) gastritis	Negative association[194,306]
NSAID gastropathy	Negative or no association[307]
Acute erosive gastritis (e.g., alcohol, aspirin)	No association[4]
Gastric ulceration	Commonly observed in patients who are not ingesting NSAIDs or aspirin[6–8,194]
Duodenal ulceration	Usually associated with idiopathic lesions (non–drug induced, non–Zollinger-Ellison syndrome)[6,8,98,308]
Gastric adenocarcinoma	Positively associated with (noncardia) cancers of the gastric body and antrum[10–13,97,212]
Gastric lymphoma	Strongly associated with MALT-type B-cell lymphomas[231,232]
Idiopathic thrombocytopenic purpura	Often associated[261–263]
Nonulcer dyspepsia	Little or no association[188–192]
Gastroesophageal reflux disease	Presence of *cag*$^+$ strains has protective association[230,237]
Barrett's esophagus	May colonize distalmost gastric epithelium in patients with gastric colonization[4]; presence of *cag*$^+$ strains has protective association[230]
Adenocarcinoma of the esophagus	Presence of *cag*$^+$ strains has protective association[97,226,227]
Childhood asthma and related allergic disorders (allergic rhinitis, eczema, and skin sensitization)	Presence of *cag*$^+$ strains has protective association[15–18]

MALT, Mucosa-associated lymphoid tumor; *NSAID,* nonsteroidal antiinflammatory drug.

Duodenal Ulceration

More than 90% of patients with "idiopathic" duodenal ulceration (i.e., not associated with nonsteroidal antiinflammatory drug use or Zollinger-Ellison syndrome) carry *H. pylori,* a prevalence significantly higher than in age-matched control subjects.[7,194] Previous *H. pylori* colonization is associated with a three- to fourfold increased risk of development of duodenal ulceration,[7] and the risk is even higher in patients colonized with *cagA*$^+$ strains.[6]

H. pylori may colonize the duodenum but only overlies metaplastic islands of gastric-type epithelium (gastric metaplasia).[4,98,195] The occurrence of *H. pylori* colonization and gastric metaplasia is highly associated with active duodenitis, a precursor lesion to ulceration,[195] and the presence of *H. pylori* in the duodenum is associated with a markedly increased risk of duodenal ulceration.[98] One model proposes that *H. pylori*–induced

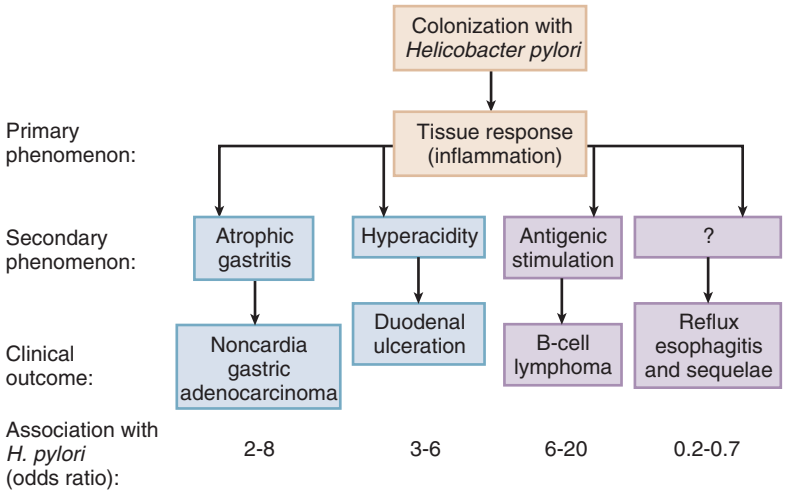

FIG. 217.2 Association of *Helicobacter pylori* colonization and disease states. After *H. pylori* acquisition, virtually all persons develop persistent colonization that lasts for life. Colonization induces tissue responses termed *chronic gastritis.* This process affects gastric physiology, including glandular structure, acid secretion, and antigen processing, which in turn affect disease risk. Colonization with *H. pylori* increases the risk of certain diseases (duodenal ulcer, gastric ulcer, noncardia gastric adenocarcinoma, B-cell lymphomas, and possibly idiopathic thrombocytopenic purpura) but decreases the risk of gastroesophageal reflux disease and its complications, including Barrett's esophagus, adenocarcinoma of the esophagus or gastric cardia, and possibly childhood asthma and related allergic disorders.

hypergastrinemia results in increased acid production, which leads to the development of gastric metaplasia in the duodenum; *H. pylori* colonization of the metaplastic gastric tissue then results in duodenitis and ulceration.

Although a significant body of evidence associating *H. pylori* colonization with idiopathic duodenal ulceration has accumulated, a causative role of *H. pylori* in ulcer disease is unproven; none of the experimental human studies has shown progression to ulceration, and why peptic ulcer disease has a remitting and relapsing course in the face of persistent colonization has never been resolved.[70] However, a large number of treatment studies using antimicrobial agents have helped define the natural history of ulcer disease. First, the use of antimicrobial agents (in the absence of acid-suppressive therapy) can heal duodenal ulcers at a rate similar to that observed with acid-suppressive therapy alone.[196,197] Second, after ulcer healing, eradication of *H. pylori* is associated with significantly lower recurrence rates than if the organism remains present.[196–198] When antimicrobial therapy that eradicates *H. pylori* is added to short-term acid-suppressive therapy, long-term ulcer relapse rates are markedly reduced, although not completely eliminated.[198,199] Altogether, these findings implicate *H. pylori* as playing a role in duodenal ulcer pathogenesis and demonstrate that antimicrobial therapy rather than long-term acid-suppressive therapy is indicated for most patients[8] because it changes the natural history of idiopathic duodenal ulcer disease. Recent studies have provided evidence that, after *H. pylori* eradication, the incidence of reflux esophagitis is doubled compared with the reflux incidence associated with failed eradication.[200,201] Thus, removal of *H. pylori* from the stomach of patients with duodenal ulceration has both benefits and costs.

Gastric Ulceration

In comparison to the high proportion of duodenal ulcer patients colonized with *H. pylori,* a smaller (50% to 80%) proportion of patients with benign gastric ulcers are colonized by *H. pylori.* The major reason is that a much higher proportion of gastric ulcers are due to nonsteroidal antiinflammatory drug or aspirin use. When such use is excluded, most of the remaining patients with benign gastric ulcers are colonized with *H. pylori,* which corresponds to a prevalence significantly higher than in age-matched control subjects.[7,194] The results of treatment of gastric ulceration with antimicrobial agents parallel the results of treatment of duodenal ulceration, changing the natural history of these diseases.[198,202]

Gastric Carcinoma

Because *H. pylori* colonization induces a tissue response (termed *chronic gastritis*) and because chronic gastritis is well known as a risk factor for the development of gastric carcinoma,[203] a role for this organism in carcinogenesis has been advanced.[97] The decreasing incidence of noncardia gastric carcinoma in developed countries is consistent with an older age of *H. pylori* acquisition[204,205] and decreasing frequency of *H. pylori* acquisition as industrialization has proceeded.[16,206] The epidemiologic characteristics of *H. pylori* colonization—including increasing prevalence at an older age; higher prevalence in blacks, Hispanics, and Asians; association with lower socioeconomic status; and early-life crowding—are all similar to the characteristics associated with noncardia gastric cancer.[65] In addition, the development of intestinal metaplasia and atrophic gastritis, two pathologic entities that are risk factors for gastric cancer, is associated with *H. pylori.*[207,208] Thus a direct role of this organism in gastric cancer is biologically plausible, and in several parts of the world there is a correlation between regions with high *H. pylori* prevalence rates and high gastric cancer rates.[209,210] Prospective studies of gastric cancer conducted in Hawaii, California, and England[211,212] and several retrospective and prospective studies each indicate that *H. pylori* is a risk factor for noncardia gastric cancer.[97] This association involves adenocarcinoma of the antrum and body of the stomach, and both the intestinal and diffuse histologic types of gastric cancer.[11–13,212] Odds ratios range from approximately 2.7 to more than 20, and the proportion of gastric cancers attributable to *H. pylori* infection ranges from about 60% to more than 90%.[12,13,210]

The presumed mechanism for adenocarcinoma involves the chronicity of *H. pylori*–induced tissue responses (inflammation), with progression to atrophic and subsequently intestinal metaplastic histology as important steps in pathogenesis. However, this process probably requires decades on average, and *H. pylori* colonization is neither necessary nor sufficient for oncogenesis. *H. pylori* alteration of signal transduction and cell-cycle kinetics in epithelial cells or gastric stem cells may predispose to neoplasia,[94,213,214] and the relationship between epithelial cell proliferation and apoptosis is probably also important.[213,215,216] *H. pylori*–induced damage to host DNA, including double-strand breaks, also may contribute to carcinogenesis.[217] Experiments in animal models suggest that CagA and the *cag* type IV secretion system (encoded by the *cag* pathogenicity island) have an important role in the pathogenesis of gastric cancer.[131,132]

There are numerous bacterial, host, and environmental factors that influence the risk of gastric cancer.[97,162] For example, the risk of gastric cancer is higher among persons colonized with *cagA*$^+$ strains than among persons colonized with *cagA*$^-$ strains[97,166] and is elevated among hosts carrying specific genetic determinants of interleukin-1β production.[168] Consumption of a high-salt diet or a diet low in fruits and vegetables has also been linked to increased gastric cancer risk. Definition of

additional host and bacterial factors that increase the risk of cancer development is an important research priority.

Gastric inflammation resolves following *H. pylori* treatment,[218] so there has been great interest in the possibility that treatment could result in regression of premalignant gastric histologic parameters or a reduced risk of gastric cancer. *H. pylori* eradication does not influence the presence of intestinal metaplasia, but several studies reported that atrophic gastritis is reversible, especially if intestinal metaplasia is not present.[219,220] Similarly, there is evidence that eradication can prevent the development of gastric atrophy.[201] Several trials reported that treatment regimens designed to eradicate *H. pylori* can reduce the risk of gastric cancer, especially in East Asian countries.[201,212,221–225]

No positive association has been observed between the presence of *H. pylori* and cancers of the gastric cardia, gastroesophageal junction, or esophagus,[11–13,211] and increasing data indicate that *H. pylori* carriage may have a protective effect against development of these types of cancer.[226,227] In parallel with the decline in *H. pylori* acquisition in developed countries, the incidence of adenocarcinomas of the gastric antrum and body has decreased over time in these countries.[228] In contrast, the incidence of several types of adenocarcinoma of the gastric cardia, gastroesophageal junction, and lower portion of the esophagus has risen dramatically.[228,229] The observed temporal relationships suggest that *H. pylori* loss may play a role in the increase of these cancers.[226,227,230] One recently recognized type of gastric cancer that occurs in the gastric corpus, primarily in women less than 50 years of age,[229] has been given the name "CYF" cancer, for gastric *c*orpus predominant, *y*oung age (<50 years old) dominant, and *f*emale dominant (rate of increase double compared with men). This type of cancer has been increasing in incidence in both Sweden and the United States, with the rise apparently beginning with the cohort born in the early 1950s. One hypothesis to explain the increasing incidence of this disease is a two-hit model, involving the loss of *H. pylori* as well as antibiotic alteration of the microbiome. Further work is needed to understand the pathogenesis of these cancers.

Gastric Lymphoma

Most gastric lymphomas arise from B lymphocytes and are termed *mucosa-associated lymphoid tumors* (MALTs or MALTomas]). *H. pylori* colonization is strongly associated with these tumors,[231,232] and eradication of *H. pylori* often leads to improvement in tumor histology.[233,234] Whether *H. pylori* eradication leads to regression of true malignancies, which are rare,[235] in contrast to the more common and more benign monoclonal lymphoid proliferation associated with *H. pylori,* is unknown. The pathogenesis of these disorders may involve chronic antigenic stimulation by *H. pylori* and subsequent induction of a polyclonal lymphoid response, a single clone of which proliferates and then undergoes neoplastic transformation.

Esophageal Diseases

Much evidence has accumulated that the incidence of *H. pylori* colonization, especially *cagA*$^+$ strains,[88] has progressively decreased in developed countries during the 20th century.[206] During this time, the occurrence of three related diseases—gastroesophageal reflux disease, Barrett's esophagus, and adenocarcinoma of the esophagus—has been increasing dramatically.[228,236] It is generally believed that Barrett's esophagus will develop in a proportion of patients with gastroesophageal reflux disease and that esophageal adenocarcinoma will develop in some of these patients. An important question is whether the loss of *H. pylori* from populations in developed countries may in some way predispose to this pathogenic sequence. Patients with gastroesophageal reflux disease are less likely to be colonized with *H. pylori* (especially *cagA*$^+$ strains) than are control subjects,[230,237] and eradication of *H. pylori* in patients with duodenal ulceration doubled the rate of gastroesophageal reflux disease development.[200] The presence of *cagA*$^+$ *H. pylori* strains is inversely associated with Barrett's esophagus and esophageal adenocarcinomas.[230,238–240] There is a clear and consistent inverse relationship between colonization with *H. pylori* (especially *cagA*$^+$ strains) and each of the disorders in the pathway (gastroesophageal reflux disease, Barrett's esophagus, and esophageal adenocarcinoma).[227] These findings suggest that *H. pylori* in the stomach is protective against the development of these esophageal disorders. The presence of decreased gastric acidity induced by long-term *H. pylori* persistence may be partially responsible.[240–243] Other potential mechanisms include *H. pylori* effects on gastric hormones, such as leptin,[244] or on the microbiota colonizing the stomach and esophagus.[245,246] In addition to the inverse relationship between *H. pylori* and the esophageal diseases described here, an inverse relationship between *H. pylori* and eosinophilic esophagitis has been reported.[247]

Asthma and Related Disorders

In recent years, asthma and related allergic disorders, including allergic rhinitis, eczema (atopic dermatitis), and skin allergies, have become markedly more prevalent, just as *H. pylori* colonization has become less common; today, less than 6% of US children are colonized with *H. pylori,*[16] a progressive decrease from the presumed near-universal presence of *H. pylori* a century ago. The results of epidemiologic studies suggest that there are specific inverse relationships between *H. pylori* and these disorders.[17] Several points have emerged about the inverse relationships. First, these associations are observed primarily with childhood-onset asthma.[15,16] Second, the effects for allergic rhinitis, eczema, and skin sensitization parallel those for asthma.[15–18] Third, the relationships have been observed not only in the United States but also in other developed countries.[18] Fourth, the strongest inverse associations are with the *cagA*$^+$ strains.[15]

These findings are consistent with the hypothesis that *H. pylori* protects against asthma and related disorders, or alternatively, *H. pylori* may be a marker for other changes in microecology that actually are the protective factors. Recent experiments have shown that *H. pylori* infection of mouse models can lead to protection against allergic asthma,[144,248] which supports the former possibility. The *H. pylori*–positive stomach includes a rich compartment of T-regulatory cells that is much better developed than in *H. pylori*–negative persons,[159,160] which could influence systemic immunity. The work in this area is in the early stages, but if *H. pylori* contributes to the protection against childhood asthma, this would influence clinical decisions related to *H. pylori* eradication.

Metabolic Disorders

In recent years, it has become clear that the stomach produces leptin, a hormone related to satiety and energy homeostasis,[249,250] and, to a greater extent, ghrelin, which has opposing effects.[251] A growing body of studies indicates that *H. pylori* status affects the levels of these hormones,[252–254] and, in particular, *H. pylori* eradication leads to an increase in ghrelin and diminution of the physiologic feeding-related decrease in serum ghrelin.[251] The data indicate that *H. pylori* colonization, by its effects on epithelial and immune cells, is involved in the regulation of these hormones.[252] It is now also clear that a generation of children in developed countries is growing up without *H. pylori* in their stomachs.[255] The full consequences of this change are unknown, but at the least, *H. pylori*–positive persons are taller.[256] Large cross-sectional studies of healthy adults have found no relationships of *H. pylori* with body mass index,[257,258] but several focused studies have observed such relationships.[259] In developed countries, there appears to be an inverse relationship between a country's *H. pylori* prevalence and its obesity prevalence.[260] This will be an important area to consider in future years.

Idiopathic Thrombocytopenic Purpura

Over the past 2 decades, there have been an increasing number of reports, chiefly from East Asia, showing an epidemiologic association between the presence of *H. pylori* and the diagnosis of idiopathic thrombocytopenic purpura (ITP).[261–265] *H. pylori* eradication has been attempted as a therapy for ITP, and although not conclusive, the results suggest that eradication can have beneficial effects in some patients.[263,264] At present, for patients diagnosed with ITP, physicians should assess *H. pylori* status, and, if positive, should consider eradicating colonization of *H. pylori* as one therapeutic approach.[263,265]

Other Inverse Associations

Several studies have reported inverse associations between the presence of *H. pylori* and inflammatory bowel disease.[266] In addition, some studies have suggested that *H. pylori* has a protective effect against diarrheal diseases and tuberculosis.[267–270]

Overview of Clinical Consequences

In summary, the presence of *H. pylori* is associated with mixed effects on human health, some favorable and some unfavorable. Although the full range of *H. pylori* effects on human health and disease is not yet completely understood, a recent study concluded that *H. pylori* status was not related to overall all-cause mortality in persons older than 40 years.[271]

DIAGNOSIS

Ascertainment of *H. pylori* colonization can be made either invasively by endoscopy and biopsy, or noninvasively by serologic analysis, breath test, or fecal antigen analysis. Properly done, each of these methodologies has a diagnostic accuracy exceeding 95%; each has advantages and disadvantages (Table 217.3).

Endoscopy with biopsy involves the most expense and is an invasive procedure, but it can yield a great deal of information, including assessment of structural lesions, such as ulcers, masses, and strictures. Biopsy specimens may be cultured for *H. pylori* on antibiotic-containing media (to diminish overgrowth by any competing flora), such as Skirrow medium, as well as with a nonselective medium, such as chocolate agar.[272] Use of two media increases the yield. Plates should be incubated for 2 to 5 days at 35° to 37°C in a moist microaerobic atmosphere (5% oxygen). Comma- or S-shaped motile organisms with catalase, oxidase, and urease activity are identified as *H. pylori*.[272] Culture enables a determination of antimicrobial susceptibilities, which may be increasingly important as antimicrobial resistance broadens. Alternatively, the organisms may be visualized on histologic sections prepared with Gram, silver, Giemsa, or acridine orange stain or by immunofluorescence or immunoperoxidase methods.[272] DNA probe and polymerase chain reaction methodologies have been developed as well, but they have no current clinical justification unless genotyping of strains or antimicrobial susceptibility testing becomes clinically important. For rapid detection of *H. pylori,* biopsy samples may be incubated at 37°C to examine for preformed urease activity. After incubation for 1 hour, the assay has a sensitivity of approximately 60%, and by 24 hours, more than 90%; bacterial overgrowth of the stomach may reduce the test's specificity, especially for longer incubations and in older patients.

High-titer, stable serum immunoglobulin G responses develop nearly universally in *H. pylori*–colonized persons.[273,274] Because serology essentially samples the entire stomach, whereas biopsy samples only a small region and the inflammatory process may be patchy, serologic analysis may be more sensitive than diagnostic methods involving biopsies.[274] With successful antimicrobial therapy, antibody levels decrease, although 3 to 6 months may be required for a noticeable effect; after ineffective therapy, high antibody levels persist.[275,276] Soon after the initial acquisition of *H. pylori,* immunoglobulin M seroconversion is noted, but levels return to baseline; with recurrence after inadequate therapy, immunoglobulin M seroconversion may again be observed.[186] A number of testing services and kits are commercially available that allow physicians to detect *H. pylori* in individual patients; the serologic assays have generally been standardized for adults, so interpretation of results in children requires caution.[277] Office-based rapid serologic tests have lower sensitivity and specificity for both adults and children.

H. pylori–positive persons also shed *H. pylori* antigens in their stools. Stool antigen assay is a relatively noninvasive means to detect positivity and to monitor therapeutic responses as early as 1 month after ending treatment. Notably, the addition of some preservatives (such as formaldehyde) to stool can cause false-negative assay results.

The high urease activity of *H. pylori* has facilitated the development of urea breath tests. Subjects fast and are then given a meal containing carbon-13 (^{13}C)-urea or carbon-14 (^{14}C)-urea; over the next hour, their breath is examined for urea-derived carbon dioxide–13 ($^{13}CO_2$) or carbon dioxide–14 ($^{14}CO_2$).[278,279] Results of these assays correlate with numbers of urease-producing *H. pylori* organisms, and can be falsely negative after therapy that suppresses but does not eradicate the organism. A negative test result 1 to 3 months after cessation of therapy usually indicates eradication of the organism.

TABLE 217.3 Modalities for *Helicobacter pylori* Diagnosis

MODALITY	ADVANTAGES	DISADVANTAGES
Endoscopy with biopsy	Permits inspection of pathology; allows detection of ulcers, neoplasms	Invasive, expensive, time consuming
Culture	Permits determination of antimicrobial susceptibilities and pathogenic features of isolates	Not optimally sensitive in most laboratories. Requires several days for results.
Histology	Generally more sensitive than culture. Allows direct visualization of organism and extent and nature of tissue involvement.	Gastritis may be patchy, and biopsy may be performed on wrong area. Insensitivity to detect small numbers of organisms. Requires several days for results.
Urease detection	Rapid; most positive results seen within 2 hr	Increased sensitivity requires longer incubation. May have false-positive results with bacterial overgrowth.
Serology	Noninvasive, rapid, quantitative, inexpensive	No determination of lesions or pathology, no antimicrobial susceptibility. Not rapidly responsive to therapy.
Urea breath tests	Relatively noninvasive, relatively rapid, quantitative, rapidly responsive to therapy. Most valuable for assessing response to eradication therapy after 4–8 wk.	Involves expensive instrumentation or administration of radioisotopes. More invasive and less convenient than serology. No determination of lesions or pathology, no antimicrobial susceptibility.
Stool antigen tests	Relatively noninvasive, relatively rapid, rapidly responsive to therapy. Most valuable for assessing response to eradication therapy after 6–8 wk.	Not quantitative. Requires stool specimen, relatively expensive for developing countries. No determination of lesions or pathology, no antimicrobial susceptibility.

THERAPY

Indications for Treatment

At present, several indications have emerged for therapy directed against *H. pylori.* For patients with peptic ulceration who are colonized with *H. pylori,* antimicrobial therapies that eradicate *H. pylori* are associated with substantially lower ulcer recurrence rates than are short-course therapies directed exclusively against gastric acidity.[198,199] Thus antimicrobial therapy now is included in the primary therapy for essentially all cases of duodenal ulceration.[8] Gastric ulcers associated with *H. pylori* can be treated in the same manner as for duodenal ulceration.[202] In patients with gastric MALTomas, antimicrobial therapy directed against *H. pylori* seems to cause tumor regression in most patients.[232] Eradication therapy also should be considered for patients with ITP who are *H. pylori* positive,[263,264] for patients who have undergone resection for early-stage gastric cancer,[220] and for patients with a high risk of gastric cancer. Data concerning the efficacy of antimicrobial therapy for *H. pylori*–associated nonulcer dyspepsia are not clear-cut; only a small proportion of patients with nonulcer dyspepsia have improvement in symptoms following eradication of *H. pylori.*[188,191,192]

Treatment Regimens

Most *H. pylori* isolates are susceptible in vitro to a variety of antimicrobial agents, including bismuth salts, amoxicillin, nitrofurans, tetracyclines, and aminoglycosides.[280,281] Primary resistance to imidazoles (such as metronidazole and tinidazole) occurs in 20% to 40% of isolates[280,282] and is most common in young women who may have received these agents for gynecologic infections or in persons from developing countries treated for parasitic infections. However, primary resistance can be present in isolates from both men and women in all age groups[283] and is associated with previous exposure to a nitroimidazole, even decades earlier.[284] Resistance to clarithromycin, other macrolides, and fluoroquinolones is increasingly common,[280,285,286] and resistance to amoxicillin and tetracycline also has been reported.[281]

Several principles of chemotherapy have emerged. First, treatment with a single agent results in apparent eradication of organisms in only a minority of cases. The cumulative effects of multiple monotherapy antibiotic courses may have hastened the disappearance of *H. pylori* from the general population, but because of its unreliability for eradication, all current treatments use combination therapy.[287] Second, some agents that are effective in vitro may be ineffective in vivo, even in combination with other agents. Erythromycin is a good example of this phenomenon.[288] The ineffectiveness of many antibiotics at an acidic pH may be responsible for the lack of activity in vivo. Third, acquired resistance frequently develops after therapy with some agents but not others. To date, resistance to bismuth salts has not been reported, and resistance to amoxicillin or tetracycline develops infrequently. In contrast, acquired resistance to fluoroquinolones is so frequent that it appears to preclude their general use. Secondary resistance to imidazoles occurs in 10% to 30% of cases, even when used in combination with other agents,[289] and the development of resistance to macrolides and rifampin can also occur. Fourth, to determine true eradication of the organism and not just temporary suppression, the patient must be shown to be free of the organism at least 1 month after the cessation of therapy if biopsy, breath test, or stool antigen test is used, and at least 6 months if serologic examination is used. Better definition of these end points is currently under investigation.

Multiple antibiotic regimens can be used successfully for eradicating *H. pylori,* but none of the regimens is 100% effective (i.e., failure rates are typically >10%) and the optimal therapeutic regimen has not been defined. Suboptimal patient compliance, antibiotic resistance, and suboptimal penetration or activity of antibiotics in the gastric environment are all factors that contribute to treatment failure. Examples of commonly used treatment regimens are shown in Table 217.4.[281,290] Proton pump inhibitors, such as omeprazole and lansoprazole, are commonly included in treatment regimens (see Table 217.4).[281,291] Proton pump inhibitors are directly inhibitory to *H. pylori,*[292] inhibit urease activity,[293] and may improve antimicrobial efficacy by inducing gastric pH neutrality. Regional antibiotic resistance patterns should potentially be considered when choosing an antibiotic regimen.[286] Tailoring therapy based on antibiotic resistance testing (e.g., analyzing 23S ribosomal RNA sequences to detect mutations associated with clarithromycin resistance) may lead to improved eradication rates.[294]

In patients from whom *H. pylori* is isolated after therapy, the organisms usually are identical to the initial isolates, thus indicating that recurrence reflects relapse rather than the acquisition of a new organism. When imidazoles or macrolides are used and fail, virtually all of the recurrent organisms are resistant. In cases of treatment failure, patients should receive antimicrobial therapy with a different regimen (see Table 217.4).[281,290,295] *H. pylori* culture and susceptibility testing should be considered before implementing second-line therapies.

TABLE 217.4 Treatment Regimens for *Helicobacter pylori*

Regimens for Initial Treatment
Bismuth quadruple therapy: PPI (standard dose twice daily) + metronidazole (500 mg three or four times daily) + tetracycline (500 mg four times daily) + bismuth four times daily (dose depends on preparation) for 14 days
Non-bismuth quadruple therapy: PPI (standard dose twice daily) + amoxicillin (1 g twice daily) + metronidazole (500 mg twice times daily) + clarithromycin (500 mg twice daily) for 14 days
PPI triple therapy[a]: PPI (standard dose twice daily) + amoxicillin (1 g twice daily) + clarithromycin (500 mg twice daily) for 14 days
Regimens Appropriate for Use After Prior Treatment Failure
Bismuth quadruple therapy: PPI (standard dose twice daily) + metronidazole (500 mg three or four times daily) + tetracycline (500 mg four times daily) + bismuth four times daily (dose depends on preparation) for 14 days
Levofloxacin triple therapy: PPI (standard dose twice daily) + amoxicillin (1 g twice daily) + levofloxacin (500 mg once daily) for 14 days

[a]Restricted to areas with low clarithromycin resistance or high eradication rates.
PPI, Proton pump inhibitor.
Modified from Fallone et al.[281] *and Malfertheiner et al.*[290]

Benefits and Risks of Antibiotic Therapy

Eradication of *H. pylori* is beneficial in patients with peptic ulcer disease, gastric MALT lymphoma, and several other conditions, and can potentially reduce the risk of gastric cancer (see "Indications for Treatment" earlier). Physicians must balance the potential benefits of antibiotic treatment with possible adverse consequences, which include development of antimicrobial resistance in *H. pylori* and other commensal bacteria,[296] medication-induced upper gastrointestinal symptoms, and less common complications, such as antibiotic-associated colitis and candidiasis. Several studies have provided evidence that, after *H. pylori* eradication, the incidence of reflux esophagitis is doubled compared with the reflux incidence associated with failed eradication,[200,201] and an increased incidence of several types of cancer involving the cardia, gastroesophageal junction, esophagus, or corpus has been linked to the absence of *H. pylori.*[228,229] If *H. pylori* protects against diarrheal diseases, as some evidence suggests,[268–270] eradication of *H. pylori* among children in developing countries could increase the risk of morbidity and mortality. Finally, if the data showing an inverse association of *H. pylori* with childhood asthma and allergic conditions and possible metabolic syndromes reflect an actual protective effect, then there should be great caution about treating young children to eradicate *H. pylori.*[255] Thus removal of *H. pylori* from the stomach has both benefits and costs. As more studies become available, physicians will need to develop criteria for treatment to optimize the therapeutic-to-toxic ratio.

Prevention of *H. pylori* Acquisition

In regions of the world with a high incidence of gastric cancer, there has been interest in the possibility that immunization might prevent *H. pylori* acquisition and *H. pylori*–associated gastric cancer.[181,182] One recent phase III study in China reported that administration of an oral recombinant vaccine to children reduced the incidence of *H. pylori* acquisition.[297] Further studies are needed to assess the long-term protective effects and benefits of vaccines.

OTHER GASTRIC HELICOBACTERS

In addition to *H. pylori,* other spiral organisms may occasionally be present in the human stomach.[298–305] These organisms are spirochetal in morphology and strongly urease positive.[298–305] Non–*H. pylori* species are much less commonly observed in the gastric mucosa than *H. pylori* and occur in perhaps 1% of persons.[300] However, these 0.5- to 1.0-µm by 4- to 8-µm spirochetes are easily visualized in specimens from colonized persons. Acquisition of some organisms (e.g., *H. felis, H. salomonis,* or *H. bizzozeronii*) probably results from contact with animals.[303] Other organisms, originally called *Gastrospirillum hominis* and more recently known as *Helicobacter heilmannii* sensu lato, have not been cultivated in vitro.[298–305] Both *H. pylori* and *H. heilmannii* may be present in the same person. The role of non–*H. pylori* species in gastric disease in humans is uncertain.

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The complete reference list is available online at Expert Consult.

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218 Enterobacteriaceae

George E. Nelson and Matthew H. Greene

SHORT VIEW SUMMARY

Definition
- The Enterobacteriaceae are a family of gram-negative, non–spore-forming facultative anaerobes that ferment glucose, reduce nitrate to nitrite, and produce catalase.

Epidemiology
- Enterobacteriaceae are the most common gram-negative pathogens isolated in microbiology laboratories and are capable of causing both community-acquired and nosocomial infection in virtually every organ system.
- They are frequently implicated in outbreak settings, especially in hospital settings.
- These organisms have numerous mechanisms of antimicrobial resistance, with increasing global spread.

Microbiology
- The family includes clinically important genera such as *Escherichia coli*, *Klebsiella*, and *Enterobacter*, among others.
- *Salmonella*, *Shigella*, and *Yersinia* are also members but are discussed in separate chapters.

Diagnosis
- Methods of diagnosis vary by pathogen but include standard culture technique as well as immunoassays, polymerase chain reaction assays, or DNA probes specific to a particular virulence factor.
- Multiplex gastrointestinal molecular assays—termed culture-independent diagnostic tests (CIDTs)—are now commonly used for diarrheal diagnostics.
- Epidemiologists employ pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) for highly discriminative strain typing during outbreaks.

Therapy
- Treatment varies according to pathogen and the severity of the clinical syndrome.
- Close attention must be given to antimicrobial resistance patterns owing to increasing prevalence and multiple mechanisms.

Prevention
- Hand and food hygiene decrease the risk of person-to-person and foodborne outbreaks.
- Strict infection control measures target nosocomial spread of antibiotic-resistant strains.
- There are no currently licensed vaccines.

The family Enterobacteriaceae belongs to the domain Bacteria, phylum Proteobacteria, class Gammaproteobacteria, and order Enterobacteriales (http://www.bacterio.net). The Enterobacteriaceae are gram-negative, non–spore-forming, facultative anaerobes that are typically motile by means of peritrichous flagella, ferment glucose, reduce nitrate to nitrite, and produce catalase but not oxidase. The family includes multiple genera implicated in human infection (Table 218.1).

This chapter begins by discussing prototypical shared structural and pathogenic features of the family Enterobacteriaceae—mostly extrapolated from the study of *Escherichia coli*— followed by more detailed descriptions of particular pathogens. *Salmonella, Shigella* (now considered an *E. coli* pathotype), and *Yersinia* are discussed elsewhere because of their distinct pathogenesis and clinical syndromes (see Chapters 223, 224, 229A, and 229B).[1]

GENERAL PROPERTIES

Epidemiology

The lower gastrointestinal tract is the predominant reservoir for Enterobacteriaceae, although these bacteria are also widely distributed in the environment. Furthermore, enterobacterial species frequently colonize the human genitourinary tract and oropharynx, especially in frequently hospitalized or immunosuppressed individuals.[2–5] Consequently, the Enterobacteriaceae are the most common gram-negative pathogens isolated in microbiology laboratories, capable of causing both community-acquired and nosocomial infection in virtually every organ system.

Antimicrobial resistance among the members of the Enterobacteriaceae family is a major global health threat.[6] Extended-spectrum β-lactamase (ESBL)–producing, metallo-β-lactamase (MBL)–producing, and carbapenem-resistant Enterobacteriaceae (CRE) isolates are frequently encountered in varied clinical settings throughout both the developed and developing world.[7–9] Plasmid-encoded resistance genes on transposons or integrons have allowed rapid and promiscuous dissemination of antibiotic resistance even between disparate enterobacterial genera.[10] Pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and whole-genome sequencing can facilitate rapid identification then containment of clonal outbreaks, including highly drug-resistant Enterobacteriaceae.[11–13]

General mechanisms of antibiotic resistance are discussed in Chapter 18.

Structural and Surface Antigenic Features

The Enterobacteriaceae are rod-shaped organisms, generally 1 to 3 μm in length and 0.5 μm in diameter, with a genome typically consisting of a single circular chromosome, although multiple plasmids may be present in the cytoplasm. As with any gram-negative organism, members of the Enterobacteriaceae family feature a peptidoglycan cell wall in the periplasmic space between an inner and outer membrane (Fig. 218.1).

Inner Membrane

The inner—or cystoplasmic—membrane is a phospholipid bilayer that maintains the proton motive force for energy storage and regulates the passage of molecules to and from the cytoplasm. The inner membrane may feature hundreds of proteins, including integral membrane proteins with transmembrane domains traversing the bilayer, lipoproteins inserted in the outer leaflet, and peripheral membrane proteins in either the inner or outer leaflet.[14] The inner membrane includes the proteins involved in electron transfer and oxidative phosphorylation, and the F_1F_0 adenosine triphosphatase that couples proton transport to adenosine triphosphate (ATP) synthesis. Furthermore, the inner membrane may feature efflux pumps, solute transporters, protein translocation systems, polysaccharide export systems, and histidine kinase signaling proteins.[15]

Periplasmic Space

The periplasmic space between the inner and outer membrane is an aqueous, oxidizing environment containing the peptidoglycan cell wall and a high concentration of proteins.[16] These proteins include oxidoreductases, isomerases, chaperones, proteases involved in protein

TABLE 218.1 Clinically Important Enterobacteriaceae

GENUS	SPECIES
Chronobacter	*sakazakii*
Citrobacter	*freundii* *koseri* *braakii*
Edwardsiella	*tarda*
Enterobacter	*cloacae* *aerogenes*
Escherichia	*coli* *albertii*
Hafnia	*alvei*
Klebsiella	*pneumoniae* *oxytoca* *granulomatis*
Morganella	*morganii*
Pantoea	*agglomerans*
Plesiomonas	*shigelloides*
Proteus	*mirabilis* *vulgaris*
Providencia	*stuartii* *rettgeri*
Salmonella	*enterica*
Serratia	*marcescens* *liquefaciens*
Shigella (now considered an E. coli pathotype)	*dysenteriae* *flexneri* *sonnei* *boydii*
Yersinia	*pestis* *enterocolitica* *pseudotuberculosis*

folding and degradation, lipoprotein-sorting proteins, detoxifying enzymes, and solute-binding proteins that ferry molecules across the periplasmic space. The periplasmic space also features enzymes involved in the biogenesis of peptidoglycan, lipopolysaccharide (LPS) and the capsule.

Peptidoglycan Cell Wall

The gram-negative cell wall is a thin layer of peptidoglycan—also called murein—composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid amino sugars joined by β-1,4 linkages with a short peptide composed of L-alanine, D-glutamic acid, L-*meso*-diaminopalmelic acid, and D-alanine attached to the carboxyl group of the muramic acid.[17] Linear strands of peptidoglycan are linked by amide bonds to adjacent strands, with murein lipoprotein anchoring the layer to the inner leaflet of the outer membrane. The peptidoglycan layer of each bacterium is believed to comprise a single contiguous molecule enveloping the organism, responsible for shape and osmotic stability yet undergoing constant remodeling as the organism elongates and divides.

Outer Membrane

The outer membrane is an asymmetrical lipid bilayer featuring a mostly phospholipid inner leaflet and an outer leaflet composed primarily of LPS. The outer leaflet also features porins—membrane proteins with a conserved β-barrel fold enclosing a central aqueous channel—that regulate the passage of hydrophilic molecules.[18] The polarity of LPS in the outer leaflet prevents penetration of lipophilic molecules, including detergents, dyes, and hydrophobic antimicrobials.[19]

Because LPS is an essential component of the outer membrane of all gram-negative bacteria, some argue it is not a virulence factor per se. Regardless, LPS will be discussed in more detail later; the repeating oligosaccharide attached to the LPS core is referred to as the O antigen, which is the basis for serogroup classification. Each species within the Enterobacteriaceae has multiple O-antigen types.

Other Surface Polysaccharides and Capsules

Other potential surface polysaccharides of the Enterobacteriaceae family include enterobacterial common antigen, colonic acid, and a wide variety of polysaccharide envelopes known as capsules. Enterobacterial common antigen is remarkably conserved throughout the family, suggesting an important although poorly understood function.[20] Many of the Enterobacteriaceae—including *E. coli* strains—feature a colonic acid layer, which resembles a subset of capsule types. True capsules are linked to either LPS or α-glycerol phosphate and are the basis of the K-antigen serotyping scheme.[21] Some enterobacterial capsules can be quite luxuriant, imparting the highly mucoid colonial morphology to *Klebsiella* and *Enterobacter* species, for example.[22]

Flagella

Flagella are flexible, rotating surface appendages that propel bacteria through liquid environments. Most members of the Enterobacteriaceae are motile by way of flagella, often emanating from all sides of the organism (Fig. 218.2A). Even nonmotile Enterobacteriaceae sometime retain genes specific to flagellar expression and are then capable of motility under certain conditions.[23] Like LPS, flagellin is recognized by host innate immune system pattern recognition receptors, which can lead to neutrophil recruitment and initiation of an inflammatory response.[24–26]

Flagellar biogenesis proceeds from base to tip and is orchestrated by a complex regulatory network, responding to a variety of extracellular signals. The flagellar filament is composed of a hollow helical array of the protein flagellin whose amino and carboxyl termini is highly conserved within and across a species. In contrast, the surface-exposed middle of the flagellin molecule is highly variable as a result of both diversifying mutations and recombination after horizontal gene transfer.[27] This diversity is represented in the H-antigen typing scheme, the third component of O:K:H serotyping.

Pili

Most of the Enterobacteriaceae produce thin nanofilaments extending from the bacterial surface called pili—also known as fimbriae—that mediate autoaggregation and adhesion to host cells.[28] Furthermore, pili facilitate bacterial conjugation and are often encoded by plasmids—capable of harboring virulence and antimicrobial resistance genes—to mediate intercellular contact and genetic exchange.[29] The family Enterobacteriaceae features a variety of pilus types, which differ in morphology and function. An individual strain may produce multiple different pili even of the same type.[30–32]

Chaperone-usher type pili are common among the Enterobacteriaceae and include the ubiquitous type 1 pili (see Fig. 218.2B). Assembly begins with secretion machinery exporting subunits of the major structural protein pilin to the periplasmic space to bind with chaperones to prevent premature subunit interactions. The pilin-chaperone complexes are then delivered across the outer membrane—in a specific order starting with the tip—by a membrane channel protein known as the usher.[33,34] Most chaperone-usher type pili feature a rigid rod composed of a helical array of pilin joined end to end with a thinner, more flexible tip.[35,36] The tip often features adhesin proteins that serve as critical virulence factors.

Type IV pili are also widespread among the Enterobacteriaceae, often forming ropelike bundles expressed at the poles of the organism[37–41] (see Fig. 218.2C). The type IV pilin protein is processed to its mature form by a dedicated prepilin peptidase that also *N*-methylates the amino-terminal residue.[42] Type IV pili are retractable, which facilitates aggregation and disaggregation and a type of locomotion called "twitching motility."[43]

Virulence Factors

The Enterobacteriaceae relevant to the human host include a wide variety of organisms, ranging from commensal bacteria to highly virulent

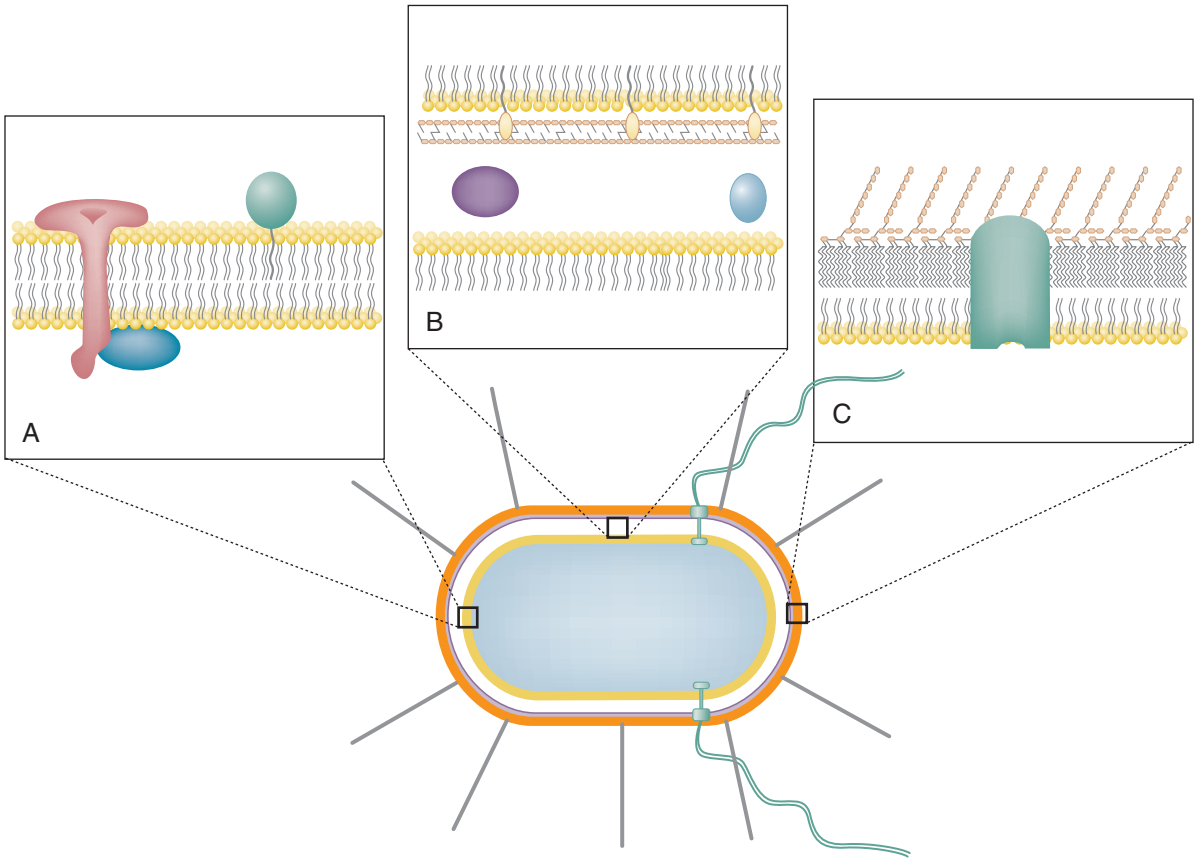

FIG. 218.1 Architecture of an enterobacterial cell. The cytoplasm is depicted in blue, the inner—or cytoplasmic—membrane in yellow, the periplasmic space in white, the peptidoglycan layer in purple, and the outer membrane in orange. Pili—also called fimbriae—are depicted in gray as linear organelles extending from the outer membrane, and flagella are shown in green as hollow, flexible organelles emanating from an assembly and secretion apparatus that spans both membranes and the periplasmic space. Insets show closer views of the envelope. (A) The cytoplasmic membrane has a phospholipid bilayer, transmembrane *(red)* proteins, peripheral membrane *(blue)* proteins, and lipoproteins *(green).* (B) The periplasmic space is surrounded by the inner and outer membranes and includes soluble proteins (*purple* and *blue*). The peptidoglycan layer, composed of disaccharide and peptide cross-linkages, is associated with the inner leaflet of the outer membrane through covalent attachment to lipoprotein *(yellow).* (C) The outer membrane layer has an inner phospholipid leaflet and an outer lipopolysaccharide leaflet. Traversing the membrane are porin proteins that regulate the passage of molecules *(green).*

pathogens. This spectrum of pathogenicity reflects the variable expression of virulence factors in a given strain. Although debate persists about what constitutes a true virulence factor, the principle of "molecular Koch's postulate" provides a helpful conceptual framework for discussing the general features of the Enterobacteriaceae discussed here. This principle defines a virulence factor as causing disease whose severity is attenuated by mutation of the gene encoding this factor, which is then restored by reintroduction of the wild-type allele.[44] As with all microbes, the common themes of pathogenicity include entry, establishment and multiplication, host defense avoidance, tissue damage, and exit.[45]

Adhesins

Many adhesins of the Enterobacteriaceae—including pili, outer membrane proteins, and even surface carbohydrates—have proven indispensable to infection.[46] An organism may feature a number of adhesins at once or in sequence in response to environmental cues or as a result of random phase variation.

Type 1 pili are the prototypical enterobacterial adhesin, composed of a rigid rod of repeating subunits of the protein FimA arranged in a helical array. The pilus tip contains a short fiber composed of FimG and FimH subunits—the so-called fimbrial adhesins—joined end-to-end with the FimA rod.[35] FimH binds to mannose residues found in glycoproteins and glycolipids on the surface of host cells.[47,48] Type 1 pili expression is subject to phase variation because of the invertible DNA element flanking the promoter of the *fim* operon, resulting in variable pili production depending on the organism's environment.[49] Furthermore, type 1 pili adhesion is enhanced by shear force such as the flow conditions of the genitourinary tract.[50] In addition to being well-established as virulence factors, type 1 pili are also important in colonization and transmission.[51,52] Furthermore, a given enterobacterial strain may produce multiple other chaperone-usher type pili and type IV pili, discussed in more detail later, depending on the particular species.

The invasin-intimin family of proteins are the best characterized outer membrane adhesins, described from studies of *Yersinia pseudotuberculosis* and enteropathogenic *E. coli* (EPEC).[53,54] These proteins—inserted into the bacterial outer membrane—share common structural features with similar amino-acid sequences, especially in their amino-terminal and central domains. The membrane portion is connected by a flexible hinge region to a rigid rod of repeating subunits similar in structure to portions of an immunoglobulin molecule. The carboxyl-terminal adhesin domain shares similarities to calcium-binding lectin molecules.[55–57]

Toxins and Secretion Systems

Many members of the Enterobacteriaceae family produce toxins released into the environment or directed to host cells. Interesting to note, despite having proven activity in animal models or tissue culture cells, many enterobacterial toxins do not fully reproduce disease in the absence of the bacteria itself, which suggests that some toxins play only an ancillary or even an unknown role in pathogenesis.

Because of the formidable barrier of the inner and outer membrane, toxin export often requires specific secretion machinery.[58] The autotransporter proteins produced by many gram-negative bacteria are a prime example.[59] Typical signal sequences at the amino termini of these proteins are cleaved as they are exported across the inner membrane

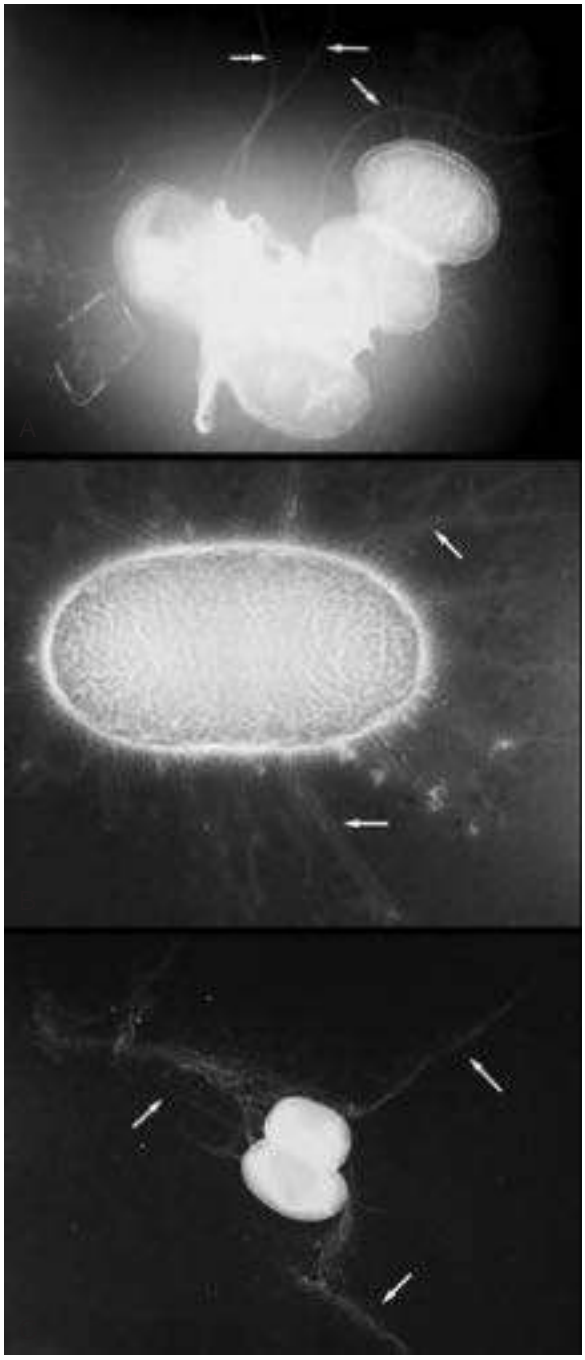

FIG. 218.2 Transmission electron micrographs of *Escherichia coli* cells. (A–B) An extraintestinal pathogenic *E. coli* strain grown under conditions favoring the expression of type 1 pili demonstrates both flagellae (*arrows* in A) and pili (*arrows* in B). (C) An enteropathogenic *E. coli* strain grown under conditions favoring expression of bundle-forming type IV pili *(arrows). (Courtesy Eric Buckles and Paula J. Fernandes.)*

then inserted into the outer membrane by the β-barrel structure of their mature amino termini. The passenger domain at the carboxyl terminus is then cleaved off and released. A subset of autotransporter proteins—termed serine protease autotransporters of the Enterobacteriaceae (SPATE)—are well characterized in *E. coli* and *Shigella* pathotypes.[60,61]

Hemolysins—named for inducing zones of clearing on blood agar plates—are toxins capable of lysing host cells. *E. coli* hemolysin is the best described and also introduces the concept of the secretion system. *E. coli* hemolysin is secreted in a single step across both the inner and outer membrane by an apparatus referred to as a type I secretion system (T1SS), composed of integral inner membrane proteins—HlyB and HlyD—that bind hemolysin. The HlyB-HlyD-hemolysin complex then engages a tunnel-channel protein, TolC, that spans the periplasmic space and outer membrane, followed by hemolysin release.[62,63] TolC serves a similar function for other toxin export, including *E. coli* heat-stable enterotoxin.[64] Hemolysin inserts into the host cell membrane with subsequent pore formation, and induces low-frequency intracellular calcium oscillations that stimulate signaling through nuclear factor kappa B (NF-κB).[65,66]

Most of the Enterobacteriaceae also feature a type II secretion system (T2SS), a multiprotein complex spanning the periplasmic compartment. T2SSs can serve a variety of functions including extracellular secretion of toxins in addition to hydrolytic enzymes.[67] For example, enterohemorrhagic *E. coli* (EHEC) O157:H7 features a plasmid-encoded T2SS that secretes a metalloprotease capable of degrading the C1 esterase inhibitor involved in complement activation.[68]

Several important enterobacterial pathogens have a type III secretion system (T3SS) that not only exports proteins through the bacterial membrane but also injects them into the host cell membrane.[69,70] The complex T3SS machinery is similar to the apparatus required for flagella assembly, featuring a "needle" extending from the bacterial outer membrane. Proteins secreted through this needle form a pore in the host cell membrane through which a variety of "effector" proteins pass, which subvert host cell pathways through structural mimicry.[71]

Lipopolysaccharide and Capsules

LPS is an essential component of the outer membrane of all gram-negative bacteria that can also function as a virulence factor. LPS has different chemical compositions depending on the organism, with varying biologic activity and potency.[72] The three major domains of LPS are the lipid A—or endotoxin—backbone, the core phosphorylated oligosaccharide, and the repeating oligosaccharide side chains.[73]

Lipid A is composed of a β-1,6-disaccharide of glucosamine that is phosphorylated and substituted with saturated hydroxylated acyl chains. The acyl groups of lipid A are inserted into the outer leaflet of the outer membrane. The core oligosaccharide is composed of a pair of 8-carbon sugars known as KDO linked to lipid A, which are in turn linked to additional sugars, forming a branched chain. The repeating oligosaccharide—or O antigen—attached to the LPS core are highly variable and, again, are the basis of Kauffman O:K:H serotyping. Genes responsible for O antigen synthesis are subject to interspecies lateral transfer, with *E. coli* alone featuring more than 170 different O-antigen serotypes.[21,74]

LPS, often bound to the host acute-phase protein LPS-binding protein (LBP), binds to CD14, where lipid A is recognized by the host pattern recognition receptor Toll-like receptor 4 (TLR4). The TLR4-MD2 complex activates NF-κB by the MyD88 pathway to initiate transcription of proinflammatory cytokines including tumor necrosis factor (TNF), chemokines, and major histocompatibility complex (MHC) receptors. This inflammatory immune response is responsible for septic shock but is also critical to host recovery. For example, mice deficient in TLR4 fail to control gram-negative infection, and TLR4 polymorphisms in humans are associated with more severe gram-negative infection.[75,76]

Enterobacterial capsules can help the organism survive in human serum by avoiding phagocytosis.[77–79] Capsules from certain strains contribute particular pathogenic features—for example, the K1 capsule, implicated in neonatal meningitis–associated *E. coli* (NMEC), or the capsule responsible for the mucoid morphology of *Klebsiella pneumoniae.*[80,81]

Iron Acquisition

Virtually all organisms require iron as a metal cofactor for critical enzymatic processes. Many enterobacterial pathogens have developed highly efficient iron scavenger systems to compete with host iron-binding proteins, controlled by the ubiquitous transcription factor ferric uptake regular (Fur).[82,83] Enterobacterial siderophores—such as enterobactin and aerobactin—are low-molecular-weight compounds with high affinity for iron that are synthesized, secreted, and recaptured by the organism.[84–86] The cytoplasmic membrane protein TonB is common to all of these iron uptake systems, spanning the periplasmic space to siderophore receptors and other gated porins in the outer membrane.[87,88]

SPECIFIC ORGANISMS

Escherichia

The genus *Escherichia* is named for Theodore Escherich who described *Escherichia coli* in 1885 during pioneering studies of the neonatal fecal microbiota.[89,90] *E. coli*, the type genus of the family Enterobacteriaceae, is the most common facultative anaerobe in the human gastrointestinal tract.[91] Although most strains are commensal organisms in the human intestine, pathogenic *E. coli* causes a variety of diseases. The genus *Escherichia* contains other species that rarely cause human infection, such as *E. blattae, E. fergusonii, E. hermanii, E. marmotae, E. vulneris,* and *E. albertii* (an *eae*-positive species often misidentified as EHEC or EPEC, with some strains previously misclassified as *Hafnia alvei* or *Shigella boydii*).[92–97]

Most *E. coli* are motile and ferment lactose and other sugars to produce indole. The average genome of *E. coli* includes approximately 5000 genes, although only about 1500 are shared among all *E. coli*, reflecting the diversity of individual factors for a given pathogenic strain.[32,91,98–100] *E. coli* acquired many of its virulence factors from horizontal gene transfer, and these are now encoded by bacteriophages, on plasmids, or within stretches of chromosome known as pathogenicity islands, which were first described in *E. coli*.[101–104]

E. coli has historically been classified by pathotypes causing diarrheal syndromes associated with characteristic virulence factors and adhesion patterns[105–107] (Table 218.2). The term *diarrheagenic E. coli* (DEC) incorporates five pathotypes discussed here—enterotoxigenic *E. coli* (ETEC); EPEC; Shiga toxin–producing *E. coli* (STEC), which includes EHEC; enteroaggregative *E. coli* (EAEC); and enteroinvasive *E. coli* (EIEC). Two additional putative enteric *E. coli* pathotypes—diffusely adherent *E. coli* (DAEC) and adherent-invasive *E. coli* (AIEC)—are also discussed.[100,108]

In outbreak settings, laboratories still use the classic Kauffman serotyping scheme to identify *E. coli* based on antigenic variation of the O (somatic) polysaccharides, H (flagellar), and K (capsular) surface antigens (e.g., *E. coli* 0157:H7). However, *E. coli* lineages elaborated through whole-genome sequencing and the discovery of hybrid strains—such as the Shiga toxin–producing EAEC O104:H4 serotype—increasingly confound classification based on pathotype, serotype, or even phylogenetic grouping.[102,109] This ambiguity reflects the evolutionary complexity of *E. coli* in the context of ongoing lateral transfer of virulence factors between strains.[110,111]

Beyond diarrheal illness, *E. coli* has the capability to cause extraintestinal disease, including infection of the peritoneum, liver, and biliary system. Furthermore, *E.* coli is the single most common cause of urinary tract infection (UTI) and a major pathogen causing meningitis, septicemia, pneumonia, cellulitis and even bone and joint infection. The term *extraintestinal pathogenic E. coli* (ExPEC)—including

TABLE 218.2 Epidemiology, Clinical Syndrome, Characteristic Virulence Factors, and Treatment Considerations for Diarrheagenic *Escherichia coli* Pathotypes

PATHOTYPE	EPIDEMIOLOGY	CLINICAL SYNDROME	CHARACTERISTIC VIRULENCE FACTORS	TREATMENT CONSIDERATIONS[a]
ETEC	Pediatric diarrhea, particularly in the developing world Traveler's diarrhea Foodborne outbreaks	Acute nonbloody diarrhea	Heat-labile (LT) and/or heat-stable toxin (ST) Heterogeneous adhesins; also called colonization factors (CFs)	Traveler's diarrhea[b]
EPEC	Pediatric diarrhea, particularly in the developing world Atypical EPEC (aEPEC) increasingly recognized to cause diarrhea regardless of age Traveler's diarrhea Nosocomial and child care center outbreaks	Acute nonbloody diarrhea Associated with vomiting and sometimes protracted disease	Attaching and effacing lesions (A/E) (see Fig. 218.3) Typical EPEC (tEPEC) defined by type IV bundle-forming pili (BFP)	Traveler's diarrhea[b] Antimicrobial therapy for severe endemic disease, including nosocomial outbreaks
STEC (including EHEC)	Foodborne outbreaks; person-to-person and animal contact transmission	Acute, often bloody diarrhea Can progress to hemolytic-uremic syndrome (HUS)	Shiga toxin EHEC produces attaching and effacing lesions (A/E) like EPEC (see Fig. 218.3)	Antimicrobial and antimotility agents associated with increased risk of HUS
EAEC	Increasingly recognized to cause diarrhea in all ages, including developed countries Associated with growth impairment in children from developing countries Traveler's diarrhea Foodborne outbreaks	Acute nonbloody diarrhea Chronic diarrhea in some children and HIV patients	Aggregative adherence fimbriae (AAF) Enteroaggregative heat-stable toxin 1 (EAST-1) and several SPATE proteins	Traveler's diarrhea[b] Case reports support antimicrobial therapy for chronic disease in HIV patients
EIEC	Pediatric diarrhea, particularly in the developing world Foodborne outbreaks; person-to-person transmission	Acute, sometimes bloody diarrhea Clinically indistinguishable from shigellosis, although typically less severe	Cellular invasion then cell-to-cell spread orchestrated by *pInv* plasmid	Antimicrobial therapy recommended for severe cases, but must first exclude STEC
DAEC	Pediatric diarrhea in somewhat older children Not an established cause of diarrhea in adults	Acute nonbloody diarrhea	Afa/Dr family adhesins	
AIEC	Associated with Crohn disease pathogenesis, although not with diarrhea per se		Adherence to M cells of Peyer patches by type I and long polar fimbriae Cell invasion facilitated by membrane-bound protein OmpA	

[a]Volume resuscitation is the most important treatment for diarrhea regardless of etiology. Most diarrheagenic *E. coli* infections are self-limiting with supportive care.
[b]Data support the use of antimicrobial therapy for shortening the duration of traveler's diarrhea with loperamide in combination with single-dose azithromycin, levofloxacin, or rifaximin.
AIEC, Adherent-invasive *E. coli*; *DAEC,* diffuse-adhering *E. coli*; *EAEC,* enteroaggregative *E. coli*; *EHEC,* enterohemorrhagic *E. coli*; *EIEC,* enteroinvasive *E. coli*; *EPEC,* enteropathogenic *E. coli*; *ETEC,* enterotoxigenic *E. coli*; *HIV,* human immunodeficiency virus; *SPATE,* serine protease autotransporters of the Enterobacteriaceae; *STEC,* Shiga toxin–producing *E. coli.*

uropathogenic *E. coli* (UPEC), NMEC, and sepsis-associated *E. coli* (SEPEC)—has been used to describe nondiarrheagenic strains.[112]

Epidemiologists have often used PFGE for highly discriminative strain typing during outbreaks. MLST has emerged as another common means of identifying pathogenic strains by their allelic profile, allowing global comparison.[113] Future typing schemes will likely incorporate the clinical syndrome, virulence factors historically associated with a given pathotype, serotyping, and whole-genome sequence data to characterize pathogenic *E. coli* most fully.

General Clinical Principles Related to Diarrheagenic *Escherichia coli*

DEC has been associated with seasonal increases in temperature.[114,115] Recent meta-analysis has suggested as much as an 8% increased incidence of diarrhea due to *E. coli* for every 1°C increase in mean monthly temperature, regardless of season.[116] Anticipated increases in the global burden of pathogenic *E. coli* underscore the need for optimal strain characterization to prevent transmission and treat infection effectively, especially in regions most affected by climate change.

E. coli strains are implicated in many diarrheal syndromes, including traveler's diarrhea, which are discussed in detail elsewhere (see Section J, "Gastrointestinal Infections and Food Poisoning," and Chapter 319). Recent guidelines emphasize the diagnostic and therapeutic strategies for suspected infectious diarrhea for both endemic disease and the returning traveler.[117–119]

The advent of multiplex gastrointestinal molecular assays—termed culture-independent diagnostic tests (CIDTs)—has helped in optimizing antibiotic therapy and identifying possible DEC outbreaks.[120,121] Guidelines suggest limiting testing to patients with diarrhea who are experiencing fever, bloody or mucoid diarrhea, severe abdominal cramping, or signs of sepsis or immunocompromised hosts.[117] Additional criteria include diarrhea lasting >7 days or epidemiologic investigation during known or suspected diarrheal outbreaks.[119]

DEC is a predominant pathogen in traveler's diarrhea.[118,122–124] Data support the use of antimicrobial therapy for shortening the duration of traveler's diarrhea with loperamide in combination with single-dose azithromycin, levofloxacin, or rifaximin.[118,125–128] However, antibiotic therapy introduces adverse side effects and increases risk for colonization with ESBL-producing Enterobacteriaceae.[128,129] Debate continues as to the severity of traveler's diarrhea warranting antibiotic treatment.[130,131]

Clinicians continue to recommend strict avoidance of contaminated food and water to prevent traveler's diarrhea, although the data are mixed as to whether this counseling reduces infection, given practical limitations of adherence.[119,132–135] Prophylactic bismuth can reduce the risk of traveler's diarrhea, but further research is needed to delineate who would clearly benefit from prophylactic antimicrobial therapy.[119,131,136,137]

Antimicrobial therapy for DEC should be directed by the identification of a clinically plausible organism.[117] Treatment specific to particular *E. coli* pathotypes will be discussed later. As a general principle, regardless of pathotype, volume resuscitation is the cornerstone of therapy for all diarrheal illness. Empirical antimicrobial therapy is discouraged in immunocompetent children and adults pending the results of appropriate diagnostic tests. Empirical therapy is recommended for infants <3 months of age with suspected bacterial diarrhea, immunocompromised patients with severe and bloody diarrhea, and ill-appearing immunocompetent patients or returning travelers with fever and/or signs of sepsis with suspected enteric fever.

Enterotoxigenic *Escherichia coli*

ETEC infection results from ingestion of contaminated water or food and is a common cause of diarrhea in the developing world, especially in children, and in returning travelers.[105,132,138] For example, ETEC producing heat-stable toxin (with or without heat-labile toxin) is one of the leading causes of pediatric diarrheal disease in sub-Saharan Africa and south Asia.[185] ETEC foodborne outbreaks occur in developed countries often as a consequence of improper sanitation.[139,140]

The ETEC incubation period ranges from hours to 2 days. The severity of diarrhea ranges from mild to cholera-like, although typically it occurs without fever, vomiting, or bloody stool. Symptoms usually last less than 5 days.[141]

ETEC pathogenesis starts with adherence, then colonization of the small intestinal mucosa, which is mediated by adhesins—or colonization factors (CFs)—on the bacterial surface.[124,142] More than 20 distinct CFs have been described, including chaperone-usher type and type IV pili, thinner fibrillae, and even afimbrial factors.[143–145] This heterogeneity of CFs may partly account for ETEC recurrence during childhood in the developing world followed by eventual immunity.[146,147] The degree to which exposure to a particular CF, toxin, or other antigen confers protection remains an important debate for vaccine development.[148]

Nonpilus adhesins, such as Tia and TibA invasins and the secreted protein EtpA, also promote bacterial adherence to intestinal cells.[149] The SPATE protein EatA has been shown to degrade EtpA, modulating adhesion and actually accelerating toxin delivery.[150]

ETEC produces heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST); these toxins are responsible for secretory diarrhea. About a third of ETEC strains produce both toxins.[151] LT has an almost identical quaternary structure and mechanism of action as cholera toxin.[152] LT features a single catalytic A subunit and a pantameric B receptor-binding subunit, secreted by a T2SS.[153] The B subunit binds to the apical membrane of enterocytes and, after endocytosis, the A subunit finds its target on the basolateral membrane—the regulatory subunit of a heterotrimeric G protein ($G\alpha_s$)—and locks it in its active form. $G\alpha_s$ then constitutively activates its target, adenylyl cyclase, increasing intracellular cyclic adenosine monophosphate (cAMP) and ultimately protein kinase A, which activates the cystic fibrosis transmembrane conductance regulator (CFTR). This cascade results in active secretion of chloride then resultant sodium and water loss into the small intestinal lumen.[154,155] LT has impressive genetic diversity with varying expression in clinical syndromes, which suggests that a particular LT type confers variable virulence.[156–159]

ST is produced by 75% to 80% of ETEC isolates and is associated with more severe disease than ETEC strains that produce LT only.[124,151] ST is a small peptide resembling the mammalian peptide hormone guanylin and binds to the guanylin receptor on the enterocyte apical membrane.[160] Traditional dogma has attributed ST-induced secretory diarrhea to receptor binding resulting in elevated cyclic guanosine monophosphate (cGMP) and protein kinase G activation then CFTR activation with resultant chloride, sodium, and water secretion.[161] However, in vivo experiments with ST in rat models have questioned this doctrine.[162,163] As with LT, new ST variants continue to be reported with varying impact on severity.[164]

The diagnosis of ETEC diarrhea can be confirmed with immunoassays, polymerase chain reaction (PCR) assays, or DNA probe assays designed to detect genes encoding ETEC toxins. Furthermore, multiplex PCR assays have been developed for diarrhea suspected to be due to *E. coli,* which includes ETEC toxin or virulence factor detection.[165–168] Less expensive approaches in development include single-chain variable fragments (scFv) against LT and ST.[169]

Antimicrobial therapy for ETEC diarrhea has been studied mostly with respect to traveler's diarrhea, in which ETEC is implicated in 20% to 40% of cases.[118,122–124] Evidence for antibiotic efficacy in traveler's diarrhea was discussed earlier (see also Chapter 319), although clinicians should consider regional differences in ETEC resistance patterns.[170]

ETEC vaccine efforts have been thwarted by antigen heterogeneity and the poor immunogenicity of ST. Killed oral vaccine that produced an immune response to ETEC CFs reduced the rate of severe diarrhea in returning travelers but proved ineffective otherwise.[171,172] A cutaneous LT vaccine successfully induced LT-specific immunoglobulin, but clinical results proved disappointing.[173,174] Research is ongoing for an oral multivalent vaccine composed of four ETEC strains overexpressing CFs and an LT toxoid[175] in addition to various vaccine formulations exploiting EtpA immunogenicity to prevent host colonization.[150,176–179]

Enteropathogenic *Escherichia coli*

EPEC was the first pathotype of DEC described, reported after devastating neonatal outbreaks in the 1940s.[114,180,181] EPEC is characterized as either typical (tEPEC) or atypical (aEPEC); aEPEC lacks the type IV bundle-forming pili (BFP) encoded by the EPEC adherence factor (EAF) plasmid.[182,183]

The EPEC incubation period is rapid, with diarrhea starting as early as 3 hours after ingestion in human volunteers and with protracted disease lasting as long as 2 weeks.[105,184] tEPEC causes substantial childhood diarrhea in the developing world and is the pathogen associated with the highest risk of death in infants aged 0 to 11 months.[185] aEPEC is a highly heterogeneous group found commonly in asymptomatic children, although increasingly it is recognized as an emerging cause of diarrhea in the developed world, affecting children, adults, and the immunocompromised.[100,183,186–195] Because transmission occurs principally through person-to-person contact, EPEC is a significant contributor to nosocomial and child care center outbreaks.[196–198]

EPEC infection produces watery nonbloody diarrhea, mostly affecting children younger than 6 months.[154,190] EPEC diarrhea is often associated with vomiting, resulting in severe dehydration and with protracted disease capable of yielding malnutrition and considerable morbidity.[199–203]

The ropelike BFP (see Fig. 218.2C) of tEPEC forms three-dimensional microcolonies on tissue culture cells in a pattern termed localized adherence (LA) (see Fig. 218.4A later).[106,107,204] BFP are involved in biofilm formation but are then able to retract after host cell binding, enhancing infectivity through more efficient colonization and effector translocation.[205–207] EPEC classification has been complicated somewhat by the discovery of strains—including both tEPEC and aEPEC—displaying diffuse or aggregative adherence patterns.[190,208] tEPEC strains with a hybrid localized and aggregative-like adherence phenotype may cause more severe and persistent diarrhea owing to augmented biofilm formation.[209]

The hallmark feature of EPEC is the formation of attaching and effacing (A/E) lesions on the apical surface of intestinal epithelial cells (Fig. 218.3).[210] A/E is orchestrated by genes on a pathogenicity island known as the locus of enterocyte effacement (LEE), which encodes a T3SS, the adhesin intimin—encoded by the *eae* gene—and the translocated intimin receptor (Tir) and other EPEC-secreted protein (Esp) effectors.[101,211,212] Other important effector proteins are encoded outside the LEE.[213,214]

The protein EspA is a filamentous extension of the EPEC T3SS that connects and forms a canal to the host cell.[215] Tir transits the EspA canal, enters a pore in the host cell membrane formed by the proteins EspB and EspD, and then inserts into the host membrane to function as a receptor for intimin.[54] Each intimin receptor dimer binds two intimin molecules protruding from the bacterial surface, activating the N-WASP and Arp2/3 actin-nucleating and filament machinery,[216] resulting in host cell microvilli loss and the formation of a cuplike actin and cytoskeletal pedestal (see Fig. 218.3).[56,216–221] Other effector proteins injected by the EPEC T3SS interrupt the NK-κB pathway to inhibit the host's innate immune response.[222–224] Both intimin and EspB are proven EPEC virulence factors,[184,225] and triple-mutant tEPEC strains that do not express BFP, EspA, or intimin fail to adhere to intestinal epithelial cells.[226]

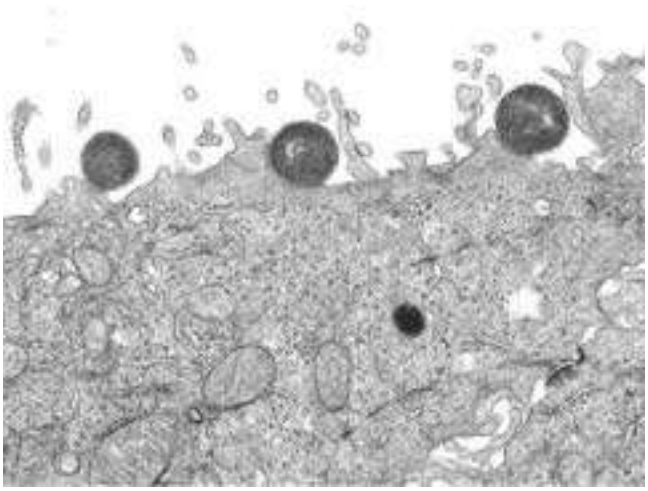

FIG. 218.3 Transmission electron micrograph of the attaching and effacing effect of enteropathogenic *Escherichia coli* on a tissue culture cell. Note the loss of microvilli, the intimate attachment of bacteria to the cell, and the cuplike pedestal composed of cytoskeletal proteins to which the bacteria are attached. Attaching and effacing effects are also seen with enterohemorrhagic *E. coli* strains and *Escherichia albertii* strains.

The precise mechanism of EPEC diarrhea is not fully understood, although it does not appear to be mediated by toxin production.[100] It has been shown that EspF—another protein secreted by the EPEC T3SS, although not involved directly in A/E lesion formation—participates in protein-protein interactions including sorting nextin 9 (SNX9), N-WASP, and the tight-junction protein occludin, resulting in decreased intestinal barrier function.[227–230] Other TSS3 proteins, including EspG and EspG2, have been implicated in inhibiting luminal chloride transport.[231] Furthermore, research shows that malnutrition resulting from protracted disease may be worsened by localized EPEC inhibition of thiamine uptake.[208]

EPEC diagnostics typically rely on probes targeting genes encoding hallmark EPEC genetic elements, including *eae* +/− *bfpA* but lacking *stx*.[232] A rapid agglutination test specific to EspB was developed for use in low income countries.[233]

Like ETEC, EPEC is a significant pathogen contributing to traveler's diarrhea (discussed earlier and in Chapter 319).[118,122,123,128] Data suggest that antibiotics are warranted in severe endemic EPEC disease, including nosocomial outbreaks,[234,235] although EPEC resistance to multiple antibiotic classes is a formidable concern.[236–240]

Human breast milk contains factors—including antibodies against BFP, intimin, EspA, EspB, and O antigens—that inhibit EPEC adherence.[198,241–249] Vaccines targeting BFP, EspB, and intimin have been explored, and EPEC strains transformed into "bacterial ghosts" by lysis plasmids elicit protective immunity in mouse models.[250] Unfortunately, immunogenicity data from human studies have proven underwhelming, presumably owing to antigen heterogeneity.[191,225,251–254]

Shiga Toxin–Producing *Escherichia coli* Including Enterohemorrhagic *Escherichia coli*

STEC incorporates any of the more than 400 *E. coli* serotypes that produce Shiga toxin, sometimes referred to as verotoxin.[255] STEC infection results in watery, often bloody diarrhea potentially complicated by hemolytic-uremic syndrome (HUS). STEC hemorrhagic colitis is often clinically mistaken as noninfectious hematochezia because of lack of fever.

HUS—a thrombotic microangiopathy resulting in anemia, thrombocytopenia, and renal failure—is the leading cause of renal failure in children. Thrombosis from HUS can lead to organ failure elsewhere including ischemic bowel, peripheral necrosis, and neurologic complications with cerebrovascular accident.[256–258] Approximately 30% of patients develop long-term sequelae including cardiac, renal, gastrointestinal, neurologic, and even endocrine complications.[259,260] HUS is more likely to complicate pediatric or elderly infections, with mortality ranging from 0.5% to 11% depending on the strain and patient age.[260–264]

STEC includes the pathotype EHEC, which has historically been responsible for large HUS outbreaks, including those due to the infamous O157:H7 serotype. This being said, the largest HUS outbreak to date was caused by an enteroaggregative O104:H4 STEC strain in northern Germany.[265,266] The prevalence and virulence of other STEC serotypes has varied considerably by geography.[265,267,268]

Large herbivorous mammals, such as cattle, are the primary natural reservoir for STEC.[269,270] STEC biofilm formation—observed on bovine hides, carcasses, and even abiotic surfaces—affords certain strains the ability to persist in the environment.[271,272]

STEC infection results from ingesting a small inoculum—as few as 100 organisms—from undercooked beef or any contaminated produce, water, or other beverage. A multistate outbreak of STEC serotypes O121 and O26 was attributed to raw flour consumption from a common production facility.[273] Fecal-oral transmission has also been documented from farm animal exposure, petting zoos, and person-to-person contact.[274–284]

The median incubation period for STEC infection is approximately 3 days, with bloody diarrhea typically developing after 2 days.[285] Most STEC cases resolve spontaneously after 1 week, although 4% to 22% of patients may progress to develop HUS, depending on infecting strain

and patient age.[262,264,266,285–287] In one large person-to-person child care outbreak, STEC shedding continued for a median duration of 30 days and up to 52 days from symptomatic individuals.[288]

Shiga toxins are a group of cytotoxic virulence factors—including Stx1 and Stx2 variants— with structural similarities to *Shigella dysenteriae* toxin that readily spread by transfection as *stx* genes are encoded on bacteriophages.[289] Shiga toxin is composed of a pentameric B subunit that binds to globotriaosylceramide and glycosphingolipids on the surface of host cells. After endocytosis, a single A subunit catalyzes the depurination of an adenine residue from the 28S rRNA of the host ribosome, rendering it nonfunctional.[290,291] Shiga toxin also affects cell signal transduction, prompting inflammation and apoptotic responses.[292,293] Furthermore, Shiga toxin can bind to leukocytes, facilitating dissemination through the bloodstream where endothelial cells elsewhere are then intoxicated, increasing procoagulant expression and subsequent microvascular thrombosis.[294–296]

EHEC is considered distinct from other STEC strains for producing the A/E lesions on intestinal cells also observed in EPEC (see Fig. 218.3).[297] As detailed earlier regarding the pathogenesis of EPEC, EHEC A/E lesion formation is orchestrated by the pathogenicity island LEE. However, in contrast to EPEC stains, genomic research shows that EHEC has a much larger effector repertoire, with the EHEC T3SS linked to a complex phage "metagenome," reflecting the complexity of LEE-positive STEC virulence, especially for O157:H7 serotypes.[298–300] Increasingly, LEE-negative STEC strains are recognized agents of diarrhea and even HUS, with virulence factors suspected to be similar to those of EAEC.[110]

Suspicion of STEC should be triggered by careful clinical history, physical examination, and laboratory parameters corresponding with HUS, such as schistocytes on peripheral smear. Sorbitol-MacConkey agar plates can be used to detect O157:H7 colonies but will fail to reveal other STEC serotypes; the microbiology laboratory should be notified of any concern for STEC because PCR assays and other immunoassays exist to readily detect *stx* genes.[117,301–304] The delayed occurrence of HUS—sometimes more than a week after onset of diarrhea—can confound proper diagnosis because STEC may no longer be detectable in stool.[154] STEC or HUS cases should prompt immediate alert of public health officials to the threat of an outbreak.[261]

Swift STEC diagnosis is critically important because proper identification will alter therapeutic management. Cellular stress—observed after exposure to fluoroquinolones and trimethoprim—induces high levels of Shiga toxin, which then lyses STEC strains.[305] Despite in vitro studies suggesting that certain antibiotics—such as azithromycin—may reduce Shiga toxin levels, antibiotic therapy is not recommended for STEC based on clinical data associating treatment with higher risk of HUS, observed across multiple antibiotic classes.[149,262,306,307]

The cornerstone of STEC treatment remains volume resuscitation and avoidance of antimotility agents.[308–310] Intensive care including renal replacement therapy may be required depending on the severity of disease or progression to HUS, although there is no evidence to support plasmapheresis for HUS due to STEC.[266,311,312] Case series have suggested improved outcomes with eculizumab—a monoclonal antibody against complement factor C5—in cases of HUS due to STEC, but this benefit was not observed during the German O104:H4 outbreak.[266,313–315]

Preventing diarrhea from STEC relies on safe food handling and consumption, including cooking ground beef to at least 155°F (68.3°C) (until "the juices run clear") and avoiding cross-contamination from undercooked meat.[278,316] Unpasteurized juices and milk also carry risk for STEC infection.[317] Proper hygiene after exposure to water in public swimming areas and animals at farms or petting zoos also reduces disease risk.

Research into STEC treatment other than antimicrobial therapy continues, including work with monoclonal antibodies against Stx1 and Stx2.[318–325] Oral toxin receptor analogues showed promise in protecting mice from sequelae of STEC infection but failed to reduce the severity of disease in children in a clinical trial.[326] STEC vaccine development is ongoing, targeting O157 antigens, Shiga toxin, intimin, and other T3SS secreted proteins.[327–332] Construction of STEC "bacterial ghosts" has shown promise as a vaccine candidate, as has Shiga toxin neutralization by a receptor mimic.[333,334]

Enteroaggregative *Escherichia coli*

The term *enteroadherent-aggregative E. coli* was initially coined to characterize strains isolated from the diarrhea of Chilean children with non-EPEC patterns of adherence observed in HEp-2 cells.[335] This group was ultimately separated into two pathotypes: EAEC and DAEC.[154,335,336]

EAEC strains are defined by their characteristic "stacked brick" aggregative adherence (AA) pattern on tissue culture cells, which has since been observed on intestinal mucosa and even abiotic surfaces (Fig. 218.4B).[337,338] EAEC was first described in 1987 and is increasingly recognized as a major cause of diarrhea in both developed and developing countries.[335,339,340] In large prospective studies in the United States, EAEC was the most common bacterial pathogen isolated from diarrheal samples.[187,341]

EAEC transmission is via the fecal-oral route, with different strains accounting for a wide variety of pathogenicity ranging from asymptomatic carriage to the severe European HUS outbreak attributed to fenugreek sprouts contaminated with the Shiga toxin–producing O104:H4 strain.[286,342–346] EAEC has been implicated in large foodborne outbreaks and traveler's diarrhea—with a frequency rivaling ETEC—and in postinfectious irritable bowel syndrome.[118,122,347–351] EAEC infection contributes significant morbidity to endemic cases of chronic diarrhea in children and HIV patients.[352–358]

Even in the absence of diarrhea, children in resource-poor environments with EAEC isolated in their stool have significant growth impairment, associated with increased interleukin (IL)-8 production[359,360] regulated through mitogen-activated protein kinases (MAPKs) that induce transcription factors NFκB and AP-1.[361] Several single nucleotide polymorphisms (SNPs)—including an SNP in the IL-8 gene promoter—have been linked to susceptibility to EAEC diarrhea.[362–365]

EAEC causes mucosal damage after adherence, resulting in microvilli loss and cell death, although the heterogeneity of a given EAEC strain—observed even in a single outbreak—defies a unifying account of pathogenesis.[338,349] EAEC aggregative adherence has been linked to the expression of chaperone-usher type adhesins encoded on plasmids, known as aggregative adherence fimbriae (AAF), which are related to the Dr family of adhesins found in DAEC and some UPEC strains.[366–369] AggR—a positive regulator of the AAF operon—has been proposed as the defining characteristic of typical EAEC, responsible for expression of multiple virulence factors associated with disease, including AAF, the surface protein dispersin, and the Aai T6SS.[91,370–372] Nevertheless, so-called atypical EAEC strains without AggR are still widely associated with diarrheal illness, reflecting the diversity of EAEC virulence yet to be described.[373]

EAEC strains can form a thick biofilm on the intestinal mucosa and on abiotic surfaces, which can harbor organisms in thick bacterial aggregates and hamper host nutrient absorption.[374–376] Pic—a mucinolytic SPATE protein—helps EAEC penetrate this biofilm but also induces mucus hypersecretion by host intestinal cells, suggesting a role in the creation of mucoid diarrhea.[377]

Several EAEC toxins have been described, including the enteroaggregative heat-stable enterotoxin 1 (EAST-1), also found in many non-EAEC strains.[378] EAST-1 shares structural similarities with the heat stable toxins of ETEC, and its pathogenesis also begins with increase of cGMP.[379,380] Some EAEC strains produce other SPATE family toxins such as plasmid-encoded enterotoxin (Pet), which causes intestinal crypt dilation and cell damage.[381,382]

The diagnosis of EAEC can be made by specific clump-formation tests, although most laboratories rely on tissue culture adhesion assays.[335,383,384] PCR and DNA probes for AggR have proven useful but are not commercially available.[385] Regardless, confirming EAEC as the causative pathogen is complex, given the spectrum of clinical disease and asymptomatic colonization.

The bulk of data regarding EAEC antimicrobial therapy is limited to the study of traveler's diarrhea, suggesting benefit in appropriate cases, although small case reports also support antibiotics to treat chronic diarrhea attributed to EAEC in HIV patients.[118,128,386,387]

In contrast to most EAEC strains, antimicrobial management was well studied during the 2011 Shiga toxin–producing EAEC outbreak in Germany. Whereas worse outcomes have been associated with antibiotic therapy for STEC diarrhea, one medical center provided

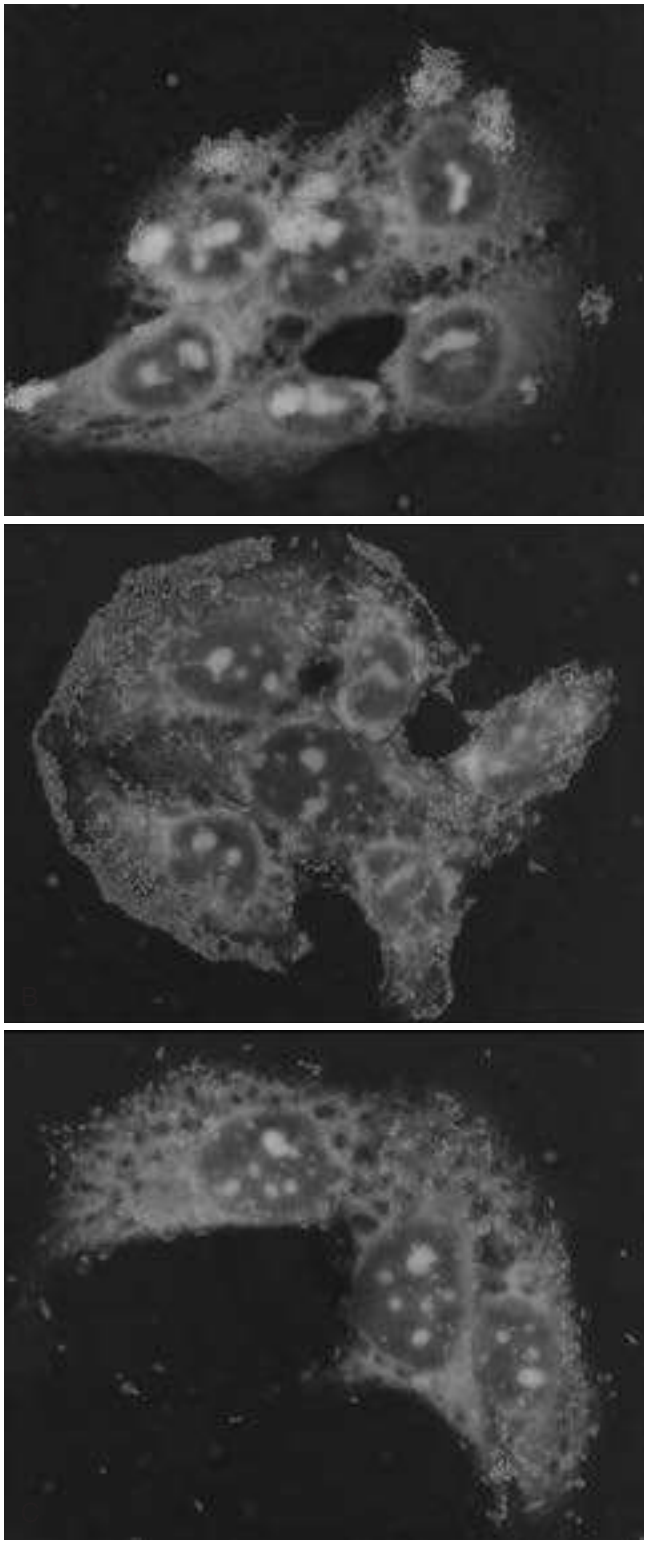

FIG. 218.4 Patterns of cellular adherence displayed by various diarrheagenic *Escherichia coli* pathotypes. (A) The localized adherence pattern exhibited by typical strains of enteropathogenic *E. coli* is characterized by discrete three-dimensional microcolonies of bacteria. (B) The aggregative adherence pattern exhibited by enteroaggregative *E. coli* strains is characterized by two-dimensional "stacked brick" aggregates. (C) Diffuse adherent *E. coli* strains adhere individually at random to tissue culture cells.

aggressive antimicrobial therapy to patients with HUS due to the German O104:4 strain, resulting in shorter duration of EAEC excretion, fewer seizures, and fewer deaths compared with historical patients untreated with antibiotics.[266] Patients studied elsewhere during the outbreak who received azithromycin for meningitis prophylaxis as part of a regimen including eculizumab were less likely to have long-term O104:H4 carriage, suggesting a role for antibiotics to prevent colonization.[388] Antibiotic therapy for this unique EAEC strain and the risk for HUS remain controversial.[389,390] Treatment data from the O104:H4 outbreak should not be widely extrapolated to less idiosyncratic EAEC strains.[110,391] Furthermore, any empirical antibiotic regimen for EAEC infection is potentially confounded by reports of widespread multidrug resistance, including ESBL and AmpC variants.[392]

Early vaccine studies against ETEC heat-labile toxin incidentally showed decreased burden of EAEC in the treatment group, although this ultimately failed to protect travelers from diarrhea due to any organism in a phase III clinical trial.[174,393]

Enteroinvasive *Escherichia coli*

The pathogenesis of EIEC infection is quite similar to that of *Shigella* and is discussed in more detail elsewhere (see Chapter 224). Although EIEC and *Shigella* represent distinct genetic lineages within the genus *Escherichia,* their similarities reflect convergent evolution, including plasmid exchange and the loss of antivirulence factors—termed pathoadaptation—through deletion, insertion, and point mutations.[1,394–396] *Shigella* retains its genus for historical reasons but is now considered to belong to *E. coli* based on phylogenetic data.[397] Like *Shigella*, EIEC are typically not motile, cannot ferment lactose, and are lysine decarboxylase negative.[398]

EIEC infection—first described in the 1940s—is clinically indistinguishable from shigellosis, although the inoculum required for disease appears considerably higher and typically results in less severe diarrhea.[399,400] EIEC diarrhea produces watery stools that can progress to dysentery, especially affecting children in the developing world.[400–405] Person-to-person and foodborne outbreaks—implicating contaminated cheese, milk products, and beef—occur in the developed world, with large outbreaks in Europe attributed to ingestion of lettuce contaminated with the motile serotype O96:H19, not previously reported in EIEC or *Shigella*.[406–408]

The hallmark feature of EIEC is cell invasion, orchestrated by a large plasmid—*pInv*—that encodes a T3SS, allowing the organism to escape macrophage phagocytosis, invade the intestinal epithelial cell, and usurp the host actin-filament machinery to spread from cell to cell.[409–411] EIEC is as capable of invasion as *Shigella,* but subsequent expression of virulence genes is lower.[412,413]

Historically, *Shigella* and EIEC were characterized by a positive Sereny test result wherein the isolate was observed to cause keratoconjunctivitis in guinea pigs.[414] Because of their close genetic relationship, distinguishing *Shigella* from EIEC is difficult. A combination PCR and biochemical assay strategy has been proposed, although often MLST analysis is required to confirm the diagnosis.[415]

EIEC diarrhea is usually self-limiting, but antibiotic therapy is warranted for severe cases. However, the clinician must be careful to first exclude the possibility of STEC infection.[416] As with other DEC strains, multidrug-resistant (MDR) EIEC strains have been reported, prompting consideration of local resistant patterns when empirical therapy is initiated.[417]

Preventing EIEC infection relies on proper hygiene and sanitation to avoid ingestion of contaminated food or water. *Shigella* vaccine development is addressed in Chapter 224.

Diffusely Adherent *Escherichia coli*

Diffuse adherence (DA) in tissue culture was observed in the initial adherence assays for non-EPEC strains, but this pattern was not clearly associated with diarrhea[335,336,418] (see Fig. 218.4C). Adult volunteers challenged with DAEC did not develop infection.[419] DAEC is now generally accepted as a DEC pathotype based on its association with watery diarrhea typically affecting somewhat older children worldwide.[100,154,420–424] Although virulence has been well established in UTI, the role of DAEC in diarrhea outside of childhood remains unclear.[425]

DAEC organisms are frequently isolated in asymptomatic children and adults, and molecular testing may have overestimated DAEC detection in diarrhea because of DNA probe cross-reactivity with EAEC strains.[425–427]

The characteristic diffuse adherence of DAEC has been attributed to both fimbrial and afimbrial adhesins from the Afa/Dr family, described initially in a diffusely adherent UPEC strain.[425,428,429] Operons of the *afa* family produce heterogeneous adhesins, with some recognizing the Dr blood group antigen as a receptor on human decay-accelerating factor (DAF), termed Afa/Dr+ adhesins.[430] Afa/Dr+ adhesins remain the defining feature of so-called typical DAEC, whereas diarrheagenic DAEC strains not expressing Afa/Dr have been grouped into the atypical EPEC pathotype.[100]

Afa/Dr+ adhesin binding to DAF on the brush border of epithelial cells increases permeability due to cytoskeletal changes, defective brush border proteinsm and microvilli loss.[431–437] DAEC flagellar stimulation of Toll-like receptor 5 (TLR5) induces IL-8 secretion then transepithelial migration of neutrophils, with subsequent inflammation further promoting DAF expression.[438–440] The presence of ileal DAEC and increased expression of DAF in patients with Crohn disease (CD) has led to speculation about the role for DAEC in CD pathogenesis.[428,441,442]

Diarrheal DAEC strains from a Brazilian pediatric case-control study showed genetic expression of a wide variety of previously described toxins—including EAST-1, ShET1 (Shigella enterotoxin 1), and hemolysin—which has made reliable laboratory detection and meaningful vaccine development challenging.[422] Antimicrobial and vaccine strategies for extraintestinal DAEC—namely, uropathogenic strains—are discussed later.

Adherent-invasive *Escherichia coli*

The term *adherent-invasive E. coli* was coined in 1999 to describe *E. coli* strains isolated from resected ileal tissue in patients with CD. AIEC does not express any traditional DEC virulence factors but can adhere and efficiently invade intestinal epithelial cells.[441,443]

Concordance studies have long implicated environmental factors in CD pathogenesis with many organisms identified as potential culprits, including viral and mycobacterial agents.[444,445] Whereas AIEC is found in less than 10% of healthy ileums, AIEC is isolated from approximately 30% of CD ileal lesions, suggesting its role as a possible pathobiont in the multifactorial etiology of CD.[446–448] AIEC is a diverse group, although particular strains such as those from the B2 phylogroup—associated with extraintestinal infection—have been isolated with higher prevalence from CD patients.[449–452]

AIEC adherence involves type I fimbriae and long polar fimbriae (LPF) adhering to M cells at the surface of the Peyer patches (PPs) and CAECAM6 on host intestinal epithelial cells.[453] Invasion is then facilitated by the expression of a membrane-bound protein, OmpA, that promotes fusion of outer membrane vesicles (OMVs) with Gp96—a host ER stress response chaperone—that is overexpressed in the ileum of CD patients.[454–456] AIEC then replicates in macrophages of the lamina propria, inducing TNF-α but not provoking macrophage death.[457,458]

Although CD treatment is beyond the scope of discussing AIEC alone, it is worth noting that CD symptoms improve when luminal bacteria burden is decreased, leading clinicians to use antibiotics targeting enteric bacteria during CD flares.[459]

Extraintestinal Pathogenic *Escherichia coli*

ExPEC strains have distinct epidemiologic, phylogenetic and virulence factor profiles relative to commensal *E. coli* and DEC.[460,461] ExPEC strains are capable of infection—community acquired and nosocomial—involving almost any organ or anatomic site.[112] Based on characteristic virulence factors and on MLST and serotyping data, particular ExPEC variants are often described as *uropathogenic E. coli, neonatal meningitis–associated E. coli,* or *sepsis-associated E. coli.*[462]

Despite such syndromic classification, ExPEC strains can share virulence factors irrespective of their anatomic niche.[462] For example, prototypical UPEC strains have been isolated from cases of pneumonia, and characteristic NMEC strains frequently cause UTI.[463,464] Certain serotypes—such as *E. coli* O18:K1:H7—have been recovered from urine, feces, and CSF, and these isolates are clearly clonal derivatives.[465] Furthermore, *E. coli* isolates thought to represent commensal strains can express ExPEC virulence factors and cause infection—including bacteremia—in the appropriate clinical scenario, including immune compromise, retained nidus of infection, or obstruction.[102,113,466–469]

ExPEC strains are typically derived from the B2 or D phylogroup with virulence genes usually encoded on plasmids or within pathogenicity islands (PAIs) distinct from DEC.[112] Some researchers have defined ExPEC as expressing at least two of the following genes: *papA* or *papC* (P fimbriae major subunit and assembly), *sfa/foc* (S and F1C fimbriae), *afa/draBC* (Dr-binding adhesins), *kpsM II* (group 2 capsule), and *iutA* (aerobactin receptor).[470] ExPEC virulence genes are detected in approximately 10% of healthy individuals, supporting the concept that the intestinal microbiota serves as a reservoir for ExPEC strains.[469]

The pandemicity of hypervirulent and MDR strains underscores the importance of further characterization of ExPEC.[471,472] Particular ExPEC sequence types—such as ST101, ST131, and ST405—are associated with extensive antimicrobial resistance, including plasmid-mediated AmpC β-lactamases, CTX-M ESBLs, NDM MBLs, and other carbapenemases.[473–479] General principles of antibiotic resistance are discussed elsewhere (see Chapter 18).

Uropathogenic *Escherichia coli*

E. coli causes the majority of UTIs and pyelonephritis.[480] The host vaginal or fecal microbiota is the most common source for these strains—termed *uropathogenic E. coli*—although UPECs are not necessarily the predominant clones from these reservoirs.[481,482] UPEC clonal outbreaks have prompted investigation into common virulence factors particularly suited for genitourinary colonization and infection.[483–489] Murine and primate models have improved understanding of urinary tract pathogenesis, although UPEC strain heterogeneity continues to inspire research into novel virulence factors and potential vaccine targets.[490–494]

Many adhesins have been implicated in UPEC pathogenesis, but the ubiquitous *E. coli* type 1 fimbria—encoded by the *fim* operon—has proven essential to infection.[32,46,493,495–497] The apical layer of the normally impermeable bladder transitional epithelium features "umbrella cells" composed of highly ordered plaques featuring lipid components and uroplakin particles.[498] UPEC type 1 fimbriae bind to uroplakin, where the innate defense system induces host epithelial cell exfoliation followed by bacterial penetration through lipid rafts.[499–501]

UPEC expression of type 1 fimbriae genes is controlled by an invertible promoter capable of phase variation, allowing the virulence of the organism to change depending on the stage of infection.[49,502–504] Flow chamber models show that expression and adhesion of type 1 fimbriae actually increase with shear stress after they settle on bladder epithelial cells or even catheter surfaces, reflecting more pathogenic sessile subpopulations.[505–507] Furthermore, UPEC type 1 fimbriae have been implicated in decreased ureteral contractility in rat models, suggesting a role in ascending infection.[508]

The chaperone-usher type P fimbriae—encoded by *pap* genes—was the first virulence factor to be discreetly associated with UPEC infection, especially pyelonephritis.[509,510] P fimbriae bind to both galactosyl-galactose moieties on erythrocytes of the P blood group and glycosphingolipids on the surface of renal epithelial cells. Mutations in *papG*—encoding the PAP adhesin on the P fimbrial tip—resulted in less virulent pyelonephritis in a cynomolgus monkey model.[511–515] *E. coli* has higher prevalence of vaginal colonization in women who do not secrete histo-blood group antigens, which increases risk for recurrent cystitis.[516]

Although described earlier in the discussion of DAEC pathogenesis, the family of Afa/Dr adhesins was first characterized in UPEC strains associated with recurrent urogenital infections, especially in pregnant women.[488,517–521] Whereas Afa/Dr+ adhesins are the defining characteristic of DAEC, both Afa/Dr+ and Afa/Dr– adhesins have been described in cystitis, pyelonephritis, and sepsis from a urinary source.[430] Afa/Dr+ adhesins bind to type IV collagen, resulting in tropism for the renal basement membrane, observed to cause chronic pyelonephritis in a mouse model.[522,523] UPEC also shares virulence factors with EAEC, which is thought to be a recent common ancestor.[524,525] For example, a large Danish community-acquired EAEC UTI outbreak confirmed the urovirulence of AAF.[526]

After adherence, UPEC strains are capable of invading and replicating in host epithelial cells, forming intracellular bacterial communities

(IBCs), which affords protection from host defenses and antimicrobial therapy and potentially serves as a reservoir for recurrent UTI.[527–531]

UPEC strains produce hemolysin—the predominant toxin found in ExPEC—which creates pore formation and induces low-frequency calcium oscillations in the renal epithelium, stimulating IL-6 and IL-8.[66,532] Hemolysin results in uroepithelial sloughing and rapid bladder hemorrhage in mouse models, with induction of caspase-dependent inflammatory death observed in human urothelial cells.[533,534] A third of UPEC strains also produce cytotoxic necrotizing factor 1 (CNF1), which inactivates Rho GTPases, resulting in reorganization of actin assembly and multinucleation in culture cells.[535,536] CNF1 results in submucosal edema in the mouse bladder with attenuated disease observed in CNF1-negative strains.[533,537]

UPEC pathogenicity is augmented by multiple iron acquisition systems. UPEC strains with mutated TonB outer membrane receptors failed to utilize the siderophores enterobactin and aerobactin and are less infective than wild-type strains.[87] Many other putative virulence factors have been suggested by experiments showing attenuated disease in mutated UPEC strains, including mutations in the O antigen, K capsule and various regulatory genes.[538–543]

Owing to the ubiquity and common recurrence of UPEC infection, prophylactic therapeutics other than antimicrobials continue to be an active area of research.[544] Data supporting prophylactic *Lactobacillus* vaginal suppositories—intended to alter the colonizing UPEC reservoir—have not proven convincing, although they may confer some protection when administered immediately after antibiotic treatment for cystitis.[545–547] Anecdotal reports of cranberry product ingestion as prophylaxis against UPEC were supported by in vitro evidence showing inhibition of both type 1 and P pilus-mediated adherence, but, unfortunately, results from placebo-controlled trials are largely disappointing.[548–552] Meta-analyses have suggested benefit from short-term prophylactic methenamine hippurate in preventing UTI in patients without renal tract abnormalities, and vaginal estrogen in preventing recurrent UTI in postmenopausal women.[4,553–555] Oral FimH antagonists have shown success in preventing UTIs in mouse models.[556,557] The general clinical approach to UTI otherwise is discussed elsewhere (see Chapter 72).

Vaccine development to prevent UPEC infection has been ongoing since the 1950s, although success has been limited by the overwhelming heterogeneity of strains.[544,558,559] Iron acquisition systems—such as the yersiniabactin receptor FyuA—appear to be a compelling vaccine target, with success demonstrated in protecting mice against pyelonephritis.[560,561] Vaginal delivery of a multivalent whole-cell vaccine—featuring strains from many uropathogenic bacteria—seemed to reduce recurrent UPEC cystitis, particularly in sexually active women younger than 52 years.[562] An intramuscular biconjugate vaccine composed of O antigens from four UPEC serotypes induced significant immunoglobulin G (IgG) response and appeared safe in human phase I trials.[563] Finally, an oral vaccine composed of bacterial extracts from multiple UPEC strains is available in Europe and may have some possible benefit as prophylaxis against recurrent UTI, although the quality of evidence is low.[564–566]

Neonatal Meningitis–Associated *Escherichia coli*

E. coli is the most common gram-negative pathogen recovered from neonatal meningitis, typically preceded by high-grade bacteremia.[567,568] *E. coli* sepsis in the newborn is presumed to result from immature host immune defenses; deaths from NMEC infection are associated with preterm birth.[569,570]

Approximately 80% of NMEC strains feature the K1 polysialic acid capsule, which is immunochemically identical to the capsular polysaccharides of group B *Neisseria meningitidis* that confer protection from phagocytosis.[569,571,572] Early rat models suggested that the K1 capsule was critical in the development of *E. coli* meningitis, with both K1+ and K1− strains recovered in the cerebrospinal fluid (CSF) but only K1+ strains remaining viable, which suggests that the capsule is not necessary for invasion but facilitates survival after bacterial cells cross the blood-brain barrier (BBB).[80,573]

The NMEC outer membrane protein A (OmpA) allows survival in the serum by binding C4b-binding protein (C4bp) to inhibit complement activation.[574] Both type 1 and type S fimbriae and a number of other NMEC proteins—including OmpA and invasion of brain endothelial cell (Ibe) proteins—bind to particular epitopes on human brain microvascular endothelial cells (HBMECs), then facilitate invasion and loss of BBB integrity.[575–582]

NMEC strains are capable of expressing hemolysin and CNF—both encoded by a common pathogenicity island described in UPEC pathogenesis—and cytolethal distending toxin, which is associated with diarrheal disease.[575,583–586] Multiple NMEC gene mutations—including *cnf* and *clb* mutants—produce strains less capable of invading HBMECs, implicating a variety of virulence factors in central nervous system (CNS) disease worthy of ongoing research.[587–589] A French study of 325 NMEC cases recently characterized the largest number of isolates described to date, with more than 75% of the 141 different strains expressing genes implicating iron-uptake systems as a virulence factor, including *fyuA* (yersiniabactin), *iucC* (aerobactin) and *iroN* (salmochelin).[569] This study also revealed an association between disease severity and *pap*GII expression, previously linked with pyelonephritis but not bacteremia.[462,590]

The clinical approach to meningitis is discussed elsewhere (see Section H, "Central Nervous System Infections"), but clinicians should be cautious in choosing empirical antimicrobial therapy because epidemiologic data show increasing rates of drug resistance in NMEC isolates compared with UPEC, even of the same sequence type.[591]

Sepsis-Associated *Escherichia coli*

E. coli is the most common pathogen in gram-negative bacteremia, featuring a wide variety of strains sometimes described as *sepsis-associated E. coli.*[592] Compared with rectal strains from uninfected controls, *E. coli* virulence factor profiles—including expression of *pap* (P fimbriae), *hyl* (hemolysin), *fyuA* (yersiniabactin), *kpsM* II (capsule) and *ompT* (outer membrane protein)—serve as a better predictor of bloodstream infection than phylogenetic grouping.[593] However, in a study of more than 1000 adult patients with *E. coli* bacteremia, no single bacterial virulence factor predicted severity of disease. Age, cirrhosis, immune compromise and a cutaneous portal of entry were predictive of death, whereas *E. coli* bacteremia from UTI carried a more favorable prognosis.[594]

The usefulness of the encompassing term *sepsis-associated E. coli* to characterize these strains is confounded by the fact that the majority of *E. coli* bacteremia in adults results from UPEC. Likewise, NMEC is frequently isolated in the context of bacteremia. The pathogenesis attributed to UPEC and NMEC was described earlier in this chapter. Research is ongoing into *E. coli* virulence factors specific to bacteremia from gut translocation in the absence of other identifiable sources.[595,596]

Possible mechanisms contributing to *E. coli* gut translocation include intestinal overgrowth, host immune deficiency, and damage to the intestinal mucosa.[597] *E. coli* gut translocation is observed frequently in patients with hematologic malignancy. Up to 70% of leukemic patients with *E. coli* bacteremia do not have an identifiable source, although gut translocation is suspected based on DNA similarity between blood and bowel isolates.[598] A subsequent analysis implicated multiple virulence genes contributing to bowel-blood translocation.[599] A murine chemotherapy-induced translocation model showed that *E. coli* with *fimH* deletion were less capable of translocation, suggesting a role for FimH in gut translocation.[600]

E. coli translocation is also frequently observed, although poorly investigated, in infants. One study defined *E. coli* from infantile gut translocation as a bloodstream isolate with the same virulence genotype and serotype as that recovered from gastric fluid or stool without a source identified elsewhere, including a negative urine culture. Analysis of 100 such isolates appeared similar to UPEC strains determined with MLST, but gut translocation isolates more frequently expressed *ibeA,* which has been implicated in neonatal meningitis, although only one infant had meningitis in the study.[601]

Considering the increasing frequency of ExPEC infection—including UPEC, NMEC, and SEPEC—in the setting of increasing antimicrobial resistance, researchers are actively identifying antigen targets for vaccine development.[602] Subtractive reverse vaccinology identified more than 200 ExPEC antigens absent in nonpathogenic *E. coli,* with a subset of these antigens showing promise in a mouse challenge model.[603]

Klebsiella

The genus *Klebsiella* was named by Trevisan to honor the microbiologist Edwin Klebs. *K. pneumoniae, K. oxytoca,* and *K. granulomatis* are the primary species associated with illness in humans. *K. planticola, K. ornithinolytica*, *K. terrigena, and K. variicola* are rarely associated with human clinical disease and are now classified in the genus *Raoultella*[604,605]; these organisms have been associated with scrombroid fish poisoning. *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* are considered non-fermenting subspecies of *K. pneumoniae* based on DNA homology studies and exhibit characteristic clinical manifestations. With those exceptions, strains within this genus ferment lactose, most produce highly mucoid colonies on plates, and all are nonmotile. *Klebsiella* organisms are found commonly in the environment, but also can asymptomatically colonize the human nasopharynx, gastrointestinal tract, and, less frequently, other sites.[606,607] Gastrointestinal colonization may precede abdominal infections, increasing risk of abdominal infection up to sevenfold, with increasing colonization rates in hospitalized patients, those with prolonged lengths of stay, and those with prior antibiotic receipt.[606] Given higher rates of intraabdominal *Klebsiella* infections in East Asian countries, especially Taiwan, ethnicity was believed to confer increased risk, but intestinal colonization rates that differ by geography account for increased disease.[608,609] Seasonal variation has been noted in *Klebsiella* bacteremia, similar to *E. coli*, but not *Enterobacter* or *Serratia*.[116,610,611]

Most *K. pneumoniae* infections are nosocomially acquired,[612–615] particularly in ICU settings,[616] but they can also occur in otherwise healthy people. UTIs, pneumonia, and bacteremia are the most common infections encountered, followed by liver abscess and other intraabdominal infections, wound infections, infections of intravascular and other invasive devices, and meningitis and postneurosurgical infections. Patients with various underlying conditions including alcohol use, diabetes, malignancy, end-stage renal disease, and immune suppression are at greatest risk.[608,617,618] Nosocomial outbreaks involving *K. pneumoniae* and, less commonly, *K. oxytoca* have frequently been reported.[613,618,619] Nosocomial meningitis, especially with preceding neurosurgical procedures, varies based on procedure type[620,621] and prophylactic antibiotic regimen.[622] *Klebsiella* is an infrequent cause of community-acquired meningitis,[623,624] with the notable exception of metastatic disease related to *Klebsiella* spp. liver abscess (KLA). Only *E. coli* is more common than *Klebsiella* in spontaneous bacterial peritonitis in both the hospital and community.[625,626] Splenic abscesses are often related to KLA (see following text). Geographic variations exist for both infectious syndrome and phenotype of recovered *Klebsiella* isolate, with most variation seen in KLA and its sequelae.

K. pneumoniae is a significant cause of primary, invasive, community-acquired liver abscesses, often associated with metastatic spread.[627–631] Initially primarily reported in Taiwan, this syndrome remains widespread in East Asian countries but is emerging globally where it likely is underrecognized. KLA is often monomicrobial, commonly associated with preexisting hepatobiliary disease, and typically manifests with single, unilobular, and multiloculated fluid collections.[608,630,632] Prior antibiotic exposure increases KLA risk.[633] Isolates exhibiting hypermucoviscosity and a hypervirulent phenotype are often implicated in metastatic infection. Metastatic spread is more common with KLA than non-KLA infections, likely because of a combination of a variety of virulence factors observed both in KLA and severe *Klebsiella* spp. infections (see discussion of virulence, later). The most common metastatic manifestations are endophthalmitis, meningitis, and brain abscess, although other sites have been described.[634–637] Treatment of KLA requires parenteral antibiotic therapy (which is often prolonged) and often drainage. When endophthalmitis is present, severe visual deficits predominate, and when suspected, prompt evaluation, antibiotics, ophthalmologic consultation, and use of intravitreal antibiotics should be considered.[608]

K. pneumoniae can cause UTIs in individuals with normal or abnormal urinary tracts or with indwelling catheters. Pathogenesis of *Klebsiella* UTIs is similar to that related to other Enterobacteriaceae. Uncomplicated UTIs, ascending acute pyelonephritis, emphysematous genitourinary infections, perinephric abscess, and prostatic involvement caused by *K. pneumoniae* do not have distinguishing clinical features from those caused by other bacterial species. *K. pneumoniae* is second only to *E. coli* as a cause of both all-cause gram-negative bacteremia and bacteremia from a urinary source.[612,638,639] Community-onset bacteremia can occur and is often severe, necessitating ICU admission, partly because of overlap with the hypervirulent phenotype seen in community syndromes, which is associated with septic shock and poor outcomes.[640–643]

Pneumonia caused by *K. pneumoniae* classically invoked particular distinguishing features, warranting the eponym *Friedländer disease.* Among these classic features are its severity, frequency in alcoholics, propensity to affect the upper lobes, production of "currant jelly" sputum resulting from hemoptysis, the bulging fissure sign on radiography from edematous lobar consolidation, and tendency for abscess formation. Despite these compelling descriptions, pneumonia caused by *K. pneumoniae* cannot be reliably distinguished clinically from other organisms, and many of the aforementioned features may be the result of misdiagnosis, inferred from culture of expectorated sputum, of anaerobic pulmonary infections.[644] CAP is infrequently caused by *K. pneumoniae* in the United States,[645,646] although higher rates have been seen in Southeast Asia,[647] and carries with it increased mortality.[648] In geriatric populations, *Klebsiella* also has been implicated in aspiration pneumonia.[649]

Klebsiella has less frequently been described in various syndromes, including uncomplicated skin and soft tissue infections indistinguishable from those caused by typical pathogens, and necrotizing fasciitis, spinal infections, septic arthritis, cardiac infections, mycotic aneurysm, and infected grafts.

Several virulence factors have been extensively investigated for *K. pneumoniae* including its luxuriant polysaccharide capsule, mucoviscous phenotype, and siderophore regulation. At least 77 capsular antigenic varieties have been described, with important geographic variation. Capsular virulence is due to phagocytosis inhibition (see earlier discussion) and to protection from bactericidal serum factors.[608,627] Capsular types K1 and K2 appear to be more virulent and cause more severe human disease,[627,650] as supported by increased lethality in animal models.[651] This increased severity is partly related to a hypermucoviscous phenotype; conversely, mutant acapsular strains exhibit reduced virulence.[652] The microbiologic observation of the mucoid phenotype as indicated by a "positive string test" (Fig. 218.5) correlates with this hypermucoviscous phenotype and clinically invasive strains.

There is not a direct relationship between virulence factors and *Klebsiella* strain identification or capsular serotype, challenging initial concepts of virulence.[627] *Klebsiella* spp. exhibiting a hypermucoviscosity phenotype have long been thought to be more invasive and implicated in KLA,[653–656] but the variety of virulence factors and observance of nonmucoid KLA isolates make *hypervirulent phenotype* a more



FIG. 218.5 Mucoid phenotype demonstrated by positive "string test." Viscous strings >5 mm in length are produced by stretching colony by loop on growth agar. *(From Siu LK, Yeh KM, Lin JC, et al. Klebsiella pneumoniae liver abscess: a new invasive syndrome.* Lancet Infect Dis. *2012;12:881–887.)*

appropriate term.[657,658] Poor glycemic control has been suggested, although not universally accepted, as a KLA risk factor, given impaired neutrophil phagocytosis of K1/K2 capsular serotypes.[659] Strains isolated from patients with invasive syndrome have distinct virulence features compared with less invasive, comparator strains.[660] These factors have genetic similarity with invasive disease isolates despite geographic diversity.[661] Capsular type K1 has emerged as an important factor in KLA and demonstrates a distinct link to a specific clonal complex, CC23, that involves a virulence plasmid; several of the factors discussed later portend higher community acquisition rates, KLA formation, and metastatic spread.[627,661–663]

The K1/2 serotypes demonstrate high rates of hypermucoviscous phenotype,[650] but hyperviscous phenotype is not limited to only the K1/2 serotypes. Presence of *magA* (mucoviscosity-associated gene), ubiquitous in K1 isolates, was initially proposed as a risk factor for both KLA and metastatic infection,[664] but it appears to be associated with K1 capsule and not a driver of virulence.[650,665,666] The regulator of mucoid phenotype *(rmpA)* gene increases mucoid phenotype for most isolates.[634,650,667–670]

K. pneumoniae can produce a variety of fimbrial types, including types 1 and 3 pili, aiding host cell adherence.[671] Aerobactin, a siderophore, increases *K. pneumoniae* virulence,[651,672–674] with increased production in hypervirulent phenotypes supporting its role in invasive disease.[675,676] Much like *E. coli,* KLA isolates may use several iron acquisition systems, of which a majority are regulated by *tonB*.[673]

All strains of *K. pneumoniae* are resistant to ampicillin as a result of the presence of a chromosomal gene encoding a penicillin-specific β-lactamase.[677] Preceding ampicillin use has been shown to increase risk of KLA[633] and postneurosurgical infections.[622] In addition, nosocomial isolates are frequently resistant to numerous other antibiotics as a result of the acquisition of MDR plasmids. For example, *K. pneumoniae* is one of the most common organisms to carry plasmids encoding ESBLs and carbapenemases, and bacteremia with such strains is associated with higher rates of treatment failure and death.[678,679] After the initial description of the novel carbapenemase, KPC-1, in a *Klebsiella* isolate in the United States, *Klebsiella* is an archetype of emerging resistance.[680] Therapeutic options for infections caused by non–MDR strains include first-generation cephalosporins, penicillin/β-lactamase inhibitor combinations, trimethoprim-sulfamethoxazole, fluoroquinolones, and aminoglycosides. For MDR strains, especially those expressing ESBLs, treatment options are often limited to fourth-generation cephalosporins or carbapenems. Carbapenemase-producing strains leave scarce options.[7]

The *K. pneumoniae* subspecies *rhinoscleromatis* is the causative agent of respiratory scleroma, also known as rhinoscleroma, a chronic granulomatous infection of the nasal passages and other parts of the respiratory tract.[627,681] In contrast to *K. pneumoniae*, *K. rhinoscleromatis* is not found outside human hosts.[682] The disease it causes is characterized by chronic inflammation and remains difficult to diagnose because of reliance on biopsy, lack of early symptom specificity, and variation of routine biochemical properties between strains.[683] Often, rhinoscleroma presents in advanced stages with disfigurement and respiratory compromise following a progressive clinical pathway from atrophic through granulomatous to fibrotic stages.[684] The disease is found primarily in impoverished areas of Central and South America, Africa, and Asia or in immigrants from these areas[681,682] and is transmitted by close contact and affected by genetic control of the host immune responses.[682] Respiratory scleroma is characterized by nodules and masses often involving the nasal passages and, less commonly, other areas of the respiratory tract. Occasionally, the infection can erode through bone and invade the CNS. The differential diagnosis includes tuberculosis, leprosy, fungal infections, granulomatosis with polyangiitis, malignancies, and sarcoidosis. Biopsy specimens show granulomatous inflammation with foamy macrophages (Mikulicz cells) containing intracellular organisms (Fig. 218.6).[685] The bacteria can be grown on ordinary laboratory media. Prolonged antibiotic therapy, often for months, and, occasionally, surgical débridement are required for cure, but relapses are common.[682] Traditionally, streptomycin or tetracycline has been used, but more recent reports suggest that trimethoprim-sulfamethoxazole or fluoroquinolones could be alternatives.[682]

The *K. pneumoniae* subspecies *ozaenae* can colonize the nasopharynx of healthy individuals. This subspecies has been associated with chronic atrophic rhinitis (*ozena* means "stench") marked by bone resorption and thick, foul-smelling discharge that often forms crusts, but its etiologic role is controversial. The organism has only rarely been isolated from patients with a variety of infections, including otitis media and mastoiditis, cystitis and pyelonephritis, soft tissue infections, bacteremia associated with neutropenia, pneumonia, and meningitis.[686,687] It is usually susceptible to multiple antibiotics except when isolated from hospitalized patients who have received previous antimicrobial therapy.



FIG. 218.6 Photomicrograph of Mikulicz cells in a patient with rhinoscleroma. The periodic acid–Schiff–positive structures within the macrophage *(arrows)* are the causative organism, *Klebsiella rhinoscleromatis. (From ID Images. © The Infectious Diseases Society of America. Case presented by Danielle Osterholzer, Tom E. Davis, and Stephen D. Allen.)*

K. oxytoca, like *K. pneumoniae,* can cause a variety of nosocomial infections and is distinguished from *K. pneumoniae* based on its ability to produce indole from tryptophan. *K. oxytoca* is rarely isolated in stool specimens of asymptomatic patients and is not believed to be a constituent of the normal bowel microbiota.[688–690] There is evidence that *K. oxytoca* has increased cytotoxin production not found in other *Klebsiella* species, which may account for its ability to induce antibiotic-associated hemorrhagic colitis with histopathologic findings similar to those of STEC or *Shigella*.[691,692] It may also cause outbreaks and a variety of nosocomial infections and may be resistant to multiple antibiotics.

K. granulomatis (formerly *Calymmatobacterium granulomatis*) is a fastidious member of the genus that causes granuloma inguinale or donovanosis, a chronic genital ulcerative disease (see Chapter 235).

Enterobacter Species, *Pantoea agglomerans,* and *Chronobacter sakazakii*

Enterobacter species have undergone reclassification. *Enterobacter cloacae* is responsible for most *Enterobacter* infections in humans. *Enterobacter aerogenes* was renamed *Klebsiella aerogenes* owing to genetic similarity.[692a,692b] *Enterobacter sakazakii* was reclassified into the genus *Chronobacter,* which includes at least seven distinct species.[693] Several less common *Enterobacter* spp. have been reorganized into the new genera of *Lelliottia*, *Pluralibacter*, and *Kosakonia*.[694] *P. agglomerans* (formerly *Enterobacter agglomerans*) is also a common isolate and is grouped with the *Enterobacter* spp. here. These bacteria ferment lactose, are motile, and form mucoid colonies. *Enterobacter* strains are part of the intestinal microbiota and rose to notoriety with a large nationwide outbreak[695] and continue to be implicated in clonal outbreaks.[696] The normal colonization may become infection, especially in patients who have received antimicrobial therapy or who are admitted to the ICU.[697] *Enterobacter* spp. may cause a wide variety of nosocomial infections,[612,698,699] including pneumonia, UTIs, wound and burn infections, and infections of intravascular and other prosthetic devices. Other than neonates and postneurosurgical patients, *Enterobacter* rarely causes meningitis. There do not seem to be distinguishing characteristics among infections caused by *E. cloacae* and *E. aerogenes*, although *E. cloacae* may have higher

rates of antimicrobial resistance, and *E. aerogenes* may have poorer outcomes.[700]

Given the considerable difficulty in correctly identifying *Chronobacter* spp., not all reports of *E. sakazakii* prior to reclassification in 2007 can be directly attributed to *Chronobacter.*[694] Given those limitations, we know that *Chronobacter* may cause bacteremia, necrotizing enterocolitis, and meningitis, primarily in neonates of low birth weight, and in such infants is associated with consumption of powdered milk formula,[701–705] with mortality rates in excess of 40%.[706,707]

P. agglomerans, a plant pathogen, is often associated with contaminated catheters or penetrating trauma.[708]

Enterobacter, *Pantoea*, and *Chronobacter* use virulence factors typical of other Enterobacteriaceae.[709–712] *Enterobacter* strains associated with illness may deploy a variety of mechanisms, including heavy metal resistance and additional siderophore assembly kits to gain a fitness advantage against other microbes, that facilitate their survival in diverse environments.[711] Efflux pumps are very active in the wide array of antimicrobial resistance in *Enterobacter* but may also help explain *E. cloacae* competitive virulence.[712] *C. sakazakii* can adhere to and invade brain microvascular endothelial cells, similar to NMEC.[713–715] The outer membrane of *C. sakazakii* is encoded by *ompA,* its best-described virulence marker.[716] *C. sakazakii* resists desiccation, which may explain its role in contaminating powdered milk, where it can survive for more than 12 months.[703,717–721] *C. sakazakii* is the only *Chronobacter* species that can metabolize sialic acid, which is found in breast milk, infant formula, the gastrointestinal tract, and the brain, possibly explaining the specific association with these syndromes.[722]

Strains belonging to the genus *Enterobacter* often show antimicrobial resistance, earning them a place in the ESKAPE mnemonic.[723,724] *E. cloacae, E. aerogenes,* and most strains of *Chronobacter* are intrinsically resistant to ampicillin and first- and second-generation cephalosporins as a result of an inducible AmpC chromosomal β-lactamase that is controlled by both positive and negative regulators.[725] Furthermore, mutants that constitutively produce high levels of β-lactamase, conferring resistance to third-generation cephalosporins, arise at frequencies of 10^{-4} to 10^{-7}, often as a result of mutations in the regulatory loci, such that resistant mutants may already be constitutively present before initiation of therapy. As with other members of the Enterobacteriaceae, *Enterobacter* spp. may carry plasmids encoding MDR phenotypes[698,726,727] (see Chapter 18 on antimicrobial resistance). Therapy must therefore be tailored to local resistance patterns and results of individual isolate susceptibility testing. Clinicians must be aware that emergence of stably derepressed resistant mutants may lead to treatment failure when third-generation cephalosporins are used, even if isolates appear susceptible at initial testing.[728] Therefore, fourth-generation cephalosporins and carbapenems may be empirically chosen for severe infections.

Serratia Species

Of the many species in the genus *Serratia, Serratia marcescens* is the one most commonly isolated from human infections, and *Serratia liquefaciens* is occasionally grown. *Serratia* strains are motile, rarely ferment lactose, and produce an extracellular DNase. The organism is widespread in the environment but not a commonly recognized component of the human fecal microbiota; thus most infections appear to be acquired exogenously. Many environmental and some clinical strains of *S. marcescens* produce a red pigment (Fig. 218.7), prodigiosin. Bartolemeo Bizio, an Italian pharmacist, first described the organism in 1819 as the cause of red discoloration of polenta, thereby discrediting the claim that the growth was due to the miraculous appearance of blood.[729] He named the bacterial genus to honor Serafino Serrati, an Italian physicist, and its species name *(marcescens)* for the Latin word for "to decay" because of the tendency of the pigment to change color as the colonies age.[730] The production of prodigiosin and the misconception that the bacterium was harmless led to its frequent use as a biologic marker to study, among other things, the transmission of bacteria through speech and contact, ascending bladder colonization from urinary catheters, and the dissemination of aerosolized bacteria after experimental release into the environment in models of biologic warfare.[731,732] It is now appreciated that *S. marcescens* can cause a wide variety of infections, ranging from UTIs, bacteremia, pneumonia, and CNS infections, to other less common infections, including ocular infections. The most common site of infection is the urinary tract, but the organism is frequently isolated from the respiratory tract and wounds.[732]



FIG. 218.7 *Serratia marcescens* colonies producing prodigiosin responsible for characteristic red pigmentation. *(From Mahlen SD. Serratia infections: from military experiments to current practice.* Clin Microbiol Rev. *2011;24:755–791.)*

In large epidemiologic surveys, *Serratia* species account for only a small percentage of pneumonia and bacteremia. *Serratia* is typically considered a nosocomial pathogen, but in a population-based study in Australia conducted over 10 years, nearly half of the cases of *Serratia* bacteremia was found to be of community onset.[733] A similar laboratory-based surveillance study from Canada found that nearly two-thirds of incident isolates were of community onset; however, the investigators were unable to distinguish community-acquired from health care–associated isolates.[734] Such data suggest that community infections may play a larger role than previously thought, and that infections may arise from endogenous (microbiota) strains, in addition to being transmitted nosocomially.

There have been more than 200 reported *S. marcescens* outbreaks[732] implicating sources such as compounded drugs,[735] prefilled syringes,[736] contaminated soaps and water,[737–739] medical devices,[740] and total parenteral nutrition.[741,742] *Serratia* outbreaks are of particular concern in pediatric ICUs.[737] *S. liquefaciens* has been noted to be a cause of transfusion-related outbreaks.[743,744]

In addition, *Serratia* species have a historical association with infections in injection drug users who are at particular risk for *S. marcescens* infections, including endocarditis[731,745] and infections at other sites after hematogenous spread.[731] *Serratia* spp. have a predilection for ocular infection, accounting for up to 20% of nosocomial *Serratia* infections, second only to respiratory infections in a retrospective review.[746] *Serratia* can cause keratitis, corneal ulceration, and endophthalmitis, which may follow hematogenous dissemination, and is second only to *Pseudomonas* spp. in the frequency of gram-negative ocular infections and rivals it with regard to infections associated with contact lens wear.[747–749]

Potential virulence factors of *Serratia* have not received as much attention as those of *E. coli* or *Klebsiella,* but *Serratia* likely uses many of the common virulence factors present in other Enterobacteriaceae. The ability to degrade macrophages has been proposed as a possible virulence attribute for *S. liquefaciens.*[750] This process is independent of internalization, but because proximity and contact are required, extracellular toxins or other bacterial effectors may be implicated. More recently, the RssAB-FlhDC-ShlBA pathway has been considered as a complex host invasive pathogenic pathway involving numerous factors, including quorum sensing and swarming.[752,754] Through this pathway, ShlA both exerts cytotoxic properties and contributes to urinary pathogenesis. ShlA, codependent on its activator, ShlB, requires cellular contact consistent with the effects on macrophages noted earlier.[752] RssAB is a regulatory system that decreases ShlA production and thereby swarming.[753] FlhDC is important for quorum sensing and positively regulates

swarming, but negatively affects biofilm formation.[754,755] *Serratia* colonization, invasiveness, and persistence may be aided by biofilm formation[756] and are likely, in part, regulated by quorum sensing.[732,757] However, biofilm formation was not evident in *Serratia* keratitis infections; accordingly, exoenzyme production may be important.[758]

Serratia isolates are resistant to ampicillin and first-generation cephalosporins because of an inducible, chromosomal AmpC β-lactamase similar to that of *Enterobacter.*[759,760] *Serratia* have been associated with nearly all mechanisms of β-lactamase production seen in other gram-negative organisms,[761] including KPC enzymes conferring resistance to carbapenems (see Chapter 18 on antimicrobial resistance). Given the variety of resistance mechanisms found, treatment of infections caused by *S. marcescens* can be difficult, and every effort should be made to identify the sources of outbreaks to control the spread of the organism in susceptible populations.

Citrobacter Species

Members of the genus *Citrobacter* are named for their use of citrate as their sole carbon source. Of the more than 10 recognized species, *Citrobacter freundii* and *Citrobacter koseri* (formerly *Citrobacter diversus*) are the most important human pathogens. They are differentiated by their ability to convert tryptophan to indole, ferment lactose, and use malonate.[762] *C. freundii* produces hydrogen sulfide and therefore can be confused with *Salmonella,* with which it was classified at one time. The urinary tract is the most frequent site from which *Citrobacter* is cultured, often in association with an indwelling catheter, and *Citrobacter* may be a frequent cause of nosocomial UTIs[763] and outbreaks.[764] These bacteria may also be seen in intraabdominal and pulmonary infections.[765,766] Bacteremia from *C. freundii* may have an intraabdominal source more commonly than from the urinary tract, especially in patients with polymicrobial bacteremia and those with underlying malignancy.[765,768] Neonatal meningitis due to *C. koseri* often (approximately 75%) causes multiple brain abscesses associated with high mortality and severe neurologic sequelae in survivors.[769] The predilection for CNS infection has been postulated to be due to a combination of a unique outer membrane in *C. koseri* and infiltration of macrophages infected with *C. koseri* resistant to phagolysosomal effects across the BBB.[769,770] Nosocomial outbreaks have been accompanied by high rates of intestinal colonization by the organism in infants and by carriage of the bacteria on the hands of health care workers.[764,771] A carbapenemase-expressing *C. freundii* outbreak was considered to have a potential foodborne source.[772]

C. freundii strains, like strains of *Enterobacter* and *Serratia,* have inducible *AmpC* genes encoding resistance to ampicillin and first-generation cephalosporins that can be produced constitutively at high levels after mutations selected by prior exposure. Furthermore, isolates of *Citrobacter* may be resistant to multiple other antibiotics as a result of plasmid-encoded resistance genes. The exposure of commensal *Citrobacter* spp. of low virulence to repeated antibiotic courses may select for accumulation of multiple resistance genes, leading to extensive resistance profiles.[773]

Hafnia alvei

H. alvei (formerly *Enterobacter hafniae*) was previously considered to be the sole species in the genus *Hafnia.* Through more recent 16S rRNA gene studies, a novel species, *H. paralvei*, now has been confirmed.[774] There are not clear virulence differences between the species, although *H. alvei* may be more cytotoxic.[775] As with most of the disease-implicated members of the Enterobacteriaceae, *Hafnia* reside in the gastrointestinal tract of humans and many animal species as part of the normal microbiota.[776] *Hafnia* are motile but do not ferment lactose. Although *H. alvei* may be cultured from various sites, it is rarely implicated in monomicrobial infections[777]; this finding suggests that its pathogenicity may be quite low, with the exception of rare case reports of isolated bacteremia. Most infections with *H. alvei* occur in patients with severe underlying illness, including malignancy, trauma, and recent surgery. There have been numerous reports linking diarrhea to the isolation of *H. alvei* from stool specimens and several studies have implicated *H. alvei* in outbreaks, although the evidence is not strong.[776] Furthermore, those strains initially reported to produce A/E lesions similar to EPEC (as described earlier) were reclassified as *E. albertii.*[94] However, in a case-control study the prevalence in stools of *H. alvei* was significantly greater in those with diarrhea than without, consistent with a possible etiologic role.[778] These strains, though, were all negative for the *eae* gene encoding intimin, found in EPEC and *E. albertii.*[94] A majority of *Hafnia* strains tested in vitro produced a cytolytic toxin that has been implicated in gastroenteritis.[775] *H. alvei* are naturally resistant or of intermediate susceptibility to tetracyclines, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, narrow-spectrum cephalosporins, azithromycin, and fosfomycin.[779] Treatment of serious *H. alvei* infections is guided by antimicrobial susceptibility testing. High rates of colistin resistance have been described, given the close phylogenetic relationship with other intrinsically colistin-resistant Enterobacteriaceae.[780] Multiple resistance patterns have been noted.[779,781]

Proteus, Providencia, and *Morganella*

The genera *Proteus, Providencia,* and *Morganella* are related members of the Enterobacteriaceae that are lactose negative and motile and produce phenylalanine deaminase. *Proteus mirabilis* and *Proteus vulgaris* account for most clinical *Proteus* isolates. Both produce urease and hydrogen sulfide, and the latter is indole positive. In contrast to most Enterobacteriaceae, these bacteria may express large numbers of fimbriae and flagella, transforming them into highly elongated rods that translocate rapidly across the surface of agar plates, resulting in a characteristic "swarming" motility. This motility produces a characteristic pattern on growth plates (Fig. 218.8) from sequential rounds of swarm cell differentiation and can overwhelm accompanying organisms. This swarming motility is regulated through a complex network acting on the flagellar transcription regulator, *flhDC.*[782] The name *Proteus* follows from the character in Homer's *Odyssey* who is capable of changing form.

Providencia stuartii is the most common species of its genus isolated from clinical specimens, but *Providencia rettgeri* is occasionally grown. These bacteria can be differentiated from *Proteus* and *Morganella* based on their ability to use citrate and ferment D-mannitol.[783] *Morganella morganii* is, at present, the only member of its genus and is citrate negative.

Proteus spp. are common causes of UTIs, occasionally in normal hosts[784] but more often in those with indwelling catheters or anatomic or functional urinary tract abnormalities.[785,786] *Proteus* are common among the gram-negative bloodstream isolates, with most secondary to UTI and often associated with urinary catheters.[639,787–789]



FIG. 218.8 Characteristic bull's eye pattern of sequential rounds of swarming and consolidation frequently seen with *Proteus*. *(From Schaffer JN, Pearson MM. Proteus mirabilis and urinary tract infections.* Microbiol Spectr. *2015;3[5].)*

Community-acquired *Proteus* UTIs in the presence of hydronephrosis or urolithiasis have increased risk of bacteremia.[790] *Proteus* has a remarkable ability to persist in the urinary tract despite antibiotics and catheter exchange, likely due to immune evasion and the protective reservoir that urinary stones provide. Infections not related to the urinary tract or bloodstream have been reported with much less frequency.

The pathogenesis of UTIs caused by *P. mirabilis* has received considerable attention.[782,791] These microorganisms have multiple features devoted to adherence and motility[792] and may produce several types of pili, the most important of which are mannose-resistant *Proteus*-like (MR/P) fimbriae.[793,794] which have been shown to contribute to bladder colonization in a murine model.[795] Ascending UTIs are common, and fimbriae and adhesins are necessary, but not sufficient, to produce infection.[782] Potent urease production contributes to colonization, stone formation, and obstruction,[786,796–798] without which the infectivity of *Proteus* is diminished.[799,800] By hydrolyzing urea to form CO_2 and ammonia, urease leads to the alkalinization of urine, which leads to the precipitation of struvite, formation of calculi, and subsequent urinary catheter obstruction. Urinary pH ≥7 suggests infection with a urease-positive bacterium, most often a *Proteus* species. *P. mirabilis* can cause monomicrobial UTIs but is frequently found in polymicrobial catheter-associated urinary tract infections. In coinfection models with *P. stuartii*, urolithiasis and bacteremia are promoted in a urease-dependent fashion, and the presence of the other uropathogens regardless of their own urease production enhances the urease activity of *Proteus*.[801–803]

Given the increased severity of *Proteus* UTIs and the propensity of this organism to cause bacteremia in community-acquired infections and patients with hydronephrosis or urolithiasis, radiographic studies such as renal sonography or computed tomography should be considered in patients with severe UTIs, especially those complicated by bacteremia.[790] Obstruction due to calculi must be promptly relieved to avert severe clinical outcomes and to avoid persistent sources that can lead to recurrent infection.[785] Most *Proteus* strains are susceptible to commonly used antibiotics, except for nitrofurantoin and tetracycline.[783] Like other members of Enterobacteriaceae, MDR strains exist and are increasing; strains of *P. vulgaris* are generally more resistant.

P. stuartii and *P. rettgeri* are relatively uncommon clinical isolates, except in elderly patients with long-term indwelling urinary catheters,[797,804,805] and are emerging in hospital settings.[806] These infections are sometimes complicated by bacteremia and may cause death.[804,807] *Providencia* uses many of the same virulence factors as *Proteus,* although they are less often studied. Biofilm production, weak urease production, and urease tolerance are proposed virulence mechanisms[797,808] and have been implicated in the organism's ability to disseminate in a hospitalized setting.[809] *P. stuartii* and *P. rettgeri* are often resistant to multiple antibiotics, including gentamicin, first-generation cephalosporins, and ampicillin,[810] although MDR phenotypes have been described.[811–814] Therapy is guided by susceptibility testing.

M. morganii is an infrequent nosocomial isolate, usually isolated from urine or wounds.[783,815] *M. morganii* bacteremia most often is due to a urinary or hepatobiliary port of entry and is often community acquired.[816,817] *Morganella* spp. also may be present in polymicrobial infections and in wounds, which are the next most common source in bacteremic cases.[816] As with other members of this group, *M. morganii* may cause nosocomial outbreaks.[818] *M. morganii* strains possess inducible AmpC β-lactamases and therefore are intrinsically resistant to ampicillin and first-generation cephalosporins; spontaneous derepressed mutants resistant to ESBLs similar to those described previously for *Enterobacter* spp. may arise.[819]

Other Genera

The majority of clinically relevant Enterobacteriaceae are discussed in the previous sections, although as sequencing studies and in-depth biochemical analyses increase, the number of distinct genera within the Enterobacteriaceae continues to expand. Descriptions of clinical disease limited to case-level data include *Averyella*, *Buttiauxella, Cedecea, Kosakonia, Leclercia, Leminorella, Moellerella, Pluralibacter, Rahnella, Tatumella,* and *Yokenella,* in addition to those highlighted here.[694]

Edwardsiella tarda is found in freshwater environments, has been associated with diarrhea, and can cause wound infections, abscesses, and bacteremia, sometimes linked to marine exposure, including in patients "noodling" or hand-fishing of catfish.[820,821] In patients with liver disease and iron overload, the mortality rates from *Edwardsiella* infections are high.[822] Genes related to iron scavenging have shown adaptive evolution, which may facilitate virulence in *Edwardsiella* to a broad range of hosts.[823]

Plesiomonas shigelloides is another organism that is found in water and has been associated with diarrhea and rarely with extraintestinal infections.[824] *P. shigelloides* was previously grouped with vibrios, given their shared oxidase production and polar flagellae, but has been reassigned based on phylogenetic studies based on ribosomal RNA gene sequencing.[825,826] *P. shigelloides* is an infrequent isolate from patients with gastroenteritis. Although the pathogenicity of *P. shigelloides* was questioned initially, it is now an accepted enteric pathogen based on case-control studies and numerous diarrheal illness outbreaks, with strong epidemiologic evidence.[824] *P. shigelloides*–associated diarrhea manifests most commonly with secretory gastroenteritis but can also cause grossly bloody stool or chronic diarrheal illness. Risk factors include recently ingested raw shellfish or travel exposure.[827]

Ewingella americana, named after William Ewing, who made many contributions to our understanding of the microbiology of the Enterobacteriaceae, is a rare cause of nosocomial bacteremia, peritonitis associated with peritoneal dialysis, and conjunctivitis.[828–830] Most isolates are highly sensitive to antibiotics, although multidrug resistance has been reported.[831]

Infections caused by organisms belonging to the genus *Kluyvera,* which closely resembles *E. coli,* are rare. These bacteria have been recovered from urine, sputum, and wounds; and in many cases, the pathologic significance of their presence is unclear. However, pyelonephritis, bacteremia, and abdominal and soft tissue infections caused by *Kluyvera* spp. have occurred, particularly in immunosuppressed patients, with some infections being fatal.[832–834] Chromosomal genes in *Kluyvera* spp. are likely the origin of CTX-M ESBLs, which have become an increasing cause of MDR and have demonstrated global spread.[835–837] Although bacteria belonging to the genus *Photorhabdus* are fascinating bioluminescent nematode symbionts, the nonluminescent species *Photorhabdus asymbiotica* is the only one recovered in humans.[694,838,839]

ACKNOWLEDGMENT

The authors thank Dr. Michael Donnenberg for his many contributions to the field, and his work on this chapter in the 6th, 7th, and 8th editions of this book.

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The complete reference list is available online at Expert Consult.

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219 *Pseudomonas aeruginosa* and Other *Pseudomonas* Species

Rafael Araos and Erika D'Agata

SHORT VIEW SUMMARY

Epidemiology

- *Pseudomonas* species are gram-negative bacteria that inhabit diverse environments, including soil, water, plants, insects, and animals.
- *P. aeruginosa* is the most important *Pseudomonas* species affecting humans, often causing serious infections associated with substantial morbidity and mortality.
- Other *Pseudomonas* species, including *P. fluorescens, P. luteola, P. putida,* and *P. stutzeri,* are less virulent but are still implicated in a wide variety of infections, primarily occurring among immunocompromised patients.
- *P. aeruginosa* has many virulence factors, including pili, flagella, enzyme secretion systems, and quorum-sensing molecules.
- *P. aeruginosa* is one of the most frequent pathogens implicated in hospital-acquired infections, including ventilator-associated pneumonia and catheter-associated urinary tract infections. Long-term acute care hospitals have very high rates of infections caused by *P. aeruginosa.*
- Characteristic infections caused by *P. aeruginosa* include ecthyma gangrenosum, malignant otitis externa, hot hand-foot syndrome, and hot tub folliculitis.
- Resistance to single and multiple antimicrobial agents is rising rapidly.

Microbiology

- *P. aeruginosa* is an aerobic gram-negative, rod-shaped bacteria.
- Growth occurs in a variety of culture media, forming smooth round colonies with a characteristic grapelike or "corn-taco" odor and a green-blue coloration.
- Identification is based on colony morphology, coloration, oxidase positivity, and growth at 42°C.

Therapy

- The majority of evidence suggests that empirical treatment with combination therapy is indicated if antimicrobial resistance rates are high.
- Streamlining to a single antimicrobial agent, once antimicrobial susceptibility profiles are available, should then be considered.
- Effective antimicrobials include quinolones, carbapenems, and broad-spectrum cephalosporins ± β-lactamase inhibitors (Table 219.3)

Pseudomonas species are ubiquitous gram-negative bacteria capable of inhabiting a wide variety of diverse environments, including soil, water, plants, insects, and animals. Among all *Pseudomonas* species, *P. aeruginosa* is the most important species affecting humans and is responsible for serious debilitating and life-threatening infections.

P. aeruginosa infections were noted in the literature in the 1800s when physicians began to report a "condition" causing a blue-green discoloration on bandages and associated with a "peculiar" odor. The cause of the discoloration was first characterized by Fordos in 1869, who extracted the blue crystalline pigment called pyocyanin.[1] In 1882, Gessard verified "the parasitic origin of this phenomenon" using Pasteur's cultures and isolated the organism, which was originally called *Bacillus pyocyaneus.* Initially, this pathogen was regarded as "a curiosity without any influence upon human pathology," and "old surgeons looked upon blue pus on their dressings as rather a favorable sign."[1] In 1894, Williams provided one of the first reviews of case reports of *B. pyocyaneus* infections.[1] He described septic patients with "hemorrhagic spots of a port-wine color" and pustules, with recovery of the organism from these skin lesions. Subsequently, more case reports of infections caused by *B. pyocyaneus* appeared in the literature.[1–3] In the 1940s, Haynes provided detailed microbiologic characteristics of *P. aeruginosa* that would distinguish it from *Pseudomonas fluorescens.* During the Vietnam War, *P. aeruginosa* was recorded as one of the three most common wound pathogens.[4,5] By the mid-1990s, *P. aeruginosa* became of great concern as a pathogen associated with burn infections and war-related wounds. *P. aeruginosa* is now considered to be of most concern because it causes a variety of infections associated with considerable morbidity and mortality, usually occurring among immunocompromised hosts. Furthermore, single-drug and multidrug resistance rates are particularly high for this pathogen, which severely limits the therapeutic options available to treat infected patients.

MICROBIOLOGY

The pseudomonads are aerobic gram-negative, motile rods. They are ubiquitous in soil, water, plants, and animals and have numerous important ecologic roles (Fig. 219.1). The German botanist Walter Migula first used the term *Pseudomonas,* which is derived from the Greek *pseudo,* meaning "false", and *monas,* meaning "unit". Although the etymology was never explained, it has been postulated that Migula created this name because the bacteria resembled the cells of nonflagellate *Monas* in size and motility.[6] *P. aeruginosa* is an obligate aerobic rod-shaped bacterium measuring 0.5 to 1.0 μm in width and 1 to 3 μm in length. It grows as a single bacterium, although it can occur in short chains. It grows on many types of culture media, forming smooth round colonies with a characteristic grapelike or "corn-taco" odor and green-blue coloration. The coloration is due to the production of pyocyanin (blue) and pyoverdin (green). This distinct color explains the species name of "*aeruginosa,*" a Latin word meaning "verdigris" or "copper rust." Some strains produce other pigments, including pyorubin (dark red) and pyomelanin (black). Isolates from patients with cystic fibrosis (CF) may have a distinct mucoid appearance. *P. aeruginosa* is oxidase positive and grows at 37°C to 42°C. Growth at 42°C allows differentiation from other *Pseudomonas* species, including *P. fluorescens* and *P. putida.* Identification of *P. aeruginosa* is based on colony morphology, coloration, oxidase positivity, and growth at 42°C.

Strains of *P. aeruginosa* can produce an extracellular polysaccharide, referred to as alginate. Overproduction of this substance leads to the formation of a mucoid colony phenotype, which is usually present among

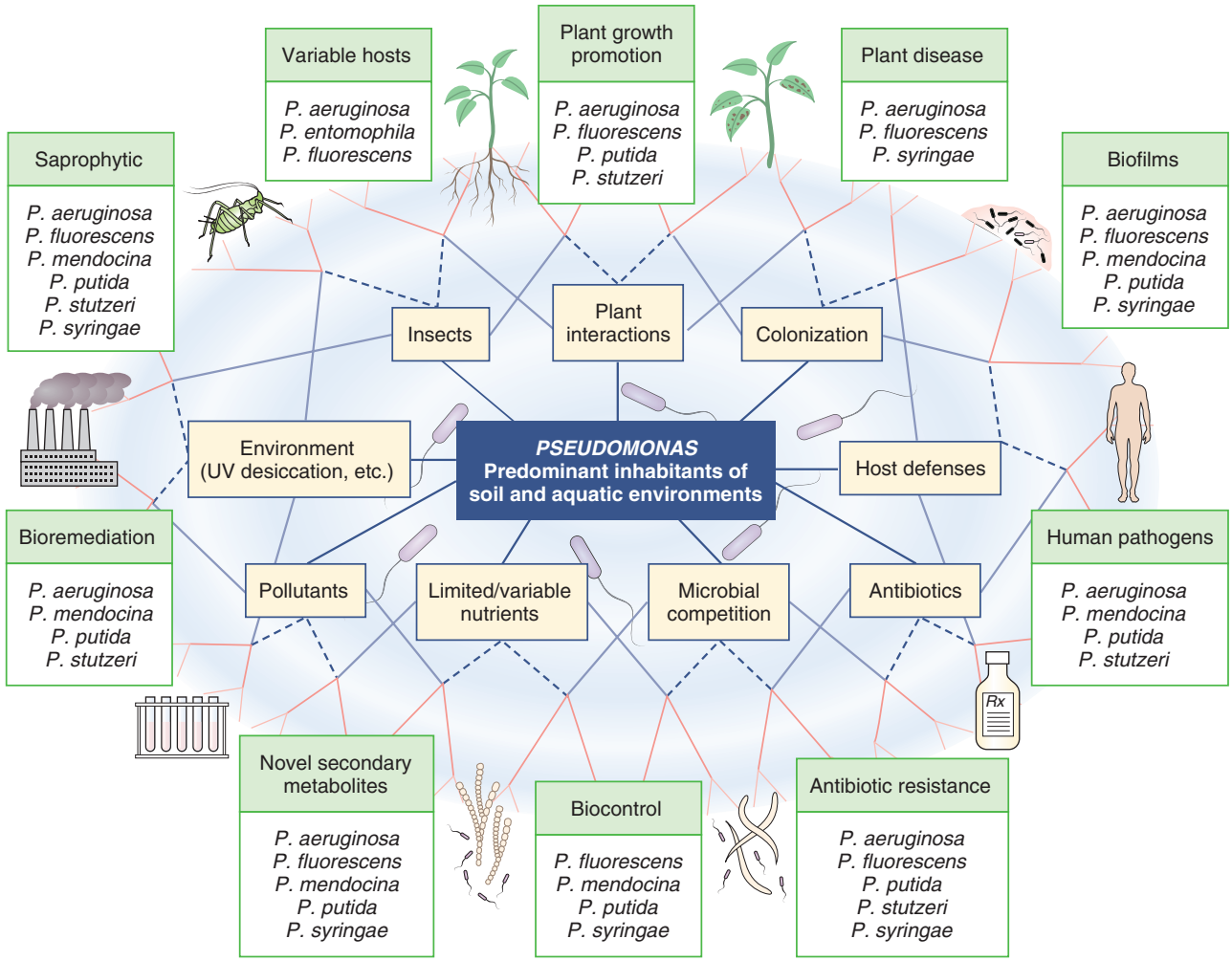

FIG. 219.1 Functional and environmental range of *Pseudomonas* species. The *Pseudomonas'* common ancestor has encountered a wide range of abiotic and biotic environments, which has led to the evolution of a multitude of traits and lifestyles with significant overlap among species. *UV,* Ultraviolet. *(Modified from Silby MW, Winstanley C, Godfrey SA, et al.* Pseudomonas *genomes: diverse and adaptable.* FEMS Microbiol Rev. *2011;35:652–680.)*

isolates recovered from patients with CF and other chronic infections. Isolates recovered from the environment or those causing nosocomial infections are usually nonmucoid.[7–9]

The *Pseudomonas aeruginosa* Genome

The *P. aeruginosa* genome is large (over 6 million base pairs)[10] and complex. In contrast to other large bacterial genomes, the *Pseudomonas* genome does not contain an abundance of gene duplication events but instead contains numerous distinct gene families. This finding explains the great genetic and functional diversity of this pathogen.[11,12]

The genome is composed of a relatively invariable core genome, which contains 90% of the total genome and includes the conserved gene sequences that encode metabolic and pathogenic factors present in the majority of *P. aeruginosa* strains. The accessory genome is highly variable and includes genes found only in certain *P. aeruginosa* strains. The genetic elements in the accessory genome result in distinct *P. aeruginosa* phenotypes, with niche-specific adaption. These genetic elements include virulence factors, resistance genes, and genes encoding specific catabolic pathways that allow persistence in harsh environments (pollutants, pesticides).[11]

Pseudomonas aeruginosa and the Human Microbiota

The Human Microbiome Project, established by the National Institutes of Health, has begun to characterize the human microbial communities colonizing healthy individuals, using 454 FLX Titanium platform pyrosequencing of 16S ribosomal RNA genes. Analysis of the human microbiota among 242 healthy adults from five major body areas (oral cavity and oropharynx, stool, vagina, nares, and skin [inner elbows and behind the ears]) revealed that *P. aeruginosa* was completely absent from both the skin and nares and that *Pseudomonas* species (not identified at the species level) were present in miniscule abundance in the stool and oral cavity, although more abundant in the latter. These data suggest that *P. aeruginosa* is not a common bacterium that inhabits the human microbiota among healthy hosts.[13]

Alterations in the microbiome lead to a decrease in colonization resistance (increasing colonization with antimicrobial-resistant bacteria) and a decrease in resilience (the microbiome's ability to recover). The absence or near absence of *P. aeruginosa* in the healthy human microbiome strongly suggests that for detectable long-term colonization to occur, a perturbation of the microbiome is necessary. Antimicrobial agents, other medications (e.g., anticholinergics), gastrointestinal diseases, and diet may be some of the necessary factors required for *P. aeruginosa* colonization.

VIRULENCE FACTORS

An abundance of virulence factors have been identified among various *P. aeruginosa* strains. These factors are either present on the bacterial cell surfaces or are secreted (Table 219.1). The majority of virulence factors, however, have been identified in cell lines or animal models

TABLE 219.1 Virulence Factors of *Pseudomonas aeruginosa* and Their Role in Pathogenesis

VIRULENCE FACTOR	ROLE IN PATHOGENESIS
Pili and flagella[14]	Attachment to host cells, motility, biofilm formation
Type I secretion system (alkaline protease)[15]	Delivery of toxins to extracellular spaces
Type II secretion system (elastase, exotoxin A, phospholipase A, protease IV)[15]	Cytotoxicity, inflammation, colonization
Type III secretion system (exotoxins S, T, U, and Y)[16,17]	Tissue injury
Endotoxin (lipopolysaccharide)	Resisting host innate defenses
Alginate[21]	Antiphagocytic activity, resists opsonic killing
Pyocyanin[22]	Tissue damage, inhibition of lymphocyte proliferation
Pyoverdin[22]	Binds iron
Quorum-sensing molecules[18,19]	Cell-to-cell communication regulating virulence and biofilm formation

and therefore their role in human disease has not been clearly established. Major virulence factors are discussed in the following sections. Virulence factors associated with CF are discussed in Chapter 71.

Pili

Pili allow the bacteria to adhere to cell surfaces, are involved in biofilm formation, and mediate motility. Five *pilA* alleles have been identified (groups I to V) among *P. aeruginosa* strains. Type IV pili (T4P) has been extensively investigated.[14] This group of pili is unique in that its members can mediate motility independent of flagella. Many *P. aeruginosa* T4P-expressing strains exhibit "twitching motility," a jerky movement with an estimated velocity of approximately 1 mm/h (equivalent to 500 cell lengths per hour, assuming the average length of a *P. aeruginosa* cell is 2 μM).[14] The functions of twitching motility include biofilm formation and exploration of surfaces. Given their role in adherence, T4P are deployed during the early stages of acute infection.[14] Production of pili is frequently lost in chronic infections, such as CF, with selection of strains with other phenotypes better suited for that environmental niche.

P. aeruginosa possesses a single polar flagellum, which plays an important role in motility, colonization, and biofilm formation, similar to pili.

Type I and II Secretion Systems

The type I secretion system (T1SS) secretes toxins in a one-step process into extracellular spaces. The most important toxin studied in the T1SS is alkaline protease, which inhibits fibrin formation and promotes dissemination of *P. aeruginosa*. Secretion of toxins by type II secretion system (T2SS) is a two-step process whereby toxins are synthesized as precursor proteins and then cleaved. Toxins excreted by this system include exotoxin A, phospholipase C, protease IV, and elastase, which mediate cytotoxic effects and inflammatory processes and promote colonization.[15]

Type III Secretion Systems

The type 3 secretion system (T3SS) is a complex secretory system that directly injects exotoxins into the cell cytoplasm. Four proteins have been identified. Exotoxin U (ExoU) is a phospholipase, which induces apoptosis as well as causes necrosis of phagocytes and parenchymal cells. Exotoxin Y (ExoY) is an adenylate cyclase, which may disrupt the barrier function of pulmonary endothelial cells. Exotoxins T and S (ExoT and ExoS) are bifunctional proteins that affect target cell growth by inhibiting DNA synthesis and inducing changes in the cytoskeleton and cellular morphology, thus affecting adherence. Although all strains harbor T3SS genes, only a few are capable of secreting these effector proteins under the conditions tested. The expression of the T3SS in *P. aeruginosa* isolates may confer a worse clinical outcome among humans than nonexpressors.[16,17]

Quorum-Sensing Molecules

Quorum sensing (QS) allows cell-to-cell communication and involves signaling molecules called autoinducers. The usual steps of QS involve the production of autoinducers followed by their active or passive release into the environment. These autoinducers are then recognized by specific receptors, resulting in changes in gene regulation.[18] This complex signaling network allows the "community" of *P. aeruginosa* bacteria to react to different signals and thereby adapt to different niches. There are three QS systems present in *P. aeruginosa* isolates: two are referred to as the LuxI/LuxR-type QS circuits, and the third is referred to as the *Pseudomonas* quinolone signal system.[18,19] These QS systems control the expression of virulence factors, including elastase, exotoxin A, and proteases.

QS also controls biofilm formation (see Chapter 71). Biofilms are a type of growth mode that results in clusters of bacterial colonies, encased in a biopolymer matrix, that attach to surfaces. Biofilms are predominantly formed on implantable devices or during chronic infections, such as osteomyelitis and CF. One of the main functions of biofilms is to reduce the efficacy of antimicrobial agents by impeding the agents' ability to reach the bacteria.[19,20]

Other Virulence Factors

A variety of other virulence factors produced by *P. aeruginosa* have been described. Endotoxin, or lipopolysaccharide, is a virulence factor located on the outer portion of the outer membrane and provides resistance to host defenses. Pyoverdins are siderophores that compete with host proteins for iron chelation. Pyocanin reacts with oxygen to form oxygen radicals, causing tissue damage, and inhibits both lymphocyte proliferation and cilia function. Lastly, alginate is an extracellular polysaccharide, which has antiphagocytic activity and resists killing by opsonization. It is a scavenger of free radicals that are released by macrophages and inhibits neutrophil chemotaxis and complement activation. Its secretion results in a mucoid morphology seen on culture plates.[21,22]

EPIDEMIOLOGY

P. aeruginosa is implicated in both community- and hospital-acquired infections, although it is much more common in the latter. In the United States, *P. aeruginosa* is the 6th most common pathogen implicated in all hospital-acquired infections, as reported in 2016 by the National Healthcare Safety Network (NHSN).[23] It is the second most common pathogen associated with ventilator-associated pneumonia[24] and ranks third among causes of catheter-associated urinary tract infections. *P. aeruginosa* is the 5th most common pathogen causing surgical site infections and 10th most common pathogen causing catheter-related bloodstream infections (BSIs). Patients at high risk for *P. aeruginosa* hospital-acquired infections include those admitted to an intensive care unit (ICU) and those with burns, neutropenia, or CF. These patient populations are discussed in this section and also in Chapter 71.

Rates of *P. aeruginosa* implicated in infections in long-term acute care hospitals (LTACHs) are even higher than rates reported from hospital settings. LTACHs are defined as health care facilities that are accredited as acute care hospitals with an average annual length of stay of at least 25 days for Medicare patients, as per the Centers for Medicare and Medicaid Services. Patients admitted to these LTACHs usually require prolonged care after hospitalization, including hemodialysis, mechanical ventilation, intravenous medication, and wound care. In contrast to the hospital setting, *P. aeruginosa* is the most common cause of catheter-associated urinary tract infections and ventilator-associated pneumonia in LTACHs.[25] Even when rates of *P. aeruginosa* infections in LTACHs are compared with those from ICUs, *P. aeruginosa* is still a more frequent pathogen implicated in LTACH infections. For example, in 2010, 19% of catheter-associated urinary tract infections in patients in LTACHs were caused by *P. aeruginosa* compared with 9% to 12% of those occurring in ICUs. Ventilator-associated pneumonia caused by *P. aeruginosa* was also more frequent in LTACHs (35%) compared with that occurring in ICUs (17%–20%). The higher occurrence of *P. aeruginosa* infections

is likely due to an older, more debilitated patient population with excessive health care exposure.[25]

Transmission Dynamics of *Pseudomonas aeruginosa* and Reservoirs

Acquisition of *P. aeruginosa* can occur both exogenously and endogenously. Exogenous acquisition occurs through contaminated hands of health care workers and environmental surfaces. An in-depth epidemiologic study characterized the exogenous transmission of multidrug-resistant (MDR) *P. aeruginosa* in six ICUs.[26] The investigators obtained cultures from hands, gloves, and gowns of health care workers during routine patient care activities, surveillance cultures from patients, and environmental samples from sinks, bedrails, vital sign monitors, supply carts, door handles, intravenous pumps, ventilators, and floors. Molecular typing, using pulsed-field gel electrophoresis, was performed to determine clonal relatedness among strains. In that study, MDR *Acinetobacter* species were the most common pathogens to contaminate health care workers' gloves and hands, occurring among 33% of interactions between health care workers and patients. *P. aeruginosa* was the second most common MDR pathogen to contaminate health care workers, which occurred during 17.4% of health care worker–patient interactions. Independent risk factors associated with health care worker contamination were presence of environmental contamination, duration in patients' room greater than 5 minutes, performing a physical examination, and contact with the mechanical ventilator. Environmental contamination was also very common: *P. aeruginosa* was recovered from 22% of rooms.[26] This study and many others emphasize that exogenous transmission plays a major role in the nosocomial acquisition of *P. aeruginosa* and that environmental contamination is central to its transmission to patients and health care workers.

Endogenous acquisition of a resistant strain of *P. aeruginosa* is defined as colonization with an antimicrobial-susceptible strain that subsequently becomes resistant primarily through antimicrobial selective pressure within the host. A study of imipenem-resistant *P. aeruginosa* transmission demonstrated that, among events that could be determined, endogenous acquisition accounted for 19% of identified acquisition events and that exogenous acquisition accounted for 31% of events.[27]

As outlined earlier, environmental reservoirs contribute substantially to the spread of *P. aeruginosa.* The most common sites either have high moisture or humidity or are water related (Table 219.2). In the hospital setting, outbreaks of *P. aeruginosa* have been linked predominantly to water sources, including potable water, showerheads, and sinks (see Table 219.2).[28,29] Other sources have included health care workers' artificial or long nails, intraocular lens solution, ultrasound transmission gel during transesophageal echocardiography, retained tissue in surgical instruments,[30] and soap dispensers. The ability of *P. aeruginosa* to form biofilms on surfaces increases its ability to survive on inanimate surfaces and makes it difficult to eradicate. Biofilms are microbial communities held together by structural polysaccharides (slime), which attach strongly to surfaces. Biofilms produced by *P. aeruginosa* lead to antimicrobial tolerance and impede eradication by environmental cleaning agents.[19,28]

TABLE 219.2 Environmental Reservoirs of *Pseudomonas aeruginosa*

Hospital Reservoirs of *P. aeruginosa*
Sinks, taps, showerheads
Potable water
Respiratory therapy equipment
Flower vases, ice makers
Hydrotherapy pools
Cleaning equipment (mops, buckets)
Bronchoscopes, endoscopes
Resuscitators
Water baths
Multidose vials
Community Reservoirs of *P. aeruginosa*
Home humidifiers
Whirlpools, hot tubs, spas
Swimming pools
Water-damaged homes

True community-acquired infections among people without any prior exposure to a health care setting are rare. The rarity of community-acquired infections reflects the fact that *P. aeruginosa* is not part of the healthy human microbiota[13] and that colonization occurs predominantly after hospitalization and antimicrobial exposure. Community-acquired infections, however, have been reported, including outbreaks from contaminated community reservoirs and among intravenous drug users. As with the hospital setting, water-related reservoirs are the main sources of *P. aeruginosa* in the community and include whirlpools, hot tubs, contact lenses, home humidifiers, water-damaged houses, swimming pools, loofah sponges, and even holy water.[31,32] Contaminated recreational water, however, is among the most common sources of *P. aeruginosa* outbreaks.[33,34] A review of outbreaks associated with recreational water, from 1971 to 2000, identified *P. aeruginosa* as the second most frequent causative pathogen, after *Cryptosporidium* species. *P. aeruginosa* was implicated in 36 outbreaks during the study period, with most of the outbreaks associated with whirlpool baths, hot tubs, and swimming pools. The main presentation is that of a superficial folliculitis that is pruritic and maculopapular and progresses to vesiculopustular within hours to days after exposure. It remits spontaneously. Conjunctivitis and otitis externa were also reported.[33] Common nonmedical terms associated with community-acquired *P. aeruginosa* infections include "hot tub rash," "swimmer's ear," and "hot hand-foot syndrome" (see "Skin and Soft Tissue Infections," later).

Food products may also be sources of *P. aeruginosa* in both community and hospital settings, because this pathogen has been isolated in a variety of vegetables, including lettuce, mushrooms, and carrots, and is present in soil.[35–37] A definitive link between the presence of *P. aeruginosa* in vegetables and subsequent colonization or infection, however, has not been clearly established. Nevertheless, it is plausible that ingestion of *P. aeruginosa*–contaminated raw vegetables by high-risk individuals, including neutropenic patients and other immunocompromised hosts and those with decreased gastric acidity or oropharyngeal invasive devices,[38,39] may increase the risk for colonization.

ANTIMICROBIAL RESISTANCE

P. aeruginosa possesses a plethora of different resistance mechanisms. It is therefore not surprising that rates of antimicrobial resistance and multidrug resistance are among the highest in these organisms compared with other common human pathogens. Of even greater concern is the paucity of novel antimicrobial agents being developed to combat *P. aeruginosa* infections. The US Food and Drug Administration (FDA), however, approved ceftolozane-tazobactam in 2014. It has enhanced affinity for *P. aeruginosa* penicillin-binding proteins and appears to be unaffected by loss of porin channels or upregulation of efflux pumps. Over 80% of isolates resistant to ceftazidime or meropenem retain susceptibility to this novel agent. Currently, it is approved for use in urinary tract and intraabdominal infections,[40] and several reports suggest that it may also be useful for treating other severe infections caused by MDR *P. aeruginosa* with confirmed susceptibility to ceftolozane-tazobactam[41,42] (see Chapter 21).

Rates of antimicrobial resistance among *P. aeruginosa* hospital isolates recovered from different types of health care–associated infections in the United States in 2014, as reported by the NHSN, were as follows: aminoglycosides, 7% to 21%; extended-spectrum cephalosporins, 10% to 27%; fluoroquinolones, 12% to 33%; carbapenems, 8% to 28%; and piperacillin ± tazobactam, 7% to 19%. Multidrug resistance, defined as resistance to three or more of these antimicrobial classes, was present among 4% to 20% of isolates.[23] Similarly to previous reports, isolates from ventilator-associated pneumonia had the highest rates, and those from surgical site infections had the lowest rates.

The 2016 Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) reported rates of *P. aeruginosa* resistance to piperacillin ± tazobactam, ceftazidime, fluoroquinolones, aminoglycosides, and carbapenems. Overall, resistance rates to ceftazidime increased from 2013 to 2016. Conversely, the mean percentages of resistance for fluoroquinolones, aminoglycosides, and carbapenems significantly decreased over the same period of time, whereas resistance

to piperacillin ± tazobactam remained stable. In 2016, 33.9% of isolates were resistant to one or more of the five antimicrobial classes, 13.6% were resistant to three or more, and 4.4% were resistant to all five classes.[43] Resistance rates to three or more groups of antimicrobials varied considerably between European countries, with lowest percentages reported from Iceland, Denmark, Luxembourg, the United Kingdom, the Netherlands, and Norway (<3%) and highest percentages from Bulgaria and Romania (36%–49%). Percentages of single-drug resistance among *P. aeruginosa* isolates obtained from Latin America and the Asia-Pacific rim range from 23% to 41%.[44,45]

In the United States in 2010, resistance rates in LTACH units were reported to be even higher than in ICUs. For example, MDR *P. aeruginosa* was more common among patients with catheter-associated urinary tract infections in LTACHs (25%) compared with those occurring in patients in ICUs (12%). Similarly, rates of multidrug resistance in patients with catheter-associated BSI were higher among those in LTACHs (16%) than those in ICUs (2%–9%).[25]

Resistance Mechanisms

P. aeruginosa harbors numerous resistance mechanisms that either decrease penetration to the target site, alter the target site, or inactivate the antimicrobial agent using bacterial enzymes. These mechanisms can be broadly categorized as intrinsic,[46–48] acquired, or adaptive, with overlap between categories.

Intrinsic Resistance

Decreased Permeability of the Outer Membrane

The semipermeable outer membrane of *P. aeruginosa* allows important nutrients to enter the cell through channels present on the cell membranes called porins. Numerous antimicrobial agents, including β-lactams, aminoglycosides, tetracyclines, fluoroquinolones, and carbapenems, enter the bacterial cell through these porins. Several families of porins have been characterized, including OprF, OprD, OprM, and TonB. Although OprF is a major porin in the outer cell membrane, allowing transport of large substrates, its role in antimicrobial resistance has not been definitively proven because loss of OprF porins in mutant strains does not alter antimicrobial penetration. Among the remaining three families, OprD has been the most studied. This channel allows entry of carbapenems but not of other β-lactams. Loss of OprD, however, may not have an equal impact on minimal inhibitory concentration increases for all carbapenems equally. Lastly, some porins in the family of OprM are presumed to be part of efflux systems and are discussed in the next section.

Not all antimicrobial agents use porins to enter the cell but instead decrease cell membrane stability by binding to lipopolysaccharides on the outer cell membrane. Examples of such agents include aminoglycosides and polymyxins.

Efflux Pumps

As the name implies, efflux pumps actively pump antimicrobial agents out of the bacteria. These pumps confer resistance to the great majority of antimicrobial agents (with the exception of polymyxins) and are the predominant systems for multidrug resistance among *P. aeruginosa*. Five superfamilies of efflux pumps have been identified, of which the resistance-nodulation-division (RND) family is among the most common. MexAB-OprM and MexXY-OprM efflux systems of the RND family confer intrinsic multidrug resistance to numerous antimicrobial agents, including fluoroquinolones, aminoglycosides, β-lactams, tetracyclines, tigecycline, and chloramphenicol. MexAB-OprM also confers resistance to meropenem but not imipenem, thus explaining differences in susceptibility patterns among different carbapenems. Similarly, the MexXY-OprM efflux system removes cefepime but not ceftazidime.[49]

Antimicrobial-Modifying Enzymes

The majority of antimicrobial-modifying enzymes are acquired on plasmids and are discussed later in the chapter,[46] with the exception of AmpC, a chromosomally encoded inducible cephalosporinase. AmpC confers resistance to all β-lactams except fourth-generation cephalosporins and carbapenems. When AmpC is overproduced, through mutations, resistance may also be conferred to these antimicrobial classes. Therapeutic failure due to the emergence of resistance during appropriate therapy can occur in over 50% of patients, especially those with serious *P. aeruginosa* infections and those with neutropenia or CF.

Acquired Resistance

Acquired resistance genes predominantly confer resistance to β-lactams and aminoglycosides.[47] Extended-spectrum β-lactamases (ESBLs) are plasmid mediated and confer resistance to penicillins, narrow- and extended-spectrum cephalosporins, aztreonam, and sometimes carbapenems. ESBL-producing *P. aeruginosa* strains have rapidly spread worldwide. ESBL families identified in *P. aeruginosa* include PER, VEB, GES, TEM, SHV, and CTX-M enzymes. GES-type enzymes also extend their activity to carbapenems. These enzymes have been recovered from isolates from China, South Africa, Brazil, and France.[47] Oxacillinase β-lactamases (OXAs) can either be narrow spectrum or broad spectrum and are weakly inhibited by clavulanic acid. Carbapenemase-hydrolyzing oxacillinases, which can be either acquired or naturally occurring, have also been identified in *P. aeruginosa* isolates, although less frequently than in *Acinetobacter* species. Resistance to carbapenems can also occur via metallo-carbapenemases and include the Verona integron-encoded metallo-β-lactamase (VIM), IMP, and New Delhi metallo-β-lactamase (NDM) families. These enzymes are of great concern because they are active against penicillins and cephalosporins as well as carbapenems. VIM-type isolates were first reported in *P. aeruginosa* isolates recovered in Italy in 1997 and subsequently spread into members of the Enterobacteriaceae, especially *Klebsiella pneumoniae*. Isolates with NDM enzymes may also carry aminoglycoside and fluoroquinolone resistance genes and remain susceptible only to colistin and polymyxin B.[50] Another enzyme active against carbapenems, present in *P. aeruginosa* isolates, is the *K. pneumoniae* carbapenemase (KPC) type. KPC-producing strains were first identified in Colombia and have since spread to Puerto Rico, China, and the United States, and most recently Brazil.[51] Carbapenem-resistant isolates, regardless of the mechanism of resistance, result in serious infections. A longitudinal study from 1989 to 2006 demonstrated that rates of imipenem-resistant *P. aeruginosa* isolates increased from 13% to 20% and that infections caused by these resistant pathogens were associated with higher in-hospital mortality. Prior exposure to carbapenems increased the risk for imipenem-resistant *P. aeruginosa* infection by almost eightfold.[52]

Aminoglycoside-Modifying Enzyme

Aminoglycoside-modifying enzymes are carried on multiple different genetic mobile elements, such as plasmids, transposons, and integrons. They confer resistance to all aminoglycosides, although, in general, amikacin may be less susceptible to these enzymes. The most common aminoglycoside-modifying enzymes are aminoglycoside nucleotidyltransferase (2′)-I, which confers resistance to gentamicin and tobramycin, and aminoglycoside acetyltransferase (6′)-II, which also confers resistance to netilmicin.

TREATMENT OF *PSEUDOMONAS AERUGINOSA* INFECTIONS

Treatment of *P. aeruginosa* infections, especially BSIs, centers around the controversy of monotherapy versus combination therapy. A few earlier studies support the use of combination therapy since mortality rates were lower when two antimicrobial agents, instead of a single agent, were used to treat BSI caused by *P. aeruginosa*.[53] The main limitation of these studies is that in many the monotherapy study arm consisted of only an aminoglycoside, which is suboptimal for the treatment of *P. aeruginosa* BSI.[53] In a meta-analysis, which also concluded that combination therapy is superior to monotherapy, four of the five included studies used aminoglycosides in the monotherapy study arm.[54] There are numerous other limitations with older studies that support the use of combination therapy, including lack of double blinding and randomization, different sources of BSI, retrospective lack of adjustment for time to start of appropriate antimicrobial therapy, and duration of follow-up. Confounding by indication, whereby the severely ill patients receive combination therapy, is another limitation of studies addressing this issue. Another reason that is often cited for supporting combination therapy is the potential synergy between the two antimicrobial agents,

usually a β-lactam and an aminoglycoside. Although in vitro and animal studies show benefit of this combined regimen, clinical studies have provided conflicting data.[55,56] Preventing the emergence of antimicrobial resistance is another reason often cited for supporting the use of combination therapy, but there are minimal data to support this statement. Administering the appropriate dose, at the correct frequency and for the optimal duration, is likely more important in preventing the emergence of resistance than combination therapy. Prompt initiation of the appropriate antimicrobial agents is also key to a successful outcome, as is removal of invasive devices if implicated.

Overall, the great majority of more recent studies do not show a survival benefit between combination therapy and monotherapy for definitive therapy.[57–61] However, the main conclusion by most investigators is that large randomized clinical trials are needed to definitively answer the question of efficacy between combination therapy and monotherapy.

Use of combination therapy should be strongly considered in the severely ill patient for the empirical treatment of *P. aeruginosa* BSI, especially in those health care institutions with patients with a high rate of multidrug resistance. Using combination therapy will thus ensure that at least one antimicrobial agent is effective against the infecting *P. aeruginosa* strain. Deescalating to a single antimicrobial agent, once antimicrobial susceptibility profiles are available, should then be considered. Narrowing to a single agent is especially relevant when the combination therapy includes an aminoglycoside, because this regimen is associated with increased nephrotoxicity.[60,61] Other adverse events that are more likely to occur with combination therapy, compared with monotherapy, include an increased risk for *Clostridioides difficile* (formerly *Clostridium difficile*) infection, further alterations in the protective effects of the human microbiota against colonization by other MDR organisms, and fungal infections.

Antimicrobial agents effective against *P. aeruginosa* and appropriate doses are listed in Table 219.3. Polymyxins (colistin, polymyxin B) should be reserved for MDR *P. aeruginosa,* when other alternatives are not available, because this class of agents is inferior to other available antipseudomonal agents.[62] Emergence of resistance during treatment has been documented for *K. pneumoniae* BSI and likely also occurs for *P. aeruginosa* infections.[63] High-dose continuous infusion of β-lactams has also been successfully used in the treatment of BSI caused by MDR *P. aeruginosa.* Moriyama and associates reported three patients infected with MDR *P. aeruginosa* strains who were successfully treated with a continuous infusion of ceftazidime (6.5–16.8 g/day) or aztreonam (8.4 g/day) and tobramycin.[64] The rationale behind this approach is that antibacterial activity of β-lactams depends on the time that the antimicrobial concentration is above the minimal inhibitory concentration of the bacteria. Using continuous infusion ensures that the concentration of the β-lactam will be above the minimal inhibitory concentration for the entire dosing interval, whereas intermittent dosing may cause the concentration to fall below the minimal inhibitory concentration. Future clinical studies, however, are required to further validate this treatment regimen.

Novel antipseudomonal agents are reviewed in detail by Wright and colleagues.[65] Several studies indicate that ceftolozane-tazobactam and ceftazidime-avibactam are effective in the treatment of MDR–*P. aeruginosa* BSI and other infections, although resistance can develop even with short courses of therapy.[41,42,66,67] The combination of ceftazidime and the novel non–β-lactam β-lactamase inhibitor avibactam has been approved for the treatment of complicated urinary tract infections and intraabdominal infections.[65] The addition of avibactam to ceftazidime allows this combination to inhibit Ambler class A β-lactamases (including KPC), AmpC β-lactamases, and OXA-type Ambler class D β-lactamases, making this drug an excellent therapeutic option for infections caused by MDR gram-negative bacteria, including *P. aeruginosa.*[67] The recommended dose for patients with normal renal function is 2 g of ceftazidime and 500 mg of avibactam every 8 hours. Resistance to ceftazidime-avibactam among *P. aeruginosa* has been described, with higher rates among MDR isolates involving efflux mechanisms or metallo-carbapenemases.[68,69]

TABLE 219.3 Intravenous Antimicrobial Agents Effective Against *Pseudomonas aeruginosa*

ANTIMICROBIAL AGENT	INTRAVENOUS DOSE
Penicillins Plus β-Lactamase Inhibitor	
Ticarcillin-clavulanate	3.1 g q4h
Piperacillin-tazobactam	4.5 g q6h or 3.375 g q4h
Broad-Spectrum Cephalosporins ± β-Lactamase Inhibitor	
Ceftazidime	2 g q8h
Cefepime	2 g q8–12h
Ceftolozane-tazobactam	1.5g q8h
Ceftazidime-avibactam	2.5 g q8h
Fluoroquinolones	
Ciprofloxacin	400 mg q8h
Levofloxacin	750 mg q24h
Carbapenems	
Imipenem	500 g q6h
Meropenem	1–2 g q8h
Doripenem	500 mg q8h
Monobactam	
Aztreonam	2 g q8h
Aminoglycosides	
Tobramycin	2 mg/kg loading dose, then 1.7 mg/kg q8h *or* 4–7 mg/kg q24h
Gentamicin	As for tobramycin
Amikacin	7.5 mg/kg q12h or 15 mg/kg q24h

For a review of novel antimicrobials with potential activity, refer to Wright et al.[65]

INFECTIONS CAUSED BY *PSEUDOMONAS AERUGINOSA*

Bloodstream Infections

BSIs are among the most serious infections caused by *P. aeruginosa,* with mortality rates reaching 60%. The nationwide Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE), which included data from 49 US hospitals, reported that from 1995 to 2002 the incidence of *P. aeruginosa* nosocomial BSI was 2.1 per 10,000 hospital admissions. *P. aeruginosa* was the third most common gram-negative bacteria causing nosocomial BSI and accounted for 4.3% of all cases. In the ICUs, *P. aeruginosa* was the fifth most common isolate implicated in BSI, accounting for 4.7% of all cases, and was the seventh most common isolate in non-ICU wards, accounting for 3.8% of cases.[70] Outside the United States, *P. aeruginosa* is implicated in even more cases of nosocomial BSI.[71] In a surveillance study that collected data from 16 Brazilian hospitals from 2007 to 2010 and used the same methodology as the SCOPE study, 8.9% of all nosocomial BSI were caused by *P. aeruginosa.*

Risk factors for *P. aeruginosa* BSI include immunodeficiency, prior hospitalization, previous antimicrobial exposure, advanced age, prior surgery, and invasive devices.[72–74] Many of these risk factors represent an association with BSI, irrespective of the implicated pathogen.

Mortality rates for nosocomial BSI caused by *P. aeruginosa* are among the highest. The great majority of reported crude mortality percentages from large surveillance studies range from 39% to 60%. These percentages are similar to those caused by *Candida* species.[70,71] Some studies, however, report lower mortality rates, ranging from 12% to 30%.[75–77] The large range in mortality rates from different studies reflects the multitude of factors that affect outcomes associated with BSI. For *P. aeruginosa* BSI, advanced age, high Acute Physiology and Chronic Health Evaluation II (APACHE II) score, sepsis, poor functional status, polymicrobial bacteremia, and inappropriate initial antimicrobial therapy have all been associated with an increased risk for mortality.[73,76–79]

Multidrug resistance is also a risk factor for increased mortality. Rates of mortality in infections due to MDR *P. aeruginosa* strains are

twofold to threefold higher compared with non-MDR strains.[75,80] Because inappropriate initial empirical therapy is a major contributor to these higher mortality rates, combination therapy for empirical treatment is warranted when multidrug resistance rates are high (see later).[81]

The predominant distinguishing feature of *P. aeruginosa* BSI is the occurrence of ecthyma gangrenosum. Although not pathognomonic for *P. aeruginosa,* the presence of these characteristic skin lesions should raise high suspicion for this pathogen (see "Skin and Soft Tissue Infections" later).

Infective Endocarditis

P. aeruginosa accounts for 3% of all cases of infective endocarditis (IE).[82] Among non-HACEK pathogens (species other than *Haemophilus* species, *Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens,* or *Kingella* species), *P. aeruginosa* is the second most common gram-negative pathogen causing IE after *Escherichia coli.*[83] There are no unique clinical characteristics of IE caused by *P. aeruginosa,* although the presence of ecthyma gangrenosum should raise suspicion. Complications are common, and mortality rates are high, ranging from 36% to 60%.[83]

Intravenous drug users are the patient population at highest risk for *P. aeruginosa* IE. The great majority have used tripelennamine and pentazocine with contaminated water or paraphernalia. Patients predominantly present with right-sided endocarditis. Complications are frequent and include sepsis, embolization, and congestive heart failure.[84] In this patient population, polymicrobial IE, with both *P. aeruginosa* and *Staphylococcus aureus,* can also occur.[84]

Left-sided IE in patients without intravenous drug use also occurs, although infrequently. It is predominantly a nosocomial infection occurring after invasive procedures, including cardiac or urogenital procedures. Infected intravascular catheters have also been implicated in IE. Of note, valvular disease is not a necessary predisposing risk factor for *P. aeruginosa* left-sided IE.[85] Splenic abscesses, neurologic sequelae, and ring and annular abscesses are frequent complications.[86]

The American Heart Association recommends, as per expert opinion, that medical treatment of *P. aeruginosa* IE should include an extended-spectrum penicillin (ticarcillin, piperacillin) and ceftazidime or cefepime in full doses in combination with high-dose tobramycin (8 mg/kg/day IV or IM in once-daily doses) with peak concentrations of 15 to 20 μg/mL and trough concentrations of less than or equal to 2 μg/mL. This combination should be given for at least 6 weeks. Use of ciprofloxacin in combination with an aminoglycoside should be used with caution because ciprofloxacin resistance can occur during therapy. Other regimens that have been successful in a small number of patients include the combination of imipenem and an aminoglycoside.[87]

Case reports have also shown success with other combinations of antimicrobial agents. Among two renal transplant recipients with no valvular heart disease who developed *P. aeruginosa* nosocomial IE, 6 weeks of therapy with imipenem and ciprofloxacin was successful in the first patient and 2 weeks of imipenem and amikacin followed by 6 weeks of imipenem alone was successful for the second patient (amikacin was stopped early owing to nephrotoxicity). Both patients' conditions were stable at 6 months of follow-up.[88]

The need for surgical intervention differs between right- and left-sided IE. Although medical treatment is usually sufficient for right-sided native valve IE, in refractory cases surgical intervention with partial tricuspid valvectomy or "vegectomy" without valve replacement may be necessary.[87] Left-sided IE and prosthetic valve IE usually require early surgical intervention, owing to the high risk for complications and high failure rates associated with medical therapy alone. Survival rates with medical therapy alone among patients with left-sided IE, for example, are only 14%.[85]

Pneumonia

Pneumonia Associated With Hospital Exposure

Three categories of *P. aeruginosa* pneumonia can occur as a result of hospital exposure: (1) hospital-acquired (nosocomial) pneumonia, defined as a pneumonia that occurs 48 hours or more after hospital admission and that was not incubating at the time of admission; (2) ventilator-associated pneumonia, defined as a hospital-acquired pneumonia that develops more than 48 to 72 hours after endotracheal intubation; and (3) health care–associated pneumonia, defined as a pneumonia that occurs among nonhospitalized patients who (a) have had an acute care hospitalization for 2 or more days within 90 days of the infection, (b) live in a nursing home or long-term care facility, (c) received intravenous antimicrobial therapy, chemotherapy, or wound care in the previous 30 days of the current infection, or (d) attended a hospital or dialysis clinic in the previous 30 days of the current infection.[89] These definitions were developed for the 2005 American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) guidelines.[89]

Hospital-Acquired (Nosocomial) Pneumonia

P. aeruginosa is the most common gram-negative bacteria implicated in hospital-acquired pneumonia.[23] The great majority of *P. aeruginosa* hospital-acquired pneumonia cases are late onset, occurring after the fifth day of hospitalization, similar to hospital-acquired pneumonia caused by methicillin-resistant *S. aureus.*[90,91] Factors that predispose hospitalized patients to hospital-acquired pneumonia include older age, duration of mechanical ventilation, prior antimicrobial exposure, transfer from a medical unit or ICU, and admission to a ward with a high prevalence of *P. aeruginosa,* reflecting colonization pressure.[91] Mortality rates are extremely high, ranging from 42% to 87%. As with BSI, the wide range reflects the presence of comorbidities, inadequate initial therapy, severity of illness at presentation, and multidrug resistance. The presence of bacteremia is associated with particularly poor outcomes. Hospital-acquired pneumonia can be associated with mechanical ventilation or not, although the former is much more common.

Hospital-Acquired Pneumonia Among Ventilated Patients

P. aeruginosa is the second most common pathogen implicated in ventilator-associated pneumonia after *S. aureus,* as per the 2011 to 2014 NHSN data, which reported rates of hospital-acquired infections from 4515 hospitals in the United States.[23] Among all gram-negative bacteria, *P. aeruginosa* is the most common cause of ventilator-associated pneumonia.[23,90]

Colonization with *P. aeruginosa* is a prerequisite for developing a *P. aeruginosa* ventilator-associated pneumonia. Once the patient becomes colonized, breaches in integrity of the airway mucosa caused by intubation result in tissue invasion by the bacteria, leading to pneumonia. Sources for *P. aeruginosa* colonization are either endogenous or exogenous. Endogenous sources include *P. aeruginosa* colonizing the oropharynx and stomach with subsequent aspiration.[92] Exogenous sources include health care workers carrying the organism and inanimate surfaces. Contaminated water sources are a particularly common reservoir for exogenous acquisition of *P. aeruginosa* implicated in ventilator-associated pneumonia. Numerous *P. aeruginosa* outbreaks have been traced to contaminated faucets, sinks, and tap water. Contaminated bronchoscopes have also been implicated as a source of *P. aeruginosa* ventilator-associated pneumonia. These cases were linked to inadequate disinfection procedures, contaminated disinfecting machines, and manufacturing defects of bronchoscopes.[93]

Antimicrobial resistance among *P. aeruginosa* strains implicated in ventilator-associated pneumonia is high. Reported percentages from US hospitals in 2014 are as follows: aminoglycosides, 18.2%; cefepime-ceftazidime, 25.7%; ciprofloxacin-levofloxacin, 31.9%; imipenem-meropenem, 28.4%; and piperacillin-tazobactam, 19.4%. Multidrug resistance, defined as resistance to three or more antimicrobial classes, was also frequent, occurring in 19.9% of strains.[23] These high rates support the use of combination therapy as empirical treatment for ventilator-associated pneumonia to ensure that at least one antimicrobial agent is active against *P. aeruginosa.* In a study of 100 consecutive patients with bacteremic *P. aeruginosa* pneumonia, adequate empirical combination therapy was associated with significantly lower 28-day mortality rates.[94]

The ATS/IDSA guidelines recommend empirical antimicrobial therapy for ventilator-associated pneumonia with an antipseudomonal β-lactam plus either an antipseudomonal quinolone or an aminoglycoside. The choice of specific antimicrobial agents should take into account the local antimicrobial susceptibility patterns and avoiding agents to which

the patient was recently exposed. If aminoglycosides are chosen, consideration should be given toward a short (5-day) course, to minimize nephrotoxicity, when used in combination with a β-lactam to treat *P. aeruginosa* pneumonia.[89] Once susceptibility profiles are available, monotherapy also should be considered because data supporting combination therapy are lacking.[89,94] Aerosolized antimicrobial agents (aminoglycosides, polymyxins) can be used as adjunctive therapy to systemic antimicrobial agents in patients with highly resistant *P. aeruginosa* ventilator-associated pneumonia.[95]

Duration of antimicrobial therapy for *P. aeruginosa* ventilator-associated pneumonia should be longer than for other pathogens. A prospective randomized, double-blind study evaluated the efficacy of 8 days versus 15 days of antimicrobial therapy for ventilator-associated pneumonia and showed that, for ventilator-associated pneumonia caused by *P. aeruginosa,* an 8-day course was suboptimal and was associated with higher recurrence rates.[96] A 15-day course is therefore recommended.[89]

Hospital-Acquired Pneumonia Among Nonventilated Patients

P. aeruginosa is also the most common gram-negative bacteria implicated in pneumonia among nonventilated hospitalized patients, accounting for 9% of all cases in this category.[90] These high percentages warrant antipseudomonal antimicrobial agents in the empirical treatment of these types of infections, similar to that used for patients with ventilator-associated pneumonia.[89]

Health Care–Associated Pneumonia

Health care–associated pneumonia develops outside the hospital among patients who have had recent substantial exposure to the health care setting. *P. aeruginosa* is implicated in 2% to 25% of health care–associated pneumonia cases.[97–99] Surveillance studies report that it is the most common or second most common gram-negative pathogen causing health care–associated pneumonia.[98,99] Because *P. aeruginosa* strains implicated in this type of pneumonia are more likely to be resistant to antimicrobial agents because of prior health care exposure, the ATS/IDSA guidelines recommend that treatment regimens should follow those for health care–associated pneumonia, as outlined previously.[89] However, these recommendations may not apply in health care settings and countries with low rates of antimicrobial resistance; therefore treatment regimens recommended for community-acquired pneumonia may be more appropriate in these settings.[97]

A retrospective, observational study compared characteristics of health care–associated and community-acquired pneumonia among patients admitted to a tertiary care hospital in South Korea from 2008 to 2010. Among 31% of patients with pneumonia in whom a pathogen was identified, *P. aeruginosa* was the second most common gram-negative bacteria, after *K. pneumoniae,* and accounted for 20% of health care–associated pneumonia and 11% of community-acquired pneumonia. These percentages were not statistically different. The percent of bacterial species, including *P. aeruginosa,* that were "potentially drug-resistant" was significantly higher among the health care–associated group (32%) than the community-acquired group (15%).[99] The investigators included the following bacteria as "potentially drug-resistant": methicillin-resistant *S. aureus, Pseudomonas* species, *Acinetobacter* species, *Stenotrophomonas maltophilia,* and extended-spectrum β-lactamase–producing Enterobacteriaceae. Actual antimicrobial susceptibility profiles, however, were not determined.

Compared with community-acquired pneumonia, patients presenting with health care–associated pneumonia are more likely to be older and have more comorbidities, a higher pneumonia severity index, and a worse functional status at presentation.[97,98] Mortality percentages are subsequently higher for health care–associated pneumonia compared with community-acquired pneumonia and range from 17% to 20%.[98,99]

Community-Acquired Pneumonia

Reported percentages of *P. aeruginosa* implicated in community-acquired pneumonia vary from 0.3% to 17%.[97–100] Although *P. aeruginosa* is not usually considered to be a common cause of community-acquired pneumonia, the high percentages reported by some studies suggest otherwise.[98,99] A surveillance study on the microbiology of pneumonia in 59 US hospitals from 2003 to 2004 found that *P. aeruginosa* was the causative pathogen for 17% of community-acquired pneumonia cases, a percentage similar to those for community-acquired pneumonia caused by *Streptococcus pneumoniae* (16.6%) and *Haemophilus* species (17.1%). In this study, the percentage for pneumonia caused by *P. aeruginosa* was similar for community-acquired pneumonia and health care–associated pneumonia.[98]

Studies have identified numerous risk factors for *P. aeruginosa* community-acquired pneumonia, including chronic obstructive pulmonary disease, bronchiectasis, alcoholism, smoking, and frequent antibiotic therapy.[101,102] An immunocompromised state, due to malignancy, transplantation, or immunosuppressive drugs, is also frequently associated with community-acquired pneumonia.[102,103] Of note, many of these studies were performed before the inclusion of health care–associated pneumonia as a new category of pneumonia, and, therefore, many of these factors likely reflect an increased risk for health care–associated pneumonia and not true community-acquired pneumonia.

The ATS/IDSA guidelines for *P. aeruginosa* community-acquired pneumonia recommend an antipseudomonal β-lactam antimicrobial agent plus either ciprofloxacin, levofloxacin, or an aminoglycoside. An alternative recommended regimen is an aminoglycoside plus either ciprofloxacin or levofloxacin.[102] Tapering to monotherapy once antimicrobial susceptibilities are available should then be considered. Studies defining the optimal duration of antimicrobial therapy are lacking. The ATS/IDSA guidelines state that longer durations of 3 to 7 days are rarely necessary but may be warranted if there are pulmonary cavities or other evidence of tissue necrosis.[102]

Mortality rates associated with *P. aeruginosa* community-acquired pneumonia are higher than those for pneumonia caused by other pathogens. In one study specifically addressing *P. aeruginosa* community-acquired pneumonia, 28% of patients died, compared with 10% who died of community-acquired pneumonia caused by other pathogens.[101]

Cystic Fibrosis

CF and bronchiectasis are two pulmonary diseases that lead to chronic colonization with *P. aeruginosa* and recurrent pneumonia. CF is a genetic disease that affects multiple systems to varying degrees. Mutations in the CF transmembrane conductance regulator (*CFTR*) gene result in abnormal ion transport, leading to inefficient mucociliary clearance and an increased risk of bacterial infections.[104–106] *P. aeruginosa* is one of the main bacterial pathogens associated with CF.[107] Initial events of *P. aeruginosa* infection in CF patients can occur early during childhood. Subsequently, the infection dynamics usually follow a model of recurrent episodes of infections that eventually lead to the establishment of a chronic infection in which *P. aeruginosa* can be continuously cultured from respiratory secretions.[106] The transition from an intermittent to a chronic infection state may occur in up to 80% of adult CF patients, and is frequently associated with the emergence of *P. aeruginosa* isolates with a mucoid phenotype, characterized by overproduction of the polysaccharide alginate.[106,108] Environmental factors, such as oxidative stress and antimicrobial selective pressure, are major drivers of the changes in gene expression that lead to the mucoid-type *P. aeruginosa.* The chronic infection caused by mucoid *P. aeruginosa* isolates is a strong predictor of pulmonary function decline, and represents a significant clinical challenge because it is associated with biofilm formation and multidrug resistance.[109]

Bone and Joint Infections

Bone and joint infections can occur via contiguous spread, direct inoculation, or secondary seeding from bacteremia, and occur frequently among intravenous drug users. Although *P. aeruginosa* is an infrequent pathogen implicated in bone and joint infections, certain specific presentations are more often seen with this pathogen. These entities include sternoclavicular septic arthritis, septic arthritis of the symphysis pubis with osteomyelitis, vertebral osteomyelitis, skull base osteomyelitis, and osteomyelitis associated with nail punctures. *P. aeruginosa* is also implicated in a majority of combat-related osteomyelitis cases.

Osteomyelitis caused by *P. aeruginosa* has a poor prognosis when compared with that caused by other pathogens. A study of 454 patients with osteomyelitis characterized risk factors for poor outcomes. *P.*

aeruginosa was implicated in 4.4% of all cases. *P. aeruginosa* osteomyelitis was associated with a twofold higher risk for recurrence compared with osteomyelitis caused by *S. aureus,* and amputation was required among 15% of patients compared with 3% to 7% when other pathogens were implicated.[110]

Sternoclavicular Septic Arthritis

P. aeruginosa accounts for approximately 10% of all cases of sternoclavicular septic arthritis. Risk factors include intravenous drug use, diabetes mellitus, trauma, and infected central venous lines. Before 1981, intravenous drug use was the main risk factor for *P. aeruginosa* sternoclavicular septic arthritis and accounted for 82% of all cases in this patient population. During this period, pentazocine with tripelennamine was a popular combination of opiate abuse. The pills required crushing and dissolving with water before injection. This form of injection drug use favored contamination by *P. aeruginosa* owing to the use of nonsterile water for dissolving the pills as well as possible favored growth of *P. aeruginosa* in the tripelennamine component.[111] After 1981, pentazocine was combined with naloxone, a narcotic antagonist when injected parenterally. This reformulation ended the epidemic of pentazocine-tripelennamine abuse and is believed to explain the subsequent decrease in cases of *P. aeruginosa* sternoclavicular septic arthritis.[112] *P. aeruginosa* now accounts for only 14% of cases among intravenous drug users.[112]

Common presenting symptoms include chest and shoulder pain, with fever present among 65% of patients. Over two-thirds have concomitant bacteremia. Complications from sternoclavicular septic arthritis include osteomyelitis, abscess formation, and mediastinitis.

Septic Arthritis of the Symphysis Pubis

Approximately 24% of cases of septic arthritis of the symphysis pubis are caused by *P. aeruginosa.* The great majority of cases are seen among intravenous drug users. As with septic arthritis of the sternoclavicular joint, cases of arthritis of the symphysis pubis caused by *P. aeruginosa* have decreased dramatically after 1981. Other patient populations at high risk are patients who have undergone urologic, pelvic, or gynecologic procedures.[113] Common presenting signs and symptoms include pain on walking or hip motion, fever, and pubic or groin pain. Osteomyelitis is present in the great majority of patients.

Vertebral Osteomyelitis

P. aeruginosa is the most common pathogen implicated in vertebral osteomyelitis among intravenous drug users, accounting for over two-thirds of cases.[114] Patients present after 1 to 3 months of symptoms, which include neck or back pain, with fever present in fewer than half. Any part of the spine can be involved, but lumbar and cervical osteomyelitis are most common, being present among 53% and 27% of patients, respectively. *P. aeruginosa* vertebral osteomyelitis can also occur as a complication of epidural anesthesia.[115]

Skull Base Osteomyelitis

Skull base osteomyelitis occurs predominantly among immunocompromised patients, especially the elderly with diabetes mellitus. *P. aeruginosa* is the most common causative pathogen, implicated in 50% of all cases. Skull base osteomyelitis is a complication of otitis externa and media, as well as infections of the mastoid and sinuses. If due to ear infections, patients will present with severe otalgia and unilateral otorrhea. If there is extension of the local infection, lower cranial nerve palsies ensue, especially involvement of the facial nerve, which is present in 60% of patients. Severe cases can also present as bilateral skull base osteomyelitis and bilateral cranial nerve palsies.[116] Blood cultures are usually negative. Mortality rates approach 15%.[117,118]

Treatment should follow similar principles as for osteomyelitis at other body sites; however, a longer duration of therapy (12 weeks) has also been used.[117]

Osteomyelitis Related to Nail Puncture Wounds

Nail puncture wounds to the plantar surface of the foot caused by *P. aeruginosa* are a result of inoculation of this pathogen through contaminated sneakers (also called tennis shoes). Coinfection with *S. aureus* has been reported. Surgical débridement is usually necessary. Osteomyelitis occurs in 1% to 2% of patients and if present should be treated with ciprofloxacin (750 mg orally twice a day) or levofloxacin (750 mg orally once a day) for a minimum of 6 weeks. Alternative antimicrobial agents include ceftazidime (2 g IV every 8 hours) or cefepime (2 g IV every 12 hours).[119,120] Plain radiographs are necessary to exclude the presence of foreign bodies, and tetanus immunization should be updated.

Combat-Related Osteomyelitis

P. aeruginosa and *Acinetobacter* species are gram-negative bacteria that are frequently recovered from combat-related cases of osteomyelitis. The microbiology of causative pathogens, however, may differ depending on whether the osteomyelitis is a new or recurrent infection. A retrospective study of casualties occurring in Operation Iraqi Freedom and Operation Enduring Freedom identified 110 cases of osteomyelitis occurring from 2003 to 2006. Gram-negative bacteria, including *P. aeruginosa, Acinetobacter* species, and *K. pneumoniae,* were more likely to be recovered during the initial episode of infection as opposed to a recurrence, in which gram-positive bacteria, such as *S. aureus,* was more likely to be recovered.[121–123]

Antimicrobial Therapy for Arthritis and Osteomyelitis

No clinical trial has evaluated whether combination therapy versus monotherapy is the optimal treatment for *P. aeruginosa* arthritis or osteomyelitis. As with other types of infections, a reasonable approach is to begin with combination therapy if *P. aeruginosa* is suspected and there is a high rate of antimicrobial resistance. Streamlining therapy to a single agent to which the pathogen is susceptible, including an antipseudomonal β-lactam antimicrobial agent, ciprofloxacin, or levofloxacin, should then be considered. The IDSA 2013 guidelines for prosthetic joint infections caused by *P. aeruginosa* recommend treatment with cefepime (2 g IV every 12 hours) or meropenem (1 g IV every 8 hours). Alternative antimicrobial agents to consider include ciprofloxacin (750 mg orally twice a day or 400 mg IV every 12 hours) or ceftazidime (2 g IV every 8 hours) for 4 to 6 weeks. These guidelines state that use of aminoglycosides is optional but that use of two active drugs should be considered based on the clinical circumstances of the patient.[124] Until specific guidelines are available for osteomyelitis, it is likely that these recommendations for the treatment of prosthetic joint infections can be extended to the treatment of osteomyelitis.

Skin and Soft Tissue Infections

Pseudomonal infections of the skin are usually the result of excessive local moisture, including swimming, laundry work, or hiking in wet areas, and are often self-limited. More serious skin conditions, such as ecthyma gangrenosum, are usually seen among the immunocompromised population. *P. aeruginosa* has a major impact on burn wound victims, as discussed in Chapter 314.

Paronychia and Green Nail Syndrome

Paronychia presents as erythema, edema, and tenderness at the adjoining nail plate and occurs as a result of inflammation or infection of the nail folds after breaches in the skin integrity between the nail fold and nail plate. Foci of purulence can also develop. Acute paronychia is primarily caused by *Pseudomonas* species, as well as *Staphylococcus* species and *Streptococcus* species. Ongoing exposure to water, as well as repeated trauma, psoriasis, and eczema, leads to chronic paronychia. Green nail syndrome is an extension of chronic paronychia and presents as a classic triad of proximal chronic paronychia, distolateral onycholysis, and bluish-green discoloration of the nail plate. This discoloration is due to the pyocyanin and pyoverdin pigments produced by *Pseudomonas* species (Fig. 219.2). Treatment consists of cessation of water exposure and application of a 2% sodium hypochlorite solution on the nail bed or acetic acid soaks twice a day. Topical antimicrobial agents, including tobramycin otic or ophthalmic drops placed under the nail bed,[125] are also effective in conjunction with the recommendations about water exposure and pH management.



FIG. 219.2 Green nail syndrome caused by *Pseudomonas aeruginosa.* *(Modified from Wu DC, Chan WW, Metelitsa AI, et al.* Pseudomonas *skin infection: clinical features, epidemiology, and management.* Am J Clin Dermatol. *2011;12:157–169.)*



FIG. 219.3 *Pseudomonas aeruginosa* folliculitis of the trunk region. *(Modified from Wu DC, Chan WW, Metelitsa AI, et al.* Pseudomonas *skin infection: clinical features, epidemiology, and management.* Am J Clin Dermatol. *2011;12:157–169.)*

Interdigital Infections

Although foot intertrigo, or toe-web folliculitis, is usually caused by dermatophytes and yeast, superinfection by *Pseudomonas* species can occur. This inflammatory process involves the interdigital spaces and can extend to surrounding areas in severe cases. Lesions are usually erythematous, exudative, and malodorous, with marked maceration. Other gram-negative bacteria have been associated with this skin infection and include *E. coli, Proteus mirabilis,* and *Morganella morganii.*[126]

Hot Tub Folliculitis

Hot tub folliculitis is an infection of the infundibulum (upper segment) of the hair follicle, just beneath the skin surface. It is a recreational skin infection associated with immersion in contaminated swimming pools, hot tubs, and whirlpools. Contaminated bath toys, loofah sponges, creams, and diving suits have also been implicated. The folliculitis is characterized by the sudden onset of painful, pruritic pustules or papules within 24 to 48 hours after exposure to the contaminated water. Affected areas involve those exposed to the water and usually involve the trunk, upper arms, and buttocks. Folliculitis usually resolves spontaneously within 7 to 14 days without any therapy.[125]

The extent of the folliculitis depends on the level of contamination, duration of exposure, and preexisting skin conditions. Serious cases of folliculitis with both skin (hemorrhagic bullae) and systemic progression can occur among immunocompromised hosts and can be life-threatening infections (Fig. 219.3).

Hot Hand-Foot Syndrome

This syndrome is characterized by painful red nodular lesions on the soles of the feet or on the hands. It occurs as a result of abrasions incurred from pool floors in which there are high concentrations of *P. aeruginosa* in the water, and usually develops within 1 to 2 days after the abrasion. It is a benign disorder that is self-limited in previously healthy hosts, with recovery occurring within 7 to 10 days. Hot hand-foot syndrome is a public health hazard. Prevention requires superchlorination of pool water, reducing the abrasiveness of pool floors, and using quaternary ammonium compounds when scrubbing pool floors and water pipes. Ozone treatment is also required to remove biofilms on pool surfaces. Several outbreaks have been described, especially among children using wading pools (Fig. 219.4).[125,127]

Body Piercing

Infection is the most common complication after body piercing, occurring in up to 20% of cases. The most common implicated pathogens are *P. aeruginosa* and *S. aureus.* Transcartilaginous piercing is associated with more serious infections than piercing of the earlobe, owing to decreased vascularity. Complications include hematoma formation, cartilage

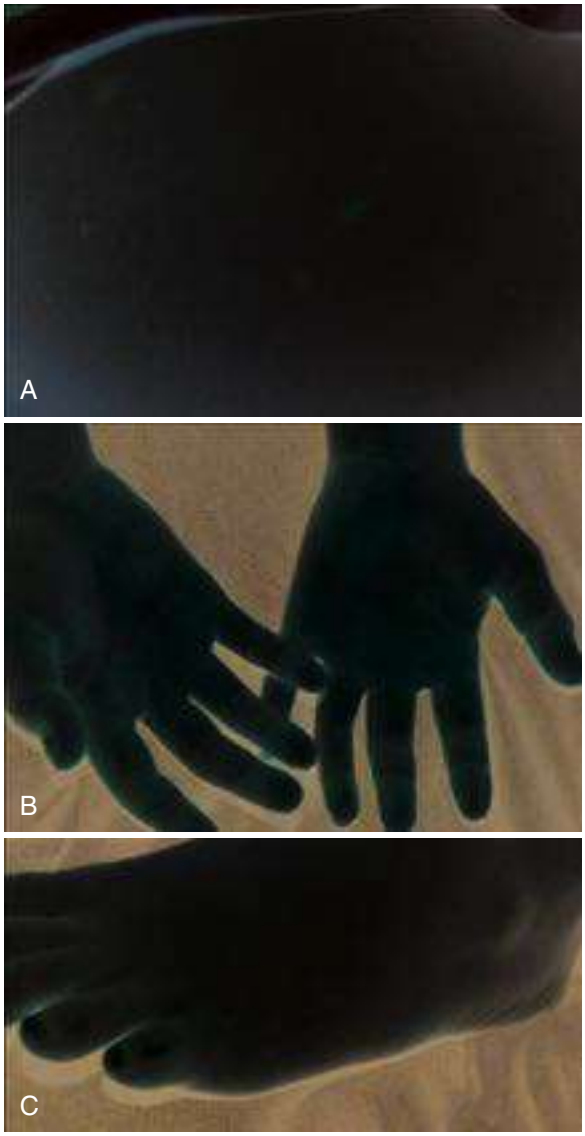

FIG. 219.4 Lesions developing after exposure to a hot tub/pool. (A) Erythematous pustules involving the abdomen. (B) Tender erythematous nodular lesions on the palms. (C) Erythema and swelling over the lateral surface of the toe. *(Modified from Yu Y, Cheng AS, Wang L, et al. Hot tub folliculitis or hot hand-foot syndrome caused by* Pseudomonas aeruginosa. J Am Acad Dermatol. *2007;57:596–600.)*



FIG. 219.5 Ecthyma gangrenosum in 67-year-old man with chronic lymphocytic leukemia. *(Modified from Walls AC, Frangos JE, Goralnick E. Ecthyma gangrenosum in a 67-year-old man with chronic lymphocytic leukemia.* J Emerg Med. *2012;43:339–341.)*

ischemia, perichondral auricular abscesses, and deformity, referred to as a "cauliflower ear." Several outbreaks of *P. aeruginosa* infections associated with body piercing have been reported. Sources included repeated use of a single-use disinfectant bottle, use of open-piercing guns, and contaminated aftercare solutions.[128,129]

Ecthyma Gangrenosum

Ecthyma gangrenosum is a characteristic manifestation of *P. aeruginosa* bacteremia among immunocompromised patients, especially those with neutropenia. Ecthyma gangrenosum, however, can also be caused by numerous other gram-negative bacteria, fungi, and viruses and has also been documented to occur among immunocompetent hosts, but with much lower incidence.[130,131]

The characteristic lesions are usually due to hematogenous dissemination, but primary skin lesions, without bacteremia, can also occur. Lesions begin as single or multiple red macules progressing to vesicles and later bullous or pustular lesions. Hemorrhage and necrosis in the central part ensues, followed by the development of a gray-black eschar with surrounding erythema (Fig. 219.5). All body parts can be involved, but ecthyma gangrenosum most commonly affects the perineal and gluteal regions and the extremities. On histology, there is epidermal and upper dermal necrosis with a mixed inflammatory cell infiltrate surrounding the infarcted area along with necrotizing vasculitis and vascular thrombosis. If bacteremia is present, *P. aeruginosa* is isolated from skin biopsy specimens as well as blood. Treatment of the bacteremia and local wound care are indicated.

Ear Infections

Simple Otitis Externa (Swimmer's Ear)

Otitis externa is an inflammation of the external auditory canal that is usually associated with infection. The most common implicated pathogens are *P. aeruginosa* and *S. aureus.* High-risk groups include swimmers, people living in humid environments, and those with narrow ear canals. Other risk factors include trauma, use of hearing devices, and presence of eczema. Patients present with tenderness and pruritus of the external canal, which are exacerbated by movement of the pinna. Erythema, swelling, and purulent discharge may also be visible. Topical antibacterial agents, such as tobramycin otic, ofloxacin, or a combination of ciprofloxacin and dexamethasone otic or aluminum acetate drops, are beneficial.[132]

Malignant Otitis Externa (Necrotizing Otitis Externa)

Malignant otitis externa is an aggressive, life-threatening infection originating in the cartilage of the external auditory canal and progressing to involve the soft tissues and bones of the skull base, resulting in temporal bone osteomyelitis. Palsies of cranial nerves VII to XII can occur with progression. The most common cranial nerve affected is the facial (VII) nerve when the infection extends to the temporal bone. As the infection progresses farther toward the jugular foramen and hypoglossal canal, lower cranial nerve palsies develop. Involvement of blood vessels can lead to septic thromboembolism.[133,134]

The majority of cases are caused by *P. aeruginosa,* followed by *S. aureus.* Other gram-negative bacteria and *Aspergillus* spp. have also been implicated. Elderly patients and those with diabetes mellitus are at highest risk, although malignant otitis externa has been described among patients with a variety of immunocompromised states as well as occasionally among immunocompetent hosts.

Severe prolonged otalgia and otorrhea are the most common presenting symptoms. Aural fullness and hearing loss may also occur. As the infection progresses, headaches, temporomandibular joint pain, trismus, and cranial nerve palsies ensue. Polypoidal granulation tissue arising from the external auditory canal can be present.

Both bacterial and fungal cultures should be obtained from the ear canal, and radiologic imaging should be performed. Antipseudomonal antimicrobial agents are required for at least 6 weeks. Initial therapy with intravenous antimicrobial agents should be considered in severe cases; otherwise, oral ciprofloxacin at high doses (750 mg twice a day) is sufficient, as long as the strain is susceptible. Aggressive débridement of necrotic tissue is also indicated. The use of hyperbaric oxygen therapy has been advocated by some, but its benefit has never been formally tested. If the patient has diabetes, then control of blood glucose concentration is crucial.[133,134]

In a retrospective study of 57 patients with malignant otitis externa treated at a tertiary care hospital from 1990 to 2008, 20% of patients had persistent or aggressive infection (>3 months) despite prolonged appropriate therapy. The risk factors for these patients were facial nerve paralysis, bilateral disease, and extent of disease (temporomandibular joint destruction, infratemporal fossa or nasopharyngeal soft tissue involvement). Outcomes were poor, with a 5-year survival of only 40% to 55%. Patients younger than 70 years had a significantly higher 5-year survival of 75%.[135]

Eye Infections

Keratitis

Keratitis is an inflammation or infection of the cornea usually caused by minor trauma. Contact lenses, especially the extended-wear type, are the main risk factor for *P. aeruginosa* keratitis, likely owing to contamination of lens solution or the use of tap water.[136,137] Patients present with a sensation of "scratchiness" during blinking, pain, and visual blurriness. There is usually excessive tearing and redness. Mild cases can be successfully treated with topical antipseudomonal antimicrobial agents, such as tobramycin ophthalmic (see Chapter 113).

Endophthalmitis

Endophthalmitis is a severe, rapidly progressive infection involving the aqueous and/or vitreous humors of the eye that often results in loss of vision. Patients present with severe eye pain, decreased visual acuity, and a hypopyon (a layering of white blood cells in the anterior chamber). Infections are predominately due to exogenous sources, including penetrating injuries to the eye, or occur as a complication of ocular surgery, especially cataract surgery. Endogenous endophthalmitis from bacteremia is rare, occurring in 2% to 6% of all cases, with other foci of infection usually present. Outcomes of endogenous endophthalmitis are very poor, with 32% of patients having "count fingers" vision, 44% becoming blind, and 25% requiring evisceration or enucleation.[138]

Numerous nosocomial outbreaks after cataract surgeries have been reported. Symptoms usually occur within 1 to 14 days postoperatively. Sources have included contaminated solutions and surgical instruments, breeches in infection control practices, and contamination from conjunctival and lid flora.[139–141]

Endophthalmitis is a medical emergency requiring intravitreal antipseudomonal antimicrobial agents. Vitrectomy is necessary for severe cases and those that do not respond to antimicrobial agents alone in the first 24 to 48 hours. The use of intravenous antipseudomonal antimicrobial agents has not been studied in clinical trials but is recommended by some experts in severe cases of endophthalmitis (see Chapter 114).[142]

Urinary Tract Infections

P. aeruginosa is among the major pathogens implicated in nosocomial urinary tract infections.[143] Mortality rates are high, with up to 17.7% and 33.9% of deaths at 30 and 90 days, respectively.[144] These high rates

likely reflect a host with numerous comorbidities, including chronic liver disease and diabetes mellitus.[144] Community-acquired urinary tract infections caused by *P. aeruginosa* are rare unless there are underlying urologic issues, including prostatitis, urinary tract obstruction, a history of urologic procedures or a neurogenic bladder, and prior treatment. Many of these risk factors suggest that the patients have had health care exposure and, therefore, these infections may not be community acquired but are likely health care associated.

In the NHSN surveillance study from 2011 to 2014, *P. aeruginosa* was the third most common pathogen implicated in catheter-associated urinary tract infections, after *E. coli* and *C. albicans,* accounting for 10.3% of all cases.[23] Surveillance studies from the Asia-Pacific region reported that *P. aeruginosa* was the third most common gram-negative pathogen, causing 7.1% of all nosocomial urinary tract infections in 2009 to 2010.[145] Studies focusing on nosocomial urinary tract infections occurring in the ICU report even higher rates of *P. aeruginosa.* In the national French nosocomial surveillance study, 16% of urinary tract infections were caused by *P. aeruginosa.*[146] Independent risk factors reported in that study were male gender, length of stay and antimicrobial exposure at ICU admission, and transfer from another ICU.[146] Patients in LTACHs also have extremely high rates of urinary tract infections caused by *P. aeruginosa.* In 2010, the NHSN reported rates of catheter-associated urinary tract infections from 104 LTACHs; *P. aeruginosa* was the most common pathogen and accounted for 19% of all of the reported infections.[25] These percentages were higher than those reported in ICU settings, which ranged from 9% to 12%.[25]

Antimicrobial resistance rates are very high among *P. aeruginosa* strains causing nosocomial urinary tract infections.[25,145,147,148] In the surveillance study of the Asia-Pacific region, approximately 40% of isolates were resistant to imipenem, ceftazidime, cefepime, and ciprofloxacin.[145] In the NHSN LTACHs study, 52% of *P. aeruginosa* isolates were resistant to ciprofloxacin, compared with 25% to 37% among ICU isolates. Rates of multidrug resistance were also substantially higher among LTACH isolates compared with ICU isolates (25% vs. 12%–16%, respectively).[25] In countries other than the United States, rates of resistance among ICU isolates are even higher. A large surveillance study of ICU isolates from 422 ICUs from 36 countries throughout Africa, Asia, Latin America, and Europe reported that 51% of strains were resistant to ciprofloxacin, 42% were resistant to piperacillin or piperacillin-tazobactam, 50% were resistant to cefepime, and 37% were resistant to carbapenems.[148] The FDA has approved a novel agent, ceftolozane-tazobactam, for the treatment of complicated urinary tract infections. This antimicrobial has excellent intrinsic activity for *P. aeruginosa,* which remains susceptible (>80 of isolates) to those strains resistant to ceftazidime or meropenem.[40]

Several outbreaks of urinary tract infections caused by *P. aeruginosa* have occurred in health care settings. One outbreak was traced to a transducer used for urodynamic studies that was contaminated with an MDR *P. aeruginosa* strain susceptible only to colistin.[149] A pseudo-outbreak was also reported from contamination of an automated urine analyzer.[150]

Treatment includes removal of the urinary catheter and correction of underlying urologic problems, if present. Avoiding catheter insertion is also the predominant approach to preventing catheter-associated urinary tract infection. Oral ciprofloxacin (500 mg twice a day) for 3 to 5 days for an uncomplicated urinary tract infection is usually sufficient. Prolonging therapy to 2 to 3 weeks is indicated for complications, including urosepsis and pyelonephritis. It is important to note that the presence of pyuria without urinary tract symptoms does not warrant therapy unless the patient is pregnant or is undergoing a transurethral prostate resection or other urologic procedure for which mucosal bleeding is expected.[151]

PSEUDOMONAS SPECIES OTHER THAN *P. AERUGINOSA* OF MAJOR CLINICAL SIGNIFICANCE

The genus *Pseudomonas* includes more than 140 species, which inhabit a variety of niches in soil and water. Genotypic-based analysis has facilitated the identification and phylogenetic assignment of *Pseudomonas* spp.[152] A subset of species other than *P. aeruginosa* cause human infection, usually among immunocompromised hosts, and are discussed in this section. Treatment of these infections should target the site of infection. The great majority of strains are susceptible to third-generation cephalosporins, piperacillin, carbapenems, and ciprofloxacin. However, antimicrobial susceptibility patterns should always guide antimicrobial therapy.

Pseudomonas fluorescens

P. fluorescens belongs to the *fluorescens* group of *Pseudomonas* species, which also includes *P. aeruginosa* and *P. putida.* This pathogen has been implicated in catheter-associated BSI. Because it can grow at 4°C and can therefore survive in blood products, transfusion-related outbreaks due to *P. fluorescens* have occurred. Outbreaks due to contaminated heparin flush solutions, drinking water dispensers in a bone marrow transplant unit, and ice baths used for cardiac output determinations have also been reported. Susceptibility to third-generation cephalosporins, piperacillin, and ciprofloxacin has been reported, but antimicrobial susceptibility profiles are always warranted to guide therapy.[153,154]

Pseudomonas fulva

P. fulva has been recovered from rice seed samples from the Philippines, from rice and petroleum fields and oil brine from Japan, and from the gills of mollusks. *P. fulva* is antagonistic to many bacterial and fungal pathogens of rice, and therefore rice seed can be used as a biologic control agent. *P. fulva* is also used in plant pathogenic fungi elimination in tomato cultures. A VIM-1–producing strain causing meningitis in a 2-year-old girl after placement of a drainage system for a neuroectodermal tumor has been reported.[155] A second case causing human infection was reported in a 56-year-old man with *P. fulva* bacteremia who was hospitalized after trauma incurred at a construction site.[156]

Pseudomonas luteola

P. luteola is another uncommon pseudomonal opportunistic pathogen. It has been previously referred to as *Chryseomonas luteola* and CDC group Ve-1. *P. luteola* is found in water, soil, and damp environments. A survey of 242 cooked ready-to-eat foods from popular roadside cafeterias and retail outlets in South Africa recovered *P. luteola* from 2.4% of food samples (vegetables, rice, beef, and pies). These food areas had no running water, and utensils and hands were washed in one bucket without regular changing of the water.[157] Reported human infections include prosthetic valve endocarditis, bacteremia, peritonitis, meningitis, and osteomyelitis.[158–160] Chronic infection of the index finger after trauma and a cutaneous abscess with bacteremia in a previously healthy man have also been reported.[161,162] Infections can be associated with central venous and peritoneal dialysis catheters.

Pseudomonas mendocina

P. mendocina rarely causes human infection. It was first isolated from soil and water samples from Mendoza, Argentina. Endocarditis, bacteremia, and spondylodiskitis have been reported.[157,158] *P. mendocina* has also been recovered from leg ulcers and urine specimens. Contamination of a reagent used for stem cell assays was responsible for a pseudo-outbreak.[163]

Pseudomonas mosselii

P. mosselii was described as a new species in 2002. Previous strains may have been identified as *P. fluorescens.*[164] Very few cases of *P. mosselii* human infection have been reported. One report concerned a 70-year-old woman with prosthetic valve endocarditis who received intravenous antimicrobial agents but was not a surgical candidate for valve replacement. The patient died of a cardiac arrest several months later, the cause of which was not reported. If *P. mosselii* was misidentified as *P. fluorescens* before recognizing it as a new species, more human infections may have occurred. VIM-producing isolates have been described.[165]

Pseudomonas oryzihabitans

This *Pseudomonas* species was previously referred to as CDC Group Ve-2 or as *Flavimonas oryzihabitans.* It is also an opportunistic pathogen, causing a variety of infections usually associated with foreign devices. Postoperative bacteremias after intraabdominal surgery, coronary artery bypass, and craniotomy have also been reported.[166–168]

P. oryzihabitans causing a cutaneous ulcer after an octopus bite was reported from Germany. A 9-year-old boy was bitten by *Octopus vulgaris* while snorkeling. Two days after the bite, a 2- to 3-cm black ulcerative lesion with a surrounding erythematous area developed. Owing to lack of healing with topical agents over the ensuing months, the lesion was excised, resulting in complete healing without the need for intravenous antimicrobial agents.[169]

Pseudomonas putida

P. putida is an opportunistic pathogen and a member of the *fluorescens* group of *Pseudomonas* species. Catheter-related bacteremia is one of the most common infections. Other infections include cholangitis associated with biliary drainage tubes, cholecystitis, pneumonia, urinary tract infections, and war wounds.[170,171] Infections predominantly occur in immunocompromised hosts, although case reports among healthy individuals have been published.[172] The majority of infections are cured with appropriate antimicrobial therapy and removal of invasive devices. Mortality rates range from 8% to 40%.[170,173]

Outbreaks of *P. putida* due to contaminated fluids or blood have been reported. Pseudo-outbreaks have also occurred as a result of contaminated urine collection kits and an automated spiral platter used to process respiratory specimens.[174]

In recent years, antimicrobial resistance rates have increased among *P. putida* isolates. In one study of nosocomial isolates recovered from 2005 to 2011, up to 20% of isolates were carbapenem resistant.[173] The majority of metallo-β-lactamase–producing *P. putida* cases reported in the literature are due to VIM and IMP enzymes.[173,175–177] KPC-2–producing *P. putida,* resistant to all antimicrobial agents except polymyxin B, has also been reported in a child with bacteremia.[178]

Pseudomonas stutzeri

P. stutzeri is an uncommon opportunistic pathogen found in the environment. Approximately 1% to 3% of *Pseudomonas* species recovered from hospital isolates are identified as *P. stutzeri.* Human infection caused by this *Pseudomonas* species was first reported in 1973 in a person with a tibial infection. Since then, numerous case reports have appeared in the literature documenting a variety of infections that affect most body sites. These infections include osteomyelitis, arthritis, bacteremia, endocarditis, endophthalmitis, pneumonia, empyema, urinary tract infections, and meningitis.[179,180] The majority of patients had underlying disorders, including chronic liver failure, chronic renal disease, or immunosuppression. Infections in otherwise healthy patients have also been reported (i.e., brain abscesses, pneumonia, empyema, and vertebral osteomyelitis). Prior surgery is also a risk factor. Mortality is rare given the low virulence of *P. stutzeri.*

Antimicrobial resistance is not as frequent among *P. stutzeri* isolates compared with *P. aeruginosa* isolates. Many *P. stutzeri* strains are susceptible to quinolones; extended-spectrum penicillins, with and without β-lactamase inhibitors; and carbapenems. However, among those *P. stutzeri* isolates recovered from immunocompromised hosts, resistance rates are higher, likely owing to prior antimicrobial and hospital exposure.[180] A recent case report of a metallo-β-lactamase–producing *P. stutzeri* was reported from a 7-year-old girl from Brazil. This patient had a brain tumor requiring an external ventricular drain. Postoperatively, she developed meningitis, and *P. stutzeri* was recovered from the cerebrospinal fluid. The isolate was resistant to all tested β-lactam antimicrobial agents and only susceptible to amikacin, gentamicin, and polymyxin B. Metallo-β-lactamase production and the class 1 integron carrying $bla_{\text{IMP-16}}$ were later identified.[177]

NOVEL THERAPEUTIC STRATEGIES AGAINST *P. AERUGINOSA* INFECTIONS

The rapid emergence and spread of multidrug resistance among *P. aeruginosa* isolates have led to an intense exploration toward developing therapeutic strategies that do not involve antimicrobial agents.[20,181,182] Numerous innovative approaches have and are being investigated for the treatment or prevention of *P. aeruginosa* infection (Table 219.4). These strategies target key processes involved in colonization, quorum sensing, and related biofilm formation. For example, several strategies, including using natural sources from plant-derived compounds, are being investigated for their antibiofilm properties (Table 219.5).[183] Unfortunately, although many new strategies show in vitro or in vivo promise, developing an effective novel strategy that can be used in humans is unlikely to occur in the near future.

TABLE 219.4 Potential Targets for the Prevention and Management of *Pseudomonas aeruginosa* Colonization and Infection

TARGET	TARGETED EFFECT ON *P. AERUGINOSA*
Biofilms	Decreased resistance to immune defenses and efficacy of antimicrobial agents
Quorum-sensing system	Interference with cell-to-cell communication
Type III secretion system	Prevention of cytotoxin release
Iron chelation	Inhibition of siderophore (pyoverdin) production leading to inhibition of virulence factors and extent of niche occupancy
Bacteriocins (pyocins)	Possible effect on niche establishment
Phage therapy	Infection of *P. aeruginosa* bacteria causing lysis
Immunotherapy	Eradication and prevention of infection
Immunization	Prevention of infection

Data from Fothergill et al.,[20] Carpenter et al.,[171] and Toru et al.[172]

TABLE 219.5 Therapeutic Strategies Against the Quorum-Sensing (QS) System

AGENT	COMMENTS
Macrolides and aminoglycosides	Inhibit QS-regulated factors; inhibit alginate production and biofilm formation Several azithromycin trials in cystic fibrosis patients showed beneficial effects for lung function
Plant-derived products	Inhibit biofilm formation
S-adenosylhomocysteine	Blocks the production of signaling molecules to prevent accumulation of signal, leading to inhibition of virulence genes activation
QS vaccine	Specific antibody to 3-oxo-C12-HSL, which plays a protective role in acute *P. aeruginosa* infection
Degrading enzymes	Degrade QS signals by attacking the lactone ring or the side chains, rendering them ineffective
Synthetic analogues (e.g., furanones)	Inhibit QS-regulated factors and biofilm formation
Plant extracts	Inhibit LasA protease, LasB elastase, and biofilm formation by several different plant extracts
Fungal products (e.g., patulin)	Thought to bind the RhlR protein and inhibit QS

Modified from Fothergill JL, Winstanely C, James CE. Novel therapeutic strategies to counter Pseudomonas aeruginosa *infections.* Expert Rev Anti Infect Ther. *2012:10;219–235.*

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220 *Stenotrophomonas maltophilia* and *Burkholderia cepacia* Complex

David Greenberg

SHORT VIEW SUMMARY

Definition
- *Stenotrophomonas maltophilia* and species of the *Burkholderia cepacia* complex (BCC) are opportunistic gram-negative pathogens that cause a variety of clinical infections in various immunocompromised hosts including individuals with host genetic defects and individuals with weakened immune systems.

Epidemiology
- *S. maltophilia* has had increasing rates of isolation worldwide and has caused nosocomial infections associated with medical equipment and solutions. It also is known to cause invasive infections in patients with cancer and has been increasing in frequency in patients with cystic fibrosis (CF).
- BCC species have caused nosocomial outbreaks associated with contaminated solutions and can cause significant morbidity and mortality in patients with CF and chronic granulomatous disease. *Burkholderia cenocepacia* and *Burkholderia multivorans* are two of the major species that are found to cause disease in patients with CF. Patient-to-patient transmission has been demonstrated in patients with CF.

Microbiology
- *S. maltophilia* and BCC spp. are gram-negative aerobic bacteria that are motile.
- As free-living organisms, they reside in a broad range of environments including aquatic environments.
- They are catalase-positive, they do not ferment glucose, and many BCC members are oxidase-positive.
- BCC spp. comprise a number of phenotypically similar but genotypically distinct species; currently there are at least 20 species in the BCC.
- *S. maltophilia* is found worldwide and has significant genetic diversity among isolates.

Diagnosis
- Accurate diagnosis is critical, as these pathogens have high levels of intrinsic antibiotic resistance. Newer methods have been used for successful identification of both BCC members and *S. maltophilia* including 16S ribosomal RNA sequencing and matrix-assisted desorption/ionization time-of-flight mass spectrometry.

Therapy
- Given that both pathogens have high levels of intrinsic resistance to antibiotics, choice of antibiotics should be driven by antibiotic susceptibility testing.
- Trimethoprim-sulfamethoxazole is the preferred antibiotic for both *S. maltophilia* and BCC, although resistance has been reported and is increasing in certain areas. Combination therapy is frequently used.
- Fluoroquinolones such as moxifloxacin show activity in *S. maltophilia,* but resistance has been reported during single-drug therapy.
- Other drugs that have been shown to have in vitro and in vivo activity in both pathogens include minocycline, ceftazidime, and newer agents such as ceftazidime-avibactam. Carbapenems have activity in BCC, and ticarcillin-clavulanate and ampicillin-sulbactam can retain activity in *S. maltophilia.*

Prevention
- Nosocomial outbreaks require strict infection control measures including isolation measures.
- Surveillance of hospital water supplies is important for preventing iatrogenic infections due to these pathogens.

Stenotrophomonas maltophilia and the *Burkholderia cepacia* complex (BCC) cause infections in a variety of vulnerable patient populations including hospitalized patients such as patients with cancer and patients with specific genetic diseases such as cystic fibrosis (CF) and chronic granulomatous disease (CGD). The high level of inherent and acquired resistance to antibiotics makes treating these infections particularly challenging.

MICROBIOLOGY

Both *S. maltophilia* and BCC species are aerobic gram-negative bacteria. These free-living organisms are present in a variety of aquatic and humid environments.[1] The presence of *S. maltophilia* has been detected in hospital drinking water.[2] These organisms do not ferment glucose and can grow on a variety of enriched bacterial media. Many BCC species are oxidase-positive, whereas *S. maltophilia* is not. Small colony variants can be found on specialized media that may not be appreciated on routine culture.[3–5] These organisms are catalase-positive and motile.[6] Historically, these bacteria were thought to be various *Pseudomonas* spp., and there have been a number of name changes over the years, further separating these out as distinct genera.

Burkholderia cepacia Complex Species

The BCC comprises a number of phenotypically similar but genotypically distinct species. The number of validly determined species has been increasing. There are currently at least 20 different species that are within the BCC.[7,8] Given the phenotypic similarity of many of these species, multiple phenotypic and genetic approaches have been used for identification and typing purposes including sequencing of 16S ribosomal RNA and *recA* genes, multilocus sequence typing, average nucleotide identity, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and biochemical tests. Despite the ever-increasing number of BCC spp., a limited number cause most human disease. An analysis of 151 isolates from an Italian CF center revealed that greater than 70% of isolates were due to three species: *Burkholderia cenocepacia* (38%), *Burkholderia stabilis* (19%), and *Burkholderia mulitvorans* (14.2%).[9] A large study from the United Kingdom that looked at >1000 *Burkholderia* isolates from both patients with CF and patients without CF showed that 56% of patients had *B. multivorans* and 15% had *B. cenocepacia* IIIA.[10]

Stenotrophomonas Genotypes

S. maltophilia is found worldwide and has been isolated from numerous environmental sources.[6] Given this, there is a large amount of genetic diversity among strains. One study illustrated the genetic diversity of clinical isolates; 80 typeable strains from 18 geographically distinct hospitals showed no predominant sequence type, and genogroup 6 represented about 40% of isolates.[11] A study in a pediatric hospital in Serbia also demonstrated high genetic diversity among both CF and non-CF isolates.[12]

PATHOGENESIS

S. maltophilia and BCC are capable of attaching and invading a number of host cells. Both pathogens have the ability to trigger an inflammatory

response. They also have a number of virulence factors and have the ability to evade host defenses. Given their ability to survive in a variety of environments, these pathogens have a number of factors that allow them to exist in various host tissues or on various abiotic surfaces.

Lipopolysaccharides, Adhesion, and Invasion

Both BCC species and *S. maltophilia* produce lipopolysaccharides (LPSs) that are capable of stimulating cytokine-driven inflammatory responses and allow for resistance to complement-mediated bacterial clearance. *B. cenocepacia* LPSs can activate immune cells through Toll-like receptor 4–mediated signaling.[13] Blood mononuclear cells that have been activated by BCC LPSs release various cytokines including tumor necrosis factor-α, interleukin-6, and interleukin-8.[14] In *S. maltophilia*, lipid A has been shown to stimulate tumor necrosis factor-α in both blood mononuclear cells and alveolar macrophages leading to airway inflammation.[15] In addition, interleukin-8 stimulates recruitment of polymorphonuclear neutrophils to sites of infection, further driving a robust inflammatory response. Divergent Toll-like receptor/MD2 signaling has been shown to occur due to changes in the inner core of BCC LPSs.[16]

B. cepacia has been shown to be able to bind to various epithelial cell receptors such as cytokeratin 13 (CK13) through an adhesin that is expressed on cable pili that helps to anchor bacteria.[17,18] Various epidemiologic studies have provided evidence for the importance of cable pili in establishing colonization as well as allowing for patient-to-patient transmissibility in the CF lung milieu.[19,20] In one study, chronic inflammation enhanced CK13 expression in patients with CF, and in regions that had high CK13 expression this corresponded with an enhanced *B. cenocepacia* burden.[21] BCC species not only can invade respiratory epithelial cells but also have been demonstrated to be able to survive and multiply within epithelial cells.[22–24]

Siderophores and Secreted Enzymes

BCC species have a number of mechanisms for scavenging iron. BCC species possess at least four iron-binding siderophores—pyochelin, ornibactin, cepaciachelin, and cepabactin—for iron chelation and uptake.[25,26] Likewise, *S. maltophilia* uses catechol-type iron-binding siderophores,[27] and these siderophores can be both positively and negatively regulated.[28,29]

S. maltophilia produces a variety of extracellular enzymes including alkaline serine proteases (at least one encoded by *StmPr1*), DNAse, RNase, gelatinase, and lipases that degrade tissue fat.[30–32] Many of these proteases are resistant to inhibitors such as α_1-antitrypsin and α_2-macroglobulin.[32] These exoenzymes allow for tissue necrosis and hemorrhage.[32] Various BCC species produce proteases, lipases, and nonhemolytic phospholipase C.[33,34]

Chronic Infection and Intracellular Survival

BCC species can invade and survive in a number of host cells. Once internalized into respiratory epithelial cells, growth can occur, and microcolonies can be seen (Fig. 220.1).[22] Intracellular survival helps facilitate immune evasion. Although autophagosomes appear to form, BCC species appear to block the full autophagocytic pathway in both epithelial cells and macrophages and use the endoplasmic reticulum for multiplication.[22,35,36] A number of mechanisms are thought to play a role in intracellular survival including blocking enzyme production, degradation of enzymes, and melanin pigment–associated superoxide quenching.[37–41] The BCC type III secretion system assists in delaying the maturation of bacteria-containing phagosomes. The failure of *B. cenocepacia* to fuse with lysosomes allows for prolonged survival in patients with CF.[42,43] Although less is known about the intracellular life cycle of *S. maltophilia,* there are data to support that some strains can survive in human monocyte-derived dendritic cells.[44]

Biofilm and Quorum Sensing

Biofilms normally contain aggregates of bacteria in extrapolymeric substances and can form on numerous foreign objects, on avascular necrotic tissue, and in certain host environments such as the lung affected by CF. Quorum sensing systems and the ability to form biofilm are

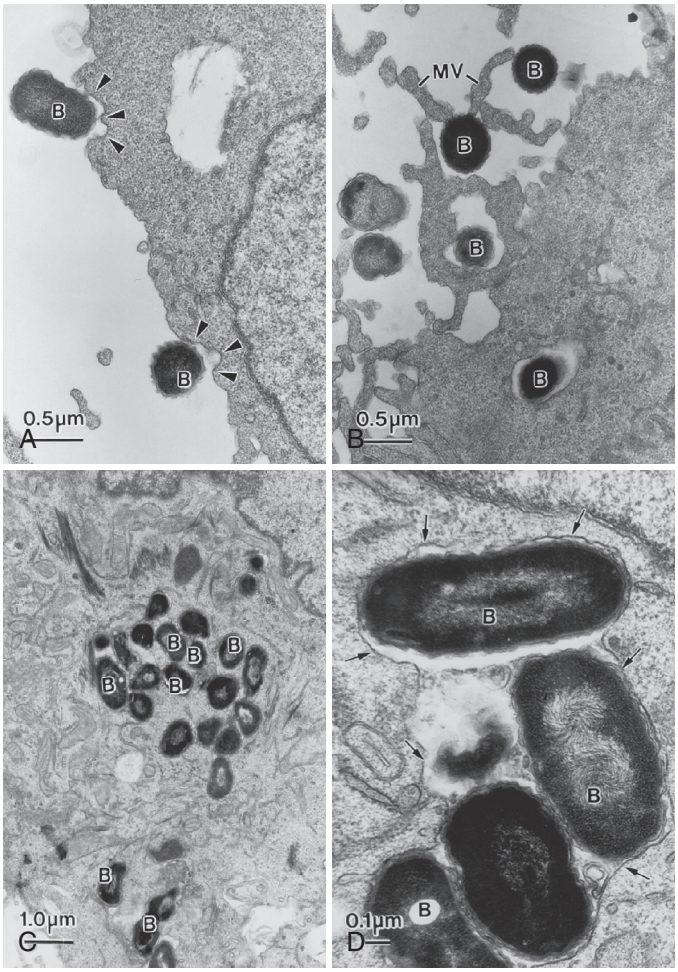

FIG. 220.1 Pathogenesis of *Burkholderia cenocepacia.* Electron micrographs showing invasion of an A549 respiratory epithelial cell monolayer by *B. cepacia* 249-2. All four micrographs show the same epithelial cell monolayer following a 2-hour incubation with the bacteria. (A) Initial contact of bacterial cells *(B)* with the surface of A549 cells. Thickening is seen at the site of contact *(arrowheads)*. (B) Apparent endocytosis of bacteria in contact with microvilli *(MV)* at the cell surface. A single bacterium is present within each membrane-bound vacuole. (C) Lower-power view of bacteria present within the cell cytoplasm. Although apparently ingested singly, organisms appear in clumps within the cytoplasm. (D) Higher-power view of bacteria within the cytoplasm. The organisms are enclosed within membrane-bound vacuoles *(arrows)*. *(Courtesy Dr. A. Griffith. From Burns JL, Jonas M, Chi EY, Clark DK, Berger A, Griffith A. Invasion of respiratory epithelial cells by* Burkholderia (Pseudomonas) cepacia. Infect Immun. *1996;64:4054–4059.)*

traits of both BCC species and *S. maltophilia*.[28,45–47,48,49] The presence of biofilm allows for the ability to withstand both immune cell activity and antibiotic efficacy. In *S. maltophilia,* regulation of biofilm occurs through the production of diffusible signaling factor, and diffusible signaling factor mutants show a number of phenotypes including loss of motility, increased susceptibility to antibiotics, and reduced production of proteases.[50,51] *B. cenocepacia* has been shown to use *N*-acyl homoserine lactones and *cis*-2-dodecenoic acid to regulate biofilm.[52]

EPIDEMIOLOGY AND AT-RISK POPULATIONS

Stenotrophomonas maltophilia

The rate of *S. maltophilia* isolation and infections has increased in multiple regions of the world. There is a broad environmental range where *S. maltophilia* has been isolated, including from animals.[53] It is not clear whether increased numbers of infections in vulnerable patient populations are due to enhanced lifesaving medical advances or an overall increase in incidence. However, there has also been an increase in the isolation

of this pathogen from various medical devices and solutions. One study of gastrointestinal endoscopes found *S. maltophilia* among a group of potential pathogens isolated after routine cleaning of the instruments.[54,55] Outbreaks have been described from other equipment sources including from contaminated bronchoscopy suction valves.[56] Nosocomial infections have been described through contamination of treated hospital water supplies.[57,58]

Health care–associated infections due to *S. maltophilia* have been described in both cancer and noncancer patient populations. Infections at two different hospitals in patients with cancer illustrate this point. At a comprehensive cancer hospital, *S. maltophilia* increased among gram-negative bacterial infections during the period 1986–2002, and at the same hospital there was an increase in moderate-to-severe *S. maltophilia* bacteremias.[59,60] A cluster of bacteremia cases among stem cell transplant patients at a large German hospital illustrated that environmental contamination leading to documented patient transmission was rare, as most patients had prior colonization.[61] Studies from various regions of the world support the increasing prevalence of infections. For the years 2000–06, there was a 93% increase in the annual number of bloodstream isolates in the United Kingdom, and for 1999–2004, there was an 83% increase in a large tertiary hospital in Taiwan.[62,63] *S. maltophilia* has also been increasing in frequency in respiratory specimens from patients with CF, although there is variability of rates based on the specific CF center.[64] In one study, having *S. maltophilia* correlated with more severe lung disease in patients with CF.[65] It remains unclear what the impact of *S. maltophilia* colonization is in both the pretransplant setting and the posttransplant setting. There was no difference in lung function decline in CF patients in the 3 years prior to acquiring *S. maltophilia* and up to 2 years after.[66–68] Although *S. maltophilia* is frequently considered a nosocomial pathogen, the high genetic diversity that has been demonstrated suggests that host colonization is likely a frequent source of the infection.[64]

Factors that have been associated with a high risk of infection include critical illness associated with pulmonary disease, prolonged use of broad-spectrum antibiotics, prolonged assisted ventilation, and prior respiratory tract colonization with *S. maltophilia*.[59,64,69–72] In immunocompromised individuals, such as patients with cancer and transplant recipients, risk factors include prolonged neutropenia, recent or current use of broad-spectrum antibiotics (including carbapenems, third- and fourth-generation cephalosporins, and fluoroquinolones), indwelling medical devices, prolonged hospitalization, hyperalimentation, and the presence of mucositis.[59,64,73,74]

Burkholderia cepacia Complex

BCC species are found widely in the environment, although a limited number of species contribute to the bulk of human disease. Nosocomial outbreaks have involved various contaminated solutions including nebulizers, bronchodilator vials, water, and chlorhexidine-cetrimide solutions.[75–77] However, immunocompromised individuals, such as patients with CF or CGD, are particularly vulnerable to infection. Although not the most frequent pathogen isolated, approximately 3.5% of patients with CF become colonized with BCC by 18 years of age. Not all BCC species cause disease to equal degrees in patients with CF, with some studies showing greater than 90% of isolated strains belonging to *B. cenocepacia* and *B. multivorans*.[78,79,80–83] Epidemiologic studies have demonstrated patient-to-patient transmission of BCC strains.[84,85] Patients with CF can become colonized with BCC and then retain that strain for long periods of time. This does not seem to be the case in patients with CGD, as such patients have been found to have recurrent infections with different strains over time.[86]

CLINICAL MANIFESTATIONS

Stenotrophomonas maltophilia

Bacteremia and Endovascular Infections

Most bloodstream infections seem to be linked to the presence of infected indwelling vascular catheters.[59,74] In transplant patients, catheter-related bacteremia has been described during the nonneutropenic period and often has been associated with catheter manipulations.[59,74,87] In addition, in severely immunocompromised patients, polymicrobial infections have been seen in up to half of cases.[74,87,88] In most cases, removal of the catheter along with a course of antibiotics has resulted in a cure. However, relapses have been described, and the prevention of relapse is related to prompt removal of the catheter (within 72 hours of the diagnosis).[89–91]

Bloodstream infections not associated with catheters can occur and lead to significant morbidity and mortality.[74,87,92,93] Patients who become bacteremic frequently have profound neutropenia that has lasted for greater than 10 days, and hematogenous dissemination usually arises from initial lung or soft tissue infections.[87,94–97] Additional risk factors for bacteremia in a neutropenic patient include hospitalization lasting >3 weeks, antineoplastic therapy, and treatment with broad-spectrum antipseudomonal antibiotics.[94,95] Poor outcomes in patients with cancer and *S. maltophilia* bacteremia include neutropenia, pneumonia, shock, severe thrombocytopenia, and initial discordant therapy.[94–96] Sepsis syndrome due to *S. maltophilia* has also been described in patients who have had mucositis that was prolonged (>3 weeks) or gingival disease.[98,99] Severe oral pain that is unresponsive to narcotics and extensive oral ulceration or bleeding or both should warrant consideration of empirical therapy to cover the possibility of *S. maltophilia*.

Although rare, endocarditis due to *S. maltophilia* has been described, and many affected patients have had septic emboli as a complication.[100–103] Other cardiac-related infections include pacemaker infections that occur late after the time of implantation.[104,105] Prompt removal of the device or prosthetic valve is an important determinant of treatment success; however, mortality remains high (approximately 40%).[100–103] A persistent endovascular focus of infection should be considered in a severely immunosuppressed patient with fever and persistent or recurrent *S. maltophilia* bacteremia.

Pulmonary Infections

The lung is a frequent site of infection, and colonization of the respiratory tract often occurs before overt clinical infection.[106–108] In an immunosuppressed patient, overt clinical signs of active disease may be diminished, and given the tendency of uncontrolled tissue necrosis and bleeding, pulmonary hemorrhage may be a presenting sign of *S. maltophilia* pulmonary disease.[106,109,110] Lobar consolidation and cavitary lesions can be seen on imaging; however, these features can be difficult to distinguish from other pathogens that cause disease in the immunocompromised host. Mortality due to *S. maltophilia* results from disease progression or hemorrhage.[108,109,110,111]

Community-acquired pneumonia has also been described in patients who do not have the traditional risk factors for infection.[112] Certain risk factors such as hematologic malignancy, simultaneous *S. maltophilia* bacteremia, carbapenem exposure, and need for critical care unit stay are less common in these patients compared with patients who acquired *S. maltophilia* from a nosocomial source.[112] Almost one-third of patients with community-acquired *S. maltophilia* pulmonary infections can be treated with oral antibiotics. Being admitted to a critical care unit is considered predictive of a poor outcome.[112] *S. maltophilia* as a pulmonary pathogen in CF is discussed in "*Stenotrophomonas maltophilia*" under "Epidemiology and At-Risk Populations."

Skin and Soft Tissue Infections

Cutaneous infections have been reported including in patients with burn injury who had exposure to nonsterile water.[113] Hematogenous seeding of the skin has been reported in neutropenic patients but is rare.[114] These lesions can manifest as nodular lesions with a violaceous center that ultimately undergoes necrosis and resemble ecthyma gangrenosum. Tissue necrosis can also occur in neutropenic patients at sites of indwelling catheters (Fig. 220.2).[115] Given that these patients are usually immunocompromised, other disseminated infectious causes of similar skin lesions include *Pseudomonas, Fusarium, Candida,* and *Mycobacterium* spp.[116] In individuals who are not immunocompromised, *S. maltophilia* skin infections are usually reported as due to traumatic wounds.[116] Skin biopsy plays an important role in diagnosing skin lesions due to this pathogen.

Head, Neck, and Central Nervous System Infections

Bacterial meningitis due to gram-negative pathogens is a known complication of neurosurgical procedures, and *S. maltophilia* is included in this



FIG. 220.2 ***Stenotrophomonas maltophilia*** **skin infection.** (A) Skin lesion in profoundly neutropenic Vietnamese girl with treatment-refractory aplastic anemia who had undergone related haploidentical stem cell transplantation following cytoxan and fludarabine pretransplant conditioning. (B) Gram-negative bacteria lining the tissue blood vessels (Brown and Hoppes stain of skin biopsy from the right leg lesion, ×1000). *(Courtesy Dr. Fedorko and Dr. John Bennett.)*

list. In addition to neurosurgical procedures, chronic sinusitis and intracranial hemorrhage are also risk factors.[117–119] In patients with infection after a neurosurgical procedure that does not respond to broad-spectrum antibiotics, in addition to other multidrug-resistant pathogens, *S. maltophilia* should be considered as a possible cause.[120] *S. maltophilia* rarely is a cause of chronic sinusitis.[121,122] *S. maltophilia* can cause both corneal infection and endophthalmitis after penetrating eye injury. In addition, it has been found not infrequently in contact lens solutions.[119,123,124]

Genitourinary, Abdominal, and Other Uncommon Infections

Both urinary tract colonization and invasive disease have been described.[125] Peritonitis is also possible and has been seen in patients undergoing peritoneal dialysis in cases where removal of the dialysis catheter is needed when there is no response to antimicrobial therapy.[126–129] Other gastrointestinal infections such as biliary sepsis, necrotizing pancreatitis, and liver abscess have been rarely reported.[130–132] Bone and joint infections are rare but have been described as consequences of trauma, after spinal surgery, and in nonimmunosuppressed patients presenting with community-acquired septic arthritis and bursitis.[133–137]

Burkholderia cepacia Complex

Respiratory Infections in Cystic Fibrosis

Although new species continue to be added to the BCC, *B. cenocepacia* and *B. multivorans* remain the most frequently isolated species from the lungs of patients with CF. Patient-to-patient transmission has been thought to play an important role in colonizing patients with CF.[138] A study from Australia showed that 85% of patients were infected with a unique BCC strain.[139] Compared with *Pseudomonas aeruginosa,* BCC infection can be associated with an accelerated decline in lung function.[140,141,142,143] Another unique feature associated with BCC infections in patients with CF is the development of a severe, rapidly progressive disease with systemic dissemination termed *cepacia syndrome*.[144] This syndrome has been reported with *B. cenocepacia, B. cepacia,* and *B. multivorans* and can occur many years after the initial infection.[140,145–147] A variety of therapeutic approaches have been attempted to treat this syndrome including using appropriate antibiotics and antiinflammatory agents such as corticosteroids and cyclosporine.[148–150]

Lung Transplantation

It has been shown that being colonized with BCC in the pretransplant period is associated with a very high risk of death after posttransplant.[151,152] In one study, there was a 37% absolute difference in long-term survival in patients who were colonized with BCC compared with patients who were not colonized.[153] In transplant recipients in Canada, the hazard ratio for death in the first year after transplant was 6.29 in recipients who had BCC compared with recipients who did not.[154]

Bacteremia and Pneumonia

Although infections of intravascular devices are an uncommon cause of bacteremia due to BCC, nosocomial outbreaks have been described involving contamination of heparin flushes and solutions made in compounding pharmacies.[155–158] Other outbreaks that included bacteremia presentations were related to contaminated liquid docusate sodium and washing gloves.[159,160] When bloodstream infections occur in patients who do not have CF, they are frequently associated with pneumonia.[161,162] Other BCC pneumonias have been reported in susceptible hosts receiving nebulizer-associated therapy.[163] Risk factors for developing nosocomial pneumonia are similar to those for *Stenotrophomonas* and include mechanical ventilation, prolonged intensive care unit stay, and broad-spectrum antibiotics.[77,164]

Other Rare Manifestations

BCC involvement of the skin and joints has been described only in case reports. Infections have been seen in patients in burn units who had exposure to unsterilized skin moisturizers.[165] In patients with cancer or who are immunosuppressed, disseminated skin lesions can resemble ecthyma gangrenosum,[166] and a mixed infection causing necrotizing fasciitis was seen in a nonimmunocompromised individual.[167] Septic arthritis from hematogenous spread is also rare but described.[168] Urinary involvement from colonization of a urinary catheter[169] and infection after renal transplant has been described.[170]

THERAPY

Stenotrophomonas maltophilia

One theme that connects both *S. maltophilia* and the BCC is their inherent resistance to a variety of antibiotics. *S. maltophilia* demonstrates inherent resistance to most β-lactams, aminoglycosides, and various disinfectants.[171,172] *S. maltophilia* also shows resistance to certain heavy metals such as silver.[173] *S. maltophilia* encodes a number of resistance genes including numerous resistance-nodulation-division efflux pumps[172,174] and the efflux pump MfsA.[175] In addition, resistance to quinolones administered for treatment of bacteremia has been described and was linked to a repressor for one of these efflux pumps, SmeDEF.[176] Other studies have shown that approximately 33% of clinical isolates have multidrug resistance to fluoroquinolones, tetracycline, erythromycin, and chloramphenicol through overexpression of SmeDEF.[177,178] Many resistance mechanisms beyond efflux have been found in *S. maltophilia* including β-lactamases, aminoglycoside-modifying enzymes, and reduced permeability of the outer membrane.[179] For many classes of antibiotics, resistance rates have been increasing over time.[180]

Trimethoprim-sulfamethoxazole (TMP-SMZ) has been a mainstay of therapy for *S. maltophilia,* as it has historically had the greatest in vitro potency against clinical isolates.[179,181,182] The vast majority of isolates in early reports showed susceptibility to TMP-SMZ; however, drug resistance has increased worldwide, in part due to the incorporation

of class 1 integrons carrying the *sul1* gene.[179,183] The global spread of this mobile gene has been linked to increased use of TMP-SMZ in patients.[184,185] For systemic infections, treatment frequently involves combination therapy with TMP-SMZ as a backbone. Combination therapy has been suggested for non–catheter-related bacteremia, endovascular infections, bone and joint involvement, central nervous system infection, systemic infection in patients with severe neutropenia or immune defects, and multifocal lung disease.[60,135,186] Some combinations including TMP-SMZ and ticarcillin-clavulanic acid have shown in vitro activity.[187] In one pediatric study, combination of TMP-SMZ with ciprofloxacin or minocycline extended survival.[188]

If resistance arises to TMP-SMZ or it cannot be tolerated, a number of other agents have been shown to have activity. The newer fluoroquinolones such as moxifloxacin demonstrate in vitro activity in *S. maltophilia* even in isolates that are resistant to TMP-SMZ.[189] However, resistance can develop after single-drug therapy, which can be prevented by using various drug combinations with ticarcillin-clavulanic acid or TMP-SMZ or both.[190–192] Although studies are limited, monotherapy with a fluoroquinolone compared with TMP-SMZ has shown similar treatment failure rates and development of resistance during therapy, although the numbers studied have been low.[193,194] In vitro, levofloxacin was shown to have decreased activity in *S. maltophilia* biofilms.[195] Other monotherapy studies included comparing TMP-SMZ with minocycline; although there were no significant differences in the treatment failure rate between the two groups, greater than 30% of the patients in both groups failed single-drug therapy.[58,196] There are no good data to support any unique therapeutic approaches for the treatment of *S. maltophilia* in patients with CF.[197]

A number of other antibiotics have been used to treat *S. maltophilia* infections including some more recently approved agents.[104] Tigecycline was compared with TMP-SMZ over a 3-year period in a Turkish hospital and showed no significant difference in 30-day mortality or clinical response rates between the two groups.[198] Newer agents have shown mixed results with both ceftolozane-tazobactam and meropenem-vaborbactam showing poor activity[199,200] and the siderophore cephalosporin cefiderocol showing good in vitro activity.[201] Ceftazidime-avibactam was shown to clear a very prolonged bacteremia in a patient with end-stage renal disease.[202]

Burkholderia cepacia Complex

BCC species can display significant antibiotic-resistant phenotypes. Mechanisms of resistance include the expression of efflux pumps, antibiotic degrading or modifying enzymes, and altered membrane function.[203] Although the carbapenems, TMP-SMZ, minocycline, and chloramphenicol are frequently active against BCC isolates, the final antibiotic choice is usually driven by results of antimicrobial susceptibility testing.[204] In one large study of 2621 BCC strains from patients with CF, it was found that minocycline (38%), meropenem (26%), and ceftazidime (23%) were the most active.[205] Various antibiotics and antibiotic combinations have had some activity in the setting of biofilm,[46,206] but this was not universal. Tobramycin is frequently used because it has been shown to have activity in biofilms and has been delivered via aerosolization.[207,208]

Given increasing rates of drug resistance, newer therapies or approaches have been tried. Newer cephalosporin combinations show promising results.[209] Ceftazidime combined with the non–β-lactam β-lactamase inhibitor avibactam was active in more than 90% of strains tested (70% of which were multidrug-resistant isolates).[210] A case of persistent BCC bacteremia in an infant was ultimately cleared once ceftazidime-avibactam was started.[211] Ceftolozane-tazobactam has also been shown to have activity in a large percentage of strains.[212] Therapeutic approaches in the discovery stage include narrow-spectrum therapy with bacteriophages and antisense molecules.[213–215] In addition, there has been broad interest in peptide-based therapeutics, as not only can these agents have inherent antimicrobial activity but they can also have antibiofilm activity, which is particularly helpful in patients with CF. The α-helical peptides BMAP-27, BMAP-28, and P19(9/B) were shown to be active in *S. maltophilia*.[216] Frequently active antibiotics and mechanisms of resistance for these pathogens are summarized in Table 220.1.

TABLE 220.1 Antimicrobial Activity and Mechanisms of Resistance in *Stenotrophomonas maltophilia* and *Burkholderia cepacia* Complex

PATHOGEN	ANTIBIOTICS WITH IN VIVO OR IN VITRO ACTIVITY[a]	COMMON MECHANISMS OF RESISTANCE
Stenotrophomonas maltophilia	TMP-SMZ[b] Quinolones (moxifloxacin, ciprofloxacin, levofloxacin) Minocycline or doxycycline Tigecycline Ceftazidime Ceftazidime-avibactam Ceftriaxone Ticarcillin-clavulanate Ampicillin-sulbactam	RND efflux pumps MfsA efflux pump Sme-related efflux pumps *sul* genes (TMP-SMZ resistance) *dfrA* genes (TMP-SMZ resistance) β-Lactamases Aminoglycoside modifying enzymes
Burkholderia cepacia complex	TMP-SMZ[b] Ceftazidime Ceftazidime-avibactam Meropenem or imipenem Minocycline	β-Lactamases (bla_{Pen}-like) RND efflux pumps Polymyxin resistance (altered LPSs) Quinolone resistance (gyrase mutations)

[a]Antimicrobial susceptibility testing should be performed for these pathogens to help guide therapy.
[b]Antibiotic of choice if sensitive.
LPS, Lipopolysaccharide; *RND,* resistance-nodulation-division; *TMP-SMZ,* trimethoprim-sulfamethoxazole.

PREVENTION STRATEGIES, IMMUNIZATION, AND IMMUNOTHERAPY

As with other pathogens, outbreaks of *Stenotrophomonas* and BCC require strict infection-control policies. BCC has been shown to have patient-to-patient transmission in CF populations.[217] Surveillance of the hospital water supply remains an important infection-control issue to prevent hospital-related infections from occurring.

Immunization and Immunotherapy

There are currently no existing vaccines for either *S. maltophilia* or BCC species, although there is interest in developing them.[218] Outer membrane proteins in both *B. cenocepacia* and *B. multivorans* have shown promise as vaccine targets in various animal models.[219,220] Flagellin has been used in *S. maltophilia* as a possible immunization target. In mouse studies, flagellin reduced bacterial colonization and had greater resistance to pulmonary infection.[221] Other strategies have included immunotherapy using antibodies to a broadly conserved surface polysaccharide poly-β-(1-6)-*N*-acetylglucosamine.[222]

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The complete reference list is available online at Expert Consult.

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221 *Burkholderia pseudomallei* and *Burkholderia mallei*: Melioidosis and Glanders

Bart J. Currie

SHORT VIEW SUMMARY

Definition
- Melioidosis is a disease of humans and animals resulting from infection with the soil and water bacterium *Burkholderia pseudomallei.*
- Glanders is a disease primarily of horses resulting from infection with *Burkholderia mallei.*

Epidemiology: Melioidosis
- The major endemic regions for melioidosis are Southeast Asia and northern Australia. Infections in travelers are most commonly in visitors to Southeast Asia, usually Thailand.
- Cases have occurred in other tropical and subtropical locations, such as the Indian subcontinent, China, Africa, the Caribbean, and Central and South America.
- Most cases occur during the rainy season, and clusters can follow severe weather events.
- Exposure occurs through percutaneous inoculation, inhalation, and ingestion (more common with glanders).
- Zoonotic and nosocomial infections are exceedingly rare with melioidosis (more common with glanders).
- Disease and outcomes are tightly linked to risk factors, especially diabetes (see Table 221.1).

Microbiology
- *B. pseudomallei* is a small, gram-negative, oxidase-positive, motile, aerobic bacillus.
- *B. mallei* is a small, gram-negative, oxidase-positive, nonmotile aerobic bacillus.
- *B. mallei* does not persist in the environment outside its equine host; genetic studies suggest that *B. mallei* evolved in animals from the environmental pathogen *B. pseudomallei.*

Diagnosis: Melioidosis
- Most infections with *B. pseudomallei* are asymptomatic.
- Of cases, 85% are acute illnesses after recent infection, and 11% are chronic infections (sick more than 2 months); activation from latency is rare but can occur decades after infection.
- More than half of cases are bacteremic, and up to one-quarter manifest with septic shock with high mortality.
- Presentation is pneumonia in about half of cases, but there are various other presentations (see Table 221.2).
- Culture is required for diagnosis using blood, sputum, urine, or pus, and it is facilitated by inoculating swabs from pus, throat, and rectum directly onto selective medium.
- Identification of *B. pseudomallei* and *B. mallei* by various methods can be problematic.
- Locally developed antigen and DNA detection techniques are available in some locations. Serology has poor specificity because of background positivity in endemic regions.

Therapy
- Ceftazidime or a carbapenem with or without trimethoprim-sulfamethoxazole (TMP-SMZ) is given initially, followed by TMP-SMZ (see Tables 221.3 and 221.4).
- Therapy for glanders is as for melioidosis, although gentamicin, azithromycin, or clarithromycin may have a role.

Prevention
- Melioidosis: Education is needed in endemic areas about minimizing exposure to wet season soils, surface water, and potential aerosols during windy monsoonal rains, especially for diabetics.
- Patients with cystic fibrosis should consider avoiding travel to high-risk areas.
- Glanders: Control of glanders in the equine species and strict precautions to prevent laboratory-acquired infection are crucial.
- Vaccines are under development for both melioidosis and glanders.

The genus *Burkholderia* is currently composed of many species, but only three are notable pathogens for humans or animals: the former *B. cepacia* complex (described in Chapter 220), *B. pseudomallei* (the agent of melioidosis), and *B. mallei* (the agent of equine glanders). All three are aerobic, nonsporulating, straight or slightly curved gram-negative bacilli that were formerly placed in the genus *Pseudomonas.*

MELIOIDOSIS

Melioidosis is a disease of humans and animals; it has enormous clinical diversity, spanning asymptomatic infection, localized skin ulcers or abscesses, chronic pneumonia mimicking tuberculosis, and fulminant septic shock with abscesses in multiple internal organs. Most disease is from recent infection, but latency with subsequent activation is well recognized and can occur decades after exposure. Most cases are reported from Southeast Asia and northern Australia, but melioidosis is increasingly being recognized in other tropical and subtropical locations and in people infected in an endemic region who return or travel to Europe and the United States (Fig. 221.1).[1] More recent modeling suggested that melioidosis is greatly underdiagnosed in most of the 45 countries in which it is known to be endemic and predicted that it may be present in a further 34 countries where it has not yet been reported.[2] The modeling estimated 165,000 infections and 89,000 deaths worldwide annually. Targeted surveillance and support for improved regional microbiology facilities are needed to determine the accuracy of these predictions.[3] The causative bacterium, *B. pseudomallei,* is also considered a potential biological warfare agent.

History

In 1912 Whitmore and Krishnaswami[4] described cases of a newly recognized septicemic disease in morphine addicts in Rangoon, Burma. Fatal cases were characterized by widespread caseous consolidation of the lung and abscesses in liver, spleen, kidney, and subcutaneous tissues. The bacillus isolated from tissues was similar to that causing glanders *(B. mallei)* but was motile. Whitmore and Krishnaswami[4] noted the clinical similarity to glanders, and Stanton and Fletcher[5] subsequently proposed the name *melioidosis,* derived from the Greek *melis* (distemper of asses). Various names were used for the causative bacterium, including *Bacillus whitmori* and, for many years, *Pseudomonas pseudomallei.*[6] In 1992 seven *Pseudomonas* spp. were moved to a new genus, *Burkholderia. B. cepacia* is the type species in the genus, which includes the organisms causing melioidosis *(B. pseudomallei)* and glanders *(B. mallei).*

Etiology

B. pseudomallei is a small, gram-negative, oxidase-positive, motile, aerobic bacillus with occasional polar flagella. On staining, a bipolar

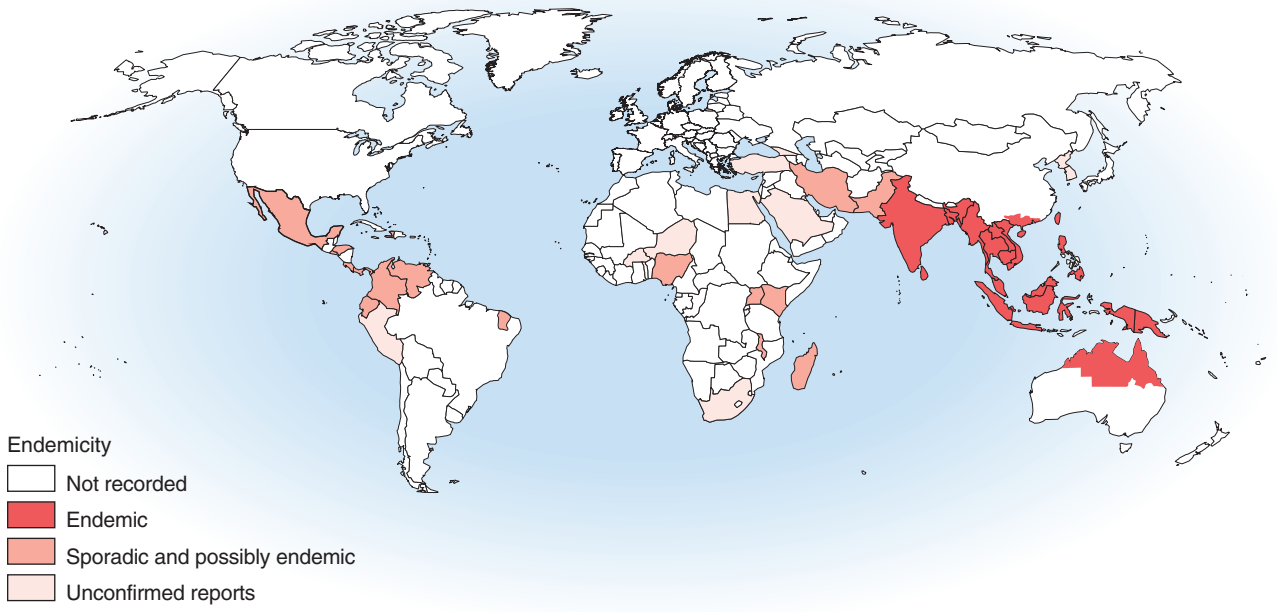

FIG. 221.1 **Known global distribution of melioidosis.**

"safety pin" pattern is seen. The organism is easily recovered on standard culture medium and must be distinguished from the closely related soil saprophytes *B. thailandensis, B. oklahomensis,* and *B. humptydooensis,* as well as from *Pseudomonas stutzeri* and other *Pseudomonas* species.

The organism is present in soil and surface water in endemic regions. Humans and animals are infected by percutaneous inoculation, inhalation, aspiration, or ingestion. Occasional laboratory-acquired infections are described, but person-to-person spread and zoonotic infection are very uncommon.

Epidemiology

After the initial account in Burma, melioidosis was documented in humans and animals in Malaysia and Singapore from 1913, in Vietnam from 1925, and in Indonesia from 1929.[7] Thailand has reported the largest number of cases,[5–8] with an estimated 2000 to 3000 cases of melioidosis each year.[9] Melioidosis is also common in Malaysia[10] and Singapore.[11,12] Other countries in the region where melioidosis is recognized in humans and animals include China (especially Hong Kong), Taiwan, Brunei, Vietnam, Cambodia, and Laos.[13–18,19] Melioidosis is also likely to occur in the Philippines.[9,20] Melioidosis has been increasingly recognized in India, although reports that some of the "plague" scares of 1994 may have been cases of melioidosis have been disproved.[21,22] Cases have been reported from Sri Lanka, Bangladesh, and Pakistan.[20] Despite the early documentation of melioidosis in Burma and Indonesia, more recent cases were not reported from Indonesia until after the 2004 Asian tsunami.[23] Cases of melioidosis have also been documented from Papua New Guinea, Fiji, and New Caledonia,[24] but the extent of endemicity in the Pacific Islands remains to be defined.

Cases of melioidosis are increasingly being documented from outside the classic endemic region of Southeast Asia, Australasia, the Indian subcontinent, and China. These include sporadic human or animal cases or environmental isolates of *B. pseudomallei* from the Middle East, Africa, the Caribbean, and Central and South America. Regional endemicity of the disease in Puerto Rico has been documented.[25] Although some of these reports are from incorrect species diagnosis, others are confirmed, making the endemic limitations of melioidosis very unclear.[20] Sporadic cases and occasional case clusters have recently occurred in Brazil and elsewhere in the Americas.[26,27] Despite more recent cases from Madagascar and Malawi,[28,29] the true extent and magnitude of the presence of *B. pseudomallei* in Africa remain entirely unknown. Global warming may result in expansion of the endemic boundaries of melioidosis.[30,31] A global phylogeny study has supported the origin of *B. pseudomallei* to be Australia, with subsequent spread to Southeast Asia and then to Africa.[32] Spread from Africa to the Americas was calculated to have been much more recent, potentially linked to the times of the slave trade.

The two locations where melioidosis is arguably the most important bacterial pathogen for humans are some northeast provinces in Thailand and the Top End of the Northern Territory of Australia. In northeast Thailand, 20% of community-acquired septicemic cases are caused by melioidosis, which accounts for 39% of fatal septicemias[33] and 36% of fatal community-acquired pneumonias.[34] In the Top End of the Northern Territory, melioidosis has been the most common cause of fatal community-acquired bacteremic pneumonia.[35]

In addition to endemic melioidosis, there are several documented situations where melioidosis became established in nontropical locations. In France in the 1970s cases of melioidosis occurred in animals in a Paris zoo, with spread to other zoos and equestrian clubs.[36] In addition to fatal animal and human cases, there was extensive soil contamination persisting for some years. *B. pseudomallei* is considered likely to have been introduced by importation of infected animals. A cluster of cases occurred over a 25-year period in southwestern Western Australia (31° S) involving animal cases and one human infection in a farmer. Ribotyping of the farm animal and human isolates and one isolate from the soil showed identical patterns.[37] This supported the suggestion of clonal introduction of *B. pseudomallei,* probably via an infected animal, with environmental contamination, local dissemination, and persistence. In 2017, further cases in animals with the same *B. pseudomallei* genotype were again documented in that location following a storm with near record rainfall.[30] This was 26 years after the previous case and demonstrates more than 50 years of persistence of *B. pseudomallei* in this temperate region.

Melioidosis was an important cause of morbidity and mortality in foreign troops fighting in Southeast Asia. Dance[36] noted that at least 100 cases occurred among French forces in Indochina between 1948 and 1954. By 1973, 343 cases had been reported in American troops fighting in Vietnam. Concerns of reactivation of latent infection in soldiers returning from Vietnam, with estimates from serology studies of approximately 225,000 potential cases, resulted in melioidosis being called the "Vietnamese time bomb."[38] However, although occasional cases of reactivation of *B. pseudomallei* still occur in Vietnam veterans, it is rare compared with the numbers of troops exposed.

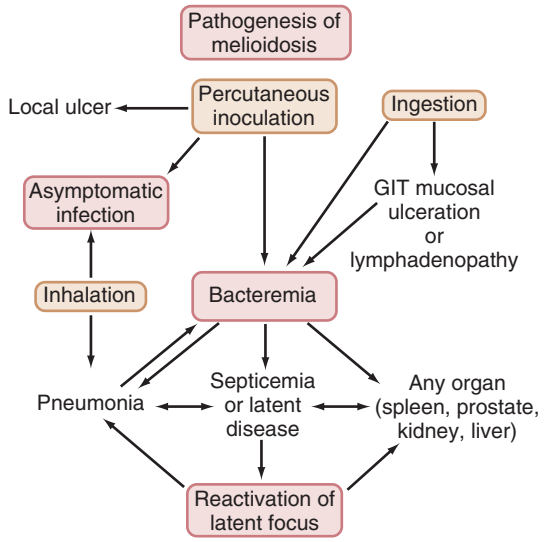

FIG. 221.2 Natural history of infection with *Burkholderia pseudomallei*. *GIT,* Gastrointestinal tract.

Transmission

Fig. 221.2 summarizes the natural history of infection with *B. pseudomallei.* Studies from Malaysia, Thailand,[39] and Australia[40] have found that the organism is more common in cleared, irrigated sites such as rice paddies and farms. It has been suggested that the increase in melioidosis cases in Thailand may be partly due to the increased number of bacteria in such environments and partly due to increased exposure to bacteria resulting from changes in behaviors such as farming techniques.[13] In Australia, *B. pseudomallei* has been found most commonly in clay soils to a depth of 25 to 45 cm, and it has been proposed that the bacteria move to the surface with the rising water table during the wet season.[41] Exposure to mud in an endurance challenge (10-km or 21-km obstacle course including river crossing and mud crawl) has been associated with melioidosis in the Northern Territory of Australia.[42] An alternative explanation for the variable bacterial presence found is that during times of stress such as in prolonged dry seasons, *B. pseudomallei* may persist in soil in a viable but nonculturable state.[43] Differential gene activation may allow such environmental bacteria to respond and adapt to different environmental conditions. This possibility is also relevant to the pathogenicity, latency, and reactivation of infection with *B. pseudomallei* in humans. Melioidosis endemic locations may vary in their specific ecologic niches for *B. pseudomallei.*[44] *B. pseudomallei* colonizes and thrives in the rhizosphere and aerial parts of native and imported grasses in northern Australia, raising implications for global epidemiology and potential dispersal.[45] The role of biofilms in the persistence of *B. pseudomallei* in the environment as well as in animal and human hosts requires further study.[46]

In most endemic regions, there is a close association between melioidosis and rainfall. In northeast Thailand[47] and northern Australia,[7] 75% and 81% of cases, respectively, have occurred in the wet season. In a study of cases in Singapore, increased incidence was associated with humidity and rainfall.[48] Although early animal studies showed infection with *B. pseudomallei* through oral or nasal exposure and from ingestion, more recent reviews have considered that most human cases are from percutaneous inoculation of *B. pseudomallei* after exposure to muddy soils or surface water in endemic locations.[6,20,35] Ingestion and sexual transmission have been suggested as unusual modes of transmission of *B. pseudomallei.* Nevertheless, a more recent study from Thailand raised the possibility that ingestion of water contaminated with *B. pseudomallei* may be a more common infecting event than previously thought, especially in endemic regions with unchlorinated water supplies.[49] Presentations of melioidosis pneumonia after presumptive inoculating skin injuries have been documented in patients with soil-contaminated burns and are common in tropical Australia.[35] This suggests hematogenous spread to the lung rather than inhalation or spread from the upper respiratory tract. However, under certain epidemiologic conditions, the inhalation route may predominate, as suggested for soldiers exposed to dusts raised by helicopter rotor blades in Vietnam.[50] Melioidosis after near drowning is well documented, with the probable infecting event being aspiration.[14] Intensity of rainfall is an independent predictor of melioidosis manifesting as pneumonia and of a fatal outcome,[51] suggesting that heavy monsoonal rainfall and winds may result in a shift toward inhalation as the mode of infection with *B. pseudomallei.* At the present time the overall proportion of melioidosis cases resulting from inhalation rather than percutaneous inoculation remains uncertain.[1,52] Several outbreaks of melioidosis in Australia have been linked to contamination of potable water with *B. pseudomallei.*[53,54] The water supplies involved were unchlorinated, or the chlorination was below standard. The contamination of the water supply has been attributed to soil disturbance during excavations. Drinking water was also identified as a likely source of infections in the more recent study from Thailand.[55]

The incubation period for melioidosis is influenced by inoculating dose, mode of infection, host risk factors, and probably differential virulence of infecting *B. pseudomallei* strains. Onset of melioidosis within 24 hours has been seen in presumed aspiration after near drowning and, in some cases, after severe weather events. In 25 cases of acute melioidosis in which a clear incubation period could be determined between the inoculating injury and the onset of symptoms, the incubation period was 1 to 21 days (mean, 9 days),[56] which is consistent with a series of nosocomial cases from Thailand in which the incubation period was 3 to 16 days (mean, 9.5 days).[57]

In a review of 72 published cases in travelers, 73% acquired the infection in Southeast Asia, most commonly Thailand, and 19% were infected in Central America.[58] In this review and a separate review of 82 published cases, traveling during heavy rains or floods and adventure travel were common.[59] Most patients presented with sepsis, pneumonia, or abscesses. Onset of illness was an average of 23 days after return (range, 1–360 days).[58]

Pathogenesis

Serology studies have shown that most infections with *B. pseudomallei* are asymptomatic.[60,61] In northeast Thailand, most of the rural population is seropositive by indirect hemagglutination (IHA),[47] with most seroconversion occurring between 6 months and 4 years of age.[61] Although melioidosis occurs in all age groups, severe clinical disease, such as septicemic pneumonia, is seen mostly in patients with risk factors such as diabetes, renal disease, and hazardous alcohol use.

In addition to infection by inhalation, bacterial load on exposure (inoculating dose) and virulence of the infecting strain of *B. pseudomallei* are likely to influence the severity of disease. However, it has been noted that despite the large bacterial load in severely ill patients with septicemic pulmonary melioidosis, person-to-person transmission is extremely unusual. This, together with the rarity of fulminant melioidosis in healthy people, supports the primary importance of host risk factors for development of melioidosis. Furthermore, although it is clear from laboratory studies of isolates of *B. pseudomallei* from animals, humans, and the environment that virulence differs among *B. pseudomallei* isolates,[62] the importance of this variation in virulence in determining clinical aspects of melioidosis remains uncertain. Molecular typing that shows clonality of isolates in animal and human clusters has revealed that the same outbreak strain can cause different clinical presentations, with host factors being most important in determining the severity of disease.[54] Whole-genome sequencing and subsequent molecular studies have shown that *B. pseudomallei* has two chromosomes, with a complex accessory genome that includes multiple genomic islands that are variably present in different strains and have a great propensity for horizontal gene transfer.[63,64] Further studies are required to unravel the global phylogeny and evolutionary history of *B. pseudomallei* and related species and to determine which genes or gene clusters may be critical for pathogenesis and disease presentation and outcome.[1,65] Bacterial genome-wide association studies have begun to show presence or absence of specific genes linked to neurologic and cutaneous melioidosis.[66]

B. pseudomallei is a facultative intracellular pathogen that can invade and replicate inside various cells including polymorphonuclear leukocytes

and macrophages and some epithelial cell lines.[67] Resistance to human serum (conferred by lipopolysaccharide [LPS])[68] and the ability of *B. pseudomallei* to survive intracellularly (conferred in part by capsular polysaccharide) appear to be critical in the pathogenesis of melioidosis.[69,70] Type III and type VI secretion systems in *B. pseudomallei* have also been found to be important in cell invasion and intracellular survival.[71,72] Quorum sensing may play an important role in many aspects of virulence of *B. pseudomallei* including cell invasion, cytotoxicity, and antimicrobial resistance.[46,67,73] Other putative virulence factor candidates include flagella; type IV pili and other adhesins; a siderophore; and secreted proteins such as hemolysin, lipases, and proteases.[67] *Burkholderia* lethal factor 1 is similar to *Escherichia coli* cytotoxic necrotizing factor 1 and interferes with initiation of translation, leading to alteration of the actin cytoskeleton and ultimately cell death.[74]

Intracellular survival of *B. pseudomallei* in human and animal hosts is likely to explain the ability for latency. After internalization, *B. pseudomallei* escapes from endocytic vacuoles into the cell cytoplasm, and induction of actin polymerization at one bacterial pole leads to membrane protrusions, with cell-to-cell spread involving these actin tails.[75] An additional survival factor for *B. pseudomallei* is the ability for phenotypic switching with a change in colony morphology resulting in changes in the expression of putative virulence factors such as biofilm and flagella.[76]

A number of studies have shown elevated levels of various endogenous inflammatory mediators and cytokines to be associated with severity and outcomes of melioidosis. Nevertheless, whether these elevated cytokines are a cause or result of severe disease is not established. In Thailand, there was an association of severe melioidosis with tumor necrosis factor-α (TNF-α) gene allele 2, which is linked to higher constitutive and inducible production of TNF-α.[77] However, in a mouse model of melioidosis, neutralization of TNF-α or interleukin-12 increased susceptibility to infection in vivo, and interferon-γ (IFN-γ) was found to be important for survival, with mice treated with monoclonal anti–IFN-γ dying more quickly.[78] A role for Toll-like receptors in the innate immune response in melioidosis has been proposed.[79,80] Therefore there are important host protective mechanisms against *B. pseudomallei* in cytokine responses as well as potentially detrimental ones, with the timing of cytokine release and the balance between proinflammatory and antiinflammatory responses likely to determine the severity of disease and outcome of infection.[67,81] The extent to which host polymorphisms in immune response contribute compared with differences in organism virulence, infecting dose of *B. pseudomallei,* and defined host risk factors such as diabetes remains to be clarified. Nevertheless, the predominant association with fatal melioidosis is the presence of defined patient risk factors.

Although a vigorous cell-mediated immune response may protect against disease progression,[82] there is no definitive evidence for the development of immunity from melioidosis after natural exposure to *B. pseudomallei*. Moreover, reinfection can occur with a different strain of *B. pseudomallei* after successful treatment of melioidosis.[83]

Table 221.1 summarizes the risk factors for melioidosis. The most important risk factors are diabetes, hazardous alcohol use, and renal disease.[6,7,84] In Thailand, the adjusted odds ratios for diabetes and renal disease (chronic renal impairment or renal or ureteric calculi) in cases of melioidosis versus control subjects were 12.9 (95% confidence interval [CI], 5.1–37.2) and 2.9 (95% CI, 1.7–5.0), respectively.[84] Other risk factors for melioidosis include chronic lung disease (including cystic fibrosis), thalassemia[85] (odds ratio in Thailand, 10.2; 95% CI, 3.5–30.8), malignancies, steroid therapy, iron overload, and tuberculosis.[84] Severe disease and fatalities are uncommon in patients without risk factors in whom infection is diagnosed early and early treatment is administered, with only two deaths in 106 patients without risk factors in one study.[7] Risk factors are less common in children than in adults.[86,87] A study from Sabah, Malaysia, demonstrated the importance of iron overload in thalassemia as a risk factor for melioidosis in children, with no cases after introduction of regular iron chelation therapy.[88]

Evidence suggests that the predisposition to melioidosis in patients with diabetes, hazardous alcohol use, or chronic renal disease may reflect impairment of innate immune function, especially neutrophil and other phagocytic cell functions such as mobilization, delivery, adherence, ingestion, and killing.[7,89] Melioidosis has also been described in chronic granulomatous disease.[90]

TABLE 221.1 Risk Factors for Melioidosis

RISK FACTOR[a]	THAILAND (% OF CASES)[b]	AUSTRALIA (% OF CASES)[c]
Diabetes	23–60	37
Alcohol excess	12	39
Renal disease	20–27	10
Chronic lung disease	NR	27
Thalassemia	7	0
No risk factors	24–36	20

[a]Not listed: malignancy, steroid therapy, iron overload, cardiac failure.
[b]Thailand data from Punyagupta S. Melioidosis: review of 686 cases and presentation of a new clinical classification. In: Punyagupta S, Sirisanthana T, Stapatayavong B, eds. *Melioidosis*. Bangkok: Bangkok Medical; 1989:217–229; Chaowagul W, White NJ, Dance DA, et al. Melioidosis: a major cause of community-acquired septicemia in northeastern Thailand. *J Infect Dis*. 1989;159:890–899; Suputtamongkol Y, Chaowagul W, Chetchotisakd P, et al. Risk factors for melioidosis and bacteremic melioidosis. *Clin Infect Dis*. 1999;29:408–413; and Limmathurotsakul D, Chaowagul W, Chierakul W, et al. Risk factors for recurrent melioidosis in northeast Thailand. *Clin Infect Dis*. 2006;43:979–986.
[c]Australia data from Currie BJ, Fisher DA, Howard DM, et al. Endemic melioidosis in tropical northern Australia: a 10-year prospective study and review of the literature. *Clin Infect Dis*. 2000;31:981–986.
NR, Not reported.

Clinical Manifestations

The earliest descriptions of melioidosis documented the fulminant end of the clinical spectrum, with abscesses throughout both lungs and in many organs.[4] At the other end of the spectrum are asymptomatic infections and localized skin ulcers or abscesses without systemic illness. Howe and colleagues[50] classified melioidosis as acute, subacute, and chronic. The Infectious Disease Association of Thailand summarized 345 cases in these categories, as follows[6,91]:

1. Multifocal infection with septicemia (45% of cases, 87% mortality)
2. Localized infection with septicemia (12% of cases, 17% mortality)
3. Localized infection (42% of cases, 9% mortality)
4. Transient bacteremia (0.3%)

Bacteremia and overall mortality rates, respectively, have been reported as 60% and 44% in Thailand,[47] 55% and 14% in Australia,[7] and 43% and 39% in Singapore.[20]

Table 221.2 summarizes the clinical manifestations in patients with melioidosis in northern Australia. Pneumonia is the most common clinical manifestation in patients with melioidosis in all studies, accounting for about half of cases. Secondary pneumonia after another primary presentation occurs in about 10% of cases. Acute melioidosis pneumonia has a spectrum from fulminant septic shock (mortality up to 90%) (Figs. 221.3 to 221.6) to mild undifferentiated pneumonia, which can be acute or subacute in nature, with little mortality.[92] Septicemic patients present acutely unwell with high fevers and prostration and often little initial cough or pleuritic pain. On chest radiographs, diffuse nodular infiltrates often develop throughout both lungs, and they coalesce, cavitate, and progress rapidly, consistent with the caseous necrosis and multiple metastatic abscess formation seen at autopsy. Nonsepticemic patients with pneumonia and some patients with septicemic pneumonia have a more predominant cough, with productive sputum and dyspnea, and their chest radiographs show discrete but progressive consolidation in one or more lobes (Fig. 221.7). In endemic regions, acute pneumonia with upper lobe consolidation warrants consideration of melioidosis, although lower lobe infiltrates are also common.

In 11% of cases in northern Australia, patients present with chronic melioidosis, defined as illness with symptoms for longer than 2 months' duration on presentation. Many of these patients have features mimicking tuberculosis, with fevers, weight loss, productive cough (sometimes with hemoptysis), and classic upper lobe infiltrates, with or without cavitation on chest radiographs (Fig. 221.8). In these patients, disease

TABLE 221.2 Clinical Presentations and Outcomes of Melioidosis in Northern Australia

	TOTAL		BACTEREMIC		NONBACTEREMIC	
	Number	Deaths (Mortality)	Number	Deaths (Mortality)	Number	Deaths (Mortality)
Septic shock present	116 (21%)	58 (50%)	103	48 (47%)	13	10 (77%)
Pneumonia	88	43 (49%)	78	35 (45%)	10[a]	8 (80%)
No evident focus	13	8 (62%)	12	7 (58%)	1[b]	1 (100%)
Genitourinary	10	5 (50%)	9	4 (44%)	1[c]	1 (100%)
Osteomyelitis/septic arthritis	4	2 (50%)	4	2 (50%)	0	0 (0%)
Soft tissue abscess	1	0 (0%)	0	0	1	0 (0%)
Not septic shock	424 (79%)	19 (4%)	195	13 (7%)	229	6 (3%)
Pneumonia	190	12 (6%)	89	9 (10%)	101	3 (3%)
Skin infection	68	0 (0%)	1	0 (0%)	67	0 (0%)
Genitourinary	66	2 (3%)	41	2 (5%)	25	0 (0%)
No evident focus	52	2 (4%)	47	2 (4%)	5	0 (0%)
Soft tissue abscess	18	0 (0%)	4	0 (0%)	14	0 (0%)
Osteomyelitis/septic arthritis	16	0 (0%)	10	0 (0%)	6	0 (0%)
Neurologic	14	3 (21%)	3	0 (0%)	11	3 (27%)
Total	540	77 (14%)	298 (55%)	61 (20%)	242 (45%)	16 (7%)

[a]Seven blood cultures not done; three blood cultures negative.
[b]Culture was positive for *Burkholderia pseudomallei* only from rectal swab, although fatal septic shock.
[c]Blood culture not done.
From Currie BJ, Ward L, Cheng AC. The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20-year Darwin prospective study. PLoS Negl Trop Dis. *2010;4:e900.*

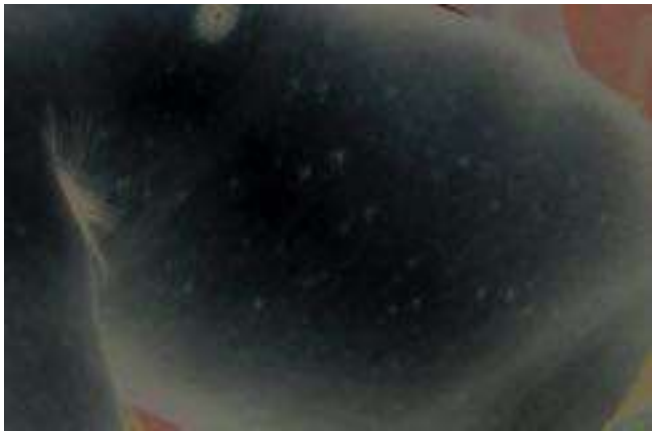

FIG. 221.3 Multiple pustules in a 46-year-old diabetic man with fatal septicemic melioidosis.



FIG. 221.5 Computed tomography scan of the chest of a 26-year-old woman with fatal melioidosis showing large pulmonary abscess.



FIG. 221.4 Chest radiograph of patient in Fig. 221.3 showing multiple pulmonary abscesses.

can be remitting and relapsing over many years, sometimes with an initial misdiagnosis of tuberculosis. Although acute deterioration with septicemia may occur, mortality in this group is low.

Previously it was thought that a colonization state did not exist for *B. pseudomallei,* with presence in sputum or the throat always reflecting disease. However, it has more recently become evident that *B. pseudomallei* can both colonize airways and cause disease in patients with cystic fibrosis (CF) and bronchiectasis.[93,94] The similarity to infection with *B. cepacia* complex in CF is concerning, given the association of *B. cepacia* complex with more rapid deterioration in lung function. Furthermore, likely transmission of *B. pseudomallei* between two siblings with CF has been reported.[95] Patients with CF traveling to locations where



FIG. 221.6 **Multiple lung abscesses seen at autopsy of a patient with acute melioidosis pneumonia.**

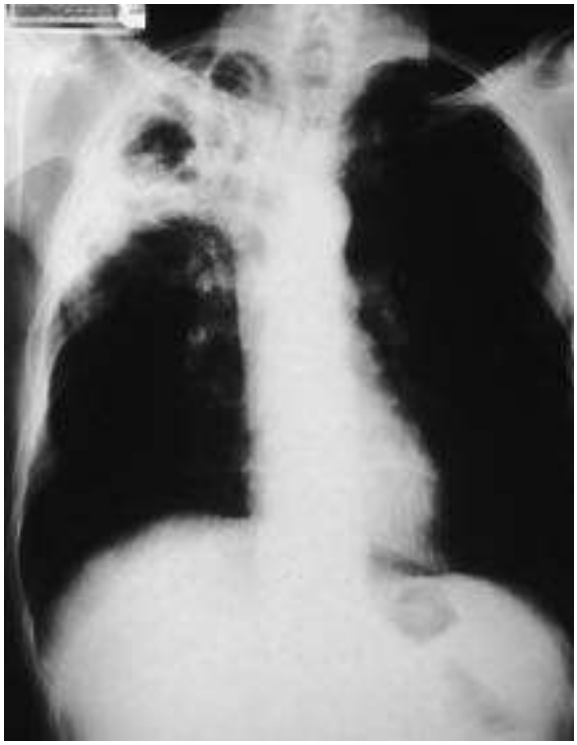

FIG. 221.8 **Radiograph of a 62-year-old man with nonfatal chronic melioidosis showing right upper lobe cavitation.**



FIG. 221.7 **Extensive left upper lobe consolidation in a 54-year-old man with fatal melioidosis pneumonia.**



FIG. 221.9 **Cutaneous melioidosis seen on the right forearm of a 50-year-old man.**

melioidosis is endemic should be warned of the risk of melioidosis, which should be considered if they become sick after returning.

It is common for patients to present with skin ulcers or abscesses (Figs. 221.9 and 221.10).[96] Occasionally a patient presents with septic arthritis or osteomyelitis, or one of these conditions can develop after the patient has presented with another primary diagnosis, usually pneumonia (Fig. 221.11). Also well recognized, regardless of the clinical presentation, are abscesses in internal organs, especially spleen, kidney, prostate, and liver (Figs. 221.12 to 221.16). Where available, abdominopelvic computed tomography (CT) scanning is useful in all patients with melioidosis to detect internal abscesses, with ultrasound an alternative for children and women of childbearing age to avoid radiation.

Three differences have been noted between Thailand and tropical Australia. First, suppurative parotitis accounts for 40% of melioidosis in children in Thailand[19,86,97] but is very rare in Australia. Second, prostatic melioidosis is well recognized but uncommon except in Australia, where routine abdominopelvic CT scanning of all patients with melioidosis has shown prostatic abscesses to be present in 18% of all male patients with melioidosis (Fig. 221.17).[98] Some prostatic abscesses were incidental in patients presenting with pneumonia or septicemia, but a primary genitourinary presentation was common with fevers, abdominal discomfort, dysuria, and sometimes diarrhea and urinary retention. Third, neurologic melioidosis accounts for about 4% of cases in northern Australia, with the distinctive clinical features being



FIG. 221.10 Cutaneous melioidosis of the right thigh in an 11-year-old boy.

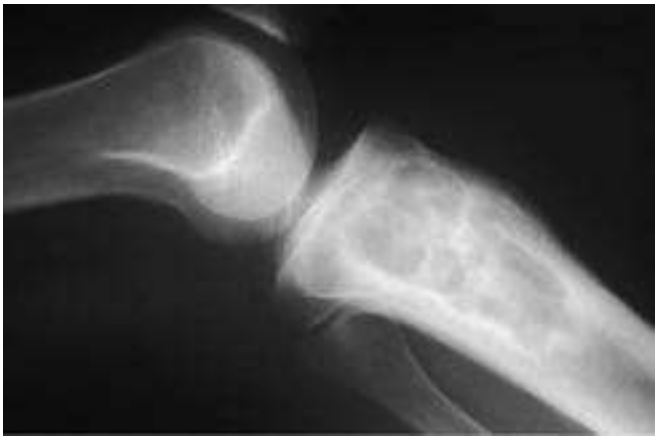

FIG. 221.11 Radiograph of a 43-year-old man showing large lucent areas in proximal tibia.



FIG. 221.12 Right psoas abscess in a 17-year-old girl with melioidosis.



FIG. 221.13 The patient in Fig. 221.12 developed an L3 osteomyelitis 6 weeks later.



FIG. 221.14 Computed tomography scan of a 60-year-old diabetic patient showing splenic abscesses.



FIG. 221.15 Abscesses seen at splenectomy performed on the patient in Fig. 221.14.

brainstem encephalitis, often with cranial nerve palsies (especially the seventh nerve), together with peripheral motor weakness or occasionally just flaccid paraparesis alone. CT scan is often normal, but dramatic changes are seen on magnetic resonance imaging, most notably a diffusely increased T2-weighted signal in the midbrain, brainstem, and spinal cord (Fig. 221.18).[87,99] Direct bacterial invasion of the brain and spinal cord occurs melioidosis encephalomyelitis.[100,101] Neurologic melioidosis is occasionally seen outside Australia, although mostly as macroscopic brain abscesses.[91,102]

Unusual foci of melioidosis infection described in case reports or case series include mycotic aneurysms, lymphadenitis resembling tuberculosis, mediastinal masses, pericardial collections, and adrenal abscesses.

It has long been recognized that *B. pseudomallei,* similar to tuberculosis, has the potential for reactivation from a latent focus, usually in the lung—hence the concern of the "Vietnamese time bomb" in returned soldiers. The longest latency period from exposure to *B.*



FIG. 221.16 Melioidosis orchitis and scrotal ulcer in a 49-year-old man.

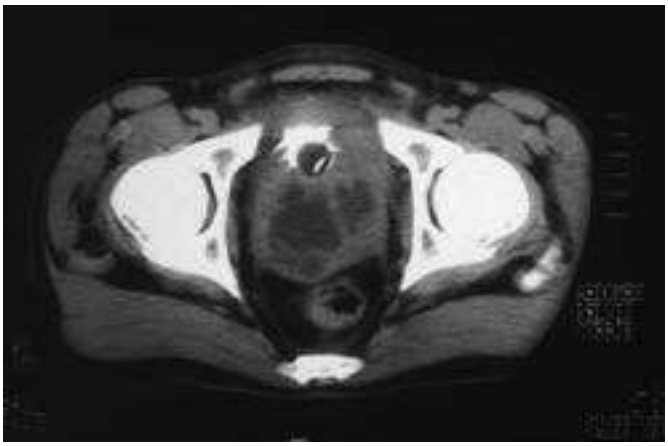

FIG. 221.17 Melioidosis prostatic abscesses in a 31-year-old man who presented with fever and urinary retention.

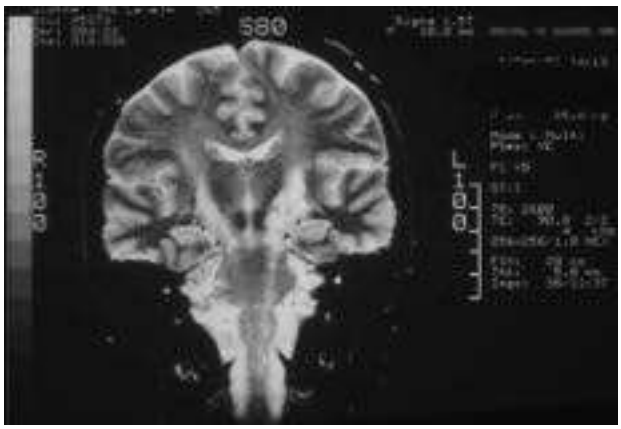

FIG. 221.18 Melioidosis encephalomyelitis in a 24-year-old man. Magnetic resonance imaging shows increased T2-weighted signal extending through the brainstem and into the spinal cord.

pseudomallei in an endemic region to onset of melioidosis in a nonendemic region was previously documented as being 62 years.[103] Importantly, this case was refuted by recent genotyping, which showed a sequence type not linked to Southeast Asia, the presumptive location of infection in the case report.[104] Cases of reactivated *B. pseudomallei* appear to be very uncommon, accounting for only 4% of cases in northern Australia. The vast majority of cases of melioidosis occur in the monsoonal wet seasons of the various endemic regions, supporting the concept that in endemic areas most patients with melioidosis have recent infections that appear with acute illness. Reactivation of melioidosis has been associated with influenza, other bacterial infections, and development of known melioidosis risk factors such as diabetes. What proportion of asymptomatic seropositive people actually have latent infection with the potential for reactivation is unknown.

Laboratory Diagnosis

Definitive diagnosis of melioidosis requires a positive culture of *B. pseudomallei*.[105] Melioidosis must be considered in febrile patients in or returning from endemic regions to enable appropriate samples to be tested. *B. pseudomallei* readily grows in commercially available blood culture media, but it is not unusual for laboratories in nonendemic locations to misidentify the bacteria as a *Pseudomonas* spp. or other *Burkholderia* spp., especially because some commercial identification systems are poor at identifying *B. pseudomallei*.[106] Experience is accumulating with identification of *B. pseudomallei* by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry systems.[107] Culture from nonsterile sites increases the likelihood of diagnosis but can be problematic. The rate of successful culture is increased if sputum, throat swabs, ulcer or skin lesion swabs, and rectal swabs are placed into Ashdown medium, a colistin-containing liquid transport broth that results in the selective growth of *B. pseudomallei,* or plated onto Ashdown agar or a commercial *B. cepacia* medium.[108,109] Addition of ampicillin, norfloxacin, and polymyxin B to Ashdown medium has been studied as a selective medium to enhance recovery of *B. pseudomallei* from heavily contaminated specimens including stool.[110] *B. pseudomallei* can be identified by combining the commercial API 20 NE or 20 E biochemical kit with a simple screening system involving Gram stain, oxidase reaction, typical growth characteristics, and resistance to certain antibiotics.[111]

There are a variety of locally developed antigen and DNA detection techniques used in endemic regions for early identification of *B. pseudomallei* in culture media and patient blood or urine, but these are not yet widely available.[111,112,113,114,115] IHA, various enzyme-linked immunosorbent assays (ELISAs), and other serologic assays are available.[114,116,117] In endemic areas, their usefulness is limited by high rates of background antibody positivity. In acute septicemic melioidosis, IHA and ELISA are often initially negative, but repeat testing may show seroconversion. Positive IHA or ELISA in a tourist returning from a region where melioidosis is endemic is useful in supporting the possibility of melioidosis, but definitive diagnosis still requires a positive culture.

Therapy

B. pseudomallei is characteristically resistant to penicillin, ampicillin, first-generation and second-generation cephalosporins, gentamicin, tobramycin, and streptomycin. Before 1989, conventional therapy for melioidosis consisted of a combination of chloramphenicol, trimethoprim-sulfamethoxazole (TMP-SMZ), doxycycline, and sometimes kanamycin, given for 6 weeks to 6 months.[6,118] However, there were also reports of the successful use of TMP-SMZ alone and tetracycline or doxycycline alone. These conventional antibiotics are bacteriostatic rather than bactericidal, and in vitro studies have shown various combinations to be antagonistic.

Subsequent studies have shown *B. pseudomallei* to be susceptible to various β-lactam antibiotics, especially ceftazidime, imipenem, meropenem, piperacillin, amoxicillin-clavulanate, ceftriaxone, and cefotaxime, with various degrees of bactericidal activity. Table 221.3 summarizes recommended antibiotic treatment[1,119]

Initial Intensive Therapy

The most important therapeutic study for melioidosis was an open-label randomized trial in Thailand comparing ceftazidime (120 mg/kg/day) with conventional therapy, which showed that ceftazidime is associated with a 50% lower overall mortality in severe melioidosis.[118] Ceftazidime then became the drug of choice for initial intensive therapy for melioidosis. Another study from Thailand showed similar results when ceftazidime was used in combination with TMP-SMZ.[120] Two randomized controlled trials in Thailand studied whether TMP-SMZ

TABLE 221.3 Antibiotic Therapy for Melioidosis

Initial Intensive Therapy (Minimum of 10–14 Days; see Table 221.4)

Ceftazidime (50 mg/kg, up to 2 g) q6h
or
Meropenem (25 mg/kg, up to 1 g) q8h
or
Imipenem (25 mg/kg, up to 1 g) q6h
Any one of the three may be combined with TMP-SMZ (6/30 mg/kg, up to 320/1600 mg) q12h (recommended for neurologic, cutaneous, bone, joint, and prostatic melioidosis)

Eradication Therapy (Minimum of 3 Months; see Table 221.4)

TMP-SMZ (6/30 mg/kg, up to 320/1600 mg) q12h

TMP-SMZ, Trimethoprim-sulfamethoxazole.

TABLE 221.4 Duration of Antibiotic Therapy for Melioidosis

ANTIBIOTIC DURATION–DETERMINING FOCUS		MINIMUM INTENSIVE PHASE DURATION (WEEKS)[a]	ERADICATION PHASE DURATION (MONTHS)
Skin abscess		2	3
Bacteremia with no focus		2	3
Pneumonia	Without lymphadenopathy[b] or ICU admission	2	3
	With either lymphadenopathy[b] or ICU admission	4	3
Deep-seated collection[c]		4[d]	3
Osteomyelitis		6	6
CNS infection		8	6
Arterial infection[e]		8[d]	6

[a]Clinical judgment to guide prolongation of intensive phase if improvement is slow or if blood cultures remain positive at 7 days.
[b]Defined as enlargement of any hilar or mediastinal lymph node to greater than 10-mm diameter.
[c]Defined as abscess anywhere other than skin, lungs, bone, CNS, or vasculature; septic arthritis is considered a deep-seated collection.
[d]Intensive phase duration is timed from date of most recent drainage or resection where culture of the drainage specimen or resected material grew *Burkholderia pseudomallei* or where no specimen was sent for culture; clock is not reset if specimen is culture-negative.
[e]Most commonly manifesting as mycotic aneurysm.
CNS, Central nervous system; *ICU,* intensive care unit.

added to ceftazidime is superior to ceftazidime alone.[121] Although the addition of TMP-SMZ conferred no survival benefit, the excellent tissue penetration of TMP-SMZ is the rationale for recommending combination therapy in neurologic, cutaneous, bone and joint, and prostatic melioidosis.

After initial favorable reports of use of amoxicillin-clavulanate, another randomized comparative trial in Thailand showed that initial therapy with high-dose intravenous amoxicillin-clavulanate is as effective as ceftazidime in preventing deaths in patients with severe melioidosis.[122] However, when amoxicillin-clavulanate was continued as eradication therapy (see "Subsequent Eradication Therapy"), treatment failure was more frequent.

The carbapenems imipenem and meropenem have the lowest minimal inhibitory concentrations against *B. pseudomallei.* Furthermore, in vitro time-kill studies to measure the rate of bacterial killing showed the carbapenems to perform better against *B. pseudomallei* than ceftazidime.[123,124] High-dose imipenem was shown in another comparative trial from Thailand to be at least as effective as ceftazidime for severe melioidosis, with no differences in mortality between the groups and with fewer treatment failures in patients given imipenem.[125] Observational data from Australia suggested that meropenem produces better outcomes in severe melioidosis than ceftazidime, which led to the recommendation that meropenem be the drug of choice for melioidosis septic shock.[126,127]

The duration of initial intensive therapy should be at least 10 to 14 days, with longer treatment required for critically ill patients or for patients with extensive pulmonary disease, deep-seated collections or organ abscesses, osteomyelitis, septic arthritis, and neurologic melioidosis. Even with the newer regimens, the therapeutic response can be slow, with median time to defervescence up to 9 days and longer times seen in patients with deep-seated abscesses.

A more recent analysis of outcomes of therapy based on duration of the intravenous phase of therapy supports recommending a longer minimum intensive phase duration for many cases of melioidosis.[128] The current Australian recommendations for the duration of initial intensive intravenous therapy are listed in Table 221.4.

Ceftazidime infusions (6 g over 24 hours, adult dose) through a peripherally inserted central catheter (PICC line) using an elastomeric infusion device (Baxter, Sydney, Australia) have enabled early hospital discharge for in-home therapy.[129] The absence of any postantibiotic effect with ceftazidime gives such a continuous infusion a theoretical advantage over intermittent dosing.

Subsequent Eradication Therapy

After initial intensive therapy using ceftazidime, imipenem, or meropenem, possibly in combination with TMP-SMZ, subsequent eradication therapy is considered necessary for preventing recrudescence or later relapse of melioidosis. Earlier molecular typing of isolates from patients with recurrent melioidosis showed that most cases were true relapses from failed eradication rather than new infection.[83] However, a more recent study from northern Australia has documented that over the past decade, recurrent melioidosis has become very uncommon, and molecular typing of isolates from recurrent melioidosis shows a reversal of attribution for recurrent melioidosis from predominantly relapse to predominantly reinfection. The decrease in relapse cases was largely attributed to longer duration of the initial intravenous therapy for many of the patients with more severe disease.[130]

Reasons for failure of eradication therapy include the following:

1. One important factor responsible for recrudescences or relapses of melioidosis is poor compliance with eradication therapy.
2. Relapses were found to be 4.7 times (95% CI, 1.6–14.1) more common in patients with severe disease than in patients with localized melioidosis.[131] This emphasizes the importance of a sufficiently long duration of preceding intravenous intensive therapy. Positive blood cultures and multifocal disease were also associated with relapse.[83]
3. Use of ceftazidime in initial intensive therapy was also associated with a halving of relapses.[131]
4. Duration of eradication therapy is also crucial; relapses after oral therapy of 8 weeks or less are more likely than if eradication therapy is given for longer than 12 weeks.[83,131]
5. The choice of agents for eradication therapy is important. Both amoxicillin-clavulanate and oral quinolones (ciprofloxacin or levofloxacin) have been found to be less effective in preventing relapse than previous conventional eradication therapy with chloramphenicol (given usually only for the first 4–8 weeks), TMP-SMZ, and doxycycline.[83] Amoxicillin-clavulanate is recommended for eradication in patients in the first trimester of pregnancy, patients intolerant of TMP-SMZ, or patients with a *B. pseudomallei* isolate confirmed as resistant to TMP-SMZ, with dosing guidelines published.[132] Quinolones should not be considered as first-line agents for melioidosis, with in vitro susceptibility testing generally showing resistance or intermediate results. A trial of eradication therapy involved a comparison of doxycycline alone versus conventional chloramphenicol (first 4 weeks only), TMP-SMZ, and doxycycline combination therapy.[133] Relapses were significantly more common in the doxycycline alone group, resulting in a recommendation that doxycycline not be used alone as first-line eradication therapy.

A randomized trial found no benefit in adding chloramphenicol to TMP-SMZ plus doxycycline for the eradication phase.[134] It has been suggested that TMP-SMZ is the critical component for eradication

therapy, and prospective studies in Australia using TMP-SMZ alone have supported this because relapses occurred almost exclusively in noncompliant patients. A randomized, multicenter, double-blind trial in Thailand confirmed that it is not beneficial to add doxycycline to TMP-SMZ and that TMP-SMZ alone is generally the eradication therapy of choice for melioidosis.[135] Interpretation of disk diffusion sensitivity testing has been problematic for TMP-SMZ, and agar dilution methods have confirmed that the vast majority of *B. pseudomallei* isolates are sensitive to TMP-SMZ. Studies from Thailand, Laos, and Cambodia have confirmed very low rates of resistance to TMP-SMZ, reversing prior perceptions of higher resistance rates in Southeast Asia than in Australia.[136,137] Therefore eradication therapy with TMP-SMZ alone can now be considered the global recommendation. Current Australian recommendations for the duration of oral eradication therapy are listed in Table 221.4.[128]

Adjunctive Therapy

Surgical drainage of large abscesses is indicated, but this is usually not necessary or possible for multiple small abscesses in the spleen and liver. Parotid abscesses require careful incision and drainage. Prostatic abscesses can often be drained under ultrasound guidance using a rectal probe, with transurethral resection reserved for failures of the simpler procedure.

State-of-the-art intensive care management has resulted in decreased mortality in patients with melioidosis septic shock. The possible primary role of neutrophil function in containing *B. pseudomallei* has led to empirical use of granulocyte colony-stimulating factor (G-CSF) in patients with strictly defined septic shock, with observational data from Australia showing a significant improvement in survival with G-CSF.[138] Nevertheless, a randomized controlled trial in Thailand has shown no survival benefit of G-CSF in that location.[139]

Prevention

Primary prevention involves education in endemic areas about minimizing exposure to wet season soils, surface water, and potential aerosols during windy monsoonal rains, especially for patients with diabetes and patients on immunosuppressive therapy, most importantly high-dose or prolonged corticosteroids. Footwear and gloves while gardening are recommended in northern Australia, but preventing occupational exposure in rice farmers may be unrealistic in Southeast Asia. Patients with cystic fibrosis should consider avoiding travel to high-risk areas.

Laboratory-acquired infections, person-to-person spread, and zoonotic infection all are very uncommon, but secondary prophylaxis with TMP-SMZ, doxycycline, or amoxicillin-clavulanate could be considered for exceptional circumstances, especially if the exposed person is diabetic or has other risk factors for melioidosis. Guidelines for management of accidental laboratory exposure have been published.[119,140] Isolation of patients is recommended only for patients with severe suppurative pneumonia with productive sputum.

Concerns of possible bioterrorism using the bacterium or its virulence components in genetically engineered constructs and of exposure of military personnel to *B. pseudomallei* have driven funding for research. Development of a melioidosis vaccine could also have substantial benefits for people living in endemic regions and for commercial livestock, although cost will be a major impediment to availability. Preliminary studies have included various conjugate, live-attenuated, and heterologous vaccine candidates.[1,141,142]

GLANDERS

Glanders is a highly communicable disease of solipeds (horses, donkeys, and mules) that is caused by *B. mallei*. It can be transmitted to other animals and to humans.

History

Glanders was described by Hippocrates and has long been recognized as an occupational risk for horse handlers, veterinarians, equine butchers, and laboratory workers. Together with anthrax, glanders was involved in the first modern use of microbes as weapons when German agents targeted horses in the United States, Romania, Spain, Norway, and Argentina between 1915 and 1918.[143]

Etiology

B. mallei is a small, gram-negative, oxidase-positive, aerobic bacillus. In contrast to *B. pseudomallei,* it is nonmotile. It is a host-adapted pathogen that, in contrast to *B. pseudomallei,* does not persist in the environment outside its equine host. The *Burkholderia* genome projects and multilocus sequence typing have supported the idea that *B. mallei* evolved in animals from the environmental pathogen *B. pseudomallei.*[144,145]

Epidemiology, Transmission, and Pathogenesis

With quarantine and other control measures, glanders has been eradicated from most countries. Enzootic foci continue in the Middle East, Asia, Africa, and South and Central America, with increasing outbreaks in some locations over the last decade. In addition to disease in equines, glanders has occurred in cats and other carnivores eating infected horse meat. Inhalation and percutaneous inoculation also occur. Discharges from the horse respiratory tract and skin are highly infectious. *B. mallei* has much greater potential for zoonotic transmission than *B. pseudomallei,* and the risk of laboratory-acquired infection also appears to be greater for *B. mallei.*[146,147] The incubation period can range from 1 to 2 days (e.g., with inhalation) to many months, and as occurs with melioidosis, reactivation from a latent focus after many years has been described.

There are many parallels with the pathogenesis of *B. pseudomallei,* with studies showing the *B. mallei* extracellular polysaccharide capsule to be a critical determinant of virulence.[70,148,149] There is differential susceptibility among animals, and although it is likely that diabetic patients are more susceptible to infection and disease progression with *B. mallei,*[147] the human host risk factors are less well defined than for melioidosis.

Clinical Manifestations

Knowledge of the disease in horses is useful for understanding the potential for zoonotic transmission to humans. In acute glanders in horses, fever is accompanied by necrotic ulcers and nodules in the nasal passages that result in copious, infectious, sticky yellow discharges. Neck and mediastinal lymph nodes are enlarged, and pneumonia with nodular abscesses and dissemination to internal organs can accompany the progressive deterioration. In cutaneous glanders (known as farcy), nodular lymphatic or skin abscesses (0.5–2.5 cm) occur and ulcerate, discharging infectious, oily yellow pus.[150]

Human glanders, similar to melioidosis, can be acute or chronic, with mode of infection, inoculating dose, and host risk factors determining the clinical course. With inhaled organisms (respiratory inoculation), acute febrile illness with ulcerative necrosis of the tracheobronchial tree can occur, with mucopurulent discharge involving the nose, lips, and eyes. Lobar or bronchial pneumonia, neck and mediastinal lymphadenopathy, pustular skin lesions, and septicemia with dissemination to internal organs can follow.[151] Historically, without antibiotics, death within 10 days usually occurred, but a more chronic pneumonic illness was also recognized after inhalation of *B. mallei.*[146]

After percutaneous inoculation, local skin nodules that can suppurate and regional lymphadenopathy occur, often accompanied by fever, rigors, and malaise.[147,151] Regional lymphadenopathy is much more common than with melioidosis. Lymphatic tract nodules and suppurating abscesses in the lymph nodes are common after several weeks in untreated cases. Dissemination at 1 to 4 weeks can result in infection in almost any tissue, with spleen and liver abscesses, pneumonia, lung abscesses, pleural nodules, and multiple subcutaneous and muscle abscesses all being common (Fig. 221.19). Central nervous system infection can also occur.

Laboratory Diagnosis

Definitive diagnosis of glanders requires a positive culture of *B. mallei.* Blood, exudates, and pus from abscesses should be cultured on standard media. Organisms are often very scanty in exudates and pus and are morphologically indistinguishable from *B. pseudomallei* organisms. As occurs with *B. pseudomallei,* some commercial identification systems may misidentify *B. mallei* as a *Pseudomonas* spp., and 16S ribosomal RNA gene–sequenced analysis or a *B. mallei*–specific polymerase chain reaction assay may be required for confirmation.[147] Currently available complement fixation tests and ELISA serologic assays cannot distinguish



FIG. 221.19 Computed tomography scan of a patient with laboratory-acquired glanders showing abscesses *(arrows)* in the liver and spleen. *(From Srinivasan A, Kraus CN, DeShazer D, et al. Glanders in a military research microbiologist.* N Engl J Med. *2001;345:256–258.)*

B. mallei from *B. pseudomallei,* but new assays based on recombinant proteins show promise in distinguishing the two.[152] The mallein skin test has been used extensively in animal control programs and has been modified for human diagnosis but has poor specificity.[146]

Therapy

The antibiotic susceptibility profile of *B. mallei* resembles that of *B. pseudomallei* except that gentamicin and newer macrolides (e.g., clarithromycin, azithromycin) are active against *B. mallei,* but not *B. pseudomallei.*[153] Although response to treatment with older regimens was often slow, rapid improvement occurred in a US military researcher with laboratory-acquired infection who was treated with imipenem and doxycycline.[147] This was the first reported case of glanders in the United States in more than 50 years. Recommended treatment and duration are the same as for melioidosis.[119]

Prevention

Prevention depends on control of glanders in the equine species and strict precautions to prevent laboratory-acquired infection.[146,147] In contrast to the case with melioidosis, isolation of all infected persons is recommended to prevent person-to-person spread. Guidelines for the management of accidental laboratory exposure have been published.[119,140] As with melioidosis, much research is being done toward a vaccine to prevent disease in humans.[154,155]

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222 *Acinetobacter* Species

Sarah Hochman and Michael Phillips

SHORT VIEW SUMMARY

Definition and Epidemiology

- *Acinetobacter* species are ubiquitous in soil and water, and are increasingly recognized to infect animals and ectoparasites, raising potential new sources of human exposure.
- *Acinetobacter baumannii* and the closely related and phenotypically indistinguishable *Acinetobacter pittii* and *Acinetobacter nosocomialis* cause the bulk of human infections and typically are acquired in the health care setting.
- *Acinetobacter* species readily incorporate multiple resistance mechanisms, and pan-resistant strains have established within the health care setting.

Therapy

- Treatment is based on susceptibility testing.
- Bacteriophages show promise as a novel form of therapy.

Prevention

- Prevention of *Acinetobacter* transmission in health care settings requires a multifactorial approach with environmental disinfection and hand hygiene as the cornerstone.
- The emergence of *A. baumannii* strains resistant to essentially all potent antimicrobial agents, coupled with the dearth of antibiotics in development, constitutes a significant public health threat.
- Vaccines, immunotherapy, or both will be important measures in the era of pan-resistant *Acinetobacter* strains.

Acinetobacter, an aerobic, catalase-positive, oxidase-negative, gram-negative coccobacillus, was first described in 1911, but the initial description of the taxonomy of this diverse species was not published until 1986.[1,2] Ubiquitous in nature, the 54 species of the genus *Acinetobacter* are associated with a specific ecologic niche that shapes their genomic contents.[3–6] (Table 222.1) *Acinetobacter baumannii* is the most virulent species and causes the bulk of human infections, but *Acinetobacter pittii*, *Acinetobacter nosocomialis, Acinetobacter lwoffii,* and *Acinetobacter radioresistens* are also significant nosocomial pathogens.[5] In the late 1980s, *A. baumannii* emerged as an important human pathogen exhibiting increased antimicrobial resistance.[7,8] Whole-genome sequencing analysis has suggested that the rapid spread of multidrug-resistant *A. baumannii* was associated with the ability to incorporate virulence and resistance determinants[9] (Fig. 222.1). The establishment of multidrug-resistant *A. baumannii* within the health care ecosystem has a tremendous cost, both financially and on patient safety. Among the gram-negative pathogens causing bacteremia, *A. baumanii* exhibits the highest rate of nonsusceptibility, with over 18% of isolates resistant to all first-line agents, including the carbapenems, β-lactams, and fluoroquinolones.[10] In the United States, over 12,000 *Acinetobacter* infections are estimated to occur annually, causing over 1300 deaths and costing society $1.6 billion dollars.[11] When compared to other multidrug-resistant gram-negative bacteria and methicillin-resistant *Staphylococcus aureus*, *Acinetobacter* infections had the highest risk for mortality at 30 and 90 days after isolation from culture.[12] These statistics, coupled with a paucity of potent antimicrobials in phase II or III of development, emphasize the importance of infection prevention efforts and the need to develop novel therapeutics and vaccines.

EPIDEMIOLOGY

Health Care–Associated Infections

Health care–associated infections represent the most substantial public health impact of *Acinetobacter,* given the rapid spread of strains resistant to all first-line antimicrobials. The application of molecular typing methods has revealed that a limited number of widespread clonal lineages of *A. baumannii* are responsible for hospital outbreaks worldwide.[13–15] Although increasing globally, the prevalence of carbapenem-resistant *A. baumannii* is decreasing over time in developed countries, suggesting that patients presenting for care after travel from areas with higher endemic rates should be considered for screening.[16–23] *A. baumannii* causes the bulk of health care–associated infections due to *Acinetobacter,* but a variety of species, including *A. pittii, A. nosocomialis, A. lwoffii,* and *Acinetobacter ursingii,* are emerging as nosocomial pathogens, especially in immunocompromised hosts.[3,5,24] Ventilator-associated pneumonia is the most frequent health care–associated *A. baumannii* infection, implicated in 3% to 7% of cases.[25,26] Among patients requiring mechanical ventilation for more than 5 days, the frequency of *Acinetobacter* increases dramatically, accounting for 26% of respiratory infections in one series.[27] Other nosocomial manifestations of *Acinetobacter* include bloodstream infections associated with intravascular catheters, surgical site infections, urinary tract infections, meningitis after neurosurgery, and soft tissue infections after burns.[5,25,28–30]

The factors that promote the emergence and transmission of *A. baumannii* in health care settings include hospitalization of patients at high risk for colonization, such as long-term care residents; breaches in environmental cleaning and disinfection; and antibiotic utilization, especially third-generation cephalosporins, fluoroquinolones, or carbapenems.[8,31–36] The ability of *Acinetobacter* species to survive for weeks on surfaces within the hospital environment leads to prolonged outbreaks, and patient movement between health care facilities without the intervention of adequate communication results in regional spread.[37–39] Essentially any surface within a patient care area can become contaminated with *Acinetobacter* and serve as a reservoir for ongoing transmission; these include sinks, faucets, humidifiers, hydrotherapy pools, curtains, pillows, and bedrails, as well as equipment such as supply carts, infusion pumps, and equipment control touch pads.[40–45] Patients with either recent or remote history of infection can remain colonized and able to contaminate their surrounding environment.[40]

Transmission of *Acinetobacter* within the health care setting occurs after lapses in proper hand hygiene, and failure to disinfect mobile medical equipment and surfaces within patient care areas.[46–48] Units with multiple-bedded rooms and susceptible patients, such as neonatal intensive care units (ICUs), are at high risk for outbreaks.[49–52] Procedures that result in a spray of contaminated fluids, such as pulsatile lavage of wounds or bronchoscopy, may also lead to heavy environmental contamination and transmission.[53,54] In addition to contaminated surfaces,

TABLE 222.1 Named *Acinetobacter* Species

SPECIES	TYPICAL HABITAT
A. albensis	Water, soil
A. antitratus	Animals
A. antiviralis	Plants
A. baumannii	Water, soil, humans, animals, food
A. baylyi	Water
A. bereziniae	Soil, food
A. bohemicus	Water, soil
A. bouvetii	Water
A. brisouii	Soil
A. calcoaceticus	Water, soil, humans, animals, food
A. calcoaceticus–A. baumannii complex	Water, soil
A. gerneri	Water
A. grimontii	Water
A. guangdongensis	Soil
A. guillouiae	Food
A. gyllenbergii	Food
A. haemolyticus	Soil, humans
A. indicus	Soil
A. johnsonii	Water, soil, humans, animals, food
A. junii	Water, soil, humans
A. kookii	Soil
A. kyongiensis	Water
A. lwoffii	Water, soil, humans, animals, food
A. nosocomialis	Soil, food
A. oleivorans	Plants, soil
A. pakistanensis	Water
A. parvus	Soil, food
A. pittii	Soil, food
A. populi	Plants
A. puyangensis	Plants
A. qingfengensis	Plants
A. radioresistens	Soil, animals, food
A. rudis	Water
A. schindleri	Animals, soil
A. seifertii	Food
A. seohaensis	Water
A. soli	Soil, food
A. tandoii	Water, soil
A. tjernbergiae	Water
A. towneri	Water
A. ursingii	Humans, food
A. venetianus	Water

Modified from Adewoyin MA, Okoh AI. The natural environment as a reservoir of pathogenic and non-pathogenic Acinetobacter *species.* Rev Environ Health. *2018;33:265–272.*

airborne particles are believed to play a role in transmission of *Acinetobacter,* either by spread through open units with multiple beds or through contamination of internal air filters of medical equipment.[55–57] An increase in health care–associated *Acinetobacter* infections during warmer, more humid months has been reported, potentially due to contamination of air handling systems.[58,59]

The integration of whole-genome sequencing with epidemiologic data such as patient movement and health care environment exposures is needed to determine transmission routes in an outbreak, and should become the standard.[60] In order to appropriately utilize whole-genome sequencing in an outbreak, an understanding of the endemic strains within a facility is needed.[61]

Community-Associated Infections

Acinetobacter pneumonia is a rare but serious cause of community-acquired pneumonia in tropical regions during the summer months, and often presents with respiratory failure and shock.[62–66] Community-onset bacteremia is typically associated with respiratory infections, and is also associated with a worse outcome compared with hospital-onset infections.[67] Community-acquired *Acinetobacter* meningitis in patients without underlying medical compromise has been rarely reported; outcomes in those who received prompt therapy were favorable.[68,69] *A. baumannii* skin colonization and invasive soft tissue infections have been associated with warfare, natural disasters, and societal disruptions.[70–73] The environmental source of these community infections, which typically present in tropical or warm regions, is unknown. In addition to soil, there is an increasing appreciation for the potential role of contaminated food, infected head and body lice, colonized pets or other animals, and hospital wastewater as environmental reservoirs of *Acinetobacter.*[14,74–87]

DIAGNOSIS

Acinetobacter is readily isolated with standard culture media, but differentiation of species based on phenotype alone is difficult, leading to the term *Acinetobacter calcoaceticus–Acinetobacter baumannii complex.*[88] The use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry allows rapid identification of *Acinetobacter* species and of the presence of resistance mechanisms. Once species are identified, a rapidly expanding number of polymerase chain reaction assays are commercially available to identify the presence of β-lactamase and carbapenemase genes.[89,90] The use of colorimetric assays and quantitative real-time polymerase chain reaction has also been used to detect antimicrobial resistance in *A. baumannii.*[91–93] The presence of heteroresistance to carbapenems has been identified in *A. baumannii,* raising the potential for breakthrough or relapsed infection.[94,95]

CLINICAL MANIFESTATIONS

Acinetobacter is a leading cause of ventilator-associated pneumonia, with increased mortality rates seen in infections caused by carbapenem-resistant strains and patients receiving initial inappropriate therapy.[96,97] When *Acinetobacter* is isolated from a pulmonary specimen, differentiation between colonization and pneumonia is critically important to avoid unnecessary antibiotic treatments with the attendant risk of emergence of toxicity as well as antimicrobial resistance.[98] The measurement of volatile organic compounds may prove to be a useful diagnostic tool and allow for differentiation between colonization and invasive infection, although this method remains in the early stages of investigation.[99] *A. baumannii* was independently associated with increased mortality compared to *A. nosocomialis* in patients with health care–associated pneumonia complicated by bacteremia, in spite of their close genetic relatedness.[100]

Acinetobacter species account for 1% to 2% of all bloodstream infections and are typically associated with intravascular devices or pneumonia, with the majority caused by *A. baumannii,* followed by *A. nosocomialis* and *A. pittii.*[101,102] The mortality associated with *A. baumannii* bacteremia is the highest, followed by *A. nosocomialis* and *A. pittii* bacteremia; other species such as *A. lwoffii* and *Acinetobacter junii* have low bacteremia-associated mortality.[101,103–106]

Acinetobacter causes about 1% of urinary tract infections, most of which are caused by strains with the ability to form biofilms on urinary catheters.[25,107] Outbreaks of *A. baumannii* meningitis in postsurgical neurosurgery patients secondary to breaches in infection prevention measures have been reported.[28,108,109] Community-associated *Acinetobacter* meningitis in immunocompetent patients without surgical procedures have rarely been reported.[68,69] *A. baumannii* skin and soft tissue infections after burns and natural and wartime traumatic injuries occur and can be related to exposure at the time of injury or to exposure after

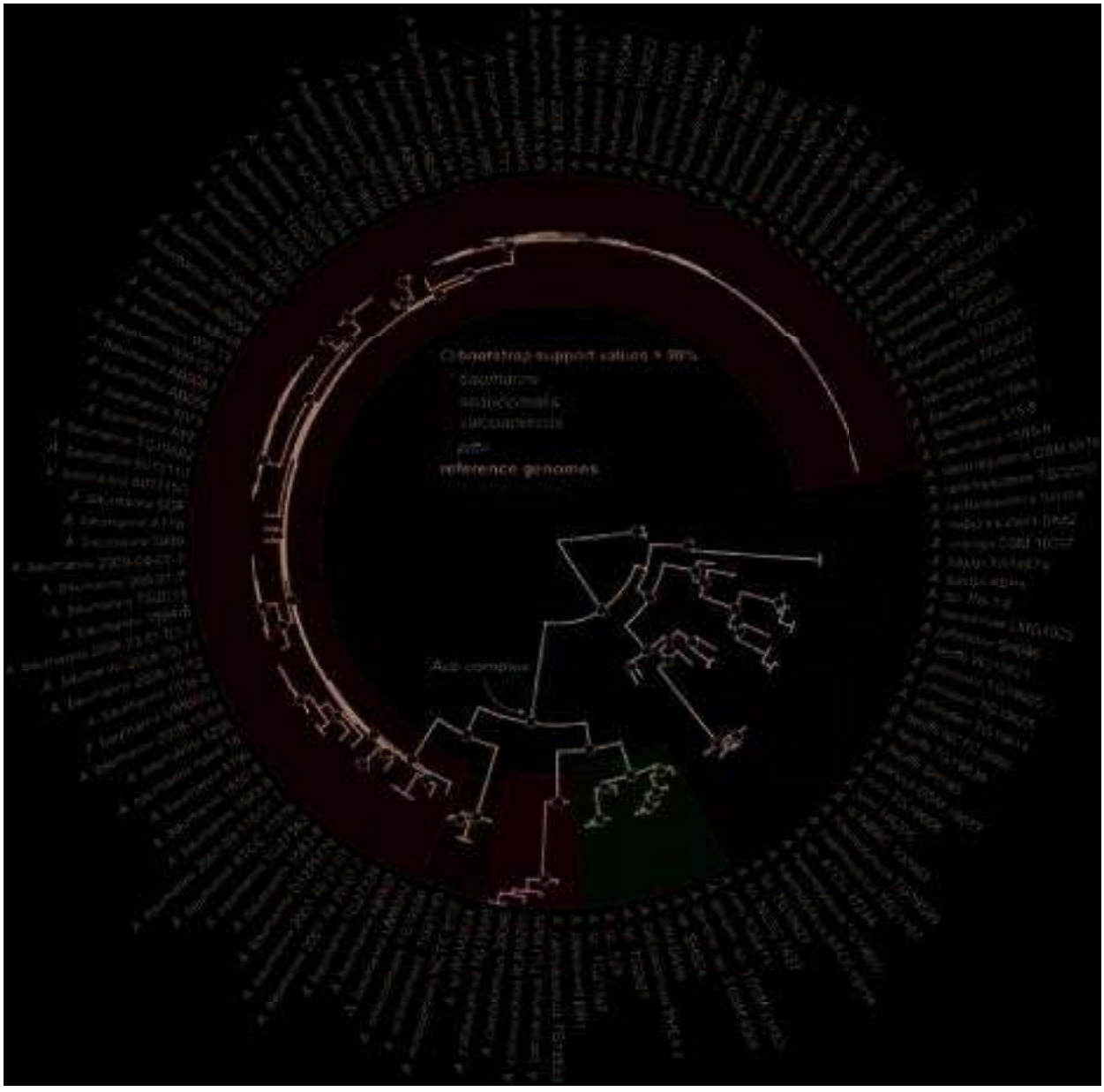

FIG. 222.1 Whole-genome phylogeny of 136 sequenced genomes in the genus *Acinetobacter.* The phylogeny was inferred with FastTree2 on a single nucleotide polymorphism (SNP) matrix alignment calculated with kSNP and filtered with noisy. The phylogeny was rooted with *A. radioresistens.* Genomes in the *Acinetobacter calcoaceticus-baumannii* (Acb) complex are colored by clade. *(From Sahl JW, Gillece JD, Schupp JM, et al. Evolution of a pathogen: a comparative genomics analysis identifies a genetic pathway to pathogenesis in* Acinetobacter. PLoS One. *2013;8:e54287.)*

hospitalization.[29,70,71,110,111] High rates of asymptomatic nasal colonization have been identified in long-term care facilities, among elderly residents and the health care workers who care for them.[112] Cocolonization with *Neisseria* species may result in prolonged colonization with *A. baumannii,* suggesting that microbial interactions may play a role in transmission.[113]

PATHOGENESIS AND ANTIMICROBIAL RESISTANCE

The pathogenicity of *A. baumannii* relates to its ability to colonize and form biofilm on mucosal surfaces and medical devices, to survive in iron-limited environments within the host, and to acquire foreign genetic material to enhance survival and increase resistance to antimicrobial agents.[114–116] An essential precursor to biofilm formation is the generation of pili on the cell surface, leading to adhesion.[117] The production of *Acinetobacter* biofilm is controlled by environmental conditions, and a favorable milieu results in the overexpression of more than 1600 genes compared to 55 in planktonic bacteria, leading to important changes in cell metabolism, motility, iron acquisition, and quorum sensing.[118] Specific genes regulating the virulence of the bacteria, such as those that regulate creation of pili, motility, and formation of biofilm, have been described.[117,119,120] Further assessment of clinical *A. baumannii* isolates reveals a correlation between antibiotic-resistant phenotypes and the ability to form biofilms, and specific strains are associated with increased mortality.[121–123] Antibiotic exposure plays an important role in occurrence of antibiotic-resistant *A. baumannii,* especially exposure to carbapenems.[124] The spontaneous mutation rates of strains of antibiotic-resistant *A. baumannii* vary widely, but rare isolates with thousandfold higher mutation rates raise concern for heteroresistance and treatment failure.[125] Neutrophils, recruited by natural killer cells, play the predominant role in host immune response to *Acinetobacter* infection.[126,127] Host factors, such as serum albumin and vitamin D deficiency, may play a role in patient mortality.[128,129]

Acinetobacter is well known for its multitude of antimicrobial resistance mechanisms, which are associated with an increase in genome size[9] (Fig. 222.2). Genomic analysis has shown that pathogenic strains of *A. baumannii* contain genes clustered on resistance islands, whose

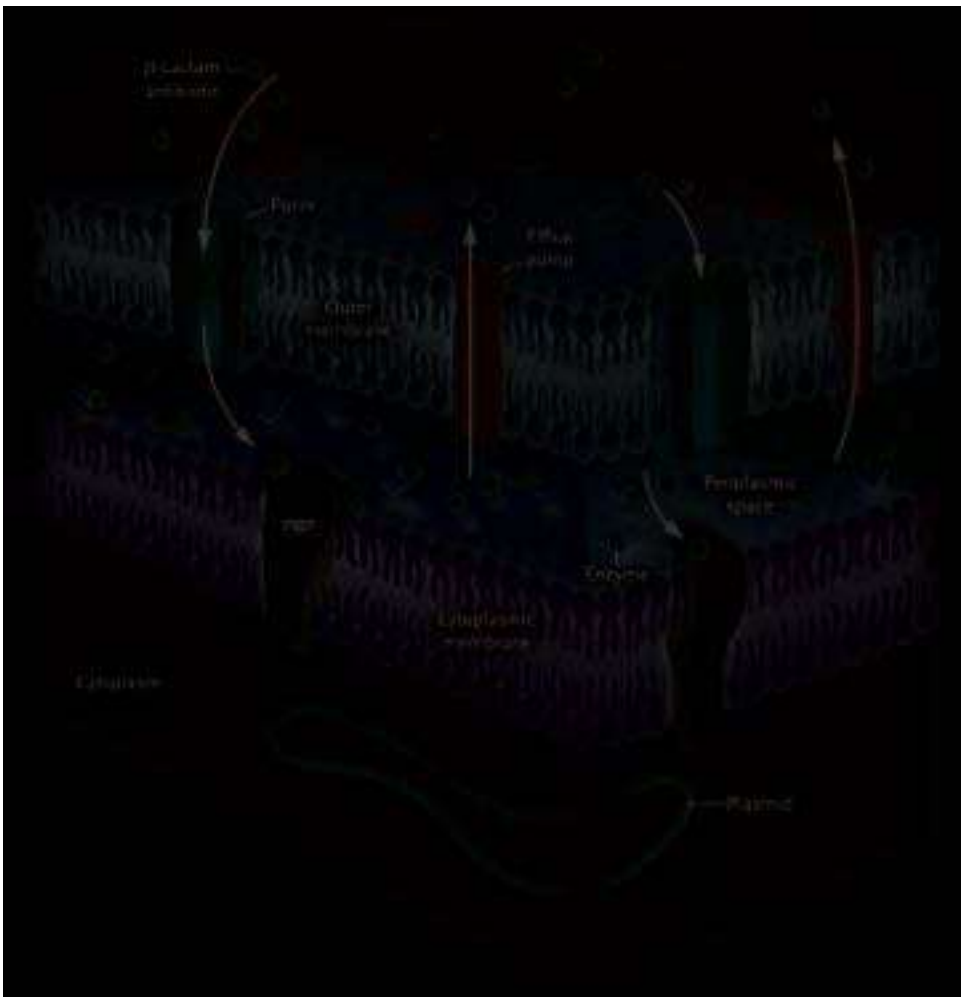

FIG. 222.2 *Acinetobacter* resistance mechanisms. *Acinetobacter,* like other gram-negative bacteria, has an outer membrane and a cytoplasmic membrane, between which (the periplasmic space) β-lactamases (carbapenemases, Ambler class C β-lactamases, and extended-spectrum β-lactamases) reside. Penicillin-binding proteins (*PBPs*), located at the level of the cytoplasmic membrane, constitute the final targets of β-lactam antibiotics. To bind to these targets, antibiotics must traverse the outer membrane through porin channels (outer membrane proteins) into the periplasmic space. Once in the periplasmic space, β-lactam antibiotics bind to PBPs or are actively expelled from the bacterial structure through efflux pumps. *Acinetobacter* can harbor integrons and transposons, genetic elements on the bacterial chromosome or on plasmids, that can carry multiple cassettes with resistant genes (e.g., extended-spectrum β-lactamases and metallo-β-lactamases). *(From Munoz-Price LS, Weinstein RA.* Acinetobacter *infection.* N Engl J Med. *2008;358:1271-1281.)*

structure may facilitate the acquisition of resistance mechanisms from other species of bacteria.[130,131] Insertion sequences such as IS*Aba1* within the *Acinetobacter* genome promote the expression of neighboring genes and result in the overexpression of several key resistance mechanisms. Additionally, it has been postulated that the ability of *Acinetobacter* to acquire resistance determinants more effectively than other bacteria may be due to the close association of several *Acinetobacter* species to the soil and water environment, which contains a large reservoir of resistance genes.[132]

Acinetobacter exerts much of its antibiotic resistance through the expression of β-lactamases. Group 1 AmpC β-lactamases are chromosomally encoded cephalosporinases that hydrolyze penicillins and first-, second-, and third-generation cephalosporins, including ceftazidime, cefotaxime, and ceftriaxone. Rates of hydrolysis of fourth-generation cephalosporins, such as cefepime, and carbapenems by AmpC enzymes are low.[133]

AmpC is not inducible in *A. baumannii* as it is in some Enterobacteriaceae, but the presence of the promoter sequence IS*Aba1* increases expression of this enzyme.[134,135] Groups 2b and 2c Ambler class A β-lactamases are encoded on genes carried by large plasmids, and confer resistance to penicillins and narrow-spectrum cephalosporins.[136,137] The emergence and rapid worldwide spread of strains containing the group 2be Ambler class A extended-spectrum β-lactamases (ESBLs) in the 1980s represented the first, rapid global spread of multidrug-resistant *A. baumannii.* These ESBLs exhibit hydrolytic activity over penicillins and all cephalosporins, thereby leading to the use of carbapenems as treatment for significant *Acinetobacter* infection; this resulted in the subsequent emergence of the group 2d Ambler class D oxacillinases. This class of oxacillinases confers resistance to carbapenems, and has resulted in a marked increase in carbapenem resistance due to the widespread distribution of the successfully spreading international clonal complexes (ICCs), ICC-I, ICC-II, and ICC-III.[138–142] The episodic emergence of clades distinct from the three ICCs associated with high virulence represents an additional threat to public health.[143] Insertion sequence IS*Aba1* is a promoter for genes encoding oxacillinases in *Acinetobacter.*[144,145] The oxacillinase bla (OXA-51-like) is intrinsic and chromosomal, while the acquired OXA subclasses, called carbapenem-hydrolyzing class D β-lactamases, are found both on the chromosome and on plasmids, and include the ubiquitous 23-like oxacillinase as well as many others.[138,146,147] The group 3 Ambler class B metallo-β-lactamases imipenemase (IMP), Verona integron–encoded (VIM), and New Delhi 1 (NDM-1) and New Delhi 2 (NDM-2) are a less frequent cause of carbapenem resistance but continue to spread worldwide.[148–151]

The second most important determinant of drug resistance in *Acinetobacter* is the presence of efflux pumps, which confer resistance to β-lactam antibiotics, chloramphenicol, macrolides, tetracyclines,

tigecycline, aminoglycosides, polymyxin, and certain antiseptics. Efflux pumps in the resistance-nodulation-division (RND) family are located on chromosomes and are overexpressed after genetic mutation and induced by tigecycline.[152,153] The predominant pump expressed in this family is AdeABC, although others have been described.[154] Non-RND efflux systems seen in *Acinetobacter* are encoded by mobile genetic elements and also play a role in antimicrobial resistance.[153] Antiseptic resistance is also encoded by efflux pumps; a small multidrug resistance (SMR) efflux pump may confer quaternary ammonium resistance, and RND efflux pumps result in biocide resistance.[155,156]

Aminoglycoside resistance in *Acinetobacter* species is determined by the presence of aminoglycoside-modifying enzymes (AMEs). These enzymes either phosphorylate, acetylate, or adenylate aminoglycoside molecules and decrease their binding affinity to the ribosomal subunit.[157] AMEs are encoded on genetically mobile elements, especially class 1 integrons, which frequently also contain genetic elements for ESBLs and metallo-β-lactamases and are reported to cause outbreaks with high mortality[158] (Fig. 222.3).

The expression of porins modifies the ability of antimicrobials to permeate the outer membrane of the bacterial cell wall; OmpAab is the principal outer membrane protein (Omp) in *Acinetobacter* and confers resistance to β-lactams and carbapenems. OmpAab has been characterized in *A. radioresistens, A. junii,* and *A. baumannii.*[159,160]

Acinetobacter resistance to fluoroquinolones is multifactorial. Mutations in the quinolone resistance-determining regions (QRDRs) lower fluoroquinolone binding to bacterial DNA gyrase and topoisomerase IV; when combined with upregulation of the AdeABC efflux pump, quinolone resistance results.[161,162]

Sulbactam, a class A β-lactamase inhibitor, has inherent antibacterial activity against *Acinetobacter* species via binding to penicillin-binding proteins. Mutations of these penicillin-binding proteins, plus additional mechanisms resulting in the overexpression of β-lactamases, likely result in sulbactam resistance.[163,164]

Resistance to the polymyxins, which include colistin and polymyxin B, is associated with both mutations in the genes encoding the two-component polymyxin regulatory system PmrA and PmrB, as well as a reduction in lipopolysaccharides in the *Acinetobacter* cell wall, resulting in less negative charge and loss of antimicrobial affinity.[165,166] The emergence of colistin resistance has resulted in pan-resistant *A. baumannii,* with evidence of regional transmission.[167]

TREATMENT

The selection of empirical and targeted therapy for *Acinetobacter* is driven by patient risk for multidrug-resistant strains, local epidemiology, site of infection, and results of antibiotic susceptibility testing. Before initiating antibiotic therapy, the clinician must determine whether the *Acinetobacter* isolated from culture represents invasive infection or colonization; this will help to reduce overutilization of antibiotics and minimize the subsequent risk of multidrug-resistant *Acinetobacter* infection.[34]

β-Lactam Antibiotics

β-Lactam antibiotics are the drugs of choice for susceptible *Acinetobacter* infections, as they are rapidly bactericidal and have a wide volume of distribution with appropriate dosing. *Acinetobacter* species frequently contain intrinsic β-lactamases that inactivate first- and second-generation cephalosporins and penicillins; however, if sensitivity testing indicates susceptibility, third- and fourth-generation cephalosporins, such as cefepime, ceftriaxone, and cefotaxime, are useful agents.[168] The widespread distribution of ESBLs has led to heavy utilization of carbapenem for hospital-acquired *Acinetobacter* infections, with resultant worldwide emergence of carbapenem-resistant strains.[138,169,170] Cefiderocol, a novel siderophore antibiotic composed of a cephalosporin molecule with a catechol moiety, is stable against all classes of β-lactamases, including carbapenemases, and offers promise of effective therapy for extensively resistant strains of *Acinetobacter.*[171–173]

β-Lactamase Inhibitors

With the widespread distribution of carbapenem-resistant *Acinetobacter,* the use of β-lactamase inhibitors should be considered as a therapeutic option, based on susceptibility testing. Sulbactam has the highest activity of the β-lactamase inhibitors, and when used alone or combined with ampicillin is effective for invasive *Acinetobacter* infections, including pneumonia, bloodstream infections, and meningitis.[174–177] Sulbactam is equally effective in treating pneumonia and bacteremia compared to carbapenems, tigecycline, colistin, and polymyxin B.[178–181] A dosage of at least 4 g of sulbactam per day in divided doses is recommended for most infections in adults, although dosages as high as 9 g in divided doses have been used.[179]

Aminoglycosides

Although susceptibility testing may show that isolates of *Acinetobacter* are susceptible to aminoglycosides, the use of these agents is limited due to low penetration into the lungs and central nervous system, concern that automated tests for *Acinetobacter* susceptibility to aminoglycosides may be inaccurate, and the detection of heteroresistant strains leading to concerns of treatment failure.[182,183]

Tigecycline

Tigecycline, a member of the glycylcycline class of antibiotics, has been successfully used to treat carbapenem-resistant *Acinetobacter* infections, but these results are based on observational studies, and the drug is frequently given in combination with other antibiotics.[184,185] Additional concerns include the large volume of distribution and low serum concentration of tigecycline, which preclude its use for bloodstream infections; reports of the development of resistance while on therapy; and increased mortality when used in combination with colistin.[186–188]

Polymyxins

Colistin and polymyxin B are frequently included in the treatment of carbapenem-resistant *Acinetobacter* infections. Although the optimal dosing of these antibiotics to treat *Acinetobacter* infection has not been determined by randomized trials, evidence suggests that a loading dose, followed by high doses with longer dosing intervals, may improve outcomes[189–191] (see Chapter 32). Colistin heteroresistance in *A. baumannii* may emerge during therapy, but resistant strains appear to be associated

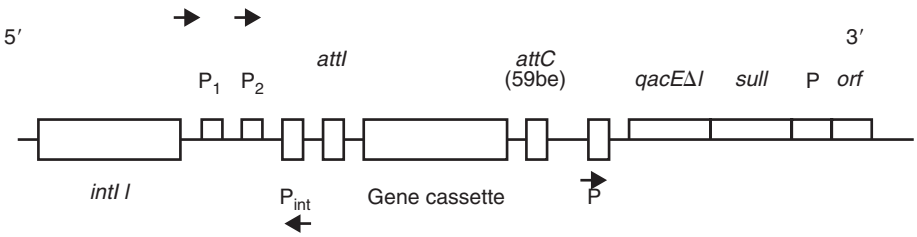

FIG. 222.3 Simplified representation of a class 1 integron. As pictured, integrons contain components of a site-specific recombination system that recognizes and captures mobile gene cassettes. Gene cassettes can be antibiotic resistance genes followed by a repeat sequence called a 59-bp element (*59be*) or *attC*. In *Acinetobacter baumannii,* gene cassettes may contain β-lactamase genes (e.g., *bla*IMP-2, *bla*IMP-4, *bla*VIM-1, and *bla*OXA). *attC,* Sequence in the gene cassette recognized by the integrase; *attI,* integration site; *orf,* open reading frame; *P,* promoter; *P1,* promoter for the gene cassette; *P2,* second promoter; *Pint,* promoter for the integrase; *qacEΔ1,* partially deleted gene that encodes resistance to a quaternary ammonium compound; *sul1,* gene for sulfonamide resistance. *(From Bonomo RA, Szabo D. Mechanisms of multidrug resistance in* Acinetobacter *species and* Pseudomonas aeruginosa. Clin Infect Dis. *2006;43:S49-S56.)*

with decreased fitness and virulence.[192,193] Innate colistin resistance is common in certain *Acinetobacter* species such as *A. junii.*[194]

Combination Treatment

Although reports of the various combinations of colistin, rifampin, carbapenems, sulbactam, minocycline, and tigecycline show promising results, prospective randomized trials are few.[195,196] One multicenter randomized trial allocated patients with carbapenem-resistant *A. baumannii* infection to colistin alone or colistin plus rifampicin. Although microbiologic eradication was significantly shorter in the combination arm, no differences in infection-related death or length of hospitalization were observed.[197] Other studies have failed to show any benefit for combination therapy and may result in increased mortality.[188,196,198]

Given limited treatment choices, the clinician should consider optimization of currently available antimicrobials by using prolonged infusion of β-lactam antibiotics for strains with intermediate susceptibility and local instillation of colistin (e.g., intrathecal or intraventricular for central nervous system infections) when the intravenous formulation provides low tissue concentration at the site of infection.[199] Extended infusion of β-lactam antibiotics allows for maximized time of drug serum concentration above minimal inhibitory concentration for the *Acinetobacter* isolate by increasing the dose and shortening the dosing interval.[200] Continuous infusion results in higher mean trough levels of β-lactams, which may result in better clinical outcomes for less susceptible *Acinetobacter* infections.[201] High-dose colistin with an extended dosing interval may provide benefit in carbapenem-resistant *Acinetobacter* infections, but the data are scant and inconsistent.[196,202] Intrathecal colistin is effective for treatment of drug-resistant *Acinetobacter* central nervous system infection.[199,203] Several studies suggest aerosolized colistin is safe and possibly efficacious for treatment of pneumonia caused by resistant *Acinetobacter* species.[204,205] Unfortunately, not many antibiotics with potency against highly resistant strains of *Acinetobacter* are in phase II or phase III of development.[206]

The use of bacteriophages to treat antimicrobial-resistant *Acinetobacter* infections has shown promise in animal studies, but human trials are needed.[207–209] Finally, development of vaccines is also evolving and provides hope for future options to prevent these infections.[210–214]

PREVENTION

Given the ability of *Acinetobacter* to survive on surfaces for weeks under dry conditions, hand hygiene and the routine disinfection of medical equipment and surfaces touched by patients and staff are the basic but essential steps to prevent transmission in the health care setting.[215–220] Factors that further increase the risk of *Acinetobacter* transmission include high colonization pressure and multiple beds within the same room.[221,222] In addition to emphasizing hand hygiene and cohorting of patients, environmental cleaning by effective disinfectants with appropriate contact times is critical to curb outbreaks.[38,47,223] Multidrug-resistant strains of *A. baumannii* expressing genes encoding porin mutations and efflux pumps resulting in reduced disinfectant efficacy have been reported; however, such strains may have reduced environmental fitness.[224] The ability of *Acinetobacter* to form biofilms on surfaces is an important step for their survival in the environment. Polycarbonate, a material frequently found in health care equipment and surfaces, allows *Acinetobacter* to form biofilm mass more readily than other materials.[225] Measuring the adequacy of the cleaning and disinfection process is important; use of an adenosine triphosphate bioluminescence assay improves performance and is helpful useful to evaluate cleaning protocols.[226,227] Environmental cultures also can play a role in outbreak evaluation, but the location and frequency of sampling may affect results.[228] Moist locations such as drains and sinks may also harbor *Acinetobacter* and serve as a source of infection; routine disinfection of drains or even removal of sinks may be required to halt outbreaks.[229–231]

Evidence of ongoing transmission should prompt consideration of alternative sources of contamination via atypical routes; air sampling in ICUs has yielded *Acinetobacter,* and fans within medical devices and ventilation systems have been implicated in transmission.[55–57,232,233] Stethoscopes may also become contaminated with *Acinetobacter* and potentially transmit infection.[234] Artificial nails on health care workers' hands are known to harbor a multitude of pathogens, including *Acinetobacter,* and should be banned from health care settings.[235,236] Additional vectors, such as contaminated external surfaces of mobile medical equipment and unused medical supplies, may play a role in transmission.[40,237] Whole-genome sequencing has proven to be a useful tool to determine the epidemiologic risk factors associated with infection.[60,238]

Good communication between health care facilities is required to prevent outbreaks due to transfer of patients colonized with multidrug-resistant *Acinetobacter.*[39] Surveillance cultures to identify patients with asymptomatic *Acinetobacter* colonization have a sensitivity between 55% and 89% depending on site and method of sampling, and may be considered when patients with epidemiologic risk factors, such as previous exposure to health care settings in countries with a high prevalence of multidrug-resistant *Acinetobacter.*[22,23] Those colonized with *Acinetobacter* upon admission to the ICU are at significantly higher risk of subsequent infection, and should prompt prevention strategies such as daily application of topical chlorhexidine gluconate.[239–242] Contact isolation precautions, composed of placing the patient in a single-bed room, dedicated equipment, and the routine use of gowns and gloves by health care workers during patient care, are also employed to reduce transmission from infected and colonized patients. *A. baumannii* is more likely to contaminate health care workers' gloves or gowns compared to other pathogens, especially after wound dressing and manipulating endotracheal tubes.[243–245] Health care workers caring for patients with *Acinetobacter* must follow standard precautions, as occupational acquisition of *Acinetobacter* is rarely reported.[246] Although antibiotic cycling has not proven effective, judicious use of antibiotics, particularly carbapenems, reduces the risk of multidrug-resistant *Acinetobacter* infection in ICU patients.[35,36,247]

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The complete reference list is available online at Expert Consult.

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223 *Salmonella* Species

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SHORT VIEW SUMMARY

Definition
- Salmonellosis includes gastroenteritis and other infections caused by nontyphoidal *Salmonella* (NTS).

Epidemiology
- There are approximately 1.2 million cases of NTS infection annually in the United States.
- Nontyphoidal salmonellosis is associated with diverse reservoirs, including fresh and prepared food items and other animal sources.

Microbiology
- There are more than 2500 *Salmonella* serotypes.
- Strains with multidrug resistance and decreased susceptibility to fluoroquinolones are increasingly prevalent.

Diagnosis
- Freshly passed stool should be plated directly on MacConkey agar or more selective media.
- Obtain blood cultures for patients suspected of bacteremia or vascular infection.
- Serogrouping is performed with commercially available antisera.

Therapy
- Antimicrobial therapy is not indicated for uncomplicated *Salmonella* gastroenteritis and prolongs the duration of fecal carriage.
- Ceftriaxone or fluoroquinolones should be administered empirically for treatment of severe gastroenteritis or when occurring in high-risk patients and for directed therapy of bacteremia or focal infections with NTS.

Prevention
- Control of foodborne outbreaks of NTS depend on a coordinated public health response and identification of controllable hazards from the farm to the table.

Salmonellae are named for the pathologist Salmon, who was involved in the first isolation (by Theobald Smith) of *Salmonella choleraesuis* from the porcine intestine.[1] *Salmonella* are effective commensals and pathogens that cause a spectrum of diseases in humans and animals, including domesticated and wild mammals, reptiles, birds, and insects. Some *Salmonella* serotypes, such as *Salmonella enterica* Typhi, *Salmonella* Paratyphi, and *Salmonella* Sendai, are highly adapted to humans and have no other known natural hosts, whereas others, such as *Salmonella* Typhimurium, have a broad host range and can infect a wide variety of animal hosts and humans. Some *Salmonella* serotypes, such as Dublin (cattle) and Arizonae (reptiles), are mostly adapted to an animal species and only occasionally infect humans. The widespread distribution of *Salmonella* bacteria in the environment, their increasing prevalence in the global food chain, and their virulence and adaptability have an enormous medical, public health, and economic impact worldwide. Salmonellae have been important organisms for the development of scientific knowledge. During the 1920s to 1940s, Kaufmann and White[2] pioneered the study of antibody interactions with the bacterial surface that resulted in agglutination assays that are the basis of serotyping today. In 1952 Zinder and Lederberg,[3] using *S.* Typhimurium, discovered the principle of genetic transduction, the transfer of genetic information from one cell to another by a virus particle (bacteriophage P22). In 1973 Ames and coworkers[4] developed the widely used Ames test, which uses *S.* Typhimurium auxotrophic mutants to test the mutagenic activity of chemical compounds. Over the last 25 years many of the important principles by which bacterial pathogenic mechanisms and host responses result in disease have been elucidated by studying salmonellae in animal and tissue culture models of mammalian infection.

CLASSIFICATION AND TAXONOMY

Salmonella is a genus of the family of Enterobacteriaceae. Before 1983 the existence of multiple *Salmonella* spp. was taxonomically accepted. Currently, as a result of experiments indicating a high degree of DNA similarity, the genus *Salmonella* is separated into two species: *Salmonella enterica*, which contains six subspecies (I, II, IIIa, IIIb, IV, and VI), and *Salmonella bongori*, which was formerly subspecies V. *S. enterica* subspecies I contains almost all the serotypes pathogenic for humans, except for the uncommon human infections with subspecies IIIa and IIIb, which were formerly designated by the genus *Arizonae*.

Members of the seven *Salmonella* spp. can be serotyped into one of more than 2500 serotypes (serovars) according to antigenically diverse surface structures: somatic O antigens (the carbohydrate component of lipopolysaccharide [LPS]) and flagellar (H) antigens (Table 223.1).[5] The name usually refers to the location where the *Salmonella* serotype was first isolated. According to the current *Salmonella* nomenclature system in use at Centers for Disease Control and Prevention (CDC) and World Health Organization laboratories, the full taxonomic designation *Salmonella enterica* subsp. *enterica* serotype Typhimurium can be shortened to *Salmonella* serotype Typhimurium or *Salmonella* Typhimurium.[6] The authors have chosen to use the abbreviated form in this chapter and will omit the "serotype," for example, designating "*Salmonella* serotype Typhimurium" as "*Salmonella* Typhimurium."

THE GENOME

The genome sequences of ≈8000 *S. enterica* strains, including *S.* Typhi; *S.* Paratyphi A, B, and C; and numerous nontyphoidal serotypes, are available in GenBank. The salmonellae genomes contain approximately 4.7 to 5.2 million base pairs, with approximately 4500 to 5400 coding sequences. Comparing sequence diversity by multilocus sequence typing suggests *S.* Typhi emerged from the *S. enterica* common ancestor around 50,000 years ago.[7] *S.* Typhi and *S.* Paratyphi A are closely related to each other but not to other *S. enterica* serotypes, and their host restriction to humans is related to loss of gene function through pseudogene formation and gene deletion.[8,9] Next-generation sequencing combined with traditional epidemiologic investigation permits a greater understanding of salmonellae evolution and spread. For example, whole-genome sequencing found that two closely related highly invasive strains of *S.* Typhimurium have recently emerged (late 20th century) and spread across sub-Saharan Africa temporally and geospatially, associated with the human immunodeficiency virus (HIV) pandemic, likely facilitated by the rapid expansion and mobility of a susceptible host population.[10] Of interest, these strains have undergone some genome reduction, similar to what has been seen in *S.* Typhi, possibly as a result of greater restriction to human hosts. However, in contrast to the speculation that these strains may have resulted in greater virulence or propensity to bacteremia,

TABLE 223.1 *Salmonella* Species, Subspecies, and Serotypes and Their Usual Habitats

SALMONELLA SPECIES AND SUBSPECIES	NO. OF SEROTYPES WITHIN SUBSPECIES	USUAL HABITAT
S. enterica subsp. *enterica* (I)	1531	Warm-blooded animals
S. enterica subsp. *salmae* (II)	505	Cold-blooded animals and the environment[a]
S. enterica subsp. *arizonae* (IIIa)	99	Cold-blooded animals and the environment[a]
S. enterica subsp. *diarizonae* (IIIb)	336	Cold-blooded animals and the environment[a]
S. enterica subsp. *houtenae* (IV)	73	Cold-blooded animals and the environment[a]
S. enterica subsp. *indica* (VI)	13	Cold-blooded animals and the environment[a]
S. bongori (V)	22	Cold-blooded animals and the environment[a]
Total	2579	

[a]Isolates of all species and subspecies have occurred in humans.
Modified from Grimont PAD, Weill F-X. Antigenic Formulae of the Salmonella Serovars 2007. *9th ed. WHO Collaborating Centre for Reference and Research on Salmonella. https://www.pasteur.fr/sites/default/files/veng_0.pdf.*

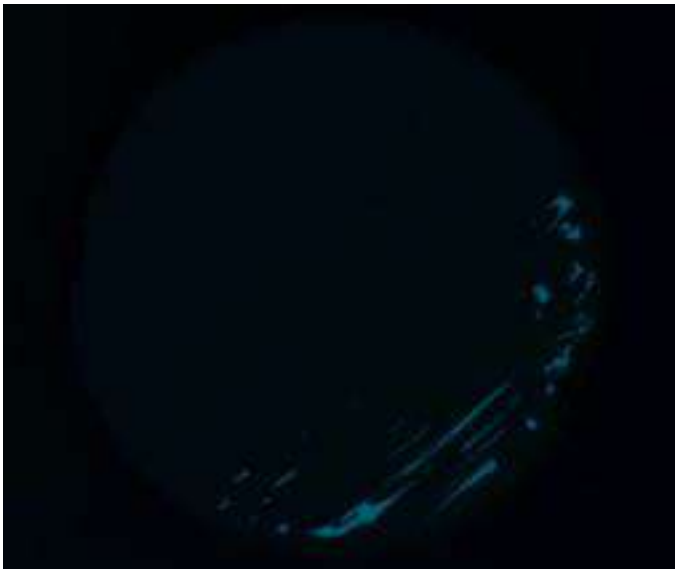

FIG. 223.1 Colonial growth pattern of *Salmonella* Arizonae grown on xylose-lysine-deoxycholate agar. Salmonellae metabolize thiosulfate to produce hydrogen sulfide, which leads to the formation of colonies with black centers and allows them to be differentiated from the similarly colored *Shigella* colonies.

if anything these strains appear less fit for resistance to innate immunity, and most usually cause simple gastrointestinal illness.[11]

MICROBIOLOGY

Salmonellae are gram-negative, non–spore-forming, facultatively anaerobic bacilli that measure 2 to 3 by 0.4 to 0.6 µm in size. Like other Enterobacteriaceae, they produce acid on glucose fermentation, reduce nitrates, and do not produce cytochrome oxidase.[12] All organisms, except *S.* Gallinarum-Pullorum, are motile as a result of peritrichous flagella, and most do not ferment lactose. However, approximately 1% of organisms can ferment lactose and therefore may not be detected if only MacConkey agar or other semiselective media are used to identify *Salmonella* based on colorimetric assay for fermentation of lactose. The differential metabolism of sugars can be used to distinguish many *Salmonella* serotypes; serotype Typhi is the only organism that does not produce gas on sugar fermentation.[12]

Freshly passed stool is preferred for the isolation of *Salmonella* and should be plated directly onto agar plates. Low-selective media, such as MacConkey agar and deoxycholate agar, and intermediate-selective media, such as *Salmonella-Shigella,* xylose-lysine-deoxycholate (Fig. 223.1), or Hektoen enteric agar, are widely used to screen for both *Salmonella* and *Shigella* spp. Selective chromogenic media, such as CHROMagar *Salmonella* (DRG International, Springfield, NJ), are more specific than other selective media, reduce the need for confirmatory testing and time to identification, and increasingly are used for the primary isolation and presumptive identification of *Salmonella* from clinical stool specimens.[13] Stool specimens can be directly inoculated into selenite enrichment broth before plating on primary media to facilitate the recovery of low numbers of organisms.[13] Highly *Salmonella*-selective media, such as selenite with brilliant green, should be reserved for use in stool cultures of suspected carriers and under special circumstances, such as outbreaks. Bismuth sulfite agar, which contains an indicator of hydrogen sulfite production and does not contain lactose, is preferred for the isolation of *S.* Typhi and can be used for the detection of the 1% of *Salmonella* strains (including most *Salmonella* serogroup C strains) that ferment lactose.[14] After primary isolation, possible *Salmonella* isolates can be tested in commercial identification systems or inoculated into screening media, such as triple-sugar–iron and lysine-iron agar.

Direct detection of enteric pathogens from stool specimens by DNA-based syndrome panels is increasingly used by clinical laboratories to allow providers to rapidly identify the cause of gastroenteritis. To ensure that outbreaks of similar organisms are detected and investigated, all specimens that test positive for nontyphoidal *Salmonella* (NTS) by culture-independent diagnostic testing and for which isolate submission is requested or required under public health reporting rules should be cultured in the clinical laboratory or at a public health laboratory.

Isolates with typical biochemical profiles for *Salmonella* should be serogrouped with commercially available polyvalent antisera or sent to a reference or public health laboratory for complete serogrouping. Salmonellae are serogrouped according to their polysaccharide O (somatic) antigens, Vi (capsular) antigens, and H (flagellar) antigens according to the Kauffman-White scheme. The Vi antigen is a heat-labile capsular homopolymer of *N*-acetylgalactosaminouronic acid that is used for the identification of *S.* Typhi strains and on occasion other *Salmonella* serotypes by slide agglutination.[15] In *S.* Typhi and *S.* Paratyphi C the polysaccharide Vi antigen can inhibit O-antigen agglutination because it is so abundant, and boiling is required to inactivate Vi antigen and to detect O antigen. Most antigenic variability occurs in the O antigen, which is composed of chains of oligosaccharide attached to a core oligosaccharide that is linked covalently to lipid A.

Although serotyping of all surface antigens can be used for formal identification, most laboratories perform a few simple agglutination reactions that differentiate specific O antigens into serogroups, designated as groups A, B, C_1, C_2, D, and E *Salmonella.* Strains in these six serogroups cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals. Although this grouping is useful in epidemiologic studies and can be used to confirm genus identification, it cannot identify whether the organism is likely to cause enteric fever because considerable cross-reactivity occurs among serogroups. For example, *S.* Enteritidis, and *S.* Typhi are both group D, and *S.* Typhimurium and *S.* Paratyphi B are both group B.

Genotyping methods frequently are used for epidemiologic purposes to differentiate strains of common *Salmonella* serotypes. These methods include ribotyping, pulsed-field gel electrophoresis, insertion sequences analysis, polymerase chain reaction–based fingerprinting, multilocus sequence typing, and increasingly whole-genome sequencing.

EPIDEMIOLOGY

In many countries the incidence of human *Salmonella* infections has increased markedly in recent decades, although good population-based surveillance data are mostly lacking, especially from sub-Saharan Africa.

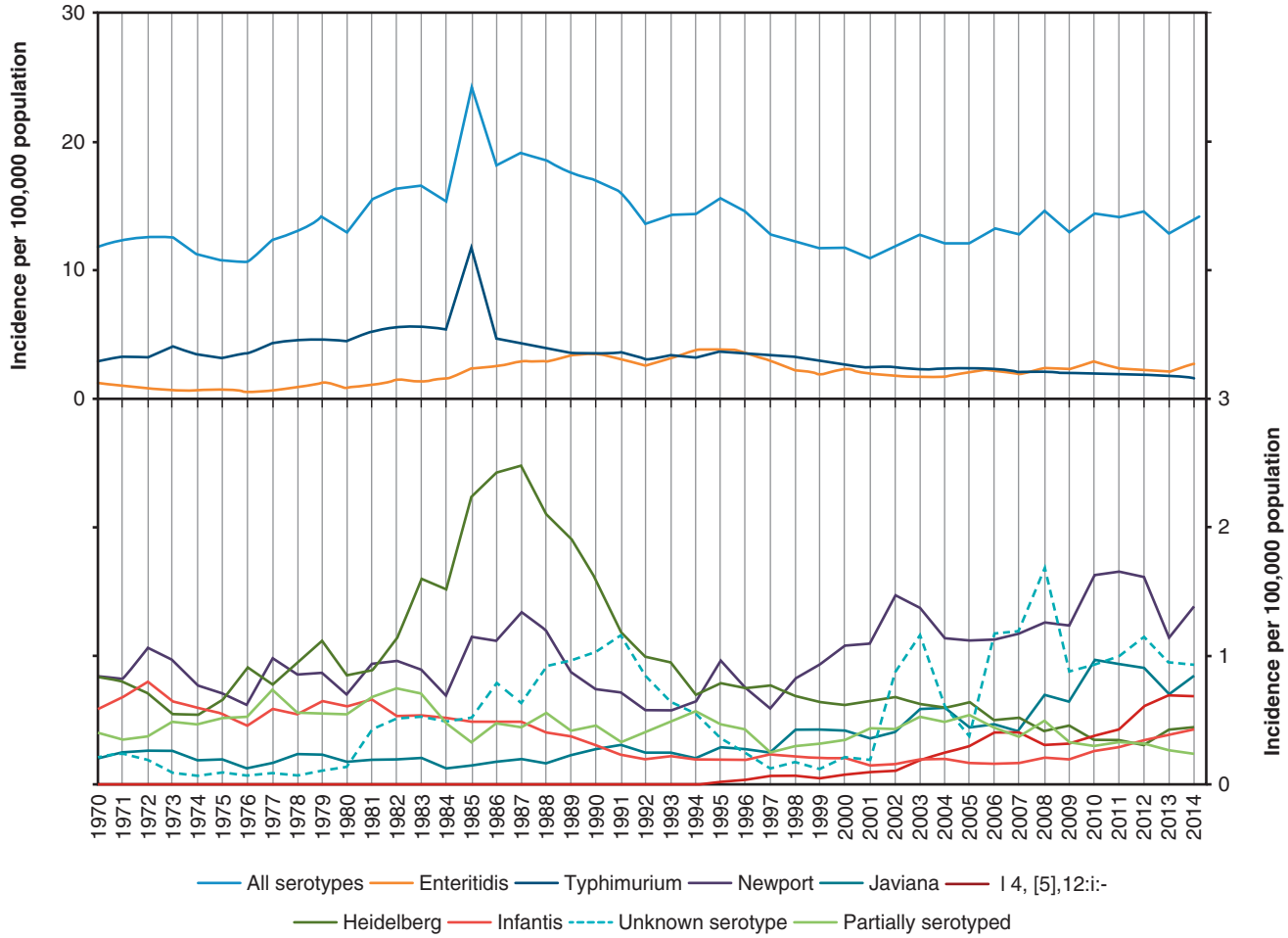

FIG. 223.2 Incidence rate of culture-confirmed human *Salmonella* infection reported to Laboratory-Based Enteric Disease Surveillance System, including all serotypes and individual serotypes with ≥1000 infections reported in 2014, by year, United States, 1970–2014. *(From Centers for Disease Control and Prevention [CDC]. National* Salmonella *Surveillance Annual Report, 2014. Atlanta, GA: US Department of Health and Human Services, CDC; 2017.* https://www.cdc.gov/nationalsurveillance/salmonella-surveillance.html.*)*

In the United States NTS species cause an estimated 1.2 million cases of foodborne illness each year, second only to noroviruses, and are associated with an estimated hospitalization rate of 2.7% and death rate of 0.5%.[16] In the United States the incidence rate of NTS infection has remained relatively stable in the last 20 years and continues to be driven largely by *S.* Typhimurium and *S.* Enteritidis[3] (Fig. 223.2).[17] In 2016 the incidence rate of salmonellosis (16.60/100,000 population) was second only to *Campylobacter* (17.43/100,000 population) among nine potentially foodborne diseases under active surveillance.[18] In comparison, during 2010–14, reported NTS incidence rates ranged between 21.4 to 25.7 per 100,000 population in the European Union.[19] Globally, *S.* Typhimurium (43.5%) and *S.* Enteritidis (17.1%) are the most common *Salmonella* serotypes, with large differences observed in the serotype distribution between regions but lesser differences between countries within the same region.[20] The incidence of NTS infection is highest during the rainy season in tropical climates and during the warmer months in temperate climates, coinciding with the peak in foodborne outbreaks.[21]

Unlike *S.* Typhi and *S.* Paratyphi, whose only reservoir is humans, NTS can be acquired from multiple animal reservoirs. Transmission of NTS to humans can occur by many routes, including consumption of food animal products, especially eggs, poultry, undercooked ground meat, dairy products, fresh produce contaminated with animal waste, contact with animals or their environment, and contaminated water. During the 1980s and 1990s, *S.* Enteritidis associated with shell eggs emerged as the predominant *Salmonella* serotype and source of foodborne disease in the United States and some other countries.[22] In the United States the rate of reported *S.* Enteritidis isolates increased from 0.6 per 100,000 population in 1976 to a high of 3.9 per 100,000 in 1994.[23] As a result of intensive surveillance and control effort, including egg farm management practices, such as rodent control and vaccination of young hens, egg quality-assurance programs on farms, egg refrigeration during storage and transport, and consumer education, the incidence of *S.* Enteritidis infection has declined in the United States and other developed countries[24] (see Fig. 223.2). However, outbreaks of *S.* Enteritidis infection associated with shell eggs continue to occur. In 2010 a national outbreak of *S.* Enteritidis infection resulted in more than 1900 reported illnesses and the recall of 500 million eggs.[25] Infection localizes to the ovaries and upper oviduct tissue and is transmitted to the forming egg before shell deposition, resulting in contamination of the albumen and yolk. Although cooking eggs until all liquid yolk is solidified kills *S.* Enteritidis, the use of pasteurized egg products remains the safest alternative for institutions and the general public.

Transmission of *S.* Enteritidis from farm to farm may be facilitated by contaminated chicken manure, insects, and rodents and by ingestion of feed contaminated with mouse droppings because *S.* Enteritidis strains cultured from the spleens of mice caught on farms have enhanced ability to contaminate eggs.[26,27] The loss of cross-immunity resulting from culling chickens infected with *S.* Gallinarum and *S.* Pullorum in the United States and United Kingdom also may have contributed to the emergence of *S.* Enteritidis.[28]

Salmonella live in the intestines of most food animals, and contamination of raw poultry and meat products can occur during slaughter and processing. Retail ground poultry and meat are at high risk of contamination with *Salmonella*, including with antimicrobial-resistant strains.[29] In 2013 18.0% of ground chicken, 15.0% of ground turkey, and 1.6%

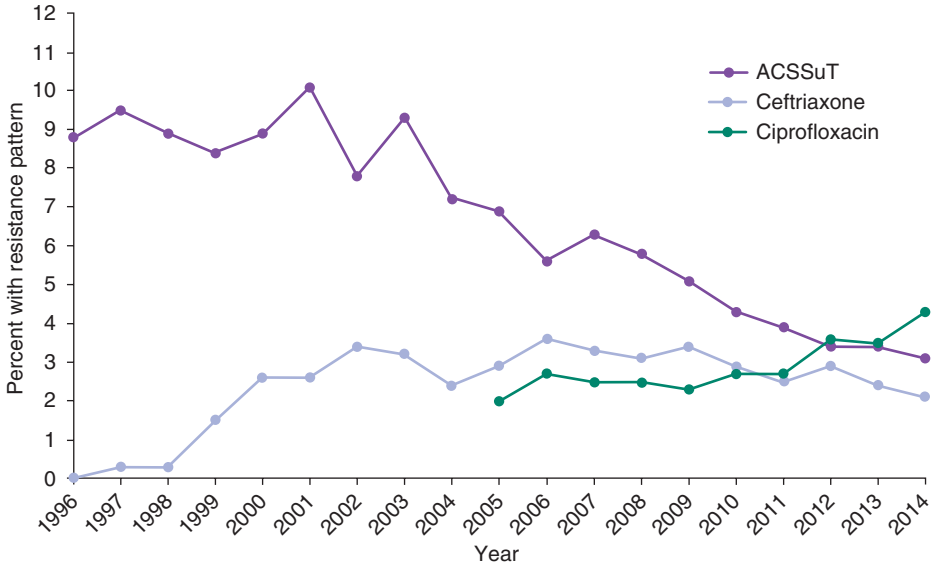

FIG. 223.3 Resistance patterns of nontyphoidal *Salmonella* isolates, United States, 1996–2014. *ACSSuT,* Resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline; *ceftriaxone,* minimal inhibitory concentration (MIC) > 4 μg/mL; *ciprofloxacin,* MIC > 0.12 μg/mL. *(From Centers for Disease Control and Prevention.* National Antimicrobial Resistance Monitoring System [NARMS] 2014 Human Isolates Surveillance Report. https://www.cdc.gov/narms/reports/index.html.*)*

of ground beef specimens sampled by the US Department of Agriculture tested positive for *Salmonella.*[30] Although raw chicken carcasses and other meats are less commonly contaminated with *Salmonella* than is ground poultry, cross-contamination of food items from handling of raw chicken and inadequate hand hygiene are risks for sporadic salmonellosis in the home.[31] There is considerable mismatch between animal and human *Salmonella* serotypes, suggesting that the risk of transmission is not equal for all food products and serotypes.[32]

Changes in food consumption and the rapid growth of international trade in agricultural food products and increasing use of manufacturing technologies have facilitated the dissemination of new *Salmonella* serotypes associated with fresh fruits and vegetables. Human or animal feces may contaminate the surface of fruits and vegetables and may not be removed by washing. Recent multistate foodborne outbreaks of salmonellosis in the United States associated with fresh produce include papayas—multiple serotypes, cantaloupe—multiple serotypes, pistachios—*S.* Montevideo, cucumbers—*S.* Poona, alfalfa sprouts—multiple serotypes, bean sprouts—*S.* Enteritidis, and tomatoes—multiple serotypes. Tomatoes can internalize *Salmonella* when immersed in water, and contamination on the tomato or melon surface can be transferred to the interior when it is cut.[33] Sprout seeds can become contaminated before sprouting, and soaking seeds with 20,000 parts per million calcium hypochlorite or other disinfectant can reduce but does not eliminate the risk of sprout-associated illness.[34] Recent salmonellae outbreaks have been associated with peanut products, including peanut butter and paste used as a food additive. Salmonellae appear capable of colonizing and adhering to the nut and can be present in raw nuts and, if inadequate processing and or roasting has occurred, in nut-related products.[35–37]

Manufactured food items pose an enormous potential hazard of foodborne salmonellosis in developed countries because of their centralized production and wide-scale distribution. Both pasteurized and unpasteurized milk and milk products, including ice cream and powdered infant formula, have been recognized as sources of *Salmonella* infections.[38,39]

Salmonellosis associated with exotic pets is a resurgent public health problem, especially from exposure to reptiles, including turtles, iguanas, lizards, and snakes, and from amphibians such as aquatic frogs.[40] Of all *Salmonella* serotypes, 40% have been cultured predominantly from reptiles and are rarely found in other animals or humans. Based on extrapolation from population-based surveillance, 6% of all sporadic *Salmonella* infections and 11% among persons younger than 21 years are attributable to contact with reptiles or amphibians.[40] The recognition of pet turtle–associated salmonellosis led to the banning of shipment of small pet turtles in the United States in 1975 and in several countries, but small turtles continue to be sold illegally and pose a health risk, especially to children.[41] Exposure to pet birds, live poultry, such as chicks and ducklings, pet rodents and hedgehogs, dogs and cats, and to pet food and pet treats made from animal parts are other reported sources of human salmonellosis, including infection with multidrug-resistant (MDR) strains.[42,43]

Multidrug resistance among human NTS isolates is increasing in both developing and developed countries.[3,44–46] A diversity of transferable resistance plasmids have been identified from MDR NTS strains and contribute to the conjugative transfer of resistance between enteric bacterial species.[47] Of particular concern has been the worldwide emergence in the 1990s of a distinct strain of MDR *S.* Typhimurium, characterized as definitive phage type 104 (DT104), that is resistant to at least five antimicrobials—ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (R-type ACSSuT).[48] All DT104 strains contain a chromosome- and integron-encoded β-lactamase (PSE-1) that appears to have been acquired from plasmids in *Pseudomonas* spp. The DT104 strain has broad host reservoirs, and its widespread clonal dissemination in domestic livestock, especially among beef and dairy cattle, likely was promoted by use of antimicrobials on farms for therapeutic uses and for growth enhancement.[49] In the United States the proportion of NTS with the ACSSuT phenotype has been decreasing since the early 2000s (Fig. 223.3). In 2014 resistance to at least ACSSuT was reported in 3.1% of NTS, including 14.5% of *S.* Typhimurium and 9.9% of *S.* Heidelberg isolates.[46] Acquisition of *S.* Typhimurium DT104 is associated with exposure to ill farm animals and to a variety of meat products, including raw or undercooked ground beef.[49] Infection with DT104 is associated with increased risk of bloodstream infection and hospitalization compared with infection with susceptible strains, likely reflecting inappropriate empirical antimicrobial therapy.[50]

Outbreaks and sporadic cases of NTS resistant to third-generation cephalosporins have been reported, and international travel and adoption may have contributed to the global spread.[51] Resistance is most commonly mediated by a transferable plasmid containing the *ampC* (bla_{CMY}) gene, although other extended-spectrum β-lactamases have been described. The bla_{CMY} gene is probably acquired by horizontal genetic transfer from *Escherichia coli* strains in food-producing animals and linked to the widespread use of the veterinary cephalosporin ceftiofur.[52] In 2014 2.1% of NTS isolates from humans in the United States were ceftriaxone resistant (minimum inhibitory concentration [MIC] ≥ 4 μg/mL).[46]

Ceftriaxone resistance is more common among NTS isolated from blood than stool and is associated with invasive infection and high case-fatality rates among African children, although most cases have concomitant malnutrition, HIV, and malaria.[53,54]

An MDR strain of *S.* Newport (MDR-AmpC), with decreased susceptibility to ceftriaxone (MIC > 2 µg/mL) and resistance to eight other human antimicrobials and ceftiofur, has emerged in the United States.[55] In 2014 MDR-AmpC was detected in 1.2% of all US NTS and 3.0% of *S.* Newport isolates.[46] Risk factors for infection with MDR-AmpC *S.* Newport include consumption of uncooked ground beef, runny eggs or omelets, and recent exposure to an antimicrobial to which the strain is resistant.[56] More recently, carbapenemase-producing NTS have been reported in Europe, North Africa, and southern Asia.

Over the last decade, strains of NTS with decreased susceptibility to ciprofloxacin (MIC 0.12–0.5 µg/mL) or ciprofloxacin resistance (MIC ≥1 µg/mL) have emerged and have been associated with delayed response and treatment failure.[57] In 2014 4.3% of NTS isolates in the United States had a ciprofloxacin MIC ≥0.12 (see Fig. 223.3), and the proportion is higher in Europe (≈6%).[46] These strains have diverse resistance mechanisms, including single and multiple mutations in the DNA gyrase genes *gyrA* and *gyrB* and mutations in the chromosomally encoded quinolone resistance-determining region and plasmid-encoded quinolone resistance genes that are not reliably detected by nalidixic acid susceptibility testing or standard ciprofloxacin disk diffusion.[57] Because commercial test systems do not contain ciprofloxacin concentrations sufficiently low to allow use of this breakpoint, laboratories need to determine the ciprofloxacin MIC by Etest or another alternative method.

In Taiwan in 2000 a high-level ciprofloxacin-resistant strain of *S.* Choleraesuis caused a large outbreak of invasive infections that was linked to the use of enrofloxacin in swine feed.[44] On the basis of increased prevalence of nalidixic acid–resistant *Salmonella* and fluoroquinolone-resistant *Campylobacter* spp. in humans, the US Food and Drug Administration withdrew approval of the use of fluoroquinolones in poultry in 2005.

Although health care–associated salmonellosis is infrequent, such infections have been associated with MDR strains, sustained transmission, and substantial morbidity and mortality.[58,59] The most frequently reported route of transmission of NTS in health care facility–associated outbreaks is foodborne.[60] Although less common, transmission of *Salmonella* from patients to health care providers has been associated with phlebotomy, handling soiled linen, noncompliance with barrier precautions, and fecally incontinent residents.[61,62] However, the risk of transmission from health care providers to patients appears to be low if infection control measures, including hand hygiene, correct use of personal protective equipment, and routine disinfection of patient-care equipment, are observed.[63] In contrast, the risk of nosocomial transmission to neonates and infants from acutely or chronically infected family members appears higher.[64] Neonates are at high risk for fecal-oral transmission of *Salmonella* because of relative gastric achlorhydria and the buffering capacity of ingested breast milk and formula. High-iron infant formula may further increase the risk of infant salmonellosis compared with breastfeeding.[65] Contaminated enteral feeding and crowding also have been associated with nosocomial transmission among pediatric patients.[66] Control of outbreaks in daycare centers may be difficult because of the need for frequent diaper changing and the higher rate and longer duration of convalescent carriage seen in the preschool-age group.[67]

Residents of nursing homes are at increased risk of foodborne salmonellosis and more severe morbidity and mortality because of poor infection control compliance and presence of comorbid illnesses, acid-suppressing medications, and waning immunity.[58,68]

PATHOGENESIS

Salmonella infections begin with the ingestion of bacteria in contaminated food or water. Estimates of the infectious dose vary substantially and depend on the method of determination. In studies involving administration of laboratory *Salmonella* strains to healthy human volunteers, the median dose required to produce disease was approximately 10^6 bacteria.[69] In contrast, investigations of point-source outbreaks suggest that as few as 200 bacteria may produce nontyphoidal gastroenteritis in many of those exposed and that the ingested dose is an important determinant of incubation period and disease severity. Discrepancies in these results may stem from use of strains attenuated by in vitro passage in the challenge experiments and from variation in disease susceptibility in the general population. Gastric acidity represents the initial barrier to *Salmonella* colonization and conditions or medications, including antacids, H2 blockers, and proton pump inhibitors, that increase gastric pH increase susceptibility to infection. On exposure to acid in vitro, salmonellae display an adaptive acid tolerance response that probably facilitates bacterial survival in the stomach and passage to the small intestine.[70]

Interactions With Intestinal Epithelium and Induction of Enteritis

Salmonellae must evade host antimicrobial factors secreted into the intestinal lumen, including antimicrobial peptides, bile salts, and secretory immunoglobulin A, and traverse a protective mucous barrier before encountering intestinal epithelial cells.[71,72] Salmonellae express an array of distinct fimbriae that contribute to tight adherence to intestinal epithelial cells in culture. It is necessary to delete multiple fimbriae synthesis genes to prevent infection in animal models, suggesting that functional redundancy exists.[73] Microscopy reveals that salmonellae invade intestinal epithelial cells by a morphologically distinct process termed *bacteria-mediated endocytosis* (Fig. 223.4).[74] Shortly after bacteria adhere to the apical epithelial surface, profound cytoskeletal rearrangements occur in the host cell, disrupting the normal epithelial brush border and inducing formation of membrane ruffles that reach out and enclose adherent bacteria in large vesicles. This process resembles the membrane ruffling and macropinocytosis induced in many cell types by growth factors and is functionally distinct from receptor-mediated endocytosis, the mechanism by which many other pathogens enter nonphagocytic cells. After the bacteria internalize, a fraction of the *Salmonella*-containing vesicles transcytose to the basolateral membrane, and the apical epithelial brush border reconstitutes. The epithelial cell type that serves as the principal portal for *Salmonella* invasion remains uncertain. In the mouse enteric fever model salmonellae preferentially adhere to and enter the specialized microfold cells (M cells) that overlie lymphoid tissue within Peyer patches.[75] In bovine and rabbit models of enteritis, however, salmonellae do not appear to interact preferentially with M cells but, instead, adhere to and invade intestinal enterocytes diffusely.[76] It is possible that M cells are the principal portal of entry in the enteric fever syndrome and that generalized invasion of enterocytes plays a greater role in the enteritis induced by NTS serotypes.

Salmonellae encode a type III secretion system (T3SS) within *Salmonella* pathogenicity island 1 (the SPI-1 T3SS), which is required for

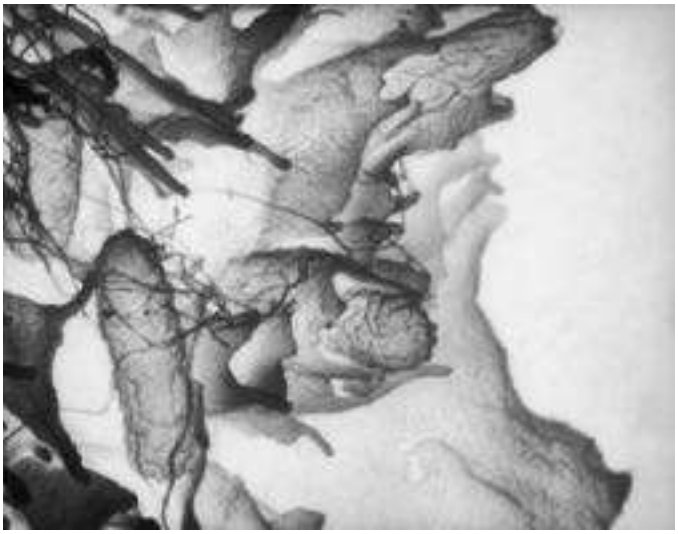

FIG. 223.4 Scanning electron micrograph showing *Salmonella* Typhimurium entering a HEp-2 cell through bacteria-mediated endocytosis. Membrane ruffles extend from the cell surface, enclosing and internalizing adherent bacteria. *(From Ohl ME, Miller SI. Salmonella: a model for bacterial pathogenesis.* Annu Rev Med. *2001;52:259–274.)*

bacteria-mediated endocytosis and intestinal epithelial invasion. T3SSs are complex macromolecular machines that have evolved to subvert host cell function through the translocation of virulence proteins directly from the bacterial cytoplasm into the host cell (see Chapter 1 for an overview). *Salmonella* mutants lacking a functional SPI-1 T3SS do not invade epithelial cells in tissue culture and are severely attenuated in animal models of infection after oral administration.[76] In the past decade, considerable attention has focused on identifying the virulence proteins translocated into epithelial cells by the SPI-1 T3SS and delineating the host cell processes these proteins target. At least five translocated proteins are essential for efficient invasion of cultured epithelial cells, although invasion in animal tissues may be more complicated and diverse.[77]

Two SPI-1 translocated proteins, SipC and SipA, promote membrane ruffling and *Salmonella* invasion through direct interactions with the actin cytoskeleton. The SipC protein inserts into the host cell plasma membrane and forms part of a protein complex that allows translocation of additional SPI-1 virulence proteins directly into the host cell cytoplasm.[78] SipC also directly nucleates actin polymerization at the site of *Salmonella* attachment and stimulates actin filament bundling.[79] The SipA protein further enhances actin polymerization through stabilization of actin filaments and reduction of the critical concentration for polymerization.[80] SipA mutants invade epithelial cells less efficiently than wild-type bacteria and induce disorganized, diffuse ruffling in host cells, in contrast to the localized ruffling induced around wild-type bacteria.

Additional SPI-1 translocated proteins contribute to *Salmonella* invasion by targeting members of the Rho family of monomeric guanosine triphosphate (GTP)-binding proteins (G proteins). Rho family members, including Cdc42, Rac, and Rho, regulate the structure and dynamics of the actin cytoskeleton and are required for formation of the membrane ruffles that mediate *Salmonella* internalization. The SPI-1 translocated proteins SopE and SopE2 directly activate Rac1 and Cdc42 in vitro by acting as guanosine diphosphate/GTP exchange factors (GEFs) and induce membrane ruffling and macropinocytosis after microinjection into epithelial cells.[81] SopB is an additional SPI-1 translocated protein that targets inositol phosphate signaling within the host cell by acting as an inositol polyphosphatase.[82] Among other effects, this activity indirectly stimulates Rho GTPases and promotes membrane ruffling.[83] This may be an important pathway for the bacteria to enter human cells, as recent data suggest that a polymorphism in VAC14, a regulator of phosphoinositide can alter entrance of bacteria into host cells and determines host susceptibility to *S.* Typhi in Viet Nam.[84]

Recent data suggest that only Rac1 and RhoG are essential for the effects of SopE, SopE2, and SopB.[85] Although mutation of *sopB, sopE,* or *sopE2* alone does not impact invasion, combined deletion of these three genes leads to a severe reduction in epithelial cell invasion.[83] Such functional redundancy among translocated proteins is an emerging theme in a variety of T3SSs. Overall, available data indicate that SipA and SipC act in concert with downstream cellular effectors of activated Rho GTPases to initiate and spatially direct the actin rearrangements that lead to *Salmonella* internalization.

Studies in mice indicate that salmonellae may also cross the intestinal epithelial border by an SPI-1–independent process involving host dendritic cells.[86,87] These cells express tight junction proteins and can intercalate between intestinal epithelial cells and access the intestinal lumen without disrupting epithelial integrity. In this manner, dendritic cells may internalize bacteria in the intestinal lumen and subsequently carry these bacteria to distant sites as they undergo their physiologic migration to lymphoid tissues. The diversity of mechanisms used by salmonellae to cross the intestinal barrier indicates the importance of this mechanism to its lifestyle within mammals.

In addition to invasion of intestinal epithelial cells, *Salmonella* serotypes clinically associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen. The SPI-1 T3SS is also required for these responses in tissue culture and animal models of enteritis. Specifically, *Salmonella* strains unable to deliver any SPI-1 virulence proteins, as a result of mutations in the secretion apparatus, fail to induce fluid secretion or neutrophil accumulation in ligated bovine ileal loops and do not cause gastroenteritis in calves.[88] In tissue culture models of enteritis translocation of SPI-1 proteins into intestinal epithelial cells leads to synthesis and polarized secretion of inflammatory mediators and neutrophil chemokines, including interleukin-8 (IL-8).[89]

Several SPI-1 translocated proteins that contribute to intestinal inflammation and fluid secretion have been identified. Stimulation of Rho GTPase signaling by SopE and SopE2 also leads to activation of microtubule-associated protein kinase pathways and movement of the proinflammatory transcription factor nuclear factor kappa B (NF-κB) to its site of action in the nucleus.[81] In addition to its role in invasion, the inositol polyphosphatase activity of SopB leads to accumulation of D-myoinositol-1,4,5,6-tetrakisphosphate in epithelial cells.[90] The increased concentration of this compound ultimately leads to an increase in cellular basal chloride secretion, with associated fluid flux. The SPI-1 translocated proteins SopA and SopD also contribute to intestinal secretory and inflammatory responses in ligated ileal loops, but the molecular basis of these effects remains unclear. Many other effector proteins that are delivered by the T3SS apparatus may also effect these or similar pathways with different targets. Individual nontyphoidal salmonellae have a diverse complement of effector proteins; for instance, many strains do not have SopE2. The association of specific effector proteins could alter the pathogenicity of specific strains and their emergence in humans from animal reservoirs.[91]

After *Salmonella* invasion, intestinal inflammation may also result from activation of the innate immune system through stimulation of proinflammatory receptors present on phagocytes and the basolateral surface of intestinal epithelia. This includes activation of Toll-like receptor 4 (TLR4) by LPS and TLR5 by bacterial flagellin.[92] The cytosolic surveillance pathway is also activated by the translocation of flagellin into the cytoplasm by the T3SS and its recognition by the inflammasome through the IL-1β converting enzyme protease-activating factor (IPAF), or NLRC4, pathway. This pathway results in the secretion of IL-1β, an important proinflammatory cytokine.[93,94] Intestinal inflammation probably contributes to fluid secretion and diarrhea through disruption of the epithelial barrier and increased water flux by an exudative mechanism. In contrast to the neutrophilic inflammation and gastroenteritis induced by NTS strains, *S.* Typhi induces monocytic inflammation in the human intestine and produces significantly less, if any, diarrhea.[95] The molecular basis of this difference in the host response remains unknown. One possibility is the presence of the Vi polysaccharide capsule in most strains of *S.* Typhi that can prevent recognition of LPS by TLR4.[96]

Several studies demonstrate that salmonellae also use the SPI-1 T3SS to deliver proteins that downregulate the host inflammatory response associated with *Salmonella* invasion. The SptP protein inactivates Rho GTPase signaling by acting as a GTPase-activating protein (RhoGAP).[97] This directly opposes the activity of SopE and SopE2 and reduces membrane ruffling and proinflammatory signaling after bacterial invasion. In addition, the SspH1 ubiquitin ligase and AvrA proteins inhibit NF-κB activation and related host cell cytokine synthesis.[98,99] These SPI-1 translocated proteins may promote bacterial persistence in the host by maintaining host cell integrity and allowing evasion of the host immune response. The presence of SPI-1 translocated proteins with opposing molecular actions (e.g., SopE and SptP) suggests that there may be temporal ordering of protein function, with initial activity of SPI-1 proteins associated with invasion and proinflammatory signaling and subsequent activity of antiinflammatory proteins. This dampening of the inflammatory response attributed to multiple bacterial effector proteins may contribute to the long period of relative asymptomatic colonization of the intestinal tract typical of NTS infection.

After inflammation is generated, an important component of salmonellae survival during gastroenteritis and its continued colonization of the intestinal tract after the resolution of disease involves the organisms' use of the sulfur-containing compound tetrathionate as an electron acceptor to promote energy metabolism in a microaerobic environment.[100] The intestinal microbiota generate toxic hydrogen sulfide gas through their metabolism, and intestinal epithelia detoxify this gas to thiosulfate. On the induction of inflammation by salmonellae and recruitment of neutrophils, reactive oxygen radicals convert the thiosulfate to tetrathionate, which only salmonellae can use for microaerophilic-based respiration to generate energy. This allows the organism to outcompete with commensals and effectively colonize the intestinal tract. The growth

advantage to salmonellae in the host conferred by tetrathionate respiration explains the utility of tetrathionate enrichment broth in the identification of salmonellae. Of interest, this process has been lost in typhoidal salmonellae that are inefficient colonizers of the intestinal tract.

Interactions With Macrophages and Systemic Infection

After crossing the epithelial barrier, salmonellae encounter and enter macrophages present in the submucosal space and Peyer patches. Macrophage invasion may occur through bacteria-mediated macropinocytosis or through phagocytosis directed by several receptors present on the macrophage. Available data in both human infection and animal models of disease indicate that the ability of *Salmonella* to survive and replicate within macrophages is essential for dissemination within the host and induction of systemic disease. In persons with enteric fever and positive blood cultures, the majority of organisms are contained within the mononuclear fraction.[101] Furthermore, the ability of *Salmonella* mutants to replicate within macrophages in tissue culture correlates with ability to produce systemic disease in the mouse typhoid model, and microscopic examination of infected mouse liver and spleen demonstrates that the majority of organisms are located within macrophages.[102,103] Although residence within the macrophage shields the bacterium from effectors of humoral immunity, it also exposes the bacterium to the microbicidal and nutrient-poor environment of the phagosome. Within the host, salmonellae induce the expression of numerous genes that allow evasion of these antimicrobial defenses.

Once in the intracellular environment, the bacteria persist within a vacuolar compartment that endures for hours to days. Salmonellae can survive within a compartment that fuses with lysosomes, and hence inhibition of phagosome fusion with lysosomes is unlikely to be a major pathogenic strategy of salmonellae. The vacuole acidifies, although its acidification may be delayed. Resistance to a variety of vacuolar bactericidal activities is essential to pathogenesis, including resistance to antimicrobial peptides, nitric oxide, and oxidative killing. This is supported by experiments demonstrating that *S.* Typhimurium mutants sensitive to these compounds are less virulent for mice and that mice deficient in these activities are more susceptible to *S.* Typhimurium.[104]

Salmonella senses the acidic environment of the *Salmonella*-containing vacuole (SCV) and activates a variety of regulatory proteins required for *Salmonella* adaptation to the intracellular environment for replication within host cells. The best studied of these is the PhoP/PhoQ two-component regulatory system. The PhoP/PhoQ system senses the intracellular environment and regulates transcription of more than 200 genes, some of which are required for survival within macrophages. PhoQ acts as the sensor protein for the phagosome environment by sensing acidic pH and antimicrobial peptides to activate gene expression.[105,106,107] Activation of the PhoP/PhoQ and other regulons leads to widespread modifications in the protein and LPS components of the bacterial inner and outer membranes.[108] As many as 900 to 1000 genes are induced in response to the phagosome environment, including many involved in remodeling of the cell surface to resist host cell killing mechanisms.[109] These surface modifications confer resistance to antimicrobial factors within the phagosome, including antimicrobial peptides, oxygen, and nitrogen radicals. PhoP/PhoQ-regulated LPS modifications include addition of aminoarabinose, ethanolamine, palmitate, and 2-hydroxymyristate to lipid A, thus altering the charge density and fluidity of the outer membrane and discouraging antimicrobial peptide insertion in the membrane.[108] Cell surface polysaccharide is also dramatically altered.[108] In addition, PhoP/PhoQ-regulated modifications in lipid A structure produce an LPS molecule with significantly less proinflammatory signaling activity and repress flagellin synthesis, which may facilitate bacterial survival within host tissues.[108] PhoP/PhoQ mutants of *S.* Typhi are avirulent in humans and are promising live typhoid vaccine candidates.[110] *S.* Typhi also modifies its surface through synthesis of the Vi capsule, a polysaccharide structure that confers resistance to phagocytosis by neutrophils and killing by complement, reduces recognition of LPS, and promotes survival within human macrophages.[111]

Another strategy for intracellular survival of Salmonellae is to slow its growth through specific mechanisms. Strains with slower growth are termed "persisters" because they have greater resistance to antimicrobials as a result of growth slowing. These persisters do not have mutations but move to a nongrowing state as a result of use of toxin-antitoxin modules that can inhibit protein translation by acetylation of transfer RNA molecules.[112,113]

Salmonella has a second T3SS that is necessary for survival in the macrophage and for establishment of systemic infection.[114] Proteins delivered by both T3SSs are important for intracellular survival. SipA delivered by SPI-1 persists on the phagosome membrane, where it promotes intracellular survival.[115] Encoded on SPI-2 is an additional T3SS that is adapted to be expressed by intracellular bacteria and translocates proteins across the membrane of the SCV into the macrophage cytosol. SPI-2 translocated proteins are hypothesized to alter trafficking to the SCV to promote bacterial growth such that useful nutrients are routed to the SCV. Most remarkably, salmonellae alter the phagosome to tubulate in a mechanism that requires SPI-2 translocated proteins. Such tubulation has been correlated with virulence because SPI-2 translocated proteins implicated in this process are required for phagosome tubulation to occur. Phagosome tubulation is dynamic and rapid and appears to be dependent on the recruitment of microtubule motors, the activation of small GTPases, and membrane lipid alteration. The mechanism by which tubulation of the phagosome promotes virulence is unknown, but it could allow bacteria or their products to specifically traffic within the phagosome to different cellular localizations to promote nutrient acquisition or cell-to-cell spread.

SPI-2 and its proteins are essential for the *S.* Typhimurium phagosome to migrate away from the nucleus after phagocytosis.[116] Several SPI-2 translocated proteins, including SifA, SifB, SseJ, SopD2, PipB, and PipB2, localize to the surface of the SCV and either contribute to tubulation or other alterations of the phagosome.[104] This also may involve manipulation of GTPases and the microtubule network, as SifA binds RhoA and a host protein, Skp, which associates with the microtubular network, and SseJ is a RhoA-dependent glycerol cholesterol transferase that alters phagosome lipids enzymatically and could alter phagosome tubulation or trafficking.[117,118] The ubiquitin ligase SspH2 and the effector SseI, which modulates host cell migration, both localize to the phagosome and to the apical cell surface membrane of polarized epithelial cells through S-palmitoylation.[119,120] Other SPI-2 translocated proteins interact with the actin cytoskeleton surrounding the SCV and probably contribute to remodeling of vacuole-associated actin networks.[121,122] SpvB is a *Salmonella* virulence protein that is secreted into the macrophage cytoplasm, possibly by the SPI-2 T3SS, and adenosine diphosphate ribosylates monomeric actin (G-actin), thus promoting disassembly of actin networks around the vacuole.[122,123]

Other SPI-2 effector proteins alter ubiquitination by functioning as ubiquitin ligases or deubiquitinases.[124,125] Although the molecular targets of their enzymatic activity are not known, currently, the mechanism by which ubiquitin ligases compete with mammalian enzymes has been shown to be by co-opting intermediates in the mammalian ubiquitin pathway.[126] Other proteins appear to localize to the Golgi apparatus, possibly to promote secretory traffic to the SCV.[104,127]

Many other bacterial factors are required for full virulence, including those required for synthesis of essential nutrients and iron acquisition and the virulence plasmids found in many NTS serotypes. The virulence plasmids of *S.* Typhimurium, *S.* Dublin, *S.* Choleraesuis, and *S.* Enteritidis all contain an 8-kilobase region that promotes dissemination beyond the intestine in animal models and bacteremia in humans.[128] This region encodes the SpvB protein and several other proteins of unknown function.

Host Response and Immunity

The innate immune system senses invasive *Salmonella* infections by using receptors that recognize conserved elements of bacterial structure. This includes recognition by plasma membrane and phagosomal membrane TLRs and cytoplasmic recognition receptors or the nucleotide oligomerization domain–like receptors (NOD-like): LPS by TLR4, bacterial lipoproteins by TLR2, flagellin by TLR5, flagellin by a signaling system that includes IPAF, and peptidoglycan by NOD1 and NOD2.[81,92,129] Activation of these receptors on phagocytes and epithelia leads to synthesis of cytokines that orchestrate the inflammatory response and instruct the subsequent antigen-specific immune response. Mice lacking a

functional TLR4 response are highly susceptible to *Salmonella* infection, confirming the importance of this initial response to infection.[130] Other studies indicate that caspase-1 and caspase-11, proteases important for secretion of IL-1 and IL-18, as well as inflammatory cell death in host cells, are important for infection in mice by the oral and systemic route, indicating that recognition and activation of the cytoplasmic sensing components by NOD family members are important for control of infection in the intestinal mucosa.[131] Recent evidence indicates this cell death and secretion of cytokines is mediated by inflammatory caspases cleaving proteins called gastrodermins, which leads to specific membrane pore formation through which cytokine release and cell death occurs.

Studies in mice further demonstrate that the initial control of *S.* Typhimurium replication in host tissue requires recruitment and activation of macrophages. In both mice and humans, macrophage activation and efficient killing of *Salmonella* are associated with production of interferon-γ (IFN-γ), IL-12, and tumor necrosis factor (TNF)-α.[132–134] Mice with targeted disruptions in the genes for these molecules are highly susceptible to infection. Rheumatoid arthritis patients treated with TNF antagonists have developed severe and fatal septicemias.[135] In addition, humans with mutations in the IFN-γ and IL-12 receptor genes develop severe infections with NTS serotypes.[136]

Although the innate immune system is able to suppress initial *Salmonella* replication, final clearance of infection and immunity to rechallenge requires a Th1-type CD4 T-cell response and production of specific antibodies by B cells.[134] *Salmonella*-specific antibodies are needed to control extracellular replication of *Salmonella* in the blood and are absent in most healthy Malawian children younger than 16 months, potentially contributing to the observed high rates of NTS bacteremia in African children younger than 2 years.[137] Both antibody and complement are required for induction of oxidative burst killing and phagocytosis of some African strains of NTS by neutrophils and monocytes.[138] Mice lacking mature CD4 cells (H2I; AB^- mice) or B cells ($Igh\text{-}6^-$ mice) are unable to control *Salmonella* infection.[134,139,140] The importance of cellular immunity in controlling *Salmonella* infection in humans is made apparent by the extreme susceptibility of individuals with HIV infection, lymphoproliferative diseases, or immune suppression after transplant.[141,142] A variety of other immunodeficiencies have been associated with *Salmonella* infection, including common variable immunodeficiency.[143] Furthermore, vaccine studies in humans demonstrate that protection against *S.* Typhi infection correlates with development of cell-mediated immunity.[144] In accord with the importance of CD4 T-cell–mediated immunity, population-based studies in humans have found an association between specific class II major histocompatibility complex alleles and susceptibility to typhoid fever.[145] CD8 T cells with cytolytic activity against *Salmonella*-infected host cells are also present in infected mice, but the importance of this activity in immunity remains unclear. Little is known about the antigen specificity of protective immune responses to *Salmonella* infection in humans, although antibodies against the Vi polysaccharide, LPS O antigen, and flagella are present in previously vaccinated or infected individuals.

CLINICAL MANIFESTATIONS

Specific *Salmonella* serotypes most often produce characteristic clinical syndromes, including gastroenteritis, enteric fever, bacteremia and vascular infection, localized infections, and the chronic carrier state, and outcomes, including case-fatality rates and hospitalization rates, differ substantially by serotype.[146]

Gastroenteritis

Infection with NTS most often results in self-limited acute gastroenteritis that is indistinguishable from that caused by many other enteric bacterial pathogens. Typically, within 6 to 48 hours after ingestion of contaminated food or water, nausea, vomiting, and diarrhea develop, but median incubation periods of up to 7 days have been reported from foodborne outbreaks of *S.* Enteritidis, likely correlating with low ingested dose.[147,148] In most cases, stools are loose, of moderate volume, and without blood. In rare cases, stool may be watery and of large volume ("cholera-like") or of small volume associated with tenesmus ("dysentery-like"). Fever (38°–39°C), abdominal cramping, nausea, vomiting, and chills frequently are reported. Headache, myalgias, and other systemic symptoms also may occur. Microscopic examination of stools shows neutrophils and, less frequently, red blood cells. NTS can cause a syndrome of pseudoappendicitis, can mimic the intestinal changes of inflammatory bowel disease, or rarely causes toxic megacolon.[147,149]

Gastroenteritis caused by NTS is usually self-limited. Diarrhea resolves within 3 to 7 days and fever within 72 hours.[147] Persistent NTS infection with relapsing diarrhea lasting up to 8 years has been described in a small fraction of patients and was associated with in-host single nucleotide mutations in key virulence regulators.[150] On occasion, patients require hospitalization because of dehydration, and death occurs infrequently. In the United States, NTS infections result in an estimated 19,336 hospitalizations and 378 deaths per year.[16] A disproportionate number of these deaths occur among the elderly, especially those residing in long-term care facilities, and among immunocompromised patients, including persons with HIV/acquired immunodeficiency syndrome (AIDS), stem cell transplantation, or those who are receiving biologic therapies.[151,152]

After resolution of gastroenteritis, the mean duration of carriage of NTS in the stool is 4 to 5 weeks in adults and 7 weeks in children.[153] Longer duration of stool carriage has been associated with random single nucleotide mutations in *S.* Typhimurium strains.[154] Antimicrobial therapy may increase the duration of carriage.[155] Acute NTS gastroenteritis has been associated with an increased risk of developing irritable bowel syndrome in several surveillance and outbreak studies with methodologic limitations.[156]

Enteric Fever

Enteric fever is a severe systemic illness characterized by fever and abdominal pain that is caused by disseminated infection with *S.* Typhi and *S.* Paratyphi A, B, and C. The definitive diagnosis of enteric fever requires the isolation of *S.* Typhi or *S.* Paratyphi from blood, bone marrow, another sterile site, intestinal secretions, or from punch biopsy of rose spots—the faint salmon-colored maculopapular skin lesions on the trunk stool that may appear at the end of the first week of illness (see Chapter 100).

Bacteremia and Vascular Infection

Up to 8% of patients with NTS gastroenteritis develop bacteremia; of these, 5% to 10% develop localized infections.[157] Bacteremia and metastatic infection are more common among persons with serotypes Choleraesuis, Dublin, Enteritidis, Heidelberg, Poona, and Schwarzengrund and among infants, the elderly, and those with comorbidities, including immunosupression.[158,159,160] Among children, NTS bacteremia usually is associated with gastroenteritis and prolonged fever, infrequently causes focal infections, and is fatal in less than 10% of cases.[161] In contrast, adults are more likely to have primary bacteremia and have a high incidence of secondary focal infections and death.[161] The mortality of NTS bacteremia increases with the duration of bacteremia and in the presence of coma or septic shock.[162,163]

In sub-Saharan Africa, NTS is among the most common causes of bacteremia, and invasive infection is associated with HIV infection in adults and among children with HIV and malaria coinfection, sickle cell disease, or malnutrition.[53] Increasingly, NTS blood isolates from sub-Saharan Africa belong to a single *S.* Typhimurium sequence type, ST313. This strain type has spread rapidly throughout the region and is associated with genome reduction that could make these strains more human adapted with direct human-to-human transmission and more likely to cause a typhoid-like illness than gastroenteritis. However, recent evidence indicates that these strains may be less fit for resistance to oxidative and nitrosative killing, indicating that the long-term colonization of individuals with these strains may have selected for less virulence for those with normal immune responses.[10,164]

NTS endovascular infection should be suspected in cases of high-grade or persistent bacteremia, especially in persons with preexisting valvular heart disease, atherosclerotic vascular disease, prosthetic vascular grafts, or aortic aneurysms (Fig. 223.5). The risk of endovascular infection complicating *Salmonella* bacteremia is estimated to be 9% to 25% in persons older than 50 years, usually involves the aorta, and most commonly results from seeding atherosclerotic plaques or aneurysms.[165,166]



FIG. 223.5 *Salmonella* Enteritidis mycotic pseudoaneurysm of the iliac artery. Contrast computed tomography scan of the abdomen and pelvis (coronary view), showing a pseudoaneurysm of the left common iliac artery *(arrow),* with mass effect on the psoas muscle and left ureter, resulting in significant hydronephrosis *(asterisk). (From Farmakiotis D, Chien KS, Shum TCT, Rodriquez-Barradas M, Musher DM. To scan or not to scan.* Clin Infect Dis*. 2013;56:1003. Used with permission.)*

Using a simple scoring system that assigns +1 point for each of four risk factors (male gender, hypertension, coronary artery disease, and serogroup C1 infection) and −1 point for each of two variables negatively associated with vascular infection (immunsuppression and malignancy), patients with NTS bacteremia can be stratified into high-risk (>1 point, sensitivity 95%, specificity 45%) and low-risk (0–1 point) vascular infection groups.[167] Mortality rates range from 14% to 60% and are lower with prompt diagnosis and combined medical and surgical therapy.[159,161] Endocarditis and arteritis are rare but are associated with potentially fatal complications, including valve perforation, endomyocardial abscess, infected mural thrombus, pericarditis, mycotic aneurysms, aneurysm rupture, aortoenteric fistula, and vertebral osteomyelitis.

Salmonellosis and HIV Infection

NTS is a leading cause of community-acquired bacteremia in HIV-infected adults, especially in sub-Saharan Africa.[168] In developed countries during the pre–antiretroviral therapy (ART) era, NTS bacteremia occurred 20 to 100 times more commonly among those with HIV infection compared with the general population, likely reflecting both increased host susceptibility and impaired clearance.[169] Among HIV-infected patients, NTS bacteremia is associated with lower CD4 lymphocyte counts and a higher risk of metastatic complications, recurrent bacteremia, and mortality despite antimicrobial therapy, especially in Africa.[168]

Recurrent NTS bacteremia is an AIDS-defining illness that apparently results from incomplete clearance of the primary infection because of impaired cell-mediated immunity and dysregulation of proinflammatory cytokines release.[170] In the pre-ART era, as many as 43% of patients with NTS bacteremia had one or more recurrent episodes.[168] Among patients receiving ART, the incidence of recurrent NTS bacteremia has declined up to 96%.[171] The risk reduction is likely due to the impact of ART on virologic suppression and immune reconstitution, a direct bactericidal activity of some antiretrovirals on *Salmonella* spp., and the prophylactic use of trimethoprim-sulfamethoxazole (TMP-SMX).[171]

Localized Infections

Extraintestinal focal infections develop in approximately 5% to 10% of persons with *Salmonella* bacteremia, and their diagnosis and management are summarized in Table 223.2.

Chronic Carrier State

The chronic carrier state is defined as the persistence of *Salmonella* in stool or urine for more than 12 months after acute infection. Chronic carriage of *Salmonella* is associated with excretion of large numbers of organisms in stool but with an absence of clinical disease, related to high levels of systemic immunity. From 0.2% to 0.6% of adults with NTS infection develop chronic carriage, most commonly associated with underlying host immunosuppression.[172] In comparison, up to 10% of untreated patients with typhoid fever excrete *S.* Typhi in the feces for up to 3 months, and 1% to 4% develop chronic carriage, especially associated with biliary abnormalities and gallstone biofilm formation.[173,174]

IMMUNIZATION AGAINST *SALMONELLA*

There is no available vaccine to protect against invasive NTS disease. Two typhoid vaccines currently are commercially available: (1) Ty21a, an oral, live-attenuated *S.* Typhi vaccine (given on days 1, 3, 5, and 7, with a booster every 5 years) and (2) a parenteral Vi capsular polysaccharide vaccine (Vi CPS), consisting of purified Vi polysaccharide from the bacterial capsule (given as a single 0.5-mL [25-µg] intramuscular dose with a booster every 2 years). Typhoid vaccines are reviewed in Chapter 100.

THERAPY

Gastroenteritis

Salmonella gastroenteritis is usually a self-limited disease, and therapy primarily should be directed to the replacement of fluid and electrolyte losses. In a large meta-analysis, antimicrobial therapy for uncomplicated NTS gastroenteritis, including short-course or single-dose regimens with oral fluoroquinolones, amoxicillin, or TMP-SMX, did not significantly decrease the length of illness, including duration of fever or diarrhea, and was associated with an increased risk of positive stool culture 1 month after treatment and adverse drug reactions.[155] Therefore antimicrobials should not be used routinely to treat uncomplicated NTS gastroenteritis or to reduce convalescent stool excretion.

Although less than 5% of all patients with *Salmonella* gastroenteritis develop bacteremia, certain patients are at increased risk for invasive infection and may benefit from preemptive antimicrobial therapy. Antimicrobial therapy should be considered for neonates (probably up to 3 months of age), those older than 50 years with suspected atherosclerosis, and for persons with immunosuppression, cardiac valvular or endovascular abnormalities, or significant joint disease. Treatment should consist of an oral or intravenous (IV) antimicrobial administered for 48 to 72 hours or until the patient becomes afebrile. Immunocompromised persons, including those with HIV infection, who develop *Salmonella* gastroenteritis may require 7 to 14 days of therapy, typically with a fluoroquinolone, to reduce the risk of extraintestinal spread.[175] For susceptible organisms, oral therapy with a fluoroquinolone, TMP-SMX, or amoxicillin is adequate. Although fluoroquinolones are not recommended for administration to children younger than 10 years, they may have a role in treating severe NTS gastroenteritis in this age group, particularly among immunocompromised patients.[176] On occasion, antimicrobial prophylaxis has been required to control institutional outbreaks, especially in long-term care facilities or pediatric wards, where compliance with infection control measures may be difficult.[177]

Bacteremia

Because of the increasing prevalence of antimicrobial resistance, empirical therapy for bacteremia or focal infection suspected to be caused by NTS should include a third-generation cephalosporin and a fluoroquinolone until susceptibilities are known. High-grade bacteremia (i.e., >50% of three or more blood cultures testing positive) should prompt evaluation for endovascular abnormalities by echocardiogram or other imaging techniques, such as computerized tomography or indium-labeled white blood cell scan. Low-grade bacteremia not involving vascular

TABLE 223.2 Extraintestinal Infectious Complications of Salmonellosis

SITE	INCIDENCE	RISK FACTORS	MANIFESTATIONS	COMPLICATIONS	MORTALITY	DIAGNOSIS	THERAPY
Endocarditis	0.2%–0.4%	Preexisting valvular heart disease	Valvular vegetation, infected mural thrombus	Valve perforation, relapse (20%–25%), pericarditis	≈70%	Blood culture, echocardiography	Early valve surgery + 6 wk P ceph 3, P ampicillin, or P, then PO fluoroquinolone
Arteritis	Rare	Atherosclerosis, aortic aneurysm, endocarditis, prosthetic graft, myelodysplasia	Prolonged fever, pain in back, chest, or abdomen	Mycotic aneurysm, aneurysm rupture, aortoenteric fistula, vertebral osteomyelitis	14%–60%	Blood culture, sonogram, MRI or CT	Early surgical intervention + 6 wk P ceph 3, P ampicillin, or P, then PO fluoroquinolone
Central nervous system	0.1%–0.9%	Infants (especially neonates)	Meningitis, ventriculitis, brain abscess, subdural empyema, encephalopathy	Seizures, mental retardation, hydrocephalus, brain infarction, relapse	≈20%–60%	CSF culture, CT or MRI	≥3 wk P ceph 3, P ampicillin, or a carbapenem
Pulmonary	Rare	Lung malignancy, structural lung disease, sickle cell anemia	Pneumonia	Lung abscess, empyema, bronchopleural fistula	≈25%–60%	Respiratory culture, chest radiograph	≥2 wk P/PO abx
Bone	<1%	Sickle cell anemia, male gender, connective tissue disease, immunosuppression	Femur, tibia, humerus, lumbar vertebrae	Relapse, chronic osteomyelitis	Very low	Bone radiograph	≥4 wk P ceph 3, P ampicillin, or P, then PO fluoroquinolone + surgery for sequestra
Joint, reactive	0.6%	HLA-B27, antimicrobial therapy	Joints (≥ three joints) involved (especially knee, ankle, wrist, and sacroiliac)	Prolonged symptoms (mean duration, 5.5 mo)	Negligible	Joint fluid examination and culture	Nonsteroidal antiinflammatory agent
Joint, septic	0.1%–0.2%	Osteoarthritis, connective tissue disease, sickle cell disease, prosthetic joint	Knee, hip, shoulder	Joint destruction, osteomyelitis	Very low	Joint fluid examination and culture	Repeated needle aspiration + ≥4 wk P/PO abx
Muscle/soft tissue	Rare	Local trauma, male gender, diabetes, HIV infection	Abscess, pyomyositis	Osteomyelitis, endovascular infection, frequent relapse	≈33%	Ultrasonography, aspiration	Drainage + ≥2 wk P abx
Hepatobiliary	Rare	Cholelithiasis, cirrhosis, amebic abscess, echinococcal cyst, hepatocellular carcinoma	Hepatomegaly, cholecystitis, hepatic abscess	Rupture with secondary peritonitis, subphrenic abscess, spontaneous bacterial peritonitis	≈10%	Ultrasonography, aspiration	Drainage + ≥2 wk P abx
Splenic	Rare	Sickle cell anemia, splenic cyst, splenic hematoma	Splenomegaly	Left pleural empyema, subphrenic abscess, rupture with secondary peritonitis	<10%	Ultrasonography, aspiration	≥2 wk P abx + percutaneous drainage or splenectomy
Urinary	0.6%	Urolithiasis, malignancy, renal transplant, elderly female	Cystitis, pyelonephritis	Renal abscess, interstitial nephritis, relapse	≈20%	Urine culture, ultrasonography	Removal of structural abnormality + 1–2 wk P abx + ≥6 wk PO fluoroquinolone or TMP-SMX
Genital	Rare	Pregnancy, renal transplant	Ovarian abscess, testicular abscess, prostatitis, epididymitis	Abscess	Very low	Ultrasonography, aspiration	Drainage of collection + 1–2 wk P abx + ≥6 wk PO fluoroquinolone or TMP-SMX
Soft tissue	<1%	Local trauma, immunosuppression	Pustular dermatitis, SC abscess, wound infection	Septic thrombophlebitis, endophthalmitis	≈15%	Drainage culture	≥2 wk P abx + drainage of collection

abx, Antibiotics; *CSF,* cerebrospinal fluid; *CT,* computed tomography; *HIV,* human immunodeficiency virus; *HLA,* human leukocyte antigen; *MRI,* magnetic resonance imaging, *P abx,* parenteral ampicillin; *P ceph 3,* parenteral third-generation cephalosporin; *PO,* oral; *P/PO abx,* parenteral or oral antibiotic (e.g., fluoroquinolone, ampicillin, TMP-SMX, or P ceph 3); *P/PO fluoroquinolone,* parenteral or oral fluoroquinolone; *SC,* subcutaneous; *TMP-SMX,* trimethoprim-sulfamethoxazole.

structures should be treated with 7 to 14 days of IV ceftriaxone or ampicillin or by IV, followed by oral, fluoroquinolone therapy. Documented or suspected endovascular infection should be treated with 6 weeks of IV ceftriaxone or IV ampicillin or by fluoroquinolone therapy (initially administered IV followed by oral when stable). Early surgical resection of infected aneurysms or other endovascular focus on infection is recommended. Alternatively, patients who are not candidates for surgical resection and vascular grafting have been treated with endovascular stenting and/or chronic suppressive oral antimicrobial therapy.[178]

Recurrent *Salmonella* Bacteremia in Persons With HIV

For HIV-infected persons with CD4 counts ≥200 cells/mm^3, a first episode of NTS bacteremia can be treated with 14 days of ceftriaxone or ampicillin or by IV, followed by oral fluoroquinolone therapy, provided that the bacteremia rapidly clears. For those persons with persistent bacteremia, metastatic complications, or CD4 count <200 cells/mm^3, 4 to 6 weeks of therapy is recommended.[23] Persons who relapse after 4 to 6 weeks of antimicrobial therapy should receive long-term suppressive therapy with an oral fluoroquinolone or TMP-SMX, based on susceptibility testing.

Focal Infections

Treatment recommendations for the management of focal *Salmonella* infections are summarized in Table 223.2. For extraintestinal nonvascular infections, antimicrobial therapy for 2 to 4 weeks (depending on the infection site) is usually recommended. In cases of chronic osteomyelitis, abscesses, and urinary or hepatobiliary infection associated with anatomic abnormalities, surgical resection or drainage may be required in addition to prolonged antimicrobial therapy to eradicate infection.

Carrier State

Treatment of persons with asymptomatic carriage of NTS is controversial. In a randomized, placebo-controlled trial conducted in Thailand among asymptomatic food workers, two 5-day regimens (norfloxacin, 400 mg twice daily, and azithromycin, 500 mg once daily) were no more effective than placebo in eradicating NTS carriage and increased the risk of reinfection with antimicrobial-resistant *S.* Schwarzengrund.[179]

Prevention and Control

The prevention and control of NTS require both an understanding of the complex cycles of transmission and ongoing surveillance to characterize trends in *Salmonella* incidence, prevalence, and antimicrobial susceptibility and to identify outbreaks. In developing countries, especially in sub-Saharan Africa, controlling the high burden of invasive NTS will require enhanced surveillance to estimate disease burden, rapid diagnostics to identify antimicrobial resistance, and clinical studies and management algorithms to improve patient outcomes.

In developed countries, control of foodborne salmonellosis requires identification of controllable hazards, monitoring, and verification to limit the introduction and multiplication of *Salmonella* from the farm to the table.[180] Recognition of foodborne outbreaks requires that clinicians have a high index of suspicion, order the appropriate laboratory test, and promptly report positive culture results to local public health departments. Vaccination of livestock and egg-laying hens, further limiting the use of antimicrobials as animal growth promoters, and improved food safety practices should further reduce the burden of foodborne salmonellosis. Active population-based surveillance for foodborne diseases has improved estimates of disease burden,[4] and use of algorithms and rapid molecular subtyping has improved the ability to detect and more rapidly control outbreaks of salmonellosis associated with widely distributed agricultural and manufactured foods.[181] Although most cases of *Salmonella* infection occur sporadically, large numbers of persons potentially may become infected when commercial kitchens serve *Salmonella*-contaminated foods that have not been sufficiently cooked or that have been mishandled. Commercial food service establishments can reduce the risk of foodborne *Salmonella* illness if they do not serve food containing raw or undercooked eggs, use pasteurized eggs whenever possible, and avoid cross-contamination of food items. Use of pasteurized eggs for all recipes calling for bulk-pooled eggs is recommended for all nursing homes and hospitals.

The most cost-effective approach to the control of salmonellosis in food handlers is attention to good personal hygiene and maintenance of time-temperature standards for food handling. Routine screening of food handlers for carriage after gastroenteritis is commonly performed before they are allowed to return to work. However, there is little justification for this approach because few outbreaks are related to specific food handlers, prolonged carriage in food handlers after gastroenteritis is rare, and the number of organisms present is small. Therefore it is reasonable to allow individuals to return to work after diarrhea is resolved. Two consecutive negative stool samples should be required only for food handlers whose work involves touching unwrapped foods that are consumed raw or served without further cooking. Routine surveillance of food handlers for asymptomatic stool carriage of *Salmonella* is not recommended.

To limit the risk of health care–associated transmission to patients and personnel, patients excreting NTS should be managed with standard precautions, including appropriate hand hygiene and the use of personal protective equipment, including gloves, when performing direct patient care or handling fecally soiled articles. Additional measures, including enhanced environmental disinfection and cohorting of ill patients may be required to control outbreaks of NTS infection in health care facilities.[182] Although NTS infection in newborns, the elderly, or the immunocompromised can be severe, the risk of transmission of *Salmonella* from health care workers to patients appears to be very small.[63] Once the health care worker is asymptomatic and passing formed stool, the individual should be allowed to return to work if standard precautions are observed. However, local and state regulations should be followed because some require work exclusion for health care workers who have salmonellosis until two or more stool cultures obtained at least 24 hours apart are negative.

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The complete reference list is available online at Expert Consult.

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224 Bacillary Dysentery: *Shigella* and Enteroinvasive *Escherichia coli*

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SHORT VIEW SUMMARY

Definition
- Diarrheal illness caused by strains of invasive forms of *Shigella* or invasive *Escherichia coli* comprises bacillary dysentery.

Epidemiology
- Being a very low-inoculum disease, shigellosis is the most infectious (communicable from person to person) of the bacterial enteropathogens.

Microbiology
- *Shigella* spp. are small gram-negative rods in the family Enterobacteriaceae.
- Similar to strains of *Shigella,* enteroinvasive *E. coli* (EIEC) possesses somatic antigens and a plasmid that controls invasiveness.

Diagnosis
- Patients passing bloody stools or those associated with cases of shigellosis should have stools processed for *Shigella.*
- The sooner a stool specimen is cultured, the higher the yield for *Shigella.*
- Once specimens have been plated on gram-negative media at 37°C and incubated overnight, lactose-negative colonies should be tested biochemically and serologically for *Shigella.*
- Nucleic acid amplification tests (NAATs) improve the sensitivity for the diagnosis of dysentery but target a gene shared by both *Shigella* and EIEC, and therefore the report does not distinguish between the two organisms. Furthermore, NAATs do not provide information on species or drug susceptibility.
- *Shigella* or EIEC identified through molecular methods should be confirmed by culture for speciation, serotyping, and drug susceptibility testing and for epidemiologic purposes.

Therapy
- Antibiotics are useful in the management of shigellosis and can be lifesaving for infection caused by the Shiga bacillus (*Shigella dysenteriae* type 1).
- The treatment of choice for adults is a fluoroquinolone antibiotic given for 3 days.
- For children, cephalosporin, ciprofloxacin, or azithromycin may be used.
- Antimicrobial resistance is an emerging problem worldwide, with increasing reports of strains that are resistant to fluoroquinolones, macrolides, and cephalosporins.

Prevention
- Because person-to-person spread is so important in *Shigella* infection, hand washing and other hygienic methods should be used to prevent spread.
- Vaccines are in development to prevent *Shigella* infection.

The term *dysentery* was used by Hippocrates to indicate a condition characterized by the frequent passage of stool containing blood and mucus, accompanied by straining and painful defecation. It was not until the end of the 19th century, when the causes of amebiasis and bacillary dysentery were determined, that the two great forms of dysentery could be accurately separated. In view of the absence of liver complications, much of the dysentery in the older historical writings is considered to be of bacillary origin (shigellosis). After the causative agents of the two types of dysentery were determined, the different epidemiologic settings were described. In 1859 in Prague, Lambl and then later Osler[1] and Councilman and Lafleur[2] helped verify the pathogenicity of *Entamoeba histolytica.* In 1906, Shiga conclusively demonstrated that a bacterium was present in the stool of many patients with dysentery and that agglutinins could be demonstrated in the serum of the infected patients.[3] At about the same time, Flexner found a similar but serologically different organism in the stools of other patients with dysentery acquired in the Philippines.[4] Rogers stated in 1913 that "epidemic dysentery in asylums, jails, or in long-occupied and unsanitary military camps during the war is almost certain to be bacillary, while sporadic cases in a warm climate are more frequently amebic."[5]

Medical writings since the beginning of recorded history have dealt with the common problems of dysentery in civilian and military populations; perhaps the greatest historical consideration is the influence that bacillary dysentery has had on military campaigns. Almost every long campaign and extended siege has produced epidemics of bacillary dysentery, particularly when sanitation and food sources could not be adequately controlled. In many military battles leading up to World War I, a heavier toll was ascribed to bacillary dysentery than to war-related injuries.[6]

MICROBIOLOGY

Shigella organisms are small gram-negative rods that are members of the family Enterobacteriaceae, tribe Escherichieae, and genus *Shigella.* They are nonmotile and nonencapsulated. Comparative genomics using whole-genome sequencing has suggested that *Shigella* and enteroinvasive *Escherichia coli* (EIEC) include diverse members with virulence mechanisms in common acquired through mobile genetic elements derived from plasmids, phage, or genomic islands. *Shigella* evolved by acquiring virulence genes from multiple lineages of *E. coli*[7]; more recently, EIEC lineages evolved independently from multiple distinct lineages of *E. coli* via the acquisition of *Shigella* virulence plasmid and in some cases *Shigella* pathogenicity islands.[8]

Shigellae are polyphyletic, meaning that they are not all descendants of a single clone. One hypothesis is that members of several *E. coli* clones acquired a mobile genetic element—the ancestor of the present-day invasion plasmid.[9] Through adaptation to the plasmid and additional convergent evolution with the acquisition of mobile genetic elements, deletions or mutations affecting gene function lead to the loss of motility and other phenotypes such as intracellular niche and the ability to spread from cell to cell that distinguish *Shigella* from *E. coli,*[10] This apparently happened multiple independent times, resulting in the various *Shigella* lineages. However, evolutionary reconstructions place *Shigella* squarely within the broad range of lineages that we call *E. coli.*[10]

Isolation Techniques

The infecting strain of *Shigella* is generally present in stool in concentrations between 10^3 and 10^9 viable cells per gram of stool, depending on the stage of illness. During the postconvalescent shedding period, counts fall to 10^2 to 10^3 viable cells per gram of stool. Recovery of the agent microbiologically is not usually difficult in the early stages of disease because of the higher counts present; it is more difficult during later stages of illness because of the lower counts of viable bacteria. Patients with shigellosis at the height of their illness can have negative stool cultures.[11] Careful selection of feces and processing on appropriate media give a higher yield of organisms. The sooner after passage the specimen is processed, the

higher is the yield. Stool that stands at room temperature for more than 24 hours has a profound drop in the number of viable cells, and recovery of the pathogen is less likely. A rectal swab obtained and seeded immediately at the bedside is the optimal way to perform a stool culture.

For bacteriologic identification of *Shigella*, a bit of blood or mucus is seeded onto at least two different media. In general, stool is plated lightly onto a medium with only mild inhibiting factors for gram-negative growth, such as MacConkey agar, xylose-lysine-deoxycholate agar, Tergitol 7, or eosin–methylene blue (EMB) agar, whereas a separate specimen is plated heavily onto a more inhibitory medium, such as *Shigella-Salmonella* medium. The more plates used, the greater the recovery yield. After overnight incubation at 37°C, lactose-negative colonies are tested biochemically and then serologically identified with *Shigella* grouping and typing antisera.

Group and Type Identification

Shigella are divided into four groups, depending on serologic similarity and fermentation reactions: group A *(Shigella dysenteriae),* with 15 serotypes; group B *(Shigella flexneri),* with 19 serotypes and subserotypes; group C *(Shigella boydii),* with 20 serotypes; and group D *(Shigella sonnei),* with a single serotype. Commercial antiserum is available for determining group- and type-specific antigenicity. *S. sonnei* accounts for 60% to 80% of the cases currently reported in the United States and other industrialized areas.

Invasive *Escherichia coli*

Certain strains of *E. coli* can cause a clinical illness indistinguishable from shigellosis and should be considered as causative agents of bacillary dysentery. Almost all the *Shigella*-like *E. coli* strains have been shown to possess somatic antigens related to *Shigella* serotypes, further demonstrating the similarity of these two groups of organisms. EIEC strains that cause bacillary dysentery have been shown to belong serologically to the following *E. coli* O groups: 28, 29, 112, 115, 124, 136, 143, 144, 147, 152, 164, and 167. Serotyping may ultimately prove to be useful in detecting these strains. At the genomic level, most EIEC strains are distributed across three lineages.[8] The classic laboratory test for determining the virulence of a bacterial isolate (*Shigella* or EIEC strain) was the Sereny test.[12] Keratoconjunctivitis develops after 1 to 7 days in guinea pigs (or rabbits) when an invasive bacterial strain (*E. coli* or *Shigella*) is dropped into the conjunctival sac of the animal (Fig. 224.1). This test is no longer used for diagnostic purposes. As discussed earlier, *Shigella* and EIEC have evolved from common ancestors and conceptually form a single pathovar within *E. coli,* with different lineages.[13]

A different form of bacillary dysentery has been shown to be caused by an O157:H7 strain of *E. coli* and other Shiga toxin (STx)–producing *E. coli* strains (see Chapter 218).

PATHOGENESIS

Communicability and Infectivity

Shigellosis is *the* most communicable of the bacterial diarrheas. Experiments in volunteers have demonstrated that shigellosis is unique among bacterial enteropathogens in that fewer than 100 viable cells can readily produce the disease in healthy adults.[14] Dose-response data obtained in volunteers for virulent strains from three species of *Shigella* are given in Table 224.1. When volunteers ingested 500 or fewer viable cells of *S. flexneri, S. sonnei,* or *S. dysenteriae* type 1 (the Shiga bacillus), essentially the same rate of clinical illness resulted—27% to 45%.[14] In general, the laboratory-passaged strains used in volunteer studies are less infectious than naturally transmitted strains, implying that the actual infectious dose in the field is considerably lower than 500 organisms. This low dose of organisms probably explains how the illness can be so readily transferred from person to person, why the secondary attack rate is so high when an index case is introduced into a family, and why recurrent bacillary dysentery is an important problem in institutionalized or crowded populations.

The reasons for this low dose response are not completely clear. One possible explanation is that virulent shigellae can withstand the low pH of gastric juice. In a study of adult Bangladeshi men admitted to the hospital with diarrhea, normal gastric acid levels were seen in patients with shigellosis, amebiasis, and pathogen-negative diarrhea, whereas patients with secretory diarrhea caused by *Vibrio cholerae* and enterotoxigenic *E. coli* had low gastric acid levels, offering evidence that *Shigella* did not require reduced gastric acidity to produce enteric disease.[15] In another study, *Shigella* isolates were able to survive at a pH of 2.5 for at least 2 hours, whereas *Salmonella* was not.[16] *Shigella* strains were also shown to be able to survive in acidic apple juice and tomato juice stored at 7°C and 22°C, respectively, for up to 14 days, showing its resistance to acid.[17] A study in Kenyan children suggested that unabsorbed iron could be a direct risk for pathogens or for intestinal dysbiosis.[18] Although EIEC and *Shigella* possess the same virulence determinates, infection with IEC requires an average dose 1000 times higher to cause infection.[19] EIEC strains have not been compared with *Shigella* to determine if relative acid susceptibility might explain the different dose response. Nonpathogenic *E. coli* strains appear to have similar acid susceptibility to strains of *Shigella,* suggesting that this is not the reason for the difference in dose response.[16]

Mucosal Invasion and Inflammation

Virulent *Shigella* and other nontoxigenic invasive *E. coli* strains produce disease after invading the intestinal mucosa via the basolateral surface of the colonic enterocyte.[20,21] Genes required for bacterial entry into epithelial cells are present on a 30-kb region of a 220-kb virulence plasmid.[22] *Shigella* infection is superficial, and only rarely does the organism penetrate beyond the mucosa, which explains the rarity of obtaining positive blood cultures in patients with shigellosis, despite the common occurrence of hyperpyrexia and toxemia. *Shigella* and EIEC invade colonic and rectal cells, including M cells of the follicle-associated epithelium, macrophages, and epithelial cells; invasion is followed by intracellular multiplication, spread of infection to adjacent cells, severe inflammation, and destruction of colonic mucosa.[23] Apoptotic destruction of macrophages in subepithelial tissue allows survival of



FIG. 224.1 Guinea pig with keratoconjunctivitis after conjunctival inoculation of invasive *Escherichia coli*. This is a positive Sereny test result but is no longer used for diagnosis.

TABLE 224.1 Response of Adult Volunteers to Experimental Challenge With Viable Virulent Strains of *Shigella*

SHIGELLA SPECIES	INOCULUM (ORGANISMS)	NO. OF VOLUNTEERS	NO. OF CASES OF CLINICAL SHIGELLOSIS (% OF TOTAL)
S. flexneri (strain 2467T)	≤180 ≥5×10^3	72 211	23 (32) 124 (59)
S. sonnei (53G)	500	58	26 (45)
S. dysenteriae 1[a]	≤200 ≥2×10^3	22 22	6 (27) 14 (64)

[a]Strains A-1 and M-131.

Modified from DuPont HL, Levine MM, Hornick RB, et al. Inoculum size in shigellosis and implications for expected mode of transmission. J Infect Dis. *1989;159:1126–1128.*

the invading shigellae, and inflammation facilitates further bacterial entry.[24] Once the organisms are intracellular, they multiply within the cytoplasm and move from cell to cell by an actin-dependent process.[23]

Pathogenic strains of *Shigella* and other bacterial enteropathogens have evolved a complex type III secretion mechanism that enables them to invade the intestinal mucosa.[25] Bacterial proteins (including toxins) are injected from the bacterial cytoplasm into the cytosol of host mucosal cells, where they modulate the functions of the host cells and dictate how the host and pathogen relate.[26–28] Each type III system consists of the secretion apparatus, secreted effector proteins, cytoplasmic chaperones (specialized for transporting the specific effector proteins), and specific transcriptional regulators.[29–31] The secreted proteins—there are approximately 20, including VirA, OspB to OspG, IpaA to IpaD, and IpgD—facilitate bacterial entry into nonphagocytic cells and induce apoptosis and are targets for vaccine development.[28] *Shigella* strains decrease production of host antimicrobial peptides, thus facilitating persistence by avoiding the host immune system.[32] Strains of bacterial pathogens that use type III secretion systems can be detected by screening for virulence genes directly.[33]

Toxigenicity

All four species of *Shigella* can produce the plasmid-borne toxin ShET2,[34] whereas the chromosomally encoded ShET1 is elaborated by *S. flexneri* 2a,[35] and STx is produced by *S. dysenteriae* 1.[36] These toxins can induce water and electrolyte secretion across the colonic epithelium. However, other enterotoxins are likely involved in secretory diarrhea because nontoxigenic strains can also cause disease. STx is similar to the STx1 and STx2 produced by enterohemorrhagic *E. coli* O157:H7 and other Shiga toxin–producing *E. coli* (STEC) strains. A high proportion of children with *S. dysenteriae* 1 develop STx-related hemolytic uremic syndrome.[37]

Anatomic Location of Infection

Studies in volunteers have helped establish the intestinal localization of bacteria in experimental shigellosis.[11] Within 12 hours after participants swallow virulent shigellae, the bacteria transiently multiply in the small bowel to concentrations of 10^7 to 10^9 viable cells/mL of luminal contents, at which time abdominal pain, cramping, and fever occur. Within a few days, the infecting strain is no longer detectable in small bowel fluid, the patient's temperature becomes lower, and pain and tenderness, generally confined to the lower abdominal quadrants, become more severe. Urgency, tenesmus, and passage of bloody mucoid stools (dysentery) often occur in the later stages of infection and correlate with a diffuse colonic localization of the bacteria. Although strains of *Shigella* appear to be resistant to acid, as discussed earlier, acid exposure may transiently inhibit the virulence properties of the organism, which may encourage transit through the small bowel to the colon, where virulence characteristics are once more produced.[38] The density of intramucosal bacteria is highest at the luminal surface and extends in decreasing concentrations to reach the lamina propria and submucosa. Microabscesses form and coalesce, becoming large abscesses that slough and produce mucosal ulcerations. In shigellosis, both humoral and cellular immune mechanisms are stimulated. Cytokine levels correlate with disease severity,[39] and a number of fecal cytokines, including interleukin (IL)-8 and IL-1β, are higher than seen with other enteric bacterial pathogens.[40]

EPIDEMIOLOGY

Hippocrates indicated that when a dry winter was followed by a rainy spring, an increase in the number of dysentery cases would follow in the summer. In general, bacillary dysentery is a summertime illness, where it is characteristically seen in children living in crowded areas with inadequate sanitation and limited water. Because of the characteristic clinical picture of bacillary dysentery, it is one of the most accurately diagnosed and reported classes of infectious diarrhea. The greatest frequency of illness is reported in infants and younger or preschool children. Disease rates and also complications and severity parallel the degree of malnutrition. Flies may be important in the transmission of bacillary dysentery,[41,42] especially in tropical climates. Dysentery in warm countries is most prevalent when the fly population is at its highest. Bacteriologic surveys of fly populations have indicated that flies can occasionally be shown to be positive for *Shigella* bacteria.[41] The low dose required for infection at least partially explains the potential for fly transmission of shigellosis. Fly control, hand washing, and breast-feeding show protective effects against the organism.[43]

Cyclic Patterns of Disease

Since the description of bacteriologic isolation procedures, cyclic epidemics of bacillary dysentery have been described, each cycle lasting 20 to 50 years.[44] In Europe, during the first 25 years of the 20th century, dysentery was generally caused by *S. dysenteriae* 1, and mortality was higher than subsequently seen when other serotypes became prevalent. Between 1926 and 1938, *S. flexneri* strains became more prevalent than the Shiga bacillus in the developing world, and *S. flexneri* remains the major *Shigella* type in these areas. *S. sonnei* has become the major cause of bacillary dysentery in European countries and the United States. Widespread epidemics in the developing world may be seen for the more virulent *S. dysenteriae* 1, resulting in deaths without proper antimicrobial therapy. In general, *S. flexneri* and *S. sonnei* represent the endemic *Shigella* species in most populations, but in situations in which hygienic standards are lower, *S. dysenteriae* 1 can cause epidemic disease. Epidemics may occur in 20- to 30-year cycles, believed to reflect the development of immunity followed by the birth every year of more and more susceptible individuals until the conditions are ripe for epidemic transmission. Shiga dysentery remains a special problem in parts of Africa, the Indian subcontinent, and Bangladesh, where epidemic strains showing greater resistance to phagocytosis cause pandemic disease.[45]

Incidence of Shigellosis by Geography and Host

The annual number of *Shigella* episodes worldwide has decreased from 80 to 165 million cases and 600,000 deaths annually in the 1990s[46] to an estimated 270,00 deaths in 2016.[47] The highest rate of *Shigella* infection (69% of cases) and the highest death rate (61% of deaths) occur in those younger than 5 years. From 2006 to 2015, between 1190 and 2308 culture-confirmed cases were reported annually in the United Kingdom.[48] In the United States 2132 cases were reported to the CDC in 2017. Of these, 31% were diagnosed with nucleic acid amplification tests (NAATs).[49] Considering that not all cases are studied, the actual number of cases may reach 500,000 in the United States.

In numerous published studies, a causative agent has been identified in 10% to 50% of pediatric diarrhea cases, depending on geographic location, severity of illness, and laboratory methods used.[50–52] Bacillary dysentery is primarily a disease of children 6 months to 10 years of age, although adults often acquire the illness from their children, or acquire it when traveling to developing countries. Bacillary dysentery does not commonly develop in children younger than 6 months but is the most common prevalent pathogen in children 24 to 59 months old in sub-Saharan Africa.[53] However, in industrialized countries, *Shigella* strains may rarely cause severe illness in newborns,[54] but in developing countries, where breastfeeding is more common, infants are resistant to shigellosis,[55] probably because of exclusion from contaminated food or drink, changes in the intestinal microbiota of breastfed children, or the presence of specific antibody in breast milk. Shigellosis has become an important problem in daycare centers for preschool children in the United States, and in religious communities with large family size.

Modes of Spread and Reservoirs in Nature

Many cases of bacillary dysentery in industrialized regions are a result of person-to-person transmission. Widespread epidemics have occurred in military or civilian populations and among persons who have ingested contaminated food or water on cruise ships. Water and food appear to be particularly important vectors of *Shigella* transmission in developing countries, where they may be the most important sources of infection.[56,57] Epidemics of waterborne shigellosis generally appear to be the result of wells contaminated with fecal material. Felson[58] found that dysentery strains could be recovered for up to 6 months from water samples maintained at room temperature. Wells are often located close to cesspools

and outhouses in developing countries, where sanitation principles are not followed. In other areas, septic tank discharge may empty into lakes, ponds, or other bodies of water close to intake lines for camp water supplies or adjacent to bathing beaches. Chlorination of water, if appropriately maintained, will remove the threat of such infections. In the United States, foodborne[59] and waterborne[60] outbreaks of shigellosis occur occasionally and represent 7% of reported cases.[47]

An epidemiologic observation has been made that when water sanitation improvements are implemented in a community, the incidence of typhoid fever falls but the prevalence of bacillary dysentery remains unchanged.[58] *S. sonnei*, the most common species in Western countries, appears to have descended from a common ancestor (monophyletic) existing hundreds of years ago that diversified into distinct lineages in Europe, followed by worldwide dissemination.[61]

Hand transmission is likely to be a common means of acquiring infection. At a custodial institution, intellectually disabled persons were studied for the prevalence of hand transmission of bacteria.[41] Finger and simultaneous fecal cultures were obtained from 268 institutionalized patients. A *Shigella* strain was isolated from the stool of 39 persons, and the fingers were positive in 4 (10% of those with a positive stool culture). In addition, fecal cultures were found to be negative in an additional 229 patients, whereas a *Shigella* strain was isolated from the hands and fingers of 2 of these patients with negative stool cultures. *E. coli* was recovered from the fingers of 82% of those studied, which demonstrates the common occurrence of fecal organisms on the hands of institutionalized persons. These institutionalized patients had adequate washroom and showering facilities and did not show evidence of decreased personal hygiene.

Outbreaks of shigellosis have been described in men who have sex with men, in some instances due to antimicrobial resistant strains[62] and spreading across several continents.[63]

Secondary transmission of *Shigella* infection is common in households, with rates influenced by the ages of family members.[41,64] After a bout of shigellosis without antimicrobial therapy, fecal excretion of the infecting strain generally lasts 1 to 4 weeks. Long-term *Shigella* carriage rarely occurs. In contrast to typhoid and cholera carriers, in whom the gallbladder or small bowel may be a site of infection, the organisms in dysentery carriage are confined to a colonic site. In the absence of coexistent parasitic infestation of the intestine, these carriers generally respond to antimicrobial therapy. The number of organisms excreted by these persons is generally less than that seen in acute dysentery, and thus the infection in such individuals is less communicable than that in active cases.

DIAGNOSIS

History

Bacillary dysentery should be considered in any patient with acute diarrheal illness associated with toxemia and systemic symptoms, particularly when the illness lasts longer than 48 hours, and when intrafamily spread occurs with an interval of 1 to 3 days between cases, fever is present, or blood or mucus is seen in stool. The occurrence of hyperpyrexia and seizures in infants and children with shigellosis has led some to the conclusion that a neurotoxin is important in the pathogenesis of clinical illness, although there is little to support this notion. In patients able to give a careful history, a descending intestinal tract infection is often described. The first symptoms may be fever and abdominal cramping, followed by voluminous watery stool during small bowel infection, followed by a decrease in fever and an increase in the number of stools passed with smaller volume ("fractional stools") as the colon becomes the site of infection. At that time, the passage of bloody mucoid stools with fecal urgency and tenesmus may develop. Abdominal pain and diarrhea occur in almost all patients with shigellosis, fever can be documented in approximately one-third of cases, and mucus is seen in the stools of 50% and gross blood in 40% of cases.[11]

Physical Examination

Findings of physical examination are nonspecific and include a variable degree of systemic toxemia, fever (which may be as high as 105°F), abdominal tenderness (especially over the lower abdominal quadrants), and hyperactive bowel sounds. Rectal examination or proctoscopy is generally painful, and an abnormally friable hyperemic rectal mucosa, increased mucus secretion, and areas of ecchymosis are generally found. Ulcerations of rectal mucosa can be seen after several days of illness. Rectal prolapse may occur with profuse stooling.

Laboratory Findings

During the acute illness, the infecting strain is present in large enough numbers that stool cultures are generally positive. In the later stages of the disease, it may be necessary first to culture material in enrichment broth before plating. Culture of colonic or rectal biopsy does not improve the efficiency of stool culture in shigellosis.[65] The key to establishing the diagnosis of shigellosis is isolation of the organism from diarrheal stool. Laboratory identification of *Shigella* was discussed earlier (see "Isolation Techniques"). In research centers where the service is available, direct fluorescent antibody microscopy may be useful in detecting the organism when present in small numbers,[66] but because of the numerous serotypes potentially responsible for the infection, this procedure does not have widespread application. Multiplexed NAATs are becoming increasingly popular for the identification of enteropathogens in stool specimens owing to their high sensitivity, rapid turnaround times, and decreased overall costs.[67] Several commercially available platforms detect *Shigella* with a high degree of sensitivity (95.9%) and specificity (99.9%) when stool culture is used as the gold standard.[68] NAATs target a gene shared by both *Shigella* and EIEC, and therefore the report does not distinguish between the two organisms. Furthermore, NAATs do not provide information on species or drug susceptibility. Thus, *Shigella* or EIEC identified with NAATs, should be confirmed by culture for speciation, serotyping, and drug susceptibility testing and for epidemiologic purposes.

The total white blood cell count demonstrates no consistent findings, although leukopenia and brisk leukocytosis are seen on occasion. A shift to the left (an increased number of band cells in comparison to segmented neutrophils) when a leukocyte differential count is performed in a patient with diarrhea suggests bacillary dysentery. The single most important laboratory test, other than stool culture, is direct microscopic examination of a stained fecal smear, which will show numerous polymorphonuclear leukocytes.[69] A wet mount preparation is made by adding stool (mucus, if present) to an equal amount of methylene blue dye. The preparation is then covered with a coverslip and examined microscopically under the high dry objective. Alternatively, the specimen can be heat fixed before staining with dilute methylene blue and examined under an oil immersion objective after drying. Numerous sheets of polymorphonuclear leukocytes are normally found in shigellosis and EIEC diarrhea (Fig. 224.2). Fecal leukocytes indicate a diffuse colitis or proctitis seen in salmonellosis, *Campylobacter* diarrhea, STx–producing invasive *E. coli* colitis, and idiopathic ulcerative colitis. Fecal lactoferrin represents a more sensitive test of mucosal inflammation than a fecal leukocyte test, and is strongly positive in most cases of shigellosis.[70]

Serologic evaluation of a patient with bacillary dysentery is not generally helpful in establishing the diagnosis because humoral antibodies do not develop before recovery. Serologic procedures are helpful as an epidemiologic tool in defining the extent of an epidemic in a population known to be infected by a known *Shigella* serotype (especially the Shiga bacillus). The humoral antibody response correlates with the severity of clinical disease.[11]

Therapy and Clinical Course

In certain patients with bacillary dysentery (particularly in infants and older adults), significant dehydration may result from excessive fluid loss through diarrhea and vomiting. The fluid losses can generally be replaced by oral intake because the diarrhea associated with bacillary dysentery is not normally associated with profound fluid and electrolyte depletion. If vomiting or extreme toxemia is a prominent feature of the illness, especially in the very young or very old, intravenous fluid replacement may be necessary. As in all diarrheal illnesses, fluid repletion is the mainstay of therapy and should be given even as antimicrobial therapies are being considered.

Antibiotics are useful in the management of shigellosis and may be lifesaving in the case of Shiga dysentery. Because the infection is normally self-limited and because antibiotic resistance commonly develops in

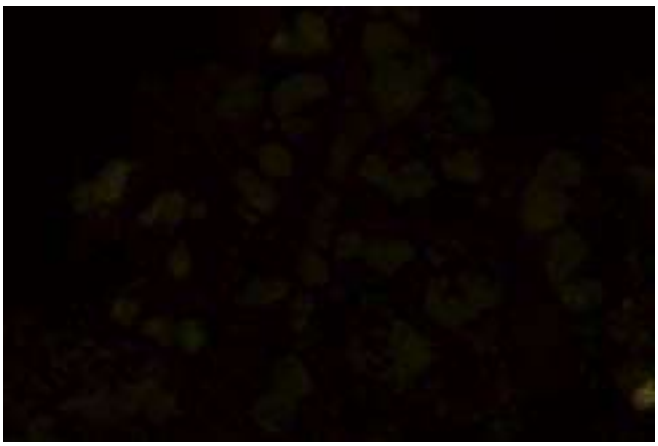

FIG. 224.2 Fecal leukocytes taken from patient with diffuse colitis (methylene blue stain). This exudative response may be seen in shigellosis, salmonellosis, *Campylobacter* infection, and colitis caused by invasive or Shiga toxin–producing *Escherichia coli.*

populations after prolonged use of drugs, some experts and the Centers for Disease Control and Prevention (CDC) recommend that antimicrobial therapy be reserved for the most severely ill patients—those with immunosuppression, with bacteremia, or with complications and those who are hospitalized; for food handlers, residents of nursing homes, or childcare providers; or for situations in which spreading and outbreaks are possible.[71,72] However, because the infection is generally transmitted from person to person and the infected or colonized person represents the major reservoir of infection, for public health reasons individuals with a positive stool culture or with known bacillary dysentery who are living or working in facilities with high risk for transmission (daycare centers, nursing homes) should be treated.

Because of the emergence of drug resistance, the efficacy of fluoroquinolones, trimethoprim-sulfamethoxazole, and macrolides in adults as empirical therapy when susceptibility is unknown has diminished. Risk factors for antimicrobial resistance include foreign travel, particularly to Southeast Asia and Africa; men who have sex with men; and HIV coinfection. For persons without these risk factors, a fluoroquinolone remains the drug of choice; for persons with these risk factors, cefixime or ceftriaxone is a reasonable alternative. For cases in which susceptibility is known, the specific drugs and dosages are indicated in Table 224.2. Trimethoprim-sulfamethoxazole had been the treatment of choice for this enteric infection, but resistance has become widespread for strains of *Shigella*.[73,74,75,76] Although 3-day therapy is generally recommended in shigellosis, single-dose fluoroquinolones may be given for milder forms of shigellosis.[77] For children, various drugs may be used. Cephalosporins have become a common form of treatment of pediatric shigellosis.[78–80] Although not approved for use in children, short-course fluoroquinolones can be safely used.[81] Amdinocillin, an unlicensed drug, has been used in Bangladesh for shigellosis.[81] Azithromycin has been used successfully for treatment of multidrug-resistant *Shigella* infection in adults[82,83] and should be useful in the management of pediatric shigellosis. Nalidixic acid may be helpful in the management of pediatric shigellosis.[84] Rifaximin should not be used to treat shigellosis. However, it has been proven to prevent shigellosis in experimental challenge studies[85] and is an option to prevent traveler's diarrhea, probably by eradicating *Shigella* infection before the organisms reach the colon and establish infection.

Intestinal motility patterns may be important in recovery from infection, and in preventing mucosal invasion by a bacterial agent.[86] In such cases, diarrhea might be viewed as a protective mechanism, and its inhibition by motility-active drugs may be counterproductive. Paregoric has occasionally been shown to worsen clinical salmonellosis[86] and, in occasional patients, antidiarrheal drugs such as diphenoxylate (Lomotil) worsen bacillary dysentery and could play a role in the development of toxic dilatation of the colon or perforation.[87] In dysenteric diarrhea, the antimotility drugs may be safely given if effective antimicrobial drugs are also administered.[88]

TABLE 224.2 Antibacterial Therapy for Patients With Shigellosis

ADULTS		CHILDREN	
Agent	**Dosage**	**Agent**	**Dosage**
Ceftriaxone	1 g IV once daily for 3–5 days	Ceftriaxone	50 mg/kg IV once daily (maximum, 2 g/day) × 5 days
Cefixime	200 mg orally twice daily for 5 days		
Ciprofloxacin[a]	500 mg bid × 3 days	Ciprofloxacin[b]	25 mg/kg/day, divided q12h × 3–5 days (daily adult dose 1–1.5 g/day)
Levofloxacin[a]	500 mg daily × 3 days		
Azithromycin	500 mg qd × 3 days	Azithromycin	10 mg/kg/day in a single daily dose × 3 days

[a]Increasing reports of decreased susceptibility to quinolones. Concern that strains that have minimal inhibitory concentrations (MICs) >0.12–1 µg/mL to ciprofloxacin should be treated with an alternate drug, and this may require revising of the Clinical and Laboratory Standards Institute (CLSI) current breakpoint of ≤1 µg/mL.[102]

[b]Not approved for use in children.

Clinical illness, if left untreated, generally lasts 1 day to 1 month, with an average of 7 days. Although mortality is unusual in shigellosis, except in malnourished children and older adults, the clinical illness is more striking and more likely to lead to hospitalization than are most other forms of infectious diarrhea. Complications, which are unusual, generally consist of severe dehydration, febrile seizures, septicemia or pneumonia from coliform organisms (and, less commonly, the infecting *Shigella* strain), keratoconjunctivitis, immune complex acute glomerulonephritis, post-*Shigella* irritable bowel syndrome and reactive arthritis, and hemolytic uremic syndrome. *S. dysenteriae* 1 characteristically produces a more serious form of diarrhea, and the mortality associated with untreated disease during epidemics may be as high as 20%. Now that oral rehydration therapy has reduced the incidence of most cases of dehydration-associated deaths from diarrhea, shigellosis represents the most important form of fatal enteric illness in areas of high endemicity.[89]

CONTROL

Environmental Control

A safe water supply is important for the control of shigellosis and is probably the single most important factor in areas with substandard sanitation facilities.[90] Chlorination is another factor important in decreasing the incidence of all waterborne enteric bacterial infections. Insecticides are useful in decreasing the vector population during peak seasons, and a decrease in the incidence of shigellosis, but not salmonellosis, may be seen after their use.[42] At other times of the year, it may be helpful to attack breeding places of insects. Garbage collection and disposal of excreta and sewage may also be useful in controlling the vectors.

In many areas of the developing world, it is necessary to examine the techniques of home preparation and storage of food. Important features may be improved, such as personal and food hygienic facilities, and refrigeration may be necessary. A major prerequisite in transmission in most cases of bacillary dysentery is the degree of contact and the level of personal hygiene between patients with disease and susceptible persons. Other factors are frequent and effective hand washing, voluntary removal of persons with diarrhea from roles as food handlers, and appropriate refrigeration and proper cooking of potentially infected foods. Breastfeeding is an important means of decreasing the incidence of bacillary dysentery in developing countries and in communities with substandard hygienic practices. Also, mothers should be taught how to prepare foods to supplement breastfeeding and to ensure the safety of the diet after weaning, thus improving sanitation and nutrition. Finally, cases of diarrhea should be adequately diagnosed and patients isolated, and antimicrobial therapy should be instituted in cases of bacillary dysentery to decrease the reservoir of virulent strains.

Immunologic Control

Epidemiologic studies have indicated that a degree of homologous immunity can be demonstrated in those who have recovered from bacillary dysentery.[91–93] These observations have supported the idea that a protective vaccine might be developed. It was shown that killed parenteral vaccines fail to protect animals against experimentally produced shigellosis[94] and to protect humans against naturally occurring illness.[95] Besredka[96] suggested that the immunity against bacillary dysentery conferred by one attack of the disease was essentially the result of sensitization of the intestinal mucosa to dysentery bacilli and that the antibodies circulating in serum had a small role or none at all in protection. After more than 90 years, Besredka's concept of intestinal immunity is still held as the primary mode through which immunologic control might be feasible. However, the nature of the intestinal immune response has not been completely characterized. In natural shigellosis, immunoglobulin A (IgA) concentrations in stool increase, as do anti-*Shigella* secretory IgA antibodies directed to homologous lipopolysaccharide.[97] Also, lymphocytes, monocytes, and granulocytes, in the absence of complement but in the presence of antibody, may serve an anti-*Shigella* function through cell-mediated mechanisms.[98]

The most successful outcome in the area of *Shigella* vaccine development was achieved by Mel and colleagues,[99] who used streptomycin-dependent mutant strains of *Shigella* as orally administered immunizing agents in Yugoslavian army soldiers and in children living in areas of hyperendemicity. These investigators demonstrated that immunization with a live-attenuated bacterial strain given orally in multiple doses (at least four) would prevent clinical disease but not alter the carrier status, provided that gastric acidity was first decreased with sodium bicarbonate swallowed just before the vaccine was given. Serotype-specific protection followed vaccination and lasted for at least 6 months, and the immunizing agent remained protective when combined as a bivalent preparation. Experiments in volunteers have demonstrated that the protective immunity imparted by oral immunization approximates that after recovery from disease.[100]

In the future, immunologic control may be possible against a limited number of serotypes of shigellae when attack rates are shown to be particularly high. Further research is being directed toward developing an immunizing strain that multiplies in the intestinal tract so that fewer doses need to be administered.[101] Attenuated bacteria can be constructed that are better adapted to host intestinal proliferation and that combine multiple serotypes. Avirulent mutants and bioengineered strains may produce anti-*Shigella* immunity. Conjugate *Shigella* vaccines are also being evaluated. It is possible that antitoxin immunity might be important to susceptibility and that a successful immunizing agent should also include a toxoid component.

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225 *Haemophilus* Species, Including *H. influenzae* and *H. ducreyi* (Chancroid)

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SHORT VIEW SUMMARY

Definition

- *Haemophilus* is a gram-negative coccobacillus with fastidious growth requirements.
- Nontypeable (nonencapsulated) *Haemophilus influenzae* is a common cause of otitis media in children and exacerbations of chronic obstructive pulmonary disease (COPD) in adults. Invasive infections due to *H. influenzae* are now more commonly due to nontypeable than typeable isolates in countries in which children are vaccinated against *H. influenzae* type b. Invasive infections with nontypeable *H. influenzae,* usually bacteremic pneumonia, most often occur in neonates or persons younger than 2 or older than 55 years with underlying comorbidities.
- Encapsulated *H. influenzae* type b causes invasive infections, including meningitis and epiglottitis, in children younger than 6 years. The other five serotypes are rare causes of invasive infection.
- *Haemophilus ducreyi* causes chancroid, a genital ulcer disease that facilitates human immunodeficiency virus transmission. Ulcers are painful and may be accompanied by inguinal lymphadenopathy, which may be suppurative. Chronic cutaneous ulcers outside the genital area occur in tropical countries.
- *Haemophilus haemolyticus* and other *Haemophilus* species are unusual causes of human disease.

Epidemiology

- The ecologic niche of *H. influenzae* is the human respiratory tract.
- Colonization of the nasopharynx by nontypeable *H. influenzae* begins in infancy, is common during childhood, and persists at a lower rate in adults.
- Invasive *H. influenzae* type b infections are rare in regions of the world where *H. influenzae* type b conjugate vaccines are used widely but are prevalent in regions where the vaccine is not used.
- The prevalence of genital chancroid appears to be decreasing, but nongenital skin ulcers have been reported in tropical countries, based on polymerase chain reaction (PCR) detection.

Microbiology

- Many members of the genus *Haemophilus* are part of the normal bacterial flora of the human upper respiratory tract.
- Fastidious growth requirements are used to distinguish the species in the laboratory. *H. influenzae* strains have six serotypes and eight biotypes. *H. ducreyi* requires special medium for culture and is better detected in genital lesions with PCR assay.

Diagnosis

- Invasive infections caused by *H. influenzae* are established by isolating the organism from sterile fluid or, with *H. influenzae* type b, by detecting capsular antigen in cerebrospinal fluid or blood. Chancroid is usually diagnosed clinically, although painful genital ulcers can be misdiagnosed as herpes simplex.

Therapy

- Otitis media and exacerbations of COPD are treated with oral antimicrobial agents, with the choice generally guided by expert guidelines.
- *H. influenzae* type b meningitis is treated with ceftriaxone, 75 to 100 mg/kg divided into 12- hour doses, or cefotaxime, 200 mg/kg/day, divided into 6-hour doses. Dexamethasone is also administered, 0.6 mg/kg/day IV in four divided doses for 4 days to children older than 2 months.
- Chancroid is treated with azithromycin, 1 g orally (single dose), or ceftriaxone, 250 mg IM (single dose). Alternatives are ciprofloxacin 500 mg twice daily for 3 days or erythromycin base 500 mg three times a day for 7 days.

Prevention

- All children should receive the *H. influenzae* type b conjugate vaccine series beginning at age 2 months as part of routine childhood vaccination.
- Incompletely immunized household contacts of patients with meningitis should receive rifampin prophylaxis (20 mg/kg once daily [600 mg maximum] for 4 days).
- Sexual contacts of patients with chancroid within 10 days of symptom onset should be treated, even if asymptomatic.

HAEMOPHILUS INFLUENZAE

Description of the Pathogen

H. influenzae is a small, nonmotile, non–spore-forming bacterium and a pathogen of humans found principally in the upper respiratory tract, first reported by Pfeiffer in 1892. The sensational claim that it was the primary agent of epidemic influenza proved fallacious; nonetheless, it has a wide range of pathogenic potential. Its requirement for growth factors, which can be supplied by erythrocytes, accounts for the generic name *Haemophilus* ("blood-loving"). In microscopic appearance, it is a small (1×0.3 μ) gram-negative bacterium. Stained organisms obtained from clinical specimens vary microscopically from small coccobacilli to long filaments. This variable morphologic appearance (pleomorphism) and inconsistent uptake of dyes (e.g., safranin) may result in erroneous interpretations of stained smears.

Aerobic growth of *H. influenzae* requires two supplements known as X factor and V factor, although neither refers to a single substance. X factor can be supplied by heat-stable iron-containing pigments that supply protoporphyrins. Porphyrin-based assays represent the most reliable methods for identifying *Haemophilus* species.[1] Because X factor is not required for anaerobic growth of *H. influenzae,* confusion may arise if *H. influenzae* is grown anaerobically (e.g., after stab inoculation). The heat-labile V factor, a coenzyme, may be supplied by nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, or nicotinamide nucleoside. Although present in erythrocytes, V factor must be released from the cell to sustain optimal growth, and thus standard blood agar is an unsatisfactory medium. *H. influenzae* exhibits satellitism around colonies of hemolytic *Staphylococcus aureus* (a source of V factor), and this technique may be used to identify *H. influenzae.* Although it

is not a strict requirement, some *H. influenzae* strains grow best in 5% to 10% carbon dioxide. Determining serotype by slide agglutination is customary, although polymerase chain reaction (PCR) assay for capsular genes has revealed false-positive slide agglutination tests.[2]

Distinguishing *Haemophilus influenzae* from *Haemophilus haemolyticus*

Strains of *H. haemolyticus* are frequently misidentified as *H. influenzae* in clinical microbiology laboratories and in published studies. The confusion results from the observation that many strains of *H. haemolyticus* are not hemolytic, and this is the sole characteristic used routinely to distinguish *H. haemolyticus* from *H. influenzae* in commercial kits and in clinical microbiology laboratories. *H. influenzae* and *H. haemolyticus* both have growth requirements for X and V factors. Analysis of 500 strains originally identified as nontypeable *H. influenzae* revealed that 27% of nasopharyngeal isolates from children and 40% of sputum isolates from adults were in fact *H. haemolyticus*.[3] *H. haemolyticus* is a commensal but does cause disease rarely.[3] Strains of *H. haemolyticus* and *H. influenzae* can be distinguished from one another through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or differences in various genetic markers, including 16S ribosomal RNA (rRNA), superoxide dismutase C, outer membrane protein (OMP) P6, protein D, fuculose kinase, and others.[3–11] Because *H. influenzae* and *H. haemolyticus* share the human upper airway as their ecologic niche and are competent for DNA exchange, there appears to be an evolutionary continuum between the two species.

Biotypes

Viability of *H. influenzae* is lost rapidly, so clinical specimens should be inoculated onto appropriate media without delay. A biotyping scheme devised by Kilian (based on indole production, urease, and ornithine decarboxylase activity) may be used to characterize individual isolates.[12] Biotype III includes *Haemophilus aegyptius,* the "Koch-Weeks bacillus." A clone of biotype IV strains is associated with neonatal and postpartum infections.

Serotypes

Colonies of *H. influenzae* are usually granular, transparent (or slightly opaque), circular, and dome shaped. On chocolate agar, most colonies attain a size of about 0.5 to 0.8 mm during the first 24 hours of growth at 37°C, enlarging to 1.0 to 1.5 mm by 48 hours. Six serotypes, designated a to f, are based on antigenically distinct capsular polysaccharide types. Colonies of encapsulated strains are mucoid (iridescent when grown on transparent media and examined with an indirect source of light) and may attain a size of 3 to 4 mm. Capsular type b strains are important invasive pathogens in humans. Strains of *H. influenzae* that lack a polysaccharide capsule are generally referred to as nontypeable because they are nonreactive with typing antisera raised against each of the six capsules. The population structure of *H. influenzae* type b is clonal, whereas nontypeable strains demonstrate substantial genetic diversity.[13] Most unencapsulated isolates are not capsule-deficient variants of extant capsule clones; they are genetically distinct from encapsulated strains of *H. influenzae.*

Epidemiology and Respiratory Tract Colonization

H. influenzae is recovered exclusively from humans; no other natural host is known. It is recovered from the upper airway and, rarely, the genital tract. Spread from one person to another occurs by means of airborne droplets or of direct contact with secretions.

Colonization in Children

Exposure to nontypeable *H. influenzae* begins after birth. Colonization of the respiratory tract is a dynamic process, with new strains of nontypeable *H. influenzae* being acquired and cleared from the respiratory tract frequently.[14] Varied patterns of colonization are evident in the first 2 years of life: brief colonization with one strain, prolonged colonization with one strain, and recurrent colonization with different strains.[14–17] Children who attend daycare centers are colonized at a higher rate than control children.[15,18] Nasopharyngeal colonization by *H. influenzae* in the first year of life is associated with an increased risk of recurrent otitis media compared with children who remain free of colonization.[19,20] The widespread administration of pneumococcal polysaccharide vaccines has caused changes in patterns of nasopharyngeal colonization. A reduction in nasopharyngeal colonization by vaccine serotypes of *Streptococcus pneumoniae,* with "replacement" of vaccine serotypes of *S. pneumoniae* by nonvaccine pneumococcal serotypes, nontypeable *H. influenzae,* and *Moraxella catarrhalis* may be occurring.[21,22] A pneumococcal conjugate vaccine that contains protein D of *H. influenzae* is being administered widely in many countries globally. The vaccine induces partial protection against otitis media caused by nontypeable *H. influenzae* but appears to have no significant effect on reducing colonization by nontypeable *H. influenzae.*[23,24] Monitoring of colonization patterns as new vaccines for bacteria that reside in the nasopharynx are developed and administered will be important.

Colonization in Adults With Chronic Obstructive Pulmonary Disease

Nontypeable *H. influenzae* frequently colonizes the lower respiratory tract in the setting of chronic obstructive pulmonary disease (COPD) and cystic fibrosis; multiple strains colonize the respiratory tract of these patients simultaneously.[25–28] Acquisition of new strains of nontypeable *H. influenzae* is associated with an increased risk of exacerbations of COPD.[4,29,30] Use of selective media improves the recovery rate of *H. influenzae* from the sputum of patients with cystic fibrosis.[31]

Based on its binding to mucin and adherence to epithelial cells, *H. influenzae* has long been considered an extracellular pathogen. Several lines of evidence, however, have established that *H. influenzae* has both an extracellular and an intracellular niche in the human respiratory tract.[32–37] Therefore, *H. influenzae* is present in the airway lumen, bound to mucin, adherent to respiratory cells, found within the interstitium of the submucosa, and found within cells of the respiratory tract. This observation has important implications for understanding the dynamics of colonization of the human respiratory tract and the human immune response to the bacterium.

Colonization and Conjugate Vaccines

Before the widespread use of conjugate vaccines, type b strains colonized the nasopharynx of children at a rate of 2% to 4%. The rate of nasopharyngeal colonization by type b strains has decreased substantially with the use of conjugate vaccines to prevent invasive infections caused by *H. influenzae* type b. Table 225.1 summarizes several features of nontypeable and type b strains.

Pathogenesis

Otitis Media

The first step in the pathogenesis of infection is colonization of the upper respiratory tract. *H. influenzae* expresses a variety of adhesin

TABLE 225.1 Comparison of Selected Features of Nontypeable and Type b Strains of *Haemophilus influenzae*

FEATURE	NONTYPEABLE STRAINS	TYPE B STRAINS
Colonization rate in upper respiratory tract	30%–80%	<1% in vaccinated populations; 2%–4% in unvaccinated populations
Capsule	Unencapsulated	PRP capsule
Pathogenesis	Mucosal infections	Invasive infections
Clinical manifestations	Otitis media, exacerbations of COPD, sinusitis	Meningitis, epiglottitis, and other invasive infections in infants and children
Evolutionary history	Genetically diverse	Clonal
Vaccine	None available; under development	Highly effective PRP conjugate vaccines

COPD, Chronic obstructive pulmonary disease; *PRP,* polyribitol ribose phosphate.

TABLE 225.2 Adhesins of *Haemophilus influenzae*

ADHESIN	MOLECULAR MASS (KDA)	OBSERVATION
Pili (fimbriae)	20–25	*hifA-hifE* gene cluster
Type 4 pilus	≈14	*pilABCD* gene cluster; mediates twitching motility
HMW1 and HMW2	120–125	Homologous with filamentous hemagglutinin of *Bordetella pertussis*
Hap	155	Homologous with IgA protease
Hsf	≈240	Surface fibrils; present in type b strains; homologue of Hia
Hia	115	Hia absent from strains that express HMW1, HMW2; present in nontypeable strains
OMP P5	≈35	Binds mucin; also called fimbrin; homologous with OMP A of *Escherichia coli*
OMP P2	36–42	Binds mucin and laminin
PE-binding adhesin	46	Binds phosphatidylethanolamine
Protein F	≈30	Binds laminin and respiratory epithelial cells
Protein E	16	Binds myeloma IgD, laminin, vitronectin and type 2 alveolar cells
Lipooligosaccharide	2.5–3.3	Adhesin for respiratory epithelial cells

Hap, Haemophilus adhesin and penetration; *Hia, H. influenzae* adhesin; *HMW,* high molecular weight; *Hsf, Haemophilus* surface fibrils; *IgA,* immunoglobulin A; *IgD,* immunoglobulin D; *OMP,* outer membrane protein; *PE,* phosphatidylethanolamine.

molecules (Table 225.2), each of which has its own specificity for host receptors.[38–44] The prevalence and distribution of adhesins vary among nontypeable strains.[42,45] In contrast to type b strains, which gain access to the bloodstream, nontypeable strains cause disease by local invasion of mucosal surfaces. The pathogenesis of otitis media involves direct extension of bacteria from the nasopharynx to the middle ear via the eustachian tube.[46] Release of lipooligosaccharide, lipoproteins, peptidoglycan fragments, and other antigens induces host inflammation.

Strains of nontypeable *H. influenzae* show differences in their pathogenic potential. A subset of strains that colonize the nasopharynx is capable of causing otitis media and has different sets of genes compared with strains that cause asymptomatic colonization. For example, otitis media strains are more likely to have the lipooligosaccharide synthesis gene *lic2B,* the histidine operon, and the urease operon than are asymptomatic colonizing strains.[47–49] Nontypeable *H. influenzae* rapidly and reversibly regulates the expression of many virulence genes through phase variation of methyltransferase proteins, which methylate DNA at sequence-specific sites, leading to changes in expression of many genes. This phasevarion switching plays an important role in the pathogenesis of nontypeable *H. influenzae* otitis media in the chinchilla model.[50]

Otitis Media With Effusion

Nontypeable *H. influenzae* has also been implicated as a cause of otitis media with effusion, a term that refers to the presence of middle ear fluid in the absence of clinical signs of acute otitis media. In addition to positive cultures of some middle ear fluids, analysis with PCR reveals the presence of microbial DNA and messenger RNA (mRNA), suggesting that *H. influenzae* is present in a viable but nonculturable form in some cases of otitis media with effusion.[51,52]

Biofilms

H. influenzae in the form of biofilms is present in the middle ear in animal models and in the middle ears of children with otitis media.[53–58] A biofilm is a community of bacteria encased in a matrix and attached to a surface. Bacteria in biofilms are more resistant to host clearance mechanisms and more resistant to antibiotics compared with planktonic bacteria.[59] Nontypeable *H. influenzae* biofilms are associated with recurrent and chronic otitis media.[53]

Exacerbations of Chronic Obstructive Pulmonary Disease

The lower respiratory tract of adults with COPD is chronically colonized by nontypeable *H. influenzae.* The course of COPD is characterized by intermittent exacerbations of the disease. Several lines of evidence implicate *H. influenzae* as the most common bacterial cause of exacerbations, including bronchoscopic sampling of the lower respiratory tract during exacerbations, analysis of immune responses to *H. influenzae* isolated from patients experiencing exacerbations, correlation of airway inflammation with sputum bacteriology, and molecular analysis of prospectively collected isolates.[29,30,60] Differences in pathogenic potential among strains in the setting of COPD are based on genome content.[61,62] A complex host-pathogen interaction most likely determines the outcome of the acquisition of a new strain of nontypeable *H. influenzae*; the determinants include virulence of the strain, degree of host impairment of innate immunity and pulmonary function, host inflammatory response, preexisting immunity, perception of symptoms, and other factors.[30] Strains of nontypeable *H. influenzae* that persist in COPD airways regulate expression of critical virulence functions with slipped-strand mispairing, mediated by changes in simple sequence repeats in multiple genes as a major mechanism for survival in the hostile environment of the human airways.[63]

Invasive Infections Caused by *Haemophilus influenzae* Type b

The importance of the type b capsule as a critical virulence factor in the pathogenesis of invasive disease has been well established with the use of genetic techniques and an infant rat model of bacteremia and meningitis.[64] Mutants lacking the polyribitol ribose phosphate (PRP) capsule do not cause invasive disease, whereas the isogenic parent strains are highly virulent in the infant rat model. The type b capsular polysaccharide is composed of PRP. The capsule enables the organism to invade the bloodstream after colonization of the respiratory tract (see later).

Immunity

Nontypeable *Haemophilus influenzae*

Immunity to infection by nontypeable *H. influenzae* is complex and not completely understood. A hallmark of noninvasive infections caused by nontypeable *H. influenzae* is their propensity for recurrence. The immune response to surface antigens of nontypeable strains is intimately involved in the pathogenesis of recurrent infection. Studies in animal models, in adults with COPD, and in children with otitis media have all demonstrated that the most prominent antibody response is directed at strain-specific determinants.[65–67] The clinical observation of recurrent infections in immunocompetent hosts (recurrent otitis media in children and recurrent exacerbations in COPD) suggests that strain-specific immune responses leave the host susceptible to recurrent infections by different strains of *H. influenzae.* A variety of membrane-associated surface-exposed determinants are immunogenic and potential targets of protective host immune responses. For example, OMP P2, the major porin protein, contains immunodominant strain-specific determinants on the bacterial surface. Adults with COPD make potentially protective antibodies to strain-specific determinants on P2 after infection. Patients remain susceptible to recurrent infections by other strains. Furthermore, the P2 genes of strains that colonize adults with COPD undergo point mutations in the human respiratory tract.[68,69] The mutations result in amino-acid changes in the surface-exposed loops of the P2 molecule. A similar phenomenon has been observed with OMP P5.[70] These variants have a selective advantage and are able to evade the host response and cause recurrent or persistent infection.

The presence of serum bactericidal antibody is associated with protection from otitis media caused by nontypeable *H. influenzae,* as is serum antibody to protein D.[66,71] Because nontypeable *H. influenzae* causes mucosal infection, mucosal immunity likely plays a role in host defense; however, the mucosal immune response to *H. influenzae* is

poorly understood. Finally, observations have suggested that cell-mediated immune responses, including Th17 responses, play a role in protection against infection.[72–74]

Haemophilus influenzae Type b

Protection against invasive *H. influenzae* type b infections is mediated by antibodies to the type b capsular polysaccharide PRP. Serum anti-PRP antibodies activate complement-mediated bactericidal and opsonic activity in vitro and mediate protective immunity against systemic infections in humans. The level of maternally acquired serum antibody to PRP declines after birth and reaches a nadir at approximately 18 to 24 months of age, the peak age incidence of meningitis caused by *H. influenzae* type b in an unimmunized child. The level of antibody to PRP then gradually rises, apparently as a result of exposure to *H. influenzae* type b or cross-reacting antigens. Systemic disease is unusual after the age of 6 years even in the absence of immunization, because of, at least in part, naturally acquired antibody to PRP.

Immunization with vaccines that are composed of PRP conjugated to carrier proteins affords protection by inducing antibodies to PRP. These vaccines are now widely used and are highly effective in preventing invasive disease caused by *H. influenzae* type b in infants and children.[75–77] In addition, these vaccines prevent colonization of the nasopharynx; this effect accounts for herd immunity by reducing the circulation of type b strains.

Clinical Manifestations of Nontypeable *Haemophilus influenzae*

Otitis Media

Nontypeable *H. influenzae* accounts for 25% to 35% of all cases of acute otitis media. Approximately 16 million episodes of otitis media occur annually in the United States. Although such episodes occur at any age, they are most common in children aged 6 months to 5 years. The typical clinical presentation of acute otitis media in infants is fever and irritability; older children also complain of ear pain. A prior viral respiratory tract infection is commonly the antecedent of an episode of otitis media. The diagnosis is made with pneumatic otoscopy. A precise causative diagnosis requires tympanocentesis, but this is not performed routinely.

Although it is not possible to determine the causative agent of otitis media in an individual child based on clinical characteristics, certain features are associated with otitis media caused by nontypeable *H. influenzae* compared with *S. pneumoniae.* Otitis media caused by nontypeable *H. influenzae* is less likely to cause fever and is less often associated with otorrhea than with pneumococcal otitis media, suggesting that the former causes a less virulent form of the disease, but substantial overlap is seen.[78,79] Nevertheless, features associated with nontypeable *H. influenzae* otitis media include a history of recurrent episodes, treatment failure, concomitant conjunctivitis, previous amoxicillin treatment, bilateral otitis media, and acute otitis media within 2 weeks of completing a course of any antibiotic.[80–83] Since 2000, infants in many countries have received the 7-valent pneumococcal conjugate vaccine, and since 2010, the 10-valent and 13-valent vaccines. These vaccines appear to have resulted in an overall decrease in otitis media and complications from otitis media in widely immunized populations.[84–86] However, an increase in the proportion of acute otitis media caused by nontypeable *H. influenzae* in children in whom initial antimicrobial therapy fails or who have recurrent episodes has occurred coincident with the widespread use of these vaccines.[87,88] Continued monitoring of the incidence and etiology of otitis media will be critical.

Exacerbations of Chronic Obstructive Pulmonary Disease

The course of COPD is characterized by intermittent exacerbations of the disease. It is estimated that approximately half of exacerbations are caused by bacteria, and nontypeable *H. influenzae* is the most common bacterial cause.[4,29] The three cardinal signs of an exacerbation are an increase from baseline of sputum production, sputum purulence (change in sputum color), and dyspnea. Fever is generally absent or low grade and infiltrates are not present on chest radiographs. A sputum Gram strain often reveals abundant gram-negative coccobacilli.

Community-Acquired Pneumonia

Nontypeable *H. influenzae* is an important cause of pneumonia in adults, particularly in older adults and those with COPD and acquired immunodeficiency syndrome.[89,90] The clinical features are indistinguishable from those of pneumonia caused by other bacteria and include fever, cough, and purulent sputum, usually of several days' duration. The chest film reveals infiltrates that may be patchy or show lobar distribution. A Gram-stained smear of the sputum shows a predominance of small gram-negative coccobacilli.

Acute Respiratory Tract Infections in Children in Developing Countries

In countries in which adverse socioeconomic circumstances are prevalent, acute pneumonia in infants is a major cause of morbidity and mortality. Nontypeable *H. influenzae* likely accounts for a significant proportion of these cases of pneumonia, but further studies are needed to establish the etiologic agents. The importance of acute respiratory tract infections as a major global health problem has led to the establishment of international programs, with the aim of enhancing the recognition, appropriate management, and prevention of respiratory tract infections.

Sinusitis

Studies that have used cultures of direct sinus aspirates have shown that nontypeable *H. influenzae* is a common cause of acute maxillary sinusitis.[91,92] Patients experience nasal obstruction, purulent nasal discharge, headache, and facial pain. As in the case of otitis media, an invasive procedure (sinus aspiration) is required to establish a causative diagnosis.

Neonatal and Maternal Sepsis

Neonatal sepsis caused by nontypeable *H. influenzae* is associated with 50% mortality overall and 90% mortality in premature infants. Many strains that cause neonatal sepsis are biotype IV and share several genotypic and phenotypic characteristics with one another.[93] Indeed, studies of the genetic relationships of these potentially invasive strains and other nontypeable strains have suggested that the invasive biotype IV strains are closely related to *H. haemolyticus.*[4]

These same biotype IV strains also cause postpartum sepsis associated with endometritis. Nontypeable *H. influenzae* is a well-documented cause of tubo-ovarian abscess or chronic salpingitis. Diagnosis is established through tubal cultures at laparoscopy or cultures of peritoneal fluid obtained with culdocentesis.

Bacteremia and Invasive Infections

Although the most common clinical manifestations of infections by nontypeable *H. influenzae* are otitis media and nonbacteremic respiratory tract infections in adults, the organism also occasionally causes bacteremia. Most invasive *H. influenzae* infections in countries in which the *H. influenzae* type b vaccines are used are caused by nontypeable strains.[94–100] Although these vaccines have changed the proportion of nontypeable strains that cause invasive disease by preventing disease from type b strains, there is no convincing evidence that an increased incidence of invasive disease from nontypeable strains has occurred.[101] However, close monitoring will be important. Population-based studies have estimated an incidence of 1.7 cases per 100,000 adults of invasive disease caused by *H. influenzae,* and overall the incidence is highest in older adults.[98,102] Most people with bacteremia have underlying conditions, such as alcoholism, cardiopulmonary disease, human immunodeficiency virus (HIV) infection, or cancer. The respiratory tract is the usual source of infection when bacteremia is present. Invasive strains of nontypeable *H. influenzae* are genetically and phenotypically diverse.[103] Bacteremic infections caused by nontypeable *H. influenzae* are associated with significant mortality.

Nontypeable *H. influenzae* is also an unusual cause of a variety of invasive infections that have been documented in case reports and small series. All the invasive diseases that are commonly caused by *H. influenzae* type b are, on occasion, caused by nontypeable strains and by types a, c, d, e, and f. These infections include adult epiglottitis, empyema, septic arthritis, cellulitis, osteomyelitis, pericarditis, cholecystitis, intraabdominal infection, and vascular graft infection.

Conjunctivitis

Nontypeable *H. influenzae* is the most common bacterial cause of conjunctivitis in children.[104,105] In contrast to the sporadic nature of other *Haemophilus* infections, conjunctivitis can occur in outbreaks, particularly in daycare centers. Clinical features include conjunctival hyperemia and purulent discharge. On occasion, nontypeable *H. influenzae* causes severe conjunctivitis that is characterized by copious, purulent discharge, lid edema, chemosis, and keratitis.

Clinical Manifestations of *Haemophilus influenzae* Type b

Meningitis

Meningitis is the most serious acute manifestation of systemic infection caused by *H. influenzae.* Antecedent symptoms of upper respiratory infection are common. Specific questioning concerning the occurrence of disease in contacts (household, daycare centers) is prudent. None of the clinical features of meningitis caused by *H. influenzae* distinguishes it from other forms of purulent meningitis. The peak age incidence varies somewhat among populations, depending in part on vaccine use, but this infection now occurs most often in those who are incompletely immunized. Cases in adults are infrequent and often involve a background of recent or remote head trauma, prior neurosurgery, paranasal sinusitis, otitis, or cerebrospinal fluid (CSF) leak. *H. influenzae* meningitis in neonates is also rare, but such cases can resemble early-onset group B streptococcal infection. The most common signs are fever and altered central nervous system function, but the young child may have few specific signs, and nuchal rigidity is often absent. More obvious manifestations, such as seizures or coma, commonly develop as the disease progresses. Subdural effusions are a common complication. Clinical suspicion should be greatest when after 2 or 3 days of adequate therapy there is a tense anterior fontanelle, seizures (particularly if focal), hemiparesis, or neurologic deterioration. In older children, the clinician looks for papilledema and altered mental status.

With appropriate management, the overall mortality rate from *H. influenzae* meningitis is less than 5%, but apparently permanent sequelae occur in many of the survivors.

Epiglottitis

Acute respiratory obstruction caused by cellulitis of the supraglottic tissues is a potentially lethal disease with a characteristically fulminating onset. Swelling of the epiglottis and aryepiglottic folds with complete obliteration of the vallecular and piriform sinuses is typical. Usually, the patient is a child (aged 2–7 years), but occurrence in adults is also well known. The onset is often explosive, with initial features being sore throat, fever, and dyspnea, progressing rapidly to dysphagia, pooling of oral secretions, and drooling of saliva from the mouth. The child is restless and anxious and adopts a sitting position, with neck extended and chin protruding to reduce airway obstruction. Abrupt deterioration commonly occurs within a few hours, resulting in death in the absence of adequate treatment. The characteristic findings are seen above the larynx. The epiglottis is red and swollen and bears a striking resemblance to a bright red cherry obstructing the pharynx at the base of the tongue. The trachea appears normal. Examination of the larynx should be performed only in a setting in which an airway can be placed, because this examination, if injudiciously performed, may lead to fatal respiratory obstruction. A lateral radiograph of the oropharynx can be useful in visualizing the swollen epiglottis.

Pneumonia and Empyema

The true frequency of primary lung infections caused by *H. influenzae* type b in children is difficult to determine with accuracy. Typically, the patient is between 4 months and 4 years of age and becomes ill in winter or spring, presenting with a consolidative pneumonia (often with pleural involvement) that is severe enough to necessitate hospitalization. In a series from England and Wales, 52% of 214 bacteremic children with nontypeable *H. influenzae* were younger than 2 years, and 52% had a comorbidity.[106] The only clinical feature that tends to distinguish *H. influenzae* pneumonia from bacterial pneumonias caused by *S. aureus* or *S. pneumoniae* is a more insidious onset. The development of severe dyspnea, tachycardia, and evidence of cardiovascular failure suggests pericarditis, an uncommon but important complication.

Cellulitis

Cellulitis is predominantly seen in young children. The clinical features are fever and a raised, warm, tender area of distinctive reddish-blue hue, most often located on one cheek or in the periorbital region. The distinctive color, its location, and the age of the child should suggest the cause. The soft tissue involvement progresses rapidly over a few hours. Some of these children have, or develop, evidence of other septic foci (e.g., meningitis) because an accompanying bacteremia is extremely common.

Bacteremia Without Localized Disease

Children, particularly those 6 to 36 months of age, may acquire bacteremia without evidence of local disease; *S. pneumoniae* is the most common cause of this syndrome, although *H. influenzae* can also be seen. Typically, fever, anorexia, and lethargy prompt the visit to a physician; the examination is nondiagnostic. This condition is appreciated most often in those with a temperature higher than 102°F (39°C) and an increased peripheral neutrophil count. Children with sickle cell disease or with a previous splenectomy are particularly susceptible. Early diagnosis and therapy are critical because these patients may worsen rapidly and develop septic shock or a localized purulent focus.

Septic Arthritis

H. influenzae causes septic arthritis in children younger than 2 years. Typically, there is involvement of a single, large, weight-bearing joint (without osteomyelitis), displaying decreased mobility, pain on movement, and swelling. Positive cultures of blood and joint fluid are usual. However, the signs and symptoms may be more subtle; for example, septic arthritis is an important cause of prolonged fever and irritability (or prolonged antigenemia) during the treatment of other systemic *H. influenzae* diseases (e.g., meningitis).

Response to systemic antibiotics is dramatic and often curative, but long-term follow-up is important because residual joint dysfunction occurs in a significant percentage of children.

H. influenzae septic arthritis also occurs in adults. A review of 29 adults with *H. influenzae* arthritis found that 14 had multiarticular disease and 15 monoarticular disease, with 6 cases being in the knee only.[107] Nineteen patients also had extraarticular infection, including meningitis, pneumonia, sinusitis, and cellulitis. Predisposing factors were found in 22, such as alcohol abuse, trauma, rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, splenectomy, multiple myeloma, lymphoma, or common variable hypogammaglobulinemia.

Clinical Manifestations of Non–Type b Encapsulated *Haemophilus influenzae*

Although non–type b encapsulated strains of *H. influenzae* (types a, c, d, e, and f) are unusual causes of invasive infection manifesting predominantly with bacteremia and pneumonia, the incidence of types a, e, and f infections has shown modest recent increases. Invasive *H. influenzae* type a infections are seen with increased frequency in indigenous populations of North America. The type a strains that cause invasive disease are clonal. Most infections due to non–type b encapsulated strains occur in the setting of underlying conditions.[108–110]

Diagnosis

Nontypeable *Haemophilus influenzae*

Because nontypeable *H. influenzae* is often present in the human upper airway in the absence of clinical disease, determining the causative pathogen in individual patients is challenging. A diagnosis of otitis media is made with pneumatic otoscopy. Culture of middle ear fluid obtained with tympanocentesis would be required in order to determine the microbial cause. However, because tympanocentesis is a relatively invasive procedure, empirical therapy with antibiotics is initiated based on predictions of the likely pathogens, which are known to be *S. pneumoniae,* nontypeable *H. influenzae,* and *M. catarrhalis* as determined in studies that have used cultures of middle ear fluid obtained with tympanocentesis. Judicious use of tympanocentesis in children with

recurrent or refractory otitis media may be indicated in order to identify the pathogen precisely in difficult cases, but this procedure is not used routinely.[111]

The cause of exacerbations of COPD and community-acquired pneumonia in individual patients is difficult to determine. The presence of nontypeable *H. influenzae* in the sputum of patients experiencing an exacerbation of COPD or pneumonia is suggestive of the diagnosis but does not establish the organism as the pathogen because it may be present in the airways in the absence of disease. Nontypeable *H. influenzae* is the most common bacterial cause of exacerbations of COPD and causes a smaller proportion of cases of community-acquired pneumonia.

Isolating the organism from a blood culture unequivocally establishes the organism as causative. Although blood cultures are invaluable when positive, most infections caused by nontypeable *H. influenzae* are not associated with bacteremia, so blood cultures are relatively insensitive.

Haemophilus influenzae Type b

A provisional diagnosis of meningitis, epiglottitis, facial cellulitis, or septic arthritis is usually prompted by the history and clinical findings. Confirmation requires microbiologic studies. Cultures of blood, CSF, and other normally sterile fluids (e.g., from joints or pleural, subdural, or pericardial spaces) are diagnostic. Even if antibiotic therapy has been started, the yield is sufficiently great to recommend that they be taken. Cultures of the inflamed epiglottis are generally positive, but specimens should be taken only when a functional airway can be guaranteed. Whenever feasible, specimens obtained for culture should also be Gram stained; in about 70% of cases of meningitis, CSF smears reveal typical organisms. Detection of capsular antigen in serum, CSF, or concentrated urine with immunoelectrophoresis, latex agglutination, or enzyme-linked immunosorbent assay may be diagnostic, and diagnosis can be made in up to 90% of culture-proven cases of meningitis. Antigen is also often detected in infected pleural, pericardial, or joint fluid and can facilitate diagnosis because it persists after antibiotic therapy.

Therapy

Nontypeable *Haemophilus influenzae*

Many infections caused by nontypeable *H. influenzae,* such as otitis media and exacerbations of COPD, can be treated with oral antimicrobial agents. Overall, approximately 30% of nontypeable strains produce β-lactamase, but substantial geographic variability in this rate is observed. Therefore, ampicillin and amoxicillin should be used only if the susceptibility of the infecting isolate is known. Mutations in the *fts1* gene, which codes for penicillin-binding protein 3, is a more recently recognized mechanism of resistance in *H. influenzae* and *H. haemolyticus* isolates.[10,112] This mechanism is common in Japan and increasing in prevalence in Europe. Although it is still uncommon in the United States, careful vigilance will be important. The clinician who manages patients with otitis media and exacerbations of COPD frequently chooses an antimicrobial agent empirically. In this circumstance, the antimicrobial agent should be active against *S. pneumoniae* and *M. catarrhalis* in addition to *H. influenzae.*

Oral antimicrobial agents that are active against nontypeable *H. influenzae* include amoxicillin-clavulanate, fluoroquinolones, macrolides (e.g., azithromycin, clarithromycin), and various extended-spectrum cephalosporins (e.g., cefixime, cefpodoxime, cefprozil, cefaclor, cefuroxime, and others).

Parenteral antibiotic therapy is indicated for more serious infections caused by nontypeable *H. influenzae.* Parenteral antimicrobial agents that are active include cephalosporins (e.g., ceftriaxone, cefuroxime, ceftazidime, cefotaxime), ampicillin-sulbactam, and fluoroquinolones.

Those with certain immunodeficiencies, especially those with primary deficiency of antibody synthesis, have increased susceptibility to infection, especially with nontypeable *H. influenzae.* They may benefit from passive infusion of immunoglobulin preparations administered intramuscularly or intravenously. This form of immunoglobulin replacement decreases the incidence of systemic infections in these persons and the number of episodes of both upper and lower respiratory tract infections caused by nontypeable *H. influenzae.*

Haemophilus influenzae Type b

Without treatment, infection caused by *H. influenzae* type b can be rapidly fatal. This is particularly true of meningitis and epiglottitis. The most favored regimen is cefotaxime or ceftriaxone. For children, cefotaxime is given as 200 mg/kg/day, divided into 6-hour doses. The pediatric dose of ceftriaxone is 75 to 100 mg/kg, divided into 12-hour doses. Adult doses are ceftriaxone, 2 g every 12 hours, or cefotaxime, 2 g every 4 to 6 hours. Treatment is continued until the patient is afebrile and without clinical or laboratory signs of infection for 3 to 5 days. The usual duration of therapy is 7 to 10 days. Patients with complications, such as endophthalmitis, endocarditis, pericarditis, or osteomyelitis, may require 3 to 6 weeks of therapy.

Administration of corticosteroids to patients with *H. influenzae* type b meningitis reduces the incidence of deafness and perhaps other neurologic sequelae (see Chapter 87). The presumed mechanism is the reduction of inflammation that results from release of bacterial cell wall fragments when bacteria are killed by antibiotics. Dexamethasone therapy (0.6 mg/kg/day IV in four divided doses for 4 days) should be administered to children older than 2 months.

Antibiotic therapy is only one facet of the management of the child with *H. influenzae* infection. Critical attention must also be given to supportive therapy, including maintaining oxygenation and adequate perfusion of tissues.

Chemoprophylaxis for *Haemophilus influenzae* Type b

In the absence of prior immunization, household contacts of those younger than 4 years with invasive *H. influenzae* type b infection have a substantial incidence of disease. Rifampin prophylaxis as 20 mg/kg once daily (600 mg maximum) for 4 days has eradicated the carrier state in approximately 95% of carriers and significantly reduced the incidence of secondary cases in household members. Rifampin comes in 150- and 300-mg capsules. The dose can be conveniently given to young children in applesauce.

Rifampin prophylaxis is recommended for all household members, including adults (except pregnant women), when there has been contact with an index case of *H. influenzae* type b disease by a household member who is younger than 48 months and whose immunization status with the conjugate vaccine is incomplete, or who is an immunocompromised child of any age. A contact is defined as a child who is a household member or who has spent 4 or more hours each day with the index patient for at least 5 of the 7 days preceding the day on which the index patient was hospitalized. Based on efficacy of the *H. influenzae* type b vaccine, chemoprophylaxis is not recommended when all household contacts younger than 48 months have completed their immunization series. Children who were immunosuppressed at the time of vaccination may not have responded and therefore should be considered unvaccinated. If rifampin is to be effective in preventing secondary cases, it should be given within 7 days after the index patient is hospitalized. The index patient should also be given rifampin if treated with regimens other than cefotaxime or ceftriaxone. Chemoprophylaxis is usually provided just before discharge from the hospital.

Rifampin prophylaxis is indicated for all attendees and personnel at a daycare center or nursery when two or more cases of invasive *H. influenzae* type b disease have occurred within 60 days if incompletely immunized children attend the facility. The duration and dose of rifampin are the same as for household contacts. Chemoprophylaxis is not indicated for a single case at a daycare center or nursery.

Active Immunization Against *Haemophilus influenzae* Type b

Conjugate vaccines for invasive *H. influenzae* type b infections in infants and children are highly effective. The vaccines induce serum antibody to the PRP capsule; this antibody is bactericidal for the organism. The protective level of serum antibody to PRP has been estimated to be approximately 0.15 μg/mL, although this estimate must be interpreted cautiously. Vaccination reduces or eliminates carriage of *H. influenzae* type b strains, and this effect has played an important role in the effectiveness of the vaccine. The widespread use of conjugate vaccines has almost eradicated invasive disease in children younger than 5 years in countries

TABLE 225.3 *Haemophilus influenzae* Type b Conjugate Vaccines

SCIENTIFIC NAME	BRAND NAME (MANUFACTURER)	CARBOHYDRATE	PROTEIN CARRIER	POLYSACCHARIDE-TO-PROTEIN RATIO	RECOMMENDED DOSE CARBOHYDRATE (μG)
PRP-T[a]	ActHIB (Sanofi Pasteur) HIBERIX (GlaxoSmithKline)	Native PRP	Tetanus toxoid	0.33	10
PRP-OMPC[a]	PedvaxHIB (Merck)	Native PRP	OMPC	0.05–0.10	15

[a]These two vaccines are also available as combination vaccines with other childhood vaccines. See Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger, United States, 2017 (www.cdc.gov/vaccines/acip).
HIB, H. influenzae type b; *OMPC,* outer membrane protein complex; *PRP,* polyribitol ribose phosphate.

with universal vaccination programs. These vaccines represent a dramatic success in disease prevention and health care cost savings.

Certain populations, including Native American and Native Alaskan children, show a persistently elevated rate of infection, even with widespread vaccination. Furthermore, localized populations with low vaccination rates contribute to the continued circulation of *H. influenzae* type b strains, despite a national vaccination rate higher than 90%. Therefore, continued surveillance, particularly in these high-risk populations, will be important. Determination of the serotypes of disease isolates will distinguish disease that results from lack of vaccination or vaccine failure from invasive disease caused by non–type b strains. A striking reduction in the incidence of invasive infections caused by *H. influenzae* type b has been seen in countries in which conjugate vaccines have been used widely. Through a global public-private partnership effort, the Vaccine Alliance, established in 2000, *H. influenzae* b (Hib) vaccine has reached an increasing number of resource-poor countries, saving millions of lives globally.

Two conjugate vaccines are currently licensed and available in the United States (Table 225.3). All children should be immunized with a conjugate vaccine beginning at 2 months of age. A primary series consisting of three doses at 2, 4, and 6 months of age (PRP-T) or two doses given at 2 and 4 months (PRP-OMPC), depending on the vaccine product, is recommended. After administration of the primary series, antibody titers decline, so an additional booster dose should be given between 12 and 15 months of age. Vaccines may be administered during visits when other vaccines are given. Adverse reactions are few; the most common are pain, redness, and swelling at the injection site.

There are no currently licensed vaccines for nontypeable *H. influenzae.* However, a randomized, prospective, placebo-controlled trial with a pneumococcal conjugate vaccine that contained protein D, a conserved surface protein of *H. influenzae* as a carrier, showed partial efficacy in prevention of nontypeable *H. influenzae* otitis media.[113] This vaccine is now used widely throughout the world. Based on active research in the area, additional progress in developing vaccines to prevent infections caused by nontypeable *H. influenzae* is anticipated.[114–118]

Haemophilus influenzae Biogroup *aegyptius*

H. influenzae biogroup *aegyptius* (the Koch-Weeks bacillus) has long been known to cause conjunctivitis. In 1984, a fulminant systemic illness characterized by purpura, peripheral necrosis, vascular collapse, and *H. influenzae* biogroup *aegyptius* bacteremia was described in a small Brazilian town.[119] The mortality was 70%, but subsequent reports described milder forms of the illness. The illness, called Brazilian purpuric fever, was described in several rural Brazilian towns in addition to two cases in Australia. The clinical illness has not been reported since the early 1990s.

H. influenzae biogroup *aegyptius* is remarkable in that the organism acquired the capacity to cause a fulminant invasive disease despite lacking a capsule, which is often associated with invasive infections. Strains of *H. influenzae* biogroup *aegyptius* are of a clonal origin, sharing several characteristics, including a unique plasmid and an identical multilocus sequence type among strains.[120,121] Analysis of genome sequence identified several novel adhesins unique to case clone strains, suggesting these adhesins may play a role in pathogenesis of Brazilian purpuric fever.[122,123]

Identifying the virulence factors that give case clone strains the ability to cause invasive disease will be important in understanding Brazilian purpuric fever. From a broader perspective, characterizing the molecular mechanisms that account for the invasive potential in an otherwise noninvasive bacterium (nontypeable *H. influenzae)* is of great interest in understanding the pathogenesis of invasive bacterial infections.

HAEMOPHILUS DUCREYI

Description of the Pathogen

H. ducreyi is the causative agent of chancroid, an infection characterized by genital ulcer and inguinal lymphadenitis. *H. ducreyi* is a highly fastidious gram-negative coccobacillus. Its microscopic appearance and its nutritional requirement for hemin account for the classification of the bacterium in the genus *Haemophilus.* Despite its name, *H. ducreyi* is not closely related to other members of the genus *Haemophilus* and will likely be reclassified in the future; this issue awaits further study.[124] The organism is a strict human pathogen, and there are no known animal or environmental reservoirs.

Epidemiology

The prevalence of chancroid has declined in the United States, with seven cases reported to the Centers for Disease Control and Prevention (CDC) in 2016. Worldwide, chancroid appears to have declined also, although infection may be occurring is some regions of Africa and the Caribbean.[125,126] However, the clinician must be cautious in interpreting prevalence data because of the difficulty in establishing a diagnosis. Like other genital ulcer disease, chancroid facilitates the transmission of HIV. This association between HIV and chancroid has generated increased interest in understanding the pathogenesis of *H. ducreyi* infection and in improving diagnostic tests for genital ulcer disease. Transmission is primarily heterosexual, and males have outnumbered females in most studies. A high proportion of infected males report sexual contact with commercial sex workers. Chancroid has been strongly associated with illicit drug use. The transmission dynamics of the organism suggest that the disease is likely to be perpetuated in highly sexually active populations, such as commercial sex workers.[127]

H. ducreyi has more recently also been recognized as an important cause of non–sexually transmitted cutaneous ulcers, particularly in the South Pacific and in Africa.[128] Strains that are isolated from cutaneous ulcers have genome sequences that are nearly identical to class I strains (of two related classes) of *H. ducreyi* that cause genital ulcers.[129]

Pathogenesis and Immune Response

Infection occurs as a result of inoculation of bacteria through breaks in the epithelium. Studies using a human model of experimental *H. ducreyi* infection have led to the identification of selected genes or gene clusters that are required for full virulence.[130–132] The bacterium colocalizes with neutrophils and macrophages but is not phagocytized, suggesting that evasion of phagocytic killing is important in pathogenesis. Ulcers contain predominantly T cells, with low numbers of B cells. Patients who have had chancroid may have repeated infections, indicating that natural infection does not confer protective immunity; a similar phenomenon is observed in the human volunteer model.[133]

Clinical Manifestations

The hallmark of chancroid is genital ulceration. The lesion often begins as a papule and evolves into an ulcer. Typical ulcers are painful, well circumscribed with ragged edges, and not indurated. The base of the ulcer

is covered with necrotic material and bleeds easily when scraped. Little or no inflammation of the surrounding skin is present. Approximately half of patients with chancroid have inguinal lymphadenopathy. These lymph nodes sometimes become fluctuant and rupture spontaneously.

Chancroid can manifest in atypical ways. Multiple ulcers may coalesce to form a giant ulcer. Ulceration may resolve before the appearance of inguinal adenopathy and suppuration, resulting in presentation as suppurative inguinal adenitis in the absence of an active genital ulcer. Multiple small ulcers may resemble folliculitis. The main differential diagnostic considerations include primary syphilis (chancre), genital herpes, lymphogranuloma venereum, donovanosis, and condyloma latum of secondary syphilis. In the presence of HIV infection, the number of ulcers at initial presentation may be greater and the duration of ulceration may be longer.

Non–sexually transmitted cutaneous ulcers caused by *H. ducreyi* have clinical features similar to those of yaws, caused by *Treponema pallidum* subsp. *pertenue,* which is endemic where *H. ducreyi* cutaneous ulcers are seen. Ulcers caused by *H. ducreyi* are less likely than yaws to show central granulating tissue and less likely to have indurated edges, but substantial overlap in clinical characteristics exists.

Diagnosis

Because a clinical diagnosis of chancroid is often inaccurate, laboratory confirmation of the diagnosis should be sought. Isolation of *H. ducreyi* from a swab of the lesion or from an aspirate of suppurative lymph nodes confirms the diagnosis. Because the organism is difficult to grow, the use of selective and supplemented media is required. No US Food and Drug Administration (FDA)–approved PCR assay for *H. ducreyi* is available in the United States. Such testing can be performed by clinical laboratories that have developed their own tests and have conducted a Clinical Laboratory Improvement Amendments (CLIA) verification study. However, PCR assay appears more sensitive than culture.[125]

A probable diagnosis of sexually transmitted chancroid for clinical and surveillance purposes can be made according to the following criteria: (1) one or more painful genital ulcers, (2) no evidence of *T. pallidum* infection at darkfield examination of ulcer exudate or with a negative result of a serologic test for syphilis performed at least 7 days after ulcer onset, (3) typical clinical presentation for chancroid, and (4) a negative result of a test for herpes simplex virus on the ulcer exudate.

Therapy

The recommendation of the CDC is a single 1-g dose of azithromycin orally. Alternative regimens include ceftriaxone (250 mg IM in a single dose), ciprofloxacin (500 mg PO twice daily for 3 days), or erythromycin base (500 mg PO three times daily for 7 days). Azithromycin and ceftriaxone have the distinct advantage of single-dose treatment.

Ulcers usually improve symptomatically by 3 days and by objective evaluation by 7 days after initiation of treatment. Lack of improvement should raise several considerations, including coinfection with another pathogen (especially genital herpes or primary syphilis), coexisting HIV infection, which is associated with a delayed response to treatment of chancroid, lack of adherence to the treatment regimen and, finally, resistance of the *H. ducreyi* isolate to the antimicrobial agent prescribed. Isolates from patients who do not respond promptly to treatment should be tested for antimicrobial susceptibility.

Contacts of patients with chancroid should be identified and treated if they had sexual contact with the patient during the 10 days before the onset of symptoms in the patient, even in the absence of clinical symptoms in the contact.

OTHER *HAEMOPHILUS* SPECIES

Description of the Pathogens

Haemophilus species other than *H. influenzae* and *H. ducreyi* are unusual causes of disease in humans. However, as a result of increased awareness of these bacteria as potential pathogens and as a result of improvements in isolating the bacteria in culture, it is now apparent that other *Haemophilus* species more commonly cause human infection than previously believed, particularly in the case of infective endocarditis. *Haemophilus* species are present as part of the normal bacterial flora of the human upper respiratory tract. *Haemophilus parainfluenzae* is the predominant species, accounting for approximately 75% of the *Haemophilus* flora of the human upper airway.

Members of the genus *Haemophilus* are small gram-negative coccobacilli with fastidious growth requirements. The growth requirements are used to distinguish among the species. *Haemophilus* require X factor (hemin), V factor (nicotinamide adenine dinucleotide), or both for growth. These are supplied by erythrocytes, but the erythrocytes must be lysed to release V factor. This growth requirement is supplied in the clinical microbiology laboratory by growing *Haemophilus* species on chocolate agar. Table 225.4 shows differential characteristics of *Haemophilus* and related species that have been documented to cause infection in humans. Species are distinguished based on their different growth requirements for X and V factor, enhancement of growth by carbon dioxide (CO_2), expression of catalase, and ability to cause hemolysis. Real-time PCR assay may be replacing the slower and more onerous identification methods.[2] Species designated as *Aggregatibacter paraphrophilus* (formerly *Haemophilus paraphrophilus*), *H. parainfluenzae,* and *Haemophilus parahaemolyticus* require V factor but not X factor for growth, whereas *Aggregatibacter aphrophilus* and *H. haemolyticus* require X and V, or X only.

Clinical Manifestations

Haemophilus species, particularly *H. parainfluenzae,* and the two species moved from *Haemophilus* to *Aggregatibacter, A. aphrophilus* and *A. paraphrophilus,* are now recognized increasingly as a cause of infective endocarditis, causing up to 5% of cases of endocarditis. *Haemophilus* species are included in the so-called HACEK *(Haemophilus* spp., *Aggregatibacter* spp. [including *Aggregatibacter actinomycetemcomitans*, formerly *Actinobacillus actinomycetemcomitans*], *Cardiobacterium* spp., *Eikenella* spp., and *Kingella* spp.) group of bacteria, which are slow-growing bacteria known to cause endocarditis. The presence of *Haemophilus* species (and others in the HACEK group) should be suspected in patients in whom endocarditis is strongly suspected clinically and who have negative blood cultures. Because these bacteria are slow growing, incubation of blood cultures for 2 weeks has been recommended. However, recent studies indicated that extended incubation does not

TABLE 225.4 Differential Characteristics of *Haemophilus* and *Aggregatibacter* Species

	GROWTH FACTOR REQUIREMENT				
ORGANISM	X	V	CO_2 DEPENDENCE	HEMOLYSIS	CATALASE
A. aphrophilus	+	–	+	–	–
A. paraphrophilus	–	+	+	–	±
H. parainfluenzae	–	+	–	–	+
H. haemolyticus	+	+	–	±	+
H. parahaemolyticus	–	+	–	+	+
H. ducreyi	+	–	±	±	–

–, Absent; +, present; CO_2, carbon dioxide.

increase recovery from standard automated blood cultures.[134,135] Most patients with endocarditis caused by *Haemophilus* and *Aggregatibacter* species have underlying valvular heart disease. Echocardiography, particularly transesophageal echocardiography, is useful in identifying vegetations and characterizing underlying valvular disease. The clinical course of *Haemophilus* and *Aggregatibacter* endocarditis tends to be subacute, and embolization is common.[136,137]

Other *Haemophilus* species are relatively rare human pathogens, presumably because of their low pathogenic potential. They have been documented as rare causes of a variety of local upper respiratory and systemic infections, including sinusitis, otitis media, conjunctivitis, dental abscess, lower respiratory tract infection, peritonitis, biliary tract infection, brain abscess, osteomyelitis, and wound infections. Most of these are documented in small series and case reports.

Therapy

Treatment should be guided by the antimicrobial susceptibility of the etiologic isolate. The antimicrobial susceptibility characteristics of other *Haemophilus* and related species are similar to those of *H. influenzae,* although fewer data on other *Haemophilus* species are available. Some strains produce β-lactamase and are thus resistant to ampicillin. Agents with generally good activity include trimethoprim-sulfamethoxazole, third-generation cephalosporins, fluoroquinolones, and aztreonam.

In view of the increasing incidence of β-lactamase production among strains of *Haemophilus,* the treatment of choice for *Haemophilus* species endocarditis is now third-generation cephalosporins (ceftriaxone or cefotaxime) or a fluoroquinlone.[138] Treatment of native valve endocarditis should be given for 4 weeks, and treatment for prosthetic valve endocarditis should continue for 6 weeks.

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The complete reference list is available online at Expert Consult.

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226 Brucellosis (*Brucella* Species)

Hanefi C. Gul and Hakan Erdem

SHORT VIEW SUMMARY

Diagnosis
- Brucellosis is diagnosed by isolation of *Brucella* from blood, bone marrow, or tissue or by serology, although the polymerase chain reaction assay is available in a few laboratories. The preferred serology is the tube agglutination test, with a titer of at least 1:160. The rose-bengal card agglutination test is useful for screening. *Brucella canis* requires a separate serology.

Microbiology
- *Brucella melitensis, Brucella suis, Brucella abortus,* and, rarely, *B. canis* cause brucellosis and are nonmotile gram-negative coccobacilli.

Epidemiology
- Although uncommon in the United States, brucellosis is a worldwide zoonosis of wild and domestic animals, principally cattle, swine, goats, and sheep. Infection is acquired by contact with infected animals, their tissues, or ingestion of unpasteurized milk or dairy products from infected cows, goats, and sheep.

Clinical Settings
- Fever, sweating, myalgia, weight loss, headache, and malaise without localizing symptoms are evident. Lymphadenopathy or hepatosplenomegaly may occur. Onset can be acute or insidious and persist for weeks or months if untreated.
- Focal infections include osteomyelitis (particularly spondylitis or sacroiliitis), epididymo-orchitis, endocarditis, meningitis, meningoencephalitis, and myeloradiculitis.

Treatment
- Doxycycline with streptomycin is the standard regimen. For alternatives and treatment of neurobrucellosis, see Table 226.1 and text.

Brucellosis is the most frequently encountered worldwide zoonotic disease, which can be acquired from sheep, goat, cattle, swine, and other animals and transmitted to humans.[1] Each year half a million new human brucellosis cases cause serious consequences on health and socioeconomic issues, particularly in underdeveloped countries.[2] The disease is under control in developed countries.[3] The disease is frequently transmitted from unsterilized milk and dairy products, and outbreaks related to unpasteurized milk are reported.[4] Brucellosis is one of the most frequent laboratory-acquired infections.[5] In addition, brucellosis is classified as a category B biological weapon by the Centers for Disease Control and Prevention (CDC) owing to the ease of facilitated transmission.[6]

HISTORY

Brucellosis is one of the earliest known diseases. Throughout history the disease has taken many different names, including Mediterranean, Maltese, or Crimean fever and Bang disease. It was named *undulant fever* after 1913 and then *brucellosis,* the name used today, from 1940 onward.[7,8]

David Bruce, a military surgeon, isolated brucellae from the spleens of the patients in Malta between 1886 and 1887. The bacterium was named *Micrococcus melitensis* after isolation. A Danish doctor, Bang, later proved that the principal cause of abortion in bovines was a miniscule bacillus that he named *Bacillus abortus.*[6] In 1914 Traum isolated the bacteria from the aborted fetuses of pigs and gave the name *Bacterium abortus suis.* In reference to Bruce's name, the bacterium was renamed under *Brucella* as *Brucella melitensis*, *Brucella abortus,* and *Brucella suis* in the 1920s. In 1953 van Drimmelen isolated *Brucella ovis* from sheep; Stoenner and Lackman recovered *Brucella neotomae* from rodents in 1957; and Carmichael and Bruner isolated *Brucella canis* from dogs in 1968.[6] In 1994 Ewalt and Ross isolated *Brucella pinnipidiae* and *Brucella cetaceae* from sea mammals, namely dolphins and seals for *B. pinnipidiae* and whales for *B. cetaceae*. These species were later named *Brucella pinnipedialis* and *Brucella ceti.*[9] Later, *Brucella microti* was identified in animals in 2008 and *Brucella inopinata* from a breast implant in 2010.[10,11]

MICROBIOLOGY

Brucella spp. are small, gram-negative, nonmotile, non–spore-forming, intracellular-reproducing, aerobic coccobacilli with a size of 0.6 to 1.5 µm in length and 0.5 to 0.7 µm in width. Three species have been divided into biovars on the basis of biologic and serologic criteria. Four species are pathogenic for humans: *Brucella abortus* (seven biovars), *B. melitensis* (three biovars), *B. suis* (five biovars), and *B. canis*. Even though all the *Brucella* spp. are catalase positive, their oxidase and urease activity, as well as hydrogen sulfide production, varies. Although most *Brucella* spp. reproduce in aerobic conditions, *B. abortus* and *B. suis* are microaerophilic and require 5% to 10% carbon dioxide.[12] Although biochemical reactions have long been used to identify *Brucella* spp., matrix-assisted laser desorption/ionization time-of-flight mass spectrometry holds promise for use in reference laboratories.[13] Most *Brucella* spp. contain two annular chromosomes of approximately 2.1 and 1.2 mega–base pairs (Mbp).[14] *B. suis* does not follow this structure and has a 3.1 Mbp single annular chromosome.[15] The guanine-cytosine (GC) content of the chromosome is 57%. Genomes of different *Brucella* spp. have large areas of homology, leading to the suggestion that this genus should be given a single species name. Sequences of more than 30 *Brucella* genomes are available, including *B. suis*, *B. melitensis, B. canis,* and *B. abortus.*[14,15,16]

Brucella does not possess any defined endotoxin. The lipopolysaccharide layer on the cell wall shows endotoxic activity. The O-chain polysaccharide on the lipopolysaccharide layer has a significant role in the bacterial virulence. The three epitopes on the O-chain, named A, M, and C, can vary according to their species. The cytoplasmic, periplasmic, and outer membrane structural proteins bear antigenic features and are recognized by the immune system.[17] Changes in genes that affect the structure of the core oligosaccharide create attenuated mutants that interact more efficiently with the innate immune system (increase dendritic cell maturation and the production of inflammatory cytokines).[18] A phospholipase A1 specific for phosphatidylethanolamine, BveA, has been identified as a factor contributing to the resistance of *Brucella* to polymyxins, an activity which, in other bacteria, may provide protection from polymyxin-producing bacteria in the rhizosphere. The construction of mutants in the *bveA* gene, which encodes a predicted

esterase, modifies the lipid content of *B. melitensis* membranes and attenuates survival, replication, and persistence in mice.[19]

The bacterium survives for 6 weeks at 4°C in cream, 30 days in ice cream, and 15 to 100 days in fresh cheese.[20,21] Boiling and pasteurizing the milk kill the bacterium. Brucellae also die when the milk goes sour or lactic acid fermentation occurs. It is safe to consume cheese after 60 to 90 days. The bacterium is also sensitive to heating, ionized radiation, and disinfectants.[22,23]

EPIDEMIOLOGY

The incidence of brucellosis across the world varies from less than 0.03 to 160 per 100,000 population.[2] Brucellosis is most commonly seen in the Mediterranean countries, the Balkans, the Persian Gulf, the Middle East, and Central and South America.[2] Since the disintegration of the former Soviet Union, the Asian continent has also emerged as a significant focus.[21] Thus brucellosis is observed more frequently in developing countries, and it is practically eradicated in developed countries.[2,7,24] As an example, Muslim and Druze populations in Israel suffered from outbreaks, with a report describing disease in children in the period 2005–11, noting that 50.8% were bacteremic.[25] Hence the disease should be suspected in returning febrile travellers.[26]

The most invasive and pathogenic type of human brucellosis is due to *B. melitensis,* followed by *B. abortus* and *B. suis*. The most common animal hosts for *B. melitensis* biovars 1 to 3 are sheep, goats, camels, and buffalo; *B. abortus* biovars 1 to 6 are seen mostly in bison and camels; *B. suis* biovars 1 to 5 are reported in pigs, reindeers, and rodents; and *B. canis* affects dogs.[6]

Contaminated sheep and goat milk with *B. melitensis* appears to be the leading source of human brucellosis worldwide.[7,25] Other routes of transmission to humans are direct contact with infected animals or their secretions through bruises and lacerations on the skin, inhalation of infected aerosols, and conjunctival inoculation.[27] A child with *B. canis* bacteremia led to a case investigation that found that the child's dog was the probable source.[25] Due to the high frequency of brucellosis among farmers, veterinarians, doctors, and laboratorians, it is recognized as an occupational disease. Laboratorians, in particular, are likely to acquire the microorganism through aerosols or direct contact,[5,25] and laboratory-induced outbreaks are also reported.[25] In a review of 71 reported laboratory-acquired cases, the median incubation period was 8 weeks.[25] Four of six pregnant laboratorians with brucellosis aborted their fetus. Antimicrobial prophylaxis in highly exposed laboratorians appeared to be effective.[25] Serologic conversion has occurred in a radiology technician whose exposure was assisting at the aspiration of a *Brucella*-infected prosthetic joint.[28] Although human-to-human transmission is quite rare, congenitally and sexually transmitted cases are reported as well.[27] Because infections related to blood transfusion and bone marrow transplantation are reported, questioning blood donors for symptoms of brucellosis and the use of diagnostic tests can be considered in endemic areas.[27]

PATHOGENESIS

Brucella is an intracellular microorganism that can survive inside the macrophages, where it has specific survival mechanisms. The bacterium is protected through immune system—evading mechanisms such as blocking macrophage apoptosis, suppressing Th1-specific immune response, and inhibiting tumor necrosis factor-α (TNF-α) production.[22] Among the significant virulence factors are the lipopolysaccharide structure on the cell membrane, VirB type-IV secretion system (T4SS), the two-component BvrR/BvrS system, and the cyclic β-1,2 glucan system.[25,29,30] In addition, virulence factors of the bacterium include the adenine and guanine monophosphate systems, which inhibit the fusion of phagolysosomes, release of myeloperoxidase, and TNF production. A superoxide dismutase detoxifies reactive oxygen intermediates, and urease protects *B. abortus* and *B. suis* from gastric acid as they pass through the stomach.[31–33]

Brucella species taken into the body arrive at local lymph nodes either inside polymorphonuclear leukocytes and macrophages or extracellular.[31] The microorganisms reproducing intracellularly spread to the neighboring cells, local lymph nodes, or reticuloendothelial organs such as liver, spleen, and bone marrow.[34,35] Brucellae form granulomas made up of epithelioid cells, polymorphonuclear leukocytes, lymphocytes, and giant cells in tissues and organs. Granulomas are known to be more frequent in *B. abortus* infections.[36] Although toxemia is commonly observed in *B. melitensis,* abscess formation in joints and spleen is more often related to *B. suis.*[37]

IMMUNE RESPONSE

Adaptive immune responses play a crucial role in controlling the infection. Cytokines such as interferon-γ (IFN-γ) and interleukin-2 (IL-2), secreted by $CD8^+$ T cells in particular, are the most significant agents in preventing the progression of the disease.[38] During infection an increase is observed in the number of γδ T cells carrying Vγ9δ T receptors. These cells not only increase bactericidal activities of macrophages but also eliminate the infected cells by cytotoxic effects.[36,39,40] Secreted cytokines, such as IL-12, IFN-γ, and TNF-α molecules, play an essential role in a natural and adaptive immune response.[39,31]

Cellular immunity has a fundamental role in controlling the disease. Although the presence of specific antibodies is of utmost importance in diagnosis, they play a limited role in the immune response.[6] The immunoglobulin M (IgM) antibodies increase in the first week and the IgG antibodies in the second.[37] After 4 weeks of rising, both immunoglobulin levels decrease rapidly after a successful treatment. Furthermore, IgG levels decrease faster than IgM levels with treatment. Even after eradication of active infection, IgM antibodies can remain positive in low titers for months or even years. A high level of IgG and IgA antibodies for longer than 6 months is a sign of chronic infection or relapse.[37,41]

CLINICAL MANIFESTATIONS

Brucellosis can involve any organ or system in the body. Hence it mimics a myriad of human disorders and is known as a "great imitator."[12] The disease is a systemic infection with diverse clinical spectra, extending from asymptomatic disease to fatal illness.[7] Clinical history of living in endemic areas, international travel, unpasteurized milk or milk products consumption, hunting, working in a laboratory, vaccinating livestock, close contact with animals or animal tissues may be suggestive of the disease. The incubation period is usually 1 to 4 weeks, although it may extend beyond several months. Although clinical differences between species are difficult to determine, *B. melitensis* infections are reported to present more acutely compared with disease caused by *B. abortus.*[24] In addition, clinical presentation and complications of *B. suis* infection in humans are reported to be similar to *B. melitensis* or *B. abortus* infections.[42]

The disease presents as either acute febrile illness or chronic infection. The onset of symptoms may be either abrupt or insidious, developing over several days to weeks. The most frequent complaints are arthralgia, fever, and fatigue seen in up to 75% to 100% of the cases, followed by sweating, malodorous perspiration, lack of appetite, myalgia, chills, and back pain.[7,12,43] Brucellosis was formerly named *undulant fever* because fever waxes and wanes in due natural course of the disease.[8] The most common clinical findings are fever and hepatomegaly in one-third to one-half of patients, followed by splenomegaly, peripheral arthritis, sacroiliitis, scrotal swelling, neck stiffness, and lymphadenopathy.[44,45] Subclassifying the patients into "acute" (<8 weeks), "subacute" (8–52 weeks), and "chronic" (>52 weeks) categories according to the onset of the disease appears to be imprecise.[46] However, fever seems to be more frequent when the onset of the disease is within 1 month.[47]

Brucellosis is characterized by frequent organ-based complications, prolonged courses of the disease, treatment failures, and relapses. When a specific organ involvement is detected, the disease is defined as the focal form of brucellosis, for which different management strategies are required. Focal involvement in due course of brucellosis is seen in more than half of the patient population.[44,48] Owing to its subtle nature, the disease is one of the leading causes of fever of unknown origin[49] and is one of the reasons for febrile neutropenia in endemic areas.[50]

A relapse in brucellosis is defined by the reappearance of clinical signs and symptoms with or without a positive culture.[7] Relapse rates are frequently around 5% to 15%, depending on the regimen used.[7,44,51,52,53] It frequently occurs within 6 months after the discontinuation of therapy and tends to be milder than the original attack.[54] Relapse is not usually due to the emergence of antibiotic-resistant strains. Rather, it is frequently

due to poor compliance with therapy.[46] Other reasons may include inappropriate antibiotic use, microbial virulence factors, and focal infections.[7]

COMPLICATIONS

Skeletal System

Osteoarticular involvement is the most common complication seen in up to half of brucellosis cases. Sacroiliitis, spondylodiskitis, and peripheral arthritis are the common types of osteoarticular lesions.[44,48] The lumbar vertebrae are frequently affected spinal sites (Fig. 226.1),[55,56] and large joints of the lower limbs, such as hips, knees, and ankles, are the most often involved joints.[6,57] Compared with tuberculous spondylodiskitis, brucellar spinal disease is a significantly less suppurative infection, requiring surgical intervention, and is characterized with less frequent spinal and neurologic complications.[56] Sacroiliitis is common in younger patients (Fig. 226.2), whereas spondylitis and peripheral arthritis are common in older patients.[12,58,59] *Brucella* infection related to joint prostheses has also been reported.[60] Paravertebral, epidural, and psoas abscesses related to spondylitis and vertebral osteomyelitis can be difficult to treat.[55,56,61]



FIG. 226.1 Sagittal fat-saturated T2-weighted magnetic resonance image of the lumbar spine showing increased bone marrow signal intensity in the L4 and L5 vertebral bodies with paravertebral soft tissue lesion. *(Courtesy Dr. Tuba Sanal, Gülhane Askeri Tip Akademisi School of Medicine.)*

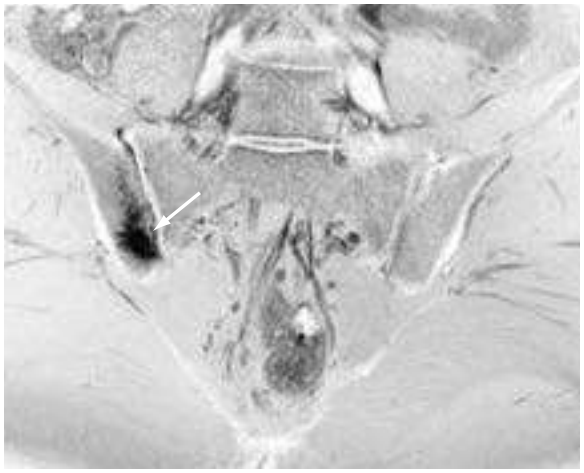

FIG. 226.2 Fat-saturated T2-weighted oblique coronal image showing osteitis at the iliac side of the right sacroiliac joint *(arrow).* *(Courtesy Dr. Tuba Sanal, Gülhane Askeri Tip Akademisi School of Medicine.)*

Plain radiographs should be reserved for spondylitis and advanced arthritis.[62] In addition, computed tomography (CT) and bone scintigraphy may have limitations owing to inadequate soft tissue resolution. Magnetic resonance imaging (MRI) appears to be the method of choice to assess the extent of osteoarticular brucellosis and in the follow-up of therapeutic responses.[62,63] The characteristic Pedro-Pons sign (a steplike erosion at the anterosuperior portion of a vertebral body) in spondylitis is noteworthy.[64]

Nervous System

Neurologic involvement occurs in approximately 10% of cases and is a serious complication of brucellosis.[44,65] Brucellosis comprises 0.5% of all community-acquired central nervous system (CNS) infections.[66] The disease can be classified into categories: acute meningitis or meningoencephalitis, chronic peripheral form (radiculoneuropathy), and chronic CNS infection (meningoencephalitis, myelitis, cerebellar involvement, cranial nerve palsies).[65] Headache and fever are observed in slightly more than half of the cases as the predominant complaints, followed by sweating, weight loss, and back pain. In addition, meningeal irritation is reported only in one-third of the cases, followed by confusion, hepatomegaly, hypoesthesia, and splenomegaly.[65] An evaluation of 263 patients with MRI or CT for CNS neurobrucellosis found that 54.3% had normal scans, and diffuse leptomeningeal inflammation was detected most frequently in one-fourth of the cases.[67] Added to that, 1.7% of *Brucella* meningitis did not display CNS pleocytosis.[68] Under these circumstances the disease has a quite subtle presentation and has a predilection to present chronic courses.[66] Some reports relate brucellosis to Guillain-Barré syndrome in endemic areas.

According to a multinational study on neurobrucellosis, half of the neurobrucellosis patients present to hospitals with serious neurologic complications. Cranial nerve involvement, mostly affecting the sixth and eighth cranial nerves, complicates one-fifth of the cases. Moreover, polyneuropathy and radiculopathy, depression, paraplegia, stroke, and abscess formation are reported. Mortality is as low as less than 1% with suitable antibiotics, although one-fifth of treated patients experience permanent sequelae. Walking difficulty and hearing loss are predominant sequelae, followed by urinary incontinence, visual disturbances, and amnesia. Roughly 4% of the patients come to neurosurgery. Moreover, the cerebral abscesses are generally small, and only one-third of patients with abscess formation appear to require surgical drainage.[52] The Thwaites and the Lancet scoring systems used to detect CNS tuberculosis may erroneously identify neurobrucellosis as tuberculous meningitis. Hence caution is indicated in the differential diagnosis.[69] In conclusion, although mortality is rare, neurobrucellosis is a serious disease with frequent sequelae despite treatment.[52,65,70]

Genitourinary Tract

Genitourinary complications are mostly seen in men and are reported in 5% to 10% of all brucellosis patients. Epididymo-orchitis is observed most frequently in males and pyelonephritis in females[48,51,57,71] Any patient with scrotal pain and swelling with coexistent arthritis and arthralgia should raise consideration for brucellar epididymo-orchitis.[51] Infection is usually unilateral, with fever or sweating in only about a third of patients.[72] The disease may simulate testicular lesions, such as tumors or tuberculosis.[73] Nephritis is mostly a complication of endocarditis. Renal, scrotal, and testicular abscesses, as well as prostatitis, caused by *Brucella* have been reported.[51,74]

Cardiovascular System

Brucella cardiovascular involvement includes endocarditis,[75] myocarditis,[76] pericarditis,[77] endarteritis,[78] thrombophlebitis,[79] and mycotic aneurysms.[80] Pancarditis leading to heart failure is known to be a serious complication.[81] Endocarditis is the most common cardiovascular complication and is reported in approximately 1% of all brucellosis cases.[48,75] Mortality in brucellosis is mostly attributed to endocarditis.[12] Echocardiography and automated blood cultures contribute significantly to diagnosis of cardiovascular brucellosis.[75] In a multicenter *Brucella* endocarditis study, aortic and mitral valves were the most frequently involved sites in the heart. Underlying cardiac valvular disorders are reported in 60% of the

patients. Although mortality is reported to be 13% despite treatment, it is significantly higher when pericardial effusion or congestive heart failure develops.[75]

Gastrointestinal System

Human brucellosis is one of the enteric fevers where systemic symptoms generally predominate over gastrointestinal (GI) complaints. GI involvement in a brucellosis patient should be considered if there are any related signs or symptoms, such as nausea and vomiting, diarrhea, constipation, and abdominal tenderness.[82] Diarrhea is reported in 3% to 6% of patients with brucellosis.[6,7,83] Although the precise reason is not known, it may be due to mucosal ulcerations in intestinal Peyer patches or other microorganisms present in contaminated raw milk.[83] Spontaneous bacterial peritonitis is one of the leading GI disorders, followed by splenic abscess and acute abdominal pain.[48] The majority of the patients with spontaneous bacterial peritonitis are reported to have underlying liver cirrhosis.[83] Acute and chronic pancreatitis,[84,85] colitis,[86] intestinal obstruction,[87] and ileitis[48] have been reported occasionally.

Hepatobiliary System

Hepatic involvement in brucellosis covers a wide spectrum, ranging from mild elevation of aminotransferases to manifest hepatitis, including granulomatous forms, and to liver abscesses.[88] Increases in aminotransferases are noted in one-fourth to one-third of brucellosis cases and are more frequent in the acute stages.[44,45,47,71] Hepatic involvement in brucellosis can present with clinical hepatitis in 3% of the cases.[44,48] In most patients with hepatic involvement, the histopathologic analysis is interpreted as reactive hepatitis.[89] Although *B. abortus* tends to establish a granulomatous form of hepatitis, *B. melitensis* may cause both diffuse and granulomatous lesions in the liver. Even necrotizing hepatic lesions are reported in the literature.[90] Cholecystitis is a rare event,[48] except with coexistent gallstones.[91] *Brucella* hepatitis may lead to liver decompensation and cirrhosis if left untreated.[88]

Respiratory System

Respiratory system involvement in brucellosis is reported in up to 1% of cases.[44,48] Bronchitis, pneumonia, and pleural effusion are the predominating pulmonary presentations.[48,92] Granulomas and solitary nodules in the lung parenchyma, as well as abscess and cavity formation, are also noted.[93,94] Miliary mottling can be present in pneumonias and may result in confusion with pulmonary tuberculosis.[95] Brucellosis is one of the most common laboratory-acquired infections, mostly due to airborne transmission.[5] Sand and airborne dust have been shown to have the potential to carry *Brucella* spp.[96] Although the interrelations between the development of pneumonia and airborne transmission are unclear, one of eight laboratory workers who experienced a brucellosis outbreak in a laboratory by airborne route had pneumonia.[97] Respiratory system brucellosis, which is usually indistinguishable from other community-acquired pneumonia forms, has a benign course and responds to treatment used for uncomplicated brucellosis.[94,98]

HEMATOLOGIC COMPLICATIONS

A wide spectrum of laboratory abnormalities related to the hematologic system in brucellosis is reported. Anemia, leukopenia, leukocytosis, thrombocytopenia, thrombocytosis, and pancytopenia are relatively common.[6,12,44,48] Bone marrow involvement shows hypercellularity, hemophagocytosis, and granulomas.[99] Although hematologic abnormalities are usually mild, disseminated intravascular coagulation can occur.[100] Hypersplenism due to brucellosis may contribute to the severity of hematologic complications.[101] In rare instances massive bleeding[102] or capillary leak syndrome, defined as unexplained capillary hyperpermeability, can occur.[103] Nevertheless, the blood profile improves with treatment. The disease may also mimic lymphoma.[104]

CUTANEOUS LESIONS

Nonspecific cutaneous and mucosal lesions are reported in 2% to 6% of brucellosis cases.[44,48,105] These manifestations are due to hypersensitivity, depositing immune complexes, or direct invasion by the organism.[106] Erythematous, papulonodular, and erythema nodosum-like lesions are the most frequent eruptions, and they usually appear at the initial stages of the infection.[105] Psoriatic lesions, palmar erythema, malar eruption, palmar eczema, petechiae, and purpura are reported in a diverse spectrum.[107,108] *Brucella* may trigger immunologic responses leading to leukocytoclastic vasculitis.[109,110]

OCULAR LESIONS

Uveitis, the most frequent eye complication, followed by optic neuritis, papilledema, keratitis, conjunctivitis, and other diverse ocular complications are observed in brucellosis. Panuveitis, in which nearly all patients had lost their vision, is reported in one-fifth of all uveitis cases.[111] Direct invasion, septic emboli from the infected site, endocardium in particular, and formation of immune complexes are reported as the mechanisms of *Brucella* eye disease.[111,112] Isolation of the microorganism from the vitreous humor has been reported.[113] Alternatively, visual improvement after treatment with steroids can be interpreted as evidence of an immune basis for the inflammation.[114]

PREGNANCY

Brucellosis manifests as a chronic infection resulting in sterility and abortion. Erythritol is considered as a contributor to the pathogenesis in animals.[115] Two reasons are known to be responsible for the lesser role of *Brucella* infection in human abortion: (1) Human placenta does not produce erythritol[7] and (2) the presence of anti-*Brucella* activity in human amniotic fluid may have protective effects.[116]

Brucellosis complicates pregnancy in one-third to one-half of women infected while pregnant, particularly in the first two trimesters. The disease is linked to intrauterine infection, fetal death, spontaneous abortion, premature delivery, and low birth weight in the neonate.[117] Congenital brucellosis is detected in 6.4% of the newborns, and the surviving newborn may experience serious sequelae.[118] Thus prompt initiation of antibiotic treatment may be lifesaving for both fetus and newborn.

DIAGNOSIS

Diagnosis of brucellosis requires the assessment of medical history, clinical evaluation, and routine laboratory and radiologic tests, combined with culture, serology, or polymerase chain reaction (PCR) assay.[119,120] Brucellar disease may easily be confused with tuberculous meningitis or spondylodiskitis in nonendemic and developed countries.[56,69] The routine laboratory tests are complete blood count, erythrocyte sedimentation rate, C-reactive protein, and liver function tests, although they are not specific for the diagnosis, and wide patient variability is known to exist.[56,69,119]

Blood and bone marrow are the most suitable specimens used in the isolation of *Brucella.* In patients receiving antibiotics, as well as patients with a chronic form of brucellosis, bone marrow culture appears more sensitive.[121] In addition, automated culture systems have improved the speed and the efficacy of *Brucella* isolation, usually within 3 days.[122] Moreover, automated culture systems are much more often positive than conventional cultures for sterile body fluids including cerebrospinal fluid (CSF).[122,123]

Numerous serologic methods are used in the diagnosis of brucellosis. Although serum agglutination test (SAT or Wright reaction) is usually recognized as the reference technique, with a positive defined as a titer of at least 1:160.[119,124] It is labor intensive and time consuming. Thus rose-bengal slide agglutination test (RBT), which uses stained killed *B. abortus* bacterial cells, offers a simple and affordable card test. The RBT has been traditionally used as a feasible format of SAT for rapid screening in emergency departments.[125,126] The Coombs antiglobulin test is used to detect nonagglutinating antibodies against *Brucella* cells. Serial dilutions up to high titers may be necessary to get beyond prozones with this process.[119] Immunocapture enzyme-linked immunosorbent assay (ELISA, Brucellacapt [Vircell; Granada, Spain]) adds patient serum to a microwell, which is coated with antibodies against human IgG, IgM, and IgA. Stained killed *Brucella* cells are added, and agglutination is observed. This method yields results comparable to the Coombs test.[127,128] Likewise, ELISA appears to be comparable to conventional serologic tests in the diagnosis of the disease.[129] However, the CDC has warned that false-positive ELISA tests for brucellosis require that positive results should be confirmed by standard agglutination tests.[130]

Negative serology does not exclude the diagnosis in brucellosis, and using more than one test is recommended in probable cases.[123,131] In addition, *Brucella* antibodies can persist long after the patient's recovery, and thus it is not always possible to distinguish patients with active disease from those with past infection.[41,132] In these cases the IgG avidity test can be valuable because high IgG avidity would suggest immune memory, not a new-onset disease. On the other hand, patients with titers lower than the diagnostic threshold and low avidity would suggest primary infection.[133] However, the PCR assay seems satisfactory in symptomatic patients with negative serology, and it provides recognition of the disease as early as 10 days after inoculation, particularly during outbreaks or relapses.[6,134,135] Of note, standard serologic testing does not detect the rare human cases of *B. canis* because of antigenic differences.[136]

TREATMENT

Antimicrobials with accumulation in phagocytes may be important for therapeutic success in brucellosis (Table 226.1).[137] Monotherapy is usually not recommended and is associated with frequent therapeutic failures or relapses.[7] However, the use of doxycycline or minocycline alone for 6 to 8 weeks can be considered in the absence of focal disease and may be cost-effective in countries with limited resources.[54] Currently, there are two recommended antibiotic combinations for the treatment of adults with uncomplicated brucellosis, both for a minimum of 6 weeks.[1,7,138] The first regimen is oral doxycycline 100 mg twice daily for 6 weeks plus intramuscular streptomycin 1 g once daily for 2 to 3 weeks. Seven days of gentamicin (5 mg/kg) combined with doxycycline appears to have similar efficacy to streptomycin plus doxycycline.[1,139,140] Although the use of aminoglycosides may produce ototoxicity and nephrotoxicity,[141] the adverse effects in due course of treatment are generally mild to moderate and rarely serious and requiring modification.[53] The second regimen is doxycycline added to 600 to 900 mg oral rifampin (15 mg/kg) once daily for 6 weeks. Although streptomycin combined with doxycycline is shown to be superior to doxycycline plus rifampin in terms of relapse and therapeutic failure,[53,142] there are clinicians who favor the use of the second combination because it is rather cheap, easy to use, and available in most countries.[1] Added to that, the use of doxycycline and an aminoglycoside showed more rapid normalization of liver aminotransferases in clinical brucellar hepatitis cases.[88]

Primary drug resistance to tetracyclines or aminoglycosides has long been known to be absent in *Brucella* isolates,[143,144] and relapses could not have been related to resistance.[143] Thus relapses usually responded well to a further course of the same antibiotics[7,143] However, decreased susceptibility or resistance to rifampin is described in some recent studies.[145–148] In addition, when patients are infected with *Brucella* RB51, a strain of *B. abortus* that is used to vaccinate cattle, rifampin should not be used because this particular strain is resistant to it.[149] Current data disclose that rifampicin combination therapy increased the risk of overall failure and relapse compared with the streptomycin-containing regimen.[149,150] Hence doxycycline combined with an aminoglycoside seems to be the first-line therapy in brucellosis. Although quinolones combined with rifampin yielded results similar to doxycycline plus rifampin,[150] quinolones are recommended only as second-line regimens, used with either rifampin or doxycycline.[1,7] They may be considered in case of antibiotic resistance or drug toxicity. Trimethoprim-sulfamethoxazole (TMP-SMX) is also associated with a high rate of relapses and should always be combined with another agent.[7] TMP-SMX is of particular importance in children because doxycycline should be avoided in children younger than 8 years due to dental complications. The relapse rates appear to be less than half with a three-drug regimen of aminoglycoside, doxycycline, and rifampin compared with doxycycline plus rifampin.[151,152] Three-drug combinations, including TMP-SMX (double-strength tablet twice daily) or an aminoglycoside, can be used to treat difficult cases, such as nonresponding or relapsing disease and focal brucellosis. There is a preliminary report on the efficacy of adding hydroxychloroquine to the standard regimen.[153]

Therapeutic strategies in focal brucellosis should be individualized according to the site involved. In addition, longer therapeutic courses are usually necessary in these cases. As an example, extension of infection through paravertebral and epidural spaces, psoas muscle, or nerve roots in due course of spondylodiskitis requires a longer treatment for a mean of 4 to 5 months.[55] In a series of 32 cases of *Brucella* spondylitis the median duration of treatment was 6 months.[63] In another report *Brucella* spondylitis appear to respond to doxycycline (for 3 months) plus streptomycin for 2 to 3 weeks.[154] The optimal treatment of brucellar meningitis seems to be 1 month of intravenous ceftriaxone 4 g daily in two divided doses combined with doxycycline and rifampin for 4 to 5 months. This regimen provides a low incidence of neurologic complications, requires shorter therapy, and is more successful when the negative outcome (relapse plus therapeutic failure) is considered.[52] The antibiotics should be continued depending on the clinical response[7] and until CSF parameters return to normal.[52] Although data are lacking on the optimal treatment of endocarditis, combinations including aminoglycosides seem rational choices.[75] Surgery contributes to survival in select endocarditis patients, such as those with congestive heart failure, valvular regurgitation, uncontrolled infection with antibiotics, embolic complications, or large vegetations.[75,155] Recurrent infection, unstable spinal column, marked kyphosis, uncontrollable pain related to spinal involvement, and focal abscesses are other probable indications for surgery in brucellosis.[52,56,156]

Early diagnosis and adequate treatment of brucellosis during pregnancy improves maternal and fetal outcomes.[157] Rifampin 900 mg once daily for at least 6 weeks is the treatment of choice during pregnancy. Adding TMP-SMX can be considered, but this option should probably be avoided preceding the 13th week and after the 36th week of gestation because of concern about teratogenicity and kernicterus.[54]

TABLE 226.1 Treatment of Brucellosis

CLINICAL FORM	PREFERRED REGIMEN	ALTERNATIVE REGIMEN
Uncomplicated	Doxycycline, 100 mg PO twice daily for at least 6 wk *Plus* Streptomycin, 1 g IM daily for 2–3 wk	Doxycycline, 100 mg PO twice daily for at least 6 wk *plus* Gentamicin, 5 mg/kg IM daily for 1 wk *or* Doxycycline, 100 mg PO twice daily for at least 6 wk *plus* Rifampin, 600–900 mg (15 mg/kg) once daily for at least 6 wk
Neurobrucellosis	Ceftriaxone, 2 g IV twice daily for at least 1 mo *Plus* Doxycycline, 100 mg PO twice daily for 4–5 mo *Plus* Rifampin, 600–900 mg (15 mg/kg) PO once daily for 4–5 mo	Trimethoprim-sulfamethoxazole, 160/800 mg PO twice daily for 5–6 mo *plus* Doxycycline, 100 mg PO twice daily for 5–6 mo *plus* Rifampin, 600–900 mg (15 mg/kg) PO once daily for 5–6 mo

[a]See text for additional information.
IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

PREVENTION

Although secure food supplies and safe laboratories are among the significant factors in prevention, controlling the disease is only possible by its eradication from domestic animals. Vaccination of domestic cattle, sheep, and goats with *B. abortus* S19, RB51, and 104M, and *B. melitensis* REV1 and vaccines plays a crucial role in control of brucellosis. No vaccine has yet been developed for *B. suis* or *B. canis*.[7,158] Nor is there a vaccine for humans.

There are limited data on prophylactic antibiotic usage subsequent to exposure to *Brucella*. In their review of laboratory-acquired brucellosis, Traxler and colleagues[159] reported that none of the 34 persons who received postexposure prophylaxis developed infection. Twenty-five of 34 received rifampin plus doxycycline. Brucellosis due to erroneous inoculation of animal attenuated-live vaccines to humans has been reported.[7] In cases of a high-risk contact, such as a laboratory exposure or accidental vaccine inoculation, rifampin plus doxycycline is recommended at standard dosages for 3 weeks. If there is a contraindication to doxycycline use, TMP-SMX prophylaxis is suggested.[160]

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227 *Francisella tularensis* (Tularemia)

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SHORT VIEW SUMMARY

Definition
- Tularemia is the zoonotic disease caused by *Francisella tularensis.*

Epidemiology
- Tularemia is widely distributed but is primarily a disease of the Northern Hemisphere.
- In the United States, the majority of cases reported in 2016 occurred in Arkansas, Colorado, Kansas, Missouri, Nebraska, Oklahoma, South Dakota, and Wyoming.
- Tularemia peaks in the late spring and summer in the United States.
- Lagomorphs and rodents are important animal reservoirs.
- Significant transmission vectors include ticks and biting flies in the United States and mosquitoes in Europe.
- Other routes of transmission include aerosol droplets, contaminated mud or water, skin contact with infected carcasses, and animal bites.
- Occupations that have an increased risk for tularemia include laboratory worker, farmer, landscaper, veterinarian, sheep worker, hunter or trapper, and cook or meat handler. Clinicians should warn laboratory personnel if tularemia is suspected.
- Tularemia spread by aerosol is a potential bioterrorist weapon.

Microbiology
- *Francisella* organisms are small, aerobic, pleomorphic gram-negative coccobacilli.
- *F. tularensis* requires cysteine or cystine (or another sulfhydryl source) for growth and therefore will not grow well on most conventional solid media.
- The three *Francisella* species that are potential human pathogens are *F. tularensis, F. philomiragia,* and *F. hispaniensis* (see Table 227.1). However, only *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* are relatively common.
- Infections caused by *F. tularensis* subsp. *tularensis* are generally more severe than those caused by *F. tularensis* subsp. *holarctica.*
- The infectious dose in humans is 10 to 50 organisms when injected intradermally or when inhaled.
- *F. tularensis* is a facultative intracellular pathogen that survives within host macrophages by impairing phagosome-lysosome fusion, and it is capable of suppressing or avoiding many other humoral and cellular host defenses.
- Recovery from infection depends on the development of host cell–mediated immunity.

Clinical Manifestations
- Tularemia starts abruptly with fever, chills, headache, anorexia, and fatigue after an average incubation period of 3 to 5 days.
- There are six significant illness patterns: ulceroglandular (see Fig. 227.3), glandular, oculoglandular, pharyngeal, typhoidal, and pneumonic (see Fig. 227.5); pneumonic and typhoidal tularemia are expected to be the primary forms resulting from a bioterrorism event.
- Secondary rashes are relatively common.
- The most common complications of tularemia are lymph node suppuration and persistent debility.

Diagnosis
- Diagnosis rests on clinical suspicion, and because of its potential danger, laboratory personnel should be notified whenever tularemia is suspected.
- *F. tularensis* is a Tier 1 select agent, and its possession and shipment are tightly restricted.
- Routine cultures and smears are often negative, and the diagnosis is frequently confirmed serologically; polymerase chain reaction tests are not widely available but offer a rapid diagnosis.

Therapy
- Streptomycin and gentamicin are the drugs of choice for all forms of severe tularemia except meningitis (see Table 227.2).
- Selected adults and children with mild to moderate disease may be treated with oral agents (see Table 227.2).
- Surgical intervention is typically limited to drainage of suppurated nodes or of empyemas.

Prevention
- The best prevention is avoiding exposure to the organism; a vaccine for tularemia is currently unavailable in the United States.
- Patients with tularemia do not need special isolation because person-to-person spread does not occur. Standard precautions for handling contaminated secretions are adequate.
- Antibiotic prophylaxis after potential exposures of unknown risk, such as tick bites, is not recommended.
- Either ciprofloxacin, 500 mg, or doxycycline, 100 mg, either given orally twice daily for 14 days is recommended for adults with suspected or proven high-risk exposure to *F. tularensis.*
- Observation without prophylactic antibiotics is appropriate for exposed children (except during a bioterrorist event) and adults with lower risk exposures.

Francisella tularensis is a gram-negative pathogen primarily of animals and occasionally of humans. The disease is now recognized as tularemia in most areas of the world, but it has been called rabbit fever, deer fly fever, Francis disease, and market men's disease in the United States; wild hare disease (yato-byo) and Ohara disease in Japan; and water-rat trappers' disease in Russia. Tularemia continues to be responsible for significant morbidity and mortality, despite the availability of numerous antibiotics active against the organism.[1]

F. tularensis infections have become a broader public health issue centering on concerns regarding potential military or terrorist uses in biological warfare. In 2000, tularemia returned to the list of reportable diseases in the United States after exclusion in 1995. With heightened surveillance and recognition of ongoing cases, attention is often quickly focused upon outbreaks of natural *F. tularensis* infection that do occur regularly throughout the world.

HISTORY

Tularemia has been so intimately linked to investigators in the United States that it has been referred to as "the first American disease" or an "American achievement."[2,3] However, its history is notable for important contributions from other areas of the world, including Japan and the former Soviet Union. Hare-associated illness compatible with tularemia

has been known in Japan since 1818, and perhaps the earliest written description of a patient with unmistakable tularemia was provided by Soken Honma in 1837.[4]

Credit for recognizing the clinical syndromes and identifying the organism belongs to American workers. In 1911, while evaluating possible plague outbreaks after the San Francisco earthquake of 1906, McCoy described a plague-like illness then prevalent in ground squirrels. With Chapin, he successfully cultured the causative bacteria in 1912.[5] They named it *Bacterium tularense* because their discovery occurred in Tulare County, California. Although the cause was unknown at the time, Pearse first clinically described human tularemia following contact with biting flies in Utah in 1911, which he termed *deer fly fever*.[6] In 1914, the first case with bacteriologic confirmation was reported in a patient from Ohio with an ocular infection.[7,8] In 1919, Dr. Edward Francis linked the deer fly as the vector of disease while recovering an unusual organism in the blood of febrile patients from Millard County, Utah, who also had suppurative lymphadenopathy[9]; he subsequently coined the human disease tularemia to emphasize the frequently accompanying bacteremia.[10] By 1928, working for the U.S. Public Health Service, Francis had firmly established the actual cause of deer fly fever as *B. tularense,* more than a decade after the organism was found in squirrels.[11] Francis also contributed to improving cultivation methods for *B. tularense,* making a serologic diagnosis, identifying tick and other reservoirs as other vectors for transmission, clarifying the clinical syndromes associated with tularemia, and emphasizing the risk to laboratory workers and consumers from infected sources. In 1974, for this lifetime of achievements, the genus in which the organism is classified was renamed *Francisella* in his honor.

In Japan, Ohara had described a rabbit-associated febrile disease in 1926.[12] He subsequently, transmitted the illness to his wife by rubbing rabbit hearts over her hand, and he recovered an organism from her lymph nodes[13]; Francis and Moore later showed that this Japanese organism was identical to *B. tularense.*[14] In 1926, tularemia was recognized in Astrakhan, Russia, and scattered outbreaks of serious human disease occurred throughout the country during the subsequent decades.[15] Scientists in the former Soviet Union also have intensively studied the disease and its causative organism.

MICROBIOLOGY

Francisella organisms are small, aerobic, catalase-positive, pleomorphic, gram-negative coccobacilli that are nonmotile and non–spore forming. They are more uniformly rod-shaped during logarithmic growth, during which they tend to exhibit bipolar staining with Gram or Giemsa methods; this staining pattern accentuates a coccoidal appearance, measuring 0.2 × 0.2 µm. The *F. tularensis* cell wall has an unusually high level of fatty acids with a unique profile. Wild-type strains possess an electron-transparent, lipid-rich capsule. Loss of the capsule may lead to loss of serum resistance and virulence, but may not diminish viability or survival within neutrophils.[13] The capsule had traditionally been thought to be directly neither toxic nor immunogenic, though recent studies have suggested that bacterial lipoproteins may interfere with neutrophil apoptosis by way of Toll-like receptor (TLR) mechanisms, and therefore foster local tissue destruction.[16]

Francisella spp. belong to the Gammaproteobacteria class in the family Francisellaceae and include the human pathogens *F. tularensis, F. philomiragia,* and *F. hispaniensis;* the fish pathogens *F. noatunensis* subsp. *orientalis* and *F. halioticida;* and environmental isolates of *F. guangzhouensis.* Other unnamed *Francisella* spp. also have been rare human pathogens. The first tick endosymbiont isolated, *Wolbachia persica,* has been reclassified as part of the genus *Francisella* based on sequencing with the new name *Francisella persica* comb. nov.[17] Molecular techniques have also defined clinical and environmental isolates, expanding by four new species designations within the genus: *Francisella opportunistica* sp. nov., *Francisella salina* sp. nov. *Francisella uliginis* sp. nov., and *Francisella frigiditurris* sp. nov.[18]

Francisella traditionally has been categorized by growth characteristics, biochemical reactions, and virulence properties (Table 227.1). The three *Francisella* spp. potentially pathogenic for humans are *F. tularensis, F. philomiragia,* and *F. hispaniensis.*[19–21] Although all three have been associated with human disease, only the *tularensis* and *holarctica* subspecies of *F. tularensis* are relatively common. *F. tularensis* subsp. *tularensis* (*nearctica*), also referred to as type A, is found almost exclusively in North America and is the most virulent species. Although previously thought to be restricted to North America, *F. tularensis* subsp. *tularensis* has been isolated in Europe and its identity verified using molecular techniques.[22]

F. tularensis subsp. *holarctica* (*palaearctica*), also referred to as type B, is found predominantly in Asia, Australia, and Europe but also in North America; it is less virulent in humans and of low virulence in rabbits. The *F. tularensis* live vaccine strains are derived from *F. tularensis* subsp. *holarctica* (see later under "Vaccination"). *F. tularensis* subsp. *novicida* is of low virulence and only rarely causes human infection, mainly in patients who are immunosuppressed.[23] Although previously classified as a separate species, its reclassification as a subspecies of *F. tularensis* was proposed based on a high degree of genetic similarity to *F. tularensis.*[19,24] However, this has been controversial, and some authorities feel it should remain a separate *Francisella* species based on phenotypic as well as genomic differences.[20,23] Strains isolated in Japan have

TABLE 227.1 Characterization of *Francisella* Species

	F. TULARENSIS SUBSPECIES[a]				
FEATURE	***tularensis***	***holarctica***	***novicida***	***F. PHILOMIRAGIA***	***F. HISPANIENSIS***
Cysteine growth requirement	+	+	–	–	–
Growth in broth plus 6% NaCl	–	–	+[b]	+[c]	NA
Motility	–	–	–	–	NA
Oxidase	–	–	–	+[c]	+
Nitrate reduction	–	–	–	–	NA
Acid from:					
Glucose	+[b]	+[b]	+[b]	+[b]	+
Glycerol	+	–	+	+	+
Gelatin hydrolysis	–	–	–	[b]	NA
Relative virulence					
Humans	High	Intermediate	Low	Low	Low
Rabbits	High	Low	Low	NA	NA

[a]The fourth *F. tularensis* subspecies, *F. tularensis* subsp. *mediasiatica,* and the *japonica* biovar of *F. tularensis* subsp. *holarctica* are described in the text.
[b]Variable or delayed.
[c]Using Kovacs test; negative using cytochrome oxidase test.
NA, Not available; *NaCl,* sodium chloride.
Data from references 19 through 21 and reference 33.

been designated *F. tularensis* subsp. *holarctica* biovar *japonica,* but their differentiation from other *F. tularensis* subsp. *holarctica* strains by traditional phenotypic testing was not possible.[21]

A fourth subspecies, *F. tularensis* subsp. *mediasiatica,* has been found only in sparsely settled regions in Central Asia and Russia.[20,25,26] An absence of human infection reports suggests that if capable of infecting, it would have low virulence.

F. philomiragia was previously called *Yersinia philomiragia.* Reclassified because it shared the unique fatty acid profile of the *Francisella* spp. and substantial DNA relatedness to this genus, it does have some unique biochemical features (see Table 227.1) and DNA hybridization patterns that distinguish it from *F. tularensis. F. philomiragia* is of low pathogenicity for humans, although it has been isolated from muskrats and a dog.[20,27,28] It is naturally found in brackish and salt water, and in liquid media it may form biofilms and interact with free-living amebas.[29] All strains originally tested produced β-lactamase and were most susceptible to aminoglycosides, cefoxitin, cefotaxime, fluoroquinolones, tetracycline, and chloramphenicol. However, an infection caused by *F. philomiragia* resistant to cefazolin and cefotaxime has been reported.[30] The most active agents against six strains in a standardized broth microdilution assay were the aminoglycosides and the fluoroquinolones.[31] The complete genome of *F. philomiragia* has been sequenced, and it encodes a class A carbapenemase.[28,32] *F. hispaniensis* was isolated from the blood and urine of a septic patient in Spain 1 month after undergoing lithotripsy.[33] The organism was classified as a separate species of *Francisella* based on its phenotypic and genotypic properties (see Table 227.1).[19]

The taxonomy of *Francisella* has been complicated because biochemical reactions may be variable, weak, or delayed and also in part because of the different terms given to organisms isolated in different areas of the world. Classification of *Francisella* has been advanced by the sequencing of the whole genome from representative strains of *F. tularensis* subsp. *tularensis* (strain Schu S4), *F. tularensis* subsp. *holarctica* (including the live-attenuated vaccine strain), and *F. tularensis* subsp. *novicida,* along with the application of various molecular typing methods.[22,24,34–37] These have included 16S ribosomal DNA gene sequence analysis, microarray analysis of the whole genome, multiple-locus variable-number tandem repeat analysis, canonical insertion-deletion markers, and single nucleotide polymorphism analysis.[22,36] Reports using these techniques have supported the currently accepted taxonomy as outlined previously and in Table 227.1, demonstrated the utility of these methods for species and subspecies typing, and identified *F. tularensis* subsp. *holarctica* biovar *japonica* as a distinct group.[37,38] Molecular typing has identified three genotypes of *F. tularensis* subsp. *tularensis* (designated A1a, A1b, and A2) and at least 10 genotypes of *F. tularensis* subsp. *holarctica.*[39] Genomic analyses have indicated that there was a common *F. tularensis* ancestor for clonal subspecies evolution, that *F. tularensis* subsp. *novicida* is the oldest, and that *F. tularensis* subsp. *tularensis* appeared before *F. tularensis* subsp. *holarctica,* which is the youngest.[40]

F. tularensis requires cysteine or cystine (or another sulfhydryl source) for growth and therefore will not grow using most routine solid media or gram-negative selective media such as MacConkey or eosin methylene blue agars. It may be recovered with the use of glucose cysteine blood agar, thioglycollate broth, chocolate agar suitable for gonococcal growth, modified Thayer-Martin medium, buffered charcoal-yeast agar, or cysteine heart agar with 9% chocolatized sheep blood.[20,21] Blood agar may support the growth of some *F. tularensis* isolates on initial plating but not on subpassage.[41] Some strains of *F. tularensis* lack an overt requirement for cysteine or enriched medium for growth, and clinically significant strains of *Francisella* have been reported that do not show the expected fastidious growth characteristics.[21] A novel brain heart infusion supplemented with 2% Vitox, 10% Fildes, and 1% histidine (BVFH) broth has been described as facilitating the early growth of *F. tularensis* compared to customary media formulations.[42]

Francisella should be suspected, however, whenever a slowly growing, small, and poorly staining gram-negative coccobacillus is isolated on chocolate agar and grows poorly or not at all on blood agar. Visible colonies take 2 to 5 days to appear. Incubation at 35°C is optimal, with or without an atmosphere of increased carbon dioxide. The recovery of *F. tularensis* from contaminated specimens may be facilitated by plating onto antibiotic-containing media known to support the growth of *F. tularensis.*[20] Virtually all *F. tularensis* strains are positive for β-lactamase, and a weak class A β-lactamase native to *F. tularensis* has been identified.[43]

Differentiation of *Francisella* from other bacteria can be accomplished using direct fluorescent antibody (DFA) staining, slide agglutination, polymerase chain reaction (PCR) assay, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) sequencing, or cellular fatty acid composition analysis.[20,44] Antisera can distinguish between *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *novicida* but not between *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica;* strains within subspecies do not have antigenic differences detectable by antisera.

It is important to note that automated laboratory identification systems should not be used for the identification of *Francisella* because they may generate aerosols and commonly misidentify *F. tularensis* as *Haemophilus* or *Aggregatibacter* species.[41] Whenever *F. tularensis* is suspected, the state public health laboratory should be notified immediately, and any local microbiologic testing should be done using a biological safety cabinet and following Biosafety Level 3 procedures.[41] Isolates that cannot be excluded as *F. tularensis* should be sent to a reference laboratory in the Laboratory Response Network, typically the state public health laboratory. Federal regulations rigorously control possession and transport of *F. tularensis* and must be followed when sending an isolate to a referral laboratory for identification.

Virulence

F. tularensis produces no known exotoxins, but many components of the *Francisella* envelope contribute to its pathogenicity, though questions remain about many putative virulence factors.[33,44,45] The lipopolysaccharide (LPS) from the live vaccine strain of *F. tularensis* possesses at least 1000-fold less endotoxin activity than the LPS from *Escherichia coli.*[46] This is in part because *F. tularensis* LPS has a different structure composed primarily of the O antigen that makes the capsule and lacks many of the traditional LPS incitatory endotoxin components. Unique features of this LPS include reduced acyl chains, longer fatty acid chains, and less lipid A phosphorylation.[47] The capsule can also block both immunoglobulin M (IgM) and complement binding with C3.[48] Many of these features are believed to contribute both to immune activation and evasion.

Unlike other gram-negative bacteria, it is less recognized by TLR4 and does not bind to LPS-binding protein.[33,45,49] However, purified *F. tularensis* LPS can stimulate human B cells and, when present together with the *F. tularensis* heat shock protein GroEL, can synergistically activate human macrophages.[50,51] The O-antigen side chains from *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* are identical but differ from that of *F. tularensis* subsp. *novicida.*[49]

Pili have been visualized on the surface of *F. tularensis* subsp. *holarctica* live vaccine strain and *F. tularensis* subsp. *novicida.* Genome sequencing has detected genes homologous to those from other bacteria that encode type IV pili in *F. tularensis* subspp. *tularensis, holarctica,* and *novicida;* and type IV pili assembly contributes to virulence in murine models.[52] Type IV pili and other bacterial surface proteins mediate adherence of *F. tularensis* to host cells, and a type IV pilus subunit of *F. tularensis* subsp. *holarctica* is a ligand for intercellular adhesion molecule 1 on rat vascular epithelial cells.[53,54]

Host immune responses are directed against numerous cell wall antigens, including membrane proteins, LPS, and carbohydrates, but previously it was not possible to identify dominant antigens. Proteomic analysis using serum from donors who have had tularemia or who have been immunized with the live vaccine strain has identified a large number of *F. tularensis* protein antigens and found possible immunodominant antigens.[55] These include many cytoplasmic and membrane proteins, as well as hypothetical proteins of unknown localization. Further proteomic analyses will be helpful to refine future diagnostic tests for tularemia and the construction of effective vaccines.[56,57]

Phenotypic correlates of virulence have included the capsule and citrulline ureidase activity. Wild encapsulated strains of *F. tularensis* are resistant to the bactericidal activity of normal serum, a capsule-deficient mutant of the live vaccine strain also is serum resistant, and the capsule-deficient mutant is less virulent in mice than its wild-type

parent.[58] The contribution of citrulline ureidase to virulence is unclear. There are pathogenic isolates that do not possess this activity. Plasmids have been found in isolates of *F. tularensis* subsp. *holarctica, F. tularensis* subsp. *novicida,* and *F. philomiragia,* but have not been found in the more virulent *F. tularensis* subsp. *tularensis* and thus are not essential for virulence. Several acid phosphatases (Acp) are present in *Francisella,* and they are important for its survival within macrophages. AcpA can inhibit the respiratory burst of neutrophils, expression of AcpA and histidine acid phosphatase (Hap) is induced by growth within macrophages, and AcpA is secreted into the cytosol of macrophages.[59,60] Although deletion of *acpA, acpB, acpC,* and *hap* from *F. tularensis* subsp. *novicida* results in a mutant strain that is impaired in its ability to survive within macrophages and to escape from the phagosome, deletion of *acpA* and the combination of *acpA, acpB,* and *acpC* deletions do not alter the virulence of *F. tularensis* type A strain Schu S4.[60,61] Mechanisms for iron acquisition are present in *F. tularensis,* and their disruption attenuates in vitro intracellular growth and organism virulence in a mouse model.[62]

Genomic and proteomic analyses have the potential to more specifically identify virulence factors in *F. tularensis.*[57,63] *F. tularensis* contains a cluster of genes involved in virulence, the *Francisella* pathogenicity island (FPI). There are two copies of the FPI in *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* and one copy in *F. tularensis* subsp. *novicida;* although largely identical, the FPI from *F. tularensis* subsp. *tularensis* differs from that in the other subspecies.[64,65] The FPI contains 19 genes required for murine virulence and intracellular growth in macrophages, including *iglABCD* and *pdpABCD.* FPI regulation involves at least six proteins that regulate many FPI genes, and some also regulate non-FPI genes.[66,67] Within macrophage cytosol the organism depends on host amino acids as the gluconeogenic sources for metabolism.[68]

Intracellular growth, iron limitation, and oxidative stress also regulate expression of these genes.[66,67] Disruption of many of these genes has been shown to impair the organism's survival within macrophages and significantly reduces virulence in animal models. Several proteins encoded by the FPI that have been linked to intracellular growth and virulence likely form part of a type VI secretion system.[66,69,70] Many other genes can also be identified that probably play a role in *Francisella* virulence.[71]

Other *Francisella* Species

A number of newly appreciated *Francisella*-like organisms have been identified by molecular testing or by culture.[72] The same novel *Francisella* species was isolated from blood in a patient with pneumonia, and from cerebrospinal fluid in a different patient with meningitis.[73] A survey of soil throughout Houston, Texas, using PCR amplification of DNA extracts identified multiple *Francisella*-like organisms that were distinct from known species of *F. tularensis* and *F. philomiragia.*[74] *Francisella*-like organisms have been identified as a cause of granulomatous diseases in several fish species. They have been cultured, and 16S ribosomal RNA gene sequencing has shown them to be related most closely to *F. philomiragia.* However, they are currently believed to represent new *Francisella* species: *F. noatunensis* comb. nov. and *F. noatunensis* subsp. *orientalis.*[75] *F. halioticida* has caused mass mortality among farmed abalone in Japan.[76]

Several endosymbiotic bacteria of ticks have been classified within the Francisellaceae family on the basis of 16S ribosomal gene sequence data. These include *Wolbachia* (*Francisella*) *persica,* an endosymbiont found in Rocky Mountain wood ticks (termed *Dermacentor andersoni* symbiont), and symbiont B of the soft tick *Ornithodoros moubata.*[21] Similar organisms can be found in other hard and soft ticks, suggesting that they may be more widely distributed than previously observed. A *Francisella*-like organism has been found as an endosymbiont of a *Paramecium* species, and a related organism has been isolated from the waters off Hong Kong.[77,78]

EPIDEMIOLOGY

Distribution

Tularemia is widely distributed, but it is primarily a disease of the Northern Hemisphere, and is most common between 30° and 71° north latitudes. It has been remarkably absent from the United Kingdom, Ireland, and South America. A single South Sudan case of *F. tularensis* bacteremia is the only report to date from Africa.[79] Reports of increased incidence in Europe, including Austria, Norway, Sweden, and Spain, have been ascribed to climate change.[80,81,82,83] Whipp and coworkers reported the first case of tularemia from Australia in 2003, caused by an *F. tularensis* subsp. *novicida*–like organism.[84]

Tularemia was very common in the United States before World War II. However, its incidence has declined steadily since the 1950s and has remained at fewer than 0.15 cases per 100,000 population since 1965.[85,86] Because of its stable and low incidence, tularemia was removed from the list of nationally reportable diseases in 1995 but was added back in 2000 in part because of the concern about its use for bioterrorism. Since 2001, the case rates have been 0.05 per 100,000 population or less.[86] Missouri, Arkansas, Oklahoma, Massachusetts, South Dakota, and Kansas reported 59% of the total US cases from 2001 through 2010, with Dukes County in Massachusetts and Buffalo and Shannon Counties in South Dakota having the highest annual incidence rates (Fig. 227.1).[85] Groups with high incidence rates include Native Americans and Alaskan Natives. Subtle changes have occurred in the geographic distribution of cases in the United States between 1965 and 1999.[87] The southern border of tularemia has shifted northward so that fewer cases have been reported from south central states in recent years. The number of reported cases in Colorado, Nebraska, South Dakota, and Wyoming dramatically increased in 2015, and this is consistent with the predicted effects of climate change on tularemia's geographic distribution.[87,88]

Environmental data also have been used to develop a model to predict risk for tularemia in specific geographic regions.[89] Detailed investigation using molecular typing found that specific *F. tularensis* strains may be geographically limited, with strain variation yielding differences in virulence and disease severity.[39] *F. tularensis* subsp. *tularensis* group A1 was found primarily in the central United States, including the states reporting the highest numbers of tularemia cases, as well as in California. *F. tularensis* subsp. *tularensis* group A2 was found primarily in the western United States at higher elevations than the A1 isolates.[90] The distribution of group A1 isolates correlated with the distribution of *Amblyomma americanum* and *Dermacentor variabilis* ticks and the eastern cottontail rabbit (*Sylvilagus floridanus*).[91] The distribution of group A2 isolates correlated with the distribution of *D. andersoni* and the deer fly *Chrysops discalis* and also with the mountain cottontail rabbit (*Sylvilagus nuttalii*). There were only a few isolates of *F. tularensis* subsp. *novicida,* but most were seen in the southeastern United States.[90] In contrast, isolates of *F. tularensis* subsp. *holarctica* were widely dispersed.[90] Testing of additional US isolates has identified two A1 genotypes, A1a and A1b. Genotype A1b isolates were significantly more likely



FIG. 227.1 Total number of tularemia cases reported in the United States, 2001 to 2010. One dot is placed randomly within county of residence for each reported case. Cases were reported from 47 states. *(From Centers for Disease Control and Prevention. Tularemia—United States, 2001–2010.* MMWR Morb Mortal Wkly Rep. *2013;62:963–966.)*

from invasive infections associated with significantly higher mortality in humans than A1a and A2 isolates.[92]

Geographic restriction of specific *F. tularensis* strains has been increasingly identified as newer molecular typing methods have been more widely applied.[39,93] Animal and human outbreaks within a single region may involve multiple *F. tularensis* types.[94,95]

Cases of *F. tularensis* subsp. *novicida* are uncommon. Almost avirulent in normal human hosts, these infections do not resemble customary tularemia, often are bacteremic, and appear to usually occur in patients with immunosuppression or significant comorbidities.[23,96] *F. tularensis* subsp. *mediasiatica* is among the least studied, found initially only in sparsely populated regions of Central Asia but more recently described in the Altai region of Russia.[25]

Incidence

Historically, the incidence of tularemia in the United States has been most frequent in June through August as well as December. The summer peak corresponds to a higher number of tick-acquired cases, whereas the smaller peak in winter reflects an increased number of hunting-associated cases. However, in recent years the number of reported infections only peaks in the late spring and summer.[97] A review of 316 available *F. tularensis* human isolates from 39 states collected between 1964 and 2004 found that 208 (66%) were *F. tularensis* subsp. *tularensis* and 108 (34%) were *F. tularensis* subsp. *holarctica.*[98] Most isolates of both subspecies occurred between May and September; a very small increase in numbers in December was noted only for *F. tularensis* subsp. *tularensis* and not for *F. tularensis* subsp. *holarctica,* and only *F. tularensis* subsp. *tularensis* was associated with lagomorph exposure.[98] Males account for the majority of cases, perhaps because of greater exposure opportunities, though tularemia can occur in individuals of any age (Fig. 227.2). In the United States, the number of reported cases are highest in children ages 5 to 9 years and in adult men ages 55 to 59 years (see Fig. 227.2).[5,85,86,99] Occupations that have been associated with an increased risk for tularemia are laboratory worker, farmer, landscaper, veterinarian, sheep worker, hunter or trapper, cook, and meat handler.

In Europe, where most cases are from type B strains, the source and clinical presentation vary by country. Ticks and other insect bites predominate, causing ulceroglandular and glandular tularemia.[83] Oropharyngeal tularemia has caused outbreaks in places with unsafe food and water,[100–102] and during wartime, such as in Kosovo.[103] Pneumonic and typhoidal tularemia are uncommon in Europe.[82]

Transmission

F. tularensis is capable of infecting hundreds of different vertebrates and invertebrates, but no more than a dozen mammalian species are important to its ecology in any geographic region. These include lagomorphs, particularly *Sylvilagus* and *Lepus* spp., and rodents such as voles, squirrels, muskrats, and beavers in North America. In Eurasia, infected animals include voles, hamsters, mice, and hares. Transmission of *F. tularensis* to humans occurs most often through the bite of an insect or contact with contaminated animal products. Other routes of transmission include aerosol droplets, contact with contaminated water or mud, and animal bites. Inoculation from freshwater fishhook injury has been described.[104] Illness may occur in families or friends because of shared activities and exposures. Nonetheless, human-to-human spread does not occur.

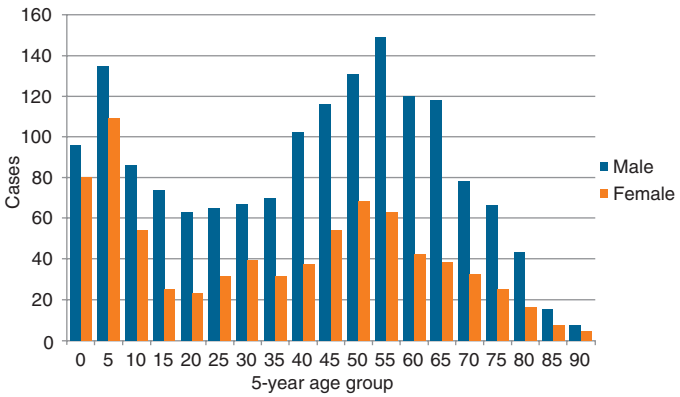

FIG. 227.2 Tularemia cases by age group and sex—United States, 2001 to 2016. Tularemia was more common in males, and occurred in persons of all ages but was most common in children. *(From Centers for Disease Control and Prevention. Tularemia: age and sex of reported cases, United States—2001–2016.* https://www.cdc.gov/tularemia/statistics/. *Accessed September 20, 2018.)*

Blood-feeding arthropods and flies are the most important vectors for tularemia in the United States. Ticks predominate in the Rocky Mountain states and eastward, whereas biting flies predominate in California, Nevada, and Utah. However, an increase in human tularemia noted in Wyoming between 2001 and 2003 was linked most often to transmission by biting flies and was associated with a simultaneous outbreak of tularemia in rabbits.[105] In contrast, mosquitoes are the most frequent insect vector in Sweden and Finland, and they are also important in the former Soviet Union. At least 13 species of ticks have been found to be naturally infected with *F. tularensis,* and transovarial passage may occur with some but not all vectors.[106] The dog tick (*D. variabilis*), wood tick (*D. andersoni*), and Lone Star tick (*A. americanum*) are commonly involved in North America. The organism may be present in tick saliva or feces inoculated either directly or indirectly into the bite wound. Tick transmission traditionally has been associated with *F. tularensis* subsp. *tularensis.* Several outbreaks of tick-borne tularemia have involved *F. tularensis* subsp. *holarctica,* although this organism is more often linked to water, rodents, and aquatic animals. Tularemia in children in the United States is now most often acquired through summertime tick exposures.

Animal contact is another important mode of acquiring tularemia. Skinning, dressing, and eating infected, undercooked animals, including rabbits, muskrats, beavers, squirrels, and birds, has transmitted tularemia, occasionally resulting in large outbreaks in hunters. Wild animals sold as pets are also potential vectors, as occurred in 2002 when infected prairie dogs were widely commercially distributed.[107] The first outbreak in Spain was associated with processing hare carcasses and hare meat, and was notable for a preponderance in women.[108] Airborne transmission has occurred from these activities, as well as from contact with water, contaminated dust, and hay.

An outbreak in 2000 of pneumonic tularemia on Martha's Vineyard was associated with mowing lawns and using a brush cutter, likely aerosolizing infected animal carcasses. Since then, cases of pneumonic tularemia have been regularly encountered on Martha's Vineyard during most summers.[109,110] An alternative explanation for pneumonic cases may rest with the finding of large numbers of *F. tularensis* bacteria within infected ticks, which may suggest that aerosolized ticks rather than animals may also contribute to cases.[91] In a highly endemic area, serologic evidence of *F. tularensis* infection among potential animal reservoirs on Martha's Vineyard found positive assays most frequently in raccoons (52.4%) and skunks (49.2%), although active infection in skunk and raccoon samples were not found by culture or PCR.[111] Domestic cats and other carnivorous animals may carry *F. tularensis* in the mouth or on claws after killing or feeding on infected prey, whether or not they are infected.[112] This is thought to be the mechanism by which occasionally domestic cats, and in one case a buzzard, transmit tularemia.[112,113] Similarly, a coyote bite caused a case of ulceroglandular tularemia in a child, and an infected dog was believed responsible for a case of pneumonic tularemia in Arizona.[114,115]

F. tularensis may survive for prolonged periods in water, mud, and animal carcasses even if frozen; however, cooking game meats thoroughly to the proper temperatures should minimize risk from ingestion. Nonetheless, contaminated food and water continue to be important environmental sources of tularemia. An outbreak of disease secondary to *F. novicida,* linked to contaminated ice, has been reported from a correctional institution in Louisiana.[96] Fresh crushed grapes were linked to an outbreak of oropharyngeal tularemia in Germany, probably contaminated with an infected rodent collected by a mechanical harvester.[116]

Disruptions of infrastructure caused by wars and natural disasters may also be significant contributing factors. In postwar Kosovo, an

epizootic increased rodent population contaminated ransacked homes and food with *F. tularensis* that led to foodborne and waterborne outbreaks among refugees returning to disrupted housing and sanitation.[117,118] Although tularemia was not reported in Kosovo before these outbreaks, it has remained endemic since.[117] The devastating effects of an earthquake in Turkey are believed to have resulted in water contamination and subsequent outbreaks of tularemia in the region.[119] During several outbreaks in Turkey, transmission from unchlorinated drinking water was confirmed using whole-genome sequencing.[120]

It has been demonstrated that *F. tularensis* subsp. *tularensis, F. tularensis* subsp. *novicida,* the live vaccine strain, and *F. philomiragia* will multiply intracellularly in *Acanthamoeba castellanii* and infect amebal cysts; *F. tularensis* subsp. *tularensis* is capable of surviving in amebal cysts for up to 21 days.[29,121,122] Such a relationship may prove relevant to natural aquatic reservoirs for *Francisella.*

PATHOGENESIS

F. tularensis is a virulent organism for susceptible species, including the accidental human host. Although the organism is reported to penetrate intact skin, most investigators believe that penetration occurs through sites of skin disruption that may be inapparent if without known cause. Organisms may remain viable in carcasses or dust for up to 136 days, and culturable organisms have remained within infected frozen rabbit meat for more than 3 years.[123] The infectious dose in humans depends on the portal of entry: 10 to 50 organisms are sufficient when injected intradermally or when inhaled, though 10^6 to 10^8 organisms are required when ingested.[123] That low numbers of bacteria can cause infection through the skin, mucous membranes, and airways helps to explain in part the extreme risk that *F. tularensis* poses to laboratory workers. In general, *F. tularensis* subsp. *tularensis* causes more severe disease than either *F. tularensis* subsp. *holarctica* or *F. tularensis* subsp. *novicida.* The specific molecular reasons underlying these differences in virulence are unclear.

During the first 3 to 5 days after cutaneous inoculation, *F. tularensis* multiplies locally and produces a papule; ulceration may begin 2 to 4 days later.[4] Organisms spread from the site of entry to regional lymph nodes and may disseminate via a lymphohematogenous route to involve multiple organs. Bacteremia is probably common in this early phase, although it is only occasionally detected. Changes in draining regional lymph nodes appear after skin lesions develop.[4] An elegant description of the pathogenesis is offered by Geyer and colleagues.[124] Infection with *F. tularensis* is characterized by an acute inflammatory response that involves fibrin, neutrophils, macrophages, and T lymphocytes. Neutrophils and macrophages surround earlier inflammatory cells, stimulated by the initial inoculum, that haves become necrotic and degenerated. Eventually, lymphocytes, epithelioid cells, and giant cells migrate into the necrotic tissue. This extensive necrosis is noted in both lung tissue and lymph nodes. As the necrotic tissue expands, adjacent veins and arteries may thrombose. The organisms are usually present at the site of the necrotic tissue but are difficult to demonstrate on routine stains. Silver impregnation techniques (Steiner, Dieterle, and Warthin-Starry silver stains) enhance the visibility of the organisms, usually found in macrophages and epithelioid cells. Granulomas develop that occasionally may caseate; for this reason, specimens may be mistaken for tuberculosis. These changes can occur in any infected site and have been found at autopsy in lung, liver, spleen, lymph nodes, and bone marrow. Coalescence of necrotic foci may yield abscess formation. *F. tularensis* may remain viable in tissues for prolonged periods. The organism's intracellular residence within the liver and other sites may help to protect it from host defenses and permit early growth. *F. tularensis* is contained within granulomas in the livers of mice infected with the vaccine strain, and granuloma formation involves hepatic natural killer cells, interferon-γ (IFN-γ) production, and expression of inducible nitric oxide synthase.[125]

Most organisms recovered from blood in a mouse model of *F. tularensis* infection were free in plasma and not in leukocytes.[126] The organisms grew well in whole blood but not in plasma, implying a requirement for host cells. These observations suggest that circulating *F. tularensis* may be taken up by leukocytes where they survive and are protected from innate humoral defenses and potentially antibiotic effects, then escape into the plasma where they can begin a cycle of reinfection. *F. tularensis* also can infect erythrocytes and persist within them, where they are protected from killing by gentamicin; this has been proposed as one reason why tularemia may relapse.[127] Intraerythrocytic *F. tularensis* also are protected from the acidic pH within the tick gut, and this enhances tick colonization.[128]

F. tularensis is a facultative intracellular pathogen that is capable of growing within several different cell types, including macrophages, dendritic cells, hepatocytes, alveolar epithelial cells, and endothelial cells.[129,130] However, macrophages are the primary site of survival and replication. Entry into macrophages occurs through a unique mechanism involving engulfment by relatively capacious and asymmetrical pseudopod loops.[129] Direct transfer from infected cells to uninfected macrophages during a contact-dependent exchange of cytosolic material associated with trogocytosis also is possible.[131] Complement receptors, mannose receptors, class A scavenger receptors, Fcγ receptors, and surface nucleolin may be utilized for *F. tularensis* uptake, and the specific receptor pathway used may affect early suppression of innate defenses and intracellular trafficking of the organism.[129,132,133] Inside the macrophage, virulent *F. tularensis* strains impair maturation of the endosome, thus avoiding phagosome-lysosome fusion. Phagosomes containing virulent *F. tularensis* are transiently acidified by the vacuolar adenosine triphosphatase pump, and organisms quickly escape into the cytosol. Bacteria proliferate in the cytoplasm, induce apoptotic cell death, and are released to further spread the infection.[134]

Immunity

Humoral immunity, directed against carbohydrate antigens, develops between the second and third weeks after infection, with the almost simultaneous appearance of IgM, immunoglobulin G (IgG), and immunoglobulin A (IgA) agglutinating antibodies. However, antibodies alone are insufficient to protect against virulent *F. tularensis* infection.[135] Opsonizing IgG and IgM antibodies are also produced, with the most efficient opsonization involving both immune serum and complement. *F. tularensis* binds activated complement C3 components to its bacterial surface, although LPS and cell wall prevent complement-mediated lysis of cells.[136] Nonetheless, the oxygen-dependent neutrophil killing of virulent wild-type strains is poor. Human neutrophils phagocytose opsonized *F. tularensis* organisms but do not kill them, as they escape into the neutrophil cytoplasm. Survival of *F. tularensis* within neutrophils is associated with inhibition of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by multiple mechanisms, failure of the respiratory burst, and impaired neutrophil responsiveness to other activating stimuli.[137] Neutrophil apoptosis is inhibited by *F. tularensis,* and survival of infected neutrophils is prolonged.[138]

Complete recovery from tularemia requires cell-mediated immunity, demonstrable approximately 1 week earlier than antibody responses and directed against protein antigens. This cell-mediated immunity is αβ T-cell dependent but may involve either $CD4^+$ or $CD8^+$ T cells. Attempts are underway to define better the critical molecular determinants that induce protective immunity. Current understanding of tularemia immunopathogenesis has largely derived from mice infected with *F. tularensis* subsp. *novicida* or other strains less virulent for humans. It is unknown how this relates to human tularemia caused by more virulent organisms. Furthermore, different routes of infection and different mouse strains may elicit dissimilar murine immune responses.[139] IFN-γ and tumor necrosis factor-α (TNF-α) activate macrophages to kill *F. tularensis* through the production of nitric oxide and other reactive nitrogen products, although alveolar macrophages may use other mechanisms to inhibit the organism.[140] Murine and human IFN-γ–activated macrophages also may inhibit growth of the virulent type A strain Schu S4 by mechanisms independent of reactive nitrogen and oxygen species.[141]

Several mechanisms are involved in the innate response that controls infection before the development of conventional cellular immunity. Early host defense against *F. tularensis* infection involves neutrophils, dendritic cells, macrophages, mast cells, TNF-α, IFN-γ, and interleukin-12, but these are not sufficient to resolve the infection. Although neutrophils are less important as a primary defense in the lung than in systemic murine infection, neutrophils recruited to the lung may contribute to lung damage.[142] Activation of natural killer T

cells in a murine model of pulmonary tularemia also contributes to the resultant inflammation and exacerbates disease.[143] The initial response to *F. tularensis* infection is dependent on membrane-associated TLR2 recognition, particularly in the lung, and cytosolic nucleotide oligomerization domain–like receptors.[139] In the mouse lung, virulent *F. tularensis* strains are capable of suppressing the expected early immune response.[144,145] TLR4 recognition of *F. tularensis* LPS is poor; interestingly, the resistance of mice to pulmonary infection with *F. tularensis* subsp. *novicida* is enhanced by pretreatment with a TLR4 agonist.[146]

For complete resolution of systemic infection, it is necessary that $\alpha\beta^+$ T cells be functional and present after the initial defenses provided by the macrophages, dendritic cells, cytokines, and neutrophils. $CD4^+$ and $CD8^+$ cells each contribute to the survival of mice infected with the vaccine strain or the virulent Schu S4 strain, and both are required to clear the infection fully.[140,147] The contribution of $\alpha\beta^+$ T cells involves TNF-α and IFN-γ production and is dependent on interleukin-12.[139] Also, it has been appreciated that both humoral and cellular immune mechanisms act together to achieve protective immunity.[148,149] Natural infection or vaccination in humans results in long-lasting memory $CD4^+$ and $CD8^+$ cells.[150] In mice, B cells contribute to protection against the attenuated and virulent strains that are not dependent on antibodies, but it is unknown if B cells play a similar role in human infection.[140,147]

F. tularensis is capable of suppressing or avoiding many aspects of humoral and cellular host defenses (see "Virulence" earlier).[151] It survives extracellularly in part because it is capable of avoiding complement-mediated lysis. It survives intracellularly by multiple mechanisms, including prevention of phagosome-lysosome fusion, escape from the phagosome, suppression of the inflammasome, alternative activation of macrophages, avoiding killing by cellular antimicrobial peptides, destabilization of host cell messenger RNA, impairment of proinflammatory cytokine production, inhibition of NADPH oxidase in neutrophils, and the induction of prostaglandin E_2 secretion, which can inhibit IFN-γ production.[137,151,152–156] It also is capable of inhibiting IFN-γ signaling and production. For example, impaired clearance of *F. tularensis* live vaccine strain from mice with pulmonary infection may result from increased local prostaglandin E_2 secretion and decreased numbers of IFN-γ–secreting T cells. Immunization of mice with *F. tularensis* may protect against subsequent challenge but not prevent persistent, low-level organ infection, and the essential determinants of sterilizing immunity remain unknown.[147]

Expansion of circulating $\gamma\delta$ T cells has been documented in patients with acute tularemia. These cells respond to phosphoantigens from many different pathogens, including *F. tularensis*. The observed increase in the levels of $V\gamma9V\delta2$ cells occurs after the first week of illness and may persist for longer than 1 year after infection.[157] However, 10 to 30 years after natural infection, long-lived memory cells responsive to *F. tularensis* heat shock proteins are $\alpha\beta$ T cells and not $\gamma\delta$ T cells.[158] Similarly, lymphocyte responsiveness to the live vaccine strain persists for up to 34 years after vaccination, and IFN-γ expression involves both $CD4^+$ and $CD8^+$ cell populations.[150]

CLINICAL MANIFESTATIONS

The clinical consequences of *F. tularensis* infection depend on the virulence of the particular organism, the portal of entry, the extent of systemic involvement, and the immune status of the host. The result can range from asymptomatic or inconsequential illness to acute sepsis and rapid death. Patients who seek medical attention usually present with at least one of six classic forms of tularemia: ulceroglandular, glandular, oculoglandular, pharyngeal, typhoidal, and pneumonic. This somewhat artificial classification emphasizes only the predominant manifestations commonly encountered, and overlapping forms may be present in many patients.

The incubation period averages 3 to 5 days, but it ranges from 1 to 21 days. Tularemia usually starts abruptly, with the onset of fever that can range to 104°F, chills, headache, malaise, anorexia, and fatigue. Other prominent symptoms may include cough, myalgias, chest discomfort, vomiting, sore throat, abdominal pain, and diarrhea. A pulse-temperature deficit was noted in up to 42% of evaluable patients in the United States, although this was found in only 5% of patients infected with *F. tularensis* subsp. *holarctica* infection in Sweden.[159,160] Fever (usually >38.3°C [101°F]) classically lasts for several days, remits for a short interval, and then recurs along with other symptoms. Without treatment, fever lasts an average of 32 days while chronic debility, weight loss, and lymphadenopathy may persist for many months longer.[161] Less virulent strains cause a milder, self-limited illness that may resolve without therapy. Systemic symptoms may abate by the time medical help is sought so that the clinical picture is dominated by one or more of the six patterns listed; this may lead to confusion regarding the correct diagnosis, particularly in the 25% to 50% of patients without an evident source of infection.

Ulceroglandular and Glandular Tularemia

Ulceroglandular tularemia has presented in the majority of cases, including in recent years those in Colorado, Nebraska, South Dakota, and Wyoming.[88] Tick bites and animal contacts are the usually recalled exposures. This form is most quickly recognized as tularemia compared to others. The initial specific complaint is often of enlarged and tender localized lymphadenopathy (Fig. 227.3). The inciting skin lesion may appear before, simultaneously with, or from one to several days after the adenopathy. It starts as a red, painful papule in a region draining into the involved lymph nodes. Vesicles also may be seen, and these may be mistaken for herpes simplex or varicella infection.[162] The papule then undergoes necrosis, leaving a tender ulcer with a raised border (see Fig. 227.3). If untreated, the ulcer may take weeks to heal and leave a residual scar. Multiple lesions may occur, particularly in those cases with animal sources.[160]

The location of the ulcer usually reflects the mode of acquisition; animal contacts tend to yield ulcers on the hands and forearms, while tick bites cause ulcers on the trunk, the perineum, the lower extremities, and the head and neck (see Fig. 227.3). The distribution of lymphadenopathy also reflects the exposure history, as illustrated in Fig. 227.4. Overall, cervical and occipital adenopathy are most common in children and inguinal adenopathy is most common in adults. Skin changes over the involved nodes occurred in 19.1% of patients in a series from Sweden,[159] which suggests underlying suppuration. Some patients have a sporotrichoid presentation with ascending subcutaneous nodules (see Fig. 227.3). Lymphangitis is rare unless there is another bacterial superinfection of the ulcer.

Glandular tularemia occurs when patients present with tender regional lymphadenopathy but without a visible cutaneous lesion. This form has traditionally accounted for one-fifth or fewer of cases in the United States. However, glandular tularemia was the most common primary clinical form in children (44%) and the second most common presentation overall among classifiable cases reported in Missouri between 2000 and 2007.[163] Glandular tularemia represents essentially the same process as ulceroglandular disease, except that a skin lesion either healed before presentation or was minimal, atypical, or overlooked.

Enlarged lymph nodes may persist for prolonged periods. In some patients, an exposure or prior febrile illness will be forgotten. For this reason, tularemia may not be considered in the initial differential diagnosis of some patients whose primary presentation is lymphadenopathy. In either ulceroglandular or glandular tularemia, the lymph nodes may suppurate (see Fig. 227.3). More than 20% will suppurate if left untreated or if treatment is delayed longer than 2 weeks.[3,164] When fluctuant, they should be needle aspirated or surgically drained. The differential diagnosis of ulceroglandular and glandular tularemia includes pyogenic bacterial infections, cat-scratch disease, syphilis, chancroid, lymphogranuloma venereum, tuberculosis, nontuberculous mycobacterial infection, toxoplasmosis, sporotrichosis, *Spirillum minus* rat-bite fever, anthrax, plague, and herpes simplex virus infection.

Oculoglandular Tularemia

Oculoglandular tularemia represents only a minority of cases. In this form, organisms have gained entry through the conjunctiva, from contaminated fingers, splashes, or aerosols. The disease may be bilateral, but this is uncommon. Early complaints may include photophobia and excessive lacrimation. Examination shows lid edema and painful conjunctivitis, with injection, chemosis, and small, yellowish conjunctival ulcers or papules in some patients. Associated tender lymphadenopathy

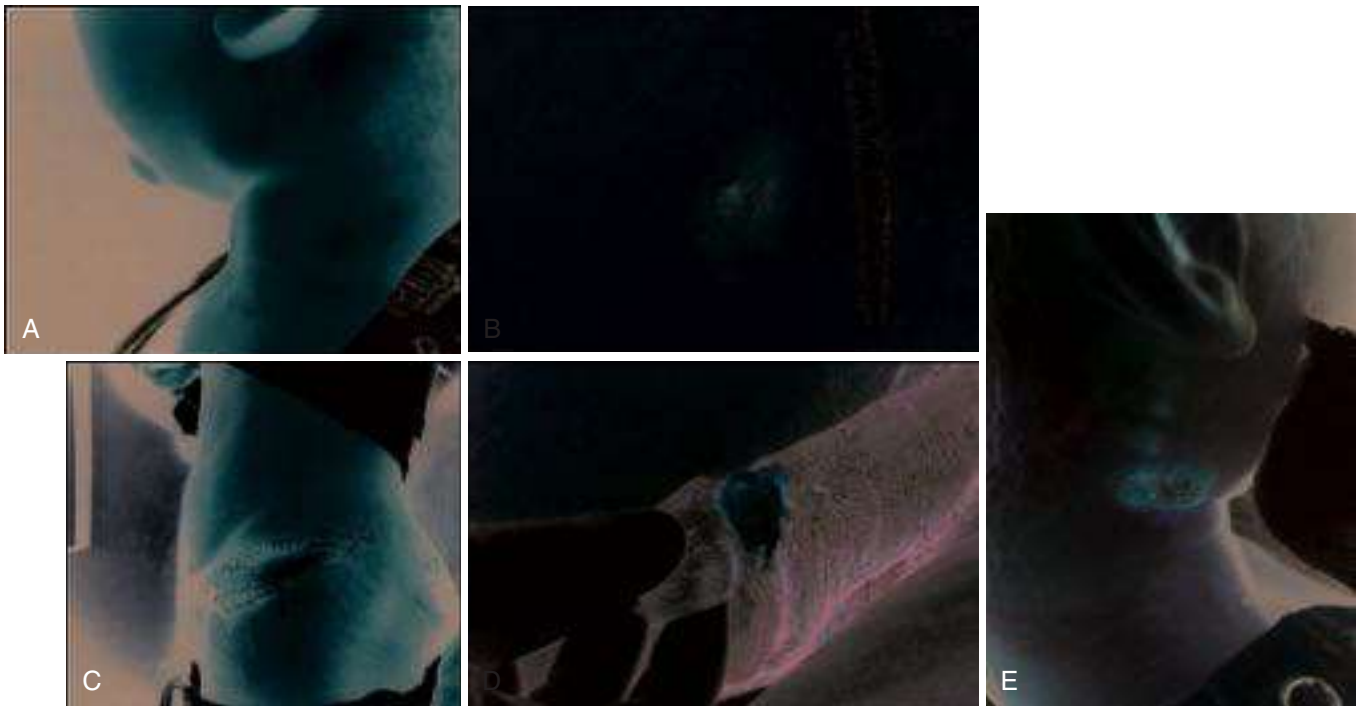

FIG. 227.3 Examples of primary lesions seen in ulceroglandular tularemia. (A) Large cervical and submandibular lymph nodes in a young child; an ulcer was found under the hairline on her forehead at the site of a tick bite. (B) Papule undergoing central necrosis with desquamation on the thigh of a middle-aged man. (C) Inguinal adenopathy and suppurative mass in a young hunter who had carried a dead hare at his side. (D) Penile ulcer that was suspected of being syphilis or another sexually transmitted disease until the history of a recent tick bite was obtained by the infectious diseases consultant. (E) Cervical ulcer and nodular adenopathy with a sporotrichoid appearance in a 6-year-old girl who had a tick removed from the area of the ulcer 2 weeks before presentation. The nodes coalesced, suppurated, and required drainage after 3 days of gentamicin therapy. Cultures of the ulcer and node drainage both grew *F. tularensis. (A and C, Courtesy Dr. Joseph A. Bocchini, Louisiana State University Health Sciences Center, Shreveport, LA. D, Courtesy Dr. John W. King, Louisiana State University Health Sciences Center, Shreveport, LA. E, Courtesy Dr. Robin Trotman, CoxHealth Infectious Diseases Specialty Clinic, Springfield, MO.)*

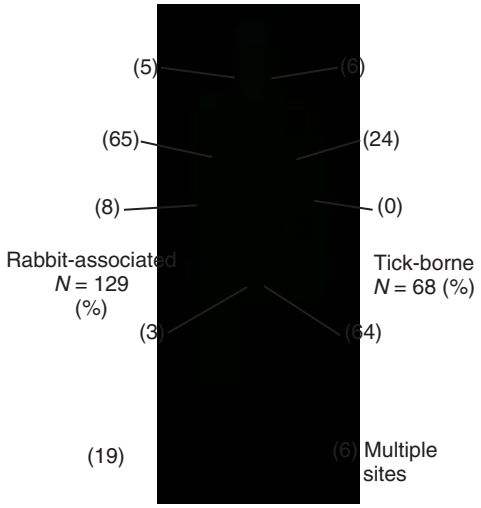

FIG. 227.4 Distribution of lymphadenopathy in rabbit-associated and tick-borne tularemia.

may occur in the preauricular, postauricular, submandibular, and cervical regions. If the adenopathy is extensive and more prominent than the eye findings, then this syndrome may be mistaken for mumps.[160]

Visual loss is rare, but complications include corneal ulceration, dacryocystitis, and nodal suppuration. Other ocular manifestations of tularemia also have been described, including cases of uveitis.[165,166] Tularemia may be an overlooked cause of Parinaud oculoglandular syndrome (conjunctivitis with ipsilateral preauricular adenopathy).[167] The differential diagnosis of oculoglandular tularemia includes pyogenic bacterial infections, adenoviral infection, syphilis, cat-scratch disease, and herpes simplex virus infection.

Pharyngeal Tularemia

Pharyngeal tularemia, another variant of ulceroglandular disease, is the result of primary invasion through the oropharynx. The source may be contaminated food, water, or droplets. This form represents few cases overall in the United States, though it has been increasingly described in other countries with outbreaks.[168–170] In a large retrospective series from Turkey, pharyngeal tularemia accounted for 85% of cases.[168] Children appear to acquire this form more often than adults, and several family members may be affected simultaneously, especially if drinking from contaminated wells or other unchlorinated water sources.[102,168]

This form must be distinguished from a sore throat that may accompany any of the other major clinical forms of tularemia. In pharyngeal tularemia, the predominant complaints are fever, severe throat pain, and a neck mass representing lymphadenopathy. Exudative pharyngitis or tonsillitis is the rule, and one or more ulcers may be seen. A pharyngeal membrane has been described in some patients that is similar to a diphtheritic membrane.[161] Cervical, pre-parotid, and retropharyngeal adenopathy may be present, occasionally with bilateral involvement or abscess formation. When there is a delay in seeking care, the dominant manifestation may be cervical adenopathy without prominent fever or pharyngotonsillitis.

The differential diagnosis includes streptococcal pharyngitis, infectious mononucleosis, adenoviral infection, diphtheria, and tuberculosis; the latter especially may be confused with tularemia when granulomatous lymphadenitis is identified on biopsy.[171] Tularemia should be suspected in an endemic area whenever a severe sore throat is unresponsive to penicillin therapy and routine diagnostic tests have been unrewarding.

Typhoidal Tularemia

Typhoidal tularemia refers to a febrile illness caused by *F. tularensis* unassociated with prominent lymphadenopathy and not fitting into any of the other primary forms. Typhoidal disease was among the most common forms recently reported in Arkansas, and is also frequently identified in states with increasing frequency of tularemia, including Colorado, Nebraska, South Dakota, and Wyoming.[88,172] The typhoidal form may result from any mode of acquisition, and it is the most difficult to diagnose. Because the portal of entry is usually inapparent clinically, a history of outdoor activities with tick, insect, or animal exposure should be pursued. Many patients have underlying severe chronic medical disorders and their presentation can be dramatic, with acute prostration and rapid death, or protracted illness.

Prominent symptoms of typhoidal tularemia may include any combination of fever with chills, headache, myalgia, sore throat, anorexia, nausea, vomiting, diarrhea, abdominal pain, and cough. The examination may reveal dehydration, hypotension, mild pharyngitis and cervical adenopathy, meningismus, and diffuse abdominal tenderness. Hepatomegaly and splenomegaly are found uncommonly in the acute stages and become more likely the longer the duration of illness. Severe disease may cause jaundice produced by cholestasis. Diarrhea is a major manifestation only in typhoidal tularemia. Bowel movements are loose and watery but only rarely bloody. Children may have more severe intestinal involvement, including focal areas of bowel necrosis.[161] Rare gastrointestinal manifestations include cholangitis, granulomatous hepatitis, and liver abscess.

Secondary pleuropulmonary involvement is fairly frequent in this form. Pulmonary infiltrates, pleural effusions, or even pulmonary nodules are described in up to 45% of subacute to chronic typhoidal cases[173,174,175]; it is even more frequent in laboratory-acquired infections. Additional findings in severely ill patients may include hyponatremia, elevated creatine phosphokinase level, myoglobinuria, pyuria, renal failure, and positive blood cultures. The differential diagnosis of typhoidal tularemia would be extensive and includes typhoid fever caused by *Salmonella* spp., brucellosis, *Legionella* infection, Q fever, disseminated mycobacterial or fungal infection, rickettsioses, malaria, endocarditis, and any other cause of prolonged fever without localizing signs.

Pneumonic Tularemia

Pneumonic tularemia refers to an illness whose initial presentation is dominated by pulmonary infection. Pneumonic tularemia was the primary clinical presentation in 39% of adults and 24% of patients overall among classifiable cases reported in Missouri between 2000 and 2007, and as common as ulceroglandular disease in Colorado, Nebraska, South Dakota, and Wyoming during 2015 when the number of reported tularemia cases in these states significantly increased.[88,163] It may result from direct inhalation of the organism or secondary hematogenous spread to the lung.

Primary pneumonic tularemia is a risk for certain occupations, including sheep shearers, farmers, landscapers, and laboratory workers. Five cases of tularemic pneumonia in Martha's Vineyard were attributed to lawn mowing and brush cutting, possibly thereby aerosolizing infected carcasses, excreta, or ticks.[176] Cases also have been described as resulting from common exposure in a more casual setting, such as a converted mill building for holiday stays.[177] This case was linked to infected dog dander exposure, as was a recent case in a patient with sarcoidosis who had no other likely exposure.[115] Pneumonia with *F. tularensis* subsp. *holarctica* bacteremia also has been described after freshwater near drowning.[178] Secondary pneumonia may occur early or after a delay of weeks to months in the course of tularemia.[3] Although secondary pneumonia may complicate any of the syndromes already discussed, Evans and colleagues[160] found pneumonia to be most frequent in typhoidal (83%) and ulceroglandular (31%) diseases. Scofield and associates[179] reported that patients with pneumonic involvement were more likely to be older, recall no exposure risk, present with typhoidal illness, have positive cultures, stay hospitalized longer, and have a higher mortality rate. From 25% to 30% of patients have infiltrates on radiographic examination without any clinical findings of pneumonia.[160] A series of 58 patients with pulmonary tularemia in Finland found 47% lacking respiratory symptoms and 7% having a normal chest radiograph, though variable findings of chest computed tomography secured a pneumonic designation.[180] Pneumonia from *F. tularensis* subsp. *tularensis* is typically a more severe disease than that caused by *F. tularensis* subsp. *holarctica,* but pneumonia caused by *F. tularensis* subsp. *holarctica* may be severe in immunocompromised patients.[181] The illness may be prolonged with either subspecies.

Common symptoms include fever, cough, no or minimal sputum production, substernal tightness, and pleuritic chest pain.[109] Hemoptysis may occur but is uncommon. Physical examination may be nonspecific or may reveal rales, consolidation, and a friction rub or signs of effusion. Some patients need mechanical ventilation, and adult respiratory distress syndrome may complicate the course of any form of tularemia. Routine examination of sputum does not help to suggest the diagnosis. However, a false-positive DFA stain for *Legionella* on bronchoscopy specimens has been reported and may be easily confused with *Legionella* pneumonia.[182,183] Infected pleural fluid is exudative and negative on Gram stain, and usually contains more than 1000 leukocytes/mm^3; cells are predominantly lymphocytes, but neutrophilic effusions may occur. Pleural effusions seen with tularemia frequently mimic those of tuberculosis. Similar findings for both include a lymphocyte-rich exudative pleural effusion and a high adenosine deaminase concentration.[184] Granulomas may be found on pleural biopsy, prompting understandable confusion with tuberculosis.

Acute radiographic changes may include subsegmental or lobar infiltrates (Fig. 227.5), hilar adenopathy, pleural effusion, and apical or miliary infiltrates; less common changes include ovoid densities, cavitation, and bronchopleural fistula. However, in some patients the initial chest radiographs are normal. Secondary pneumonias are more likely to involve the lower lobes and be bilateral, perhaps because of their hematogenous origin. Healing usually occurs without residual changes, but fibrosis and calcifications may result. Therefore tularemia may manifest as enigmatic community-acquired pneumonia that does not respond to routine therapies. The differential diagnosis of pneumonic tularemia includes *Mycoplasma* pneumonia, *Legionella* infection, *Chlamydia pneumoniae* infection, Q fever, psittacosis, tuberculosis, the deep mycoses, and many other causes of atypical or chronic pneumonias.

Patients in four consecutive cases of pneumonic tularemia reported from Switzerland presented with fever of 1 to 8 weeks' duration and were found to have one or more dense nodular pulmonary infiltrates suspicious of cancer and hilar or mediastinal lymphadenopathy.[173] ^{18}F-Fluorodeoxyglucose–positron emission tomography/computed tomography scans were highly positive in all four patients. Diagnosis was suspected by finding necrotizing granulomas in the nodes by using

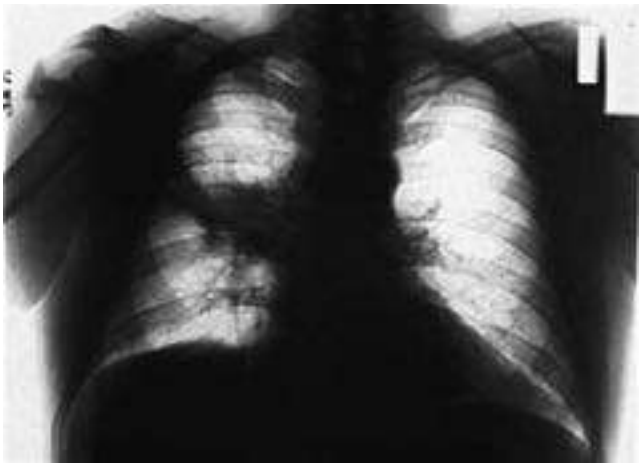

FIG. 227.5 Chest radiograph of untreated tularemia pneumonia. This patient remained symptomatic for more than 3 months. The diagnosis was established serologically when poorly developed granulomas were found in a transbronchial biopsy specimen, other causes were excluded, and the exposure history was finally obtained. *(From Penn RL, Kinasewitz GT. Factors associated with a poor outcome in tularemia.* Arch Intern Med. *1987;147:265–268.)*

transbronchial needle aspiration or thoracoscopy. Node tissue was PCR positive for *F. tularensis* in three patients; subspecies were not determined. Tularemia agglutination titers were positive in all cases, with titers of 1:320 to 1:5120. Three patients responded to ciprofloxacin. One relapsed after doxycycline and responded to gentamicin plus ciprofloxacin. A similar patient from France also responded to ciprofloxacin.[185]

Secondary Skin Manifestations

Primary skin rashes due to tularemia have been described, including on the face and elsewhere.[186] Secondary rashes are an underappreciated part of tularemia and may be found in up to 52% of cases, especially with oropharyngeal presentations.[159,187,188,189] They usually appear within the first 2 weeks of symptoms, but in a minority of cases they are delayed. The rash is more common in women than in men. Cutaneous changes may include diffuse maculopapular and vesiculopapular eruptions, pustules, erythema nodosum, erythema multiforme, acneiform lesions, and urticarial and vasculitis-like eruptions.[189] Sweet syndrome has also been reported in association with tularemia.[190] Although any secondary rash may be part of any form of tularemia, erythema nodosum has been found to occur most commonly with pneumonic tularemia, while erythema multiforme was described in 11.3% of patients in a large Turkish series, most of whom presented with oropharyngeal or glandular forms.[191]

Francisella tularensis subsp. *novicida* and *Francisella philomiragia* Infections

The clinical manifestations of infections caused by *F. tularensis* subsp. *novicida* are less well characterized than for the other subspecies. Heightened surveillance for *F. tularensis* and the availability of molecular methods for subspecies identification have led to several recent reports of *F. tularensis* subsp. *novicida* involvement in human disease. A *F. tularensis* subsp. *novicida*–like organism was isolated from an adult with a toe abscess and regional adenopathy that complicated a cut sustained in brackish water in Australia, and *F. tularensis* subsp. *novicida* bacteremia was documented in a patient from Thailand with terminal ovarian cancer.[84,192] Brett and coworkers[193] identified *F. tularensis* subsp. *novicida* bacteremia in a patient who nearly drowned while body surfing along the South Carolina coast.[193] An outbreak of three symptomatic cases of *F. tularensis* subsp. *novicida* bacteremia occurred in a Louisiana prison, and one patient died.[96] *F. tularensis* subsp. *novicida* was isolated from one of the facility ice machines, believed to be the most likely vehicle for its transmission. These worldwide reports and the molecular identification of older *F. tularensis* isolates as *F. tularensis* subsp. *novicida* after being previously classified as other subspecies suggest that infections caused by *F. tularensis* subsp. *novicida* may be underappreciated.

F. philomiragia infection has caused a skin vesicle, pneumonia, empyema, sepsis, peritonitis, splenic microabscesses, and meningitis. This organism predominantly infects patients with host defenses impaired by chronic granulomatous disease, near drowning in salt water or estuaries, myeloproliferative disorders, or renal transplantation.[194,195,196] Only a small number of cases have been documented, more from North America than Europe, and the organism has been found in tissues, blood, cerebrospinal fluid, and other body fluids.

Clinical Recognition of a Bioterrorism Event

The 2001 Tularemia Consensus Statement developed by the Working Group on Civilian Biodefense concluded that aerosolization would be the most likely method for dispersing *F. tularensis,* because inhalation of bacilli would affect the highest number of people with the most devastating manifestations of the disease.[197] Children are theorized to be more vulnerable than adults to an aerosolized agent because of their higher respiratory rate, more permeable skin, and higher skin-to-mass ratio.[198] Secondary cases may also arise following contact with contaminated environmental sources or animals.[199]

After an aerosol release and an incubation period of 3 to 5 days, patients may present with the acute onset of fever (38°–40°C [100.4°–104°F]), malaise, headache, rigors, coryza, and sore throat. The subsequent clinical syndrome depends on the immune status of the host, the inhaled inoculum, and the virulence of the released agent. It is anticipated that most patients would develop either primary pneumonic or typhoidal forms.[197] A significant number of patients may develop respiratory failure or have signs and symptoms heralding severe sepsis. Because airborne organisms also may invade through extrapulmonary sites and food and water may be contaminated, less common presentations could include oculoglandular, pharyngeal, ulceroglandular, or glandular disease.[200] Rapid recognition and reporting of a possible bioterrorist event due to tularemia is a difficult clinical challenge. It should be suggested by clustered cases of pneumonic or typhoidal disease, particularly in urban areas in patients without the usual exposure history.

COMPLICATIONS AND OUTCOME

Suppuration of involved lymph nodes is currently the most common complication of tularemia (see Fig. 227.3), and this may occur even after directed antibiotic therapy. Among the tularemia patients from Missouri with lymphadenopathy reported between 2000 and 2007, 19% required drainage of suppurative nodes. Lymph node suppuration was associated with a longer delay in starting effective antibiotic therapy.[164] Nodes that suppurate after appropriate therapy are often sterile but benefit from drainage.[201] Patients with severe disease may manifest disseminated intravascular coagulation, renal failure, rhabdomyolysis, jaundice, and hepatitis. Meningitis, encephalitis, pericarditis, peritonitis, osteomyelitis, splenic rupture, and thrombophlebitis have become very rare since antibiotic therapy has become available. Guillain-Barré syndrome rarely has been described complicating cases of ulceroglandular tularemia.[202]

Rare cases of otitis media,[203] otomastoiditis,[204] endocarditis,[205,206] aortitis,[207] prosthetic joint infection with and without bacteremia,[208,209] and peritonitis[210] caused by *F. tularensis* have been reported. Endocarditis complicated typhoidal disease in all four patients reported in a recent series.[206] Meningitis occurs rarely with ulceroglandular and typhoidal disease, and the cerebrospinal fluid in these patients almost always shows a mononuclear cell pleocytosis, with a high protein concentration and hypoglycorrhachia.[211] Brain abscesses may be seen as a complication of meningitis.[212]

Tularemia may lead to months of debility in some patients, usually associated with late lymph node suppuration or persistent fatigue. A recent outbreak of waterborne oropharyngeal tularemia in the Republic of Georgia was marked by delayed diagnosis and treatment, the frequent occurrence of neuropsychiatric symptoms, and slow resolution of adenopathy. Neuropsychiatric symptoms in this cohort included headaches, chronic fatigue, difficulty concentrating, and sleep disturbances.[213] Features that are associated with a worse prognosis include increasing age, serious coexisting medical conditions, symptoms lasting 1 month or longer before treatment, significant pleuropulmonary disease, typhoidal illness, renal failure, a delay in the diagnosis, and inappropriate antibiotic therapy.[1,179] Overall death rates in the modern antibiotic era have been 2% to 4% but may range up to 24% depending on the strain.[196] Mortality had been as high as 60% before the introduction of streptomycin as treatment.[214]

DIAGNOSIS

The diagnosis of tularemia ultimately rests on clinical suspicion. Results of routine laboratory testing are nonspecific. The leukocyte count and sedimentation rate may be normal or elevated. Thrombocytopenia, hyponatremia, elevated serum aminotransferase values, increased creatine phosphokinase level, myoglobinuria, and sterile pyuria are occasionally found.[1]

Routine Cultures and Pathology

Because of its potential danger to laboratory personnel, individuals working in the area or who may come in contact with patient specimens should be notified if tularemia is suspected. The organism is rarely seen on Gram-stained smears or in tissue biopsy specimens and it does not grow in routinely plated cultures. However, *F. tularensis* may be recovered from blood, pleural fluid, lymph nodes, wounds, sputum, and gastric aspirates when processed on supportive media. Isolations by blood culture have included the less virulent *F. tularensis* subsp. *holarctica* as well as the more virulent *F. tularensis* subsp. *tularensis.*

Guidelines for sentinel-level clinical laboratories were recently issued for *F. tularesis*.[41] For clinicians concerned about this infection, Biosafety Level 2 is sufficient for laboratory handling of routine clinical specimens, but Biosafety Level 3 should be used to process isolates suspected of being *F. tularensis*.[20] Because *F. tularensis* is a Tier 1 select agent, its possession and shipment are tightly restricted.[215] Isolates thought to be *F. tularensis* must be shipped to the state public health laboratory or a specialized registered laboratory in the Laboratory Response Network, and the remainder destroyed within 1 week. These laboratories also may provide reliable, rapid diagnostic tests. Misidentification of *F. tularensis* may occur when commonly available automated laboratory identification systems are used; it is most often reported as *Haemophilus influenzae* (satellite test or X and V factor test positive) or *Aggregatibacter* spp.

Many methods for the rapid diagnosis of tularemia have been reported, including DFA and immunohistochemical staining of smears and tissues, antigen detection in urine, detection using specific monoclonal antibodies, RNA hybridization with a 16S ribosomal probe, and various PCR techniques. DFA and PCR tests are usually available in specialized Laboratory Response Network laboratories. Nonculture methods for directly detecting *Francisella* species in clinical specimens are being explored, including real-time PCR, recombinase polymerase amplification, and direct deep DNA sequencing.[199,216–220] Ongoing research is focused on the development of field-stable PCR, recombinase polymerase amplification, proteomic, and immunologic methods for the rapid and simultaneous detection of multiple potential bioterrorism agents.[222] Newer methods that may limit the need for laboratory manipulation of unknown culture isolates and that can identify the organism to the subspecies level include real-time PCR and MALDI-TOF.[223–226]

The use of PCR analysis for diagnosis is appealing in that smears and cultures are usually negative, standard microbiologic isolation may be hazardous to laboratory personnel, serologic diagnosis may take several weeks to confirm, and the basic methodology is widely available in many clinical laboratories. Although very sensitive in artificial media, PCR assays are less sensitive when applied to biologic specimens and false-negative results may occur. A review of three reports from Sweden found that PCR assay using primers for the 17-kDa lipoprotein *F. tularensis* gene *tul4* was 77% sensitive and culture was 63% sensitive for the diagnosis of ulceroglandular tularemia.[214] PCR analysis may prove useful for diagnosing tularemia in patients several weeks into their illness and in those already receiving suppressive empirical antibiotic therapy. Real-time PCR assays with multiple targets offer promise for improved sensitivity and specificity, including for field use.[219] An automated cartridge-based PCR assay can detect *F. tularensis* bacteremia in a primate model, but is not yet commercially available.[227] However, one commercially available multiplex PCR system does include *F. tularensis* in two of its special panels.[228]

Serologic Diagnosis

Serologic studies are the most common way the diagnosis of tularemia is confirmed. Antibodies to *F. tularensis* may be demonstrated by tube agglutination, microagglutination, hemagglutination, enzyme-linked immunosorbent assay, and immunochromatographic assay; the tube agglutination and microagglutination tests are the standard methods in the United States, and enzyme-linked immunosorbent assays also are used in Europe.[20,229,230] Standard serologic tests detect infections with *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* equally well. They are usually negative with *F. tularensis* subsp. *novicida* and with *F. philomiragia*. Standard tube agglutination titers are usually negative in the first week of illness, are positive in most patients after 2 weeks, and peak after 4 or 5 weeks. The microagglutination assay is up to 100-fold more sensitive than tube agglutination.

IgM and IgG antibodies appear together, and high titers of both may persist for longer than a decade after infection, limiting the value of a single positive result.[231] A presumptive diagnosis is supported by an acute tube agglutination titer of 1:160 or more, or an acute microagglutination titer of 1:128 or more, in the presence of compatible disease, but this may also reflect remote infection. Definitive serologic diagnosis requires a fourfold or greater change in titer between acute and convalescent specimens, and at least one test must be positive.[20] Serologic studies may need to be repeated at 7- to 10-day intervals before a rise is demonstrated. Antibodies may cross react with *Brucella* spp., *Proteus* OX19, *Legionella* spp., and *Yersinia* spp., but titers to *F. tularensis* are almost always higher. False-positive heterophil agglutinins also rarely occur during tularemia.

Investigational Diagnostic Assays

Tests for cell-mediated immunity, such as whole-blood IFN-γ release assay in response to tularemia antigens, are promising and may be positive earlier than serologic studies, but they are not commercially available.[229] Gene profiling of cells in whole-blood samples obtained from patients with tularemia offers promise as a future rapid diagnostic tool.[232]

THERAPY

Antibiotic Treatment

Because tularemia circulates within zoonotic reservoirs and environmentally, not within humans, antibiotic resistance has not been reported clinically and has not evolved over time.[233] However, antibiotic-resistant organisms have been produced within laboratories and could be introduced in a bioterrorism scenario.[234,235] Antimicrobials with good in vitro activity include aminoglycosides, tetracyclines, and fluoroquinolones, though only aminoglycosides and tetracyclines are approved by the US Food and Drug Administration for treatment of tularemia. As an intracellular organism and a β-lactamase producer, not unexpectedly, there is resistance to the β-lactam antibiotic class.[43] The macrolide class also tends to have poor activity, although different susceptibilities occur between A and B strains.[236,237]

The Centers for Disease Control and Prevention and the World Health Organization recommend aminoglycosides as the drugs of choice for the treatment of severe tularemia.[238,239] Streptomycin is preferred in adults because it is efficacious with minimal relapses and has been approved in the United States for tularemia treatment by the US Food and Drug Administration. Gentamicin is an acceptable substitute that may be given intravenously, is often more readily available, and has less vestibular toxicity than streptomycin; in addition, gentamicin blood levels are usually easier to obtain in a timely fashion (Table 227.2). The recommended dosage of streptomycin that is effective therapy for tularemia is 7.5 to 10 mg/kg intramuscularly every 12 hours for 7 to 14 days. An alternative regimen is 15 mg/kg intramuscularly every 12 hours for the first 3 days, followed by half this dose to complete treatment. For patients who are very ill, 15 mg/kg every 12 hours may be given throughout a 7- to 10-day course. Doses greater than 2 g/day of streptomycin in adults do not increase efficacy and should not be given. The pediatric weight-based regimens for streptomycin are up to a maximum of the adult dose (see Table 227.2). The first few days of streptomycin rarely may induce a Jarisch-Herxheimer–like reaction, with an increase in symptoms and a transient decrease of the serum agglutination titer.

Gentamicin is preferred for children because it may be given intravenously, may be more readily available than streptomycin, and may have fewer adverse effects (see Table 227.2).[240] However, Kaya and coworkers[241] retrospectively identified 11 children with oropharyngeal tularemia who had a poor response to 7 to 10 days of gentamicin treatment; 7 patients had persistent adenopathy that improved after therapy was changed to streptomycin, and the other 4 patients developed suppurative nodes that responded to streptomycin and surgical drainage. The desired gentamicin peak serum level is at least 5 μg/mL. The efficacy of single daily gentamicin dosing has been reported for small numbers of adult cases but has not been rigorously studied.[109,242] Nonetheless, it is efficacious, and some practitioners consider once-daily gentamicin an acceptable alternative for adults that facilitates completing treatment as an outpatient.[109] However, divided dosing of gentamicin is recommended for children (see Table 227.2).[240] The doses of both streptomycin and gentamicin need to be adjusted for renal insufficiency.

Penetration of the aminoglycosides into the cerebrospinal fluid is poor and erratic, and it may be inadequate in tularemic meningitis. Pittman and colleagues[243] reported a central nervous system shunt infection caused by *F. tularensis* that was successfully treated with intrathecal gentamicin. Contentin and colleagues[244] reported a case of

TABLE 227.2 Antibiotic Therapy for Tularemia

INDICATION AND PATIENT GROUP	RECOMMENDED ANTIBIOTICS AND DOSAGES
Serious Disease	
Adults	Streptomycin,[a] 10 mg/kg IM q12h for 7–10 d (not to exceed 2 g/d), *or* Gentamicin,[a] 5 mg/kg/d IM or IV divided q8h for 7–10 d
Children	Streptomycin,[a] 15–20 mg/kg IM q12h for 7–10 d (not to exceed 2 g/d), *or* Gentamicin,[a] 5 mg/kg/d IM or IV divided q8–12h for 7–10 d
Mild to Moderate Disease	
Adults	Ciprofloxacin,[b] 500 mg orally twice daily for 10–14 d, *or* Doxycycline, 100 mg orally twice daily for 14–21 d
Children[c]	Gentamicin,[a] 5 mg/kg/d IM or IV divided q8–12h for 7–10 d, *or* Ciprofloxacin,[c] 20–40 mg/kg/d orally divided twice daily for 10–14 d (not to exceed 1500 mg/d)
Meningitis	
Adults	Streptomycin or gentamicin in the doses given for moderate to serious disease plus either ciprofloxacin, 400 mg IV every 8–12 h, doxycycline, 100 mg IV every 12 h, or chloramphenicol, 15–25 mg/kg IV q6h (not to exceed 4 g/d), for 14–21 d
Children	Gentamicin in the doses for moderate to serious disease plus either ciprofloxacin, 20–30 mg/kg/d IV divided every 8 or 12 h (not to exceed 1.2 g/d), or doxycycline, 2.2–4.4 mg/kg/d IV divided q12h (not to exceed 200 mg/d), for 14–21 d

[a]The streptomycin and gentamicin doses listed are for patients with normal renal function, and they need to be adjusted for renal impairment. See text for an alternative streptomycin regimen for very ill patients. In adults with normal renal function, once-daily administration of gentamicin also is acceptable.
[b]For adults an oral ciprofloxacin dose of 750 mg twice daily also has been used in some reports.
[c]Gentamicin is preferred for children with moderate disease; ciprofloxacin is an alternative for children with mild disease.

F. tularensis subsp. *holarctica* meningitis that was cured after 4 weeks of high-dose ciprofloxacin combined with 18 days of gentamicin and 6 days of thiamphenicol. Additional cases of tularemic meningitis have been documented, and successful treatment has included a combination of streptomycin with chloramphenicol or a combination of doxycycline with either streptomycin or gentamicin (see Table 227.2).[211,245] Chloramphenicol may be unavailable or in short supply in the United States; it should not be chosen to treat other forms of tularemia because of its potentially serious toxicity and the availability of more effective alternatives with less dangerous potential side effects.

Selected children with mild disease and adults with mild to moderate disease may be treated with one of the oral antibiotics listed in Table 227.2, either alone or after initial treatment with an aminoglycoside.[240] Doxycycline and tetracycline are bacteriostatic for *F. tularensis.* Historically, use of this class led to higher rate of relapse after treatment, which is why they are recommended to be given for at least 14 days (see Table 227.2). A recent retrospective case series from Missouri reported no relapses among 17 patients receiving either doxycycline (16 patients) or tetracyclines (1 patient) as their tularemia therapy (median treatment duration of 21 days, range 10–42 days), suggesting that selected patients treated with a tetracycline for the recommended duration may have better outcomes than previously reported failure rates of 15% to 48%.[246] Of the 16 patients treated with doxycycline, 9 had drainage of suppurative lymph nodes prior to starting therapy and 3 required a repeat drainage procedure while receiving doxycycline (one patient after 1 week and two patients after 2 weeks of treatment).[246] Doxycycline is preferred over tetracycline because it is better tolerated and provides the convenience of twice-daily dosing. Tetracycline should not be used in children younger than 8 years of age, during pregnancy, or during lactation unless the benefits clearly outweigh the risks. Doxycycline is not recommended for the treatment of tularemia in children, although it is thought not to cause tooth enamel staining under the age of 8, and is now recommended for other tick-borne infections by the American Academy of Pediatrics.[240,247,248] Relapses may follow any regimen but are more common when tetracyclines are used for less than 14 days. Although a relapse after initial treatment with doxycycline may be re-treated with doxycycline given for at least 14 to 21 days, re-treatment with a different agent such as streptomycin or gentamicin may be preferred.

In vitro susceptibility studies have found that the fluoroquinolones are active against *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica,* as well as *F. tularensis* subsp. *mediasiatica* and *F. tularensis* subsp. *novicida.*[31,249,250] Clinical experience with the fluoroquinolones as therapy for tularemia caused by *F. tularensis* subsp. *holarctica* has been favorable, even in immunocompromised hosts. Some consider fluoroquinolones to be the drugs of choice for adults with mild to moderate *F. tularensis* subsp. *holarctica* infections,[251] and Eliasson and coworkers[252] have recommended the use of gentamicin combined with a fluoroquinolone for patients with severe tularemia. However, there is less published experience using these agents in the United States or for infections caused by the more virulent *F. tularensis* subsp. *tularensis.* Among tularemia cases reported in Missouri between 2000 and 2007, 9 of 10 patients given ciprofloxacin alone or combined with ineffective agents were cured.[164] Fluoroquinolones are preferred over doxycycline as oral treatment of tularemia for appropriate adults by some authorities because of efficacy with a reported lower likelihood of relapse.[251,252] Ciprofloxacin has also been effective therapy in children with tularemia, and is a recommended alternative for children with mild disease (see Table 227.2).[187,240,253] However, the outcome with fluoroquinolone therapy may be suboptimal in some children as well as in adults.[174,254] Importantly, acquired fluoroquinolone resistance has not been documented in natural tularemia, even among patients who failed fluoroquinolone treatment.[255]

Ciprofloxacin was active in vitro against the few isolates of *F. philomiragia* tested. It was used successfully to treat a child with chronic granulomatous disease and *F. philomiragia* adenitis and lung infection, but it was part of an unsuccessful regimen in a young man with *F. philomiragia* sepsis.[31,194,256]

Drugs with well-established clinical efficacy (see Table 227.2) have exhibited achievable minimal inhibitory concentrations against *F. tularensis* on standardized in vitro susceptibility tests.[31,249,250] Other agents with relatively low minimal inhibitory concentrations have included erythromycin, rifampin, cefoxitin, cefotaxime, ceftriaxone, and ceftazidime. The effectiveness of these drugs in treating tularemia is not fully established, and ceftriaxone has failed in several patients treated as outpatients.[257] Ceftriaxone exhibited poor intracellular inhibitory activity against a strain of *F. tularensis* subsp. *holarctica* grown in macrophage-like cell monolayers, whereas aminoglycosides, doxycycline, telithromycin, fluoroquinolones, and rifampin were active in this assay.[236] However, rifampin is not recommended to treat tularemia because of its potential to induce the emergence of resistance.[238] Although erythromycin has been used successfully in a few patients who were believed to have *Legionella* infections, resistance to erythromycin is prevalent in many areas in Europe and Russia and in general the drug is considered unreliable as therapy.[31,182,238,258,259] *F. tularensis* subsp. *tularensis* and biovar II strains of *F. tularensis* subsp. *holarctica* are considered resistant to macrolides, but macrolide minimal inhibitory concentrations are lower to biovar I strains of *F. tularensis* subsp. *holarctica.*[260,261] Ikäheimo and associates[262] found that all 38 type B clinical isolates they tested were resistant to imipenem in vitro.

Pregnant and Immunosuppressed Patients

Tularemia cases occurring during pregnancy or in immunosuppressed patients have been reported only rarely, but they may be more frequently

encountered during a bioterrorism event, affecting large numbers of people without the usual risk factors.[263] Treatment of pregnant or immunocompromised patients with tularemia is challenging, and optimal antibiotic regimens are unknown. Aminoglycosides, tetracyclines, and fluoroquinolones have potential risks to the fetus when used during pregnancy; immunocompromised patients with tularemia may have an increased risk for relapse or treatment failure.

The risk to the fetus of tularemia during pregnancy is unknown; although prematurity and fetal loss may occur, healthy newborns also have been reported after maternal tularemia.[264,265] Before the availability of effective antibiotics, Pullen and Stuart[266] reported three patients with tularemia during pregnancy and all gave birth to normal children. Dienst[161] reported two pregnant patients with tularemia; one was successfully treated with streptomycin in the fifth month of pregnancy and had a normal birth, and the other spontaneously aborted a 6-week-old fetus after 35 days of illness. Four pregnant patients with tularemia and suppurative adenopathy were treated successfully with gentamicin followed by oral ciprofloxacin and lymph node drainage; all the patients had otherwise normal pregnancies, and the infants were normal at 18 months of follow-up.[267] Among three pregnant women with tularemia in Turkey, one patient was treated with gentamicin and two patients were untreated; all three patients had uncomplicated pregnancies and delivered healthy babies.[268] Tularemia in a pregnant woman in France, where macrolide-sensitive strains of *F. tularensis* subsp. *holarctica* predominate, was successfully treated with a 6-week course of azithromycin.[269] However, a review of 101 patients with tularemia in France identified two pregnant patients, and both were unsuccessfully treated with macrolides.[174]

Potentially immunocompromising illnesses were present in another 8 of the 101 French patients, but their treatments and outcomes were not specifically identified.[174] Case reports of tularemia in patients with hematopoietic stem cell transplantation, renal transplantation, acquired immunodeficiency syndrome, chronic graft-versus-host disease, and TNF inhibitor therapy have described successful treatment with at times prolonged courses of gentamicin, fluoroquinolones, or doxycycline, alone or in various combinations.[270–273,274,275,276]

Surgical Treatment

Surgical therapies are limited to drainage of abscessed lymph nodes and chest tube drainage of empyemas. Multiple nonprospective series reported fewer treatment failures when antibiotic therapy is combined with surgical drainage when feasible.[201,277]

Immunotherapy

Antibodies to *F. tularensis* and serum from vaccinated people are effective passive therapies for murine infection.[135,278,279] Proteomic analysis has been used to identify target antigens for the development of monoclonal antibodies that are effective in protecting mice from pulmonary intranasal challenge when given either intranasally or intraperitoneally.[280] Together, these results offer the future hope of immunotherapeutic agents for tularemia.

Therapy for Tularemia After a Bioterrorism Event

Recommendations for therapy for tularemia in the context of a bioterrorism event were presented in the Working Group for Civilian Biodefense Consensus Statement.[197] Specific treatment options depend on whether there are contained or mass casualties; smaller numbers of infected people permit individual medical care that would not be possible with larger numbers of patients. Thus treatment options for contained casualties are similar to those listed in Table 227.2 and as discussed earlier; in addition, the Working Group also included chloramphenicol alone as an option for both adults and children or ciprofloxacin as an option for children. For contained casualties, the Working Group recommended that streptomycin, gentamicin, or ciprofloxacin be given for 10 days and that doxycycline or chloramphenicol be given for 14 to 21 days.[197] In mass casualty situations, only oral ciprofloxacin or doxycycline for 14 days is recommended by the Working Group for both children and adults.[197] Susceptibility testing of the *F. tularensis* strain used as a bioweapon will be important because it may have been modified to select for resistance to standard antibiotic treatments. Molecular assays capable of rapidly and reliably detecting ciprofloxacin resistance in *F. tularensis* have been reported.[281]

The Working Group preferred gentamicin for the treatment of pregnant patients among contained casualties and preferred oral ciprofloxacin for pregnant patients among mass casualties.[197] The Working Group recommended treatment with either streptomycin or gentamicin for immunocompromised patients in both contained and mass casualty situations.[197]

PREVENTION

Avoiding exposure to the organism is the best prevention of tularemia. Wild animals should not be skinned or dressed using bare hands, and bare hands should not be used to handle an animal that appears ill. Gloves, masks, and protective eye covers should be worn when performing such tasks and when disposing of dead animals brought home by household pets. Wild game should be cooked thoroughly before ingestion. Wells or other waters that are contaminated by dead animals should not be used. Before mowing, checking property for animal carcasses will avoid aerosolizing potentially infected animal tissue. Treatment of community water supplies with standard chlorination protects against waterborne tularemia.[197,282,283] The most important measure to avoid tick bites in infested areas is wearing clothing that is tight at the wrists and ankles and that covers most of the body. Chemical tick repellents may also be of benefit. Frequent checks should be made for attached ticks so that they may be removed promptly; this must not be done with bare hands, and care should be taken not to crush the tick.

Hospitalized patients with tularemia do not need special isolation because person-to-person spread does not occur, and even in the preantibiotic era, secondary cases were not found. Standard universal precautions for contaminated secretions are adequate when handling drainage from wounds or eyes.

Vaccination

Vaccines prepared from killed *F. tularensis* are ineffective, in part because they only induce an antibody response. A live vaccine based on an attenuated strain of *F. tularensis* subsp. *holarctica* (LVS vaccine), originally obtained from the former Soviet Union, was developed in the United States. The LVS vaccine is an attenuated *F. tularensis* strain that occurs in two colony phenotypes, only one of which is immunogenic. This vaccine does not spread from the inoculation site, induces cell-mediated and humoral immunity, is effective in preventing typhoidal disease, and reduces the severity of ulceroglandular disease but does not prevent it.[284,285] However, the live vaccine strain of *F. tularensis* cannot provide high immune response in the presence of antimicrobial agents such as doxycycline. Importantly, the LVS vaccine has been ineffective in protecting against pulmonary challenge with virulent *F. tularensis* subsp. *tularensis*.[286] LVS vaccination was considered in the past for people who worked with *F. tularensis* and for anyone else with repeated occupational exposures. However, the LVS vaccine is not approved and is no longer available for general use in the United States. Removal was prompted by questions about its stability, basis of attenuation, efficacy after challenge with other *Francisella* strains by varied routes of exposure, usefulness in immunocompromised patients, and the need to administer it by scarification.[284,287]

A new tularemia vaccine is actively being sought using several different strategies, including developing inactivated or subunit vaccines, finding different attenuated strains with defined mutations that are immunogenic, and improving the LVS vaccine.[286,288–290] Genomic and proteomic analyses are being used to identify candidate *F. tularensis* proteins that are recognized by patients who have had tularemia for inclusion in potential subunit vaccines.[55,291] Analysis of differences in immune responses to vaccines among protected and unprotected animals may help identify human correlates of protection.[286,292] In humans and animal models, protection against pulmonary challenge is enhanced when the LVS vaccine is given by aerosol or intranasally. This and other observations suggest that mucosal immunity may contribute to vaccine-induced protection against respiratory tularemia. An LVS-based, multivectored vaccine has successfully protected mice against respiratory

challenge with virulent *F. tularensis, Bacillus anthracis,* and *Yersinia pestis*.[293]

An improved LVS vaccine was produced using accepted good manufacturing practices that had limited toxicity in rabbits; this vaccine induced IgG, IgM, and IgA antibodies that cross reacted with *F. tularensis* subsp. *tularensis* strain Schu S4. A phase I dose-escalating trial of this new LVS vaccine in humans found it to be safe and immunogenic, eliciting both antibody and IFN-γ responses.[294] A phase II double-blind, placebo-controlled trial comparing the new DVC-LVS vaccine in humans to the existing vaccine (USAMRIID-LVS) demonstrated equivalent safety and seroconversion rates with earlier antibody production at 14 days.[295] However, there are many biologic and regulatory issues that will need to be addressed before this or another vaccine is available for human use.[113,296]

Antibiotic Prophylaxis

Antibiotic prophylaxis after potential exposures of unknown risk, such as tick bites, is not recommended. In the past, intramuscular streptomycin was given for preemptive treatment of documented exposures from laboratory accidents because streptomycin successfully aborts illness when given in the incubation period after experimental inoculation. Gentamicin should be effective for this purpose as well, but this has not been confirmed. Currently, either ciprofloxacin, 500 mg, or doxycycline, 100 mg, given orally twice daily for 14 days is recommended for adults with suspected or proven high-risk exposure to *F. tularensis*.[107,197] Exposed children may be observed for fever or other signs of illness without antibiotics, except during a bioterrorist event.[107] Observation without antibiotics is also appropriate for individuals with lower-risk exposures and in vaccinated individuals. No therapy is needed for someone whose only exposure is to a patient with tularemia because human-to-human transmission does not occur.

Recovery from tularemia is believed to confer protective immunity for life, although a few recurrent infections have been documented.[297,298] Most recurrences have been clinically mild ulceroglandular disease, and systemic symptoms have been uncommon. Therefore previously infected individuals are not likely candidates for vaccination or preemptive antibiotic therapy after a known exposure.

Antibiotic Prophylaxis After a Tularemia Bioterrorism Event

All people, regardless of age or history of past tularemia, identified early in the incubation period after exposure to an *F. tularensis* bioterrorist event are candidates for antibiotic prophylaxis. The Working Group for Civilian Biodefense recommended either doxycycline or ciprofloxacin orally for 14 days for all exposed people except immunocompromised patients.[197] Mice are significantly protected from lethal *F. tularensis* subsp. *tularensis* respiratory infection by fluoroquinolones, and this is true even when the fluoroquinolone is delayed up to 72 hours after infection; however, delay of doxycycline beyond 24 hours is ineffective.[299,300] These results suggest that a fluoroquinolone may be preferred for oral prophylaxis after exposure to an *F. tularensis* bioweapon.

The doxycycline and ciprofloxacin regimens for adults are the same as those discussed previously for prophylaxis after other high-risk exposures. The Working Group believed that 14 days of either oral doxycycline or oral ciprofloxacin is an appropriate choice for children exposed to *F. tularensis* as a result of bioterrorism.[197] The doxycycline dosing recommended by the Working Group is 2.2 mg/kg twice daily for children weighing less than 45 kg and 100 mg twice daily for children weighing 45 kg or more.[197] The dosing of ciprofloxacin recommended by the Working Group for children exposed to an *F. tularensis* bioweapon is 15 mg/kg orally twice daily, not to exceed 1 g daily.

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The complete reference list is available online at Expert Consult.

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228 *Pasteurella* Species

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SHORT VIEW SUMMARY

Definition
- Frequently, *Pasteurella* infection is a cause of skin and soft tissue infections after an animal bite or scratch.
- *Pasteurella* infection often is part of a mixed bacterial infection.
- Complications include septic arthritis and osteomyelitis.
- Less frequently, *Pasteurella* infection causes pneumonia in patients with underlying lung disease.
- Other deep-seated complications are uncommon but carry high morbidity and mortality.

Epidemiology
- Distribution is worldwide.
- The principal reservoir is animals.
- Infection most often occurs after bites or scratches from dogs and especially cats.
- Infections have been reported after bites or scratches from a large number of other animals.
- Infections also occur in individuals with animal exposure without bites or scratches.

Microbiology
- Nonmotile, facultatively anaerobic, gram-negative coccobacilli
- Grows best on sheep blood and chocolate agar, not usually on MacConkey agar
- Somewhat fastidious
- Can be difficult to isolate from nonsterile specimens, especially sputum
- *P. multocida* with three subspecies: *multocida, septica, gallicida* (least common) (see Table 228.1)
- Several other related species of genus *Pasteurella* cause human disease.

Diagnosis
- Progressive skin and soft tissue inflammation after an animal bite injury, especially after puncture wounds
- Isolation of the organism from wound/tissue culture, often as part of a mixed bacterial infection
- Sputum or pleural fluid isolates from patients with underlying lung conditions presenting with lower respiratory tract infections

Therapy
- See Chapter 315 for management of bite wounds. *P.* multocida is susceptible to most penicillins, second- and third-generation cephalosporins, carbapenems, tetracycline, fluoroquinolones, and trimethoprim-sulfamethoxazole. Susceptibility is less or variable with macrolides, linezolid, aminoglycosides, clindamycin, vancomycin, and antistaphylococcal penicillins.
- Once identified as *Pasteurella* spp., a penicillin is the antimicrobial agent of choice.
- β-Lactam resistance is rare, mostly described in respiratory isolates.
- Aggressive surgical débridement of soft tissue infections is important. Penetrating bite wounds over the hands lead to tenosynovitis and are a surgical emergency, requiring incision and drainage.

Prevention
- Antimicrobial prophylaxis for selected bite wounds is usually with amoxicillin–clavulanic acid 875 mg orally twice daily for 5 days (see Chapter 315).

Pasteurella are gram-negative coccobacilli that inhabit the oral cavity and gastrointestinal tract of many animals and cause various infectious problems, including septicemia and pneumonia. In humans infection is most often caused by dog and cat bites, resulting in cellulitis, subcutaneous abscesses, and a number of other syndromes. Bacteria belonging to the genus *Pasteurella* were first isolated from birds with cholera in 1878; they were characterized 2 years later by Pasteur. In 1886 Hueppe speciated the organism *Bacterium septicemia haemorrhagica* as the cause of hemorrhagic septicemia in animals. The first human case of *Pasteurella* infection, a case of puerperal sepsis, was described by Brugnatelli in 1913. The isolation of *Pasteurella multocida* from an infection occurring after a cat bite was first described in 1930. Subsequently, as additional isolates were recovered and characterized, related species were grouped together, first as *Pasteurella septica* and then by the late 1930s as the *P. multocida* group. The complete genome of *P. multocida* was sequenced in 2001, offering an opportunity to elucidate the mechanisms of pathogenicity more accurately.[1]

DESCRIPTION OF THE PATHOGEN

The family Pasteurellaceae includes the genera *Pasteurella, Haemophilus, Actinobacillus, Aggregatibacter,* and *Mannheimia,* among others.[2,3] On the basis of the genomic sequence, it appears that *Pasteurella* and *Haemophilus* diverged approximately 270 million years ago.[1] DNA hybridization separates the *Pasteurella* spp. into two groups: (1) *Pasteurella* sensu stricto and (2) *Pasteurella*-related spp., with the latter more closely related to *Haemophilus* and *Aggregatibacter* (Table 228.1).[3] Species of the genus *Pasteurella* are nonmotile, facultatively anaerobic, gram-negative coccobacilli measuring 1 to 2 μm in length. Organisms grow in culture on a variety of commercial media, including sheep blood and chocolate agar, but not usually on MacConkey agar media. They are fastidious and can be difficult to isolate and identify from nonsterile specimens such as sputum. In one study matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) accurately identified 59 of 66 *Pasteurella* isolates and had the advantage of rapidity.[4] Most strains are catalase, oxidase, and indole positive and produce acid from sucrose. The most common human isolates belong to the *P. multocida* group and appear as smooth, iridescent, blue colonies on growth media. Encapsulated isolates typically appear mucoid. Strain differences of *P. multocida* have traditionally been identified on the basis of capsular antigens that define 5 serogroups (A–F) and somatic antigens that define 16 serovars (1–16).[5] Strains in groups B, E, and F have been rarely associated with human infection. Of the remaining strains (A, D), group A strains have been more frequently isolated as respiratory tract colonizers or pathogens, whereas non-A strains have been isolated more frequently from nonrespiratory tract specimens, including blood, cerebrospinal fluid, and abscess fluid.[6] Ribosomal DNA and other sequence-based molecular techniques offer better resolution than serotyping and have now superseded it for identification and typing of *Pasteurella*.[7,8] Because

TABLE 228.1 *Pasteurella* Species

***Pasteurella* Sensu Stricto**
P. multocida
Subsp. *multocida*
Subsp. *septica*
Subsp. *gallicida*
P. canis
P. dagmatis
P. stomatis
***Pasteurella*-Related Species**
P. aerogenes
P. bettyae
P. caballi
P. pneumotropica

Data from Zbinden R, Carroll KC, Jorgensen JH, et al, eds. Manual of Clinical Microbiology. *11th ed. Washington, DC: American Society for Microbiology Press; 2015:652–666.*

of their sometimes fastidious nature, specific guidelines for methodologies to accurately determine minimal inhibitory concentrations (MICs) of *Pasteurella* spp. have been published.[9]

EPIDEMIOLOGY

On the basis of case reports and case series of infected patients, *Pasteurella* spp., particularly *P. multocida,* appear to have a worldwide distribution. For most *Pasteurella* spp. the principal reservoir is in animals. *P. multocida* has been isolated from the upper respiratory tracts of a variety of animals, including dogs, cats and other felines, pigs, and a wide variety of domestic and wild animals. Dogs and cats have particularly high colonization rates. In most cases carriage is asymptomatic, although both upper and lower respiratory tract infections and septicemia are well known to occur in animals.[3,10] Although the reservoirs of most of the non-*multocida* spp. (*P. canis, P. stomatis, P. dagmatis, P. aerogenes,* and *P. pneumotropica*) are likely animals, notable is *Pasteurella bettyae,* a cause of neonatal infection and genitourinary infection in adults, whose reservoir is not well defined.[11] Respiratory tract colonization by *P. multocida* in humans is well known to occur. In most cases colonized patients have underlying upper or lower respiratory tract diseases, including chronic sinusitis, chronic obstructive pulmonary disease (COPD), or bronchiectasis.[12–14] Most colonized patients have a history of household or domesticated animal contact.[15]

Broadly speaking, human infection with *Pasteurella* can be divided into three types: infection occurring after animal bites, usually from dogs or cats; infection occurring after other animal exposures; and infection with no known animal contact. Infection after animal bites is the most commonly reported clinical setting for the organism (see Chapter 315).[15–22]

Among animal bites, dog bites are most common, followed by cat bites. Approximately 15% to 20% of dog bite wounds and greater than 50% of cat bite wounds become infected.[15,21] The higher incidence of infection after cat bites probably results from the fact that cat teeth are thinner and more commonly result in puncture wounds, which are known to carry a higher risk of infection. *Pasteurella* spp. are the most commonly isolated pathogens from dog and cat bites, present in 50% and 75% of cases, respectively; followed by *Staphylococcus* and *Streptococcus* spp.[19–24] Francis and associates,[20] studying bite-related *P. multocida* infections in Oregon between 1962 and 1972, noted that 76% were the result of cat bites, and the remaining 24% were from dog bites. The difference in incidence of *Pasteurella* infections in dog and cat bites also may reflect the higher rate of upper respiratory colonization in cats. *Pasteurella* infections have also been reported after bites from a variety of other animals, including pigs, rats, lions, opossums, and rabbits.[22] In addition to bites, *Pasteurella* infections have also been reported after dog and cat scratches and from the licking of open wounds by these animals.[17,25,26]

Pasteurella infections are well known to develop in patients exposed to animals but without a history of bites or scratches. These include skin and soft tissue infections, bone and joint infections, pneumonia, meningitis, endocarditis, and septicemia.[15,27,28] Persons at risk for infection from animal exposure include veterinarians, farmers, livestock handlers, pet owners, and food handlers. Although the route of infection in many reported cases is not clear, most have been presumed to result from inadvertent direct inoculation of organisms or from upper respiratory tract colonization, with subsequent dissemination to the target organ or organs. In one case report a patient with underlying bronchiectasis and diabetes was hospitalized with pneumonia caused by *P. multocida*. Pulsed-field gel electrophoresis of her sputum isolate, compared with *P. multocida* isolated from her dog's pharynx, yielded identical patterns, strongly implicating her dog as the source of her infection.[27,29]

In a significant proportion of *Pasteurella* cases, no known animal exposure or contact can be identified. In 1970 Hubbert and associates[28] reviewed what was then the world's literature; they identified 72 reported cases of *P. multocida* infection unrelated to bites and described 136 additional cases. In 16% of the reviewed cases and 31% of their additional cases, no animal exposure or contact could be identified. Once again, the spectrum of infectious complications was wide, similar to that described for patients with nonbite animal exposures. In a more recent literature review of 156 *Pasteurella* bacteremia adult cases, 20% did not have animal exposures reported. Vertical transmission of *P. multocida* has been described rarely.[30,31]

PATHOGENESIS

Despite *Pasteurella* spp. having been long established as human pathogens, the precise mechanisms of pathogenicity remain uncertain. In animals virulent *P. multocida* strains adhere to mucosal epithelial cells in the upper respiratory tract, particularly in the tonsils, and in some cases they are mediated by fimbriae.[32] Several virulence factors have been described in *Pasteurella* spp.[10] Much attention has focused on *P. multocida* toxin (PMT). PMT, produced by strains A and D, is a potent mitogen that has been shown to activate a number of intracellular signaling cascades, resulting in a multitude of deleterious effects.[33] Specifically, PMT has been shown to inhibit the migratory response of dendritic cells, thereby impairing immune surveillance.[34] Along with ToxA protein, also produced by *P. multocida,* PMT is associated with progressive atrophic rhinitis in pigs.[35] *P. multocida* lipopolysaccharide (LPS) is another virulence factor, proven by the fact that strains with truncated LPS have been shown to be attenuated in virulence.[36] In addition, most virulent *Pasteurella* strains produce polysaccharide capsules that confer many possible mechanisms of pathogenicity, including resistance to desiccation, promotion of adherence, and resistance to phagocytosis and complement-mediated killing.[37] Finally, binding of transferrin by some pathogenic *Pasteurella* strains has been demonstrated and may be a mechanism used by the bacteria to ensure an iron supply necessary for growth.[38] Indeed, on the basis of the genomic sequence data, 2.5% of the entire genome encodes for proteins involved in iron acquisition.[1]

The humoral response to *P. multocida* infection has been characterized. Antibodies to somatic and capsular antigenic determinants develop within 2 weeks after clinical infection. Capsular antibodies are more long-lasting than somatic antibodies.[39] The precise role for such antibodies in host defense in humans is not clear. Studies in healthy breeders whose livestock suffered from pasteurellosis show a high rate of seropositivity to *Pasteurella*, illustrating the limitations of serology for the diagnosis of active *Pasteurella* infection in humans.[40]

CLINICAL MANIFESTATIONS

Most reported *Pasteurella* infections in humans are caused by *P. multocida* and involve skin and soft tissues.[41] Although both subspecies *multocida* and *septica* cause skin and soft tissue infections as a result of animal bites and scratches, subspecies *multocida* has also been found to cause respiratory tract infections and systemic infection.[6,42] Other species have been described much less commonly (Table 228.2).[17] Beyond skin and soft tissues, *Pasteurella* can uncommonly cause chronic respiratory tract infection in patients with underlying pulmonary disease and invasive infection syndromes, such as bacteremia, endocarditis, and meningitis. In a retrospective case-control study seeking to identify risk factors for invasive *Pasteurella* infection, only advanced age was found to be significant in multivariate analysis,[43] but it is thought that immunocompromised hosts are at enhanced risk.

TABLE 228.2 Clinical Characteristics of 159 Strains of *Pasteurella* Species Isolated From 146 Infected Humans Over a 3-Year Period

		SITE OF INFECTION			
SPECIES	***N***	**WOUND INFECTIONS OR ABSCESSES**[a]	**BLOOD**	**CEREBROSPINAL FLUID**	**OTHER**
P. multocida subsp. *multocida*	95	85	5	1	4[b]
P. multocida subsp. *septica*	21	20		1	
P. canis	28	28			
P. stomatis	10	10[c]			
P. dagmatis	5	2[d]			3[e]

[a]Caused by dog or cat bites or wounds licked by dogs or cats.
[b]Includes three cases of infection from cut wounds unassociated with any known animal contact.
[c]In eight cases of wound infection, *P. multocida* subsp. *multocida* was also recovered.
[d]In cases of wound abscesses, *P. multocida* subsp. *multocida* and *P. canis* were also recovered.
[e]One case each of severe cellulitis, groin abscess, and throat abscess.
Modified from Holst E, Rollof J, Larsson L, et al. Characterization and distribution of Pasteurella species recovered from infected humans. J Clin Microbiol. *1992;30:2984–2987.*

Skin and Soft Tissue Infections

Infections of skin and soft tissues most commonly develop after an animal bite or scratch. Less commonly, infections develop after a dog or cat has licked an open wound. Inflammation, swelling, and tenderness develop at the site of injury, usually within 24 hours from the time of exposure.[15,18,20,44] Regional lymphadenopathy occurs in 30% to 40% of cases. Wound discharge, ranging from serosanguinous to frankly purulent, has been noted in 20% to 40% of cases; fever develops in approximately 20%. Anatomically, greater than 50% of cases of infection from both dog and cat bites occur in the upper extremities, followed by the lower extremities, head, face, and neck; multiple sites of infection are sometimes evident.[20,44–46] Abscesses and tenosynovitis are the most frequent complications of *Pasteurella* soft tissue infection, with septic arthritis and osteomyelitis being less common. Bacteremia is uncommon. Weber and colleagues[15] noted an overall complication rate of 39% among 23 patients studied. In a large study analyzing bacterial species isolated from wounds inflicted by dog and cat bites, *P. canis* was the most common isolate from dog bites, whereas *P. multocida* subsp. *multocida* and subsp. *septica* were the most common from cat bites.[23]

Bone and Joint Infections

Bone and joint infections with *Pasteurella* spp. take three different forms—septic arthritis, osteomyelitis, and combined arthritis and osteomyelitis. Ewing and associates[47,48] reported two cases each of septic arthritis and osteomyelitis caused by *P. multocida* and reviewed the literature. Among 14 cases of septic arthritis reported and reviewed, 7 (50%) involved dog or cat bites or scratches and 5 (36%) involved animal exposure without recent or known bites or scratches. In the remaining 2 cases there were no reported animal exposures. The knee was the most common joint involved (11 cases), often in the setting of rheumatoid arthritis, osteoarthritis, or joint prosthesis. Five of the 14 patients were receiving prednisone. Osteomyelitis developed as the result of direct extension of soft tissue inflammation or by direct inoculation of the periosteum at the time of the bite. Among 13 cases of osteomyelitis reported, 9 (69%) involved animal bites or scratches, 1 (8%) involved animal exposure, and 3 had no reported exposure. In contrast with septic arthritis, most cases (69%) of osteomyelitis developed in an upper extremity bone, usually the hand or wrist. Also, unlike septic arthritis, chronic medical conditions and corticosteroid therapy were not common antecedents. Finally, among 7 cases of combined septic arthritis and osteomyelitis, 6 involved bones and joints of the upper extremities, usually a phalanx and interphalangeal joint infected after a cat bite. In a pediatric case series of 14 patients with bone and joint involvement, upper extremities were most commonly affected. Immunocompromising conditions or underlying joint disease were not common. Animal bites, licks, or scratches were reported in 9 of 14 cases (64%), with 4 (29%) cases reporting animal contact without bites or scratches and 1 case with no known exposure.[49]

Several contemporary case series point to a predilection of the organism for prosthetic joints. Knee and hip prostheses are most often involved,[25,50,51] although shoulder prosthesis infection has also been reported.[52] The infection may occur many years after the joint implantation, with the mean being 7.6 years in one series.[50] A single joint is most commonly involved. In virtually all cases there is a history of animal exposure. Risk factors have included older age, rheumatoid arthritis, and corticosteroid use.

Central Nervous System Infections

Central nervous system infections with *P. multocida* have been reported infrequently, predominantly in infants or elderly patients,[30] although cases in the immunocompetent have been described.[53] Meningitis is most common. There have been rare cases of focal lesions, such as brain abscess and subdural empyema.[15,30,54,55] Of 29 cases of meningitis reported in the English language literature through 1999, most patients had animal contact, usually licking of mucosa or nonintact skin.[55–57] Bacteremia was seen in nearly two-thirds of patients. Mortality was 25% overall, with decreasing mortality among later cases.[57] In a French pediatric meningitis surveillance study, *Pasteurella* accounted for 0.15% of all pediatric meningitis cases. All occurred in infants younger than 4 months. Forty-four out of 48 cases (92%) had a suspected animal source, most commonly household cats or dogs; however, only 8% of cases had a history of traumatic animal contact. A lack of direct contact between the infant and the animal suggests a potential role of horizontal transmission from the caregiver to the patient.[30]

In *Pasteurella* meningitis the CSF characteristics are usually consistent with bacterial meningitis, with pleocytosis and neutrophil predominance, high protein, and low glucose. Gram-negative bacilli or coccobacilli are seen on Gram stain in 70% to 80% of cases,[30] which can be confused with other more common meningitis agents, such as *Neisseria* or *Haemophilus*.

Bacteremia and Endocarditis

Bacteremia can occur as a complication of *Pasteurella* infection. In a comprehensive review of the literature, Vondra and Myers[58] summarized the cases of 156 individuals with *Pasteurella* bacteremia. Fifty-seven (36.5%) had septic shock. Bacteremia was most often associated with skin and soft tissue infections (26.9%), respiratory tract infection (21.8%), endovascular infection (14.7%), intraabdominal-pelvic infection (10.3%), and bone and joint infection (9%). Most cases were associated with animal contact (80%), and most patients had underlying medical conditions, especially cirrhosis, malignancy, alcoholism, COPD, and diabetes.[59] Mortality was high, especially in immunocompromised patients, but overall mortality decreased substantially each decade starting from 1950 to 2010. *P. dagmatis*, although less frequent than *P. multocida*, has more frequently been reported causing bacteremia rather than localized infection.[60]

Infective endocarditis has been reported much less frequently than septicemia. From a group of 32 patients reported in the literature,[61–63]

preexisting heart disease was evident in 12 cases. Animal contact was evident in 50% of patients. Rare cases of prosthetic valve endocarditis with *Pasteurella* spp. (especially *P. dagmatis*) have been reported.[64–66]

Respiratory Tract Infections

Respiratory tract infections with *Pasteurella* spp. involve the upper respiratory tract, causing sinusitis and bronchitis, and the lower respiratory tract, causing both pneumonia and empyema.[67] The respiratory tract is second only to skin and soft tissue in frequency of clinical isolation of *Pasteurella*. *P. multocida* subsp. *multocida* has been reported to be the most common *Pasteurella* spp. to cause respiratory tract infections.[6,42] As noted, asymptomatic *Pasteurella* colonization of the upper respiratory tract has been reported in patients with underlying respiratory tract disease, including COPD and bronchiectasis.[12] Presumably for a subset of this patient group, the organism invades and causes disease. Chronic symptomatic exacerbations of underlying lung disease with isolation of *Pasteurella* have been described uncommonly.[14] There is nothing clinically distinguishing about upper respiratory tract infections. Pneumonia usually occurs in patients with underlying lung disease and is usually lobar, with a short prodrome. Twenty-one *Pasteurella* pleural effusion and empyema cases were reviewed by Jogani and colleagues.[68] The median age was 69 years, and 57% had comorbidities, such as COPD, bronchiectasis, or cirrhosis. In 87% of cases the pleural fluid obtained was grossly purulent. Although respiratory and constitutional complaints are dominant presenting symptoms, fever seems surprisingly uncommon.[69]

Intraabdominal Infections

Of the few reported cases of *Pasteurella* intraabdominal infections, spontaneous bacterial peritonitis and appendicitis, with or without associated peritonitis, have been the most frequent clinical syndromes. Among the reported cases of spontaneous bacterial peritonitis, almost all have had cirrhosis (usually alcoholic) and preexisting ascites.[70,71] Raffi and associates[72] reported three cases of *P. multocida* appendiceal peritonitis and identified eight additional well-documented cases of appendicitis in the literature. Accompanying peritonitis was variably present. It was postulated that the source of the organism was most likely from oropharyngeal colonization. Several cases of peritonitis have been reported in association with peritoneal dialysis.[73,74] Cats were the presumed source in most patients, but dog- and hamster-related cases have been reported, some with *P. aerogenes* and *P. pneumotropica* as the causative organism.[75,76] Poor hygiene, including manipulation or puncture of the peritoneal dialysis access, was thought to be the likely cause of infection.

Other *Pasteurella* Infections

Infections of other sites with *Pasteurella* spp. have been reported rarely and include genitourinary tract, infection,[77,78] epiglottitis,[79] keratitis,[80] and endophthalmitis.[15,81] *Pasteurella* infections have also been reported in a variety of immune deficiencies, including bronchitis in a patient with Sweet syndrome,[82] fatal sepsis in a patient with hairy cell leukemia,[83] cellulitis with bacteremia in a neutropenic host,[84] and malacoplakia in a patient with acquired immunodeficiency syndrome.[85]

THERAPY, PREVENTION, AND PROGNOSIS

See Chapter 315 for management of bite wounds. Several decades of clinical experience with *Pasteurella* and numerous in vitro studies have indicated that penicillin is the best antimicrobial agent for treating virtually all forms of infection.[15,16,20] Penicillins with good in vitro activity include penicillin G, penicillin V, ampicillin, amoxicillin, and amoxicillin-clavulanic acid. Antistaphylococcal penicillins, including oxacillin, nafcillin, dicloxacillin, and cloxacillin are not as active and are not recommended for treatment of documented *Pasteurella* infections.[86,87] Many cephalosporins demonstrate in vitro activity against *P. multocida*. In general, activity increases with later-generation cephalosporins. Goldstein and associates[88] have reported high MICs for cephalexin, cefaclor, and cefadroxil and recommended that they not be used for treatment of documented infections. The oral cephalosporins, cefuroxime and cefixime, along with parenteral agents, including ceftriaxone, cefoperazone, and ceftaroline, demonstrate excellent in vitro activity and are probably good substitutes for penicillin.[87]

Plasmid-mediated β-lactamase production has been described in *P. multocida* strains isolated from animals. Human cases of *Pasteurella* infection caused by penicillin-resistant strains have been reported. Virtually all penicillin-resistant isolates have come from respiratory tract specimens.[14,89] Among non–β-lactam antibiotics, agents with in vitro activity include tetracyclines, fluoroquinolones, macrolides, trimethoprim-sulfamethoxazole (TMP-SMX), and chloramphenicol.[90,91] A fluoroquinolone, doxycycline, or TMP-SMX should be considered as an alternative for patients with intolerance to β-lactams. Aminoglycosides have moderate-to-poor activity in vitro and probably should not be used, particularly given the paucity of clinical experience. Clindamycin and erythromycin consistently demonstrate high MICs in vitro and are not recommended. The long-acting macrolides, such as azithromycin, appear to have better activity but should be used with caution because clinical experience with these agents is limited.[91]

Because animal bite wound infections are frequently polymicrobial and may include *Staphylococcus aureus,* streptococcal species, and anaerobes in addition to *Pasteurella,* empirical antibiotic therapy should be directed at these organisms until or unless wound cultures define the specific bacteriology of the infection.[22] Outpatient treatment of documented, uncomplicated *Pasteurella* cellulitis can be undertaken with penicillin VK, amoxicillin, or ampicillin, with close follow-up.[15] Duration of therapy is not well defined, but 10 to 14 days is probably a reasonable time course. Patients with evidence of involvement of deeper structures (e.g., tenosynovitis, arthritis) should be hospitalized and treated parenterally, with β-lactam–β-lactamase combinations being the treatment of choice until the microbiology is defined. Drainage and débridement may be necessary for patients who have progressive infection with extensive suppuration. Infection of the hand tendons should be regarded as a surgical emergency for incision and drainage.[92]

Treatment of septic arthritis should consist of antimicrobial therapy along with frequent drainage of the involved joint(s).[47] Most patients recover fully. Similarly, the outcome for osteomyelitis appears to be good, although débridement in addition to antimicrobial therapy is often necessary. The outcome of septic arthritis with osteomyelitis is not as good, with residual deformity and loss of function being common. For patients with *Pasteurella* osteomyelitis, antimicrobial therapy should be continued for 4 to 6 weeks.

Patients with other end-organ forms of *Pasteurella* infection do poorly overall. High mortality has been reported for most of these cases. A third-generation cephalosporin is recommended for *Pasteurella* meningitis. For patients with bacteremia without endovascular infection, the mortality rate is high, probably as a result of the infection itself combined with the underlying medical problem, usually alcoholic cirrhosis. For endocarditis, among 32 reported patients, 8 died and 10 required valve replacement.[61–63] For patients with pneumonia, morbidity and mortality are high, almost certainly as a result of severe underlying pulmonary disease. Finally, patients with spontaneous bacterial peritonitis have an extremely high mortality rate, whereas those with appendicitis, with or without peritonitis, generally do well.[70,72]

The use of antimicrobial prophylaxis for patients presenting with animal bites shortly after the injury that are not obviously infected is controversial. The Infectious Diseases Society of America guidelines on skin and soft tissue infections recommends prophylaxis after dog or cat bites in the following groups of patients: (1) are immunocompromised; (2) are asplenic; (3) have advanced liver disease; (4) have preexisting or resultant edema of the affected area; (5) have moderate-to-severe injuries, especially to the hand or face; or (6) have injuries that may have penetrated the periosteum or joint capsule[93] adjacent to bones or joints, or wounds in certain immunocompromised hosts (e.g., splenectomy). Although a few small trials have been completed, none has been large enough or seen enough patients to reach significant end points to determine the efficacy of such therapy unequivocally.[94,95] Amoxicillin–clavulanic acid is a commonly used agent that has activity against *S. aureus,* streptococci, anaerobes, and *Pasteurella*. The combination of either cefuroxime, doxycycline, a fluoroquinolone or TMP-SMX, plus either metronidazole or clindamycin should be alternatives for patients with serious penicillin allergies. Courses of 3 to 5 days are usually recommended.[93]

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The complete reference list is available online at Expert Consult.

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229A Plague (*Yersinia pestis*)

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SHORT VIEW SUMMARY

Definition
- Life-threatening zoonosis caused by *Yersinia pestis*
- Naturally cycles among rodents and their fleas
- Potential bioterrorism agent

Epidemiology
- Globally distributed but found within distinct ecologic foci
- Vector borne, highly invasive, all ages, frequently fatal

Microbiology
- Aerobic, gram-negative member of Enterobacteriaceae, closely related to *Y. pseudotuberculosis*
- Growth at 37°C on MacConkey agar, sheep blood, and brain-heart infusion
- Automated identification systems may misidentify

Clinical Manifestations and Diagnosis
- Regional suppurative lymphadenitis, sepsis, or fulminant pneumonia with plague
- Bioterrorism mass exposure: fulminant pneumonia
- Culture of blood, bubo, or sputum
- Acute and convalescent serology

Therapy
- Immediate use of antimicrobial agents: lifesaving for plague
- Plague: streptomycin, 1 g twice daily; gentamicin, 5 mg/kg once daily; or levofloxacin, 500 to 750 mg once daily, preferred (see Table 229A.2 for options)

Prevention
- Reduce rodent harborage; control fleas on pets
- Avoid contact with rodents and sick animals
- Bioterrorism exposure: doxycycline 100 mg twice daily or levofloxacin 500 mg, once daily

The genus *Yersinia* includes at least 19 described species, of which 3 are important human pathogens.[1] *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are enteric pathogens usually acquired through ingestion of contaminated food or water. The third species, *Yersinia pestis,* causes plague. Although closely related to *Y. pseudotuberculosis, Y. pestis* has undergone a marked evolutionary shift to become a vector-borne pathogen capable of achieving the high levels of bacteremia in mammalian hosts.

HISTORY

Plague has been credited with causing at least three major pandemics over the past 1500 years.[2] The first struck the Byzantine Empire during the sixth century, killing an estimated 40 million persons in the Mediterranean basin. The second pandemic began in Central Asia and spread west along caravan routes to reach the Crimean Sea port of Kaffa (now Feodosiya in Ukraine) in 1346.[3] In what is believed to be the first use of plague as a weapon, the Mongols, who were battling the Genoese for control of the city, reportedly catapulted bodies of plague victims over the city walls. Genoese ships returning to Italy introduced the disease into several port cities, and from 1347–54, the "Black Death" spread swiftly through Europe and the British Isles, killing an estimated one-third of the population.[4] Although some historians have questioned whether the Black Death was actually caused by plague,[5] evidence from archeologic and transmission studies strongly supports the role of *Y. pestis* as the causative agent.[6–8] Plague persisted in Europe for the next 300 years, causing periodic outbreaks before dying out.

The third, or "modern," pandemic began in China's Yunnan Province in the 1850s. Infection spread along trade routes to Hong Kong, where Alexandre Yersin isolated the causative agent in 1894. Four years later Paul-Louis Simond identified the plague bacillus in the tissues of dead rats and subsequently proposed a role for fleas in transmission. Over the ensuing 20 years steamships helped disseminate *Y. pestis*–infected rats and fleas to port cities worldwide, including several in North America. Rat-associated plague was soon brought under control in most urban areas; however, infection spread to other species of rodents and became entrenched in rural areas of the Americas, Africa, and Asia. The region most affected has shifted over time from India, where more than 20 million cases occurred during the first half of the 20th century, to war-torn Vietnam during the 1960s and 1970s, and finally to sub-Saharan Africa. Collectively, Madagascar, Uganda, Tanzania, and the Democratic Republic of Congo currently account for the majority of cases reported worldwide.[9–12]

Along with its historical importance, *Y. pestis* has considerable potential as a biological weapon. During World War II the Japanese military dropped ceramic bombs with plague-infected fleas over areas of China, apparently causing outbreaks. Both the United States and the Soviet Union evaluated *Y. pestis* as a potential weapon during the Cold War era, and in the 1980s a Soviet defector to Great Britain revealed that the Russians had succeeded in preparing a powdered aerosolized form, genetically engineered to be resistant to several antibiotics. The potential for misuse by terrorists is considered an important national security threat, requiring special measures for medical and public health preparedness.[13] The plague bacillus is designated as a Tier I (formerly Category A) select agent whose handling is regulated by federal law.[14,15]

MICROBIOLOGY

Y. pestis is an aerobic, gram-negative coccobacillus that exhibits bipolar staining with Giemsa, Wright, and Wayson stains. A member of the Enterobacteriaceae, it grows well on blood or MacConkey agar and in nutrient broths, such as brain-heart infusion. When incubated at 37°C, small colonies 1 to 2 mm in diameter are visible on blood or MacConkey agar after 24 to 48 hours. At 72 hours, colonies grown on blood agar can take on a raised, irregular, "fried-egg" morphology. *Y. pestis* does not form spores and, unlike *Y. enterocolitica* and *Y. pseudotuberculosis,* is nonmotile when incubated at lower temperatures. It does not ferment lactose and is citrate, urease, and indole negative.[16]

Genomic studies indicate that *Y. pestis* evolved recently from the enteric pathogen *Y. pseudotuberculosis*.[17,18] The transition from enteric to flea-borne pathogen required the ability to survive in the flea gut and to achieve high concentrations in the blood of mammalian hosts. These traits were endowed in part through the acquisition of two plasmids encoding factors that are differentially expressed at temperatures encountered in fleas and mammals.[19–21] The 110-kilobase (kb) pMT1/pFra plasmid encodes both *Yersinia* murine toxin (Ymt), which is

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

necessary for colonization of the flea midgut, and the fraction 1 (F1) envelope antigen, which inhibits phagocytosis in mammals (Table 229A.1). The smaller 9.5-kb pPCP1 plasmid encodes a plasminogen activator protein (Pla protease) that is responsible for temperature-dependent coagulase and fibrinolysin activities. The origins of these plasmids remain uncertain; however, approximately half of the DNA sequences of the pMT1/pFra plasmid are shared with a plasmid of *Salmonella enterica* serotype Typhi.[22]

As with the other yersiniae, the plague bacillus also has an approximately 70-kb plasmid that mediates expression of virulence factors that prevent production of proinflammatory cytokines, increases resistance to phagocytosis, and enhances intracellular survival.[23] Chromosome-mediated factors include a potent lipopolysaccharide endotoxin and a pigmentation factor, the hemin storage locus *(hms),* which regulates iron uptake and enables the bacteria to form blockages of the flea gut that enhance transmission.[19,20]

Y. pestis isolates can be classified into three principal biovars based on their ability to ferment glycerol and reduce nitrate. It has been postulated that these biovars—Antigua, Medievialis, and Orientalis—reflect strains associated with the first, second, and third pandemics, respectively. Various molecular methods suggest, however, that these biovars do not correlate fully with phylogenetic relationships, and although Orientalis is associated with the third pandemic, the association of earlier pandemics with specific biovars remains uncertain.[18,24,25]

EPIDEMIOLOGY

Although not the scourge it once was, plague remains a threat in areas of Africa, Asia, and the Americas, including the western United States (Fig. 229A.1).[11,26] Before 2007, when required notification was discontinued, between 1000 and 6000 cases were reported annually to the World Health Organization (WHO) from 25 countries.[27] Nearly 80% of cases were reported from Africa, 15% from Asia, and the remainder from the Americas. During 2010–15, only about 500 plague cases were reported annually to WHO.[12] Nevertheless, the potential for cases and outbreaks persists, especially in rural areas of Madagascar, Uganda, and the Democratic Republic of Congo. In 2017 an outbreak of pneumonic plague was reported in the capital city of Madagascar, with more than 2700 suspect cases identified. Although the true number of infections

TABLE 229A.1 Key Virulence and Transmission Factors of *Yersinia pestis*

LOCATION	FACTOR(S)	PUTATIVE FUNCTION
pPCP1 plasmid (9.5 kb)	Plasminogen activator (Pla protease)	Protease: targeted activity at mammalian tissue barriers; adhesion: enhances invasion of mammalian cells; coagulase: promotes blockage of flea midgut
pCD1 plasmid (70–75 kb)	*Yersinia* outer protein (Yop) virulon	Type III secretion system inhibits phagocytosis and lymphocyte proliferation; transport of effector proteins to host cells
	V antigen	Facilitates intracellular survival; required for type III secretion system translocation pore
pMT1/pFra plasmid (≈110 kb)	Fraction 1 antigen	Expressed at higher temperatures; creates capsule that interferes with phagocytosis
	Yersinia murine toxin (Ymt)	Phospholipase D activity upregulated at lower temperatures, necessary for colonization of flea midgut and blockage formation; toxic for mice and rats
Chromosome (4.6 Mb)	Hemin storage system (Hms)	Proteins produced at lower temperatures; iron acquisition; colonization of flea proventriculus, biofilm production
	Yersiniabactin (Ybt) system	Siderophore; iron acquisition
	Yersinia Fe uptake system	Adenosine triphosphate–binding cassette transport system; iron acquisition
	Lipopolysaccharide	Temperature-dependent remodeling of lipid A structure; prevents containment by mammalian immune response
	pH 6 fimbriae antigen (Psa)	Blocks phagocytosis; pH dependent

kb, Kilobase; *Mb,* megabase.
Data from references 1, 12, and 14.

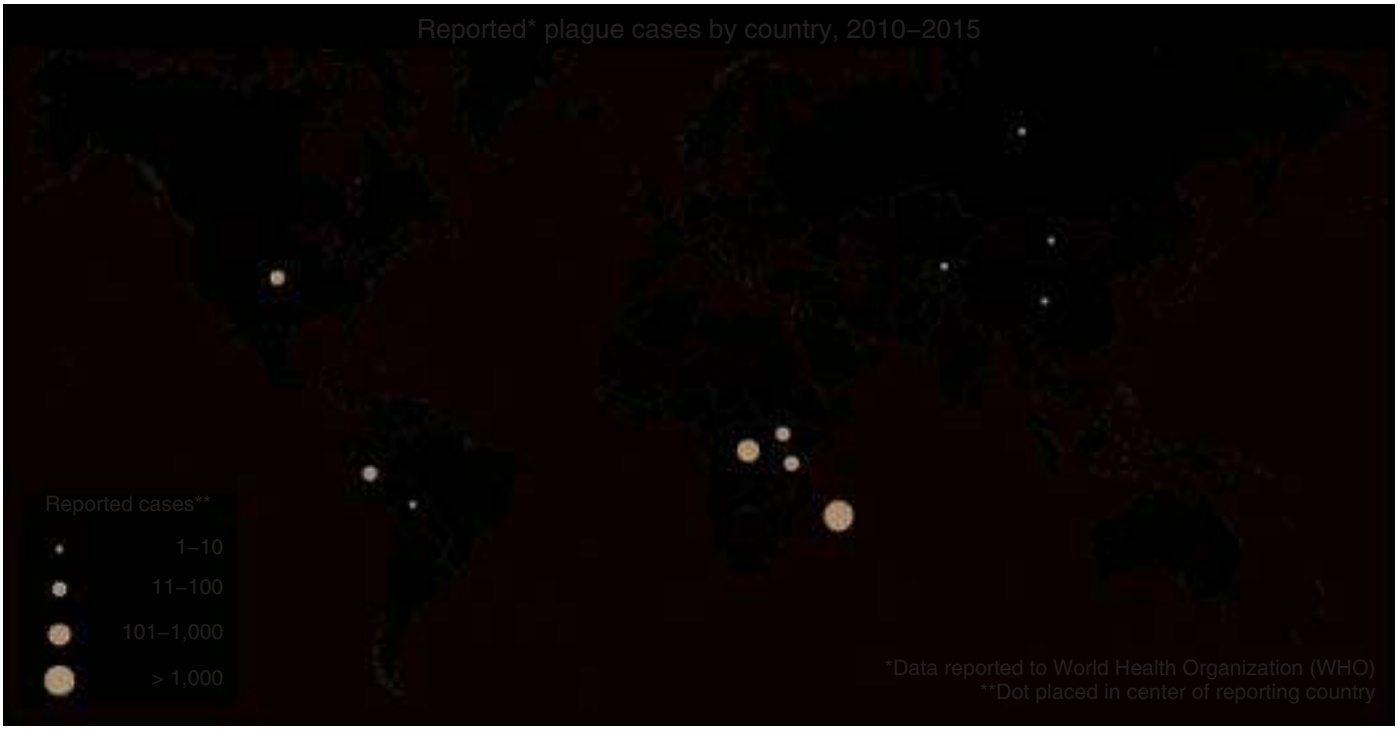

FIG. 229A.1 Worldwide distribution of plague in humans (as reported to the World Health Organization), 2010–15. Dots are placed in center of reporting country and are proportional to the number of reported cases. *(Data compiled from the World Health Organization, Centers for Disease Control and Prevention, and other sources.)*

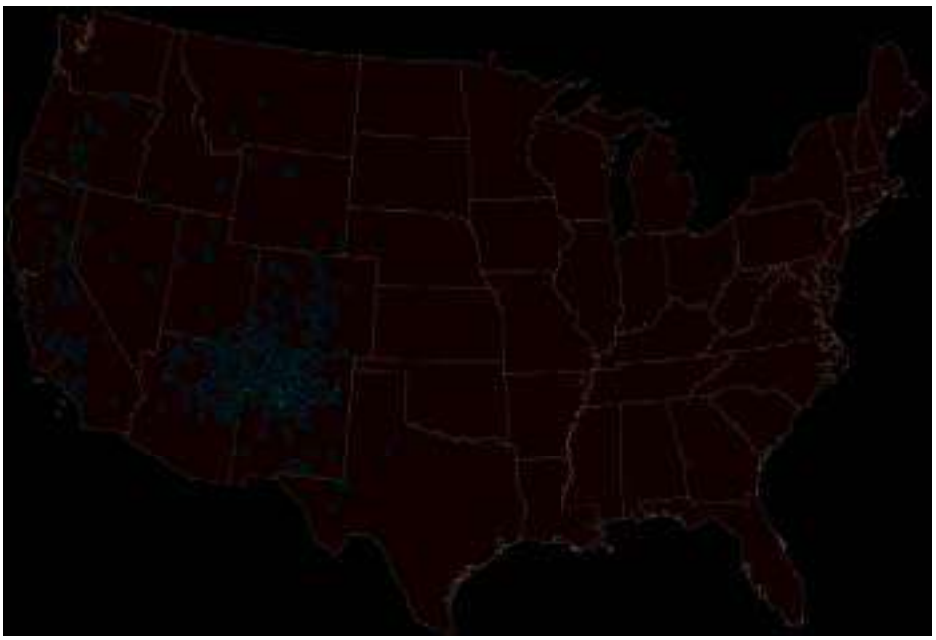

FIG. 229A.2 Cases of plague in the western United States, 1970–2016. Each dot represents one case, with the dot placed randomly in the county of exposure. *(Courtesy Centers for Disease Control and Prevention.)*

was likely far lower, the social and economic impact was substantial.[28] As demonstrated by reports of human plague cases in Algeria and Libya, the disease can reemerge in an area even after decades of quiesence.[29]

In the United States plague has been reported since 1900.[30] Initially restricted to port cities, plague is currently endemic in the 17 contiguous western states extending from the Pacific Coast to the Great Plains. A total of 437 cases in humans were reported during 1970– 2010 (≈10 cases per year), with 60 deaths (14% mortality). During 2010–16 there were 2 to 16 cases per year in the United States.[12,31] Approximately 80% of human cases occur in New Mexico, Arizona, and Colorado and 10% in California (Fig. 229A.2).[32] Human plague is most common during May through October, when fleas are active; wintertime cases are rare and often associated with hunting. Males are slightly more likely to be infected, and more than half of cases occur in persons younger than 20 years. Incidence is highest among Native Americans and Hispanics, although non-Hispanic whites account for the majority of cases.[33] Within endemic areas, elevated plague risk is associated with close contact with rodents and their feline and canine predators, the presence of harborage and food sources for wild rodents in the vicinity of homes, and possibly a failure to control fleas on pet dogs and cats.[34,35] Expanding suburban development in prime plague habitat has led to increasing cases in some areas and a shift toward higher socioeconomic groups in recent years.[36] Travelers with plague can present a diagnostic challenge when encountered outside endemic areas (peripatetic plague) and may trigger concern about possible terrorist exposures.[37]

NATURAL CYCLES

The epizootiology of plague is complex and not completely understood (Fig. 229A.3).[38] Fundamentally a flea-transmitted disease of rodents, plague is found in endemic foci scattered throughout the world. Different foci involve different rodent and flea species, each with their own ecology and predilection for disease. Perhaps triggered by climatic conditions, epizootics characterized by explosive spread and sudden mass mortality among susceptible rodents occur periodically. Epizootics promote dispersal of infected fleas and increase the risk for transmission to humans, especially when rodent species living in close proximity to humans are involved. Worldwide, the most concerning are the peridomestic rats, *Rattus rattus* and *Rattus norvegicus,* and their highly efficient flea vectors, *Xenopsylla cheopis* and *Xenopsylla braziliensis.* In the western United States epizootics occur among ground squirrels (*Spermophilus*

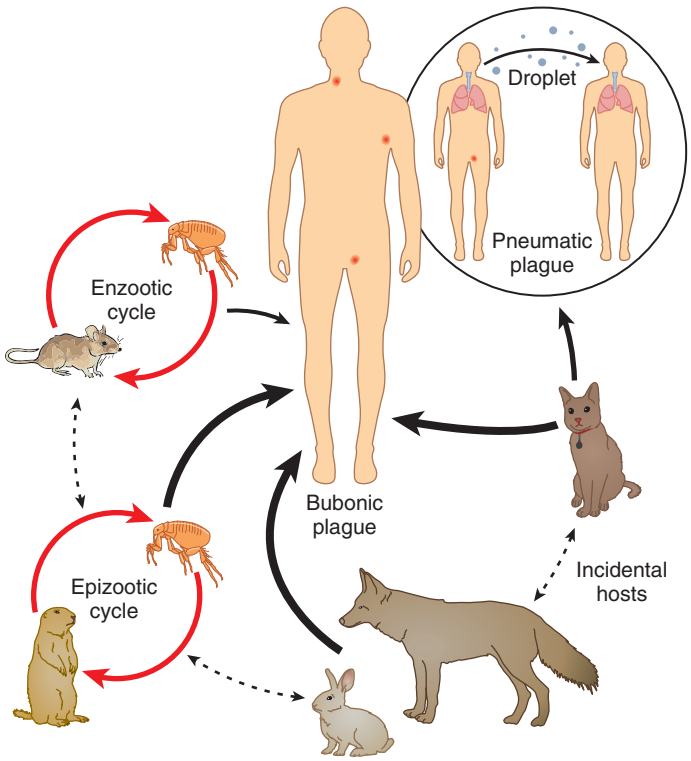

FIG. 229A.3 Ecology and transmission of plague. Red arrows indicate zoonotic cycles.

spp.), prairie dogs (*Cynomus* spp.), and chipmunks (*Tamias* spp.), as well as less familiar rodent species.

How *Y. pestis* survives in nature between epizootics is uncertain. One hypothesis holds that the organism continues to circulate slowly and undetected in enzootic cycles involving rodent species that are less susceptible to fulminant infection.[39] An alternative hypothesis holds that although the organism dies rapidly on inanimate surfaces, it may be able to survive in a niche within the soil, periodically infecting

burrowing rodent hosts.[6] This second hypothesis has received support recently by the demonstration that *Y. pestis* can replicate within certain free-living ameba,[40] an ability that may also relate to the organism's capacity as a facultative intracellular pathogen.

TRANSMISSION TO HUMANS

Humans become infected with *Y. pestis* through flea bites, direct contact with tissues or secretions of infected animals, or rarely through inhalation of infectious aerosols (see Fig. 229A.3). Flea-borne transmission is especially common during epizootics when large numbers of rodents die and their fleas seek alternative sources for a blood meal. Hunters become infected through direct inoculation while skinning or handling carcasses of infected rodents, rabbits and hares, wild cats, and coyotes.[41] Direct inoculation is associated with an increased risk for septicemia and death, possibly because the bacterium, coming directly from a warm-blooded host, is already expressing F1 antigen and thus less susceptible to phagocytosis. Consumption of uncooked meat from camels, goats, and other ungulates, which are also susceptible to infection, has been identified as the source of small outbreaks in northern Africa, the Middle East, and Central Asia.[42] Aerosols generated while handing infected animals can also pose a risk, as demonstrated by the case of a US wildlife biologist who developed primary pneumonic plague after necropsying an infected mountain lion.[43] Although generally "dead-end" hosts, humans who develop pneumonic plague can transmit the infection to close contacts through respiratory droplets.

Domestic pets are also an important source of potential exposure. Domestic cats that eat infected rodents develop pharyngeal infections that can be transmitted directly to humans through respiratory droplets, causing primary pneumonic plague.[44] Although dogs appear less likely to become clinically ill when infected, fulminant pneumonia in dogs with transmission to owners has been reported.[45] In addition, dogs may play a role in increasing human exposure by transporting rodent fleas into the home, especially if they are allowed to sleep on their owner's bed at night.[34] Laboratory infections, once common, are rare under current practices. Nevertheless, in 2009 a US laboratory researcher died of sepsis caused by an attenuated strain of *Y. pestis* that lacked the virulence genes necessary for iron absorption. Postmortem evaluation revealed that the worker had undiagnosed hemochromatosis.[46,47]

PATHOGENESIS

Fleas become infected when feeding on a bacteremic host. In the cooler environment of the flea, the bacillus expresses a variety of factors that facilitate colonization of the flea midgut, replication, and blockage of the flea intestine (see Table 229A.1).[21] Starved of sustenance, "blocked" fleas feed aggressively, regurgitating bacteria into the bite wound. Many of the inoculated bacteria are likely phagocytized and killed by polymorphonuclear lymphocytes; however, a few are taken up by mononuclear cells and carried via lymphatics to the regional lymph nodes.[19] Growing at 37°C, the bacteria begin expressing F1 envelope antigen, enhancing their ability to resist subsequent phagocytosis by polymorphonuclear lymphocytes.[2,19] Within the lymph node, the bacilli stimulate an intense inflammatory response that is detectable clinically as a bubo. Microscopic examination of the fully developed bubo reveals invasion by polymorphonuclear leukocytes, hemorrhagic necrosis with destruction of normal architecture, and dense concentrations of extracellular bacilli. Bacteremia is common and, in the absence of specific therapy, can lead to secondary pneumonia, disseminated intravascular coagulation, acute renal failure, and irreversible shock.[48] Blockage of vessels in cooler acral sites, including fingers, toes, ears, and nose, can lead to gangrene (Fig. 229A.4), a startling feature that may have given rise to the name "Black Death."[49] Pathogenesis of pneumonic plague is marked by a rapid, destructive proinflammatory phase that results in rapid death.[50]

CLINICAL MANIFESTATIONS

Plague takes several different clinical forms, depending in part on the route of exposure. In the United States 80% to 85% of patients present with primary bubonic plague, 15% with septicemic plague, and 1% to 3% with pneumonic or other forms of plague. The usual incubation period is 2 to 7 days but can be as short as 1 day for patients with primary pneumonic plague.

Bubonic Plague

Bubonic plague results from cutaneous exposure and is characterized by the sudden onset of high fever, chills, weakness, and headache. A bubo or swelling of regional lymph nodes becomes apparent in the groin, axilla, or neck within the first day. Buboes vary from 1 to 10 cm and elevate the overlying skin, which may be warm and erythematous. Palpation typically elicits extreme tenderness. Bubonic plague is distinguished from other forms of lymphadenitis by its sudden onset, the intensity of inflammation in the bubo, and the usual absence of obvious skin lesions or associated ascending lymphangitis (Fig. 229A.5). Nevertheless, careful examination distal to the bubo will occasionally reveal a small papule or scab demarcating the site of the flea bite.[48,51] Rarely, ulcers or eschars may develop, which can be confused with those of anthrax or tularemia (Fig. 229A.6). The differential diagnostic possibilities for bubonic plague include cat-scratch disease, staphylococcal or streptococcal lymphadenitis, tularemia, filarial lymphadenitis, chancroid and other sexually transmitted diseases, and strangulated hernia.

Septicemic Plague

Septicemic plague is characterized by the sudden onset of high fever without associated bubo or other obvious localizing signs. Illness is rapidly progressive, leading to overwhelming sepsis and organ failure within a few days. Typically, the diagnosis is not considered until *Y.*



FIG. 229A.4 Hand of a patient with plague displaying acral gangrene, a manifestation that may have given rise to the name "Black Death." *(Courtesy Centers for Disease Control and Prevention.)*



FIG. 229A.5 Femoral and inguinal buboes in a boy showing marked edematous swelling, erythema, and overlying desquamation. *(Courtesy Centers for Disease Control and Prevention.)*



FIG. 229A.6 Axillary bubo with ulceration and small eschar at the site of an infective flea bite. *(Courtesy Centers for Disease Control and Prevention.)*

pestis is isolated from blood cultures. Some patients have gastrointestinal symptoms of nausea, vomiting, diarrhea, or abdominal pain, which may further complicate the diagnostic workup.[52] Because of delays in diagnosis and treatment, the case-fatality rate for septicemic plague in the United States is approximately 28%, three times higher than for bubonic plague.[53,54]

Pneumonic Plague

Pneumonic plague occurs in two forms, secondary and primary, both of which are frequently fatal and potentially contagious to close contacts.[55,56] Secondary pneumonic plague is the more common form and arises through hematogenous spread of bacteria from a bubo or other source. Approximately 10% of all plague patients in the United States develop secondary pneumonic plague, usually as a result of delayed treatment of bubonic infections. Secondary pneumonic plague begins as an interstitial process with cough productive of scant, tenacious sputum, typically beginning 5 to 6 days after illness onset. Chest radiographs reveal diffuse alveolar infiltrates that are almost always bilateral and often accompanied by pleural effusions.[57] If untreated, sputum will become more copious and eventually bloody, and death will often occur within 3 to 4 days.

Primary pneumonic plague is a fulminant condition that results from direct inhalation of bacteria into the lungs. This can occur through contact with another patient with pneumonic plague, exposure to animals (especially cats) with respiratory or pharyngeal plague, laboratory exposures, or, potentially, as a result of intentional aerosol release for purposes of terrorism. Symptoms begin within 1 to 4 days after exposure. Patients experience sudden onset of fever, chills, headache, malaise, and rapidly advancing tachypnea, dyspnea, hypoxia, chest pain, cough, hemoptysis, and general signs of endotoxemia. Chest radiographs demonstrate a lobar pneumonia initially (Fig. 229A.7), followed by dense consolidation and bronchopneumonic spread to other lobes of the same or opposite lung.[44,58] Sputum is often purulent but may be watery, frothy, and copious and also may be blood tinged or grossly hemorrhagic, at which point it may contain large numbers of plague

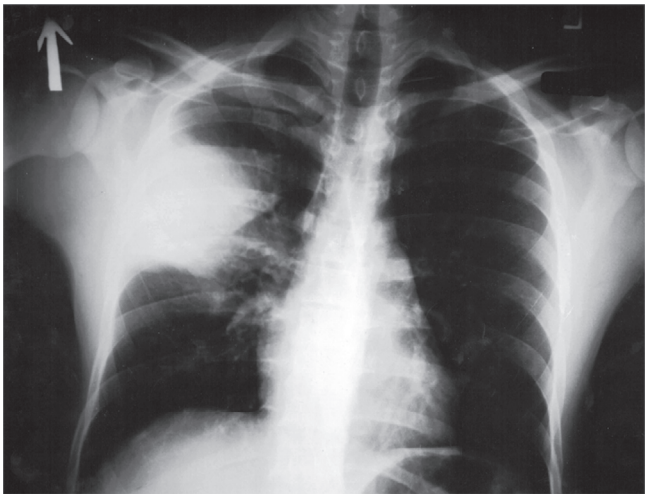

FIG. 229A.7 Chest radiograph of patient with primary pneumonic plague. *(Courtesy Centers for Disease Control and Prevention.)*



FIG. 229A.8 Photomicrograph of lung tissue from fatal case of primary pneumonic plague and septicemic plague. Note filling of alveolar spaces with inflammatory cells and debris. *(Courtesy Centers for Disease Control and Prevention.)*

bacilli.[55] Histologically, numerous bacilli and inflammatory cells fill the alveolar space[50] (Fig. 229A.8). Untreated pneumonic plague is almost always fatal, and mortality is very high in persons whose treatment is delayed beyond 24 hours after symptom onset. The case-fatality rate for pneumonic plague in the United States since 1950 approaches 25%.

To prevent person-to-person transmission, patients with suspected pneumonic plague should be managed in isolation under respiratory droplet precautions.[13,59] Strict isolation under negative pressure and filtering exhaust is not necessary because infectious dispersal by fine aerosol or dried droplet nuclei does not occur. Person-to-person transmission of pneumonic plague requires close contact, typically with a patient who is in the late stages of infection and coughing copious amounts of bloody sputum.[60] The last confirmed outbreak of person-to-person respiratory spread in the United States occurred in Los Angeles in 1924, and there have been at least nine subsequent cases of primary pneumonic plague without secondary transmission.[44] Nevertheless, in 2014 three persons in Colorado developed primary pneumonic plague after exposure to an ill, infected dog. Although it could not be proven, one of the patients may have acquired the infection while caring for an infected family member in the hospital.[45]

Other Syndromes

Plague meningitis is a rare complication that can occur acutely or as a delayed manifestation of inadequately treated bubonic plague. Symptoms include fever, headache, sensorial changes, and meningismus. Examination of cerebrospinal fluid (CSF) reveals a pleocytosis with predominance

of polymorphonuclear leukocytes. Bacteria are frequently demonstrable with a Gram or Wayson stain of CSF sediment. Another rare presentation is pharyngeal plague, which resembles acute tonsillitis. The anterior cervical lymph nodes are usually inflamed, and *Y. pestis* may be recovered from a throat culture or by aspiration of a cervical bubo. Asymptomatic pharyngeal colonization with *Y. pestis* has also been reported among close contacts of patients with pneumonic plague. Osteomyelitits involving both skull and long bones has been reported.[61]

LABORATORY FINDINGS

Laboratory findings in patients with plague are similar to those of patients with other serious gram-negative infections. Peripheral white blood cell counts generally range from 10,000 to 25,000 cells/mm^3 with a predominance of immature neutrophils. Leukemoid reactions with counts as high as 50,000/mm^3 or greater can occur. Platelet counts may be normal or low in the early stages of bubonic plague. Bacteremia is common; in one series, single blood cultures obtained at the time of hospital admission were positive in 27% of cases.[51] Without prompt treatment, patients develop such high-level bacteremia that bipolar staining bacilli can be seen in the peripheral blood smear, a finding that strongly suggests the diagnosis of plague and is associated with a poor outcome (Fig. 229A.9). Disseminated intravascular coagulation, thrombocytopenia, elevated results of liver function tests, and impaired renal function are all common in advanced stages of illness.[51]

DIAGNOSIS

Plague should be considered in any patient with an acute febrile illness and a risk for exposure to infected animals or fleas in a plague-endemic area. A careful history and thorough physical examination are required to make a timely diagnosis; a delayed or missed diagnosis of plague is associated with a high case-fatality rate. In the United States the Laboratory Response Network provides upgraded, standardized diagnostic testing for *Y. pestis* and other select agents.[14,62] All state public health laboratories have the ability to conduct rapid and confirmatory testing or, if necessary, forward materials to the CDC for confirmation and advanced procedures, including molecular subtyping.[63]

When plague is suspected, diagnostic specimens should be obtained promptly and effective antimicrobial therapy initiated immediately thereafter.[13] Chest radiographs should be obtained to rule out pneumonia. Appropriate diagnostic specimens include blood cultures and bubo aspirates, sputum, tracheobronchial washes, swabs of skin lesions or pharyngeal mucosa, and CSF, as indicated by signs and symptoms. Bubo aspirates are especially useful and can be obtained by inserting a 20-gauge needle on a 10-mL syringe containing 2 mL of sterile saline solution into the bubo and withdrawing the plunger several times until the saline becomes blood tinged.

Primary specimens can be stained with Wayson or Giemsa stain and with Gram stain, and then examined using light microscopy. In the case of pneumonic plague, stain and culture of bronchial washings or expectorated sputum may yield a putative diagnosis quickly. With Wayson staining, *Y. pestis* appears as light blue bacilli with dark blue polar bodies, giving the organisms a closed safety-pin appearance that is characteristic of, but not pathognomonic for, *Y. pestis*. If possible, the specimens should also be examined using direct fluorescent antibody (DFA) testing. Presumptive identification of *Y. pestis* can be made by polymerase chain reaction assay.[16,63,64]

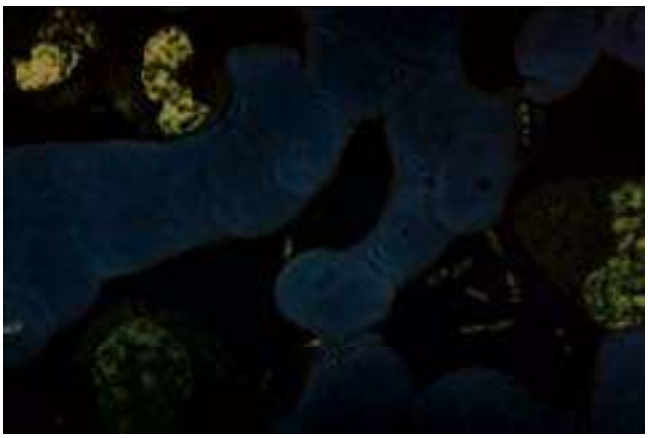

FIG. 229A.9 Peripheral blood smear of septicemic plague patient showing large numbers of bipolar-staining bacilli. *(Courtesy Centers for Disease Control and Prevention.)*

Laboratory confirmation is best achieved through the isolation of *Y. pestis* from body fluids or tissues. Samples should be inoculated onto suitable culture media (e.g., brain-heart infusion broth, sheep blood agar, chocolate agar, or MacConkey agar) and held for 5 to 7 days.[16] Plague bacilli are readily distinguished from other gram-negative bacteria by staining properties, growth characteristics, and biochemical profiles. Nevertheless, automated identification systems frequently misidentify *Y. pestis* as other species, an error that frequently delays diagnosis.[65] Isolates are confirmed in reference laboratories by lysis with *Y. pestis*–specific bacteriophage.

In patients with negative cultures, plague can be confirmed serologically by passive hemagglutination testing for antibodies to *Y. pestis* F1 antigen. A fourfold or greater change in titer between an acute serum and a convalescent serum collected 3 to 4 weeks later is considered diagnostic, as is a single titer greater than or equal to 1:128 in an unvaccinated patient with compatible illness.[16] A small percentage of plague patients will develop diagnostic antibody levels within 5 days after illness onset, most seroconvert within 1 to 2 weeks, a few seroconvert more than 3 weeks after onset, and less than 5% fail to seroconvert.[66] Rapid, handheld chromatographic assays designed to detect *Y. pestis* F1 antigen in patient samples are being evaluated for rapid presumptive diagnosis at the bedside.[67]

For diagnosis in fatal cases, samples of lymph nodes, liver, spleen, lungs, and bone marrow should be collected at autopsy for culture, DFA antibody testing, and histologic studies, including immunohistochemical staining. Cary-Blair or a similar holding medium can be used to transport swabs or tissues for culture.

THERAPY

Antimicrobial Agents

Without treatment, 50% of patients with bubonic plague and nearly all patients with septicemic or pneumonic plague will die. Effective antibiotic therapy should be given immediately after obtaining diagnostic specimens. Although fluoroquinolones may offer a less toxic alternative (see later), streptomycin has been considered the drug of choice since its introduction in the 1940s, and prompt administration can reduce the mortality rate in bubonic plague to 5% or less. Streptomycin should be administered intramuscularly twice daily, in a dose for adults of 15 mg/kg/dose (maximum, 1 g) for 7 days or at least 3 days after remission of fever and other symptoms. Most patients improve rapidly and become afebrile after approximately 3 days of therapy.[48] Streptomycin is ototoxic and nephrotoxic and should be used cautiously in pregnant women, older patients, and patients with hearing difficulty.

Although not approved by the US Food and Drug Administration (FDA) for treatment of plague, gentamicin has been proposed as an acceptable alternative based on in vitro susceptibility studies, animal models, and reports of efficacy in treating humans with plague.[13,68–72] A retrospective analysis of 50 plague patients treated in New Mexico between 1985 and 1999 suggests that gentamicin, or a combination of gentamicin and doxycycline, is at least as efficacious as streptomycin.[73] All 36 gentamicin-treated patients survived without complications. In a randomized trial of 65 patients with plague in Tanzania, 94% of patients treated with gentamicin recovered.[74] Gentamicin is considered to be safer than streptomycin for use in pregnant women and children.

For patients with contraindications to the use of aminoglycosides, tetracycline and its congeners are satisfactory alternatives. Doxycycline is the tetracycline of choice in treating plague because of the convenience of its twice-daily dose schedule, its rapid absorption from the gut, and its superior ability to achieve peak serum concentrations. Doxycycline treatment should be initiated with a loading dose, either intravenously or orally, depending on the severity of illness. In adults a loading dose of 200 mg every 12 hours on the first day *rapidly* achieves a peak serum concentration of approximately 8 μg/mL[75] and is followed by a daily dose

of 100 mg every 12 hours. Tetracycline is administered to adults in an initial loading dose of 2 g, followed by a usual dose of 2 g/day in four divided doses. Doxycycline or tetracycline can also be used to complete a course of treatment begun with an aminoglycoside. When used as principal treatment, a tetracycline should be given for 7 to 10 days or for at least 3 days after fever and other symptoms have subsided.

Levofloxacin, ciprofloxacin, and moxifloxicin have been approved recently by the FDA for treatment of plague, based on studies showing the efficacy in vitro and in animal models, including African green monkeys.[76–82] Published information on successful treatment of human plague is limited, however.[83] In a small case series from Uganda, six patients with culture-confirmed plague, including one with secondary pneumonic plague, were treated successfully with oral ciprofloxacin.[84] Based on FDA approval and pharmacologic properties, either levofloxicin or chloramphenicol should be used for conditions in which high tissue penetration is important, such as plague meningitis, pleuritis, or myocarditis.[13,68,80] It may be used separately or in combination with an aminoglycoside. Chloramphenicol is given as a loading dose of 25 to 30 mg/kg, followed by 50 to 60 mg/kg/day in four divided doses. As indicated by clinical response, the chloramphenicol dose may be reduced to a daily dose of 25 to 30 mg/kg/day to lessen the magnitude of bone marrow suppression, which is reversible. The irreversible marrow aplasia associated with chloramphenicol is so rare (estimated to occur in 1 in 40,000 patients) that its consideration should not deter its use in patients who are seriously ill with plague infection. Trimethoprim-sulfamethoxazole (cotrimoxazole) has been used successfully to treat bubonic plague, but responses may be delayed and incomplete, and it is not considered a first-line choice. Penicillins, cephalosporins, and macrolides have a suboptimal clinical effect and are not recommended for use in treating plague.

Strains of *Y. pestis* that are resistant to antimicrobial agents have only rarely been isolated from humans. Usually these strains have shown partial resistance to a single agent only and have not been associated with treatment failures. In 1995 two clinical isolates with plasmid-mediated drug resistance were recovered in Madagascar, one with high-level resistance to streptomycin and the second resistant to multiple drugs, including streptomycin, chloramphenicol, ampicillin, tetracycline, and sulfonamides.[68] Nevertheless, both patients recovered when treated per protocol with streptomycin and trimethoprim-sulfamethoxazole. Molecular studies have identified very different plasmids in the two strains, and they are believed to have arisen independently, possibly through horizontal gene transfer in the flea midgut.[68] It is unclear whether such resistance would be expected to propagate in nature in the absence of antibiotic pressure on wild rodent populations, and to date these remain the only such natural isolates identified among thousands tested worldwide. Antimicrobial resistance is not known to have emerged during the treatment of plague in humans, and relapses after recommended courses of treatment are virtually unknown.

Supportive Therapy

The hemodynamic status of patients with plague should be monitored closely and shock managed according to general principles used to combat endotoxic shock.[85] There is no evidence that corticosteroids are beneficial in treating plague. Buboes usually recede during the first week of antibiotic treatment, but it may be several weeks before they completely resolve; on occasion, they enlarge or become fluctuant, requiring incision and drainage.

PREVENTION

Infection Control

All suspected plague cases should be reported immediately to state health department authorities for assistance with confirmation of microbiologic diagnosis, epidemiologic investigation, and protection of the public's health. Patients with uncomplicated infections who are treated promptly do not present a hazard to others. Those with cough or other signs of pneumonia should be placed in isolation and managed under respiratory droplet precautions for at least 48 hours after the initiation of antibiotic therapy or until the sputum culture is negative. Respiratory droplet precautions include the use of masks, gowns, gloves, and protective eyewear when providing direct patient care.[59] Cultures of clinical materials are usually negative after 24 hours of treatment. Patients without respiratory plague can be managed under standard precautions. Potentially infective clinical fluids should be handled with gloves and with care to avoid aerosolization, as could result from dropping a specimen or by breakage of a container during centrifugation. Routine clinical specimens are managed in the laboratory under Biosafety Level 2 precautions, but manipulation of cultures should be performed in a negative-pressure hood using Biosafety Level 3 procedures.

Chemoprophylaxis

Antibiotics can be used for chemoprophylaxis against plague in persons believed to have had an infective exposure within the previous 7 days, such as family members, care providers, and others with a close and direct contact with a patient having pneumonic plague or a laboratory worker exposed to an accident that may have created an infective aerosol.[13] Doxycycline, given in an adult dose of 100 mg twice daily for 7 days, or ciprofloxacin, 500 mg twice daily for 7 days, are appropriate choices for prophylaxis.

Vaccination

Historically, two types of plague vaccine have been available for use in humans, a killed whole-cell vaccine (KWC) and a live-attenuated vaccine (LWC). Neither vaccine is currently licensed for use in the United States, although LWC is reportedly available in Russia.[86] The KWC vaccine is not effective against respiratory exposures and has no usefulness in combating epidemic disease in a setting of modern sanitation and prophylactic antibiotics.[87] Nevertheless, concern for bioterrorism and the possibility of engineered, multidrug resistance have maintained interest in the development of effective vaccines for plague. A series of candidate vaccines have been developed using recombinant capsular subunit protein F1 and the low-calcium response V antigen (LcrV), either as a mixture of the two antigens or as a fusion of the two antigens. Both types of vaccine have shown promise in animal models against subcutaneous, intranasal, or aerosol challenge, and various constructs have been evaluated in phase I and phase II trials.[86] Other recent approaches toward immunization include development of DNA vaccines, expression of protective antigens in live bacterial, viral, and plant carriers; passive immunization with aerosolized monoclonal antibodies; and vaccines based on attenuated *Y. pseudotuberculosis*.[86]

Environmental Control

Persons living in endemic areas should take precautions to reduce exposure to rodents and fleas, including rat-proofing dwellings, removing food and harborage for rodents, using repellents, and applying insecticides on their pets.[32] Local or state health authorities should be consulted for regionally appropriate advice on rodent and vector control. If killing of rodents is considered, flea control should be performed before or at the same time to reduce the chances that infected fleas will feed on humans.

BIOTERRORISM

Y. pestis is designated a Tier 1 select agent, reflecting its high potential for use as a biological weapon. The organism is found naturally in many parts of the world, is relatively easy to manipulate in the laboratory, including the introduction of antimicrobial resistance, and has been previously developed and used as a biological weapon. Furthermore, because of its rich history, plague has a cultural cachet that amplifies its effect as an agent of social disruption well beyond its direct medical impact.[88]

Intentional release of *Y. pestis* could be achieved through several routes; however, an aerosol release is generally considered the most deadly and therefore the most likely. Aerosol exposure would be expected to cause an outbreak of primary pneumonic plague, which is rapidly fatal and has the potential for secondary person-to-person transmission. WHO estimates that a release of 50 kg of *Y. pestis* over a city of 5 million people could result in 150,000 cases of pneumonic plague and 36,000 deaths.[89] Clinicians and hospital laboratorians would almost certainly be the first to recognize an outbreak and should be familiar with the clinical features of pneumonic plague (see earlier discussion).[90,91] The appearance of multiple patients with fulminant pneumonia, especially

if characterized by bloody sputum containing gram-negative rods, should raise consideration of a bioterrorist attack.

To aid in the laboratory recognition of bioterrorism events, the American Society of Microbiology, in coordination with the CDC and the Association of Public Health Laboratories (APHL), has developed protocols designed to assist Sentinel Level Clinical Microbiology Laboratories with information and techniques to evaluate microorganisms that might be suspected as agents of bioterrorism or to refer specimens to public health laboratories for confirmation.[63] In addition, the Working Group on Civilian Biodefense has published recommendations for treatment and postexposure prophylaxis of plague in the setting of a bioterrorism event (Table 229A.2).[13] These recommendations predate FDA approval of fluoroquinolones for treatment of plague, and concerns for engineered multidrug resistance may dictate the use of multiple agents until results of antimicrobial resistance testing are available. Treatment recommendations for contained casualty settings are similar to those listed earlier for naturally occurring plague. In a mass casualty setting, medication shortages may increase the need for use of non–FDA-approved agents. For example, gentamicin is recommended by the Working Group on Civilian Biodefense but has not been approved by the FDA for treatment or prevention of plague. Individual physicians can decide to use these agents from local sources off label; however, use of these drugs from the US government's Strategic National Stockpile (SNS) would require either an Emergency Use Authorization (EUA) issued by the Secretary of Health and Human Services or an Investigational New Drug (IND) application.[92] In anticipation, the CDC has submitted IND and pre-EUA applications for the use of the agents for plague in the event of a bioterrorist attack. WHO has published additional guidance for the public health officials.[93]

MASS PROPHYLAXIS

Mass chemoprophylaxis is a likely public health response after an intentional release of *Y. pestis* and could be a valuable control measure. Although prevention of illness in patients exposed to the primary release would be logistically challenging, given the short incubation period of 1 to 4 days between exposure and illness onset, prophylaxis of their contacts to prevent secondary transmission may be achievable. Nevertheless, recent modeling suggests that the risk for secondary transmission is quite low for most patients and that droplet precautions and social distancing may be quite effective at preventing subsequent transmission.[94] Plague vaccines are not currently available in the United States and, given the short incubation period, would not have a role in an immediate response. A variety of tools have been developed to assist the health care community prepare for a bioterrorism or mass casualty event.[95,96]

OCCUPATIONAL EXPOSURE

A minimum of Biosafety Level 3 PPE (i.e., Tyvek outer clothing, gloves, booties, and positive-pressure high-efficiency particulate air [HEPA]-filtered respirators) should be used for field work involving necropsy of infected animals.[97] Morticians, forensic personnel, and veterinarians should follow precaution standards published for other health care workers.[59] Recommendations on biosafety practices for laboratory workers have been published.[98]

ENVIRONMENTAL PERSISTENCE

Y. pestis does not form spores and is highly susceptible to desiccation and damage by ultraviolet radiation in sunlight. As a general rule, the majority of organisms released into the environment are expected to become nonviable within 1 hour.[89] Nevertheless, plague bacilli can survive in the soil for prolonged periods under certain circumstances and might serve as a source of infection for rodents.[99] Standard treatment of sewage would decrease but not eliminate contamination.[100] Monitoring of animal populations may be a useful tool to assess the geographic extent of environmental contamination and ongoing risk for human infection after an intentional release.

TABLE 229A.2 Working Group on Civilian Biodefense Recommendations for Treatment of Pneumonic Plague[a]

Contained Casualty Setting	
Adults	Preferred choices: Streptomycin: 1 g IM twice daily Gentamicin: 5 mg/kg IM or IV once daily or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV 3 times daily[b] Alternative choices: Doxycycline; 100 mg IV twice daily or 200 mg IV once daily Ciprofloxacin: 400 mg IV twice daily[c] Chloramphenicol: 12–25 mg/kg IV 4 times daily[d]
Children[e]	Preferred choices: Streptomycin: 15 mg/kg IM twice daily (maximum daily dose, 2 g) Gentamicin: 2.5 mg/kg IM or IV 3 times daily[b] Alternative choices: Doxycycline: if ≥45 kg, give adult dosage; if <45 kg, give 2.2 mg/kg IV twice daily (maximum, 200 mg/day) Ciprofloxacin: 15 mg/kg IV twice daily[c] Chloramphenicol: 12–25 mg/kg IV 4 times daily[d]
Pregnant women[f]	Preferred choice: Gentamicin: 5 mg/kg IM or IV once daily or 2 mg/kg loading dose followed by 1.7 mg/kg IM or IV 3 times daily[b] Alternative choices: Doxycycline: 100 mg IV twice daily or 200 mg IV once daily Ciprofloxacin: 400 mg IV twice daily[c]
Mass Casualty Setting and Postexposure Prophylaxis[g]	
Adults	Preferred choices: Doxycycline: 100 mg PO twice daily[i] Ciprofloxacin: 500 mg PO twice daily[c] Alternative choice: Chloramphenicol: 12–25 mg/kg PO 4 times daily[i]
Children[e]	Preferred choices: Doxycycline[h]: if ≥45 kg, give adult dosage; if <45 kg, give 2.2 mg/kg PO twice daily Ciprofloxacin: 20 mg/kg PO twice daily Alternative choice: Chloramphenicol: 12–25 mg/kg PO 4 times daily[i]
Pregnant women[f]	Preferred choices: Doxycycline: 100 mg PO twice daily[h] Ciprofloxacin: 500 mg PO twice daily Alternative choice: Chloramphenicol: 12–25 mg/kg PO 4 times daily[i]

[a] *Modified from Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense.* JAMA. *2000;283:2281–2290.* After publication of these recommendations, the US Food and Drug Administration (FDA) approved levofloxacin, ciprofloxacin, and moxifloxacin for treatment of plague. Therapy continued for 10 days. Oral therapy can be substituted when the patient's condition improves.

[b] Gentamicin is not FDA-approved for treatment of plague, although animal and limited human studies support its use (see text). Evidence suggests that once-daily dosing may be efficacious with lower toxicity. Aminoglycoside dosages should be adjusted for renal function.

[c] Other fluoroquinolones can be substituted at doses appropriate for age. Ciprofloxacin dosage should not exceed 1 g/day in children.

[d] See text regarding loading dose. Concentration should be maintained between 5 and 20 μg/mL; concentrations >25 μg/mL can cause reversible bone marrow suppression.

[e] In children, the ciprofloxacin dose should not exceed 1 g/day.

[f] All recommended antibiotics for plague have relative contraindications for use in children and pregnant women; however, use is justified in life-threatening situations.

[g] The duration of postexposure prophylaxis to prevent plague infection is 7 days.

[h] Tetracycline can be substituted for doxycycline.

[i] Children younger than 2 years should not receive chloramphenicol. Chloramphenicol dose should not exceed 4 g/day. An oral formulation is available only outside the United States.

IM, Intramuscular; *IV,* intravenous; *PO,* oral.

ACKNOWLEDGMENTS

The author wishes to acknowledge the contributions of David Dennis, who authored previous versions of this chapter, and the assistance of Anna Perea, Kiersten Kugeler, and Christina Nelson.

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The complete reference list is available online at Expert Consult.

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229B *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Richard R. Watkins

SHORT VIEW SUMMARY

Definition
- Zoonoses with multiple animal hosts; widespread in environment
- Display tropism for lymph nodes

Epidemiology
- Fecal-oral transmission; enteric pathogens; more common in children
- Worldwide distribution but most common in northern Europe; infections frequently occur in winter months; *Yersinia enterocolitica* serogroup O:3 causes most disease in the United States

Microbiology
- Aerobic and anaerobic growth, non–spore forming, pleomorphic, non–lactose fermenting gram-negative bacilli; members of family Enterobacteriaceae
- Motile at 25°C but nonmotile at 37°C; growth at 37°C on MacConkey agar, *Salmonella-Shigella* agar (*Y. enterocolitica*), and brain-heart infusion; CIN (cefsulodin, irgasan, novobiocin) agar is selective medium
- Distinguished from other enteric pathogens and from *Yersinia pestis* by biochemical profiles

Clinical Manifestations
- Enterocolitis, mesenteric lymphadenitis, and pseudoappendicitis are most common
- Risk for sepsis is increased in those with iron overload or treated with iron-chelating agents (e.g., deferoxamine)
- Reactive arthritis, erythema nodosum, and Graves disease may follow gastrointestinal illness

Diagnosis
- Can be cultured from stool, blood, bile, wounds, throat, mesenteric lymph nodes, cerebrospinal fluid, and peritoneal fluid
- Acute and convalescent serology; molecular methods are becoming more widespread
- Automated systems can misidentify

Therapy
- Antimicrobial agents generally unnecessary for enteritis or mesenteric lymphadenitis in immunocompetent patients; do not reduce risk for postinfectious complications
- For extraintestinal symptoms, aminoglycosides, tetracycline, and fluoroquinolones are effective; *Y. enterocolitica* often produce β-lactamases, precluding the use of penicillin and lower-generation cephalosporins

Prevention
- Follow safe food handling practices; avoid undercooked meat (especially pork), unwashed vegetables, and unpasteurized dairy products
- Vaccine development is in early stages, none is available for humans or animals

First discovered in 1894 in Hong Kong by Alexandre Yersin and Shibasaburo Kitasato, the genus *Yersinia* currently includes 18 species. Of these, only three have been identified as human pathogens: *Y. pestis, Y. pseudotuberculosis,* and *Y. enterocolitica*. While *Y. pestis* causes plague and is responsible for one of the greatest calamities in human history, the Black Death of mid-14th century Europe, infections from *Y. pseudotuberculosis* and *Y. enterocolitica* generally cause a self-limited gastrointestinal illness. Genetic analysis has shown that *Y. pseudotuberculosis* is the direct evolutionary ancestor of *Y. pestis* and the two organisms have approximately 98% identity at the DNA level.[1] The contrast in lifestyle and virulence between *Y. pestis* and *Y. pseudotuberculosis* is mainly due to the presence of two additional plasmids that *Y. pestis* possesses, as well as minor genomic differences.[2] *Yersinia pseudotuberculosis* and *Y. enterocolitica* are among the most divergent of the species and gained their pathogenicity independently, yet they share pathogenicity islands and other virulence factors and cause similar gastrointestinal diseases. Although gastroenteritis is the most common presentation of yersiniosis, other clinical features include mesenteric adenitis, terminal ileitis, septicemia, and reactive arthritis. All *Yersinia* species are zoonotic, with humans being accidental hosts. The modes of transmission are primarily fecal-oral, by hand-to-mouth transfer of organisms following handling of contaminated animals and animal carcasses, and by ingestion of contaminated food or water. Patients who are immunodeficient, recipients of blood transfusions, and those with iron overload treated with deferoxamine are at particular risk of *Yersinia* sepsis.

MICROBIOLOGY

As members of the family Enterobacteriaceae, *Y. enterocolitica* and *Y. pseudotuberculosis* are non–spore forming, pleomorphic, non–lactose fermenting gram-negative bacilli that grow under aerobic and anaerobic conditions. They are urease-positive organisms, which distinguishes them from urease-negative *Y. pestis*. Both *Y. enterocolitica* and *Y. pseudotuberculosis* are motile at 25°C but not at 37°C. They grow on brain heart infusion agar, MacConkey agar, and *Salmonella-Shigella* agar at room temperature and at 37°C and in buffered saline at 4°C. Culture on specific selective media (cefsulodin irgasan novobiocin [CIN] agar), with or without preenrichment in broth or phosphate-buffered saline at 4°C or 16°C, is one protocol for isolating *Y. enterocolitica* and *Y. pseudotuberculosis* from stool and other nonsterile sites. They form 2- to 4-mm colonies with a characteristic deep red center (bull's-eye) and a transparent margin on CIN agar. The colonies are difficult to detect after incubation for 24 hours but are easily visible at 48 hours (Fig. 229B.1). *Yersinia enterocolitica* and *Y. pseudotuberculosis* can be distinguished from other enteric pathogens and from *Y. pestis* by biochemical profiles. Strains are differentiated by combined biochemical reactions and serogroups. Six biotypes (1A, 1B, 2, 3, 4, 5) and 60 serogroups of *Y. enterocolitica* have been described; not all strains are pathogenic for humans. Clinical infections are mostly associated with serogroups O:3, O:9, and O:5,27 and biotypes 2, 3, and 4. The previously dominant serogroup O:8 has been declining in North America.[3] There is a separate system for serotyping *Y. pseudotuberculosis,* which is also based on somatic antigens. Fourteen serotypes of *Y. pseudotuberculosis* have been identified, five of which (O:1 to O:5) are considered pathogenic for humans. Serotype O:1 accounts for approximately 80% of isolates from humans.

A multitude of virulence factors allow *Y. enterocolitica* and *Y. pseudotuberculosis* to adhere to host cells and tissues and undermine immunologic defenses. The most extensively studied is the type III secretion system (T3SS) that is carried on the pYV virulence plasmid.

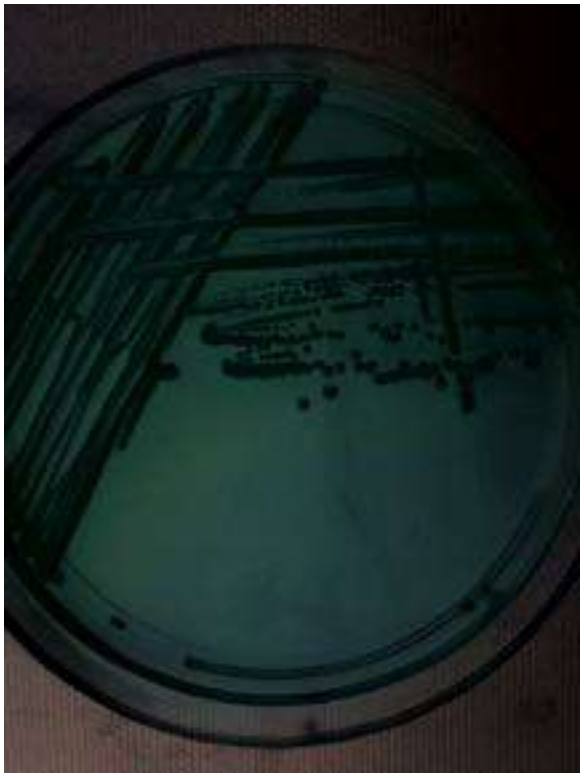

FIG. 229B.1 Yersinia enterocolitica growing on cefsulodin irgasan novobiocin agar. *(Courtesy Drs. Sandra Richter and Joshua Otiso.)*



FIG. 229B.2 Photomicrograph of Yersinia enterocolitica using a flagella-staining technique. *(From the Public Health Images Library, Centers for Disease Control and Prevention.)*

The T3SS injects *Yersinia* outer proteins (Yops) directly into the host cell, causing subversion of signaling pathways that suppresses transcription of genes of the innate immune response, inhibition of phagocytosis, and blockage of cytokine production.[4] Chromosomal virulence factors include adhesion proteins YadA (previously known as Yop1), invasin (Inv), Ail, and MyfA; the heat-stable enterotoxin Yst; and the *myf* operon that encodes genes of a fimbria similar to the CS3 fimbria of enterotoxigenic *Escherichia coli*.[4] Furthermore, 1B/O:8 strains of *Y. enterocolitica* contain a high-pathogenicity island that encodes yersiniabactin, a siderophore that enhances iron acquisition. Serogroup O:3 and O:9 isolates of *Y. enterocolitica* lack this siderophore for iron transport and are less invasive in normal hosts. However, in the setting of iron overload or treatment with therapeutic chelating agents (e.g., deferoxamine), these strains can achieve virulence similar to O:8 strains. *Yersinia enterocolitica* use flagella (Fig. 229B.2) to help establish contact with the intestinal epithelium.[4] The role that Yst plays in gastroenteritis is controversial. Yst is not detectable in diarrheal stool samples in infected animal models, and some strains carry the *yst* gene but do not produce the enterotoxin, suggesting the presence of silent genes. Nevertheless, noninvasive 1A strains that cause diarrhea often carry the *yst* gene as their only virulence factor.[5] *Yersinia pseudotuberculosis* produces *Y. pseudotuberculosis*–derived mitogen (YPM), a superantigenic toxin that induces proliferation of human T lymphocytes and causes toxic shock. The genes that code for YPM are not carried by plasmids but are inserted in an unstable locus of the genome.[6]

EPIDEMIOLOGY

The reservoirs for *Y. enterocolitica* and *Y. pseudotuberculosis* are diverse and include soil, water, and the intestinal tracts of wild and domestic animals. Most of the environmental strains are avirulent, but those in pigs and their food products are the major source for pathogenic *Y. enterocolitica* in humans.[7] Yersiniosis has a global distribution and is the third most common zoonosis in Europe. Germany accounts for over half of the reported cases, with an annual incidence of 7.2 per 100,000 population.[8] This may be due to a higher prevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* in animal reservoirs, as well as higher pork consumption in Germany compared to other European nations. In the United States, the average annual incidence of yersiniosis is 0.16 cases per 100,000 population.[9] There has been a significant decrease in infections among African Americans less than 5 years of age but an increasing incidence among whites ages 19 to 64 years and Hispanics. This decline in African-American children may be due to educational efforts in the state of Georgia.[10] Infections have been reported in other parts of the world, including South America, Africa, and Asia, but yersiniosis is not considered an important cause of tropical diarrhea. In 2014, a sustained outbreak of yersiniosis from *Y. pseudotuberculosis* occurred in all the major cities of New Zealand, with 220 laboratory-confirmed cases.[11] Genomic and epidemiologic data suggested a single point-source contamination of the food chain, with subsequent nationwide distribution of contaminated produce. Unlike other enteropathogens, *Yersinia* infections often occur during winter months and in cold climates.

The majority of cases of gastrointestinal illness occur in children under 5 years of age, whereas mesenteric adenitis and terminal ileitis are more common among older children and adults. In the United States, yersiniosis is more likely to be diagnosed in young children compared with the general population.[12] Transmission of infection occurs by ingestion of contaminated food or water and, less often, by direct contact with infected animals or patients (Fig. 229B.3). The zoonotic reservoirs of *Y. enterocolitica* are diverse and include pigs, dogs, cats, rodents, bats, sheep, deer, horses, rabbits, cows, beavers, and muskrats. Flies can also be carriers and might facilitate the spread of yersiniosis from animals to humans.[13] In France, *Y. pseudotuberculosis* tends to be more common in wild animals, particularly birds, rodents, hares, and rabbits, compared to domesticated ones.[14] *Yersinia enterocolitica* is frequently present on the tonsils and in the alimentary tract of pigs, and transmission can occur by ingesting incompletely cooked pork and by contamination of other foods by pork products. Outbreaks of yersiniosis have been associated with contaminated milk and milk products[15]; contaminated produce such as bean sprouts, carrots, and lettuce[16]; and foods contaminated with spring water.[17] The ability of the organisms to grow at 4°C means that refrigerated meats can be sources of infection. Cases of sepsis due to *Y. enterocolitica* have occurred following transfusion of blood stored between 2°C and 6°C.[18] Occupational exposure to pigs increases the risk of infection in farmers,[19] slaughterhouse workers,[19] and butchers.[20] Among pregnant Danish women with occupational exposures, elevated immunoglobulin G to *Y. enterocolitica* serogroup O:3 was not a risk factor for adverse pregnancy outcomes.[21] Household transmission likely occurs through the fecal-oral route since children with yersiniosis may excrete the organism in their stool for several weeks. The cecum may become chronically colonized and serve as a reservoir for dissemination of infection to extraintestinal sites.[22]

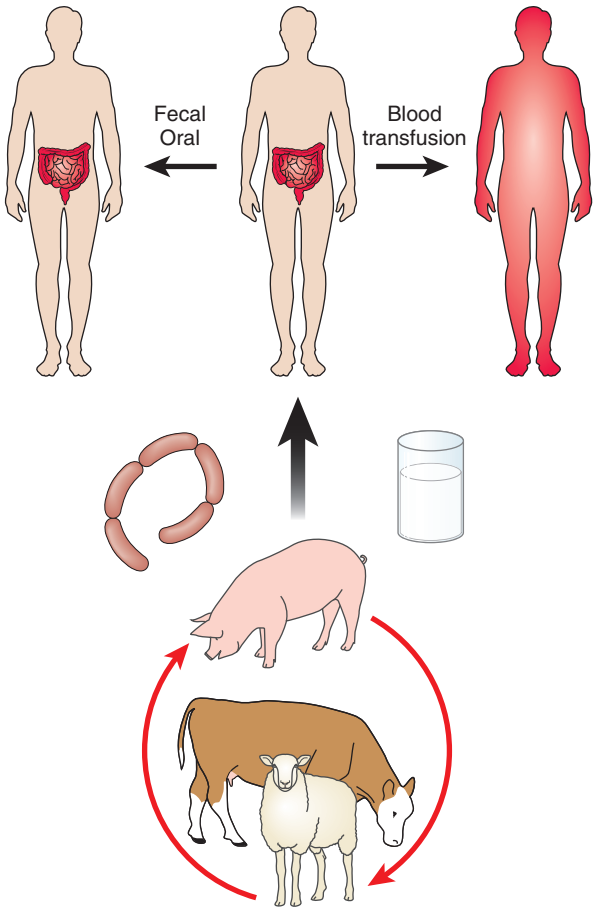

FIG. 229B.3 Transmission routes of Yersinia enterocolitica. Red circular arrows indicate zoonotic cycles. Wide black arrow indicates common routes of transmission to humans. Narrow black arrows indicate rare routes. *(Courtesy Paul Mead.)*

Systemic infections from *Y. enterocolitica* and *Y. pseudotuberculosis* are also observed, particularly in immunosuppressed patients, the elderly, and those with underlying conditions such as iron overload, alcoholism, cirrhosis, diabetes mellitus, cancer, and malnutrition.[23] The association between the treatment of iron overload with deferoxamine and *Yersinia* sepsis is well recognized in clinical practice. Deferoxamine enhances the growth of *Yersinia* and also inhibits neutrophil function.[24] Iron-loaded patients with β-thalassemia are at increased risk for severe yersiniosis, even when their body iron burden (as indicated by the serum ferritin level) is only moderately elevated and they are no longer receiving iron-chelating therapy with deferoxamine.[25]

A postinfection reactive polyarthritis can occur and has a predilection for patients with human leukocyte antigen (HLA)-B27, possibly due to molecular mimicry between HLA-B27 antigen and *Yersinia* antigens.[26] There is also some evidence for an association between *Y. pseudotuberculosis* infection and Kawasaki disease, possibly due to the YPM superantigen.[27–29]

CLINICAL MANIFESTATIONS

The incubation period after ingestion of the organism is 1 to 11 days, with diarrhea lasting for a few days to a few weeks (average of 2 weeks). Patients shed organisms in their feces and remain infectious during the symptomatic period. *Yersinia enterocolitica* presents as an invasive diarrhea characterized by fever, abdominal pain, mucus- and blood-containing stools, and the presence of fecal leukocytes.[30] Nausea and vomiting affects 15% to 40% of cases. Perforation of the ileum and rectal bleeding can occur in serious infections. Patients with mesenteric adenitis (Fig. 229B.4) or terminal ileitis have fever and right lower quadrant pain, which is more common in older children and adolescents

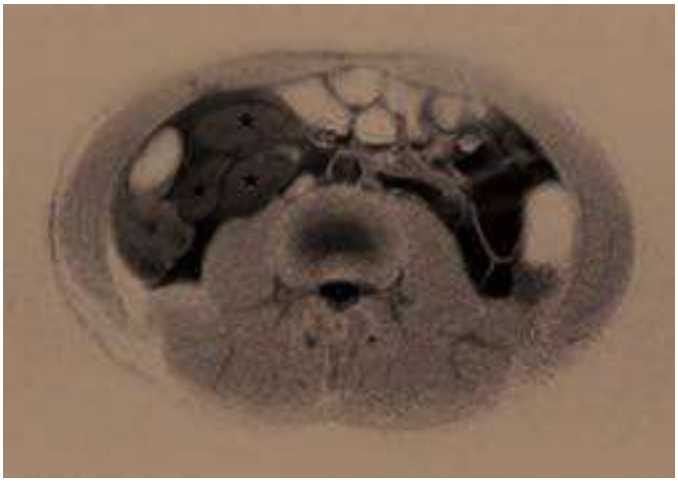

FIG. 229B.4 Abdominal MRI with *Yersinia* mesenteric adenitis. White stars indicate enlarged mesenteric lymph nodes. *(Courtesy Tara Palmore, MD.)*

and may be clinically indistinguishable from acute appendicitis (pseudoappendicitis).[31,32] When evaluating patients suspected of having acute appendicitis, computed tomographic scanning of the abdomen and pelvis may help avoid exploratory laparotomy by revealing a normal appendix and enlarged mesenteric lymph nodes.[33] Thickening and ulcerations of the terminal ileum and cecum can be seen on endoscopy, with raised round lesions on Payer patches, and can led to massive rectal bleeding.[34] Complications following gastrointestinal infections can include granulomatous enterocolitis[35] and granulomatous appendicitis.[36] Convalescent carriage of *Y. enterocolitica* in stool of untreated individuals may occasionally extend for weeks to months in a small percentage of those infected.

Yersinia pseudotuberculosis is more likely to cause abdominal pain and fever than diarrhea in all age groups. The most common manifestation in humans is mesenteric adenitis, which, like *Y. enterocolitica,* causes an acute appendicitis–like syndrome with fever and right lower quadrant abdominal pain. The infection is usually self-limited.

Acute pharyngitis can occur from *Y. enterocolitica,* with or without diarrhea, and fatalities have been reported.[37] The organism can also cause community-acquired pneumonia without accompanying diarrhea or other gastrointestinal symptoms.[38] Skin and soft tissue infections usually result from failure of the skin barrier on distal body parts, followed by spread of the infection to the regional lymph nodes from the ruptured skin.[39] Appropriate antibiotic therapy and surgical drainage are essential, because failures have occurred after common empirical antibiotics for cellulitis (e.g., vancomycin or cefazolin) were used.

Sepsis from *Y. enterocolitica* and *Y. pseudotuberculosis* is uncommon and presents as a severe illness with fever and leukocytosis. Patients are often elderly or have underlying medical conditions such as diabetes mellitus, liver disease, hemochromatosis, or iron overload. Human immunodeficiency virus infection has also been shown to be a risk factor for *Y. pseudotuberculosis* sepsis.[40,41] Septicemic patients may develop hepatic or splenic abscesses, peritonitis, septic arthritis, psoas abscesses, osteomyelitis, endocarditis, myocarditis, mycotic aneurysms, or meningitis. Rapid-onset septic shock is seen in transfusion-transmitted *Y. enterocolitica,* which is caused by a preformed endotoxin.[18] Serotype O:9 was implicated in a fatal case of posttransfusion sepsis from contaminated red blood cells following a vaginal delivery.[42]

Far East scarlet-like fever (FESLF) has been associated with certain strains of *Y. pseudotuberculosis* in eastern Russia, Korea, and Japan (Izumi fever). These strains produce the superantigenic toxin YPM (described earlier), which is integral to the pathogenesis of FESLF.[43] Six stages of FESLF have been identified[44]:

1. The incubation period, which is asymptomatic and lasts 7 to 10 days.
2. The initial onset, in which fever, rigors, headache, myalgias, arthralgias, weakness, loss of appetite, swelling of scleral vessels,

coryza, abdominal pain, and hyperemia of the face, neck, and conjunctiva occur.
3. The accrual stage, which occurs 3 days after symptom onset and is characterized by high fevers, abdominal symptoms, and a scarlet fever–like rash.
4. Remission, which usually occurs 6 days after disease onset and is characterized by a decrease in the severity of most symptoms, most notably fever, with persistence of rash and increased jaundice.
5. Recurrence with exacerbation, which typically occurs 8 days after disease onset and has an increase in symptom severity, with the exception of fever, with desquamation occurring and jaundice becoming maximal.
6. Convalescence, which usually occurs after 12 days with gradual resolution of all symptoms, including rash, desquamation, and jaundice.

POSTINFECTIOUS COMPLICATIONS

Reactive arthritis occurs after yersiniosis in approximately 10% to 20% of cases.[45,46] First described by Finnish physicians in 1969, it begins a few days to a month after the onset of diarrhea and can afflict both children and adults.[47] The typical clinical presentation is an inflammatory arthritis with a predilection for large weight-bearing joints.[48] Extraarticular inflammatory signs, including urethritis and conjunctivitis, are not uncommon. Symptoms usually resolve after 3 to 5 months, although it may take up to a year in some cases. Serologic testing of synovial fluid for *Y. enterocolitica* or *Y. pseudotuberculosis* can be helpful, although these tests may not discriminate recent infection from past exposure unless immunoglobulin M antibodies are inactivated by sample pretreatment.[49] Reactive arthritis and especially sacroiliitis is more likely to develop in people with the HLA-B27 antigen.[50] This may be a result of molecular mimicry, since YadA shares a linear tetrapeptide with HLA-B27.[51] However, not all strains associated with reactive arthritis have YadA, so it is likely that other factors also play a role.[52] For example, two major *Y. enterocolitica* antigens, Ye 19 kDa and Ye HSP60, induce synovial T-cell proliferation in patients with reactive arthritis.[53] In experimental models, tumor necrosis factor signaling alters the anti-inflammatory and proinflammatory cytokine balance and influences the development of severe chronic reactive arthritis after *Y. enterocolitica* infection.[54] Greater severity of gastrointestinal symptoms has been associated with the development of reactive arthritis.[55]

Erythema nodosum develops after yersiniosis in approximately 3% of cases.[56] Skin lesions typically appear on the legs about 2 weeks after the onset of abdominal symptoms and usually resolve spontaneously within a month (Fig. 229B.5). Women with erythema nodosum outnumber men by a ratio of 2:1 and there is no association with HLA-B27. The condition may result because of an increase in autoantibodies from polyclonal B-cell activation induced by *Yersinia*.[57]

Patients with thyroid disorders have a higher prevalence of antibodies to *Yersinia* than normal subjects or patients with other disorders.[58] *Yersinia enterocolitica* has high-affinity binding sites for thyroid-stimulating hormone and expresses antigens that cross react with thyroid-stimulating hormone receptor (TSHR). There is sequence homology between *Y. enterocolitica* outer membrane protein M and TSHR.[59] *Yersinia pseudotuberculosis* outer membrane protein FM has similarly been shown to cross react with TSHR.[60] Overall, a large number of studies have reported epidemiologic, serologic, and molecular evidence that *Yersinia* is involved in the pathogenesis of autoimmune thyroid disease and Graves disease, yet none has shown a direct correlation between infection and disorders of the thyroid. Indeed, most patients with yersiniosis do not develop thyroid disorders. One hypothesis is that the ability to produce anti-TSHR antibodies in response to *Yersinia* antigens homologous to the TSHR persists only in susceptible individuals, with *Yersinia* antigens acting as a trigger for disease development.[61]

PATHOGENESIS

Following ingestion of contaminated food or water, *Yersinia* bacteria travel to the distal part of the small bowel. There they attach to and invade the intestinal epithelium via microfold (M) cells. Adherence and invasion are facilitated at human body temperature by multiple virulence factors, including fimbriae, flagella, and the Inv and Ail proteins. Inv clusters and activates β_1 integrins on the apical surface of M cells, signaling actin cytoskeleton rearrangements that promote internalization by phagocytes.[62] The bacteria enter Peyer patches and macrophages, where they survive and multiply. Replication of *Y. enterocolitica* in Peyer patches may be facilitated by the immune regulator protein Gal-1, which has been shown to play a role in limiting bacterial clearance.[63] After an incubation period of 4 to 7 days, the phagocytized bacteria migrate to mesenteric lymph nodes, triggering an inflammatory response that leads to abdominal pain. Further spread can occur via the bloodstream to the liver and spleen. The dissemination of *Y. enterocolitica* but not *Y. pseudotuberculosis* requires C-C motif of chemokine receptor 7–dependent migration of dendritic cells and monocytes, which may be due to differences in surface adhesins/invasins, secreted factors between the pathogens, or both.[64] Once they reach the mesenteric lymph nodes, liver, and spleen, *Yersinia* form extracellular microabscesses surrounded by epithelioid granulation tissue. In severe cases, thrombosis of mesenteric blood vessels, intestinal necrosis, and hemorrhage may occur. The appendix often has a normal histologic appearance or demonstrates mild inflammation. Septicemia may lead to focal abscesses in distal organs, including the lungs and brain.



FIG. 229B.5 Erythema nodosum. *(Courtesy Dr. Gary M. White.)*

DIAGNOSIS

Fecal leukocytes are often present during acute illness, but the finding is nonspecific. *Yersinia* can be isolated from stool, blood, bile, wounds, throat, mesenteric lymph nodes, cerebrospinal fluid, and peritoneal fluid. *Yersinia,* particularly *Y. pseudotuberculosis,* can form pinpoint colonies on both blood agar and MacConkey agar in 24 hours.[65] *Yersinia enterocolitica* appears as small, lactose-negative colonies on MacConkey in 48 hours. Ornithine decarboxylase, sucrose, and sorbitol are all positive for *Y. enterocolitica* and negative for *Y. pseudotuberculosis.* Detection from stool can be problematic due to overgrowth of fecal flora, so cold enrichment followed by culture on CIN agar is a common protocol in clinical laboratories. A new type of chromogenic agar has been developed for *Y. enterocolitica* that is as sensitive as CIN but more specific and has a lower false-positive rate.[66] Recovery of organisms from otherwise sterile sites, such as blood and cerebrospinal fluid, is usually faster than recovery from stool samples.

A variety of serologic methods are available to aid in diagnosis. These include enzyme-linked immunosorbent assays, tube agglutination, and immunoblotting. *Yersinia enterocolitica* and *Y. pseudotuberculosis* cross react with one another and with other organisms, including *Brucella, Vibrio, Bordetella, Borrelia, Morganella, Salmonella,* and *E. coli.*[67–69] Agglutinin titers increase shortly after the onset of symptoms, peak in the second week, and generally disappear within 2 to 6 months, although elevated titers may persist longer in some cases. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy, which identifies bacteria according to specific protein profiles, has supplanted biochemical and phenotypic characterization in many laboratories, but this method has been challenged in differentiating *Y. pestis* and *Y. pseudotuberculosis.*[70,71] Whole-genome sequencing can identify *Yersinia* strains to the species level but is not yet widely available in the clinical microbiology laboratory setting.[72]

A number of polymerase chain reaction–based multiplex gastrointestinal pathogen identification panels are marketed for use with primary stool specimens. These assays offer substantially improved turnaround time on primary laboratory diagnosis compared with culture-based methods, although recovery of isolates from culture is still required for taxonomic classification and susceptibility testing.[65] False-positive results remain a concern, with one assay reported to have a sensitivity of only 48% for *Y. enterocolitica.*[73] Currently there are no commercially available multiplex polymerase chain reaction panels that include *Y. pseudotuberculosis* in the pathogen target list.

Patients with reactive arthritis after yersiniosis often have elevated inflammatory markers (e.g., erythrocyte sedimentation rate and C-reactive protein) and negative rheumatoid factor and antinuclear antibodies. Synovial fluid culture is sterile. There is no specific histopathologic characteristic that differentiates erythema nodosum from yersiniosis compared to other etiologies.[74]

TREATMENT

Care is primarily supportive, with hydration and proper nutrition being the mainstays of treatment. Antimotility medications should be avoided because they might increase the risk for bacteremia. Most isolates of *Y. enterocolitica* are susceptible to aminoglycosides, tetracyclines, trimethoprim-sulfamethoxazole (TMP-SMX), piperacillin, third-generation cephalosporins, and quinolones.[75] They are usually resistant to penicillin, ampicillin, and first-generation cephalosporins due to β-lactamase production. The role for antimicrobial therapy in uncomplicated acute diarrhea and mesenteric adenitis is unclear because these infections are usually self-limited. Antibiotics may be beneficial in cases of severe enterocolitis. They should be used in septicemia and in cases with extraintestinal manifestations, such as cellulitis, pneumonia, osteomyelitis, endocarditis, and meningitis. Also, select patient populations should receive antibiotic therapy, including the elderly, diabetics, cirrhotics, immunocompromised individuals, those receiving chemotherapy, and young children. Duration of therapy depends on the severity of illness, with 7 to 14 days being sufficient for many patients with enterocolitis,[76] 14 days for bacteremia,[77] and longer courses for complicated cases such as endocarditis and osteomyelitis. Deferoxamine therapy should be discontinued when an infection from *Yersinia* is suspected or confirmed.[78,79]

Yersinia pseudotuberculosis is usually sensitive in vitro to ampicillin, tetracycline, ciprofloxacin, cephalosporins, and aminoglycosides. Antibiotics are not necessary in most cases of mild disease and mesenteric adenitis. For moderate *Y. pseudotuberculosis* disease, TMP-SMX (TMP 8 mg/kg/day and SMX 40 mg/kg/day in two divided doses) can be used to treat children, and ciprofloxacin 500 mg twice daily can be used to treat adults. Patients with severe disease, including septicemia, should receive ceftriaxone (2 g daily in adults, 100 mg/kg in children, in 1 or 2 divided doses daily) plus gentamicin (5 mg/kg/day in 3 divided doses daily). The mortality rate in *Y. pseudotuberculosis* septicemia remains high despite appropriate antibiotic therapy and supportive care.[80,81]

Reactive arthritis is managed with nonsteroidal antiinflammatory drugs, intraarticular corticosteroid injections, and physical therapy. Systemic glucocorticoids can be used when the patient does not respond to nonsteroidal antiinflammatory drugs and intraarticular glucocorticoids or has a large number of involved joints. Antibiotics have not been shown to be beneficial and are not recommended.[82]

PREVENTION

Improving conditions and methods in slaughterhouses, such as avoiding head meat, is an important intervention that reduces *Y. enterocolitica*–contaminated processed pork.[83] The storage time of refrigerated but unfrozen foods should be limited. The consumption of unwashed raw vegetables, unpasteurized milk, and undercooked meats (especially pork and pork products, such as chitterlings), should be avoided especially by immunocompromised individuals. In health care settings, enteric precautions along with strict hand washing and control of environmental cross-contamination should be instituted. Blood bank personnel need to ask donors about any recent symptoms of gastroenteritis and tell them to notify the blood bank if symptoms occur after donation. Vaccine development for *Y. enterocolitica*[84] and *Y. pseudotuberculosis*[85] is underway, but currently none is approved for human or animal use.

ACKNOWLEDGMENT

The author wishes to acknowledge the contributions of Dr. Paul S. Mead, who wrote the previous version of this chapter.

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230 *Bordetella pertussis*

Valerie Waters and Scott A. Halperin

SHORT VIEW SUMMARY

Definition

- Pertussis, infection with *Bordetella pertussis,* is divided into three stages:
  - Catarrhal stage: rhinorrhea, nonpurulent conjunctivitis, occasional cough, low-grade fever
  - Paroxysmal stage: paroxysms or fits of coughing, inspiratory whoop
  - Convalescent stage: gradually diminishing cough lasting up to 8 weeks
- The clinical case definition includes cough for 14 days or longer and one or more of the following: paroxysmal cough, inspiratory whoop, or posttussive vomiting

Epidemiology

- With the introduction of whole-cell pertussis vaccine in 1940s, pertussis rates dropped dramatically.
- Peaks of disease continue to occur every 3 to 5 years.
- Most cases, hospitalizations, and deaths occur in unimmunized infants younger than 6 months.
- Outbreaks in children with high vaccine rates may be partly due to waning immunity from acellular pertussis vaccine.

Microbiology

- *Bordetella* organisms are small gram-negative coccobacilli; growth is fastidious.
- Filamentous hemagglutinin and fimbriae are two major adhesins.
- Pertussis toxin (PT) helps organisms evade host defenses and causes systemic manifestations; it also acts as an adhesin.

Diagnosis

- Diagnosis is traditionally made by means of culture from nasopharyngeal swabs or aspirates; specific swabs, transport media, and growth media should be used to enhance recovery.
- Newer polymerase chain reaction assays are more sensitive for detection of *B. pertussis* and are the procedure of choice.
- Antibodies to *B. pertussis* PT can be used for diagnosis.
- Direct fluorescent antibody testing is available but not recommended.

Therapy

- Erythromycin, 40 mg/kg/day in four divided doses, is recommended for children (maximum 2 g/day), or clarithromycin, 15 mg/kg/day in two divided doses (maximum 1 g/day) for 7 to 14 days. Azithromycin, 10 mg/kg/day for 4 days, is recommended for infants younger than 1 month. Five days of azithromycin is probably effective for treatment or prophylaxis in adults (500 mg first day, then 250 mg daily) and is better tolerated and has fewer serious drug interactions than erythromycin, 500 mg four times daily for 14 days.
- Supportive care is paramount in management of pertussis, especially in infants.

Prevention

- Immunization is the single most effective means of preventing pertussis.
- The pertussis immunization schedule in the United States and Canada for children and adolescents is as follows: DTaP (diphtheria toxoid, tetanus toxoid, acellular pertussis vaccine, pediatric formulation) at 2, 4, 6, and 18 months, with booster at 4 to 6 years; and Tdap (tetanus toxoid, diphtheria toxoid, acellular pertussis vaccine, adult formulation [reduced doses of "d" and "ap" components]) for preadolescents and adolescents.
- In the United States and Canada, it is recommended that all adults age 19 years and older receive a single dose of Tdap. The exception is pregnant women who, in the United States, are recommended to receive Tdap for each pregnancy, ideally in the 27th to 36th weeks of pregnancy, to protect their newborns from pertussis.
- All health care workers with patient contact should be given a single dose of Tdap if they have not been vaccinated as an adult, irrespective of when they received the last dose of tetanus toxoid.
- Prophylaxis can be considered for close contacts exposed within 21 days of onset of cough in the index case. Adults and adolescents are given erythromycin 500 mg four times daily for 7 to 14 days, azithromycin 500 mg on the first day and 250 mg daily for 4 additional days, or clarithromycin 500 mg twice daily for 7 days.

HISTORY

The first epidemic of whooping cough was described in 1578 by de Baillou, who wrote the following: "The lung is so irritated that, in its attempt by every effort to cast forth the cause of the trouble, it can neither admit breath nor easily give it forth again. The sick person seems to swell up, and, as if about to strangle, holds his breath clinging in the midst of his jaws."[1] This vivid clinical description of whooping cough holds true to this day. In 1679, Sydenham[2] gave this respiratory illness the name "pertussis," meaning a violent cough of any type. The organism that causes whooping cough was discovered in 1900 by Bordet and Gengou.[3] They described a new gram-negative bacillus (subsequently named *Bordetella pertussis*, after Bordet) that they had found in the sputum of a 6-month-old infant with whooping cough. By 1906, they had developed a culture medium to support the growth of the organism and described in detail its morphologic features and virulence characteristics. In 1943, Joseph Lapin,[1] a pediatrician who worked in the whooping cough clinic at the Bronx Hospital in New York City, wrote an extensive monograph on the subject of pertussis.

DESCRIPTION OF PATHOGEN

B. pertussis is the pathogen that causes whooping cough or pertussis.[4] It is one of 10 known *Bordetella* species, namely, *B. pertussis, B. parapertussis, B. bronchiseptica,* ovine-adapted *B. parapertussis, B. avium, B. hinzii, B. holmesii, B. trematum, B. petrii,* and *B. ansorpii. B. pertussis* and *B. parapertussis* are the most common *Bordetella* species causing respiratory illnesses in humans. With improved molecular testing methods, polymerase chain reaction (PCR) assays can now distinguish between *B. pertussis* and *B. holmesii* and have detected *B. holmesii* in 0.1% to 20% of patients with pertussis-like symptoms.[5–7] *B. holmesii* can colonize the respiratory tract but also can cause a pertussis-like syndrome.[8] Unlike *B. pertussis, B. holmesii* can cause bacteremia or other nonpulmonary infections. Distinction between the two species can be through biochemical reactions, the distinctive brown pigment of *B. holmesii,* or use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.[9]

Although *B. pertussis* strictly affects humans and has no known animal reservoir,[10] many of the other *Bordetella* species are recognized

primarily for the diseases they cause in animals. *B. bronchiseptica* causes kennel cough in dogs and cats, and human infections occur primarily in immunocompromised patients, often after exposure to animals.[11–13] Ovine-adapted *B. parapertussis* causes respiratory tract infections in sheep.[14] *B. avium* is a pathogen of poultry[15] but has been isolated from the ear culture of a patient with chronic otitis media.[16] Similarly, *B. hinzii* also colonizes the respiratory tract of poultry and has been isolated from the sputum of cystic fibrosis patients.[17] It has been reported to cause bacteremia in immunocompromised[18,19] and in immunocompetent patients[20] and has been described as a cause of chronic cholangitis.[21] *B. trematum* has been isolated from patients with wounds or otitis media.[22,23] *B. petrii,* originally identified from an environmental source,[24] has been isolated from patients with chronic infections.[25–27] Finally, in 2005, a novel species of *Bordetella, Bordetella ansorpii,* was described after the isolation of a gram-negative bacillus from the purulent exudate of an epidermal cyst.[28] 16S ribosomal RNA (rRNA) gene sequencing has revealed that this bacterium belongs to the *Bordetella* genus but is distinct from other *Bordetella* species. This species was subsequently isolated from an immunocompromised patient in the United Kingdom.[29]

Bordetella species are small gram-negative coccobacilli.[30] Some species are motile and, except for *B. petrii,* are strictly aerobic. All species possess catalase activity and oxidize amino acids but do not ferment carbohydrates. *Bordetella* organisms grow optimally at 35°C to 37°C. *Bordetella* species are fastidious because their growth can be inhibited by components commonly found in laboratory media. In addition, their rate of growth is inversely related to their degree of fastidiousness. *B. pertussis* is the most fastidious and slowest growing of the *Bordetella* species. Its growth is inhibited by fatty acids, metal ions, sulfides, and peroxides. Isolation of *B. pertussis* requires a medium containing charcoal, blood, or starch. Traditionally, Bordet-Gengou (BG) medium has been used and consists of a potato-starch base. Charcoal medium (Regan-Lowe [RL] medium), supplemented with glycerol, peptones, and horse or sheep blood, can also be used and may provide better isolation of *B. pertussis* than the BG medium.

PATHOGENESIS

B. pertussis infection and disease occur after four important steps: (1) attachment, (2) evasion of host defenses, (3) local damage, and (4) systemic manifestations.[4]

Filamentous hemagglutinin (FHA) and fimbriae (FIM) are two major adhesins and virulence determinants for *B. pertussis.* FHA is a 220-kDa surface-associated and secreted protein, and FIM is a filamentous cell surface structure. They are required for tracheal colonization, are highly immunogenic, and are components of certain acellular pertussis vaccines.[4] However, there is likely redundancy in the adhesion role of *B. pertussis* proteins, and it has been suggested that virulence factors such as pertactin (PRN) may mediate attachment in the absence of FHA.[31] Pertussis toxin (PT) also acts as an adhesin[32] and has specific recognition domains for human cilia.[33]

Evasion of host defenses occurs primarily through adenylate cyclase toxin (ACT) and PT.[34] ACT is a toxin secreted by *B. pertussis* that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which inhibits the migration and activation of phagocytes. It has also been shown to suppress T-lymphocyte activation and chemotaxis.[35] PT, one of the most important virulence factors of *B. pertussis,* also targets the innate immune system of the lung by inactivating or suppressing G protein–coupled signaling pathways. PT has two components, the A subunit and the B subunit. The B (binding) subunit binds to the cell surface to enable adenosine diphosphate (ADP)–ribosylation of G proteins by the A (active) subunit, thereby altering the cell.[34] Through this mechanism of action, PT delays the recruitment of neutrophils to the respiratory tract and targets airway macrophages to promote *B. pertussis* infection.[36] The virulence factors of *B. pertussis,* such as PT, are encoded by the *bvg* (or *vir*) gene. The *bvg* operon is composed of *bvgA* and *bvgS,* members of a two-component signal transduction system that controls the genetic state, or phase, of *B. pertussis.*[37] There are virulent and avirulent phases, and their expression is regulated by environmental factors.[38]

Original reports by Lapin described the local tissue damage caused by pertussis in the lung.[1] The initial pulmonary lesion is a lymphoid hyperplasia of peribronchial and tracheobronchial lymph nodes. Necrosis and desquamation of the bronchial epithelium follow, with diffuse infiltrations by macrophages (Fig. 230.1). Most of the damage to the ciliated epithelial cells is caused by tracheal cytotoxin (TCT). TCT is a disaccharide tetrapeptide derived from peptidoglycan, which triggers the production of an inducible nitric oxide (NO) synthase.[39] The synthase produces NO, which ultimately kills the tracheal epithelial cells. The induction of the NO synthase is likely caused by the cytokine interleukin-1 (IL-1), generated in response to TCT.[40] Dermonecrotic toxin (DNT), a 160-kDa heat-labile secreted toxin that activates intracellular Rho guanosine triphosphate (GTP)ases, may also have a role in local tissue damage.[41] DNT was first discovered by Bordet and Gengou and derives its name from the characteristic skin lesion produced when injected into test animals.

Unlike other bacterial diseases, there are few systemic manifestations of *B. pertussis* infection because it does not enter the circulation and disseminate. *B. pertussis* is relatively sensitive to killing by serum in vitro. However, in vivo, even serum-sensitive strains can efficiently infect mice.[42,43] *B. pertussis* has multiple mechanisms for avoiding antibody-mediated complement killing,[44] including the expression of BrkA, a surface-associated protein belonging to the autotransporter secretion system.[45] PT is the primary virulence determinant responsible for the systemic manifestations, of which the most prominent is leukocytosis with lymphocytosis.[34] Other systemic responses include sensitization to histamine and serotonin and sensitization of the beta-islet cells of the pancreas. This latter effect leads to hyperinsulinemia with resultant hypoglycemia, particularly in young infants.[1] Pertussis-associated encephalopathy is observed rarely[46]; some have suggested that it may be caused by the effect of PT on the central nervous system via monocyte chemoattractant protein-1 (MCP-1) overexpression.[47] Fatal pulmonary hypertension has also been associated with pertussis in infants.[48] Pathologic studies have demonstrated that, in addition to producing pulmonary vasoconstriction resulting from hypoxemia, pulmonary infection with *B. pertussis* triggers toxin-mediated leukocytosis, causing increased vascular resistance and subsequent refractory pulmonary hypertension.[49]

In addition to the direct effects of these virulence factors in the lung, some believe that they modulate, in a more global fashion, the immune system itself.[50] Studies investigating immunomodulation by *B. pertussis* have demonstrated skewing of the host immune response toward expansion of the Th17 subset of T lymphocytes, induced by the production of cytokine IL-23.[51] The Th17 immune response may be protective against some other gram-negative bacterial respiratory pathogens[52] but may also be associated with chronic autoimmune inflammation.[53] Some have suggested that the chronic cough seen with *B. pertussis* infection may be explained by this autoimmune phenomenon, akin to asthma,[50] although it has also been hypothesized to be a direct effect of mediators such as bradykinin released in response to tissue damage.[54]

EPIDEMIOLOGY

Despite vaccination, pertussis disease continues to be a problem in the developing and developed world (Fig. 230.2).[55] According to the World Health Organization (WHO), an estimated 24.1 million cases and 160,700 deaths occurred in 2014 in children younger than 5 years because of *B. pertussis.*[56] Case-fatality rates in developing countries may be as high as 3% in infants.[57] WHO recommended that a pertussis incidence of less than 1 case per 100,000 population be achieved in Europe by 2000. Data from countries represented in the Global Pertussis Initiative (GPI) have indicated that this incidence has not yet been achieved.[57]

Prevaccine Era

In the prevaccine era, pertussis was a major childhood illness and a leading cause of death. Pertussis disease has always been cyclic, with peaks occurring every 3 to 5 years.[58] From 1940 to 1948 in the United States, pertussis was responsible for more deaths in the first year of life than measles, scarlet fever, diphtheria, poliomyelitis, and meningitis combined.[4] Unlike the current age distribution of pertussis disease, however, pertussis affected children primarily 1 to 10 years of age. From 1918 to 1921 in Massachusetts, for example, more than 80% of pertussis

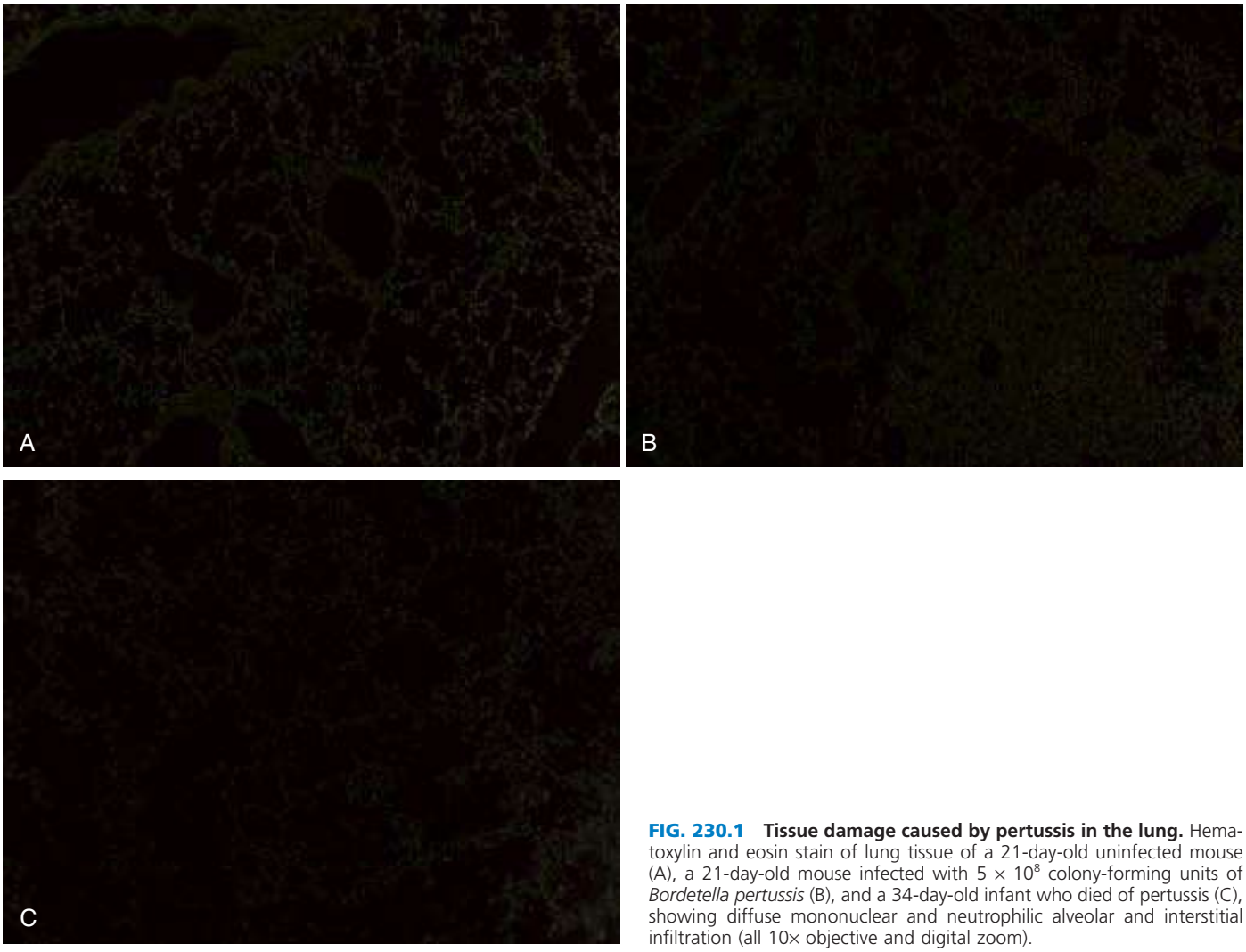

FIG. 230.1 Tissue damage caused by pertussis in the lung. Hematoxylin and eosin stain of lung tissue of a 21-day-old uninfected mouse (A), a 21-day-old mouse infected with 5×10^8 colony-forming units of *Bordetella pertussis* (B), and a 34-day-old infant who died of pertussis (C), showing diffuse mononuclear and neutrophilic alveolar and interstitial infiltration (all 10× objective and digital zoom).

cases occurred in children aged 1 to 9 years, whereas only 10% occurred in infants younger than 1 year.[59]

Vaccine Era

With the introduction of the whole-cell pertussis vaccine in the 1940s, pertussis rates dropped dramatically. They reached a nadir in the United States in the late 1970s to early 1980s, with a reported 0.5 to 1.0 cases per 100,000 population between 1976 and 1982.[59] There has been a gradual increase in pertussis rates over the last 20 years, with peaks of disease continuing to occur every 3 to 5 years.[60] The age distribution of pertussis disease has also changed, with the most cases occurring in unimmunized infants younger than 1 year. Data from the National Notifiable Diseases Surveillance System and the National (Nationwide) Inpatient Sample database in the United States have revealed that from 1993 to 2004, 86% of hospitalizations and all deaths caused by pertussis occurred in infants 3 months of age or younger.[61] Similarly, according to Canadian data from 1991 to 1997 from the Canadian Immunization Monitoring Program, ACTive (IMPACT) network, almost 80% of hospitalized patients with pertussis and all deaths secondary to pertussis occurred in children 6 months of age or younger.[62]

Current Issues Regarding Resurgence of Pertussis

In recent years, a resurgence in pertussis has been reported in many countries worldwide.[7,63–67] The reason for this resurgence is likely to be multifactorial.[64] One of the key factors is the finding that neither natural pertussis infection nor immunization produces lifelong immunity to pertussis.[68,69] Different pertussis vaccines have had varying rates of success over the years. In the 1990s, Canada experienced a resurgence of pertussis, primarily in young adolescents.[70] This was a result of the low effectiveness of the whole-cell pertussis vaccine used between 1985 and 1998 in that country. This resulted in a "marching cohort" effect, or an increase each year in the age of peak incidence by 1 year, which revealed the existence of a susceptible cohort (Fig. 230.3).[71] This was addressed with universal immunization programs to vaccinate adolescents with a more effective acellular pertussis vaccine.[70] However, despite a more effective acellular pertussis vaccine,[72] pertussis outbreaks continue to be reported in young adults and in young children who recently completed a full pertussis vaccine series.[65,73–78] In 2010, a large pertussis outbreak occurred in California, with the highest number of pertussis cases in more than 60 years.[78] Second to children younger than 6 months, the highest disease rates were observed in fully vaccinated preadolescents (7–10 years of age), as observed by others.[79] A case-control study of children 4 to 12 years of age who were PCR positive for pertussis, compared with PCR-negative and matched controls, demonstrated that PCR-positive children were more likely to have received the fifth DTaP (diphtheria toxoid, tetanus toxoid, acellular pertussis vaccine) dose earlier than controls, with an odds of acquiring pertussis increasing by an average of 42% per year.[80] This suggests that immunity after acellular pertussis vaccination may begin to decline after 4 to 5 years, indicating that a booster dose may be appropriate. Epidemiologic studies have also shown that decreasing antibody levels to PT at a population level can precede large pertussis epidemics,[63] although long-term memory B cells in vaccinated children may persist despite waning antibody levels and may provide protection

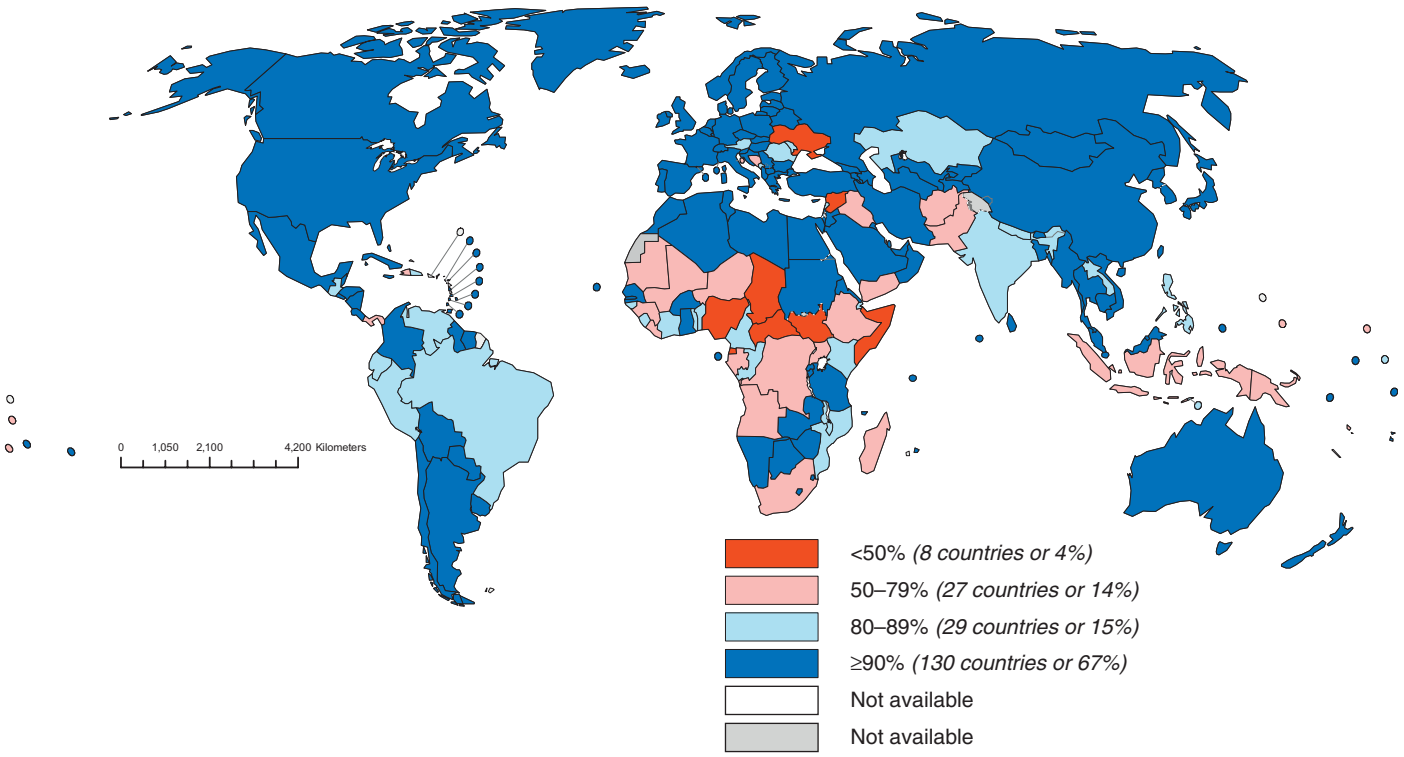

FIG. 230.2 Immunization coverage with diphtheria-tetanus-pertussis (DTP3) vaccine in infants in developing countries, 2016. *(From World Health Organization/United Nations Children's Fund coverage estimates 2016 revision, July 2017.)*

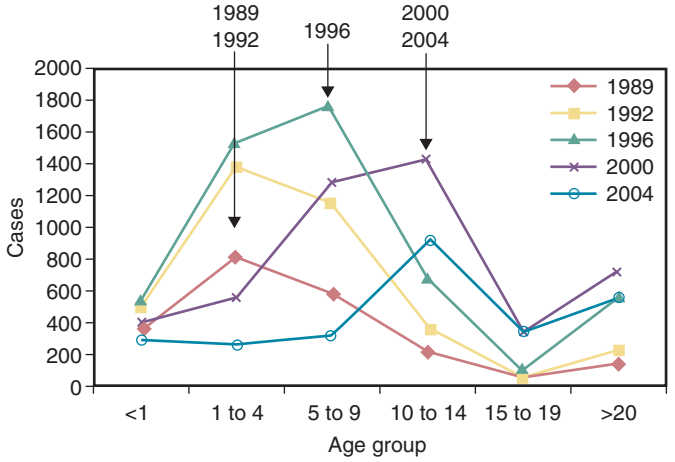

FIG. 230.3 Number of cases of pertussis reported in different age groups in Canada from 1989 to 2004. *Arrows* indicate the age group in which the incidence was highest in a given year, excluding infants younger than 1 year.

against pertussis disease.[81] Additional factors that may have contributed to the resurgence in reported pertussis include increased awareness and subsequent testing for pertussis with very sensitive molecular methods that can detect as little as one organism of *B. pertussis*, making it difficult to distinguish between colonization and disease (discussed further under "Carrier State").[82] Finally, it is possible that the bacterium itself has evolved and changed over time in response to vaccination practices; additional detail regarding strain variation among *B. pertussis* is included in the section "Molecular Diagnosis."

Carrier State

In the past, based on knowledge obtained from traditional culture methods, there was not considered to be a carrier state for *B. pertussis* in the nasopharynx. However, this may no longer be true, according to studies done with more sensitive PCR methods. In addition to circulating among adults, there may also be transient nasopharyngeal (NP) carriage of *B. pertussis* in immunized children. A case-control study[65] described a laboratory-confirmed (primarily by PCR assay) outbreak of pertussis occurring in preschool-aged children. This was not a classic pertussis, as evidenced by a lower number of cases meeting a clinical case definition, a very low hospitalization rate of unimmunized infants, and a low secondary attack rate in households. High vaccine rates may have moderated the outbreak, and, with respiratory coinfection in a significant proportion of cases, a positive PCR result may simply have reflected transient NP carriage of *B. pertussis* in the absence of evidence of seroconversion.

CLINICAL PRESENTATION

There is a spectrum of disease caused by *B. pertussis* infection, and its presentation will vary according to the patient's age, degree of immunity, use of antibiotics, and respiratory coinfection.[83]

Young Children

Joseph Lapin[1] wrote a detailed description of typical or classic pertussis in 1943 that remains true to this day. Pertussis is classically divided into three stages: the catarrhal or prodromal stage, the paroxysmal stage, and the convalescent stage. The catarrhal stage begins after a usual incubation period of 7 to 10 days, with a range of 5 to 21 days. In the catarrhal stage, children will present with signs and symptoms of a common upper respiratory tract infection, including rhinorrhea, nonpurulent conjunctivitis with excessive lacrimation, occasional cough, and low-grade fever. The catarrhal stage typically lasts 1 to 2 weeks and is followed by the paroxysmal stage. As its name suggests, the paroxysmal stage is characterized by paroxysms or fits of coughing. The child will typically have spasms of uncontrollable coughing, often 10 to 15 coughs in a row in a single expiration, the face may turn red or purple and, at the end of the paroxysm, he or she may have an inspiratory whoop. The whoop is caused by inspiring against a partially closed glottis. With the force of the coughing, the child may produce mucous plugs and often may have posttussive vomiting. Paroxysms occur more frequently at night. The paroxysmal stage lasts 1 to 6 weeks. During the end of

the first stage and beginning of the second stage, patients may exhibit signs of systemic disease such as leukocytosis with lymphocytosis, both risk factors for worse clinical outcome.[84–86] Hyperinsulinemia may also occur, although it is rarely associated with hypoglycemia.[87] Lastly, as symptoms begin to wane, the patient enters the convalescent stage. The length of the cough distinguishes pertussis from other respiratory tract illnesses. In classic pertussis, it usually lasts 1 to 6 weeks, although it can last longer; pertussis is known as the "cough of 100 days" from the Chinese. Most clinical case definitions require a cough of at least 14 days and at least one of the following symptoms: paroxysmal cough, inspiratory whoop, or posttussive vomiting.[83] The mean duration of cough in adults with pertussis is 36 to 48 days.[88] For up to 1 year after pertussis infection, it is not uncommon to have recurrences of the paroxysmal cough or inspiratory whoop with other respiratory illnesses.

Infants and Adults

Pertussis can present atypically in adults and infants. Infants are less likely to have the characteristic inspiratory whoop and a significant catarrhal stage and are more likely to present with gagging, gasping, cyanosis, or apnea and have a prolonged convalescent phase.[86,87,89] Infants often present with the nonspecific sign of poor feeding and can present with seizures. In adults, paroxysmal coughing is seen in most patients, and several studies have reported cough duration of longer than 21 days.[88] There is a wide range in the percentage of adult patients with pertussis who have whooping (8%–82%) and posttussive vomiting (17%–65%; mean, 50%). Unlike children, in adults, posttussive vomiting is strongly suggestive of pertussis.

COMPLICATIONS

Several complications are associated with pertussis. According to data from Canada and the United States, pneumonia is the most common complication of pertussis in hospitalized patients, especially among newborns younger than 1 month (10%–18%).[61,62] Pneumonia can be caused by *B. pertussis* infection itself (see Fig. 230.1) or coinfection with other respiratory pathogens. Respiratory syncytial virus (RSV) is the best described copathogen with *B. pertussis.* Rates of RSV coinfection in infants hospitalized with pertussis range from 5% to 33%.[61,89] Conversely, 8% to 16% of children with RSV infection may also be positive for *B. pertussis.*[90,91] The case-fatality rate of pertussis is highest in infants; approximately 1% of children younger than 6 months who are hospitalized with pertussis infection will die.[62] In a case-control study done by the IMPACT surveillance network, leukocytosis and pneumonia were independent predictors of death in infants hospitalized with pertussis.[84] Encephalopathy is a rare complication of pertussis.[46] It occurs more commonly in younger nonimmunized children (in 1.4% of infants younger than 2 months)[92] but can also occur in adults.[93] It typically manifests between the second and fourth weeks of cough.[94] Seizures are the most common clinical manifestation, although paresis and paraplegias, ataxia, aphasia, blindness, deafness, and decerebrate posturing have also been described. Pertussis-specific antigens may cross the blood-brain barrier and directly affect the central nervous system because high cerebrospinal fluid (CSF) antibody titers to PT and FHA have been reported in cases of pertussis encephalopathy.[46] Complications of pertussis such as pneumonia and urinary incontinence are surprisingly high in older patients and significantly more frequent in adults than adolescents, especially in those who smoke or who have asthma.[95] Other complications of pertussis caused by the forceful and persistent nature of the cough include subconjunctival hemorrhages, syncope, and rib fractures.

DIAGNOSIS

The first step in the diagnosis of pertussis is to have the appropriate index of suspicion for pertussis disease. For vaccine trials, WHO defines a case of pertussis as the presence of a paroxysmal cough for 21 days or longer and one or more of the following criteria: positive culture for *B. pertussis,* significant increases in immunoglobulin G (IgG) and IgA antibody against FHA, agglutinogen (AGG) 2 and 3 or PT, and proven contact with a culture-confirmed case.[83] This definition favors specificity of diagnosis rather than sensitivity. According to the Centers for Disease Control and Prevention (CDC),[96] a clinical case is defined as a patient with cough for 14 days or longer, in the absence of a more likely diagnosis, and at least one of the following: paroxysmal cough, whoop, or posttussive vomiting, or, in infants younger than 1 year, apnea. This is a more sensitive definition, but confirmation requires a positive laboratory finding by culture or PCR assay or a confirmed epidemiologic link.

Culture

Laboratory confirmation of pertussis has traditionally been made with culture methods.[30] Proper collection (including the timing of culture), transport, and storage of specimens are required to enhance the detection of *B. pertussis* by culture methods. Preferred patient specimens for the diagnosis of pertussis are NP aspirates and posterior NP swabs. These specimens contain the ciliated respiratory epithelial cells for which *B. pertussis* has an affinity. The advantage of NP aspirates over swabs is an increased culture positivity rate and added sample for any additional tests. If NP swabs are used, swab material should be calcium alginate, Dacron, or rayon because cotton will inhibit the growth of *B. pertussis.* Specific transport media should be used for NP specimens, including 1% acid-hydrolyzed casein or Amies medium with charcoal. Specimens may be inoculated into enrichment media such as RL transport medium, which contains half-strength charcoal agar and horse blood with cephalexin to suppress normal NP flora growth. For culture testing, specimens should then be inoculated onto BG or RL medium supplemented with glycerol, peptones, and sheep (or preferably horse) blood. Cephalexin is added to reduce the growth of normal flora but may inhibit the growth of some strains of *B. pertussis.* After incubation in ambient air at 35°C to 36°C, *B. pertussis* colonies may become visible after 3 to 4 days, although plates are typically held up to 7 days. *B. pertussis* colonies are round, domed, mercury silver in color, and shiny and produce hemolysis on BG agar. Polyclonal or monoclonal antibodies may be used to confirm identity, or *B. pertussis* can be identified with biochemical tests based on differential phenotypic characteristics.

Molecular Diagnosis

Culture method is the most specific way to diagnose pertussis. The sensitivity of culture for *B. pertussis,* however, varies widely depending on specimen transport and collection methods, as described earlier, and patient factors such as previous immunization, interval since symptom onset, antibiotic use, and age.[83] Different studies have reported culture sensitivity rates for *B. pertussis* ranging from 15% to 80%.[97–99] Many clinical microbiology laboratories now use nucleic acid detection methods such as PCR assay as a more sensitive diagnostic test for pertussis. Different PCR assays target different chromosomal regions of *B. pertussis,* including the PT promoter region, a region upstream of the porin gene, the repetitive insertion sequence IS*481,* the ACT *(cyaA)* gene, and a region upstream of the flagellin gene.[30] PCR methods for *B. pertussis* include the standard PCR gel assay and the reportedly more sensitive real-time assay.[82] The main advantages of diagnosing pertussis with PCR are its aforementioned increased sensitivity and the rapidity of testing compared with culture methods. Appropriate PCR testing permits the differentiation between *B. pertussis* and *B. holmesii*, which can also cause a pertussis syndrome.[100,101] In addition, unlike culture, which is often positive only early in the course of the disease, the PCR result will remain positive even after 21 days of antibiotic treatment in more than half of pertussis cases.[102] However, one also needs to be cautious with the use of PCR testing for pertussis. Because the PCR detects genomic material, it will detect both live and dead bacteria. As with any PCR-based method, contamination is always a concern. Although *B. pertussis* is not an environmental organism, contamination at single collection sites, with positive cultures from environmental surfaces and staff, and even from aerosolized *B. pertussis* vaccine, has been described.[103–105] Contamination may also occur in the laboratory and may be responsible for pseudo-outbreaks of pertussis.[106] A commercially available PCR-based respiratory panel includes *B. pertussis* and *B. parapertussis* (BioFire, Salt Lake City, UT). PCR methodologies and results may vary significantly between laboratories. Depending on where the crossing threshold is set for real-time PCR positivity, the sensitivity and specificity may also vary widely. Studies suggest that PCR may be too sensitive a test to use alone as a screening

method for pertussis diagnosis in an outbreak setting[73] and, in the absence of clinical, serologic, or culture confirmation, positive results may simply reflect transient NP carriage.[65]

The advent of newer molecular methods has also furthered our understanding of the genetic variation among strains of *B. pertussis*. Several investigators have used genotyping methods involving DNA sequencing to look at polymorphisms in the genes encoding for the three major *B. pertussis* antigens: pertussis toxin (PT), PRN, and FIM.[107,108] Many large molecular epidemiologic studies throughout the world have found that *B. pertussis* strains have evolved over time, frequently into dominant clones in response to widespread vaccination pressures.[109,110] For example, studies in Australia and Europe have noted the emergence of *B. pertussis* strains carrying a new allele for the PT promoter *(ptxP3),* conferring increased PT production.[111–113] In the Netherlands, the emergence of *ptxP3* strains was temporally associated with increased incidence of hospitalizations and deaths and increased lethality caused by pertussis, suggesting that genetic adaptions in *B. pertussis* may permit the organism to evade vaccine-induced immunity.[114] In addition, PRN-deficient variants have been identified worldwide, ranging in prevalence from 2.6% in Finland to 27% in Japan. In the United States, there has been an increase in the prevalence of PRN-deficient strains; in the California outbreak in 2010, only 6% of isolates were PRN deficient, whereas in the Washington outbreak in 2012, 63% of isolates were PRN deficient.[115] This increase has not been observed in other countries such as Canada.[116] Although the likelihood of disease in vaccinated persons may be higher in PRN-deficient strains, these strains are not associated with worse clinical outcomes or decreased antibiotic susceptibility and are equally capable of being detected with nucleic acid amplification testing, raising the question as to their clinical significance.[117–120]

These genetic adaptations by the organisms may partially explain the recent resurgence in pertussis disease. Although it is not entirely clear what the natural evolution of *B. pertussis* is over longer periods of time and whether this evolution is directly influenced by vaccination programs worldwide, multiple reports now address these changes over sufficient periods of time to suggest that vaccine genes are evolving and that these changes are associated with vaccine pressure.[121,122] Hence, continued culturing of *B. pertussis* isolates from respiratory samples will be necessary to ensure the ongoing genetic characterization of the bacterium.

Serology

Pertussis can also be diagnosed with serologic methods.[123] The advantage of serology is that by the time the patient presents with typical symptoms of pertussis, the antibody response is usually present. In contrast, NP cultures are usually positive only early in the course of the disease. Most laboratories use an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to *B. pertussis.* ELISAs have been developed with whole bacterial cells, PT, FHA, PRN, FIM, lipooligosaccharide, and ACT. Whole–bacterial cell ELISAs are limited by cross-reactivity with other *Bordetella* species and other bacteria. PT is the most common antigen used for *B. pertussis* serologic testing. PT is produced only by *B. pertussis*, making it a highly specific antigen, and it is an important protective antigen in the immune response to infection and immunization. Unfortunately, young infants may not produce an antibody response to PT. FHA has also been used as an ELISA antigen. However, the antibody response to FHA is not specific because all *Bordetella* species have FHA, and antibodies to *Haemophilus influenzae* and *Mycoplasma pneumoniae* have been shown to cross-react with FHA. In addition, the presence of FHA antibody has not been correlated with protection in human studies. PRN and FIM may also be used to detect a serologic response to *B. pertussis,* but antibodies to these antigens occur less consistently than those against PT. ELISAs using lipooligosaccharide and ACT have been described in only a few research laboratories. Although there are ELISAs to detect IgG, IgA, and IgM to *B. pertussis*, IgG assays are the most frequently used as a diagnostic test and are the best standardized and most widely available. IgG rises typically 2 to 3 weeks after infection or primary immunization and 1 week after booster immunization. Distinguishing between antibody responses secondary to infection and secondary to recent immunization may not be possible. Paired sera are the gold standard for serologic diagnosis, and a twofold increase is considered significant evidence of seroconversion.

The difficulty with *B. pertussis* serologic assessment, however, is that one rarely obtains acute- and convalescent-phase sera but rather obtains convalescent- and late convalescent-phase sera because pertussis is often recognized late in the course of the disease. In immunized individuals, the antibody response is rapid, and one may not see the antibody rise in convalescent serology. Single-serum antibody titers have thus been used to diagnose pertussis, and antibody cutoffs that correlate with acute infection based on population-derived antibody levels have been established.[124] Single-serum testing is particularly useful for adolescents and adults because cultures are often negative in this age group and pertussis immunizations are usually more remote. Modeling has suggested that these thresholds are still valid, even in the face of adolescent and adult vaccination with Tdap (tetanus toxoid, diphtheria toxoid, acellular pertussis vaccine, adult formulation).[125]

Direct Fluorescent Antibody

Finally, direct fluorescent antibody (DFA) testing can also be used to diagnose pertussis infection. Fluorochrome-conjugated monoclonal or polyclonal antibodies recognizing a lipooligosaccharide epitope directly detect *B. pertussis* in NP secretions.[30] However, although it is a rapid and inexpensive diagnostic test, it has poor sensitivity and specificity. The sensitivity of DFA compared with culture is reported to range from 30% to 71%.[99,126] In addition, the specificity of DFA is variable because of cross-reactivity with other organisms, such as *B. bronchiseptica, H. influenzae*, and diphtheroids.[30] DFA testing is not considered to provide laboratory confirmation of a case of pertussis for most national surveillance systems and has been replaced in large part in clinical laboratories by the other diagnostic tests described.

THERAPY

Antimicrobial Agents

There is controversy regarding the efficacy of antibiotic therapy in the different stages of pertussis disease. The difficulty is partly caused by the fact that most studies are powered for a primary outcome of NP microbial eradication rather than for a clinical outcome. The classic teaching is that antibiotics improve symptoms and ablate disease when given early in the course of the disease, during the catarrhal stage, but not when given in the paroxysmal stage (Table 230.1).[127] In an open randomized study by Bergquist and colleagues,[128] 17 patients with a positive culture for *B. pertussis* were treated with erythromycin for 10 days and compared with an untreated control group. Treatment eradicated

TABLE 230.1 Treatment for Pertussis

WHO	WHEN	WHAT
Infants <1 mo	Suspected or proven pertussis	Azithromycin 10 mg/kg/day as a single dose for 5 days
Children	Suspected or proven pertussis within 21 days of onset of symptoms	Azithromycin 10 mg/kg/day (max 500 mg) on day 1, then 5 mg/kg/day (max 250 mg/day) daily on next 4 days *or* Erythromycin 40 mg/kg/day in four divided doses (max 1–2 g/day) for 7–14 days *or* Clarithromycin 15 mg/kg/day in two divided doses (max 1 g/day) for 7 days
Adolescents and adults	Suspected or proven pertussis within 21 days of onset of symptoms	Erythromycin 2000 mg/day in four divided doses for 7–14 days *or* Clarithromycin 1000 mg/day in two divided doses for 7 days *or* Azithromycin 500 mg on day 1, then 250 mg daily on next 4 days

Modified from Kimberlin DW, Brady MT, Jackson MA, et al, eds. Red Book: 2018–2021 Report of the Committee on Infectious Diseases. *31st ed. Itasca, IL: American Academy of Pediatrics; 2018.*

the bacterium from the nasopharynx in all but 1 patient, and the treated group developed significantly fewer whoops than the control group. However, although patients were enrolled within the first 14 days of their illness, a significant proportion already complained of a whooping cough on their initial visit, suggesting that antibiotics may have an effect, albeit not a dramatic one, in the paroxysmal and the catarrhal stages of disease. The Erythromycin Study Group from Germany[129] has also reported improvement in the frequency and severity of coughing in patients in the early paroxysmal stage of pertussis when treated with erythromycin. However, a Cochrane review of 11 randomized or quasirandomized controlled trials of antibiotics for treatment of whooping cough concluded that although antibiotics are effective in eliminating *B. pertussis,* they do not alter the subsequent clinical course of the illness.[130]

Traditionally, oral erythromycin was the antibiotic of choice for treatment of pertussis.[131] Erythromycin estolate is considered superior to erythromycin ethylsuccinate or erythromycin stearate because of the higher drug concentrations achieved in the serum and respiratory secretions. The CDC recommends an erythromycin dose of 40 to 50 mg/kg/day in four divided doses for children to a maximum of 2 g/day for adolescents and adults for 14 days to treat pertussis.[127] Although the CDC recommends a 14-day treatment course, 7 days of a maximum of 1 g of erythromycin estolate treatment was found to be as effective for the eradication of *B. pertussis* and improved compliance in a randomized, controlled clinical trial in Canada.[132] The main problem with oral erythromycin treatment for pertussis is the frequency of gastrointestinal side effects. Up to 30% of patients may experience gastrointestinal symptoms such as nausea, vomiting, or diarrhea. Adults may have serious drug interactions between erythromycin and other drugs. In addition, there is an association between oral erythromycin and hypertrophic pyloric stenosis in infants younger than 1 month; azithromycin, 10 mg/kg daily for 5 days, should therefore be used in this age group.[133]

Several randomized controlled trials have assessed the role of newer macrolides in the treatment of pertussis.[134,135] A Cochrane review of these studies has determined that short-term antibiotics (azithromycin for 3–5 days or clarithromycin for 7 days) are as effective as long-term antibiotics (erythromycin for 10–14 days) in eradicating *B. pertussis* from the nasopharynx (relative risk [RR], 1.02; 95% confidence interval [CI], 0.98–1.05) but had fewer side effects (RR, 0.66; 95% CI, 0.52–0.83).[130] Despite the sparse clinical outcome data in adults, alternatives to erythromycin for treatment and prophylaxis appear to be azithromycin, 500 mg the first day, then 250 mg for 4 days, or clarithromycin, 500 mg twice daily for 7 days. Trimethoprim-sulfamethoxazole for 7 days may also be effective, although the efficacy data are not convincing.[136,137] Although fluoroquinolones have been shown to have in vitro activity against *B. pertussis,*[138] there is a lack of evidence of their clinical efficacy, and reports have described quinolone-resistant *B. pertussis* resulting from mutations in the DNA gyrase gene *(gyrA).*[139] *B. pertussis* strains are not tested routinely for antimicrobial susceptibility because there is no standardized method for antimicrobial susceptibility testing for *B. pertussis* and *B. pertussis* is often detected through molecular rather than culture methods.[131] Although erythromycin resistance, which predicts resistance to other macrolides, in *B. pertussis* strains has been identified and is likely caused by a mutation of the erythromycin-binding site in the 23S rRNA gene,[140,141] there is no evidence that erythromycin resistance in *B. pertussis* is increasing or spreading.[138] Continued surveillance through culture of *B. pertussis* isolates, however, is needed.

Supportive Care

In addition to antibiotic therapy, supportive care is paramount for the management of pertussis, especially in infants. Intubation and mechanical ventilation may be required in infants with apnea and cyanosis.[89] Physicians should also be aware of the potential for respiratory coinfection and treat patients appropriately. Adjunctive therapies such as corticosteroids, salbutamol, pertussis-specific immunoglobulin, and antihistamines have been used to manage the cough of pertussis symptomatically and are the subject of a Cochrane review.[142] Only six studies with a total of 196 participants reported data in sufficient detail to be included in the analysis. No statistically significant benefit was found for any of the interventions. Neither diphenhydramine nor salbutamol changed the frequency of coughing spells, and dexamethasone did not decrease the length of hospital stay. Pertussis immunoglobulin did not affect length of hospital stay and, although it was associated with a mean reduction in the number of whoops per 24 hours, the difference was not statistically significant. An efficacy study of pertussis immunoglobulin for the treatment of hospitalized infants with pertussis was terminated prematurely because of expiration of the immunoglobulin and unavailability of additional study product.[143]

PREVENTION

Immunization

Immunization is the single most effective means of preventing pertussis disease (Table 230.2) (see Chapter 316). The history of pertussis vaccination is long and began with attempts to develop a vaccine from the whole *B. pertussis* organism. The difficulty lay in striking the right balance between making a vaccine with enough bacteria that it was immunogenic versus making a vaccine that was too reactogenic because of additional impurities.[4] Before the 1940s, the efficacy of a vaccine could be assessed only in human trials until Kendrick and associates[144] developed the mouse potency test. In the mouse potency test, the efficacy of a vaccine was determined by immunizing a mouse intraperitoneally and then infecting it intracerebrally with live *B. pertussis.* Survival was measured at 14 days, and a potency unit was calculated according to WHO criteria. Using standardized potency vaccines, the British Medical Research Council performed multiple field trials during the 1940s and 1950s to test different vaccines and found a correlation between the results of the mouse potency test and degree of protection in children against pertussis disease.[145] Concurrently, the United States began routine immunization of children with whole-cell pertussis vaccines in the 1940s and observed a dramatic drop in the rates of pertussis. However, no formal prospective clinical efficacy trials were done to assess whole-cell pertussis vaccines, routinely given combined with diphtheria and tetanus toxoids (DTP vaccine). The best data available regarding their efficacy are from six vaccine trials done in four countries from 1990 to 1995, comparing acellular and whole-cell pertussis vaccines.[4] Except for the Connaught vaccine—thought to be less effective after attempts to make it less reactogenic—the percentage efficacy of DTP against typical pertussis ranges from 89% to 96%. WHO recommends immunization with DTP, and it continues to be used worldwide in many countries.[55] Updated WHO recommendations address the choice of vaccine and vaccination during pregnancy.[146] The problem with whole-cell pertussis vaccines is their reactogenicity. It is known that DTP vaccine causes significantly more local reactions (redness, swelling, and pain) and systemic reactions such as drowsiness, vomiting, and persistent crying than acellular pertussis vaccines and DT alone.[4] More serious adverse events such as convulsions and hypotonic and hyporesponsive episodes (HHE) are much rarer occurrences, especially after the introduction

TABLE 230.2 Vaccination for Pertussis

WHO	WHEN	WHAT
Children	2, 4, 6, and 15–18 mo Booster at 4–6 yr	DTaP or DTaP combination vaccines
Adolescents	11–18 yr, 11–12 yr preferred	Tdap
Adults	Single adult dose	Tdap
Health care workers	Single adult dose	Tdap
Pregnant women	Every pregnancy; 27–36 wk of gestation preferred	Tdap

[a]Based on US schedules.
DTaP, Diphtheria toxoid, tetanus toxoid, acellular pertussis vaccine, pediatric formulation; *Tdap,* tetanus toxoid, diphtheria toxoid, acellular pertussis vaccine, adult formulation (reduced doses of "d" and "ap" components).
Modified from Kimberlin DW, Brady MT, Jackson MA, et al, eds. Red Book: 2018–2021 Report of the Committee on Infectious Diseases. *31st ed. Itasca, IL: American Academy of Pediatrics; 2018; and Liang JL, Tiwari T, Moro P, et al. Prevention of tetanus, diphtheria, and pertussis with vaccines in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Recomm Rep. *2018;67(RR-2):1–44.*

of acellular vaccines.[147] There has also been much discussion surrounding the role of pertussis vaccine in causing neurologic injury and death.[148] From the 1940s to the 1970s, there were multiple case reports and series reporting possible vaccine complications ranging from encephalopathy and coma to death.[149,150] The ensuing controversy about pertussis vaccination affected much of Europe, Japan, the United States, the Soviet Union, and Australia. A large case-control study, the National Childhood Encephalopathy Study (NCES), investigated the relationship between DTP immunization and neurologic injury and found the risk to be very low (1 in 111,000). In 1991, the US Institute of Medicine reviewed all the literature and concluded that there was no evidence to support an association between pertussis immunization and permanent neurologic damage.[151] In addition, according to IMPACT data from 1993 to 2002 in Canada, there was no evidence of encephalopathy after more than 6.5 million doses of pertussis vaccine.[152] There was also initial concern regarding the possible association between pertussis vaccination and sudden infant death syndrome (SIDS).[153,154] However, several large controlled studies failed to find a cause-and-effect relationship between pertussis immunization and SIDS.[153,154] It is likely that any possible association between pertussis immunization and infantile seizures or SIDS is a result of the fact that these conditions occur coincidentally with the time at which the two- or three-dose primary series of DTP is administered.

Acellular Pertussis Vaccines

In response to concerns regarding reactogenicity, pertussis vaccines composed of acellular components of *B. pertussis* were developed. Sato and coworkers[155] from Japan were the first to design an acellular pertussis vaccine containing two purified hemagglutinins (HAs): FHA and leukocytosis-promoting factor HA. By removing endotoxin (lipopolysaccharide [LPS]) from the preparation, they were able to produce a vaccine that was as effective as the whole-cell vaccine and caused fewer side effects. The vaccine was used for mass immunization in Japan beginning in 1981 during an epidemic of pertussis. Although efficacy data for this first acellular pertussis vaccine were limited, its use spurred the development of further prototypes. Several different versions of acellular pertussis vaccines have since been developed and include one-, two-, three-, four-, and five-component vaccines (composed of a combination of PT, FIM types 2 and 3, PRN, and/or FHA). All are less reactogenic than their whole-cell counterparts because they do not contain LPS.[4] Their efficacies vary depending on the type of study done and the case definition used but range from 59% to 93% for pertussis cases defined according to the WHO criteria.[156] Vaccines containing PT, FHA, PRN, and FIM are likely more effective against mild disease than vaccines containing PT, FHA, and/or PRN alone, suggesting that FIM may have a role in protection against infection and/or milder disease.[157] The recently developed baboon model of clinical pertussis provides some insight into the differing immune responses to whole-cell versus acellular pertussis vaccines.[158] Baboons who clear wild-type pertussis infection mount very strong Th17 and Th1 responses and a very low Th2 response. In contrast, baboons vaccinated with acellular pertussis vaccine remain heavily colonized in the airway, can transmit pertussis, and have a strong Th2 response with low Th17 and Th1 responses. Baboons vaccinated with whole-cell pertussis vaccine fall in between these two presentations, being able to eventually clear infection with low Th17 and Th1 responses and no Th2 response.[159,160] These data may help to explain the pertussis resurgence seen in the era of acellular pertussis vaccination.

Vaccination Schedules

Most North American immunization schedules recommend pertussis vaccination (DTaP, pediatric formulation) at 2, 4, 6, and 18 months of age, with a booster at 4 to 6 years of age. Although there are many different pertussis immunization schedules throughout the world, most will have two or three doses in the first year of life, another dose in the second year of life, and a preschool booster. Childhood immunization with pertussis-containing combination vaccines has been demonstrated to be safe, immunogenic, and effective for the control of pertussis.[161] However, pertussis outbreaks continue to occur in the adult[75,162] and adolescent population.[77,163] Because adolescents and adults continue to be a source of pertussis transmission,[164,165] immunization of this group was investigated in the Adult Pertussis Trial (APERT).[166] In this study, 2781 healthy subjects were randomized to receive either a tricomponent acellular vaccine or a hepatitis A vaccine. Vaccine protection against pertussis disease was 92% during the study follow-up period (median duration, 22 months). Several prospective, randomized, controlled trials have assessed the reactogenicity and immunogenicity of Tdap in several thousand adolescents and adults.[167–169] The Tdap vaccine elicited robust immune responses and had a similar safety profile to the Td vaccine. Follow-up data from several of these studies demonstrated that antibody titers to pertussis exceeded preimmunization levels 1, 3, and 5 years later.[170] Further studies have shown that Tdap boosters (Adacel [Sanofi Pasteur, Toronto, Canada] or Boostrix [GlaxoSmithKline, Research Triangle Park, NC]; lower case letters "d" and "ap" indicate reduced dose compared with DTaP) can be given safely at any interval after prior tetanus, diphtheria, or pertussis vaccinations in adolescents.[171–173] Both cellular and humoral immune responses occur after acellular pertussis immunization of adults and adolescents, but the cell-mediated immune response may be of greater magnitude and longer duration and thus more reflective of long-term protection.[174] Both the National Advisory Committee on Immunization (NACI) and the Advisory Committee on Immunization Practices (ACIP) of the CDC have recommended routine Tdap vaccination for adolescents and to replace Td with Tdap boosters in adults.[175–177] Tdap has also been shown to be safe and immunogenic in older individuals[178] and is now also recommended for adults older than 65 years.[179] Experience in Canada[70,180,181] and in the United States[182,183] has demonstrated that an adolescent pertussis vaccine program can be implemented on a national scale, is safe, and can result in a further decrease in the incidence of pertussis. To date, however, Tdap programs for adults have been more difficult to implement.[184]

Vaccination of Health Care Workers

Health care workers are another potential source of pertussis transmission, particularly to patients. Multiple nosocomial pertussis outbreaks have been described,[185–187] with significant patient health consequences and costs incurred by the health care system.[188–190] Costs included direct medical center costs for treatment and prophylaxis, costs for personnel time, and indirect medical center costs for time lost from work. It is more cost-effective to vaccinate health care workers than to manage a nosocomial exposure of pertussis.[191] ACIP recommends that health care workers who work in hospitals or ambulatory care settings and have direct patient contact should receive a single dose of Tdap if they have not been vaccinated previously as an adult, regardless of when they received their last dose of Td.[177] Given its cost-effectiveness, health care institutions should provide universal Tdap immunization to their health care workers.

Protection of Infants

Infants are most vulnerable to severe pertussis disease, so specific strategies have been examined to protect neonates from pertussis transmission.[192] A cocoon strategy of vaccinating household members has been suggested to provide partial protection to unimmunized infants because parents are known to be important sources of infection in these cases.[177] One study showed a 70% decrease in the number of pertussis cases in infants 0 to 3 months of age in households in which a cocoon strategy had been implemented.[193] Another study demonstrated an ability to vaccinate more than 90% of postpartum women with Tdap.[194] However, there are practical and logistical difficulties in implementing vaccination programs to reach all potential infant contacts, including fathers and other family members.

Vaccination During Pregnancy

In light of the increased incidence of pertussis and known serious complications that can occur with neonatal pertussis disease, ACIP recommended the use of Tdap during pregnancy[195] after reviewing the safety data from registries and small studies.[172,196] Subsequent reviews of reports to the Vaccine Adverse Event Reporting System (VAERS) did not identify any concerning patterns in maternal, infant, or fetal outcomes after Tdap vaccination of pregnant women.[197] In October 2012, ACIP updated its recommendations, advising that Tdap also be

given during each subsequent pregnancy. Although pertussis antibodies cross the placenta, maternal antibody levels are low and decay rapidly.[198] Administering a pertussis booster dose during pregnancy may increase neonatal antibody titers but may not help with the infant's cellular immune response to infection. However, a case-control study in England and Wales estimated that vaccination during pregnancy was 93% effective in preventing pertussis during the first 2 months of life.[199] A subsequent randomized, double-blind, placebo-controlled clinical trial of Tdap immunization of pregnant women at 30 to 32 weeks' gestation demonstrated higher concentrations of maternal pertussis antibodies at delivery and higher antibodies in their infants at birth and at age 2 months compared with those whose mothers received placebo.[200] In addition, antibody responses in infants born to women who received Tdap during pregnancy were not different after the fourth dose of DTaP. There is a concern, however, that maternal immunization may interfere with an infant's ability to respond to active immunization. A large Canadian, randomized, controlled, multicenter trial in which 273 pregnant women received either Tdap or Td in the third trimester revealed that infants whose mothers had received Tdap had lower antibody levels to PT, FHA, PRN, and FIM antigens at 7 months of age (after pertussis vaccination at 2, 4, and 6 months) compared with infants whose mothers had received only Td.[201] Furthermore, antibody levels to PT, FHA, and FIM were lower in these infants when measured 1 month after the 12-month pertussis booster. Reassuringly, a retrospective cohort study of almost 150,000 newborns from 2010 to 2015 in California showed that maternal Tdap vaccination was 91.4% effective at preventing neonatal pertussis during the first 2 months of life and 69% effective at preventing pertussis during the first year of life, providing additional protection to infant DTaP immunization. As a result of ACIP recommendations, Tdap vaccination coverage during pregnancy in the United States has increased from <1% before 2009 to 54% in 2015. Attempts to further refine the optimal timing (gestational week) for vaccination continue. A prospective trial of vaccination in the third trimester showed improved cord blood antibody levels in mothers vaccinated in the early period (28–36 + 6 weeks) compared with the late period (32–38 + 6 weeks).[202] A trial initially designed to demonstrate the noninferiority of second-trimester vaccination instead showed superiority by rates of both seropositivity and concentration of infant antibody.[203] Second-trimester maternal Tdap vaccination also leads to higher birth anti-PT titers in preterm neonates compared with third-trimester maternal vaccination, providing possible improved protection for a particularly vulnerable patient population.[201] The durability of vaccination during pregnancy with Tdap suggests that antibodies wane significantly 9 to 15 months after delivery.[204]

Neonatal Vaccination

Finally, neonatal pertussis vaccination has also been studied as a method of preventing pertussis. Vaccination of newborn infants with whole-cell pertussis vaccine resulted in "immune tolerance" or reduced antibody responses to *B. pertussis* antigens compared with infants who received their first pertussis vaccine at 2 months of age.[205,206] However, early neonatal immunization with acellular pertussis vaccine was safe, was well tolerated, and accelerated the acquisition of pertussis antibodies in infants without inducing immune tolerance.[207,208] The difficulty with the current inactivated pertussis vaccines, however, is that they require interferon-γ (IFN-γ) production as part of a Th1 response to effectively induce vaccine-mediated protective immunity, and neonatal T cells produce lower levels of IFN-γ.[209,210]

Investigators have therefore developed a live-attenuated *B. pertussis* vaccine to be delivered intranasally in an attempt to replicate the long-lasting and vigorous immunity seen with natural infection in neonates. Such a live-attenuated vaccine was shown to induce significantly better protection against pertussis disease in infant mice than an injectable acellular vaccine[211] and to produce long-term cellular and humoral immune responses in mice.[212] A phase I double-blind, placebo-controlled, dose-escalating study of a nasal live-attenuated *B. pertussis* vaccine (BPZE1) was conducted in 48 healthy adult male volunteers and demonstrated that the vaccine was safe and induced colonization, particularly in the higher-dose group.[213] In addition, colonization was associated with significant increases in serum antibodies against PT, PHA, PRN, and FIM and in memory B-cell responses to PT, FHA, and PRN.[214] However, certain participants were not colonized. These individuals were noted to have significantly higher prevaccination antibody levels to FHA, PRN, and FIM, possibly due to prior infection with *B. pertussis*. This suggests that previous exposure to the organism may induce immunity, preventing BPZE1 uptake.[215] Phase Ib clinical trials of intranasal immunization with high-dose BPZE1 in healthy volunteers are ongoing (NCT02453048).

Chemoprophylaxis

In addition to immunization, chemoprophylaxis may be used to prevent the transmission of *B. pertussis*. In the United States, erythromycin prophylaxis is recommended for all household contacts and other close contacts, including those in child care.[127] In Canada, chemoprophylaxis is recommended only for infants or pregnant women (third trimester) and household contacts (Table 230.3).[216] However, erythromycin prophylaxis of pertussis contacts is not practiced uniformly throughout the world. In fact, in a Cochrane review of two randomized, controlled trials of antibiotic treatment of contacts,[217,218] the authors concluded that chemoprophylaxis of contacts older than 6 months does not significantly improve clinical symptoms or the number of cases developing culture-positive *B. pertussis*.[130] It is important to note, however, that these randomized trials did not specifically investigate the use of erythromycin in high-risk populations, such as children younger than 6 months, who might benefit the most from any added protection. Although there are reports of successful chemoprophylaxis against pertussis, these are often the results of uncontrolled or poorly controlled studies.[219,220] It is likely, in many cases, that transmission occurs before pertussis is diagnosed in the index case, thus limiting the usefulness of chemoprophylaxis. If chemoprophylaxis is to be of any benefit, it needs to be given early in the course of the disease in the index case and before the occurrence of the first secondary case.[221] Chemoprophylaxis

TABLE 230.3 Postexposure Prophylaxis for Pertussis for Close Contacts

WHO	WHEN	WHAT
Infants	Exposed to case of pertussis	Azithromycin 10 mg/kg/day as a single dose for 5 days
Children	Exposed to case of pertussis within 21 days of onset of cough in index case	Erythromycin 40 mg/kg/day in four divided doses (max 1–2 g/day) for 7–14 days *or* Clarithromycin 15 mg/kg/day in two divided doses (max 1 g/day) for 7 days *or* Azithromycin 10 mg/kg (max 500 mg) on day 1, then 5 mg/kg/day (max 250 mg) daily next 4 days *plus* Age-appropriate pertussis vaccination
Pregnant women (third trimester)	Exposed to case of pertussis	Erythromycin 2000 mg/day in four divided doses for 7–14 days *or* Clarithromycin 1000 mg/day in two divided doses for 7 days *or* Azithromycin 500 mg, then 250 mg daily next 4 days *plus* Tdap vaccination (if not already given in this pregnancy)
Other adolescents/adults	Exposed to case of pertussis within 21 days of onset of cough in index case	Erythromycin, clarithromycin, and azithromycin dosages as for pregnant women; plus Tdap vaccination

Tdap, Tetanus toxoid, diphtheria toxoid, acellular pertussis vaccine, adult formulation (reduced doses of "d" and "ap" components).
Modified from Kimberlin DW, Brady MT, Jackson MA, et al, eds. Red Book: 2018–2021 Report of the Committee on Infectious Diseases. *31st ed. Itasca, IL: American Academy of Pediatrics; 2018.*

is generally not recommended if 21 days have elapsed since the onset of cough in the index patient.[127]

Pertussis in Schools and Daycare Centers

Children and staff with pertussis should be excluded from attendance until 5 days after initiation of macrolide treatment. Those untreated should be excluded for 21 days after onset of illness. Exposed children, particularly incompletely immunized children, should be observed for 21 days after the last contact.

FUTURE DIRECTIONS

Although much has been learned about pertussis since the time of Lapin, there remain several unanswered questions. How much pertussis disease is there in adults? Do adults require multiple doses of pertussis vaccine, and will this result in less cough illness? Is there an NP carrier state for *B. pertussis*? Is maternal immunization a safe and effective way of preventing neonatal pertussis? Future research is needed in these and other areas to further our understanding of pertussis.

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The complete reference list is available online at Expert Consult.

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231 Rat-Bite Fever: *Streptobacillus moniliformis* and *Spirillum minus*

Vijayashree Mekala and Ronald G. Washburn

SHORT VIEW SUMMARY

Definition
- Rat-bite fever is an acute febrile illness characterized by rash and relapsing fever. It is caused by *Streptobacillus moniliformis* and *Spirillum minus.*

Epidemiology
- The infection is transmitted by bites or scratches from rodents or carnivores that prey on rodents or by ingestion of contaminated milk or water. Rat-bite fever is not generally reportable, and the incidence is probably higher than the literature reflects.

Microbiology
- In the United States and Europe, rat-bite fever is caused by *S. moniliformis,* a fastidious gram-negative bacillus. In Asia, the causative organism is *S. minus,* a spirochete that has never been grown in culture.

Diagnosis
- *S. moniliformis* is diagnosed by clinical presentation plus culture in enriched media or polymerase chain reaction. *S. minus* is diagnosed by clinical presentation plus direct visualization or xenodiagnosis.

Therapy
- Penicillin is the treatment of choice for both types of rat-bite fever.

Prevention
- Preventive measures include eradication of rats, avoidance of nonpasteurized milk and water, and use of gloves by laboratory workers when handling rats.

Rat-bite fever is a rare systemic febrile illness typically transmitted by the bite of a rat or other small rodent. The infection has a worldwide distribution and can be caused by either *Streptobacillus moniliformis* or *Spirillum minus,* bacteria commonly found in the oropharyngeal flora of rodents. Streptobacillary disease accounts for the vast majority of cases of rat-bite fever in the United States,[1] whereas *S. minus* infections occur mainly in Asia. Table 231.1 compares the two different forms of rat-bite fever.

Illness after rat bites has been known in India for more than 2000 years,[2] and the characteristic syndrome of rat-bite fever was recorded in the United States as early as 1839.[3] Early in the 20th century, the causative gram-negative bacillus, initially named *Streptothrix muris ratti,* was recovered from clinical material.[4,5] In 1925, a blood culture isolate from a laboratory worker with fever, rash, and arthritis was called *S. moniliformis*, based on its morphologic resemblance to a beaded necklace.[6] In 1926, a similar organism, *Haverhillia multiformis,* was grown from the blood of patients during an epidemic illness resembling rat-bite fever in Haverhill, Massachusetts.[7] Both *H. multiformis* and *S. muris ratti* were subsequently shown to be identical to *S. moniliformis,* the causative agent of streptobacillary rat-bite fever.[2]

STREPTOBACILLUS MONILIFORMIS

Bacteriology

S. moniliformis is a pleomorphic, nonmotile, nonsporulating, nonencapsulated gram-negative bacillus in the family Leptotrichiaceae,[8] measuring 0.3 to 0.7 µm wide by 1 to 5 µm long.[9] Filaments and beadlike chains up to 150 µm long may contain 1- to 3-µm-wide fusiform swellings (Fig. 231.1).[10] The organism is microaerophilic and capnophilic, requiring a partial pressure of carbon dioxide between 8% and 10% for primary isolation at 37°C. Trypticase soy agar or broth must be supplemented with 10% to 20% rabbit, sheep, or horse serum; defibrinated blood; or ascites to support optimal growth. A shell vial cell culture technique that used human endothelial cells rescued viable *S. moniliformis* after conventional culture methods failed.[11] Sodium polyanethol sulfonate, a substance sometimes added to aerobic blood culture bottles as an anticoagulant or to trypticase soy or thioglycollate broth to inhibit the antibacterial activity of human blood, impedes the growth of *S. moniliformis* in concentrations of at least 0.0125%.[12,13] Thus if rat bite fever is suspected, the laboratory should be informed and serum-supplemented broth or agar medium without sodium polyanethol sulfonate should be used.

On blood agar plates, nonhemolytic cotton-like colonies, 1 to 2.5 mm in diameter, appear after approximately 3 days of incubation. In broth media, characteristic flocculent "puffballs" are seen at the bottom of the broth after 2 to 10 days (Fig. 231.2). Penicillin-resistant L-phase variants may form spontaneously or in the presence of penicillin both in vivo and in vitro.[14] Low muramic acid content in cell envelopes may contribute to the propensity of *S. moniliformis* to produce L forms,[15] which impart a turbid appearance to broth media and a "fried egg" colony morphology on solid agar.[9] Sugar fermentation is variable but often includes galactose, glucose, maltose, and salicin. Fatty acid analysis by gas-liquid chromatography is useful for the rapid identification of *S. moniliformis* isolates.[9,16–20] In addition, sodium dodecyl sulfate–polyacrylamide gel electrophoresis patterns of cellular proteins may be useful for epidemiologic studies of the epidemic (Haverhill fever) form of the infection.[21] Guanine plus cytosine content is 24 to 26 mol%, and the complete genome sequence has been reported.[8,9]

Epidemiology

In the United States, where 50% to 100% of rats are colonized with *S. moniliformis,* persons who are at risk for percutaneous inoculation with *S. moniliformis* include animal laboratory personnel and individuals (especially children) inhabiting crowded urban dwellings or rural areas infested with wild rats.[1,22,23–27,28] Although rat bite fever is not reportable, based on an estimated 20,000 rat bites annually and a 10% bite infection rate, an incidence of more than 1000 cases per year is estimated.[9] The infection is typically transmitted by the bite or scratch of rats, mice, guinea pigs, squirrels, or carnivores that prey on those rodents, including cats, dogs, pigs, ferrets, weasels, and snakes.[22,29,30,31] One reported case followed the bite of a gerbil,[32] another the scratches of a rooster.[33] The infection may also be acquired by handling rats, without any apparent breach of intact skin[23,34,35] or with a portal of entry, such as varicella lesions.[36] Wild rats as well as laboratory and pet rats harbor *S. moniliformis* in their nasopharyngeal microbiota,[23,37–40] and they may develop otitis media.[41] In contrast, healthy laboratory mice are generally not colonized with *S. moniliformis* but are susceptible to epizootic infections characterized by polyarthritis, septicemia, pneumonia, otitis media, and high rates of abortion.[2,38,42–44] *S. moniliformis* has also been reported to cause pleuritis in a koala,[45] cervical abscesses and pneumonia in guinea pigs,[46] arthritis in turkeys and a monkey, and endocarditis in a macaque.[47]

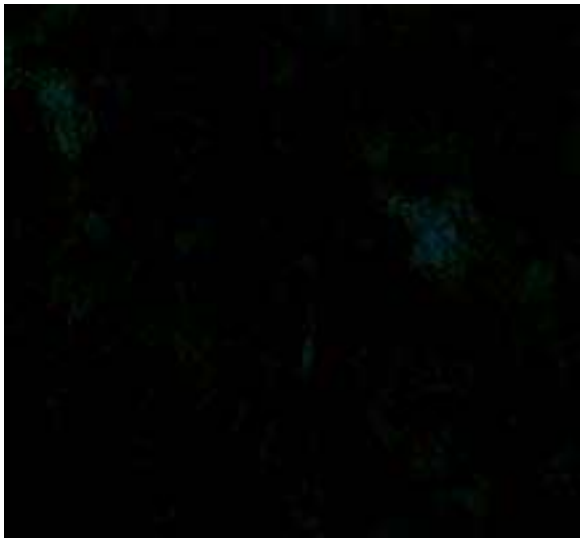

FIG. 231.1 Pleomorphic gram-negative bacilli—*Streptobacillus moniliformis.* *(From Albedwawi S, LeBlanc C, Shaw A, et al. A teenager with fever, rash and arthritis.* CMAJ. *2006;175:354.)*

TABLE 231.1 Comparison of Two Different Types of Rat-Bite Fever

PARAMETER	ORGANISM	
	Streptobacillus moniliformis	***Spirillum minus***
Organism	Gram-negative bacillus	Gram-negative coiled rod
Geographic distribution	North America, Europe	Asia
Mode of transmission	Rat bite, ingestion	Rat bite
Clinical syndrome:		
Ulceration of initial bite wound	No	Yes
Arthritis	Yes	No
Regional lymphadenopathy	No	Yes
Rash	Yes	Yes
Relapsing fever	Yes	Yes
Diagnosis	Culture, polymerase chain reaction	Direct visualization, xenodiagnosis
Therapy	Penicillin G	Penicillin G

Pathophysiology

The clinical disease is probably the consequence of failed local cutaneous defenses and bacterial dissemination. The few available published pathology reports described interstitial pneumonia, lymph node hyperplasia, erythrophagocytosis, vasculitis,[48,49] and intravascular thrombi.[50,51]

Oral ingestion of organisms has caused several epidemics of Haverhill fever (erythema arthriticum epidemicum), an illness clinically resembling rat-bite fever. Potential sources of such outbreaks include foods such as turkey, or milk or water contaminated with rat excrement.[6,7,12,23,52–54] Presumably, once ingested, *S. moniliformis* organisms gain access to the peripheral circulation by penetrating the gastrointestinal mucosa.

Clinical Manifestations

After the rat bite, a brief incubation period (1–22 days, usually <10 days), is followed by abrupt onset of fever, chills, headache, vomiting, and severe migratory arthralgias and myalgias that mark the beginning of clinical disease. By that time, the wound itself has usually already healed. The diagnosis is often initially obscured by the fact that patients are unaware of bites occurring during sleep. In contrast to *S. minus* infection, regional lymphadenopathy is minimal or absent. The peripheral white blood cell count may be as high as 30,000/mm^3 with a leftward shift, and approximately 25% of patients have false-positive nontreponemal syphilis serologies.[30,48] Within 2 to 4 days after the onset of fever, a nonpruritic maculopapular, morbilliform, petechial,[55] vesicular[53] or pustular[48,56] rash erupts over the palms, soles, and extremities (Fig. 231.3). Skin lesions may become purpuric[50,57,58] or confluent and may eventually desquamate.[22] Approximately 50% of patients develop asymmetrical polyarthritis or true septic arthritis[59–61] concurrently with the rash or within a few days thereafter.[24,62,63,64–67] The knees are most commonly involved, followed by the ankles, elbows, wrists, shoulders, and hips.[52,65,66] Typically, fever subsides after 3 to 5 days, even without specific antibiotic therapy, and the remaining symptoms gradually resolve within 2 weeks. However, fever may occasionally relapse in an irregular pattern for weeks or months,[14] producing a clinical picture of fever of undetermined origin. Alternatively, arthritis may persist for as long as 2 years.[9] Haverhill fever differs clinically from percutaneously acquired rat-bite fever chiefly in the high incidence of pharyngitis and heightened severity of vomiting.[12,22]

Reported complications of *S. moniliformis* infection include endocarditis,[23,52,64,68–75] myocarditis,[23,37] pericarditis,[2,68] sepsis,[76,77] cutaneous leukocytoclastic vasculitis,[78] systemic vasculitis,[79] meningitis,[71] pneumonia,[23,71] hepatitis,[35] septic arthritis,[24,80–83] diskitis,[84] amnionitis,[85] and anemia.[2,37] Abscesses have been observed in almost all organs, including brain,[86] liver, spleen, kidney, epidural space[87,88] and vertebrae,[31,89,90] skin,[10,91] muscle,[33] and the female genital tract.[19] In infants and young children, diarrhea and weight loss may be prominent.[23,27,71] The mortality of untreated cases ranges as high as 13%,[71] and endocarditis in the

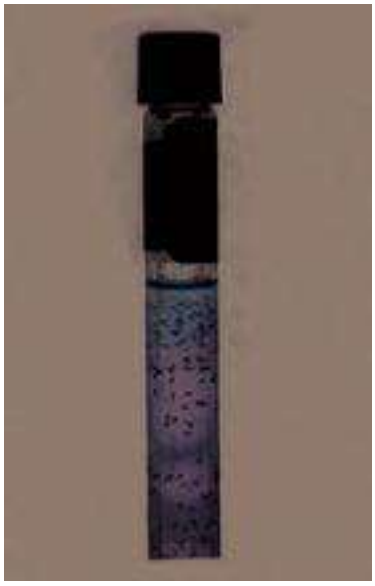

FIG. 231.2 "Puffballs" in broth culture—*Streptobacillus moniliformis.* *(From Albedwawi S, LeBlanc C, Shaw A, et al. A teenager with fever, rash and arthritis.* CMAJ. *2006;175:354.)*



FIG. 231.3 Petechial rash of rat-bite fever. *(From Albedwawi S, LeBlanc C, Shaw A, et al. A teenager with fever, rash and arthritis.* CMAJ. *2006;175:354.)*

preantibiotic era was often lethal. Most of those intravascular infections involved prosthetic materials,[74,92] or native valves that were previously damaged by rheumatic valvulitis or calcification,[52] but one case involved a previously normal mitral valve.[74]

Diagnosis

In a febrile patient with rash and recent rat exposure, the diagnosis can usually be narrowed down to rat-bite fever or leptospirosis. However, the physician caring for a laboratory worker may step into the trap of attributing a seemingly benign febrile illness to viral infection. Furthermore, without a positive exposure history, the diagnosis may be even more elusive because diagnoses such as meningococcemia, enteric fever, drug reaction, and viral exanthem enter into consideration. When the rash involves the palms and soles, rat-bite fever may mimic Rocky Mountain spotted fever[93] or secondary syphilis. The presence of oligoarticular or migratory polyarthritis heightens concerns about disseminated gonococcal infection, Lyme disease, brucellosis, septic arthritis, infective endocarditis, rheumatoid arthritis, and acute rheumatic fever.

Direct visualization of pleomorphic bacillary organisms in Giemsa-, acridine orange–, or Gram-stained smears of blood, joint fluid,[50] and pus may provide an early clue to the diagnosis. However, laboratory diagnosis ultimately rests on culturing *S. moniliformis* using enriched media.[94] An enzyme-linked immunosorbent assay has been developed for detection of specific antibodies against *S. moniliformis.*[95] More recently, polymerase chain reaction (PCR) techniques that amplify bacterial 16S ribosomal RNA have been used to detect *S. moniliformis* infection in rodents[40,96,97] and humans.[a] Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has also been used for positive identification of the organism from blood cultures.[80,101,102]

Therapy and Prevention

Both agents of rat-bite fever, *S. moniliformis* and *S. minus,* are susceptible to penicillin. In the past, procaine penicillin G was given intramuscularly (IM) as 600,000 units every 12 hours for 10 to 14 days.[2,22,62] Currently, intravenous (IV) penicillin G or ceftriaxone appear more appropriate. The Jarisch-Herxheimer reaction may complicate initial therapy of *S. minus* infections. Oral tetracycline, 500 mg every 6 hours, is preferred for penicillin-allergic patients.[2,16] Streptomycin (7.5 mg/kg) can be given IM every 12 hours, although potential ototoxicity makes this less desirable. Limited experience indicates that ceftriaxone,[50,56,67,79,88] erythromycin,[103] chloramphenicol,[12,93] or clindamycin[23,37] might also be effective.

Most patients respond promptly to therapy. For individuals who appear well after 5 to 7 days of parenteral therapy, the course can be completed with an additional week of oral penicillin V or ampicillin, 500 mg every 6 hours. Patients with mild disease can probably be successfully treated by the oral route for the entire course.

Endocarditis is so rare that optimal therapy is uncertain. It is probable that 4 weeks of IV penicillin, with or without streptomycin or gentamicin,[72,75] are adequate. A total daily dose of 20 million units has been advocated for patients whose isolates are resistant to 0.1 μg/mL.[69]

After a rodent bite, the wound should be thoroughly cleaned, and tetanus prophylaxis should be administered if warranted by the patient's immunization history. A prophylactic 3-day course of oral penicillin (2 g/day) would seem reasonable, although efficacy is unknown, and the patient should be advised to report any subsequent symptoms. Measures to limit the incidence of rat-bite fever include eradication of rats in urban areas, avoidance of nonpasteurized milk and potentially contaminated water, and the use of gloves by laboratory workers when handling rodents.

SPIRILLUM MINUS

S. minus causes a significant portion of rat-bite fever cases in Asia but rarely produces infection in the United States.[1,25] A case occurred in a traveler returning from Vietnam to Italy.[104] In Japan, the infection is called *sodoku (so,* rat; *doku,* poison).

The causative organism was discovered by Carter during the 19th century.[105] In the early years of the 20th century, specimens from patients with *sodoku* were shown to contain spirochetes capable of infecting guinea pigs. Those bacteria were initially called *Spirocheta morsus muris* or *Sporozoa muris.*[2] The organism was renamed *Spirillum minus* in 1924.[106]

Bacteriology

S. minus is a short, thick, gram-negative, tightly coiled spiral rod measuring 0.2 to 0.5 μm by 3 to 5 μm.[22,107] The organism has two to six regular helical turns. Terminal polytrichous flagella confer darting motility, which can be demonstrated with darkfield examination. The flagella can be stained with silver impregnation methods (e.g., Fontana-Tribondeau staining). Despite reports to the contrary, *S. minus* has not been cultured on artificial media, and its name derives from its appearance alone. No attempt at sequence analysis of the organism in body fluids has been reported.

Epidemiology, Pathogenesis, and Pathology

The epidemiology of *S. minus* infections is similar to that of streptobacillary rat-bite fever, with the exception that oral ingestion has not been shown to cause spirillary disease. The major route of transmission is through rat bites. Approximately 25% of tested rats were positive for *S. minus* in conjunctival and nasopharyngeal secretions, pulmonary lesions, and blood.[23] Human-to-human transmission has not been documented. The reasons for the marked geographic differences are not known.

Relapses of spirillary rat-bite fever have been postulated to be caused by seeding of blood and distant foci during periodic reactivation of the primary bite lesion. The available recorded autopsies show granulomatous inflammation at the original site of inoculation, with epithelial necrosis and mononuclear infiltration of the dermis. Regional lymph nodes are hyperplastic. Deep tissue specimens from distant areas of skin rash contain dilated blood vessels and round cell infiltrates. Liver, spleen, renal tubules, myocardium, and meninges may be hemorrhagic, with areas of necrosis in liver and kidney.

Clinical Manifestations

The initial bite wound heals promptly but then becomes painful, swollen, and purple approximately 1 to 4 weeks later, and is associated with regional lymphangitis and lymphadenitis. This local inflammatory lesion ushers in a systemic illness characterized by fever, chills, headache, and malaise. In contrast to streptobacillary rat-bite fever, arthritis and myalgias are rare in *S. minus* infection. Next, the bite wound progresses to chancre-like ulceration and induration with eschar formation. During the first week of fever, a blotchy violaceous or reddish-brown macular rash erupts over the extremities, face, scalp, and trunk, and then it fades during subsequent afebrile intervals. On occasion, the rash may be urticarial.[22] Leukocytosis with peripheral white blood cell counts in the range of 10,000 to 20,000/mm^3 may be observed, and up to 50% of patients have false-positive syphilis serologies.

Without specific antibiotic therapy, fevers lasting 3 to 4 days recur at regular intervals between afebrile periods of 3 to 9 days. Spontaneous cure usually occurs within 1 to 2 months, but in selected instances, fevers have relapsed for years.[22]

The most serious complication of untreated spirillary rat-bite fever is endocarditis. Most of these rare intravascular infections have been observed in patients with preexisting valvular disease, but one reported case occurred on a normal aortic valve.[107] The spectrum of reported complications also includes myocarditis, pleural effusions, hepatitis, splenomegaly, meningitis, epididymitis, conjunctivitis, and anemia.[22,27] Overall mortality of untreated *S. minus* infections in the preantibiotic era was 6% to 10%.

Diagnosis and Therapy

Diagnosis depends on the history of rat bite, typical clinical features, and demonstration of the organisms on examination of blood, exudate, or lymph node tissue by using Giemsa stain, Wright stain, or darkfield microscopy. Organisms have been recovered from mice or guinea pigs 1 to 3 weeks after intraperitoneal inoculation,[22,108] but the animals must be prescreened to rule out the presence of preexisting spirochete infections. No specific serologic test or PCR assay is available for *S. minus* infection. Therapy is the same as for *S. moniliformis* infection.

[a]References 11, 30, 35, 53, 57, 61, 79, 81, 83, 88, 90, 92, 98–100.

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232 Legionnaires' Disease and Pontiac Fever

Paul H. Edelstein and Craig R. Roy

SHORT VIEW SUMMARY

Definition
- Legionnaires' disease is a noncontagious type of bacterial pneumonia caused by *Legionella* spp. bacteria, most commonly *Legionella pneumophila.*
- Pontiac fever is a several-day-long, nonpneumonic, febrile, influenza-like illness associated with exposure to *Legionella* spp. that resolves spontaneously.
- Legionellosis includes legionnaires' disease, Pontiac fever, and extrapulmonary *Legionella* spp. infection not associated with pneumonia.

Epidemiology
- Legionnaires' disease is acquired by inhaling a water aerosol containing *Legionella* spp. bacteria, and possibly by microaspirating *Legionella* spp.–containing water.
- About 20 to 80 cases annually per million population occur in most developed countries, with the elderly, males, cigarette smokers, and immunosuppressed patients most at risk.
- About 1% to 5% of patients hospitalized for community-acquired pneumonia have legionnaires' disease.
- Pontiac fever is associated with inhalation of *Legionella* spp.–containing water but not necessarily caused by the bacterium. It occurs in sporadic and epidemic form at apparently low incidence but may be more common than is reported.

Microbiology
- Constituted of more than 60 known species, the *Legionella* bacteria are ubiquitous in the aqueous environment and probably moist soils.
- Optimal bacterial growth requires specialized media that contain cysteine and iron.
- The bacteria are facultative intracellular parasites of free-living amebae and human monocytes and macrophages that use hostlike proteins to masquerade as host and to avail themselves of intracellular resources.

Diagnosis
- Clinical and roentgenographic differentiation of legionnaires' disease from common causes of pneumonia is not generally possible.
- Specific and specialized laboratory testing can be helpful for disease diagnosis but is not highly sensitive in all settings. Urine antigen testing, the most commonly used test, is about 70% and 30% sensitive in community-acquired and nosocomial disease, respectively.

Therapy
- Macrolides, tetracyclines, and quinolone antimicrobials can all be used to successfully treat the disease, with azithromycin and levofloxacin being the most active. Tetracyclines may not be active for *L. longbeachae*–caused disease.
- For cure, 3 to 14 days' therapy is required, depending on disease severity, host factors, and type of therapy used.

Prevention
- No vaccine is available.
- Proper environmental design and maintenance reduce disease risk.

HISTORY

Legionnaires' disease (LD) is an acute pneumonic illness caused by gram-negative bacilli of the genus *Legionella*, the most common of which is *Legionella pneumophila (Lp).* Pontiac fever (PF) is a febrile, nonpneumonic, systemic illness closely associated with, if not caused by, *Legionella* spp. *Legionellosis* is the term that encompasses all diseases caused by, or presumed to be caused by, the *Legionella* bacteria, including LD, focal nonpulmonary infections, and PF.

LD was first recognized when it caused an epidemic of pneumonia at a Pennsylvania State American Legion convention in Philadelphia in 1976; 221 people were affected, and 34 died. Despite intensive laboratory investigation, the cause of the outbreak went undetected for many months. Epidemiologic investigation concluded that the disease was most likely airborne, and focused primarily at one convention hotel, which closed because of adverse publicity.[1,2] About 6 months later, Joseph McDade and Charles Shepard, investigators at the Centers for Disease Control and Prevention, discovered the etiologic agent, a fastidious gram-negative bacillus.[3] Because of the historical association with the American Legion convention, this disease is now called "legionnaires' disease," and the etiologic agents belong to the family *Legionellaceae,* with *Lp* being the agent responsible for the 1976 Philadelphia epidemic. Several past unsolved outbreaks of pneumonia in the 1950s to the early 1970s had been LD.[4–6] An unsolved epidemic of a nonpneumonic febrile illness in Pontiac, Michigan, was found to be associated with *Lp* exposure; this illness was termed "Pontiac fever".[7,8] Prior epidemics of PF occurred as early as 1949.[9] Bacterial isolates from the 1940s through the 1960s, previously thought to be rickettsial agents, were found to be *Legionella* bacteria.[10–15] Both the organism and the disease had been studied decades before, but major advances in technology were required to properly determine its cause.

Even with identification of *Lp* in 1977 as the cause of LD, how best to diagnose the disease and ways to abort epidemics of LD remained uncertain for several years. Epidemics of the disease, especially nosocomial ones, commonly lasted for years.[16–22] It was discovered that *Lp* and other *Legionella* spp. were naturally occurring aquatic bacteria that grew in warm water, in cooling towers, water heaters, and potable water plumbing. These discoveries led to the end of several multiyear outbreaks of the disease.[23] It is now unusual for LD outbreaks to last more than a week or two.

LD occurs in both sporadic and epidemic forms, sometimes involving many hundreds of victims.[24–27] The disease, while a relatively rare (1%–5%) cause of community-acquired pneumonia that is often easily treated and relatively mild, can cause severe, and fatal, disease.

THE ETIOLOGIC AGENT

The *Legionella* spp. are small gram-negative bacilli with fastidious growth requirements. Amino acids are the main energy source for extracellular growth, with glycerol or glucose used during intracellular growth.[28] Obligate aerobes, the bacteria grow at temperatures ranging from 20°C to 42°C. *Coxiella burnetii,* an obligate intracellular parasite and the etiologic agent of Q fever, is the closest relative of the *Legionellaceae.* L-Cysteine is required for the growth of all but one of the clinically important *Legionella* spp., and this amino acid is needed for the initial growth of all described *Legionella* spp. from environmental or clinical sources. Soluble iron is required for optimal growth, and for initial isolation of the bacterium from both clinical and environmental sources.

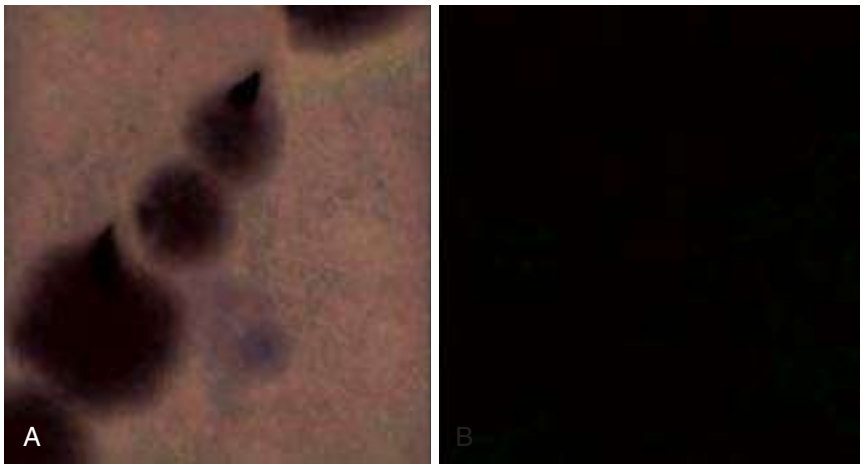

FIG. 232.1 Cultures of *Legionella pneumophila*. (A) Typical opal-like colony of *L. pneumophila* grown on BCYEα agar. (B) Gram stain of *L. pneumophila* taken from culture plate. Basic fuchsin should be used as the counter-stain as safranin stains the bacterium poorly.

A yeast extract agar containing iron, L-cysteine, α-ketoglutarate, and charcoal and buffered with an organic buffer (buffered charcoal-yeast extract [BCYEα] agar) is the preferred growth medium for clinical isolation. Clinically important *Legionella* spp. grow best at 35°C in humidified air on BCYEα medium, usually in 3 to 5 days after inoculation of plates. Up to 14 days' incubation may very rarely be required for the isolation of unusual *Legionella* spp.

More than 60 different *Legionella* spp. have been described, about half of which have been reported to infect humans.[29] *Lp* serogroup 1 (*Lp*1) caused the 1976 Philadelphia outbreak, and is the cause of 65% to 90% of all cases of LD for which there is a bacterial isolate.[30–32] *Lp*1 dominance is not universal. For example, *Lp*1 constituted only 66% of *Legionella* spp. isolates in LD patients in Ontario Province, Canada, with *Lp* serogroup 6 being a relatively common isolate in that province.[32] Also, *L. longbeachae (Llb)* causes 50% to 85% of LD cases in New Zealand, and a similar fraction in Australia.[33] *Lp*1 can be divided into multiple subtypes using a variety of serologic, other phenotypic, and genetic methods. One particular subtype of *Lp*1 causes 55% to 76% of cases of LD due to *Lp,* and 85% of cases due to *Lp*1. This "Pontiac" subtype reacts with a specific monoclonal antibody and contains the *lag-1* gene encoding a lipopolysaccharide acetyl transferase.[34,35] The most common non-*Lp* species that are isolated from humans are *Llb, L. micdadei, L. bozemanae,* and *L. dumoffii,*[31] which, with the exception of Australasia, constitute fewer than 5% of culture-proven cases.[36,37]

Most clinical microbiology laboratories should be able to identify *Legionella* bacteria to the genus level (Fig. 232.1). Identification of *Lp* and *Lp*1 can be accomplished by sophisticated laboratories. Identification of other *Lp* serogroups and other *Legionella* spp. is more difficult, requiring molecular testing for accurate identification.[38] Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry identification of bacterial colonies can be useful for the identification of common *Legionella* spp., but may not be completely accurate depending on the validity of the database, with custom databases increasing the accuracy of bacterial identification.

MICROBIAL ECOLOGY

The *Legionella* bacteria are found in our natural aqueous environment, in lakes, streams, and even coastal oceans, at temperatures ranging from 5°C to greater than 50°C.[39] Warm water (25°C–40°C) supports the highest concentration of these bacteria, with warm water being the major bacterial reservoir leading to LD. Free-living amebae in the same waters support the intracellular growth and survival of the *Legionella* bacteria[40,41] (Fig. 232.2). When faced with inimical environmental factors the *Legionella*-infected amebae encyst, allowing the survival of both the host and parasite. In both natural and man-made waters, *Legionella*-infected amebae are found in consortia of many different microorganisms, all of which exist in a biofilm.



FIG. 232.2 Gimenez stain of *L. pneumophila* growing in an ameba. Note that the bacteria are much smaller and more uniform in morphology than when the bacteria are taken from a culture plate (see Fig. 232.1B).

In addition to intraamebal survival, free-living *Legionella* bacteria can enter a low metabolic state termed "viable but not cultivatable", making them difficult to recover from the environment and biocide-resistant.[42] The *Legionella* bacteria, amebae, and other microorganisms constantly escape from the biofilm because of pressure fluxes into a freely moving phase. Environmental changes that disrupt the biofilm can result in the sudden and massive release of *Legionella* bacteria into the surrounding water. If this water is then aerosolized or aspirated, the bacteria can cause illness in a susceptible host. Almost all cases of LD result from *Legionella* contamination of warm man-made water sources. *Legionella* is one of several opportunistic pathogens that are found in the built plumbing environment.[43] Some recently reported novel water sources include rain puddles in tropical regions, tsunami-related water exposure, windshield wiper fluid, water from truck tankers used to clean or maintain roads, and corroded domestic water pipes.[44–48] One exception to water as a risk factor is that *Llb* appears to be transmitted mainly through potting soil used by gardeners.[49] Water treatment of source water to make potable water may increase the concentration of *Legionella* bacteria (and mycobacteria) in the water.[50] *Legionella* bacteria are present in very low concentrations in disinfectant-treated cold potable water, usually at levels of less than 1 bacterium per liter. The bacterial density can be amplified by growth in biofilm within water distribution pipes, especially older pipes with low or no water flow. The bacteria can be further amplified in the presence of warm conditions, such as those found in many buildings or heat rejection devices. *Legionella* bacteria concentrations in air-conditioning cooling towers range from

10^2 to 10^8 colony-forming units (CFU)/L. Up to 80% of air-conditioning cooling towers tested contain the bacterium, as do 5% to 30% of home and industrial water heaters and hot water plumbing.[39] Contaminated water that is aerosolized serves as a disseminator of the bacteria into the environment. The concentration of *Legionella* bacteria in a particular environmental site may spontaneously fluctuate over a wide range.

PATHOGENESIS

Overview

LD is initiated by inhalation, and probably microaspiration, of *Legionella* bacteria into the lungs. Although *Legionella* bacteria are ubiquitous in our environment, they rarely cause disease. A confluence of a number of factors must occur simultaneously before LD is possible. These factors include the presence of virulent strains in an environmental site; a means for dissemination of the bacteria, such as by aerosolization; and proper environmental conditions allowing the survival and inhalation of an infectious dose of the bacteria by a susceptible host. Strains of different virulence exist for the same species, and some species and serogroups are more virulent than others.[51–53] Possible strain virulence factors include aerosol stability, ability to grow within macrophages, possession of eukaryotic gene homologs, and surface hydrophobicity.

The infectious form of the bacterium is not known, but in all cases the bacteria originate from water or soil. Several possibilities exist for the infectious particle, including bacteria contained within an amebal cyst, a sporelike form,[54] a biofilm particle containing *Legionella* bacteria and other bacteria, and freely dispersed extracellular *Legionella* bacteria. Virulence increases when the bacterium is grown in amebae, in the late stationary phase in vitro, or as the sporelike form.[55–57] The bacterial inoculum required to cause LD is unknown. Guinea pigs develop asymptomatic infection, disease, and death with inocula of 10 to 100, 1000, and 10,000 bacteria, respectively.[58,59] Bacteria in amebal cysts or in a biofilm fragment contain greater than 1000 bacteria, making it possible that inhalation of an infected amebal cyst or biofilm fragment could cause disease.[60] Survival of aerosolized extracellular *Lp* is dependent on relative humidity.[61] Relative humidity may be a key factor in disease transmission.[25,62] LD is a seasonal disease in some regions, with most cases occurring in the warmer months. For example, in the northeastern United States, the majority of cases occur from June through early October. This is probably because of elevated ambient temperature and humidity.[63]

After bacteria enter the lung, they are phagocytosed by alveolar macrophages. The bacteria produce virulence factors that enhance phagocytosis and promote intracellular survival and replication (see "*Legionella pneumophila* Virulence Factors" below). After sufficient intracellular replication, the bacteria kill the macrophage, escape into the extracellular environment, and are then rephagocytosed by macrophages. The bacterial concentration in the lung increases due to amplification of the bacteria within macrophages.

Following this intracellular multiplication, neutrophils, additional macrophages, and erythrocytes infiltrate the alveoli, and capillary leakage results in edema.[64] Chemokines and cytokines released by infected macrophages help trigger the severe inflammatory response. In the mouse model of LD, the relevant proinflammatory chemokines and cytokines include keratinocyte-derived chemokine (KC), macrophage inflammatory protein-2 (MIP-2), tumor necrosis factor-α (TNF-α), interleukin (IL)-12 and IL-18, and interferon-γ (IFN-γ).[65] Humans with LD had elevated levels of TNF-α and IL-8 in relation to other bacterial pneumonias in one study.[66] Systemic spread of the bacteria may be accomplished by infection of circulating monocytes.

The mechanisms for systemic toxicity of the disease are unclear, but it involves the severe inflammatory response to virulent *Lp.* Cytokine production is mediated by detection of microbial products by receptors of the innate immune system. Toll-like receptors (TLRs) on macrophages and other cells initiate host responses to both virulent *Lp* and avirulent mutants by detecting common pathogen-associated molecular patterns (PAMPs). TLR2 detects lipoproteins and lipopeptides, TLR5 flagellin, and TLR9 bacterial DNA.[65] Replication of *Lp* is greater in the lungs of mice deficient for TLR2 or the TLR adapter protein MyD88, resulting in higher mortality; and mice lacking TLR5 or TLR9 have delayed innate immune responses but disease susceptibility is not as pronounced as in TLR2-deficient mice.[65] Epidemiologic data indicate that humans with a TLR5 stop polymorphism are more susceptible to LD; for unclear reasons human TLR4 polymorphisms are protective[67–70] even though the lipid A moiety produced by *Lp* has an atypical structure and is less stimulatory toward TLR4 than the classic lipid A molecule produced by Enterobacteriaceae.[71]

The delivery of microbial products into the macrophage cytosol by virulent *Lp* contributes significantly to the robust inflammatory response. The mouse protein caspase 11 and the human homologues caspase 4 and caspase 5 bind directly to cytosolic lipopolysaccharide (LPS) during *Lp* infection of macrophages,[72–74] and caspase 11 has been shown to be essential for septic shock, implicating this pathway as being critical for LD pathology. Many responses are activated by host nucleotide-binding oligomerization domain (NOD)–like receptor (NLR) proteins. The mouse NLR protein NAIP5 detects bacterial flagellin to activate caspase-1–dependent processing and secretion of the cytokines IL-1b and IL-18, and animals that are defective for NAIP5 are significantly more susceptible to *Lp* infection. The NLR proteins NOD1 and NOD2 respond to peptidylglycan fragments delivered into the cytosol by virulent *Lp,* and sustain activation of innate immune signaling pathways. Lastly, the proteins RIG-I and MAVS respond to bacterial nucleic acid delivered into the cytosol during *Lp* infection to activate type I interferon production.[65]

Although the severe Th1 response induced by *Lp* can be detrimental to the host under high bacterial loads, these cytokines are crucial for the clearance of *Legionella* organisms.[75] Cytokines produced by infected alveolar macrophages are essential for the recruitment of neutrophils and for stimulating IFN-γ production by natural killer cells, which are both needed for sterilization in the lung.[76] Neutrophils are efficient at clearing extracellular *Lp* from the lungs. Macrophages activated by IFN-γ kill *Lp.*[77] This change in macrophage permissiveness involves, among other things, a reduction in intracellular iron, a factor that is necessary for *Lp* replication.[78] Indeed, the majority of legionellae seen in lung samples are associated with alveolar macrophages. Furthermore, the susceptibility of an animal species correlates with the ability of *Lp* to infect its macrophages, and bacterial mutants that are impaired for in vitro infection of macrophages have reduced virulence. Antibodies develop during the course of *Lp* infection, but the humoral immune response does not appear to be critical for host defense.

It is widely believed that the adaptation of *Lp* to protozoan niches in nature engendered it with the ability to infect mammalian phagocytes.[79] *Lp* enters the macrophage by conventional or coiling phagocytosis,[80–82] processes that utilize the host cell actin cytoskeleton.[83] Opsonization with the C3 component of complement can promote phagocytosis,[84] but entry by this pathway dampens the oxidative burst and thereby may enhance bacterial intracellular survival. However, opsonin-independent phagocytosis also appears to be important.[57] Even in the event that the oxidative burst is triggered, *Lp* strains may be resistant to hydrogen peroxide, superoxide anion, and hydroxyl radicals.

After entry, legionellae reside within a nascent phagosome (Fig. 232.3) that does not fuse with endosomes or lysosomes,[85–87] thereby avoiding acidification and degradative enzymes. The phagosome subverts host vesicles in the early secretory pathway using proteins produced by *Lp.*[88] The vacuole containing *Lp* rapidly recruits membrane from the endoplasmic reticulum and develops into an organelle that resembles the host rough endoplasmic reticulum.[89–91] This specialized *Legionella*-containing vacuole supports intracellular replication. This vacuole expands during replication and ultimately fills the host cell. Upon nutrient depletion (e.g., amino acid depletion), *Lp* enters stationary phase and converts to a flagellated form that is primed to seek out and infect new host cells.[55,92] Egress of *Lp* from the expended host cell is not well understood, but in macrophages this involves pathogen-induced apoptosis that leads to cellular necrosis.[93,94]

Neither the pathogenesis nor the etiology of PF is known with certainty. PF is caused by inhalation of a disease-causing environmental aerosol derived from water containing microorganisms including *Legionella* bacteria. Thirty percent to 85% of patients with this disease have serum anti-*Legionella* antibody in higher concentrations than is found in the normally healthy population.[95] The prevalent assumption is that the illness is caused by inhalation of the *Legionella* bacteria. Since

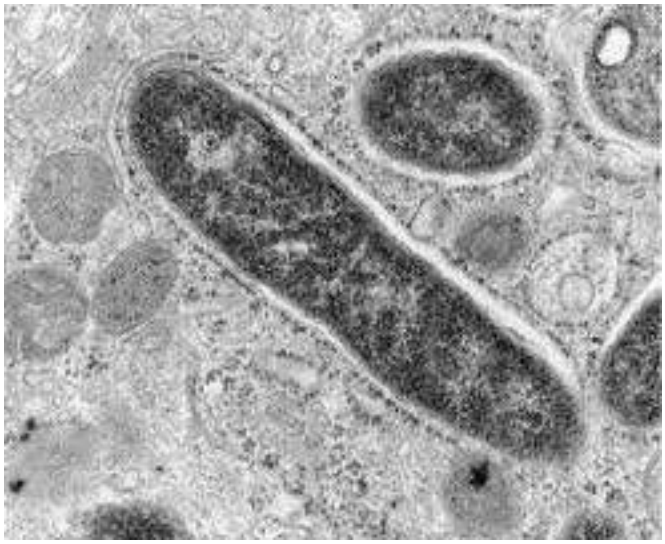

FIG. 232.3 Electron micrograph of *L. pneumophila* growing within a phagosome of an alveolar macrophage. Note the characteristic ribosomal studding of the phagosome.

the aerosols contain a mixture of microorganisms and endotoxins, it is unclear whether the disease is due to inhalation of endotoxin, of a polymicrobial aerosol, or of *Legionella* bacteria alone or to a combination of all these agents.[95] A study of the virulence for guinea pigs of the *Lp* strain that caused the 1976 Philadelphia LD epidemic and the PF environmental isolate from the Pontiac, Michigan, epidemic showed no differences between the two bacterial strains.[96] The incubation period of 4 to 6 hours of some PF patients supports a toxin-mediated illness, while the median incubation period of around 35 hours is more suggestive of initial bacterial multiplication causing illness. Infections with non-*Legionella* bacteria can produce *Legionella* antibodies, so the presence of such antibodies does not prove that PF is due solely to *Legionella* infection or intoxication. Bath water fever, a clinical syndrome thought to be due to endotoxin inhalation, is very similar to PF, suggesting that PF may also be caused by endotoxin inhalation.[95] Several reports exist in which exposure to the same environmental source led to PF in most exposed people, but to LD in a few people; whether the milder illness was really PF or mild LD is open to question, and does not help answer the question of etiology and pathogenesis.[95] Perhaps the strongest evidence implicating systemic infection with *Legionella* bacteria as the cause of PF has been the very rare reports of positive *Lp* urinary antigen tests or positive cultures in patients with PF.[95] The rarity of such cases and the rapid recovery without antibiotic therapy argue against systemic infection as the cause of PF.

Legionella pneumophila Virulence Factors

Studies of *Lp* grown in mammalian eukaryotic cells and in its natural environmental host, free-living amebae, show that the bacterium changes dramatically within cells as the intracellular infection progresses, becoming very small and highly motile as the cell loses its ability to sustain bacterial multiplication. These small motile forms, termed the "transmissive" type of bacteria, have increased virulence for eukaryotic cells. Once inside the cells, a nonmotile "replicative" phase predominates. The transmissive bacteria can be produced in vitro under nutrient-limiting conditions. Multiple bacterial factors control this transition between the transmissive and replicative phases, with some of the most important ones being production of ppGpp and activation of LetA/S and RpoS. CsrA plays an important role in the posttranscriptional regulation of this process.[97,98]

A variety of surface structures have been implicated in *Lp* pathogenesis. Type IV pili modestly promote bacterial attachment to macrophages and epithelial cells,[99] and flagella promote invasion independent of adherence.[100] The major outer membrane protein is a porin that also serves as a binding site for complement components and thus mediates opsonophagocytosis.[101] The Mip protein is a surface-exposed peptidyl prolyl isomerase that is required for the early stages of intracellular infection and for full virulence in animals.[102–104] Legionella LPS contains some endotoxic activity, and changes in LPS have correlated with increases in serum resistance, intracellular growth, and virulence.[105] Finally, the *rcp* gene, which appears to encode a lipid A–modifying enzyme, confers resistance to cationic peptides and promotes macrophage and lung infection.[106]

Lp secretes a variety of proteins, degradative enzymes, and putative toxins. The release of proteins by *Lp* into the extracellular milieu is mediated primarily by a type II secretion system.[107] Genome sequencing also suggests the existence of type I and type V secretion systems.[108] A number of enzymes and novel proteins are all secreted via the *Legionella* type II system.[107] Mutations within the genes encoding the type II secretion system diminish infectivity for macrophages, protozoa, and animals.[109,110] A secreted zinc protease is produced during infection and promotes pathology in the guinea pig model of disease as well as intracellular infection of some amebae hosts.[111,112]

A *Legionella* type IVb secretion system called Dot/Icm is essential for intracellular replication and virulence in animal models of disease.[113,114] It is essential for the ability of the *Legionella* parasite to modulate host vesicular transport to avoid delivery to lysosomes and promote vacuole biogenesis. Mutations in *dot/icm* loci lead to loss of virulence.[115,116] More than 300 proteins are secreted by the Dot/Icm system and in most cases these "effector" proteins translocate from the *Legionella*-containing vacuole into the host cell cytoplasm.[114] Some of these have been implicated in bacterial evasion of lysosome fusion.[117–119] Others, such as RalF, DrrA (SidM), LepB, LidA, and SidJ, play roles in the recruitment of endoplasmic reticulum–derived membranes to the *Legionella*-containing vacuole.[120–123] The effector SdhA is important for maintaining the integrity of the vacuole in which *Lp* resides,[124] and effectors SidF, SidP, and LepB directly modulate phosphatidylinositol phosphate signatures on the cytosolic surface of the vacuole that are important for the binding of other effectors.[125] *Lp* expresses several effectors that function as glucosyltransferases that are capable of inhibiting host cell protein synthesis (elongation factor 1A).[126,127] Although Dot/Icm is essential for *Lp* infection, a second type IV secretion system known as Lvh is found in addition to Dot/Icm in many strains; however, this system does not appear to deliver effector proteins into host cells but retains the ability to promote DNA conjugation, which could facilitate horizontal transfer of genes encoding effectors.[128] Interestingly, many of the type II and type IV secreted proteins as well as other mediators of infection (e.g., LpnE and Lpg0971) bear striking sequence similarity to eukaryotic proteins, suggesting that they were acquired by horizontal gene transfer from a eukaryotic host and use similar biochemical activities to facilitate *Lp* infection.[108,117,129–134]

Several infectivity factors have been localized to the *Lp* periplasm or cytoplasm. A Cu-Zn superoxide dismutase resides in the periplasm, affording resistance to toxic superoxide anions, and the KatB catalase-peroxidase is needed for optimal intracellular infection.[135,136] The *Legionella* phosphoenolpyruvate phosphotransferase and HtrA protein promote intracellular growth and virulence, and the phosphotransferase regulates expression of the transcriptional activator PmrA.[137–139] *Lp* iron acquisition, important for intra- and extracellular replication, involves, among other things, a secreted ferric iron chelator (the siderophore legiobactin), a secreted pyomelanin with ferric reductase activity, and an inner membrane ferrous iron transporter (FeoB).[140–143]

In addition to the identification of eukaryotic-like proteins in the *Lp* genome, another outcome of sequencing bacterial genomes is the realization that there are large segments of DNA, including plasmids and chromosomal "islands", that can vary between *Lp* strains.[108,144–146] It is possible that these variable regions of the genome will help explain differences in virulence that may exist between strains. Indeed, large deletions of the chromosome that remove individual islands can decrease *Lp* replication in specific protozoan hosts.[147]

Virulence Factors and Pathogenesis of Other *Legionella* Species

Relatively little is known about the virulence mechanisms, molecular pathogenesis, and cell biology of infections caused by *Legionella* spp. other than *Lp.* With the major exception of *Llb,* infections caused by

the other *Legionella* spp. are rare and found almost exclusively in severely immunocompromised patients. Genome sequencing of 38 different *Legionella* species revealed that there are over 5000 different effector proteins encoded by the genus and only seven Dot/Icm effector proteins that are shared by all species.[148] Thus effector plasticity, which facilitates adaptation of *Legionella* to different environmental hosts, likely influences human virulence potential. Despite unrestricted growth in otherwise nonpermissive macrophages, some non-*Lp* species fail to promote inflammatory cell death,[149] which could be correlated to a failure to cause disease in nonimmunocompromised people, although this is unstudied. *Llb* resides within a ribosome-studded phagosome, albeit with markers of late endosomal maturation, unlike *Lp,* which is able to block endosomal maturation at an early stage.[150–152] In contrast, *L. micdadei* resides in a smooth phagosome, and morphologic data suggest *L. dumoffii* grows within the cytoplasm rather than in a phagosome.[150,153–155] Analysis of the genome of several different *Llb* strains has shown that this species has and utilizes the Dot/Icm system for causing infection, that it is nonflagellated, and that it possesses a wide range of eukaryotic effectors different from those found in *Lp.*[156,157] Many of the *Llb* effectors appear to have been derived from plants and soil organisms, suggesting that it has evolved as primarily a soil-adapted bacterium. Experimental virulence of *Llb* for macrophages appears to be relatively independent of growth phase, in contrast to *Lp,*[151,158] and studies in mice and explanted human macrophages show that host antibacterial cytokines are suppressed.[157]

EPIDEMIOLOGY

Incubation Period and Contagiousness

The incubation period during most outbreaks of LD is between 2 and 10 days, with up to 10% of epidemic cases having incubation periods longer than 10 days. The median values are 4 to 6 days, with some outliers of from 1 up to 28 days.[1,159,160] A 2-month incubation period was reported for one nosocomial case.[161] The incubation period of PF is from 4 hours to 3 days, with a median of around 32 to 36 hours, although incubation periods of up to 5 days have been reported.[7,162,163] The incubation period of LD due to *Legionella* species other than *Lp* is not known with certainty; based on case reports from nosocomial cases of the disease, the incubation period after onset of immunosuppression appears to similar to that of LD due to *Lp.*[164]

Person-to-person transmission of LD or PF does not occur. A report of "probable" person-to-person transmission, the only such case ever reported, lacked sufficient scientific evidence to support this conclusion.[165] Laboratory transmission to humans has not been documented.

Patterns and Rates of Disease and Mortality

LD occurs in both sporadic and epidemic forms. For the years 2013–2014, about 3% of reported US cases were clustered, whereas in England and Wales from 2013 to 2015, about 20% of reported cases were clustered.[166–168] About 2% of reported cases are health care associated. In England and Wales for the same time periods, 40% of cases were acquired while traveling abroad. In the United States, about half the clustered cases were acquired by exposure to contaminated drinking water and the remainder by exposure to environmental water, such as from cooling towers. Underreporting of the disease is likely because of empirical treatment without laboratory testing, insensitive diagnostic tests, and the use of passive surveillance systems. Only 5166 cases of LD were reported in 2014 (16.2 per million population) to the Centers for Disease Control and Prevention (CDC). This rate is lower than that reported in Denmark, France, and Portugal in 2014, with 28.1, 20.5, and 56.4 cases per million population, respectively, and higher than that reported for England and Wales (5.8 per million).[36]

Prospective studies of both sporadic community-acquired and nosocomial LD have reported far more cases in the United States than would be expected based on the number of cases reported to the CDC. One study in Ohio of adults with community-acquired pneumonia requiring hospitalization found that 2.4% of such patients had LD, and that the disease incidence was approximately 80 per million population per year.[169] When extrapolated to the entire population of the United States, the authors estimated that between 8000 and 18,000 hospitalized cases of LD occur annually. The incidence of LD causing community-acquired pneumonia not requiring hospitalization is not known. One small regional US study estimated that the incidence of LD among outpatients treated for pneumonia was 40 to 280 per million population per year.[170] Thus somewhere between 18,000 and 88,000 cases of LD are estimated to occur per year in the United States, the majority of which are neither epidemic nor hospitalized. A German study of community-acquired LD found that the annual rate was 180 to 360 cases per million population, which if extrapolated to the United States would mean that 56,000 to 113,000 LD cases occur per year in the United States.[171] Some geographic regions appear to have more LD than others.[63] The incidence of LD in the United States and elsewhere appears to be increasing, based on the numbers of reported cases; whether this is due to more widespread use of the urine antigen test, better reporting and surveillance, or a true disease increase is unknown.[172] Estimates of LD as a cause of community-acquired pneumonia requiring hospitalization in adults range from 0.5% to 10% of all admitted pneumonia cases; an average value is probably approximately 2%, even in geographic regions with excellent diagnostic capabilities.[173–177]

LD in children is uncommon, representing 1% or less of causes of pneumonia, and occurs as a nosocomial disease of immunosuppressed children, and in neonates.[178] Nosocomial and domestically acquired cases of LD have been reported in apparently immunologically normal newborns exposed to *Legionella*-contaminated water in incubators and bath tubs, and during birth.[178–180]

Shortly after the 1976 Philadelphia outbreak, nosocomial LD outbreaks were reported in several cities throughout the United States and Europe. Because relatively little was known about the environmental ecology of *Lp,* or about optimal diagnostic methods, these outbreaks were characterized by long durations, often years in length, and high numbers of cases and fatalities. For example, the LD outbreak at the Wadsworth Veterans Administration Hospital in Los Angeles, California, resulted in more than 250 cases of disease in both patients and visitors over an 8-year period.[16,23,181] Nosocomial LD epidemics continue to occur worldwide, albeit with durations measured in weeks rather than years.[182,183] Nosocomial pneumonia usually affects a relatively small number of hospitalized patients, with attack rates less than 1% of patients.[16,184] During nosocomial outbreaks of LD, the minority (5%–11%) of patients with nosocomial pneumonia of all etiologies have been reported to have LD.[16,185–187]

Some recent large community-based LD outbreaks have been in New York City in 2015 (138 people, 16 deaths) and Flint, Michigan, in 2015 (91 people, 12 deaths). Several large outbreaks involved people visiting a town center, rather than being inside a certain building. Long-distance spread of disease from industrial aerosols has been reported.[25,188]

LD affecting travelers constitutes up to half of reported cases in some countries. In many cases, a common source outbreak has been found, but for others the cases appear to be sporadic. Multiple common source outbreaks have been uncovered in travelers by a cooperative European reporting system that collects and analyzes LD cases in Europeans.[189]

Sporadic cases of the disease are about 5-fold to 20-fold more common than linked cases of the disease. Some sporadic cases are undoubtedly the result of common source exposures. This is especially true of travelers who return to their homes during the incubation period, or while they are ill but still well enough to travel. Evidence for common source links for apparently unrelated cases comes from observations that proximity of residence to wet cooling towers is a risk factor for LD.[190,191] Residential acquisition from drinking and bathing water, which rarely results in more than a single case, accounts for less than 10% to 15% of sporadic LD.[192–194]

The reported incidences of LD in the United States rose almost fivefold from 2000 to 2016 (19 per million per year), and almost twofold from 2006 to 2016. The relative contributions of increased testing, aging of the population, increased immunosuppression of the population due to advances in medical care, and global warming are uncertain.

PF often causes explosive outbreaks of disease with high attack rates. Attack rates of 70% to 90% have been reported from several epidemics.[7,162,195–197]

LD mortality rates are variable, ranging from less than 1% to as high as 80%, depending on the underlying health of the patient, promptness of specific therapy, and whether the disease is sporadic, nosocomial, or part of a large outbreak.[30,198–201] The lowest mortality rates (approximately 1%), were observed in large outbreaks of the disease, whereas the highest mortality rates were reported in untreated nosocomial disease in patients with severe underlying diseases.[202] The average fatality rates for sporadic disease are estimated to be approximately 10% to 15%. Fatality rates of nosocomial disease have declined by more than 50% in the United States over the last 20 years; a similar but less dramatic decrease in death rates of community-acquired cases has also been observed.[30] The decline in mortality rates appears to be due to better and faster disease recognition and more widespread use of LD-effective empirical therapy for pneumonia.[30,202]

Risk Factors

Because of the ubiquity of *Legionella* spp. in our natural and man-made environments, most people encounter these bacteria frequently. Yet most of those exposed do not get the disease. This is attributed to largely unknown host immune mechanisms and to the lack of sufficient concentrations of virulent *Legionella* spp. bacteria in small aerosol particles. The known host risk factors for LD are those that result in decreased local or systemic cellular immunity, and those activities that increase the chances of exposure to an infectious aerosol or microaspiration of contaminated water. Also important are environmental and bacterial factors such as relative bacterial virulence, bacterial aerosol stability, organism growth conditions, and factors that facilitate the spread of the bacterium from contaminated water to the host, such as wind direction, relative humidity, and aerosol formation.

Male gender, cigarette smoking, chronic heart or lung disease, diabetes, end-stage renal failure, organ transplantation, immunosuppression, some forms of cancer, and age greater than 50 years are LD risk factors.[184,193,203,204] The approximately twofold greater risk for men may be due to the greater prevalence of cigarette smoking and its complications in males. As the population ages in developed countries, LD incidence will rise, based on the very high attack rates in the elderly, in the range of 800 to 1500 per million per year for those age 80 years and older.[32] Poverty, at least in large cities, is a risk factor for LD.[205] There may be racial disparities in LD incidence, with African Americans having a higher attack rate than whites, even after accounting for socioeconomic status.[205,206] Cigarette smoking and glucocorticoid administration both separately increase risk by approximately twofold to sevenfold. Anti-TNF-α therapy is a significant risk factor for LD, especially including TNF-α antibodies, but also thalidomide and lenalidomide.[207,208] Anti-CD52 therapy can result in severe LD.[209] Lung, but not gastrointestinal tract, cancer is an LD risk factor.[203] A variety of hematologic malignancies have also been shown to be important risk factors, especially hairy cell leukemia.[185,203] Small single studies of genetic predisposition to LD have shown that polymorphisms in TLR4, TLR5, and mannose-binding lectin produce minor predispositions to the disease.[67,69,210,211] Recent surgery has been an important risk factor for nosocomial disease.[212,213] Alcoholism is a predisposing condition in only some studies.[184,185,193,214] Host risk factors for non-*Lp* LD appear to be similar to those for *Lp* infection; most patients with these infections are immunosuppressed or have cancer, with the exception of LD due to *Llb*.[215,216] There appear to be no predisposing host factors for PF.

Activities that increase the chances of exposure to *Legionella* bacteria in water increase the risks of disease acquisition. Recent overnight travel, use of well water in the home, recent plumbing work inside the home, disruptions of water supply resulting in "brown" water at the water tap, and living in a water distribution network with older plumbing all increase the acquisition risk of community-acquired LD.[191,193,194,217,218] Increased risk activities include exposure to whirlpool or hot spring spas; living close to a cooling tower; and being near decorative fountains.[190,191,204,219,220] Rarely reported risks are near-drowning[10,44,220,221] and delivery by water birth.[222] Exposure risk factors for LD due to *Llb* are much different than for *Lp* infection, with handling of potting soil or compost, cigarette smoking, chronic obstructive lung disease, and not washing hands after use of the soil being major risk factors.[49]

A wide range of nosocomial exposures can result in LD. Almost all involve delivery of *Legionella*-contaminated water into the respiratory tract, and include the use of tap water filled or rinsed nebulizers, humidifiers, oxygen humidifiers, ventilator tubing, and nasogastric feedings or lavages.[223–226] Proximity to a contaminated decorative fountain has transmitted nosocomial LD in several outbreaks.[26,227] Consumption of *Legionella*-contaminated ice can also be a risk factor for nosocomial LD.[228] These are in addition to exposure to *Legionella*-contaminated air originating from a cooling tower.[229–231] Rare cases of nosocomial *Legionella* wound infection have resulted from irrigation or bathing of wounds in *Legionella*-contaminated water.[232,233]

Modes of Transmission

LD is transmitted from the environment to humans by inhalation of an infectious aerosol.[234] In an unknown fraction of cases microaspiration of contaminated water into the lungs is the mode of transmission.[226,235]

Multiple examples of exclusive aerosol transmission of LD exist, especially in epidemics having a cooling tower, water spa, water fountain, or water mister as the source of disease.[1,219,229,230,236–238] Drinking of water at the epidemic site has not been demonstrated to a be risk factor for LD.

The data supporting microaspiration of water as a major mode of transmission are less convincing. These data include examples of nosocomial disease in patients whose major risk factor was nasogastric tube irrigation with tap water, and interruption of nosocomial outbreaks by substituting sterile water for tap water for drinking and nasogastric tube irrigation.[226,235] Whether microaspiration is the major transmission mode for nosocomial disease is controversial and unproven.[239]

Rare reports of peritonitis or bowel abscesses caused by *Lp* have led to speculation that oral ingestion may be a mode of disease transmission.[240–242] In all these cases, *Lp* pneumonia or empyema occurred concurrently, making unclear which organs were the ones primarily infected. It is very unlikely that oral ingestion of *Legionella*-contaminated water is more than a very minor mode of transmission of the disease.

Outbreak Investigation

Prompt notification of public health authorities of any strongly suspected or confirmed case of LD is critically important for detecting epidemics of the disease, and is legally required in many regions. What appears to be only a single case of the disease may be part of an epidemic, or the index case.

Medical institutions should embark on extensive investigation of even a single case of nosocomial LD.[243] More cases may have occurred previously, or will appear subsequently. Retrospective review of nosocomial pneumonia cases for a 3- to 6-month period may yield more cases. Prospective laboratory testing of all patients with nosocomial pneumonia for LD for a 3- to 6-month period also can be useful. Hospital physicians should be notified to consider LD when making diagnostic and therapeutic decisions in patients with nosocomial pneumonia until it is clear that any ongoing nosocomial outbreak has been eradicated.

Investigation of both community-acquired and nosocomial outbreaks of LD requires a thorough epidemiologic investigation. Environmental testing without concurrent epidemiologic investigation can result in misleading findings, even when molecular fingerprinting is used to compare clinical and environmental isolates.[244–247] Not all environmental microbiology companies are competent in the proper collection, processing, and culturing of water specimens for *Legionella* spp. bacteria; certification is required in some countries, with a voluntary certification program available in the United States.[248] It is crucially important to obtain *Legionella* bacteria clinical isolates as part of an epidemiologic investigation; this allows molecular fingerprinting and comparison of clinical and environmental isolates. Environmental sampling protocols for outbreak investigation are available.[249,250]

Rough clues to the environmental source of an LD epidemic can be found in the pace of the outbreak and its geographic distribution. Explosive outbreaks involving tens to hundreds of people over a several-day period are most often due to a contaminated massive aerosol generator, usually a cooling tower but sometimes a whirlpool spa or misting device. Potable water–associated outbreaks may produce as many cases, but generally over a much longer period of time, such as many weeks

or months. Cooling tower–related epidemics often affect both building visitors and people who are within several hundred meters up to a 10-km distance from the tower.[25]

Environmental Decontamination for Outbreaks

All aerosol-generating sources implicated or highly suspected of being a source of epidemic LD should be taken out of operation as soon as possible. A number of guidelines exist for emergency disinfection of such sources, and these may differ according to local regulations or guidelines.[249–251] Long-term remediation can be complex and requires expert engineering and public health advice.

CLINICAL PRESENTATION

Legionnaires' Disease

LD causes acute consolidating pneumonia that cannot be accurately differentiated from pneumococcal pneumonia based on clinical or roentgenographic findings on initial presentation.[252–254] Initial clinical findings of both community-acquired and nosocomial epidemic LD in the 1970s seemed to show that a distinct clinical syndrome was observed.[255] This syndrome was characterized by fever with pulse-temperature dissociation, myalgia, nonproductive cough, few pulmonary symptoms, diarrhea, confusion, hyponatremia, hypophosphatemia, and elevated liver-associated enzymes. While this symptom complex does occur in LD, it is neither specific nor frequent enough to allow differentiation of LD from other common causes of community-acquired pneumonia. Clinical scoring systems to help increase diagnostic accuracy have not performed well enough to be used to guide proper therapy.[256–258]

The clinical findings of non-*Lp* LD do not differ significantly from those caused by *Lp*.[216,259] *Llb* LD occurs predominantly in nonimmunosuppressed patients, whereas the reverse is true for LD caused by the other non-*Lp Legionella* spp.[33] Those with immunosuppression and non-*Lp* LD tend to have a more severe course and greater likelihood of nonpulmonary LD, but this is similar to the findings of immunosuppressed patients with *Lp* LD.

A prodromal illness may occur, lasting for hours to days, with symptoms of headache, myalgia, asthenia, and anorexia. Fever accompanies this prodrome, except in severely immunocompromised patients and sometimes in the elderly. Multiple rigors may occur, as well as diarrhea and abdominal pain. Cough, with or without chest pain, may develop hours to days after onset of the prodrome; the cough produces purulent sputum in only approximately 50% of patients. The initial clinical picture may be confusing because the systemic symptoms can be more impressive than ones referable to the lower respiratory tract, leading some physicians to diagnose "influenza", a gastrointestinal illness, "sepsis", and in some cases an acute abdomen syndrome. These prodromal symptoms escalate in severity as the disease progresses, which may result in a several-day delay before presentation; the median time to presentation after onset of illness is approximately 4 days.[260] Careful physical examination of the chest, and chest roentgenography, almost always demonstrates findings of consolidating pneumonia.[261,262] Chest computed tomography findings include ground-glass opacities and consolidation.[263,264] Pleuritic chest pain, sometimes in concert with hemoptysis, can occur and may result in an incorrect diagnosis of pulmonary infarction. Headache may be the most prominent feature, and so severe as to suggest subarachnoid hemorrhage. Mental confusion is commonly reported; obtundation, seizures, and focal neurologic findings occur less frequently.[265–267] Some patients may have negative chest x-ray films on presentation but chest films taken a day later may show focal or diffuse pulmonary infiltrates; as with other causes of community-acquired pneumonia, plain chest films may be less sensitive than chest computed tomography examination at presentation.[268] Lung cavitation is seen almost exclusively in immunosuppressed patients.[269] Pleural effusion without pulmonary infiltrates is rarely observed. Bronchoscopic findings in LD patients are often remarkable for the absence of inflammation or purulent secretions in the large airways. Abdominal examination may reveal generalized or local tenderness and, in rare cases, evidence of peritonitis. Splenomegaly is uncommon. Findings of pericarditis, myocarditis, and focal abscesses are rare. No rash is associated with this disease, except that caused by other factors such as drug therapy. Symptoms of rhinorrhea, chronic afebrile fatigue, and fever without pneumonia lasting for many weeks are either not seen or so rare as to make the diagnosis unlikely.

Nonspecific laboratory test abnormalities may occur in LD[270] that are consistent with having a severe case of pneumonia. These include hyponatremia, hypophosphatemia, increased liver-associated enzymes, hyperbilirubinemia, leukopenia, thrombocytopenia, disseminated intravascular coagulation, leukocytosis, pyuria, elevated creatine kinase, and elevated lactate dehydrogenase. Patients with LD are more likely to have hyponatremia than do those with other causes of pneumonia, but the range of serum sodium values is too broad for this abnormality to be diagnostic in an individual patient.[271] Laboratory markers of pancreatitis are detected if this is a complication of LD. Renal disease caused by LD may result in the presence of urine casts and white blood cells, elevated serum creatinine levels, or both types of abnormalities. Myoglobinuria is a relatively common finding as indicated by a positive dipstick test for "blood" in the absence of significant numbers of red blood cells in the urine.

Clinical diagnosis may be more specific if the patient's clinical course after treatment has been given is taken into account, and if epidemiologic and immunologic risk factors are considered. The chances of a patient having LD are increased if an acute consolidating pneumonia fails to respond to several days of β-lactam antimicrobial therapy, or if the pneumonia is severe enough to require intensive care unit hospitalization. Important epidemiologic clues include use of a hot tub or recreational spa; travel outside the home for one or more days; recent pneumonia of a coworker, fellow conference attendee, or fellow traveler; and recent plumbing work done at home or work.

The nonspecific presentation of LD can make clinical diagnosis very difficult, and mandates empirical therapy for this disease in most patients with community-acquired pneumonia of uncertain etiology. Diagnosis of the index or sporadic case of nosocomial LD is difficult because of the rarity of nosocomial LD in most hospitals, and because laboratory testing for nosocomial LD is uncommon in such settings.

Extrapulmonary Infections

Extrapulmonary infections are rare and usually occur as metastatic complications of pneumonia in immunocompromised patients. Metastatic infection has been reported almost exclusively in immunocompromised patients, or patients with fatal LD, who may develop abscesses and other infections of the brain, the spleen or extrathoracic lymph nodes, and skeletal and myocardial muscles.[272–276] Other reported sites of metastatic infection have been the intestines and liver, the kidneys, the peritoneum, the pericardium, vascular shunts and grafts, bone marrow, joints, surgical wounds (including those related to prosthetic heart valves and the aorta), native heart valves, the perirectal area, and the skin and subcutaneous tissues.[240,277–288] Both reported cases of culture-confirmed native valve endocarditis were in immunocompromised patients with LD.[288,289] In some of these cases the onset of symptoms of the metastatic infection preceded the recognition of pneumonia by several days, and in other cases the metastatic infection presented days to weeks subsequent to onset of the pneumonia. A metastatic infection site may be the only evidence of relapse of infection. Three cases of metastatic infection have been reported in apparently previously healthy patients,[274,290,291] but otherwise immunosuppression is found in such patients.

Very rare cases of primary infection not preceded or accompanied by pneumonia have been reported. Some of these appear to be the result of direct inoculation of *Legionella*-contaminated water into various tissues, usually in hosts with immune compromise. Some infections were introduced by bathing postoperative patients with contaminated tap water, by the use of therapeutic baths, and by inadvertent tap water irrigation of the mediastinum after esophageal perforation. Culture-proven sites of such infections have been surgical or other wounds, subcutaneous tissues, prosthetic and native joints, prosthetic heart valves, the mediastinum, and the respiratory sinuses.[232,233,286,291–303] In contrast to the case of native valve endocarditis, almost all patients with prosthetic valve endocarditis were neither immunosuppressed nor had LD.[292,293,296,297,304–306] Native and prosthetic joint infections have been reported uniformly in immunocompromised patients with preexisting arthritis, with one exception of a patient who was not immunocompromised but did have

underlying arthritis.[286,291,301–303,307] With one exception all joint infections occurred in the absence of LD. Polymerase chain reaction (PCR)–positive, but culture-negative, meningoencephalitis has been reported in patients without LD.[308]

Pontiac Fever

PF is a self-limited, short-duration febrile illness, usually diagnosed only during an outbreak of the disease.[7,95,162,163] Symptom onset is 4 to 60 hours after exposure to a bacteria-contaminated aerosol, either in a workplace or some other group setting. More than 80% to 90% of exposed people become ill. Unlike LD, children commonly get the disease.[95,162,163] The sources of contaminated aerosol have included industrial processes using sprayed water, recreational spas, decorative water fountains, and cooling towers. Fever, myalgia, headache, and asthenia are the dominant symptoms. Cough, dyspnea, anorexia, arthralgia, and abdominal pain occur less frequently. Most patients are not ill enough to seek medical attention, recovering without specific therapy 3 to 5 days after disease onset. There is little information about physical examination findings in the first day of illness; examination 2 to 5 days after onset may show fever and tachypnea. Pneumonia does not occur. Fatigue and nonfocal neurologic complaints may persist for up to several months in the minority of affected patients.[7] Because the clinical findings are nonspecific, it is very difficult, if not impossible, to accurately diagnose this disease in the absence of similar illnesses in coworkers or others with a common source exposure. Inquiries regarding the health of coworkers and acquaintances may help to confirm the diagnosis, but this can be nonspecific and insensitive.[309]

LABORATORY DIAGNOSIS

Specific, but relatively insensitive, tests are available for the diagnosis of LD (Table 232.1). Culture yield depends on the severity of illness, with the lowest yield (15%–25%) for mild pneumonia and the highest yield (>90%) for severe pneumonia causing respiratory failure.[38] Prior specific antimicrobial therapy affects yield adversely, although some patients have positive sputum cultures for days to weeks after initiation of specific therapy. Expectorated sputum or, even better, endotracheal aspirates are good specimens for culture; neither bronchoscopy nor lung biopsy is required for good culture yield, assuming a good-quality sputum specimen is obtained. Sputum Gram stain is insensitive. *Legionella micdadei,* and rarely *Lp,* may be acid fast or weakly acid fast.[310] Sputum cultures may be positive despite the presence of epithelial cells and lack of leukocytes, making suspicion of LD an exception to the usual sputum adequacy screening criteria. Blood culture is positive in approximately 10% of critically ill or severely immunocompromised LD patients, and in most patients with prosthetic valve endocarditis; specialized culture techniques are required to detect the bacteremia. Culture diagnosis is not dependent on *Lp* serotype or on *Legionella* spp., a fault of all of antibody and urine testing. Culture is also often the only test positive in cases of LD caused by other *Legionella* species. Culture media and selective conditions are optimized for the detection of *Lp,* with unknown performance for the detection of non-*Lp* species. Since a clinical isolate may be required for complete investigation of the source of an outbreak, culture for *Legionella* should always be performed regardless of the results of an antigen or PCR test. Many clinical laboratories have neither the expertise nor the ability to properly perform these specialized cultures.

Urine antigen testing is the most common laboratory test ordered for LD diagnosis.[30,311] This very specific test is easily performed by those without special skills and is often positive when other tests are negative. The test is not perfect because it is most sensitive for the detection of the Pontiac subtype of *Lp*1 (up to 90%), less sensitive for other monoclonal antibody types of *Lp*1 (60%), and very poorly sensitive (<5%) for other *Lp* serogroups and other species.[312] Since the majority (approximately 90%) of cases of community-acquired LD are due to the Pontiac subtype, the average sensitivity of this test is in the range of 70% to 80%. Immunocompromised patients, patients with nosocomial LD, and patients in some geographic regions are more likely to have LD caused by other serogroups and species, and hence a negative urine antigen test.[313] Yield can be increased by urine concentration, and for one assay type by prolonging the incubation time. Rare false-positive tests may be due to rheumatoid-like factors, easily inactivated by boiling; most laboratories do not use an inactivation step. A common clinical error is to order only urine antigen testing and to stop therapy for LD if the urine test is negative, because the urine test can be insensitive. Positive tests are associated with more severe disease.[214] Patients with extensive bilateral LD may excrete urinary antigen for weeks to months after recovery; this should not cause confusion over whether a positive test is the result of new or old pneumonia in the absence of a history of recent hospitalization for severe pneumonia.

Antibody detection is insensitive and of low specificity unless paired acute and convalescent sera are tested.[314] For optimal yield, convalescent sera should be collected at 4, 6, and 12 weeks after disease onset. Only approximately 75% of patients with culture-proven LD will seroconvert at all, even with optimally timed and tested sera. Commercially available serologic tests lack specificity or sensitivity because of deviations from the standardized test methods. Only seroconversion to *Lp*1 is of high enough specificity for clinical use; measurement of antibodies to other serogroups and species is plagued by low specificity and is not recommended.

Detection of *Lp* in respiratory tract tissues and fluids using immunofluorescent microscopy (direct fluorescent antibody [DFA] test) is very specific if a monoclonal antibody to this species is used and the test is performed by experts.[315] Use of other reagents, or testing by nonexperts, can result in many false-positive test results. The test is insensitive. Most laboratories do not perform this test because of its low yield and complexity. DFA staining can be useful for the detection of LD in fixed lung specimens.

Molecular amplification (PCR) and detection of *Lp* and non-*Lp* species is available at reference and public health laboratories.[38] No commercial PCR test approved by the US Food and Drug Administration is marketed in the United States. Most evaluations have shown that the molecular methods are about as sensitive as culture, with more recent studies showing that molecular methods are approximately 30% more sensitive than culture. The addition of PCR testing for *Lp* and *Legionella*

TABLE 232.1 Specific Diagnostic Tests for Legionnaires' Disease Caused by *Legionella pneumophila*

TEST	SPECIMEN TYPES	SENSITIVITY	SPECIFICITY	NOTES
Culture	Sputum, other lower respiratory tract secretions; lung; pleural fluid; blood; extrapulmonary tissues, fluids	20%–95%	100%	May be positive up to several days after treatment; requires special media and expertise
Antigenuria	Urine	60%–95%	>99%	Highest sensitivity for *L. pneumophila* serogroup 1, Pontiac type; may remain positive for days to months
Immunofluorescent microscopy	Same as culture	20%–50%	99%	Highest specificity with monoclonal antibody; requires very high level of technical expertise
Antibody	Paired serum	20%–70%	95%–99%	Highest specificity for *L. pneumophila* serogroup 1
Molecular amplification	Sputum, other lower respiratory tract secretions; urine	70%–95%	95%–99%	Excellent performance in some reference laboratories

[a]Pertains only to *L. pneumophila* infections. The yield of diagnostic tests is lower for infection caused by other species, especially those tests based on immunoassay.

spp. increases diagnostic yield by 10% to 100% over that for urine antigen testing, and appears to be most useful for the diagnosis of milder cases of the disease and for the detection of *Llb* infection.[316] A genus-specific PCR test, which can detect all known *Legionella* spp., can be quite useful for testing specimens from immunocompromised and other patients who may not be infected with *Lp.* Some studies have reported nonspecific PCR results; some reagents and nucleic acid extraction kits may be contaminated with *Legionella* DNA. Use of PCR for clinical purposes should only be undertaken for very well-validated and controlled assays, but when they are available these assays can increase diagnostic yield. Genetic sequencing of positive PCR products, or the use of whole-genome sequencing, in the absence of a positive culture, can be used in many cases for species-level identification and molecular fingerprinting.[317,318]

Optimal test yield requires performing more than one type of test; PCR (when available), sputum culture, and urine antigen testing are the preferred tests. If these are negative, and there are clinical or epidemiologic reasons for making a retrospective diagnosis weeks to months later, then antibody testing should be ordered. For patients who may be more likely to have LD caused by *Legionella* spp. other than *Lp*1, the best laboratory tests are sputum culture and PCR rather than urine antigen and serum antibody testing.

The yield of all tests except perhaps antibody determination is diminished by specific therapy, requiring testing before, or within a few days, of the start of antimicrobial therapy. Therapy should not be withheld pending collection or testing of specimens, and should not be stopped based solely on the result of a negative laboratory test.

THERAPY AND RESPONSE TO THERAPY

Legionella bacteria are intracellular parasites of monocytic phagocytes. This means that all antimicrobial agents efficacious for LD must be concentrated and bioactive in the correct subcellular location within these cells. The macrolides, quinolones, and tetracyclines all meet these criteria for *Lp* infection. Some of the non-*Lp* bacteria (e.g., *L. dumoffii, L. feeleii, L. jordanis*) are known not to reside in a phagosome,[154] making it possible that some of these drugs, which are differentially distributed within cells,[319] may be less effective than others for infections caused by these *Legionella* species. Tet(56), a tetracycline destructase that confers tetracycline resistance, has been reported to be present and active in one strain of *Llb*.[320] Proteins with sequence homology to Tet(56) are present in other *Llb* strains, *L. clemsonensis, L. massiliensis, L. nautarum, L. feeleii, L. quinlivanii,* and *L. geestiana;* apart from *Llb,* these other *Legionella* spp. either have not been reported to cause LD or are very rare causes of the disease. The presence of Tet(56) in *Llb* suggests that a tetracycline should not be used for the therapy of LD caused by this species, and that in regions where *Llb* infection is common tetracyclines are not drugs of choice for the empirical therapy of community-acquired pneumonia suspected to be LD. None of the β-lactams, monobactams, aminoglycosides, or phenicols is active for LD.[199]

Prospective adequately sized clinical trials of antimicrobial therapy for LD have not been performed.[199,257,321] Patients treated with erythromycin or a tetracycline drug had significantly lower fatality rates than did those treated with β-lactam or aminoglycoside agents in the 1976 Philadelphia epidemic.[1] In one outbreak of nosocomial disease, immunosuppressed patients treated with erythromycin had a 24% fatality rate, in comparison to an 80% rate for otherwise-treated patients, and erythromycin-treated nonimmunocompromised patients had a 7% fatality rate in comparison to 25% for those otherwise treated.[255] Small numbers of patients with LD have been reported to responded very well to erythromycin, a tetracycline, azithromycin, dirithromycin, clarithromycin, pefloxacin, ciprofloxacin, gatifloxacin, and levofloxacin. One retrospective study of LD with severe pneumonia showed that patients treated with a fluoroquinolone antimicrobial agent within 8 hours of ICU admission had significantly better outcomes than did those treated later, or with other drugs, including erythromycin.[322] Some more recent uncontrolled studies showed that erythromycin and clarithromycin were inferior to levofloxacin therapy for LD in terms of hospital length of stay, time to become afebrile, and complications, but not mortality[323,324]; of note, no comparison with azithromycin, the most potent anti-*Legionella* macrolide, was performed. Addition of rifampin to levofloxacin led to worse outcomes than levofloxacin alone in another uncontrolled study.[325] One study showed no significant difference in outcome for 104 patients treated with either levofloxacin, clarithromycin, or azithromycin.[326] Two recent large retrospective, propensity-adjusted studies showed that there is no significant difference in outcome for hospitalized LD patients treated with either azithromycin or levofloxacin, including in critically ill patients,[327,328] and a third large retrospective study of critically ill patients showed that levofloxacin therapy reduced mortality in comparison to non-azithromycin macrolides.[329] Combination antimicrobial therapy for LD is not beneficial, even in critically ill patients.[329] The available evidence supports the use of either azithromycin (500 mg daily for 3–7 days) or levofloxacin (500–750 mg daily for 5–10 days) for hospitalized patients with LD, with neither erythromycin nor clarithromycin being preferred therapies (Table 232.2).

Acquired resistance to antimicrobial drugs used to treat LD has not been detected in multiple in vitro susceptibility studies, including in unpublished studies of isolates from patients who had poor responses to therapy. *Lp* is known to develop drug tolerance when grown in macrophages,[330] and studies in guinea pigs show evidence of drug

TABLE 232.2 Preferred Therapy for Legionnaires' Disease

CLINICAL CONDITION	FIRST CHOICES	DOSAGE[a,b]	SECOND CHOICES	DOSAGE[a,b]
Mild pneumonia inpatient or outpatient, not immunocompromised	Azithromycin ***or*** Levofloxacin ***or*** Ciprofloxacin ***or*** Moxifloxacin ***or*** Clarithromycin	500 mg qd for 3–5 d 500 mg qd for 7–10 d 500 mg bid for 7–10 d 400 mg qd for 7–10 d 500 mg bid for 10–14 d	Doxycycline[c] ***or*** Erythromycin	200 mg loading dose, then 100 mg bid for 10–14 d 500 mg qid for 10–14 d
Moderate to severe pneumonia or immunocompromised[d]	Azithromycin ***or*** Levofloxacin ***or*** 750 mg qd for 5–7 d	500 mg qd for 5–7 d 500 mg qd for 7–10 d	Ciprofloxacin ***or*** Moxifloxacin ***or*** Erythromycin	400 mg IV tid initially, then 750 mg PO bid for 10–14 d 400 mg qd for 10–14 d 750–1000 mg IV qid for 3–7 d, then 500 mg PO qid for a total course of 21 d

[a]Dosage adjustments must be made for some of these drugs for patients with renal insufficiency. Patients with mild disease may be treated entirely with oral therapy, whereas for severely ill patients parenteral therapy is advised until improvement is seen and oral absorption is sufficient. Unless otherwise noted, all dosages given are for either IV or oral routes.
[b]Therapy duration may need to be considerably longer for patients with lung abscesses, empyema, endocarditis, or extrathoracic infection.
[c]Doxycycline should not be used to treat suspected or known *L. longbeachae* infection.
[d]Severely immunocompromised patients may require courses of therapy at the high end of the duration ranges given, or even longer. Close clinical follow-up is required to detect possible relapse after antibiotics have been discontinued.

tolerance for erythromycin and several other drugs.[331–333] Drug tolerance, possibly due to induction of efflux pumps,[334] was thought to be the cause of relapses in immunocompromised patients treated with erythromycin, and the overall poorer performance of erythromycin and clarithromycin therapy in critically ill patients. More recently, the preexistence and emergence of low-level quinolone heteroresistance during treatment for LD was reported, based on molecular studies during quinolone and other therapies.[335,336] There is also one report of the isolation of a clinical isolate with elevated ciprofloxacin and levofloxacin minimal inhibitory concentrations, but the relationship between these results and the response of the patient to ciprofloxacin therapy is uncertain,[337] with no apparent outcome difference between those patients with a heteroresistant population and those without one.[335] Since quinolone therapy is quite effective for the treatment of LD, and since dual quinolone-macrolide therapy does not improve outcome in critically ill patients, it is difficult to suggest combination therapy or nonquinolone therapy to counter the emergence of resistance to quinolone therapy.

The decision regarding which antimicrobial agent to administer for LD should be guided by severity of illness, degree of immunocompromise, drug cost, drug toxicity, and drug availability (see Table 232.2). Nonimmunocompromised outpatients with mild LD can be treated with any of the drugs listed in Table 232.2, with drug cost, availability, and toxicity being the main deciding factors. Hospitalized or immunocompromised patients with LD should be treated with one of the listed quinolones or azithromycin, unless drug unavailability or cost prevents their use. Initial intravenous antimicrobial therapy may be required in severely ill patients. Even in such patients, oral antimicrobial therapy can be used as soon as there is clinical improvement and intestinal drug absorption is adequate. Immunocompromised patients treated with erythromycin or clarithromycin, with or without rifampin, may suffer relapse days to months after cessation of therapy, especially if the level of immunosuppression is subsequently increased. In cases in which severe immunosuppression is chronic, thought should be given to continued suppressive therapy with a drug such as azithromycin, which has a very long intracellular half-life.[298]

The optimal duration of antibiotic therapy for LD has not been determined conclusively.[338] Use of short courses of azithromycin (500 mg/day), ranging from 3 days to 7 days in outpatients and inpatients, respectively, has been very effective, with only one relapse reported in a severely immunosuppressed patient.[339–341] Those with extensive pneumonia or with severe immunocompromise may profit by longer administration of azithromycin. Neither erythromycin nor clarithromycin are as active, or persistent in macrophages, as is azithromycin, requiring treatment durations of 14 to 21 (if immunosuppressed) days. Five days of treatment with levofloxacin (750 mg/day) has been shown to be very effective without relapses, and as effective as a 7- to 14-day course with 500 mg/day.[342] Overall, except for severely immunocompromised patients, the duration of antibiotic therapy should follow the general guidelines for all forms of community-acquired pneumonia.[343]

Rifampin should not be used to treat LD unless there are no other options available. Coadministration of rifampin with any of the drugs listed may be harmful, and has negligible benefit.[325] Rifampin dramatically reduces serum and lung concentrations of all of the macrolide drugs due to induction of drug clearance, with the enhanced clearance persisting for weeks after stopping the rifampin.[344–346]

Most LD patients treated with one of the recommended antimicrobial agents respond promptly to the therapy, sometimes within hours. Within 12 to 24 hours, most patients have improvement or complete clearance of myalgia, confusion, headache, abdominal pain, diarrhea, nausea, vomiting, and anorexia. Four to 7 days may be required for complete resolution of fever, with a steady decrease in fever over that period and with the most improvement being seen in the first day or two. Cough, sputum production, shortness of breath, and pleuritic chest pain respond more slowly to therapy, but major improvements usually occur within the first several days. As with most types of bacterial pneumonia, convalescence may be prolonged for months and complicated by neuropsychiatric disease, including chronic fatigue[347,348]; in addition there can be persistent (months) pulmonary physiologic abnormalities that may be symptomatic.[349] Evidence of chest consolidation on physical examination, and especially by roentgenography, takes considerably longer to resolve. It is common to observe apparent increases in the sizes of the original pulmonary infiltrates despite substantial clinical improvement over the first several days of therapy; this is not a cause for alarm as long as by other measures the patient is improving.[350] Complete clearing of the chest roentgenograph may not occur for up to 4 months after initiation of specific antimicrobial therapy, with the majority of patients having complete clearing by 2 months.[261,351,352] Severely immunocompromised patients, or those with severe pneumonia requiring artificial ventilation, may take longer to improve after initiation of specific therapy, or may not respond at all because of irreversible acute respiratory distress syndrome (ARDS); Even in such patients many of the systemic signs of infection (e.g., fever) improve, although the respiratory failure itself may worsen.

Failure to respond to specific therapy for LD should bring into question the validity of the diagnosis, the possibility of coinfection or superinfection, and the possibility of extrapulmonary disease complicating the LD. Up to 10% of patients with LD have coinfections or superinfections with other respiratory pathogens or other pathogens, such as *Pneumococcus, Haemophilus influenzae, Staphylococcus aureus, Enterobacteriaceae, Listeria, Nocardia, Pneumocystis, Aspergillus,* tuberculosis, *Cryptococcus,* and a variety of viruses.[257,353,354] Superinfection with opportunistic pathogens is generally seen in severely immunocompromised patients and in those in nosocomial LD, whereas coinfection with common respiratory pathogens may be seen in patients with community-acquired LD, based on positive blood or sputum cultures at the time of diagnosis of LD.[353–357] Drug fever, pancreatitis, myocarditis, hepatitis, pericarditis, metastatic infection, and pleural empyema may rarely be complications of LD, or its treatment, and causes of prolonged fever or poor response to therapy.

Ancillary therapy of LD has not been studied systematically. Correction of fluid and electrolyte abnormalities, and hypoxemia, is useful. Corticosteroid therapy for LD-caused ARDS is advocated by some,[257] but is of unproven benefit and could be detrimental to control of the infection.[260,358,359] A clinical response to administration of azithromycin or a quinolone is mandated before the administration of such immunosuppressive therapy, as well as continuation of the antimicrobials during and after steroid therapy. Corticosteroid therapy may be indicated for postpneumonic lung diseases such as cryptogenic organizing pneumonia, and perhaps pulmonary fibrosis.[360–362] Extracorporeal membrane oxygenation therapy can be lifesaving in patients with severe LD.[363]

PREVENTION

Immunization and Chemoprophylaxis

No human vaccine for LD exists, and prior infection does not necessarily prevent reinfection.[354] Antibiotic prophylaxis has prevented LD in immunocompromised patients during nosocomial epidemics of the disease.[364,365]

Engineering Modifications and Maintenance

Proper building, cooling tower, and plumbing design and construction can reduce the frequency and intensity of *Lp* contamination of potable water systems.[366] Risk assessment and a water management program are important parts of environmental control, and required by many governmental organizations.[367–369]

Use of monochloramine to treat public drinking water reduces *Legionella* spp. colonization of water, as well as rates of nosocomial LD.[370–372] Secondary disinfection with monochloramine also appears to be highly effective.[373,374]

Environmental Cultures for *Legionella* Bacteria

There is little national or international consensus regarding the benefit of routine environmental cultures to prevent LD. The *Legionella* bacteria present in our aqueous environment almost never cause disease. That, combined with natural fluctuations, imprecision in measuring bacterial concentrations, and extreme heterogeneity in *Legionella* environmental concentrations, make it difficult to define a specific and sensitive bacterial

concentration target for remediation. Many, but not all, government and other organizations recommend against the routine use of environmental cultures in risk assessment.[243,375–378]

Many bodies recommend that hospital water systems supplying wards housing immunocompromised patients be cultured for *Lp,* and that remediation be carried out if any positive cultures are found in these areas. Other organizations suggest that all hospital water supplies be checked.[377–380] Evidence from several LD nosocomial outbreaks shows that detection of any *Legionella* spp. in a hospital water distribution may be a cause for concern, and that a guideline previously used by some for remediation if 30% or more of cultured sites are positive is insensitive.[381,382]

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The complete reference list is available online at Expert Consult.

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233 *Capnocytophaga*

J. Michael Janda and Richard B. Clark

SHORT VIEW SUMMARY

Definition
- Endogenous or zoonotic-associated infections have been reported, depending on the species of *Capnocytophaga* involved.
- Symptoms range from sepsis (most often) with and without central nervous system involvement to ocular disease; illnesses associated with pregnancy; and illnesses involving bones, joints, and soft tissues.

Epidemiology
- Endogenous infections arise in children or adults with blood dyscrasias and significant neutropenia.
- Sepsis arises from human oral species seeding into the circulatory system through abraded or damaged gums or gingival pockets in the oral cavity.
- Mortality rate is low (<3%) with appropriate treatment.
- Zoonotic infections arise primarily in male patients from a dog bite or close contact with dogs.
- Persons most at risk include dog owners, breeders, veterinarians, kennel workers, and hunters.
- Patients at highest risk for developing serious life-threatening infections with disseminated intravascular coagulation include patients who have undergone splenectomy or functionally asplenic patients and patients with ethanol abuse or immunosuppression.
- Reported mortality rates range from 13% to 33%.

Microbiology
- Bacilli are gram-negative with tapered ends.
- They are slow growing and fastidious, requiring 3 to 10 days of incubation on supportive media.
- No standardized method of determining drug susceptibilities exists, but testing should be attempted, if possible.

Diagnosis
- Is history compatible with *Capnocytophaga* infection (dog bite)?
- Does Gram or Wright-Giemsa stain of blood or other sterile specimens show bacilli with tapered ends sometimes within polymorphonuclear leukocytes?
- Conventional tests that include catalase, oxidase, and arginine dihydrolase are slow but reliable.
- Rapid molecular tests include 16S ribosomal RNA gene sequencing for rapid diagnosis on specimens or isolated organisms, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for identification of isolated organisms, and whole-genome sequencing.

Therapy
- Carbapenems and penicillin/β-lactamase inhibitor combinations are the first-choice drugs in serious infections caused by human oral *Capnocytophaga*. Resistance to a number of drug classes including β-lactams is frequently observed owing to β-lactamase production by human oral *Capnocytophaga*.
- A broader range of treatments is available for zoonotic infections. *Capnocytophaga canimorsus* recovered from dogs is not known to produce β-lactamases.
- Acquired drug resistance has been reported in some isolates.

Prevention
- Clinicians must be made aware of these organisms, the diseases they cause, and the patients at risk.
- Laboratories may require extra incubation time, a specialized incubation atmosphere, and richer media to isolate these organisms; clinicians should inform laboratory personnel about clinical suspicion so that appropriate culture methods are used.
- Rapid testing methods should be used to decrease time to diagnosis.

The genus *Capnocytophaga* consists of nine or more gram-negative fastidious species that typically reside as normal flora in the oral cavities of humans and other vertebrates including dogs and cats. Members of this genus can cause serious life-threatening infections in humans including septicemia and meningitis as a direct consequence of inapparent injuries or penetrating trauma resulting from animal contact or bites, most often involving dogs. Persons with underlying conditions including asplenia, alcoholism, and immunosuppression (neutropenia) are at greater risk of developing serious and fulminant systemic disease due to *Capnocytophaga*. The primary pathogen of this genus is *Capnocytophaga canimorsus,* formerly known as dysgonic fermenter 2 (DF-2). The number of *Capnocytophaga* infections may be increasing in the general population due to many factors including increasing pet ownership, animal-associated occupations, and a greater number of immunocompromised persons.

TAXONOMY

The genus *Capnocytophaga* consists of fermentative capnophilic bacteria that morphologically appear as fusiform thin to slender gram-negative bacilli with tapered ends. The genus currently consists of nine species—*C. canimorsus, C. canis, C. cynodegmi, C. gingivalis, C. granulosa, C. haemolytica, C. leadbetteri, C. ochracea, C. sputigena*—that can be subdivided into two major groups associated with the oral microbiota of humans and animals.[1,2] A tenth and eleventh reputed species, *C. stomatis* and *C. endodontalis,* have been recovered from animals and human infections[1] and a periapical abscess,[3] respectively. All validated species or taxa are recognized human pathogens or implicated in causing disease.[1,2] Phylogenetically, the genus *Capnocytophaga* resides in the family Flavobacteriaceae. The complete genomes of six different *Capnocytophaga* spp. have been sequenced more recently.[4]

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Few studies have focused on which virulence factors are important in oral-associated *Capnocytophaga* infections. Oral-associated *Capnocytophaga* organisms produce a number of cell-bound or extracellular factors that may promote progression of periodontitis by enhancing the growth of bacteria in plaques and subgingival pockets or by evading host immune responses. Such factors include immunoglobulin A1 (IgA1) protease, phospholipase A_2, aminopeptidases, and type IX secretion system as well as the ability to form biofilms and chemotaxis-guided (gliding) motility.[5,6]

Progress has been made in understanding potential pathogenicity mechanisms functional in zoonosis-associated invasive infections typified by fulminant *C. canimorsus* septicemia.[7] These mechanisms can be broadly categorized into four areas: (1) failure of *C. canimorsus* to trigger

innate immune responses based on resistance to complement-mediated lysis and evasion of phagocytosis by macrophages; (2) inability of Toll-like receptor 4 to respond to *C. canimorsus* with activation of proinflammatory responses including generation of tumor necrosis factor, cytokines (interleukin-6), chemokines (interleukin-8), and nitric oxide[8]; (3) expression of cell surface lipoproteins (polysaccharide utilization loci) and sialidase, which deglycosylate host glycoproteins (including human IgG), leading to bacterial growth and persistence in vivo; and (4) a modified lipid A and lipopolysaccharide architecture that may affect endotoxicity and binding to human myeloid differentiation factor 2.[9,10]

More recent studies indicate that only a subset of *C. canimorsus* strains may be highly pathogenic for humans.[11] Of nine known capsular serotypes of *C. canimorsus,* serotypes A, B, and C predominate, causing almost 90% of human infections. In contrast, these serotypes are found at a much lower frequency (7.6%) in the oral microbiota of dogs.[12] This fact may help account for the relatively low frequency of *C. canimorsus* infections in people after dog bites.

Human *Capnocytophaga* infections can be broken down into five major categories: (1) septicemia, (2) diseases of the central nervous system (CNS), (3) eye infections, (4) illnesses associated with pregnancy, and (5) miscellaneous complications including infections of bone and tissue. Although most *Capnocytophaga* infections occur in individuals with impaired immune function or with significant underlying disease, infections in healthy individuals have been well documented.[13–15]

Human Oral-Associated Species

Oral *Capnocytophaga* spp. are residents of the subgingival sulcus and of supragingival plaque and have been implicated as opportunistic pathogens involved in gingivitis, periodontal disease, and oropharyngeal mucositis.[16] Carriage rates of these fastidious bacteria in the oral cavity have been reported as high as 100% with species distribution and prevalence varying from study to study.[16] One study using two different molecular approaches found *C. ochracea* and *C. granulosa* to be the two most common species associated with subgingival plaque.[17] These results closely mirror findings from a multicenter study in which *C. ochracea* predominated as the cause of *Capnocytophaga* bacteremia in patients with cancer.[18] A 2016 review of all oral-associated *Capnocytophaga* infections reported between 2000 and 2016 found *C. ochracea* as the etiologic agent in 59% of cases and *C. sputigena* as the etiologic agent in 29%, with the remaining illnesses (12%) divided among several species.[16]

The most common serious infection associated with oral *Capnocytophaga* infection is septicemia. Sepsis is most often observed in patients with underlying hematologic malignancies including acute and chronic myelogenous leukemia, non-Hodgkin lymphomas, Hodgkin disease, and multiple myeloma (Table 233.1).[18–20] Onset of sepsis typically coincides with the initiation of profound neutropenia (<500 granulocytes/mm^3) induced by chemotherapy or after hematopoietic stem cell transplantation.[21] Oral ulcerations such as severe mucositis, gingival hyperplasia, esophagitis, and periodontitis appear to serve as portals of entry for systemic invasion. Maury and associates[19] found all 24 patients with *Capnocytophaga* bacteremia in their study to be neutropenic; 88% of these patients had severe oral mucositis or periodontitis. Warren and Allen[22] suggested that pediatric patients are more prone to developing *Capnocytophaga* sepsis than adults; Campbell and Edwards[23] reviewed the literature on pediatric infections caused by *Capnocytophaga* spp. and found bacteremia as a common feature of infection in all 16 immunocompromised children. In contrast, Jolivet-Gougeon and coworkers[24] found that the frequency of oral carriage of *Capnocytophaga* organisms by children hospitalized on an oncology service ranged from 16% to 61%, and no cases of systemic disease attributed to *Capnocytophaga* were detected during this 10-year period. These latter results suggest that although oral carriage of *Capnocytophaga* organisms by children may be high, the risk for developing severe disease is relatively low.

Bloodborne disease caused by human oral *Capnocytophaga* spp. is typically monomicrobial (85%–90%). When polymicrobial infections occur, they most often involve viridans streptococci, anaerobes, or aerobic or facultatively anaerobic rods that represent other oral microbiota. *C. ochracea* and *C. sputigena* are the most commonly implicated species in sepsis, with most cases occurring in immunocompromised persons.[16,18,19] However, serious *C. ochracea* sepsis and purpura fulminans were reported in a healthy 46-year-old man 2 weeks after an uneventful dental extraction.[25] Rare cases of *C. gingivalis* or *C. haemolytica* bacteremia have been reported.[16] The overall attributable mortality rate associated with *Capnocytophaga* bacteremia ranges from 16% to 42%; recent investigations have reported lower values, approaching 0%.[18,19,22,23] Fatal cases of *Capnocytophaga* bacteremia caused by oral-associated species continue to be described on occasion.[20]

TABLE 233.1 Salient Features Distinguishing Human-Associated From Animal-Associated *Capnocytophaga*

CHARACTERISTIC	HUMAN-ASSOCIATED	ANIMAL-ASSOCIATED
Patient Population		
Children	+	–
Adults	+	+
Underlying Diseases[a]		
Leukemia, lymphoma	+	–
Asplenia	–	+
Ethanol abuse	–	+
Risk Factors		
Neutropenia and chemotherapy	+	–
Dental manipulations	+	–
Animal bites	–	+
Laboratory Tests		
Catalase	–	+
Oxidase	–	+
Arginine dihydrolase	–	+

[a]Predisposing for invasive disease, such as septicemia.

Oral species are less frequently implicated in CNS infections than their zoonotic counterparts. In contrast to septicemia, CNS illnesses most often manifest in immunocompetent individuals, with the major risk factor being dental manipulations (see Table 233.1). Cases of *Capnocytophaga* subdural empyema and frontal brain abscess, sinusitis, and pleuropneumonitis have been described in healthy individuals who underwent various dental procedures including tooth extraction and orthodontia.[16] Brain abscesses and extremely rare cases of meningitis also have been reported in pediatric patients with blood dyscrasias.

Ocular infections can run the gamut from blepharoconjunctivitis to keratitis, endophthalmitis, and corneal ulceration. People prone to developing ocular disease are older than 70 years of age, immunosuppressed, or involved in intravenous or crack cocaine drug use. In one series of 10 patients with keratitis, risk factors associated with infection included corneal epithelial defects, previous ocular infections, topical corticosteroid therapy, and intraocular surgery.

Chorioamnionitis is the most common clinical presentation associated with pregnancy and *Capnocytophaga* infection. Mild-to-severe illnesses can develop in women, resulting in premature contractions, labor, or fetal death. *Capnocytophaga* spp. can be recovered from infected placenta, meconium, cervix, and endometrium as well as amniotic fluid. Perinatal illnesses may ensue via an ascending route of infection or through hematogenous spread.[16,26] Many cases of chorioamnionitis involve recent oral sex as the primary risk factor with a report of *Capnocytophaga* spp. recovered from dental plaque of a patient's partner.[27] Contemporary studies suggest the possibility of an increasing incidence of *C. sputigena* bacteremia in neonates or infants. Such perinatal infections are typically found in low-birth-weight (790–1820 g) preterm infants with gestational ages ranging from 22 to 29 weeks.[27,28] Maternal findings in reported cases are consistent with chorioamnionitis. The neonatal mortality rate reported for 15 cases was 6.7% in one study.[27]

Unusual monomicrobial or mixed infections involving oral *Capnocytophaga* spp. include peritonitis, pyogenic arthritis, vertebral osteomyelitis, cervical and liver abscesses, aspiration pneumonia, pleuropneumonitis, pleural effusion and empyema, pyonephrosis, and soft tissue infections such as pyomyositis of the iliopsoas muscle.[16]

Infections Associated With Zoonotic Species

The zoonotic species *C. canimorsus* and *C. cynodegmi* are normal inhabitants of the oral cavity of dogs and cats.[29,30] Between 58% and 70% of dogs harbor *C. canimorsus* in their oral cavity,[11,31] whereas comparable numbers in cats vary from 15% to 57%.[7] In many case reports, predisposing risk factors and apparent routes of infection are not identified, although Dilegge and associates[29] found that several *Capnocytophaga* spp. could be recovered from the mouths of dogs.

Most life-threatening illnesses associated with these two zoonoses are attributed to *C. canimorsus* (>90%), which seems to have a higher predilection for causing serious disease than *C. cynodegmi*. Zoonotic-associated infections (septicemia, meningitis) arise via exogenous introduction of bacteria into wounds from penetrating trauma (dog bite) or by unapparent inoculation of bacilli into surfaces or tissues via close contact with pets. Such complications can result from innocuous activities associated with pet ownership including close petting, intimate contact (kissing), minor scratches, or the licking of minor abrasions or apparently intact skin. Invasive diseases resulting from such exposures are associated with higher mortality rates than endogenously acquired infections.

Symptoms associated with *C. canimorsus* septicemia are nonspecific and similar to symptoms of other gram-negative pathogens including fever, diarrhea, abdominal pain, vomiting, headache, and confusion.[32] A prominent diagnostic feature, found in 20% to 40% of cases of *C. canimorsus* septicemia, is a rash on the trunk or extremities or both that may vary from a macular or maculopapular eruption to a more severe and rapidly fatal form such as purpura fulminans with petechial lesions, retiform purpura, urticarial lesions, or symmetrical gangrene.[33–35] The overall case-fatality rate for *C. canimorsus* septicemia varies from 13% to 33%,[36,37] but mortality rates can approach 60% to 80% in patients with septic shock and multiple organ failure.[11,38] A review of more than 400 cases of *C. canimorsus* bacteremia estimated the overall case-fatality rate to be 26%.[7]

Meningitis is the second most common presentation, and the reported mortality rate is very low (3%) compared with cases of septicemia without CNS involvement.[7,39] At least 96% of patients with meningitis present with two of four symptoms including headache, fever, neck stiffness, and altered mental status.[39] *C. canimorsus* has also been linked to endocarditis, with a fatality rate approaching 25%.[40] Other miscellaneous infections have been described including brain abscess, sacral epidural abscess, eye and bone or joint infections, cellulitis, glomerulonephritis, renal failure, and pneumonia.[7,33,41,42]

EPIDEMIOLOGY

Several studies have estimated the annual incidence of *C. canimorsus* infections at 0.5 to 1.0 infection/1 million population per year,[36–38] or about 150 to 300 cases in the United States each year. *C. canimorsus* sepsis most commonly occurs in men (male-to-female ratio 2.7–3.8:1) older than 50 years of age (70%–90%) with one or more underlying conditions (62%–89%).[33,43] Past medical history often reveals recent dog bite exposure or incidental contact with dogs (60%–84%),[7,37,44–46] with the onset of symptoms occurring 1 to 8 days after exposure.[7,45] People at risk for developing bloodborne disease include dog owners, veterinarians, breeders, kennel workers, mail carriers, and hunters. On at least six occasions, cat bites or scratches have been implicated as the source of *C. canimorsus* or related species septicemia, with one reported fatality.[2,47] More than 400 cases of systemic infections involving *C. canimorsus* have been published in the literature, with one retrospective study reporting on 55 cases of invasive disease over a 32-year period.[7,32] Some surveys suggest that detection of systemic disease caused by *C. canimorsus* may be increasing because of several factors including increasing pet ownership, more opportunities for animal contact, a higher number of immunocompromised persons in the general population, and better molecular methods in the laboratory for detecting infections.

Patients who have undergone splenectomy or functionally asplenic patients are more prone to developing sepsis with disseminated intravascular coagulation than individuals with intact spleens, and multiple case reports describing overwhelming postsplenectomy infections accompanying *C. canimorsus* sepsis have been reported.[48] The incidence of patients with *C. canimorsus* bacteremia and asplenia is 13% to 33%.[7,33,37] Other underlying conditions associated with aggressive *C. canimorsus* infections include ethanol abuse (20%–31%), immunosuppression (3%–6%), rituximab, and corticosteroid therapy.[7,33,37,49] Job and associates[50] estimated that patients with underlying medical problems are three times more likely to contract *Capnocytophaga* disease than healthy individuals, although fatality rates are not appreciably different. In addition to disseminated intravascular coagulation, other complications associated with *C. canimorsus* septicemia include Waterhouse-Friderichsen syndrome, Stevens-Johnson syndrome, thrombotic thrombocytopenic purpura, and hemolytic-uremic syndrome.[51–53] In 30% to 40% of infections, no recognizable predisposing factor is evident,[38] and up to 40% of serious life-threatening *C. canimorsus* illnesses can occur in apparently healthy people.[43,54]

Although *C. cynodegmi* is a common resident of the mouths of dogs and cats, it rarely causes disease.[46] Differences in pathogenicity between *C. canimorsus* and *C. cynodegmi* appear to be unrelated to complement-mediated lysis, as both species are readily killed by human serum.[55] A fatal case of *C. cynodegmi* sepsis and meningitis in a 72-year-old woman who had previously undergone splenectomy and who was bitten on the hand by her pet dog has been described.[56] She rapidly developed signs of sepsis, with facial purpura and a progressive macular rash, and died of infection within 48 hours of admission. *C. cynodegmi* peritonitis has been reported in a 67-year-old man[57] and in a 39-year-old woman on continuous or intermittent peritoneal dialysis.[58] In both cases a pet cat was suspected as the source of infection.

DIAGNOSIS AND LABORATORY IDENTIFICATION

Presumptive diagnosis of *Capnocytophaga* sepsis can be made by examination of the patient's peripheral whole blood or buffy coat using Gram or Wright-Giemsa stain.[59] The presence of *Capnocytophaga* should be strongly suspected if slender, medium to long, gram-negative rods with tapered ends are observed (Fig. 233.1). Pers and colleagues[60] reported seeing bacteria on initial microscopic examinations of cerebrospinal fluid (CSF), whereas another report described slender, fusiform bacilli in conjunctival scrapings.[61] 16S ribosomal RNA (rRNA) gene sequencing performed directly on a clinical sample or a positive blood culture or CSF can decrease the turnaround time to diagnosis,[59,62] and polymerase chain reaction (PCR) assay using species-specific primers can detect *Capnocytophaga* in mixed culture in dental plaque samples.[63]



FIG. 233.1 Gram stain of *Capnocytophaga* exhibiting long, slender rods. *(Courtesy Dr. Edward J. Bottone.)*

Despite the widespread application of molecular techniques to identify organisms from clinical samples, most clinically significant isolates of *Capnocytophaga* are detected by culture. Clinical suspicion of *Capnocytophaga* septicemia should prompt a request to the laboratory to incubate blood cultures for up to 10 days or longer to enhance recovery of this fastidious, often slow-growing organism.[64] Recovery of capnocytophagae from CSF or blood typically takes 3 to 7 days. Some *Capnocytophaga* spp. appear to be sensitive to the anticoagulant sodium polyanethole sulfonate used in some blood culture bottles. One report cited recovery of *C. canimorsus* after 25 days of incubation.[40] Performing a Gram stain on blood culture sediment at the first indication of growth can yield a presumptive identification, although there is a small chance that the Gram stain can be misread.[65] *Capnocytophaga* spp. can be recovered from other clinical specimens including CSF, abscess material, respiratory secretions, amniotic fluid, the urogenital tract, joint fluid, and various tissues. Prolonged incubation (>7 days) of these cultures may be required to recover capnocytophagae.

Capnocytophaga spp. are considered fastidious bacteria because they grow slowly on blood-enriched media and generally require an increased carbon dioxide atmosphere (5%–10%). Microaerobic and anaerobic atmospheres also have been described as conducive to isolation of these organisms from clinical samples. They grow on blood agar and often on chocolate agar but not on MacConkey or heart infusion agars. Several reports have described successfully using enriched basal media such as heart infusion, brain heart infusion with 5% blood, or basic media enriched with rabbit serum.[31,40] Other authors have reported success isolating strains from mixed cultures using selective media containing gentamicin, bacitracin, polymyxin B, vancomycin, or trimethoprim. *Capnocytophaga* isolates are reported to grow on laked blood with kanamycin and vancomycin agar medium with a reduced concentration of kanamycin (2 μg/mL), on Thayer-Martin media, on Martin-Lewis media, on Columbia agar media, and in thioglycolate broth.[66]

Colonies may be visible at 24 hours but often require 48 to 72 hours to reach 2 to 4 mm in diameter. They are convex and smooth and can show irregular edges, indicating what is described as gliding motility (Fig. 233.2). Colonies of the human oral strains can have a slight yellow pigment on initial growth, which becomes darker yellow to orange with age. Colonies are also described as having a bluish purple hue or a metallic sheen on blood agar medium.[57] Others describe colonies as being yellowish, pinkish, or bluish speckled.[66] The colonies of the zoonotic strains are not usually pigmented.

A number of methods have been employed to identify these isolates to species including conventional biochemical tests, protein profiles, multilocus enzyme electrophoresis, serotyping of IgA1 proteases, DNA probes, 16S rRNA PCR restriction fragment length polymorphism analysis, broad-range PCR from apparently nonviable organisms,[67] matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, 16S rRNA gene sequencing, and whole-genome sequencing.[17,68,69] Most current commercial identification kits are unable to identify this organism to genus and species levels. One study showed that only 11 of 24 test *Capnocytophaga* spp. were identified correctly by an automated identification system to the genus level.[70] One package insert cautions the laboratorian to consider specimen source, atmospheric preferences, Gram stain characteristics, and growth on selective agar when using their product. Table 233.1 lists some biochemical tests that can help differentiate the main *Capnocytophaga* groups. A combination of basic morphologic and conventional biochemical tests with 16S rRNA gene sequencing has proved useful in identifying these organisms to the species level.[32]

Reports have described success in identifying *Capnocytophaga* either to genus level or to genus and species level using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.[70] One study identified 22 of 24 human oral *Capnocytophaga* isolates correctly,[69] whereas another identified 22 of 23 correctly as either *C. canimorsus* or *C. cynodegmi*.[70]



FIG. 233.2 Gliding motility exhibited by *Capnocytophaga* on blood agar–based medium. *(Courtesy Dr. Edward J. Bottone.)*

THERAPY

There are no guidelines at the present time for antimicrobial susceptibility testing of *Capnocytophaga* published by either the European Committee on Antimicrobial Susceptibility Testing or the Clinical and Laboratory Standards Institute. The inherent slow growth of *Capnocytophaga* spp. isolates makes antibiotic susceptibility difficult to perform. Owing to the lack of a standardized method, many different susceptibility assays have been published for these organisms including agar dilution,[71–75] broth microdilution,[76,77] Etest,[78] and agar diffusion.[79] These antimicrobial susceptibility tests also used different media, incubation times, and atmosphere of incubation (anaerobic vs. increased carbon dioxide) (5%–10%)[77–82]; thus for certain drugs (see further on), results among individual laboratories may not agree.

Early reports of antibiotic susceptibility testing revealed almost universal susceptibility to the β-lactam antibiotics (Table 233.2).[71,75,76] Before 1987, there were only four reported β-lactamase strains among *Capnocytophaga* spp.[19,76] However, the detection of β-lactamase production became more common as studies were published in the 1980s and 1990s.[73,81,83,84] Aside from being resistant to both penicillin and amoxicillin, many of these isolates showed resistance to third-generation cephalosporins such as ceftazidime and cefotaxime. They remained susceptible to β-lactam/β-lactamase inhibitor combinations such as amoxicillin-clavulanate, piperacillin-tazobactam, and ampicillin-sulbactam.[72–74,82,84] It was not unusual for more than 50% of the published study isolates to produce a β-lactamase.[19,72] One study reported that the ceftazidime resistance of 195 human oral *Capnocytophaga* spp. ranged from 37% to 100% in the years 1993–2002.[24]

Investigators soon discovered that the *Capnocytophaga* isolates produced several different types of β-lactamases including the newly discovered *Capnocytophaga sputigena* 1 (CSP-1) β-lactamase.[80–82] CSP-1 is associated with resistance to both the penicillins (amoxicillin, piperacillin) and some second-generation and third-generation cephalosporins (cefotaxime, ceftazidime) but is inhibited by clavulanic acid. Thus CSP-1-harboring *Capnocytophaga* organisms are still highly susceptible

TABLE 233.2 Antibiotic Susceptibilities of *Capnocytophaga*

HIGHLY SUSCEPTIBLE	INTERMEDIATELY SUSCEPTIBLE[b]	RESISTANT OR INCONSISTENT
Imipenem[19,72,77,82,87]	Ciprofloxacin[71,73,74,80,79]	Gentamicin[73,74,88]
Meropenem[91]	Clindamycin[73,75,77,79,80,84]	Vancomycin[75]
Piperacillin/tazobactam[72,82]	Erythromycin[75,80,84]	Polymyxin B[74,80]
Amoxicillin/clavulanate[16,82,84]	Tetracycline[75,84]	Trimethoprim[71]
Tigecycline[88]	Aztreonam[71,72,82]	Ceftazidime[16,19,24,77,84] Cefotaxime[16,19,77,84] Amoxicillin[16,72,82,84,87]

[a]These susceptibility groups are predominantly useful for treating human oral *Capnocytophaga* spp.; zoonotic *Capnocytophaga* spp. remain more susceptible to antibiotics.
[b]It is recommended to perform antibiotic susceptibility in relation to these drugs before using them to treat patients infected with *Capnocytophaga* spp.

in vitro to β-lactamase inhibitor combinations such as amoxicillin-clavulanate and piperacillin-tazobactam. In addition, organisms producing CSP-1 are still susceptible to the carbapenem imipenem. Other β-lactamases commonly found in oral *Capnocytophaga* spp. are CfxA2 and CfxA3 β-lactamases. According to one study, these class A β-lactamases may be responsible for up to 70% to 80% of β-lactam resistance in *Capnocytophaga* spp.[84,85]

Both CfxA2 and CfxA3 β-lactamases are predominately cefuroximases that hydrolyze penicillins and extended-spectrum cephalosporins but are still susceptible to inhibition by clavulanic acid.[84] CfxA2 and CfxA3 β-lactamases may be chromosomal or plasmid based.[86] Different variants of CfxA have been described.[86] Other β-lactamases produced by *Capnocytophaga* include the extended-spectrum M-17 derived from *bla*TEM$_{-1a}$. As with many other extended-spectrum β-lactamases,[87] this enzyme is able to effectively hydrolyze both ceftazidime and cefotaxime, although cefotaxime is less affected. The rare chromosome-based *bla*OXA-347 has been described in *Capnocytophaga* isolates using whole-genome sequencing; this carbapenemase has been detected in *C. cynodegmi* chromosomes and is responsible for resistance to imipenem and to second-generation and extended-spectrum cephalosporins.[88]

For other non–β-lactam drugs, the resistance patterns depend on the specific drug. Most *Capnocytophaga* organisms are resistant to gentamicin,[73,88] polymyxin B,[74,80] and trimethoprim.[71] However, for drugs such as gentamicin, the minimal inhibitory concentrations range from being highly susceptible levels (0.05 μg/mL)[77,80] to complete high-level resistance (128 μg/mL).[73,74,88] These data may indicate a lack of standardization among the various described methods.[89]

For clindamycin, some studies indicate rare resistance,[73,75,77,79,80] but others note an increase in resistance approaching 30%.[84] Erythromycin resistance seems to parallel clindamycin resistance.[75,80,84] Resistance to both of these drugs is associated with the *erm*(F) and *erm*(C) genes.[81] Quinolone resistance is also becoming more common[77,79] even though early reports[71,73,74,76,77,79] indicated a lack of resistance to this class of drugs. Tetracycline resistance is not common in published studies.[75,80,84]

For oral therapy of milder infections, clindamycin, doxycycline, or a fluoroquinolone can be used. Carbapenems may be indicated in mixed soft tissue infections with more resistant organisms than *Capnocytophaga* spp. Aminoglycosides, antistaphylococcal penicillins, colistin, and first-generation cephalosporins are not considered useful.

Initial therapy in most *Capnocytophaga* cases is empirically determined and involves treatment with broad-spectrum antibiotics based on presenting diagnosis. When either a gram-negative rod or a *Capnocytophaga* sp. is suspected, more specific antimicrobial treatment is prescribed. Because it may take several days before a *Capnocytophaga* sp. can be presumptively recognized, clinicians should keep this organism in mind in cases in which there is a history of dog or cat bite or with neutropenic patients who have oral mucositis or periodontitis. This is especially true if the patient is asplenic or functionally asplenic or has a history of alcohol abuse.

Differences in outcome of serious *Capnocytophaga* infections have been documented. In a Denmark study of 39 cases of dog bites or associated with dog bites, 12 patients died.[60] All but 2 of the 39 patients were infected with *C. canimorsus* and were treated with penicillin, which may be an inferior drug therapy. No data on β-lactamase positivity rates were provided, even though to our knowledge, no β-lactamase–producing *C. canimorsus* organisms have been detected.

In a study of 11 cases of *Capnocytophaga* bacteremia,[19] *C. ochracea* and *C. sputigena* were important causes of fever in neutropenic patients, especially where β-lactam therapy has failed. In this study, eight patients were infected with β-lactamase–positive *Capnocytophaga*. In these cases, the introduction of imipenem would be the preferred option. A second option would be a fluoroquinolone or clindamycin.

Another study of a series of 28 patients with cancer concluded that *Capnocytophaga* bacteremia is usually an uncomplicated condition.[90] The outcomes were good after antibiotic therapy; however, only one of their isolates produced a β-lactamase even though 50% of their tested isolates were resistant to ciprofloxacin. Consequently, the authors recommended that these cases be managed with a carbapenem or a ureidopenicillin with a β-lactamase inhibitor.

Clinicians should be aware of the increased β-lactam resistance of *Capnocytophaga* to second-generation and extended-spectrum cephalosporin antibiotics. A case of *C. sputigena* fatal bacteremia was described in which the patient was treated with both amikacin and ceftazidime, to which the organisms were resistant.[81] Another case of CfxA3 β-lactamase–positive *C. sputigena* in an immunocompromised pediatric patient has been described.[91] Treatment was altered to both meropenem and clindamycin, and the patient recovered. It may be difficult to obtain a *Capnocytophaga* susceptibility test in a timely manner, as most laboratories have not validated this organism for susceptibility testing and would send out to a reference laboratory for assays of this type. Etest incubated in increased carbon dioxide or anaerobically may be the easiest test to perform, as very few laboratories perform agar dilution.

PREVENTION

Clinician awareness and patient education are the most effective ways to prevent *Capnocytophaga* infections. Clinicians should keep in mind patient risk factors associated with these infections. Clinical laboratories should know how to handle specimens and identify isolates effectively and quickly when *Capnocytophaga* organisms are suspected. This awareness can reduce the time to diagnosis and result in more specific antimicrobial treatment. Rapid presumptive diagnosis may result in the patient receiving better targeted antibiotics for these organisms before a conclusive diagnosis is confirmed. Individuals with enhanced susceptibility to infection, particularly patients who have undergone splenectomy or are asplenic, should be made aware of activities such as pet (dog or cat) ownership that increase their risk for developing *Capnocytophaga* sepsis.[38,92,93] Asplenic patients should not take care of a neighbor's dog or cat.[94] Several authors suggest the controversial step of clinicians prophylactically treating all patients in high-risk groups for possible *Capnocytophaga* infections after a dog bite.[2] One group is reportedly working on developing a standard test to detect more dangerous strains,[36] whereas another group is trying to understand why some individuals are more prone to developing overwhelming disease.[38,74]

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The complete reference list is available online at Expert Consult.

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234 *Bartonella*, Including Cat-Scratch Disease

Stacey R. Rose and Jane E. Koehler[a]

SHORT VIEW SUMMARY

Epidemiology and Microbiology

- Fastidious, facultatively intracellular, pleomorphic gram-negative bacilli
- Transmission via infected feces of arthropod vectors by direct inoculation into nonintact human skin
- *Bartonella bacilliformis:* endemic to Andes mountains; reservoir—humans; vector—sand flies
- *Bartonella henselae:* globally endemic; reservoir—cats; vector—cat flea; transmitted to humans via cat scratch and inoculation of wound with flea feces
- *Bartonella quintana:* sporadic outbreaks worldwide; associated with homelessness and other conditions of poor sanitation; reservoir—humans; vector—human body louse; transmitted to humans via inoculation of louse feces into louse bite wound

Clinical Features

- Chronic bloodstream infections (for months) is a hallmark of *Bartonella* infections
- *B. bacilliformis:* in immunocompetent patients causes biphasic illness—Oroya fever (acute phase) characterized by fever, hemolytic anemia and high fatality rate when untreated; verruga peruana (late phase), characterized by crops of vascular proliferative skin lesions that evolve through stages
- *B. henselae*: in immunocompetent patients causes cat-scratch disease (CSD)—self-limited regional lymphadenopathy, more common in pediatric population and in warm, humid climates that favor feline flea infestation
- *B. henselae:* in advanced human immunodeficiency virus (HIV) infection and other immunocompromising conditions, causes vascular proliferative lesions—bacillary angiomatosis (BA) and hepatic and splenic bacillary peliosis (BP)
- *B. henselae:* blood culture-negative endocarditis—usually occurs in patients with preexisting native valve lesion or prosthetic valve; may be associated vasculitis/glomerulonephritis, including positive immune markers (cytoplasmic-antineutrophil cytoplasmic antibody (c-ANCA) and/or anti–proteinase 3 antibodies (anti-PR3)
- *B. henselae:* in immunocompetent and immunocompromised patients (HIV-infected and transplant recipients) causes fever of unknown origin (FUO), disseminated infection
- *B. quintana:* in immunocompetent homeless patients causes trench fever—usually self-limited febrile illness with bacteremia
- *B. quintana:* blood culture-negative endocarditis—can occur in patients with normal heart valves; may be associated with c-ANCA– or anti-PR3–positive vasculitis/glomerulonephritis
- *B. quintana:* in advanced HIV infection and other immunocompromising conditions causes vascular proliferative lesions—BA, usually cutaneous, subcutaneous, osseous (but not hepatic)
- *B. quintana:* in immunocompromised HIV-infected (but not transplant) patients causes FUO, disseminated infection

Diagnosis

- Extremely fastidious and difficult to culture
- Serology (indirect immunofluorescence assay [IFA])—mainstay for diagnosis, but cross-reactivity among *Bartonella* spp. common, and IFA may be negative for weeks early in disease course; sensitivity is highest in immunocompetent patients with CSD; slightly lower in solid-organ transplant (SOT) recipients and lowest in late-stage acquired immunodeficiency syndrome patients
- Histopathology: CSD—granulomatous inflammation; BA/BP—vascular proliferation; Warthin-Starry or Steiner stain demonstrates *Bartonella* bacilli in tissue, especially in BA/BP lesions
- Polymerase chain reaction amplification of DNA extracted from tissue or blood increasingly important and available

Therapy

- *B. bacilliformis:* Oroya fever, first line—ciprofloxacin plus ceftriaxone for 14 days, although fluoroquinolone resistance has been reported; alternative: chloramphenicol, coadministered with a β-lactam antibiotic
- *B. bacilliformis:* verruga peruana, first line—azithromycin for 7 to 14 days; alternatives—rifampin for 21 to 28 days or ciprofloxacin for 14 days
- *B. quintana* and *B. henselae:* infection in immunocompromised hosts (e.g., in HIV-infected and SOT recipients with BA/BP)—doxycycline is first line; erythromycin or azithromycin is second choice (doxycycline is preferred due to tolerability issues and drug-drug interaction potential with macrolides); duration of 3 months or longer for cutaneous BA disease and 6 months or longer for visceral, extensive cutaneous, or relapsing *Bartonella* infection
- *B. quintana:* bacteremia without endocarditis (trench fever)—doxycycline for 4 weeks plus gentamicin for the first 2 weeks
- *Bartonella* spp.: endocarditis—doxycycline for 6 weeks plus gentamicin for first 2 weeks *OR* both doxycycline plus rifampin for at least 6 weeks (doxycycline + rifampin preferable to doxycycline plus gentamicin in setting of *Bartonella* endocarditis-associated renal insufficiency, which occurs frequently)
- *B. henselae:* CSD lymphadenopathy in immunocompetent host—no treatment (self-limited); possibly azithromycin for extensive CSD lymphadenitis
- *B. henselae:* CSD disseminated or CNS infection in immunocompetent host (e.g., encephalitis, neuroretinitis, hepatosplenic involvement, osteomyelitis)—two-agent therapy required, doxycycline (or azithromycin) +/– rifampin for 4 to 6 weeks

Prevention

- *B. henselae:* control of flea infestation of cats is most important; avoid cat scratches of humans
- *B. quintana:* eradicate and prevent body lice infestation of humans

[a]Dr. Koehler was supported by funding from a Pew Scholar Award, a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research, a California HIV Research Program Award, and by National Institutes of Health/National Institute of Allergy and Infectious Diseases grants R01AI52813 and R01AI103299.v.

BACKGROUND AND CLASSIFICATION OF *BARTONELLA* SPECIES

The genus *Bartonella* is a member of the class Alphaproteobacteria and family Bartonellaceae, and it is closely related to the genera *Brucella* and *Agrobacterium*; members of the family Rickettsiaceae are more distantly related. On the basis of genetic similarity,[1,2] unification of the genera *Bartonella* and *Rochalimaea* as a single genus and the removal of the family Bartonellaceae from the order Rickettsiales were proposed in 1993.[2] The similarity of *Bartonella* to the pathogen *Brucella* has been further substantiated through whole-genome sequencing showing that *Bartonella* contains a reduced version of the chromosomal elements of *Brucella melitensis*.[3]

The genus *Bartonella,* synonymous with *Bartonia,* was described in 1913 and referred to the human erythrocyte-adherent organisms originally described by Dr. A. L. Barton in 1909.[4,5] The type species is *Bartonella bacilliformis.* Limited to the Andes mountain regions of South America, *B. bacilliformis* infection received little attention outside its endemic zone until related bacteria, originally classified in the genus *Rochalimaea,* were found to be pathogens in individuals with acquired immunodeficiency syndrome (AIDS) in the early 1990s.

The former genus *Rochalimaea,* previously grouped with *Bartonella* in the order Rickettsiales, had long contained only two member species: *Rochalimaea vinsonii,* the "Canadian vole agent," and *Rochalimaea quintana*,[6] the agent of trench fever, a debilitating but self-limited human illness so named after it affected numerous military personnel in World War I.[7–9] Except for sporadic outbreaks, trench fever was rarely reported after World War I. However, *R. quintana* reemerged in the 1990s as a pathogen causing a debilitating and fatal disease in patients with AIDS and in urban homeless individuals.[10–13,14] Two new species pathogenic to humans, originally named *Rochalimaea henselae* and *Rochalimaea elizabethae,* also were identified.[15–18]

In 1995 a further merger of species in the genus *Grahamella* (also erythrocyte-associated bacteria, infecting rodents, birds, fish, and other animals) into the genus *Bartonella* was proposed.[19] Numerous *Bartonella* spp. and subspp. have been identified subsequently,[20–30] and some of these *Bartonella* spp. cause infrequent infections in humans.[28–36] Although *Bartonella* spp. have been characterized recently as "emerging" pathogens, DNA analysis of dental pulp from ancient human remains provides evidence that *B. henselae* and *B. quintana* have infected humans since antiquity.[37]

A list of validated members of the genus *Bartonella* is provided in Table 234.1. A continuously updated list of validated *Bartonella* spp. can be found at http://www.bacterio.net/bartonella.html.

TABLE 234.1 Currently Recognized *Bartonella* Species and Their Identified Potential as Human Pathogens

Documented as Common Human Pathogens
Bartonella bacilliformis
Bartonella henselae
Bartonella quintana
Uncommon or Suspected as Human Pathogens
Bartonella alsatica
Bartonella ancashensis
Bartonella clarridgeiae
Bartonella doshiae
Bartonella elizabethae
Bartonella grahamii
Bartonella koehlerae
Bartonella rochalimae
Bartonella schoenbuchensis
Bartonella tribocorum
Bartonella vinsonii subsp. *arupensis*
Not Identified as Human Pathogens
Bartonella acomydis
Bartonella apis
Bartonella birtlesii
Bartonella bovis
Bartonella callosciuri
Bartonella capreoli
Bartonella chomelii
Bartonella coopersplainsensis
Bartonella florencae
Bartonella fuyuanensis
Bartonella heixiaziensis
Bartonella jaculi
Bartonella japonica
Bartonella pachyuromydis
Bartonella peromysci
Bartonella queenslandensis
Bartonella rattaustraliani
Bartonella senegalensis
Bartonella silvatica
Bartonella talpae
Bartonella taylorii
Bartonella vinsonii subsp. *berkhoffii*
Bartonella vinsonii subsp. *vinsonii*

EPIDEMIOLOGY OF THE COMMON HUMAN-PATHOGENIC *BARTONELLA* SPECIES

Bartonella spp. are primarily infectious agents of nonhuman mammals. Humans are incidental hosts for most *Bartonella* spp., with stercorarian transmission via inoculation of *Bartonella*-infected feces excreted from arthropod vectors into nonintact human skin. The exceptions are *B. quintana* and probably *B. bacilliformis*, for which the definitive mammalian reservoir is believed to be humans.

Bartonella bacilliformis Epidemiology

Natural transmission of *B. bacilliformis* infection is mainly confined to the Andes mountain range in Peru, Ecuador, and Colombia at altitudes between 500 and 3200 m.[38] This is presumed due to the limited regional distribution of the sand fly vectors (genus *Lutzomyia* [formerly *Phlebotomus*]) of *B. bacilliformis*. Even in the modern antibiotic era, focal outbreaks continue,[39] and new geographic areas of *B. bacilliformis* infection have been reported recently.[39] In addition to outbreaks in new geographic areas, outbreaks in areas of endemicity continue to occur; for instance, 191 cases were reported from 2003 in a community with endemic disease in Peru.[40] Cases of *B. bacilliformis* infection, one possibly from vertical transmission and the other after blood transfusion, have been reported.[41,42] No nonhuman vertebrate reservoirs have been identified for *B. bacilliformis.*

Bartonella quintana Epidemiology

B. quintana is globally distributed, and the only identified vector of *B. quintana* is *Pediculus humanus,* the human body louse. Outbreaks of trench fever (also known as Wolhynia fever, Meuse fever, His-Werner disease, shin bone fever, shank fever, and quintan or 5-day fever) have been focal and widely separated. Clusters of trench fever cases usually are associated with conditions of poor sanitation and personal hygiene and are significantly associated with exposure to body lice.[43] A survey of published reports of zoonotic and vector-borne infections, as assessed by seroprevalence in the homeless and very poor in the United States and Europe from 1990–2014, identified *B. quintana* as the most commonly reported vector-borne infection in the homeless.[44] The prevalence of *B. quintana* in body lice can be high: *B. quintana* was detected in 33.3% of body lice pools removed from infested homeless persons in San Francisco.[45] *B. quintana* has been isolated from captive nonhuman primates in China and the United States and, more recently, from wild-caught Japanese macaques, suggesting that nonhuman primates may serve as a natural reservoir for *B. quintana*.[46–48]

Bartonella henselae Epidemiology

B. henselae is globally endemic and has been detected in 800-year-old French cats[49]; serologic studies indicate that infection of domestic cats occurs worldwide, with the prevalence of antibodies in cats being higher in warm, humid climates, where fleas are more prevalent.[50] Prevalence of bacteremia in cats varies[51,52–54] but tends to be higher among feral animals in any particular locale. The *B. henselae* colony-forming units (CFU)/mL of blood in infected cats can reach extremely high levels of

≥10^6; yet these cats are usually asymptomatic. Free-ranging and captive wild felids in California also have a substantial prevalence of antibodies reactive with *B. henselae*,[55] and *B. henselae* has been isolated from the blood of free-ranging mountain lions *(Puma concolor)* and bobcats *(Lynx rufus)* in California.[56]

B. henselae bacteremia in pet cats is significantly associated with development of bacillary angiomatosis (BA)[43,51] or typical cat-scratch disease (CSD) in their human contacts[57]; transmission of *B. henselae* to humans also is linked to domestic cats by serologic and epidemiologic studies.[58,59] A recent review of cases of CSD in the United States from 2005–13 suggests an incidence of 4.5 outpatient diagnoses and 0.19 inpatient admissions per 100,000 population, with a higher incidence among children (age 5–9 years) and women, and in southern regions (Fig. 234.1).[60] For epidemiologic studies, diagnosis of human *B. henselae* infection has been confirmed by detection of anti-*Bartonella* antibodies, culture recovery of *B. henselae* from lymph nodes with the pathologic findings of CSD lymphadenitis,[53,61] and by detection of *B. henselae* DNA by polymerase chain reaction (PCR) in tissue from cases of CSD lymphadenitis,[62,63] CSD skin test antigen,[64] and BA lesions.[43,51] For *B. henselae*, one potential case of human-to-human transmission has been reported: donor-derived *B. henselae* infection in the recipient of an orthotopic liver transplant.[65] The major arthropod vector of *B. henselae* is the cat flea, *Ctenocephalides felis,* as demonstrated by epidemiologic associations,[43,53,58,59] identification of *B. henselae* by culture and DNA amplification from cat fleas,[51,52] and transmission of *B. henselae* to cats by infected fleas under controlled experimental conditions.[66] Cat fleas appear to serve as efficient vectors for cat-to-cat transmission[66]; their contribution to human infection likely occurs after inoculation of *B. henselae*–infected flea feces into the human during a cat scratch. Additional *Bartonella* spp. also have been identified in cat fleas.[67] Other types of fleas, as well as ixodid and *Dermacentor* ticks, have been found to harbor various *Bartonella* spp., but transmission to humans via ticks has not yet been demonstrated.[68–73]

Epidemiology of Other *Bartonella* Species Associated With Human Infection

In addition to *B. henselae*, *Bartonella clarridgeiae*[20–22] and *Bartonella koehlerae*[74] also cause asymptomatic, persistent bloodstream infection in cats. Although *B. koehlerae* is infrequently isolated from domestic cats, *B. clarridgeiae* is quite prevalent, especially in European domestic cats.[21,75] Of 50 *Bartonella* isolates recovered from 94 stray cats in France, Heller and colleagues[21] found that 34% were *B. henselae* genotype I, 36% were *B. henselae* genotype II, and 30% were *B. clarridgeiae.* Of interest, despite the high prevalence of *B. clarridgeiae* in cats, this species has neither been isolated from, nor DNA amplified from, lymph nodes of an immunocompromised or immunocompetent human with CSD. *B. koehlerae* is occasionally transmitted to humans and represents an uncommon cause of endocarditis.[32] Because *Bartonella elizabethae* has been isolated only once from a human,[18] little is known of its epidemiology, except that it has been cultured from the blood of rodents,[76] and its DNA has been amplified from the blood of rodents,[76] fleas from rodents,[76] and dogs.[77] Recently, a new species, *Bartonella ancashensis*, was isolated from the blood of two patients with verruga peruana in Ancash, Peru. The patients, age 3 and 12 years, had clinical features typical of verruga peruana (multiple chronic skin nodules). Sequencing of this blood isolate revealed a *Bartonella* spp. that was phylogenetically related to, but distinct from, *B. bacilliformis.*[78] Another recently described human pathogen, *Candidatus* Bartonella tamiae, was isolated from three patients during a study of people presenting with febrile illness in Thailand; it has been proposed as a new *Bartonella* spp. but is not yet formally recognized.[35] To date the definitive mammalian reservoir for *Candidatus* Bartonella tamiae has not been identified, although rodents and bats have been implicated.[36,79] *Bartonella rochalimae* was isolated from the blood of a patient with a febrile illness after travel to Peru; subsequently, this organism was isolated from gray foxes *(Urocyon cinereoargenteus)* in northern California[34] and detected by PCR in fleas *(Pulex irritans)* from dogs in Peru,[69] implicating a canid reservoir.



FIG. 234.1 Geographic distribution of cat-scratch disease by US census division, United States, 2005–2013. Rates are reported as average incidence per 100,000 population per year. During the study period there were <10 cases in Alaska and <10 cases in Hawaii. *(Reproduced with permission from Nelson CA, Saha S, Mead PS. Cat-scratch disease in the United States, 2005–2013.* Emerg Infect Dis. *2016;22:1741–1746.)*

Bartonella vinsonii is generally not considered a human pathogen, although *B. vinsonii* subsp. *arupensis* and *B. vinsonii* subsp. *berkhoffii* have been associated with isolated cases of endocarditis in humans by serology and DNA amplification; these *Bartonella* subspp. have not yet been directly isolated from a human with endocarditis.[28–30] *B. vinsonii* subsp. *arupensis* was isolated from the blood of an otherwise healthy rancher from the western United States who had fever without endocarditis,[27] and this strain was implicated as the cause of endocarditis in another patient.[28] *B. vinsonii* subsp. *berkhoffii* also can cause bacteremia and endocarditis in dogs.[23,24] *Bartonella alsatica* has been associated with endocarditis in humans,[31,80] and wild rabbits (a known reservoir of *B. alsatica*) were a possible source of infection in these two patients.

CLINICAL MANIFESTATIONS OF THE COMMON HUMAN PATHOGENIC *BARTONELLA* SPECIES: *B. BACILLIFORMIS, B. HENSELAE,* AND *B. QUINTANA* (Table 234.2)

Bartonella Bacilliformis Clinical Manifestations: Oroya Fever and Verruga Peruana

The long-suspected link between Oroya fever and verruga peruana was confirmed tragically in 1885 by Daniel Carrión, a medical student who injected himself with blood from a verruga peruana lesion and subsequently died of Oroya fever.[81] The eponym "Carrión disease" has since

TABLE 234.2 Characteristic Features of the Most Common Human Infections With *Bartonella* Species

BARTONELLA SPECIES	RISK FACTORS	VECTOR	RESERVOIR	DISTRIBUTION	CLINICAL FEATURES (VARIES BY HOST IMMUNE STATUS)	PATHOLOGIC FEATURES
Bartonella bacilliformis	Living in endemic area; poor housing/ exposure to vector	Sand fly	Humans	Endemic in Andes mountains (Peru)	**Biphasic illness:** Acute phase (Oroya fever): fever, malaise, hemolytic anemia; high mortality if untreated **Late phase (verruga peruana):** eruptions of nodular skin lesions	**Acute phase:** Intraerythrocytic organisms on blood smear **Late phase:** Vascular proliferative pattern
Bartonella henselae	Exposure to cats (especially kittens); CSD more common in pediatric population; immunocompromised patients (HIV, transplant recipients) at risk for disseminated disease	Cat flea (transmitted to humans via cat scratch)	Cats (especially kittens)	Worldwide; more common in warm, humid climates	**Immunocompetent:** CSD: self-limited, regional lymphadenopathy Atypical CSD, including Parinaud oculoglandular syndrome (granulomatous conjunctivitis with ipsilateral regional lymphadenopathy) Ophthalmic manifestations including neuroretinitis FUO in children Culture-negative endocarditis (in patients with pre-existing valvular abnormality) Chronic endocarditis: vasculitis/ glomerulonephritis **Immunocompromised:** Bacteremia FUO Systemic/erythematous/ violaceous skin lesions (BA) Hepatic/splenic lesions (BP)	**Immunocompetent:** Necrotizing granulomatous inflammation **Immunocompromised:** Usually vascular proliferative (BA/BP) process in patients with severe immune compromise (e.g., advanced AIDS, early period post–solid-organ transplant), although histologic appearance may be more suppurative in those with moderate immunosuppression (e.g., several years post–solid-organ transplant)
Bartonella quintana	Homelessness, body lice, conditions of poor sanitation	Human body louse	Humans	Worldwide	**Immunocompetent:** Trench fever: self-limited febrile illness Culture-negative endocarditis (with or without preexisting valvular abnormality) **Immunocompromised:** Bacteremia FUO Skin, subcutaneous, osseous vascular lesions (BA)	No specific histopathology in setting of trench fever/endocarditis/ bacteremia **Immunocompromised:** vascular proliferative process (BA)

AIDS, Acquired immunodeficiency syndrome; *BA,* bacillary angiomatosis; *BP,* bacillary peliosis; *CSD,* cat-scratch disease; *FUO,* fever of unknown origin; *HIV,* human immunodeficiency virus.

denoted the full spectrum of *B. bacilliformis* infection. Oroya fever, an acute hematologic disease resulting from primary *B. bacilliformis* bacteremia and erythrocyte invasion, develops 3 to 12 weeks after cutaneous inoculation with infected sand fly feces.[82] In its mildest insidiously developing form, a febrile illness can last less than a week and go unrecognized, giving rise to subsequent cutaneous manifestations that are the first-recognized clinical findings.[83–85] When illness onset is abrupt, high fever, chills, diaphoresis, anorexia, prostration, headache, and mental status changes are associated with rapidly developing, profound anemia resulting from bacterial invasion and destruction of erythrocytes.[4,85,86] Intense myalgias and arthralgias, abdominal pain and emesis, jaundice, lymphadenopathy, thrombocytopenia, and complications such as seizures, delirium, meningoencephalitis, obtundation, dyspnea, hepatic/gastrointestinal dysfunction, and angina pectoris can occur during this stage,[85,87,88] believed to be a consequence of the anemia and microvascular thrombosis, with subsequent end-organ ischemia.

Without antimicrobial therapy, the fatality rate is high for the severe, abrupt form of bacteremic illness, Oroya fever.[87] With appropriate treatment in the modern era, mortality is reported to be less than 10%.[85] For survivors, convalescence is associated with a decline of fever and disappearance of bacteria on blood smears, but also a temporarily increased susceptibility to subsequent (opportunistic) infections, such as salmonellosis[85,88,89] or toxoplasmosis.[85,90,91] Asymptomatic persistent bacteremia with *B. bacilliformis* infection can occur in up to 15% of survivors of acute infection.[92] It has been suggested that initial infection may be asymptomatic, or only mildly symptomatic, more often than was previously thought.[84,93] Both the Oroya fever survivors and asymptomatically bacteremic individuals are suspected to serve as the reservoir for *B. bacilliformis*.

Verruga peruana lesions, the eruptive phase of *B. bacilliformis* infection, usually become evident within weeks to months after resolution of untreated acute infection. This late-stage manifestation is characterized by crops of skin lesions marked by an evolution of stages[85]: miliary, then nodular (Fig. 234.2), and subsequently what are called mulaire lesions (Fig. 234.3). Of the eruptive manifestations, mulaire lesions are the most superficial and obviously vascular: often bulbous, engorged with blood, and prone to ulceration and bleeding. Mucosal and internal lesions also can occur. The nodules may develop at one site while receding at another. Healing at a particular skin site is often punctuated by recurrences and usually takes place over several weeks to 3 or 4 months. Subsequently, fibrosis of mulaire lesions can occur. Histology of active lesions demonstrates vascular proliferation with occasional bacteria evident in interstitial spaces. Bacterial invasion of/replication within endothelial cells (long believed to be the cause of cytoplasmic inclusions first described by Rocha Lima) is actually rare.[94]



FIG. 234.2 Multiple nodular subcutaneous lesions of verruga peruana in an inhabitant of the Peruvian Andes. Nodular verruga peruana eruptions are localized around the flexures of the elbows and knees, as well as on the thighs and legs. *(Courtesy Dr. J.M. Crutcher, Oklahoma State Department of Health, Oklahoma City, OK.)*



FIG. 234.3 A single, large, mulaire lesion of verruga peruana on the leg of an inhabitant of the Peruvian Andes. Mulaire lesions frequently develop superficial ulceration, and copious bleeding can occur as a result of their highly vascular nature. Ecchymosis of the skin surrounding the lesion is also evident. *(Courtesy Dr. J.M. Crutcher, Oklahoma State Department of Health, Oklahoma City, OK.)*

Bartonella henselae and *Bartonella quintana*: Clinical Manifestations Depend on the Infecting *Bartonella* Species and Immune Status of Host

The clinical manifestations of infection with these two *Bartonella* spp. depend on a combination of species-specific microbiologic and host-specific immunologic factors (Fig. 234.4). Species-specific epidemiologic risk factors, such as exposure to cats and cat fleas for *B. henselae*, and homelessness and exposure to lice for *B. quintana*, are applicable to both immunocompetent and immunocompromised hosts. In immunocompetent hosts, infection with *B. henselae* typically manifests as CSD: regional, granulomatous lymphadenitis (Fig. 234.5A). Both *B. henselae* and *B. quintana* can present as fever of unknown origin (FUO). Endocarditis, usually due to *B. quintana,* most commonly occurs in immunocompetent hosts, often in the absence of a history of valvular abnormalities.[95–98,99] *B. henselae* endocarditis is a less frequent cause of endocarditis and most often occurs in patients with underlying valvular pathology or congenital heart disease (CHD).[100,101] Immunocompromised solid-organ transplant (SOT) recipients and persons living with human immunodeficiency virus (HIV; CD_4^+ cell count <100/mm^3) who are infected with either *B. henselae* or *B. quintana* can develop BA (see Fig. 234.5B–D) but also can present with other clinical manifestations, for instance, bacillary peliosis (BP) hepatis (*B. henselae*), bacteremia (*B. quintana* or *B. henselae*), endocarditis (*B. quintana* or *B. henselae*), and/or FUO (*B. quintana* or *B. henselae*).[43,102,103] Additional host/immunologic risk factors for more severe *Bartonella* infection, such as the use of biologic disease-modifying antirheumatic drugs, have been reported, although a clear association between the use of these agents and a particular clinical manifestation of *Bartonella* infection is not clearly defined.[104]

Bartonella henselae and *Bartonella quintana:* Bacteremic Illness

Acute mortality resulting from bacteremia with non-*bacilliformis Bartonella* spp., even when persistent, is uncommon in immunocompetent hosts in the absence of endocarditis. In recent years, *B. quintana* bacteremic infection in immunocompetent individuals ("trench fever") has been identified sporadically and in small clusters, predominantly in homeless people in North America and Europe.[14,105,106] Trench fever is characterized by a self-limited fever, often with a 5-day periodicity (quintan pattern).[7,8] The incubation period may span 3 to 38 days before the usually sudden onset of chills and fevers. In the most limited form of trench fever, a single bout of fever lasts 4 or 5 days. In the more typical periodic form, there are three to five, and sometimes up to eight, febrile paroxysms, each approximately

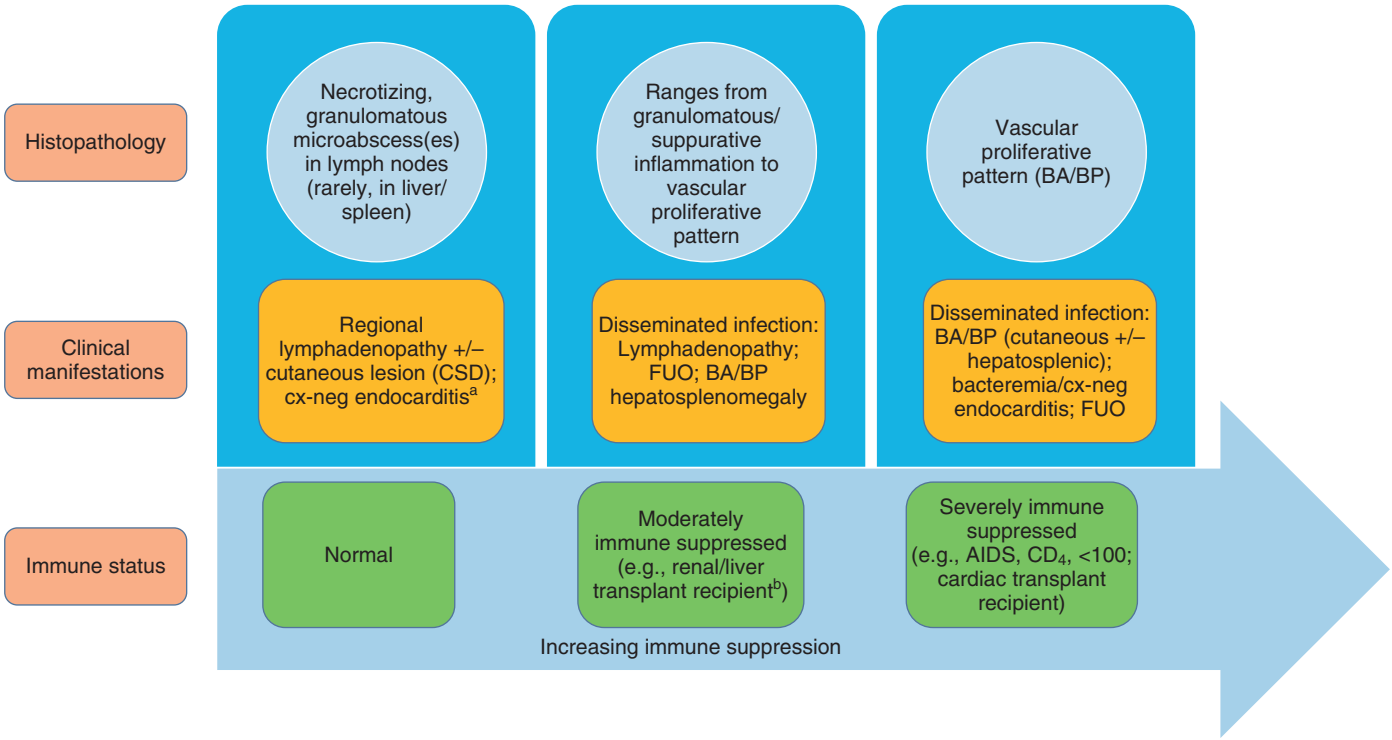

FIG. 234.4 Host immune status is associated with different clinical and pathologic manifestations of *Bartonella henselae* or *Bartonella quintana* infection. [a]Endocarditis can occur in healthy hosts with underlying valvular pathology (e.g., *B. henselae*) or on native, normal valves (e.g., *B. quintana*). [b]Among transplant patients, disseminated infection and vascular proliferative pattern on pathology occur earlier in the posttransplant period, likely due to higher levels of immunosuppression. *AIDS,* Acquired immunodeficiency syndrome; *BA/BP,* bacillary angiomatosis/bacillary peliosis; *CSD,* cat-scratch disease; *cx-neg,* culture-negative; *FUO,* fever of unknown origin.

5 days apart. Patients are often asymptomatic between febrile paroxysms. The continuous form is manifested by 2 or 3 weeks, and up to 6 weeks, of uninterrupted fever. Afebrile infection with *Bartonella* is very common; one study found 8 of 10 homeless individuals with active *B. quintana* bacteremia were afebrile.[107] Other nonspecific symptoms and signs, such as headache, retro-orbital pain, conjunctival injection, myalgias, arthralgias, bone pain, hepatosplenomegaly, rash, leukocytosis, and albuminuria can accompany *B. quintana* bacteremia. *B. quintana* bacteremia has the potential to evolve into long-term persistence if not treated appropriately.[106,108] *B. henselae* bacteremia in immunocompetent hosts is rare, and localizing symptoms or physical findings are unusual.[108] However, *B. henselae* bacteremia can present with abrupt onset of isolated fever or, rarely, accompany CSD lymphadenitis.[109] Aseptic meningitis concurrent with *B. henselae* bacteremia also has been documented.[108,109] *B. henselae* bacteremia is much less likely to persist or become relapsing than *B. quintana* in immunocompetent people.

HIV-infected patients with advanced immunosuppression (CD_4^+ cell count <100/mm^3) are more likely to develop severe and prolonged bacteremia with *B. quintana* or *B. henselae.*[102,103] In these patients *Bartonella* bacteremia has been associated with cutaneous BA (*B. henselae* or *B. quintana*), hepatic and splenic BP *(B. henselae),* granulomatous hepatitis *(B. henselae),* infiltration of the bone marrow *(B. henselae),*[110] FUO (*B. quintana* and *B. henselae*), and occasionally endocarditis (*B. quintana* much more frequently than *B. henselae*).[11,102,103] In one area of high HIV prevalence, evidence of *B. henselae* or *B. quintana* infection was found in 18% of people evaluated for acute or persistent unexplained fever, 97% of whom were HIV infected and had a median CD_4^+ cell count of 35 per mm^3 and no antiretroviral exposure.[103] In a study of 49 patients with documented BA/BP (92% had HIV infection), 50% of patients sampled had bacteremia with either *B. quintana* or *B. henselae.*[43] The clinical presentation of *Bartonella* bacteremia often is characterized by insidious development of malaise, body aches, fatigue, weight loss, progressively higher and longer recurring fevers, and sometimes headache.[43,103] Hepatosplenomegaly and/or splenomegaly may occur, but localizing symptoms or physical findings can be lacking. Secondary hemophagocytic lymphohistiocytosis has been associated with *B. henselae* in both HIV-infected and transplant hosts, heralded by hepatosplenomegaly and cytopenias.[111,112]

Bartonella henselae and *Bartonella quintana:* Endocarditis

Bartonella spp. are recognized as one of the most important causes of blood culture-negative endocarditis.[11,13,95–101,113] Two large European studies of patients with blood culture-negative endocarditis found *Bartonella* spp. to be the second most frequently identifiable cause, after *Coxiella burnetii.*[99,113] In the United States, where *Coxiella* endocarditis is less common than in Europe, *Bartonella* spp. are quite likely the most common cause of culture-negative endocarditis. Patients with *B. quintana* endocarditis are usually homeless, often substance abusers, and have exposure to body lice, whereas people with *B. henselae* endocarditis more commonly have cat exposure and preexisting valvular heart disease.[99] *B. henselae* endocarditis is infrequent in humans; despite the frequency of CSD in the pediatric population, *B. henselae* endocarditis has been recognized only rarely.[97] The reports of pediatric endocarditis usually involve patients with CHD.[101] The more common cause of human endocarditis, *B. quintana,* is extremely rare in pediatric practice because of the less frequent exposure of children to risk factors associated with *B. quintana:* homelessness and body lice.

Patients with *Bartonella* endocarditis typically present with a prolonged (weeks to months) history of nonspecific symptoms, such as fever, fatigue, and weight loss. In a retrospective study of 101 patients with *Bartonella* endocarditis,[98] ≈83% were febrile and 43% had evidence of embolic phenomena at the time of presentation. Fifty-eight patients (57%) had previously known valvular heart disease, 58 (57%) had involvement of the aortic valve, and 18 (18%) had involvement of multiple valves. Irrespective of antimicrobial therapy, 76 patients had severe valvular damage and required valvular surgery. Twelve patients ultimately died; two were cured only after treatment for a relapse, and the remaining 87% were cured with the initial therapy.[98]



FIG. 234.5 The degree of host immunocompromise determines the clinical cutaneous manifestations of *Bartonella henselae* infection. (A) Primary inoculation lesion of *B. henselae* causing cat-scratch disease (CSD) in an immunocompetent host; this erythematous lesion is typically transient, and usually the lesion and lymphadenopathy resolve without antimicrobial therapy. (B) CSD in a moderately immunocompromised host (5 years after cadaveric kidney transplant), presenting as a chronic, single, violaceous nodule on the dorsum of the hand with a flat, necrotic eschar on top and poorly formed granulomas on histopathology (see Fig. 234.7A1–A3). (C) Bacillary angiomatosis (BA) in a more immunocompromised host (6 years after combined pancreas-kidney transplant) appearing as a 4-mm vascular nodule on the lip *(arrow);* shave biopsy demonstrated vascular proliferation consistent with BA. Patient also had bacilllary peliosis hepatis (see Fig. 234.8F) with severe liver failure. (D) BA in a patient with advanced AIDS (CD_4^+ cell count <100/mm^3), who presented with a 2-month-old friable, vascular BA finger lesion and a 2-week-old vascular scrotal lesion. *(Image A courtesy Dr. Anna Sander, Merian Schule, Freiburg, Germany. Image B reprinted with permission from Koehler JE, Duncan LM. Case records of the Massachusetts General Hospital. A 56-year-old man with fever and axillary lymphadenopathy.* N Engl J Med. *2005;353:1387–1394. Image C reproduced with permission from Freed MM, Ramirez-Valle F, Chandran S. Fever, hepatosplenomegaly and a skin nodule in a kidney-pancreas transplant recipient.* Am J Transplant. *2012;12:2556–2558. Image D reproduced with permission from Koehler JE, LeBoit PE, Egbert BM, Berger TG. Cutaneous vascular lesions and disseminated cat-scratch disease in patients with the acquired immunodeficiency syndrome [AIDS] and AIDS-related complex.* Ann Intern Med. *1988;109:449–455.)*

It is important to be aware of the rapidly increasing number of reports of vasculitis associated with *Bartonella* endocarditis. Patients with *B. henselae* and *B. quintana* endocarditis can present with clinical features of a systemic, small vessel vasculitis associated with a positive cytoplasmic antineutrophil cytoplasmic antibody (c-ANCA), anti–proteinase 3 antibodies (anti-PR3), and/or kidney injury with glomerulonephritis.[101,114–117] In these patients, detection of *Bartonella* infection is critical to avoid exposure to immunosuppressive therapy for vasculitis and to institute timely antimicrobial therapy for *Bartonella* endocarditis. In c-ANCA–positive patients the c-ANCA titers may be useful for clinical monitoring of endocarditis treatment,[115] and antimicrobial treatment of the underlying endocarditis usually results in resolution of the vasculitis, without steroids.[101,114,115]

As noted earlier, in addition to antimicrobial therapy (see "Treatment") *Bartonella* endocarditis requires surgical intervention in the majority of cases. Delays in surgical intervention have been associated with morbid complications, such as stroke[30] and acute myocardial infarction.[118]

The diagnosis of culture-negative endocarditis due to *Bartonella* has been established with serology, amplification of *Bartonella* DNA from blood or valve tissue, immunohistochemistry, or a combination of these modalities. A series of 106 cases from a single center in France revealed the sensitivity of two of their diagnostic tests: immunoblotting with patient serum (100%) and PCR of DNA extracted from valvular tissue (92%). Serology testing for *Bartonella* immunoglobulin G (IgG) antibodies by immunofluorescence was positive (defined as ≥1:100) in 91% of *Bartonella* endocarditis patients; an IgG titer ≥1:800 had a positive predictive value of 94% for endocarditis.[100] In a review of *Bartonella* endocarditis among 13 patients with CHD, serology was positive in 100% of cases, and PCR on DNA extracted from valvular tissue detected *Bartonella* in all 11 patients who underwent surgery.[101] Evaluation of homeless individuals or SOT recipients with fever and glomerulonephritis, cutaneous small vessel vasculitis, and/or positive c-ANCA or anti-PR3 should prompt a clinical workup for *Bartonella* endocarditis.

Other *Bartonella* Species: Endocarditis and Bacteremia

B. elizabethae has been isolated from a single patient with bacteremia and endocarditis.[18] *B. vinsonii* subsp. *arupensis* and *B. vinsonii* subsp. *berkhoffii* have been associated with a few isolated cases of endocarditis in humans.[28–30] *B. vinsonii* subsp. *arupensis* also was isolated from a US rancher who had bacteremia and fever.[27] In addition, *B. koehlerae* and *B. alsatica* have been associated with endocarditis in humans.[31,32,80] *B. rochalimae* caused a febrile bacteremic illness with splenomegaly in an immunocompetent traveler returning from Peru, who had sustained numerous arthropod bites.[33] A novel species, *Candidatus* Bartonella tamiae, was isolated from the blood of three patients being evaluated

for fever in Thailand.[35] It is evident that many *Bartonella* spp. associated with wild and peridomestic animals can infect humans, albeit rarely, especially patients with abnormal heart valves or CHD.

Bartonella henselae and *Bartonella quintana* Infections in Immunocompromised Patients: Bacillary Angiomatosis/Bacillary Peliosis

BA (also referred to as bacillary epithelioid angiomatosis) is a manifestation of *B. quintana* or *B. henselae* infection involving vascular proliferation in skin and regional lymph nodes, which was initially described in patients with advanced HIV-infection (CD_4^+ cell count <100/mm^3).[102,103,119–121] Subsequently, BA has been identified in a variety of internal organs, including liver, spleen, bone, brain, lung, bowel, and uterine cervix.[121–127] BA has been increasingly identified in other hosts with compromised immunity (such as SOT and chemotherapy recipients)[128–135] and, very rarely, immunocompetent hosts.[136,137] *B. henselae* and *B. quintana* have been found to be a cause of BA both by direct culture[10,17,51,138] and by PCR amplification of *Bartonella*-specific DNA sequences from tissue.[10,43,128,139] Either species can cause cutaneous BA lesions, but subcutaneous and osseous lesions are more often associated with *B. quintana* and hepatic and splenic BP only with *B. henselae.*[43] In addition, patients with *B. henselae* genotype I infection may be more likely to have hepatic and splenic peliosis, whereas patients with *B. henselae* genotype II are more likely to develop BA lesions involving the skin or lymph node, or both.[140]

Cutaneous BA lesions often arise in crops (Fig. 234.6D and E), but both the temporal pattern of development and the gross morphologic characteristics can vary. Of interest, the clinical and histopathological appearance of verruga peruana lesions caused by *B. bacilliformis* in immunocompetent hosts closely resembles the cutaneous BA lesions caused by *B. henselae* and *B. quintana* in immunocompromised hosts. BA lesions also can resemble pyogenic granuloma, but the major clinical differential diagnosis in HIV-infected patients is Kaposi sarcoma.[141] In gross appearance, BA skin lesions can be subcutaneous (see Fig. 234.6F); form dermal nodules (see Fig. 234.6C); or form single or multiple papules that are flesh colored, red, or purple (see Fig. 234.6E).[142] Skin lesions also can display ulceration (see Fig. 234.6D), serous or bloody drainage, and crusting (see Fig. 234.6B). Lesions can range in diameter from millimeters to centimeters (see Fig. 234.6C), number from a few to hundreds (see Fig. 234.6E), be fixed or freely mobile, be associated with enlargement of regional lymph nodes, involve mucosal surfaces or deeper soft tissues (see Fig. 234.6A), occur in a variety of distributions, and bleed copiously when incised. Osteomyelitis also can be associated with BA lesions (see Fig. 234.6F). Visceral BA lesions can be quite dramatic as well, in both their number and heterogeneity of gross appearance.

Although BA can closely resemble other neovascular tumors clinically, BA can be distinguished from other vascular proliferative tumors histologically.[142,143] BA consists of lobular proliferation of small blood vessels containing plump, cuboidal endothelial cells, interspersed with mixed inflammatory cell infiltrates having a neutrophil predominance (Fig. 234.7B1). Endothelial cell atypia, mitoses, and necrosis may be present. Fibrillar- or granular-appearing amphophilic material is often present in interstitial areas when stained by hematoxylin and eosin (H&E) stain. Warthin-Starry staining or electron microscopy shows this material to be clusters of bacilli (see Fig. 234.7B2 and B3).

When cutaneous BA lesions are absent, diagnosis is often delayed because of the nonspecific features associated with hepatic or splenic BP (fever, lymphadenopathy, hepatomegaly, splenomegaly, anemia, pancytopenia, and serum alkaline phosphatase elevation).[102] BP, originally described as a process involving the liver, sometimes occurs in the spleen and occasionally lymph nodes in HIV-infected people[144,145] and has since been identified in other immunocompromised people.[129,133] Molecular epidemiologic investigation has revealed that *B. henselae* (and not *B. quintana*) appears to be the only *Bartonella* spp. eliciting this host response of BP.[43] Involved organs contain numerous blood-filled cystic spaces (peliosis) that can range from microscopic to several millimeters in size. Hepatic and splenic BP appear as multiple hypodense or low-attenuation lesions on ultrasonographic or computed tomographic (CT) abdominal scanning (Fig. 234.8C).[146] H&E-stained tissue reveals that the peliotic spaces (see Fig. 234.8F) are partially lined with endothelial cells and often separated from surrounding parenchymal cells by fibromyxoid stroma containing a mixture of inflammatory cells, dilated capillaries, and clumps of granular material. Such clumps are composed of aggregates of bacilli that stain with Warthin-Starry stain.[144] Presenting clinical features of BP hepatis are often nonspecific, although there is a dramatic report of an HIV-infected patient presenting with fever and abdominal pain, who developed massive hemoperitoneum from hepatic BP.[147]

B. henselae–related inflammatory reactions in immunocompromised hosts, in the absence of associated angiomatosis or peliosis, have been reported in liver (see Fig. 234.8B), spleen, lymph nodes, heart, lung, and bone marrow.[112,129,148–152] They are characterized by nodular collections of lymphocytes and nonepithelioid histiocytes that can become centrally necrotic, containing aggregates of neutrophils and karyorrhectic debris suggestive of microscopic abscess formation (see Fig. 234.8E).[148,149] These may represent a clinical-pathologic link with CSD and are more likely to occur in HIV-infected patients who have less severe immunosuppression or SOT recipients with a later disease presentation (mean, 5.2 years) posttransplantation.[129]

Bartonella henselae: Cat-Scratch Disease

Cat-Scratch Disease Background

The various clinical manifestations of CSD have been recognized for more than a century, but "la maladie des griffes de chat" was not defined as a syndrome until 1950.[153] CSD remained an infection in search of an agent for more than 40 years after that. Thus most cases have been identified by clinical-pathologic criteria, supplemented by reactions to unstandardized skin test antigens before identification of *B. henselae*. Historically, the diagnosis of a case of typical CSD required fulfillment of three of the four following criteria (all four were necessary in an atypical case): (1) history of an animal (usually cat or dog) contact, with the presence of a scratch or primary skin or eye lesion; (2) aspiration of "sterile (culture-negative)" pus from the lymph node, or culture and other laboratory testing that excluded other etiologic possibilities; (3) a positive CSD skin test; and (4) a lymph node biopsy revealing pathology consistent with CSD. Skin test antigen, originally described by Hanger and Rose, was prepared by heating saline-diluted "sterile (culture-negative)" pus aspirated from CSD lymphadenitis at 56°C for 72 hours. It was never standardized or produced commercially. It is of historic interest because of its confirmatory role in diagnosis of CSD before the identification of *B. henselae* as the CSD agent. However, its potential for transmission of hepatitis viruses, HIV, and prions is a major contemporary concern, even if the source patients are well screened. Its use in the era of other methods of diagnosis is no longer warranted.

Among the *Bartonella* spp., CSD has been associated nearly exclusively with *B. henselae.* Evidence indicating the central role of *B. henselae* includes the serologic response of people with CSD to *B. henselae* antigens[59]; the identification of *B. henselae* in CSD lymphadenitis by culture,[61] PCR-based DNA amplification,[62,63,154–157] and immunocytochemistry[158]; detection of *B. henselae* in CSD skin test antigens by PCR[64]; and the recovery of *B. henselae* isolates from the blood of healthy cats (which can be persistently bacteremic)[51,53,159] and from cat fleas.[51]

In the United States an estimated 12,500 to 25,000 CSD cases occur annually.[60,160] Of interest, veterinary care personnel do not have evidence of notably higher levels of infection than the general population.[161] It is reasonable to ascribe the vast majority of CSD cases to *B. henselae* on the basis of the numerous lines of evidence developed in recent years. Yet it remains possible that other agents can, very rarely, cause "typical" CSD cases, such as was reported with *Afipia felis* and *Bartonella grahamii*.[162–164] CSD is the most commonly recognized manifestation of human infection with *Bartonella*.

Typical Cat-Scratch Disease

"Typical CSD" represents 88% to 89% of CSD cases overall. A primary cutaneous papule or pustule develops approximately 3 to 10 days after an animal contact (most commonly a kitten or feral cat with flea infestation) at a site of inoculation (usually from a scratch) (Fig. 234.9A),[165–167] and it may last for 1 to 3 weeks. Regional lymphadenopathy ipsilateral to the inoculation site (usually head, neck, or upper extremity) that develops in 1 to 7 weeks (see Fig. 234.9B and C), is the most prominent and common



FIG. 234.6 Diverse manifestations of bacillary angiomatosis (BA) caused by *Bartonella henselae* or *Bartonella quintana* in severely immunocompromised AIDS patients. In severely immunocompromised patients with AIDS, infection with *B. henselae* or *B. quintana* can manifest as BA, a vascular proliferative process. Cutaneous lesions are most common, but deeper soft tissue, musculoskeletal, or visceral masses can occur. Diagnosis is often delayed due to the diversity of clinical appearances and broad differential diagnoses in this patient population. (A) Magnetic resonance imaging shows a 7 × 4 × 5-cm lobulated, highly vascular, soft-tissue mass *(arrow)* anterior to the femoral vessels, 8 months before diagnosis of BA. (B) Cutaneous BA on the lower leg (pretibial region) of an AIDS patient (CD_4^+ cell count 20/mm^3). The lesion is protuberant, without erythema, firm and dry, with a collarette of hyperkeratotic skin covered with serous crust. (C) 3.5 × 3.5-cm pedunculated, vascular cutaneous BA lesion on the thigh of a woman with AIDS, systemic symptoms, and inguinal adenopathy. (D) Cutaneous BA, presenting with numerous, painful angiomatous nodules within an indurated, erythematous plaque that developed over the biopsy site of the right thigh mass in the patient shown in (A). *B. quintana* was cultured from the cutaneous lesions. (E) Cutaneous BA in a man with AIDS and multiple comorbid opportunistic infections. Reddish-purple dome-shaped papules, 0.2 to 1 cm in diameter, were noted on the face, trunk, and upper extremities and were initially thought to represent Kaposi sarcoma, but histopathology demonstrated BA. (F) A man with AIDS presented with a 4-month history of a painful, 5 × 3-cm firm, erythematous nonpulsatile right wrist mass *(left image);* a radiograph showed a bony defect in the distal radius *(right image, arrows).* Histopathology confirmed BA. *(Images A, B, and D reprinted with permission from Koehler JE, Quinn FD, Berger TG, LeBoit PE, Tappero JW. Isolation of* Rochalimaea *species from cutaneous and osseous lesions of bacillary angiomatosis.* N Engl J Med. *1992;327:1625–1631. Image C reproduced with permission from Koehler JE, Glaser CA, Tappero JW.* Rochalimaea henselae *infection. A new zoonosis with the domestic cat as reservoir.* JAMA. *1994;271:531–535. Image E reproduced with permission from Cockerell CJ, Whitlow MA, Webster GF, Friedman-Kien AE. Epithelioid angiomatosis: a distinct vascular disorder in patients with the acquired immunodeficiency syndrome or AIDS-related complex.* Lancet. *1987;2:654–656. Image F reproduced with permission from Koehler JE, LeBoit PE, Egbert BM, Berger TG. Cutaneous vascular lesions and disseminated cat-scratch disease in patients with the acquired immunodeficiency syndrome [AIDS] and AIDS-related complex.* Ann Intern Med. *1988;109:449–455.)*

manifestation (>90% of typical cases) and the one that usually prompts medical evaluation. Even at the time of such presentation, an inoculation site (scratch, bite, or primary papule or pustule) can be detected in more than two-thirds of patients when actively sought. One-third to 60% of patients may have low-grade fever lasting several days. One-fourth of patients report malaise or fatigue, and approximately 10% report headache or sore throat. Transient rash occurs in approximately 5% of patients. Erythema nodosum (erythematous nodules on the lower legs) has been described as a another rare dermatologic manifestation of CSD in immunocompetent patients.[168] Transient, mild leukocytosis, with increased neutrophils and sometimes eosinophils, as well as elevated erythrocyte sedimentation rate, can occur.

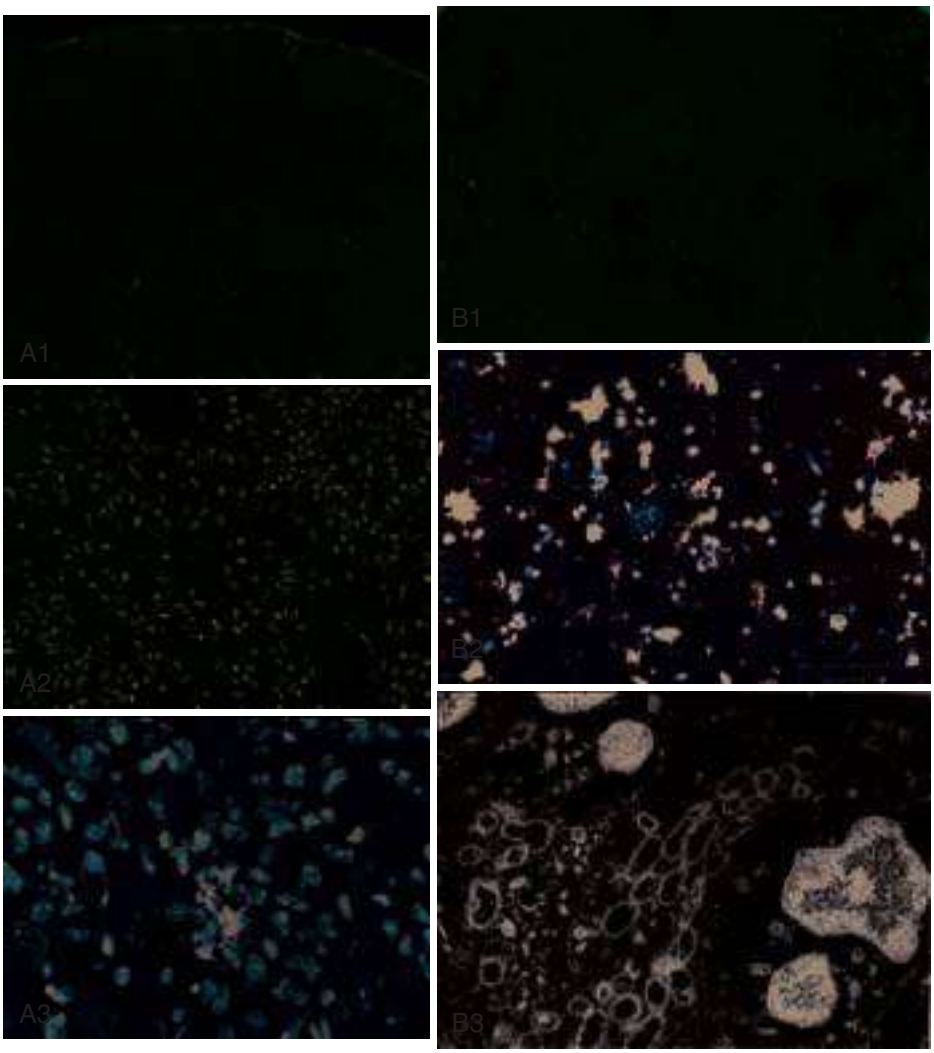

FIG. 234.7 Histopathologic manifestations of cutaneous cat-scratch disease (CSD) and bacillary angiomatosis (BA) in patients with compromised immunity and infection with *Bartonella henselae*. (A) Chronic CSD (cutaneous) in a moderately immunocompromised host (5 years postcadaveric kidney transplant; patient's clinical lesion shown in Fig. 234.5B). (A1) Histopathology (hematoxylin and eosin stain [H&E]) demonstrates poorly formed granulomas. Epidermal hyperplasia is observed at low magnification, with an underlying dense inflammatory infiltrate that extends throughout the dermis and into the subcutis. (A2) At higher magnification, the inflammatory infiltrate is noted to be composed of histiocytes, lymphocytes, neutrophils, and plasma cells. (A3) Warthin-Starry silver staining shows clumped and solitary rods *(black-stained organisms),* consistent with *Bartonella* spp. (B) BA (cutaneous) in a severely immunocompromised patient with AIDS. (B1) H&E staining of a biopsied cutaneous BA lesion demonstrates a dermal vessel. The vessel is lined with protuberant endothelial cells surrounded by myxoid connective tissue containing neutrophils and amphophilic granular material in close proximity to the vascular lumen. (B2) Warthin-Starry staining of cutaneous BA tissue reveals multiple clumps of tangled, dark silver-staining bacillary organisms (bar = 10 μm). (B3) transmission electron micrograph of cutaneous BA lesion tissue shows multiple trilaminar cell-walled bacillary organisms. *(Images in A reprinted with permission from Koehler JE, Duncan LM. Case records of the Massachusetts General Hospital. A 56-year-old man with fever and axillary lymphadenopathy.* N Engl J Med. *2005;353:1387–1394. Images in B reprinted and modified with permission from Koehler JE, Tappero JW. Bacillary angiomatosis and bacillary peliosis in patients infected with human immunodeficiency virus.* Clin Infect Dis. *1993;17:612–624.)*

Nearly half of typical CSD patients have a single lymph node involvement, another 20% have multiple node involvement at one site, and the remaining one-third have node involvement at multiple sites. Up to one-sixth of patients with typical CSD develop lymph node suppuration. Ultrasonography may assist in the assessment of lymph node size and suppuration,[53,169] and it can be used to direct needle aspiration of pus (usually done to relieve discomfort). Node enlargement usually persists for 2 to 4 months but may last considerably longer; spontaneous resolution is the rule, regardless of whether the patient is treated with antibiotics. The histopathology of nodes includes a mixture of nonspecific inflammatory reactions, including granulomata and stellate necrosis (see Fig. 234.9D and E). However, out of 100 cases evaluated with molecular confirmation of *B. henselae* infection, 43 lacked microabscesses, the classic diagnostic feature of CSD; in addition, sometimes PCR-positive CSD lymph nodes demonstrated features mimicking other infectious lymphadenopathies. This underscores the importance of *B. henselae* molecular testing.[170] Bacilli are best demonstrated by Warthin-Starry and possibly Dieterle or Steiner silver staining. Uncommonly, hypercalcemia may complicate CSD lymphadenopathy as a result of endogenous overproduction of active vitamin D associated with granuloma formation.[171]

Musculoskeletal Manifestations of Cat-Scratch Disease

Although previously considered to be uncommon in CSD, some musculoskeletal manifestations actually occur in greater than 10% of cases, as defined in an 11-year surveillance study involving 913 patients with compatible clinical presentation and confirmatory PCR or serologic test, or both, for *B. henselae*.[172] Myalgia occurred in 5.8% of surveyed patients, had a median duration of 4 weeks, and was often severe. Arthropathy (arthralgia or arthritis, or both) occurred in 5.5%, involving mainly the medium and large joints, for a median of 5.5 weeks, and was characterized as moderate to severe in intensity in more than half of these patients. In a small proportion chronic symptoms persisted for more than 1 year and could be debilitating. Age older than 20 years

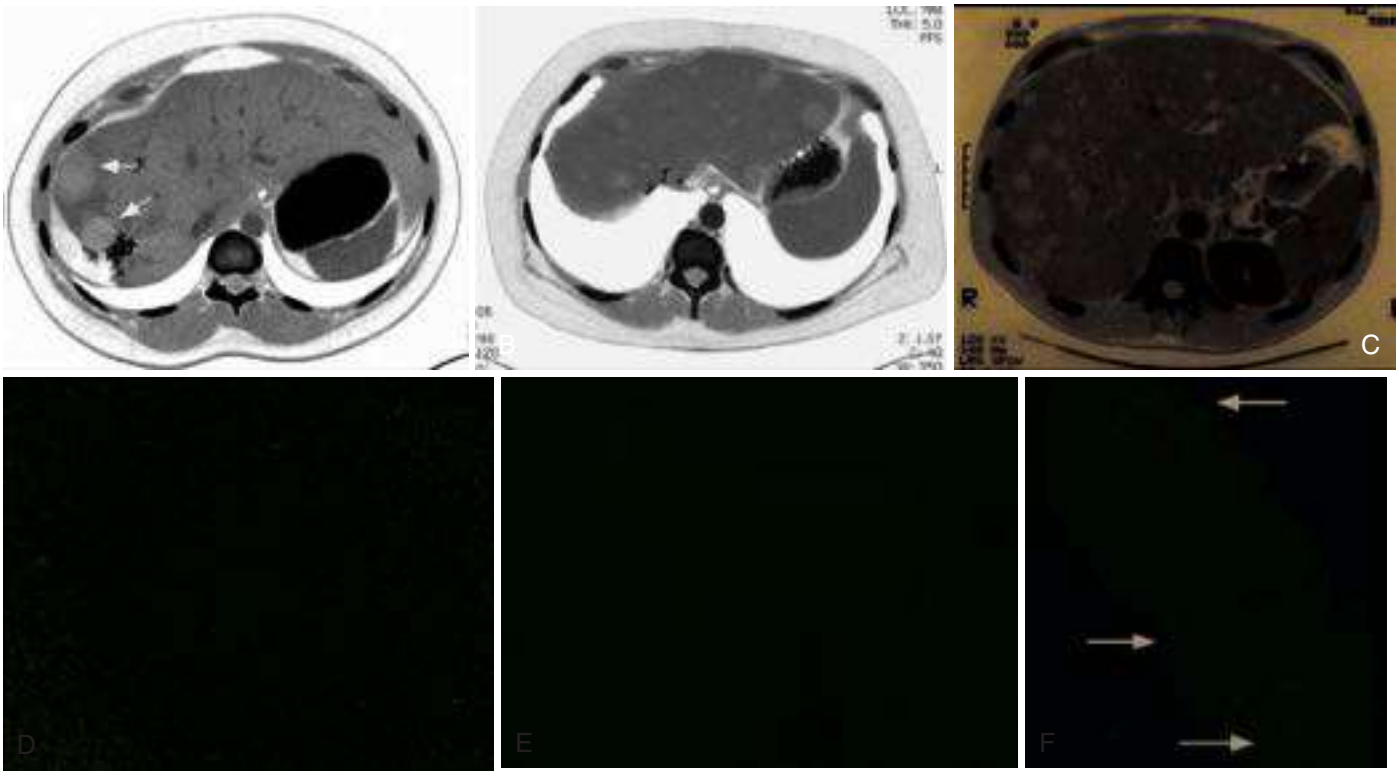

FIG. 234.8 Hepatic manifestations of *Bartonella henselae* occur often when infection disseminates in either immunocompetent or immunocompromised patients, but histopathologic characteristics differ markedly depending on host immune status. Although hepatic lesions caused by *B. henselae* infection can have a similar appearance on CT imaging, biopsy and histopathologic examination reveal markedly different manifestations, depending on the immune status of the host. Histopathology of hepatic lesions from immune competent hosts typically demonstrates necrotizing granulomatous lesions with few *B. henselae* bacilli, whereas hepatic lesions from severely immunocompromised patients usually reveal bacillary peliosis (BP) hepatis with numerous bacilli. In patients with moderate immunosuppression, such as solid-organ transplant recipients, the spectrum of pathology is quite variable, from granulomatous hepatitis to BP hepatis. (A) Abdominal CT scan showing several hypodense liver lesions *(arrows)* in an immunocompetent patient. (B) Abdominal CT scan showing discrete hypodense masses in a liver transplant patient. (C) Abdominal CT scan showing multiple hypodense liver lesions in a patient with advanced AIDS and disseminated *B. henselae* infection; biopsy demonstrated BP hepatis. (D) Resected liver lesion in an immunocompetent patient with granulomatous hepatitis due to *B. henselae* infection, documented by molecular diagnostics. Histopathology shows necrotizing granulomatous inflammation with giant cells and characteristic palisading histiocytes. (H&E, 100×). (E) Hepatic granuloma in a moderately immunocompromised host (2.7 years post–liver transplant); H&E histopathology on the resected liver lesion shows a necrotizing granuloma with palisading histiocytes and central coagulative necrosis. (F) *B. henselae* BP hepatis in an immunocompromised host (6 years after combined pancreas-kidney transplant). Transjugular liver biopsy stained with H&E showed numerous randomly distributed, cystic, blood-filled spaces *(arrows)* between areas of normal parenchyma. *(Images A and D reproduced with permission from VanderHeyden TR, Yong SL, Breitschwerdt EB, Maggi RG, Mihalik AR, Parada JP, Fimmel CJ. Granulomatous hepatitis due to* Bartonella henselae *infection in an immune competent patient.* BMC Infect Dis. *2012;12:17. Images B and E reproduced with permission from Thudi KR, Kreikemeier JT, Phillips NJ, Salvalaggio PR, Kennedy DJ, Hayashi PH. Cat scratch disease causing hepatic masses after liver transplant.* Liver Int. *2007;27:145–148. Image C reproduced with permission from Koehler JE, Tappero JW. Bacillary angiomatosis and bacillary peliosis in patients infected with human immunodeficiency virus.* Clin Infect Dis. *1993;17:612–624. Image F reproduced with permission from Freed MM, Ramirez-Valle F, Chandran S. Fever, hepatosplenomegaly and a skin nodule in a kidney-pancreas transplant recipient.* Am J Transplant. *2012;12:2556–2558.)*

increased the risk of having any of these symptoms, and female gender also was associated with increased risk of arthropathy.[172] Less commonly, tendonitis, neuralgia, and osteitis were identified, each at a rate of less than 1%. A review of 47 patients with skeletal manifestations associated with CSD found a median age of 9 years. The most frequently involved sites were the vertebral column (42%) and the pelvic girdle (27%).[173]

Atypical Manifestations of Cat-Scratch Disease

The nonspecific nature and rarity of many of the atypical manifestations can result in a delay of accurate diagnosis until a history of cat exposure, a positive serology, or suggestive findings on histopathology prompt specific evaluation for *B. henselae* infection.[165] "Atypical" manifestations of CSD[174] include Parinaud oculoglandular syndrome, self-limited granulomatous hepatitis/splenitis,[175] atypical pneumonitis,[176] osteitis,[173,177] and neurologic syndromes (predominantly encephalopathy and neuroretinitis). A syndrome of prolonged FUO in children also has been described.[178] Because of the insidious and nonspecific nature of the fever and abdominal pain of CSD hepatitis/splenitis, diagnosis may be delayed until a history of cat exposure prompts serologic testing and ultrasonographic or CT abdominal imaging, which usually demonstrates multiple hypodense lesions (see Fig. 234.8A).[146,179–181] Histopathologic examination usually demonstrates necrotizing granulomatous inflammation with giant cells and palisading histiocytes (see Fig. 234.8D).

Encephalopathy Complicating Cat-Scratch Disease

Encephalopathy, a dramatic but infrequent manifestation, was first reported within a few years of the description and naming of CSD. Encephalopathy probably occurs in 2% to 4% of all diagnosed CSD cases, although estimates range widely from 1% to 7%.[182] In a state-wide study to systematically identify the etiology of encephalitis in California, *Bartonella* was identified as the most common bacterial cause of encephalitis (7/203 total cases).[183] All patients required ICU care, and brain MRI scans and CSF analyses were normal. Currently, a history of cat exposure and presence of anti–*B. henselae* antibodies in serum provide the best evidence for diagnosis of CSD encephalitis. Adolescents and adults may represent a greater proportion of cases of CSD encephalopathy than they do of CSD overall.[184] Although encephalopathy usually follows the development of lymphadenopathy, it also has been reported to precede lymph node involvement or to occur in its absence. Persistent, generalized headache is a common part of the

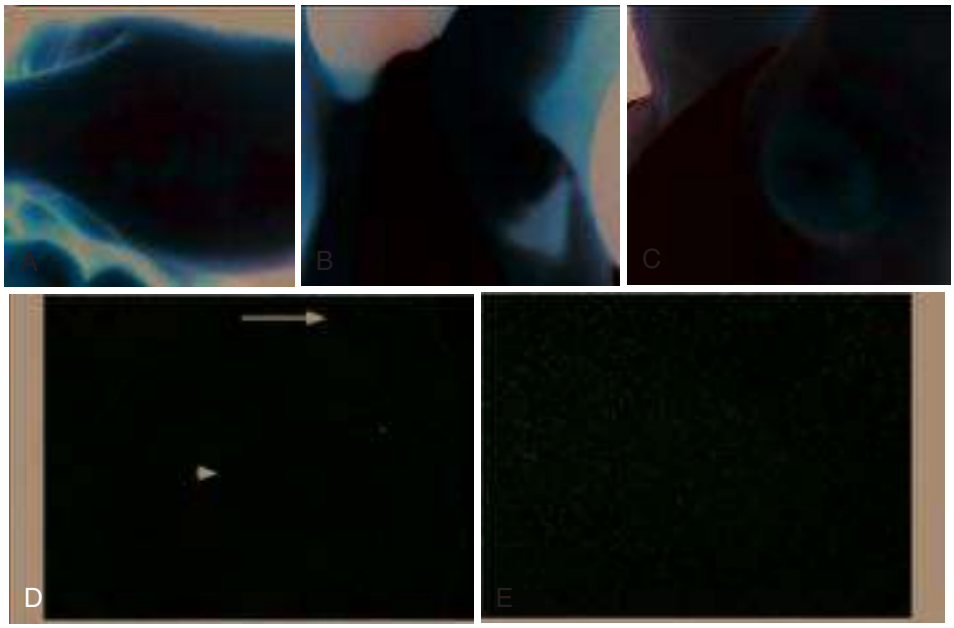

FIG. 234.9 Clinical and histopathologic features of typical cat-scratch disease (CSD) in the immunocompetent host. In an immune competent host, the typical presentation of CSD involves (A) a cutaneous lesion at the site of inoculation (7–10 days after a cat scratch and before development of ipsilateral lymphadenopathy), and (B and C) progressive regional lymphadenopathy. (D) Shows the typical H&E-stained histopathologic appearance of suppurative granulomatous lymphadenitis of CSD in a low-power view, with a large, ovoid, pale-appearing granuloma *(arrowhead),* in contrast to a normal germinal center *(arrow).* (E) A higher-power view of a granuloma shows eosinophilic epithelioid histiocytes and small lymphocytes forming the rim, with a necrotic central core that contains numerous neutrophils. *(Image A courtesy Dr. Timothy Berger, University of California–San Francisco. Images B and C reproduced with permission from Dr. Anna Sander from Katzenkratzkrankheit und andere* Bartonella*-Infektionen. In: Hofmann F.* Handbuch der Infektionskrankheiten*. 8th ed. Landsberg, Germany: ecomed Verlagsgesellschaft AG; 2004 (Ergänzungslieferung). Images D and E reproduced with permission from Dr. Linda Ernst, North Shore Medical Group, Evanston, IL, published in Florin TA, Zaoutis TE, Zaoutis LB. Beyond cat scratch disease: widening spectrum of* Bartonella henselae *infection.* Pediatrics*. 2008;121:e1413–e1425.)*

history, but fever is an inconsistent finding. Patients may become restless, and combativeness occurs often. Nearly half of patients develop seizures that range from focal to generalized and from brief and self-limited to status epilepticus. Short-term anticonvulsant therapy may be required, as well as supportive therapy when there is obtundation or coma. Concurrent acute neurologic manifestations can be present transiently (e.g., nuchal rigidity, pathologic reflexes, pupillary dilatation). When they occur, neurologic deficits, such as aphasia, cranial nerve palsy, paresis, hemiplegia, and ataxia, also are usually self-limited, although time to resolution may span weeks to months to as long as 1 year. However, persistence of cognitive impairment and of seizures has been reported uncommonly.[185–187]

Laboratory studies, such as cerebrospinal fluid (CSF) analysis and culture, generally do not add specific diagnostic findings to the clinical picture of CSD encephalopathy but can exclude other processes. Elevations of CSF protein concentration and leukocytes occur in only approximately one-third of patients but do not necessarily coincide in the same patients; lymphocytes predominate and hypoglycorrhachia is rare. CSF cultures have been consistently negative, even after the identification of *B. henselae* as the causative agent of CSD. Studies of the brain with CT or magnetic resonance imaging, or both, are usually normal, but a few cases of persistent structural abnormalities have been reported.[186,187] Electroencephalography during the acute phase of CSD encephalopathy commonly reveals diffuse slowing, yet another nonspecific feature that resolves with clinical recovery.

The pathogenesis of CSD encephalopathy and other central nervous system (CNS) manifestations associated with CSD remains unclear. It is unknown whether these rare complications are attributable to direct invasion of the CNS by *B. henselae* or to other mechanisms, such as vasculitis or host immune response. At autopsy of a patient who died from CSD meningoencephalitis, there was marked cerebral edema with no gross evidence of acute meningitis. Microscopic examination revealed multiple granulomatous lesions, as well as meningitis and encephalitis.[188] Warthin-Starry silver stain of the brain and liver revealed pleomorphic rod-shaped bacilli consistent with *B. henselae*. Analysis of brain tissue with PCR confirmed the presence of *B. henselae* DNA.[188] Autopsy of another rare case of a 6-year-old child who died from disseminated CSD with encephalitis demonstrated intracerebral histologic findings of perivascular lymphocytic infiltrates and microglial nodules.[189]

Ocular Manifestations of Cat-Scratch Disease

Ocular manifestations of CSD include Parinaud oculoglandular syndrome (an atypical form of CSD lymphadenitis), neuroretinitis, vascular-occlusive events, retinitis, choroiditis, and optic nerve granuloma.[190–192] Up to half of the 11% or 12% of CSD cases that are atypical represent Parinaud oculoglandular syndrome, a self-limited granulomatous conjunctivitis with ipsilateral, usually preauricular, lymphadenitis (Fig. 234.10).[193,194] After its description in 1970,[192] neuroretinitis remained solely a clinical diagnosis for years, but development of a serologic test for anti–*B. henselae* antibodies has facilitated diagnosis.[59] Neuroretinitis associated with CSD[192,195,196] subsequently has been associated with *B. henselae* infection by serology.[109,190] *B. grahamii* neuroretinitis was identified in a single patient by PCR amplification and sequence analysis of DNA in the intraocular fluid of an HIV-seronegative patient with bilateral neuroretinitis and behavioral changes.[197] *B. elizabethae* has been implicated as the pathogen in another patient with neuroretinitis, although only by serologic antibody studies.[198]

Neuroretinitis often manifests with a sudden loss of visual acuity, usually unilaterally, and sometimes preceded by an influenza-like syndrome or development of unilateral lymphadenopathy. The most striking retinal manifestation is papilledema associated with macular exudates in a star formation (Fig. 234.11), first associated with CSD in 1984.[195] Although this manifestation is characteristic of CSD neuroretinitis, other types of inflammation/infection also can cause this finding. In a retrospective study of 24 CSD patients with 35 affected eyes,[190] isolated foci of retinitis or choroiditis were the most common ocular manifestation, identified in 83% of eyes and 83% of patients. Optic disk swelling was the second most common finding (46% of



FIG. 234.10 Atypical cat-scratch disease: Parinaud oculoglandular syndrome, a granulomatous conjunctivitis, is associated with ipsilateral regional lymphadenopathy (usually preauricular and, less commonly, submandibular).

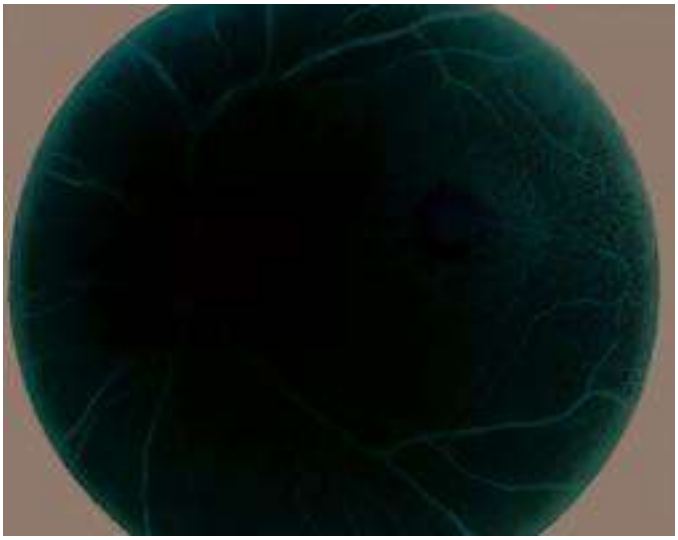

FIG. 234.11 Atypical cat-scratch disease (CSD): neuroretinitis is one of the more common complications of CSD. The striking papilledema associated with stellate macular exudates is among the most common manifestations of CSD neuroretinitis.

eyes and 63% of patients), followed by a macular star (43% of eyes and 63% of patients), and vascular-occlusive events (14% of eyes and 21% of patients). Final visual acuity was 20 of 25 or better in 26 of 35 eyes (74%) and was similar in both treated and untreated patients.[190] Although patients with neuroretinitis or retinochoroiditis can have concomitant anterior segment inflammation, the association of isolated uveitis (without optic disk edema or focal retinochoroiditis) with *Bartonella* infection is not as well established; in one study,[199] 17 of 51 patients with uveitis had positive *Bartonella* serology, compared to 1 of 101 controls; however, the presence of *Bartonella* could not be confirmed with PCR testing of surgical specimens (lens mass, intraocular fluid) obtained from 33 patients.[199] Unlike many other causes of neuroretinitis, *B. henselae* neuroretinitis usually spontaneously resolves with a favorable visual outcome, although prospective follow-up of some recent, well-documented cases reveals that some have mild residual visual deficits.[109,196] The utility of antimicrobial or corticosteroid therapy, or both, continues to be debated.[196]

Differential Diagnosis of Cat-Scratch Disease

The differential diagnosis of typical CSD includes many causes of (unilateral) lymphadenopathy, among which are typical or atypical mycobacterial infection, tularemia, plague, brucellosis, syphilis, sporotrichosis, histoplasmosis, coccidioidomycosis, toxoplasmosis, infectious mononucleosis syndromes, lymphoma, and other neoplasms.[200] In SOT recipients, posttransplant lymphoproliferative disease also would be included in the differential diagnosis. In the inguinal area tender adenopathy in the absence of a genital lesion suggests *Staphylococcus aureus*, CSD, lymphogranuloma venereum, and, in the febrile patient with tick exposure, tularemia. The diagnosis of CSD can be overlooked if the clinician fails to obtain an adequate history, especially in patients with atypical CSD syndromes, but also in adults with typical CSD who are seen by internists lacking experience with diagnosing CSD (in contrast to pediatricians). In elderly adults (older than 60 years) manifestations tend to be less typical, further confounding the diagnosis.[201] With domestic cats representing the largest category of companion animals in the United States, obtaining an accurate history regarding animal exposure is critical when evaluating a patient with findings consistent with CSD. Fortunately, spontaneous resolution occurs in most cases of CSD, whether typical or atypical.

Bartonella Infections in Solid-Organ Transplant Recipients

Background/Risk Factors for Acquisition of *Bartonella* in Solid-Organ Transplant Recipients

SOT recipients represent an important emerging population that develops serious and potentially life-threatening *Bartonella* infections; the majority of cases have been reported since 2012. When *Bartonella* was first identified in the United States in the 1990s, the infections occurring in immunocompromised individuals were almost exclusively identified in patients with late-stage HIV infection. As increasingly active antiretroviral agents were identified, the incidence of *Bartonella* infections in HIV-infected individuals decreased dramatically, whereas the incidence increased substantially in SOT recipients. In addition to the temporal difference in recognition of *Bartonella* infections in these two immunocompromised populations, the infecting *Bartonella* sp. also differs: in AIDS patients with *Bartonella* infection in the 1990s, approximately half were infected with *B. henselae* and the other half with *B. quintana*.[43] In contrast, SOT recipients almost always are infected with *B. henselae*,[129] presumably because SOT recipients are housed, not homeless, and thus often exposed to pet cats (*B. henselae*) but not to body lice (*B. quintana*). Indeed, most SOT recipients with *Bartonella* infection either owned a cat or had exposure to cats.[129] One patient had a potential donor-derived *Bartonella* infection.[65] This patient developed a localized liver infection 2 months after liver transplantation and did not have a history of cat exposure. The donor serum IFA IgG for *B. henselae* was 1:64 (lowest positive titer).

The recent numerous reports of *Bartonella* infection in SOT recipients provides documentation of the increasing frequency and wide spectrum of disease manifestations in this patient population.[112,129–133,150–152] In a review of 29 SOT recipients with *B. henselae* infection, 28% of patients had typical CSD with regional lymphadenopathy, and 72% had disseminated disease, which included BA (cutaneous, lymph node), hepatic or splenic BP, granulomatous or suppurative tissue reaction (liver, lymph node, bone, lung, or bone marrow), endocarditis, and bilateral chorioretinitis.[129] Representative of the typical distribution of SOTs, two-thirds of the patients had received a kidney transplant, one-fourth a liver transplant, and one patient each had received a lung and heart transplant.[129] Of note, eight patients were receiving trimethoprim-sulfamethoxazole (TMP-SMX) (for *Pneumocystis* prophylaxis) when the *Bartonella* infection occurred, providing further evidence that this agent is not an acceptable treatment for bartonellosis.

Clinical Features of *Bartonella* Infection in Solid-Organ Transplant Recipients

As noted earlier for HIV-infected patients, the clinical manifestations in SOT recipients depend on the degree of immunocompromise (see

Fig. 234.4 and Table 234.2). Prominent clinical features included fevers (93% of patients) and lymphadenopathy (41%),[129] emphasizing the importance of considering *B. henselae* infection when evaluating an SOT recipient with FUO or lymphadenopathy. The majority of patients (69%) had abnormal findings on CT scan, including lymphadenopathy, splenomegaly, hepatomegaly, and low-attenuation hepatic and splenic lesions (see Fig. 234.8B), a lytic bone lesion, or a lung nodule.[129]

Cases of ocular bartonellosis in transplant recipients also have been reported; in one case the ocular disease was manifested as panuveitis and retinal detachment, and the patient also was found to have endocarditis and ring-enhancing cerebral lesions, emphasizing that ocular manifestations should prompt workup for disseminated *Bartonella* infection in an immunocompromised host.[131]

Diagnosis of *Bartonella* Infections in Solid-Organ Transplant Recipients

Histopathologic examination, serologic testing, and PCR testing for *B. henselae* provided important methods for establishing the diagnosis of *Bartonella* infection.[129] PCR testing on tissue (lymph node, liver, bone marrow, lung) or serum was positive in 12 of 14 patients in whom it was performed.[129] Serologic testing was positive in 6 of 6 of CSD patients and 17 of 21 patients with disseminated disease who underwent testing, providing reassurance that most SOT recipients are capable of mounting an antibody response to *Bartonella* infection.[129] Serologic testing should be performed in a validated reference laboratory, and because serology may be negative initially, acute and convalescent serologic testing is important and may increase the diagnostic accuracy.[129,132,133,150] Note that a negative *Bartonella* serology does not exclude the diagnosis, as reported in two SOT recipients with cutaneous BA but negative acute and convalescent serologies.[130]

Pathologic Findings and Relation to Duration of Time Posttransplant

Granulomatous or suppurative inflammation (see Figs. 234.7A1–A3 [cutaneous] and 234.8E [hepatic]), the histopathology characteristic of CSD, occurs more often than BA/BP (see Fig. 234.8F [hepatic]) in SOT recipients with *Bartonella* infection.[129] Patients with BA/BP developed infection on average 3 years earlier posttransplant than patients with granulomatous or suppurative inflammation. Similarly, patients with disseminated bartonellosis developed disease approximately 3 years earlier after transplantation than patients who had typical CSD. These findings may reflect a greater degree of immunosuppression closer to the time of transplantation. A clear relationship between a specific immunosuppressive regimen and development of disseminated disease or BA/BP was not identified, possibly due to the limited number of patients and retrospective nature of the study.[129]

Prognosis for Solid-Organ Transplant Recipients With *Bartonella* Infection

The majority of SOT recipients with *Bartonella* infection responded to antibiotic therapy, but several patients experienced a relapse of CSD[129] or BA,[130] and two patients died from endocarditis.[129] In another patient, who presented 2 years postrenal transplantation with fever, lymphadenopathy, and pauciimmune necrotizing glomerulonephritis, antimicrobial therapy was unsuccessful, and the patient ultimately required retransplantation. However, experience in treatment of other immunocompromised hosts has demonstrated that the short treatment duration (1 week of azithromycin) would be of insufficient duration to cure an immunocompromised host.[151]

CELLULAR AND MOLECULAR PATHOGENESIS ASSOCIATED WITH *BARTONELLA* INFECTIONS

In cats and other mammalian hosts, *Bartonella* appears to be a nearly perfectly adapted parasite, capable of causing long-term or cyclic, high-grade, intraerythrocytic bacteremia, largely in the absence of illness in its cognate host.[51,57,66,202–205] Each *Bartonella* spp. has adapted to, and infects, only one or a few mammalian reservoir hosts.[206] Elucidation of the mechanisms of this host-pathogen relationship is a focus of current research. Fundamental knowledge about the pathogenic mechanisms of *Bartonella* spp. in humans is growing, primarily focused on entry mechanisms into endothelial cells and erythrocytes, induction of angiogenesis (e.g., in lesions of BA), and mechanisms of *Bartonella* survival in the mammalian host and arthropod vector. The pathogenic mechanisms of *Bartonella* spp. involve complex host-pathogen interactions. In vitro studies demonstrate that *B. henselae* can invade a diverse repertoire of host cells, including endothelial cells, monocytes, macrophages, dendritic cells, hematopoietic progenitor cells, and epithelial cells.[207–212] Pathogenic mechanisms have been reviewed in detail elsewhere.[207,208,213,214]

Adhesion to host cells is an important initial step in infection of the host, and a number of surface proteins and structures have been implicated in this adhesion process. The *B. quintana* variably expressed outer membrane proteins (Vomps) and the *B. henselae* ortholog, *Bartonella* adhesion A (BadA) protein, are nonfimbrial, nonpilus, outer membrane trimeric autotransporter adhesion (TAA) proteins that play an important role in attachment to host cells.[215,216] The Vomps in *B. quintana* are encoded by a family of four genes and are necessary for infection of the host.[217] Each Vomp is expressed on the *B. quintana* surface, and each has a unique region that mediates binding to different host cell substrates or to other *Bartonella* bacilli (autoaggregation).[215] The Vomps also undergo phase variation during prolonged bloodstream infection, which can facilitate persistence in the mammalian host.

During endothelial cell invasion by *B. bacilliformis,* the bacterium induces rearrangement of the host cytoskeleton.[218] Entry of *B. henselae* into endothelial cells in vitro can occur via two mutually exclusive pathways in vitro.[211] One route of entry involves uptake of a single or a small group of bacteria into *Bartonella*-containing vacuoles (BCVs). BCVs appear to avoid lysosomal fusion and, instead, accumulate in the perinuclear space.[210,211] The second route of entry involves bacterial aggregation on the cell surface, followed by engulfment and internalization of the bacterial aggregate by a unique structure, the invasome.[207,208,211] Invasome-mediated uptake is dependent on *Bartonella* effector proteins (Beps), which are translocated into endothelial cells via the VirB/D4 type IV secretion system (T4SS), and bidirectional signaling through integrin receptors.[207–209,219]

Adherence and invasion of erythrocytes by *Bartonella* spp. are likely specific to each species' cognate host(s), and establishment of high-density bacteremia is important to ensure continuous transmission.[206,207] There is in vitro evidence that human-specific *B. bacilliformis* and *B. quintana* can invade human erythrocytes[220,221]; host specificity for the *Bartonella* spp., designated lineage 4 (which includes most modern *Bartonella* spp., including *B. henselae, B. quintana, B. vinsonii,* and *Bartonella tribocorum*), is governed by the Trw T4SS and is essential for erythrocyte attachment and invasion.[206,207,208] *B. bacilliformis,* the sole representative of an ancestral lineage, does not have a Trw T4SS but does have a unipolar tuft of flagella that confer motility and may participate in erythrocyte attachment and invasion.[207,208,222] Of interest, the *Bartonella* Trw system has been investigated for use in genetic engineering, given its potential for facilitating cellular entry and DNA integration.[223] Erythrocyte invasion by *B. bacilliformis* and *B. henselae* may involve an extracellular deformin protein and proteins encoded by the invasion-associated locus *(ialAB)*.[207,208,222,224–227] Deformin may induce invaginations of the erythrocyte membrane, which could serve as points of entry for *Bartonella*.[207,208] After entry into erythrocytes in vitro, *B. bacilliformis* can replicate within, and occasionally escape from, the erythrocytes.[220] In an elegant in vivo study by Schülein and colleagues,[228] *Bartonella* infection of erythrocytes was followed over time by confocal imaging and flow cytometry, demonstrating the replication of *Bartonella* in erythrocytes during bloodstream infection.

B. bacilliformis has been demonstrated to stimulate endothelial cell proliferation both in vitro and in vivo, possibly through a stimulatory factor shed from, or actively secreted from, the bacterium.[229–231] Several in vitro studies indicate that the proliferative effects of *B. henselae* and *B. quintana* may be partially mediated by the adhesins BadA and Vomp, which are required for bacterial attachment to endothelial cells.[215,216,232,233] BadA also may promote angiogenesis through activation of hypoxia inducible factor-1 and nuclear factor kappa B, which induces the secretion of vascular endothelial growth factor (VEGF), and interleukin-8 (IL-8), respectively.[207,232] In *B. quintana* expression of the Vomp is associated

with induction of VEGF expression from human macrophage and epithelial cell lines.[233]

Another family of important virulence factors in *B. henselae* and *B. quintana* is that of the hemin binding proteins (Hbps), porin-like outer membrane proteins that bind hemin and play a role in heme acquisition, which is crucial for *Bartonella* growth.[234] Overexpression of one protein in the *B. henselae* Hbp family, HbpC, results in increased resistance to heme toxicity, implicating HbpC in protection of *B. henselae* from the toxic levels of heme present in the gut of the arthropod vectors that transmit *Bartonella* spp.[235] In contrast, other members of the *B. henselae* Hbp family are hypothesized to maintain a heme reservoir and increase the efficiency of heme uptake when the *Bartonella* bacteria occupy their other niche, the mammalian bloodstream, where iron and hemin are extremely scarce.[235] In addition, *B. henselae* Hbp knockdown mutants have a decreased ability to counteract reactive oxygen species, have decreased capacity to invade endothelial cells, and are more rapidly cleared in flea feces.[236] These findings highlight the role Hbps have in facilitating endothelial cell invasion by *B. henselae,* resistance against oxidative stress, and survival of *B. henselae* in the flea vector.

Probably the most important and most studied virulence factors are the *Bartonella* Beps.[237] The *B. henselae* and *B. quintana* Beps are translocated into the host cell by the VirB/VirD T4SS, where the Bep target processes in the host and modulates it to the advantage of the invading bacterium. The *B. henselae* and *B. quintana* BepAs are necessary and sufficient to inhibit vascular endothelial cell apoptosis by causing elevated intracellular levels of cyclic adenosine monophosphate (cAMP), resulting in expression of cAMP-responsive genes.[238] This BepA activity may contribute to the endothelial cell proliferation observed in the focal lesions of verruga peruana and BA. BepC is important for invasome formation, inducing uptake of *Bartonella* at the host cell surface; it also effects host cell fragmentation.[237] It has been proposed that BepE, which protects endothelial cells from BepC-mediated cell fragmentation, is necessary for infection of the reservoir host dendritic cells during intradermal inoculation.[239] Further study of Beps will likely provide novel insight into the pathogenesis mechanisms used by *Bartonella* to survive and persist in the reservoir host.

Immune evasion and attenuation of the host inflammatory response are important strategies adopted by *Bartonella* for survival in the host. The invasion of erythrocytes provides a sequestered niche to protect *Bartonella* from the adaptive and innate immunity of the reservoir host.[207] Multiple studies have demonstrated that the helper T-1 response, which stimulates the production of interferon-γ and tumor necrosis factor-α, probably plays a central role in the elimination of *B. henselae*.[207,240,241] The inflammatory response elicited by *B. henselae* in CSD leads to the formation of B-cell–rich suppurative granulomas, which appear to be mediated by the effect of the organism on dendritic cells.[212] However, *Bartonella* also can attenuate the host immune response and promote persistence of infection by stimulating IL-10 production. IL-10 is known to suppress the function of multiple immune cells, including helper T cells, monocytes, macrophages, and dendritic cells. This mechanism was suggested by an investigation of the immune response in homeless people with chronic asymptomatic *B. quintana* bacteremia, which identified a specific increase in IL-10 secretion with a concomitant dampening of the inflammatory response.[242] *B. quintana* also may dampen the immune response by blocking Toll-like receptor 4, thus inhibiting the downstream inflammatory response.[243]

LABORATORY DIAGNOSIS OF *BARTONELLA* INFECTIONS

The major impediments to diagnosis of *Bartonella* infections are (1) omission of *Bartonella* from the differential diagnosis because of the nonspecific manifestations and the widely varying presentations as the result of host immune status; (2) failure to obtain a history of risk factors for *Bartonella* infection; and (3) the extremely fastidious nature of *Bartonella* spp., resulting in a subsequent lack of a reproducible, sensitive, specific, and widely available diagnostic tests.

To include *Bartonella* infection in the differential diagnosis, a crucial initial step is obtaining a history of cat and flea exposure (*B. henselae*), homelessness and body louse exposure (*B. quintana*), or exposure to an area endemic for *B. bacilliformis*. Diagnostic approaches that are available at many clinical and reference laboratories include serology, direct PCR-based DNA detection assays, and sometimes culture. However, to ensure the most reliable results, testing for *Bartonella* infection should be performed in Clinical Laboratory Improvement Amendments (CLIA)-approved laboratories, using well-validated tests, such as IFA testing or PCR using broad-range bacterial or *Bartonella*-specific 16S ribosomal DNA (rDNA) primers at established reference laboratories. Use of validated tests is particularly important for diagnosis of patients presenting with atypical symptoms or with a potential new presentation of *Bartonella* infection. Laboratories with extensive experience include, but are not limited to, the Centers for Disease Control and Prevention (CDC),[59] University of Washington Molecular Diagnostics Laboratory (UW-MDL),[244] and Unité des Rickettsies in Marseille, France.[245]

If direct isolation of *Bartonella* is not possible (generally impractical because of the highly fastidious nature of *Bartonella*), a diagnosis of *Bartonella* infection can be achieved by PCR amplification of DNA extracted directly from involved tissues or blood, in the absence of additional manipulation of samples, and using appropriate PCR controls and a low number of PCR cycles. Amplification of *Bartonella*-specific DNA also may identify the infecting *Bartonella* spp. Although isolation or molecular identification of a *Bartonella* spp. provides a definitive diagnosis, when these are not possible, fulfillment of two or more positive criteria among epidemiologic, serologic, and histologic tests can provide a presumptive diagnosis. Analysis of serum antibodies using IFA testing is the most widely available laboratory test for diagnosis of *Bartonella* infection, with the caveat that there is extensive cross-reactivity among the different *Bartonella* spp., the IgM assay lacks sensitivity, and many late-stage AIDS patients never develop anti-*Bartonella* antibodies. Additional diagnostic approaches include direct examination of blood smears *(B. bacilliformis)* or histopathologic materials stained with H&E or Warthin-Starry silver stain (typically for CSD and BA/BP). Although serologic testing remains the mainstay of diagnosis for most *Bartonella* clinical syndromes, molecular diagnostics are increasingly used. A detailed discussion of diagnostic techniques for *Bartonella* is available in other reference sources.[246,247]

Direct Examination of Blood Smears and Histopathologic Specimens

Giemsa-stained blood films are commonly used in endemic locales of South America to detect *B. bacilliformis* in patients with Oroya fever. A wide morphologic range is seen in such smears, with the organisms appearing as red-violet rods or rounded forms, occurring singly or in groups, and associated with erythrocytes. Bacillary forms are most typically seen, measuring 0.25 to 0.5 × 1.0 to 3.0 μm; rounded organisms measure approximately 0.75 μm in diameter. The cells are often curved and may show unipolar or bipolar enlargement and granules. Although *B. bacilliformis* bacteria appear to be adherent to erythrocytes by light microscopy, they have also been observed within erythrocytes when viewed by electron microscopy.[248] Except for *B. bacilliformis,* blood smears rarely detect *Bartonella* during bacteremia in humans. The low titers of bacteremia associated with *B. henselae* and *B. quintana* during human infection make direct observation of bacteria in blood smears difficult; however, direct immunofluorescence[249] and acridine orange staining[12,33] may occasionally be positive.[249]

Bartonella spp. are gram negative, are not acid fast, and stain poorly or not at all in tissue, except by silver deposition stains (e.g., Warthin-Starry stain). *B. henselae* and *B. quintana* are demonstrable by Warthin-Starry staining in BA/BP; *B. henselae* bacilli may be observed by silver staining during the early stages of lymphadenopathy in CSD but typically not during the later granulomatous stage of inflammation. Species-specific direct detection of organisms in tissue by immunocytochemical labeling also has been described,[97,158,250] but reagents for such labeling are not widely available and not well characterized.

Specimen Collection and Handling for Culture

Isolation of *Bartonella* spp. is most commonly attempted from blood and tissue. The time interval from collection to processing should be minimized. If storage of specimens is necessary, the specimens should be immediately frozen. A controlled study of the effects of blood collection

and handling methods showed that blood specimens from *B. henselae*–infected cats, collected in either ethylenediaminetetraacetic acid (EDTA) or Isolator (Alere Isolator Microbial Tubes; Abbott, Abbott Park, IL) blood-lysis tubes, yielded good recovery and that blood collected in tubes containing EDTA could be plated after 26 days of storage at −65°C with no loss of culture sensitivity.[251] Whenever possible, specimens should be collected before antimicrobial therapy, especially before receiving even a single dose of a tetracycline or macrolide. Growth of *B. henselae* also is inhibited by concentrations of sodium polyanethol sulfonate (SPS) that are used in blood culture media.[246] Lytic blood culture systems (e.g., Isolator Tubes) combine the advantageous effects of neutralizing SPS toxicity by hemoglobin freed from erythrocytes, in addition to releasing intracellular *Bartonella* organisms.

Culture of *Bartonella* Species

All *Bartonella* spp. can be cultured on cell-free media, unlike members of the order Rickettsiales. Recovery of *Bartonella* spp. (except *B. quintana* and *B. koehlerae*) is optimized by using freshly prepared (<7 days old) heart infusion agar and 5% fresh defibrinated rabbit blood plates, poured with double the usual agar volume.[43,252] Various other formulations of blood or chocolate agar will support *B. henselae* growth, with the best results dependent on media freshness, presence of a hemin or hemoglobin source, and adequate moisture. *B. quintana,* and especially *B. koehlerae,* are probably the most fastidious *Bartonella* spp. and grow optimally on chocolate agar. Note that *Bartonella* isolation from humans requires more than 7 days of incubation before colonies can be detected on agar.[43,103] Therefore routine bacterial culture protocols rarely allow *Bartonella* spp. to be detected, and sensitivity is further compromised by prior antibiotic exposure.[43] Protocols designed to yield other slowly growing organisms (e.g., *Histoplasma capsulatum* or *Mycobacterium avium* complex on noninhibitory media) also can result in recovery of *Bartonella* spp. if incubation time is prolonged (at least 14–28 days). Cultures are not recommended to diagnose cases of CSD; the sensitivity is at best only 20% compared with PCR assays.[253] Attempted isolation of *Bartonella* spp. may be useful in the settings of (1) FUO after cat or body louse exposure; (2) fever, lymphadenitis, neuroretinitis, or encephalitis of unknown origin in the immunocompromised patient; (3) endocarditis without recovery of typical pathogens (i.e., culture negative); and (4) BA/BP. The highest yield of *Bartonella* spp. from blood culture is in patients with BA/BP (50%)[43] and homeless individuals with body louse exposure.[107]

Ideally, inoculated plates should be incubated at 35° to 37°C under conditions of 5% to 10% carbon dioxide (CO_2) and greater than 40% humidity; this environment can be created in a candle jar.[10] The candle jar should be soaked in a bleach solution, cleaned thoroughly, and then wiped with 70% ethanol before inoculated plates are placed in the candle jar. Because adequate ambient moisture is critical, and isolation of *Bartonella* spp. from human blood or tissue rarely occurs before 8 days of incubation, the candle jar should not be opened until 8 days after inoculation and then only weekly thereafter. This also decreases the likelihood of contamination of the rich agars during the prolonged (up to 4 weeks) incubation time before *Bartonella* is detected.[10,43,103] Note that *B. bacilliformis,* and possibly some strains of *B. clarridgeiae,* have a lower optimal temperature (25°–30°C) for growth. It is ideal to perform all culture and screening of inoculated plates in a biosafety cabinet to further reduce chances of agar contamination. As an alternative to candle jars, plates can be sealed after 24 hours of incubation with plastic film or shrink wrap to preserve moisture content of the media and reduce contamination.

Although *Bartonella* spp. usually grow best on solid or semisolid media, there are alternative approaches to plating blood collected in an EDTA tube or Isolator tube directly onto agar. These include broth-based blood culture systems, chemically defined fluid media,[254] or eukaryotic cell coculture systems.[10,255] The sensitivity of a shell vial culture assay may be slightly better than that of agar plate techniques.[245] In the automated, continuously monitored blood culture systems, *Bartonella* spp. rarely produce turbidity or convert enough oxidizable substrate for these CO_2 detection–based systems to indicate growth. However, several isolates of *Bartonella* have been detected initially using BACTEC (Becton Dickinson, Sparks, MD) or resin-containing media, followed by acridine orange staining at the termination of a 7- or 14-day incubation period, with subsequent recovery of *Bartonella* colonies after subculture to solid media.[11,12,33,95] Another CO_2 detection–based blood culture system, BacT/Alert (bioMérieux, Durham, NC), yielded a positive growth algorithm in several cases of *B. henselae* bacteremia.[256] Although Gram stains of the broth and routine 72-hour subcultures proved negative, acridine orange and Warthin-Starry staining demonstrated bacilli, and *B. henselae* was ultimately subcultured on semisolid media.[256] Extended incubation of blood culture vials also resulted in recovery of *B. quintana* in prosthetic valve endocarditis.[257]

Bartonella spp. have been isolated from liver, spleen, lymph node, and skin after homogenization, either by direct plating of tissue homogenate or aspirate[10,17,53,61,109] or by cocultivation with various cell lines.[10,13] *B. henselae* and *B. quintana* grow in endothelial cell cultures as elongated pleomorphic organisms visible in Gimenez-stained preparations 72 hours after inoculation of the cell cultures. The cocultivation method is not practical for most microbiology laboratories, although it may result in recovering isolates not cultured in cell-free media. In the absence of coculture, more than 1 month of incubation often is necessary to yield colonies from tissue specimens.[53,61] Isolation of *Bartonella* spp. from potentially contaminated biopsy sites (e.g., skin) can be facilitated by using selective media that exploits the observation that *Bartonella* spp. infecting humans are instrinsically resistant to nalidixic acid and cefazolin. These two antibiotics can be incorporated into chocolate agar (nalidixic acid, 20 µg/mL and cefazolin, 2 µg/mL).[217]

Identification of *Bartonella* Species

The primary isolate of a given *Bartonella* spp. can have several morphologic colony types, for instance, *B. henselae*: (1) irregular, raised, whitish, rough, and dry in appearance (characterized as "cauliflower," "molar tooth," or "verrucous"), with agar pitting and strong adherence to the agar or (2) flat, circular, moist in appearance, and less adherent. Both are often present in the same primary culture (Fig. 234.12A).[43] Colony heterogeneity varies by species. *B. henselae* typically displays a greater proportion of rough colonies, and primary cultures of *B. quintana* colonies are virtually always



FIG. 234.12 Appearance of colonies from primary isolation of *Bartonella henselae* and *Bartonella quintana* from biopsied tissue of cutaneous bacillary angiomatosis (BA) lesions from AIDS patients. (A) Primary isolation of *B. henselae* from a cutaneous BA lesion reveals colonies that are predominantly uniform in size, elevated, rough, gray, and deeply embedded in the chocolate agar. (B) Primary isolation of *B. quintana* from a cutaneous BA lesion on chocolate agar reveals colonies that are flat, round, smooth, shiny, opaque, of heterogeneous size, and demonstrating neither a rough appearance nor pitting of the agar. *(Images A and B reproduced with permission from Koehler JE, Sanchez MA, Garrido CS, et al. Molecular epidemiology of* Bartonella *infections in patients with bacillary angiomatosis-peliosis.* N Engl J Med. *1997;337:318.)*

uniformly smooth, although very heterogenous in size (see Fig. 234.12B).[43] Serial *B. henselae* subcultures on agar tend to have a progressively increasing proportion of smooth colonies. *B. bacilliformis* colonies are flat, small, and translucent or slightly white. Gram-stained *Bartonella* from colonies appear as small, gram-negative, slightly curved rods, sometimes resembling *Campylobacter* or *Helicobacter*. Twitching motility of *B. henselae* is evident in wet mounts. *B. bacilliformis, B. clarridgeiae, B. ancashensis*, and *B. rochalimae* bacteria possess polar flagella.

A preliminary identification of *B. henselae* or *B. quintana* can be made if the following are observed: (1) a prolonged incubation time of >7 days before the appearance of colonies, (2) a Gram stain appearance of small curved gram-negative bacilli, and (3) negative catalase and oxidase reactions.[247,252] Isolates should be referred to a laboratory experienced with *Bartonella* spp. for confirmatory identification; definitive diagnosis requires molecular testing (discussed later).

The use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been proposed as a potentially useful tool for rapid identification of *Bartonella* spp. from a positive clinical culture. This technique permits bacterial identification based on unique patterns of peptidic spectra. In a proof-of-concept study, successful identification of 36 of 39 "blind-tested" *Bartonella* strains was demonstrated.[258] Subsequently, peptide mass reference spectra were constructed from *Bartonella* and other zoonotic pathogens and added to a reference database, the MALDI-TOF MS Biotyper System (Bruker, Billerica, MA), for research purposes.[259] Test results are rapid (within minutes); limitations of this testing modality include the lack of available MALDI-TOF MS technology at most clinical laboratories and that testing requires isolation of a positive culture from a clinical specimen.[258,259]

Molecular Methods for *Bartonella* Identification and Characterization

Numerous PCR assays, some using real-time DNA detection technology,[128,260,261] have been developed and become increasingly important for direct detection of *Bartonella* spp. in pus, skin lesions, tissue, blood, or serum. *Bartonella* DNA is rarely detected in sera from CSD lymphadenitis patients[262]; however, in the majority of patients with CSD, a *B. henselae* gene fragment specific for the citrate synthase, heat shock protein, or the *groEL* or 16S rRNA gene can be amplified from lymph node tissue by PCR.[63,155–157,261] Amplification of rRNA gene segments with universal primers, followed by direct nucleotide sequence analysis of the amplification products, is used in a number of reference laboratories to identify and speciate *Bartonella* isolates.[157] Improved detection of *Bartonella* DNA in histologic or other material, such as sera from culture-negative endocarditis, has been reported using nested PCR amplification techniques.[263,264]

PCR testing of excised cardiac valves has become one of the most important modalities for diagnosis of *Bartonella* culture-negative endocarditis.[99,100,101,113] In the case of an AIDS patient with aortitis of unknown etiology, broad-range bacterial 16S rRNA gene primers were used to detect *B. quintana* from DNA extracted from a paraffin-embedded sample of biopsied aortic tissue; the result was subsequently confirmed by serologic testing.[244] PCR amplification from a variety of tissues also has proved useful for the diagnosis of both regional and disseminated bartonellosis in SOT recipients.[129] A direct blood PCR-based method also identified *B. bacilliformis* in a subset of patients that had clinically suspected Carrión disease with a negative thin blood smear.[265] Use of PCR in the diagnosis of *B. bacilliformis* from dried blood spots also has been reported.[266]

PCR-based typing techniques have proven useful for epidemiologic characterization of *Bartonella* isolates, and some studies suggest a greater diversity of restriction fragment length polymorphism (RFLP) types in the blood of bacteremic HIV-infected people than in the CSD lesions of immunocompetent hosts.[267,268] Strain typing can be performed using PCR-based RFLP,[267–269] pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST),[270] repetitive extragenic palindromic PCR (REP-PCR),[20,271] enterobacterial repetitive intergenic consensus PCR (ERIC-PCR),[54] and multilocus variable-number tandem-repeat analysis (MLVA).[272] When these and other techniques are compared with PFGE, the latter tends to be less reliable than other typing methods for *Bartonella* spp. that have been studied.[270,273]

Serologic Testing for Detecting Infection With *Bartonella* Species

For *B. bacilliformis*, early testing using a simple sonicated lysate as antigen in an IgG immunoblot assay was reported to have 70% and 94% sensitivity in people with acute and chronic infection, respectively, but with suboptimal specificity.[274] An IFA had 82% and 93% sensitivity in people with acute and chronic infection, respectively, but at the expense of substantial cross-reaction with other *Bartonella* spp.[275] IFA also detected IgM in patients with early acute *B. bacilliformis* infection. When used in the endemic region to assess patients with syndromes compatible with *B. bacilliformis* disease, the low specificity of these tests may be relatively less important than for the geographically widespread *Bartonella* spp. An enzyme-linked immunosorbent assay for detecting *B. bacilliformis* infection in endemic areas used recombinant *B. bacilliformis* Pap31 protein,[276] resulting in a sensitivity of 84.5% and a specificity of 94% in 302 sera from at-risk Peruvian patients.[277]

A number of IFA[59,278–280] and enzyme immunoassays (EIAs) [252,281,282] have been described for *B. henselae* and *B. quintana* diagnosis. They most commonly have been used to demonstrate anti-*Bartonella* antibodies in people with CSD[59,278,280–282] or endocarditis[13,96–98] and in some cases of retinitis.[109] The CDC developed an IFA that uses *B. henselae* or *B. quintana* bacteria cocultivated with Vero cells on slides and then fixed.[59,278] This is the most widely adopted test for diagnosis of *Bartonella* infection, but preparation of slides, dilution of serum, and time-consuming reading of immunofluorescence on each slide affect the ability to make rapid, reproducible, and cost-effective diagnosis of *B. henselae* and *B. quintana*. In general, the sensitivity of this IFA is highest in the setting of CSD (≈84%,[59] and up to 95% when using the strictest clinical criteria for CSD[278]). The sensitivity is substantially lower among late-stage AIDS patients with culture-proven bartonellosis (≈75%).[103] In SOT recipients, this IFA appears to be at least 80% sensitive in the setting of disseminated disease and even higher in patients with regional lymphadenitis.[129] The *Bartonella* IFA sensitivity and specificity vary substantially for different commercial laboratories in the United States.

Depending on the laboratory, an IFA IgG titer of 1:64 for *B. quintana* or *B. henselae* indicates possible infection, and a titer greater than 1:256 strongly suggests recent or acute infection.[278] A fourfold rise in titer between acute and convalescent specimens is definitive.[283] For patients with culture-negative endocarditis, a very high serologic titer (IFA IgG ≥1:1024 in US laboratories or ≥1:800 at Unité des Rickettsies in France) provides supportive evidence of *Bartonella* endocarditis and has a high positive predictive value.[96] However, IFA assays performed only one time for a particular subject appear to be variably, and sometimes suboptimally, sensitive and specific.[280,281,284] Some patients with CSD can have low or negative titers that subsequently increase as late as 3 weeks after presentation[278]; thus obtaining repeat serology 3 to 4 weeks after presentation can increase the IFA sensitivity. It is important to note that the *Bartonella* IFA IgM is frequently negative, even in documented acute infection, and cannot be relied on to diagnose acute *Bartonella* infection. Also of importance, IFA tests often show extensive cross-reactivity between antibodies to *B. quintana* and *B. henselae,* and often other *Bartonella* spp.[278] Furthermore, in some of these assays there may be serologic cross-reaction with antibodies to *Chlamydia* spp. and *Coxiella burnetii,* two other potential agents of "culture-negative endocarditis" in humans; however, the *Bartonella* titer usually is much higher than the cross-reacting genus titer.[13,96,279,284] Despite the challenges in interpretation outlined earlier, serology, typically IFA, remains the mainstay of diagnosis for most clinical syndromes of bartonellosis.

EIA and IFA methodologies that use partially,[97,252,285] highly purified,[286] or recombinant[276,287] antigen preparations ultimately may prove more species sensitive and specific. In addition, the use of specific fractions or purified proteins may enhance the standardization, interpretation, and efficiency of serological testing for *Bartonella*.[288]

Antimicrobial Susceptibility Testing of *Bartonella* Isolates

It is important to note that in vitro susceptibility testing of *Bartonella* isolates is often not relevant to the in vivo patient treatment response, and thus there is a well-demonstrated lack of correlation between the in vivo and in vitro susceptibilities. In addition, susceptibility testing

methods have not been standardized for *Bartonella* due to its extremely fastidious nature. In vivo, *B. henselae* and *B. quintana* infections respond rapidly to erythromycin, doxycycline, and rifampin. If required, antimicrobial susceptibility testing can be performed by incorporation of antimicrobial agents into either blood or chocolate agar and then testing by the agar dilution technique.[15,289] *Haemophilus* test medium with a broth microdilution technique also has been described, but the Etest (bioMérieux, France) may be the most practical means to assess in vitro susceptibility of *B. quintana* and *B. henselae* isolates.[290–292] Testing in other types of systems also has been reported.[293–296] In vitro, *B. henselae* isolates appear to be susceptible to many antibacterial agents tested but are intrinsically resistant to nalidixic acid and cefazolin, which can be useful for preparing selective agar.[217] *B. quintana* has a similar susceptibility pattern to *B. henselae* in vitro. In summary, *Bartonella* infections in patients are most reliably responsive to tetracycline, macrolide, and rifamycin classes.

TREATMENT AND PREVENTION OF *BARTONELLA* INFECTIONS

Consensus recommendations for treatment of *Bartonella* spp., based on the best available evidence at the time, were published in 2004.[297] However, the increasing identification of small vessel vasculitis, glomerulonephritis, and renal compromise associated with *Bartonella* endocarditis necessitate great caution in, or avoidance of, using gentamicin (or other potentially nephrotoxic antimicrobials) as an adjunct antimicrobial in the first 2 weeks of doxycycline treatment of *Bartonella* endocarditis, although suggested in previous guidelines (see later).[114,115,117]

Treatment of *Bartonella bacilliformis* Infections

For acute *B. bacilliformis* infection in South America (Oroya fever), oral ciprofloxacin with ceftriaxone for 14 days is recommended for first-line treatment,[39,297–299] although some reports of intrinsic and in vivo fluoroquinolone resistance suggest that fluoroquinolones may not provide optimal treatment.[300–302] Alternative antibiotic options for Oroya fever include chloramphenicol (adults: 500 mg orally [PO] or intravenous [IV] every 6 hours; children: 50 to 75 mg/kg/day PO or IV divided into four doses) for 14 days, coadministered with a β-lactam antibiotic (e.g., amoxicillin-clavulanate).[297,298] Parenteral therapy can be substituted for severe illness or if oral intake or bowel absorption is impaired. Currently, in Peru the primary agent of choice in the treatment of eruptive-phase lesions (verruga peruana) is oral azithromycin for 7 to 14 days (<14 years of age, <45 kg: single dose of 10 mg/kg/day; ≥14 years of age, >45 kg: single dose of 500 mg/day).[298] Alternative agents include rifampin for 21 to 28 days or ciprofloxacin for 14 days.[85,297–299]

Treatment of *Bartonella* Infections in the Immunocompromised Host

For immunocompromised patients with HIV infection, periodically updated guidelines for prevention and treatment of opportunistic infections in adults and adolescents have been developed under the auspices of the National Institutes of Health, CDC, and Human Immunodeficiency Virus Medicine Association of the Infectious Diseases Society of America.[303] This information can be accessed online at https://aidsinfo.nih.gov/guidelines/html/4/adult-and-adolescent-opportunistic-infection/329/bartonellosis. For *B. henselae* or *B. quintana*, in vitro susceptibilities often do not predict in vivo response to therapy, as noted earlier in "Antimicrobial Susceptibility Testing of *Bartonella* Isolates." Indeed, in contrast to in vitro sensitivities, CSD and BA/BP have developed in, and *Bartonella* has been cultured from, HIV-infected patients or SOT recipients while receiving ongoing therapy with TMP-SMX, β-lactam antibiotics, or fluoroquinolone antibiotics.[43,121,129,303] Thus these antibiotics are not recommended for empirical treatment of *B. quintana* or *B. henselae*. In contrast, therapy with rifampin, tetracyclines, or macrolides (even a single dose) dramatically reduced culture recovery of *B. quintana* or *B. henselae* from blood and BA/BP lesions. In addition, treatment or prophylaxis with a macrolide appears to protect against development of *Bartonella* infections.[43] In particular, the routine use of rifabutin, clarithromycin, or azithromycin for the prevention of *M. avium* complex infections in people with late-stage AIDS appears to have reduced the incidence of *Bartonella* infections.[103]

On the basis of observed clinical effectiveness in immunocompromised patients with culture-proven *Bartonella* infections, the recommended initial agent of choice to treat *Bartonella* infections is doxycycline (100 mg PO or IV every 12 hours) or erythromycin (adults: 500 mg PO or IV every 6 hours; pediatric: 40 mg/kg/day in four divided doses, maximum total daily dose, 2 g/day) for a minimum of 3 months.[43,297,303–305] For severe illness, rifampin can be added to doxycycline, although interactions with antiretroviral therapy or immunosuppressants should be considered. Azithromycin (500 mg PO daily) or clarithromycin (500 mg PO twice a day) is recommended as an alternative treatment.[303] In addition, azithromycin can be considered for patients at risk for antibiotic nonadherence.[303]

In SOT recipients with disseminated bartonellosis, treatment regimens consisting of doxycycline, or macrolides (azithromycin, erythromycin, or clarithromycin), or a combination of doxycycline and a macrolide has been clinically effective.[129] However, due to the poor tolerability of erythromycin and potential drug-drug interactions with antiretroviral and immunosuppressive agents, doxycycline is often preferred over erythromycin in transplant recipients and HIV-infected individuals.

Relapse occurs frequently in immunocompromised patients; to prevent relapse, immunocompromised patients should receive at least a 3-month course of initial therapy with doxycycline or a macrolide for any manifestation of *Bartonella* infection. For immunocompromised patients with widely disseminated disease (e.g., BA/BP), an initial treatment duration of 6 months should be considered. If relapse occurs after initial treatment of 3 to 6 months, treatment should be extended for a minimum of another 3 months. If subsequent relapse occurs, chronic suppressive therapy with doxycycline or erythromycin should be considered. HIV-infected patients starting antiretroviral therapy should be treated for *Bartonella* until the CD_4^+ cell count is >200/mm^3 for 6 months or longer.[303]

Treatment of *Bartonella* Bacteremia and Endocarditis

In World War I trench fever (*B. quintana* bacteremia without endocarditis) appeared to spontaneously resolve without antibiotic treatment, although many soldiers experienced one or more relapses before ultimate resolution.[7,8] Treatment of modern-day trench fever was studied in an open, randomized trial enrolling homeless patients in France.[106] One group received doxycycline for 4 weeks plus gentamicin for the first 2 weeks, and the other group did not receive antimicrobial therapy. Clearance of *B. quintana* occurred in 7 out of 7 treated but only 2 of 9 untreated patients. One patient in the treatment group developed increased creatinine.[106] The current recommendation for treating isolated *Bartonella* bacteremia in immunocompetent individuals (after endocarditis has been ruled out) is with 4 weeks of doxycycline plus gentamicin in the first 2 weeks.[106,297]

For confirmed *Bartonella* endocarditis, parenteral therapy is indicated initially. Some published guidelines recommend IV gentamicin for the first 2 weeks, in combination with 6 weeks of doxycycline (100 mg PO or IV every 12 hours).[297,306] This recommendation is based largely on retrospective, observational data suggesting improved outcomes of patients with *Bartonella* endocarditis who received an aminoglycoside.[98] However, it is important to note that before *Bartonella* endocarditis is suspected and diagnosed, it usually has been present for many months or even a year, and thus renal failure is frequently present at presentation in patients with *Bartonella* endocarditis (or develops shortly thereafter), as the result of small vessel vasculitis and/or deposition of immune complexes in the kidneys. In one study 44% of patients with *B. quintana* and 50% of patients with *B. henselae* endocarditis had renal failure.[99] In addition, the number of reports of *Bartonella* endocarditis-associated vasculitis and glomerulonephritis has increased dramatically recently, prompting caution about the use of any nephrotoxic antibiotics for *Bartonella* endocarditis treatment.[114–117] Furthermore, in a recent meta-analysis the combination of gentamicin and doxycycline was not significantly more effective than other regimens.[307] There are reports of successful therapy for endocarditis with rifampin-containing regimens, most commonly paired with doxycycline for adults (doxycycline 100 mg PO or IV every 12 hours), or a macrolide for children.[101,115] Thus rifampin,

which has excellent intracellular penetration, can be added to doxycycline for 6 weeks (adults: 300 mg PO or IV every 12 hours; pediatric: 10 mg/kg PO or IV every 12 hours, maximum dose 600 mg/day), and is the preferred substitute for gentamicin, which penetrates cells poorly.

Relapsing infection is the hallmark manifestation of *Bartonella*, and treatment of *Bartonella* endocarditis is often extended beyond 6 weeks of antibiotic therapy. Because anti-*Bartonella* antibody titers are often very high in patients with endocarditis, obtaining titers diluted to end point every 6 to 8 weeks to ensure a decrease may be useful for determining duration of therapy. For HIV-infected and other immunocompromised patients, ≥3 months of treatment is appropriate,[297] as well as in immunocompetent patients when fever or bacteremia is persistent or recurrent.[109] Valve surgery is required for *Bartonella* endocarditis in the majority of cases.[98]

Treatment of Cat-Scratch Disease

There have been anecdotal reports of the utility of various agents[308,309] in the treatment of CSD. However, in the immunocompetent host, only azithromycin has been demonstrated to accelerate the resolution of typical CSD lymphadenopathy in a placebo-controlled, double-blind study.[169] The value of antibiotic therapy for CSD in immunocompetent patients remains uncertain because of the usually benign outcome of most manifestations[308] and the risk of adverse drug reactions and potential for generation of antimicrobial resistance. If antimicrobial treatment is contemplated for treating extensive CSD lymphadenitis, oral azithromycin (adults: 500 mg on day 1 and then 250 mg PO once daily on days 2–5; pediatric: 10 mg/kg PO on day 1 [maximum dose 500 mg] and 5 mg/kg [maximum dose 250 mg] PO once daily on days 2–5) should be the agent of first choice.[297] Limited data in children with hepatosplenic CSD has shown favorable clinical outcomes with rifampin therapy.[175] Due to the risk of developing rifampin resistance with monotherapy, combining rifampin with azithromycin for 14 days is the preferred treatment if rifampin is used.

Treatment of Neuroretinitis, Encephalopathy, Osteomyelitis, and Parenchymal Cat-Scratch Disease

Although the utility of antibiotic therapy in altering the course of neurologic and ophthalmologic manifestations of CSD has not been studied rigorously, two-agent therapy for neuroretinitis appears to accelerate resolution in comparison with untreated historical control cases. The agents used in such cases have usually been erythromycin or doxycycline (with or without combined rifampin) or, alternatively, azithromycin or clarithromycin.[109,190] Currently, treatment with 4 to 6 weeks of oral doxycycline (adults: 100 mg PO or IV every 12 hours) or azithromycin (pediatric: 10 mg/kg PO on day 1 [maximum dose 500 mg] and then 5 mg/kg [maximum dose 250 mg] PO once daily thereafter) plus rifampin therapy (adults: 300 mg PO or IV every 12 hours; pediatric: 10 mg/kg PO or IV every 12 hours [maximum dose 600 mg/day]) is recommended for neuroretinitis.[297] Systemic corticosteroid therapy is often administered by ophthalmologists as an adjunct to antimicrobials; no definitive advantage or disadvantage to such an approach has been demonstrated. For other neurologic manifestations of CSD, the combination of doxycycline and rifampin also is recommended for HIV-infected patients[303] and immunocompetent individuals.[297] For immunocompetent patients with CSD and bony or parenchymal (liver, spleen) involvement, initial parenteral therapy for several weeks may be advantageous, and oral therapy is then often continued for 4 to 6 weeks total.

Prevention of *Bartonella* Infections

Prevention of *B. bacilliformis*, *B. henselae,* and *B. quintana* infections is probably best achieved by avoiding the locales or circumstances in which one is exposed to the arthropod vectors harboring *Bartonella*. Eradication of body louse infestation reduces the risk of *B. quintana* infection.[303] Prevention of *B. henselae* (and possibly *B. koehlerae)* infection entails avoidance of interactions with cats that might result in scratches, bites, or licks and exposure to cat fleas or flea feces.[303] Feral cats, cats that are allowed outdoors, cats with fleas, and kittens (<12 months of age) all have a higher risk of being infected with *B. henselae*.[310] The role of cat fleas in the direct transmission of *B. henselae* to humans is not known. However, treatment of pet cats for flea infestation is extremely important because flea feces carry large numbers of *B. henselae* organisms, and infected flea feces can readily contaminate the claws of cats, resulting in transmission of *B. henselae* to humans during a scratch. Human cat owners or contacts who are immunosuppressed should be especially careful to avoid cat scratches and to eliminate cat fleas from their cats and home. Antibiotic therapy does not durably eliminate bacteremia in cats implicated in CSD transmission or cats with demonstrated *B. henselae* bacteremia,[51,311–313] and thus treatment of pet cats with antibiotics is not recommended.[303] Direct contact with cat feces or urine does not appear to present a risk for *B. henselae* infection in humans. In general, removal of cats from the household of immunocompetent or immunocompromised humans is unnecessary as long as the flea control and contact precautions described earlier are maintained.[303]

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The complete reference list is available online at Expert Consult.

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235 *Klebsiella granulomatis* (Donovanosis, Granuloma Inguinale)

Bradley P. Stoner and Hilary E.L. Reno

SHORT VIEW SUMMARY

Definition
- Donovanosis is a chronic ulcerative sexually transmitted infection caused by the intracellular bacterium *Klebsiella granulomatis.*

Epidemiology
- Donovanosis is generally restricted to developing countries in tropical locations; it is rare in developed countries.

Microbiology
- *K. granulomatis* is a gram-negative encapsulated intracellular bacterium that demonstrates bipolar densities on staining.

Diagnosis
- The diagnosis is confirmed by visualizing Donovan bodies on microscopic examination of biopsy specimens or crush preparation smears prepared from active ulcerative lesions.

Therapy
- The treatment of choice is azithromycin, 1 g weekly (or 500 mg daily) for at least 3 weeks or until lesions have healed and complete epithelialization has occurred.

Donovanosis is a chronic, progressive ulcerative disease of the skin and subcutaneous tissues that is caused by the encapsulated, gram-negative bacterium *Klebsiella* (formerly *Calymmatobacterium*) *granulomatis.* The infection is also commonly referred to as granuloma inguinale, but because this term can easily be confused with lymphogranuloma venereum (caused by the invasive L-serovars of *Chlamydia trachomatis*), many experts now recommend *donovanosis* as the preferred term. McLeod[1] is credited with providing the first description of donovanosis based on his work in India in 1881; this was followed by the discovery of the causative organism by Donovan[2] in 1905. Donovanosis is uncommon in developed, nontropical settings, and cases are sporadically reported from Papua New Guinea, South Africa, India, Brazil, and Australia.[3]

BIOLOGY OF CAUSATIVE ORGANISM

K. granulomatis is an encapsulated intracellular pleomorphic gram-negative bacillus (dimensions approximately 1–2 µm long by 0.5–0.7 µm wide) that resides in cytoplasmic vacuoles of large mononuclear cells.[4] The bacteria are described as having bipolar densities when stained (Donovan bodies), which give the appearance of closed safety pins on microscopic examination. The bacteria multiply intracellularly and are subsequently released on rupture of mature intracytoplasmic vacuoles leading to infection of surrounding cells. Ultrastructurally the organisms are described as having a clearly defined capsule and the absence of flagella, along with small surface projections resembling pili or fimbriae and electron-dense granules (35–45 µm in diameter) in the cell periphery.[5] These granules had previously been thought to represent evidence of bacteriophage infection; however, this hypothesis remains controversial.[6]

In the 1940s investigators reported successful culture of the organism in chick embryo yolk sacs[7] and subsequently in egg yolk–based media and defined liquid media.[8] However, no pure isolates were stored for further study, hampering further characterization of the organism. In the 1990s renewed efforts to isolate the bacterium from clinical specimens were successful using human monocyte cultures[9] and Hep-2 cell monolayers.[10] In 1999 detailed molecular analyses of *Calymmatobacterium granulomatis* phylogeny ultimately resulted in its proposed reclassification as a *Klebsiella* species.[11,12]

GEOGRAPHIC DISTRIBUTION AND EPIDEMIOLOGY

Donovanosis is a relatively rare disease in developed countries. In the United States, it is estimated that less than 100 cases occur annually, although epidemiologic tracking is difficult because it is not a nationally reportable condition. Donovanosis is recognized as a cause of genital ulcer disease in parts of India, Papua New Guinea, the Caribbean, and South America (particularly Brazil) and has been identified in Zambia, Zimbabwe, South Africa, Southeast Asia, and among Aboriginals and Torres Strait Islanders in Australia.[3,13] The fact that these are all tropical locations raises the possibility of ecologic tropism, that is, preferential organismal survival and infectivity in tropical environments, although this has not been proved. The reported incidence has decreased significantly in recent years in Australia,[14] Papua New Guinea,[15] India,[16] South Africa,[17] India, and Jamaica,[18] owing to either greater recognition of donovanosis as a public health problem and the implementation of appropriate measures for prevention and control or general improvements in living standards and health service provision in endemic areas. Still, cases of donovanosis continue to be encountered in parts of the world remote from these endemic regions (e.g., metropolitan France[19]) as a result of immigration to developed countries or alternatively through increased global travel to predominantly tropical areas.[20] In most cases the disease is considered to be sexually acquired: infection rates of 52% have been reported among steady sex partners of individuals with donovanosis.[21] However, the possibility of nonsexual transmission remains a controversial hypothesis. In the 1960s Goldberg[22,23] postulated that the causative organism may be a gastrointestinal tract commensal and that the vagina may be infected through autoinoculation. Nonsexual transmission is further suggested by lesions in very young children and the occurrence of infection in extragenital sites (e.g., mastoiditis and meningitis).[24] However, the age distribution in endemic areas, the common co-occurrence of other sexually transmitted infections, and the high frequency of genital tract lesions support the assertion that donovanosis is predominantly transmitted through sexual contact.

CLINICAL MANIFESTATIONS

The primary lesion of donovanosis begins as a small indurated nodule or painless papule that develops at the site of exposure following an incubation period estimated to be between 3 and 40 days based on epidemiological observations.[25] The lesion soon ulcerates to form a painless, exuberant, beefy red, granulomatous ulcer with rolled edges that bleeds easily on contact (Fig. 235.1). Multiple smaller lesions may coalesce into large areas of ulceration, and new ulcers may appear via autoinoculation. The disease extends into subcutaneous tissues and may become progressively more destructive (Fig. 235.2). Spontaneous healing may result in scar formation and tissue deformities (Fig. 235.3). In severe cases, lymphatic blockage may occur resulting in lymphedema

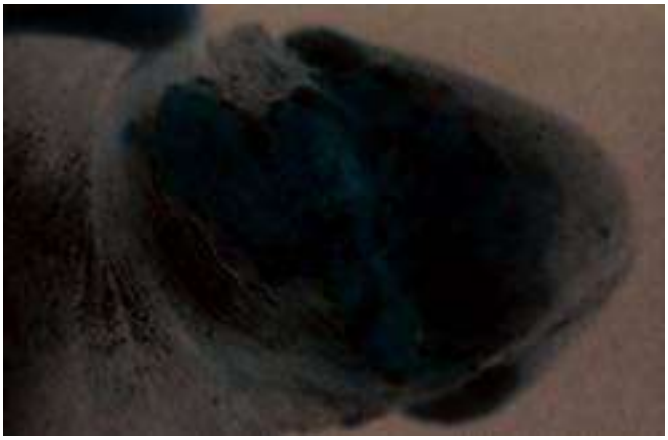

FIG. 235.1 Early lesion of donovanosis.



FIG. 235.2 Extensive active lesions of donovanosis.



FIG. 235.3 Late lesions of donovanosis showing extensive scarring and subcutaneous spread.

and external genital elephantiasis.[26] The prepuce, coronal sulcus, and penile shaft are commonly reported sites of infection in men. In women the labia and fourchette are commonly involved sites, and vaginal wall and cervical lesions may also occur. Subcutaneous inguinal spread may lead to pseudobubo formation without lymph node involvement. Hypertrophic lesions have been reported during pregnancy,[27] raising concern that pregnancy may exacerbate disease outcomes.[28] However, increases in diagnoses during pregnancy may simply reflect the detection of asymptomatic disease during routine prenatal pelvic examination. Rectal lesions may be associated with receptive anal intercourse,[29] and oral lesions have been reported in association with orogenital contact.[30]



FIG. 235.4 Scraping from an active lesion of donovanosis showing typical Donovan bodies in large mononuclear cells (Giemsa stain, ×100).

Systemic disease is rare but appears to be more common in women with primary lesions of the cervix.[31] Hematogenous spread of infection is rare but has been documented, resulting in the formation of pelvic granulomas, bone and joint involvement, and lymphadenitis.[32] Constitutional symptoms are generally absent except in cases with coinfection with other sexually transmitted agents, secondary bacterial infection, or extensive spread. Donovanosis should be distinguished from other causes of genital ulcer disease, regional lymphadenopathy, and genital elephantiasis. The lesions most likely to resemble donovanosis clinically include cases of pseudogranulomatous chancroid, ulcerating genital warts, primary and secondary syphilis, and squamous cell carcinoma. Multiple infectious agents have been isolated from a single ulcerative lesion, raising the possibility of coinfection with other sexually transmitted pathogens.[17,33] Early reports suggested a link between donovanosis and squamous cell carcinoma of the external genitalia.[34] This hypothesis remains unproven; however, more recent case reports of donovanosis with malignant transformation again raise this concern.[35,36] The possible coexistence of donovanosis and oncogenic human papillomavirus may be one explanation for this phenomenon. There have been no confirmed cases of congenital donovanosis infection, but two cases have been described in infants younger than 6 months presenting with Donovan body–positive external auditory canal polyps, otitis media, and mastoiditis.[37] The infants in these two cases were born to mothers with human immunodeficiency virus infection with genital donovanosis lesions.

DIAGNOSIS

Clinical evaluation provides the basis for a presumptive diagnosis of donovanosis: classic-appearing, slow-growing, relatively painless, bright red hypertrophic lesions that bleed easily on contact.[4] Wherever possible, confirmation of the diagnosis should be obtained through histologic examination of clinical specimens from active lesions. These may include punch biopsy specimens or scrapings taken from the edges of active lesions or crush preparation smears of granulation tissue taken by scalpel or forceps after cleaning with physiologic saline.

Although biopsy specimens are mandatory to exclude malignancy, crush preparation smears are adequate in most cases for diagnosis of acute active disease.[38] Ideally the smear preparations should be made immediately with fresh moist tissue and should be fixed and stained with Giemsa, Leishman, or Wright stain before being examined microscopically.[4,39] Although the use of rapid Giemsa techniques may provide an immediate definitive diagnosis,[40] most experts prefer standard Giemsa or silver stains for histologic examination of fixed, embedded tissue specimens.[4] The demonstration of typical bipolar-staining intracellular Donovan bodies in stained smears obtained from lesions (Fig. 235.4) remains the gold standard for diagnosis of donovanosis. Donovan bodies may also been detected in Papanicolaou-stained smears obtained from women with cervical lesions.[41–43]

Intradermal skin tests and complement fixation serologic tests for donovanosis lack sufficient sensitivity and specificity and are not routinely performed in clinical practice.[4] A sensitive and specific indirect immunofluorescence serologic test has been developed using tissue sections from proven cases of donovanosis as the antigen.[44] However, a lack of suitable clinical material that may be used as the antigen limits widespread use of this test. The ability to culture *K. granulomatis* in monocytes[9,45] and Hep-2 cells[10] may ultimately lead to the development of additional serologic tests in the future and offers the possibility of developing in vitro antimicrobial susceptibility testing of clinical isolates to generate more rational therapeutic decision-making approaches.

Using DNA extracted from biopsy material, Australian researchers demonstrated a high degree of molecular homology between *K. granulomatis* and other *Klebsiella* spp. by sequencing a region of the *phoE* (phosphatase porin) gene of *K. granulomatis*.[46] Although there appears to be a high degree of homology between *phoE* genes of *K. granulomatis* and other *Klebsiella* spp., researchers were able to amplify a 700-bp region demonstrating two base changes that occur only in *K. granulomatis,* thereby differentiating it from other *Klebsiella* spp. This product was subsequently digested with the *Hae*III restriction endonuclease to yield a 167-bp fragment unique to *K. granulomatis*.[47] This molecular approach was later refined to include a colorimetric detection system.[48,49] This work offers a foundation for development of future diagnostic methods for donovanosis and may ultimately provide a viable routine alternative to culture or microscopy.

THERAPY

Prompt initiation of antimicrobial therapy slows progression of lesions and limits further tissue destruction. However, there is no global consensus about the ideal treatment for donovanosis because most antibiotics have been evaluated in open trials, with few data available from comparative, microbiologically controlled studies. Azithromycin (1 g once per week orally or 500 mg daily orally for at least 3 weeks) is now endorsed as the first-line recommended agent by US and European treatment guidelines based on successful outcomes in numerous cases series and individual case reports.[3,50] The optimal duration of treatment cannot be stated categorically because larger lesions appear to require a longer duration of therapy.

With successful therapy, lesions begin to heal from the edges toward the center. Experts suggest that treatment should be continued until complete epithelialization has occurred, which can take several weeks; otherwise relapse may occur. Historically, tetracycline (500 mg four times daily orally) or doxycycline (100 mg twice daily orally) were the treatments of choice for the disease, although many treatment failures or relapses were recorded.[51] Similarly, trimethoprim-sulfamethoxazole (trimethoprim 80 mg/sulfamethoxazole 400 mg, two tablets twice daily orally) proved effective in many cases, but relapse was common (presumably due to inadequate duration of treatment).[4,13] Erythromycin (500 mg four times daily orally) has also been used extensively, especially in pregnancy (with or without the addition of an aminoglycoside such as gentamicin).[4,15] However, gastrointestinal side effects are commonly reported.

Studies of azithromycin demonstrate its clinical effectiveness,[52] although randomized controlled trials are lacking. Outcomes data indicate that 1 g taken orally weekly for 4 to 6 weeks may be the most effective strategy.[53] This antibiotic may be particularly appropriate for the treatment of donovanosis because it concentrates within macrophages and exhibits a long tissue half-life. An added advantage of azithromycin is that it may also have activity against other sexually transmitted bacteria—notably *Haemophilus ducreyi, Treponema pallidum,* and *C. trachomatis.* Other medications that have proved effective include quinolone antibiotics,[54] chloramphenicol and thiamphenicol,[55] ceftriaxone,[56] and the aminoglycosides gentamicin and streptomycin, which have often been used to supplement tetracycline therapy in severe cases.[55,57] In contrast, penicillin appears to be ineffective for treatment of the disease. Anecdotal evidence suggests that lesions may be more extensive and that prolonged periods of therapy may be required for patients with donovanosis who are coinfected with human immunodeficiency virus.

ACKNOWLEDGMENT

The authors thank Dr. Ronald C. Ballard for his contributions to the field and for writing the chapter in the eighth edition of this text.

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The complete reference list is available online at Expert Consult.

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236 Other Gram-Negative and Gram-Variable Bacilli

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SHORT VIEW SUMMARY

Definition
- This chapter includes gram-negative and gram-variable aerobic bacilli that are less commonly encountered as causes of infection and are not discussed in other chapters.
- The taxonomy of many of these organisms has been and continues to be in a state of flux.
- The organisms discussed are broadly divided into those that ferment glucose and those that weakly ferment or do not ferment glucose. Specific genera discussed include the glucose fermenters (*Actinobacillus* and *Aggregatibacter, Aeromonas, Cardiobacterium, Chromobacterium, Dysgonomonas, Kingella,* zoonotic *Neisseria* species, *Plesiomonas*), and the glucose nonfermenters and weak fermenters (*Achromobacter* and *Alcaligenes, Chryseobacterium* and *Elizabethkingia, Comamonas* and *Delftia, Eikenella, Flavobacterium* and *Myroides, Ochrobactrum, Oligella,* less common species of *Pseudomonas, Ralstonia* and *Cupriavidus, Rhizobium, Roseomonas, Shewanella, Sphingobacterium, Sphingomonas,* and *Weeksella* and *Bergeyella,* as well as Centers for Disease Control and Prevention Groups NO-1, WO-1, WO-2, O-1, O-2, and O-3 and *Gardnerella* and *Mobiluncus*).

Epidemiology
- Some of these gram-negative bacilli are ubiquitous in the environment.
- Many are generally considered to be of low virulence but may be opportunistic pathogens under certain circumstances.
- Some infections have been linked to hospital sources, particularly unclean water sources, nonsterile environmental surfaces, or contaminated solutions.

Microbiology
- Many of these organisms are fastidious and difficult to grow.
- Identification of glucose-nonfermenting gram-negative bacilli poses a challenge to clinical laboratories because conventional biochemical systems frequently fail to provide accurate identification. Accurate identification, especially to species level, often requires cell wall fatty acid analysis, 16S ribosomal RNA gene sequencing–based technologies, or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Diagnosis
- The types of infections caused by these organisms are quite varied and are diagnosed by analysis of cultures of samples obtained from the infection site.
- Because these organisms are infrequently encountered and are often of low virulence, interpretation of culture results must be correlated with clinical findings.

Therapy
- The small number of patients reported and the variety of antibiotic regimens used do not permit identification of the optimal therapeutic regimen for most of these organisms.
- Methods for antibiotic susceptibility testing for many of these organisms are not standardized and Clinical and Laboratory Standards Institute breakpoints for interpretation may not be available.
- Some of the environmental bacteria are of concern because they carry resistance genes on transferable genetic elements and could serve as reservoirs of resistance genes when introduced into the clinical setting.
- Some of these infections may be difficult to eradicate when the causal bacteria exist in well-developed biofilms.

Prevention
- Attention should be given to practices to prevent device-related infections, particularly in immunocompromised individuals.

A large number of gram-negative aerobic bacilli have been reported to cause human infection. In this chapter, selected gram-negative and gram-variable organisms are discussed that have not been described in other chapters and are important in certain clinical or epidemiologic circumstances, are newly described, or present special problems of diagnosis or therapy. For some of the bacteria considered here, taxonomy is in a state of flux as classifications based on phenotypic characteristics are replaced by contemporary measures of genetic relationship, including 16S ribosomal RNA (rRNA) sequencing studies. Current nomenclature and previous designations are listed in Table 236.1.

Identification of some of these organisms is difficult; the automated systems used by many microbiology laboratories cannot identify some of these bacteria and often misidentify others. Consequently, clinical laboratories sometimes use a general description (e.g., gram-negative nonfermenter) rather than the genus and species name. The clinical site of infection (as shown in Table 236.2), colony morphology, and the ability of the organism to metabolize carbohydrates by fermentation provide clues that can suggest a particular organism or group of organisms. This information can help select the most effective way to provide definitive identification because for some of these organisms, special procedures for recovery, characterization, or antimicrobial susceptibility testing are required. The decision to use alternative diagnostic methods is often based on the perceived clinical significance of the isolate, economic considerations, and available expertise. Cell wall fatty acid analysis and molecular methods, such as 16S rRNA gene sequencing, have been used to identify difficult organisms, but these methods are not available in most clinical laboratories. The introduction of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry for identification of microorganisms in clinical laboratories may overcome some of the limitations of biochemical-based identification and allow for identification of microorganisms that are difficult to identify using traditional biochemical methods.

Because complete identification is often not pursued, infections caused by some of these uncommon pathogens may go unrecognized. In addition, there are no published methodologic guidelines or interpretive breakpoints for susceptibility testing for most of these organisms. Consequently, reported susceptibility test results from the literature can be difficult to interpret, especially if methods and interpretive criteria are not specified. For susceptibility testing of organisms for which there are no US Food and Drug Administration or Clinical and Laboratory Standards Institute (CLSI) interpretive breakpoints, microbiology reports are generally limited to the minimal inhibitory concentration (MIC) value, and an interpretation is not provided.

GLUCOSE FERMENTERS

Actinobacillus and *Aggregatibacter* Species

Actinobacillus and *Aggregatibacter* species are coccoid to small gram-negative bacilli in the family Pasteurellaceae. These organisms are normal microbiota of the oral cavity, and less frequently the urogenital tract,

TABLE 236.1 Current Nomenclature and Previous Names of Gram-Negative Bacteria

CURRENT DESIGNATION	PREVIOUS NAMES
Glucose Fermenters	
Actinobacillus spp. *A. ureae*	*Pasteurella ureae, Pasteurella haemolytica* var. *ureae*
Aeromonas spp. *A. hydrophila* *A. caviae* *A. veronii* biotype *sobria*	*A. sobria*
Aggregatibacter actinomycetemcomitans	*Actinobacillus actinomycetemcomitans, Bacterium actinomycetemcomitans*
Cardiobacterium spp. *C. hominis* *C. valvarum*	
Chromobacterium violaceum	
Dysgonomonas capnocytophagoides	CDC DF-3
Kingella spp.	
Neisseria animaloris, Neisseria zoodegmatis	CDC EF-4a, CDC EF-4b
Plesiomonas shigelloides	*Aeromonas shigelloides, Pseudomonas shigelloides*
Glucose Nonfermenters (or Weak Fermenters)	
Achromobacter spp. *A. xylosoxidans* *A. denitrificans*	*Alcaligenes denitrificans* subsp. *xylosoxydans, Alcaligenes xylosoxidans* subsp. *xylosoxidans, Alcaligenes xylosoxidans* *Alcaligenes denitrificans, Alcaligenes xylosoxidans* subsp. *denitrificans*
Alcaligenes faecalis	*A. odorans,* CDC VI
Bergeyella zoohelcum[a]	*Weeksella zoohelcum,* CDC IIj
Chryseobacterium spp. *C. indologenes*	*Flavobacterium indologenes*
Comamonas spp. *C. testosteroni*	*Pseudomonas testosteroni*
Cupriavidus spp.[b] *C. pauculus* *C. gilardii*	*Wautersia paucula, Ralstonia paucula,* CDC group IVc-2 *Wautersia gilardii, Ralstonia gilardii*
Eikenella corrodens	*Bacteroides corrodens*
Elizabethkingia meningoseptica[c]	*Chryseobacterium meningosepticum, Flavobacterium meningosepticum*
Methylobacterium mesophilicum and *M. extorquens*[d]	*Pseudomonas mesophilica*; *Protomonas extorquens, Vibrio extorquens, Bacillus extorquens, Pseudomonas extorquens, Flavobacterium extorquens, Protaminobacter rubra,* "the pink phantom"
Myroides spp. *M. odoratus* *M. odoratimimus*	*Flavobacterium odoratum*
Ochrobactrum spp. *O. anthropi* *O. intermedium*	CDC Vd, *Achromobacter* groups A and D *Achromobacter* group C
Oligella spp. *O. ureolytica* *O. urethralis*	CDC IVe *Moraxella urethralis,* CDC M-4
Pseudomonas spp. *P. fluorescens* *P. putida* *P. stutzeri* *P. oryzihabitans* *P. luteola*	*Flavimonas oryzihabitans, Chromobacterium typhiflavum,* CDC Ve-2 *Chryseomonas luteola, Chryseomonas polytrichia,* CDC Ve-1
Ralstonia spp. *R. pickettii* *R. mannitolilytica*	*Pseudomonas pickettii, Burkholderia pickettii* *R. pickettii* biovar 3/"*thomasii,*" *Pseudomonas thomasii*
Rhizobium radiobacter	*Agrobacterium radiobacter, Bacillus radiobacter, Bacterium radiobacter, Rhizobium radiobacter, Achromobacter radiobacter, Alcaligenes radiobacter, Pseudomonas radiobacter, Agrobacterium tumefaciens,* CDC Vd-3
Roseomonas spp.	CDC pink coccoid groups I through IV
Shewanella putrefaciens	*Pseudomonas putrefaciens, Alteromonas putrefaciens, Achromobacter putrefaciens,* CDC Ib-1, Ib-2
Sphingobacterium spp. *S. multivorum* *S. spiritivorum*	*Flavobacterium multivorum,* CDC IIk-2 *Flavobacterium spiritivorum,* CDC IIk-3
Sphingomonas paucimobilis	*Pseudomonas paucimobilis,* CDC IIk-1
Weeksella virosa	*Flavobacterium genitale,* CDC II-f

[a]See "*Weeksella* and *Bergeyella* Species" in text.
[b]See "*Ralstonia* and *Cupriavidus* Species" in text.
[c]See "*Chryseobacterium* and *Elizabethkingia* Species" in text.
[d]See "*Roseomonas* Species and Other 'Pink-Pigmented' Gram-Negative Bacilli" in text.
CDC, Centers for Disease Control and Prevention.

TABLE 236.2 Classification of Selected Gram-Negative Aerobic Bacilli by Likely Site of Infection

	MOST LIKELY CLINICAL SETTINGS AND SITES OF INFECTION								
ORGANISM	**Bloodstream**	**Device Associated**	**Intestine**	**Soft Tissue**	**Osteoarticular**	**Bite Wound**	**Urine**	**CSF**	**Nosocomial Clusters**
Glucose Fermenters									
Aeromonas	X		X	X					
Aggregatibacter	X			X		X			
Cardiobacterium	X								
Chromobacterium	X			X					
Dysgonomonas			X						
Elizabethkingia	X							X	X
Kingella	X				X				
Neisseria animoralis, N. zoodegmatis (CDC group EF-4)						X			
Plesiomonas			X						
Glucose Nonfermenters (or Weak Fermenters)									
Achromobacter	X	X							X
Bergeyella						X			
Chryseobacterium	X						X		X
Comamonas	X							X	X
Cupriavidus	X								
Eikenella	X			X	X	X			
Methylobacterium	X	X							
Myroides	X								X
Ochrobactrum	X	X							X
Oligella							X		
Pseudomonas	X	X							X
Ralstonia	X								X
Rhizobium	X	X							
Roseomonas	X	X							
Shewanella	X			X					X
Sphingobacterium	X								
Sphingomonas	X	X						X	
Weeksella							X		

CDC, Centers for Disease Control and Prevention; *CSF,* cerebrospinal fluid.

in humans. They also colonize animals, which can serve as reservoirs for opportunistic human infections. The genus *Aggregatibacter* was created based on the phylogenetic similarity of *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus, Haemophilus paraphrophilus,* and *Haemophilus segnis.*[1] On transfer to the new genus, *Aggregatibacter aphrophilus* and *Aggregatibacter paraphrophilus* were combined into one species, *Aggregatibacter aphrophilus.*[1] The genus name reflects a propensity of these organisms to aggregate with other bacteria.

Aggregatibacter actinomycetemcomitans (formerly *Actinobacillus actinomycetemcomitans*) is the best known pathogen of this group. *A. actinomycetemcomitans* was first described as a human pathogen in 1912 and was initially called *Bacterium actinomycetem comitans.* Early isolates were recovered only in conjunction with *Actinomyces israelii* (hence the species designation), leading to speculation that *A. actinomycetemcomitans* was not itself capable of causing disease. After the introduction of penicillin, it was observed that *A. actinomycetemcomitans* sometimes could be recovered from persistent lesions of actinomycosis after *A. israelii* was eradicated. By the early 1960s, recovery of this organism in pure culture from blood and other normally sterile body fluids was reported widely. The organism is best known as a cause of endocarditis but has also been isolated in pure culture from patients with meningitis, brain abscess, endophthalmitis (with and without concomitant endocarditis), soft tissue infections, parotitis, septic arthritis, osteomyelitis, spinal epidural abscess, urinary tract infection, pneumonia, empyema, and pericarditis.[2,3] Soft tissue infections most commonly involve the cervicofacial area, although they can occur elsewhere, including the chest and abdomen. There are reports of *A. actinomycetemcomitans* mimicking actinomycosis and causing pneumonia with chest wall invasion.[4]

Although the organism is part of the endogenous microbiota of the mouth and can be recovered from about 20% of teenagers and adults, it (along with *Porphyromonas gingivalis*) is one of the major pathogens in adult and juvenile forms of periodontitis. Extraoral infections are believed to occur due to hematogenous dissemination from lesions in the oral cavity. *A. actinomycetemcomitans* is present in the periodontal pockets of more than 50% of adults with refractory periodontitis and 90% of patients with localized aggressive periodontitis (formerly called *localized juvenile periodontitis*), a destructive form of periodontitis characterized by loss of the alveolar bone of the molars and incisors. Clonal spread of the organism within families has been demonstrated using polymerase chain reaction (PCR)–based typing systems.[5]

A. actinomycetemcomitans is classified into seven serotypes (a through g). The prevalence of different serotypes and their association with

periodontal disease varies among geographic and ethnic populations. The JP2 strain of serotype b has enhanced virulence and is associated with significantly higher prevalence of periodontitis in people of African and Mediterranean descent. Serotype c is the most prevalent subgingival type in Asian individuals as well as in Brazil and the United States.[6] Serotypes a, b, and c are detected most frequently in German patients, and c and d are found in Korean patients.[7]

A. actinomycetemcomitans is a successful pathogen with well-characterized virulence factors, including two exotoxins: leukotoxin and cytolethal distending toxin (Cdt). The leukotoxin selectively binds to β_2-integrin and destroys leukocytes by inducing apoptosis or lysis.[8] Cdt is prevalent among certain gram-negative bacteria and acts by damaging DNA, which produces growth arrest and subsequent apoptosis of a wide variety of eukaryotic cell types.[8] Other virulence factors include proteins Aae and ApiA, which allow the organism to adhere to epithelial cells and become internalized. Production of didanosine tetraphosphate may enhance bacterial survival within the cytoplasm.[9] Induction of cytokines and other factors contribute to tissue destruction and resorption of alveolar bone.[10] Intracellular survival allows the organism to evade the host immune response, penetrate the epithelial cell layer, and reach the underlying connective tissues. *A. actinomycetemcomitans* is further able to evade host immune responses by inducing increased expression of Toll-like receptor 2, leading to phagocytosis and apoptosis of macrophages via p38 mitogen-activated protein kinase activation and tumor necrosis factor-α production.[11] *A. actinomycetemcomitans* in biofilms is strongly associated with loss of periodontal tissue attachment. The ability of this organism to adhere to abiotic surfaces and form biofilms has been attributed to type IVb–like fimbriae that are primarily composed of fimbrial lower-molecular-weight protein (Flp).

A. actinomycetemcomitans is one of the HACEK organisms, along with *Haemophilus parainfluenzae,* other *Aggregatibacter* spp. (*A. aphrophilus* [formerly *Haemophilus aphrophilus*] and *A. segnis*), *Cardiobacterium* spp. (*C. hominis, C. valvarum*), *Eikenella corrodens,* and *Kingella* spp. (*K. kingae, K. denitrificans*), which have in common that they are part of the normal oral microbiota, and have slow growth in culture, the need for incubation in an atmosphere enhanced with CO_2 for recovery in culture, and a predilection for causing endocarditis. The onset of endocarditis is usually insidious, with a mean time to diagnosis of about 3 months. In comprehensive reviews of *A. actinomycetemcomitans* endocarditis, almost half of patients had periodontal disease or recent dental work and over 60% had underlying native valvular disease (33%) or prosthetic valves (28%).[2,12] Fever was present in fewer than 50%; peripheral manifestations and splenomegaly each occurred in about one-third. Therapy was successful in 85% to 91%, but significant embolization was common (39%) and 23% required valve replacement.[2]

Prosthetic valve endocarditis with *A. actinomycetemcomitans* was usually recognized earlier than native valve endocarditis (42 vs. 106 days), which probably was attributable to a higher index of suspicion. This earlier diagnosis may account for the high cure rate achieved with antibiotics alone and a relatively low rate of embolization reported.

Culture isolation of *A. actinomycetemcomitans* is the usual means of diagnosis, and the fastidious, slow-growing nature of the organism makes this difficult. Cultures must be incubated in an enhanced (5%–10%) CO_2 atmosphere. By 18 to 24 hours, a few colonies (punctate, nonhemolytic) may be apparent on blood or chocolate agar, but the organism grows slowly and incubation for at least 48 hours is needed. After further incubation, a starlike structure tends to form in the center of the mature colony. The organism grows poorly on MacConkey agar. In broth or blood cultures, the organism often grows only in small "granules" adherent to the sides of the tube or bottle, with the medium remaining clear. Although the mean duration for incubation using continuously monitored blood cultures until detection is 3 to 5 days, up to 30 days may be required, especially if the patient has received prior antibiotic therapy.[12] This finding underscores the need to hold blood culture bottles for a prolonged time if endocarditis caused by a fastidious organism is suspected. The appearance of the organism on Gram stain is coccoid to coccobacillary, similar to *Haemophilus* species. *A. actinomycetemcomitans* is urease negative and indole negative, reduces nitrate, and usually is oxidase negative. It is catalase positive, which helps differentiate it from *A. aphrophilus*.[1] Most of the HACEK organisms are included in current MALDI-TOF mass spectrometry databases, which allows for rapid and more accurate identification.

A. actinomycetemcomitans usually is susceptible to cephalosporins (especially third-generation agents), rifampin, trimethoprim-sulfamethoxazole, aminoglycosides, fluoroquinolones (including ciprofloxacin and moxifloxacin), tetracycline, azithromycin, and chloramphenicol.[2,13] In vitro susceptibility to penicillin and ampicillin is variable, but test results do not necessarily correlate with the clinical outcome. In general, treatment of actinomycosis with penicillin and surgical drainage (when necessary) is sufficient, even when mixed infection is present. Vancomycin, erythromycin, and clindamycin have little activity against *A. actinomycetemcomitans.* The organism displays variable susceptibility to metronidazole, and in vitro synergy between metronidazole and both β-lactams and ciprofloxacin has been reported.[14] Because of strain-to-strain variability, testing of clinical isolates is recommended. CLSI provides conditions and breakpoints for broth microdilution susceptibility testing.[15] The bioMérieux Etest (bioMérieux, Inc., Hazelwood, MO) can be used with supplemented Mueller-Hinton agar, *Haemophilus* test medium, or *Brucella* agar supplemented with 5% sheep blood, hemin, and vitamin K incubated at 5% CO_2 for 24 to 72 hours. In the past, penicillin or ampicillin combined with an aminoglycoside was the usual treatment for endocarditis caused by this organism. Because of the potential for β-lactamase production, reports of failures with penicillin therapy, and difficulties with susceptibility testing, third-generation cephalosporins are now considered the drugs of choice. For endocarditis caused by HACEK organisms, the American Heart Association guidelines endorsed by the Infectious Diseases Society of America recommend ceftriaxone or ampicillin-sulbactam as initial therapy.[16] A fluoroquinolone may be used to treat patients with β-lactam allergy, but clinical data are limited.

A. actinomycetemcomitans endocarditis has developed after dental procedures despite the prophylactic use of penicillin, erythromycin, or vancomycin. Severe *A. actinomycetemcomitans*–associated periodontitis is usually treated with mechanical débridement in combination with oral tetracycline therapy. Tetracycline failures occur, however, and a report suggests that the combination of metronidazole and amoxicillin is effective in suppressing subgingival infection.[17]

Five species of *Actinobacillus*—*A. lignieresii, A. equuli, A. suis, A. hominis,* and *A. ureae*—are rare causes of human disease. The first three are commensals and opportunistic pathogens in animals, whereas the latter two are commensals of the human upper respiratory tract.[1] *A. lignieresii, A. suis,* and *A. equuli* rarely can cause infections after bite wounds from farm animals.[18] These infections can be polymicrobial. One report has described a boar hunter who developed endocarditis caused by an *Actinobacillus* organism that resembled *A. suis* and *A. hominis* biochemically.[19] Another report described 46 clinical *A. hominis* isolates acquired over a 22-year period, mostly from Copenhagen, Denmark.[18] Before this report, there were only a few case reports of human infections caused by this organism. Most of the isolates were from the respiratory tract; 18 of 33 respiratory isolates were reported to be pure cultures of *A. hominis.* The remaining respiratory cultures contained at least one other common respiratory pathogen. All the patients in this series had underlying diseases, including alcoholism, cardiovascular disease, drug addiction, chronic obstructive pulmonary disease, and cancer. Most patients had fever and pulmonary infiltrates, and 9 of 36 patients for whom clinical information was available died, including 1 of the 2 patients with bacteremia. The identification of the *A. hominis* isolates was confirmed by ribotyping and DNA hybridization. In this and other reports, automated systems had difficulty identifying *Actinobacillus* species. Fatal *A. hominis* bacteremia has also been reported in two patients with severe underlying liver disease.[20] *A. ureae* is a rare cause of bacteremia and meningitis. Nine of 14 cases of *A. ureae* meningitis were posttraumatic, and another occurred after neurosurgery.[21] Several patients had underlying chronic illnesses, including alcoholism and human immunodeficiency virus (HIV) infection.

Identification of *Actinobacillus* species is problematic. At the genus level, these organisms are biochemically similar to *Pasteurella* species. Species identification can be difficult without DNA hybridization studies.

A. ureae meningitis has been treated successfully with penicillin and third-generation cephalosporins.[21]

Aeromonas Species

Aeromonads are ubiquitous inhabitants of fresh and brackish water. They have also been recovered from chlorinated tap water, including hospital water supplies. They occasionally cause soft tissue infections and sepsis in immunocompromised hosts and increasingly have been associated with diarrheal disease and other infections in immunocompetent individuals.

Taxonomy of the aeromonads has been revised over the past few decades and continues to be in transition. Aeromonads are broadly divided into the mesophilic group, with optimal growth temperatures of 35°C to 37°C and associated with human infection, and the psychrophilic group, with optimal growth temperatures of 22°C to 25°C and associated with disease in fish. The species designations within the mesophilic group are currently largely based on DNA hybridization studies, but new information based on full-genome sequencing and microarray analysis will likely result in further taxonomic revisions.[22] *A. hydrophila, A. caviae* (synonym, *A. punctata*), and *A. veronii* biovar *sobria* are reported most frequently in human infections.[22] The pathogenic potential of *Aeromonas* species has been attributed to several virulence factors that are very heterogeneously present among clinical isolates. The pathogenicity of *A. hydrophila* has been attributed to the ability of the bacterium to produce the cytotoxic enterotoxin Act and cytotonic enterotoxins Ast and Alt, as well as a variety of proteases and type III secretion systems and surface structures, including pili and S-layer, lateral, and polar flagella, which allow the organism to attach to cells and enter tissue.[22] Carriage of multiple toxins appears to be a property of *A. hydrophila* but not other *Aeromonas* species. The aerolysin/hemolysin group of toxins, including Act, are important virulence factors in *A. caviae* and *A. veronii* biovar *sobria.* Alt and lateral flagella are notably significantly less prevalent in these species.[22]

Aeromonas was first isolated more than 70 years ago, but evidence implicating this genus as a cause of gastrointestinal disease has been amassed only since the early 1980s. Reports from diverse geographic locations have associated *Aeromonas* species with diarrheal disease in humans; in some locales, they are recovered as commonly as *Shigella* or *Campylobacter.* Many laboratories do not routinely culture stool for *Aeromonas,* so the incidence of *Aeromonas*-associated diarrhea may be underestimated. Evidence supporting a causative role in diarrheal disease includes (1) a higher carriage rate in symptomatic compared with asymptomatic individuals; (2) an absence of other enteric pathogens in most symptomatic patients harboring *Aeromonas* species; (3) identification of *Aeromonas* enterotoxins[22]; (4) improvement of diarrhea with antibiotics active against *Aeromonas* species and clinical worsening with antibiotics ineffective against the organism; and (5) evidence of a specific secretory immune response coincident with diarrheal disease.[23] Most of this information refers to *A. hydrophila* and *A. cavie*; the extent of clinical information about the other species in relation to diarrheal disease is limited.

Aeromonas caviae is the predominant isolate from diarrheal stools, but in some geographic areas, *A. hydrophila* and *A. veronii* biovar *sobria* are frequently isolated as well.[22,23] Other *Aeromonas* species appear to cause asymptomatic carriage only.[22] *Aeromonas*-associated diarrhea usually occurs during the summer, when the concentrations of aeromonads in water are the highest. Most cases are sporadic. An epidemiologic study was unable to implicate the drinking water supply as the source of diarrheal isolates; *Aeromonas* isolates from diarrheal stool were genetically unrelated to those from water supplies.[24] *Aeromonas* is increasingly being recognized as a cause of diarrhea in travelers returning from Asia, Africa, and Latin America.[22] Daycare center outbreaks have been reported, although in one study, molecular typing did not suggest clonal spread.[22] The clinical manifestations of *Aeromonas*-associated diarrhea are varied. Diarrhea is usually watery and self-limited, but some persons develop fever, abdominal pain, and bloody stools. Fecal leukocytes may be present. Occasionally, diarrhea may be severe or protracted, and hospitalization may be necessary. Rare cases of ischemic colitis associated with *Aeromonas* have been reported in healthy children and adults, and chronic colitis developing after acute *Aeromonas*-associated diarrhea has been reported in adults.[22,25] Although no controlled trials have validated antimicrobial therapy for *Aeromonas*-associated diarrhea, clinical improvement has occurred with antibiotics active against the organism. Hemolytic-uremic syndrome associated with *Aeromonas* enterocolitis has been described in infants and adults.[22] *Aeromonas*-associated diarrhea has been shown to be more prevalent in individuals with concurrent rotavirus infection. The relevance of this finding is supported by in vitro studies demonstrating that preinfection of enterocyte-like cells with rotovirus can increase the capacity of some *Aeromonas* strains to adhere to enterocytes.[26]

In contrast, the evidence for pathogenic roles of aeromonads in extraintestinal infections is much more clear-cut. Most *Aeromonas* soft tissue infections are caused by *A. hydrophila.* Trauma followed by exposure to fresh or brackish water (and not salt water, even though aeromonad density in seawater is similar to that in fresh water) usually, but not invariably, precedes infection.[22] Cellulitis develops within 8 to 48 hours, and systemic signs are common.[27] Suppuration and necrosis around the wound are frequent, and surgical débridement is often necessary. Fasciitis, myonecrosis (occasionally associated with gas formation), and osteomyelitis may develop. In the setting of a rapidly progressive cellulitis after an injury related to water exposure, *Aeromonas* and *Vibrio* species infections should be considered in the differential diagnosis. *Aeromonas* soft tissue infections can develop after exposure to soil, in association with crush injuries, and as a complication of burns, typically when initial management of the burn included immersion in natural water sources.[22] There is one reported outbreak of *A. hydrophila* wound infections in participants of a mud football competition in Australia. The field was "prepared" with water from an adjacent river but DNA fingerprints of the river isolates did not match those of the human isolates.[28] *Aeromonas* was second only to *Staphylococcus aureus* in one study of bacteria causing secondary infection in untreated Buruli ulcer lesions.[29] *Aeromonas* soft tissue infection is a recognized complication of the use of medicinal leeches in conjunction with reimplantation or flap surgery.[30] *A. hydrophila* and other *Aeromonas* species are normal inhabitants of the foregut of leeches. Leeches lack the requisite proteolytic enzymes and are dependent on the symbiotic *Aeromonas* to digest the blood meal. *Aeromonas* infection has developed in about 12% of patients treated with leeches.[28] Prophylactic antibiotics, particularly ciprofloxacin or cefotaxime, have been recommended at the time of leech application.[30,31] Infections have developed despite prophylaxis, with the isolated strains determined to be resistant to ciprofloxacin.[30,31] The onset of infection after the application of medicinal leeches ranges from 1 day to more than 10 days. Mild wound infection, loss of flap, myonecrosis, and sepsis may ensue.

Aeromonas bacteremia and sepsis are uncommon, but in the largest series reported to date, 143 *Aeromonas* bacteremias, including 104 that were monomicrobial, occurred in one institution in Taiwan over a 10-year period.[32] *A. hydrophila* caused 60% of the bacteremias; most of the other isolates that were identified by species were *A. veronii* subtype *sobria* and *A. caviae.*[32] Most patients in this series were immunocompromised, including 54% who were cirrhotic and 21% who had an underlying malignancy. Spontaneous bacterial peritonitis was common in cirrhotic patients with abdominal pain. There was a similar distribution of *Aeromonas* species in a study of 53 *Aeromonas* blood isolates collected from 27 medical centers in the United States over a 10-year period.[33] Most patients were immunocompromised, and underlying malignancy was much more common than liver disease in this series. Most patients with *Aeromonas* sepsis do not present with diarrhea. Interestingly, about one-third of *Aeromonas* bacteremias are hospital acquired.[34] *Aeromonas* has been recovered from hospital water supplies, and clusters of *Aeromonas* bacteremia have been described.[35] In some series, the hospital-onset cases were not epidemiologically linked and endogenous gut microbiota was the presumed source.[34] The mortality rate for *Aeromonas* sepsis is 33% or higher.[22] Other species—*Aeromonas jandaei, Aeromonas veronii* biovar *veronii,* and *Aeromonas schubertii*—have rarely been isolated from the blood.[22] A variety of other infections caused by *Aeromonas* species have been reported, including intraabdominal abscess, pancreatic abscess, hepatobiliary infection, spontaneous bacterial peritonitis in patients with cirrhosis, meningitis, endocarditis, suppurative thrombophlebitis, osteomyelitis, urinary tract infection, prostatitis, pneumonia (including near-drowning–associated pneumonia), empyema, lung abscess, tonsillitis, epiglottitis, keratitis, and otitis media.[22] *A. hydrophila* epididymitis and bacteremia developed in a healthy man 24

hours after he had sexual intercourse with his wife in their swimming pool. Cultures obtained from the pool grew *A. hydrophila.*[36]

Aeromonas organisms are gram-negative, nonsporulating facultative anaerobic rods that usually are β-hemolytic on blood agar and ferment carbohydrates with acid and gas production. The organisms grow well on MacConkey agar (some strains are lactose fermenters and some are not), but growth on thiosulfate citrate–bile salts–sucrose medium is variable. Selective techniques are often necessary for the isolation of *Aeromonas* species from mixed cultures. The organisms are more difficult to identify in stool cultures because enteric media may be inhibitory for some *Aeromonas* species. Either blood agar that contains ampicillin (10 or 30 μg/mL) or cefsulodin irgasan novobiocin agar can be used as a selective medium.[22] Growth of colonies on plates usually occurs within 24 hours. *Aeromonas* species are oxidase positive, helping to distinguish these organisms from Enterobacteriaceae. Identification of *Aeromonas* to the genus level is generally not difficult, but misidentifications, particularly as *Vibrio* species, may occur with automated systems.[22] Identification to species can be difficult and many clinical laboratories proceed no further, reporting an *Aeromonas* isolate as "*Aeromonas* species" or "*Aeromonas hydrophila* complex." MALDI-TOF mass spectrometry can provide rapid and accurate identification. CLSI document M45-A2 provides interpretive criteria for disk diffusion and MIC testing for several species of *Aeromonas.*[15] The clinically relevant *Aeromonas* species are uniformly resistant to penicillin and ampicillin, are often resistant to cefazolin and ticarcillin, and are usually but not invariably susceptible to third-generation cephalosporins, aztreonam, and carbapenems.[22] Resistance to cefotaxime has developed on therapy.[32] Sensitivity to piperacillin and ticarcillin-clavulanate is variable. *Aeromonas* species can produce serine β-lactamases, including an Ambler class D penicillinase, class C cephalosporinase, and, less frequently, Temoniera (TEM) family extended-spectrum β-lactamases.[22] Some isolates exhibit coordinated expression of these β-lactamases after both induction and selection of derepressed mutants.[22] *Aeromonas* can also harbor chromosomal CphA metallo-β-lactamases that have narrow substrate profiles and specifically hydrolyze carbapenems.[22] Metallo-β-lactamases of the Verona integron–encoded (VIM) and imipenemase (IMP) families that confer broader β-lactam resistance have been described in strains of *A. hydrophila* and *A. caviae,* encoded on an integron and a plasmid, respectively.[22] There are reports of increasing resistance to tetracycline and trimethoprim-sulfamethoxazole. In one report, tigecycline was active against 200 of 201 isolates.[37] Aminoglycosides are usually active, with resistance to tobramycin being more common than resistance to gentamicin or amikacin.[38] Fluoroquinolones are highly active against *Aeromonas* species, although the existence of chromosomal mutations and plasmid-mediated quinolone resistance in environmental *Aeromonas* strains raise concern that fluoroquinolone resistance could easily develop.[39] *Aeromonas* species harboring a conjugative plasmid that confers multiple antibiotic resistance have been identified.[40] A cephalosporin or fluoroquinolone is generally recommended for treatment of *Aeromonas,* with the addition of an aminoglycoside for severe infections.[38,39] Because of emerging resistance, polymicrobial therapy may be considered for empirical treatment until in vitro susceptibility results are available.

Cardiobacterium Species

Cardiobacterium hominis and *Cardiobacterium valvarum* are the only two species in the genus *Cardiobacterium.* Unlike the other HACEK organisms, these organisms rarely cause disease other than endocarditis. *Cardiobacterium* species have been described as *Pasteurella*-like organisms; they are part of the microbiota in the nose, mouth, and throat and are present occasionally on other mucous membranes as well as in the gastrointestinal tract.

There are more than 80 reported cases of *C. hominis* infection, and all but a few have involved the heart valves. Most patients have had underlying anatomic defects (e.g., rheumatic heart disease, ventricular septal defect, congenital bicuspid valve) or prosthetic cardiac valves.[41,42] Many patients with endocarditis have had severe periodontitis or prior dental procedures without antimicrobial prophylaxis. *C. hominis* endocarditis occurring after upper gastrointestinal endoscopy has been reported.[43] A subacute presentation, with an insidious onset (mean of 2–5 months before diagnosis) and an absence of fever at the time of diagnosis, is common.[44] Some of the patients have splenomegaly, anemia, immune-mediated glomerulonephritis, and hematuria, consistent with a long period between infection and diagnosis. Large vegetations, and large vessel emboli, are characteristic. The mortality rate is about 10%, and valve replacement is needed in about 30% of cases.[12] Septic arthritis, vertebral osteomyelitis, mycotic aneurysms (intracranial and mesenteric), and neurologic involvement are reported complications of *C. hominis* endocarditis.[44]

Almost all clinical isolates come from blood, although meningitis associated with endocarditis has been described. In one of the very rare cases of infection without endocarditis, a patient with adenocarcinoma of the kidney invading the cecum developed an abdominal abscess and bacteremia; abscess and blood cultures grew *C. hominis* and *Clostridium bifermentans.*[45] There is also a case report of *C. hominis* pacemaker lead infection without valvular involvement.[46] Because of phenotypic similarities, it is suspected that some clinical isolates identified as *C. hominis* may actually have been *C. valvarum. C. valvarum* has caused several endocarditis cases worldwide with a spectrum of presentations similar to *C. hominis,* including insidious infection, ability to cause embolism, and the need for valve replacement in the majority of cases.[47] Most cases for which details were provided were associated with periodontitis or an antecedent dental procedure without antimicrobial prophylaxis.[47] This species was first described as *Cardiobacterium* species strain B from dental plaque and has also been described among the etiologic agents in advanced lesions of children with noma.[48]

Cardiobacterium species are pleomorphic gram-negative rods; morphology varies considerably depending on culture conditions. They often have swelling of one or both ends and retain the crystal violet dye at the poles during the Gram stain procedure. Microscopically, the organisms sometimes form rosettes, but short chains, teardrops, pairs, and clusters are also common. Supplementation of the medium with yeast extract results in a loss of the pleomorphism, and most organisms become sticklike, gram-negative rods with rounded ends. Incubation in high humidity and 3% to 5% CO_2 maximizes recovery of the organism. Most strains grow better on sheep blood agar than chocolate agar and will grow on Mueller-Hinton agar or trypticase soy agar without blood, but grow poorly on MacConkey agar or similar selective media. Colonies of *C. hominis* are 1 to 2 mm in diameter on sheep blood agar, usually by 48 to 72 hours after incubation at 37°C under increased CO_2. However, with some systems, incubation for 5 to 7 days before growth can be confirmed is not unusual, and cultures should be held for this period or longer if *C. hominis* is suspected. *C. valvarum* is considered to be more fastidious than *C. hominis,* with tiny visible colonies, 0.2 to 0.8 mm in diameter, appearing on blood agar after 72 to 96 hours of incubation. Colonies of *C. valvarum* are nonhemolytic; however, colonies of *C. hominis* produce slight α-hemolysis after 3 to 4 days of incubation and develop a rough appearance, with a serpentine pattern of growth from the edge to adjacent colonies.[49] *Cardiobacterium* organisms are oxidase positive and catalase negative, and they produce indole (although positivity is weak in many strains of *C. hominis* and absent in some oral strains of *C. valvarum*). *Cardiobacterium* species may be misidentified as *Pasteurella multocida* when using the API 20NE identification strip (bioMérieux, Inc., Hazelwood, MO).[49] The phenylphosphonate reaction can be used to separate *C. hominis* (positive) from *C. valvarum* (negative).[47] MALDI-TOF mass spectrometry also successfully identifies *Cardiobacterium* and distinguishes the two species.[50] PCR amplification of 16S ribosomal DNA from heart valve and arterioembolic tissue has detected *C. hominis* sequences in cases of culture-negative endocarditis.

Susceptibility tests are difficult to perform because of the slow growth of the organism and unusual nutritional requirements, although the Etest appears to be useful.[49,51] When tested, the organism is usually broadly susceptible to β-lactam drugs, fluoroquinolones, chloramphenicol, rifampin, and tetracycline.[51] Susceptibility to aminoglycosides, erythromycin, and clindamycin is variable. Isolates with the ability to produce β-lactamase have been reported.[44] Current American Heart Association guidelines recommend treating endocarditis caused by HACEK organisms with a 4-week course of ceftriaxone, ampicillin-sulbactam, or a fluoroquinolone.[16] In a review of cases, most patients

were treated successfully with penicillin alone, ceftriaxone alone, or penicillin and aminoglycosides, with the duration of therapy ranging from 25 to 63 days.[47] Although microbiologic cure is usually achieved, complications frequently arise during the course of therapy. Systemic embolization, mycotic aneurysm, or progressive cardiac failure has necessitated valve replacement in a number of cases.

Chromobacterium Species

Chromobacterium violaceum is a rare opportunistic human pathogen but can cause life-threatening sepsis with metastatic abscesses. The organism is a common soil and water inhabitant in tropical and subtropical areas. Most cases of human infection have come from Southeast Asia, although more than 35 cases have been reported in the United States, almost all from the Southeast (primarily Florida). Cases have also been reported from Australia and South America. Although not considered a normal inhabitant of the human gastrointestinal tract, *C. violaceum* was present in the feces of 3 of 65 children whose stool was cultured at the time of admission to a hospital in Atlanta.[52]

C. violaceum infection occurs in infants, children, and adults, almost always in the summer months and usually after exposure of nonintact skin to contaminated water (often stagnant) or soil. Two cases followed near drownings. Symptoms include pain at a local site of infection, fever, nausea, vomiting, abdominal pain, and diarrhea. Local cellulitis, pustules, ulcers with necrotic bases, or lymphadenitis commonly precedes evidence of systemic infection. Septic shock develops rapidly, as can pneumonia and visceral abscesses involving the liver, spleen, and lungs. This presentation can be confused with septicemic melioidosis, which is more common than *C. violaceum* infection in Southeast Asia, where both diseases are endemic. The mortality rate for reported cases in the United States is about 60%. Urinary tract infection, conjunctivitis, orbital cellulitis, retropharyngeal infection with prevertebral abscess, neutropenic sepsis, osteomyelitis, brain abscess, meningitis, puerperal sepsis, and internal jugular vein thrombophlebitis have been reported. There are also a few case reports in the pediatric literature of *C. violaceum*–associated diarrhea. A report from Brazil of one confirmed and two suspected cases in siblings is the first cluster of suspected *C. violaceum* infections linked to a common source.[53] *C. violaceum* infection is more common in patients with chronic granulomatous disease (CGD), but cases occur in the apparently normal host.[54] There appears to be a higher survival rate in persons with CGD compared with patients without known neutrophil dysfunction. This may reflect a selection bias because *C. violaceum* infection can be the initial manifestation of CGD, with the diagnosis of CGD being established only after recovery from the infection. Deficiency of polymorphonuclear leukocyte glucose-6-phosphate dehydrogenase and neutrophil dysfunction also were present in a 3-year-old patient who died with *C. violaceum* sepsis. Most strains produce an antioxidant pigment, violacein, that protects the organism against oxidative stress induced by the host response to infection. Other pertinent virulence factors based on the study of only one clinical and one environmental isolate include greater endotoxicity of the outer membrane and enhanced resistance to phagocytosis in the virulent strain.[55] Diagnosis is made by culture of blood, abscess fluid, or skin exudate.

C. violaceum organisms are long gram-negative bacilli; occasionally, the organisms are slightly curved and can be confused with vibrios. The organisms are facultatively anaerobic, with versatile and adaptable pathways for energy generation, and grow readily in 18 to 24 hours on common laboratory media containing tryptophan, which include sheep blood agar, chocolate agar, Mueller-Hinton agar, trypticase soy broth, and MacConkey agar. Incubation at 37°C usually is effective, although growth is enhanced if incubation occurs at 25°C. Most strains produce violacein, an insoluble pigment that imparts a violet-black color to the colonies on solid media under aerobic conditions, hence the species' name (Fig. 236.1). There are a few reports of infection caused by nonpigmented strains. Violacein can induce apoptosis in leukemia cell lines and is being investigated as a potential chemotherapeutic agent.[56] The color may be lost on subculture or after therapy is begun. The organisms produce hydrogen cyanide, so a faint cyanide smell may be present. The oxidase reaction is usually positive but may be difficult to detect in pigmented strains. Demonstration of oxidase can be enhanced by incubating the culture anaerobically, which inhibits pigment formation.[53]

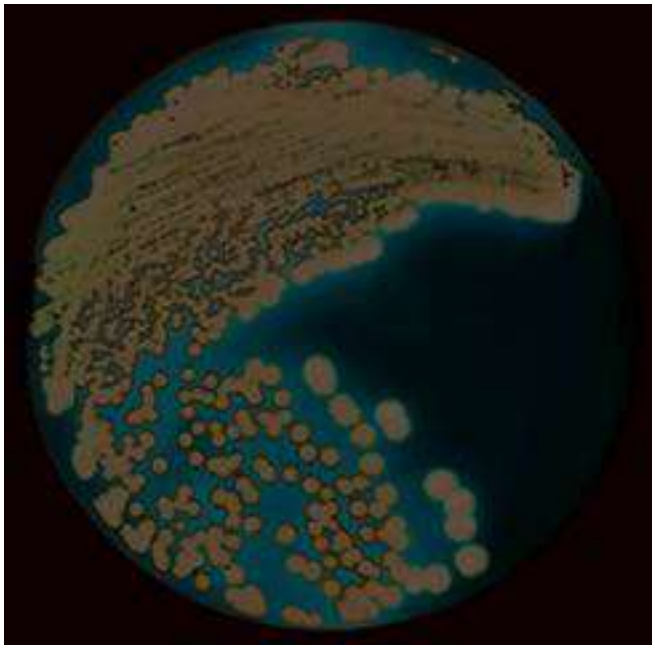

FIG. 236.1 Violet-black colonies of *Chromobacterium violaceum* from production of the pigment violacein.

Antibiotics having the greatest activity against *C. violaceum* generally include fluoroquinolones, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, imipenem, and gentamicin.[57] The ureidopenicillins are often active, but resistance to cephalosporins is common. *C. violaceum* is also resistant to colistin. Although aztreonam is a natural product of some strains of *C. violaceum,* most clinical isolates are susceptible to this agent.[58] Because of the rarity of infection, the often fulminant course, and the high mortality rate, the optimal antibiotic therapy is unknown. Ciprofloxacin is the most active antibiotic in vitro, and there are recent case reports of successful treatment with fluoroquinolones, often in combination with other agents. Most survivors of this infection were treated with chloramphenicol or a penicillin (carboxypenicillin or a ureidopenicillin) in combination with an aminoglycoside. Relapse has occurred more than 2 weeks after the completion of therapy and apparent cure, presumably because of a residual suppurative focus.[53] Oral trimethoprim-sulfamethoxazole, doxycycline, or ciprofloxacin has been used after 2 to 4 weeks of intravenous therapy with other antibiotics, with the oral regimen continued for several weeks to a few months to prevent relapse. Antibiotics at subinhibitory concentrations, such as occur during the postantibiotic phases of clinical therapy, have been shown to enhance quorum-sensing–related virulence factors, including violacein production, chitinase production, and biofilm formation.[59]

Chromobacterium haemolyticum, a species newly described in 2008, has been reported to cause pediatric bacteremia, proctocolitis in children, pneumonia, and necrotizing fasciitis.[60–62] These cases have been reported from the United States, Japan, and Thailand. There was known aquatic exposure in two of the cases. *C. haemolyticum* differs from *C. violaceum* in that it is nonpigmented and has strong β-hemolytic activity on sheep blood agar. Although there are also differences in several biochemical reactions, misidentifications of *C. haemolyticum* as nonpigmented *C. violaceum* occur with currently available biochemical and MALDI-TOF mass spectrometry systems; 16S rRNA sequencing is required for definitive identification. Overall, *C. haemolyticum* is more resistant than *C. violaceum,* with higher MICs for most drugs. Isolates are typically susceptible to fluoroquinolones and resistant to β-lactam antibiotics.

Dysgonomonas Species

The genus *Dysgonomonas* taxonomically clusters in the *Bacteroides-Prevotella-Porphyromonas* group and presently contains four species—*D.*

gadei, D. capnocytophagoides, D. mossii, and *D. hofstadii*—that have been isolated from human sources. The type species is *D. gadei* but *D. capnocytophagoides* has been reported more frequently. *D. capnocytophagoides* and *D. mossii* were originally members of the Centers for Disease Control and Prevention (CDC) dysgonic fermenter (DF)-3 group, indicating an organism that ferments glucose and has difficulty growing on routine media. Isolates from the genus *Dysgonomonas* are rare but have been recovered from blood, wounds, urine, peritoneal fluid, umbilicus, stools, and gallbladder.

D. capnocytophagoides has been isolated from diarrheal stools of patients with immune deficiencies, including common variable hypogammaglobulinemia, HIV infection, diabetes with chronic renal failure, and lymphoreticular and other malignancies, and from patients receiving immunosuppressive agents, but its role as a gastrointestinal pathogen remains controversial.[63,64] With the use of selective media, this organism was isolated from 11 of 690 (1.6%) stools submitted for bacterial culture at the National Cancer Institute.[64] In another prospective study of the role of *D. capnocytophagoides* in diarrheal disease, the organism was recovered from 2 of 178 specimens (1.1%) submitted for *Clostridioides difficile* (formerly *Clostridium difficile*) toxin assay and from 3 of 129 (2.3%) stool specimens from patients with HIV infection. These data suggest that the paucity of reports of recovering *D. capnocytophagoides* from stool specimens may not be attributable to its rarity (as a colonizer or pathogen) but rather to the inability to recover the organism on conventional media. Antibiotic therapy directed at *D. capnocytophagoides* produced a therapeutic response in some of these patients, including 4 of 11 in the first study. Some of the responders had diarrhea of several months' duration, with prompt resolution after antibiotic therapy was initiated. In other patients, the clinical significance of the organism was unclear; eradication of the organism from the stool was not accompanied by resolution of diarrhea, or the diarrhea resolved without specific therapy. *D. capnocytophagoides* has also been isolated from the urine as a cause of biliary sepsis,[65] from a polymicrobial thigh abscess in a patient with insulin-dependent diabetes,[66] from liver abscesses and blood after radiofrequency ablation in a patient with hepatocellular carcinoma,[67] and from patients with neutropenia, including the blood and stool of a patient with acute myelocytic leukemia.[68] *D. gadei* and *D. mossii* have been isolated from the gallbladders of patients with cholecystitis.[69] *D. mossii* has also been recovered repeatedly from intestinal fluid in a patient with pancreatic cancer but was not associated with obvious infection.[69] *D. hofstadii* was isolated from a postoperative abdominal wound.[70]

Organisms in the genus *Dysgonomonas* are coccobacillary to short gram-negative, nonmotile, facultative rods. Growth occurs on blood agar and chocolate agar after 1 to 3 days of incubation in ambient, CO_2-enriched, or anaerobic atmospheres, but growth is less on blood agar and no growth occurs on MacConkey agar. Routine enteric media do not support growth of *D. capnocytophagoides.* Selective *Campylobacter* media do support growth when incubated at 37°C, but not at 42°C, which is the routine incubation temperature for *Campylobacter.* Selective media such as cefoperazone vancomycin amphotericin B blood agar inhibit normal microbiota and allow recovery of *D. capnocytophagoides* from stool specimens.

Dysgonomonas colonies are gray-white and nonhemolytic with a slight sweet aromatic odor. X factor is required for growth, nitrate is not reduced, and oxidase and catalase tests are negative. Identification can be made using the API Rapid ID 32A system (bioMérieux, Durham, NC) or the VITEK 2 system (bioMérieux, Durham, NC) or by whole-cell fatty acid gas chromatography.[66,70]

Despite a lack of established breakpoints, the Kirby-Bauer disk diffusion and MIC methods have been used for antimicrobial susceptibility testing.[69,71] *Dysgonomonas* species appear to be resistant to most β-lactam drugs, fluoroquinolones, aminoglycosides, metronidazole, vancomycin, erythromycin, and gentamicin. Many strains are susceptible to chloramphenicol, trimethoprim-sulfamethoxazole, clindamycin, and tetracycline. Tetracycline or clindamycin was used in the few reported cases of diarrheal disease that responded promptly to antibiotic administration. Despite a Kirby-Bauer zone size suggesting susceptibility, imipenem failed to clear *D. capnocytophagoides* from the bloodstream in the one reported bacteremic patient; the bacteremia resolved after therapy with trimethoprim-sulfamethoxazole was initiated.

Kingella Species

Kingella species are members of the family Neisseriaceae. They are normal microbiota of the human oropharynx and also are occasionally found in the oral cavity in other animals. Five species—*K. kingae, K. denitrificans, K. oralis, K. potus,* and *K. negevensis*—have been described. *Kingella kingae* is the most frequently recognized member of the genus and has been isolated from invasive infections with the oropharynx implicated as the source. *Kingella kingae* is the most common cause of bone and joint infections in young children and belongs to the HACEK group of fastidious gram-negative organisms associated with endocarditis (see Chapter 213).

Kingella denitrificans has been implicated in cases of endocarditis, bacteremia, empyema, corneal ulcers, chorioamnionitis, granulomatous disease secondary to acquired immunodeficiency syndrome (AIDS), retropharyngeal abscess, and peritonitis in peritoneal dialysis patients.[72,73] The one reported pediatric peritonitis case was polymicrobial and the source of the organisms was presumed to be the patient's dog.[74]

The other *Kingella* species have been reported much less frequently. *K. oralis* has been isolated from subgingival plaque in patients with and without periodontitis, and its relationship to disease is unclear.[75] The only reported infection attributed to *K. potus* was in a forearm wound in a zookeeper as a result of a kinkajou bite.[76] *K. negevensis* is described as one of the oropharyngeal microbiota of healthy children but has not been studied in other populations.[77] In the only reported case of invasive infection to date, PCR targeting the GroEL molecular chaperone protein gene identified *K. negevensis* in a culture-negative osteoarticular specimen from a child.[78]

Kingella spp. are coccoid to medium-sized gram-negative rods that tend to resist decolorization and do not grow on MacConkey agar. The organisms are notoriously fastidious, and growth is enhanced in the presence of 5% to 10% CO_2. *K. denitrificans, K. potus,* and *K. oralis* colonies are low, convex, and 1 to 2 mm in diameter after 48 hours of incubation. They are friable and nonhemolytic and have a nondiffusible yellow pigment. *K. negevensis* colonies are said to resemble a small colony variant of *K. kingae* and are opaque, pinpoint, and faintly β-hemolytic. All species are catalase negative and oxidase positive. All species except *K. potus* ferment glucose. Automated biochemical identification systems can misidentify *Kingella* as *Haemophilus* spp., *Gardnerella vaginalis, Neisseria* spp., or *Moraxella* spp.[79] Current MALDI-TOF mass spectrometry databases include *K. kingae* and *K. denitrificans,* and these organisms have been correctly identified using this technology in several reports. Amplification and sequencing of the 16S rRNA gene or species-specific nucleic acid amplification tests targeting either the *rtx* operon or the *groEL* gene have been useful for detection of *Kingella* in some bone and joint specimens in which cultures have not revealed the pathogen.[80]

Approved breakpoints for broth microdilution susceptibility testing of the HACEK group have been published by the CLSI.[81] *Kingella* spp. are generally susceptible to a wide range of antibiotics, including β-lactams, macrolides, tetracyclines, co-trimoxazole, and quinolones. β-Lactamase–positive isolates have been reported to be susceptible to combinations with β-lactam inhibitors.

Neisseria animaloris and *Neisseria zoodegmatis*

The bacteria previously known as CDC eugonic fermenter (EF)-4 have been renamed *Neisseria animaloris* and *Neisseria zoodegmatis* based on polyphasic taxonomic studies, including 16S rRNA gene sequence analysis[82] These bacteria are nonmotile, oxidase-positive, fastidious gram-negative rods that produce acid from glucose (*eugonic fermenter*) but have relatively few other reactions and display very slow growth in anaerobic conditions. They are distinguished from *Cardiobacterium* and *Kingella* by the capacity to produce catalase and lack of indole production. Two biotypes were recognized based on the presence (EF-4a) or absence (EF-4b) of arginine hydrolase activity, DNA guanine-cytosine content, cellular fatty acid analysis, and whole-cell protein analysis. Organisms previously recognized as EF-4a are now *Neisseria animaloris* and EF-4b organisms are *Neisseria zoodegmatis.* These bacteria are normal inhabitants of the oral cavity of dogs and cats. Most human infections occur after dog bites, although infections associated with cat

bites or scratches occur as well.[83] The organism can be isolated from bite wounds that do not demonstrate signs of inflammation, but cellulitis, abscess formation, and fever may develop. Systemic infection or infection not involving skin or skin structures is extremely rare. Endophthalmitis caused by *Pasteurella multocida* and EF-4 occurred after a cat scratch in an 8-year-old girl.[84] There is one report of bloodstream infection occurring in a patient with hepatic carcinoid who denied being bitten by a dog or cat.[85] An otherwise healthy man whose dogs often licked him in the ears developed chronic EF-4 otitis media that required mastoidectomy.[86]

These bacteria usually appear as short rods on Gram stain but can also appear as small coccoid forms or long chains. The organisms grow well on blood agar and chocolate agar within 24 hours, but grow poorly or not at all on MacConkey and similar agars. Incubation in 5% CO_2 does not noticeably enhance growth. The colonies are small, may be slightly yellow-orange, and are smooth; some strains have a popcorn-like odor.

Penicillin G and ampicillin are active against these *Neisseria* species at concentrations attainable with oral administration, so amoxicillin-clavulanate, which is recommended for bite wounds, is adequate for coverage.[87,88] Fluoroquinolones and tetracycline can also be used as oral therapy. Chloramphenicol and aminoglycosides have activity, whereas cephalosporins, particularly first-generation agents, are less active in vitro.[87,88]

Plesiomonas shigelloides

Plesiomonas shigelloides, a ubiquitous freshwater inhabitant, has been implicated as a cause of acute diarrhea and, rarely, serious extraintestinal disease.[89] The name *Plesiomonas,* from the Greek word for "neighbor," was chosen because the organism was believed to be closely related to *Aeromonas.* Its classification has been a matter of some debate; it was previously classified in the family Vibrionaceae but is currently classified in the Enterobacteriaceae.[90] *P. shigelloides* is the only species in the genus. The organism was originally isolated in 1947 and given the name C27. It has also been named *Pseudomonas shigelloides, Aeromonas shigelloides,* and *Fergusonia shigelloides.*

P. shigelloides is a water- and soil-associated organism that replicates at temperatures above 8°C. It is found primarily in freshwater or estuary environments within temperate and tropical climates but can exist in seawater during the warm-weather months. Asymptomatic carriage of *P. shigelloides* is very rare among healthy persons. The usual vehicles of transmission of plesiomonads to humans are water; food such as oysters, shrimp, or chicken[89]; and a variety of animals that may be colonized with the organism.[91] The organism has been acquired during foreign travel. *P. shigelloides* is associated with gastroenteritis and has been identified as a cause of outbreaks, but the failure to identify an enteropathogenic mechanism, the lack of an animal model, and unsuccessful attempts to induce disease in volunteers make it impossible to firmly establish a causal relationship. Thus the clinical significance of finding the organism in a diarrheal stool is uncertain. An epidemiologic study in Ecuador found stronger evidence that *Plesiomonas* diarrhea was associated with rotavirus coinfection than single infection.[92] Potential virulence factors include β-hemolysins, cytotoxins, exoenzymes, and adherence factors, but their significance is unknown.[93]

The clinical presentation of individuals in whom *P. shigelloides* is isolated from diarrheal stool in the absence of detection of other pathogens varies from a mild self-limited illness to mucoid, bloody diarrhea with fecal leukocytes. A predominance of a secretory-type diarrhea has been noted,[89] but other series have found a high percentage with a clinical illness compatible with enteroinvasive disease featuring abdominal pain, fever, bloody diarrhea, and fecal leukocytes.[94] Most symptomatic patients have either traveled abroad or been exposed to potentially contaminated water or food. Outbreaks have been reported, particularly from Japan. The role of antibiotics for *Plesiomonas*-associated diarrhea is uncertain. Antimicrobial therapy did not shorten the duration of fever or diarrhea in Thai children with *Plesiomonas*-associated diarrhea.[95] On the other hand, in a small nonrandomized Canadian study of patients who developed *Plesiomonas*-associated diarrhea after travel abroad, 8 of 9 treated patients were asymptomatic within 2 weeks, compared with 6 of 15 controls ($P < .05$).[94]

Most descriptions of extraintestinal disease come from individual case reports. These reports include cases of osteomyelitis, septic arthritis, endophthalmitis, spontaneous bacterial peritonitis, pancreatic abscess, splenic abscess, biliary disease,[96] cholecystitis, cellulitis, pyosalpinx,[97] epididymo-orchitis, and pneumonia.[98] About 10 cases of neonatal sepsis with meningitis have been described.[99] Bacteremia is rare and usually occurs in immunocompromised hosts. In a case series of *Plesiomonas* bacteremia from Hong Kong, all seven patients were elderly; four had biliary tract disease, and three had underlying malignancy.[100] Bacteremia accompanying gastroenteritis has been reported in a healthy 15-year-old girl.

P. shigelloides is a motile, facultatively anaerobic, gram-negative, oxidase-positive bacillus. It is readily isolated from some enteric agars such as MacConkey agar but does not grow well on thiosulfate citrate bile salts sucrose medium. Selective techniques may be necessary for isolation of the organism from mixed cultures, such as the use of bile peptone broth or trypticase soy broth with ampicillin.[101] The organism grows well at 35°C and produces visible colonies (nonhemolytic) within 24 hours. The organism can now be identified on gastrointestinal multiplex molecular panels, but its clinical significance is uncertain.[102]

P. shigelloides is usually susceptible to chloramphenicol, trimethoprim-sulfamethoxazole, quinolones, cephalosporins, and imipenem.[95,103] Because of β-lactamase production, most isolates are now resistant to penicillins, including ureidopenicillins, although the β-lactamase inhibitor combinations appear to be active. Susceptibilities to aminoglycosides and tetracycline are variable. Antimicrobial therapy for established enteric infections is the same as for *Shigella* and generally includes a fluoroquinolone or azithromycin.[95] Systemic infections have been successfully treated with fluoroquinolones, carbapenems, or β-lactam/inhibitor combinations such as piperacillin-tazobactam.[97–100]

GLUCOSE NONFERMENTERS OR WEAK FERMENTERS

Achromobacter and *Alcaligenes* Species

The taxonomic designations for *Achromobacter* and *Alcaligenes* species have been particularly confusing. *Achromobacter xylosoxidans* was renamed *Alcaligenes xylosoxidans* subsp. *xylosoxydans,* but 16S rRNA sequence analysis and guanine-cytosine content studies support placement of this organism back in the genus *Achromobacter.*[104] Other *Alcaligenes* species—*A. ruhlandii, A. piechaudii,* and *A. denitrificans*—have also been transferred to *Achromobacter.* Organisms formerly considered *Achromobacter* groups A, C, and D (and before that, CDC groups Vd-1 and Vd-2) are now named *Ochrobactrum anthropi* and are considered separately (see "*Ochrobactrum* Species" later). *Achromobacter* species are nonfermenting gram-negative bacilli found in soil and water, including swimming pools, well water, municipal and hospital water supplies, dialysis solutions, ultrasound gel, and chlorhexidine solutions. They can occasionally be recovered from the respiratory and gastrointestinal tracts, primarily in persons with health care contact. Infection results when organisms are introduced into wounds or colonize those with compromised host defenses. Clinically relevant species include the asaccharolytic species *A. dentrificans, A. piechaudii,* and *Alcaligenes faecalis* (this organism remains in the genus *Alcaligenes*); the saccharolytic species *A. xylosoxidans*; and the unnamed *Achromobacter* group F. Although sometimes considered a contaminant, *Achromobacter* group B (now classified along with *Achromobacter* group E as *Pannonibacter phragmitetus*) has been recovered from the blood of patients with clinical sepsis and endocarditis.[105]

A. xylosoxidans is the most clinically important of these organisms. It probably is part of the endogenous microbiota of the ear and gastrointestinal tract and is a common contaminant of fluids.[106] The organism has been implicated in outbreaks of nosocomial infection associated with contaminated solutions (e.g., intravenous fluids, hemodialysis fluid, irrigation fluids, mouthwash), pressure transducers, incubators and humidifiers, and contaminated soaps and disinfectants.[107] Contamination of well water was the apparent source of bacteremia in one case. Clinical illness that is caused by *A. xylosoxidans* has involved isolates from blood, peritoneal and pleural fluids, urine, respiratory secretions, and wound exudates. Bacteremia, often related to intravascular

catheters, is the most commonly reported infection and is frequently polymicrobial in patients with underlying malignancies.[108,109] Biliary tract sepsis, meningitis (sometimes with lymphocytic predominance in cerebrospinal fluid), pneumonia (nosocomial and community acquired), peritonitis (including spontaneous bacterial peritonitis and peritonitis in patients on continuous ambulatory peritoneal dialysis), urinary tract infection, conjunctivitis, osteomyelitis, prosthetic knee infection, mesh infection, infected necrotizing pancreatitis, and prosthetic valve endocarditis have been reported.[106–114] Patients often have an immunosuppressed state such as cancer or HIV infection, but this is not always the case, especially in nosocomial outbreaks.[109,113] *A. xylosoxidans* as well as other *Achromobacter* species have been recovered with increasing frequency from respiratory secretions of persons with cystic fibrosis.[114] Colonization has been associated with exacerbation of respiratory symptoms, possibly as a result of exacerbation of the inflammatory response caused by lipopolysaccharide and a cytotoxic factor produced by *Achromobacter.*[114,115] Several case-control studies have not shown more rapid deterioration in clinical or pulmonary function status among cystic fibrosis patients chronically colonized with *Achromobacter* or *Alcaligenes* species, except in a subgroup of patients with a rapid increase in specific precipitating antibodies to *A. xylosoxidans.*[116] Recovery of *A. xylosoxidans* in neonatal infection may result from perinatal transfer from the mother.

A. denitrificans has been recovered as a single pathogen from blood, cerebrospinal fluid, and other normally sterile body fluids, as well as from a renal abscess and in mixed culture from sites usually containing normal microbiota.[117] Few recent publications have addressed the pathogenic role of these organisms. *A. piechaudii* was believed to cause chronic otitis in a diabetic patient[118] and has also been recovered from blood in a patient with a hematologic malignancy and an infected Hickman catheter who had recurrent bacteremia.[119]

Alcaligenes faecalis can be recovered in a variety of clinical settings. Most isolates of *A. faecalis* from blood or respiratory secretions are related to the contamination of hospital equipment or fluids with the organism, with resulting human colonization or infection. The urine is the other common site of recovery, although *A. faecalis* infrequently causes symptomatic urinary tract infection. It also has been recovered from corneal ulcers, ear discharges, wound drainage, peritoneal fluid, and feces.[120,121] It is rarely recovered in pure culture from any of these sites.

Phylogenetically and biochemically, *Alcaligenes* and *Achromobacter* are closely related to the genus *Bordetella. Achromobacter* species grow well on standard microbiologic media, including MacConkey agar. They produce flat, spreading, and rough colonies and have peritrichous flagella, features that help distinguish them from pseudomonads. The majority of strains will also grow on *Burkholderia cepacia* selective agar. The organisms are oxidase positive and catalase positive and oxidize glucose to produce acid, but are urease negative and indole negative. *A. xylosoxidans,* as the species name indicates, oxidizes xylose readily, which distinguishes it from other species in the genus. Distinguishing the organisms and confirming the identification is made difficult by their lack of reactivity in many biochemical or assimilation tests. MALDI-TOF mass spectrometry offers accurate identification of this group of organisms and will play an increasing role as the technology becomes more widely utilized.

An isolate of *A. xylosoxidans* can easily be mistaken for a non–*P. aeruginosa* strain of *Pseudomonas* or for a strain of the *B. cepacia* complex, but the unusual susceptibility pattern suggests the correct identity. Methods for susceptibility testing are not standardized. Piperacillin-tazobactam and carbapenems are active in vitro and would be appropriate initial therapy for serious *A. xylosoxidans* infections, whereas trimethoprim-sulfamethoxazole may be used for urinary tract and other infections not requiring parenteral therapy. Ceftazidime also has activity, although strains are generally resistant to other cephalosporins, narrow-spectrum penicillins, aztreonam, and aminoglycosides. Susceptibility to the fluoroquinolones is variable, and high rates of resistance to ciprofloxacin and aminoglycosides have been noted in strains isolated from cystic fibrosis patients. High concentrations of colistin inhibit most strains. Resistance mechanisms include a constitutive oxacillinase and acquired β-lactamases, as well as multidrug efflux pumps.[122] The presence of imipenemase (IMP)-, Verona integron–encoded (VIM)-, or Tripoli (TMB)-type metallo-β-lactamase genes carried on transferable class 1 integrons can produce carbapenem resistance and hold the potential for horizontal transfer.[123,124]

A. faecalis strains produce a distinctive odor resembling that of sweet apples and are usually susceptible to trimethoprim-sulfamethoxazole, ureidopenicillins, carbapenems, and (unlike *Achromobacter* species) most cephalosporins.[120] Results vary for aztreonam, fluoroquinolones, and aminoglycosides, with resistance to gentamicin being common.[120] Extended-spectrum β-lactamase production has also been described in *A. faecalis.*

Chryseobacterium and *Elizabethkingia* Species

Chryseobacterium species are inhabitants of soil and water and can be recovered from a variety of foods. They can be found in municipal water supplies despite adequate chlorination and have been recovered from the hospital environment, often in conjunction with clusters of clinical isolates. *Chryseobacterium* species are organisms of low virulence, and their presence in clinical specimens usually represents colonization and not infection. *C. indologenes* (formerly *Flavobacterium indologenes*) is the most frequently isolated species but is a rare cause of human disease. Most of the published cases have originated in Taiwan, with a few cases reported from Australia, Europe, India, and the United States. The majority of reported infections have been hospital acquired, and the vast majority of patients had undergone invasive procedures and had underlying conditions, such as neoplasms, diabetes mellitus, stem cell or solid-organ transplantation, or prolonged use of antibiotics. Reported infections include bacteremia, ventilator-associated pneumonia, cellulitis, peritonitis, indwelling device–associated infection, urinary tract infections, biliary tract infection, lumboperitoneal shunt infection, ocular infections, central nervous system infection, and surgical and burn wound infections. Infections have often been associated with a high mortality rate.[125]

Based on phylogenetic and phenotypic data, *C. meningosepticum* and *C. miricola* have been placed in the genus *Elizabethkingia* and are now known as *E. meningoseptica* and *E. miricola.*[126] In 2011, *E. anophelis* was described as a new species.[127] Cases of human infection due to *E. miricola* are rare. *E. miricola* has been isolated from respiratory and blood cultures of a stem cell transplant recipient with relapse of mantle cell lymphoma and from the urine of a pediatric patient with multiple comorbidities.[128,129]

E. meningoseptica has historically been the most frequently isolated species in the genus and is clinically significant in up to one-half of the adults and in about two-thirds of the neonates from whom it is recovered.[130] It is associated with both outbreaks and sporadic infections. In pediatric patients, neonatal meningitis is the most common presentation of *E. meningoseptica,* especially in premature infants during the first 2 weeks of life.[131] Clusters of neonatal meningitis have been linked to many sources, including contaminated saline solution for flushing eyes, respiratory equipment, and sink drains.[125,132] Neonatal meningitis is fatal in more than half the cases, and brain abscesses and other severe sequelae are common. Most *E. meningoseptica* infections in adults are hospital acquired and occur in immunocompromised hosts. The respiratory tract is the most common site of infection, and outbreaks have been linked to contaminated ventilator tubing and aerosols.[125] In outbreaks, respiratory tract colonization occurs more often than infection. Bacteremia is the second most common presentation of *E. meningoseptica* infection. In one cluster of bloodstream infections related to a contaminated anesthetic, the bacteremia was transient and systemic signs of infection resolved without specific antibiotic therapy, attesting to the low virulence of this organism in adults. *E. meningoseptica* has also caused endocarditis (including prosthetic valve endocarditis), cellulitis, wound infection, sepsis after extensive burns, abdominal abscess, dialysis-associated peritonitis, and endophthalmitis.[125,130] Other contaminated sources include contaminated syringes in ice chests, vials, sink drains, sink taps, tube feedings, flush solutions for arterial catheters, pressure transducers, and antiseptic solutions.[125,130,132] Infections including cellulitis, septic arthritis, community-acquired respiratory tract infection, keratitis, and bacteremia have been reported in the absence of underlying diseases.[125,130,133]



FIG. 236.2 **Pale yellow colonies of *Elizabethkingia meningosepticum* on blood agar plate.**

E. anophelis was first described in 2011 and reported to cause neonatal meningitis in 2013. Since then, several infections and outbreaks have been reported, including a 2015–2016 outbreak of 66 laboratory-confirmed infections in 63 patients in the United States, primarily in Wisconsin, and a separate report of 12 isolates from 11 patients in Illinois.[134] Most isolates were cultured from blood and caused disease in older patients with underlying comorbidities.[135]

Chryseobacterium and *Elizabethkingia* species may be long, thin, slightly curved, and occasionally filamentous on Gram stain. *C. indologenes* colonies usually form a dark-yellow to orange pigment in culture as a result of the production of the pigment flexirubin, whereas *E. meningoseptica* colonies are smooth, large, and pale yellow (Fig. 236.2). Both organisms grow well and form colonies within 24 hours on blood or chocolate agar and grow at a much slower rate, if at all, on MacConkey agar. They are not motile and produce positive catalase and oxidase reactions. *Chryseobacterium* can be distinguished from other nonfermenters by the ability to produce indole in tryptophan broth, but the reaction is often very weak. *Chryseobacterium* species produce proteases and gelatinase, which may contribute to virulence and are responsible for the greenish discoloration around the colonies on blood agar. *E. meningoseptica* has the ability to create biofilms, particularly in nutrient-rich conditions; isolates that produce higher quantities of biofilm have been associated with poor outcomes.[136] MALDI-TOF may be helpful in identification of *Elizabethkingia* isolates to the species level, but in one study species identification required amending the MALDI-TOF database.[137]

Chryseobacterium species and *E. meningoseptica* are resistant to most antibiotics, and the use of inactive drugs as empirical therapy may contribute to the poor outcome in many infections. Results of susceptibility testing vary when different methods are used; disk diffusion methods especially are unreliable, and broth microdilution should be employed, if possible.[138] Etest has also been suggested as a possible alternative for testing certain antibiotics.[139] *E. meningoseptica* and *Chryseobacterium* organisms produce β-lactamases and are naturally resistant to most β-lactam drugs, including carbapenems and aztreonam.[138,140] This resistance in *C. indologenes* has been shown to be due to chromosomally encoded class A extended-spectrum β-lactamase CIA, in addition to class B metallo-β-lactamase IND variants (IND-1 to IND-7 and IND-2a).[140,141] Three β-lactamase genes have been identified in *E. meningoseptica,* a class D serine-β-lactamase CME conferring resistance to cephalosporins and two unrelated wide-spectrum metallo-β-lactamases, BlaB (subclass B1) and GOB (subclass B3) with carbapenemase activity.[142] *E. meningoseptica* is unique in being the only reported microorganism with two intrinsic chromosomally encoded metallo-β-lactamases genes.[142] Cefepime has poor activity against *E. meningoseptica,* and only modest activity against *C. indolgenes.*[143] Tigecycline and piperacillin-tazobactam had similar activity against *E. meningoseptica,* with 88.5% of isolates being susceptible using US Food and Drug Administration breakpoints for Enterobacteriaceae.[144] *E. meningoseptica* is typically resistant to aminoglycosides, chloramphenicol, erythromycin, and colistin.[144] Fluoroquinolones are usually active in vitro, and sparfloxacin, cinafloxacin, and levofloxacin are somewhat more active than ciprofloxacin.[145] In two studies, minocycline was the only agent active against all *E. meningoseptica* strains.[130,138] Doxycycline and trimethoprim-sulfamethoxazole susceptibility was variable. Rifampin is active against most strains and has been used as part of combination therapy to clear persistent infection. Vancomycin, alone or in combination with other agents, including rifampin, has been successful in the treatment of meningitis in infants.[146] In some reported cases of meningitis treated successfully with vancomycin, the MICs of vancomycin were 8 to 12 μg/mL.[147] However, two groups reported that vancomycin was inactive in vitro (MICs of 16 to <64 μg/mL) and called into question the usefulness of vancomycin against *E. meningoseptica.*[130,138] Thus there is no optimal regimen for *E. meningoseptica* meningitis, and therapy should be based on properly performed susceptibility testing. Possible regimens include rifampin in combination with trimethoprim-sulfamethoxazole, a fluoroquinolone, or minocycline.

Comamonas and *Delftia* Species

Comamonas species are common environmental bacteria that occasionally cause human disease. Although these organisms are of low virulence and clinical significance is sometimes difficult to establish, some of their obscurity may be due to the inability of clinical laboratories to identify them; isolates may be reported as being nonfermentative gram-negative bacilli that could not be further identified.

Comamonas testosteroni, formerly *Pseudomonas testosteroni,* is the most common pathogen of the genus. In a review of 33 reported cases, the most common sites of infection were the bloodstream (13 cases), the peritoneal cavity (10 cases), and cerebrospinal fluid (3 cases).[148] Unusual sites of infection include urine, vitreous fluid, an infected animal bite wound, the embryonic cord of a stillborn infant of an intravenous drug–abusing mother, and pneumonia in a patient with immunodeficiency syndrome.[148] Many of these infections are polymicrobial.

Infections caused by *Delftia acidovorans* (formerly *Comamonas acidovorans* or *Pseudomonas acidovorans*) have been reported in immunocompetent and immunocompromised individuals as well as those with underlying disease.[149] These include keratitis and other ocular infections, bacteremia (including catheter-related bacteremia), endocarditis associated with intravenous drug use, otitis externa, peritonitis in a patient receiving peritoneal dialysis, hospital-acquired pneumonia, empyema, and urinary tract infection.[149] An outbreak of *D. acidovorans* bacteremia was linked to contaminated pressure-monitoring devices, but clinical and epidemiologic information was not provided.[148] Another species, *Delftia tsuruhatensis,* has also been reported as a cause of catheter-related infection.

Comamonas are strictly aerobic, motile, nonpigmented, oxidase-positive, gram-negative bacilli that grow well on routine bacteriologic media. Biochemical characteristics include accumulation of β-hydroxybutyrate, acetamide hydrolysis, and reduction of nitrate to nitrite. Most currently available identification systems will identify *Comamonas* to genus level, if at all. Species are distinguished by carbon compound use patterns. *Delftia* is phenotypically similar to *Comamonas.* Key differentiating characteristics include oxidation of fructose and mannitol and resistance to 10-μg colistin disks and 300-U polymyxin B disks.[150] There are no guidelines for antibiotic susceptibility testing for *Comamonas* or *Delftia* species. Published cases appear to indicate that *C. testosteroni* is more susceptible to common antibiotics than is *D. acidovorans.* Aminoglycosides, fluoroquinolones, carbapenems, piperacillin-tazobactam, and ceftazidime are potentially active. *D. acidovorans* is more resistant to aminoglycosides than *C. testosteroni* but is generally susceptible to broad-spectrum cephalosporins, piperacillin, aztreonam, carbapenems, quinolones, and trimethoprim-sulfamethoxazole.[150] Development of resistance to broad-spectrum penicillins and cephalosporins during antibiotic treatment has occurred.[150]

Eikenella Species

Eikenella corrodens is a fastidious facultative anaerobic gram-negative bacillus that is present as endogenous microbiota in the mouth and upper respiratory tract as well as on other mucosal surfaces. Although

it is recovered most often as a component of polymicrobial infection, commonly coexisting with streptococci, it has been recovered from sterile sites in pure culture.[151] Characteristic of *Eikenella* infection is an indolent course, generally taking more than 1 week from the time of injury to clinical manifestation of disease.[152] Many patients with *Eikenella* infection have underlying diseases, especially head and neck malignancies.[152] In case series and literature reviews, the head and neck were the most common sites of *Eikenella* infections in both adults and children.[151,152] Other common clinical manifestations include respiratory tract infections and human bite infections, infections among chronic finger or nail biters, and "clenched-fist injuries" following altercations. Because of the proximity of bone and joint spaces, these hand infections may lead to osteomyelitis and septic arthritis. The bacillus has been reported as a cause of ulceration after a human bite to the penis.[153] *Eikenella* has also caused infection in insulin-requiring diabetic patients and drug-abusing "skin poppers" who lick their needles,[154] and has caused necrotizing fasciitis after elective hernia repair.[155] Severe soft tissue infection, with or without underlying osteomyelitis, may be slow to resolve. Suppuration due to *Eikenella* infections is foul smelling, mimicking an anaerobic process. Pulmonary infections, including empyema, pneumonia, and septic emboli, in conjunction with internal jugular vein thrombosis (postanginal sepsis), can occur, typically in patients with underlying chronic illnesses or intrathoracic malignancies. Acute suppurative thyroiditis has occasionally been reported in adults and rarely in children. Gynecologic infections have been reported, including chorioamnionitis resulting in preterm labor and fetal demise and infection associated with intrauterine contraceptive devices.[156] *Eikenella* has also been recovered in pure culture from synovial fluid, bone, cerebrospinal fluid, brain, subdural and visceral abscesses, pleuropulmonary infection, and blood.[157,158] *E. corrodens* is another of the HACEK organisms, which have in common the need for incubation in an atmosphere enhanced with CO_2 for recovery in culture and a predilection for infecting the heart valves. Endocarditis caused by *E. corrodens* typically has an indolent course, but acute presentations are reported.[159] Endocarditis usually occurs after intravenous drug use or in patients with abnormal heart valves, including prosthetic valves, but infection of a structurally normal heart valve in a patient without predisposing risk factors has been reported.[12,160]

E. corrodens is a gram-negative, small straight rod that at times can appear pleomorphic or coccobacillary. It grows in either aerobic or anaerobic environments. It is nonmotile and non–spore forming and does not have a capsule. Cell surface components vary from strain to strain, and these differences may relate to virulence.[161] On blood or chocolate agar, even aided by the presence of 3% to 10% CO_2, the organism grows slowly, and it often requires 2 days or more to recognize the typical pinpoint colonies. Colonies are small and grayish (older colonies may become light yellow), produce a slight greenish discoloration on the blood agar, and elaborate an odor resembling that of bleach (hypochlorite). About half produce the pitting ("corroding") of the agar that is considered characteristic. The organism grows poorly on MacConkey agar. Strains do not form acid from carbohydrates; are oxidase positive, catalase negative (a few strains are weakly catalase positive), urease negative, and indole negative; and reduce nitrate to nitrite. Ampicillin, ureidopenicillins, second- and third-generation cephalosporins, and tetracyclines are active against *E. corrodens* in vitro and have been effective clinically.[156,162] The organism is susceptible to fluoroquinolones and azithromycin; however, it is uniformly resistant to clindamycin, erythromycin, and metronidazole and often resistant to aminoglycosides.[156,162] Because *Eikenella* infections are often polymicrobial, initial therapy with ampicillin-sulbactam or amoxicillin-clavulanate is appropriate in many cases, whereas ampicillin can be used for monomicrobial infections. Ceftriaxone is recommended for treatment of endocarditis caused by HACEK organisms because some of these gram-negative organisms produce β-lactamases. Although β-lactamase production in *Eikenella* is uncommon at present, these enzymes have been described.[163]

Flavobacterium and *Myroides* Species

The genus *Flavobacterium* consisted of a heterogeneous group of yellow-pigmented bacteria that did not prove to be closely related when subjected to genotypic analysis. Consequently, many *Flavobacterium* species, including the clinically important species, have been reclassified to other genera and are discussed elsewhere in this chapter. *Flavobacterium meningosepticum,* formerly the most important species, is now a member of the genus *Elizabethkingia,* whereas *Flavobacterium indologenes* is now in the genus *Chryseobacterium. Flavobacterium multivorum* and *Flavobacterium spiritivorum* now reside in the genus *Sphingobacterium. Flavobacterium odoratum,* an uncommon clinical isolate, has been placed in the genus *Myroides* and divided into two species, *M. odoratus* and *M. odoratimimus. Myroides* spp. are common in soil and water and are often not considered pathogenic. However, there are reports of clinical infections, including urinary tract infection, endocarditis, ventriculitis, cutaneous infections, pneumonia, bacteremia, septic shock, and soft tissue infections. These infections typically occur in severely immunocompromised patients, although rare severe infections have occurred in normal hosts.[164] Hospital-acquired infections are usually believed to originate from a hospital water source.[164] One outbreak of catheter-related bloodstream infections was traced to ampules of water contaminated with *M. odoratus* and *B. cepacia.*[165]

Myroides species are obligately aerobic and grow on most media, including MacConkey agar. Colonies are yellow and produce a fruity odor similar to that of *A. faecalis.* The organisms are nonmotile and are oxidase, catalase, urease, and gelatinase positive. They reduce nitrite and do not produce indole. Isolates are successfully identified to the genus level using VITEK 2 and MALDI-TOF, which is able to distinguish *M. odoratus* and *M. odoratimimus.*[164,166] *Myroides* are broadly resistant to β-lactams, including carbapenems, with variable susceptibility to aminoglycosides, quinolones, and sulfamethoxazole.[164,166] Cutaneous as well as systemic infections have been successfully treated with a quinolone, carbapenem, or trimethoprim-sulfamethoxazole based on the results of in vitro susceptibility testing.[164] Analysis of the genome of an *M. odoratimimus* strain isolated from a patient with urinary tract infection revealed several resistance genes.[167]

Additional *Myroides* species isolated from clinical cultures but of unclear significance include *Myroides phaeus,* isolated from the saliva of a student in China,[168] and a newly described species, *Myroides injenensis,* isolated from urine.[169]

Ochrobactrum Species

Organisms formerly called CDC group Vd and *Achromobacter* groups A, C, and D were renamed *Ochrobactrum anthropi* (Gr. *ochros,* "pale yellow").[170] Studies suggest that *Achromobacter* group C and some group A strains belong to a distinct species now designated as *O. intermedium.*[170] *O. anthropi* has been recovered from the environment and clinical sources. Published reports suggest that this organism is an emerging pathogen in immunocompromised patients and that infections caused by this organism may be increasing in frequency.

Intravascular catheter–related bacteremia is the most common infection associated with *O. anthropi.*[171,172] This organism has contaminated biologic products, which have been the source of small outbreaks. Five bloodstream infections occurred in organ transplant recipients who received contaminated rabbit antithymocyte globulin.[173] Consistent with the low virulence of this organism, bacteremia resolved in four of five immunosuppressed patients in this series without antibiotic administration. Three patients developed *O. anthropi* urinary tract infections following transrectal ultrasound–guided prostate biopsies.[174] Three cases of postoperative meningitis in neurosurgical patients were traced to cadaveric pericardial patches possibly contaminated during processing.[175] *O. anthropi* has been cultured from tap water at a hematology unit in association with a small outbreak.[176] It has been reported to cause bacteremia in patients on hemodialysis, in patients with AIDS, and in liver transplant recipients and peritonitis in patients undergoing continuous ambulatory peritoneal dialysis.[177–180] *Ochrobactrum* endophthalmitis has occurred after hematogenous spread and postoperatively, including a cluster of nine cases after cataract extraction with lens implantation.[181] Other reported infections include infection of pacemaker leads, prosthetic valve endocarditis, pancreatic abscess, pelvic abscess complicating appendicitis, necrotizing fasciitis, septic arthritis, and osteochondritis after a puncture wound.[182] It has also been recovered from bile, urine, wounds, stool, throat, and vagina.[183] There is a single case report of *Ochrobactrum tritici* causing

bacteremia and cholecystitis. This isolate was misidentified by both MALDI-TOF and biochemical tests as *O. anthropi,* with the species identification made by 16S rRNA sequencing.[184]

O. anthropi is an oxidase-positive, non–lactose-fermenting gram-negative bacillus that grows readily on MacConkey agar. The organism oxidizes glucose and xylose, but 72 hours or more of incubation may be required before this is apparent. *O. anthropi* is motile by means of peritrichous flagella, which helps to differentiate it from pseudomonads and *Chryseobacterium.* The organism is similar to *A. xylosoxidans* subsp. *xylosoxidans* in biochemical characteristics, but it can hydrolyze urea and grows poorly on cetrimide agar.[107] *O. anthropi* and *O. intermedium* are closely related to *Brucella* species. Routine biochemical tests and automated identification systems are not reliable and are prone to misidentification; at best, these systems provide identification to the genus level.[185] *O. anthropi* is usually susceptible to trimethoprim-sulfamethoxazole and fluoroquinolones, both of which should be considered appropriate initial therapy. Isolates are variably susceptible to gentamicin, amikacin, netilmicin, imipenem, and tetracycline and generally resistant to β-lactams, including most cephalosporins and penicillins, at least in part as a result of the presence of an AmpC β-lactamase.[185] Failures with imipenem therapy have been reported.

Oligella Species

The genus *Oligella* was named for the small size of the bacilli on Gram stain and contains two species, *Oligella urethralis* (formerly *Moraxella urethralis* and CDC group M4) and *Oligella ureolytica* (formerly known as CDC group IVe). *O. urethralis* is a commensal of the genitourinary tract, and most clinical isolates are from the urine, predominantly from men. Although symptomatic infections are rare, bacteremia, septic arthritis mimicking gonococcal arthritis, and peritonitis in two patients receiving chronic ambulatory peritoneal dialysis[186] have been described. *O. ureolytica* is also primarily found in the urine, usually from patients with long-term indwelling urinary catheters or other urinary drainage systems. These patients have a propensity to develop urinary stones that may be related to the ability of the organism to hydrolyze urea and alkalinize the urine, leading to precipitation of phosphates. Bacteremia has been reported in patients with obstructive uropathy.[187] *O. ureolytica* bacteremia has been reported in a patient with AIDS and infected decubitus ulcers.

Oligella species, especially *O. urethralis,* resemble *Moraxella* and appear coccobacillary on Gram stain. Most strains will grow on blood or MacConkey agar but require extended incubation (2–4 days) before growth can be detected. *O. urethralis* is nonmotile, whereas most strains of *O. ureolytica* are motile by peritrichous flagella. The rapidity of the urease reaction (within 5 minutes on a Christensen urea agar slant) is a distinctive feature of *O. ureolytica.* These organisms are oxidase positive and catalase positive and reduce nitrate to nitrite. Contemporary data on antimicrobial susceptibilities are sparse. Strains of *O. urethralis* are usually susceptible to β-lactam antibiotics, but β-lactamase–producing strains, as well as strains resistant to ciprofloxacin, have been reported.[186] Resistance to β-lactam antibiotics is due to acquisition of chromosomally encoded AmpC β-lactamases, either ADC-7 or ABA-1, derived from *Acinetobacter baumannii.*[188,189]

Pseudomonas Species

The genus *Pseudomonas* has been modified considerably and now contains the organisms previously known as *Flavimonas oryzihabitans* and *Chryseomonas luteola.*[190] These organisms are included in the nonfluorescent group of pseudomonads that includes *P. stutzeri* and other rarely encountered species. The fluorescent group contains *P. fluorescens, P. putida,* and *P. aeruginosa. P. aeruginosa* is the only member of the genus that possesses significant virulence factors and is an important human pathogen; it is discussed in Chapter 219. Members of the fluorescent group produce pyoverdin, a yellow-green pigment that fluoresces under ultraviolet light. Pseudomonads are environmental organisms and have a predilection for moist environments. They can contaminate solutions such as distilled water, disinfectants, and intravenous solutions. Not surprisingly, many of the infections caused by these organisms are health care–associated. *P. fluorescens* species complex contains ~20% of the *Pseudomonas* species.[191] *P. fluorescens* is considered to be of low virulence and an uncommon cause of human infection. Most infections have been hospital acquired and have involved immunocompromised patients. Reported outbreaks include catheter-associated bacteremia, pseudobacteremia due to contaminated blood collection tubes, peritonitis in peritoneal dialysis transplant patients, and febrile neutropenia associated with a contaminated drinking water dispenser in a bone marrow transplantation unit.[192] Contaminated heparin flush solution caused a large multistate outbreak of *P. fluorescens* catheter-related bacteremia; in some exposed patients with implanted ports, diagnosis was delayed for many months after exposure to the solution.[193] This organism can grow at 4°C, allowing it to proliferate in contaminated blood products and occasionally cause transfusion-related sepsis.[194] *P. fluorescens* can be misidentified by commercial laboratory systems. Because isolation of this organism can reflect pseudobacteremia, proper identification is important to avoid unnecessary antimicrobial therapy.

P. putida is also an occasional cause of health care–associated bacteremia in patients with cancer, pneumonia, peritonitis, urinary tract infections, and neonatal sepsis.[195,196] In an outbreak of *P. putida* catheter-related bacteremia caused by a contaminated flush solution, infection was cured without catheter removal in most patients.[197] Isolation of this organism from clinical specimens can reflect contamination; it has also been associated with pseudo-outbreaks. *P. stutzeri* is another uncommon clinical isolate that has been reported to cause bacteremia, nosocomial brain abscess, and meningitis in immunocompromised hosts.[198] Rare cases of community-acquired osteomyelitis, septic arthritis, conjunctivitis, pneumonia, and peritoneal dialysis–related peritonitis have also been reported.[199] *P. stutzeri* has also been implicated as a cause of pseudobacteremia and of delayed-onset endophthalmitis after cataract surgery as well as an unusual case of relapse of endocarditis 4 years after the initial episode.[199,200] *Pseudomonas mendocina* has occasionally caused bacteremia and endocarditis.[201]

Pseudomonas oryzihabitans (L. *oryza* + *habitans,* "inhabiting rice") is the current name for the organism that at various times has been called *Chromobacterium typhiflavum, Flavimonas oryzihabitans,* and CDC group Ve-2.[202] It is an infrequent cause of infection with characteristics similar to those of *P. luteola. P. oryzihabitans* is normally found in soil, water, and damp environments such as rice paddies. In the hospital setting, it has been recovered from sink drains and respiratory therapy equipment.[202] Central venous catheter–associated bloodstream infection is the most commonly reported infection. In an 8-year study from a major cancer center, 21 of 22 episodes of *P. oryzihabitans* bacteremia were catheter related.[203] In this series, most infections were non–hospital acquired, polymicrobial infections were common, and most bacteremias could be treated without catheter removal. In contrast, in another series, all *P. oryzihabitans* bacteremias were hospital acquired and the implicated intravascular devices were removed in most cases.[204] The organism has also been associated with other foreign bodies, such as peritoneal dialysis catheters, ventriculostomy tubes, vascular grafts, prosthetic joints, and intraocular lenses.[205,206] Soft tissue infections, postoperative wound infections, splenic abscesses, and meningitis have been reported.[207,208] Although most patients with *P. oryzihabitans* infection are immunocompromised, the infections are indolent and recovery is the rule. *Pseudomonas luteola* is another uncommon opportunistic pathogen. It was previously known as CDC group Ve-1 and *Chryseomonas luteola. P. luteola* infections are often associated with foreign bodies such as central venous and peritoneal dialysis catheters. Reported infections include bacteremia, peritonitis (associated with appendicitis and colon cancer as well as catheters), osteomyelitis, endocarditis, leg ulcers, cellulitis, postoperative endophthalmitis, and meningitis and brain abscesses.[205,207,209,210]

Pseudomonas species are aerobic, non–spore-forming, gram-negative rods. They are motile owing to the presence of one or more polar flagella. They are lactose nonfermenters and grow well on MacConkey agar. Most clinical isolates (except *P. luteola* and *P. oryzihabitans*) are oxidase positive. In addition to the negative oxidase reaction, these two species produce yellow-pigmented colonies on MacConkey agar that help distinguish them from other pseudomonads. Unlike other fluorescent pseudomonads, including *P. aeruginosa, P. fluorescens* and *P. putida* do not reduce nitrate and oxidize xylose. *P. stutzeri* colonies are brown, dry, and wrinkled on primary isolation media.

There are limited antimicrobial susceptibility data for these pseudomonads. *P. putida* can show broad resistance to β-lactam antibiotics, and some isolates of this organism produce a metallo-β-lactamase that can readily hydrolyze carbapenems.[211] *P. oryzihabitans* is usually susceptible in vitro to ureidopenicillins, third-generation cephalosporins, aztreonam, imipenem, aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole but shows resistance to earlier-generation cephalosporins.[203,212] Clinical isolates of *P. luteola* are often resistant to first- and second-generation cephalosporins, tetracyclines, ampicillin, and trimethoprim-sulfamethoxazole but are susceptible to third-generation cephalosporins, imipenem, aminoglycosides, and quinolones.[205] A high proportion of clinical *P. putida* strains harbor metallo-β-lactamases on transposable elements that can potentially disseminate and contribute to multidrug resistance in other organisms.[211]

Ralstonia and *Cupriavidus* Species

The genus *Ralstonia* was established in 1995 and initially contained one recognized pathogen, *Ralstonia pickettii* (formerly *Pseudomonas,* then *Burkholderia, pickettii*). Subsequently, several other clinically relevant species were added to the genus, including *Ralstonia paucula* (formerly designated as CDC group IVc-2), *Ralstonia gilardii,* and, most recently, *Ralstonia mannitolilytica* (formerly *Pseudomonas thomasii,* then *R. pickettii* biovar 3/*"thomasii"*). There was extensive taxonomic revision of the genus in 2004, with *R. pickettii* and similar organisms remaining in the genus *Ralstonia,* but species in the *R. eutropha* lineage, including *R. paucula* and *R. gilardii,* were transferred to the new genus *Wautersia.* 16S rRNA profiles quickly revealed that the newly named *Wautersia* organisms were synonymous with the existing genus *Cupriavidus,* which, according to nomenclature rules, has priority over the genus name *Wautersia,* and all species that had been placed in the genus *Wautersia* were transferred to the genus *Cupriavidus. Ralstonia* and *Cupriavidus*[213,214] species are environmental gram-negative, nonfermentative bacilli of low virulence. *Cupriavidus* have also been found as abnormal microbiota in the respiratory tract of patients with pulmonary tuberculosis and on the skin of patients with psoriasis.[213,214] Reported infections caused by *Ralstonia* and *Cupriavidus* are related to contaminated infusates or occur in immunocompromised hosts, including transplant recipients and patients with HIV infection or leukemia.[215]

R. pickettii can grow in saline and other fluids and has been the cause of many outbreaks related to contaminated infusates and pseudo-outbreaks related to contaminated solutions used in laboratory diagnosis.[216,217] The contamination of solutions has occurred during the manufacturing process and by extrinsic manipulation. In addition to bacteremia from contaminated intravenous products, airway colonization has been caused by contaminated respiratory therapy solutions.[217] In one outbreak due to a contaminated saline solution, only 1 of 19 patients with *R. pickettii* airway colonization received antimicrobial therapy, consistent with the low virulence of the organism.[217] Several hospital-associated outbreaks attributed to *R. mannitolilytica* have also been described.[218,219] Clinical isolates of other *Ralstonia* and *Cupriavidus* species are less common. A number of *Ralstonia* and *Cupriavidus* species have been isolated from sputum cultures of cystic fibrosis patients.[220,221] Most of the reported human infections of *C. pauculus* and *C. gilardii* are intravascular catheter–related bloodstream infections.[222] *C. pauculus* has been recovered in cases of peritoneal dialysis–associated peritonitis, tenosynovitis after a cat bite,[223] pneumonia in immunocompetent patients, a pseudo-outbreak from contaminated culture swabs, and two reports of bacteremia in patients on extracorporeal membrane oxygenation with a contaminated thermoregulatory reservoir as the source.[224] Reports of infection caused by *C. metallidurans* include septicemia in a patient with multiple comorbidities and catheter-related bacteremia.[225,226] Most patients have responded well to antibiotic therapy.

Ralstonia and *Cupriavidus* species grow on routine media, although growth may be slow and require more than 72 hours of incubation to visualize colonies. *Ralstonia* species have one or more polar flagella in motile species, produce acid from glucose and several other carbohydrates, and are resistant to colistin, whereas *Cupriavidus* species have peritrichous flagella, do not produce acid from glucose, and are susceptible to colistin. Extensive biochemical testing is required for identification, and misidentification of these organisms by commercially available systems is common.[219] Identification may also be confused because *R. pickettii, R. mannitolilytica,* and *R. insidiosa* are able to grow on selective media intended for isolation of *B. cepacia.* 16S rRNA PCR and MALDI-TOF mass spectrometry have been useful means of identification.[220,221]

There are no validated in vitro susceptibility testing methods for *Ralstonia* species or *Cupriavidus* species. *R. pickettii,* but not other *Ralstonia* or *Cupriavidus* species, produces a chromosomally encoded class D oxacillinase β-lactamase, OXA-22, that confers resistance or reduced susceptibility to aminopenicillins, narrow-spectrum cephalosporins, and aztreonam.[227] An inducible chromosomal oxacillinase β-lactamase, OXA-60, that hydrolyzes imipenem is also widespread.[227] Isolates of *R. pickettii* have been reported to be generally susceptible to the ureidopenicillins, ciprofloxacin, and trimethoprim-sulfamethoxazole, with varied susceptibility to aminoglycosides.[228] *R. mannitolilytica* is often resistant to ampicillin, aminoglycosides, and aztreonam. *C. pauculus* is reportedly susceptible to many β-lactams, along with quinolones and tetracycline, but is often resistant to aminoglycosides.[215,222]

Rhizobium (Formerly *Agrobacterium*) Species

Based on 16S ribosomal DNA analysis, organisms previously known as *Agrobacterium, Allorhizobium,* and *Rhizobium* are now unified into a single genus, *Rhizobium.* These organisms are well-known plant pathogens; most contain a large tumor-inducing plasmid, and infection produces neoplastic growth in many plant species. They are present in soil and plants and have a worldwide distribution. Although most clinical isolates appear nonpathogenic, there have been more than 50 reported cases of human disease caused by *Rhizobium* species, primarily *R. radiobacter* and, more rarely, *Agrobacterium tumefaciens.* However, *A. tumefaciens* and *R. radiobacter* differ only by the presence or absence of the tumor-inducing plasmid, and they are now combined into a single species, *Rhizobium radiobacter.* Recent reports suggest that the newly named species, *Rhizobium pusense,* distinguishable from *R. radiobacter* only by 16S rRNA sequencing, is the main human pathogen of this genus.[229,230]

More than half of the reported cases of *R. radiobacter* infection are intravascular catheter–related bloodstream infections in compromised hosts, primarily patients with malignancies.[231] Most of these infections were not hospital acquired. However, there is a report of three cases of *R. radiobacter* (reported as *Agrobacterium* spp.) bacteremias occurring at a single institution in patients with tunneled intravenous catheters.[232] Two of these cases were epidemiologically linked, and the isolates had common pulsed-field gel electrophoresis patterns suggesting nosocomial transmission.[232] Other opportunistic infections associated with *R. radiobacter* include pneumonia, urinary tract infections, peritonitis, cellulitis, wound infections, cerebral abscess, and endocarditis.[233] Most of these infections occurred in hospital settings or in immunosuppressed patients or involved a device, the removal of which has been necessary in some cases to effect a cure. *R. radiobacter* has also caused pseudobacteremia resulting from contaminated citrated tubes used for clotting factor studies.[234] Several cases of postoperative endophthalmitis occurred in patients, some of whom reported outdoor activities such as gardening or golfing that may have exposed them to soil bacteria.[235] In most cases, the source of the infecting organisms is unknown. Consistent with this organism being an opportunistic pathogen of low virulence, all patients have survived.

The organism readily grows on blood agar and MacConkey agar when incubated aerobically. Colony appearance varies for the different species. Flagellar stains show peritrichous distribution. Organisms are oxidase positive and catalase positive, and they produce gas from a variety of carbohydrates, including lactose. Rapid hydrolysis of urea and slower hydrolysis of esculin are key features that help to distinguish this organism from *Alcaligenes* species and *Pseudomonas* species, which it otherwise closely resembles. Ambiguous or erroneous identifications occur with standard identification systems, and identification often requires 16S rRNA sequencing. Clinical as well as environmental isolates have been reliably identified using MALDI-TOF mass spectrometry. Clinical isolates have been variably susceptible to antibiotics and display variations in susceptibility patterns within classes of antibiotics. Reported *R. radiobacter*

strains have universally been susceptible to fluoroquinolones, cefepime, and carbapenems, and these agents should be considered for initial therapy. Acquired resistances are common for other β-lactam antibiotics and aminoglycosides, with gentamicin being more active than tobramycin. Monobactams are produced by some soil strains; not surprisingly, clinical isolates are often resistant to aztreonam.

Roseomonas Species and Other "Pink-Pigmented" Gram-Negative Bacilli

The group of organisms previously known as CDC "pink coccoid" groups I through IV have been placed in the genus *Roseomonas* (L. *roseus* + *monas,* "a rose-colored or pink bacterium"). Although *Roseomonas* species can be recovered from the environment, most named *Roseomonas* species, including *R. gilardii* subsp. *gilardii, R. gilardii* subsp. *rosea, R. cervicalis,* and *R. mucosa,* have been isolated from clinical specimens.[236] *Roseomonas* appears to cause more clinical disease than the related pink-pigmented bacterium *Methylobacterium.*[237] *Methylobacterium* species, which are so named because of their ability to facultatively use methane, were previously classified under such names as *Pseudomonas mesophilica, Protomonas extorquens, Protaminobacter rubra,* "the pink phantom," and *Vibrio extorquens.*[238] The two most clinically relevant species, *M. mesophilicum* and *M. zatmanii,* are very similar phenotypically, and some reference laboratories limit identification to the genus level only.[238]

R. gilardii is usually recovered in pure culture and, in one retrospective series, appeared to cause clinical illness more often than not.[237] Infections are often community acquired. Bloodstream infection is the most common presentation and may be related to the presence of intravascular catheters[239] or may be secondary to processes at other sites, including intraabdominal abscesses, and respiratory tract or urinary tract infections. These infections usually, but not invariably, occur in patients with underlying medical illnesses such as malignancies, AIDS, chronic renal disease, or diabetes. Device removal may be necessary to clear intravascular catheter–related bacteremia. Peritoneal dialysis–associated peritonitis, vertebral osteomyelitis, ventriculitis, septic bursitis, soft tissue infections, epiglottitis, postoperative endophthalmitis, and postoperative septic arthritis have also been reported.[237,240–244] Thirty-six episodes of *Roseomonas* bacteremia occurred in one referral cancer center over a 12-year period, with *R. mucosa* causing 61% of these infections.[245] Most patients had central venous catheters; line removal was necessary in six patients to clear the bloodstream infection. Most of the isolates were initially misidentified or unidentifiable; *Roseomonas* infection was confirmed by supplemental testing, including 16S rRNA genotypic studies. *R. fauriae* is rarely isolated from clinical specimens but has been reported to cause peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis.[246] *R. mucosa* septic arthritis has been reported in a patient with rheumatoid arthritis undergoing treatment with infliximab.

Methylobacterium has also caused intravascular catheter–related bacteremia, peritonitis in patients receiving continuous ambulatory peritoneal dialysis, and soft tissue infections.[247] A pseudo-outbreak of *Methylobacterium* respiratory tract infections was traced to contaminated tap water in the bronchoscopy suite.[248]

Roseomonas species are plump gram-negative rods or coccobacilli. In contrast, *Methylobacterium* species do not stain well and can appear gram variable, and also have intracellular vacuoles. Colonies are pink pigmented and are sometimes mucoid. Both these organisms can appear weakly oxidase positive and are catalase positive and urease positive. *Roseomonas* can be distinguished from *Methylobacterium* by the inability to oxidize methanol, the inability to assimilate acetamide, and the absence of long-wave ultraviolet light absorption. *Methylobacterium* has been isolated after 1 week of incubation on medium ordinarily used for the isolation of mycobacteria.[241]

Carbapenems, aminoglycosides, and tetracyclines are the most active antibiotics against *Roseomonas* species.[242,245,249,250] These organisms generally have high MICs to penicillins and cephalosporins, with the exception of β-lactamase inhibitor combinations, which are frequently but not invariably active. *Roseomonas* species usually have low MICs to fluoroquinolones but high MICs to trimethoprim-sulfamethoxazole.[250]

Methylobacterium grows slowly, and susceptibility testing is not always possible.[238] Many *Methylobacterium* isolates produce a β-lactamase, and the organisms have high MICs to penicillins and many cephalosporins. Aminoglycosides, ciprofloxacin, and trimethoprim-sulfamethoxazole are active.

Shewanella Species

Shewanella putrefaciens (formerly *Pseudomonas putrefaciens, Alteromonas putrefaciens,* or CDC group Ib) is widely distributed in the environment and has infrequently been implicated as a cause of human disease. *Shewanella* can be recovered from a variety of water sources, natural gas and petroleum reserves, dairy products, meat, and fish. Most reported human *Shewanella* infections have been attributed to *S. putrefaciens.* However, the automated and semiautomated identification systems used have not been able to differentiate between *S. putrefaciens* and *S. algae* because *S. algae* is not included in the databases.[251] When additional phenotypic or genotypic characteristics are considered, most human infections are realized to be caused by *S. algae.*[251] Cases of human infection attributed to *S. haliotis* and *S. xiamenensis* have also been reported.[252] *Shewanella* is frequently isolated as part of a polymicrobial infection, most often with enteric organisms, and its pathogenic role is often unclear. *Shewanella* is most commonly isolated from intraabdominal specimens, skin and soft tissue specimens, blood, and sputum.[253–255] Malignancy, hepatobiliary disease, diabetes mellitus, and colonization of the biliary tract are common underlying conditions. Cellulitis of the lower extremity in association with chronic ulcers or after burns is one of the more commonly described presentations.[253] Exposure to seawater is also a commonly reported risk factor.[256,257] An unusual case of *Shewanella* wound infection occurred following a cobra snakebite and required amputation of the infected finger.[258] *Shewanella* bacteremia, which also is frequently polymicrobial, can accompany soft tissue infection or biliary tract disease or occur in compromised hosts.[254] Compromised hosts are more likely to have accompanying signs of sepsis and have a poor outcome. A common source outbreak that led to 31 patients infected or colonized by *Shewanella* on a surgical ward was traced to a contaminated shared measuring cup.[259] In this outbreak, blood, bile, and ascitic fluid were the most common culture isolation sources. Bacteremia and respiratory distress have been described in neonates and premature infants.[254] Bacteremia following consumption of raw fish in a patient with end-stage renal disease has been reported.[260] Less commonly reported infections include peritonitis, pneumonia, empyema, purulent pericarditis, meningitis, brain abscess, osteomyelitis, otitis, urinary tract infection, endophthalmitis, keratitis, infected aortic aneurysm, and flexor tenosynovitis.[251,253–255,261]

On Gram stain, *Shewanella* is a short to long gram-negative rod and can be filamentous. It grows readily and produces small to medium-sized colonies that have a yellow-orange or brown-to-tan soluble pigment that causes greenish discoloration of the medium. Colonies may be mucoid and have a fishlike smell. *Shewanella* is oxidase positive and is the only nonfermenter that produces hydrogen sulfide on triple sugar iron agar, a key feature that allows easy identification in the laboratory. *S. algae* can be distinguished from *S. putrefaciens* by growth at 42°C, growth in 6.5% NaCl, mucoid colonies, weak β-hemolysis on sheep blood agar, reduction of nitrite, and the inability to produce acid from sucrose, maltose, and L-arabinose.[251] With current databases, MALDI-TOF mass spectrometry accurately identifies isolates to the genus level, but species identification is currently problematic.[262]

Putative virulence factors in *S. algae* that enable severe infection include production of a hemolysin and exotoxin, ability to adhere to epithelial cells, and biofilm formation.[256] *Shewanella* is also resistant to many heavy metals and antiseptic agents, which allows survival in harsh environments.[256]

β-Lactamases and multidrug efflux pumps have been detected that contribute to high MIC values for some antibiotics.[256] *Shewanella* is reported as resistant to penicillin and cefazolin but susceptible to most third- and fourth-generation cephalosporins and piperacillin.[255] The organisms are also usually susceptible to aminoglycosides, chloramphenicol, erythromycin, and quinolones but less predictably susceptible to tetracycline and trimethoprim-sulfamethoxazole.[251,253,254,263,264] Carbapenem-resistant strains have been reported.[264] *Shewanella* may

also contain a chromosomally encoded gene, *qnr3,* that confers resistance to quinolones by protecting DNA gyrase and probably also topoisomerase IV.[264] Because *Shewanella* is often recovered as part of a polymicrobial intraabdominal infection, piperacillin-tazobactam is reasonable initial therapy. However, development of resistance while on treatment has been reported with piperacillin-tazobactam and imipenem.[263]

Sphingobacterium Species

The genus *Sphingobacterium* includes organisms previously classified as *Flavobacterium* species. The organisms that were transferred to this genus contain large amounts of sphingophospholipid compounds in their cell membranes and have other taxonomic features that distinguish them from flavobacteria. Most isolates from humans are *S. multivorum* or *S. spiritivorum*. Most cases of *S. multivorum* infection are hospital acquired, but the natural habitat of the organism is not well defined. The reported cases of *S. multivorum* have been associated with peritonitis, septicemia, bacteremia, chronic respiratory infection in patients with underlying conditions, and colonization of the airway in patients with cystic fibrosis. It has also been recovered from contact lens storage cases from patients with corneal infiltrative events.[265] Also reported is an unusual case of necrotizing fasciitis and septic shock in an immunocompromised patient following a scratch to the leg by the patient's dog.[266] *S. spiritivorum* has been rarely recovered from clinical specimens, primarily urine and blood.[267] Cellulitis presumably from soil contact with secondary bacteremia and dialysis catheter–related bacteremia have been reported, as has hypersensitivity pneumonitis related to a water source harboring *S. spiritivorum*.[268–270]

S. multivorum and *S. spiritivorum* are straight gram-negative rods that are strictly aerobic; grow on blood agar but are limited or absent on MacConkey agar; are DNAase, oxidase, catalase, and urease positive and indole negative; and produce light-yellow colonies. Automated systems and the API 20 NE system (bioMérieux, Durham, NC) produce reliable identifications. *Sphingobacterium* species are intrinsically resistant to many commonly employed antibiotics and can grow in some antiseptics and disinfectants.[271] *S. multivorum* can produce an extended-spectrum β-lactamase and a metallo-β-lactamase conferring resistance to third-generation cephalosporins and carbapenems, respectively.[272] Trimethoprim-sulfamethoxazole and quinolones appear to be active. The combination of trimethoprim-sulfamethoxazole and perfloxacin produced cure in a bacteremic patient.[271] A bacteremic patient receiving hemodialysis improved clinically after receiving ampicillin and one dose of tobramycin, despite in vitro testing showing ampicillin resistance. The combination of surgical débridement and amoxicillin-clavulanate was effective in the reported necrotizing fasciitis case.[266] *S. spiritivorum* tends to be more susceptible, with ceftazidime, carbapenems, trimethoprim-sulfamethoxazole, and quinolones appearing active in vitro.

Sphingomonas Species

The genus *Sphingomonas* contains at least 95 species, of which only one, *Sphingomonas paucimobilis,* is an occasional human pathogen.[273] This organism, formerly known as *Pseudomonas paucimobilis* and CDC group IIk-1, is widely distributed in soil and water, including water sources in the hospital environment. Most *Sphingomonas* infections are hospital acquired and typically occur in immunocompromised individuals. Central catheter–related bacteremia, bacteremia due to a contaminated intravenous medication, peritoneal catheter–associated peritonitis, meningitis, ventriculoperitoneal shunt infection, brain abscess, soft tissue infection, wound infection, postoperative endophthalmitis, adenitis, urinary tract infection, and a variety of visceral abscesses have been reported.[274–279] Although ventilator-associated pneumonia has been described,[280] airway colonization was much more common than infection in intensive care unit outbreaks.[280,281] *S. paucimobilis* is considered to be an organism of low virulence, likely owing to the absence of endotoxins, but infection can lead to septic shock. Sporadic reports of unusual invasive and severe infections include septic arthritis and osteomyelitis, respiratory tract infections in cystic fibrosis patients, and necrotizing soft tissue infections.[277,282] Diabetes mellitus and alcoholism appear to be risk factors for community-acquired *S. paucimobilis* infections, including bacteremia.[278]

S. paucimobilis is a polymorphic gram-negative rod and is strictly aerobic, weakly oxidase positive, and catalase positive. Colonies grow on blood agar but not MacConkey agar and produce a yellow pigment. Despite the presence of a single polar flagellum, a low percentage of cells are actively motile, and motility can be difficult to demonstrate in the laboratory (thus the name *paucimobilis*).[283] *Sphingomonas* may be misidentified by conventional identification systems but is correctly identified by MALDI-TOF mass spectrometry.

The most effective antibiotics appear to be broad-spectrum β-lactam antibiotics, β-lactam–β-lactamase inhibitor combinations, cephalosporins, fluoroquinolones, and carbapenems.[282] Patients have been noted to respond well, even debilitated hosts and in cases when empirical treatment did not correlate with subsequent susceptibility tests.[276] The importance of removing the catheter to ensure complete eradication of the organism and to prevent recurrence of intravascular catheter–associated bloodstream infections is stressed in several reports.[276]

Weeksella and *Bergeyella* Species

The genus *Weeksella,* when proposed in 1986, contained two species, *W. zoohelcum* (CDC group IIj) and *W. virosa* (CDC group IIf), that differed from most nonfermentative gram-negative bacilli in being susceptible to penicillin. *W. zoohelcum* has been moved to the new genus *Bergeyella. Bergeyella zoohelcum* (Gr. "animal" + "wound") is part of the normal oral microbiota of dogs and other animals, and most clinical isolates come from bite wounds.[284–286] The organism was also isolated from a soft tissue infection in an injured tsunami survivor.[287] There are a few case reports of invasive *B. zoohelcum* infections, including meningitis, infective endocarditis, and bacteremia; some have occurred after dog bites or lengthy exposure to pets.[285,288] A 44-year-old woman developed *B. zoohelcum* bacteremia 1 day after eating a meal prepared with goat blood.[289] A proposed new species, *B. cardium,* was isolated from patients with infective endocarditis in Korea.[290] *W. virosa* has been isolated predominantly from the genital tract and urine of women and is usually not a pathogen. There are case reports of dialysis-associated peritonitis, spontaneous bacterial peritonitis, presumed dialysis catheter–related bacteremia and sepsis, overwhelming sepsis and pneumonia, and intracranial infection.[291] All infected patients had significant comorbidities.

Both *Bergeyella* and *Weeksella* generally grow well on blood agar, but some strains of *Bergeyella* grow better on chocolate agar. Both organisms do not grow on MacConkey agar. They are oxidase positive, catalase positive, indole positive, and nonpigmented. In contrast to *W. virosa, B. zoohelcum* produces urease and is resistant to polymyxin. *W. virosa* (L., "slimy") forms mucoid colonies that stick tenaciously to agar surfaces. Both species have low MICs to β-lactam antibiotics, including penicillin, chloramphenicol, and fluoroquinolones, and have variable MICs to tetracycline and trimethoprim-sulfamethoxazole; many of these agents have been used successfully in case reports. *W. virosa* is usually resistant to one or more aminoglycosides. The combination of penicillin susceptibility and aminoglycoside resistance is a clue to the identification of this organism. *Bergeyella* has been misidentified by automated commercial systems, but MALDI-TOF mass spectrometry has been successfully used to identify these organisms.[285]

Centers for Disease Control and Prevention Groups

The CDC Special Bacteriology Reference Laboratory receives unusual isolates from state laboratories and other reference laboratories. Some of these isolates are unnamed and are grouped by growth characteristics. Each of these groups represents one or more species. Although many of the isolates are from sterile sites, clinical information is often limited, and the pathogenic role of these organisms is uncertain. Some of the CDC groups of gram-negative rods or coccobacillary organisms include the following:

1. CDC group NO-1 (NO for nonoxidizer) consists of at least 22 strains of fastidious gram-negative bacilli isolated from human wounds, most of which were related to dog or cat bites.[292,293] These organisms are similar to asaccharolytic strains of *Acinetobacter* but have a negative *Acinetobacter* transformation assay, have different cellular fatty acid profiles, and, unlike

most *Acinetobacter* organisms, reduce nitrate. They are susceptible to many antimicrobial agents, including β-lactams, aminoglycosides, fluoroquinolones, and tetracycline.

2. CDC group WO-1 (WO for weak oxidizer) includes 96 oxidase-positive, motile gram-negative rods, most of which were isolated from clinical specimens.[294] One third of the clinical isolates were from blood, and 10% were from cerebrospinal fluid.[294] Signs of sepsis were present in some of the patients, but the clinical significance of this group of organisms remains unclear.
3. CDC group WO-2 isolates now reside in the genus *Pandoraea,* which has five named and at least three unnamed species. These organisms can colonize the airways of patients with cystic fibrosis and rarely can cause clinical disease, including bacteremia.[295–297] *Pandoraea* species are often resistant to ampicillin, extended-spectrum cephalosporins, and aminoglycosides and are variably susceptible to fluoroquinolones.[296]
4. CDC groups O-1, O-2, and O-3 (O for oxidizer) are phenotypically similar; they are oxidase-positive, curved gram-negative rods that do not grow on MacConkey agar but grow on *Campylobacter*-selective media. One case of group O-1 pneumonia complicated by bronchopulmonary fistula and bacteremia has been reported.[298] One group O-3 isolate that was submitted to the CDC had been identified as a *Campylobacter* species, indicating the potential for misidentification of the O-3 group.[299] The CDC collection of group O-3 includes isolates from a variety of clinical sources, including blood, lymph nodes, joint fluid, bone, and lung. They were resistant to most β-lactam antibiotics, except imipenem; all were susceptible to aminoglycosides and trimethoprim-sulfamethoxazole but not ciprofloxacin.
5. Fifteen strains of an oxidase-positive, gram-negative rod biochemically resembling *Neisseria weaveri* (CDC group M-5) are currently designated Gilardi rod group 1 by the CDC.[300] Most of the strains were isolated from human wounds of the extremities or blood cultures.

Gardnerella and *Mobiluncus* Species

Gardnerella vaginalis is difficult to characterize in terms of its microbiologic designation and its clinical relevance. By 16S rRNA sequence analysis, it is sufficiently distinct to merit its own genus but is somewhat closely related to *Bifidobacterium* species, which are anaerobic gram-positive rods.[301] It is a facultatively anaerobic, oxidase- and catalase-negative, nonsporing, nonencapsulated, nonmotile, pleomorphic, gram-variable rod (see the excellent review by Catlin.[302]) *G. vaginalis* has a thin cell wall, which does not retain the crystal violet/iodine complex on decolorization, accounting for the gram-variable or gram-negative appearance of the organism. However, the preponderance of evidence suggests that *G. vaginalis* has a gram-positive heritage.

The natural habitat for *G. vaginalis* is the human vagina, where culture-independent methods have shown that it is essentially universally present in women without signs or symptoms of vaginal infection.[303] During the development of bacterial vaginosis, the normal vaginal microbiota composition changes. Bacterial vaginosis is characterized by a decrease in numbers of lactobacilli and a predominance of *G. vaginalis* and a mixture of other anaerobic bacteria (see Chapter 108).[304] *G. vaginalis* strains associated with bacterial vaginosis have been shown to produce a cytolysin and are efficient biofilm producers.[305] However, the role of *G. vaginalis* and other organisms in the pathogenesis of bacterial vaginosis remains unclear. Molecular analyses of the vaginal microbiota of women with this disorder have discovered novel species of unculturable bacteria; some of these bacterial species are highly specific for bacterial vaginosis.[306]

Extravaginal infections caused by *G. vaginalis* are uncommon. Bacteremia is seen almost exclusively in women and is usually associated with postpartum endometritis, postpartum fever, chorioamnionitis, septic abortion, or infection after cesarean section.[307] It is a relatively infrequent urinary tract isolate, and its clinical significance can be difficult to ascertain. However, it has been recovered from suprapubic bladder aspirates from pregnant women.[308] Rare osteoarticular infections have been reported in women with or without joint prostheses and no concurrent genital infection.[309] *G. vaginalis* has also been recovered from the male urogenital tract, but definitive evidence of sexual transmission is lacking.[310] It has occasionally been associated with disease in men, often in association with urologic procedures or underlying urologic problems; reported infections include bacteremia, balanitis, urethritis, urinary tract infections, and an unusual case of urosepsis and septicemia with infective endocarditis and septic emboli.[302,311] Oral metronidazole, intravaginal metronidazole gel, or intravaginal clindamycin cream is the recommended treatment for bacterial vaginosis.[312] The utility of these agents may reflect the importance of the mixed anaerobic microbiota in bacterial vaginosis. Treatment of the sexual partner has not been shown to influence a woman's response to therapy or relapse rate, and the routine treatment of sexual partners is not recommended.[312] β-Lactams have been used to treat extravaginal infections caused by *G. vaginalis.*

Mobiluncus species are slowly growing, curved, gram-variable, motile, anaerobic bacteria predominantly found in the human vagina and also have been associated with bacterial vaginosis. *Mobiluncus* has been isolated from the vagina of as many as 97% of women with bacterial vaginosis[313] but in a minority of healthy controls.[314] However, the role of *Mobiluncus* in the pathogenesis of bacterial vaginosis is unclear. *Mobiluncus* species, more commonly *Mobiluncus curtisii,* have been associated with upper genitourinary tract infections and adverse pregnancy outcome.

Extra–genitourinary tract infections have included nonpuerperal breast abscesses, umbilical and mastectomy wounds, and a polymicrobial hepatic abscess.[315] There are several reported cases of *Mobiluncus* bacteremia, including one in a previously healthy woman who developed septic shock with coagulopathy, acute respiratory distress syndrome, and renal failure.[316] There is a single case report of a man with *Mobiluncus* bacteremia and underlying ulcerative colitis.[317] *Mobiluncus* species are usually susceptible to penicillins, ampicillin, cefoxitin, clindamycin, erythromycin, imipenem, and vancomycin,[318] but can be resistant to metronidazole.

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The complete reference list is available online at Expert Consult.

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237 Syphilis (*Treponema pallidum*)

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SHORT VIEW SUMMARY

Definition
- Syphilis is a chronic, multistage sexually transmitted infection caused by the spirochete *Treponema pallidum.*

Epidemiology
- According to the World Health Organization, globally, approximately 17.7 million persons aged 15 to 49 years had venereal syphilis in 2012; an estimated 5.6 million new cases occur each year.
- Globally, more than 1 million pregnant women are estimated to be infected each year; approximately one-third of these infections will result in stillbirths or other adverse outcomes of pregnancy.
- In developing countries, transmission is largely heterosexual, whereas transmission among men who have sex with men predominates in industrialized nations.
- Genital ulcers caused by syphilis are a major cofactor for bidirectional transmission of human immunodeficiency virus (HIV).

Microbiology
- *T. pallidum* subspecies *pallidum, pertenue,* and *endemicum* cause venereal syphilis, yaws, and endemic syphilis, respectively. *Treponema carateum* causes pinta.
- Until recently, the pathogenic treponemes were considered to be uncultivatable. Continuous long-term culture of *T. pallidum* subsp. *pallidum* by cocultivation with rabbit epithelial cells was just reported.
- All pathogenic treponemes are indistinguishable with routine clinical laboratory tests.
- Humans are the only natural hosts for *T. pallidum* subsp. *pallidum.* Infection with *T. pallidum* subsp. *pertenue* has been well documented in nonhuman primates in sub-Saharan Africa.
- *T. pallidum* possesses both inner and outer membranes; its outer membrane lacks lipopolysaccharide and contains a paucity of integral membrane proteins and surface-exposed lipoproteins, hence its impressive capacity for immune evasion and its designation as "the stealth pathogen."
- *T. pallidum* must acquire essentially all nutrients from its obligate human host and generates adenosine triphosphate primarily by glycolysis.
- *T. pallidum* disseminates early during the course of infection and invades the central nervous system in a substantial percentage of persons with early syphilis.

Diagnosis
- Because *T. pallidum* cannot be cultivated in vitro, diagnosis of syphilis depends on demonstrating treponemes in clinical samples or by demonstrating reactivity in serologic tests, or both.
- Darkfield (DF) microscopy and polymerase chain reaction are useful for detecting treponemes in exudative lesions, principally chancres.
- Serodiagnosis of syphilis involves two types of serologic tests: nontreponemal and treponemal. The former detects antibodies against lipoidal antigens (primarily cardiolipin), whereas the latter detects antibodies against *T. pallidum* proteins.
- Serodiagnosis of syphilis has traditionally used an algorithm in which nontreponemal antibodies are used for screening and the results are then confirmed with treponemal antibodies. In recent years, the so-called "reverse algorithm," in which an automatable treponemal test is used to screen followed by confirmation with a nontreponemal test, has gained popularity.
- Because nontreponemal titers usually decline with therapy, nontreponemal antibody tests are used as indicators of disease activity. However, it is now recognized that nontreponemal tests remain reactive in a substantial percentage of treated patients.
- Treponemal tests usually remain reactive for life.

Therapy
- Penicillin G is the preferred form of therapy for all stages and types of syphilitic infection.
- The use of 2.4 million units of intramuscular benzathine penicillin (single dose) is the regimen recommended by the Centers for Disease Control and Prevention and the World Health Organization for early syphilis, regardless of HIV status.
- Doxycycline is the preferred alternative for nonpregnant, penicillin-allergic patients.
- Penicillin-allergic pregnant females should be desensitized.

Prevention
- Prophylactic treatment is indicated for partners exposed to known active cases within the past 90 days regardless of whether lesions are present or serologic tests are reactive in the exposed individual.
- Early identification and treatment of gestational syphilis prevent congenital infection.

Syphilis is a complex systemic illness caused by the highly invasive, noncultivable spirochete *Treponema pallidum.* It holds a special place in the history of Western medicine because of its prevalence in modern times before the advent of penicillin, the many historical personages who had or are presumed to have had the disease, and its protean clinical manifestations, for which it came to be known as "the great imitator" or "the great impostor."[1–5] The first designated and recognized medical specialists were syphilologists. Special clinics were established in Europe and North America to care for the enormous number of persons with this disorder, and its ubiquity spawned one of the first specialized medical journals, the *American Journal of Syphilis, Gonorrhea and Venereal Disease.* The identification of the causative agent, initially named *Spirochaeta pallida,* in 1906 was a milestone in biomedical research.[4]

Venereal syphilis is usually transmitted by sexual contact and, unlike most other bacterial infections, is never diagnosed in routine clinical

practice by isolation of the causative organism. Instead, diagnosis depends on detection of *T. pallidum* or *T. pallidum* DNA in patient specimens or, most commonly, reactivity in serologic assays.[5–7] The inability to isolate *T. pallidum* from patient specimens, with the consequent dependence on serologic findings, is the root cause of most of the management dilemmas that continue to surround this disorder.[8,9] Confusion and controversies also arise from the disease's protracted natural history, which is classically divided into the following stages: (1) an incubation period lasting up to 90 days (average of 3 weeks); (2) a primary stage characterized by an ulcer, the *chancre,* at the site of inoculation, often associated with regional lymphadenopathy; (3) a florid, disseminated stage (secondary syphilis), typically characterized by generalized skin rash, mucocutaneous lesions, and lymphadenopathy, but capable of involving any organ system, including the central nervous system (CNS); (4) an asymptomatic latent period lasting years, detectable only through reactive serologic tests; and, (5) in approximately one-third of untreated persons, a recrudescent, tertiary stage involving the ascending aorta (cardiovascular syphilis) or the CNS (neurosyphilis) or causing necrotizing granulomatous lesions (gumma) in almost any organ.

HISTORY

The origins and spread of venereal syphilis, and the evolutionary differentiation of the syphilis spirochete from the causes of the nonvenereal treponematoses, particularly yaws, have been long-standing sources of debate and controversy. Phylogenetic trees constructed from reference genes and complete genomic sequences demonstrate that the pathogenic treponemes form distinct clusters.[10–12] These findings definitively disprove the Unitarian hypothesis, championed by Hudson,[13,14] that the treponematoses are caused by the same bacterium but with varying modes of transmission and clinical manifestations determined by climate and demographic factors. The debate currently centers around whether venereal syphilis was imported into Europe by shipmates of Christopher Columbus in 1493 or was an established entity that erupted in epidemic form throughout Europe as a consequence of urbanization and social upheaval.[15–19] The controversy over these two mutually exclusive theories is fueled by the imprecision and uncertainties inherent in the historical record, radiocarbon dating, and paleopathologic findings believed to represent the signature of treponemal disease in skeletal remains. Unfortunately, the inability of investigators, despite exhaustive efforts, to use polymerase chain reaction (PCR) to amplify ancient treponemal DNA from pre-Columbian archeological specimens precludes definitive resolution of this controversy.[20–23] Moreover, given the high degree of similarity between the pathogenic treponemes,[10] sequence data from numerous loci, perhaps complete genomes, will be needed to resolve this issue on a genetic basis.[24] Lacking direct molecular proof, the proponents of the "New World" or "Columbian" hypothesis point to the extensive osteologic record of presumptive treponemal disease in New World pre-Columbian populations dating back 7000 years as opposed to the sporadic skeletal evidence from Old World pre-Columbian excavation sites. In a comprehensive evaluation of all extant osteologic data, Harper and colleagues[16] concluded that no case of Old World treponemal disease has a radiocarbon date that places it firmly in the pre-Columbian period. These same investigators used sequence analysis of multiple loci in modern *T. pallidum* subspecies isolates to buttress their argument that syphilis spirochetes originated in the New World,[25] although others have questioned the robustness of their phylogenic scheme.[26,27] Indeed, analysis of genetic differences in *T. pallidum* supspecies based on estimated mutation rates leads to the conclusion that the agents of yaws and venereal syphilis diverged thousands of years ago during human prehistory.[27,28] The discovery of yaws-like treponemes that infect nonhuman primates (NHPs) in Africa[29–31] strongly supports another key tenet of the "pre-Colombian" hypothesis as first proposed by Hackett[14]—that the human treponematoses originated as a zoonosis that spilled over from an NHP reservoir. *T. pallidum* subsp. *pallidum* presumably evolved during the subsequent human diaspora from Africa.

Universally agreed on is that an epidemic known as the Great Pox (as distinguished from smallpox) ravaged Europe shortly after the return of Columbus from his first voyage of discovery.[4,32] This plague broke out during the invasion of Italy in 1494 by Charles VIII of France, whose army included numerous mercenaries, some from Spain, and was accompanied by a horde of civilians, many of whom were prostitutes.[4,32] Although it cannot be proved with certainty that *T. pallidum* was the cause of this scourge, the first clear descriptions of the serious ailment dubbed "the French Disease" by contemporary physicians and chroniclers noted its sexual mode of transmission and traced its origins to the West Indies and members of Colombus's expedition.[4,32] From these primary historical accounts the Colombian hypothesis took root. The name "syphilis" derives from an epic poem, *Syphilis sive morbus Gallicus (Syphilis or the French Disease),* published in 1530 by Girolamo Fracastoro, a Veronese physician, poet, and philosopher. In his treatise, Fracastoro provided a detailed description of the clinical features of syphilis along with recommendations for treatment, ending with the allegory of Syphilus, a shepherd who contracted an illness as punishment for offending the sun god Apollo.[4] During the pandemic of the early 16th century, syphilis in adults manifested as a fulminant, often fatal, illness that progressed rapidly from genital ulceration to gummas. Whether the comparatively mild nature of the present-day ailment reflects a change in the virulence of *T. pallidum,* an adaptation of the human host, or the disappearance of a concomitantly occurring but unknown illness is an enduring mystery. A remarkable phylogenetic comparison of genomic sequences of geographically widespread *T. pallidum* strains, many obtained directly from clinical samples, has added to this conundrum.[33] This study revealed that all modern-day syphilis spirochetes diverged from a common ancestor less than 500 years ago, a time frame roughly congruent with the explosive emergence of syphilis on the European scene. Variation in outer membrane proteins (OMPs),[34] which was not examined by these investigators,[33] driven by herd immunity as the spirochete gained traction within European populations[35,36] might explain changes in its virulence properties over the ensuing centuries.

One of the difficulties in sorting through medical writings from the 17th and 18th centuries is that unequivocal clinical distinctions between syphilis, gonorrhea, and other venereal diseases did not emerge until the late 18th century. John Hunter's misguided self-inoculation experiment with urethral pus containing both *Neisseria gonorrhoeae* and *T. pallidum* served only to prolong the misconception that syphilis and gonorrhea are the same disease.[4] By the mid-19th century, however, syphilis had been distinguished from gonorrhea and its principal clinical features defined, although not without some erroneous teachings. One prominent example is the dogma of Ricord, the greatest syphilologist of the 19th century, that material from secondary syphilis lesions is noncontagious.[4]

In 1903, Metchnikoff launched the era of modern syphilology when he observed that a female chimpanzee inoculated on the clitoris with material from a syphilitic lesion developed a chancre followed by secondary syphilis. Two years later, Schaudinn and Hoffmann discovered *S. pallida* in chancre exudates and adjacent lymph node aspirates. By 1906, Wassermann had developed his complement fixation test using an extract from the liver of a syphilitic stillborn baby. Subsequently, it was shown that extracts from uninfected beef livers and hearts were equally sensitive and that a phospholipid extracted from beef heart (cardiolipin) reproduced the Wassermann reaction when mixed with lecithin and cholesterol. In 1910, Ehrlich introduced his 606th arsenic preparation, arsphenamine or salvarsan, for syphilotherapy, hailed at the time as "the magic bullet." In 1912, Nichols and Hough[37] used rabbit inoculation to isolate the organism from the cerebrospinal fluid (CSF) of a patient who developed neurosyphilis after salvarsan therapy for secondary syphilis. In addition to being the first demonstration of early CNS invasion by *T. pallidum,* their findings suggested that the CNS may serve as a sanctuary for the spirochete during inadequate therapy of early syphilis, a concern to this day.[38] Also noteworthy is that the "Nichols" isolate became the reference laboratory strain of *T. pallidum* and was the source of DNA for the *T. pallidum* genome sequencing project.[39] Of note, another interesting observation from genomic sequencing is that the phylogenetic cluster (clade) containing the Nichols strain greatly diminished in prevalence during the latter part of the 20th century and is being replaced by a second clade represented by the high-level erythromycin-resistant street-strain 14 (SS14).[12,33,40] In 1927, Dr. Julius Wagner von Jauregg was awarded the Nobel Prize in Medicine for describing malarial fever as treatment for neurosyphilis. In 1937, Thomas Parran, the US Surgeon

General from 1936 to 1948, published his landmark book *Shadow on the Land,* in which he estimated that 10% of Americans would be infected by syphilis during their lifetimes.[41] In 1943, John Mahoney of the US Public Health Service treated the first syphilis patients with penicillin,[42] the true magic bullet, work that won him the Lasker Award in 1947. Not surprisingly, syphilis rates plummeted over the next decade. Despite alarming comebacks, most notably the "drugs for sex" cocaine-fueled epidemic of the late 1980s to early 1990s[43] and the substantial upswing in cases among men who have sex with men (MSM) beginning in the late 1990s,[44] syphilis rates have never approached pre–World War II levels.

The enduring impact of syphilis on the biomedical research community extends beyond its ongoing threat to public health. In an effort to better understand the natural history of syphilis, from 1932 to 1972 the US Public Health Service conducted the "Tuskegee Study of Untreated Syphilis in the Negro Male" in which 412 indigent African-American men with latent syphilis in rural Macon County, Alabama were followed without treatment along with 192 black males without serologic evidence of syphilis.[45,46] The revelation 4 decades after the study's inception that treatment had been withheld for years despite the proven efficacy of penicillin sparked outrage and charges of abuse of authority and racial exploitation on the part of the US government and the medical profession.[47] It has also been argued that the continuance of the study was more a reflection of the lack of data and consensus about the efficacy of penicillin therapy for late syphilis than of racial bias.[46] Questions of racial bias aside, the egregious violations of patient rights brought to light the need for much more stringent regulation of research involving human subjects and led to the 1979 Belmont Report, the establishment of the National Human Investigation Board, and the requirement for institutional review boards. In 2010, the public health and medical communities were stunned by disclosures of bioethical violations even more serious than those of the Tuskegee Study.[48–50] Susan Reverby, a Wellesley College historian investigating Dr. John Cutler, one of the physicians involved in the implementation of the Tuskegee Study, inadvertently discovered that from 1946 to 1948 the US Public Health Service conducted studies in Guatemala in which male prisoners were unknowingly inoculated with *T. pallidum* and allowed to transmit syphilis to their sexual partners as part of a plan to evaluate prophylactic treatment regimens. Among the many disturbing revelations was that Parran had approved funding for the studies and appears to have followed their progress. The National Archives has posted more than 12,000 pages of documents related to this study on its website (www.archives.gov/research/health/cdc-cutler-records). A full report by the Presidential Commission for the Study of Bioethical Issues can be found at https://bioethicsarchive.georgetown.edu/pcsbi/node/5896.html.

ETIOLOGY

The causal agent of venereal syphilis is *T. pallidum* subsp. *pallidum,* which belongs to the order Spirochaetales, the family Spirochaetaceae, and the genus *Treponema.* Other members of the genus *Treponema* that can infect humans are *T. pallidum* subsp. *pertenue* (yaws), *T. pallidum* subsp. *endemicum* (bejel or endemic syphilis), and *Treponema carateum* (pinta). In addition to the pathogenic spirochetes, commensal treponemes have been isolated from humans, particularly from the oral cavity and the prepuce of uncircumcised men, and they have been identified by amplification of 16S ribosomal RNA genes from the gut microbiomes of hunter-gatherer and traditional agrarian populations.[51,52] At one time, investigators believed that commensal treponemes were nonpathogenic variants of *T. pallidum.*[53] Miao and Fieldsteel[54,55] disproved this notion by showing with DNA-DNA hybridization that *T. pallidum* subsp. *pallidum* and *pertenue* were indistinguishable and that there was no discernible homology between the DNAs of pathogenic and cultivatable treponemes. As a result, the agents of venereal syphilis, endemic syphilis, and yaws were reclassified as subspecies of *T. pallidum*; *T. carateum* retained its status as a separate species because no isolates were available for study (as is true today). The pathogenic treponemes are believed to have evolved by reductive evolution from a commensal treponeme; comparative genomics, using the *Treponema denticola* genome as a frame of reference, suggests that the divergence from a common progenitor occurred millions of years ago.[56,57] In contrast, as noted earlier, the divergence of the *T. pallidum* subspecies postdates the emergence of modern man, probably occurring within the past 20,000 years.[27,56,57]

All four pathogenic treponemes are morphologically indistinguishable and induce antibodies detected with the routine serologic tests used to diagnose venereal syphilis.[7,58] In clinical situations, distinction among the corresponding infections rests on geography, clinical manifestations, patient age, and other demographic features.[7,58] Of the four pathogens, only *T. pallidum* subsp. *pallidum* is transmitted routinely by sexual contact.[59] Because the other three are transmitted nonsexually, the diseases they cause are collectively referred to as the *endemic* or *nonvenereal treponematoses.*[58] *T. pallidum* subsp. *pallidum* is considered the most virulent because it is the only subspecies capable of regularly breaching both the blood-brain and maternal-fetal barriers.[59,60] At the opposite end of the virulence spectrum is *T. carateum,* which disseminates only to cutaneous sites and causes the least severe skin lesions. A relatively small number of nucleotide differences in the *T. pallidum* subsp. *pallidum* and *pertenue* genomes, which are 99.8% identical, appear to be responsible for profound differences in tissue tropisms and clinical manifestations.[61,62] For many years it was thought that *T. pertenue* is not transmissible by sexual activity; the discovery of genital ulceration, often severe, in NHPs infected with *T. pertenue*–like organisms[29,31,63] argues that the mode of transmission is not determined solely by genetic factors. Until recently, the pathogenic treponemes were considered to be uncultivatable.[64] In what appears to be a major breakthrough, continuous long-term culture of three *T. pallidum* subsp. *pallidum* strains by cocultivation with rabbit epithelial cells has been reported.[65] Intratesticular inoculation of rabbits, rabbit infectivity testing (RIT), remains the only means of recovering strains from clinical specimens.[66,67]

T. pallidum is approximately 0.2 μm in diameter, has tapering ends, and ranges in length from 6 to 20 μm (Fig. 237.1). Because of their small diameter, pathogenic treponemes cannot be visualized with brightfield microscopy and are best visualized with darkfield (DF) or phase-contrast microscopy (see Fig. 237.1A). Although these organisms are often described as spiral or coiled, high-resolution time-lapse microscopy has confirmed earlier reports that, like the Lyme disease spirochete *Borrelia burgdorferi, T. pallidum* has a flat-wave morphology (see Fig. 237.1A).[65,68] Within the periplasmic space are the flagellar filaments, which extend from motors at each cell pole and overlap in the middle of the cell cylinder (see Fig. 237.1B–C).[68,69] The rotation of the filaments, driven by the motors, propagates an undulating wave along the length of the bacterium capable of propelling it through complex fluids, tissue matrices, and intercellular junctions.[70,71] *T. pallidum* is often described as gram negative. In addition to this description being erroneous from a phylogenetic standpoint,[72] the syphilis spirochete lacks the genes for synthesis of lipopolysaccharide (LPS),[39] the hallmark glycolipid of gram-negative organisms, and does not take up Gram stain. Other major differences in the molecular architecture and composition of their cell envelopes further underscore the phylogenetic gulf between *T. pallidum* and gram-negative bacteria.[34,73] The outer membrane of *T. pallidum* contains an extraordinarily low density of integral membrane proteins (see Fig. 237.1D)[74–76] that, as a whole, have little sequence homology with OMPs of gram-negative bacteria.[73,77] In gram-negative bacteria, the peptidoglycan resides directly beneath the outer membrane; in *T. pallidum,* the murein layer is found midway in the periplasmic space, beneath the flagellar filaments, which rotate against it in order to transfer their motive force to the cell cylinder.[68,69] For many years, it was believed that *T. pallidum* possesses a coat of serum proteins and mucopolysaccharides that shields it from the host's immune system.[78] It is now widely accepted that the paucity of proteins and pathogen-associated molecular patterns (PAMPs) on the spirochetal surface is the basis for the bacterium's impressive capacity for immune evasion, which has earned for it the name "stealth pathogen."[34,73,79]

The genomes of pathogenic treponemes consist of single circular chromosomes of approximately 1.1 MB,[39] near the low end of the spectrum for eubacteria and only half the size of the genomes of oral treponemes.[80,81] The absence of plasmids, pathogenicity islands, transposable elements, and restriction-modification systems indicates that the organism has limited capacity for uptake of exogenous DNA, perhaps explaining why it has remained exquisitely sensitive to penicillin for more than 7 decades. The syphilis spirochete is the only pathogenic

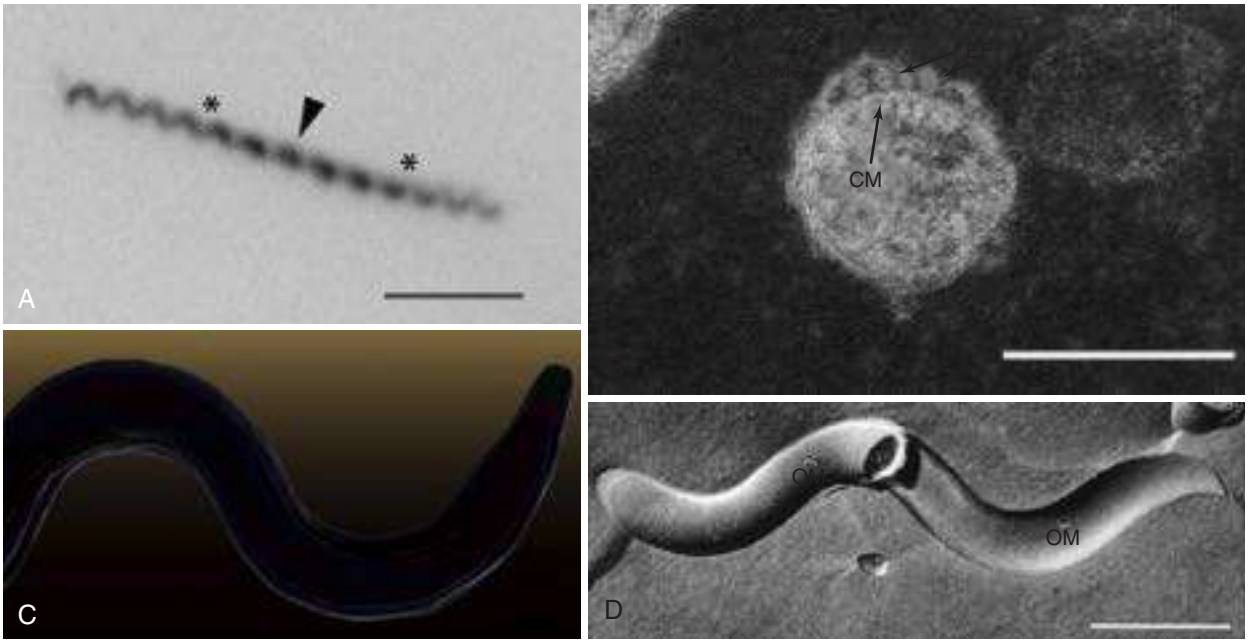

FIG. 237.1 (A) Darkfield micrograph showing the flat wave morphology of *Treponema pallidum*. Asterisks and arrowhead indicate segments oriented 90 degrees from each other. *Bar,* 10 µm. (B) Ultrathin section of *T. pallidum* showing the outer and cytoplasmic membranes (*OM* and *CM,* respectively) and flagellar filaments (endoflagella *[EF]*). *Bar,* 200 nm. (C) Top view of a surface-rendered model of *T. pallidum* generated from cryoelectron tomograms showing the outer and cytoplasmic membranes *(transparent yellow),* flagellar motors (basal bodies, *dark lavender*), flagellar filaments *(light lavender),* cytoplasmic filaments *(orange),* cap *(green),* and cone *(pink). Bar,* 200 nm. (D) Freeze-fracture electron microscopy reveals rare outer membrane proteins (particles) in the *T. pallidum* outer membrane. Convex and concave leaflets of the outer membrane are indicated. *Bar,* 500 nm. *(A courtesy Ms. Carson Karanian and Ms. Morgan Ledoyt. C from Izard J, Renken C, Hsieh CE, et al. Cryo-electron tomography elucidates the molecular architecture of* Treponema pallidum, *the syphilis spirochete.* J Bacteriol. *2009;191:7566–7580. D from Radolf JD, Bourell KW, Akins DR, et al. Analysis of* Borrelia burgdorferi *membrane architecture by freeze-fracture electron microscopy.* J Bacteriol. *1994;176:21–31.)*

treponeme whose physiology has been examined in detail, although there is no reason to believe that it differs substantively from those of the other subspecies.[34] *T. pallidum* subsp. *pallidum* replicates slowly (doubling time of approximately 30 hours in rabbits)[82] and poorly tolerates desiccation, elevated temperatures, and high oxygen tensions.[83] Because optimal replication under in vitro conditions occurs in ambient oxygen concentrations of 3% to 5%, the organism, once considered anaerobic, is now classified as a microaerophile.[83] During the course of reductive evolution, *T. pallidum* has dispensed with a vast amount of the biosynthetic machinery found in other bacterial pathogens. It is unable to synthesize fatty acids, nucleotides, enzyme cofactors, and most amino acids and must therefore scavenge all of these nutrients from its obligate human host by using a complex repertoire of cytoplasmic membrane transporters after they traverse the outer membrane via porins and other channels.[34,39,73] Lacking the components for oxidative phosphorylation, *T. pallidum* relies on glycolysis as its primary means for production of adenosine triphosphate (ATP), exploiting the abundant supply of glucose in blood and interstitial fluids. Additional ATP is likely generated through a recently discovered flavin-dependent Na/H^+ redox pump working in tandem with an A-type ATP synthase.[34] Identification of stable, genetically variable loci within the *T. pallidum* genome has made possible the development[84] and further refinement[85] of a scheme for typing syphilis strains, an essential tool for molecular epidemiologic studies. A meta-analysis of global typing studies reported the existence of at least 57 subtypes, with wide variation in geographic distribution, although a small number of subtypes predominate overall.[86] Recent studies emphasizing vaccine development have pointed to the need for a typing system that takes into account sequence variation in OMPs in *T. pallidum* strains circulating in clinical populations.[87]

EPIDEMIOLOGY

Despite the availability of effective and inexpensive antibiotic treatment, venereal syphilis continues to cause significant morbidity and mortality globally. According to the World Health Organization (WHO), globally, approximately 17.7 million persons aged 15 to 49 years had venereal syphilis in 2012, with an estimated 5.6 million new cases.[88] Although syphilis has recently reemerged in the United States and Europe[89–92] most individuals (>90%) who acquire syphilis reside in less affluent regions of the world.[93]

The number of new syphilis cases in the United States has cycled continuously since active reporting began in 1941. The advent of penicillin treatment and the establishment of aggressive syphilis-control public health campaigns toward the end of World War II led to notable decreases in the total number of new syphilis cases. As shown in Fig. 237.2, the incidence of venereal syphilis steadily decreased over several decades, with periodic outbreaks between 1986 and 1994 and between 2002 and 2011. In 2016, the rate of reported cases of primary and secondary syphilis in the United States was 8.7 cases per 100,000 population (27,814 total cases), more than four times the 2.1 per 100,000 rate at the historical nadir in 2000.[94] The upswing in the total number of early syphilis cases in the late 1980s, ascribed principally to heterosexual transmission, as would be expected, led to increases in gestational and congenital syphilis (CS) cases. The reappearance of syphilis during this time period was linked epidemiologically to the exchange of sex for drugs, especially crack cocaine.[95] The most recent surge has occurred chiefly among gay and bisexual men and other MSM,[96] a high-risk group that accounted for 58.1% of all primary and secondary syphilis cases reported to the Centers for Disease Control and Prevention (CDC) in 2016.[94] However, it is important to highlight that during the period from 2013 to 2016, syphilis rates in the United States increased in both men and women, in every region of the country, in every age group among those aged 15 years or older, and in every racial and ethnic group. The reasons for this latest increase are likely multifold and include lack of sexual inhibition resulting from the availability of effective treatment for human immunodeficiency virus (HIV), the use of the Internet to meet partners, the practice of HIV serosorting, and the increase in oral sex as a form of "safe sex."[96] Some have suggested that, risk factors notwithstanding, the oscillating pattern of syphilis outbreaks reflects changes in levels of immunity to *T. pallidum* within susceptible populations.[35] In the United States, syphilis is currently characterized by low-level endemicity with

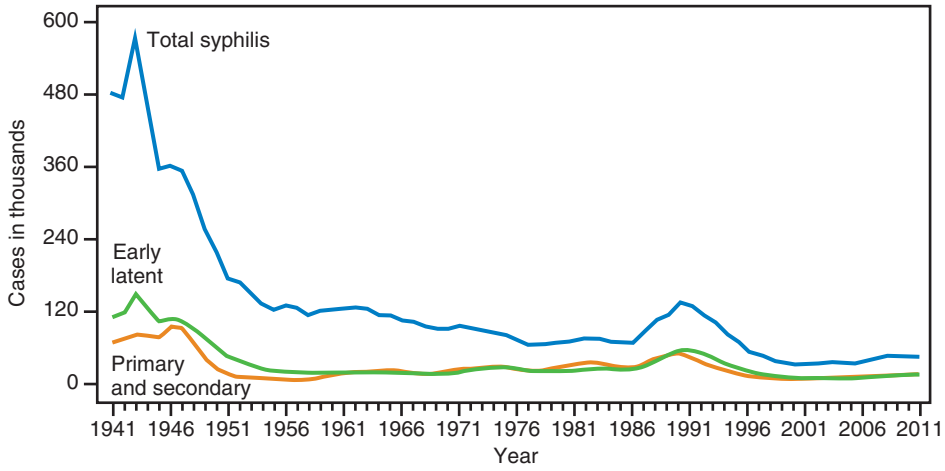

FIG. 237.2 Syphilis epidemiology in the United States: reported cases by stage of infection, 1941–2011. *(Modified from Centers for Disease Control and Prevention. STD Surveillance 2010, National Profile.* http://www.cdc.gov/std/stats10/figures.*)*

concentration among population subgroups with high rates of partner change, poor access to health services, social marginalization, or low socioeconomic status. The highest incidence of syphilis in the United States is evidenced in MSM from poor, underserved minority communities in southern states, from Maryland to Florida to eastern Texas and also in urban centers in California.[90] In addition, several other metropolitan areas throughout the country continue to report a high incidence of venereal syphilis in HIV-infected MSM.

In European and European Union member states, 24,541 new cases of syphilis were reported in 2014, with an overall incidence rate of 5.1 per 100,000 population. Similar to trends shown earlier in this chapter for the United States, close to two-thirds (63%) of reported syphilis cases were diagnosed in men who reported having sex with other men (MSM). The highest rate of syphilis was observed in Malta (11.5 per 100,000 population), followed by Lithuania (8.7), Iceland (7.7), and Spain (7.7). Rates below 2.5 per 100,000 population were observed in Croatia, Cyprus, Greece, Italy, and Slovenia.[97,98] The increase in syphilis cases in European/European Union states is also linked to changes in sexual behavior among MSM, in addition to more complete reporting and improved case detection through more rigorous testing as recommended in current international HIV management guidelines.

The social changes and disruption of medical services that followed the breakup of the Soviet Union in the late 1980s led to important increases in syphilis rates in Eastern Europe and Russia.[99,100] Although improved prevention strategies have led to a steady decrease in the overall disease burdens in this region of the world, Eastern European countries and Russia continue to report higher burdens of syphilis than their counterparts in the West. Findings from a recent review reveal that country-specific rates vary widely, with a mean syphilis incidence rate for Eastern European countries of 12.5 per 100,000 population, ranging from a high of 41 per 100,000 in Belarus to a low of 1.15 per 100,000 in Albania.[100] A report from the Russian Federation revealed that although the overall syphilis incidence declined nationally from 79.4 per 100,000 in 2004 to 28.9 per 100,000 in 2013, rates remain very high in Far Eastern, Siberian, and Northwestern Federal districts.[101] In the Tuva Republic, located in southern Siberia, the incidence rate of syphilis was estimated to be 177 per 100,000.

In China, a country where syphilis was virtually eradicated in the 1950s, the incidence and prevalence of the disease have more than quadrupled in the past several years.[102] In 2008 there were more syphilis cases in the coastal province of Guangdong than were reported in the entire European/European Union region in the same time period. Although the causes are not entirely clear, this increase in syphilis rates has been attributed to migration from rural communities to urban environments, leading to earlier sexual debut, later marriage, more lifetime partners, and increased commercial sex, including in MSM. These factors are compounded by limited syphilis screening, lack of adequate partner notification, and an overall unwillingness by the general population to access sexually transmitted disease (STD) health care services.[103]

Endemic rates of syphilis transmission have been stubbornly persistent in the resource-limited public health systems of Latin America and the Caribbean. Because syphilis is not rigorously reported, and often not recognized by health care providers in the region, country-specific disease prevalence and incidence rates most likely underestimate the true magnitude of the problem. Syphilis has been shown to be a leading cause of genital ulcerative disease in both Peru and the Dominican Republic,[104] second only to genital herpes. Likewise, in another study from Peru, the prevalence of venereal syphilis was estimated to be 10.5% among MSM and 2.0% in socially marginalized men and women.[104] In a Brazilian study, high syphilis prevalence rates were the norm in prisoners, commercial sex workers, and MSM.[105] High syphilis prevalence rates have also been documented for MSM (5% and 13%) and female sex workers (6.8% and 15.3%) in Honduras and Guatemala, respectively.[105a] In one study describing the epidemiology of venereal syphilis in Colombia, 10% of female sex workers in Bogota had serologic and clinical evidence of the disease.[106] In another study, seroprevalence rates in sexually active men and women between the ages of 18 and 24 were close to 6%.[106,107] Overall, studies from this region consistently document high syphilis prevalence among MSM, particularly in Peru, Argentina, Costa Rica, El Salvador, Honduras, Nicaragua, and Guatemala.[108–110] Throughout the region, transgender persons had the highest syphilis rates of all groups, which in this population were linked to high vulnerability as a result of low self-esteem and marginalization, and a lack of legal recognition and labor opportunities, which often leads to engagement in commercial sex work.

Regardless of geographic location, the worldwide epidemiology of syphilis has been greatly influenced by developments in the HIV epidemic. A statistical association between the two diseases became apparent at the outset of the acquired immunodeficiency syndrome (AIDS) epidemic in the early 1980s.[111] Since that time, numerous studies have documented the high rate of HIV infection among patients with syphilis.[96,112,113] Not surprisingly, this increase has disproportionately affected large metropolitan areas with well-established populations of HIV-coinfected MSM, including San Francisco, where the incidence of primary and secondary syphilis increased more than 600% (26 cases per 100,000).[114] This increase has been attributed to increased reports of unprotected sex among HIV-positive MSM and increased HIV prevalence. Simultaneously, there also has been a resurgence of traditional sexual marketplaces such as saunas and cruising grounds, together with a new and rapid growth of Internet chat rooms, increasing the opportunity for rapid and easy access to new sexual partners. The overall effect has been to join previously isolated sexual networks, increasing the size of the sexual networks and thereby reducing the time taken for the spread of syphilis to evolve. In addition, the idea that oral sex is "safer" sex and rarely associated with HIV transmission

may explain the role of oral sex in syphilis transmission.[115] During the period from 2000 to 2016, the rise in the rate of reported primary and secondary syphilis was primarily attributable to increased cases among men and, specifically, among gay and bisexual men and other MSM.[116] Rates of HIV coinfection among MSM with newly diagnosed early syphilis are as high as 50%.[117] In one study from Florida, 21% of men diagnosed with early syphilis in 2003 had acquired HIV infection by the end of 2011.[118] Although the association between syphilis and HIV infection initially was thought to simply reflect similar behavioral risk factors for acquisition and transmission, it has become apparent over the years that complex biologic relationships exist between these two diseases.[96,119,120] The presence of a syphilitic chancre can theoretically facilitate HIV transmission by either increasing the host's susceptibility to infection with the virus or the HIV-infected host's infectiousness to discordant sexual partners. The former is associated with the disruption of the protective epithelial and mucosal barriers present in genital chancres and the known enrichment of the lesion with activated lymphocytes, macrophages, and dendritic cells (DCs), all of which are potential targets and donors for HIV[121–124] and which differentially increase expression of key HIV coreceptors (i.e., CCR5 and DC-SIGN) in untreated patients.[124,125] With respect to increased infectiousness, the inflammatory response elicited by *T. pallidum* is capable of inducing HIV gene expression, thereby promoting viral replication.[126] Not surprisingly, in HIV-infected patients, CD4 counts decrease and HIV viral loads increase in individuals with untreated syphilitic coinfection.[127]

Nearly 1.0 million pregnant women are estimated to be newly infected with syphilis every year, and approximately half of pregnant women who are left untreated will experience adverse outcomes in their pregnancies. These include early fetal loss (20–28 weeks' gestation) and stillbirth (>28 weeks' gestation), neonatal death, low-birth-weight infants, and infants with clinical evidence of infection.[128] In 2008, syphilis in pregnancy contributed to 305,000 stillbirths and fetal and neonatal deaths, and an additional 215,000 infants were at increased risk of dying from low birth weight, prematurity, or complications of infection related to syphilis.[128] Of particular concern, in sub-Saharan Africa, syphilis contributes to approximately 20% of all perinatal deaths. After decreasing from 10.5 to 8.4 reported CS cases per 100,000 live births during 2008–2012, the rate of reported CS in the United States subsequently increased each year during 2012–2016. In 2016, there were a total of 628 reported cases of CS, including 41 syphilitic stillbirths, and the national rate was 15.7 cases per 100,000 live births. This rate represents a 27.6% increase relative to 2015 (12.3 cases per 100,000 live births) and a 86.9% increase relative to 2012 (8.4 cases per 100,000 live births).[129]

PATHOGENESIS

Studying syphilis pathogenesis poses a formidable and unique set of challenges. First and foremost is that *T. pallidum* has, until very recently, defied all attempts at continuous cultivation in artificial media[64,65] and therefore cannot be genetically manipulated. A second serious limitation is the lack of a facile murine model for dissecting the complex host response to this pathogen.[130,131] Although the outbred rabbit model recapitulates many facets of human disease, rabbits do not develop typical secondary lesions after intradermal inoculation, nor do they develop neurologic complications or neuropathologic effects comparable to those of humans, even when inoculated intravenously.[132,133] Investigators who wish to circumvent these deficiencies by studying infection in humans must cope with an enormous spectrum of disease manifestations, person-to-person variability in immune responses, high rates of HIV coinfection, and the fact that results from blood and skin (the two most accessible sites) represent snapshots from just two compartments of a dynamic, systemic process.[134,135] A third obstacle is the fragility of *T. pallidum,* particularly its outer membrane, a physical property that has greatly hampered efforts to identify and examine molecules that function at the host-pathogen interface.[136,137] Lastly, transcriptional and proteomic analysis of syphilis spirochetes has thus far been confined to organisms extracted from inflamed rabbit testes,[138,139] a circumstance unlikely to represent the expression profiles in human tissues during the various stages of disease. Given all of these impediments, it is not surprising that our current understanding of the microbiologic and immunologic factors that determine the outcome of human infection lags far behind that of most other common bacterial infections.

Person-to-person transmission of spirochetes initiates the pathogenic sequence. With venereal syphilis, this typically occurs when treponemes are transferred during intimate contact, usually sexual (oral-genital as well as genital-genital, rarely kissing), with an actively infected partner. In their human inoculation studies conducted at Sing Sing Prison in the 1950s, Magnuson and colleagues[140] (see discussion of ethics) found that as few as 10 organisms of the Nichols strain could cause lesions. Thus, only miniscule amounts of infectious exudate need be exchanged when microenvironmental conditions are conducive to survival of this fastidious pathogen. Individuals with venereal syphilis are most infectious during the primary and secondary stages of disease (including secondary relapses of early latency) when moist, mucocutaneous lesions are present. Persons with early syphilis may be infectious, however, even if they lack open lesions. Micrographs showing that spirochetes are abundant within the epidermis and superficial layers of the dermis in secondary syphilitic lesions[141] suggest how minute abrasions created during sexual activity might result in infection. Transfusion syphilis, a well-recognized nonsexual mode of transmission before World War II, no longer occurs in the United States[142] but remains a significant concern in underdeveloped countries.[143] The agents of yaws, pinta, and endemic syphilis are transmitted by nonsexual contact with open lesions, usually during childhood.[58]

Syphilis spirochetes rapidly gain entry into their new host either by directly penetrating mucous membranes or via abrasions and cracks in skin. Remarkably, treponemes applied to the preputial mucosa of rabbits migrate into the subepithelium within several hours.[144] Once below the epithelial surface, they begin to multiply locally and disseminate through lymphatics and blood vessels. Why some members of the bacterial population remain localized whereas others respond to the same environmental signals by migrating away from the inoculation site is a fascinating and unresolved question. The experimental and clinical evidence that *T. pallidum* disseminates systemically early during the course of infection is overwhelming.[59,145] Brown and Pearce[146] recovered *T. pallidum* from inguinal lymph nodes and blood of rabbits within 48 hours of intratesticular inoculation, well before the onset of orchitis; these findings have been replicated with quantitative PCR.[147] Numerous reports in the early 20th century documented transmission following the transfusion of blood from seronegative donors (i.e., during the incubation period).[148] Almost any organ in the body can be invaded during the spirochetemia of early syphilis, including the CNS.[1,67,149] Using both RIT and PCR, investigators in the molecular era have confirmed that substantial percentages of patients with early syphilis without neurologic signs or symptoms harbor *T. pallidum* in their CNS.[150,151]

It is widely believed that *T. pallidum* must adhere to epithelial cells and extracellular matrix components in order to gain the "foothold" needed to establish infection[152,153]; spirochetes subsequently disseminate within tissues by coordinating adherence with motility in a series of "stop and go" movements.[71,154] Binding studies suggest that fibronectin and laminin are important substrates for this attachment-mediated form of motility.[155–157] Putative *T. pallidum* adhesins for these host molecules have been identified[152,158–160] and, in two cases (TP0435 [Tpp17] and TP0751 [pallilysin]), crystal structures have been obtained.[161,162] Light micrographs showing *T. pallidum* attached end-on to cell surfaces suggested years ago that the tip of the bacterium functions as an attachment organelle[163]; this notion has been buttressed by recent cryoelectron tomographic analyses (see Fig. 237.1C).[68,69] Chemoreceptor arrays near the cell ends translate environmental cues (chemotactic signals) into a chain of phosphorylation events that determine whether the flagellar motors turn clockwise or counterclockwise.[69,164] Directed motility does not occur unless the motors at each end of the cell turn in opposite directions.[164] How a spirochete coordinates the direction of rotation of the motors at opposite ends of the cell is a mystery.[164] Circulating treponemes bind to vascular endothelium within target organs, reaching the parenchyma by negotiating their way through the tight junctions separating endothelial cells, a process called *interjunctional penetration.*[70] Use of the Lyme disease spirochete, *B. burgdorferi,* as a

heterologous host identified TP0751 (pallilysin) as a putative adhesin for vascular endothelium.[165]

Although rare examples of intracellular treponemes have been reported,[166,167] the syphilis spirochete is believed to be incapable of establishing long-term residence in phagocytic or nonphagocytic cells.[59,168] There is also now a consensus among syphilologists that clinical manifestations result from the inflammatory processes driven by the presence of treponemes and treponemal constituents within infected tissues.[168,169] How does the spirochete flourish within the extracellular space in the face of the increasingly vigorous humoral and cellular responses it elicits as the disease progresses? Current thinking is based on an infection model that pits innate and adaptive immunity against a motile invader structurally equipped to "fly" beneath the host's "immunologic radar," perhaps even manipulating the host response to facilitate dissemination and immune evasion.[79,134,170]

Although lacking LPS, *T. pallidum* contains abundant lipoproteins, which are capable of activating macrophages and DCs via CD14- and Toll-like receptor 1/2 (TLR1/2)–dependent signaling pathways.[79,169] However, because of the bacterium's unique cell envelop architecture,[34,73,76] the vast majority of these PAMPs are not readily accessible to TLRs or other pattern recognition receptors (PRRs) expressed on the surface of innate immune cells. As a result, spirochetes can replicate at the site of inoculation, disseminate hematogenously, and then replicate at metastatic sites virtually unchecked by innate surveillance systems. At some point, and through poorly understood mechanisms, pathogen sensing is triggered and organisms are taken up by tissue-based DCs, which then traffic to draining lymph nodes to present cognate treponemal antigens to naïve B and T cells.[171] The production of opsonic antibodies enhances the uptake and degradation of spirochetes by phagocytes, allowing spirochetal PAMPs to gain access to PRRs lining the phagosomal vacuole and stimulating the production of proinflammatory cytokines, while production of interferon-γ (IFN-γ) by recruited, locally activated natural killer (NK) and $CD4^+$ and $CD8^+$ T cells bolsters macrophage-mediated clearance and inflammation.[134,170,172–174] Although these adaptive responses help to shift the balance in favor of the host, the spirochete is by no means without countermeasures. The paucity of antigenic targets on its surface,[73,76,77,160] the copious production of antibodies against subsurface lipoprotein "decoys,"[73,77,175] and the emergence of antigenically variant subpopulations via intergenomic recombination and phase variation of OMPs[176–180] collectively enable a subpopulation of bacteria to avoid opsonophagocytosis[181] and other forms of antibody-mediated clearance, prolonging disease manifestations and fueling the relapses of early latency. How, then, is the pathogen eventually contained, bringing about the quiescent stage of disease called late latency? Possibly, as infection proceeds, the antibody repertoire broadens and intensifies sufficiently to the point at which the spirochete's antigen-poor surface is overwhelmed, its capacity for antigenic variation is exhausted, or both. Nevertheless, although no longer capable of disseminating, organisms survive for years in a substantial proportion of untreated individuals, establishing niduses of inflammation that set the stage for recrudescent (i.e., tertiary) disease when, for reasons unknown, the balance shifts back in favor of the pathogen (see "Natural Course of Untreated Syphilis," later).

PATHOLOGIC FEATURES

Perivascular infiltrates composed of lymphocytes, histiocytes (macrophages), and plasma cells, accompanied by varying degrees of endothelial cell swelling and proliferation, are the histologic hallmarks of syphilis regardless of anatomic site or stage of disease (Fig. 237.3A–B).[182] Spirochetes are abundant in early syphilis lesions and often are observed in and around blood vessels (see Fig. 237.3C), occasionally even protruding into the lumen in histologic specimens (i.e., "caught in the act" of disseminating).[182–184] In contrast, with the exception of paresis (see later), spirochetes are not easily demonstrable in tertiary syphilis lesions.[182,185]

In primary syphilis, progression of the vasculopathic changes to frank endarteritis obliterans causes tissue necrosis, ultimately giving rise to a genital ulcer, the chancre. In secondary syphilis skin lesions, a wide variety of histologic patterns, including granuloma during later phases, can be superimposed on the characteristic features noted earlier.[186–188] Cutaneous infiltrates are most intense in the papillary dermis but often extend into the reticular dermis, assuming a bandlike (lichenoid) distribution. Follicles and sweat glands are frequently sleeved with inflammatory cells and, when extensive and severe, cause syphilitic alopecia. Epithelial abnormalities in secondary skin lesions include exocytosis, keratinocyte necrosis, acanthosis, microabscesses, and hyperkeratosis. Edema, papillation, and hyperplasia of the epidermis are distinctive features of condylomata lata, the highly infectious, intertriginous excrescences of secondary syphilis. Lymph nodes in secondary syphilis exhibit marked follicular hyperplasia coupled with lymphocyte depletion and histiocytic infiltration of the paracortical zones.[189] Although circulating immune complexes are detectable in patients with secondary syphilis,[190] immune complexes are not typically observed in skin.[123] Glomerular deposition of immune complexes, however, has been demonstrated in patients with nephrotic syndrome, a rare complication of secondary syphilis.[191,192]

A gumma is a circumscribed mass of granulation tissue, so named because of its rubbery or gummy gross consistency. The gumma of tertiary syphilis histologically is composed of a dense infiltrate of lymphocytes, plasma cells, epithelioid cells, and multinucleated giant cells surrounding a caseous, necrotic core; proliferating fibroblasts and fibrosis also may be present.[193] Endarteritis and perivascular inflammation help to distinguish syphilitic gummas from those caused by tuberculosis. Obliterative endarteritis involving the vasa vasorum, the nutrient vessels of the aortic adventitia, is the key pathologic lesion in cardiovascular syphilis.[194] The ascending aorta and arch are most frequently affected because the vasa vasorum are most plentiful in these regions of the aorta.[194,195] These changes eventually give rise to intimal thickening and wrinkling (producing a distinctive "tree bark" appearance), patchy medial necrosis, and adventitial scarring with destruction of elastic fibers and weakening of the aortic wall.

Asymptomatic neurosyphilis and syphilitic meningitis are due to diffuse leptomeningitis (Fig. 237.4A).[149] The pathologic features of meningovascular syphilis explain the syndrome's variable mixture of focal neurologic signs with superimposed encephalitis.[149,196,197] In general, there is diffuse thickening and lymphocytic infiltration of the meninges with two kinds of arteritis: (1) *Heubner* endarteritis, affecting large and medium-sized arteries, characterized by crescentic collagenous thickening of the intima, thinning of the media, and dense, inflammatory infiltrates (lymphocytes and plasma cells) within the adventitia (see Fig. 237.4B), and (2) *Nissl-Alzheimer* endarteritis of small vessels, characterized by the proliferation of endothelial and adventitial cells. *Paresis* and *tabes dorsalis* involve poorly understood spirochete-driven, neurodegenerative processes of brain tissue, hence their designation as "parenchymatous." In paresis, diffuse meningovascular inflammatory changes are associated with striking, progressive loss of cerebral cortical neurons, resulting in gross cerebral atrophy (greatest in the frontal and temporal lobes), and proliferation of astrocytes and glial cells. Microglial cells are hypertrophied and elongated and often contain abundant iron. Spirochetes are often readily detectable, usually in the gray matter, with little correlation between the clinical picture and the location and distribution of organisms. Tabes dorsalis (Greek for "consumption of the back") is characterized by demyelination of dorsal root ganglia with secondary wallerian degeneration of the posterior columns of the spinal cord (see Fig. 237.4C). In early tabes, the leptomeninges and dorsal roots are heavily infiltrated with lymphocytes and plasma cells. These inflammatory changes diminish as the disease becomes chronic, eventually disappearing in so-called burnt-out cases. The degenerative changes in the tabetic spinal cord can be so severe that the posterior surface of the cord is concave, rather than convex.

Immunohistochemical (IHC) analysis of the cellular infiltrates in primary and secondary syphilis lesions has provided valuable insights into the pathogenic mechanisms operative during infection. Staining with macrophage markers has confirmed that these professional phagocytes are universally present.[123,134,170,198] A long-standing point of dogma in the syphilis field, based on work done with the rabbit model, is that the cellular response to *T. pallidum* is a classic, delayed-type hypersensitivity reaction.[78] Immunostaining has revealed that this is not the case in humans. Whereas lesional T cells in rabbits are predominantly $CD4^+$ lymphocytes,[199] $CD8^+$ T cells are prominent in humans.[123,134,170,200,201] This finding has implications for both the ontogeny of the adaptive

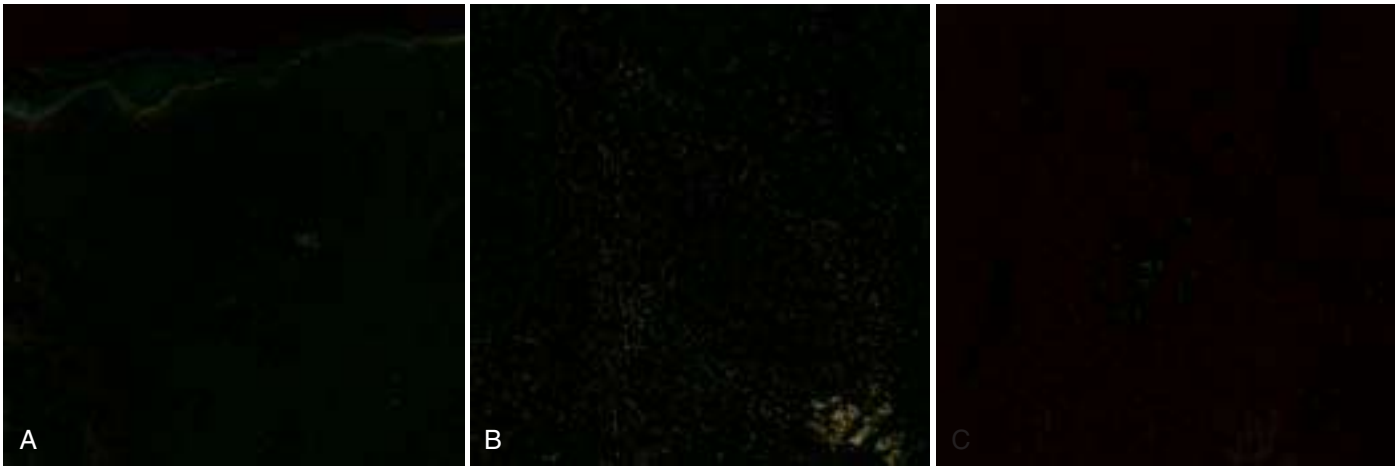

FIG. 237.3 Characteristic histopathology from a secondary syphilis skin lesion. (A) Punch biopsy specimen obtained from secondary syphilis skin lesion demonstrates a mixed infiltrate composed of lymphocytes, histiocytes, and plasma cells, accompanied by varying degrees of endothelial cell swelling and proliferation. (Hematoxylin-eosin stain, ×100.) (B) Characteristic obliterative endarteritis, ×400. (C) Immunohistochemistry staining reveals abundant spirochetes, labeled in *red,* in and around the wall of a blood vessel, ×400. *(Courtesy Drs. Adriana Cruz and Rodolfo Trujillo, Centro Internacional de Entrenamiento e Investigaciones Médicas, Cali, Colombia.)*

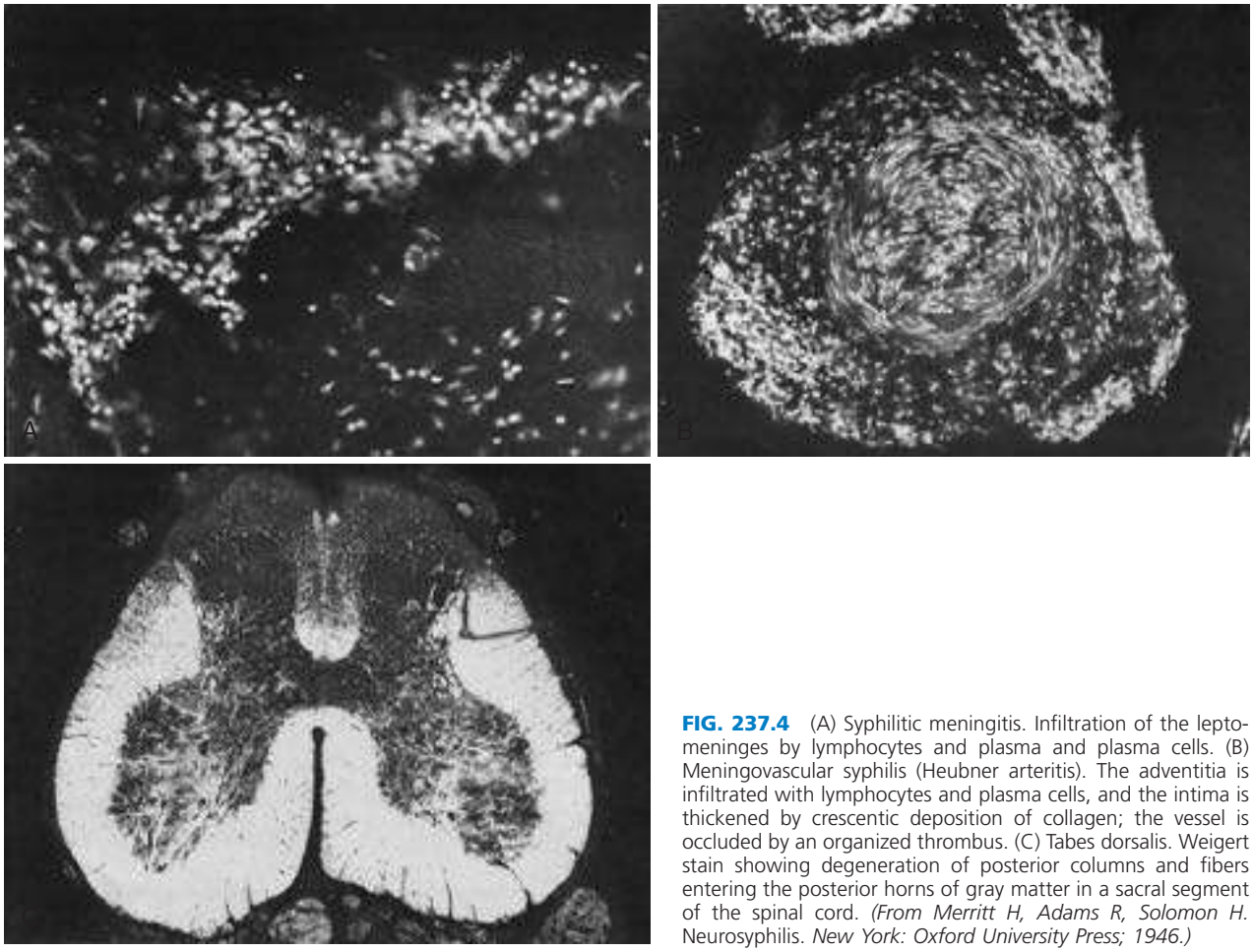

FIG. 237.4 (A) Syphilitic meningitis. Infiltration of the leptomeninges by lymphocytes and plasma and plasma cells. (B) Meningovascular syphilis (Heubner arteritis). The adventitia is infiltrated with lymphocytes and plasma cells, and the intima is thickened by crescentic deposition of collagen; the vessel is occluded by an organized thrombus. (C) Tabes dorsalis. Weigert stain showing degeneration of posterior columns and fibers entering the posterior horns of gray matter in a sacral segment of the spinal cord. *(From Merritt H, Adams R, Solomon H.* Neurosyphilis. *New York: Oxford University Press; 1946.)*

cellular response and its role in clearance of organisms. Because *T. pallidum* is an extracellular pathogen, priming and activation of $CD8^+$ T cells (resulting in local production of IFN-γ) would have to occur by cross-presentation; whether this is advantageous for the spirochete is an open question. NK cells, another potential source of IFN-γ, also have been identified in secondary syphilis skin biopsy specimens.[134,170] Evidence has been provided that *T. pallidum* promotes the development in secondary syphilis lesions of regulatory T cells, which could facilitate spirochetal persistence by producing antiinflammatory molecules.[201,202]

NATURAL COURSE OF UNTREATED SYPHILIS

Of the numerous investigations that have contributed to our understanding of syphilis, the "Oslo Study" stands out as the single greatest source of information about the natural course of the disease.[203] The study was born from the clinical impression of Boeck at the University of Oslo that the mercury-based antisyphilitics of the late 19th century were more harmful than efficacious, coupled with his astute insight that "the immune mechanisms of the body are the most important in combating the disease." In 1891, he began a prospective study of 1978 untreated patients with early syphilis who were hospitalized and closely monitored until all signs and symptoms had disappeared; enrollment ended in 1910 when arsphenamine became accepted syphilotherapy. In 1925, his successor, Bruusgaard, recognizing that Boeck's material "is hardly to be duplicated anywhere else," conducted follow-up on 473 persons (309 living and 164 dead) from the original cohort. Concerns over potential biases and shortcomings in Bruusgaard's data prompted his successor, Gjestland, in 1948 to undertake a painstaking reexamination of the available records for the 1404 patients who lived near Oslo. Because Boeck had enrolled his cohort before the availability of both DF microscopy and the Wassermann test, his diagnoses rested entirely on clinical criteria; during their reexamination, Gjestland and collaborators concluded that the great majority of individuals had, in fact, been correctly diagnosed. Among the key findings of Gjestland's comprehensive analysis was that 24% of enrollees developed one or more secondary relapses within 5 years of discharge. Two-thirds of the relapses took place within 6 months of resolution of secondary lesions, whereas nearly 90% occurred within 1 year. These observations form the basis for the 1-year time point used clinically and epidemiologically to distinguish early latent from late latent syphilis. (As of January 2018, the CDC had renamed early and late latent syphilis as *early syphilis, non-primary, non-secondary* and *syphilis, unknown duration or late,* respectively.) Twenty-eight percent eventually developed some type of late manifestation: 10% developed cardiovascular syphilis, 6.5% developed symptomatic neurosyphilis; and 16% developed benign (i.e., gummatous) tertiary disease. Many of these patients had more than one "late" complication. Of those on whom autopsy was performed, 35% of the men and 22% of the women had evidence of cardiovascular involvement, especially aortitis. Syphilis was considered the primary cause of death in 15% of the men and 8% of the women. Thus, another important finding of the Oslo Study was that untreated syphilis can be uneventful and that many persons appear to spontaneously self-cure.

In 1932 the US Public Health Service began a prospective comparative study (the infamous Tuskegee study of 1932 to 1972, see earlier) involving 412 African-American men with untreated syphilis of 3 or more years' duration and 192 uninfected, matched controls.[45,46] It should be noted that when this study was initiated, there was no consensus about the usefulness of arsenicals for treatment of persons with latent syphilis or even the need to treat such individuals, especially older than the age of 50. Indeed, as noted earlier, a consensus on the need to treat latent syphilis was slow to develop even after the efficacy of penicillin for early syphilis became well established.[204] Serial reports from this study underscored the excess cardiovascular morbidity and mortality resulting from untreated syphilis.[45,194] In his analysis of autopsy data from the first 20 years of the study, Peters and colleagues[205] found that 50% of patients who had been infected for 10 years had cardiovascular involvement and that cardiovascular and neurosyphilis were the primary causes of death among the 40% of syphilitic patients who had died during this period. Of the 41% of survivors at 30 years of follow-up, 12% had evidence of late, predominantly cardiovascular, syphilis.[206]

In a third large study published in 1946, Rosahn[207] used a novel approach, postmortem examination, to assess the outcome of untreated syphilis. Of a total of 3907 cases encompassing all diseases, 380 were found to involve clinical, laboratory, or postmortem evidence of syphilis. Only 77 (39%) of the 198 untreated cases demonstrated anatomic changes consistent with syphilis; in 31 of these, syphilis was not the cause of death. Twenty percent of the untreated individuals, therefore, were thought to have died from complications of late syphilis. Rosahn believed that the sizable percentage of individuals who appeared to have self-cured mirrored the findings from the Oslo Study (only the Bruusgaard data were available at the time). Of the anatomic lesions attributable to syphilis, 83% were cardiovascular, 8% were neurologic, and 9% were gummas. Cardiovascular syphilis was almost certainly overrepresented in this series because it involved hospitalized persons who came to autopsy. An interesting sidelight was the observation that continued reactivity of serologic tests, what we now call the serofast state,[208] can occur without apparent histologic evidence of disease.

A schematic depicting the course of untreated syphilis is shown in Fig. 237.5.[182,209–211] The incubation period is a median of 3 weeks but may vary from 10 to 90 days depending on the size of the inoculum.[212] The primary stage begins with the appearance of the chancre at the site of inoculation. This usually painless, solitary lesion does not develop in every case or may go unnoticed, particularly in women or MSM. The secondary or disseminated stage becomes evident 4 to 10 weeks after the appearance of the chancre and is associated with the highest treponemal burdens in the bloodstream and tissues. After weeks to several months, the untreated patient then enters a period of latency during which the diagnosis can be made only by serologic testing (unless a relapse occurs). As per the revised terminology described earlier, latency is divided into *early syphilis, non-primary, non-secondary* (infectious relapses and/or spirochetemia common) and *late syphilis of unknown duration* (relapses, spirochetemia, or both unlikely) stages.

Late syphilis refers to both clinically inapparent latent infection and the tertiary syndromes (benign gummas, cardiovascular syphilis, and neurosyphilis) that develop in approximately one-third of untreated cases. Cardiovascular syphilis typically involves the aortic arch.[194] The skin, bones, liver, CNS, and spleen are the most commons sites in which gummas develop.[1] As noted earlier, although invasion of the CNS occurs during early syphilis, individuals usually do not develop neurologic complications at this stage. Asymptomatic neurosyphilis may resolve spontaneously, persist indefinitely, or progress to one of the "early" neurosyphilis syndromes, meningitis and meningovascular syphilis, within the first 1 to 10 years of infection, or one of the "late" parenchymatous syndromes, general paresis and tabes dorsalis, 10 or more years after infection.[149,213,214]

Whether coinfection with HIV worsens the manifestations of syphilis or accelerates the course of the disease, or both, has been a contentious issue for more than 2 decades. Since the late 1980s, a number of case reports and small series have documented ophthalmologic and neurologic complications, as well as unusual or highly destructive nonneurologic syphilis syndromes in HIV-infected patients.[215–217] The sheer volume of cases led many authorities to conclude that infection with HIV poses a profoundly increased risk of complications, particularly neurologic, during active syphilis.[96,216,217] To the surprise of many, a multicenter prospective study sponsored by the CDC did not bear this out.[151,218] The differences in clinical presentation between HIV-infected and HIV-uninfected patients were marginal. Although HIV-infected patients with primary syphilis tended to present with more genital ulcers and genital ulcers were present more frequently in HIV-infected patients with secondary syphilis, manifestations of disseminated infection, including neurologic and ophthalmologic complications, were not worsened by concomitant HIV infection. Thus, if atypical and aggressive presentations of syphilis do occur more frequently among HIV-infected patients, they likely represent a small percentage of total cases, an assessment shared by several groups.[96,211,219,220] The effectiveness of current antiretroviral regimens should further mitigate concerns about the ability of HIV infection to adversely affect the natural course and manifestations of syphilis.[221] Interesting to note, no correlation has been observed between HIV status and isolation or detection of *T. pallidum* in CSF,[150,151] suggesting that the bacterium's inherent invasiveness, rather than the

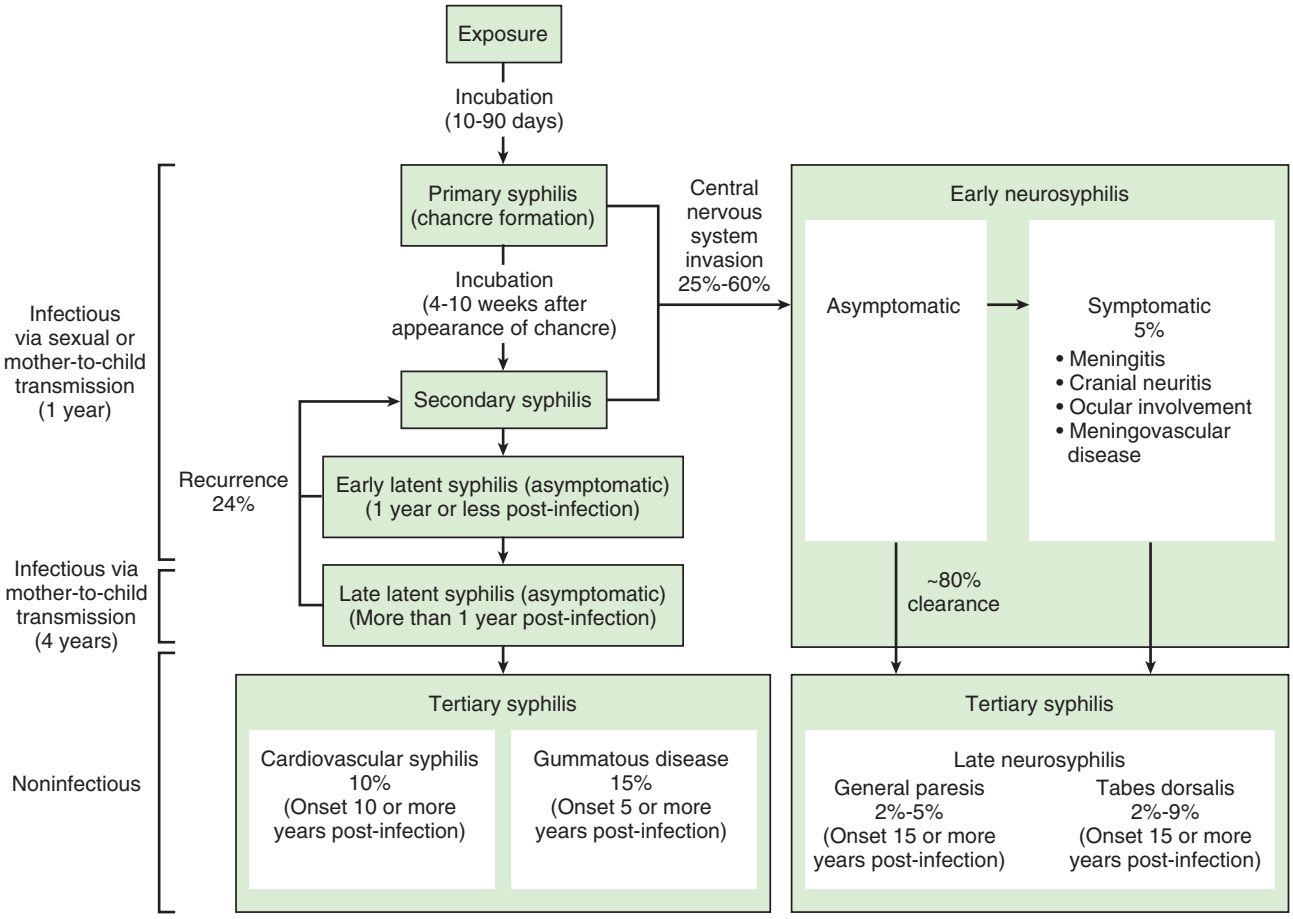

FIG. 237.5 Natural course of untreated syphilis. As of January 2018, the Centers for Disease Control and Prevention renamed early and late latent syphilis as *early syphilis, non-primary, non-secondary* and *syphilis, unknown duration or late,* respectively. *(Modified from Golden MR, Marra CM, Holmes KK. Update on syphilis: resurgence of an old problem.* JAMA. *2003;290:1510–1514.)*

integrity of host immune defenses, is the primary determinant of CNS invasion during early syphilis.

The effect of early syphilis on HIV viral load also has been examined by several groups.[222–224] Most,[222–225] but not all,[226,227] studies found that active syphilis causes transient increases in viral loads and decreases in CD4 count that return to baseline with effective antimicrobial therapy. Besides diminishing the dangers of untreated syphilis to the coinfected individual, lowering viral loads in semen by treatment of syphilis has important public health implications for this at-risk population.

CLINICAL MANIFESTATIONS

Several excellent summaries of the spectrum of clinical manifestations in acquired syphilis are available.[209–211,228]

Primary Syphilis

Syphilis commences clinically when spirochetes replicating at the site of inoculation induce a local inflammatory response sufficient to generate a macule, which over the course of 1 to 2 weeks becomes papular and then ulcerates, producing the defining lesion of primary syphilis, the chancre.[1,182,230] The base of the typical chancre (Fig. 237.6A) is smooth, clean, and without exudate; the borders are raised and have a characteristic, cartilaginous consistency. Unlike the exquisitely painful genital ulcers caused by *Haemophilus ducreyi* (chancroid) and herpes simplex virus (herpes genitalis), the classic chancre is painless and nontender on examination; spirochetal infiltration of cutaneous sensory nerves may explain this phenomenon. Multiple chancres frequently occur, especially in persons who are coinfected with HIV.[218] Chancres that do not conform to the classic features are extremely common, representing more than half of the cases in one large series.[230]

The chancre forms wherever spirochetes are inoculated. The external genitalia are most frequently involved. In one study, penile lesions in men (presumably heterosexual) were located, in decreasing order of frequency, on the prepuce, coronal sulcus, shaft, corona, glans, frenum, and urethral meatus.[230] In women, lesions were located on the labia majora, labia minora, fourchette, and perineum[230]; occasionally chancres occur on the cervix, presenting as a painless discharge. Among heterosexuals, a much greater percentage of men present with primary syphilis because intravaginal chancres tend to go unnoticed; a male-to-female ratio of 9:1 was noted in one large study.[231] Similarly, chancres in MSM often go unnoticed because they occur within the rectum. Chancres in the anal area may be exquisitely painful and mistaken for anal fissures. As with anogenital ulcers, sexual orientation is a determining factor for the location of oral chancres. In heterosexuals, they typically occur on the upper lip in men and on the tongue in women, whereas in MSM they tend to be located on the tongue (see Fig. 237.6B).[232] Genital chancres are often accompanied by inguinal adenopathy, frequently bilateral, consisting of moderately enlarged, discrete, painless nodes, whereas cervical adenopathy often accompanies chancres in the oral cavity (see Fig. 237.6C). The chancre heals on its own within 3 to 6 weeks, leaving either no trace or a thin atrophic scar; the lymphadenopathy may persist longer.

Primary syphilis must be differentiated principally from herpes simplex virus infections, chancroid, and traumatic suprainfected genital lesions. Primary genital herpes usually begins as a painful erythematous rash that develops into clusters of vesicles accompanied by regional lymphadenopathy and systemic symptoms. It runs a 10- to 14-day course in immunocompetent patients. Recurrent genital herpes is less florid and is characterized by mild to moderately painful vesicles and no lymphadenopathy. Chancroid is characterized by one or more extremely



FIG. 237.6 (A) Classic penile chancre. (B) Oral chancre. (C) Bilateral cervical lymphadenopathy in same patient as in (B). *(B and C courtesy Dr. Kevin Dieckhaus. Copyright 2013.)*

painful, exudative, indurated ulcers associated with tender, eventually suppurative, lymphadenopathy. The ulcer has irregular, overhanging edges and bleeds easily (e.g., when scrapings are collected for a DF examination). Early venereal warts, granuloma inguinale, lymphogranuloma venereum, tuberculosis, atypical mycobacterial infections, tularemia, sporotrichosis, anthrax, rat-bite fever, or any genital ulcer may resemble early primary syphilis.

Secondary Syphilis

The manifestations of secondary syphilis, the often florid, systemic stage of the infection, are protean and are largely responsible for the disease's legendary reputation as "the great imitator."[1,209–211,229,233,234] The vast majority (>95%) of cases involve the skin and mucous membranes. With rare exceptions, secondary syphilis rashes are macular, papular, papulosquamous, annular, or pustular, or various combinations thereof.[229] Although often described as nonpruritic, in one series 40% of patients complained of itch, which could be severe.[235] The rash of secondary syphilis usually begins on the trunk and proximal extremities as bilateral, pink to red, discrete macular lesions 3 to 10 mm in diameter that are usually overlooked by the patient. These lesions often evolve from macules into brownish red papules (hence the term *maculopapular*); in a few patients, they progress to pustular lesions termed *pustular syphilids*. In one study of more than 200 patients, 94% and 66% of patients with maculopapular and papular lesions, respectively, presented within 4 weeks of the onset of rash as opposed to only 1 of 11 patients with pustular lesions.[236] Scaling is minor with most papular lesions but can be pronounced enough to resemble guttate psoriasis; hyperkeratotic plaques also are not unusual. Lesions are generally widespread and symmetrically distributed, although it is not uncommon for the rash to be anatomically limited. Involvement of palms and soles (Fig. 237.7A–B), either as part of the generalized eruption or as an isolated finding, is a characteristic feature of the maculopapular rash (see Fig. 237.7C) that helps in distinguishing secondary syphilis from psoriasis and pityriasis rosea. When the hair follicles are involved, patchy alopecia or thinning and a loss of eyebrows and beard may develop (see Fig. 237.7D). Lesions teeming with spirochetes, referred to as *mucous patches,* may develop on labial (see Fig. 237.7E), lingual, gingival, buccal, palatal, or pharyngeal mucosa, as well the moist surfaces of the genitalia. The typical mucous patch is an oval-shaped, shallow ulcer with a slightly raised border covered by a grayish-white or silvery membrane. In warm, moist intertriginous areas, the papules can enlarge, coalesce, and erode to produce painless, broad, moist, gray-white to erythematous papillary excrescences termed *condylomata lata* (see Fig. 237.7F). Generalized lymphadenopathy with firm, nontender nodes is a common physical finding in secondary syphilis; enlargement of the epitrochlear lymph nodes should always suggest the diagnosis.[1] Constitutional symptoms are also common and include low-grade fever, malaise, pharyngitis, anorexia, weight loss, arthralgias, and myalgias. During relapses of secondary syphilis, the skin lesions tend to be less florid, asymmetrically distributed, and more infiltrated.

Lues maligna is an unusual and severe variant of secondary syphilis eruption. Patients with this form of the disease may look systemically ill and present with rapidly developing polymorphic, ulcerating, rupioid (i.e., with heaped-up crusts) lesions sparing the palms and soles. Long recognized to affect debilitated persons, the condition has recently been reported most often in patients with HIV infection.[237–240]

Secondary syphilis can involve virtually any organ system, often causing diagnostic delay or confusion when not accompanied by telltale mucocutaneous signs. Subclinical elevations of liver enzymes, principally alkaline phosphatase, occur in up to 50% of patients.[241] The rare,

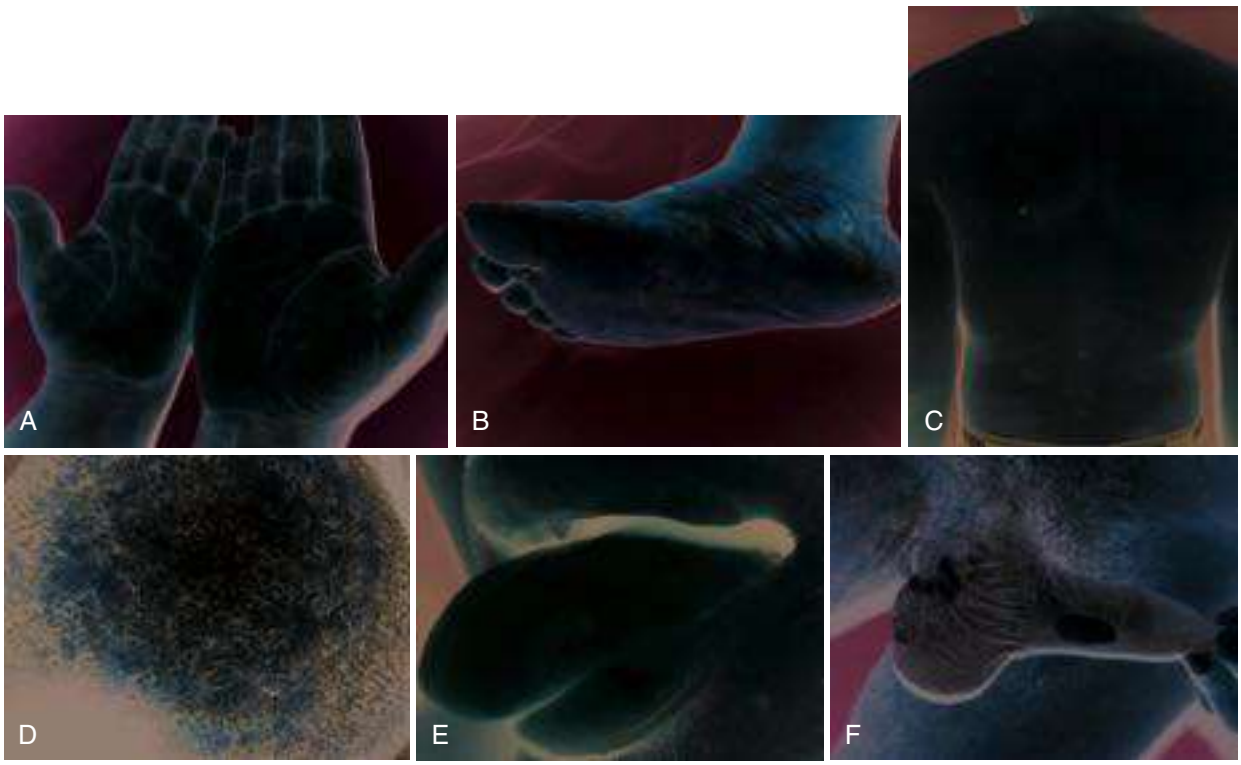

FIG. 237.7 Secondary syphilis lesions. (A) Characteristic palmar rash. (B) Plantar rash with hyperkeratosis. (C) Syphilitic exanthem—posterior trunk. (D) Syphilitic patchy alopecia. (E) Syphilitic oral mucous patch. (F) Male genital condylomas. *(Courtesy Dr. Adriana Cruz, Centro Internacional de Entrenamiento e Investigaciones Médicas, Cali, Colombia.)*

symptomatic hepatitis is characterized by a disproportionately elevated serum alkaline phosphatase level, mildly increased aminotransferases, and usually normal bilirubin. The histologic picture includes portal inflammation, mild hepatocellular necrosis, noncaseating granulomas, and rarely cholestasis. The gastrointestinal tract, particularly the stomach, may also become extensively infiltrated, ulcerated, or both, and the condition can be mistaken for lymphoma or carcinoma.[242,243] Epigastric or abdominal pain, early satiety, and nausea and vomiting are the most common gastrointestinal symptoms. All of the structures of the eye may be involved in secondary syphilis: the cornea (interstitial keratitis), the anterior chamber (iritis and anterior uveitis), the vitreous and choroid (posterior uveitis and chorioretinitis), and the optic nerve (optic neuritis).[244–247] Vitritis can be so dense that it obscures visualization of the retina.[248] The presence of cotton-wool spots in a patient with HIV infection can result in confusion between chorioretinitis due to syphilis and cytomegalovirus (CMV); retinal hemorrhage, a characteristic feature of CMV, does not occur with syphilis. Otosyphilis manifests with sudden or progressive sensorineural hearing loss, tinnitus, vertigo, and dysequilibrium.[249] The most common type of skeletal lesion is periostitis, most frequently involving the tibia, but also the sternum, skull, and ribs. Rarely, destructive bony lesions occur.[250,251] Symmetrical tenosynovitis and/or arthritis involving wrists, knees, and ankles also have been reported.[252] Syphilitic meningitis and stroke syndromes are well-recognized neurologic complications of secondary syphilis (see later). Although pneumonia is most typically associated with severe CS (see later), rare cases of pulmonary involvement in adults have been reported in the pre- and post-HIV eras.[253–255]

Latent Syphilis

Latent syphilis is, by definition, the stage during which serologic tests are reactive without clinical manifestations. Important to note, the term does not mean that the disease process is quiescent, only that clinical signs and symptoms are not evident. Stokes described syphilis as "the relapsing disease par excellence," recognizing that the immune system may require years before it can contain the spirochete, even if unable to fully eradicate it in many instances.[1] The Oslo Study is only one of several in the prepenicillin era that documented a high rate of relapse in early syphilis. Although the percentages differ, they concurred insofar as the preponderance of relapses occurred during the first 1 to 2 years,[1] observations that led to the somewhat arbitrary 1-year demarcation between early latent and late latent syphilis. However, the fact that asymptomatic pregnant women can transmit the infection to their infants in utero 5 or more years after infection clearly demonstrates that recurrent episodes of "silent" spirochetemia occur for prolonged periods.[1,60] In reality, there is no clear biologic demarcation between early latency, when the disease is still systemically active, and late latency, when it is active but anatomically contained.

Mucocutaneous relapses are by far the most common form of infectious relapse and the ones with greatest public health significance because of their potential for disease transmission.[229] The cutaneous lesions of secondary relapses tend to be less florid than initial secondary outbreaks, asymmetrically distributed, and often confined to the mouth, genital, and anal regions. Relapsing lesions have a greater tendency to assume annular forms; mucous patches and condylomata lata also are common. Late mucocutaneous relapses can manifest as localized destructive lesions, resembling gummas. A small percentage of relapses involve noncutaneous sites, such as bone (usually tibial periostitis), eye (usually iritis), liver, other viscera, and the CNS. These are probably flare-ups of infectious foci established earlier.

Tertiary Syphilis

Tertiary syphilis is a (usually) slowly progressive, destructive inflammatory process that can affect any organ in the body to produce clinical illness 5 to 30 or more years after the initial infection.[145,182,210] It is generally subdivided into neurosyphilis, cardiovascular syphilis, and gummatous syphilis. Although customary and convenient, inclusion of neurosyphilis among the tertiary syndromes is technically inaccurate because, as noted earlier (also see Fig. 237.5), not all forms of neurosyphilis occur as late disease. Since the introduction of penicillin, once-common forms of tertiary disease have become so rare that most

practitioners have never seen a case. These changes partly reflect the diminished prevalence of syphilis in the United States and other industrialized countries in the postantibiotic era, but also are attributed to the widespread use of antibiotics for unrelated conditions with the consequent "inadvertent" treatment of latent syphilis. In an oft-cited series of 241 cases published in 1972, Hooshmand[256] proposed that patients no longer presented with the classic neurosyphilitic syndromes as a result of widespread exposure to antibiotics. Most authorities now believe that the laboratory criteria used in that study for the diagnosis of these putative "forme frustes" of neurosyphilis were inadequate.[214] In fact, since that series many cases of patients with identifiable neurosyphilis syndromes have been published. Nevertheless, there is no question that the presentation of neurosyphilis has undergone a significant shift in recent years as paresis and tabes dorsalis have been replaced by meningeal and meningovascular syndromes.[210,211,257]

Neurosyphilis

The neurosyphilis syndromes are arguably the most confusing to practitioners for several reasons (for an excellent recent review, see Ghanem[258]). One is the baffling array of signs and symptoms *T. pallidum* can inflict on the CNS, which pose a diagnostic challenge to even the most experienced and well-trained clinician. Another is the unpredictable nature of the disease. Syphilologists have long pondered the question of why some individuals develop neurosyphilis and others do not.[149] Whether the answer(s) lie in the putative neuroinvasive properties of particular strains of *T. pallidum*[133,259] or in polymorphisms within the human genome that influence the responses elicited by spirochetes once within the CNS,[260] or a combination of the two, remains to be determined. A third reason is that neurosyphilis belies the entrenched concept that syphilis proceeds via an orderly and stereotypical temporal sequence. To the contrary, because the CNS is invaded during the spirochetemic episodes of early syphilis, neurologic manifestations can occur during any stage of the disease. In addition, clinicians often have trouble grasping the fact that asymptomatic CNS infection (often termed "neuroinvasion") is a frequent occurrence in early syphilis that does not usually require specific management, and that *T. pallidum* can be present in the spinal fluids of patients with early syphilis in the absence of neurologic symptoms and CSF abnormalities.[8,9,211,258,261] Lastly, the classification scheme for neurosyphilis developed by Merritt and colleagues[149] in the 1940s, still widely used today,[258] tends to convey the impression that patients always present with distinct neurologic syndromes. The reality, however, recognized by the scheme's creators[149] and underscored by contemporary neuroimaging modalities,[262] is that overlap syndromes with combinations of meningeal, vascular, and parenchymatous features frequently occur.

Asymptomatic Neurosyphilis

Asymptomatic neurosyphilis is defined by the presence of one or more CSF abnormalities (pleocytosis, elevated protein concentration, or reactive CSF Venereal Disease Research Laboratory [VDRL] test) in persons with serologic evidence for syphilis but no neurologic signs or symptoms. The recognition that neurologically asymptomatic patients with early syphilis can have abnormal spinal fluids and that these changes are potential harbingers of symptomatic neurosyphilis predates the discovery of *T. pallidum*. Rabbit isolation studies in the 1920s and 1930s,[1,149,263,264] since confirmed,[150,151] established definitively that CSF abnormalities are the consequence of CNS invasion by *T. pallidum* during early syphilis. Studies using rabbit inoculation from this same period,[263] also confirmed in the modern era,[150,151] revealed that up to 25% of ostensibly normal CSF samples from patients with early syphilis can harbor *T. pallidum*. The cumulative incidence of CSF abnormalities in untreated early syphilis peaks at somewhere between 30% and 50% of patients during the first 12 to 18 months of infection.[149,265]

Asymptomatic CNS infection can follow three distinct, not easily predictable paths.[185,258,266–268] In a substantial percentage of patients with either early or late syphilis, the abnormalities resolve spontaneously; in the remainder, they either persist without development of overt neurologic symptoms or worsen with the eventual appearance of a neurosyphilitic syndrome. Hence, the general trend over time is for asymptomatic neurosyphilis to decrease in frequency, due to either progression or resolution, while the proportion of symptomatic neurosyphilis increases. Accordingly, on the basis of examining more than 2200 patients, Merritt and colleagues[149] maintained that the incidence of late asymptomatic neurosyphilis was in the vicinity of 10%, well below the peak values noted earlier for early syphilis. Several tenets about the complex relationship between asymptomatic and symptomatic neurosyphilis emerged from the preantibiotic era and continue to be useful today: (1) asymptomatic neurosyphilis is always the forerunner of meningeal, vascular, and parenchymatous syndromes; (2) asymptomatic neurosyphilis during late syphilis carries a worse prognosis than asymptomatic neurosyphilis during early infection; (3) the likelihood of progression from asymptomatic to symptomatic neurosyphilis increases in rough proportion to the degree of CSF abnormalities; and (4) if the spinal fluid remains normal during the first 2 years of infection, or if it is abnormal but reverts to normal, symptomatic neurosyphilis will not develop.

Although the clinical importance of diagnosing asymptomatic neurosyphilis was well appreciated in the preantibiotic era, there was a diversity of opinion as to when lumbar puncture should be done,[1] just as there is today.[9,269] The Europeans, influenced by Ravaut, argued for later spinal fluid examination to avoid overtreating persons whose CNS infections were going to resolve spontaneously. Americans, led by Moore, advocated early examination of the spinal fluid to identify persons with severe CSF abnormalities (the so-called "paretic formula") who would benefit from more intensive arsenical therapy. The issue faded into the background as it became evident that neurorelapse was uncommon in patients with early syphilis given intramuscular benzathine penicillin G (BPG). However, as discussed later, it has resurfaced in the HIV era as a result of numerous case reports suggesting that single-dose intramuscular BPG predisposes to CNS relapse in the absence of a fully competent immune system,[215] along with clinical studies suggesting a higher prevalence of asymptomatic neurosyphilis among patients with early syphilis who are coinfected with HIV.[270–272]

Syphilitic Meningitis

Syphilitic meningitis typifies the ambiguity of syphilis staging for CNS disease because it is a true overlap syndrome, occurring as either an early or a late manifestation (see Fig. 237.5). Approximately 10% of cases occur in persons with a rash of secondary syphilis, and most occur during the first 2 years of infection. Even when meningitis is the sole presenting manifestation, more than half of patients recall having a chancre or a secondary syphilis rash, or both, an unusual occurrence in patients with parenchymatous syndromes. On the other hand, there are many documented instances of patients presenting with this form of neurosyphilis in late syphilis or in which it complicates late forms of neurosyphilis, or both. Although headache and meningismus mimicking many other causes of aseptic meningitis are not uncommon in patients with secondary syphilis, the full-blown meningeal syndrome is rare, representing less than 10% of all cases of neurosyphilis.[213] In their classic series, Merritt and Moore[213] could find only 80 well-authenticated cases over a 15-year period in three general hospitals, and they estimated, on the basis of their survey of the literature, that it may complicate between 0.3% and 2% of early syphilis cases (probably an overestimate, even at the low end, in our experience). In addition to signs and symptoms indicative of increased intracranial pressure (e.g., nausea, vomiting, headache), which occurred in nearly all cases, these authors distinguished three syndromic variants: (1) acute hydrocephalus without focal signs unrelated to increased intracranial pressure; (2) meningitis of the vertex, presenting with seizures, focal neurologic deficits (e.g., hemiplegia, aphasia), and changes in sensorium; and (3) basilar meningitis with cranial nerve palsies, especially of nerves III, VI, VII, and VIII. Eighth cranial nerve involvement can be unilateral or bilateral and can affect either or both the acoustic and vestibular nerves. The differential diagnosis is broad and includes viral causes of aseptic meningitis and meningoencephalitis; neuroborreliosis; tuberculous and fungal meningitis; parameningeal processes (e.g., abscess); and noninfectious processes such as sarcoidosis and carcinomatous meningitis. Reactive serologic tests for syphilis in blood and CSF, along with the subacute onset of signs and symptoms, help to distinguish meningeal syphilis from the numerous other infectious and noninfectious entities in the differential diagnosis.

Meningovascular Syphilis

Meningovascular syphilis comprises those syndromes in which focal neurologic signs due to infarction are the dominant presenting feature.[149] Like syphilitic meningitis, meningovascular syphilis represents an early-late overlap syndrome, although weighted more toward the late end of the temporal spectrum (see Fig. 237.5). The spinal cord, brainstem, or cerebrum may be involved separately or together, although the majority of patients in the large series by Merritt and colleagues[149] had vascular syndromes referable to the middle cerebral artery. Accordingly, the most common focal findings were contralateral hemiplegia or hemiparesis, homonymous hemianopsia, and aphasia. Recent case reports underscore the potentially catastrophic outcomes resulting from involvement of the posterior cerebral circulation.[273,274] In the 42 cases reviewed by Merritt and colleagues,[149] only 25% of patients gave a history of primary or secondary syphilis. These authors attributed 3% of their neurosyphilis cases at Boston City Hospital to meningovascular syphilis. This proportion is likely much lower than the current incidence. In a large series from India in which magnetic resonance imaging (MRI) was used, 11 of 35 patients were classified as having focal neurologic symptoms attributable to ischemia.[262] The relatively young age of patients with meningovascular syphilis (30–50 years) helps to distinguish this syndrome from atherosclerotic stroke, hence the clinical dictum that a stroke in a young or middle-aged individual always should raise suspicions of syphilis. As with atherosclerotic stroke, onset may be sudden ("syphilitic apoplexy"). However, a substantial proportion of patients have prodromal symptoms, such as headache, vertigo, insomnia, irritability, and personality and behavioral changes, weeks to months before the thrombotic event. Merritt and colleagues[149] attributed these affective and cognitive changes to the leptomeningeal component of the underlying pathologic condition. MRI showing significant cortical atrophy in many cases suggests that paretic-type changes are also contributory.[262] In the series by Merritt and colleagues,[149] only 5 of the 42 patients had neurologic deficits, suggesting involvement of more than one blood vessel. In recent case reports in which MRI and magnetic resonance angiography were used, however, imaging revealed diffuse and often bilateral involvement,[257] as one might expect with a CNS vasculitis. Meningovascular syphilis may also rarely involve the spinal cord, resulting in infarction of the anterior or, less commonly, posterior spinal arteries. In contrast to patients with involvement of cerebral vessels, the spinal variant lacks prodromal symptoms and is usually sudden in onset. Because of its semiacute course and association with global alterations in cerebral function, meningovascular syphilis has to be differentiated from chronic meningitides, as well as rheumatologic causes of cerebral vasculitis, particularly systemic lupus erythematosus, Wegener granulomatosis, and polyarteritis nodosa, and, in patients with a history of genital ulcers, Behçet disease.

Parenchymatous Syndromes

The parenchymatous syndromes, general paresis and tabes dorsalis, the last to occur in the neurosyphilis temporal sequence (see Fig. 237.5), involve distinct, poorly understood, neurocytotoxic mechanisms in addition to the vascular-based inflammatory and ischemic changes that cause symptomatology in meningeal and meningovascular syphilis.[185,258] In the preantibiotic era, these two syndromes accounted for one-half to two-thirds of all cases of neurosyphilis, and both were fourfold to sevenfold more common in men than women. As one indicator of how common they were, according to Stokes,[1] paresis accounted for 11% of neuropsychiatric admissions in the United States and 5% to 7% of cases of mental disease in the French, German, American, and Russian armies. The virtual disappearance of tabes dorsalis, which is disproportionate to the decline in the incidence of paresis, is one of the most striking changes in the epidemiology of neurosyphilis. Because the American Association of Neurology no longer recommends screening for paresis as part of a routine dementia evaluation,[275] it is important to maintain vigilance for this relatively rare but still encountered dementing illness. In case reports,[276–279] and in the experience of the authors, a substantial degree of reversibility can be seen when paresis is promptly recognized and treated.

Merritt and colleagues[149] described general paresis as a chronic, spirochetal meningoencephalitis that severely disturbs the structure and function of the cerebral cortices, particularly the frontal and temporal lobes. In one of the largest case series in the postantibiotic era (from the United Kingdom), the interval between infection and admission varied between 4 and 41 years (mean, 21 years).[280] The typical clinical picture is a slow, often insidious, onset of neuropsychiatric disturbances coupled with progressive deterioration in cognitive function. The presentation, however, may be abrupt. In the British series, the duration of symptoms varied between 24 hours and 5 years and approximately 20% of patients had seizures.[280] As the disease worsens, patients experience loss of motor control to the point of paralysis along with worsening loss of bowel and bladder control. Untreated paresis is fatal. The constellation of signs and symptoms can be remembered with the mnemonic PARESIS: *p*ersonality (emotional lability, paranoia); *a*ffect (carelessness in appearance); *r*eflexes (hyperactive); *e*ye (Argyll Robertson pupils [Fig. 237.8])[281]; *s*ensorium (illusions, delusions, especially megalomania, hallucinations); *i*ntellect (decreased recent memory, judgment, insight); and *s*peech (slurred). The dramatic postmortem pathologic findings described by Merritt and colleagues[149] have been demonstrated on a number of occasions in patients with MRI, now an essential tool for making the diagnosis.[276,277,282,283] In several instances, subacute changes in personality and cognition in concert with temporal abnormalities at MRI have been confused with herpes encephalitis (Fig. 237.9).[284–287] Kodama and colleagues[276] concluded from a small series ($n = 7$) that mesial temporal lobe atrophy at MRI carries a poor prognosis for improvement after antimicrobial therapy.

The demyelinating process in the posterior spinal cords of patients with tabes dorsalis eventually results in the development of an ataxic, wide-based gait and foot slap; paresthesias; shooting or lightning pains (sudden onset, rapid radiation, and disappearance); bladder disturbances; fecal incontinence; impotence; loss of position and vibratory sense; absent ankle and knee jerk reflexes; and loss of deep pain and temperature sensation. The characteristic "lightning" or lancinating pains experienced by at least 75% of patients are usually present at the outset of the disease, typically affecting the lower extremities, and occurring episodically.



FIG. 237.8 Argyll Robertson pupils in a patient with tabes dorsalis. A 47-year-old man with a history of human immunodeficiency virus (HIV) infection presented with an 8-month history of severe paroxysmal shooting pains in his legs, progressive difficulty in walking, tinnitus, and urinary incontinence. He had a fully suppressed HIV viral load and a $CD4^+$ count of 400 cells/mm^3 while receiving antiretroviral therapy. The physical examination showed Argyll Robertson pupils, which are nonreactive to bright light but briskly constrict when focusing on a near object (see video at https://www.nejm.org/doi/10.1056/NEJMicm1507564?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dwww.ncbi.nlm.nih.gov). Magnetic resonance imaging showed high signal changes in the dorsal columns of the thoracic spine, and samples of blood and cerebrospinal fluid were positive for syphilis on Venereal Disease Research Laboratory (VDRL) testing and *Treponema pallidum* particle agglutination assay. The patient was treated with intravenous penicillin for 14 days, and gabapentin was started for the neuropathic leg pains. Tabes dorsalis is a form of neurosyphilis that is characterized by degeneration of the nerves in the dorsal columns of the spinal cord. Along with Argyll Robertson pupils, the condition is associated with ataxia and loss of proprioception. After treatment, the patient's symptoms and mobility slowly improved, although the shooting pains in his legs have continued despite pharmacotherapy. The serum VDRL level fell appropriately, and the result on VDRL testing of the cerebrospinal fluid was negative. *(From Osman C, Clark TW. Tabes dorsalis and Argyll Robertson pupils.* N Engl J Med. *2017;375:e40.)*

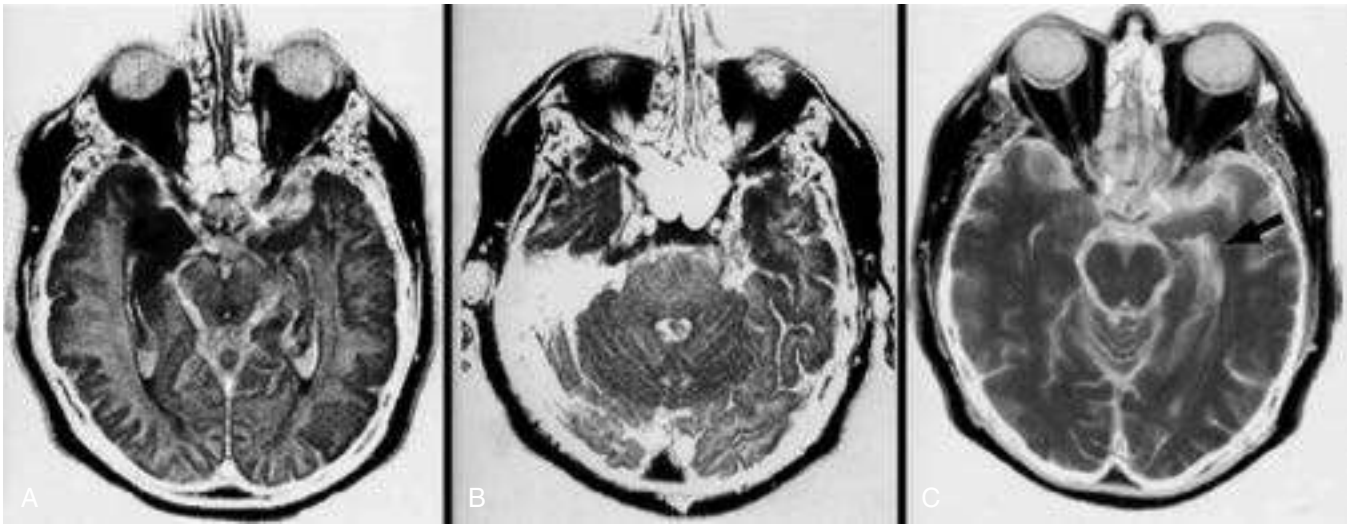

FIG. 237.9 Magnetic resonance images of general paresis in a 50-year-old man with a 3-month history of progressive dementia and seizures. (A) Axial fluid-attenuated inversion recovery (FLAIR) image obtained at midbrain level. (B) Axial FLAIR image obtained at the level of the pons. Asymmetrical bilateral signal hyperintensity in the mesial temporal lobes is greater on the right side than on the left. (C) Axial T1-weighted image obtained at the level of the low midbrain showing mild left temporal lobe atrophy, evidenced by dilatation of the temporal horn *(arrow). (From Bash S, Hathout GM, Cohen S. Mesiotemporal T2-weighted hyperintensity: neurosyphilis mimicking herpes encephalitis.* Am J Neuroradiol. *2001;22:314–316.)*

Some patients, 10% to 20%, experience visceral crises. In the most common gastric form, the individual experiences recurrent episodes of sudden, agonizing epigastric pain with nausea and vomiting that can last for days and mimic surgical emergencies. Intestinal, rectal, and laryngeal crises can also occur. Decrease or loss of tendon and patellar reflexes with preservation of muscle strength is a common, relatively early, neurologic finding. Ataxia was regarded as such a cardinal symptom of tabes that it was once called "progressive locomotor ataxy"; between 50% and 80% of patients exhibit a positive Romberg sign. Trophic degenerative joint disease, Charcot joints, and traumatic ulcers or sores on the lower extremities and feet resulting from the loss of proprioception and sensation were once prominently featured in textbooks. Degenerative ocular changes are also common components of the tabetic syndrome. The Argyll Robertson pupil (see Fig. 237.8), although not limited to tabes or even syphilis, is a characteristic late feature.[281] Primary optic atrophy occurs over a period of months to years, beginning peripherally and proceeding to the center of the nerve, producing progressive concentric constriction of the visual fields with retention of normal vision, referred to as "gun barrel" sight.[288]

Central Nervous System Gumma

The CNS gumma, the rarest form of neurosyphilis, was uncommon even in the preantibiotic era. In a review of 2203 brain tumors published in 1932, Cushing[289] found only 12 cases, and Merritt and colleagues[149] found only 5 well-documented cases over a 15-year span at the Boston City Hospital. Nevertheless, sporadic reports continue to appear in the modern literature.[193,290,291] Because of the likelihood that a CNS gumma will initially be mistaken for a tumor or other type of space-occupying lesion (e.g., toxoplasmosis in an AIDS patient), this entity well exemplifies the reputation of syphilis as the great mimicker. The pathologic features of gumma within the CNS are no different than those occurring outside it. Merritt and colleagues[149] considered gumma of the CNS to be a chronic, localized form of syphilitic meningitis that extends from the pia mater into the adjacent brain or spinal cord. In a comprehensive review of the literature, Fargen and colleagues[193] found that two-thirds of the lesions occurred in the cerebral convexities, of which the majority were located in the frontal lobes or frontoparietal region. The pituitary was the most common site outside the cerebral hemispheres.

Ocular Syphilis

Ocular complications may occur as part of a neurosyphilis syndrome, often asymptomatic neurosyphilis, or as an isolated manifestation.[149,244,246,247,292] Anterior or posterior uveitis or panuveitis, the most common abnormalities, can occur during either early or late syphilis (see Chapter 115). Other ocular syndromes include episcleritis, vitritis, retinitis, papillitis, interstitial keratitis, acute retinal necrosis, and retinal detachment. Primary optic atrophy is unique to late syphilis and is most associated with tabes dorsalis.[149,288] The differential diagnosis includes tuberculosis, rheumatoid arthritis, sarcoidosis, toxoplasmosis, histoplasmosis, and ocular *Toxocara canis* infections. The presence of pupillary abnormalities distinguishes syphilis from these other processes. Unless scarring has occurred, improvement with treatment can be dramatic.

Otosyphilis

Syphilis can cause hearing loss via two mechanisms during early or late infection. One is osteitis of the temporal bone with destructive changes in the membranous cochlea and labyrinth. The other is inflammation and atrophy of cranial nerve VIII, typically insidious and often bilateral.[293] Merritt and colleagues[149] found that 10 of their 80 cases of syphilitic meningitis had involvement of the acoustic or vestibular branches of the eighth cranial nerve, or both, whereas 57 of their 203 cases of tabes dorsalis had hearing impairment. Involvement of cranial nerve VIII typically begins with high-frequency hearing loss and progresses to a complete unilateral or bilateral loss of cochlear and vestibular function.[294] Syphilitic labyrinthitis has been known to mimic Meniere disease. In the postantibiotic era, otosyphilis has become liberally defined as an unexplained sensorineural hearing loss in the presence of a reactive treponemal serologic test.[295,296] As noted by Pletcher and Cheung,[297] this definition lacks a clear causal relationship between syphilis and the clinical symptoms. Nevertheless, in such cases, treatment is usually indicated because there are no diagnostic measures that can reliably eliminate the possibility of syphilis.

Cardiovascular Syphilis

In the preantibiotic era, asymptomatic aortitis was considered to be highly prevalent; in fact, Moore[298] maintained that the majority of patients with long-standing syphilis have subclinical aortitis undetectable on radiographs or at clinical examination. Despite the marked decline in incidence since World War II, syphilitic involvement of the aorta continues to occur, although the diagnosis is often not suspected clinically or made before surgery or postmortem examination.[194,299,300] Dilation of the proximal portion of the aorta and linear calcification of the anterolateral wall of the ascending aorta are suggestive radiographic signs. Weakening of the aortic valve ring and distortion of the cusps

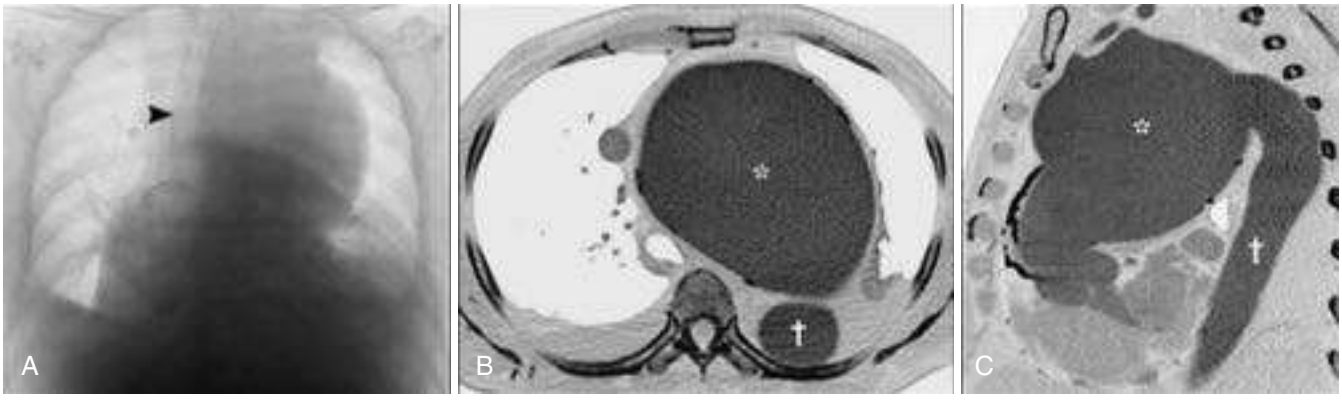

FIG. 237.10 Cardiovascular syphilis in a 76-year-old man with progressive dyspnea and leg swelling. (A) Chest radiograph showing a mediastinal mass with tracheal deviation *(arrowhead).* (B) Contrast-enhanced computed tomogram of the chest showing a large saccular aortic aneurysm. (C) Sagittal reconstruction image. Asterisks in (B) and (C) indicate aneurysm; daggers show normal descending aorta. *(From Rajab TK, Gallegos RP. Images in clinical medicine. Giant syphilitic aortic aneurysm.* N Engl J Med. *2011;364:1258.)*

results in aortic regurgitation, the most common complication of syphilitic aortitis, and in approximately 20% of these cases, coronary ostial stenosis. Fibrosis of the media also contributes to the development of ostial stenosis and, rarely, can cause severe coronary occlusion even in the absence of dilation of the aortic root.[301] Surprisingly, syphilitic aneurysm is the least common complication, occurring only one-third as frequently as aortic insufficiency and producing clinically apparent, albeit often devastating, manifestations in only 5% to 10% of patients with aortitis. A recent case report emphasized the importance of considering syphilis in patients who present with aortic regurgitation and myocardial infarction.[302] Syphilitic aneurysms are usually saccular, although they may be fusiform; because of the diffuse scarring in the aortic wall, they tend not to dissect (Fig. 237.10). Approximately 50% of aneurysms occur in the ascending aortic arch and have been designated the "aneurysm of signs" because they attain great size with few symptoms. In a recent surgical series, Roberts and colleagues[303] stated that the key to recognizing cardiovascular syphilis at surgery was the diffuse nature of the involvement of the tubular portion of the ascending aorta with complete or virtual sparing of the sinus portion. Aneurysm of the innominate artery is a rare complication of ascending aortitis.[299,304] Next most frequently involved (30%–40%) is the transverse arch, designated the "aneurysm of symptoms" because of its location in proximity to a number of mediastinal structures. The minority of aneurysms, 10% to 15%, occur in the descending arch; a small percentage occur below the sixth vertebral body or, even more rarely, below the thorax.[305]

Late Benign (Gummatous) Syphilis

The gumma is a necrotizing granulomatous lesion that occurs in late syphilis. These indolent lesions are most commonly found in the skin, om mucocutaneous surfaces, and in the skeletal system but can develop in any organ. They may be single or multiple and vary in size from small defects to large tumor-like masses (Fig. 237.11). They are of clinical importance principally because of their local destructiveness or cause of masslike effects, or both.[306] The cutaneous manifestations range from superficial nodules to deep granulomatous lesions, which may break down to form punched, ragged ulcers resembling pyoderma gangrenosum.[307] Involution is followed by the development of thin, atrophic, noncontractile scars arranged in arciform patterns. Gummatous hepatitis may cause low-grade fever, epigastric pain, and tenderness and eventually cirrhosis *(hepar lobatum).* Gummas of the gastrointestinal tract can ulcerate or cause leathery fibrotic changes in the bowel wall, simulating carcinoma and causing stricture.[308] Gummas of bone, originating from the metastasis of spirochetes to the periosteum during early syphilis, may result in fractures and joint destruction of long bones and radicular signs and symptoms when occurring in vertebra.[309–311] Gumma of the upper respiratory tract can lead to perforation of the nasal septum or palate.[1,182] Gummas must be distinguished from other infectious and noninfectious causes of granuloma (tuberculosis, deep fungal infections, and sarcoidosis) and neoplasm. Penicillin treatment results in rapid improvement and thus is diagnostic.



FIG. 237.11 Cutaneous syphilitic gumma. *(From Chudomirova K, Chapkanov A, Abadjieva T, Popov S. Gummatous cutaneous syphilis.* Sex Transm Dis. *2009;36:239–240.)*

LABORATORY DIAGNOSIS

Diagnosis of syphilis is stage dependent and requires a combination of clinical and laboratory criteria as shown in Table 237.1.[5–7,211] Because *T. pallidum* cannot be cultivated on artificial media, laboratory diagnosis relies on direct detection of the pathogen in patient specimens or reactivity in serologic tests, or both. Direct detection of *T. pallidum* in clinical material is the only means of establishing a microbiologic diagnosis. At one time, direct detection was limited to visualization of live (i.e., motile) treponemes with DF microscopy and silver staining of treponemes in paraffin-embedded tissues. IHC detection has largely supplanted silver staining, and PCR has greatly expanded the range of clinical specimens in which treponemes can be detected, although it is still not a routine diagnostic tool, and no US Food and Drug Administration–approved PCR test for syphilis is on the market. Serologic tests are unquestionably the mainstay of syphilis diagnosis. In addition to supplementing direct detection, they are the only means of establishing a probable diagnosis when lesional specimens are not available or cannot be obtained. Syphilis serodiagnosis depends on the use of assays measuring two distinctly different types of antibody reactivities, the so-called "nontreponemal" and "treponemal" tests. Traditionally, serodiagnosis of syphilis has been performed by screening for nontreponemal antibodies and confirming reactivity with a treponemal test. Reverse-sequence algorithms in which sera are first tested by an automated treponemal assay and, when reactive, followed by a nontreponemal test have transformed the landscape of syphilis serodiagnosis while also creating unforeseen dilemmas, particularly when screening low-prevalence populations.[312,313] Nontreponemal

TABLE 237.1 Clinical and Laboratory Criteria for Diagnosis of Syphilis

Presumed Incubating Syphilis Requiring Treatment

Persons who have had sexual contact within the last 90 days with a person diagnosed with early syphilis
Persons who have had sexual contact >90 days previously with a person diagnosed with early syphilis if serologic test results are not immediately available and the opportunity for follow-up is uncertain
Sex partners of persons with late latent syphilis or syphilis of undetermined duration who have nontreponemal test titers >1 : 32

Early Syphilis
Primary

Confirmed (Requires 1 and 2 or 3)
1. One or more chancres (ulcers)
2. Identification of Treponema pallidum in lesion exudate by DF microscopy
3. Detection of T. pallidum DNA in lesion exudate with PCR

Probable (Requires 1 and Either 2 or 3)
1. One or more lesions compatible with chancres
2. Reactive nontreponemal test
3. Reactive treponemal test

Secondary

Confirmed (Requires 1 and Either 2, 3, or 4)
1. Localized or diffuse mucocutaneous lesions consistent with secondary syphilis
   a. Macular, papular, follicular, papulosquamous, or pustular rash
   b. Condylomata lata (anogenital region or mouth)
   c. Mucous patches (oropharynx or cervix)
2. Identification of T. pallidum in lesion exudates with DF microscopy
3. Identification of T. pallidum in skin biopsy with silver or immunohistochemical staining
4. Detection of T. pallidum DNA in tissue with PCR

Probable (Requires 1 and 2)
1. Skin or mucous membrane lesions consistent with secondary syphilis
2. Reactive nontreponemal test titer ≥4 and a reactive confirmatory treponemal test

Early Syphilis, Non-Primary, Non-Secondary

(formerly early latent; no confirmed case classification)

(Requires: A. 1, 2, 4, 5; B. 1, 3, 4, 5; C. 4, 5 and 6, 7, or 8)
1. Absence of signs and symptoms of syphilis
2. No prior history of syphilis
3. A prior history of syphilis
4. Documented seroconversion or fourfold or greater increase in nontreponemal test titer during the previous 12 months
5. Documented seroconversion of a treponemal test during the previous 12 months
6. A history of symptoms consistent with primary or secondary syphilis during the previous 12 months
7. A history of sexual exposure to a partner who had confirmed or probable primary or secondary syphilis or probable early latent syphilis (documented independently as duration <1 year)
8. Reactive nontreponemal and treponemal tests in a person whose only possible exposure occurred within the preceding 12 months

Syphilis, Unknown Duration or Late

(formerly *late latent*; initial infection occurred greater than 12 months previously or insufficient evidence to conclude that infection was acquired during the previous 12 months; *no confirmed case classification*)

(Requires 1, 2, and Either 3 or 4)
1. Absence of signs and symptoms of syphilis
2. A reactive nontreponemal and treponemal test
3. No prior history of syphilis
4. A prior history of syphilis and a current nontreponemal test titer demonstrating fourfold or greater increase from the last nontreponemal test titer

Late Syphilis
Benign (Gummatous) and Cardiovascular

Confirmed (Requires 1 and 2 or 3)
1. Clinically compatible case (e.g., inflammatory lesions of the skin, bones, or cardiovascular system)
2. Identification of treponemes in tissue sections with silver or immunohistochemical staining
3. Detection of T. pallidum DNA in tissue with PCR

Probable (Requires 1, 2, and 3)
1. Clinically compatible case
2. A reactive serum treponemal test
3. Absence of clinical signs or symptoms consistent with neurosyphilis

Neurosyphilis

Confirmed (Requires 1, 2, and Either 3, 4, or 5)
1. Clinical signs consistent with neurosyphilis
2. A reactive serum treponemal test
3. A reactive VDRL in CSF
4. Detection of T. pallidum DNA in CSF or tissues with PCR
5. Identification of treponemes in tissue with silver or immunohistochemical staining

Probable (Requires 1, 2, and 3)
1. Clinical signs consistent with neurosyphilis
2. A reactive serum treponemal test
3. Elevated CSF protein or leukocyte count in the absence of other known causes

Continued

TABLE 237.1 Clinical and Laboratory Criteria for Diagnosis of Syphilis—cont'd

Congenital Syphilis: Neonatal

Confirmed (Requires 1 and 2 or 3)

1. Clinically compatible case (e.g., hepatosplenomegaly, rash, condylomata lata, jaundice, anemia)
2. Demonstration of T. pallidum by microscopic examination of specimens from lesions, amniotic fluid (antenatal), placenta, umbilical cord, nasal discharge, or autopsy material
3. Detection of T. pallidum DNA in lesions, tissue, blood, CSF, or a combination of these, by PCR

Probable (Requires 1 or 2 and 3)

1. Infant born to a mother who had untreated or inadequately treated syphilis at delivery, regardless of findings in the infant
2. An infant or child with a reactive treponemal test result
3. One of the following additional criteria
   a. Clinical signs or symptoms of congenital syphilis on physical examination
   b. Evidence of congenital syphilis on radiographs of long bones
   c. Abnormal CSF cell count or protein without other cause
   d. Reactive VDRL in CSF
   e. Reactive treponemal antibody absorbed—19S-IgM antibody test or IgM enzyme-linked immunosorbent assay

Syphilitic Stillbirth

1. A fetal death that occurs after a 20-week gestation
2. Fetus weighs >500 g and the mother had untreated or inadequately treated syphilis at delivery

CSF, Cerebrospinal fluid; *DF,* darkfield; *IgM,* immunoglobulin M; *PCR,* polymerase chain reaction; *VDRL,* Venereal Disease Research Laboratory.

Modified from the Centers for Disease Control and Prevention. STD Surveillance Case Definitions. https://wwwn.cdc.gov/nndss/conditions/syphilis/case-definition/2018/. Accessed January 27, 2018. Reproduced from Jorgensen JH, Pfaller MA, Carroll KC, et al, eds. Manual of Clinical Microbiology. *11th ed. Washington, DC: ASM Press; 2015. © 2015 American Society for Microbiology. Used with permission. No further reproduction or distribution is permitted without the prior written permission of American Society for Microbiology.*

tests have a unique role in management because they are used as surrogate markers for disease activity during screening and gauging the adequacy of therapeutic response. For detailed information on diagnostic tests for syphilis, the reader is referred to work by Sena and colleagues.[7] A report by the Association of Public Health Laboratories (APHL) summarizing the findings of an expert consultation panel on syphilis diagnostics convened by the CDC in 2009 can be found at https://www.aphl.org/programs/infectious_disease/std/Documents/ID_2009Jan_Laboratory-Guidelines-Treponema-pallidum-Meeting-Report.pdf.[314]

Direct Examination for Spirochetes Darkfield Microscopy and Direct Fluorescent Antibody Test for *Treponema pallidum*

DF microscopy is a simple, rapid method for detecting live *T. pallidum* in clinical specimens.[7,315] In experienced hands, DF microscopy is capable of detecting organisms in suspensions containing a minimum of 10^4 to 10^5 spirochetes per milliliter. The method is most applicable to moist, exudative lesions of primary and secondary syphilis, which typically contain high concentrations of treponemes. Other specimens, such as lymph node aspirates, amniotic fluid, and nasal discharge from infants with suspected CS, also may contain spirochetes at sufficient concentration for successful DF examination. Detailed descriptions of the DF procedure can be found in Sena et al.,[7] Larsen et al.,[315] and Wheeler et al.[316] Briefly, the surface of the lesion should be cleansed with nonbactericidal saline and gently abraded with dry gauze. The serous exudate is then transferred to a glass slide, covered with a cover slip, and examined; the suspension can be thinned by adding a drop of nonbacteriostatic saline. Because the test relies on observation of motile treponemes, the specimen should be examined as rapidly as possible, ideally within 20 minutes. Visualization of a single motile treponeme is considered diagnostic. DF microscopy should not be performed on oral lesions because *T. pallidum* cannot be distinguished easily from commensal oral spirochetes. Given the many biologic and technical factors that determine the success of the technique, it must be emphasized that a negative result does not rule out the diagnosis of syphilis. DF microscopy also cannot distinguish *T. pallidum* from the other pathogenic treponemes, although this is rarely a cause for diagnostic uncertainty. In recent years, the DF technique has fallen into disfavor because it requires a microscope outfitted with a special condenser and a highly trained microscopist. A 2014 survey of national reference and large clinical laboratories in Latin America and the Caribbean revealed that only 2 of 69 participating facilities still performed DF microscopy.[317] Nevertheless, the fact that at least 15% of patients with DF-positive primary syphilis have nonreactive serologic tests[6,318] underscores the continued need for proficiency with DF microscopy, an opinion endorsed by an expert panel convened by the CDC.[314]

The direct fluorescent antibody test for *T. pallidum* (DFA-TP) identifies *T. pallidum* in touch preparations by immunostaining with either a fluorescein isothiocyanate (FITC)–conjugated polyclonal or monoclonal antibody.[7,315,319] Because motility is not required for interpretation of DFA-TP, slides can be fixed and processed at a later time. DFA-TP with a *T. pallidum*–specific monoclonal antibody can be performed on all mucocutaneous lesions of primary and secondary syphilis, including specimens from anal and oral sites. Although DFA-TP is at least as sensitive as DF microscopy and more specific, it has never gained wide acceptance because of its technical complexity, its instrumentation requirements, and the poor availability of antibody conjugates. In fact, the most recent European guidelines recommend against DFA-TP testing.[320]

Visualization of *Treponema pallidum* in Tissues

Silver impregnation is the traditional method for detection of *T. pallidum* in formalin-fixed tissues. The Dieterle technique is believed to be more sensitive than Warthin-Starry staining.[321] Although used with great success in countless cases, silver staining is prone to artifacts, has limited sensitivity, and is not specific for *T. pallidum.* Spirochetes other than *T. pallidum* will stain with this method; *B. burgdorferi,* which induces similar histopathologic changes, is the one most likely to cause diagnostic confusion in regions where the endemic treponematoses do not occur and Lyme disease is common.[320,322] In 1992, Ito and colleagues[323] introduced the use of an FITC-labeled *T. pallidum*–specific monoclonal antibody for immunostaining of processed tissues. As with the DFA-TP, the technique has never become popular. Recently, impressive results have been reported with use of commercially available polyclonal anti–*T. pallidum* antibodies and avidin-biotin immunoperoxidase staining to detect treponemes in paraffin-embedded skin biopsy specimens.[134,141,170,183,324] In head-to-head comparisons, immunohistochemistry compares favorably with PCR for detection of treponemes in tissue samples[325] and has the advantage of enabling assessment of the spatial distribution of spirochetes in samples and their relationship to matrix, vascular, and cellular components of syphilitic infiltrates.[183,325,326]

Polymerase Chain Reaction Detection of *Treponema pallidum*

Despite the numerous studies published since the first use of PCR for molecular detection of *T. pallidum* in the early 1990s,[327,328] a commercially available or approved, standardized test has yet to be introduced within

or outside the United States.[5,7] Of the many gene targets, *polA (tp0105)* and *tpn47 (tp0574)* have been the most extensively used and appear to be more or less comparable in sensitivity and specificity when properly optimized.[329,330] Although PCR can detect *T. pallidum* in a wide variety of specimen types, including embedded and archived tissues, its usefulness, not surprisingly, appears to be greatest for genital ulcers and other exudative lesions, which typically contain high concentrations of treponemes.[7] A meta-analysis found that the sensitivity and specificity of PCR for genital ulcer swabs were approximately 80% and 95%, respectively, with DF microscopy used as the gold standard; these authors concluded that PCR was more useful for confirming than excluding a diagnosis of primary syphilis.[329] It is important to note, however, that head-to-head comparisons have repeatedly demonstrated that PCR can detect spirochetes in DF-negative samples from patients with a high suspicion of primary syphilis.[331–336] Studies using conventional and real-time PCR indicate that PCR analysis of blood, even during early syphilis, has limited diagnostic usefulness,[329] probably because of variability in the concentrations of circulating organisms and the limited amount of blood that can be processed for amplification; degradation of DNA during frozen storage of blood may also be a factor.[234] In a study from China, *T. pallidum* was detected in the serum or plasma of 50% of patients with secondary syphilis compared with 21% and 13% for individuals with primary and early latent disease, respectively.[337–339]

Serologic Tests

Syphilitic infection elicits two different types of antibody responses, designated as "nontreponemal" and "treponemal."[6,340] The term *nontreponemal,* derived from the long-held belief that the inciting antigens are lipids liberated from inflamed tissues, is probably a misnomer given that cardiolipin is a major phospholipid constituent in *T. pallidum.*[341] In addition to lacking sensitivity in primary and late syphilis, nontreponemal antibodies can be elicited in conditions with no relationship to syphilis, so-called biologic false positives (BFPs; see later). Beginning with the fluorescent treponemal antibody absorption (FTA-ABS) test, treponemal tests were developed to address the lack of specificity of the nontreponemal tests.[6,340] The need to achieve higher throughput with decreased laboratory costs prompted the development of automatable immunoassays that use recombinant antigens instead of *T. pallidum* lysates. Rapid point-of-care (RPOC) tests have been developed to meet the need for on-site testing in developing countries with limited public health and laboratory infrastructures. None of the currently available serologic tests can distinguish venereal syphilis from the endemic treponematoses. Serum is the specimen of choice for both nontreponemal and treponemal tests; however, plasma may also be used in the rapid plasma reagin (RPR) card test and the toluidine red unheated serum test (TRUST).[342,343] Although syphilis screening in low-risk adults has been largely abandoned, systematic reviews provide convincing evidence in favor of syphilis screening for pregnant women and adults and adolescents at increased risk of infection.[5,344]

Nontreponemal Tests

The nontreponemal antibody tests in widespread use today are the descendants of the original Wassermann test. The standard nontreponemal test is the VDRL slide test in which heat-inactivated serum is tested for its ability to flocculate or agglutinate a standardized suspension of a cardiolipin, cholesterol, and lecithin and read microscopically at ×100.[5] Most laboratories and blood banks now use the RPR card test, which uses finely divided charcoal particles as a visualizing agent, for routine screening and following the response to therapy, whereas VDRL testing is used exclusively for CSF. As a general rule, RPR titers on the same serum specimens tend to be higher than VDRL titers. TRUST is a macroflocculation assay in which the charcoal is replaced with toluidine red; its sensitivity is equivalent to that of the RPR test, whereas its specificity is slightly higher.[342,343]

Nontreponemal tests are reported as the highest dilution giving a fully reactive result.[315] A four-fold change in titer using the same nontreponemal test method is necessary to demonstrate a significant difference and should be performed in the same laboratory and, if possible, on the same day. Sera with extremely high nontreponemal titers may give weakly reactive, atypical, or even negative "rough" reactions at low dilutions because antibody excess prevents the agglutination reaction. Most laboratories circumvent this "prozone" phenomenon, which occurs in approximately 1% to 2% of sera from patients with secondary syphilis and occasionally in HIV-infected patients,[315,345] by routinely titrating all samples to at least 16 dilutions. In untreated patients, the degree of reactivity of nontreponemal tests roughly correlates with the stage of disease and inversely with treponemal burdens. Approximately 30% of patients with early primary syphilis have nonreactive nontreponemal tests.[318] The greater sensitivity of treponemal tests for detecting early infection is a principal argument for screening using the "reverse algorithm" (see later).[346,347] Nearly all patients with secondary syphilis will have nontreponemal test titers of at least 1 : 8. Although there have been rare case reports of HIV-reactive syphilis patients with nonreactive nontreponemal tests,[348,349] a nonreactive nontreponemal test (i.e., one without a prozone) essentially rules out the diagnosis of secondary syphilis. Approximately one-third of patients with tertiary syphilis will have nonreactive nontreponemal tests; these are presumed to be cases in which the inflammatory response is largely burned out, but nevertheless these patients still require stage-specific treatment (see later).

Conditions other than treponemal infection can elicit antilipoidal antibodies that cause reactivity in a nontreponemal test with a negative treponemal test result, termed a *biologic false-positive* reaction.[350] Acute BFP reactions, defined as lasting less than 6 months, are associated with transient diseases or conditions such as malaria, brucellosis, and mononucleosis. Recently, smallpox vaccination was shown to produce BFP reactions.[351] Causes of chronic BFP reactions, defined as lasting more than 6 months, include autoimmune diseases, particularly systemic lupus erythematosus, HIV infection, intravenous drug use, and leprosy. Patients with hepatitis C infection are noted to be five times more likely to have BFP reactions than hepatitis C–negative controls.[352] Acute BFP titers tend to be higher than chronic BFPs. Aging and pregnancy have also been cited as causes of BFP reactions, but it is likely that other underlying conditions were responsible for the false-positive test results. For example, in a Swedish study of 5170 pregnant women, eight of nine BFPs were positive for reasons other than pregnancy.[353] A BFP is not necessarily indicative of a disease state. A study of 19,067 Jamaicans revealed that 0.59% of the general population had false-positive VDRL results.[354] The relationship between the anticardiolipin (i.e., nontreponemal) antibodies of syphilis and those associated with the antiphospholipid (aPL) syndrome, a cause of BFP tests for syphilis, was once a source of confusion. It is now recognized that aPL "autoimmune" antibodies are distinct and directed against β_2-glycoprotein I, a cardiolipin-binding hemostasis control protein.[208,211,261,355–360]

Treponemal Tests

Conventional treponemal tests (i.e., the FTA-ABS and *T. pallidum* particle agglutination [TPPA] tests) and the majority of newer commercially available treponemal assays measure both immunoglobulin G (IgG) and IgM without distinguishing the immunoglobulin class responsible for reactivity. This property accounts for their high level of sensitivity for syphilitic infection of all stages but also their inability to distinguish active from inactive disease. It is important to remember, therefore, that a reactive treponemal test usually remains reactive for life. On the other hand, treponemal tests should always be performed when primary syphilis is suspected because of their greater sensitivity than nontreponemal tests (86% vs. 70%), becoming reactive 1 to 2 weeks after the appearance of the chancre.[5,318] Treponemal tests also should be performed for suspected tertiary syphilis even when nontreponemal tests are nonreactive. Although reactive treponemal tests in this context are sufficient evidence for treatment, they are not definitive evidence that syphilis is the underlying process.

The formats of treponemal tests have changed substantially over the years.[7,315] The first to come into widespread use was the FTA-ABS test. In this test, a serum sample, adsorbed with an extract of the cultivatable treponeme *Treponema phagedenis* Reiter (Sorbent) to remove cross-reactive antibodies generated against commensal microbiota, is used to immunolabel treponemes fixed to glass slides. Labeled organisms are demonstrated by means of an FITC-conjugated antihuman immunoglobulin antibody, and the sample is scored by eye based on fluorescence intensity. Because of the subjectivity involved and the need for expensive microscopy equipment, the CDC no longer regards the FTA-ABS test as

the gold standard treponemal test and does not recommend it for routine usage.[361] The microhemoagglutination–*T. pallidum* (MHA-TP) assay is a passive hemagglutination assay of formalinized, tanned erythrocytes sensitized with *T. pallidum* antigen that can be used to test preabsorbed patient sera. The TPPA test, which uses gelatin particles sensitized with *T. pallidum* antigens, has supplanted the MHA-TP. Since the 1980s, numerous enzyme-linked immunoassays (EIAs) and chemiluminescent immunoassays (CIAs) have been developed for syphilis diagnosis, the majority of which use recombinant *T. pallidum* antigens to detect IgM or IgG, usually both. In head-to-head comparisons, the TPPA compares favorably with other treponemal tests currently on the market, including EIAs and CIAs using recombinant antigens. Unlike the immunoassays, the TPPA assay is not automatable and, because it is read manually rather than spectrophotometrically, tends to be subjective and cannot be readily digitized. Detailed information on the newer formats used for treponemal tests can be found in the work of Sena and colleagues.[362]

Many laboratories handling large patient volumes have switched to a "reverse algorithm" in which screening is performed with an automatable treponemal EIA or CIA followed by a nontreponemal test when the former is reactive.[5,344,346,361,363–366] Head-to-head comparison of the traditional and reverse sequence indicates that the latter is more sensitive, particularly for patients with primary and latent disease. However, individuals with so-called 'discordant' serologic test results (i.e., immunoassay reactive, nontreponemal test nonreactive) also may have treated infection. Taking a careful clinical history is important because previously treated asymptomatic individuals will require no further management. For persons with discordant serologic findings and without a history of treatment, a TPPA test or comparably sensitive immunoassay should be performed.[361,364] If a second treponemal test result is positive, persons with a history of previous treatment will require no further management unless sexual history suggests likelihood of reexposure. In this instance, a repeat nontreponemal test in 2–4 weeks is recommended to evaluate for early infection, and those without a history of treatment for syphilis should be offered it. If the second treponemal test is nonreactive, the clinician may decide that no further evaluation or treatment is indicated or that treatment is indicated for individuals at high risk. Practitioners should be aware of data, based on use of the TPPA assay as the confirmatory treponemal test, indicating that discordant test results in individuals at low risk of syphilis have a high likelihood of being immunoassay false positives. This situation is commonly encountered during prenatal screening. Mmeje and colleagues[367] found that 80% of women in a low-risk clinical setting with discordant prenatal serologic findings had nonreactive TPPA tests and that a majority of these individuals had nonreactive repeat CIA tests. These data support CIA retesting of low-risk patients with discordant serologic findings and negative TPPA test results, reserving treatment for those with a reactive follow-up test.[261] Given these caveats, the CDC and the US Preventive Services Task Force have been reluctant to endorse the reverse algorithm and have opted instead to provide guidance for clinicians and facilities that use it.[261,344]

Syphilis Serologic Tests in HIV Infection

There is now consensus that serologic tests for syphilis perform well in persons coinfected with HIV.[261] However, some caveats should be borne in mind. First, HIV-infected individuals may have a higher incidence of false-positive nontreponemal test results.[368,369] Second, nontreponemal test titers in HIV-infected individuals tend to be higher at presentation (including prozone phenomena) and remain persistently elevated after treatment.[112,151,370,371] These serologic findings may reflect the B-cell dysregulation associated with HIV infection. Third, there are the well-documented, although extremely rare, cases of HIV-infected patients with secondary syphilis and nonreactive syphilis serologies.[348,372] If serologic test results are negative in an HIV-infected individual with suspected secondary syphilis, skin biopsy for histopathologic examination and direct detection of *T. pallidum* should be performed.

Rapid Point-of-Care Tests

Resource-poor countries often cannot meet the personnel and laboratory demands for reliable syphilis serology testing. In addition, when syphilis serologic assays are performed off site, delays in diagnosis can result in missed opportunities for treatment and intervention, a grave concern in regions with high rates of CS. RPOC tests address these problems.[5,373,374] WHO established the ASSURED criteria to define the characteristics of an ideal RPOC test: *a*ffordable, *s*ensitive, *s*pecific, *u*ser-friendly, *r*apid and robust, *e*quipment free, and *d*eliverable to those who need them.[375] Most of the commercially available RPOC tests for syphilis are immunochromatographic strip assays that use recombinant *T. pallidum* antigens to detect IgM and IgG antibodies in whole blood from fingerstick specimens and can be performed in approximately 20 minutes. Numerous rapid tests have been evaluated in diverse clinical and community settings and shown to fulfill the ASSURED criteria and reduce disease burden[5,376] An RPOC test that simultaneously detects nontreponemal and treponemal antibodies has shown excellent sensitivity and specificity with archived samples and performed well on site in China.[377,378] Rapid, dual HIV and syphilis tests are now available and have been shown to be more cost-effective than single tests and to prevent more adverse pregnancy outcomes.[379]

Tests for Neurosyphilis

Contributing to the complexity of diagnosing neurosyphilis is deciding which patients should undergo lumbar puncture. As discussed earlier, CSF abnormalities are common in patients with early syphilis without neurologic symptoms, and a substantial proportion of patients with early syphilis have treponemes in their CNS, yet (see later) randomized trials have failed to demonstrate a benefit of enhanced therapy in patients either without or with HIV coinfection.[151,380] Consequently, the CDC does not recommend routine lumbar puncture in patients with early syphilis, regardless of HIV status.[261] Several studies have suggested that serum RPR titers greater than or equal to 32 or CD4 counts below 350 in HIV-infected patients indicate a higher risk of asymptomatic neurosyphilis[270,381,382]; the long-term benefits of lumbar puncture and more intensive therapy in this patient subset remain unproven.[9] Because of the low yield, the CDC no longer recommends routine lumbar puncture in patients with late syphilis to identify asymptomatic neurosyphilis.[261] Of course, patients with reactive serologies and neurologic symptoms or findings should always undergo lumbar puncture regardless of disease stage.[261]

Unfortunately, no gold standard exists for the laboratory diagnosis of neurosyphilis.[38] Although a reactive CSF VDRL test constitutes definitive evidence of neurosyphilis, a presumptive diagnosis can be based solely on the presence of an elevated CSF protein or pleocytosis, or both. Given the limited and conflicting data on performance,[383,384] the CSF RPR test is currently not recommended in place of the CSF VDRL test. The CSF FTA-ABS assay has high sensitivity but low specificity because reactivity may be due to the passive transfer of IgG antitreponemal antibodies across the blood-brain barrier rather than intrathecal production of antibodies.[385] Because of the lack of specificity, the CDC does not recommend performing treponemal tests on CSF, although it is worth noting that a nonreactive treponemal test rules out the diagnosis. Methods for discriminating between intrathecal production and passive diffusion of treponemal antibodies have been devised but are cumbersome, are difficult to interpret, and cannot be used to assess therapeutic response.[386,387] In a study of 40 patients diagnosed with symptomatic neurosyphilis (30 patients with early syphilis, 10 with late), only 17 had PCR-positive spinal fluids.[338] As is often the case in studies of neurosyphilis, the criteria used by these investigators to diagnose neurosyphilis have been called into question.[339] In any event, a positive result of PCR assay on CSF in a symptomatic patient provides strong support for a diagnosis of neurosyphilis, whereas a negative PCR does not rule it out.

THERAPY

Principles of Syphilotherapy

There is no evidence that the susceptibility of *T. pallidum* to penicillin has diminished over the 70 years it has been used. Parenterally administered aqueous penicillin G is the preferred therapy for all forms and stages of syphilis. However, as discussed later, the preparation used, dosage, and duration of therapy vary with the stage of disease and manifestations to be treated. Table 237.2 presents guidelines for the treatment of syphilis based on current CDC recommendations.[261] Clement and coworkers[388] have published an extensive literature review evaluating the quality of evidence for the CDC guidelines.

TABLE 237.2 Recommended Therapy for Syphilis (Based on 2015 Centers for Disease Control and Prevention Sexually Transmitted Disease Treatment Guidelines[1])

STAGE	PATIENTS NOT ALLERGIC TO PENICILLIN	PATIENTS ALLERGIC TO PENICILLIN[a]	ALTERNATIVE REGIMENS[a]
Early syphilis[b] (primary, secondary, early latent), adults	BPG 2.4 MU IM in a single dose	Doxycycline, 100 mg PO bid for 14 days *or* Tetracycline hydrochloride, 500 mg PO qid for 14 days *or* Desensitization to penicillin in pregnant women, treat with BPG	Ceftriaxone, 1–2 g IM or IV daily for 10–14 days *or* Azithromycin[c] 2 g PO
Late latent, syphilis of unknown duration, adults, or nonneurologic tertiary syphilis	BPG 2.4 MU IM at weekly intervals × 3 (7.2 MU total)	Doxycycline, 100 mg PO bid for 28 days *or* Tetracycline hydrochloride, 500 mg PO qid for 28 days	Ceftriaxone (dosage and duration unknown)
Neurosyphilis, otic syphilis, and ocular syphilis, adults[d]	Aqueous crystalline penicillin G, 18–24 MU per day given as 3–4 MU IV q4h or continuous infusion for 10–14 days *or* Procaine penicillin G, 2.4 MU IM, plus probenecid, 0.5 g PO qid, daily for 10–14 days	Ceftriaxone, 2 g IM or IV daily for 14 days	
Pregnancy[c]	Stage-appropriate treatment with penicillin as for nonpregnant patients; penicillin-allergic patients should be desensitized and treated with penicillin		
Congenital syphilis	Aqueous crystalline penicillin G, 100,000–150,000 units/kg/day, administered as 50,000 units/kg/day IV q12h during the first 7 days of life and q8h thereafter for a total of 10 days *or* Procaine penicillin G, 50,000 units/kg IM daily for minimum of 10 days	Not applicable	
Older infants (>30 days old) and children with a diagnosis of congenital syphilis	Aqueous crystalline penicillin G 200,000–300,000 units/kg/day IV, administered as 50,000 units/kg q4–6h for 10 days In selected cases, treatment can be given with benzathine penicillin G,[b] 50,000 units/kg/day, up to 2.4 MU IM in a single dose or procaine penicillin G 50,000 units/kg IM daily for minimum of 10 days		

[a]Therapeutic regimens other than penicillin have not been well studied, especially in patients with syphilis duration longer than 1 year; therefore, careful follow-up is mandatory. Ceftriaxone should be used cautiously in patients with a well-documented history of penicillin allergy. There are insufficient data to recommend ceftriaxone for penicillin-allergic pregnant patients.
[b]HIV-infected and HIV-uninfected patients. Available data indicate that additional doses of BPG, amoxicillin, or other antibiotics in early syphilis do not enhance efficacy, regardless of HIV status.
[c]Azithromycin as a single 2-g dose PO appears to be as effective as 2.4 MU IM BPG for early syphilis. However, mutations in *Treponema pallidum* that confer resistance to azithromycin are geographically widespread. For this reason, azithromycin should be used with caution only when treatment with BPG or doxycycline is not feasible. *Azithromycin should not be used in pregnant women or in men who have sex with men.*
[d]In light of the extensive track record with the use of high-dose intravenous penicillin for neurosyphilis, penicillin-allergic patients probably should be desensitized. Data for the use of ceftriaxone as alternative therapy for neurosyphilis are limited. If concern exists about the safety of ceftriaxone, skin testing and, if necessary, desensitization should be performed.
BPG, Benzathine penicillin G; *HIV,* human immunodeficiency virus; *MU,* million units.

Because *T. pallidum* cannot be cultivated, it has never been possible to use conventional in vitro methods to assess its susceptibility to antimicrobials or rationally design therapeutic regimens. Some success in defining the spirochete's susceptibility to antimicrobials other than penicillin has been achieved in vitro by inhibiting incorporation of radiolabeled amino acids into protein[40] or replication during coculture with mammalian cells[40,389] and by assessing response to treatment in the rabbit model.[390–393] Beginning with the initial observations of the effectiveness of penicillin in 1943,[42] the principles underlying successful treatment of syphilis with this mainstay antimicrobial have had to be extrapolated from pharmacokinetic data, animal experimentation, and clinical trials.[394,395] Early investigations established that *T. pallidum* is exquisitely sensitive to penicillin but that the determinants of cure differed from those observed with other penicillin-sensitive organisms (e.g., streptococci). To be effective, a minimal serum concentration of 0.02 μg/mL had to be maintained for at least 7 to 10 days. Maintaining adequate serum levels of penicillin without prolonged interruptions was found to be more important than the total dose administered. As elegantly stated by Eagle and colleagues,[396] "*Treponema pallidum* is … one of the most sensitive, as well as one of the most resistant of all bacteria to the action of penicillin. It is one of the most sensitive in terms of the smallest concentration, which is bactericidal; it is one of the most resistant in terms of the time for which it must be exposed to that concentration in order to be killed." This unusual dose-time relationship can be attributed directly to the bacterium's extraordinarily slow rate of replication in mammalian tissues, rather than a lack of targets for β-lactams.[39,82,397] Once the principles for successful therapy had been deduced, the rationale for the use of depot formulations of penicillin G for treatment of individual patients became evident, as did the possibility of using them as the basis for public health control programs.[394,398] Of the various long-acting

preparations developed in the early penicillin era, BPG was found to provide the most sustained levels of antibiotic. A single intramuscular injection of 2.4 million units of BPG yields treponemicidal concentrations lasting as long as 3 to 4 weeks.[394]

Incubating or Early Syphilis

Presumptive or epidemiologic treatment should be given to anyone who has been exposed to a patient with primary, secondary, or early latent syphilis within the preceding 90 days, even if he or she is seronegative and has no evidence of infection at examination. Persons without physical findings who were exposed more than 90 days before the diagnosis of infectious syphilis in a sex partner should be treated presumptively if serologic test results are not immediately available and follow-up is uncertain.[261] If serologic test results in such individuals are negative, no treatment is necessary. Eagle and colleagues[399] showed in the rabbit model that much smaller doses of penicillin are necessary to abort incubating syphilis than to eradicate established infection. However, to ensure adequate safety margins, prophylactic treatment schedules are the same as those used for patients with clinically evident early infection, 2.4 million units of BPG. This regimen has been reported to be 100% effective for preventing infection in contacts of persons known to have early syphilis.[400,401]

Therapy for patients with early syphilis needs to accomplish two objectives: (1) resolution of infectious mucocutaneous lesions, critically important from a public health standpoint, and (2) eradication of spirochetes to resolve manifestations and prevent sequelae, including tertiary complications. Initial studies of penicillin using miniscule doses by today's standards made clear the drug's remarkable effectiveness in accomplishing the first objective. Although they do not meet contemporary standards for clinical trials, studies of single-dose BPG for early syphilis conducted in the 1950s and 1960s were well enough conducted and involved large enough numbers of patients for meaningful conclusions to be drawn regarding the second.[9,394,401,402] On the whole, investigators reported excellent clinical and serologic responses with posttreatment follow-up periods up to several years. Treatment failures were uncommon (collectively, approximately 5%) and usually were due to lack of reversion of nontreponemal test titers, a serologic phenomenon no longer considered unequivocally to be indicative of failure to eradicate treponemes. Rare, overt (i.e., clinical) failures were usually judged to be reinfections. In a large comparison of multiple therapeutic regimens, Schroeter and colleagues[401] found a single intramuscular injection of 2.4 million units of BPG to be equivalent to 4.8 million units of procaine penicillin G given daily for 8 days or in three divided doses over 6 days. As discussed at length earlier, syphilologists have long been keenly aware of the potential for the CNS to serve as a reservoir for relapse. Particularly important, therefore, were follow-up studies showing that patients with early syphilis who were given single-dose BPG had normal CSF values 2 years after treatment.[403–405] On the basis of these results, clinicians dispensed with routine examination of spinal fluid 2 years after treatment, an important milestone in syphilis management.

Even before the advent of HIV, some authorities had begun to question the use of single-dose BPG therapy for early syphilis on the basis of isolated case reports of neurorelapse of primary or secondary syphilis in patients treated with BPG, along with evidence that 2.4 million units of intramuscular BPG fails to achieve detectable levels of penicillin in CSF.[400,406,407] However, as noted earlier, serious reservations emerged in the late 1980s as a result of numerous case reports and small series suggesting that the incidence of BPG treatment failures in HIV-infected patients exceeded the norms from the pre-AIDS era.[215–217] To address this urgent therapeutic issue, the CDC conducted a multicenter, randomized controlled trial comparing 2.4 million units of intramuscular BPG with enhanced therapy (2.4 million units of intramuscular BPG plus oral amoxicillin-probenecid) in a cohort of 541 neurologically asymptomatic patients with early syphilis (101 and 440 persons, respectively, with and without HIV infection). The findings were reassuring. Not only were the presenting clinical manifestations similar in the HIV-infected and HIV-uninfected patients, but serious CNS or eye complications following treatment were not observed in patients randomized to either regimen. Important to note, although serologic responses were poorer overall among the HIV-infected patients, there was no evidence that enhanced therapy improved clinical or serologic outcomes in patients with early syphilis either with or without HIV infection.[151] Critics of the study have maintained that it was underpowered and did not include a long enough follow-up period and that the comparison should have included high-dose intravenous penicillin G, the standard regimen for neurosyphilis.[408] However, a systematic review of the literature published in 2011 found that no data from well-controlled trials supporting the viewpoint that HIV-infected patients with early syphilis should routinely receive more intensive therapy.[409] Indeed, the only other large-scale treatment trial deemed to be of good quality,[408] a comparison of azithromycin and BPG conducted in Tanzania,[410] also failed to observe a difference in treatment response among HIV-infected and HIV-uninfected patients given 2.4 million units of BPG for early syphilis during 9 months of follow-up. Since then, two studies comparing a single dose versus three doses of BPG also failed to establish superiority of the multidose regimen in patients with syphilis who were coinfected with HIV.[380,411] Thus, there is now more than ample evidence,[412] backed by extensive clinical experience, for the CDC's recommendation of a single intramuscular injection of 2.4 million units of BPG as primary treatment of early syphilis in all individuals without neurologic or ophthalmologic findings regardless of HIV status.[261] At the same time, the authors acknowledge that there are many practitioners who believe that treatment of uncomplicated early syphilis in patients with HIV coinfection requires, at a minimum, three weekly injections of 2.4 million units of BPG.[8]

Latency

Inasmuch as patients with latent syphilis by definition lack signs and symptoms, the primary objective of treatment during latency is to prevent secondary relapses in early latency and late sequelae in all. In women of childbearing years, prevention of congenital infection is also an important therapeutic goal.[60] In early latency, spirochetes are actively replicating, although outbreaks are muted or contained, or both, compared with secondary disease, presumably because of an increasingly effective immune response. The rationale for using single-dose BPG to treat early latent syphilis, therefore, is the same as for primary and secondary infection. Nevertheless, assessing therapeutic efficacy in early latency is difficult because there is no way to determine which patients are at risk for secondary relapses, and years of follow-up would be required to establish whether treatment has prevented late complications. Despite limited published data,[402,413] decades of clinical experience suggest that a single dose of BPG is extremely effective at preventing secondary relapses.

Assessing therapeutic efficacy in late latency is even more problematic because the decline in nontreponemal tests can be extremely slow and, as has long been recognized, does not occur in a substantial percentage of patients.[208,394,414] Although no microbiologic data exist, it is assumed that spirochetes in late syphilis are replicating slowly (if at all) and therefore require more prolonged contact with penicillin than in early disease[395]—hence the recommendation for three weekly injections of 2.4 million units of BPG for treatment of all nonneurologic forms of late syphilis. Appropriately, Clement and coworkers[388] evaluated the evidence for this regimen, as well as those described for all forms of tertiary syphilis, as based primarily on expert consensus. According to CDC guidelines, for the purposes of partner notification and presumptive treatment of exposed sex partners, patients with syphilis of unknown duration who have high nontreponemal serologic test titers (i.e., >1:32) should be assumed to have early syphilis.[261] However, the CDC cautions against using serologic titers to differentiate early latent from late latent syphilis. When the duration of infection cannot be ascertained, caregivers should opt for the late latent treatment regimen.

Tertiary Syphilis

The CDC recommends that all persons with tertiary syphilis should be tested for HIV and receive a CSF examination before the initiation of therapy; individuals with CSF abnormalities should be treated with a neurosyphilis regimen (see later).[261]

Cardiovascular and Gummatous Syphilis

As with late latent syphilis, spirochetes in tertiary lesions are presumed to be replicating slowly.[395] Consequently, the rationale for using 7.2

million units of intramuscular BPG in three divided doses in non-neurologic forms of tertiary syphilis is the same. As one would anticipate, there is a paucity of data supporting the efficacy of this regimen for tertiary disease. An additional problem in assessing responses in late syphilis is that only inflammation, not destruction and scarring that already have occurred, will resolve with therapy. The primary treatment objective for tertiary syphilis, therefore, is to eradicate spirochetes so as to prevent further tissue damage. The literature contains a number of case reports documenting the penicillin responsiveness of gumma, although in many of these, penicillin formulations other than BPG were used. Consistent with the authors' experience, two reports[415,416] show prompt resolution of gumma in response to single-dose intramuscular BPG. Although penicillin therapy for cardiovascular syphilis has been accepted since the late 1940s and there are data from as far back as the 1950s to support its efficacy in arresting disease progression and symptomatic deterioration,[194,298] there are no trials supporting the use of BPG. There is also global disparity in treatment guidelines. Although US, European, and WHO guidelines recommend 7.2 million units of intramuscular BPG, Australian guidelines call for either intravenous penicillin for 15 days or daily intramuscular procaine penicillin for 20 days.[300] The CDC acknowledges that some clinicians elect to treat cardiovascular syphilis with high-dose intravenous penicillin.[261]

Neurosyphilis

Although recommendations for treatment of neurosyphilis once included 7.2 million units of intramuscular BPG and the large majority of patients (at least 80%) do, in fact, respond to this regimen, accumulated evidence for treatment failures, as well as the inability of BPG to achieve treponemicidal levels in the CSF, led the CDC to stop recommending it.[400] Aqueous penicillin G, 18 to 24 million units daily, administered as 3 to 4 million units intravenously every 4 hours or as a continuous infusion for 10 to 14 days, is the currently recommended regimen; failures with this regimen are virtually nonexistent. If compliance can be ensured, procaine penicillin 2.4 million units intramuscularly daily with probenecid 500 mg four times a day for 10 to 14 days can be considered as an alternative. Although not mandatory, CDC guidelines allow for follow-up of intravenous therapy with three divided doses of BPG to ensure prolonged penicillinemia for comparable treatment of nonneurologic tertiary syphilis. Otosyphilis and ocular syphilis, both frequently associated with neurosyphilis, should be treated as such regardless of the results of lumbar puncture.

Penicillin Allergy

Penicillin allergy presents one of the most serious challenges in the routine management of syphilis because it deprives the practitioner of the most powerful weapon for combating the disease. A careful history should always be obtained, therefore, before one decides that penicillin is contraindicated, and if necessary and possible, consultation with a subspecialist should be sought. Penicillin-allergic patients whose compliance with therapy or follow-up is questionable should be desensitized and treated with BPG. Doxycycline and tetracycline have long-established track records as alternatives to penicillin with a reasonable amount of published data, some recent and including HIV-infected persons, indicating efficacy comparable to that of BPG.[400,401,417–420] Clement and coworkers[388] gave alternative tetracycline and doxycycline regimens a rating indicative of support from randomized clinical trials, although lower in overall quality than the evidence for BPG. Doxycycline is preferred both because of its twice-daily administration, ensuring better compliance, and its lipophilicity, enabling it to cross the blood-brain barrier. Pharmacokinetics data and limited clinical studies suggest that ceftriaxone is effective for treating both early and late syphilis, including neurosyphilis,[419,421–423] although the optimal dose and duration of therapy for either have not been determined. Clinicians opting for ceftriaxone must be mindful of a risk, albeit probably small, of cross-reactivity in persons with anaphylactic-type hypersensitivity to penicillin.[424,425] Enthusiasm for azithromycin, which can be as efficacious as BPG when administered orally as a single 2-g dose,[410] has been tempered by the discovery of geographically widespread (including the United States) macrolide-resistant strains of *T. pallidum* associated with A2058G or A2059G mutations in the bacterium's 23S rRNA genes.[426–431] In light of this disappointing development, azithromycin should be used cautiously, with careful follow-up, and not at all in pregnant patients or in MSM.[261]

Gestational Syphilis

Syphilis in pregnancy poses great risk to both the mother and her unborn child.[5,60,432–434] Thus, treatment objectives are twofold: resolution of infection in the mother and prevention of in utero infection of the fetus. Every case of CS represents an opportunity missed to make the diagnosis antenatally.[435] All women should be screened serologically during the early stages of pregnancy and, for those at high risk, at delivery as well. Infected pregnant patients should receive penicillin at dosage schedules appropriate for the stage of syphilis as recommended for nonpregnant patients. Prospective studies have demonstrated the efficacy of BPG to prevent CS,[436] although pharmacokinetics data and case reports do raise some doubt as to whether one intramuscular injection of BPG is sufficient once the fetus is already infected.[437] Some clinicians administer a second injection of BPG, although there are no controlled data to support the practice. Consultation with an obstetrician or maternal-fetal subspecialist is strongly recommended in the event of sonographic evidence of syphilitic infection in the fetus because complications (a form of Jarisch-Herxheimer [JH] reaction, see later), such as preterm labor and fetal distress, can be precipitated by treatment. There is no satisfactory alternative to penicillin for the treatment of syphilis during pregnancy. If the patient has a well-documented or skin test–proven penicillin allergy, desensitization is strongly recommended, given penicillin's track record in preventing CS, the inability to use tetracycline derivatives in this setting because of their adverse effects on the fetus, the well-recognized risk for treatment failure with macrolides, and the paucity of data for ceftriaxone.[261]

JARISCH-HERXHEIMER REACTION

The JH reaction is a systemic reaction resembling bacterial sepsis that usually begins 6 to 8 hours after the initial treatment of syphilis with effective antibiotics, especially penicillin.[298,438,439] It is particularly common when secondary syphilis is treated but can occur at any stage. On the basis of work done with relapsing fever, another spirochetal infection in which severe JH reactions commonly occur,[440,441] this phenomenon is likely a cytokine storm caused by the abrupt release of lipoproteins and other PAMPs from lysed treponemes. The JH reaction in syphilis consists of the abrupt onset of fever, chills, myalgias, headache, tachycardia, hyperventilation, vasodilation with flushing, and mild hypotension. It lasts from 12 to 24 hours and occurs with varying degrees of severity. Localized forms of the JH reaction also may occur in patients with ocular syphilis, neurosyphilis, and cardiovascular syphilis.[298,442,443] In the case of cardiovascular syphilis, a JH reaction can result in sudden death due to aneurysmal rupture or coronary occlusion. Patients should be warned of the possibility of the reaction before treatment; in cardiovascular syphilis, an argument can be made for prophylactic treatment. The JH reaction can be prevented or treated with an antiinflammatory agent such as aspirin every 4 hours for a period of 24 to 48 hours. Prednisone can also abort the reaction, though no data exist as to when it should be used.[439]

Response to Therapy

Once disease manifestations have resolved after treatment, typically within weeks, decline in nontreponemal test titers is the only means of monitoring therapeutic response over prolonged periods. At one time, it was believed that nontreponemal test results decline rapidly (i.e., within 3 months) and become nonreactive ("serorevert") in most appropriately treated patients with primary and secondary syphilis.[357,394,444–446] Romanowski and coworkers[447] deserve much credit for showing definitively that the nontreponemal antibody response to therapy of early syphilis is often slow and incomplete. Their report, along with the serologic data presented in the randomized treatment trial published by Rolfs and colleagues[151] several years later, led to less stringent criteria for an adequate serologic response. This is currently defined as a fourfold decrease in nontreponemal titer no later than 1 year after therapy for early syphilis and 2 years for late latent syphilis.[261] Even so, serologic titers do not change substantially (i.e., remain positive at the initial titer or decline only one dilution) in 15% to 20% of patients with early

syphilis and in an even higher, although not well defined, proportion of patients treated for latent syphilis.[211] Serologic cure is primarily associated with younger age, higher baseline nontreponemal titers, and earlier syphilis stage.[208,448] A major question is whether patients who do not demonstrate serologic response benefit from additional treatment. Sena and coworkers[449] found that only 27% of 82 HIV-negative "serofast" patients exhibited serologic response after retreatment during an additional 6 months of follow-up. CSF examination to exclude neurosyphilis should be considered for patients who do not achieve a fourfold decline during the appropriate interval after therapy,[261] especially those in whom close follow-up and serologic monitoring cannot be ensured. Although some experts doubt that many serofast patients will turn out to have neurosyphilis (the authors' experience as well),[450] a recent report from China found CSF abnormalities considered indicative of asymptomatic neurosyphilis in 34% of serofast patients.[358] Many patients will have persistent low-level titers despite a fourfold or greater decline after treatment. Evaluation of serologic data from a multicenter treatment trial treatment[359] found, quite remarkably, that only 17% of patients with an appropriate decline in nontreponemal titer at 6 months achieved seroreversion 12 months after therapy, with the seroreversion rate highest for primary infection.[360] There is no evidence that such individuals benefit from lumbar puncture or additional therapy,[261] nor are there anecdotal reports suggesting frequent neurologic relapses in this group.[450] More than six decades after the demonstration of the efficacy of penicillin therapy, the biologic significance of persistent, low nontreponemal titers is unknown. It has been proposed that this phenomenon represents a failure of the immune response to halt production of antilipoidal antibodies as a result of B-cell tolerance[208] rather than the failure of therapy to eradicate treponemes hiding within a CNS niche, as was once believed.[208,451]

Pleocytosis, long regarded as the hallmark of an active inflammatory process,[1,149] should resolve within weeks to months after appropriate therapy for neurosyphilis. When positive, CSF VDRL findings should be monitored quantitatively but may not normalize. An elevated protein may, likewise, persist indefinitely.

Congenital Syphilis

Although early diagnosis and treatment of women with gestational syphilis can prevent CS, persistently high rates of venereal syphilis in the general population combined with a lack of access to prenatal care in many regions of the world have seriously hampered efforts to control the disease.[128,452–455] Globally, the magnitude of CS rivals that of HIV infection in neonates.[452] WHO estimates have indicated that 1.0 million pregnant women have active syphilis and that up to one-third of such pregnancies result in early fetal death (22–28 weeks' gestation), stillbirth (>28 weeks), neonatal death, preterm or low-birth-weight infants, and newborns with congenital infection.[5,128] In the United States, the incidence of CS increased by 38% (8.4–11.6 cases per 100,000) from 2012–2014, reversing a decade long decline in its overall incidence. In 2015, the CDC confirmed that the incidence of CS has continued to increase, reaching a two-decade high at 12 cases per 100,000 live births.[456] The rise in the incidence of CS has been most pronounced among whites, with a 69% increase. However, the overall prevalence remained highest in African Americans (35.2 cases per 100,000), Hispanics (15.5 cases per 100,000), and Native Americans (10.3 cases per 100,000). A total of 19 states, including California, Florida, Louisiana, Michigan, and New York, have reported dramatic increases in new cases of CS. The most common risk factor for developing CS in the United States continues to be lack of prenatal care (around 21% of affected pregnancies) or inadequate care.[456]

Although the problem of CS in sub-Saharan Africa and, more recently, China and the United States has received a good deal of attention, CS in Latin America and the Caribbean has been largely unnoticed, in spite of what appears to be a public health problem of alarming dimensions. In 2012, Colombia's National Institute of Health Surveillance System (Sistema Nacional de Vigilancia en Salud Pública [SIVIGILA]) reported rates of GS/CS many times greater than those in the United States and Western Europe, and in a retrospective study in a predominantly black, indigent population of Colombia's Pacific coastal region the reported rate rivaled rates in sub-Saharan Africa.[457] A similar problem exists in Haiti and other Caribbean nations.[458] The picture of both venereal syphilis in the general population and pregnant women and, by extension, CS is not one of a vanishing disease. Rather, this potentially deadly GS/CS dyad requires continued monitoring, early and effective screening, and treatment strategies to help reverse this trend of increasing disease burden.

The vast majority of CS cases are believed to arise in utero.[459] The fetus can be infected during any stage of syphilitic infection in the mother, but it is most likely to occur during the spirochetemia associated with untreated primary and secondary syphilis,[460–462] and less frequently during early latency (60%–90% vs. 40% in early latent and <10% in late latent syphilis).[460,461] Findings from a PCR-based study underscored the pathogenic and prognostic importance of spirochetemia during pregnancy; the investigators found that miscarriage, stillbirth, and neonatal death, respectively, occurred in 81%, 73%, and 83% of syphilitic mothers who were PCR positive in blood.[463] Although fetal syphilis can occur during the first trimester,[464] the risk to the offspring increases significantly during the second and third trimesters. Fetal infection is characterized by early hepatic dysfunction and placental involvement, followed by spread of the spirochete into the amniotic fluid and the appearance of severe hematologic anomalies.[462] In mothers who acquire syphilis during pregnancy, compromise of the fetus can be more severe and, in some instances, lead to multiorgan compromise.[462] Not surprisingly, up to 7% of fetuses with CS are stillborn.[465–467] Death in the newborn period is usually the result of liver failure, severe pneumonia, hypopituitarism, or pulmonary hemorrhage.

No other form of syphilis better exemplifies the remarkable invasiveness of *T. pallidum* than CS. The presence of spirochetes in the placenta and umbilical cord supports transplacental invasion of maternal bloodborne spirochetes as the major route of transmission to the infant.[468] Macroscopically, the infected placenta is generally large, thick, and pale, weighing up to one-third as much as the fetus.[469,470] Histologically it reveals focal proliferative villitis with necrosis and focal infiltration of maternal lymphocytes and plasma cells, endothelial and adventitial proliferation of villous vessels leading to small blood vessel obliteration, large and immature villi, extensive stromal hyperplasia, and, in some cases, multiple small gumma indicative of miliary spread of the bacterium.[469–472] Spirochetes can often be detected in the placenta with silver stain, immunohistochemistry, or PCR.[468,473] Intrauterine growth retardation, a commonly associated finding in the infected newborn, is thought to reflect inadequate nutrition of the fetus as a result of syphilitic placentitis.[60,474–476] Necrotizing funisitis, an inflammatory process involving the matrix of the umbilical cord, characterized by perivascular inflammation and obliterative endarteritis, is, for all practical purposes, pathognomonic of CS.[477] Indeed, CS should be suspected clinically whenever the umbilical cord is swollen and discolored red, white, and blue, resembling a "barber's pole."[477,478]

The clinical, laboratory, and radiographic features present in the newborn period or within the first 2 years of life, or both, comprise early CS, whereas those that manifest after 2 years of age comprise late CS (Table 237.3). Depending on the degree of systemic involvement, the clinical presentation of early CS can range from life-threatening disease (i.e., fetal hydrops) to an otherwise normal-appearing newborn with only minimal laboratory and radiographic anomalies.[60] The earliest sign of CS is usually rhinitis (snuffles), which is soon followed by a diffuse, maculopapular, desquamative rash with extensive sloughing of the epithelium, particularly on the palms, on the soles, and about the mouth and anus (Fig. 237.12). In contrast to acquired syphilis in the adult, a vesicular rash and bullae, also known as *pemphigus syphiliticus,* may develop. These lesions are teeming with spirochetes and histologically have the characteristic obliterative endarteritis and perivascular mononuclear cuffing on microscopic examination that are found in lesions of acquired syphilis. Newborn hair may be brittle and sparse; infantile alopecia, especially affecting the eyebrows, is considered suggestive of CS. At the nares, lips, and anus, the initial lesions may be indistinguishable from the mucous patches of secondary syphilis but then become deeply fissured and hemorrhagic, leading to Parrot radial scars of late CS, which are also known as *rhagades.*[479]

Multiple organs can be affected in newborns with early CS.[480,481] Liver involvement is common; indeed, more than 30% of infected

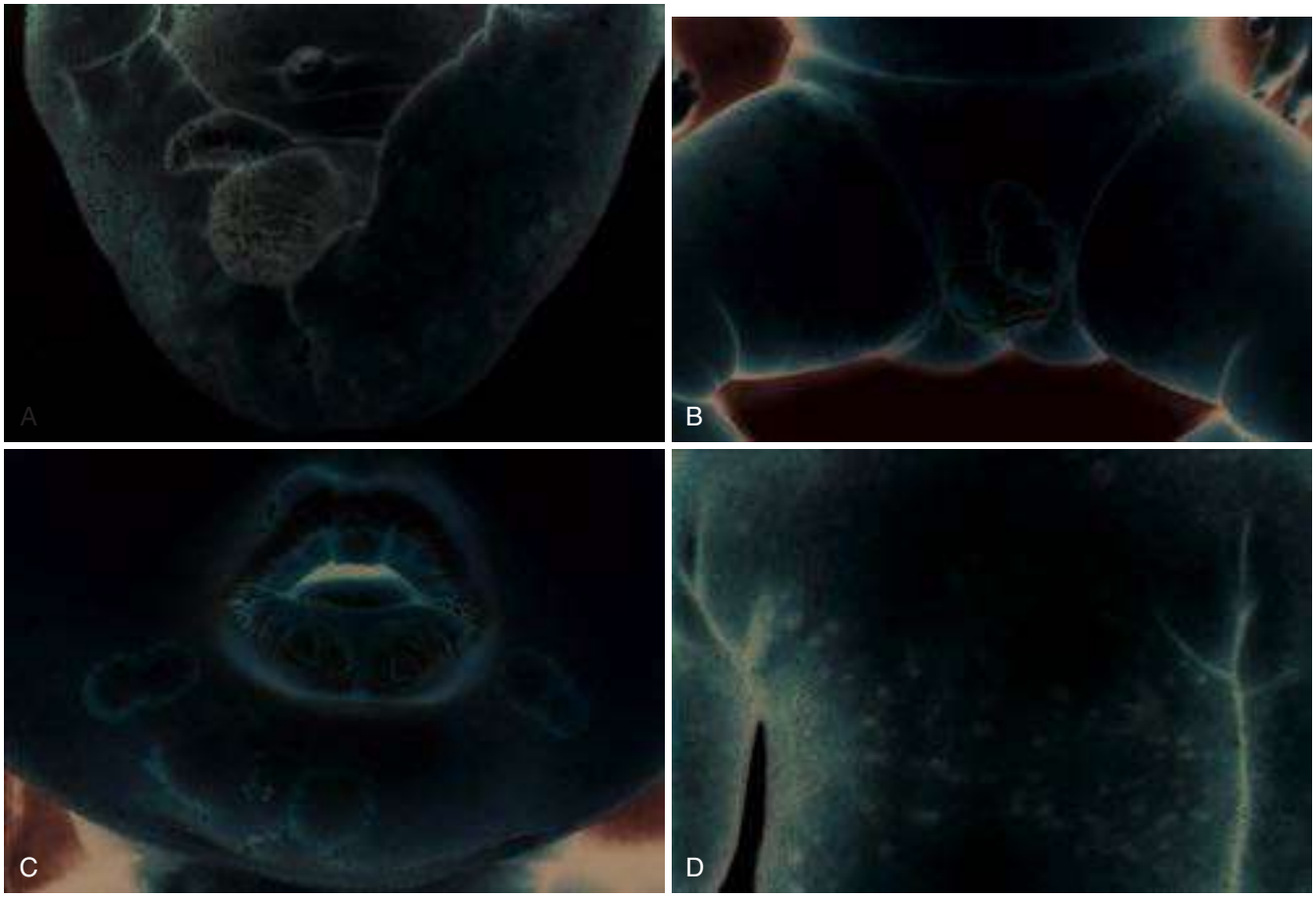

FIG. 237.12 Congenital syphilis. (A) Scaling annular rash of congenital syphilis. (B) Ulceration of perineal region in newborn with congenital syphilis. (C) Face of the same infant reveals perioral papules, ulcerous lesions, and labial fissures. (D) Dusky macular rash extending over the anterior trunk of a newborn infant with congenital syphilis. *(Courtesy Dr. Leon Chameides, Hartford, CT.)*

TABLE 237.3 Clinical Signs of Congenital Syphilis

Early Congenital Syphilis
Osteochondritis, periostitis
Snuffles, hemorrhagic rhinitis
Bullous lesions, palmar/plantar exanthem
Hemolytic anemia
Hepatosplenomegaly
Jaundice
Central nervous system involvement: elevated cell count or elevated protein in cerebrospinal fluid
Generalized lymphadenopathy
Pneumonitis
Nephrotic syndrome
Nonimmune hydrops fetalis
Intrauterine growth retardation
Late Congenital Syphilis
Frontal bosses
Short maxillas
Saddle nose
Protruding mandible
Interstitial keratitis
Eighth nerve deafness
High palatal arch
Hutchinson incisors
Mulberry molars
Sternoclavicular thickening (Higoumenaki sign)
Clutton joints (bilateral painless swelling of knees)
Saber shins
Flaring scapulas

Modified from Kampmeier RH. Essentials of Syphilology. *3rd ed. Philadelphia: JB Lippincott; 1943; Stokes JH, Beerman H, Ingraham NR.* Modern Clinical Syphilogy: Diagnosis, Treatment: Case Study. *3rd ed. Philadelphia: Saunders; 1945; and Patton ME, Su JR, Nelson R, et al; Centers for Disease Control and Prevention. Primary and secondary syphilis—United States, 2005–2013.* MMWR Morb Mortal Wkly Rep. *2014;63:402–406.*

neonates will manifest both direct and indirect hyperbilirubinemia, as well as elevated transaminases. The major hematologic features of CS are anemia, leukocytosis or leukopenia, in some cases monocytosis, and thrombocytopenia.[462,482] Renal involvement with an immune complex glomerulonephritis, evidenced by the appearance of nephrotic syndrome, may develop and usually occurs at about the fourth month of life.[483] A generalized osteochondritis and perichondritis or periostitis may affect the architecture of all bones of the skeletal system but are most prominent in the long bones and can be easily discerned on radiographs.[484,485] CNS involvement is common, occurring in at least 22% of infected neonates,[486] ranging from asymptomatic invasion by *T. pallidum* to acute syphilitic leptomeningitis. When untreated, neurosyphilis in the infant can lead to a chronic meningovascular process, which results in hydrocephalus, cranial nerve palsies, and even cerebral infarction. Asymptomatic CNS involvement is inferred from the presence of abnormal CSF studies, such as pleocytosis (defined as >25 white blood cells/μL for infants <1 month), high CSF protein levels (defined as >150 mg/dL in term infants <1 month of age and >170 mg/dL in preterm infants <1 month of age, although some experts use a threshold of >40 mg), a reactive CSF VDRL test, or a combination of these, in a healthy-appearing infant. Asymptomatic CNS syphilis occurs in approximately 40% of infants who have clinical, laboratory, or radiographic abnormalities of CS but is infrequent in infants without such manifestations. Because CSF studies are of low sensitivity to detect spread of spirochetes into the brain, in some cases asymptomatic disease can be diagnosed only by serum or CSF IgM immunoblotting or by PCR detection of spirochetal DNA.[486] Hypopituitarism manifesting with persistent neonatal hypoglycemia has been observed among some infants with CS.[487] Although rare, chorioretinitis, glaucoma, and uveitis have also been described in newborns with early CS.[60]

With a few exceptions, the untreated child who survives the first 6 to 12 months of life will develop manifestations of late CS.[60] The generalized osteochondritis, perichondritis, and periostitis may result in deformities of the nose (saddle nose)[488] and the metaphyses of the lower extremities (anterior bowing, or saber shin).[60] Cardiovascular syphilis is rare, whereas interstitial keratitis is common.[489] Photophobia, pain, circumcorneal inflammation, and superficial and deep vascularization of the cornea may occur at any time between the ages of 5 and 30 years.[489] Asymptomatic late congenital neurosyphilis occurs in up to one-third of perinatally infected children, whereas symptomatic disease is rare and fits the adult patterns of tabes dorsalis, syphilitic encephalitis, and local gummas. Eighth nerve compromise with resulting sensorineural deafness is particularly common.[484] Other late characteristic stigmata include recurrent arthropathy and bilateral knee effusions (Clutton joints)[425]; centrally notched, widely spread, peg-shaped upper central incisors (Hutchinson teeth)[490]; frontal bossing; and poorly developed maxillas.[491]

A complete evaluation of the neonate suspected of having CS should include a complete physical examination and DF microscopic examination or DFA staining of suspicious lesions or body fluids (e.g., nasal discharge). Conventional blood tests, including a complete blood count and liver and renal function tests, along with HIV testing and long bone radiographs, should be done in all infants. Nontreponemal tests (VDRL or RPR) and, if available, IgM immunoblotting are recommended on serum and CSF (VDRL only on CSF). The CDC no longer recommends obtaining umbilical cord blood because of the potential for contamination with maternal blood and because RPR testing of umbilical cord blood is prone to false positives owing to contamination with Wharton jelly.[492] The nontreponemal test that is performed on the infant should be the same as that done on the mother so that the maternal and infant titers can be directly compared. Other tests should be performed as clinically indicated (i.e., chest radiography, abdominal ultrasonography, ophthalmologic examination, auditory brainstem response, and neuroimaging studies). It is important to note that a diagnosis of acquired syphilis in an older child is suggestive of sexual abuse, although some cases might be attributable to progression of CS.[493]

To maximize treatment of children potentially infected with *T. pallidum,* including those who are asymptomatic at the time of initial presentation, in 1996 the CDC issued a revised case definition for CS (see Table 237.2).[494] The criteria include all infants with clinical evidence of active syphilis, as well as normal-appearing neonates and stillbirths delivered to women with untreated or inadequately treated syphilis. According to these guidelines, a confirmed diagnosis of CS requires laboratory demonstration of *T. pallidum* in tissues or lesions of infected infants (see Table 237.2). Amniotic fluid, placental tissue, cytobrushings or sections of umbilical cord, nasal fluid, scrapings from a rash, tissue impressions, and lymph node aspirates are examples of specimens that can be examined for spirochetes.[473,495–497] DF microscopy, DFA-TP, immunohistochemistry, and silver staining can all be used to visualize *T. pallidum.* PCR can reveal small amounts of *T. pallidum* DNA in lesion exudates or tissues but cannot be used to confirm the presence of living organisms.[497–499] Because most of these detection methods are difficult to perform in most clinical settings, a confirmed diagnosis is achieved only in a small proportion of newborns, with most affected children receiving a diagnosis of presumptive CS. CS is highly probable if the neonate has any of the following: abnormal physical examination findings consistent with CS; a serum quantitative nontreponemal serologic titer that is greater than or equal to fourfold (two dilutions) the corresponding maternal titer; a reactive serum VDRL/RPR test and abnormal CSF (see earlier); and reactive treponemal antibody test after 15 months of age.

As per Table 237.2, the diagnosis of presumptive CS is made on the basis of (1) identification of syphilis in the mother; (2) adequacy of maternal treatment; (3) presence of clinical, laboratory, or radiographic evidence of syphilis in the infant; and (4) comparison of maternal (at delivery) and infant nontreponemal serologic titers obtained using the same test and preferably performed in the same laboratory.[500] Infants younger than 1 month who have normal physical examination findings and serum VDRL or RPR titers less than fourfold the maternal titer are presumed to have CS if their mother was not treated or received inadequate or suboptimal therapy. Some experts would also presume infants to have CS if their mothers had contact with a person with primary or secondary syphilis within 90 days before delivery and were not treated or were inadequately treated, even if the mother had nonreactive serology. A diagnosis of CS is unlikely if the infant has normal physical examination findings and a nonreactive VDRL/RPR test and the mother was adequately treated during pregnancy and had an appropriate serologic response, or if the neonate has normal physical examination findings and serum VDRL or RPR titer the same or less than fourfold the maternal titer and the mother was adequately treated before pregnancy and her titers remained low (VDRL <1:2; RPR <1:4) and stable before and during pregnancy and at delivery. Because at least one-third of the mothers who give birth to syphilitic children have not had prenatal care, and about half have had a nonreactive serologic test during the first trimester of pregnancy, serologic testing of the mother is always warranted at delivery, especially in high-risk patients. No infant or mother should leave the hospital unless the maternal serologic status has been documented at least once during pregnancy, and at delivery in communities and populations in which the risk for CS is high.

Parenteral penicillin is the drug of choice for the treatment of CS (see Table 237.2).[60,501] Infants 4 weeks of age or younger who have confirmed or presumptive disease should be treated with either aqueous crystalline penicillin G, 50,000 units/kg IV every 12 hours for the first 7 days of life and every 8 hours beyond 1 week of age for a total of 10 days, or aqueous procaine penicillin G, 50,000 units/kg IM once daily for 10 days (see Table 237.2). The levels of penicillin that are achieved in the CSF after intramuscular administration of procaine penicillin are lower than those with intravenous administration of aqueous penicillin.[502] However, the clinical significance of this observation is unclear because there have been no treatment failures reported after treatment with procaine penicillin.[461] If more than 1 day of penicillin therapy is missed, the entire course should be restarted. Single-dose therapy with intramuscular injection of BPG (50,000 units/kg) can be considered in the infant with normal physical examination findings, normal CSF, and normal radiographic and laboratory studies and whose nontreponemal test titer is the same or less than the maternal titer, under the following circumstances: (1) The mother was treated with the appropriate regimen for 30 or fewer days before delivery; (2) the mother received her recommended penicillin therapy for the stage of infection during the pregnancy but the nontreponemal titer did not decrease fourfold at the time of delivery; and (3) the mother has untreated syphilis or her treatment status is undocumented. Children who are diagnosed with CS after 1 month of age (including those with late CS) and children with acquired syphilis should be treated with aqueous penicillin G (50,000 units/kg IV every 4 to 6 hours for 10 days). In addition, for children with CS or findings compatible with CNS involvement, some experts suggest that the 10-day course of aqueous penicillin be followed with a single dose of intramuscular BPG (50,000 units/kg).

Follow-up of children treated for CS after the newborn period should be conducted as is recommended for neonates. The risk of infection for the infant is minimal if the mother has received adequate penicillin treatment during pregnancy. Nevertheless, the child must be examined monthly after delivery and until the nontreponemal test or PCR assay result becomes negative. Penicillin treatment should never be withheld to prove the diagnosis, and every neonate born to a syphilitic mother should be promptly treated unless adequate, serologically proven effective treatment with penicillin can be documented more than 1 month before delivery. Because giving penicillin to the neonate is almost risk free, all neonates born to syphilitic mothers should be treated, regardless of whether the mother was treated during pregnancy. Adequate treatment of the mother usually but not always ensures that the fetus will not be infected. Infants born to mothers who are coinfected with syphilis and HIV should receive the same evaluation and treatment as those whose mothers do not have HIV infection.[501] There is insufficient evidence to determine whether such infants require different evaluation, treatment, or follow-up.

IMMUNITY

For nearly 200 years, syphilologists have intensely pondered and debated the existence, timing, and nature of protective immunity in acquired

syphilis.[169,503,504] In the 19th century, it was believed that individuals became immune to reinfection during primary syphilis, a notion referred to by the term "chancre immunity." This flawed concept was based largely on "autoinoculation" experiments in which material taken from presumptive chancres failed to cause lesions when inoculated elsewhere on the same patient. Using his primate model, Neisser concluded that chancre immunity is dependent on the presence of active infection and that cure of the disease leads to the disappearance of immunity.[169] Chesney,[505] in contrast, correctly concluded from data generated using the rabbit model that syphilitic infection does indeed elicit lasting immunity, provided the initial infection is of sufficient duration. Important to note, he found substantially less cross-immunity to challenge with heterologous strains of *T. pallidum,* findings confirmed years later, also in rabbits, by Turner and Hollander.[132,505] In a meticulously executed study involving nearly 300 rabbits, which took advantage of the recent availability of penicillin, Magnuson and Rosenau[506] showed definitively that immunity to homologous challenge in the animal model was dose and time dependent and could be achieved in the absence of concurrent infection. To a significant extent, Magnuson and coworkers[140] replicated these results in humans several years later in a remarkable (and unrepeatable) series of experiments conducted at Sing Sing Prison. Although these investigators went to great length to explain that all participants were volunteers, the authors of this chapter must state unequivocally that they do not condone experimentation in incarcerated populations even if it was considered acceptable in the era in which it was performed. In any event, the Magnuson group showed that patients with treated primary and secondary syphilis were susceptible to challenge with the Nichols strain of *T. pallidum,* whereas those with treated or untreated late latent disease were resistant. Interpretation of these experiments is clouded by the extraordinarily high challenge inoculum used (1×10^5), more than 2000-fold greater than the median infective dose (ID_{50}) determined in the same study, compounded by the likely heterologous challenge in the majority of "volunteers." However, there is also abundant clinical experience that patients successfully treated for primary and secondary syphilis can be reinfected.[508,509] It seems reasonable to conclude, therefore, that the immunity that develops during early syphilis is limited, even more so against heterologous infection, and that it dissipates rapidly after treatment. In a controversial paper, Grassly and coworkers[35] used mathematical models derived from epidemiologic data to argue that the oscillatory pattern of syphilis epidemics over an approximate 11-year cycle is due to the buildup of immunity in untreated populations, followed by the waning of immunity after large-scale treatment. Their hypothesis deserves reconsideration given recent technical advances in sequencing genomes of syphilis spirochetes in lesions[33,180] and our much improved knowledge of *T. pallidum*'s OMP repertoire,[34,73] the presumptive targets of protective antibodies.

The lack of an inbred animal model has greatly hindered efforts to dissect the components of the immune response that confer protection. Because *T. pallidum* is an extracellular pathogen, one would expect passive administration of antibodies from immune animals to protect against homologous challenge. Such experiments have repeatedly shown that administration of large amounts of serum or purified immunoglobulins yields only modest lesion attenuation in rabbits challenged intradermally and that dissemination of spirochetes is not prevented.[169] In other words, passive immunization is at best only partially protective. The paucity of antigenic targets on the surface of the syphilis spirochete, coupled with other mechanisms for antibody avoidance (e.g., limited production of antibodies against surface-exposed epitopes, antigenic variation, and differential expression of OMPs; see earlier), is an attractive explanation for these results.[73]

The inability of public health measures to curtail the explosive increase in syphilis cases worldwide, with particular concern for the global burden of maternal syphilis and CS, has called renewed attention to the need for an effective vaccine.[5,434,503,504,510] The feasibility of eliciting a protective immune response against *T. pallidum* was demonstrated nearly 50 years ago. In a hallmark study, Miller[507] reported complete and long-lasting (1 year) protection of rabbits immunized over a 37-week period with *T. pallidum* rendered noninfectious by gamma irradiation. Important to note, this regimen did not protect against challenge with the Haiti B strain, thought at the time to be subspecies *pertenue* but now known to be subspecies *pallidum.* Until recently, efforts to replicate this stunning result by immunization with recombinant *T. pallidum* proteins believed to contain surface-exposed epitopes yielded either no or only partial degrees of protection. In what appears to be a major advance, Lithgow and colleagues[511] showed that immunization of rabbits with TP0751 reduced spirochete dissemination. The high degree of conservation of TP0751 in *T. pallidum* strains suggests that a vaccine containing this lipoprotein could be useful on a global scale.

ACKNOWLEDGMENTS

We are deeply indebted to Ms. Carson Karanian and Ms. Morgan Ledoyt for their assistance with myriad aspects of manuscript production, referencing, and figure generation.

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The complete reference list is available online at Expert Consult.

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238 Endemic Treponematoses

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SHORT VIEW SUMMARY

Definition
- The endemic treponematoses include yaws, endemic syphilis, and pinta. All are cutaneous diseases caused by treponemes closely related to *Treponema pallidum,* the causative agent of syphilis.

Microbiology
- The treponemal species that cause the endemic treponematoses cannot be easily cultured for diagnosis. Microbiologic diagnosis is most often based on nontreponemal and treponemal serologic reactivity in tests designed for syphilis diagnosis.

Epidemiology
- The endemic treponematoses are generally spread only from human to human by direct contact. After global eradication campaigns in the 1950s and 1960s, yaws rates have again increased worldwide. Endemic syphilis and pinta remain uncommon, but the prevalence is unknown because of the lack of surveillance data.

Clinical Manifestations
- Similar to syphilis, initial lesions of endemic treponematoses resolve spontaneously; recurrent secondary lesions are typically cutaneous or involve bone or cartilage.
- A latent (asymptomatic) stage may occur in individuals infected with yaws or endemic syphilis, and a minority of untreated patients progress to bone, soft tissue, and cartilaginous destruction.

Diagnosis
- Diagnosis of endemic treponematoses is based on clinical recognition of compatible skin lesions and confirmed by serologic testing.
- The development of a point-of-care immunochromatographic lateral flow assay that simultaneously tests for treponemal and nontreponemal antibodies facilitates rapid diagnosis and treatment.

Therapy
- Benzathine penicillin G was the mainstay of therapy of the endemic treponematoses. More recently, oral azithromycin has been proven to be an easy-to-administer alternative to penicillin injections for therapy of yaws.

Prevention
- Yaws appears to be amenable to mass treatment as a control and prevention measure.
- In 2012 the World Health Organization announced plans to embark on mass treatment initiatives designed to eradicate yaws by 2020.

The endemic treponematoses comprise yaws, endemic syphilis, and pinta and are caused by *Treponema pallidum* subsp. *pertenue, Treponema pallidum* subsp. *endemicum,* and *Treponema carateum*. Nearly eliminated in the 1960s by an eradication campaign led by the World Health Organization (WHO), these relatively uncommon diseases are now often seen among children living in low-income and middle-income countries. The bacteria that cause the endemic treponematoses are morphologically and serologically indistinguishable from *T. pallidum* subsp. *pallidum,* the causative agent of sexually transmitted syphilis, and important parallels are found between the natural history of these diseases and syphilis. Similarly, the tools for management are adapted almost entirely from tools historically used as part of syphilis control efforts. Nonetheless, the endemic treponematoses differ from syphilis in terms of epidemiology, clinical manifestations, and at the level of the bacterial genome.[1–3,4,5]

Disease rates have again increased more recently.[1,6] Based on the successful eradication of yaws in India and the potential for recently described oral azithromycin therapy to facilitate control and prevention, the WHO announced a new effort to eradicate yaws.[7,8]

MICROBIOLOGY

Within the genus *Treponema,* four bacteria are presently recognized as human pathogens: *T. pallidum* subsp. *pallidum, T. pallidum* subsp. *pertenue, T. pallidum* subsp. *endemicum,* and *T. carateum.* Much remains to be learned about these organisms. They cannot be cultured in vitro, and current understanding of their biology is based on the careful study of relatively few clinical isolates, most often propagated in laboratory animals (e.g., rabbits, hamsters, and guinea pigs).[4,5] Although sequencing of the *T. pallidum* genome showed more than 99% DNA homology,[3] several genetic loci have been identified that may permit differentiation of *T. pallidum* subsp. *pallidum* from the other subspecies (*T. pertenue* and *T. endemicum*).[4,5] *T. carateum* isolates are not available for study at the present time, and any molecular similarities and differences have yet to be compared with the *T. pallidum* subspecies.[9] In the future, application of evolving molecular diagnostic methods to the study of human treponemes promises to provide new insights into the biology of these organisms.

Morphology

T. pallidum and the treponemes causing endemic treponematoses are long, thin (8–13 × 0.15 μm), motile bacteria that cannot be seen with Gram stain and are best seen in specimens of a lesion exudate with a Warthin-Starry silver stain, darkfield microscopic examination, or fluorescent antibody techniques. Their regular, spiral morphology and characteristic corkscrew motility are helpful for recognition in clinical specimens.

Antibiotic Sensitivity

Based on clinical and serologic response to therapy as well as studies performed in experimental animals, these treponemes are sensitive to penicillins and tetracyclines. Because the organisms cannot be propagated in vitro, data on minimal inhibitory and bactericidal concentrations are unavailable. Sulfa drugs and fluoroquinolone antimicrobials have not been found to be active against *T. pallidum* subsp. *pallidum*.[10,11] Clinical and laboratory resistance (mutations of 23S ribosomal RNA gene) to erythromycin and other macrolide antibiotics has been shown for multiple isolates of *T. pallidum* subsp. *pallidum* from North America and Western Europe,[12] but similar resistance among the endemic treponematoses was not documented until 2018.[13] With the adoption by the WHO of azithromycin as the preferred treatment for yaws,[14] close monitoring for macrolide resistance will be important.

EPIDEMIOLOGY

The endemic treponematoses are diseases of low-income and middle-income countries, with varied geographic distribution. However, the true scope of disease is unknown because endemic treponematoses are

not notifiable diseases in most countries. Yaws appears to have worldwide distribution (Central and South America, Asia, Africa, and Pacific Islands) and is found in warm, humid climates with heavy rainfall including tropical regions and rainforests. Of 13 endemic countries that reported to the WHO between 1990 and 2014, prevalence of active yaws disease ranged from 0.31% to 14.54%, and prevalence of latent yaws ranged from 2.45% to 31.05% with a higher burden of infection in the Pacific Islands.[15] Endemic syphilis is less common than yaws and occurs primarily in dry, arid areas among nomadic and seminomadic rural populations. The disease has been most common in North Africa, Southwest Asia, and the eastern Mediterranean region. In contrast to the global distribution of yaws and endemic syphilis, pinta is limited to the Western Hemisphere. The disease has been described in Mexico, Caribbean islands (including Cuba), and Central and South American countries.[6,9] The prevalence of pinta is unknown at the present time because of the lack of surveillance data. However, substantial numbers of cases including a case series of more than 200 patients have been described in rural Brazil.[16] Within these regions, each of the endemic treponematoses disproportionately impacts impoverished people living in remote areas. Limited access to hygienic facilities has been associated with increased infection rates.

Transmission

Similar to syphilis, the endemic treponematoses are acquired and spread through direct contact with infectious exudates from patients with active infection or who have relapsed after latent infection (infected but asymptomatic). In the case of endemic syphilis, transmission is possible via fomites (see "Endemic Syphilis [Bejel]"). However, in contrast to syphilis, little evidence exists that transmission of endemic treponematoses occurs via blood, blood products, or transplacentally from untreated pregnant women to unborn children. Protective immunity induced by infection has been shown only in rabbit experimental models conducted in the early 20th century; however, a later study in hamsters concluded that immunity was not absolute.[1]

CLINICAL MANIFESTATIONS

The endemic treponematoses can be characterized by several common clinical characteristics as well as characteristics that distinguish them from one another.[17] Although there are several important differences, there are numerous parallels between the well-described course of untreated syphilis (*T. pallidum* subsp. *pallidum* infection) and the natural history of the endemic treponematoses. Similar to syphilis, each is a chronic bacterial infection that progresses through a series of well-described clinical stages, predictably progressing from an early, localized stage to a later, more widespread stage. Early in the course of infection, even before primary lesions develop, the treponemes spread hematogenously throughout the body; without treatment, they subsequently give rise to secondary and late manifestations of infection. Once primary lesions develop, regional lymphadenopathy follows. As in the case of syphilis, primary lesions of yaws and pinta resolve spontaneously without treatment; then in the secondary stage, skin lesions may recur up to 5 years, with the frequency of these recurrences declining with time.[1,4] Recurrent secondary manifestations of early endemic treponematoses are primarily dermatologic; however, a substantial proportion of persons also develop osteitis or other bony or cartilaginous lesions. Primary and secondary lesions may resolve, and a patient's infection may become latent, where the patient harbors infection without exhibiting signs and symptoms.[13]

Late Complications and Sequelae

In a minority of untreated patients with yaws and endemic syphilis, late complications of infection occur. The lesions of late infections tend to be more destructive than the early lesions and most commonly manifest either as ulcerative or hyperkeratotic cutaneous lesions or as bone and joint involvement. A distinguishing element of the endemic treponematoses is that, in contrast to syphilis, little evidence exists of cardiovascular or neurologic sequelae in late or untreated infection.

Differential Diagnosis

The differential diagnosis varies between the endemic treponematoses and their stages. As the clinical presentation of endemic treponematoses can be mistaken for sexually transmitted syphilis, it is difficult to distinguish between the two diseases especially in countries where both are endemic. Early yaws may present similar to impetigo, scabies, molluscum contagiosum, lichen planus, and cutaneous leishmaniasis. The differential diagnosis for endemic syphilis includes orofacial herpes simplex and noninfectious aphthous ulcers. Early lesions of pinta may be confused with eczema, psoriasis, and leprosy.

Yaws

Yaws, one of the 20 neglected tropical diseases according to the WHO, is a chronic infection caused by *T. pallidum* subsp. *pertenue* and is the most common of the endemic treponematoses. After years of relatively low levels of prevalence (see "Prevention"), there has been a resurgence of disease.[1,6,7] Yaws is most common in children 2 to 15 years of age. Human-to-human transmission of yaws is thought to be facilitated by skin breaks (e.g., lacerations, insect bites), crowding, and a relative lack of hygiene. These circumstances are generally more common in children than in adults and may explain why most initial infections occur in children and adolescents. Notably, yaws-like strains have been identified from skin lesions of African monkeys, baboons, and gorillas suggesting that *T. pallidum* subsp. *pertenue* may also cause the same disease in nonhuman primates.[2] To date, there has been no evidence of transmission from nonhuman primates to humans, but it is possible that nonhuman primates may be a potential reservoir for the bacteria.

A primary papule forms at the site of inoculation (commonly the legs, feet, or buttocks) after an incubation period of 9 to 90 days (mean, 21 days). Over a period of 3 to 6 months, the primary papule of yaws may increase in size and then heal spontaneously. Primary lesions are typically pruritic, facilitating autoinoculation; tender regional lymphadenopathy, fever, and constitutional symptoms may also develop. At about the time the primary papule heals, secondary lesions near or distant from the initial lesion often occur. These lesions may also be papular and are thought to be a consequence of local (autoinoculation) and hematogenous or lymphatic spread of infection (Fig. 238.1). In the secondary stage, lesions of yaws predominately involve the skin near the nose and mouth and bones or cartilage of the extremities and, even if untreated, generally heal without scarring (Fig. 238.2). Similar to the primary lesions, lesions of secondary yaws resolve spontaneously without therapy, and patients enter a latent stage that, if untreated, may last a lifetime.[4] Occasionally, secondary infection or ulceration of cutaneous yaws lesions occurs and results in more pronounced lesions and scarring.

About 10% of untreated patients develop late lesions of yaws characterized by hyperkeratotic plaques, destructive bony lesions, or gummata. In the 1820s, Spaniards first coined the term *gangosa* (nasal voice) to describe these disfiguring chronic manifestations, as they destroy bone,



FIG. 238.1 Initial papillomatous yaws lesion on upper thigh (also called primary frambesioma, mother yaw, *chancre pia nique*). Initial lesion usually commences as a papule on lower extremities and slowly enlarges to form a raspberry-like lesion. *(From Ferine PL, Hopkins DR, Niemel PLA, et al.* Handbook of Endemic Treponematoses. *Geneva: World Health Organization; 1984.)*



FIG. 238.2 Early ulceropapillomatous yaws on the leg (also called *ulcère post-chancreux*). *(From Ferine PL, Hopkins DR, Niemel PLA, et al.* Handbook of Endemic Treponematoses. *Geneva: World Health Organization; 1984.)*



FIG. 238.3 Gangosa. *(From Ferine PL, Hopkins DR, Niemel PLA, et al.* Handbook of Endemic Treponematoses. *Geneva: World Health Organization; 1984.)*

cartilage, and soft tissue of the mouth and nose (Fig. 238.3). Bowing of the anterior tibiae (saber shins) may likewise be a late manifestation of yaws arising from infectious periostitis.

Healing is promoted with therapeutic administration of azithromycin or penicillin. Complete healing may occur without sequelae in patients receiving early treatment. Yaws infections may be prevented with prophylactic administration of azithromycin in exposed patients (see "Therapy" and "Prevention").

Endemic Syphilis (Bejel)

Endemic syphilis is caused by *T. pallidum* subsp. *endemicum*.[1,6] Similar to yaws, endemic syphilis is a disease of childhood and adolescence, with most cases occurring in individuals 2 to 15 years of age. Not only does transmission of endemic syphilis occur via direct contact, but transmission via fomites (i.e., on shared eating or drinking utensils) also may occur because early lesions are often mucosal. The primary lesions of endemic syphilis are typically mucous patches on oral pharyngeal mucosa or lesions at the angles of the lips (angular stomatitis) and may go unobserved. These painless lesions may resolve without therapy, but secondary lesions may appear 3 to 6 months later. Secondary lesions of endemic syphilis manifest as rashes, as mucosal lesions, or with bony and cartilaginous involvement. Similar to secondary syphilis, endemic syphilis rashes in the secondary stage may display a wide variety of morphologies including disseminated papular lesions and condylomata lata in moist areas of the skin accompanied by regional lymphadenopathy.

After a period of latency shorter than for yaws (6 months to several years), late complications of endemic syphilis are relatively common.[4] These complications may manifest as gummatous lesions or chronic ulcerative skin lesions in 25% to 50% of patients. In contrast to early lesions, late lesions tend to be destructive, chronic progressive lesions, some of which progress to deforming bony and cartilaginous facial lesions referred to as *gangosa* (see "Yaws"). In addition to the ulcerative lesions of endemic syphilis, bony involvement may occur and is manifested as osteoperiostitis of the long bones and hands causing disability and deformity.

Pinta

Pinta is the endemic treponematosis caused by a unique treponemal species, *T. carateum*.[6] The peak age prevalence for pinta is older than for yaws or endemic syphilis, with most individuals 15 to 30 years of age. Pinta is also thought to spread between individuals through direct lesion contact. An important difference between pinta and the other endemic treponematoses is that without treatment the lesions tend to persist. The classic initial lesion of pinta is a papule or erythematous epithelial plaque. These lesions tend to occur on parts of the body that are not typically clothed, most often the leg, foot, forearm, or back of the hands where skin to skin contact is common. These lesions gradually enlarge through local extension to form hyperkeratotic pigmented lesions. The lesions of pinta are also accompanied by regional lymphadenopathy. Disseminated, small lesions (pintids) may occur distal to the initial lesion 3 to 9 months after infection and slowly enlarge and coalesce. Over time, the lesions of pinta become pigmented taking on a darker color described as slate blue. Late pinta is characterized further by additional pigmentary cutaneous changes; lesions may include dyschromic treponeme-containing lesions and achromic treponeme-free lesions. The depigmentation process of pinta occurs at different rates in the same lesion, giving the lesions a mottled appearance. No other disability or late complications of pinta have been described.

Attenuated Disease

A mild presentation of both yaws and endemic syphilis has been described in regions of lower prevalence. In highly endemic areas the papillomatous stage of yaws tends to last months to years, but in areas of low endemicity the stage is shortened and characterized by fewer, dry crops of lesions in the intertriginous regions.[1,6] Similarly in areas of lower prevalence, endemic syphilis has been characterized by fewer early and late lesions lasting a shorter period of time. Multiple factors are thought to lead to attenuated disease including improved hygiene, nutrition, and access to treatment.[6]

DIAGNOSIS

Diagnosis of individual cases of the endemic treponematoses in large part depends on recognition of appropriate clinical findings and is confirmed with serologic testing with tests for syphilis.[6]

Direct Visualization

Although not widely available, demonstration of characteristic treponemes with silver staining or darkfield microscopy of lesion exudates or immunofluorescent antibody stains for treponemes can provide immediate and highly specific diagnosis of the endemic treponematoses. However, facilities capable of performing these methods are limited in low-income and middle-income countries where endemic treponematoses are common. Therefore these methods are rarely used for diagnosis.

Serology

No serologic tests have been specifically developed for diagnosis of endemic treponematoses; however, because the humoral antibody response to these diseases is indistinguishable from the response to syphilis, serologic tests for syphilis are important for confirmation of clinically suspected infections. Serologic tests for syphilis and the endemic treponematoses are divided into nontreponemal and treponemal tests.[18] The nontreponemal assays are based on cross-reactivity of cardiolipin-cholesterol-lecithin antigens with antibodies to *T. pallidum* and include tests such as the rapid plasma reagin (RPR) card test and Venereal Disease Research Laboratory (VDRL) tube tests. These tests provide quantifiable results that are useful not only for screening for infection but also for evaluation of response to therapy after treatment. In contrast, the treponemal assays are based on the reactivity of antibodies to either recombinant *T. pallidum* antigens or antigens from *T. pallidum* propagated in laboratory animals. The treponemal tests are available in a variety of formats including fluorescent treponemal antibody absorption, *T. pallidum* hemagglutination, or several different commercial enzyme-linked immunosorbent assays. False-positive results sometimes occur with both nontreponemal and treponemal tests. Because treponemal and nontreponemal tests are unrelated, use of an unrelated test to confirm an initial test result (i.e., confirmatory testing of reactive RPR or VDRL test results with a treponemal test) greatly increases the specificity of test results.

Six months after effective treatment, most patients with active endemic treponematoses have a fourfold (two dilutions) or greater decline in RPR or VDRL titers. Reinfection or relapse may be indicated with a VDRL or RPR titer that increases two or more dilutions. Reversion of treponemal serologic test results to nonreactive is considerably less common than for nontreponemal tests. Early in the disease, patients may have negative serologic tests.[16] Patients with latent infections will have positive serologic tests. Patients with advanced disease may have nontreponemal tests that have low titers or become nonreactive.[2]

Point-of-Care Syphilis and Endemic Treponematosis Test

A simple, point-of-care test based on simultaneous detection of both treponemal and nontreponemal antibodies has been developed for rapid diagnosis and treatment of syphilis and endemic treponematoses.[19] This immunochromatographic lateral flow assay detects both immunoglobulins (IgM and IgG) against a recombinant *T. pallidum* antigen (T1) and a nontreponemal antigen (T2). This assay may be used for testing serum, plasma, or whole blood; has a 1-year shelf life at room temperature; distinguishes between past and active infection; confirms the diagnosis in real time for patients with skin lesions; and can be performed outside of clinics, providing remote communities access to diagnostic testing. In addition, the test is useful for estimation of population prevalence of endemic treponematoses and detection of continued transmission after control efforts (e.g., mass treatment programs). A meta-analysis of 7267 tests showed good performance, but sensitivity was higher in patients with high-titer (RPR ≥1:16) infection compared with low-titer (RPR <1:16) infection for both the T1 component (98.2% vs. 90.1%, $P < .0001$) and the T2 component (98.2% vs. 80.6%, $P < .0001$).[20] As the number of active cases declines after a mass treatment campaign, a diagnostic test with higher sensitivity or repeat testing may be needed to confirm that all cases have been identified and treated.

Polymerase Chain Reaction

The use of polymerase chain reaction (PCR) for surveillance has been proposed to improve diagnostic accuracy of skin ulcers, especially in settings where other causative pathogens may be coendemic and cause similar lesions.[21] Treponemal DNA is detected with use of a PCR assay alone, which applies a qualitative or quantitative amplification-based method to detect single genes or with reverse-transcriptase PCR followed by the use of labeled primers to target amplicons from *T. pallidum* subsp. *pallidum* 16S ribosomal RNA. Specific areas in the *T. pallidum* genome that distinguish it from other bacterial organisms have been identified including *tpf-1, bmp, tmpA,* 47-kDa lipoprotein, and *polA*.[4] However, PCR testing is expensive, requiring electricity and trained laboratorians at equipped laboratory facilities to perform the test (which may not be available in field conditions); access and delivery of specimens from remote areas may also be difficult. The only reliable method at the present time to distinguish *T. pallidum* subspecies (*pallidum, pertenue,* and *endemicum*) is the combined use of PCR and DNA sequencing, which identifies molecular differences between subspecies (e.g., *gpd, tprC, tprI, arp,* IGR19), but availability is limited to a few reference laboratories.[5]

THERAPY

Benzathine penicillin G had been the preferred drug for treatment of the endemic treponematoses.[1,2,6] The need for refrigerated storage of penicillin, the inconvenience of injections for patients, the potential for allergic reactions to β-lactam antibiotics, and the risk of needlestick injuries to health care providers are drawbacks for many countries. A randomized trial in 2012 demonstrated that a single oral dose of 30 mg/kg of azithromycin (up to a maximal dose of 2 g) was noninferior to benzathine penicillin G for treatment of early yaws. Cure rates are similar with recommended doses of azithromycin or benzathine penicillin G.[14] Based on these landmark findings, the WHO now recommends azithromycin as the preferred treatment for endemic treponematoses.[8]

Observational studies in the Pacific region where endemic treponematoses and trachoma occur concurrently suggest that lower doses of azithromycin (20 mg/kg, up to maximal dose of 1 g) typically used to treat trachoma may also be effective against endemic treponematoses.[22,23] Studies comparing efficacy of the two doses (30 mg/kg and 20 mg/kg) of azithromycin for treatment are ongoing. If the infected individual is intolerant to azithromycin, WHO recommends a single dose of benzathine penicillin G, 600,000 U for children younger than 10 years of age or 1.2 million U for individuals 10 years of age or older, may be administered for treatment. Despite the lack of formal studies, extrapolation from experience with sexually transmitted syphilis suggests that tetracycline or doxycycline given for 14 days may also be effective.

Response to therapy may be determined through resolution of early lesions or, in patients with late infection, arrest of progression. Serologic response to therapy may also be documented by declines in RPR or VDRL titers of two or more dilutions 6 months following treatment (as with sexually transmitted syphilis). After treatment, however, titers may not revert to seronegativity.

PREVENTION

In the 1950s it was estimated that more than 50 to 160 million people worldwide were infected with yaws, of which 40 million may have had permanent disfigurement of the face, joints, bones, and soft tissues. Because of the large geographic distribution and disease burden, the WHO led a global control program from 1952 to 1964 using active serologic screening and staged treatment in 46 countries.[6,7] In this effort, about 300 million examinations were performed, and more than 50 million people were treated. Prevalence of endemic treponematoses was reduced by more than 95%. However, as disease prevalence waned, so did resources and commitment for continued surveillance and control efforts.[2,24] Infection rates of yaws once again rebounded[6,7] with global prevalence of disease estimated at about 460,000 cases in the late 1990s; however, this is likely an underestimate.[1]

In the past decade, progress has been made to eliminate yaws. The potential effectiveness of community-based control efforts has been demonstrated in India, where reimplementation of the control measures reduced the number of reported new cases from 3571 in 1996 to 0 cases in 2004 followed by negative serologic surveys among children younger than 5 years old for 3 consecutive years. In 2006 yaws was eliminated from India and formally declared eradicated by the WHO in 2016.[24] Ecuador also reported elimination of yaws through a mass treatment program.[25]

Based on the confirmed success in India and the potential to simplify provision of therapy for entire communities, in 2012 the WHO announced a new, aggressive roadmap that targets global elimination of yaws by 2020.[7,8] In contrast to the staged treatment strategy of the 1950s, two new treatment strategies will simplify case detection and treatment of active and latent disease. Total community treatment is the treatment of an entire community regardless of active cases. This strategy first aims for 100% treatment coverage of a confirmed endemic village or

community followed by surveillance and, if necessary, incorporates mop ups with additional treatment of identified cases. Total targeted treatment, the treatment of all active clinical cases and contacts, is used every 3 to 6 months to detect and treat remaining active cases.[8] Pilot total community treatment programs with azithromycin in some endemic countries (Solomon Islands, Papua New Guinea, and Ghana) demonstrated the potential for eradication of endemic treponematoses.[22,26,27] A longitudinal study followed the 16,092 inhabitants of a Papua New Guinea island (Lihr Island) in which nearly 84% received a dose of azithromycin. Although prevalence of yaws initially declined, it began steadily increasing 2 years later, likely due to relapse of untreated latent infections and reintroduction of yaws through in-migration. This study is the first to identify a point mutation in a yaws strain that conferred resistance to macrolides, previously unreported among endemic treponematoses. For successful eradication, the WHO now recommends treatment coverage rates should exceed 90%, and if rates are less, another round of mass treatment should be considered along with close monitoring for macrolide resistance.[28]

Scientific advances including development of point-of-care and molecular diagnostics will aid in achieving the 2020 goal. However, further studies are needed to continually improve these eradication strategies. The future of endemic treponemal research lies in improving surveillance and geographic mapping, integrating point-of-care testing, determining etiology of nontreponematoses skin ulcers by using advanced molecular diagnostics, monitoring for macrolide resistance, and looking for *T. pallidum* infections in nonhuman primates.[25]

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239 *Leptospira* Species (Leptospirosis)

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SHORT VIEW SUMMARY

Definition
- Leptospirosis is caused by infection with pathogenic spirochetes of the genus *Leptospira.*

Epidemiology
- Leptospirosis is a zoonosis of global distribution.
- Leptospirosis is maintained in nature by chronic renal infection of carrier animals, especially rodents.
- Infections typically occur after occupational or recreational exposure to water or soil contaminated with rodent urine.
- In developing countries with poor housing standards, leptospirosis outbreaks occur regularly in urban settings after heavy rainfall and flooding.

Microbiology
- Twenty-three named leptospiral species have been described, of which 10 are known to be pathogenic (see Table 239.1).

Diagnosis
- Clinical diagnosis requires a high index of suspicion based on epidemiologic exposure. The signs and symptoms of early leptospirosis are nonspecific.
- Serologic tests are helpful when positive but may have poor sensitivity during the first week of illness. Polymerase chain reaction assay of serum and urine appears to be a promising diagnostic technique. Culture can be done using blood early in the course and urine later on but requires special media.

Therapy
- Antibiotic therapy (see Table 239.4) should be initiated as early in the course of the disease as suspicion allows. For mild disease, doxycycline, amoxicillin, or oral ampicillin is recommended. For more severe disease, intravenous ceftriaxone, ampicillin, or penicillin is used.
- Patients with early renal disease with high-output renal dysfunction and hypokalemia should receive aggressive volume repletion and potassium supplementation.
- Patients who progress to oliguric renal failure should undergo rapid initiation of hemodialysis or peritoneal dialysis, which is typically required on only a short-term basis.

Prevention
- The most effective preventive strategy is to reduce direct contact with potentially infected animals and indirect contact with urine-contaminated soil and water.
- Vaccination of agricultural and companion animals is practiced, and vaccines for humans are used in France, Cuba, and parts of Asia.

HISTORY

A syndrome of severe multisystem disease, presenting with profound jaundice and renal function impairment, was described by Weil in Heidelberg in 1886. Other descriptions of disease that probably represent leptospirosis were made earlier, but the etiology cannot be definitively ascribed to leptospiral infection.[2] Leptospires were first visualized in autopsy specimens from a patient thought to have had yellow fever[3] but were not isolated until several years later, almost simultaneously in Germany and Japan.[4] Diagnostic confusion between severe icteric leptospirosis and yellow fever continued, with prominent researchers such as Stokes and Noguchi dying in their attempts to discover the etiologic agent.[4] Several authoritative reviews have been published.[2,4,5,6,7,8]

ETIOLOGY

"Leptospira" derives from the Greek *leptos* (thin) and Latin *spira* (coiled). Aptly named, the leptospires are a mere 0.1 µm in diameter by 6 to 20 µm in length. The cells have pointed ends, one or both of which is usually bent into a characteristic hook (Fig. 239.1). Motility is conferred by the rotation of two axial flagella underlying the membrane sheath, which are inserted at opposite ends of the cell and extend toward the central region.[9] Because of their small diameter, leptospires are best visualized by darkfield microscopy, appearing as actively motile spirochetes (Fig. 239.2). Leptospires are readily cultured in polysorbate-albumin media if specimens are obtained before initiation of antibiotic therapy.[10]

Historically, the genus *Leptospira* was classified into two species, *L. interrogans* and *L. biflexa,* composed of pathogenic and nonpathogenic strains, respectively. Within each species, large numbers of serovars were differentiated using agglutinating antibodies. Serovar specificity is conferred by lipopolysaccharide (LPS) O-antigens.[11] More than 250 serovars of pathogenic leptospires have been described; because of the large number of serovars, antigenically related serovars were grouped into serogroups for convenience in serologic testing.

Leptospires are now classified into a number of species defined by their degree of genetic relatedness, determined by DNA reassociation.[12,13] There are currently 23 named species, including pathogens (e.g., *L. interrogans*), nonpathogenic saprophytes (e.g., *L. biflexa*), and species of indeterminate pathogenicity (e.g., *L. inadai*) (Table 239.1).[14] This classification is supported by 16S RNA gene sequencing (Fig. 239.3)[15] but is quite distinct from the former serologic classification.[5]

The system of serogroup nomenclature has no taxonomic standing but is retained because presumptive serogroup determination by serologic testing has some epidemiologic value. However, serologic responses may bear surprisingly little relationship to the infecting serovar in individual patients.[16]

The genome sequences of several *Leptospira* spp. and strains have been determined,[17,18,19,20,21] and sequencing of another 200 strains is under way through the *Leptospira* Genomics and Human Health Project. The availability of these genome sequences has already led to better understanding of leptospiral pathogenesis.[22]

EPIDEMIOLOGY AND TRANSMISSION

Globally, leptospirosis is a leading zoonotic cause of morbidity and mortality that disproportionately affects vulnerable populations, such as rural subsistence farmers and urban slum dwellers. Human infections

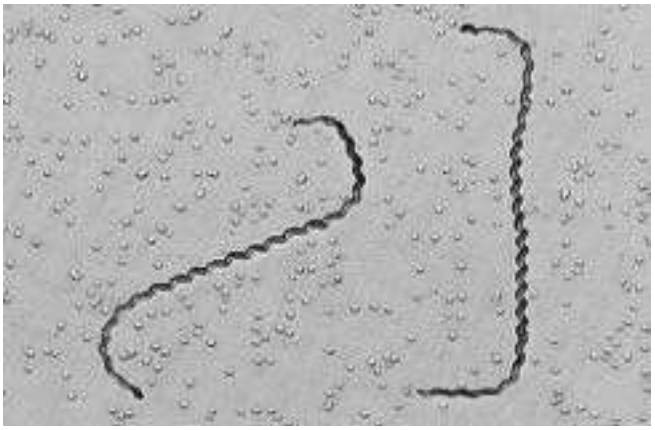

FIG. 239.1 Scanning electron micrograph of cells *leptospira interrogans* showing helical structure and curved (hooked) ends (original magnification ×60,000). *(Courtesy Rob Weyant, Centers for Disease Control and Prevention.)*

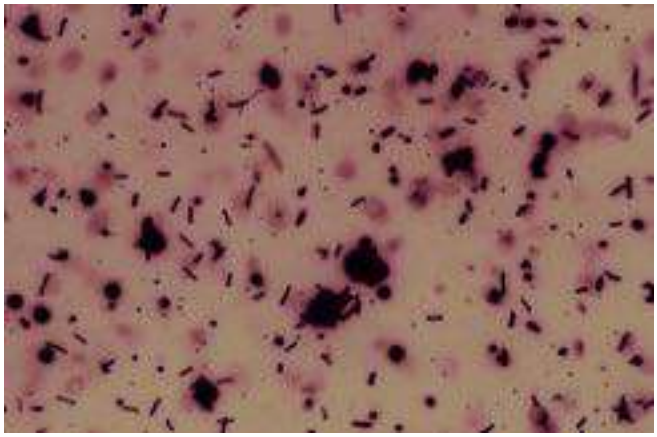

FIG. 239.2 Leptospires viewed by darkfield microscopy (original magnification ×100). *(Courtesy Mildred Galton, Public Health Image Library, Centers for Disease Control and Prevention.)*

are endemic in most regions, and the peak incidence occurs in the rainy season in tropical regions and the late summer to early fall in temperate regions.[23] In developing countries with poor urban housing standards, leptospirosis outbreaks occur regularly after heavy rainfall and flooding.[24] In the United States the highest incidence is found in Hawaii; active surveillance in 1992 detected an annual incidence of approximately 128 per 100,000.[25] Based on a systematic review of 80 published studies and databases from 34 countries, it is estimated that there are 1.03 million cases and 58,900 deaths each year.[26] Morbidity and mortality are greatest in the poorest regions of the world and in areas where surveillance is not routinely performed. It is likely that these figures still underestimated the burden because leptospirosis patients are commonly misdiagnosed with dengue, malaria, and other infections. More important than incidence, estimates suggest that globally ~2.90 million of disability adjusted life-years (DALYs) are lost per annum from the approximately 1.03 million cases.[27] This estimate of DALYs from leptospirosis is more than four times greater than the ~0.83 million DALYs due to dengue.[28]

Leptospirosis is maintained in nature by chronic renal infection of carrier animals. The most important reservoirs are rodents and other small mammals, but livestock and companion animals are also significant sources of human infection. Infection of carrier animals usually occurs during infancy, and once infected, animals may shed leptospires in their urine intermittently or continuously throughout life.

Infection occurs through direct or indirect contact with urine or tissues of infected animals. Direct contact is important in transmission to veterinarians, workers in milking sheds on dairy farms, abattoir

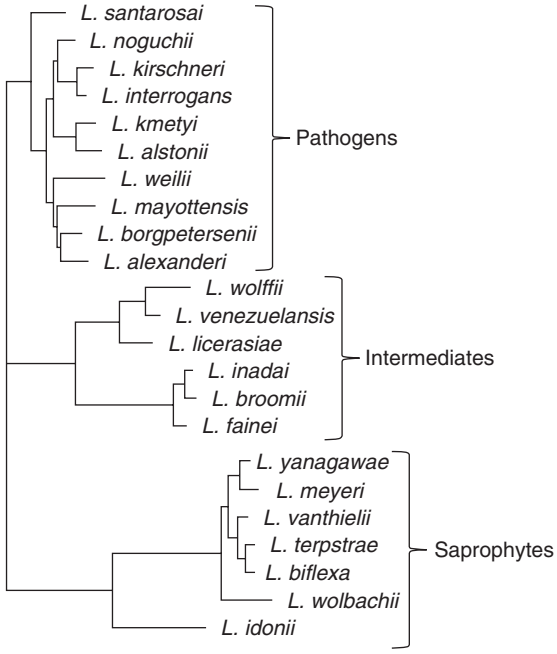

FIG. 239.3 Unrooted phylogenetic tree based on 16s RNA gene sequences of the leptospiraceae obtained from genbank. Species composed of pathogens (see Table 239.1) cluster separately from saprophytes (nonpathogenic species) and species whose pathogenicity is intermediate.

TABLE 239.1 Species of *Leptospira* and Some Pathogenic Serovars

SPECIES	SELECTED PATHOGENIC SEROVARS
L. interrogans	Icterohaemorrhagiae, Copenhageni, Canicola, Pomona, Australis, Autumnalis, Pyrogenes, Bratislava, Lai
L. kirschneri	Bim, Bulgarica, Grippotyphosa, Cynopteri
L. borgpetersenii	Ballum, Hardjo, Javanica
L. mayottensis	Kenya, Mini
L. santarosai	Bataviae
L. noguchii	Panama, Pomona
L. weilii	Celledoni, Sarmin
L. alexanderi	Manhao 3
L. alstonii	Sichuan
L. kmetyi	Manilae
L. inadai	Intermediate
L. fainei	Intermediate
L. broomii	Intermediate
L. wolffii	Intermediate
L. licerasiae	Intermediate
L. venezuelensis	Intermediate
L. wolbachii	Nonpathogen
L. biflexa	Nonpathogen
L. vanthielii	Nonpathogen
L. meyeri	Nonpathogen
L. terpstrae	Nonpathogen
L. yanagawae	Nonpathogen
L. idonii	Nonpathogen

workers, butchers, hunters, and animal handlers; transmission to children handling puppies and to dog handlers has been reported. Males are predominantly affected, with approximately 80% of the total burden.[27] Indirect contact is more common and is responsible for disease after exposure to wet soil or water. The great majority of cases are acquired by this route in the tropics, either through occupational exposure to water, as in rice or taro farming, or through exposure to damp soil and water during avocational activities. Overseas operations place the US military, including military dogs, at risk of leptospirosis. This is particularly true of Special Operations Forces, who work closely with local military.[29]

Recreational exposures have become relatively more important, often in association with adventure tourism to tropical endemic areas. Several large point-source waterborne outbreaks involving attack rates as high as 42% have occurred recently after athletic events.[30,31] In recent years there has been an increase in leptospirosis cases among dogs in the eastern regions of North America and in the Midwest,[32] associated with a shift in the predominant serovars causing disease.[33,34] Veterinary vaccine manufacturers have responded to this problem by licensing new canine vaccines containing these emerging serovars.

PATHOGENESIS

Leptospires enter the body through cuts and abrasions, mucous membranes or conjunctivae, and aerosol inhalation of microscopic droplets. Swallowing contaminated lake water was the only behavioral risk factor identified in a case-control study of a large leptospirosis outbreak at the 1998 Springfield Triathlon.[30] However, the oral mucosae are probably a more important route of entry after ingestion than the intestinal tract. On entering the body there is widespread hematogenous dissemination, with levels as high as 10^6 leptospires per milliliter of blood documented by quantitative polymerase chain reaction (PCR).[35] Organisms readily penetrate tissue barriers, resulting in invasion of the central nervous system and aqueous humor of the eye. Transendothelial migration of spirochetes is facilitated by a systemic vasculitis, accounting for a broad spectrum of clinical illness. Severe vascular injury can ensue, leading to pulmonary hemorrhage, ischemia of the renal cortex and tubular-epithelial cell necrosis, and destruction of the hepatic architecture, resulting in jaundice and liver cell injury with or without necrosis.[36]

The mechanisms by which leptospires cause disease are not clearly understood. Potential virulence factors include immune mechanisms, toxin production, adhesins, and other surface proteins. Human susceptibility to leptospirosis may be related to poor recognition of leptospiral LPS by the innate immune system.[37,38] Human Toll-like receptor 4 (TLR4), which responds to extremely low concentrations of gram-negative LPS (endotoxin), appears to be unable to bind leptospiral LPS,[38,39] perhaps because of the unique methylated phosphate residue of its lipid A.[40] In contrast to human TLR4, mouse TLR4 can recognize leptospiral LPS and has been shown to play an important role in preventing fatal leptospirosis infection in mice.[39,41] Direct tissue damage may also be due to production of hemolytic toxins, which may act as sphingomyelinases, phospholipases, or pore-forming proteins, and collagenases.[42–44]

Immune-mediated mechanisms have been postulated as one factor influencing the severity of symptoms.[45] Investigation of the same triathlon outbreak mentioned earlier identified the human leukocyte antigen (HLA) DQ6 as an independent risk factor for leptospirosis.[46] The structural location of HLA-DQ6 polymorphisms associated with disease suggested that leptospires produce a superantigen that can cause nonspecific T-cell activation in susceptible individuals. Other immune mechanisms, including circulating immune complexes, anticardiolipin antibodies, and antiplatelet antibodies, have been proposed, but their significance is unproven. Although immune or autoimmune mechanisms may play a role in some cases of leptospiral uveitis,[47] a high percentage of such patients have leptospiral DNA in the aqueous humor by PCR.[48]

Much recent work has focused on the roles of surface lipoproteins in leptospiral pathogenesis.[49] The major outer membrane lipoprotein LipL32 is highly conserved among pathogenic serovars.[50] LipL32 is a major target of the human immune response[51] and appears to be involved in pathogenesis of tubulointerstitial nephritis.[52] Virulent leptospires respond to the increased osmolarity of host tissues by inducing expression of the multifunctional Lig surface proteins that mediate interactions with a variety of host proteins, including extracellular matrix factors.[53] The Lig proteins are early antigens; IgM antibodies to their immunoglobulin-like repeats develop early in infection, offering an approach to improved detection of acute infection.[54] The endostatin-like LenA protein binds the complement regulatory protein, factor H, and pathogenic strains exhibit proteolytic activity that cleaves complement, suggesting an important role in serum resistance.[55,56]

CLINICAL MANIFESTATIONS

Leptospiral infection is associated with a broad spectrum of severity, ranging from subclinical illness, followed by seroconversion to two clinically recognizable syndromes: a self-limited, systemic illness seen in roughly 90% of infections and a severe, potentially fatal illness accompanied by any combination of renal failure, liver failure, and pneumonitis with hemorrhagic diathesis.[2,6,7] In some patients the disease has two distinct phases: an initial septicemic stage that is followed by a temporary decline in fever, followed by an immune phase in which the severe symptoms occur. However, in many severe cases the distinction between these two phases is not apparent; in addition, many patients present only with the onset of the second phase of the illness.

The mean incubation period is 10 days, ranging from 5 to 14 days; determination of precise exposures may be difficult, leading to significant imprecision in estimated incubation times. The acute, septicemic phase of illness begins abruptly with high, remittent fever (38°–40°C) and headache, chills, rigors, and myalgias; conjunctival suffusion (redness without exudate); abdominal pain; anorexia, nausea, and vomiting; diarrhea; and cough and pharyngitis. A pretibial maculopapular cutaneous eruption occurs rarely (Table 239.2). Conjunctival suffusion and muscle tenderness, most notable in the calf and lumbar areas, are the most characteristic physical findings but may occur in a minority of cases (see Table 239.2). Other less common signs include lymphadenopathy, splenomegaly, and hepatomegaly. The acute phase lasts from 5 to 7 days. Routine laboratory tests are nonspecific but indicative of a bacterial infection. Leptospires can be recovered from blood and cerebrospinal fluid (CSF) during the acute phase of illness, but meningeal signs are not prominent in this phase. Leptospires may also be recovered from urine, beginning about 5 to 7 days after the onset of symptoms (Fig. 239.4). Urinalysis reveals mild proteinuria and pyuria, with or without hematuria and hyaline or granular casts. Death is rare in the acute phase of illness.

The immune phase of illness generally lasts from 4 to 30 days (see Fig. 239.4). The disappearance of leptospires from the blood and CSF coincides with the appearance of immunoglobulin M (IgM) antibodies.[7,57] The organisms can be detected in almost all tissues and organs and in urine for several weeks, depending on the severity of the disease. In addition to the acute-phase symptoms described in the preceding paragraph, the immune phase may be characterized by any or all of the following signs and symptoms: jaundice, renal failure, cardiac arrhythmias, pulmonary symptoms, aseptic meningitis, conjunctival suffusion with or without hemorrhage; photophobia; eye pain; muscle tenderness; adenopathy; and hepatosplenomegaly (see Table 239.2). Abdominal pain is not uncommon and may be an indication of pancreatitis.

Aseptic meningitis, with or without symptoms, is characteristic of the immune phase of illness, occurring in up to 80% of cases. In endemic areas a significant proportion of all aseptic meningitis cases may be caused by leptospiral infection.[58] Symptomatic patients present with an intense, bitemporal and frontal throbbing headache with or without delirium. A lymphocytic pleocytosis occurs, with total cell counts generally less than 500/mm.[3] CSF protein levels are modestly elevated, between 50 and 100 mg/mL; the CSF glucose concentration is normal. Severe neurologic complications, such as meningoencephalitis, hemiplegia, transverse myelitis, cerebral venous thrombosis, or Guillain-Barré syndrome, occur only rarely.[5,59] Uveitis is reported in horses and humans and is associated with the second phase of illness, perhaps accounting for underreporting.[60,61] An unusual case of chronic meningoencephalitis in an immunocompromised child was diagnosed only by next-generation sequencing.[62]

The most distinctive form of severe illness that may develop after the acute phase of illness is Weil disease, characterized by impaired hepatic and renal function. More severe cases may progress directly

TABLE 239.2 Signs and Symptoms on Admission in Patients With Leptospirosis in Large Case Series

PERCENT WITH:	PUERTO RICO, 1963[150] (*N* = 208)	CHINA, 1965[151] (*N* = 168)	VIETNAM, 1973[152] (*N* = 93)	KOREA, 1987[153] (*N* = 150)	BARBADOS, 1990[63] (*N* = 88)	SEYCHELLES, 1998[154] (*N* = 75)	BRAZIL, 1999[24] (*N* = 93)	HAWAII, 2001[155] (*N* = 353)	INDIA, 2002[156] (*N* = 74)
Jaundice	49	0	1.5	16	95	27	93	39	34
Anorexia	—	46	—	80	85	—	—	82	—
Headache	91	90	98	70	76	80	75	89	92
Conjunctival suffusion	99	57	42	58	54	—	28.5	28	35
Vomiting	69	18	33	32	50	40	—	73	—
Myalgia	97	64	79	40	49	63	94	91	68
Arthralgia	—	36	—	—	—	31	—	59	12
Abdominal pain	—	26	28	40	43	41	—	51	—
Nausea	75	29	41	46	37	—	—	77	—
Dehydration	—	—	—	—	37	—	—	—	—
Cough	24	57	20	45	32	39	—	—	—
Hemoptysis	9	51	—	40	—	13	20	—	35
Hepatomegaly	69	28	15	17	27	—	—	16	—
Lymphadenopathy	24	49	21	—	21	—	—	—	15
Diarrhea	27	20	29	36	14	11	—	53	—
Rash	6	—	7	—	2	—	—	8	12

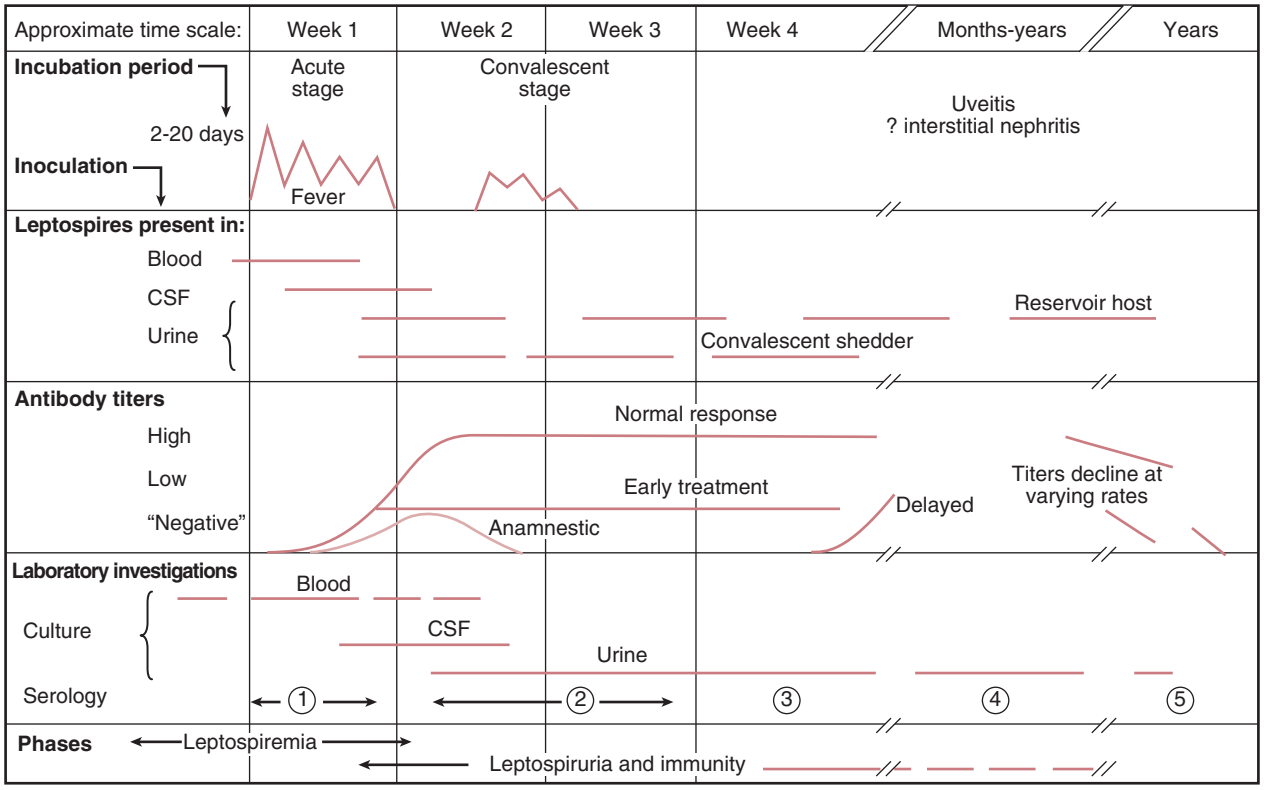

FIG. 239.4 Biphasic nature of leptospirosis and relevant investigations at different stages of disease. Specimens 1 and 2 for serology are acute-phase specimens; 3 is a convalescent-phase sample, which may facilitate detection of a delayed immune response; and 4 and 5 are follow-up samples that can provide epidemiologic information, such as the presumptive infecting serogroup. *CSF,* Cerebrospinal fluid. *(Modified from Turner LH. Leptospirosis.* Br Med J. *1969;1:231–235; and Levett PN. Leptospirosis.* Clin Microbiol Rev. *2001;14:296–326.)*

from the acute phase without the characteristic brief improvement in symptoms to a fulminant illness, with fever greater than 40°C and the rapid onset of liver failure, acute renal failure, hemorrhagic pneumonitis, cardiac arrhythmia, or circulatory collapse.[7] Mortality rates in patients developing severe disease have ranged from 5% to 40%.[2,5,6,63] In a study of 840 hospitalized patients with severe leptospirosis (14% case-fatality rate), risk of death was found to increase with age, especially in adults 40 years of age or older.[24,64] Altered mental status has been found to be the strongest predictor of death.[24,65] Other poor prognostic signs include acute renal failure (oliguria, hyperkalemia, serum creatinine >3 mg/

dL), respiratory insufficiency (dyspnea, pulmonary rales, chest radiograph infiltrates), hypotension, and arrhythmias.[24] In jaundiced patients disturbance of liver function is out of proportion to the rather mild and nonspecific pathologic findings. Conjugated serum bilirubin levels may rise to 80 mg/dL, accompanied by more modest elevations of serum aminotransferases, alanine aminotransferase and aspartate aminotransferase, which rarely exceed 200 IU/L.[66] This is in marked contrast to viral hepatitis. Jaundice is slow to resolve, but death due to liver failure almost never occurs in the absence of renal failure. At autopsy, degenerative changes are seen in hepatocytes, Kupffer cells may be hypertrophied, cholestasis is evident, and erythrophagocytosis and mononuclear cell infiltrates are observed.[67] Hepatocellular necrosis is absent.

Kidney involvement is initially characterized by a unique nonoliguric hypokalemic form of renal insufficiency. Hallmarks are impaired sodium reabsorption, increased distal sodium delivery, and potassium wasting. The impairment in sodium reabsorption appears to be due to selective loss of the epithelial sodium channel in the proximal tubular epithelium. The blood urea nitrogen level is usually less than 100 mg/dL, and the serum creatinine level is usually less than 2 to 8 mg/dL during the acute phase of illness.[68] Thrombocytopenia occurs in the absence of disseminated intravascular coagulation and may accompany progressive renal dysfunction.[69] Renal biopsy reveals acute interstitial nephritis; immune-complex glomerulonephritis may also be present.[70] If electrolyte and volume losses are not replaced, patients progress to oliguric renal failure. In fatal cases the kidneys are swollen and yellow, with prominent cortical blood vessels.[36] Histologic findings include a diffuse, mixed tubulointerstitial inflammatory cell infiltrate of lymphocytes, plasma cells, macrophages, and polymorphonuclear leukocytes, accompanied by focal areas of tubular necrosis.[67] Acute kidney injury in leptospirosis is associated with elevated markers of endothelial injury.[71]

Severe pulmonary hemorrhage syndrome (SPHS) can be a prominent manifestation of infection and may occur in the absence of hepatic and renal failure.[72] Frank hemoptysis can arise simultaneously with the onset of cough during the acute phase of illness.[73] However, hemorrhage is often inapparent until patients are intubated; clinicians should suspect SPHS in patients with signs of respiratory distress whether or not they have hemoptysis. With progressive pulmonary involvement, radiographic abnormalities seen most frequently in the lower lobes evolve from small nodular densities ("snowflake-like") to patchy alveolar infiltrates; confluent consolidation is uncommon but may occur.[74] The pathophysiology of SPHS is consistent with acute respiratory distress syndrome (ARDS) with diffuse lung injury, impaired gas exchange, and hemodynamic changes indicative of septic shock.[75] At autopsy, the lungs appear grossly congested and demonstrate focal areas of hemorrhage.[67] Histologically, damage to the capillary endothelium leads to congestion with foci of interstitial and intraalveolar hemorrhage, diffuse alveolar damage, and severe airspace disorganization.[76] Although inflammatory infiltrates are usually absent, a community-acquired pneumonia has been described in Indonesia, an area of high prevalence, from four patients who presented with fever, dry cough, dyspnea, and conjunctival suffusion or scleral icterus and crackles on physical examination. Infiltrates on chest radiograph were present in all cases, and the diagnosis of leptospiral pneumonia was supported by real-time PCR from multiple specimens and serologic confirmation.[77]

Congestive heart failure occurs rarely. However, nonspecific electrocardiographic changes are common.[78] In more than half of patients receiving continuous cardiac monitoring, cardiac arrhythmias may occur, including atrial fibrillation; flutter and tachycardia; and cardiac irritability, including premature ventricular contractions and ventricular tachycardia.[78] Atrial fibrillation is associated with more severe disease.[79] Cardiovascular collapse with shock can develop abruptly and in the absence of aggressive supportive care can be fatal. At autopsy, interstitial myocarditis with inflammatory involvement of the conduction system is seen[80]; acute coronary arteritis and aortitis are also common at postmortem examination.[81]

LABORATORY DIAGNOSIS

Direct Detection Methods

Direct visualization of leptospires in blood or urine by darkfield microscopic examination has been used for diagnosis (Table 239.3). However, artifacts are commonly mistaken for leptospires, and the method has both low sensitivity (40.2%) and specificity (61.5%).[82] A range of staining methods has been applied to direct detection, including immunofluorescence staining, immunoperoxidase staining, and silver staining. These methods are not widely used because of the lack of commercially available reagents and their relatively low sensitivity.

TABLE 239.3 Common Diagnostic Tests for Leptospirosis

TEST	SENSITIVITY[a,b]	SPECIFICITY[a,b]	COSTS[c]	ADVANTAGES	DISADVANTAGES[d]	REFERENCE
Culture	5%–50%	100%	++	Provides definitive evidence. Applicable to human and veterinary diagnosis.	Slow and requires significant expertise	Levett; WHO[113]
Darkfield microscopy (DFM)	10^4 bacteria/mL	Low; confusion with protein fibers	+	Quick and early diagnosis. Applicable to human and veterinary diagnosis.	Unreliable, requires confirmation	Levett, 2001[5]; WHO[113]
Microscopic agglutination test (MAT)	90%	>90%	+++	Gold standard.[e] Applicable to human and veterinary diagnosis.	Requires a panel of live antigens, difficult (expertise), laborious. Problems with seronegative carrier animals.	Levett, 2001[5]; WHO[113]
IgM–enzyme-linked immunosorbent assay (ELISA)	>90%	88%–95%	++	Cost effective and relatively rapid (1–2 h)	Serology, needs confirmation by MAT	WHO[113]
Latex agglutination test	82%	95%	++	Easy, quick (30 s), cost effective	Serology, needs confirmation by MAT	WHO[113]
Lateral flow test	81%	96%	++	Easy, quick (10 min), finger prick blood, cost effective	Serology, needs confirmation by MAT	WHO[113]
Real-time polymerase chain reaction (PCR)	100%	93%	+++	Early diagnosis. Applicable to human and veterinary diagnosis.	Few tests validated, sophisticated, expensive equipment, requires expertise	Ahmed et al, 2011[90]

[a]Sensitivity and specificity largely depend on a number of factors: stage of illness, type and producer of test, and panel of clinical materials used for testing.[5,157]
[b]Sensitivity of culture depends mainly on the route and transport time to the laboratory. MAT is the gold standard and should be 100%. However, when comparing with culture, this gold standard appears not optimal. Sensitivities of other serologic tests are compared with MAT.
[c]Excluding costs for equipment.
[d]Serology has the disadvantage that it detects antibodies 7–10 days after the onset of the disease. This is too late for antibiotic treatment, which should start within the first 4 days.
[e]Serologic confirmation requires testing of both acute and convalescent serum samples for seroconversion or significant titer rise.
Modified from Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clin Microbiol Infect. *2011;17:494–501.*

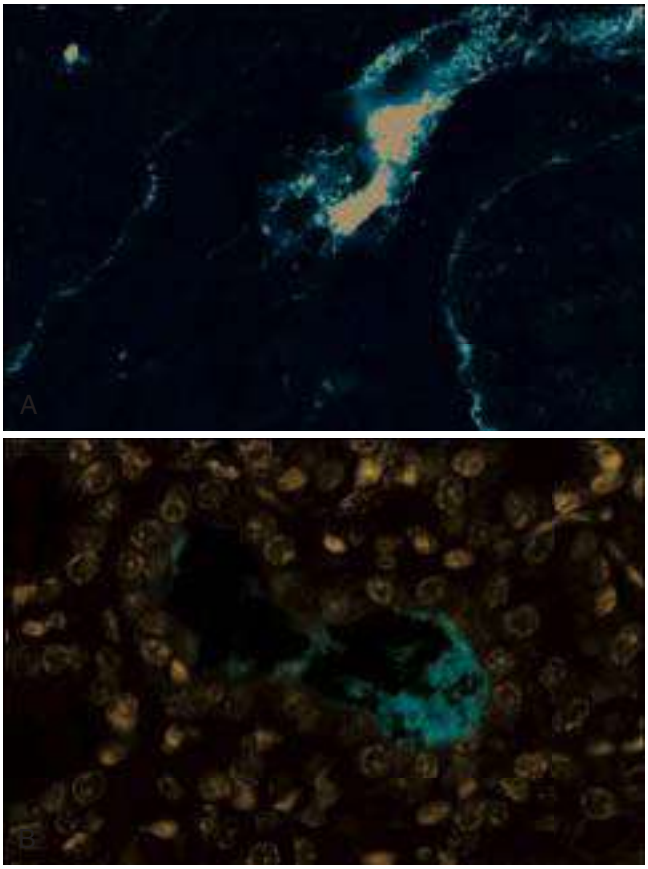

FIG. 239.5 Kidney sections stained by silver staining (A) and immunohistochemical staining (B), showing presence of multiple leptospires in tubules. *(A from Barnett JK, Barnett D, Bolin CA, et al.* Infect Immun. *1999;67:853–861.[149] B from Haake DA, Chao G, Zuerner RL, et al.* Infect Immun. *2000;68:2276–2285.)*

Detection of leptospiral antigen in blood or urine has been attempted but without significant success. Many PCR assays[83–85] and several loop-mediated isothermal amplification assays[86–88] have been developed for the detection of leptospires, but relatively few have been evaluated in prospective clinical studies,[88,89] and there have been no multicenter studies of multiple molecular diagnostic methods. The chief advantage of PCR is the prospect of confirming the diagnosis during the early acute (leptospiremic) stage of the illness, before the appearance of IgM antibodies, when treatment is likely to have the greatest benefit.[90] For early diagnosis, serum or plasma might be the optimal specimen, but urine has been often positive, even early in the course.[91] In fulminating cases, in which death occurs before seroconversion, PCR may be of great diagnostic value.[83,92] Histologic diagnosis (Fig. 239.5) traditionally relied on silver impregnation staining,[3] but immunohistochemical staining offers greater sensitivity and specificity.[93,94]

Isolation and Identification

Leptospires can be isolated from blood, CSF, and peritoneal dialysate fluids during the first 10 days of illness. Specimens should be collected while the patient is febrile and before antibiotic therapy is initiated. One or two drops of blood should be inoculated directly into culture medium at the bedside. Survival of leptospires in commercial blood culture media or refrigerated blood samples for several days has been reported.[95,96] Urine can be cultured after the first week of illness. Specimens should be collected aseptically into sterile containers without preservatives and must be processed within a short time of collection; best results are obtained when the delay is less than 1 hour because leptospires do not survive well in acidic environments.[10]

Cultures are performed in albumin-polysorbate media such as EMJH (Ellinghausen-McCullough-Johnson-Harris),[97] which is available commercially. Primary cultures are performed in semisolid (0.2% agar) medium, to which 5-fluorouracil is usually added as a selective agent. Cultures are incubated at 30°C for several weeks because initial growth may be slow.

Isolated leptospires are identified to serovar level by traditional serologic methods.[98,99] These techniques are limited in availability to a few reference laboratories. However, molecular methods such as pulsed-field gel electrophoresis show promise for rapid identification of most isolates.[100] Identification to species level is accomplished by sequencing the 16S ribosomal RNA gene of isolates[15] or by sequencing the products of PCR directly.[90,101] Powerful molecular techniques, such as multiple locus sequence typing and multilocus variable-number tandem-repeat analysis, are being applied to the epidemiologic analysis of leptospirosis.[102–105] As more genomic data become available, these approaches are becoming more standardized.[106–109] In addition to molecular typing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has been investigated for identification of *Leptospira* isolates.[110]

Indirect Detection Methods

The majority of leptospirosis cases are diagnosed by serology. The reference standard assay is the microscopic agglutination test (MAT), in which live antigens representing different serogroups of leptospires are reacted with serum samples and then examined by darkfield microscopy for agglutination.[10] Due to regional variations in infecting strains, it has been considered important to include local serovars in a laboratory's MAT panel. Recently, this view has been challenged.[111] The MAT is a complex test to maintain, perform, and interpret, and participation of the laboratory in a proficiency testing program is essential to maintaining accuracy.[112] Use of the MAT is restricted to a few reference laboratories.

A serologically confirmed case of leptospirosis is defined by a fourfold rise in MAT titer to one or more serovars between acute-phase and convalescent serum specimens run in parallel.[113] A single titer of at least 1:800 in the presence of compatible symptoms is strong evidence of recent or current infection.[114] Suggestive evidence for recent or current infection includes a single titer of at least 1:200 obtained after the onset of symptoms.[115] Delayed seroconversions are common, with up to 10% of patients failing to seroconvert within 30 days of the clinical onset. Cross-reactive antibodies may be associated with syphilis, relapsing fever, Lyme disease, viral hepatitis, human immunodeficiency virus infection, legionellosis, and autoimmune diseases.[116]

The interpretation of the MAT is complicated by cross-reaction between different serogroups, especially in acute-phase samples.[5] Cross-reactivity in acute samples is attributable to IgM antibodies, which may persist for several years.[117] The MAT is a serogroup-specific assay and should not be used to infer the identity of the infecting serovar.[16] However, knowledge of the presumptive serogroup may be of epidemiologic value in determining potential exposures to animal reservoirs.

Diagnostic application of the MAT is limited by the relatively low sensitivity when acute serum samples are tested.[118] Other agglutination assays that detect total immunoglobulins, such as the indirect hemagglutination assay, suffer from similarly low sensitivities on acute specimens but have high case sensitivities when acute and convalescent specimens are tested.[119] IgM antibodies are detectable after about the fifth day of illness, and IgM-detection assays are available in several formats.[119–122] Use of these assays as screening tests offers the potential to enhance the diagnostic capacity of many laboratories, particularly in developing countries, where most cases of leptospirosis occur.[123] The modified Faine criteria scoring system, which combines serologic results with clinical history and epidemiologic factors, may be useful in some settings.[124] The persistently unsatisfactory state of diagnostics, especially in early disease, is summarized in a recent study and a recent review, which highlight the general problem of lacking a gold-standard diagnostic assay by which to assess the utility of tests that are affordable and require less extensive laboratory capacity.[125,126]

THERAPY

Antibiotic therapy should be initiated as early in the course of the disease as suspicion allows. There have been few randomized or placebo-controlled trials,[127,128,129,130] and these have produced conflicting results.

TABLE 239.4 Antimicrobial Agents Recommended for Treatment and Chemoprophylaxis of Leptospirosis

INDICATION	COMPOUND	DOSAGE
Chemoprophylaxis	Doxycycline	200 mg PO orally once per week
Treatment of mild leptospirosis	Doxycycline Ampicillin Amoxicillin	100 mg bid PO 500–750 mg q6h PO 500 mg q6h PO
Treatment of moderate-to-severe leptospirosis	Penicillin Ceftriaxone Ampicillin	1.5 MU IV q6h 1 g IV q24h 0.5–1 g IV q6h

bid, Twice daily; *IV,* intravenously; *MU,* million units; *PO,* orally.

Therapeutic benefits of antibiotics may be difficult to demonstrate in populations where patients present for medical care with late or severe disease, or both. Nevertheless, severe disease is usually treated with intravenous penicillin and mild disease with oral doxycycline (Table 239.4). Once-daily ceftriaxone has been shown to be as effective as penicillin.[131] Jarisch-Herxheimer reactions have been reported in patients treated with penicillin.[132] Patients receiving penicillin should be monitored because of the increased morbidity and mortality of such reactions.

Supportive therapy is essential for hospitalized patients. Patients with early renal disease with high-output renal dysfunction and hypokalemia should receive aggressive volume repletion and potassium supplementation to avoid severe dehydration and acute tubular necrosis.[68] In patients who progress to oliguric renal failure, rapid initiation of daily hemodialysis reduces mortality, particularly in critically ill patients, and is typically required on only a short-term basis. Renal dysfunction caused by leptospirosis is typically completely reversible.[133,134] Patients requiring intubation for SPHS have decreased pulmonary compliance and should be managed as cases of ARDS. Protective ventilation strategies involving low tidal volumes (<6 mL/kg) to avoid alveolar injury due to high ventilation pressures have been shown to dramatically improve survival rates.[135]

PREVENTION

Prevention of leptospirosis may be achieved by avoidance of high-risk exposures, adoption of protective measures, immunization, and use of chemoprophylaxis, in varying combinations depending on the environmental circumstances and the degree of human activity.

High-risk exposures include immersion in fresh water, as in swimming, and contact with animals and their body fluids.[2] Removal of leptospires from the environment is impractical, but reducing direct contact with potentially infected animals and indirect contact with urine-contaminated soil and water remains the most effective preventive strategy available. Consistent application of rodent control measures is important in limiting the extent of contamination. Appropriate protective measures depend on the activity but include wearing boots, goggles, overalls, and rubber gloves. In tropical environments walking barefoot is a common risk factor.[136] In an urban area of Thailand with high environmental contamination and unavoidable exposure to flood waters, a single dose of doxycycline, 200 g, was considered to have protective efficacy, particularly for flood victims with lacerations, in whom efficacy was estimated to have been 92%.[137]

Immunization of agricultural and companion animals with killed vaccines is widely practiced both to prevent disease and to reduce human exposure. Vaccination of animals at an early age before exposure is crucial in achieving optimum reduction of shedding.[138] Periodic (usually annual) boosters are required to maintain immunity.[139] Current bovine and porcine vaccines used in the United States contain serovars Icterohaemorrhagiae, Canicola, Grippotyphosa, Pomona, and Hardjo, whereas canine vaccines contain all except serovar Hardjo. New vaccines for bovine use stimulate a type 1 cell-mediated immune response against serovar Hardjo[140,141] and appear to protect against renal colonization and urinary shedding.[142]

Human immunization is not widely practiced. A vaccine containing serovar Icterohaemorrhagiae is available in France for workers in high-risk occupations,[143] and a vaccine has been developed for human use in Cuba.[144] Immunization has been more widely used in Asian countries to prevent large-scale epidemics in agricultural laborers.

For individuals who will be unavoidably exposed to leptospires in endemic environments, chemoprophylaxis is recommended (see Table 239.4). Weekly doxycycline (200 mg) has been shown to be effective in military personnel without previous exposure who underwent jungle training.[145] The use of doxycycline prophylaxis after excess rainfall in local populations in endemic areas has been studied.[146,147] Symptomatic disease was significantly reduced in one study, but serologic evidence of infection was found equally in subjects and controls.[146] Limitations of doxycycline are its photosensitivity, high frequency of gastrointestinal side effects, dietary calcium restrictions, and contraindication in pregnant women and children. In vitro susceptibility of leptospires to azithromycin[148] and its longer serum half-life suggest that this agent would be a reasonable alternative to doxycycline; however, clinical trials are necessary to validate this approach.

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The complete reference list is available online at Expert Consult.

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240 Relapsing Fever Caused by *Borrelia* Species

James M. Horton

SHORT VIEW SUMMARY

Definition
- Relapsing fever is caused by spirochetes of the *Borrelia* genus.
- The illness is characterized by relapsing fevers with spirochetes evident on a blood smear.

Organism
- *Borrelia* spp. are divided between *Borrelia burgdorferi,* which causes Lyme disease (see Chapter 241), and the *Borrelia* spp. that cause relapsing fever.
- The relapsing fever species are divided between louse-borne and tick-borne species.

Epidemiology
- Tick-borne relapsing fever occurs on almost every continent, but in the United States it is endemic in the Rocky Mountains.

Pathophysiology
- The spirochete changes surface antigens about every 7 days, causing the cyclic, relapsing fever.

Clinical Manifestations
- The patient presents with fever for 3 days alternating with afebrile periods lasting about 7 days.

Diagnosis
- Spirochetes can be seen on the peripheral blood smear during febrile periods.

Therapy
- Doxycycline or penicillin is effective treatment.
- Jarisch-Herxheimer reactions are common, and the patient should be observed in a clinic or hospital for at least 3 hours after starting antibiotic therapy.

Prevention
- Postexposure therapy with doxycycline is preventive.

Relapsing fever is characterized by recurrent fevers with spirochetemia caused by organisms of the *Borrelia* genus.[1] The spirochetes are broadly divided between endemic tick-borne species (tick-borne relapsing fever [TBRF]) and epidemic louse-borne species (louse-borne relapsing fever [LBRF]). TBRF occurs in North and South America, Europe, Africa, and Asia and can be caused by about 20 different species.[2,3] In the United States, relapsing fever is endemic in the Rocky Mountain regions of the western states but can also occur in travelers returning from other endemic areas.[4,5] LBRF was epidemic in the early 20th century but has subsided as a result of improved sanitation. Currently LBRF is found only on the Horn of Africa and possibly among the homeless in Europe.[6]

ORGANISM

The genus *Borrelia* belongs to the family Spirochaetaceae. It consists of two groups: species that cause Lyme disease and species that cause relapsing fever (Table 240.1).[7] The relapsing fever spirochetes are genetically diverse pathogens that are broadly divided between the louse-borne *Borrelia recurrentis* and the tick-borne species. The TBRF agents are maintained by soft ticks of the family Argasidae. Some species such as *Borrelia lonestari* and *Borrelia miyamotoi* group genetically with the classic relapsing fever spirochetes but are spread by other tick species.[7] *B. lonestari* is spread by the Lone Star tick, *Amblyomma americanum.* In one study it was associated with southern tick–associated rash illness by polymerase chain reaction (PCR) assay, but further studies were unable to confirm that result.[8] *B. miyamotoi,* which is spread by *Ixodes dammini* or *Ixodes scapularis,* has been described in Japan, Europe, Russia, and the United States. Molloy and coworkers[9] described 97 cases across the northeastern United States diagnosed by PCR (see Table 240.1).[8,10]

The pathogens are spirochetes that are 8 to 30 µm long and 0.2 to 0.5 µm wide.[2] The spirochetes have spontaneous antigenic variation of the outer membrane proteins called the variable major proteins (*vmp*).[11] These proteins determine the serotypes and are the mechanism for the relapsing disease. *Borrelia* strains can grow in artificial media (Barbour-Stoenner-Kelly medium),[12] although the diagnosis is usually confirmed by microscopic examination of a blood smear. The spirochetes are readily visualized with Wright or Giemsa stains, but species cannot be distinguished by the staining characteristics. The spirochetes can survive in the tick vectors for up to 12 years; ticks remain the optimal means of maintaining organisms.[13]

Ticks are divided between the soft-bodied Argasidae ticks and the hard-bodied Ixodid ticks. Soft-bodied ticks of the Argasidae family are vectors for most TBRF and are strongly associated with rodents. Ticks become infected during a blood meal on a vertebrate with spirochetemia. Humans become infected by tick bites via salivary gland secretions.[2] The hard-bodied ticks are the vectors for *B. miyamotoi*.[10]

In the Rocky Mountain western states, relapsing fever is caused predominantly by *Borrelia hermsii,* but it can also be caused by *Borrelia parkeri, Borrelia turicatae,* and *Borrelia mazzotti.* Each TBRF *Borrelia* spp. is associated with one species of transmitting soft-bodied ticks of the *Ornithodoros* genus. In the western United States, *B. hermsii* is transmitted by *Ornithodoros hermsi.*[2]

LBRF is caused only by *B. recurrentis* and is spread by the human body louse *(Pediculus humanus).* The spirochetes do not penetrate through the louse intestine, so the disease is spread by crushing or squeezing the louse, releasing the organisms on the skin.[2,15]

EPIDEMIOLOGY AND TRANSMISSION

TBRF and its host, the *Ornithodoros* tick, occur on every continent except Australia and Antarctica (see Table 240.1).[2] Although the host and habitat of each *Ornithodoros* tick species may vary, they have some characteristics in common. The ticks are obligate blood feeders, and the spirochetes from a blood meal invade all tissues of the tick within hours. The spirochetes can persist for years in the tick salivary glands, facilitating the transmission during the short feeding period of 20 minutes. Infection of humans occurs when saliva or excrement is released during feeding. Animal reservoirs include chipmunks, squirrels, rabbits, rats, mice, owls, and lizards. The ticks inhabit rodent burrows, decaying wood, cabins, animal shelters, and caves.[16]

Intrusion of humans into the tick's environment creates the opportunity for disease transmission. In the United States, the largest epidemic occurred in 1973 when 62 campers became ill after sleeping in log cabins in Arizona.[17] In North America, TBRF occurs in the Rocky Mountain regions (Fig. 240.1) of the western states at altitudes above 1500 feet, and most cases occur during the summer months, although cases have been reported in the winter.[4,17] In Texas, most cases occur

TABLE 240.1 *Borrelia* Species That Cause Relapsing Fever

SPECIES	DISEASE	ARTHROPOD VECTOR	GEOGRAPHIC DISTRIBUTION	RESERVOIR
B. hermsii	TBRF	*Ornithodoros hermsi*	Western United States and Canada	Rodent
B. turicatae	TBRF	*Ornithodoros turicata*	Southwestern United States	Rodent
B. parkeri	TBRF	*Ornithodoros parkeri*	Western United States and Baja California	Rodent
B. mazzottii	TBRF	*Ornithodoros talaje*	Mexico and Central America	Rodent
B. venezuelensis	TBRF	*Ornithodoros rudis*	South America	Rodent
B. crocidurae	TBRF	*Ornithodoros erraticus*	Middle East, North Africa	Rodent
B. hispanica	TBRF	*Ornithodoros marocanus*	Iberian peninsula and North Africa	
B. recurrentis	LBRF	*Pediculus humanus*	Eastern Africa, previously worldwide	
B. miyamotoi[2]	Meningitis,[a] febrile illness	*Ixodes dammini* *Ixodes scapularis*	United States, Europe and Asia	Rodent
B. lonestari[a]	Illness association unclear[8]	*Amblyomma americanum*	Southern United States	Deer

[a]Presumed pathogen, Koch's postulates not fulfilled.
LBRF, Louse-borne relapsing fever; *TBRF,* tick-borne relapsing fever.
Modified from Southern PM, Sanford JP. Relapsing fever: a clinical and microbiological review. Medicine. *1969;48:129–149; and Barbour A, Hayes S. Biology of* Borrelia *species.* Microbiol Mol Biol Rev. *1986;50:381–400; based on Gugliotta JL, Goethert HK, Berardi BS, et al. Meningoencephalitis from* Borrelia miyamotoi *in an immunocompromised patient.* N Engl J Med. *2013;368:240–245.*



FIG. 240.1 Cases of tick-borne relapsing fever—United States, 1990–2011. One dot is placed randomly within county of exposure where known; shading indicates states where tick-borne relapsing fever was reportable. *(From Forrester JD, Kjemtrup AM, Fritz CL, et al. Tickborne Relapsing fever—United States 1990-2011.* MMWR Morb Mortal Wkly Rep. *2015;64:58–60. With permission from the Centers for Disease Control and Prevention.)*

during the winter and are associated with spelunking.[14]) Outbreaks have been reported in Arizona, California, Colorado, Montana, New Mexico, Washington, and British Columbia; however, most illness is sporadic.[5,12,18] Most patients have a history of exposure to a cabin or woodpile with rodent infestation. The ticks feed for less than 1 hour, and bites are painless, so many patients give no history of a tick bite.[17] The ticks feed nocturnally. TBRF occurs more commonly in male patients, and most patients are younger than 20 years.[1] TBRF has also been transmitted by transfusion, transplacentally, and by laboratory exposure.

Because TBRF occurs on almost every continent (see Table 240.1), the diagnosis should be considered in a febrile patient returning from

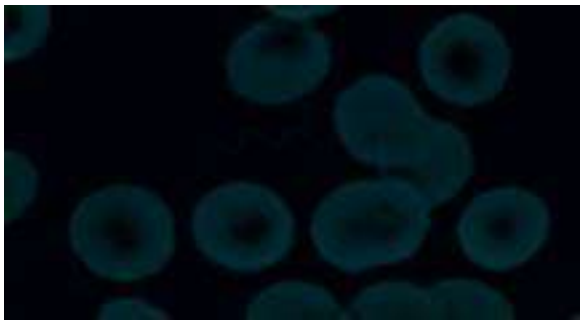

FIG. 240.2 Peripheral blood smear from a patient with tick-borne relapsing fever acquired in Idaho (wright stain). *(From Rhee KY, Johnson WD. Borrelia species (relapsing fever). In Mandell GL, Bennett JE, Dolin R, eds.* Principles and Practice of Infectious Diseases. *6th ed. Philadelphia: Churchill Livingstone; 2005.)*

Central America, Africa, the Middle East, or southern Europe. In West Africa, as the number of cases of malaria has decreased, the percentage of febrile patients with TBRF has increased to 7.3%. In some districts, cases of TBRF exceed malaria.[43]

LBRF was epidemic in the early 20th century throughout Eurasia and Africa. The last major epidemic occurred during World War II in Africa and Europe when an estimated 50,000 people died of the infection under conditions of crowding, such as in displaced-person camps. Today it is largely confined to Ethiopia and the surrounding area, where an estimated 10,000 people a year have LBRF.[20,21] LBRF has been suspected to cause illness in homeless people in France and has been described among refugees from northeast Africa to Europe.[19,22]

PATHOPHYSIOLOGY

Perhaps the most interesting aspect of this disease is the relapsing pattern of fever and the antigenic changes in the spirochete. During the febrile periods, spirochetes are dividing rapidly and become visible on the blood smear (Fig. 240.2).[1] With LBRF, concentrations of more than 100,000 organisms/mm^3 of blood have been described.[24] With the development of a specific immune response to the dominant *vmp*, the organisms are cleared from the blood, resulting in the afebrile period; however, the spirochete then express a different *vmp*, resulting in recurrent spirochetemia and fever. Type-specific immunity suppresses this new antigenic pathogen, and the cycle of fever and afebrile periods begins again. The cyclic process of antigenic variation followed by specific antibody production is responsible for the relapsing course of the illness.[1,2]

TABLE 240.2 Summary of Clinical Features of Relapsing Fever

FEATURE	MEAN VALUE OR INCIDENCE	
	Louse-Borne Disease	Tick-Borne Disease
Case-fatality rate	4%–40%	2%–5%
Incubation period	8 days (range, 4–18 days)	7 days (range, 4–18 days)
Duration of first febrile attack	5.5 days	3 days
Duration of afebrile interval	9 days	7 days
Duration of relapses	2 days	2–3 days
Number of relapses	1–2 (range, 1–5)	3 (range, 0–13)
Maximal temperature	101°F–102°F	105°F
Splenomegaly	77%	41%
Hepatomegaly	66%	17%
Jaundice	6%	7%
Rash	8%	28%
Respiratory symptoms	34% (cough)	16%
Central nervous system involvement	30%	9%

Modified from Southern PM, Sanford JP. Relapsing fever: a clinical and microbiological review. Medicine. *1969;48:129–149.*

TABLE 240.3 Treatment of *Borrelia* Relapsing Fever

ORGANISM	TREATMENT	DOSE	DURATION
LBRF[a]	Tetracycline	500 mg	Once
	Doxycycline	200 mg	Once
	Procaine penicillin G	400,000–800,000 U IM	Once
	Erythromycin	500 mg	Once
TBRF[b]	Tetracycline	500 mg qid	10 days
	Doxycycline	100 mg bid	10 days
	Erythromycin	500 mg qid	10 days
TBRF involving central nervous system[c]	Penicillin G	3 million U IV q4h	10–14 days
	Ceftriaxone	2 g/day IV or 1 g bid IV	10–14 days

[a]Based on clinical trials.[21,39]
[b]Based on review of sporadic cases.[4,16]
[c]Based on case reports and extrapolation from other spirochetal diseases of the central nervous system.[27]
IM, Intramuscular; *IV,* intravenous; *LBRF,* louse-borne relapsing fever; *TBRF,* tick-borne relapsing fever.

The antigenic shift is due to a "loose form of programming," with the antigens usually occurring in the same sequence.[25] With the third episode, the spirochete has mixtures of serotypes.[25] The *vmp* genes reside on linear plasmids, and activation of a new *vmp* results from recombination between different plasmids.[25]

Pathologic findings at autopsy include hepatitis, miliary splenic abscesses, and central nervous system hemorrhages with perivascular infiltrates. Hemorrhagic gastrointestinal and renal lesions have been described.[1]

CLINICAL MANIFESTATIONS

After a median incubation period of 7 days (range, 2–18 days), the patient has fever lasting about 3 days (range, 2–7 days), alternating with afebrile periods lasting about 7 days (range, 4–14 days) (Table 240.2).[1] If untreated, a patient with TBRF can have 30 relapses of the febrile illness, whereas LBRF is usually associated with only 1 relapse. Most patients have at least two relapses of fever before the disease is diagnosed.[16] The febrile episodes can be accompanied by myalgias, arthralgias, headache, dizziness, and vomiting.[16] Lymphadenopathy, hepatosplenomegaly, and a rash occur in less than one-third of patients.[1] Neurologic complications include lymphocytic meningitis, altered mental status, Bell palsy, other cranial nerve palsies, paralysis, and seizures.[1,26,27] Uveitis and endophthalmitis have been reported.[28] Acute respiratory distress syndrome from relapsing fever has been reported in the United States.[29] Myocarditis occurs infrequently and can cause arrhythmias.[5] Systemic inflammatory response syndrome has been reported and can be associated with abnormal liver enzymes, azotemia, hypoxia, and acute respiratory distress syndrome.[5] In some cases the patient presents with a sepsis syndrome from a Jarisch-Herxheimer reaction after receiving an empirical antibiotic such as ciprofloxacin or amoxicillin for a presumed bacterial infection.[30,31] Usually, each febrile relapse becomes less severe. Relapsing fever during pregnancy is associated with increased maternal and fetal mortality and has been associated with spontaneous abortions.[1,4]

TBRF must be differentiated from Colorado tick fever, brucellosis, tularemia, plague, juvenile rheumatoid arthritis, leptospirosis, and occult malignancy.[32] Relapsing fever and Lyme disease share similar neurologic manifestations[33]; the serologic assays cross react, making the differentiation between these two diseases difficult.[16] In one series, 7 of 16 patients with TBRF in Idaho had false-positive test results for Lyme disease.[16]

For LBRF, the differential diagnosis includes typhus, malaria, typhoid fever, leptospirosis, hepatitis, and dengue. In patients from tropical countries, relapsing fever must be differentiated from malaria, and the two illnesses can occur simultaneously.[34] A coexisting louse-borne typhus epidemic can make early diagnosis difficult. The relapsing fever, epidemiology, and spirochetes on smear will confirm the LBRF diagnosis.[1]

On laboratory testing, the leukocyte count ranges from 3000 to 16,000 cells/mm^3.[1] In patients with neurologic symptoms, the cerebrospinal fluid has a pleocytosis (15–2200 cells/mm^3 with a protein elevation up to 160 mg/dL).[1] Spirochetes are found in the cerebrospinal fluid on microscopy in 12% of patients with neurologic symptoms.[1] Elevated lactate dehydrogenase and creatine kinase levels have been reported.[35]

DIAGNOSIS

Relapsing fever is diagnosed by identifying the spirochetes on a Giemsa or Wright blood smear (see Fig. 240.2).[1] An acridine orange stain can also identify the spirochetes on the smear.[4,36] The diagnosis of TBRF is sometimes first suspected by the hematology laboratory technician.[17] The sensitivity of the blood smear during the febrile period is about 70% in TBRF.[1] The sensitivity of the blood smear is less during the afebrile periods, and thick smears might have a higher susceptibility.[1]

Negative blood smears, even when repeated, do not exclude relapsing fever.[31] In LBRF the number of spirochetes may be too low to be detected by routine blood smear; an acridine orange stain of the buffy coat technique was more sensitive in one report.[37] The spirochetes were concentrated in the red blood cell layer.[37]

Acute and convalescent serologic testing with indirect fluorescent antibody and enzyme-linked immunosorbent assay can be used in patients with negative blood smears.[20] Cross-reactivity of the serology tests with *Borrelia burgdorferi* has been reported in one case.[26] Positive serologic tests for syphilis (Venereal Disease Research Laboratory test) occur in 5% of patients with relapsing fever and are thought to represent false-positive results.[1] PCR assay has been used to diagnose *B. hermsii* bloodstream infection.[38] The diagnosis can also be confirmed by mouse inoculation or culture in modified Kelly medium.[12]

THERAPY

Doxycycline, tetracycline, and penicillin are the preferred antibiotic agents for relapsing fever.[1,16] The in vitro susceptibilities indicate that the minimal inhibitory concentrations to penicillin and tetracycline are less than 0.1 mEq/mL.[31] Macrolides, cephalosporins, and chloramphenicol have in vitro activity, but clinical data on their use are limited. The spirochetes are usually resistant in vitro to fluoroquinolones, sulfonamides, and aminoglycosides (Table 240.3).

Antibiotic selection for TBRF is based on case reports and retrospective series.[4,16] Most patients with TBRF are treated for 7 to 10 days with doxycycline (100 mg twice a day) or tetracycline (500 mg four times a day). If tetracyclines are contraindicated, erythromycin (500 mg four times a day) may be used. Relapses may occur with shorter courses of

antibiotics.[1,4] Animal studies suggest that relapses might be due to residual spirochetes in the central nervous system. The placenta can be a reservoir for spirochetes in pregnant patients.[4] Meningitis or encephalitis should be treated with parenteral antibiotics such as penicillin G or ceftriaxone for 14 days. The spirochetes should clear from the blood within 8 hours.

LBRF treatment is based on larger clinical trials.[39] Tetracycline in a single oral dose (0.5 g) is the preferred therapy for LBRF.[21,39] Doxycycline (200 mg) or penicillin G procaine (400,000–800,000 U intramuscularly) has been used.[21,39] For pregnant women or small children, erythromycin, 0.5 g in a single dose, is also effective.[21] Again, the spirochetes should clear from the blood within 8 hours.

Jarisch-Herxheimer reactions with rigors, fever, and leukopenia occur in about half of the patients after the first antibiotic dose.[4,16] Although the reaction usually occurs within 2 hours of the first antibiotic dose, it can occur more than 3 hours later, so all patients should be observed for several hours after the initial antibiotic dose.[4,16] This complication can be accompanied by hypotension, respiratory distress syndrome, and death.[4,16,24] One study reported that patients with microhematuria are less likely to have a Jarisch-Herxheimer reaction, but the pathophysiology of this association is not known.[16] Other factors such as age, sex, proteinuria, the presence of juvenile (band) neutrophils on the white blood cell differential, and choice of antibiotic do not affect the risk for Jarisch-Herxheimer reactions in TBRF.[16] One study found that low-dose penicillin was less likely than tetracycline to provoke a Jarisch-Herxheimer reaction in LBRF, but the patients had a higher relapse rate.[40] Prior administration of hydrocortisone does not prevent the reaction. Persistence of fever in a patient with relapsing fever from a tropical country should prompt a search for a coinfection such as malaria or typhoid fever.[33,34]

Without treatment, the mortality rate of TBRF approaches 10%,[17] but the average mortality rate is about 3%. Untreated epidemic LBRF has a mortality of 40%.[1] The prognosis is worse in LBRF, with altered consciousness, jaundice, pulmonary edema, rash, Jarisch-Herxheimer reaction, vomiting, and delay in treatment.[34]

PREVENTION

Prevention of relapsing fever requires avoidance or elimination of the arthropod vectors. In developed countries, avoidance of cabins and woodpiles with rodents, elimination of rodent nests, and use of insect repellents containing DEET (*N,N*-diethyl-*m*-toluamide) can prevent TBRF.[17] Fumigation with pyrethrin or permethrin has been used, but failures to prevent disease have been reported.[17] Good personal hygiene and, if necessary, delousing procedures prevent LBRF. DDT (dichlorophenyltrichloroethane) was widely used during World War II, but DDT-resistant louse strains have subsequently developed. Other insecticides such as dimethyl dithiophosphate (malathion) are now used for delousing (see Chapter 292).[41] LBRF can be controlled by basic hygiene including washing of clothes of refugees from endemic countries.[23] Postexposure prophylaxis with doxycycline, 200 mg on day 1 followed by 100 mg daily for 4 days, has been effective in preventing TBRF.[42]

Borrelia miyamotoi

B. miyamotoi is genetically similar to the other relapsing fever spirochetes but has significant differences in clinical presentation and diagnosis. It is transmitted by the hard-shelled *Ixodes* ticks that also transmit Lyme disease.[44] It is associated with the small rodents that are also associated with Lyme disease in eastern North America, Asia, and Europe[45,46] and so occurs mostly in the spring and summer. Cases have been reported in the United States, Europe, Russia, and Japan.[47] In areas endemic for Lyme disease, antibodies to *B. miyamotoi* are found in 1% of healthy blood donors and 3% of patients with suspected Lyme disease.[48] In The Netherlands, 10% of forestry workers are seropositive for *B. miyamotoi*.[49] The incubation period is 12 to 16 days followed by symptoms of nonspecific fever, myalgia, arthralgias, and headache.[50] Less common presentations include lymphadenopathy, relapsing fever, neurologic symptoms, and cognitive symptoms.[23,47] A relapsing fever course was present in 11% of patients.[50] Nine of 48 patients (20%) had erythema migrans skin lesions, but some may have had coinfection with Lyme disease.[50] Laboratory findings include mild leukopenia and transaminase elevation.[50] Patients with central nervous system involvement can have a lymphocytic pleocytosis on cerebrospinal fluid analysis.[7] In most immunocompetent patients, *B. miyamotoi* has no long term sequelae, but two cases of cognitive decline and gait disturbances over months have been described.[7,47] Coinfections with other *Ixodes*-transmitted pathogens have occurred including Lyme disease, babesiosis, human granulocytic anaplasmosis, and relapsing fever.[47] The diagnosis is confirmed by PCR testing or immunoassay (enzyme-linked immunosorbent assay or bead assay). The spirochete has been observed in blood smears during the febrile illness, but the sensitivity of the test is not known.[7] The spirochete has been seen on Giemsa staining of a cytospin of the cerebrospinal fluid.[47] Based on case reports, the treatment consists of doxycycline 100 mg twice a day for 14 days.[47] If the patient has central nervous system involvement, ceftriaxone 2 g daily for 14 to 28 days or penicillin G (24 million U/day) has been reported to be successful in case reports.[7,47] A Jarisch-Herxheimer reaction was observed in 15% of patients; in one case the reaction occurred within 9 hours.[7,50] Prevention consists of taking measures to avoid tick bites.[47] Patients with *B. miyamotoi* live in areas of Lyme disease, so they often receive doxycycline prophylaxis after a tick bite. There are no randomized trials of the efficacy of doxycycline prophylaxis for *B. miyamotoi* infection.

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The complete reference list is available online at Expert Consult.

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241 Lyme Disease (Lyme Borreliosis) Due to *Borrelia burgdorferi*

Allen C. Steere

SHORT VIEW SUMMARY

Definition
- A tick-borne infection caused by the spirochete *Borrelia burgdorferi* sensu lato leading to a multisystem illness primarily in the skin, joints, nervous system, heart, or a combination of these.

Epidemiology
- The vectors of Lyme disease are 14 closely related ixodid tick species that are part of the *Ixodes ricinus* complex.
- The disease occurs in parts of North America, Europe, and Asia.
- The disorder in the United States occurs primarily in three distinct foci: in the Northeast from Maine to North Carolina; in the Midwest in Wisconsin, Minnesota, and Michigan; and in the West, primarily in northern California.
- The peak onset of early infection is in the summer months.
- About 30,000 cases are reported yearly in the United States, but the actual number is estimated to be ~300,000 cases annually, mostly from the northeastern United States.

Clinical Characteristics
- Without antibiotic therapy, Lyme borreliosis generally occurs in stages, with different clinical manifestations at each stage, although patients may not express all stages.
- Stage 1 usually consists of a slowly expanding skin lesion, erythema migrans, which may be accompanied by flulike symptoms.
- Stage 2, which occurs weeks later includes manifestations of early disseminated infection, particularly neurologic or cardiac involvement.
- In the United States, stage 3, which occurs usually occurs months after disease onset, is commonly characterized by intermittent or persistent arthritis in one or a few joints, most commonly the knees, over a period of several years, whereas in Europe, acrodermatitis chronica atrophicans is the most common late manifestation of the illness.

Microbiology
- The genus *Borrelia* currently includes 20 closely related species known collectively as *Borrelia burgdorferi* sensu lato (*B. burgdorferi* in the general sense).
- The human infection is caused primarily by three pathogenic species: *B. burgdorferi* sensu stricto (*B. burgdorferi* in the strict sense) is the sole cause in the United States, whereas *Borrelia afzelii* and *Borrelia garinii* are the primary causes of the infection in Europe.

Diagnosis
- Culture of *B. burgdorferi* in Barbour-Stoenner-Kelly medium permits definitive diagnosis but has been possible reliably only from biopsies of erythema migrans skin lesions, an early disease manifestation.
- Diagnosis is usually based on characteristic clinical findings and a positive immunoglobulin G serologic test result, using a two-test approach of enzyme-linked immunosorbent assay and Western blot.

Therapy
- For early infection, appropriate oral antibiotic therapy (usually doxycycline, 100 mg twice daily or amoxicillin, 500 mg three times daily) for 14 to 21 days is usually successful.
- For some patients with neurologic involvement or arthritis, intravenous antibiotic therapy (often ceftriaxone, 2 g daily) for 28 days may be necessary.

Prevention
- Personal protection methods to avoid tick bites and carry out tick checks after exposure in endemic areas are the major prevention strategies.
- A vaccine for Lyme disease consisting of recombinant OspA with adjuvant was shown to be safe and effective, but the vaccine is not now available.

Lyme disease or Lyme borreliosis, which is caused by the tick-borne spirochete *Borrelia burgdorferi* (sensu lato), occurs in temperate regions of North America, Europe, and Asia.[1,2] It is now the most common vector-borne disease in the United States and Europe.[3] The illness usually begins in summer (stage 1) with a characteristic expanding skin lesion, called erythema migrans (EM), which occurs at the site of the tick bite.[1,4] Within several days to weeks (stage 2), the spirochete may spread to other sites, particularly to other skin sites, the nervous system, heart, or joints. After months to years (stage 3), sometimes following periods of latent infection, the spirochete may cause persistent disease, most commonly affecting the joints, nervous system, or skin. Serologic testing is the most practical laboratory aid in diagnosis. All stages of the disorder are usually curable by appropriate antibiotic therapy, but in patients who develop postinfectious syndromes, complete recovery may be delayed.

Lyme disease was recognized as a separate entity in 1976 because of close geographic clustering of affected children in Lyme, Connecticut, who were thought to have juvenile rheumatoid arthritis.[5] However, parts of the illness were recognized previously in Europe and were given different names, including erythema chronicum migrans, Bannwarth syndrome, or acrodermatitis chronica atrophicans.[4] These syndromes were linked conclusively in 1982 and 1983 with the recovery of a previously unrecognized spirochete from the tick vector[6] and from infected patients.[4] The basic outlines of the disease are similar worldwide, but there are regional variations, primarily between the illness found in America and that in Europe and Asia.[1]

CAUSATIVE ORGANISM

The agents of Lyme borreliosis belong to the eubacterial phylum of spirochetes, which are vigorously motile, corkscrew-shaped bacteria. The spirochete cell wall consists of a cytoplasmic membrane surrounded by peptidoglycan and flagella and then by a loosely associated outer membrane (Fig. 241.1).[7] Of the *Borrelia* spp., *B. burgdorferi* is the longest (20–30 μm) and narrowest (0.2–0.3 μm), and it has fewer flagella (7–11). The complete genome of *B. burgdorferi* has been sequenced. *B. burgdorferi* strains have a small, linear chromosome (≈950 kilobases)[8] and 17 to 21 linear and circular plasmids, which comprise about 40% of its DNA.[9]

The remarkable aspect of the *B. burgdorferi* genome is the large number of sequences for predicted and known lipoproteins,[8,9] including the plasmid-encoded outer-surface proteins (Osp) A through F. These and other differentially expressed outer-surface proteins presumably help the spirochete adapt to, survive in, and persist in markedly different arthropod and mammalian environments. In addition, during the disseminated phase of the infection, another surface-exposed lipoprotein,

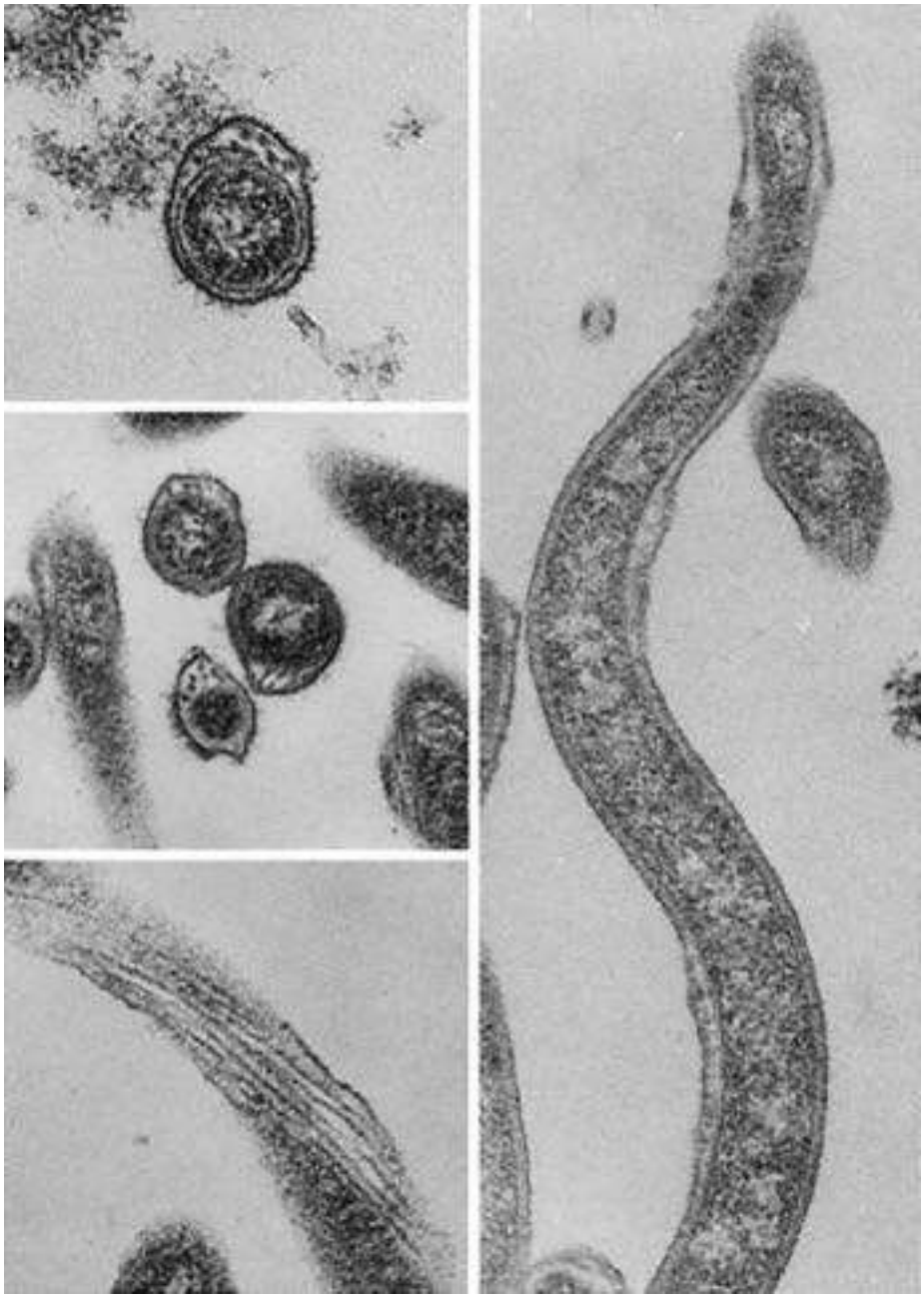

FIG. 241.1 Electron micrographs of *Borrelia burgdorferi.* The spirochetes have a transverse diameter of about 0.2 mm and 7–11 flagella, which are shown *(left)* in cross section in the upper and middle pictures and in tangential section in the lower picture. In longitudinal section *(right),* the organism has an apparent slime layer, an outer membrane, flagellae, a cell wall, and cytoplasmic constituents; its length is 11–39 mm (×40,000, except upper left [×60,000]). *(From Steere AC, Grodzicki RL, Kornblatt AN, et al. The spirochetal etiology of Lyme disease.* N Engl J Med. *1983;308:733–740. Copyright © 1983, Massachusetts Medical Society. All rights reserved.)*

called VlsE, undergoes extensive antigenic variation.[10] The organism has few proteins with biosynthetic activity and apparently depends on the host for much of its nutritional requirements.[8,9] The genome contains no homologues for systems that specialize in the secretion of toxins or other virulence factors. The only known virulence factors of *B. burgdorferi* are surface-exposed lipoproteins that allow the spirochete to attach to mammalian cells.

The genus *Borrelia* currently includes 20 closely related species known collectively as *Borrelia burgdorferi* sensu lato (*B. burgdorferi* in the general sense).[3,11] However, only three pathogenic species commonly cause Lyme borreliosis.[12] To date, all North American strains have belonged to the first group, *B. burgdorferi* sensu stricto (*B. burgdorferi* in the strict sense), hereafter called *B. burgdorferi.* All three groups have been found in Europe, but most isolates there have been group 2 *(Borrelia garinii)* and 3 *(Borrelia afzelii)* strains. Only the latter two groups have been found in Asia. These differences may well account for regional variations in the clinical picture of Lyme borreliosis. *B. burgdorferi* grows best at 33°C in a complex liquid medium called Barbour-Stoenner-Kelly (BSK) medium.[7]

Strains of *B. burgdorferi* have been subdivided according to several typing schemes, one based on sequence variation of OspC,[13] a second based on differences in the 16S-23S ribosomal RNA (rRNA) intergenic spacer (IGS) region (or RST [ribosomal RNA intergenic spacer type]),[14] and a third based on eight chromosomal housekeeping genes (multilocus typing).[15] Among *B. burgdorferi* strains in the northeastern United States, the genes encoding OspC or the IGS are in strong linkage disequilibrium, suggesting a clonal structure of strains in this geographic region.[16] From these typing systems, information is emerging concerning differential

pathogenicity of strains of *B. burgdorferi*.,[13,14] OspC type A (RST 1) strains, which account for 30% to 50% of infections in the northeastern United States, are particularly virulent. The OspC types of midwestern *B. burgdorferi* strains are more diverse than strains in the Northeast; OspC type H strains are most abundant (18%), and RST 1 strains account for only 3% of the infections there.[17] *B. burgdorferi* strains in the United States represent distinct genotypes from *B. burgdorferi* strains in Europe. However, the clinical features of *B. burgdorferi* infection in Europe appear similar to those for *B. afzelii* and *B. garinii* infection, the common *Borrelia* spp. in Europe, suggesting that strains within a particular regional environment accrue similar characteristics as other strains that share the same ecologic niche.[18]

VECTOR OF TRANSMISSION AND ANIMAL HOSTS

The vectors of Lyme borreliosis are closely related ixodid tick species that are part of the *Ixodes ricinus* complex (also called the *Ixodes persulcatus* complex).[19] In the northeastern and midwestern United States the deer tick, *Ixodes scapularis* (also called *Ixodes dammini;* Fig. 241.2), is the vector, and *Ixodes pacificus* is the vector in the West. In Europe the sheep tick, *Ixodes ricinus,* and in Asia, the taiga tick, *Ixodes persulcatus,* are the primary vectors.

Ixodid ticks have larval, nymphal, and adult stages, and they require a blood meal at each stage.[12] The peak questing periods for adult *I. scapularis* are spring and fall; for nymphs, May through July; and for larvae, August and September. In the northeastern United States, from Maine to North Carolina and in the north central states of Wisconsin and Minnesota, a highly efficient, horizontal cycle of *B. burgdorferi* transmission occurs among larval and nymphal *I. scapularis* ticks and certain rodents, particularly white-footed mice and chipmunks.[20] This cycle results in high rates of infection among rodents and nymphal ticks, and primarily nymphal tick bites cause many new human cases of Lyme disease during the late spring and summer months. White-tailed deer, which are not involved in the life cycle of the spirochete, are the preferred host of adult *I. scapularis*, and they seem to be critical for the survival of ticks.

The vector ecology of *B. burgdorferi* is different on the West Coast, where the frequency of Lyme disease is low. There, two intersecting cycles are necessary for the transmission of the disease,[21] one involving the dusky-footed wood rat and *Ixodes spinipalpus* (also called *Ixodes neotomae*) ticks, which do not bite humans and that maintain the cycle in nature, and the other involving wood rats and *I. pacificus* ticks, which are less often infected but do bite humans.

In the southeastern United States, immature *I. scapularis* ticks feed primarily on lizards rather than rodents, and lizards are not susceptible to *B. burgdorferi* infection. Therefore *B. burgdorferi* infection occurs rarely in that part of the country. Instead, in the southern United States, in the mid-Atlantic states, and now moving into northeastern states, a rash resembling erythema migrans, called southern tick-associated rash illness (STARI), has been associated with the bite of the Lone Star tick *(Amblyomma americanum).*[22] Although a novel *Borrelia* spp. called *Borrelia lonestari* has been found in these ticks, it is not clear that they are the cause of the disease. STARI may be accompanied by nonspecific systemic symptoms, but it is not known to cause chronic infection (see Chapter 298 for further details).

In Europe there is still debate about the preferred animal hosts of *I. ricinus*. These ticks feed on more than 300 animal species, including small mammals, birds, and reptiles.[23] Because the *Borrelia* species differ in their resistance to complement-mediated killing, small rodents are important reservoirs for *B. afzelii,* whereas birds are strongly associated with *B. garinii.*[24]

EPIDEMIOLOGY

Since surveillance was begun by the Centers for Disease Control and Prevention (CDC) in 1992, the number of reported cases of Lyme disease has increased dramatically in the United States. Currently, more than 30,000 cases have been reported yearly, making Lyme disease the most common vector-borne infection in the United States.[3] Moreover, the CDC estimates that the actual number of cases is closer to 300,000 annually.[25] The disorder occurs primarily in three distinct foci: in the Northeast from Maine to North Carolina; in the Midwest in Wisconsin, Minnesota, and Michigan; and in the West, primarily in northern California. The infection has now spread to Canada. Lyme borreliosis also occurs in temperate regions of the Northern Hemisphere in Europe and Asia. There, the highest reported frequencies of the infection are in middle Europe, particularly in Germany, Austria, Slovenia, and also in Sweden.[26]

During the past 40 years the infection in the United States has continued to spread, particularly in the Northeast. It has caused focal outbreaks in some coastal areas, and it now affects suburban locations near Boston, New York, Philadelphia, Baltimore, and Washington—the most heavily populated parts of the country.[27] Within these areas the occurrence of Lyme disease is highly focal. In Connecticut, which has one of the highest reported frequencies of Lyme disease in the United States, cases have been noted in all parts of the state, but most of the cases are still clustered in two counties in the southeastern part of the state, where the original epidemiologic investigation took place in the town of Lyme. In a large, 2-year vaccine trial, the yearly incidence of the disease in such highly endemic areas was greater than 1 per 100 participants.[28]

Why did Lyme disease emerge in the northeastern United States during the latter part of the 20th century?[12] The infection has probably been in North America for thousands of years, but ecologic conditions were altered during the European colonization of North America. Woodlands were cleared for farming, and deer were hunted practically to extinction. However, during the 20th century, farmland reverted to woodland, deer proliferated, white-footed mice and other rodents were plentiful, and the deer tick thrived. Soil moisture and land cover, as found near rivers and along the coast, were favorable for tick survival. Moreover, these areas became heavily populated with both humans and deer, as more rural wooded areas became wooded suburbs in which deer were without predators and hunting was prohibited. Finally, the spread of a particularly virulent spirochetal strain, *B. burgdorferi* OspC type A, may have contributed to the rise in the incidence of the infection.[29]

PATHOGENESIS

To maintain its complex enzootic cycle, *B. burgdorferi* must adapt to two markedly different environments: the tick and the mammalian or avian host. The spirochete survives in a dormant state in the nymphal tick midgut during the fall, winter, and spring, where it expresses primarily OspA and certain other proteins.[30] When the tick feeds during the late spring or summer, these proteins are downregulated, and another set of proteins, including OspC, is upregulated.[31,32] OspC binds mammalian plasminogen and its activators present in the blood meal, which facilitates spreading of the organism in the tick.[33] In addition, within

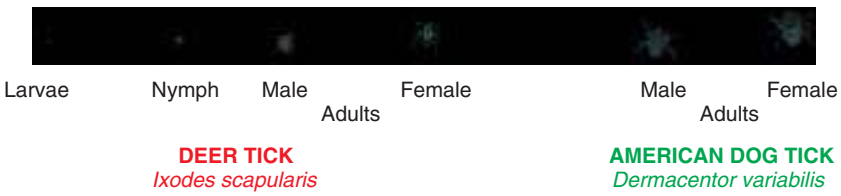

FIG. 241.2 The deer tick, *Ixodes scapularis,* is the primary vector of Lyme disease in the United States. The nymph stage is most frequently implicated. For comparison, the dog tick, *Dermacentor variabilis,* is shown, but this tick does not transmit Lyme disease. Shown actual size. *(Courtesy Massachusetts Department of Public Health Division and the Cape Cod Cooperative Extension.)*

the tick salivary gland, OspC binds a tick salivary gland protein (Salp 15), and coating of the spirochete in this tick protein is essential for initial immune evasion in the mammalian host.[34] The spirochete, which has few proteins with biosynthetic activity,[8] appears to meet its nutritional requirements by infection of a mammalian or avian host.

After injection of *B. burgdorferi* by the tick (and a clinical incubation period of 3–32 days), the spirochete usually first multiplies locally in the skin at the site of the tick bite. In most patients immune cells first encounter *B. burgdorferi* at this site. Dendritic cells isolated from the dermis readily engulf *B. burgdorferi* in vitro.[35] During the initial infection *B. burgdorferi* induces potent proinflammatory and compensatory antiinflammatory responses in cells in EM lesions,[36,37] and *B. burgdorferi*–stimulated peripheral blood mononuclear cells (PBMCs) produce primarily proinflammatory cytokines, particularly interferon (IFN)-γ.[38] Thus both innate and adaptive cellular elements are mobilized to fight the infection.

Within days to weeks, *B. burgdorferi* strains in the United States often disseminate to many distant anatomic sites. During this period the spirochete has been recovered from blood and cerebrospinal fluid (CSF),[6,7,39] and it has been seen in small numbers in specimens of myocardium, retina, muscle, bone, spleen, liver, meninges, and brain.[40] To disseminate, *B. burgdorferi* adheres to integrins, proteoglycans, or glycoproteins on host cells or tissue matrices. As in the tick, spreading through the skin and other tissue matrixes may be facilitated by the binding of plasminogen and its activators to the surface of the spirochete.[33] During dissemination, a 66-kilodalton (kDa) spirochetal protein binds the platelet-specific integrin $\alpha_{IIb}\beta_3$ and the vitronectin receptor ($\alpha_v\beta_5$).[41] A 26-kDa glycosaminoglycan binding protein binds heparan sulfate and dermatan sulfate,[42] which are expressed on endothelial cells. A 47-kDa fibronectin-binding protein (BBK32) binds fibronectin,[43] a ubiquitous extracellular matrix protein. Finally, decorin-binding proteins A and B (DbpA and DbpB) of the spirochete bind decorin,[44] a proteoglycan on the surface of collagen, which may explain the alignment of spirochetes with collagen fibrils in the extracellular matrix in the heart, nervous system, or joints.[40]

All affected tissues show an infiltration of lymphocytes and plasma cells.[40] Some degree of vascular damage, including mild vasculitis or hypervascular occlusion, may be seen in multiple sites, suggesting that spirochetes may have been in or around blood vessels. Although *B. burgdorferi* has been identified inside cultured cells in vitro, it has not been seen in intracellular locations in histologic sections of infected tissues from patients with Lyme disease.

Despite an active immune response, *B. burgdorferi* may survive during dissemination by changing or minimizing antigenic expression of surface proteins and by inhibiting certain critical host immune responses. Two linear plasmids (lps) seem to be essential, including lp25, which encodes a nicotinamidase,[45] and lp28-1, which encodes the VlsE lipoprotein,[10] the protein that undergoes extensive antigenic variation.[46] In addition, the spirochete has a number of highly homologous, differentially expressed lipoproteins, including OspE/F paralogs, which further contribute to antigenic diversity.[47] Finally, *B. afzelii* and, to a lesser degree, *B. burgdorferi* have complement regulator-acquiring surface proteins that bind complement factor H and factor H–like protein 1.[48,49] These complement factors inactivate C3b, which protects the organism from complement-mediated killing.

Both innate and adaptive immune responses are required for optimal control of spirochetal dissemination. Membrane lipoproteins are mitogenic for B cells.[50] The specific immunoglobulin M (IgM) response is often associated with polyclonal activation of B cells, including elevated total serum IgM levels,[51] circulating immune complexes,[52] and cryoglobulins.[51] In murine *B. burgdorferi* infection, CD1d presentation of monogalactosyl diacylglycerol (MgalD, BbGL-II) to natural killer T cells may be important in the early innate immune response, possibly as an initial source of IFN-γ.[53] CD1d-deficient mice do not control the infection as well as their wild-type counterparts.[54]

In the human infection the adaptive IgG response develops gradually over weeks to months to an increasing array of spirochetal proteins[55] and two borrelial glycolipids.[56] Using protein arrays that expressed approximately 1200 or more *B. burgdorferi* proteins, antibody responses were detected to a total of more than 100 proteins in a population of patients with early or late Lyme disease, particularly plasmid-encoded outer-surface lipoproteins.[57] Spirochetal killing seems to be accomplished primarily by bactericidal B-cell responses,[58] which promote spirochetal killing by complement fixation and opsonization.[59] As shown in mice, the primary purpose of *B. burgdorferi*–specific $CD4^+$ Th1 cells, which secrete mainly IFN-γ, is to prime T-cell–dependent, B-cell responses.[60] *B. burgdorferi*–specific $CD8^+$ T cells and NK cells are other important sources of IFN-γ.[61,62] In patients with EM a dichotomy was noted between Th1 and Th17 responses. High Th1-associated responses correlated with more effective immune-mediated spirochetal killing, whereas high Th17-associated immune responses correlated with post-Lyme symptoms.[63]

In the enzootic infection *B. burgdorferi* spirochetes must survive this immune assault for only the summer months before returning to the larval ticks to begin the cycle again the next year. In contrast, infection of humans is a dead-end event for the spirochete. Within several weeks to months, innate and adaptive immune mechanisms, even without antibiotic treatment, control widely disseminated infection, and generalized systemic symptoms wane.[1] However, without antibiotic therapy, spirochetes may survive in localized niches for several more years. *B. burgdorferi*, the sole cause of the infection in the United States, may cause persistent arthritis or, in rare cases, a subtle encephalopathy or polyneuropathy accompanied by minimal, if any, systemic symptoms.[1] Patients with Lyme arthritis have high antibody responses to many spirochetal proteins that are suggestive of hyperimmunization due to recurrent waves of spirochetal growth.[55] Even without antibiotic treatment, the number of patients who continue to have attacks of arthritis decreases by about 10% to 20% each year, and few patients have had attacks for longer than 5 years.[64] Thus immune mechanisms seem to succeed eventually in the eradication of *B. burgdorferi* from selected niches, including the joints or nervous system.

CLINICAL CHARACTERISTICS

As with other spirochetal infections, human Lyme borreliosis generally occurs in stages, with remissions and exacerbations and different clinical manifestations at each stage.[4] Early infection consists of stage 1 (localized EM), followed within days or weeks by stage 2 (disseminated infection). Late infection, or stage 3 (persistent infection), usually begins months to years after the disease onset, sometimes following long periods of latent infection. In an individual patient, however, the infection is highly variable, ranging from brief involvement in only one system to chronic, multisystem involvement of the skin, nerves, or joints. In the United States about 10% of individuals have asymptomatic *B. burgdorferi* infection and seem to cure the infection without antibiotic therapy.[65]

Early Infection: Stage 1 (Localized Infection)

In about 70% to 80% of patients EM develops at the site of the tick bite (Fig. 241.3A and Table 241.1).[66,67] However, because of the small size of nymphal *I. scapularis*, most patients do not remember the tick bite. During the first several days the lesion often has a homogeneous red appearance.[68] In addition, the centers of early lesions sometimes become intensely erythematous and indurated, vesicular, or necrotic. As the area of redness around the center expands, most lesions continue to have bright-red outer borders (usually flat, but occasionally raised) and partial central clearing. In some instances migrating lesions remain an even, intense red; several red rings are found within the outside one; or the central area turns blue before it clears. Although the lesion can be located anywhere, the thigh, groin, and axilla are particularly common sites. If EM is on the head, only a linear streak might be seen to emerge from the hairline. The lesion is hot to the touch, and patients often describe it as burning or occasionally itching or painful.

In Europe EM is often an indolent, localized infection, whereas in the United States, the lesion is associated with more intense inflammation and signs and symptoms that suggest dissemination of the spirochete.[1] In one US study spirochetes were cultured from plasma samples in 50% of patients with EM.[69] In a recent study the EM skin lesions of *B. burgdorferi*–infected US patients expanded faster, were associated with more symptoms, and had higher messenger RNA levels of



FIG. 241.3 (A) An early erythema migrans skin lesion is seen 4 days after detection. (B) Four days after the onset of the initial skin lesion, secondary lesions have appeared, and several of their borders have merged. *(From Steere AC, Bartenhagen NH, Cratt JE, et al. The early clinical manifestations of Lyme disease.* Ann Intern Med. *1983;99:76–82.)*

macrophage-associated chemokines and cytokines than did EM lesions of *B. afzelii*–infected Austrian patients.[70]

Early Infection: Stage 2 (Disseminated Infection)

Within several days to weeks of the onset of the initial EM lesion, patients, particularly in the United States, may develop multiple annular secondary skin lesions (see Fig. 241.3B and Table 241.1),[1,67] a sign of hematogenous dissemination. Although their appearance is similar to that of the initial lesions, they are generally smaller, migrate less, and lack indurated centers; they are not associated with previous tick bites. Individual lesions sometimes appear and fade at different times, and their borders sometimes merge. During this period some patients develop malar rash, conjunctivitis, or, rarely, diffuse urticaria. EM and secondary lesions usually fade within 3 to 4 weeks (range, 1 day–14 months).

EM is often accompanied by malaise and fatigue, headache, fever and chills, generalized achiness, and regional lymphadenopathy.[1,67] In about 18% of patients[66] these symptoms are the presenting picture of the infection.[71] In addition, patients sometimes have evidence of meningeal irritation with episodic attacks of excruciating headache and neck pain, mild encephalopathy with difficulty with mentation, migratory musculoskeletal pain, hepatitis, generalized lymphadenopathy or splenomegaly, sore throat, or rarely, nonproductive cough.[67] A few patients have had microscopic hematuria, sometimes with mild proteinuria (dipstick). During the first days of illness headache and neck stiffness are not associated with a spinal fluid pleocytosis or objective neurologic deficit. Except for fatigue and lethargy, which are often constant, the early signs and symptoms are typically intermittent and changing. For example, a patient might experience predominantly headache and a stiff neck for several days. After a few days of improvement, musculoskeletal pain might begin.

After several weeks to months, about 15% of untreated patients in the United States develop frank neurologic abnormalities, including meningitis, encephalitis, cranial neuritis (including bilateral facial palsy), motor and sensory radiculoneuritis, mononeuritis multiplex, cerebellar ataxia or myelitis—alone or in various combinations.[72] The usual pattern consists of fluctuating symptoms of meningitis with superimposed cranial (particularly facial palsy) or peripheral radiculoneuropathy.

On examination such patients usually have neck stiffness only on extreme flexion; Kernig and Brudzinski signs are not present. Facial palsy may occur alone,[73] and in rare instances, it may be the presenting manifestation of the disease. In children the optic nerve may be affected by inflammation or increased intracranial pressure, which may lead to blindness.[1] In Europe the most common neurologic manifestation is Bannwarth syndrome, which consists of neuritic pain, lymphocytic pleocytosis without headache, and sometimes cranial neuritis.[1]

In patients with meningitis, CSF typically has a lymphocytic pleocytosis of about 100 cells/mm,[4] often with an elevated protein but a normal glucose level.[72] Specific IgG, IgM, or IgA antibody to the spirochete is produced intrathecally,[74] and *B. burgdorferi*–specific oligoclonal bands may be present. Electrophysiologic studies of affected extremities suggest primarily axonal nerve involvement.[75] Histologically, the lesions show axonal nerve injury with perivascular infiltration of lymphocytes and plasmocytes around epineural blood vessels. Stage 2 neurologic abnormalities usually last for weeks or months, but they may recur or become chronic.

Within several weeks after the onset of illness, about 5% of untreated patients develop cardiac involvement.[76] The most common abnormality is fluctuating degrees of atrioventricular block (first-degree, Wenckebach, or complete heart block). However, some patients have evidence of more diffuse cardiac involvement, including electrocardiographic changes or a gallium scan, compatible with acute myopericarditis, radionuclide evidence of mild left ventricular dysfunction, or, rarely, cardiomegaly. No patients have had heart murmurs. The duration of cardiac involvement is usually brief (3 days–6 weeks), and the insertion of a permanent pacemaker is usually considered unnecessary.[77] However, fatal cases have been reported.[78,79] In Europe *B. burgdorferi* has been isolated from endomyocardial biopsy samples from several patients with chronic dilated cardiomyopathy.[80] However, this complication has not been observed in the United States.[81]

During this stage migratory musculoskeletal pain is common in joints, tendons, bursae, muscle, or bones.[1] In addition, a few patients have been described with osteomyelitis, myositis, panniculitis, or fasciitis. Conjunctivitis is the most common eye abnormality in Lyme disease, but deeper tissues in the eye may be affected as well.[1,82] There are case reports of iritis, followed by panophthalmitis, choroiditis with exudative retinal detachments, or interstitial keratitis, similar to that seen in syphilis. In Europe *Borrelia* lymphocytoma, a subacute skin lesion, typically on the ear or breast, may occur during this period.[83] These lesions have dense infiltrates of B cells, which often form follicular structures, sometimes with the appearance of germinal centers. *Borrelia* lymphocytomas usually last for months but may persist for 1 year or longer.

Late Infection: Stage 3 (Persistent Infection)

Months after the onset of the illness, within the context of strong cellular and humoral immune responses to *B. burgdorferi,* about 60% of patients in the northeastern United States begin to experience intermittent attacks of joint swelling and pain, primarily in large joints, especially the knee, usually one or two joints at a time (see Table 241.1).[1,64] However, both large and small joints may be affected. Joint fluid white blood cell counts range from 500 to 110,000 cells/mm,[4] most of which, in patients with

TABLE 241.1 Manifestations of Lyme Disease by Stage

	EARLY INFECTION		LATE INFECTION
System[b]	**Localized Stage 1**	**Disseminated Stage 2**	**Persistent Stage 3**
Skin	Erythema migrans	Secondary annular lesions Malar rash Diffuse erythema or urticaria Evanescent lesions Lymphocytoma	Acrodermatitis chronica atrophicans Localized scleroderma-like lesions
Musculoskeletal		Migratory pain in joints, tendons, bursae, muscle, bone Brief arthritis attacks Myositis[c] Osteomyelitis[c] Panniculitis[c]	Prolonged arthritis attacks Chronic arthritis Peripheral enthesopathy Periostitis or joint subluxations below acrodermatitis
Neurologic		Meningitis Cranial neuritis, facial palsy Motor or sensory radiculoneuritis Subtle encephalitis Mononeuritis multiplex Pseudotumor cerebri Myelitis[c] Cerebellar ataxia[c]	Chronic encephalomyelitis Spastic paraparesis Ataxic gait Subtle mental disorders Chronic axonal polyradiculopathy
Lymphatic	Regional lymphadenopathy	Regional or generalized lymphadenopathy Splenomegaly	
Heart		Atrioventricular nodal block Myopericarditis Pancarditis	
Eyes		Conjunctivitis Iritis[c] Choroiditis[c] Retinal hemorrhage or detachment[c] Panophthalmitis[c]	Keratitis
Liver		Mild or recurrent hepatitis	
Respiratory		Nonexudative sore throat Nonproductive cough	
Kidney		Microscopic hematuria or proteinuria	
Genitourinary		Orchitis[c]	
Constitutional systems	Minor	Severe malaise and fatigue	Fatigue

[a]The staging system provides a guideline for the expected timing of the different manifestations of the illness, but this may vary in an individual case.
[b]The systems are listed from the most to the least commonly affected.
[c]Because the inclusion of these manifestations is based on one or a few cases, they should be considered possible but not proven manifestations of Lyme disease.
From Steere AC. Lyme disease. N Engl J Med. *1989;321:586–596. Copyright © 1989, Massachusetts Medical Society. All rights reserved.*

high white blood cell counts, are polymorphonuclear leukocytes. Although the total number of patients who continue to have recurrent attacks of arthritis decreases by about 10% to 20% each year, attacks of knee swelling sometimes become longer during the second or third year of illness, sometimes lasting 1 year or longer. However, even in untreated patients, intermittent or persistent arthritis usually resolves completely within several years.

Although most patients with either intermittent or persistent arthritis respond to oral or intravenous (IV) antibiotic treatment, a small percentage of patients have persistent joint inflammation in a knee for months or even several years after 1 to 2 months or longer of oral antibiotics and 1 month or longer of IV antibiotics. This illness is defined as postinfectious, antibiotic-refractory Lyme arthritis.[84] In these patients the synovial lesion, which is similar to that seen in other forms of chronic inflammatory arthritis, including rheumatoid arthritis, shows synovial cell hyperplasia; vascular proliferation; an infiltration of mononuclear cells, particularly T cells; and upregulation of adhesion molecules.[85] In some patients obliterative microvascular lesions are seen, which appears to be a unique feature of Lyme synovitis.[86]

Antibiotic-refractory Lyme arthritis, which has been noted primarily in the northeastern United States, is associated with a high frequency of infection with highly inflammatory RST 1 strains.[87] However, persistent synovitis in the postantibiotic period is not thought to result from persistent infection. The results of polymerase chain reaction (PCR) testing are usually negative in synovial fluid after antibiotic therapy, and both culture and PCR results have been uniformly negative in synovial tissue obtained at synovectomy more than 1 year after the conclusion of antibiotic treatment.[88] This suggests that joint inflammation may continue in some patients after the near or total eradication of live spirochetes from the joint with antibiotic therapy. In MyD88$^{-/-}$ mice, which have a high pathogen load, spirochetal antigens are retained near cartilage surfaces after antibiotic therapy, and patellae homogenates from these mice induce macrophages to secrete tumor necrosis factor (TNF)-α.[89] However, patients with either antibiotic-responsive or antibiotic-refractory Lyme arthritis have low pathogen loads during the infection.[88] Moreover, in human patients with refractory arthritis, T-cell and B-cell responses to *B. burgdorferi* decline after antibiotic treatment,[90,91] whereas the levels of inflammatory cytokines in synovial fluid often increase,[92] suggesting that *B. burgdorferi* antigens are not the sole driver of postinfectious immune responses.

Multiple host factors are also thought to be important in antibiotic-refractory Lyme arthritis. Along with spirochetal factors, these host factors may lead to excessive inflammation, immune dysregulation, and infection-induced, site-specific autoimmunity. Patients with refractory arthritis who are homozygous for a specific Toll-like receptor 1 polymorphism (1805GG), particularly when infected with RST 1 strains of *B. burgdorferi*, have significantly higher levels of proinflammatory cytokines in synovial fluid, including TNF-α and IFN-γ, compared with the levels in patients with antibiotic-responsive arthritis.[93] Moreover, in patients with refractory arthritis, the CD4^{+} CD25 high T-cell population often has lower frequencies of T-regulatory cells, higher expression of

activation coreceptors, and less effective inhibition of proinflammatory cytokines than this cell population in patients with antibiotic-responsive arthritis.[94]

Furthermore, the greatest known risk factors for antibiotic-refractory Lyme arthritis are specific human leukocyte antigen (HLA)-DR alleles, which are risk factors for many autoimmune diseases. In antibiotic-refractory arthritis the alleles that are increased in frequency (such as the DRB1*0101 and 0401 alleles) encode HLA-DR molecules that bind and present an epitope of *B. burgdorferi* outer-surface protein A ($OspA_{165–173}$).[95] OspA presentation by the DRB1*0401 molecule appears to stimulate a particularly strong Th1 response,[96] which may contribute to excessive joint inflammation. However, an immune response to OspA is commonly found only in patients with Lyme arthritis in the United States, where arthritis is a more frequent manifestation of the disease than in other locations.[97] In support of the hypothesis that autoimmune phenomena may play a role in persistent arthritis after antibiotic therapy, four autoantigens have now been identified: endothelial cell growth factor (ECGF), matrix metalloproteinase-10 (MMP-10), apolipoprotein B-100 (apoB-100), and annexin A2, which are targets of T-cell and B-cell responses in a subset of patients with antibiotic-refractory arthritis.[98–101] Autoantibodies to ECGF correlate with the presence of obliterative microvascular lesions in synovial tissue in these patients.[86] However, within months to several years, the adaptive immune response to autoantigens, in the absence of live spirochetes, seems to eventually regain homeostasis, and the arthritis resolves.

In rare instances, along with or after episodes of Lyme arthritis, patients may develop chronic neurologic manifestations of the disorder.[1,102] In both the United States and Europe a chronic axonal polyneuropathy may develop, manifested primarily as spinal radicular pain or distal paresthesias. Even though sensory symptoms are often localized, electrophysiologic testing frequently shows a diffuse axonal polyneuropathy affecting both proximal and distal nerve segments.[75] In Europe *B. garinii* may cause chronic encephalomyelitis, characterized by spastic paraparesis, ataxia, cognitive impairment, bladder dysfunction, and cranial neuropathy, particularly of the seventh or eighth cranial nerve, accompanied by intrathecal antibody production of IgG antibody to *B. burgdorferi*.[1]

In the United States a mild, late neurologic syndrome has been reported, called Lyme encephalopathy, manifested primarily by subtle cognitive disturbances.[102] Although there are no inflammatory changes in CSF, intrathecal antibody production to the spirochete can often be demonstrated. Neither neuropsychological tests of memory nor single-photon emission computed tomography scanning of the brain has sufficient specificity to be helpful in diagnosis. Acrodermatitis chronica atrophicans, which sometimes follows years after EM, has been observed primarily in Europe and Asia in association with *B. afzelii* infection.[83] Acrodermatitis chronica atrophicans begins with red violaceous lesions that become sclerotic or atrophic. These lesions, which may be the presenting manifestation of the disease, may last for many years, and *B. burgdorferi* has been cultured from such lesions as many as 10 years after their onset.

POST–LYME DISEASE SYMPTOMS OR SYNDROME

The term "post-Lyme symptoms" probably consists of more than one syndrome, and the pathogenetic mechanisms that account for these symptoms may not be the same in all patients. At one end of the spectrum, one or a few subjective symptoms, such as malaise and fatigue or minor joint symptoms, may persist for several months after antibiotic treatment of EM.[103] At the far end of the spectrum, patients may have disabling joint and muscle pain, neurocognitive difficulties, incapacitating fatigue, and sleep disorder for years after Lyme disease.[104] This syndrome is similar to or indistinguishable from chronic fatigue syndrome or fibromyalgia, which is thought to be a centralized pain syndrome.[105] Patients with these conditions do not have evidence of joint inflammation; they have normal neurologic test results; and they may have a greater degree of anxiety and depression than unaffected persons.[106] In contrast, late well-recognized manifestations of Lyme disease, including arthritis, encephalopathy, or neuropathy, are usually associated with minimal systemic symptoms. A subset of patients with Lyme disease who develop post–Lyme disease symptoms after erythema migrans have elevated interleukin-23 levels.[107] There is currently no evidence that persistent subjective symptoms after recommended courses of antibiotic therapy for Lyme disease are caused by active *B. bergdorferi* infection.[108]

A counterculture has emerged that ascribes pain and fatigue syndromes to "chronic Lyme disease" when there is little or no evidence of *B. burgdorferi* infection.[108] In such patients the term chronic Lyme disease, which is equated with chronic *B. burgdorferi* infection, is a misnomer, and the use of prolonged antibiotic treatment has not been shown to be beneficial and may lead to substantial cost and serious harm.[109] Patients should be counseled that the proposed therapies have never been proven to be effective and can carry significant side effects, including fatalities.

CONGENITAL INFECTION

In the mid-1980s the transplacental transmission of *B. burgdorferi* was reported in two infants whose mothers had Lyme borreliosis during the first trimester of pregnancy.[1] Both infants died during the first week of life. In both, spirochetes were seen in various fetal tissues stained with the Dieterle silver stain, but cultures and serologic testing were not performed. However, in subsequent prospective studies, no cases of congenital infection have been linked to the Lyme disease spirochete.[110]

COINFECTION

I. scapularis ticks transmit not only *B. burgdorferi,* the Lyme disease agent, but also other infectious agents. These include *Babesia microti* (a red blood cell parasite)[111]; *Anaplasma phagocytophilum* (formerly referred to as "the agent of human granulocytic ehrlichiosis")[111,112]; *Borrelia miyamotoi,* which is more closely related to relapsing fever *Borrelia* than *B. burgdorferi*[112,113]; *Borrelia mayonii* and *Ehrlichia* sp. Wisconsin,[113,114] which are newly recognized species that occur in the upper midwestern United States; and Powassan (deer tick) encephalitis virus,[115,116] which is closely related to European tick-borne encephalitis virus. Babesiosis, anaplasmosis, and *B. miyamotoi* may each cause nonspecific systemic symptoms during summer, and *B. miyamotoi* may cause meningoencephalitis in immunocompromised patients.[117] However, neither *A. phagocytophilum, B. microti*, nor *B. miyamotoi*, is known to cause chronic infection, as with untreated *B. burgdorferi* infection. Fatal cases of infection with deer tick encephalitis virus have been described.[116]

In Europe and Asia *I. ricinus* and *I. persulcatus* ticks, the vectors of *B. burgdorferi* (sensu lato), also transmit tick-borne encephalitis virus.

LABORATORY DIAGNOSIS

Culture of *B. burgdorferi* from patient specimens in BSK medium permits definitive diagnosis. However, positive cultures have been obtained mainly early in the illness, primarily from biopsies of EM lesions,[28] less often from plasma samples,[69] and only occasionally from CSF samples in patients with meningitis. Later in the infection, PCR testing is greatly superior to culture in the detection of *B. burgdorferi* in joint fluid.[88] *B. burgdorferi* has not been isolated from the CSF of patients with chronic neuroborreliosis, and *B. burgdorferi* DNA has been detected in CSF samples in only a small number of such patients.[118]

Because of limited utility of microbiologic techniques, diagnosis is usually based on the recognition of a characteristic clinical picture, exposure in an endemic area, and, except in those with EM, a positive antibody response to *B. burgdorferi*.[119] For serologic testing in the United States, the CDC currently recommends a two-test approach in which samples are first tested by enzyme-linked immunosorbent assay (ELISA), and those with equivocal or positive results are tested by Western blotting (Fig. 241.4).[120] These tests are usually performed with *B. burgdorferi* sonicates, most often obtained from strain B31, an RST 1 strain.

According to the CDC criteria,[119] an IgM Western blot is considered positive if two of the following three bands are present: 23, 39, and 41 kDa; however, the combination of the 23- and 41-kDa bands may still be a false-positive result. An IgG blot is considered positive if 5 of the following 10 bands are present: 18, 23, 28, 30, 39, 41, 45, 58, 66,

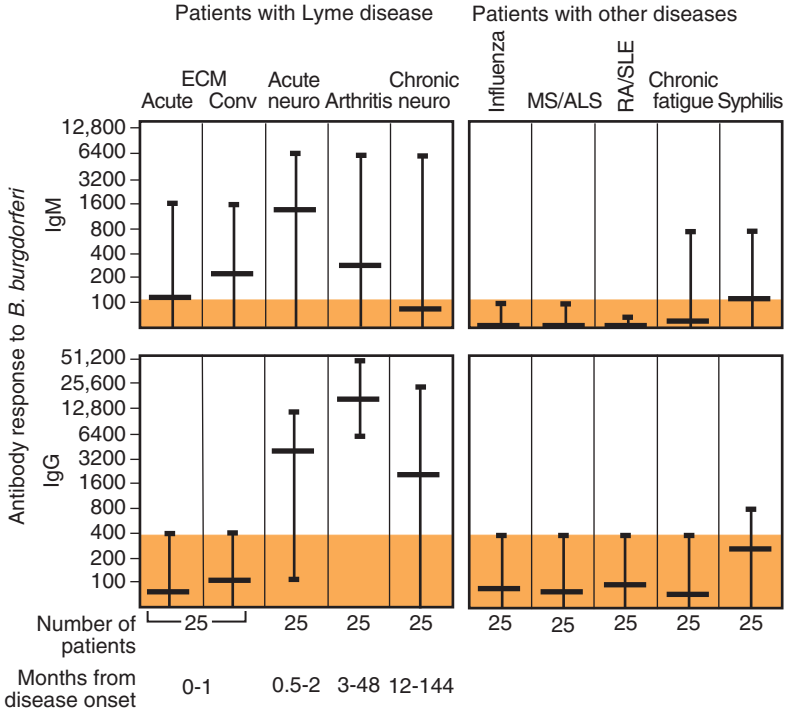

FIG. 241.4 Antibody titers to *Borrelia burgdorferi* by enzyme-linked immunosorbent assay in patients with various manifestations of Lyme disease and in control subjects. Horizontal bars = mean; vertical bars = range; orange hatched area = normal range. Normal range was derived from sera from 50 healthy control subjects. *Acute neuro,* Meningitis; *ALS,* amyotrophic lateral sclerosis; *chronic neuro,* encephalopathy or polyneuropathy; *Conv,* convalescent phase; *ECM,* erythema chronicum migrans; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *MS,* multiple sclerosis; *RA,* rheumatoid arthritis; *SLE,* systemic lupus erythematosus. *(From Dressler F, Whalen JA, Reinhardt BN, et al. Western blotting in the serodiagnosis of Lyme disease.* J Infect Dis. *1993;167:392–400.)*

and 93 kDa (Fig. 241.5). Approximately half of the normal population has IgG reactivity with the 41-kDa flagellar antigen of the spirochete, and this response, by itself, has no diagnostic significance. In Europe no single set of criteria for immunoblot interpretation gives high levels of sensitivity and specificity in all countries.[121]

Serodiagnosis is insensitive during the first several weeks of infection. During this period approximately 30% of patients with EM in the United States have positive responses in acute phase samples, usually of the IgM isotype, but by convalescence 2 to 4 weeks later, about 65% to 75% have seroreactivity, even after antibiotic treatment.[122,123] In patients with classic EM (a large erythema with a bull's eye center, partial central clearing, and a redder outer border), serologic testing is not necessary. However, in patients with less characteristic EM, serologic testing may be helpful. However, it is often necessary to test both acute and convalescent serum samples at the conclusion of 2 to 3 weeks of antibiotic therapy to have reasonable serologic sensitivity. Because these tests are usually performed with an RST 1 strain, the sensitivity of Western blotting may be somewhat lower when the patient is infected with an RST 2 or 3 strain.[14] After 4 to 8 weeks, patients with active infection have positive IgG antibody responses, regardless of the RST of the strain causing the infection. In persons with illness for longer than 4 to 8 weeks, a positive IgM test alone is likely to be a false-positive result, and thus a positive IgM response should not be used to support the diagnosis after the first 2 months of infection.

In patients with acute neuroborreliosis, especially those with meningitis, intrathecal production of IgM, IgG, or IgA antibody to *B. burgdorferi* may often be demonstrated. However, an additional step must be taken to account for the fact that IgG antibody can diffuse across the blood-brain barrier. One method involves dilution of serum and CSF samples according to the total IgG concentrations in these fluids prior to ELISA determinations.[124] Another method, antibody capture enzyme immunoassay, compares the proportion of total-to-specific antibody in both serum and CSF.[74] CSF testing is not a part of the CDC case definition for neuroborreliosis because intrathecal antibody production can be demonstrated primarily in patients with meningitis and not reliably in those with other neurologic manifestations of the disorder or in those with chronic neuroborreliosis.

After antibiotic treatment, antibody titers decline, but IgG and even IgM responses may persist for many years after treatment.[11] However, the Western blot, a nonquantitative test, does not change much after treatment or the number of positive bands decreases very slowly. Thus even a positive IgM response cannot be interpreted as showing recent infection or reinfection unless the appropriate clinical picture is present. In a large vaccine trial in the United States, *B. burgdorferi* caused asymptomatic infection in about 10% of infected patients.[65] In seroprevalence surveys in Europe, more than half of the subjects who were seropositive by ELISA did not remember symptoms of Lyme borreliosis.[125] If patients with past or asymptomatic infection have symptoms caused by another illness, the danger is that the symptoms may be attributed incorrectly to Lyme disease.

The most promising second-generation serologic test is an IgG ELISA that uses a 26-mer peptide of the sixth invariant region of the VlsE lipoprotein of *B. burgdorferi,* called the C6 peptide ELISA.[122,123,126] Similar results were obtained with this test and the standard two-test approach of sonicate IgM and IgG ELISA and Western blot. The principal advantage of the C6 peptide ELISA is the early IgG response, and therefore an IgM test is not necessary. However, the C6 ELISA is not quite as specific as sonicate Western blot.

For this reason, an alternative two-tier test strategy has been proposed that uses two enzyme immunoassays—a sonicate ELISA followed by an IgG VlsE C6 peptide enzyme immunoassay.[127,128] This method eliminates the need for IgM testing; it has nearly comparable sensitivity to the VlsE C6 peptide ELISA alone, and it has better specificity than the C6 ELISA, comparable with that of the conventional two-tiered sonicate ELISA and Western blot assays. The most valuable use for this test is to support the diagnosis of early Lyme disease. Moreover, the information provided to the clinician is straightforward; the patient is either seropositive or seronegative. However, a two-test approach that includes Western blot remains valuable for assessing more complex

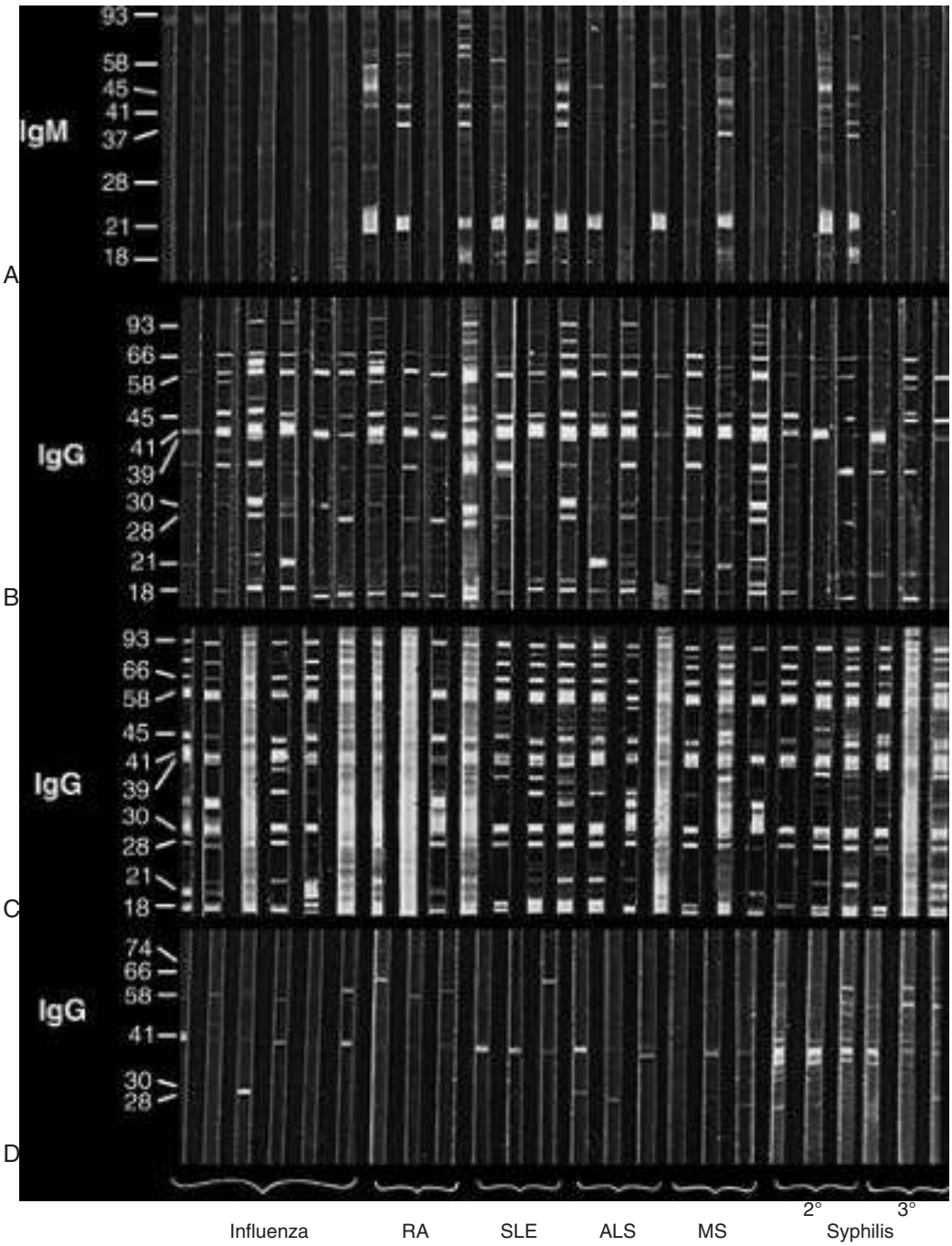

FIG. 241.5 Western blots. (A) Acute-phase sera from 25 patients with erythema migrans. (B) 25 patients with Lyme meningitis or facial palsy. (C) 25 patients with Lyme arthritis. (D) 24 representative patients (controls) who had influenza vaccinations, rheumatoid arthritis *(RA),* systemic lupus erythematosus *(SLE),* amyotrophic lateral sclerosis *(ALS),* multiple sclerosis *(MS),* or secondary or tertiary syphilis. Molecular masses (kDa) are at left. *(From Dressler F, Whalen JA, Reinhardt BN, et al. Western blotting in the serodiagnosis of Lyme disease.* J Infect Dis. *1993;167:392–400.)*

cases because the blot can provide information about the duration of the infection or whether reinfection has occurred. As with the sonicate tests, the response to the VlsE peptide may persist for months or years after successful antibiotic treatment; therefore persistence of the anti-VlsE antibody response cannot be equated with spirochetal persistence in Lyme disease.[122]

Can the infection acquired in Europe be detected with standard two-tiered serologic testing, using assays designed for use in the United States? European assays outperformed analogous US assays in a conventional two-tiered testing algorithm. However, a VlsE C6 peptide ELISA used as a stand-alone test or in the second tier of a two-tiered algorithm performed comparably with conventional two-tiered testing using European assays and can be used for evaluation of any patient, regardless of travel history.[129]

DIFFERENTIAL DIAGNOSIS

Early in its course, a small, homogeneous EM lesion may resemble the red papule of an uninfected tick bite. If an erythema expands rapidly after a tick bite, it is more likely an allergic reaction to tick saliva than an EM lesion, which expands slowly (≈1 cm/day). Patients with secondary annular EM lesions may be thought to have erythema multiforme, but Lyme disease is not associated with blistering, mucosal lesions, or involvement of the palms and soles. Facial palsy caused by *B. burgdorferi* differs from that associated with herpes simplex type 1 virus (Bell palsy)

or varicella-zoster virus (Ramsey-Hunt syndrome) by its seasonal onset (usually June through September), frequent association with EM, and positive IgM and IgG antibody responses to *B. burgdorferi*. Lyme arthritis is most like reactive arthritis or peripheral spondyloarthropathy in an adult or the pauciarticular form of juvenile idiopathic arthritis in a child. Patients with Lyme arthritis usually have high *Borrelia*-specific IgG antibody titers by ELISA, with responses to many spirochetal proteins by Western blot.

The most common mistake is to confuse chronic fatigue syndrome or fibromyalgia with chronic Lyme disease. Although subjective pain or fatigue symptoms may follow Lyme disease, the active infection usually affects one system at a time, and the patient has objective measures of involvement in that system. Moreover, in late disease, systemic symptoms are usually minimal, if present at all.

THERAPY

Evidence-based treatment recommendations for Lyme disease have been presented by the Infectious Diseases Society of America.[130] In brief, the various manifestations of Lyme disease can usually be treated with oral antibiotic therapy, except for patients with certain, objective neurologic abnormalities and occasional patients with Lyme arthritis who may require IV therapy for successful treatment of the infection (Table 241.2). For early localized or disseminated infection, doxycycline for 14 to 21 days is recommended in persons age 8 years or older, except for pregnant women. An advantage of doxycycline is its efficacy against *A. phagocytophilum*, a possible coinfecting agent. Amoxicillin, the second-choice alternative, should be used in children or pregnant women. In case of allergy cefuroxime axetil is a third-choice alternative. Erythromycin or its cogeners, which are fourth-choice alternatives, are recommended only for patients who are unable to take doxycycline, amoxicillin, or cefuroxime axetil. Approximately 15% of patients with disseminated infection experience a Jarisch-Herxheimer–like reaction during the first 24 hours of therapy. In vitro, *B. burgdorferi* is sensitive to tetracycline, penicillin, erythromycin, and their congeners and to third-generation cephalosporins, but it is resistant to rifampin, ciprofloxacin, and the aminoglycoside antibiotics.[130,131]

In multicenter studies of patients with EM, similar results were obtained with doxycycline, amoxicillin, and cefuroxime axetil, and more than 90% of patients had satisfactory outcomes.[132,133] Although some patients had subjective symptoms after treatment, objective evidence of persistent infection or relapse was rare, and re-treatment was usually not necessary. In a recent study 10 days of doxycycline therapy was as effective as 20 days of treatment in the majority of patients with EM, and adding one 2-g dose of parenteral ceftriaxone to the beginning of a 10-day course of doxycycline did not enhance therapeutic efficacy.[134] IV ceftriaxone, although effective, was not superior to oral agents in patients with EM in the absence of objective neurologic involvement.[135] In contrast with second- and third-generation cephalosporin antibiotics, first-generation cephalosporins, such as cephalexin, were ineffective.

For patients with objective neurologic abnormalities, 2- to 4-week courses of IV ceftriaxone are most commonly given.[102,136] Parenteral therapy with cefotaxime or penicillin G may be a satisfactory alternative. In Europe oral doxycycline gave equivalent results to IV therapy in both children and adults with acute neuroborreliosis.[137,138] Although not tested systematically in the United States, oral doxycycline is being used more for this purpose in the United States, particularly in patients who have facial palsy alone or other more localized involvement of the nervous system. With antibiotic treatment the signs and symptoms of acute neuroborreliosis usually resolve within weeks, but those of chronic neuroborreliosis improve slowly over a period of months. Objective evidence of relapse is rare after a 4-week course of therapy. In patients with high-degree atrioventricular nodal block, IV therapy for at least part of the course and cardiac monitoring are recommended, but insertion of a permanent pacemaker is not necessary.

Either oral or IV regimens are usually effective for the treatment of Lyme arthritis.[136,139] Oral therapy is easier to administer; it is associated with fewer side effects, and it is considerably less expensive. Therefore the recommendation is to treat with oral doxycycline or amoxicillin for 30 days.[139] In those with minimal or no response, IV therapy for 2 to 4 weeks is recommended, although improvement does not often begin until the third or fourth week of therapy. Despite treatment with either oral or IV antibiotic therapy, a small percentage of patients in the United States have persistent joint inflammation for months or even several years after 2 months or longer of oral antibiotics or 1 month or longer of IV antibiotics, termed postinfectious, antibiotic-refractory Lyme arthritis.[84] If patients have persistent arthritis despite this treatment, such patients may be treated with nonsteroidal anti-inflammatory agents, disease-modifying antirheumatic drugs, or arthroscopic synovectomy.[84]

TABLE 241.2 Treatment Regimens for Lyme Disease

	EARLY INFECTION (LOCAL OR DISSEMINATED)
Adults	Doxycycline, 100 mg orally twice a day for 14–21 days Amoxicillin, 500 mg orally three times a day for 14–21 days *Alternatives in case of doxycycline or amoxicillin allergy:* Cefuroxime axetil, 500 mg orally twice a day for 14–21 days Erythromycin, 250 mg orally four times a day for 14–21 days
Children (age 8 years or younger)	Amoxicillin, 250 mg orally three times a day or 20 mg/kg/day in divided doses for 14–21 days *Alternatives in case of penicillin allergy:* Cefuroxime axetil, 125 mg orally twice a day for 14–21 days Erythromycin, 250 mg orally three times a day or 30 mg/kg/day in divided doses for 14–21 days
Arthritis (intermittent or persistent)	Doxycycline, 100 mg orally twice a day for 30–60 days Amoxicillin, 500 mg orally four times a day for 30–60 days *or* Ceftriaxone, 2 g IV once a day for 14–28 days Penicillin G, 20 million U IV in four divided doses daily for 14–28 days
Neurologic abnormalities (early or late)	Ceftriaxone, 2 g IV once a day for 14–28 days. Penicillin G, 20 million U IV in four divided doses daily for 14–28 days *Alternative in case of ceftriaxone or penicillin allergy:* Doxycycline, 100 mg orally three times a day for 14–28 days[b]
Facial palsy alone	Oral regimens may be adequate
Cardiac abnormalities First-degree AV block (P-R interval >0.3 s) High-degree AV block	Oral regimens, as for early infection Ceftriaxone, 2 g IV once a day for 14–21 days[c] Penicillin G, 20 million U IV in four divided doses daily for 28 days[c]

[a]Treatment failures have occurred with any of the regimens given, and a second course of therapy may be necessary.
[b]In the author's experience, this regimen is ineffective for the treatment of late neurologic abnormalities of Lyme disease.
[c]Once the patient has stabilized, the course may be completed with oral therapy.
AV, Atrioventricular; *IV,* intravenous.

After appropriately treated Lyme disease, approximately 10% of patients (although the percentage is highly variable in different studies) continue to have subjective symptoms, primarily musculoskeletal pain, neurocognitive difficulties, or fatigue, in some instances, for years.[103,130] Among such patients, five double-blind, placebo-controlled trials failed to show a benefit from additional courses of antibiotic treatment.[140,141,142] In the largest of these studies, patients with post–Lyme disease syndrome received IV ceftriaxone for 30 days, followed by oral doxycycline for 60 days or IV and oral placebo preparations for the same duration. However, there were no significant differences between the groups in the percentage of patients who felt that their symptoms had improved, worsened, or remained the same.[140] However, there was considerable evidence of adverse effects from antibiotic therapy or the IV catheter, including catheter-induced sepsis, pulmonary embolus, anemia, or allergic reactions.[140,141,142] Moreover, prolonged IV antibiotic therapy for unsubstantiated Lyme disease has resulted in biliary complications, *Clostridioides difficile* (formerly *Clostridium difficile*)–associated colitis, autoimmune hemolytic anemia, granulocytopenia, septic shock, vertebral osteomyelitis, paraspinal abscess, and in several instances, death.[109,130] Patients with post–Lyme disease syndrome are best treated symptomatically rather than with prolonged courses of antibiotic therapy.

Although it has not been studied systematically, patients with asymptomatic infection are often given a course of oral antibiotics. Because the risk of maternal-fetal transmission seems to be low, standard therapy for the stage and manifestation of the illness may be sufficient for pregnant patients, except that doxycycline should be avoided.[130] Reinfection may occur in patients who are treated with antibiotics for EM, an early disease manifestation.[143] However, the author has not observed reinfection in a patient with the expanded immune response associated with Lyme arthritis.

PREVENTION

When possible, people should avoid tick-infested areas.[144] If not, insecticides containing DEET (*N,N*-diethyl-meta-toluamide) or permethrin effectively deter ticks, but permethrin can be applied only on clothing, and DEET, although generally safe, can cause serious side effects when excessive amounts are applied directly to the skin.[144] Therefore insecticides may be valuable for the occasional hike in the woods but are less helpful for people living in endemic areas who have daily tick exposures. After exposure in tick-infested areas, tick checks are important. Immature *I. scapularis* ticks usually stay within a few inches of the ground; they often transfer to the lower extremities of the host and attach to moist parts of the body, such as the groin or axillae. In small children they may also be found on the head and neck, which are unusual sites for tick attachment in adults. Because 24 to 72 hours of tick attachment is necessary before transmission of the spirochete occurs, removal of a tick within 24 hours of attachment is usually sufficient to prevent Lyme disease. However, if an engorged nymphal *I. scapularis* tick is found, a single, 200-mg dose of doxycycline usually prevents Lyme disease when given within 72 hours after the tick bite occurs.[130,145]

Environmental control of ticks over widespread areas is difficult.[144] Methods that may be helpful include application of acaricides, landscaping to provide desiccating barriers between tick-infested areas and lawns, and in some settings, removal or exclusion of deer. New methods of tick control, including host-targeted acaricides against rodents and deer, are being developed and may provide help in the future. A commercial Lyme disease vaccine consisting of recombinant OspA with adjuvant was marketed in 1999,[28] but it was withdrawn in 2002. A second-generation OspA vaccine for Lyme disease has been tested in Europe.[146,147] Although a vaccine for Lyme disease is not available now, previous experience proved that vaccination is feasible for the prevention of this infection.

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242 Anaerobic Infections: General Concepts

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SHORT VIEW SUMMARY

Definition
- An anaerobe is an organism that requires reduced oxygen for growth, failing to grow on the surface of solid media in 10% carbon dioxide in air.
- Anaerobes that commonly cause human infections (*Bacteroides, Prevotella*, and *Fusobacterium* spp.) are generally aerotolerant.

Epidemiology
- Anaerobic bacteria are the predominant forms of life in the human body.
- Anaerobes are dominant commensals in mucosal surfaces, such as the oral cavity and the gastrointestinal (GI) and female genital tracts.
- Infections involving anaerobes are often polymicrobial and usually result from the disruption of mucosal surfaces by surgery, trauma, tumors, or ischemia and the subsequent infiltration of resident microbiota.
- Commensal anaerobes have been implicated as crucial mediators of several physiologic, metabolic, and immunologic functions in the mammalian host.

Microbiology
- Despite the number of anaerobic species represented in the normal human microbiota, relatively few are involved in human infections.
- Among the anaerobic gram-negative bacilli, the *Bacteroides fragilis* group is most commonly isolated from human infections.
- Other important gram-negative bacilli involved in human infections include *Prevotella, Porphyromonas*, and *Fusobacteria* spp.

Diagnosis
- Because of their fastidious nature, anaerobes are difficult to isolate and therefore are often overlooked.
- Their isolation requires appropriate methods for collection, transport, and cultivation of specimens.

Clinical Setting
- Anaerobic bacteria generally cause endogenous infections after the breakdown of mucosal barriers and the leakage of indigenous aerobic and anaerobic microbiota into normally sterile sites.
- Infections that are likely to involve anaerobes as important pathogens include oral and dental infections; brain abscess; human and animal bites; aspiration pneumonia and lung abscess; peritonitis after a perforated viscus; infections of the female genital tract, infections after surgeries involving the GI, oral, or female genital tract; and necrotizing infections of soft tissues and muscles.
- The hallmark of infection caused by anaerobic bacteria, mostly gram-negative bacilli, is abscess formation.

Therapy
- Successful therapy for anaerobic infections generally involves the administration of appropriate antimicrobial agents combined with surgical management or percutaneous drainage.
- The antibiotics used to treat anaerobic infections should be active against both aerobic and anaerobic organisms because many of these infections are of mixed etiology. Antibiotic regimens are usually selected empirically on the basis of the type of infection.
- Antibiotic susceptibility testing of anaerobic bacteria is rarely performed in clinical laboratories.
- The antibiotics with the greatest activity against nearly all anaerobic bacteria include carbapenems, β-lactam/β-lactamase inhibitor combinations, metronidazole, and chloramphenicol.
- Antibiotic resistance is increasingly reported among anaerobic bacteria. The major changes have involved the activity of clindamycin, cephamycins, and moxifloxacin against *B. fragilis* and related strains.

Anaerobic bacteria are a major component of the normal human microbiota (formerly termed the *normal flora*) residing on mucous membranes and predominate in many infectious processes, particularly those arising from mucosal sites. These organisms generally cause disease after the breakdown of mucosal barriers and the leakage of indigenous flora into normally sterile sites. The predominance of anaerobes in certain clinical syndromes can be attributed to the large numbers of these organisms residing on mucous membranes, the elaboration of a variety of virulence factors, the ability of some anaerobic species to resist oxygenated microenvironments, synergy with other bacteria, and resistance to certain antibiotics.

Clinicians have become more aware in the past few decades of the types of infections caused by anaerobic bacteria. However, difficulty in handling specimens in which anaerobes may be important and technical difficulties in cultivating and identifying these organisms in clinical microbiology laboratories continue to lead to many cases in which the anaerobic etiology of an infectious process remains unproven. The importance of anaerobes in certain infections is further enhanced by the failure to provide appropriate antibiotic coverage for anaerobes in mixed aerobic/anaerobic infections and an increase in the number of anaerobes that have become resistant to antimicrobial agents. These various factors combine to make it crucial to understand the types of infections in which anaerobes can play a role, to use appropriate microbiologic tools to identify the organisms in clinical specimens, and to choose the most appropriate treatment, including antibiotics and surgical drainage or débridement of the infected site.

This chapter focuses on infections caused by nonsporulating anaerobic bacteria and does not include the clostridial infections and syndromes (see Chapters 243 to 246).

DEFINITION OF AN ANAEROBE

An anaerobe is an organism that requires reduced oxygen for growth and fails to grow on the surface of solid media in 10% carbon dioxide (CO_2) in air. In contrast, facultative organisms can grow in the presence

or absence of air, and microaerophilic bacteria can grow in 10% CO_2 in air or under aerobic or anaerobic conditions. As opposed to several anaerobic species inhabiting human bodily surfaces, which can survive only under strict anaerobic conditions (<0.5% oxygen), anaerobes that commonly cause human infections (*Bacteroides fragilis, Prevotella melaninogenica*, and *Fusobacterium nucleatum*) are generally aerotolerant (i.e., they tolerate 2%–8% oxygen) and can survive for sustained periods, but cannot replicate, in an oxygenated atmosphere.

ANAEROBES IN THE NORMAL HUMAN MICROBIOTA

Anaerobic bacteria are the predominant forms of life in the human body. Several hundred species of anaerobic organisms have been identified in the human microbiota[1,2]; however, many of them cannot be characterized by cultivation in vitro, which has been the cornerstone of microbiology since the 19th century. In an analysis of 13,555 prokaryotic ribosomal RNA (rRNA) gene sequences from the colon, most bacteria identified were considered uncultivated and novel microorganisms.[3] New technologies based on DNA analyses expanded researchers' knowledge, and two main projects, the Human Microbiome Project, carried out by the National Institutes of Health,[4] and the European Metahit, aim to characterize the normal microbiota in healthy individuals.

Increasing evidence indicates that human microbiota composition is influenced by diet, geography, and environmental exposure.[5] Anaerobes are dominant in mucosal surfaces, such as the oral cavity and the gastrointestinal (GI) and female genital tracts. These sites account for 99% to 99.9% of the culturable microbiota. The microbial species and concentrations vary at different sites (Table 242.1). It is interesting that anaerobes also inhabit areas of the body that are exposed to air: skin, nose, mouth, and throat. It has been hypothesized that the ability of anaerobes to withstand oxygen at these sites is due in part to the presence of aerobes and facultative organisms that consume oxygen and reduce the oxidation-reduction potential. In addition, anaerobes are believed to reside in the portions of these sites that are relatively well protected from oxygen, such as gingival crevices.

Anaerobes normally abound in the oral microbiota, with concentrations ranging from 10^2/mL in saliva (*Veillonella parvula* being predominant) to 10^{12}/mL in gingival scrapings. The ratio of anaerobic to aerobic bacteria ranges from 1:1 on teeth to 1000:1 in the gingival crevices. The indigenous oral anaerobic microbiota primarily comprises *Prevotella* and *Porphyromonas* spp., with *Fusobacterium* and *Bacteroides* (non–*B. fragilis* group; see later) present in lower numbers. Until recently the lower respiratory tract was considered sterile below the larynx; however, more recent studies using culture-independent techniques have documented the presence of a respiratory tract microbiota that extends from the nasal passages to the alveoli.[6]

Low numbers of anaerobic bacteria are present in the normally acidic conditions of the stomach and upper intestine. In people with decreased gastric acidity, the microbiota of the stomach resembles that of the oral cavity. The upper intestine contains relatively few organisms until the distal ileum, where the microbiota begins to resemble that of the colon. A stagnant proximal, small intestinal segment caused by stricture, obstruction, diverticulum, or blind loop results in colonic concentrations of bacteria with a predominance of anaerobes. In the colon there are up to 10^{12} organisms per gram of stool, with anaerobes outnumbering aerobes by approximately 1000:1 and accounting for 99.9% of the total bacterial burden. The predominant culturable anaerobes are *Bacteroides* spp. (principally members of the *B. fragilis* group, including *B. fragilis, B. thetaiotaomicron, B. ovatus, B. vulgatus, B. uniformis,* and *Parabacteroides distasonis*) and *Clostridium, Peptostreptococcus,* and *Fusobacterium* spp. However, many of the bacteria in the human colon cannot be cultivated by current laboratory methods.

The normal female genital tract is colonized by 10^7 to 10^9 bacteria per mL, with an anaerobic-to-aerobic ratio of 1:1 to 10:1. The predominant anaerobic species are *Prevotella, Bacteroides, Fusobacterium, Clostridium*, and the anaerobic *Lactobacillus* spp. *Bacteroides* spp. are found in the genital tract of approximately 50% of women, with *B. fragilis* making up less than 15% of this microbial population.[7] The skin microbiota contains anaerobes as well, the predominant species being *Cutibacterium* (formerly *Propionibacterium*) *acnes* and, to a lesser extent, other species of *Cutibacterium* and *Peptostreptococcus.*

TABLE 242.1 Comparison of the Anaerobic Human Microbiota at Mucosal Surfaces

ANATOMIC SITE	SAMPLED SITE	TOTAL BACTERIAL NUMBERS (per g/mL)	ANAEROBE: AEROBE RATIO
Upper airways	Nasal washings	10^3–10^4	3–5:1
	Saliva	10^8–10^9	1:1
	Tooth surface	10^{10}–10^{11}	1:1
	Gingival crevices	10^{11}–10^{12}	10^3:1
Gastrointestinal tract	Stomach	0–10^5	1:1
	Jejunum/ileum	10^4–10^7	1:1
	Terminal ileum and colon	10^{11}–10^{12}	10^3:1
Female genital tract (endocervix and vagina)		10^7–10^9	1–10:1

THE MICROBIOME IN HEALTH AND DISEASE

Commensal bacteria in general and commensal anaerobes in particular have been implicated as crucial mediators of several physiologic, metabolic, and immunologic functions in the mammalian host (see also Chapter 2). The occupation of distinct ecologic niches within the intestinal environment that would otherwise be filled with potentially pathogenic organisms is among the most important roles that anaerobes serve as components of the normal colonic microbiota. In what is termed *colonization resistance,*[8] the presence of anaerobes effectively interferes with colonization by potentially pathogenic bacterial species through the depletion of oxygen and nutrients, the production of enzymes and toxic end products, and the modulation of the host's intestinal innate immune response. For example, *Bacteroides thetaiotaomicron* stimulates Paneth cells to produce RegIIIγ, a bactericidal lectin that can result in killing of gram-positive bacteria.[9] The normal colonic microbiota plays an important role in protection against *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea or colitis, a toxin-mediated, potentially life-threatening disease that results when *C. difficile* spores in the colon transform to vegetative forms with toxin production because of antibiotic elimination of critical components of the competing colonic microbiota and a consequent decrease in microbiota diversity. Changes in the microbiota are accompanied by changes in the metabolome that support *C. difficile* germination and growth (see Chapter 243).[10]

A large body of evidence, including randomized controlled trials, systematic reviews, and meta-analyses, provides clear evidence that fecal microbiota transplantation is a highly effective treatment against recurrent *C. difficile* infection.[11,12] Beyond the treatment of *C. difficile* infection, fecal microbiota transplantation has been investigated in other disorders associated with alteration of the gut microbiota, in particular, ulcerative colitis and metabolic syndrome, but at present there are no definitive conclusions.

The anaerobic component of the intestinal microbiota is also responsible for the production of secreted products that are helpful in human health. The production of vitamin K by anaerobes in the intestine is beneficial to the host, and the production of bile by these organisms is useful in fat absorption and cholesterol regulation. The gut microbiota is integral to the host's digestion and nutrition. Microbiota components can generate nutrients from substrates that are otherwise indigestible by the host. For example, carbohydrate fermentation by *Bacteroides* and other intestinal bacteria results in the production of volatile fatty acids that are reabsorbed and used by the host as an energy source.[13] The capacity of gut microbial digestion of xyloglucans, commonly found in dietary vegetables, has been mapped to a single locus in a certain species of *Bacteroides*.[14]

The microbiota contributes to the development and homeostasis of the immune system. The anaerobic intestinal microbiota influences the development of an intact mucosa and of mucosa-associated lymphoid tissue. Germ-free animals exhibit reductions in vascularity, digestive enzyme activity, and muscle wall thickness as well as undeveloped gut-associated lymphoid tissue. Colonization of these mice with a single species, *B. thetaiotaomicron,* affects the expression of various host genes that influence nutrient uptake, metabolism, angiogenesis, mucosal barrier function, and development of the enteric nervous system.[15] Through its symbiosis factor polysaccharide A (PSA), *B. fragilis* influences the development and function of the immune system[16] and protects mice against colitis in a model of inflammatory bowel disease.[17] In addition, PSA can confer protection both prophylactically and therapeutically, restrain inflammatory processes, and ameliorate disease in an extraintestinal site in a mouse model of multiple sclerosis.[18,19]

Although the gut microbiota confers many benefits (as discussed previously), its dysregulation, termed *dysbiosis*, may play a role in the pathogenesis of diseases characterized by inflammation and aberrant immune responses, such as inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, asthma, and type 1 diabetes.[20,21] Furthermore, the gut microbiota has been associated with obesity, metabolic syndrome,[22,23] cardiovascular disease,[24] and even mental illness.[25]

ETIOLOGY AND MICROBIOLOGY OF ANAEROBIC CLINICAL INFECTIONS

Despite the number of anaerobic species represented in the normal human microbiota, relatively few are involved in human infections. Infections involving anaerobes are often polymicrobial and usually result from the disruption of mucosal surfaces by surgery, trauma, tumors, or ischemia and the subsequent infiltration of resident microbiota. Cecal contents are the source of microorganisms in the case of intraabdominal infections after disruption of intestinal continuity and contamination of the peritoneal cavity. Infections of the head and neck are caused by the commensal microbiota of the mouth. After contamination of previously sterile sites by the mucosal microbiota, the relatively few anaerobic bacteria that survive in the infected site are those that have resisted changes in oxidation-reduction potential and host defense mechanisms.

Table 242.2 shows the gram-negative and gram-positive anaerobes most commonly isolated from clinical specimens.

TABLE 242.2 Anaerobes Commonly Found in Human Infections

Gram-Negative Bacilli
Bacteroides fragilis group: *B. fragilis, B. thetaiotaomicron, Parabacteroides distasonis, B. ovatus, B. vulgatus*
Porphyromonas spp.: *P. asaccharolytica, P. gingivalis*
Prevotella spp.:
Pigmented: *P. intermedia, P. melaninogenica, P. corporis, P. denticola, P. loescheii, P. nigrescens*
Nonpigmented: *P. bivia, P. disiens, P. oralis*
Fusobacterium spp.: *F. nucleatum, F. necrophorum, F. varium*
Bilophila spp.: *B. wadsworthia*
Sutterella spp.
Gram-Positive Cocci
Peptostreptococcus spp.: *P. magnus, P. asaccharolyticus, P. anaerobius, P. prevotii, Parvimonas micra* (formerly *Peptostreptococcus micros*)
Others: *Coprococcus, Peptococcus, Ruminococcus*
Microaerophilic streptococci: (not true anaerobes)
Gram-Positive Non–Spore-Forming Bacilli
Cutibacterium (formerly *Propionibacterium*) spp.: *C. acnes*
Bifidobacterium spp.: *B. dentium*
Lactobacillus spp.
Eubacterium spp.: *E. lentum*
Actinomyces spp.: *A. israelii, A. naeslundii, A. odontolyticus, A. viscosus*
Gram-Positive Spore-Forming Bacilli
Clostridium spp.: *C. perfringens, C. difficile, C. sporogenes, C. sordellii, C. septicum, C. tertium, C. ramosum, C. novyi, C. histolyticum, C. bifermentans, C. innocuum, C. tetani, C. botulinum*

Anaerobic Gram-Negative Bacilli

Among the anaerobic gram-negative bacilli, the *B. fragilis* group is most commonly isolated from human infections.[26] Of this group, *B. fragilis* is the species most often isolated from clinical infections, particularly those emanating from the lower intestine. Other members of this group frequently recovered include *B. thetaiotamicron, B. distasonis, B. ovatus, and B. vulgatus*. These species are all part of the normal GI microbiota and predominate in intraabdominal infections and other conditions that originate from the gut microbiota, such as decubitus ulcer. In the oral cavity the pigmented anaerobes, mainly *Prevotella (P. melaninogenica, P. intermedia), Porphyromonas (P. asaccharolytica),* and nonpigmented *Prevotella (P. oralis, P. oris),* are recognized as species with higher pathogenic potential. They are the predominant anaerobes isolated from respiratory infections and their complications, such as aspiration pneumonia, lung abscess, chronic otitis media, and chronic sinusitis. These species are also isolated from intracranial infections arising from the oral cavity, such as brain abscess.

The fusobacteria *F. nucleatum, F. necrophorum,* and *F. varium,* which normally reside in the oral cavity and the intestinal tract, are often isolated from sites of necrotizing pneumonia and abscesses, including brain abscess. *Prevotella bivia* and *Prevotella disiens* colonize the vagina and are the organisms most frequently isolated from infections arising at this site.

Anaerobic Gram-Positive Cocci

Anaerobic gram-positive cocci are part of the oral, upper respiratory tract, intestinal tract, and skin microbiota and are isolated from anaerobic infections arising from these sites, including chronic sinusitis, mastoiditis, aspiration pneumonia, lung abscess, and necrotizing soft tissue infections (see Chapter 248).

Anaerobic Gram-Positive Non–Spore-Forming Rods

These organisms are part of the microbiota of the gingival crevices, GI tract, vagina, and skin. They include *Cutibacterium* (formerly *Propionibacterium*), *Eubacterium*, *Bifidobacterium*, *Lactobacillus*, *Arcanobacterium*, *Actinomyces*, *Atopobium*, *Mobiluncus*, and *Pseudoramibacter*. The organisms can be isolated from intracranial abscesses, aspiration pneumonia, and peritonitis. *Cutibacterium* spp., of which *C. acnes* is commonest, are ordinarily nonpathogens but can cause infections in implanted prostheses and central nervous system (CNS) shunts and drains.

Anaerobic Spore-Forming Bacilli

Clostridia are the main pathogens among this group. These organisms are commonly isolated from wounds, abscesses, and blood. The most common isolates from clinical infections are *C. perfringens*, *C. septicum*, *C. ramnosum*, *C. novyi*, *C. sordellii*, *C. histolyticum*, *C. fallax*, *C. bifermentans*, and *C. innocuum*.[27] *C. difficile,* which can cause severe intraluminal infections, usually is not invasive.

CLINICAL SYNDROMES CAUSED BY ANAEROBES

Anaerobes are remarkable in their ability to cause a variety of infections at a number of different anatomic sites. Table 242.3 summarizes the types of infections caused by these organisms. Because anaerobes colonize sites that are home to aerobes and also facultative organisms, many infections from which anaerobes are isolated also involve these other bacteria. Fig. 242.1 shows a Gram-stained specimen from a site of mixed infection in a patient with Meleney gangrene, a form of cellulitis involving *Staphylococcus aureus* and anaerobic streptococci.

Certain infections are likely to involve anaerobes as important pathogens, and the presence of these organisms should be assumed. Such infections include oral and dental infections; brain abscess; human and animal bites; aspiration pneumonia and lung abscess; peritonitis after a perforated viscus; infections of the female genital tract (e.g., endometritis, amnionitis, septic abortion, and tubo-ovarian abscess); infections after surgeries involving the GI, oral, or female genital tract; and necrotizing infections of soft tissues and muscles.

The hallmark of infection caused by anaerobic bacteria (mostly gram-negative bacilli) is abscess formation. Typically, abscesses form

TABLE 242.3 Infections Commonly Caused by Anaerobes

Mouth, Head, and Neck
Chronic sinusitis
Dental infection and dental abscess
Periodontitis, periodontal abscess
Gingivitis
Perimandibular infection
Thoracic Cavity
Aspiration pneumonitis
Necrotizing pneumonia
Empyema
Lung abscess
Abdominal Cavity
Peritonitis
Appendicitis
Biliary tract infection
Wound infection
Liver abscess
Intraabdominal abscess
Female Genital Tract
Vulvar and Bartholin gland abscesses
Pelvic inflammatory disease and adnexal abscess
Pelvic abscess
Postsurgery and postpartum infection
Septic pelvic thrombophlebitis
Endometritis, amnionitis, septic abortion
Intrauterine device–associated infection
Bacterial vaginosis
Central Nervous System
Brain abscess
Subdural empyema
Epidural abscess
Skin and Soft Tissue
Diabetic foot ulcer
Decubitus ulcer
Cutaneous abscess
Gas gangrene
Bite wound infection
Bones and Joints
Arthritis
Osteomyelitis
Blood
Bacteremia



FIG. 242.1 Gram-stained specimen from a patient with meleney gangrene. This mixed infection involving *Staphylococcus aureus* and anaerobic streptococci usually occurs around surgical wounds, stomas, and cutaneous fistulas. The infection spreads slowly and often results in skin ulceration but lacks the severe systemic toxicity observed with necrotizing fasciitis. *(Courtesy Dr. Andrew Onderdonk.)*

at sites of direct bacterial contamination, although distant abscesses resulting from hematogenous spread are not uncommon with the more virulent anaerobes.

Anaerobic Infections of the Mouth, Head, and Neck

Anaerobes contribute to infections associated with periodontal disease and to disseminated infections arising from the oral cavity and spreading to adjacent structures in the head and neck[28] (see Chapter 64). The organisms isolated reflect the contiguous normal microbiota, among which *Fusobacteria, Prevotella, Porphyromonas, Bacteroides* (non-*fragilis*) and *Peptostreptococcus* spp. predominate.

Anaerobes are involved in dental infections, including pulpitis, periapical or dental abscess, and perimandibular space infection. In the gingival crevices and gums anaerobes are involved in gingivitis, periodontitis, and periodontal abscess. Formation of dental plaque, which is influenced by oral hygiene and other host factors, leads to the acquisition of pathogenic bacteria and development of these infections. Chronic periodontitis is the most common form of periodontitis. It is plaque induced and a major cause of tooth loss throughout the world. In the healthy periodontium the sparse microbiota consists mainly of gram-positive organisms, such as *Streptococcus sanguinis* and *Actinomyces* spp. In the presence of gingivitis the predominant subgingival microbiota shifts to a greater proportion of anaerobic gram-negative bacilli, with dominance of *Prevotella intermedia*. In well-established periodontitis the microbiota further increases in complexity. The predominant isolates are *Porphyromonas gingivalis, P. intermedia, Aggregatibacter actinomycetemcomitans, Treponema denticola,* and *Tannerella forsythensis.*

Infections of the periodontal area may extend into the mandible, causing osteomyelitis of the maxillary sinuses or infection of submandibular spaces.

Gingivitis may become a necrotizing ulcerative process (trench mouth, Vincent stomatitis). This disease is usually sudden in onset and is associated with tender bleeding gums, foul breath, and a bad taste. Patients may be systemically ill with fever, cervical lymphadenopathy, and leukocytosis. The infection may spread and cause destruction of bone and soft tissue or acute necrosis of the pharynx.

Noma, or cancrum oris, is a necrotizing infection of the oral mucous membranes. It is characterized by destruction of soft tissue and bone and evolves rapidly from gingival inflammation to orofacial gangrene. Noma occurs most frequently in young children ages 1 to 4 years with malnutrition or systemic disease. It occurs worldwide but is most common in sub-Saharan Africa.[29]

Perimandibular infections arise from the spread of organisms originating in the upper airways to potential spaces formed by the fascial planes of the head and neck. Two life-threatening perimandibular infections are Ludwig angina and Lemierre syndrome. Ludwig angina is a bilateral infection of the sublingual and submandibular spaces that results in marked local tissue swelling, tongue displacement, and potential airway compromise.

Lemierre syndrome,[30] usually caused by *F. necrophorum*, is an infection of the posterior compartment of the lateral pharyngeal space with secondary septic thrombophlebitis of the internal jugular vein, bacteremia, and frequent metastasis, most commonly to the lungs. Although *F. necrophorum* is the most common organism reported to cause Lemierre syndrome, other bacteria implicated include *Bacteroides*, *Eikenella*, *Streptococcus*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Proteus*, *and S. aureus.* Peritonsillar abscess is another type of deep neck space infection that is frequently caused by anaerobic bacteria, such as *F. necrophorum* and *Peptostreptococcus* spp. *F. necrophorum* has been indicated as a possible important cause of acute pharyngitis.[31]

Although anaerobic bacteria play little role in acute sinusitis, they have been implicated in chronic sinusitis in both children and adults and are found in 0% to 52% of cases, depending on the specimen collection method. These infections are usually caused by a mixture of aerobic and anaerobic bacteria. The predominant anaerobic bacteria

include *Peptostreptococcus*, *Fusobacterium*, pigmented *Prevotella* spp., and *C. acnes*.[32] Anaerobic bacteria have been isolated in a large percentage of cases of chronic suppurative otitis media in children.[33] The role of anaerobes in acute otitis media is less clear.

Central Nervous System Infections

CNS infections associated with anaerobic bacteria include brain abscess, epidural abscess, and subdural empyema. Anaerobic meningitis is rare and usually suggests a parameningeal collection or shunt infection. Anaerobic bacteria are common constituents of brain abscesses.[34,35] These organisms generally originate from the normal mouth microbiota and are most commonly associated with solitary brain abscesses originating from otorhinolaryngeal infections, such as otitis, sinusitis, or tooth infection. However, intraabdominal or pelvic infections can occasionally lead to bacteremia with an anaerobic organism that seeds the cerebral cortex. The anaerobes in such cases usually reflect the colonic or female genital tract microbiota. A single anaerobic species, or a mixture of anaerobic and aerobic bacteria, may be found in brain abscesses; prominent among the anaerobes are anaerobic or microaerophilic gram-positive cocci and *Prevotella*, *Cutibacterium*, *Fusobacterium*, *Eubacterium*, *Veillonella*, *Bacteroides* (including *B. fragilis*), and *Actinomyces*. Fig. 242.2 shows a computed tomographic scan of a left parietal brain abscess.

Pleuropulmonary Infections

Pleuropulmonary infections[36] are most commonly associated with the aspiration of frequent or large volumes of oropharyngeal material by patients with predisposing conditions, such as dysphagia due to neurologic or esophageal disorders or transiently impaired consciousness due, for example, to alcohol or drug abuse, seizures, head trauma, or cerebrovascular accident. Severe periodontal or gingival disease predisposes to anaerobic pleuropulmonary infections as well (see Chapter 64). The anaerobes most common in pleuropulmonary infections are indigenous to the oral cavity, especially the gingival crevice, and include pigmented and nonpigmented *Prevotella, Peptostreptococcus, Bacteroides* spp., and *F. nucleatum*. Many of these infections are mixed aerobic and anaerobic, and the predominant aerobes from community-acquired aspiration pneumonias are microaerophilic streptococci. A study from Japan using vigorous culture techniques in 212 patients with community-acquired lung abscess showed aerobic and microaerophilic streptococci to be the most common pathogens (60% of patients) and anaerobes to be the second most common (26%).[37] In a study on aspiration pneumonia from a long-term care facility, gram-negative bacilli were the most common isolates (49%), followed by anaerobes (16%) and *S. aureus* (12%).[38] Nosocomial aspiration pneumonia commonly involves a mixture of anaerobes and gram-negative bacilli or *S. aureus*.



FIG. 242.2 Computed tomographic image of a left parietal brain abscess. Area outlined in *white* demarcates the walled-off abscess. *(From Mandell G, ed.* Atlas of Infectious Diseases. *Philadelphia: Churchill Livingstone; 1995.)*

Four major clinical syndromes can develop: aspiration pneumonitis, which can be complicated by necrotizing pneumonia, lung abscess, and empyema. In contrast to the abrupt course of acute pneumonias (e.g., pneumococcal pneumonia), aspiration pneumonitis has an indolent course, evolving over days or weeks instead of hours. Patients usually present with subacute and chronic pulmonary symptoms and manifestations of chronic disease, including weight loss and anemia. Chills are uncommon. The lobes of the lung that are affected are those that were dependent, according to the position of the patient when the aspiration occurred. The sputum is not foul smelling initially but can become malodorous with prolonged infection, and Gram stain reveals a mixed flora. Sputum samples are not reliable for culture because they contain the normal oral flora, but cultures of samples obtained by transtracheal or transthoracic aspiration, which currently are rarely used, may be of value. Protected brush or bronchoalveolar lavage samples obtained by bronchoscopy are controversial because of possible contamination and difficulty associating specific microbes with disease etiology.

Necrotizing pneumonia is characterized by the development of many small abscesses within the pulmonary parenchyma. Lung abscesses most often arise secondary to the development of periodontal disease, and, as would be expected, oral anaerobes predominate. Empyemas are a result of long-term anaerobic pulmonary infection complicated by bronchopleural fistula, foul-smelling sputum, and pleuritic chest pain.

Intraabdominal Infections

Intraabdominal infections, mainly peritonitis (generalized or localized) and abscesses, are usually polymicrobial and result from a breach in the continuity of the mucosal surface and spillage of the normal microbiota into the sterile peritoneal cavity. The cause of the breach can be appendicitis, diverticulitis, neoplasm, inflammatory bowel disease, surgery, or trauma. In infections originating from colonic sites, specimens yield, on average, four to six species, with a predominance of coliforms, anaerobes, and streptococci/enterococci. The most common isolates are *Escherichia coli* and *Bacteroides* spp., among which *B. fragilis* is predominant (see Chapter 74). Other anaerobes commonly isolated from this type of infection include *Peptostreptococcus, Prevotella,* and *Fusobacterium* spp. The involvement of clostridia can lead to severe infections. The dominance of four to six bacterial species out of more than 500 different colonic mucosal species is related to both virulence factors of these species and the inability of clinical laboratories to grow in culture many other species residing in the colonic mucosa.

Disease originating from proximal bowel perforation reflects the microbiota of that site, with a predominance of aerobic and anaerobic gram-positive bacteria and *Candida*. Anaerobic bacteria have been implicated in enterocolitis (typhlitis), an infection of the cecum or the entire bowel in the setting of neutropenia. *Clostridium septicum,* other clostridia, and mixed anaerobes have also been implicated.

B. fragilis has been associated with watery diarrhea in case-control studies of children with undiagnosed diarrheal disease. Enterotoxin-producing strains are more prevalent in patients with diarrhea than in control groups. An etiologic relationship between enterotoxin-producing *B. fragilis* strains and diarrhea has been suggested. [39]

Female Genital Tract Infections

The female genital tract is a major reservoir for anaerobes, which outnumber aerobes at this site by 10:1. Anaerobes are encountered in nearly all infections that are not caused by sexually transmitted agents, including vulvar and Bartholin gland abscesses, pelvic inflammatory disease and adnexal abscesses, septic pelvic thrombophlebitis, intrauterine contraceptive device–associated infection, endometritis, amnionitis, septic abortion, and postoperative or postpartum infections (see Chapter 109). The major isolates from these infections are *P. bivia*, *P. disiens,* and *Peptostreptococcus*, *Porphyromonas*, and *Clostridium* spp. *Actinomyces* and *Eubacterium* spp. are isolated from infections associated with intrauterine devices. Like intraabdominal infections, most infections of the female genital tract are of mixed etiology, involving both anaerobes and aerobes.

Bacterial vaginosis, a disease process in which anaerobes predominate, is characterized by malodorous discharge and inflammation. Although the etiology is not clear, a change in bacterial ecology, with consequent overgrowth of certain bacterial species that replace the *Lactobacillus*-dominated normal microbiota, has been suggested. The anaerobic bacteria involved include *Gardnerella vaginalis, Prevotella*, *Mobiluncus*, and *Peptostreptococcus* spp. A study based on 16S rRNA identification found other anaerobes that were more common in cases than in controls: *Atopobium, Leptotrichia, Megasphaera,* and *Eggerthella*.[40]

Skin and Soft Tissue Infections

Anaerobic infections in the skin and soft tissue can be caused by the cutaneous anaerobic microbiota, mainly *Peptostreptococcus,* but are most often caused by contamination with the flora from adjacent mucosal surfaces.[41] Examples include infected sebaceous and pilonidal cysts, paronychia, breast abscesses, surgical wound infections, human or animal bites, diabetic foot ulcers, and decubitus ulcers. Aspirates from wounds and subcutaneous tissue infections and abscesses below the waist (e.g., decubitus ulcer and diabetic foot infection) yield colonic mixed microbiota, including aerobes and anaerobes, mainly the *B. fragilis* group and *Clostridium* spp., together with Enterobacteriaceae and *Enterococcus* spp. Infections around the oropharynx or infections that originate from that site (paronychia, bites) harbor oral microbiota, such as *Prevotella, Porphyromonas, Fusobacterium,* and *Peptostreptococcus* spp. Anaerobes can also be found in deep soft tissue infections, such as necrotizing fasciitis, synergistic cellulitis, crepitant cellulitis, and gas gangrene, usually as part of a mixed anaerobic/aerobic etiology. This type of infection usually occurs at sites that can be contaminated from oral secretions or feces; the disease can spread rapidly and can be destructive. Gas may be found in the infected tissues. The major pathogens in these deep infections include a combination of aerobes and anaerobes, mainly group A β-hemolytic streptococci, clostridia, peptostreptococci, and *Bacteroides* spp. Fournier gangrene, a form of cellulitis that involves the scrotum, perineum, or anterior abdominal wall and results in extensive loss of skin, may require aggressive surgical intervention.

Bone and Joint Infections

Many patients with osteomyelitis due to anaerobic bacteria have evidence of an anaerobic infection elsewhere in the body. Infected adjacent soft tissue sites are the source of the organisms involved in the osteomyelitis. Examples are diabetic foot ulcers and decubitus ulcers that may be complicated by mixed aerobic/anaerobic osteomyelitis. Cranial and facial bone osteomyelitis is usually caused by oral microbiota that spread from a contiguous soft tissue source or from sinus, ear, or dental infection. Hematogenous seeding of bones with anaerobic bacteria is uncommon. The predominant anaerobes in osteomyelitis of all sites are *Bacteroides* and *Peptostreptococcus* spp. *Prevotella*, *Fusobacterium*, and *Porphyromonas* spp. are prevalent in skull and bite infections. *Clostridium* spp. are found in osteomyelitis associated with wound contamination after trauma or exposure to gut microbiota.

Most cases of anaerobic arthritis, in contrast to anaerobic osteomyelitis, involve a single isolate, and most cases are secondary to hematogenous spread, trauma, or a prosthetic joint. The predominant anaerobes in arthritis are the *B. fragilis* group, *Fusobacterium* spp., and *Peptostreptococcus* spp.[42]

Cutibacterium is occasionally identified in infections involving prosthetic devices, especially shoulder devices.[43]

Anaerobic Bacteremia

Anaerobes are an important cause of bloodstream infections and have accounted for approximately 5% (range, 0.5%–12%) of cases of clinically significant bacteremia, but the rate of anaerobic bacteremia (AB) decreased from the 1970s through the early 1990s. Recent reports present conflicting data regarding rates of AB. A study from the Mayo Clinic compared three periods (1993–96, 1997–2000, 2001–04) and found a 74% increase in the mean incidence of AB.[44] In contrast, a report from Switzerland compared two periods (1997–2001 and 2002–06) and found decreases in both the number of anaerobe-positive blood cultures and the proportion of all blood culture isolates that were anaerobes.[45]

The majority of cases of AB are due to gram-negative bacilli, mainly from the *B. fragilis* group; *B. fragilis* is the anaerobe most commonly isolated from these infections (60%–80% of cases).[46] Reported fatality rates are high, ranging from 25% to 44%, and *B. fragilis* group bacteremia contributes to morbidity and mortality.[47] Other species causing AB include *Clostridium* spp. (10%), *Peptostreptococcus* spp. (10%), and *Fusobacterium* spp. (5%). AB is usually secondary to an infectious process that has emanated from an intraabdominal source, the female genital tract, the respiratory tract, or soft tissue. Debilitating diseases such as malignancies, diabetes, organ transplantation, and abdominal and pelvic surgeries are among the predisposing factors for AB.[48]

In a retrospective nested case-control study, diabetes was found as a risk factor for AB when the source of bacteremia was unknown.[49]

PATHOGENESIS OF ANAEROBIC INFECTIONS

Infections caused by anaerobes are generally a result of the breakdown of a mucosal barrier and the subsequent leakage of the indigenous polymicrobial flora into previously sterile closed spaces or tissue. The introduction of many species of bacteria into otherwise sterile sites leads to a polymicrobial infection in which certain organisms predominate. Tissue ischemia, trauma, surgery, perforated viscus, shock, and aspiration provide anaerobes with the opportunity to penetrate mucosal barriers and enter tissue with a lowered oxidation-reduction potential conducive to proliferation. Three major factors are involved in the pathogenesis of anaerobic infections: virulence factors of the organisms, bacterial synergy, and mechanisms of abscess formation. The predominant gram-negative anaerobes in these infections include *B. fragilis*, *Prevotella*, *Fusobacterium*, and *Porphyromonas* spp. Although some of these organisms are numerically dominant in the normal microbiota, others make up a much smaller proportion (e.g., *B. fragilis*, 0.5%); thus their predominance among clinical isolates indicates that they possess one or more virulence factors that enhance their ability to cause disease. Typically, virulence factors associated with anaerobes confer the ability to evade host defenses, adhere to cell surfaces, produce toxins and enzymes, or display surface structures, such as capsular polysaccharides, that contribute to pathogenic potential. Table 242.4 lists some of the virulence factors associated with anaerobic organisms commonly isolated from clinical infections.

TABLE 242.4 Colonization Factors Associated With Pathogenic Anaerobes That Contribute to Virulence of Medically Important Anaerobes

Bacteroides Fragilis
Capsular polysaccharides
Neuraminidase
Proteases
Enterotoxin
Hemagglutinin
Porphyromonas Gingivalis
Proteases (gingipains)
Lipopolysaccharides
Capsule
Hemolysin
Fusobacterium Necrophorum
Leukotoxin
Hemolysin
Lipopolysaccharides
Phospholipase
Proteases
Fusobacterium Nucleatum
Lipopolysaccharides
Adhesins
Proteases
Leukotoxin
***Prevotella* Spp.**
Lipopolysaccharides
Proteases

The ability of anaerobic bacteria to act synergistically during polymicrobial infection contributes to the pathogenesis of anaerobic infections. The phenomenon of microbial synergy in these infections remains poorly characterized. It has been postulated that facultative organisms function in part to lower the oxidation-reduction potential in the microenvironment and that this change allows the propagation of obligate anaerobes. Additional studies indicate that anaerobes can produce compounds such as succinic acid and short-chain fatty acids that inhibit the ability of phagocytes to clear facultative organisms. Studies in experimental models demonstrate that facultative and obligate anaerobes synergistically potentiate abscess formation.

Although it constitutes only 0.5% to 1% of the normal colonic microbiota, *B. fragilis* is the anaerobe most commonly isolated from intraabdominal infections and bacteremia. The high frequency of abscess formation associated with *B. fragilis* led to studies of this organism's pathogenic potential in relevant animal models of disease. In an animal model of intraabdominal sepsis the capsular polysaccharide was identified as the major virulence factor of *B. fragilis;* this polymer plays a specific, central role in the induction of abscesses.[50] A series of detailed biologic and molecular studies of this virulence factor showed that *B. fragilis* produces at least eight distinct capsular polysaccharides,[51] far more than the number reported for any other encapsulated bacterium. *B. fragilis* can exhibit a wide array of distinct surface polysaccharide combinations by regulating the expression of these different capsules in an on-off manner through the reversible inversion of DNA segments containing the promoters for their expression. Structural analysis of two of these polysaccharides, PSA and PSB, revealed that each polymer consists of repeating units with positively charged free amino groups and negatively charged groups. This structural feature is rare among bacterial polysaccharides, and the ability of PSA and, to a lesser extent, PSB to induce abscesses in animals depends on this zwitterionic charge motif.[52]

Mechanistic studies of the pathogenesis of intraabdominal abscess formation by *B. fragilis* revealed a multifunctional role for its capsular polysaccharides in this process. PSA was found to activate host $CD4^+$ T cells and promote the release of interleukin-17 (IL-17), interferon-γ, and chemokines[53] through a Toll-like receptor 2–dependent mechanism.[54] In addition, the capsule induces the release of the proinflammatory cytokines tumor necrosis factor-α and IL-1β from peritoneal macrophages. These cytokines potentiate the increase of cell adhesion molecules, such as intracellular adhesion molecule-1 on mesothelial cell surfaces, which in turn leads to an increase in the binding of neutrophils to these cells and initiates abscess formation.[55] The capsules of *B. fragilis* also facilitate binding of the organism to mesothelial cells lining the surface of the peritoneal cavity.

B. fragilis produces other virulence factors that allow it to predominate in disease. Although the lipopolysaccharide (LPS) of *B. fragilis* possesses little biologic activity, this organism synthesizes pili, fimbriae, and hemagglutinins that aid in attachment to host cell surfaces. In addition, *Bacteroides* spp. produce many enzymes and toxins that contribute to pathogenicity. Enzymes such as neuraminidase, protease, glycoside hydrolases, and superoxide dismutases are all produced by *B. fragilis*. An enterotoxin termed *B. fragilis* toxin (BFT) has also been identified in *B. fragilis*. This toxin is a metalloprotease that is cytopathic for intestinal epithelial cells and induces fluid secretion and tissue damage in ligated intestinal loops of experimental animals. Strains producing the toxin have been recovered from patients with diarrhea and from healthy controls. An association of BFT-positive *B. fragilis* with clinical episodes of diarrhea in children and adults has been suggested. There is evidence from mouse models that enterotoxin-producing strains of *B. fragilis* may play a role in colon carcinoma.

A prominent etiologic agent in adult periodontitis, *P. gingivalis,* relies on a broad range of virulence factors to cause disease.[56] These include extracellular proteases (cysteine proteinases) that can cause attachment, degradation, or cleavage of host cell proteins and surface receptors and can modulate the host immune response; adhesins such as fimbriae and hemagglutinins; and a putative invasin (halo acid dehalogenase family phosphoserine phosphatase). The capsular polysaccharide of *P. gingivalis* is a potent virulence factor that facilitates the spread of infection in mice to an extent greater than that seen with unencapsulated strains. The LPS of *P. gingivalis* has strong proinflammatory activity and has been implicated in the initiation and development of periodontal disease. *P. gingivalis* has been shown to invade and replicate within host cells, a mechanism that may facilitate its spread. It also evades the host immune response through modulation of innate immune function.

F. necrophorum causes numerous necrotic conditions and human oral infections. Toxins such as leukotoxin, endotoxin, and hemolysin have all been implicated as virulence factors, with leukotoxin and endotoxin playing an important role in the pathogenesis of disease. *F. nucleatum* has been isolated frequently from cases of periodontitis and contributes significantly to gingival inflammation. This organism coaggregates with other oral bacteria to promote attachment to plaque; in addition, it produces several adhesins that facilitate attachment. Both *F. nucleatum* and *F. necrophorum* produce a potent LPS that is responsible for the release of numerous proinflammatory cytokines and other inflammatory mediators that may play a pathogenic role in periodontal disease; in addition, this LPS presumably accounts for the severity of illness in Lemierre syndrome.

Virulence factors associated with *Prevotella* spp. are poorly defined. The organisms' ability to interact with other anaerobes has been reported. Among their prominent virulence traits is the production of proteases and metabolic products such as volatile fatty acids and amines. This group of organisms is particularly noted for secretion of immunoglobulin A (IgA) proteases. The degradation of IgA produced by mucosal surfaces allows *Prevotella* to evade this first line of host defense. *P. intermedia* can invade oral epithelial cells, and antibody specific for fimbriae from this organism inhibits invasion.

Among the most clearly identified virulence factors for anaerobic bacteria are the exotoxins produced by clostridial species, including botulinum toxins, tetanus toxin, *C. difficile* toxins, and five toxins produced by *C. perfringens* (as well as many other clostridial species). These are among the most virulent bacterial toxins known, as demonstrated in mouse lethality assays.

DIAGNOSIS OF ANAEROBIC INFECTIONS

Anaerobic organisms can be difficult to culture, and their identification can be expensive, time consuming, or even misleading because of confusion with normal microbiota in some cases. Therefore many anaerobic infections are diagnosed because the presence of anaerobes is suspected. There are few clinical clues to the probable presence of anaerobic bacteria at infected sites. Infections at various sites, especially those proximal to mucosal surfaces with indigenous anaerobic microbiota, are indicative of the presence of anaerobes, particularly in the GI tract, female genital tract, or oral cavity. Anaerobes are often associated with tissue necrosis and abscess formation at all anatomic locations, including cerebral, dental, peritonsillar, lung, intraabdominal, tubo-ovarian, and cutaneous sites.

A foul odor or gas is highly suggestive of anaerobes as well, although the absence of these factors does not rule out anaerobic infection. In fact, a putrid odor may be a relatively late feature and develops in only approximately one-third to one-half of patients. Furthermore, gas in tissue can sometimes be produced by aerobic bacteria such as *E. coli*. A patient's failure to respond to antibiotics that are not active against anaerobes suggests anaerobic infection. Because anaerobic infections are often polymicrobial, Gram stain of exudates showing a polymicrobial flora and organisms with morphologic features of anaerobes is indicative of anaerobic infection. For example, *Bacteroides* spp. are small, delicate gram-negative bacilli; *F. nucleatum* is a fusiform bacteria with pointed ends; *F. necrophorum* is a long, "ropy" gram-negative bacilli, and *Clostridium* spp. are large "boxcar"-like gram-positive bacilli. By contrast, *Peptostreptococcus* cannot be distinguished from aerobic gram-positive cocci on the basis of Gram stain appearance.

When samples from suspected anaerobic infections are cultured, it is imperative that they be properly collected and transported. Samples should be collected so as to avoid contamination by indigenous flora of mucosal surfaces. The optimal specimens are normally sterile fluids (e.g., blood, pleural, and peritoneal fluids, and aspirates) or biopsy samples from normally sterile sites. In general, liquids or tissues are preferable to swab specimens. The optimal way to transport specimens is by immediate delivery to permit prompt microbiologic processing

or with anaerobic transport tubes. Suitable commercially available anaerobic-transport media should be used. Although many anaerobes are aerotolerant, exposure to oxygen, even for the briefest period, can interfere with culture of some organisms. Selective and nonselective media should be used for culture to identify the clinically relevant anaerobes. It is also important to remember that prior antibiotic therapy reduces cultivability of these bacteria. Specimens should be processed as quickly as possible and handled appropriately in the clinical microbiology laboratory; they should be subjected to Gram staining, and the results should be compared with those of culture. It is not uncommon for specimens to yield no growth on culture but for numerous organisms (both gram-positive and gram-negative) to be evident on Gram staining, a result suggesting that anaerobic organisms are present. Sterile pus may indicate anaerobic infection with failed collection or identification methods. Recent advances in direct detection of anaerobes from clinical samples include 16S rRNA gene–based methods, DNA hybridization, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) Biotyper, multiplex polymerase chain reaction, and oligonucleotide array technologies.[57]

TREATMENT OF ANAEROBIC INFECTIONS AND ANTIBIOTIC RESISTANCE

Successful therapy for anaerobic infections generally involves the administration of appropriate antimicrobial agents combined with surgical management or percutaneous drainage. Because anaerobic infections can cause severe tissue damage or result in abscess formation, débridement of necrotic tissue, drainage of abscesses, restoration of airspaces, resection, maintenance of blood supply, or a combination of these interventions is necessary.

The antibiotics used to treat anaerobic infections should be active against both aerobic and anaerobic organisms because many of these infections are of mixed etiology. Antibiotic regimens are usually selected empirically on the basis of the type of infection, the species of organisms usually present in such cases, Gram stain results, and knowledge of antimicrobial resistance patterns. Other factors influencing the selection of antibiotics include the need for bactericidal activity and for penetration into certain organs (such as the brain), the risk of toxicity, and consideration of the impact on the normal microbiota. Antibiotic susceptibility testing of anaerobic bacteria is rarely performed in clinical laboratories because of inadequate anaerobic culture techniques, difficulty in obtaining results within a useful time frame, and poor quality control of in vitro susceptibility results. It is accepted that testing is important for patients with serious or prolonged infections or in cases in which antibiotics have not had an impact. Testing is also helpful in monitoring the activity of new drugs and recording current resistance patterns among anaerobic pathogens. A correlation between the antimicrobial resistance of an anaerobic pathogen and a poor clinical outcome has been reported in several retrospective trials. A study of antibiotic-treated patients with *Bacteroides* isolates from blood found mortality rates of 45% among those whose isolates were deemed resistant to the agent used and 16% among those whose isolates were deemed sensitive.[58] Notwithstanding this report, antibiotic regimens are still often selected empirically with good results.

The antibiotics with the greatest activity against nearly all anaerobic bacteria include carbapenems, β-lactam/β-lactamase inhibitor combinations, metronidazole, and chloramphenicol (Table 242.5). Antibiotic resistance is increasingly reported among anaerobic bacteria.[59] Resistance rates vary widely among different geographic regions and institutions. The major changes have involved the activity of clindamycin, cephamycins, and moxifloxacin against *B. fragilis* and related strains (*P. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus*). Of interest are the higher resistance rates found among the "other *Bacteroides*" (non–*B. fragilis*) than in *B. fragilis,* even though the "other *Bacteroides*" are less frequently isolated from clinical cases.

Penicillin G is the drug of choice when the infecting strains are susceptible. Most *Clostridium* strains and *Peptostreptococcus* spp. remain susceptible to penicillin. However, the medically important *Bacteroides* spp. are typically resistant to penicillin G. Other strains that may exhibit resistance to penicillin include pigmented and nonpigmented *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., and microaerophilic streptococci. Anaerobes manifest three major mechanisms of resistance to β-lactam antibiotics: inactivating enzyme production (mainly β-lactamases such as penicillinase and cephalosporinase), low-affinity penicillin-binding protein expression, and decreased permeability through porin channel alteration. The production of β-lactamases is the most common mechanism among anaerobes, especially among the *B. fragilis* group; some other *Bacteroides* spp.; and *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp.

Penicillin G, ampicillin, and amoxicillin are equally active against anaerobic strains that do not produce β-lactamase, but the semisynthetic penicillins (methicillin, nafcillin, and oxacillin or dicloxacillin) have unpredictable activity and frequently are inferior to penicillin G. The semisynthetic penicillins—carboxypenicillins (carbenicillin, ticarcillin) and ureidopenicillins (piperacillin, azlocillin, mezlocillin)—generally are administered in high doses and display good activity against most anaerobes in these concentrations; however, up to 30% of *B. fragilis* strains are resistant to these agents.

Cephalosporins have limited use because many anaerobes, including the *B. fragilis* group and many *Prevotella*, *Porphyromonas*, *and Fusobacterium* spp., produce cephalosporinases. The cephalosporinases has little or no activity against the cephamycins cefoxitin and cefotetan, which display greater activity against anaerobic organisms, Approximately 85% of *B. fragilis* isolates are susceptible to cefoxitin, but the other *B. fragilis* group species are more resistant.[59] Cefotetan is less effective than cefoxitin against *B. fragilis* and other members of the *B. fragilis* group.

β-Lactam/β-lactamase inhibitor antibiotic combinations, such as piperacillin-tazobactam and ampicillin-sulbactam, are usually a good option against β-lactamase–producing anaerobes, including the *B. fragilis* group. Resistance rates reported from most countries are still low: 0.5% to 3% in the United States, 3% to 10% in Germany, and 1% to 8% in Argentina. However, up to 48% of *B. fragilis* isolates in Taiwan were found to be nonsusceptible to ampicillin-sulbactam, and a significant increase in resistance to ampicillin-sulbactam among other *Bacteroides* spp., *Prevotella* spp., and *Fusobacterium* spp. was found as well.[60]

Ceftazidime-avibactam, one of the newer β-lactam/β-lactamase inhibitor antibiotic combinations, has limited action against anaerobic bacteria. A study of 396 samples demonstrated the inefficiency of this combination against anaerobes, such as *B. fragilis, Fusobacterium* spp., *Prevotella* spp., and *Porphyromonas* spp.[61] For treatment of intraabdominal infections, ceftazidime-avibactam should be combined with metronidazole.

Carbapenems (ertapenem, doripenem, meropenem, and imipenem) are equally active against anaerobes, with <1% of *B. fragilis* strains showing resistance in the United States and Europe. Higher rates of nonsusceptibility are being reported from some countries (5% in Germany, 8% [to doripenem] in Canada, and 7%–12% in Taiwan).

Metronidazole is active against gram-negative anaerobes, including the *B. fragilis* group; resistance is rare (<1%) in both Europe and the United States but has been reported. Resistance to metronidazole is more prevalent among gram-positive anaerobes, including *C. acnes, Actinomyces* spp., lactobacilli, and anaerobic streptococci. This antibiotic is well tolerated, reaches significant levels in serum, and penetrates abscesses well.

Clindamycin is active against many anaerobes. Rates of resistance to clindamycin among the *B. fragilis* group have increased in the United States from 3% in 1982 to 16% in 1996 and 26% in 2000, with rates as high as 40% to 50% in some series. Resistance has also increased in many non-*Bacteroides* anaerobes. Up to 10% resistance was found in *Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Peptostreptococcus spp*.

C. acnes isolates have also become more resistant to clindamycin, especially in association with previous therapy for acne.

Fluoroquinolones such as moxifloxacin have the potential to treat mixed aerobic/anaerobic infections. However, a survey in the United States found 38% resistance to moxifloxacin among the *B. fragilis* group; in Europe 14% of isolates were resistant, and in Taiwan 7% to 25% of anaerobes isolated from blood cultures were resistant to moxifloxacin.

The macrolides erythromycin, azithromycin, and clarithromycin have moderate-to-good in vitro activity against anaerobic bacteria other than the *B. fragilis* group and other gram-negative anaerobes, but the published clinical experience is limited.

TABLE 242.5 Antimicrobial Agents Effective Against Medically Important Anaerobes

ANTIMICROBIAL AGENT	COMMENT
Nearly Always Active	
Carbapenems (imipenem, meropenem, doripenem)	Resistant to most *Bacteroides* β-lactamases
Metronidazole	Bactericidal against most gram-negative anaerobic strains; inactive against *Cutibacterium* spp., *Actinomyces* spp., peptostreptococci, and microaerophilic streptococci such as *Streptococcus anginosus*
β-Lactam/β-lactamase inhibitor combination (ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid)	Good option against β-lactamase–producing anaerobes, including the *Bacteroides fragilis* group; low resistance rates
Chloramphenicol	Despite excellent in vitro activity against all clinically important anaerobes, this drug is less desirable than other active drugs because of documented clinical failures and bone marrow toxicity
Variably Active	
Clindamycin	Resistance among the *B. fragilis* group has increased in recent years, up to 60% of strains in some reports. Clindamycin no longer recommended for complicated intraabdominal infections
Cephamycins (cefoxitin, cefotetan)	Variable and increasing resistance. Cefotetan no longer recommended for complicated intraabdominal infections
High-dose antipseudomonal penicillins (carbenicillin, ticarcillin, piperacillin, azlocillin, mezlocillin)	Good activity against most anaerobes; however, up to 30% of *B. fragilis* strains are resistant to these agents
Tigecycline	Active against nearly all anaerobes, including *Bacteroides* spp. Resistance has been reported
Variably Resistant	
Penicillin	Inactive versus some or most penicillinase-producing anaerobes, including most of *B. fragilis* group and many strains of *Prevotella*. Most *Clostridium* strains and *Peptostreptococcus* spp. are susceptible
Cephalosporins (excluding cephamycins)	Limited utility because many anaerobes, including the *B. fragilis* group and many *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp., produce cephalosporinases
Tetracyclines (doxycycline and minocycline)	Limited use because of significant resistance
Vancomycin	Active against gram-positive anaerobes; inactive against gram-negative anaerobes
Macrolides	Moderate-to-good in vitro activity against anaerobic bacteria other than the *B. fragilis* group and other gram-negative anaerobes; however, published clinical experience limited
Fluoroquinolones (moxifloxacin)	*B. fragilis* group shows increasing resistance, and moxifloxacin no longer recommended in the IDSA guidelines for complicated intraabdominal infections
Resistant	
Aminoglycosides	—
Trimethoprim-sulfamethoxazole	—
Monobactams (aztreonam)	—

IDSA, Infectious Diseases Society of America.

Among the tetracyclines, doxycycline and minocycline are of limited use because of significant resistance. Tigecycline, a glycylcycline, is active against anaerobic bacteria, including most *Bacteroides* spp., peptostreptococci, and *Cutibacterium, Prevotella*, and *Fusobacterium* spp. Tigecycline has been used as a single-agent treatment for complicated intraabdominal infections; however, resistance (≈6%) among *Bacteroides* and non-*Bacteroides* spp. has been reported.

Despite excellent in vitro activity against all clinically important anaerobes, chloramphenicol is less desirable than other active drugs for the treatment of anaerobic infection because of documented clinical failures and bone marrow toxicity.

Anaerobic bacteria are intrinsically resistant to aminoglycosides and trimethoprim-sulfamethoxazole.

As mentioned previously, resistance among some anaerobes has increased significantly over the past 3 decades. Recently, cases of multidrug-resistant *B. fragilis* have been reported; isolates were resistant to metronidazole, carbapenems, piperacillin-tazobactam, and clindamycin, among other agents.

In clinical situations specific regimens must be tailored to the initial site of infection. Antibiotic treatment for intraabdominal infections needs to be directed against *Bacteroides* spp. and the gram-negative aerobic flora of the bowel. Single agents suitable for patients with mild-to-moderate community-acquired intraabdominal infections include the carbapenems and β-lactam/β-lactamase inhibitor combinations, such as piperacillin-tazobactam or ticarcillin-clavulanate. The Surgical Infection Society/Infectious Diseases Society of America 2010 guidelines also list cefoxitin, moxifloxacin, and tigecycline as options, but these agents should usually be avoided because of substantial rates of in vitro resistance to cefoxitin and fluoroquinolones among *Bacteroides* spp. and coliforms and concern about greater mortality associated with tigecycline than with other antibiotics for various infections, including intraabdominal infections. Moxifloxacin may still be cautiously used for intraabdominal infections, provided that the patient has mild-to-moderate disease and has not been exposed to fluoroquinolones recently.

A two-drug regimen is an alternative, with one drug active against coliforms and the other against anaerobes (e.g., a third-generation cephalosporin or a quinolone with metronidazole). In addition, if the clinician suspects that gram-positive facultative organisms, such as enterococci, are involved, therapeutic regimens should include ampicillin or vancomycin. Although clindamycin and cefotetan were previously considered acceptable options for intraabdominal infections involving anaerobes, these drugs are no longer recommended because of escalating rates of resistance in the *B. fragilis* group. (Ampicillin-sulbactam is not recommended because of high rates of resistance among community-acquired *E. coli*, not because of resistance in anaerobic bacteria).[62]

Mixed aerobic/anaerobic infections of oral origin must include drugs active against both the gram-positive aerobic microbiota and the anaerobic flora of the mouth, which usually does not include the *B. fragilis* group. β-Lactam drugs (penicillins and cephalosporins) alone are not suitable because of β-lactamase production by anaerobic strains

that are usually isolated from infections originating above the diaphragm (*Fusobacterium, Prevotella,* and *Peptostreptococcus*). Suitable regimens for these infections include clindamycin, β-lactam/β-lactamase inhibitor combinations, or penicillin together with metronidazole.

The failure of antibiotic therapy against an anaerobic infection should prompt consideration of surgical drainage or débridement of the infected site. In addition, the possibility of coinfection with one or more drug-resistant aerobic organisms should be considered. In these situations isolation of the organisms should be attempted to determine antibiotic susceptibility.

ACKNOWLEDGMENT

The authors wish to acknowledge the contributions of Arthur O. Tzianabos, PhD, to this chapter in earlier editions of this book.

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243 *Clostridioides difficile* (Formerly *Clostridium difficile*) Infection

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SHORT VIEW SUMMARY

Definition
- *Clostridioides difficile* infection (CDI) is an acute diarrheal illness due to colitis that is most often preceded by antimicrobial use and is caused by toxins of an anaerobic spore-forming bacterium, *Clostridioides difficile* (formerly *Clostridium difficile*).

Epidemiology
- *C. difficile* is found in water, soil, meats, and vegetables and is particularly common in health care environments, where the spores are difficult to eradicate. Patients are exposed to and ingest *C. difficile* spores in the health care setting from contact with the environment or health care workers who do not practice good hand hygiene.
- If the patient has taken antibiotics recently, the normal microbiota is likely to be disrupted, and in this disrupted microbiota the ingested spores, which are resistant to stomach acid, can germinate.

Pathogenesis
- Vegetative cells proliferate in the colon, producing two major toxins: A (primarily an enterotoxin) and B (primarily a cytotoxin). The two toxins produce a marked neutrophilic inflammatory response in the colon, resulting in diarrhea, erosion of the mucosa, and formation of pseudomembranes composed of necrotic cells and proteinaceous material that extends over the mucosal surface.
- Recurrence of diarrhea following treatment occurs in 25% of patients and requires re-treatment.

Microbiology
- *C. difficile* are obligate gram-positive anaerobic bacteria that can survive in environments under aerobic conditions by forming spores.
- The majority of strains produce two toxins, A and B, thought to be responsible for many of the disease characteristics. A third toxin, binary toxin, is produced by recent epidemic strains that have caused outbreaks with increased severity and mortality.
- Some strains of *C. difficile* lack the genes for toxin production and are nontoxigenic, and are not thought to cause CDI.

Diagnosis
- Diagnosis of CDI is based on the presence of clinical symptoms (usually defined as >3 watery, loose, or unformed stools within a period of 24 hours or less) coupled with a diagnostic test (usually of a stool specimen) that detects the presence of either the *C. difficile* organism or its toxin genes, or detection of *C. difficile* toxin using an enzyme immunoassay or cell cytotoxin assay.

Therapy
- The first steps in effective treatment are to stop the offending antibiotic and provide fluid and electrolyte support, if needed.
- Specific treatment consists of vancomycin or fidaxomicin orally for mild-to-moderate disease (metronidazole may also be used but is less effective), vancomycin or fidaxomicin orally for severe disease (white blood cell count >15,000/mL, or creatinine >1.5 × baseline), and vancomycin orally or by nasogastric tube plus intravenous metronidazole for severe complicated (also called fulminant) CDI that presents with hypotension, shock, ileus, or megacolon.
- Patients with recurrent CDI may benefit from oral vancomycin in a taper and pulse dose, fidaxomicin, or the monoclonal antibody bezlotoxumab. Multiple CDI recurrences are effectively treated by fecal microbiota transplant (FMT).

Prevention
- Prevention in the health care environment is focused on preventing patient exposure to spores of *C. difficile* by utilizing isolation, cohorting, gloves and gowns, and hand washing (alcohol rubs are ineffective against spores). Bleach is used in the environment to eradicate spores.
- It is exceedingly difficult to prevent spore exposure, so antimicrobial stewardship programs to reduce unnecessary antimicrobial use are extremely effective in limiting the number of susceptible patients.
- A number of new preventive approaches are undergoing clinical investigation, including vaccines to induce antibodies to toxins A and B, and biotherapeutics that are live bacterial organisms such as FMT or spores of nontoxigenic *C. difficile*.

The administration of antibiotics can be complicated by a number of unintended consequences, among which gastrointestinal side effects are quite common. Gastrointestinal symptoms occur in up to 25% to 50% of patients depending on the specific antimicrobial agent, patient population, and epidemiology.[1] While the majority of these side effects are mild, consisting of minor antibiotic-associated diarrhea (AAD) without systemic signs of illness, some patients can develop frank colitis and severe clinical manifestations, including toxic megacolon, intestinal perforation, sepsis, and death. Infection with *Clostridioides difficile* (formerly *Clostridium difficile*) is thought to be responsible for about 25% of all cases of AAD and is the underlying etiology in nearly all cases of severe disease and pseudomembranous colitis (PMC).

While Koch's postulates were fulfilled for *C. difficile* 40 years ago,[2] we still have an incomplete understanding of how disruption of the normal indigenous gut microbiota leads to *C. difficile* colonization and frank disease. Additionally, while adequate antimicrobial therapy for *C. difficile* infection was described shortly after the etiologic role for the pathogen was established,[3] a recent increase in the apparent severity, prevalence, and recurrence of *C. difficile* infection (CDI)[4,5] has prompted additional interest in understanding the pathogenesis of this infection.

HISTORICAL OVERVIEW

Prior to 1978 the disease pseudomembranous colitis and the organism *Clostridioides difficile* had never been linked. PMC was described in humans by Finney in 1893,[6] and in animals in 1943 by Hambre and colleagues, following administration of penicillin that resulted in rapid animal death.[7] PMC occurred in patients prior to the antibiotic era, perhaps consistent with the lack of prior antibiotic use in a small subset of current CDI patients.[8] However, it was the antibiotic era that was accompanied by an increase in PMC, particularly in association with use of lincomycins.[9,10] It was the publication by Tedesco and coworkers[10] in 1974 that started the search for a cause for PMC linked to clindamycin use, at the time called *clindamycin-associated colitis*. Of

200 clindamycin-treated patients, 21% were found to have diarrhea and nearly half of those (10%) were found to have PMC, including 38% of patients who inexplicably had onset of diarrhea after clindamycin had been stopped.

After 4 years of intensive research, in 1977 and 1978 a succession of papers leading to the identification of *C. difficile* and its toxins as the cause of clindamycin-associated colitis or PMC and of vancomycin as an effective treatment were published. The laboratories of Larson in the United Kingdom, and Bartlett, Fekety, and Finegold in the United States were largely responsible for these discoveries, using the hamster as an essential animal model for CDI.[11–17]

In retrospect, the rapidity and thoroughness of the CDI research discoveries of the 1970s may have engendered an early perception that there was little else to learn. This may have led to a clinical perception of CDI as a nuisance disease that was not even monitored as a health care–associated infection in many hospitals. This perception of CDI changed markedly in the early 21st century when CDI epidemics in North America and Europe with high mortality occurred that were caused by a previously little-known *C. difficile* strain that has been variously described as North American pulsed-field gel electrophoresis type 1 (NAP1), restriction endonuclease analysis (REA) type BI, and PCR ribotype 027, collectively referred to as NAP1/BI/027.[5,18]

It should be noted that prior to the realization that *C. difficile* was the etiologic agent for the majority of cases of PMC, other infectious agents had been implicated as causative agents of PMC and AAD (reviewed by Gorkiewicz[19]). *Staphylococcus aureus* was one of the first bacteria associated with pseudomembranous enteritis following the administration of antibiotics.[20,21] Experimental evidence was provided by studies that demonstrated that antibiotic-treated chinchillas would develop enterocolitis after infection with *S. aureus.*[22] Although *C. difficile* is responsible for most cases of severe antibiotic-associated colitis, enterotoxigenic *S. aureus* strains can still be isolated from *C. difficile*–negative cases.[23] Enterotoxigenic *Clostridium perfringens* strains have also been isolated from patients with AAD.[24] Another form of severe antibiotic-associated colitis (associated with hemorrhage, but not pseudomembrane formation) appears to be due to infection with *Klebsiella oxytoca.*[25] These findings indicate that a proportion of cases of antibiotic-associated colitis and AAD that are due to infection are not secondary to *C. difficile,* but this is only in the minority of cases. Additionally, it needs to be restated that infection (including infection with *C. difficile*) accounts only for about a quarter of cases of AAD. In noninfectious cases of AAD, disruption of the normal composition and function of the indigenous gut microbiota alone, as detailed in the following section, is thought to be the underlying cause.[26]

PATHOGENESIS

The Indigenous Gut Microbiota and Antibiotics

The pathogenesis of antibiotic-associated colitis and AAD, whether or not they are associated with *C. difficile* infection, is thought to center on the disruption of the normal, indigenous gut microbiota by antimicrobial drug administration. It is important to note that the concept of the indigenous gut microbiota playing a central role in human health has existed for at least 100 years. Recent scientific and technologic advances have permitted the study of the role that the gut microbiome plays in human health and disease,[27,28] including the development of antibiotic-associated colitis and AAD.

The indigenous gut microbiota are thought to play a number of critical roles in host homeostasis and for the purposes of CDI, the function of importance has been called "colonization resistance."[29] Colonization resistance refers to the ability of the normal gut microbial community to resist the ingrowth of pathogenic microbes. A number of different mechanisms are speculated to be important for maintaining colonization resistance, including competition for nutrients, occupancy of ecologic and physical niches, production of antimicrobial products, and signaling through the host immune system (Fig. 243.1).[30] Regardless of the specific mechanisms, therapeutic antibiotic administration can profoundly disrupt the indigenous gut microbiota community and therefore disrupt colonization resistance. Studies in human and animal systems indicate that antibiotics can have long-lasting effects on the gut microbiome structure, and thus alter gastrointestinal function.[31–34] With regard to *C. difficile* the altered gut microbiome structure and function can directly influence the biology of the pathogen in terms of growth and pathogenesis. In the following sections, as specific topics in the pathogenesis and virulence of *C. difficile* are discussed, potential influences of the indigenous microbiota are highlighted.

Sporulation and Germination in *Clostridioides difficile*

As with many anaerobic bacteria, during periods of environmental stress vegetative *C. difficile* bacteria initiate the sporulation program, which results in the production of the spore form of the organism.[35,36] This physically robust form is stable to oxygen stress, temperature extremes, and desiccation. Within the hospital environment, this includes resistance to the effects of alcohol-based hand sanitizers.[37] The spore form thus represents a reservoir for the organism, which can persist in the environment.

Following ingestion of spores, germination is apparently triggered by the environment that exists within the gastrointestinal tract.[38] It has been shown that primary bile acids, including taurocholic acid, can serve as potent germinants in vitro, with glycine functioning as a cogerminant.[39–41] Interestingly, members of the indigenous microbiota efficiently metabolize primary bile acids through the process of deconjugation and dehydroxylation to produce secondary bile acids,[42–44] some of which have been shown to be inhibitory to *C. difficile.*[40,41,45] Therefore one mechanism by which the disruption of the indigenous microbiome by antibiotics can lead to CDI is through alteration of in vivo bile acid metabolism.[30,46] This leads to a situation that favors germination of ingested spores and the subsequent vegetative outgrowth of the pathogen. The key role of spore germination in the pathogenesis of *C. difficile* may serve as an attractive therapeutic target, as has been recently demonstrated with the use of a synthetic bile salt analogue that inhibits germination and has a protective effect in animal studies.[47] Similarly, restoration of bile salt metabolism by administration of a bacterium with 7-α-dehydroxylase activity could restore colonization resistance against *C. difficile.*[48]

Toxin Production

The histopathologic hallmark of *C. difficile* infection is damage to the mucosal epithelium with generation of an acute, neutrophil-predominant inflammatory response with the formation of a pseudomembrane consisting of sloughed epithelial cells, inflammatory cells, and a fibrinous exudate (Fig. 243.2). Damage to the epithelium is caused by the most well-characterized *C. difficile* virulence factor, the large glucosyltransferase toxins TcdA and TcdB.[49–52] Members of the large clostridial toxin family, TcdA and TcdB are produced within the gastrointestinal lumen during the stationary phase of vegetative growth of *C. difficile.* Following binding to a number of potential cell surface receptors,[53–56] these toxins are taken up by the cells of the mucosal epithelium through the process of receptor-mediated endocytosis.[57,58] Within the endosome the toxin undergoes autocleavage to release the catalytic subunit, which is transferred to the host cell cytoplasm.[59] Subsequently, the active subunit glycosylates host cell guanosine triphosphatases belonging to the Rho family of cytoskeletal regulatory proteins.[60,61] Intracellular activation of Rho guanosine triphosphatases leads to disruption of the actin cytoskeleton, ultimately leading to apoptotic and necrotic cell death. In cultured epithelial cells, cytoskeletal disruption results in cell rounding and cytotoxicity. In vivo these effects are manifested by loss of cell-cell tight junctions with subsequent increase in epithelial permeability.[62,63] TcdA and TcdB also induce the secretion of cytokines in host cells, including interleukin-8, which likely leads to the acute neutrophilic inflammatory infiltrate that characterizes *C. difficile* infection.[64] Recent data suggest that these two related toxins cause cell death and tissue damage via distinct pathways.[65,66]

Other Virulence Factors

Some strains of *C. difficile,* including the NAP1/BI/027 strains that are responsible for the recent global outbreaks of CDI, secrete a toxin that is a member of the clostridial binary toxins exemplified by the iota toxin from *Clostridium perfringens.*[67] *C. difficile* binary toxin (CDT) is

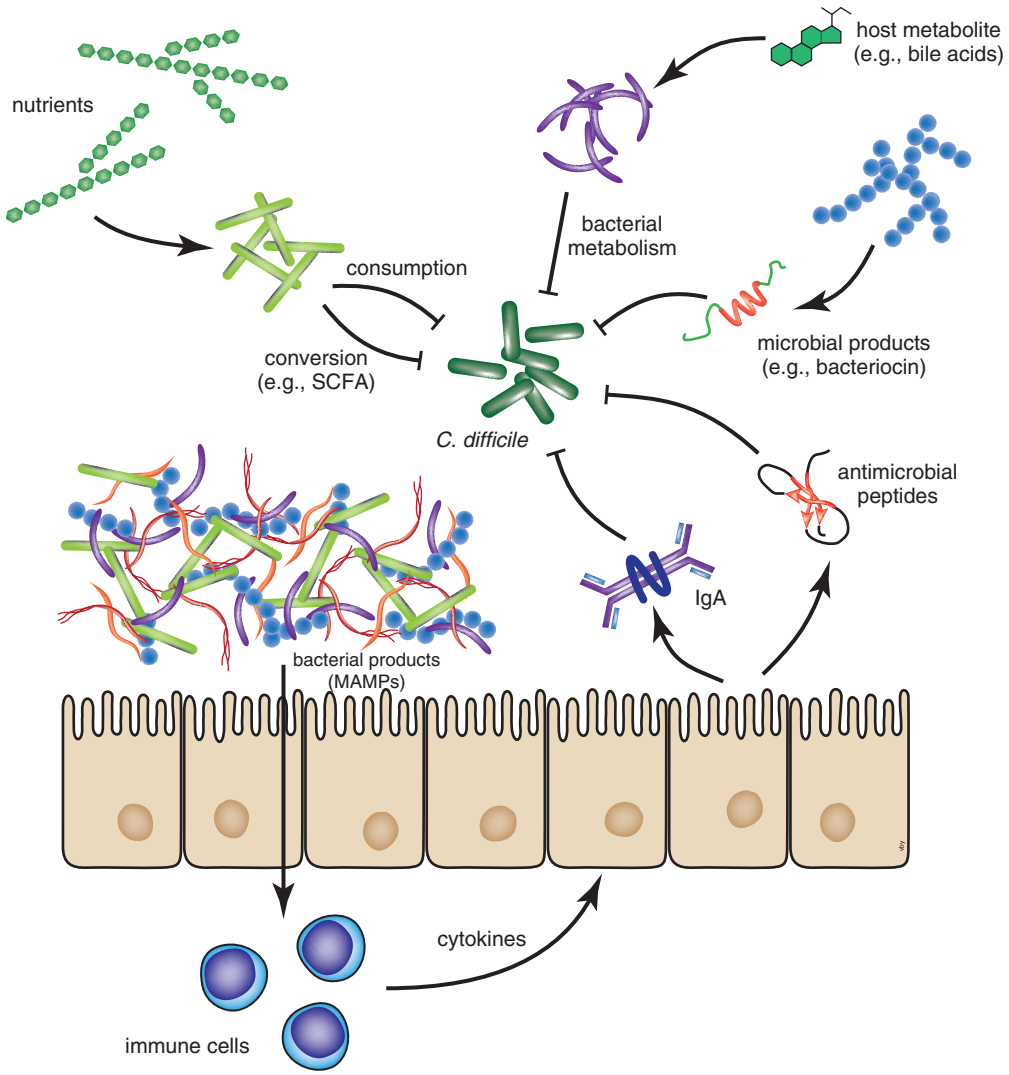

FIG. 243.1 Mechanisms by which the indigenous microbiota can mediate colonization resistance against *Clostridioides difficile*. *IgA,* Immunoglobulin A; *MAMPs,* microbial-associated molecular patterns; *SCFA,* short-chain fatty acid. *(From Britton RA, Young VB. Interaction between the intestinal microbiota and host in* Clostridium difficile *colonization resistance.* Trends Microbiol. *2012;20:313–319.)*



FIG. 243.2 Histopathology of pseudomembranous colitis. There is significant polymorphonuclear infiltrate, a fibrinous exudate, and epithelial damage.

a two-part toxin that is an ADP-ribosyltransferase specific for actin monomers, thus disrupting the actin cytoskeleton.[67] CDT is produced by only a small fraction of *C. difficile* strains, and thus its role in pathogenesis is unclear.[68] Although some reports suggest that infection with a CDT-producing *C. difficile* strain results in more severe clinical disease, this is not a universal finding and the actual significance of infection with CDT-producing strains is not clearly defined.[69–72]

In addition to the three toxins, multiple other potential virulence factors for *C. difficile* have been studied, including surface layer proteins, surface polysaccharides, flagella, and various adhesins.[73] To date, the specific roles of any of these factors in *C. difficile* virulence have not been delineated, but ongoing work on these factors may lead to new strategies to prevent and treat CDI.

Host Response to *Clostridioides difficile* Infection

Patients infected with *C. difficile* can mount adaptive immune responses to the toxins TcdA and TcdB.[74] The level of antibody response to the *C. difficile* toxins is inversely correlated with the relative risk of developing recurrent disease.[75] This finding served as the basis for the development of toxin-specific monoclonal antibodies for the prevention of CDI recurrence.[76] Additionally, because the pathogenesis of *C. difficile* is tightly linked to pathogen expression of TcdA and TcdB, an alternative approach to immunotherapy in the form of toxin-based vaccines is being explored.[77]

Additional work has been done examining the role of innate immune responses in the pathogenesis of CDI.[78] In animal models, mice that are deficient in Toll-like receptor signaling, including MyD88 knockout mice, were shown to be more susceptible to experimental CDI.[79,80] Similarly, animals deficient in signaling through the intracellular

pattern-recognition pathway involving Nod1 are more susceptible to *C. difficile* infection.[81] It appears that signaling through the innate immune system early during infection is important in host defense against *C. difficile*. Increased interleukin-23 signaling is encountered in humans with acute CDI, and this finding is replicated in murine models of infection.[82] Protection against acute disease apparently reflects the balance of several innate immune responses to *C. difficile* infection. Innate lymphoid cells (ILCs) of the ILC-1 type and to a lesser extent of the ILC-3 type are required for recovery from acute disease in a murine model.[83] Different studies demonstrated that eosinophils, stimulated by the microbiota, also provide protection in murine models of acute CDI.[84] Interestingly, these same investigators demonstrated that expression of binary toxin may increase virulence of *C. difficile* by suppressing this eosinophilic response.[85] These studies illustrate the complex system of interactions among the microbiota, host responses, and pathogen in the pathogenesis of CDI. Importantly, this may lead to novel treatment strategies for CDI, particularly severe acute disease.

Pathogenesis of Recurrent *Clostridioides difficile* Infection

While the majority of patients with symptomatic CDI respond to antibiotic therapy directed against the pathogen, up to 25% of patients will have recurrent symptoms following the discontinuation of *C. difficile* therapy for an initial episode of disease.[86,87] The risk of recurrence increases with each episode, resulting in significant morbidity and mortality in this population of patients. A number of factors have been investigated with regard to the pathogenesis of recurrent infection. As noted previously, the adaptive host response may play a role in determining the risk of recurrent disease.[75]

Several studies have indicated that pathogen characteristics may contribute to the risk of recurrence. Infection with the epidemic NAP1/BI/027 *C. difficile* strain is associated with an increased risk of recurrence.[88] The reasons for this are unclear, but may have to do with the intrinsic antibiotic resistance, the dynamics of spore biology of the strain, or the presence of an additional toxin, binary toxin.

Because alteration of the indigenous gut microbiota by antibiotics is a prerequisite for the majority of cases of CDI, the role of the gut microbiome in recurrence has been examined. It has been shown that patients with recurrent CDI exhibit decreased microbial diversity of their indigenous gut microbes.[89] Presumably, if the gut microbiota is unable to return to its baseline state, the patient is at increased risk of reinfection or regrowth of residual *C. difficile* following therapy. This has led to the exploration of restoring microbiome diversity through the administration of probiotic bacteria or the transplantation of feces from normal donors (see "Treatment" later).

Altered Virulence in Specific *Clostridioides difficile* Lineages

The increase in the incidence and apparent severity of CDI over the past 15 years has been associated with the widespread appearance of the NAP1/BI/027 strain of *C. difficile*. This has prompted a number of investigators to determine if this particular strain of *C. difficile* is actually associated with worse outcomes in infected patients and whether or not this strain has specific virulence determinants not widespread in other strains of the pathogen.

Several factors are characteristic of NAP1/BI/027 strains. These strains typically are resistant to newer fluoroquinolone antibiotics.[5] Furthermore they possess the binary toxin CDT, and also harbor a characteristic mutation in the anti-sigma factor *tcdC,* which is theorized to play a role in the regulation of toxins TcdA and TcdB.[5] This mutation in *tcdC* introduces a frameshift that results in a truncated, nonfunctional protein.[90] Because studies have demonstrated that these epidemic strains can produce increased levels of toxin in vitro, it has been proposed that this characteristic mutation is responsible for this phenotype, although this has been questioned by reconstitution of *tcdC* that showed no effect on toxin production.[80] There is in vitro evidence that the TcdC regulator can inhibit in vitro expression of the large *C. difficile* toxins,[90] but the presence of alterations within this regulatory gene does not directly correlate with increased production of toxins within clinical isolates.[91] One group demonstrated that complementation of the *tcdC* mutation in *trans* will decrease the amount of toxin produced by a NAP1/BI/027 clinical isolate and reduce the virulence of this strain in the hamster model of infection.[92] However, a second group that utilized a genetic system that allowed correction of the *tcdC* mutation directly on the chromosome failed to show an association between *tcdC* genotype and the level of toxin production.[93]

Additional in vitro studies suggested that NAP1/BI/027 strains had an increased ability to sporulate,[94] which could contribute to the ease with which these strains can spread within the health care environment. However, this observation has also been disputed by measuring sporulation in vitro in multiple *C. difficile* strains, including multiple NAP1/BI/027 strains that did not reveal increased sporulation of these epidemic strains.[95] Perhaps most important to the enhanced virulence of these strains is the presence of a third toxin, binary toxin CDT, an ADP-ribosylating toxin that has been shown to enhance the lethality of toxin A in the hamster model and to cause a modest degree of hamster mortality in the absence of toxins A and B.[96] It is interesting to note that this constellation of characteristics has also arisen within an unrelated epidemic strain of polymerase chain reaction (PCR) ribotype 078 that was identified in Europe (and that arose in swine)[97] and together with ribotype 027 was found to exhibit increased 14-day mortality in CDI patients.[98]

Despite this evidence, there is still debate over the relative importance of these genetic and phenotypic characteristics of the epidemic *C. difficile* strains in the pathogenesis of the organism.[99] In spite of these conflicting data, it is likely that our understanding of the basic pathogenesis of CDI will result in the development of more effective strategies for prevention and treatment of this infection.

EPIDEMIOLOGY

C. difficile is a ubiquitous organism, able to survive for long periods in the environment through sporulation. al Saif and Brazier showed the widespread presence of *C. difficile* in environmental sites such as surface water, drinking water, swimming pools, and soil, and in a wide variety of animals, including dogs, cats, horses, sheep, and pigs.[100] In addition, some foods have been shown to be contaminated at a low level with *C. difficile* spores that are of the same ribotype that cause clinical disease. This includes beef, pork, turkey, and a variety of vegetables, but there has been no evidence of foodborne transmission to date. It is highly likely that humans ingest *C. difficile* spores frequently, but remain asymptomatic (and uncolonized) as a result of the colonization resistance of an intact gut microbiota.

Health Care–Associated *Clostridioides difficile* Infection

It has been known since 1979 that the hospital environment is contaminated with *C. difficile* spores.[101] In addition, *C. difficile* contamination of the hands of hospital personnel and of the home environment of patients, and spore persistence for up to 20 weeks in the environment, have been known since 1981.[102] This environmental contamination is presumed to be the result of the repeated CDI diarrheal episodes of hospitalized patients and resistance of spores to killing by environmental cleaners and disinfectants other than bleach. In 1986, the first prospective case-control study of CDI was published and showed 87% of 149 cases to be health care associated (and hospital associated).[103] In addition, *C. difficile* was found asymptomatically in the stool of 21% of control patients. Both the specific use of clindamycin and use of multiple antibiotics for the treatment of infection were identified as risk factors. McFarland and colleagues[104] in a landmark paper used rectal swab specimens to identify hospital acquisition of *C. difficile* by 21% of sampled patients, 63% of whom were asymptomatic. Johnson and coworkers[105] also used weekly rectal swab cultures to document health care (in this case hospital) acquisition of *C. difficile* by 21% of patients; in this study, 82% of patients were asymptomatic. The use of REA typing identified 18 unique REA types of *C. difficile*, but symptomatic disease (CDI) was caused only by two closely related REA types, B1 and B2, which also caused asymptomatic colonization in some patients, but CDI in others.

It has also been shown that admission of asymptomatic patients carrying *C. difficile* in their stool to a hospital ward preceded the

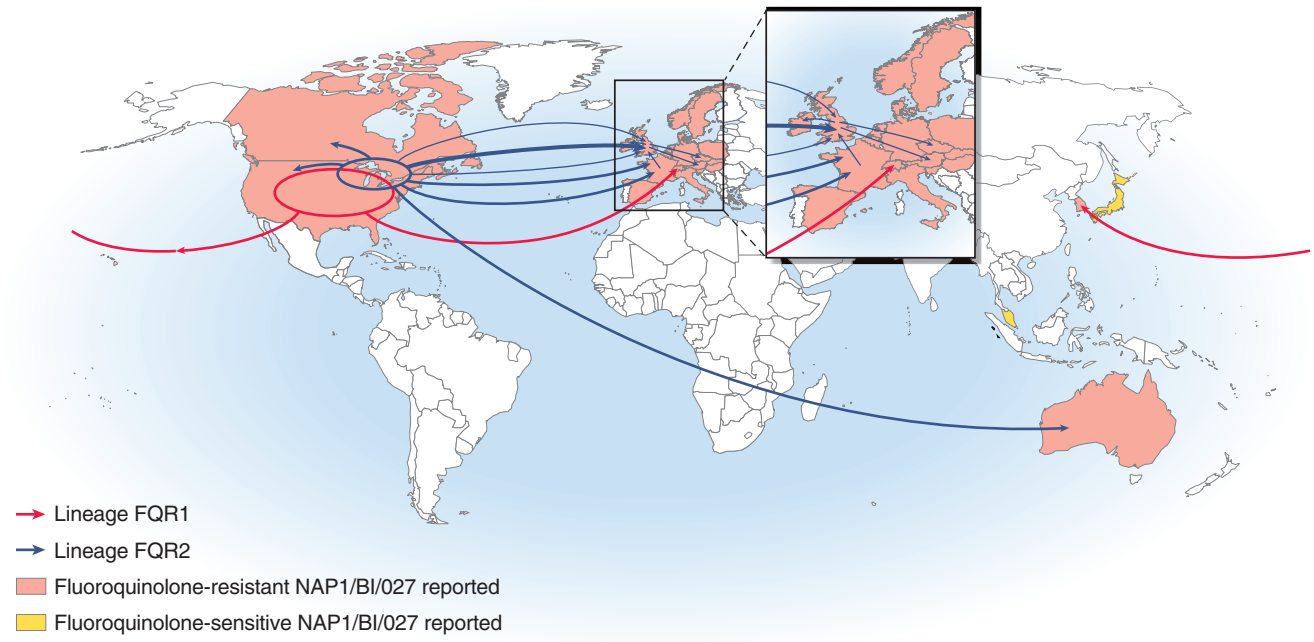

FIG. 243.3 Inferred global spread of the NAP1/BI/027 strain of *Clostridioides difficile*. *(From He M, Miyajima F, Roberts P, et al. Emergence and global spread of epidemic healthcare-associated* Clostridium difficile. Nat Genet. *2013;45:109–113.)*

acquisition of that specific strain of *C. difficile* by other patients on the same ward in 85% of acquisitions.[106] Although asymptomatic patients colonized with *C. difficile* may be a source of transmission to other patients, contrary to intuitive thinking, they have a significantly lower risk of developing CDI themselves compared to uncolonized patients on the same hospital wards at the same time.[107] It is presumed that these asymptomatic colonized patients are protected from CDI either because they harbor nontoxigenic strains of *C. difficile* (which lack toxin genes and do not cause symptoms) or they are protected from the effect of *C. difficile* toxins through adaptive immunity (most likely an anamnestic antitoxin antibody response) if they are colonized by toxigenic strains of *C. difficile*.

The health care epidemiology of CDI changed markedly in the early years of this century when multiple hospitals, first in the United States and then in Canada, reported widespread outbreaks of CDI of particularly high severity, causing high mortality and requiring increased use of colectomy to treat patients refractory to medical management.[5,18,108] These outbreaks were caused by a specific group of *C. difficile* strains designated NAP1/BI/027 that rapidly spread to the United Kingdom and European Union countries. Although unknown at the time, there were two distinct lineages of NAP1/BI/027, designated FQR1 and FQR2, that acquired fluoroquinolone resistance (FQR) mutations and a highly related conjugative transposon.[109] The FQR1 lineage almost certainly originated in the United States and was widely disseminated there, with spread to Asia and Switzerland (Fig. 243.3). The FQR2 lineage was found in both Montreal, Canada and multiple US sites and spread more widely than FQR1 to the United Kingdom and Europe. Health care–associated CDI incidence rose to as high as 22.5 CDI cases per 1000 discharges in the Montreal NAP1/BI/027 outbreak, with 30-day attributable mortality of 6.9%.[18] More recent reports suggest that the current hospital incidence in the United States is in the range of 5 to 10 CDI cases per 10,000 days of care.[110]

Community-Associated *Clostridioides difficile* Infection

There has never been a question that CDI occurs in the community setting, but whether the incidence of community-associated CDI versus community-onset CDI is increasing is an active epidemiologic question that is likely being affected by shorter inpatient stays and increased health care delivery in the outpatient setting. In particular, the use of antibiotics in the outpatient setting, calculated to amount to 258 million courses in 2010,[111] is likely to create enormous pressure for the development of community-associated CDI. Hirschhorn and colleagues[112] found the rate of community-associated CDI to be 7.7 cases/year per 100,000 population in a health plan database in Boston published in 1994. No antibiotic exposure was found in a surprising 35% of patients in the previous 42 days. More recently the US Centers for Disease Control and Prevention (CDC) has shown that community-associated CDI rates vary by geographic area and that independent predictors of higher community-associated CDI incidence are older age, white race, female gender, and use of nucleic acid amplification testing (NAAT). After adjusting for these predictors, community-associated CDI rates ranged from 30.7 to 41.3 per 100,000 population.[113] Conflicting community rates have been found in other large databases. One of these, the UK General Practice Research Database, found that CDI increased from less than 1 case per 100,000 in 1994 to 22 cases per 100,000 in 2004. Only 39% of these patients had prior antibiotic exposure documented within 90 days prior to CDI.[113] A reanalysis of the same data using a case definition of community-associated CDI as treatment with oral vancomycin found an association with prior antibiotic exposure in 55% of patients.[114] Using a more definitive methodology, a UK study of positive stool cultures from community-onset diarrhea patients showed 2.1% of stools positive for *C. difficile* toxin by cell cytotoxin assay in urban and rural patient cohorts, corresponding to rates of 29.5 and 20.2 cases per 100,000 population, respectively, much closer to more recent US rates.[115] Only 52% of these patients had antibiotic exposure in the previous 4 weeks.

A more recent report of community CDI epidemiology conducted by the CDC through its Emerging Infections Program has clarified a number of aspects of CDI in the community and health care settings.[110] In contrast to the 1986 report of 87% hospital-onset CDI, the more recent population-based report from the CDC of 10,342 CDI cases in 111 acute-care hospitals and 310 nursing homes revealed only 25% hospital-onset CDI, a similar proportion with nursing home onset, and 45% with community-onset CDI (Fig. 243.4). Among all CDI cases, 94% had either inpatient or outpatient health care exposure within the previous 12 weeks, leaving only 6% of all CDI cases with no prior health care exposure.

In a related study, of 989 community-associated CDI patients, only 18% had no outpatient health care exposures, 41% had low-level, and 41% had high-level outpatient exposures.[116] Of these, 358 (36%) did not receive antibiotics, but the incidence of antibiotic exposure was

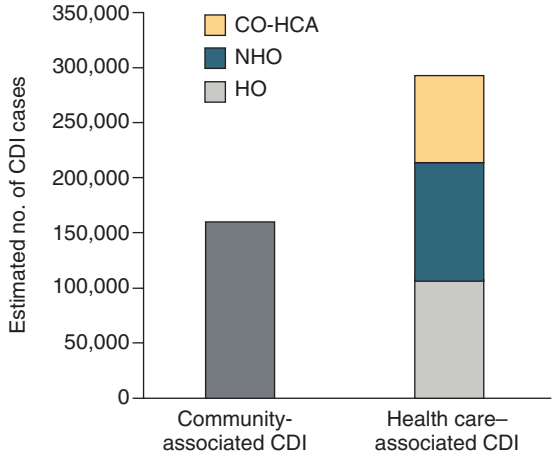

FIG. 243.4 Estimated burden of *Clostridioides difficile* infection *(CDI)* in the United States, 2011. Cases are divided into community-associated versus health care–associated cases. Of the community-associated cases, 82% were estimated to be associated with outpatient health care exposure. The health care–associated cases are divided into community-onset health care–associated cases *(CO-HCA),* hospital onset cases *(HO),* and nursing home–onset cases *(NHO). (From Lessa FC, Mu Y, Bamberg WM, et al. Burden of* Clostridium difficile *infection in the United States.* N Engl J Med. *2015;372:825–834.)*

significantly higher in the groups with health care exposure than in those without ($P < .01$), an indication that health care exposure predisposes to CDI via exposure to antibiotics. The frequency of exposure to proton pump inhibitors (PPIs) was not significantly higher in patients without antibiotic exposure than in patients with antibiotic exposure, suggesting that PPI use is not an independent or surrogate (for antibiotic use) risk factor for community-associated CDI. CDI patients with low-level health care exposures were more likely to be exposed to infants <1 year of age (odds ratio, 2.1; 95% confidence interval, 1.1–4.5) and household members with CDI (odds ratio, 6.9; 95% confidence interval, 5.6–56.5) compared to patients with high-level exposures, suggesting that these are *C. difficile* exposure sources. There was no association between food or animal exposure and level of health care exposure, leaving the source for acquisition of *C. difficile* undetermined for most patients.

Molecular Epidemiology of *Clostridioides difficile* Infection and Importance of Strain Type

The lack of a universally sensitive and reproducible typing system has slowed progress in the molecular epidemiology of CDI, but PCR ribotyping, pulsed-field gel electrophoresis, and REA are the most frequent typing systems in use. REA was used to document the surprising finding that approximately 50% of isolates from second episodes of CDI were different from the original isolate, suggesting an environmental source for recurrent CDI at least half of the time[117]; this observation has shifted recently toward more frequent identification of the same strain as the cause of recurrence (>80% of instances).[118] REA was also used to document the wide diversity of *C. difficile* strains in one hospital (55 different types) during an endemic period.[119] The REA J group found in multiple US hospitals in the 1990s was shown by PCR ribotyping in the United Kingdom to be ribotype 001.[120,121] These J/001 strains were clindamycin resistant, and increased CDI incidence was associated with clindamycin exposure in the patients. Molecular typing was instrumental in identifying the current epidemic *C. difficile* toxinotype III strains both by pulsed-field gel electrophoresis (type NAP1) and by REA (group BI) in hospitals in the United States and Canada, and confirmed in the United Kingdom and Europe as PCR ribotype 027.[5,18] These strains are highly resistant to fluoroquinolone antibiotics and are associated with fluoroquinolone use as a risk factor.[18] Typing using whole-genome sequencing suggests that there is a very diverse population of *C. difficile* strains causing CDI in hospitals.[122]

TABLE 243.1 Risks for Development of *Clostridioides difficile* Infection

Any antibiotic versus no antibiotic:
Number of antibiotics (risk increases with number)
Days of antibiotics (increased risk with increased days)
Type of antibiotic:
Highest risk: clindamycin, fluoroquinolones, cephalosporins of second generation and higher
Moderate risk: penicillins, macrolides, penicillin β-lactamase inhibitors, carbapenems, vancomycin, metronidazole
Lower risk: aminoglycosides, tetracyclines, trimethoprim, sulfonamides, rifampin
Proton pump inhibitors and histamine type 2 blockers
Patient age (increased risk with increased age of the patient)
Prior hospitalization
Severity of underlying illness
Abdominal surgery
Nasogastric tube
Duration of hospitalization
Long-term care residency

Clostridioides difficile Infection in Children

Hall and O'Toole first described *C. difficile* in 1935 as a colonizing organism in newborn infants.[123] Ironically this continues to be a contentious diagnostic issue in children up to the age of 1 to 2 years because the frequent asymptomatic colonization of these young children confounds the diagnosis of diarrhea; if tested they frequently will be positive for toxigenic strains of *C. difficile,* but the frequency of stool positivity for *C. difficile* in these children with diarrhea is no higher than that of children of the same age without diarrhea.[124] It is not known how very young children can tolerate colonization by toxigenic *C. difficile* without developing diarrhea or other symptoms. Data from newborn rabbits suggests that there may be insufficient toxin A receptors in these young animals that allows them to tolerate toxin, but similar observations in humans have not been made.[125] When children are sampled on a monthly basis over the first year of life, all are found to at some time be colonized with *C. difficile,* both toxigenic and nontoxigenic strains, and they are noted to frequently change strains over time.[126] Because of the high rate of colonization in children under the age of 1 to 2 years, it is not recommended that they be tested for CDI.[125] Beyond the age of 3 years the colonization rate of children resembles that of adults (<3%) and risk factors for CDI are also similar to those in adults, but CDI rates are generally much lower in children than in adults, especially adults over age 65 years.[124,127] Health care–associated CDI in children is associated with prolonged hospitalization and increased mortality, whereas community-associated CDI is not.[128]

Risk Factors

Risk factors have been identified for CDI, for recurrent CDI, for CDI severity, and for CDI caused by the epidemic NAP1/BI/027 strain.

Risk for *Clostridioides difficile* Infection

The overall risk for developing CDI is dominated by antimicrobial exposure, including duration, number, and class of antimicrobials (Table 243.1).[129] Risk is highest during therapy and in the first month after cessation of antimicrobial therapy and decreases between 1 and 3 months posttherapy.[130] In addition, stomach acid–inhibiting agents such as PPIs and histamine type 2 antagonists have also been associated with increased risk for CDI, although some studies fail to make this association.[131] It is likely that the combination of an antimicrobial and a PPI increases the risk for CDI.[132] Patient factors are also important CDI risks, with advanced age, immunosuppression, prior hospitalization, and severity of underlying illness contributing to increased risk.[133] Absence of antitoxin antibodies and waning antibody levels with increasing age are likely additional risks for CDI. A meta-analysis and strain risk comparisons have identified fluoroquinolone exposure and age over 65 years as risks for CDI caused by NAP1/BI/027.[134,135]

Risk for Recurrent *Clostridioides difficile* Infection

Risk factors for recurrent CDI are similar to those for an initial episode but are particularly predictable because one antecedent episode of CDI increases risk with each subsequent recurrence (Table 243.2). Additional antimicrobial treatment either during or after the initial CDI episode markedly increases risk of recurrence, and as with primary CDI, the most elderly patients and those with the most severe underlying disease are at highest risk of recurrence.[136–138]

Risk for Severe *Clostridioides difficile* Infection

There has been an ongoing search for a clinical prediction rule or score that would predict at the time of diagnosis which patients are destined for a severe CDI outcome, usually defined as death, need for intensive care unit admission, megacolon, perforation, shock, or requiring colectomy for treatment. A number of patient predictors, such as a white blood cell (WBC) count >15,000 or 20,000/μL and a rise in creatinine of >1.5 times baseline (or a serum creatinine ≥1.5 mg/dL), have correlated with more severe disease.[4,139,140] Low serum albumin and elevated WBC count have been found to predict CDI severity independent of infecting strain type, but higher 14-day mortality has been observed with infection caused by two strains of *C. difficile*—NAP7-8/BK/078 and NAP1/BI/027—as well as with the presence of specific patient biomarkers: elevated WBC and C-reactive protein, and low serum albumin.[99,141] Additionally, infection with the NAP1/BI/027 strain of *C. difficile* has resulted in lower cure rates and higher recurrence rates compared to other *C. difficile* strains when treated with either fidaxomicin or vancomycin.[142] Although several studies have demonstrated the association between infection with NAP1/BI/027 strains and increased disease severity, this has not been a universal finding.[99]

Infection Control and Prevention

Infection control practices for CDI are of two primary types: prevention of *C. difficile* transmission to the patient, and reduction of the likelihood of CDI developing in the event that a patient encounters *C. difficile* or its spores (Fig. 243.5). Current guidelines for prevention of CDI in hospitals focus on patients with symptomatic infection and emphasize the importance of optimizing basic practices before considering addition of special approaches with less supporting evidence or greater potential for undesirable outcomes.[143] For prevention of transmission, the basic practices include contact precautions (i.e., personnel wear gloves and gowns for any contact with the patient or environment and patients are placed in a single-patient room with dedicated noncritical patient care equipment) and efforts to ensure adequate cleaning and disinfection of equipment and the environment.[143] Gloves are recommended because alcohol hand sanitizers are ineffective in reducing spores of *C. difficile* on hands.[37,144,145] In addition, there is evidence that requiring health care workers to wear gloves when handling the body substances of patients can reduce the incidence of CDI.[146] Sporicidal disinfectants (e.g., bleach, peracetic acid–based products) are recommended, particularly in outbreak or hyperendemic settings, based upon multiple studies reporting reductions in CDI when bleach was substituted for nonsporicidal disinfectants.[147] The recommendation for dedicated noncritical patient care equipment (e.g., thermometers, stethoscopes) for CDI cases is based on evidence that such equipment may frequently become contaminated with spores.[148] Moreover, replacement of electronic thermometer use (the handles become contaminated with spores of *C. difficile*) by disposable thermometers has been associated with significant reductions in CDI rates.[149]

If basic practices are unsuccessful in controlling CDI, health care facilities may consider adding special approaches that address plausible sources of transmission. Patients with acute CDI shed large numbers of spores,[150] and delays in diagnosis are not uncommon.[151] In addition, many patients with CDI continue shedding spores as asymptomatic carriers for several weeks after treatment[152] (see Fig. 243.5). Based on these findings, many hospitals preemptively isolate patients with orders for CDI testing, or extend the duration of contact precautions to hospital discharge, or both. The potential benefit of expanded use of contact precautions must be balanced against the potential for adverse effects.[143] Given that up to 25% of initial CDI cases will develop a recurrence, extending the duration of contact precautions also ensures that many patients with recurrent CDI will be under contact precautions when their symptoms recur. Monitoring of environmental cleaning with feedback to cleaning personnel, daily disinfection of surfaces in CDI rooms, and adjunctive use of no-touch disinfection technologies (e.g., ultraviolet light room decontamination devices, hydrogen peroxide vapor) can reduce spore contamination in patient rooms, and have been associated with reductions in CDI in quasi-experimental studies.[147,153,154] However, in two recent multicenter randomized trials, interventions to improve cleaning and disinfection through monitoring and feedback or ultraviolet light room decontamination did not reduce

TABLE 243.2 Risk Factors for Development of Recurrent *Clostridioides difficile* Infection

Any prior episodes of *C. difficile* infection, particularly severe infection
Antibiotic use (concomitant or post–*C. difficile* infection treatment, or both)
Age ≥65 years
Prolonged or recent stay in health care facility
High severity of Horn index for underlying illness
Immunosuppression
Proton pump inhibitor use
Infection with NAP1/BI/027 strain
Absence of an antitoxin A antibody response

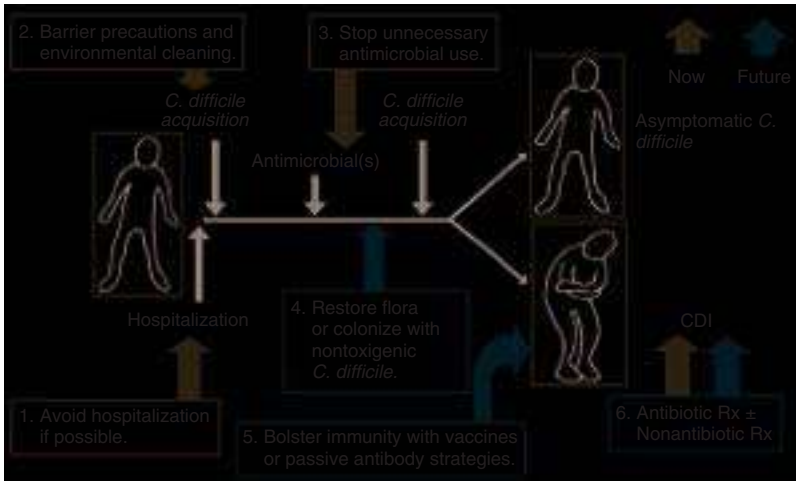

FIG. 243.5 Sites of attack for present and future prevention and management of *Clostridioides difficile* infection. *(From Gerding DN, Johnson S. Management of* Clostridium difficile *infection: thinking inside and outside the box.* Clin Infect Dis. *2010;51:1306–1313.)*

health care–associated CDI in comparison to standard cleaning with bleach.[155,156] In a secondary analysis of one of these trials, addition of ultraviolet light room decontamination to standard cleaning in isolation rooms was associated with a significant but modest reduction in hospital-wide incidence of CDI, but no reduction was seen when ultraviolet light was added to bleach disinfection.[157]

One limitation of current approaches for prevention of *C. difficile* transmission is that asymptomatic carriers of toxigenic *C. difficile* are not addressed. Two recent studies suggest that asymptomatic carriers may be an important source of transmission.[158,159] Moreover, in a controlled quasi-experimental study in a Canadian acute-care hospital, detecting and isolating asymptomatic carriers was associated with a significant decrease in the incidence of health care–associated CDI.[160] Given the difficulties involved in implementing screening programs for *C. difficile* carriage, additional high-quality studies are needed to evaluate the impact of routine screening and isolation. A more feasible approach might be to extend the duration of contact precautions after CDI symptoms resolve because patients with prior CDI are an easily identified subset of asymptomatic carriers (~20% of all carriers).[143,161]

Preventing patients from encountering and ingesting *C. difficile* while in the hospital is extremely difficult given the survival durability of spores and their resistance to commonly used hand sanitizers and disinfectants. Rendering the patient host less susceptible to disease if spores are ingested is an alternative method to prevent infection. The most effective method to reduce the risk for CDI is to reduce overuse of all antimicrobials or of specific high-risk agents, including cephalosporins, clindamycin, and fluoroquinolones. In a recent systematic review and meta-analysis, implementation of antimicrobial stewardship interventions was associated with a 32% overall reduction in the incidence of CDI.[162] Restriction of clindamycin or fluoroquinolones has been associated not only with significant reductions in CDI, but also with elimination of clindamycin- or fluoroquinolone-resistant epidemic strains causing outbreaks, including NAP1/BI/027 epidemic strains.[163,164] Current guidelines recommend that all health care facilities encourage appropriate use of antimicrobials as a basic CDI prevention practice, and consider more intensive stewardship interventions, including restriction of high-risk agents based on local CDI epidemiology.[143]

It is anticipated that a number of experimental measures will be effective in the future in preventing CDI, including *C. difficile* vaccines and biotherapeutics (use of live organisms to restore colonization prevention) given to patients who have taken antibiotics (see Fig. 243.5).[165] Both of these approaches are undergoing human clinical trials and are expected to become available for general use.[166–169] Recently, monoclonal antibodies directed against *C. difficile* toxins have become available as a potential approach to reduce the risk for recurrence of CDI in patients with primary or recurrent CDI.[76]

Use of probiotics (available as nutritional supplements) to prevent AAD and CDI has had a mixed experience. Efficacy in reducing AAD has been demonstrated in a number of studies, particularly in children; however, the wide diversity of organism content and dosage of available products has made it difficult to recommend specifically effective agents or combinations. Randomized trials of various probiotic products that have demonstrated benefit have either been underpowered or poorly designed, with rates of CDI in control groups so high that they challenge credibility.[170,171] *Saccharomyces boulardii* was shown to be marginally effective in preventing recurrent CDI when combined with high-dose (2 g/day) vancomycin but not when combined with lower vancomycin doses or with metronidazole, and was more effective in recurrent CDI than primary CDI, where it was ineffective in preventing infection and recurrent episodes.[172,173] The subject of preventive effectiveness of probiotics has undergone multiple meta-analyses, the most recent of which suggests that there may be a benefit in prevention of primary CDI in patients taking antibiotics.[174,175] However, the field needs rigorous, well-powered studies using well-defined products to provide an adequate scientific basis for a practice that is poorly documented.

CLINICAL AND PATHOLOGIC MANIFESTATIONS

Infection with a toxin-producing strain of *C. difficile* can be asymptomatic, especially in certain populations, including infants and patients in hospitals and chronic care facilities.[102,176,177] In patients who develop symptomatic disease during a course of therapeutic antibiotics, symptoms can occur concurrently with the initiation of antibiotics or even several weeks after the discontinuation of therapy.

Diarrhea due to CDI can range in severity from minimal, self-limited disease to profuse, with 20 or more episodes a day. About 20% to 25% of patients can be expected to resolve symptoms by stopping the offending antibiotics without using specific CDI treatment. Associated signs and symptoms can include fever, abdominal pain, and tenesmus.[178] Leukocytosis is a common feature of CDI, and a significant leukemoid reaction can be seen with peripheral WBC counts >25,000/mm^3 and exceeding 100,000/mm^3 in rare cases. Significant WBC count elevation can serve as a marker of severe disease, along with hypoalbuminemia and elevation of baseline serum creatinine levels.[179] Complications of the most severe forms of CDI include hypotension or shock, the development of toxic megacolon (suggested by radiographic evidence of acute dilatation of the colon to >6 cm), intestinal perforation, and acute peritonitis. Extraintestinal manifestations of CDI are surprisingly rare, including very infrequent evidence of bacteremia or peritonitis in the absence of perforation.

By endoscopic examination, the initial lesions of PMC initially appear as 1- to 2-mm whitish-yellow plaques that appear on a background of normal-appearing mucosa (Fig. 243.6).[178] As the disease progresses, these plaques coalesce and, as they become confluent, form the typical pseudomembrane covering the entire colon wall. Generally the entire colon is involved, but 10% of patients have rectal sparing. Although endoscopy is not part of the typical diagnostic strategy for diagnosis of CDI (see "Diagnosis" next), it can be useful when there is an acute abdomen or coexisting conditions such as inflammatory bowel disease.[180]

DIAGNOSIS

Diagnosis of CDI is based on the presence of clinical symptoms (usually defined as ≥3 watery, loose, or unformed stools within a period of 24 hours or less) coupled with a diagnostic test (usually of a stool specimen) that detects the presence of either the *C. difficile* organism or its toxin genes, or detection of *C. difficile* toxin using an enzyme immunoassay or cell cytotoxin assay. Diagnosis can also be made in patients with diarrheal symptoms by the use of lower gastrointestinal endoscopy to visualize pseudomembranes in the colon, but this method is used sparingly and is far more costly and less sensitive than a variety of stool diagnostics (Table 243.3). The presence of appropriate symptoms prior to stool testing is critical because *C. difficile* can be carried asymptomatically, especially by hospitalized patients.[181] Since the pathogen and its toxins can be readily detected with most of the available tests, detection of *C. difficile* in the absence of symptoms does not meet the criteria for a CDI diagnosis.

There is considerable controversy surrounding the most appropriate stool test to use for CDI diagnosis. The first test used, and one of the gold standards, was the cell cytotoxicity assay, which was developed contemporaneously with the discovery of *C. difficile* and its toxins as the cause of CDI.[182] The test is performed by taking dilutions of supernatants of stool, placing them on cell culture lines (a variety of cell types can be used) and incubating them for 24 to 48 hours and then observing microscopically for cell rounding, an indication of the presence of toxin B (and occasionally toxin A). Confirmation that cell rounding is due to *C. difficile* toxin is done by neutralizing the assay with *C. difficile* or *Clostridium sordellii* antitoxin (the latter of which cross reacts with *C. difficile* toxins).

Soon after the cell cytotoxicity assay was developed, a selective media containing cycloserine and cefoxitin for the culture of *C. difficile* from stool was described, paving the way for the second gold standard test, stool culture for toxigenic *C. difficile*.[183] The first enzyme immunoassay (EIA) for toxin A was described in 1983, followed by development of a monoclonal antibody for toxin A that set the standard for CDI testing for decades to come. Although not nearly as sensitive as the two gold standard tests, cell cytotoxicity assay and toxigenic culture (culture of *C. difficile* from stool and confirmation of toxin production in vitro), EIA was less labor intensive and provided more rapid turnaround of test results.[184,185]

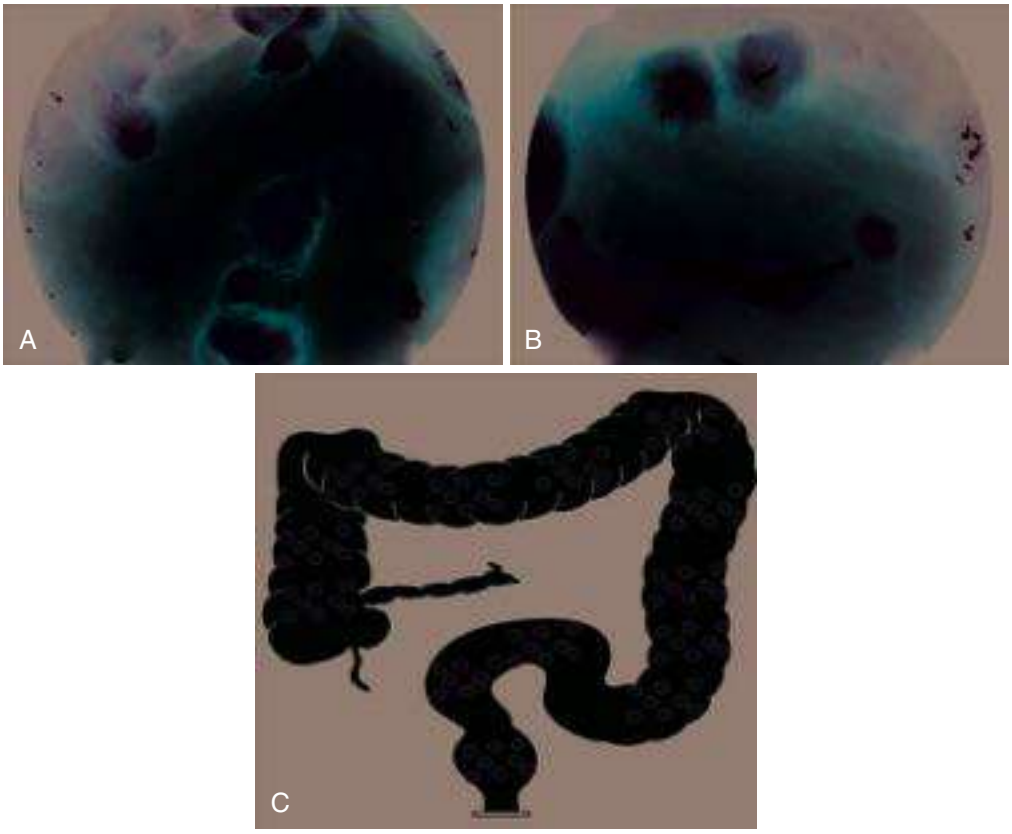

FIG. 243.6 Colonoscopic view of pseudomembranous colitis. (A and B) Discrete lesions surrounded by normal-appearing mucosa are seen early in disease. Hemorrhage is seen at the edges of some pseudomembranes (A) in this patient with underlying leukemia. (C) The anatomic location of the lesions.

TABLE 243.3 Endoscopic and Stool Diagnostic Tests for *Clostridioides difficile* and Its Toxins

TEST	SENSITIVITY (%)	SPECIFICITY (%)	COMMENT
Colon endoscopy	~50	100	Sensitivity and specificity are for detection of PMC
Cell cytotoxicity	77–86	97–99	The less sensitive of two gold standards compared with toxigenic culture
EIA for toxin A	67–92	93–99	Versus cell cytotoxicity
EIA for toxin B	60–89	93–99	Versus toxigenic culture
EIA for GDH	71–100	67–99	Compared with stool culture for *C. difficile*
Toxigenic culture for *C. difficile*	95–100	96–100	The more sensitive of two gold standards compared with cell cytotoxicity
Nucleic acid amplification test (PCR and LAMP)	88–100	88–97	Versus toxigenic culture Most sensitive rapid single test available but also expensive
Two-step GDH testing[a]	56–90	81–97	Discrepancy between GDH and toxin test is 13%–19%
Three-step GDH testing[b]	83–100	93–100	

EIA, Enzyme immunoassay; *GDH,* glutamate dehydrogenase; *LAMP,* loop-mediated isothermal amplification; *NAAT,* nucleic acid amplification test; *PCR,* polymerase chain reaction; *PMC,* pseudomembranous colitis.
[a]Two-step GDH testing: EIA for GDH and EIA for toxins A and B.
[b]Three-step GDH testing: EIA for GDH and EIA for toxins A and B, arbitrated by NAAT for discrepancies.

After decades of use, the toxin A EIA test became obsolete after it failed to detect increasingly frequent clinical outbreaks of CDI in multiple hospitals caused by *C. difficile* A−/B+ organisms that do not produce toxin A but cause CDI.[186] The toxin A EIA test was subsequently replaced with a variety of toxin A/B EIA tests utilizing a monoclonal antibody for detection of toxin A and polyclonal antibodies for detection of toxin B. These assays have been found to be relatively insensitive when compared with the cell cytotoxicity assay and toxigenic culture of *C. difficile* (see Table 243.3).[187,188] Not only are the EIA assays less sensitive than cell cytotoxicity assays, but cell cytotoxicity assays are less sensitive than toxigenic culture.[189,190]

A latex agglutination test developed in the 1980s reputedly for the detection of toxin A was subsequently found to detect another protein, glutamate dehydrogenase (GDH), which is not specific to *C. difficile* and is present in both toxigenic and nontoxigenic *C. difficile* isolates.[191] However, when GDH was converted from a latex agglutination to an EIA platform, the sensitivity was markedly improved, and the use of GDH testing (also known as common antigen) as part of a two-step or three-step test algorithm (GDH requires pairing with one or more tests for toxin to identify toxigenic *C. difficile*) has become one of the choices to replace toxin EIA alone with more sensitive tests. Utilizing the high negative predictive value of the GDH EIA test and the level of test positivity in the laboratory, 80% to 95% of specimens can be reported as *C. difficile* negative with rapid turnaround of results in the laboratory.[192] However, confirmation that a positive GDH specimen is due to a toxigenic strain requires doing a cell cytotoxin assay of the

stool to confirm presence of *C. difficile* toxin, and this adds 2 to 3 days to the test reporting. Because of the poor sensitivity of the EIA assays for toxins A and B, a number of two-step algorithms have been devised by the European Society for Clinical Microbiology and Infectious Diseases utilizing EIA testing for GDH and for toxins A and B.[193] To simplify the test process and improve the specificity of these two-step algorithms, manufacturers have designed tests that combine both EIA assays in a single test.[194] When results of GDH and toxin A/B EIA are discrepant (13%–19% of tests), the difference can be resolved by NAAT, which results in rapid turnaround of results (the so-called three-step algorithm) and is less expensive than testing all specimens with NAAT.

Improved CDI test sensitivity can also be obtained by using NAAT technologies alone, which are targeted to amplify conserved regions of either toxin A or toxin B genes using either PCR or loop-mediated isothermal amplification technology. NAAT detects the presence of a toxin gene in stool and thus is a test for detection of the presence of toxigenic *C. difficile,* similar to doing a toxigenic culture, but is not a test for toxin in the stool. GDH testing also detects the presence of *C. difficile,* but does not distinguish toxigenic from nontoxigenic strains. Use of PCR as a stand-alone test for CDI is increasing rapidly in laboratories in the United States and Europe and because of the increased sensitivity, apparent rates of CDI have increased by 50% or more in hospitals that have converted to its use. NAAT is the most sensitive rapid single test for *C. difficile* currently available and has sensitivity of 88% to 100% when compared to toxigenic culture (see Table 243.3).[190] Although NAAT is increasingly being employed by laboratories, because of high cost many laboratories are testing first with a GDH EIA or a GDH/toxinA/B EIA (two-step algorithm) and resolving discrepant GDH and toxin A/B EIA results with NAAT to reduce test costs (three-step algorithm) while retaining rapid test result reporting (see Table 243.3).

The increased rate of CDI diagnosis by NAAT has led some investigators to question whether NAAT is too sensitive as a stand-alone test.[195] The concern is that there is no test for toxin in the stool when NAAT is used alone, and that NAAT may be detecting *C. difficile*–colonized patients who have diarrhea for some other reason. Several studies have found that when NAAT is used alone, it detects more CDI cases than a three-step algorithm using GDH/toxinA/B EIA and cell culture cytotoxicity for discrepancies.[196,197] When patients who were diagnosed by NAAT alone ($N = 29$) were compared to patients diagnosed by both NAAT and the three-step algorithm ($N = 56$), it was found that patients diagnosed with NAAT alone had significantly lower rates of mortality, intensive care unit admission, readmission for CDI, and colectomy than patients diagnosed with the three-step algorithm ($P < .001$).[196] Similarly, in a recent larger study, 293 of 1416 patients were found to be NAAT positive (PCR+) but only 131 to be both PCR+ and toxin+ by EIA. The rates of complications (colectomy, megacolon, intensive care unit care) and CDI-related death at 30 days were significantly higher in the PCR+/toxin+ patients than in the PCR+/toxin− patients ($P < .001$).[197] The critical question is whether patients diagnosed by NAAT alone require treatment. They may have mild CDI that may or may not require treatment, or they may be asymptomatic carriers and require no treatment but should be isolated nonetheless because they may transmit *C. difficile,* particularly while having diarrhea. In the United Kingdom a two-step algorithm utilizing GDH EIA or NAAT for initial testing followed by a sensitive EIA for toxin A/B has been guideline recommended based on evidence of greater all-cause 30-day mortality and CDI complications when toxin is detected in stool compared to detection of only a toxigenic *C. difficile* organism.[198] The specific vulnerability of this algorithm is the reliance on an EIA test for detection of toxin in stool, a somewhat insensitive test in past experience. The 2017 Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) CDI Guidelines in the United States recommend that hospitals base the diagnostic test used on the presence of 3 or more diarrheal stools within 24 hours prior to testing, in which case either NAAT or a toxin-containing algorithm is recommended. If hospitals and laboratories do not agree to a diarrhea definition prior to testing, only an algorithm utilizing toxin testing is recommended, but not NAAT alone.[199]

The advent of more sensitive tests for CDI, such as NAAT or GDH algorithms, has made the use of repeated stool testing for diagnosis unnecessary.[200] A single test is sufficient to make or rule out the diagnosis of CDI, and tests should not be repeated for at least 7 days unless the clinical presentation changes. In addition, because these tests are so sensitive, they should not be used to evaluate response to therapy—the so-called test of cure—because the majority of successfully treated CDI patients will continue to have detectable *C. difficile* in stools posttreatment for 6 weeks or more.[152,201] Presence of continued *C. difficile* in stool is not an indication of failed therapy. Successful treatment is measured by the clinical resolution of symptoms, not microbiologic elimination of the pathogen.

TREATMENT

Before effective treatment for CDI was known, symptomatic patients were treated by stopping the offending antibiotic and giving fluid and electrolyte support if needed. About 20% to 25% of patients resolved their symptoms when this was done. When effective antimicrobial treatment was discovered, the practice of stopping the offending antibiotic and giving supportive treatment for 24 to 72 hours to see if the patient would respond before initiating treatment with vancomycin or metronidazole was continued.[202] This practice changed in the early years of the 21st century when previously unrecognized fulminant CDI with ileus, shock, and rapid progression to death occurred frequently and required rapid institution of specific treatment to save the life of the patient.[5,18,108]

Effective treatment of CDI with vancomycin was demonstrated as early as 1981; however, it was also shown that recurrences were frequent.[203] Because the cost of vancomycin was quite high at the time, alternative treatments were sought and a prospective randomized trial was conducted that showed vancomycin and metronidazole were comparable for CDI treatment and markedly reduced the cost of treatment.[202] Thus as early as 1983, two effective drugs for treatment of CDI were identified and used extensively with comparable results until the end of the 20th century, although metronidazole has never been approved for CDI treatment by the US Food and Drug Administration (FDA).[204] Vancomycin dosing for CDI was typically 500 mg 4 times a day initially, but to reduce cost a comparison study of 125 mg 4 times a day was conducted and the lower dose was found to be as effective as the higher dose.[205] There ensued considerable debate over the years as to whether vancomycin or metronidazole was the superior treatment agent for CDI despite small comparative trials that showed no difference. A randomized, blinded, stratified prospective trial published in 2007 showed metronidazole and vancomycin to be similar in treatment response for mild CDI, but that vancomycin was superior for severe CDI ($P < .02$).[206] This study confirmed the increasing clinical impression that metronidazole was not as effective in treating CDI as vancomycin. Despite numerous attempts, the reduced clinical effectiveness of metronidazole could not be attributed to *C. difficile* resistance to metronidazole. More recently, publication of two phase III randomized blinded clinical trials of the toxin-binding polymer tolevamer, which had two comparator treatment arms of vancomycin and metronidazole, also showed in a subanalysis of the largest such study to date that vancomycin was superior to metronidazole for treatment of all CDI patients using a multivariate analysis ($P = .034$). The 2017 IDSA/SHEA CDI Guidelines no longer recommend metronidazole as first-line therapy for CDI, replacing it with vancomycin or fidaxomicin.[199,207]

The importance of treating only symptomatic patients became apparent when it was discovered that asymptomatic patients colonized with *C. difficile* were commonly found in hospitals. They were thought at the time to be at risk of developing CDI, and a decolonizing treatment trial of asymptomatic patients was conducted comparing placebo versus vancomycin versus metronidazole.[208] In 9 of 10 patients treated with oral vancomycin, *C. difficile* organisms could not be detected during and immediately after treatment, compared with 3 of 10 patients treated with metronidazole ($P = 0.02$) and 2 of 10 patients given placebo ($P = 0.005$).[208] This initial effect could be explained because the mean fecal concentration of the unabsorbed vancomycin was over 1400 μg/g feces, whereas metronidazole, which is well absorbed in the absence of diarrhea, was not detectable in the feces of 9 of 10 patients. However, at the 2-month follow-up, 8 of 9 evaluable patients who received vancomycin remained colonized, 5 with new strains of *C. difficile,* compared to 2 of 10 placebo patients ($P < .005$) and 3 of 10 metronidazole patients (P

TABLE 243.4 Current Therapy for *Clostridioides difficile* Infection Based on Severity and Recurrence

CDI TYPE	ANTIBIOTIC	DOSE[a]	ALTERNATIVES
C. difficile (nonsevere)	Vancomycin, *or* Fidaxomicin	125 mg PO qid × 10 d 200 mg PO bid × 10 d	If vancomycin or fidaxomicin is not available or contraindicated, metronidazole 500 mg PO tid × 10 d
C. difficile (severe[b])	Vancomycin, *or* Fidaxomicin	125 mg PO qid × 10 d 200 mg PO bid × 10 d	None
C. difficile (severe complicated or fulminant)	Vancomycin + Metronidazole	500 mg PO qid × 10–14 d 500 mg IV q8h × 10–14 d	Tigecycline, 50 mg IV bid × 10–21 d in place of metronidazole Additional vancomycin via rectal retention enema, 500 mg in 100 mL normal saline q6h if complete ileus present Surgical colectomy or ileostomy
C. difficile (first recurrence)	Vancomycin, *or* Vancomycin taper and pulse, *or* Fidaxomicin	125 mg PO qid × 10 d if metronidazole was used for the initial episode 125 mg PO qid × 10 d, bid for 1 wk, qd for 1 wk, and then every 2 or 3 d for 2–8 wk if standard vancomycin was used for the initial episode 200 mg PO bid × 10 d if vancomycin was used for the initial episode	None
C. difficile (>one recurrence)	Vancomycin taper and pulse, *or* Vancomycin/rifaximin, *or* Fidaxomicin, *or* Fecal microbiota transplantation[c]	125 mg PO qid × 10 d, bid for 1 wk, qd for 1 wk, and then every 2 or 3 days for 2–8 wk Vancomycin 125 mg PO qid × 10 d followed by rifaximin 400 mg PO tid × 20 d 200 mg PO bid × 10 d	None

[a]All randomized trials have compared 10-day treatment courses, but some patients (particularly those treated with metronidazole) may have delayed response to treatment and clinicians should consider extending treatment duration to 14 days in those circumstances.
[b]The criteria proposed for defining severe or fulminant CDI are based on expert opinion. These may need to be reviewed in the future upon publication of prospectively validated severity scores for patients with CDI.
[c]The opinion of the panel is that appropriate antibiotic treatments for at least two recurrences (i.e., three CDI episodes) should be tried prior to offering fecal microbiota transplantation.

< .019). For asymptomatic *C. difficile* colonization, which poses little risk to the patient, vancomycin is effective as a temporary decolonizer, and metronidazole is ineffective, but colonization seems to be a transient phenomenon that resolves without any treatment and is only prolonged with vancomycin treatment, which presumably further disrupts the restoration of colonization resistance by the normal microbiota.[107] As such, routine treatment of asymptomatically colonized patients is not recommended.

Treatment recommendations for CDI have been developed for mild, moderate, severe, and severe complicated (also called fulminant) CDI, and for first and multiple recurrences of CDI. Recommendations adapted from the IDSA/SHEA Guidelines are shown in Table 243.4 together with alternative treatments that may also be effective.[140] For all listed treatments, the primary goal of successful treatment is the elimination of symptoms, and the secondary goal is prevention of recurrent CDI. Microbiologic persistence of *C. difficile* in stool occurs in the majority of patients following successful treatment, peaking at about 2 weeks posttreatment and persisting in about 5% for 6 months.[209] Repeat stool testing following treatment as a "test of cure" should *not* be done because it is not a predictor of either treatment success or failure, and will lead to unnecessary treatments, testing, and likely morbidity.

Mild-to-Moderate *Clostridioides difficile* Infection

Either vancomycin or fidaxomicin can be used to treat mild-to-moderate CDI using criteria established by the 2017 IDSA/SHEA Guidelines.[199] Metronidazole is inexpensive and may be used for first-episode mild-to-moderate CDI if vancomycin or fidaxomicin is contraindicated or not accessible, but cure rates are inferior and prolonged treatment beyond 10 to 14 days may be required. Vancomycin is also inexpensive if the intravenous form of the drug is formulated for oral administration in flavored syrups to disguise the unpleasant taste, as is done in most hospital pharmacies. Vancomycin capsules for oral administration are available from multiple generic suppliers in the United States; however, their cost remains high compared to the liquid preparations.[206,210] Other agents have been used for CDI treatment, including nitazoxanide, rifaximin, teicoplanin, and bacitracin, but none is FDA approved for CDI nor has demonstrated any advantage over vancomycin or fidaxomicin, and some are not available for use in the US market.

Severe *Clostridioides difficile* Infection

Although there is no general agreement about which patients have indications of severe disease at treatment onset, the most predictive biomarkers are WBC >15,000/μL and increase in serum creatinine >1.5 times baseline or an absolute creatinine >1.5 mg/dL if prior creatinine level is not available.[140,199] Additional biomarkers include low serum albumin (<2.5 mg/L) and elevated C-reactive protein.[99,141] Severe outcomes are also more common with infection caused by the NAP1/BI/027 and NAP7-8/BK/078 strains of *C. difficile,* but it is not recommended that treatment decisions be based on strain type, since patients exhibit a full range of severity with these strains, from asymptomatic colonization to fulminant CDI, and treatment decisions should be based on the biomarkers of the individual patient, not the strain type of the infecting organism. Vancomycin is the preferred treatment agent for patients with severe CDI. Fidaxomicin may be a suitable alternative to vancomycin because it demonstrated comparable treatment response to vancomycin and lower rates of recurrence; however, the cost of fidaxomicin is considerably higher than vancomycin, particularly if the inexpensive liquid vancomycin formulation is used.[210] Both vancomycin and fidaxomicin have lower cure rates and higher recurrence rates with patients infected by the NAP1/BI/027 strain of *C. difficile* compared to other *C. difficile* strains, so there appears to be no advantage to either drug for treating CDI caused by these strains.[142]

Severe Complicated or Fulminant *Clostridioides difficile* Infection

Severe complicated or fulminant CDI is defined as a CDI patient with hypotension or shock, ileus, or toxic megacolon. These patients are the most difficult to treat and have the highest risk of mortality or need for surgical intervention to manage CDI. Recommendations for treatment of these patients in the 2017 IDSA/SHEA Guidelines are supported by only low-to-moderate evidence (see Table 243.4). If possible, these patients should be cared for in an intensive care unit for fluid and

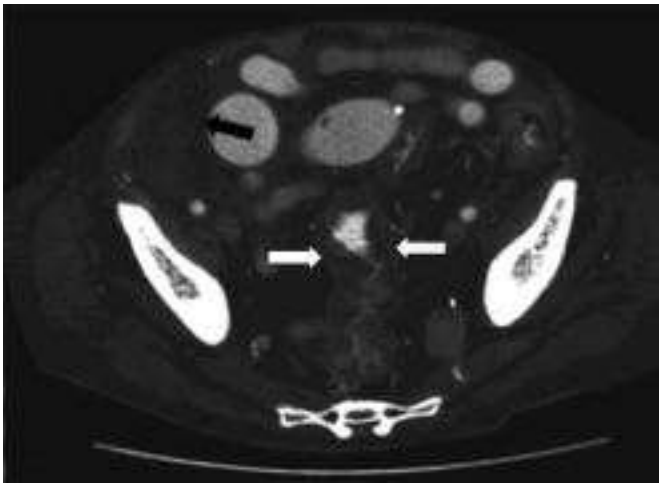

FIG. 243.7 Computed tomography scan of patient with pseudomembranous colitis demonstrating ascites *(black arrow)* and mucosal thickening of the colon *(white arrows).*

blood pressure support and have surgical consultation early in the course of illness to prepare the surgical team for the possible need for urgent colectomy or ileostomy in the event medical management is not successful. These patients often have an acute abdomen, and computed tomography scanning reveals typical findings of a diffusely thickened colonic wall and ascites (Fig. 243.7). Because of the presence of ileus in many of these patients, there is concern that vancomycin given orally or through a nasogastric tube may not reach the colon. As a result, the recommended dose of vancomycin is increased to 500 mg 4 times a day and addition of intravenous metronidazole is also recommended. Additional vancomycin can also be given via rectal retention enema (500 mg in 100 mL normal saline every 6 hours), if complete ileus is present. Anecdotal data indicate that tigecycline intravenously may be an effective alternative to intravenous metronidazole. The tigecycline minimal inhibitory concentrations range from 0.016 to 0.25 mg/L for *C. difficile,* and through primary biliary excretion of unchanged drug, up to 59% of the intravenous dose is recovered in feces. The number of successfully treated patients is very small, there has already been a failure reported, and tigecycline is not FDA approved for treatment of CDI.[211,212] Other strategies for fulminant CDI not responding to treatment include intravenous immunoglobulin, for which there has never been a randomized trial and for which evidence of benefit is poor.[213]

Usual surgical indications for CDI have included perforation, toxic megacolon, and acute abdomen, but failed medical management of patients with hypotension or shock is also an indication for colectomy. Patients should have WBC and lactate levels monitored at least daily as an indicator of possible need for surgical intervention. Surgical outcome with subtotal colectomy is more successful if patients undergo resection before the serum lactate rises to 5 mmol/L or the WBC count to 50,000/μL.[214] The mortality following subtotal colectomy for CDI often exceeds an unacceptably high 50%, especially if surgery is performed in patients with lactate levels >5 mmol/L or WBC counts >50,000/μL.[214,215] As a substitute for colectomy, a diverting ileostomy procedure followed by colonic lavage with warmed polyethylene glycol solution and daily vancomycin via the ileostomy has been evaluated in 42 patients. This operation was accomplished laparoscopically in 35 patients (83%). Ileostomy resulted in reduced mortality compared to a historical control population who had undergone colectomy (19% vs. 50%; odds ratio, 0.24; $P = .006$). Preservation of the colon was achieved in 39 of 42 patients (93%).[215] These early results are encouraging because of not only the possibility to reduce mortality, but also colon preservation.

First Recurrence of *Clostridioides difficile* Infection

First recurrences of CDI have usually been treated with the same antimicrobial regimen as used for the primary CDI episode, and responses to metronidazole and vancomycin are comparable, with 33.3% risk of a subsequent recurrence.[216] Recurrence is not thought to reflect an antimicrobial failure, but rather is a failure of the host to either reconstitute his or her microbiota to establish colonization prevention, or failure of the host to develop a protective antitoxin antibody immune response during the initial infection, or both. The 2017 IDSA/SHEA Guidelines recommend treatment of first recurrence of CDI with either vancomycin for 10 days, vancomycin for 10 days followed by taper and pulse dosing, or fidaxomicin for 10 days[199] (see Table 243.4). All are weak recommendations with low-to-moderate quality evidence, reflecting the lack of definitive data for management of recurrent CDI. Fidaxomicin has been shown to be comparable to vancomycin for treatment of first CDI recurrences, with a response rate of >90% for each following a 10-day treatment course, but fidaxomicin was superior to vancomycin with a significantly reduced rate of subsequent recurrence within 28 days (vancomycin, 35.5% recurrence; fidaxomicin, 19.7% recurrence; $P = .045$).[217] This study requires confirmation in a larger population given the marginally significant probability, but avoidance of a subsequent recurrence occurred in over 80% of fidaxomicin-treated patients, which is encouraging and could justify the higher acquisition cost of fidaxomicin. More recently, the standard dose of fidaxomicin 200 mg twice a day for 10 days has been changed to 200 mg twice a day for 5 days followed by 200 mg every other day for an additional 20 days for treatment of both first episode and first recurrence of CDI, and although overall sustained cure (treatment success without recurrence) was not improved over standard dosing, the recurrence rate was reduced to less than 5%.[218]

Multiple Recurrences of *Clostridioides difficile* Infection and Fecal Microbiota Transplantation

For patients who have had a second recurrence of CDI, there is no proven effective treatment regimen. Guidelines advise avoiding metronidazole because of the potential for development of neuropathy following prolonged use, and recommend a vancomycin-tapering regimen, one example of which is listed in Table 243.4. Although there is no standardized regimen for duration or rapidity of tapering or pulse dosing, the addition of every-third-day pulse dosing has been shown in an observational study to have a higher success rate than every-other-day pulse dosing (81% vs. 61%; $P = .03$).[219] Most strategies for prevention of recurrence in patients who have had multiple recurrences utilize a longer than usual treatment course with gradual reduction of dose, or additional treatment following 10 days of vancomycin with an antimicrobial that will have a presumably reduced impact on the normal microbiota, such as rifaximin or fidaxomicin.[220,221] Although results are encouraging with these so-called chaser regimens (vancomycin followed by rifaximin or fidaxomicin), there are no controlled, randomized, or blinded studies on which to compare efficacy.

Interest in the use of microbiota replacement therapy, typically referred to as fecal microbiota transplantation (FMT), has increased dramatically in the past few years. The initial randomized comparative study of FMT was done in comparison to treatment with vancomycin at a high dose (500 mg 4 times/day for 14 days, and the same vancomycin dose plus bowel lavage).[222] The study was discontinued early due to the superiority of the FMT regimen, which was given via nasoduodenal tube. Patients in the study had experienced a median of two to three (range, one to nine) previous recurrences of CDI. The first FMT resulted in resolution of further CDI episodes in 81% of patients. In comparison, patients treated with vancomycin had a 31% response ($P < .008$), and those treated with vancomycin with bowel lavage had a 23% response ($P < .003$).[222]

A systematic review in 2015 summarized 2 randomized controlled trials, 28 case series, and 5 case reports that described FMT for treatment of *C. difficile* infection.[223] For the 516 patients who were treated for recurrent CDI, FMT was highly effective, showing disease resolution in 85% of cases. In 2017, another group focused on the 10 randomized controlled trials that examined FMT for recurrent CDI, 5 of which compared FMT to either placebo or vancomycin treatment and the other 5 of which compared FMT delivery modalities.[224] Again, FMT was shown to be more effective than placebo or vancomycin, although

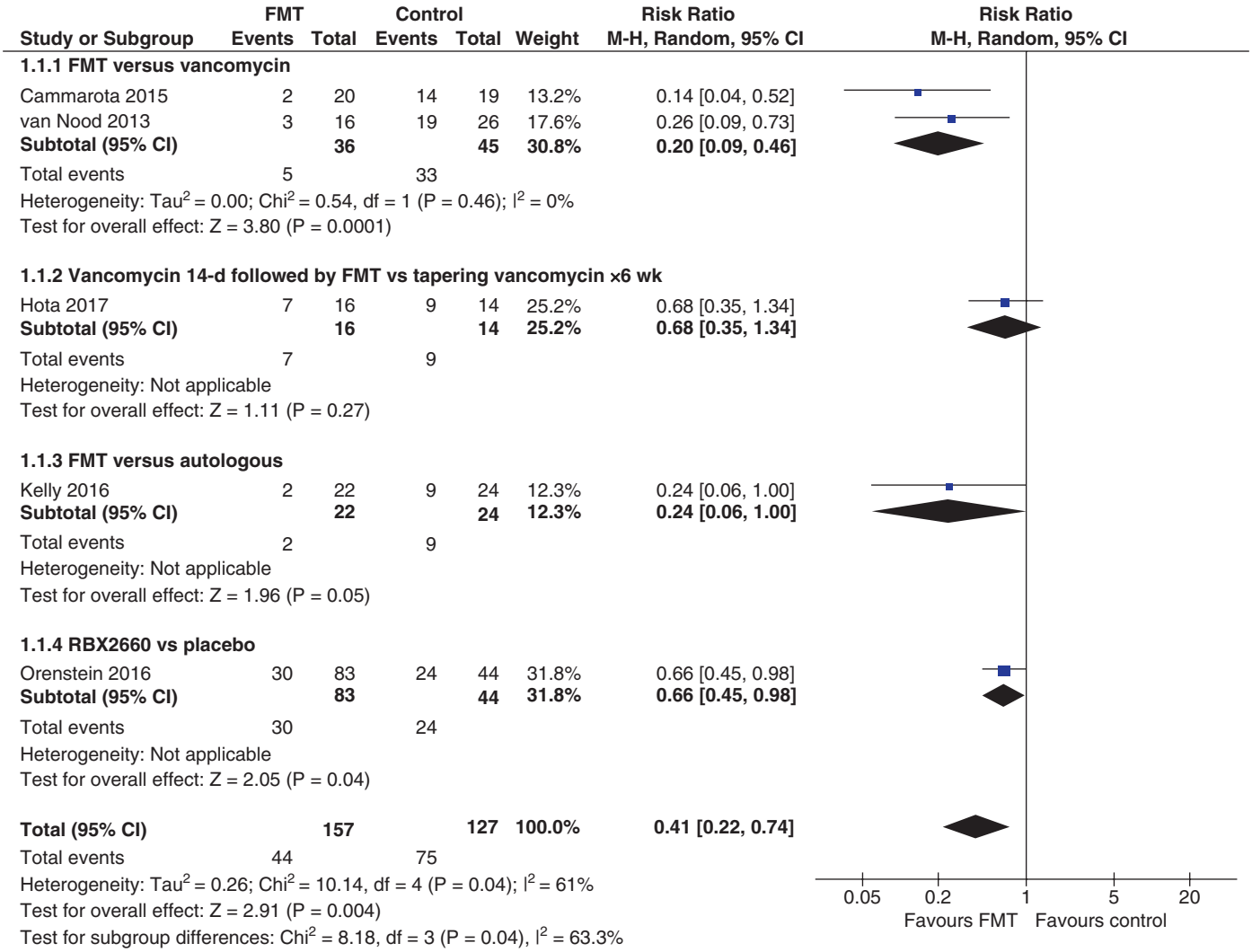

FIG. 243.8 Results of fecal microbiota transplantation *(FMT)* for recurrent *Clostridioides difficile* infection. A recent systematic review of controlled trials of FMT for recurrent *C. difficile* infection concluded that there was moderate-quality evidence supporting the use of FMT over vancomycin or placebo. *(From Moayyedi P, Yuan Y, Baharith H, Ford AC. Faecal microbiota transplantation for* Clostridium difficile*-associated diarrhoea: a systematic review of randomised controlled trials.* Med J Aust. *2017;207:166–172.)*

there was considerable variation between studies (Fig. 243.8). This study as well as another recent meta-analysis showed that both fresh and frozen fecal material had equal potency when used for FMT.[225] Lyophilized fecal preparations also appear to be another effective alternative for the preparation of material for FMT.[226] It should be noted that significant questions remain regarding methodology for FMT. In part this is due to the fact that many reports fail to describe key methodologic components in detail.[227]

The results of a European consensus conference on the clinical use of FMT was published in early 2017.[228] Although this group considered the use of FMT for other clinical indications, they concluded that they could only recommend FMT for CDI. FMT was recommended not only for multiple recurrences of CDI (defined as return of symptoms within 8 weeks after the onset of a previous episode that resolved after completion of initial treatment) but also for CDI not responding to standard therapy (vancomycin) for at least a week, and severe *C. difficile* colitis with no response to standard therapy after 48 hours.[229]

The clinical success of FMT reinforces the treatment benefit of reestablishing the indigenous microbiota in patients, thus restoring colonization resistance. Following FMT, it has been demonstrated that the gut microbiome of the recipient rapidly changes and resembles that of the donor.[230,231] Because recurrent CDI is associated with decreased overall diversity of the gut microbiome,[89] it is instructive to note that successful treatment with FMT is accompanied by an increase in microbiome diversity.[230,232]

The FDA has determined that the use of FMT requires filing an investigational new drug application, although there is a statement that there will be enforcement discretion in the setting of FMT for the clinical indication of treating recurrent CDI. So-called stool banks are providing stool preparations from screened donors for physician purchase and use for FMT CDI treatment under enforcement discretion. There have also been efforts to prepare fecal material for FMT utilizing clinical good-manufacturing processes to produce a stable, standardized, and reproducible product for clinical use. One of these formulations (RBX2660) was used in a prospective, multicenter open-label study and shown to be effective in treating recurrent CDI.[233] In an alternate approach, another commercial entity has prepared feces by treatment with ethanol to eliminate potential pathogens and to select for spore-forming organisms. This product (SER-109) was used to successfully treat patients with recurrent CDI in an open-label trial but failed to show superiority over placebo in a randomized blinded trial.[234]

As an alternative to FMT, there is also work on the development of treatment in the form of specific cultivars of the indigenous gut microbiota for the treatment of recurrent CDI. Initial work in continuous-flow cultures and germ-free mice demonstrated that cultivars from the hamster cecum could successfully compete with *C. difficile*.[235] More recent work

in murine models of CDI have shown that individual members and defined combinations of the indigenous mouse gut microbiome can restore colonization resistance against CDI.[236,237] The value of this approach was demonstrated in two patients who were given a mixture of 33 bacterial cultivars isolated from a healthy donor for the successful treatment of recurrent CDI.[238]

Monitoring Outcome of Treatment

It is important to monitor response to treatment at a minimum daily basis for mild, moderate, and severe CDI patients, and multiple times per day for fulminant CDI patients. A clinical response is usually evident within the first 3 days of treatment and includes improvement in abdominal pain, reduced frequency of stools, decreasing WBC count if elevated, and resolution of fever if present. If patient symptoms are worsening or have not improved by day 5 or 6 of treatment, a change of therapy is indicated. Usually the therapy change is from metronidazole to vancomycin, but there is no advantage to using both agents together orally. Stool frequency should decrease to ≤3 per day for at least 2 days by end of therapy. Successful treatment is measured by resolution of clinical symptoms, and not by microbiologic retesting of stools, which in the majority of successfully treated patients will still harbor *C. difficile* for 6 or more weeks following treatment. The administration of additional antibiotics for non–*C. difficile* infections should be avoided if at all possible during and after CDI treatment because they lead to a decreased response to treatment and an increased risk of recurrence.[136]

After successful treatment, patients should be informed of the possibility of recurrence of symptoms, usually within the first month following treatment, and to call their health care provider if diarrhea symptoms (>3 loose or unformed stools per day) recur, since recurrent CDI can be severe or fulminant and require rapid diagnosis and treatment. Stool testing should be repeated to document toxin in stools if possible, because retention of the organism (and NAAT and GDH test results) will commonly be positive following CDI treatment. Re-treatment should follow the recommendations in Table 243.4. Finally, irritable bowel symptoms may occur following CDI and can be difficult to distinguish from recurrent episodes.[239]

CONCLUSIONS

The epidemiology of CDI continues to evolve as it is now apparent that transmission of *C. difficile* strains occurs internationally and that strains of *C. difficile* possessing binary toxin in addition to toxin A and B are the cause of higher patient mortality. The appearance of new *C. difficile* strains is a dynamic process, with introductions to health care facilities of these new strains occurring frequently, reflecting in part the unremitting selective pressure of extensive antibiotic use. It should be anticipated that this will result in new health care–associated outbreaks caused by previously unrecognized strains and emphasizes the need to maintain a high level of vigilance through molecular typing of strains to detect these events.

Diagnosis, prevention, and treatment of CDI are presently in a state of flux, with promising new modalities on the horizon. Availability of highly sensitive NAAT and GDH algorithms should improve infection-control efforts by identifying the correct patients for isolation; however, further study to determine the best diagnostic testing algorithm is needed so that we do not unnecessarily treat patients who may be colonized with *C. difficile* but have diarrhea for another reason.

Improved treatment and prevention is being approached through biotherapeutics, immunologics, and vaccines (see Fig. 243.5). These include use of live bacterial organisms to restore colonization resistance, such as nontoxigenic *C. difficile* to prevent primary and recurrent CDI and use of FMT as an effective treatment of CDI to restore the microbiota. It is anticipated that at some point a subset of bacterial organisms in the microbiota will be identified that will provide the same benefit as FMT and will be available for oral administration. As early as 1989 a combination of bacterial organisms was identified that was effective in treating recurrent CDI.[240]

We now have available an intravenous monoclonal antibody, bezlotoxumab, directed at toxin B of *C. difficile* that in two large phase III clinical trials in 2655 adults with first-episode or recurrent CDI was shown to reduce recurrence of CDI from 27% to 17% ($P < .001$).[76] Actoxumab, a monoclonal antibody directed against toxin A, was ineffective alone and was not significantly better than bezlotoxumab alone when used in combination with bezlotoxumab. Patients with predefined high risks for recurrent CDI (age ≥65 years, severe CDI, immunosuppression, history of CDI) were most likely to benefit from bezlotoxumab, but those lacking any risk factors did not benefit.[241] Subjects with multiple risk factors benefitted most. Finally, multiple vaccines are being developed for prevention of CDI utilizing antigens derived from *C. difficile* toxins. These vaccines appear to be safe and are highly effective in animal models, but a phase III CDI vaccine clinical trial has recently been discontinued for futility following an interim analysis, suggesting that a successful vaccine for CDI may be more difficult to achieve than anticipated.

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244 Tetanus (*Clostridium tetani*)

Torrey Boland Birch and Thomas P. Bleck

SHORT VIEW SUMMARY

History
- Tetanus is a nervous system disease caused by the toxin tetanospasmin produced by *Clostridium tetani*.
- Vaccination for tetanus has been widely available in developed countries since the 1940s.

Epidemiology
- Acute injury and injection drug use are risk factors for tetanus.
- Tetanus is rare in developed countries due to the availability of effective vaccines.
- In developing countries neonatal tetanus can occur when there is failure of aseptic technique and mothers are inadequately immunized.
- In the United States tetanus is more common in people older than 65 years, likely due to waning immunity.

Pathogenesis
- *C. tetani* is an obligately anaerobic bacillus that produces two toxins: tetanospasmin and tetanolysin.
- It develops a terminal spore that is extremely stable in the environment, retaining the ability to germinate and cause disease indefinitely.
- Tetanospasmin enters the nervous system through the lower motor neurons and is carried to the brain and spinal cord via retrograde transport.
- Tetanospasmin prevents release of neurotransmitters from inhibitory cells leading to increased muscle tone and a hypersympathetic state.

Clinical Manifestations
- Tetanus is divided into four clinical types: *generalized, localized, cephalic,* and *neonatal*.
- Hallmarks of generalized tetanus include trismus (lockjaw, or masseter rigidity) and risus sardonicus (increased tone in the orbicularis oris)

Diagnosis
- Diagnosis of tetanus relies on history and examination findings.
- Culturing *C. tetani* is difficult and not helpful.

Treatment
- The airway must be secured at the time of presentation.
- Benzodiazepines provide the mainstay of symptomatic therapy.
- Passive immunization with human tetanus immune globulin (HTIG) shortens the disease course and may lessen disease severity.
- Antibiotic therapy with metronidazole may improve outcomes.

Prevention
- All children should be vaccinated against tetanus through the diphtheria-tetanus-acellular pertussis (DTaP) vaccine series.
- All pregnant women should receive a dose of the tetanus-reduced diphtheria toxoid-acellular pertussis (Tdap) vaccine with each pregnancy
- Adults should receive a tetanus booster (Td) every 10 years. One of the Td doses should be replaced with Tdap.
- Wound management of minor, clean wounds should include completion of tetanus immunization if incomplete or a booster dose of vaccine (Td) if the last dose was given more than 10 years before. Patients with serious contaminated wounds should also receive HTIG.

HISTORY

Tetanus was well known to the ancients; descriptions by Egyptian and Greek physicians survive to the present. They recognized the frequent relationship between injuries and the subsequent development of fatal spasms. Gowers[1] provided the quintessential description of tetanus in 1888.

Tetanus is a disease of the nervous system characterized by persistent tonic spasm, with violent brief exacerbations. The spasm almost always commences in the muscles of the neck and jaw, causing closure of the jaws (trismus, lockjaw), and involves the muscles of the trunk more than those of the limbs. It is always acute in onset, and a very large proportion of those affected die.[1]

Nicolaier[2] isolated a strychnine-like toxin from anaerobic soil bacteria in 1884. Six years later, Behring and Kitasato[3] described active immunization with tetanus toxoid. Inactivation of tetanus toxin was developed in the early 20th century and was widely used for treatment and prevention by the 1940s. This latter discovery should have reduced tetanus to a historical curiosity, but we still fail to fulfill this promise.

EPIDEMIOLOGY

Between 2001 and 2008 the Centers for Disease Control and Prevention (CDC) received reports of 233 tetanus cases, with an overall annual incidence of 0.10 cases per 1 million persons and an incidence of 0.23 cases per 1 million persons 65 years or older.[4] Data through 2008 are summarized in Fig. 244.1. Globally, 13,505 cases of tetanus were reported in 2016.[5] United States and global statistics likely represent underreporting. Most reported cases are in patients older than 60 years,[6] indicating that waning immunity is an important risk factor.[7] This may be a particularly serious problem in older women.[8,9] Changes in patterns of immigration may increase the number of unimmunized or inadequately immunized patients presenting for care in developed countries.[10] Injection drug abuse places patients at risk for tetanus,[11] as do other potentially unsterile practices that allow inoculation of spores.[12]

Causes of Tetanus

C. tetani germinates in low oxygen conditions, particularly in wounds. Acute injuries account for about 70% of US cases, evenly divided between punctures and lacerations.[13] Other identifiable conditions are noted in 23%, leaving about 7% of cases without an apparent source. Other studies cite rates of cryptogenic tetanus as high as 23%. Outbreaks of tetanus are common after natural disasters in developing countries, when people are likely to suffer from open wounds, fractures, and crush injuries.[14] Neonatal tetanus is rare in the United States.

Mortality

In developing countries, mortality rates due to tetanus are as high as 28 per 100,000. Until recently, primary tetanus immunization programs in these countries were ineffective. As a result, 800,000 to 1 million annual deaths were attributed to tetanus during the 1980s.[15] Two-thirds of cases worldwide occurred in sub-Saharan Africa, where more than

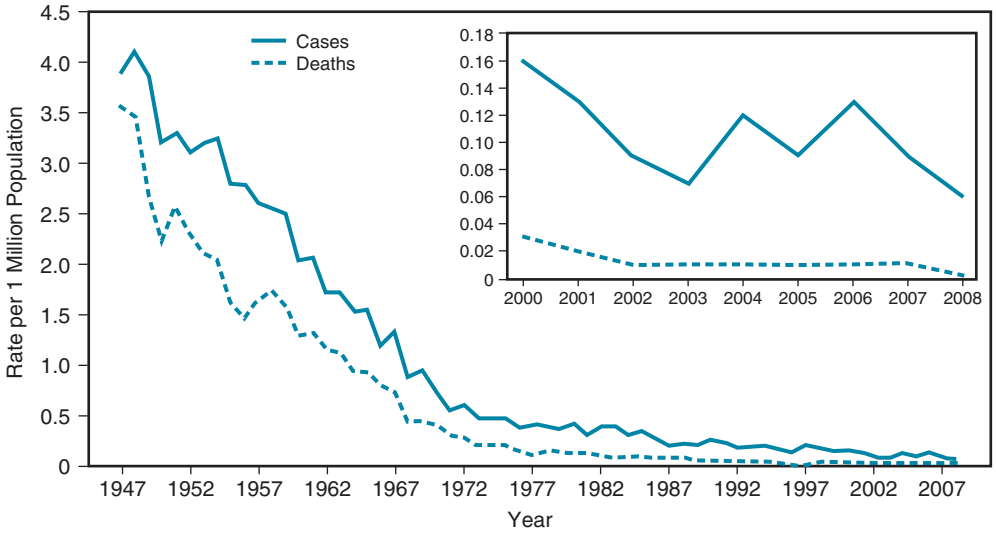

FIG. 244.1 Reported cases and deaths from tetanus in the United States, 1947–2008. *(Data from Centers for Disease Control and Prevention. Tetanus surveillance—United States, 2001–2008.* MMWR Morb Mortal Wkly Rep. *2011;60:365–369.)*

40% of tetanus is a result of neonatal infection[15,16]; nearly one-third of these infants were born to mothers of a previously afflicted child, highlighting a failure to immunize.[17]

In 1989 a worldwide commitment to the elimination of neonatal tetanus by the World Health Assembly[18,19] resulted in a decline of more than 50%, and a resurgent effort in 1999,[20] the Maternal and Neonatal Tetanus Elimination Program,[21] met with additional success. In 2015 the World Health Organization (WHO) estimated that there were 34,019 neonatal deaths due to tetanus, representing a 96% reduction in neonatal tetanus deaths compared with the 1980s.[22] The Global Immunization Vision and Strategy, launched by WHO and United Nations Children's Fund in 2005, continues to target tetanus as a preventable cause of neonatal death by promoting routine tetanus toxoid administration in hard-to-reach, previously underserved areas.[23] As of 2014, it was estimated that globally, 86% of infants had received at least three doses of the diphtheria-tetanus-pertussis vaccine.[24]

MICROBIOLOGY OF *CLOSTRIDIUM TETANI*

Clostridium tetani is an obligately anaerobic bacillus that is gram positive in fresh cultures but may have variable staining in older cultures or tissue samples.[25] It is present in soil, especially heavily manured soil, and in the intestinal tract and feces of various animals. It is believed to be transiently present in the human gastrointestinal (GI) tract, perhaps dependent on recent ingestion.[26] The complete genome of the organism has been sequenced, and its products were recently compared with other clostridia.[27] During growth the bacilli possess abundant flagella and are sluggishly motile. Two toxins, tetanospasmin (commonly called *tetanus toxin*) and tetanolysin, are produced during this phase. Tetanospasmin is encoded on a plasmid that is present in all toxigenic strains.[28] Tetanolysin is of uncertain importance in the pathogenesis of tetanus. Mature organisms lose their flagella, develop a terminal spore, and begin to resemble a squash racquet (Fig. 244.2).[29] The spores are extremely stable in the environment, retaining the ability to germinate and cause disease indefinitely. They withstand exposure to ethanol, phenol, or formalin but can be rendered noninfectious by iodine, glutaraldehyde, hydrogen peroxide, or autoclaving at 121°C and 103 kilopascals (15 psi) for 15 minutes. Growth in culture is optimal at 37°C under strictly anaerobic conditions, but culture results are of no diagnostic value. Antibiotic sensitivity is discussed later.

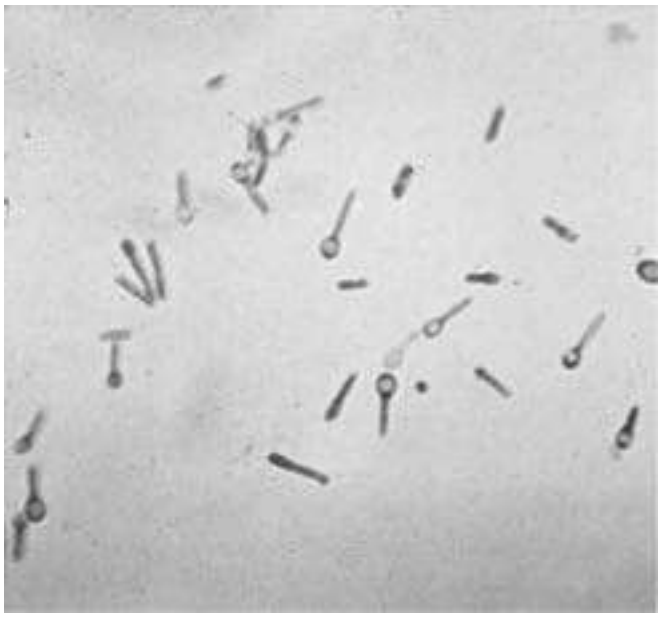

FIG. 244.2 Gram stain of a culture of *Clostridium tetani*. *(From Bleck TP, Brauner JS. Tetanus. In: Scheld WM, Whitley RJ, Marra CM, eds.* Infections of the Central Nervous System. *3rd ed. New York: Lippincott Williams & Wilkins; 2004:625–648. Courtesy Paul C. Schrechenberger, PhD, and Alex Kuritza, PhD.)*

PATHOGENESIS

The clostridial toxins that produce both tetanus and botulism are similar in structure and function despite the almost diametrically opposed clinical manifestations of the diseases. These toxins are zinc-dependent matrix metalloproteinases, a category encompassing a diverse group of enzymes ranging from normal human cellular constituents necessary for cellular remodeling,[30] through determinants of neoplastic cell function,[31] to exotoxins of other microorganisms such as *Bacteroides fragilis*.[32] Tetanospasmin is synthesized as a single 151-kilodaltons (kDa) chain that is cleaved extracellularly by a bacterial protease into a 100-kDa heavy chain and a 50-kDa light chain (fragment A), which remain connected by a disulfide bridge.[33] The heavy chain can be further divided into fragments B and C by pepsin. The heavy chain appears to mediate binding to cell surface receptors and transport proteins, whereas the light chain produces the presynaptic inhibition of transmitter release that produces clinical tetanus. The nature of the receptor to which tetanospasmin binds, previously thought to be a ganglioside, has been debated.[34] Recent research suggests that the extracellular matrix proteins nidogen-1 and nidogen-2 serve as the tetanospasmin receptor.[35] The

toxin enters the nervous system primarily through the presynaptic terminals of lower motor neurons, where it can produce local failure of neuromuscular transmission. It then exploits the retrograde axonal transport system and is carried to the cell bodies of these neurons in the brainstem and spinal cord, where it expresses its major pathogenic action.[36]

Once the toxin enters the central nervous system (CNS), it diffuses to the terminals of inhibitory cells, including both local glycinergic interneurons and descending γ-aminobutyric acid–ergic (GABAergic) neurons from the brainstem. The toxin degrades synaptobrevin, a protein required for docking of neurotransmitter vesicles, with their release site on the presynaptic membrane.[37] By preventing transmitter release from these cells, tetanospasmin leaves the motor neurons without inhibition. This produces muscular rigidity by raising the resting firing rate of motor neurons and also generates spasms by failing to limit reflex responses to afferent stimuli. Excitatory transmitter release in the spinal cord can also be impaired, but the toxin appears to have greater affinity for the inhibitory systems. The autonomic nervous system is affected as well; this is predominantly manifested as a hypersympathetic state induced by failure to inhibit adrenal release of catecholamines.

Toxin binding appears to be an irreversible event. At the neuromuscular junction, initial recovery from botulism depends on sprouting a new axon terminal; this is probably the case at other affected synapses as well. Later, the new synapses are removed when the original ones reestablish their connections.[38]

CLINICAL MANIFESTATIONS

Tetanus is classically divided into four clinical types: *generalized, localized, cephalic,* and *neonatal.* These are valuable diagnostic and prognostic distinctions but reflect host factors and the site of inoculation rather than differences in toxin action. Terms describing the initial stages of tetanus include the *incubation period* (time from inoculation to the first symptom) and the *period of onset* (time from the first symptom to the first generalized spasm). The shorter these periods, the worse the prognosis.[39] The incubation period ranges from 3 to 21 days.[40] Various rating scales are available.[41] Certain portals of entry (e.g., compound fractures) are associated with poorer prognoses. Tetanus may be particularly severe in narcotics addicts, for unknown reasons.[42] *Generalized tetanus* is the most commonly recognized form and often begins with *risus sardonicus* (increased tone in the orbicularis oris) and *trismus* (lockjaw, or masseter rigidity) (Fig. 244.3). Abdominal rigidity may also be present. Progression of the disease typically occurs in a descending pattern. The *generalized spasm* resembles decorticate posturing and consists of opisthotonic posturing with flexion of the arms and extension of the legs (Fig. 244.4). The patient does not lose consciousness and experiences severe pain during each spasm. The spasms are often triggered by sensory stimuli. During the spasm the upper airway can be obstructed, or the diaphragm may participate in the general muscular contraction. Either of these compromises respiration, and even the first such spasm may be fatal. In the modern era of intensive care, however, the respiratory problems are easily managed, and autonomic dysfunction, usually occurring after several days of symptoms, has emerged as the leading cause of death.[43] Symptoms of autonomic hyperactivity, such as hypertension, tachycardia, and hyperthermia, may also be present.

The illness can progress for about 2 weeks, reflecting the time required to complete the transport of toxin, which is already intraaxonal when antitoxin treatment is given. The severity of illness may be decreased by partial immunity.[44] Recovery takes an additional month and is complete unless complications supervene. Rare cases have been described lasting several months.[45] Lower motor neuron dysfunction may not be apparent until spasms remit, and recovery from this deficit in neuromuscular transmission may take additional weeks.[46] Recurrent tetanus may occur if the patient does not receive active immunization because the amount of toxin produced is inadequate to induce immunity.[47] *Localized tetanus* involves rigidity of the muscles associated with the site of spore inoculation. This may be mild and persistent and often resolves spontaneously. Lower motor neuron dysfunction (weakness and diminished muscle tone) is often present in the most involved muscle. This chronic form of the disease probably reflects partial immunity to tetanospasmin.[48] Localized tetanus is more commonly a



FIG. 244.3 Risus sardonicus and trismus. (A) Risus sardonicus. Note the straightened upper lip at rest. (B) Trismus. The patient is opening his mouth as fully as possible. *(From Bleck TP. Tetanus. In: Scheld WM, Whitley RJ, Durack DT, eds.* Infections of the Central Nervous System. *New York: Raven Press; 1991:603–624.)*



FIG. 244.4 Opisthotonus. *(From Bell C.* Essays on the Anatomy and Physiology of Expression. *2nd ed. London: J. Murray; 1824.)*

prodrome of generalized tetanus, however, which occurs when enough toxin gains access to the CNS. *Cephalic tetanus* is a special form of localized disease affecting the cranial nerve musculature, almost always after an apparent head wound (Fig. 244.5). Although earlier reports linked cephalic tetanus to a poor prognosis, more recent studies have revealed many milder cases. A lower motor neuron lesion, frequently producing facial nerve weakness, is often apparent.[49] Extraocular muscle involvement is occasionally noted. *Neonatal tetanus* (Fig. 244.6) follows infection of the umbilical stump, most commonly caused by a failure of aseptic technique if mothers are inadequately immunized.[50] Cultural practices may contribute.[51] The condition usually presents with generalized weakness and failure to nurse; rigidity and spasms occur later. The



FIG. 244.5 Cephalic tetanus. Right facial paresis is present in addition to the grimace. *(From Veronesi R, Focaccia R. The clinical picture. In: Veronesi R, ed.* Tetanus: Important New Concepts. *Amsterdam: Excerpta Medica; 1981:183–206.)*



FIG. 244.6 Neonatal tetanus. *(From Veronesi R, Focaccia R. The clinical picture. In: Veronesi R, ed.* Tetanus: Important New Concepts. *Amsterdam: Excerpta Medica; 1981:183–206.)*

mortality rate exceeds 90%, and developmental delays are common among survivors.[52] Poor prognostic factors include age younger than 10 days, symptoms for fewer than 5 days before presentation to hospital, and presence of risus sardonicus or fever.[53] Apnea is the leading cause of death among neonatal tetanus patients in the first week of life, and sepsis in the second week.[54] Bacterial infection of the umbilical stump leads to sepsis in almost half of infants with neonatal tetanus, which contributes to the substantial mortality despite treatment.[55]

DIAGNOSIS

Tetanus is diagnosed by clinical observation and has a limited differential diagnosis. Laboratory testing cannot confirm or exclude the condition and is primarily useful for excluding intoxications that may mimic tetanus. Electromyographic studies are occasionally useful in questionable cases. Such testing becomes more important when no portal of entry is apparent. Antitetanus antibodies are undetectable in most tetanus patients, but many reports document the disease in patients with antibody levels above the commonly cited "protective" concentration of 0.01 IU/L.[56] Rarely, patients apparently develop antibodies that are not protective.[57]

Attempts to culture *C. tetani* from wounds are not useful in diagnosis because (1) even carefully performed anaerobic cultures are frequently negative; (2) a positive culture does not indicate whether the organism contains the toxin-producing plasmid; and (3) a positive culture may be present without disease in patients with adequate immunity.[58]

Strychnine poisoning, in which glycine is antagonized, is the only condition that truly mimics tetanus; toxicologic studies of serum and urine should be performed when tetanus is suspected, and tetanus should be considered even if strychnine poisoning appears likely. Because the initial treatments of tetanus and strychnine intoxication are similar, therapy is instituted before the assay results are available. Dystonic reactions to neuroleptic drugs or other central dopamine antagonists may be confused with the neck stiffness of tetanus, but the posture of patients with dystonic reactions almost always involves lateral head turning, which is rare in tetanus. Treatment with anticholinergic agents (benztropine or diphenhydramine) is rapidly effective against dystonic reactions. Dental infections may produce trismus and should be sought, but they do not cause the other manifestations of tetanus.

TREATMENT

The patient with tetanus requires simultaneous attention to several concerns. Attention to the airway and to ventilation is paramount at the time of presentation, but the other aspects of care, especially passive immunization, must be pursued as soon as the respiratory system is secure. Table 244.1 presents a suggested management protocol.

Stabilization

Tetanic spasms sometimes demand that the airway be secured before other lines of therapy are possible. An orotracheal tube can be passed under sedation and neuromuscular junction blockade; a feeding tube should be placed at the same time. Because the endotracheal tube may stimulate spasms, an early tracheostomy is usually beneficial.[59]

Management of Muscle Spasms

Benzodiazepines have emerged as the mainstay of symptomatic therapy for tetanus.[60] These drugs are $GABA_A$ agonists and thereby indirectly antagonize the effect of the toxin. They do not restore glycinergic inhibition. The patient should be kept free of spasms and may benefit from the amnestic effects of the drugs as well. Diazepam has been studied most intensively, but lorazepam and midazolam appear equally effective. Tetanus patients have unusually high tolerance for the sedating effect of these agents and commonly remain alert at doses normally expected to produce anesthesia.[61]

The intravenous (IV) formulations of both diazepam and lorazepam contain propylene glycol. At the doses required to control generalized tetanus, this vehicle may produce lactic acidosis.[62] Nasogastric delivery of these agents is often possible, but some tetanus patients develop GI motility disorders and do not absorb drugs well. IV midazolam (5–15 mg/h or more) is effective and does not contain propylene glycol, but it must be given as a continuous infusion because of its short half-life.[63] Propofol infusion is also effective,[64] but the amount necessary to control symptoms may produce the propofol infusion syndrome, characterized by metabolic acidosis, rhabdomyolysis, and cardiac arrhythmias, or exceed the patient's tolerance of the lipid vehicle. When the symptoms of tetanus subside, these agents must be tapered over at least 2 weeks to prevent withdrawal symptoms. Intrathecal baclofen, a $GABA_B$ agonist, is also effective in controlling tetanus but has no clear advantage over benzodiazepines. Neuroleptic agents and barbiturates, previously used for tetanus, are inferior for this indication and should not be used except in settings where benzodiazepines are unavailable. Magnesium sulfate infusion may reduce the need for additional medications to control muscle spasms and cardiovascular instability but does not appear to reduce the need for mechanical ventilation.[65]

Rarely, patients cannot be adequately controlled with benzodiazepines alone. Neuromuscular junction blockade is indicated in such patients,

TABLE 244.1 Suggested Management Protocol for Generalized Tetanus

I. Diagnosis and Stabilization: First Hour After Presentation

A. Assess airway and ventilation. If necessary, perform endotracheal intubation using benzodiazepine sedation and neuromuscular blockade (e.g., vecuronium, 0.1 mg/kg).
B. Obtain samples for antitoxin level, strychnine and dopamine antagonist assays, electrolytes, blood urea nitrogen, creatinine, creatine kinase, and urinary myoglobin determination.
C. Determine the portal of entry, incubation period, period of onset, and immunization history.
D. Administer benztropine (1–2 mg, IV) or diphenhydramine (50 mg, IV) to rule out a dystonic reaction to a dopamine-blocking agent.
E. Administer a benzodiazepine IV (diazepam in 5-mg increments, or lorazepam in 2-mg increments) to control spasm and decrease rigidity. Initially, use a dose that is adequate to produce sedation and minimize reflex spasms. If this dose compromises the airway or ventilation, intubate using a short-acting neuromuscular-blocking agent. Transfer the patient to a quiet, darkened area of the intensive care unit.

II. Early Management Phase: First 24 Hours

A. Administer human tetanus immunoglobulin, 500 U, IM; as an alternative, consider IV pooled immune globulin.
B. At a different site, administer IM adsorbed tetanus toxoid such as tetanus-diphtheria vaccine (0.5 mL) or diphtheria-pertussis-tetanus vaccine (0.5 mL), as appropriate for age. Adsorbed tetanus toxoid without diphtheria toxoid is available for patients with a history of reaction to diphtheria toxoid; otherwise, the correct combination for the patient's age should be used.
C. Begin metronidazole, 500 mg, IV, q6h, for 7–10 days.
D. Perform a tracheostomy after placement of an endotracheal tube and under neuromuscular blockade if spasms produce any degree of airway compromise.
E. Débride any wounds as indicated for their management.
F. Place a soft, small-bore nasal feeding tube or a central venous hyperalimentation catheter, and begin feeding. Patients receiving total parenteral nutrition should also be given parenteral histamine-2 blockade or other gastric protection.
G. Administer benzodiazepines as required to control spasms and produce sedation. Consider magnesium sulfate infusion. If adequate control is not achieved, institute long-term neuromuscular blockade (e.g., vecuronium, 0.8–1.7 μg/kg/min); continue benzodiazepines for sedation with intermittent electroencephalographic monitoring to ensure somnolence. Neuromuscular junction blockade should be discontinued daily to assess the patient's physical examination and to decrease the possibility of excessive accumulation of the blocking agent.

III. Intermediate Management Phase: Next 2–3 Weeks

A. Treat sympathetic hyperactivity with labetalol (0.5–2 mg/min) as needed for blood pressure control) or morphine (0.5–1 mg/kg/h by continuous infusion). Magnesium sulfate infusion may be considered, as well as epidural blockade with a local anesthetic. Avoid diuretics for blood pressure control because volume depletion will worsen autonomic instability.
B. If hypotension is present, initiate saline resuscitation. Place a pulmonary artery catheter and an arterial line, and administer fluids, dopamine, or norepinephrine as indicated.
C. Sustained bradycardia usually requires a pacemaker. Atropine or isoproterenol may be useful during pacemaker placement.
D. Begin prophylactic heparin.
E. Use a flotation bed, if possible, to prevent skin breakdown and peroneal nerve palsies. Otherwise, ensure frequent turning and use antirotation boots.
F. Maintain benzodiazepines until neuromuscular blockade, if used, has been terminated, and the severity of spasms has diminished substantially. Then taper the benzodiazepine dose over 14–21 days.
G. Begin rehabilitation planning.

IV. Convalescent Stage: 2–6 Weeks

A. When spasms are no longer present, begin physical therapy. Many patients require supportive psychotherapy.
B. Before discharge, administer another dose of tetanus-diphtheria vaccine or diphtheria-pertussis-tetanus vaccine.
C. Schedule a third dose of toxoid to be given 4 wk after the second.

IM, Intramuscularly; *IV,* intravenously.
Modified from Bleck TP, Brauner JS. Tetanus. In: Scheld WM, Whitley RJ, Marra CM, eds. Infections of the Central Nervous System. *3rd ed. New York: Lippincott Williams & Wilkins; 2004:625–648.*

with the caveat that sedation is still required for psychological reasons. All the available drugs have side effects, including the potential for prolonged effect after the drug is discontinued. Vecuronium or cisatracurium (by continuous infusion) and pancuronium (by intermittent injection) are adequate choices. These agents should be stopped at least once daily to assess the patient's progress and to observe for possible complications. Electroencephalographic monitoring is a useful adjunct for this purpose.[66]

Wound Management

Most tetanus patients still have the portal of entry apparent when they present. If the wound requires surgical attention, this may be performed after spasms are controlled. However, the course of tetanus is not affected by wound débridement.

Passive Immunization

Passive immunization with human tetanus immune globulin (HTIG) shortens the course of tetanus and may lessen its severity. A dose of 500 units appears as effective as larger doses.[67] A meta-analysis of the benefits of intrathecal HTIG therapy was inconclusive.[68] However, in a randomized trial, the administration of intrathecal HTIG with intramuscular HTIG resulted in shorter duration of spasms, shorter hospital stay, and decreased respiratory assistance demands compared with intramuscular HTIG alone.[69] Pooled IV immune globulin has been proposed as an alternative to HTIG,[70] although this should be approached with caution.[71] Active immunization must also be initiated.

Antimicrobial Therapy

The role of antimicrobial therapy in tetanus remains debated. The in vitro susceptibilities of *C. tetani* include metronidazole, penicillins, cephalosporins, imipenem, macrolides, and tetracycline. A study comparing oral metronidazole to intramuscular penicillin showed better survival, shorter hospitalization, and less progression of disease in the metronidazole group.[72] This may reflect a true advantage of metronidazole over penicillin, but it more likely corresponds to a negative effect of penicillin, a potent $GABA_A$ antagonist. Topical antibiotic application to the umbilical stump appears to reduce the risk for neonatal tetanus.[7]

Management of Autonomic Dysfunction

Autonomic dysfunction generally reflects excessive catecholamine release and may respond to combined α-adrenergic and β-adrenergic blockade with IV labetalol.[73] β-Blockade alone is rarely used because the resulting unopposed α effect may produce severe hypertension. If β-blockade is chosen, the short-acting agent esmolol should be used.[74] Other approaches to hypertension include morphine infusion,[75] magnesium sulfate infusion,[76] and epidural blockade of the renal nerves.[77] Hypotension is less common but, if present, may require norepinephrine infusion. Myocardial dysfunction is also common[78] and may represent a further reflection of catecholamine excess.[79]

Nutritional Support

Nutritional support should be started as soon as the patient is stable. The volume of enteral feeding needed to meet the exceptionally high caloric and protein requirements of these patients may exceed the capacity of the GI system.

The mortality rate in mild and moderate tetanus at present is about 6%; for severe tetanus, it may reach as high as 60%, even in expert centers.[80] A well-designed protocol for the critical care of tetanus patients can substantially reduce morbidity and mortality.[81] Among adults, age has little effect on mortality, with octogenarians and nonagenarians faring as well as middle-aged patients.[82] Tetanus survivors often have serious psychological problems related to the disease and its treatment that persist after recovery and may require psychotherapy.[83]

Prevention

Tetanus is preventable in almost all patients, leading to its description as the "inexcusable disease."[84] Tetanus toxoid (TT), a heat-inactivated toxin, was developed in 1924.[85] The vaccine was initially used among military personnel in World War II. As a result, tetanus accounted for

only 12 of nearly 3 million hospitalizations during the war; five cases were fatal.[86,87]

The Advisory Committee on Immunization Practices (ACIP) recommends a primary tetanus vaccination in combination with diphtheria and pertussis (DTaP) at 2, 4, 6, and 12 to 18 months, and at 4 to 6 years.[88] In 2005 the US Food and Drug Administration approved the use of a new vaccine formulation: tetanus, reduced diphtheria toxoid, and acellular pertussis (Tdap).[89] Tdap should be administered once to all adolescents at age 11 to 18 years (also see Chapter 316).[88]

Serologic analysis of the US population suggests that tetanus immunity wanes with age.[90,91] Although 80% of patients aged 6 to 39 years were noted to have protective antibodies to tetanus, only 28% of patients older than 70 years were seropositive.[90] Therefore it is recommended that all adults receive a tetanus diphtheria toxoid (Td) booster every 10 years. Between the ages of 19 and 64 years, one of these Td boosters should be replaced with a single dose of Tdap. Tdap was previously recommended only for adults older than 65 years who had not previously received Tdap and who anticipated close contact with infants aged younger than 1 year; however, in 2012 ACIP recommended Tdap for all adults aged 65 years and older.[92] That same year, ACIP also made a recommendation that all pregnant women receive Tdap during each pregnancy, regardless of prior vaccination status.[93] All patients who seek medical attention for a wound should have their tetanus immunization history reviewed. Patients with minor, clean wounds who have not received at least three doses of a tetanus toxoid–containing vaccine or whose last dose was 10 years or more prior should receive a tetanus toxoid–containing vaccine; which vaccine is administered depends on patient's age and vaccine history. Patients with more serious or contaminated wounds who have an incomplete or unknown tetanus vaccine history should receive a tetanus toxoid–containing vaccine and HTIG. HTIG binds directly to toxin, providing temporary immunity. HTIG should be given at a different site and with a different syringe than vaccine. Patients with serious wounds who have previously received at least three doses of a tetanus toxoid–containing vaccine but whose last dose was 5 or more years ago should receive a tetanus booster without HTIG.[94] Tetanus-prone wounds are characterized by devitalized tissue, such as a crush injury, or by a wound with potential contamination with dirt or rust.

Some patients with humoral immune deficiencies may not respond adequately to toxoid injection[95]; such patients should receive passive immunization for tetanus-prone injuries regardless of the period since the last booster. About half of patients lose tetanus immunity after chemotherapy for leukemia or lymphoma.[96] Patients who have undergone bone marrow or stem cell transplantation require revaccination after the procedure.[97] It is recommended that three Td boosters be given, the first of which should be administered 6 to 12 months after transplantation. One of the three Td doses should be replaced with Tdap.[98] Antibody production by the transplanted immune cells may play a minor role in subsequent host immunity.[99] Most young patients with human immunodeficiency virus (HIV) infection appear to retain antitetanus antibody production if their primary immunization series was completed before they acquired HIV[100]; however, only a minority respond adequately to booster immunization.[101] Vitamin A deficiency interferes with the response to tetanus toxoid.[102] There have been rare reports of patients with protective antitoxin antibodies in their serum from immunization developing tetanus as well.

Neonatal tetanus may occur because of inadequate immunization of the mother. Although a full series of maternal immunizations is ideal, even one or two doses of tetanus toxoid confer substantial protection against neonatal tetanus.[103] Application of topical antimicrobial agents to the umbilical cord stump markedly decreases the incidence of neonatal tetanus when maternal immunization is insufficient.[104] Mild reactions to tetanus toxoid (e.g., local tenderness, edema, low-grade fever) are common. More severe reactions are rare and likely are due to a hypersensitivity response to the preservative thimerosal.[105] Although there have been reports suggesting a connection between tetanus immunization and Guillain-Barré syndrome, a careful epidemiologic analysis did not confirm an association.[106]

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245 Botulism (*Clostridium botulinum*)

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SHORT VIEW SUMMARY

Definition
- Botulism is a toxin-mediated paralytic illness caused by *Clostridium botulinum.* It is classified as foodborne botulism, infant botulism, wound botulism, iatrogenic botulism, botulism of undetermined etiology, or inhalational botulism.

Epidemiology
- Foodborne botulism occurs in outbreaks, whereas other forms are sporadic.
- Foodborne botulism is associated with home-canned or fermented foods.
- Infant botulism historically is associated with honey ingestion.
- Wound botulism is associated with injection drug use of "black-tar" heroin.
- Botulinum toxins A and B are used for therapeutic and cosmetic purposes and may cause iatrogenic botulism.
- Botulism is a potential bioterrorism agent deployed by aerosol or ingestion.

Microbiology
- *C. botulinum* is a gram-positive, strictly anaerobic bacillus that forms a subterminal spore.
- *C. botulinum* produces seven distinct toxins, designated types A through G.

Diagnosis
- Presumptive diagnosis is based on clinical presentation: acute, bilateral cranial neuropathies with symmetrical descending weakness.
- Mouse bioassay is the gold standard for botulinum toxin.
- Culture of serum, stool, and environmental samples requires strict anaerobic conditions and is low yield.
- Characteristic electrophysiologic study findings are suggestive of botulism.

Therapy
- Supportive care remains the mainstay of botulism treatment.
- Heptavalent botulinum antitoxin is available for noninfant botulism in the United States.
- Human botulinum immune globulin (BabyBIG) is available for the treatment of infant (younger than 1 year) botulism. To obtain it, contact the California Department of Health Services, Infant Botulism Treatment and Prevention Program (510-231-7600; www.infantbotulism.org).

Prevention
- Proper food preparation prevents foodborne botulism.
- There is no currently available vaccine.

Botulism and tetanus result from intoxication with the protein neurotoxins elaborated by two related species of *Clostridium.* The toxins are very similar in structure and function but differ dramatically in their clinical effects because they target different cells in the nervous system. Botulinum neurotoxins predominantly affect the peripheral neuromuscular junction and autonomic synapses and primarily manifest as weakness. In contrast, although tetanus toxin can affect the same systems, its effects reflect tropism for inhibitory cells of the central nervous system (CNS) and primarily manifest as rigidity and spasm. Both conditions have potentially high fatality rates, and both are preventable through education and public health measures.

Clostridium botulinum produces most cases of botulism, with a few other clostridial strains accounting for the remainder. Botulinum toxins are designated types A through G based on antigenic differences.[1] Type C/D hybrid toxins have also been described.[2] Types A, B, E, and F produce human disease, whereas types C and D are almost exclusively confined to animals.[3] Type G toxin has not been associated with naturally acquired disease. In 2014, a possible new toxin was discovered as a result of a case of infant botulism in which the toxin produced could not initially be neutralized by any of the existing anti-A through anti-G antitoxins in mouse bioassay.[4] There remains some uncertainty regarding whether this toxin should be classified as a distinct toxin. Although it was originally designated as a distinct toxin labeled as type H, the Centers for Disease Control and Prevention (CDC) has used the term "F/A Hybrid" because it can be neutralized by existing serotype A antitoxin, albeit at higher doses.[5–7] For this reason, most do not consider this to be a distinct toxin. A novel monoclonal antibody directed against this toxin has been described but is not commercially available.[8] In addition, a novel toxin has recently been identified through genomic sequencing of a *C. botulinum* strain, although the clinical implications of this finding are unclear.[9] The clinical forms of botulism include *foodborne botulism, infant botulism, wound botulism*, and *botulism of undetermined etiology.* Botulinum A toxin has achieved prominence as a therapeutic modality in conditions that result from excessive muscle activity (e.g., torticollis), leading to rare cases of *iatrogenic botulism.* Botulinum toxin has also been developed as a weapon, which could be used to contaminate food or beverage supplies or could be aerosolized.

HISTORY OF BOTULISM

The term *botulism* derives from the Latin word *botulus,* or sausage. Outbreaks of poisoning related to sausages and other prepared foods occurred in Europe in the 19th century. Justinus Kerner, a district health officer in southern Germany, recognized the connection between sausage and the paralytic illnesses of 230 patients in 1820 and made sausage poisoning a reportable disease.[10] At about the same time, physicians in Russia recognized a disease with similar symptoms, which they termed *fish poisoning.*[11] In 1897, van Ermengen published the first description of *C. botulinum* and showed that the organism elaborated a toxin that could induce weakness in animals.[12] This was subsequently shown to be type A toxin; type B was discovered in 1904.[13] Wound botulism was described in 1943,[14] and infant botulism in 1976.[15] The occurrence of sporadic cases without an apparent etiology, many related to gastrointestinal colonization, was first reported in 1986.[16] Type A toxin was isolated and purified in 1946.[17]

EPIDEMIOLOGY

Foodborne botulism is most frequently recognized in outbreaks, whereas the other forms are sporadic. Although commercially canned foods were commonly the source of toxin in the early part of this century, home-canned vegetables, fruits, and fish products are now the most common sources. In some cultures, such as among Alaskan Natives, preferred food preparation practices involving fish fermentation commonly lead to botulism.[18] In China, homemade fermented beans are the leading cause.[19] Commercial foods and restaurants are still occasional sources.[20,21]

Consumption of peyote for religious reasons has resulted in botulism.[22] Pruno, an illicit, prison-brewed alcoholic beverage, has also emerged as a cause of botulism.[23] In the United States, 263 cases occurred from 1990 to 2000 because of 163 foodborne botulism events (17–43 cases per year).[24] In 2015 there were 39 cases of confirmed foodborne botulism in the United States, primarily associated with five outbreaks.[25]

Infant botulism primarily occurs with toxin types A, B, or F. In the past, infections were attributed to honey ingestion,[26] but other sources have emerged as feeding honey to infants has been discouraged.[27] In the absence of competing microbiota found in children and adults, *C. botulinum* colonizes the intestine of infants (ages 6 days to 12 months). Infection occurs as a consequence of absorption of toxin produced by *C. botulinum* in situ.[28] From 1992 to 2006, 2419 cases of infant botulism were identified in the United States (average, 2.1 cases per 100,000 live births).[29] Two infants without other exposures are believed to have contracted botulism through soil contamination.[30] Rare cases of infant botulism have been associated with *Clostridium baratii*[31] or *Clostridium butyricum*.[32]

Wound botulism may be caused by either type A or type B organisms. In such cases, *C. botulinum* spores contaminate the wound, leading to subsequent germination and toxin production. Almost exclusively associated with injection drug use of "black-tar" heroin, wound botulism was first reported in the United States in the 1990s. Spore contamination of heroin during preparation can lead to infection, particularly in patients who inject by "skin-popping" (i.e., drug injection into tissue rather than the vein).[33,34] The majority of wound botulism cases have been reported in California. However, black-tar heroin–related cases have also been described in Europe, including 12 cases in Germany in 2005.[35–37]

Adult botulism of unknown etiology usually involves type A toxin, but types B and F have also been implicated.[38] Affected adults become colonized with and subsequently infected by toxin-producing clostridia. Adults at risk include those with loss of bowel microbiota because of anatomic abnormalities, functional disorders, or antibiotic use.[39,40–42] In the setting of adult botulism of unknown etiology attributed to type F the disease was caused by *C. baratii*.[42]

Botulinum toxin types A and B are approved by the US Food and Drug Administration (FDA) for cosmetic and therapeutic purposes (e.g., blepharospasm, strabismus, cervical dystonia). Iatrogenic botulism cases are uncommon but have been reported with the therapeutic[43] and unlicensed cosmetic use of botulinum toxin A.[44,45]

Rare cases of inhalational botulism have been associated with the intranasal use of contaminated cocaine.[46] Inhalation is also one of the potential routes of a bioterrorist attack with botulinum toxin. *C. botulinum* has been considered a high enough probability for use in bioterrorism that it has been targeted by a blue ribbon panel for special research emphasis. A bioterrorist attack with this toxin could cause intoxication via ingestion or as an aerosol. In the event of intentional contamination of food with botulinum toxin, the signs and symptoms of the victims of such an attack would be indistinguishable from a natural outbreak of botulism, except that epidemiologic investigation might reveal that the common food ingested was not typically associated with botulism or that different foods in the same area were all contaminated. Introduction of toxin into milk trucks or other large, closed food or beverage transports would produce sporadic cases. In such a circumstance, individual clinicians would be unlikely to recognize an attack early in its development. Automated systems for the collection of epidemiologic data are required for this purpose.[47]

Predicting the consequences of dissemination into the environment is more problematic because there are no data regarding the stability of the toxin in water or sunlight. One CDC expert estimated that an aerosol release of toxin could affect 10% of people within 500 meters.[48] Once in the atmosphere, the decay rate of the toxin is estimated to be 1% to 4% per minute. Modeling an aerosol exposure suggests that substantial inactivation could take up to 2 days but would be accelerated by extremes of temperature and humidity.[48]

MICROBIOLOGY OF *CLOSTRIDIUM BOTULINUM*

C. botulinum is a large, gram-positive, strictly anaerobic bacillus that forms a subterminal spore.[49] The species is divided into four physiologic groups. Group I organisms are proteolytic in culture and can produce toxin types A, B, and F. Group II organisms are nonproteolytic and can produce toxin types B, E, and F. Group III organisms produce toxin types C and D, and group IV produces type G. A single strain almost always produces only one toxin type. Group II organisms grow optimally between 25°C and 30°C, and the other groups grow best between 30°C and 37°C. Although each strain of the organism typically contains several plasmids, only type G toxin is encoded on a plasmid (unlike *Clostridium tetani,* in which the toxin apparently is always encoded on a plasmid).[50]

C. botulinum spores are found throughout the world in soil samples and marine sediments.[51] These spores are able to tolerate 100°C at 1 atm for several hours; because boiling renders solutions more anaerobic, it may actually favor the growth of *C. botulinum*.[52] Proper preparation of food in a pressure cooker will render spores inert.

PATHOGENESIS

In foodborne botulism, toxin is ingested with the food in which it was produced. It is absorbed primarily in the duodenum and jejunum and passes into the bloodstream, by which it reaches peripheral cholinergic synapses (including the neuromuscular junction). Infant botulism and probably adult botulism of unknown etiology have a somewhat different pathogenesis in that they are acquired through the ingestion of spores rather than preformed toxin. The infant's intestinal microbiota is thought to be particularly permissive for the germination of spores, which leads to the production of toxin. The spores are acquired from environmental sources contaminated with soil in which botulinum spore counts are high.[53] In adults, achlorhydria and antibiotic use may predispose to gastrointestinal colonization with *C. botulinum*. In cases of wound botulism, spores are introduced into a wound, where they germinate and produce toxin. Lastly, in inhalational botulism, the toxin crosses through the pulmonary alveolar epithelium to gain access to the bloodstream.[54] The clinical manifestations of botulism depend on the type of toxin produced, rather than the site of its production.

Botulinum toxin is synthesized as a single polypeptide chain of low potency; the molecular weight varies from 150 to 165 kDa, depending on the toxin type. The botulinum toxins are zinc-dependent metalloproteinases,[55] as is tetanospasmin (the neurotoxin associated with *C. tetani)*. The toxin is then nicked by a bacterial protease to produce two chains, with the light chain constituting about one-third of the total mass. As with tetanospasmin, the chains remain connected by a disulfide bond. The nicked toxin type A becomes, on the basis of molecular weight, the most potent toxin found in nature. In contrast to the spores, the toxin is heat labile. Different toxin types may undergo different postsynthetic processing.[56]

In the laboratory, the clostridial toxins have provided a major tool for understanding the mechanisms of neurotransmitter release. Once present at the synapse, the toxins prevent the release of acetylcholine (ACh). This appears to result from a three-stage process.[57] The heavy chain of the toxin mediates binding to presynaptic receptors. The nature of these receptors is uncertain; different toxin types bind to different receptors, with type B receptors outnumbering type A receptors by a factor of four.[58] The toxin enters the cell by receptor-mediated endocytosis.[59] Once inside the neuron, the toxin types differ in the mechanisms by which they inhibit ACh release.[60] The release of synaptic vesicles by an action potential is initiated by an abrupt rise in the intracellular free Ca^{2+} concentration, mediated by voltage-dependent calcium channels (Fig. 245.1).[61] This increase in free calcium triggers an interaction between synaptotagmin (in the vesicle membrane) and syntaxin (on the presynaptic cell membrane), clamping the vesicle to the presynaptic membrane. Synaptobrevin (also referred to as *vesicle-associated membrane protein*[62]) also binds to syntaxin and appears to dock the vesicle to the membrane at the proper location for fusion. There are different isoforms of synaptobrevin within neurons; a protein termed *cellubrevin* performs a similar function in nonneuronal secretory cells.[63] Synaptophysin, the third major component of this mechanism, probably forms the fusion pore that allows release of the vesicle contents into the synaptic cleft.[64]

Clostridial neurotoxins inhibit vesicle release by cleaving peptide bonds in these proteins.[65] Each toxin has a specific locus of activity. Tetanospasmin, along with botulinum neurotoxins B, D, F, and G, cleaves synaptobrevin.[66,67] Tetanospasmin and botulinum neurotoxin B appear

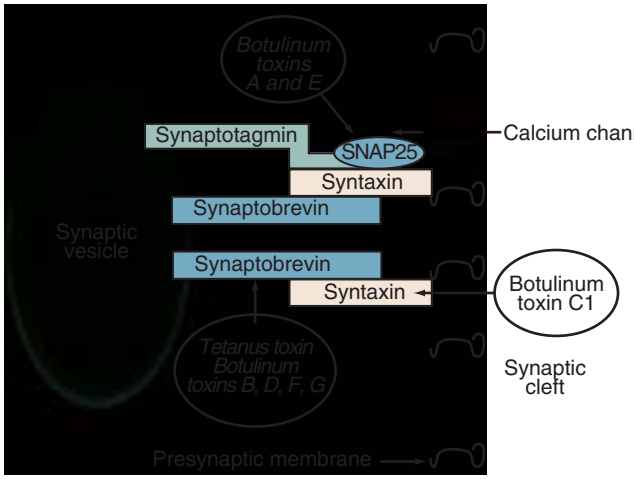

FIG. 245.1 Components of the transmitter release mechanism. SNAP25, synaptosomal-associated protein-25. *(From Bleck TP, Brauner JS. Tetanus. In: Scheid WM, Whitley RJ, Durack DT, eds.* Infections of the Central Nervous System. *2nd ed. New York: Raven Press; 1997:629–653.)*

to share the same cleavage site on synaptobrevin.[68] In contrast, botulinum toxins A[69] and E act on the 25-kDa synaptosomal-associated protein 25 (SNAP25),[70] and botulinum toxin C1 affects syntaxin. The toxins affect only the free proteins; once they have complexed to cause transmitter release, they are not subject to attack.[71] Synaptobrevin and synaptotagmin cleavage also occurs normally, as an effect of an endogenous protease, and these proteins are probably involved in organelle recycling.[72] The endogenous protease does not appear homologous to the clostridial toxins. However, the result is that stimulation of the presynaptic cell (e.g., the alpha motor neuron) fails to produce transmitter release, thus producing paralysis in the motor system or autonomic dysfunction when parasympathetic nerve terminals or autonomic ganglia are involved.

Once damaged, the synapse was originally thought to be rendered useless. Because of the widespread interest in the therapeutic use of botulinum toxin, substantial research into the mechanisms of recovery is underway. The initial recovery of function in type A botulism requires sprouting of the presynaptic axon and the subsequent formation of a new synapse. Later, the original synapse recovers, and the newer ones are pruned away.[73] With type F botulism, recovery is substantially faster, suggesting that the original synapse regains function more rapidly.[74]

Botulinum toxin is transported within nerves in a manner analogous to tetanospasmin and can thereby gain access to the CNS. However, symptomatic CNS involvement is rare.[75]

CLINICAL MANIFESTATIONS

The classic presentation of botulism is that of a patient who develops acute, bilateral cranial neuropathies associated with symmetrical descending weakness. The CDC suggests attention to these cardinal features: (1) fever is absent (unless a complicating infection occurs), (2) the neurologic manifestations are symmetrical, (3) the patient remains responsive, (4) the heart rate is normal or slow in the absence of hypotension, and (5) sensory deficits do not occur (except for blurred vision).[42] The first two features were important for the exclusion of poliomyelitis. Atypical findings such as unilateral symptoms or ascending paralysis have been described in up to 7% of reported cases.[76]

Foodborne botulism usually develops 12 to 36 hours after toxin ingestion. The patient initially reports nausea and a dry mouth, and diarrhea may occur at this stage. If diarrhea does occur, it is due to other substances in the ingested material; botulinum toxin itself will produce constipation when it affects parasympathetic autonomic ganglia. Evidence of cranial nerve dysfunction most commonly starts with the eyes, reflecting parasympathetic involvement (blurred vision as a result of pupillary dilation) or involvement of cranial nerves III, IV, or VI.[77] Pupillary reactions may remain abnormal for months after motor recovery.[78] Nystagmus is occasionally noted, usually in type A disease. Lower cranial nerve dysfunction manifests as dysphagia, dysarthria, and hypoglossal weakness. Weakness then spreads to the upper extremities, the trunk, and the lower extremities. Respiratory dysfunction may result from either upper airway obstruction (the weakened glottis tends to close during attempted inspiration) or diaphragmatic weakness. Patients who need mechanical ventilation require mean periods of 58 days (type A) and 26 days (type B) for ventilatory weaning.[79] Recovery may not begin for up to 100 days.[80] Autonomic problems may include gastrointestinal dysfunction, alterations in resting heart rate, loss of responsiveness to hypotension or postural change, hypothermia, and urinary retention.[81]

Hughes summarized published reports to analyze differences in the clinical findings of intoxication with different toxin types (Table 245.1).[82] Type A is significantly more commonly associated with dysarthria, blurred vision, dyspnea, diarrhea, sore throat, dizziness, ptosis, ophthalmoplegia, facial paresis, and upper extremity weakness. Types B and E appear to produce more autonomic dysfunction. None of these differences is diagnostic of the toxin type, however. It is important to note that the pupils are either dilated or unreactive in less than 50% of patients; although these are useful signs when present, their absence in no way diminishes the likelihood of botulism.

Infants with botulism present with constipation, which may be followed by feeding difficulties, hypotonia, increased drooling, and a weak cry.[83] Upper airway obstruction may be the initial sign[84] and is the major indication for intubation.[53] In severe cases, the condition progresses to include cranial neuropathies and respiratory weakness, with ventilatory failure occurring in about 50% of diagnosed patients. The condition progresses for 1 to 2 weeks and then stabilizes for another 2 to 3 weeks before recovery starts.[85] Relapses of infant botulism may occur.[86]

Wound botulism lacks the prodromal gastrointestinal disorder of the foodborne form but is otherwise similar in presentation. Fever, if present, reflects wound infection rather than botulism. The wound itself may rarely appear to be healing well while neurologic manifestations are occurring. Conversely, *C. botulinum* infection may produce abscesses[87]; botulism has also been reported as a result of sinusitis with this organism after cocaine inhalation.[88] The reported incubation period varies from 4 to 14 days.

The signs and symptoms exhibited by victims of inhalational botulism are the same as those seen with ingestion. The latency between exposure and clinical disease after inhalation appears to be between 12 hours and 3 days, with maximal disease by about 5 days.[48]

Botulinum toxin has been used to treat a variety of chronic pain syndromes, achalasia, and anal fissures.[89] It has also achieved widespread notoriety for its use in cosmetic procedures. In 2004, four patients developed clinical symptoms of botulism after the unlicensed cosmetic use of botulinum toxin A.[44]

DIAGNOSIS

A history appropriate to the type of botulism suspected is the most important diagnostic test. If others are already affected, the condition is easily recognized. However, because the toxin may not be evenly distributed in foodstuffs, the absence of other patients does not eliminate the diagnosis. A screening tool has been suggested to help facilitate timely diagnosis of botulism, which is intended to aid physicians in identifying patients who may have botulism, although it is not intended for diagnosis. This tool uses three criteria: (1) afebrile status; (2) at least one of the following symptoms: blurred vision, double vision, difficulty speaking, change in sound of voice, dysphagia, or thick tongue; and (3) at least one of the following signs: ptosis, extraocular palsy, facial paralysis, fixed pupils, or descending paralysis. Based on a review of 241 cases, this tool showed a sensitivity of 87%.[90] This tool would likely also pick up many botulism mimics; however, it could facilitate earlier treatment.

Botulism has a limited differential diagnosis. Myasthenia gravis and Lambert-Eaton myasthenic syndrome (LEMS) each share some of the characteristics of botulism, but are rarely fulminant, and myasthenia lacks autonomic features. An edrophonium test may be considered, but an improvement in strength is not pathognomonic of myasthenia gravis and has been reported in botulism[91]; however, edrophonium is currently out of production. Tick paralysis is excluded by a careful physical examination because the *Dermacentor* tick will still be attached.

TABLE 245.1 Symptoms and Signs in Patients With the Common Types of Human Botulism

	TYPE A (%)	TYPE B (%)	TYPE E (%)
Neurologic Signs and Symptoms			
Dysphagia	96	97	82
Dry mouth	83	100	93
Diplopia	90	92	39
Dysarthria	100	69	50
Upper extremity weakness	86	64	NA
Lower extremity weakness	76	64	NA
Blurred vision	100	42	91
Dyspnea	91	34	88
Paresthesias	20	12	NA
Gastrointestinal Signs and Symptoms			
Constipation	73	73	52
Nausea	73	57	84
Vomiting	70	50	96
Abdominal cramps	33	46	NA
Diarrhea	35	8	39
Miscellaneous Symptoms			
Fatigue	92	69	84
Sore throat	75	39	38
Dizziness	86	30	63
Neurologic Findings			
Ptosis	96	55	46
Diminished gag reflex	81	54	NA
Ophthalmoparesis	87	46	NA
Facial paresis	84	48	NA
Tongue weakness	91	31	66
Pupils fixed or dilated	33	56	75
Nystagmus	44	4	NA
Upper extremity weakness	91	62	NA
Lower extremity weakness	82	59	NA
Ataxia	24	13	NA
DTRs diminished or absent	54	29	NA
DTRs hyperactive	12	0	NA
Initial Mental Status			
Alert	88	93	27
Lethargic	4	4	73
Obtunded	8	4	0

DTRs, Deep tendon reflexes; NA, not available.
Data from Tacket CO, Rogawski MA. Botulism. In: Simpson LL, ed. Botulinum Neurotoxin and Tetanus Toxin. *San Diego, CA: Academic Press; 1989:351–378; Hughes JM. Botulism. In: Scheld WM, Whitley RJ, Durack DT, eds.* Infections of the Central Nervous System. *New York: Raven Press; 1991:589–602; and Weber JT, Hibbs RG, Darwish A, et al. A massive outbreak of type E botulism associated with traditional salted fish in Cairo.* J Infect Dis. *1993;167:451–454.*

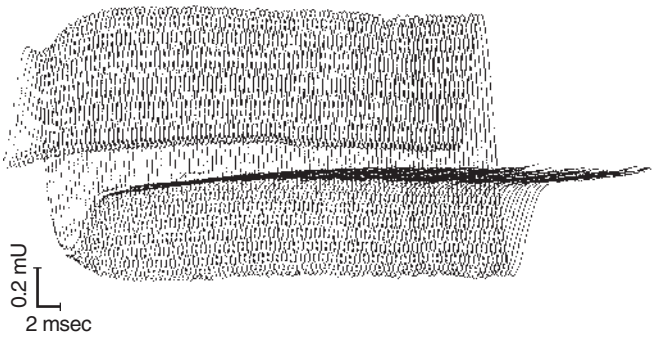

FIG. 245.2 Repetitive nerve stimulation in infant botulism. Note the increment in response amplitude during the initial stimulations. *(Courtesy Vern Juel, MD, Department of Neurology and Laboratory of Electromyography, University of Virginia, Charlottesville, VA.)*

Classic acute inflammatory demyelinating polyneuropathy (Guillain-Barré syndrome) frequently begins with sensory complaints, rapidly produces areflexia, rarely begins with cranial nerve dysfunction, and does not alter pupillary reactivity. Patients with botulism do not become areflexic until the affected muscle group is completely paralyzed. The Miller Fisher variant of Guillain-Barré syndrome presents with oculomotor dysfunction and may produce other cranial neuropathies but includes a prominent ataxia that is lacking in botulism. Patients with polio are febrile on presentation and have asymmetrical weakness. Magnesium intoxication may mimic botulism.[92] Rarely, botulism may be confused with diphtheria, organophosphate poisoning, or brainstem infarction.[93]

Conventional diagnosis of botulism relies on the demonstration of toxin in serum, gastric secretions, stool, or food samples. The most sensitive means of botulism toxin detection has traditionally been the mouse bioassay.[94] After receiving an injection of sample, mice are followed for the development of symptoms. Toxin type may be determined by injecting infected mice with type-specific botulism antitoxin. Botulism symptoms are absent from infected mice that receive the appropriate antitoxin. Confirmation and toxin typing are obtained in almost 75% of cases.[95] The mouse bioassay is labor and resource intensive, and therefore the testing is performed in a limited number of public health laboratories. A novel assay based on mass spectroscopy has been reported to have greater sensitivity than the mouse bioassay and detected botulinum toxin in an infant in whom polymerase chain reaction, bacterial cultures, and mouse bioassay were negative.[96,97] In addition, this assay can be performed in 7 to 8 hours, compared with the mouse bioassay, which may take several days. A Biosafety Level 2 containment facility is a minimum requirement for *C. botulinum* detection and evaluation, given its potency. Testing should be performed under the direction of local state or health departments. If consultation is required after hours, the regional Poison Center (800-222-1222) or the CDC's Emergency Operations Center (770-488-7100) may be contacted.

Anaerobic cultures of serum, stool, and the implicated food, if available, may assist in making the diagnosis. However, samples rarely yield *C. botulinum* because strict anaerobic conditions are required for growth, and competing fecal microbiota or nontoxigenic *C. botulinum* strains can make isolation difficult. Toxin excretion may continue up to 1 month after the onset of illness, and stool cultures may remain positive for a similar period.

Enzyme-linked immunosorbent assay has been used to detect botulinum toxin in clinical specimens and in contaminated food samples, such as fish fillets, canned salmon and corned beef, pasta products, and canned vegetables.[98–101] Techniques based on the polymerase chain reaction and on mass spectrometry are also being explored as potential diagnostic tools.[102,103,104]

Electrophysiologic studies reveal normal nerve conduction velocities; the amplitude of compound muscle action potentials is reduced in 85% of cases, although not all motor units may demonstrate this abnormality.[105] Repetitive nerve stimulation at high rates (20 Hz or greater, compared with the 4-Hz rate used in the diagnosis of myasthenia gravis) may reveal a small increment in the motor response (Fig. 245.2), as opposed

to the decrement expected in myasthenia. This test is very uncomfortable and should not be requested unless botulism or LEMS is a serious consideration. Botulism can be distinguished electrophysiologically from LEMS.[106] In infant botulism, the increments may be very dramatic. In questionable cases, single-fiber electromyography studies may be useful. There is currently some debate regarding the sensitivity of electrodiagnostic techniques in cases of infant botulism.[107] The therapeutic use of botulinum A toxin for dystonic disorders can produce electrophysiologic evidence of toxin dissemination to distant sites.[108]

If botulinum toxin is used as a biologic weapon, the diagnosis would depend on the route of exposure. Contaminated food or beverages would result in an epidemic resembling that of a natural foodborne outbreak. A deliberate release of botulinum toxin should be suspected if patients with acute flaccid paralysis and prominent bulbar palsies present in large numbers. An unusual toxin type (such as C, D, F, G, or F/A Hybrid) or symptoms among patients with a common geographic location may suggest an act of bioterrorism.[48] The amount of inhaled toxin–producing disease would probably not produce measurable toxin in blood or other patient samples, except perhaps for nasopharyngeal secretions. Therefore, current approaches to the diagnosis of botulism and the detection of botulinum toxin would be of limited value during an attack. The diagnosis can be confirmed most rapidly with electromyography.

THERAPY

The importance of supportive therapy for botulism is underlined by the progressive improvement in mortality rates with advances in critical care, especially ventilatory support. The decision to intubate should be based on (1) bedside assessment of upper airway competency and (2) changes in vital capacity. (In general, an appropriately performed vital capacity measurement of <12 mL/kg frequently indicates intubation. However, the facial weakness of botulism may preclude a tight seal on the spirometer mouthpiece, invalidating the test.) One should not wait for the partial pressure of arterial carbon dioxide ($Paco_2$) to rise or the oxygen saturation to fall before intubating the patient. In contrast to tetanus, the autonomic dysfunction of botulism is rarely life-threatening, and patients who receive appropriate airway and ventilator management should recover unless complications supervene. Patients intubated with high-volume, low-pressure endotracheal tubes should not automatically undergo tracheostomy, regardless of the duration of intubation, unless required for mechanical reasons.[109] If contaminated food may still reside in the gastrointestinal tract, purgatives may be useful unless ileus has occurred. The detailed critical care management of botulism patients is beyond the scope of this text; Tacket and Rogawski have presented a useful approach.[52]

In March 2010, the CDC announced the availability of a new, heptavalent botulinum antitoxin (HBAT). HBAT contains equine-derived antibody to the seven known botulinum toxin types (A through G).[110] It replaced the trivalent (types A, B, and E) equine serum and was FDA approved in 2013. It is the only antitoxin available for noninfant botulism in the United States and has been found to be relatively safe and effective in adults, particularly when administered within 2 days of symptom onset,[111] although there is a 1% to 2% risk of anaphylactic reaction.[112] Because of the risk of serious adverse reactions and the potential for lifelong sensitization to equine proteins, HBAT is not approved for administration in infants younger than 1 year.[113] Human botulinum immune globulin (BabyBIG) was approved by the FDA in 2003 for the treatment of infant (younger than 1 year) botulism. Use of BabyBIG has resulted in decreased length of intensive care unit stay, length of mechanical ventilation, and overall length of hospital stay.[114] These benefits are greatest when BabyBIG is administered within the first 3 days of hospitalization.[115] The current recommended dose is 50 mg/kg as an intravenous infusion, although the recommended dose may vary with each manufactured lot and should be confirmed. For suspected cases of infant botulism in any state, the California Department of Health Services, Infant Botulism Treatment and Prevention Program should be contacted (510-231-7600; www.infantbotulism.org). In the event of intentional dissemination of botulinum toxin, heptavalent antitoxin may be dispensed by the Department of Defense.[48]

Patients with wound botulism should also undergo débridement, even if the wound appears to be healing well. Anaerobic cultures should be obtained at the time of surgery. The value of local instillation of antitoxin is unknown. The role of antibiotic treatment is untested, but penicillin G (10–20 million units daily) is frequently recommended. Metronidazole may be an effective alternative. Aminoglycosides and tetracyclines, which can impair neuron calcium entry, worsen infant botulism.[116] Lysis of *C. botulinum* in the gut by antibiotics may also increase the toxin available in infant botulism.[117] This effect has not been reported in adult cases but should be considered when gastrointestinal infection is suspected. Aminopyridines promote neurotransmission from intoxicated nerve terminals and may have a role in the treatment of the paralysis associated with botulism.[118]

In the event of a bioterrorist attack, several logistical issues would make treatment problematic. Based on the limited information available, a large-scale attack (either foodborne or by aerosol) would probably not begin to produce symptomatic victims for more than a day, thereby delaying diagnosis and containment. Furthermore, the supply of antitoxins is small, reflecting the low incidence of the natural disease. With a large-scale attack, the available antitoxin would be quickly exhausted. Thus, airway protection and ventilation may be the only viable treatment options. To minimize additional exposures, exposed skin and clothing should be washed with soap and water, whereas contaminated surfaces should be cleaned with 0.1% hypochlorite bleach solution if they cannot be avoided for the hours to days required for natural degradation.[48]

Although the greatest improvement in muscle strength occurs in the first 3 months of recovery from botulism, patients still show improvements in strength and endurance for up to 1 year after disease onset.[48,119] With prompt attention and supportive care, the mortality rate for botulism ranges from less than 5% to 8%.[119] The mortality rate for infant botulism is less than 1%.[28]

Long-term consequences of botulism were detailed in an evaluation of 211 patients from the Republic of Georgia from 1998 to 2003. Patients interviewed at least 6 months after illness reported higher rates of fatigue, weakness, and dyspnea on exertion when compared with controls. Affected patients had limitations in functional capacity and impaired psychosocial well-being.[120]

PREVENTION

The most important aspect of botulism prevention is proper food handling and preparation. It is impractical or undesirable to treat many foods in a manner to eliminate *C. botulinum* spores; hence, methods for the control of botulism focus on the inhibition of bacterial growth and toxin production.[117] Because the toxin is heat labile, terminal boiling or similarly intense heating of contaminated food will inactivate it. Food containers that appear to bulge may contain gas produced by *C. botulinum* and should not be opened. Other foods that appear to be spoiled should not be tasted.

In the event of an outbreak, foods suspected of being contaminated should be refrigerated until retrieval by public health personnel. Laboratory testing for botulism in the United States is available only at the CDC and several state and city public health laboratories. According to the Working Group on Civilian Biodefense, persons with potential exposure in a foodborne botulism outbreak should be monitored closely for the development of signs and symptoms; antitoxin should be administered promptly at the first signs of illness.[48]

Immunity to botulinum toxin does not develop even with severe disease, and the repeated occurrence of botulism has been reported.[121] An investigational pentavalent toxoid (ABCDE) vaccine was previously available for use among high-risk laboratory workers and military personnel in the United States.[122] However, in 2011 the CDC discontinued use of the vaccine because of data suggesting waning immunogenicity and frequent local reactions.[123] New vaccine strategies, including recombinant toxin A/B vaccines and inhalational vaccines, are currently being investigated.[54,124]

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246 Diseases Caused by *Clostridium*

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SHORT VIEW SUMMARY

CHARACTERISTICS OF *CLOSTRIDIUM* SPECIES

- Member of the phylum Firmicutes
- Anaerobic gram-positive rods capable of forming endospores
- Ubiquitous in nature
- Traditional phenotypic classification methods rely on carbohydrate fermentation, detection of short-chain fatty acid end products of fermentation, Gram stain morphology, colony morphology, and detection of specific toxins.

CLOSTRIDIOIDES (FORMERLY CLOSTRIDIUM) DIFFICILE INFECTION (CDI)

- Clinical manifestations range from a self-limiting diarrheal disease that disappears when antibiotics are discontinued to fulminant presentations with characteristic pseudomembranes within the large intestine and progression to toxic megacolon and fatal complications.
- The initiating event involves the disruption of the intestinal microbiome during antibiotic treatment, followed by the germination of *C. difficile* spores and production of an enterotoxin (TcdA) and a cytotoxin (TcdB).
- Highly virulent toxin-producing strains possess a deletion of TcdC, which normally downregulates toxin production.
- Treatment includes metronidazole in less serious cases, vancomycin for serious and/or progressive disease, and fecal transplantation for recurrent disease.
- Diagnosis: enzyme immunoassays for the detection of TcdA and/or TcdB, membrane immunoassay to detect the antigen glutamate dehydrogenase and toxin, and polymerase chain reaction.
- Infection control measures, including rapid diagnosis of CDI, the use of contact precautions for positive patients, appropriate therapy, and rigorous cleaning of rooms to effectively kill spores, must be implemented with the hospital or nursing home environment.

CLOSTRIDIUM PERFRINGENS AND CLOSTRIDIAL MYONECROSIS (GAS GANGRENE)

- *Clostridium perfringens* produces a variety of toxins (see Table 246.2). All strains produce α-toxin, a lecithinase that causes cell membrane damage.
- Most common following traumatic crushing injures that result in lowered tissue oxygen levels, penetrating trauma involving foreign bodies contaminated with soil, gastrointestinal or biliary tract surgery, and septic abortion.
- Diagnosis: Initial symptoms include severe pain, redness at the wound site followed by rapidly spreading brown to purple discoloration, edema and gas, and serosanguineous discharge with a characteristic "mousy" odor. Progression to full-blown sepsis with hypotension, renal failure, and metabolic acidosis occurs rapidly.
- Treatment involves prompt surgical débridement of infected tissues, including amputation for extremities or hysterectomy in uterine gas gangrene. Antibiotic treatment with penicillin, metronidazole, clindamycin, or the carbapenems.

FOOD POISONING CAUSED BY *CLOSTRIDIUM PERFRINGENS*

- *Clostridium perfringens* type A is involved in most cases.
- Involves the ingestion of at least 10^8 viable enterotoxin-producing cells in food products that are not properly cooked or stored.
- The incubation period is 7 to 15 hours, and cases resolve spontaneously within 24 to 48 hours.

OTHER CLOSTRIDIAL INFECTIONS

- Bacteremia—Clostridia account for 1% of all positive blood cultures. Significant risk factors include hemodialysis, intestinal malignancy, and inflammatory bowel disease.
- Biliary tract infections—Clostridia can be isolated from over 20% of diseased gallbladders, and *C. perfringens* accounts for 50% of these. Radiographic detection of gas within the biliary tract requires surgical intervention and prompt antibiotic treatment.
- Female genital tract infections—Clostridia are present in up to 20% of non–sexually transmitted disease genital infections and may be present as constituents of bacterial vaginosis. *Clostridium perfringens* and *Clostridium sordellii* can be isolated from postpartum and postabortion infections.
- Pleuropulmonary infections—Clostridia are recovered from up to 10% of pulmonary infections, with *C. perfringens* accounting for the majority.

The genus *Clostridium* includes over 200 described species. Members of this genus participate in a variety of invasive and toxigenic infections. They can cause disease that is strictly toxin mediated, such as antibiotic-associated colitis (AAC) and foodborne botulism, or contribute to invasive infections, including bacteremia, clostridial myonecrosis (gas gangrene), and other suppurative infections driven by histotoxins and enzymes that destroy soft tissue. Historically, clostridial infections were recognized as discrete clinical syndromes well before the germ theory of disease was proposed. The clinical features of tetanus were well described by some of the earliest medical writers, such as Hippocrates, and the toxic nature of this species was noted as early as the 1870s.[1] Clostridia are often isolated as part of a mixed microbiota during suppurative infections that occur as a result of fecal or soil contamination of otherwise sterile tissues. Prior to 1977, the most commonly reported clostridial infections and intoxications were those caused by *Clostridium perfringens*. Other species, most notably *Clostridium tetani* in nonimmunized individuals and *Clostridium botulinum*, also generated considerable interest due to the severity and often fatal nature of the intoxication they caused.

With the discovery of the etiology of AAC first in an animal model[2] and subsequently in humans,[3] it soon became clear that in the antibiotic era *Clostridioides difficile (formerly Clostridium difficile)* was the most common clostridial species associated with the human disease formerly called C. *difficile*–associated diarrhea and currently called *C. difficile* infection (CDI). Within the hospital setting, CDI has become a significant worldwide nosocomial infection problem[4] resulting in both toxin-mediated diarrheal disease and more fulminant presentations such as pseudomembranous enterocolitis and toxic megacolon. Recent recognition that more virulent strains of *C. difficile* occur in health care settings has provoked an increased awareness of this nosocomial infection and has prompted a demand for both rapid methods for diagnosis and more aggressive treatment of CDI and AAC.[5–7] While the well-recognized

pathogenic members of the genus *Clostridium* continue to participate in a broad array of infectious processes, it is also important to note the important role that previously obscure species, such as *C. difficile,* play in human disease.

CHARACTERISTICS OF *CLOSTRIDIUM* SPECIES

Microbiology

Members of this genus are phenotypically characterized as anaerobic, gram-positive rods capable of forming endospores. *Clostridium* spp. are ubiquitous in nature, found in soils and sediments throughout the world and as members of the intestinal microbiome of humans and most other animals. Over 70% of humans are colonized with clostridia at concentrations of 10^8 to10^9 organisms per gram of feces.[8] Clostridia can also be isolated as part of the vaginal microbiome of healthy women,[9] although they tend to be transient members of this microbiome, occurring in low numbers as a result of contamination by intestinal microbiota, rather than as part of the autochthonous community. Most members of this genus are obligate anaerobes, while strains of a few *Clostridium* species, such as *C. tertium, C. histolyticum, C. innocuum,* and *C. perfringens,* are aerotolerant and can be confused with members of the genus *Bacillus* during laboratory diagnosis. Based on 16S ribosomal DNA (rDNA) sequence data, members of the genus *Clostridium* are part of the phylum Firmicutes, a diverse group of gram-positive organisms including both spore-forming and non–spore-forming genera. Based on 16S rDNA sequence analysis, the clostridia can be divided into 11 homology groups, with most of the clinically significant species belonging to homology group 1.[10] Traditional phenotypic classification methods for the clostridia rely on carbohydrate fermentation profiles, detection of short-chain fatty acid end products of fermentation, Gram stain morphology, colony morphology on agar media, and detection of specific toxins. More recently, proteomic analysis using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry has been employed for identification of certain species.[11,12] Although many different species have been isolated from human clinical material, only a small number of species are regularly associated with human disease (Table 246.1).

Microscopically, the vegetative cells of *Clostridium* species are rod-shaped, often pleomorphic, and found as short chains, as clusters, or in pairs. The cells of most species have rounded ends. This may vary, with some species showing more pointed ends (*Clostridium ramosum*). Some species form long chains (*Clostridium spiroforme*), which may be tightly packed to form coils. Clostridia usually stain gram positively in young cultures, with some species losing this staining characteristic in older cultures. Species such as *Clostridium clostridioforme* and *C. ramosum* rarely show the typical gram-positive appearance and present as gram-variable or gram-negative rods (Fig. 246.1). When spores are present, they tend to be ovoid or spherical, with the spore often distending the vegetative cell to produce a "club-shaped" appearance. Spores may be located centrally, subterminally, or as terminal structures, depending on the species. Spore location is used as part of the phenotypic identification process. Most clostridia are motile by virtue of peritrichous flagellae, with the notable exception of the common clinical isolates *C. perfringens* and *C. ramosum*.[10]

Clostridium spp. have diverse metabolic pathways and can be saccharolytic, proteolytic, both, or neither. *Clostridium* spp. are not known to reduce sulfate. The end products of fermentative metabolism are mixtures of short-chain fatty acids and alcohols, a characteristic that can be used for identification purposes in the clinical laboratory. Aerotolerant strains of clostridia do not form spores in the presence of oxygen, are catalase negative, and grow more abundantly under anaerobic conditions. Clostridia do not have a complete cytochrome system and are therefore oxidase negative.[10] Most strains are catalase and superoxide dismutase negative, although trace amounts of activity have been reported for some species. Clostridia produce a variety of biologically active proteins, including hemolysins, proteolytic enzymes, and other toxins. It is the protein toxins produced by clostridia that account for their importance in human disease. Clostridia produce a greater diversity of toxins than any other genera of bacteria.[10] These include neurotoxins, enterotoxins, collagenases, proteases, necrotoxins, lecithinases, lipases, DNases, and neuraminidases. The potency of some of these toxins, such as botulinum neurotoxin (BoNT) and tetanus neurotoxin (TeNT), render them among the most lethal substances yet described; less than 0.2 ng of purified TeNT is fatal in mice.

Pathogenesis

Invasive infections caused by clostridia are invariably due to organisms that are either part of the normal intestinal and vaginal microbiome or acquired by a traumatic injury breaching the skin that becomes contaminated with soil, unsanitary water, or fecal material. Intoxications can occur either in response to endogenous toxin production, such as that associated with CDI, or by ingestion of preformed toxins

TABLE 246.1 Clostridial Species Commonly Associated With Human Disease

SPECIES	SPORE LOCATION	LECITHINASE PRODUCED	LIPASE	ENTEROTOXINS PRODUCED	HISTOTOXINS, HEMOLYSINS, PROTEASES	NEUROTOXINS PRODUCED
Tissue Infections						
C. perfringens	ST, C	+	–	Yes	Yes	No
C. ramosum	T	–	–	No	Yes	No
C. septicum	ST	–	–	No	Yes	No
C. sordellii	ST	+	–	No	Yes	No
C. bifermentans	ST	+	–	No	Yes	No
C. tertium	T	–	–	No	Yes	No
C. sphenoides	ST	–	–	No	Yes	No
C. baratii	ST	–	–	No	Yes	No
C. novyi	ST	+	+	No	Yes	No
C. histolyticum	ST	–	–	No	Yes	No
Intoxications						
C. difficile	ST	–	–	Yes	Yes	No
C. botulinum	ST, T	–	+	No	Yes	Yes
C. tetani	T	–	–	No	Yes	Yes

C, Centrally; *ST,* subterminally; *T,* terminally.

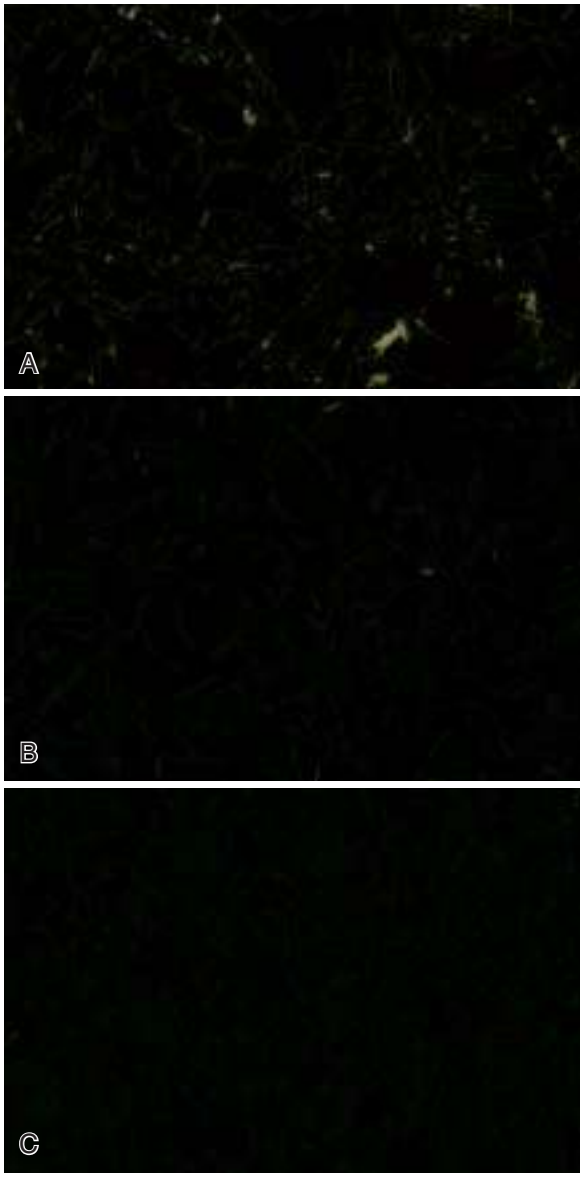

FIG. 246.1 Gram stain characteristics of *Clostridium* species. (A) *Clostridium perfringens.* (B) *Clostridium novyi.* (C) *Clostridium ramosum.*

contaminating food, as is the case for noninfant botulism. Apart from environmental spread of *C. difficile* within a susceptible population, such as hospitalized patients on broad-spectrum antibiotic therapy and residents in nursing homes, clostridia rarely cause infection through person-to-person contact.

The spores of clostridia account for their persistence in hostile environments and their exogenous acquisition by humans. In addition to their long-term survival in soil or food, clostridial spores may spread via aerosol transmission as part of naturally occurring dust clouds. *C. difficile* is of concern because this species may be part of the intestinal microbiome of an individual or may be acquired through contact with individuals or contaminated surfaces and equipment within the hospital environment harboring spores. The vegetative cells of clostridia are generally susceptible to routinely used disinfectants; however, spores can survive hostile environments, including heat, desiccation, and exposure to many commonly employed disinfectants.[13–15] This allows pathogenic clostridia to persist in the environment, even following routine disinfection procedures. Methods for eliminating clostridial spores from some environments, such as *C. difficile* in the hospital setting, include finding methods to promote germination of the spores so that the vegetative cells can be destroyed.[13,16]

Clostridium spp., particularly members of clusters IV and XIVa derived from the gut microbiota, have recently been shown to have beneficial effects on the immune system. One can speculate that this may be why almost all children have *C. difficile* present in their intestine during the first year of life. Specifically, a consortia of *Clostridium* species promoted accumulation of colonic regulatory T-cell development in mice.[17,18] Inoculation of these *Clostridium* spp. into mice increased their resistance to chemically induced colitis and blunted their allergic responses. This finding casts this genus in a new light, suggesting its members are not only fearsome pathogens but also may hold therapeutic potential for autoimmune and allergic conditions.

MAJOR INFECTIONS AND INTOXICATIONS

Clostridioides difficile Infection

Historical Perspective

Until it was identified as the primary cause of AAC in 1977, *C. difficile* was not regarded as a particularly common or important pathogen; however, the association of *C. difficile* with CDI and AAC has brought this organism to prominence as the most common clostridial species associated with human disease. Hall and O'Toole published the first description of *C. difficile* in 1935 and suggested that it might be involved in intestinal disease in children.[19] Interestingly, the clinical description of pseudomembranous colitis dates to the 1890s. An animal model for antibiotic-associated intestinal disease was first reported in the 1940s, with several additional observations on the induction of bowel inflammation by antibiotics in hamsters, guinea pigs, and rabbits made in the 1950s. The occurrence of pseudomembranous colitis in patients receiving broad-spectrum antibiotics prior to the 1970s was not uncommon. Based on laboratory analysis, it was often attributed to *Staphylococcus aureus,* one of the major nosocomial pathogens of the antibiotic era. Cultures of stool from these patients often yielded high levels of *S. aureus;* however, obligately anaerobic organisms were not evaluated in these early studies. Given the common isolation of *S. aureus* from stool samples obtained from healthy individuals, these earlier observations were something of a self-fulfilling prophecy. While certain strains of *S. aureus* produce potent enterotoxins that may be responsible for some cases of AAC, there is little evidence to suggest that this species is a common cause of pseudomembranous colitis.

In 1974, investigators in St. Louis noted that about 20% of patients receiving the lincosamide antibiotic clindamycin developed diarrhea, and half of these patients had pseudomembranous colitis when examined endoscopically.[20] Publication of these observations set the stage for more detailed examination of the role of antibiotics in the occurrence of pseudomembranous colitis and led to the search for an etiologic agent. The breakthrough that ultimately led to identification of *C. difficile* as the causative agent of CDI involved a hamster model of AAC demonstrating that vancomycin prevented the occurrence of AAC induced by clindamycin, suggesting that a gram-positive organism was involved in the hamster disease.[21] Armed with this information and the knowledge that the disease appeared to be toxin-mediated, based on molecular size filtration studies, these same investigators isolated clostridial species from the ceca of hamsters with AAC and showed that one species, *C. difficile,* can cause disease in other hamsters as pure cultures or culture filtrates in the absence of prior antibiotic exposure. The link to human disease was made when the same toxin isolated from the hamster model was found in stools of AAC patients using a combination of cytotoxicity assays and an anti-clostridial antibody capable of neutralizing the cytotoxic effect.[22] Vancomycin remains the antibiotic of choice for treating serious CDI in humans (see Chapter 243 for a more extensive discussion of treatment alternatives), and the same cytotoxicity assay used to correlate animal and human disease remains the gold standard for evaluating other diagnostic assays.

Clinical Manifestations

The clinical manifestations of CDI range from a self-limiting diarrheal disease that disappears when antibiotics are discontinued to fulminant presentations with characteristic pseudomembranes within the large intestine and progression to toxic megacolon, often with fatal complications.[23] Pseudomembranes, while present in 97% of CDI, are not

pathognomonic for CDI and may also occur in antibiotic-associated diarrhea not caused by *C. difficile.*[24,25] Symptoms may occur while patients are receiving antibiotics, usually after 5 to 10 days of therapy, or can occur 2 to 10 weeks after antibiotic therapy has been completed.[26] All classes of antibiotics have been associated with CDI, including the penicillins, cephalosporins, macrolides, lincosamides, and aminoglycosides. Diarrhea accompanied by fever occurs in most patients and resembles the symptoms caused by many other intestinal pathogens. In severe cases, bloody diarrhea may be present. Diarrhea is often accompanied by bloating and cramping. Leukocytosis is not uncommon. CDI is more common in the elderly and in hospitalized patients receiving broad-spectrum antibiotic therapy. When surgical intervention is required for severe disease, colostomy carries a substantial risk of mortality regardless of the age of the patient.[27]

Molecular Pathogenesis

The initiating event for CDI involves the disruption of the intestinal microbiome and the normal mechanisms controlling clostridial populations for clostridia such as deconjugated bile acids during treatment with antibiotics. While it is not uncommon for mild diarrhea to occur simply due to the disruption of the microbiome when broad-spectrum antibiotics are used, the presence of *C. difficile* in the intestine can lead to more serious toxin-mediated disease. Since *C. difficile* spores can survive even high concentrations of antibiotics, germination of spores and rapid growth of the organism may occur as antibiotic levels in the lumen of the bowel decrease below inhibitory concentrations for *C. difficile* prior to the recovery of the normal microbiome.

The virulence of *C. difficile* is due to production of two high-molecular-weight toxins, *C. difficile* toxin A (TcdA) and *C. difficile* toxin B (TcdB), originally described as an enterotoxin and a cytotoxin, respectively. Intestinal epithelial cells endocytose the toxins and, once internalized, the toxins irreversibly glucosylate members of the Rho family of small GTP-binding proteins. As a result, there are major alterations in the actin cytoskeleton. These changes lead to the characteristic cytotoxic effects noted in cell culture by compromising the cell's ability to internalize nutrients and carry out vesicular transport, both of which may lead to cell death. The genes for TcdA, TcdB, and an RNA polymerase sigma factor, *C. difficile* toxin R (TcdR), are carried as a pathogenicity locus. In addition, *tcdC,* a gene that downregulates toxin production by interfering with TcdR, is part of this locus.[5,28–30] Hypervirulent toxin-producing strains, with a deletion within the *C. difficile* toxin C gene (*tcdC*), have been reported and account for the current concerns about more virulent CDI. Deletions in the *tcdC* gene can contribute to unregulated toxin production, resulting in much higher levels of toxin being produced. A particular outbreak-associated ribotype, O27/NAP1, is attributed with causing many cases of severe disease. Alterations in the *tcdC* gene have been noted in multiple ribotypes, with the O27 ribotype currently the most commonly associated with *tcdC* deletions.[31] In addition to the two major toxins TcdA and TcdB, a binary ADP ribosylating toxin—*C. difficile* toxin (CDT), a common A-B toxin motif for many clostridial species—has been reported to contribute to the occurrence of more severe CDI.[31–34] Not all strains of *C. difficile* produce all three toxins (TcdA, TcdB, and CDT). Surveillance studies indicate that production of both TcdA and TcdB is most common, occurring in about 65% of strains, with TcdB (cytotoxin) produced by 97% of all strains evaluated. This likely accounts for the utility of the cytotoxicity assay as a diagnostic method before the full range of toxins produced by *C. difficile* was appreciated.

Treatment and Diagnosis

Less serious presentations of CDI have been treated with metronidazole since the late 1990s, with vancomycin reserved for treatment of serious and/or progressive disease.[35] However, given the occurrence of rapidly progressing severe disease in some patients, vancomycin or fidaxomicin (see later in this chapter and see Chapter 243) may be used in cases of moderate disease.[36] Adjunct therapies using probiotics to reconstitute the normal microbiome of the intestine or anion exchange resins that bind toxin have been proposed, but appear to be of limited value and may be harmful for the patient.[35,37]

Of particular concern is the recurrence of CDI once appropriate therapy has been discontinued. This is thought to be due to the survival of the biologically inert spores during therapy and regrowth of the vegetative cells once therapy is discontinued in a microbiologic environment that has been damaged by CDI and by the subsequent antibiotic therapy. It has been shown in gnotobiotic mice that the concentrations of *C. difficile* can be suppressed by vancomycin, but that low levels of spores survived during treatment. The concentration of *C. difficile* returned to pretreatment levels once therapy was discontinued, indicating that the spores survived antibiotic treatment and germinated when antibiotic levels declined.[38] A relatively new antibiotic—fidaxomicin, an RNA polymerase inhibitor—has been shown in clinical trials to reduce the rate of recurrent CDI by almost 50%.[39–41]

Approximately 20% of patients treated for CDI develop recurrent disease. Current recommendations include repeating initial therapy. For a second recurrence, a pulsed dosing of vancomycin is recommended; however, the successful use of fecal microbiota transplants is increasingly becoming the treatment of choice for multiple recurrences of CDI.[36,42–46] Published reports suggest a greater than 90% efficacy in patients with recurrent disease.[47] There are currently a number of ongoing clinical trials of fecal microbiota transplantation for various inflammatory bowel diseases.[47–51] Alternatives to fecal microbiota transplantation and fidaxomicin for recurrent disease also include the use of bezlotoxumab, a monoclonal antibody directed at TcdB during primary CDI to prevent recurrent disease.[52]

Laboratory diagnosis of CDI is generally accomplished by detection of toxin in the stool specimens of patients suspected of having clinical disease. While culture can be performed to isolate *C. difficile,* the presence of the organism does not necessarily mean CDI is present because 4% to 10% of overtly healthy adults may carry this organism as part of their normal intestinal microbiome, and simply detecting the organism by antigen, toxin gene polymerase chain reaction (PCR), or "toxigenic culture" does not necessarily correlate with clinical disease. Initially, the cytotoxicity assay for TcdB was used to diagnose CDI. In this assay, a monolayer cell culture is exposed to a filtrate of stool for 24 to 48 hours and examined for the characteristic cytopathic effect of TcdB. If detected, a portion of the filtrate is mixed with antibody to TcdB and a second cell culture used to determine if the cytopathic effect can be neutralized (Fig. 246.2). While this is a very sensitive assay, the time required to make a definitive diagnosis is often 48 to 72 hours. Currently, there are a variety of enzyme immunoassays (EIAs) available to provide rapid detection of TcdA, TcdB, or both. Assay sensitivity ranges from less than 50% to better than 93%, depending on the assay used and the quality of the specimen, while assay specificity is usually greater than 90%.[53,54] Most EIAs include detection of both TcdA and TcdB, since it is known that some strains produce one toxin but not the other. While the prevalence of CDI varies depending on patient demographics, one large Boston teaching hospital performed over 8000 EIAs in 2012, with over 10% yielding positive results for one or both toxins (unpublished data).

PCR assays designed to detect the genes have also become popular for detection of CDI. However, these assays detect one or more of the toxin genes, but detection of the toxin genes does not necessarily mean that toxin is being produced. Toxin production can only be measured by looking directly for the toxins or the transcription products of the toxin genes *tcdA* and *tcdB.* The current algorithm used in many diagnostic laboratories includes preliminary testing for an antigen, glutamate dehydrogenase (GDH), produced by most strains of *C. difficile* and the presence of toxin using a membrane immunoassay. If antigen is present but no toxin is detected, a PCR assay that detects *tcdA* and *tcdB* is currently recommended to confirm CDI. If the PCR assay is positive, then it is presumed that a symptomatic patient has CDI. This approach is likely to change because the PCR assay only confirms that *C. difficile* is present, but not that toxin is being produced. Many patients positive for GDH and PCR with negative results for toxin are being treated for CDI when the cause of their disease may be another agent/toxin that is not being assayed. Recent studies in England have pointed out the problems with the existing algorithm employed in the United States and note that the best clinical correlation in symptomatic patients is to test for *C. difficile* with either GDH or PCR testing and a sensitive toxin assay.[55,56]

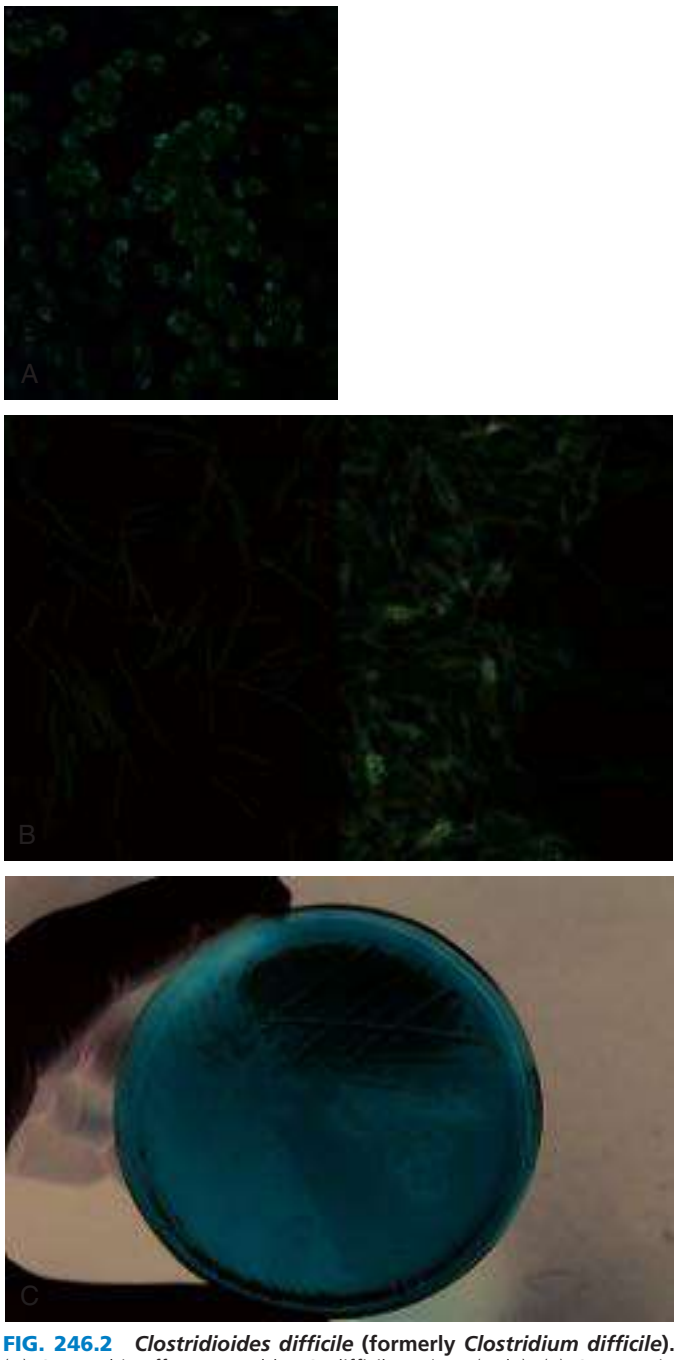

FIG. 246.2 ***Clostridioides difficile*** **(formerly** ***Clostridium difficile*****).** (A) Cytopathic effect caused by *C. difficile* toxin B (TcdB). (B) Gram stain of *C. difficile*. (C) Growth of *C. difficile* on blood agar.

While *C. difficile* is responsible for many cases of antibiotic-associated diarrhea, it is not the only cause. Other clostridial species, such as *C. perfringens* and *Clostridium sordellii,* are also capable of producing enterotoxins under similar circumstances that lead to diarrheal disease during antibiotic therapy.[25] Therefore a negative assay for *C. difficile* toxins does not preclude the involvement of other clostridial species in the clinical manifestations.

When cultured on selective agar media, *C. difficile* can be readily isolated from stool samples (see Fig. 246.2). The colonies are generally flat, 4 to 6 mm in diameter, and gray in color, with irregular margins and a ground-glass appearance. A distinctive "barnyard" odor is often detected from cultures grown on blood agar media because of production of *p*-cresol. Gram stain of colony growth usually shows gram-positive to gram-variable, somewhat slender rods, with subterminal spores when present. Definitive identification requires both fermentation profiles and analysis of short-chain fatty acid end products of fermentation or the use of 16S rDNA sequencing.

It is extremely important for appropriate infection control measures to be implemented within the hospital or nursing home environment for patients diagnosed with CDI. These measures include the rapid diagnosis of CDI in patients on antibiotic therapy, contact precautions for patients with CDI, appropriate therapy, and rigorous cleaning of rooms between patient admissions using disinfectants that effectively kill spores.[6]

TABLE 246.2 Toxins Produced by *C. perfringens*

TOXIN	STRAIN TYPES	BIOLOGIC ACTIVITY
α	All strains	Lecithinase
β	B and C	Necrotoxin, necrosis of the bowel
ε	B and D	Lethal, hemorrhagic
ι	E	ADP ribosylating; lethal
cpe	A, C, and D	Cytopathic
Neuraminidase	All strains	Hydrolyses *N*-acetylneuraminic acid
δ	B and C	Hemolysins
κ	All strains	Collagenase
λ	B, D, and E	Protease
μ	All strains	Hyaluronidase
ν	All strains	DNase

cpe, C. perfringens enterotoxin.

CDI: an Emerging Public Health Crisis

The incidence of hospital-based CDI diagnoses has skyrocketed. There was a 117% increase in the listing of CDI on hospital discharges in the Healthcare Cost and Utilization Project website from 2000 to 2005.[57,58] CDI-related death rates have steadily increased as well over the past decade. Redelings and colleagues reported a 35% increase in mortality rates from 1999 (5.7 per million US population) to 2004 (23.7 per million US population).[59] Similar mortality trends have been observed in Great Britain.[60] Numerous recent studies underscore the importance of the observation that CDI represents a major public health concern requiring increased prevention, surveillance, and reporting.[61–64]

Clostridium perfringens and Clostridial Myonecrosis (Gas Gangrene)

Clostridial myonecrosis, or gas gangrene, is most often caused by a traumatic injury that becomes contaminated with clostridial spores from species that cause myonecrosis, most commonly those of *C. perfringens.* Throughout history, cases of gas gangrene have increased during times of human conflict due to the numbers of traumatic injuries provoked during violent conflict. Although other clostridial species can and do cause gas gangrene, the prevalence of *C. perfringens* in such infections marks it as one of the major clostridial pathogens. *Clostridium perfringens* can be isolated from soil and sediment samples from any geographic region. It is also one of the most common clostridial species isolated from the intestinal tract of humans and other animals. The ability of *C. perfringens* to cause myonecrosis is due to the production of a variety of protein toxins. Since strains of *C. perfringens* vary in their ability to produce some of the major toxins, this attribute differentiates this species into strain types (Table 246.2). Epidemiologically, *C. perfringens* type A is most common in human stool,[65] with other types more commonly associated with food poisoning and other enterotoxigenic disease (see later). Gas gangrene, while most often associated with *C. perfringens,* can be caused by other *Clostridium* species, including *C. septicum, C. sordellii, C. novyi, C. bifermentans,* and *C. histolyticum.* The common denominator for these species is the production of exotoxins, most often lecithinase, that devitalize tissue and promote invasive disease and myonecrosis.



FIG. 246.3 ***Clostridium perfringens.*** (A) Lecithinase production by *C. perfringens* on egg yolk agar. Note the opaque zone surrounding the colony. (B) Hemolysin production by *C. perfringens* on blood agar.

Pathogenesis

All strains of *C. perfringens* produce α-toxin, a lecithinase (Fig. 246.3) that causes damage to cell membranes. During severe sepsis with *C. perfringens,* rapid hemolytic anemia may occur due to hemolysis caused by the α-toxin. The α-toxin is considered the major lethal toxin produced by *C. perfringens* during gas gangrene. In addition to the α-toxin, most strains produce additional hemolysins, proteases, collagenase, hyaluronidase, DNase, and neuraminidase.

Gas gangrene is most common following traumatic injuries that result in lowered tissue oxygen tension, such as crushing or penetrating injuries; the presence of foreign bodies, including soil or pieces of the object causing penetrating trauma; and mixed infections containing other organisms capable of reducing the oxygen levels at the site of infection. Studies of wounds occurring on the battlefield indicate that most are contaminated by clostridial spores, yet only a small percentage of these contaminated wounds result in clostridial myonecrosis. In the civilian population, approximately 10% of crushing wounds occurring as a result of automobile accidents have been shown to contain clostridial spores.[66] Clostridial myonecrosis may also occur following surgery, most often of the gastrointestinal (GI) or biliary tract, and following septic abortions. Contamination of lesions in which vascular insufficiency is present (e.g., diabetic foot ulcers), the occurrence of damaged tissue associated with burns, and underlying neoplastic disease may also contribute to the occurrence of gas gangrene.[67] Infections of the central nervous system are rare, but do occur.[68]

Diagnosis and Treatment

Clinically, clostridial myonecrosis generally begins within 24 to 72 hours after traumatic injury or surgery. Initial symptoms may include severe pain in the absence of obvious physical findings, suggesting a deep tissue infection. When traumatic injuries penetrate the skin, redness at the site of the wound, followed by a rapidly spreading brown to purple discoloration of the skin, is often seen. The progression of gas gangrene is rapid, and within hours of the initial symptoms, edema and gas may be detected within the underlying tissues by physical examination, ultrasound, or radiographic evaluation. Hemorrhagic bullae may occur, along with a serosanguineous discharge and characteristic odor often described as "mousy." The odor is distinct from the putrid odor, due to the production of volatile amines, associated with gram-negative anaerobic infections. Gram staining of the discharge often reveals the typical gram-positive boxcar-shaped rods characteristic of *C. perfringens* (see Fig. 246.1A). Neutrophils are often not seen on Gram stain due to the lethal nature of α-toxin for these polymorphonuclear cells.[69] Gas gangrene leads to profound ischemia resulting from obstructed microvascular circulation caused by platelet aggregation within vessels and fibrin deposition. Unlike most soft tissue infections, in which the inflammatory process increases blood flow, lesions resulting from clostridial myonecrosis do not bleed readily.[70] These proischemic properties are attributed to the α-toxins of *C. perfringens* and *C. septicum,* both of which have similar biologic activity. The θ-toxin of *C. perfringens,* perfringolysin O, may also act synergistically with α-toxin to decrease microvascular perfusion. The toxins can also result in systemic hematologic derangements such as disseminated intravascular coagulation. Fever is often minimal during the early stages of disease; however, progression to full-blown sepsis with its classic hypotension, renal failure, and metabolic acidosis may set in rapidly.

Rapid diagnosis of clostridial myonecrosis, a true medical emergency, is critical for proper therapeutic intervention. Clinical signs such as severe pain at the site of the traumatic injury, tachycardia in the absence of fever and obvious systemic toxicity accompanied by edema, discoloration of the skin, appearance of hemorrhagic bullae, and gas detected within the tissues are typical findings. Gram staining of fluids or exudates showing the typical boxcar-shaped gram-positive rods and few polymorphonuclear cells is often the earliest laboratory findings. Spores are not seen in Gram stains of clinical specimens. Growth of *C. perfringens* on blood agar plates is rapid, with colonies often detected within 12 to 16 hours of inoculation. Colonies are yellowish to gray and opaque, and 4 to 8 mm in diameter with irregular borders, and exhibit a characteristic double zone of hemolysis with an inner zone of β-hemolysis surrounded by an outer zone of partial hemolysis (see Fig. 246.3B). The lecithinase or α-toxin can be detected by growth on egg yolk agar medium, which is rich in triglycerides (see Fig. 246.3A). An obvious white precipitation surrounding colonies is evidence of lecithinase production. Neutralization of this reaction by specific antisera (the Nagler reaction) is presumptive evidence for the identification of *C. perfringens.* Most clinical microbiology laboratories use a combination of fermentation reactions and detection of short-chain fatty acid end products for definitive identification of *Clostridium* spp. Although not widely used at present by clinical microbiology laboratories, rapid PCR methods for identification of *C. perfringens* are available.[71] Methods such as MALDI-TOF mass spectrometry may be used for rapid identification of organisms, including *Clostridium* spp.[12,72–75]

The most important part of therapy for clostridial myonecrosis is emergent surgical débridement of infected tissues. When the abdominal wall is involved, débridement of the affected muscle with generous resection margins of apparently healthy tissue is important to prevent recurrence or progression of infection. For extremities, amputation or extensive débridement is appropriate. Uterine gas gangrene most often requires emergency hysterectomy. Early antibiotic intervention is essential for survival, and penicillin remains the most commonly employed antibiotic.[76] Although there is some concern regarding the continued susceptibility of *C. perfringens* to penicillin, most strains tested are susceptible to easily achieved levels. Other agents, including metronidazole, clindamycin, and the carbapenems, can also be used for treating clostridial myonecrosis. *Clostridium perfringens* remains susceptible to most front-line antimicrobial agents, although resistance to antibiotics

such as clindamycin has been reported.[77] The role of hyperbaric oxygen treatment as adjunctive therapy remains controversial,[76,78] as a result of the nature of the compromised tissue perfusion attributable to clostridial-driven microvascular damage. Some clinicians argue that hyperbaric oxygen treatment makes it easier to identify viable tissue, which decreases the need for more extensive surgical débridement. Other adjunctive therapy includes the use of granulocyte colony-stimulating factor to stimulate hematopoietic proliferation. While some cases of clostridial myonecrosis have been successfully treated with surgical débridement and hyperbaric oxygen alone, inclusion of aggressive antibiotic therapy early in the course of treatment increases survival rates, particularly when combined with aggressive and early surgical débridement, and is considered the best therapeutic intervention.[76]

Clostridium botulinum and Botulism

Botulism refers to a group of organisms that all can produce one or more potent neurotoxins (BoNT) that cause paralysis. *Clostridium botulinum* is the most common species associated with botulism, but strains of other *Clostridium* species, including *C. baratii, C. butyricum,* and *C. argentinense,* have been shown to produce BoNT as well.

Botulism occurs as one of four clinical syndromes: foodborne intoxication, infant botulism, wound infection/intoxication, and intestinal colonization/intoxication in adults. According to the Centers for Disease Control and Prevention, there were 199 reported cases of botulism in the United States in 2015, the latest year for which data are available. Of these cases, 141 were infant botulism, 15 were wound infections, and 39 were food related. The major differentiating characteristic among strains producing BoNT is the serologic group of the neurotoxin produced (Table 246.3). Botulism in humans is caused by serotypes A, B, and E almost exclusively. Toxin serotypes C and D have been associated with disease in domestic and wild animals. Rarely, disease caused by types C, D, and F have been reported in humans. Strains that produce toxin type G are phenotypically different from other *C. botulinum* strains. Strains producing serotype G toxin have therefore been identified as *C. argentinense.* No cases of human disease due to serotype G toxin have been reported to date.

Infant botulism was first recognized in the United States in 1976, but has now been reported throughout the world in countries that have reporting systems for botulism.[79,80] Originally associated with the addition of honey to baby formula, there are now other recognized sources of spores associated with infant botulism.[81,82] The serotype distribution for these cases is similar to the serotypes that can be isolated from the soil of specific geographic areas. In the United States, approximately half of reported cases of infant botulism are from California, one of the states with an aggressive program for recognition and treatment of infant botulism. The disease results from the germination of ingested spores within the GI tract, with vegetative cells rapidly proliferating and producing BoNT, which is then absorbed and distributed via the peripheral circulation. Most cases occur in infants between 3 weeks and 6 months of age; however, cases have been reported to occur in infants a few hours after birth. Symptoms are like those described for adults, but may be harder to discern initially. Often constipation followed by lethargy and difficulty in feeding are the earliest signs of distress. There appears to be a spectrum of disease severity ranging from self-resolving cases that do not require treatment to life-threatening intoxications requiring hospitalization, supportive care, and specific antitoxin therapy. Treatment of infant botulism with human immune globulin that neutralizes BoNT (BabyBIG) has been approved by the FDA.[10]

Wound botulism occurs almost exclusively in users of illicit injectable drugs. Many of the reported cases involve the subcutaneous injection of drugs ("skin popping"), with a subsequent local infection with BoNT-producing clostridia. Absorption of BoNT into the peripheral circulation results in the flaccid paralysis characteristic of botulism.

Foodborne botulism occurs when BoNT is ingested with contaminated food. Historically, foodborne botulism was associated with improperly preserved meat products and canned foods. Today, foodborne botulism is extremely rare in the United States. Most current cases still occur as a result of ingestion of improperly prepared home-canned foods or sausage.

Adult intestinal botulism is similar to infant botulism in that ingested spores of BoNT producing clostridial species germinate and the vegetative cells proliferate and produce toxin that is absorbed by the host, resulting in characteristic symptoms. This particular form of botulism is exceedingly rare, with only an occasional report of affected adults.

Botulism is a paralytic disease process affecting the nerves stimulating skeletal muscle contraction. All BoNT serotypes block the release of acetylcholine at the neuromuscular junction, resulting in blockage of innervation of muscle activity. All serotypes of BoNT are heterodimeric proteins of approximately 150 kDa in size. In the native state, BoNT often is associated with other nontoxic proteins that serve to stabilize the toxin molecule. Once absorbed from the GI tract, the neurotoxin is rapidly distributed via the peripheral circulation. BoNT crosses the plasma membrane by receptor-mediated endocytosis. The light chain (molecular weight, 50 kDa), the catalytic domain of the molecule, is internalized into the nerve cell through a protein channel. BoNT serotype A is a zinc-dependent metalloprotease that blocks the release of acetylcholine by cleaving synaptosomal-associated protein of 25 kDa (SNAP-25), a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein necessary for exocytosis of this neurotransmitter.[10,83]

Botulism classically presents as an acute flaccid paralysis beginning with impairment of the cranial nerves that innervate the eyes, head, face, and pharynx. Paralysis descends in a symmetric fashion to involve the muscles of the thorax and extremities. When ingested, BoNT is often associated with GI symptoms, including abdominal cramps, nausea, vomiting, and diarrhea. In some cases, constipation may precede the descending paralysis. Death may result from paralysis of the tongue and pharyngeal muscles, leading to upper airway compromise, or from respiratory failure secondary to diaphragmatic and intercostal muscle paralysis.

Diagnosis of botulism is most often made by detection of the specific BoNT in food, vomitus, intestinal contents, or other bodily fluids by PCR and EIA or the definitive mouse neutralization assay. Testing is most often performed by public health laboratories rather than clinical microbiology laboratories. Culture of suspect foods, soil, and/or dust samples may also result in the isolation of *C. botulinum* or other BoNT-producing clostridia. Confirmation of culture identification generally requires a toxin neutralization assay, since there are many clostridial species that are phenotypically similar to *C. botulinum* that do not produce toxin (e.g., *C. sporogenes, C. baratii*).

Treatment of botulism requires intensive supportive care, including ventilator-assisted respiration for extended periods of time until the flaccid paralysis resolves. Specific antitoxin can neutralize circulating BoNT, lessening the effects of the toxin if given early, but does not neutralize toxin that is already bound to nerve tissue. Thus paralysis does not immediately subside with antitoxin therapy and continued supportive care is necessary.

TABLE 246.3 Characteristics of *Clostridium* sp. Producing Botulinum Neurotoxin (BoNT)

GROUP	DISEASE	TOXIN TYPES	SACCHAROLYTIC	PROTEOLYTIC	LIPASE
I	Humans, animals	A, B, F	Yes	Yes	Yes
II	Humans, animals	B, E, F	Yes	No	Yes
III	Birds	C, D	Yes	+/–	Yes
IV	Humans, animals	G	No	Yes	No

Clostridium tetani and Tetanus

Tetanus is caused by *C. tetani* and is most often associated with contamination and growth of *C. tetani* within puncture wounds, usually not considered as serious injuries. A potent neurotoxin is responsible for disease in the absence of neutralizing antibodies. In the United States, there are only about 50 cases of tetanus reported to the Centers for Disease Control and Prevention each year, because most people in Western countries are immunized with tetanus toxoid. Tetanus is a disease of nonimmunized people, usually in developing countries where vaccination against the neurotoxin is not widely practiced. The organism is found in the soil and intestinal contents of most animal species. Tetanus intoxication is similar to botulism and is caused by the neurotoxin tetanospasmin (TeNT). Unlike botulism, wherein the toxin does not affect the central nervous system, TeNT traverses the nerve terminal through the nerve body, with axonal transport to the spinal cord and brainstem. TeNT is synthesized as a single inactive polypeptide chain that is cleaved by a protease to produce an active di-chain consisting of a heavy chain (100 kDa) and a light chain (50 kDa) linked by a disulfide bond. The heavy chain facilitates binding to the neuron and the light chain enters the cell through endocytosis. The light chain is a zinc endopeptidase that cleaves the SNARE protein in the same place that BoNT acts, preventing exocytosis of specific neurotransmitters.[10]

The diagnosis of tetanus is usually made by clinical observation, since the organism is rarely cultured from infected sites. Confirmation of prior vaccination makes the diagnosis of tetanus extremely unlikely in vaccinated individuals. Treatment of tetanus generally occurs in intensive care settings with the administration of agents to control spasms, reduce anxiety, and promote muscle relaxation. Because pulmonary complications are common, respiratory support for extended periods of time is often necessary. Although rare in the United States and despite the availability of appropriate supportive care, the mortality from tetanus ranges from 25% to 50%, with pulmonary complications often as the cause of death. Recovery in uncomplicated cases takes 3 to 6 weeks.

Food Poisoning Caused by *Clostridium* Species

Clostridium perfringens type A causes virtually all cases of clostridial food poisoning throughout the world. Contaminated food products that are not properly cooked or stored allow for the proliferation of large numbers of vegetative *C. perfringens* cells. Once consumed, the vegetative cells sporulate within the small intestine, releasing an enterotoxin that causes the characteristic symptoms. The enterotoxin appears to be a cytotoxin in that it causes demonstrable damage to mammalian cells. The enterotoxin is a small polypeptide with a molecular weight of about 35 kDa. The toxin causes fluid accumulation in the rabbit ileal loop assay, provokes vomiting with orogastric challenge in experimental animals, and produces a characteristic cytopathic effect in Vero and other cell lines.[24,84] The *C. perfringens* enterotoxin gene *cpe* can be used to detect disease-causing strains. There is some evidence that the *cpe* gene can be carried on a plasmid and spread from one *C. perfringens* strain to another. More recently, the association between some cases of presumed AAC and *C. perfringens* enterotoxin have been reported.[25]

Clostridium perfringens food poisoning requires the ingestion of at least 10^8 viable enterotoxin-producing cells and occurs most often following the consumption of inadequately cooked and stored food. Meat and meat-containing foods are the most commonly implicated sources. Most cases occur as a common source outbreak, usually involving commercially prepared foods, and on average involve more than 20 affected individuals. Unlike *S. aureus* food poisoning and salmonellosis, home outbreaks appear to be uncommon. However, this may only reflect the fact that individual illnesses are not as likely to be identified as food poisoning when compared to group illnesses involving large numbers of people.

The incubation period for *C. perfringens* food poisoning is short, generally between 7 and 15 hours with a range of 6 to 24 hours. Symptoms include watery diarrhea, abdominal cramping, vomiting, and fever. Cases resolve spontaneously within 24 to 48 hours. When disease is suspected, culture of suspected food or stool from affected individuals is recommended, with quantitative determination of the number of *C. perfringens* present serving as a presumptive diagnostic indicator. If the isolated organisms are present at concentrations exceeding 10^6 cfu/g and the toxin gene is detected, *C. perfringens* type A is presumed to be the cause of the outbreak. Detection of the enterotoxin using a cytopathic toxin assay with neutralization can also be used as a diagnostic test. Some public health laboratories also employ latex agglutination or EIA for detecting the enterotoxin.

OTHER CLOSTRIDIAL INFECTIONS

Bacteremia

Although obligate anaerobes are infrequently isolated from bacteremic patients, clostridia are second only to *Bacteroides* among anaerobes isolated from blood cultures, accounting for approximately 1% of all positive cultures.[85,86] Blood culture data for a large Boston teaching hospital show that, over a 5-year period from 2003 to 2007, over 29,000 positive blood cultures were detected, with over 200 positive for obligate anaerobes (unpublished data). Of the positive cultures, 135 were positive for *Bacteroides fragilis* and 58 were positive for *Clostridium* spp. Of the *Clostridium* species isolated, *C. perfringens* accounted for 42 isolates, *C. septicum* for 12 isolates, and *C. difficile* for 4 isolates. Significant risk factors associated with isolation of clostridia from blood include hemodialysis, intestinal malignancy, and inflammatory bowel disease.[85] Patients undergoing treatments that render them neutropenic are also at increased risk for clostridial bacteremia; *C. perfringens,* followed by *C. septicum,* are the most common species isolated from blood. While isolation of clostridia from blood may reflect either a transient bacteremia due to infection at another site or contamination, isolation of certain species, such as *C. septicum,* may reflect the presence of an underlying intestinal malignancy (Fig. 246.4). If not treated promptly, the toxins produced by clostridia during septicemia can result in severe disease and devastating clinical outcomes.

Abdominal Infections

Infections resulting from contamination of the peritoneal cavity with intestinal contents are most often polymicrobial, and clostridia are often isolated from purulent material accompanying such infections, along with other obligate anaerobes and facultative species. Although a clear role in the infectious process has been established for organisms such as *B. fragilis* and the Enterobacteriaceae, the pathogenic significance of clostridia during these mixed infections is not clear. Gangrenous infections of the abdominal wall caused by *C. perfringens* have been well documented and usually are associated only with the presence of clostridia.

Biliary Tract Infections

Clostridia can be isolated from over 20% of diseased gallbladders and represent contamination of the biliary tract by normal intestinal bacteria. Biliary tract infections may either involve a single clostridial pathogen or reflect a mixed microbiota. *Clostridium perfringens* accounts for 50% of the clostridial species isolated. In some cases, gas may be present in the gallbladder lumen along with purulent material.[1] Gas gangrene of the abdominal wall is a rare complication of gallbladder surgery, resulting in contamination of the peritoneal cavity by the diseased biliary tract tissue. Demonstration of gas within the biliary tract by radiographic methods is an indication for emergent surgical intervention and prompt antibiotic treatment directed at both obligate anaerobes, including clostridia and facultative enteric organisms.

Female Genital Tract Infections

Clostridia can be isolated from the genital tract of approximately 10% of women as part of the normal vaginal microbiota. Clostridia are present in up to 20% of non–sexually transmitted disease genital infections and may be present as part of bacterial vaginosis.[87] Although not common, postpartum and postabortion infections caused by clostridia, particularly *C. perfringens* and *C. sordellii,* can be severe.[88,89] Clostridial uterine infections start as localized chorioamnionitis as a result of infection of the fetus and placental tissues. Often a gaseous vaginal discharge is present. The infection may spread to the uterine wall and endometrial



FIG. 246.4 *Clostridium septicum*. (A) *Clostridium septicum* growth on blood agar. Discrete colonies are not seen, and growth appears as a swarming film on the surface of the agar plate. (B) Gram stain of *C. septicum* demonstrating the gram-variable nature and the pleomorphic appearance of this species.

tissues, and in the most severe cases uterine necrosis accompanied by sepsis ensues.[1]

Pleuropulmonary Infections

Clostridia have been recovered from up to 10% of anaerobic pulmonary infections, with *C. perfringens* accounting for the majority of isolates.[1] Clostridia are rarely isolated as pure cultures from these specimens, suggesting that their presence as part of a mixed microbiota does not necessarily reflect a causative role in the disease process. The most common sources for clostridia in such infections include oral microbiota and aspirated stomach contents. The clinical features of infections from which clostridia can be isolated are similar to those for other obligate anaerobes. On rare occasions, gas within the pleural space can be detected in association with the presence of clostridia.

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The complete reference list is available online at Expert Consult.

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247 *Bacteroides, Prevotella, Porphyromonas,* and *Fusobacterium* Species (and Other Medically Important Anaerobic Gram-Negative Bacilli)

Wendy S. Garrett and Andrew B. Onderdonk

SHORT VIEW SUMMARY

Definition

- *Bacteroides, Porphyromonas, Prevotella,* and *Fusobacterium* spp. account for most infections caused by gram-negative anaerobic rods. *Bilophila* and *Sutterella* spp. also cause human infections, although they are less frequently encountered.

Epidemiology

- These organisms are part of the normal human microbiome and can be isolated from the oral cavity, gastrointestinal tract, and vaginal vault of humans.

Microbiology

- The organisms are obligately anaerobic, gram-negative, non–spore-forming rods. Members of this group can be proteolytic, saccharolytic, or both. Some species produce catalase and superoxide dismutase in low concentrations. Some species have capsular polysaccharides that have been shown to be potent immunomodulators, whereas other species produce abundant proteases that can degrade tissue.

Diagnosis

- Identification in the clinical laboratory includes colony morphology, Gram stain, pigment production visualized in natural light and as fluorescence emission after exposure to ultraviolet light, and numerous biochemical tests (e.g., Vitek). More recently the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry proteomic identification methods have been employed for some species.

Therapy

- Treatment is directed by culture and sensitivity test results (see Table 247.2). In general, β-lactam antibiotics in conjunction with β-lactamase inhibitors show excellent activity against *Bacteroides fragilis* group members and *Prevotella, Porphyromonas, Fusobacterium, Bilophila,* and *Sutterella* spp. First-generation cephalosporins are ineffective against the *B. fragilis* group and *Prevotella* and *Porphyromonas* spp. Clindamycin is a highly effective antibiotic against *Prevotella, Porphyromonas,* and *Fusobacterium* spp.; however, there is significant resistance among the *B. fragilis* group and *Sutterella* spp.

OVERVIEW

The genera *Bacteroides, Porphyromonas, Prevotella,* and *Fusobacterium* account for most infections caused by gram-negative anaerobic rods (GNARs). *Bilophila* and *Sutterella* also cause human infections, although they are less frequently encountered in clinical practice. These obligately anaerobic gram-negative bacteria colonize the oropharynx, gastrointestinal tract, and urogenital tract of humans. Several species from some of these genera are useful symbiotic bacteria, facilitating host metabolism and favorably shaping immune responses. However, many of these microbes act opportunistically, causing infections when they gain access to otherwise sterile tissues. When tissue barriers are breached, GNARs have a predilection for abscess formation, with the most common sites being the oropharynx, abdominal cavity, lungs, and female genital tract. These bacterial species also present clinical challenges because they are often resistant to commonly used antibiotics. A few strains from the *Fusobacterium, Bilophila,* and *Sutterella* genera have been associated with either inflammatory bowel disease or colon cancer, although causative roles remain to be robustly established outside of preclinical models.

HISTORY

The end of the 19th century was a fertile time for the discovery of GNARs. The recognition of GNARs as important pathogens first occurred in animals in 1884 and is attributed to Loeffler. Schmorl is credited with identifying GNARs as opportunists in humans in 1891. Identification of GNARs as members of the human microbiota dates back to 1898, when Halle published his discovery of *Fusobacterium* colonizing the female genital tract. At the same time, GNARs were also isolated by Veillon and Zuber from clinical material from pelvic, appendiceal, and brain abscesses.[1]

The *Bacteroides* spp. as a group were first described in the late 1890s, and for many years *Bacteroides* was a physiologically disparate genus of pleomorphic, obligately anaerobic, gram-negative rods. Until the early 1960s, competing taxonomic systems and confusion regarding nomenclature made it difficult to determine the role of specific members of this group of organisms during infectious processes. Holdeman and colleagues[2] brought some conformity to the phenotypic classification of this group of organisms, which allowed other investigators to identify *Bacteroides fragilis* as a clinically significant abscess-inducing pathogen. With the advent of uniform taxonomic classification and the use of 16S ribosomal RNA phylogenetic-driven taxonomic classifications, several species were reclassified. *Bacteroides* has been further subdivided into two additional genera—*Porphyromonas* and *Prevotella*—both of which primarily colonize the oral cavity. The genus *Fusobacterium* was identified in the early 20th century and is well documented in studies by microbiologists at the Virginia Polytechnic Institute and Pasteur Institute.

MICROBIOLOGY

Bacteroides

Bacteroides is a genus of gram-negative, non–spore-forming, obligately anaerobic, rod-shaped bacteria. More than 30 species of *Bacteroides* have been recognized. The strictest taxonomic definition of *Bacteroides* limits this census to less than a dozen separate species. Within the Bacteroidaceae family, *Bacteroides* organisms are distinctive in their DNA guanine-cytosine composition—40 to 48 mol%. The principal products of their saccharolytic metabolism are acetate, succinate, and

isovalerate. The saturated anteiso-methyl and isomethyl branched-chain fatty acids are used for their long-chain fatty acid–based identification. *Bacteroides* organisms express hexose monophosphate shunt–pentose phosphate pathway enzymes. Their sphingolipid-rich membranes also possess menaquinones, particularly MK-10 and MK-11. *Bacteroides* peptidoglycan contains meso-diaminopimelic acid. Several *Bacteroides* spp. express numerous capsular polysaccharides. These glycoantigens are of interest biologically for their immunomodulatory potential, particularly in the case of *B. fragilis* polysaccharide A.[3]

GNARs are differentiated from one another in the clinical laboratory by use of standard techniques: colony morphology, Gram stain, pigment production visualized in natural light and as fluorescence emission after exposure to ultraviolet light, and numerous biochemical tests. Short-chain fatty acid analysis can also be used for species-level *Bacteroides* discrimination. The *B. fragilis* group is of special medical importance for several reasons. It is often the predominant GNAR in polymicrobial infections, and members of this group have the potential to express β-lactamase. *B. fragilis* group bacteremias are also associated with a high mortality rate of 27%.[4] Consequently, identification of the *B. fragilis* group by the clinical laboratory is often critical for appropriate therapeutic intervention. On blood agar, *B. fragilis* forms circular, entire, white or gray, 2- to 3-mm colonies that are shiny and smooth. The *B. fragilis* group can be pleomorphic on Gram stain, forming straight rods of varying length as well as coccobacilli (Fig. 247.1A). When grown in liquid medium, cells develop bipolar vacuoles and show a characteristic "safety pin" appearance. A useful characteristic of the *B. fragilis* group is bile tolerance compared with other GNARs, enabling its growth on *Bacteroides* bile-esculin agar. In addition, *B. fragilis* is highly resistant to the antibiotics kanamycin, vancomycin, and colistin. The use of a simple disk diffusion assay for these antibiotics is often part of the identification process for GNARs (Table 247.1).

Prevotella and *Porphyromonas*

Both *Prevotella* and *Porphyromonas* were previously considered to be part of the genus *Bacteroides*. These pigmented GNARs can be distinguished from one another metabolically as the saccharolytic *Prevotella* spp. and the asaccharolytic *Porphyromonas* spp. Approximately 20 *Prevotella* spp. have been implicated in causing human disease. *Prevotella* forms circular, convex, 1- to 2-mm, shiny, gray colonies. On Gram stain, they form short gram-negative rods and may assume coccobacilli forms (Fig. 247.1B). *Prevotella* grows well on laked blood agar with kanamycin and vancomycin (LKV) and has variable resistance to colistin. Although *Prevotella* spp. are largely regarded as pigmented GNARs, they can be nonpigmented as well. Pigmented *Prevotella* spp. form brown or black colonies after a week of growth on LKV. Before this brown or black pigment develops, *Prevotella* may fluoresce a dark red on exposure to a Wood lamp (long-wave ultraviolet light). By colony morphology and Gram stain, *Porphyromonas* spp. tend to form smaller colonies and appear as shorter rods or coccobacilli on Gram stain but can be difficult to distinguish from *Prevotella*. *Porphyromonas* usually grows as pigmented colonies, initially forming gray colonies that darken to black colonies within a week after plating on laked blood agar. *Porphyromonas* does not grow on LKV media because of its sensitivity to vancomycin, but it is resistant to colistin.

Fusobacterium

Fusobacterium is a genus of obligately anaerobic filamentous gram-negative rods that are members of the phylum Fusobacteria, in contrast to the *Bacteroides, Prevotella,* and *Porphyromonas* genera, which are members of the phylum Bacteroidetes. On blood agar, *Fusobacterium* forms pinpoint colonies that can be circular or irregular, with some species, such as *Fusobacterium nucleatum,* forming umbonate "fried egg" colonies after 3 to 5 days of incubation. Depending on the strain, they can be hemolytic, and some strains hemagglutinate. *Fusobacterium* spp. can be variable on Gram stain and display a range of cellular morphologies from coccoid, pleomorphic spherules *(Fusobacterium necrophorum)* to rod shaped. Rods can be short with rounded ends or long and thin with pointed ends *(F. nucleatum)*, arrayed end to end (Fig. 247.1C). As a genus, *Fusobacterium* is sensitive to both kanamycin and colistin and resistant to vancomycin. It can be distinguished by its bile sensitivity and metabolism of threonine to propionate. Most species are indole positive and produce butyric acid during the fermentation of glucose.

Symbiosis

The human microbiome is dominated by anaerobes. GNARs colonize the mucosal surfaces of the oropharynx, gastrointestinal tract, and female urogenital tract, and *Bacteroides* and *Prevotella* are among the most abundant genera present. Mutualism and opportunism are important features of the symbiotic relationship between human hosts and colonizing species of the *Bacteroides, Prevotella, Porphyromonas,* and *Fusobacterium* genera.

TABLE 247.1 Techniques and Properties to Differentiate Medically Important Gram-Negative Anaerobic Rods

	ANTIBIOTIC SENSITIVITY (PENCILLIN [P], VANCOMYCIN [V], COLISTIN [C], KANAMYCIN [K])	GROWTH IN 20% BILE	PIGMENT	BRICK RED FLUORESCENCE
Bacteroides fragilis group	$P^RV^RC^RK^R$	Y	N	N
Prevotella	$P^SV^RC^VK^R$	N	Y	V
Porphyromonas	$P^SV^SC^RK^R$	N	Y	V
Fusobacterium	$P^SV^RC^SK^S$	V	N	N

N, No; *R,* resistant; *S,* sensitive; *V,* variable; *Y,* yes.



FIG. 247.1 Gram stains of selected gram-negative anaerobic rods. (A) *Bacteroides fragilis.* (B) *Prevotella intermedia.* (C) *Fusobacterium nucleatum.*

Gastrointestinal Tract

The human colon is colonized by 10 trillion to 100 trillion bacteria, making it the largest repository for bacteria in the body. The *Bacteroides* genus constitutes 30% of the total colonic bacteria. *Bacteroides vulgatus, Bacteroides thetaiotaomicron, B. fragilis,* and *Bacteroides ovatus* are the most commonly encountered *Bacteroides* spp. in the human colon.[5] There is significant variability among humans regarding the colonic colonization, with different *B. fragilis* group members as assessed by stool culture and 16S ribosomal RNA gene amplicon sequencing (Comstock and Onderdonk, unpublished). The colonization of the gastrointestinal tract occurs during descent through the vaginal canal or postnatally for infants delivered by cesarean section. In particular, children born by cesarean section appear to have a delay in reaching their full *Bacteroides* complement.[6] Both breast milk and exposure to environmental factors are important forces in shaping colonization. Many studies over the past several decades have been undertaken to examine this process in detail, with genomic-based technology taking center stage more recently.

SYMBIOSIS AND MUTUALISM IN IMMUNITY AND METABOLISM

There has been an explosion of interest in clinical correlations between microbiota profiles including the presence or absence of specific species and human health and disease. The pioneering work of Björkstén[7] and others examined correlations between infectious diseases and atopic and allergic diseases for many decades. One study suggested that *B. fragilis* colonization at younger than 3 weeks of age increases the risk of asthma later in life.[8] The basic science behind these clinical observations rests in the hypothesis that certain bacterial factors not only instruct immune cell development but also tune immune responsiveness. A large body of work in preclinical models over the past decade supports that the *B. fragilis* zwitterionic polysaccharide A (PSA) reinforces homeostatic immune function both in the gut and systemically despite earlier work that focused on the abscessogenic potential of *B. fragilis*. Investigators have demonstrated that PSA is a symbiosis factor that balances T-cell subset population size and function and may be a beneficial microbe for the treatment of chronic inflammatory diseases such as inflammatory bowel disease and multiple sclerosis.[9]

The immunomodulatory effects of *Bacteroides* spp. are not exclusive to *B. fragilis; B. thetaiotaomicron* also has immunomodulatory properties with the potential to dampen proinflammatory responses to commensal, intestinal bacteria. *B. thetaiotaomicron* targets the RelA subunit of the transcription factor nuclear factor kappa B, a master regulator of proinflammatory immune responses. This process is dependent on a nuclear receptor and transcription factor called *peroxisome proliferator-activated receptor gamma* and is independent of the nuclear export receptor CRM1.[10] However, the mechanism by which *B. thetaiotaomicron* drives this process remains to be fully understood. Strains of *B. thetaiotaomicron* with sulfatase activity have also been shown to contribute to intestinal inflammation in genetically susceptible mice.[11,12] *Bacteroides* and other intestinal commensals also contribute to host immunity through the process of colonization resistance, the concept that the entrenched presence of gut symbionts provides protection from invading pathogens. The most central and well-understood function of *Bacteroides* spp. involves the mutualistic function of metabolism. The human gut at its core is a bioreactor. The saccharolytic *Bacteroides* spp. process diverse dietary and host polysaccharides for their own metabolic needs and in doing so aid in human digestion and nutrient liberation for their human hosts. A well-nuanced understanding of the biochemistry and genomics of glycan foraging by *B. thetaiotaomicron* has emerged over the past several years. In addition, numerous studies support the potential role of these bacteria in human energy balance with implications for the human obesity epidemic.[13]

Female Urogenital Tract

The female urogenital tract, particularly the vagina, is densely colonized by anaerobes. Although gram-positive anaerobic lactobacilli are the predominant colonizers, GNARs may also be present in significant numbers. Vaginal colonization is dynamic, with variations not only intrinsic to the female reproductive life cycle from the premenarchal through postmenopausal years but also with significant shift over a given menstrual cycle.[14] Pregnancy and parturition result in substantial microbiota population shifts in both the urogenital tract and the gastrointestinal tract as well.[15,16]

There is also intraindividual genus-level diversity in colonization with *Bacteroides, Fusobacterium, Prevotella,* and *Porphyromonas* genera. In addition, distinct microbial community differences exist among the labia, the urethral vestibular region, the length of the vagina, and the cervix. Frequent isolates from the vaginal vault include *Bacteroides urealyticus* and members of the *B. fragilis* group; *Prevotella bivia, Prevotella disiens, Prevotella buccalis, Prevotella melaninogenica,* and *Prevotella corporis; Porphyromonas asaccharolytica;* and *F. nucleatum.* Most research on the vaginal microbiota and symbiotic effects has focused on *Lactobacillus,* with the view that GNARs may contribute to vaginal dysbiosis, negative consequences for fetal outcomes, and host mycotic and bacterial infections.

Oropharynx

The oropharynx is a diverse niche for both aerobes and anaerobes. Within the mouth, the teeth, gingival crevices, saliva, and posterior pharyngeal structures provide distinctive milieus with diverse pH and oxygen tensions. Gingival scrapings are particularly dense in bacteria, with estimated concentrations on the order of 10^{11-12} CFU/mL. Similar to the colonization of the lower gastrointestinal tract, colonization of the oropharynx starts at birth. Among the anaerobes, *Lactobacillus* and *Peptostreptococcus* are the earliest colonizers. Fusobacterial populations emerge with the eruption of the first teeth and increase with establishment of full juvenile dentition. Colonization with *Prevotella* and *Porphyromonas* spp. emerges after colonization by *Fusobacterium* spp. Of the GNARs, *Porphyromonas gingivalis* and *P. endodontalis; F. nucleatum; Prevotella intermedia, P. melaninogenica, Prevotella denticola,* and *Prevotella loescheii;* and *Tannerella forsythia* all commonly populate dental plaque. Poor dentition, gingivitis, and other periodontal diseases correlate with increased numbers of GNARs, as do hospitalization, residence in a long-term care facility, and multiple medical comorbidities.

Opportunism

Symbiosis should be viewed as a host-microbe relationship spanning the spectrum from mutualism through opportunism. Most, if not all, symbiotic GNARs have pathogenic potential. *B. fragilis* warrants special consideration because although *B. fragilis* itself may not be the most abundant GNAR cultured from the gastrointestinal tract, it is the most common GNAR identified in clinical isolates from both blood and abscesses.[17] Multiple virulence factors underpin this observation and account for the observed opportunism of *Prevotella, Porphyromonas, Fusobacterium,* and *Bilophila.* Virulence factors produced by these bacteria include capsular polysaccharides, outer membrane proteins, lipopolysaccharide (LPS) endotoxins, attachment factors (fimbriae/pili/adhesins), toxins, and numerous enzymes. Synergism is another important concept that explains the presence of GNARs in many polymicrobial infections. Several facultative anaerobes and aerobes can provide favorable environments that can promote the growth of GNAR. For example, the production of superoxide dismutase by facultative species protects obligate anaerobes that do not produce this enzyme constitutively against the highly lethal superoxide anion. Without the presence of these other microbes, the oxygen tension would not be favorable for GNARs. In many polymicrobial infections, facultative anaerobes and aerobes function mutualistically for GNARs, providing strong synergy for their expansion, especially for *B. fragilis.* In turn, the *B. fragilis* group with its frequent β-lactamase activity can provide a protective environment for normally β-lactam-sensitive facultative anaerobes and aerobes. Such cooperativity can also translate into an interdependency that may explain why antimicrobial therapy that is not effective against every member of a polymicrobial infection is nevertheless able to successfully cure some infections.

Endotoxic Lipopolysaccharide

Endotoxic LPS is a powerful mediator of systemic inflammation and a driver of septic shock. LPS can differ in its endotoxic potential. Because of the structure of lipid A in *Bacteroides,* it lacks C14-2OH fatty acid,

and the LPS does not elicit a strong host inflammatory response. However, LPS immunogenicity of gut microbiome GNARs such as *Bacteroides* spp. may be of considerably more biologic significance than previously appreciated, as levels have been associated with susceptibility to autoimmune diseases including type 1 diabetes in human populations.[18] *Porphyromonas* and *Prevotella* spp. appear to have a similarly attenuated LPS. *P. gingivalis* LPS may be able to antagonize the proinflammatory effects of other LPS in mixed infections, as suggested by in vitro experiments on primary human monocytes. In contrast, *Fusobacterium* LPS elicits a more inflammatory host response because its lipid A contains C14-2OH fatty acid.

Capsular Polysaccharides

B. fragilis produces eight distinct polysaccharides, PSA to PSH, and there is complex phase variation in the expression of these polysaccharides. There is also substantial variability in these polysaccharides across *B. fragilis* strains.[19] Over the past 30 years, the work of Kasper, Onderdonk, Comstock, and Mazmanian has led to a nuanced understanding of the role of these polysaccharides in abscess formation and immunomodulation. The capsular polysaccharide complex facilitates binding of *B. fragilis* to mesothelial cells lining the peritoneum providing a nidus for abscess formation and in the intestinal mucosa helps to reinforce balanced T-cell immune responses. Numerous *Porphyromonas* and *Prevotella* spp. including *Prevotella oralis* and *P. melaninogenica* as well as *Fusobacterium* spp. are also encapsulated and are abscessogenic.

Pili and Fimbriae

In addition to capsular polysaccharide complexes promoting adherence, *Bacteroides* and *Porphyromonas* spp. can adhere to the epithelium via pili. There are great variations in cellular adherence of different *Bacteroides* spp. and strains. Pili are more frequently observed in pathogenic clinical isolates than in fecal samples from healthy subjects.[20] Pili have been observed specifically in *B. fragilis* and *B. ovatus* strains as well as in *P. gingivalis* and *F. necrophorum,* and other *Fusobacterium* spp. express numerous adhesins.

Enzymes and Toxins

Numerous enzymes and toxins facilitate GNAR host mucosa invasion and immune system evasion. *Bacteroides, Prevotella, Porphyromonas,* and *Fusobacterium* spp. can also express numerous extracellular enzymes (collagenase, chondroitin sulfatase, hyaluronidase, neuraminidase, deoxyribonuclease, phosphorylase, and proteases) that can facilitate epithelial barrier breach. Enterotoxigenic strains of *B. fragilis* (enterotoxigenic *B. fragilis* [ETBF]) cause diarrhea in both children and adults.[21] The toxin Btf is a 20-kDa zinc-containing metalloprotease, and there are at least three isoforms. Btf is posited to cleave e-cadherin in the zonula adherens, resulting in intestinal barrier compromise.

Metabolic End Products

Numerous *Bacteroides* and *Prevotella* spp. produce significant amounts of succinate as an end product of metabolism. Several studies have suggested that succinate inhibits polymorphonuclear leukocyte (PMN) phagocytosis. In addition, a succinate-rich environment may inhibit PMN migration and perhaps drive PMN apoptosis.

Host Immune Response

Cellular and noncellular innate and adaptive immune responses play important roles in defending the host from infections from the GNAR. Neutrophils are an important first line of defense. Phagocytic and oxygen radical–generating abilities are not the only defense of the neutrophil against pathogenic GNARs. Neutrophils also produce a number of antimicrobial peptides that are bactericidal for GNARs. GNAR-positive abscesses are often neutrophil rich, and neutrophils can kill *Bacteroides* spp. and other GNARs under anaerobic conditions. Further support for the role of neutrophils in protecting the host from GNAR infections comes from studies of the prevalence of gram-negative bacteremia in neutropenic patients. The complement system is another innate immune mechanism important in the clearance of these infections. Both the classical and the alternative complement pathways opsonize several *Bacteroides* and *Prevotella* spp. Most discussions of dendritic cells and *Bacteroides* spp. have not revolved around host defense but instead have focused on how dendritic cell–internalized glycoantigens can direct T-cell responses. In mouse models, immunization with PSA has been shown to protect from *B. fragilis*–induced abscesses. The protective effects of immunization were shown to be dependent on $CD4^+$ T cells, despite the fact that polysaccharides are regarded as T-cell–independent antigens. Furthermore, recombinant interleukin-2, a potent cytokine for T-cell proliferation, was protective against *B. fragilis* abscess formation in a dose-dependent manner, further substantiating a role for T cells in host-adaptive immunity to *Bacteroides* spp. B-cell deficiency has been shown to be a risk factor in mice for disseminated anaerobic infections, and passive immunity has been shown to be protective in mouse models. In rat models, passive transfer has not been shown to be protective. In general, there is little direct clinical evidence linking B-lymphocyte and T-lymphocyte function to host immunity to GNARs.

INFECTIONS

On primary evaluation few initial signs and symptoms implicate GNARs specifically. The clinical signs and symptoms of GNAR infection depend on the location of the infected site. A careful history with consideration of potential sites of translocation or portal of entry may raise the suspicion for involvement of GNARs (e.g., bacteremia in a patient with an intraabdominal process). Systemic infections such as bacteremia involving GNARs are often polymicrobial. Most of these infections arise endogenously from GNARs already colonizing the mucosal surfaces. Common clinical signs and symptoms associated with anaerobic infections in general include physical proximity to a mucosal site, foul-smelling purulent discharge, tissue necrosis, and palpable or imaged gas in tissues. In the present age of molecular diagnostics, clinicians should remember to engage their senses and in particular their noses in patient evaluations, as anaerobic empyema and peritonitis can often be diagnosed by smell at the bedside quickly and at no cost.

Bacteremia

The mortality of GNAR bacteremia has been reported to range from 15% to 60%, with the upper range of this mortality reflecting untreated infections.[22] In a retrospective, multicenter study from France, anaerobes accounted for 0.5% to 9% of all bacteremias, of which 60% were *Bacteroides* spp. and 22% were *Clostridium* spp. These figures are consistent with prior data over the past several decades.[23] In the United States, the estimated prevalence of GNAR bacteremia over the past 3 decades has fluctuated between 0.5% and 15%. Until more recently, the data suggested a trend toward a decreased incidence. A study from the Mayo Clinic reported that, at this large center at least, cases of anaerobic bacteremia are on the rise, with 53 cases per year reported during the years 1993–96, 75 cases per year reported during the years 1997–2000, and 91 cases per year reported during the years 2001–04.[24] During the period 2008–12 in one Boston hospital, approximately 55,000 blood cultures were performed per year, and approximately 10% were positive for growth; 0.08% of these were positive for GNARs, and 0.05% were *B. fragilis* cases (Onderdonk, unpublished observation). Because of the lack of specific signs and symptoms of GNAR bacteremia, clinicians are highly dependent on the clinical laboratory for diagnosis. At most US hospitals, blood samples from potentially bacteremic patients are routinely collected into culture systems, allowing the evaluation of anaerobes. There should be a low clinical threshold for broad empirical coverage in critically ill patients with histories consistent with GNAR bacteremic sources.

Of all the GNARs, *B. fragilis* is most commonly isolated in bacteremias, and there is variation in mortality rates reported among *B. fragilis* group members. Mortality rates of 24% and 31% have been associated with *B. fragilis,* rates of 50% have been associated with *Parabacteroides distasonis,* and rates of 100% have been associated with *B. thetaiotaomicron.*[25,26] Rapid susceptibility testing and administration of appropriate antibiotic therapy are essential for optimal clinical outcomes, as substantiated by an observational study of *Bacteroides* bacteremia that demonstrated 16% mortality with optimal therapy and 45% mortality without.[27] *Fusobacterium* bacteremias represent less than 1% of all bacteremias and less than 10% of anaerobic bacteremias. Of the *Fusobacterium* spp. causing bacteremia, *F. nucleatum* and *Fusobacterium mortiferum* are

the most common. Subclinical and transient bacteremia with *Prevotella* and *Fusobacterium* spp. has been documented after dental cleaning. Clinically significant bacteremia with *Prevotella* spp. has been reported after both periodontal and obstetric procedures. GNAR bacteremias may occur as secondary infections in the setting of other primary GNAR infections. Intraabdominal infections are the most frequent source of these bacteremias. Frequent intraabdominal processes associated with GNAR bacteremia include abscesses, surgical procedures, intestinal perforation, obstruction, colorectal cancer, and other malignancies. The upper or lower respiratory tract is often the primary site for fusobacterial bacteremia. The female genital tract, oropharynx, skin ulcers, and skeletal and soft tissues should be thoroughly investigated as sources of bacteremia because they are the associated source in 5% to 25% of cases.

Skeletal Infection

Gram-negative anaerobes can infect both joints and bones, as reviewed in depth by Brook.[28] Septic arthritis caused by GNARs generally results from either a hematogenous seeding of the joint space or direct extension of the bacteria via the skin. The large lower extremity joints—hips and knees—are the most common sites, followed by the shoulders and elbows. Surgery, trauma, and multiple medical comorbidities all are risk factors. Most cases are associated with single species rather than a polymicrobial infection, as is often seen with abscesses. In a classic review of both pediatric and adult cases of septic arthritis, Finegold[29] reported on more than 1200 anaerobic joint infections and found that *F. necrophorum* was the most common isolate recovered.[30] *B. fragilis* group bacteria can also cause septic arthritis, and these infections are often secondary and distant from the primary site. Anaerobic osteomyelitis tends to be polymicrobial. *Bacteroides* and *Fusobacterium* spp. are most frequently cultured. Not surprisingly, *Prevotella* and *Porphyromonas* spp. have been recovered from osteomyelitis cases involving bites. Mastoid bone infections often involve these oropharyngeal commensals as well. Patients with sickle cell disease, who are at increased risk for osteomyelitis secondary to sickle-related bony infarction, are the subject of several case reports of GNAR osteomyelitis involving both the axial and the appendicular skeleton.[31] Patients with diabetes and peripheral vascular disease are another risk group for anaerobic osteomyelitis; these infections often develop in the setting of chronic foot ulcers.

Skin and Soft Tissue Infection

GNARs are important pathogens in surgical wound infections, bites, ulcers, and infected pilonidal cysts. Following intraabdominal or gynecologic surgery, wounds can become infected with *Bacteroides* and *Prevotella* spp., resulting in infections of proximal skin and soft tissues. Human, dog, and cat bites can result in GNAR skin infections. *Prevotella* is a common anaerobe that infects dog bite wounds. With routine availability of anaerobic culture and non–culture-based identification methods, anaerobes are increasingly being recovered and detected from infected human and animal bites, especially bites complicated by abscesses. Pilonidal cysts and sacral decubitus ulcers can readily become contaminated by feces and subsequently infected by GNARs. Similarly, cutaneous abscesses, carbuncles, and furuncles in the perineal region can become infected with GNARs. *B. fragilis* is frequently cultured from decubitus ulcers in both elderly and pediatric populations as well as from foot ulcers from patients with peripheral vascular disease or diabetes.

Central Nervous System Infection

The most common central nervous system (CNS) infection is meningitis; however, anaerobic meningitis is extremely rare. In Finegold's historical analysis of anaerobic infections,[29] 298 cases of meningitis were reviewed. However, more than half were without substantiated culture data. In a later review of pediatric populations by Law and Aronoff,[32] 271 cases were reviewed, of which the vast majority—more than 85%—were in the setting of brain abscesses. Anaerobic culture of cerebrospinal fluid is not routinely performed, and given the rarity of these infections, there should be a compelling reason to do so. Of the GNARs reported, *B. fragilis* and *F. necrophorum* are the most commonly isolated. In several of these cases, the upper respiratory tract or intestinal tract was the primary source that resulted in hematogenous spread in patients with medical comorbidities compromising the integrity of the blood-brain barrier. Chronic and acute otitis media have also been implicated in several of these rare cases.

Anaerobic infections of the CNS typically manifest as abscesses and are frequently polymicrobial. When abscesses develop outside of the brain parenchyma and around the dura mater, they are referred to as *subdural empyemas* or *epidural abscesses,* depending on the location. The location of the brain abscess correlates with the source of the infecting organism, often arising in adjacent structures (e.g., frontal lobe abscess and sinus infections, temporal lobe abscess, mastoiditis). A brain abscess stemming from bacteremia in the absence of focal trauma can arise throughout the lobes as a focal or multifocal process. Recent neurosurgery, trauma, and the presence of ventricular shunts are risk factors for GNAR abscesses. The site of primary infection not only informs abscess location but also narrows the spectrum of causative organisms. *Bacteroides, Fusobacterium, Prevotella,* and *Porphyromonas* spp. all have been isolated from brain abscesses.

Infections of the Aerodigestive Tract

Oropharyngeal Infections

GNARs are frequent culprits in infections of the teeth and peridontium. Most odontogenic infections arise in the setting of dental caries. Once opportunists have established themselves in a dental plaque, they can cause local infections or disseminate and seed locoregional sites via extension or distant sites via hematogenous spread. Extension to and infection of the sublingual, submandibular, and perimandibular spaces can result in Ludwig angina, a rapidly progressing infection of the floor of the mouth that without rapid surgical intervention can lead to death by asphyxiation. Although *Actinomyces israelii* is the most commonly identified microorganism, among the GNARs, *Fusobacterium* has been commonly isolated. Tongue piercing, a popular trend, also increases the risk of Ludwig angina.[33,34] Locoregional spread can result in infection of the maxillary sinus, cavernous sinus, or brain parenchyma. Severe facial cellulitis, such as periorbital cellulitis, is another complication of anaerobic dental caries infection. More distal sites of dental infections involving hematogenous spread include endocarditis, mediastinal or pleuropulmonary abscesses, or orthopedic infections.

Poor dental hygiene results in gingivitis, which can lead to more severe periodontal disease. Acute necrotizing ulcerative gingivitis, or Vincent angina, is the most severe manifestation of gingivitis. *F. necrophorum, P. melaninogenica, P. intermedia, F. nucleatum,* and *P. gingivalis* all have been implicated as causative agents in acute necrotizing ulcerative gingivitis as well as odontogenic infections. *B. ureolyticus* and *T. forsythia,* but not the *B. fragilis* group, have also been identified as causative agents in oral infections.

GNARs can result in peritonsillar abscess formation. Both *P. melaninogenica* and *F. necrophorum* are frequent GNAR isolates. A study of patients with sore throats presenting to a university clinic reported detection of *F. necrophorum* in 20.5% of patients and 9.4% of asymptomatic students.[35] An accompanying editorial emphasized the challenge in interpretation of these data.[36] One feared complication of peritonsillar abscesses that involves *F. necrophorum* is Lemierre syndrome—jugular vein septic thrombophlebitis. These septic emboli can seed the lungs and result in multiple systemic abscesses. Although infection begins in the throat, symptoms of pharyngitis are often resolving when symptoms and signs of Lemierre syndrome develop. Tenderness in the anterior cervical triangle is often present. Bacteremia is universally present. Septic emboli may cavitate or cause empyema. Contrast-enhanced computed tomography is useful in showing jugular vein thrombosis. Clinical presentation of sore throat in conjunction with pneumonia, lateral neck pain, or symptoms of septicemia should raise suspicion for Lemierre syndrome in children and adults in good health. Although cases of Lemierre syndrome seemed to diminish with the introduction of antibiotics for streptococcoal pharyngitis starting in the 1940s, after the 1990s the reported incidence started to increase for unclear reasons. Whereas 4 to 6 weeks of appropriate antibiotic coverage is a cornerstone of treatment, the use of anticoagulation has remained controversial with a paucity of evidence-based data to support its use.[37]

GNARs play a role in chronic sinusitis and acute exacerbations of chronic sinusitis but not in acute sinusitis, which in the absence of a

viral etiology is caused by *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis.* The predominant anaerobes recovered in chronic sinus infections are *Prevotella, Porphyromonas, Fusobacterium,* and *Peptostreptococcus spp*. Rare complications of chronic sinusitis include CNS abscesses.

Salivary Gland Infections

Infection of the salivary glands, usually the parotid glands, can result from viral or bacterial pathogens. *Staphylococcus aureus* is the most frequent organism associated with acute suppurative parotitis, and mumps virus can be a cause of acute parotitis. GNARs are the predominant anaerobes implicated in non–*S. aureus* parotitis.[38] *Prevotella, Porphyromonas,* and *Fusobacterium* spp. all have been reported.

Ear Infections

Aerobes, *S. pneumoniae* and *H. influenzae*, are the predominant causative organisms in bacterial acute and chronic otitis media in the pediatric population. Anaerobes have been found in serous effusions and transmeatal biopsy specimens from patients with chronic otitis media and acute exacerbations in the setting of chronic otitis media. In a study by Brook and Finegold,[39] culturing serous effusions from 114 patients with otitis media yielded data from approximately 40% of samples; aerobes predominated over polymicrobial anaerobic and aerobic populations, followed by single anaerobic isolates in 15%. *Prevotella* spp. were the second most commonly cultured anaerobes. Le Monnier and colleagues[40] published a retrospective study of their institution's experience with 25 pediatric acute otitis media cases caused by *F. necrophorum*. Of these patients, 44% had uncomplicated otitis media, 40% had acute mastoiditis, and 16% (4 patients) had Lemierre syndrome. In the classic study of the microbiology of chronic otitis media by Brook and Finegold,[39] the *B. fragilis* group and *P. melaninogenica* were the predominant anaerobes cultured.

Thoracic Infections

Anaerobic infections of the lung parenchyma and pleural space are relatively common. More specifically, these clinical infections include community-acquired and nosocomial pneumonias, lung abscesses, and pleural empyemas. Anaerobes can also result in acute mediastinitis in the setting of severe oropharyngeal infections or perforations in the upper gastrointestinal tract. Poor dentition, gingivitis, chronic obstructive pulmonary disease, cystic fibrosis, and neuromuscular diseases all are medical comorbidities that increase the risk of anaerobic pleuropulmonary infections. Smoking, alcoholism, conditions associated with impaired consciousness, and the inability to clear oral secretions (seizure disorder, dementia, severe cerebrovascular disease) all increase the risk of aspiration, which is a key inciting event in these pneumonias and empyemas. Obtaining good-quality sputum samples, those not contaminated with saliva, can be a clinical challenge that confounds identification of the causative organisms in these pneumonias.

Pleuropulmonary infections linked with aspiration events are commonly polymicrobial with both aerobic and anaerobic isolates. Viridans-group streptococci members are frequently cultured aerobes in these infections. Regarding the GNARs, *Prevotella buccae, P. disiens, P. intermedia,* and *P. melaninogenica; B. urealyticus* and *T. forsythia;* and *F. nucleatum* all have been associated with these infections. Notably, mixed streptococcal and anaerobic pleural infectious processes have a lower associated mortality than staphylococcal, enterobacterial, or polymicrobial aerobic infections.[41] Sputum of patients with cystic fibrosis is a favorable niche for many bacteria in the absence of overt infection. *Prevotella* spp. have been cultured in relatively high numbers from sputum of patients with cystic fibrosis, and *Pseudomonas aeruginosa* colonization correlates with increased anaerobic colonization.[42]

Cardiovascular Infections

Although GNARs are relatively uncommon causes of endocarditis and pericarditis, they are clinically important because of their antibiotic resistance and high associated mortality of 21% to 43%.[43] Of the GNARs, *B. fragilis* group bacteria are the most common causative agent for endocarditis. However, *Fusobacterium* spp., specifically *F. necrophorum,* and other *Bacteroides* spp. have been cultured in endocarditis. Primary infectious sources include the gastrointestinal tract, head and neck, and genitourinary tract, with hematogenous spread to the cardiac valves. Anaerobic endocarditis is similar to aerobic endocarditis in terms of its valvular pattern, male predominance, and risk factors (e.g., intravenous drug use). However, a prior clinical history of heart disease is not as common in GNAR endocarditis, and thromboembolic complications are more common than in aerobic endocarditis. Specifically, *B. fragilis* is associated with large valvular vegetations and subsequent diffuse thrombolic phenomena. *B. fragilis* expression of heparinase and other fibrinolytic enzymes may underpin these findings.[44] In addition to the classic thromboembolic phenomena of endocarditis, temporal lobe and renal emboli and portal vein thrombosis have been observed. *B. fragilis* group and *Fusobacterium* spp. are the GNARs associated with pericarditis. Cardiac surgery, trauma, gastrointestinal fistulas and perforations, and concomitant pleuropulmonary infections all are risk factors.

Intraabdominal Infections

Intraabdominal abscesses can occur after frank perforation stemming from a trauma, surgical procedures of the intestine or biliary tract, or intestinal cancer. Abscesses also form in the setting of inflammatory or infectious processes such as appendicitis, inflammatory bowel disease, diverticulitis, cholecystitis, or pancreatitis. *B. fragilis* is the prototypic anaerobe associated with intraabdominal abscesses. *Escherichia coli* is also a common isolate. The facultative anaerobe *E. coli* and *B. fragilis* can act synergistically, and both are often isolated from intraabdominal abscesses. The host response to the capsular polysaccharides of *B. fragilis* results in abscess formation. Studies in mouse models using intraperitoneal injection of *B. fragilis* have provided valuable insight into how adaptive and innate immune cell subsets as well as mesothelial cells contribute to intraabdominal abscess formation. It remains a scientific curiosity why *B. fragilis,* which makes up less than 0.5% of the intestinal microbiota, is the cause of the vast majority of intraabdominal abscesses.

Peritonitis

Patients with end-stage renal disease on long-term peritoneal dialysis are at increased risk for peritonitis, a cause of great morbidity and mortality in these patients. More than 80% of peritoneal samples in such cases of peritonitis are culture positive, and of these only 2.5% are associated with anaerobic organisms.[45] Peritonitis secondary to trauma such as gunshot wounds or perforation in the setting of intraabdominal surgery or an intraabdominal inflammatory process is more likely to involve a GNAR, especially the *B. fragilis* group. In perforation peritonitis the GNARs most frequently cultured are *B. fragilis, Fusobacterium* spp., *Prevotella* spp., *Porphyromonas* spp., and *Bilophila wadsworthia.*[46] *B. wadsworthia* is frequently isolated in peritoneal inflammation and abscesses arising in the setting of appendiceal perforation or gangrene. In neonates, peritonitis and abscesses most often occur in the setting of necrotizing enterocolitis.

Enteritis

ETBF has been implicated in enteritis, in acute diarrhea in children and adults, and as an inciting agent in inflammatory bowel disease. Diarrhea in these cases is severe, nonhemorrhagic, and accompanied by marked abdominal pain. Observational studies have reported on the constellation of symptoms and prevalence of ETBF in adult and pediatric populations and hospital-acquired diarrhea.[21] Intriguing links are also emerging between ETBF and human inflammatory bowel disease and colorectal cancer.[47]

Urogenital Tract Infections

GNARs play a central role in numerous non–sexually transmitted diseases of the urogenital tract. The bulk of these infections involve the female reproductive organs and include bacterial vaginosis, Bartholin cyst abscess, pelvic inflammatory disease, tubo-ovarian abscess, endometritis, chorioamnionitis, and wound infections secondary to gynecologic or obstetric procedures. Bacterial vaginosis is common with vaginal microbiota blooms of *Gardnerella vaginalis, Bacteroides* spp., *Prevotella* spp., *Mobiluncus* spp., and genital mycoplasmas. The instigating events of bacterial vaginosis—in particular the relative importance of anaerobes versus *G. vaginalis* in disease causation—remain controversial (see

Chapter 108). Bacterial vaginosis is a risk factor for preterm labor; as such, GNAR colonization of the vagina and reproductive structures may pose a risk for adverse pregnancy outcomes. GNARs also play a pathogenic role in acute and chronic prostatitis, prostatic and scrotal abscesses, and scrotal gangrene (Fournier gangrene). In these male urogenital infections, *B. fragilis, Prevotella* spp., and *Porphyromonas* spp. have been isolated, and antibiotic therapy directed at these organisms is part of successful treatment regimens.

RECENT ASSOCIATIONS OF GRAM-NEGATIVE ANAEROBIC RODS AND DISEASE

For several GNARs, metagenomics technology is finding or disproving associations with diseases. Numerous studies now support an association between *F. nucleatum* and colorectal cancer using human colorectal tissue metagenomics and metatranscriptomics data.[48,49] Follow-up studies are yielding insight into the potential mechanisms of causality of *F. nucleatum* in colorectal carcinogenesis.[50–53] In contrast, potential associations between *Sutterella wadsworthensis* and inflammatory bowel disease have not been proven with metagenomics or metatranscriptomics.[54] *B. wadsworthia* has garnered interest in inflammatory bowel disease pathogenesis on the basis of a study investigating links between a high-fat diet and experimental colitis using a mouse model.[55] The explosion of interest in the microbiome and its members is clearly creating a resurgence of research in GNARs.

THERAPY

Although antimicrobials are the mainstay of treatment for GNAR infections, treatment regimens may also involve interventional and surgical approaches as well as adjunctive therapies to facilitate healing and recovery. Selection of empirical antimicrobial therapy for infections involving GNARs is guided by a few general principles. Intraabdominal infections should include coverage for both anaerobes and coliform bacteria; either two-drug regimens or single agents are appropriate (specific antibiotics are discussed later). Urogenital tract infections are usually polymicrobial, involving coliforms, anaerobes, and streptococci, and broad-spectrum monotherapy or two drugs are appropriate. Similarly, skin and soft tissue infections are polymicrobial, involve both aerobes and anaerobes, and require broad coverage. CNS infections, particularly brain abscesses, should be treated with metronidazole, which has good CNS penetration, and a β-lactam–β-lactamase combination or a third-generation cephalosporin for streptococcal coverage. In addition to combating infection, antibiotics have a clear prophylactic role in surgery (see Chapter 318). Especially in the case of colorectal surgery, prophylactic antibiotics improve patient outcomes by reducing postoperative infections.[56] Coverage for both aerobes and anaerobes has led to a significant decrease in surgical wound infections.

Surgical Treatment

Surgical treatment is an essential modality for many GNAR infections. Incision and drainage are usually necessary for treatment of abscesses. Because necrosis is often a feature of infections complicated by GNAR, débridement of necrotic tissues is necessary for resolution of infections. Interventional radiologists frequently use fluoroscopy, ultrasound, or computed tomography guidance to place percutaneous drains that effectively drain abscesses. In many cases, surgical drainage is performed if relatively less invasive measures are unsuccessful. Location, size, and attendant procedural risk all are prominent factors in clinical management of abscesses.

Antibiotic Therapy

Treatment of many GNAR infections is empirical because of the polymicrobial nature of these infections and the time delays associated with anaerobic culture, identification, and susceptibility testing. Antibiotics that provide coverage across the aerobic and anaerobic spectrum should be administered when there is clinical suspicion of mixed infections. Many GNARs are resistant to a number of antimicrobials as a result of extended β-lactamase resistance and metronidazole resistance, making these infections a clinical challenge (Table 247.2).

For decades, penicillin G was the drug of choice for numerous GNAR infections. The *B. fragilis* group is penicillin resistant and, as such, penicillin G is used for GNAR infection outside of the abdominopelvic cavity. However, penicillin treatment failure has emerged secondary to β-lactamase production by certain GNARs. Penicillin resistance has been reported for *P. bivia* and *P. disiens, Porphyromonas* spp., *Bacteroides splanchnicus,* and *B. wadsworthia.* β-Lactamase-producing strains of *F. nucleatum,* both intraoral and extraoral, have been reported, and *F. necrophorum* is often penicillin resistant.[57] Although penicillin G at one time was considered a reasonable choice for minor odontogenic infections, it is not recommended as empirical coverage for severe oropharyngeal or pleuropulmonary GNAR infections because of concerns about resistance.

β-Lactam antibiotics in conjunction with β-lactamase inhibitors (e.g., ticarcillin-clavulanate or piperacillin-tazobactam) show excellent activity against *B. fragilis* group members as well as *Prevotella*, *Porphyromonas, Fusobacterium, Bilophila,* and *Sutterella* spp. The first-generation cephalosporins are not effective against the *B. fragilis* group, *Prevotella* spp., and *Porphyromonas* spp. because these organisms all produce cephalosporinase. Regarding the second-generation cephalosporins, there is resistance among the *B. fragilis* strains to cefoxitin, and other *B. fragilis* group members are resistant to cefotetan and cefmetazole. There is variable resistance to third-generation cephalosporins such as ceftizoxime. *B. fragilis* and *B. wadsworthia* are usually resistant.

The carbapenems are a highly effective class of antibiotics for the GNARs, and several have broad-spectrum activity against both aerobic and anaerobic bacteria. Imipenem, meropenem, ertapenem, and doripenem all are US Food and Drug Administration (FDA)–approved carbapenems. Both doripenem and ertapenem provide excellent empirical coverage for complicated intraabdominal infections. However, resistance is emerging, and nonsusceptibility to carbapenems has been reported for *B. fragilis, Fusobacterium* spp., and *Prevotella* spp. isolates.[58] Although chloramphenicol has excellent in vitro activity against GNARs, its attendant toxicities have limited its use.

Clindamycin is a highly effective antibiotic against *Prevotella, Porphyromonas,* and *Fusobacterium* spp. However, there is significant resistance among the *B. fragilis* group (5%–35%) and *Sutterella* spp. (25%–35%).[59] Therefore although clindamycin's activity against aerobic

TABLE 247.2 Antibiotic Sensitivities of Medically Important Gram-Negative Anaerobic Rods

	BACTEROIDES FRAGILIS GROUP	*PREVOTELLA* SPECIES	*PORPHYROMONAS* SPECIES	*FUSOBACTERIUM* SPECIES
Piperacillin-tazobactam	S	S	S	S
Amoxicillin-clavulanic acid	S	S	S	S
Clindamycin	R (V)	S	S	S
Carbapenem	S	S	S	S
Metronidazole	S	S	S	S
Moxifloxacin	S	S	S	S
Penicillin	R	R	R	S

R, Resistant >30%; *S,* sensitive <5%; *V,* variable >5%, <30% sensitivity.

gram-positive cocci makes it an appealing choice for the treatment of polymicrobial infections, its lack of activity against important anaerobes limits its use as a sole drug in anaerobic infections.

Tigecycline, a glycylcycline, is currently the only FDA-approved member of its class. Tigecycline has outstanding activity against a range of clinical isolates including gram-positive and gram-negative anaerobes and gram-positive aerobes.[60] The broad spectrum of tigecycline makes it attractive for empirical therapy for complicated skin, soft tissue, and intraabdominal infections,[61] although the FDA has issued a warning about excessive mortality associated with its use.

Resistance among GNARs to the quinolone antibiotics varies. Although all GNARs are highly susceptible to moxifloxacin, resistance rates for levofloxacin and ciprofloxacin are as high as 50%.[62]

Metronidazole has been a highly effective agent for all GNARs except *Sutterella* spp. for approximately 50 years. Anaerobes convert this prodrug into its active form, which then inhibits their nucleic acid synthesis. The rates of metronidazole resistance among the GNARs remain low—less than 1%. However, there is concern for emerging resistance. The *nim* resistance genes, of which six have been identified, confer resistance to metronidazole by encoding a reductase that prevents the conversion of metronidazole into its active form. A study of more than 1500 *B. fragilis* clinical isolates from Europe detected *nim* gene expression in 2% of samples, raising concern for evolving resistance.[63] Use of metronidazole as a single agent is limited for many infections involving GNARs (e.g., pleuropulmonary infection) because of the resistance of aerobic or microaerophilic streptococci.

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The complete reference list is available online at Expert Consult.

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248 Anaerobic Cocci and Anaerobic Gram-Positive Nonsporulating Bacilli

Eija Könönen

SHORT VIEW SUMMARY

Microbiology and Epidemiology

- Gram-positive anaerobic cocci (*Anaerococcus, Finegoldia, Parvimonas, Peptoniphilus, Peptostreptococcus*) are frequent findings in clinical specimens with anaerobic growth.
- Clinically relevant anaerobic gram-positive nonsporulating bacilli include members of *Cutibacterium* and *Pseudopropionibacterium* (formerly *Propionibacterium*), *Lactobacillus, Actinotignum* (formerly *Actinobaculum*), *Atopobium,* and *Eggerthella*- and *Eubacterium*-like taxa.
- Typical infectious sites with anaerobic cocci or gram-positive anaerobic nonsporulating rods include the central nervous system, eye, oral cavity, respiratory tract, abdomen and intestine, genital tract, bone and joints, wounds, and blood.

Diagnosis

- Proper specimen collection and transport techniques are essential for the successful recovery of strictly anaerobic bacteria.
- Some automated blood culture systems may adversely affect recoveries of gram-positive anaerobic cocci (*Finegoldia magna, Peptostreptococcus anaerobius*) from blood.
- *Actinotignum schaalii* may be overlooked as a uropathogen in routine (aerobic) urine cultures.
- Use of advanced diagnostic methods such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, in particular, and sequencing of the 16S ribosomal RNA gene, improves considerably the identification of fastidious organisms and reveals their clinical significance.

Clinical Significance

- Anaerobic bacteremia varies markedly by pathogen; if patients have an underlying condition and are not treated for infection with anaerobes, the mortality rate can be high. In this context, bacteremia with *Lactobacillus* and *Eggerthella*-like species is of special concern.
- Among gram-positive anaerobic cocci, *F. magna* has the highest pathogenic potential. It is involved in skin, soft tissue, and joint infections. *F. magna* and *Peptoniphilus harei* are among the principal species in diabetic foot, venous leg, and decubitus ulcers, which are chronic biofilm-associated infections. *Parvimonas micra* is a common finding in brain abscesses, suggesting an oral source. *Peptostreptococcus anaerobius* is isolated from ulcer and skin specimens of the lower extremities and pus specimens of the genitourinary tract.
- Of gram-positive anaerobic nonsporulating rods, cutaneous propionibacteria, especially *Cutibacterium acnes,* are a clinically relevant finding in cases of implantation of foreign bodies and late postoperative infections. High concentrations of *Atopobium vaginae* are typical for bacterial vaginosis. *Eggerthella lenta* recoveries come from blood, abscesses, wounds, obstetric and genitourinary tract infections, and intraabdominal infections.

Treatment

- Among gram-positive anaerobic cocci, the common resistance to clindamycin, in particular, is a global phenomenon. Some strains resistant to penicillin have been reported. Susceptibilities between different genera and species vary; *P. anaerobius* and *F. magna* are of main concern. *Veillonella* species show increasing resistance to clindamycin.
- For anaerobic gram-positive bacilli, the most active antimicrobial agents are the penicillins and carbapenems. Species- and strain-related resistance to glycopeptides among lactobacilli is common, and the use of vancomycin in the treatment of *Lactobacillus* bacteremia is not advisable. Susceptibility rates of *Eggerthella lenta* to penicillin are consistently poor and to amoxicillin-clavulanate, cefoxitin, and metronidazole good. *Cutibacterium, Bifidobacterium,* and *Lactobacillus* are mostly and *Atopobium, Mobiluncus, Eggerthella,* and *Eubacterium* occasionally resistant to metronidazole. Uropathogenic and bacteremic *Actinotignum* strains show high minimal inhibitory concentration values to trimethoprim-sulfamethoxazole and fluoroquinolones.
- Chronic wounds and foreign body and oral infections are typical biofilm-associated infections for which antimicrobial therapy alone is ineffective, and mechanical intervention is needed in their management.

Both anaerobic cocci and anaerobic gram-positive nonsporulating bacilli belong to the commensal microbiota of the digestive tract, and some are members of the microbiota of the urogenital tract and skin. When the environment changes due to trauma, immunosuppression, or antimicrobial therapy, they can cause damage in a susceptible host and result even in life-threatening infections.[1,2] The major genera of anaerobic cocci and gram-positive anaerobic nonsporulating bacilli are listed in Table 248.1. Other anaerobic bacteria are discussed in Chapters 242 through 247.

TAXONOMY

Gram-positive anaerobic cocci (GPAC) belonging to the family Peptostreptococcaceae in the phylum Firmicutes are strictly anaerobic and vary in their cell size according to the species. This heterogeneous group has confronted remarkable taxonomic revisions in recent years. In 2001, a radical revision among the genus *Peptostreptococcus* was made when saccharolytic species and nonsaccharolytic species, which use peptone as their energy source and produce butyrate as a major metabolite of their glucose fermentation, were separated from each other by reclassifying them as *Anaerococcus* and *Peptoniphilus,* respectively.[3] Since then, several novel species with clinical origin have been added especially to the genus *Peptoniphilus.*[1] In addition, many candidate species of *Anaerococcus* and *Peptoniphilus* are suggested based on a culturomics approach, however, they still wait for validation and further information on their clinical significance. Currently, the original genus *Peptostreptococcus* includes two human species (*P. anaerobius* and *P. stomatis*), while the genera *Finegoldia, Parvimonas,* and *Murdochiella* are each represented by a single species (*F. magna, P. micra,* and *M. asaccharolytica,* respectively) so far.[1] In the genus *Peptococcus, P. niger* has remained the only human species. All of the previously mentioned genera harbor clinically relevant species. Within the family Lachnospiraceae, a strictly anaerobic gram-positive diplococcus, *Ruminococcus gnavus,* has gained interest as being a dominant part of the human gut microbiota but also having clinical relevance.

Among gram-negative anaerobic cocci, the genera *Anaeroglobus, Megasphaera, Negativicoccus,* and *Veillonella* belong to the family

TABLE 248.1 Genera of Anaerobic Bacteria Covered in This Chapter

Gram-Positive Anaerobic Cocci
Anaerococcus
Finegoldia
Murdochiella
Parvimonas
Peptococcus
Peptoniphilus
Peptostreptococcus
Ruminococcus
Gram-Negative Anaerobic Cocci
Acidaminococcus
Anaeroglobus
Megasphaera
Negativicoccus
Veillonella
Gram-Positive Anaerobic Nonsporulating Bacilli
Firmicutes
Bulleidia
Eisenbergiella
Eubacterium
Filifactor
Lactobacillus
Mogibacterium
Moryella
Oribacterium
Pseudoramibacter
Solobacterium
Turicibacter
Actinobacteria
Actinotignum (formerly *Actinobaculum*)
Actinomyces (not covered in this chapter)
Alloscardovia
Atopobium
Bifidobacterium
Catabacter
Collinsella
Cutibacterium (formerly *Propionibacterium*)
Eggerthella
Gordonibacter
Mobiluncus
Pseudopropionibacterium (formerly *Propionibacterium*)

Veillonellaceae, while the genus *Acidaminococcus* was moved to the family Acidaminococcaceae, with both families located in the phylum Firmicutes.[4] Within the genus *Veillonella,* six species (*V. atypica, V. denticariosi, V. dispar, V. parvula, V. rogosae,* and *V. tobetsuensis*) are isolated mainly from the human oral cavity,[5,6] whereas *V. montpellierensis*[7] and the novel *V. seminalis*[8] are found at genital sites. The genus *Acidaminococcus* includes two species (*A. fermentans* and *A. intestini*)[9] and the genera *Anaeroglobus* and *Negativicoccus* one species each (*A. geminatus* and *N. succinivorans,* respectively).[4,10] Of the human *Megasphaera* species, *M. micronuciformis* has been isolated from human clinical specimens.[11] Although the pathogenicity of gram-negative anaerobic cocci is generally considered low, they may be of potential importance especially in mixed infections.

Anaerobic gram-positive nonsporulating bacilli are widely distributed among two phyla, Actinobacteria and Firmicutes.[2] Within the family Actinomycetaceae of the phylum Actinobacteria, the genera *Actinomyces, Actinotignum* (formerly *Actinobaculum*), *Trueperella* (formerly *Arcanobacterium*), and *Varibaculum* include facultatively anaerobic, coccoid or branching rods with a positive Gram stain reaction, while *Mobiluncus* species are motile and strictly anaerobic, curved bacilli with variable Gram reactions.[2,12] Recent attempts to revise the heterogeneous genus *Propionibacterium* led to the transfer of cutaneous propionibacteria (*P. acnes, P. avidum,* and *P. granulosum*) to a novel genus, *Cutibacterium.*[13] Furthermore, *Propionibacterium propionicum* was reclassified as being a single species in the novel genus *Pseudopropionibacterium.* These species are anaerobic and aerotolerant, variable-shaped organisms of which *Cutibacterium acnes,* in particular, can be encountered in clinical material.[2,14] A novel *Propionibacterium, P. namnetense,* which is closely related to *C. acnes,* has clinical relevance.[15] Members of the family Bifidobacteriaceae are strictly anaerobic or occasionally microaerophilic, pleomorphic rods. Human *Bifidobacterium* species reside in the intestine in particular, while two former *Bifidobacterium* species, *Scardovia inopinata* and *Parascardovia denticolens,* and the novel *Scardovia wiggsiae* are mainly isolated from oral sites.[2,16] The closely related *Alloscardovia omnicolens* has been found in various types of human infectious material.[17] The genera *Atopobium* and *Olsenella,* with variable, often coccoid cells, have been created from the so-called anaerobic lactobacilli belonging to the family Coriobacteriaceae.[2] *Atopobium* species with clinical relevance in humans include *A. minutum, A. rimae, A. parvulum,* and *A. vaginae.* In addition, the novel *A. deltae* strain was found as an etiologic agent of sepsis.[18] The genus *Olsenella* includes two human species, *O. uli* and *O. profusa,* isolated from the oral cavity.[2] Although most *Eubacterium* species belong to the Firmicutes, a number of former *Eubacterium* species with a high guanine-cytosine content have been renamed and removed to the phylum Actinobacteria. Among those are *Eggerthella lenta* and *Collinsella aerofaciens* from the gut and *Slackia exigua* from the oral cavity.[2] *Eggerthella sinensis, Paraeggerthella* (formerly *Eggerthella*) *hongkongensis, Gordonibacter pamelaeae,* and the motile and catalase-producing *Catabacter hongkongensis,* in particular, have been found in blood.[19–21]

The other major phylum of gram-positive anaerobic nonsporulating bacilli is the Firmicutes. Within this phylum, the genus *Lactobacillus* constitutes a branch subdivided into several groups, whereas some former *Lactobacillus* species were reclassified and moved to other genera (e.g., *Atopobium* and *Olsenella*).[2] *Eubacterium* and *Eubacterium*-like species are widely distributed among the Firmicutes. Clinically important, mainly in oral infections, are *Eubacterium brachy, E. infirmum, E. minutum, E. nodatum, E. saphenum, E. sulci, Filifactor alocis, Mogibacterium* species, *Pseudoramibacter alactolyticus, Bulleidia extructa,* and *Solobacterium moorei.*[22] Other anaerobic gram-positive nonsporulating bacilli within the Firmicutes with potential clinical relevance are *Turicibacter sanguinis, Oribacterium sinus,* and *Moryella indoligenes,* as well as the novel, gram-variable *Eisenbergiella tayi.*[2,23,24]

Members of the Commensal Microbiota

Among gram-negative anaerobic cocci, *Veillonella* species are considered mainly harmless, or even beneficial, colonizers of the mouth from the early years of life onward.[25] *Acidaminococcus* and *Megasphaera* species reside the human intestine at young age; however, geographic or ethnic differences, or both, in their abundance are significant.[26]

Among GPAC organisms, *R. gnavus* plays an important role as an early colonizer of the human gut.[27] Although members of the genera *Anaerococcus, Finegoldia, Parvimonas, Peptoniphilus,* and *Peptostreptococcus* are commonly present as part of the human microbiota, residing the mouth, intestine, and both the female and male genital tract as well as the surfaces of the eye, nasal cavity, and skin,[1,28–31] only limited knowledge exists about their role as commensal organisms.

Anaerobic gram-positive nonsporulating bacilli belong to the commensal microbiota of the digestive tract, and some members are part of the microbiota of the urogenital tract and skin. The latter environment is widely colonized by cutaneous propionibacteria, *C.* (formerly *P.*) *acnes,* in particular.[14] *Bifidobacterium* and *Collinsella* species are important in the development of the gut microbiota.[26,32] In elderly persons, a decrease in the levels of intestinal *Bifidobacterium* populations is seen with aging.[33] Lactobacilli, such as *Lactobacillus rhamnosus, L. paracasei, L. plantarum,* and *L. salivarius,* form an important component on mucosal surfaces of the gastrointestinal tract.[26,34] The most prevalent lactobacilli in health-associated vaginal biofilms are *L. crispatus, L. gasseri,* and *L. jensenii,* while *L. iners* can also be found in intermediate and dysbiotic states of the vaginal microbiota.[35] Hydrogen peroxide production by these species, except for *L. iners,* may help to control the overgrowth of bacterial vaginosis–associated organisms and to protect from obstetric infections during pregnancy.[36] With variable results, beneficial *Lactobacillus* and *Bifidobacterium* strains have been used as probiotics, aiming to protect the homeostasis or to alter the dysbiotic composition of the microbiota.[37,38]

Clinical Significance

In infectious lesions, the organisms covered in this chapter often appear together with other anaerobic or facultative bacteria in polymicrobial consortia typical at or close to their natural anatomic site. Hematogenous spread of anaerobes from the commensal microbiota and local infections along the local veins and through the general circulation expose the host to systemic infections, such as brain abscesses and endocarditis.[39,40] The clinical significance of anaerobic bacteremia varies markedly by pathogen; if patients have an underlying condition and are not treated with drugs active against anaerobes and drainage when appropriate, the mortality rate can be high.[41–47] According to data from the Mayo Clinic (Rochester, MN), the increased prevalence of anaerobic bacteremia was especially shown for "peptostreptococci", and their increasing resistance to metronidazole was of concern.[41] However, in a population-based monitoring of anaerobic bloodstream infections in the Province of Alberta, Canada, no increase in resistance rates of anaerobes was observed from 2000 to 2008.[45] Moreover, a decrease in the incidence of anaerobic bacteremia was reported from a Belgian university hospital over a 10-year period between the years 2004 and 2013.[46] Besides bacteremia, typical infectious sites where anaerobic cocci or gram-positive anaerobic nonsporulating rods can be involved include the central nervous system, eye, oral cavity, respiratory tract, abdomen and intestine, genital tract, and wounds.[1,2]

GRAM-POSITIVE ANAEROBIC COCCI

With the incorporation of advanced methods into the clinical microbiology arsenal, the impact of GPAC (formerly known as peptostreptococci) as causative agents in a variety of human infections has increased considerably. For example, in anaerobic bacteremias, GPAC organisms are among the most frequent findings,[45,46] and their importance in pathogenic biofilms of chronic ulcers has been emphasized. The most common GPAC species isolated from various types of clinical specimens is *Finegoldia magna,* but *Parvimonas micra, Peptoniphilus harei, Peptostreptococcus anaerobius,* and *Anaerococcus vaginalis* are not rare.[1,39,48]

Anaerococcus

Typical infectious sites for the genus *Anaerococcus* include the female genital tract; *A. hydrogenalis, A. lactolyticus, A. prevotii, A. tetradius,* and *A. vaginalis* have been isolated from vaginal discharges and ovarian abscesses, while *A. octavius* recoveries come from the skin, nasal cavity, and vagina.[3] *A. prevotii, A. vaginalis,* and *A. lactolyticus* are also among the prevalent findings in chronic wound samples.[49–51] In addition, the involvement of *A. prevotii* in mixed surgical infections and skin infections[52,53] and endodontic infections with abscesses[54] has been reported. *A. murdochii* has been recovered from polymicrobial infections, mainly in the lower extremities but also in the neck and a sternal wound.[55,56] Recently, two novel *Anaerococcus* species with clinical relevance have been suggested: *A. degenerii* (four strains isolated from toe tissue, from drainage fluid after rectal carcinoma surgery, from a pelvic abscess, and from an intraabdominal abscess after rectal carcinoma surgery)[57] and *A. nagyae* (three strains isolated from an infectious lesion of the groin, from a nephrourecterectomy wound infection, and from blood).[58]

Finegoldia

Among GPAC organisms, *F. magna* has the highest pathogenic potential and, subsequently, it is found in a variety of human infections.[1,28,48,52] This high pathogenicity is explained by its specific virulence properties.[1,59] Although GPAC species usually appear in polymicrobial infections, *F. magna* can be identified as the only infectious finding.[28,60] Most typically, *F. magna* is involved in wound, soft tissue, and bone and joint infections.[28] In addition, there are reports on its involvement in endocarditis, breast abscesses, pleural empyema, mediastinitis, chronic balanitis, and bacterial vaginosis.[1,61] *F. magna* is one of the principal species in diabetic foot, venous leg, and decubitus ulcers, which are chronic, polymicrobial biofilm-associated skin and skin structure infections.[1,49–51] According to a report of 61 bone and joint infections with anaerobic organisms,[56] *F. magna* was present in 21% of the cases, which all were polymicrobial and were located in the lower extremities, especially in the ankle. There is increasing evidence for *F. magna* as a causative agent in orthopedic implant–associated infections.[56,60,62]

Parvimonas

P. micra is the far most prevalent GPAC species in the mouth, where it is considered a putative pathogen within subgingival biofilms connected to severe periodontitis,[63] infected root canals with or without abscesses,[54,64] failing dental implants,[65] and odontogenic infections.[66] Strikingly, in a Norwegian study, most brain abscesses proved to be infected by *P. micra* or other *Parvimonas* sp.,[39] suggesting an oral origin. Some recoveries come from bacteremias[46] and bone and joint infections.[56,67] Furthermore, *P. micra* has been shown, for the first time, to be capable of causing bacterial meningitis.[68] This organism is also found among the bacterial vaginosis–related microbiota exposing to chronic genital inflammation.[69]

Peptoniphilus

In clinical specimens with GPAC organisms, *P. asaccharolyticus* appears as a frequent finding[28,52]; however, some doubt has been raised about the correct identification of the species.[55,70,71] Instead, a biochemically similar species, *P. harei,* seems to cover a considerable proportion of clinically relevant GPAC findings.[46,48,55,70] *P. asaccharolyticus/P. harei* was frequently detected among 61 bone and joint infections.[56] *P. harei* is one of the primary findings in biofilms of venous leg ulcers, and *P. harei, P. ivorii,* and *P. indolicus* in pressure ulcers.[1,51] *P. lacrimalis* has been isolated from a lacrimal gland abscess[3] and osteoarticular and vaginal specimens[1] but is also detected as a rather common finding in decubitus ulcer samples.[50] Novel *Peptoniphilus* species have been isolated from polymicrobial human infections below the waistline, including *P. coxii* (leg infections, back cyst, flank abscess, endometrial fluid, and tonsil biopsy), *P. duerdenii* (vaginal abscess), *P. gorbachii* (lower extremities), *P. koenoeneniae* (buttock abscess), *P. olsenii* (lower extremities), *P. rhiniditis* (chronic rhinosinusitis), and *P. tyrrelliae* (leg infection, ischial wound, and perirectal abscesses).[72–75]

Peptostreptococcus

P. anaerobius has long been considered an important human pathogen. A similar species, *P. stomatis,* was not described until 2006[76]; therefore the term *P. anaerobius sensu lato* is used when citing earlier literature. There are reports on its involvement in various types of infections all over the body, including the head, respiratory tract, gastrointestinal tract, genitourinary tract, skin and soft tissue, bone and joints, and cardiovascular sites.[28] *P. anaerobius sensu lato* is frequently isolated from abscesses of various anatomic sites, mixed surgical infections, diabetic wounds, and cutaneous wounds caused by dog or cat bites.[28,49,52,77] In the original description of *P. stomatis,* it was considered an oral species, whereas *P. anaerobius* was suggested to harbor sites below the waistline.[76] Both *P. anaerobius* and *P. stomatis* (oral clone CK035) have been detected in infected root canal specimens and bronchoalveolar lavage samples from patients with ventilator-associated pneumonia.[54,78] In a study of 64 *P. anaerobius sensu lato* strains, *P. stomatis* strains originated not only from oropharyngeal but also gastrointestinal specimens, whereas *P. anaerobius* strains originated mainly from ulcer and skin specimens of the lower extremities and pus specimens of the genitourinary tract.[79]

Murdochiella

M. asaccharolytica represents this novel genus as a single species, which has been isolated from an abdominal abscess and a pilonidal cyst aspirate in humans.[80]

Ruminococcus

Some case reports are currently available on *R. gnavus* in human infections.[81–84]

GRAM-NEGATIVE ANAEROBIC COCCI

Acidaminococcus

A. intestini has been isolated from mixed cultures of specimens of the gastrointestinal area in particular, such as abdominal and peritoneal fluids, inguinal and anal abscesses, and the rectum, but also from maxillary necrosis, axillary abscess, and pressure ulcers.[9]

Anaeroglobus

Few reports are available on *A. geminatus.* According to its original description, the characterized strain was recovered from a postoperative

fluid sample collected after gastrectomy.[10] Oral *A. geminatus* recoveries come from persistent root canal infections[85] and subgingival samples of periodontitis patients, especially those suffering from rheumatoid arthritis.[86] Other infectious lesions with *A. geminatus* include pneumonia with empyema and brain abscess.[39,87]

Megasphaera

M. micronuciformis has been isolated from a liver abscess and pus from a fingertip, as part of mixed growth.[4] Other *Megasphaera* recoveries, though less characterized, are from bacterial vaginosis.[69,88]

Negativicoccus

The only species of this genus, *N. succinivorans,* has been isolated from skin and soft tissue specimens in mixed cultures,[4] and from a bacteremia case with an intestinal source.[89]

Veillonella

Although *Veillonella* species are rarely seen as causative infectious agents, they can be involved even in severe infections, such as anaerobic bacteremia and endocarditis, identified as *V. atypica, V. parvula/V. alcalescens, V. dispar,* and *V. montpelliernsis.*[45,46,90] In addition, *V. parvula* has been detected in meningitis and diskitis with secondary bacteremia,[91] osteomyelitis,[92] and prosthetic knee infections,[93] while *V. montpelliernsis* has been isolated from gastric fluid of a newborn and amniotic fluid samples[7]. The novel *V. seminalis* has been recovered from clinical semen samples, Bartholin gland and perianal abscesses, and a groin wound.[8]

GRAM-POSITIVE ANAEROBIC NONSPORULATING RODS

Propionibacteria

Cutibacterium (*Propionibacterium*) *acnes* is currently recognized as an important pathogen in bone and joint infections, especially in joints with implants.[14,56,94] In a study dealing with 276 patients, the shoulder had a propensity for developing *C. acnes* arthritis compared to lower limbs.[94] This may make sense, because the chest area has a high content of sebaceous glands where cutaneous propionibacteria dominate. Postoperative spondylodiskitis (spinal osteomyelitis) with an involvement of *C. acnes* differs from that caused by aerobic bacteria; it mainly appears in middle-aged subjects, it is rarely complicated by epidural abscess, and there is usually a long incubation period between the preceding invasive operation and infection.[95] Moreover, in the case of anaerobic spondylodiskitis, blood cultures often remained negative. *C. acnes* has been detected in connection to intervertebral disk degeneration,[14,96] and it was possible to demonstrate the high prevalence of *C. acnes* biofilms in the disk tissue.[96] Of the cutaneous propionibacteria, *Cutibacterium* (*Propionibacterium*) *avidum* has also caused attention as a potential pathogen; recent reports have connected this organism to prosthetic hip joint infections[97,98] and soft tissue infections[99–101] after surgical procedures at close proximity to a deep skin crease. Infectious cases with *Cutibacterium* (*Propionibacterium*) *granulosum* have been reported in infective endocarditis in a native mitral valve[102] and in a prosthetic hip.[103] The novel *Propionibacterium namnetense,* which is closely related to *C. acnes,* has been isolated from infected bone (tibia) and surrounding tissue.[15] A few cases of severe skull base osteomyelitis with *C. acnes* as a single isolate have been reported recently.[104] Implantation of medical devices such as intraocular lenses (see Chapter 115), spinal and ventriculoperitoneal shunts (see Chapter 92), and prosthetic heart valves exposes the recipient to infections by cutaneous propionibacteria.[14] Indeed, most endocarditis cases caused by *C. acnes* involve prosthetic cardiac devices in middle-aged men.[105] There is also a risk for further destruction, such as aortic root abscess.[106] Typically, *C. acnes* is isolated from sebaceous follicles, causing lesions of acne vulgaris, where the susceptibility of the host seems to be a dominant factor.[107] Specimens from central nervous system infections, endodontic infections, and infected dog and cat bite wounds can harbor propionibacteria, usually *C. acnes* or, in few cases, *C. granulosum.*[64,77,108] In addition, *Propionibacterium acidifaciens* has been detected in cariotic teeth and persistent endodontic infections.[64,109] *Pseudopropionibacterium* (*Propionibacterium*) *propionicum* resembles phenotypically *Actinomyces israelii,* and is involved in actinomycotic lesions and abscesses, infections of the eye, and endodontic infections.[12]

Lactobacilli

Lactobacillus species not only are beneficial organisms but can act as causative agents in serious infections, especially in immunocompromised individuals with prolonged hospitalization.[40,44,45,110–112] Among the clinically most relevant species are *L. rhamnosus, L. salivarius, L. paracasei, L. casei, L. fermentum,* and *L. acidophilus.* Intraabdominal infections form the main focus of lactobacilli.[44] Their presence in blood specimens is of medical importance because bacteremia and endocarditis with involvement of lactobacilli carry a relatively high mortality rate.[40,44,45,111] *L. rhamnosus,* which is typically vancomycin resistant, is among the most frequent *Lactobacillus* findings in blood.[40,110,111,113] A report from Taiwan, however, found *L. salivarius* as the dominant species in bacteremias caused by lactobacilli.[44] Whether probiotic *Lactobacillus* strains could be of harm has been speculated.[112,114] In a recent study,[115] it was shown that the probiotic strain *L. rhamnosus* GG differs from clinical blood isolates, of which some were able to form biofilms or to induce platelet aggregation. Lactobacilli are among major recoveries from advanced dental caries lesions,[113] and, interestingly, a dental procedure or condition was suggested as a potential predisposing factor in half of the cases of *Lactobacillus* endocarditis.[40] In addition to bacteremia and endocarditis, lactobacilli can be found in infections such as meningitis, empyema, cholecystitis, peritonitis, pyelonephritis, and prosthetic knee infection.[113] Different from other vaginal *Lactobacillus* species, *L. iners* has been observed to dominate in dysbiotic vaginal communities that are susceptible to bacterial vaginosis.[35]

Bifidobacteria

Except for the involvement of *Bifidobacterium* sp. in dental caries, they are generally considered nonpathogenic, and some strains (e.g., *B. breve, B. longum*) are used as probiotics. In the current literature, however, there is an increasing awareness of the potential pathogenicity of bifidobacteria. In pediatric and adult bacteremic cases, with or without preceding probiotic use, the recognized species were *B. infantis, B. adolescentis, B. breve, B. longum,* and *B. dentium* (in the older literature named as *B. eriksonii*).[116] Several types of infections caused by bifidobacteria have been reported.[117] Occasional infectious findings include also *B. scardovii.*[118] In the closely related genus *Alloscardovia, A. omnicolens* was named referring to its presence everywhere in the human body.[17] Clinical *A. omnicolens* isolates originate from blood, urine, genital, pulmonary, oral, and abscess specimens.[17,119] In a study in which 15 putative bifidobacterial isolates from sterile sites or those present in significant numbers were identified to the species level, four species were identified: *A. omnicolens, B. breve, B. longum,* and *B. scardovii* from the gastrointestinal or genitourinary tract, and *B. breve* from blood.[120] *Scardovia wiggsiae,* a novel species, has been linked to severe early childhood caries.[121]

Atopobium and *Olsenella*

A. vaginae is involved in infections of the female genital tract. High concentrations of *A. vaginae* are typical for bacterial vaginosis and, thus, a diagnostically valuable marker of this dysbiotic state.[88] Moreover, *A. vaginae* has been reported from uterine endometritis and bacteremia cases connected to invasive infections of the female genital tract.[122] *A. minutum* has been isolated from various infections of the lower part of the body, *A. parvulum* from respiratory specimens and endodontic infections, and *A. rimae* from severe odontogenic infections.[22,123] *Olsenella* species, especially *O. uli,* have been detected in various oral infections.[54,123] In addition, *A. rimae* and *O. uli* have been reported as causative organisms in clinically significant bacteremia.[110,124]

Actinomyces-Like Organisms

The genus *Actinomyces* is discussed in the chapter on actinomycosis (see Chapter 254). Of the three species of the genus *Actinotignum* (formerly *Actinobaculum*), *A. schaalii* has emerged as an important uropathogen in the elderly, but pediatric cases have also been reported.[12] Cystitis, pyelonephritis, and urosepsis are typical infections caused by

A. schaalii. Most cases of urosepsis have been patients with a predisposing condition, such as renal stones and lithotripsy or other instrumentation. In addition, *A. schaalii* has been found in blood and abscess specimens as well as those from cellulitis, spondylodiskitis, bladder necrosis, epididymitis, and endocarditis.[12,125–127] *Actinotignum* bacteremia patients are typically older males, and a relatively high mortality (16%) has been reported.[127] All strains of the two *Varibaculum* species, *V. cambriense* and the novel *V. anthropi,* have been isolated from clinical specimens.[12,128] Typically, they were involved in abscesses at various body sites, but also in infections of the female genital tract.

Mobiluncus

Of the two *Mobiluncus* species, *M. curtisii* appears to be more virulent than *M. mulieris. M. curtisii* has been connected to bacterial vaginosis, in which its presence is seen as vibrio-like organisms in smears of vaginal fluid and the treatment failure as persistence of the organism.[88] In addition, *M. curtisii* has been occasionally isolated from endometrial smears and pus specimens of the female genital tract.[129] Some reports on extragenital infections and bacteremias with *Mobiluncus* species have been presented.[130]

Eggerthella and Related Species

This group of closely related species is known to possess particular pathogenic potential, causing severe invasive infections. Of these, *Eggerthella lenta* is most common and has been isolated especially from mono- and polymicrobial bacteremias, often with a significant mortality rate.[43,131,132] Other clinically relevant isolations come from abscesses, wounds, spondylodiskitis, osteomyelitis, obstetric and genitourinary tract infections, and intraabdominal infections.[133–135] A retrospective analysis of 33 *E. lenta* bacteremias (January 2000–September 2013) was performed in a large health care network in Australia.[132] A variety of comorbidities at intraabdominal sites, in particular, were found as a background factor, underlining the importance of abdominal diagnostics when *E. lenta* is identified in blood. This was supported by an analysis from a Swedish tertiary hospital with 18 *E. lenta* bloodstream infections.[136] It was recently shown that *E. lenta* can be found in blood of oncologic patients with advanced disease but without infection, indicating translocation of this gut bacterium due to damage of the intestinal barrier.[137] In a tertiary medical center in Taiwan, all "*Eubacterium*"-associated bacteremias in the years 2001–2010 were analyzed[43]; of the 16 patients found, 7 harbored *E. lenta* and 3 *Paraeggerthella hongkongensis;* furthermore, 9 had polymicrobial bacteremia in which *Bacteroides* species were the most common concomitant findings. The gastrointestinal and female genital tracts were suggested as the main entries of these bacteria to blood.[43] In a hospital in Detroit, 25 bacteremia cases with *E. lenta* from 1999 to 2010 were retrospectively reviewed; half of the bacteremias were monobacterial, and the main sources were abdominal and complicated soft tissue infections.[131] Also, *Catabacter hongkongensis* and *Gordonibacter pamelaeae* have been found in blood samples in pure culture, and bacteremia had mainly a gastrointestinal source.[42,138,139] An oral species, *Slackia exigua,* is found in odontogenic and human bite infections,[22,140] but can seemingly be translocated to the pulmonary area; it was reported to be the causative organism of a severe empyema in a patient with multiple cariotic lesions in her teeth.[141] Other extraoral infections with *S. exigua* include bacteremia, and abscesses and wounds at various sites of the body.[46,142]

Eubacterium and Related Taxa

Various *Eubacterium* sp. (e.g., *E. brachy, E. nodatum, E. saburreum,* and *E. sulci*) and related species (e.g., *Filifactor alocis, Mogibacterium timidum, Mogibacterium vescum,* and *Pseudoramibacter alactolyticus*) residing the oral cavity have been recovered from periodontal, endodontic, and odontogenic infectious lesions.[2,22,54] Conceivably, organisms of this group are among the anaerobic recoveries from infected human bite wounds.[140] In a Norwegian multicenter study, *E. brachy* was among the main findings in 37 spontaneous brain abscesses, and *F. alocis* also was found in two cases.[39] A recent report presented a case initially suspected as thoracic actinomycosis due to its chronicity but where *E. brachy* proved to be the causative organism.[143] In addition to being isolated from infections in the upper part of the body, *E. nodatum* has been found in infections of the female genital tract.[144] *E. limosum, E. callanderi,* and *E. tenue* have been detected in clinically significant bacteremias.[43,110] Also, few bacteremia cases of *Solobacterium moorei* have been reported in connection to various comorbidities.[145] Some isolates of *S. moorei* come from wound infections together with other bacteria, especially anaerobes.[146] *Bulleidia extructa* has been found in odontogenic infections, a brain abscess, and a periprosthetic hip joint infection.[22,39,147] Among other clinically relevant Firmicutes taxa, *Turicibacter sanguinis* has been isolated from human blood, *Oribacterium sinus* from pus of a human sinus, *Moryella indoligenes* from abscesses below the waistline, and *Eisenbergiella tayi* from blood and appendix tissue.[2,23,24,148]

MICROBIOLOGIC ASPECTS

Proper specimen collection and transport techniques are essential for the successful recovery of strictly anaerobic bacteria. Avoidance of contamination by commensals of the surrounding skin, mucous membranes, and nonsterile secretions is crucial; therefore mucosal or cutaneous swabs are not appropriate for specimen collection, although they are still used.[149] Instead, samples collected by tissue biopsy, wound curettage, or aspiration should be used as specimens. Transport media should be specifically designed for the survival of anaerobic bacteria.

It is likely that the involvement of anaerobic bacteria in infections is underestimated because many of these organisms are slow-growing and fastidious. Noteworthy is that the automated blood culture system may affect some GPAC recoveries from blood.[1] For instance, *Peptostreptococcus* species are sensitive to sodium polyanethol sulfonate,[79] which is a common component in automated blood culture sets. Also, the detection of *Finegoldia magna* may be affected by some commonly used blood culture systems.[1] Another concern is the potential misinterpretation of isolated organisms such as cutaneous propionibacteria from blood specimens or *Actinotignum* from urine as insignificant contaminants.[105,126]

In the laboratory, clinical specimens should be processed without delay using rich, nonselective media and prolonged incubation (up to 10–14 days).[2] In implant-associated infections, sonication of the removed implant (or part of it) and multiple conventional cultures are recommended.[14] In mixed cultures, many anaerobic organisms can be easily overgrown by facultatives, leading to biased estimates of their true numbers and clinical significance. Currently, *Actinotignum schaalii* is considered a significant uropathogen[12] but it is often missed in routine (aerobic) urine cultures; therefore advanced methods are required for blood isolates from urosepsis. Notably, for some taxa within the phylum Actinobacteria, polymerase chain reaction–based methods with standard primers may result in less successful detection than anaerobic culture. Accurate identification of anaerobic cocci and *Eubacterium*-like organisms, in particular, is limited if only phenotypic tests are used.[22,55] Diagnostic methods such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), in particular, and sequencing of the 16S ribosomal RNA gene are increasingly used for identification in clinical microbiology laboratories. With these methods, clinically relevant but fastidious organisms (e.g., *Eggerthella lenta* or *Actinotignum schaalii*) can be reliably identified.[126,132,136] However, refined technical procedures as well as comprehensive databases are needed for an excellent performance of MALDI-TOF MS for gram-positive anaerobes; for instance, *Bifidobacterium longum, Finegoldia magna,* and *Peptoniphilus ivorii* have been shown to require specific extraction steps in MALDI-TOF MS,[150] while an expanded number of main spectral profiles has improved the correct identification of GPAC organisms.[151] Adoption of culture-independent methods, together with an increased awareness regarding the infectious role of anaerobes, on one hand, will help to reveal the clinical relevance of fastidious organisms covered in this chapter. On the other hand, the more precise identification brings a challenge to clinicians to recognize a variety of bacterial names given for findings in clinical specimens.

TREATMENT

Initial diagnosis of infection and choice of antimicrobial therapy is often based on empirical information, while awaiting laboratory test results. Susceptibility profiles can significantly differ between countries or even between institutions; therefore it would be of benefit to conduct periodically local susceptibility surveys for clinically relevant anaerobes

to guide the therapy. Unfortunately, hospital laboratories seldom perform susceptibility testing for anaerobic isolates and, if performed, very few antimicrobials are tested.[149]

Tables 248.2 and 248.3 present susceptibilities of major gram-positive anaerobic cocci and bacilli to a number of antimicrobials. Among GPAC organisms, the common resistance to clindamycin, in particular, is a global phenomenon.[152–157] In addition, some GPAC strains resistant to penicillin have been reported.[48,153,154] Susceptibilities between different genera or even closely related species vary; for instance, *Peptostreptococcus anaerobius* constantly shows higher minimal inhibitory concentration (MIC) values when compared to those of *Peptostreptococcus stomatis*.[79] Also the susceptibility of *Finegoldia magna* as the most common GPAC recovery from infectious material is of special concern.[1,59,153] Because metronidazole is the drug of choice in many anaerobic infections, metronidazole-resistant strains can impair the treatment outcome. In a multicenter survey from the United States, 98% and 96% of GPAC organisms were susceptible to metronidazole according to the Clinical and Laboratory Standards Institute breakpoints (MIC ≤8 μg/mL), in the years 2007–2009 and 2010–2012, respectively.[158] In contrast, nearly 30% of the 14 strains identified as GPAC were interpreted as resistant to metronidazole (MIC >4 μg/mL) using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint in a Croatian report.[159] A high prevalence of nitroimidazole resistance genes has been found among GPAC species, especially within *P. anaerobius* isolates, and two highly resistant strains of *F. magna* were also detected.[160] Quinolone resistance of *F. magna* is common globally, and two *F. magna* strains resistant to chloramphenicol have been reported in Korea.[59] Among gram-negative cocci, high resistance rates to penicillin have been reported for *Veillonella* species.[152,156,158] Between the periods of 2007–2009 and 2010–2012 reported from the United States, clindamycin resistance increased from 7% to 34%, respectively, among *Veillonella*.[158]

In general, the antimicrobial agents most active against anaerobic gram-positive bacilli are the penicillins and carbapenems. It is notable that species- and strain-related resistance to glycopeptides is frequent among the genus *Lactobacillus*.[40,44,113] Therefore the use of vancomycin for the treatment of bacteremia or infections with involvement of lactobacilli is not advisable. Also cephalosporins are not consistently effective in the treatment of *Lactobacillus* bacteremia. Bifidobacteria and the closely related *Alloscardovia omnicolens,* a rare pathogen from various clinical specimens, are susceptible to β-lactams.[119,152,161] According to data on uropathogenic and bacteremic *Actinotignum* strains, MIC values to β-lactams and vancomycin are low but those to trimethoprim-sulfamethoxazole and fluoroquinolones, especially ciprofloxacin, are high.[125,127] Susceptibility rates of *Eggerthella lenta* to penicillin were consistently poor and those to amoxicillin-clavulanate, cefoxitin, and metronidazole good, while other resistance rates varied considerably between studies—for example, those for piperacillin-tazobactam ranged from 0 to 83%, and for clindamycin from 0 to 18%.[132,136,152] Although clindamycin has good bone penetration, its use in bone- and joint-related infections caused by *Cutibacterium acnes* may be of concern due to its increasing resistance.[14] Nearly complete resistance of *C. acnes* to metronidazole has been shown to be independent of geography,[14,152,156–158] and metronidazole-resistant strains are common also among other facultatively anaerobic/microaerophilic organisms such as *Bifidobacterium* and *Lactobacillus*.[113,152,161]

Diverse bacteriology of intraabdominal infections recalls antimicrobial therapy that covers both aerobic and anaerobic bacteria, such as cephalosporin or fluoroquinolone combined with metronidazole or monotherapy with carbapenem or tigecycline.[162] Some novel drugs, among those tigecycline and linezolid, exhibit potential activities against anaerobic bacteria; however, concern has been raised regarding the efficacy and safety issues of tigecycline therapy.[163] When anaerobic bacteria are involved in biofilm infections, as is the case typically with oral infections, various chronic wounds, and foreign bodies, antimicrobial therapy alone is ineffective despite in vitro susceptibility of the potential causative organism(s) to antibiotics.[164] Due to biofilm structure, mechanical intervention (drainage, surgery) or removal of foreign bodies is needed in the management of these infections.

TABLE 248.2 Susceptibilities of Anaerobic Cocci to Antimicrobials

	ANAEROCOCCUS	*FINEGOLDIA*	*PEPTONIPHILUS*	*PEPTOSTREPTOCOCCUS*	*PARVIMONAS*	*VEILLONELLA*
Penicillin	≤0.002–1	0.016–1	0.016–4	0.016–16	0.016–0.25	≤0.002–1
Amoxicillin	ND	<0.016–0.5	<0.016–0.125	ND	<0.016–2	0.023–2
Amoxicillin-clavulanate	0.016–0.5	0.016–0.5	0.016–0.25	0.016–8	0.016–0.125	<0.016–16
Piperacillin-tazobactam	≤0.015–0.125	0.015–0.25	ND	ND	≤0.015–0.015	≤0.015–1
Cefoxitin	≤0.015–0.5	0.06–1	ND	ND	≤0.015–2	0.12–8
Imipenem	0.002–0.125	0.002–0.25	0.002–0.125	0.002–1	0.002–0.125	0.004–2
Meropenem	ND	0.03–0.12	ND	ND	0.004–0.12	0.015–2
Clindamycin	≤0.015–>256	0.016–>256	0.016–>256	0.016–256	≤0.015–>256	≤0.015–256
Metronidazole	0.016–0.5	0.016–4	0.016–2	0.016–1	≤0.015–1	<0.016–4

ND, No data available.
[a]Range of minimal inhibitory concentrations (MICs;mg/L) of tested strains to indicated antimicrobial agents.[152,155,156]

TABLE 248.3 Susceptibilities of Gram-Positive Anaerobic Bacilli to Antimicrobials

	ACTINOTIGNUM	*ALLOSCARDOVIA*	*BIFIDOBACTERIUM*	*COLLINSELLA*	*EGGERTHELLA*	*EUBACTERIUM*	*LACTOBACILLUS* (VANCOMYCIN-RESISTANT)	*LACTOBACILLUS* (VANCOMYCIN-SUSCEPTIBLE)	*PSEUDOPROPIONIBACTERIUM*
Penicillin	0.004–0.5	≤0.01–0.12	0.015–2	0.002–8	0.004–4	0.003–2	0.125–>32	≤0.06–0.5	≤0.004–2
Amoxicillin-clavulanate	ND	0.06–0.5	ND	ND	≤0.06–2	ND	0.12–>32	0.25–0.5	ND
Piperacillin-tazobactam	ND	0.12–0.5	≤0.015–0.5	0.015–0.125	≤0.03–64	≤0.015–0.03	0.12–>64	0.25–4	0.015–2
Cefoxitin	≤0.015–1	ND	0.12–>256	ND	0.15–16	0.06–>256	16–>128	8–>128	0.015–4
Ertapenem	ND	ND	ND	ND	0.06–2	ND	0.5–>16	0.5–8	≤0.06–0.75
Imipenem	ND	≤0.01–0.12	ND	ND	ND	ND	≤0.015–16	0.03–1	≤0.03–2
Meropenem	0.004–0.06	ND	0.015–2	≤0.002–0.12	≤0.06–4	0.007–0.12	ND	ND	0.06–1.5
Vancomycin	ND	0.12–1	ND	ND	0.25–8	ND	4–>128	0.5–2	0.016–2
Daptomycin	ND	≤0.01–16	ND	ND	ND	ND	0.125–4	0.5–>32	0.125–2
Linezolid	ND	0.12–1	ND	ND	ND	ND	1–8	1–>32	0.016–2
Tigecycline	ND	≤0.01–0.06	ND	ND	≤0.06–1	ND	ND	ND	0.016–0.064
Moxifloxacin	ND	0.06–4	ND	ND	0.03–>32	ND	ND	ND	0.125–0.5
Levofloxacin	ND	0.25–>32	ND	ND	ND	ND	0.25–>32	0.5–>32	0.125–≥256
Clindamycin	≤0.015–>256	≤0.01–>256	≤0.015–8	<0.015–64	≤0.015–>256	≤0.012–32	≤0.03–0.5	≤0.03–4	≤0.016–>256
Metronidazole	0.06–>256	≥32	0.12–>256	0.03–>256	≤0.016–>64	≤0.015–0.5	ND	ND	0.06–>256

ND, No data available.
[a]Range of minimal inhibitory concentrations (MICs;mg/L) of tested strains to indicated antimicrobial agents. [14,44,113,119,132,152]

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The complete reference list is available online at Expert Consult.

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249 *Mycobacterium tuberculosis*

Daniel W. Fitzgerald, Timothy R. Sterling, and David W. Haas

SHORT VIEW SUMMARY

Microbiology

- Humans are the only reservoir for *Mycobacterium tuberculosis.*
- The organism is an acid-fast, aerobic bacillus with a high cell wall content of high-molecular-weight lipids.
- Incomplete necrosis produces cheesy, acellular material (i.e., caseous necrosis).
- Pulmonary cavities contain huge numbers of organisms.

Epidemiology

- *M. tuberculosis* infects one-quarter of the world's population, with 10.4 million new cases reported each year.
- Almost all infections are due to inhalation of droplet nuclei.
- Key determinants of infection are closeness of contact and infectiousness of the source.
- The strongest risk factor for progression to active tuberculosis (TB) is acquired immunodeficiency syndrome (AIDS).
- The highest case rates are in countries heavily affected by human immunodeficiency virus (HIV)/AIDS.
- Drug resistance can be primary or secondary.
- Multidrug-resistant tuberculosis (MDR-TB) indicates resistance to both isoniazid (INH) and rifampin (RIF).
- Extensively drug-resistant tuberculosis (XDR-TB), which indicates resistance to INH, RIF, a fluoroquinolone, and a second-line injectable drug, is of immense concern.

Immunology and Pathogenesis

- Infection requires a cellular immune response.
- Airborne droplet nuclei reach the terminal air spaces, where they are ingested by alveolar macrophages and then carried by lymphatics to regional lymph nodes.
- Occult preallergic lymphohematogenous dissemination occurs to the lung apices and elsewhere.
- Granulomas form when antigen load is small and tissue hypersensitivity is high.
- Age influences likelihood and pattern of disease.
- TB in advanced AIDS is characterized by middle or lower lung field location, absence of cavitation, increased extrapulmonary disease, and a negative test for *M. tuberculosis* infection (e.g., tuberculin skin test or interferon-γ release assay).

Tuberculin Skin Testing and Interferon-γ Release Assays

- To detect *M. tuberculosis* infection, a positive tuberculin test is defined by induration. Interpretation depends on the diameter of induration.
- Interferon-γ release assays may be used instead of skin testing. They are more specific for *M. tuberculosis* than tuberculin skin testing. Results are positive, negative, or indeterminate. T-Spot results can also be borderline.
- Testing for *M. tuberculosis* latent infection is recommended for persons at high risk for developing TB if untreated.
- Test results (particularly the skin test) are negative in at least 20% of active TB cases.

Diagnosis

- Culture is the gold standard.
- Visible growth takes 3 to 8 weeks on solid media, and a mean of 10 days for smear-positive and 20 days for smear-negative specimens on liquid media.
- An estimated 10,000 organisms per milliliter are required for sputum smear positivity. Nucleic acid amplification testing (NAAT; e.g., Xpert MTB/RIF and Xpert MTB/RIF Ultra) have sensitivities and specificities that approach those of culture and can be completed in 1 day.
- The use of three sputum specimens increases sensitivity.
- A radiograph showing a patchy or nodular infiltrate in the lung apices is highly suggestive, especially if the infiltrate is cavitary.
- Pulmonary TB can occur in persons with normal chest radiographs, particularly in HIV-positive persons with advanced immunosuppression.

Therapy (see Tables 249.7 and 249.8)

- Multidrug regimens, typically starting with at least four active drugs, are recommended for initial treatment.
- At least 6 months of therapy is usually required.
- INH is the cornerstone of therapy; RIF, the second major antituberculous agent, causes many drug-drug interactions; 2 months of pyrazinamide is essential for 6-month regimens.
- Adjustment of doses of antiretroviral agents during treatment of TB with RIF and rifabutin is shown in Chapter 39, Tables 39.3 and 39.5. The time to initiate antiretroviral therapy during TB treatment is shown in Chapter 39, Table 39.2.
- Directly observed therapy is crucial.
- Susceptibility testing is important to guide therapy. NAAT and DNA sequencing are offering more rapid approaches to susceptibility testing.
- With appropriate chemotherapy, patients usually become noninfectious within 2 weeks.
- Treatment of XDR-TB is usually associated with poor outcomes.
- Bedaquiline is an approved drug for MDR-TB.

Therapy for Latent Infection

- Short-course regimens are replacing 9 months of INH for latent infection, particularly in low–TB incidence settings.
- Twelve weeks of directly observed, once-weekly INH plus rifapentine, and 4 months of daily rifampin, are as effective as 9 months of INH.

Prevention

- Case finding and treatment comprise the most effective method of control of TB.
- Hospitalized HIV-positive patients with respiratory symptoms should be admitted to negative-pressure isolation rooms. N95 masks are used for health care workers.
- Childhood bacillus Calmette-Guérin vaccination in high-burden countries decreases incident TB. Other vaccines are in clinical trial.

The term *tuberculosis* describes a broad range of clinical illnesses caused by *Mycobacterium tuberculosis* (or, less commonly, *Mycobacterium bovis*). Tuberculosis (TB) is the leading cause of death due to a single infectious agent worldwide, and ranks ninth among all causes of death. In 2014 the World Health Assembly embraced a resolution to reduce deaths from TB by 95% by the year 2035. In 2016 there were an estimated 10.4 million new cases of TB and 1.7 million deaths from TB worldwide.[1] TB can affect virtually every organ, most importantly the lungs, and is typically associated with granuloma formation.

HISTORY

There is evidence of spinal TB in Neolithic, pre-Columbian, and early Egyptian remains. However, TB did not become a major problem until the Industrial Revolution, when crowded living conditions favored its spread. In the 17th and 18th centuries, TB caused one-fourth of all adult deaths in Europe. Before antimicrobial agents became available, the cornerstone of treatment was rest in the open air in specialized sanatoria. Sanatorium regimens probably benefited some patients who were diagnosed before cavitation but had little impact on cavitary disease. When it became clear that cavitation was the pivotal event in progressive pulmonary TB, most special therapies focused on cavity closure.

The modern era of TB began in 1946 with demonstration of the efficacy of streptomycin (STM). In 1952, the availability of isoniazid (INH) made TB curable in most patients, and the addition of rifampin (RIF) in 1970 allowed for even more effective combination therapy. With drug coverage, it became possible to successfully resect tuberculous tissue, but with drug treatment, resection was rarely necessary. Bed rest and collapse therapy added nothing to chemotherapy; treated patients rapidly became noninfectious; and specialized sanatoria ultimately disappeared. The duration of chemotherapy progressively decreased from approximately 2 years before the availability of RIF, to 9 months with INH plus RIF, and to 6 months with multidrug therapy including INH, RIF, and pyrazinamide (PZA). With INH it also became practical to treat asymptomatic people believed to harbor tubercle bacilli based on positive tuberculin test results.

In the United States, reported cases of TB had declined nearly every year since accurate statistics became available. However, in 1985, case rates began increasing, driven largely by human immunodeficiency virus (HIV) infection. TB-control programs in some large cities were not equipped to manage this emerging problem. The often-interrelated factors of illicit drug use, homelessness, and HIV infection predispose to reactivation of remote TB, to the acquisition and spread of new disease, and, because of irregular adherence to drug therapy, to the development and spread of drug-resistant strains. Epidemics involving strains that were resistant to at least INH and RIF (i.e., multidrug-resistant [MDR] strains) emerged in these populations and spread to HIV-negative persons, including health care workers. Many outbreaks were caused by the Beijing strain, with "strain W" dominating in New York City. Estimates based on data from 2006 through 2008 suggest that 80% of cases in the United States are due to reactivation.[2]

Treatment programs failed because of drug resistance, medication nonadherence, and nosocomial transmission of *M. tuberculosis*. Since 1992, however, TB incidence rates in the United States have progressively declined and in 2016 reached the lowest in history.[3] This attests to the success that can be achieved with intensified diagnostic, treatment, and prevention efforts, and with control of HIV-induced immunocompromise by means of antiretroviral therapy (ART).

The global situation has, unfortunately, not been as successful. The HIV pandemic fueled increased TB case rates in resource-limited countries worldwide, especially in sub-Saharan Africa. Scant resources and fragile infrastructure, together with a high prevalence of HIV infection and acquired immunodeficiency syndrome (AIDS), have driven the global burden of TB. As in the United States, multidrug-resistant tuberculosis (MDR-TB) emerged and spread. In response, the World Health Organization (WHO) has worked diligently to expand TB treatment services, including directly observed therapy, short-course (DOTS) programs. In parallel, second-line medications, including fluoroquinolones, were made increasingly available worldwide. Since 2011, WHO has recommended that MDR-TB be treated using mainly ambulatory care, which appears to be more effective than hospitalization.[4]

With widespread use of second-line agents, selection for *M. tuberculosis* resistant to both first- and second-line drugs was inevitable. Extensively drug-resistant tuberculosis (XDR-TB), defined as resistance to at least INH, RIF, a fluoroquinolone, and a second-line injectable drug (kanamycin, capreomycin, amikacin), first occurred as early as 2001 in KwaZulu-Natal, South Africa,[5,6] and by 2016 it had been reported in at least 121 countries worldwide.[1] Although application of established TB public health principles, supported by ample funding, may ultimately control this dire situation,[7] the immensity of this challenge is daunting. There are added challenges in reaching vulnerable populations, including those who experience inequality, prejudice, marginalization, and limits on their social, economic, cultural, and other rights.[8]

MICROBIOLOGY

The *M. tuberculosis* complex comprises at least nine species in the genus *Mycobacterium,* family Mycobacteriaceae, and order Actinomycetales that are causes of human TB and zoonotic disease. The *M. tuberculosis* complex species share 99.9% sequence identity and likely evolved from a single clonal ancestor.[9] The species *M. tuberculosis* sensu stricto causes the vast majority of human TB worldwide. *Mycobacterium africanum* causes human TB in West Africa, where it accounts for up to 50% of cases.[10] *Mycobacterium canettii* is an extremely rare cause of human TB in the Horn of Eastern Africa. *M. bovis* causes disease in cattle and spreads to humans through animal contact and consumption of unpasteurized milk. An investigation of six TB cases in the United Kingdom demonstrated that *M. bovis* can be transmitted by aerosol from patients with pulmonary lesions.[11] *Mycobacterium caprae,* another cattle pathogen, *Mycobacterium microti,* a pathogen for rodents, and *Mycobacterium pinnipedii,* a pathogen for seals, have been reported to cause zoonotic TB in humans. *Mycobacterium orygis* (antelope) and *Mycobacterium mungi* (mongoose) have been described in animals but have not been reported in humans.

Advances in genetic analysis, including whole-genome sequencing (WGS), have shed new light on the phylogenetics of the *M. tuberculosis* complex.[12,13] These studies show that *M. tuberculosis* sensu stricto and *M. africanum,* the predominant causes of human disease, can be further divided into seven phylogenetic lineages, L1 to L7. This is a rapidly changing field of study, with multiple nomenclatures in use. For example, the L2 lineage is also called the East Asian lineage or the Beijing strain. There is some evidence that different lineages vary in virulence, host adaptation, transmissibility, or ability to acquire drug resistance, but further research is needed to clarify the clinical importance of such differences.

Humans are the only reservoir for the species *M. tuberculosis,* although many animals are susceptible to infection.[14] Some have postulated that an ancient ancestor of *M. tuberculosis* infected hominids in East Africa 3 million years ago and has since coevolved with its human host.[15] *M. tuberculosis* is an aerobic, non–spore-forming, nonmotile bacillus with a high cell wall content of high-molecular-weight lipids. Growth is slow, the generation time being 15 to 20 hours, compared with much less than 1 hour for most common bacterial pathogens, and visible growth takes from 3 to 8 weeks on solid media. The organism tends to grow in parallel groups, producing the colony characteristic of serpentine cording. In radical contrast to other bacteria, a very large portion of *M. tuberculosis* genes encode enzymes involved in lipogenesis and lipolysis.

A wide spectrum of laboratory techniques has been developed to diagnose active TB. No single test is perfect, and, unfortunately, some diagnostics on which clinicians still rely were developed over 100 years ago. Advantages and limitations of various methods are presented in Table 249.1.

Acid-Fast Staining

The term *acid-fast bacilli* (AFB) is practically synonymous with mycobacteria, although *Nocardia* and some other organisms are variably acid fast. In the Ziehl-Neelsen stain, a fixed smear covered with carbol-fuchsin is heated, rinsed, decolorized with acid-alcohol, and counterstained with methylene blue. The Kinyoun stain is modified to make heating unnecessary. The organisms appear as slightly bent, beaded rods 2 to 4 μm long and 0.2 to 5 μm wide. In sputum they often lie parallel, or

TABLE 249.1 Comparison of Assays Used in the Diagnosis of Active Tuberculosis

CLINICAL LABORATORY QUESTION	DIAGNOSTIC ASSAY	ADVANTAGES	LIMITATIONS
Are mycobacteria present in a clinical specimen?	Culture on solid media (Löwenstein-Jensen egg-based or Middlebrook agar-based media)	Gold standard for isolating *Mycobacterium tuberculosis*; detects 10–100 organisms/mL; shows colony morphology, detects mixed infection, allows quantification of growth; provides organisms for speciation, strain identification, susceptibility testing	Visible growth takes 3–8 wk
	Culture in liquid broth	Sensitivity and specificity similar to solid media; automated systems decrease workload; provides organisms for speciation, strain identification, and susceptibility testing; turn positive in a mean of 10 days for smear-positive and 20 days for smear-negative specimens	Does not show colony morphology, detect mixed cultures, or quantify growth
	Acid-fast stain (Ziehl-Neelsen, Kinyoun, auramine rhodamine)	Same-day results; simple technology; inexpensive light microscope; fluorescence microscope required for auramine-rhodamine stain	Less sensitive than culture, requiring 10,000 organisms/mL; cannot distinguish *M. tuberculosis* from other mycobacteria
	Nucleic acid amplification (e.g., PCR)	Same-day results; sensitivity intermediate between acid-fast stain and culture; identifies organisms as members of *M. tuberculosis* complex	Requires advanced laboratory techniques; cannot distinguish dead from viable organisms; culture still needed for speciation, strain identification, and susceptibility testing
	Nucleic acid amplification with GeneXpert MTB/RIF and Xpert MTB/RIF Ultra	Sensitivity of Xpert MTB/RIF roughly 100 CFUs/mL, requires 100 min, detects rifampin resistance mutations with sensitivity of 95% and specificity of 98% Xpert MTB/RIF Ultra is more sensitive than Xpert MTB/RIF but with slightly less specificity	Expensive equipment; detects dead bacilli; culture needed for other susceptibility testing
	Urinary antigen detection (e.g., ELISA for LAM)	Point-of-care testing; relatively high sensitivity in advanced untreated AIDS (<50 CD4 T cells/mm^3)	Sensitivity very low in situations other than advanced AIDS
Is a mycobacterium isolated from a clinical specimen a member of *M. tuberculosis* complex? (*M. tuberculosis, Mycobacterium bovis, M. bovis*–BCG, *Mycobacterium africanum, Mycobacterium microti,* or *Mycobacterium canettii*)	Nucleic acid amplification	(See above)	(See above)
	Nucleic acid probes	Results available in 2 h; sensitivity and specificity approach 100%; does not require amplification	Requires at least 10^5 organisms; most useful for pure culture, not directly on clinical specimen; cannot distinguish among members of *M. tuberculosis* complex
	BACTEC *p*-nitroacetyl-aminohydroxypropiophenone assay (NAP)	Provides preliminary identification of *M. tuberculosis* complex (NAP susceptible)	Need for paired cultures increases cost (growth with and without NAP)
	High-performance liquid chromatography (HPLC)	Same-day results; sensitivity and specificity approach 100%; can distinguish *M. bovis* BCG from other members of *M. tuberculosis* complex	Requires HPLC technology; only useful with pure culture
To which species of *M. tuberculosis* complex does a clinical isolate belong?	Colony morphology and biochemical assays (e.g., niacin test, heat-sensitive catalase, nitrate reduction, pyrazinamide monodrug resistance)	Classic approach for speciation of *M. tuberculosis*	Time-consuming and labor-intensive
	PCR genomic analysis	May rapidly distinguish among *M. tuberculosis* complex species	Not yet commercially available for this purpose
Do different *M. tuberculosis* isolates represent the same strain?	Genotyping by restriction fragment length polymorphism analysis, spoligotyping, mycobacterial interspersed repetitive unit analysis, and whole-genome sequencing	The CDC offers free strain typing through the National Tuberculosis Genotyping and Surveillance Network	Sophisticated assay available only at specialized centers
Is an *M. tuberculosis* isolate drug resistant?	Agar proportion method	Quantifies the proportion of organisms resistant to a drug	Requires as long as 8 wk
	Liquid BACTEC method	Results within 5–14 days	Does not quantify proportion of resistance
	Molecular line probe assays and whole-genome sequencing for chromosomal mutations associated with drug resistance (see above for GeneXpert MTB/RIF)	Allow same-day determination of drug resistance; line probe assay can be performed directly on smear positive sputum samples or culture isolates; can detect resistance to isoniazid, rifampin, quinolones, and second line injectibles	Need to be validated in multiple clinical settings and not yet FDA approved

AIDS, Acquired immunodeficiency syndrome; BCG, bacillus Calmette-Guérin; *CDC,* Centers for Disease Control and Prevention; CFU, colony-forming unit; *ELISA,* enzyme-linked immunosorbent assay; *FDA,* US Food and Drug Administration; *LAM,* lipopolysaccharide lipoarabinomannan; *PCR,* polymerase chain reaction.

two organisms adhere at one end to form a V. An estimated 10,000 organisms per milliliter of sputum are required for smear positivity, and detection of at least 10 organisms on a slide is optimal; a single organism on a slide is highly suggestive. The sensitivity of sputum acid-fast bacillus smear when compared with culture is approximately 60%.[16] Sensitivity is significantly lower with noncavitary disease and HIV infection. Sensitivity increases by approximately 10% with the collection of a second sputum sample, and 2% with a third.[17] Sputum processing with bleach and concentration before acid-fast staining also increases sensitivity.[18] Most laboratories in the United States now use a fluorochrome stain with phenolic auramine or auramine-rhodamine, a slightly modified acid-alcohol decolorization step, and potassium permanganate counterstaining. Because the mycobacteria are easily seen with a 20× or 40× low-magnification objective, fluorescence microscopy requires less technician time and may increase the sensitivity over conventional acid-fast bacillus smears.[19] Advances in ultrabright light-emitting diode (LED) microscopes make the technology more robust for use in resource-poor settings.[20] WHO recommends that LED microscopy replace conventional fluorescence microscopy and that it be phased in as an alternative for conventional Ziehl-Neelsen microscopy.[21] As with all laboratory procedures, strict quality control is needed for acid fast staining, or rates of false positives and negatives quickly rise.[22]

Any biologic fluid or material can be examined directly (e.g., pleural fluid, cerebrospinal fluid [CSF], urine, gastric lavage fluid), although thin fluids are best examined after sedimentation by centrifugation. Positive smears from concentrated gastric aspiration material are usually due to *M. tuberculosis* and are especially important in young children from whom sputum collection may not be possible.

Culture Methods for *Mycobacterium tuberculosis*

Culture is the gold standard for detecting mycobacteria in clinical specimens. Samples of sputum or tissue require initial decontamination to remove fast-growing nonmycobacterial organisms and liquefaction to allow access of decontaminants to nonmycobacterial organisms and media nutrients to surviving mycobacteria. Decontamination-liquefaction is most commonly done using *N*-acetyl-L-cysteine as a mucolytic in 1% sodium hydroxide solution. Mycobacteria are relatively protected during this procedure by a fatty acid–rich cell wall. However, normally sterile tissues or fluids such as CSF or pleural fluid should not be decontaminated, because some loss of mycobacterial viability does occur. The sample is then neutralized and centrifuged, and the sediment is inoculated onto media.

Three types of media may be used for culture of mycobacteria: solid egg-based media (e.g., Löwenstein-Jensen), solid agar-based media (e.g., Middlebrook 7H11), and liquid broth (e.g., Middlebrook 7H12). Media are made selective for mycobacteria by adding antibiotics. Nonselective media, on which growth is more rapid, are available. Growth is more rapid in 5% to 10% carbon dioxide. Liquid broth cultures require a mean of 10 days of incubation for smear-positive and 20 days for smear-negative specimens for detection of organisms, as compared with solid media, which require 3 to 8 weeks. However, solid media allow examination of colony morphology, detection of mixed cultures, and quantification of growth. Furthermore, occasional strains of mycobacteria may grow only on solid media. For these reasons, experts suggest using liquid and solid media in conjunction, with inoculation of at least one solid medium culture.[23]

Commercial automated liquid broth systems greatly facilitate mycobacterial culture. They monitor mycobacterial growth through detection of CO_2 production or O_2 consumption with radiometric, fluorometric, or colorimetric indicators. The BACTEC mycobacterial growth indicator tube (MGIT) system (Becton Dickinson Microbiology Systems, Sparks, MD), which detects growth in 1 to 3 weeks by means of a fluorometric method, is widely used.[24,25]

A noncommercial liquid broth assay was developed in which mycobacteria are cultured in liquid media on a multiwell plate and then examined microscopically for characteristic serpentine cording. The addition of antimicrobial agents to the media allows drug susceptibility testing to be performed simultaneously. This microscopic-observation drug-susceptibility (MODS) assay yields results in 7 to 10 days, with sensitivity and specificity similar to those of commercially available liquid broth systems.[26] Sensitivity is diminished (65.4%) when the source of diagnostic material is not sputum (lymph node sampling).[27] An automated MODS (Auto-MODS) has been compared with conventional culture in Thailand. With 95.5% sensitivity and 97.1% specificity and a time to culture positivity of 10 days (interquartile range, 8–13 days), this test appears to be effective and attractive in resource-limited settings.[28] In addition, a commercial kit (the Hardy TB MODS Kit; Hardy Diagnostics, Santa Maria, CA) now provides results comparable to those of the conventional MODS, with the ability to detect INH and RIF resistance in a Biosafety Level 2 setting and 97.9% concordance with indirect proportion susceptibility testing.[29]

Nucleic Acid Amplification

Nucleic acid amplification testing (NAAT) offers another technique for the direct detection of *M. tuberculosis* in clinical specimens. The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) is a US Food and Drug Administration (FDA)–approved automated molecular test for detection of *M. tuberculosis* with sensitivity and specificity that approach those of culture.[30] The test is simple to perform and gives results in 100 minutes. It uses real-time polymerase chain reaction (PCR) amplification of an *M. tuberculosis* gene for detection. GeneXpert MTB/RIF assay had an overall sensitivity of 89% and specificity of 99% for detection of *M. tuberculosis* in sputum samples compared with gold standard culture testing. Sensitivity was 98% for smear- and culture-positive cases and 67% for smear-negative culture-positive cases.[31] Analysis of three sputum samples further increases sensitivity.[32] These initial reports continue to be modified under the influence of factors such as prevalence of disease, prevalence of HIV coinfection, and variable performance on smear-positive and smear-negative samples.[33,34] The GeneXpert MTB/RIF simultaneously detects RIF resistance (see "Drug Susceptibility Testing" later).

The Xpert MTB/RIF assay has also been used on nonrespiratory samples (e.g., pleural fluid, CSF, peritoneal fluid, urine, fine-needle aspirates), but the performance in nonrespiratory specimens varies by population, TB prevalence, and type of fluid sampled. Detection in pleural fluid in a report from India revealed a sensitivity of only 54.8%.[35]

The assay has been endorsed by WHO and is rapidly being deployed to countries with high rates of endemic TB. A pooled testing strategy, which might ameliorate some of the cost burden, has been reported with encouraging results.[36] With increased use, the examination of the clinical utility now extends to the test's impact on mortality, and in South Africa no reduction associated with the use of the Xpert MTB/RIF was found compared with microscopy.[37] FDA approval has been extended to an indication for use of the test in removing patients from airborne isolation after one or two negative test results.[38] A single-institution analysis of 207 airborne-isolation cases found that the efficiency (time to result and sensitivity) was greatest with a two-test strategy, and a one-test strategy missed one case.[39] A multisite (Burkino Faso, Cambodia, Cameroon, and Vietnam) assessment comparing Xpert MTB/RIF testing of nasopharyngeal aspirates and stool versus culture in HIV-infected children younger than 13 years yielded a sensitivity on all samples of 79.3% and 75.8%, respectively, on the alternative sources.[40]

The confounding issue of detection of residual DNA not representative of replication-competent bacteria has been addressed in South Africa, where patients with re-treatment cases were significantly more likely to have false-positive results (14%) than those with newly diagnosed cases.[41]

A new version, the Xpert MTB/RIF Ultra assay (Cepheid, Sunnyvale, CA) has been developed. Initial studies suggest that it improves sensitivity by approximately 15% in smear negative-culture positive pulmonary TB; however, there is a small decrease in specificity from 99% to 98%.[42,43] The newer Xpert MTB/RIF Ultra assay may also improve sensitivity in nonrespiratory samples. In a study in Uganda of 129 HIV-infected patients with suspected meningitis, the Xpert MTB/RIF Ultra had a sensitivity of 70% versus a clinical case definition of tuberculous meningitis compared with a sensitivity of 43% for BACTEC culture and 43% for the older Xpert MTB/RIF.[44] WHO has recommended that the Xpert Ultra assay be adopted in settings with high rates of HIV and

TB such as sub-Saharan Africa. Given the lower specificity, there may be more false-positive results in low-HIV and low-TB settings.[45]

Another commercially available and FDA-approved NAAT assay that can be used directly on respiratory samples is the Amplified *M. tuberculosis* Direct (MTD) test (Hologic, San Diego, CA). The sensitivity is intermediate between acid-fast staining and culture. For smear-positive specimens, the sensitivity is 98% and the specificity is 99%. For smear-negative cases, sensitivity ranges from 60% to 80% and the specificity remains 99%.[46–48]

A number of other NAAT tests use novel technologies but are not FDA approved.[49] The COBAS TaqMan MTB assay (Roche Molecular Diagnostics, Pleasanton, CA) is a real-time PCR test that targets *M. tuberculosis* ribosomal RNA. The loop-mediated isothermal amplification assay (TB-LAMP; Eiken Chemical Company, Tokyo, Japan) does not require a thermal cycler or other sophisticated laboratory equipment. It can be performed manually in less than 1 hour, with results read with the naked eye.

Nucleic acid amplification testing complements but does not replace clinical judgment, acid-fast smear, and culture in the diagnosis of TB.[50] In sputum acid-fast smear-positive individuals, positive nucleic acid amplification indicates the presence of *M. tuberculosis* complex and confirms active TB. When there is a high clinical index of suspicion for pulmonary TB but with a negative acid-fast smear, a positive NAAT result is highly predictive of TB and allows early initiation of therapy. NAAT assays perform less well when the clinical index of suspicion for TB is low, in which case the frequency of false-positive tests may approach that of true positives.[51]

Detection of Mycobacterial Antigens in Urine

Detection of *M. tuberculosis* antigens in urine may have some usefulness in diagnosing TB in patients with advanced AIDS, particularly in countries where coinfection is highly prevalent. The Alere Determine lateral flow urine lipoarabinomannan (LF-LAM) assay (Abbott, Abbott Park, IL) is a commercially available point-of-care test for the diagnosis of active TB.[52] In a review of 12 studies of the LF-LAM test for TB diagnosis in HIV-infected patients, the pooled sensitivity was 45% and the specificity was 92%.[53] The combination of microscopy and urinary LAM testing provides incremental diagnostic sensitivity in HIV-infected adults. Sensitivity for TB in HIV-uninfected persons is less than 25%.

Speciation of Mycobacteria

Once mycobacteria have been identified in a clinical specimen, speciation is necessary for clinical diagnosis and epidemiologic investigation. For example, speciation may be important in immunocompromised patients at risk for nontuberculous mycobacterial infection, in localities where *M. bovis* transmission from animals to humans is possible, or in bladder cancer patients receiving bacillus Calmette-Guérin (BCG) immune stimulatory therapy. Speciation generally involves two steps: First, mycobacteria are identified as members of the *M. tuberculosis* complex (e.g., *M. tuberculosis, M. bovis, M. africanum*) or as *Mycobacterium* species other than tuberculosis (MOTT). Subsequently, if necessary, mycobacteria can be speciated within the *M. tuberculosis* complex. NAAT assays, growth in selective antibiotic media, nucleic acid probes, and high-performance liquid chromatography are all used to place mycobacteria within the *M. tuberculosis* complex.[54] Each has advantages and limitations that are detailed in Table 249.1. Identifying individual species within the *M. tuberculosis* complex is more challenging. *M. tuberculosis* grows slowly, lacks pigment, produces niacin, reduces nitrates, has weak catalase activity that is lost by heating to 68°C at pH 7.0, does not demonstrate monodrug resistance to PZA, is resistant to thiophen-2-carboxylic acid hydrazide, and prefers aerophilic conditions. Other members of the complex show different patterns with these tests. Next-generation sequencing technology is rapidly making WGS of clinical isolates routine and also enables identification of species within the *M. tuberculosis* complex.[55]

Genotyping of *Mycobacterium tuberculosis*

Characterizing the particular strain of *M. tuberculosis* is important for epidemiologic purposes, such as tracing transmission from person to person, distinguishing exogenous reinfection from endogenous reactivation in cases of recurrent TB, and identifying laboratory cross-contamination of cultures. The Centers for Disease Control and Prevention (CDC) offers free strain typing through the National Tuberculosis Genotyping Service based on four typing methods: restriction fragment length polymorphism (RFLP) analysis, spacer oligonucleotide typing (spoligotyping), mycobacterial interspersed repetitive unit (MIRU) analysis, and WGS.[56] In RFLP analysis, DNA cleavage fragments generated using *pvuII* are separated by means of electrophoresis and visualized with use of a probe to a repetitive DNA sequence, insertion sequence (IS) *6110.* Because numerous copies of IS*6110* are present at variable chromosomal locations in most *M. tuberculosis* isolates, identical RFLP patterns represent the same strain. Spoligotyping detects variability in the direct repeat (DR) region in *M. tuberculosis* genome. There are direct repeats of a conserved 36-bp sequence, separated by multiple spacer sequences. Different *M. tuberculosis* strains are distinguished by the presence or absence of 43 unique spacers. Each possible combination of spacers is designated by a unique numeric code that identifies a unique spoligotype strain. In MIRU analysis, 12 different DNA sequences, each of which can be tandemly repeated in the *M. tuberculosis* genome, are analyzed. The number of tandem repeats for each of the 12 sequences (or loci) is determined by means of PCR to create a 12-number code. Each code corresponds to a unique MIRU strain.

WGS of clinical isolates is rapidly supplanting prior typing technology. In the United States, many state public health laboratories and the National TB Molecular Surveillance Center routinely perform WGS on isolates from every TB case. Many other countries are developing similar systems. WGS provides data on species, strain type, and drug susceptibility (see later). The WGS bioinformatics pipeline and reporting system are being standardized, and clinicians should anticipate WGS reports on every culture-positive TB case in the near future.[57]

Drug Susceptibility Testing

Testing of *M. tuberculosis* isolates for drug susceptibility is important to guide therapy. There are two broad methods to test an *M. tuberculosis* isolate's drug susceptibility: phenotypic testing, which relies on culturing *M. tuberculosis* in media with varying concentrations of antibiotics, and genotypic testing, which correlates specific mutations in the isolate's genome with known drug susceptibility patterns.

In the United States, the agar proportion method is most commonly used to determine phenotypic drug resistance. The absolute concentration method and resistance ratio method are used less commonly. The agar proportion method compares growth of appropriately diluted inocula on drug-containing media versus growth on drug-free media and is reported as proportion resistant. For most drugs, resistance is significant when growth on drug-containing media exceeds 1% of control; 6% to 10% resistance or more indicates that the drug will add nothing to multidrug therapy. Liquid broth systems, such as BACTEC, can also be used and provide results in 5 to 14 days, but they do not give the proportion of resistant organisms.[58,59]

As noted earlier, the MODS method shows promise as an inexpensive and rapid method for culture and drug susceptibility testing.[26]

Molecular testing to detect *M. tuberculosis* chromosomal mutations associated with mycobacterial drug resistance is an exciting development.[32,60–63] Most commonly used are tests for RIF resistance, which predicts poor treatment outcomes and is a surrogate marker for MDR-TB. Assays detect mutations in the 81-base-pair *rpoB* gene, which encodes the β-subunit of RNA polymerase and correlates with greater than 96% of RIF resistance. Resistance to INH is more complex and is encoded by multiple genes, including the catalase peroxidase gene *katG,* the *inhA* gene involved in fatty acid biosynthesis, the *ahpC* gene, the *oxyR* gene, and the *kasA* gene.[64] Mutations associated with resistance to PZA, ethambutol (EMB), second-line injectable drugs, and fluoroquinolones have also been identified.

The GeneXpert MTB/RIF assay detects mutations in the *rpoB* gene and has a sensitivity of 95% and specificity of 98% for detection of RIF resistance in respiratory samples that are RIF resistant with culture techniques. However, use of the test in populations with high rates of HIV infection and drug resistance, such as in Swaziland, revealed that the test failed to detect a high-prevalence rpoBI491F outbreak strain.

This RIF-resistance–conferring mutation was present in 30% of MDR strains.[65] The drug-resistance assay is performed on sputum simultaneously with detection of organisms, and results are available within 2 hours. Three line probe assays are also commercially available: the INNO-LiPA Rif. TB kit (Innogenetics, Zwijndrecht, Belgium), which detects resistance to RIF in culture isolates; the Genotype MTBDRplus assay (version 2.0) (Hain Lifescience, Nehren, Germany), which detects resistance to INH and RIF in culture isolates and smear-positive sputum samples, and thereby can enable identification of MDR-TB; and the Genotype MTBDRsl assay (Hain Lifescience), which detects resistance to fluoroquinolones and second-line injectable drugs in culture isolates and smear-positive sputum samples and thereby can be used to identify XDR-TB.[66–69] WHO has recommended widespread use of these molecular assays.[70] The clinical impact of rapid diagnosis and drug-resistance detection has been reported from Tbilisi, Georgia, where the MTBDRplus assay was associated with selection of drug regimens that provided higher and more rapid rates of culture conversion at 24 weeks.[71]

WGS is also becoming a powerful tool for the detection of drug-resistance mutations in the *M. tuberculosis* genome.[72,73] Currently it can be performed only on culture isolates, and the bioinformatics pipelines, databases cataloguing drug-resistance mutations, and reporting systems are being standardized for routine clinical use.

EPIDEMIOLOGY

General Considerations

M. tuberculosis infects 1.7 billion people or approximately one-quarter of the world's population and caused 10.4 million new cases of TB and approximately 1.7 million deaths in 2016.[1,74,75] TB is the leading cause of death worldwide resulting from a single infectious agent, ranking above HIV since 2014.[76] Immunocompromise due to HIV infection is a risk factor for TB, and 374,000 TB deaths are in HIV-infected individuals. Drug-resistant TB is emerging globally, with approximately 490,000 new MDR-TB cases in 2016. Two factors essential for the rapid spread of *M. tuberculosis* are crowded living conditions and a population with little native resistance. In the 19th century, TB caused more than one-fourth of all adult deaths in Europe, eliminating those with the least native resistance. A downward trend had been established before the turn of the 20th century. Epidemiologists once believed the disease would eventually disappear based on the assumptions that 1 in 20 infections result in active cavitary disease of the lung (i.e., become contagious). Thus, each cavitary case would have to infect 20 persons for case rates to be maintained.[77] In Holland in the early 1900s, one infectious case produced only 13 new infections.[77] The annual decrement in mortality and morbidity from TB was approximately 5% in developed countries owing to progressively higher natural residual resistance in those who survived infection and to living conditions less conducive to airborne spread. This rate of decline approximately doubled after chemotherapy became widespread.

In the 1980s and 1990s, the high incidence of TB in Africa, Asia, Eastern Europe, and South America, the HIV coepidemic, and burgeoning MDR-TB demonstrated that predictions of the disappearance of TB were premature, and WHO declared TB a global public health emergency. Since then, tremendous gains have been made in reducing the number of new TB cases, now in decline for the past several years. The integration of multiple modalities, such as genotyping combined with a series of spatial analysis tools, might improve the efficiency of targeted transmission control.[78]

Recent Morbidity and Mortality Trends

In the United States, the incidence of TB has decreased steadily over the past 25 years, from 10.5 reported cases per 100,000 population in 1992 to 2.8 cases per 100,000 population in 2017, the lowest in recorded history.[2,79] Recent estimates of latent infection in the United States suggest a prevalence of latent infection in 2011–12 of 4.4% by the tuberculin skin test (TST) and 4.8% by the QuantiFERON-TB Gold In-Tube test (QFT-GIT; Cellestis Limited, Carnegie, Victoria, Australia).[80] In the United States the TB rate in foreign-born persons is 15 times higher than in US-born persons, and foreign-born persons now account for almost 70% of reported cases in the United States (Fig. 249.1).[79] This reflects increased immigration from high-prevalence countries, especially

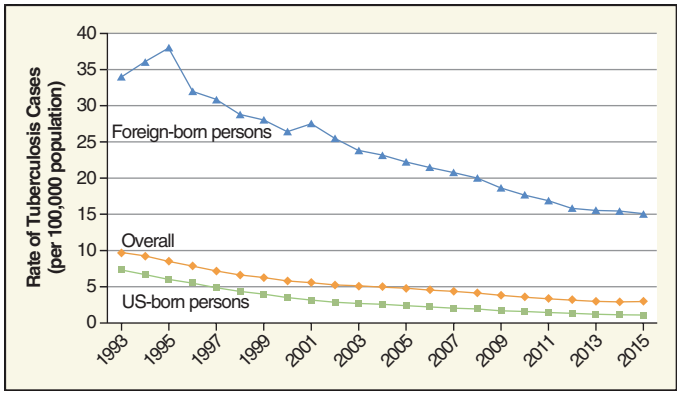

FIG. 249.1 Rates of tuberculosis cases among US-born versus foreign-born persons, United States, 1993–2015. *(Modified from Tuberculosis in the United States: National Tuberculosis Surveillance System highlights from 2015. Slide 17.* https://www.cdc.gov/tb/statistics/surv/surv2015/default.htm.*)*

Mexico, the Philippines, India, Vietnam, and China, from which immigrants account for more than half of the TB cases in foreign-born individuals.[81] Strain genotyping suggests that TB among foreign-born persons is from reactivation of latent infection acquired before arrival in the United States. Furthermore, the risk declines with duration of residency, also suggesting that most infections were acquired before immigration.[82] Screening of immigrants to the United States with a culture-based, rather than a smear-based, algorithm is justified by the high rate (54.4%) of smear-negative and culture-positive cases.[83]

TB has also become concentrated in certain ethnic and racial minorities and medically underserved populations, often occurring in contact-based microepidemics. In 2017, rates among US-born blacks was seven times higher than in non-Hispanic US-born whites; the rate in Native Americans was nine times higher than in whites.[79] A number of outbreaks have affected the urban poor, alcoholics, injection drug users, the homeless, migrant farm workers, and prison inmates.

The age distribution of TB reflects the degree of ongoing transmission in a given population. Disease in the elderly is generally due to reactivation of infection acquired in the remote past, whereas TB in young children indicates ongoing active transmission in the community. In this regard, 75% of childhood cases in the United States occurred in patients who had TB exposure through foreign-born parents or prior residence outside the United States.[84,85]

TB in the United States is most frequent in geographic regions and demographic groups where AIDS is prevalent, notably in urban blacks and Hispanics between 25 and 45 years of age.[86] Persons with active TB are more frequently HIV positive than is the general population. Approximately 8% of all TB cases in the United States occur in HIV-infected persons.[3]

Despite a predominantly urban epidemiology, large TB outbreaks have also affected small communities.[87,88] One well-characterized outbreak began in 1988 in a coastal Maine village where TB had not been reported in the previous 3 years.[88] A shipyard worker with cavitary TB was the source of 21 subsequent active cases and 697 new tuberculous infections. In retrospect, the source patient had repeatedly sought medical attention for cough, sore throat, and hoarseness during 8 months before TB was diagnosed and treated. This report highlights the need for vigilance regarding all segments of the population, not just those known to have high TB case rates.

The prevalence of positive TST results in US Navy recruits offers insights into trends of latent tuberculosis infection (LTBI) over time and risk factors for infection. From 1958 through 1969, more than 1 million US Navy recruits underwent TSTs, and in 5.2% the results were positive. In the 1980s and 1990s, the rate of tuberculin reactivity dropped to approximately 1.5% of new Navy recruits. The prevalence was greater in blacks (5%), Hispanics (5%), and Asian/Pacific Islanders (26%) than in whites (0.8%). Tuberculin positivity was more than 10-fold more prevalent among foreign-born recruits.[89–91] In a survey

TABLE 249.2 Reported Tuberculosis Case Rates in Immigrants According to Country of Birth: Stratified by Time Since Entry Into the United States

COUNTRY	CRUDE RATE (PER 100,000 PERSON-YEARS)[a] US ENTRY ≤2 YEARS	US ENTRY >2 YEARS
Somalia	889	179
Ethiopia and Eritrea	562	82
Vietnam	319	47
Cambodia	307	65
Philippines	283	38
Ecuador	194	31
Haiti	189	40
Honduras	177	28
Peru	159	32
Guatemala	111	21
India	106	33
China	74	26
El Salvador	73	11
Mexico	52	14
Korea	40	19
All foreign-born individuals	75	16

[a]Data provided for the 15 most commonly reported countries of birth.
Modified from Cain KP, Benoit SR, Winston CA, et al. Tuberculosis among foreign born persons in the United States. JAMA. *2008;300;405–412.*

TABLE 249.3 Estimated Incidence of Tuberculosis Cases in Countries That Accounted for 75% of Cases Worldwide, 2016

COUNTRY	CASES	RATE PER 100,000 POPULATION
India	2,790,000	211
Indonesia	1,020,000	391
China	895,000	64
Philippines	573,000	554
Pakistan	518,000	268
South Africa	438,000	781
Democratic Republic of Congo	425,000	323
Nigeria	407,000	219
Bangladesh	360,000	221
Myanmar	191,000	361
Ethiopia	182,000	177

Modified from World Health Organization. Global Tuberculosis Report 2017. http://www.who.int/tb/publications/global_report/gtbr2017_annex4.pdf.

of noninstitutionalized civilians in the United States, the prevalence of tuberculin reactivity among persons 25 to 74 years of age decreased from 14.4% in 1972 to 5.6% in 2000 and to 4.4% in 2011.[80,92]

Immigrants for the most part retain the tuberculin positivity and TB rates of their country of origin (Table 249.2).[93,94] Other groups, such as injection drug users, patients with end-stage renal disease or diabetes, health care workers in endemic countries, residents of institutions for the homeless, and, to a lesser degree, nursing home residents, demonstrate morbidity rates greatly in excess of the general population.[95–103]

On a global scale, TB has a devastating impact in lower- and middle-income nations, with 10 countries accounting for nearly 70% of all prevalent cases (Table 249.3).[104] In 1993, WHO declared TB a global public health emergency and intensified major initiatives to address the problem. An important aspect of this strategy is supervised treatment, which may include DOTS.[105] WHO subsequently reported decreasing incidence rates of TB in all six regions of the world, including Southeast Asia, the Western Pacific, and Africa. In China, access to DOT was rapidly expanded from 31% of TB cases in 2001 to 80% in 2005, and from 1990 to 2010 the prevalence of smear-positive disease decreased from 170 to 59 cases per 100,000, in a population of greater than 1.3 billion.[106,107] Similar progress has also been made in India, the country with the greatest number of TB cases.[108,109] Challenges remain; molecular epidemiologic data from China from 2009 through 2012 indicate that recent transmission still accounts for a substantial proportion of new cases and that sputum smear–negative cases may be responsible for 30% of secondary cases.[110] In 2014 WHO launched its End TB Strategy with the goal of reducing global TB deaths by 90% and TB incidence by 80% by 2030. Major expansions of current activities and new prevention and treatment strategies will be needed to achieve this goal.[1]

Over 35 million persons worldwide are now living with HIV/AIDS.[111] The potential for continued interaction between HIV and TB is therefore immense. In some developing countries where most persons harbor tubercle bacilli before adulthood, the prevalence of HIV infection becomes the only determinant of coinfection. The situation is worst in sub-Saharan Africa, where the incidence of TB has risen in parallel with the incidence of HIV infection. Between 1990 and 2005, the incidence of TB more than doubled, from 149 to 343 per 100,000 population. TB is the leading cause of death in HIV-infected people, and in 2016, 22% of the 1.7 million TB deaths were in HIV-coinfected people. Seventy-five percent of the world's HIV-positive TB deaths occurred in Africa.[1] The continued scale-up and integration of HIV and TB programs are necessary to decrease the deaths in these populations.

Drug-Resistant Tuberculosis

Drug-resistant TB poses an immense challenge for TB control. Resistance to antituberculous agents can be either *primary,* that is, present before initiation of therapy and due to transmission of a drug-resistant *M. tuberculosis* strain, or *secondary,* indicating emergence of resistance after antituberculosis therapy. Risk factors for infection with drug-resistant TB are listed in Table 249.4. Strains resistant to at least INH and RIF are defined as MDR-TB. Strains resistant to at least INH, RIF, a fluoroquinolone, and second-line injectable drugs are defined as XDR-TB.[112]

WHO has regularly reported global drug-resistance rates since 1994.[113–116] In the United States, the rate of primary resistance to any antituberculosis drug has remained stable at about 12%, with that of MDR-TB at about 1%.[115] Globally, 3.3% of new TB cases and 20.1% of previously treated TB cases are MDR.[116] In 2016, there were an estimated 490,000 new cases of MDR-TB worldwide. Parts of Eastern Europe and central Asia, including Belarus, Estonia, Latvia, the Russian oblasts of Ivanovo and Tomsk, and Henan Province in China, have rates of MDR in newly diagnosed patients with TB that exceed 18%.[114–118]

Prior antituberculosis treatment is the most important risk factor for drug-resistant TB. Additional historical clues that increase the likelihood of drug resistance include infection acquired in regions where resistance is prevalent and known contact with a drug-resistant case. One study from southern California recorded resistance in 71% of patients with TB who had been previously treated and had cavitary disease.[119] Of immense concern is transmission of XDR-TB.[112] Cases of XDR-TB have been reported in 105 countries worldwide, and it is estimated that 9.7% of MDR-TB is XDR-TB.[116]

Mode of Spread

Almost all infections with *M. tuberculosis* are due to inhalation of droplet nuclei—infectious particles from a person with pulmonary TB aerosolized by coughing, sneezing, or talking—which dry while airborne, remain suspended for long periods, and reach the terminal air passages. A cough can produce 3000 infectious droplet nuclei, talking for 5 minutes an equal number, and sneezing many more than that. Accordingly, the air in a room occupied by a person with pulmonary TB may remain

TABLE 249.4 Epidemiologic Circumstances in Which an Exposed Person Is at Increased Risk for Infection With Drug-Resistant *Mycobacterium tuberculosis*

- Exposure to a person who has known drug-resistant tuberculosis
- Exposure to a person with active tuberculosis who has had prior treatment for tuberculosis (treatment failure or relapse) and whose susceptibility test results are not known
- Exposure to persons with active tuberculosis from areas in which there is a high prevalence of drug resistance
- Exposure to persons who continue to have positive sputum smears after 2 months of combination chemotherapy
- Travel in an area of high prevalence of drug resistance

[a]This information is to be used in deciding whether to add a fourth drug (usually ethambutol) for children with active tuberculosis, not to infer the empirical need for a second-line treatment regimen.
From Centers for Disease Control and Prevention. Treatment of tuberculosis. American Thoracic Society, CDC and Infectious Diseases Society of America. MMWR Recomm Rep. *2003;52(RR–11):1–88.*

infectious for approximately 30 minutes even after his or her absence. Although in theory one droplet nucleus may be sufficient to establish infection, prolonged exposure and multiple aerosol inocula are usually required. Strains may vary widely in their transmissibility,[87] but infection does not generally occur outdoors because *M. tuberculosis* is killed by ultraviolet light. Although homes are the focus of TB contact investigations, evidence is mounting that in high-TB settings, approximately 85% of transmission occurs in other social spaces including medical facilities, public transportation vehicles, workplaces, schools, churches, bars, and other areas where people congregate.[120]

Large drops of respiratory secretions and fomites are unimportant in transmission, and special housekeeping measures for dishes and bed linens are unnecessary. Other modes of transmission are rare. Infection with *M. bovis* from ingestion of contaminated milk was once commonplace. Skin inoculation of *M. tuberculosis* from contamination of an abrasion occurs in pathologists and laboratory personnel (prosector's wart), and venereal transmission has been recorded. Although the source is pulmonary in the vast majority of cases, aerosolization of organisms during irrigation of cutaneous lesions or at autopsy has caused spread to health care workers.[121,122] The special case of organ-donor–transmitted infection should be noted because transplant recipients have a higher frequency of TB than the general population, and high mortality.[123] Because donor-derived disease develops late (>90 days) in the majority of cases, and many donors have identifiable risk factors for tuberculous infection, it has been proposed that screening protocols using donor histories that would heighten surveillance of recipients, in addition to cultures and smears, and testing for latent TB in donors should be undertaken.[124]

Risk for Infection

The most important determinants of infection of tuberculin-negative persons are closeness of contact and infectiousness of the source. From 2002 to 2011, 26 outbreaks in the United States were associated with index cases with a substantial burden of substance abuse, incarceration, and homelessness.[125] Cases with positive smears are highly infectious; those positive only on culture are less so. The degree of sputum positivity and pattern of coughing are important. Compared with measles, one case of which will infect 80% of susceptible casual contacts, TB is only moderately infectious in most circumstances. In the United Kingdom, a study of 111 cases with 825 contacts found that a shorter interval to liquid culture positivity identified patients at high risk of transmitting infection and was also superior to detection by means of smear.[126]

TB morbidity in a population is determined both by the risk for infection and the risk for acquiring active disease once infected. In Holland in the 1970s, 50% of newborn to 14-year-old household contacts of individuals with smear-positive cases became tuberculin positive, but only 5% did so when the contact case was culture positive but smear negative.[77] In the United States, approximately 27% of household contacts of individuals with smear-positive cases become infected, although rates as high as 80% occur in closed environments.[127] A large epidemiologic investigation in San Francisco from 1991 to 1996 estimated that 17% of new active TB cases arose from smear-negative, culture-positive index cases.

Persons coinfected with HIV do not appear to be more infectious than HIV-negative source cases and because they are more likely to be smear negative than are HIV-negative patients, they may be less infectious. In one large study from the Democratic Republic of Congo, household contacts of HIV-positive patients with pulmonary TB were no more likely to become infected with *M. tuberculosis* than were household contacts of HIV-negative TB patients.[128] A study in Uganda demonstrated that transmission from an HIV-infected index case to contacts was dependent on the index patient's sputum smear positivity and presence of a cavity on chest radiograph.[129] A meta-analysis of six studies involving exposed health care workers supports this finding, as does a study of household contacts in Peru.[130,131]

Influence of Chemotherapy on Spread of Infection

Patients receiving appropriate chemotherapy promptly become noninfectious as cough subsides and the concentration of organisms in sputum decreases. The time required to become noninfectious depends on the patient's burden of organisms, but there is indirect evidence that this occurs within 2 weeks in patients with drug-sensitive TB.[132] Thus, case finding and treatment comprise the most effective method of TB control.

Risk for Progression From Infection to Active Disease

In general, 3% to 4% of infected individuals acquire active TB during the first year after tuberculin conversion and an additional 5% do so thereafter, although the uniformity of these rates has been challenged in a study from Victoria, Australia, in which the cumulative hazard for progression to disease after infection was 14.5%, with most risk occurring within the first 5 months and greater risk in children younger than 5 years (50.6%).[133,134] These estimates are based on heavy exposures during disease-prone periods of life. Persons infected with small inocula or during disease-resistant periods probably have a much smaller risk,[135] whereas the risk for progression in immunocompromised persons is greater. In one study of 12,876 unvaccinated adolescents, 10.4% of those who converted their tuberculin tests acquired clinical TB, 54% of these within 1 year and 78% within 2 years.[77] The three periods of life during which infection is most likely to produce disease are infancy, ages 15 to 25 years, and old age. (The effect of age on disease progression is discussed in "Influence of Age on Tuberculous Infection.")

The likelihood of active disease developing varies with the intensity and duration of exposure. Persons with intense exposures are most at risk not only for infection but also for disease.[127] The degree of tuberculin positivity has some predictive value. Malnutrition, renal failure, and immunosuppression all favor progression of infection to active disease, but by far the strongest risk factor is HIV infection. Among tuberculin-positive, HIV-positive injection drug users in one methadone clinic population, 8% per year acquired active TB.[136]

Institutional Spread of Tuberculosis

Hospitals

TB has long been a recognized risk to health care workers and hospitalized patients. In the 1980s, numerous explosive outbreaks of TB occurred among HIV-infected patients in specialized wards and hospices in the United States and Europe.[137–141] In the first reported outbreak on an HIV ward, the index patient had fever, cough, a normal chest radiograph, and negative acid-fast smears, but a positive sputum culture for *M. tuberculosis*.[138] On the same ward, 39% of other HIV-positive patients acquired active TB within 60 days. Major factors contributing to these outbreaks included (1) delays in diagnosis, especially in HIV-infected patients with atypical chest radiographs; (2) inadequate negative-pressure ventilation inpatient rooms; (3) use of aerosol-generating procedures such as bronchoscopy and sputum induction; (4) rapid progression to active TB in HIV-positive patients; and (5) in the case of MDR-TB, prolonged infectivity despite antituberculous chemotherapy.[142] In high-income countries, decreased numbers of AIDS hospitalizations, decreased TB incidence, low rates of MDR, and improved hospital infection-control

programs have generally diminished these outbreaks. In middle- and lower-income countries, with higher rates of HIV- and TB-related hospital admissions and less resources for infection control, nosocomial transmission remains a major public health problem. An investigation of 404 cases of XDR-TB in South Africa demonstrated the continued importance of nosocomial transmission.[143,144]

In reaction to outbreaks of MDR-TB, the CDC in 1994 established very stringent criteria for removing from respiratory isolation patients who were suspected to have pulmonary TB and had not begun empirical treatment. These criteria now include three consecutive negative sputum smears on good-quality specimens obtained at least 8 hours apart.[145] Studies have demonstrated that two negative sputum Xpert MTB/RIF tests improve sensitivity and specificity for identifying patients who will be found to be culture and smear negative. Use of the Xpert MTB/RIF will decrease time in isolation and will be more cost-effective than use of three AFB smears.[146,147]

Shelters for the Homeless

Approximately 1% of the US population experiences homelessness in a given year, and these people account for a disproportionately high percentage (6%) of all the TB cases in the country annually. Poor nutrition, injection drug use, alcoholism, lack of access to TB screening and treatment, and crowding increase the risk for both endogenous reactivation of remote infection and acquisition of new (exogenous) infection in homeless shelter clients.[96] A review of cases between 1994 and 2003 showed that 6% of TB cases in the United States occurred in homeless individuals, of whom 87% were men and 34% were HIV positive and in whom alcohol and drug abuse was common.[148] A 2015 CDC-sponsored workshop with homeless shelter staff and public health officials from across the country defined key measures to prevent TB transmission in shelters including strict infection-control procedures, active case finding, screening for latent TB, cooperation between shelters and public health authorities, education of shelter staff and clients, data sharing, and prevention of stigma so that clients with symptoms will freely come forward.[149]

Correctional Facilities

The incidence of TB is markedly higher in incarcerated persons than in the general population.[150] In the United States, high-risk populations—including young black and Hispanic men, injection drug users, and HIV-infected persons—are overrepresented in prison populations.[151] Although most prison cases are due to reactivation of old infections, outbreaks of MDR-TB have shown that transmission of new (exogenous) infection also occurs. Although difficult for obvious reasons, preventive and curative services in correctional facilities are a high public health priority for everyone.[151,152] Transmission in prisons and jails may serve as an amplifier for spread to the community. In a study in a medium-size city in Brazil between 2009 and 2013, 25% of TB cases occurred among prisoners, who represented <1% of the city's population, and an additional 25% of the TB cases in the city were in ex-prisoners who had recently been released into the community. The remaining 50% occurred in community members. *M. tuberculosis* strain typing demonstrated clear clonal links between the prison cases, cases in ex-prisoners, and community cases.[153]

Controlling Nosocomial Spread

Spread of TB in the health care setting has raised justifiable concern. Tuberculin conversion rates as high as 50% among health care workers on HIV wards were reported early in the AIDS epidemic.[139] Delays in diagnosis and initiation of therapy are critical for both outcome and infectiousness to others.[142,154] Accordingly, TB must be considered in any HIV-positive patient with subacute or chronic pulmonary symptoms or symptoms compatible with extrapulmonary TB. Screening with NAAT tests such as Xpert MTB/RIF may expedite diagnosis.

Hospitalized HIV-positive patients with respiratory symptoms or people with suspected TB should be admitted to negative-pressure isolation rooms (so that air flows from the corridor into the room and is safely exhausted to the outside) with six air changes per hour. Procedures that stimulate coughing, such as sputum induction or bronchoscopy, should be carried out in negative-pressure rooms or special booths. The use of particulate respirator masks (N95), with appropriate training and fitting, further reduces risk and is recommended by the CDC. Ultraviolet irradiation of the air— either with the air pulled by a fan through a radiation chamber or with the ultraviolet beam directed into the uppermost parts of the room so as to avoid direct irradiation of personnel—is also advised. The CDC has published extensive guidelines for control of transmission of TB in diverse health care settings.[155] Further evidence from a guinea pig model, in which the animals breathed untreated air from a TB ward, supports the use of air disinfection based on ultraviolet treatment.[156] WHO has also published TB infection-control guidelines for health care facilities with strategies that may be attainable for low- and middle-income countries.[157]

IMMUNOLOGY

TB is the prototype of infections that require a cellular immune response for their control (see Chapter 6). An effective immune response against *M. tuberculosis* infection relies on $CD4^+$ T cells and the cytokines interleukin (IL)-12, interferon-γ, and tumor necrosis factor (TNF).[158] Conversely, *M. tuberculosis* has adapted to host immunity and likely depends on the cellular immune response to facilitate tissue damage, formation of pulmonary cavities, and its own aerosol transmission. WGS of *M. tuberculosis* shows that the 491 major human T-cell epitopes expressed by the mycobacterial genome are highly conserved, suggesting that *M. tuberculosis* is no longer evolving to evade the human immune response but rather has reached an equilibrium with it.[159]

After initial inhalation, droplet nuclei with *M. tuberculosis* must bypass mechanical barriers, ciliated respiratory epithelial cells, and mucins in the upper airways to arrive in the alveolar spaces.[160] In the alveoli, *M. tuberculosis* is phagocytized by the alveolar macrophage. Entry into macrophages involves interactions with complement receptors, mannose receptors, and Fc receptors. Various *M. tuberculosis* molecules are recognized by multiple pattern recognition receptors (PRRs) at the cell surface, in the phagosome, or in the cytosol of the macrophage. Other immune cells that participate in the initial host response include neutrophils, dendritic cells, λδ T cells, mucosal-associated invariant T (MAIT) cells, CD1-restricted T cells, and natural killer (NK) cells.[161]

The alveolar macrophage may successfully kill the mycobacteria through phagosome maturation, fusion with the lysosome, or autophagy. Alternatively the mycobacteria may release virulence factors that delay phagosome maturation or result in phagosome rupture. *M. tuberculosis* uses several strategies to survive within macrophage phagosomes while delaying or preventing effective immune responses. Mycobacterial urease helps prevent acidification of the phagosome, thus limiting the effectiveness of bactericidal enzymes. In addition, by remaining within the phagosome, the organism does not initially elicit T-cell responses via the proteosomal pathway of antigen presentation. The organism also secretes abundant superoxide dismutase, catalase, thioredoxin, and other antioxidants that detoxify reactive oxygen species generated by phagocytes. Microbial antioxidants not only provide direct protection against host-generated oxidants but also suppress early oxidant-mediated immune responses needed for efficient antigen presentation, including the activation and apoptosis of macrophages.[162] This both ensures the organism's continued survival within its host cell and delays the development of strong adaptive T-cell responses. The recent elucidation of such pathogenic mechanisms has fostered rational strategies toward vaccine development (see "Vaccination").

If the initial infection is successful, unrestrained replication proceeds for weeks, both in the initial focus and in lymphohematogenous metastatic foci. The development of adaptive cellular immunity is delayed and takes approximately 4 to 8 weeks and ultimately supervenes. Tissue hypersensitivity is florid in comparison with other intracellular infections, perhaps fueled by the adjuvant activity of mycobacterial lipids.

$CD4^+$ Th1 cells, which secrete interferon-γ, are central to the protective immune response against *M. tuberculosis*. *M. tuberculosis* antigen-specific $CD4^+$ Th1 cells are activated by dendritic cells via the IL-12 pathway in the lymphatic tissue, clonally expand, and then migrate to the site of infection. $CD4^+$ T-cell production of interferon-γ activates macrophages at the site of antigen. Activated macrophages accumulate high concentrations of lytic enzymes and reactive metabolites that greatly increase their mycobactericidal competence, causing a decrease in the

mycobacterial load. Activated macrophages also secrete regulatory molecules (e.g., TNF-α and transforming growth factor-β), which in concert with lymphocyte secretory proteins (e.g., interferon-γ, migration-inhibitory factor) determine the character of the pathologic and clinical response. Epithelioid cells, characteristic of the tuberculous granuloma, are highly stimulated macrophages. The Langhans giant cell consists of fused macrophages oriented around TB antigen with the multiple nuclei in a peripheral position, representing the most successful type of host-tissue response. Cytotoxic $CD8^+$ T cells are also generated during infection and may directly lyse infected mononuclear phagocytes.

When the population of activated lymphocytes reaches a certain size, cutaneous delayed reactivity to tuberculin manifests, generally within 3 to 9 weeks after initial infection. At the same time, enhanced macrophage microbicidal activity appears. The pathologic features of TB are the result of the degree of hypersensitivity and the local concentration of antigen. When the antigen load is small and tissue hypersensitivity is high, organization of lymphocytes, macrophages, Langhans giant cells, fibroblasts, and capillaries results in granuloma formation. Foci characterized by the resulting hard tubercles are said to be *proliferative* or *productive* and constitute a successful tissue reaction with containment of infection, healing with eventual fibrosis, encapsulation, and scar formation. When both antigen load and hypersensitivity are high, epithelioid cells and giant cells are sparse or entirely lacking; lymphocytes, macrophages, and granulocytes are present in a less organized fashion; and tissue necrosis may be present, a tissue reaction that has been called *exudative*. In the absence of necrosis, exudative lesions may heal completely or tissue necrosis may occur. Necrosis in TB tends to be incomplete, resulting in solid or semisolid acellular material referred to as *caseous* because of its cheesy consistency. The chemical environment and oxygen tension in solid caseous material tend to inhibit microbial multiplication. However, caseous necrosis is unstable, especially in the lungs, where it tends to liquefy and discharge through the bronchial tree, producing a tuberculous cavity and providing conditions in which bacterial populations reach very high titers. Cavities may contain 10^7 to 10^9 organisms compared with only 10^2 to 10^4 in areas of caseous necrosis.[163] Infectious material sloughed from a cavity creates new exudative foci in other parts of the lung (bronchogenic spread). A cross section of a pulmonary cavity demonstrates all these pathologic reactions, from the least to the most successful in terms of containment of infection. The central cavity, which contains myriad bacilli, is surrounded by a layer of caseous material with fewer organisms, a more peripheral layer of macrophages and lymphocytes with little organization and still fewer organisms, an area that is even more peripheral with epithelioid cells and giant cells in which the bacterial content is quite low, and, most peripherally, a bacillus-free layer of encapsulating fibrosis.

When the degree of hypersensitivity is very low, the tissue reaction may be nonspecific, consisting of polymorphonuclear leukocytes and mononuclear cells with huge numbers of tubercle bacilli, so-called *nonreactive tuberculosis.*[164] The immunologic spectrum from florid hypersensitivity to little or no specific tissue reaction is similar to that seen in leprosy and is recapitulated in HIV-infected persons as the $CD4^+$ T-cell count declines.[165]

Epidemiologic studies suggest that in a very small subgroup of individuals exposed to *M. tuberculosis,* the initial innate immune response may be able to prevent *M. tuberculosis* infection. These individuals have been termed "resistors" and likely represent <10% of the population.[166] Of those who become infected with *M. tuberculosis* and develop a cellular $CD4^+$ T-cell response, the adaptive immune response will be successful in containing the infection and preventing TB disease 90% to 95% of the time.[158] Active disease from *M. tuberculosis* does not generate a durable immunity against the occurrence of a second TB disease episode. To the contrary, a first episode of active TB is a significant risk factor for a second episode of TB.[167–169] Immune correlates of these successful and unsuccessful immune responses are not fully known and are an area of intense investigation.

Testing for Latent Tuberculosis Infection

Individuals infected with *M. tuberculosis* with no clinical evidence of disease have LTBI. They have been infected with *M. tuberculosis* but do not have TB disease. To test for LTBI, a skin or blood test is performed to detect a cellular immune response to tuberculous antigens, indicating prior infection. The CDC suggests testing people who are at increased likelihood for infection and progressing to active TB, and who would therefore benefit from preventive therapy. The CDC also recommends stratifying patients' risk of developing TB when interpreting TST results. The TST is the classic test, but blood interferon-γ release assays are increasingly recommended by expert panels.[170]

TUBERCULIN SKIN TEST

The TST is used to determine whether an individual is infected with *M. tuberculosis.* Koch's tuberculin (old tuberculin) was an extract of a boiled culture of tubercle bacilli. In 1934, Siebert made a simple protein precipitate (purified protein derivative [PPD]) of old tuberculin, which became the preferred reagent in most areas. In 1941, a large single lot was adopted as the biologic standard (PPD-S) to which other preparations are now standardized. A 5-tuberculin unit (TU) dose of PPD is equivalent to 0.0001 mg of PPD-S protein in 0.1 mL of solution.[171]

DOSAGE

The sensitivity and specificity of the 5-TU dose were derived in populations in which the incidence of TB was accurately known. A 5-TU dose of tuberculin clearly separated groups with 100% infection, such as sanatorium patients, from groups with a very low incidence of TB, such as infants from noninfectious environments. In the former, tuberculin reactions peaked at 16 to 17 mm; in the latter, 0- to 5-mm reactions were elicited.

Technical Aspects

The TST is performed by means of intradermal injection of 5 TU of PPD in 0.1 mL of solution, usually on the volar aspect of the forearm. The injection is made with a short, beveled 26- or 27-gauge needle with the bevel facing upward (Mantoux test). Correct injection produces a raised, blanched, 6- to 10-mm wheal. Deeper injections may be washed out by vascular flow, resulting in false-negative results. The loss of potency that occurs when PPD adsorbs to glass surfaces is prevented by the addition of the detergent polysorbate 80 (Tween 80). Tween-stabilized tuberculin in solution is light sensitive and must be refrigerated. The skin reaction is usually read in 48 to 72 hours. A positive test result is defined by the diameter of induration, not erythema, in response to 5 TU. The diameter should be read across the forearm and can be measured by viewing the reaction tangentially against a light background. An alternative is to use a medium-point ballpoint pen to draw a line starting 1 to 2 cm away from the skin reaction and moving toward its center. The pen is lifted when resistance is felt, the procedure repeated from the opposite direction, and the distance between opposing line ends measured.

Targeted Tuberculin Testing

The American Thoracic Society (ATS), Infectious Diseases Society of America (IDSA), and CDC guidelines for LTBI testing recommend targeted testing of persons at increased likelihood of infection and/or at high risk for developing TB and with the intent for treatment of LTBI if the test is positive.[170,172] This includes persons at high risk of infection (e.g., recent immigrants, household TB contacts) and persons with clinical conditions that increase the risk for TB disease (e.g., those with HIV/AIDS, organ transplant recipients, patients taking TNF inhibitors). Testing of persons at lower risk of infection and disease progression is discouraged. Initial testing is also recommended for persons whose activities place them at increased risk for exposure, such as employees at medical and correctional facilities.

Interpretation

Based on sensitivity and specificity of tuberculin skin testing, three cutoff levels have been recommended for defining positive reactions: 5 mm, 10 mm, and 15 mm (see "Treatment of Latent Tuberculous Infection").[170,172] The 5-mm cutoff is used for close contacts, children younger than 5 years, people who have HIV infection, those who are undergoing immunosuppressive therapy, those with an abnormal chest radiograph consistent with prior TB, and patients with silicosis. The 10-mm cutoff is used for people who are likely to be infected

but at are low-to-intermediate risk of disease progression. The 15-mm cutoff is for people at low risk, although current guidelines for targeted testing suggest that low-risk persons not be tested. Ninety percent of persons with 10 mm of induration and virtually all with greater than 15 mm of induration to 5 TU are infected with *M. tuberculosis*. Induration of less than 10 mm may reflect cross reactions caused by infection with other mycobacterial species or prior BCG vaccination. However, even 5- to 10-mm reactions are suggestive of tuberculous infection in geographic areas substantially free of other mycobacteria, such as the northeastern United States, and in persons with immune suppression, such as HIV-infected persons.[173,174] Because of the potential for false-positive TST results in people who have received BCG vaccine, the ATS, IDSA, and CDC recommend an interferon-γ release assay rather than the TST for people from countries that vaccinate with BCG.[170,175]

Booster Effect

Although tuberculin cannot sensitize an uninfected person, it can restimulate remote hypersensitivity that has deteriorated. This booster effect (a positive tuberculin test result after a negative one) develops within several days after a first injection and may be persistent. This causes interpretative problems, because a negative test result followed by a positive test result approximately 10 weeks later may be a product of either a recent infection or a booster effect. This problem is circumvented by retesting nonreactors 1 to 3 weeks after the initial test. If the second test result is positive, this indicates boosting rather than recent tuberculin conversion. In early rheumatoid arthritis patients, with baseline increased risk of TB compounded by the use of TNF-α inhibitor therapy, the use of a booster PPD skin test increased LTBI detection from 31.3% to 46.5% in early rheumatoid arthritis and 21.7% to 28.8% in late rheumatoid arthritis patients.[176]

False-Positive and False-Negative Reactions

False-positive reactions represent nontuberculous mycobacterial infection. False-negative reactions occur in at least 20% of all persons with known active TB. In one study, 25% of 200 patients with active TB were nonreactive to 5 TU and 10% were also nonreactive to 250 TU.[177] Most false-negative test results in patients with TB are attributed to general illness and become positive 2 to 3 weeks after effective treatment is initiated. Protein malnutrition diminishes all cutaneous delayed hypersensitivity reactions. Sarcoidosis may cause false-negative TST results. Intercurrent viral infections (including HIV-1 infection with <200 $CD4^+$ T cells/mm^3), vaccination with live-virus vaccines (measles, smallpox), reticuloendothelial disease, and corticosteroid therapy may cause false-negative tuberculin reactions. Attempts to correlate negative tuberculin test results with generalized anergy (e.g., negative skin test responses to mumps, *Candida,* and tetanus toxoid) have not been illuminating, and such "anergy testing" is therefore not recommended (see "Tuberculin Skin Testing and HIV Infection").[178] Intraobserver reliability in reading reactivity may vary by as much as 3 mm, causing some classification uncertainty if induration is close to the cutoff value.[179] TST results are negative during the first 3 to 9 weeks of initial infection.

Variant ("Delayed") Tuberculin Reactivity

An unusual form of tuberculin response (so-called delayed reactivity) has been described among Indochinese immigrants. This involves induration of less than 10 mm at 48 to 72 hours, which increases to greater than 10 mm when the skin test is read again at 6 days.

Loss of Tuberculin Reactivity

Earlier in the 20th century, lifelong tuberculin positivity was maintained by frequent reexposure to tubercle bacilli or continued active disease. However, a positive tuberculin test will revert to negative unless restimulated by new aerosol inocula or persisting infection. In one tuberculin survey, 8.1% of positive reactors reverted to true negative when retested 1 year later (Table 249.5).[180] Persons with a history of a positive skin test result can be safely retested. Two negative tests a week apart (to exclude boosting) indicate true negativity.

TABLE 249.5 Annual Tuberculin Conversion Rates (Positive to Negative) According to Age Groups, Victoria County, Canada, 1959–1962

AGE GROUPS	POSITIVE REACTORS RETESTED AFTER 1 YEAR	NO. OF REVERSIONS TO NEGATIVE	REVERSION RATE (%)
0–19	99	22	22.2
20–39	200	16	8.0
40–59	525	25	4.8
60 and older	377	34	9.0
TOTAL	1201	97	8.1

From Grzybowski S, Allen EA. The challenge of tuberculosis in decline: a study based on the epidemiology of tuberculosis in Ontario, Canada. Am Rev Respir Dis. *1964;90:707–720.*

Tuberculin Skin Testing and HIV Infection

During HIV infection, tuberculin reactivity decreases as the CD4 cell count falls. One study of patients with active TB demonstrated 10 mm or greater induration in response to 5 TU in only 60% of persons with HIV infection and in 35% of those with AIDS.[181] Induration of 5 mm in persons with HIV infection is sufficient to warrant treatment of LTBI (see "Treating Latent Tuberculous Infection in Persons With HIV Infection").[182] Testing simultaneously for cutaneous anergy with ubiquitous antigens such as mumps, tetanus toxoid, and *Candida* is not recommended. Because of lack of standardization and reproducibility, and variable risk for TB among anergic persons, cutaneous anergy does not predict *M. tuberculosis* infection, and responsiveness to control antigens but not PPD does not exclude tuberculous infection (see "Treatment of Latent Tuberculous Infection").

Interferon-γ Release Assays for Latent *M. tuberculosis* Infection

Three interferon-γ release assays are FDA approved to detect latent *M. tuberculosis* infection. These tests measure host cellular immune response to *M. tuberculosis* antigens in whole-blood samples. These tests overcome several limitations of the TST, including false-positive results from environmental mycobacterial exposure or BCG vaccination, operator-dependent variability in test placement and reading, and the need for a follow-up visit to examine skin induration. Unlike the TST, blood tests do not require a follow-up visit for determination of results. The CDC recommends that these tests be used preferentially for most populations rather than the TST to detect LTBI.[170,183] In a patient population at increased risk for tuberculous disease, such as candidates for organ transplantation, serial testing with interferon-γ release assays before transplant has revealed conversion and reversion rates of reactivity that undermine the ability to determine true-positive rates.[184] These conversion and reversion rates have also been studied in South African adolescents. Concordance with the development of disease was good for conversion but not reversion, and incident TB was eightfold higher in reverters than in those with consistently negative results.[185] In a study in 17 European centers, TST, QFT-GIT test, and T-SPOT.TB test (Oxford Immunotec, Abingdon, United Kingdom) (see later) were compared for their performance and comparability in immunocompromised patients (HIV infection, chronic renal failure, rheumatoid arthritis, solid-organ and hematopoietic stem cell transplant patients) versus immunocompetent controls. TB incidence was low but higher in HIV-infected persons with a positive TST result than with either interferon-γ release assay. Treatment was effective in preventing disease.[186]

The QFT-GIT test quantifies release of interferon-γ from lymphocytes in whole blood incubated overnight with three *M. tuberculosis* antigens: the early secretory antigen target-6 (ESAT-6), culture filtrate protein-10 (CFP10), and TB7.7. These three proteins are absent from BCG and from most other nontuberculous mycobacterial species. Blood must be tested within 12 hours of collection. The FDA has approved the QFT-GIT test, and the CDC has established guidelines for its use to detect latent

TB.[183] According to the CDC, the QTF-GIT may be used in all circumstances in which TST is used and is often the preferred test.

The next-generation QuantiFERON-TB Gold Plus (QFTPlus) test has been FDA approved and is starting to be rolled out in the United States and Europe. QTFPlus contains a fourth tube with short peptides from CFP-10, which are designed to elicit increased $CD8^+$ T-cell production of interferon-γ. Furthermore, the next generation assay does not use the TB7.7 antigen. Four milliliters of blood are divided into four tubes. After incubation, if either antigen tube produces more interferon-γ than the nil (no antigen) tube, the result is positive. If the mitogen tube does not produce more interferon-γ than the nil tube, the result is indeterminate. There are only a few studies comparing the QFT-GIT and QTFPlus, and these suggest comparable results or perhaps slightly improved sensitivity of the QTFPlus in elderly or immunocompromised populations.[187–189] Larger studies in diverse populations are needed.

Another assay, the T-SPOT.TB (Oxford Immunotec, Abingdon, United Kingdom) is an ELISPOT assay that also detects T-cell responses to *M. tuberculosis* antigens ESAT-6 and CFP10.[190] It has been approved for use by the FDA in the United States. In an investigation of a large school outbreak of TB in the United Kingdom, the ELISPOT had 89% agreement with the TST and correlated more closely with exposure to the index case than the TST. Its performance for health care worker screening in US hospitals demonstrates high concordance among serial tests and high test completion rates.[191]

Data on the performance of the interferon-γ release assays in children are limited, and guidelines differ on the optimal test. The ATS/IDSA/CDC guidelines recommend use of the same criteria as in adults for children aged 5 years and older. For children younger than 5 years, these guidelines recommend the TST, recognizing the limited data. Of note, 5 mL of blood is required for the interferon-γ release assay, which may be excessive for small children.[183] The American Academy of Pediatrics states that the TST is recommended for children younger than 2 years. Either the TST or an interferon-γ release assay is acceptable for children aged 2 years and older, but for BCG-vaccinated children aged 2 years and older the γ release assay is preferred.[192] Of note, a negative TST or γ release assay does not exclude active disease, and children are at high risk of rapid progression to TB disease.

PATHOGENESIS

Airborne droplet nuclei containing tubercle bacilli reach the terminal air spaces where multiplication begins. The initial focus is usually subpleural and in the midlung zone (the lower parts of the upper lobes and the upper parts of the lower and middle lobes), where greater airflow favors deposition of bacilli. (Very rarely, nonpulmonary initial foci will involve abraded skin, the intestine, the oropharynx, or the genitalia, all associated with foci in regional lymph nodes.)

The initial pulmonary focus is typically single, although multiple foci are present in about one-fourth of cases. The bacteria are ingested by alveolar macrophages, which may be able to eliminate small numbers of bacilli. However, bacterial multiplication tends to be mostly unimpeded, destroying the macrophage. Bloodborne lymphocytes and monocytes are attracted to this focus, the latter differentiating into macrophages, which ingest bacilli released from degenerating cells, and pneumonitis slowly develops. Infected macrophages are carried by lymphatics to regional (hilar, mediastinal, and sometimes supraclavicular or retroperitoneal) lymph nodes, but in the nonimmune host may spread hematogenously throughout the body. During this occult preallergic lymphohematogenous dissemination, some tissues favor retention and bacillary multiplication. These include the lymph nodes, kidneys, epiphyses of the long bones, vertebral bodies, and juxtaependymal meningeal areas adjacent to the subarachnoid space, but, most important, the apical-posterior areas of the lungs. Before the development of hypersensitivity (tuberculin reactivity), microbial growth is uninhibited, both in the initial focus and in metastatic foci, providing a nidus for subsequent progressive disease in the lung apices and in extrapulmonary sites, either promptly or after a variable period of latency.

Evolution of the Primary Infection

Tuberculin positivity appears 3 to 9 weeks after infection and marks the development of cellular immunity and tissue hypersensitivity. In most instances the infection is controlled, with the only evidence of infection being a positive skin test result. In a minority of cases, antigen concentration in the primary complex, consisting of the initial pulmonary focus (the Ghon focus) and the draining regional nodes, will have reached sufficient size that hypersensitivity results in necrosis and radiographically visible calcification, producing the Ranke complex (parenchymal and mediastinal calcific foci). Much less commonly, pulmonary apical and subapical metastatic foci contain sufficient bacilli that necrosis ensues with the onset of hypersensitivity, producing tiny calcific deposits (Simon foci) in which viable bacilli may persist.

The onset of tuberculin hypersensitivity may be associated with erythema nodosum or phlyctenular keratoconjunctivitis (a severe unilateral inflammation of the eye), although these manifestations are unusual in the United States. The primary complex may progress. In children, large hilar or mediastinal lymph nodes may produce bronchial collapse with distal atelectasis or may erode into a bronchus and spread infection distally. Also, typically in children but also in those infected in advanced age[97] and HIV-infected patients,[193] the primary focus may become an area of advancing pneumonia, so-called progressive primary disease, which may cavitate and spread via the bronchi. Again, typically in the very young, preallergic lymphohematogenous dissemination may progress directly to hyperacute miliary TB as a result of caseous material directly reaching the bloodstream, either from the primary complex or from a caseating metastatic focus in the wall of a pulmonary vein (Weigert focus). Hematogenous dissemination in the very young is often followed within weeks by tuberculous meningitis. In adolescents and young adults, the subpleural primary focus may rupture, delivering bacilli and antigen into the pleural space to produce serofibrinous pleurisy with effusion. Overwhelmingly, the most important consequence of preallergic lymphohematogenous dissemination is seeding of the apical-posterior areas of the lung, where disease may progress without interruption or after a latent period of months or years, resulting in pulmonary TB of the adult or reactivation-type TB (endogenous reinfection).

Primary (Childhood) and Reinfection (Adult) Tuberculosis

The traditional terms *primary* or *childhood pulmonary tuberculosis* and *reinfection* or *adult pulmonary tuberculosis* followed radiographic observations early in the 20th century when initial (primary) infection in childhood was believed to be universal.[194] Children's radiographs characteristically demonstrated large mediastinal or hilar lymph nodes with inconspicuous pneumonitis in the lower or middle lung field, whereas in adolescents and adults, apical or subapical infiltrates, often with cavitation and no hilar adenopathy, were the rule. These clinical and radiographic differences are due to age-related immunologic factors. Although many primary infections in adolescents and adults resemble primary infection in childhood, in others in this age group, an apical-posterior, metastatic pulmonary focus progresses within weeks to "adult"-type pulmonary disease, whereas the initial focus in the lower lung field and hilar nodes involutes undetected.

CHRONIC PULMONARY TUBERCULOSIS

Apical Localization

In adults, apical localization of pulmonary TB has often been attributed to the hyperoxic environment of the apices and the aerobic nature of the organism. A more plausible theory attributes it to deficient lymphatic flow at the lung apices, especially the posterior apices, where the pumping effect of respiratory motion is minimal. Deficient lymph traffic would favor retention of bacillary antigen and, when hypersensitivity ensues, tissue necrosis. Apical-posterior localization with a tendency to cavitation and progression is characteristic of pulmonary TB in adolescents and adults. In contrast, infection acquired by the elderly often causes nondescript lower lobe pneumonia similar to progressive primary infection of childhood.[97]

Endogenous Versus Exogenous Reinfection

In countries where the level of contagion is low, most cases of active TB reflect reactivation of latent foci.[194] However, when contagion is high, exogenous reinfection may be more common.[77,195] The temporal dynamics of relapse versus reinfection in high-prevalence settings suggest

that most relapse occurs early after completion of therapy, but reinfection becomes predominant 1 year after completion of therapy, accounting in total for at least half of recurrence.[196] In a large study in Malawi, 85% of recurrent TB cases in HIV-infected people were due to reinfections with a new *M. tuberculosis* strain, and HIV-infected adults were 20 times more likely to develop disease from reinfection than HIV-negative people.[197] Airflow in the apical-posterior areas of the lung is low, but when inhaled droplet nuclei reach that location, as is more likely with high levels of contagion, bacillary multiplication will be favored by the same local factors that enhance multiplication of bloodborne organisms. Support for this comes from a study from India that showed that disease in household contacts of active cases was most common in the middle-aged and elderly, who were certain to have been previously infected,[198] and from molecular epidemiology data.[96] Repeated inhalational exposures to tubercle bacilli maintain tissue hypersensitivity and cellular immunity, making superinfection more difficult; however, when the airborne inoculum is large, or in immunocompromised hosts, superinfection may occur.

Influence of Age on Tuberculous Infection

Many of the best clinical descriptions of TB come from the preantimicrobial era, when infection occurred early in life and cellular immunity was maintained by frequent exposure to tubercle bacilli. However, in industrialized countries, infection more often occurs later in life and cellular immunity may wane in the absence of restimulation. Accordingly, clinical patterns have changed. At one time, most patients were adolescents and young adults with apical cavitary disease. In developed countries, the incidence of TB (cases per 100,000) is now greatest in older persons, in whom hypersensitivity is less marked and in whom the clinical manifestations may be different and more subtle. Hypersensitivity and cellular immunity likely become less vigorous with age (see "Epidemiology").

Infection in Infancy and Childhood

Infection in infants often results in disease with local progression and dissemination (miliary-meningeal disease). The younger the patient, the greater the risk for progressive disease until the age of 5 years. From age 5 until puberty is a time of relative resistance to progressive disease, although not to infection. When disease occurs, it is usually the childhood type of pulmonary TB. Involvement of lymph nodes, bones, and, less commonly, other progressive extrapulmonary foci may develop, but TB confined to the lung in this age group usually heals spontaneously. The short-term prognosis in these individuals is good even if untreated, but there is a high frequency of relapse with chronic cavitary TB when the more disease-prone periods of adolescence and young adulthood arrive.[199]

Infection in Adolescence and Young Adulthood

Clinical disease developing after infection in adolescence or young adulthood may resemble childhood infection (lower lung field pneumonitis, hilar adenitis) but with less parenchymal and hilar calcification (Fig. 249.2). This is particularly the case in immunocompromised patients, including those living with HIV.[194] Rarely, the radiographic picture may be mixed, with features of childhood disease subsiding while chronic upper lobe (adult) disease progresses. However, disease in this age group frequently first appears as chronic upper lobe TB with no clinical manifestations of childhood disease. The tendency toward apical cavitation soon after the initial infection appears soon after puberty and is marked in young adults.[172] Evidence suggests that adolescents (and adults) may have a subclinical tuberculous pneumonia for months prior to the onset of overt disease.[200,201] Because most young people in industrialized countries are tuberculin negative (Fig. 249.3), most pulmonary TB in adolescents and young adults is due to recent initial infection rather than to late progression of childhood infection.

Infection in Midadulthood

Infection acquired during the middle years has a much better immediate and probably long-term prognosis than infection acquired in the teens and early 20s, presumably because of a reduced tendency to tissue necrosis.[199,202] One study demonstrated progression from infection to cavitary TB in 23% of patients infected from 15 to 19 years of age, 13% of those infected from 20 to 24 years of age, 4% of those infected from 25 to 29 years of age, and only 2% of those infected after 30 years of age. Progression occurred in 3 months in many and within 1 year in most.[203] (Elderly individuals were not included in the study.)



FIG. 249.2 Chest radiograph showing marked right hilar lymphadenopathy and lower lobe opacity in a 58-year-old woman with primary tuberculosis.

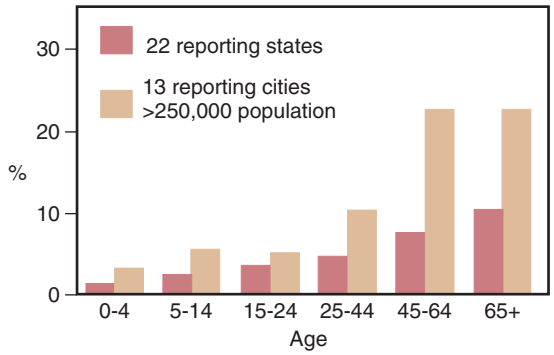

FIG. 249.3 Percentage of positive tuberculin reactors by age, selected areas, United States, 1979. *(From Centers for Disease Control and Prevention [CDC], Tuberculosis Control Division. Tuberculosis in the United States, 1979. Atlanta, GA: CDC; 1981:4–31.)*

Infection in Old Age

In the elderly, infection acquired years earlier can progress as age compromises immunity, producing typical apical-posterior disease. Studies of TB in nursing homes, however, have demonstrated that elderly patients are often tuberculin negative, either because they had never been infected or because remote past infection had been completely cleared, with a loss of tissue hypersensitivity. Such tuberculin-negative persons are susceptible to new infection; and if this occurs, they acquire active disease with a frequency similar to that of adolescents. This is typically a nondescript, poorly resolving pneumonitis in the lower or middle lobes or anterior segments of the upper lobes, sometimes with pleural effusion and resembling primary infection in children except with much less hilar-mediastinal lymphadenopathy.[97] Even with prompt diagnosis and treatment, TB after age 65 appears to be more frequently associated with death.

Late Hematogenous Tuberculosis

Chronic TB is probably always associated with recurrent abortive episodes of hematogenous spread. However, when aging or other factors compromise cellular immunity, such episodes may become progressively more frequent, producing the subtle and often fatal syndrome of late hematogenous or progressive generalized TB.

Intercurrent Events

General stress, poor health, and malnutrition favor progression of infection. Therapy with corticosteroids or other immunosuppressive agents compromises host defenses, as do hematopoietic-reticuloendothelial diseases, particularly malignancies. TB in complicating myeloproliferative disorders may cause confusion because disseminated TB can cause aplastic anemia, thrombocytopenia, leukopenia, and leukemoid reactions that may mimic leukemia. However, most patients with TB and hematologic findings that suggest leukemia will have both diseases. Inflammatory diseases such as rheumatoid arthritis and systemic sclerosis are often accompanied by immunologic derangements that may decrease immunocompetence even prior to the use of immunosuppressive therapies. Patients with systemic sclerosis in Taiwan had a significantly higher risk for TB and pulmonary TB, independent of systemic sclerosis treatment, compared with matched control subjects drawn from the National Health Insurance database (incidence rate ratios, 2.81 and 2.53, respectively).[204] Biologic TNF-α inhibitors (e.g., infliximab, etanercept, and adalimumab) that are prescribed for rheumatoid arthritis and other inflammatory diseases increase the likelihood of reactivation TB, including extrapulmonary and disseminated disease.[205–209]

The postgastrectomy state, jejunal-ileal bypass surgery, and end-stage renal disease are all risk factors for progression (see "Treatment of Latent Tuberculous Infection"). Viral illnesses, particularly in children, may predispose to progression of infection. Destructive local pulmonary processes such as lung abscess, carcinoma, cavitary histoplasmosis, and pulmonary resection occasionally are followed by activation of previously quiescent pulmonary foci. The development of bone and joint TB after physical injury and late generalized hematogenous TB after major trauma both illustrate that the balance between host and infection can be altered by both systemic factors and local physical disturbance.

Tuberculosis in People With HIV Infection

The earliest descriptions of TB with AIDS emphasized the very great risk for reactivation of remote infection as a result of progressively compromised cellular immunity. Among Haitians, all of whom were likely infected with *M. tuberculosis* in childhood, HIV infection was associated with development of active TB in 60%.[103] Subsequent studies of HIV-positive and tuberculin-positive methadone clinic patients in New York City showed that active TB developed in 8% yearly.[136]

TB remains the leading cause of death in HIV infection in reports from highly endemic areas, despite the introduction of ART.[210] As discussed in "Epidemiology," HIV-infected patients are predisposed not only to reactivation of remote infection but also to rapid progression of recently acquired infection.[137,138] It is unclear whether AIDS increases susceptibility to acquisition of new infection. Provision of ART restores immune function and decreases risk for TB, although not to levels seen among HIV-negative individuals.[211]

PULMONARY TUBERCULOSIS

Primary Tuberculosis in Childhood

The initial focus of pulmonary TB in children occurs most frequently in the midlung zones but may develop anywhere. At the time of tuberculin conversion, fever and lassitude and rarely erythema nodosum or phlyctenular keratoconjunctivitis may be present briefly. Clinical manifestations of the initial infection depend on the age of the patient. It is most often symptomatic in childhood because of an age-related tendency to extensive regional lymphadenitis. This may compress central bronchi, causing a brassy cough or atelectasis of a segment or lobe, or may rupture into a bronchus, seeding infection distally and causing pneumonia. In the very young, there is a tendency to progressive lymphohematogenous dissemination with miliary-meningeal disease. Uncommonly, again more in infants, local progression of the initial pneumonia results in progressive primary disease, which may spread via the bronchial tree or the bloodstream. However, most infections during the relatively disease-resistant period of childhood (ages 5–12 years) are usually nonprogressive, with healing by involution and encapsulation.[199] Progression, if any, occurs in extrapulmonary metastatic foci or with the development of apical-posterior pulmonary TB, usually when the patient reaches puberty or young adulthood.

Postprimary (Adult-Type) Pulmonary Tuberculosis

Primary infection in adolescents and adults (1) may occur without symptoms and signs, (2) may produce a typical primary complex, or (3) may result in typical chronic pulmonary TB without a demonstrable primary complex. Any pneumonic infiltrate, especially if associated with a hilar or mediastinal node, may represent primary infection. These lesions may undergo caseation, liquefaction, and bronchogenic spread just as with classic chronic pulmonary TB.

Postprimary pulmonary TB in adults is usually asymmetrical and characterized by caseation, fibrosis, and frequently cavity formation. It begins as a patch of pneumonitis in the subapical-posterior aspect of an upper lobe, usually just below the clavicle or first rib (Fig. 249.4). A less frequent location is the apex of the lower lobe, where it may be obscured by the heart and hilum on a chest radiograph but readily visible on a chest CT image. The inflammatory response in the sensitized host produces a fibrin-rich alveolar exudate containing a mixture of inflammatory cells. Serial radiographs may demonstrate waxing and waning and sometimes complete regression. If the process accelerates, however, an area of caseous necrosis surrounded by epithelioid cells, granulation tissue, and eventually fibrosis develops. This may arrest by inspissation of the caseous area, fibrous encapsulation, and healing. Caseation, however, tends to liquefy and drain into the bronchial tree, spreading bacillary contents by coughing. The cavity is prevented from collapsing by the fibrous capsule and the inelasticity of the surrounding lung. The pulmonary cavity favors bacillary multiplication to enormous titers, 5 to 6 logs greater than in noncavitary lesions. The progressive nature of pulmonary TB is due to (1) the tendency of apical caseous foci to liquefy, (2) the enormous concentrations of organisms in the resulting pulmonary cavities, and (3) spread of this bacilli-rich material through the bronchial tree. Progression from minimal infiltrate to far-advanced cavitary disease can occur within a few months (Fig. 249.5).



FIG. 249.4 Chest radiograph showing a right apical infiltrate in a patient with moderately advanced postprimary tuberculosis.



FIG. 249.5 Chest radiograph showing far-advanced bilateral apical cavitary pulmonary tuberculosis in a 32-year-old woman from Ethiopia.

Coughing aerosolizes infectious cavity secretions that may distribute widely throughout the lung (bronchogenic spread). New foci eventually develop that, in turn, may undergo caseation, fibrosis, and healing or slough, resulting in new cavities. The segment or lobe containing the initial cavity is typically involved first with scattered patchy disease, but the contralateral apex is often secondarily involved with progressive disease. Bronchogenic spread may establish foci of infection in the lower lobe and anterior portions of the upper lobe, producing a polymorphous mottling on a chest radiograph, but these are usually nonprogressive and heal with fibrosis. Although hematogenous spread from an established pulmonary focus can occur, it is usually limited by hypersensitivity-induced thrombosis.

The highly infectious secretions from a cavity always cause some degree of endobronchial inflammation and ulceration, which may be extensive. Ulcerative tuberculous laryngitis is an extension of this process, as is local disease throughout the upper airways, mouth, middle ear, and gastrointestinal tract.

Mechanisms of healing are the same whether spontaneous or under the influence of chemotherapy. Without drug therapy, solid caseous foci surrounded by contracting fibrous tissue occasionally arrest. However, viable bacilli almost always persist in such lesions and can later reactivate. Before drug therapy, healing of persisting cavities never occurred, and some large, thick-walled cavities in shrunken fibrotic lobes could persist for years with minimal symptoms while remaining highly infectious (chronic fibroid TB). With drug therapy, cavities may resolve or they may heal but remain open, sometimes with complete reepithelialization. The major risk for such persistent cavities is superinfection with organisms such as *Aspergillus* or nontuberculous mycobacteria.

Lower Lobe and Endobronchial Tuberculosis

These terms *lower lobe tuberculosis* and *endobronchial tuberculosis* are not appropriate for chronic pulmonary TB of the ordinary kind that happens to involve the apex of the lower lobes. In adults, *lower lung field tuberculosis* describes three different but often associated processes: (1) progressive lower lobe pneumonia in recently infected older individuals; (2) endobronchial TB, often with parenchymal consolidation-collapse; and (3) TB complicating AIDS. These three processes differ from postprimary TB radiographically, and the former two have a low bacterial content.

Progressive Lower Lobe Disease in Older Persons

TB in an older individual frequently causes a nonspecific, non-resolving pneumonitis in the lower or middle lobes or anterior segments of the upper lobes, similar to primary infection in childhood, except with less hilar and mediastinal adenopathy.[127] TB should be considered in any slowly or non-resolving pneumonitis in an older patient.

Endobronchial Tuberculosis

In the past, superficial endobronchial lesions resulting from infectious secretions were common, sometimes spreading to the larynx and beyond or causing obstructive atelectasis with collapse. These superficial lesions responded quickly to chemotherapy. Now endobronchial disease is most frequently caused by rupture of an adjacent node into the bronchial tree, or less frequently by direct spread from parenchymal TB.[212] The chest radiograph typically reveals collapse-consolidation but may be normal in as many as 20% of cases, although a chest computed tomography (CT) scan may be abnormal. Sputum smear results are usually negative, but the bronchial lavage result is frequently positive.[212]

The usual bronchoscopic findings are mucosal edema, ulceration, and narrowing, but in 30% of cases, bulky granulation tissue may resemble bronchogenic carcinoma. Endobronchial involvement is usual in lower lung field TB,[213] and endobronchial ulcers occasionally produce positive sputum smears with normal chest radiographs. Large parenchymal cavities may be present, at times associated with an air-fluid level resulting from intermittent obstruction and poor drainage. Bronchial perforation by tuberculous nodes with endobronchial mass formation and lower lobe consolidation has been observed in patients with AIDS.

Calcified nodes can erode into the bronchial tree and cause hemoptysis, expectoration of calcific material (lithoptysis), or spread of previously quiescent bacilli. The atelectatic pneumonitis, which may result with or without new active disease, is most frequently seen in the anterior segment of the upper lobe and medial segment of the middle lobe.

Pulmonary Tuberculosis in AIDS

TB as first described in persons with advanced AIDS was characterized by middle or lower lung field location, absence of cavitation, a greatly increased incidence of extrapulmonary disease, and usually a negative TST.[214,215] It resembled childhood TB except for a negative TST and less prominent hilar and mediastinal lymphadenopathy. A later study of TB in a much less ill population of persons in TB clinics unaware of their HIV infection found a clinical picture no different from ordinary reactivation TB in HIV-negative patients, with apical, often cavitary, disease and tuberculin positivity being the rule. The clinical picture of TB during HIV infection is determined by the degree of immunocompromise (Table 249.6). Although HIV-positive persons may have symptoms similar to those seen in HIV-uninfected persons, presentations can also be atypical or patients can be asymptomatic.[216]

HIV-positive persons may also acquire new infection from others in their environment, a risk that was first observed among patients with advanced AIDS living in HIV wards and domiciles. The clinical picture in these patients can include diffuse, rapidly progressive, noncavitary disease that is often fatal.[137,138] It is unclear whether HIV-infected persons are more likely to become infected after exposure to *M. tuberculosis* than HIV-uninfected persons, but once infected, they are more likely to progress to active disease. HIV-infected persons do not appear to be more likely to transmit *M. tuberculosis* than HIV-uninfected persons,[130] but TB disease, and therefore *M. tuberculosis* transmission, can occur even when the source patient has a normal chest radiograph or a negative acid-fast sputum stain.[217,218] The Cepheid GeneXpert MTB/RIF or the more recent Ultra version should assist in rapid detection of *M. tuberculosis* in sputum of such patients.

It is important to consider TB in HIV-positive individuals with respiratory failure in the intensive care unit. Patients may have adult respiratory distress or sepsis syndrome with multiple organ system failure. The diagnosis can be made readily by stain and culture of sputum or blood or both.

TABLE 249.6 Clinical Manifestations of Active Tuberculosis in Early Versus Late HIV Infection

	EARLY HIV INFECTION	LATE HIV INFECTION
Tuberculin test result	Usually positive	Usually negative
Adenopathy	Common	Unusual
Pulmonary distribution	Upper lobe	Lower and middle lobe
Cavitation	Often present	Typically absent
Extrapulmonary disease	10%–15% of cases	50% of cases

[a]For practical purposes, "early" and "late" may be defined as $CD4^+$ cell counts greater than 300 cells/mm^3 and less than 200 cells/mm^3, respectively.
HIV, Human immunodeficiency virus.

Tuberculomas

Asymptomatic rounded lesions may develop as the parenchymal residua of the initial infection or as an upper lobe caseous lesion encapsulates (Fig. 249.6). These are ordinarily static, but larger ones may cavitate to produce new spread of disease. In some persons, excessive fibrosis occurs with small caseous or granulomatous residua becoming surrounded by concentric layers of fibrous tissue, at times with central or concentric calcification resembling histoplasmomas. Most such lesions are stable, but important in that they can be confused with cancer.



FIG. 249.6 Chest radiograph demonstrating multiple bilateral pulmonary tuberculomas in an asymptomatic 35-year-old man from Poland.

Symptoms

Initial infection with *M. tuberculosis* is usually asymptomatic, although some persons may have a relatively brief period of symptoms.[219,220] Early pulmonary TB is asymptomatic and may be discovered by chance on a chest radiograph. As the bacillary population grows, however, nonspecific constitutional symptoms such as anorexia, fatigue, weight loss, chills, fever, and night sweats may ensue. A productive cough is usually present. Coughing to clear cavitary secretions is usually mild and well tolerated but may become bothersome when bronchial involvement is extensive. The mucopurulent sputum is nonspecific, and both cough and sputum may be ignored by patients with chronic bronchitis. Hemoptysis resulting from caseous sloughing or endobronchial erosion is usually minor but connotes advanced disease. Sudden massive hemoptysis resulting from erosion of a pulmonary or bronchial artery by an advancing cavity (Rasmussen aneurysm) was an occasional terminal event in the predrug era but is now seldom seen. In inactive disease, brisk hemoptysis may be due to *Aspergillus* superinfection of residual cavities (aspergilloma). Chest pain is usually due to extension of inflammation to the parietal pleura. Pleural involvement adjacent to an established cavity tends to cause visceral-parietal pleural symphysis without effusion (dry pleurisy). Serofibrinous pleurisy with effusion is often an early postprimary event but may also complicate chronic pulmonary TB. Rarely, chest pain leads to discovery of tuberculous empyema. Symptoms often pertain to site of disease, such as painful pharyngeal ulcers; indolent and nonhealing ulcers of the mouth or tongue; hoarseness and dysphagia that are due to laryngeal involvement; tuberculous otitis media; gastrointestinal symptoms that are due to enteric ulceration, perforation, or mass formation; or anal pain that is due to tuberculous perirectal abscess and fistula formation. Lower lobe TB resulting from bronchial lymph node perforation may be associated with lithoptysis (stone spitting) and characteristically produces symptoms of severe endobronchial disease with serious cough and often hemoptysis.

Physical Examination

Physical findings are not specific, and in general do not reflect the extent of the illness and may be absent in spite of extensive disease. Dullness with decreased fremitus may indicate pleural thickening or fluid. Crackles may be appreciated only when the patient breathes in after a short cough (posttussive rales) and may persist long after healing, owing to permanent distortion of small airways. With large lesions, signs of consolidation with open bronchi (whispered pectoriloquy, tubular breath sounds) can be heard. Distant hollow breath sounds heard over cavities are called *amphoric,* like the sound made by blowing across the mouth of a jar (amphora).

Radiographic Findings

The chest radiograph is central to diagnosis, determination of the extent and character of disease, and evaluation of the response to therapy. Chest CT is a valuable adjunct to routine chest radiography. Certain patterns are highly suggestive, although not diagnostic, of TB. A patchy or nodular infiltrate in the apical- or subapical-posterior areas of the upper lobes or the superior segment of a lower lobe is highly suggestive of early chronic TB, especially if bilateral or associated with cavity formation (see Fig. 249.4). Cavities may be more apparent with CT or magnetic resonance imaging (MRI). On routine chest radiography, cavitation in the apical segment of the lower lobe may be obscured by the heart shadow and, in the lateral view, by the dorsal spine. Air-fluid levels are uncommon in upper lobe TB (less than 10%) but occur more frequently in lower lobe cavities. Fresh bronchogenic spread from recent spillage of infectious cavity contents appears as multiple, discrete, soft, fluffy infiltrates, or a confluent infiltrate adjacent to a cavity, or in the middle or lower lung field on the same or opposite side. These latter types of spread are seldom progressive and heal by rounding up into more discrete lesions with regular borders.

Both chronicity and histopathologic features can be estimated based on the chest radiograph. Granulomatous lesions tend to be small, nodular, and sharply defined, indicating few organisms and a good host response. Exudative lesions (pneumonic) tend to have soft, indistinct borders and are more unstable. Fibrotic scars have sharp margins and tend to contract. Caseation causes increased density. Healing exudative lesions first become smaller and less dense and then, as scarring develops, become more sharply defined. Lower lobe TB is nonspecific radiographically. Other patterns include poorly resolving pneumonia, atelectasis, mass lesions, and large cavities with air-fluid levels; initial misdiagnosis is the rule. Pneumonia associated with hilar adenopathy should always suggest primary TB, regardless of the lung fields involved and patient age. Pulmonary TB can also occur in persons with a normal chest radiograph; up to 6% of HIV-seronegative and 22% of HIV-seropositive persons with pulmonary TB have a normal chest radiograph.[217,221–223]

Laboratory Findings

Normocytic, normochromic anemia, hypoalbuminemia, and hypergammaglobulinemia are characteristic of advanced disease. The white blood cell count is usually normal but may be between 10,000 and 15,000

cells/mm^3. Many HIV-negative patients with active TB have CD4$^+$ T-cell counts much lower than 500 cells/L, which return toward normal with treatment.[224] Monocytosis is seen in less than 10% of cases. Hematuria or sterile pyuria should suggest coexisting renal TB. Hyponatremia with features of inappropriate secretion of antidiuretic hormone is characteristic of tuberculous meningitis but also occurs with isolated pulmonary involvement. Hyponatremia should also suggest associated Addison disease due to adrenal TB. Hypercalcemia is also seen during pulmonary TB, usually in the first weeks of therapy.

Diagnosis

A strong presumptive diagnosis can often be made based on the radiographic pattern. A positive sputum smear, usual in extensive disease, provides additional evidence in support of a diagnosis. However, an intercurrent cancer or lung abscess, particularly in the apices, may erode a quiescent focus of TB and cause brief shedding of tubercle bacilli without causing active disease. The best diagnostic sputum specimen is an early morning sample. Although two sputum specimens are sufficient in some settings, three specimens are recommended because of greater sensitivity.[225] Aspiration of gastric contents, obtained early in the morning to sample sputum swallowed during sleep, is an alternative when sputum is not produced. The specificity of gastric aspiration is diminished by the presence of nontuberculous mycobacteria but may be higher in children than adults. Sputum induction by hypertonic saline aerosols is also an effective substitute in ambulatory patients; the yield is comparable to that of fiberoptic bronchoscopy.[226] Pulmonary TB in patients with AIDS is often noncavitary and therefore may have a lower bacillary burden than in HIV-seronegative persons. The high prevalence of smear-negative TB in HIV-infected persons underscores the importance of obtaining sputum culture, even in resource-poor settings.[227] Positive sputum smears are much more likely to indicate *M. tuberculosis* than *Mycobacterium avium* complex, even in areas where both diseases are common.[228] NAAT, described previously, can provide a rapid distinction between the two infections in smear-positive respiratory secretions. (Additional diagnostic assays, including NAAT and testing for *M. tuberculosis* antigens in urine, are discussed under "Microbiology.")

A negative tuberculin reaction or interferon-γ release assay does not exclude TB; skin test anergy and negative assays can occur in the setting of active disease.[177,229,230] The TST and interferon-γ release assays are insensitive in immunocompromised persons, such as HIV-infected persons with less than 100 CD4$^+$ T cells/mm^3.[230,231] Granuloma formation at histologic examination, even with AFB, is still only strong presumptive evidence, because similar findings may be produced by MOTT. Granulomas in the absence of AFB can be seen with other infectious diseases (e.g., histoplasmosis) and noninfectious causes (e.g., sarcoidosis, autoimmune disease). Definitive diagnosis requires culture and speciation.

Fiberoptic Bronchoscopy

Diagnostic fiberoptic bronchoscopy with transbronchial biopsy and bronchoalveolar lavage should be considered when sputum tests are inconclusive and the suspicion of TB remains high. In AIDS patients with pulmonary TB but negative smears, bronchoscopy yields a rapid diagnosis (based on smears and histologic features) in only one-third of cases.[232–234] Thus, a negative acid-fast stain at bronchoscopy does not exclude TB, although such cases are certainly less contagious.

Tuberculosis Diagnosed at Autopsy

From 1985 through 1988, 5.1% of all reported TB cases in the United States were diagnosed at death.[235] Usually, the patient was elderly and had underlying diseases. Both nonresolving pulmonary processes and extrapulmonary TB, particularly chronic miliary and meningeal disease, are often present in such patients. The usual reason for failure to diagnose TB in such patients is failure to look for it; delays in TB diagnosis, as can occur when persons receive fluoroquinolones, can also contribute.[236]

Tuberculosis and Cancer

It has been estimated that 1% to 5% of TB patients also have cancer, most being male smokers. It is possible that cancer can arise in tuberculous scars, and it is certain that cancer can erode old quiescent tuberculous foci, causing active disease. However, in many patients the diseases will be anatomically remote. No one cancer cell type predominates.

When TB and cancer occur together, diagnosis of the latter is often difficult but should be kept in mind in older men with TB who smoke, and sputum cytologic studies should be performed. There are certain radiographic findings that suggest concomitant cancer, such as progression of one area while the remainder of the lesion is regressing, a large (>3 cm) mass lesion admixed with infiltrative disease, the presence of hilar nodes in adult chronic pulmonary TB, and postobstructive atelectasis.[237]

THERAPY

Before effective drugs were available, 50% of patients with active pulmonary TB died within 2 years and only 25% were cured.[238] With the advent of chemotherapy, successful treatment became a reasonable goal in all adults. In practice, failures occur because of drug resistance or an inappropriate regimen, or, most important, because of nonadherence to therapy. For this reason the responsibility for adequate treatment has been shifted from the patient to the prescribing physician and to the health department, emphasizing the importance of directly observed therapy (DOT).[239]

Antituberculous Drugs

Additional information on dosage, adverse effects, drug-drug interactions, and other aspects of the pharmacology of antituberculous drugs is provided in Chapter 39.

Isoniazid

INH is the cornerstone of therapy and should be included in all regimens unless the *M. tuberculosis* strain is INH resistant. Occasionally, when the *M. tuberculosis* strain is resistant to low-level INH but susceptible to higher INH levels, and there is resistance to other antituberculosis drugs, INH is included in the regimen (although not considered to have full activity). The most important adverse effect of INH is hepatitis, the risk for which increases with age and underlying liver disease. Although rare, this complication can be fatal.[240] INH continues to be a cause of significant drug-induced liver injury, which may be associated with a lack of adherence to guidelines that recommend cessation of therapy based on symptoms (nausea, abdominal pain, jaundice, or unexplained fatigue) or alanine aminotransferase levels. In one study, 70% of patients who died or underwent liver transplantation continued INH for more than 7 days after meeting stopping criteria.[241]

Rifampin

RIF is the second major antituberculous agent. The most important complication of RIF is hepatitis, which is usually cholestatic. Although the risk for hepatitis is lower with RIF than INH,[242,243] hepatitis occurs more frequently in regimens containing both INH and RIF than in those containing INH alone.[244]

Of special concern is that RIF, by inducing hepatic P-450 cytochrome oxidases, causes many drug-drug interactions. Examples of drugs whose levels are reduced in the presence of RIF include warfarin, hormonal contraceptives, azole antifungal agents, methadone, corticosteroids, cyclosporine, tacrolimus, nonnucleoside reverse transcriptase inhibitors, and HIV-1 protease inhibitors. This can result in subtherapeutic levels of these drugs, necessitating either increases in their dosage or use of an antituberculosis drug other than RIF. The interaction between RIF and HIV-1 protease inhibitors can lead to substantially lower HIV-1 protease inhibitor levels, inadequate control of viral replication, and emergence of drug-resistant virus. In this setting, RIF may be replaced by rifabutin, which has comparable antituberculous activity but is a weaker enzyme inducer.[245] Adjustment of antiretroviral drugs with treatment of TB is shown in Tables 39.3 and 39.5.

Rifapentine

Rifapentine is a rifamycin antibiotic with a longer half-life than RIF or rifabutin, which allows once-weekly administration.[239,246] Once-weekly therapy, together with INH or moxifloxacin, may be considered in HIV-seronegative persons in the continuation phase of TB treatment of noncavitary pulmonary disease[239,247]; however, the rifapentine-INH regimen is not generally recommended in the recent ATS/CDC/IDSA

guidelines.[239] Once-weekly rifapentine should not be used to treat HIV-infected persons with active TB owing to the high risk for acquired rifamycin resistance.[248]

Pyrazinamide

PZA is an essential component of 6-month regimens. Early studies of PZA using high doses recorded such serious hepatotoxicity that it was largely abandoned. At currently recommended doses, PZA is associated with higher rates of hepatotoxicity and rash than other first-line drugs.[249] Cohort and case-control analyses found that adding PZA to INH and RIF increased the risk for hepatotoxicity appreciably.[250] In addition, severe hepatic injury and deaths have been reported among predominantly HIV-negative adults receiving short-course RIF plus PZA for LTBI.[251] The beneficial effect of PZA is limited to the first 2 months in regimens containing both INH and RIF.[252] Additional side effects include hyperuricemia, mild nongouty polyarthralgias that respond to nonsteroidal antiinflammatory agents, and gout. *M. bovis* is uniformly resistant to PZA.[253]

Ethambutol

EMB is included in initial treatment regimens until drug susceptibility results are returned and resistance to the other first-line drugs has been excluded, at which time it can be discontinued. It is given at a daily dose of 15 to 20 mg/kg. At 15 mg/kg the risk for ocular toxicity is low, but assessment of visual acuity and color discrimination should be performed at baseline and monthly while the patient is on therapy.

Streptomycin

STM, the first major antituberculous drug, was promptly replaced by INH as the cornerstone of therapy. Its activity is similar to that of EMB when either drug is given with INH, RIF, and PZA. Its use is limited by relatively high rates of resistance (particularly in high-incidence countries), parenteral administration, nephrotoxicity, and ototoxicity.

Fluoroquinolones

In vitro activity and some favorable trial results suggest that fluoroquinolones, particularly later-generation agents such as levofloxacin and moxifloxacin, are effective antituberculosis drugs.[254–256] However, fluoroquinolones should not be used as first-line therapy but rather should be reserved for patients who are intolerant of first-line drugs or who have drug-resistant TB, as part of a well-designed multidrug regimen. In the treatment of INH-resistant infection, a retrospective cohort study found more frequent favorable outcomes with regimens that contained a fluoroquinolone, without regard to the other components of standard TB therapy.[257] In countries where fluoroquinolones are easily available, use of quinolones by persons with undiagnosed TB is likely contributing to increased resistance in *M. tuberculosis.* In a high-prevalence country such as India, rates of quinolone resistance are already high both in new and previously treated cases.[258] The potential role of fluoroquinolones as first-line antituberculosis therapy is currently under evaluation in clinical trials. A 6-month regimen in South Africa, Zimbabwe, Botswana, and Zambia using daily (2 months) and then weekly moxifloxacin in place of INH and weekly rifapentine was as effective as a control regimen.[247] A dose of moxifloxacin 10 mg/kg/day may be insufficient for children 7 to 15 years of age being treated for MDR-TB.[259]

Unfortunately, multiple phase III trials have not supported the use of fluoroquinolones in regimens meant to shorten the duration of therapy to 4 months. In sub-Saharan Africa, a 4-month gatifloxacin-containing regimen was inferior to standard therapy.[260] The previously referenced trial in South Africa, Zimbabwe, Botswana, and Zambia contained an arm based on 4-month therapy that was inferior to standard therapy, and a trial in nine countries on three continents also found that a 4-month regimen replacing EMB or INH with moxifloxacin was inferior to standard 6-month therapy.[247,261]

Bedaquiline

Bedaquiline (Sirturo) is a diarylquinoline; its mechanism of action is adenosine triphosphate synthase inhibition. It has potent activity against *M. tuberculosis.* In persons with MDR-TB, there were improved 2-month sputum culture conversion rates among those who received bedaquiline plus optimal background therapy, compared with optimal background therapy alone (48% vs. 9%, respectively).[262] Potential toxicities include nausea and QT interval prolongation. The terminal elimination half-life is 164 days; in one study almost all patients had detectable plasma levels 96 weeks after the last dose.[263] In December 2012, bedaquiline was approved by the FDA for treatment of MDR-TB, but with a black box warning because of a greater number of deaths in the bedaquiline arm compared with those who received optimal background therapy only. Safety will be monitored closely in future trials. A report of 35 French patients who received bedaquiline for at least 1 month did not encounter drug-associated deaths.[264] With data now reported in the follow-up of patients through 120 weeks from baseline, the addition of bedaquiline led to faster culture conversion and more conversions at the end of observation. There were 10 deaths in the 79-patient bedaquiline group compared with 2 in the 81-patient placebo group, although FDA analysis of these differences noted that both deaths in the placebo group and 5 in the bedaquiline group were due to progression of disease.[265,266]

A large retrospective observational study among 428 MDR-TB patients treated with bedaquiline noted good safety and tolerability, and only one death.[267] The recommended dosage for bedaquiline is 400 mg once daily orally for 2 weeks, followed by 200 mg three times a week for 22 weeks taken orally with food to maximize absorption. Pharmacokinetic analysis suggests that penetration into the CSF is poor.[268] High cost may be a significant barrier to use of bedaquiline. Drug interactions will require further study; phase I data indicate that RIF and rifapentine increase bedaquiline clearance (4.78- and 3.96-fold, respectively).[269] Interactions with HIV therapy that includes the potent CYP3A4 inhibitor ritonavir suggest that concomitant administration with bedaquiline might require dose adjustment.[270] A provisional guideline for use of bedaquiline has been published.[271]

Second-Line Agents

Second-line agents are less efficacious or more toxic, or both, than first-line drugs. These include ethionamide, cycloserine, terizidone (available overseas), amikacin, kanamycin, capreomycin, thiacetazone, para-aminosalicylic acid (PAS), and other agents discussed in Chapter 39.

Third-Line Agents

Third-line agents have even less activity against *M. tuberculosis* than second-line agents and are usually given only as adjunctive therapy to persons with XDR-TB. These drugs have generally not been evaluated in a systematic manner for the treatment of TB. Such drugs include amoxicillin-clavulanate, clarithromycin, and linezolid (see "Treatment of Multidrug-Resistant Tuberculosis" and "Therapy for Extensively Drug-Resistant Tuberculosis"). Clofazimine is also in this group, but is now being evaluated as a more prominent treatment option given its important role in shortening treatment of MDR-TB.[272,273]

Agents Under Development

There are several new drugs that are currently under investigation for potential use in MDR-TB. These include the oxazolidinones (e.g., sutezolid [Sequella] and LCB01-0371 [LegoChem Biosciences]), nitroimidiazoles (e.g., delamanid [Otsuka] and pretomanid [Global Alliance for TB Drug Development]), and diamines (e.g., SQ-109 [Sequella]).[274] Delamanid, when given with optimal background therapy for MDR-TB, was associated with significantly higher rates of 2-month sputum culture conversion.[275] However, it did not improve cure and survival compared with optimal background therapy in a phase III trial, so WHO has recommended that it be used when other, more effective drugs, cannot be used.[276] Of note, delamanid significantly prolongs the QT interval.[277] Pretomanid (PA-824) is a prodrug, as is delamanid, and in phase II trials seems to have had its greatest efficacy in combinations containing PZA.[277,278] A phase IIb trial in drug-susceptible and drug-resistant TB of a regimen of moxifloxacin, pretomanid, and PZA had an acceptable safety profile. Bactericidal activity was superior at the 8-week assessment, although the predictive utility of that end point remains controversial.[279]

Selecting a Drug Regimen

Before RIF was available, excellent results in drug-sensitive infections were obtained with INH plus either PAS or EMB given for 18 to 24

months, "reinforced" in extensive disease by STM for the first 6 to 12 weeks. Relapse rates were unacceptably high with shorter courses. However, demonstration that RIF was equal to INH in efficacy led to studies of shorter treatment regimens. In definitive studies, drug-sensitive TB responded as effectively to 9 months of INH and RIF as to 18- to 24-month regimens not containing RIF.[280,281] It was subsequently demonstrated that 6-month regimens based on an initial 2-month intensive "bactericidal phase" of INH, RIF, PZA, and either STM or EMB, followed by a "continuation phase" of INH and RIF for 4 more months, performed as well.[282–284] Next it was shown that neither STM nor EMB improved results over a three-drug regimen (INH, RIF, and PZA) during the first 2 months of intensive therapy when the isolate was fully susceptible.[282] This 6-month three-drug regimen is acceptable for drug-sensitive disease. However, given concerns about resistance, EMB should be included until susceptibility testing results are known. A 6-month continuation phase of INH and EMB is inferior to a 4-month continuation phase of INH and RIF.[285]

Standard Regimens Based on Isoniazid and Rifampin

The combination of INH (5 mg/kg; maximum 300 mg), RIF (10 mg/kg; maximum 600 mg), PZA (25 mg/kg, maximum 2 g), and EMB (15 mg/kg), all given once daily by mouth, should be initiated in persons suspected of having TB.[239,286,287] Therapy should be given daily throughout the entire course of treatment.[239] If intermittent therapy is considered, it should be no less frequent than three times per week, and given only in persons at low relapse risk.[239] Intermittent therapy should be provided under direct observation. These four drugs should be continued for 2 months; EMB can be discontinued when susceptibility results are received, noting that the infecting organism is susceptible to the other three drugs.

At the end of 2 months, PZA and EMB can be discontinued and INH and RIF continued to complete a 6-month course. Several treatment regimens have been endorsed by the CDC, ATS, IDSA,[239] and WHO.[288] Several well-studied regimens are presented in Tables 249.7 and 249.8.[239] As an alternative in persons with drug-susceptible disease, INH and RIF can be given daily during the first 2 months, followed by 7 months of either daily or intermittent therapy, with no less than thrice-weekly administration preferred.[239,289] When given thrice or twice weekly, the RIF dose remains the same and the INH dose is increased to 15 mg/kg (900 mg maximum). In persons with a cavity on initial chest radiograph and positive sputum cultures after 2 months of therapy, TB relapse risk is high[290]; INH and RIF should be continued for an additional 3 months (9-month total course) to decrease the relapse risk.[239]

In persons with resistance to or intolerance of INH, a 6-month regimen of RIF, PZA, and EMB can be used. Results of all 6-month regimens in patients with initial resistance to RIF are poor, and such cases probably require 18- to 24-month courses, as was the case before RIF was available.[291]

When hepatitis (defined as aminotransferase levels greater than five times the upper limit of normal regardless of symptoms or greater than three times the upper limit of normal in persons with symptoms of hepatitis) occurs in patients receiving INH, RIF, and/or PZA, all drugs should be discontinued until hepatic aminotransferase levels normalize and symptoms resolve. Drugs may then be cautiously reintroduced in a stepwise fashion while serum aminotransferase levels are monitored. If persons are intolerant of INH, a regimen of RIF, PZA, and EMB can be given to complete a 6-month course. If persons are intolerant of RIF, a more prolonged (18- to 24-month) regimen based on INH and at least one companion drug (e.g., EMB and possibly a fluoroquinolone) can be continued.

Directly Observed Treatment

Poor adherence to antituberculosis therapy over the entire course of treatment, with resultant development of drug-resistant TB and low rates of TB cure and treatment completion, has led to the recommendation to use DOT whenever feasible.[239] Important to note, DOT is

TABLE 249.7 Drug Regimens for Culture-Positive Pulmonary Tuberculosis Caused by Drug-Susceptible Organisms

INITIAL PHASE			CONTINUATION PHASE			
REGIMEN[a,b,c]	DRUGS[d]	INTERVAL AND DOSES[e] (MINIMAL DURATION)	REGIMEN	DRUGS[d]	INTERVAL AND DOSES[e,f] (MINIMAL DURATION)	RANGE OF TOTAL DOSES (MINIMAL DURATION)
1	INH RIF PZA EMB	7 days/wk for 56 doses (8 wk) or 5 days/wk for 40 doses (8 wk)[g]	1	INH/RIF	7 days/wk for 126 doses (18 wk) or 5 days/wk for 90 doses (18 wk)[g]	182–130 (26 wk)
2	INH RIF PZA EMB	7 days/wk for 56 doses (8 wk), or 5 days/wk for 40 doses (8 wk)[g]	2	INH/RIF	3 times/wk for 54 doses (18 wk)	110–94 (26 wk)
3	INH RIF PZA EMB	3 times/wk for 24 doses (8 wk)	3	INH/RIF	3 times/wk for 54 doses (18 wk)	78 (26 wk)
4	INH RIF PZA EMB	7 days/wk for 14 doses (2 wk), then 2 times/wk for 12 doses (6 wk)	4	INH/RIF	2 times/wk for 36 doses (18 wk)	62 (26 wk)

[a]Regimen effectiveness is greatest for Regimen 1 to least for Regimen 4. Regimen 1 is preferred for newly diagnosed pulmonary tuberculosis. Regimen 2 is a preferred alternative regimen when more frequent DOT during the continuation phase is difficult to achieve. Regimen 3 should be used with caution in patients with HIV and/or cavitary disease; missed doses can lead to treatment failure, relapse, and acquired drug resistance. Regarding Regimen 4, twice-weekly regimens should be avoided in HIV-positive patients and patients with smear-positive and/or cavitary disease; if doses are missed, therapy is equivalent to once weekly, which is inferior.
[b]If the patient's isolate is susceptible to both INH and RIF, EMB is not necessary, and the intensive phase can consist of INH, RIF, and PZA only.
[c]Variations of the preferred regimen may be acceptable in certain clinical and/or public health situations, as described elsewhere.[239]
[d]Pyridoxine (vitamin B_6), 25–50 mg/day, is given with INH to all persons at risk of neuropathy (e.g., pregnant women; breastfeeding infants; persons with HIV; patients with diabetes, alcoholism, malnutrition, or chronic renal failure; or patients with advanced age). For patients with peripheral neuropathy, experts recommend increasing pyridoxine dose to 100 mg/day.
[e]When directly observed therapy is used, drugs may be given 5 days/wk and the necessary number of doses adjusted accordingly. Although there are no studies that compare five with seven daily doses, extensive experience indicates this would be an effective practice.
[f]Patients with cavitation on an initial chest radiograph and positive cultures at completion of 2 months of therapy should receive a 7-month (31-week) continuation phase.
[g]Five-day-a-week administration is always given by DOT.
DOT, Directly observed therapy; *EMB,* ethambutol; *HIV,* human immunodeficiency virus; *INH,* isoniazid; *PZA,* pyrazinamide; *RIF,* rifampin.
Modified from Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, et al. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. Clin Infect Dis. *2016; 63:e147–e195.*

TABLE 249.8 First-Line Tuberculosis Medications

DRUG DOSE (MAXIMUM)	MAJOR ADVERSE REACTIONS	RECOMMENDED MONITORING	DOSAGE FORMS	COMMENTS
Isoniazid[a] (INH)				
Daily C: 10–15 mg/kg (300 mg) A: 5 mg/kg (300 mg) *Once weekly* C: Not recommended A: 15 mg/kg (900 mg) *Twice weekly* C: 20–30 mg/kg (900 mg) A: 15 mg/kg (900 mg) *Three times weekly* C: Not recommended A: 15 mg/kg (900 mg)	Hepatic enzyme elevation, peripheral neuropathy, hepatitis, rash, CNS effects, increased phenytoin, (Dilantin), and disulfiram (Antabuse) levels	Baseline hepatic enzymes Repeat monthly if baseline abnormal, risk factors for hepatitis, or symptoms of adverse reactions	*Scored tablets:* 50 mg, 100 mg, and 300 mg *Syrup:* 50 mg/5 mL *Aqueous solution (IV/IM):* Scarce and may not be available	Hepatitis risk increases with age and alcohol consumption. Overdose may be fatal. Aluminum-containing antacids reduce absorption. Pyridoxine (vitamin B_6) may decrease peripheral neuritis and CNS effects.
Rifampin[a] (RIF)				
Daily C: 10–20 mg/kg (600 mg) A: 10 mg/kg (600 mg) *Once weekly* C: Not recommended A: Not recommended *Twice weekly* C: 10–20 mg/kg (600 mg) A: 10 mg/kg (600 mg) *Three times weekly* C: Not recommended A: 10 mg/kg (600 mg)	Hepatitis, fever, thrombocytopenia, flulike syndrome, rash, gastrointestinal upset, renal failure Reduces levels of many drugs, including methadone, warfarin (Coumadin), birth control pills, theophylline, dapsone, ketoconazole, PIs, and some NNRTIs Orange discoloration of secretions (sputum, urine, sweat, tears) and may permanently stain soft contact lenses	CDC no longer recommends routine monitoring tests; however, many clinicians continue to order baseline CBC, platelets, hepatic enzymes Repeat if baseline abnormal, risk factors for hepatitis, or symptoms of adverse reactions	*Capsules:* 150 mg and 300 mg *Syrup:* can be formulated from capsules by pharmacy *Aqueous solution (IV/IM):* Scarce and may not be available	Patients on methadone will need an increased dose of methadone (average 50%) to avoid opiate withdrawal. Interaction with many drugs leads to decreased levels of one or both. May make glucose control more difficult in diabetics. Contraindicated for patients taking PIs and some NNRTIs. Women on birth control pills need a barrier method while on rifampin.
Pyrazinamide (PZA)				
Daily C: 15–30 mg/kg (2 g) A: 15–30 mg/kg (2 g) *Once weekly* C: Not recommended A: Not recommended *Twice weekly* C: 50 mg/kg (2 g) A: 50–70 mg/kg (4 g) *Three times weekly* C: Not recommended A: 50–70 mg/kg (3 g)	Gastrointestinal upset, hepatotoxicity, hyperuricemia, arthralgias, rash, gout (rare)		*Scored tablets:* 500 mg	May complicate management of diabetes mellitus. Treat increased uric acid only if symptomatic. Most common reason for TB patients experiencing GI upset.
Ethambutol[b] (EMB)				
Daily C: 15–20 mg/kg (1000 mg) A: 15–25 mg/kg (1600 mg) *Once weekly* C: Not recommended A: Not recommended *Twice weekly* C: 50 mg/kg (2.5 g) A: 50 mg/kg (4 g) *Three times weekly* C: Not recommended A: 25–30 mg/kg (2400 mg)	Decreased red-green color discrimination, decreased visual acuity (optic neuritis), rash	Baseline tests of visual acuity and color vision Monthly testing for patients taking >15–25 mg/kg, for those taking EMB for >2 mo, and for patients with renal insufficiency	*Tablets:* 100 mg and 400 mg	Optic neuritis may be unilateral; check each eye separately. Not recommended for children too young for monitoring of vision unless drug resistant. Use lowest possible dose in range (except for drug-resistant patients). EMB should be discontinued immediately and permanently if any signs of visual toxicity occur.
Rifabutin (RBT)				
Daily C: Not recommended A: 5 mg/kg (300 mg) *Once weekly* C: Not recommended A: Not recommended *Twice weekly* C: Not recommended A: 5 mg/kg (300 mg) *Three times weekly* C: Not recommended A: 5 mg/kg (300 mg)	Hepatitis fever, thrombocytopenia, neutropenia, leukopenia, flulike symptoms, hyperuricemia Orange discoloration of secretions (sputum, urine, sweat, tears) and may permanently stain soft contact lenses Reduces levels of many drugs, including methadone, warfarin (Coumadin), birth control pills, theophylline, dapsone, ketoconazole, PIs, and NNRTIs With increased rifabutin levels, severe arthralgias, uveitis, leukopenia occur	Baseline hepatic enzymes Repeat if baseline values abnormal, risk factors for hepatitis, or symptoms of adverse reactions	*Capsules:* 150 mg	Patients on methadone may need an increased dose to avoid opiate withdrawal. Interaction with many drugs leads to decreased levels of one or both. May make glucose control more difficult in diabetics. Women on birth control pills need to use a barrier method while on rifabutin. In combination with NNRTIs or PIs, dosages change significantly.

TABLE 249.8 First-Line Tuberculosis Medications[a]—cont'd

DRUG DOSE (MAXIMUM)	MAJOR ADVERSE REACTIONS	RECOMMENDED MONITORING	DOSAGE FORMS	COMMENTS
Rifapentine (RPT)				
Once weekly only C: Not approved for use in children A: 10 mg/kg (600 mg) *(Data indicate that 900 mg of RPT is well tolerated and CDC is recommending this higher dose for latent tuberculosis when given once weekly with INH.)*	Hepatitis, thrombocytopenia, neutropenia, leukopenia, hyperuricemia, flulike syndrome Reduces levels of many drugs, including methadone, warfarin (Coumadin), birth control pills, theophylline, dapsone, ketoconazole, PIs, and NNRTIs Orange discoloration of secretions (sputum, urine, sweat, tears) and may permanently stain soft contact lenses	Baseline hepatic enzymes, CBC, and platelets. Repeat if baseline values are abnormal, risk factors for hepatitis are present, or there are symptoms of adverse reactions	*Tablets (film-coated):* 150 mg *Indications:* Pulmonary TB patients who are HIV negative, noncavitary, not pregnant, with organisms pan-sensitive and culture negative at 2 months (two consecutive negative cultures) Administered once weekly with INH during the continuation phase of treatment.	See drug interactions with rifampin.

[a]Combination drugs are recommended in the rare instance in which a patient is placed on self-administered therapy: IsonaRif contains INH, 150 mg, RIF, 300 mg; Rifamate contains INH, 150 mg, RIF, 300 mg; Rifater contains INH, 50 mg, RIF, 120 mg, and PZA, 300 mg.
[b]In 2003, the CDC recommended dosing based on weight ranges for PZA and EMB. After reviewing available data, the Maryland TB Expert panel recommended that the previously recommended dosage ranges be used, advising use of the lowest possible dose in the dose range.
A, Adult; *C,* child; *CBC,* complete blood cell count; *CDC,* Centers for Disease Control and Prevention, *CNS,* central nervous system; *GI,* gastrointestinal; *HIV,* human immunodeficiency virus; *NNRTIs,* nonnucleoside reverse-transcriptase inhibitors; *PIs,* protease inhibitors.
Modified from the 2007 Maryland Guidelines for Prevention and Treatment of Tuberculosis. http://phpa.dhmh.maryland.gov/OIDPCS/CTBCP/CTBCPDocuments/tbguidelines.pdf.

cost-effective, especially when considering the cost of MDR-TB cases that are prevented.[292] Because all doses are observed, compliance is improved and the likelihood of emergence of resistance minimized. The ability of mandatory DOT to control drug resistance in a community is well established.[293,294] In some cases, recalcitrant patients must be detained for completion of therapy.

Regimens of Less Than 6 Months for Minimal Disease

Extent of disease can be quantified by the mycobacterial content of sputum, with smear- and culture-positive sputum representing most severe disease, smear-negative and culture-positive sputum representing intermediate disease, and smear- and culture-negative sputum representing the least amount of disease. The possibility that the early bactericidal activity of moxifloxacin or gatifloxacin in combinations might permit 4-month regimens in smear-positive, culture-positive cases has, to date, not been confirmed (see "Fluoroquinolones").[247,257,260] Good results have been obtained with as little as 4 months of therapy in patients with less extensive TB.[295] A 4-month course of treatment (2 months of INH, RIF, PZA, and EMB followed by 2 months of INH and RIF) is recommended for HIV-seronegative persons with smear-negative, culture-negative pulmonary TB.[239] In HIV-seropositive persons with culture-negative TB, a 6-month regimen is recommended.

Fixed-Dose Combination Tablets

Fixed-dose preparations are available; these contain 150 mg of INH and 300 mg of RIF (Rifamate); INH 50 mg, RIF 120 mg, and PZA 300 mg (Rifater); or INH 75 mg, RIF 150 mg, PZA 400 mg, and EMB 275 mg (Rifafour). These prevent the patient from omitting drugs and taking monotherapy and therefore decrease the risk for resistance. Fixed-dose combinations are particularly useful when DOT cannot be provided.

Treatment of Multidrug-Resistant Tuberculosis

Surprisingly, studies of four-drug, 6-month chemotherapy demonstrated that initial INH or STM resistance did not compromise outcome, but results were very poor (>50% lack of conversion or relapse) when initial RIF resistance was present.[291] In a meta-analysis, treatment failure and relapse were substantially higher in the presence of initial drug resistance.[296] A critical component in combatting the development of extensively resistant strains in patients with MDR-TB is access to effective second-line agents, with increased sputum culture, susceptibility testing, and genotypic data. In 832 patients with MDR disease followed by the Green Light Committee (GLC), the mandate of which is to facilitate increased access to these interventions in middle- and low-income countries, 8.9% without baseline resistance to second-line agents still acquired XDR disease, and as baseline drug resistance increased, the risk of new cases with acquired XDR TB increased also. There was less resistance acquired during treatment in GLC-approved sites.[297] Additional data from the United States support the need for increased monitoring for treatment failure associated with the acquisition of resistance to second-line drugs, with mortality significantly higher (26.5% vs. 10.0%) in patients with acquisition of resistance versus control patients.[298] Acquisition of resistance may impart greater risk than baseline resistance to the same drugs.[299] It is important to note retrospective results from the Russian Federation, where an "aggressive" regimen of at least five likely effective drugs was associated with a decreased risk of death and failure during MDR-TB treatment.[300]

For therapy for TB that is resistant to both INH and RIF, susceptibility testing for second-line drugs should be performed and treatment individualized according to the susceptibility test results. Support for this recommendation has been provided in a meta-analysis of studies including almost 9000 patients, in whom susceptibility testing for EMB, PZA, and second-line agents was associated with higher odds of treatment success.[301] In some settings, standardized second-line regimens are used. If a suboptimal regimen is prescribed, resistance to additional drugs may emerge and the opportunity for success may be lost. In a study from Denver, Colorado, only one-half of 171 HIV-negative patients with MDR-TB ever converted sputum cultures to negative despite prolonged administration of carefully selected regimens (not including fluoroquinolones).[302] In a follow-up study from the same institution, the long-term success rate was 75%.[303] In a study from Latvia, 66% of MDR-TB patients completed therapy or were cured.[304] In contrast, two smaller studies among HIV-seronegative patients from New York City and San Francisco noted remission in virtually all evaluable HIV-negative patients treated for MDR-TB.[305,306] A study from Bangladesh found that at least 9 months of gatifloxacin, clofazimine, EMB, and PZA, supplemented with prothionamide, kanamycin, and high-dose INH during an initial intensive phase of at least 4 months, resulted in an 88% relapse-free cure rate.[272] Recent preliminary results of the STREAM trial, in which the "Bangladesh" regimen was compared with standard therapy, demonstrated similar effectiveness of the 9-month regimen, although noninferiority was not demonstrated.[307] A randomized trial of the addition of clofazimine to "individual-based" drug regimens in China found a significant increase in treatment success in the clofazimine

group versus the control group (74% vs. 54%; $P = .035$).[308] In a CDC report, 38% of MDR isolates were resistant to PZA, so the role of PZA may be limited in MDR-TB.[309] For TB that is INH and RIF resistant but fluoroquinolone susceptible, a fluoroquinolone should always be administered along with other drugs to which the organism is susceptible. In this setting, 2-month sputum culture conversion rates are improved by adding bedaquiline.[262] The risk for treatment failure is increased if the *M. tuberculosis* isolate is also resistant to fluoroquinolones.[310,311] Levofloxacin may be preferred over ofloxacin, but moxifloxacin has the greatest in vitro activity against *M. tuberculosis*. Companion drugs may include aminoglycosides (STM, kanamycin, or amikacin) or capreomycin, ethionamide, and cycloserine.[305,312–315] The injectable agents are particularly important for good outcomes, although nephrotoxicity and ototoxicity are concerns. The uncertain efficacy of newer therapies is underlined by the report of the development of resistance to bedaquiline and delamanid during treatment of MDR-TB.[316] In a small observational study of imipenem/clavulanate added to an optimized background regimen, either indifferent or worse outcomes were noted, suggesting that adding imipenem-clavulanate in the setting of treatment failure was not likely to improve outcome.[317]

Therapy for Extensively Drug-Resistant Tuberculosis

Therapy for XDR-TB, which is defined as resistance to INH, RIF, any fluoroquinolone, and at least one of three injectable second-line drugs (amikacin, kanamycin, or capreomycin),[112] is difficult and usually associated with poor outcomes. The risk for treatment failure and death has been higher than in patients with MDR-TB in some series,[318,319] but not all.[320] Cure rate in a report from South Africa was 5%, and mortality was 78% at 60 months.[321] Treatment with at least five drugs to which the organism is susceptible is recommended. In a study of 41 patients with treatment-refractory XDR-TB, linezolid was associated with sputum culture conversion, but 82% of patients had clinically significant adverse events attributable to linezolid.[322] The cure rate 1 year after the end of treatment was 78%.[323] A similar trial in China also found a significantly higher cure rate (70% vs. 34% in the control group), but again with an adverse event rate in the linezolid group of 82%.[324] A retrospective report in a predominantly HIV-infected South African population noted improved culture conversion with clofazimine (40% vs. 29% in the comparator group), with only minor adverse effects.[325]

Course of Therapy and Duration of Observation

At least three sputum samples should be submitted for smear and culture before beginning treatment. If available, specimens for GeneXpert should also be obtained. In patients with presumed TB, treatment should be initiated immediately; a few days of antituberculous treatment will not interfere with bacteriologic diagnosis. If cultures are negative and there are no alternative diagnoses, clinical and radiographic response to therapy after 2 months of treatment is consistent with a diagnosis of TB. Beginning 1 month after initiation of therapy, three sputum cultures should be obtained monthly to monitor conversion to negative or, if sputum cultures remain positive, to detect treatment failure and the possible emergence of drug resistance. Sputum cultures should convert to negative within 2 months. In a minority of patients, sputum smears remain positive after cultures turn negative. Sporadic positive smears for long periods presumably represent inactive bacilli released from caseous foci. When cultures remain positive after 4 months of treatment, it is considered treatment failure. Causes of treatment failure include drug resistance, noncompliance with therapy, and malabsorption of antituberculosis drugs. Sensitivity testing should be performed and consideration given to adding at least two new drugs to which the organism was sensitive at the outset of treatment, at least until sensitivities are known. Addition of only one drug risks development of resistance to the added drug. Adherence to therapy should be ensured and serum drug levels considered to assess absorption.

Patients receiving INH, RIF, and PZA should be asked monthly about symptoms of hepatitis, and hepatic aminotransferase levels should be checked in symptomatic persons. In persons with abnormal hepatic aminotransferase levels at baseline, such laboratory findings should be monitored regularly, particularly early in the course of therapy. Patients receiving EMB should be regularly questioned regarding visual symptoms; their visual acuity should be measured (Snellen chart) and red-green color discrimination assessed monthly. Patients receiving STM should be examined for balance and high-frequency hearing loss, and their renal function should be monitored closely.

Relapse after adequate treatment of drug-sensitive infections is infrequent (2%–5%). Prolonged follow-up of appropriately treated patients is not necessary except in the case of unusually extensive disease, slow bacteriologic response to treatment, suspicion of poor compliance, drug-resistant disease, or high-risk patients with intercurrent diseases. In high-incidence settings, an additional 12 months of INH after completion of a 6-month RIF-containing regimen reduces the TB recurrence rate among HIV-infected adults.[326,327] However, such a practice is often not performed owing to logistical constraints.

Retreatment

Recurrent TB may be due to either relapse (same *M. tuberculosis* strain as the original episode) or reinfection (different *M. tuberculosis* strain). Genotyping (RFLP, MIRU, and spoligotyping) or WGS may be used to distinguish *M. tuberculosis* isolates. Clinical judgment based on experience is critical in re-treatment cases, and testing of susceptibility to first- and second-line drugs is required.[239] Some generalizations concerning re-treatment can be made:

1. In a patient with drug-susceptible TB who receives rifamycin-based DOT, relapse is likely due to a drug-susceptible organism. Such patients usually respond again to the initial regimen.
2. If compliance has been irregular, particularly if the patient has not received DOT, resistant organisms will probably be present.
3. When drug resistance is suspected, the treatment regimen should include INH, RIF, PZA, EMB, a fluoroquinolone, and an injectable agent (e.g., capreomycin), pending susceptibility results.
4. Capreomycin or amikacin can replace STM. Kanamycin is less effective and more toxic and is used as a last resort. There is usually no cross-resistance between capreomycin and STM, amikacin, or kanamycin, but amikacin and kanamycin are usually cross-resistant.
5. TB resistant to INH and RIF (i.e., MDR-TB) should be treated with a fluoroquinolone, ethionamide (or protionamide), PZA, and probably an injectable agent plus either cycloserine or PAS. The intensive phase of treatment is for 8 months, and the total treatment duration is 20 months.[314,315,328] For those who meet the criteria, the 9-month short-course regimen should be considered.[315]
6. TB resistant to INH, RIF, an injectable agent, and a fluoroquinolone (i.e., XDR-TB) should be treated with at least four second-line antituberculosis drugs likely to be effective, in addition to PZA during the intensive phase of treatment.[315,320] Surgical resection may be required. A prolonged course of treatment is necessary, but the optimal duration is unknown.

Other Forms of Treatment

Bed rest does not influence outcome when effective chemotherapy is given. Resection still has a role in the salvage of patients in whom treatment fails and who have localized, resectable disease, and extensive drug resistance.

Corticosteroids

Corticosteroids in conjunction with antituberculosis therapy improve neurologic outcome and mortality in persons with tuberculous meningitis. Use of corticosteroids is therefore recommended in this situation. For all other clinical manifestations of TB, however, there is no definite long-term benefit of adjunctive corticosteroids and therefore no therapeutic role.[329]

Treatment of Tuberculosis in HIV-Infected Patients

The treatment of HIV-related TB is complicated by major drug-drug interactions between antituberculosis drugs and ART. These interactions

affect the choice of therapy (and appropriate doses) for both diseases. Invaluable websites that provide extensive advice and information regarding treatment options for HIV-related TB, including managing and avoiding drug-drug interactions, are maintained by the Department of Health and Human Services[245] and the CDC.[330] These websites are updated regularly and should be consulted routinely because new antiretroviral agents and interactions are being discovered. Chapter 39 includes tables from the CDC website about use of antiretroviral agents together with RIF or rifabutin.

The rifamycins interact with many HIV-1 protease inhibitors, nonnucleoside reverse transcriptase inhibitors, and integrase strand transfer inhibitors.[245] Among the rifamycins, RIF has the most interactions; rifapentine appears to have a similar effect,[331] but rifabutin has less profound interactions. RIF is contraindicated for use with HIV-1 protease inhibitors (e.g., saquinavir, indinavir, nelfinavir, fosamprenavir, atazanavir, darunavir, tipranavir) and the nonnucleoside reverse-transcriptase inhibitors etravirine and rilpivirine. RIF also lowers nevirapine levels, so there are concerns that these two drugs should not be given in combination.[245] Maraviroc can be used with RIF if the maraviroc dose is increased to 600 mg twice daily. The RIF pharmacokinetic interaction with saquinavir-ritonavir and lopinavir-ritonavir may be overcome with increased protease inhibitor dosages.[332,333] However, several healthy volunteer studies were prematurely terminated when individuals receiving RIF promptly developed marked hepatotoxicity with addition of ritonavir-boosted lopinavir,[332] saquinavir,[333] or atazanavir.[334] Accordingly, these combinations are generally not used. Rifabutin may be used in combination with protease inhibitors, with appropriate dose modification.[245] Unfortunately, rifabutin is not presently available in most resource-limited countries.

RIF modestly lowers plasma levels of efavirenz and, to a greater extent, nevirapine. However, among patients receiving multidrug regimens for TB, RIF has been shown to paradoxically increase plasma levels of efavirenz, particularly among *CYP2B6* slow metabolizers[335]; NAT2 slow metabolizers of INH may also contribute to increased efavirenz levels.[336] RIF does not appear to affect the antiviral efficacy of efavirenz, and they can be administered concomitantly with the efavirenz dose maintained at 600 mg daily, particularly in persons <60 kg. There are greater concerns about concomitant administration of RIF and nevirapine. Several small studies have demonstrated favorable clinical and virologic responses, but toxicity is greater than when RIF is given with efavirenz.[337] In persons with concurrent TB at the start of ART, failure to achieve virologic suppression was more common in persons who received nevirapine than efavirenz (both given with RIF).[338] RIF lowers raltegravir levels by 40%; the raltegravir dose should be increased to 800 mg twice daily and virologic response followed closely. No dosage adjustments are needed when raltegravir is given with rifabutin. RIF lowers maraviroc levels by 64%. Although it is preferred to not coadminister these drugs, if it is done, the dose of maraviroc should be increased to 600 mg twice daily. Additional interactions and drug dose adjustments are given in Chapter 39.

Despite this extensive list of interactions, it is not recommended that treatment of TB be delayed or that highly active ART be avoided. Because antimycobacterial drugs other than the rifamycins do not have substantial interactions, an alternative regimen with INH, STM, PZA, and EMB can be considered. However, in HIV-infected persons, regimens that are not rifamycin based may be less effective than rifamycin-based regimens.

Acquired rifamycin resistance in the setting of TB relapse is seen almost exclusively in patients with advanced AIDS who receive highly intermittent antituberculosis therapy, such as once-weekly INH plus rifapentine or twice-weekly INH plus rifabutin.[248,339] Additional data suggest that the risk of acquired RIF resistance is also increased in HIV-infected persons treated with thrice-weekly therapy.[340] It is therefore now recommended that HIV-infected persons should receive daily antituberculosis therapy in both the intensive and continuation phase of TB treatment.[239]

Patients with HIV-related enteropathy may not respond to chemotherapy because of inadequate absorption of oral agents, and, in some cases, pharmacokinetic monitoring may be necessary.[341]

Immune Reconstitution Inflammatory Syndrome

Immune reconstitution inflammatory syndrome (IRIS) results from rapid restoration of immune responses to opportunistic pathogens, most importantly *M. tuberculosis.* This has most often been described in patients receiving ART. It has additionally been seen in other immunodeficiencies, as in a report of a severe inflammatory reaction that occurred during therapy of disseminated TB in a Thai woman who had anti–interferon-γ antibodies.[342] Characteristic features include initiation of virologically effective ART within the previous 3 weeks (the period of most rapid immune recovery), clinical deterioration with exaggerated inflammation, and absence of an alternative explanation such as drug resistance, drug hypersensitivity, other infections, or lymphoma. TB-associated IRIS comprises two main categories: (1) onset in patients who are already being treated for TB (termed *paradoxical IRIS*) and (2) onset in patients with previously unrecognized, untreated TB (termed *unmasking IRIS*). During paradoxical IRIS, patients have typically been responding to TB therapy but develop recurrent, new, or worsening manifestations, such as fever, cough, lymph node enlargement, or radiographic abnormalities after starting antiretroviral agents.[343,344] Such patients demonstrate robust peripheral blood T-cell responses to PPD and increased proinflammatory cytokines.[345] Paradoxical IRIS is most likely to occur in patients with more advanced AIDS, disseminated and extrapulmonary TB, and a good immunologic and virologic response to antiretroviral agents and in those who initiate antiretroviral agents soon after starting antituberculous drugs.[346,347] Reported frequencies range from 8% to 43%.[346] Although symptoms of paradoxical IRIS are usually self-limited and last a median of 2 months,[348,349] morbidity can be substantial and mortality can occur but is rare. A 4-week course of prednisone improved symptoms and decreased the need for hospitalization in one clinical trial.[350] Paradoxical reactions such as lymph node enlargement or cerebral tuberculomas infrequently affect HIV-negative individuals or HIV-positive individuals not receiving antiretroviral agents. In one study, paradoxical reactions affected 36% of HIV-infected patients receiving therapy for both TB and HIV but only 2% of HIV-negative patients and 7% of HIV-infected patients not on ART.[343] Paradoxical reactions after the initiation of ART tend to be more severe and more likely to involve multiple organs. Paradoxical reactions in previously healthy patients can be confused with uncontrolled infection.

Less is understood about unmasking IRIS. In resource-limited settings, TB is frequently diagnosed during the initial months of ART, but underlying mechanisms vary.[351] Some represent new or progressive TB due to persistent immunodeficiency, some reflect active TB that was present but undiagnosed before ART was started, and others reflect subclinical TB that was truly unmasked by restoration of robust immune responses to *M. tuberculosis.* Consensus case definitions have been published to facilitate research into TB-associated IRIS in resource-limited settings.[346]

The optimal timing of ART initiation in HIV-infected persons with TB has been evaluated in three clinical trials.[352–354] Among persons with fewer than 50 $CD4^+$ T cells/mm^3, initiation of ART within 2 to 4 weeks of starting antituberculosis therapy was associated with improved AIDS-free survival compared with delayed initiation of ART. Among persons with more than 50 $CD4^+$ T cells/mm^3, initiation of ART 8 to 12 weeks after initiation of antituberculosis therapy was associated with a lower risk for IRIS and adverse events without increasing the risk for AIDS or death.[353,354] The most recent TB treatment guidelines recommend the timing of ART initiation in these patient groups according to these findings.[239,288]

Duration of Therapy

The duration of antituberculosis therapy is determined by relapse risk. Several observational studies noted comparable relapse rates in HIV-infected and HIV-uninfected persons after 6 months of rifamycin-based antituberculosis therapy. Based on these data, the current recommendation is to treat for the same duration regardless of HIV status, particularly if the HIV-positive patient is receiving ART.[239,286,288] However, some observational studies have noted higher recurrence rates in HIV-infected than HIV-uninfected persons who receive 6 months of treatment.[355,356] A recent meta-analysis found that ART decreased the risk for TB relapse;

rifamycin-based therapy for 8 months or more and daily administration in the intensive phase were also associated with improved treatment outcomes.[357] In the only clinical trial of 6 versus 9 months of antituberculosis treatment in HIV-infected persons, TB recurrence risk was lower in those who received treatment for 9 months, but mortality rate did not differ; all persons received antituberculosis treatment three times a week, and none received ART.[358] The most recent ATS/CDC/IDSA guidelines recommend extending the duration of antituberculosis treatment to 9 months in HIV-positive persons who do not receive concomitant ART.[239]

Other Special Treatment Circumstances

Childhood

Pulmonary TB in childhood should be treated with INH, RIF, and PZA for 2 months, followed by INH and RIF for 4 months. The inability to monitor visual acuity limits the use of EMB in very young children, although it can be given if the bacillary burden is high, drug resistance is suspected, or both.[239]

Pregnancy

Treatment should not be deferred during pregnancy. For drug-sensitive TB, INH, RIF, and EMB comprise the regimen of choice. STM should not be used during pregnancy because of potential eighth nerve toxicity in the fetus. Although PZA is routinely recommended by international organizations, use has not been uniformly recommended in the United States because of inadequate teratogenicity data; its use should be considered on a case-by-case basis.[239]

Uremia and End-Stage Renal Disease

Dosages of INH and RIF need not be adjusted for renal failure but should be administered after dialysis, and pyridoxine supplementation should be routine. In patients with creatinine clearance less than 30 mL/min and those on hemodialysis, EMB should be administered at 20 to 25 mg/kg, and PZA at 25 to 35 mg/kg, both given three times per week (after dialysis for those on hemodialysis). Biochemical monitoring of hepatotoxicity during renal failure may be complicated by abnormally low aminotransferase levels in uremia.

Liver Disease

The selection and dosage of antituberculous agents do not need to be modified in most patients with underlying liver disease, but hepatic aminotransferase and bilirubin levels must be followed closely. In persons intolerant of INH owing to hepatotoxicity, a 6-month regimen of RIF, PZA, and EMB can be used. If PZA cannot be tolerated, a regimen of INH, RIF, and EMB for 2 months, followed by 7 months of INH and RIF, should be given.[239] For persons with extensive TB and severe hepatitis who should not have a prolonged treatment interruption, "bridging" regimens that include EMB, fluoroquinolone, and STM could be considered until a more standard regimen can be instituted.[239] Preexisting liver disease (e.g., hepatitis C virus infection) may complicate the detection of drug-related hepatotoxicity; accordingly, clinical and biochemical supervision should be assiduous.

Patients Receiving Immunosuppressive Drugs

TB that develops during immunosuppressive treatment of another disease should be treated with the same regimens used to treat immunocompetent hosts. Immunosuppressive therapy need not be discontinued. Given the increased predisposition to TB among persons receiving a TNF-α inhibitor (e.g., etanercept, infliximab, adalimumab), discussion between all of the patient's medical providers (e.g., rheumatologist, gastroenterologist, pulmonologist, and infectious disease specialist) may be helpful to determine a rational treatment strategy.

Treatment of Latent Tuberculous Infection

Soon after INH became available, it became widely used in the United States to treat not only persons with active disease (as part of combination therapy) but also *M. tuberculosis* infection, to prevent progression to active TB. In contrast, many other countries have used a TB-prevention strategy based primarily on BCG vaccination at birth rather than treatment of LTBI. The current strategy in the United States is to test for *M. tuberculosis* infection using the TST or an interferon-γ release assay in persons who are at high risk for progressing to active TB, and then to treat all persons with a positive test result regardless of age. Criteria for TST positivity and groups for whom treatment of LTBI is indicated are listed in Table 249.9.[172,359]

TABLE 249.9 Criteria for Tuberculin Positivity by Risk Group

REACTION ≥5 MM OF INDURATION	REACTION ≥10 MM OF INDURATION	REACTION ≥15 MM OF INDURATION
HIV-positive persons	Recent immigrants (within 5 yr) from high-prevalence countries	Persons with no risk factors for tuberculosis
Recent contacts of tuberculosis case patients	Injection drug users	
Fibrotic changes on chest radiograph consistent with prior tuberculosis	Residents and employees of high-risk congregate settings (prisons and jails, nursing homes, hospitals and other health care facilities, residential facilities for patients with AIDS, and homeless shelters)	
Patients with organ transplants and other immunosuppressed patients (receiving equivalent of ≥15 mg/day of prednisone for at least 1 mo)	Children <4 yr of age, or infants, children, and adolescents exposed to adults at high risk	

AIDS, Acquired immunodeficiency syndrome; *HIV,* human immunodeficiency virus.
Modified from centers for disease control and prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. American thoracic society. MMWR Morb Mortal Wkly Rep. *2000;49(Rr-6):1–51.*

Drug Regimens

Nine months of INH, up to 300 mg daily, is a recommended regimen for treatment of LTBI in adults and children, regardless of HIV status.[172,360] Although 90% efficacious, its effectiveness is limited by low treatment completion rates. When necessary, supervised intermittent treatment of LTBI with INH, 15 mg/kg (up to 900 mg) twice weekly, can be used. Pyridoxine supplementation, 10 to 50 mg daily, is recommended to prevent peripheral neuropathy in persons older than 65 years; pregnant and breastfeeding women; persons with diabetes mellitus, chronic renal failure, or alcoholism; persons undergoing treatment with anticonvulsants; and persons who are malnourished. A 3-month regimen of once-weekly INH plus rifapentine given to adults and children under direct observation is as effective as 9 months of INH.[361,362] Implementation of this 3-month regimen led to a 31% increase in completion of preventive therapy in New York City health clinics.[363] The weekly dose of both drugs for adults weighing more than 50 kg is 900 mg. This regimen is an equal alternative to 9 months of INH for persons 12 years of age and older who are at increased risk for progressing from latent infection to active TB; this includes HIV-infected persons who are not receiving ART.[364–366] It is important to exclude active TB in HIV-infected patients on this regimen. Permanent drug discontinuation due to an adverse event was more common with the 3-month regimen than with 9 months of INH alone (4.9% vs. 3.7%, respectively). The most common side effect was a flulike syndrome that has been seen before with intermittent RIF therapy, and with INH.[367] Another option is 4 months of RIF (10 mg/kg; 600 mg maximum).[172] This regimen is effective and well tolerated.[242,243,368] A 3-month regimen of daily INH and RIF is also effective. Although there have been limited head-to-head comparisons, a recent network meta-analysis demonstrated similar safety and effectiveness of 3 months of weekly INH plus rifapentine, 3 months of daily INH plus RIF, and 4 months of daily RIF.[369]

A 2-month regimen of daily RIF-PZA is as effective as a 12-month daily regimen of INH in HIV-infected adults.[370] Unfortunately, initial enthusiasm for this 2-month regimen waned after numerous reports of severe liver injury and death, primarily in HIV-negative individuals.[251] This regimen is no longer recommended.[371]

Optimal treatment of LTBI when resistance to both INH and RIF (i.e., MDR-TB) is likely is not known. Regimens of PZA plus either EMB or a fluoroquinolone for 6 to 12 months have been recommended, but such regimens are poorly tolerated and their effectiveness has never been studied.[172]

Risk for Isoniazid Hepatotoxicity During Treatment of Latent Tuberculous Infection

Use of INH is associated with an increased risk for hepatotoxicity; the risk in a recent clinical trial of 9 months of INH was 2.7%.[361] A US Public Health Service survey found that the incidence of probable INH-associated hepatitis increased with age: Rates per 1000 persons were 0 for those younger than age 20, 3 for ages 20 to 34, 12 for ages 35 to 49, 23 for ages 50 to 64, and 8 for age older than 64.[240] The incidence in daily drinkers of alcohol was also high (26.5 per 1000). The number in the elderly was probably falsely low because of a small sample size. A much larger experience recorded hepatitis in 4.6% of patients older than 65.[372] A large European study reported a hepatitis incidence of 520 per 100,000 population; the figure was 280 per 100,000 for those younger than 35 years and 770 per 100,000 for those older than 54 years. Most hepatitis develops within the first 3 months, and the risk for death, once clinical hepatitis develops, is substantial.[240,373] Biochemical monitoring will likely prevent some deaths, because there is a subclinical phase of at least several weeks. Byrd and colleagues recorded increased serum aminotransferase levels in 18.3% of patients taking INH but no deaths in a biochemically monitored population. Many patients with severe biochemical hepatitis would not have been detected with monitoring of symptoms only.[374] In contrast, one public health clinic reported only 11 cases of clinical hepatotoxicity and no deaths among more than 11,000 persons receiving INH over a 7-year period.[375] Based on this and other considerations, emphasis is now placed on clinic monitoring for signs and symptoms of adverse effects, with prompt evaluation if these develop. Current recommendations advocate routine baseline and follow-up laboratory monitoring only for persons with HIV infection, pregnant and early postpartum women, and persons with chronic liver disease or who use alcohol regularly.[172]

Snider and Caras analyzed 177 cases of fatal hepatitis from various sources[376] and estimated a case rate of 14 per 100,000 of those starting and 23 per 100,000 of those completing therapy. Sixty-nine percent were female, with clustering around pregnancy.

Treatment of Contacts of Active Cases

The US Public Health Service contact study showed that treatment of LTBI decreased the incidence of subsequent TB among contacts of active cases from 1550 to 610 cases per 100,000, a 61% reduction over 10 years.[377] In those who were tuberculin negative when first surveyed, the figures (per 100,000) were 510 without and 150 with chemoprophylaxis, a 59% reduction. Estimates of the risk to contacts in some smaller studies are much higher. One year of INH therapy is about 70% effective in preventing disease, with failures most often caused by nonadherence.[378] INH prophylaxis may also fail in drug-resistant infections. It appears unlikely that prophylactic INH monotherapy taken reliably by contacts results in subsequent INH resistance.[360,379]

Treatment of LTBI is indicated for persons found to be interferon-γ release assay or TST positive (5 mm of induration for the latter) after contact with an active case. In children younger than 5 who are close contacts of an active case, the TST is preferred over the interferon-γ release assay[380]; if the TST result is negative, preventive therapy should be initiated and the test repeated after 3 months. If the result of the second test is positive, a preventive therapy course should be completed; if it is negative, treatment can be discontinued.

To address the risk to health care workers inadvertently exposed to TB, Stead reviewed 33 previously investigated hospital and nursing home outbreaks.[174] In this setting, on discovering that exposures have occurred, a list of all exposed personnel and their TST or interferon-γ release assay results before exposure should be assembled. Persons with a negative test result should be retested 8 weeks after the exposure to allow time for TST or interferon-γ release assay conversion. However, if exposure is particularly heavy, or in HIV-positive health care workers, preventive therapy should be started even before retesting. Treatment can later be discontinued in HIV-negative persons if they remain TST or interferon-γ release assay negative.[155]

Treatment of Quiescent, Previously Untreated Pulmonary Tuberculosis

Tuberculin-positive patients with fibrotic upper lobe lesions and patients who had active TB before drugs were available are at increased risk for developing TB.[378] Treatment is the same as for LTBI (see earlier discussion). The longer the lesion has been stable, the lower is the risk for relapse.

Treatment of Individuals With Recent Infection

The first 2 years after *M. tuberculosis* infection is the period of greatest risk for development of active disease. Most authorities recommend treatment of LTBI for any person known to have become infected within 2 years, regardless of age. This is documented by conversion of the TST or interferon-γ release assay result from negative to positive within 2 years.

Treating Latent Tuberculous Infection in Persons With HIV Infection

In the era before highly active ART, TST- and HIV-positive injection drug users had an annual risk for active TB of approximately 8% per year.[136] As indicated in Table 249.10, the CDC recommends that a TST showing greater than 5 mm induration or a positive interferon-γ release assay result is an indication for treating LTBI in persons with known or suspected HIV infection in whom active TB has been ruled out.[172] WHO recommends treating LTBI in HIV-positive persons with a positive or unknown TST; treatment options include 6 to 9 months of INH, 3 months of INH plus rifapentine, 3 to 4 months of INH plus RIF, or 3 to 4 months of RIF.[381] Due to concerns regarding reinfection, WHO recommends at least 36 months of INH in HIV-positive persons living in TB-endemic countries.[381] In Côte d'Ivoire, both immediate initiation of ART and completion of 6 months of INH preventive therapy demonstrated independent decreases in the risk of death or

TABLE 249.10 Miliary Tuberculosis

FACTOR OR FINDING	STUDY			
	BIEHL[400]	MUNT[401]	MAARTENS ET AL.[402]	KIM ET AL.[403]
No. of cases	69	68	109	38%[a]
Mean age	51	50	—	60%[a]
Nonwhite race	85%	87%	94%	79%[a]
Predisposing factors	15%	31%	42%	66%[a]
Weeks of symptoms	2-16	3-24	1-52	—
Meningitis	17%	19%	22%	—
Tuberculin positive	61%	84%	43%	28%
Miliary radiograph	93%	97%	—	91%[a]
Other foci of tuberculosis	32%	23%	—	—
Positive sputum smear	—	39%	33% (21/64)	36% (12/33)
Marrow diagnostic[b]	—	20%	41% (9/22)	9% (2/22)
Transbronchial biopsy diagnostic[c]	—	—	76% (39/51)	63% (5/8)

[a]This percentage includes interstitial and diffuse alveolar patterns.
[b]Marrow diagnostic if caseating granuloma or acid-fast bacilli are seen.
[c]Transbronchial biopsy diagnostic if any granuloma or acid-fast bacilli are seen.

severe HIV-related disease among HIV-positive persons with >500 $CD4^+$ lymphocytes/mm^3.[382]

Anergy testing in immunocompromised persons is unreliable, and treatment of latent TB in anergic HIV-infected persons with latent TB does not decrease the risk of reactivation. Therefore, since 1997 the CDC has recommended against routine anergy testing for HIV-positive persons at risk for TB (see "Tuberculin Skin Testing and HIV Infection").[383] The failure of predictive tests in high TB incidence areas is underlined by experience in Khayelitsha, South Africa, where there was no association between the benefit of INH preventive therapy and TST or interferon-γ release assay result, leading to the recommendation that all patients receiving ART in moderate- to high-incidence areas should receive INH preventive therapy.[384]

Mycobacterium tuberculosis Infection in Persons With Additional Risk Factors

Treatment of LTBI is advised for persons with *M. tuberculosis* infection who are from groups with a known high incidence of TB, including immigrants from developing countries, injection drug users, the homeless, prisoners, and residents of long-term care facilities.[172] An argument has been made for treating latent tuberculous infection in patients after gastrectomy and jejunoileal bypass surgery for obesity. There is a greatly increased incidence of TB in patients undergoing chronic renal dialysis[100] and in renal transplant patients. Treatment of LTBI is also recommended for infected persons with silicosis,and for those those with myeloproliferative disorders and hematologic malignancies, especially when corticosteroids are given. Prolonged treatment with high doses of corticosteroids undoubtedly predisposes to activation of latent TB. Latently infected individuals who are to receive anti–TNF-α monoclonal antibodies (e.g., infliximab, etanercept, or adalimumab) should receive treatment of latent tuberculous infection.[205] As much treatment as possible should be received before the patient starts immunosuppressive therapy; ideally a treatment course would be completed, and this may be facilitated by short-course regimens.

Hepatotoxicity of INH has made preventive therapy difficult in patients with severe liver disease or after liver transplant. Fluoroquinolones have been considered as an option, with recognition of issues such as tendon rupture, although data to support this use are sparse.[385,386]

Pregnant Women

Because INH treatment for latent tuberculous infection may be associated with a slightly increased risk for maternal hepatitis, added caution with respect to INH-induced hepatotoxicity is indicated. There are insufficient safety data for the other preventive therapy regimens in pregnancy.

The Nursing Home Problem

A major analysis by Stead and colleagues showed that 3.8% of men and 2.3% of women who were tuberculin positive at admission to nursing homes acquired active disease and that this could be decreased 10-fold with treatment of latent tuberculous infection.[372] Treatment of latent tuberculous infection was clearly beneficial in patients who tuberculin converted after admission, with 11.6% of men and 7.6% of women acquiring active disease without treatment of latent tuberculous infection, but only 0.2% with treatment.

Vaccination

BCG, a live-attenuated vaccine derived from a strain of *M. bovis,* is used in young children throughout much of the world. Vaccination of children results in a 60% to 80% decrease in the incidence of TB,[387] but efficacy has varied widely. Its use is reasonable in high-prevalence situations, greater than those that now exist in the United States and most industrialized nations. It should be administered only to tuberculin-negative persons. Although BCG vaccine does not prevent infection, it usually prevents progression to clinical disease and effectively prevents disseminated disease in young children. The risk for disseminated BCG infection after vaccination in infants born to HIV-positive mothers is small. BCG should not be given to persons known to be infected with HIV. Prior BCG vaccination does not alter guidelines for TST interpretation, particularly if at least 10 years have passed since vaccination.

The effect of BCG vaccination on tuberculin reactivity depends on the age at vaccination and interval before skin testing. In a study in Montreal, children vaccinated once with BCG before the age of 1 year had a 7.9% prevalence of positive TST results 10 to 25 years later, comparable to those who never received BCG.[388] Prevalence of positive TST results was 18% among those vaccinated between 1 and 5 years of age and 25.4% among those vaccinated after age 5. Although tuberculin reactivity wanes after infant BCG vaccination, later skin testing can cause a booster effect, a potential source of confusion. Interesting to note, there is no relationship between tuberculin reactivity after BCG vaccination and protection against development of active TB.[175]

Intravesicular BCG, used to treat bladder cancer, is a rare cause of miliary granuloma in the liver or lung, psoas abscess, or osteomyelitis.[389–391] This mycobacteriosis responds to treatment with INH and RIF.

The development of a better vaccine for TB is a high priority.[392] Some candidates are subunit vaccines wherein immunodominant antigens are expressed by viral vectors or formulated with adjuvant. Live-attenuated vaccines have the potential advantage of inducing responses to a broader array of antigens, and the current vaccine against TB—BCG—has been modified to produce greater amounts of immunodominant antigens.

The Aeras Foundation is a not-for-profit organization based in Rockville, Maryland, dedicated to the development and testing of new TB vaccine candidates. Candidate vaccines furthest along in the Aeras Foundation pipeline for testing in clinical trials include the following: M72/AS01E (a fusion protein derived from *M. tuberculosis* antigens MTB32A and MTB39A plus adjuvant AS01E); H4:IC31 (a fusion protein derived from antigens 85B and TB10.4 plus adjuvant IC31); H56:IC31 (a fusion protein derived from antigens 85B, ESAT-6, and Rv2660c plus adjuvant IC31); and MTBVAC (a live-attenuated vaccine based on a human *M. tuberculosis* isolate); MVA85A (Ag85A expressed by modified Vaccinia Ankara virus); Aeras-402 (adenovirus expressing Ag85B and TB10.4); M72F (a fusion of Rv1196 and Rv0125 delivered in AS01 adjuvant); H56 (a fusion of Ag85B, ESAT-6, and Rv2660 delivered in IC31 adjuvant); ID93 (a fusion of Rv1813, Rv2608, Rv3619, and Rv3620 delivered in GLA-SE adjuvant); and Hybrid-4 (a fusion of Ag85B and TB10.4 delivered in IC31 adjuvant).

In the first infant efficacy trial of any TB vaccine for nearly a half century, more than 2700 infants (given BCG as neonates) were enrolled, with 1399 infants given a boosting inoculation with MVA85A within 6 months after receiving BCG. Although MVA85A was well tolerated and induced modest cell-mediated immune responses, it unfortunately did not provide significant protection against the development of TB.[393] In contrast, a trial of M72/AS01E in HIV-negative adults with latent TB in Kenya provided 54% protection against reactivation.[394]

An improved understanding of host pathways for presenting antigens of intracellular pathogens and mechanisms by which *M. tuberculosis* suppresses antigen presentation may foster novel and effective live-attenuated vaccine candidates. The Stop TB Partnership regularly updates an online document describing new vaccine candidates.[395]

EXTRAPULMONARY TUBERCULOSIS

Extrapulmonary TB can be divided into three groups based on pathogenesis. The first comprises superficial mucosal foci resulting from the spread of infectious pulmonary secretions via the respiratory and gastrointestinal tracts. Such lesions were once almost inevitable complications of extensive cavitary pulmonary disease but are now rare. The second group comprises foci established by contiguous spread, such as from a subpleural focus into the pleural space. The third group comprises foci established by lymphohematogenous dissemination, either at the time of primary infection or, less commonly, from established chronic pulmonary or extrapulmonary foci.

AIDS and Extrapulmonary Tuberculosis

Before 1985, cases of pulmonary TB in the United States decreased each year, whereas the number of extrapulmonary cases remained stable at about 4000 per year. The percentage of cases caused by extrapulmonary disease subsequently increased, largely as a result of coinfection with HIV. Unlike in non-AIDS patients, concomitant pulmonary and extrapulmonary disease is very common in AIDS patients with TB.

Cases of HIV-associated pulmonary and extrapulmonary TB have declined in the United States since 1992.[396] There are certain distinguishing features of AIDS-associated extrapulmonary TB. The frequency of disseminated disease (more than one focus or progressive hematogenous disease) is high—38% in one series—and rapidly progressive forms with diffuse pulmonary infiltrates, acute respiratory failure, and disseminated intravascular coagulation have been observed. TB pleuritis, when it occurs, is often bilateral and part of a disseminated process. Visceral lymphadenopathy, both mediastinal and abdominal, is frequent, and a contrast-enhanced CT scan showing nodes with central low attenuation suggests the diagnosis. Abscesses of the liver, pancreas, prostate, spleen, chest, abdominal wall, and other soft tissues have also been described. Among 320 cases of extrapulmonary TB from 1995 to 2007 managed at Grady Memorial Hospital in Atlanta, HIV coinfection was associated with a decreased likelihood of the pleural space as a site of extrapulmonary disease; among HIV-infected patients with extrapulmonary TB, those with fewer than 100 $CD4^+$ T cells/mm^3 were more likely to have central nervous system (CNS), meningeal, and/or disseminated TB.[397] Extrapulmonary TB appears to be predictive of an increased risk for developing paradoxical IRIS when ART is initiated (see "Immune Reconstitution Inflammatory Syndrome").[346]

General Comments on Treatment of Extrapulmonary Tuberculosis

Extrapulmonary foci usually respond to treatment more rapidly than does cavitary pulmonary TB, owing to the lower burden of organisms in the former. Therapy with four-drug regimens (INH, RIF, PZA, and EMB) for 2 months, followed by INH and RIF for 4 months, is advised in most cases caused by drug-sensitive organisms. The exceptions include bone and joint disease (6–9 months) and tuberculous meningitis (9–12 months, although optimal duration is unknown).[239] Adjunctive corticosteroids are recommended for persons with pericardial or CNS TB.

Diagnostic difficulty and delay can have a devastating impact on survival in critically ill patients. In a study from Uganda, a highly endemic country, empirical therapy for TB, despite negative sputum smears, led to improved survival in patients presenting with "danger signs," including fever greater than 39°C, tachycardia greater than 120 beats/min, or tachypnea greater than 30 breaths/min.[398] However, among HIV-positive persons with <50 $CD4^+$ lymphocytes/mm^3 starting ART, empirical four-drug therapy did not improve survival compared with INH preventive therapy alone.[399]

Miliary Tuberculosis

The term *miliary tuberculosis,* first used to describe its pathologic resemblance to millet seeds, now describes any progressive disseminated hematogenous TB. Miliary TB can be roughly divided into three groups: (1) acute miliary TB associated with a brisk and histologically typical tissue reaction; (2) cryptic miliary TB, a more prolonged illness with subtle clinical findings and an attenuated histologic response; and (3) nonreactive TB characterized by huge numbers of organisms, little organized tissue response, and often a septic or typhoidal clinical picture.[164]

Usual (Acute) Miliary Tuberculosis

In the prechemotherapy era, miliary TB occurred either soon after primary infection in children or young adults or as a terminal event in untreated chronic organ TB. In children, the illness is acute or subacute, with high intermittent fevers, night sweats, and occasional rigors. Pleural effusion, peritonitis, or meningitis occurs in as many as two-thirds of affected persons. The illness in young adults is usually more chronic and initially less severe. However, miliary TB is now more frequently observed in older individuals, often with underlying illnesses or conditions that may confuse diagnosis.

Four large series in the chemotherapy era[400–403] have emphasized the frequency of miliary TB in minority racial groups and the importance of underlying conditions such as alcoholism, cirrhosis, neoplasm, pregnancy, rheumatologic disease, and treatment with immunosuppressive agents (see Table 249.10).[400–403] There is usually no prior history of TB, and the onset is often subtle. Generalized symptoms of fever, anorexia, weakness, and weight loss are nonspecific. Headache may indicate meningitis; abdominal pain may be due to peritonitis; and pleural pain may result from pleuritis. Physical findings are likewise usually nonspecific, but a careful search for cutaneous eruptions, sinus tracts, scrotal masses, and lymphadenopathy may yield a prompt biopsy diagnosis. A miliary infiltrate on a chest radiograph is the most helpful finding and the usual reason miliary TB is suspected (Fig. 249.7). Unfortunately, many patients, particularly the elderly, succumb to miliary TB before the chest radiograph becomes abnormal.[404] The white blood cell count is usually normal, and anemia is the rule. Hyponatremia with the laboratory features of inappropriate secretion of antidiuretic hormone is frequent, particularly with meningitis.[401] Addison disease should be considered as a cause of hyponatremia. Increased levels of alkaline phosphatase and aminotransferases are common, as are hypoxemia, hypocapnia, and impairment of pulmonary diffusion capacity. Cultures of sputum, gastric contents, urine, and CSF are positive in some combination in most cases, but smears of sputum and pulmonary secretions alone are positive in fewer than one-third. Immediate diagnosis often results from examination of tissue (lymph nodes, scrotal masses when present, liver biopsy, or bone marrow specimens). Mycobacterial blood cultures may also be positive. Transbronchial biopsy is an excellent way to obtain tissue and should be performed promptly when the diagnosis is suspected.[405] The finding of caseating granulomas or AFB is virtually diagnostic.

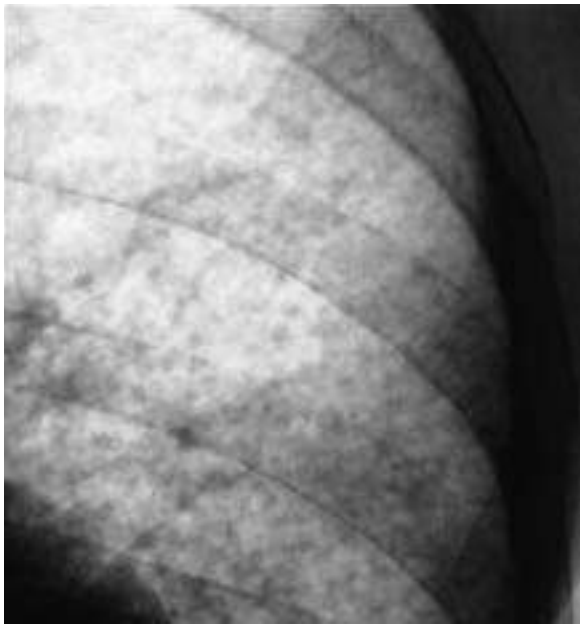

FIG. 249.7 **Detail of a chest radiograph (left midlung zone) showing countless 0.5- to 1.0-mm nodules typical of miliary tuberculosis.**

Rapid diagnosis is mandatory. However, treatment should be initiated immediately based on strong clinical suspicion, because mortality from miliary TB is most often due to delays in treatment. Response may be prompt or may take several weeks. Fulminant miliary TB may be associated with severe refractory hypoxemia and disseminated intravascular coagulation.

Cryptic Miliary Tuberculosis and Late Generalized (Chronic Hematogenous) Tuberculosis

Chronic organ TB is probably always associated with intermittent, nonprogressive seeding of the bloodstream. In some individuals, however, especially as age or other factors compromise immunity, this becomes continuous and produces progressive hematogenous TB long after the primary infection.[406] The term *cryptic miliary tuberculosis* usually describes older patients with miliary TB in whom the diagnosis is obscure because of normal chest radiographs, negative TST results, and often confounding underlying illnesses to which symptoms are

mistakenly attributed[407]; this term has also been applied to miliary TB diagnosed at autopsy.[404]

The foci responsible for late generalized TB are often clinically silent—for example, renal, genitourinary, osseous, or visceral lymph nodes.[406] Chronic pulmonary foci are at times involved but are rarely the only source. The clinical picture is frequently fever of unknown origin, often with a normal chest radiograph and a negative TST result. Fever may be absent, and in one series antemortem diagnosis was made in only 15% of cases.[406] Late generalized TB may be associated with major hematologic abnormalities.

Nonreactive Tuberculosis

The histologic appearance in this rare form of disseminated hematogenous TB shows nonspecific necrosis containing disintegrating neutrophils and enormous numbers of tubercle bacilli.[190] In the typical case, granulomas and epithelioid cells are lacking, although intermediate cases have areas more typical for TB. The gross pathologic findings are soft abscesses from minute to 1 cm, which always involve the liver and spleen, usually the marrow, commonly the lungs and kidneys, but never the meninges. The clinical picture may be overwhelming sepsis, with splenomegaly and often an inconspicuous diffuse mottling on the chest radiograph. Major hematologic abnormalities are common (see "Miliary Tuberculosis and Hematologic Abnormalities").

Miliary Tuberculosis and Hematologic Abnormalities

Some patients with late generalized TB and most with nonreactive TB have serious hematologic abnormalities, including leukopenia, thrombocytopenia, anemia, leukemoid reactions, myelofibrosis, and polycythemia.[408] Leukemoid reactions may suggest acute leukemia, although most patients in whom hematogenous TB coexists with the clinical picture of leukemia have both diseases. Disseminated TB should be considered when pancytopenia is associated with fever and weight loss or as a cause of other obscure hematologic disorders.

Primary Hepatic Tuberculosis

Rarely, miliary TB may mimic cholangitis, with fever, liver function test abnormalities suggestive of obstructive disease, and little evidence of hepatocellular disease. Diagnosis is made through liver biopsy.

Miliary Tuberculosis in AIDS

In AIDS patients, 10% with TB and 38% with extrapulmonary TB have miliary disease.[232,409] Major constitutional symptoms and hectic fevers are characteristic. The chest radiograph is abnormal in 80% and may include typical miliary mottling. Only 10% of patients are tuberculin positive.[232] The sputum smear is positive in only 25%,[409] but cultures of many materials will be positive, including blood in 50% to 60%. Biopsy specimens during life show typical tuberculous histologic appearance but with more stainable organisms than in non-HIV miliary TB. In fatal cases, in contrast, the histologic picture is often nonreactive TB.[232]

Abscesses of various soft tissue and visceral organs have been described in patients with AIDS and TB, usually with other evidence of disseminated disease. Locations include the liver, spleen, pancreas, psoas muscle with or without spinal involvement, mediastinum, neck, chest wall, abdominal wall, and prostate.[232,409,410] Diagnosis is usually made by means of CT or ultrasonography and confirmed with needle or catheter aspiration. Clinical response to chemotherapy and drainage is usually good. An abscess may appear or reappear during therapy and respond to repeated aspiration.

Central Nervous System Tuberculosis: Tuberculous Meningitis

This condition is usually caused by rupture of a subependymal tubercle into the subarachnoid space rather than direct hematogenous seeding. Meningitis complicating miliary disease usually develops several weeks into the illness. In childhood, meningitis is an early postprimary event, and three-fourths of these persons have a concurrently active primary complex, pleural effusion, or miliary TB. Subependymal foci may remain quiescent indefinitely before rupturing. This may follow head trauma or be associated with general depression of host immunity as a result of alcohol abuse or other factors.

Pathologic Features

Meningeal involvement is most pronounced at the base of the brain. In long-standing cases, a gelatinous mass may extend from the pons to the optic nerves, being most prominent adjacent to the optic chiasm. In more chronic cases, fibrous tissue may encase cranial nerves. Vasculitis of local arteries and veins may lead to aneurysm, thrombosis, and focal hemorrhagic infarction. Perforating vessels to the basal ganglia and pons are most often involved, producing movement disorders or lacunar infarcts; involvement of branches of the middle cerebral artery may cause hemiparesis.

Clinical Manifestations

The usual illness begins with a prodrome of malaise, intermittent headache, and low-grade fever, followed within 2 to 3 weeks by protracted headache, vomiting, confusion, meningismus, and focal neurologic signs. The clinical spectrum is broad, ranging from chronic headache or subtle mental status changes to sudden, severe meningitis progressing to coma. Fever may be absent, and the peripheral white blood cell count is usually normal. Mild anemia is usual, and hyponatremia resulting from inappropriate antidiuretic hormone secretion is common. Evidence of concomitant extrameningeal TB is present in roughly three-fourths of cases,[411] with miliary shadowing on the chest radiograph being most suggestive. In many cases, however, there are no clinical or historical clues to suggest TB.

The cornerstone of diagnosis is examination of the CSF. The cell count generally ranges from 0 to 1500/mm^3, the protein level is increased, and the CSF glucose is characteristically low. A lymphocytic predominance is usual, although one-fourth of cases have a polymorphonuclear pleocytosis, usually early in the course. Identifying bacilli often requires examination of large volumes of fluid from repeated lumbar punctures. In one study, stains of sediment revealed AFB in 37% of cases on initial examination but in 90% when fluids from four large-volume lumbar punctures were examined.[411] Initial atypical findings such as neutrophilic pleocytosis, a normal glucose concentration, or even entirely normal CSF indices evolve to more typical mononuclear cell predominance with hypoglycorrhachia over time. Commercial kits for detecting *M. tuberculosis* in respiratory specimens by means of amplification technology (see Table 249.1) can also be used on CSF. Overall, the sensitivity of PCR assay for examination of the CSF is low. The CSF is often culture negative for *M. tuberculosis,* but the CSF PCR assay has been reported to be positive in roughly 60% to 90% of CSF samples that eventually are culture positive.[412,413] Results of testing CSF with the Xpert MTB/RIF Ultra assay were positive in 16 of 23 cases of proven or probable tuberculous meningitis, making this the preferred rapid test for this infection.[414]

In patients with meningitis, CT or MRI may reveal rounded lesions presumed to be tuberculomas, basilar arachnoiditis, cerebral infarction, or hydrocephalus (Fig. 249.8). Contrast-enhanced MRI frequently reveals concomitant spinal cord and radicular involvement (transverse and longitudinal myelitis, spinal tuberculoma, or abscess and other manifestations), which may lead to disabling complications.[415]

Prognosis is influenced by age, duration of symptoms, and neurologic deficits. Mortality is greatest in patients younger than age 5 (20%), in those older than age 50 (60%), or in those in whom illness has been present for more than 2 months (80%).[411] Patients without neurocognitive impairment, focal neurologic signs, or hydrocephalus at the start of therapy are likely to recover, whereas approximately half of patients who are stuporous or have dense paraplegia or hemiplegia die or recover with severe residual neurologic defects.[411] A prognostic score derived from cases in 43 centers in 14 countries found that altered consciousness, diabetes mellitus, immunosuppression, neurologic deficits, hydrocephalus, and vasculitis predicted an unfavorable outcome in culture-proven tuberculous meningitis.[416] Concomitant HIV infection does not appear to alter the clinical and laboratory manifestations or the prognosis of tuberculous meningitis, except that CNS mass lesions are more likely.[417]



FIG. 249.8 Multiple cerebral cortical densities on computed tomography scan of a patient with tuberculous meningitis.

Therapy

In the presence of meningeal inflammation, both INH and PZA reach concentrations in the CSF equaling those in blood. RIF penetrates the blood-brain barrier less well but still adequately. In some cases, PZA is given beyond the first 2 months of treatment because of its excellent CNS penetration, although its activity after 2 months of treatment is unclear. The RIF dose is sometimes increased owing to its poor CNS penetration, particularly with concomitant administration of corticosteroids. Otherwise, the dosages are as for pulmonary TB. A recent study demonstrated that higher-dose RIF (13 mg/kg IV vs. the standard 10 mg/kg oral), together with INH, PZA, and corticosteroids, was associated with improved 6-month survival and neurologic recovery.[418] However, a trial of intensification with high-dose RIF (15 mg/kg/day) and levofloxacin for the first 8 weeks, added to a standard 9-month regimen, in Vietnam failed to improve survival.[419]

Most authorities recommend adjunctive corticosteroids in TB meningitis, particularly stage 2 (objective neurologic findings) and stage 3 (stupor-coma) patients, beginning prednisone at 60 to 80 mg daily. This may be gradually reduced after 1 to 2 weeks and discontinued by 6 to 8 weeks, as guided by symptoms. Symptoms and CSF abnormalities may rebound transiently as corticosteroids are tapered. Ventricular shunting may be beneficial if symptomatic hydrocephalus supervenes.

Tuberculomas

Intracranial tuberculomas are space-occupying lesions that may manifest as seizures. They are most frequently multiple, appearing on imaging studies as avascular masses with surrounding edema. Corticosteroids reduce edema and decrease symptoms, and chemotherapy prevents spread of infection in cases diagnosed at operation.

Tuberculous Spinal Meningitis

Infrequently, TB causes spinal meningitis with or without intracranial involvement. In advanced cases, the cord may be completely encased in a gelatinous exudate. An intramedullary tuberculoma or an extradural granulomatous mass can cause symptoms without meningeal involvement. Nerve root or cord compression causes pain, bladder or rectal sphincter weakness, hypesthesia, anesthesia, paresthesias in the distribution of a nerve root, or paralysis. Subarachnoid block may cause CSF protein concentrations to be extremely high, with or without cells.

Tuberculous Pleurisy (Serofibrinous Pleurisy With Effusion)

Types of Tuberculous Pleurisy

Early Postprimary Pleurisy With Effusion

When infection occurs early in life, tuberculous pleurisy with effusion follows the primary infection within weeks or months. The pathogenesis is rupture of a large subpleural component of the primary infection and delivery of infectious, antigenic material into the pleural space, with inflammation and seeding of foci over the visceral and parietal pleura. In the past, this affected mostly adolescents and young adults and, rarely, older adults. Immediate prognosis was excellent, with resolution of the effusion within several months in as many as 90% of cases. However, studies of soldiers during World War II (before chemotherapy) demonstrated that 65% relapsed with chronic organ TB within 5 years.[420] Early postprimary serofibrinous pleurisy with effusion identifies quantitatively large primary infections with a relatively poor long-term prognosis.

Pleurisy With Effusion Complicating Chronic Pulmonary Tuberculosis

In contrast to early studies,[420] an increased proportion of pleurisy with effusion since the early 1980s occurs in older individuals with chronic pulmonary TB, often with complicating illnesses such as cirrhosis or congestive heart failure to which the effusion is mistakenly attributed. In one study, one half of pleurisy cases occurred in the setting of established chronic pulmonary TB.[421]

Pleurisy With Effusion Complicating Miliary Tuberculosis

Pleural effusions occur in 10% to 30% of cases of miliary TB.[400,402] These may be associated with other progressive extrapulmonary foci and involvement of other serous membranes. Cases with coexistent pleural (at times bilateral), peritoneal, and pericardial TB have been referred to as *tuberculous polyserositis.*

Clinical Manifestations and Diagnosis

The clinical presentation may be low grade and subtle or abrupt and severe, easily confused with acute bacterial pneumonia. Cough and pleuritic chest pain are usual, and fever may be high. The effusion is usually less than massive and almost always unilateral except when associated with miliary TB. The pleural fluid typically contains 500 to 2500 white blood cells/mm^3, with more than 90% lymphocytes in two-thirds of cases. However, 38% of cases in one series had predominantly neutrophils and 15% had more than 90% neutrophils on the first tap.[422] Repeated taps demonstrate a shift to lymphocytic predominance. Mesothelial cells, characteristic of neoplastic effusions, are sparse or absent, eosinophils are rarely present, and less than 10% of effusions are serosanguineous. The pleural fluid protein level usually exceeds 2.5 g/dL, the glucose concentration is usually moderately low compared with serum values but rarely less than 20 mg/dL, and the pH is almost always 7.3 or lower and may be as low as 7.0. Increased pleural fluid adenosine deaminase levels have been suggested to be highly sensitive and specific for tuberculous pleuritis.[423,424] High pleural fluid adenosine deaminase levels with other conditions (e.g., neoplasia) limit its diagnostic utility in countries with a low prevalence of TB,[425] although levels with such conditions infrequently exceed the suggested cutoff for TB.[426,427] A significant negative association of age and pleural fluid adenosine deaminase suggests the need for a lower cutoff in patients older than 45 years, to decrease false-negative results.[428] Bloody pleural fluid suggests either TB or malignancy. In the usual case of early postprimary pleurisy with effusion, the acid-fast stain of the fluid sediment is seldom positive, the culture is positive in 25% to 30%, pleural needle biopsy yields granulomas in 75%, and culture of a needle biopsy specimen may be positive even in the 25% of cases with nonspecific pleuritis on histologic examination. Cases complicating chronic pulmonary TB more often have positive pleural acid-fast smears (50%) and positive cultures (60%) but are less likely (25%) to demonstrate granulomas on pleural biopsy. Repeat pleural biopsy may be necessary to establish the diagnosis, and a small open pleural biopsy or thoracoscopy is diagnostic in virtually all cases. Smears of sputum or gastric fluid are rarely positive in early

postprimary cases, and cultures are positive in 25% to 33%. In contrast, sputum smear is positive in 50% and the culture is positive in 60% of "reactivation" cases.[421] TB is often not considered as the cause of a pleural effusion in an older person with complicating illnesses such as cirrhosis or congestive heart failure.[422] When pleural effusion complicates miliary TB, findings associated with the latter condition usually dominate the clinical picture.

Therapy

Early postprimary pleural effusions spontaneously resolve in 2 to 4 months. Chemotherapy does not hasten resolution but prevents active disease elsewhere in the body, which will otherwise occur in 65% of cases.[420] Therapy is as described for pulmonary TB. Multiple thoracenteses are not necessary once the diagnosis is established and treatment initiated. A small minority heals with pleural fibrosis. Corticosteroid therapy hastens symptomatic improvement and fluid resorption, but no long-term benefit has been shown and therefore it is not recommended.

Tuberculous Empyema and Bronchopleural Fistula

Tuberculous empyema occurs when a major cavity ruptures into the pleural space. This often catastrophic illness is usually associated with bronchopleural fistula formation and frank pus. Before antituberculous drugs were available, tuberculous empyema was almost always rapidly fatal. It virtually never occurs in patients being treated with chemotherapy.

Tuberculous Pericarditis

Tuberculous pericarditis is most often caused by extension from a contiguous focus of infection, usually mediastinal or hilar nodes but also the lung, spine, or sternum. Less commonly, it occurs during miliary TB. It sometimes develops during the course of otherwise effective drug therapy. In the United States, tuberculous pericarditis is an uncommon complication of AIDS; but in two series from sub-Saharan Africa, the vast majority of effusive pericarditis cases were tuberculous and almost all patients were HIV positive.[429,430]

Clinical Manifestations and Diagnosis

The onset may be abrupt, resembling acute idiopathic pericarditis, or insidious, resembling congestive heart failure. Symptoms of infection or cardiovascular compromise may be present. Individual cases may manifest as chronic constrictive pericarditis and may be mistaken for cirrhosis with ascites. As many as 39% of patients also have a pleural effusion, providing a convenient source for diagnostic fluid and tissue.[431,432] Echocardiography demonstrates effusion when present and may reveal multiple loculations suggestive of TB.

Pericarditis with effusion is usually quickly diagnosed based on physical findings and radiologic examination, but establishing that it is tuberculous in nature is often difficult. The TST result may be negative, and evidence of extrapericardial TB lacking. In areas of high endemicity, a presumptive diagnosis is often correctly made.[431,432] In the United States, however, many cases are initially misdiagnosed as idiopathic, uremic, or rheumatoid pericarditis.[433]

Pericardiocentesis (ideally performed in a cardiac catheterization laboratory) is indicated for hemodynamic compromise. However, because pericardiocentesis carries risk, and because 90% of acute pericarditis in the United States is idiopathic (presumed viral) and subsides spontaneously in 2 to 3 weeks, some authorities advise against early pericardiocentesis. If improvement has not occurred by that time, a subxiphoid pericardial window can be performed. This provides both fluid and tissue for diagnosis, although in some cases the biopsy demonstrates only nonspecific inflammation.[431,432] Tuberculous pericardial fluid demonstrates many of the characteristics of tuberculous pleural fluid, with acid-fast smears being rarely positive and cultures being positive in approximately 50% of cases. Bloody fluid suggests either TB or malignancy. The usefulness of adenosine deaminase determinations on pericardial fluid is not certain, but PCR assay for *M. tuberculosis* may be diagnostic, although sensitivity is low.

Therapy

Antibiotic treatment is the same as for pulmonary TB. In a large study from South Africa from the 1980s, treatment with corticosteroids (60 mg/day for 4 weeks, 30 mg/day for 4 weeks, and 15 mg/day for 2 weeks) decreased mortality from 11% in controls to 4% in treated patients. Pericardiectomies were also less frequently necessary in patients given corticosteroids (30% in controls vs. 11% in corticosteroid-treated patients). However, a larger comparative trial of immunotherapy with either *Mycobacterium indicus pranii* (1250 patients) or prednisolone (1400 patients), for definite or probable tuberculous pericarditis, failed to demonstrate a significant effect on a composite score of death, cardiac tamponade requiring pericardiocentesis, or constrictive pericarditis.[434] The incidence of constrictive pericarditis and hospitalization was decreased in prednisolone-treated patients, and rates of adverse events, especially cancer, were elevated in HIV-infected patients. Because the size of this study was much larger than that in previous reports, current recommendations for the use of adjunctive corticosteroids in the management of tuberculous pericarditis have been revised: They should not be routinely used.[239] However, selective use of corticosteroids in persons at the highest risk of inflammatory complications should be considered. Surgical drainage via a subxiphoid pericardial window at the outset did not decrease either mortality or the eventual need for pericardiectomy, although it provided diagnostic tissue and obviated the need for recurrent pericardiocenteses. However, 2% surgical mortality was associated with the procedure.[431,435]

When hemodynamic compromise persists for 6 to 8 weeks, pericardiectomy is usually indicated, and this should probably be performed earlier rather than later. Approximately two-thirds of patients, however, do well without surgery.

Skeletal Tuberculosis: Pott Disease (Tuberculous Spondylitis)

One-third of cases of skeletal TB involve the spine, as a result of past hematogenous foci, contiguous disease, or lymphatic spread from pleural disease. The earliest focus is the anterior superior or inferior angle of the vertebral body. This usually spreads to the intervertebral disk and adjacent vertebra, producing the classic radiographic picture of anterior wedging of two adjacent vertebral bodies with destruction of the intervening disk and the physical finding of a tender spine prominence or gibbus. The lower thoracic spine is involved most frequently, followed by the lumbar spine (Fig. 249.9).

In endemic countries, Pott disease usually occurs in older children and young adults, but in developed countries it has become a disease of older persons.[436] In a country such as Denmark, with large immigrant populations from endemic areas, spinal TB accounted for more than half of bone and joint disease and was mainly seen in younger immigrants.[437] Evidence of other foci of TB and systemic symptoms are often absent, early complaints may be back pain or stiffness with an initially normal radiograph, and diagnosis may be delayed until signs of advanced disease such as paralysis, deformity, or sinus formation develop. Bacilli are sparse, and smear and culture of pus or tissue are positive in only one-half of cases. Histologic studies reveal granulomas with or without caseation in three-fourths of cases.

Clinical Manifestations

Abscess and Sinus Formation

Paraspinal cold abscesses develop in 50% or more, in some cases appearing after treatment has been initiated, and in some cases visible only with CT or MRI. The pus, confined by tight ligamentous investments, can dissect along tissue planes for long distances to present as a mass or a draining sinus in the supraclavicular space, above the posterior iliac crest in the Petit triangle, or in the groin, the buttock, or even the popliteal fossa. The abscess can spread infection to distant vertebral bodies, sometimes without affecting the intervening vertebrae. Epidural or psoas abscess can also complicate tuberculous spondylitis.

Spinal Tuberculosis Without Bony Involvement on Plain Films

In high TB/HIV prevalence settings, radiculomyelitis may be a common manifestation of spinal TB, and often manifests as a paradoxical reaction.



FIG. 249.9 **Magnetic resonance image showing extensive destruction of L1 and L2 vertebral bodies and the intervening disk with posterior extension in a Pakistani man with Pott disease.**

Among 274 mostly HIV-positive South African patients who were referred to a center for patients with spinal disease but without bony involvement on plain films, MRI imaging revealed radiculomyelitis in 76%, spondylitis in 39%, subdural abscess in 15%, and intramedullary tuberculoma in 12%.[438]

Pott Paraplegia

In approximately half of cases, weakness or paralysis of the lower extremities is present or develops after treatment has begun, perhaps owing to arachnoiditis and vasculitis.[436] Less frequently, it will be due to compression of the cord by an inflammatory mass or rarely pressure in the abscess producing ischemic changes in the subjacent cord. Inflammatory thrombosis of the anterior spinal artery can occur, and sudden cord compression may result from marked spinal instability.

Therapy

A 6- to 9-month course of therapy that contains INH and RIF is at least as effective as 18-month regimens that include INH plus PAS and EMB.[439,440] Adjunctive surgical débridement or resection of the involved bone plus bone grafting did not improve outcome compared with antituberculosis therapy alone. Thus, current recommendations are to treat spinal TB with a 6- to 9-month regimen that includes INH and RIF, with PZA and EMB for the first 2 months.[239] Surgical intervention may be necessary in patients who do not respond to therapy or those with cord compression with neurologic deficits or spine instability.

Peripheral Osteoarticular Tuberculosis

Older reports described peripheral tuberculous arthritis as a chronic, slowly progressive monoarthritis in 90% of cases,[441] often without systemic symptoms or extraskeletal TB, and most frequently in the hip or knee. A history of trauma was common, followed weeks or months later by indolent progressive inflammation. More recent reports suggest a shift to an older population with a different clinical picture, including more systemic symptoms, multiple joint involvement, and periarticular abscess formation.[442] Tenosynovitis of the hand, arthritis of the wrist, and carpal tunnel syndrome can be caused by TB. Clinical confusion occurs when TB superinfects joints previously involved with other arthritides.

The earliest manifestation of tuberculous arthritis is pain, which may precede signs of inflammation and radiographic changes by weeks or months. Radiographs initially may show soft tissue swelling but later demonstrate osteopenia, periarticular bony destruction, periosteal thickening, and eventually destruction of cartilage and bone. Cold abscesses and draining sinuses often develop in chronic cases.

In the absence of coexistent extraarticular TB, diagnosis almost always requires biopsy. Histologic features compatible with TB warrant chemotherapy, although other chronic infections (fungi, nontuberculous mycobacteria) can cause identical clinical and histologic pictures. For early cases, prolonged chemotherapy results in complete resolution. Surgery is necessary only when serious joint instability requires fusion, and then only after chemotherapy has failed.

Tuberculous osteomyelitis can affect any bone, including the ribs, skull, phalanx, pelvis, and long bones.[443] Other causes of osteomyelitis of the rib are rare, and TB is the most common infectious cause of single or multiple osteomyelitic rib lesions. Tuberculous osteomyelitis outside the vertebral body manifests as a cold abscess, with swelling and only modest erythema or pain.

TABLE 249.11 Clinical Manifestations of Renal Tuberculosis in Two Series of Patients

FACTOR OR FINDING	STUDY: SIMON ET AL.[445]	STUDY: CHRISTENSEN[446]
No. of patients	102	78
Primarily genitourinary symptoms	61%	71%
Back and flank pain	27%	10%
Dysuria, frequency	31%	34%
Constitutional symptoms	33%	14%
Abnormal urine, no symptoms	5%	20%
Abnormal urinalysis	66%	93%
Abnormal intravenous pyelogram	68%	93%
Tuberculin positive	88%	95%
Abnormal chest radiograph	75%	66%
Active pulmonary tuberculosis	38%	7%
Other old or active extrapulmonary disease	5%	20%
Urine culture positive		
For tuberculosis	80%	90%
For routine pathogens	45%	12%
Epididymitis, orchitis	19%	17%
Chronic prostatitis	6%	6%

Genitourinary Tuberculosis: Renal Tuberculosis

Asymptomatic renal cortical foci may occur during all forms of TB. An autopsy study of pulmonary TB revealed unsuspected renal foci in 73% of cases, usually bilateral; in 25% of miliary cases, patients have positive urine cultures.[444] Cortical foci tend to be stable unless they penetrate to the medulla, where local factors favor accelerated infection. Most patients have evidence of concomitant extragenitourinary disease, usually pulmonary and most frequently inactive. In normal hosts, the interval between infection and active renal disease is usually years and sometimes decades. Local symptoms predominate, and advanced tissue destruction may occur long before the diagnosis is made.

The clinical manifestations in two large series of cases are presented in Table 249.11.[445,446] Although sterile pyuria is typical of renal TB, positive cultures for routine bacterial pathogens may lead to misdiagnosis, sometimes for years. The contrast-enhanced abdominal CT is usually abnormal. Early findings are nonspecific, but later changes may be more suggestive, including papillary necrosis, ureteral strictures, "pipestem" changes, "corkscrewing," "beading," hydronephrosis, gross parenchymal cavitation, and autonephrectomy. Focal calcification is particularly suggestive. The clinical disease is usually unilateral, although microscopic

changes are probably always bilateral. Culture of three morning urine specimens for mycobacteria establishes the diagnosis in 80% to 90% of cases. When a renal abnormality is present but urine cultures are negative, cytologic studies and culture of material obtained by fine-needle biopsy may be diagnostic. Ureteral cicatrization and obstruction may occur after otherwise effective chemotherapy, but surgery is rarely required.

Hypertension is not a feature of renal TB, and renal function is usually preserved. However, a rare condition called *tuberculous interstitial nephritis* may cause renal failure.[447] It is characterized by interstitial granulomas and normal-sized kidneys, usually in the presence of active extrarenal TB. AFB have been seen but not cultured from renal biopsy specimens, and renal dysfunction responds to corticosteroid therapy but not antituberculous chemotherapy alone. It is unclear that tuberculous interstitial nephritis is actually caused by tuberculous infection.

Male Genital Tuberculosis

Eighty percent of male genital TB is associated with coexistent renal disease, and most advanced renal TB is associated with some male genital focus.[448] Spread of infection from renal foci involves the prostate, seminal vesicles, epididymis, and testis, in that order. The usual clinical finding is a scrotal mass that may be tender or associated with a draining sinus. Calcified foci may form within the prostate during treatment of prostatic TB. Genital foci not associated with renal disease can be established by lymphohematogenous spread and usually present as a painful testicular or scrotal mass. Diagnosis may be suggested by the presence of epididymal or prostatic calcification, although the latter also occurs with nontuberculous chronic prostatitis. The diagnosis is usually established by biopsy, and response to chemotherapy is excellent.

Genitourinary Tuberculosis in AIDS

In a study of 79 HIV-positive patients with TB, 77% had positive urine cultures, usually as an incidental finding. Only two had male genital involvement, none had symptoms of renal disease, and in only 4% was the genitourinary tract the only apparent site of TB.[409]

Female Genital Tuberculosis

Female genital TB begins with a hematogenous focus in the endosalpinx, from which it may spread to the endometrium (50%), ovaries (30%), cervix (10%), and vagina (1%).[449] In the cervix, a granulomatous ulcerating mass may resemble carcinoma. Common complaints are infertility or local symptoms consisting of menstrual disorders and abdominal pain. The clinical picture may suggest pelvic inflammatory disease that is unresponsive to therapy. Spread to the peritoneum may also occur. Systemic symptoms are uncommon, and evidence of old TB need not be present. Pregnancies that occur in the presence of pelvic TB are often ectopic. Although cultures of menstrual blood or endometrial scrapings may be positive, the diagnosis is usually made by examination of tissue removed at operation. Response to chemotherapy is excellent, and surgery is needed only for residual large tubo-ovarian abscesses.

Gastrointestinal Tuberculosis

Before effective chemotherapy was available, 70% of patients with advanced pulmonary disease acquired gastrointestinal TB from swallowing infectious secretions and usually developed diarrhea and abdominal pain. Although most cases at present are likely due to swallowed respiratory secretions, radiographic evidence of pulmonary TB is less frequent, the diagnosis being made unexpectedly with surgery or endoscopy.[450]

Any location from mouth to anus can be involved. Nonhealing ulcers of the tongue or oropharynx and nonhealing sockets after tooth extraction may be due to TB. Esophageal disease is most frequently caused by an adjacent caseous node, which leads to stricture with obstruction or tracheoesophageal fistula formation and rarely to fatal hematemesis from an aortoesophageal fistula. Stomach involvement may be ulcerative or hyperplastic and may cause gastric outlet obstruction. Isolated duodenal disease can produce symptoms of peptic ulcer or obstruction. Small bowel involvement may lead to perforation, obstruction, enteroenteric and enterocutaneous fistulas, massive hemorrhage, and severe malabsorption. Small bowel lesions are frequently multiple. The ileocecal area is the most typical site of enteric TB, producing pain, anorexia, diarrhea, obstruction, hemorrhage that may be severe, and often a palpable mass. Clinical, radiographic, endoscopic, and even operative findings may suggest carcinoma. A successful diagnosis is usually made by means of colonoscopy. In a study of 50 cases, ileocecal involvement with or without involvement of other areas was found in 35 cases, isolated segmental colonic disease was found in 13 cases, and pancolitis was initially misdiagnosed as ulcerative colitis in 2 cases. Evidence of pulmonary TB was present in only 18 cases.[451] The response of gastrointestinal TB to chemotherapy is excellent. Once the diagnosis is established, surgery should be deferred if possible until the results of chemotherapy have been assessed.

Pancreatic TB may manifest as an abscess or as a mass involving local nodes and resembling carcinoma. The biliary tract may be obstructed by tuberculous nodes, and tuberculous ascending cholangitis has been described. TB is a frequent cause of granulomatous hepatitis. This is usually asymptomatic but may be associated with an increased alkaline phosphatase level that is out of proportion to bilirubin levels with normal aminotransferase levels. Very rarely, tuberculous granulomatous hepatitis causes jaundice without evidence of extrahepatic TB. This is called *primary tuberculosis of the liver. Focal hepatic tuberculosis* describes single or multiple tuberculous abscesses. These appear to occur most frequently in racial groups with little natural immunity to TB and in children.[452]

Gastrointestinal Tuberculosis in AIDS

Bowel involvement is not a common feature of extrapulmonary TB in AIDS patients. One series reported bowel fistulas in less than 4% of such cases,[409] another reported CT evidence of gastrointestinal abnormalities in 4 of 23 cases,[453] and a third study noted positive stool cultures for *M. tuberculosis* in 4 of 10 cases.[454] Tuberculous visceral abscesses, including hepatic, splenic, and pancreatic, may occur in AIDS patients. Pain and fever are usually present. Diagnosis is often made by means of CT or ultrasonographically guided drainage procedures. Chemotherapy alone has not been effective in all cases.[410]

Tuberculous Peritonitis

Tuberculous peritonitis results either from spread of adjacent tuberculous disease such as an abdominal lymph node, intestinal focus, or fallopian tube, or during miliary TB. In a summary of 11 series, evidence of associated pleuropulmonary TB was present in 25% to 83% of cases and the TST result was positive in 30% to 100% of cases.[455] Pleural effusion is the most frequent associated finding, but evidence of TB in other sites is often present. AIDS patients do not have an increased frequency of peritonitis.[409]

The clinical picture has been divided into *plastic* and *serous* types. The less common plastic type is characterized by tender abdominal masses and a "doughy abdomen." Serous effusions present as ascites with or without signs of peritonitis. Symptoms of fever, abdominal pain, and weight loss are common.[455] The onset may be insidious, although acute presentations resembling bacterial peritonitis also occur. In the past, diagnosis was often made at surgery for a mass or an acute abdominal condition. Tuberculous peritonitis often goes undiagnosed in patients with concomitant cirrhosis with ascites.[456] Of 20 patients with both conditions, the diagnosis of tuberculous peritonitis was suspected before death in only 11. Tuberculous peritonitis has been reported in peritoneal dialysis patients with the clinical picture of bacterial peritonitis unresponsive to routine antibiotics.[457]

The peritoneal fluid is exudative, usually containing 500 to 2000 cells. Lymphocytes typically predominate, although in some cases neutrophils are more abundant early in the process. Acid-fast smear of peritoneal fluid is seldom positive, and culture is positive in only 25% of cases. An increased adenosine deaminase level in ascitic fluid has been reported to have high sensitivity and specificity,[458] although among 140 patients in India the positive predictive value was only 25%.[459]

Analysis of peritoneal fluid with PCR, such as the Xpert MTB/RIF or Xpert MTB/RIF Ultra assay, may provide rapid diagnosis. In the absence of other foci of TB, peritoneal tissue may need to be obtained in order to make the diagnosis. Histologic examination of peritoneal biopsy specimens obtained with a Cope needle were positive in 64%

of cases and those obtained by peritoneoscopy in 85% in one series.[460] Fatal hemorrhages after both Cope needle biopsy and peritoneoscopy have been recorded.[455]

Treatment is the same as for pulmonary TB. There is some evidence that adjunctive corticosteroids decrease the likelihood of late intestinal obstruction,[460] but pending definitive studies, the routine use of adjunctive corticosteroids cannot be recommended.[461]

Tuberculous Lymphadenitis (Scrofula): Peripheral Nodes

Lymphadenitis is the most frequent form of extrapulmonary TB. In HIV-negative persons, it is usually unilateral and cervical in location.[462] The most common site is along the upper border of the sternocleidomastoid muscle, where it presents as a painless, red, firm mass. It is seen most frequently in young adult females of non-European ancestry, although it can affect any age or race. Children often have an ongoing primary infection, but in other age groups evidence of extranodal TB and systemic symptoms are usually absent. Lymphadenopathy outside the cervical and supraclavicular area indicates more serious TB, usually with systemic symptoms. The TST result is almost always positive. Fine-needle aspiration demonstrates cytologic evidence of granuloma, but smears or cultures are usually negative.[463] Biopsy with culture is often required for diagnosis, because nodes with a nonspecific histologic appearance have been positive for *M. tuberculosis* on culture, and material with typical histologic features may be due to other mycobacteria or fungi. Complete excision of involved nodes with no drain left in place is recommended in order to diminish the possibility of postoperative fistula formation. Untoward events such as node enlargement with pain, suppuration, sinus formation, and appearance of new nodes occur in 25% to 30% of cases, both during and after chemotherapy, and do not indicate failure of drug treatment. These likely represent reactions to retained tuberculous antigens rather than uncontrolled infection; they usually subside spontaneously, and short courses of corticosteroids may be beneficial when the problem persists.[464]

Conversely, in individuals with AIDS, peripheral tuberculous lymphadenitis is almost always multifocal and associated with major systemic symptoms such as fever, weight loss, and evidence of TB in the lungs (parenchyma, nodes, or pleura) or elsewhere (Fig. 249.10).[463] In an early series from New York City, TB caused 57% of generalized lymphadenopathy in HIV-positive injection drug users.[465] In contrast to non–HIV-infected persons, material removed with fine-needle aspiration is positive on acid-fast stain in the great majority of cases—as frequently as it is with culture. However, both cytologic and histologic findings are less specific than in HIV-negative persons.[466]

Mediastinal Tuberculous Lymphadenopathy

Mediastinal adenopathy during primary infection is often visible radiographically, especially in children. In African Americans, mediastinal adenopathy resulting from TB may also be seen in young adults, and cases in very old persons have been reported.[467] Associated systemic symptoms may or may not be present, causing confusion with other mediastinal masses such as histoplasmosis, lymphoma, and carcinoma. The finding of low-density areas in the nodes on CT scan suggests TB, but diagnosis usually requires mediastinoscopy. In HIV-infected persons with TB, in contrast, mediastinal lymphadenopathy is frequent. Multiple nodes are usually involved, coalescing into large mediastinal masses with low-density centers, peripheral contrast enhancement, and no calcification.[468,469]

Fibrosing Mediastinitis

TB can cause fibrosing mediastinitis, although less commonly than histoplasmosis. Patients present with dyspnea on exertion resulting from compression of pulmonary veins and arteries or, less commonly, superior vena cava syndrome. Hilar adenopathy or active pulmonary disease is rarely found. A perfusion lung scan helps define the extent of pulmonary vascular compression, but thoracotomy is required for diagnosis. Mediastinoscopy is either contraindicated because of superior vena cava syndrome or unsuccessful because of fibrosis.



FIG. 249.10 Axillary lymphadenitis caused by *Mycobacterium tuberculosis* in a patient with acquired immunodeficiency syndrome.

Mesenteric Tuberculous Lymphadenitis

In HIV-negative persons, isolated symptomatic mesenteric lymphadenitis without bowel disease or peritonitis is rare. It may cause abdominal pain, fever, a palpable mass, or symptoms of partial small bowel obstruction. In AIDS patients with TB, abdominal lymphadenopathy is common and may be massive.[469,470] Involvement is more often intraabdominal than retroperitoneal, and occasionally obstruction of the biliary tract, ureters, or bowel is observed. As with thoracic disease, the nodes often are low density or have low-density centers and peripheral enhancement. Other abnormalities on CT may include abscesses in the liver, spleen, pancreas, or kidney; local ileal thickening; extraluminal bowel gas indicating fistula formation; and ascites.

Cutaneous Tuberculosis

In the past, a number of cutaneous conditions were associated with TB elsewhere in the body, although *M. tuberculosis* could not be identified in the lesions. These have been considered allergic reactions to the infection and termed *tuberculids*. They include erythema induratum of Bazin, papulonecrotic tuberculids, and others. This association has been questioned, and some have attributed tuberculids to other processes, such as sarcoidosis.[471] *M. tuberculosis* DNA has been detected in erythema induratum skin lesions with PCR.[472] Erythema nodosum has been attributed to primary TB, although organisms cannot be cultured from the lesions.

The pathogenesis of cutaneous involvement in TB is varied. Skin involvement may result from exogenous inoculation (which in the previously nonsensitized host is associated with regional lymphadenitis). TB verrucosa cutis, arising from direct inoculation, has also been called prosector's wart, referring to inoculation of a hand at time of autopsy of a patient with TB. Infection may also spread from an adjacent focus to the overlying skin (as from lymphadenitis, osteomyelitis, or epididymitis), and hematogenous spread from a distant focus or as a part of the generalized hematogenous dissemination. This last is seen in patients with AIDS and tuberculous bacteremia.[473] The clinical picture of all cutaneous mycobacterial infections, including TB, is highly variable, and any unexplained skin lesion, especially if it has nodular or ulcerative components, may be due to TB, particularly in AIDS patients.

Tuberculous Laryngitis

In the prechemotherapy era, laryngeal TB occurred in more than a third of patients dying of pulmonary TB, often associated with painful ulcers of the epiglottis, pharynx, tonsils, and mouth, and with middle

ear involvement. Laryngeal disease was highly infectious and often caused terminal widespread bronchogenic dissemination throughout the lungs. At present, however, more than one-half of laryngeal TB cases are due to hematogenous seeding. Such cases are still highly contagious. Lesions vary from erythema to ulceration and exophytic masses resembling carcinoma.[474] Symptoms include cough, wheezing, hemoptysis, dysphagia, odynophagia, and otalgia.

Tuberculous Otitis

Tuberculous otitis media is rare and frequently misdiagnosed. Half of the cases have no other evidence of present or past TB. The classic clinical picture is painless otorrhea with multiple tympanic perforations, exuberant granulation tissue, early severe hearing loss, and mastoid bone necrosis. The diagnosis has been missed for years by excellent otolaryngologists, even when tissue was available. Tuberculous otitis may be complicated by facial nerve paralysis. Response to drug therapy is excellent, and surgery is usually not required.[475]

Miscellaneous Conditions

TB of the aorta with or without aneurysm formation can be caused by spread from contiguous diseased nodes, pericarditis, spondylitis, paravertebral abscesses, or empyema. Extensive hematogenous dissemination or aortic rupture may occur. TB produces various ocular syndromes, including choroidal tubercles, uveitis, iritis, and episcleritis (see Chapter 115). An interesting observation has been made in patients with uveitis (most suggestive of sarcoidosis) who had positive interferon-γ release assays to TB. Fifteen of the 20 patients had lived in an area endemic for TB before moving to the Netherlands. Despite the absence of documented tuberculous infection elsewhere in the body, antituberculosis therapy was associated with remission in 91% of those treated.[476] During disseminated TB in areas of high TB prevalence, choroidal TB (usually without symptoms) may occur in 5% to 20% of patients.[477] TB may also involve the breast, producing abscesses, sclerosing lesions resembling carcinoma, and multiple nodules. Destructive nasal lesions resembling Wegener granulomatosis both clinically and histologically have been caused by TB.[478] TB of the adrenal glands may cause adrenal enlargement with or without calcification, as may histoplasmosis, but granulomatous adrenal TB may cause Addison disease without either calcification or adrenal enlargement.[479]

Mycobacterium bovis and Bacillus Calmette-Guérin

M. bovis is a member of the *M. tuberculosis* complex. Isolation requires that decontamination and culture procedures used for TB be optimized. Sodium pyruvate is often substituted for glycerol in the culture medium. *M. bovis* may be mistaken as *M. tuberculosis* clinically, but molecular methods including WGS can distinguish the two. The BCG strain of *M. bovis* is used to vaccinate against TB. BCG Tice and BCG Connaught are the most widely used strains in North America and Europe, but very many other strains have been used to treat bladder cancer.[480]

Unlike *M. tuberculosis, M. bovis* largely infects humans as a zoonosis. Cattle are the primary reservoir, but a large variety of warm-blooded animals may be infected. In developing countries, infection is acquired from close contact with infected cattle or ingestion of infected dairy products.[481,482] From 2006 to 2013, *M. bovis* caused approximately 1% of TB cases in the United States, with higher prevalence among infants and children, foreign-born patients, Hispanics, females, and persons living near the Mexican border.[480] *M. bovis* infection may also complicate intravesicular BCG administered to treat intraepithelial bladder cancer.[483,484]

Intravesicular BCG is given as a series of bladder injections through a catheter, with each instillation containing as many as 8×10^8 bacilli. Symptoms of cystitis are common during treatment. Infections within the genitourinary tract can appear during the following weeks and may manifest as ulcers, plaques, or nodules in the bladder or urethra. Epididymo-orchitis or prostatitis may also occur months later. Spread beyond the urinary tract affects 3% to 7% of recipients.[483] A sepsis-like syndrome after intravesicular BCG manifests as fever, hypotension, and dyspnea. This can occur in the first few days after injection or appear with insidious onset of malaise, low-grade fever, weight loss, and dyspnea many weeks later.[485] A miliary pattern may affect the lungs. Granulomas may be seen in bone marrow, but cultures of blood and bone marrow are usually negative. Focal lesions can appear weeks or even years after intravesicular therapy, including thoracolumbar spondylodiscitis, granulomatous hepatitis, arthritis, uveitis, and mycotic aneurysm.[484] In a series of 20 cases of mycotic aortic aneurysm, the peak incidence was at 6 weeks after treatment, with declining incidence over 7 years.[486] In another series of mycotic aneurysms after intravesicular BCG, average time to diagnosis was 19 months.[487] Vascular grafts and prosthetic joints may also become infected. Culture isolation of *M. bovis* has occurred in only 41% of reported cases, although PCR may be positive at culture-negative sites.[483] Disruption of mucosal integrity from a recent urologic procedure or during the bladder treatment is suspected but unproven as the predisposing factor.[483]

Treatment largely includes RIF, INH, and EMB for 2 to 3 months, followed by 6 months of INH and RIF.[483,484] Most strains of *M. bovis*, including BCG strains, are PZA resistant. Fluoroquinolones have successfully replaced INH or RIF in some cases. Results are generally favorable except with mycotic aneurysm, which has a poor outcome.[486,487]

Ingestion of *M. bovis* in unpasteurized dairy products can cause cervical lymphadenitis.[488] Pulmonary *M. bovis* infection resembles typical TB and is acquired from exposure to infected animals or, rarely, humans.[489] In North America, reactivation of *M. bovis* acquired in other countries may occur. *M. bovis* accounts for 0.3% of diagnosed pulmonary TB cases in the Americas.[481] Isolates acquired from animals or humans, and rarely from vaccine, may be resistant to INH or RIF.[490]

In countries where BCG vaccine is routinely given to children, unrecognized primary immune deficiencies such as severe combined immunodeficiency or mutations in the IL-12/interferon-γ axis may predispose to disseminated *M. bovis* infection. In this setting, infection occurs at the injection site, contiguous axillary lymph nodes, and distant sites including bones. Restoration of immune response by means of allogeneic hematopoietic stem cell transplantation may be required for cure.[491]

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250 Leprosy (*Mycobacterium leprae*)

Kathryn Dupnik

SHORT VIEW SUMMARY

Epidemiology

- Leprosy is an underrecognized and underdiagnosed disease. Early diagnosis is key to decreasing disability at the individual level, and decreasing risk of *Mycobacterium leprae* transmission in the community.
- Of the people who were diagnosed with leprosy in 2016, 95% were from only 22 countries.
- Experts hypothesize that most of the world's population has a natural immunity to *M. leprae,* although the factors that make some people more susceptible are incompletely understood.
- The primary route of transmission is likely by the respiratory route, either from respiratory droplets or nasal secretions.
- Nine-banded armadillos in the southeastern United States carry *M. leprae,* and genetic epidemiology studies suggest zoonotic transmission.

Microbiology

- *M. leprae* is an obligate intracellular pathogen with tropism for macrophages and Schwann cells. It cannot be propagated in culture.
- *M. leprae* is acid-fast positive. The best staining method to detect *M. leprae* in human specimens is the Fite stain.
- *M. leprae* can stain acid-fast positive even when they are no longer viable, although the morphology and staining characteristics change (see Fig. 250.5).

Diagnosis (Fig. 250.3)

- On clinical examination, leprosy skin lesions can be macules, papules, nodules, or patches. Classically, the skin lesions have decreased touch sensation.
- Nondermatologic symptoms can include uveitis, neuritis, nasal septal perforation, and paresthesias in hands or feet.
- A person with hypoesthesia may present with burns, ulcers, or other traumatic lesions and a report of not having felt the injury.
- *M. leprae* infection is confirmed by histopathology and Fite staining of biopsy material. The biopsy should be full thickness, including the deep dermis.
- The leprosy subtype should be determined using the Ridley-Jopling criteria.
- Assistance is available from the US National Hansen's Disease Program (NHDP) in Baton Rouge, LA.

Therapy (Table 250.1)

- *M. leprae* infection is not an indication for isolation of people with leprosy in any clinical form or at any stage of disease.
- Care for people with leprosy is with standard precautions.
- By the US NHDP guidelines, people with paucibacillary leprosy receive dapsone and rifampicin. People with multibacillary leprosy receive dapsone, rifampicin, and clofazimine.
- Alternative antibiotics that can be substituted for components of recommended multidrug therapy include clarithromycin, ofloxacin, and minocycline.
- If a person develops neuritis, reversal reaction, or erythema nodosum leprosum during multidrug therapy, the antibiotics should be continued.
- Treatment for neuritis with or without associated reversal reaction is with high-dose corticosteroids.
- Treatment options for erythema nodosum leprosum include thalidomide (with appropriate precautions for dispensing this teratogenic agent), high-dose clofazimine, and corticosteroids.

Prevention

- Bacillus Calmette-Guérin (BCG) vaccination of household contacts of people with leprosy may decrease the risk of developing clinical disease. If a contact does develop leprosy after BCG vaccination, vaccination decreases the chance that the leprosy will be multibacillary.
- Studies on single-dose rifampicin prophylaxis of contacts of people with leprosy have been inconclusive. It is part of the WHO guidelines but has not been adopted by the US NHDP as of this writing.

Leprosy, or Hansen disease, is a curable infectious disease caused by *Mycobacterium leprae*. Widespread implementation of multidrug antibiotic therapy for leprosy in the 1980s dramatically decreased the burden of leprosy worldwide. There is no indication for physical or social isolation of people with leprosy. Sequelae of leprosy, including nerve damage, muscle weakness, and physical deformity, are related to delayed diagnosis and pathologic immune reactions that complicate the course of disease. Early diagnosis and prompt initiation of multidrug therapy are crucial for improving outcomes for people with leprosy.

HISTORY AND SOCIETY

Descriptions of leprosy have been found in texts written over 2000 years ago in China, India, and Egypt.[1,2] Skeletal remains from centuries ago show bony changes consistent with leprosy and *M. leprae* DNA is detectable in skeletal remains from the 4th century. Genetic epidemiology studies show correlation of leprosy strain types with patterns of human migration and trade routes, and confirm the ancient origin of this bacterium.[3]

M. leprae was discovered in 1873 by Gerhard Henrik Armauer Hansen in Norway, in a time when leprosy incidence was increasing. Hansen visualized bacteria in tissue from a nodule, but was unable to culture the bacteria or infect animals, which would have been proof of disease causation. Hansen inoculated the eye of a leprosy patient with a knife that had incised a leprous nodule from another patient. He admitted doing this without her consent, and lost physician privileges but remained the Chief Medical Officer for Leprosy in Norway.[4]

Fear of leprosy led to compulsory isolation of people suspected to have leprosy. There is no indication for isolation of people with leprosy at any stage of disease.[5] The current goals of the World Health Organization (WHO) for 2020 are zero new cases of leprosy in children where deformity is present at diagnosis, reduction in the rate of leprosy with disability at diagnosis in all ages, and zero countries with laws permitting discrimination against leprosy ("Triple Zero Campaign").[6] WHO guidelines for eliminating discrimination against people with leprosy recommend that discriminatory language, including the term *leper,* be removed from government publications. *Leper* is a stigmatizing term and should not be used to refer to someone with *M. leprae* infection.[5]

EPIDEMIOLOGY

Leprosy is an underrecognized and underdiagnosed disease. Challenges to prompt diagnosis include reluctance of patients to seek care because of leprosy-related stigma and failure of physicians to recognize the

disease at the time of clinical presentation. Additionally, there are no definitive laboratory tests to determine who has had exposure, has infection, or is at risk for progressing from infection to clinical leprosy. Experts hypothesize that most of the world's population has a natural immunity to leprosy, and it is unknown what factors make some people more susceptible to *M. leprae* infection and subsequent development of clinical leprosy. The prolonged incubation periods between exposure, infection, and development of symptoms are challenging for studies of *M. leprae.*

Studies on *M. leprae* transmission are also limited by the inability to propagate *M. leprae* in culture and the lack of an animal model that replicates human leprosy and leprosy pathologic immune reactions. It is thought that transmission occurs with close, long-term exposure from a person with a disseminated leprosy to a person who has an innate susceptibility to the infection. The primary mode of transmission is thought to be by the respiratory route, from either respiratory droplets or nasal secretions.

Rees and McDougall showed that athymic mice could be infected with *M. leprae* when the respiratory exposure was 60 minutes to *M. leprae* aerosolized in a 1.2×10^8 *M. leprae*/mL suspension. Even in a laboratory setting with high concentrations of *M. leprae,* only 33% of mice had any tissues positive for *M. leprae* 14 to 24 months after exposure.[7] *M. leprae* DNA is detectable in the nasal secretions of people with leprosy and in those who live in leprosy-endemic areas, but the role of this carriage in transmission has not been definitively established.[8] After respiratory introduction of *M. leprae,* the bacteria likely disseminate throughout the body. One hypothesis is that this is due to reprogramming of infected cells into stem cells, which migrate and affect immune response.[9] In untreated lepromatous leprosy with high bacillary load, *M. leprae* bacteremia can be detected.[10]

Antibodies to *M. leprae* have been detected in cord blood, although *M. leprae* bacteremia in cord blood has not been reported.[11] Studies of placentas from mothers with lepromatous leprosy do not show pathologic changes associated with leprosy, although small numbers of acid-fast bacilli (AFB) have been found in placental tissues.[12] Thus vertical transmission of leprosy from a mother with lepromatous leprosy is possible, but extremely unlikely if she has been treated. Skin-to-skin contact as a route of transmission has not been confirmed.[13] Multiple publications have reported leprosy lesions developing in the area of a tattoo, suggesting inoculation leprosy from unsterilized needles.[14]

Genetic Epidemiology

Leprosy cases can be clustered within communities and families. A series of studies have been conducted looking for a genetic explanation for susceptibility to leprosy. Early genetic linkage studies from Brazil reported associations of human leukocyte antigen (HLA) class II and tumor necrosis factor (TNF) genes and a cluster of genes at chromosome 17q11.2 with leprosy.[15,16] A genome-wide association scan in Han Chinese found five genes associated with susceptibility to leprosy: *TNFSF15, HLA-DRB1, RIPK2, NOD2,* and *LRRK2.*[17] Single nucleotide polymorphism (SNP) types in the *NOD2* pathway have been found to be associated with leprosy in several populations.[18,19] An additional chromosome location found to be associated with leprosy was chromosome 6q25, in which risk alleles in the Parkinson disease gene *PARK2* were associated with risk of leprosy in Vietnamese and Brazilian populations.[20,21] There are no confirmed SNPs associated with a person's risk of developing either multibacillary or paucibacillary leprosy; a review of genetic studies that address these designations has been compiled by Gaschignard and colleagues.[22]

Zoonotic Potential

There are several animal reservoirs for *M. leprae.* Nine-banded armadillos (*Dasypus novemcinctus*) are a probable source of leprosy in the southeastern United States. A strain of *M. leprae* circulating in armadillos from at least four different states is identical to the *M. leprae* strain in people with leprosy in these states who never traveled outside the United States.[23] Additionally, a new armadillo-related *M. leprae* strain has been identified in both armadillos and humans. A recent study found that over 40% of people with leprosy referred to the US National Hansen's Disease Program (NHDP) for leprosy diagnosis in 2007 to 2012 had one of two armadillo-associated strains.[24] Genetic typing suggests that these armadillo-associated strains were introduced into North America during migrations from Europe.

Recently, leprosy in red squirrels (*Sciurus vulgaris*) from Scotland and England has been described. The causative bacterium for squirrel leprosy in England is *M. leprae.* This *M. leprae* is closely related to *M. leprae* from a human skeleton buried 700 years ago in Britain, as well as to *M. leprae* in US armadillos.[25] In Scotland red squirrels, *M. lepromatosis* has been isolated as the cause of squirrel leprosy. There are no reported transmissions of leprosy from red squirrels to humans in either of these countries.[25] Non–experimentally acquired leprosy has also been described in chimpanzees and macaques.[26,27]

There are no known insect vectors that transmit *M. leprae.* A recent study showed experimental infection of the kissing bug *Rhodnius prolixus* with transmission of infection to the mouse footpad by *R. prolixus* feces,[28] but this has yet to be replicated and is an area for further investigation. Molecular studies have detected *M. leprae* DNA from soil and water, and water-based organisms have been experimentally infected with *M. leprae.* Lymphocyte-free *rag1* mutant zebrafish form granulomas after experimental *M. leprae* infection.[29] Currently, there is no strong evidence supporting an environmental source for transmission of *M. leprae* to humans.[30]

Nosocomial Infections

People who are caring for a person with *M. leprae* infection should use standard precautions.[31] There is no indication for contact or respiratory isolation precautions for people with treated or untreated leprosy. Persons treated with multidrug therapy become noninfectious in a matter of days.[32]

Global Epidemiology

The adoption of multidrug therapy for leprosy in 1981 dramatically decreased the rate of leprosy new case detection worldwide. In 2016, the WHO reported 214,783 new cases of leprosy diagnosed worldwide, a new case detection rate of 2.9 per 100,000 population (Fig. 250.1); 22 countries accounted for 95.03% of new cases. The greatest number of cases are reported from India (63%), Brazil (12%), and Indonesia (8%). Six percent of new cases already have severe disability with visible deformity, an indicator of significant delay in diagnosis.[6] Millions of people worldwide are living with sequelae of leprosy.

Leprosy is an underdiagnosed disease.[33] This is related to the chronic nature of the infection with insidious onset of symptoms, fear and stigma deterring contact with the health care system, or delay in diagnosis when signs and symptoms are not identified as being compatible with leprosy. In places with endemic leprosy, active case-finding activities diagnose more leprosy than would be expected from the baseline new case detection rate. Delay in diagnosis results in increased physical and functional disability from further nerve damage. Delay in diagnosis may also increase risk of transmission of *M. leprae* from someone with multibacillary leprosy with a high bacterial index. Awareness among medical professionals and communities is essential for timely diagnosis and initiation of treatment.

Epidemiology in the United States

Since 1894, 13,950 cases of leprosy have been registered in the United States. The NHDP estimates that approximately 9000 people who have or had leprosy are alive, many with long-term sequelae of leprosy and who could benefit from specialized care. There were 178 new cases reported in 2015, which continues a trend of increased case reporting. Most cases were reported from Florida, California, Texas, Louisiana, Hawaii, and New York. Fig. 250.2 shows the distribution of reported leprosy cases from 2005 to 2014. There are "indigenous foci" of leprosy transmission in Hawaii, Puerto Rico, and the region of the western Gulf of Mexico. There were 96 cases reported from Texas, Louisiana, Arkansas, Mississippi, Alabama, Georgia, and Florida. These are states where *M. leprae* has been isolated from nine-banded armadillos; 65% of cases from these states were in people who were born in and had never lived outside the United States. Country-wide, 57% of people with leprosy reported being born outside of the United States, with most cases in people coming from the South Pacific region. In 2015, new cases ranged



FIG. 250.1 Geographic distribution of new cases of leprosy diagnosed in 2016. *(From World Health Organization. Global leprosy update, 2016: accelerating reduction of disease burden.* Wkly Epidemiol Rec*. 2017;92:501–519; reprinted with permission.)*



FIG. 250.2 Average number of new cases of leprosy diagnosed per year (2005–2014) in the United States. *(Reprinted with permission from the US Human Resources and Services Administration. Originally published as Figure 3 in* A Summary of Hansen's Disease in the United States—2015.*)*

in age from 7 to 95 years and were 66% male. Over half of new cases had lepromatous leprosy, the most disseminated form of leprosy, which increases risk for complications and sequelae.[34]

MICROBIOLOGY

M. leprae is not culturable on standard laboratory growth media. Isolation and propagation of *M. leprae* in the laboratory requires experimental infection of armadillos or footpads of immunodeficient mice.[35] The doubling time of *M. leprae* is 14 days during the logarithmic growth phase, and up to 40 days during mouse footpad infection.[36] *M. leprae* are Gram stain positive and carbol fuschin acid-fast stain positive. They are straight or slightly curved, 2.1 µm long, and 0.25 to 0.3 µm in width. The cell wall of *M. leprae* is rich in lipids and glycolipids, including phenolic glycolipid 1 (PGL-1). Antibody to PGL-1 is used to assess exposure to *M. leprae* and sometimes as part of diagnostic evaluation. There is no evidence of plasmids or bacteriophages that contribute to *M. leprae* pathogenesis.

M. leprae is an obligate intracellular pathogen, with macrophages and Schwann cells most frequently infected. The genome of *M. leprae* is much smaller than *Mycobacterium tuberculosis* (3.27 vs. 4.41 Mb, respectively), and appears to have undergone reductive evolution with loss of portions of the oxidative, microaerophilic, and anaerobic respiratory chains, such that it is unable to utilize certain carbon sources.[37] Protein-coding genes comprise only half of the *M. leprae* genome.[37] The *M. leprae* cell wall components are less diverse than in *M. tuberculosis,* and *M. leprae* has fewer enzymes available to facilitate scavenging of host cell lipids.[37] It lacks many of the enzymes used by *M. tuberculosis* to survive in macrophages.[37] *M. leprae* has an extremely conserved genome, with 99.995% sequence identity in *M. leprae* strains from four geographically distant countries.[3] The frequency of SNPs is very low at 1 in 28,400 base pairs. A survey of 84 SNPs in *M. leprae* from 28 geographic regions separate *M. leprae* into 4 SNP types and 16 SNP subtypes.[1,3] These SNP subtypes have been used for phylogeographic studies charting the dissemination of leprosy with human migration routes throughout history.

Recently, a new species of mycobacteria, *M. lepromatosis,* has been implicated as a cause of leprosy in humans. It is reported to have association with diffuse lepromatous leprosy, and with erythema necroticans, also known as Lucio phenomenon, a potentially fatal complication of leprosy.[38] *M. lepromatosis* was first characterized in two patients from Mexico who had diffuse lepromatous leprosy and erythema necroticans that was fatal.[39] In one study, 6 of 227 leprosy biopsies collected worldwide had *M. lepromatosis*; all were from people from Mexico.[40] *M. lepromatosis* has also been identified in red squirrels in the United Kingdom that had leprosy-like syndromes.[25,41] *M. lepromatosis* and *M. leprae* genomes are similar sizes and have 93% nucleotide sequence identity in protein-coding genes, but only 82% in pseudogenes. To date, no mutations have been identified in *M. lepromatosis* that correspond to drug resistance mutations in *M. leprae.*[40,42]

MECHANISMS OF IMMUNITY

Leprosy is not an easily transmitted infection. Many people may be resistant to infection by *M. leprae* even during heavy exposure. Other people may be infected with *M. leprae* but never develop clinical leprosy, suggesting that either the cell-mediated immune response has cleared the infection or the infection is controlled or becomes latent. People who do not have and will not develop leprosy may be positive on tests of granulomatous (Mitsuda lepromin skin test) or humoral (anti–PGL-1 serology) immune response to *M. leprae.* There is likely an innate immune response that protects these people from disease development.

The likely first interaction between *M. leprae* and the human immune system is with resident macrophages and dendritic cells in mucosal surfaces via pathogen recognition receptors. The impact of this interaction on proinflammatory or antiinflammatory cytokine release may be what determines the subsequent course of infection.[43] Toll-like receptors and nucleotide-binding oligomerization domain (NOD)–like receptors recognize *M. leprae* antigens and have been associated with the development of leprosy per se and paucibacillary versus multibacillary phenotypes in genetic studies.[22]

People with a strong cell-mediated immune response to *M. leprae* have localized infection with formation of granulomas, manifested as paucibacillary leprosy.[44] People without a strong cell-mediated response have more disseminated disease, higher bacterial load, antibodies to the organism, and, clinically, lepromatous leprosy. Transcriptome studies of leprosy skin lesions have confirmed distinct gene expression profiles in tuberculoid versus lepromatous leprosy lesions. Tuberculoid lesions have higher expression of genes associated with antigen processing and proinflammatory cytokines, while lepromatous lesions have higher expression of genes associated with antiinflammatory cytokines and B cells.[45] A recent systematic review on the role of human genetics in polarization to tuberculoid or lepromatous leprosy identified 28 genes with haplotypes or SNPs associated with paucibacillary versus multibacillary leprosy, including genes encoding pathogen recognition receptors.[22] Additional pathways associated with different clinical manifestations of leprosy are type I interferon-induced pathways inducing increased cell-mediated immune response[46] and inhibition of autophagy pathways leading to higher macrophage *M. leprae* burden in multibacillary leprosy.[47] Most immunologic studies in leprosy can describe the "how" of the immune status of people with different clinical forms of leprosy, but to date we do not understand the "why" of what causes a person to develop a strong cell-mediated versus strong humoral immune response to *M. leprae.*

CLINICAL MANIFESTATIONS OF LEPROSY

> If immunology is the root of the tree, its fruit is the knowledge of infectivity, prognosis, and management of the patient.[48]

A wide variety of clinical presentations can result from *M. leprae* infection. In 1953, the "Madrid classification" was proposed, which included a tuberculoid form, a lepromatous form, and "dimorphous" leprosy that was intermediate between the tuberculoid and lepromatous forms. An "indeterminate" classification was also included, which is the initial lesion of leprosy; it theoretically can either heal or develop to a lepromatous or a tuberculoid form, depending on host immune response.[49] Dimorphous leprosy is considered a manifestation of immunologic instability, wherein leprosy will eventually transition to a more tuberculoid or more lepromatous form.

A more rigorous classification system incorporating clinical findings, histopathology, bacterial index, and immunologic parameters was proposed by Ridley and Jopling in 1962 (Fig. 250.3).[50] Recognizing that leprosy occurs on a continuous spectrum, it considers a person's "resistance" to *M. leprae* infection and subdivides leprosy into five types.[48] The Ridley-Jopling classification includes polar tuberculoid (TT) and polar lepromatous (LL), but subdivides dimorphous leprosy into three borderline types: borderline tuberculoid (BT), mid-borderline (BB), and borderline lepromatous (BL) (see Fig. 250.3). The tuberculoid pole is characterized by a strong cell-mediated immune response to *M. leprae,* with histopathology of skin biopsies showing well-formed granulomas and infiltration of nerves and few or no bacilli. The lepromatous pole is characterized by a poor cell-mediated immune response to *M. leprae,* and skin biopsies have foamy macrophages and large numbers of bacilli. The borderline forms are more unstable and have components of both cell-mediated and humoral immune responses to *M. leprae.*

The WHO developed a classification system for leprosy diagnosis and treatment recommendations that could be implemented in resource-limited settings. Presumptive diagnosis of leprosy is based on hypopigmented or reddish skin lesion with loss of sensation, peripheral nerve symptoms, or skin smear with AFB. The WHO schema contains two groups, paucibacillary and multibacillary leprosy, which determines length of recommended antibiotic regimen. If a person has five or fewer hypopigmented, hypoesthetic skin lesions, the designation is paucibacillary. If a person has more than five skin lesions, the designation is multibacillary, which necessitates a longer course of multiantibiotic treatment for leprosy[51] (Table 250.1). The Ridley-Jopling classification system provides additional information that can be helpful in determining appropriate treatment duration, risk for development of leprosy immune reactions, and monitoring response to treatment and should be utilized when possible.



	TT	BT	BB	BL	LL
Types of lesions	macule plaque raised border annular	macule plaque raised border satellite lesions	macules plaques "targetoid"	macules papules plaques nodules asymmetric	macules plaques nodules diffuse symmetric
Sensation in lesions	markedly diminished or absent		slightly to moderately diminished		slightly diminished or normal
Nerve enlargement	asymmetrical single or few nerves		more likely to be symmetrical several nerves		"stocking and glove" anesthesia possible
Risk for immune reactions	very rarely RR	RR	RR	RR ENL	ENL Lucio phenomenon

FIG. 250.3 Immunopathologic spectrum of leprosy, representing the five histopathologic subtypes of leprosy in the Ridley-Jopling classification. *Top,* Hematoxylin and eosin–stained sections (magnification ×63) showing well-formed epithelioid granulomas in TT progressing to disorganized histiocytes in LL. *Middle,* Fite-stained sections (magnification ×1000) showing scarce organisms in TT progressing to very high bacillary load in LL. *Bottom,* Representative photos are examples of clinical presentations for each of the Ridley-Jopling subtypes. *Table,* Characteristics of skin lesions and nerves on clinical examination and the risk for the pathologic immune reactions of leprosy. *AFB,* Acid-fast bacilli; *BB,* mid-borderline; *BL,* borderline lepromatous; *BT,* borderline tuberculoid; *ENL,* erythema nodosum leprosum; *LL,* polar lepromatous; *RR,* reversal reaction; *TT,* polar tuberculoid. *(Histopathology and Fite stain figures from Scollard DM, Adams LB, Gillis TP, et al. The continuing challenges of leprosy.* Clin Microbiol Rev. *2006;19:338–381; reprinted with permission. Clinical photographs courtesy Dr. Mauricio Nobre, Hospital Giselda Trigueiro, Natal, Rio Grande do Norte, Brazil.)*

TABLE 250.1 Recommended Regimens to Treat Leprosy in Adults

	WHO RECOMMENDATIONS[a]			US (NHDP) RECOMMENDATIONS[b]		
	Medication	**Dosage**	**Duration**	**Medication**	**Dosage**	**Duration**
Paucibacillary or "tuberculoid" leprosy (TT, BT, <5 lesions)	Dapsone Rifampicin Clofazimine Clofazimine	100 mg daily 600 mg once a month 50 mg daily 300 mg once a month	6 months 6 months 6 months 6 months	Dapsone Rifampicin	100 mg daily 600 mg daily	12 months 12 months
Multibacillary or "lepromatous" leprosy (BB, BL, LL, ≥5 lesions)	Dapsone Rifampicin Clofazimine Clofazimine	100 mg daily 600 mg once a month 50 mg daily 300 mg once a month	12 months 12 months 12 months 12 months	Dapsone Rifampicin Clofazimine	100 mg daily 600 mg daily 50 mg daily	24 months 24 months 24 months

[a]WHO treatment regimen guidelines from https://apps.who.int/iris/bitstream/handle/10665/274127/9789290226383-eng.pdf?ua=1.
[b]US Department of Health and Human Services HRSA treatment regimen guidelines from https://www.hrsa.gov/hansens-disease/diagnosis/recommended-treatment.html.
BB, Mid-borderline; *BL,* borderline lepromatous; *BT,* borderline tuberculoid; *HRSA,* Health Resources & Services Administration; *LL,* lepromatous leprosy; *NHDP,* National Hansen's Disease Program; *TT,* tuberculoid; *WHO,* World Health Organization.

Indeterminate leprosy is the earliest visible manifestation of *M. leprae* infection. Indeterminate leprosy is a single macule that is usually hypopigmented, and may have slight decrease in sensation. There is generally still hair in the area of the lesion, but it may be drier than the surrounding skin. Biopsy of the lesion can be nonspecific, although perineural cuffing is typical of indeterminate leprosy. There may or may not be bacilli present in the lesion. The immune response to *M. leprae* has not been fully developed in people with indeterminate leprosy, and a person may self-resolve, progress to tuberculoid leprosy, or progress to lepromatous leprosy.

CLINICAL PRESENTATION

People with leprosy may present for medical evaluation with a wide variety of symptoms, related to the type of leprosy. During the initial assessment, a thorough travel and social history is essential. Risk factors for leprosy in the United States include history of residence in a country with a high burden of leprosy or in a state in which zoonotic transmission of leprosy is documented. Family history of leprosy is important both as a potential source of *M. leprae* infection and as an indicator of underlying genetic risk factors. On physical examination, a full skin survey should be completed to assess macules, papules, plaques, subcutaneous nodules, and infiltrative skin changes. Leprosy skin lesions are uncommon on the perineum and scalp and in the axillae, but otherwise can develop anywhere. Since the lesions themselves are asymptomatic, a person may not report lesions when they are in a nonvisible area of the body. Peripheral nerves should be assessed for enlargement and tenderness to palpation (Fig. 250.4). Symptoms such as numbness, tingling, weakness, and shooting pains should be specifically assessed for. Sensation should be tested in the hands and feet to detect subclinical neurologic involvement, which could necessitate additional treatments or interventions.

Skin lesions are a prominent feature of leprosy and can be macules, patches, papules, or nodules. Lesions may be reddish or hypopigmented. The earliest sensory change in leprosy skin lesions is compromised perception of the difference between warm and cold temperatures.[52,53] Affected skin may lack or have decreased hairs. Decreased sweating is related to autonomic cutaneous nerve compromise, but is more difficult to detect on clinical examination. Nerve dysfunction is most severe in people with strong cell-mediated immune response to *M. leprae.* People with leprosy may or may not have neurologic symptoms or subjective complaints at initial presentation. However, reduction or absence of sensation in a skin lesion is a very useful indicator to distinguish leprosy from other similar-appearing skin lesions.

Severe loss of sensation can lead to trauma and an initial presentation with burns, contusions, and lacerations, often in the region of skin lesions and without the expected associated pain. These are particularly common in the hands and feet, and can be associated with activities of daily living such as cooking, farming, or even holding a hot beverage. In extreme circumstances, people may present with acute or chronic localized muscle weakness, or even paralysis of an extremity. A hand in a claw position or a foot that drags on the ground while walking are strongly suggestive of leprosy, and warrant skin survey and investigation for other signs and symptoms suggestive of leprosy and leprosy immune reactions.

Skin lesions of lepromatous leprosy or diffuse leprosy are more subtle. The lack of cell-mediated immunity means that destruction of cutaneous nerves, hair follicles, and melanocytes are not prominent features, which changes the clinical presentation. Rather than discrete lesions, some people with polar lepromatous leprosy have infiltrative skin changes including loss of normal skin contours and skin thickening. One place to assess for this change is in the forehead. In advanced disease, a person may have loss of lateral eyebrows (superciliary madarosis) or eyelashes (ciliary madarosis) or both. There may be deformities of the nose (so-called saddle nose) or perforation of the nasal septum. Symmetrical enlargement and nodularity of the ears is also a classic finding for lepromatous leprosy. Diffuse involvement of the face in lepromatous leprosy results in infiltration and subcutaneous nodules (lepromas) with abundant *M. leprae.* Leprosy is in the differential diagnosis for "leonine facies."

The pathologic immune reactions of leprosy, reversal reaction and erythema nodosum leprosum (ENL), are significant contributors to morbidity related to leprosy. They may occur prior to, during, or after antibiotic therapy for *M. leprae* and are an indication for urgent evaluation and initiation of appropriate treatment. These reactions are discussed in following sections.

Infection of the eye by *M. leprae* and the impact of *M. leprae*–associated inflammation of muscles and nerves cause significant morbidity in leprosy. Direct damage can occur from chronic *M. leprae* infection per se, because *M. leprae* can enter the eye during initial dissemination of the infection. In lepromatous leprosy, there can be lepromas in the eyebrows and upper eyelids. In the eye, the cornea may have visible discrete particles and there may be white microlepromata on the surface of the iris. These are signs of chronic *M. leprae* infection with a high bacterial load. Additionally, both reversal reaction and ENL immune reactions can cause acute, serious ocular damage. In reversal reaction, nerve damage of the trigeminal nerve or facial nerve can cause lagophthalmos and defective blink reflex. The sequelae include permanent lagophthalmos, keratitis, corneal opacities, ectropion, entropion, and trichiasis. ENL's ocular manifestations include episcleritis, scleritis, and iridocyclitis. In cases of chronic inflammation in the eye, uveitis can cause nerve damage that leads to iris atrophy, more than one pupil, and low intraocular pressure.[54] Ocular complications can occur even after multidrug therapy is completed.[55] An additional consideration in people with leprosy and immune reaction on long-term steroids is the increased risk for developing steroid-related cataracts.

There is a specific type of leprosy called diffuse lepromatous leprosy, or Latapí lepromatosis. This form of leprosy is most common in Mexico, and may be associated with either *M. leprae* or *M. lepromatosis* infection. In a study of 300 cases of diffuse leprosy in Mexico diagnosed between 1970 and 2004, 90.5% were from the Pacific Coast states of Mexico.[56] People with diffuse lepromatous leprosy are at risk for necrotic vasculopathy,

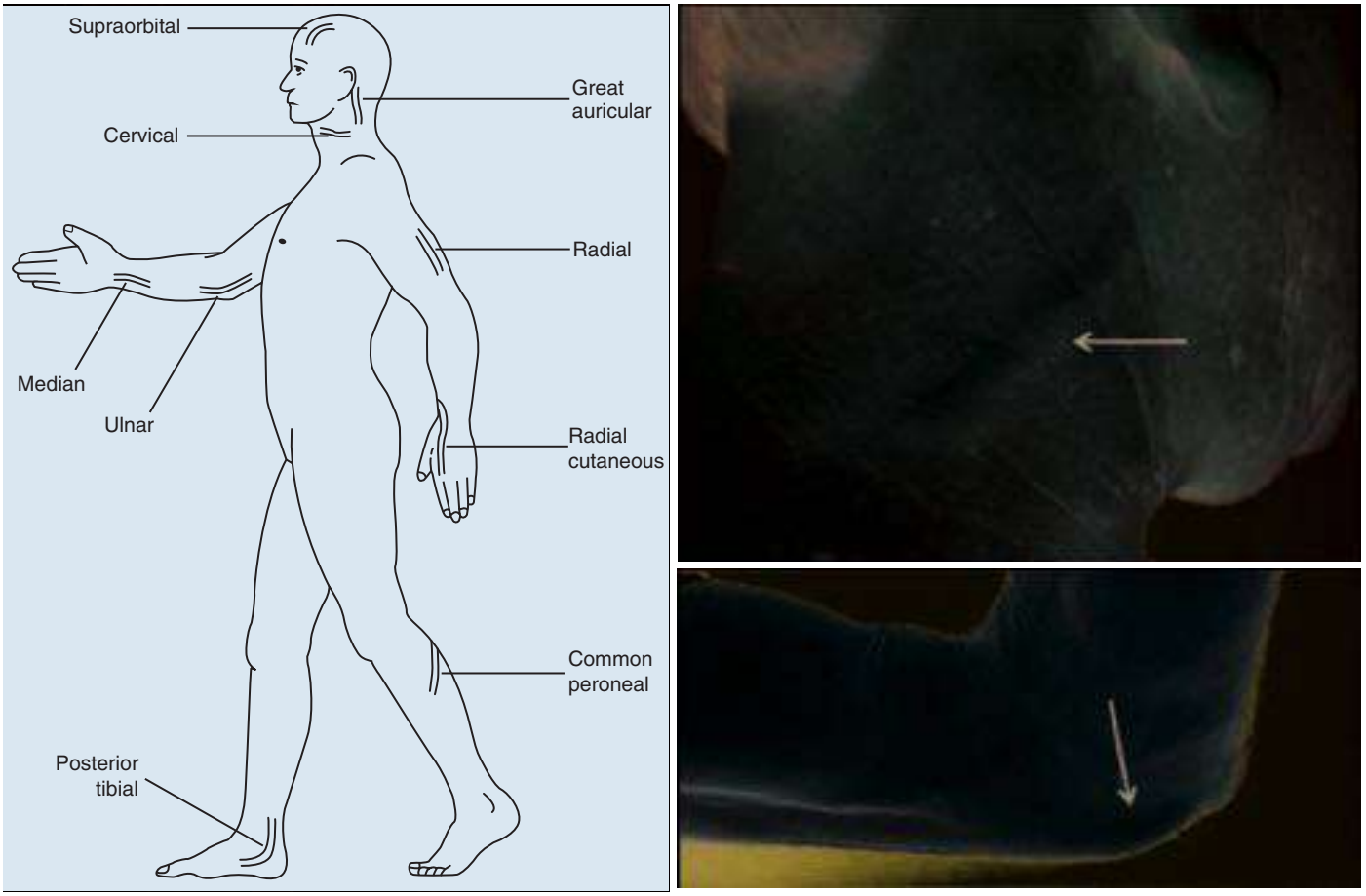

FIG. 250.4 Palpation of peripheral nerves to assess size, nodularity, and tenderness. Palpation of peripheral nerves is a key component of the physical examination of a person suspected of having leprosy. *Left,* Map of the palpable nerves that can be affected during leprosy. *Top right,* Enlarged right auricular nerve *(arrow). Bottom right,* Enlarged left ulnar nerve *(arrow)* in a patient with reversal reaction and neuritis. *(Left image from Hastings RC.* Leprosy. *2nd ed. New York: Churchill Livingstone; 1994, reprinted with permission. Right images courtesy Dr. Mauricio Lisboa Nobre, Hospital Giselda Trigueiro, Natal, Rio Grande do Norte, Brazil.)*

referred to as Lucio phenomenon. This vasculopathy can progress to multiple cutaneous infarcts that may be fatal, so early recognition of this form of leprosy can be lifesaving.[38] The skin lesions of diffuse lepromatous leprosy are much less prominent. The infiltration of *M. leprae* is so diffuse that in the early stages the skin appears smooth and shiny. After subcutaneous tissue has been destroyed by infection, the skin is looser and wrinkled. Nodules, if present, are palpable subcutaneously but generally not visible as in lepromatous leprosy or ENL. One retrospective study of diffuse lepromatous leprosy treated in California over a period of 35 years found that the median delay in diagnosis was 3 years, and in many people the diagnosis was only reached when they presented with necrotic vasculitis.[57] This delay in diagnosis was also reported in the aforementioned study from Mexico, where the time from appearance of first sign of leprosy and the diagnosis of leprosy varied from 1 week to 12 years, with a median of 3 years.[56] Prompt treatment of the underlying mycobacterial infection in diffuse lepromatous leprosy can prevent this complication. Associated findings are madarosis and chronic rhinitis, which can be the presenting complaints of diffuse lepromatous leprosy. With the appropriate history, leprosy should be included in the differential diagnosis.

Prominent pathologic features of diffuse leprosy are in the cutaneous vasculature, with AFB visible in endothelial cells and fibrin clots in blood vessels. In early stages, the vessel lumen can decrease because of intimal thickening. As the infection progresses, AFB are visible in more blood vessels and the vessel lumen size decreases further. Eventually the vessel lumen is no longer visible and there may be foamy macrophages around the blood vessels. When ischemic necrosis develops, the epidermis and superficial dermis develop necrosis and vascular occlusion caused by thrombus or intimal thickening. There may be fibrin deposition and an inflammatory infiltrate with many types of immune cells.[56]

Primary neural leprosy is another diagnostic challenge in leprosy.[58] In this form of leprosy, the presentation can be of nerve pain, paresthesias, weakness, or paralysis. On examination, there is a lack of leprosy skin lesions. The affected nerve is generally enlarged and tender to palpation, with palpation eliciting both localized pain and shocklike pain that radiates in the distribution of the nerve. Additional findings can include muscle weakness, atrophy in the muscles innervated by the affected nerve, decrease in sensation in the area of innervation, or paralysis. People may present with acute neuritis, and should be considered for acute initiation of corticosteroids to decrease nerve swelling and associated neurologic compromise. Primary neural leprosy can affect one or multiple nerves, and treatment duration is generally a reflection of the number of nerves involved—paucibacillary course for single nerve and multibacillary course for multiple nerves. Nerve biopsy can assist in diagnosis by demonstrating AFB and inflammatory changes, although visualization of AFB is not required for a diagnosis of neural leprosy. However, there are risks of nerve damage with nerve biopsy. Electromyography of affected nerve(s) can be consistent with demyelinating mononeuropathy.[58–60]

DIAGNOSTIC TESTING

Histopathology of skin biopsy is most helpful for confirming a diagnosis of leprosy and to determine the Ridley-Jopling clinical form. It is very important to select an appropriate lesion and to biopsy in the appropriate area. Generally, the most active margin of the most active

lesion should be biopsied at the edge of the lesion. In most lesions, the infection is most pronounced at the border. This is especially important for targetoid lesions since the central area will have different pathologic findings and bacillary loads from those of the border. Skin biopsies can be of the "punch" variety, optimally full thickness and including the deep dermis. The pathologist should be advised as to the clinician's suspicion for leprosy, with a special request for acid-fast staining, in particular the Fite stain, which is the stain of choice for detecting *M. leprae.* The Fite staining methodology includes oil in the deparaffinizing reagent to help maintain the integrity of the *M. leprae* cell wall such that it remains acid fast during the staining procedure, thus increasing the sensitivity of the stain. Standard Ziehl-Neelsen stains may be negative for AFB, even when AFB are seen with the Fite stain.[61] Additionally, standard hematoxylin and eosin staining is crucial for histopathologic characterization of the type of immune infiltrate and involvement of cutaneous nerves. It is important to note that a leprosy lesion may be negative for AFB. This is particularly true in the polar tuberculoid and borderline tuberculoid forms of the disease. In these cases there are classic histopathologic signs of leprosy, including perineural inflammation, which are nearly pathognomonic for the diagnosis.

Histopathologic findings of leprosy can be focal and variable in a single biopsy. In paucibacillary leprosy, there may be very small numbers of or no *M. leprae* visible in polar tuberculoid disease. Typical pathologic findings are presented in the earlier section on "Clinical Manifestations of Leprosy" and in Fig. 250.3. The Fite staining method is not specific for *M. leprae,* and confirmation of *M. leprae* requires a molecular diagnosis. At the time of this writing, biopsies containing AFB on staining with negative mycobacterial cultures can be reviewed by the NHDP. All biopsies reviewed by NHDP are tested for *M. leprae* by polymerase chain reaction (https://www.hrsa.gov/hansens-disease/diagnosis/biopsy.html). *M. leprae* can be detected in areas other than the skin and nerves, but this is not sensitive for leprosy. If *M. leprae* were present, it would be only in cases of lepromatous leprosy.[62] Even if present, *M. leprae* in tissues can reflect sporadic, transient dissemination of *M. leprae* or organ involvement.

Slit-skin smear testing is one method to confirm that leprosy is multibacillary. For accurate and precise results, smear collection and interpretation of the stained material should be completed by trained personnel.[63,64] The NHDP recommends diagnostic skin smear from 6 sites: bilateral earlobes, elbows, and knees, and from the margin of one or more skin lesions. The NHDP has a protocol for collection of the skin smears (https://www.hrsa.gov/hansens-disease/diagnosis/skin-smears.html). Briefly, a 3- to 5-mm long incision is made 2 to 3 mm deep to the skin surface. Pressure is maintained internal to the incision to decrease vascularity temporarily. The blade is used to scrape the incision, which collects dermal tissue and fluid. This material is placed on a slide and dried at room temperature for later Fite acid-fast staining. The NHDP can perform Fite staining and determination of the bacterial index, at no cost to the patient. The bacterial index is determined by counting the number of bacilli per oil immersion field. The *M. leprae* bacterial index is on a semi-logarithmic scale from +1 (1–10 bacilli per 100 fields) to +6 (over 1000 bacilli per field). This method detects both viable and nonviable bacteria. Clearance of *M. leprae* by immune cells is a very slow process. On serial slit-skin smear tests, the bacterial index will decrease an average of 1 point per year. Consecutive monitoring of bacterial index can be helpful to document response to antibiotic treatment. A consistently increasing bacterial index can be a sign that bacteria is resistant to the antibiotics, the infection has recurred, or that a cured patient has been reinfected.

When a skin lesion is consistent with leprosy, but sensation testing does not reveal a definitive deficit in touch or temperature sensation, one option to assist with diagnosis is to assess response to histamine.[65] Histamine skin prick tests are used as a positive control for allergy skin testing panels. In normal skin, histamine introduction results in a "triple response" of (1) local redness, followed by (2) a more disseminated flare, and (3) the wheal. Since the flare results from reflex dilation of arterioles, leprosy lesions do not have a flare because *M. leprae* infection and immune response to it have compromised the sensory nerves that generate the flare response. An "incomplete" histamine response, in which the flare is absent in the area of the lesion but present in unaffected skin, is specific for leprosy.[66]

The lepromin or Mitsuda skin test is an assessment for granulomatous response to *M. leprae* antigen.[67] Killed *M. leprae* is injected subcutaneously, and assessed after 4 weeks for development of palpable induration at the site of injection, and for granuloma formation on biopsy. The lepromin skin test is of limited utility as part of a diagnostic workup. It is not available in the United States and is not US Food and Drug Administration (FDA) approved. It is not in widespread use worldwide.

Serologic tests for leprosy are in development, including as a potential diagnostic or community screening tool for leprosy. The most studied serologic target is PGL, found in the cell wall of *M. leprae,* reviewed by Penna and colleagues.[68] A positive anti–PGL-1 serology can be found in people with lepromatous forms of leprosy and high antibody levels. However, healthy community controls can also have positive PGL-1 serologies, which may be related to prior exposure or subclinical infection, or may be a false positive. In addition, results are negative in most tuberculoid (TT and BT) patients, which means that the test misses a large percentage of cases of leprosy. A recently developed serologic assay of antibody response to a multiantigen fusion protein, termed Leprosy IDRI Diagnostic (LID)-1, is in use in Brazil and is available for research purposes only in the United States. However, the sensitivity and specificity of this test in leprosy per se precludes its use as a stand-alone diagnostic tool.

TREATMENT OF LEPROSY

Antibiotics

Leprosy is curable with multidrug antibiotic therapy. This has been the standard of care in the United States since 1981 and worldwide since 1982. At the time of this writing, multidrug therapy is available for free to any patient diagnosed with leprosy, either through the WHO or from the NHDP for people in the United States. Multidrug therapy has dramatically improved prognosis for people diagnosed with leprosy, and has resulted in significant decline in the rates of leprosy worldwide.[69] The current dosing regimens recommended by the WHO and NHDP are shown in Table 250.1. "Uniform multidrug therapy" in which paucibacillary and multibacillary leprosy are both treated with a three-drug, 6-month regimen, has been evaluated, although at this time it is not part of national or global recommendations.[70–72]

The antibiotics routinely used in leprosy multidrug therapy are dapsone, rifampin, and clofazimine. In leprosy treatment programs that use WHO-distributed blister packs, multidrug therapy for leprosy is often administered as modified directly observed therapy, where medications are dispensed once a month. At this time, a monthly dose supplemented with rifampin and a higher-dose clofazimine is administered under supervision. For people who are unable or unwilling to take certain components of the standard multidrug therapy regimen, there are alternative antibiotics that can be substituted for individual components of multidrug therapy. These include clarithromycin, ofloxacin, and minocycline.[51,73]

The first antibiotic found to be effective for leprosy was dapsone in the 1940s.[74] Dapsone monotherapy is no longer appropriate given the development of resistance when dapsone is used as a single agent, but it remains a key component of multidrug therapy. People with glucose-6-phosphate dehydrogenase (G6PD) deficiency are at risk for dapsone-induced methemoglobinemia, so screening for G6PD deficiency before starting dapsone is recommended unless in a resource-limited setting where G6PD testing is not available. Anemia is a common side effect of dapsone, even in people without G6PD deficiency, and is a sign that alternative multidrug therapy regimens should be considered.[75] Severe side effects include potentially fatal blood dyscrasias such as agranulocytosis and aplastic anemia and dapsone hypersensitivity syndrome. The hypersensitivity syndrome is characterized by exfoliative dermatitis, lymphadenopathy, fever, and hepatosplenomegaly and has a mortality rate of almost 10%. A genome-wide association study of dapsone hypersensitivity syndrome in leprosy in China found an association with HLA-B*13:01 haplotype.[76]

Rifampin is the most bactericidal agent in the multidrug therapy regimen. In mouse studies, a single dose of a rifampin-containing

multidrug regimen decreased viable *M. leprae* almost to the limit of detection as determined by inoculation of a normal mouse footpad.[32] Rifampin is included in both paucibacillary and multibacillary treatment regimens with monthly (WHO guideline) or daily (NHDP guideline) dosing. Rifampin should not be used as monotherapy, because of the risk of development of resistance. Rifampin induces multiple components of the cytochrome P-450 system, resulting in significant drug interactions, including with oral contraceptives and corticosteroids. Use of a drug interactions database at initiation of multidrug therapy and with any changes in regimen are recommended to decrease likelihood of adverse reactions related to interactions. Rifampin can cause orange discoloration of bodily fluids, hepatotoxicity, and hypersensitivity reactions, including DRESS (*d*rug *r*eaction with *e*osinophilia and *s*ystemic *s*ymptoms) syndrome. People taking rifampin may also report a flulike syndrome, which may be related to intermittent dosing or dosage, or both.[77]

Clofazimine is a bacteriostatic antibiotic that also has antiinflammatory activity. It is used in the treatment of multibacillary leprosy, and also for treatment of ENL at higher doses. Clofazimine was FDA approved in 1986, but is no longer commercially available. Clofazimine is obtainable in the United States from the NHDP as part of an investigational new drug application from the FDA for leprosy treatment. Clofazimine is generally well tolerated at the doses used to treat leprosy. It is lipophilic, and a major side effect is hyperpigmentation from deposition of clofazimine in the skin, particularly in areas of inflammation. The hyperpigmentation is worsened with sun exposure, in addition to other symptoms of clofazimine-associated photosensitivity. The hyperpigmentation is not harmful and resolves after stopping clofazimine, but the symptom can be very distressing to the patient. Often this is because the pattern of hyperpigmentation is recognized in a person's community as a sign of leprosy. There have been reports of depression and suicide in people taking clofazimine, thought to be related to distress about the skin changes.[78] Uncommonly, and especially when used at higher doses than those used in standard multidrug therapy, clofazimine can cause abdominal discomfort and enteropathy related to clofazimine crystal deposition.[79] Alternatives to clofazimine are detailed in leprosy treatment recommendations.[51,73,80]

Drug Resistance

Resistance of *M. leprae* to multidrug therapy is thought to be uncommon. Resistance testing is generally done as part of national and global surveillance to assess continued efficacy of multidrug therapy for leprosy on a population level,[81] and is not currently the standard of care for individual patients. Gold standard resistance testing requires successful inoculation and propagation of *M. leprae* in the mouse footpad, which is not feasible in most cases. Molecular methods can also be used to deduce drug resistance from mutations in *folP1* (dapsone), *rpoB* (rifampin), and *gyrA* (ofloxacin). Even with molecular testing, there needs to be enough bacteria in the specimen for DNA isolation, amplification, and sequencing, which limits its use in tuberculoid leprosy. In the United States, molecular testing for mutations associated with resistance to dapsone, rifampin, and quinolones may be available through the NHDP.

Surgical Intervention

There are some situations in acute and chronic management of leprosy when surgical intervention may be indicated, including neuritis with neurologic compromise that has not improved with steroids. The first-line therapy for acute neuritis and nerve function impairment is high-dose corticosteroids. If, despite this intervention, the nerve remains compressed, the resulting neurologic deficit(s) can be permanent. In these and some other special cases, surgical decompression of the nerve can be considered to maximize return of function and for pain control.[82,83] A Cochrane review of decompressive surgery for nerve damage in leprosy determined that the randomized control trials published up to 2012 were not of high enough quality to assess effectiveness of this intervention for ulnar neuropathy.[84] People with footdrop from posterior tibial nerve involvement and decreased sensation in the sole of the foot are at increased risk for foot ulcers related to altered gate and increased pressure on the forefoot. Tibialis posterior transfer surgery can improve gait and weight-bearing distribution to decrease risk of foot injury and deformity.[85]

Laryngeal involvement by *M. leprae* can cause dysphonia, stridor, and in rare cases airway obstruction.[86] Airway obstruction from laryngeal leprosy requiring emergency tracheostomy has been reported.[87] Fortunately, this complication has become very rare in an era of curative multidrug therapy for *M. leprae*.

After leprosy cure, reconstructive surgery may be desired by a patient to reverse physical changes that occurred due to the infection. These may include surgical intervention for lagophthalmos, reconstruction of nasal bridge in saddle nose deformity, release of contractures, or resection of hypertrophied earlobes. The role of surgery for complications of leprosy has been reviewed by Virmond and coworkers.[88]

Physical Therapy and Occupational Therapy Interventions

At the time of diagnosis of leprosy, a person should have a full sensory, motor, and neurologic function evaluation. This evaluation should be repeated at the termination of leprosy treatment, in the case of reactions, or for changes in a patient's signs or symptoms. The neurologic changes from leprosy may be visible or silent.[89] If any compromise is noted, evaluations by a physical therapist and an occupational therapist are indicated, with practitioners experienced in peripheral neuropathies if available. Guidance for self-care should include (1) inspection for skin factors that predispose to wounds, (2) knowledge about potentially dangerous activities, (3) prevention such as using protective footwear, and (4) basic wound management.[90] The Innovative Care for Chronic Conditions model has been applied to self-care programs in leprosy, where physicians and health care professionals are advised to "help people to create plans that will help them achieve their goals."[91]

The International Federation of Anti-Leprosy Associations (ILEP) has published a series of learning guides (https://www.leprosy-information.org) that can be helpful in orienting the medical team and patient to self-care activities to prevent disability.[92] The sequelae of leprosy are chronic, and the interventions to improve functioning and decrease development of additional morbidity from trauma and infections related to sensory and autonomic damage in the extremities often need to be lifelong. People with disability at diagnosis, with neuritis, and with more disseminated disease have increased risk for developing additional disability in the years following leprosy cure,[93] and may benefit from more frequent follow-up or reinforcement of self-care principles. The NHDP has developed a multidisciplinary program to decrease lower extremity amputations in people with decreased protective sensation in the feet, called Lower Extremity Amputation Prevention (LEAP). LEAP includes (1) annual foot screenings to identify sensation changes, (2) education, (3) daily self-inspection of feet, (4) appropriate footwear, and (5) prompt intervention for foot problems when they develop.[94]

Monitoring Treatment Response

Clinical response to treatment can be very slow. Patients may become discouraged when they do not see rapid improvement, so additional social support may be needed to maintain compliance with the regimen. Additionally, the development of reactions can be seen by care providers and patients as a worsening brought on by antibiotics, when in fact the reactions are merely a sign of reactivity to *M. leprae*. Notably, *M. leprae* are highly resistant to degradation in the tissues, even when no longer viable. Histologic demonstration of AFB in tissue sections is not necessarily evidence of active disease. Minimal reduction of the bacterial load may be seen in the first 2 years, even though multidrug therapy is highly effective in killing the bacilli. Fragmented and degenerating bacilli may be seen in Fite stains for several years after discontinuing multidrug therapy (Fig. 250.5).[35] Mouse studies have shown that these bacteria are not viable.[95]

There are some situations in which leprosy lesions can appear to worsen or there are new skin lesions during or after multidrug therapy. The most common cause of these worsened or new lesions is leprosy immune reactions. Although most common in the first months after starting multidrug therapy for leprosy, reversal reaction can develop at any time, including after multidrug therapy has been completed and *M. leprae* infection is cured. Immune reaction is much more common than relapse as the cause of worsening skin lesions in leprosy. When relapse occurs, it is generally in people who had a high bacterial load



FIG. 250.5 Fite stains for *Mycobacterium leprae* remain positive for years after the bacteria are no longer infectious. These Fite stains (magnification ×1000) of biopsy material were obtained yearly from a person with lepromatous leprosy from the time of diagnosis (top panel: *t* = 0). At 1 year, the bacteria do not stain as solid, indicating that the bacteria have begun to degenerate. In subsequent years, the bacterial index continues to decrease as the bacteria degrade or are cleared by the immune system, or both. This patient was treated for 2 years with multidrug therapy according to the NHDP guidelines, and did not have a relapse. This figure illustrates that a biopsy may stain positive for bacteria for years after a person is no longer infectious and has been cured of the infection. *(From Scollard DM, Stryjewska B. Treatment and prevention of leprosy. In: von Reyn CF, ed. UpToDate:* Nontuberculous Mycobacterial Infections*; reprinted with permission. Last updated December 1, 2016.)*

at the time of starting multidrug therapy. This may be related to *M. leprae* in immunologically protected sites where antibiotics do not penetrate, such as the eye or testis. Relapse due to multidrug resistance is even more uncommon. This may be related to drug resistance of the initial infecting *M. leprae* strain, or to the emergence of drug resistance during multidrug therapy. Because drug resistance is so rare, the WHO recommendation is to re-treat *M. leprae* relapse with a standard multidrug therapy course.[80] Leprosy caused by reinfection with a new strain of *M. leprae* is rare, and may be related to a household contact with untreated multibacillary leprosy.[96]

PREVENTION OF LEPROSY

The current focus of leprosy prevention and control efforts is for early diagnosis of leprosy cases so that treatment can be initiated promptly. This has the dual benefit of decreasing leprosy-related disability for the patient, as well as decreasing the time that a person could potentially transmit *M. leprae.* There is currently no FDA-approved vaccine for leprosy. In India, a vaccine with *M. indicus pranii* is in use for prevention and as an immunotherapeutic.[97–99] An *M. leprae*–specific candidate vaccine (LEP-F1 + GLA-SE) is being evaluated in a phase I safety and immunogenicity trial as of this writing (NCT03302897 at https://clinicaltrials.gov).

It is recommended that household contacts of people with newly diagnosed leprosy be evaluated for symptoms and signs of leprosy on examination. Additionally, this interaction with the health care system provides opportunities for education about leprosy and symptoms for which medical advice should be sought. Additional interventions are used in some countries to decrease risk of developing leprosy in these contacts, as summarized by Gillini and colleagues.[100] One intervention that can provide partial protection is bacillus Calmette-Guérin (BCG) vaccination.[101] In a Brazilian study of over 5000 contacts of leprosy patients, BCG vaccination or revaccination at the time of identification as a leprosy contact gave 56% protection compared with contacts who did not receive BCG.[102] For multibacillary leprosy, protection with BCG vaccination reached 88%. One precaution to note with BCG vaccination is that in a recent field study, researchers found that 0.4% of contacts who received BCG vaccine presented with paucibacillary leprosy in the 12 weeks after vaccination.[103] Almost half of these early diagnoses postvaccination had neuritis or reversal reaction. It is unclear if BCG vaccination decreased the incubation period or changed the pathogenesis of *M. leprae* infection.

Postexposure prophylaxis of household contacts with single-dose rifampicin has been studied as an intervention to decrease leprosy in regions hyperendemic for leprosy. Concerns have been raised about potential induction of rifampicin resistance in *M. tuberculosis* from single-dose rifampicin, but emergence of rifampin-resistant *M. tuberculosis* in a community that has received rifampicin for leprosy postexposure prophylaxis has not been documented.[104] In French Polynesia, 2751 residents were treated with single-dose rifampicin. Comparing 10 years of posttreatment new leprosy case detection rates to those from the prior 20 years showed a 70% rate of protection.[105] In a double-blind, cluster-randomized, placebo-controlled study, 21,711 household and neighborhood contacts of 1037 people with leprosy in northwest Bangladesh received a single dose of rifampicin as postexposure prophylaxis.[106] Of note, only 28% of the leprosy index cases had multibacillary leprosy, which is the form of leprosy in which risk of transmission is highest. The reduction in incidence in the rifampicin prophylaxis group was 56.5% 2 years later, and 34.9% at 4 years. The number needed to treat to prevent one new case of leprosy at 4 years was 297. Efficacy of single-dose rifampicin was highest in contacts of paucibacillary leprosy, females, serology (anti–PGL-1) negative contacts, those with no history of BCG vaccine, and more distant contacts of the case.[106] Persons with the closest contact (e.g., immediate household members) had minimal benefit from single-dose rifampin. A secondary analysis, using a presumed BCG scar as a marker for perinatal BCG vaccination, found a combined 80% protection for single-dose rifampicin in people who had received perinatal BCG vaccination. The study protocol for a cluster-randomized controlled study (Netherlands clinical trial NTR3087) of single-dose rifampicin plus new or booster BCG immunization for postexposure prophylaxis for contacts has been published,[107] although results on efficacy are not available at the time of this writing.

PATHOLOGIC IMMUNE REACTIONS OF LEPROSY

Reactions are a significant cause of additional morbidity for people with leprosy.[108] Neurologic sequelae from immune reactions can be irreversible. Reactions can occur prior to, during, or after treatment for leprosy. Reactions are characterized as extension or worsening of skin lesions and symptoms due to immunologic responses to *M. leprae;* they are not drug reactions nor are they signs that *M. leprae* infection is worsening. For patients who are still on multidrug therapy, it is very important to continue the therapy for the full recommended course. Even when bacteriologic cure has been reached, reactions can persist. Reactions are often recurrent and can remit and recur for years, with long-term corticosteroids or other immunomodulatory medications required to control symptoms.

Type 1 Reversal Reaction

People with the unstable borderline forms of leprosy (BT, BB, and BL) are at risk for reversal reaction. This reaction is characterized by increased erythema and induration of preexisting lesions, and is the result of spontaneous enhancement of cell-mediated immunity in these lesions (Fig. 250.6). The most serious manifestation of reversal reaction is neuritis with sudden loss of nerve function. There is a validated severity scale for reversal reaction, but the components that comprise the scale require



FIG. 250.6 Reversal reactions are characterized by erythema and edema of existing leprosy skin lesions and appearance of new lesions. Reversal reactions may also be associated with neuritis. *(Courtesy Dr. Mauricio Lisboa Nobre, Hospital Giselda Trigueiro, Natal, Rio Grande do Norte, Brazil.)*

evaluation by proficient practitioners.[109] The scale includes cutaneous signs of reversal reaction, voluntary muscle testing, and muscle power.

Neuritis during reversal reaction can present as facial palsy, ulnar nerve palsy resulting in inability to extend at the wrist, and peroneal nerve palsy with inability to flex at the ankle (footdrop). Patients will generally report shooting, shocklike pains in the distribution of the nerve. These pains can be reproduced by palpation on examination. Affected nerves will commonly be enlarged relative to the contralateral nerve (see Fig. 250.4). Acute neuritis with loss of function needs to be treated emergently with corticosteroids. With prompt initiation of corticosteroids and resultant decrease in nerve swelling within the nerve sheath, function can return. Recommended starting dose is prednisone 1 mg/kg with slow taper off when symptoms have improved.[110] In a small case-control study with 21 people, comparing prednisone at 2 mg/kg with 1 mg/kg starting dose for ulnar neuropathy, the higher dose resulted in faster results in the first month, but both dosing regimens were ultimately equally effective.[111] A fixed dosing schedule for reactions has been recommended by the WHO. A study comparing a fixed prednisolone treatment schedule of 20 weeks with a longer treatment of 32 weeks did not show additional improvement in nerve function impairment with the longer duration of prednisolone. The authors recommended a 20-week course of prednisolone with additional prednisolone in those patients for whom it is indicated.[112] When close monitoring by a specialist is possible, tailoring of corticosteroid dosing to symptoms and with consideration for steroid side effects is preferred.[113] Blood glucose and blood pressure monitoring should be continued during steroid treatment. In regions endemic for *Strongyloides,* treatment for *Strongyloides* should be considered prior to starting high-dose steroids.[114] Cases of fatal *Strongyloides* hyperinfection in people receiving corticosteroids for leprosy reactions have been reported.[115]

In addition to initiation of corticosteroids, the inflamed nerve should be immobilized, such as with a sling in the case of ulnar neuritis, to decrease irritation of the nerve and minimize further swelling. Patterns of sensory loss, in addition to range of motion and strength, should be monitored during steroid treatment and the evolution of the immunologic reaction. For reversal reactions that do not improve as expected with high-dose corticosteroids, treatment of concurrent infections may decrease systemic inflammation and lessen the severity of the reaction.[115a]

It is important to note that many studies that do not show benefit of corticosteroids in improving sensory or nerve function are conducted in people who have only mild nerve impairment or whose nerve impairment is chronic. Acute neuritis and neuritis during reversal reaction are different entities that are definite indications for corticosteroids. A Cochrane review on steroid use in leprosy addresses these issues.[116] Alternative immunomodulatory medications have been tested for their impact on improvement of nerve function impairment. In a randomized controlled trial of people with reversal reaction with acute neuritis, reactional skin lesions, or both who received prednisolone and placebo versus prednisolone and azathioprine for 24, 36, or 48 weeks, adding azathioprine to prednisolone did not improve outcomes.[117]

At this time there are no predictive biomarkers for risk of developing reversal reaction. Low-dose prophylactic prednisone (20 mg/day) for 4 months from the time of diagnosis in people with borderline leprosy was not effective at reducing development of nerve impairment or immune reactions at 1 year after starting multidrug therapy for leprosy.[118]

Neuritis

Neuritis can occur as part of reversal reaction or ENL, or independently. People with acute neuritis usually present with shooting pains in the distribution of the affected nerve as a chief complaint. Pain may be aggravated by movement or by certain actions. Nerves may be enlarged (compared to contralateral nerve) or nodular on physical examination, and tenderness to palpation can be exquisite (see Fig. 250.4). Prednisone should be started at 1 mg/kg/day as for reversal reaction.[110] Immobilization, as with a sling, can provide symptomatic comfort and decrease irritating movement. Minimizing movement during acute neuritis will decrease further inflammation and swelling of the nerve. There should be a full assessment for nerve function impairment, including both sensory and motor deficiencies, with comparison to baseline assessments. Patients should be followed with monitoring for symptom improvement and improvement in objective measures of nerve function.

Type 2 Erythema Nodosum Leprosum

ENL occurs in people with polar lepromatous or borderline lepromatous leprosy. The exact etiology of ENL is unknown; immune complex formation has been associated with ENL, although its role in pathogenesis is unclear.[119] People with ENL generally present with systemic symptoms including fever and malaise, which distinguishes ENL from reversal reaction, which is not associated with fever. ENL is characterized by tender, indurated nodules that number from a few to hundreds (Fig. 250.7). During ENL, there can be end-organ effects, presumably due to the immune reaction occurring at areas of mycobacterial deposition in internal organs. These can include the liver, kidneys, spleen, (rarely) adrenal glands, and testes. Episcleritis, scleritis, and uveitis can occur as part of ENL, which merits an emergency consultation with an ophthalmologist, in addition to corticosteroids and treatment for ENL.[54] Neuritis with nerve function impairment can be part of ENL,



FIG. 250.7 Erythema nodosum leprosum is characterized by tender, erythematous nodules that can become confluent. *(Courtesy Dr. Barbara Stryjewska, National Hansen's Disease Program, Baton Rouge, LA.)*

either as a neuritis or from ENL within the nerve itself. If the ENL includes a component of neuritis, corticosteroids are indicated to decrease perineural swelling and nerve compression. There is a validated clinical severity scale for ENL, in use primarily in research settings, which includes fever, characteristics of the skin lesions, joint inflammation, and pain.[120]

Treatment of ENL varies depending on the severity of the ENL and what medications are available in a particular country.[121] Thalidomide is an antiangiogenic agent with anti-TNF properties that causes severe birth defects, including phocomelia. However, thalidomide can dramatically improve ENL's systemic symptoms and lesions within 1 to 3 days. A study comparing thalidomide 300 mg daily and prednisolone 40 mg daily for moderate and severe ENL found that the mean time to resolution of cutaneous lesions was 10 days shorter in the thalidomide group and mean remission period was 5 times as long (10.9 vs. 2.2 months).[122] A slowed taper of thalidomide results in less reemergence of ENL.[123] However, thalidomide has proven teratogenic effects and cannot be used in anyone who is pregnant or who could become pregnant. Men taking thalidomide must use barrier protection during any sexual contact with women of childbearing potential, and should not donate sperm.[124] In some countries, thalidomide is prohibited because it is teratogenic. Because of the teratogenic effects, the WHO does not recommend use of thalidomide in ENL. When thalidomide is available, prescription by a physician, dispensing by a pharmacist, and dosing by the patient are tightly regulated.[125] Counseling and consent regarding thalidomide need to include risks of inadvertent or intentional diversion.[126] Additional side effects of thalidomide include drowsiness, constipation, and peripheral neuropathy.[124]

Another significant caution with using thalidomide for ENL is the risk of deep vein thrombosis (DVT). The risk of thrombosis is highest when thalidomide is coadministered with corticosteroids, and is most often seen during ENL treatment when thalidomide and prednisone are being cross-tapered.[127–129] In a 2012 review of the literature that included five cases of leprosy treated with thalidomide and prednisone, the mean time to DVT was 6 weeks (range, 2–10 weeks) after starting thalidomide.[129] In people with multiple myeloma receiving thalidomide and low-dose dexamethasone, the addition of a thromboprophylactic agent can decrease rates of venous thrombosis.[130,131] The impact of thromboprophylaxis on thromboembolism risk in people with ENL on thalidomide and prednisone is not known.

Non-thalidomide treatments for ENL include pentoxifylline, corticosteroids, and high-dose clofazimine.[121,132] Pentoxifylline reduces blood viscosity and inhibits the synthesis of TNF. A double-blind trial on the efficacy of pentoxifylline and thalidomide for ENL found that thalidomide was superior, with only 5% of people on thalidomide still having skin lesions at 7 days, compared with 41% of people on pentoxifylline. Pentoxifylline can be considered for people who have contraindications to thalidomide.[133] In a pilot study of ENL in Ethiopia, 33 people were randomized to prednisolone plus cyclosporine or prednisolone alone. The prednisolone dosing and taper were different for the two groups. People with chronic ENL on cyclosporine and prednisolone had twice as many severe flare-ups as the prednisolone group. People on prednisolone alone needed more prednisolone to treat ENL recurrence, but this was likely related to the differences in prednisolone dosing and taper in the two groups.[134] There are case reports in the literature of ENL treated with anti-TNF monoclonal antibodies such as infliximab or a TNF antagonist such as etanercept,[135,136] but these agents are associated with reactivation of *M. tuberculosis* and increased risk of infections, and so could be high-risk in many clinical situations.



FIG. 250.8 Lucio phenomenon is recognized clinically by erosions and ulcerations secondary to vasculitis.

Necrotic Vasculitis (Lucio Phenomenon)

"Erythema necroticans" or necrotic vasculitis, also called Lucio phenomenon, is one of the most severe complications of leprosy, with high fatality rates. It is most common in people with diffuse lepromatous leprosy. Diffuse lepromatous leprosy and Lucio phenomenon have been described mostly from Mexico or in people who could have been infected with *M. leprae* or *M. lepromatosis* in Mexico, with isolated cases reported from Asia, Africa, and elsewhere in the Americas.

One case series described clinical and histologic findings in 30 people with diffuse lepromatous leprosy and Lucio phenomenon treated in California between 1969 and 2004. Although this period of time spans different approaches to leprosy multidrug therapy, the series illustrates the diagnostic dilemma encountered in diagnosing diffuse lepromatous leprosy because of its atypical clinical signs, and of determining that the acute presentation of necrotic vasculitis is related to *M. leprae* infection.[57] Although Lucio phenomenon is rare among people with leprosy, it can be fatal, and prompt consideration of leprosy and Lucio phenomenon as part of the differential diagnosis for necrotic vasculitis is essential.[38] The ulcers of Lucio phenomenon are hemorrhagic, indurated, and marginated (Fig. 250.8). The borders are irregularly serrated and the ulceration is concave to the skin. Before ulceration, early lesions may be light blue with an erythematous halo. The number and distribution of infarcts is variable. Earlier in the course of disease, hemorrhagic infarcts tend to be up to 5 cm in diameter and ovoid, whereas later the infarcts are up to 10 cm and more round. Interestingly, in this case series the peripheral leukocyte count was not dramatically elevated.[57] Histologically, biopsies of the skin lesions show foamy macrophages and few lymphocytes. Fite stain generally shows a very high bacterial load. The most helpful histologic feature is the presence of fibrin clots. If neutrophils are present, they are likely associated with necrosis and not with the vascular changes. Superficial dermal vessels are congested with endothelial proliferation, often with thrombosis in the vessels.[57,137]

Immediate treatment for Lucio phenomenon includes supportive care, consideration for sepsis because superinfection of the infarcts is common, and initiation of multidrug therapy for leprosy—including rifampin if not contraindicated, and high-dose corticosteroids. Thrombi and necrosis requiring amputation have been reported. Immunosuppressants other than corticosteroids have been used in some case reports of Lucio phenomenon, but their efficacy has not been proven. Plasmapheresis has been used in cases of necrotic lepromatous vasculitis that do not respond to corticosteroids and supportive care.[138,139] Long-term sequelae of Lucio phenomenon can include scarring, venous insufficiency, and chronic ulcers in addition to the sequelae from leprosy.

Considerations for People With Altered Immunity

Human immunodeficiency virus (HIV) infection dramatically increases the risk of progressing from latent *M. tuberculosis* infection to active tuberculosis, but no such association has been seen for *M. leprae* infection in people with HIV.[140] People with HIV can develop the full spectrum of leprosy disease,[141] although interestingly, studies have suggested that people with HIV may be more likely to present with paucibacillary disease than people without HIV.[141] The timing of clinical disease and development of reversal reactions can be related to immune reconstitution after initiating antiretroviral therapy.[142] In a review of 16 cases of leprosy developing after starting antiretroviral therapy, 10 were characterized as borderline tuberculoid leprosy with reversal reaction. The time from initiation of antiretroviral therapy to diagnosis with leprosy was between 1 and 6 months, the approximate timeframe for development of immune reconstitution.[143] There are no recommended modifications to the leprosy multidrug therapy due to HIV infection, although consideration needs to be made for potential interactions between anti–*M. leprae* and anti-HIV medications. In the case of neuritis occurring independently or as part of reversal reaction, corticosteroids should be started at standard dosing to reduce the risk of irreversible nerve damage.[144]

Anti-TNF agents are used to treat several autoimmune diseases. There are cases of people who developed clinical leprosy after administration of an anti-TNF monoclonal antibody, presumably from the decrease in cell-mediated immune response to the *M. leprae*. Discontinuation of the anti-TNF monoclonal antibody can lead to reversal reaction as the immune system recovers,[145] leading to an immune reconstitution-like reaction. Leprosy and leprosy immune reactions have also been described after solid-organ transplantation[146,147] and during conditioning or immunosuppression for allogeneic stem cell transplantation.[148]

Several immunologic changes related to gestation can alter the course of infections during pregnancy and the postpartum period. Of note, many published studies on leprosy during pregnancy were conducted prior to the use of curative multidrug therapy for leprosy. As such, the natural history of leprosy during pregnancy may have looked different prior to the standardization of multidrug therapy in 1982 than it does in the 21st century. Women may be more likely to be diagnosed with leprosy during pregnancy.[149] This can be related to a decrease in Th1 response to *M. leprae*. One prospective study from Addis Ababa, Ethiopia conducted from 1975 to 1978 reported that of 68 pregnant women with previously diagnosed BL or LL leprosy, 56% had worsening disease during pregnancy and of these, 89% had documented increase in the *M. leprae* bacterial index.[150] Postpartum, the bacterial index reverted in about half of these women. For women who developed ENL, the first episode occurred during pregnancy. For women who developed reversal reaction, the first episode occurred postpartum.[150] WHO guidelines recommend treatment of leprosy with standard multidrug therapy during pregnancy.[80] These medications are known to cross the placenta, and specific risks and benefits should be discussed with the patient before initiating leprosy multidrug therapy.

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251 *Mycobacterium avium* Complex

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SHORT VIEW SUMMARY

Microbiology and Epidemiology

- *Mycobacterium avium, Mycobacterium intracellulare,* and *Mycobacterium chimaera* are closely related organisms grouped into the *Mycobacterium avium* complex (MAC).
- MAC is found in water, soil, and animals but is not spread from person to person.
- MAC most commonly causes pulmonary disease followed by lymphadenitis and disseminated disease, although rare disease presentations are possible, especially in immunocompromised hosts.
- Pulmonary disease typically occurs in older patients with bronchiectasis, chronic obstructive pulmonary disease, or other structural lung disease and is more common in the setting of corticosteroid use. In younger patient populations, pulmonary disease is most commonly seen in the setting of cystic fibrosis.
- Lymphadenitis occurs mostly in children younger than 5 years of age.
- Disseminated disease occurs almost exclusively within immunosuppressed patients, including those with acquired immunodeficiency syndrome (AIDS) and $CD4^+$ cell counts less than $50/mm^3$ as well as individuals with malignancy, persons with various autoantibody states or functional cytokine deficits, and those using immune-suppressing medications.

Clinical Manifestations and Diagnosis

- Pulmonary disease diagnosis requires a triad of clinical symptoms, radiographic abnormalities, and positive respiratory cultures.
- Pulmonary disease typically has a chronic presentation with indolent symptoms developing over months to years. Cough is the most common symptom, but low-grade fatigue, anorexia, shortness of breath, fever, and sweats can also be seen.
- Radiographically, pulmonary disease typically has nodular infiltrates with or without cavities. At times it can mimic tuberculosis.
- Lymphadenitis is usually cervicofacial, unilateral, and painless. Diagnosis is via lymph node aspiration or excision. It can be self-limited over time.
- Disseminated disease usually manifests as fever, weight loss, anorexia, and night sweats. Many patients will have acid-fast blood cultures positive for MAC, especially if they are human immunodeficiency virus (HIV) positive. Generalized lymphadenopathy, intraabdominal lymphadenopathy, and lower gastrointestinal tract involvement can be seen.

Treatment (See Tables 251.2 and 251.3)

- Pulmonary disease
  - Diagnosis does not necessitate treatment because many patients can be managed for months to years with pulmonary hygiene, increased caloric intake, and exercise.
  - Treatment for pulmonary disease usually includes a macrolide (azithromycin is favored over clarithromycin), plus ethambutol and a rifamycin (typically rifampin). In cavitary disease or more severe disease, clinicians should consider the addition of an aminoglycoside, usually intravenous amikacin.
  - Treatment duration is based on clinical response and tolerability, but typically pulmonary disease is treated until cultures have been negative for at least 12 months; the usual total duration is 18 to 24 months.
  - Cure of pulmonary disease is difficult, and relapse and reinfection are both common, a context that argues for a chronic disease approach to management.
  - Hypersensitivity pneumonitis may be treated with avoidance of exposure, short-term steroids, or both. It rarely requires antimicrobial therapy.
- Lymphadenitis can be treated with surgical excision or, if this is not possible, with multidrug therapy. In some cases it can be monitored clinically for self-resolution.
- Disseminated disease should be treated with a macrolide plus ethambutol and usually a rifamycin. Individuals with HIV should have HIV therapy initiated as soon as possible. Immune reconstitution inflammatory syndrome may complicate recovery. Consideration should be given to including intravenous amikacin in at least the first 8 weeks of therapy, especially in the non-HIV population. Treatment duration varies based on immune system recovery.
- Skin and soft tissue or bone disease should be treated similarly to disseminated disease, although treatment duration is often shorter and débridement may be needed to provide source control for deeper seeded infections.

Prevention

- HIV/AIDS
  - The classic teaching is that HIV-infected persons with fewer than 50 $CD4^+$ cells/mm^3 should be given weekly azithromycin to prevent disseminated MAC. This can be stopped once $CD4^+$ cell counts are sustained above $100/mm^3$.
  - In the new area of effective antiretroviral therapy (ART) for HIV, the duration of $CD4^+$ cells/mm^3 remaining less than 50 after the start of ART is limited, and there is reasonable consideration for holding MAC prophylaxis and only starting HIV treatment.
- Pulmonary disease
  - In individuals with structural lung disease and especially active MAC pulmonary infection, it can be worth avoiding exposure to settings at particularly high risk of aerosolized MAC, such as hot tubs, prolonged soil exposure, and prolonged shower exposure. How much this reduces risk of invasive disease is unclear.
  - When possible in patients with active pulmonary MAC disease or pulmonary MAC colonization, it is worth limiting exposure to systemic corticosteroids, and perhaps even inhaled corticosteroids.
- All disease states
  - Like other mycobacterial diseases, risk of infection and more aggressive disease progression is increased by systemic exposure to anti–tumor necrosis factor-α therapeutics, and these should be avoided when possible in at-risk individuals.

Mycobacterium avium complex (MAC) is the most common of all the nontuberculous mycobacteria (NTM) to cause human infection and pulmonary infection.[1] MAC contains genetically diverse strains with different reservoirs and pathogenicity for humans.[2] The three most common are *M. avium, M. intracellulare,* and *M. chimaera.*[1,3–5] *M. avium* and *M. intracellulare* are often not microbiologically separated and are referred to as *M. avium-intracellulare* (MAI). Two major disease syndromes are produced by MAC in humans: (1) pulmonary disease, usually in adults with some underlying lung disease but whose systemic immunity is generally intact; and (2) extrapulmonary disease, most commonly disseminated disease in patients with advanced immunosuppression such as human immunodeficiency virus (HIV) infection, or localized

cervical lymphadenitis, usually in immunocompetent children.[6] Rarely, MAC can cause disease at other sites, such as cutaneous disease or bone and joint disease.[6] The frequency of MAC pulmonary disease seems to be increasing, especially in older populations, but it is unclear if increases are due to improved microbiologic and radiographic techniques or increased clinician and population awareness leading to increased detection versus true increase in disease incidence.[7–13] Meanwhile, the incidence of disseminated MAC disease, which increased precipitously during the HIV pandemic, has declined with the introduction of effective antiretroviral therapy (ART).[13,14] MAC treatment remains difficult, with prolonged treatment courses and fairly modest success rates, especially in pulmonary disease, for which lung surgery is sometimes needed.[6,15–17] New antimicrobials, antimicrobial combinations, or treatment paradigms are clearly needed for this infection, which has surpassed tuberculosis in incidence and prevalence in many economically developed countries.[6,15–17]

EPIDEMIOLOGY

Reservoir and Route of Acquisition

MAC is composed of environmental organisms thought to be acquired by inhalation, microaspiration, or ingestion.[6] Perhaps due to the low virulence described later, person-to-person spread has not been observed.[6,17] Environmental sites harboring MAC are diverse; they include water, soil, and animals.[6,18–20] MAC has been found to colonize natural water sources as well as indoor water systems, pools, and hot tubs.[19,21] Although specific sites from which individuals acquire MAC are only rarely identified, exposure to recirculating water systems has been described as a specific source of acquisition in human disease, especially in persons with HIV or acquired immunodeficiency syndrome (AIDS).[21–24] However, only a minority of cases can be traced to this source, and this type of connection between exposure and disease has been less clearly documented in the HIV-negative population, suggesting that other environmental reservoirs are important.[4,22,25] Aerosols of natural fresh and salt water as well as dirt particles may contain MAC, and these have been proposed as vehicles leading to transmission of MAC respiratory disease, although aerosolized fluids were not associated with the acquisition of MAC in a case-control study, and other studies have shown a less clear connection between what is in household water and the lungs.[4,18,19,25] Cutaneous MAC infection has been described to occur specifically in relation to hot tub/Jacuzzi use.[6,26] Aerosols from heater-cooler units of heart-lung machines have been specifically documented to transmit *M. chimaera* during open heart surgery, causing a notable global outbreak of these infections, including cases of disseminated disease.[27–29] There is also a strong and growing association between MAC pulmonary disease and gastric reflux disease, and an association with microaspiration, suggesting that this may be the proximate route of entry in many individuals.[30–32] Overall, the ubiquitous presence of MAC in nature makes it difficult to ascertain where an individual patient acquired his or her disease, and except in specific situations this makes avoidance strategies or investigations to identify a source of limited utility.[6]

Pulmonary Disease

MAC pulmonary disease is seen worldwide, although for a variety of reasons prevalence is higher in developed countries, likely in part due to increased life span.[1,3,11,12,33] In the United States and Canada, regional studies have shown an overall disease prevalence of 5 to 19 per 100,000 persons, with prevalence rates potentially as high as 20 to 45 per 100,000 persons in the elderly.[3,8,9,34] Incidence of disease seems to be increasing in many countries, including the United States, Canada, the United Kingdom, and Denmark, and shows substantial geographic variability within countries.[a] Few countries have a national database to accurately evaluate the true occurrence of MAC, although in Denmark where there is complete capture of pulmonary mycobacterial cultures in a national database, the incidence of NTM lung disease (of which 57% are MAC) in 2008 was 1.08 per 100,000 persons.[10] The average age of patients with MAC pulmonary disease in the United States is greater than 60 years, and most patients above this age are women.[5,7,34] However, younger persons with cystic fibrosis (CF) are at significantly increased risk for MAC pulmonary disease, and a causal role has been proposed for this organism in the pulmonary tissue destruction in CF.[37–39] Some specific risk factor(s) for MAC pulmonary disease are present in most cases; these include airway diseases (e.g., bronchiectasis), damage from prior pulmonary infections, structural lung diseases (e.g., chronic obstructive pulmonary disease), and processes that affect the immune systems, such as the use of corticosteroids and other immunosuppressive agents.[9,32] Chronic bronchiectasis is a particularly interesting entity in this regard because it is strongly associated with MAC, but it is still unclear if it is the result of the disease or a predisposition to the disease, as is discussed further later.[9,32,40] These factors, even taken together, do not explain the overall increase in cases of MAC over the past decade.[8]

Extrapulmonary Disease

The most well-known form of extrapulmonary MAC, disseminated MAC (dMAC), was extremely rare before 1980.[41] The heightened susceptibility of patients with HIV/AIDS to this process led to a marked increase, and in 1994 an estimated 37,000 cases of dMAC were seen in patients with AIDS, making this the most common clinical manifestation of MAC at that time.[42–44] With the introduction of effective ART and the increase in ART access, the number of patients with AIDS has declined and with it the number with dMAC.[44–46] The greatest risk for MAC in patients with HIV is with the severe depression of the $CD4^+$ cell count, with dMAC rarely seen in patients with greater than 100 $CD4^+$ cells/mm^3 and the median $CD4^+$ cell count among patients with dMAC and AIDS around 10 cells/mm^3.[47,48] However, there is a spectrum of risk for dMAC that increases as the $CD4^+$ count declines, and the prior occurrence of another opportunistic condition increases the risk for dMAC at any given $CD4^+$ cell level, as does a detectable HIV viral load.[48–50] Children with AIDS have a risk for MAC similar to that of adults, and rates of dMAC have also fallen dramatically in this age group as HIV treatment has improved.[51]

dMAC can also be seen in patients with disease states or medications that cause a mycobacterial-specific immune defect, such as anti–tumor necrosis factor-α (anti–TNF-α) inhibitors, corticosteroids, interferon-γ (INF-γ) pathway defects, *GATA2* mutations, or primary immunodeficiency diseases, such as interferon-γ receptor 1 (IFNγR1) or interleukin-12 receptor β-1 (IL-12Rβ1) deficiency (Table 251.1).[52] While disseminated disease due to innate immunodeficiencies is rare, disseminated disease due to iatrogenic immunosuppression is growing with increasing use of corticosteroids, inhibitors of TNF-α, chemotherapeutic agents, and organ transplants. The combination of anti–TNF-α agents and steroids seems

TABLE 251.1 Genetic Defects Predisposing to Severe Mycobacterial Infections

GENE INVOLVED	INHERITANCE MECHANISM	IMPAIRMENT IN THE IMMUNE RESPONSE
IFNGR1	AD, AR	Impaired IFN-γ response[278]
IFNGR2	AR	Impaired IFN-γ response[279]
STAT1	AD, AR	Impaired IFN-γ and IFN-α response[280,281]
IL12RB1	AR	Impaired T and NK cell function[282]
IL12RB2	AR	Impaired T and NK cell function[283]
ISG15	AR	Decreased IFN-γ production[284]
IRF8	AD, AR	Impaired T and NK cell function[285]
IKBKG	X-linked	Impaired IL-12 production[286]
GATA2	AD, sporadic	Decreased monocytes and dendritic cells[287]
CYBB	X-linked	Decreased oxidative bursts in monocytes and macrophages[288]

AD, Autosomal dominant; *AR,* autosomal recessive; *IL-12,* interleukin-12; *IFN-α,* interferon-α; *IFN-γ,* interferon-γ; *NK,* natural killer.
Modified from National Jewish Health. NTM-TB insights. https://www.nationaljewish.org/NJH/media/ProEd/NTM-TB-INSIGHTS-March-2016.pdf. Accessed May 13, 2019.

[a]References 1, 7, 8, 10, 35, 36.

to confer the most MAC-specific (and mycobacterial disease–specific in general) risk for disease dissemination in comparison to their risk of triggering dissemination of other infections, although pulmonary MAC infection is still the most common entity with anti–TNF-α use.[53–55]

Although less well known than disseminated disease, MAC lymphadenitis is a more common clinical entity in older studies and one that is likely underdiagnosed because many cases of lymphadenitis are not cultured, fail to grow an organism, or never come to medical attention.[56] Before 1980, most nontuberculous lymphadenitis in the United States was due to *Mycobacterium scrofulaceum,* but more recently, MAC has been the cause.[57] MAC cervical adenitis is largely a disease of children younger than age 3 years on the basis of reports from Europe, North America, and Australia.[57–59] A report from children in the Netherlands estimated the incidence of MAC lymphadenitis at 51 cases per 100,000.[59] MAC lymphadenitis is also seen in HIV-infected persons, particularly as "unmasking IRIS" a manifestation of the immune reconstitution inflammatory syndrome (IRIS).[60–62]

MAC skin and soft tissue infection or deeper tendon/joint/bone infection are relatively uncommon entities for which good epidemiologic data are lacking, and are usually related to direct inoculation after puncture wounds, trauma, or surgical incisions.[6] Due to MAC presence in the water and soil, and the association with puncture/trauma, this form commonly involves the hands, and when it does, has a predilection for causing tenosynovitis.[6]

The epidemiology of the relatively newly recognized MAC subspecies, *M. chimaera,* warrants mention here. Although it is generally considered a minimally pathogenic environmental organism, it is now identified as a cause of intravascular infection, primarily as postoperative infections of prosthetic material stemming from a global outbreak, identified in Germany, in which the source of infection was heater-cooler units used in cardiac surgery.[25,27,29] The long incubation time from exposure to disease made diagnosis difficult, with many of the cases diagnosed 1 to 4 years after initial surgery.[27,63] Although the individual risk of infection after such surgery is low, thousands of patients in Europe and the United States were exposed to the contaminated Stöckert 3T heater-cooler devices, prompting a massive effort to locate and treat infected patients.[64] Decontamination of the heater-cooler devices has proven difficult, and it is not completely clear if the outbreak is resolved.[65]

PATHOGEN: CLASSIFICATION AND MICROBIOLOGY

Organisms

Like other mycobacteria, MAC organisms are aerobic, non–spore-forming, nonmotile bacilli. Their cell walls include mycolic acid–containing, long-chain glycolipids, glycopeptidolipids, or both that protect the facultative intracellular organisms from lysosomal attack and give the organisms their "acid-fast" staining characteristic.[6] MAC are classified as "slow-growing" nontuberculous organisms, generally taking 10 to 35 days to grow on solid media and producing smooth-transparent, smooth-opaque, and rough colony variants that are usually light tan in color, although there are variations of uncertain significance.[66] MAC can be cultured on solid or liquid media, with liquid media more sensitive and yielding growth more quickly but not allowing quantification of load.[6] Glycolipid typing has divided MAC into 28 serovars: 1 through 6, 8 through 11, and 21 are *M. avium,* and 7, 12 through 20, and 25 are *M. intracellulare.*[67] There is considerable diversity in the strains of MAC that infect patients, with multiple strains sometimes present in the same patient.[68,69] Advances in molecular genetics have increased the number of subspecies, but the clinical relevance is poorly understood. Distinguishing between MAC species and subspecies is done by using polymerase chain reaction with DNA sequencing, hybridization with specific DNA probes, or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, although depending on the strength of the library there can be some errors seen with MALDI-TOF mass spectrometry.[2,70,71] The identification of *M. chimaera* is relevant in that it does add a prediction of lower virulence for this organism than for MAI in pulmonary infection.[25,72] It is less clear whether there is a relevant difference between *M. avium* and *M. intracellulare,* and since the subspecies cannot be differentiated from each other by traditional biochemical tests, most laboratories group them together as MAI.[6,73]

Virulence

All members of the MAC complex are relatively low-virulence organisms, with *M. chimaera* the least virulent.[6,25,72] Supporting the lower virulence, patients who actually develop true *M. chimaera* pulmonary disease are more likely to be immunosuppressed, have cancer, have received a transplant, or have high inoculum exposure due to infected cardiac surgery equipment.[29,72] There seems to be a difference in virulence between MAC serovars because serovars 1, 4, and 8 are uncommon in the environment but cause most cases of disseminated disease in persons with AIDS.[67] Possible virulence factors include adherence to intestinal epithelial cells, production of catalase, failure to acidify vesicles, and inhibition of phagosome-lysosome fusion.[74–76] MAC has the ability to change colony types in vivo, and this seems related to virulence because clinical isolates from disseminated disease are usually the smooth-transparent colony type, rather than the domed or opaque type.[66,77] Colonies that are smooth and transparent are more likely to replicate in vivo, induce cytokines, and cause more infection in animal models and usually have decreased in vitro susceptibility to antimycobacterial agents.[77–80] There may also be synergy with the HIV virus itself, because isolates from humans with MAC disease can increase cell lysis and stimulate HIV replication in vitro relative to the abilities of animal MAC isolates.[81] It is unclear whether there is a meaningful pathogenicity difference between *M. avium* and *M. intracellulare,* although the largest study in this regard, reviewing 590 patients in South Korea with MAC lung disease, showed statistically more symptomatic, radiographically advanced, and sputum smear–positive disease in the *M. intracellulare* group, as well as less optimal response to treatment.[82–85] However, it is worth noting that this group was also older and had lower body mass index values, suggesting a sicker host.

Pathogenesis

MAC infection starts from primary acquisition of the organism by inhalation/aspiration, ingestion, or direct inoculation. There are specific characteristics of pathogenesis to consider depending on the site of infection.

Pulmonary Disease

MAC pulmonary disease may develop after infection via inhalation/aspiration of MAC from the oropharynx. The duration from acquisition of infection to disease is unknown but presumably occurs over months to years. More than one distinct MAC isolate can be recovered from some patients, suggesting that infection, superinfection, and colonization may occur concomitantly.[69] Grossly, tissue lesions are usually localized, well-circumscribed nodules that at times display cavitary changes. Histologic analysis typically shows poorly formed to well-formed granulomas with surrounding acute and chronic inflammation.[86] Giant cells are seen frequently, and in some cases there is either caseating or noncaseating necrosis.[86] Thoracic lymph node involvement is uncommon.[87] Granulomatous pleuritis, bronchitis, vasculitis, and interstitial pneumonia have also been reported.[86]

Structural lung disease plays an exceedingly important role in the pathogenesis of invasive MAC pulmonary disease.[6,17] The unifying theme in almost all cases is some structural lung abnormality that leads to increased microbial access from the upper to the lower airway, compromised vascular supply and impaired immune surveillance in lower airway segments, and an environment conducive to biofilm formation. This creates a context where an organism of limited pathogenicity can develop to a critical mass necessary to cause invasive disease. Of importance is the role of larger airway disease, with bronchiectasis being a very common comorbidity, seen in up to 85% of patients in recent case series with higher rates in studies with more rigorous radiographic methodology.[9,82,87–90] There is much debate about whether bronchiectasis comes first and predisposes to MAC or whether subclinical MAC infection causes bronchiectasis, but the strong association between the two suggests that the physiology of the both is tightly linked. Once MAC infection is established in bronchiectatic lungs, impaired mucociliary clearance and biofilm formation support infection persistence.[91–93] A handful of known but uncommon causes of bronchiectasis should be considered, including CF, immunoglobulin G deficiency, pulmonary ciliary disorders, connective tissue disease, toxin exposure, and allergic

bronchopulmonary aspergillosis.[94] Most nonbronchiectatic cases of pulmonary MAC are in the setting of obstructive lung diseases (e.g., emphysema) or fibrotic lung diseases treated at least intermittently with immunosuppression.[6,9,90] In these cases, MAC preferentially infects damaged lung, with cavity-like superinfection of bullous emphysematous areas a common finding. Inhaled corticosteroids probably play an additive role by causing airway immunosuppression, with recent data suggesting an association between use and pulmonary MAC disease.[95–97]

Extrapulmonary Disease

Disseminated disease usually starts by ingestion of MAC, followed by localized disease in the lung or gut, with the gut more common. Dissemination then ensues from one of these locations over several months.[98] The organisms penetrate the gut wall and subsequently are phagocytized by macrophages and other reticuloendothelial cells.[99,100] Most disseminated disease is due to a single strain, but multiple distinct isolates have been recovered from up to 15% of patients.[68] Histologically, epithelial cells show only mild inflammatory changes and ulceration is uncommon.[100] Foamy macrophages fill the lamina propria, where these massively infected cells packed with bacilli may expand the intestinal villi, giving an appearance similar to Whipple disease.[100] The resulting thickening of the bowel wall can rarely lead to intussusception, gastrointestinal hemorrhage, or obstruction. Mesenteric adenopathy ensues with poorly formed granulomas and at times neutrophilic necrosis.[101,102] Subsequent hematologic dissemination can then occur and while any organ can be seeded, the most common sites are liver, spleen, and bone marrow.[102,103] The mycobacterial burden in bacteremia is variable, ranging from 1 to greater than 10^5 colony-forming units/mL.[104,105] MAC bacteremia leads to elevated serum levels of TNF-α and interleukin-6, which are likely responsible for the predominant symptoms of fever, night sweats, and cachexia.[106,107] The mechanism of the severe anemia frequently seen in dMAC is not well understood because bone marrow involvement is not always present, erythropoietin levels are variable, and clinical response to exogenous erythropoietin is unpredictable.[108] A unique pathophysiologic abnormality that can be seen with dMAC is asymptomatic but marked elevation of serum alkaline phosphatase with little elevation of transaminases, bilirubin, or other parameters of hepatic function.[109,110] Liver histology in these cases is unremarkable, suggesting interference with enzyme metabolism rather than hepatic destruction. In the setting of HIV/AIDS, when ART is instituted, a vigorous granulomatous response results in elimination of bacilli from the tissues, but also causes a period of marked lymph node enlargement while this process occurs—IRIS, as seen in MAC and HIV/AIDS.[60–62,111,112] Untreated, dMAC ultimately leads to death by generalized wasting.

The other common form of extrapulmonary MAC, cervical lymphadenitis, is likely acquired through ingestion of MAC and spread to local lymph nodes, where disease can develop in the setting of relative but not extensive immunosuppression, such as childhood.[56] Lesions again reveal granulomas. Ulceration and cutaneous fistula formation are frequent complications, particularly when nodes have been aspirated or biopsied.[113] In the immunologically "normal" host, nodes are often single and dissemination of disease does not occur. Without treatment, the nodes usually go through a process of purulent drainage after 4 to 8 weeks followed by self-resolution by the 1-year mark in most, leaving only a small scar at the site of infection.[114] In the less common skin, joint, or bone infections, direct inoculation leads to infection followed by local granulomatous response and adjacent spread of disease. Again, dissemination in this setting is uncommon.

Host Immunity

Pulmonary Disease

The fact that many cases of pulmonary MAC disease occur in persons with a history of airway disease suggests that impaired pulmonary clearance mechanisms predispose to disease. These patients typically do not have a profoundly impaired immune response and show acceptable delayed hypersensitivity to MAC antigens as well as adequate humoral and cell-mediated immune responses.[115] The TNF-α pathway appears to have significant importance in pulmonary disease control given the increased rates of pulmonary infection in hosts taking TNF-α inhibitors.[54,55] Although there is an increased risk of dissemination with these agents, especially in combination with corticosteroids, this is still rare and most disease remains localized.[54,55]

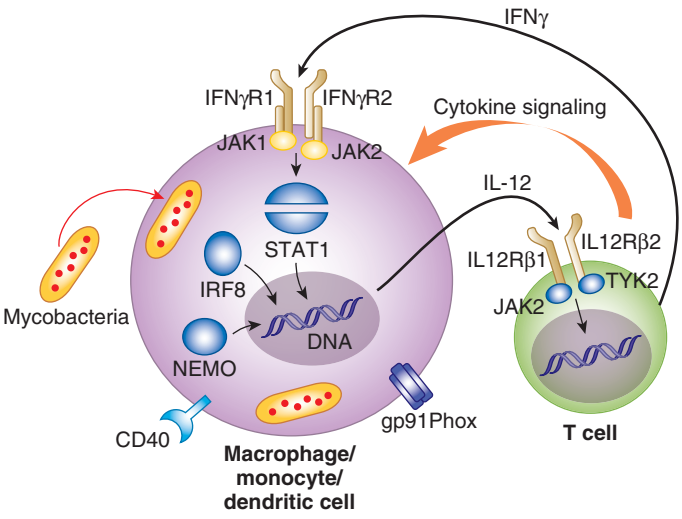

FIG. 251.1 Impaired cytokine production by T cells in patients with HIV/AIDS leads to failure of macrophage activation to eliminate intracellular *Mycobacterium avium* complex. *CD40,* Cluster of differentiation 40; *gp91Phox,* glycosylated polypeptide 91^{phox}; *IFNγ,* interferon-γ; *IFNγR1,* interferon-γ receptor 1; *IFNγR2,* interferon-γ receptor 2; *IL-12,* interleukin-12; *IL12Rβ1,* interleukin-12 receptor β-1; *IL12Rβ2,* interleukin-12 receptor β-2; *IRF8,* interferon regulatory factor 8; *JAK1,* Janus kinase 1; *JAK2,* Janus kinase 2; *NEMO,* nuclear factor kappa B essential modulator; *STAT1,* signal transducer and activator of transcription 1; *TYK2,* tyrosine kinase 2. *(Modified from National Jewish Health. NTM-TB insights. https://www.nationaljewish.org/NJH/media/ProEd/NTM-TB-INSIGHTS-March-2016.pdf. Accessed May 13, 2019.)*

Extrapulmonary Disease

In addition to the increased risk of disseminated infection due to TNF-α inhibitors, systemic steroids, or both, individuals with inherited defects in the IFN-γ signaling pathway are exquisitely susceptible to dMAC, confirming the importance of this cytokine in host defense against MAC (see Table 251.1). Currently known defects include the IFN-γ receptor ligand-binding chain, the IFN-γ signal-transducing chain, defective IL-12–mediated modulation of IFN-γ production, and signal transducer and activator of transcription 1 (STAT1) abnormalities, which also impair IFN-γ, as well as TNF-α, response.[52] Additionally, as mentioned previously, *GATA2* mutations and primary immunodeficiency diseases, such as IFNγR1 or IL-12Rβ1 deficiency, predispose to poor mycobacterial disease control and disseminated infection.[52]

In patients with HIV/AIDS, cytokine production by T cells is severely impaired leading to failure of macrophage activation to eliminate intracellular MAC (Fig. 251.1).[116,117] While MAC-specific T cells develop during disseminated disease in AIDS, they do not control the pathogen.[103] Cytotoxic $CD4^+$ cells are also important in inhibiting intracellular replication of MAC, but their function is impaired in HIV infection.[118] Additionally, antibodies against MAC are produced in response to disease in normal hosts but not in patients with AIDS.[49] Although the role of these antibodies is unclear, they do increase MAC killing in vitro.[119] Other nondisseminated extrapulmonary disease states, such as lymphadenitis and skin and soft tissue infections, demonstrate generally intact humoral and cell-mediated immunity and robust noncaseating granulomatous response to infection.

CLINICAL MANIFESTATIONS

Pulmonary Disease

The clinical presentation of pulmonary MAC disease is nonspecific and can be confused with other chronic pulmonary diseases.[6] Features of pulmonary disease vary only slightly between patients whose respiratory cultures grow *M. avium* and *M. intracellulare,* but *M. chimaera* is more often a transient colonizer.[72] The classic presentation of pulmonary

MAC is one of a subacute-to-chronic illness occurring in individuals with a known or previously unknown history of underlying pulmonary pathology.[6,73] Other features that may be identified on a chest radiograph in patients with pulmonary MAC are pectus excavatum and scoliosis, with pectus excavatum 3 to 10 times and scoliosis 2 to 3 times as common as in the general population.[6,120,121] Pleural effusions are very rare.[122,123] MAC pulmonary disease is bilateral in most cases and while the percentage with cavitary disease varies by cases series, more recent data suggests 15% to 35% of cases have some cavitary component.[124–127] The cavities in patients with MAC are more likely to be thin walled than in tuberculosis, may be quite large (most 2–5 cm), and may initially have been caused by another disease before being superinfected by MAC (Fig. 251.2).[122,128] Tree-in-bud opacities are seen often, but these are not specific for MAC. Bronchiectasis or other underlying lung disease is increasingly recognized as present in most individuals, as discussed further later (Fig. 251.3). Since bronchiectasis also predisposes this patient population to colonization with other microbes that can act as pathogens, flares in disease due to these organisms can mimic flares due to MAC.[6,17] Initial data out of the bronchiectasis registry suggest that *Pseudomonas* colonization in particular portends a more difficult bronchiectasis course, and interestingly, *Pseudomonas* is less commonly found in patients with MAC than bronchiectatic patients without NTM.[32,129] Other NTM can also be found with *Mycobacterium gordonae,* a common colonizer that is also almost never a pathogen, and *Mycobacterium abscessus* complex, found in roughly 20% to 30% of cases, sometimes as a coinfection.[130,131]

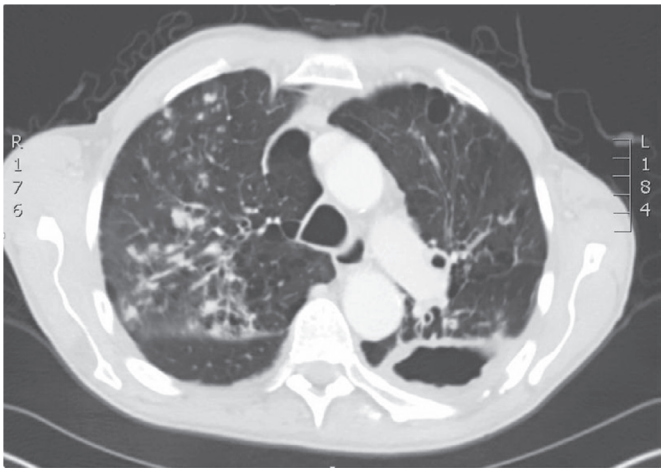

FIG. 251.2 Fibrocavitary pulmonary *Mycobacterium avium* complex in a male patient with a smoking history predisposing to underlying emphysema and bronchiectasis. Pleural thickening, nodular tree-in-bud opacities, and bullous changes of emphysema are also present.

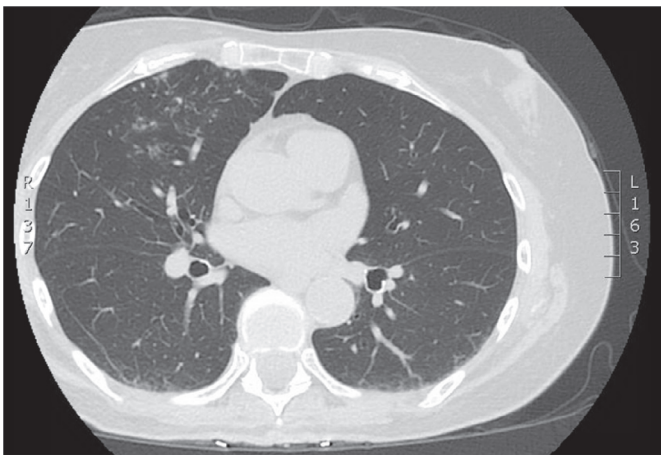

FIG. 251.3 Nodular bronchiectatic pulmonary *Mycobacterium avium* complex in an elderly woman with bronchiectasis showing the tree-in-bud opacities than are often seen in this disease.

There seem to be two general phenotypes in this disease, although the understanding is evolving and there is significant overlap. The more common phenotype is in thin, middle-aged to elderly women with no previously known lung disease, although bronchiectasis is often found during the evaluation.[9,34,90,132–134] This syndrome is sometimes referred to as "the Lady Windermere syndrome," for the principal character in Oscar Wilde's play, although this phenotype has been more recently described in males as well.[133,135] The syndrome has been increasingly recognized over the last 30 years, likely due to a combination of increased physician awareness, improved diagnostics, and increased awareness in the general population.[136] Patients with this syndrome usually present with chronic cough, but other constitutional symptoms, such as weight loss, fever, sweats, and fatigue, can be mild and indolent.[6,82] Chest radiography may have minimal abnormalities that often wax and wane in extent over years.[122,137–139] Most patients have underlying bronchiectasis that is identified much more readily on chest computed tomography (CT) than chest radiography, but even on CT the findings can wax and wane with time.[122,126,137,138,140–142] The CT finding of bronchiectasis combined with multiple nodular infiltrates most prominently in the right middle lobe and lingula is particularly suggestive of MAC pulmonary disease (Fig. 251.4).[6,122]

The less common earlier described phenotype is shifted toward middle-aged men, frequently with a history of heavy smoking and usually with previously known and significant underlying structural lung disease.[6] The clinical picture is also one of a chronic disease, but the symptoms are often more overt, including productive cough in most, weight loss or weakness in a substantial number, and a minority with fever or night sweats.[6,127,143] Inflammatory markers are more often elevated.[128] Chest radiographs in this chronic form of pulmonary MAC typically show upper lobe fibronodular and cavitary disease, which may be associated with pleural thickening (see Fig. 251.2).[6,122,127] Rates of cavitation can be higher than with tuberculosis, likely due to superinfection of bullae or damaged lung parenchyma that is already present.[6] Of note, the all-cause mortality in this disease form seems to be much higher than in the nodular bronchiectatic disease described earlier, likely due to a sicker host.[6,125,127]

Patients with CF are frequently colonized with MAC or other NTMs, but the clinical importance of MAC in individual patients can be difficult to discern. In one multicenter prevalence study of 986 patients with CF, 13% had at least one of three sputum samples positive for NTM, most (72%) with MAC, and similar findings have been replicated in other studies.[37–39,144] Overall, individuals with CF and MAC had similar pulmonary function tests compared with patients without MAC, suggesting that it is non–disease-causing colonization in at least some individuals.[145] Serial CT scans provide important information because

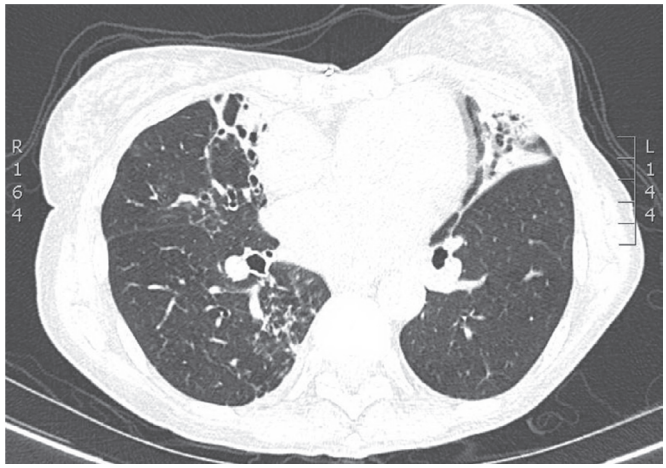

FIG. 251.4 Dense bronchiectasis of the right middle lobe and lingula that is classic for pulmonary *Mycobacterium avium* complex in a woman with a longer history of nodular bronchiectatic disease. The areas of dense bronchiectasis and surrounding inflammation can be difficult to penetrate with antimicrobial therapy and sometimes require lung surgery to adequately address.

patients with MAC identified by culture and progression of CT abnormalities are more likely to have clinical decline than other CF patients with MAC.[145] CT abnormalities suggestive of progressive MAC in CF include of areas of cystic or cavitary disease, subsegmental or larger areas of consolidation, pulmonary nodules, and tree-in-bud opacities.[145] The current standard is to use the same diagnostic and treatment criteria for MAC as are used in patients without CF, although the popularity of chronic azithromycin treatment in CF has raised additional concern about azalide/macrolide resistance.[38,146,147]

Hot tub lung—another rare, although well-known, pattern of MAC pulmonary disease—is less infectious and more inflammatory in nature, typically occurring in persons exposed to pools of heated water containing MAC.[148–151] It is presumed that in these cases inhalation of aerosolized MAC results in a hypersensitivity pneumonitis.[148–151] Individuals present with mild-to-moderate dyspnea and dry cough, with or without fever. Chest radiographs and CT scans show patterns similar to other hypersensitivity pneumonitides, including bilateral alveolar infiltrates, centrilobular nodules, and ground-glass opacities.[152,153]

Extrapulmonary Disease

dMAC occurs exclusively in persons with advanced immunosuppression, with dMAC in HIV/AIDS being the classic example, and if untreated is fatal. A natural history study of patients with HIV infection found that MAC bacteremia developed at a median $CD4^+$ count of 13 cells/mm^3, almost always in patients with an uncontrolled viral load, and the median survival after diagnosis was only 134 days.[48] In such cases, it can be difficult to separate features directly attributable to MAC from abnormalities attributable to advanced HIV/AIDS, but some clue is provided by a study evaluating patients at risk, in which prospective monthly blood cultures for MAC showed that most changes occurred in the first 2 months of positive MAC blood cultures.[154] The presentation generally includes high fever, weight loss, night sweats, and severe anemia (hematocrit <25%).[109,154,155] Other features associated with MAC include abdominal pain, diarrhea, intraabdominal lymphadenopathy, hepatosplenomegaly, leukopenia, and an elevated serum alkaline phosphatase level.[109,155] Many different organs can be involved, with a comprehensive autopsy series of 44 patients with HIV/AIDS and dMAC showing most common involvement of the spleen, lymph nodes, liver, intestines, colon, and bone marrow and less common involvement of the lungs, adrenal glands, and stomach and rarely the central nervous system.[156] Patients with HIV/AIDS and dMAC only rarely have pulmonary disease with parenchymal lung involvement, occurring in less than 10% of patients.[98,157] dMAC can also be seen in individuals with underlying immunodeficiencies (e.g., Table 251.1) or in those on immunosuppressive therapy, especially high-dose corticosteroids, TNF-α, or both, and the clinical presentation is generally similar.[41,52,54,158,159] Given declines in AIDS incidence in many developed countries, as well as increased use of immunosuppressive drugs, these may become the more common forms of dMAC in some populations. Due to the nonspecific manifestations with dMAC, the differential diagnosis is broad and depending on the host and epidemiology, disseminated disease due to other mycobacterial and fungal granulomatous diseases as well as lymphoma and, in HIV patients, AIDS wasting syndrome should be considered.

Local manifestations of dMAC may occur in patients with severe immunosuppression who have immune reconstitution. This usually happens either in the case of ART initiation in HIV patients or in the case of withdrawal of immunosuppressive therapy in other patients, and leads to IRIS or a "paradoxical reaction" as described earlier in this chapter.[60–62,111] Especially in HIV/AIDS, this can manifest as painful lymphadenopathy occurring in the first 3 months of initiating ART, while abdominal pain, hepatosplenomegaly, and pulmonary-thoracic disease have been reported to occur in roughly 25% of cases.[62,111] IRIS symptoms differ from those of dMAC itself; fever may be present, but constitutional symptoms (e.g., weight loss, night sweats) are usually absent, and blood cultures do not grow MAC.[61,62,111] Since these patients are at risk for many etiologies of these symptoms, biopsy may be required to establish an accurate diagnosis.

Cervicofacial lymphadenitis is the most common localized extrapulmonary manifestation of MAC and is usually seen in children, with at least 80% of individuals between 1 and 5 years of age.[57,59,160] Tuberculosis as a cause of lymphadenopathy is much more common than MAC in adults.[161] MAC lymphadenitis is usually painless or minimally painful unilateral enlargement of a node in the submandibular or high jugular region.[57,59,160,162] Nodes are often firm, varying from 1 to 7 cm in diameter, with the mean size in one series reported as 2.5 × 3 cm.[161,162] Bilateral disease is rare and multiple nodes are involved in less than 20%. The disease almost always stays localized absent significant immunocompromise, and systemic symptoms such as fever are uncommon.

MAC, while uncommon, is still the most commonly reported NTM to cause cutaneous disease.[163] Cutaneous MAC occurs in both immunocompetent and immunocompromised hosts and is difficult to differentiate from other chronic skin lesions.[164,165] Cutaneous MAC is generally due either to direct inoculation of the skin by trauma, surgery, or injection or to cutaneous seeding in disseminated disease.[166–168] When it occurs, cutaneous lesions may be indolent ulcers, nodules, or plaque with local swelling, erythema, tenderness, and infection of deeper tendons (especially with hand involvement), but little local lymphadenopathy and few systemic symptoms.[165,168] MAC is also a rare cause of osteomyelitis, mastoiditis, renal disease, prostatitis, peritonitis, corneal ulceration, mastitis, septic arthritis, and synovitis. MAC osteomyelitis can rarely develop in HIV-infected patients with dMAC, and vertebrae are the most common bone involved.[169] Osteomyelitis may clinically present later as patients develop immune reconstitution with initiation of ART.[169] In the non-HIV population, cases of osteomyelitis are even more rare, often vertebral, and usually either due to direct inoculation or associated with medication-induced immunosuppression.[170,171]

DIAGNOSIS

Pulmonary Disease

Diagnosis of MAC pulmonary disease is difficult because of the frequency of positive sputum cultures among persons without overt disease.[6] A single sputum culture that grows MAC has a marginal positive predictive value for clinical disease and as a result the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) have developed a generally accepted case definition for pulmonary MAC disease.[6,9,17] In this definition's criteria, patients must have symptoms compatible with MAC pulmonary disease, have abnormalities on chest imaging compatible with MAC pulmonary disease, and have one of the following microbiologic features: (1) two positive MAC sputum cultures; (2) a bronchoalveolar lavage with a positive culture for MAC; or (3) acid-fast bacilli (AFB) or granulomas, or positive MAC culture from a histopathologic specimen (if MAC is not cultured from the biopsy, MAC should be present in at least one sputum or bronchoalveolar lavage sample).[6]

AFB sputum smears are neither sensitive nor specific in the diagnosis of pulmonary MAC disease because they are rarely positive and, when positive, sometimes represent other acid-fast organisms.[6] Cultures usually turn positive by 35 days when performed on solid media and by 10 to 21 days when performed in broth, but growth time depends on the inoculum size and cultures cannot be classified definitively as negative until 6 weeks.[6] Cultures contaminated with bacterial or fungal overgrowth before 6 weeks cannot be interpreted and should be repeated. Patients who do not meet the diagnostic criteria should be followed and reevaluated because clinical disease may develop over time.[39,125,126,172–174] Occasionally treatment should be pursued without meeting all diagnostic criteria if there are very compelling clinical features to support either aggressive treatment or chronic suppressive therapy (i.e., profound immunosuppression, other severe lung disease with limited pulmonary reserve).

For hypersensitivity pneumonitis due to MAC, diagnosis is usually clinical based on a combination of exposure history, chest imaging, and sometimes lung pathology. Patients usually give a history of recent and sometimes extensive exposure to aerosols from pools or hot tubs.[6,153] Chest radiographs may be normal, may show nodules or infiltrates, or in the acute presentation, can show total opacification of the lung fields.[148,152,153] In the acute stages, lung biopsy may show neutrophilic or lymphocytic infiltrates; later, noncaseating granulomas may be seen.[153] Patients with hypersensitivity pneumonitis may grow MAC from sputum, bronchoalveolar lavage, or lung biopsy specimens and this entity may be a combination of early infection and hypersensitivity, but patients

frequently do not develop chronic infection with MAC.[6] No test for serum precipitins to MAC antigens analogous to that for allergy-mediated *Aspergillus* pulmonary disease is currently available.

Extrapulmonary Disease

Disseminated extrapulmonary MAC is diagnosed by recovery of MAC from blood or another usually sterile site (e.g., bone marrow, liver, or spleen).[6] Since growth from lymph nodes may represent localized disease and growth from sputum, bronchial washings, gastrointestinal biopsy specimens, or stool can represent either colonization or localized disease, in such cases positive cultures from more than one organ are necessary to establish a diagnosis of disseminated disease.[6] Isolation of MAC from stool by itself does not indicate disease and should not be used in clinical diagnosis.[6] In patients with HIV/AIDS, blood is the preferred specimen for diagnosis of dMAC and is the method of diagnosis in more than 90% of cases, with one specimen having a diagnostic yield around 90% and two specimens a yield near 99%.[6,175,176] One caveat is that in early disease, bone marrow biopsy with culture may be more sensitive.[103] Blood cultures are also the test of choice for disseminated disease in non–HIV infected hosts, but less is known about the test characteristics in this setting. Blood culture technique should be that of AFB cultures because MAC will not grow in standard blood culture media, and lysis-centrifugation systems to quantify bacterial load have limited clinical utility.[6,176] When the burden of bacteremia is high, mycobacteria may be seen on Kinyoun or auramine stain of buffy coat smears, but this is neither sensitive nor specific. Direct detection of MAC in blood by polymerase chain reaction may be as sensitive and specific as culture, but this assay is not widely available.[177,178]

The diagnosis of MAC lymphadenitis requires growth of MAC from the involved node and a compatible clinical syndrome.[6] Excision is preferred to needle biopsy because fistula formation is common after needle biopsy.[6,113,114] Histologic examination is important and typically reveals noncaseating granulomas with or without AFB, but should be interpreted within the clinical context.[6,113,179] Isolated lymphadenopathy has a large differential diagnosis, as mentioned previously, so workup for other entities is often warranted. Lymph nodes reactive to local bacterial infections, concurrent viral infections, other granulomatous infection, and malignancy should be considered.

Diagnosis of MAC disease in other sites, such as skin, soft tissues, bones, and joints, may be suggested by the finding of AFB or tissue granulomas, but confirmation requires recovery of MAC by culture of the affected site and a compatible clinical syndrome since MAC has the potential to be a nonpathogenic colonizer of nonsterile body sites, as mentioned earlier.[6,179]

TREATMENT AND PREVENTION

Principles of Treatment

Successful treatment of MAC is a challenge and is highly influenced by the site of infection, the immune status of the host, and the host's ability to tolerate antimicrobial therapy. As with therapy of other mycobacterial infections, the use of at least two, and usually three, active drugs is essential to prevent emergence of resistance and improve outcomes.[6,17,180] Therapy is made difficult by the paucity of antibiotics that are highly active against MAC, by the frequency of adverse effects associated with available antibiotics, and by some lack of clarity around what outcome metric best represents "success".[181] In pulmonary MAC disease, given the difficulty providing curative therapy and the prolonged antibiotic courses needed, patient-related quality of life and the way this is affected by both the disease and the therapeutic interventions are important considerations.[6,182] At times a less effective antibacterial approach may be the most appropriate choice for an individual patient, although in general effective antimicrobial treatment seems to improve quality of life in this population.[6,182–184] Since disseminated disease carries much higher and more rapid morbidity and mortality, every effort should be made to provide aggressive curative therapy and reduce the immune defect that predisposed to dissemination.[6]

Specific Antimicrobials

Macrolides and their subclass azalides (hereafter all referred to as macrolides) exhibit excellent in vitro activity against MAC and have revolutionized the treatment of MAC disease.[185–189] Although clarithromycin has lower minimal inhibitory concentrations (MIC), azithromycin is at least as active, if not more active, in vivo than clarithromycin (likely due to better tissue penetration), and the two have equivalent clinical outcomes.[6,17] Additionally, azithromycin has fewer drug-drug interactions, its levels are less affected by rifamycins, and it is dosed once daily versus twice daily for clarithromycin, generally making it the macrolide of choice for MAC.[190,191] An important point about macrolides is they are the only drug class for MAC treatment with proven correlation between drug susceptibilities and clinical outcomes, making macrolide drug susceptibility testing an important part of management.[6,17,192] Roughly 99% of strains of MAC from patients not previously given a macrolide are susceptible to clarithromycin or azithromycin.[193–195] Prior macrolide exposure, especially macrolide monotherapy, has implications because significant resistance can develop in this setting.[194,196] This resistance develops more quickly in disseminated than pulmonary disease, likely due to higher disease burden and more metabolically active organisms in disseminated disease.[193,197] Treatment outcomes are significantly worse if resistance develops, so efforts to mitigate macrolide resistance are a central tenant of MAC management.[6]

Other drugs have been shown to have clinical activity against MAC as well. Ethambutol has in vitro activity against MAC, has proven efficacy in reducing mycobacteremia for HIV/AIDS patients with dMAC, and is part of most recommended MAC regimens.[6,185,198] Ethambutol is probably the second most important drug in MAC regimens because it is more effective than any other class, including rifamycins, in protecting against the development of macrolide resistance.[199–202] Ethambutol in combination with a macrolide is the only two-drug regimen allowed by the 2007 ATS/IDSA guidelines for treatment of certain forms of MAC lung disease and a pragmatic randomized, multicenter clinical trial comparing this two-drug therapy with standard three-drug therapy for pulmonary MAC has been funded.[6,191] Every effort should be made to include ethambutol, dosed at 15 mg/kg/day, or 25 mg/kg three times/week, in MAC combination therapy.[6] Rifamycins are active in vitro against MAC, and clinical outcomes in both pulmonary MAC disease and dMAC have improved with addition of rifamycins to the regimen.[185,201,203,204] Given their long-term tolerability and increased efficacy data compared to other options, they are the third drug added to MAC treatment regimens that make up guideline-directed therapy.[6,17] Although it is not proven that MICs of ethambutol or rifamycins are associated with clinical outcomes, there are some suggestive emerging data and we would suggest taking their MICs into account.[205] In many ways aminoglycosides, with amikacin usually the most active of the class, are the most effective MAC therapeutics against susceptible isolates and improve treatment outcomes when used.[6,192,206] However, aminoglycoside toxicity and the lack of oral formulations limit use.[6,207] For more severe disease in which the debilitation of illness offsets the risk of toxicity and need for prolonged intravenous access, they become the additional drug of choice for an induction phase of therapy as discussed in more detail later.[6] When used for induction therapy, aminoglycosides can generally be given three times weekly, which makes this therapy more feasible and tolerable for many patients.[6,207]

The most relevant additional oral antibiotics in cases of failure or intolerance of first-line regimens include clofazimine, fluoroquinolones (FQs), oxazolidinones (linezolid and tedizolid), and bedaquiline.[208–213] Clofazimine is the most intriguing agent in this group, with the most reliably low in vitro MICs and an acceptable long-term safety profile known from the leprosy treatment experience.[190] However, early clinical data from dMAC treatment in HIV/AIDS patients showed higher mortality rates in clofazimine-containing arms, raising concern about this drug and leading to a black box warning for use in HIV/AIDS.[200,214,215] When this literature is critically assessed, the patients in the clofazimine arms in these trials may have been sicker or clofazimine may have been combined with less effective antibiotics versus the comparator groups, and there is no strong physiologic basis for the worse outcomes; therefore there is reason to think this risk may be overstated, especially since other studies show no difference in outcomes.[216] However, it is still generally recommended that the drug not be used for dMAC in HIV/AIDS.[6,217] There are promising case series using clofazimine for other forms of MAC, especially pulmonary disease, but robust, controlled

clinical data are currently lacking, although one monotherapy randomized controlled trial for pulmonary MAC is ongoing (NCT02968212).[208,210,218,219] It is important to note that if clofazimine is used at standard dosing of 50 to 100 mg daily, the long half-life means it takes roughly 60 days to reach therapeutic steady state.[220,221] FQs have a robust body of supporting literature in tuberculosis, but MICs for MAC are generally high due to baseline resistance, and they should not be used unless there are favorable MICs from a reputable laboratory.[190,222] Moxifloxacin has the most in vitro activity of all the FQs against MAC and is the FQ of choice if they are to be used.[190,222] There are concerns that FQs do not adequately protect macrolides against the development of resistance and may even have some antagonism with macrolides.[6,223] As such, FQs should never be used in combination with macrolides as a two-drug combination in the treatment of MAC disease.[6] A third active drug must be included. Oxazolidinones and bedaquiline are used in treatment of resistant tuberculosis and generally have in vitro activity against MAC. There are now limited but promising data from case series in their use for MAC, especially pulmonary disease.[209–211,224,225] Given the smaller body of data for their use, and their often treatment-limiting toxicities, they are generally reserved for use in macrolide-resistant pulmonary MAC or salvage pulmonary MAC cases, as described later.

In the pre-macrolide era, quoted "success rates" for pulmonary MAC treatment were no better than 50%, with longer treatment regimens and higher rates of drug toxicity.[6,226,227] HIV/AIDS patients with dMAC also responded poorly, with high mortality, to non–macrolide-containing regimens.[228,229] Even with therapy in the macrolide era, response rates are still lower than ideal and treatment courses are long, probably in part because we still do not understand optimal pharmacokinetic and pharmacodynamic considerations for this disease or the optimal way to assess treatment response.[181,190,230] In pulmonary MAC over the last 20 years, patients who have tolerated 6 or more months of macrolide therapy show sputum conversion rates ranging from 60% to 90%, with other clinical end points such as durable culture conversion and radiographic resolution less common.[186,187,231–234] Most patients in these studies received ethambutol, a rifamycin, and sometimes an aminoglycoside in addition to the macrolide. Even in the macrolide era, treatment outcomes for dMAC in HIV/AIDS can still be poor in the initial year after diagnosis, likely influenced by socioeconomic and racially based disparities in resources and among those who get the disease, but in those who survive the first year, long-term good outcomes can be achieved with MAC treatment and durable ART.[235,236]

Antimicrobial Tolerability

The maximal dose of clarithromycin is 500 mg twice daily, and higher doses have been associated with poorer clinical outcomes and should not be used unless guided by therapeutic drug monitoring.[193,216] Many patients have difficulty tolerating 500 mg twice daily of clarithromycin because of gastrointestinal side effects and dosing frequency, which is an advantage of 250 mg of daily azithromycin, for which the side effect profile and dosing schedule are more favorable.[237] When gastrointestinal side effects occur, they are often time limited, but if not, they can sometimes be mitigated by decreasing dosing frequency to three times weekly, at an increased dose of 500 mg, although this is not usually recommended in severe or disseminated disease (Tables 251.2 and 251.3).[233] Azithromycin also causes fewer drug-drug interactions than clarithromycin, making it more feasible to use in individuals on multiple medications.[238] Ethambutol is well tolerated by most patients, although at higher doses, gastrointestinal intolerance may occur. The main toxicity concern with ethambutol is optic neuritis, making baseline evaluation and serial symptom monitoring for ocular symptoms while on therapy warranted, although we do not think serial ophthalmologic examination is needed in the absence of symptoms with standard lower dosing (15–20 mg/kg/d or 25 mg/kg three times/week).[6,239] Since rifabutin is associated with higher rates of gastrointestinal distress, liver function abnormalities, neutropenia, and less commonly uveitis and severe arthralgias, rifampin has become the rifamycin of choice for MAC treatment due to better tolerability.[6,240] The most common side effect with rifampin is still gastrointestinal intolerance, although this often improves after the first few weeks on therapy, and can be managed with antinausea therapy or by taking the medication with food (even though drug absorption is slightly impaired by this).[6] The most problematic issue with rifamycin use is drug interactions, as discussed later. All the rifamycins turn body fluids (particularly urine) a reddish-orange color—a benign issue that will resolve once therapy is completed.[6]

TABLE 251.2 Initial Regimens for Pulmonary *Mycobacterium avium* Complex

MILD TO MODERATE NODULAR/ BRONCHIECTATIC DISEASE	CAVITARY OR ADVANCED/SEVERE DISEASE
Azithromycin 250 mg PO daily or 500 mg PO three times weekly[b]	Azithromycin 250 mg PO daily[c]
Ethambutol 15 mg/kg PO daily or 25 mg/kg PO three times weekly	Ethambutol 15 mg/kg PO daily
Rifampin 10 mg/kg (600 mg max.) PO daily or three times weekly[d]	Rifampin 10 mg/kg (600 mg max.) PO daily[e] Amikacin 10–15 mg/kg IV three times weekly[f]

[a]See text for full dosing recommendations.
[b]Alternative: clarithromycin 500 mg PO twice daily or 500 mg three times weekly.
[c]Alternative: clarithromycin 500 mg PO twice daily.
[d]Alternative: rifabutin 300 mg PO daily or 300 mg PO three times weekly.
[e]Alternative: rifabutin 300 mg PO daily.
[f]Alternative: Streptomycin 10–15 mg/kg IV or IM three times weekly.
Modified from Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. *2007;175:367–416.*

TABLE 251.3 Optimal Serum Drug Levels for Antibiotics Used in the Treatment of MAC

ANTIMICROBIAL	OPTIMAL SERUM LEVEL (mcg/mL)[b]
Azithromycin	0.2–0.7
Clarithromycin	2.0–7.0
Rifampin	8.0–24.0
Rifabutin	0.3–0.9
Ethambutol	2–6
Amikacin (IV)	C_{max} 3–5 times greater than isolate MAC MIC

[a]Recommended levels from Alsultan and Peloquin.[248]
[b]Measured at 2 h postdose unless specified.
C_{max}, Peak serum concentration; *MAC, Mycobacterium avium* complex; *MIC,* minimal inhibitory concentration.

Aminoglycosides have no oral formulation and in almost all cases must be administered parenterally, although streptomycin is usually given intramuscularly.[6] However, patients frequently become intolerant of the injections associated with long-term streptomycin treatment, so the availability of long-term intravenous access makes amikacin the preferable agent.[6] Administration of aminoglycosides twice weekly or three times weekly is adequate for treatment of MAC in most forms and lessens inconvenience as well as drug toxicity.[207] The major toxicities of aminoglycosides are hearing loss, vestibular dysfunction, and renal function impairment. When amikacin, kanamycin, or streptomycin is given daily at 15 mg/kg or three times weekly at 25 mg/kg, ototoxicity has occurred in roughly 35%, nephrotoxicity in 15%, and vestibular toxicity in 10% of MAC patients.[207] We recommend starting at 10 to 15 mg/kg three times weekly to limit these toxicities further and increasing the dose until the peak serum concentration–to-MIC (C_{max}/MIC) ratios are in the estimated optimal range of 3 to 5.[6] Given the complexity, expert consultation is recommended to achieve optimal dosing.[6] Aminoglycoside hearing loss is usually permanent, so audiometry should be performed at baseline and repeated monthly in all patients on systemic aminoglycoside therapy for MAC.[6] Similarly, serum creatinine levels should be followed either weekly or every other week.

Clofazimine toxicity is most commonly gastrointestinal, although almost all patients will also experience darkening of the skin as is well described in the leprosy literature.[6,208,218] Prolonged FQ use likely increases the already known chances of quinolone-associated tendinopathy,

especially in the elderly population and patients on systemic steroids, and this effect combined with some predilection for causing delirium in older patients, limits its use since these scenarios apply to many patients with MAC disease. The cytopenia-related toxicity with the oxazolidinones can be mitigated without lowering efficacy by using daily dosing instead of the twice-daily dosing used for other bacterial infections, but neuropathy or optic neuritis often is a limiting factor after 4 to 5 months of therapy.[241,242]

Drug Interactions

Neither azithromycin nor ethambutol has significant drug interactions, a major advantage of these agents.[6] Clarithromycin inhibits cytochrome P-450 isoenzyme 3A4 and interferes with the metabolism of drugs that use this isoenzyme, raising their serum levels.[238] Increased serum concentrations of drugs such as carbamazepine, omeprazole, and warfarin may be seen. Serum levels of clarithromycin are also increased when the drug is coadministered with the common drugs fluconazole or ranitidine. Even more importantly, the metabolism of clarithromycin is increased, and thus its serum drug level lowered, when administered with rifamycins.[190,191] In the largest pharmacodynamic study on this topic, the C_{max} of clarithromycin was decreased by 68% compared to a 23% decrease in azithromycin C_{max} with concurrent rifampin administration, leading to peak levels below target range more frequently for clarithromycin (56% vs. 35%).[190] Rifamycins have drug-drug interactions with many medications in addition to macrolides, usually increasing clearance and lowering levels of other drugs by induction of the hepatic microsomal enzyme cytochrome P-450 pathway.[6,190,243] Many drugs can be affected, including clarithromycin, methadone and other opiates, warfarin and almost all other anticoagulants, estrogens, and several classes of HIV antiretrovirals (especially protease inhibitors and non-nucleoside reverse-transcriptase inhibitors).[243,244] When possible, serum levels of these agents should be monitored when coadministered with rifamycins.[243] FQs have a number of drug-drug interactions, and importantly moxifloxacin, the most MAC-active FQ, cannot be used with rifampin due to its metabolism by rifampin causing significantly decreased levels, a problem that does not affect the other FQs.[245] Additionally, there is the possibility of additive QT interval prolongation when FQs and macrolides are used in combination, making consideration of QTc interval monitoring warranted.[6] Coadministration of fluoroquinolones with divalent or trivalent cations (e.g., calcium, ferrous sulfate) can lead to decreased absorption and decreased serum FQ levels, and patients should be counseled to either not take these supplements or separate them by at least 2 to 3 hours from FQ dosing. The primary drug interaction to consider with oxazolidinones is the risk of serotonin syndrome when linezolid is given concurrently with other serotonin-active medications.[246]

Drug Dosing in Impaired Renal Function

Clarithromycin, ethambutol, most FQs, and aminoglycosides are excreted by the kidneys, and doses of these agents should be reduced in patients with renal insufficiency.[6] Rifampin, rifabutin, azithromycin, and the FQ moxifloxacin are excreted largely by the liver and do not require dose reduction when given to patients with renal insufficiency, although consideration should be given to rifamycin dose reduction in end-stage renal disease.[6]

Serum Drug Level Monitoring

The significance of serum levels of the drugs used to treat MAC is largely unclear.[6] Two pharmacologic studies totaling more than 600 persons treated for MAC showed a substantial number of these persons to have serum drug levels below the target range.[190,247] This raises concern for the possibility of monotherapy in certain situations, especially when only two-drug therapy is being utilized. It may also explain suboptimal therapeutic response in some patients.[190,230] Although there are no data clearly correlating serum drug levels or use of drug level monitoring to clinical outcomes, we believe the previously noted findings provide some rationale for drug level monitoring, especially in cases with suboptimal therapeutic response, atypical treatment regimens, complicated drug-drug interactions, or reasons to suspect poor drug absorption.[6] For the first-line MAC agents, serum drug level monitoring should be performed 2 hours after the dose is administered, although sometimes the peak can be delayed in individuals with slowed gastrointestinal absorption.[248] The theoretical goal levels for these drugs largely come from tuberculosis literature, and the application to MAC therapy is still controversial (see Table 251.3).[248] As mentioned previously, when intravenous amikacin is used, therapeutic drug monitoring should always be performed, targeting C_{max}/MIC ratios of 3 to 5.[6]

Macrolide-Resistant *Mycobacterium avium* Complex Disease

Isolates resistant in vitro to clarithromycin are uniformly cross-resistant to azithromycin.[249] There may still be a rationale for continuing the macrolide in treatment of pulmonary MAC disease given immunodulatory benefits, activity against concomitant pathogens, and the possibility of mixed MAC populations (discussed previously). Treatment outcomes for macrolide-resistant disease are generally poor, as discussed earlier. Most experts recommend a four-drug regimen comprising a rifamycin, ethambutol, an FQ (if susceptible) or clofazimine, and an aminoglycoside as initial therapy, although the long-term durability of the aminoglycoside is problematic.[6] Clofazimine in particular has been used with good effect in salvage treatment regimens for MAC, and given this and its more reliably low MICs in vitro than FQs, as well as synergy with the amikacin, which is often used in these cases, clofazimine may be a more optimal agent than FQs in this situation.[250,251] Newer agents for the treatment of tuberculosis, such as linezolid, tedizolid, bedaquiline, and delamanid, have activity against MAC and are probably the best options after quinolones and clofazimine are entertained. The clinical evidence behind these drugs is limited, especially in macrolide-resistant disease, so while their use should be considered, we cannot provide a strong recommendation for their use at this time.[6] When used, linezolid is best dosed as 600 mg daily. Bedaquiline is dosed as 400 mg twice daily for 2 weeks, followed by 200 mg three times weekly.[209,241] Ethionamide and cycloserine can at times have activity but have substantial toxicities that limit their use practically, and they should only be used in consultation with a specialist. Other mycobacterial drugs such as isoniazid and pyrazinamide are minimally active in vitro with no known clinical benefit, although they are still recommended in certain situations by some experts.[17,252]

Immunomodulatory Treatment of *Mycobacterium avium* Complex Disease

MAC disease in patients with inherited immune defects may have a better response to antimycobacterial therapy when treatment to restore or circumvent immune defects is undertaken. When inherited defects in the IFN-γ signaling pathway are present, subcutaneously administered IFN-γ may overcome these defects and may lead to clinical improvement.[253] IFN-γ has not been shown to be of benefit in pulmonary MAC disease without defined immune defects and is not recommended in this setting, although one small and currently unreplicated trial has shown some benefit.[6,254] In patients with either inherited or acquired immunoglobulin deficiencies, there is sometimes a role for intermittent intravenous immunoglobulin replacement to help with disease control, although clinical studies to prove benefit are lacking.

Specific Organ System Treatment Considerations

Pulmonary *Mycobacterium avium* Complex

Treatment Choice and Monitoring

As discussed earlier, the decision to treat for pulmonary MAC is made difficult by the long duration of therapy, drug toxicity, the difficulty of clearing dense infection from areas of bad underlying bronchiectasis or structural lung disease, and the risk of reinfection after effective treatment is completed. Treatment choice is also guided by severity of the pulmonary disease, with more extensive or cavitary disease or both portending a more severe disease state that warrants more intensive treatment (Table 251.4).[6,17,255] In this setting an aminoglycoside, usually intravenous amikacin, should be added three times weekly to the oral backbone regimen for the initial 2 to 4 months of treatment, with specific monitoring as discussed previously.[6,17,207] For the most common disease form, nodular/bronchiectatic disease, the standard regimen involves

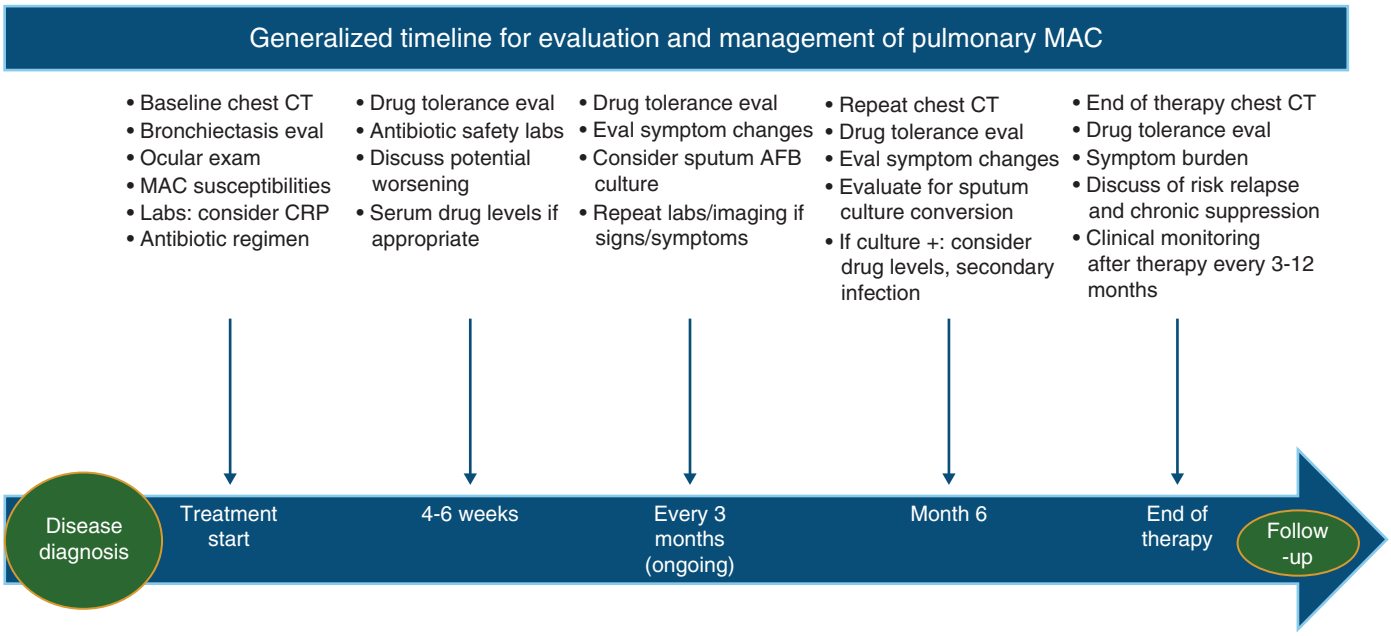

FIG. 251.5 Schematic to guide longitudinal management of individuals with pulmonary *Mycobacterium avium* complex *(MAC)* on therapy. Individual patient characteristics and goals of care should be important considerations in this process. *AFB,* Acid-fast bacilli; *CRP,* C-reactive protein; *CT,* computed tomography; *eval,* evaluation.

TABLE 251.4 Antibiotic Regimens for Disseminated *Mycobacterium avium* Complex

PREFERRED REGIMEN	ALTERNATIVE REGIMEN OR OPTION
Azithromycin 500 mg PO daily *plus*	Clarithromycin 500 mg PO twice daily *plus*
Ethambutol 15 mg/kg PO daily *plus*	Ethambutol 15 mg/kg PO daily *with or without a rifamycin*[b]
Rifampin 10 mg/kg (600 mg max.) PO daily *consider adding*	Rifabutin 300 mg PO daily
Amikacin 10–15 mg/kg IV three times weekly *for the first 4–12 wk*	

[a]See text for full dosing recommendations.
[b]Alterative acceptable regimen: macrolide (azithromycin/clarithromycin) + only ethambutol.
Modified from Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. *2007;175:367–416.*

azithromycin 250 mg daily or clarithromycin 500 mg twice daily, ethambutol 15 mg/kg/day, and rifampin 600 mg daily or rifabutin 300 mg daily, with azithromycin the favored macrolide and rifampin the favored rifamycin for reasons mentioned previously.[6,17] For patients with milder, noncavitary disease, who cannot tolerate the initial regimen, one option is changing from daily to three-times-weekly dosing with dosing adjustments to azithromycin and ethambutol as outlined in Table 251.2.[231,234] This three-times-weekly regimen can also be considered for initial therapy in patients with mild-to-moderate nodular bronchiectatic disease, although the benefits of starting with daily therapy in this setting include more options for deintensifying therapy in response to side effects and, for patients with more advanced noncavitary disease, potentially enhanced efficacy.

We recommend clinical evaluation of the patient every 4 to 6 weeks until stable and then thereafter every 3 to 4 months to evaluate for clinical response, adverse events, and adherence to the drug regimen. Although guidelines suggest treating for 12 months after sputum culture conversion, most patients convert by month 6, and many are not able to produce sputum after the first two or three months.[186,231,234] As a result, while repeating sputum every 1 to 3 months until negative is reasonable to attempt, in the patients in whom it is not feasible, a target treatment duration of 15 to 18 months is appropriate. Shorter durations may be reasonable for patients with minimal disease who show a rapid clinical response to treatment, although data here are limited. In our experience, some patients even have an initial worsening 4 to 12 weeks into treatment due to increased lung inflammation in the setting of mycobacterial death, a clinical situation with faint echoes of the paradoxical response to the initiation of therapy for tuberculosis. As discussed earlier, interval chest radiographs have limited sensitivity for nodular bronchiectatic disease, but we think obtaining chest CT scans roughly 6 months into therapy, at the end of therapy, and at a time of a sustained clinical worsening has utility to guide management. Patients who do not show clinical improvement, whose sputum does not clear of MAC after 6+ months, or who have radiographic worsening after 6 months, should be evaluated for adherence to the regimen, subtherapeutic drug levels, and potential concomitant infections (Fig. 251.5).[6] Although almost all MAC isolates from untreated patients are susceptible to macrolides, resistance can develop during treatment, and since antecedent macrolide exposure is common in individuals with lung disease and resistance to macrolides significantly affects prognosis, macrolide susceptibility testing at the start of therapy is vital.[6,17] As discussed previously, although drug susceptibilities for nonmacrolide antibiotics have never been shown to correlate closely with clinical outcomes, given the less consistent MIC profiles for drugs such as rifamycins, ethambutol, quinolones, aminoglycosides, and linezolid, we recommend this testing at a specialty mycobacterial laboratory before starting therapy and for patients on treatment who remain culture positive after prolonged therapy.

It can be difficult to tell the difference between relapse and reinfection, both of which can occur months or years after treatment, and patients should be followed intermittently after therapy due to this.[6,128,256] When relapse happens, it most commonly happens in the first 9 to 12 months after therapy cessation, while reinfection remains a lifelong risk, especially in those with nodular bronchiectatic disease.[128,256,257] Given the frequency of relapse and reinfection and the difficulty of initial cure, some individuals require either chronic suppressive treatment or intermittent treatment courses throughout life.[6] As mentioned earlier, this dynamic also raises the question of whether "cure" is the appropriate paradigm within which to consider this disease and argues for a more chronic disease management approach focusing on a combination of symptoms, quality of life, and long-term lung function.

Because most individuals with pulmonary MAC disease have either bronchiectasis or other structural lung disease, airway clearance therapies

and other lung disease treatment form an important part of their management.[258,259] Many individuals with milder disease can be managed with aggressive airway clearance/pulmonary hygiene alone and do not require antibiotic therapy.[6,136] Even when antibiotics are necessary, these other interventions should not be forgotten because they are synergistic with antimicrobial therapy.[6,136] Because the physiology predisposing to pulmonary MAC also allows other organisms to gain easier access to the respiratory tree, individuals with MAC disease are usually colonized with and sometimes infected with other pathogens, including other mycobacteria.[129,130,260,261] MAC treatment choice sometimes needs to take these other organisms into account, or clinicians need to consider a superinfection with these other organisms as one of the possible reasons for worsening in a MAC patient.[136,262] *Pseudomonas aeruginosa* in particular is implicated in a more severe disease phenotype in individuals with bronchiectasis, worse quality of life, and exacerbation metrics in pulmonary MAC.[32,129] There is a poorly understood interplay of the lung microbiome here, and in some patients, treatment of MAC sometimes allows other pathogens to flare (and vice versa) as organisms compete for resources within the airway.

Surgery

Surgical lung resection is a possibility for select patients who do not respond to medical therapy or who have focal dense bronchiectatic disease or lung cavities where tissue penetration of antibiotics may be limited.[6] Although some of the surgical procedures for MAC were performed in the pre-macrolide era, surgical resection of MAC-infected lungs continues to be successfully used in the area of macrolide-based regimens.[263–266] Occasionally, patients need surgery to control complications of MAC, such as pneumothorax, severe bronchiectasis, or hemoptysis. Careful patient selection is important because surgery can have significant complications such as pneumothorax, prolonged air leaks, and bronchopulmonary fistulas. Thoracoscopic intervention, although it requires more technical expertise, helps lower the complication rates and should generally be the first-line method of surgical intervention.[264] With careful selection and in experienced surgical hands, outcomes are good, but given the infrequency with which these interventions are performed at most centers, we recommend referral to a center with good experience in the resection of MAC-infected lung tissue.[263–265]

Hypersensitivity Pneumonitis

Most patients with MAC hypersensitivity pneumonitis (hot tub lung) respond to short-term interventions, and some patients are cured simply by avoiding the exposure source.[149,153] When progressive pulmonary symptoms are present, a short (roughly 2 months) course of prednisone with or without antimycobacterial drugs for 3 to 6 months has been shown to be effective, suggesting this is mostly an inflammatory/allergic process and not a deep infection.[153]

Extrapulmonary Disease

Disseminated Disease

For dMAC, antimycobacterial treatment should be initiated promptly for all patients with confirmed disease.[6] In HIV/AIDS patients, empirical treatment is not recommended because clinical suspicion is a poor predictor of dMAC disease, and if pursued the clinician should evaluate for other illness if the cultures remain negative after 6 weeks.[103] All dMAC isolates from patients with HIV/AIDS should have initial susceptibility testing performed because macrolide resistance has been reported to be as high as 15% to 20%.[196] HIV-infected patients should not be treated if they are only colonized with MAC in the sputum or gastrointestinal tract but have no evidence of active infection, although patients should be followed carefully because a high percentage may develop MAC bacteremia within 1 year if effective ART is not instituted.[98] Treatment should involve at least a macrolide (azithromycin 500 mg daily or clarithromycin 500 mg twice daily) and ethambutol dosed at 15 mg/kg/day, although most experts recommend adding a rifamycin as well (see Table 251.4).[201,217] Given that early clinical outcomes are still poor, aminoglycosides are highly effective at more rapidly decreasing disease burden, and that induction with an IV aminoglycoside is usually pursued in severe lung dMAC in the non-HIV population, we recommend considering adding intravenous amikacin for the first 8 to 12 weeks to the above regimen.[217,235,236] Perhaps the most important tenant of therapy for dMAC in HIV/AIDS is the initiation of ART, because patients have improved clinical outcomes and decreased MAC relapse if ART for HIV disease is administered concurrently.[217] ART in this setting should be started as soon as possible.[217] ART can be complicated by drug interactions between the rifamycins and the protease inhibitors and nonnucleoside reverse-transcriptase inhibitors, but in the modern area, anti-HIV integrase inhibitors allow concurrent administration of rifamycins, which is preferred to stopping the rifamycin. Of the integrase inhibitors, raltegravir should be given at double dose of 800 mg twice daily and dolutegravir should be given at a double dose of 50 mg twice daily when administered with rifampin, while neither require dose adjustment when used with rifabutin.[217] It is currently recommended that elvitegravir and bictegravir not be coadministered with either rifampin or rifabutin. When effective antimycobacterial therapy for dMAC in HIV/AIDS patients is instituted, fevers and night sweats usually resolve within 2 to 4 weeks, and mycobacteria are cleared from the blood in 4 to 8 weeks.[217] The response of anorexia, fatigue, and severe anemia can be more variable. Follow-up blood cultures are not necessary for all patients with clinical improvement but should be done for patients who fail to improve after 4 to 8 weeks.[217] MAC isolates from patients failing therapy should be submitted for drug susceptibility testing, although most isolates remain sensitive.[154,200]

The duration of antimycobacterial therapy for dMAC depends on immune status.[6] In HIV/AIDS patients, therapy should be continued indefinitely for those with fewer than 100 $CD4^+$ cells/mm^3, whereas therapy may be discontinued in patients who have received MAC therapy for at least 12 months and have had cell counts of 100 $CD4^+$ cells/mm^3 or greater for at least 6 months.[217] This recommendation is based on several large series of HIV/AIDS patients with dMAC who have had a significant elevation of $CD4^+$ cell counts after starting ART and had antimycobacterial therapy stopped successfully.[267,268] Patients should continue to be followed because there have been occasional reports of patients relapsing with systemic MAC after discontinuation of antibiotics. In general, therapy for dMAC in the non-HIV/AIDS population should follow a similar course. However, since there may not be an immunodeficiency to address with ART, antimycobacterial treatment duration may differ based on clinical response.[6] For situations in which disseminated disease was predisposed by immunosuppressive medications, these should be promptly discontinued or at least the dose should be reduced as much as possible (especially when anti-TNF-α agents or corticosteroids are used).

Some patients beginning ART for HIV/AIDS or withdrawing predisposing immunosuppressive medications experience IRIS, as mentioned previously.[62,111] Most patients with IRIS improve with continuation of therapy.[217] For patients with severe symptoms, a short course (4–8 weeks) of steroids (e.g., prednisone, 0.5 mg/kg daily, tapered as signs and symptoms permit) may relieve symptomatic discomfort, but if the earlier-noted symptoms are persistent or recurrent, workup for additional causes should be undertaken before multiple courses of steroids are administered, although IRIS symptoms can last 3 to 6 months in some cases.[112,217] IRIS can also occur in dMAC in non-HIV/AIDS patients if there was a predisposing immunosuppressive medication that is stopped. In rare situations where this form of IRIS is severe enough, a low dose of the prior immunosuppressive may even have to be reinstituted until symptoms abate.

Prevention. Patients with HIV/AIDS, not on ART, and with $CD4^+$ cell counts of fewer than 50 cells/mm^3 are at high risk of developing dMAC disease, roughly 20% each year.[48,49] Given the high mortality associated with dMAC, primary chemoprophylaxis of MAC can be helpful for patients with $CD4^+$ cell counts below 50 cells/mm^3, with clarithromycin, azithromycin, and rifabutin all showing efficacy and with macrolides more effective in comparison studies.[217,269–272] There does not appear to be added benefit to using combination therapy as prophylaxis. Azithromycin 1200 mg once weekly is the preferred regimen, with azithromycin 600 mg twice a week, clarithromycin 500 mg twice daily, and rifabutin 300 mg daily as alternatives.[217] Before beginning antimycobacterial prophylaxis, patients with symptoms of dMAC should have a mycobacterial blood culture performed to rule out dMAC. Patients on ART whose $CD4^+$ count rises to greater than 100 cells/mm^3 for

greater than 3 months are no longer at increased risk for MAC, and controlled studies have shown the safety of discontinuing prophylaxis in this population.[217,273,274] One more recent change in this field is that, with the introduction of more potent ART, viral load suppression and $CD4^+$ cell count rise to greater than 100 cells/mm^3 happen so quickly that they have often occurred by the time the 6- to 8-week time period at which to document negative AFB blood cultures has elapsed. As a result, there is now a compelling argument against the need for dMAC primary prophylaxis in HIV/AIDS if ART is promptly initiated.[275] In the non-HIV population, there are no immunosuppressive medications at high enough risk for dMAC for primary prophylaxis to be warranted, and this should be avoided in this population.

Other Localized Disease

In the management of MAC lymphadenitis, surgical excision is the treatment of choice.[57,162,276] When complete excision of the node is performed as a diagnostic and therapeutic intervention, concomitant antibiotics are often not required.[276] For individuals for whom surgery poses a high risk, therapy with a macrolide-containing regimen may be successful, although the optimal duration of therapy is unclear.[6,57,277] As mentioned previously, some cases are also self-limited, so intervention is not required in every case.

For skin and soft tissue infection as well as deeper bone and joint infection, optimal therapy usually involves a combination of surgical débridement and antibiotics.[6] There is almost no literature to guide decision making, but we favor induction therapy with two or three oral drugs plus an intravenous aminoglycoside for the first 2 to 4 months, especially in bone and joint disease. Optimal duration of therapy for skin and soft tissue infection is to treat until lesions resolve, usually 4 to 6 months, while in bone and joint infection a duration of 6 to 12 months is probably appropriate, although this should be influenced by adequacy of initial débridement as well as clinical response.[6]

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The complete reference list is available online at Expert Consult.

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252 Infections Caused by Nontuberculous Mycobacteria Other Than *Mycobacterium avium* Complex

Barbara A. Brown-Elliott and Richard J. Wallace, Jr.

SHORT VIEW SUMMARY

DEFINITION

- The category of nontuberculous mycobacteria is composed of species other than *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium leprae*. For *Mycobacterium avium* complex, see Chapter 251.
- Previously known as "atypical mycobacteria" or "mycobacteria other than *M. tuberculosis*."
- More than 170 species of nontuberculous mycobacteria (NTM).
- Divided into rapidly, intermediately, and slowly growing species.

EPIDEMIOLOGY

- Most species are worldwide and ubiquitous in the environment, including household water, potting soil, vegetable matter, dust, animals, and birds.
- NTM include pathogens and nonpathogens.
- Cases in chronic pulmonary infections involve patients with underlying disease, such as bronchiectasis, cystic fibrosis, and chronic obstructive pulmonary disease.
- Other cases involve extrapulmonary sites, including skin and soft tissue, bones, joints, bursae, tendon sheaths, lymph nodes, eyes, ears, blood, brain, and cerebrospinal fluid.

MICROBIOLOGY

- Acid-fast bacilli (AFB) stain poorly with Gram staining.
- Identification of definitive species level is only accomplished by molecular or proteomic methods currently.

Rapidly Growing Mycobacteria

- Rapidly growing mycobacteria (RGM) produce mature colonies on solid media within 7 days.
- Routine culture media, such as blood agar, chocolate agar, trypticase soy agar, most MTBC media (Middlebrook 7H10 or 7H11 agar and Lowenstein-Jensen agar), and various broths, including rapid broth detection systems, support the growth of most species.
- Preference is for 28° to 30°C incubation for some species, although many species also grow at 35°C.
- Some RGM, especially *Mycobacterium abscessus,* are adversely affected by decontamination methods that are used for isolation of MTBC.
- Currently, six groups of pathogenic species are defined based on presence or absence of pigment and genetic relatedness.

Intermediately Growing Mycobacteria

- Most MTBC media, including Middlebrook 7H10 or 7H11 agar and Lowenstein-Jensen agar, support growth of species.
- They require 7 to 10 days to reach mature growth.
- Primarily, two pigmented species are involved:
  - *Mycobacterium marinum* (pathogen) grows optimally at 28° to 30°C and is associated with marine water and marine animals.
  - *Mycobacterium gordonae* (nonpathogen) grows optimally at 35° to 37°C and is a common tap water contaminant.

Slowly Growing Mycobacteria

- Produce mature colonies in more than 7 days on solid media.
- Most MTBC media, including Middlebrook 7H10 or 7H11 agar, Lowenstein-Jensen agar, Middlebrook broth, and rapid detection broth systems, support growth of species, except for fastidious species, such as *Mycobacterium haemophilum* (requires hemin or iron) and *Mycobacterium genavense* (requires mycobactin J).
- Most species, except *M. haemophilum* (28°–30°C), *Mycobacterium xenopi* (42°–45°C), and some environmental species, grow optimally at 35° to 37°C.

DIAGNOSIS

Pulmonary

- Signs and symptoms of NTM lung disease are variable and nonspecific.
- Patients often present with chronic cough or throat clearing, with or without sputum production and severe fatigue. Less frequently, malaise, dyspnea, fever, hemoptysis, and weight loss may occur.
- Clinical diagnosis depends on multiple positive microbiologic cultures of respiratory samples for AFB.
- Follow the American Thoracic Society guidelines for diagnosis.
- High-resolution chest computed tomography is useful.
- Routine chest radiographs are recommended.
- Single positive sputum cultures are not definitive for NTM disease.
- Positive bronchoalveolar lavage with known lung (NTM) pathogen considered definitive for NTM disease.
- Expert consultation is required for patients with infrequently encountered or highly drug-resistant species of NTM.

Extrapulmonary

- Infection may involve fever, drainage, bacteremia, granulomatous systemic infections, necrosis, and so forth, depending on the site of infection.
- Clinical diagnosis depends on positive microbiologic smears and cultures for mycobacteria of drainage, biopsy tissue, or body fluid, leading to isolation of specific NTM.

THERAPY

Rapidly Growing Mycobacteria

- *Pulmonary disease*
  - Most pulmonary disease is caused by *M. abscessus* subsp. *abscessus* or *M. abscessus* subsp. *massiliense.*
  - Antimicrobial therapy alone is generally unsuccessful for microbiologic eradication of *M. abscessus* subsp. *abscessus* in the presence of a functional *erm* gene.
  - Drugs used for slowly growing mycobacteria, including MTBC, such as rifampin, rifabutin, ethambutol, isoniazid (INH), streptomycin, and pyrazinamide are not effective.
  - Multidrug regimens of macrolides (azithromycin or clarithromycin), high-dose intravenous (IV) cefoxitin (8–12 g/day in divided doses) or imipenem (1 g twice daily), and low-dose intravenous amikacin (peaks in the 20–25 μg/mL range on once-daily dosing) is probably optimal for adults with *M. abscessus.* Once-daily IV tigecycline is often included; dosage adjustment is usually necessary for clinical improvement, not cure. The role of inhaled routine or liposomal amikacin is not well established.
  - Patients with pulmonary *M. abscessus* subsp. *abscessus* and a functional *erm* gene are treated for several months until clinically improved and may require several courses of treatment.
  - Macrolides may be useful for isolates of *M. abscessus* subsp. *massiliense* but not most *M. abscessus* subsp. *abscessus, Mycobacterium fortuitum,* and *Mycobacterium smegmatis* isolates that contain functional inducible *erm* genes.

SHORT VIEW SUMMARY—cont'd

- Other antimicrobials are often useful for species other than *M. abscessus* and *M. chelonae* (minocycline, doxycycline, trimethoprim-sulfamethoxazole [TMP-SMX], quinolones, linezolid, and, for *M. chelonae,* tobramycin).
- *Extrapulmonary disease*
  - In general, clinicians prescribe a multidrug regimen based on in vitro susceptibility testing, including a macrolide (except in case of isolates with functional *erm* genes).
  - Rifampin, rifabutin, ethambutol, and INH are not effective.
  - Patients with significant disease are treated for 6 months, depending on the severity of infection.
  - Other antimicrobials often useful for species of the *M. fortuitum* group are minocycline, doxycycline, TMP-SMX, quinolones, linezolid, and, for *M. chelonae,* tobramycin.

Intermediately Growing Mycobacteria

- *M. marinum* is found on skin and in soft tissue infections (usually hands).
- Treatment options include macrolides, rifampin, TMP-SMX, or a combination of rifampin and ethambutol.

Slowly Growing Mycobacteria

- *Pulmonary disease*
  - Antibiotic treatment generally follows the regimen for *Mycobacterium avium* complex: rifampin, ethambutol, and a macrolide (clarithromycin or azithromycin) with adjustments depending on in vitro antimicrobial susceptibility testing.
  - Patients are generally treated for 12 months of culture negativity.
- *Extrapulmonary disease*
  - Treatment is the same as for pulmonary disease, except that it is usually for 6 months depending on the severity of infection.

PREVENTION

- Recent suggestion of person-to-person spread of a genotype of *M. abscessus* subsp. *massiliense.*
- Tap (household) water derived from community water systems is considered the major reservoir for most common NTM species.
- Biofilms may render NTM less susceptible to disinfectants and antimicrobials.
- Public health concerns are increasing.

OVERVIEW

The recent advances in mycobacterial culture techniques and the increasing utility of modern molecular techniques for identification of previously unidentified organisms have produced a major resurgence of interest in disease caused by the nontuberculous mycobacteria (NTM). In addition, there has been an increasing appreciation of the defects in lung structure and immune response that predispose to NTM.[1,2] This group of mycobacteria is composed of species other than the *Mycobacterium tuberculosis* complex (MTBC), which consists of *M. tuberculosis, M. africanum, M. bovis, M. canettii, M. caprae* (caprine), and several other lesser known species (*M. pinnipedii, M. microti, M. suricattae* [meerkat], *M. orygis,* and *M. mungi* [mongoose]).[3,4] Although *M. leprae* is not a member of the MTBC, it is usually considered separate from the NTM as it is here (see Chapter 250). Previous names for this group of organisms include "atypical mycobacteria" or "mycobacteria other than *M. tuberculosis*."[5] Currently, there are more than 170 species of NTM, of which more than half are considered to be pathogens or potential sources of human or animal disease. Approximately 20 of these species have been described since 2014.[4,6,7] *Mycobacterium avium* complex (MAC) is described extensively in Chapter 251. By tradition, NTM have been categorized into different groups based on characteristic colony morphology, growth rate, and pigmentation (the Runyon system of classification). This system is now considered outdated as we focus predominantly on rapid molecular systems of diagnostics. However, growth rates and colony pigmentation continue to provide practical means for grouping species of mycobacteria within the laboratory and are thus still useful.[5,7]

Rapidly Growing Mycobacteria

The group of organisms called *rapidly growing mycobacteria* (RGM) includes nonpigmented and pigmented species that produce mature growth on media plates within 7 days. There are currently six groups or complexes of RGM based on pigmentation and genetic relatedness. Nonpigmented pathogenic species within the *M. fortuitum* group now include approximately 10 species: *M. fortuitum, M. peregrinum, M. senegalense,*[4,7–9] *M. setense,*[10] and former members of the third biovariant complex, including *M. septicum,*[11,12] *M. porcinum,*[12] *M. houstonense, M. boenickei, M. brisbanense,* and *M. neworleansense.*[12] A newly described, closely related species, *M. aquaticum,* has been recovered from hemodialysis water but not yet considered pathogenic.[13] In addition, there are six validated species within the second group, the *M. chelonae-abscessus* group (*M. chelonae, M. saopaulense* [a pathogen described in humans and fish],[14] *M. salmoniphilum,*[4] *M. franklinii* [multiple pulmonary and sinus infections],[15,16] *M. immunogenum,*[2] and the recently emended three subspecies of *M. abscessus: M. abscessus* subsp. *bolletii, M. abscessus* subsp. *massiliense,* and *M. abscessus* subsp. *abscessus*, previously designated as *M. bolletii, M. massiliense,* and *M. abscessus,* respectively).[17,18] *M. salmoniphilum* has been revived as a fish pathogen but as yet has not been recovered in human samples.[4] The *M. mucogenicum* group includes three species: *M. mucogenicum* (formerly *M. chelonae*–like organism) and two newer-described species, *M. aubagnense* and *M. phocaicum.*[6,7] A fourth group of pathogenic organisms within the RGM is the *M. smegmatis* group.[7,19] Isolates within this group include two late-pigmenting species: *M. smegmatis* (formerly *M. smegmatis* [sensu stricto]) and *M. goodii.*[7,19] All of these species, including the newly described species, have been recovered from clinical specimens on multiple occasions.[7,19]

The fifth group, (early) pigmenting RGM, contains several species that have been implicated in clinical disease, including *M. flavescens, M. neoaurum, M. vaccae, M. phlei, M. canariasense, M. cosmeticum, M. monacense, M. psychrotolerans,* the thermophilic species *M. thermoresistibile, M. bacteremicum,*[20] and *M. iranicum.*[4] Recent additions include *M. celeriflavum,*[4] *M. hippocampi* (a marine pathogen),[4] and *M. anyangense* (a cattle pathogen).[21] *M. mageritense* (formerly in the *M. fortuitum* group) and *M. wolinskyi* (formerly in the *M. smegmatis* group) have been suggested to comprise a sixth group of nonpigmented species, which are genetically closely related to each other.[7,22,23]

Slowly Growing Mycobacteria

This group includes species of mycobacteria that require more than 7 days to reach mature growth. Some species may also require nutritional supplementation of routine mycobacterial media.[5] The major clinically important established species within this group include the MAC, which is discussed in Chapter 251; *M. kansasii; M. xenopi; M. simiae* complex (*M. simiae, M. lentiflavum*, *M. triplex,* and the newly described pigmented species, *M. europaeum*); *M. szulgai; M. malmoense;* and *M. scrofulaceum.*[4,5] In addition, the *M. terrae–M. nonchromogenicum* complex is now composed of several clinically significant species associated with tenosynovitis, including *M. arupense*[6,24,25]; *M. kumamotonense*[4,5,25]; *M. hiberniae*[2]; *M. heraklionense*[4,24,25]; *M. longobardum*[24]; and most recently, *M. virginiense,*[25] and one pink pigmented species, *M. engbaekii,*[4,24] that, like *M. nonchromogenicum,* has not yet been established as pathogenic.[25] *M. asiaticum,*[26] *M. florentinum,*[6] *M. senuense,*[5] and *M. montefiorense,*[5,6] a pathogen in eels,[5,6] were previously described. Recently described nonpigmented species also include human pathogens and potential pathogens: *M. kyorinense*[4]; *M. noviomagense*[27]; *M. shinjukuense*[4]; *M. sherrisii*[4,6]; *M. koreense*[4]; and *M. riyadhense,*[4] a species related to *M. malmoense* and *M. szulgai,* which was originally identified as MTBC due to a false-positive commercial line probe. Two other species, *M. stomatepiae* and *M. angelicum,* genetically related to *M. szulgai,*[28] have been reported in fish. *M. algericum,*[4] genetically related to the *M. terrae* complex, was described as a pathogen in goats. *M. minnesotense,* described in early 2013, has not been recovered from clinical samples to date.[29] *M. paraterrae, M. paragordonae,* and *M. parakoreense* have rarely been described in patients and still have uncertain clinical significance.[4] Pigmented newly described species include *M. europaeum,*[4]

M. paraseoulense,[4] *M. shigaense,*[4] and *M. parafficum*.[4] *M. persicum*, a pigmented species described in 2017 and related to *M. kansasii,* has been reported in pulmonary samples from four unrelated patients in Iran.[30]

Other previously described pigmented organisms in this group include *M. nebraskense,*[5,6] *M. parascrofulaceum,*[6] *M. parmense,*[6] *M. saskatchewanense,*[6] *M. seoulense,*[6] and *M. pseudoshottsii* (a fish pathogen related to *M. shottsii*).[6]

In Africa and Australia *M. ulcerans* continues to be a major pathogen. Cultivation of this species is difficult because it requires up to several months to grow, so molecular detection and identification are currently more optimal than culture techniques.[26,31,32] Other organisms that require special nutritional supplements include *M. haemophilum,* which requires hemin for growth (hence its name), and *M. genavense,*[26,31–33] which requires mycobactin J and prolonged incubation in broth culture. Most of these slowly growing mycobacteria grow best at 35° to 37°C, with the exception of *M. haemophilum,* which prefers lower temperatures (28°–30°C), and *M. xenopi,* which usually grows well at 42° to 45°C.[26,32,34]

Intermediately Growing Mycobacteria

This group of organisms includes *M. marinum, M. gordonae,* and *M. intermedium*,[2] and a newly described cattle pathogen, *M. bourgelatti*, related to the latter species, may also belong to this group but has not yet been described in humans.[4] These organisms are pigmented and require 7 to 10 days to reach mature growth. *M. marinum* grows optimally at 28° to 30°C, whereas *M. gordonae* prefers 35° to 37°C.[31] *M. intermedium,* an NTM of uncertain clinical significance has an optimal temperature between 31° and 37°C.[2]

Nontuberculous Mycobacteria and the Environment

Most NTM species are readily recovered from the environment. Isolates have been recovered from samples of soil, water, animals, plant material, and birds.[5,23] A few fastidious species that are known to cause disease, such as *M. haemophilum* and *M. ulcerans,* have rarely been recovered from the environment.[32] Although an association with an environmental source may be present, a direct link to the environment has not been proven except for health care–associated disease and pseudooutbreaks. Recently, the possibility of person-to-person spread of a strain of *M. abscessus* subsp. *massiliense* has been reported among patients in a US cystic fibrosis center.[35] Isolates that genetically match the strain have been seen in geographic areas outside the United States, including the United Kingdom and South America.[36–38] Community drinking water systems are considered the major reservoirs for most common human NTM pathogens and thus are of increasing public health interest. Slowly growing NTM species, other than MAC, isolated from household tap water include *M. gordonae, M. kansasii, M. xenopi, M. simiae, M. arupense,* and the newly described *M. aquaticum.*[5,13]

Among the RGM, *M. mucogenicum* and the closely related *M. phocaicum* are common tap water isolates.[7,26] Other RGM species from tap water include *M. porcinum, M. immunogenum,* and *M. chelonae.* Recent studies of household water have shown that biofilms, which are the filmy layers at the solid and liquid interface, are recognized as a source of growth and possibly a mode of transmission for mycobacteria.[5,7,39] Moreover, biofilms may serve to render mycobacteria less susceptible to disinfectants and antimicrobial therapy.[5,39] Biofilms appear to be present in almost all collection and piping systems, so mycobacteria may often be recovered from these sites. The persistence of pathogenic NTM in water and biofilms has important implications in the epidemiology of infections related to water.[5,39]

Nontuberculous Mycobacteria and Clinical Disease

NTM produce six major clinical disease syndromes (Table 252.1), which are reviewed in the following sections.

PULMONARY DISEASE

Geography of Common Nontuberculous Mycobacteria Species

Chronic pulmonary disease in a human immunodeficiency virus (HIV)-negative host is the most common localized clinical disease caused by NTM.[26] In the United States MAC, followed by *M. kansasii,* is the most frequently recognized pathogen.[26] In Canada, some parts of the United Kingdom, and Europe, *M. xenopi* ranks third, whereas *M. malmoense* is second after MAC in Scandinavia and northern Europe.[5,26,32] In southeast England *M. xenopi* and *M. kansasii,* known to be present in local water supplies, are both more common than MAC.[5] A recent study in Ontario, Canada showed that *M. xenopi* was the second most frequently isolated NTM after MAC. The study also revealed that patients with *M. xenopi* disease have higher rates of pulmonary cavitation than MAC and are often associated with chronic obstructive pulmonary disease (COPD) and significant mortality rates.[40] In the United States the third most common cause of NTM pulmonary disease is *M. abscessus* complex, which produces 80% of pulmonary infections caused by RGM.[26] (This study antedated recognition of the subspecies *bolletii* and *massiliense.*) Intriguingly, the proportion of *M. abscessus* subsp. *massiliense* varies geographically.[7,41–43] Reports from Korea and Japan have indicated, inexplicably, that the ratio of *M. abscessus* to all NTM is much higher in South Korea than in other Asian countries, including Japan.[42,43] Reports from the National Institutes of Health in the United States show *M. abscessus* subsp. *massiliense* in 28% of 40 patients with lung disease due to NTM, 21% of 39 isolates in the Netherlands, 22% of 50 patients with cystic fibrosis in France, and 55% of 150 patients and 26% of 102 patients in South Korea and Japan, respectively.[42] Bronchiectasis was found to be significantly more frequent in *M. abscessus* subsp. *abscessus* than in *M. abscessus* subsp. *massiliense.*[42] Recently, *M. abscessus* subsp. *massiliense* has been increasingly recognized in respiratory samples of patients, including patients with cystic fibrosis.[26,44–46] Studies in Korea and Japan and, more recently, the United States (for in vitro MIC studies) have emphasized major differences in macrolide susceptibility patterns and clinical response rates between *M. abscessus* subsp. *abscessus,* of which approximately 80% are resistant to macrolides, and *M. massiliense,* which are usually macrolide susceptible; thus patients with *M. abscessus* subsp. *massiliense* respond favorably to clinical treatment with macrolides, unlike *M. abscessus* subsp. *abscessus.*[7,42,43,47]

Nontuberculous Mycobacteria Species Associated Infrequently With Pulmonary Disease

Among the newly described RGM, pulmonary infection has been reported with *M. iranicum*[4] (from Italy, Iran, and Turkey), the newly validated species *M. franklinii*, and *M. celeriflavum.*[4,15] Less commonly, *M. fortuitum, M. goodii, M. abscessus,* and *M. smegmatis* have been associated with lipoid pneumonia[7,8,19] and achalasia.[7,8,19,26] Patients with achalasia exhibit a bilateral subacute to acute alveolar disease with high fevers, striking leucocytosis count higher than 20,000/μL, cough, and mucus production; acute illness is common. The histopathology shows a combination of lipoid disease and acute/granulomatous infection.[26] Other NTM that are infrequently associated with pulmonary disease include *M. szulgai,*[26,31] *M. simiae,*[26,31] *M. celatum,*[26,31] *M. lentiflavum,*[26,31] *M. asiaticum,*[26,31] *M. heckeshornense,*[26,31] *M. florentinum,*[6] *M. arupense,*[6,26] *M. kumamotense,*[4,25] *M. nebraskense,*[4,26] and rarely, *M. gordonae,*[26,31] *M. saskatchewanense,*[6,31] *M. senuense,*[31] and *M. seoulense.*[4] Recently described species *M. kyorinense,*[4] *M. noviomagense,*[4] *M. paraseoulense,*[4] *M. europaeum,*[4] (recently validated, but not recently described), *M. shinjukuense,*[4] *M. koreense,*[48] *M. sherrisii,*[2,4] and the aforementioned new species in the *M. terrae* complex[4,24] have also been associated with pulmonary disease. Some isolates originally described as *M. nonchromogenicum* and thought to be pathogenic have recently been identified as *M. heraklionense.*[4] Rarely, isolates of *M. persicum,*[30] *M. parakoreense,*[4] *M. paraense,*[49] and *M. talmoniae*[50] have also been recovered from pulmonary samples.

Pulmonary Syndromes Associated With Nontuberculous Mycobacteria Other Than *Mycobacterium avium* Complex

Clinical disease with *M. kansasii* produces upper lobe fibrocavitary disease and nodular disease similar to MAC in the same setting. The *M. abscessus* complex also produces nodular disease in the setting of bronchiectasis. Pulmonary NTM disease is rare in children, except for those with cystic fibrosis.[26,44] *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* have been increasingly recovered from respiratory samples collected from patients with cystic fibrosis.[41] The

TABLE 252.1 Major Clinical Syndromes Associated With Nontuberculous Mycobacterial Infection Other Than *Mycobacterium avium* Complex

SYNDROME	MOST COMMON CAUSES (OTHER THAN MAC)	LESS FREQUENT CAUSES
Chronic nodular lung disease (adults with bronchiectasis; cystic fibrosis)	*M. xenopi, M. malmoense, M. kansasii, M. abscessus* subsp. *abscessus, M. abscessus* subsp. *massiliense, M. abscessus* subsp. *bolletii*	*M. szulgai, M. smegmatis, M. celatum, M. simiae, M. goodii, M. asiaticum, M. heckeshornense, M. branderi, M. lentiflavum, M. triplex, M. fortuitum, M. abscessus* subsp. *bolletii, M. florentinum, M. nebraskense, M. saskatchewanense, M. seoulense, M. senuense, M. paraseoulense, M. europaeum, M. algericum* (goats), *M. sherrisii, M. kyorinense, M. noviomagense, M. celeriflavum, M. franklinii, M. fragae, M. insubricum, M. iranicum, M. llatzerense, M. shinjukuense, M. koreense, M. heraklionense, M. parascrofulaceum, M. parakoreense, M. paraense, M. persicum, M. talmoniae*
Cavitary lung disease	*M. abscessus* subsp. *abscessus*	*M. europaeum, M. riyadhense, M. xenopi*
Cervical or other lymphadenitis (especially children)	*M. malmoense* (northern Europe), *M. lentiflavum,*	*M. scrofulaceum* (rarely), *M. abscessus, M. fortuitum, M. tusciae, M. palustre, M. interjectum, M. elephantis, M. heidelbergense, M. parmense, M. bohemicum, M. haemophilum, M. europaeum, M. florentinum, M. triplex, M. asiaticum, M. kansasii, M. heckeshornense, M. bourgelatii* (cattle)
Skin and soft tissue disease	*M. fortuitum* group, *M. chelonae, M. abscessus, M. marinum, M. ulcerans* (Australia, tropical countries only)	*M. kansasii, M. haemophilum, M. porcinum, M. smegmatis, M. genavense, M. lacus, M. novocastrense, M. houstonense, M. goodii, M. immunogenum, M. mageritense, M. abscessus* subsp. *massiliense, M. monacense, M. bohemicum, M. branderi, M. shigaense, M. szulgai, M. asiaticum, M. xenopi, M. kumamotense, M. setense, M. montefiorense* (eels), *M. pseudoshottsii* (fish), *M. salmoniphilum* (salmonids), *M. shottsii* (fish), *M. hippocampi* (sea horses), *M. iranicum, M. llatzerense*
Skeletal (bone, joint, tendon) infection	*M. marinum, M. fortuitum* group, *M. abscessus, M. chelonae*	*M. haemophilum, M. heckeshornense, M. smegmatis, M. wolinskyi, M. goodii, M. lactus, M. triplex, M. xenopi M. terrae* complex *(M. arupense, M. heraklionense, M. kumamotense, M. longobardum, M. virginiense)*
Disseminated infection		
HIV-seropositive host	*M. kansasii,*	*M. marinum, M. simiae, M. fortuitum, M. conspicuum, M. celatum, M. lentiflavum, M. triplex, M. sherrisii, M. heckeshornense, M. genavense, M. haemophilum, M. xenopi*
HIV-seronegative host	*M. abscessus, M. chelonae*	*M. marinum, M. kansasii, M. haemophilum, M. xenopi M. conspicuum, M. shottsii* (fish), *M. pseudoshottsii* (fish)
Catheter-related infections	*M. fortuitum, M. abscessus, M. chelonae*	*M. mucogenicum, M. phocaicum, M. immunogenum, M. mageritense, M. septicum, M. porcinum, M. bacteremicum, M. brumae, M. neoaurum*
Hypersensitivity pneumonitis	Metal workers Hot tub	*M. immunogenum*

HIV, Human immunodeficiency virus; *MAC, Mycobacterium avium* complex.
Too little information is available for selected pathogens such as *M. xenopi, M. malmoense, M. szulgai, M. celatum,* and *M. asiaticum* and the newly described species. The role of *M. scrofulaceum* is unclear. With the advent of newer molecular identification methods, the numbers of this species have decreased.

majority of patients with the *M. abscessus* complex are younger and have more severe disease.[44] Patients with cystic fibrosis also have bronchiectasis in addition to chronic recurrent airway and parenchymal infections that may predispose them to NTM infections.[44]

Clinical Presentation of Nontuberculous Mycobacterial Lung Disease

Because the signs and symptoms of NTM lung disease are often variable and nonspecific, disease with NTM is difficult to diagnose without positive respiratory cultures (Table 252.2).[26] Patients often present with chronic cough, a "throat clearing" with or without sputum production, and fatigue. Less frequently, complaints of malaise, dyspnea, fever, hemoptysis, and weight loss may also be present. Clinical studies should include microbiologic cultures for acid-fast bacilli (AFB) and routine chest radiographs. High-resolution chest computed tomography (CT) is essential in patients suspected of having nodular bronchiectasis. Recovery of NTM from a single sputum sample is not proof of NTM disease, especially when the AFB smear is negative and NTM are present in low numbers.[26,51] The American Thoracic Society statement on the diagnosis and treatment of NTM[26] has revised the diagnostic criteria to determine lung disease caused by NTM (Table 252.3).[26] For NTM disease due to organisms other than MAC, these criteria may need to be adjusted because inadequate data are available to evaluate these criteria. Expert consultation should be obtained when NTM that are infrequently encountered are recovered.[26]

Treatment of *Mycobacterium avium* Complex Lung Disease

This issue is discussed separately in Chapter 251.

Treatment of *Mycobacterium kansasii* Lung Disease

Treatment of lung disease caused by *M. kansasii* has traditionally been less difficult than that for MAC since the introduction of rifampin.[26] A regimen of daily rifampin, 600 mg, isoniazid (INH), 300 mg, and ethambutol, 15 mg/kg, has been widely accepted in the United States and is still recommended by the American Thoracic Society (Table 252.4).[26,45] In HIV-positive patients receiving a protease inhibitor or nonnucleoside reverse-transcriptase inhibitor, rifampin should be replaced by rifabutin, although the dosage depends on the antiretrovirals. The rifabutin dose should be decreased to 150 mg daily in patients receiving amprenavir, atazanavir, nelfinavir, and ritonavir. Rifabutin should be increased to 450 mg daily or 600 mg thrice weekly in patients receiving efavirenz. Maraviroc dose should be doubled if it must be used with rifabutin. Patients taking elvitegravir-cobicistat-tenofovir-emtricitabine should not be given rifabutin. No rifabutin dose adjustment is required for patients taking raltegravir, nevirapine, or etravirine.

TABLE 252.2 Clinical Settings for Nontuberculous Mycobacterial Lung Disease (Other Than *Mycobacterium avium* Complex)

RADIOGRAPHIC DISEASE	SETTING	USUAL PATHOGEN[a] (RARE PATHOGEN)
Upper lobe cavitary	Male smokers, often abusing alcohol, usually early 50s	*M. kansasii*
Right middle lobe, lingular nodular bronchiectasis	Female nonsmokers, usually older than 60 yr	*M. abscessus, M. abscessus* subsp. *massiliense (M. kansasii)*
Localized alveolar, cavitary disease	Prior granulomatous disease (usually tuberculosis) with bronchiectasis	*M. abscessus*
Reticulonodular or alveolar bilateral lower lobe disease	Achalasia, chronic vomiting secondary to gastrointestinal disease, exogenous lipoid pneumonia (mineral oil aspirations, etc.)	*M. fortuitum (M. abscessus, M. smegmatis, M. goodii)*
Reticulonodular disease	Adolescents with cystic fibrosis, HIV-positive hosts, may be prior bronchiectasis secondary to *Pneumocystis* pneumonia or other cause	*M. abscessus* subsp. *abscessus,*[b] *M. abscessus* subsp. *massiliense*
Hypersensitivity pneumonitis	Metal workers Indoor hot tub	*M. immunogenum*

[a]Too little information is available for selected pathogens such as *M. xenopi, M. malmoense, M. szulgai, M. celatum,* and *M. asiaticum* and the newly described species.
[b]If a functional *erm* gene is present, treatment with clarithromycin may not be warranted.
HIV, Human immunodeficiency virus.

TABLE 252.3 American Thoracic Society Diagnostic Criteria for NTM Lung Disease

The minimum evaluation of a patient for NTM lung disease should include:
1. Chest radiograph or, when no cavitation is present, HRCT
2. At least three sputum or respiratory samples for AFB culture
3. Exclusion of other disease such as tuberculosis

Clinical diagnosis of NTM is based on pulmonary symptoms, presence of nodules or cavities as seen on chest radiograph or an HRCT scan with multifocal bronchiectasis with multiple small nodules, and exclusion of other diagnoses.

Microbiologic diagnosis of NTM:

At least two expectorated sputa (or at least one bronchial wash or lavage) with positive cultures for NTM or transbronchial or other lung biopsy showing the presence of granulomatous inflammation or AFB with one or more sputum or bronchial washings that are culture positive for NTM.

AFB, Acid-fast bacilli; *HRCT,* high-resolution computed tomography; *NTM,* nontuberculous mycobacteria.
Data from Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment and prevention of nontuberculous mycobacterial diseases. American Thoracic Society Statement. Am J Respir Crit Care Med. *2007;175:367–416.*

Patients should be treated with at least 12 months of culture negativity.[26] For patients resistant or intolerant to rifampin, clarithromycin is a reasonable alternative agent.[26,45,52]

The usefulness of INH in this regimen is controversial, with a macrolide offering a much better alternative. In the United Kingdom INH is omitted from the regimen. A recent study in Japan showed little difference in the addition of INH in a small number ($n = 14$) of patients.[53] An intermittent regimen (three times weekly) using clarithromycin in place of INH (the same drugs and drug concentration as for MAC) suggests that intermittent therapy for *M. kansasii* can be highly effective, although it has been reported only in a limited number of patients.[52] This regimen is currently preferred by some experts.[26,52]

Untreated strains of *M. kansasii* are susceptible to low concentrations of rifampin, rifabutin, ethambutol (although in vitro susceptibility results [MICs] may appear resistant due to technical difficulties with testing ethambutol), linezolid, streptomycin, sulfonamides, clarithromycin, and the quinolones. Information on the clinical utility of drugs other than rifampin or ethambutol is limited.[26,52] Acquired mutational resistance of *M. kansasii* to rifampin can occur, but this organism is readily treated with multidrug regimens.[26,52]

Treatment of *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *massiliense* Lung Disease

Treatment of *M. abscessus* (with a functional *erm* gene, which inactivates the macrolides) lung disease with antimicrobials alone has generally been unsuccessful.[26,45] Courses of therapy in adults with clarithromycin, high-dose IV cefoxitin (8–12 g/day in two to three divided doses) or imipenem (1 g twice daily), and low-dose parenteral amikacin (peaks in the 20- to 25-μg/mL range on once-daily dosing) produce clinical improvement with limited toxicity but usually do not result in microbiologic cure.[7,26] Studies using an IV glycylcycline antibiotic, tigecycline, have shown good in vitro activity (minimal inhibitory concentrations [MICs], ≤1 μg/mL) against most species of clinically important RGM, including the *M. abscessus* complex,[54,55] and this drug is now often included in multidrug therapy, especially in patients who have developed amikacin auditory toxicity. A recent clinical trial reported successful treatment of three patients with *M. abscessus* lung disease using amikacin liposome inhalation suspension (ALIS).[56] However, no data are available to compare ALIS with nonliposomal administration, and further large studies are needed to assess the effectiveness of this new agent.[57] Inhaled commercial (IV) amikacin has also been used but with limited reported results.[56] There are preliminary data that bedaquiline may be useful in *M. abscessus* infection, but large clinical trials and in vitro MIC studies are needed.[58]

A concern for the usefulness of the macrolides for *M. smegmatis, M. fortuitum*, and the *M. abscessus* subsp. *abscessus* is that the majority of these species, including approximately 80% of the isolates of *M. abscessus* subsp. *abscessus*, contain functional inducible *erm* genes (*erm38, erm39,* and *erm41*, respectively), which induce macrolide resistance and are likely responsible for treatment failure in macrolide-containing regimens against the *M. abscessus* complex other than *M. abscessus* subsp. *massiliense*.[47] In addition to inducible macrolide resistance, acquired macrolide resistance due to mutations in the 23S ribosomal RNA (rRNA) gene can develop during antibiotic treatment.[59]

Isolates of *M. abscessus* subsp. *massiliense* and approximately 15% of subsp. *abscessus* isolates do not contain functional *erm* genes and hence are intrinsically macrolide susceptible. These isolates usually show good clinical response to clarithromycin-containing regimens, with response rates up to 85%.[47,60–66]

Treatment of Other Nontuberculous Mycobacterial Lung Disease

A recommended or standardized treatment for lung disease caused by other slowly growing mycobacteria, such as *M. simiae, M. szulgai, M. xenopi, M. malmoense,* and the newly described species, has not been established.[26,45] Drug combinations similar to those used with MAC, such as clarithromycin, ethambutol, rifabutin, and perhaps an aminoglycoside with 12 months of negative cultures, seem reasonable.[26,32,34,45]

Pseudooutbreaks of pulmonary disease have been described, usually related to contamination of bronchoscopes or the automated endoscope washing machine.[26,39] *M. immunogenum* is the most common species recovered in this setting, followed by *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*.[7,39] *M. immunogenum* has also been associated with hypersensitivity pneumonitis associated with occupational exposure to metalworking fluids.[67]

LYMPHADENITIS

Localized cervical lymphadenitis is the most common NTM disease in children, with a peak incidence between 1 and 5 years of age.[26] NTM-affected lymph nodes are usually in the anterior cervical chain

TABLE 252.4 Frequently Used Treatment Regimens for Common Nontuberculous Mycobacterial Pathogens Other Than *Mycobacterium avium* Complex

SPECIES	DISEASE[a]	DRUG	DAILY ADULT DOSES[b]	THREE TIMES WEEKLY ADULT DOSE	DURATION
M. kansasii	Pulmonary				
	(United States)	Isoniazid *or*	300 mg	—	Culture negative at least 12 mo
		Clarithromycin *plus*	500 mg bid	500 mg bid	
		Rifampin *plus*	600 mg	600 mg	
		Ethambutol	15 mg/kg	25 mg/kg	
	(United Kingdom)	Rifampin *plus*	600 mg	600 mg	9–12 mo
		Ethambutol	15 mg/kg	15 mg/kg	
	Disseminated	Same as pulmonary			
	HIV-positive	Same as pulmonary (United States) but replace rifampin (rifampin inactivates HIV drugs) with rifabutin *or*	150 mg		Same as pulmonary (United States)
		Clarithromycin[c]	500 mg bid		
M. abscessus subsp. *abscessus* (80% of isolates harbor functional *erm* gene)	Pulmonary (adults)	Amikacin IV (peaks in low 20s μg/mL)	7.5–10 mg/kg single dose daily[d]	NA	2 wk (designed to improve, not cure)
			500 mg daily inhaled amikacin or ALIS		ALIS: not determined
M. abscessus subsp. *massiliense* (no functional *erm* gene)		*Plus* imipenem IV *or*	1 g bid		
		Cefoxitin IV *or*	8–12 g/day (divided into 2–3 doses)		2 wk
		Tigecycline 25–50 mg IV once daily			
		Plus clarithromycin[e]	500 mg bid		6 mo
	Cutaneous localized	Clarithromycin	500 mg bid[f]		6 mo
	Disseminated or extensive cutaneous	Same three drugs as above[g]		NA	Not established
M. marinum	Cutaneous	Clarithromycin *or*	500 mg bid	NA	3 mo minimum for all regimens
		Minocycline *or*	100 mg bid		
		Rifampin *plus*	600 mg		
		Ethambutol	15 mg/kg		

[a]HIV-negative host unless otherwise stated.
[b]Drugs by mouth unless otherwise stated.
[c]Patients on HIV medicines inactivated by rifampin.
[d]Based on age, weight, and renal status (American Thoracic Society).
[e]If a functional *erm* gene is present, treatment with clarithromycin may not be warranted.
[f]Patients with complicated lesions may require surgical débridement and amikacin plus cefoxitin or imipenem.
[g]Amikacin plus clarithromycin plus imipenem or cefoxitin or tigecycline.
ALIS, Amikacin liposome inhalation suspension; *bid,* twice daily; *HIV,* human immunodeficiency virus; *IV,* intravenous; *NA,* not applicable.

and are unilateral and painless. The nodes may enlarge rapidly with the formation of fistulas to the skin, and prolonged drainage may occur. On occasion, other nodes outside the head and neck, such as the mediastinal lymph nodes, may be involved.[5,26] A definitive diagnosis of NTM lymphadenitis is made by recovery of the etiologic organism from lymph node cultures. The tuberculin skin test is often weakly positive (5–10 mm), but it may be more than 10 mm.[26] Routine biopsy or incision and drainage should be avoided because these procedures often result in the formation of fistulas and chronic drainage. Fine-needle aspiration with cytology and culture has been used increasingly with apparently few associated problems.

The Italian Society of Preventive and Social Pediatrics has recently proposed a practice algorithm using a multidisciplinary approach to define categories of patients, including those to "watch/wait," those requiring empirical antibiotic therapy, and those needing prompt diagnostic workup, considering the risk for severe underlying disease for the management of pediatric cervical lymphadenopathy.[68]

Treatment of NTM cervical lymphadenitis is still evolving. The potential role of macrolide treatment regimens without surgery or as a supplement to surgery in complicated or recurrent disease is being considered with increasing frequency. Clarithromycin combined with ethambutol or rifabutin is the usual suggested regimen (see Table 252.4). However, the established treatment of routine NTM cervical lymphadenitis (from the premacrolide era) remains surgical excision without antimicrobial treatment.[26]

Since the early 1980s, 80% of cases of culture-positive NTM lymphadenitis in children in the United States have been caused by MAC.[5,26] The remainder of the cases in Australia and the United States were caused by *M. scrofulaceum,* and only about 10% of the cases have been caused by *M. tuberculosis*.[5,26] The incidence of *M. scrofulaceum* has declined since the 1980s and is now a rare cause of the lymphadenitis. Although not yet described in lymph node culture, some isolates that were previously identified as *M. scrofulaceum* by phenotypic methods have been found to be a genetic match for *M. parascrofulaceum,* suggesting that the prevalence of this new species may have previously been underestimated. In parts of northern Europe, including Scandinavia and the United Kingdom, *M. malmoense* has become the second most common pathogen after MAC.[5,26,32,69] *M. lentiflavum* appears to be an increasing cause of cervical lymphadenitis in selected geographic areas.[5,70] The same is true for *M. haemophilum* because a recent report from Israel showed that the rate of isolation of *M. haemophilum* in cervical lymphadenitis since 1996 has been 51%.[32] Rarely, other species are recovered, including RGM,[5,8,26] *M. heckeshornense, M. asiaticum, M. florentinum, M. kansasii, M. interjectum, M. parmense, M. palustre, M. tusciae, M. heidelbergense, M. elephantis, M. triplex, M. bohemicum,* and *M. europaeum*.[2,5,6] For cases that fail to grow NTM, DNA sequencing has emerged as a means to provide an etiology in these cases.[71]

A report from Thailand of 128 HIV-negative adult patients described disseminated NTM infection with bilateral lymphadenitis in 89% of the patients. The majority of infections were caused by RGM, including *M. abscessus, M. fortuitum, M. chelonae,* and *M. thermoresistibile.* Unlike the more common form of NTM lymphadenitis in children, all but 1 of 129 patients were adults whose infection spread to other organ involvement.[72] Subsequent studies revealed these patients to have acquired autoantibodies to interferon-γ. In addition, a rare case of systemic *M. abscessus* complex (no subspecies identification was provided) lymphadenitis in a Japanese patient with leukemia was recently published.[73]

LOCALIZED CUTANEOUS, JOINT, AND SOFT TISSUE INFECTIONS

Although most pathogenic species of NTM have been incriminated in cutaneous NTM disease, the most common etiologic agents are *M. marinum, M. ulcerans,* and the RGM.[7,8,26,45,74]

Intermediately Growing Mycobacteria

Mycobacterium marinum

M. marinum causes an infection historically recognized as "swimming pool" or "fish tank" granuloma.[26,45,74] This common name is derived from the epidemiologic niche of the organism. Most infections occur 2 to 3 weeks after contact with contaminated water from one of these sources. The lesions are most often small violet papules on the hands and arms that may progress to shallow, crusty ulcerations and scar formation. Lesions are usually singular. However, multiple ascending lesions resembling sporotrichosis ("sporotrichoid disease") can occasionally occur.[26,74] Most patients are clinically healthy, with a previous local hand injury that becomes infected while cleaning a fish tank, or patients may sustain scratches or puncture wounds from saltwater fish, shrimp, fins, and other marine life contaminated with *M. marinum*. Swimming pools seem to be a risk only when nonchlorinated. Diagnosis is made from culture and histologic examination of biopsy material, along with a compatible history of exposure. No treatment of choice is recognized for *M. marinum* (see Table 252.3). However, successful treatments have traditionally been a two-drug combination of rifampin plus ethambutol or monotherapy with doxycycline, minocycline, clarithromycin, or trimethoprim-sulfamethoxazole (TMP-SMX) given for a minimum of 3 months. Clarithromycin has been used increasingly because of good clinical efficacy and minimal side effects, although published experience is limited.[26,45,74]

Community-Acquired Extrapulmonary Infections Due to Rapidly Growing Mycobacteria

The rapidly growing species *M. abscessus, M. fortuitum*, and *M. chelonae* are the most common NTM involved in cases of community-acquired infections of skin and soft tissue in the United States.[7,8,26,45] The *M. fortuitum* group is responsible for 60% of localized cutaneous infections in previously healthy individuals. Unlike infections with the *M. chelonae–M. abscessus* complex, the patient with *M. fortuitum* localized infection usually has no predisposing immune suppression.[5,7,45] In a series of 42 patients for whom clinical history was available, the majority of infections involved some type of traumatic injury, such as metal puncture wounds from stepping on a nail (48%), motor vehicle accidents (26%), and injuries involving the foot or leg (≈40%).[5,7,45] Open lacerations or fractures were common. *M. setense,* a newer member of the *M. fortuitum* group, has been described in a case of osteomyelitis.[10]

In contrast, localized infections with *M. chelonae* are seen primarily in patients who are immunosuppressed, especially on long-term corticosteroids. Autoimmune diseases, such as rheumatoid arthritis and systemic lupus, are often predisposing factors.[7] In a study by Wallace and colleagues,[8,26,45] 35% of the *M. chelonae* with nonpulmonary infections were seen in localized wound infections.

Disease due to the *M. abscessus* complex occurs in normal hosts and those with immune suppression. Examples of localized wound infection with *M. abscessus* include soft tissue infection of the cheek after an insect bite and vertebral osteomyelitis.[7,26]

Since 2002 several outbreaks of lower-extremity folliculitis due to RGM (*M. fortuitum, M. abscessus,* and *M. mageritense* disease), associated with nail salons ("foot-spa disease"), have been reported.[5,7,39] Leg hair removal by wax stripping, followed by NTM-contaminated foot baths has resulted in indolent folliculitis.

Recent outbreaks of RGM infections, especially with *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* after cosmetic surgeries and mesotherapy, have also been described.[7,39,75]

Slowly Growing Mycobacteria

On occasion, localized community-acquired infections of the skin, soft tissue, synovium, or bone may involve slowly growing species, such as MAC, *M. szulgai, M. xenopi, M. asiaticum,* and *M. kansasii*. Rarely, species such as *M. novocastrense, M. lacus, M. branderi, M. bohemicum,* and *M. monacense* are involved.[4,26] Among the newer described species, *M. shigaense* has been recovered from skin biopsies of both immunocompromised and immunocompetent patients.[4] Hand infections involving tendons and/or joints (tenosynovitis) has long been associated with *M. terrae* complex. Recent sequencing studies have shown the major species in this group causing tenosynovitis are *M. arupense, M. kumamotonense, M. heraklionense,* and the newly described species *M. virginiense,* but not *M. nonchromogenicum,* as was previously thought.[25]

Health Care–Associated Infections

Sporadic cases of health care–associated skin and soft tissue disease have also been described. These cases include infections of long-term intravenous (IV) or peritoneal catheters; postinjection abscesses; surgical wound infections, such as after cardiac bypass surgery; and augmentation mammoplasty and cosmetic procedures, including tattooing.[26,39,45] Infections after insertion of prosthetic devices, including prosthetic heart valves and joints, have also been reported.[76]

A cluster of 12 cases involving *M. fortuitum* and *M. porcinum* in postaugmentation mammoplasty surgical site infections was recently described in Brazil.[5,7,39] Clustered outbreaks or pseudooutbreaks of mycobacterial skin, soft tissue, or bone infections have been described and usually result from contaminated fluids, such as ice made from tap water, irrigation, exposure to tap water, injectable medicines, and topical skin solutions/markers.[5,7,26,39]

The contamination of benzalkonium chloride (a quaternary ammonium commonly used as an antiseptic) with *M. abscessus* was responsible for a serious outbreak of *M. abscessus* after steroid injections and serves to emphasize the limitations of disinfectants against mycobacteria, especially quaternary ammonium compounds ("quats").[7,39] There have been reports of eye disease due to RGM, including postkeratoplasty and after laser-assisted in situ keratomileusis (LASIK) surgery for correction of myopia.[8,77] A cluster of *M. chelonae* keratitis was associated with hyperopic LASIK using a contact lens mask. Thirty-one of 43 additional cases of keratitis between 2000 and 2001 were part of this outbreak, whereas the 12 other reported cases were sporadic.[5,77]

Other outbreaks involving NTM have involved contamination of liposuction equipment with *M. chelonae,* with the same disease strain found in tap water used for rinsing suction tubing.[39] Most of the skin and soft tissue disease outbreaks have involved the rapidly growing species *M. fortuitum* and *M. abscessus*.[7,8,39,75] However, an outbreak of disease in four patients with alcohol-resistant mycobacterial species (two with *M. chelonae* and two with *M. nonchromogenicum*) was reported in Hong Kong after acupuncture treatments from 1999 to 2000.[39] In addition, between 2003 and 2004, an outbreak of *M. abscessus* occurred in patients from the United States who visited the Dominican Republic for cosmetic surgery for fat removal (known as "lipotourism").[7,39] In 2013 21 patients were found to have NTM wound infections after cosmetic surgery in the Dominican Republic.[7,78] Thirteen were at a single clinic, and all except one of the cultures were *M. abscessus* complex. The average time postoperatively for swelling and pain to develop was 3 weeks. Although no water samples or environmental samples were available for testing in either outbreak, the reservoir for these types of outbreaks has historically been municipal or hospital water supplies.[39]

Outbreaks of species including *M. cosmeticum* and *M. abscessus* subsp. *massiliense* have also been described recently from postinjection abscess after influenza or cold antibiotic injections, cosmetic procedures, and laparoscopic surgeries in Korea, Brazil, Venezuela, and Ohio.[18,39,75,79,80] Of major concern, a single strain of *M. abscessus* subsp. *massiliense* was incriminated in an epidemic of surgical site infections in Brazil since 2004.[81] This strain has been the subject of several reports. Additional outbreaks have involved *M. haemophilum* and *M. chelonae* in patients who received tattoos.[7,39,82]

Recently, a large outbreak of hospital-associated *M. abscessus* was reported in the United States. Approximately one-half of the cases were lung transplant patients with respiratory cultures positive for *M. abscessus*. Elimination of tap water exposure among high-risk patients decreased the incidence rate to baseline numbers. The investigation also noted that one-half of the cases occurred in cardiac surgery patients with invasive infections. Intense disinfection protocols and use of sterile

water for heater-cooler units of cardiac bypass machines, along with specific engineering strategies, mitigated the outbreak.[83]

SKIN/SOFT TISSUE

Diagnosis and Treatment

Diagnosis of all types of mycobacterial skin and soft tissue infections is made by culture of specific NTM from drainage material or tissue biopsy. Treatment may include amikacin, cefoxitin, ciprofloxacin, moxifloxacin, clarithromycin, doxycycline, linezolid, sulfonamides, and imipenem for the *M. fortuitum* group. Only amikacin, cefoxitin, tigecycline, imipenem, and sometimes linezolid and clarithromycin have activity against *M. abscessus*. Only amikacin, imipenem, tigecycline, tobramycin, clarithromycin, and sometimes linezolid, moxifloxacin, and doxycycline have activity against *M. chelonae*.[7,8,26,45] Approximately 25% of strains of *M. chelonae* also show susceptibility to doxycycline and quinolones, including moxifloxacin.[7] Clarithromycin is generally the drug of choice for localized disease (but not for disseminated disease) caused by *M. chelonae* and macrolide-susceptible isolates of *M. abscessus*.[7,26,45] However, the efficacy of macrolide treatment for the majority of *M. abscessus* subsp. *abscessus* (and the *M. fortuitum* group) is likely diminished by recent recognition that they harbor functional *erm* genes that confer inducible resistance to macrolides.[45,47,60–63] The duration of therapy is usually 4 to 6 months.

Antituberculous agents, other than ethambutol for *M. smegmatis,* have no efficacy against any of the RGM and should not be used.[7,26,45,84] Monotherapy with quinolones is not recommended because of the high risk of mutational resistance of RGM to these agents. Treatment of slowly growing species is similar to that for chronic lung disease, except that the duration of therapy may only be 6 to 12 months, depending on the severity of the disease.[26,45]

Two unusual species causing skin and soft tissue infections in select situations are *M. ulcerans* and *M. haemophilum*. *M. ulcerans* is not endemic in the United States, but it is endemic in northern areas of Australia and tropical locations of the world, where it is commonly known as the "Buruli ulcer."[26,32,85] *M. ulcerans* is extremely slow growing, with the average incubation time around 8 to 12 weeks, and *M. haemophilum* may take up to 3 to 4 weeks to grow on primary culture.[26,32,45,84] Thus newer molecular techniques for identification of these organisms have expedited diagnosis of infection with the organisms.[26,45] The *M. ulcerans* infection progresses from an itchy nodule, most often on the extremities, to a necrotic lesion that may result in severe limb deformity. Treatment success is common in early disease with excisional surgery. Regimens have included streptomycin and dapsone with or without ethambutol and combinations of rifampin, sulfonamides, ethambutol, and clarithromycin. However, for advanced ulcerative disease, therapeutic response has generally been poor.[26,45] Surgical débridement and skin grafting then become the usual therapeutic measures of choice.[26,45] *M. haemophilum* causes cutaneous infections (primarily of the extremities) in immunosuppressed patients, especially in the setting of organ transplantation, long-term high-dose steroid use, or HIV.[26,31,32,45] The majority of cases involve skin and soft tissue infections.[32] *M. haemophilum* has a special growth requirement for hemin or iron and may present some diagnostic difficulties if iron- or hemin-supplemented media and lower temperatures (incubation at 28°–30°C) are not used.[26,32,45] A surprising number of specimens are AFB smear positive and culture negative, so a presumptive diagnosis is often based on typical caseating granulomas and a negative culture for *M. tuberculosis* in the common clinical setting. Therapy for this species usually includes combinations of clarithromycin and rifampin or rifabutin for 12 to 24 months. The addition of granulocyte-macrophage colony-stimulating factor with reduction of immunosuppression therapy has been associated with a successful outcome in a cardiac transplant patient.[5,26,32,45] Of note, *M. montefiorense* and *M. pseudoshottsii,* as well as *M. stomatepiae,* have been recovered from granulomatous lesions in eels and fish, respectively.[2]

INFECTION OF TENDON SHEATHS, BONES, BURSAE, AND JOINTS

Both rapidly growing and slowly growing species of NTM have been implicated in chronic granulomatous infections involving tendon sheaths, bursae, bones, and joints after direct inoculation of the pathogen through accidental trauma, surgical incisions, puncture wounds, or injections.[8,26,45,86] Most patients have no underlying immune suppression, but high risk for some pathogens, such as *M. chelonae, M. haemophilum,* and *M. xenopi,* is seen in patients who are immunosuppressed.[26,32,39,45] *M. marinum* and *M. heckeshornense* have been described as causing tenosynovitis of the hand,[26,45] although the RGM, *M. kansasii,* and *M. terrae* complex (especially *M. arupense*, *M. heraklionense*, and *M. virginiense*) have also been associated with a chronic type of disease.[5,24–26,45,87,88] Osteomyelitis of the sternum caused by *M. fortuitum* and *M. abscessus* has also been found in clustered outbreaks and sporadic cases after cardiac surgery.[7,26,39,83] Other species—*M. goodii, M. wolinskyi, M. porcinum,* and *M. setense*—have been isolated from posttraumatic osteitis and osteomyelitis. In addition, *M. haemophilum* has a tendency to involve bones and joints, usually with concurrent draining skin lesions and bacteremia.[31,32,45] A newly described species, *M. decipiens,*[86,89] has also been associated with tenosynovitis. *M. lacus* has been recovered from infected synovial fluid.[2] Recently, an outbreak of 58 cases of bone and joint infections in immunocompetent patients in a French hospital involved *M. xenopi*.[39]

Management of mycobacterial rheumatologic infections often requires surgical débridement for both diagnosis and therapy, especially for the closed spaces of the hand and the wrist and for patients with infected bones, such as fractured long bones or the sternum after cardiac surgery. Drug therapy for the specific pathogen is also essential.[5,26]

DISSEMINATED DISEASE

In the setting of advanced HIV infection, most disseminated NTM disease is due to *M. avium.* However, other NTM, including *M. kansasii, M. genavense, M. haemophilum, M. simiae, M. celatum, M. malmoense, M. marinum,* and RGM, have also been recovered.[8,26,32] Disseminated disease among *M. triplex, M. lentiflavum, M. heckeshornense*, *M. conspicuum,* and *M. sherrisii* has also been reported.[22,26,31,32]

After MAC, disseminated *M. kansasii* is the second most frequent cause of disseminated NTM disease in the setting of acquired immunodeficiency syndrome.[26,90] Pulmonary and cutaneous manifestations have occurred[26,90] in patients with chronic lymphocytic leukemia, after organ transplantation, and in those infected by HIV. One study reported five patients with disseminated *M. kansasii* infection, including three patients with pulmonary and extrapulmonary involvement and two patients with exclusive extrapulmonary involvement. All patients had $CD4^+$ lymphocyte counts less than 200 cells/μL. The most common clinical manifestation was pulmonary disease with thin-walled cavitary lesions.[5,26] Before the advent of antiretroviral therapy, *M. genavense* was the second most frequently isolated species after *M. avium* in some geographic areas.

Disseminated disease in non-HIV patients has been reported and is most frequently caused by *M. chelonae.*[7] Disseminated disease involving *M. chelonae* is primarily cutaneous and typically presents as a chronic syndrome with multiple painful, draining, red nodules, usually involving the lower extremities.[5,7,26] Almost all patients are immunosuppressed, usually from corticosteroid therapy for disease such as rheumatoid arthritis. Other types of immune suppression occur in patients with autoimmune disease, leukemia, or transplanted organs. Although the disease is presumably a consequence of hematogenous spread, a portal of entry is rarely evident, and septicemia is rare.[7,26,45]

Although the majority of disseminated cutaneous disease is due to *M. chelonae, M. abscessus* complex has been reported in approximately 20% of the cases. Disease involving sites other than the skin is rare, except in severely immunosuppressed patients. Disseminated disease with *M. abscessus* is a serious disease and can be difficult to treat.[5,26,45,73] Other NTM, such as *M. haemophilum,* produce similar clinical syndromes in similar settings with infection, most often in the lower extremities in immunosuppressed patients.[26,32]

CATHETER-RELATED INFECTIONS

Currently, catheter-related infections are the most common health care–associated NTM infections encountered.[7,8,20,26] Infections are seen most often with long-term central IV catheters, but they may also occur with peritoneal or shunt catheters. The usual pathogens are RGM, especially *M. fortuitum* and *M. mucogenicum* (see Table 252.1). These infections may be manifested as fever, local catheter site drainage, bacteremia, or, occasionally, lung infiltrates or granulomatous hepatitis. The usual treatment is catheter removal combined with appropriate antibiotics for 6 to 12 weeks.[5,7,26,91]

An outbreak of *M. phocaicum,* a member of the *M. mucogenicum* group, was reported in central venous catheters in an oncology unit of a Texas hospital.[5,39,45] Isolates of the RGM species *M. neoaurum, M. brumae, M. mageritense,* and the recently described *M. bacteremicum* have also been incriminated in catheter-related sepsis.[2,20,22]

MISCELLANEOUS INFECTIONS

Less commonly, NTM have been associated with other types of infections, including central nervous system (CNS) and ocular infections.[26,44,77,92] NTM, including *M. genavense, M. kansasii, M. malmoense,* and RGM, primarily *M. fortuitum,* have been identified with CNS disease.[26,45,92] The first known case of *M. abscessus* meningitis was described in 2001 in a 59-year-old woman who had no significant medical history but had sustained a knife wound to the neck months before her symptoms.[92]

Recently, the newly described *M. iranicum*[93] and *M. doricum*[2] were recovered from cerebrospinal fluid cultures of patients, although the latter species was considered of uncertain significance.

Ocular infections have most often involved the RGM, especially the *M. abscessus–M. chelonae* group.[26,45,77] The number of infections has increased to include postkeratoplasty and post-LASIK surgery. As previously discussed, infections have been associated with outbreaks and in sporadic fashion.[26,77] Treatment for patients with corneal infections due to RGM is usually complicated by the lack of available effective antimicrobials.[26,77] For patients who do not respond to topical antimicrobials, surgical interventions are recommended.[45,77]

RGM can cause mastoiditis and chronic otitis media of insidious onset, usually in children with a perforation or ventilation tube in the tympanic membrane.[5] Infection appears with ear drainage or polyps in the external auditory canal. CT shows opacification of the mastoid air cells, with or without destruction of bony septae. Mastoidectomy tissue is full of granulomas, but AFB are not seen. Cultures of the mastoid tissue grow RGM, usually *M. abscessus* complex. Treatment involves surgical removal of infected tissue and months of antimycobacterial treatment.

LABORATORY ASPECTS

Stain and Culture

The methods used for staining and culture of *M. tuberculosis* generally work well for the NTM, although the yield of some NTM, especially the RGM, may be adversely affected by the decontamination methods that are standard for *M. tuberculosis*.[26,33,51] Middlebrook 7H10 or 7H11 agar and the rapid broth systems all support growth of the commonly encountered NTM.[26,51,84] Cultures of skin and soft tissue should be plated at 28° to 30°C, as well as at 35°C, because species such as *M. marinum, M. chelonae,* and *M. haemophilum* grow only at low temperatures on primary isolation.[23,26,31] *M. genavense* (broth for 6–8 weeks)[5,26,31,45] and *M. haemophilum* (iron or heme in the media and lower temperatures)[5,26,32] have special growth requirements.[5,26,31,45,94] If *M. ulcerans, M. genavense,* or *M. malmoense* is suspected, cultures should be held up 10 to 12 weeks before discarding.[26,32] Due to the difficulty often encountered in growing *M. ulcerans* and *M. genavense* on solid media, molecular techniques are usually necessary to identify these species directly from broth.[5,26,32,33]

Identification

Identification of NTM increasingly focuses and depends on the use of rapid diagnostic systems. High-performance liquid chromatography, which assesses the patterns of long-chain fatty acids (mycolic acids) has generally been replaced by molecular methods such as gene sequencing of multiple genes, including 16S rRNA, *hsp65*, *rpoB,*[4,6,26,95] and commercially available molecular probes. The latter probes for RNA are currently available for *M. tuberculosis*, *M. avium, M. intracellulare,* MAC, *M. gordonae,* and *M. kansasii*.[5,26] For most of the newer species, partial gene sequencing of the 16S rRNA gene, *rpoB* gene, or both, are important or essential for species identification.[2,4,6,26] Molecular methods, including the INNO-LiPA multiplex probe assay has been introduced into the United States. This assay targets the 16S-23S internal transcribed spacer (Innogenetics; Ghent, Belgium), whereas the GenoType *Mycobacterium* assay targets the 23S rRNA gene (Hain Lifescience; Nehren, Germany). These two methods have been widely used in Europe for identification of multiple species of NTM.[2,4,31,96]

Other methods of identification include pyrosequencing, a short sequence analysis method based on detection of pyrophosphate during DNA synthesis,[31,97] and mass spectral identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).[4,7] To date, MALDI-TOF has reliably identified some species of NTM but not the rarer species.[98] Moreover, it cannot differentiate the closely related members of the *M. fortuitum* group and the subspecies of the *M. abscessus* complex. The efficacy of this method greatly depends upon the availability of quality updated databases.[33] Thus further study of this technique is warranted.

Biochemical testing to determine carbohydrate use is slow and inadequate and should be replaced by molecular methods for more definitive identification.[4,7] It should also be emphasized that NTM species identification obtained before the use of modern molecular techniques should be suspect until identification is confirmed by molecular methods.[4,7]

Susceptibility Testing: Rapidly Growing Mycobacteria

The three most widely used methods of susceptibility testing of RGM include agar disk elution, broth microdilution, and the E-test gradient MIC.[26,45,84] In 2003 the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards) published a document for global standardization of susceptibility testing of mycobacterial species, including the NTM.[23,45,84] The only CLSI-recommended method for susceptibility testing with RGM is the broth microdilution technique.[45,84] Antituberculous agents are not effective against these species. The document was revised in 2011 to include recommendations for moxifloxacin, meropenem, and detection of the inducible *erm* gene in RGM.[84] Testing of tigecycline has not been addressed by the CLSI, yet, although it appears to be a useful antimicrobial against most RGM.[55] There are no recommended mycobacterial breakpoints.[7,54,84] Minimal recommendations for testing the RGM include clarithromycin (used as a class representative agent for the new macrolides), amikacin, cefoxitin, imipenem, doxycycline, linezolid, ciprofloxacin, TMP-SMX, and tobramycin (for *M. chelonae* only).[26,84]

Susceptibility Testing: Slowly Growing Nontuberculous Mycobacteria

The proportion method in agar, broth microdilution, E-test, and automated broth systems, including the MGIT (mycobacteria growth indicator tube) (Becton, Dickinson and Company; Franklin Lake, NJ) and the VersaTrek/ESP (Thermofisher, formerly Trek Diagnostics; Cleveland, OH) techniques, have been used for determining MICs of the slowly growing NTM.[45,84] However, only the broth microdilution susceptibility testing is currently recommended by the CLSI for all slowly growing species.[45,84] Susceptibility testing of rifampin and clarithromycin (used as the class agent for the macrolides, including azithromycin), rifabutin, minocycline, amikacin, linezolid, quinolones (ciprofloxacin and moxifloxacin), and TMP-SMX for most slowly growing NTM species (other than MAC) is recommended.[5,45,84] Ethambutol MICs are not reproducible and MIC results may be misleading, potentially unnecessarily eliminating an effective agent from the treatment regimen.[84] Susceptibility testing to pyrazinamide is not recommended because it has no efficacy against NTM. Currently, susceptibility testing to rifampin and clarithromycin only is recommended for isolates of *M. kansasii.* Isolates of *M. kansasii* that are resistant to rifampin should be tested against the aforementioned panel of antimicrobials.[84] Isolates of *M. kansasii* that are susceptible to rifampin will also be susceptible to rifabutin and should require no additional testing.[26,45,84]

If considered clinically significant, isolates of slowly growing NTM (other than MAC) should be tested against all of the aforementioned drugs.[5,26,45,84] Streptomycin and INH may be useful clinically, but the CLSI has not addressed breakpoints for these antimicrobials.[26,45,84]

Isolates of *M. marinum* may not require susceptibility testing unless the patient fails to respond to treatment after several months.[26,45,84] However, recent resistance trends among isolates of this species may necessitate more frequent testing.

The role of antimicrobial susceptibility testing for some NTM remains uncertain.[99] With the increasing clinical significance of NTM disease, further testing, especially of the newly described species, is warranted.

Strain Comparison

Molecular methods, such as random amplified polymorphic DNA polymerase chain reaction (RAPD), pulsed-field gel electrophoresis (PFGE), repetitive sequence-based PCR (rep-PCR), multilocus sequence typing (MLST), and more recently, whole-genomic sequencing (WGS) have been used for strain comparison, ("DNA fingerprinting") of NTM (non-MAC) outbreaks.[26,31,39,41,100] PFGE has been the most widely used technique applicable to essentially all species of NTM and has been considered the gold standard for definitive strain comparison of the NTM. With recent modifications to the technique to eliminate DNA degradation, strain typing is feasible for all species of mycobacteria.[100,101] Variable number tandem repeat (VNTR) has been useful in some outbreak studies of *M. abscessus*,[83] whereas the potential of WGS, especially core genomics, remains largely untapped among many NTM species. Genomic sequencing may potentially not only aid in the identification of species but can potentially identify genetic determinants that characterize species diversity and strain specificity that may provide insight into the aspects of environmental survival, antibiotic resistance, and ultimately the pathogenicity of strains or species. Although WGS is generally not widely available in clinical and reference laboratories, it is emerging as an important tool in the study of NTM.[102,103]

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The complete reference list is available online at Expert Consult.

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253 *Nocardia* Species

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SHORT VIEW SUMMARY

Definition

- Nocardiosis results from infection by members of the genus *Nocardia,* which are ubiquitous environmental saprophytes that cause localized or disseminated disease in humans and animals.

Microbiology and Epidemiology

- Microscopically, *Nocardia* appear as gram-positive, beaded, weakly acid-fast, branching rods.
- Molecular speciation has revolutionized taxonomy by identifying several new species and reassigning species, especially from the commonest pathogenic group, the former *Nocardia asteroides* complex.
- Infection arises by direct inoculation through the skin or by inhalation.
- Mycetomas from *Nocardia* spp., most often caused by *N. brasiliensis,* primarily affect immunocompetent hosts in tropical countries.
- Immunocompromise, alcoholism, and certain lung diseases predispose patients to pulmonary and disseminated nocardiosis, most often due to *N. cyriacigeorgica, N. nova,* or *N. farcinica.*

Clinical Manifestations

- Primary skin infection may be manifested as superficial cellulitis or pyogenic abscess(es), lymphocutaneous (spirotrichoid) infection, or chronically progressive, destructive disease with sinus tract formation (mycetoma), usually on a distal limb.
- Presentation of lung disease may be subacute or chronic, with productive or nonproductive cough, dyspnea, hemoptysis, and fever, and other systemic symptoms. Cavity formation within the pneumonia or spread to the central nervous system (CNS), or both, are suggestive of nocardiosis. Isolated CNS lesions also occur, and their presentation can be insidious.

Diagnosis

- Cerebral imaging, preferably magnetic resonance imaging, should be performed in all cases of pulmonary and disseminated nocardiosis to rule out insidious CNS disease.
- The microbiology laboratory should be informed of suspected nocardiosis because it may not be detected by routine laboratory methods. Respiratory secretions, skin biopsies, or aspirates from deep collections are the most useful diagnostic specimens and are typically positive on Gram stain. Modified acid-fast stain of sputum or pus is helpful in suggesting the diagnosis. Growth of *Nocardia* spp. may take 48 hours to several weeks but usually 3 to 5 days.
- Species identification may be predictive of antimicrobial susceptibility; it often requires molecular identification based on nucleic acid technology (NAT, DNA sequencing).
- Recently, mass spectrometry analysis using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has been reported to be a reliable and more rapid alternative to NAT, but species identification is contingent on a reliable mass spectral database.

Therapy and Follow-Up

- Trimethoprim-sulfamethoxazole (TMP-SMX) is the mainstay of treatment, and monotherapy is usually successful in patients with isolated skin infection or mycetomas that are not extensive (see Table 253.3).
- In infection spread beyond the lung, speciation and/or susceptibility testing results should guide definitive combination therapy. TMP-SMX is usually included in initial therapy.
- Empirical therapy with TMP-SMX plus amikacin (with imipenem or meropenem) is recommended in immunocompromised patients or those with disseminated disease but no brain involvement.
- For isolated cerebral disease, empirical TMP-SMX plus imipenem or meropenem are suitable.
- When additional sites are involved or the patient has life-threatening disease, empirical three-drug regimens, such as TMP-SMX, meropenem or imipenem, and amikacin (or ceftriaxone in patients with renal failure), are preferred. Initial adjunctive therapy with linezolid is an option, especially if one of these classes of drug is contraindicated.
- Clinical improvement is generally evident within 3 to 5 days or, at the most, 7 to 10 days after initiation of appropriate therapy.
- Prolonged therapy is necessary to prevent relapse.
- Surgical excision or drainage of pus may be required, depending on the extent and site of the lesions or the response to medical therapy, or both.
- Patients with deep-seated infection should be monitored clinically and radiologically during and for up to 12 months after cessation of therapy.

Nocardia is a genus of aerobic actinomycetes responsible for localized or disseminated infections in animals and humans. The genus is named after Edmond Nocard, who in 1888 described the isolation of an aerobic actinomycete from cattle with bovine farcy. The first human case of nocardiosis was reported by Eppinger in 1890. Cases of human disease have increased substantially in the past 2 decades, in association with an increasing population of immunocompromised hosts and improved methods for detection and identification of *Nocardia* spp. in the clinical laboratory. In parallel, an increasing number of novel species of *Nocardia* has been recognized as human pathogens.

CLASSIFICATION

The aerobic actinomycetes are a large and diverse group of gram-positive bacteria[1] that appear on microscopy as branching, filamentous cells. Members of the group are often only distantly related phylogenetically. A subgroup, classified in the suborder Corynebacterineae, is the most important cause of human and veterinary infection and includes the genera *Mycobacterium, Corynebacterium, Nocardia, Rhodococcus, Gordonia,* and *Tsukamurella.*[1] All members of the group have cell walls containing meso-diaminopimelic acid, arabinose, galactose (type IV cell wall[1]), and mycolic acids of various chain lengths. The latter are

responsible for varying degrees of acid fastness on modified acid-fast staining. In this chapter the genus *Nocardia* is discussed in the context of human infection.

Previous taxonomic classifications have relied on traditional phenotypic methods to assign nocardiae to both genus and species. *Nocardia* spp. are characterized by an ability to form aerial hyphae and to grow in media containing lysozyme and by an inability to grow at 50°C.[1,2] Speciation using biochemical reactions has been largely superseded due to their frequent inability to distinguish between species, especially those that are phylogenetically closely related.[1,2]

Molecular Identification and Taxonomy

Molecular techniques are now preferred for accurate species determination. In addition, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has proven useful for identification of clinically relevant *Nocardia* spp.[3,4]

The application of molecular methods[2,5,6] has greatly expanded the spectrum of pathogenic nocardiae and has led to significant taxonomic changes and species reassignment within the genus. This is particularly evident among isolates formerly assigned to the *Nocardia asteroides* complex.[2] More than 100 *Nocardia* spp. have now been identified (National Center for Biotechnology Information—taxonomy for *Nocardia:* www.ncbi.nlm.nih.gov/Taxonomy/, and www.bacterio.net/nocardia.html), many of which have been implicated in human disease (Table 253.1).[2,6,7] The nomenclature of isolates formerly in the *N. asteroides* complex is summarized in Table 253.2 and includes *Nocardia cyriacigeorgica,*[8] *Nocardia abscessus,*[9] the *Nocardia nova* complex, and the *Nocardia transvalensis* complex. Other major human pathogens include *Nocardia*

TABLE 253.1 Current Classifiable *Nocardia* Species Names

NOCARDIA SPECIES[a]	FREQUENCY[b]	*NOCARDIA* SPECIES[a]	FREQUENCY[b]
Nocardia abscessus	22	*Nocardia donostiensis*	
Nocardia acidovorans		*Nocardia elegans*	12
Nocardia africana		*Nocardia endophytica*	
Nocardia alba		*Nocardia exalbida*	4
Nocardia alboflava		*Nocardia farcinica*	160
Nocardia altamirensis		*Nocardia flavorosea*	
Nocardia amamiensis		*Nocardia fluminea*	
Nocardia amikacinitolerans		*Nocardia fusca*	
Nocardia anaemiae		*Nocardia gamkensis*	
Nocardia aobensis	5	*Nocardia globerula*	
Nocardia araoensis	1	*Nocardia goodfellowii*	
Nocardia argentinensis		*Nocardia grenadensis*	
Nocardia arizonensis		*Nocardia harenae*	
Nocardia artemisiae		*Nocardia heshunensis*	
Nocardia arthritidis	4	*Nocardia higoensis*	1
Nocardia asiatica	21	*Nocardia ignorata*	
Nocardia asteroides	2	*Nocardia inohanensis*	1
Nocardia beijingensis	24	*Nocardia interforma*	
Nocardia bhagyanarayanae		*Nocardia iowensis*	
Nocardia blacklockiae		*Nocardia jejuensis*	
Nocardia boironii		*Nocardia jiangxiensis*	
Nocardia brasiliensis	71	*Nocardia jinanensis*	
Nocardia brevicatena		*Nocardia kruczakiae*	
Nocardia caishijiensis		*Nocardia lasii*	
Nocardia callitridis		*Nocardia levis*	
Nocardia camponoti		*Nocardia lijiangensis*	
Nocardia canicruria		*Nocardia lillensis*	
Nocardia carnea	3	*Nocardia mexicana*	
Nocardia casaurinae		*Nocardia mikamii*	
Nocardia caverna		*Nocardia miyunensis*	
Nocardia cerradoensis		*Nocardia neocaledoniensis*	
Nocardia coeliaca		*Nocardia niigatensis*	4
Nocardia concava	3	*Nocardia ninae*	
Nocardia coubleae		*Nocardia niwae*	
Nocardia crassostreae		*Nocardia nova*	81
Nocardia cummidelens		*Nocardia novocastrensa*	
Nocardia cyriacigeorgica	60	*Nocardia otitidiscaviarum*	14
Nocardia devorans		*Nocardia paucivorans*	1

TABLE 253.1 Current Classifiable *Nocardia* Species Names—cont'd

NOCARDIA SPECIES[a]	FREQUENCY[b]	*NOCARDIA* SPECIES[a]	FREQUENCY[b]
Nocardia pigrifrangens		*Nocardia takedensis*	
Nocardia pneumoniae		*Nocardia tartaricans*	
Nocardia polyresistens		*Nocardia tenerifensis*	
Nocardia pseudobrasiliensis	2	*Nocardia tengchongensis*	
Nocardia pseudosporangifera		*Nocardia terpenica*	1
Nocardia pseudovaccinii		*Nocardia testacea*	2
Nocardia puris	4	*Nocardia thailandica*	1
Nocardia rayongensis		*Nocardia thraciensis*	
Nocardia rhamnosiphila		*Nocardia transvalensis*	13
Nocardia rhizosphaerae		*Nocardia uniformis*	
Nocardia rhizosphaerihabitans		*Nocardia vaccinii*	
Nocardia roseoalba		*Nocardia vermiculata*	
Nocardia salmonicida		*Nocardia veterana*	3
Nocardia salmonicolor		*Nocardia vinacea*	3
Nocardia seriolae		*Nocardia violaceofusca*	
Nocardia shimofusensis		*Nocardia vulneris*	
Nocardia sienata	1	*Nocardia wallacei*	10
Nocardia soli		*Nocardia xestospongiae*	
Nocardia speluncae		*Nocardia xishanensis*	
Nocardia strombolensis		*Nocardia yamanashiensis*	
Nocardia sungurluensis		*Nocardia zapadnayensis*	
Nocardia sylvodorifera			

[a]Species names in http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi. Accessed January 22, 2018.
[b]Frequency of classifiable isolates at Chiba University, Medical Mycology Center, 1999–2007.
Data from Mikami U. [Recent progress in taxonomic studies on pathogenic Nocardia *and usefulness of the bacteria for the studies on secondary metabolites and antibiotic resistant mechanisms].* Nihon Ishinkin Gakkai Zasshi. *2010;51:179–192 [in Japanese].*

TABLE 253.2 *Nocardia asteroides* Complex: Major Changes in Taxonomic Categories

FORMER SPECIES OR SPECIES GROUP ASSIGNMENT	CURRENT SPECIES GROUP DESIGNATION	CURRENT SPECIES DESIGNATION
N. asteroides drug pattern I	—	*N. abscessus*
N. asteroides drug pattern II	*N. paucivorans*/*N. brevicatena* complex	*N. paucivorans*[b] *N. brevicatena*[b]
N. asteroides drug pattern III	*N. nova* complex[c]	*N. nova* sensu stricto, *N. africana* *N. aobensis* *N. elegans*, *N. kruczakiae*, *N. veterana*
N. asteroides drug pattern IV[d]	*N. transvalensis* complex	*N. wallacei*, *N. transvalensis* sensu stricto, *N. blacklockiae*
N. asteroides drug pattern V		*N. farcinica*
N. asteroides drug pattern VI		*N. cyriacigeorgica*

[a]*N. brevicatena* and *N. paucivorans* are not new species names; they have been reclassified.
[b]*N. asteroides* sensu stricto is rarely pathogenic.
[c]It is uncertain to which species the former *N. asteroides* drug pattern III isolates now correspond.
[d]Only *N. wallacei* is designated as the former "*N. asteroides* drug pattern IV." The other members of the *N. transvalensis* complex are previously either separate as "*N. asteroides* complex" or are recently identified species.
Data from Conville PS, Witebsky FG. Nocardia, Rhodococcus, Gordonia, Actinomadura, Streptomyces, *and other aerobic actinomycetes. In: Jorgensen JH, Pfaller MA, Carroll KC, et al., eds.* Manual of Clinical Microbiology. *Washington, DC: American Society for Microbiology Press; 2015.*

otitidiscaviarum, Nocardia farcinica, and *Nocardia brasiliensis.*[2] Some more recently described or reclassified species have been reported to cause human infection. They include *Nocardia paucivorans* (*Nocardia brevicatena/paucivorans* complex[2,10]), *Nocardia africana,*[11] *Nocardia veterana*[12] (*N. nova* complex[2]), *Nocardia wallacei*, and *Nocardia blacklockiae* (*N. transvalensis* complex).[13] The terminology "*N. asteroides* spp. complex" is no longer used because it encompasses such a heterogenous group of organisms.

ECOLOGY AND EPIDEMIOLOGY

Nocardia spp. are ubiquitous environmental saprophytes, occurring in soil, organic matter, and aquatic habitats, including in waste-water systems.[1,2] Human infection usually arises from direct inoculation of the skin or soft tissues or by inhalation. *N. brasiliensis* is the commonest cause of mycetoma due to nocardial infection in immunocompetent hosts reported from tropical regions of the southern United States, Mexico, Central and South America, and Australia. Worldwide, respiratory and disseminated infections occur predominately in immunosuppressed hosts, and although species distribution varies with geographic region, infections are most often due to *N. cyriacigeorgica*, *N. nova, N, abscessus*, *N. brasiliensis,* and *N. farcinica.*[1,2,14,15]

Nocardia spp. are well-recognized causes of infection in animals, with bovine mastitis being the most common.[2] There are no reports of animal-to-human transmission nor of person-to-person transmission. However, clusters of invasive nocardiosis acquired by patients in oncology and transplantation units, presumed to be associated with inhalation of contaminated air or dust, have been described.[2] Transmission via the hands of staff or contaminated fomites appeared likely in one outbreak.[2] Hospital construction work may have been a risk factor in separate clusters of postsurgical wound infections due to *Nocardia* spp.[2,16] Cases of indwelling intravenous (IV) line–associated bloodstream



FIG. 253.1 Pulmonary nocardiosis. Photomicrograph of direct Gram-stained smear from a patient with pulmonary nocardiosis, showing typical branching rods.

infection, mostly due to members of the *N. asteroides* spp. complex or *N. nova,* have been reported occasionally in immunosuppressed patients.[17] A community cluster of *N. cyriacigeorgica* infection associated with unlicensed cosmetic procedures has also been described.[18] In a recent study, hospital environmental reservoirs of *Nocardia*, including dust on window frames and equipment and potable water sources were described.[19] Pulsed-field gel electrophoresis,[16] random amplification of polymorphic DNA fingerprinting,[20] and multilocus sequence typing[18] have been used to confirm clusters and define common sources.

PATHOLOGY

Sections of tissues infected with *Nocardia* usually show an acute pyogenic inflammatory reaction. Gram stains (Fig. 253.1) of specimens may reveal branching, beaded, filamentous bacteria, similar to those seen in smears taken from cultures, within abscesses. "Sulfur granules" (bacterial macrocolonies), similar to those seen in actinomycosis, may be found in nocardial mycetomas. *Nocardia* spp. usually stain acid-fast in tissue sections if a method such as that of Fite-Faraco is used, whereas *Actinomyces* spp. do not.[1]

PATHOGENESIS

Disease manifestations of nocardiosis are determined by the portal of entry, tissue tropism, growth rates in vivo, ability to survive phagocyte attack, the nature of the host immune reaction, and the characteristics of the infecting strain.

Immune Response to *Nocardia* Infection

After IV inoculation into mice, virulent *Nocardia* are cleared from the blood within a few hours and localize in a number of organs (lung, brain, kidneys, liver, and spleen). Innate immune function is important in the initial response to infection. In an intranasal mouse model of pulmonary nocardiosis, effective clearance was dependent on early neutrophil recruitment initiated by interleukin-17 (IL-17) production by γδT lymphocytes.[21] Early neutrophil mobilization appears to retard the process until lymphocyte-mediated cytotoxicity and activated macrophages effect a definitive response.[22,23] In human infection *Nocardia*-induced granulocyte-macrophage colony-stimulating factor (GM-CSF) production may also be a key cytokine response mediator.[24] Protective immune responses to *Nocardia* spp. are primarily T-cell–mediated. Nocardiosis is more problematic in patients with impaired cell-mediated immunity, eliciting little in the way of an effective humoral response.[22] Healing is associated with strong, sustained rises in interferon-γ (IFN-γ) in animal models, and IFN-γ may have a therapeutic role in humans with chronic granulomatous disease.[25]

Specific Virulence Determinants

The nocardial envelope of the Corynebacterineae suborder, which includes *Mycobacterium* spp. and *Nocardia* spp., is an asymmetrical bilayer composed of inner-leaflet mycolic acids and assorted noncovalently bound outer-layer glycolipids. A substantial proportion of the cell wall mass is composed of peptidoglycan.[26] Mycolic acid polymers are found in many actinomycetes, including *Nocardia* spp., and are associated with virulence.[27] Outer-layer lipids induce production of proinflammatory cytokines IL-1β and IL-6 by macrophages and are likely to be responsible for the powerful granulomatous reaction to *N. brasiliensisis* infection,[28] but they are also implicated in the immunosuppressive microenvironment generated later.[29]

Nocardia spp. contain no cell wall lipopolysaccharide, exopolysaccharide capsule, or surface fimbriae. Strain-dependent specific adhesins and invasive properties influence the outcome of infection in animal models.[30,31] Specific toxins, including hemolysins and proteases, have been identified, but these are not thought to be widespread or particularly significant virulence factors.[22,32,33] Highly pathogenic members of the *N. asteroides* complex secrete superoxide dismutase into growth media, whereas nonpathogenic *Nocardia* spp. do not.[34] Catalases, superoxide dismutase, and two types of putative determinants of mammalian cell entry, secreted siderophores and toxins, are present in the genomes of pathogenic *Nocardia*.[35]

Host Cell–*Nocardia* Interactions

Virulent strains of *N. asteroides* are relatively resistant to neutrophil-mediated killing.[36] Patients with specific defects in the phagocyte oxidative burst (e.g., chronic granulomatous disease)[14,25] or with anti–GM-CSF neutralizing antibodies in their serum may be more vulnerable to this infection.[24]

Virulent *Nocardia* inhibit phagosome-lysosome fusion more successfully in vitro,[37] giving rise to cell wall–deficient forms (L-forms) that persist within macrophages.[38] Such forms ("filterable *Nocardia*") are readily cultured from in vitro broth filtrates, especially when supplemented with erythrocytes,[39] and L-forms have been isolated from animal infections.[40,41]

Ciliated epithelia appear relatively resistant to invasion by *Nocardia* spp. However, a range of susceptible lung- and airway-associated cell types has been observed in rat models.[31] Tropism for cerebral tissue is evident experimentally, but neuroinvasiveness and macrophage penetration vary significantly between strains. Electron microscopic studies of infected macrophage and astrocytoma-derived or astrocytoma-related cell lines suggest that the penetration competence of invasive *N. asteroides* spp. complex is localized to the bacterial apex.[32] Specific lectins have been shown to determine site specificity in the murine brain,[32] intrinsic differences in expression of which may contribute to variations in host susceptibility.[42]

Biofilms

Although IV catheter–associated bloodstream infections are rare in clinical practice, nocardiae promote heavy growth of biofilms, both on the surface of central venous catheter segments in vitro and in a biofilm model.[17] When embedded in such a matrix, the organisms are resistant to antimicrobial drugs unless exposed to very high local concentrations, such as can be achieved with intraluminal antimicrobial lock therapy.[17]

CLINICAL EPIDEMIOLOGY

Nocardia Species and Disease Associations

Members of the former *N. asteroides* spp. complex are responsible for about 80% of noncutaneous invasive disease and for most systemic and central nervous system (CNS) disease.[22,43] *N. farcinica* is also an important pathogen, notable for its relatively greater resistance to antibiotics. There is also evidence from mouse models that it may be more virulent than other *Nocardia* spp.[44] *N. brasiliensis* is the most often reported cause of cutaneous and lymphocutaneous disease, particularly in tropical areas. *N. pseudobrasiliensis,* a species now separated from *N. brasiliensis,* appears to be associated with disseminated, including CNS, infections.[1,45] Pulmonary disease is the most frequent presentation of nocardiosis caused by the less common pathogens *N. transvalensis*[46] and *N. otitidiscavarum,*[22] although both may cause severe cutaneous infection.[47] Superficial nocardiosis after implantation is not necessarily

associated with compromised cell-mediated immunity but may progress to disseminated disease in that setting.[47]

Immunocompromise as a Risk Factor for Nocardiosis

Immunocompromise is a well-established risk factor for nocardiosis. *Nocardia* spp. may therefore be considered as opportunistic pathogens, which cause serious and disseminated disease in settings such as organ transplantation and lymphoreticular neoplasia. The relative risk for progressive disease reflects the level of immunosuppression. A compilation of more than 1000 randomly selected cases from the literature in the early 1990s showed that greater than 60% of all reported cases of nocardiosis were associated with preexisting immune compromise, ranging from alcoholism and diabetes to chronic granulomatous disease, organ transplantation, and acquired immunodeficiency syndrome (AIDS).[22] In a recent northern Australian study, greater than one-third (36%) of patients with nocardiosis were immuncompromised.[43] Among recipients of solid-organ transplants with nocardiosis, significant risk factors include receipt of high-dose corticosteroids at time of onset, cytomegalovirus disease within the preceding 6 months, high serum trough levels of calcineurin inhibitors within the preceding 30 days, use of tacrolimus, patient age, and length of stay in the intensive care unit postoperatively.[15,48] Use of low-dose trimethoprim-sulfamethoxazole (TMP-SMX) for *Pneumocystis* prophylaxis, such as one double-strength tablet twice a week, did not prevent nocardiosis in either solid-organ or hematopoietic stem cell transplant (HSCT) recipients.[48–50] Breakthrough nocardiosis remains susceptible to TMP-SMX.[49] The use of anti–tumor necrosis factor-α agents has been associated with disseminated nocardial infections.[51,52] Although cases of nocardiosis have been described in patients with AIDS, the overall incidence is low and not fully explained by the use of sulfonamide prophylaxis against *Pneumocystis jirovecii* pneumonia.[53]

Chronic Lung Disease as a Risk Factor for Nocardiosis

Persons with chronic lung disorders, such as pulmonary alveolar proteinosis, and almost any condition requiring long-term corticosteroid use are also at risk. Other chronic airway conditions that may predispose to colonization with *Nocardia,* with the potential, albeit low, for subsequent infection include cystic fibrosis (CF) and non-CF bronchiectasis. In one study the incidence of nocardiosis among patients with bronchiectasis rose significantly between 1996 and 2013.[54]

CLINICAL MANIFESTATIONS

Primary Cutaneous Nocardiosis

Primary cutaneous nocardiosis may manifest as superficial cellulitis or abscess, lymphocutaneous (spirotrichoid) infection, or mycetoma. Unlike other forms of nocardiosis, this usually develops in immunocompetent hosts.[55] Superficial infection often follows relatively trivial inoculation injuries (Fig. 253.2), which may vary from insect and animal bites to puncture wounds and contaminated abrasions. The lymphocutaneous form includes a rare variant, cervicofacial nocardiosis, which is associated with prominent localized lymphadenitis.[56] Members of the former *N. asteroides* complex more commonly cause superficial infections, whereas *N. brasiliensis* is the most common cause of progressive cutaneous and lymphocutaneous disease.[22] Because the initial response to *Nocardia* is pyogenic, localized skin lesions may initially be treated as staphylococcal or streptococcal in origin; however, nocardial disease is usually more indolent. In advanced disease a mycetoma can develop with sinus tract formation. Mycetomas are a chronically progressive, destructive disease, occurring days to months after inoculation, and are typically located distally on the limbs. Eumycetoma (of fungal etiology) and actinomycetoma (due to actinomycetes) are equally prominent in the literature, the epidemiology varying with geographic location (see Chapter 261). Overall, *Streptomyces* and *Actinomadura* spp. appear to be of equal or greater importance than *Nocardia* spp. as causative agents of actinomycetoma. Suppurative granulomas, progressive fibrosis and necrosis, sinus formation with destruction of adjacent structures, and macroscopically visible infective granules (grains) are regular features of nocardial mycetoma.



FIG. 253.2 Skin lesions. Nocardial skin lesions due to direct inoculation in an immunosuppressed landscape gardener.

Pulmonary Disease

Pulmonary disease is the predominant clinical presentation of nocardiosis and is acquired through inhalation of organisms from the environment.[15,22] Any species may cause lung infection, although the most common are *N. cyriacigeorgica*, *N. nova,* and *N. farcinica*. Onset of symptoms may be subacute or chronic and include one or more of productive or nonproductive cough, dyspnea, hemoptysis, and fever and other systemic symptoms. In patients with malignancy, radiologically evident pulmonary infiltrates commonly herald the presence of nocardiosis.[57] Established infection may include endobronchial inflammatory masses, pneumonia, lung abscess, and cavitary disease with contiguous extension to surface and deep structures, including effusion and empyema.

Radiologic Manifestations of Pulmonary Nocardiosis

These include irregular nodules (usually cavitating when large), reticulonodular or diffuse pneumonic infiltrates, and pleural effusions (Fig. 253.3). High-resolution computed tomography (CT) of pulmonary lesions most often shows them to be dense, well-circumscribed nodules or masses, often with central cavitation. Interlobar septal thickening around the lesion or ground-glass infiltrates may also be seen.[58] The "halo sign," considered characteristic of aspergillosis in neutropenic patients, has been described. Progressive fibrotic disease may develop in the immunocompetent host, and diagnosis is often difficult. Pulmonary nocardiosis may occasionally complicate advanced human immunodeficiency virus (HIV) infection (most commonly when the CD4 count is <200/mm^3), where it often presents with alveolar infiltrates that progress during therapy rather than as cavitary disease.[59]

Differential Diagnosis of Pulmonary Nocardiosis

Nocardiosis should always be considered in the differential diagnosis of indolent pulmonary disease, particularly in the setting of cellular immune compromise, along with other actinomycetes (e.g., mycobacteria, *Actinomyces* spp.) and fungi (e.g., *Cryptococcus neoformans, Aspergillus* spp.). Pneumonia may have subacute presentation, resembling staphylococcal pneumonia. Clues to a nocardial etiology include spread to contiguous structures, especially with soft tissue swelling or external fistulae, and to the CNS. Invasive diagnostic procedures, including bronchoalveolar lavage for pneumonia, should be considered early in the immunocompromised host because disease may be rapidly progressive; in patients with severe immunodeficiency, coexisting pathology with similar clinical characteristics (e.g., aspergillosis, tuberculosis, malignancy) is well documented.[60]

Central Nervous System Nocardiosis

CNS involvement was recognized in greater than 44% of cases of all systemic nocardiosis in an early survey[22] compared with 4% to 33% in

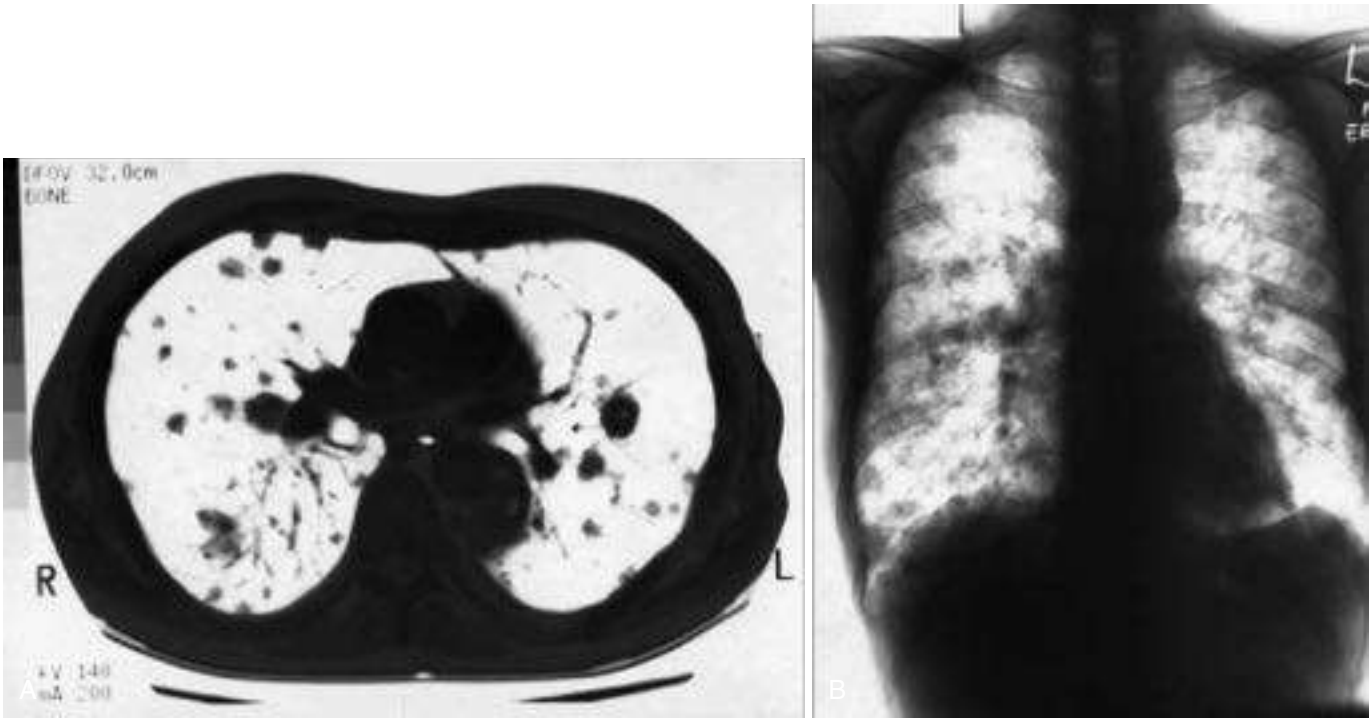

FIG. 253.3 Pulmonary nodules. Multiple pulmonary nodules, demonstrated by computed tomography (A) and chest radiograph (B), in an immunosuppressed patient with disseminated nocardiosis.

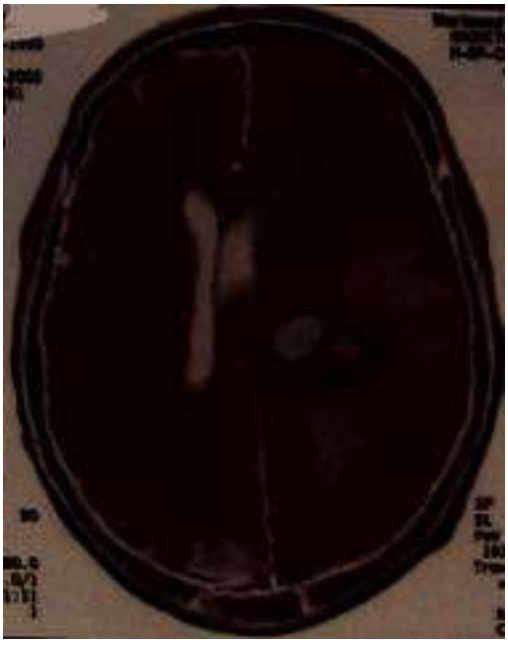

FIG. 253.4 Brain abscess. Magnetic resonance image showing *Nocardia* brain abscess.



FIG. 253.5 Skin lesion. Skin lesion from disseminated *Nocardia farcinica* infection.

more recent series.[48,57,61] Clinical manifestations usually result from local effects of granulomas or abscesses in the brain and, less commonly, the spinal cord or meninges (Fig. 253.4). These include headache, focal neurologic signs, seizures, confusion, and depressed consciousness. Multiple brain lesions are common. CNS nocardiosis should always be considered in patients with pulmonary or disseminated disease. Indeed, clinically silent brain abscess is sufficiently common that cerebral imaging, preferably magnetic resonance imaging (MRI), should be performed routinely in such cases.[48,49] Isolated CNS disease can also occur.[17,62,63] Insidious presentations are often mistaken for neoplasia because of the paucity of clinical and laboratory signs of inflammation; silent invasion and persistence make diagnosis and management more difficult.[14,22] Tissue diagnosis of a cerebral mass in the setting of proven pulmonary nocardiosis is not always necessary.[14] However, cerebral biopsy or aspiration should be considered early in the immunocompromised patient because of the higher incidence of serious coexisting pathology and a more aggressive course than that ascribed traditionally to cerebral nocardiosis. *N. farcinica* has a particular association with CNS (and skin) disease.[48,57]

Disseminated Nocardiosis

Disseminated infection is characterized by sites widespread abscess formation. The most commonly reported sites include the CNS (see earlier) and eye (particularly the retina), skin and subcutaneous tissues, kidneys, joints, bone, and heart (Fig. 253.5). Occasional cases of IV catheter–associated bacteremia have also been reported. Bacteremia is uncommon. *N. nova* was the predominant bloodstream pathogen in cancer patients and recipients of HSCT.[49,57]

Keratitis

Nocardia keratitis is well described in Asia and has been reported in travelers returning from Asia.[62,64] Although uncommon in most parts of the world, the entity is increasingly recognized, largely due to the availability of molecular diagnostic methods in many laboratories (see later). It is an aggressive ocular infection, typically after corneal trauma or minor surgical procedures to the eye. If the diagnosis is delayed, the infection can lead to corneal scarring.[62] Although the most commonly identified agents are from the former *N. asteroides* complex and *N. brasiliensis*, a broad range of species have now been implicated.[63] With appropriate therapy, keratitis resolves with good visual outcomes.[62]

Colonization

Occasional instances of transient colonization of the respiratory tract and skin by *Nocardia* spp. have been reported and appear to represent aerosol contamination or soil-derived contamination. Colonization of the sputum may be found in patients with underlying pulmonary pathology (e.g., bronchiectasis) or cystic fibrosis who are not receiving steroid therapy and requires no specific therapy. To be considered significant, *Nocardia* should be visible on Gram or modified acid-fast stain, produce a pure or predominant growth in culture, and be isolated repeatedly from clinical specimens. However, the extent to which spontaneously resolving or subclinical pulmonary infection occurs in the population is ill-defined, and at least one leading authority warns against dismissing positive sputum cultures as harmless.[22] The isolation of *Nocardia* from any site in an immunocompromised patient must never be ignored and should prompt appropriate investigations for invasive infection.

LABORATORY DIAGNOSIS

Nocardia spp. are most frequently isolated from respiratory secretions, abscess aspirates, and biopsy specimens. In pulmonary nocardiosis the diagnostic yield is increased by testing repeated sputum specimens, bronchoalveolar lavage, and biopsies.[1] For cutaneous nocardiosis, tissue biopsies are preferred for culture.[1] Specimens should be transported in sterile containers, and the microbiology laboratory should always be informed when nocardiosis is suspected, as the diagnosis may be missed by routine laboratory methods.

Direct smears from such specimens typically show gram-positive, beaded, fine, right-angled branching filaments (<1 µm diameter). Filaments may fragment to form rods and cocci of varying sizes. They are characteristically acid fast by methods such as the modified Kinyoun technique.[2] *Nocardiae* can be identified directly from clinical specimens using nucleic acid tests,[65,66] which may be particularly useful when there has been preexisting antimicrobial therapy or when biopsy specimens have been embedded in paraffin for histopathology.

Standard blood culture media support the growth of nocardiae, but prolonged incubation (up to 2 weeks) and blind subcultures may be required for their detection.[1] Bacteremia, as demonstrated by positive blood cultures, is rare in patients with nocardiosis.[2] In general, *Nocardia* spp. will grow on nonselective media used routinely for culture of bacteria, fungi, and mycobacteria. However, in specimens containing mixed flora (e.g., respiratory secretions), *Nocardia* colonies are easily obscured by those of more rapidly growing bacteria, and the yield is increased by use of selective media, such as Thayer-Martin agar with antibiotics[67] or paraffin agar.[68] Buffered charcoal-yeast extract medium, which is commonly used for selective growth of *Legionella* spp., may also be used for the isolation of *Nocardia* spp. from respiratory specimens.[69] Decontamination methods used for mycobacterial culture are too harsh for *Nocardia* spp. and may substantially reduce the numbers of viable organisms present in the specimen.[70]

Growth of *Nocardia* spp. on solid media take 48 hours to 14 days, but typical colonies are usually seen after 3 to 5 days. They appear as either buff or pigmented, waxy, cerebriform colonies (Fig. 253.6) or have a dry, chalky-white appearance if aerial hyphae are produced.[1] They have a characteristic earthy odor. Smears from cultures often show greater fragmentation of filaments than direct smears from clinical specimens.

Presumptive *Nocardia* isolates were previously classified on the basis of biochemical tests that were relatively expensive, slow, and limited by their inability to differentiate between members of the *N. asteroides* and *N. nova* complexes or to identify newly described species.[1,2] Molecular testing and MALDI-TOF MS can be used to identify *Nocardia* to a genus or species level and, where necessary, isolates should be referred to a reference laboratory.

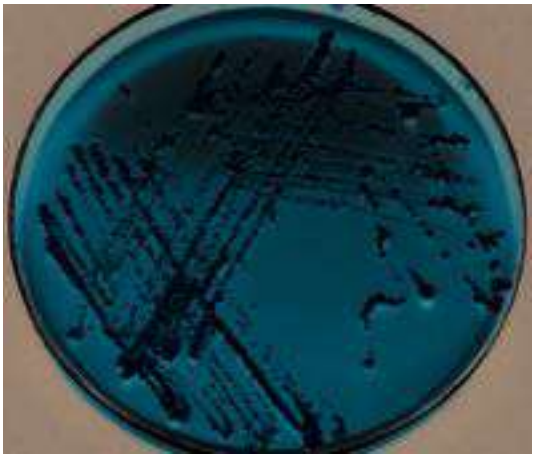

FIG. 253.6 Culture. Typical colonies of *Nocardia* spp. growing on selective media.

Identification of *Nocardia* Species

Molecular techniques can provide definitive identification of most *Nocardia* isolates and recognize new species. An accurate species identification allows the prediction of antimicrobial susceptibilities in many cases.[1] Polymerase chain reaction–amplified DNA can be characterized, analyzed, and compared with archived sequences. Although sequencing of the first 500 to 606 base pairs (bp) of the 5′ end of the 16S ribosomal RNA (rRNA) gene contains sufficient sequence variability for species identification,[2,5,6] sequence analysis of longer gene fragments (e.g., 999–1400 bp) is necessary to distinguish between closely related species, most notably *N. abscessus*/*N. asiatica*/*N. arthritidis, N. africana*/*N. elegans,* and *N. veterana*.[5] Although guidelines of the Clinical and Laboratory Standards Institute (CLSI) recommend that greater than 99.6% similarity is needed for species identification, Conville and associates[1,71] recommend greater than 99.8% homology to differentiate between some species, including those within the *N. nova* complex.

Although housekeeping gene sequences, such as *hsp65, secA1, gyrB, rpoB,* and *sodA,* can improve phylogenetic resolution,[1,72,73] the accuracy of species identification is also dependent on the quality of sequences in gene repositories,[74] and inaccuracies have been found in public databases.[75] Thus comparison with sequences of well-characterized type strains of a species and/or with those in a curated database (e.g., the SmartGene [SmartGene GMbH, Zug, Switzerland]) is necessary for correct identification. Errors may also arise because multiple but different 16S rRNA genes are present in certain *Nocardia* spp., such as *N. nova*.[74–76] Multiple copies of the *gyrB* and *rpoB* genes have also been identified.[77]

Multilocus sequence analysis (MLSA) is a molecular identification technique that uses concatenated sequences from a number of gene targets to clarify the phylogenetic relationships of closely related species.[1] This is regarded as a more effective method of identification, as single-gene targets may be susceptible to recombination, gene transfer, and stochastic variation.[77] MLSA has been useful for the identification of known *Nocardia* spp. and demonstrated the clustering of previously uncharacterized clinical isolates.[4,77–79] Although whole-genome analysis of clinical isolates would be the gold standard in terms of *Nocardia* identification and allow the detection of resistance genes, current limitations to routine laboratory usage include cost, access to next-generation sequencing, and the need to manipulate and analyze large datasets.[80]

MALDI-TOF MS compares the spectra produced by bacterial proteins with a library generated from well-characterized organisms, allowing rapid identification. In a European study, performed after optimization of the spectral library, MALDI-TOF MS identified 91% of 171 isolates

to the species level and 6% to the genus level; species were confirmed by molecular testing.[81] In a multicenter trial the use of three combined libraries resulted in identification at species level in 84% and at genus level in 6%.[3] Reextraction of specimens with a low MALDI-TOF MS identification score (<2.0) and modification of extraction methods have also improved identification.[3,82] In two studies, where in-house libraries were developed, identification to species level was made in 95% and 90.6% of isolates and to genus level in an additional 5% and 9.4%, respectively.[4,82] A strategy for improved *Nocardia* identification would be to use MALDI-TOF MS technology for rapid identification of the majority of isolates and, when identification is uncertain, perform nucleic acid–based molecular identification.[82]

MANAGEMENT

Antimicrobial Selection

Successful management of *Nocardia* infection requires the use of antimicrobial drugs, often in combination, and, where appropriate, surgical drainage or débridement. As there are no randomized trials to determine optimal regimens, recommendations are based on retrospective and observational data, animal studies, in vitro susceptibility profiles, and expert opinion.

Initial selection of a therapeutic regimen should be based on the species of *Nocardia*, the site and severity of infection, the host immune status, and potential drug interactions or toxicity. As clinical isolates of *Nocardia* exhibit variable resistance to antimicrobial agents, antimicrobial susceptibility testing is advised. Although there is a lack of data directly correlating in vitro susceptibility with clinical outcome, with the exception of CNS disease,[83] therapy based on in vitro susceptibility is often effective.

In Vitro Susceptibility Testing

The CLSI has approved broth microdilution methods for antimicrobial susceptibility testing of aerobic actinomycetes and has set interpretive breakpoints for commonly used antimicrobials.[84] Other available methods include the E-test (AB Biodisk; Solna, Sweden) and BACTEC radiometric methods. Both correlate well with broth microdilution results and are easier to use in the routine clinical laboratory.[85]

Susceptibility profiles for commonly isolated *Nocardia* spp. are summarized in Table 253.3. Although resistance patterns can be predicted for some *Nocardia* spp., geographic variability has been reported, and new species for which susceptibility data is lacking continue to be identified.[86]

Discrepancies in susceptibility testing between laboratories have also been well-documented, particularly with testing of TMP-SMX.[86–90] These are largely due to technical issues inherent in broth microdilution methodology and include inoculum preparation, end-point determination, and lack of standardized interlaboratory proficiency testing programs.[87,91] For these reasons, referral of isolates to a specialized reference laboratory is recommended in the following circumstances: if local susceptibility testing is performed and yields data contrary to that expected from published literature, for patients with deep-seated or disseminated infection, for patients intolerant of treatment with TMP-SMX, and for those who fail therapy or relapse after therapy. Isolates of newly described species and of *Nocardia* spp. known to be more inherently resistant should also be considered for susceptibility testing in a reference laboratory.[86,91]

Antimicrobial Regimens

Sulfonamides, most recently in the form of TMP-SMX, have been the mainstay of therapy for *Nocardia* infection since their introduction in the 1940s and have substantially improved outcomes. In cases of disseminated (including CNS) disease and in immunosuppressed patients, mortality rates with sulfonamide monotherapy are greater than 50%,[92–94] and combination antimicrobial therapy is recommended. This should be commenced empirically pending susceptibility results. Amikacin combined with imipenem or meropenem, or three-drug regimens consisting of TMP-SMX, amikacin, and a carbapenem or third-generation cephalosporin, have been used for high-risk patients.[14,15] Increasing experience with linezolid suggests it is effective in combination with the above agents and may have a useful role in initial empirical management of disseminated or CNS *Nocardia* infection pending susceptibility results.[92–94] TMP-SMX, linezolid, carbapenems, and ceftriaxone may be preferred to amikacin-containing regimens in solid-organ transplant populations because of the high incidence of renal impairment in this group.[15] Other agents that have been used in combination regimens include broad-spectrum quinolones, minocycline, and tigecycline.

Trimethoprim-Sulfamethoxazole

TMP-SMX, available in the fixed ratio of 1:5, is the sulfonamide preparation preferred for management of *Nocardia* infection. Synergistic activity between the two drug components has been demonstrated against the majority of *Nocardia* spp. in vitro,[95,96] giving this formulation an advantage over older sulfonamide agents, such as sulfadiazine and sulfisoxazole. Although it is not known whether synergy occurs in vivo, optimal ratios of TMP to SMX for demonstration of synergy in vitro vary from less than 1:5 to 1:10.[96,100] Drug levels in serum and CSF are estimated to reach 1:20[95] and 1:7 or less in tissues and pus, including cerebral abscesses.[96,97]

The recommended dose of TMP-SMX in adults with normal renal function and localized disease is 5 to 10 mg/kg/day TMP and 25 to 50 mg/kg/day SMX given in two to four divided doses, depending on extent of disease.[96] Although use of 5 mg/kg/day (TMP component), together with surgical débridement, is considered sufficient for effective

TABLE 253.3 Antimicrobial Susceptibility of Selected *Nocardia* Species

	N. cyriacigeorgica	*N. farcinica*	*N. nova complex*	*N. transvalensis complex*	*N. brasiliensis*
Trimethoprim-sulfamethoxazole (TMP-SMX)	S	S[a]	S	S	S
Amoxicillin clavulanate	R	S	R	V (0%–53% R)	S
Ceftriaxone	S	R	V (25%–53% R)	V (15%–37% R)	V (13%–66% R)
Imipenem	V (3%–57% R)	V (4%–67% R)	S	R	R
Amikacin	S	S	S	R	S
Linezolid	S	S	S	S	S
Moxifloxacin	R	S,?V[b]	R	S	S
Clarithromycin	R	R	S	R	R
Minocycline	V (15%–95% R)	R	V (44%–88% R)	R	V (23%–76% R)
Tigecycline	V	R	V	ND	S

[a]Recent studies suggest resistance rates of 0.5%–6% to TMP-SMX in *N. farcinica;* however, rates of 45% have been described in Spain.
[b]Generally active with variable resistance in some reports.
ND, No data; *R,* generally inactive; *S,* generally active (some isolates may be resistant); *V,* variable resistance in vitro (published ranges in parentheses).

management of primary cutaneous infection,[96] doses of 15 mg/kg/day TMP and 75 mg/kg/day SMX are used in severe or extensive infection, disseminated infection, cerebral abscess, or AIDS.[60,95] Lower doses of TMP-SMX have been used successfully to treat infection in some immunocompromised patients; for example, 5 mg/kg/day of the TMP component given daily in two doses has successfully treated pulmonary infection in renal transplant recipients. In these cases, however, TMP-SMX was usually prescribed as only one component of a combination therapy regimen.[83,99] Lower-dose regimens using 10 mg/kg/day[100,101] or less[102] of the TMP component have also successfully cured cases of cerebral abscess. Dose reduction of TMP-SMX is recommended in renal impairment.

Therapeutic drug monitoring of serum sulfonamide levels may have a role where absorption from the gastrointestinal tract is uncertain, in patients who require high doses of TMP-SMX and are at risk of dose-related toxicity (renal failure and bone marrow impairment), and in cases of poor therapeutic response. The recommended therapeutic level of sulfonamide, measured 2 hours after an oral dose at steady state, is 100 to 150 mg/L[103]; however, there are minimal data equating sulfonamide levels to toxicity or efficacy.[98]

The decision to use sulfonamides in therapy will in part depend on the causative species of *Nocardia*. Although sulfonamide-resistant *Nocardia* have been reported (see Table 253.3), resistance of *Nocardia* spp. overall to TMP-SMX appears to be between 2% to 3%.[78,86,87] Alternative antimicrobial agents should be considered in infections caused by *Nocardia* demonstrated to be sulfonamide-resistant, in patients who are clinically failing sulfonamide therapy, and in those intolerant of sulfonamide-containing regimens due to hypersensitivity or toxicity. Sulfonamide intolerance has been reported in 3.4% to 8% of exposed non–HIV-infected patients[104,105] and in up to 55% of AIDS patients.[106] Desensitization to TMP-SMX may be considered for those with hypersensitivity, to enable ongoing treatment with sulfonamides. Patients receiving other myelosuppressive agents, such as azathioprine,[107] or nephrotoxic agents, such as calcineurin inhibitors, are at increased risk of sulfonamide-induced myelotoxicity and nephrotoxicity, respectively.

Other Regimens

The choice of alternative therapeutic agents is based on in vitro susceptibility data; efficacy in animal models, primarily short-term murine models of pulmonary and cerebral nocardiosis[108,109]; and published case reports and case series. Assessment of the efficacy of these regimens is complicated by the relative rarity of infection, the diversity of agents that have been used either in combination or sequentially, the variability in host populations studied, and the variable chronic course of *Nocardia* infection.

Amikacin and Carbapenems

Most clinical experience to date has been with amikacin and imipenem. Amikacin exhibits excellent activity in vitro against most species of *Nocardia*, with the exception of *N. amikacinitolerans*, *N. pseudobrasiliensis*, and *N. transvalensis complex,* with resistance to amikacin demonstrated in 57%, 31%, and 72% of isolates, respectively, in one study.[86] Imipenem has good in vitro activity against *N. nova* and many other *Nocardia* spp.; however, resistance has been reported in up to 100% of strains of *N. pseudobrasiliensis* and *N. brasiliensis* and in moderate-to-high numbers of isolates within the *N. transvalensis* complex, *N. otididiscaviarum*, *N. farcinica*, *N. cyricageorgica,* and *N. abscessus*.[86,110] Synergy between amikacin and TMP-SMX has been demonstrated in vitro; with imipenem, the effectiveness is primarily additive.[111]

Amikacin and imipenem have been successfully used, together or in combination with sulfonamides or other agents, in both immunocompromised patients and in patients with *Nocardia* infection involving different body sites.[15,112,113] In one study cure was achieved in seven of eight patients who received amikacin in combination with agents that demonstrated synergy in vitro.[113] Amikacin and imipenem in combination have been recommended as initial parenteral therapy in pulmonary *Nocardia* infection[114] and in severe infection,[14] particularly where there may be concerns regarding TMP-SMX susceptibility or toxicity; however, there are no clinical trials to support this approach. Once-daily dosing regimens of amikacin make it attractive for in-home IV therapy programs; however, the potential for amikacin to cause ototoxicity and nephrotoxicity makes its use problematic in many risk groups that have underlying poor renal function.

Limited data are available on the use of alternative carbapenems (meropenem, ertapenem) in *Nocardia* infection. Meropenem is clinically appealing as it is associated with a lower incidence of seizures compared with imipenem, achieves good CSF penetration, and is active in vitro activity against several *Nocardia* spp.[110,115] Of note, susceptibility of *Nocardia* isolates to meropenem cannot be inferred from susceptibility to imipenem. Imipenem appears to be the most active carbapenem in vitro against *N. farcinica* and *N. otidiscaviarum*,[110,115] whereas meropenem has demonstrated more activity than imipenem in vitro against *N. abscessus* and the *N. transvalensis* complex.[110] Ertapenem demonstrates poor activity against *Nocardia* spp. generally, although some have observed that *N. nova* isolates may be susceptible to all carbapenems, including ertapenem in vitro.[110] A single study of 51 isolates of *Nocardia* spp. concluded that overall meropenem is 4 times less active and ertapenem 16 times less active than imipenem against the species tested.[116] Published reports of the use and efficacy of meropenem in nocardiosis have been in the setting of combination therapy.[15,117–119]

Third-Generation Cephalosporins

The third-generation cephalosporins ceftriaxone, cefotaxime, and cefuroxime exhibit in vitro activity against a variety of *Nocardia* spp., but *N. farcinica* isolates are typically resistant, and variable levels of resistance have been demonstrated in isolates of the *N. transvalensis* complex, *N. pseudobrasiliensis,* and *N. otidiscaviarum*. Greater than 10% of isolates of *N. brasiliensis* and the *N. nova* complex are resistant to ceftriaxone in vitro (see Table 253.3). Synergy between cefotaxime and imipenem has been demonstrated against susceptible strains of *Nocardia* in a murine model of cerebral *Nocardia* infection, where the combination of cefotaxime and imipenem was more effective at reducing colony counts at 72 hours than either agent given alone.[120] Synergistic activity has also been described in vitro between cefuroxime and amikacin.[113]

Oxazolidinones: Linezolid and Tedizolid

The oxazolidinone linezolid exhibits excellent in vitro activity against all known species of *Nocardia*.[86,121,122] and has excellent oral bioavailability and good CNS penetration.[123] Increasing clinical experience suggests that it is efficacious when used in combination with another anti-*Nocardia* agent[92–94,124]; however, its toxicity profile precludes long-term usage. When given for more than 14 days, linezolid is associated with an increased risk of hematologic toxicity,[125] lactic acidosis, retrobulbar optic neuritis, peripheral neuropathy, and the serotonin syndrome. There is some evidence that therapeutic drug monitoring of plasma linezolid levels may reduce the incidence of toxicity; however, results are conflicting.[126–128] In 18 cases of solid-organ transplant recipients treated for *Nocardia* infection, in whom 17 of 18 received TMP-SMX and 15 of 18 received linezolid, the median duration of therapy with linezolid was 21 days.[94] Ten of the 15 (67%) ceased linezolid due to adverse events; thrombocytopenia was noted in 14 of 15 (93%) and anemia in 9 of 15 (60%). However, these rates were similar to those in patients who did not receive linezolid.

Given its potential for toxicity, linezolid may not be appropriate for long-term therapy of *Nocardia* infections. However, it may be useful in initial and empirical management of infection or in combination with other agents, particularly when amikacin may not be an option due to nephrotoxicity, while results of susceptibility tests are pending.

Limited available data suggest that tedizolid, a second-generation oxazolidinone, has greater efficacy than linezolid in vitro with minimal inhibitory concentrations twofold to threefold lower than linezolid against many *Nocardia* spp.[129] Although phase III studies comparing tedizolid with linezolid demonstrated a lower incidence of thrombocytopenia with tedizolid (3.2% vs. 5.6%),[130] there is insufficient clinical experience to recommend its use.[131]

Minocycline and Amoxicillin-Clavulanate

The most frequently used oral alternatives to sulfonamides include minocycline and amoxicillin-clavulanate. Minocycline has the best in

vitro activity of the tetracyclines and has shown efficacy when used alone, in combination with other antimicrobials, as sequential therapy, and in patients intolerant of sulfonamides.[132–134] Reports of failure when used as monotherapy in immunocompromised patients, however, have also been reported.[135]

Amoxicillin-clavulanate has been effective in individual cases when used in combination with other agents or as sequential therapy.[136,137] It may be particularly useful in the treatment of cutaneous infection due to *N. brasiliensis*, a consistent β-lactamase producer, and is the preferred oral agent for this species in patients who cannot tolerate sulfonamides.[138] A case of acquired resistance to β-lactam and β-lactamase inhibitor antibiotics has been described, however, resulting in relapse during therapy of *N. brasiliensis* with amoxicillin-clavulanate.[139] Other species with susceptibility to amoxicillin-clavulanic acid include *N. abscessus* and *N. farcinica*.[2] The use of amoxicillin-clavulanate should be guided by in vitro susceptibility results; demonstration of β-lactamase production is not necessarily predictive of resistance to β-lactam drugs, and isolates of the *N. nova* complex can test ampicillin susceptible/intermediate but may be resistant to amoxicillin-clavulanic acid.[2]

Fluoroquinolones, Macrolides, and Tigecycline

In vitro susceptibility results should also be used to guide the choice of alternative agents for which few clinical data are available, such as the macrolides, fluoroquinolones, and tigecycline. Moxifloxacin demonstrates activity against several *Nocardia* spp., including *N. farcinica*, the *N. transvalensis* complex, *N. pseudobrasiliensis,* and *N. brasiliensis*, and has greater activity in vitro than ciprofloxacin.[86,140] Published clinical experience in patients treated with moxifloxacin has mainly been as salvage therapy in infections due to *N. farcinica*, or in combination with other agents or surgical débridement. Results have been mixed, with both successful and poor outcomes reported.[141,142]

Clarithromycin is the macrolide most commonly used for treatment of *Nocardia* infections and exhibits good activity in vitro against *N. nova*.[143] This agent has been used successfully to treat *N. nova* infections as part of combination therapy, as sequential therapy, and in cases of sulfonamide intolerance.[144,145] Limited in vitro data on use of the IV drug tigecycline suggest that it has activity against a variety of *Nocardia* spp., including imipenem-resistant and TMP-SMX–resistant isolates; however, no clinical efficacy data are available.[116,146]

Superficial Infection and Mycetoma

Localized or isolated cutaneous disease in the immunocompetent host may be treated with monotherapy. Oral TMP-SMX is preferred; however, other agents, including minocycline, amoxicillin-clavulanate, fluoroquinolones, and macrolides, have been used depending on isolate susceptibility.

Nocardia Intravascular Catheter-Related Bloodstream Infection

Nocardia central line–associated bloodstream infections are rare and occur primarily in immunocompromised patients. Early line removal, together with combination antimicrobial therapy, is recommended.[17]

Severe Infection

Empirical Therapy

Although TMP-SMX as monotherapy may be successfully used in mild pulmonary infection with no evidence of dissemination, combination antimicrobial therapy with at least two agents is recommended for immunocompromised hosts, those with more than one site of infection not involving the CNS, and any patient with severe pulmonary involvement or isolated CNS disease. Recommended regimens include amikacin and imipenem or meropenem, or amikacin and TMP-SMX. Although two-drug therapy may be suitable in cases of isolated cerebral disease, when additional sites are involved, or infection is life threatening, three-drug regimens that cover all likely pathogenic *Nocardia* spp. are recommended, for instance, TMP-SMX plus imipenem or meropenem, plus amikacin or ceftriaxone (or linezolid in patients with renal failure). Empirical therapy should be modified after *Nocardia* speciation and susceptibility results.

Surgical Management

The need for surgical management of *Nocardia* infection depends on the site and extent of disease.

Surgery in Extraneural Nocardiosis

In extraneural infection, indications for aspiration, drainage, or excision of abscesses are similar to those for other chronic bacterial infections. Thick-walled, multiloculated abscesses are unlikely to be managed successfully with therapeutic aspiration alone.[147]

Surgery in Cerebral Nocardiosis

Surgical management is advised for brain abscesses that are accessible and relatively large, if the patient's condition deteriorates or lesions progress within 2 weeks of therapy, or when there is no reduction in abscess size within a month.[148] Stereotactic aspiration is useful for decompression of cerebral abscesses, but cure is often achieved only after craniotomy and complete excision of the abscess.[147] Small cerebral abscesses may be cured by prolonged antimicrobial therapy. As cerebral abscesses can progress despite appropriate therapy, all patients should be monitored with regular cerebral CT scans or MRI. Surgical intervention may be required for successful management of empyemas and mediastinal collections. Pericarditis complicating pulmonary *Nocardia* infection is often fatal unless pericardial drainage is performed.[149]

Keratitis and Other Eye Infections

Keratitis

In localized *Nocardia* keratitis, such as infection after LASIK (laser-assisted in situ keratomileusis) surgery, topical amikacin (1.5%–2.5% solution) has most commonly been used[62] due to its activity in vitro against *Nocardia* spp., its good corneal penetration with high local concentrations in the eye, and its safety profile.[55] In a series of 111 cases of culture-proven *Nocardia* keratitis in India, 89.7% of isolates were reported as amikacin susceptible.[150] Treatment consisted of topical amikacin 2.5% eye drops administered hourly with the addition of oral TMP-SMX in cases where response was slow or symptoms worsened; 82% resolved with medical treatment alone. The average duration of treatment for keratitis was 38 days.

Other antimicrobials that have been used topically in *Nocardia* keratitis include TMP-SMX preparations, tobramycin, ciprofloxacin (0.3%), and moxifloxacin (0.5%).[151] Pretreatment of *Nocardia* keratitis with topical quinolone preparations has been associated with increased resistance to moxifloxacin in *Nocardia* spp. in vitro.[152] Topical corticosteroids should be used with caution in *Nocardia* keratitis. In a randomized, placebo-controlled trial comparing topical corticosteroid use with placebo in bacterial keratitis, corticosteroids were associated with worse clinical outcomes at 3 months and with larger scar size at 12 months in patients with *Nocardia* keratitis compared with those who did not receive topical prednisolone.[153]

The use of systemic (oral or IV) antimicrobial therapy depends on the clinical context.

Endophthalmitis

Nocardia is a rare cause of endophthalmitis, either exogenous from surgery or trauma, or, even less commonly, hematogenous. Relatively poor outcomes have been noted after use of intravitreal amikacin in patients with *Nocardia* endophthalmitis despite susceptibility of the causative species to amikacin in vitro (100% and 90%, respectively).[62] Thus systemic TMP-SMX is usually given. Antimicrobial choice depends on species and results of susceptibility testing (see Table 253.3). Intravitreal amikacin carries with it a small risk of macular infarction.[154]

Duration of Therapy and Prognosis

Clinical Responses to Therapy

Clinical improvement is generally evident within 3 to 5 days[108] or, at most, 7 to 10 days after the initiation of effective therapy.[101] Parenteral therapy can usually be safely changed to an oral regimen after 3 to 6 weeks, depending on clinical response. Initial high doses of TMP-SMX may also be reduced at this time. Patients with extensive infection, necrotic foci not amenable to surgery, or those who respond slowly may benefit from prolongation of parenteral and subsequently oral therapy.[14]

Causes of Therapeutic Failure

Lack of response to initial therapy may be due to primary drug resistance; inadequate penetration of drug into sites of infection (dependent on dose, bioavailability of oral drugs, abscess location and pathology, and patient compliance); the presence of a sequestered abscess requiring surgical drainage; and, in an immunocompromised host, overwhelming nocardial infection or a coexisting or secondary opportunistic infection.

Management of Immunosuppressive Drug Therapy in Patients With Nocardiosis

Reduction or cessation of immunosuppressive drugs may be required if *Nocardia* infection is uncontrolled or progressive despite therapeutic serum levels of antimicrobial agents.

Duration of Therapy in Immunocompetent Hosts

Recommendations for the duration of therapy are empirical and based primarily on reports of relapse after sulfonamide therapy of different durations.[a] Isolated cutaneous infection, including sporotrichoid nocardiosis and superficial ulcers, can be cured with 1- to 3-month courses of therapy.[96] Prolonged therapy is required in patients with mycetoma.[147] Immunocompetent patients with pulmonary or systemic *Nocardia* infection and without CNS involvement should be treated for a minimum of 6 months. Those with CNS infection should be treated for 12 months.[14]

Duration of Therapy in Immunosuppressed Hosts

HIV-negative, immunosuppressed patients with isolated pulmonary disease should be treated for at least 6 months and those with disseminated disease for 6 to 12 months, depending on the degree of immunosuppression and response to therapy. Therapy should be continued for 12 months or longer if there are intercurrent increases in immunosuppression (e.g., due to episodes of graft rejection). For patients who must be maintained on steroid or cytotoxic therapy after successful treatment of *Nocardia* infection, prolonged low-dose, maintenance antimicrobial therapy or secondary prophylaxis may be required (see section "Prophylaxis").

In patients with AIDS, early institution of a prolonged primary course of antinocardial therapy is essential, as treatment of patients with late presentations or of those with relapsed nocardial infection has usually been unsuccessful.[59] Although secondary prophylaxis has traditionally been recommended in patients with AIDS at the completion of *Nocardia* therapy, restoration of the immune system with antiretroviral therapy has been associated in one report with clinical cure of *Nocardia* infection in the absence of appropriate therapy, suggesting immune restoration may abrogate the need for secondary prophylaxis.[157]

Short-Course Therapy

Cure of extrapulmonary abscesses has been described in a few cases treated with a short course of amikacin (7–8 weeks) with amikacin and surgical drainage[43,158] or, in a case of cerebral nocardiosis, amikacin plus ceftriaxone.[159]

Prophylaxis

Primary Prophylaxis

Primary prophylaxis against *Nocardia* infection is not usually recommended for immunosuppressed patients posttransplantation as the incidence of infection is low. Of note, prophylaxis against *P. jirovecii* infection is often prescribed in this patient group using TMP-SMX two or three times weekly. This regimen does not prevent infection with *Nocardia,* and substantial numbers of patients develop nocardiosis after solid-organ or stem cell transplantation while on such prophylaxis.[15,48,146,160,161]

Secondary Prophylaxis

The efficacy of secondary prophylaxis to prevent relapse or recurrence of *Nocardia* infection has not been determined. It may be considered for patients with persisting immunosuppression, once therapy for *Nocardia* infection is completed. TMP-SMX at a dose of one double-strength tablet daily (800 mg SMX component) has been used; however, there are no efficacy or outcome data to support this approach.[102,116]

[a]References 59, 95, 96, 100, 155, 156.

Clinical Outcomes

The clinical outcome of *Nocardia* infection depends on underlying host factors, site and extent of disease, the infecting *Nocardia* spp., and duration of therapy.

Worse outcomes have been reported in immunosuppressed patients compared with nonimmunosuppressed patients. A mortality of 70% was described in one study of 27 HIV patients with advanced immunosuppression; however, all but one patient had received monotherapy for their *Nocardia* infection.[59] Mortality rates of 55% have been reported in immunocompromised patients with CNS disease compared with 20% in nonimmunocompromised patients.[148] *Nocardia* infection of the CNS has been associated with worse outcomes in general when compared with other sites of infection. Mortality rates of 47.8% have been reported in CNS infection compared with 7.6% with pulmonary infection alone.[156] Cure rates of up to 80% were described in disseminated disease compared with 60% in CNS disease in another study.[95] Isolated cutaneous disease generally has a 100% cure rate. Mortality rates are higher in immunocompromised patients with *Nocardia* infection than in matched controls without *Nocardia* infection. A case-control study of 35 solid-organ transplant recipients demonstrated a 6-month mortality rate of 14% compared with 4% in non-*Nocardia* control subjects. Cure rates of 89% were described in the study regardless of site of infection, although those with disseminated infection received prolonged therapy (median, 7.5 months) compared with those with nondisseminated infection (median, 6 months).[15] A recent case-control study of 117 solid-organ transplant recipients showed a mortality rate of 16.2% at 1 year compared with 1.3% in non-*Nocardia* control subjects.[119] A multivariate analysis of this population revealed that a history of tumor, invasive fungal infection in the preceding 6 months, and donor age were independent risk factors for mortality from *Nocardia* infection at 1 year (rather than site, dissemination, or antimicrobial regimen). Of interest, a prior episode of acute rejection was associated with improved survival from *Nocardia* infection. Exposure to high-dose steroids, cytomegalovirus infection in the preceding 6 months, and high median calcineurin levels within 30 days have previously been identified as independent risk factors for the development of *Nocardia* infection in organ transplant recipients.[15]

Although immunosuppressive therapy increases the risk of pulmonary and disseminated nocardiosis in recipients of organ transplants, it is not clear to what extent continuation of immunosuppressive therapy during treatment of nocardiosis has an impact on outcome. Most patients can be cured with appropriate antimicrobial therapy even if immunosuppressive drugs are continued, provided that the diagnosis is made early and appropriate full-dose antinocardial therapy is continued for an adequate period of time.[136,160] On the other hand, delay in diagnosis and early cessation of therapy have been associated with increased rates of relapse and worse outcomes.[59,96,156]

Effect of *Nocardia* Species on Outcome

Some *Nocardia* spp. may be more virulent and associated with worse clinical outcomes. Isolates of *N. farcinica* were more virulent than other species in a mouse model,[44] and in a series of 20 microbiologically confirmed cases of CNS nocardiosis, 6 of 7 deaths reported at 1 year were attributed to *N. farcinica*.[162] Multidrug-resistant isolates may also be predicted to have worse outcomes, depending on the availability of effective antimicrobial choices.

Summary of Management

The choice and dose of antimicrobial drugs and the duration of therapy depend on the sites and extent of infection, underlying host factors, the species of *Nocardia*, and the clinical response to initial management. TMP-SMX remains the mainstay of therapy. Initial combination therapy is indicated in patients who are immunosuppressed or who present with brain involvement or disease involving multiple sites. Oral maintenance therapy can be initiated after 4 to 8 weeks, depending on the severity of infection and the clinical response. Newer, orally active drugs may be of value as short-term adjunctive or salvage therapy (linezolid), or as salvage or maintenance therapy when supported by susceptibility testing (late-generation quinolones or macrolides).

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The complete reference list is available online at Expert Consult.

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254 Agents of Actinomycosis

Thomas A. Russo

SHORT VIEW SUMMARY

Definition of Actinomycosis
- Actinomycosis is an indolent infection caused by *Actinomyces* and closely related species.
- Characteristics include chronicity, crossing of tissue boundaries, and masslike features.
- Bulky disease mimics malignancy.
- Sinus tract(s) may develop, resolve, and recur.
- Refractory or relapsing infection may occur after a short course of therapy.
- Not all infections due to *Actinomyces* spp. manifest with the clinical syndrome of actinomycosis.

Microbiology
- *Actinomyces* is a genus of gram-positive, filamentous bacteria.
- Most species are anaerobes; a few are microaerophilic.
- "Companion organisms" are usually present.

Epidemiology and Pathogenesis
- Actinomyces is a human commensal organism of the oral, gastrointestinal, and pelvic mucosa.
- Disruption of the mucosal surface is the initiating factor for infection.
- Individuals of all ages and normal hosts may be infected.
- Actinomycosis is associated with intrauterine devices and bisphosphonates.

Clinical Manifestations
- All organs and sites can be involved.
- Oral-cervicofacial manifestations are the most common.
- A classic presentation is a painless mass at the angle of the jaw.
- Alternative presentations are myriad.

Diagnosis
- The diagnosis of actinomycosis is challenging. The condition is often missed or mistaken for cancer.
- Sulfur granules (grains) are distinctive clusters of organisms is tissue.
- The laboratory should be informed about suspected actinomycosis.
- Prior antibiotics can inhibit growth on culture.
- There is an increasing role for polymerase chain reaction assays and perhaps matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.
- The diagnosis is often made from the appearance of grains at pathologic evaluation after surgery that may have been unnecessary.

Therapy
- Therapy is based on clinical experience and is individualized.
- Treatment involves a high-dose and prolonged course of antibiotics.
- A standard regimen involves administration for 2 to 6 weeks intravenously, often penicillin G 3 to 4 million units intravenous every 4 hours, followed by 6 to 12 months orally.
- Imaging is helpful in defining duration.
- The most extensive experience has been with penicillin, tetracyclines, and erythromycin (see Table 254.1).
- A short course of treatment directed against companion organisms may be warranted.
- Medical therapy usually is curative, even with extensive disease.
- Interventional radiology is valuable for drainage of accessible abscesses.
- Surgery is reserved for critical sites (e.g., central nervous system) and refractory disease.

Actinomycosis is an indolent, slowly progressive infection caused by anaerobic or microaerophilic bacteria, primarily from the genus *Actinomyces,* that normally colonize the mouth, colon, and vagina. Disruption of mucosa may lead to infection of virtually any site. When the organisms invade tissue, they form tiny, sometimes visible clumps, called grains or "sulfur granules," named for their yellow color. Lesions of actinomycosis are purulent foci surrounded by dense fibrosis. Classic presentations that should prompt consideration of this unique infection are (1) the combination of chronicity, progression across tissue boundaries, and masslike features, which mimics malignancy (with which it is often confused); (2) the development of a sinus tract, which may spontaneously resolve and recur, from which sulfur granules (grains) may be seen in the discharge or at histopathologic/cytologic assessment; and (3) a refractory or relapsing infection after a short course of therapy, because cure of established actinomycosis often requires prolonged treatment.

Although actinomycosis was common in the preantibiotic era, today its incidence is diminished.[1,2] Even when actinomycosis was more common, it was stated to be "the most misdiagnosed disease" and that "no disease is so often missed by experienced clinicians."[3] Actinomycosis remains a diagnostic challenge because it is uncommon, physician experience with its clinical manifestations is limited, and laboratory cultivation and identification are challenging.[4,5] However, this infection is usually curable with medical therapy alone. Therefore an awareness of myriad presentations can expedite diagnosis and treatment and can minimize unnecessary surgical interventions, morbidity, and mortality that all too often occur with actinomycosis.

ETIOLOGIC AGENTS

Actinomycosis is most commonly caused by the gram-positive higher bacterium *Actinomyces israelii*.[6] Additional species that are established but are less common causes of actinomycosis include *Actinomyces naeslundii,*[7] *Actinomyces viscosus,*[8] *Actinomyces odontolyticus,*[9] *Actinomyces gerencseriae,*[10] *Actinomyces graevenitzii,*[11] and *Actinomyces meyeri*, which has been increasingly described in some studies.[12,13] *Pseudopropionibacterium propionicum* (formerly *Propionibacterium propionicum* and *Arachnia propionica*) has been described as a cause of actinomycosis,[6,14,15] but most reports primarily describe this pathogen as causing lacrimal canaliculitis[16] and endodontic infections.[10]

Until recently, classification of *Actinomyces* spp. was based on differences in the results of phenotypic testing (see "Diagnosis" later). However, advances in microbiologic taxonomy, using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and genotypic methods such as comparative 16S ribosomal RNA (rRNA)[16,17] or sequencing of alternative genes,[18] have led to the identification of many new *Actinomyces* species from both human and animal specimens.[10] Many *A. naeslundii* isolates are now reclassified as *Actinomyces oris.* These methods have also led to the reclassification of certain *Actinomyces* species as *Trueperella (Arcanobacterium), Actinotignum (Actinobaculum),*[19] or *Cellulomonas.*[10,16,20,21] Presently, 47 species and 2 subspecies have been recognized.[22] Twenty-five species have caused infections in humans,[10] although the "classic" syndrome of actinomycosis has been variably described.[23,24] *Actinomyces europaeus,*[16,20,25,26,27] *Actinomyces neuii,*[26,28–31] *Actinomyces radingae,*[16,20,26,32,33]

Actinomyces turicensis,[16,20,24,26,32] *Actinomyces georgiae,*[34] *Actinomyces urogenitalis,*[35] *Actinomyces funkei,*[26,36,37] *Actinomyces hongkongensis,*[38] *Actinomyces houstonensis,*[26] *Actinomyces massiliensis,*[39] *Actinomyces timonensis,*[40] and *Actinomyces cardiffensis*[23,41] are capable of causing a variety of human infections. *A. neuii* most commonly has been reported to cause endovascular infection and abscesses.[42] *Trueperella (Arcanobacterium) pyogenes*[43] and *Trueperella (Arcanobacterium) bernardiae*[44,45] are additional causes of human infection.

When an adequate bacteriologic evaluation is performed, most if not all actinomycotic infections are polymicrobial in nature.[1,6] *Aggregatibacter (Actinobacillus) actinomycetemcomitans, Eikenella corrodens, Fusobacterium, Bacteroides, Capnocytophaga, Staphylococcus, Streptococcus,* and Enterobacteriaceae have been isolated in various combinations, depending on the site of the infection. The contribution of these additional isolates to the pathogenesis of actinomycosis is difficult to assess; however, it seems reasonable to consider them as being potential copathogens when one is designing therapeutic regimens.

EPIDEMIOLOGY

The agents of actinomycosis have been clearly established as members of the endogenous flora of mucous membranes. The frequency of oral cavity colonization with *Actinomyces* is nearly 100% by 2 years of age.[46] It is also often cultured from the gastrointestinal tract, bronchi, and female genital tract. It has never been cultured from nature, and there are no documented cases of person-to-person transmission.[47] Although the normal habitats for the more recently identified *Actinomyces* spp. have not been optimally defined, data available to date suggest that these species are also members of the endogenous oral, gastrointestinal, and genital flora.[26] Possible transmission via a lamb bite has been described in a single report.[48]

Actinomycosis has no geographic boundaries. Infection may occur in individuals of all ages. The peak incidence of actinomycosis is reported to be from 30 to 60 years, with cases in individuals younger than 10 and older than 60 years being less frequent.[6,49] Nearly all series have reported males to be infected more frequently than females, at a ratio of approximately 3:1.[1,6,49,50] Plausible but unproven explanations for this discordance include poorer dental hygiene and increased oral trauma in males.[49]

Studies on the occurrence of actinomycosis estimated a yearly incidence of 1:100,000 in the Netherlands and Germany in the 1960s and 1:300,000 in the Cleveland area during the 1970s, making this disease uncommon but not rare.[49] Its frequency has undoubtedly diminished since the preantibiotic era, when this disease was not only common but more malignant in nature. Improved dental hygiene and early antimicrobial treatment of infections before the development of a characteristic classic actinomycotic syndrome are likely contributing factors. However, individuals or populations that do not have access to dental or medical care, or both; prolonged use of an intrauterine contraceptive device (IUCD) (see "Pelvic Disease" later); and bisphosphonate use (see "Oral-Cervicofacial Disease" later) are associated with higher risk. Furthermore, many unrecognized cases probably occur, especially oral-cervicofacial disease, owing to early successful empirical therapy.

PATHOGENESIS AND PATHOLOGY

A pivotal step in the pathogenesis of actinomycosis is disruption of mucosal or epithelial barriers enabling entry of colonizing *Actinomyces* or related genera. Oral and cervicofacial disease is frequently associated with dental procedures, trauma, oral surgery, head and neck radiotherapy, or oncologic surgical procedures.[51] Pulmonary infections often arise in the setting of aspiration, and abdominal infection is usually preceded by conditions that result in loss of mucosal integrity, such as surgery, diverticulitis, appendicitis, or foreign bodies (e.g., fish bones).[1,49] Recognition of factors that enable bacterial entry into deep tissues, however, may be absent.[50] The lack of such a history should not prevent consideration of this disease when the clinical circumstance is appropriate.

Other bacterial species concomitantly present have been designated as "companion organisms." They may serve as copathogens by aiding in the inhibition of host defenses or by reducing oxygen tension. The difficulty in establishing an animal model of infection with *Actinomyces* alone and enhancement of infection by coinoculation of *E. corrodens* support the concept that additional organisms are important for the initiation of infection.[52] Furthermore, coaggregation of *Actinomyces* and *Streptococcus* spp. results in increased resistance to phagocytosis and killing.[53]

An acute inflammatory phase manifesting with a painful cellulitic reaction is occasionally observed with oral-cervicofacial disease or with soft tissue infection elsewhere in the body. The chronic phase of this disease is more often seen.[50] "Classic" disease is characterized by a densely fibrotic lesion that undergoes slow, contiguous spread that ignores tissue planes. However, the factor(s) responsible for the unique pathogenesis of this disease remain undefined. Lesions usually appear as either single or multiple indurated swellings. Over time, central fluctuance and suppuration develop. The fibrous walls of the mass have been characteristically described as "wooden" and, in the absence of suppuration, have been frequently confused with neoplasms. This extensive fibrosis, which is one of the hallmarks of this disease, may be minimal, especially in pulmonary and central nervous system (CNS) lesions. Given time, sinus tracts will often extend from the abscess to either the skin or adjacent organs or bone, depending on the location of the lesion. Sinus tracts can spontaneously close and then reform. Overlying skin may assume a red to bluish hue. Lymphatic spread and associated lymphadenopathy are uncommon. Hematogenous dissemination can occur from local sites, which occasionally results in fulminant infection, although in the antibiotic era this clinical syndrome has become rare.

Microscopically, lesions have an outer zone of granulation, consisting of collagen fibers and fibroblasts. There is a central purulent loculation that contains neutrophils that surround the sulfur granules present. Granules are conglomerations of organisms and are virtually diagnostic of this disease. Bacterial biofilms may contribute to granule formation.[54] One to six may be present per loculation, and they range from microscopic to macroscopic in size (see "Diagnosis" later). As many as 50 loculations may be present per lesion, and these loculations are separated by granulation tissue or foamy macrophages and may undergo coalescence. Lymphocytes and plasma cells are usually present, and eosinophils are seen in 15% of abscesses. Multinucleated giant cells are occasionally seen, primarily in pulmonary lesions, but they have also been described in disease elsewhere.[50] Suppuration is a constant feature of active disease but may not be present in all areas of the lesion. Grains are usually surrounded by neutrophils and may be sporadic in biopsy tissue; additional sections may be required for them to be seen.

Foreign bodies appear to facilitate infection. This association has been most commonly observed with IUCDs and pelvic actinomycosis.[55] Associations with actinomycosis and foreign material elsewhere are less strong. Aspirated, ingested, or implanted foreign bodies may contribute to pathogenesis via facilitating the growth and survival, and biofilm formation.

Cases of actinomycosis have been described in the settings of steroid use,[56] anti–TNF-α agents,[57,58] bisphosphonate treatment,[59] radionecrosis,[59,60] acute leukemia during chemotherapy,[61] organ transplantation,[62] common variable immunodeficiency,[48] chronic granulomatous disease,[63] and human immunodeficiency virus (HIV) infection.[64] Ulcerative mucosal lesions (herpes simplex virus, cytomegalovirus, chemotherapy) and abnormalities in host defenses likely facilitated the development of actinomycosis in some HIV-associated cases; however, it remains unclear which arm(s) of the host defense are critical in preventing or controlling this infection and the degree to which the incidence of infection is increased in these settings.

CLINICAL MANIFESTATIONS

Oral-Cervicofacial Disease

Actinomycosis most commonly occurs and is best recognized in the oral-cervicofacial location, with a mean of 55% of cases.[49]

Oral-cervicofacial disease can manifest as a soft tissue swelling, an abscess, a mass lesion,[49,65] or, occasionally, an ulcerative lesion.[66] The diagnosis of actinomycosis not only should be considered in the classic setting of a painless mass at the angle of the jaw (Fig. 254.1) but should be included in the differential diagnosis of any lesion in the head and neck region. When lesions appear solid, neoplasm is the usual diagnostic consideration. Pain, fever, and leukocytosis are variably present.[49] Soft



FIG. 254.1 Submandibular actinomycotic abscess.

tissue infections of the head and neck may also manifest as chronic, recurring abscesses, particularly if the treatment course is short.

Periapical and endodontic infection caused by *Actinomyces* probably occurs far more frequently than is recognized.[67] Appropriate dental intervention and antibiotic therapy usually result in cure before more extensive disease develops.

The most common location for diagnosed actinomycosis is the perimandibular region, but the cheek, submental space, retromandibular space, and temporomandibular joint also may be affected.[68,69] As noted, a hallmark of this disease is the potential for contiguous extension. Spread to the skin may result in sinus tract(s) formation, and these can spontaneously close and open elsewhere. The overlying skin often develops a bluish or purplish red hue. Involvement of the muscles of mastication frequently occurs, resulting in trismus.[1] Associated mandibular periostitis or osteomyelitis may also be present but is surprisingly infrequent.[70] A lytic lesion, rarefaction with sclerosis, or sclerosis alone may be seen, and this latter pattern may be confused with tumor.[71] Maxillary disease, including osteomyelitis, occurs less frequently.[72] Maxillary and ethmoid sinusitis may occur as isolated disease or can be concomitant with infection of the maxilla.[73,74] The hard palate may also be involved, with presentation as a mass lesion.[1,75]

Two clinical settings more recently have been recognized for contributing to an increased incidence of actinomycotic infection of the mandible and maxilla.[59] The first is infected osteoradionecrosis, a complication of radiation therapy used in the treatment of head and neck cancer. The second has been termed *bisphosphate-associated osteonecrosis* (Fig. 254.2). Bisphosphonates are increasingly used to reduce bone disease, particularly due to multiple myeloma and for the prevention of osteoporosis. Although our understanding of the pathogenesis of these syndromes remains incomplete, it appears that the first insult is radiation therapy or bisphosphonates, or both, altering the local host defenses, followed by disruption of the mucosa, usually from dental procedures, thereby enabling *Actinomyces* to access and infect the gingiva and bone.[76,77]

Isolated masses or ulcerative lesions can also occur in the tongue,[78] vallecula,[79] nasal cavity,[80] nasopharynx,[81] soft tissues of the head and neck,[49,82] salivary glands,[83] patent thyroglossal duct,[84] thyroid,[85] branchial fistula or cleft cyst,[86] and hypopharynx, larynx, or both.[87,88] Although grains are frequently seen in tonsillar clefts at histopathologic assessment of excised tonsils, they appear not to be the cause of recurrent tonsillitis or obstructive tonsillar hypertrophy. The absence of a pathologic tissue reaction around the grain makes a causal role for both of these entities unlikely.[89–92]

Actinomycosis is also an uncommon but important cause of otitis media; untreated cases may result in fatal extension into the mastoid and then the CNS. It is characterized by numerous episodes of otitis media that transiently respond to conventional short-course therapy and resistance to myringotomy. Diagnosis can be made through pathologic and microbiologic examination of infected material from the affected middle ear that may appear to be a cholesteatoma.[93] Infection of the external ear and temporal bone may occur from the spread of middle ear disease.

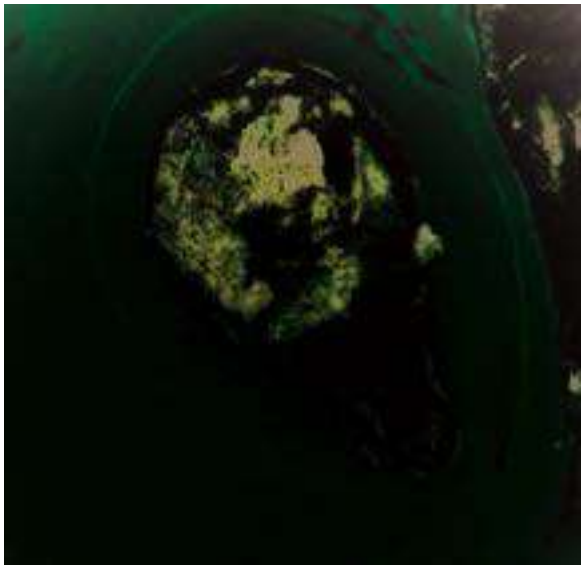

FIG. 254.2 Maxillary osteomyelitis due to *Actinomyces viscosus* in a patient with multiple myeloma who was treated with bisphosphonates and underwent several dental procedures. Pictured is a sulfur granule within necrotic maxilla.

Actinomyces and *P. propionicum* can cause lacrimal canaliculitis.[94,95] *Actinomyces* also has caused postoperative endophthalmitis after intraocular lens implant[96] or intravitreal injection,[97] and keratitis after laser-assisted in situ keratomileusis.[98]

The scenario of temporary improvement with a short course of empirical antibiotic therapy, followed by relapse, should always arouse suspicion for actinomycosis, regardless of the location. As the disease spreads to adjacent structures, there is little regard for normal tissue planes. Computed tomography (CT) scans or magnetic resonance images usually reveal an infiltrative, well- or ill-defined mass with inflammatory changes; associated lymphadenopathy is uncommon.[99,100] Extension to any contiguous structure may occur, including the carotid artery, orbital cavity, cranium, cervical spine, trachea, or thorax.[101,102,103,104]

Thoracic Disease

Thoracic involvement comprises approximately 15% of cases of actinomycosis.[105] Aspiration of organisms from the oropharynx is the usual source of infection, which may be facilitated by foreign material or foreign bodies. Direct extension can occur from disease in either the head and neck or the abdominal cavity; however, such secondary spread has become increasingly uncommon since the advent of efficacious antimicrobial therapy.

The most common clinical presentation is an indolent, slowly progressive process that involves some combination of the pulmonary parenchyma and pleural space. Chest pain, fever, weight loss, and, less commonly, hemoptysis are prominent symptoms, and a cough, when present, is variably productive.[105,106] A relapsing pneumonia after a short treatment course should make actinomycosis a consideration. There are no specific radiographic manifestations, and any lobe may be involved. Cavitary disease may develop and is more readily detected with CT scans because multiple small cavities are more common than large ones.[107] Hilar or mediastinal adenopathy may be present. The usual appearance is either a mass lesion or pneumonia with or without pleural involvement (Fig. 254.3).[100,108] Pleural thickening, effusion, or empyema is present in more than 50% of cases of thoracic actinomycosis (see Fig. 254.3B). Rarely, actinomycosis may manifest as an isolated effusion. The extension of an empyema into or through the chest wall (empyema necessitatis) should raise suspicion for this disease (see Fig. 254.3A).[109] Pulmonary disease that extends across fissures or pleura (see Figs. 254.3C and 68.2A), involves the mediastinum, or has contiguous bony disease should suggest actinomycosis.[108] In the absence of these classic scenarios, however, thoracic actinomycosis is almost never suspected. It is mistaken



FIG. 254.3 Thoracic actinomycosis. (A) Chest wall mass. (B and C) Chest radiograph and computed tomography demonstrating the pulmonary infiltrate, pleural effusion, and pleural and chest wall extension *(arrow).* (D) Purulent pleural fluid obtained by means of aspiration. *(Courtesy Dr. C.-B. Hsiao.)*

either for malignant disease, with the diagnosis made by the pathologist after resection, or for an empyema or pneumonia secondary to more usual causes. Tuberculosis, nocardiosis, histoplasmosis, blastomycosis, cryptococcosis, mixed anaerobic infection, bronchogenic carcinoma, lymphoma, mesothelioma, and pulmonary infarction are among the entities confused with pulmonary actinomycosis.[106]

Less common manifestations of thoracic actinomycosis include multiple pulmonary nodules, miliary disease, infection of surgical bronchial stumps, and endobronchial lesions, which may be associated with aspirated foreign bodies.[110,111,112] Primary breast disease either manifests as a persistent or recurring abscess(es) or mimics malignancy.[113] Breast abscess is usually unilateral and nonlactional. The *Actinomyces* species are often not those typical of classic actinomycosis. Infection of a mammary prosthesis has also been described.[114]

Endocarditis, Pericarditis, and Mediastinal Disease

Mediastinal actinomycosis is an uncommon event. The structures within the superior, anterior, middle (heart), or posterior mediastinum can be involved alone or in combination, resulting in a diverse array of clinical presentations. Infection usually results from contiguous spread from the thorax but can arise from perforation of the esophagus, chest trauma, or extension of head and neck or abdominal disease.[115] Involvement of cardiac structures represents the majority of mediastinal infections reported. Pericarditis is most common and may be initially asymptomatic or hemodynamically insignificant; but if it is allowed to progress, cardiac tamponade and constrictive or adhesive pericarditis will develop.[4] Less frequently, myocardial, endocardial, or valvular infection occurs, either via extension from the pericardium[116] or by hematogenous seeding.[117] Involvement of the left common carotid, left subclavian, and aortic arch can occur with superior mediastinal disease.[118] Anterior mediastinal involvement may manifest as an isolated mass or, rarely, superior vena cava syndrome.[119] Concomitant anterior chest wall infection is common, but associated sternal disease is rare. Posterior mediastinal involvement results variably in paraspinous muscle and soft tissue disease, esophageal fistula or encasement, or vertebral body infection. Because of the slow progression of the disease, both vertebral body destruction and new bone formation occur, resulting in a mottled, saw-toothed, or honeycombed appearance of bone on radiograph. The transverse processes, and with disease progression the pedicles and spinous processes, are similarly involved as the bodies, in contrast to their usual sparing with tuberculosis. The corresponding posterior ends of ribs are usually involved, and a typical wavy periostitis may be present, but, unlike in tuberculosis, vertebral body collapse and disk space narrowing are not

usually seen. Extension to the epidural space with spinal cord compression may occur.[120] Primary esophageal disease with and without HIV infection has also been described.[64]

Abdominal Disease

The proportion of reported cases involving the abdomen averages 20%.[49] Any disease, traumatic event, or surgical intervention that allows the agents of actinomycosis to breach the gastrointestinal mucosa has the potential to be complicated by this infection. Appendicitis, especially with perforation, is the most common predisposing event and is associated with 65% of the cases of actinomycosis originating in the abdomen. As a result, the right iliac fossa is the most frequent primary site of abdominal disease, and right-sided abdominal infection is more common than left. It is also one of the potential inciting events for tubo-ovarian infection. Diverticulitis or foreign body perforation of the transverse or sigmoid colon tends to be associated with left-sided disease and accounts for 7.3% of cases, a surprisingly low percentage considering the incidence of diverticulitis. The loss of gastric mucosal integrity from peptic ulcer disease, gastrectomy, or gastric bypass is associated with 4.4% of cases.[121] Additional associations include antecedent bowel surgery, spillage of bile or gallstones during laparoscopic cholecystectomy,[122] typhoid fever, amebic dysentery, chicken or fish bones, trauma, and hemorrhagic pancreatitis. Interesting to note, actinomycosis rarely develops as a consequence of Crohn disease or ulcerative colitis.[123] In a number of cases, the inciting condition is not always apparent. It is important to keep in mind that ascension of IUCD-associated pelvic actinomycosis has become an increasingly recognized source of abdominal disease.[124] Hematogenous dissemination and extension from the thorax are other portals of entry.

As a consequence of the flow of peritoneal fluid, direct extension of primary disease, or both, virtually any abdominal organ, region, or space can be involved, either alone or in combination, regardless of the initial site of infection. Abdominal actinomycosis is perhaps the greatest diagnostic challenge. This infection is rarely considered before the clinical laboratory or pathologist establishes the diagnosis. Months to years usually pass from the time of the inciting event to clinical recognition of this indolent disease.[50] Associated symptoms are generally nonspecific, with fever, weight loss, change in bowel habits, abdominal pain, or a sensation of a mass being most common. Abdominal actinomycosis usually manifests either as an abscess or as a firm-to-hard mass lesion that is often fixed to the underlying tissue and mistaken for tumor.[125] Sinus tracts or extension to either the abdominal wall or perianal region or between the bowel and other organs may develop and mimic inflammatory bowel disease (Fig. 254.4). CT findings usually demonstrate a mass lesion with focal areas of decreased attenuation or a thick-walled cystic mass; enhancement is most often heterogeneous, and adjacent bowel is thickened. Lesions often appear invasive, suggesting a tumor, but associated lymphadenopathy is uncommon.[100,126,127] With colonic involvement, mucosal nodules with associated inflammation may be observed.[128]

Perirectal or perianal disease may result from extension of pelvic infection or, less commonly, abdominal disease. Primary disease occurs with either local mucosal damage or infection of anal crypts. The most common presentation is single or multiple perianal abscesses, sinus or fistula tract formation, or both. Infiltrating masses may develop in the buttock, posterior thigh, scrotum, or inguinal region.[129] Recurrent disease over months to years or wounds that fail to heal after drainage or fistulotomy are clues that should suggest actinomycosis, particularly in the absence of documented inflammatory bowel disease. Strictures of the rectum can also occur and cause an alteration of bowel habits, mimicking primary bowel or metastatic prostatic or pelvic tumors.[130] This presentation is most often due to extension of pelvic disease.

Hepatic infection was present in 5% of all cases of actinomycosis.[50] Spread to the liver occurs via extension from a contiguous abdominal focus or hematogenously from more distant but established abdominal or extraabdominal foci. Most commonly, abdominal surgery occurred several months before the recognition of hepatic actinomycosis.[131] A case associated with a pancreatic stent has also been described.[132] Hepatic involvement is common in disseminated actinomycosis. The entity of primary or isolated disease is presumably hematogenous seeding from cryptic foci. Single or multiple abscesses or lesions suggesting neoplasia are the usual presentation (Fig. 254.5).[133] In general, a more indolent course is observed compared with the more usual causes of pyogenic hepatic abscesses, but companion organisms may contribute to a more

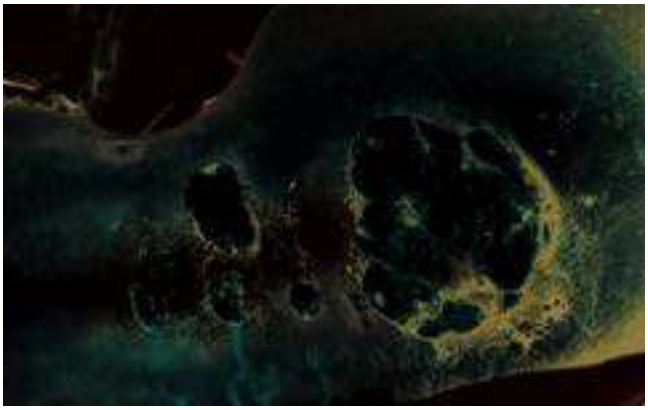

FIG. 254.4 Multiple draining sinuses of the right flank secondary to intraabdominal actinomycosis associated with appendicitis.



FIG. 254.5 Hepatic-splenic actinomycosis. (A) Computed tomogram showing multiple hepatic abscesses and a small splenic lesion due to *Actinomyces israelii* (*inset:* Gram stain of abscess fluid demonstrating beaded filamentous gram-positive rods). Arrow demonstrates extension outside of the liver. (B) Subsequent formation of a sinus tract. *(From Saad M: Actinomyces hepatic abscess with cutaneous fistula.* N Engl J Med. *2005;353:e16.)*

acute presentation.[134] Symptoms and laboratory findings often point to the right upper quadrant, but liver functions test results may be normal. The presently available imaging modalities and percutaneous diagnostic techniques have allowed for an increasing number of cases to be diagnosed without surgery. The intrahepatic mass is usually mistaken on imaging for a malignancy.[131,135] Percutaneous biopsies may fail to be diagnostic because sulfur granules are in scattered foci.[135] Uncommon presentations or sequelae of hepatic actinomycosis include cholangitis, portal vein occlusion or thrombosis, cholestasis, and extension into the thorax.[136,137] Splenic infection is less common.[138]

Actinomycosis of the gallbladder manifesting as cholecystitis or suspected neoplasm can occur but is exceedingly rare,[4] as is pancreatic involvement.[139] "Primary" actinomycosis of the omentum, abdominal wall, and retroperitoneum has been reported, but the majority of these cases are likely due to secondary spread from a cryptic or obscured abdominal source.[140] Isolated peritonitis associated with peritoneal dialysis has also been reported.

All levels of the urogenital tract can be infected by the agents of actinomycosis. Renal involvement can occur as either a result of hematogenous dissemination from a cryptic or defined focus or via direct extension within the pelvis, peritoneum, or thorax. The disease usually manifests as pyelonephritis, renal carbuncle, or perinephric abscess, and is usually mistaken for tuberculosis, neoplasm, or infection from the usual bacterial agents.[141,142] Hematuria and pyuria are often present, and *Actinomyces* can be successfully detected in the urine with appropriate stains and anaerobic cultures. Renal arteriography usually demonstrates a normal or diminished pattern of vascularization. Extension from pelvic disease or rectal disease may result in (1) ureteric obstruction, (2) infiltration, compression, or encasement of the bladder that can lead to hydronephrosis and renal failure, (3) a pubic mass, or (4) vesicocolic, ileovesical, vesicouterine, or vesicocutaneous fistulas.[143,144] Prostatic, testicular, primary vesical, penile, penile prosthesis, pilonidal sinus, and urachal involvement have been described.[42,145–148] Secondary membranous glomerulopathy that resolved with treatment of pelvic actinomycosis has also been described.[149]

Pelvic Disease

Actinomycotic involvement of the pelvis may occur as a consequence of an intraabdominal inciting event, such as appendicitis or rectal disease. However, the most common portal of entry is ascension from the uterus in association with the presence of any type of IUCD, a widely recognized risk factor for pelvic infection.[150,151] Pelvic infection also has been described with oocyte retrieval,[35] with endometrial curettage, and with other pelvic foreign bodies, including pessaries, an endocervical contraceptive device, retained cervical cerclage, a retained hairpin used for an abortion, surgical mesh, and a retained intrauterine fetal bone.[152–154] Although disease rarely develops within the first year after IUCD placement, the risk of infection likely increases with time.[150,151] On average, an IUCD is in place for 8 years in pelvic actinomycosis-associated cases. Pelvic actinomycosis also has manifested after IUCD removal, thereby warranting diagnostic consideration in this setting.[155] Although the precise risk of IUCD-associated actinomycosis has not been quantitated, it appears to be small.

Presentation is typically indolent, with fever, weight loss, abdominal pain, and abnormal vaginal bleeding or discharge being common symptoms. The earliest form of pelvic actinomycosis associated with an IUCD may be endometritis. A pelvic mass or unilateral or bilateral tubo-ovarian abscess represents the next stage of disease progression (Fig. 254.6), which if untreated evolves into a "frozen pelvis" mimicking malignancy. Disease frequently involves the ureters, bladder, or both, resulting in hydroureter and hydronephrosis. Rectal involvement is also common, and entrapment or impingement of small or large bowel may cause fistula or bowel obstruction.[156] Extension to the abdominal wall may lead to sinus tract development. In contrast to malignancy and tuberculosis, ascites and lymphadenopathy are uncommonly observed with pelvic actinomycosis.[157] CA-125 levels may be elevated, especially with more extensive disease, further contributing to deception.[24,156,157] However, CT and magnetic resonance imaging (MRI) may suggest actinomycosis[158] and, if considered, a minimally invasive diagnostic procedure can be performed, thereby ensuring that surgical intervention is appropriately curtailed.[159] Rarely, the presentation of acute peritonitis, disseminated peritoneal lesions, pelvic bone involvement, extension to the thorax, or hematogenous dissemination may occur.[150,160,161]



FIG. 254.6 Intrauterine contraceptive device (IUCD)–associated pelvic actinomycosis. An IUCD encased by endometrial fibrosis *(solid arrow)*, paraendometrial fibrosis *(open arrow),* and an area of suppuration *(open arrowhead)* can be appreciated.

Actinomyces-like organisms (ALOs) are observed on average in 7% of Papanicolaou-stained cervical specimens of women who use an IUCD.[162] This finding alone has a low positive predictive value for diagnosing pelvic infection. The detection of ALOs in the absence of symptoms warrants patient education and close follow-up but not removal of the IUCD, unless an equally suitable means of contraception can be agreed on.[162,163,164,165] Considering the overall woman-years of IUCD use and the limited number of reported cases of pelvic actinomycosis, the risk appears to be small, but the consequences of infection are significant. Therefore, in the absence of more data, it would appear prudent to remove IUCDs if symptoms of pain, abnormal bleeding, or discharge cannot be attributed to other pathogens, regardless of whether *Actinomyces* or ALOs are detected. A 14-day course of a penicillin or tetracycline should be given for treatment of possible actinomycotic endometritis.

Central Nervous System Disease

Actinomycosis of the CNS is rare. The source may be hematogenous, or it may develop through extension of oral-cervicofacial disease. Individuals with hereditary hemorrhagic telangiectasia are at increased risk for brain abscess, with *Actinomyces* organisms being potential etiologic agents.[166] In one report, the mean duration of symptoms before diagnosis was 2.1 months, longer than with most causes of CNS infections.[167] Headache and focal neurologic findings are the most common clinical features. Fever is variably present.

Single or multiple brain abscesses are the usual disease manifestation, most commonly appearing on CT or MRI scans as a ring-enhancing lesion(s) with a thick wall that may be irregular or nodular.[168] Multiloculation, edema, and contiguous areas of low attenuation may be present. These findings are also consistent with brain abscess and tumor.[100,102] Less commonly, solid nodular or mass lesions termed *actinomycetomas* or *actinomycotic granuloma* occur. Magnetic resonance perfusion and spectroscopy findings have also been described.[169]

A chronic meningitis may develop as a consequence of a parameningeal focus, primarily the middle ear or paranasal sinuses. Presentation may be acute, particularly with rupture of an abscess into the subarachnoid space, or chronic, with the cerebrospinal fluid having a normal or low glucose level, elevated protein, lymphocytic pleocytosis, and negative culture. Primary meningitis has also been described.[170] Diagnosis can be made by means of microscopic examination or, rarely, culture of cerebrospinal fluid.

Extension of disease from foci of cranial osteomyelitis, paranasal sinus, or middle ear disease can result in cranial epidural or subdural infection or both.[171] Spinal epidural disease may occur from direct extension of abdominal, thoracic, or cervical disease; is usually associated with a contiguous osteomyelitis; and may result in spinal cord compression.[172] Cavernous sinus syndrome, spinal intrathecal, and ventriculoperitoneal shunt infection have also been reported.[30,173–175]

Musculoskeletal Disease

Actinomycotic infection of the bone is usually a result of an adjacent soft tissue infection but may also be incited by trauma (e.g., fracture of the mandible) or hematogenous spread. Presently, the mandible and maxilla, in association with and without osteoradionecrosis and bisphosphonate therapy, are the most frequent sites of involvement[59,76] (see "Oral-Cervicofacial Disease" earlier). In the preantibiotic era, the unchecked spread of thoracic and abdominal disease resulted in vertebral infection being the most common site for osseous actinomycosis (see "Thoracic Disease" earlier). Actinomycosis of the skull, ribs, clavicle, sternum, scapula, or pelvis may also occur from extension of oral-facial, thoracic, or abdominal disease. The clinical and radiographic features of infection in these locations were discussed earlier.

Infection of the extremities, although uncommon, often poses diagnostic difficulties. Blunt or penetrating trauma, injections, surgery, and hematogenous dissemination from apparent or cryptic foci are initiating events.[12,176,177,178,179] Skin, subcutaneous tissue, muscle, and bone may be involved alone or in various combinations. Cutaneous sinus tracts or abscesses are present in the majority of cases, as is bony involvement in the form of periostitis or acute or chronic osteomyelitis. Presentation is usually indolent. A mycetoma (Madura foot) is an indolent soft tissue infection, usually of the foot, that progresses over months to years. Bacteria or fungi are introduced from soil or vegetation into the soft tissue by minor trauma. When bacterial agents are the cause, the term "actinomycetoma" may be used, whereas the term "eumycetoma" may be used when fungi are the offending agents. Despite the name being suggestive of *Actinomyces* as a causative agent, actinomycetoma is usually caused by *Nocardia* or *Actinomadura* sp.[180] (see Chapter 261).

Actinomycotic infections of hip and knee prostheses have been described in several reports.[181,182] Early presentations suggest that *Actinomyces* may be introduced perioperatively, whereas late presentations suggest hematogenous seeding from a cryptic distant site. Actinomycotic septic arthritis of the knee has developed in association with trauma or injection of hyaluronate,[183] or as a consequence of hematogenous seeding. Popliteal cyst infection has also been described.[184] Actinomycosis is rarely a result of a closed-fist injury.

Disseminated Disease

Although uncommon, all of the agents of actinomycosis are capable of hematogenous dissemination resulting in multiorgan involvement. *A. meyeri* appears to have the greatest capability of causing this syndrome. Disease in any location may serve as the source for spread. The lungs and liver are the most commonly affected organs, and the presentation of multiple nodules mimics disseminated malignancy. The kidneys, brain, spleen, skin, soft tissues of the extremities, and, less commonly, the heart valves may also be infected in various combinations. The clinical presentation may be surprisingly indolent when the extent of disease is appreciated.[12,177,185]

DIAGNOSIS

The diagnosis of actinomycosis, particularly when it mimics malignancy, is rarely considered. All too often the first mention of actinomycosis is from the pathologist after extensive surgery has been performed. However, an increasing body of evidence supports that medical therapy alone is usually sufficient for cure, including extensive invasive disease.[159,167,186,187] Therefore the challenge for the clinician is to consider the possibility of actinomycosis so that this unique infection can be diagnosed in the least invasive fashion and unnecessary surgery can be avoided.[188] Clinical or radiographic presentations that suggest actinomycosis have been discussed previously.[100] Of note, actinomycotic disease has demonstrated hypermetabolism on ^{18}F-fluorodeoxyglucose–positron emission tomography (FDG-PET).[189] Aspirations and biopsies (with or without CT or ultrasound guidance) are being successfully used to obtain clinical material for diagnosis, although surgery may be required.[167,190–192,193] However, transbronchial biopsies have been less successful in providing diagnostic material for thoracic actinomycosis.[112]

The single most helpful diagnostic maneuver for actinomycosis is the demonstration of sulfur granules—a microscopic or macroscopic in vivo matrix of bacteria, calcium phosphate, and host material—in pus (Fig. 254.7A), via cytopathologic evaluation,[194] or in a histologic section of a tissue specimen (Fig. 254.7B–C). Microscopically, granules are round, oval, or horseshoe shaped. Although bacilli within the granule are rarely visible with hematoxylin-eosin stain, the use of tissue Gram stains, Gomori methenamine silver, and Giemsa stains will demonstrate gram-positive, filamentous, branching bacteria at its periphery (Fig. 254.7). On hematoxylin-eosin stain the granules may be eosinophilic or variably surrounded by a radiating fringe of eosinophilic clubs. This eosinophilic, proteinaceous coating around organisms in tissue has been called the *Splendore-Hoeppli phenomenon.* It represents an ill-defined host response but may be accounted for, in part, by eosinophil granule major basic protein. In tissue sections, granules are most commonly found within microabscesses. Although they may be abundant, they are usually scanty. Only a single granule was identified from 26% of specimens in one series.[50] The examination of additional histopathologic sections and the use of positively charged slides to optimize adhesion increases diagnostic sensitivity.[195] Macroscopic granules are usually yellow (hence their name, sulfur granule) but may be white, pinkish gray, gray, or brown. Granules also may be identified grossly from draining sinus tracts, other purulent material, or sputum, but they may easily escape notice unless sought. When pus is poured down the side of a glass tube, granules will adhere and are identified more readily. A magnifying glass may aid in their identification.

A combination of the clinical scenario and stains of the granule can be used to distinguish actinomycotic sulfur granules from others. The Splendore-Hoeppli phenomenon is not specific for actinomycosis and can also be seen in other chronic infections, such as schistosomiasis, orbital pythiosis, cutaneous larval migrans, sporotrichosis, basidiobolomycosis, aspergillosis, blastomycosis, *Malassezia* folliculitis, mycetoma, botryomycosis, nocardiosis, and some noninfectious entities.[196] Therefore a special stain (e.g., Gram, silver) is needed to show that the granule is composed of branching bacteria and not fungi (eumycetoma), cocci, or bacilli (botryomycosis). Botryomycosis is a chronic bacterial soft tissue and rarely visceral infection that produces loose clumps of bacteria that resemble granules. Etiologic agents include *Staphylococcus, Streptococcus, Escherichia, Pseudomonas,* and *Proteus,* which are easily distinguished from the agents of actinomycosis by the presence of cocci or nonbranching bacilli.[197] If branching bacteria are seen on staining of the granule and the infection did not originate in subcutaneous tissue (a characteristic of actinomycetoma) or tonsillar tissue (see "Oral-Cervicofacial Disease" earlier), then the diagnosis of actinomycosis is established. Clinically, nocardiosis may closely resemble actinomycosis but does not form granules in visceral lesions.[198] However, when *Nocardia* is the causative agent of actinomycetoma, granules are formed. On Gram stain, the branching gram-positive bacilli are indistinguishable from *Actinomyces,* but they may be stained by a modified Fite acid-fast stain, whereas *Actinomyces* is not.[194] The granules formed by the fungal agents of eumycetoma show branching hyphae on periodic acid–Schiff or Gomori methenamine silver stain (see Chapter 261). Specimens obtained from mucus-producing locations, such as the endocervix, the bronchus, or ventricular colloid cysts, may possess pseudoactinomycotic radiate granules. If hematoxylin-eosin stain alone is used, they may mimic actinomycotic granules, as a central region is bordered by the Splendore-Hoeppli phenomenon. However, special stains will reveal the absence of microorganisms.[199]

The bacteriologic identification of one of the agents of actinomycosis from a sterile site will confirm the diagnosis. However, the microbiologic identification occurs in only a minority of cases.[50] This is due, in part, to the fact that the agents of actinomycosis are exceedingly sensitive to a wide variety of agents, and even a single dose can interfere with their isolation. Therefore the most important step for optimal microbiologic yield is the avoidance of any antimicrobial therapy before obtaining the specimen. Because these organisms are normal inhabitants of the oral

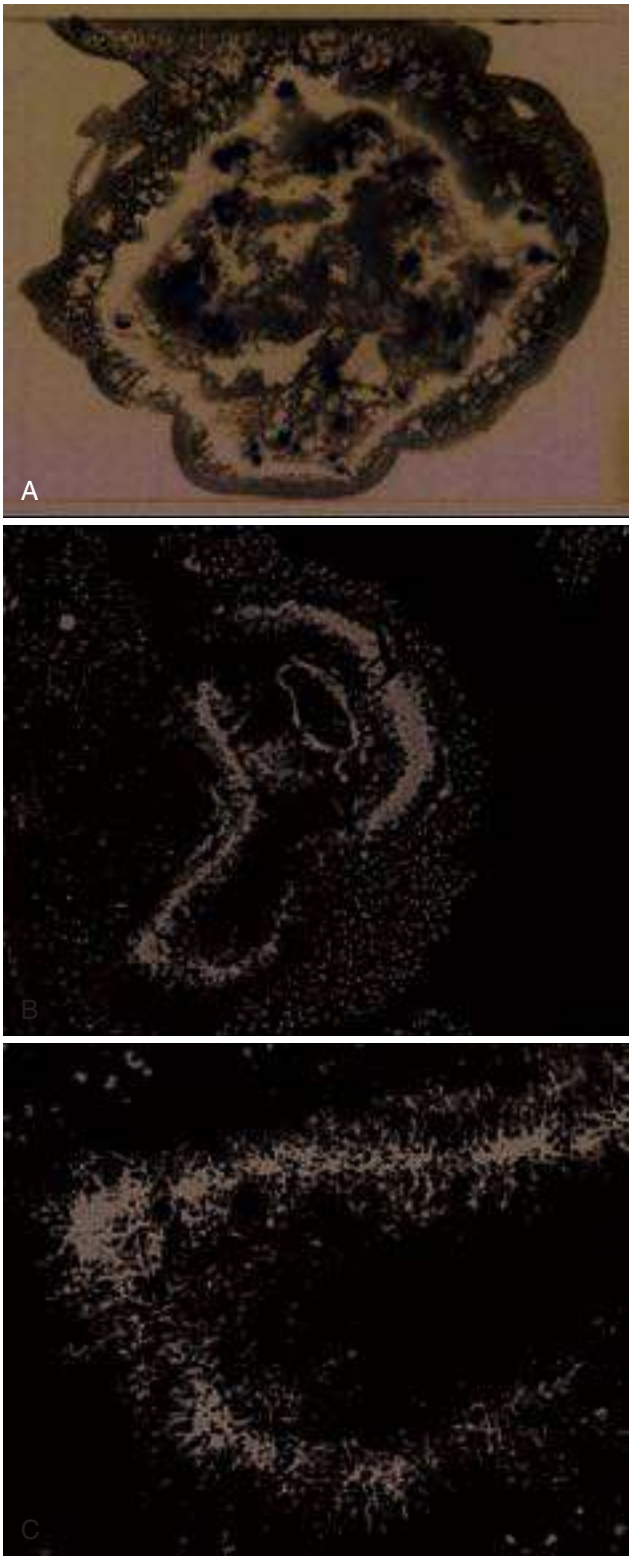

FIG. 254.7 (A), Macroscopic actinomycotic sulfur granules demonstrated by placing pus between two slides. (B) Microscopic actinomycotic sulfur granule surrounded by inflammatory cells. (Hematoxylin-eosin stain, ×250.) (C) Actinomycotic granule. Brown and Brenn stain (×1000) demonstrates the delicate, branched filaments of *Actinomyces.*

cavity and female genital tract, the identification of organisms alone, in the absence of sulfur granules or an appropriate clinical syndrome, from sputum, bronchial washings, and cervicovaginal secretions is of little significance. Isolation of *Actinomyces* from blood cultures in the absence of defined infectious focus may represent contamination or transient bacteremia from a mucosal site of colonization, in which case treatment may not be necessary.[200]

Although most strains of *Actinomyces* are microaerophilic (except *A. meyeri* and *A. neuii,* which can grow aerobically), strict anaerobic processing and anaerobic growth should be used for primary isolation. The laboratory should receive specimens expeditiously or in anaerobic transport media. Tissue, pus, or sulfur granules are ideal, and swabs should be avoided. The microbiology laboratory should be alerted before receiving any specimen that could harbor the agents of actinomycosis. A Gram stain of the specimen is usually more sensitive than culture, especially if the patient has received prior antibiotics. If granules are identified, they should be washed and crushed between two slides for examination (see Fig. 254.7A). The agents of actinomycosis are non–spore-forming, branching, filamentous rods, except for *A. meyeri* and *A. neuii,* which are small and nonbranching. Growth usually appears within 5 to 7 days, but primary isolation may take up to 2 to 4 weeks. Although specialized media are not required, the use of semiselective media may increase isolation rates of *Actinomyces,* particularly when more rapidly growing organisms are also present.[201] *A. israelii* characteristically forms a "molar tooth" colony on agar and grows as clumps within broth. *A. odontolyticus* colonies usually appear rust-brown or red colored. *Actinomyces* are indole negative. Traditional identification is based on these features in combination with tests for urease, catalase, gelatin hydrolysis, and fermentation of cellobiose, trehalose, and arabinose. However, classification of *Actinomyces* based on phenotypic tests may result in misidentification.[16] Likewise, identification with the Vitek 2 ANC card is not yet optimal.[202] Sequencing or restriction analysis of amplified 16S ribosomal DNA can assist in resolving ambiguities in identification of the non–spore-forming, gram-positive rods.[16,203,204]

Actinomycosis should not be excluded if not cultured or the characteristic pathologic findings are not present, especially when only small quantities of tissue are available. Sulfur granules are easily missed and only fibrosis with or without inflammation may be identified. As noted, multiple sections may increase diagnostic sensitivity. The use of 16S rRNA gene amplification and sequencing by clinical microbiology laboratories is increasing, thereby enhancing diagnostic sensitivity and specificity.[205,206] MALDI-TOF mass spectrometry holds similar promise, but databases are still in the process of being optimized.[10,206] A combination of appropriate microbiologic, molecular, and pathologic studies will maximize the chances of success.

THERAPY

The discovery and use of penicillin in the treatment of classic actinomycosis has dramatically altered the course of this disease. Two principles of therapy, based on the clinical experience of the past 70 years, have evolved. It is necessary to treat this disease both with high doses and for a prolonged period of time. The need for intensive treatment is presumably due to the drugs' poor penetration of the thick-walled masses common in this infection and/or the sulfur granules themselves, which may represent a biofilm. Although therapy needs to be individualized, daily treatment with 18 to 24 million units of penicillin given intravenously for 2 to 6 weeks, followed by oral therapy with penicillin or amoxicillin for 6 to 12 months, is a reasonable guideline for serious infections and bulky disease. Interesting to note, a study on the treatment of thoracic actinomycosis had a 22% (5/23) failure rate with medical therapy, but these patients received only 0 to 2 days of initial parenteral therapy.[207] If the duration of therapy is extended beyond the resolution of measurable disease, then relapses, one of the clinical hallmarks of this infection, will be minimized. CT and MRI studies are generally the most objective modalities to accomplish this goal. MRI scans are often more sensitive than CT scans for detecting residual infection and should be employed if possible, particularly in areas where the consequences of relapse are particularly significant (e.g., the CNS).[208] In one case, PET appeared to be more sensitive than CT for monitoring disease resolution.[111]

Cases with less extensive involvement, particularly in the oral-cervicofacial region, may be cured with a shortened duration of therapy.[209,210] Furthermore, in some cases of low bulk, oral-cervicofacial disease therapy can be initiated with an oral agent. The treatment course for nonclassic actinomycotic infections—that is, the absence of tissue

TABLE 254.1 Appropriate and Inappropriate Antibiotic Therapy for Actinomycosis

Group 1: Extensive Successful Clinical Experience[b]
Penicillin (3–4 million units IV q4h or amoxicillin 500 mg PO q6h)[c]
Erythromycin (500–1000 mg IV q6h or 500 mg PO q6h)
Tetracycline (500 mg PO q6h)
Doxycycline (100 mg IV q12h or 100 mg PO q12h)
Minocycline (100 mg IV q12h or 100 mg PO q12h)
Clindamycin (900 mg IV q8h or 300–450 mg PO q6h)[d]
Group 2: Anecdotal Successful Clinical Experience
Ceftriaxone[c]
Ceftizoxime
Imipenem-cilastatin
Piperacillin-tazobactam
Group 3: Agents Predicted to Be Efficacious Based on in Vitro Activity
Vancomycin
Linezolid
Quinupristin-dalfopristin
Ertapenem[c]
Azithromycin[c]
Tigecycline
Group 4: Agents That Should Be Avoided
Metronidazole
Aminoglycosides
Oxacillin
Dicloxacillin
Cephalexin
Fluoroquinolones

[a]Additional coverage for concomitant "companion organisms" may be required.
[b]Controlled evaluations have not been performed. Dose and duration require individualization, depending on the host site and extent of infection. As a general rule, a maximum antimicrobial dose for 2 to 6 weeks of parenteral therapy followed by oral therapy for a total duration of 6 to 12 months is required for classical actinomycosis (e.g., tissue invasive, bulky disease), whereas a shorter duration may suffice for less extensive diseases, particularly in the oral-cervicofacial region. Monitoring therapeutic effect with computed tomography or magnetic resonance images is advisable when appropriate.
[c]Agents to consider for home parenteral therapy (see text for details).
[d]Recent in vitro data demonstrated a >20% resistance rate in the majority of species tested.

changes—especially for the more recently described *Actinomyces* spp. is unclear. In the absence of data, a syndrome-based treatment plan is most appropriate.

Extensive successful clinical experience also has been reported for amoxicillin, erythromycin, tetracycline, doxycycline, minocycline, and clindamycin, although it should be noted that in vitro data have demonstrated >20% resistance to clindamycin in most species.[13,211] Anecdotal successes with imipenem-cilastatin,[212,213] ceftriaxone,[214] ceftizoxime,[215] and piperacillin-tazobactam[216] have been reported (Table 254.1). In vitro data demonstrate that vancomycin, quinupristin-dalfopristin, linezolid, rifampin, ertapenem, tigecycline, and azithromycin are active against *Actinomyces* spp.,[13,211,217,218–221,222] although some variation in in vitro susceptibility exists among species. In vitro data also suggest that fluoroquinolones, oxacillin, dicloxacillin, cephalexin, metronidazole, and aminoglycosides should be avoided.[217,223]

For home parenteral therapy, the ease of once-a-day dosing makes ceftriaxone appealing in certain circumstances; however, a greater body of literature supporting its efficacy would be desirable. The availability of portable infusion pumps for home therapy allows for both the appropriate dosing and practical administration of intravenous penicillin. For infections in critical sites (e.g., the CNS), this approach remains the safest until more information regarding other agents is available. The pharmacokinetic properties, availability of oral and parenteral formulations, and potential efficacy of azithromycin and ertapenem also make these agents appealing. Unfortunately, few in vitro and no clinical data exist on their use to treat actinomycosis.

It is unclear whether other bacteria frequently coisolated with the etiologic agents of actinomycosis require treatment, but many of them are pathogens in their own right. Designing a therapeutic regimen that includes coverage for these organisms during the initial treatment course is reasonable. If microbiology is not available, it is important to consider the site of infection when designing empirical coverage. For example, *A. actinomycetemcomitans, E. corrodens, Fusobacterium,* and *Capnocytophaga* are more likely to be coisolates in head and neck infection, whereas the Enterobacteriaceae and *Bacteroides* are more commonly coisolated in abdominal infection.

In the preantibiotic era, surgical removal of infected tissue was the only beneficial treatment. Despite the advent of efficacious antimicrobial therapy, combined medical and surgical therapy is still advocated in some reports. However, the literature supports the approach of initially attempting a cure with medical therapy alone.[159,167,186,187] Successes with antibiotics alone have been reported in cases of extensive disease that initially appeared to be incurable. CT and MRI should be used to monitor the response to therapy.[127,224] In most cases surgery can be avoided altogether or a less extensive procedure will be required. This approach is particularly important when the possibility of sparing critical organs is involved, such as the bladder or reproductive organs in women of childbearing age. In a patient with disease in a critical location (e.g., epidural space, selected CNS disease), with significant hemoptysis,[225] or in whom suitable medical therapy fails, surgical intervention may be appropriate. In the setting of actinomycosis manifesting as a well-defined abscess, percutaneous drainage in combination with medical therapy is a reasonable approach.[133,226]

Actinomycosis has been described in association with HIV infection, steroid use, and lymphoproliferative tumors. Whether these infections were due to disease-associated disruptions of mucosa (e.g., cytomegalovirus infection with HIV infection), host defense abnormalities, immunosuppressive therapy, or some combination of these is unclear. From a treatment perspective, it is reasonable to initially use the same approach as that for noncompromised hosts. Aggressive treatments directed against HIV and minimizing immunosuppressive therapy are also desirable if possible. Although prospective controlled data are not available, when *Actinomyces* organisms are identified in the setting of bisphosphonate-related osteonecrosis of the jaw (BRONJ), a prolonged course of antimicrobial therapy is reasonable and appears to be efficacious. The role of surgical débridement for BRONJ is less clear, but resection of at least necrotic bone seems prudent.[76,227–229]

Usually actinomycosis responds well to medical therapy. However, refractory or perceived refractory disease has been described in HIV-infected individuals and overtly normal hosts. In this setting, basic principles of infectious disease apply. First, exclude infection elsewhere (e.g., intravenous catheter–acquired bacteremia, *Clostridioides difficile* (formerly *Clostridium difficile*) colitis), noninfectious causes, or both (e.g., drug fever, unrelated disease) as being responsible. Next, confirm that high-dose parenteral therapy is being used for initial treatment. Identify and drain significant purulent collections associated with the actinomycotic infection. Consider the possibility that untreated coisolates (companion organisms) may be responsible. Although penicillin-resistant strains or evolution of resistance during therapy has not yet been clearly documented in vivo, this possibility should be considered when more likely scenarios are excluded. Finally, surgery should be considered when infection is refractory to medical therapy, although as stated previously, this can usually be avoided, at least initially.

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The complete reference list is available online at Expert Consult.

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255 Introduction to Mycoses

John E. Bennett[a]

The advent of the human immunodeficiency virus epidemic and the ever-increasing use of immunosuppressive drugs has dramatically increased the incidence of deep mycoses and substantially broadened the range of fungi causing potentially lethal disease. Clinicians caring for highly immunosuppressed patients are rarely able to ignore a fungal isolate because the species is not "pathogenic." An additional complication is that the rapidly moving field of diagnostic microbiology is providing names of species that clinicians have not encountered before (see "Cryptic Species" later), and centralization of facilities has moved the laboratory away from where the clinician works, complicating communication.

CHANGES IN HOW FUNGI ARE IDENTIFIED

Identification by DNA Sequence

DNA sequence is replacing appearance (morphology) for identifying fungi and showing relatedness between different fungal genera.[1] Sequencing has provided a powerful tool for discovering new relationships (e.g., *Penicillium marneffei* should be in the genus *Talaromyces*), for identifying newly discovered infections (*Emergomyces* species; see Chapter 268),[2] and for discovering unexpected groups, called "cryptic species," within existing single species. For example, cryptic species have been found in *Aspergillus, Coccidioides, Cryptococcus, Paracoccidioides, Sporothrix,* and many other genera. Despite these important contributions to taxonomy, limitations to basing phylogeny on DNA sequences alone include the following: (1) less than 1% of an estimated 1.5 million fungal species have been sequenced; (2) the extent of variability *within* a monophyletic species is not known for many fungi pathogenic for humans because too few isolates have been sequenced; (3) the distinction between one species and another based on sequence alone is fundamentally arbitrary; (4) no single area of the genome is suitable to distinguish all fungi; (5) published sequences of species are known to contain errors in species identification and sequence; (6) instability of fungal names is detrimental to medicine, making it difficult for the clinician to find precedent for managing an infection with a fungus given a new name; and (7) sequence-derived distinctions do not necessarily translate into differences in biology of medical importance, such as epidemiology, pathogenesis, or response to treatment.[3]

Cryptic Species

New fungal species are being described by their DNA sequence alone. Although sequences between the genes coding for ribosomal RNA (internal transcribed spacer [ITS] region, D1/D2 region) are most commonly used, genes coding for proteins, such as β-tubulin or calmodulin, are added, depending on the genus. These species are called "cryptic species" because they cannot be identified by their appearance in culture or by biochemical tests. An increasing number are also being identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which compares the spectrum of small proteins extracted from the organism with a database of many other organisms.[4–6] The machine reports what species matches the spectrum of the isolate being analyzed, with a number indicating how similar the spectra are. A lower number may indicate the genus is correct but the species may not be. As more correctly identified fungi are put into the database, this technique is more useful. Despite the high cost of the machine, laboratories like the simplicity of sample preparation and analysis, inexpensive reagents, and rapid completion. The end result is that more cryptic species will be appearing in culture reports.

Frequency of cryptic species within isolates of a genus depends on the genus and geographic location. For example, cryptic species within *Aspergillus* isolates vary from 10% to 26%.[7] Laboratories not equipped to identify cryptic species will report the identification using traditional techniques, so that *Aspergillus lentulus* will be reported as *Aspergillus fumigatus* or, more correctly, *Aspergillus fumigatus* complex.[8] As data accumulate, it will become more clear if some cryptic species differ significantly from other species in the complex in terms of response to specific antifungal drugs, virulence, or habitat. For example, *A. lentulus* is more resistant to azoles in vitro than some other species in the *A. fumigatus* complex, though it is unclear if this impacts response to azole treatment.

One Fungus, One Name

For decades, fungi could have had one name for the fungus sporulating in its "sexual state," called its teleomorph, and another name when it was growing only "asexual" spores (anamorph). Sexual spores are formed when the fungus mates (combines haploid DNA), usually with another isolate; forms a diploid structure; and then undergoes meiosis, producing haploid spores that are recombinants of the parental isolates. There are a few species in which an isolate can mate with itself and form sexual spores. Most cultures in the diagnostic laboratory form only asexual spores, which are produced by mitosis. Almost all the fungal names that clinicians recognize come from the anamorph rather than the teleomorph, such as *Aspergillus,* not *Neosartorya,* and *Blastomyces dermatitidis,* not *Ajellomyces dermatitidis.* Now, medical mycologists have agreed that each fungal species should have a single name and that the old schema of having a different name for the sexual form and the asexual form was unnecessarily confusing. Which of the two names should be retained is still a matter of debate for some species. A more contentious issue for medical mycologists is whether sequence differences alone should be used to carve out a new "cryptic" species from within an existing species. Some mycologists believe that sequence differences alone are not enough to define a cryptic species and believe that evidence of biologic differences is needed. Some of the more recently identified cryptic species do differ from other isolates formerly in the same species. *Sporothrix brasiliensis,* isolates of which were formerly assigned to *Sporothrix schenckii,* appears to be more geographically confined and more virulent than isolates still classified as *S. schenckii* (see Chapter 259). There are epidemiologic differences between *Coccidioides immitis* and *Coccidioides posadasii* and between *Paracoccidioides brasiliensis* and

[a]The chapter was written by Dr. Bennett in his personal capacity. The views expressed herein do not necessarily represent the views of the National Institutes of Health, the Department of Health and Human Services, or the United States.

Paracoccidioides lutzii (see Chapters 265 and 267). In these three examples, the clinical disease is similar enough that clinical information about the older species is sufficient to guide diagnosis and management.

COMMON FEATURES OF PATHOGENIC FUNGI

Some specialized terms used in this chapter are listed in Table 255.1.

TABLE 255.1 Lexicon of Mycology Terms for the Clinician

Aleurioconidia/aleuriospore—spore growing at the end of a specialized hypha. The spore is released by breakage of the hypha adjacent to the spore.
Anamorph—a fungus forming only asexual spores.
Arthrospore—a spore formed by a hypha breaking at a septum.
Asexual spores—spores formed by mitosis, a form of cell division that creates an exact copy of the original cell.
Basidiomycete—one of the four major classes of fungi; includes mushrooms and *Cryptococcus neoformans.*
Basidiospore—a sexual spore that arises on a specialized structure, usually club shaped, in a basidiomycete (e.g., *C. neoformans* forms basidiospores in its sexual state).
Blastospore—an asexual spore formed by budding (e.g., *C. neoformans, Candida* spp.).
Conidiophore—specialized hyphae that bear a conidium (spore) on the end.
Conidium (plural, conidia)—an asexual spore usually produced at the tip or side of a hypha.
Dematiaceous mold—dark-colored mold. Same as phaeohyphomycetes.
Dimorphic—capable of producing both hyphae and yeast (e.g., the agents of coccidioidomycosis, blastomycosis, histoplasmosis, sporotrichosis, and chromoblastomycosis).
Diploid—having two sets of chromosomes.
Endemic fungi—fungi having a limited geographic distribution (e.g., blastomycosis, histoplasmosis, and coccidioidomycosis).
Endospore—spore formed within a larger cell, such as a *Coccidioides* spherule.
Entomophthoramycosis—infections caused by molds of the order Entomophthorales, including species of *Conidiobolus* and *Basidiobolus.*
Germ tube—a hypha emerging from a yeastlike structure, characteristic of *Candida albicans* cells placed on specialized culture medium.
Haploid—having a single set of chromosomes.
Heterothallic—a fungus that can mate only between different colonies of an opposite mating type.
Homothallic—a fungus in which mating can take place within the same colony (e.g., *Pseudallescheria boydii*).
Hyaline—colorless, transparent.
Hyalohyphomycosis—infection caused by molds with light-colored colonies. This term includes most of the pathogenic molds and is so broad that it has not proven useful.
Hypha (plural, hyphae)—the tubular element that forms the body of a fungus.
Imperfect state—fungus producing only asexual spores.
Meiosis—process in a dividing cell that allows reassorting of chromosomes and reduces the number of chromosomes by half, from diploid to haploid.
Mitosis—process in a dividing cell that produces two genetically identical copies of the original cell.
Mold—filamentous fungus. A colony on agar generally appears fuzzy, rather than smooth.
Morphology—appearance of the fungus.
Mucormycosis—infection by molds of the subphylum Mucormycotina.
Mycelium (pleural, mycelia)—the mass of hyphae making up a fungal colony.
Perfect state—fungus capable of producing sexual spores.
Phaeohyphomycosis—infection caused by molds with dark-colored colonies caused by pigmentation in the hyphae. Individual hyphae may not have enough pigment to be dark colored under the microscope. A colony can be dark colored because of the spores, such as *Sporothrix schenckii,* and may not be an agent of phaeohyphomycosis.
Phenotype—genetically determined properties that help distinguish an organism from otherwise similar organisms (e.g., requirement for exogenous uracil is a useful phenotype in some yeast mutants).
Pseudohyphae—a string of budding cells (e.g., those formed by most *Candida* spp.).
Sexual spores—spores formed by meiosis, a form of division in which the number of chromosomes is reduced by half.
Spherule—large round cell of *Coccidioides* species that forms spores inside.
Sporangium—a sacklike structure with asexual spores (sporangiospores) inside. Spores are released when the sack breaks.
Spp.—abbreviation for species (plural).
Teleomorph—a fungus forming sexual spores.
Thallus—the vegetative body of a fungus, such as a fungal colony.
Yeast—technically, a fungus of the family Saccharomycetaceae, including *Saccharomyces cerevisiae* (baker's yeast). The terms *yeast form* or *yeastlike* are generally used to denote fungi that reproduce by budding.

Yeasts and Molds

It is important for all infectious disease specialists to understand the distinction between yeasts and molds. Even at the first recognition in a diagnostic laboratory that a fungus has been found in a smear or culture, the laboratory can distinguish between a yeast and a mold. Yeasts are typically round or oval; generally form smooth, flat colonies; and reproduce by budding. Biochemical tests are important for identification of yeasts, although MALDI-TOF mass spectrometry is increasingly being used. Molds are composed of tubular structures called hyphae and grow by branching and longitudinal extension. Mold colonies typically appear fuzzy. However, not all pathogenic fungi can be categorized neatly by their appearance in tissue as yeasts or molds. *Coccidioides* species, *Rhinosporidium seeberi*, and *Pneumocystis jirovecii* are round in tissue but do not bud. Instead, the cytoplasm divides to form numerous internal spores that, on rupture of the "mother" cell, are released to form new spherical structures.

Dimorphic Fungi

Some fungi can grow either as a yeast or as a mold. In candidiasis and tinea versicolor, the fungus is often seen in both tubular and rounded forms but is not commonly considered to be dimorphic. The so-called dimorphic fungi grow in the host as yeastlike forms but grow at room temperature in vitro as molds. These fungi include the agents of histoplasmosis, blastomycosis, sporotrichosis, coccidioidomycosis, paracoccidioidomycosis, chromoblastomycosis, talaromycosis (see Chapter 268), and emergomycosis (see Chapter 268).

DIAGNOSIS OF MYCOSES

Fungi can often be identified in tissue, even in the absence of culture, by taking into account the clinical findings, body site, inflammatory response, and fungal appearance (Fig. 255.1; Table 255.2). Culture diagnosis is potentially more accurate than diagnosis by histologic features, but many smaller laboratories encounter difficulties in isolating and identifying fungi. The histologic features of a biopsy specimen can be more rapidly diagnostic than culture when mycoses are caused by slow-growing fungi. Biopsy slides are more readily mailed to consultants than cultures, which may arrive nonviable or contaminated. Finally, biopsy may provide proof that the fungus is invading tissue and is not just a contaminant or saprophyte growing on debris in a lung cavity or skin ulcer. Ideally, both histologic examination and culture should be done together. Detection of fungal DNA by polymerase chain reaction has proved useful for identifying fungi in tissue and clinical specimens, such as bronchoalveolar lavage, although currently that is most often confined to research settings. Also, luminescent DNA probes for hybridization to fungal RNA are commercially available and valuable for identifying colonies of *Histoplasma capsulatum, Coccidioides* spp., *B. dermatitidis*, and *Cryptococcus neoformans.* Identification of fungi in tissue by immunohistochemistry remains experimental.

MICROSCOPIC APPEARANCE OF FUNGI

Tissue Stains

Brown and Brenn stain (tissue Gram stain) results in fungi that may appear gram-positive or gram-negative. *Actinomyces* and *Nocardia* are gram-positive, but other stains are preferred for visualizing fungi in clinical material.

Grocott-Gomori methenamine silver (GMS) stains all fungal walls brownish-black and is the most sensitive stain for fungal cells. As an exception, some agents of mucormycosis, such as *Mucor velutinosus,* do not stain or stain poorly with GMS. The usual counterstain in GMS, such as fast green, does not allow adequate visualization of the inflammatory response but shows the fungi in strong contrast. *Leishmania,* which does not stain with GMS and has a cytoplasmic dot (a kinetoplast), should be distinguished from *H. capsulatum* and *P. jirovecii.* A black dot is sometimes visible in the cell wall of *Pneumocystis* cysts on GMS. Artifacts caused by deposition of silver oxide around other structures can resemble yeasts or hyphae and are a common source of diagnostic confusion with GMS. *Actinomyces* may stain with GMS in a grain from a patient with actinomycosis or actinomycetoma, although the Brown and Brenn stain is preferred for these organisms in tissue.

Hematoxylin and eosin (H&E) stains some fungal cells purple, but other fungal cells may be visible only as refractile clear structures. H&E is most valuable for studying the inflammatory response in the tissue.

Mayer mucicarmine stains cryptococcal cells burgundy red. Staining ranges from deep to negligible in the same section and may not be detectable at all in some tissues. *Rhinosporidium seeberi* also stains positive, but the huge size, endospores, and lack of budding prevent confusion. Although *B. dermatitidis* sometimes takes up mucicarmine faintly, a positive mucicarmine stain is helpful in distinguishing cryptococci from other yeasts. Although mucicarmine stains only the capsule, the capsule shrinks around the cryptococcal cell wall during fixation so that the cell wall may appear to be stained.

Masson-Fontana (or Fontana-Masson) stains melanin brown in fungal cell walls. This stain is not highly specific but can be useful for distinguishing hyphae of agents of phaeohyphomycosis from hyphae of agents of hyalohyphomycosis, such as *Aspergillus, Fusarium,* and *Scedosporium. Cryptococcus neoformans* and *Cryptococcus gattii* usually stain positive by Masson-Fontana. Mucicarmine is a more specific but less sensitive stain for cryptococci.

Periodic acid–Schiff (PAS) stains all fungal cell walls red. PAS also stains some microsporidia, *Tropheryma whipplei,* and many polysaccharide-containing structures within tissue. In the Splendore-Hoeppli phenomenon of basidiobolomycosis and sporotrichosis, the concretions around the hyphae stain with PAS. *Histoplasma* within old caseous foci may stain with GMS and not PAS.

Fresh Clinical Specimens

Calcofluor white stains chitin-containing structures so that they fluoresce bright white under ultraviolet light in a fluorescent microscope. This stain in the microbiology laboratory has replaced the KOH (potassium

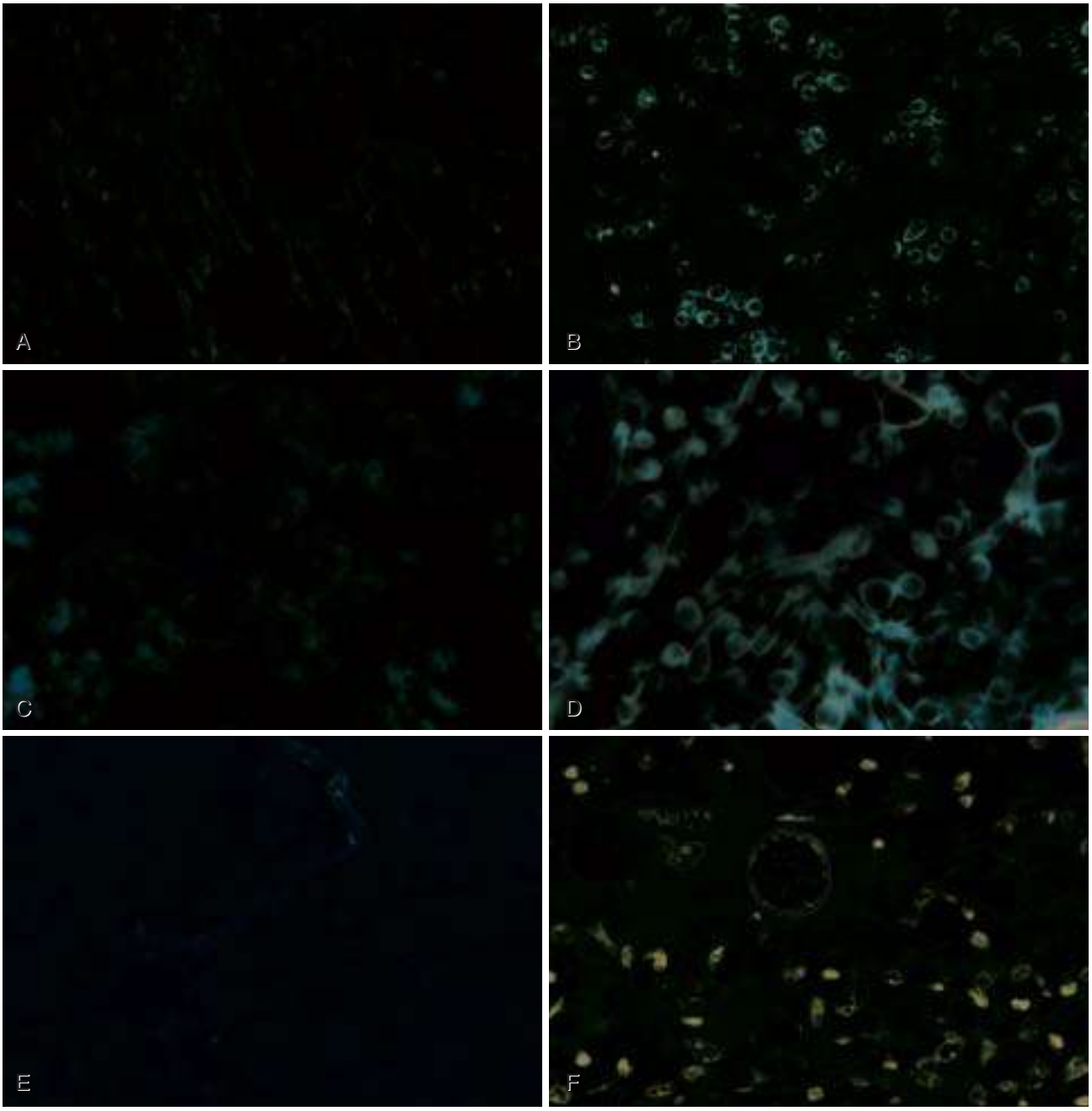

FIG. 255.1 Appearance of fungi in tissue. (A) *Aspergillus* sp.; periodic acid–Schiff (PAS) stain. (B) *Histoplasma capsulatum;* Gomori methenamine silver (GMS) stain. (C) *Candida glabrata* yeast cells; PAS stain. (D) *Candida albicans;* GMS stain. (E) *Rhizomucor* sp.; GMS stain. (F) *Coccidioides* sp.; hematoxylin and eosin (H&E) stain.

Continued

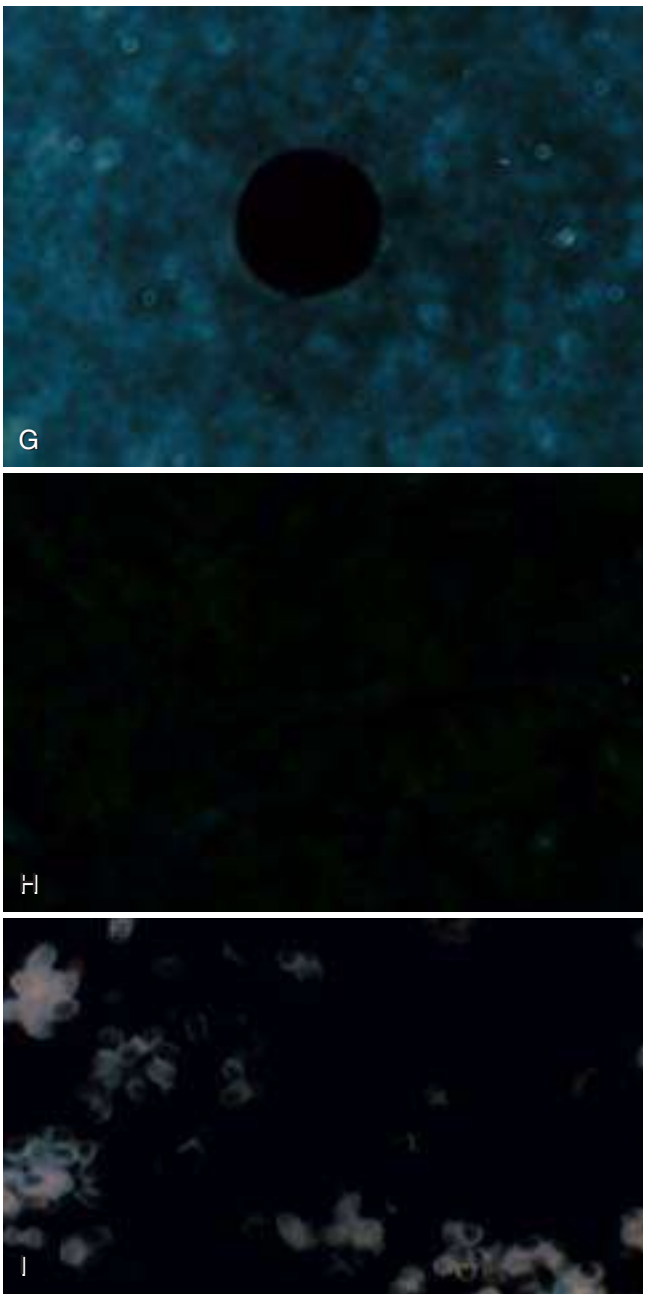

FIG. 255.1, cont'd (G) *Cryptococcus neoformans;* India ink smear of cerebrospinal fluid. (H) *Cladophialophora bantiana;* H&E stain. (I) *Talaromyces marneffei;* GMS stain.

hydroxide) wet mount because the contrast speeds examination. *Pneumocystis,* microsporidia, *Cryptosporidium,* and some parasitic cysts also are calcofluor positive.

With *Gram stain, Candida* yeast cells and pseudohyphae often appear gram-positive on clinical specimens. Some technologists can identify cryptococci in cerebrospinal fluid (CSF) by Gram stain, but Gram positivity may be only a few dots on the cell or not seen at all.

With *India ink,* suspension of colloidal carbon particles mixed with CSF will show the capsule around a cryptococcal cell. Capsule size may be too small to be visible on cultured cells. India ink staining should not be done on pus, sputum, or bronchial lavage specimens because viscous material surrounds many structures and can resemble a capsule.

SEROLOGIC DIAGNOSIS

Despite a rich history of serologic studies of mycoses, the only mycosis for which serodiagnosis has an established role is coccidioidomycosis (see Chapter 265). Even with this infection, lack of standardization of tests among laboratories and among methods has made it difficult for the clinician to interpret the results. The situation is even worse for histoplasmosis and blastomycosis, for which the most promising test in the literature, complement fixation, has been considered too labor intensive and has been replaced in commercial laboratories by tests of unknown significance. Serodiagnosis for any mycosis should be used with great caution and with knowledge of the technique and laboratory performing the test.

Diagnosis by antigen detection has proved very useful in disseminated histoplasmosis and cryptococcosis. Severe cases of aspergillosis, coccidioidomycosis, and blastomycosis may also be amenable to diagnosis by antigen detection. An enzyme immunoassay using a rat monoclonal antibody that detects fungal polysaccharide in serum and bronchoalveolar lavage specimens has been used in the diagnosis of invasive aspergillosis and talaromycosis[9] (see Chapters 257 and 268). Sensitivity and specificity depend on the cutoff used for positivity, the patient population being tested, and prior use of mold-active antifungals. However, in high-risk

TABLE 255.2 Typical Appearance of Fungi in Tissue

FUNGI	DESCRIPTION
Yeastlike Fungi	
Histoplasma capsulatum	2–3 × 3–4–μm oval, budding uninucleate cells; often intracellular; granulomatous inflammation. Caseous necrosis can occur. Cells in African histoplasmosis (var. *duboisii*) are 6–15 μm in diameter.
Talaromyces marneffei	2–3 × 2–6–μm oblong yeasts, some with central cross septum; often intracellular except in areas of necrosis.
Pneumocystis jirovecii	3.5- to 7-μm cysts stain black on methenamine silver and may show a central black dot. White on calcofluor. No budding. Clusters of cysts occur in alveoli surrounded by eosinophilic amorphous material.
Candida glabrata	2.5–3 × 4–5–μm oval budding cells; pyogenic necrosis.
Cryptococcus neoformans/C. gattii	4- to 6-μm round uninucleate cell with large surrounding capsule; narrow pore between mother and daughter cell; daughter cell detached while small. Stains red with mucicarmine.
Sporothrix schenckii/S. brasiliensis/ S. mexicana	1–3 × 3–10–μm cigar-shaped cell or 2- to 10-μm round budding cell; pyogenic and granulomatous inflammation.
Blastomyces dermatitidis/B. gilchristii	8- to 15-μm round multinucleated cell with large pore between mother and daughter cell; daughter cell remains attached until almost the size of mother cell; pyogenic and granulomatous inflammation.
Paracoccidioides brasiliensis/P. lutzii	2- to 30-μm multiply budding, round cells with tiny pore between mother and daughter cell; daughter cell released when small.
Coccidioides posadasii/C. immitis	5- to 60-μm thick-walled, nonbudding, round cells that may contain endospores. Lung cavities may contain hyphae.
Agents of chromoblastomycosis	4- to 12-μm round or oval, brown, thick-walled cells, often in clumps; hyphal forms may be seen in superficial crusts.
Molds	
Aspergillus spp.	2- to 5-μm–wide hyphae, frequently septate, even diameter, Y-shaped branching; propensity for vascular invasion; necrosis.
Agents of mucormycosis	4- to 15-μm–wide hyphae, rarely septate, uneven diameter, often branch at broad angles; propensity for vascular invasion; necrosis.

patients with prolonged neutropenia or allogeneic stem cell transplantation, the galactomannan test has proven useful in preemptive treatment strategies, often in conjunction with high-resolution chest computed tomography (see Chapter 307). Tests are commercially available for detecting β-glucan in serum. β-Glucan is a cell wall component of many different molds and yeasts, including *P. jirovecii*. Problems of insensitivity and false-positive tests have complicated interpretation of this test, and it has yet to find its niche in the armamentarium of diagnostic tests.

EPIDEMIOLOGY

Mycoses are acquired through two major routes: inhalation of airborne fungal spores into the lungs and paranasal sinuses, or direct contact. *Malassezia* spp. become part of the skin microbiome during infancy through contact with colonized humans. *Candida albicans* is acquired in the intestinal and mucosal microbiome from passage through the birth canal or later in life by contact with colonized persons. Ringworm is acquired by contact with infected animals, infected persons, or soil. Inoculation of saprophytic fungi on vegetation or soil through minor trauma can lead to sporotrichosis, mycetoma, or chromoblastomycosis. Ingestion has not proven to be a portal for pathogenic fungi, although that has been suspected for gastrointestinal basidiobolomycosis. Contact with water is suspected to transmit rhinosporidiosis.

Agents of histoplasmosis, blastomycosis, coccidioidomycosis, and cryptococcosis grow in natural sites, are inhaled, and initiate infection in the lung. A restricted reservoir in nature accounts for the geographic restriction of these mycoses. *Talaromyces marneffei* probably is acquired by inhalation, although the reservoir in nature is not well understood. Molds that infect immunosuppressed patients, such as those causing aspergillosis, mucormycosis, and fusariosis, are saprobes that are widely distributed in nature.

High-efficiency particulate air (HEPA) filtration of air in hospital rooms is used to protect highly immunosuppressed patients from invasive mold infections. The necessity of moving such patients out of the protected air for imaging and other procedures has limited the efficacy of air filtration. Anecdotal evidence has connected hospital construction with clusters of aspergillosis cases in immunosuppressed patients.

With rare exception, mycoses are not transmissible from patient to patient. Standard precautions for hospitalized patients are all that is indicated. Ringworm of the scalp in children is transmissible to other children, so caps and combs should not be shared by infected children and playmates. Airborne transmission of *P. jirovecii* has been documented, but reactivation also accounts for a large percentage of cases. Bandages or casts that become contaminated with draining pus from patients with coccidioidomycosis require care to ensure that the fungus does not remain on the fomite for several days because, at room temperature, the fungus will grow as the infectious, spore-bearing mold form.

The diagnostic laboratory should be alerted when specimens from patients suspected of having coccidioidomycosis or histoplasmosis are sent for culture. Once these cultures grow in the mold form, they can be hazardous to laboratory personnel.

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256 *Candida* Species

Michail S. Lionakis and John E. Edwards, Jr.

SHORT VIEW SUMMARY

Microbiology
- Budding yeast found abundantly in nature worldwide.
- Normal human commensals.
- Human-to-human transmission of disease is rare.
- Multiple species are pathogenic: *Candida albicans* and *Candida glabrata* most common.

Epidemiology and Pathogenesis
- Normally not pathogenic in humans until host defense mechanisms become compromised.
- Disease of modern medicine: broad-spectrum antibiotics, indwelling intravenous catheters, prosthetic devices, hyperalimentation fluids, cancer treatment with cytotoxic agents, use of immunosuppressives for organ transplantation.
- Has emerged as the fourth most common organism recovered from blood in hospitalized patients. A health care–associated infection. Can disseminate widely when entering the bloodstream.
- Emerging incidence, especially in developing countries where modern advances in medical therapeutics are being introduced.
- Organism can infect nearly every organ and nearly all types of prosthetic materials. Female genital tract and oral mucosa are the most common mucocutaneous sites infected. Kidney, brain, heart, and eye are most commonly infected with dissemination.

Diagnosis
- Diagnosis is by culture in normally sterile body fluids or by visualizing the organism in specimens of mucosa or skin using Gram stain, calcofluor white, or fungal stains, preferably confirmed by culture.

Treatment
- Topical agents exist for mild to moderate mucosal or cutaneous candidiasis. For deep tissue infections, systemic treatment with amphotericin B, an azole, or an echinocandin is used (see Tables 256.3 to 256.5).

Written descriptions of oral lesions that were probably thrush date to the time of Hippocrates and Galen. Langenbeck, in 1839, found fungi in oral lesions of a patient.[1] In 1861, Zenker described the first well-documented case of deep-seated *Candida*. The first case of *Candida*-induced endocarditis was described in 1940.[2]

The most interesting period in the history of *Candida* infections began in the 1940s, when the widespread use of antibiotics was introduced. Since then, the incidence of practically all forms of *Candida* infections has risen abruptly. *Candida* spp. have been the fourth most common organisms recovered from blood of hospitalized patients in the United States during recent decades.[3] Between 2000 and 2005 the incidence of candidemia-related hospitalizations per 100,000 population rose by 52%.[4] A subsequent decrease in the incidence of candidemia has been noted in the late-2000s and early-2010s in the United States, which may reflect the successful implementation of prevention strategies for the decrease of central line–associated bloodstream infections.[5,6] The decreased incidence may also reflect a decrease in blood cultures. The burden of this illness in terms of morbidity, mortality, and expense is considerable. Estimates of the cost of candidemia in the United States are at least 2 billion dollars per year.[5,7] A small sample of the numerous reviews detailing the continued emergence of *Candida* as a common pathogen, the evolution of the disease in developing countries, the shift to non-*albicans* species, and antifungal resistance is available.[8–13] The emerging *Candida* infections include not only bloodstream infection but also arthritis, osteomyelitis, endophthalmitis, myocarditis, pericarditis, pacemaker-induced endocarditis, ventricular assist device infection, meningitis, peritonitis, myositis, pancreatitis, and others that are elaborated upon in detail in their respective sections of this chapter. The increasing incidence of human immunodeficiency virus type 1 infection, the use of therapeutic modalities for advanced life support, and certain surgical procedures, such as organ transplantation and the implantation of prosthetic devices, have expanded the incidence of *Candida* infections (Table 256.1).

Two interesting trends are continuing to develop with the extensive, rapidly evolving literature on *Candida* infections. First, as developing countries have introduced advanced medical care, including primarily more complex surgical procedures and more comprehensive cancer treatments, their increasing reports of the epidemiology and predisposing factors for *Candida* infections have recapitulated those that have been noted during the past two decades from countries with advanced medical care. Second, there has been a steady and significant increase in reports on the incidence and manifestations of *Candida* infections caused by non-*albicans* species.[8]

PATHOGEN

Candida organisms are yeasts, that is, fungi that exist predominately in a unicellular form. They are small (4–6 μm), thin-walled, ovoid cells (blastospores) that reproduce by budding. They grow well in vented routine blood culture bottles and on agar plates and do not require special fungal media for cultivation. Several automated blood culture methods offer more rapid detection of *Candida*. Yeast forms, pseudohyphae, and hyphae may be found in microscopic examination of clinical specimens; identification of the hyphae and pseudohyphae is facilitated with 10% potassium hydroxide, which clears the epithelial cells, and with fluorescent microscopic examination of calcofluor white–stained smears.[14] The organism also stains gram-positive (Fig. 256.1).

Candida organisms form smooth, creamy white, glistening colonies that may resemble staphylococcal colonies. A rapid, presumptive identification of *C. albicans* can be made by placing the organism in serum and observing formation of germ tubes, small projections from the cell surface that appear within 90 minutes. However, both false-negative and false-positive germ tube formation may occur. The remainder of the identification and speciation procedures are based primarily on physiologic parameters rather than on morphologic characteristics. Metabolic tests include carbohydrate assimilation and fermentation reactions, nitrate utilization, and urease production. Chlamydospore formation is also used to identify *C. albicans*. Because of variation in species pathogenicity, speciation is desirable. There are more than 150 species of *Candida*, but only a small percentage are regarded as frequent pathogens for humans. They are *C. albicans, C. guilliermondii, C. krusei,*



FIG. 256.1 Morphology of *Candida* seen on Gram stain. *(From Anaissie EJ, McGinnis MR, Pfaller MA. The laboratory and clinical mycology. In: Anaissie EJ, McGinnis MR, Pfaller MA, eds.* Clinical Mycology. *2nd ed. New York: Churchill Livingstone; 2009:55–77.)*

TABLE 256.1 Predisposing Factors for Disseminated Candidiasis

Exposure to broad-spectrum antibiotics
Indwelling intravenous catheters
Hyperalimentation fluids
Gastrointestinal surgery
Thoracic surgery
Neutropenia
Low-birth-weight neonates
Burn patients
Intravenous heroin use
Solid-organ transplant patients on immunosuppressives

C. parapsilosis, *C. tropicalis, C. pseudotropicalis, C. lusitaniae, C. dubliniensis,* and *C. glabrata* (formerly classified as *Torulopsis glabrata*). *C. dubliniensis* is a relatively newly described species that was formerly included within *C. albicans.*[15] *C. dubliniensis* forms germ tubes and chlamydospores and is identified as *C. albicans* by the most common methods. However, it will not grow at 45°C, is darker green when initially isolated on CHROMagar Candida (BD Diagnostics, Heidelberg, Germany), and hybridizes poorly to the Ca3 probe. Because it is not yet clear how the clinical features may differ from those of *C. albicans,* if at all, the two are considered synonymous in this chapter. Infections by other species are being reported with increasing frequency, such as the azole-resistant species *Candida inconspicua* and newer *Candida* species such as *C. orthopsilosis* and *C. metapsilosis.*[16] The API 20C AUX yeast strip (bioMérieux, Durham, NC) is a commercial kit that gives accurate identification of most *Candida* spp. in 2 to 5 days. Rapid methods have been developed for speciation, such as the AdvanDx PNA FISH (OpGen, Gaithersburg, MD), but they are not widely used at present.[17]

Nucleotide sequencing and protein analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) are identifying different groups within some *Candida* spp., now being described as potentially different species within a larger species complex. Examples are *Candida africana* within the *C. albicans* complex, *C. nivariensis* and *C. bracarensis* within the *C. glabrata* complex, *C. metapsilosis* and *C. orthopsilosis* within the *C. parapsilosis* complex, and *C. duobushaemulonii* and *C. haemulonii* var. *vulnera* in the *C. haemulonii* complex.[18,19] These cryptic species are not identified by most routine testing, and their clinical relevance is as yet unclear.[18,19] Problems in defining these cryptic species include inadequate definition of variability within the cryptic species, both in nucleotide sequence and in protein patterns, as well as the need to develop an efficient and reproducible means of identifying cryptic species. Two commercially developed MALDI-TOF MS systems are now available.[20]

Of extreme importance is the evolution of the species of *Candida* designated as *C. auris,* which was recovered from the ear of a patient in Japan in 2009 and has now spread globally. Multiple excellent reviews are now available, the details of which are beyond the scope of this chapter.[21–25] The following selected practical points can be made at this time:

1. The organism has now been found on five continents and appears to be spreading relatively rapidly.
2. It arises from separate clades.
3. Certain strains of the species are resistant to all classes of antifungal drugs.
4. Candidemia with *C. auris* has been associated with mortality rates greater than 50%.
5. Current fungal speciation methods have been associated with misidentification and classified as incorrect *Candida* species.
6. In-hospital outbreaks have occurred, and eradication of the organism from fomites in patients' rooms has been difficult to impossible.
7. The precise epidemiology of this species is not known at this time.

Many authors have named this organism the "*Candida* Superbug."

EPIDEMIOLOGY AND ECOLOGY

C. albicans organisms have been recovered from soil, animals, hospital environments, inanimate objects, and food. Non-*albicans* species may live in animal as well as non-animal environments. Only rarely are *Candida* spp. laboratory contaminants. That principle has not been generally appreciated historically, and interpretation of positive cultures as laboratory or skin contaminants has led to important errors in patient management.

The organisms are normal commensals of humans and are commonly found on skin, throughout the entire gastrointestinal (GI) tract, in expectorated sputum, in bronchoalveolar lavage fluid, in the female genital tract, and in the urine of patients with indwelling Foley catheters. There is a relatively high incidence of carriage on the skin of health care workers. Although the vast majority of *Candida* infections are of endogenous origin, human-to-human transmission is possible. Examples are thrush of the newborn, which may be acquired from the maternal vagina, and balanitis in the uncircumcised man, which may be acquired through sexual contact with a partner having *Candida* vaginitis. There is also important, emerging evidence that *Candida* infection can be acquired from the hospital environment. Molecular biology tools are improving considerably the understanding of *Candida* epidemiology.[26–29]

PATHOGENESIS AND IMMUNOLOGY

The most important predisposing factors to *Candida* infections are iatrogenic. The introduction of newer therapeutic modalities for advanced life support into clinical medicine has been primarily responsible for the dramatic change in the incidence of nearly all forms of candidiasis in recent decades. Of these factors, probably the most important have been the introduction of antibiotics, the use of myeloablative chemotherapy for neoplastic diseases, and the widespread use of indwelling intravenous catheters. Antibiotics suppress normal bacterial flora and allow *Candida* organisms to proliferate, especially in the GI tract.[30] The factors that predispose patients to disseminated candidiasis, which have been mentioned in countless reviews, are listed in Table 256.1.

An extensive discussion of the virulence factors, pathogenesis, and immunity of *Candida* infections is beyond the scope of this chapter and can be only highlighted herein. Virulence factors responsible for the capability of the organism to damage the host have been studied and reviewed extensively.[14,31] Of these, the switch of *C. albicans* from yeast to hyphal phase, in vivo, has been the most intensively investigated trait through the years. In infected organs, the hyphal phase dominates. Locking the organism in yeast phase by genetic engineering renders it nonpathogenic.[32] Yet, the precise role of this phenotypic switch in conferring *Candida* pathogenicity in humans remains elusive, since *C. glabrata* can cause lethal infections but does not undergo hyphal transformation. Gene disruption studies have identified well over 150 factors that promote *Candida* virulence.[14] Biofilm formation has been studied extensively and is important for *Candida* pathogenicity on both human tissues and prosthetic materials.[33,34] Animal models of both deep visceral and mucocutaneous candidiasis have examined mechanisms of adherence to host constituents and prosthetic devices. *Hwp1* and genes within the *ALS* gene family have received the greatest attention for conferring

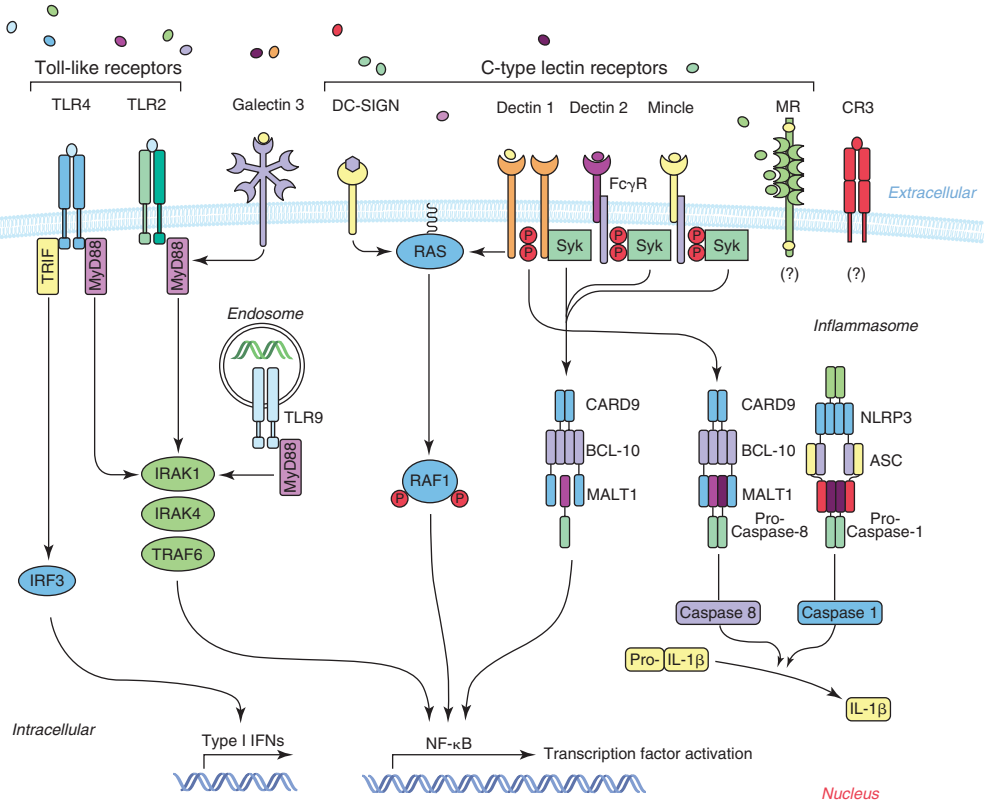

FIG. 256.2 Pathways involved in immune defense against *Candida*. *(Modified from Lionakis MS, Netea MG. Candida and host determinants of susceptibility to invasive candidiasis.* PLoS Pathog. *2013;9:e1003079.)*

adherence; however, several other genes also operate as adhesins.[35] Secreted aspartyl proteases and phospholipases,[36] capable of directly damaging tissue during invasion, have received considerable attention also.[37] Candidalysin, a cytolytic peptide toxin secreted by *C. albicans,* was recently shown to be critical for mucosal epithelial damage and host fungal recognition.[38]

Perhaps the greatest expansion in knowledge of *Candida* pathogenesis in recent years has been in the area of host defense mechanisms and genetic susceptibility to infection. *Candida* is a normal human commensal and becomes pathogenic mainly due to immune system perturbations. For a more complete review of this topic the reader is directed to comprehensive, contemporary, and excellent reviews (only a partial list).[39–43] An important concept in *Candida* host defense is that the constituents of effective immunity against mucosal and systemic disease cleanly segregate. Thus innate immunity via phagocytes is critical for control of systemic candidiasis, whereas adaptive immunity via T lymphocytes is important for control of mucosal candidiasis.

Fig. 256.2 summarizes current concepts regarding the pattern recognition receptors (PRRs) involved in *Candida* recognition by the innate immune system. Among PRRs, C-type lectin receptors (CLRs) have emerged as the most critical in humans. Dectin-1, the prototypic CLR, recognizes *Candida* β-glucan, while dectin-2 and dectin-3 recognize *Candida* mannans. The caspase recruitment domain–containing protein 9 (CARD9) signaling pathway, shown in Fig. 256.2, is downstream of CLR engagement and activates the immune system via production of cytokines and chemokines and via phagocyte recruitment and fungal uptake and killing.[43] Inherited deficiency in *CLEC7A,* which encodes dectin-1, is associated with recurrent vulvovaginitis and onychomycosis, but not systemic candidiasis.[44] Conversely, inherited deficiency in *CARD9* causes deeper immune defects and is the only known congenital immunodeficiency that predisposes to both mucosal and systemic candidiasis. The latter has a unique predilection for the central nervous system (CNS) due to impaired neutrophil recruitment to the *Candida*-infected CNS.[45] Beyond candidiasis, CARD9-deficient patients develop deep-seated dermatophytosis, extrapulmonary aspergillosis, and phaeohyphomycosis.[46–48]

A primary anti-*Candida* defense mechanism is intact skin and mucosal membranes. Any process causing skin maceration or mucosal damage leaves the involved site susceptible to *Candida* invasion, even in healthy individuals. In recent years, the importance of interleukin-17 (IL-17) signaling in protecting against *Candida* invasion at mucocutaneous barriers has become apparent. Mechanistically, IL-17 produced by $CD4^+$ Th17 cells, γδ T cells, and innate lymphoid cells protects via mediating the production of antimicrobial peptides that inhibit mucosal *Candida* growth. Several inherited disorders that impair human IL-17 immunity and cause chronic mucocutaneous candidiasis (CMC) have been discovered and are described later in this chapter.[49–51] Of note, IL-17 increases in humans in response to vaccination with the encoded product of the N-terminus of Als3.[52]

After *Candida* invades the bloodstream and disseminates into deep tissues, myeloid cells such as neutrophils, monocytes, and macrophages are key components of defense via effective uptake and killing of *Candida*.[43] Indeed, a strong predisposing factor for disseminated candidiasis is iatrogenic neutrophil deficiency, usually caused by cytotoxic chemotherapy. In addition, patients with defective oxidative cytotoxicity, such as those with chronic granulomatous disease due to NAPDH oxidase deficiency, or patients with complete myeloperoxidase deficiency are at risk for developing systemic (but not mucosal) candidiasis.[50] However, the majority of these patients do not develop candidiasis during their lifetime, underscoring the importance of nonoxidative fungal killing mechanisms that can compensate in the absence of oxidative burst. Importantly, recent studies have uncovered polymorphisms in immune-related genes in intensive care unit (ICU) patients that increase their risk for candidemia and disseminated candidiasis.[43,53–55] These studies show promise for identifying ICU patients at high risk for candidiasis, in whom personalized strategies for prophylaxis and treatment may help improve outcomes. Although well recognized for their protective roles, neutrophils may rarely cause tissue damage during candidiasis. Indeed, some *Candida*-infected patients develop worsening symptoms upon neutrophil recovery, necessitating corticosteroid administration.[56] Therefore identification of molecules that mediate neutrophil immunopathology in the mouse model of candidiasis, such as the chemokine

receptor Ccr1 and others, show promise for developing immune-based therapies for selected patients.[57]

CLINICAL MANIFESTATIONS

As the frequency of diseases due to *Candida* has increased, a relatively large number of manifestations, which were previously either not recognized or extremely infrequent, have become well documented. The discussion of these clinical manifestations is facilitated by their subdivision into mucocutaneous and deep organ involvement.

Mucous Membrane Infections

Thrush

Oral *Candida* infections are common and have been reviewed extensively.[58–62] The term *thrush* is applied to a specific form of oral candidiasis characterized by creamy white, curdlike patches on the tongue and other mucous membranes (Fig. 256.3); the patches are removable by scraping and leave a raw, bleeding, and painful surface. The patches are actually pseudomembranes consisting of *Candida,* desquamated epithelial cells, leukocytes, bacteria, keratin, necrotic tissue, and, in the mouth, food debris. The formation of *Candida* biofilm,[63] as well as epithelial cell invasion,[64] is important in the establishment of oropharyngeal candidiasis. The diagnosis is made by the clinical appearance of the lesion and confirmed by scraping, using either a potassium hydroxide smear or Gram stain to show masses of hyphae, pseudohyphae, and yeast forms. Simple culturing does not solidify the diagnosis because *Candida* grows easily from normal mouths. In addition to the classic lesions, which have been described by Lehner,[65] other manifestations include (1) acute atrophic candidiasis, a nonspecific atrophy of the tongue that is thought to be a sequela of acute pseudomembranous candidiasis; (2) chronic atrophic candidiasis or "denture sore mouth," which is a chronic inflammatory reaction and epithelial thinning under the dental plates; (3) angular cheilitis, an inflammatory reaction at the corners of the mouth (not due exclusively to *Candida*); and (4) *Candida* leukoplakia, which is firm, white plaques affecting the cheek, lips, and tongue that have a protracted course (and, in rare instances, may be precancerous). Since the introduction of inhaled steroids for the treatment of asthma, especially in children, oral thrush has been reported extensively in patients treated with these agents. The incidence has ranged from 0% to 77%. Thrush developing in patients who use inhaled steroids usually resolves spontaneously without a change in the dosage of the agent or is successfully managed with topical nystatin or clotrimazole. Other patients with a high incidence of thrush are cancer patients and those with acquired immunodeficiency syndrome (AIDS). Patients with thrush for no obvious reason should be evaluated for AIDS. Because of the introduction of potent antiretroviral therapy, the incidence of colonization and symptomatic infection with thrush has declined somewhat in patients with AIDS, but remains common.[66] Certain patients may have chronic thrush, and thrush may be a feature of inherited CMC syndromes (see later).

Candida Esophagitis

Although there have been a small number of reports of *Candida* esophagitis occurring in patients with no known underlying illness, it is more commonly associated with treatment of malignancy of the hematopoietic or lymphatic systems (Fig. 256.4) and in AIDS patients.[67,68] Additionally, omeprazole has been implicated as a risk factor. Esophageal disease was believed to occur by direct spread from oral disease (thrush), but reviews have shown that *Candida* esophagitis may occur frequently without thrush; this is an important clinical concept. The most common symptoms of *Candida* esophagitis include painful swallowing, a feeling of obstruction on swallowing, and substernal chest pain. Nausea and vomiting may also occur. The diagnosis is made definitively by biopsy during endoscopy (Fig. 256.5). However, the appropriate clinical settings, associated with the endoscopic appearance of white patches resembling thrush that show masses of hyphae and pseudohyphae on scraping, are enough evidence to initiate therapy without a histopathologic demonstration of the organisms invading the mucosa. It is important to recognize that *Candida* esophagitis can occur simultaneously with herpes simplex virus or cytomegalovirus infection in severely immunocompromised patients. Radiographic examination may be helpful in making a clinical diagnosis; irregularity of the esophageal mucosa as a result of ulcerations may be seen, as well as shoulder defects, diverticulae, fistulas, and dilatation of the esophagus from denervation. Endoscopy is the preferred procedure for definitive diagnosis, however. The pseudomembrane that forms may become so extensive that it causes intraluminal protrusions and partial esophageal obstruction. Perforation of the esophagus due to esophageal candidiasis is very rare. Generally, if perforation occurs, it is in the lower two-thirds of the esophagus. Some patients have had extensive esophageal disease and been almost asymptomatic, probably as a result of denervation of the esophagus from the disease. Other complications include bleeding and, presumably, dissemination.



FIG. 256.3 Typical oral thrush with curdlike white patches over the tongue. *(Courtesy Dr. Arnold Gurevitch.)*

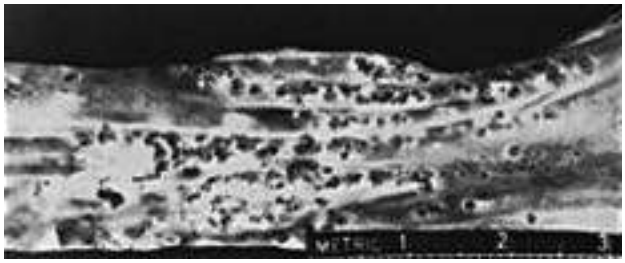

FIG. 256.4 Severe *Candida* esophagitis at autopsy.

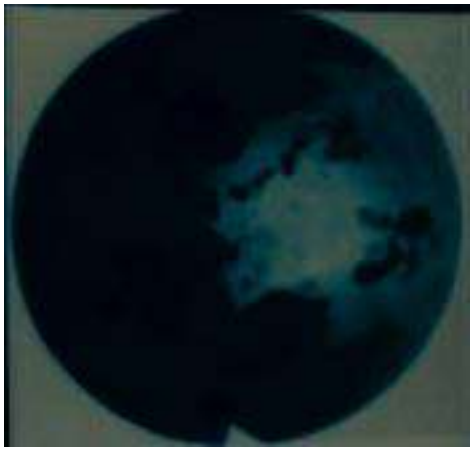

FIG. 256.5 *Candida* esophagitis in an AIDS patient. Numerous white plaques are seen.

Nonesophageal, Mucous Membrane, Gastrointestinal Candidiasis

The most common clinical setting for GI tract candidiasis is in patients with neoplastic disease. The esophagus is the most common site, followed by the stomach and small intestines. The most frequent lesions are single or multiple ulcerations containing *Candida* deep in the ulcer beds. *Candida* can also invade ulcers caused by other diseases, such as peptic ulcer and malignant gastric ulcer. As in other mucous membrane *Candida* infections, white plaques may be seen on endoscopy of the duodenum, and there may be thickening of mucosal folds in the duodenum and jejunum. Equal in frequency to the involvement of the small bowel is involvement of the large bowel, which again may be characterized by ulceration, superficial erosions, pseudomembrane formation, penetrating ulcers, and, rarely, perforation.

Reviews of defensive mechanisms of mucosal candidiasis have been published.[69,70] It is highly likely that hematogenous dissemination from the GI tract occurs in neutropenic patients with GI mucosal damage due to cytotoxic chemotherapy (Fig. 256.6).[71,72]

Candida Vaginitis

Candida has assumed the role of the most common cause of vaginitis, with higher frequency rates than those of *Trichomonas* or bacterial vaginosis. This common infection is most frequently seen in the setting of diabetes mellitus; antibiotic therapy, particularly with β-lactams; and pregnancy. In addition, although controversial, the use of certain birth control pills may be a predisposing factor. Estimates are that 75% of women have an episode of candidal vaginitis during their lifetime, 50% of them will have at least one recurrence, and 8% will have recurrent vulvovaginal candidiasis (>3 episodes/year).[73] Recent investigations have shown that certain polymorphisms in innate immune genes and the interleukin-22 (IL-22) signaling axis may account for recurrence in this subset of patients.[42,74,75]

Of interest, in contrast to oral and esophageal disease, vaginal candidiasis is not more common or more refractory to treatment in patients with AIDS, indicating that cells other than CD4 T lymphocytes are critical for local vaginal anti-*Candida* host defense. Conversely, use of antibiotic therapy is an important factor responsible for the emergence of *Candida*-induced vaginitis, whereas antibiotics do not predispose to oral or esophageal disease. Antibiotics can have effects by disrupting local IL-17/IL-22 protective immunity, via promoting biofilm formation by *Candida,* or both.[76,77] Excellent reviews of the current trends in the epidemiology and pathogenesis of vaginal candidiasis are available.[73,76–79] In these reviews, the rising incidences of non-*albicans* *Candida* species and of azole resistance in the species of *Candida* recovered are emphasized.[80,81]

Although *Candida*-induced vaginitis may be accompanied by a thick, curdlike discharge, scanty discharge may instead characterize the infection. Edema and intense pruritus of the vulva are almost always present. The discharge consists of epithelial cells and masses of hyphae and pseudohyphae, accompanied by lymphocytes and neutrophils.[82] The vagina and labia are usually erythematous, and extension onto the skin of the perineum can occur (Fig. 256.7). In addition, endometritis due to *Candida* has been reported, and the urethra may become secondarily infected.

Cutaneous Candidiasis Syndromes

Generalized Cutaneous Candidiasis

This condition is an unusual form of cutaneous candidiasis and is characterized by widespread eruptions over the trunk, thorax, and extremities with increased severity in the genitocrural folds, anal region, axillae, hands, and feet (Fig. 256.8). The process begins as individual



FIG. 256.7 Extension of *Candida* vaginitis onto the perineum.



FIG. 256.8 Generalized cutaneous candidiasis. *(Courtesy Dr. Victor Newcomer.)*

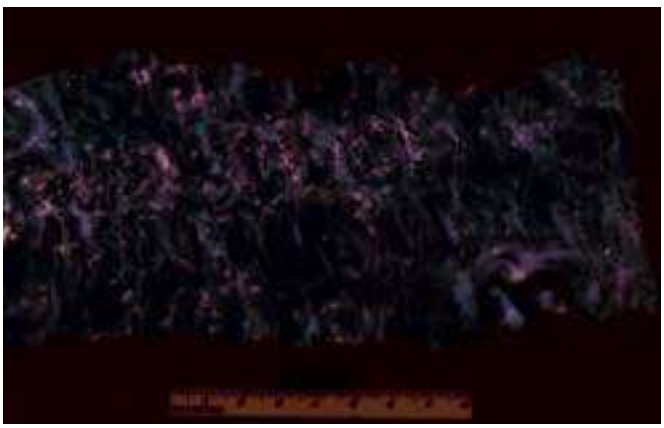

FIG. 256.6 Numerous small intestine ulcerations with *Candida* and disseminated candidiasis in a neutropenic patient at autopsy.

lesions that spread into large confluent areas. It is more common in newborns and children but does occur in adults.[83,84] It should not be confused with *Candida* folliculitis or the cutaneous macronodular lesions of widespread hematogenously disseminated *Candida* (see later).

Erosio Interdigitalis Blastomycetica

This term applies to *Candida* infection occurring between the fingers or toes (Fig. 256.9). It has a red base, may extend onto the sides of the digits, is painful, and is predisposed to by maceration.[85,86]

Candida Folliculitis

Infection with *Candida* at the hair follicles can occur (Fig. 256.10). Rarely, the condition may become extensive. It must be distinguished from folliculitis caused by the dermatophytes and tinea versicolor. This folliculitis has been described in immunocompromised hosts and intravenous drug abusers. Its incidence is increased in obesity.[87,88]

Candida Balanitis

This process begins as vesicles on the penis that develop into patches resembling thrush and are accompanied by severe itching and burning. *Candida* is one of the more common causes of balanitis.[89] It may spread to the thighs, gluteal folds, buttocks, and scrotum. It can be acquired through sexual intercourse with a partner who has vaginal candidiasis.[90]



FIG. 256.9 Erosio interdigitalis blastomycetica. *(Courtesy Dr. Arnold Gurevitch.)*



FIG. 256.10 Severe *Candida* folliculitis in beard distribution. *(Courtesy Dr. Victor Newcomer.)*

Cutaneous Lesions of Disseminated Candidiasis

Four distinct types of lesions associated with disseminated candidiasis have been described. The macronodular lesions (Fig. 256.11) are 0.5 to 1 cm in diameter, are pink to red, and may either be single or occur widely distributed over the entire body. The most accurate method of making a specific diagnosis is by punch biopsy[91,92] and demonstration of organisms on histologic section. Most patients with these lesions are neutropenic, and all have disseminated candidiasis, not local inoculation. Additionally, lesions resembling ecthyma gangrenosum,[93,94] purpura fulminans,[95] and leukocytic vasculitis[96] have been described. Chronic lesions of pyoderma gangrenosa may become superinfected with *Candida* and delay their definitive diagnosis.

Intertrigo

This common skin condition affects any site in which skin surfaces are in close proximity and provide a warm, moist environment.[97] It begins as vesicopustules, which enlarge and rupture, causing maceration and fissuring. The area of involvement has a scalloped border with a white rim consisting of necrotic epidermis, which surrounds an erythematous, macerated base. Frequently, satellite lesions are found that may coalesce and extend the affected area. A variant form of cutaneous candidiasis in the intertriginous region has a miliary appearance resembling miliaria rubra with erythematous macules or vesicopustules.

Paronychia and Onychomycosis

Candida is one of the most common causes of paronychia. Species other than *albicans* may be causative and may cause osteomyelitis of the distal phalanx.[98,99] Many skin bacteria, as well as *Candida,* can usually be recovered by culture of the infected area. The appearance of the reaction is that of a relatively well-localized area of inflammation that becomes warm, glistening, and tense and may spread extensively under the nail (Fig. 256.12). Unless the disease process is stopped, secondary thickening, ridging, and discoloration occur, and nail loss may develop.

Candida paronychia occurs in association with frequent immersion of the hands in water. People who may contract paronychia include dishwashers, laundry workers, and parents of babies. There is also a higher incidence of paronychia among diabetic patients than in the nondiabetic population. Specific diagnosis is made by Gram stain or potassium hydroxide preparation and culture showing predominantly *Candida* organisms.

Candida can cause onychomycosis, usually in patients with paronychia.[100,101]

Diaper Rash

Candida is a common cause of diaper rash in infants.[102] The condition generally starts in the perianal area and spreads over the perineum in the region of diaper contact (Fig. 256.13). The process is facilitated by maceration caused by wet diapers. The probable origin is the GI tract. Diagnosis is made by scraping the area and demonstrating the organisms on potassium hydroxide preparation.



FIG. 256.11 Macronodular lesions of disseminated candidiasis. *(Courtesy Dr. Richard Meyer.)*



FIG. 256.12 **Chronic mucocutaneous candidiasis of the fingers and nails.**



FIG. 256.13 **Severe *Candida* diaper rash.** *(Courtesy Dr. Victor Newcomer.)*

Perianal Candidiasis

Although numerous organisms and combinations of organisms have been associated with pruritus ani either alone or in combination, *Candida* is a frequent cause.[103] The perianal skin develops marked erythema and progresses to maceration (Fig. 256.14). Intense pruritus results. Complications include involvement of the anal canal and extensive spread over the perineum. It can occur among men who have sex with men.[104]

Chronic Mucocutaneous Candidiasis

The term *chronic mucocutaneous candidiasis* (CMC) describes a heterogeneous group of *Candida* infections of the skin, mucous membranes, hair, and nails that have a protracted and persistent course despite what is usually adequate therapy. The major complication is disfiguring lesions of the face, scalp, and hands. *Candida* esophagitis can be a long-term complication, and can cause esophageal stenosis and, in some instances, carcinoma. Alopecia in areas of infection is common and may be permanent. The subject has been reviewed comprehensively in a classic publication.[105] Most forms of CMC begin in infancy or within the first 2 decades; rarely, the onset may be after the age of 30 years. The first manifestation is usually oral thrush followed by nail infections. There is a broad spectrum of severity, ranging from chronic involvement of an isolated nail to a severely disfiguring form (*Candida* granuloma) (Fig. 256.15).

In recent years, an explosion in the discovery of several inborn errors of IL-17 immunity has dramatically expanded our understanding of the pathogenesis of and genetic susceptibility to CMC. The topic has been reviewed comprehensively.[50] Briefly, deficiency in the following genes may present with CMC: *IL17RA, IL17RC, IL17F, ACT1, RORC, AIRE, STAT3, DOCK8, CARD9, CLEC7A, STAT1, IL12RB1,* and *STK4.* The direct defects of IL-17 signaling (i.e., mutations in *IL17RA, IL17RC, IL17F,* and *ACT1*) primarily manifest with CMC and staphylococcal skin infections without noninfectious manifestations. Other genetic perturbations result in CMC together with syndromic manifestations; two of these are sufficiently important to briefly highlight. Autoimmune polyendocrinopathy-candidiasis–ectodermal dystrophy (APECED) is an autosomal-recessive disease caused by *AIRE* mutations. Patients manifest with selective infection susceptibility to CMC (~85% prevalence) along with a variety of endocrine and nonendocrine autoimmune manifestations, and ectodermal dystrophy.[106] CMC is typically the first disease manifestation during infancy and is associated with the presence of neutralizing autoantibodies to IL-22, IL-17A, and IL-17F.[106] Job syndrome or autosomal-dominant hyperimmunoglobulin E syndrome is caused by *STAT3* mutations. Affected patients manifest with CMC



FIG. 256.14 **Perianal candidiasis.** *(Courtesy Dr. Victor Newcomer.)*



FIG. 256.15 ***Candida* granuloma.** *(Courtesy Dr. Victor Newcomer.)*

(~70% prevalence), skin and pulmonary staphylococcal infections, skeletal abnormalities, and elevated serum immunoglobulin E. CMC develops in Job syndrome patients because *STAT3* is critical for the differentiation of CD4 T cells into the Th17 lineage.[107]

Deep Organ Involvement

Central Nervous System Candidiasis

Candida infects both parenchymal brain tissue and the meninges, usually as a complication of hematogenously disseminated candidiasis. Of note, CNS involvement is more frequent in neonates with candidemia. Approximately 50% of patients with *Candida* meningitis have had disseminated disease in other organs. When infection occurs in brain parenchyma, it generally forms multiple microabscesses and small macroabscesses scattered throughout the tissue. Rarely, larger abscesses have occurred and may be visualized by computed tomography. Mechanisms by which this organism localizes to the brain in the mouse model have been elucidated.[108] As mentioned previously, CARD9 deficiency predisposes to CNS candidiasis due to insufficient neutrophil recruitment into the brain. The diagnosis should be considered in putatively immunocompetent individuals who present with CNS *Candida* disease.[45]

Virtually all patients with *Candida* meningitis have had cerebrospinal fluid pleocytosis. Fifty percent have had a lymphocyte pleocytosis with an average count of 600 cells/mm^3. Sixty percent have had hypoglycorrhachia and elevated protein levels; organisms have been present on wet mount or Gram stain in approximately 40%. *C. albicans* has been the responsible pathogen in 90% of cases. Occasional cases due to *C. tropicalis* have been reported.

The clinical manifestations of CNS involvement with diffuse microabscesses may be variable. If the patient is comatose or noncommunicative, detection of abnormalities may be exceptionally difficult. When meningitis is present, the signs of meningeal irritation (headache, stiff neck, irritability), typical of any meningeal infection, are frequently present. In the newborn, particularly the very-low-birth-weight neonate, diagnosis is often difficult and delayed, leading to permanent neurologic sequelae. Lumbar puncture should be considered when the blood cultures of such infants contain *Candida*.

In addition to occurring as a complication of disseminated candidiasis, *Candida* meningitis may result from infection of a ventricular shunt, or may be introduced by lumbar puncture, trauma, or neurosurgery,[109] or may complicate bacterial meningitis. The signs and symptoms are nonspecific. Untreated, the mortality rate is very high; it is reduced substantially with antifungal therapy. Hydrocephalus is a frequently occurring complication of the infection. The number of cases of *Candida* meningitis reported in neonates has been increasing.

Respiratory Tract Candidiasis

In general, *Candida* pneumonia occurs in two forms: (1) either local or diffuse bronchopneumonia originating from endobronchial inoculation of the lung, a very rare event; or (2) a hematogenously seeded, finely nodular, diffuse infiltrate that in its early stages may be difficult to distinguish from congestive heart failure or *Pneumocystis* pneumonia. Other forms of *Candida* pneumonia are very rare; those that have been described are necrotizing pneumonia, *Candida* as a fungus ball in the lung, and transient infiltrates due to *Candida*. Radiographic and computed tomographic findings are nonspecific, and definitive diagnosis depends on biopsy-proven fungal invasion of pulmonary tissue. Because of a relatively high prevalence of yeasts colonizing the respiratory tract, especially in ill patients, a definitive diagnosis of *Candida* pneumonia cannot be made on radiographic findings and recovery of yeasts from sputum, bronchoalveolar lavage, or endotracheal tube aspirate. A contemporary discussion of the significance of *Candida* in sputum in ill patients is now available.[110] *Candida* has also caused bronchial infection, laryngitis, epiglottitis, and infection of laryngeal prostheses. The entity of "fungal empyema thoracis" has been described as an emerging clinical entity, usually a combination of bacteria and *Candida* in the empyema.[111] Hospitalized patients who develop bacterial pneumonia, are treated with broad-spectrum antibiotics, and then begin growing *Candida* from the sputum only rarely have *Candida* pneumonia, that is, pneumonia that is predominantly caused by *Candida*.

Cardiac Candidiasis

In addition to causing endocarditis, *Candida* infects both the pericardium and the myocardium. *Candida* myocarditis occurs as diffuse microabscesses scattered throughout the myocardium with normal intervening myocardial tissue. The relatively high incidence of myocarditis has been stressed by Franklin and coworkers,[112] who found that 62% of their 50 patients with disseminated candidiasis had myocardial involvement. Other retrospective autopsy studies have shown a range from 8.4% to 93%. *Candida* myocarditis has also occurred in AIDS patients. Autopsy series of disseminated candidiasis reveal a surprisingly high incidence of myocarditis (without associated valvular involvement) and point to the importance of thorough cardiac evaluation in patients who may have disseminated candidiasis. Of interest has been the emergence of *Candida* organisms as a cause of pericarditis. A review of purulent pericarditis spanning the years 1960 to 1974 revealed that *Candida* organisms were either the single cause or combined with *Aspergillus* in 15% of the 26 cases.[113] A more recent review is available.[114]

Candida Endocarditis

Fungi are the cause of between 1% and 10% of all forms of endocarditis. In a detailed review of 319 cases of fungal endocarditis, *Candida* accounted for 67% of the cases. In the last several decades, there have been several hundred reported cases. The entity of *Candida* endocarditis has been reviewed extensively.[115–117] Additional cases have been reported in children.[118] *Candida* endocarditis on native or prosthetic valves occurs mainly in association with six clinical factors: (1) underlying valvular heart disease; (2) heroin addiction[119]; (3) cancer chemotherapy; (4) implantation of prosthetic valves[120]; (5) prolonged use of intravenous catheters (endocarditis, right atrial fungal masses, and infection of atrial myxomas have all been described); and (6) preexisting bacterial endocarditis, on which it is superimposed. Of these associations, by far the most frequent is post–cardiac surgery, accounting for approximately 50% of the prosthetic valve infections. Two-thirds of the cases of *Candida* endocarditis are nosocomially acquired.[121] Of interest is the frequency of species other than *C. albicans* that have caused endocarditis; a minimum of 41% of the cases have been due to organisms of non-*albicans* species, some of which have been very rarely recovered species. Newer species continue to be reported regularly. In heroin addicts, *C. parapsilosis* has been the most common causative organism.[122]

The pathogenic mechanisms for fungal endocarditis are not fully understood, but patients who undergo cardiac surgery are at risk for candidemia by being exposed to multiple antibiotics, prolonged intravenous fluid administration, and intravenous plastic catheters. Both the damaged endocardium and prosthetic material apparently serve as foci for the localization of *Candida* organisms. Also, contamination of suture material has been implicated in cases reported with concentration along the suture line. Contamination of homografts and heterografts before insertion has also been documented.

The valves most commonly involved in *Candida* endocarditis have been the aortic and the mitral (Fig. 256.16). In postoperative *Candida*

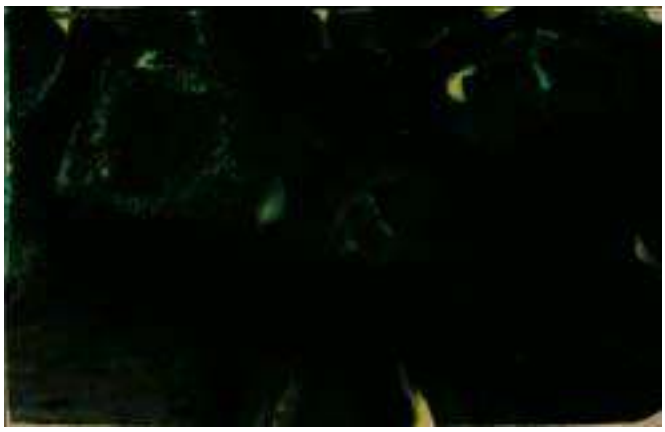

FIG. 256.16 ***Candida parapsilosis*** **vegetations on an aortic valve.**

endocarditis, the type of surgery has not been as important as the length of the postoperative course and complications during the postoperative period. *Candida* infection has been seen in simple valvulotomies and in prosthetic material placement, heterografts, and homografts. Pacemaker endocarditis has also been described.[123] The physical findings and usual symptoms of *Candida* endocarditis are not significantly different from those of bacterial endocarditis with the exception of the occurrence of large emboli to major vessels. Osler nodes, Janeway lesions, splinter hemorrhages, hepatosplenomegaly, hematuria, proteinuria, pyuria, and urinary casts all can occur. In addition, although the lesions of hematogenous ocular candidiasis have been described much more frequently in the setting of disseminated candidiasis without endocarditis, they may also be seen with endocarditis.

The complications of *Candida* endocarditis are very similar to those of bacterial endocarditis and include valve perforation, myocarditis, congestive heart failure, and major emboli. Although most cases of postoperative prosthetic valve *Candida* endocarditis occur in the first 2 postoperative months, some have occurred later, and some patients who have been treated have had recurrent active disease after 2 years, and perhaps as long as 8 years. Therefore in following patients treated for postoperative endocarditis, careful follow-up must be extended over a prolonged period.

Most patients with *Candida* endocarditis have positive blood cultures. Modern blood culture methods, such as nucleotide detection systems, are likely to provide better sensitivity and specificity. The largest prospective experience with various serum diagnostic tests for *Candida* endocarditis was published in 2012.[124] Detection of serum (1,3)-β-D-glucan and antimannan antibody had a very high negative predictive value. Studies of serodiagnostic tests were inconclusive regarding their impact on treatment. Echocardiography is becoming progressively more helpful, and vegetations may be detected with this technique. False-negative results are common, especially in cases of mural endocarditis without valvular involvement. Transesophageal echocardiography has improved the sensitivity, particularly in the mitral valve.

The therapy for *Candida* endocarditis is discussed in detail in the section on therapy. Before the introduction of surgical procedures for the management of *Candida*-induced endocarditis, the mortality rate from this disease was approximately 90%. With combined surgical and medical therapy, this high mortality rate has dropped to approximately 45%. Because of the introduction of newer antifungals, there has been a greater propensity for their use in chronic suppression for selected patients.

Candida endocarditis has been seen in association with bacterial endocarditis as a polymicrobial infection. In general, *Candida* has been a superinfection introduced by prolonged intravenous catheterization for antibiotic administration. Interesting investigations are developing showing how *Candida*-bacteria interactions enhance the pathogenicity of a coinfection.[125,126]

Urinary Tract Candidiasis

This topic has been reviewed comprehensively and definitively.[127–134] The presence of *Candida* in the urine is common in hospitalized patients and usually does not indicate urinary tract infection. Antibiotics, diabetes mellitus, and Foley catheters have been associated with the acquisition of candiduria, usually asymptomatic. Although the use of colony counting in urine has been attempted to separate colonization from infection, it has not been reliable. Most patients with health care–associated asymptomatic candiduria have spontaneous resolution; however, long-term persistence of candiduria may follow bladder catheter removal, particularly in patients with diabetes mellitus or bladder outlet obstruction with residual urine or urinary diversion structures, such as ileal conduits and orthotopic neobladders. The cystoscopic appearance of *Candida* cystitis is that of a chronic nonspecific mucosal inflammation, though thrushlike patches are occasionally seen. Hematogenous dissemination may occur, usually following an operative procedure on the bladder.

Candida infection of the upper urinary tract has been classified into two distinct forms: that developing along an ascending route, and that resulting from hematogenous spread. Ascending infection can arise after a ureteral stent or transcutaneous catheter is placed in the renal pelvis to relieve obstruction. If *Candida* gains access to the renal pelvis through these procedures, subsequent partial or complete obstruction of the urinary drainage predisposes to *Candida* invasion of the renal papillae, followed by papillary necrosis. This can happen when the stent or catheter is removed and the ureter is still partially obstructed. Imaging may show a mass, often called a fungus ball, within the pelvis, composed of a sloughed papilla and inflammatory debris. The mass can obstruct the ureteropelvic junction, catheters, or stents. Relief of obstruction and systemic antifungals are required to prevent progressive kidney destruction and possibly perinephric abscess. If the renal pelvis had been drained percutaneously, *Candida*-infected urine can pass from the pelvis into the perinephric space through the catheter tract.

The hematogenous form of the disease is by far the most common. The pathologic changes are those of multiple microabscesses or renal cortical abscesses large enough to be seen on imaging. Emphysematous pyelonephritis may occur, particularly in insulin-dependent diabetics.

Isolated urethral candidiasis can occur in both men and women. In men, it usually results from sexual contact with women with *Candida* vaginitis. In women, it is generally thought to be acquired from extension of *Candida* vaginitis. *Candida* prostatic infection has also been reported. A history of previous antibiotic use has been frequent.

Candida Arthritis, Osteomyelitis, Costochondritis, and Myositis

Sites of localization for hematogenous *Candida* osteomyelitis include the spine (vertebrae and intervertebral disks [Fig. 256.17]), wrist, femur, cervical spine, and costochondral junctions of the ribs, scapula, and proximal humerus.[135,136] Prosthetic joints may also be infected. Blood cultures have usually been negative, and diagnosis has been made by percutaneous needle aspiration of the involved area. In children the long bones are generally affected, whereas in adults the axial skeleton predominates. Spinal involvement may be accompanied by disk infection. Bone infection may require surgical débridement of bone and drainage of contiguous abscess. Osteomyelitis as a result of contiguous spread from the skin has also been documented but is rare.



FIG. 256.17 *Candida* spinal osteomyelitis. *(From Edwards JE, Turkel SB, Elden HA, et al. Hematogenous candida osteomyelitis.* Am J Med. *1975;59:89–94.)*

Candida arthritis of native joints occurs most frequently as a complication of disseminated candidiasis.[135] It can also occur from trauma, surgery, or intraarticular injections of steroids; as a complication of heroin injection; and in patients with rheumatoid arthritis or prosthetic joints, in very-low-birth-weight neonates, or patients with AIDS. Culture of joint fluid may grow only a few colonies, which may be misinterpreted as contamination. In *Candida* arthritis occurring unassociated with disseminated candidiasis, non-*albicans* species have been the most common. Although the majority of cases of *Candida* arthritis have been acute, chronic *Candida* arthritis has been reported, especially in leukemic patients. *Candida* costochondritis can occur from hematogenous seeding, particularly to the sternoclavicular joint cartilage, or as a complication of median sternotomy wound infection.

Candida infection of muscle has been described. The majority of patients have been neutropenic, had hematogenously disseminated candidiasis, and had pain in the involved muscle. The organisms may be seen on biopsy of the involved muscle. Cases have also been reported in drug addicts. Generally, the muscle involvement is diffuse. However, a discrete muscle abscess may occur.

Intraabdominal Candidiasis: *Candida* Infection of Peritoneum, Liver, Spleen, and Gallbladder

Candida infection of the peritoneum is a complication of peritoneal dialysis, GI surgery, abdominal trauma, perforation of an abdominal viscus, and organ transplantation.[137–142] Prior antibiotic administration has been an important predisposing factor. The peritoneal process usually remains localized to the abdomen; the incidence of dissemination is approximately 25% in patients acquiring the disease from GI tract perforation or leaking intestinal anastomosis. In patients with peritonitis caused by chronic ambulatory peritoneal dialysis, dissemination is distinctly uncommon.

Other GI organs infected with *Candida* that have been reported include the gallbladder, the liver and spleen, the spleen alone, and the pancreas. Fungus balls may form in the gallbladder and bile ducts.

Hepatosplenic candidiasis has emerged as an important clinical problem in immunocompromised hosts and may require prolonged therapy.[143] Most of these hepatosplenic infections have occurred in severely immunocompromised patients and become manifest during their recovery from neutropenia. Fever may last weeks or months, long after cultures of liver biopsy are culture negative.[143] When the liver and spleen are involved, there is frequently involvement of other organs also, such as the kidney. Computed tomography, ultrasonography, or magnetic resonance imaging may visualize liver, kidney, or spleen abscesses (Fig. 256.18). Shrinkage of lesions on imaging can take weeks or months. Laparoscopic or transcutaneous biopsy can show necrotic lesions with abortive pseudohyphae readily misidentified as *Aspergillus.* The incidence of this entity has diminished in recent years, probably as a function of the increased use of antifungal prophylaxis and empirical therapy.

Candidemia

Current automated blood culture systems detect *Candida* species after roughly 40 hours of incubation, depending on the system, with *Candida glabrata* taking a day longer. Unlike other *Candida* species, the anaerobic bottle may become positive before the aerobic bottle with *C. glabrata.* Identification of the yeast varies between laboratories but may use MALDI-TOF MS, a multiplex polymerase chain reaction (PCR) panel that includes the five most common *Candida* species (FilmArray Blood Culture Identification; Biofire, Salt Lake City, UT), fluorescence in situ hybridization, or automated or manual biochemical panels or, to identify *C. albicans,* a germ tube test may be used. A PCR-based system that uses magnetic resonance technology to detect the five most common *Candida* species in whole blood within 3 to 5 hours has been marketed (T2Candida Panel; T2 Biosystems, Lexington, MA); susceptibility testing requires culture. Technology in this area is changing rapidly.

Including all species of *Candida* together, they constitute the fourth or fifth most common cause of bloodstream infections in hospitalized patients. Approximately 80% occur in patients with a venous catheter as the likely source. Formation of a biofilm in the lumen and around the catheter tip is thought to contribute to the difficulty in sterilizing the catheter by antibiotic lock therapy or infusion through the catheter or, if there is one, its subcutaneous injection port.[144] Catheter removal is almost always required for cure, but the difficulty in removing implanted catheters in thrombocytopenic patients has prompted delay in selected, hemodynamically stable patients receiving their systemic antifungal through the catheter. Suppurative thrombophlebitis can occur in insertion sites of short peripheral catheters or in arteriovenous fistulae in hemodialysis patients.

Ocular Candidiasis

Candida spp. can enter the eye from the bloodstream (endogenous) or directly into the eye from trauma or surgery (exogenous). Endogenous ocular infection usually begins in the choroid and spreads rapidly to the retina (chorioretinitis) and then may extend into the vitreous humor. Exogenous ocular infection is uncommon but may follow intraocular lens implantation, corneal transplantation, or other procedures that introduce *Candida* into the anterior chamber. The term *endophthalmitis* includes infection in either aqueous or vitreous humor but has been used to include chorioretinitis alone as well (Fig. 256.19).

Through the 1970s there was increased reporting of hematogenous ocular candidiasis and an actual increase in incidence of this complication of candidemia.[145–149] Case reports have described ocular candidiasis as a complication of tattooing, childbirth (in nonimmunocompromised women), abortion, therapy for human immunodeficiency virus (HIV) infection,

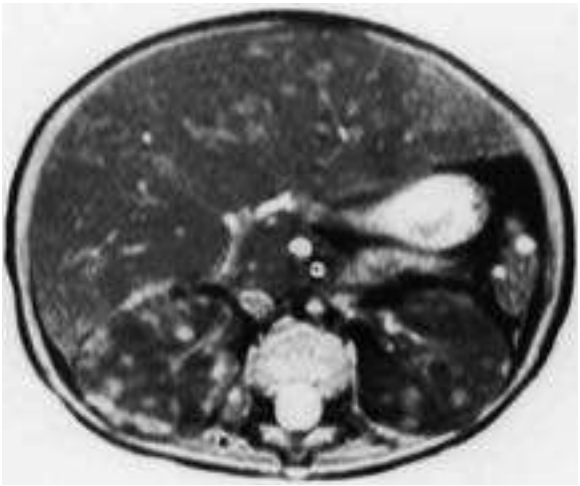

FIG. 256.18 *Candida* abscess in the liver, kidney, and spleen on magnetic resonance imaging.

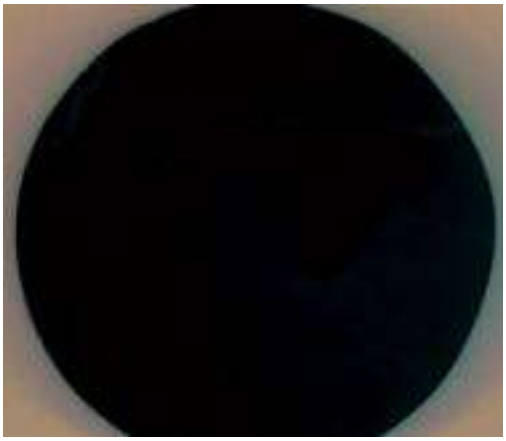

FIG. 256.19 Advanced hematogenous *Candida* endophthalmitis. *(From Fishman LS, Griffin JR, Sapico FL, et al. Hematogenous Candida endophthalmitis—a complication of candidemia.* N Engl J Med. *1972;286: 675–681.)*

urinary tract infection, and intravenous administration of contaminated dextrose infusion solution for minor ailments in a rural setting.

Endogenous Ocular Candidiasis

The bloodstream infection leading to endogenous *Candida* endophthalmitis can be transient or prolonged. Candidemia may result from an intravenous catheter (the most common source), intravenous drug abuse, or a focus in deep tissue. Incidence of ocular lesions in candidemic patients has varied widely, with 2.5%, 8.9%, and 16.2% in three different publications. *C. albicans* is the cause of endogenous ocular candidiasis in 90% or more of patients, with *C. tropicalis* accounting for most of the remainder. *C. glabrata* and *C. parapsilosis,* the second and third most common causes of candidemia after *C. albicans,* seem to have less potential for invading the retina, a feature also observed in experimental ocular candidiasis in rabbits. Early lesions in the retina are not specific and have an appearance similar to lesions from diabetes mellitus, hypertension, HIV, cytoid bodies of systemic lupus erythematosus and chorioretinitis from cryptococcosis, coccidioidomycosis, histoplasmosis, aspergillosis, and other mycoses. The lesions slowly resolve in most patients, if appropriate systemic antifungal therapy is given. Progression into the vitreous begins with vitreous haze, called *vitritis,* and can form masses of neutrophils, lymphocytes, and pseudohyphae floating in the vitreous humor ("fungus balls"). The lesions are not actually "fungus balls," since they are dominantly inflammatory cells surrounding relatively few organisms. A retinal lesion is not always seen, in part because a dense vitritis may obscure the retina, or the lesion may be in the anterior portion of the posterior chamber, such as the ciliary body. Initial symptoms are blurred vision or "floaters," though many patients are too ill or cognitively impaired to relate the symptoms. Deep bulbar pain is a late symptom. The importance of funduscopic examination during the first week of therapy in every candidemic patient, or in patients suspected to have disseminated candidiasis who are not candidemic, is to detect ocular lesions and assure that they do not progress. Early diagnosis depends on dilated funduscopic examination of both eyes, in that about half the patients have lesions in only one eye at the time of diagnosis. Extension of infection into the anterior chamber may be followed by circumlimbal vascular dilation, called a ciliary flush, that may be mistaken for conjunctivitis, particularly in intubated patients with exposure keratitis from inadequate lid closure.

Diagnosis can be confirmed by smear and culture of a vitreous aspirate. When a pars plana vitrectomy is done, the undiluted first specimen, called a "vitreous biopsy," is preferred to the diluted vitrectomy specimen. The latter should be centrifuged and a smear done on the sediment.

Exogenous Ocular Candidiasis

The indolent appearance of anterior chamber inflammation, with cells and protein in the aqueous humor, may follow intraocular lens implantation, corneal transplantation, other surgery, or trauma. A variety of *Candida* species have been reported. Diagnosis is usually made by smear and culture of fluid aspirated from the aqueous humor.

"Chronic Candidiasis Syndrome" or "the Yeast Connection"

This entity was popularized by William G. Crooke, MD, in 1979, with the publication of a book written by him entitled, "The Yeast Connection." The entity has been the subject of several lay books describing a multitude of symptoms, generally related to fatigue, GI discomfort, and memory dysfunction (described by some as "brain fog"), thought to be attributable to *Candida* organisms colonizing mainly mucous membrane tissues. Currently, there is considerable information about this disorder on the Internet and a host of different remedies are suggested, as well as support groups for afflicted patients and their families. What would be considered a direct scientific connection between the presence or overgrowth of the organism and these symptoms has not been established to date. Some individuals have claimed amelioration or resolution of their symptoms with various remedies, including special diets; however, the beneficial effects are generally temporary, and the claims of benefit have not been endorsed by the US Food and Drug Administration.[150]

TREATMENT AND PROPHYLAXIS

General Comments

Treatment strategies for nearly all forms of *Candida* infections have been reviewed in comprehensive detail in both the United States and Europe for both neutropenic and nonneutropenic patients, pediatric patients, and patients with HIV infection.[151–155] This summary has been formulated from these combined sources and is presented in condensed form in Tables 256.2 to 256.5. Complete dosing recommendations for both adults and pediatric patients will not be discussed herein, but are detailed in the current references.

Granulocyte transfusions have been given for systemic *Candida* infection, but they are not used on a wide-scale basis, and their efficacy has not been clearly established from the limited experience to date. Granulocyte colony-stimulating factor and other cytokines or immunomodulators have not been sufficiently evaluated in clinical settings, but are under investigation, and may be an option in desperate situations.[156]

Antifungal sensitivity testing is being used with significantly increased frequency for directing management of *Candida* infections, especially in refractory cases. The use of susceptibility testing for *Candida* in general has been reviewed[157]; considerable progress has been made by both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) on developing standardized sensitivity testing. The need for routine sensitivity testing on all isolates has not developed to this point, but work to improve the clinical relevance of specific species and drug breakpoints continues.[158]

For prophylaxis of *Candida* infection in stem cell transplant recipients, fluconazole has remained the drug of choice, with micafungin as an alternative. Prophylaxis in high-risk ICU patients is controversial, with the 2016 Infectious Diseases Society of America (IDSA) guidelines having a weak recommendation for fluconazole with an echinocandin as an alternative[157] (Table 256.2). It should be noted that fluconazole prophylaxis does not provide protection against molds. Table 256.3 summarizes current IDSA recommendations for empirical therapy. Empirical therapy of fever and neutropenia, including the possibility of *Candida* and mold infections, is discussed in Chapter 306.

TABLE 256.2 *Candida* Prophylaxis

POPULATION	RECOMMENDATION
Intensive care unit high-risk patients in units with high rates of invasive candidiasis (>5%)	Fluconazole: 400 mg (6 mg/kg) daily
Neonates with birth weights <1000 g in neonatal intensive care units with high rates of invasive candidiasis (>10%)	Fluconazole: 3–6 mg/kg twice daily for 6 wk
Patients with acute leukemia and remission-induction chemotherapy–induced neutropenia (from induction to end of neutropenia for all regimens)	Fluconazole: 400 mg PO (6 mg/kg) daily *or* Posaconazole tablets: 300 mg PO twice daily for two doses, then 300 mg PO daily *or* Voriconazole: 6 mg/kg PO twice daily for two doses, then 4 mg/kg PO twice daily *or* Caspofungin: 70-mg loading dose, then 50 mg IV daily *or* Micafungin: 100 mg IV daily Itraconazole: 200 mg PO once daily
Stem cell transplant recipients	Fluconazole: 400 mg (6 mg/kg) daily *or* Micafungin: 50 mg daily (if concern for molds, or fluconazole side effects) (for all: until engraftment or day 100 and immunosuppressives discontinued)

An 800-mg (12 mg/kg) loading dose for fluconazole should be given in adults.
Modified from Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. *2016;62:e1–e50.*

TABLE 256.3 Empirical Therapy

NONNEUTROPENIC PATIENTS	NEUTROPENIC PATIENTS
Fluconazole: 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily *or* Caspofungin: 70-mg loading dose, then 50 mg daily *or* Anidulafungin: 200-mg loading dose, then 100 mg daily *or* Micafungin: 100 mg daily (Echinocandins are preferred if there has been previous exposure to azoles or there is a high risk for *Candida glabrata* or *Candida krusei* infection)	LFAMB: 3–5 mg/kg daily *or* Caspofungin: 70-mg loading dose, then 50 mg daily *or* Micafungin: 100 mg daily *or* Voriconazole: 6 mg/kg twice daily for 2 doses, then 4 mg/kg twice daily
Alternatives	
LFAMB, 3–5 mg/kg daily, is an alternative for intolerance or unavailability of other antifungals	Fluconazole: 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily

LFAMB, Lipid formulation of amphotericin B.
Modified from Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis. *2016;62:e1–e50.*

Systemic Drugs for Candidiasis

Polyenes

Amphotericin B (AMB) generally remains the cornerstone of therapy for disseminated and deep organ *Candida* infection, especially those infections that may be rapidly fatal or refractory to azoles or to echinocandins. In the overall longitudinal experience of treatment of *Candida* infections, most of the experience has been with amphotericin B deoxycholate (AMB-D). However, lipid formulations of amphotericin B, including liposomal amphotericin B (LAMB) and amphotericin B lipid complex (ABLC), have been approved by regulatory agencies and are used extensively now. While evidence does not exist that LAMB or ABLC is more efficacious than AMB-D for the management of *Candida* infections, their use has become very popular, due mainly to their lower level of renal toxicity.

Triazoles

The triazoles currently available for the treatment of *Candida* infections include fluconazole, itraconazole, voriconazole, and posaconazole. Isavuconazole is a recently approved, extended-spectrum triazole that has excellent activity against *Candida* species, but is currently undergoing studies in candidemia. All of the triazoles have diminished activity against *C. glabrata, C. auris,* and *C. krusei.* Among the triazoles, fluconazole remains the primary choice, and the majority of experience has been with it. It has excellent oral absorption, which is not affected by food consumption. It is considered the standard of therapy for oropharyngeal candidiasis and esophageal and vaginal infection.[157] It also has high penetration into the cerebrospinal and ocular fluids and the highest level of renal excretion. The aggressiveness for treating patients with candidemia was changed dramatically when fluconazole was found to have equal efficacy to AMB in nonneutropenic patients.[159,160] The considerably less toxicity associated with fluconazole compared to AMB, and the lack of reliable diagnostic criteria for establishing the presence or absence of disseminated candidiasis in candidemic patients, made the risk-benefit ratio of treating highly in favor of treatment rather than watchful waiting. The use of the other triazoles for *Candida* infections is applied mainly to situations in which they may have a specific advantage regarding the susceptibility of an isolate, when fluconazole has failed, or when it is desirable to cover mold infections in addition to treating *Candida.* It does have an indication for prophylaxis of *Candida* infections.

Echinocandins

Three echinocandins are now approved by regulatory agencies for treatment of *Candida* infections and are the recommended treatment of candidemia in nonneutropenic and neutropenic patients, with fluconazole now indicated as an alternative.[157] The recommended echinocandins are caspofungin, micafungin, and anidulafungin. These drugs have the advantage of lower minimal inhibitory concentrations (MICs) for a broader spectrum of species of *Candida,* including *C. glabrata* and *C. krusei. C. parapsilosis* has a higher MIC, but echinocandins are now considered to be active for this species and the MIC cutoff has been adjusted. The currently marketed echinocandins have the disadvantage of not being available in an oral preparation to date.

The echinocandins have had a favorable toxicity profile. They have highly comparable pharmacodynamics. In patients with moderate to severe hepatic dysfunction, a dose reduction is recommended for caspofungin, but not for the others. In candidemia, the three echinocandins are considered to have equal efficacy. Many clinicians prefer to use these drugs as first-line therapy for patients with candidemia until the species of *Candida* is identified, especially if their recovery of *C. glabrata* is at high rates in their institutions. However, a retrospective analysis of initial treatment of *C. glabrata* sepsis found no difference in mortality whether fluconazole or an echinocandin was used.[161] Catheter removal may be as important as initial choice of antifungal.[150]

With increased echinocandin use in hospitals, *C. albicans* and *C. glabrata* strains with mutations in the *FKS1* and *FKS2* genes and decreased echinocandin susceptibility are being encountered, although this remains a rare event.[142,162,163] Most, but not all, of the patients with these strains have been receiving echinocandins. Whether patients infected with these strains are more likely to fail echinocandin therapy is controversial at present, largely because so many other clinical factors influence the outcome of deep candidiasis.

Flucytosine

Flucytosine, also known as 5-fluorocytosine (5-FC), is virtually never used alone for management of *Candida* infections. It is sometimes used in combination with AMB-D, ABLC, or LAMB for *Candida* endocarditis, meningitis, hepatosplenic candidiasis, and occasionally progressive endophthalmitis. However, there are no controlled studies demonstrating an advantage with the combination therapy, and it is a toxic agent with respect to bone marrow depression. It is rarely ever used currently. It has broad-spectrum activity against the *Candida* species, except *C. krusei.*

Candidemia in Nonneutropenic Patients

The decision to administer treatment for candidemia has been discussed previously in this chapter, and the strong consensus that all candidemic patients should be treated with antifungals was described. For nonneutropenic patients, an echinocandin is recommended as initial therapy.[152] If the isolate is susceptible to fluconazole and the patient is clinically stable, the echinocandin should be switched to fluconazole. Although voriconazole is effective for candidemia, it is recommended primarily when additional mold coverage is desired or as step-down oral therapy for candidemia due to *C. krusei* or cases due to voriconazole-susceptible *C. glabrata.* A lipid formulation of AMB is an alternative if the patient is intolerant to other antifungals or has an isolate resistant to other antifungals.[152] Although controversial, removal or at least changing of intravenous lines is recommended.

Candidemia in Neutropenic Patients

For most neutropenic patients, an echinocandin is recommended for initial therapy.[152] A lipid formulation of AMB is an effective but less well tolerated alternative to echinocandins. For patients less ill, and in whom no fluconazole prophylaxis has been given, fluconazole is an alternative. Fluconazole can be used as a step-down therapy in clinically stable patients who had a fluconazole-susceptible isolate and have cleared their blood cultures.[152] For *C. krusei* infections, an echinocandin is preferred, but LAMB, ABLC, or AMB-D or voriconazole may be used. Continuation of treatment for 2 weeks beyond the documentation of the clearance of the neutropenia is recommended. If possible, intravenous lines should be removed or changed. Granulocyte colony-stimulating factor–mobilized granulocyte transfusions may be used if prolonged neutropenia is expected, although no conclusive benefit has been demonstrated.

Candida Infections of the Cardiovascular System

Once the diagnosis of *Candida*-caused endocarditis is made, the procedure of choice is to initiate LAMB, with or without 5-FC, or an echinocandin.[121,152,164] If an echinocandin is chosen, the 2016 IDSA guidelines recommend using an increased dose, although the evidence was said to be of low quality (Table 256.4).[152] Valve replacement has long been recommended to be performed as soon as possible, although this has not been shown to reduce the high mortality.[121,164] The desirability of waiting for an effect of antifungal therapy before surgery is unknown, and currently it is recommended to perform surgery as soon as it is logistically feasible. After surgery, antifungal therapy should be given for at least 6 to 10 weeks because of the significant incidence of relapse. If there is a perivalvular abscess, or other evidence of residual *Candida,* extended suppressive therapy beyond the 6 to 10 weeks should be administered. Some patients with *Candida* endocarditis have had relapses years after surgery. Patients with *Candida* endocarditis should be monitored carefully for a minimum of 2 years postoperatively. Fluconazole is commonly used as long-term suppressive therapy. Occasional cases of successful nonsurgical therapy are reported in both adults and children.[165,166]

For prosthetic valve endocarditis, the recommendations are the same as for native valve disease. Suppressive therapy should be lifelong if it is not feasible to remove the valve. For other forms of cardiovascular infection, such as pericarditis or myocarditis, in general, prolonged administration of an echinocandin is recommended. For suppurative thrombophlebitis, incision and drainage of the infected vein and removal of the catheter should be done in combination with administration of the antifungal. For pacemaker and defibrillator infections, removal of the device with administration of antifungals for similar durations as those used for endocarditis is recommended. If only the generator or pocket is infected, 4 weeks of therapy following removal of the device is recommended, whereas, if the wire is infected, at least 6 weeks of therapy following the wire removal is recommended. For ventricular assist devices that cannot be removed, treatment is the same as for endocarditis.

Central Nervous System *Candida* Infection

LAMB, with or without 5-FC therapy, is the recommended treatment for both meningitis and diffuse parenchymal infection.[152] This recommendation is based entirely on observational studies. There is experimental evidence that LAMB may have a higher brain concentration than other formulations.[167] The clinical significance of this evidence remains to be established, but most experts use LAMB in documented CNS infection (authors' observations). In exceptionally severe cases, intrathecal antifungals should be considered. Removal of infected ventricular devices is recommended if feasible. If not, they should be changed if possible. Step-down therapy with fluconazole (400 to 800 mg daily) can be used once infection is controlled.

TABLE 256.4 *Candida* Treatment Strategies

INDICATIONS	PRIMARY TREATMENT	ALTERNATIVE	COMMENTS
Candidemia			
Nonneutropenic patients	An echinocandin[a]	Fluconazole: 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily *or* LFAMB[b] *or* Voriconazole: 6 mg/kg twice daily for 2 doses, then 4 mg/kg twice daily	Fluconazole for patients who are clinically stable and have no prior azole exposure. Fluconazole can also be step-down therapy for patients who have susceptible isolates and blood cultures converted to negative.
Neutropenic patients	An echinocandin[a] *or* LFAMB[b]	Fluconazole: 800-mg (12-mg/kg) loading dose, then 400 (6 mg/kg) daily *or* Voriconazole: 6 mg/kg twice daily for 2 doses, then 4 mg/kg twice daily	An echinocandin is preferred. Fluconazole is step-down therapy for clinically stable patients with susceptible isolates and negative blood cultures. Voriconazole is used when additional coverage for mold is preferred.
Cardiovascular endocarditis	LFAMB[b] with or without 5-FC, 25 mg/kg qid *or* An echinocandin[c]	Step-down to fluconazole 400–800 mg (6–12 mg) daily for susceptible organism, patient clinically stable, negative blood cultures	Valve replacement is strongly recommended.
Central nervous system	LFAMB[b] with or without 5-FC, 25 mg/kg qid for several weeks	Fluconazole: 400–800 mg (6–12 mg) daily for susceptible organism after clinical improvement	Removal of prosthetic devices is strongly recommended.
Urinary tract			
Asymptomatic candiduria	Not indicated except in neonates and instrumentation of infected urinary tract		
Symptomatic cystitis	Fluconazole: 200 mg (3 mg/kg) daily for 2 wk	AMB-D: 0.3–0.6 mg/kg for 1–7 days *or* 5-FC: 25 mg/kg qid	Bladder irrigation with AMB-D, 50 mg/L, can be used for fluconazole-resistant species.
Renal parenchymal candidiasis	Fluconazole: 200–400 mg (3–6 mg/kg) daily for 2 wk *or* An echinocandin[a]	LFAMB[b] or AMB-D: 0.5–0.7 mg/kg daily with or without 5-FC 25 mg/kg qid[d] *or* 5-FC alone for 2 wk	
Ocular: chorioretinitis with or without vitritis	Fluconazole: 400–800 mg (6–12 mg/kg) daily *or* Voriconazole: 6 mg/kg IV q12h for 2 doses, then 4 mg/kg IV q12h	LFAMB: 3–5 mg/kg IV daily, with or without 5-FC, 25 mg/kg qid	Intravitreal AMB-D, 5–10 µg, or voriconazole, 100 µg. Often vitrectomy is used if 3–4+ vitritis or macular involvement is present.

[a]Once-daily micafungin, 100 mg; caspofungin, 70-mg loading, then 50 mg; or anidulafungin, 200-mg loading, then 100 mg.
[b]Liposomal amphotericin B (AmBisome), 3–5 mg/kg daily, or amphotericin B lipid complex (Ablecet), 5 mg/kg daily.
[c]For initial therapy of *Candida* endocarditis, the 2016 guidelines[157] recommend caspofungin, 150 mg; micafungin, 150 mg; or anidulafungin, 200 mg once daily.
[d]Strength of recommendations for 5-FC is weak.
AMB-D, Amphotericin B deoxycholate; *5-FC,* 5-fluorocytosine; *LFAMB,* lipid formulation of amphotericin B.
Modified from references 150–152, 154, 155, 157, and 162.

Intraabdominal Candidiasis, *Candida* Peritonitis, Gallbladder Infection

The topic of when to begin therapy in patients with an abdominal drain that grows *Candida* on culture and the controversy regarding therapy have been discussed and reviewed in detail.[138,168] In general, there has been a lowering in the threshold for treating such patients, but the impact of treatment on their clinical outcome remains to be fully defined. In most instances, recovery of *Candida* from existing drains does not require treatment, while recovery of the organism from "freshly placed" drains does require therapy. Discovery of *Candida* in ascites from an undrained abdomen usually means that therapy is required. Hematogenous dissemination from the peritoneum can occur.

The topic of empirical therapy for nonneutropenic patients in the ICU has been reviewed extensively.[157] For those patients at high risk for intraabdominal infection (probable leaking intestinal anastomosis, fever with no known source, surrogate markers for invasive candidiasis, positive cultures from nonsterile sites), empirical therapy with the same recommendations for candidemia as in nonneutropenic patients should be used. Patients who do not have subsequent evidence of candidiasis and have negative cultures after 4 to 5 days should have the antifungal therapy stopped. Source control, with drainage of potential intraabdominal abscesses, is pivotal.

For chronic disseminated (hepatosplenic) candidiasis, LAMB, ABLC, or an echinocandin is recommended.[152] After several weeks of treatment, step-down therapy with fluconazole can be considered in patients who are stable and no longer neutropenic. Adjuvant corticosteroid therapy has been advocated for patients with prolonged and disabling fever.[56]

Candida-caused cholecystitis may respond to an echinocandin, a lipid formulation of AMB, or fluconazole.[169] In candidiasis of the gallbladder or biliary tract, drainage may be necessary[170] in addition to antifungal therapy. *Candida* may complicate necrotizing pancreatitis and has been an increasing cause of pancreatitis. This entity should be treated with an echinocandin until the patient is stable. *Candida* pancreatic abscess has been successfully drained with computed tomography–guided percutaneous aspiration in addition to systemic antifungal therapy.

Urinary Candidiasis

Postcatheterization asymptomatic candiduria usually resolves without specific antifungal therapy. Current recommendations are to not treat it, unless the patient is symptomatic or in a group at high risk for dissemination. These groups include symptomatic patients, renal transplant patients, very-low-birth-weight infants, patients who are undergoing urinary tract instrumentation, and patients with obstruction of the urinary tract. If the goal is to treat, relief of obstruction and removal of urinary stents and catheters are the primary goals. Fluconazole is the only azole with urine bioactivity and would be reasonable for susceptible *Candida* species. Local amphotericin (a solution of 50 mg of amphotericin B in 1 L of sterile water infused at 40 mL/hr through a Foley catheter) was used extensively in the past but has become unpopular due to the efficacy of oral fluconazole (200–400 mg/day for 7–14 days). However, it may be useful still for patients with resistant *Candida,* especially *C. glabrata.* For fluconazole-resistant isolates, LAMB or AMB-D with or without 5-FC or voriconazole is an alternative.[171] Treatment should be continued until cultures are negative and the patient is asymptomatic. Selected patients may require irrigation with AMB through nephrostomy tubes placed directly in the collecting systems. If fungus balls form in the urinary tract, they require surgical removal. For papillary necrosis or renal abscess, an echinocandin or intravenous LAMB or ABLC is indicated. However, oral fluconazole may be an alternative in patients with mild to moderate infection. 5-FC is another alternative, but is less popular, especially in patients with renal insufficiency, and resistance develops rapidly.

Eradication of candiduria in patients who require a persistent indwelling Foley catheter is unnecessary, with the exception of patients undergoing bladder or prostate surgery. A placebo-controlled trial found that fluconazole at 200 mg/day for 14 days resulted in eradication of the candiduria, but there was a high rate of recurrence, suggesting the futility of antifungal therapy.[172] A large observational study has also verified the futility of antifungal therapy.[173]

Mucocutaneous Candidiasis

Oral thrush should be treated with topical agents whenever possible (Table 256.5). A 7- to 14-day course of clotrimazole 10-mg oral troches given five times per day or a miconazole mucoadhesive 50-mg tablet applied once daily for 7 to 14 days to the gum on the canine fossa is recommended.[152] For mild disease, nystatin suspension (100,000 U/mL), swish and swallow, 4 to 6 mL four times daily or 1 to 2 nystatin pastilles (200,000 U each) dissolved slowly in the mouth four times daily can be used. For more severe or refractory disease, oral fluconazole at 100 to 200 mg daily is recommended. For cases that are refractory to fluconazole, itraconazole solution and posaconazole suspension or voriconazole are alternatives, although control of HIV with antiretroviral therapy is usually sufficient. Therapy for denture sore mouth is the same as that for thrush, with the addition of meticulous cleaning of the dentures and correction of ill-fitting plates. Angular chelitis, which is frequently associated with denture sore mouth, should be treated with either topical clotrimazole or miconazole cream on the corners of the mouth.

The diagnosis of *Candida* esophagitis is best made on esophagoscopy but less reliably can be made on the basis of the presence of oropharyngeal thrush and symptoms of esophagitis in patients with AIDS or cancer. Topical therapy with clotrimazole troches or nystatin suspension usually fails. Treatment with fluconazole is preferred.

For refractory esophagitis, itraconazole solution is considered the first-choice strategy. Posaconazole suspension and voriconazole are alternatives. If necessary, an intravenous echinocandin may be used. In refractory esophageal infections, low-dose (10–20 mg/day) AMB has been successful. Long-term suppressive therapy may be necessary in patients with AIDS who have not responded to combination antiretroviral therapy.

Candida intertrigo is most successfully managed by decreasing the moisture of the involved area and by the application of topical azole cream or lotions, such as miconazole or clotrimazole. Management of *Candida* diaper rash has been successful with nystatin powder or cream in combination with a corticosteroid, such as Mycolog-II cream. The same agents used for diaper rash are generally successful for pruritus ani.

Uncomplicated *Candida* vaginitis responds to short courses of topical or oral therapy in the vast majority of patients. The following regimens used from 1 to 7 days are considered comparable: over-the-counter clotrimazole, butoconazole, miconazole, and tioconazole ointment; and terconazole (prescription only). For uncomplicated cases, oral fluconazole (150 mg once) is effective.[157] Other regimens include nystatin (100,000 units daily for 1–2 weeks) and boric acid (600 mg in a gelatin capsule once daily vaginally for 14 days).[174] For severe acute *Candida* vulvovaginitis, oral fluconazole (150 mg every 72 hr for 2–3 doses) is recommended.[152] Recurrent *Candida* vaginitis requires eradication of causal factors as much as possible. Then treatment for 2 weeks with topical or oral azoles should be used, followed by 6 months of fluconazole; 150 mg orally per week is recommended.[157,175]

Candida-caused paronychia is best managed by preventing immersion of the hands in water as much as possible and applying clotrimazole or miconazole cream twice daily. Drainage of any paronychial pus is also important.

Chronic Mucocutaneous Candidiasis

Topical therapy with azoles or polyenes applied to skin and mucous membranes achieves only slight improvement when used to treat acute infection and is best suited as step-down suppressive treatment in patients already treated successfully with a systemic antifungal. Azole, intravenous AMB, or echinocandin therapy has been effective, but nearly all patients relapse when treatment stops. Oral 5-FC is typically not effective. The most important advance in the therapy of this disease is systemically administered azoles: fluconazole, voriconazole, or posaconazole.[105] Fluconazole is recommended as the primary therapy.[171] Numerous reports illustrate successful treatment, although therapy for months or years may be necessary. Development of azole resistance with long-term therapy is a common problem affecting up to 40% to 50% of patients. In these cases, initial induction treatment with an echinocandin is necessary. Therefore initial induction for at least 4 weeks with fluconazole or an echinocandin (if treating an azole-resistant strain), followed by

TABLE 256.5 *Candida* Treatment: Other Indications

INDICATION	PRIMARY TREATMENT	ALTERNATIVE	COMMENT
Peritonitis	An echinocandin[a] *or* Fluconazole: 400–800 (6–12 mg/kg) daily *or* Voriconazole: 6 mg/kg q12h IV or PO, then 4 mg/kg q12h	LFAMB: 3–5 mg/kg daily	*Candida* recovered from an existing drain does not necessarily indicate peritonitis. Drains may become colonized. *Candida* from "freshly placed" drains usually requires treatment. Infected peritoneal dialysis catheters must be removed.
Chronic mucocutaneous candidiasis	Fluconazole *or* Itraconazole *or* Posaconazole	LFAMB: 3–5 mg/kg daily *or* An echinocandin (standard doses)	In severe cases, LFAMB may be necessary. After induction with a systemic antifungal for at least 4 wk, step-down suppressive therapy can be used with topical polyenes.
Oral thrush (mucocutaneous candidiasis)	Clotrimazole troches: 10 mg, dissolved slowly in mouth 5 times daily *or* Miconazole mucoadhesive buccal tablet: 50 mg placed on gum near canine tooth once daily for 7–14 d *or* Nystatin suspension: 100,000 U/mL, swish and swallow 4–6 mL 4 times daily; *or* 1–2 nystatin pastilles: 200,000 U each, dissolved slowly in mouth qid for 14 d *or* Fluconazole: 100–200 mg daily	Itraconazole solution: 200 mg daily *or* Posaconazole oral suspension: 100 mg bid on day 1, then 100 mg daily *or* Voriconazole: 200 mg bid *or* IV echinocandin[a] *or* AMB-D: 0.3 mg/kg daily	IV agents are reserved for refractory cases.
Candida esophagitis	Fluconazole: 200–400 mg IV or PO (3–6 mg/kg) daily for 14–21 days *or* An echinocandin[a] for 14–21 d	Itraconazole oral solution: 200 mg daily for 14–21 d *or* Posaconazole suspension: 400 mg bid for 14–21 d *or* Voriconazole: 200 mg bid *or* AMB-D: 0.3–0.7 mg/kg daily	IV agents are for refractory patients.
Candida vulvovaginitis	Multiple topical agents are available *or* Fluconazole: 150-mg single dose for mild disease; for severe acute vulvovaginitis, repeat 150 mg q72h for 2–3 doses	*Candida glabrata* unresponsive to azoles; boric acid gel capsule: 600 mg daily for 14 d	Fluconazole, 150-mg single dose weekly, has been used for recurrent disease.

[a]Once-daily micafungin, 100 mg; caspofungin, 70-mg loading dose, then 50 mg; or anidulafungin, 200-mg loading dose, then 100 mg.
AMB-D, Amphotericin B deoxycholate; *LFAMB,* lipid formulation of amphotericin B.
Modified from references 150–152, 154, 155, 157, and 162.

chronic suppressive topical polyene administration, offers the best outcomes while decreasing the chances of resistance (authors' experience). Concomitant ringworm infection of the skin or nails is common and may benefit from terbinafine therapy.

Ocular Candidiasis

Treatment of *Candida* endophthalmitis has been reviewed in detail.[148] *Candida* chorioretinitis usually responds to systemic antifungal drugs in the absence of significant (grade 3–4 of 5) vitritis (see Table 256.4). Vitritis requires both systemic and intravitreal antifungals, a decision always made in consultation with an ophthalmologist. Intravitreal injections of conventional AMB (5 to 10 μg) or intravenous formulations of voriconazole (100 μg) have been used (see Chapter 114). When intravitreal therapy is done intraoperatively at the time of vitrectomy, the antifungal is injected into the vitreous cavity at the end of the procedure. Intravitreal injections may need to be repeated if infection is not controlled, although the antifungal may increase the vitritis transiently.

Exogenous ocular candidiasis in the anterior chamber is treated systemically, though intracameral, subtenon, or subconjunctival antifungal injections or a topical antifungal is sometimes given for refractory cases. Antifungal eyedrops are given every 2 hours initially, to facilitate diffusion into the anterior chamber.

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257 *Aspergillus* Species

George R. Thompson III and Thomas F. Patterson

SHORT VIEW SUMMARY

Definition
- Infection is caused by hyaline mold *Aspergillus*.
- Syndromes range from colonization, such as fungus ball due to *Aspergillus* (aspergilloma); allergic responses to *Aspergillus*, including allergic bronchopulmonary aspergillosis; and semiinvasive or invasive infections, from chronic necrotizing pneumonia to invasive pulmonary aspergillosis and other invasive syndromes.

Epidemiology
- Highest incidence of infection in patients undergoing hematopoietic stem cell transplant or solid-organ transplantation
- Infection more likely in patients with extensive immunosuppression or those with relapse or recurrence of underlying malignancy
- Improved survival with early diagnosis and newer therapies, but mortality rates in severely or persistently immunosuppressed patients is substantial

Microbiology
- Culture-based diagnosis useful to establish specific diagnosis
- *Aspergillus* spp. complexes with distinct antifungal susceptibilities so that culture-based diagnosis is clinically relevant
- Molecular analysis required to establish species-level identity
- Increasing rates of antifungal resistance are reported in some settings.

Diagnosis
- Proven infection is established by culture of the organism.
- Assay of biomarkers, such as galactomannan (GM) and β-D-glucan, and polymerase chain reaction are useful as criteria for establishing a probable diagnosis but are not diagnostic alone
- Serial assessment of biomarkers may be useful for measuring response to therapy.

Therapy
- Voriconazole or isavuconazole is recommended for primary therapy in most patients (see Table 257.4).
- Liposomal amphotericin B can be used as primary therapy in patients in whom voriconazole or isavuconazole is not tolerated or contraindicated because of drug interactions or other reasons.
- Alternative agents for salvage therapy include amphotericin B lipid complex, the echinocandins (caspofungin, micafungin, or anidulafungin), posaconazole, or itraconazole.
- Combination therapy of voriconazole plus an echinocandin is not recommended for routine use, but there may be some subgroups of patients, such as those with early diagnosis of infection based on detection of GM or those with failure or primary therapy with a single agent, who may benefit from such an approach.

Prevention
- Antifungal prophylaxis with posaconazole or possibly voriconazole is used for high-risk patients
- Risk-benefit ratio of prophylaxis in individual patients should be considered.
- Infection control is important to reduce risk in hospitalized patients, but long duration of risk (>180 days) in high-risk hematopoietic stem cell transplant (HSCT) or solid-organ transplantation patients makes community-acquired infection likely.

Invasive aspergillosis (IA) remains a major cause of morbidity and mortality in the immunosuppressed population. This infection is caused by *Aspergillus*, a hyaline mold, and is responsible for a variety of noninvasive or semiinvasive conditions. These syndromes range from colonization, such as a fungus ball due to aspergillosis (also known as aspergilloma); allergic responses to *Aspergillus*, including allergic bronchopulmonary aspergillosis (ABPA); and semi-invasive or invasive infections, from chronic necrotizing pneumonia to invasive pulmonary aspergillosis and other invasive syndromes.

Aspergillosis syndromes are a major focus in clinical mycology due to the dramatic increase in the number of patients affected and the associated morbidity and mortality of this infection.[1] The increased number of *Aspergillus* infections has coincided with the growing immunosuppressed population. Patients with established IA have poor outcomes.[2-5] Successful therapy depends not only on an early diagnosis but also on reversal of underlying immune defects. Even when therapy is begun promptly, outcomes are often poor, particularly in patients with disseminated or central nervous system (CNS) disease and in those who remain profoundly immunosuppressed.[1] New diagnostic approaches and new management strategies have been established. In this chapter clinical mycology, epidemiology, pathogenesis, clinical presentation, diagnosis, treatment, and prevention of aspergillosis are described.

MYCOLOGY

The genus *Aspergillus* was first recognized in 1729 by Micheli, in Florence, who noted the resemblance between the sporulating head of an *Aspergillus* sp. and an aspergillum used to sprinkle holy water.[6] In 1856 Virchow published the first complete microscopic descriptions of the organism.[7] *Aspergillus flavus* was formally named by Link in 1809.[8] Thom and Church first classified the genus in 1926 with 69 *Aspergillus* spp. in 11 groups. The term "group" is now more correctly referred to as "section," but this reporting is not commonplace in clinical mycology laboratories.[9] Because phenotypic methods and internal transcribed spacer sequencing identify *Aspergillus* isolates within a section and not individual species, it has been recommended that isolates should be reported as members of a "species complex."[10] With the recent use of molecular techniques to characterize pathogenic fungi, aspergilli have now increased dramatically to include more than 250 species in 8 subgenera (*Aspergillus*, *Fumigati*, *Circumdati*, *Candidi*, *Terrei*, *Nidulante*, *Warcupi*, and *Ornati*) that are subdivided into multiple sections and species complexes.[10,11]

Most species of *Aspergillus* reproduce asexually, but a teleomorph (or sexual form) has been identified for a number of species, including pathogenic species such as *Aspergillus nidulans* (teleomorph, *Emericella nidulans*); *A. flavus (Petromyces flavus); A. udagawae (Neosartorya udagawae);* and the most common pathogen, *A. fumigatus (Neosartorya fumigata);* and many others.[12–14] Even though the correct taxonomic nomenclature would rename these organisms using the sexual form, generally the generic name *Aspergillus* has been retained to simplify nomenclature regardless of their teleomorphs, rather than separating the organisms into unfamiliar species based on discovery of a sexual state.[15] As with other pathogenic fungi, the taxonomy of *Aspergillus* has undergone extensive reclassification with use of molecular studies,

TABLE 257.1 Characteristics of *Aspergillus* Species Associated with Invasive Infection

ASPERGILLUS SPECIES	FREQUENCY OF SPECIES COMPLEX ISOLATED IN CLINICAL INFECTION (%)	COLONY CHARACTERISTICS	MICROSCOPIC FEATURES	CLINICAL SIGNIFICANCE
A. fumigatus (see Fig. 257.1A and B)	50–67	Smoky gray green; may have pale yellow or lavender reverse; grows at 50°C	Columnar; uniseriate; smooth to finely roughened conidia 2–3.5 µm	Most common invasive species; most pathogenic
A. flavus (see Fig. 257.2A and B)	8–14	Olive to lime green	Radiate to loosely columnar; uniseriate or biseriate; rough conidiophore; conidia 3–6 µm	Sinusitis; skin infection; produces aflatoxin
A. terreus (see Fig. 257.3A– C)	3–5	Beige to cinnamon buff	Columnar; biseriate; globose; small 2–2.5 µm conidia; globose accessory conidia along hyphae	Increasingly detected; resistant to amphotericin B; more susceptible to newer azoles
A. niger. (see Fig. 257.4A and B)	5–9	Initially white, rapidly turning black with yellow reverse	Radiate; biseriate; globose, black, very rough conidia 4–5 µm	Uncommon in invasive infections; superficial agent of otic disease; colonization

Data from Patterson TF, Kirkpatrick WR, White M, et al. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group. Medicine (Baltimore). *2000;79:250–260 [invasive infections: United States]; Balajee SA, Kano R, Baddley JW, et al. Molecular identification of Aspergillus species collected for the Transplant-Associated Infection Surveillance Network.* J Clin Microbiol. *2009;47:3138–3141 [transplant-associated infections: United States]; Alastruey-Izquierdo A, Mellado E, Cuenca-Estrella M. Current section and species complex concepts in Aspergillus: recommendations for routine daily practice.* Ann NY Acad Sci. *2012;1273:18–24 [invasive infections: Spain].*

such as sequencing of ribosomal, β-tubulin, calmodulin, or rodlet A genes, which has allowed more natural subgroupings of ascomycetous fungi.[10,11,16–18] It is important to note that with identity established by means of molecular sequencing, the result of a familiar species may be reported as an unfamiliar teleomorph, which has led to assigning one name to one fungus to clarify this potential confusion in clinical mycology.[19,20]

The genus *Aspergillus* is an anamorphic member (asexual form) of the family Trichocomaceae. The teleomorphs of *Aspergillus* spp. are classified in eight genera in the order Eurotiales in the phylum Ascomycota. Identification of the genus and of common pathogenic species is usually not difficult, but species-level identification of less common members can be laborious, and misidentification of atypical or "cryptic" members of sections, such as poorly sporulating forms, is common using solely phenotypic methods.[11,18,21]

The most common species causing invasive infection is *Aspergillus fumigatus*, the most common pathogen in the section *Fumigati*, which historically has made up a vast majority of invasive isolates: *A. flavus; Aspergillus terreus*; and, less commonly for invasive infection, *Aspergillus niger*.[3] Recent studies have shown emergence of less common species, including *A. terreus* and unusual less pathogenic species as the etiologic agents of invasive infection, including many cryptic species that are identifiable only by molecular studies.[18,21–23] With more prolonged and profound immunosuppression, along with molecular identification of cryptic species within a species complex, the list of rare species causing invasive infection continues to increase, including *A. alabamensis, A. alliaceus* (teleomorph *Petromyces alliaceus*), *A. avenaceus, A. caesiellus, A. candidus, A. carneus, A. clavatus, A. calidoustus, A. flavipes, A. glaucus, A. granulosus, A. insuetus*, *A. keveii*, *A. lentulus, A. nidulans (Emericella nidulans), A. novofumigatus, A. ochraceus, A. puniceus, A. pseudodeflectus, A. restrictus, A. sydowii, A. quadrilineatus, A. tamarii, A. tanneri*, *A. udagawae (Neosartorya udagawae), A. tubingensis*, *A. versicolor, A. viridinutans*, *A. vitus* (teleomorph *Eurotium amstelodami*), *A. wentii, A. thermomutatus* (teleomorph *Neosartorya pseudofischeri*), and many others, although the authenticity of at least some of these has been questioned and perhaps misidentified before molecular studies.[9,11,18,24–28]

Pathogenic *Aspergillus* spp. are easily cultured from pathologic samples and grow rapidly (24–72 hours) on a variety of media. Blood cultures are uncommonly positive and usually reflect contamination rather than invasive disease.[29] A distinguishing characteristic of pathogenic *Aspergillus* spp. is their ability to grow at 37°C. *A. fumigatus* is able to grow at 50°C, a feature that, in addition to morphology, can also be used to identify this species and can help distinguish *A. fumigatus* from cryptic *Aspergillus* spp. within the *A. fumigatus* complex.[21,25] Most species initially appear as small, fluffy white colonies on culture plates within 48 hours. Presumptive identification of an *Aspergillus* species complex is usually accomplished by appearance of the fungus on gross and microscopic inspection of the colony, which provides typical sporulation, although specific species-level identification requires molecular confirmation so that laboratories should report isolates identified phenotypically as a species complex.[11]

Microscopic features and colony morphology for the most common clinical isolates—*A. fumigatus, A. flavus, A. terreus*, and *A. niger*—are described in Table 257.1 and shown in Figs. 257.1 to 257.4. Species-level identification of *Aspergillus* has become increasingly important due to antifungal drug susceptibility differences.[11,30]

Aspergillus fumigatus is the most frequent species in invasive infection, constituting >90% in some series, although, recently, a prevalence of 50% to 67% has been described.[3,11,18,29] Colonies of *A. fumigatus* are typically gray-green with a wooly to cottony texture (see Fig. 257.1A). Like other species of *Aspergillus*, hyphae are hyaline (lightly pigmented), have septa, and are usually branched at acute (typically 45-degree) angles. The conidial head is columnar with conidiophores that are smooth walled and uncolored, or darkened in the upper portion near the vesicle. This species is uniseriate (a term describing phialides that are attached directly to the vesicle), with closely compacted phialides borne only on the upper portion of the vesicle (see Fig. 257.1B). Conidia are smooth to finely roughened and are 2 to 3.5 µm in diameter. The fruiting head (the conidiophore and conidia) is not commonly seen in clinical specimens, although it may be detected in sites exposed to air, such as wounds or lung cavities. Like other *Aspergillus* spp., it is widespread in nature—found in soil, on decaying vegetation, in the air, and, more recently, in water supplies.[31,32]

Other cryptic members of the section *Fumigati*, meaning identification by molecular methods, have been described that are human pathogens, including *A. lentulus, A. calidoustus, A. tubingensis, A. novofumigatus, A. thermomutatus* (teleomorph *N. pseudofischeri*), *A. viridinutans*, and others.[11,18,21,33–35] These species are frequently poorly sporulating and fail to grow at 50°C.[21,25] Of importance, some of these species, such as *A. lentulus*, *A. viridinutans*, and *A. thermomutatus*, may exhibit decreased antifungal susceptibility and can be associated with a poor clinical outcome.[11,30,36] When these species are identified only phenotypically, they should be referred to as members of the *Aspergillus fumigatus* species complex.

Aspergillus flavus is a common isolate in sinusitis, skin, and other invasive infections. This species, which produces an aflatoxin, is found in soil and decaying vegetation.[37] Colonies are olive to lime green and grow at a rapid rate (see Fig. 257.2A). Some isolates are uniseriate but are more typically biseriate. In biseriate species sterile cells known as metulae are attached to the vesicle, and these, in turn, support the

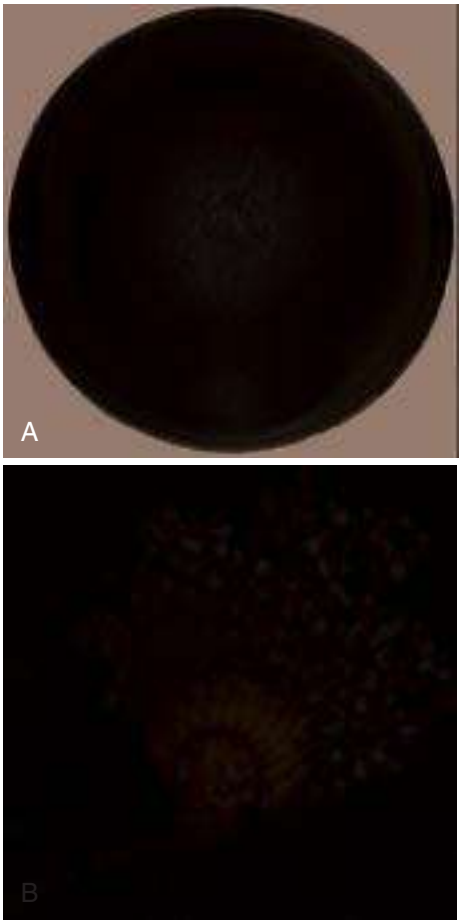

FIG. 257.1 Aspergillus fumigatus. (A) Gray-green colony morphology on potato flakes agar. (B) Uniseriate conidiophore with columnar conidia (all photomicrograph magnifications ×420). *(Courtesy Dr. Deanna Sutton.)*

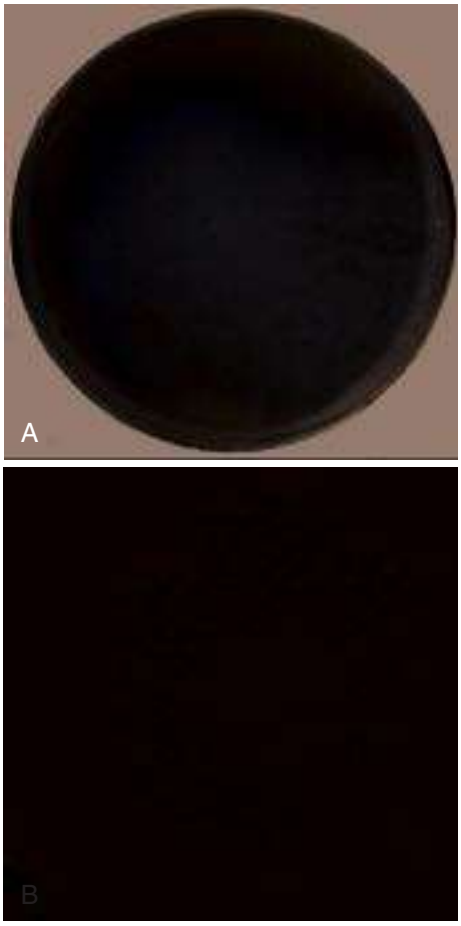

FIG. 257.2 Aspergillus flavus. (A) Olive–lime green colony on potato flakes agar. (B) Radiate, biseriate conidia. *(Courtesy Dr. Deanna Sutton.)*

phialides. *A. flavus* has noticeably rough conidiophores and smooth conidia (3–6 µm) (see Fig. 257.2B). If characterized by morphology alone, *A. flavus* and other species in the section *Flavi* should be referred to as members of the *Aspergillus flavus* species complex. Other species, such as *A. alliaceus*, have been reported to cause clinical infection and may have decreased susceptibility to polyenes.[11,38]

Aspergillus terreus is a common soil-related isolate that has been increasingly reported in invasive infection in immunocompromised hosts.[22] *A. terreus* conidia are small (2.0–2.5 µm), and the colony color and fruiting structures are characteristic for this species (see Fig. 257.3). Colonies range in color from buff to beige to cinnamon (see Fig. 257.3A).[11] Conidiophores are smooth walled and hyaline, and the conidial heads are biseriate and columnar (see Fig. 257.3B). A distinguishing feature of this species is the presence of globose accessory conidia that are produced on hyphae. These accessory conidia can be detected in histopathologic samples, which may be used to establish presumptive identification of the species.[39] Identification of this species has become increasingly important because of its resistance to antifungals, including amphotericin B, although improved susceptibility with newer azoles has been reported.[40–42] Molecular characterization has shown *A. terreus* is also a species complex with species such as *A. alabamensis* and *A. carneus* causing infection in immunocompromised hosts and, like *A. terreus*, is less susceptible to polyene antifungals.[11,28,43]

Aspergillus niger (see Fig. 257.4A and B) is found in soil, on plants, and even in food and condiments, such as pepper. Colonies are initially white but quickly become black, with the production of the pigmented fruiting structures (see Fig. 257.4A). It grows rapidly with a pale yellow reverse. Conidial heads are biseriate and cover the entire vesicle. Conidia are brown to black and are very rough (4–5 µm) (see Fig. 257.4B), although the hyphae are hyaline. The species may produce oxalate crystals in clinical specimens.[44] The role of *A. niger* in invasive infection is less well established, with its decreased pathogenicity perhaps due in part to the fact that its larger conidia do not readily reach deep into lung tissues. It is a common colonizing isolate and can cause superficial infection, such as otitis externa.[29,45] This species complex also contains several related species, including *A. tubingensis*, which is one of the more common cryptic aspergilli identified, suggesting it may have a role in invasive infection.[11,30,46]

Other species of *Aspergillus* are less common in invasive infection but are increasingly reported, perhaps reflecting increased awareness and molecular techniques confirming their identity.[3,11,18,29] *Aspergillus nidulans* (section *Nidulantes*) and *A. tanneri*, (section *Circumdati*, species complex *terreus*), for example, have been reported as causes of infection in patients with chronic granulomatous disease and are species that may be resistant to amphotericin B.[26,30,47] *Aspergillus calidoustus* is a species in the section *Ustus*, which grows at 37°C (formerly called *A. ustus*, a species that fails to grow at 37°C) and exhibits high minimum inhibitory concentrations (MICs) to several classes of antifungal agents.[48–50] Thus even previously "nonpathogenic" *Aspergillus* spp. must be considered potentially clinically significant in an appropriate clinical setting and host.[9,18]

EPIDEMIOLOGY

Aspergillus is ubiquitous worldwide—found in soil, water, food, and air—and is particularly common in decaying vegetation. The inoculum for infection is not known, but hosts with normal pulmonary host defenses rarely develop disease despite exposure to the organism with normal daily living through airborne conidia, including foodstuffs like pepper, herbs, and so forth.[51] Patients with altered host immunity, particularly those with reduced pulmonary host defenses (e.g., those



FIG. 257.3 Aspergillus terreus. (A) Buff-cinnamon colony on potato flakes agar. (B) Columnar, biseriate smooth conidia. (C) Globose, sessile accessory conidia along hyphae. *(Courtesy Dr. Deanna Sutton.)*

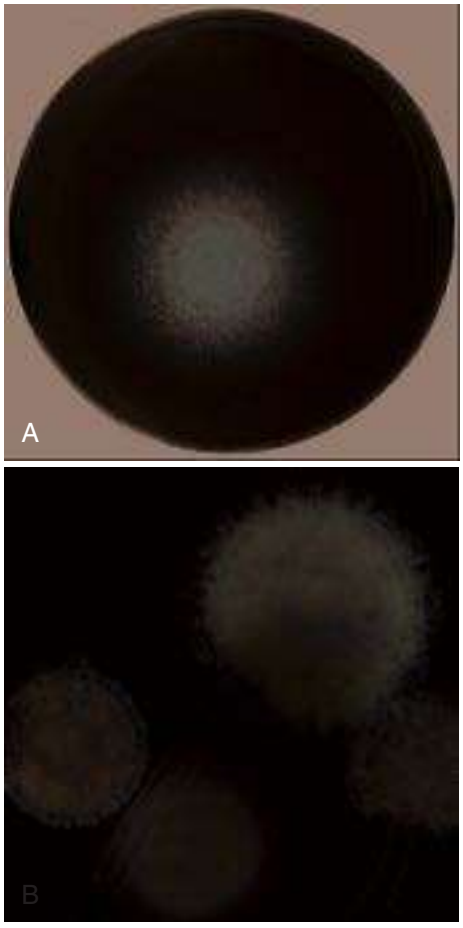

FIG. 257.4 Aspergillus niger. (A) Black colony on potato flakes agar. (B) Large, radiate, biseriate and uniseriate conidia. *(Courtesy Dr. Deanna Sutton.)*

who use corticosteroids) that inhibit the activity of pulmonary macrophages or those who are neutropenic, have increased susceptibility to these organisms.[52]

Patients with prolonged and profound neutropenia are at high risk for IA, but changing treatment patterns in chemotherapy and transplantation, along with the use of growth factors, have limited the numbers of persistently neutropenic patients.[53,54] It is important to recognize that, even among high-risk patients with hematologic malignancy, there is substantial heterogeneity of risk.[55,56] In patients with acute myelogenous leukemia, the incidence of IA has been reported to range from as low as 1% to 2% to rates as high as 25% to 28% or more, with mean incidence in most series at 4% to 7%.[54,57–59] Most cases of IA occur in the period of neutropenia after primary induction chemotherapy or in patients with refractory or recurrence of malignancy, with the risk of aspergillosis during consolidation chemotherapy very low.[55,56]

In patients undergoing HSCT or bone marrow transplantation, a recent increase in the incidence of IA has been reported, and the epidemiology of infection has changed (Table 257.2).[54,60] In patients undergoing HSCT the major periods of risk are bimodal, with peak incidence occurring fewer than 40 days but more than 100 days after transplantation, with mean time to diagnosis more than 180 days in some series.[53,54,61–63] In the Transplant-Associated Infection Surveillance Network (TRANSNET) study the median time to onset of IA was 99 days, with 22% and 53% of 335 IA cases occurring 1 and 4 months after transplantation, respectively.[54] One of the factors in the changing epidemiology in this patient population is the use of nonmyeloablative transplant procedures, which has shifted the major risk factor in these patients from neutropenia to that of the use of high doses of corticosteroids or other immunosuppressive therapy for the treatment of acute or chronic graft-versus-host disease (GVHD).[53] Only 31% of the HSCT recipients with IA reported by Wald and colleagues[53] were neutropenic. Acute or chronic GVHD may occur late after HSCT and increases the risk for *Aspergillus* infection.[53,54,61]

TABLE 257.2 Incidence and Mortality of Invasive Aspergillosis in Transplantation

TYPE OF TRANSPLANT	INCIDENCE (%)		12-WEEK MORTALITY (%)
	RANGE	MEAN	
Allogeneic stem cell	2.3–11	7	40–75
Autologous stem cell	0.5–2	1	50
Lung	2.4–9	6	5–21
Liver	1–8	4	45–59
Heart	0.3–6	2	15–21
Kidney	0.1–2	0.5	25–48

Data from references 54, 62, 63, 65–67, and 134 .

Other immunosuppressed patients are also at risk for IA, including patients undergoing organ transplantation, particularly lung transplantation (see Table 257.2).[64,65] The increased risk in lung transplantation is because the transplanted organ is constantly exposed to the environment, ciliary clearance is reduced, and many of these patients are colonized with *Aspergillus* in either the native or transplanted lung.[66] In most organ transplant recipients the diagnosis of IA is a late occurrence; the mean time to diagnosis is typically more than 180 days and may occur a year or more after the organ transplant, although the median onset in liver transplants appears shorter (~100 days).[65,67] Other patients at

higher risk for IA include patients receiving corticosteroids or biologic agents, including the tumor necrosis factor-α (TNF-α) antagonists, such as infliximab, etanercept, adalimumab, and others, including ibrutinib.[68] Newer biologic agents have variable risks for host acquisition of invasive fungal infections, and risks remain incompletely defined. IA occurs in other immunosuppressed patients, including patients with pulmonary diseases, acquired immunodeficiency syndrome (AIDS), chronic granulomatous disease, and other hereditary immunodeficiency syndromes.[3,52] IA can occur in critically ill patients in intensive care units (ICUs) independent of traditional risk factors, suggesting additional cumulative risk factors for disease in this setting, although the overall impact of IA in that population is unclear and varies in different series.[69,70]

Outbreaks of IA have occurred in patients exposed to aerosols of *Aspergillus* conidia in association with construction and other environmental risks.[71–74] In severely immunosuppressed patients aspergillosis may occur from other exposures as well, including aerosols of contaminated water.[32,75] Aspergillosis may also occur as endogenous reactivation from prior infection or colonization. Therefore it may not be possible to prevent all cases by reducing environmental exposures within the hospital setting.[76] It should also be noted that, with the prolonged period of risk, these infections become very difficult to prevent with protective environments because much of their health care will occur in a non–hospital-based setting.[76]

PATHOGENICITY AND HOST DEFENSES

The usual route of infection for IA is through inhalation of *Aspergillus* conidia into the lungs. Although less common, invasive infection may also follow local tissue invasion, such as through surgical wounds or contaminated intravenous (IV) catheters or armboards, leading to cutaneous infection.[77]

In the absence of effective host defenses after pulmonary exposure, the inhaled small resting conidia enlarge and germinate, resulting in transformation into hyphae with subsequent vascular invasion and eventual disseminated infection. The incubation period for conidial germination in pulmonary tissue is variable, estimated to range from 2 days to months and may even vary by species and strain.[39] The growth rate at 37°C may be one determinant of the rate of disease progression and possible pathogenicity of the organism. Hydrocortisone significantly increases the growth rates of *Aspergillus*, further enhancing the role of corticosteroids as a risk factor for invasive disease.[78] The process of hyphal growth and tissue invasion results in a hallmark feature of IA—vascular invasion (Fig. 257.5) and pulmonary infarction (Fig. 257.6), which are classic features of invasive pulmonary aspergillosis due to the angioinvasive nature of the organism.

Although infection in apparently normal hosts can occur, IA is uncommon in immunocompetent hosts.[3] Normal pulmonary defense mechanisms are usually able to contain the organism in a host with intact pulmonary defenses. The first line of defense against *Aspergillus* is ciliary clearance of the organism from the airways and limited access to the alveoli due to conidial size. This feature is one reason for the increased pathogenicity of *A. fumigatus* compared with other species of *Aspergillus*.[79] After conidia reach the alveoli, the major line of defense becomes the pulmonary macrophages, which are capable of ingesting and killing *Aspergillus* conidia.[80] After the cells germinate, polymorphonuclear leukocytes act to extracellularly kill both swollen conidia and hyphae.[81] Efficacy of host defenses against the organism may be enhanced by opsonization of conidia with complement or other molecules, such as mannose-binding protein and surfactant proteins.[82] *A. fumigatus* possesses a hydrophobic external component, which inhibits fungal recognition[83]; produces melanin, which inhibits host phagocytosis[84]; and a complement inhibitor, which may increase its pathogenicity.[85] Antibodies against *Aspergillus* are common because of the ubiquitous nature of the organism, although they are not protective, nor are they useful in the diagnosis of infection in high-risk patients owing to the lack of consistent seroconversion after exposure or infection.[86]

Many *Aspergillus* spp. produce toxins, including aflatoxins, ochratoxin A, fumagillin, and gliotoxin, the last of which may reduce macrophage and neutrophil function, although the role of these toxins as major virulence factors is not established.[87] *Aspergillus* spp. possess other potential virulence factors, including production of proteases, phospholipases, and metabolites, although strains deficient in those genes are still capable of producing experimental infection.[88] These metabolites are additionally under investigation for diagnostic utility.[89]

T-helper (Th) cytokines have vital roles in innate and adaptive defense against *Aspergillus*. Studies in experimental aspergillosis have shown that a Th1 response is associated with a favorable response.[90] Pathogen recognition receptors, including Toll-like receptors (TLRs) and dectin-1, also mediate defense against *A. fumigatus*.[91,92] Recognition of *Aspergillus* motifs by TLR2 and dectin-1 results in activation of intracellular



FIG. 257.5 **Lung tissue section showing narrow, acutely branching septated hyphae on hematoxylin and eosin stain showing vascular invasion (original magnification ×420).**



FIG. 257.6 **Infarcted lung tissue due to *Aspergillus* angioinvasion on gross lung specimen.**

pathways, leading to proinflammatory cytokine production.[93,94] These events are needed for an effective initial antifungal defense and bridge the gap between the innate and acquired immunity.[95] Polymorphisms in host genes mediating innate immune defenses influence susceptibility to IA. TLR4 haplotypes and polymorphisms in plasminogen gene alleles have both been shown to be associated with increased risk of IA in HSCT patients.[96,97] Additional pathways have been recently identified in large-scale genomic studies, and these polymorphisms may have implications on transplant donor screening and the risk stratification of recipients.[98]

In contrast to the deficient host responses that lead to invasive *Aspergillus* infections, the pathogenesis of ABPA and allergic fungal sinusitis relates to aberrant inflammatory host responses to the organism.[99] The immune response to *Aspergillus* antigens in ABPA, as in both asthmatic patients and those with cystic fibrosis, is a Th2 response.[79] ABPA begins with an allergic inflammatory response that follows after *Aspergillus* conidia are inhaled into the bronchi, where they germinate and form hyphae.[99] These mycelial cells release allergens that are processed by antigen-presenting cells bearing human leukocyte antigen (HLA)-DR2 or HLA-DR5 and presented to T cells within the bronchoalveolar lymphoid tissue. The T-cell response to these allergens favors a Th2 response, with release of cytokines interleukin (IL)-4, IL-5, and IL-13.[79] The inflammatory response in the bronchial submucosa leads to excessive mucin production, extravasation of eosinophils into the bronchial mucin, intermittent bronchial obstruction with atelectasis, and, over time, to bronchiectasis in some patients. Allergic fungal sinusitis is also characterized by submucosal inflammation and eosinophil-rich mucin in the sinus cavity.

Aspergilloma, called *fungus ball of the lung*, is a mass of hyphae in a preexisting cavity. *Aspergillus* causes a brisk immunoglobulin G (IgG) antibody response to the organism even though invasion of the cavity wall is rarely observed. Increased risk for chronic forms of pulmonary aspergillosis and noninvasive forms of pulmonary aspergillosis have been linked to subtle immune defects, including polymorphisms in mannose-binding protein and TLR polymorphisms.[100,101]

CLINICAL PRESENTATION

The spectrum of clinical syndromes associated with aspergillosis is diverse, ranging from allergic responses, asymptomatic colonization, superficial infection, and acute or subacute invasive disease. The clinical presentation reflects the underlying immune defects and risk factors associated with each patient group, with greater immune suppression correlating with increased risk for invasive disease.

Allergic Manifestations of Disease

Allergic Bronchopulmonary Aspergillosis

ABPA is a long-term allergic response to *Aspergillus* that is characterized by mucoid impaction of the bronchi, eosinophilic pneumonia, and transient pulmonary infiltrates due to atelectasis. Central bronchiectasis occurs in some patients after several years of disease.[99] The incidence of ABPA is estimated to range from 1% to 2% in patients with persistent asthma and is approximately 7% (range, 2%–15%) in patients with cystic fibrosis.[79,99,102] Diagnostic criteria for ABPA include: (1) asthma, (2) central bronchiectasis on chest computed tomography (CT), (3) immediate skin test reactivity to *Aspergillus* spp. (or *A. fumigatus)*, (4) total serum immunoglobulin E (IgE) concentration > 417 IU/mL (1000 ng/mL), (5) elevated serum IgE and/or IgG antibody to *A. fumigatus*, (6) fleeting infiltrates on chest radiography, (7) serum precipitating antibodies to *A. fumigatus*, and (8) peripheral blood eosinophilia.[103] Other clinical features may be present that can be used to support the diagnosis, including positive sputum cultures for *Aspergillus* or smears with hyphae consistent with *Aspergillus*, brown mucus plugs with degenerated eosinophils (Charcot-Leyden crystals) in sputa, and chest radiographic findings suggesting bronchial inflammation.[104] These latter chest radiographic findings include the "ring sign," indicating bronchial thickening without mucus plugs, and "parallel lines" or "tram tracks," suggesting bronchiectasis, which contrasts to tapering seen in the normal bronchus.[102]

The diagnosis of ABPA in cystic fibrosis may be particularly difficult because many of the diagnostic criteria overlap with common manifestations of cystic fibrosis (Table 257.3).[79] In these patients eosinophilia is not a useful diagnostic tool because the patients may have elevated peripheral blood eosinophils from other causes, or it may be suppressed from corticosteroid therapy.

ABPA typically progresses through a series of remissions and exacerbations but can eventually lead to pulmonary fibrosis and chronic pulmonary aspergillosis, which is associated with a poor long-term prognosis.[102,104,105] Management of ABPA is directed at reducing acute asthmatic symptoms and avoiding end-stage fibrosis. Corticosteroid therapy is commonly used for treating exacerbations, although few randomized trials have been conducted for their use.[106] Guidelines suggest that worsening diagnostic or clinical parameters may warrant a trial of corticosteroid therapy.[1] The role for antifungal therapy was evaluated with a randomized, double-blind, placebo-controlled trial that showed itraconazole at 200 mg/day for 16 weeks significantly reduced daily corticosteroid use, reduced levels of IgE, and improved exercise tolerance and pulmonary function.[107] In patients with severe asthma and fungal sensitization, itraconazole therapy resulted in significant clinical improvement in approximately 60%.[108] The efficacy of voriconazole, posaconazole, and isavuconazole have not been evaluated extensively in this setting but resulted in approximately 70% responses in ABPA or asthma patients who had previously failed itraconazole.[109]

TABLE 257.3 Criteria for Diagnosis and Management of Allergic Bronchopulmonary Aspergillosis in Patients with Cystic Fibrosis

Diagnostic Criteria[103]	
Predisposing Conditions (One Must Be Present)	
Asthma Cystic Fibrosis	
Obligatory Criteria (Both Must Be Present)	
Aspergillus skin test positivity or elevated IgE levels to *Aspergillus fumigatus* Elevated total IgE (typically >1000 IU/mL)	
Other Criteria (At Least Two Must Be Present)	
Precipitating serum antibodies to *A. fumigatus* or elevated serum *Aspergillus* IgG Radiographic abnormalities consistent with ABPA Total eosinophil count >500 cells/μL (may be historical)	
Recommendations for Screening for ABPA	
Maintain clinical suspicion for diagnosis	Especially in patients older than 6 years
Determine total serum IgE annually	If total IgE is greater than 500 IU/mL, consider skin test or measure IgE to *Aspergillus*
If total IgE 200–500 IU/mL, repeat if clinical suspicion is high	Consider retesting with disease exacerbation and perform other diagnostic tests
Therapy for Exacerbations of ABPA	
Corticosteroids: 0.5–2 mg/kg/day oral prednisone equivalent (maximum 60 mg/day) for 1–2 weeks, tapered over 2–3 months	Recommended for disease exacerbation in all patients except those with steroid toxicity
Antifungal therapy (itraconazole or other azole with *Aspergillus* activity—voriconazole, posaconazole)	Slow steroid response or toxicity; itraconazole 5 mg/kg/day up to 400 mg/day[108]—levels necessary (voriconazole or posaconazole may be effective when itraconazole fails but not extensively studied)[109]; monitor liver function tests; duration 3–6 months
Adjunctive therapy: inhaled corticosteroids; bronchodilators; environmental manipulation	No evidence for efficacy in ABPA but may be useful in asthma; reasonable to search for source of extensive mold exposure

ABPA, Allergic bronchopulmonary aspergillosis; *IgE,* immunoglobulin E; *IgG,* immunoglobulin G.
Data also from Stevens DA, Moss RB, Kurup VP, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis. *2003;37(suppl 3):S225–S264.*

Other Allergic Manifestations

Aspergillus is an occasional cause of allergic fungal sinusitis, although most cases in the United States are due to melanized molds.[110] This entity occurs in patients with a history of chronic allergic rhinitis, often with hyperplastic nasal mucosa forming nasal polyps. A mass of inspissated mucus forms in a sinus cavity with *Aspergillus* hyphae and Charcot-Leyden crystals. The sinus mucosa is hyperplastic but not invaded.[111] Management is directed at aerating the sinus and ensuring that tissue invasion is not present. The benefit of treating with either intranasal steroids or systemic antifungal agents has not been shown.[1]

Saprophytic Colonization and Superficial Aspergillosis

Fungus Balls Due to *Aspergillus*

A pulmonary fungus ball due to *Aspergillus*, or aspergilloma, is a solid mass of hyphae growing in a previously existing pulmonary cavity. Typically, *Aspergillus* fungus balls of the lung develop in preexisting cavities in the pulmonary apex of patients with chronic lung disease, such as bullous emphysema, sarcoidosis, tuberculosis, histoplasmosis, congenital cyst, bacterial lung abscess, or, very rarely, in a pulmonary bleb from *Pneumocystis* pneumonia in patients with AIDS.[112] On chest radiograph a pulmonary aspergilloma appears as a solid round mass in a cavity. The detection of *Aspergillus* in sputum cultures or detection of high titers of *Aspergillus* antibodies are further evidence that the radiographic findings are consistent with a diagnosis of fungus ball due to *Aspergillus* so that a biopsy is not usually necessary except to diagnose the underlying lung disease.[113]

In many patients the fungus ball due to *Aspergillus* remains asymptomatic, but in a significant number of patients hemoptysis occurs and can be fatal.[113] Surgical resection is considered the definitive therapy, but the dense pleural adhesions adjacent to the fungus ball and the poor pulmonary reserve of most patients make surgery hazardous. Contamination of the pleural space with *Aspergillus* and the common complication of bronchopleural fistula in the postoperative period can lead to chronic *Aspergillus* empyema. Dense adhesions make pleural drainage difficult, often requiring pleural stripping, further compromising lung function.[113]

Aspergillus can also be associated with fungus balls of the sinuses without tissue invasion.[110] The maxillary sinus is the most common site for a sinus aspergilloma to occur.[114] Clinical presentation is similar to that for any chronic sinusitis. CT of the sinus can be used to confirm the fungus ball, along with cultures of *Aspergillus*, usually *A. flavus* or *A. fumigatus.* Management is usually directed at surgical removal and a generous maxillary antrostomy for sinus drainage, along with confirmation that invasive disease has not occurred.

Denning and colleagues[115] have described a spectrum of chronic pulmonary aspergillosis ranging from chronic cavitary pulmonary aspergillosis, characterized by the formation and expansion of multiple cavities and the presence of fungal balls, to chronic fibrosing or necrotizing aspergillosis, in which slowly progressive infection occurs, usually in a single thin-walled cavity with demonstration of hyphae invading tissue. To differentiate chronic cavitary pulmonary aspergillosis from a simple *Aspergillus* fungus ball, patient symptoms, radiographic evidence of inflammation, and radiographic stability are useful parameters. The global burden of chronic pulmonary aspergillosis after tuberculous is thought to be substantial, which is important because of the potential response to antifungal agents in some patients.[116] In all of these conditions the diagnosis is suggested by radiologic and clinical features, and the role of therapy remains speculative, although it appears that long-term antifungal therapy may be beneficial in a subset of patients.[115,117]

Other Superficial or Colonizing Syndromes of Aspergillosis

Otomycosis is a condition of superficial colonization typically due to *A. niger*.[117] The clinical features include findings similar to other causes of external otitis, with the external auditory canal potentially revealing mold growing on cerumen and desquamated epithelial debris. *A. fumigatus* looks greenish, and *A. niger* forms a black tuft. Treatment is to focus on the underlying chronic otitis externa and débridement rather than the fungus. In immunocompromised patients, invasive otitis externa resembles that due to *Pseudomonas aeruginosa* clinically and may respond to antifungal therapy.[45,118]

Onychomycosis due to *Aspergillus* is another superficial condition that, although rare, can become chronic and responds poorly to antifungal agents.[119] Antifungal agents in the setting of nail infection may have a spectrum of activity limited to yeasts; thus a nail culture can be useful in patients with nonresponsive disease to establish a specific fungal etiology so that appropriate therapy can be initiated.

Aspergillus is an occasional etiology of keratitis, particularly after trauma or corneal surgery (see Chapter 113).[120,121] The diagnosis can be established with smears demonstrating hyphae, which may be indistinguishable from other molds, such as *Fusarium,* that also cause keratitis, but culture results are usually positive and confirm the diagnosis. Therapy consists of topical antifungal agents, usually topical natamycin 5% or topical voriconazole, although studies demonstrating efficacy of antifungal therapy are limited.[117,120–123] Surgical intervention may be required for deep lesions or those nonresponsive to medical therapy.[117] Amphotericin B has been injected intracamerally when corneal penetration has occurred.[124]

Invasive Syndromes Caused by *Aspergillus*

IA most frequently begins in the lung after inhalation of *Aspergillus* conidia. Invasion of hyphae into pulmonary vasculature is common, occurring in as many as a third of patients with invasive pulmonary aspergillosis. Disseminated disease occurs either by hematogenous spread to distant sites or by contiguous extension from the lung. Hematogenous dissemination to the CNS or other organs, including the thyroid, liver, spleen, kidney, bone, heart, and skin, is common in patients with severe immunosuppression, such as those undergoing HSCT, and heralds a poor prognosis.[3]

Invasive Pulmonary Aspergillosis

The most common manifestation of IA is invasive pulmonary aspergillosis (IPA). IPA rarely manifests before 10 to 12 days of profound neutropenia, which is a major risk factor for infection.[125] The incubation period for developing IPA after inhalation of conidia is not known but in patients with neutropenia has been estimated at approximately 15 days.[126] A significant number of patients have manifestations of IPA on admission or within the first 2 weeks of hospital admission, suggesting that community-acquired exposure is common.[76,127]

Symptoms of IPA include progressive cough, dyspnea, pleuritic chest pain, fever despite coverage with broad-spectrum antibiotics, and pulmonary infiltrates. These symptoms may be reduced in patients who are unable to mount an inflammatory response owing to profound neutropenia. In addition, although fever is common, it may be absent in those receiving high doses of corticosteroids. Other clinical features of IPA include hemoptysis, pleural effusion, and pneumothorax. However, all the physical findings are nonspecific and may lag significantly behind the disease process. Clinical characteristics may resemble a pulmonary embolism with pleuritic chest pain, hemoptysis, and dyspnea, which reflect the angioinvasive nature of the organism. Laboratory studies are also nonspecific but may include elevation in bilirubin, lactate dehydrogenase, and C-reactive protein, as well as coagulation abnormalities. Life-threatening hypoxia may occur in patients with extensive or progressive infection.

In extensive infection multiple diffuse nodular pulmonary infiltrates are readily seen on chest radiographs or chest CT (Fig. 257.7). Although these are not diagnostic, dense nodular lesions are associated with a poorer prognosis.[128,129] Other pulmonary radiographic findings of IPA include the classic pleural-based, wedge-shaped densities or cavitary lesions, although the former are not commonly detected, and the latter are present late in the course of infection.[128,130] Pleural effusions are more common than previously considered, but whether they are a specific manifestation of IPA is not established.[128] The presence of a "halo" of low attenuation surrounding a nodular lesion on CT is an early finding in IA (Fig. 257.8A).[128,130] Later in the course of infection, these nodular lesions may cavitate, usually in temporal association with recovery of neutrophils, forming an "air-crescent" sign (see Fig. 257.8B). These radiographic features are characteristic of IPA, but similar findings can

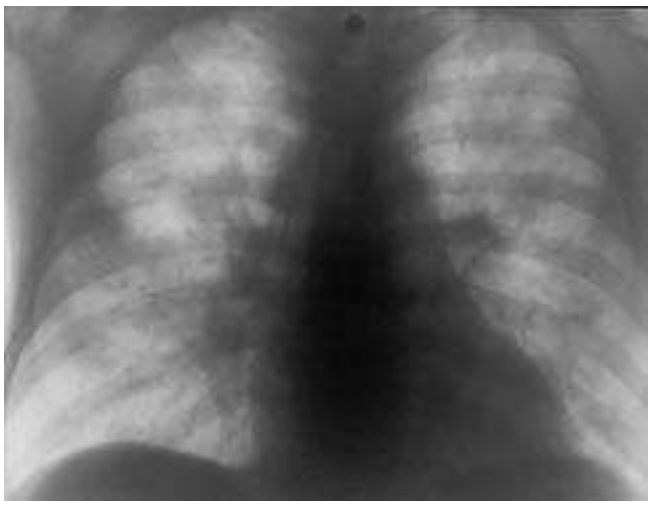

FIG. 257.7 Chest radiograph showing diffuse pulmonary infiltrates of invasive pulmonary aspergillosis.

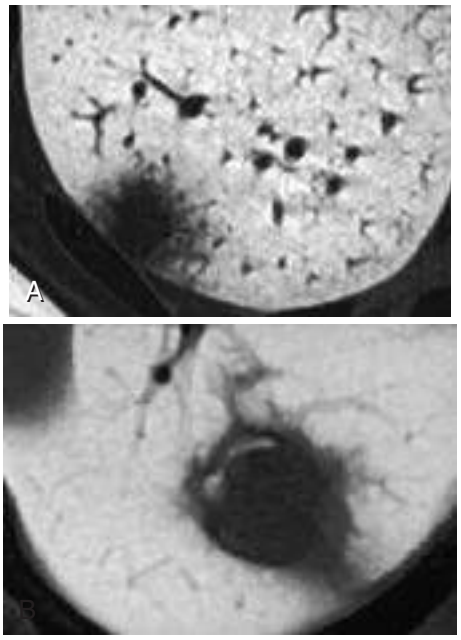

FIG. 257.8 Computed tomography of chest. (A) "Halo" sign of low attenuation surrounding a nodular lung lesion detected in early pulmonary aspergillosis. (B) "Air-crescent" sign in a nodular lung lesion found in late disease. *(Radiographs courtesy Dr. Reginald Greene.)*

also occur with other angioinvasive organisms, including *Mucorales, Fusarium*, and *Scedosporium*, as well as bacterial pathogens.

Tracheobronchitis

Aspergillus in the airways can range in significance from colonization, which is common in lung transplantation, to ulcerative tracheobronchitis.[66] The syndrome of *Aspergillus* tracheobronchitis typically occurs in patients undergoing lung transplantation and in patients with AIDS and is characterized by extensive pseudomembranous or ulcerative lesions due to *Aspergillus*. In patients undergoing lung transplantation the infection often occurs at the suture line of the lung transplant and can lead to dehiscence of the anastomotic site. Symptoms of tracheobronchitis are nonspecific and include dyspnea with associated pulmonary function abnormalities, cough, chest pain, fever, or hemoptysis. Symptoms may be mild and can be confused with other causes, including rejection. Results of plain radiographs may be normal so that clinical suspicion is needed to establish the diagnosis, which is accomplished by bronchoscopy with biopsy to document tissue invasion. A prolonged course of a systemic antifungal agent therapy is usually required for treatment, although aerosols of liposomal formulations of amphotericin B have been used for localized disease.[117]

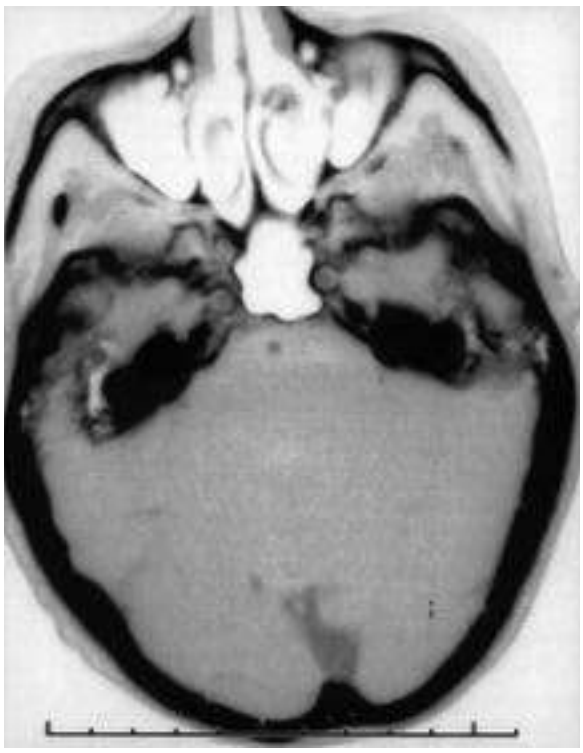

FIG. 257.9 Computed tomography of sinuses showing sinus wall thickening.

Sinusitis

Aspergillus infection of the sinuses and nasal cavities in immunocompromised patients manifests as acute invasive rhinosinusitis often in association with invasive pulmonary aspergillosis.[110,111] The clinical manifestations are not specific to *Aspergillus* and include fever, cough, epistaxis, sinus discharge, and headaches. Clinical signs are also nondiagnostic, but findings of an ulcerative nasal lesion with an eschar or nonsensitive area may be a clue to a fungal diagnosis.[131] Presence of epistaxis or unexplained fever in a high-risk patient may warrant endoscopy and biopsy of a nasal mucosal lesion. In patients with progressive infection the disease spreads to contiguous paranasal sinuses, palate, orbit, or brain. The mortality in invasive cases is high, ranging from 20% in patients with leukemia in remission to up to 100% in patients with relapsed leukemia or those undergoing bone marrow transplantation.[117] Plain radiographs are not diagnostic, may be falsely negative, and do not distinguish fungal etiologies from other causes of sinusitis. Sinus CT scans are useful for establishing extent of infection and determining local tissue invasion (Fig. 257.9). Routine surveillance cultures of the nose have been advocated, but these lack specificity and sensitivity. Cultures from sinus aspirates are useful to demonstrate the presence of *Aspergillus*, but a biopsy with tissue invasion is needed for definitive diagnosis.[132] Therapy for these infections is often difficult and requires long-term administration of antifungal medications.[117] The role of surgery is controversial. Efficacy of antifungal prophylaxis has not been demonstrated, but attempts at reduction of environmental exposures in high-risk patients may be beneficial.[133]

Disseminated Infection

Progressive invasive pulmonary aspergillosis often results in disseminated IA, a complication associated with an extremely high mortality.[2] In patients with severe and ongoing immunosuppression, disseminated infection can occur with mortality rates historically >90%.[2,3] Even with advances in antifungal therapy, mortality rates in

disseminated disease occurred in approximately 50% in the TRANSNET study.[134]

Other Invasive Syndromes

Cerebral Aspergillosis

Cerebral aspergillosis is associated with the highest mortality of IA syndromes, with mortality rates of >90% in most series.[3,134,135] The incidence of cerebral aspergillosis is difficult to determine because the diagnosis is often unsuspected, but it has been estimated to occur in 10% to 20% of all cases of IA, usually in patients with persistent immunosuppression and disseminated disease.[3,136] In one series of patients undergoing HSCT *Aspergillus* was found in 58% of biopsied cerebral mass lesions.[137] The diagnosis may occur an extended time after transplant (>100 days) and is nearly always associated with extensive immunosuppression, such as therapy for GVHD.[138] Concomitant pulmonary infection is usually, but not always, present.[137] Isolated cerebral aspergillosis can occur in immunocompetent patients or in the setting of injection drug use, in which case it may be associated with a slightly better prognosis, provided the diagnosis is made and surgical drainage or removal is performed.[136] *Aspergillus* meningitis is rare. The clinical presentation of cerebral aspergillosis is nonspecific and is characterized by focal neurologic signs, alteration in mental status, and headaches.[139] On CT of the brain the appearance is nonspecific and is similar to that of other infectious causes of brain abscess, with ring enhancement of the abscess, along with surrounding edema (Fig. 257.10) and may be hemorrhagic.[140] Magnetic resonance imaging (MRI) scans may reveal additional lesions, but the findings are still nonspecific. Confirmation of the diagnosis requires biopsy, but the diagnosis is often presumed. In patients without a clear diagnosis biopsy is recommended because the differential diagnosis is extensive, including other fungi and an extensive array of opportunistic diseases. Until recently the outcome of this infection has been almost universally fatal, although voriconazole has been associated with favorable responses in approximately 30% of patients.[135,136]

Bone Aspergillosis

Aspergillus osteomyelitis is an uncommon finding of IA. Vertebral osteomyelitis can result from local extension of an *Aspergillus* empyema. *Aspergillus* osteomyelitis can also be seen as a complication of disseminated infection or as a primary infection in certain risk groups, such as chronic granulomatous disease, in IV drug users, or after spinal procedures.[141] Vertebral osteomyelitis is the most common site for hematogenous spread to bone, usually involving the lumbar region.[142] The lesions can be seen on plain radiographs (Fig. 257.11) and on a CT or an MRI scan, which can be useful to stage the infection and to guide needle biopsy of the lesion. Favorable responses with voriconazole in *Aspergillus* osteomyelitis of the spine exceeded 60% in one review, although the need for long-term therapy and surgical intervention in medically nonresponsive patients is noted.[143] Infection of an intervertebral disk is a rare complication of hematogenous spread or surgery on the disk.

Cutaneous Infection

Skin involvement by *Aspergillus* can represent either disseminated hematogenous infection or local inoculation of infection that may arise around an IV catheter insertion site or the surrounding areas covered by adhesive dressings.[77] Whereas most lesions occur in patients with neutropenia or in other immunocompromised patients, *Aspergillus* can also invade patients with burns or surgical wounds. Clinically, the lesion is an area of rapidly increasing erythema with a necrotic, often ulcerated, center (Fig. 257.12). The lesions may resemble pyoderma gangrenosum. Pathologically, invasion of blood vessels and cutaneous ulceration occurs. Cutaneous disease can also be a manifestation of widespread disseminated disease, and in that setting a skin biopsy can be a relatively easy method to obtain tissue to establish the diagnosis of IA.

Other Sites

IA has also been reported in anecdotal cases to cause infection in virtually all body sites, including the heart, kidney, esophagus, intestine, and

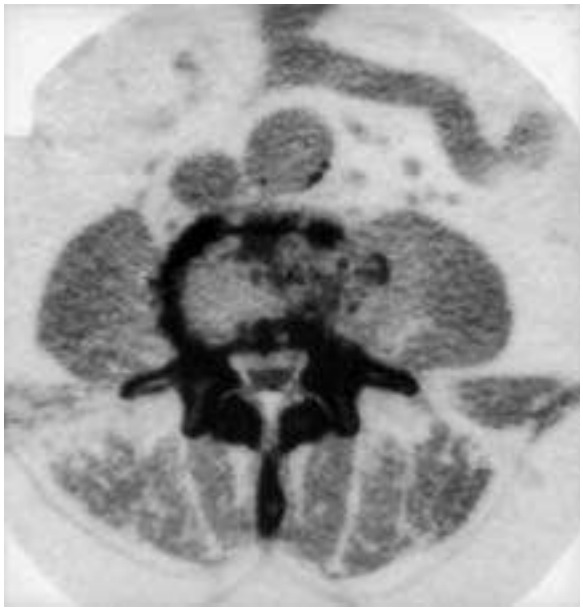

FIG. 257.11 **Radiograph of spine showing bone destruction associated with spinal aspergillosis.**

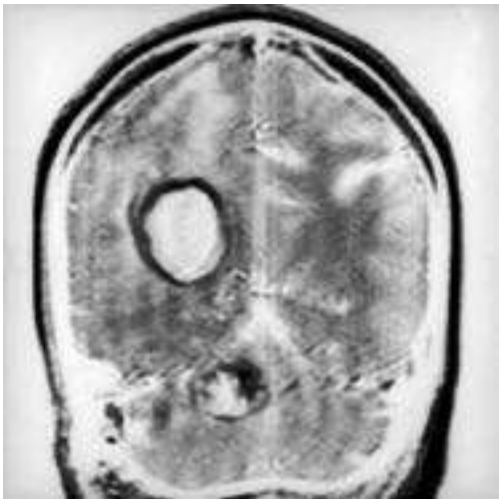

FIG. 257.10 **Brain abscess of invasive aspergillosis with ring enhancement and extensive edema.**



FIG. 257.12 **Necrotic skin lesion of cutaneous aspergillosis.**

others.[117] *Aspergillus* endocarditis can occur in either native or prosthetic heart valves.[144] Diagnosis is difficult because blood cultures usually remain negative even with extensive disease.[145] Even with surgical intervention, long-term survival is limited, although favorable outcomes with newer agents, particularly voriconazole, have been reported.[146,147] *Aspergillus* pericarditis is also associated with disseminated infection but can occur because of local extension of invasive pulmonary aspergillosis and can be complicated with cardiac tamponade. Some of these uncommon syndromes appear more common in certain epidemiologic settings. Renal infection occurs in patients with AIDS or with a history of injection drug use.[117]

DIAGNOSIS AND ANTIFUNGAL RESISTANCE

A proven diagnosis of IA requires a tissue biopsy showing invasion with hyphae and a positive culture for *Aspergillus*.[132] The diagnosis can also be established with positive cultures from a normally sterile site, such as a needle biopsy or cerebrospinal fluid (CSF), although blood cultures are rarely positive.[148] Tissue biopsies may not be possible because of potential risks, although *Aspergillus* hyphae are easily seen with common fungal stains, such as Gomori methenamine silver or periodic acid–Schiff. *Aspergillus* hyphae are hyaline, septate, acute-angle branched, even diameter, and 3 to 6 μm in width. Although these features usually distinguish *Aspergillus* from agents of mucormycosis, they are not distinguishable from a number of other opportunistic molds, including *Fusarium, Lomentospora* (formerly *Scedosporium*), *Paecilomyces*, and others so that a positive culture is needed to confirm the diagnosis.[149]

Cultures for *Aspergillus* in respiratory samples in high-risk patients, particularly if obtained via bronchoalveolar lavage (BAL), can support the diagnosis of probable IA.[132] *Aspergillus* can also be cultured from patients in whom no clinical illness is apparent, and positive cultures in patients with a low risk for IA should be interpreted with caution.[29]

Radiographic findings can also be used in the diagnosis and management of invasive pulmonary aspergillosis. Plain chest radiographs are of limited diagnostic utility because they are insensitive and findings are nonspecific.[128] A "halo" of low attenuation surrounding a nodular lesion on CT imaging is an early finding in invasive pulmonary aspergillosis and has been used as a marker for initiating early antifungal therapy.[4,128,150] The volume of lesions may increase over the first 7 days of infection, even when therapy is successful, so that early radiologic progression should be interpreted cautiously.[130] The CT findings of IPA have been validated in high-risk, neutropenic, and bone marrow transplant recipients, but in other patients, including solid-organ transplant recipients and those with nontraditional risk factors in the ICU, the CT findings are not as well defined.

Non–culture-based methods have been used to establish a rapid diagnosis of IA.[151] Antibody detection is of limited utility because immunosuppressed hosts fail to mount an antibody response even with invasive infection.[86] Detection of galactomannan (GM) by enzyme immunoassay has contributed substantially to the diagnosis of probable IA.[152] This assay has been validated in a variety of patient groups, particularly with serial measurements. Studies have suggested a sensitivity as high as 89% and a specificity of 92% in high-risk HSCT patients not receiving antimold prophylaxis.[153] Other studies have found the assay to be less sensitive (40%–50%), reflecting the impact of prior antifungal therapy in reducing the level of circulating GM, a limited number of samples per patient, extent of infection, and other variables.[152–154] This has resulted in recommendations for a lower cutoff for a positive test result, especially in high-risk patients with greater probability of infection.[153,155] A meta-analysis using an optical density index cutoff of ≥0.5 showed a sensitivity of 78% and a specificity of 81%.[156] False-positive results have been reported in some children, including in some neonates, which may be due to dietary intake or the presence of cross-reacting antigens with bacteria such as *Bifidobacterium*, and in patients receiving therapy with piperacillin-tazobactam—which now appears to be uncommon—and other antibiotics.[152,157] GM detection has also has been used for other body fluids, such as CSF, and in BAL fluid.[152,158] GM results from BAL fluid testing demonstrate sensitivity that is greater than that seen in serum testing and may be less affected by antifungal therapy.[159–161] A negative test result may be useful in ruling out a diagnosis of IPA.[160] Some colonized patients, such as those with chronic obstructive pulmonary disease or those undergoing lung transplantation, may have a positive GM result and not have evidence of invasive infection.[162] Serial assessments of GM may offer prognostic value in outcomes of infection. Other potential markers also include the nonspecific fungal marker (1→3)-β-D-glucan.[163,164]

Several reports demonstrate the potential for using polymerase chain reaction (PCR) as an early diagnostic marker for IA, which may be more sensitive that other methods.[165] These assays have not been traditionally standardized, and it was thus problematic to determine their diagnostic utility.[166,167] More recently, PCR has become commercially available, allowing standardization and quality control schemes to be implemented. The specificity of BAL PCR appears to be greater than GM testing, providing better utility for a diagnostic test.[168]

Susceptibility testing for *Aspergillus* has been standardized, but correlation with clinical responses has not been well established.[169] Antifungal resistance to itraconazole has been reported developing after exposure to antifungal therapy and is associated with point mutations in the *cyp51A* gene target of triazoles antifungals.[170] That resistance mechanism, which correlated with lack of efficacy in an animal model, has not been a common finding. However, resistant isolates have also been detected in azole-naïve patients.[171,172] In the initial report all cases were observed since 1999, and the prevalence of resistant *A. fumigatus* ranged from 1.7% to 6%. The authors speculated that resistance in *A. fumigatus* could be related to the widespread use of agricultural triazole fungicides.[172] These isolates have a particular genetic alteration consisting of a 34–base pair tandem repeat in the promoter coupled with a point mutation in the *cyp51A* target gene: an amino-acid substitution at codon 98 (TR34/L98H), causing multi–azole resistance. In contrast to the point mutations that occur in resistant isolates after azole exposure, no isogenic strains demonstrating this mechanism of resistance have been shown, suggesting a potential environmental source of infection. This mechanism has been most prevalent in the Netherlands, where agricultural azole fungicides are common, and it has also been demonstrated in several other European countries, India, and Asia.[173–175] More recently, other similar but distinct mechanisms of resistance (TR46/Y121F/T289A) have been reported in clinical isolates.[176] It is not clear how frequently these mutations occur clinically, but the lack of azole responsiveness in patients with these isolates suggests it may become prudent to consider that possibility. Different mutations induce differential triazole susceptibility, with some mutations causing resistance to voriconazole and isavuconazole, whereas others cause resistance to posaconazole and itraconazole, and others cause panazole resistance.[177] Other species, such as *Aspergillus terreus* and cryptic *Aspergillus* spp., may be resistant to amphotericin B or other antifungals, so testing of those species may be warranted.[11,30,178]

THERAPY (Table 257.4)

Historical efficacy of antifungal therapy in IA has been extremely poor, with favorable responses in less than 40% of patients and overall mortality rates of 60% or more.[2,3] Although mortality rates in high-risk patients continue to be unacceptably high, mortality rates have decreased.[63,134] Response to antifungal therapy depends on several factors, including the immune status of the host and the extent of infection at time of diagnosis.[3] In severely immunosuppressed patients, such as those undergoing allogeneic HSCT, and in patients with CNS or widely disseminated infection, extremely poor outcomes are seen, with mortality rates >75% in those groups.[a]

Primary Antifungal Therapy

Voriconazole

Voriconazole is a potent, broad-spectrum triazole that has become the recommended primary therapy for most patients with IA.[1] Voriconazole can be given either orally or intravenously (see Chapter 40B). The recommendation for voriconazole for primary therapy is based on a randomized trial that compared voriconazole with amphotericin B deoxycholate,

[a]References 3, 65, 117, 134, 136, 179, 180.

TABLE 257.4 Antifungal Agents for Invasive Aspergillosis

AGENT	CLASS	ROUTE OF ADMINISTRATION	DOSE	COMMENTS
Primary Therapy				
Voriconazole	Azole	IV/PO	6 mg/kg (IV) q12h × 2 doses, followed by 4 mg/kg (IV) q12h or 200 mg (PO) q12h. Some experts advise oral dosing as 4 mg/kg (PO) q12h.	Recommended for primary therapy in most patients due to randomized trial demonstrating improved survival compared with amphotericin B deoxycholate[4,117,181]; caution for use in patients with potential liver toxicity and for drug interactions; measurement of serum levels for efficacy and avoidance of toxicity[187]
Alternative Primary Therapy				
Isavuconazole	Azole	IV/PO	372 mg q8h × 6 doses, IV or PO followed by 372 mg daily (372 mg isavuconazonium sulfate = 200 mg isavuconazole)	Well tolerated, efficacy in treatment of pulmonary aspergillosis, and fewer adverse events than voriconazole-treated patients[194]
Liposomal amphotericin B	Polyene	IV	3–5 mg/kg/day	Well tolerated; minimal infusion reactions or nephrotoxicity; initial doses of 10 mg/kg/day more toxic and not more effective[199]
Other Agents				
Amphotericin B	Polyene	IV	1–1.5 mg/kg/day	Previous gold standard; significant toxicity in higher doses; limited efficacy in high-risk patients[3,200,201]
Amphotericin B lipid complex	Polyene	IV	5 mg/kg/day	Indicated for patients intolerant or refractory to standard therapy; case-controlled data suggest better efficacy than amphotericin B deoxycholate[203]
Amphotericin B colloidal dispersion	Polyene	IV	3–6 mg/kg/day	More infusion-related toxicity than other lipid formulations; efficacy similar to amphotericin B in primary treatment[204]
Posaconazole	Azole	IV/PO	Investigational in United States for *Aspergillus* treatment: tablet or IV, 300 mg q12h × 2 doses, followed by 300 mg daily	Oral and IV formulation; efficacy in salvage therapy and prophylaxis[58,207,208]
Itraconazole	Azole	PO	200 mg twice daily (PO) or 65 mg twice daily of SUBA-itraconazole	Erratic bioavailability improved with oral solution and new 65-mg capsule, serious cardiac adverse events with higher doses, drug interactions common; IV formulation not currently available[3]
Caspofungin	Echinocandin	IV	70 mg first day then 50 mg/day	Approved for refractory infection and intolerance to standard therapy; well tolerated[213]; limited efficacy as monotherapy for primary infection[180]; preclinical and anecdotal data showing improved efficacy in combination with azoles[210,217]
Micafungin	Echinocandin	IV	Investigational for *Aspergillus* treatment (United States) (50–100 mg/day)	Efficacy for prevention and salvage treatment of aspergillosis[214,245]
Anidulafungin	Echinocandin	IV	Investigational for *Aspergillus* (200 mg first day then 100 mg/day)	Combination trial data showing improved outcomes in patients with galactomannan diagnosis of invasive pulmonary aspergillosis

IV, Intravenous; *PO,* orally.

with each agent followed by other licensed antifungal therapy if needed for intolerance or progression of disease.[4] In this trial voriconazole was successful in 52% of patients versus 31% in those receiving amphotericin B deoxycholate. Superiority of voriconazole was demonstrated in patients at high risk for mortality, including those undergoing HSCT and in those with disseminated and CNS infection.[136,181] A survival benefit of voriconazole was also shown.[4] Voriconazole has also demonstrated efficacy in very difficult-to-treat clinical conditions, with responses of approximately 34% in CNS infection and in 52% of patients with osteomyelitis.[136,143]

Although voriconazole is generally adequately tolerated and exhibits a favorable pharmacokinetic profile, there are a number of issues to consider, including drug intolerance and drug interactions, especially those with immunosuppressive agents, such as cyclosporine, tacrolimus, and sirolimus, the latter of which is contraindicated for use with voriconazole. Other drug-drug interactions are common with medications metabolized through cytochrome P-450 (CYP)3A4, CYP2C9, or CYP2C19 enzymes. The most common adverse event has been a transient and reversible visual disturbance that has been reported in approximately 30% of patients receiving the drug.[4] This effect is dose related and described as an altered or increased light perception that is temporary and not associated with pathologic sequelae. Other adverse events have been less common, including dose-limiting liver abnormalities in 10% to 15%, skin rash in 6%, nausea and vomiting in 2%, and anorexia in 1%. Other serious adverse events have also been associated with long-term voriconazole use, including alopecia (which appears reversible with discontinuation of the drug), periostitis (perhaps related to the fluoride molecules in the compound), skin malignancies, and potentially severe peripheral neuropathies.[182–185] Therapeutic drug monitoring is recommended for voriconazole to improve efficacy[186] and limit adverse events such as liver toxicity and hallucinations, which are perhaps more common, with levels >6 μg/mL, and decreased efficacy with trough levels <1 μg/mL.[187–189] Weight-based dosing may increase the likelihood of achieving target drug levels with voriconazole, but considerable interpatient and intrapatient variability occurs.[190–192]

Alternative Primary Therapy

Isavuconazole

Isavuconazole is also a broad-spectrum triazole and an alternative first-line agent in the treatment of pulmonary aspergillosis.[1] Isavuconazole can be given orally or intravenously (see Chapter 40B). After administration, the prodrug, isavuconazonium, is cleaved by plasma esterases to the active agent isavuconazole.[193] Isavuconazole is an alternative primary therapeutic agent based on a single, comparative phase 3, randomized

controlled trial. This study found isavuconazole noninferior to voriconazole in the primary treatment of invasive mold infection. In patients with proven or probable aspergillosis there was no difference in all-cause mortality at day 42 or 84.[194] Patients with disseminated infection were not included in this trial. Adverse events from isavuconazole were less frequently observed compared with the voriconazole group (hepatobiliary disorders 9% vs. 16%, eye disorders 15% vs. 27%, and skin disorders 33% vs. 42%, respectively).

Isavuconazole is generally well tolerated and has been found a useful agent in patients intolerant of other mold-active triazoles.[195] Drug-drug interactions occur with this agent as well, and caution with coadministration of agents metabolized by CYP3A4 is needed. Adverse reactions included hepatotoxicity (16%), infusion-related reactions, and hypokalemia (15%).[194,196] In contrast to other triazoles isavuconazole has been associated with shortening of the QT interval, although the significance of this remains unclear.[197] An association of serum drug levels with efficacy or toxicity has not been found,[198] and there are currently no recommendations for monitoring serum concentrations of isavuconazole,[1]

Lipid Amphotericin Formulations

In patients in whom voriconazole is contraindicated based on drug interactions, liver dysfunction, intolerance, or other contraindication, liposomal amphotericin B is recommended as an alternative primary therapy.[1] Liposomal amphotericin B offers the advantage of less toxicity than amphotericin B deoxycholate and allows administration of higher doses of therapy.[199] This recommendation is based on a clinical trial that compared high initial doses of liposomal amphotericin B (10 mg/kg/day) versus 3 mg/kg/day for 2 weeks, followed by standard antifungal regimens. In this trial similar efficacy of 50% and 46% was seen in the two groups, respectively.[199] These results suggest that standard doses of liposomal amphotericin are adequate as primary therapy in some patients.[117]

Other Antifungal Agents

Amphotericin B Deoxycholate

For more than 4 decades, amphotericin B deoxycholate was the gold standard therapy for patients with IA.[117] Several studies documented the limited efficacy and substantial toxicity of amphotericin B deoxycholate in high-risk patients.[4,200,201] The overall response rates of amphotericin B deoxycholate are <25%, with substantial drug-related toxicities.[3,4,201] Similar findings were documented by Bates and colleagues,[200] who found that renal toxicity occurred in approximately 30% of patients receiving amphotericin B deoxycholate and that this toxicity was associated with a sixfold increase in mortality and a dramatic increase in hospital costs. Thus, with unacceptably high mortality rates and significant toxicity, amphotericin B deoxycholate is not recommended for IA.[117]

Other Polyenes

Amphotericin B lipid complex is a lipid formulation of amphotericin B approved for IA salvage therapy.[202,203] It is generally used in doses of 5 mg/kg/day and is better tolerated than amphotericin B deoxycholate. Another study evaluated amphotericin B colloidal dispersion for primary therapy for IA, which showed similar efficacy, with reduced renal toxicity of the lipid formulation but with serious pulmonary toxicities so that this preparation offers little advantage for its use.[204] Meta-analysis of studies using lipid formulations of amphotericin B show reduced nephrotoxicity and favorable results as salvage therapy with these agents for IA.[205]

Other Triazoles

Itraconazole is approved for use as salvage therapy of aspergillosis, but its utility in invasive infection has been limited because of an unfavorable pharmacokinetic and tolerability profile. Although an IV formulation obviated bioavailability concerns, that formulation is no longer clinically available. Itraconazole is more frequently used in less immunosuppressed patients who are able to take oral therapy and for use as sequential oral therapy and for those patients with saprophytic or allergic conditions.[3,108] A new formulation of itraconazole (SUBA-itraconazole) has been developed and may offer pharmacokinetic advantages over conventional itraconazole.[206]

Other extended-spectrum triazoles, including posaconazole and ravuconazole, have activity in vitro against *Aspergillus*. Posaconazole is approved for prophylaxis of invasive fungal infection, including aspergillosis, and has demonstrated efficacy in salvage therapy.[58,207,208] As with voriconazole, drug level monitoring of posaconazole is recommended, particularly in patients with GVHD.[209]

Echinocandins

The echinocandins are an additional class of antifungals with *Aspergillus* activity.[180,210,211] These agents, which are administered intravenously, target glucan synthase, which is needed for production of β-1,3-glucan in fungal cells walls.[212] These effects are not fungicidal but significantly alter the growing fungal cell wall. Included in these agents are caspofungin, micafungin, and anidulafungin. Caspofungin is approved for treating patients refractory to, or intolerant of, standard therapies for IA but is not recommended for primary therapy.[180,213] In one prophylaxis study micafungin reduced the number of *Aspergillus* infections compared with standard prophylaxis with fluconazole and has activity in salvage therapy of the disease.[214] Anidulafungin is another echinocandin with *Aspergillus* activity and exhibits a favorable toxicity profile similar to those of the other echinocandins.

Combination Antifungal Therapy

The rationale for combination therapy is to maximize treatment by targeting multiple targets or metabolic pathways, or different points in the same pathway, to improve efficacy by an additive or synergistic effect. Antifungal drug combinations have been evaluated in vitro and in animal models in a number of prior studies with variable results.[215–219] Antagonism has been demonstrated in some but not all studies, particularly between polyenes and triazoles; however; the combination of a triazole and an echinocandin exhibits synergistic to additive interactions.[219–222] A randomized clinical trial has been completed comparing voriconazole with the combination of voriconazole plus anidulafungin. This trial in HSCT patients failed to demonstrate superiority of the combination regimen for the primary study end point of fungal-free survival. However, in the subgroup of patients diagnosed by GM detection (presumably those with early disease), mortality was significantly reduced. Guidelines do not currently recommend routine use of combination primary therapy, but these data suggest that combination therapy may be beneficial in some patients.[1]

Adjuvant Therapy

Adjuvant therapies including surgery, granulocyte transfusions, and growth factors have been used to augment antifungal therapy, although their utility has not been established. Surgical resection of isolated pulmonary nodules has been shown to improve outcome of infection,[129,223] although favorable outcomes with triazoles suggest that in some patients surgical resection may not be necessary. Surgical resection should also be considered in patients with severe hemoptysis or lesions near the hilar vessels or pericardium.

Other adjuvant therapies, including granulocyte and granulocyte-macrophage colony-stimulating factors, interferon-γ, and granulocyte transfusions,[224] have all been shown in anecdotal reports to improve outcomes but are not generally recommended for routine use, given little demonstrated efficacy.[1,225]

Approach to Therapy

Guidelines for treating IA have been published by the Infectious Diseases Society of America and other groups internationally, with similar recommended approaches to therapy.[226–228] There are few randomized trials to support evidence-based approaches to therapy and heterogeneity of underlying host factors, and diagnostic criteria further impact clinical outcomes in this disease. A prompt diagnosis and effective initial therapy are both critical in improving the outcome of this infection.[229] Radiography and use of biomarkers, such as GM, may facilitate an early detection of aspergillosis in high-risk patients.[230,231] Most patients should receive primary therapy with voriconazole, which has been shown to be superior to amphotericin B deoxycholate and has been used in a variety of clinical

syndromes since its availability for use in this disease.[4] In patients who are intolerant of voriconazole, have a contraindication to the drug, or have progressive infection, alternative agents include isavuconazole or lipid formulations of amphotericin B, the echinocandins, or another triazole.[199,207,213,214] Primary use of combination therapy in all patients is not recommended at the present time because of the lack of encouraging clinical trial data, but combination therapy in some groups of patients, such as those with an early diagnosis of infection, may prove beneficial. In a salvage setting the addition of another agent or a switch to another antifungal class may be considered due to the poor outcomes of a single agent in progressive infection.[117] Although the optimal duration of antifungal therapy is not known, improvement in underlying host defenses is crucial to successful therapy.

PREVENTION AND PROPHYLAXIS

Prevention of IA in high-risk patients is difficult. Nosocomial outbreaks of aspergillosis have been linked to construction, contaminated ventilation systems, and possibly to contaminated water.[75,232,233] For high-risk patients, such as those undergoing HSCT, the use of high-efficiency particulate air filters, frequent air exchanges, and positive pressure ventilation has been recommended to limit exposures in the hospital setting.[71–74] Infection control measures, such as construction barriers, will limit exposure to aerosols.[127] Attention to routine maintenance and cleaning of showers and water systems may further reduce risk.[72,234] Some patients will still develop infection with these precautions, and an increasing number of immunosuppressed patients receive care outside of the hospital setting so that community-acquired infection is common.[76]

Efficacy of antifungal prophylaxis has been limited because of the toxicity of amphotericin B and the limited activity of other oral agents against *Aspergillus.* Agents evaluated in this setting include low-dose amphotericin B, low doses of lipid formulations of amphotericin B, and nasal and aerosolized forms of amphotericin B, none of which has demonstrated conclusively to be beneficial in a large randomized clinical trial. Studies of aerosolized formulations of lipid amphotericin B have demonstrated its safety and potential efficacy in lung transplant recipients and in patients with prolonged neutropenia who are at high risk for IA.[235,236] Itraconazole has been suggested to have benefit against prevention of molds, but its poor tolerance in high-risk patients has also limited its use,[237,238] although a newer formulation may be useful in this setting.[206] In a long-term study of fungal prophylaxis in patients with chronic granulomatous disease, itraconazole reduced the incidence of serious fungal infections, including those due to *Aspergillus*.[239]

Two randomized trials have established the safety and efficacy of posaconazole in high-risk patients. Posaconazole was compared with fluconazole in a randomized, double-blind trial in patients undergoing HSCT with GVHD. In that trial posaconazole significantly reduced the number of breakthrough fungal infections, including IA.[208] In the companion trial posaconazole was compared with either fluconazole or itraconazole for patients with acute myelogenous leukemia or myelodysplastic syndrome.[199] In that trial prophylaxis with posaconazole reduced breakthrough fungal infections and significantly prolonged survival. More serious adverse drug events occurred with posaconazole therapy so that a risk-benefit analysis should be considered when recommending posaconazole prophylaxis.[57] Guidelines recommend the use of posaconazole prophylaxis in patients with acute myelogenous leukemia or with GVHD, who are at high risk for IA.[b] Voriconazole prophylaxis has been evaluated in two randomized trials, the first being a strategy of comparing fluconazole with voriconazole, with GM monitoring for fungal infection.[242] The primary end point of improved fungal free survival was not met, although there were fewer *Aspergillus* infections in the voriconazole arm and improved fungal-free survival in patients with acute myeloid leukemia. The second trial compared voriconazole with itraconazole in allogeneic HSCT and demonstrated better efficacy of voriconazole due to improved tolerability compared with itraconazole.[243] A meta-analysis of antimold active prophylaxis showed fewer episodes of IA but more adverse events and no impact on overall survival.[244] In high-risk patients the consideration for antimold active prophylaxis should be considered, but in patients at lower risk careful monitoring and preemptive therapy may be an appropriate course of action.

[b]References 1, 117, 179, 226, 240, 241.

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The complete reference list is available online at Expert Consult.

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258 Agents of Mucormycosis and Entomophthoramycosis

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SHORT VIEW SUMMARY

Definition

- Mucormycosis is an aggressive, angioinvasive fungal infection, caused by filamentous fungi in the subphylum Mucoromycotina, order Mucorales, that afflicts immunocompromised patients with severe metabolic conditions, such as uncontrolled diabetes mellitus. Skin and soft tissue infections in immunocompetent patien hosts may be encountered in patients with severe soft tissue trauma (e.g., from tornadoes, combat injuries, or burns).
- Entomophthoramycosis is a rare infection of the paranasal sinuses, subcutaneous tissues, or gastrointestinal (GI) tract caused by filamentous fungi in the subphylum Entomophthoramycotina that are principally encountered in the tropics.

Epidemiology

- Mucormycosis is acquired primarily via inhalation of environmental sporangiospores in immunocompromised hosts or through direct inoculation during trauma. The infection may break through antifungal prophylaxis regimens used to reduce the risk of aspergillosis.
- Entomophthoramycosis typically is an indolent subcutaneous infection localized to the sinuses, head and face (conidiobolomycosis), or trunk and arms (basidiobolomycosis) and acquired by inhalation or minor trauma. GI basidiobolomycosis has occurred in Arizona and the Near East and is perhaps acquired by ingestion.

Microbiology

- Most common culture-confirmed cases reported in the literature are *Rhizopus* spp. (47%), *Mucor* sp. (18%), *Cunninghamella bertholletiae* (7%), *Apophysomyces elegans* (5%), *Lichtheimia (Absidia)* spp. (5%), *Saksenaea* spp. (5%), and *Rhizomucor pusillus* (4%), with other species (8%) accounting for the remaining cases.
- *Conidiobolus coronatus* and *Conidiobolus incongruous* cause conidiobolomycosis. *Basidiobolus ranarum* causes basidiobolomycosis.

Diagnosis

- A high index of suspicion in immunocompromised patients is essential because most signs, symptoms, and radiographic signs of mucormycosis are nonspecific. Cultures have poor sensitivity. Diagnosis is typically established by histopathologic documentation of "ribbon-like" angioinvasive hyphae in tissue, although this is prone to error.

Treatment

- Lipid formulations of amphotericin B are the drugs of choice. Oral posaconazole, or perhaps isavuconazole, is used for treatment once infection is clinically stable and if absorption is adequate.

Prevention

- Given the rarity of mucormycosis and entomophthoramycosis, primary prophylaxis is not recommended. Secondary or potentially indefinite prophylaxis should be considered for immunocompromised patients with a previous episode of mucormycosis, depending on the status of underlying immunosuppression.

The disappearance of the class Zygomycetes from current taxonomy has made the term *zygomycosis* obsolete. The term *zygomycosis* encompassed both mucormycosis and entomophthoramycosis. These two infections are so different that no new name has been proposed to include both infections. Most of this chapter discusses mucormycosis, a group of filamentous fungi in the subphylum Mucoromycotina that belong to the order Mucorales. Mucorales can cause life-threatening infections in humans, especially in immunocompromised hosts. The first documented report of human mucormycosis is credited to Paltauf,[1] who in 1885 reported a disseminated infection in a patient with rhinocerebral involvement caused by angioinvasive, ribbon-like hyphae that he termed *Mycosis mucorina*. Subsequent descriptions of the infection in the following decades relied on tissue morphology and, as often is the case today, were infrequently confirmed by culture. Hence the findings of coenocytic (aseptate or pauciseptate) fungal hyphae in tissue invading blood vessels was assumed to be due to *Mucor* spp. and have become synonymous with the clinical term *mucormycosis* or, simply, *Mucor* infection. This terminology is further justified by the fact that all but the rare *Mortierella* spp. are within the order Mucorales.[2] However, the number of species causing human mucormycosis has expanded considerably in the past 2 decades with improvements in culture-based morphologic identification and the application of more precise molecular diagnostics and sequencing for fungal identification (Table 258.1). In fact, members of the genus *Rhizopus,* not *Mucor,* are reported as the most predominant cause of human infections, although in Europe *Mucor* and *Lichtheimia* account for approximately one-quarter of reported cases.[3–5]

ETIOLOGY

Agents of mucormycosis are ubiquitous fungi in the environment that are commonly found in decaying organic substrates, including bread, fruits, vegetable matter, soil, compost piles, and animal excreta.[6] These fungi characteristically produce large, ribbon-like hyphae that are irregular in diameter with only occasional septae, hence the characterization of these organisms as aseptate fungi. Identification can be confirmed by observing the characteristic, saclike fruiting structures (sporangia), which produce internally spherical yellow or brown spores (sporangiospores) (Fig. 258.1).[6] Spores ranging from 3 to 11 μm in diameter are easily aerosolized and dispersed and cause infections in humans when inhaled or introduced through a cutaneous or percutaneous route.[6]

Although several species of the order Mucorales have been reported to predominate as causes of human mucormycosis, culture recovery of these fungi from infected tissue is suboptimal and may skew the current understanding of the etiologic spectrum of mucormycosis. The widening application of molecular diagnostic techniques (i.e., polymerase chain reaction [PCR]) in culture-negative cases may expand the current understanding of the prevalence and etiology of this infection. In a global review of more than 900 reported cases of mucormycosis, *Rhizopus* spp. (47%) were the most frequently reported causes of culture-confirmed mucormycosis, followed by *Mucor* spp. (18%), *Cunninghamella bertholletiae* (7%), *Apophysomyces elegans* (5%), *Lichtheimia (Absidia)* spp. (5%), *Saksenaea* spp. (5%), and *Rhizomucor pusillus* (4%), with a variety of other uncommon species representing the remaining 8% of culture-confirmed cases.[3]



FIG. 258.1 Mucormycosis agents. Illustration of the major differentiating morphologic features of three of the most common agents of mucormycosis isolated from patients. Note the presence and location of the rhizoids and columella, as well as the shape of the sporangia. The infectious spores reside within the sporangia. *(Illustration by Lori Messenger.)*

TABLE 258.1 Taxonomic Organization of the Most Common Agents of Mucormycosis and Entomophthoramycosis

Mucormycosis
Rhizopus rhizopodoformis
Rhizopus arrhizus (Rhizopus oryzae)
Rhizopus microsporus
Rhizomucor pusillus
Rhizopus stolonifer
Cunninghamella bertholletiae
Apophysomyces elegans
Saksenaea vasiformis
Lichtheimia (Absidia) corymbifera
Mucor circinelloides
Mucor velutinosus
Syncephalastrum racemosum
Actinomucor elegans
Cokeromyces recurvatus
Mortierella wolfii
Entomophthoramycosis
Conidiobolus coronatus
Conidiobolus incongruus
Basidiobolus ranarum

Seasonal variations may affect the incidence of mucormycosis, with most infections occurring during periods of higher temperatures and decreased precipitation.[7,8] Likewise, major weather events have also been associated with infections with less frequently isolated species, such as *Syncephalastrum racemosum* from respiratory samples after Hurricane Katrina, *Apophysomyces trapeziformis* soft tissue infections in patients with traumatic injuries associated with the Joplin, Missouri tornado,[9] or posttsunami wound infections due to *Apophysomyces elegans*.[10] Combat wound infections are also influenced by environmental factors, as in Afghanistan, where wounds sustained in the southern regions, with lower elevation, warmer temperatures, and greater isothermality, had greater mold contamination.[11]

Insights From Genomic Sequencing

The first published genomic sequence of an agent of mucormycosis was *Rhizopus arrhizus* strain 99-880 (subsequently reclassified as *Rhizopus delemar*), which was isolated from a diabetic patient with fatal cerebral mucormycosis.[12] Sequencing revealed the presence of abundant transposable elements accounting for 20% of the genome and evidence of a whole-genome duplication event during the evolution of the fungus.[12] The whole-genome duplication event resulted in duplication of nearly all subunits of the protein complexes involved in respiratory electron transport chains and ubiquitin-proteasome systems, as well as multiple gene families related to cell growth and signal transduction and known fungal virulence factors, including secreted aspartic and subtilase protein families.[13] Sequencing also revealed duplication of the ergosterol biosynthetic pathway (e.g., lanosterol 14α-demethylase), the major target of azoles including voriconazole, isavuconazole, and posaconazole.[14] These genetic features may explain why Mucorales have a uniquely aggressive capacity for rapid growth in patients, even in the face of exposure to antifungal agents and the host immune response.[15] More recent sequencing studies of representative isolates from Mucorales and noninvasive Entomophthorales have identified differences in gene copy numbers that encode spore coat (Cot[H]) proteins, which facilitate invasion of blood vessel endothelial cells.[16] The most commonly isolated Mucorales (*Rhizopus, Mucor,* and *Lichtheimia*) contain three to seven copies of the *cotH* gene, whereas those that occasionally cause disease in humans (*Apophysomyces, Cunninghamella, Saksenaea,* and *Syncephalastrum*) contain only one to two copies. Notably, the *cotH* gene was lacking in Entomophthorales, which are taxonomically similar to Mucorales but do not cause blood vessel invasion.[16]

Acquisition of Infection

The primary mode of acquisition of mucormycosis is inhalation of spores from environmental sources.[17] Trauma, penetrating wounds, burns, and direct injection of sporangiospores can cause infection through a cutaneous or percutaneous route. Gastrointestinal (GI) mucormycosis, although less common, has been reported in both immunocompetent and immunocompromised patients with repeated ingestion of spores during periods of severe malnutrition, ingestion of nonnutritional substances (pica), ingestion of contaminated pharmaceutical products, prepackaged foods, fermented porridges and alcoholic drinks prepared from corn, or eating with contaminated chopsticks.[18,19] More recently, an outbreak of food poisoning was linked to intake of Greek yogurt contaminated with *Mucor circinelloides*.[20] It is unclear whether the fungus itself, which could survive transit through the GI tract and retain virulence, or a secondary metabolite or toxin was the cause of clinical symptoms experienced by the consumers.

Patient Populations at Risk

Agents of mucormycosis are unique among filamentous fungi in their ability to infect a broader, more heterogeneous population of human hosts compared with other opportunistic molds. The epidemiology of mucormycosis is similar in adult and pediatric patients.[21] Although most cases of mucormycosis are community acquired, nosocomial acquisition or pseudooutbreaks[22] have been linked to contaminated bandages and bandage tape,[23–25] needles,[26,27] and tongue depressors used to construct splints for intravenous (IV) and arterial cannulation sites in preterm infants.[28,29] An outbreak of invasive mucormycosis in a pediatric hospital was linked to contaminated bed linens resulting from improper storage, packaging, and transport from an offsite laundry service.[23,30]

The populations most commonly at risk for mucormycosis include patients with poorly controlled diabetes mellitus, prolonged neutropenia, high-dose corticosteroid treatment,[31,32] or immunosuppressive therapy associated with transplantation, and/or elevated levels of free iron, which enhances fungal growth.[33] The importance of iron overload in the pathogenesis of mucormycosis was first noted in patients with end-stage renal disease in the preerythropoietin era, where regular transfusions were required for persistent anemia, resulting in iron overload.[34] Treatment of these patients with deferoxamine to lower their iron levels was linked to the development of disseminated, rapidly fatal mucormycosis.[35,36] It was subsequently discovered that although deferoxamine is an iron chelator for the human host, it also acts as a siderophore in Mucorales, directly delivering iron to the fungus.[34] Less commonly, mucormycosis is a cause of infection in the setting of renal failure, diarrhea, and malnutrition in low-birth-weight infants and rarely in human immunodeficiency patients.[3]

Patients who develop mucormycosis in the absence of underlying disease or immunosuppression at the time of infection frequently have histories of penetrating trauma, burns, surgery, or illicit IV drug use before the infection. For example, a case series of mucormycosis at a nononcology, tertiary care center found that traumatic wounds or surgical sites were the most common infection sites (31%), followed by rhinocerebral (25%) and disseminated (12.5%) infections.[37] Severe

soft tissue infections from blast injuries in combat have also caused mucormycosis.[37,38]

Mucormycosis is infrequently identified as a complication associated with primary immunodeficiency. This complication has occurred in a case of severe congenital neutropenia associated with HAX-1 (HCLS1 [hematopoietic cell-specific Lyn substrate 1]–associated protein X-1) deficiency, or with a newly identified genetic abnormality, such as in a patient with a STAT-1 (signal transducer and activator of transcription-1) gain-of-function mutation who presented with disseminated *Apophysomyces trapeziformis* infection.[39,40]

Incidence of Mucormycosis

Mucormycosis is not a reportable disease. The incidence of mucormycosis is probably underestimated in many epidemiologic series given the inherent challenges associated with antemortem diagnosis and the declining rate of autopsies in high-risk populations. A recent estimate of the disease burden of mucormycosis in US hospitals from 2005–15 reported a prevalence of 0.12 to 0.16 cases per 10,000 discharges, depending on the International Classification of Diseases–9 code definitions used to define the disease.[41] Cases of mucormycosis were associated with an average hospital length of stay of 17 days, with 23% dead at discharge and more than one-third of patients requiring readmission to the hospital. The average cost per hospital stay was $112,419 (2014 US dollars).[41] Surveys from Europe have reported incidence rates of mucormycosis ranging from 0.43 to 1.2 cases per million people.[42,43] In autopsy series of high-risk hematology populations, mucormycosis has accounted for 8% to 13% of all reported invasive fungal infections.[44–46]

Mucormycosis is an important cause of fungal infections in patients with hematologic malignancy, recipients of allogeneic hematopoietic stem cell transplant (HSCT), and, less commonly, patients undergoing solid-organ transplantation (SOT) (Fig. 258.2).[47] Data from the Centers for Disease Control and Prevention (CDC) Transplant-Associated Infection Surveillance Network (TRANSNET) acquired from prospective surveys of 25 US transplantation centers from 2001–06 reported 1-year cumulative incidence rates for mucormycosis of 0.29% in allo-HSCT and 0.07% in SOT,[45,48] accounting for 8% and 2%, respectively, of fungal infections diagnosed in these populations. Of concern, some mucormycosis cases have presented as breakthrough infection on antifungal prophylaxis or treatment effective against *Aspergillus* but not Mucorales (i.e., voriconazole, echinocandins)[49] or because of potentially insufficient absorption of posaconazole.[50,51] Collectively, these data suggest that mucormycosis should be considered in any high-risk hematologic malignancy patient whenever fungal sinusitis develops on *Aspergillus*-active antifungal prophylaxis, especially in patients with prolonged immunosuppression and underlying hyperglycemia associated with prolonged corticosteroid use.

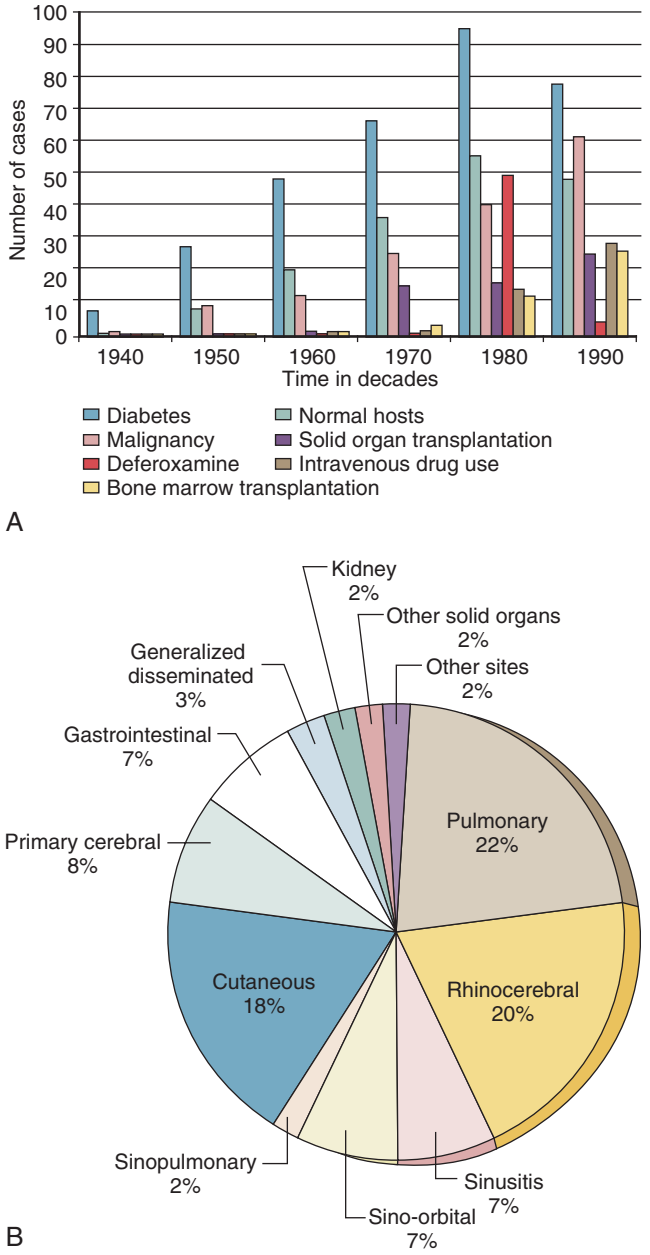

FIG. 258.2 Mucormycosis incidence. Incidence of mucormycosis over 6 decades by host population (A) and site of infection (B). *(Modified from Roden MM, Zaoutis TE, Buchanan WL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases.* Clin Infect Dis. *2005;41: 634–653.)*

PATHOGENESIS (Fig. 258.3)

Most Mucorales sporangiospores are sufficiently small to evade host upper airway defenses and reach the distal alveolar spaces after inhalation.[6] Larger spores (>10 µm) may lodge in the nasal turbinates, predisposing patients to sinusitis.[6] Inhalation of a high spore inoculum, which can occur with excavation, construction, or work in contaminated air ducts, can lead to a slowly progressing pulmonary mucormycosis even in immunocompetent hosts.[52,53] In the case of primary cutaneous mucormycosis, subcutaneous inoculation of spores is the most common event leading to infection in immunocompetent hosts. Cutaneous mucormycosis in immunocompetent hosts usually follows massive soft tissue injury but has been described with even minor trauma, including insect bites[54] and tattoos.[47]

Relatively little is known about initial steps of how Mucorales sporangiospores attach to the respiratory or GI epithelium.[55] *Rhizopus* sporangiospores can adhere to extracellular matrix proteins, such as laminin and type IV collagen,[56] which may be exposed after epithelial damage caused by cytotoxic chemotherapy, infection, diabetes, or trauma. Toxins elicited by Mucorales during germination may contribute to epithelial cell damage.[57] A recent outbreak of food poisoning linked to intake of Greek yogurt contaminated with *M. circinelloides* identified secondary metabolites produced by the contaminating strain that were toxic to the GI mucosa.[20]

Mucorales appear to possess a unique mechanism for adhering to and invading endothelial cells by specific recognition of host receptor–glucose regulator protein 78 (GRP78).[58] Expression of GRP78 on the endothelial cell surface increases as a stress response after exposure to elevated concentrations of β-hydroxy butyrate (BHB), glucose, and iron, similar to those found in patients with diabetic ketoacidosis.[59] Mucorales germlings, but not sporangiospores, bind to GRP78, initiating endothelial cell–mediated fungal endocytosis, resulting in host cellular death.[58] Suppression of GRP78 expression or blocking its function by antibodies suppresses fungal invasion of host endothelial cells by Mucorales, but not *Aspergillus fumigatus* or *Candida albicans*.[58] Endothelial cell uptake is also enhanced through activation of host platelet-derived growth factor pathways (PDGF)[16] because treatment with small-molecule inhibitors of PDGF reduces endothelial cell damage caused by *Rhizopus*.

Proteins from the CotH spore protein coat family are the key ligands that bind GRP78 expressed on the host endothelium.[60] Blocking CotH

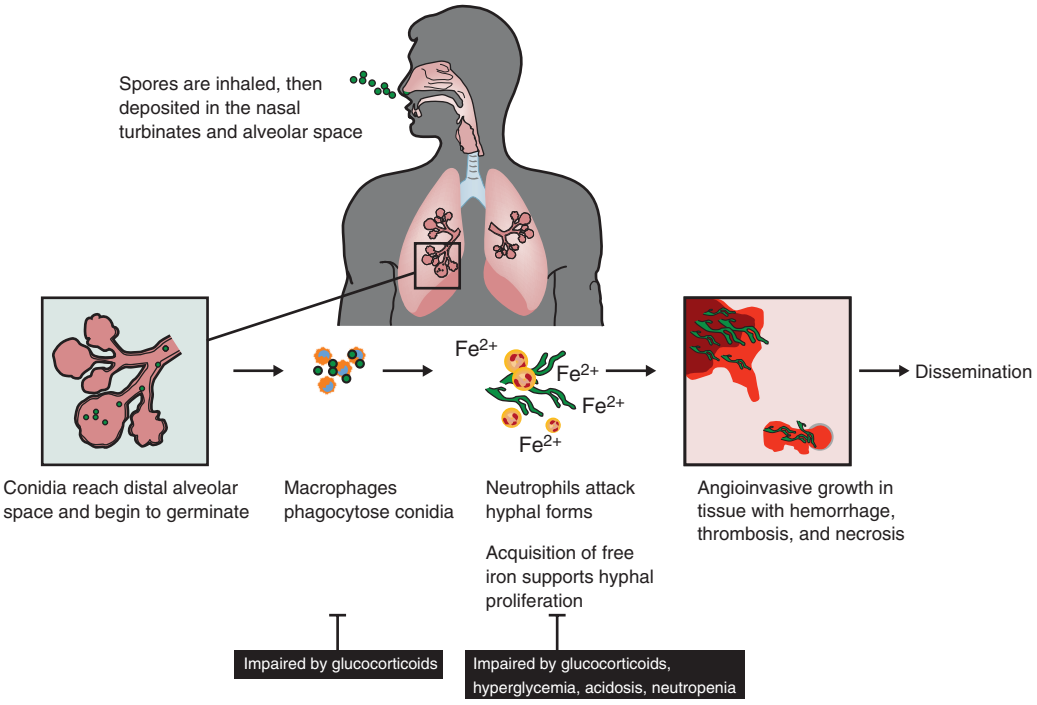

FIG. 258.3 **Pathogenesis of invasive mucormycosis.**

protein function with antibodies reduces the ability of Mucorales to invade and injure endothelial cells in vitro and improves survival in mice.[60] However, it is still unknown whether some hosts may be more susceptible to developing mucormycosis due to single nucleotide polymorphisms in GRP78, which could result in enhanced binding affinity for certain sequences of CotH proteins expressed in specific Mucorales.[61]

Role of Iron Uptake in Mucormycosis Pathogenesis

Anecdotal case reports and experimental data from the 1980s suggested that hemochromatosis is a predisposing risk factor for mucormycosis.[33,62] Fungi can acquire iron from the host by using low-molecular-weight iron chelators (siderophores) or high-affinity iron permeases, such as ferrirhizoferrin.[63] Of the two mechanisms, it is believed that high-affinity iron permeases play the more critical role for adaptive survival of the fungus in the human host. Iron overload in organs such as the liver has also been reported to enhance fungal virulence.[64]

Patients with diabetic ketoacidosis are particularly susceptible to developing rhinocerebral forms of mucormycosis, perhaps because of diminished capacity of transferrin to bind and sequester free iron at a pH less than 7.4.[33,65] The growth of *R. arrhizus* is markedly different in sera collected from patients with diabetic acidosis compared with healthy control subjects. Artis and colleagues[33,65] reported that normal human serum is not capable of supporting the growth of *R. arrhizus* even with the addition of free iron. However, under acidic conditions (pH < 7.4), the addition of exogenous iron markedly enhances the growth of *R. arrhizus* hyphae. Similarly, sera collected from patients with ketoacidosis supports exuberant growth of *R. arrhizus* without exogenous iron, provided the pH was maintained at less than 7.4, suggesting that acidosis disrupts the ability of transferrin to bind and sequester free iron.[33,65]

Historically, patients with severe hemochromatosis or aluminum toxicity received treatment with the metal chelator deferoxamine, which, paradoxically, is associated with increased susceptibility for developing disseminated mucormycosis.[34,66] Subsequent experimental models demonstrated that *R. arrhizus* can use deferoxamine as a xenosiderophore to form the ferrioxamine complex, which will make iron available for use previously unavailable to the fungus.[34,67] Specifically, *Rhizopus* can bind deferoxamine complexes and strip away free iron through a reductive process that allows intracellular uptake of free iron through the enzyme iron permease.[62] Of interest, uptake of radiolabeled iron in the presence of deferoxamine is 8- to 40-fold lower in *Candida* and *Aspergillus* compared with *R. arrhizus,* suggesting this mechanism is a relatively unique pathogenic trait of this fungus. This observation has been confirmed in animal models where administration of deferoxamine worsens survival of guinea pigs infected with *R. arrhizus* but not *Candida albicans.*[67]

Unlike deferoxamine, newer iron chelator agents, such as deferiprone and deferasirox, have not been associated with increased risk for mucormycosis because of their limited capacity to act as xenosiderophores for *Rhizopus* spp. Indeed, both deferiprone and deferasirox have shown protective effects in murine[68] and guinea pig[66] models of mucormycosis, with several case reports suggesting a possible benefit of adjunctive deferasirox therapy in human mucormycosis.[69] However, a small nonrandomized study found no survival benefit of adding deferasirox in combination with liposomal amphotericin B (L-AMB) in patients with hematologic malignancies.[70] Deferasirox may be more effective adjunct therapy for mucormycosis in the setting of diabetic ketoacidosis versus patients with neutropenia. Nevertheless, adjunctive iron chelation therapy cannot be recommended in patients with mucormycosis until more convincing clinical evidence of its benefit is available.[71]

Host Immune Response Against Mucormycosis

Both mononuclear and polymorphonuclear phagocytes, including natural killer cells, prevent germination of Mucorales spores and damage hyphal forms of the fungus.[72–74] After encountering *R. arrhizus* hyphae, human polymorphonuclear neutrophils activate robust proinflammatory gene expression through activation of Toll-like receptor 2 and nuclear factor kappa B pathways.[75] Hyphal damage is elicited by oxidative mechanisms after monocyte or neutrophil attachment to hyphal fragments.[72,76] Platelets may also play a role in host antifungal defenses against Mucorales by adhering to hyphae forms and secreting microbiocidal proteins.[77]

Defects in phagocytic activity associated with decreased cell numbers (i.e., neutropenia) or function (i.e., associated with glucocorticoids, hyperglycemia, or acidosis) allow unimpeded growth of the hyphal form. Glucocorticoids are known to impair the migration, attachment, ingestion, and phagolysosome fusion of bronchoalveolar macrophages essential for clearing spores from the alveoli.[78] Neutrophils collected from patients with severe hyperglycemia and diabetic ketoacidosis, burn-stress pseudodiabetes, or glucocorticoid-treated graft-versus-host disease (GVHD) often have impaired chemotaxis and diminished

oxidative and nonoxidative fungicidal mechanisms against Mucorales spores and hyphae.[79] In the setting of these impaired host immune defenses, rapidly growing Mucorales can outcompete the impaired immune response and rapidly spread through tissues and blood vessels.[55]

Mucorales-specific $CD4^+$ or $CD8^+$ T cells that produce interferon (IFN)-γ, interleukin (IL)-10, and, to a lesser extent, IL-17 can be detected early in infection in patients with hematologic malignancies and decrease with resolution of the infection.[80] As such, detection of such cells may support the diagnosis of mucormycosis. Mucorales have also been reported to activate human dendritic cells through Dectin-1, resulting in the production of IL-23 and induction of proinflammatory Th17 responses.[81] Elevated concentrations of glucose, iron, and BHB encountered in patients with diabetic ketoacidosis impair T-cell proliferation, IFN-γ production, and phagocyte-mediated killing.[59,82–85]

Evidence from animal models suggests that Mucorales can persist in granulomatous clusters, which reactivate during periods of intensive immunosuppression or metabolic dysfunction, such as diabetic ketoacidosis.[86] The possibility of a preexisting latent infection stage raises questions concerning the possibility of screening for latent mucormycosis in patients before undergoing intensive immunosuppressive therapy.

Histopathology

Mucorales have an exceptional capacity to invade blood vessels, resulting in necrosis of vessel walls and mycotic thrombi.[87] Thrombosis of vessels leads to infarction and hematogenous dissemination. Infected tissue typically reveals extensive necrosis with diffuse infiltration of polymorphonuclear leukocytes. However, in areas with ischemic necrosis, inflammation is sometimes minimal despite the presence of numerous hyphae.[88,89] In the otherwise healthy host a pyogenic or pyogranulomatous response without angioinvasion is more common. Reactivation from granulomas, that is, granulomatous inflammation, is possible during periods of subsequent immunosuppression or metabolic dysfunction (e.g., hyperglycemia, ketoacidosis).

CLINICAL MANIFESTATIONS OF MUCORMYCOSIS

The clinical presentation of mucormycosis is broad, depending on the underlying immune status and comorbidities of the host (Table 258.2). The signs and symptoms of mucormycosis are nonspecific, emphasizing the importance of a high index of suspicion in susceptible patient populations. In the immunocompromised host, mucormycosis can present as a fulminant angioinvasive infection that frequently disseminates with fatal consequence. The clinical manifestations of mucormycosis can be generally grouped into one of six syndromes with some overlap: (1) rhinocerebral infections and (2) pulmonary, (3) cutaneous, (4) GI, (5) disseminated, and (6) unusual presentations of mucormycosis.

TABLE 258.2 Patterns of Mucormycosis by Host Population

PREDISPOSING CONDITION	PREDOMINANT SITES OF INFECTION
Diabetes mellitus	Rhinocerebral, sino-orbital, cutaneous
Malignancy (typically hematologic malignancy)	Pulmonary, sinus, cutaneous, sino-orbital, disseminated
Hematopoietic stem cell transplantation	Pulmonary, sinus, rhinocerebral, disseminated
Solid-organ transplantation	Sinus, cutaneous, pulmonary, rhinocerebral, disseminated
Intravenous drug use	Cerebral, endocarditis, cutaneous, disseminated
Malnutrition	Gastrointestinal, disseminated
Deferoxamine therapy	Disseminated, pulmonary, rhinocerebral, cerebral, cutaneous, gastrointestinal
Trauma	Cutaneous, ocular

Rhinocerebral Infections

Rhinosinusitis, rhino-orbital, and rhinocerebral infections are classic manifestations of human mucormycosis. Infection is initially localized to the nasal turbinates and paranasal sinuses after inhalation of spores but can rapidly progress to the orbit (sino-orbital) or brain (rhinocerebral), particularly in patients with diabetic ketoacidosis or profound neutropenia.[42,90] Patterns of progression for the infection demonstrate some host predilection (see Table 258.2). The rhino-orbital form occurs more frequently in patients with poorly controlled diabetes, whereas patients with underlying leukemia or lymphoma are more likely to present with pulmonary infections (Fig. 258.4). Indeed, rhino-orbital mucormycosis is sometimes the first manifestation of undiagnosed diabetes mellitus, especially in patients from developing countries.

Clinical Presentation

Initial symptoms of sinus invasion by mucormycosis are indistinguishable from other more common causes of sinusitis. Sinus pain, congestion, headache, mouth or facial pain, otologic symptoms, and hyposmia or anosmia are common. A concomitant nonproductive cough often reflects lung involvement. Involved tissues become red, then violaceous, and finally black with thrombosis and tissue necrosis. Necrotic eschars of the nasal cavity and turbinates, facial lesions around the nose, and exophytic or necrotic lesions of the hard palate extending from the maxillary sinus are signs of rapidly progressing infection.[91] Careful endoscopic inspection of the nasal cavity and biopsy of suspicious lesions is helpful for early diagnosis. However, the absence of lesions or necrotic eschars does not rule out the possibility of rhinocerebral infection because necrotic nasal or palate lesions may be seen in only 50% of patients within 3 days of the onset of infection.[91]

Extension of sinus disease is primarily into contiguous structures. Maxillary sinus infection extends into the hard palate, nasal cavity, and ethmoid sinus. Sphenoid disease invades the cavernous sinus, contiguous temporal lobe, and internal carotid artery in the siphon. Septic emboli from the carotid artery into the frontal and parietal lobes can occur. Ethmoid sinus disease may invade the face or frontal lobe but easily crosses the lamina papyracea into the orbit.[92] The frontal sinus is an uncommon primary site. Invasion of the orbit is typically unilateral (see Fig. 258.4). Periorbital edema, ptosis, proptosis, chemosis, and preseptal and orbital edema are early signs of orbital extension. Pain and blurring or loss of vision often indicate invasion of the globe or optic nerve. Infraorbital facial numbness follows invasion of the infraorbital nerve within the orbit.[92] Patients with extensive rhino-orbital or rhinocerebral disease may present with trigeminal and ocular motor nerve palsy after cavernous sinus invasion (cranial nerves III, IV, and VI, and the superior two branches of the fifth nerve).[93] A bloody nasal discharge may be an early sign of nasal mucosal invasion.[94,95] Intracranial complications include epidural and subdural abscesses, cavernous, and, less commonly, sagittal sinus thrombosis.[96–98] Frank meningitis in patients with mucormycosis is rare.[17]

Radiology

Radiographic imaging is often suggestive of severe sinusitis but lacks the specificity to diagnose rhinocerebral mucormycosis. Patients with fungal or surgical disruption of the dura mater may present with superimposed bacterial meningitis, or bacterial sinusitis may complicate postoperative management.[99] Computed tomography (CT) of the sinuses typically reveals mucosal thickening, air-fluid levels, and bony erosion (Fig. 258.5).[100] Highly immunosuppressed patients often present with pansinusitis that is highly suggestive of an aggressive fungal infection.[50] Orbital thickening may also be detected on CT scans but can be detected earlier by magnetic resonance imaging (MRI).[101] CT and MRI scans of the orbits may be unremarkable during the initial stages of the infection, highlighting the importance of serial radiographic imaging for monitoring disease progression.[101] The frequency of radiographic imaging is patient dependent but may be required every 2 to 3 days in patients with suspected progression. Therefore rhinoscopy or nasal endoscopy is critical for confirming tissue ischemia and the extent of disease.[99] Extraorbital muscle thickening is often the first sign on CT or MRI of orbital involvement and should prompt empirical antifungal therapy until surgical exploration or biopsy of the sinus and orbits can be

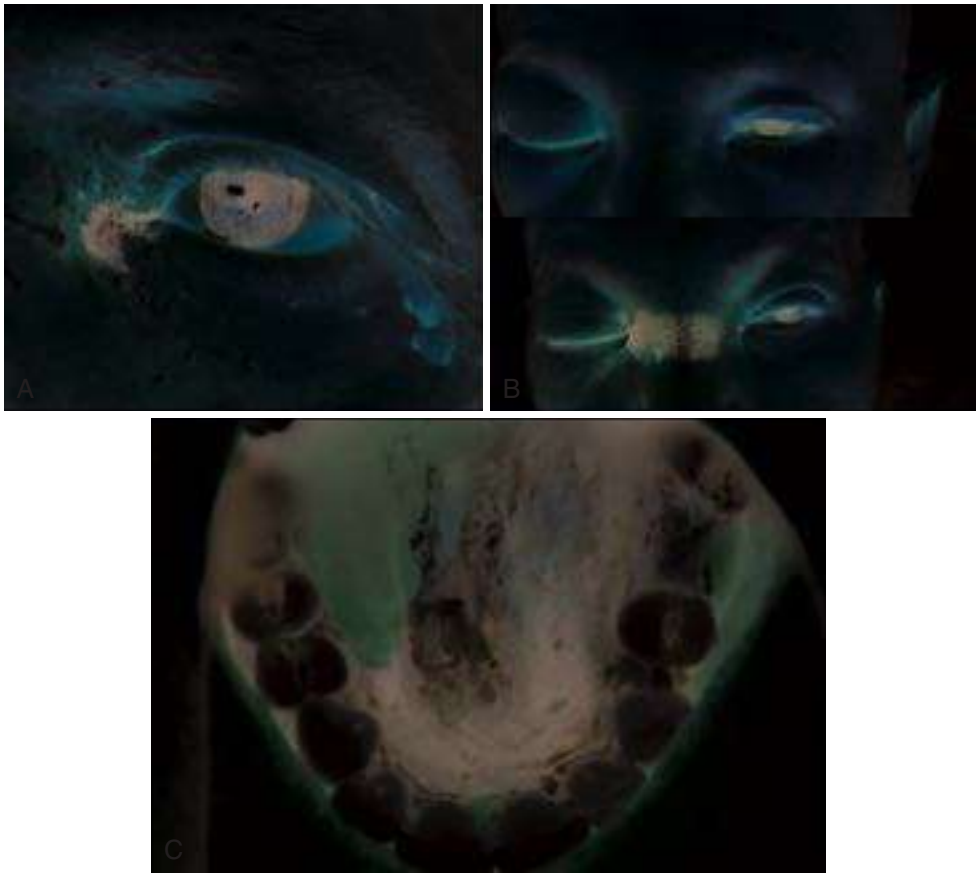

FIG. 258.4 Sino-orbital involvement of mucormycosis. (A) Orbital involvement in cancer patient. Note the periorbital ecchymosis and sanguineous discharge from the eye. (B) Rapid progression of orbital involvement with necrosis of nasal bridge in less than 24 hours. (C) Necrotic eschar on the hard palate of a cancer patient with rhinocerebral mucormycosis. *(Courtesy Drs. Gerald Bodey, George Viola, and Mona Shiekh Sroujieh, University of Texas, MD Anderson Cancer Center, Houston, TX.)*

performed, which should be done as soon as possible.[100] Every effort should be made to establish an early definitive diagnosis of mucormycosis by biopsy and culture of necrotic lesions and rapid histologic assessment of frozen sections.[102] Impression smears from the biopsy or surgical margin may also reveal hyphae consistent with mucormycosis.

Pulmonary Infections

Pulmonary mucormycosis is most commonly encountered in patients with prolonged neutropenia, recipients of hematopoietic stem cell or solid-organ transplantation, and patients receiving deferoxamine therapy.[3,17,103] The infection frequently occurs concomitantly with sinus infection.[17] However, the clinical manifestations of the infection are indistinguishable from more common opportunistic molds, such as invasive pulmonary aspergillosis (IPA). Therefore timely diagnosis is a critical factor in the outcome on the infection because first-line antifungals typically used for aspergillosis, such as voriconazole, lack activity against Mucorales. A case series of 61 leukemia and transplant patients with evidence of fungal pneumonia found that 84% of patients who were eventually documented to have pulmonary mucormycosis were receiving ineffective antifungal therapy at the time of their diagnosis.[104,105] Similarly, an analysis of 70 hematologic malignancy patients with pulmonary mucormycosis demonstrated that a delay in the administration of appropriate antifungal therapy (typically an amphotericin B formulation) of as few as 6 days was associated with a doubling of 4-week (35.1% vs. 66.6%; $P = .006$) and 12-week (48.6% vs. 82.9%; $P = .029$) crude mortality rates.[106] Data to support imaging findings that might heighten the suspicion for pulmonary mucormycosis are reported in a retrospective analysis of neutropenic leukemia patients. In the first 5 days of clinical symptoms, fever or chest pain, a reverse halo sign (focal ground-glass attenuation surrounded by a ring of consolidation) was present in 15 of 16 patients with proven pulmonary mucormycosis.[107,108] However, the reverse halo sign is not specific for mucormycosis but, rather, may be suggestive of invasive aspergillosis, mucormycosis, or organizing pneumonia.[109,109a]

Clinical Presentation

Clinical symptoms of pulmonary mucormycosis are subtle and nonspecific even at late stages in the infection, especially in patients receiving therapies that suppress immune responses (i.e., high-dose glucocorticoid therapy, anti–T-cell antibodies).[106] Patients frequently present with refractory fever on broad-spectrum antibiotics, nonproductive cough, progressive dyspnea, and pleuritic chest pain.[50,106] Pulmonary mucormycosis can traverse tissue planes in the lung, invading through the bronchi, diaphragm, chest wall, and pleura.[103] A pleural friction rub on auscultation is present in some patients. Hyphal invasion of blood vessels results in necrosis of the surrounding parenchyma, ultimately leading to cavitation or potentially fatal hemoptysis.[110–112] In patients with hematologic malignancies, clues for distinguishing pulmonary mucormycosis from IPA may include the presence of severe sinusitis; history of prophylaxis with antifungals that possess activity against aspergillosis but not mucormycosis (i.e., voriconazole or echinocandin prophylaxis); suggestive CT imaging findings, such as reverse halo sign and multiple nodular infiltrates with or without pleural effusion; and possibly the repeated absence of detectable *Aspergillus* galactomannan antigen in the serum or in bronchial alveolar lavage (BAL) fluid.[50,113–115] Unfortunately, it is common in debilitated patients to have a concomitant polymicrobial pneumonia, which can further confound early diagnosis of pulmonary mucormycosis.[103,110]

Radiology

The radiographic presentation of pulmonary mucormycosis is broad, including focal consolidation with nonspecific infiltrates, cavitary lesions, or even diffuse opacities that evolve quickly, depending on the underlying

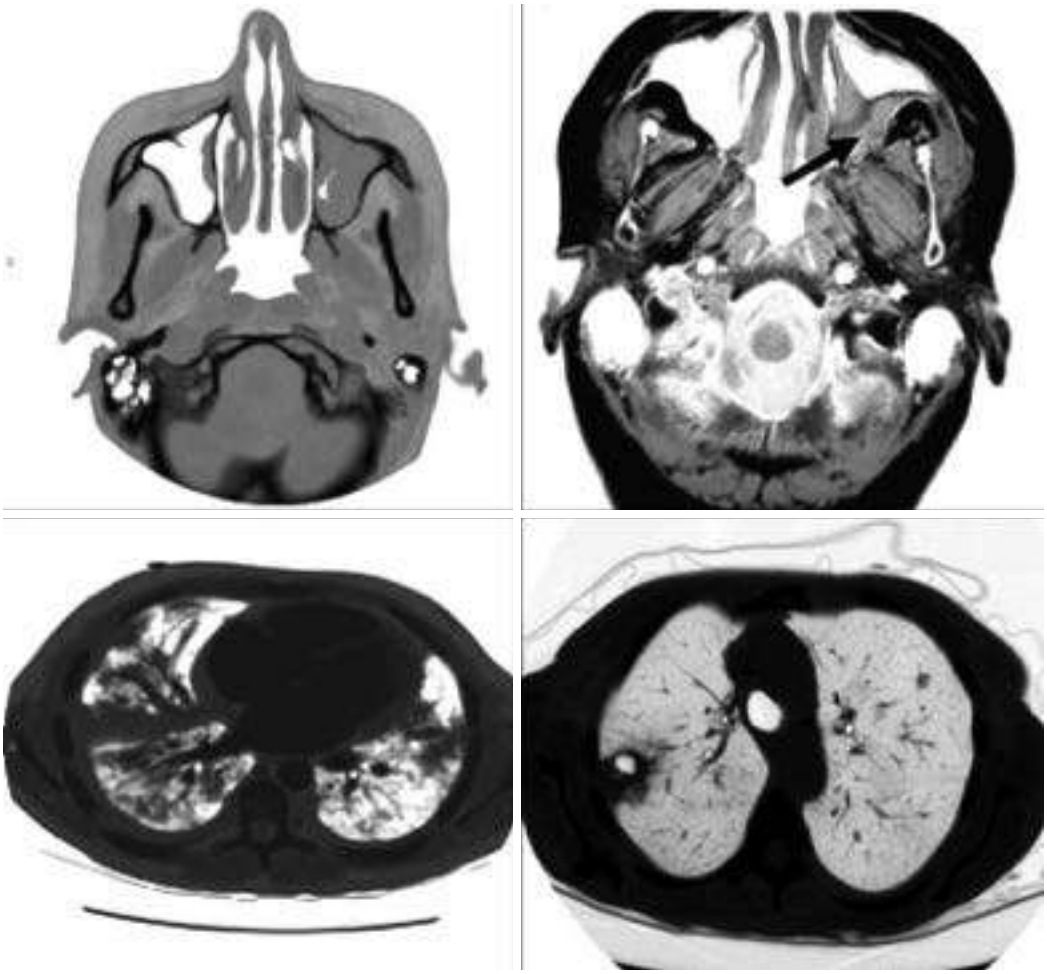

FIG. 258.5 Radiographic findings in sinopulmonary mucormycosis. (A) Left maxillary sinus air-fluid level is evident in this computed tomography (CT) scan that is indistinguishable from bacterial sinusitis. (B) Magnetic resonance image reveals T2 signal hyperintensity in the left pterygoid musculature *(arrow)* in conjunction with a left maxillary sinus air-fluid level. (C) Multiple heterogeneous nodular and consolidative lesions with a large pulmonary vessel infarct *(wedge)* and modest pleural effusions are shown in a cancer patient with pulmonary mucormycosis. (D) Contrast-enhanced CT scan demonstrates a cavity within a dense infiltrate in a patient with acute myelogenous leukemia and pulmonary mucormycosis. *(Courtesy Dr. Edith Marom, University of Texas, MD Anderson Cancer Center, Houston, TX.)*

immune status of the patient (see Fig. 258.5).[103,116] Thrombosis of pulmonary vessels with angioinvasion often leads to large wedge-shaped infarcts.[103] Earlier studies of pulmonary mucormycosis suggested a predilection for upper lobar disease in 55% to 84% of cases.[117,118] However, any part of the lung may be involved, and bilateral disease is common.[103]

Like IPA, high-resolution chest CT is the best method of determining the extent of pulmonary mucormycosis and typically demonstrates evidence of the infection before its appearance on standard chest radiographs (see Fig. 258.5). Although nodular opacities without an air bronchogram indistinguishable from aspergillosis are the most common finding on CT scan, the presence of multiple nodules (≥10), pleural effusion, or both, may favor the diagnosis of pulmonary mucormycosis.[116] Halo and air crescent signs are encountered less frequently in leukemic patients with pulmonary mucormycosis compared with pulmonary aspergillosis.[116] However, centrally located lesions demonstrating the air-crescent sign are often associated with an increased risk for pulmonary artery erosion and massive hemoptysis.[102] Three small case series have suggested that a reverse halo sign, a focal round area of ground-glass attenuation surrounded by a ring consolidation, may be a more common early radiographic finding in patients with invasive pulmonary mucormycosis compared with aspergillosis.[107,108,119,120]

Pulmonary mucormycosis rapidly spreads to the contralateral lung and distal organs if not promptly treated. Although patients with pulmonary mucormycosis usually die from disseminated disease before respiratory failure occurs, dissemination is rarely detected antemortem.[121] The overall mortality rate of pulmonary mucormycosis ranges from 50% to 70% but can increase up to 95% in patients with extrathoracic dissemination.[121]

PULMONARY MUCORMYCOSIS IN LESS SEVERELY IMMUNOCOMPROMISED PATIENTS

In more immunocompetent hosts, pulmonary mucormycosis may present with more atypical, slowly progressing forms.[6] Mycotic pulmonary artery aneurysms and pseudoaneurysms, bronchial obstruction, and even asymptomatic solitary nodules have been described without clear underlying immune dysfunction. Patients with diabetes mellitus have a predilection for developing endobronchial lesions that present with a less fulminant course than pulmonary mucormycosis encountered in the neutropenic or transplant population.[117] On occasion, endobronchial lesions may lead to obstruction of the major airways or erosion of major pulmonary blood vessels and fatal hemoptysis.

Like *Aspergillus* spp., Mucorales in rare instances can form mycetomas in preexisting lung cavities or cause slowly necrotizing pneumonia and hypersensitivity syndromes. *Rhizopus* spp. have also been implicated in an allergic alveolitis described in farm workers and Scandinavian sawmill workers (wood-trimmer's disease).[122,123]

Skin and Soft Tissue Infections

Cutaneous mucormycosis is typically the result of direct spore inoculation or exposure of skin already compromised by burns or extensive trauma or, less commonly, after insect or spider bites.[124,125] Necrotizing soft

tissue infections with cutaneous mucormycosis have been reported in survivors with traumatic injuries after volcanic eruptions, tsunamis, and tornadoes.[9,10] Mucorales-contaminated bandages, needles, and bed linens have also been implicated in outbreaks of soft tissue infections in hospitalized patients.[23–27,30]

Cutaneous mucormycosis typically starts as erythema and induration of the skin at a puncture site and progresses to necrosis with a black eschar (Fig. 258.6). Cutaneous infections can quickly extend into the deep fascia and muscle layers. Necrotizing fasciitis has been reported in patients with progressive cutaneous mucormycosis and is associated with an extremely poor prognosis.[126–131] Neutropenic patients, in particular, are susceptible to lymphatic and blood vessel invasion, infarction, and necrosis with eventual dissemination. In patients with massive soft tissue trauma the mold invades the gray necrotic tissue at the margins of the wound and spreads to contiguous viable tissue, enlarging the wound. Unlike many other molds (*Aspergillus, Fusarium, Scedosporium,* fungi causing phaeohyphomycoses), the skin appears to be a less common site of secondary involvement in disseminated mucormycosis.[17] Atypical dermatologic manifestations have also been reported in less immunosuppressed leukemic patients and solid-organ transplant recipients, mimicking erythema nodosum or panniculitis.[132,133]

Skin biopsy is critical for diagnosis because necrotic skin lesions in neutropenic patients have a broad differential diagnosis.[134] Biopsy specimens taken from the center of the lesion down to the subcutaneous fat are most likely to reveal hyphae invading the blood vessels of the dermis and subcutis. Excision and wide débridement of cutaneous lesions, coupled with systemic antifungal therapy and, according to some, hyperbaric oxygen therapy, can further reduce mortality rates (see Chapter 50).

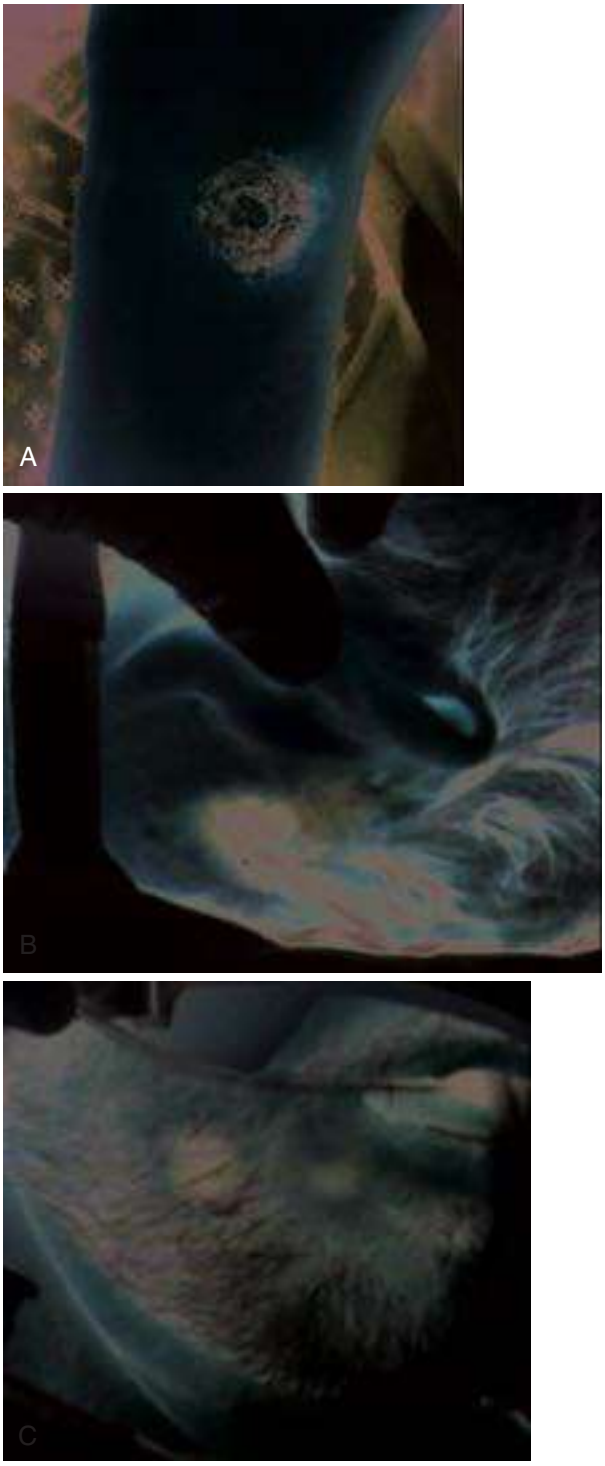

FIG. 258.6 Cutaneous presentation of mucormycosis. (A) Chronic, nonhealing ulcer with necrosis after traumatic inoculation. Cutaneous ecthyma gangrenosum lesions behind the ear (B) and face (C) of a neutropenic patient with disseminated mucormycosis. *(Courtesy Drs. Gerald Bodey and Saud Ahmed, University of Texas, MD Anderson Cancer Center, Houston, TX.)*

Osteomyelitis

Mucorales are an uncommon cause of bone and joint infections that are typically diagnosed late after initial symptom onset. A review of 34 individual cases from 30 publications[135] found that bone and joint infections were most frequently associated with trauma, automobile accidents with fracture, and puncture of the knee or penetrating wounds. Direct inoculation was the main mechanism of infection in 56% of cases, particularly in patients with prior trauma or surgery. Hematogenous dissemination occurred in 24% of cases in patients with hematologic malignancy or immune impairment, whereas another 21% of cases occurred from contiguous spread of the infection. Fever was uncommon, but patients frequently experience local pain and tenderness in the affected joint with occasional cellulitis with elevated inflammatory markers, such as elevated erythrocyte sedimentation rate, white blood cell counts, and possibly elevated C-reactive protein. Diagnosis was most frequently established by open surgical biopsy.[135] Mucorales osteomyelitis requires both antifungal treatment and surgical intervention that may include débridement, bone grafting/fixation procedures, full excision, or amputation if required. The overall mortality rate in a published case series was 24%.[135]

Gastrointestinal Mucormycosis

Primary GI mucormycosis is a rare infection, with protean manifestations occurring primarily in malnourished patients and premature infants, where it can present as necrotizing enterocolitis.[19] The infection often starts with an ulcer, sometimes in the stomach, but can involve any compartment of the GI tract.[136] Patients may present with peritonitis after the fungus has invaded through the gastric mucosa and bowel wall.[137] Liver abscesses have also been described after ingestion of herbal products contaminated with *Mucor indicus*.[138] In neutropenic patients seeding of the GI tract is probably more common than previously thought because only a few of the infections are identified antemortem. Patients may present with subtle findings of fever, enterocolitis, or hematochezia that can progress to colonic ischemia with transmural necrosis and perforation of the gut. Masslike appendiceal or ileal lesions have also been described.[137] Unfortunately, GI mucormycosis is often diagnosed late because of the nonspecific presentation and a high degree of suspicion is required for early diagnosis by endoscopic biopsy.[137]

Disseminated Mucormycosis

Disseminated mucormycosis is rarely apparent before death. The symptoms vary depending on the site of dissemination, degree of vascular invasion, and affected organs. The patient groups classically at risk for this infection have received treatment with deferoxamine for iron overload, are persistently neutropenic patients with active leukemia, or are allogeneic stem cell transplant recipients with GVHD receiving high-dose glucocorticoid therapy or anti–T-cell antibodies.[6,17] Pneumonia is common in patients with disseminated mucormycosis and is assumed

to be the primary source in most patients even when not detectable radiologically.[121] Because of the poor sensitivity of blood and respiratory cultures for diagnosing disseminated mucormycosis, biopsy of the suspected sites is critical for diagnosis of the infection. Patients with hematologic malignancies or diabetic patients with disseminated mucormycosis have presented with acute myocardial infarction or bowel ischemia after arterial occlusion by fungi.[139–141]

Less Common Presentations of Mucormycosis

Although rare, peritonitis has been described in patients undergoing continuous ambulatory peritoneal dialysis.[142–144] The infection tends to have a slowly progressive course, although the attributable mortality rate in patients who received delayed or inappropriate therapy can exceed 60%.[142] In peritoneal dialysis, catheter-related mucormycosis, prompt removal of the catheter, and several weeks of systemic antifungal therapy are essential.

Isolated reports of mucormycosis of the trachea,[145] mediastinum,[146] bone,[147] heart,[148,149] thyroid, and kidney[150] have been described. Other manifestations, such as otitis externa,[151] corneal infection, and superior vena cava syndrome,[152] have also been reported. IV drug abusers are particularly vulnerable to central nervous system manifestations of mucormycosis, often presenting as a brain abscess involving the basal ganglia in conjunction with infective endocarditis.[153–157]

DIAGNOSIS

Because of the ubiquitous nature of the fungus in the environment, positive cultures may occasionally reflect culture contamination rather than true infection. However, discovery of hyphae in a specimen from an immunocompromised host is an important diagnostic clue that should be confirmed whenever possible with histopathologic documentation of fungal invasion.[158] Not surprisingly, the site of infection has a major impact on the likelihood of histopathologic confirmation.[6] The ease of accessibility of the skin or sinuses allows more definite diagnosis of infections at these sites. Tissue swabs and cultures of sputum, sinus secretions, nasal mucosa, and BAL fluid are usually nondiagnostic but may be an important indication of disease in immunocompromised patients.[159] Blood cultures rarely grow Mucorales despite the angioinvasive nature of these pathogens.

In tissue, Mucorales hyphae can often be distinguished from other more common opportunistic molds, such as *Aspergillus* and *Fusarium,* by their broad (uneven diameter, 3–25 μm), empty, thin-walled, mostly aseptate hyphae.[87] Frequently, these hyphae have focal bulbous dilation and nondichotomous irregular branching at occasional right angles. Tissue sections may show a variety of mixed hyphal forms that include folded, twisted, or compressed hyphae that may be mistaken for septae or, when transected, large empty spherules of *Coccidioides immitis.* Reproductive hyphal structures containing spores (sporangia) are rarely observed in deep tissue, even in well-aerated sites of infection. Mistaken histologic identification is relatively common and can lead to inappropriate therapy.

A variety of stains, including hematoxylin and eosin, Grocott-Gomori methenamine silver, and periodic acid–Schiff stains, will reveal characteristic hyphal elements in tissue. Failure of hyphae to stain with Grocott-Gomori methenamine silver is observed in some patients. Perineural invasion is found in 90% of tissues that contain nerves.[93] The inflammatory process can range from neutrophilic, granulomatous, or pyogranulomatous to minimal inflammation with hemorrhage, depending on the degree, chronicity, and type of underlying immune deficit.[87] Fungal hyphae can also be examined directly with a potassium hydroxide preparation of the tissue specimen or BAL fluid. Staining with fluorescent dyes, such as calcofluor white, Blankofluor, or Uvitex, may enhance detection of hyphal elements during ultraviolet microscopic examination and improve the discrimination between septate and aseptate molds in biopsy specimens.[104,105,160]

Species Identification

Identification of Mucorales to the genus and species level requires cultivation of the fungus in culture to examine reproductive fruiting structures of the fungus. Most species grow rapidly on fungal media, such as Sabouraud dextrose agar incubated at 25° to 30°C. The level of development of the rhizoids, the shape of the sporangium, and the location of the sporangiospores are the morphologic features used to identify different genera of Mucorales (see Fig. 258.1). Nucleic acid sequencing of PCR is being used increasingly to identify cultures without typical morphologic features.[161] Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry may also aid identification and is becoming more available in reference laboratories, although reference databases used to aid interpretation are still limited.[162,163] Recent applications of PCR-based identification have suggested that up to 20% of species may be misidentified at the species level by morphology alone. Unfortunately, culture recovery of the agents of mucormycosis from tissue is inherently poor owing to the friability of the nonseptated hyphae, making them more susceptible to damage during tissue manipulation. Recovery from tissue can be improved by mincing (not homogenizing) tissue and using culture techniques that simulate in vivo growth, including incubation at 35° to 37°C in semianaerobic conditions.[164,165]

Differentiating Mucormycosis From Other Molds

The importance of early differentiation of mucormycosis from other mold infections has generated interest in the development of non–culture-dependent or non–histopathology-dependent diagnostic tests, such as detection of specific antigens or nucleic acid by PCR. Unfortunately, molecular techniques are in use only in research laboratories for the diagnosis of mucormycosis but have shown some promise when evaluated retrospectively in histopathologic or culture-confirmed cases.[166,167] Antigen tests for *Aspergillus* (galactomannan) and other fungal species (β-D-glucan) are not useful for mucormycosis. Several studies have attempted to improve early diagnosis through detecting nucleic acid in serum by using PCR assays or in situ hybridization techniques.[161,168–170] To date these techniques have shown the greatest utility as adjunctive diagnostics, for molecular typing in epidemiologic studies, or for confirming the presumptive genus of the pathogen when histopathology is positive, but cultures are negative.[171] In a prospective study of CT-guided percutaneous lung biopsy samples from nonthrombocytopenic patients with suspected fungal pneumonia, Lass-Florl and colleagues[105] demonstrated that the rapidity of diagnosis and differentiation of mucormycosis from aspergillosis could be improved by using a three-step analysis approach for biopsy specimens: (1) calcofluor white staining to rapidly distinguish septate versus aseptate hyphae, (2) *Aspergillus* galactomannan and PCR testing for rapid identification, and (3) PCR testing of DNA in select biopsy specimens in which aseptate hyphae were observed or *Aspergillus* markers were negative. These promising results will require further confirmation in a wider range of patients before PCR becomes a standard-of-care adjunctive diagnostic test for mucormycosis.

Antifungal Susceptibility Testing

Susceptibility testing of Mucorales isolates is not routine in most clinical microbiology laboratories. Although standardized methods applicable for testing Mucorales isolates have been proposed by both the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST), minimal inhibitory concentration (MIC) results obtained using these two methods often differ.[172,173] Moreover, MIC results often increase by at least two dilution steps with incubation beyond 24 hours.[174] Agar-based methods, such as the Etest, have shown relatively good reproducibility and agreement (70%–100%) with broth microdilution methods, depending on the isolate and Mucorales tested.[175] Nevertheless, the performance of many commercially available tests has not been systematically compared with reference methods for Mucorales. Interpretive MIC breakpoints have not been defined for the Mucorales. Therefore the role of MIC testing in the management of mucormycosis remains uncertain.

Amphotericin B is considered the most active drug against Mucorales and is active in vitro against most species within this order with MIC values of 0.03 to 2 μg/mL (Table 258.3).[174] However, susceptibility does vary by species, with higher MICs observed for *Cunninghamella* spp.[176] Epidemiologic cut-off values (ECVs) for amphotericin B have

TABLE 258.3 Summary of in Vitro Antifungal Activity of Different Antifungal Drugs Against Mucorales (Data Are Adapted From Dannaoui)[174]

CLASS	MOLECULE	MIC_{50} (μg/mL)	INTERPRETATION
Allylamines	Terbinafine	0.06 to >16	Variable activity, depending on species
Pyrimidine analogues	Flucytosine	>16	Inactive
Polyenes	Amphotericin B	0.03 to 2	Active
Azole	Fluconazole	>16	Inactive
	Itraconazole	0.25 to 16	Variable activity depending on species
	Voriconazole	2 to >16	Not active
	Posaconazole	0.03 to 2	Active, depending on species
	Isavuconazole	0.25 to 8	Active, depending on species
Echinocandins	Caspofungin	>8	Inactive
	Micafungin	>8	Inactive
	Anidulafungin	>8	Inactive

been proposed using CLSI methods for *Lichtheimia corymbifera*, *M. circinelloides*, and *Rhizopus microsporus* (2 μg/mL) and for *R. arrhizus* (4 μg/mL).[176] The frequency of non–wild-type isolates based on these ECVs ranges from 0%–2.15% depending on the species. One small case series reported that Mucorales infections associated with an amphotericin B MIC <0.5 μg/mL was significantly associated with better 6-week outcomes.[177]

Posaconazole and, perhaps, isavuconazole are considered to have clinically useful anti-Mucorales activity (see Table 258.3). The MIC_{50} of posaconazole ranges from 0.25 to 2 μg/mL for *Rhizopus* spp., 0.5 to 2 μg/mL for *Mucor* spp., and 0.125 to 1 μg/mL for *Lichtheimia* spp.[172,173,178] When evaluated in neutropenic murine model of invasive pulmonary mucormycosis, the posaconazole serum area under the concentration-time curve (AUC)/MIC ratio was most predictive of antifungal effect, with the greatest reduction in lung fungal burden measured at an AUC/MIC greater than 100.[179] For *R. arrhizus* (MIC, 2 μg/mL), an AUC/MIC target >100 was achieved when serum trough posaconazole exposures in the animals exceeded 4 μg/mL.[179]

Isavuconazole susceptibility is species dependent, with MIC_{50s} ranging from 0.25 to 1 μg/mL for *Lichtheimia* spp. and *Rhizomucor* spp., 1 μg/mL for *Rhizopus* spp., but significantly higher for *M.* circinelloides (8 μg/mL).[172] Although MICs in general are one to three dilutions higher for isavuconazole versus posaconazole, slightly higher serum drug exposures with isavuconazole might possibly compensate for these modest differences in potency.[172]

Itraconazole exhibits variable species-specific antifungal activity in Mucorales, similar to isavuconazole, but itraconazole's unpredictable absorption does not make it a reliable triazole for treatment of invasive mucormycosis. Fluconazole lacks activity against Mucorales (see Table 258.3), and voriconazole lacks sufficient activity in vitro and in vivo to treat mucormycosis at clinically achievable concentrations.[172,173,178] The lack of sufficient voriconazole activity in vivo is also reflected by frequent cases of breakthrough mucormycosis in patients on voriconazole prophylaxis or therapy.[51,115,121]

Although 1,3-β-D-glucan synthase target is present in Mucorales, 1000-fold higher echinocandin concentrations are required to inhibit the enzyme complex in *R. arrhizus* compared with *Candida albicans* or *Aspergillus fumigatus*.[180] In consequence, echinocandins are not considered effective agents in vivo and have demonstrated only limited activity in animal models as monotherapy at lower but not higher doses.[180–182]

Combination Therapy

In vitro and preclinical in vivo studies of antifungal combination therapy (lipid amphotericin B formulations, triazoles, echinocandins, flucytosine, and terbinafine, as well as non-antifungal agents) have revealed mostly indifferent interactions with occasional synergy but no reports of antagonism for combinations of triazoles, amphotericin B, echinocandins, terbinafine, and, on occasion, non-antifungal agents.[174,183] A combination of lipid amphotericin B plus an echinocandin demonstrated improved survival in *R. arrhizus* rodent infection models compared with lipid amphotericin B alone.[181,184] Combinations of posaconazole or isavuconazole with either echinocandins or lipid amphotericin B formulations generally display indifferent results with respect to either triazole or lipid amphotericin B monotherapy.[174,185,186]

Deferasirox, the iron chelator with antifungal effects against Mucorales, has been evaluated in combination with L-AMB[68] and in triple combination with micafungin.[184] Both of these combination regimens, which were administered in diabetic mice, were more effective at prolonging survival compared with L-AMB alone. Modest synergistic effects were also noted in a neutropenic murine models of *R. arrhizus* invasive pulmonary mucormycosis with a combination of posaconazole and deferasirox.[179] However, combination regimens (posaconazole plus L-AMB, posaconazole plus an echinocandin, or posaconazole plus deferasirox) were only as effective as maximally dosed posaconazole.[179] Nevertheless, when deferasirox combined with L-AMB was evaluated in a double-blind multicenter study of neutropenic patients with mucormycosis, no survival benefit was found in the combination therapy group.[70]

TREATMENT

Successful treatment of mucormycosis relies on timely diagnosis, reversal of underlying predisposing factors, early surgical débridement of infected tissue, and rapid initiation of effective high-dose systemic antifungal therapy.[187] Early diagnosis is critical to the outcome of mucormycosis because small focal lesions can be surgically resected before the lesions progress to involve critical structures or distal organs.[50,121] Patients often have an indolent clinical presentation until extensive invasion or dissemination of the infection. Delays in the administration of systemic antifungal therapy increase the probability of patient death due to disseminated infection.[106]

Patients with suspected rhinocerebral mucormycosis should undergo a thorough examination and "staging" of their disease, including CT of the paranasal sinuses and lungs as well as endoscopic examination of nasal turbinates with biopsy of any suspicious lesions or necrotic eschars. Patient outcome can be improved if initial treatment decisions are based on frozen tissue samples from biopsy rather than waiting for tissues to be fixed and stained for histology.[188] Rapid correction of predisposing conditions, such as control of hyperglycemia, reversal of ketoacidosis, and rapid tapering of glucocorticoid therapy, are critical for reversing conditions that favor fungal virulence and dissemination.[59] In rare cases, correction of diabetic ketoacidosis was sufficient to allow recovery from cavitary pulmonary mucormycosis without antifungal treatment.[6] In neutropenic patients granulocyte transfusions may be beneficial as a temporary approach until granulocyte recovery.[150]

Antifungal Therapy

No prospective randomized studies of the primary treatment of mucormycosis have been performed, owing to the rarity and heterogeneous nature of this mycosis. Most evidence concerning the activity of existing antifungals has come from retrospective small case series, case registries, prospective open-label studies, and animal models of infection. Because both surgical and medical interventions are simultaneously or sequentially performed, it is difficult to ascertain the relative efficacy of drug therapy alone.[17] Historically, the drug of choice for the treatment of mucormycosis was conventional amphotericin B deoxycholate (ABD), administered

at the maximal tolerated doses of 1 to 1.5 mg/kg/day.[189] Unfortunately, high doses of conventional amphotericin B are usually not tolerated for more than several days before renal function deteriorates, especially in patients with diabetes or receiving concomitant nephrotoxic therapies.

Lipid Amphotericin B Formulations

Lipid formulations of amphotericin B are safer than ABD for long-term administration and, in our opinion, are the preferred first-line treatment for severe mucormycosis.[187,190] Most experience in the treatment of mucormycosis has been with either liposomal amphotericin B (L-AMB) or amphotericin B lipid complex (ABLC). Although no comparative studies have been performed, outcomes with the use of the two lipid amphotericin B formulations are similar to those historically reported for conventional ABD, albeit with lower rates of nephrotoxicity.[189]

In one of the few published case series, 24 patients with mucormycosis and diabetes as the predominant underlying risk factors were treated with ABLC after failure or intolerance of the conventional ABD formulation.[190,191] The overall response rate (improvement or cure of infection) was 71% (17/24 patients), with few reported toxic effects, even in patients with preexisting renal dysfunction. Several case series have reported the successful treatment of mucormycosis with the liposomal formulation of amphotericin B, sometimes administered at high doses (i.e., 10 mg/kg/day) for prolonged treatment courses.[192,193]

The optimal dosing approach for L-AMB in patients with mucormycosis is frequently debated. Preclinical pharmacokinetics/pharmacodynamics studies in murine models of pulmonary mucormycosis that simulated human dosing have suggested that ABLC at 5 mg/kg/day or L-AMB at 10 mg/kg/day results in rapid antifungal accumulation in the lung, reduced fungal burden, and improved survival.[194] However, these animal models focus on short treatment periods and do not routinely assess nephrotoxicity of higher-dose L-AMB regimens. Both clinical and observational studies have suggested an early "window" of 10 to 14 days when nephrotoxicity risk is lowest with L-AMB, which may be shortened in patients receiving concomitant nephrotoxic agents or aggressive diuresis.[195–197]

In one of the few randomized trials that compared standard doses of L-AMB (3 mg/kg/day) to a higher dose-regimen (10 mg/kg/day for 14 days, then 3 mg/kg/day) for invasive aspergillosis, patients randomized to the higher-dosed L-AMB regimen failed to achieve higher response rates but experienced significantly higher rates of nephrotoxicity and severe hypokalemia.[198]

The feasibility of high-dose (10 mg/kg/day) L-AMB treatment for the initial treatment of mucormycosis was explored in a multicentric prospective French study of 40 patients with invasive mucormycosis.[199] The planned treatment of 10 mg/kg/day was administered as an infusion of at least 2 hours for 4 weeks. In patients in whom there was doubling of serum creatinine levels, compared with baseline, it was recommended that the L-AMB dose be reduced to 7.5 mg/kg/day. If creatinine values did not improve within 3 days, L-AMB dosing was further reduced to 5 mg/kg/day. Among the 33 patients who could be analyzed at 4 weeks after starting therapy or end of therapy, a favorable response was documented in 12 of 33 (36%) at week 4 and 14 of 31 (45%) by week 12. Most patients (24/31, 71%) underwent at least one surgical procedure. Of importance, although 4 weeks of high-dose L-AMB was planned, the average treatment duration at this dose was 13.5 days (range, 0–28), with 40% of patients experiencing a doubling in the baseline serum creatinine and severe hypokalemia (serum potassium <3 mmol/L). Therefore considerations for using doses of L-AMB higher than 5 mg/kg/day should take into account that a sizable proportion of patients will develop renal injury requiring dosage reduction or possibly a switch to triazole therapy after the first 1 to 2 weeks of therapy. Daily infusions of a liter of saline are used in some centers to reduce L-AMB nephrotoxicity in adults who can tolerate an increased sodium load.

Triazoles

The prospects for using posaconazole in the treatment of mucormycosis has improved with the introduction of an extended-release tablet formulation with improved bioavailability compared with the older suspension, and an IV formulation solubilized in sulfobutyl ether β-cyclodextrin. These newer formulations are dosed differently than the oral suspension, which has to be administered two to four times daily in smaller doses to maximize absorption. The extended-release tablet and IV formulation of posaconazole are the preferred formulations of posaconazole for most patients due to their more reliable pharmacokinetics properties.[200]

Most of the published clinical experience concerning mucormycosis treatment with posaconazole has used the older suspension formulation administered at 800 mg/day in divided doses. In an open-label study evaluating posaconazole as salvage therapy (not initial therapy), the overall success rate of posaconazole (800 mg/day) was 70% in 24 patients, and it was well tolerated with only minimal GI side effects.[201,202] Similarly, a retrospective survey of posaconazole-based salvage therapy in 91 patients with refractory mucormycosis indicated an overall success rate of 61%, including 65% in patients with pulmonary mucormycosis.[203] Among patients who were not categorized as treatment successes, 21% had evidence of stable disease after 12 weeks of treatment. At present the US Food and Drug Administration (FDA) has not approved posaconazole for primary or salvage therapy of mucormycosis, indicating the need for further studies.

The extended-release tablet formulation of posaconazole is administered at a dose of 300 mg (three 100-mg delayed-release tablets) twice a day on the first day, then 300 mg daily. Although the absorption of the tablets is improved with food, the tablets have adequate bioavailability even in patients with poor dietary intake or receiving acid-suppression therapy, such as a proton pump inhibitor.[204] The IV formulation of posaconazole is dosed at 300 mg IV twice daily on day 1, followed by 300 mg daily thereafter, and rapidly achieves plasma concentration exposures of greater than 500 ng/mL in 95% of patients, which often took more than a week in patients receiving posaconazole suspension.[205] Although therapeutic drug monitoring has been recommended for posaconazole in *Aspergillus* treatment guidelines to ensure serum trough levels greater than 1.5 mg/L during the treatment of infection,[206,207] a similar relationship between serum trough levels of posaconazole and outcome of mucormycosis has not been reported.

In 2015 the FDA approved isavuconazole for treatment of invasive mucormycosis based on an open observational report by the manufacturer[208] of 21 patients who received isavuconazole as primary therapy for proven or probable disease; 7 had the drug discontinued, 2 were still on therapy when reported, 6 died during isavuconazole treatment, and 6 were alive when isavuconazole treatment was considered completed. Results with salvage therapy in 16 patients were also considered favorable. The small numbers and diverse results make comparisons with amphotericin B and posaconazole difficult.[209] Isavuconazole may be a possible alternative to posaconazole or L-AMB, particularly for longer-term therapy. An advantage of isavuconazole is that it is administered as a prodrug formulation (isavuconazonium) in either an IV and oral formulation with excellent bioavailability that is not affected by dietary intake or acid-suppression therapy. Isavuconazole is dosed with a 372-mg loading dose administered every 8 hours for 6 doses (48 hours), then a 372-mg maintenance dose daily. Isavuconazole is generally well tolerated, making it a possible alternative for patients who are clinically stable and have limiting drug interactions with posaconazole or cannot tolerate the nephrotoxic effects of L-AMB. However, some species, such as *M. circinelloides,* exhibit MICs greater than achievable serum levels of patients with currently recommended isavuconazole dosing.[172] We have also observed cases of breakthrough mucormycosis during isavuconazole treatment in patients with prolonged neutropenia and relapsed hematologic malignancies.

Combination Therapy

Successful treatment of mucormycosis with combinations of amphotericin B, terbinafine, rifampicin, L-AMB, posaconazole, and echinocandins has also been described in small case series and case reports.[183,210] Interpretation of these studies is confounded by small numbers, retrospective nature, and confounding factors such as neutrophil recovery and surgery.[210,211] One of the largest case-control studies evaluating the possible benefits of combination therapy for invasive mucormycosis was performed at MD Anderson Cancer Center,[212] which identified 47 patients who received early L-AMB monotherapy and 59 patients who received combination treatment. The most common combination regimens were L-AMB plus an echinocandin (46%), L-AMB plus

posaconazole (27%), or triple combination therapy (27%). A propensity score analysis for combination therapy was used to attempt to adjust for confounding variables associated with prescription of combination therapy. The investigators could not identify any survival benefit for combination therapy over timely administration of L-AMB, even after propensity score adjustment (odds ratio, 0.8; 95% confidence interval, 0.3 to 2.4; $P = .69$) or if patients were stratified into groups for low versus high risk for mortality.[212]

Treatment Duration

The duration of treatment required for mucormycosis is highly individualized to the patient. Near normalization of radiographic imaging, negative biopsy specimens, and cultures from the affected site and recovery from immunosuppression are indicators that a patient is a candidate for stopping antifungal therapy. Late relapses of mucormycosis after successful treatment have been reported several years after discontinuation of secondary posaconazole prophylaxis or onset of new immunosuppression.[213] In one case relapsing disease was accompanied by increased ^{18}F-fluorodeoxyglucose uptake in lesions detected by positron emission tomography–CT.[213] Therefore continued follow-up of patients is critical in any patient who discontinues treatment for mucormycosis.

Prophylaxis

Because mucormycosis is a relatively rare infection, primary prophylaxis is generally not recommended. Secondary prophylaxis is often desired in patients requiring further immunosuppression after treatment for mucormycosis. Posaconazole, and possibly isavuconazole, appears to be a safe option for patients who require continuous, oral long-term antifungal therapy because they remain at high risk for relapsing infection.[213]

Surgical Management

In rhinosinusitis surgical débridement of infected tissue is a crucial component of therapy and should be urgently performed to limit the aggressive spread of infection to contiguous structures. Repeated removal of necrotic tissue or radical surgical resection (e.g., exenteration of the orbit) with subsequent reconstructive surgeries may be required for lifesaving control of rapidly evolving infection.[118] However, rhinosinusitis has been treated successfully in select patients without radical resection, particularly when multimodality treatment options are used with careful follow-up.[214] Extension to the brain usually portends a fatal outcome. Decisions regarding the extent of surgical débridement are highly individualized to the patient. Intraoperative frozen sections and MRI can help determine the extent of involved tissue and tissue margins. Conditions such as low platelet counts and other bleeding problems must be corrected with sufficient transfusions before surgical intervention. Unfortunately, bleeding risks may limit surgical options in some patients with profound thrombocytopenia.

In patients with pulmonary mucormycosis, surgical treatment in conjunction with systemic antifungal therapy has been shown to significantly improve survival compared with antifungal therapy alone.[118,215] One large case series reported a mortality rate of 55% in patients who received systemic antifungal therapy alone compared with 27% in patients who received antifungal therapy plus surgical intervention.[110] Although the reported outcomes may reflect a selection bias in offering surgery to less severely ill patients with unifocal disease, removal of infected or devitalized tissue early, when the infection is localized, provides the greatest benefit. Cavitation of lesions near the hilum has been followed by fatal hemoptysis, providing an incentive for resection in selected patients. Repeated surgeries are also necessary in some cases. The benefit of pulmonary resection of a dominant lung lesion (debulking) is unknown in patients with multifocal or disseminated mucormycosis.

Adjunctive Therapies

Patients with profound neutropenia and progressive mucormycosis despite optimal therapy may be candidates for neutrophil transfusion. With currently available techniques, more than 10^{10} granulocytes can be infused, resulting in an immediate postinfusion absolute neutrophil count usually exceeding 1000/μL. Repeated infusions are needed every few days and can lead to diminishing response in the patient's neutrophil count due to allosensitization. Success of this bridging procedure in treating mucormycosis is still uncertain but likely depends ultimately on the patient's marrow recovery.

Several other adjunctive measures have been explored for improving tissue viability, impeding fungal proliferation, and improving host immunity. Hyperbaric oxygen therapy was reported to be a beneficial adjunct to standard surgical and antifungal therapy for mucormycosis, particularly for diabetic patients with rhinocerebral disease.[216–218] Although this is not one of the approved uses of hyperbaric oxygen (see Chapter 50), the increased oxygen pressure achieved with hyperbaric oxygen may improve neutrophil activity and the putative oxidative killing effects of polyene antifungals.[219] High oxygen concentrations have also been reported to inhibit growth of Mucorales in vitro and to improve the rate of wound healing by increasing the release of tissue growth factors.[220] Lack of convincing clinical evidence supporting the benefit of hyperbaric oxygen therapy, however, limits its recommendation for routine clinical use for this expensive, controversial, and logistically difficult intervention for mucormycosis.[71]

Multiple immune-augmentation strategies have been proposed for mucormycosis, including administration of cytokines that enhance phagocytic activity, such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, or IFN-γ alone or in combination with granulocyte transfusions. Various combinations of these approaches have been reported with some favorable outcomes in case reports.[219,221] However, many of these immune-augmentation strategies carry some risk for enhancing inflammatory lung injury[222,223]; therefore the relative benefits of such adjunctive strategies must be balanced against the risk for increased harm to the patient.

Prognosis

The site of infection and underlying host factors are the key prognostic determinants of mucormycosis outcome. Active hematologic malignancy, allogeneic HSCT, and disseminated infection are associated with poor outcome.[166] In our experience, most patients with hematologic cancer who develop mucormycosis die within 12 weeks of diagnosis.[121,212,224] However, earlier diagnosis of the disease and aggressive treatment have been associated with improved survival rates in recent series.[166,212] Correction of underlying immune impairment (e.g., rapid tapering of glucocorticoids), combined with aggressive multimodality treatment approaches, offer the best chance for patient survival.

ENTOMOPHTHORAMYCOSIS

Infections caused by fungi in the subphylum Entomophthoramycotina, called *entomophthoramycosis,* include both conidiobolomycosis and basidiobolomycosis. These are rare infections of the paranasal sinus and subcutaneous tissues, principally encountered in the tropics, that rarely affect other tissues.[225] In contrast to mucormycosis, entomophthoramycosis is usually a chronic, nonangioinvasive infection in relatively immunocompetent individuals.[226,227] Although rare, case clusters of invasive disease have been reported in both immunocompromised and immunocompetent patients.[228,229] Conidiobolomycosis affects primarily the head and face, whereas basidiobolomycosis is often localized to the subcutaneous tissues of the trunk and arms or the GI tract.[226,227] The infections are characterized by slow-growing, tumor-like masses in infected tissues that can remain indolent for years. Both fungi are common inhabitants of the soil throughout the world, including the United States. However, most cases of conidiobolomycosis are found in tropical Africa, South America, Central America, and Asia.[226] Similarly, reports of cutaneous basidiobolomycosis are primarily concentrated in tropical areas of Africa and Southeast Asia and in the tropical and subtropical regions of Asia, Australia, and South America.[227] Entomophthoramycosis also demonstrates some age specificity: conidiobolomycosis is uncommon in children, but 88% of basidiobolomycosis cases occur in patients younger than 20 years.[230]

Conidiobolomycosis

Subcutaneous rhinofacial conidiobolomycosis is the most common manifestation of infection caused by *Conidiobolus coronatus.* Symptoms typically begin with nasal discharge, epistaxis, unilateral nasal obstruction,

sinus tenderness, and extensive and persistent facial swelling that may result in disfiguration. The infection slowly progresses with granulomatous inflammation in the subcutaneous tissue without bone involvement or ulceration of the skin.[231] Systemic symptoms are rare, but disseminated conidiobolomycosis has been observed.[226] Infections with *Conidiobolus incongruus* are extremely rare but often more aggressive.[159]

Basidiobolomycosis

Infections caused by *Basidiobolus ranarum* often begin as a nodular subcutaneous lesion on the trunk, arms, or buttocks. The mode of transmission for *B. ranarum* is assumed to be through minor trauma and insect bites. Fungal spores are found in the bristles of mites and are probably carried by other insects. *B. ranarum* has been theorized to be present on "toilet leaves" used for skin cleaning after a bowel movement, resulting in direct inoculation of the perineum.[232] The predominance of lesions in the buttocks, thighs, and perineum would appear to support this theory. The subcutaneous lesions elicited by *B. ranarum* are typically firm but not painful, with edema around the involved sites. Deeper invasion of muscle underlying the subcutaneous tissue has been reported.[227] Several cases have been reported of otherwise healthy persons with GI tract basidiobolomycosis, most often in the colon,[233] that may present as obstruction or mimic the presentation of Crohn disease.[232,234,235] Clinical features of GI basidiobolomycosis include abdominal pain, nausea, vomiting, diarrhea, or abdominal mass.[235] Peripheral blood eosinophilia and high serum immunoglobulin E concentrations are usual. Most cases are slowly progressive locally and may be mistaken for Crohn disease or form large abdominal masses mimicking malignancy. Chronic fibrotic-appearing lesions, including angioinvasive disease reminiscent of mucormycosis seen in diabetic and immunocompromised patients, have also been described.[229]

Diagnosis

In areas where entomophthoramycosis is common the diagnosis is often suspected from the clinical appearance of the patient and characteristic lesions or swelling.[232,235] Definitive diagnosis requires biopsy of the involved site, with characteristic findings of broad, sparsely septated hyphae surrounded by eosinophilic granular material (Splendore-Hoeppli phenomena).[232,236,237] Tissue eosinophilia and granulomatous inflammation are usual. Peripheral eosinophilia may also be present, but cultures from the infected site are often negative. PCR assay of DNA extracted from tissue or from the isolated fungus, followed by sequencing, may be the most useful confirmatory diagnostic test.[13,233]

Differential Diagnosis

Eumycetomas have some clinical similarities to entomophthoramycosis, presenting as a chronic granulomatous fungal disease that affects the limbs, most commonly the feet (mycetoma pedis or Madura foot), and on occasion the abdomen, chest, and head, typically with draining sinuses. Eumycetomas can be differentiated by the presence of grains surrounded by neutrophils on biopsy and by culture. Pythiosis is usually a disease of horses, cattle, dogs, and cats but is a rare cause of subcutaneous disease in humans that can resemble entomophthoramycosis or can cause GI or disseminated infection resembling mucormycosis. Although the histologic appearance of the hyphae is similar, presence of vascular invasion and the appearance of motile zoospores on water culture of the causative organism, *Pythium insidiosum,* can distinguish the entities.[13]

Therapy and Prevention

Conidiobolus spp. are generally more resistant to systemic antifungals than *Basidiobolus* spp.[238] In the older literature agents used to treat entomophthoramycosis included potassium iodide, trimethoprim-sulfamethoxazole, ketoconazole, itraconazole, and amphotericin B with varying success and clinical outcome.[226,227,239–241] At present the preferred drugs for rhinofacial conidiobolomycosis appear to be a combination of a saturated solution of potassium iodide and itraconazole.[241] Surgical removal and reconstructive surgery for grossly swollen or disfigured tissues, combined with medical therapy, often provides the best chance for complete recovery. Subcutaneous basidiobolomycosis has been reported to respond to itraconazole.[242] Response of GI basidiobolomycosis to mold-active azoles has generally been favorable in small numbers of cases, including itraconazole, posaconazole, or voriconazole.[241,243–245]

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The complete reference list is available online at Expert Consult.

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259 *Sporothrix schenckii*

John H. Rex and Pablo C. Okhuysen

SHORT VIEW SUMMARY

Microbiology and Epidemiology

- *Sporothrix schenckii* sensu lato, a group of closely related species, are dimorphic fungi that cause sporotrichosis with budding yeast in tissue and a mold in culture. Fungal cells in tissue may be oval, spherical, or elongate (cigar-shaped), with variable size in the same lesion.
- Worldwide distribution, especially in tropical and subtropical regions.
- Acquisition is associated with exposure to soil, plants, plant products (hay, straw, sphagnum moss), and a variety of animals (especially cats).
- Grows slowly on fungal culture from involved tissues.

Diagnosis

- Lymphocutaneous disease: presentation with an indolent papulonodular lesion, sometimes ulcerating, should suggest the diagnosis. Secondary lesions along lymphangitic channels are characteristic. Biopsy will show pseudoepitheliomatous hyperplasia with pyogranulomas and microabscesses in the dermis. Yeastlike cells can be seen with Gomori methenamine silver stain in one-third of cases but are not seen with hematoxylin and eosin stain. Culture will be positive.
- The most common extracutaneous form is osteoarticular. Pulmonary disease (usually cavitary) and meningeal disease are also well described. All are diagnosed by culture. Organisms can be seen in tissue with Gomori methenamine silver stain but may not be sufficiently distinctive to permit diagnosis of sporotrichosis.
- Multifocal dissemination may also occur, in immunocompromised subjects: cultures of skin lesions and joints are usually positive, whereas blood, spinal fluid, and bone marrow cultures are only occasionally positive.

Therapy

- Itraconazole at 100 to 200 mg/day (or 65 mg to 130 mg/day if using the enhanced bioavailability SUBA-itraconazole formulation; see Chapter 40B) orally is the treatment of choice for lymphocutaneous disease, with 3 to 6 months of therapy typically needed for complete resolution. Terbinafine 250 mg daily has also been successful in some cases of cutaneous disease.
- Therapy with a saturated solution of potassium iodide is also effective for lymphocutaneous disease, although it is associated with many side effects.
- Itraconazole is also active in extracutaneous disease, but extended therapy may be required. In difficult settings, amphotericin B is also employed. Success has also been reported in a few cases with terbinafine. Improvement of immune status (e.g., introduction of antiretroviral therapy in subjects with human immunodeficiency virus coinfection, reduction of immunosuppressive agents) is useful.

MYCOLOGY

Sporothrix schenckii sensu lato (meaning in the broadest sense) is a dimorphic fungus that exists in a hyphal form in vitro at temperatures less than 37°C. Colonies are initially white but gradually become brown to black due to the production of pigmented conidia. In vivo or at 37°C on rich media such as brain heart infusion agar, the organism converts to an oval- or cigar-shaped budding yeast. Along with the characteristic morphology of the sporulating mold, identification is based on demonstration of this conversion to a yeast form. DNA sequencing has shown that *S. schenckii* sensu stricto (meaning in a narrow sense) is a cluster of several closely related species.[1–5] Identification to the species level is currently done by partial sequencing of the calmodulin gene. Identification of species by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry appears promising.[6] There are small morphologic and biochemical differences between some species, but these are not sufficient for identification. As an exception, *Sporothrix luriei* has been rarely isolated from humans and differs by producing a variety of unusual shapes in vivo.[7] The most common species is *S. schenckii* sensu stricto, which is found in America, Asia, and Africa. *Sporothrix brasiliensis* has so far been found only in Brazil, where it has often caused disseminated infection. Erythema nodosum or erythema multiforme has accompanied skin lesions in some of the cutaneous *S. brasiliensis* cases.[8] *Sporothrix mexicana* has been isolated from a few environmental sources and infections in Mexico, Brazil, Italy, and Portugal. *Sporothrix pallida,* which may be nonpathogenic, and *Sporothrix globosa* have been more broadly distributed geographically.[5] Because most of the information about sporotrichosis has not distinguished the cryptic species within the *Sporothrix schenckii* complex, the term *S. schenckii* is understood for the remainder of this chapter to include these closely related cryptic species.[4]

EPIDEMIOLOGY

Sporotrichosis has been reported from locations around the globe, but most case reports come from the tropical and subtropical regions of the Americas.[2,9] Most of the cases in the United States so far have been identified as *S. schenckii.*[10] Regions of hyperendemicity are known.[9,11] *Sporothrix schenckii* is most often isolated from soil, plants, or plant products such as straw, wood, sphagnum moss, and thorny plants, though the fungus is not a plant pathogen. Scratches on exposed skin of florists, rose gardeners, horticulturalists, farmers, miners, and armadillo hunters have increased risk of infection.[2,12,13] Because most cases appear to be due to occupational or avocational exposure to these materials, typically in the form of gardening or farming, patients with suggestive syndromes should be asked about these activities. Cases of animal-to-human transmission involving squirrels, horses, dogs, cats, pigs, mules, insects, and birds have been described.[14,15] An ongoing epidemic of sporotrichosis in Brazil has its origin in thousands of urban cats infected with *Sporothrix brasiliensis,* transmitting infection to thousands of humans and dogs.[11,16] Finally, sporotrichosis was apparently transmitted from the infected cheek of a mother to her infant[17] and to a lung transplant recipient via the transplanted lung.[18]

CLINICAL SYNDROMES

Infections due to *S. schenckii* can be divided into several syndromes. The lymphocutaneous forms are the most common.

Lymphocutaneous Sporotrichosis

Cutaneous disease arises at sites of minor trauma and inoculation of the fungus into the skin. The initial lesion is most often on a distal extremity, but almost any site may be involved, including such central locations as the nose and the ocular adnexa.[2,19,20] This preference



FIG. 259.1 Sporotrichosis of the fifth finger in a gardener. Three nodular lesions are visible on the hand and arm.



FIG. 259.2 Sporotrichosis of the bones of the wrist.

for cooler parts of the body corresponds to the known intolerance of some strains of *S. schenckii* to growth at 37°C.[21] Initial lesions are papulonodular, often erythematous, and range in size from a few millimeters to 2 to 4 cm. The lesions may be smooth or verrucous, and they often ulcerate and develop raised erythematous borders.[19] Lesions often develop proximally along lymphatic channels—these secondary lesions evolve in the same fashion as the primary lesion (Fig. 259.1). Secondary lesions do not usually involve a lymph node, although lymphadenopathy may develop. The lesions are typically painless, even after they ulcerate. The fixed, or plaque, form of sporotrichosis differs by not demonstrating any tendency to spread locally. Although spontaneous resolution of fixed sporotrichosis has been described,[19] the lesions of sporotrichosis usually wax and wane over months to years. The patient will not have systemic symptoms, and laboratory examinations will be normal.

The indolent progression and physical examination features suggesting both lymphocutaneous and fixed sporotrichosis are also produced by a number of other organisms (Table 259.1). Cultures of the drainage from skin lesions are occasionally helpful, but culture of biopsy material is preferred and is diagnostic when positive. Microscopic examination will reveal pseudoepitheliomatous hyperplasia in the epidermis with pyogranulomas and granulomas, often with areas of liquefactive necrosis in the mid and upper dermis.[22] Gomori methenamine silver stain will detect yeast cells in about one-third of cases, particularly if multiple sections are examined.[23]

Extracutaneous Sporotrichosis

Osteoarticular involvement is the most common form of extracutaneous sporotrichosis.[2,24] Involvement is of the major joints of the extremities—wrist (Fig. 259.2), elbow, ankle, and knee (Fig. 259.3); the hip, shoulder, and spine are not involved.[25] Most patients present with involvement of a single joint without previously having had sporotrichosis at any other site. The joint is swollen and painful on motion, an effusion is present, and a sinus tract may develop. The overlying skin may or may not be erythematous. Systemic symptoms are minimal and, other than elevation of the erythrocyte sedimentation rate, laboratory examinations are unrevealing. Untreated, other joints may become involved. Tenosynovitis associated with carpal tunnel syndrome or nerve entrapment has been reported.[26] The radiologic changes of osteomyelitis develop slowly and include loss of articular cartilage, periosteal reaction, and periarticular osteopenia and cystic changes. Failure to consider the diagnosis has resulted in an average 25-month delay before diagnosis.[27] Repeated culture of fluid from joint aspiration, as well as culture and microscopic examination of tissue from synovial biopsies, is often required to make the diagnosis. Differential considerations include pigmented villonodular synovitis, tuberculosis, gout, osteoarthritis, and rheumatoid arthritis.

TABLE 259.1 Differential Diagnosis of Sporotrichoid Lesions

Lesions Resembling Lymphocutaneous Sporotrichosis
(papulonodular lesions with or without central ulceration and with one or more nodules in the proximal skin along paths of presumed lymphatic spread)

- Nocardiosis due to *Nocardia brasiliensis*
- Cutaneous leishmaniasis
- Mycobacterial infection due to:
  - *M. tuberculosis* (tuberculosis cutis verrucosa)
  - *M. marinum*
  - *M. chelonae*
  - *M. kansasii*
  - *M. fortuitum*
- Infections that may rarely resemble lymphocutaneous sporotrichosis:
  - *Mycobacterium leprae*
  - Cowpox
  - *Staphylococcus aureus*
  - *Streptococcus pyogenes*
  - *Francisella tularensis*
  - *Scedosporium apiospermum*
  - *Fusarium* species

Lesions Resembling Plaque Sporotrichosis
(chronic, indurated hyperkeratotic plaques)

- Infections—as for lymphocutaneous disease and also:
  - Blastomycosis
  - Paracoccidioidomycosis
  - Chromoblastomycosis
  - Lobomycosis
- Neoplasms
  - Squamous carcinoma
  - Basal cell carcinoma
  - Mycosis fungoides
- Other
  - Psoriasis
  - Lupus vulgaris
  - Pyoderma gangrenosum

Modified from Kostman JR, DiNubile MJ. Nodular lymphangitis: a distinctive but often unrecognized syndrome. Ann Intern Med. *1993;118:883–888; and Smego RA Jr, Castiglia M, Asperilla MO. Lymphocutaneous syndrome—a review of non-sporothrix causes.* Medicine. *1999;78:38–63.*

Pulmonary sporotrichosis is well described and may present with multifocal noncavitary disease as part of the syndrome of multifocal extracutaneous infection (see later) or primary cavitary disease following fungal inhalation.[28,29] The typical patient with primary cavitary disease is a 30- to 60-year-old-male. Approximately one-third of the patients are alcoholic; one-third have a concomitant medical illness such as pulmonary tuberculosis, diabetes mellitus, sarcoidosis, and steroid use; and one-third are apparently normal. Patients are occasionally asymptomatic but will usually have a productive cough, low-grade fever, or weight loss. Other than elevation of the erythrocyte sedimentation rate, laboratory abnormalities are minimal. The chest radiograph reveals unilateral or bilateral cavitary lesions, usually with an associated parenchymal infiltrate



FIG. 259.3 Sporotrichosis of the knee with formation of a Baker cyst. *(From Kwon-Chung KJ, Bennett J.* Medical Mycology. *Philadelphia: Lee & Febiger; 1992:712.)*

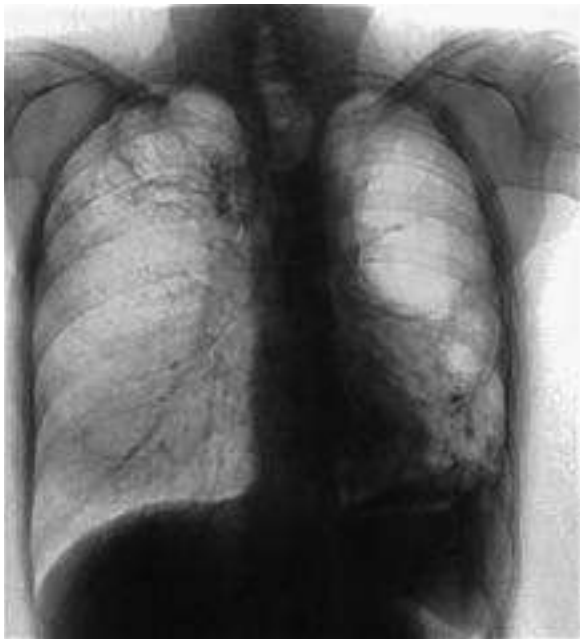

FIG. 259.4 Chest roentgenogram demonstrating extensive bilateral cavitation due to sporotrichosis.

(Fig. 259.4). Pleural effusions and hilar lymphadenopathy are occasionally noted. Gram stain or cytologic examination of sputum or bronchial washings will sometimes reveal elongated budding yeast,[30] and sputum culture will usually yield the organism. With some patients, however, repeated cultures and long-term follow-up are necessary in order to make the diagnosis.[31] Untreated, the cavities of pulmonary sporotrichosis gradually enlarge and produce progressive pulmonary dysfunction. A single case of spontaneous resolution of noncavitary infection has been reported.[32] The differential diagnosis includes mycobacterial infections (due to both *Mycobacterium tuberculosis* and other mycobacteria), histoplasmosis, and coccidioidomycosis.

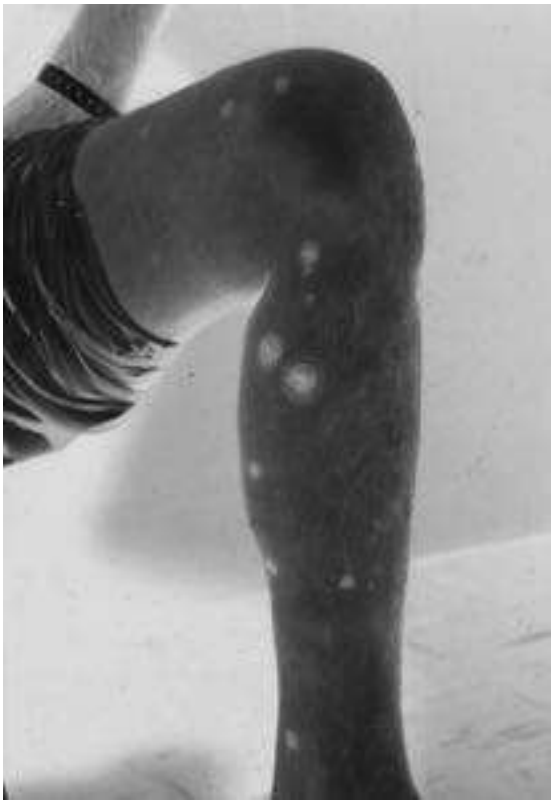

FIG. 259.5 Hematogenously disseminated skin lesions scattered over the skin of a previously normal male farmer who also had joint lesions.

An indolent meningitis due to *S. schenckii* has been described, with skin lesions or other extra–central nervous system foci noted in about half of cases.[33–36] Cerebrospinal fluid (CSF) analysis demonstrates a lymphocytic pleocytosis, an elevated protein, and hypoglycorrhachia. Cultures of the CSF are positive, although repeated cultures of large volumes of CSF or serologic studies may be required to make the diagnosis. Infections of a variety of other sites have been reported but are uncommon. Involvement of the ocular adnexa, sometimes with spread to the eye, has been described.[20,37] Endophthalmitis may even occur without prior trauma or other evidence of sporotrichosis.[38] Cases of isolated involvement of the sinuses, kidney, testes, and epididymis have also been reported.[24,39]

Multifocal Extracutaneous Sporotrichosis

In otherwise normal patients with extracutaneous sporotrichosis, the lesions are generally restricted to a single site and are only locally progressive. Occasionally a patient with osteoarticular sporotrichosis will have involvement of several joints, but the presentation is otherwise identical to that in patients with involvement of only a single joint. A much smaller group of patients, on the other hand, present with weight loss or variable low-grade fever and often have several widely scattered cutaneous lesions without necessarily showing a single primary distal extremity lesion with the pattern of lymphangitic spread (Fig. 259.5). Mild anemia, leukocytosis, and elevation of the erythrocyte sedimentation rate may be present. Osteolytic bone lesions and arthritis are common, and spread to the palate, eyes, and central nervous system may develop.[24,40] Noncavitary lung lesions may also be seen.[41] Untreated infection is ultimately fatal. Patients with this form of sporotrichosis almost always have some form of immunosuppression, commonly hematologic malignancy,[24,40] or human immunodeficiency virus (HIV) infection (see later). Cultures of skin lesions and joints are usually positive, whereas blood and bone marrow cultures are occasionally positive. Immunosuppressed patients with what appears to be simple cutaneous sporotrichosis should be examined for other sites of infection, and a technetium pyrophosphate bone scan or positron

emission tomography/computed tomography (PET/CT) should be performed.[41a]

CLINICAL MANIFESTATIONS OF SPOROTRICHOSIS IN THE HIV-INFECTED PATIENT

Infection with HIV predisposes to invasive, atypical, or disseminated manifestations of sporotrichosis. When $CD4^+$ counts are relatively well preserved, localized infection may follow direct cutaneous inoculation in a pattern analogous to that in immunocompetent patients.[42] However, widespread lymphocutaneous sporotrichosis or multifocal extracutaneous disease may be seen in patients with more advanced HIV infection. In a review of patients with acquired immunodeficiency syndrome (AIDS) with disseminated sporotrichosis,[43] almost all had fewer than 100 $CD4^+$ T cells/μL. Multiple ulcerative skin lesions are usually present. Sporotrichosis may also present as multifocal tenosynovitis and arthritis with or without overt cutaneous disease or systemic dissemination and thus may resemble disseminated gonococcal infection or the seronegative spondyloarthropathies such as reactive arthritis or psoriatic arthritis (Fig. 259.6), which are seen with a higher frequency in the setting of AIDS.[44] Widespread visceral dissemination also occurs, as evidenced by reports of meningitis with parenchymal brain lesions[35,36]; lung abscess, liver, and spleen involvement[45]; endophthalmitis[46]; and fungemia with spread to esophagus, colon, testes, bone marrow, and lymph nodes.[46] Dissemination may manifest as part of the immune reconstitution inflammatory syndrome.[47] Sinusitis with invasion of the contiguous bone and soft tissues has also been described,[48] and emphasizes the potential for the respiratory tract as the initial focus of infection in HIV-infected patients.

DIAGNOSIS

Diagnosis is best made by culture of the affected site, though repeated attempts at culture may have to be made. A positive culture from any site is ordinarily diagnostic of infection, although a case of saprophytic involvement of the respiratory tract has been described.[49] A positive blood culture strongly suggests the multifocal form of sporotrichosis seen in immunocompromised hosts, and the lysis-centrifugation system may be more sensitive in detecting fungemia in nonimmunocompromised patients.[50] Serodiagnosis has been described[51] but has often been complicated by the presence of antibodies in individuals without evidence of sporotrichosis[52] and by cross-reactivity with other fungi.[2] Molecular diagnostic techniques such as polymerase chain reaction have also been described.[53]

Examination of biopsy specimens reveals a pyogranulomatous response and is diagnostic if characteristic 1- to 3-μm × 3- to 10-μm cigar-shaped yeast forms are seen. Unfortunately, the yeast may be difficult to detect unless multiple sections are examined,[23] although lesions from immunocompromised hosts may contain numerous yeasts (Fig. 259.7). In addition, *S. schenckii* often assumes a more rounded tissue form, making the biopsy suggestive but not diagnostic. The organisms may be surrounded by a stellate, periodic acid–Schiff-positive, eosinophilic material known as an *asteroid body.* In the brain or eye, a capsule has sometimes been demonstrable around the yeastlike cells.

As with other immunosuppressed patients, individuals with advanced AIDS may have a high fungal load that results in positive smears and cultures.[30] Skin biopsy may reveal fungal elements with a limited inflammatory response, and this should prompt the clinician to initiate a search for a systemic immunodeficiency.[54]

THERAPY

Treatment guidelines for sporotrichosis have been proposed.[55] Due to its convenience and consistent efficacy,[56] itraconazole at 100 to 200 mg/day (or 65 mg to 130 mg/day if using the enhanced bioavailability SUBA-itraconazole formulation; see Chapter 40B) has become the therapy of choice for cutaneous sporotrichosis.[55] Therapy with itraconazole is given for 2 to 4 weeks beyond complete resolution of all lesions and usually requires 3 to 6 months to effect a clinical cure. Relapse has been observed on occasion after cessation of therapy. Should relapse develop, a higher dose of itraconazole (200 mg twice daily), or terbinafine or iodide, may be tried. Terbinafine has been effective in adults at doses of 250 to 1000 mg/day for 12 to 24 weeks,[37,57] but direct comparative data do not exist and the limited data suggest more frequent and more durable cures with higher doses. Further, the maximum US Food and Drug Administration–approved terbinafine regimen is 250 mg daily for 12 weeks; the safety of extended higher-dose therapy has not yet been extensively validated. Iodides are an effective and inexpensive but poorly tolerated therapy for cutaneous sporotrichosis[58] and have been effective in cases of therapeutic failure of itraconazole.[59] Iodide therapy is prescribed as a saturated solution of potassium iodide (SSKI), with therapy begun at 5 to 10 drops taken orally three times per day. The dose is gradually advanced to 25 to 40 drops three times daily (for children) or 40 to 50 drops three times daily (for adults). SSKI has a bitter taste and is made more palatable by taking it in milk, juice, or a carbonated beverage. Side effects include nausea, anorexia, diarrhea, parotid or lacrimal gland enlargement, and an acneiform rash. These side effects will remit with reduction of the dose of SSKI or temporary



FIG. 259.6 Extensive multifocal sporotrichosis with tenosynovitis of the toes, arthritis of the ankles, and associated lymphedema in a patient with advanced acquired immunodeficiency syndrome. This patient also had tenosynovitis of the wrists and hands along with arthritis of the wrists and knees.

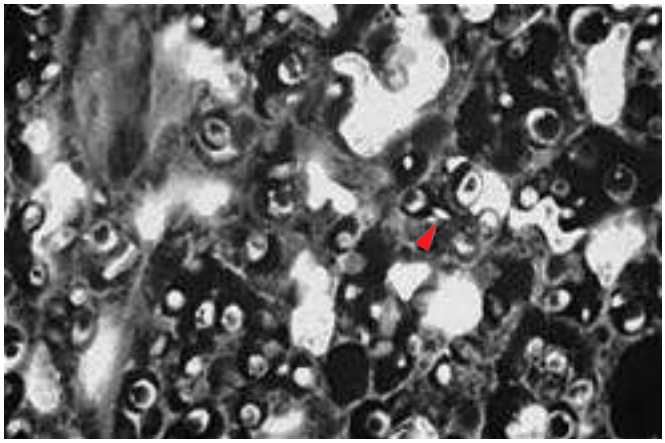

FIG. 259.7 Numerous yeasts of sporotrichosis in a cutaneous lesion from an immunosuppressed patient. In the normal host, organisms are usually difficult to locate. Although a single cigar-shaped form is present *(arrowhead),* most of the yeasts have a rounded form that is consistent with, but not diagnostic of, sporotrichosis. *(Courtesy Dr. Ronald Rapini, Houston, TX.)*

cessation of therapy. For both terbinafine and iodide, therapy should be continued until 2 to 4 weeks after the cutaneous lesions have resolved, a process that usually takes 3 to 6 months. Some patients are allergic to iodides, and in others cutaneous disease may respond slowly to iodide therapy or rarely fail to respond at all.

Ketoconazole has not proven to be effective, and amphotericin B is too toxic to be used in this setting. Consistent with its limited in vitro activity, fluconazole has only modest clinical activity and should be used only if other therapies are not tolerated.[60–62] Clinical data with the newer azoles are sparse,[63] and elevated minimum inhibitory concentrations have been reported with both voriconazole and posaconazole.[60–62,64] Due to the temperature sensitivity of this organism, local application of heat is a useful adjunct therapy and on occasion has been curative.[65] Given the toxicity of both the azoles and the iodides in pregnant women (skeletal bone deformities and goiter, respectively),[66] use of heat may be especially valuable during pregnancy.[55,67]

Therapy of extracutaneous sporotrichosis is often difficult. Osteoarticular sporotrichosis has been treated with intravenous amphotericin B (a lipid-associated preparation is recommended due to the improved safety profile of these formulations),[55] but itraconazole at 200 mg twice daily for at least 1 year is the preferred approach. Ketoconazole (400–800 mg/day) and fluconazole (200–400 mg/day) appear less efficacious, and ketoconazole is hepatotoxic.[55,68] As noted previously, very few clinical data are available with the newer azoles (voriconazole and posaconazole), and the echinocandins do not appear consistently active.[2,60,61,69] Intraarticular amphotericin B is sometimes given, although its role has not been clearly defined. Surgical débridement is often employed, but its utility is also uncertain. SSKI has rarely been reported to be effective but usually is not.

If diagnosed prior to the development of cavities, pulmonary sporotrichosis may be treated with itraconazole, SSKI, or amphotericin B.[28,29] Cure of cavitary disease typically requires pulmonary resection plus a perioperative course of itraconazole or amphotericin B.[55] Treatment failure is often associated with incomplete resection.[4]

Sporothrix schenckii meningitis does not consistently respond to amphotericin B, and the addition of 5-fluorocytosine may be warranted. Limited data suggest that itraconazole might be useful as suppressive or step-down therapy.[55] The number of reported cases of involvement of other specific sites is too limited to permit generalization. Extracutaneous sporotrichosis in the immunocompromised host usually responds at least partially to either amphotericin B or itraconazole, although relapse is common.

Therapy of Patients With Acquired Immunodeficiency Syndrome

Therapy of sporotrichosis in AIDS should be tailored to the presenting syndrome. Itraconazole appears to be the drug of choice, and individuals with limited cutaneous disease can be treated with 200 mg twice daily. Amphotericin B should be used as initial therapy of disseminated disease, with lipid-associated formulations preferred over amphotericin B deoxycholate.[55] Based on the observation of frequent relapse and dissemination, chronic suppressive therapy with itraconazole appears warranted following initial control of infection. Although unsupported by direct data, it is appears reasonable to discontinue therapy in individuals who have been treated for itraconazole for at least 1 year and whose $CD4^+$ cell counts have been about 200 cells/μL for at least a year.[55]

Both anecdotal and published experience suggest that multifocal extracutaneous disease in HIV-infected patients may respond poorly, if at all, to current therapies.[70,71] Therapy should be initiated with amphotericin B followed by lifetime suppression with itraconazole. Progression may occur despite amphotericin therapy. Because disseminated disease has been reported to develop despite ongoing fluconazole being given for other indications,[72] fluconazole is not a first-line choice. As has been demonstrated for other opportunistic pathogens, the use of highly active antiretroviral therapies may also assist in clearing the infection.[73]

Itraconazole Blood Level Monitoring

When itraconazole is used for noncutaneous infection or in HIV-infected subjects, confirmation of adequate blood levels is recommended (see Chapter 40B). In HIV in particular, levels may be reduced by achlorhydria, malabsorption, or diarrhea due to other opportunistic pathogens. As for other fungal infections, target levels of the parent (unmetabolized) itraconazole molecule of at least 500 ng/mL by high-performance liquid chromatography generally appear adequate. The increased bioavailability of itraconazole cyclodextrin suspension is helpful in achieving such blood levels.

PROGNOSIS

Cutaneous sporotrichosis responds well to therapy and has an excellent prognosis. Osteoarticular sporotrichosis may require prolonged therapy but is not life threatening. Other forms of extracutaneous sporotrichosis can be difficult to treat and may have substantial morbidity and mortality.

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260 Agents of Chromoblastomycosis

Duane R. Hospenthal

SHORT VIEW SUMMARY

Definition
- Chromoblastomycosis (chromomycosis) is a chronic fungal infection limited to the skin and subcutaneous tissue.
- It produces scaly nodular, tumorous, verrucous, plaque or cicatricial lesions, typically affecting the lower extremities.
- It is defined by the microscopic presence of muriform cells.

Epidemiology
- Chromoblastomycosis has a worldwide distribution, with most cases occurring in tropical and subtropical regions.
- The largest numbers of cases are reported from Madagascar, Brazil, Mexico, Venezuela, and Costa Rica.
- The disease has a male predominance and is more prevalent in those aged 40 to 69. It is commonly associated with outdoor activities such as farming or woodcutting and with absence of footwear.

Microbiology
- Infection is most commonly caused by species of *Fonsecaea (F. pedrosoi, F. monophora, F. nubica)* or *Cladophialophora (C. carrionii).*
- Less commonly it is caused by *Exophiala, Phialophora,* or *Rhinocladiella* species.
- Other dark-walled fungi are rarely reported as causative agents.

Diagnosis
- The presence of muriform cells (also called sclerotic, "copper penny," or Medlar bodies) is pathognomonic.
- Muriform cells are observed microscopically in skin scrapings prepared with potassium hydroxide or in routinely stained skin biopsy specimens.
- Culture on standard mycologic media is possible but not always necessary.

Therapy
- No proven treatment has been identified.
- Small lesions may be treated successfully with surgical excision, liquid nitrogen or topical heat application, or photocoagulation. Use of curettage and electrocautery is discouraged because of reports of disease spread after their use.
- Oral itraconazole 200 to 400 mg daily or terbinafine 250 to 500 mg daily in combination with local liquid nitrogen is the most effective therapy.
- Posaconazole has been used successfully in a small number of patients with disease that is refractory to itraconazole or terbinafine.

Prevention
- No vaccine is available.
- The need for footwear and proper protective clothing should be stressed.

Chromoblastomycosis (chromomycosis) is a chronic, localized fungal infection of the skin and subcutaneous tissue that produces raised scaly lesions, usually of the lower extremities. The lesions of chromoblastomycosis are frequently warty or cauliflower-like in appearance, with pathognomonic muriform cells (also called "copper penny" or sclerotic bodies) found at histologic examination. This disease of tropical and subtropical distribution is produced by inoculation of the infecting fungi in association with minor trauma. Alexandrino Pedroso, for whom the major etiologic agent is named, first noted the disease in 1911, although the first publication to describe what was likely chromoblastomycosis appeared in 1914, authored by Max Rudolph.[1,2] The first reports to include identification of the fungal cause of this disease were published 1 year later by Medlar and Lane, who described a patient with disease acquired not in the tropics but in the United States.

ETIOLOGIC AGENTS

Infection is caused by one of several dark-walled (dematiaceous) fungi found in the soil and in association with cacti, thorny plants, and other live or decaying vegetation. *Fonsecaea pedrosoi* is the most common cause of chromoblastomycosis. Other *Fonsecaea* species (*F. monophora* and *F. nubica*) and *Cladophialophora carrionii* are also common etiologic agents. *Phialophora verrucosa* and *Rhinocladiella aquaspersa* are less commonly reported. In the largest reports from Brazil,[3] Mexico,[4] Sri Lanka,[5] India,[6] and Japan,[7] *F. pedrosoi* has been responsible for 66% to 96% of all infections. With the more recent recognition of *F. monophora* and *F. nubica* as new species, identification of these organisms as the cause of both new cases and old cases (with reidentification of isolates previously identified as *F. pedrosoi*) of chromoblastomycosis continues to increase.[8–12] *Fonsecaea compacta* is currently believed to be a variant of *F. pedrosoi* and not a distinct species. Rare or isolated reports of disease caused by *Botryomyces caespitosus, Chaetomium funicola, Cladophialophora arxii, Cladophialophora boppii, Cyphellophora ludoviensis, Exophiala (Wangiella) dermatitidis, Exophiala jeanselmei, Exophiala spinifera, Fonsecaea pugnacius, Phaeosclera dermatioides, Rhinocladiella phaeophora, Rhinocladiella similis, Rhinocladiella tropicalis,* and *Rhytidhysteron* species have been published.[1,2,13–19]

EPIDEMIOLOGY

Chromoblastomycosis has been described to occur throughout the world, although most cases arise in tropical and subtropical regions, especially those with high annual rainfall. Large numbers of cases have been described from Madagascar, Brazil, Mexico, Venezuela, and Costa Rica. Disease is more prevalent in males (4:1 ratio), in those aged 40 to 69,[3] in association with outdoor activities such as farming and woodcutting, and in the absence of footwear. In Madagascar, a unique epidemiology has been described in what is probably the largest focus of endemic disease.[20] Madagascar has two distinct foci of infection, with disease secondary to *F. pedrosoi* occurring in the humid, rainy, northern evergreen forest region and disease secondary to *C. carrionii* found in the arid southern desert region. In a study of 1343 cases of disease over 40 years in that country, the prevalence of 1 case per 1920 inhabitants in the southern desert region has been described, with an incredible 1 in 910 prevalence in a single district of that region.

PATHOLOGY AND PATHOGENESIS

Traumatic inoculation of these fungi results in a mixed chronic suppurative and granulomatous host response.[21] The epidermis typically becomes thickened in a process called pseudoepitheliomatous hyperplasia,



FIG. 260.1 Sclerotic bodies of chromoblastomycosis. *(From Beneke ES, Rogers AL.* Medical Mycology and Human Mycoses. *Belmont, CA: Star Publishing; 1996.)*



FIG. 260.2 Chromoblastomycosis of the foot. *(From Beneke ES, Rogers AL.* Medical Mycology and Human Mycoses. *Belmont, CA: Star Publishing; 1996.)*

a histologic morphologic appearance that may be misidentified as malignancy by more inexperienced microscopists. Foci of polymorphonuclear cells and microabscesses are seen in both the epidermis and dermis. In the dermis, granulomas that include multinucleated giant cells and epithelioid cells are present, along with varying amounts of fibrosis. Fibrosis is increased in older lesions and can extend into the subcutaneous tissue, although disease rarely extends deep into the subcutaneous tissue. The hallmark of chromoblastomycosis, the muriform cells—also called sclerotic, copper penny, or Medlar bodies—may be found intracellularly in macrophages or extracellularly in abscesses. These are darkly pigmented (brown-golden), thick-walled, rounded cells, 4 to 12 µm in diameter, with cross walls in one or two planes (Fig. 260.1). Hyphae may also occasionally be seen, usually in the epidermis. The host response to these structures results in a process termed *transepithelial elimination,* in which fungi and damaged tissue are expelled through the epidermis, a process similar to that seen in calcinosis cutis.[22] The immunologic response of the host in this mycosis is still not well understood. Disease appears to be associated with an ineffective immune response to the organism, with chronic inflammation produced in response to persistence of the fungi in tissue. The cell-mediated immune response appears to play a central role. High interleukin-10 (IL-10) levels, low interferon-γ levels, and inefficient T-cell proliferation have been noted in patients with severe disease, with the opposite seen in those with milder chromoblastomycosis.[23] This ineffective response has been linked to lack of recognition of these fungi by Toll-like receptors (TLRs) in a murine model.[24] Antibody responses have shown association with disease chronicity and extent but do not appear to provide any degree of protection in this infection.[25]

CLINICAL MANIFESTATIONS

Weeks to months after inoculation of the causative organisms through minor trauma, subjects typically develop a small scaly papule on the lower extremity at the site of the trauma (Figs. 260.2–260.4). This lesion slowly develops into a superficial nodule, commonly with an irregular friable surface. Frequently, these nodules later spread out to become purplish, irregular, raised plaques. In descriptions by Carrion,[26] lesions of chromoblastomycosis were categorized into five types: (1) early nodular lesions, described as soft and pink-violaceous, with smooth, verrucous, or scaly surfaces; (2) tumorous lesions—large, papillomatous, often lobulated masses with crusting, sometimes described as cauliflower-like; (3) verrucous lesions with prominent hyperkeratosis; (4) plaque lesions; and (5) cicatricial lesions. Most lesions have black dots associated with their outer surface that are composed of fungi and necrotic debris, the products of transepithelial elimination. Although not typically painful, lesions may be associated with pruritus, are easily traumatized, and bleed readily. Ulceration is generally limited to those lesions with bacterial superinfection. Large lesions may become hyperkeratotic, and limb distortion, including elephantiasis, can occur as a result of blockage of normal lymphatic drainage.



FIG. 260.3 Chromoblastomycosis with multiple verrucous nodules. *(From McGinnis MR, Chandler FW. Chromoblastomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*



FIG. 260.4 Chromoblastomycosis of the lower leg with lobulated, confluent nodules with focal ulcerations. *(From McGinnis MR, Chandler FW. Chromoblastomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

Although lesions have been described to occur chiefly on the lower extremities (80%–85%) in most regions of the world,[3,27] an exception to this pattern is reported from Japan. Evaluation of 290 lesions from that country found that chromoblastomycosis occurs most commonly on the upper extremities of male subjects and on the face or neck of females.[7]

Persistence of the lesions of chromoblastomycosis for 30 years has been reported, and delays in diagnosis of 1 to 3 years are not unusual. Although most lesions remain localized without spread to deeper structures, localized dissemination may occur via autoinoculation or via the lymphatics. Hematogenous spread has only rarely been described but does include reports of dissemination to the central nervous system.[7] The occurrence of secondary bacterial infection has been reported to affect as many as 63% of persons with chromoblastomycosis.[4] Although apparently rare, at least 14 cases of squamous cell carcinomas and 1 case of melanoma have been reported; all occurred after 8 or more years of disease.[28,29]

The differential diagnosis of chromoblastomycosis includes psoriasis, other mycoses (e.g., blastomycosis, coccidioidomycosis, lobomycosis, mycetoma, paracoccidioidomycosis, cutaneous phaeohyphomycosis, sporotrichosis, tinea), cutaneous tuberculosis, leprosy, leishmaniasis, protothecosis, keratoacanthoma, squamous cell carcinoma, and sarcoidosis.

DIAGNOSIS

Chromoblastomycosis should be suspected in persons with chronic scaly or friable lesions of the extremities, especially in rural tropical climates. Microscopic examination of skin scrapings can provide a rapid diagnosis of chromoblastomycosis because the characteristic muriform cells can be seen in potassium hydroxide preparations, especially those containing black dots (see Fig. 260.1). These unique structures may also be readily observed with standard staining of skin punch biopsy specimens with hematoxylin and eosin (Fig. 260.5). Although not absolutely necessary, culture can be performed to identify the specific cause of infection. Standard mycologic media (Sabouraud glucose agar), with and without cycloheximide, should be used and cultures incubated for at least 4 weeks. In culture, the fungal agents of chromoblastomycosis appear as dark molds. Under standard culture conditions, these fungi may be identified by the microscopic appearance of hyphae and reproductive structures. The muriform structures seen in tissue have been produced in vitro with low pH and the addition of propranolol, but this is not necessary for clinical diagnosis.[30] Exoantigen testing of the fungus has been developed to aid in identification, although this is not commonly used.[31] Serologic and skin tests have also been developed, but their use in this rare disease is limited to specialized centers in more endemic regions of the world.

THERAPY

Although spontaneous resolution has been reported,[32] this is only a rare occurrence, and most chromoblastomycosis is a chronic indolent infection. When caused by its most common agent, *F. pedrosoi,* it is difficult to eradicate, even with prolonged therapy. Multiple modalities have been used to treat patients with chromoblastomycosis, including surgery, local (physical) treatments, and antifungal agents.[33] Unfortunately, no single reproducibly successful treatment strategy has been identified. Surgical removal of small lesions appears to be effective, as does local application of liquid nitrogen, topical heat, and photocoagulation. Local curettage or electrocautery has been reported sometimes to result in disease spread and is to be discouraged. Heat therapy (42°C–46°C) with pocket warmers and other devices providing prolonged daily warmth directly to the lesions has been described as effective with 2 to 12 months of treatment.[34] Cryotherapy with liquid nitrogen sprays or applied with soaked cotton swabs or balls has been successful in the cure of small early lesions and may be used effectively in combination with antifungal medications on larger lesions. Topical imiquimod has been used successfully in four cases of disease secondary to *F. pedrosoi.*[35] The use of topical ajoene (a garlic extract) and 5-fluorouracil has been reported to be effective against disease secondary to *C. carrionii.*[36]

Currently, the best therapy appears to be itraconazole or terbinafine, perhaps with adjunctive cryotherapy with liquid nitrogen or other local treatments.[37,38] Other antifungal agents, including amphotericin B (intravenous or intralesional), 5-fluorocytosine, ketoconazole, and fluconazole, have been used with poor to mixed success, alone or in combination.

Itraconazole has been reported to be effective in many patients in uncontrolled, nonrandomized studies. Early study with lower doses (100 to 200 mg daily) of itraconazole documented high response rates with this azole antifungal agent, but the numbers of cures were small (3 of 10 patients treated for 12 to 24 months).[39] Treatment of chromoblastomycosis caused by *C. carrionii* with itraconazole has met with much greater success than treatment of that caused by other agents. One study reported cure in 2 of 5 patients with disease secondary to *F. pedrosoi* and 8 of 9 patients with *C. carrionii,* all given 100 to 400 mg of the drug daily for 4 to 8 months.[40] Queiroz-Telles and colleagues have reported the cure of 42% of patients (8 of 19) treated with 200 to 400 mg of itraconazole for a median of 7 months.[41] Another group described the therapy for 10 patients with disease secondary to *F. pedrosoi,* 4 of whom had experienced failure of prior therapy with ketoconazole.[42] All patients received 200 to 400 mg of itraconazole daily, and 8 also received monthly cryotherapy with liquid nitrogen. Nine patients were cured with 3 to 12 months of therapy; 2 of these responded with sustained cures after only 3 months of itraconazole at the lower dose of 200 mg daily. The remaining patient had marked improvement without cure. Recurrence was noted in a single patient who had been cured with a 6-month course of itraconazole (400 mg daily) and cryotherapy. Decreased in vitro susceptibility to itraconazole has been reported in one study of sequential clinical isolates of *F. pedrosoi,* potentially accounting for treatment failures.[43] Bonifaz and associates described good success with smaller lesions treated with itraconazole or cryosurgery and in larger lesions treated with itraconazole followed by cryosurgery.[44] In that study, which included 4 patients in each group and dosage of itraconazole at 100 mg three times daily for 5 to 14 months, 8 of 12 patients (67%) were cured and the remaining 4 showed improvement. In addition to daily therapy, success with itraconazole given as pulse therapy, 400 mg daily for 7 days/month for 6 to 12 months, has also been reported.[45] If the better-absorbed SUBA-itraconazole forumation is used, 65-mg capsules would be equivalent to 100-mg capsules of the older formulation.

The allylamine antifungal agent terbinafine has produced excellent results in the treatment of chromoblastomycosis. In the largest study to date, terbinafine, 500 mg daily, was given to 35 patients for up to 12 months.[46] In that study, 16 patients had experienced failure of thiabendazole in the past and almost half had lesions of longer than 10 years' duration. Improvement, defined as lack of bacterial superinfection and resolution of edema, was seen after 2 to 4 months of therapy and, after 12 months, 86% obtained mycologic cures (72% with clinical cures).[27] Patients with *C. carrionii* infections were noted to respond more quickly than those with *F. pedrosoi* infection in that study. Unexpectedly, partial reversal of fibrosis of the lesions of chromoblastomycosis has also been reported to occur with terbinafine therapy. This reversal has been suggested to be independent of mycologic cure of infection in those receiving terbinafine.[47,48] Terbinafine used in alternate-week or

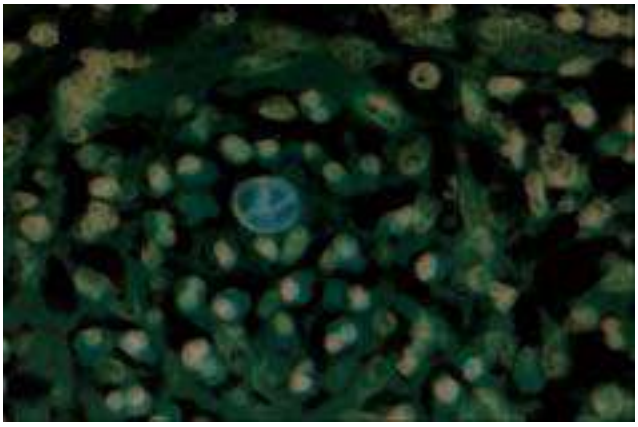

FIG. 260.5 Dermal abscess with quadrate cluster of organisms in a mixture of neutrophils, macrophages, eosinophils, and a giant cell (hematoxylin and eosin). *(From McGinnis MR, Chandler FW. Chromoblastomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

combination therapy with itraconazole to treat 4 patients with resistant infections successfully has also been reported.[49]

The newer broad-spectrum azole antifungal agents may potentially be useful in this disease. In vitro testing has shown that the minimum inhibitory concentrations of voriconazole for *F. pedrosoi* and *F. compacta* are lower than those seen with itraconazole.[50] *F. pedrosoi* has also been shown to be susceptible to the echinocandin caspofungin in one small in vitro study.[51] Posaconazole (800 mg/day of the suspension in divided doses for 6 to 12 months) has been used to successfully treat a small number of patients (five of six) with disease refractory to itraconazole and terbinafine.[52]

No vaccinations exist to prevent chromoblastomycosis. Proper protective clothing, especially footwear, and early treatment of the lesions are the only available preventive measures against this disease.

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261 Agents of Mycetoma

Duane R. Hospenthal

SHORT VIEW SUMMARY

Definition

- Mycetoma is an infection of the skin and subcutaneous tissue characterized by a triad of localized swelling, draining sinuses, and grains (aggregates of infecting organisms). Unlike actinomycosis, which also forms grains in lesions, mycetoma enters the body through the skin.
- It most commonly affects a single site, typically involving the lower extremity and especially the foot.
- Eumycetoma (eumycotic mycetoma) is mycetoma caused by fungi, most commonly *Madurella mycetomatis.*
- Actinomycetoma (actinomycotic mycetoma) is mycetoma caused by bacteria, most commonly *Nocardia brasiliensis.*

Epidemiology

- Distribution is worldwide, with most cases occurring in tropical and subtropical regions.
- Largest numbers of cases are reported from Africa, Latin America, and the Indian subcontinent.
- Mycetoma occurs predominantly in men, ages 20 to 40, typically with occupations that expose them to the environment.

Microbiology

- *Madurella* spp., *M. mycetomatis, Trematosphaeria grisea,* and *Scedosporium apiospermum* complex are the most common causes of eumycetoma, although *Falciformispora senegalensis, Falciformispora tompkinsii, Exophiala jeanselmei,* and many other genera and species of fungi have been reported as etiologic agents.
- *Nocardia brasiliensis, Actinomadura madurae, Streptomyces somaliensis,* and *Actinomadura pelletieri* are the most common causes of actinomycetoma, although disease secondary to other species of *Actinomadura, Nocardia,* and *Streptomyces* has been described.

Diagnosis

- Clinical presentation of the classic triad of chronic, painless soft tissue swelling with draining sinuses that discharge grains is pathognomonic.
- Microscopic examination of the grains can differentiate between eumycetoma and actinomycetoma.
- Culture of causative agent from grains can better direct selection of antimicrobial therapy.
- Radiographic techniques (plain radiographs, ultrasound, computed tomography, and magnetic resonance imaging) can be used adjunctively in making (or excluding) the diagnosis and determining its extent.

Therapy

- Small lesions may be treated successfully with surgical excision alone.
- Actinomycetoma is typically treated with medical therapy alone.
- Eumycetoma commonly requires combined medical and surgical therapy, but results are poor.
- No single therapy has proved most effective for either form of mycetoma.
- Most actinomycetoma regimens include parenteral aminoglycosides and oral sulfa drugs.
- Less severe actinomycetoma may be treated with 6 to 24 months of trimethoprim-sulfamethoxazole.
- Eumycetoma is typically treated with a regimen of an oral azole antifungal drug for 6 to 24 months, perhaps combined with debulking surgery.

Prevention

- No vaccine is available.
- Use of footwear and proper protective clothing should protect against this infection.

Mycetoma is a chronic progressive granulomatous infection of the skin and subcutaneous tissue most often affecting the lower extremities, typically a single foot. The disease is unique from other cutaneous or subcutaneous diseases in its triad of localized swelling, underlying sinus tracts, and production of grains or granules (composed of aggregations of the causative organism) within the sinus tracts. These infections may be caused by fungi and termed *eumycotic mycetoma* or *eumycetoma*, or by filamentous higher bacteria and termed *actinomycotic mycetoma* or *actinomycetoma.* The term *mycetoma* can also be found in the literature incorrectly referring to a fungus ball found in a preexisting cavity in the lung or within a paranasal sinus, most often caused by *Aspergillus* spp. Grain formation by infecting organisms is restricted to the diseases mycetoma, actinomycosis (see Chapter 254), and botryomycosis. Actinomycosis is a disease produced by the anaerobic and microaerophilic higher bacteria that normally colonize the mouth and gastrointestinal and urogenital tracts. The portal of entry in actinomycosis is from those colonized sites, whereas in mycetoma the portal is the skin and subcutaneous tissue into which the organism was inoculated by minor trauma. Botryomycosis is a chronic bacterial infection of soft tissues in which the causative organism, often *Staphylococcus aureus,* is found in loose clusters among the pus.[1] In a rare form of ringworm called *dermatophyte mycetoma,* there are also loosely compacted clusters of hyphae in subcutaneous pus.[2] In contrast, mycetoma grains are dense clusters of organisms.

ETIOLOGIC AGENTS

The agents of mycetoma are fungi and aerobic filamentous bacteria that have been found on plants and in the soil.[3,4] The predominance of bacterial versus fungal causes of mycetoma varies among geographic locations. Eumycotic (true fungal) disease is caused by a variety of fungal organisms. These can be divided into those that form dark grains and those that form pale or white grains (Table 261.1). Color distinctions are made by observing unstained specimens. Among the fungi causing dark-grained mycetoma, the most common are *Madurella mycetomatis, Falciformispora* (formerly *Leptosphaeria*) *senegalensis,* and *Trematosphaeria grisea.* Other agents include *Corynespora cassicola, Curvularia geniculata, Curvularia lunata, Emarellia grisea, Emarellia paragrisea, Exophiala jeanselmei, Exophiala oligosperma, Falciformispora* (formerly *Leptosphaeria*) *tompkinsii, Madurella fahalii, Madurella pseudomycetomatis, Madurella tropicana, Phialophora verrucosa, Plenodomas avramii, Pseudochaetosphaeronema larense, Rhinocladiella atrovirens, Medicopsis* (formerly *Pyrenochaeta*) *mackinnonii, Biatriospora* spp., *Roussoella* spp., *Rhytidhysteron* spp., and *Medicopsis* (formerly *Pyrenochaeta*) *romeroi. Scedosporium apiospermum* complex species are the most common cause of pale-colored grains. Other fungi in that category include *Fusarium* (formerly *Acremonium*) *falciforme, Sarocladium* (formerly *Acremonium*) *kiliense, Acremonium recifei, Aspergillus flavus, Aspergillus hollandicus, Aspergillus nidulans, Phialophora* (formerly *Cylindrocarpon*) *cyanescens, Cylindrocarpon destructans, Diaporthe phaseolorum, Fusarium solani,*

TABLE 261.1 Typical Morphologic Features of Mycetoma Grains

GRAIN COLOR	CAUSATIVE AGENT
Eumycetoma (Eumycotic Mycetoma)[a]	
Black grains	*Madurella* spp., *Biatriospora* spp., *Trematosphaeria* spp., *Pseudochaetosphaeroma* spp., *Roussoella* spp., *Rhytidhysteron* spp., *Curvularia* spp., *Exophiala* spp., *Falciformispora* spp., *Medicopsis* spp., *Phaeoacremonium* spp., *Phialophora verrucosa*
Pale grains (white to yellow)	*Scedosporium apiospermum* complex, *Aspergillus* spp., *Diaporthe phaseolorum, Fusarium* spp., *Neotestudina rosatii, Pleurostomophora ochracea*
Actinomycetoma (Actinomycotic Mycetoma)[b]	
Pale grains (white to yellow)	*Actinomadura madurae, Nocardia* spp.
Yellow to brown grains	*Streptomyces somaliensis*
Red to pink grains	*Actinomadura pelletieri*

[a]Hyphae of 2- to 5-μm diameter are observed within grain.
[b]Filaments of 0.5- to 1-μm diameter are observed within grain.

Fusarium moniliforme, Fusarium keratoplasticum, Fusarium pseudensiforme, Neotestudina rosatii, Phaeoacremonium spp., *Pleurostomophora ochracea,* and *Polycytella hominis.*[5–13]

Actinomycetoma is caused by members of the order Actinomycetales, most commonly *Nocardia brasiliensis, Actinomadura madurae, Streptomyces somaliensis,* and *Actinomadura pelletieri.* Cases have been reported that were caused by *Actinomadura latina, Nocardia aobensis, Nocardia farcinica, Nocardia harenae, Nocardia otitidiscaviarum* (formerly *N. caviae*), *Nocardia mexicana, Nocardia transvalensis, Nocardia veterana, Nocardia yamanashiensis,* and *Nocardiopsis dassonvillei.*[4,14–18] Actinomycetoma grains are typically white or pale yellow, except those caused by *Actinomadura pelletieri,* which are red to pink.

Some reports use species names that are not currently recognized, leaving in doubt the identification.[19] The modern use of molecular techniques, including polymerase chain reaction, DNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), has both assisted in better identifying and classifying the agents of mycetoma, and made comparisons between current and previous pathogen identification difficult to track.[20–22]

EPIDEMIOLOGY

The oldest description of this disease appears to date back to the ancient Indian Sanskrit text *Atharva Veda,* in which reference is made to *pada valmikam,* translated to mean "anthill foot."[5] More modern descriptions from Madras, India, in the 19th century led to this disease initially being called "madura foot," or *maduromycosis,* a term still used by some today to describe eumycotic mycetoma. Mycetoma is most commonly found in tropical and subtropical climates, with the highest incidence reported from endemic areas in the Indian subcontinent, the Middle East, Africa, and Central and South America. One of the largest current groups of cases is in Sudan. Only scattered reports describe cases originating in the United States, Europe, and Japan. Disease occurs around five times more frequently in males, commonly in the 20- to 40-year-old age range. Disease is more common in agricultural workers and outdoor laborers but is not exclusively seen in rural areas. Disease occurs sporadically throughout most areas of the world, and some postulate that the increased numbers in tropical regions may also result in part from less use of protective clothing, chiefly shoes, in the warmer, poorer endemic regions.

The causative agents of mycetoma vary from region to region and with climate. Worldwide, *M. mycetomatis* is the most common cause of this disease, but *A. madurae, M. mycetomatis,* and *S. somaliensis* are more commonly reported from drier regions, whereas *S. apiospermum* complex spp., *Nocardia* spp., and *A. pelletieri* are more common in those areas with higher annual rainfall. In India, *Nocardia* spp. and *T. grisea* are the most common causes of mycetoma; in the Middle East, *M. mycetomatis* and *S. somaliensis;* in West Africa, *F. senegalensis;* and in East Africa, *M. mycetomatis* and *S. somaliensis.* In Central and South America, *T. grisea* and *Nocardia* spp. are the common causes of mycetoma, and in the United States, *S. apiospermum* complex spp. are the most commonly recovered causative agent.[23]



FIG. 261.1 Mycetoma of the foot. *(From Beneke ES, Rogers AL.* Medical Mycology and Human Mycoses. *Belmont, CA: Star Publishing; 1996.)*

PATHOLOGY AND PATHOGENESIS

Infection follows inoculation of organisms, frequently through thorn punctures, wood splinters, or preexisting abrasions or trauma. After inoculation, these normally nonpathogenic organisms grow and survive through the production of grains (also called granules or sclerotia), structures composed of masses of mycelial fungi or bacterial filaments and a matrix component. The matrix material has been shown to be host derived with some pathogens. In eumycetoma, hyphal elements often have thickened cell walls toward the periphery of grains, potentially conferring protection against the host immune system.[24] Grains are seen in histopathology within abscesses containing polymorphonuclear cells. Complement-dependent chemotaxis of polymorphonuclear leukocytes has been shown to be induced by both fungal (*M. mycetomatis* and *S. apiosperumum* complex) and actinomycotic (*S. somaliensis*) antigens in vitro.[25] Cells of the innate immune system attempt to engulf and inactivate these organisms, but in disease they ultimately fail to accomplish this goal. Abscesses containing grains are seen in association with granulomatous inflammation and fibrosis. *Nocardia brasiliensis* has been shown to be resistant to human neutrophil peptides.[26] Three types of immune responses have been described in response to the grains of mycetoma.[27] The type 1 response is seen as neutrophils degranulate and adhere to the grain surface, leading to gradual disintegration of the grain. Type 2 response is characterized by the disappearance of neutrophils and arrival of macrophages to clear grains and neutrophil debris. Type 3 response is marked by the formation of epithelioid granuloma. This host response does not appear to be able to control infection but likely accounts for the partial spontaneous healing that is seen in the disease.

It is not clear whether persons who develop mycetoma have predisposing immune deficits.[4] Disease does not appear to be more common in immunocompromised hosts, and early studies of immune function in persons with mycetoma have not clearly documented a common deficit.[28,29] Recent work examining genes responsible for innate immune functions has identified polymorphisms that appear to predispose people to this infection, which may be linked with neutrophil function.[30] It has been suggested that the greater frequency of disease in men is not completely explained by increased frequency of exposure to soil and plant material. Progesterone has been shown in vitro to inhibit the growth of *M. mycetomatis, M. romeroi,* and *N. brasiliensis.*[31,32] In the study of *N. brasiliensis,* estradiol limited disease produced in animals.[31]

CLINICAL MANIFESTATIONS

More than 75% of persons with mycetoma have a lesion of a lower extremity, most commonly the foot (70%) (Figs. 261.1 and 261.2). Next



FIG. 261.2 Mycetoma of the leg (seen from back of knee). *(Courtesy Dr. Glenn W. Wortmann.)*

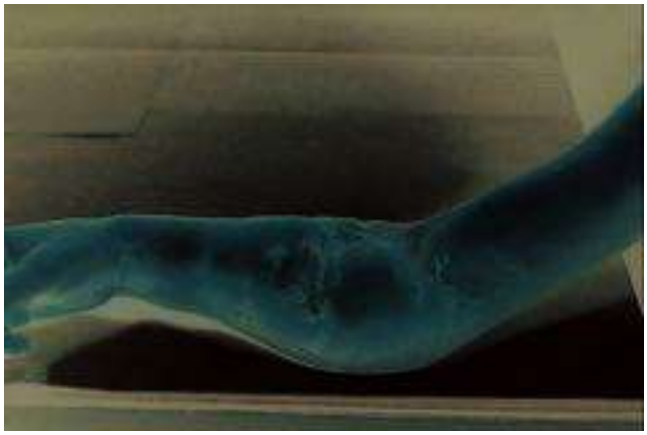

FIG. 261.3 Mycetoma of the arm caused by *Madurella mycetomatis.* *(From Chandler FW, Ajello L. Mycetoma. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997:1035–1044.)*

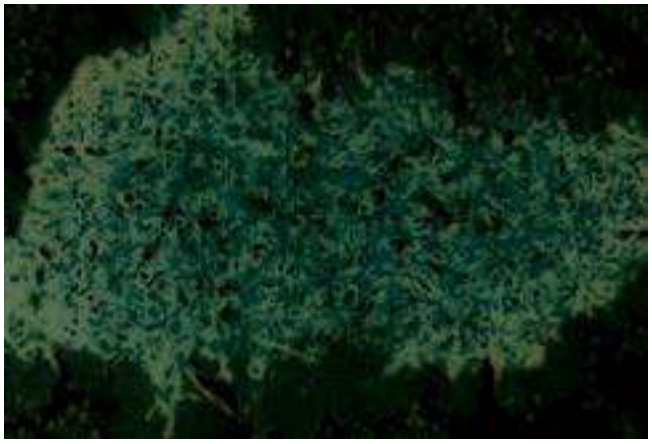

FIG. 261.4 Eumycetoma grain of *Fusarium (Acremonium) falciforme.* (Gomori methenamine silver and hematoxylin and eosin stains.) *(From Chandler FW, Ajello L. Mycetoma. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997:1035–1044.)*



FIG. 261.5 Eumycetoma grain of *Scedosporium apiosperumum–Pseudallescheria boydii* complex. (Hematoxylin and eosin stain.) *(From Chandler FW, Ajello L. Mycetoma. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997:1035–1044.)*

in frequency is disease of the hand (15%), followed by the upper extremities and other areas of the body that may be exposed by carrying firewood or thorny brush, including the upper back and adjacent neck, top of the head, and, rarely, the face (Fig. 261.3). Lesions in more than one anatomic site are extraordinarily rare. Disease begins in most cases as a single, small, painless subcutaneous nodule. This nodule slowly increases in size, becomes fixed to the underlying tissue, and ultimately develops sinus tracts beneath the lesion. These tracts open to the surface and drain purulent material with grains. Grains are several millimeters in diameter and may be seen by close inspection of a gauze bandage covering the sinus tract. Progression to draining sinus tracts can take weeks, months, and even years, occurring more rapidly in actinomycetoma. In a study of patients in India, the average time to presentation with disease from history of probable inciting trauma was 3 years for *N. brasiliensis,* 7 years for *A. madurae,* and 9 years for *T. grisea.*[33]

Disease can affect the skin, subcutaneous tissue, and eventually contiguous bone, spreading along fascial planes. Overlying skin appears smooth and shiny and is commonly fixed to the underlying tissue. Skin may be hypopigmented or hyperpigmented, with signs of both old healed and active sinuses, displaying the cycle of spontaneous healing of older sinus tracts and simultaneous spread of infection to new areas that is typical of this disease. Swelling is often firm and nontender, and the overlying skin is not erythematous. Muscle, tendons, and nerves are generally spared direct infection, but extensive local damage may lead to muscle wasting, bone destruction, and limb deformities. Lymphatic spread is rare, although it may follow surgical manipulation. Hematogenous spread has not been documented. This disease and its effects are generally localized, and thus no signs or symptoms of systemic illness are usually seen in mycetoma unless secondary bacterial infection occurs. When left untreated, disease continues to progress, and bacterial superinfection can lead to increased morbidity from local abscess formation, cellulitis, bacterial osteomyelitis, and, rarely, septic death.

Differential diagnosis includes botryomycosis, chronic bacterial osteomyelitis, tuberculous osteomyelitis, chromoblastomycosis, phaeohyphomycosis, and soft tissue or bone tumor.

DIAGNOSIS

A diagnosis of mycetoma can be made by the classic triad of painless soft tissue swelling, draining sinus tracts, and extrusion of grains. Diagnosis of the causative organism can be made by microscopic observation and culture of a grain. Deep biopsy with histopathology and culture is usually not necessary, although obtaining a deep tissue biopsy avoids the bacterial contamination of surface cultures. Grains may not be seen in any one histopathologic section because they are scattered along the tracts. When a grain is present in the section, its large size and surrounding cluster of neutrophils make it difficult to miss, even without fungal or bacterial stains (Figs. 261.4 through 261.9). Organisms are usually not seen outside the grain. An alternate diagnostic strategy is the ultrasound-guided needle aspiration of grains directly

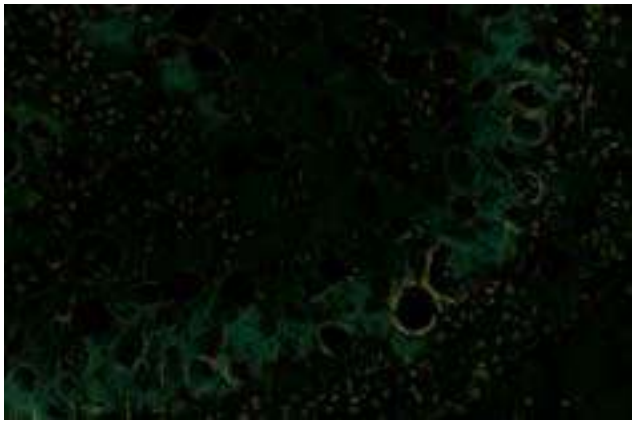

FIG. 261.6 Eumycetoma grain of *Curvularia geniculata*. (Hematoxylin and eosin stain.) *(From Chandler FW, Ajello L. Mycetoma. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997:1035–1044.)*



FIG. 261.7 Eumycetoma grain of *Neotestudina rosatii*. (Gomori methenamine silver and hematoxylin and eosin stains.) *(From Chandler FW, Ajello L. Mycetoma. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997:1035–1044.)*



FIG. 261.8 Actinomycetoma grain. (Gridley stain.) *(From Beneke ES, Rogers AL.* Medical Mycology and Human Mycoses. *Belmont, CA: Star Publishing; 1996.)*

from an unopened sinus tract for microscopic observation and culture.[34] Evaluation of spontaneously extruded grains may not allow diagnosis because these grains are often composed of dead organisms and contaminated with skin surface bacteria that outgrow the mycetomatous agent in culture.

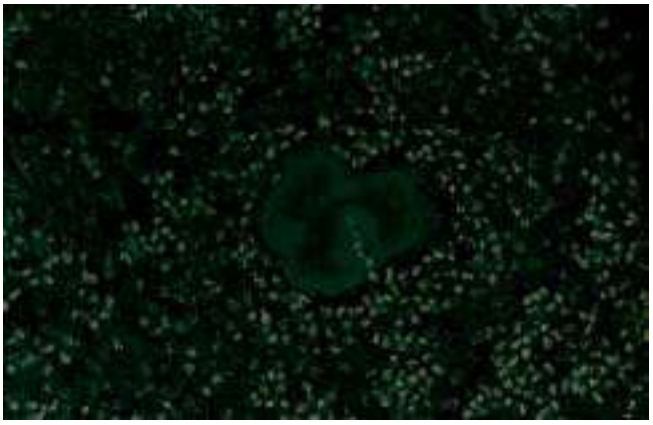

FIG. 261.9 *Nocardia brasiliensis* grain. (Hematoxylin and eosin stain.) *(From Chandler FW, Ajello L. Mycetoma. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997:1035–1044.)*

The grains (or granules or sclerotia) of mycetoma are usually 0.2 to 5 mm in diameter and thus may be observed grossly, without magnification. Microscopic evaluation of crushed grains prepared with potassium hydroxide or stained with Gram stain is useful in differentiating fungal from bacterial causes. On inspection, actinomycetes are recognized by the production of 0.5- to 1-μm-wide filaments and fungi by 2- to 5-μm-wide hyphae. Many reports and reviews have detailed the use of grain color, size, and consistency to diagnose the specific cause of mycetoma, but recovery of the causative agents in culture is more accurate and of greater clinical usefulness when resources are available.

Culture of grains recovered from aspirated material or biopsy specimens can be used to diagnose the specific cause of mycetoma. If extruded grains are used, most experts suggest rinsing these in 70% alcohol or with antibiotic-containing saline solutions to decrease bacterial contamination. Specimens should be cultured on mycologic and mycobacteriologic media and held for at least 4 weeks.

The role of radiology in the management of mycetoma is that of adjunctive assessment of disease extent and involvement of bone, and perhaps long-term follow-up of disease regression or progression. Radiographic studies can help define the extent of disease and aid in the differentiation of mycetoma from other disease. Standard radiographic studies can reveal bony involvement such as periosteal erosion secondary to invasion, osteoporosis, and changes consistent with osteomyelitis, including lytic lesions. Ultrasonography has been used successfully in the differentiation of mycetoma from osteomyelitis or tumor. In a study of 100 patients with foot swelling who underwent ultrasonography before surgical excision, these lesions were found to have characteristics that distinguished them from other diseases.[35] Eumycetoma were found to produce single or multiple thick-walled cavities, without acoustic enhancement, with grains represented as distinct hyperreflective echoes. Actinomycetoma produced similar results, except grains produced fine echoes that were found at the bottom of the cavities. Magnetic resonance imaging (MRI) and computed tomography have also been evaluated in the management of mycetoma. Both modalities provide accurate assessment of disease extent when compared with surgical findings, especially in the soft tissues.[36] When compared directly, computed tomography appears to be more sensitive for detecting early changes consistent with bone involvement. A *dot-in-circle sign* has been described as a potentially specific diagnostic finding seen with MRI.[37,38] The dots are tiny hypointense foci (believed to be grains) within spherical, high-intensity lesions (the circle) surrounded by low-intensity matrix on T2-weighted imaging, which represent granulomas scattered in areas of fibrosis. T1-weighted, fat-saturated, postgadolinium images may also produce this appearance. An MRI grading system has been developed by the Mycetoma Research Centre (Khartoum, Sudan) for use in the diagnosis and management of mycetoma.[39]

The use of serology in the diagnosis and long-term management of this disease has been advocated by some authorities. Of the tests

described, counterimmunoelectrophoresis has been the most commonly used. Lack of standardization or widespread availability limits the use of these tests to centers that see a large volume of such patients. In the United States, the infrequency of the diagnosis and the diverse number of pathogens render serology of no practical use.

THERAPY

Treatment of mycetoma has proved to be difficult and typically includes prolonged courses of antimicrobial agents, often with surgical debulking.[40–42] Surgery alone has a limited role in the treatment of mycetoma.[43] In addition to limb amputation, surgical excision of smaller lesions may be successful as monotherapy. Typically, surgery is employed adjunctively in fungal mycetoma to debulk large lesions after weeks to months of azole antifungal therapy has been given. Because chemotherapy varies for actinomycetoma and eumycetoma, at a minimum the clinician must differentiate whether a mycetoma is caused by actinomycetes or fungi. Ideally, recovery of the causative organism can allow identification of species, and perhaps even susceptibility testing, to guide therapy. Treatment regimens are currently based on expert opinion because no randomized controlled trials have been performed. Duration of therapy is also not defined, and most patients require 3 to 24 months of therapy to obtain an adequate response.

The most commonly described regimens for actinomycetoma include parenteral aminoglycosides combined with oral sulfonamide drugs or dapsone. Therapy with 5-week cycles of oral trimethoprim-sulfamethoxazole (TMP-SMX) (2 double-strength tablets twice daily) with parenteral amikacin (15 mg/kg/day divided into 2 daily doses) given during the first 3 weeks has been reported to produce successful results in all treated patients.[4,14,44] More than half of 56 patients treated with this regimen were cured with one or two cycles; none required more than four cycles, and only one relapsed. The single patient with relapse was treated successfully with three cycles of TMP-SMX combined with netilmicin, an aminoglycoside. Other aminoglycoside-sulfonamide regimens include gentamicin or streptomycin in combination with either TMP-SMX or dapsone.[40,41,44,45] TMP-SMX alone may be successful in the treatment of smaller lesions. Other regimens that have been used include streptomycin with either sulfadoxine-pyrimethamine or rifampin; a combination of penicillin, gentamicin, and TMP-SMX followed by TMP-SMX and amoxicillin; and regimens that include amoxicillin-clavulanate, tetracycline, rifampin, fusidic acid, clindamycin, or imipenem-cilastatin.[a] Success with linezolid therapy has also been reported in one patient.[4,14]

Antifungal therapy for eumycetoma most commonly includes the use of azole antifungals because amphotericin B has not been effective in producing long-term cures. The first-line agent in the treatment of this disease is considered to be itraconazole (200–400 mg/day). Cure rates with this drug are quite variable, and it is suggested that all patients be evaluated for surgical debulking after their disease is controlled with azole therapy, typically after a year of therapy.[41,48,49] In vitro, all three of the newer azoles—isavuconazole,[50] posaconazole, and voriconazole—have good activity against many of the causative agents of eumycetoma. Case reports of successful therapy with voriconazole have been published,[51–53] as has a small case series of successful therapy for previously azole-refractory disease that responded to posaconazole.[54] Because of its susceptibility pattern, mycetoma secondary to the *S. apiospermum* complex should be treated with voriconazole. Successful therapy with terbinafine, an allylamine antifungal, has also been reported. Improvement or cure was seen in 16 of 20 patients who completed 24 to 48 weeks of terbinafine therapy (500 mg twice daily).[55] There are very little safety data on doses this high. In vitro, terbinafine has limited activity against *M. mycetomatis* compared with that observed with itraconazole, ketoconazole, or posaconazole.[56]

PREVENTION

No preventive vaccine is available against any of the causative agents of mycetoma. Disease prevention is best accomplished by reduction of the incidence of the traumatic inoculation of the causative organisms. Wearing shoes and clothing to protect against splinters and thorn pricks should be stressed. Debilitating disease can be prevented by early identification and treatment of lesions, usually with minor surgery and chemotherapy.

[a]References 4, 14, 40, 41, 46, 47.

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262 Cryptococcosis (*Cryptococcus neoformans* and *Cryptococcus gattii*)

John R. Perfect

SHORT VIEW SUMMARY

Life Cycle
- The life cycle represents both asexual (clinical) and sexual (recombination) cycles, with the impact of the sexual cycle on pathogenesis.

Taxonomy
- There are 19 cryptococcal species with two major pathogenic species, *Cryptococcus neoformans* and *Cryptococcus gattii.*
- At present, the following taxonomic divisions have been proposed, but this area continues to evolve in the molecular era: *C. neoformans* var. *grubii* (serotype A), with five genotypes (VNI, VNII, VNBI, VNBII, and the hybrid VNIII); *C. neoformans* var. *neoformans* (serotype D or genotype VNIV); and five other cryptic species: *Cryptococcus gattii, Cryptococcus bacillisporus, Cryptococcus deuterogattii, Cryptococcus tetragattii,* and *Cryptococcus decagattii* (serotypes B/C or VGI–VGV).[1]

Identification
- Biochemical tests, including urease production, melanin formation, and appearance of a capsule
- Antigen tests, cultures, DNA-based tests, or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)

Ecology
- *C. neoformans* (serotypes A and D) found in pigeon guano or associated with certain trees (mopane trees)
- *C. gattii* found around trees from eucalyptus to coniferous species (landscape of niche may be changing related to climate changes)

Epidemiology
- Not a routine constituent of human microbiota but can colonize humans without evidence of disease
- Widespread asymptomatic infections occur in the exposed populations
- Risk factors for disease: acquired immunodeficiency syndrome (AIDS), corticosteroid treatment, transplantation, cancer, monoclonal antibodies (from anti–tumor necrosis factor to anti-CD52), sarcoidosis, autoantibodies to granulocyte-macrophage colony-stimulating factor, idiopathic CD4 lymphocytopenia, and new tyrosine kinase inhibitors for cancer treatment; identifies primarily cell-mediated immunity defects but humoral perturbations may also be a factor (see Table 262.1)
- Antiretroviral therapy (ART) impact on appearance of clinical cryptococcosis is impressive in patients with AIDS. However, even widespread availability of ART does not eliminate high numbers of cryptococcosis in some populations.
- A million new cases of cryptococcosis per year, with more than 600,000 deaths worldwide at the peak of the human immunodeficiency virus (HIV) epidemic; now reduced although not yet eliminated with ART availability, with an estimated present census of over 200,000 new cases per year worldwide with over 100,000 deaths per year.

Pathogenicity
- Virulence factors (examples include but not limited to: capsule, melanin, high temperature growth, urease, phospholipase)
- Many understandings of cryptococcal disease at the genetic and molecular level have been acquired.

Host Responses
- Efficient protection requires intact and coordinated cell-mediated, innate, and humoral immunity networks.

Pathogenesis
- Three factors: (1) host defenses, (2) virulence of strains, and (3) size of infectious inocula
- Cryptococcosis is considered primarily a reactivation clinical disease but disease can occur with the initial infection.

Clinical Manifestations (see Table 262.2)
- Major body sites for infection are the central nervous system and lung.
- Other body sites with unique infection considerations are the skin, prostate, peritoneum, and eye.
- Cryptococci have produced infection within any organ of the human body.
- Disease primarily depends on host immunity—either too little or too much (immune reconstitution inflammatory syndrome [IRIS]).

Laboratory Diagnosis
- Microscopy: India ink and histopathologic stains, including a general fungal Gomori methenamine silver stain, or the specific capsular polysaccharide stains (mucicarmine or alcian blue)
- Culture of cerebrospinal fluid (CSF), blood, bronchoalveolar lavage
- Cryptococcal antigen detection in CSF and serum is sensitive and specific as determined by either latex agglutination assay, enzyme-linked immunosorbent assay, or a lateral flow assay.

Management
- The Infectious Diseases Society of American Guidelines in 2010 established road maps for therapies and strategies (https://academic.oup.com/cid/article/50/3/291/392360); most of its principles are still intact but a few updates can be made.
- Direct antifungal drug resistance uncommonly arises during therapy, but relapse/persistent isolates should have in vitro antifungal susceptibility testing performed and compared to original isolate.
- Treatment strategies are outlined in this chapter for (1) cryptococcal meningitis in AIDS patients, transplant recipients, and non-AIDS, nontransplant patients and (2) pulmonary infections.
- Treatment has been best studied for cryptococcal meningitis in HIV-infected patients (2018 US Department of Health and Human Services guidelines at https://aidsinfo.nih.gov/guidelines/html/4/adult-and-adolescent-oi-prevention-and-treatment-guidelines/333/cryptococcosis). Treatment is initiated with amphotericin B, preferably with the liposomal formulation at 3 to 4 mg/kg daily, the alternatives being the lipid complex at 5 mg/kg or conventional amphotericin B at 0.7 mg/kg/day. Any one of these can be used plus flucytosine 25 mg/kg every 6 hours (100 mg/kg/day with normal renal function) for at least 2 weeks and until clinically improved. In cryptococcal meningitis, delaying ART during these 2 weeks may prevent IRIS from complicating management and increased early deaths. Patients who respond to induction-phase combination antifungal therapy may then be switched to fluconazole, 400 to 800 mg/day, for 8 to 10 weeks as a consolidation phase. Finally, a suppressive phase is begun with fluconazole 200 mg once daily. Antifungal therapy in HIV-infected patients can be stopped after 1 to 2 years in patients who respond to ART with a CD4 count above 100/μL for at least 3 months, a nondetectable viral load, and a negative or low serum cryptococcal antigen. In non–HIV-infected patients who improve, therapy is also generally extended for 1 year.
- Complications of cryptococcal meningitis requiring special attention are (1) early increased intracranial pressure, which can lead to blindness, permanent dementia, and death; (2) development of hydrocephalus, which may require placement of a ventriculoperitoneal shunt; and (3) IRIS, which may be mistaken for therapeutic failure.

Cryptococcus neoformans and *Cryptococcus gattii* are encapsulated, heterobasidiomycetous fungi that have progressed from being rare human pathogens, with just over 300 cases of cryptococcosis reported in the literature before 1955, to becoming a common worldwide opportunistic pathogen as immunocompromised human populations have dramatically increased over the past 2 decades.[2] Cryptococcosis crosses the entire spectrum of patient populations, from the apparently immunocompetent host without an underlying disease to those severely immunocompromised from infection with the human immunodeficiency virus (HIV), an organ transplantation, or a malignancy and its treatment.[3,4] Furthermore, it has a wide range of clinical presentations, which can vary from asymptomatic colonization of the respiratory airways to dissemination of infection into any part of the human body. *Cryptococcus* enters the host primarily through the lungs but has a special predilection for invading the central nervous system (CNS) of the susceptible host. Pulmonary infections are common and may have multiple clinical presentations and management issues. On the other hand, cryptococcal meningitis represents the primary life-threatening infection for this fungal pathogen and has required the most clinical attention.

HISTORY

The first identification of *Cryptococcus* from an environmental source was made by Sanfelice in 1894, from peach juice in Italy.[5] Within a year, Busse and Buschke[6] independently reported the first human case of cryptococcosis in a young woman who developed a chronic ulcer over the skin above her tibia, with yeasts identified in the tissue and later at autopsy. This yeast was also found to have spread to multiple organs in her body. By 1914, Versé described a human case of cryptococcal meningitis,[7] and in 1916 Stoddard and Cutler gave a complete description of the CNS pathology for this infection, including in their report that the yeast forms had surrounding areas of clearing within the tissue.[8] This finding was the first description of the signature structure for this yeast, the polysaccharide capsule. During the early years of clinical cryptococcosis, the names of this yeast were several and included *Saccharomyces neoformans, Cryptococcus hominis,* and *Torula histolytica*. In 1950 Benham attempted to categorize these poorly defined yeasts based on morphology, fermentation, and serologic studies.[9] She named one yeast *C. hominis* and its disease "cryptococcosis." The name was later changed to *C. neoformans* based on temporal priority because Sanfelice had first used the species name of *neoformans.* However, despite Benham's proposal, it took another 25 years before "cryptococcosis" became the primary nomenclature for this infection rather than "torulosis." In 1976 Kwon-Chung discovered and characterized the sexual stage of *Cryptococcus,* and the teleomorphs were named *Filobasidiella neoformans* and *Filobasidiella bacillispora.*[10] It was proposed in 2002 that there be two varieties—*C. neoformans* var. *neoformans* (serotype D) and *C. neoformans* var. *grubii* (serotype A)—and another species, *Cryptococcus gattii* (serotypes B and C).[11] In 2005 the genome sequence of *C. neoformans* was released,[12] and now hundreds of strains, including strains of *C. gattii,* have been sequenced for comparisons[13–15] as we have entered the genome age for fungal pathogen discoveries. With whole-genome sequence information of over a thousand strains, the *Cryptococcus* species complex has now been further divided into genotypes and species, and taxonomic issues are dynamic.[1,16,17]

MYCOLOGY

Life Cycle and Genetics

The life cycles of both *C. neoformans* and *C. gattii* involve two distinct forms: asexual and sexual. The asexual stage exists as encapsulated yeast cells that reproduce by simple, narrow-based budding. The haploid (occasionally diploid in nature), unicellular yeasts are the primary forms recovered from environmental sources and human infections. The asexual or yeast forms represent the primary structures seen in host tissue and recovered from cultures during clinical disease. However, this fungus has a more complex life cycle, with a bipolar mating system that can be observed under certain in vitro conditions and even on plants.[18] For example, yeasts exist in one of two mating types, "alpha" or "a." When two strains of opposite mating types are physically placed together on specific, nutrient-poor media such as V-8 juice agar, the cells undergo conjugation, producing filaments with true clamp connections. At the ends of these filaments, basidia form, and within these basidia, meiosis occurs and chains of basidiospores are produced. The 1- to 2-micron basidiospores, with their size and shape, have been hypothesized to be the infectious propagules.[19] They may deposit in the lung, where the spores rapidly convert to yeasts. However, the sexual stage at present remains a laboratory observation, and the sexual structures, such as basidiospores, have yet to be identified in nature, but clearly, sexual recombination appears to be occurring in nature.[20] Studies have made great progress in precisely understanding the molecular signaling networks that control the sexual cycle, and in some cases genes in these mating pathways have been linked to both morphogenesis and virulence of the yeast.[21]

In the 1980s, an interesting epidemiologic observation was made and confirmed by others that in most areas of the world, more than 95% of environmental and clinical *C. neoformans* isolates appear to contain only the alpha mating locus.[22] The reason for this genetic bias remains unclear, but two factors may be important. First, it has been discovered that under certain environmental conditions *C. neoformans* undergoes haploid fruiting.[23] Haploid fruiting occurs when haploid yeast strains under specific conditions produce hyphae and basidiospores without mating and the exchange of genetic information through meiosis. It is possible that this haploid fruiting with sporulation allows wider dissemination of the fungus in the environment and thus more environmental exposure, leading to more clinical disease. Second, it has now been shown that sexual reproduction can occur between partners with the same mating type,[24] and this allows recombination and improved fitness of progeny. The alpha mating strains are much more likely to produce haploid fruiting structures or perform same-sex matings than the "a" mating strains. An alternative explanation for the alpha mating locus bias is that the approximately 100-kilobase locus or its adjacent genomic areas contain virulence genes that make these mating-type strains more fit in the environment (or in the host). Initial studies with congenic strains of *C. neoformans* var. *neoformans* differing primarily in the mating locus did suggest that the alpha mating strain was more virulent in mice.[24] On the other hand, the alpha and "a" mating loci have been identified in *C. neoformans* var. *grubii,* and experiments with two congenic strains in this variety differing only in the mating locus appear to be identical in virulence.[25] It is still uncertain how much the mating loci types contribute to the virulence of this yeast, and the alpha mating–type bias is not yet precisely explained. However, as witnessed in the Vancouver Island *C. gattii* outbreak, sexual recombination in nature between strains can play an important role in the evolution of pathologic fitness for strains.[20] Furthermore, in certain areas within Botswana, environmental and clinical isolates do have similar numbers of both mating types,[14] so that simple exposure may be cause for the mating-type bias.

Taxonomy

The genus *Cryptococcus* comprises 19 species, loosely characterized as a variety of encapsulated yeasts. There continues to be occasional reports of human infections with several of these non-*neoformans* and non-*gattii* species, such as *Cryptococcus albidus* and *Cryptococcus laurentii.*[26–28] However, such clinical reports are uncommon, and the disease is occasionally poorly documented with these rare cryptococcal species. Therefore any human infection with a cryptococcal species other than *C. neoformans* or *C. gattii* needs rigorous histopathology and cultural proof of invasive disease.

For several decades, *C. neoformans* strains had been grouped into two varieties that included five serotypes based on their capsular structure. *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* included strains with serotypes A, D, and AD, and *C. gattii* contained strains with serotypes B and C. The serotype classification (A–D) describes antigenic differences in the structure of the polysaccharide capsule; these differences are detected by antibodies from rabbit sera[29] or by specific monoclonal antibodies.[30,31]

The stable taxonomic classification of these varieties and serotypes has now evolved through new genomic analyses, and several new changes have been proposed.[32] With the use of specific DNA typing methods and other physiologic factors, it has been proposed that the serotype A strains be classified into a separate variety, *C. neoformans* var. *grubii.*[33]

Serotype D isolates are to remain in the variety *neoformans.* The varietal status of serotype AD hybrid strains has not been proposed. It has also become clear that most of the serotype AD strains represent stable diploid strains, possibly occurring as incomplete genetic crosses between varieties *neoformans* and *grubii.* However, genetic mapping of A and D strains suggests that these varieties biologically diverged from each other more than 18 million years ago.[34] Thus with whole-genome sequencing of many cryptococcal strains, *C. neoformans* var. *grubii* (serotype A) can be divided into five genotypes (VNI, VNII, VNBI, VNBII, and VNIII), with a separate variety, *C. neoformans* var. *neoformans* (serotype D), as genotype VNIV. *C gattii* (serotypes B and C) has been proposed to be divided into five cryptic species: *Cryptococcus gattii*, *Cryptococcus bacillisporus*, *Cryptococcus deuterogattii, Cryptococcus tetragattii,* and *Cryptococcus decagattii* (genotypes VGI–VGV).[7]

As rapid advances in the understanding of genetic population diversity are made among the *Cryptococcus* complex during the genome era, taxonomic relationships and nomenclature will remain in some flux. It has been suggested that the group be designated "*Cryptococcus* complex species" until further widespread genetic and phenotypic studies are completed and analyzed.[16] However, at present, for clinicians the standard serotype classification used for half a century and the split into two varieties, var. *neoformans* and var. *grubii,* and two species, *C. neoformans* and *C. gattii,* still remain useful nomenclature for describing the clinical strain differences in epidemiology, pathogenesis, and clinical features. As further research continues, the taxonomic name of a strain may become less important to the clinician than the identification of its specific genetic structure. In this chapter, the terms "var. *grubii*" and "var. *neoformans*" will continue to be appended to the designation of serotype A and serotype D, respectively, even though serotyping sera are no longer available, because so much of the literature is based on serotype.

The anamorph (yeast or asexual stage) dominates clinical discussion of this encapsulated yeast. On the other hand, the teleomorph, with its more complex structure and its genome sequences, places this fungus within the basidiomycete family, and its teleomorph genus name is *Filobasidiella.* Thus the teleomorph of serotypes A and D strains is called *Filobasidiella neoformans* and the teleomorph of serotypes B and C strains is designated *Filobasidiella bacillispora;* however, these teleomorphic names are not used in clinical practice.

Identification

On most routine laboratory agar media, colonies of *C. neoformans* and *C. gattii* appear within 48 to 72 hours after plating a specimen. Some selective fungal media containing cycloheximide inhibit the growth of this yeast and thus should not be used. For blood cultures, the lysis-centrifugation method works well for isolating *Cryptococcus* but is no longer necessary because automated blood culture methods can reliably detect cryptococcemia, which is commonly observed in severely immunosuppressed patients such as patients with acquired immunodeficiency syndrome (AIDS) and disseminated disease.[35] In some populations of the world with high rates of HIV infection, cryptococcemia is a common finding in patients during the workup of fever with blood cultures. However, cryptococcemia rarely produces symptoms of hypotension or septic shock.

On agar plates, the yeast colony grows as a white-to-cream–colored, opaque colony several millimeters in diameter. The colonies typically become mucoid with prolonged incubation, reflecting increased polysaccharide capsule formation. Colonies occasionally develop sectors that differ in pigmentation or exhibit morphologic changes (e.g., smooth or wrinkled). In fact, *C. neoformans* has been shown to possess the ability to produce a morphologic switching colony phenotype, which explains the variety of colony shapes in some strains and emphasizes the plasticity of the cryptococcal genome.[36,37] The optimal environmental growth temperature for the majority of *C. neoformans* strains is between 30° and 35°C, with a maximum tolerated temperature for most strains at 40°C. Serotype A strains tend to tolerate higher environmental temperatures better than serotype D and serotype B/C strains.[38] *C. neoformans* and *C. gattii* strains generally grow well at 37°C, with doubling generation times of 3 to 6 hours, and this high-temperature growth characteristic is a primary virulence phenotype that separates them from other cryptococcal species that generally do not either grow or survive well at mammalian body temperatures and thus are rarely found to be human pathogens.

In the clinical laboratory, *C. neoformans* and *C. gattii* can be readily differentiated from other yeasts on the basis of their morphology and biochemical tests. The specific identification can be confirmed by a battery of biochemical tests available commercially in kits.[39,40] Recently the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry system has been effective in identifying and distinguishing both *C. neoformans* and *C. gattii* yeasts.[41] However, there are three direct tests that predict that a yeast may be *C. neoformans* or *C. gattii.* First, placing the yeast into an India ink preparation for microscopy may reveal the encapsulation of the yeast. The capsule is generally better seen in direct clinical specimens from the host and may not be as apparent in wet mounts made from in vitro cultures. This finding occurs because capsule production is induced by certain environmental cues, such as elevated carbon dioxide concentrations, serum, urea, or limited iron conditions. In fact, the mammalian host environment provides an ideal environment for capsule production.

Second, a rapid urease test is positive for most *Cryptococcus* species. *Cryptococcus* species, unlike *Candida* species, possess urease, an enzyme that hydrolyzes urea to ammonia and increases the ambient pH. A positive urease test can be detected in the laboratory within minutes.[42] Several nonpathogenic yeasts can produce abundant urease, and *Trichosporon* species may be weakly urease positive.

Third, *C. neoformans* is one of the few yeast species that possesses prominent activity of laccase,[43] an enzyme that allows the conversion of diphenolic compounds into melanin. Detection of this unique biologic characteristic is possible with media containing niger seed (birdseed), caffeic acid, or dopamine. Yeast colonies that turn brown to black on these special agars are identified as melanin positive. In a clinical specimen, such a yeast colony will likely be either *C. neoformans* or *C. gattii.* However, other cryptococcal species in the general environment also possess laccase, but these selective agar assays are still particularly helpful when attempting to identify pigmented cryptococcal colonies from environmental samples contaminated by other fungi or bacteria species, or both.

Histopathologically, *C. neoformans* and *C. gattii* have several characteristic features. In most clinical cases, *Cryptococcus* in tissue exhibits a prominent capsule. Microscopically, most clinical isolates appear as spherical, narrow-based, budding, encapsulated yeast cells in both tissue and culture. Short hyphal or pseudohyphal structures may exist in vivo, or under certain stress conditions in vitro, but these structures are rarely observed unless certain in vitro nutrient conditions for mating or haploid fruiting are met. The yeast cells vary in size from 5 to 10 microns in diameter, and they exhibit single or multiple buds. Because the buds are readily detached from their parental cells, the majority of yeast cells in both tissue and culture lack buds. In tissue, *Cryptococcus* has the ability to produce large (titan) cells of 50 to 100 μm, which possess features such as aneuploidy and large antiphagocytic capsules that promote their survival.[44] Finally, the size of the capsule under direct observation dynamically varies with the individual strain and its immediate environment.

There are three methods for identifying the four serotypes. First, commercial antibodies had been used to distinguish differences in the capsular structures but presently are not available. Second, there are known differences between the biochemical pathways of the serotypes. Most serotypes B and C (*C. gattii*) isolates assimilate glycine as a sole carbon source, whereas serotypes A and D (*C. neoformans*) isolates generally do not. An agar containing L-canavanine, glycine, and bromothymol blue (CGB) uses a color change to separate serotypes A and D from B and C. Third, analysis of DNA base composition is extremely accurate. Comparison of sequenced genomes of these serotypes shows an approximately 6% to 8% overall difference in nucleotide sequences between serotype A and D strains and an even greater difference between these strains and serotype B and C strains.[13] A variety of molecular methods, including random amplified polymorphic DNA (RAPD), karyotypes, polymerase chain reaction (PCR) fingerprinting, multilocus sequencing typing (MLST),[45] and direct sequencing of strains, can be used to readily identify an isolate as belonging to a certain serotype or clade.[46,47] Furthermore, strains are classified into

specific genotypes by PCR fingerprint patterns and sequence. There are presently multiple distinct genotypes with VNI, VNII, VNBI, VNBII, VNBIII, and VNIV for *C. neoformans* and VGI to VGV for *C. gattii,* and within these genotypes there are subgroup genotypes such as VGIIa to VGIIc, identified by MLST,[45] which possess epidemiologic and maybe pathobiologic relevance.[48] Along with genome sequencing, MALDI-TOF mass spectrometry has also become a precise and facile method for distinguishing cryptococcal strains in the laboratory.[41]

ECOLOGY

C. neoformans and *C. gattii* are saprobes in nature.[49] *C. neoformans* was first described in fruits, but after years of investigation it is clear that it also has an environmental niche or habitat associated with certain trees and rotting wood. A second consistent finding is that *C. neoformans* has frequently been isolated from soil contaminated by guano from birds.[50,51] On the other hand, *C. gattii* clearly has an environmental association with trees, from eucalyptus to a variety of coniferous trees, but not bird guano.[52]

Cryptococcus neoformans Serotypes A, D, and AD (*grubii* var. or *neoformans* var.)

In the 1950s, Emmons first isolated *C. neoformans* from soil and from the droppings and nests of pigeons.[50,51] Since the original reports, the fungus has been found in soil samples from around the world. However, the soils most enriched in *C. neoformans* are those that are frequented by birds, especially pigeons, turkeys, and chickens. Guano from other birds, such as canaries and parrots, has also yielded the yeast. Despite this consistent ecologic observation, the precise link between the yeast's natural habitat and the birds is still not certain. Occasionally, birds develop disease that involves *C. neoformans,* but this is relatively unusual. The resistance of birds to disease may result from their very high body temperature, which is not conducive to growth of *C. neoformans,* and possibly even their innate protective immunity. However, the yeasts may transiently colonize the gastrointestinal tract of the birds. The most common environmental niche for this species remains rotting vegetation or wood of certain trees, such as coniferous trees and particularly mopane trees. The birds may simply represent vectors, spreading the fungus from these vegetations into the soils and dusts of human traffic.

Cryptococcus gattii Serotypes B and C

Unlike *C. neoformans, C. gattii* has never been cultured from bird guano. Furthermore, there appears to be a certain geographic limitation to the occurrence of infections with this variety. With this knowledge base, investigators were initially able to culture *C. gattii* from vegetation around and associated with the river red gum trees (*Eucalyptus camaldulensis*) and forest red gum trees (*Eucalyptus tereticornis*) in Australia.[53] Because these trees were exported to other areas of the world where *C. gattii* is also observed, it was reasoned that *Eucalyptus* species may be a vector for infection. It was suggested that the poorly encapsulated yeasts or basidiospores might be released in relationship to the flowering of these trees, but this has not yet been proved. However, despite the association of these trees with *C. gattii,* the outbreak of cryptococcosis on Vancouver Island, British Columbia, revealed that other trees such as firs, maples, and oaks may also be an ecologic niche for specific strains of *C. gattii.*[54] It is proposed that these new geographic locations and specific environmental niches for *C. gattii* could be due to recent climate changes.[52]

Another ecologic factor that may be important to the human pathogenicity of this fungus is its association with other organisms. For example, it has been found in soil associated with a variety of bacteria, amebas, mites, worms, and sow bugs. The stress of this biotic area with its abundant predatory scavengers may have selected for a yeast species that can survive such harsh conditions. In fact, studies have shown that *C. neoformans* can survive within amebas, which in some respects may provide an environment similar to that in a human macrophage.[55] Furthermore, nonpathogenic cryptococci can act as food for the nematode *Caenorhabditis elegans,* but *C. neoformans* can actually kill the worm, and several invertebrate models (worms, grubs, and amebae) have been used in studies of cryptococcal pathogenesis and treatment experiments.[56,57]

EPIDEMIOLOGY

C. neoformans is not generally considered to be a routine constituent of the human microbiota. There are clinical reports of *Cryptococcus* being isolated from nonsterile body sites on patients with no signs or symptoms of cryptococcosis.[58] It can also be detected as a commensal in cats and dogs. Furthermore, endobronchial colonization is more frequently observed in humans in the presence of an underlying chronic pulmonary disease. When *C. neoformans* is isolated from nonsterile clinical specimens, the clinician must examine the patient for evidence of disease and analyze risk factors for the potential development of disease before planning further management strategies. Several methods have been used to study the existence of prior infection with *C. neoformans* without evidence for disease. Research has shown that patients with cryptococcosis have delayed hypersensitivity to cryptococcal antigens,[59] and the prevalence of positive skin test reactions in pigeon fanciers and laboratory workers engaged in research activities with this yeast has been reported to be high.[60] Unfortunately, there is no established skin test for routine clinical use in patients with cryptococcosis today, and this reduces our ability to assess the magnitude of this infection. However, most adults possess specific antibodies to *C. neoformans* antigens; for instance, in New York City, most children acquire antibodies to cryptococcal antigens before the age of 10 years.[61,62] These observations suggest that there are frequent asymptomatic infections. These studies examining serologic evidence of cryptococcal exposure in children do emphasize that in some respects there are certain areas of high exposure to this fungus and other geographic sites with much lower exposure.[63] Although exposure to this yeast is likely limited in certain areas of the world, cryptococcal disease has been reported worldwide.

The vast majority of patients with symptomatic disseminated cryptococcosis have a clearly identified underlying immunocompromised condition (Table 262.1). The most common underlying conditions worldwide include AIDS, prolonged treatment with corticosteroids, organ transplantation, advanced malignancy, diabetes, and sarcoidosis. In fact, occurrence of cryptococcosis may identify an underlying idiopathic CD4 lymphocytopenia[64] or the development of certain autoantibodies, or be associated with the use of the specific immune-modifying monoclonal antibodies, such as alemtuzumab, infliximab, etanercept, and adalimumab.[65,66] Finally, it has been estimated that approximately 20% of patients who have cryptococcosis without HIV infection have no apparent underlying disease or risk factor.[67] The genetic susceptibility to cryptococcal disease has been clearly determined in murine models but needs more studies in humans. However, recent studies on associations with polymorphisms in immunoglobulin genes and mannose-binding protein gene might be the start of understanding the group of patients with cryptococcal disease without an apparent immunosuppressive event,

TABLE 262.1 Conditions Known to Be or Possibly Associated with Predisposition to *Cryptococcus neoformans* Infections

HIV infection
Lymphoproliferative disorders
Sarcoidosis
Corticosteroid therapy
Hyper-IgM syndrome
Hyper-IgE syndrome
Autoantibodies to GM-CSF
Monoclonal antibodies (e.g., infliximab, entanercept, adalimumab, alemtuzumab)
Systemic lupus erythematosus[a]
HIV-negative CD4$^+$ T-cell lymphocytopenia
Diabetes mellitus[b]
Organ transplantation[a]
Peritoneal dialysis
Cirrhosis

[a]Immunosuppressive therapy may account for the predisposition.
[b]Diabetes mellitus has historically been considered a risk factor for cryptococcal infection. However, diabetes is a common disease, and it is unclear whether this condition is truly a specific risk factor for cryptococcosis.
GM-CSF, Granulocyte-macrophage colony-stimulating factor; *HIV,* human immunodeficiency virus; *IgE,* immunoglobulin E; *IgM,* immunoglobulin M.
Modified from Casadevall A, Perfect JR. Cryptococcus neoformans. *Washington, DC: ASM Press; 1998:410.*

or how these associations might add to the risk of an immunosuppressed individual.[68–70] Furthermore, recent studies have suggested a link between autoantibodies to the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) and risk for cryptococcosis.[71,72]

The best estimates for rates of cryptococcosis in the United States in the pre-AIDS era predicted an overall incidence of 0.8 case per 1 million persons per year. In 1992, during the peak of the AIDS epidemic in the United States, the rate reached almost 5 cases of cryptococcosis per 100,000 persons per year in several large cities. In the mid-1990s, before highly active antiretroviral therapy (ART) but with widespread use of fluconazole for oral candidiasis, the rate was reduced, and it stabilized in the cities at approximately 1 case per 100,000 persons per year.[73,74] With the widespread use of ART in developed countries by the beginning of the 21st century, the incidence of cryptococcosis has declined further and appears to have reached a stable number of new infections.[75] In the AIDS population in developed countries, it now generally represents an infection that identifies a disadvantaged patient or an untreated and undiagnosed HIV infection. Thus cryptococcosis in patients with AIDS identifies a group as having or wanting less access to medical care, or both.[76]

In less medically resourced countries with major ongoing epidemics of HIV, such as in sub-Saharan Africa, cryptococcosis reached high prevalence. Some reports estimate that 15% to 45% of individuals with advanced HIV infection succumb to cryptococcosis.[77,78] In a population-based surveillance study for cryptococcosis in an antiretroviral-naïve South African population, the overall incidence in HIV-infected patients was 95 cases per 100,000 patients, and those with AIDS had a rate of 14 cases per 1000 patients.[79] A recent sobering study in Botswana with generally excellent access to ART showed an initial drop in cases of cryptococcosis, but over the last 4 to 5 years the incidence has stabilized at a still very high level.[80] A general report from the US Centers for Disease Control and Prevention emphasized that at the peak of the AIDS epidemic, there were annually almost a million new cases of disseminated cryptococcosis with more than 600,000 deaths per year, and in sub-Saharan Africa mortality is higher than with infections caused by tuberculosis in similar areas.[3] In many African medical centers, cryptococcosis represents the most common cause of culture-proven meningitis, even surpassing *Neisseria meningitidis* and *Streptococcus pneumoniae* meningitis.[81] In fact, the risk of cryptococcosis appears higher for African-born individuals even when they move to industrialized nations.[82] Increasing cases of cryptococcosis have consistently followed the pattern of HIV infections, and in countries such as Thailand, blood cultures done before widely available ART frequently contained this yeast. However, with increased availability of ART in sub-Saharan Africa and Asia, the magnitude of these observations has changed.[83] An updated assessment of cryptococcosis during widespread ART now predicts over 200,000 cases per year with over 100,000 deaths per year from cryptococcosis.[84] However, the incidence of cryptococcosis will not be dramatically reduced further secondary to undiagnosed HIV infections, noncompliance with ART, or antiretroviral drug resistance.

The varieties or species of *Cryptococcus* that are identified as causing disease differ by geographic location and by whether the patient has a concomitant HIV infection. Before the AIDS epidemic, Kwon-Chung and Bennett found that at least 80% of clinical isolates worldwide were *C. neoformans* serotype A (var. *grubii*).[85] *Cryptococcus gattii* serotype B was almost exclusively found in tropical and subtropical areas, such as southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa. Serotype C was rare in all localities but seemed to follow the same geographic distribution as serotype B. However, with outbreaks in Vancouver and the Pacific Northwest in the United States and even isolated reports of *C. gattii* infections east of the Mississippi River and in Europe,[86,87] the epidemiology is clearly changing with this species.[52] *C. neoformans* serotype D was predominantly isolated from Europe, especially Denmark, Germany, Italy, France, and Switzerland, and some strains of this variety were found in the United States.[85,88,89] In AIDS patients, the vast majority of isolates are serotype A, although serotype D has constituted a significant percentage of isolates in several areas of France. A small, measurable portion of cases in AIDS patients have been reported to be caused by *C. gattii* and specifically by VGIII and VGIV genotypes.[90–92] On the other hand, the numbers of *C. gattii* infections remain small even in areas where this species was commonly observed to cause disease in the pre-AIDS era, and clinical presentations and outcomes remain similar to *C. neoformans* infections in a comparable risk group.[93,94]

Cryptococcosis has a measurable rate of infection in two other major risk groups: cancer patients and recipients of solid-organ transplants. Since the 1950s, it has been known that patients with lymphoproliferative disorders and certain hematologic malignancies, such as chronic lymphocytic leukemia, were at higher risk than the general population for cryptococcosis.[95–98] A retrospective analysis of case reports from a single large cancer center from 1989 to 1999 reported that the incidence of cryptococcosis was 18 cases per 100,000 admissions, and the occurrence is predicted to increase with further frequent use of cell-mediated immune inhibitors, such as alemtuzumab, ibrutinib, and fludarabine, in the management of certain malignancies.[99,100] On the other hand, checkpoint inhibitors to programmed cell death protein-1 for cancer treatment may actually be preventive with their actions against cryptococcosis.[101] Because of their profound and prolonged immunosuppression, organ transplant recipients have also been a prime target for this infection. In one cohort, cryptococcosis occurred in 2.8% of all solid-organ transplant recipients.[102] Kidney and liver transplant recipients appear to have the highest risk for cryptococcosis.[102–104] In contrast, in bone marrow transplant recipients, who have a high incidence of fungal infections, cryptococcosis is not common.[105] In rare circumstances, the transplanted organ (e.g., cornea, kidney, lung) has been shown to carry the cryptococcal infection into a susceptible recipient.[106–108] This clinical scenario represents one of the few instances of person-to-person transmission of this infection.

Sarcoidosis, with or without corticosteroid therapy, predisposes to cryptococcosis and can be a diagnostic dilemma. The lung, skin, bone, and CNS lesions of the two diseases overlap clinically and by histopathology. Despite uncertain pathophysiology, diabetes as an underlying disease or cofactor is frequently mentioned in those patients without HIV or transplant recipient risk factors in most reviews.

There has always been a small but consistently higher rate of cryptococcosis in males than in females, and at times there are subtle differences in immunologic responses between sexes, suggesting hormonal influences on cryptococcal disease.[109,110] Cryptococcosis can occur before puberty, but even in children with several known risk factors the incidence is uncommon, and in an area of high HIV prevalence only 2% of cryptococcosis cases were in children.[111] Interestingly, there have been several reports of cryptococcosis in children with a hyperimmunoglobulin M syndrome.[112,113] In adults, idiopathic $CD4^+$ T-cell lymphocytopenia may be identified by the development of disseminated cryptococcosis, and paradoxically this underlying condition with cryptococcosis actually may have a good prognosis for treatment outcome.[64,114] With much less precision, it has been suggested that smoking and outdoor activities may increase the risk of cryptococcosis,[73,115] and that recent intravenous drug abuse might provide risk even without HIV infection.[116]

There is general agreement that most cryptococcal infections are acquired primarily by inhalation of infectious propagules, but there are occasional cases of direct traumatic inoculation through contaminated environmental projectiles or laboratory/clinical accidents such as needlesticks.[117,118] However, neither the environmental source of infection nor the infectious form of *C. neoformans* has been precisely established in most cases of cryptococcosis. It is hypothesized that either dehydrated, poorly encapsulated yeast cells or basidiospores (<5 μm) are needed as infectious propagules for alveolar deposition in the lungs.[19] Studies at sites with contaminated soils or trees have found that the surrounding air contains the correct size of propagules for airway infection.[119–121] Molecular typing methods have confirmed that clinical isolates can be indistinguishable from environmental isolates.[122–124] Although associations between infection and environmental exposure have been reported for many of the classic dimorphic fungi, this association is rare for *C. neoformans.* However, the outbreak of *C. gattii* infections on Vancouver Island and the Pacific Northwest has convincingly linked human and animal infections to common environmental exposures.[54] There has not been a consistent seasonal association for the occurrence of cryptococcosis worldwide, although one study did observe more cases in the fall

and winter.[125] These uncertain observations likely reflect the influence of the host-reactivation pathophysiology of this disease in many cases, or differences in environmental humidity for aerosol production. Recent fundamental niche mediation models have begun to trace and map the best environmental conditions for certain cryptococcal species' survival.[126]

Human-to-human transmission of cryptococcosis has not been reported except in cases of contaminated transplant tissue.[51,106,107] Many species of animals, including dogs and cats, can develop cryptococcosis,[127–129] but there is infrequent evidence of zoonotic transmission between them and humans. In one case, *C. neoformans* isolated from the cage of a pet cockatoo was molecularly linked with the strain that caused infection in a transplant recipient who was exposed to the cage.[130] Also, several cryptococcal cases have been linked to intense bird exposures,[131] and even a possible nosocomial outbreak of cryptococcosis in an intensive care unit has been reported.[132]

PATHOGENICITY

The encapsulated yeast *C. neoformans* has been studied extensively for more than 50 years. In the past 2 decades, genetic and molecular biologic research, in concert with well-established and robust animal models, has rapidly increased our understanding of its pathobiology.[133] Progress in cryptococcal molecular biology has led to the use of karyotypes, repetitive elements, transposons, and whole-genome sequencing to identify yeast strains through a variety of analyses, including restriction fragment length polymorphism, RAPD, PCR fingerprints, and MLST. Recently, the entire sequenced genomes of many strains of *C. neoformans* and *C. gattii* have been published,[12,14,15,47,134,135] and with present sequencing methods many more strains will have their entire genomes sequenced and analyzed. Several transformation systems are available for introducing DNA into this yeast, and site-specific gene disruptions and replacements are routine; a comprehensive whole-genome deletion library has even been created.[136] Dozens of specific null mutants in a variety of pathways or for specific enzymes have been produced to examine their impact on the virulence composite of the yeast in several robust animal models,[133,137] including zebrafish, in which pathogenesis can visually be observed.[133,137,138] Furthermore, differential display PCR, complementary DNA subtraction techniques, serial analysis of gene expression, microarray analysis, and RNA sequencing have been used to document and understand *C. neoformans* transcriptional profiles.[55,139–142] Proteomic and metabolomic approaches have also been used to study its pathophysiology.[143–145] It is also observed that the cryptococcal genome shows plasticity and rapid changes, and variations can influence its virulence and resistance to drugs.[46,60,61,146–149] With the use of whole-genome sequencing and following persistent or relapse isolates in the human host, the rates and location of genetic and epigenetic changes have been reported in the human host.[150–152]

All these molecular tools have been employed to determine the components and mechanisms that make this yeast such an efficient and deadly pathogen. The following paragraphs describe its most prominent virulence phenotypes.

Capsule

The most distinctive feature of *C. neoformans* and *C. gattii* is a polysaccharide capsule containing an unbranched chain of α-1,3-linked mannose units substituted with xylosyl and β-glucuronyl groups. The serotype specificity appears to be determined by structural differences in the glucuronoxylomannan (GXM) related to the number of xylose residues and the degree of *O*-acetylation of hydroxyl groups. The capsular polysaccharide has a highly negative cell surface charge and is attached to the cell wall by α-1,3-glucan residues. However, it is easily released into the immediate growth media or tissue. Capsular thickness, which varies between isolates, is regulated by several environmental cues, including ambient partial pressure of carbon dioxide, serum, urea, and low iron concentrations, which increase capsular size in many strains. These environmental signals appear to augment the yeast's ability to produce disease and may help explain why the capsule may be small in in vitro cultures and the general environment but is much larger when observed within the mammalian host. Mutant cryptococci that are made to be hypocapsular or acapsular are dramatically less virulent than their parental strains in animal models.[153] However, infections caused by capsule-free or poorly encapsulated strains have been rarely observed in the mammalian host.[154,155]

The impact of the capsular polysaccharide on host immunity can be profound at many pathophysiologic levels. For example, it has been shown to produce the following effects on the host[156]: (1) it acts as an antiphagocytosis barrier, (2) it depletes complement, (3) it produces antibody unresponsiveness, (4) it dysregulates cytokine secretion, (5) it interferes with antigen presentation, (6) it produces brain edema, (7) it creates selectin and tumor necrosis factor receptor loss, (8) it allows a highly negative charge around yeast cells, (9) it extrudes itself into the intracellular environment with the potential for local toxicity on cellular organelles, and (10) it enhances HIV replication. Furthermore, it confers resistance to oxidative stress, which may improve its intracellular survival.[157] When the GXM is shed into the host environment, it affects host immunity at many levels, but fortunately, its detection in host fluids permits a very successful diagnostic test.

The biochemistry of this imposing structure remains poorly understood, but new insights continue to occur for this structure.[158] For example, multiple genes related to capsule synthesis have been identified.[159] Through creation of specific null mutants, it has been shown that any disturbance in efficient capsular synthesis (e.g., reduced formation, secretion, or elimination of the structure) attenuates the ability of the mutated yeast to produce disease.[153] Furthermore, there have been new insights into the many molecular signaling pathways that control expression of the capsule. For example, one critical pathway necessary for efficient capsular production uses a G protein that signals through a cyclic adenosine monophosphate–mediated pathway. Down-regulation of this pathway with a concomitant reduction in capsule size produces an attenuated virulence phenotype, but if a mutation in the pathway upregulates capsule production, the mutant yeast becomes hypervirulent.[160]

Melanin

The production of melanin is observed in many fungi, including some pathogenic species.[43] *C. neoformans* possesses a laccase, an enzyme that catalyzes the conversion of diphenolic compounds such as L-3,4-dihydroxyphenylalanine (DOPA), norepinephrine, epinephrine, and other related aromatic compounds to quinones, which rapidly autopolymerize to form melanin. The production of this pigment can help identify the yeast in the laboratory, but it is also a major virulence factor for the yeast. Laccase is bound to the inner aspect of the yeast's cytoplasmic membrane, and a site-directed mutant for the gene encoding for it has been created. This laccase-negative or albino mutant has been attenuated for virulence in animal models.[161]

One proposed mechanism by which melanin may protect the yeast is through its ability to act as an antioxidant, and it has been shown that yeast cells without the ability to form melanin are more susceptible to oxidative stress. Other potential mechanisms by which melanin protects the yeast from host damage involve the following: (1) cell wall support or integrity, (2) alteration in cell wall charge, (3) interference with T-cell response, (4) abrogation of antibody-mediated phagocytosis, and (5) protection from temperature changes and antifungal agents.

It remains unclear whether the catecholamine-rich CNS, with its excellent substrates for melanin formation, provides some tissue tropism or a rich environment that enhances this yeast's ability to produce disease. For instance, it has clearly been shown that melanin is formed in yeast cells within the brain.[162,163]

High-Temperature Growth

A basic trait of all pathogenic fungi is their ability to grow well at mammalian body temperature. For example, *C. neoformans* and *C. gattii* are the only cryptococcal species to grow consistently well at 37°C, and when mutants are made that cannot grow well at this body temperature, they are avirulent even when they possess the ability to make a capsule and produce melanin. There appears to be some evolutionary drift in high-temperature growth, in that isolates of *C. gattii* and serotype D *C. neoformans* (var. *neoformans*) generally appear to be more sensitive to growth inhibition and killing at high temperatures than serotype A

C. neoformans (var. *grubii*), and these less heat-tolerant yeast isolates quickly lose viability at temperatures of 40°C and above.[38]

There has been progress in understanding the genetic controls for high-temperature growth in *C. neoformans*. First, signaling pathways such as calcineurin, RAS, and trehalose have been associated with the yeast's ability to grow at mammalian body temperatures, and these are linked to its virulence composite.[164–167] It is also clear that vacuolar adenosine triphosphatase activity[168] is also important for high-temperature growth of this yeast. *C. neoformans* has evolved a series of molecular pathways and mechanisms to withstand host temperature stresses, and these thermal responses are a major reason it is a pathogen.[169]

Other Pathogenicity Factors

Detailed research has focused on the three classic virulence factors of *C. neoformans* (capsule, melanin, and growth at 37°C), but this complex pathogen has many other tools to produce disease. First, individual clinical or environmental strains vary in their ability to produce disease in animal models despite possessing all known major virulence factors. Second, strains can also rapidly change their virulence potential by passage through animals. Third, although many strains are clonal, there have been recombination events in nature to produce serotype AD hybrids and progeny that are more fit or virulent than the parental strains.[20] Fourth, future research will need to determine genetic or epigenetic controls over the subtle changes that make both environmental and clinical strains vary individually in their virulence potentials; even clinical isolates can vary in their virulence in animals despite already producing human disease.[170] The future challenge for research will be to link genetic changes in strains with clinical outcome.

A series of specific genetic loci have been associated with the virulence composite.[3] Phospholipase activity has been linked to virulence by a gene knockout of the phospholipase B1 gene (*PLB1*). Null mutants of *PLB1* are hypovirulent[171] and may have an impact on the immunologic response of the host to infection.[5] *C. neoformans* makes large amounts of urease, and if the *URE1* gene encoding for this enzyme is disrupted, infection with the mutant is attenuated in mice, where it has a blood-brain barrier entry defect,[172] but not in rabbits when inoculated directly into the CNS compartment.[6,173] A vacuolar adenosine triphosphatase gene is an example of a locus that appears to have an impact on several virulence phenotypes, and the absence of the encoding gene attenuates virulence of the strain.[9,168] There are now many examples in *C. neoformans* in which a gene or pathway controls multiple virulence phenotypes.[174] In addition to melanin as an antioxidant, several other genes, such as those for superoxide dismutase,[175] alternative oxidase,[176] and flavohemoglobin,[177] are associated with oxidative or nitrosative stress and have been linked to the virulence composite of this yeast.

It is clear that mechanisms for stress responses are important for the yeast to establish a robust infection, but there is also some redundancy in these systems because the yeast can at times still survive in the host without these protective features.[23] The sexual cycle and virulence of fungi are linked together in pathogenesis.[21] The alpha mating locus has been linked to the virulence of *C. neoformans* serotype D (var. *neoformans*) strains by the demonstration that the alpha mating strain was more virulent than its congenic "a" mating pair.[24] These results suggest that the mating locus, which is more than 100 kilobases in size, may contain some virulence genes. On the other hand, studies have found that congenic *C. neoformans* serotype A (var. *grubii*) strains had no apparent difference in virulence between "alpha" and "a" mating strains in several animal models.[178] This illustrates an important principle, which has also been shown with multiple genetic loci: virulence genes in one variety are not used by another variety or species, and vice versa. Thus both micro and major evolutionary drift may explain the variability and complexity of the entire virulence composite of *C. neoformans* and *C. gattii* strains.

HOST RESPONSES

Because serologic and skin hypersensitivity studies frequently identify cryptococcal infections and yet the incidence of cryptococcosis is low, it has been concluded that host immunity in humans is generally effective in controlling infection after initial exposure to this yeast.[179,180] In fact, the vast majority of cryptococcal infections are diagnosed in patients with a compromised cell-mediated immunity.[179] Furthermore, there is general agreement among clinicians that a strong cellular immune response producing granulomatous inflammation is essential for containment of infection.[180–183] Because granuloma formation is a result of a helper T cell 1 (Th1)–polarized response, cytokines such as tumor necrosis factor, interferon-γ, and interleukin (IL)-2 are required.[184,185] Proinflammatory cytokines, such as IL-12 and IL-18, and chemokines, such as monocyte chemotactic protein-1 and macrophage inflammatory protein-1α, are critically important for recruitment of inflammatory cells to the site of this infection.[186,187]

Several immune cell populations, such as natural killer cells, and certain types of lymphocytes have been shown to possess direct anticryptococcal effects. Human lymphocytes (CD4, CD8) inhibit growth of *C. neoformans* by direct contact.[188] A primary effector cell against *C. neoformans* is the macrophage, which produces anticryptococcal activity when it is "activated."[189] Other professional phagocytes, such as monocyte-derived macrophages, microglial cells, and polymorphonuclear neutrophils, may kill or at least significantly inhibit *C. neoformans,* but the alternative macrophage responses enhance infection.[190] It has been shown that a major factor in the infectivity of *C. neoformans* is its ability to survive inside cells and that this yeast can actually be extruded from professional phagocytes to infect other cells or be contained in vacuoles with virulence proteins.[191–193]

It is not only the state of cellular activation or the type of host cell but also the number of cells at the site of infection that appear to provide an effective host immune response. It is clear from natural history studies in patients with AIDS that the risk of cryptococcal disease dramatically increases as total CD4 lymphocyte counts drop below 50 to 100 cells/μL of blood.[194] In these patients, the paucity of inflammatory cells at the site of infection, such as the subarachnoid space, is impressive. Animal studies further confirm the importance of cell-mediated immunity by showing that T-cell–depleted mice are dramatically more susceptible to infection.[195]

A series of innate factors, such as the anticryptococcal activity of saliva and serum, may discourage active infection or disease with *C. neoformans.* Although surfactant A, dectin-1, and Toll-like receptors appear to have little influence on cryptococcosis,[196–198] there are other innate factors, such as surfactant D, that appear to have a critical impact on infection, and the yeast can actually co-opt this host material to improve its survival.[199] Phagocytosis of *C. neoformans* is optimally performed in the presence of complement or antibody. The intracellular fate of yeasts depends on cytokines such as interferon-γ or GM-CSF to improve intracellular inhibition or killing of the yeasts by either host oxidative or nonoxidative mechanisms. Human studies have consistently correlated a positive outcome with the presence and quantity of cytokines such as interferon-γ at the site of infection.[201] Human genome and immunogenetic studies in the future should reveal whether patients who have cryptococcosis, despite having an apparently normal immune system and no risk factors, might have subtle defects in innate or acquired immunity to this yeast. For example, it has already been shown that long-term survivors of cryptococcal meningitis have measurable persistent specific cell-mediated defects against *C. neoformans,* and recently it was shown that there is an association of common functional genetic polymorphisms in low-affinity Fcγ receptors with cases of cryptococcosis.[69,202,203]

It appears that *C. neoformans* has both extracellular and intracellular components or stages of infection. The results of histologic examination of tissues and fluids range from virtual absence of an inflammatory reaction to intense granulomatous inflammation with caseous necrosis. The immune reaction appears to be primarily a function of the host status, but the yeast can participate in the inflammatory response because switch variants in a single strain can produce vastly different histologic responses.[36] The immune response may be influenced by not only the shedding of polysaccharide into tissue but also other yeast factors such as mannitol,[204] melanin,[205] and prostaglandins,[206] which may have profound direct effects on immunomodulation of these infections.

There is substantial evidence that the humoral immunity arm can contribute to an effective immune response,[207,208] including an immunoglobulin M response.[209] Several groups have shown that preinfection monoclonal antibody strategies directed against the polysaccharide capsule can reduce the burden of yeasts and improve survival in animal models.

In fact, a polysaccharide–tetanus toxoid conjugate vaccine was shown to elicit high titers of antibody to the capsule and subsequently to protect against an intravenous inoculation of cryptococci in mice.[210] These antibodies provide for (1) efficient phagocytosis, (2) enhanced natural killer cell function, and (3) improvement in clearing capsular polysaccharide. Antibodies to other structural components such as melanin[211] and glucosylceramide in the cell wall[212] have also been able to improve the host's ability to fight infection. Sophisticated serologic studies of the host have suggested that there are both qualitative and quantitative differences in the individual types of immunoglobulins that may predispose to disseminated cryptococcal disease.[213] Finally, one pilot study in humans has been performed to determine the safety and pharmacodynamics of a monoclonal antibody for treatment of cryptococcosis.[214]

PATHOGENESIS

The pathogenesis of cryptococcosis is determined by three broad factors: (1) the status of the host defenses, (2) the virulence of the strain of *C. neoformans* and *C. gattii,* and (3) the size of the inoculum. The relative importance of each factor as a determinant of clinical disease remains uncertain, but it is clear that the complexities of these interactions together produce the ultimate presentation.

A reasonable scenario for the pathophysiology of cryptococcosis is that the susceptible host comes into contact with cryptococci from the environment through inhalation of infectious propagules. In the alveoli, the yeasts contact the alveolar macrophages, which recruit other inflammatory cells through cytokines/chemokines, and a proper Th1 response and granulomatous inflammation is elicited. The infection can then take one of three pathways:

1. In an immunosuppressed host, the yeast continues to proliferate and disseminate, causing clinical disease.
2. The effective immune response completely eliminates the yeast from the host.
3. The yeasts produce a small pulmonary lymph node complex and remain dormant in tissues but are not dead.

The third scenario may be a common occurrence. Baker, in elegant postmortem studies of asymptomatic individuals, showed the existence of pulmonary foci and hilar nodes containing yeasts in individuals with no antecedent complaints.[215,216] The yeasts remain dormant and the host is clinically asymptomatic until loss of local immunity occurs through, for example, corticosteroid use or progression of an HIV infection. Then the yeasts begin to replicate in the pulmonary lymph node complex and eventually disseminate into organs outside the lung. This pathophysiology is similar to the scenario proposed for reactivation of tuberculosis and histoplasmosis. Studies in France have given epidemiologic support for this concept of reactivation. In African expatriates who lived in Europe for many years before their development of cryptococcosis, the infecting strain possessed a genotype consistent with strains from an African origin.[217] Although this evidence is indirect, there is also little evidence that cryptococcosis arises from a recent exposure, and only rare case clusters following a group exposure have been reported.

In careful autopsy studies it has been shown to more accurately call the CNS pathogenesis features "meningoencephalitis," but because of common usage this chapter primarily uses the more restrictive term "meningitis."

CLINICAL MANIFESTATIONS

The two common sites for infection with this encapsulated yeast, the lung and the CNS,[218] were emphasized in a large review of cryptococcosis in HIV-negative patients. In this cohort, 109 patients (36%) were diagnosed with only pulmonary involvement and 157 (51%) presented with initial evidence of CNS disease.[67] Three other sites of infection (skin, prostate, and eye) have clinical features that are worthy of mention. However, it should be noted that *C. neoformans* has been found to infect any organ of the human body (Table 262.2), and in the severely immunosuppressed patient cryptococcosis may present with involvement of multiple body sites.

Cryptococcosis demonstrates a few differences depending on whether the patient has or does not have an underlying HIV infection.[125,219–222] HIV-infected patients present with more CNS and extrapulmonary infections, higher rates of positive India ink examinations, higher polysaccharide antigen titers, more frequent positive blood cultures, and fewer cerebrospinal fluid (CSF) inflammatory cells. These clinical distinctions are primarily a function of the severity of immunosuppression and the resulting high burden of yeasts. They most likely do not reflect a specific interaction between HIV and *C. neoformans* growth.

Lung

The respiratory tract is the most common portal of entry for this yeast, and symptoms range from asymptomatic colonization of the airway[223] to life-threatening pneumonia with evidence of an acute respiratory distress syndrome.[224–226] In at least one-third of normal hosts, the infection is asymptomatic on presentation and is detected by an abnormal chest radiograph. On the other hand, patients can present with acute symptoms of fever, chest pain, cough, weight loss, and sputum production.[227] Common and unusual pulmonary presentations are listed in Table 262.2. Cryptococcosis occasionally occurs with another pathogen; coinfections of the lung have been reported with tuberculosis, nocardiosis, and echinococcosis.[228–230] There are rare cases of cryptococcosis associated with pulmonary alveolar proteinosis and autoantibodies against GM-CSF.[231,232] Also, *C. neoformans* may be isolated from the sputum repeatedly over months and years in patients with prior chronic lung disease but no immunosuppression, no evidence of active pulmonary parenchymal disease, negative serum cryptococcal antigen, and negative fungal cultures from urine and CSF. These patients are considered to have chronic endobronchial colonization.

In normal hosts, chest radiographs commonly show well-defined, noncalcified single (Fig. 262.1) or multiple nodules. An initial presentation may be that of a radiographic lesion (or more than one) that is worrisome for a lung malignancy but then is proved by lung biopsy to be a cryptococcal infection. Other radiographic characteristics include indistinct masslike infiltrates, hilar lymphadenopathy, lobar infiltrates (Fig. 262.2), pleural effusions, and lung cavitation.[233] When infection is limited to the lung, the test for serum cryptococcal antigen is often negative. If there is pulmonary cryptococcosis with a positive test for serum cryptococcal antigen, it is prudent to consider an extrapulmonary source of infection, although workups may still be negative for another infection site (e.g., blood, skin, urine, CSF). When *C. neoformans* has been isolated from the lung in patients at high risk for dissemination due to an underlying immunosuppressive disease or treatment, a lumbar puncture should be considered to rule out CNS infection, even in the absence of symptoms. Although it is rare, early, asymptomatic spread to the CNS may be manifested only by a positive CSF fungal culture, with otherwise normal CSF and a negative antigen test. The number of cryptococci may be so low that several milliliters of CSF must be cultured for a positive culture to be obtained. Because positive CSF cultures are infrequent, some clinicians advocate treating previously normal patients with disease apparently limited to the lung who are asymptomatic and have no apparent underlying disease with long-term fluconazole and omitting the lumbar puncture.[234,235]

In the severely immunosuppressed host with AIDS or receiving high-dose corticosteroids, cryptococcal pneumonia can progress more rapidly (over days instead of weeks).[224,226] Unlike immunocompetent hosts, most immunosuppressed individuals have constitutional symptoms such as fever, malaise, chest pain, shortness of breath, and weight loss. In these patients, pneumonia can progress to features of acute respiratory compromise even without evidence of CNS involvement. However, because of the ability of the yeast to disseminate outside the primary lung focus to the CNS, these very-high-risk patients frequently present with a meningeal rather than a pulmonary syndrome. In AIDS patients, cryptococcal pneumonia may not be symptomatic, and more than 90% may present with concomitant CNS infection at the initial diagnosis. Chest radiographs in these immunocompromised hosts are similar in their range of presentations to those of immunocompetent hosts. However, alveolar and interstitial infiltrates are particularly common and thus might be confused with *Pneumocystis* infection. Because the severely immunosuppressed patient with pulmonary cryptococcosis and AIDS generally has a CD4 count substantially below 100 cells/μL, it is always prudent to consider the possibility of coinfection with other opportunists such as typical and atypical mycobacterium, cytomegalovirus, *Nocardia,* and *Pneumocystis.*

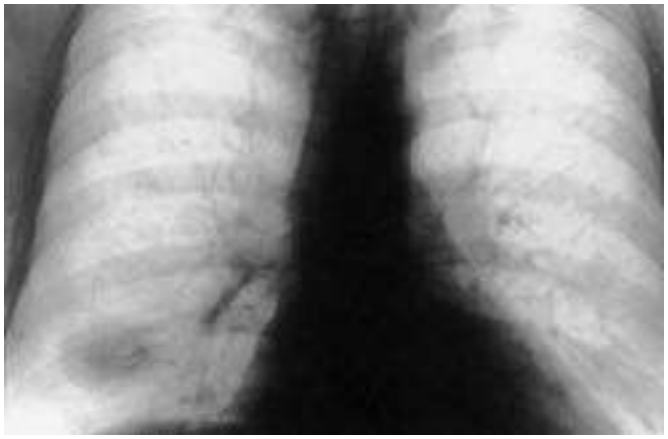

FIG. 262.1 Cryptococcal nodule. Previously healthy, asymptomatic patient with a right lung nodule.

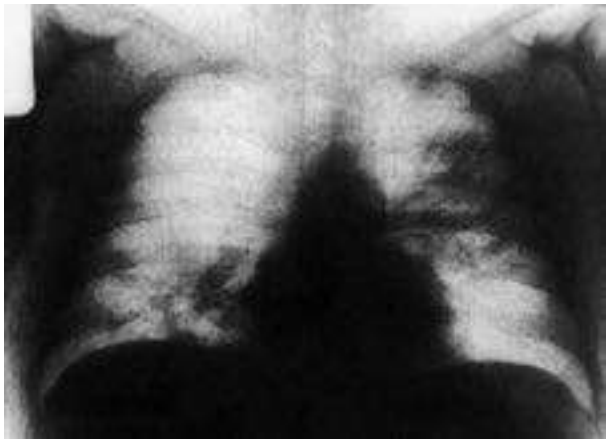

FIG. 262.2 Cryptococcal pneumonia. Previously healthy patient with fever, cough, shortness of breath, and left lobar infiltrate.

TABLE 262.2 Clinical Manifestations of Cryptococcosis

Central Nervous System

Acute, subacute, chronic meningitis
Cryptococcomas of brain (abscesses)
Spinal cord granuloma
Chronic dementia (from hydrocephalus)

Lung

Nodules (single or multiple)
Lobar infiltrates
Interstitial infiltrates
Cavities
Endobronchial masses
Endobronchial colonization
Acute respiratory distress syndrome
Mediastinal adenopathy
Hilar adenopathy
Pneumothorax
Pleural effusions/empyema
Miliary pattern

Skin

Papules and maculopapules
Subcutaneous abscess
Vesicles
Plaques
Cellulitis
Purpura
Acne
Draining sinuses
Ulcers
Bullae
Dermatitis herpetiformis–like
Molluscum contagiosum–like

Eye

Papilledema
Extraocular muscle paresis
Keratitis
Chorioretinitis
Endophthalmitis
Optic nerve atrophy

Genitourinary Tract

Prostatitis
Renal cortical abscess
Positive urine culture from occult source
Genital lesions

Bone and Joints

Osteolytic lesion (single or multiple sites)
Arthritis (acute/chronic)

Muscle

Myositis

Heart, Blood Vessels

Cryptococcemia
Endocarditis (native and prosthetic)
Mycotic aneurysm
Myocarditis
Pericarditis
Infected vascular graft

Gastrointestinal Tract

Esophageal nodule
Nodular or ulcerated lesions in stomach or intestines (may resemble Crohn disease)
Hepatitis
Peritonitis
Pancreatic mass

Breast

Breast abscess
Lymph nodes
Lymphadenopathy

Thyroid

Thyroiditis
Thyroid mass

Adrenal Gland

Adrenal insufficiency
Adrenal mass

Head and Neck

Gingivitis
Sinusitis
Salivary gland enlargement

Modified from Casadevall A, Perfect JR. Cryptococcus neoformans. *Washington, DC: ASM Press; 1998:409.*

Central Nervous System

Most patients with cryptococcosis of the CNS present with signs and symptoms of subacute meningitis, such as headache, fever, cranial nerve palsies, lethargy, coma, or memory loss over several weeks (see Table 262.2).[218] Symptoms may not be typical, and patients may present with acute (several days) symptoms of severe headaches, with intermittent headaches, or even with no headache but with altered mental status.

HIV-infected patients with cryptococcal meningitis exhibit few differences at presentation from those without HIV. However, several clinical aspects may be more prominent in patients with AIDS.[67] First, the

burden of yeasts is generally higher, and this may be reflected in higher polysaccharide antigen titers, slower conversion of CSF to sterilization during treatment, and a tendency toward a higher incidence of increased intracranial pressure. Second, there is a greater likelihood of finding the yeast in extracranial locations during the initial workup. Third, the possibility is greater that a second CNS event may occur, such as infection with *Toxoplasma gondii* or development of a lymphoma. Fourth, the use of ART in AIDS patients has created a distinct immune reconstitution inflammatory syndrome (IRIS) in cryptococcal infections.[236–238] It appears in two forms: unmasking and paradoxical. In the unmasking form, after starting ART, some patients develop acute symptoms of cryptococcal meningitis or pain and swelling in peripheral, hilar, or mediastinal lymph nodes. In the paradoxical form, this syndrome may occur during treatment of cryptococcal meningitis in the first few weeks or months after ART is introduced. It appears to correlate with a significant drop in HIV load, but there may be only a modest rise in the number of CD4 cells.[239] It is hypothesized that as immunity improves with ART, silent or latent cryptococcal infections are made clinically apparent as inflammation is mobilized to interact with the yeasts or polysaccharide antigen. During treatment for cryptococcal meningitis, IRIS may be marked by increasing headaches, new neurologic signs, appearance of more inflammatory cells in the CSF, and possibly increased intracranial pressure.[240] Distinction between immune reconstitution and progressive infection can be difficult, but cultures from the CSF and lymph node aspirates are generally negative in IRIS, even though cryptococci may be present on a smear and cryptococcal antigen titers tend to be lowering.

There are still limited precise data that relate the severity of the meningitis to the particular infecting strain, but this is an area of active investigations.[20,170,241] In most cases the host defense responses determine the clinical manifestations. However, some clinical presentations may depend on the particular infecting strain. For example, in areas of the world where patients have infections with both *C. neoformans* serotypes A, D, and AD (var. *neoformans* and var. *grubii* hybrid) and *C. gattii,* cerebral cryptococcomas (Fig. 262.3) and hydrocephalus with or without large pulmonary mass lesions in immunocompetent hosts were found more commonly with the *C. gattii* infections.[242–244] Although patients infected with this species may have higher survival rates, a subgroup of patients with *C. gattii* infections have brain parenchymal lesions by scan, complications of hydrocephalus and increased intracranial pressure, cranial neuropathies, and a poor response to therapy with substantial morbidity. These observations suggest that some *C. gattii* strains may have a greater propensity for invading the brain parenchyma. Another example of a specific strain affecting disease production is from the Vancouver outbreak; it was found that a recombinant strain had been created in nature that was more virulent in animal models than one of the parental strains and, furthermore, this strain (genotype VGIIa) had taken over most of the environmental and clinical isolates within this outbreak.[20]

Skin

C. neoformans can produce almost any type of skin lesion (see Table 262.2). A common lesion is a papule or maculopapule with a soft or ulcerated center (Fig. 262.4). A draining sinus usually originates in an underlying bone lesion or occasionally a subcutaneous abscess. Some lesions in severely immunosuppressed patients are easily mistaken for molluscum contagiosum, and lesions can mimic acne vulgaris, squamous carcinoma, or basal cell carcinoma. After pulmonary and CNS sites of infection, the skin is the third most common organ for appearance of infection. Skin manifestations can be extraordinarily varied.[245–248] For example, in severely immunosuppressed patients, skin infections may present as a cellulitis (Fig. 262.5) or an abscess that mimics a bacterial skin infection in both appearance and rapidity of onset.[249,250] Because of the variety of skin manifestations, a correct diagnosis requires a biopsy with proper histopathology and culture. This is extremely important in the immunocompromised host.

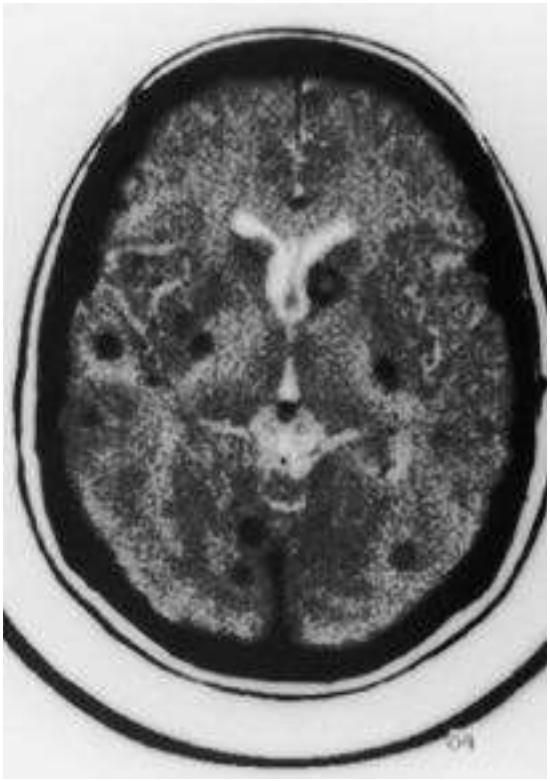

FIG. 262.3 **Computed tomography scan of previously healthy patient with multiple cryptococcomas.**



FIG. 262.4 **Forehead ulcer in a human immunodeficiency virus–infected host with *Cryptococcus neoformans* seen in histopathology.**

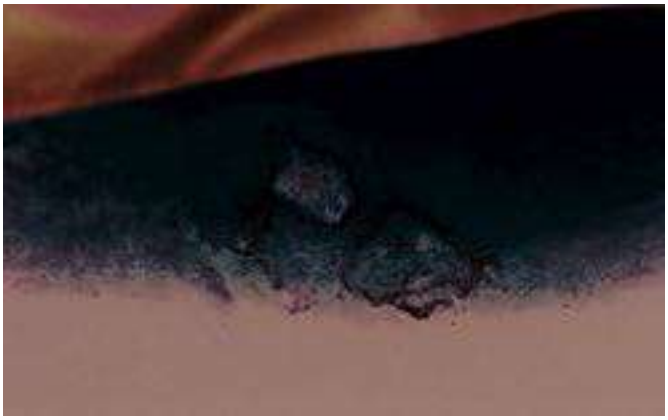

FIG. 262.5 **Severely immunosuppressed patient with cellulitis of the arm caused by *Cryptococcus neoformans*.**

In most cases, the skin lesions represent a sentinel finding for disseminated cryptococcal infection. In fact, severely immunosuppressed patients can present with both cutaneous cryptococcosis and another pathogenic fungus in the skin as a manifestation of disseminated fungal disease.[251] However, there is strong evidence that rare cases of skin cryptococcosis represent primary cutaneous cryptococcosis from direct inoculation or exposure rather than being a marker of disseminated disease. In a large retrospective review of patients with cutaneous findings, a series of immunocompetent patients had: (1) solitary skin lesion(s) on unclothed areas of the skin; (2) a history of skin injury, participation in outdoor activities, or exposure to bird droppings; (3) isolation of *C. neoformans;* and (4) no evidence of disseminated disease.[252] There are reports of direct inoculation of yeasts into skin by laboratory or clinical accidents and defined episodes of trauma.[117,118] In these cases, there has been no evidence for dissemination of infection from this body site of infection except for one case of a needlestick.[118]

Involvement of skin may be influenced by several factors. First, some strains of *C. neoformans* have been described as being dermatropic in animal models.[253] Second, an observation made in a cohort of solid-organ transplant recipients suggests that patients receiving tacrolimus appeared to develop a higher ratio of skin and soft tissue infections to CNS infections when compared with previous immunosuppressive regimens.[107] Because tacrolimus has anticryptococcal activity at temperatures of 37° to 39°C[254] but loses its anticryptococcal activity at environmental temperatures, the skin involvement might result from the lower temperatures at this body site.

Prostate

Like *Blastomyces* and *Mycobacterium tuberculosis, C. neoformans* can invade the prostate gland, and in most cases of cryptococcal infection this involvement is asymptomatic.[255] In fact, asymptomatic or silent prostate infection may first be identified during urologic surgery and may spread into the bloodstream during surgery.[256] For *C. neoformans,* this gland was considered an important site for sanctuary of this yeast from antifungal treatment in HIV-infected patients before ART.[257,258] Frequently, in follow-up of patients with AIDS and cryptococcal meningitis after initial antifungal therapy, cultures of urine (with or without prostatic massage) or seminal fluid were positive for the yeast. In many patients the location of relapse after therapy remains uncertain, but the prostate is clearly a site that requires prolonged therapy to clear infection in severely immunosuppressed patients. Besides the prostate, penile[259] and vulvar[260] lesions with *C. neoformans* have been reported, but there has been no evidence for conjugal spread of this yeast, and isolated cryptococcuria is a marker for disseminated disease.

Eye

In the early reviews of cryptococcal meningitis, ocular signs and symptoms were reported in 45% of the cases.[261] The most common manifestations are ocular palsies and papilledema. Small white retinal exudates, without overlying vitritis, are probably the next most common finding. In severely immunosuppressed patients, several features of ocular involvement have arisen. First, cryptococcal eye infections can occur simultaneously with other pathogens such as HIV and cytomegalovirus.[262] Second, the presence of extensive retinal lesions, particularly with vitritis, frequently leads to blindness, and only occasionally is it successfully managed.[263,264] Third, there are reports of catastrophic loss of vision without evidence for endophthalmitis.[265,266] In these cases of blindness, which may occur while receiving therapy, two pathogenic processes have been identified. First, there is a visual loss secondary to an optic neuritis produced by infiltration of the optic nerve with yeasts, and, as for endophthalmitis, there are few options for successful management. Second, other patients present with visual loss in one or both eyes during antifungal therapy. In these patients, symptoms are probably related to the development of cerebral edema and unrelieved high intracranial pressure. The probable pathogenesis is compression of the ophthalmic artery within the optic sheath. Treatment is decrease of CSF pressure by repeated lumbar punctures, CSF shunting, or perhaps slitting the optic sheath within the posterior orbit. Once blindness has occurred, return of visual acuity is rare. A central scotoma or optic atrophy may be the only sequela of cured ocular cryptococcosis.

Other Body Sites

C. neoformans can produce infection in most areas of the body (see Table 262.2), and several require further discussion. Cryptococcemia occurs during severe immunosuppression and when there is a high burden of yeasts in the body, and it is a common finding in advanced AIDS. Cryptococcemia rarely produces vascular instability, and only a few cases of native or prosthetic valve endocarditis have been described.[267]

Before the AIDS epidemic, bone lesions were reported in up to 5% of disseminated cases.[268] Bone lesions are typically one or more well-circumscribed osteolytic lesions in almost any bone and may have a contiguous soft tissue abscess ("cold abscess"). Bone lesions of sarcoidosis resemble cryptococcal lesions on radiographs but are more often on the hands or feet and have no contiguous soft tissue abscess.[269] In AIDS patients, yeasts may be found in bone marrow biopsy cultures.

Cryptococcal peritonitis can present in two distinct patient groups: (1) those receiving chronic ambulatory peritoneal dialysis and (2) those with underlying liver disease and cirrhosis.[270,271]

Rare body sites for cryptococcosis (less than a dozen reported cases) include genital and urinary tracts (renal cortical abscess, positive urine culture from an occult site); muscle (myositis); heart (native and prosthetic valve endocarditis, mycotic aortitis or aneurysm, myocarditis, pericarditis, vascular foreign body); thyroid (thyroiditis, mass); adrenal gland (adrenal insufficiency); head and neck (gingivitis, sinusitis, salivary gland enlargement); gastrointestinal tract (gastrointestinal nodules or ulcers); liver (hepatitis); breast (inflammatory mass); and lymph nodes (lymphadenopathy).

Immune Reconstitution Inflammatory Syndrome

IRIS and cryptococcosis are well described with HIV infection and its treatment,[237] but it also clearly occurs as a complication in solid-organ transplant recipients[272] and normal hosts.[273,274] IRIS may occur within a few days to a few months after the introduction of ART with manifestations of fever, organ inflammation (by symptoms, radiographs), increased intracranial pressure,[275] or a combination of these, with a pattern of immunologic biomarkers carefully described.[276,277] In solid-organ transplant recipients, it occurs with those on potent antirejection regimens that have been reduced after initiation of antifungal therapy. It occurs at a mean period of 6 weeks after starting antifungal therapy and may be associated with organ graft loss. An elegant series of cryptococcal spinal arachnoiditis cases was described with impressive features of a dysregulated immune system post–infectious inflammatory response syndrome and response to corticosteroid therapies.[278] IRIS in cryptococcosis with its dysregulated immune response needs to be identified by the clinician, because there are no specific tests for it, and the diagnosis relies on clinical guidelines and expert judgment.[279] It is so critically important to recognize IRIS because its management is different than for relapse or persistence of cryptococcosis.

LABORATORY DIAGNOSIS

Microscopic Examination

The simple procedure of mixing together India ink and biologic fluids to identify the 5- to 10-micron-diameter encapsulated yeasts remains a rapid and effective method for diagnosing cryptococcal meningitis (Fig. 262.6). Approximately 50% of non-AIDS patients with cryptococcal meningitis and over 80% of patients with AIDS have a positive India ink examination of the CSF. Experience is required to distinguish an encapsulated yeast from a lymphocyte with surrounding proteinaceous debris. India ink smears of urine, sputum, and bronchoalveolar lavage specimens are almost impossible to interpret. With calcofluor white and a fluorescent microscope, yeasts can be detected in a specimen when numbers are reduced. With routine histopathologic stains such as hematoxylin and eosin, the yeasts are surrounded by empty spaces, which reflect the capsule. The polysaccharide capsule can be identified with stains such as mucicarmine and alcian blue (Fig. 262.7), and its ability to produce melanin allows it to be stained with the Fontana-Masson stain. Gomori methenamine silver fungal stain identifies the narrow-based budding yeast in tissue (Fig. 262.8), and a Gram stain usually reveals a poorly stained gram-positive yeast. Both biopsies and

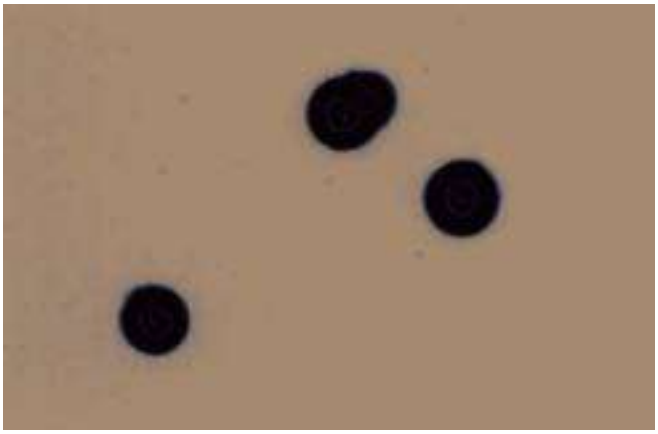

FIG. 262.6 India ink preparations from cerebrospinal fluid of patient with meningitis. Note encapsulated yeasts.



FIG. 262.8 Gomori methenamine silver stain shows narrow-based yeast with a faint capsule.



FIG. 262.7 Alcian blue stain of lung tissue from patient with cryptococcal pneumonia. Note blue stain of polysaccharide capsule.

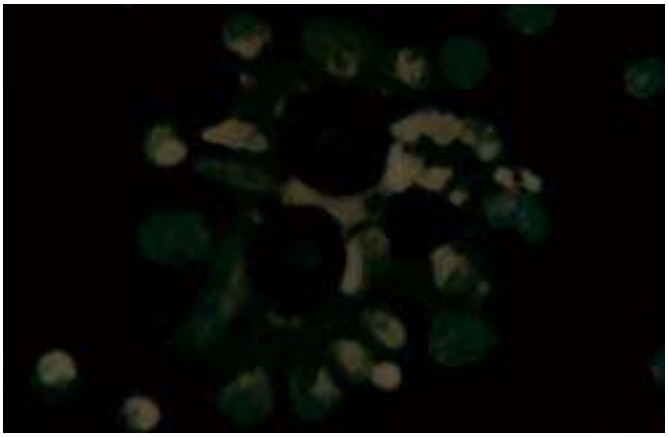

FIG. 262.9 Cytospin preparation of a host with cryptococcal meningitis showing encapsulated yeast surrounded by a mixed inflammatory reaction.

cytologies (Fig. 262.9) can be extremely helpful in the diagnosis of cryptococcosis.

Cultures

C. neoformans can grow on most bacterial and fungal media. Both automated and lysis centrifugation methods are effective in detecting cryptococcemia, and the finding of positive blood cultures has been more common during the AIDS epidemic. Most *C. neoformans* isolates from untreated patients can be detected in culture 3 to 7 days after the specimen is collected and placed into or on culture media. In some advanced CNS infections there can be more than a million colony-forming units of yeasts per milliliter of CSF. Quantitative CSF yeast counts have been used for evaluation of antifungal therapy.[280,281] Initially, this counting of viable CSF yeasts was an effective research tool to determine the impact of various treatments on the burden of yeasts, but recently this quantitative CSF culture strategy has begun to show clinical relevance and correlation with outcome.[282–284] It may become attractive to utilize in individual therapeutic strategies.[285]

Isolates can be identified by biochemical reactions,[39,40] MALDI-TOF mass spectrometry,[41] or DNA-based methods.[286,287] Other low-tech methods to presumptively identify the yeasts are to perform a rapid urease test or to inoculate the yeast onto Staib medium (birdseed agar),[288] DOPA, or caffeic acid media (in which colonies will produce melanin and turn brown to black). However, some other yeasts produce urease, and the highly mucoid colonies of some strains of *C. neoformans* may not produce enough melanin to be clearly positive.

The identification of *C. neoformans* and *C. gattii* can be made by several methods: (1) a color reaction on concanavalin-glycine-thymol agar, which distinguishes *C. neoformans* from *C. gattii*[289]; (2) an antibody kit for serotyping, which presently is not commercially available[290]; and (3) fingerprinting with DNA-based methods, which can further separate strains of the cryptococcal complex into multiple genotypes.[291–293] Reports show that the MALDI-TOF mass spectrometry platform can rapidly distinguish the two species.[294] However, with our present knowledge base it is not yet clinically apparent that the clinician needs to know the species, variety, or genotype to initially manage the patient with cryptococcosis.

Antigen Detection

The tests for detection of cryptococcal polysaccharide antigen in serum and CSF are extremely accurate for the diagnosis of invasive disease.[295] Both latex agglutination and enzyme-linked immunosorbent assay tests are greater than 90% sensitive and specific.[296] The Meridian CALAS latex agglutination test (Meridian Bioscience, Inc., Cincinnati, OH) has provided positive tests in sera and CSF, with negative Meridian Premier enzyme immunoassay (EIA) results, and usually from samples with lower antigen titers that follow treatment.[297] With the proper treatment of serum specimens (boiling and pronase/2-mercaptoethanol treatment), false-positive latex agglutination tests are not common.[298] False-positive latex agglutination tests are usually negative by EIA, and vice versa. An occasional false-positive test is observed when there is a cross-reactive antigen in the specimen, and this may occur with microorganisms such

as *Trichosporon asahii* (*beigelii*)[299] or other infections.[300] The false-negative tests may be present in early asymptomatic meningitis and in chronic, indolent meningitis. The clinical experience with, and preciseness of, antigen detection has been carefully studied and validated in sera and in CSF for clinical practice, and it is not recommended to detect polysaccharide antigen in the urine and bronchoalveolar lavage fluid, despite some reports to the contrary.[301] Recently, a new lateral flow assay (LFA) has been introduced into clinical practice. It is cheap, rapid, and simple. The LFA has been found to be more sensitive than the Meridian Premier EIA and at least as sensitive as the Meridian CALAS latex agglutination test.[302] The LFA has major indications for preemptive management of HIV-infected patients in resource-poor environments. A drop of fingerstick blood can be used to screen high-risk HIV-infected patients and can provide a bedside test with CSF.[303] Although dilutions of sera or CSF can be tested by LFA and titers determined, the quantitative results have differed from those obtained by latex agglutination.[304,305] Like other tests for cryptococcal polysaccharide antigen, false-positives can be observed in sera from the rare cases of *T. asahii* infection.[305,306]

A number of clinical issues are related to the use of cryptococcal polysaccharide antigen. Serum cryptococcal polysaccharide antigen tests have been used successfully to screen high-risk, febrile AIDS patients, particularly those with headache, in areas where the incidence of cryptococcal meningitis is high,[307] but these tests may be less useful in areas where the prevalence of infection is low.[308] In patients with cryptococcal infection of the lung who have a positive serum polysaccharide antigen test, there is heightened concern that the infection has become extrapulmonary. It is also unlikely that this high-molecular-weight molecule can cross the blood-CSF barrier, and thus its detection in CSF probably confirms the presence of the yeast in this compartment. In fact, there are occasional cases of meningitis in which CSF antigen is detected early in infection before there is a high enough yeast colony count for the routine clinical laboratory to detect a positive culture, particularly with small volumes of CSF. There are also isolated cases of serum cryptococcal polysaccharidemia in asymptomatic HIV-infected patients with negative fungal cultures from CSF and urine. Management of these asymptomatic antigenemia cases can be confusing, but in these high-risk patients it is wise to start preemptive antifungal therapy because many of them will eventually develop cryptococcosis, and their prognosis is poor.[309–312] Many preemptive strategies in multiple countries are being studied or instituted to save lives with early antifungal therapy intervention in cryptococcal antigen-positive HIV patients, and with the new LFA it is becoming more clinically feasible in resource-limited areas.[313] As a strategy in the clinic where lumbar punctures are limited, recent studies suggest that a positive serum antigen titer of 1:160 or greater reflects a clinically relevant risk of CNS involvement in asymptomatic patients and demands an aggressive approach to manage cryptococcal CNS disease.[314]

Despite its excellence as a diagnostic test, the cryptococcal polysaccharide antigen test is not sufficiently accurate to use in making specific decisions during treatment. In fact, serial polysaccharide antigen titers are imprecise and should not be used to develop treatment guidelines.[315] The cryptococcal polysaccharide antigen titer, however, does give general prognostic information. Initial high titers (≥1:1024) demonstrate a high burden of yeasts in the host, poor host immunity, and a greater chance of therapeutic failure. Furthermore, it is encouraging when consideration of an IRIS diagnosis is being made that the cryptococcal antigen titer is stable or dropping. Finally, cryptococcal antigen titers can be used in follow-up when maintenance antifungal regimens are discontinued in HIV-infected individuals. Antibodies to *C. neoformans* may be detected during infection and are helpful for epidemiologic studies, but because many of these patients with disease are immunosuppressed, the titers are inconsistent and not generally used for diagnostic or therapeutic decisions.

Radiology

The chest radiograph of pulmonary cryptococcosis can show a variety of characteristics, including local or diffuse infiltrates, nodules, hilar lymphadenopathy, cavitation, and pleural effusion(s).[316–320] In AIDS patients, the diffuse interstitial infiltrates may be confused with coexistent *Pneumocystis* infection.[321,322]

Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain are frequently used in the management of cryptococcal meningitis.[323–327] Approximately half of CT scans are normal during CNS infection. However, a CT scan can reveal hydrocephalus, gyral enhancement, or single or multiple nodules that may or may not be enhancing. Cryptococcomas may be single or multiple, and in some populations, such as those with *C. gattii* infection, they can occur in more than 25% of non-AIDS and apparently immunocompetent patients. In patients with AIDS, the CT scan differs only in that approximately one-third of patients demonstrate cortical atrophy from the underlying HIV infection. The MRI scans are more sensitive than CT scans for detecting abnormalities in cryptococcal meningitis. MRI findings can include numerous, clustered foci that are hyperintense on T2-weighted images and nonenhancing on postcontrast T1-weighted images in the basal ganglia or midbrain. Rarely, there may also be multiple miliary enhancing parenchymal and leptomeningeal nodules.

There are several points to emphasize with regard to CNS radiology. First, there is no pathognomonic scan, and patients with cryptococcal meningitis may simply present with evidence of idiopathic hydrocephalus.[328] Second, in AIDS patients, CNS parenchymal lesions may represent lymphoma or a second infection such as toxoplasmosis or nocardiosis. Third, follow-up scans may actually show worsening of lesions, with enlargement, new lesions, or persistence of cryptococcomas or more leptomeningeal enhancement. These findings are not necessarily a sign of treatment failure. They may simply represent enhancement by inflammation as microscopic yeast foci are being eliminated. Especially with the use of ART and the potential for immune reconstitution, these radiographs need to be judged carefully in the context of the patient's cultures and clinical signs and symptoms before deciding that the radiograph signifies treatment failure. Lesions on MRI scans may not decrease in size for months or years despite resolution of disease.[329]

MANAGEMENT

The management of cryptococcosis has been the subject of a series of evidence-based studies. Infectious Diseases Society of America guidelines have been established for therapy and were revised in 2010.[330,331] The general recommendations should be helpful to clinicians as a reference point for individual patient management, but there remain many unanswered questions, and unique decisions will need to be made in certain cases. It is clear that cryptococcal meningitis is uniformly fatal without antifungal treatment. However, before the availability of antifungal agents, there were several reports of patients who survived for years before succumbing to infection.[332] In contrast, with the severe immunosuppression of HIV infection and if adequate treatments are not available, a high percentage of untreated patients die within the first 2 weeks of hospitalization.[333] The rapidity of the progression is likely to depend on host factors. On the other hand, it has been shown that some individuals without an immunosuppressive underlying disease have asymptomatic endobronchial colonization with no detectable pulmonary lesions on CT scan, negative serum cryptococcal antigen, and negative fungal cultures from CSF and urine. These patients without disease may not need antifungal treatment. In a review of non-AIDS patients with positive pulmonary cultures for *C. neoformans,* approximately 20% did not receive treatment.[67] Previously, healthy patients with cryptococcosis confined to the lung healed spontaneously. However, because pulmonary cryptococcosis in immunosuppressed patients or those to be immunosuppressed can disseminate or become a chronic disease in the lung, all such patients should be treated.

In Vitro Drug Susceptibility Analysis

Methods for in vitro susceptibility testing of *C. neoformans* and *C. gattii* have been modified and standardized, and the epidemiologic cutoff values for the cryptococcal complex to azoles have been established.[334,335] Most initial isolates have low minimal inhibitory concentrations (MICs) to amphotericin B, flucytosine, and azoles but high MICs to echinocandins. By in vitro susceptibility testing, isolates have been detected in treated patients who are resistant to flucytosine, azoles, and polyenes. In fact, there appears to be some correlation between MICs and clinical resistance,[336–338] and by molecular typing methods, most of the refractory cases represent relapse isolates rather than reinfection.[339,340] In fact, they

possess MICs that are similar to those of the primary isolates.[341] Some of the findings may be due to the known unstable drug heteroresistance characteristics of *C. neoformans* in vivo.[342] However, in some cases it was suggested by molecular techniques that reinfection occurred with a novel strain.[343,344] When MICs of isolates rise while the patient is being treated, or when the MICs were initially greater than 16 μg/mL for fluconazole or 128 μg/mL or greater for flucytosine, failure of treatment might possibly be related directly to drug resistance.[345] However, there is as yet no specific therapeutic MIC breakpoint for antifungal drugs validated for cryptococcosis. In fact, in HIV-associated cryptococcal meningitis within Africa where fluconazole is used to treat mucocutaneous candidiasis and as primary therapy for cryptococcal disease, positive relapse episodes have been associated with isolates that had reduced susceptibility to fluconazole (MIC ≥64 μg/mL).[150,346] *C. neoformans* strains that possess known drug resistance mechanisms—from drug target amplifications or changes in the ergosterol pathway to augmentation of drug efflux pumps through genetic mutations/duplications in ergosterol-target genes or high-level expression of drug efflux genes—have been identified.[342,345] Furthermore, plasticity in the genome can allow chromosome instability, such as aneuploidy and thus duplications of specific drug target(s) or efflux drug pump(s), to produce a lower response to fluconazole in vivo.[347] However, unstable drug resistance with aneuploidy formation during in vivo stress may be lost in vitro and thus might be missed with in vitro cultures and direct drug susceptibility testing.[60] In fact, the differences between in vivo and in vitro yeast cells with a plastic genome may contribute to the apparent fungistatic behavior of fluconazole in the treatment of high-burden yeast infections.

Treatment Strategies

In 2010 the Infectious Diseases Society of America updated their Clinical Practice Guidelines for the management of cryptococcal disease,[127] and their principles and direct recommendations are incorporated in the following discussion with updated opinions.

Cryptococcal Meningitis

Amphotericin B remains the cornerstone of induction therapy for cryptococcal meningitis, and from the early studies when it was used alone[348] to its use in combinations, it has performed reasonably well for this infection, with successes in the non-AIDS era of between 60% and 75%.[349,350] With polyene therapy, two issues have been noted. First, recent studies have suggested that higher daily doses of amphotericin B might be more effective in sterilization of the CSF.[351] A standard induction dose for amphotericin B deoxycholate has now been established at 0.7 mg/kg/day. Second, liposomal amphotericin B (AmBisome) at 3 to 6 mg/kg/day and probably amphotericin B lipid complex at 5 mg/kg/day have had treatment successes similar to that of amphotericin B deoxycholate, with reduced toxicity.[352,353] In patients with renal dysfunction or at risk for renal dysfunction, the lipid products of amphotericin B are frequently recommended. For example, they are used as primary therapy in transplant recipients.[331] In fact, lipid formulations of amphotericin B may also be advantageous in all patients with cryptococcal meningitis in that they are less likely to require adjustments of the induction regimen that can produce a negative impact on treatment outcome.[354,355] Also, in resource-limited areas, there is both animal[356] and now human experience[357] that supports limited doses of liposomal amphotericin B with azole in induction therapy as effective.

Flucytosine has been used alone in treatment of cryptococcal meningitis,[358] but frequent development of direct drug resistance on monotherapy has meant that it cannot be recommended as a single agent for treatment of this infection. It is primarily used in combination therapy with conventional amphotericin B or lipid formulations of amphotericin B,[349,350,359–362] and doses in patients with normal renal function are typically 100 mg/kg/day but will need to be adjusted in those with renal dysfunction. Drug levels should be monitored to keep 2-hour postdose levels under 100 μg/mL,[363] or careful follow-up of complete blood counts should be performed, to reduce the development of bone marrow depression in those with risk for this toxicity, such as patients with renal dysfunction or those receiving high doses of polyenes. One report concluded that adding flucytosine to amphotericin B reduced the rates of relapse during itraconazole maintenance compared with amphotericin B monotherapy,[364] and another retrospective report showed reduced rates of failing in severe cryptococcal meningitis with the combination compared with other regimens[365]; finally, a direct survival benefit from this combination therapy has been reported in two large randomized studies.[280,366] Azoles have been used effectively in the management of cryptococcal meningitis. For instance, fluconazole has been used extensively in cryptococcal meningitis because of its excellent CSF pharmacokinetics and long-term oral safety.[367–371] Clinical trials have shown that it penetrates well into CSF and is excellent for use in the consolidation and suppressive phases of cryptococcal meningitis management.[372,373] However, it tends to be fungistatic and is probably best used in the stage of infection in which there is a low burden of yeasts in the CSF, and thus is not recommended for the primary induction phase of therapy for meningitis when a polyene is available for use.

Itraconazole, despite its poor CSF penetration and inconsistent oral bioavailability, has been successfully used in the treatment of cryptococcal meningitis.[374,375] However, it has been shown to be inferior to fluconazole for the suppressive phase of treatment.[364] Its place in therapy for cryptococcal meningitis is probably as a less-than-ideal alternative to first-line therapy with fluconazole in the consolidation and suppressive phases of management if the drug levels are monitored. It is not recommended for primary induction-phase therapy. Other azoles have been studied for treatment of cryptococcosis, but miconazole and ketoconazole are no longer used. The relatively new triazoles—voriconazole, posaconazole, and isavuconazole—have been studied in a small number of refractory cases of cryptococcosis with moderate success,[314,376,377] but it is not clear whether they possess any advantage over fluconazole.

The antifungal class of β-glucan synthase inhibitors, such as caspofungin, micafungin, and anidulafungin, does not possess reliable anticryptococcal activity and is not to be used for management of cryptococcal infections.

Combination therapy for the management of cryptococcal meningitis has been extremely well studied. The combination of amphotericin B and flucytosine has become the standard therapy for meningitis, and in patients without AIDS it usually sterilizes CSF after 2 weeks of therapy. In fact, it clears CSF yeast counts significantly faster than amphotericin B alone, amphotericin B plus fluconazole, or all three agents together.[378] In areas without access to flucytosine, amphotericin B plus fluconazole has reasonable anticryptococcal induction success, and the 800-mg/day dose of fluconazole is probably superior to the 400-mg/day dose when added to amphotericin B.[379] Flucytosine and fluconazole together have been studied in animals and in open clinical trials for induction therapy with some benefit, and the combination is not inferior to the polyene combination regimens,[380] but this combination remains an alternative regimen when only oral therapy can be given pending further studies.[381] Finally, successful three-drug regimens for induction therapy have occasionally been successfully reported with a polyene, an azole, and flucytosine,[382] but the added benefit of a three-drug regimen has not consistently been proven to be better than the two-drug regimens.

A standard algorithm for the management of cryptococcal meningitis in patients with HIV is a three-stage regimen.[360] Induction-phase treatment is initiated with amphotericin B 0.7 mg/kg/day plus flucytosine 100 mg/kg/day for at least 2 weeks. In resource-limited environments, a 1-week induction combination of a polyene plus flucytosine appears to be effective, with a better outcome than prolonged polyene therapy.[380] Patients who have responded clinically may be switched to fluconazole 400 to 800 mg/day for 8 to 10 weeks as a consolidation phase. Finally, a suppressive phase is begun with fluconazole 200 mg once daily. The use of suppressive- or maintenance-phase therapy for cryptococcal meningitis became a concept during the pre-ART AIDS epidemic, when 50% to 60% of patients relapsed after therapy was stopped. With the use of fluconazole, daily suppression was better than that obtained with intermittent amphotericin B or itraconazole, and there was a reduction in the relapse rates to less than 5%.[372,373] Recent data in several studies have shown that administration of ART, with its ability to produce immune reconstitution (rising CD4 counts and lower HIV loads), allows antifungal therapy to be stopped after 1 to 2 years in patients with a CD4 count above 100/μL for at least 3 months, a nondetectable viral load, and a negative or low serum cryptococcal antigen.[383,384]

However, these patients should be followed off therapy with serum cryptococcal antigen titers for rising titers and/or development of new symptoms.

Patients without AIDS can be given either a 4-week induction-phase regimen of amphotericin B, with or without initial flucytosine, or, more commonly, the previously mentioned regimen used for AIDS patients of 2 weeks. If induction therapy includes only a polyene, this phase should be extended at least another 2 weeks. In transplant recipients with known renal toxicity issues, a lipid polyene formulation is favored with flucytosine for a 2-week induction period. These patients can then receive a consolidation phase of fluconazole 400 to 800 mg/day for 8 weeks and finally be placed on suppressive doses of fluconazole 200 mg/day. Criteria for stopping therapy are not well defined but include resolution of initial symptoms, negative CSF cultures from several milliliters of CSF, normal CSF glucose, and a prolonged asymptomatic period of 6 months to 1 year. A negative CSF or serum cryptococcal antigen does not appear to be required to discontinue therapy. Patients with continuing high-level immunosuppression may benefit from prolonged fluconazole therapy after these criteria are met because relapse rates are measurable. Because there was a 15% to 25% relapse rate before the AIDS epidemic, primarily occurring in the first year after stopping therapy, most patients will receive at least 12 months of suppressive fluconazole therapy.

The site of infection may modify the treatment recommendation.[330] Any presentation of disseminated cryptococcosis should probably follow the recommendations for cryptococcal meningitis. On the other hand, cryptococcosis confined to the lung in previously healthy persons responds well to fluconazole at 200 to 400 mg/day for 3 to 6 months.[67,368,369] Nonimmunosuppressed patients with endobronchial colonization but without radiologic evidence of pulmonary parenchymal disease do not require antifungal treatment. However, if the patient is symptomatic, immunocompromised, or at risk for immunosuppression, treatment should be started. CNS cryptococcomas are treated with a prolonged combination of amphotericin B and flucytosine for the induction phase and then tend to be treated for longer periods with fluconazole, but they rarely need surgical removal.[385] MRI scans of the brain may not show a decrease in lesion size for many months after treatment is started and even stopped. Edema around a lesion, if present, decreases more rapidly.[329]

Identifying a relapse or persistent infection can be difficult in patients with cryptococcal infections. The two clearest signs of relapse after at least 4 weeks of an established antifungal regimen, which suggest a change in management, are (1) development of new clinical signs and symptoms and (2) repeat positive cultures. The persistence of a positive India ink examination or changing versus fixed polysaccharide antigen titers are not precise indications of relapse. IRIS in cryptococcosis must be considered.[239] It is marked by a rapid return of an inflammatory response that may produce new symptoms, such as fever, headaches with or without increasing intracranial pressure, and increased number of host cells in the CSF. This syndrome may occur from several weeks to months to a year after beginning ART or after immunosuppressive dose reduction in transplant recipients. It is still not certain when it is best to initiate ART during the treatment of cryptococcal meningitis to prevent this syndrome, but recommendations range from 2 to 10 weeks after the start of antifungal therapy, and at present it is beneficial to not start ART during the induction phase of treatment.[331] In resource-limited settings ART may need to be started around the 4- to 5-week period. This recommendation is supported by the Cryptococcal Optimal ART Timing (COAT) trial, which showed a worse outcome with early ART administration (during the first 2 weeks of cryptococcal therapy) compared with delayed ART.[386] It is important to recognize that IRIS is not an indication of direct antifungal failure and might be improved with empirical corticosteroid therapy in seriously ill patients with CNS disease.

A critical management issue in cryptococcal meningitis is the role of increased intracranial pressure.[387,388] Patients with severe infection often present with CSF opening pressures in excess of 250 mm of CSF and rapidly progressing signs of cerebral edema. Clinicians need to particularly respond to high opening pressures and symptoms rather than a specific elevated opening pressure measurement. Symptoms include confusion, somnolence, severe headache, emesis, cranial nerve palsies, and fading vision. The pathophysiology for this elevated subarachnoid pressure even as antifungal treatment is started remains uncertain, but there is a suggestion of CSF outflow obstruction through the arachnoid villi by clumping of yeasts.[388] Furthermore, cerebral edema is obvious at autopsy, with uncal grooving, midbrain compression, and herniation of the cerebellar tonsils. Control of increased intracranial pressures with external drainage, such as by repeated lumbar punctures with large-bore needles and ventricular or lumbar drains, may be necessary during the early treatment phase, and frequent lumbar punctures may be especially important in resource-limited settings.[390,391] Persistent, symptomatic, high CSF pressures may warrant placement of a permanent CSF shunt. In retrospective reviews, corticosteroid treatment for elevated intracranial pressure without IRIS was not found to be generally useful.[387,388] In fact, a recent study confirms the lack of value for routine early use of corticosteroids in cryptococcal meningitis during induction therapy, with more disabilities, adverse events, and reduced antifungal killing of yeasts with the use of corticosteroids.[392] Thus cases need to be examined on an individual basis for use of corticosteroids during the diagnosis of IRIS. Unfortunately, despite interventions, blindness, permanent dementia, or death may still result.

It is vital to distinguish cerebral edema from classic hydrocephalus. The latter is diagnosed by the presence of dilated cerebral ventricles and dementia, with or without gait ataxia or urinary incontinence. CSF pressure may or may not be elevated. A loculated temporal horn of the lateral cerebral ventricle may present as a space-filling mass and cause transfalciform herniation. Symptoms of hydrocephalus need to be identified in the follow-up management period and can occur months after the initial diagnosis. A shunt for hydrocephalus can be placed successfully during effective therapy for cryptococcal meningitis.[328] No evidence suggests that a shunt placement after institution of appropriate antifungal therapy presents a foreign body that impairs cure, but it needs to be placed after the start of the antifungal regimen.

Every attempt to improve the immunity of the patient with cryptococcosis should be made. For example, a goal is to reduce the routine daily dose of prednisone to less than or equal to 20 mg/day during therapy. Adjunctive cytokine therapies, such as with granulocyte colony-stimulating factor, GM-CSF, and interferon-γ, have in vitro support, and preliminary human studies with interferon-γ as adjunctive therapy have been positive.[393,394] However, immunomodulation for management of cryptococcosis awaits further definitive clinical studies, and clinicians must be wary of rapidly pushing too far with immune stimulation into IRIS. At present, immunomodulation should probably be considered as adjunctive therapy for culture-positive refractory cases. Specific monoclonal antibodies for treatment remain in early clinical trials.[214] Finally, ART has a major influence on improving immunity and has made a significant impact on a patient's long-term prognosis with cryptococcal meningitis.[395] ART should be instituted and monitored in all HIV-infected patients after induction treatment for cryptococcal meningitis. It is essential to gain control of the HIV infection for long-term success.

PROGNOSIS

The most important prognostic factor for success in the treatment of cryptococcosis remains the ability to control the patient's underlying disease. In fact, it has been shown that cancer victims have shorter survival than patients with AIDS because of the inability to control their underlying neoplasm.[396] In another major group of patients with cryptococcosis, those who received solid-organ transplants, the results are conflicting. Studies have shown an outcome similar to that in patients without an underlying disease,[67] but another study reported a death rate of 42%.[366] Ironically, the group with the worst prognosis is the non-HIV, nontransplant recipient group, which likely represents the variety of underlying diseases or delay in making the diagnosis, or both.[397]

Several studies have examined the prognostic features of cryptococcal meningitis,[42,349,359] and a summary of the different populations, treatment modalities, and end point evaluations suggests that there are three major prognostic findings: (1) burden of yeasts at presentation, (2) poor inflammatory response, and (3) level of the patient's sensorium at presentation. For example, a poor prognosis is indicated by a strongly positive India ink examination, a high polysaccharide antigen titer (≥1:1024), and a poor inflammatory response in the CSF (<20 cells/μL).[398]

In one cohort, cryptococcosis was more severe in males, HIV-seropositive patients, and serotype A infection.[399] In a *C. gattii* cohort, both neurologic deterioration sufficient to require intensive care unit admission and the presence of underlying immunosuppressive diseases were associated with a poor prognosis.[400] Patients who present with a lucid sensorium have a better prognosis than those who are stuporous or in a coma. An abnormal brain imaging at baseline is associated with reduced survival. The prognosis is also influenced by the ability to manage the underlying disease and to detect and treat elevated intracranial pressure. For instance, poor prognostic underlying conditions are severe liver disease or a hematologic malignancy.[10] Multiple studies have identified the poor prognosis of patients with liver diseases, and this likely reflects both end-organ disease and delayed diagnosis.[401] Identification of these high-risk patients so that failure and relapse can be predicted may allow the clinician to design a specific antifungal regimen for this refractory subset. Although the host appears to be the primary factor in outcome, the infecting cryptococcal strain may have some impact on outcome, and more studies are warranted in this area.[402] In most cases in developed countries, the immediate mortality rate (at 6 months to a year) of cryptococcal meningitis unfortunately still remains at 10% to 25%.[103,403] In underdeveloped countries with limited resources, the mortality rate at 6 months can reach from 50% to 100%.[333]

PREVENTION

Four potential methods for preventing infection in high-risk patients exist. First, in the pre-ART era, fluconazole prophylaxis in patients with AIDS and CD4 counts under 100 cells/μL has been shown to be effective in reducing the incidence of cryptococcosis.[404–406] However, both the use of ART and concern about fluconazole resistance with its widespread use have reduced enthusiasm for this general approach in most settings. Second, active immunization with a vaccine in high-risk patients has been considered. A cryptococcal GXM–tetanus toxoid conjugate vaccine that protected mice has been developed,[407] and several new potential protective antigens have been identified.[408] However, human trials have not yet been conducted, and parameters for the use of a vaccine in nonimmunosuppressed populations with potential risk factors for disease are hard to define. Third, the use of protective serotherapy with specific monoclonal antibodies[409,410] could be considered in high-risk patients, but protection would require repeated injections. Fourth, a preemptive treatment strategy in areas of high cryptococcal prevalence and HIV infection, by using cryptococcal antigen screening with ART introduction, may be cost-effective in managing or preventing cryptococcal disease.[411] Finally, high-risk patients can attempt to avoid high-risk environments, such as sites where large numbers of yeasts might be aerosolized from bird droppings.

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263 *Histoplasma capsulatum* (Histoplasmosis)

George S. Deepe, Jr.

SHORT VIEW SUMMARY

Definition
- Histoplasmosis is the most frequent cause of fungal respiratory infection and has a broad spectrum of clinical manifestations ranging from a self-limited, acute, influenza-like illness to a progressive disseminated infection that is life-threatening.

Epidemiology
- The fungus is typically found in the midwestern and southeastern United States and in Central and South America.
- Indigenous cases have been reported worldwide.
- The fungus thrives in decaying bird guano (starlings and blackbirds) and bat guano.
- Patients with acquired immunodeficiency syndrome (AIDS) or who are receiving immunosuppressive drugs, including tumor necrosis factor-α inhibitors, are predisposed to disseminated infection.

Microbiology
- The fungus exists in the mycelial form in nature.
- The mycelia produce two sizes of conidia—microconidia and macroconidia. The former are thought to induce disease because they are small enough to reach the bronchioles and alveoli.
- Conversion to the yeast phase is driven by temperature.

Diagnosis
- Serology is useful for all but AIDS patients. Complement fixation titers of 1:32 or greater are indicative of active disease. The presence of the H immunodiffusion band signifies active disease. An M band does not discriminate between current or remote infection.
- The *Histoplasma* antigen test, either from urine or from serum, is particularly useful in diagnosis of extrapulmonary disease. It is also useful for following response to therapy.
- Tissue and buffy coat examination is useful for detecting the organism.
- See Table 263.2 for diagnostic tests.

Therapy
- Treatment regimens are as follows:
  - Acute pulmonary histoplasmosis: mild to moderate—no treatment or itraconazole 200 mg 3 times a day for 3 days followed by 200 mg twice a day for 6 to 12 weeks; moderate to severe—lipid-formulated amphotericin B, 3 to 5 mg/kg or deoxycholate amphotericin B, 0.7 to 1 mg/kg, for 1 to 2 weeks, followed by itraconazole for a total of 10 to 11 weeks; chronic cavitary—itraconazole as described above
  - Disseminated disease: moderate to severe—same as moderate-to-severe pulmonary disease; in children, can use deoxycholate amphotericin B, 1 mg/kg for 4 to 6 weeks
  - Rheumatologic manifestations—nonsteroidals
  - Mediastinal lymphadenitis—no treatment or, if symptomatic, itraconazole as described above
- See Table 263.3 for treatment options.

Prevention
- For prophylaxis for immunosuppressed patients, itraconazole 200 mg daily is administered.
- Construction sites, spelunking, and remodeling of unoccupied homes or farm habitat should be avoided.

Histoplasma capsulatum is one of the more common causes of infection in the US Midwest and Southeast. Histoplasmosis, acquired through inhalation of mycelial fragments and microconidia, is most often self-limiting but can cause potentially lethal infection in patients with preexisting conditions. It remains a frequent cause of opportunistic infection in patients whose immune system is impaired by pharmaceutic agents or by the human immunodeficiency virus (HIV). This accelerating trend is unlikely to abate because the reservoir of *H. capsulatum* (soil) will never disappear.

HISTORY

The discovery of *H. capsulatum* was made in December 1905, when Samuel Darling, a pathologist stationed in Panama, examined visceral tissues and bone marrow from a young man from Martinique whose death was originally attributed to miliary tuberculosis.[1] Peering through his microscope, Darling was struck by the presence of many small bodies, most of which were intracellular. Having been influenced by reports from Leishman and Donovan, he mistakenly thought that this organism was a protozoan. Because it lacked a kinetoplast, Darling assumed that it was a different *Leishmania* species. He termed this new species *Histoplasma capsulatum* because it seemingly exhibited a capsule. It was not until 1912, after reviewing tissue specimens, that da Rocha Lima suggested that the organism resembled a yeast rather than a protozoan.[2] More than 20 years later, the organism was finally isolated on artificial medium and observed to grow as a mold at room temperature and as a yeast at 37°C.[3]

For many years, the presence of pulmonary calcifications had become synonymous with healed tuberculosis by physicians. Amos Christie, a pediatrician at Vanderbilt University, dispelled that dictum.[4,5] The presence of cutaneous reactivity to a skin test reagent, prepared from the mycelial phase of the organism, in an infant with disseminated histoplasmosis prompted large-scale testing during the 1930s. This endeavor unearthed the surprising finding that histoplasmosis was highly prevalent in the Ohio and Mississippi River Valleys.[5] Moreover, many cases of presumed tuberculosis that were based on the presence of calcified nodules on chest radiographs were determined to be histoplasmosis instead.[6] Eventually, many individuals residing in tuberculosis sanatoriums in the midwestern and southeastern United States were recognized to have been mistakenly admitted. They had histoplasmosis, not tuberculosis. Some of these individuals contracted tuberculosis while housed in open wards with patients who had active pulmonary tuberculosis.

ECOLOGY AND EPIDEMIOLOGY

Cases of histoplasmosis have been reported from every continent except Antarctica. *H. capsulatum* is a soil-based fungus that has been isolated from many regions of the world and is most often associated with river valleys; as mentioned earlier, the most highly endemic regions are the Ohio and Mississippi River Valleys (Fig. 263.1).[6] The conditions that favor the growth of this fungus in soil are a mean temperature of 22°C to 29°C, an annual precipitation of 35 to 50 inches, and a relative humidity of 67% to 87%. These conditions are typically found in the temperate

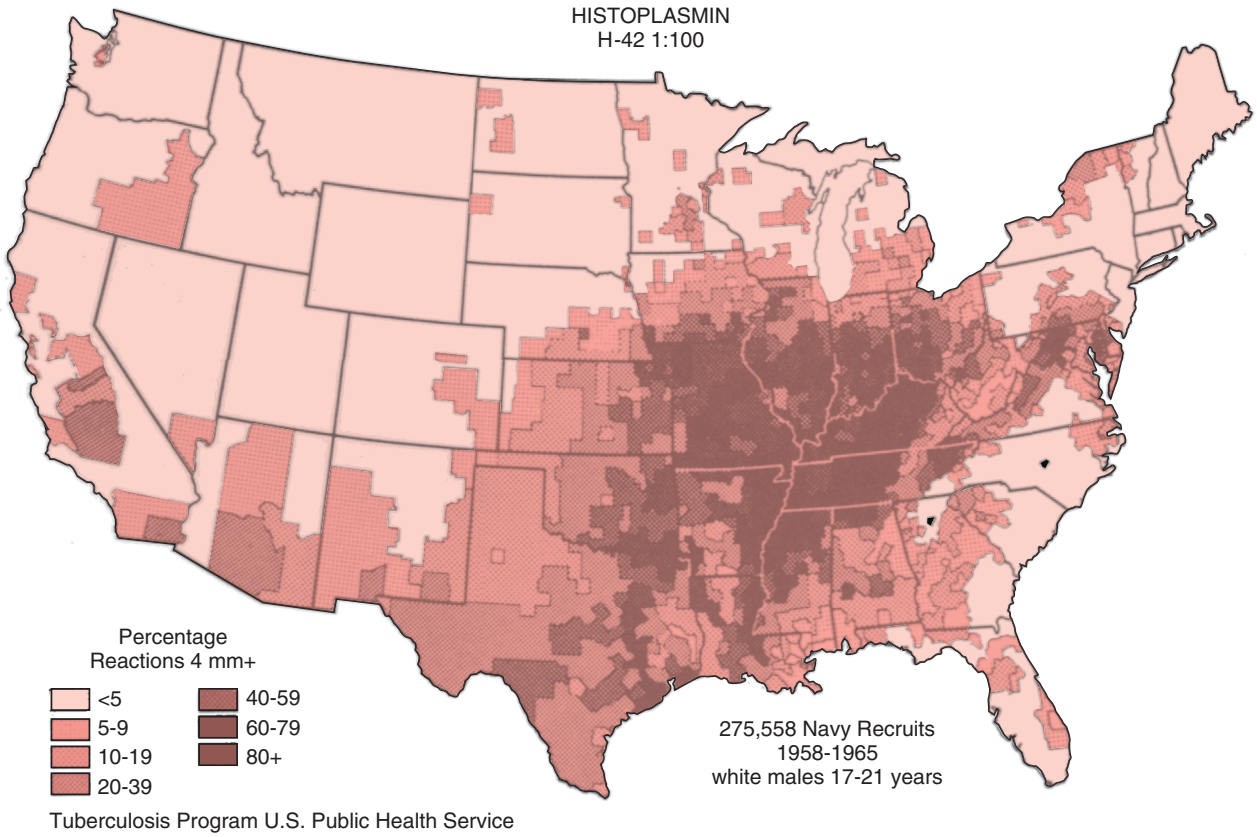

FIG. 263.1 Histoplasmin reactivity in the continental United States among naval recruits. *(From Edwards LB, Acquaviva FA, Livesay VT, et al. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States.* Am Rev Respir Dis. *1969;99:1–111.)*

zone between latitudes 45 degrees north to 30 degrees south.[7] The organism is usually found within 20 cm of the surface, and it prefers soil that is acidic, has a high nitrogen content, and is moist. In areas where avians roost, the fungus is found most often where the guano is decaying and mixed with soil.[8] In such areas, infectious particles can exceed 10^5/g of soil. Fresh guano is less likely to contain any infectious particles. There is a strong association between the presence of bird and bat guano and the presence of *H. capsulatum*. In fact, the first isolation of the organism from an environmental source was from an area adjacent to a chicken house. Birds are not infected by the fungus, and attempts to isolate *H. capsulatum* from their cloaca have been unsuccessful. Bats, on the other hand, carry the fungus in their gastrointestinal tracts and shed it.[9]

Disruption of the soil by excavation or construction is one of the most common means of releasing infectious elements that are inhaled and eventually settle into the lungs. Individuals involved in recreational or work activities that expose them to disrupted soil are at highest risk for infection. Persons at risk include spelunkers who roam caves where bats reside and those who are engaged in agriculture, outdoor construction, or rehabilitation of buildings that have been inhabited by birds or bats. Human-to-human transmission via the pulmonary route has not been reported.

H. capsulatum contains five to seven chromosomes. Differences in numbers of chromosomes are evident among strains. Originally, the organism could be distinguished by two chemotypes, but the advent of molecular biology has improved methods to distinguish strains of *H. capsulatum*. Eight clades of this fungus have been identified through molecular analysis[10]—two North American, two Latin American, and one each of Australian, Indonesian, Eurasian, and African clades. The spread of this fungus appears to have originated from Latin America between 3 and 13 million years ago. Interesting to note, many of the isolates recovered from acquired immunodeficiency syndrome (AIDS) patients in St. Louis were found to be in clade 1, and these isolates are much less virulent in mice.[11] Genetic differences can be associated with varied clinical manifestations. *H. capsulatum* from specific regions of South America often produce skin lesions, whereas isolates from North America do not. The findings suggest that *H. capsulatum* is highly diverse at the genetic level, perhaps based on the fact that the fungus undergoes sexual recombination in nature, thus allowing for exchange of genetic material.

The impact of histoplasmosis on health care costs is not precisely known because it is not a reportable disease. Hospitalizations for histoplasmosis in the United States showed an upward trend from 2001 to 2012. Over this time period, in-hospital mortality was 4.9%. The median cost for hospitalization was approximately $72,000, and total charges in 2012 were estimated to be $371 million.[12] Another analysis of mortality spanning the years 1980 to 1997 demonstrated that the incidence of mortality in the United States from histoplasmosis ranged from 0.1 to 0.15 per 100,000 population.[13] Earlier data indicated that disease develops in males more frequently than in females by a 4:1 ratio. This information was likely skewed because of the association of chronic pulmonary histoplasmosis with smoking, which for many years was a male-dominated activity. That sex bias has not been observed in recent years.

MYCOLOGY

H. capsulatum is classified as a member of the family Ascomycetes and has a heterothallic form designated *Ajellomyces capsulatum* (see Chapter 255). Mating types (+) and (−) have been described, and when combined onto sporulating medium, they produce fruiting bodies containing asci. Isolates from patients carry the (−) mating type two to seven times more frequently than the (+) type, although the ratio of mating types in soil is 1:1.[14]

The organism has two morphotypes: the mycelial and yeast phases. The former is present at ambient temperature, and the latter at 37°C or higher. The saprobic or mycelial phase can be divided into two colony

types: brown (B) and albino (A). The A type grows more rapidly in culture and loses the capability to produce spores after prolonged subculturing. The B type generates a brown pigment. Yeast cells from the B type are more virulent in mice than those from the A type.

The basic elements of the nutritional needs of the organism are poorly defined because of the lack of a standardized medium. The organism requires vitamins, thiamine, biotin, and iron. Sulfhydryl groups in the form of cysteine or cystine are necessary for growth and maintenance of the yeast phase. The mycelial and yeast phases differ in their requirements for calcium. Chelation of this element from medium inhibits the growth of the mycelial but not the yeast phase.[15]

Microscopic evaluation of the mycelial phase reveals two types of conidia. Macroconidia are large ovoid bodies that span 8 to 15 μm in diameter. The surface is decorated with slender protrusions that are referred to as *tuberculate.* Microconidia are small, smooth oval bodies with a diameter ranging from 2 to 5 μm (Fig. 263.2). These forms are believed to be the infective phase because their size is small enough to lodge in the terminal bronchioles and alveoli.

The transition from the saprobic to the yeast phase is a critical step in infectivity of the fungus. On exposure to 37°C, the organism undergoes genetic, biochemical, and physical alterations that result in the production of yeast cells that are uninucleate. These forms are small, typically 2 to 5 μm in diameter, and reproduce by multipolar budding (Fig. 263.3). The stimulus for the transition is heat, and the shift in temperature may be sensed by a change in the fluidity of the yeast membrane. Analysis of the conversion using microarrays has revealed numerous alterations in gene expression. The shift was associated with induction of genes contributing to conidiation, cell polarity, and melanin.[16] Using insertional mutagenesis, a transcription factor termed *Ryp1* has been found to be essential for growth of yeast cells at 37°C. In addition, three other genes, *Ryp2, Ryp3, Ryp4,* that are crucial for the transition have been identified. Hence, there is a complex regulatory network that dictates the conversion to yeast cells when the temperature is elevated.[17] Three biochemical stages have been identified during the conversion after exposure to 37°C. Stage 1 is characterized by an uncoupling of oxidation-phosphorylation and a decrease in RNA and protein synthesis. In stage 2, no respiration is detectable, and in stage 3 there is a resumption of respiration. Chitin and α- and β-glucan content differ between the two phases.

Within tissues, yeast cells may possess a morphologic appearance that differs from the usual ovoid shape. Misshapen or large yeasts have been observed in tissues and epithelial cells. These allomorphs may contain less α-1,3-glucan and appear to be less virulent in mice than oval-shaped yeasts.



FIG. 263.2 Mycelial phase of *Histoplasma capsulatum.* Both macroconidia and microconidia are evident.

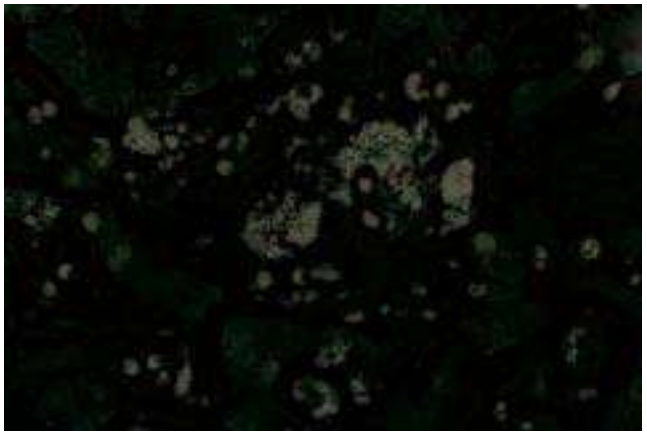

FIG. 263.3 Yeast cells of *Histoplasma capsulatum* in a section of liver. (Gomori methenamine silver, ×1000.)

PATHOGENESIS

The study of the pathogenesis of this fungus has accelerated as a result of technologic advances, including a transformation system to delete genes; silencing RNA; and insertional mutagenesis using *Agrobacterium tumefaciens.* These tools create the foundation for examining the influence of genes or gene regulators on the pathobiology of *H. capsulatum.*[18,19]

The transition from the mycelial to the yeast phase is the most critical determinant in the establishment of infection.[20] This contention is supported by several findings. First, it is rare to find mycelial particles in tissues of humans or mammals with established infection. Rather, yeast cells are commonly detected. Second, exposure of *H. capsulatum* mycelia to *p*-chloromercuriphenylsulfonic acid (PCMS), a sulfhydryl inhibitor, irreversibly blocks the conversion to yeasts but does not alter growth of yeasts or mycelia. PCMS-treated mycelia fail to infect animals.

Iron and zinc are vital elements required for survival of *H. capsulatum.*[21] The organism acquires iron from the intracellular environment by three means: release of iron-scavenging siderophores, production of a ferric reductase, and modulation of pH to remove iron from transferrin. α-1,3-Glucan has been found to be a key virulence factor in the pathogenesis of *H. capsulatum.*[18] Synthesis of this carbohydrate, which is regulated by an amylase, blocks β-glucan binding to Dectin-1 and thereby suppresses generation of important proinflammatory cytokines.

After conidia settle into the alveoli, they bind to the CD11-CD18 family of integrins and are engulfed by neutrophils and macrophages.[22] It is likely that the conversion of mycelia to the yeast phase transpires, at least partially if not entirely, intracellularly. The duration of the phase transition ranges from hours to days. After transformation of conidia into yeasts in the lungs, yeasts migrate, presumably intracellularly, to local draining lymph nodes and subsequently to distant organs rich in mononuclear phagocytes (e.g., liver, spleen). The yeasts grow within resting macrophages. Activation of cellular immunity is necessary for restricting growth, and in primary infection this arm of immunity matures by 2 weeks.

Innate Immunity

In experimental pulmonary infection, neutrophils constitute one of the prominent cell populations that emigrate early into infected foci of lungs.[23] These cells are capable of inhibiting the growth of yeast cells. Constituents from the azurophilic granules express fungistatic activity, and defensins also inhibit the growth of yeast cells.[24] Neutrophils mount a respiratory burst in response to the fungus, but the oxygen intermediates are trapped intracellularly. Despite the burst, there is little evidence that toxic oxygen intermediates contribute to the anti-*Histoplasma* activity of these phagocytes. Resistance to these molecules is mediated in large part by superoxide dismutase 3, a copper/zinc-dependent enzyme.[25]

Macrophages and dendritic cells are the principal effector cells in host resistance to this fungus.[22,26] The fate of yeast cells in each of these cell populations differs. Yeasts proliferate within resting mononuclear phagocytes, but this form is killed by dendritic cells. As noted, macrophages engulf yeast via CD11-CD18 receptors, whereas dendritic cells use the fibronectin receptor. Engagement of two disparate receptors may explain in part the different fates within these cell populations. The central importance of monocytes or macrophages, or both, in controlling *H. capsulatum* is highlighted by the discovery of individuals

with congenital monocytopenia (*GATA 2* deficiency) who present with disseminated histoplasmosis.[27]

In murine macrophages, a high percentage of yeast cells are located within phagolysosomes. Binding to the CD11-CD18 receptors and subsequent entry into human macrophages are mediated by heat shock protein 60 expressed on the surface of yeast.[28] The fungus must contend with the adverse contents (e.g., acid proteinases) of this intensely hostile environment. A mechanism whereby yeasts survive is by alkalinization of the phagolysosome.[29] Yeast cells raise the pH of the phagocytic compartment from 6 to 6.5. One reason for maintaining the pH within a narrow range is that yeast cells require iron to grow, and if the pH exceeds 6.5, they cannot acquire iron from the host.[22]

Nitric oxide produced by activated murine macrophages is a major mediator of anti-*Histoplasma* activity. The ability of this nitrogen intermediate to oxidize iron may explain its potent fungicidal activity.[30] However, its influence in human infection remains unknown because human macrophages infected with *H. capsulatum* have not been reported to produce nitric oxide.

Macrophages from HIV-infected individuals manifest defective activity in their interaction with *H. capsulatum*. These cells bind fewer yeasts than cells from uninfected individuals, and a direct correlation exists between the $CD4^+$ T-cell count and the capacity of macrophages to bind yeast cells. On entry into cells, yeasts grow more rapidly within macrophages from HIV-infected individuals or in macrophages that have been infected in vitro with a macrophage-tropic strain of HIV. The envelope glycoprotein 120 from the virus is responsible for the inhibition of binding yeasts to macrophages[22] but not the altered growth characteristics of the yeasts in phagocytes.

Adaptive Immunity

Within the elements of the acquired immune response, T cells are pivotal in clearance of the fungus. Experimental studies indicate that neither B cells nor antibodies influence host resistance, although the data are limited. $CD4^+$ cells are extremely important in controlling primary infection in mice.[31] The central role of this T-cell subset in this species is supported by the finding that in HIV-infected individuals, most cases of histoplasmosis develop when the $CD4^+$ cell count is lower than 200/μm.[32] Mice deficient in $CD8^+$ cells are impaired in their ability to reduce the fungal burden, but they can eventually eliminate the fungus.[31] Vaccination of mice with yeast cells gives rise to a $CD8^+$ interleukin (IL)-17^+ T-cell population that confers protective immunity. The outgrowth of this population provides an additional arm to adaptive immunity that can combat infection.[33] In secondary infection, the absence of $CD4^+$ or $CD8^+$ cells diminishes the efficiency of yeast elimination, but mice survive. The loss of protective immunity develops only when both subsets are eliminated.

The primary contribution of T cells to host defense is the release of cytokines that eventually activate mononuclear phagocytes. Neutralization of endogenous interferon-γ (IFN-γ) or mice congenitally deficient in this lymphokine are exceptionally susceptible to infection.[31] Other cytokines in mice that are necessary for host clearance are IL-12 and tumor necrosis factor-α (TNF-α). Blockade of endogenous production of either of these leads to the death of mice. The effect of IL-12 is mediated through the induction of IFN-γ.[31] Interesting to note, IL-12 is important in primary infection but not in reexposure histoplasmosis.[31] TNF-α and IFN-γ are both necessary for controlling primary infection, and the former is required for secondary infection.[31] Their importance in humans has been highlighted by the finding that TNF antagonists or individuals with defective IFN-γ signaling manifest an enhanced susceptibility to disseminated infection.[34,35]

In vitro, recombinant IFN-γ activates murine peritoneal macrophages to inhibit the growth of yeast cells. Macrophages from other tissue sources are either nonresponsive to this stimulus or require costimulation with lipopolysaccharide.[21] The anti-*Histoplasma* action of IFN-γ is mediated by limiting iron acquisition, and this effect can be reversed through exposure to additional iron.[29] Human macrophages, on the other hand, do not respond to human recombinant IFN-γ to inhibit yeast cell growth.[22] The cytokines that activate these cells are macrophage colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and IL-3.[22] Granulocyte-macrophage colony-stimulating factor appears to inhibit intracellular growth of yeast cells by limiting access to zinc in phagosomes.[36] Although the infection is limited by cell-mediated immunity, tissues are not sterilized. Tissues of infected individuals contain yeasts, some of which may remain viable for many years. The dormant organisms pose little risk unless the individual becomes immunosuppressed as a result of potent immunosuppressive agents used to combat various clinical conditions or immunosuppressive viruses such as HIV. The metabolic state of *H. capsulatum* in tissues is unknown. It is likely that some of the yeasts remain viable because individuals who moved from endemic to nonendemic areas many years ago may have reactivated infection. The cascade of immunologic events that leads to activation of this form of the infection remains largely unknown. A murine model of reactivation histoplasmosis has been developed, and it should facilitate studies of the organism and the host in this form of infection. In mice, $CD4^+$, $CD8^+$, and a Thy-1.2, $CD4^-$, $CD8^-$ cell must be eliminated to achieve progressive infection. B cells also appear to be important in the severity of reactivation disease.

Genetic Predisposition to Disease

The emerging field of human genetic susceptibility to infectious diseases has extended to histoplasmosis. Several underlying genetic alterations are now known to predispose to more aggressive histoplasmosis. The X-linked hyper–immunoglobulin M syndrome is associated with defective IL-17 generation, and the majority of these individuals with disseminated histoplasmosis manifest gastrointestinal invasion, thus indicating the central influence of IL-17 on mucosal host defenses.[37] Disseminated histoplasmosis in individuals with mutations in the IFN-γ receptor and IL-12 pathway has been recognized.[38] Others have reported that patients with disseminated histoplasmosis or coccidioidomycosis have a gain-of-function mutation in the transcription factor signal transducer and activator of transcription 1 (STAT1), which is important in the signaling cascade of type 1 and type 2 IFNs.[39] Although these cases are uncommon, one should consider the possibility of a genetic predisposition in individuals in whom preexisting immunologic defects are not known.

Granulomas

The hallmark of the tissue response to this fungus is the development of caseating or noncaseating granulomas in which calcium may be deposited (Fig. 263.4). The granuloma consists of an admixture of mononuclear phagocytes and lymphocytes, principally T cells. The putative function of the granuloma is to contain fungal growth. Although IFN-γ and TNF-α are important in the generation of granulomas formed in response to other microbes, neutralization of these two cytokines does not prevent their formation in response to *H. capsulatum*. The organization of the *Histoplasma* granuloma has been characterized in mouse livers and lungs. $CD4^+$ and $CD8^+$ cells are present in the granulomas of mice. T-cell composition is polyclonal, and these cells are the source of IFN-γ and IL-17, whereas macrophages are the principal source of TNF-α.[40]

Organized granulomatous inflammation is typically observed in self-limited disease. Conversely, in progressive disseminated histoplasmosis



FIG. 263.4 Granuloma in the lung of a patient with histoplasmosis.

TABLE 263.1 Spectrum of *Histoplasma capsulatum*–Induced Disease

MANIFESTATIONS	ACUTE PULMONARY DISEASE	CHRONIC CAVITARY PULMONARY DISEASE	PROGRESSIVE DISSEMINATED DISEASE
Clinical	Often asymptomatic	Fever, productive cough, chest pain	Fever, weight loss, hepatosplenomegaly, hematologic disturbances[a]
Immunologic			
Positive skin test	>90%	70%–90%	30%–55%
Lymphocyte transformation	+++	+ to +++	±
Antibody to *Histoplasma capsulatum*[b]	25%–85%[c]	75%–95%	70%–90%
Antigenuria	20%[c]	40%	60%–90%
Pathologic			
Positive culture from lungs	<25%	5%–70%	50%–70%
Histology	Caseating and noncaseating granulomas, few yeasts, giant cells	Noncaseating granulomas, interstitial fibrosis, necrosis, yeasts, cavities, few to moderate yeasts	Diffuse macrophage proliferation, abundant few giant cells

[a]Hematologic disturbances include anemia, leukopenia, and thrombocytopenia.
[b]Complement fixation titer of greater than or equal to 1:8.
[c]Higher incidence in those with symptomatic infection.
+, Indicates a proliferative response to antigen or mitogen that is 3- to 5-fold higher than background; ++, 5- to 10-fold higher than background; +++, more than 10-fold higher.
From Deepe GS Jr, Bullock WE. Histoplasmosis: a granulomatous inflammatory response. In: Gallin JI, Goldstein IM, Synderman R, eds. Inflammation: Basic Principles and Clinical Correlates. *2nd ed. New York: Raven Press; 1992:943.*

(PDH), the more common histopathologic appearance of tissue is a massive influx of macrophages with scattered lymphocytes. Well-circumscribed granulomas are infrequently present, and the lack of an organized inflammatory response is indicative of a perturbed cellular immune response. Occasionally, the inflammatory response in mediastinal lymph nodes is exaggerated, resulting in excessive granuloma formation followed by fibrosis. The progressive scarring may affect the patency of the airways and major blood vessels.[41]

Delayed-Type Hypersensitivity

In experimental infection, either cutaneous or in vitro delayed-type hypersensitivity responses to *H. capsulatum* antigens are detected approximately 2 weeks after exposure.[31] In humans, delayed-type hypersensitivity responses manifest within 3 to 6 weeks after exposure.[42] These values are simply approximations because the precise time at which individuals are exposed in endemic areas is exceptionally difficult to determine. Reexposure to *H. capsulatum* in previously sensitized individuals is characterized by a more rapid tissue response. This finding is not surprising because *H. capsulatum* induces a memory response in which the immune system reacts in a much shorter time frame.

Infection with *H. capsulatum* produces a broad array of clinical and pathologic manifestations that must be recognized in order to diagnose and treat individuals afflicted with this fungus correctly. The clinicopathologic manifestations are summarized in Table 263.1.

PULMONARY HISTOPLASMOSIS

Acute Infections

Acute Primary Infection

Symptoms

The vast majority of primary infections (>90%) go unrecognized medically. Usually, they are asymptomatic or result in mild influenza-like illness for which individuals do not seek medical attention. However, there is a small proportion of patients who become overtly ill. The major determinant for the development of symptoms is likely to be the inoculum size, although differences in strain virulence cannot be excluded.[43,44] Other contributing factors include age and underlying diseases. Thus, older adults, children younger than 2 years, and individuals whose immune systems are compromised are more likely to develop progressive disseminated disease symptoms.

In those who become ill, the typical incubation time is 7 to 21 days, and most individuals manifest symptoms by day 14.[43,44,45] Fever that may reach 42°C, headache, nonproductive cough, chills, and chest pain are the most common symptoms noted. Chest pain is described usually as a substernal discomfort, although in an outbreak in children it was more often located in the anterior chest.[45] Pleuritic chest pain is uncommon. The chest pain is believed to be caused by enlargement of the mediastinal or hilar lymph nodes, or both. Malaise, weakness, fatigue, and myalgias are observed in a distinctly smaller percentage of patients. Most symptoms resolve within 10 days, but they can persist for several weeks if there is an exposure to a heavy inoculum. Acute pulmonary infection can be accompanied by a number of rheumatologic manifestations. Arthralgias, erythema nodosum, and erythema multiforme are present in approximately 6% of patients, most of whom are women.[46] In some, these manifestations of histoplasmosis may be the presenting complaint. Frank arthritis is distinctly uncommon.



FIG. 263.5 Chest radiograph of patient with acute pulmonary histoplasmosis.

Clinical Findings

Physical findings in acute pulmonary histoplasmosis are minimal. Crackles may be detected on auscultation of the lungs and, rarely, hepatosplenomegaly. The common radiographic features are characterized by a patchy pneumonitis that eventually calcifies and hilar lymphadenopathy (Fig. 263.5). If a heavy exposure has transpired, numerous patches of pneumonitis that calcify may develop, and these produce the so-called buckshot appearance on the chest radiograph.[35] Pleural effusions are

distinctly uncommon. The white blood cell count is usually within the normal range, but approximately 30% of patients will have leukocytosis or leukopenia during the course of this infection. Another laboratory abnormality is a transient increase in the serum alkaline phosphatase level. Pulmonary function studies, performed in only a few patients, have demonstrated reversible restrictive defects, impaired diffusing capacity of lung for carbon monoxide (DL_{CO}), and obstructive defects.[47]

Pericarditis

At least 6% of patients who acquire histoplasmosis develop acute pericarditis.[48] This figure may underestimate the true incidence because only those most seriously affected seek medical attention. Precordial chest pain and fever are frequent. A high proportion of patients report a respiratory illness approximately 6 weeks before the onset of the pericarditis. A pericardial friction rub is auscultated in more than 75% of patients with pericarditis, and pulsus paradoxus is also present in more than 75%. An enlarged cardiac silhouette is usually seen on chest radiographs. Electrocardiographic abnormalities indicative of pericarditis—for example, ST segment elevation—are often observed. Only a small percentage of individuals develop cardiac tamponade. The likely cause of the pericarditis is not direct invasion of the organism because it is rarely found in tissue specimens or in pericardial fluid, but rather the granulomatous inflammatory response that is mounted in mediastinal lymph nodes adjacent to the pericardium.

Differential Diagnosis

Acute pulmonary histoplasmosis must be distinguished from influenza and other forms of community-acquired pneumonia.[49] This task is difficult unless a thorough exposure history is obtained. Of greater concern, however, are patients who present with mediastinal lymphadenopathy. This finding is often considered to be caused by a hematologic malignancy rather than histoplasmosis. In such cases, patients may undergo unnecessary surgical procedures in an attempt to establish a diagnosis. Sarcoidosis also should be considered, and distinguishing between these conditions can be difficult. Both may have similar histopathologic features, and serum angiotensin-converting enzyme levels are elevated in each disease.[50] Thus, in all patients who present with mediastinal or hilar lymphadenopathy, it is critically important that histoplasmosis be considered in the differential diagnosis in patients who reside or have recently been in an endemic region.

A Ghon complex and pulmonary calcifications are common in healed pulmonary histoplasmosis. Another characteristic feature of resolved primary infection is the presence of splenic or liver calcifications. In fact, the presence of these on a routine radiograph should be considered evidence of resolved histoplasmosis if the patient has been in an endemic area. Although splenic and liver calcifications are also noted in healed tuberculosis, the most likely cause of these findings remains histoplasmosis because the incidence of tuberculosis in the United States is much lower than that of histoplasmosis. Positron-emission tomography studies performed to diagnose malignant pulmonary nodules are reported to be falsely positive in healed histoplasmosis lesions, especially if there is active inflammation. One assessment that distinguishes cancer from histoplasmosis is that in the latter, the fluorodeoxyglucose signal intensity is often higher in lymph nodes than in the lung lesion[51]

Acute Reinfection

It is not uncommon for those who reside in endemic areas to be exposed more than once to *H. capsulatum*. Those who are reexposed to a large inoculum in heavily endemic areas present with a milder influenza-like illness. The onset can begin within 3 days, which is shorter than in primary infection. The characteristic chest radiograph is one of numerous small nodules that are diffusely scattered throughout both lung fields. This feature has been referred to as *miliary granulomatosis*. Hilar or mediastinal lymphadenopathy is absent. The duration of illness is often briefer than in primary infection.[43,44,52]

Complications of Primary Histoplasmosis

Histoplasmoma

An infrequent complication of primary histoplasmosis is the development of a mass lesion that resembles a fibroma.[53] When it arises, it is found most often in the lung. Instead of resolving, a nidus of infection gradually enlarges over years to form a concentric mass. Presumably, the growth is caused by persistent antigenic stimulus from the yeasts. It is composed of active inflammation at the periphery and fibrous tissue within the inner sphere, and eventually the central portion calcifies. Radiographically, the histoplasmoma may have a central core of calcium or rings of calcium, and these findings are useful in distinguishing it from a neoplastic growth.

Mediastinal Granuloma and Fibrosis

Another complication of primary infection is a massive enlargement of the mediastinal lymph nodes that is caused by the granulomatous inflammation mounted in response to the fungus.[54] The diameter of these nodes can reach 8 to 10 cm. The nodes are caseous and contain a fibrotic shell that may be up to 5 mm thick. Often, this process is asymptomatic. Occasionally, however, the nodes may impinge on major airways and impair gas exchange. During the healing process, the fibrotic tissue can cause retraction of the airways, leading to postobstructive pneumonias, hypoxemia, and bronchiectasis. The fibrosis also may constrict the esophagus or the superior vena cava, resulting in dysphagia, superior vena cava syndrome, or both.[53,54]

Calcific deposits that originate within the lungs occasionally produce lithoptysis. More common, however, is the penetration of enlarged, calcified nodes into the airways and the generation of particles of calcium that can be expectorated. If the calcified mass is large, airway obstruction may ensue. Another consequence of enlarged nodes is the creation of sinuses or fistulas between the airways and pericardium or esophagus.[53]

A rare but dire consequence of mediastinal involvement is mediastinal fibrosis.[55] This syndrome is similar to that observed with tuberculosis, in which the infection leads to a massive deposition of fibrotic tissue within the mediastinum (Fig. 263.6). The mechanism underlying this exuberant immune response is unknown. However, it appears not to be triggered by massive numbers of yeast because they are observed infrequently in lesions. The reaction to the antigen or antigens from *H. capsulatum* must be host specific, based on its infrequent development. Genetic susceptibility loci for this entity have not been identified. The fibrosis encroaches on all the structures of the mediastinum, including the major airways, superior vena cava, and esophagus. The symptoms that arise from the fibrotic process are attributable to the narrowing of the patency of these structures. Hypoxemia, shortness of breath, superior vena cava syndrome, and dysphagia may ensue as the fibrotic process progresses.

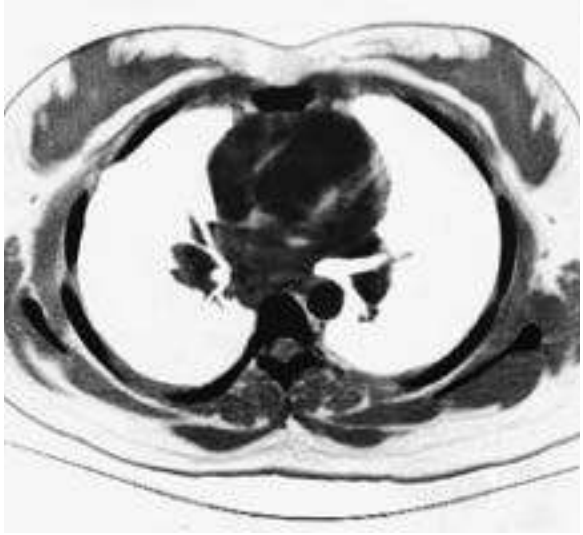

FIG. 263.6 Computed tomography scan of the mediastinum in a patient with mediastinal fibrosis.

CHRONIC PULMONARY HISTOPLASMOSIS

Cavitary pulmonary histoplasmosis and chronic pulmonary histoplasmosis have been considered for many years to be synonymous terms. However, one review called for reconsideration of this usage. Although the precise incidence was not known because of the sporadic nature of the disease, approximately 8% of individuals developed fibrocavitary disease following two large epidemics in Indianapolis.[56] Cavitary lesions were found in the upper lobes in more than 90% of cases (Fig. 263.7). Men older than 50 years with preexisting chronic lung disease, usually emphysema, were reported to constitute the highest proportion of patients, and it was quite unusual in those younger than age 40 (<5% of all cases).[56,57] However, a recent review has called this association into question. Among a group of patients with pulmonary symptoms that extended for at least 6 weeks, nearly 50% were women and 20% had chronic obstructive pulmonary disease (COPD). Cavities were detected in less than 40%.[58] The causes for the changing face of this illness are not clearly defined, but the decrease in tobacco use is certainly a contributing factor. It also suggests that there are at least two forms of chronically active lung disease, cavitary and noncavitary.

Symptoms

In the classic form of cavitary disease, the most frequent symptoms are low-grade fever, productive cough, dyspnea, and weight loss of insidious onset. Night sweats, chest pain, hemoptysis, and malaise are less common. Hemoptysis is rare. Completely asymptomatic intervals with radiologic stability are interspersed with periods of recurrent symptoms and radiologic progression. Both an early and a late stage have been described. The major difference between the two forms is the symptoms. In the early stage, the illness, characterized by chest pain, productive cough, fever, and weakness, begins abruptly and persists for several weeks before medical attention is sought. In the late stage, the proportion of patients experiencing productive cough and hemoptysis is much higher, whereas chest pain and fever are much less frequent. Bronchogenic transmission from one segment of the lung to another may occur during cough or aspiration.[57]

Laboratory Findings

Radiographically, patchy infiltrates appear and develop areas of dense consolidation that progress to cavitation. Over months or years, extensive fibrosis, retraction, and areas of compensatory emphysema appear unless effective treatment is given. The most common location is the upper lobes. Almost all patients have a history of heavy cigarette use or exhibit evidence of emphysema coexisting with COPD, and therefore the cavities must be distinguished from preexisting bullae. Thin-walled or thick-walled cavities may form in response to *H. capsulatum.* Enlarged hilar or mediastinal lymph nodes are notably absent, and distinctive laboratory features are not present. Leukocytosis and elevated alkaline phosphatase levels are detected in about one-third of symptomatic patients, and anemia is present in half.[56,57]



FIG. 263.7 Chest radiograph of a patient with cavitary histoplasmosis.

Pathology

The earliest lesion that appears at histopathologic assessment is an interstitial pneumonitis. The inflammatory infiltrate is composed primarily of lymphocytes and macrophages and is often found adjacent to bullae. The alveolar walls are thickened, and the peribronchial lymphatics contain a similar type of inflammatory infiltrate. Subsequently, necrosis develops, and it resembles that caused by infarction. Vascular compromise, as denoted by subintimal thickening and vessel obliteration, is present in inflamed regions. Proteinaceous exudate can be found in bullae, and yeasts are present in the necrotic lining of a cavity or in small encapsulated necrotic lesions. Areas of infarction are slowly replaced by scarring of the involved parenchyma. The healing phase is characterized by fibrosis and retraction, some leaving central areas of caseous necrosis surrounded by epithelioid cells, lymphocytes, and giant cells. Neighboring bullae may enlarge from compensatory emphysema. After healing, recurrence of cavitary lesions will develop in approximately 20% of patients, but the prognosis of recurrence is no different from that of the initial infection.[57]

Spontaneous resolution of thin-walled and thick-walled cavities ranges from 10% to 60%, and thin-walled cavities have a higher healing rate. Despite shrinking and fibrosis of individual lesions, new lesions continue to appear and radiologic progression occurs in an estimated 79%. In individuals with COPD, cavitary histoplasmosis can exacerbate the existing pulmonary dysfunction, and the destructive nature of the inflammation irreversibly compromises pulmonary function. Death caused by cavitary histoplasmosis is distinctly unusual but is attributable to respiratory failure, cor pulmonale, or secondary bacterial pneumonia.

The association between the presence of COPD and chronic cavitary histoplasmosis suggests that the anatomic defect present in these lungs predisposes patients to this clinical form of infection and promotes the formation of cavities. This intriguing postulate has been proposed and is most likely correct, although no experimental data exist to support it.[57] The difficulty in testing this hypothesis is that a suitable animal model has not been developed. In addition, it does not explain the development of cavities in other patients. At present, *H. capsulatum* is not known to elaborate any elastinolytic or proteolytic enzymes that digest collagen.

Noncavitary Chronic Pulmonary Disease

The most common symptoms of those with the redefined chronic pulmonary histoplasmosis who did not necessarily have cavitary lesions are cough, weight loss, and fever and chills. Radiographic changes commonly include nodules, infiltrates, and lymphadenopathy. Less common are fibrosis, thickened pleura, and volume loss. Arthralgias, dyspnea, fatigue, and chest pain are observed in less than 50% of patients. The most striking difference is that in those without cavities, positive cultures are highly unusual. Most patients in a series were treated with antifungal therapy, and infection resolved in 70% of those. Of the remaining patients who received therapy, 10% manifested a protracted illness, 15% had a recurrence, and in 5% the infection did not resolve. Among the group that did not receive antifungal therapy (13 of 46), 2 manifested progressive infection and 1 did not clear the infiltrate.[58]

PROGRESSIVE DISSEMINATED HISTOPLASMOSIS

Incidence

Although all primary infections can be considered disseminated because yeast cells migrate from the lungs to organs rich in mononuclear phagocytes, the term *progressive disseminated histoplasmosis* refers to the relentless growth of the organism in multiple organ systems. The estimated incidence is 1 in 2000 cases of histoplasmosis. After the two Indianapolis epidemics, 8% of clinically recognized cases of histoplasmosis were PDH.[59] The major risk factors in those two epidemics for this manifestation of histoplasmosis were age older than 54 years and immunosuppression. In the pre–antiretroviral therapy era, the incidence approached 25% in AIDS patients from endemic regions. The outcome

of infection with *H. capsulatum* was poor among HIV-infected individuals, including the presence of a chronic medical condition and a history of herpes simplex virus infection. Conversely, the use of antiretrovirals and triazoles was associated with a decreased risk. In an analysis of 1074 renal allograft recipients, 0.4% developed clinically recognized PDH over a 25-year span.[60] This value is moderately different from the one reported during the Indianapolis epidemic, in which 2.1% of renal allograft recipients exhibited PDH.[61] Others have reported an incidence of 0.1%. Another risk factor for acquiring PDH is the use of TNF-α antagonists.[34] Disseminated histoplasmosis in hematopoietic stem cell transplant recipients appears to be infrequent. This finding seems unusual given the severity of immunosuppression but may support the concept that many cases of disseminated disease are reinfections.

Reactivation Versus New Exposure

The exposure leading to PDH is inapparent, without an antecedent episode of acute pulmonary histoplasmosis. PDH can develop by reexposure to the fungus or by reactivation of dormant endogenous foci. The precise distribution of cases that arise from endogenous reactivation is unknown. In endemic areas, it is impossible to distinguish reactivation versus reinfection. Autopsy studies in the 1950s revealed that the fungus could be detected in lymph nodes by means of silver stain, yet the tissues were sterile. This finding certainly suggests that reinfection is more important in endemic areas. Reactivation of latent infection can develop from transplanted organs.[62] In one such case, kidney transplantation from one donor was associated with infection in recipients who were not residents of an endemic area. These types of cases are difficult to discern in endemic areas. Most cases of PDH are now observed in immunosuppressed individuals.[59,63] However, there still exist cases in previously normal individuals, often at the extremes of age, who were not known to exhibit preexisting immunologic dysfunction. The perturbations that cause a breach in the integrity of the immune system and therefore lead to reactivation of quiescent infected foci have not been delineated.

Pathology

Although infection with *H. capsulatum* produces a broad range of disease, PDH also can be categorized according to clinical and pathologic manifestations. There is an acute form associated with a fulminant course. Histopathologically, massive macrophage infiltration and scattered lymphocytes are apparent. Tissue macrophages are engorged with yeast cells, and tests of cellular immunity often reveal poor to absent responses. At the other extreme is the chronic form, characterized by an indolent course and the presence of well-circumscribed granulomas in involved tissues. In tissues, few numbers of yeast are seen, and delayed-type hypersensitivity responses are intact in a high proportion of individuals.

Acute Progressive Disseminated Histoplasmosis

In the era before aggressive immunosuppressive or cytotoxic therapy, this entity was principally seen in infants—hence its moniker, the infantile form. To date, however, it is most often observed in those who are severely immunosuppressed, especially those with AIDS and hematologic malignancies such as Hodgkin and non-Hodgkin lymphoma. When it occurs in infants and young children, it is believed that this form of histoplasmosis is a progression of a primary exposure or reinfection because pulmonary symptoms dominate the early phases of illness. The onset is usually abrupt, extending over just a few days. Fever and malaise are the two most common manifestations, followed by weight loss, cough, and diarrhea. Physical findings include hepatosplenomegaly in almost all patients, lymphadenopathy, especially of the cervical chain, in about 30%, and crackles. Jaundice is observed in a minority, and oropharyngeal ulcers develop in less than 20%.[63]

Laboratory Abnormalities

Hematologic disturbances are frequent. Anemia is present in more than 90% of cases, and most have a hematocrit lower than 20%. Leukopenia and thrombocytopenia are observed in more than 80% of children. Serum levels of the liver enzymes alanine aminotransferase and alkaline phosphatase are elevated in a high proportion. Chest radiographs most often reveal a patchy pneumonitis with mediastinal and hilar node enlargement. This finding supports the contention that acute PDH in children represents an extension of an exogenous exposure. Untreated, the mortality is 100%; before the introduction of effective antifungal agents, most children died within 5 to 6 weeks after onset of symptoms. Terminal events include disseminated intravascular coagulation; gastrointestinal hemorrhage, probably resulting from severe thrombocytopenia; and secondary bacterial sepsis associated with profound granulocytopenia.

Progressive Disseminated Histoplasmosis and HIV

In HIV-infected individuals, the risk factors for the development of histoplasmosis are a $CD4^+$ cell count lower than 200 cells/mL, history of exposure to chicken coops, and a known positive serologic finding of complement-fixing (CF) antibodies before illness.[64] In the era of antiretroviral therapy, the incidence of disease in HIV-infected individuals has dropped substantially. Most AIDS patients who develop PDH have had at least one opportunistic infection. On seeking medical attention, almost all patients manifest evidence of a systemic illness. Fever and weight loss are found in more than 90% of those with AIDS and PDH. The most common physical findings include rales, hepatosplenomegaly, and lymphadenopathy. Mucosal ulcers are distinctly uncommon, but as many as 10% of patients will exhibit cutaneous lesions.[65] The common cutaneous findings are a maculopapular eruption, petechiae, or ecchymosis. The maculopapular rash does not display any unique pattern of distribution. The finding of skin lesions is much more common in those infected with the South American strains of *H. capsulatum.* In a small study, up to 66% of South American AIDS patients with disseminated histoplasmosis manifested skin lesions. The most common appearance is a papular eruption with crusting. Less frequent are nodular or purely pustular lesions. Histopathologic evaluation of skin lesions reveals necrosis circumscribing the superficial dermal vessels. There is perivascular cuffing with lymphocytes and neutrophils, but the number of cells is few. Yeasts are present both intracellularly and extracellularly. In addition to the skin findings, a number of other unusual manifestations have been reported, including colonic masses, perianal ulcers, chorioretinitis, meningitis, and encephalitis. It is estimated from results of one series that up to 20% of patients with PDH will have central nervous system (CNS) involvement.[66] The more aggressive forms include encephalitis, acute meningitis, and encephalopathy in acute PDH. Histoplasmoma of the CNS and chronic meningitis are manifestations of a more indolent form of PDH.

Laboratory Abnormalities in Coinfection

Anemia, thrombocytopenia, and leukopenia are common laboratory features of PDH in the immunosuppressed population. In AIDS patients, the alteration in the peripheral blood counts may be attributable in part to the disease or to the drugs that they are receiving. Elevated serum levels of hepatic enzymes are frequently detected. Again, concomitant drugs may obscure the laboratory abnormalities caused strictly by *H. capsulatum.* Chest radiographs typically demonstrate widely scattered nodular opacities or a diffuse reticular pattern (Fig. 263.8). However, a substantial percentage of patients (30%) may present with a normal radiograph.[67]

The fatality rate of acute PDH in the immunocompromised patient is 100% if untreated. With therapy, survival rates of the acute episode exceed 80%. Infrequently, patients exhibit a sepsis-like syndrome characterized by disseminated intravascular coagulation, encephalopathy, acute respiratory distress syndrome, vascular collapse, and, subsequently, multiorgan failure. In some patients, bone marrow biopsy has demonstrated the presence of histiocytes phagocytosing erythrocytes. This form of PDH has been termed the *reactive hemophagocytic syndrome* and, despite aggressive management and therapy, the outcome is usually catastrophic.

Subacute Progressive Disseminated Histoplasmosis

Subacute PDH is distinguished from the acute form primarily by the more prolonged nature of the symptoms before the patient seeks medical attention. Fever and weight loss are common at some time during the

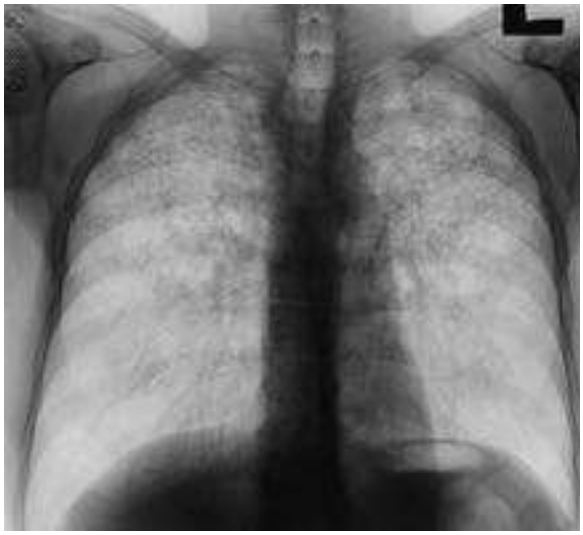

FIG. 263.8 **Diffuse infiltrates in patients with progressive disseminated histoplasmosis and acquired immunodeficiency syndrome.**

course of infection, but fever is a presenting complaint in only about 50%. Physical findings include hepatosplenomegaly and oropharyngeal ulcers. In contrast to the ulcers observed in acute PDH, these are deeper and more likely to be confused with malignancy. Laboratory abnormalities are much less striking than in acute PDH. Although anemia and leukopenia are noted in up to 40%, the percentage of patients with severe depression of the hematocrit or leukocyte count is low. Thrombocytopenia is evident in about 20% and is usually mild. Rarely is the platelet count lower than 20,000/μL.[63]

Clinical Features

One of the notable features of subacute PDH is the presence of focal lesions in various organ systems, including the gastrointestinal tract, endovascular structures, CNS, and adrenal glands.[63,68] Aside from the liver and spleen, the gastrointestinal tract is one of the most common organs affected in subacute PDH. Yeast cells can be found in the bowel mucosa in up to 70% of autopsy cases. Macroscopic ulcerations of the small and large bowel are present in about 40%, and perforation from a penetrating ulcer has been reported. The terminal ileum and cecum are the sites most frequently involved. Symptoms referable to the bowel are not frequent, but if present, diarrhea and crampy abdominal pain are typical complaints. Intestinal obstruction of the ileum has also been reported.

Endovascular Infection

Endocarditis and infection of other vascular structures may be a manifestation of subacute PDH.[69] The aortic and mitral valves are affected more commonly than right-sided valves, and the aortic valve is the single most common valve involved. In about 50% of cases, there is prior evidence of valvular disease, such as a bicuspid aortic valve. Echocardiography shows that the lesions tend to be extensive, and large-vessel embolization can be the presenting symptom. Clumps of yeasts embedded in a fibrin mesh are the characteristic histopathologic feature. Occasionally, allomorphs as large as 20 μm in diameter have been observed. In addition, hyphal forms of *H. capsulatum* have been detected in valvular lesions. If untreated, death usually ensues. Other endovascular manifestations include prosthetic valve endocarditis, infection of abdominal aortic aneurysms, and prosthetic vascular grafts. Previous reports have indicated that blood cultures are rarely positive. However, those reports preceded improved methods for isolating *H. capsulatum* from blood.

Central Nervous System Involvement

CNS infection involves all age groups and causes a number of manifestations, including chronic meningitis, mass lesion, and cerebritis. Among these, chronic meningitis is the most frequent.[66] Symptoms of CNS histoplasmosis may antedate medical attention for several weeks, and they include headache, altered sensorium, and cranial nerve deficits. Seizures, ataxia, meningismus, and other focal deficits constitute much of the remaining symptoms. It must be emphasized that only half of patients may complain of symptoms localized to the CNS. Associated physical findings consist of hepatosplenomegaly in about one-third, lymphadenopathy, and mucocutaneous lesions.

Pleocytosis of the cerebrospinal fluid (CSF) is present in all patients. Cell counts usually range from 10 to 100/μL with a preponderance of lymphocytes. Hypoglycorrhachia and elevated protein levels are detected in 80%. Histopathology of the brain parenchyma and meninges characteristically reveals granulomatous inflammation. A perivenous granulomatosis in which parasitized macrophages are observed beneath the intima of parenchymal and meningeal veins is commonly seen. The basilar meninges are the most severely affected area of the CNS. Hydrocephalus may contribute to the symptoms.

An intracerebral granulomatous mass, or histoplasmoma, causes a mass effect and may be mistaken for a malignancy or abscess on computed tomography (CT) images because it exhibits ring enhancement with the administration of contrast. Dense fibrotic tissue surrounds a caseous center in which yeasts are detected. Histoplasmomas may be associated with meningitis but often are independently present. CSF pleocytosis is common, but hypoglycorrhachia is not.

Although symptoms arising from involvement of adrenal glands are not frequent, autopsy series indicate that yeasts invade this organ system in approximately 80% of cases.[70] Macrophages containing yeasts are found scattered throughout the parenchyma of the adrenal gland. There is no particular predilection for the cortex or the medulla. The severity of infection ranges from focal areas containing parasitized macrophages to diffuse involvement of the adrenal parenchyma. The former is most commonly detected. Tissue necrosis is seen but usually involves only a small portion of the gland. Grossly, the adrenal glands are enlarged. This postmortem discovery has been supported by findings on CT in which a high percentage of patients with subacute PDH display enlarged adrenals. Overt Addison disease is uncommon, occurring in less than 10%.

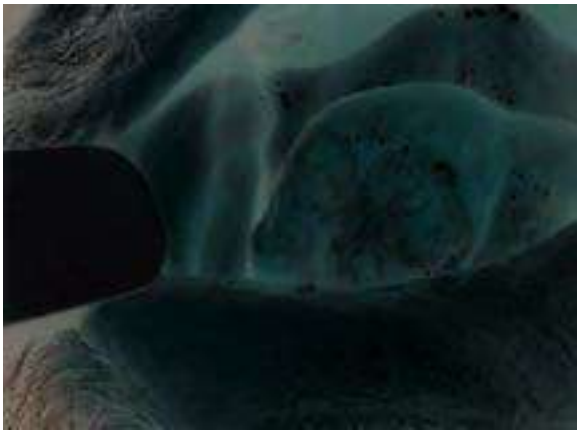

FIG. 263.9 **Tongue ulcer in a patient with chronic disseminated histoplasmosis.**

Chronic Progressive Disseminated Histoplasmosis

Clinical Findings

Chronic PDH can be distinguished from subacute PDH by the prolonged chronicity of symptoms that are often mild. This form is seen almost exclusively in previously normal adults. Malaise and lethargy stand out as the most frequent complaints. Fever is much less frequent (<30%) and is often low grade. The most common physical finding (50%) is an oropharyngeal ulcer that is well circumscribed, indurated, and usually deep and painless (Fig. 263.9).[63] The tongue, buccal mucosa, larynx, gums, and lip are the most affected structures. Occasionally the lesion is on the labia or glans penis. These lesions are often confused with

squamous carcinoma oral malignancy. Hence, it is incumbent on the clinician to consider the diagnosis of histoplasmosis; otherwise, tissue will be sent only for histologic evaluation. Histopathologically, the center of the lesion contains macrophages with many yeasts, but the number of such macrophages decreases in the periphery of the lesion. Unlike the histologic reaction in other viscera, the response in the mucosa is an admixture of acute and chronic inflammation. Thus, plasma cells, lymphocytes, eosinophils, and granulocytes are found infiltrating the ulcer, and fibrosis is a characteristic feature. Areas of intact mucosa may show hyperplasia that can be confused with squamous carcinoma on superficial biopsy. Well-circumscribed granulomas are typically found, usually at the periphery.

Other symptoms include hepatosplenomegaly in about one-third of patients. Chronic meningitis or chronic granulomatous hepatitis may be the only manifestation of infection. Unlike subacute PDH, there is a notable absence of disease involvement of other organ systems, including CNS, heart, and adrenals. Bone infection, Addison disease, and endocarditis all have been described, but these entities are uncommon. Hematologic abnormalities are distinctly uncommon and often not significant. This illness may persist for years, with periods of spontaneous improvement in symptoms, without being recognized. On occasion, there may be an abrupt worsening caused by involvement of a particular organ such as the CNS, adrenals, or heart.[63] Usually, however, the illness remains undiagnosed until symptoms arising from a single organ are observed. Without appropriate therapy, infection progresses to death.

OCULAR HISTOPLASMOSIS

Two different syndromes of ocular involvement are described. The less common is a uveitis or panophthalmitis in association with active PDH. Granulomas are present in the uvea, and yeasts are recovered from lesions. Much more frequent is the presumed ocular histoplasmosis syndrome (POHS), which consists of a posterior uveitis or choroiditis in individuals who manifest skin test positivity to histoplasmin and intrathoracic calcifications.[71] However, it must be stressed that a skin test and the presence of intrathoracic calcifications do not prove cause and effect.

Typically, in POHS there are peripheral atrophic scars and a lack of vitreous or anterior segment inflammation. The scars or "histospots" are located posterior to the equator of the eye. They range in size from 0.2 to 0.7 disk diameters and can vary in number from 1 to 70 in a single eye. Involvement of both eyes is uncommon (<10%). Most individuals are between 20 and 50 years of age when this syndrome is diagnosed, and the prevalence may be as high as 10% in endemic regions. The major destructive consequence of this lesion is macular hemorrhage, which develops 10 to 20 years after the appearance of scars. Neovascularization and scarring can lead to loss of vision in up to 60% of patients.[71] Because neovascularization can exert such devastating effects, efforts have been made to understand its cause. It has been shown that the integrin is expressed on blood vessels from patients with POHS.

The histopathology of POHS reveals a lymphocytic infiltration in the scarred areas. Yeasts are rarely observed in the eye or elsewhere. A model for this syndrome has been developed in primates to define the cellular immunopathology. Chronic lesions contain a preponderance of B and $CD4^+$ cells. As in affected human eyes, yeasts are not found in the lesions. Within the choroidal lesions, there is an increase in the percentage of $CD4^+$ cells and macrophages. There is no definitive proof that *H. capsulatum* causes the scars that are observed in humans, although the primate model establishes that this fungus can produce choroidal scars. The pathogenesis appears to be an exuberant cellular immune reaction to inert fungal antigens, thus somewhat resembling the tissue response in mediastinal fibrosis. Corticosteroid treatment of POHS does not activate latent histoplasmosis.

AFRICAN HISTOPLASMOSIS

In Africa, the classic *H. capsulatum* var. *capsulatum* coexists with *H. capsulatum* var. *duboisii*. The yeast form of the latter is typically much larger, with a diameter of up to 15 µm, and has a thicker wall. The mycelial form of both is indistinguishable. The pathogenesis of this fungus is presumed to be inhalation from the soil, although a primary pulmonary infection has not been demonstrated. Cutaneous inoculation is certainly an alternative mode of acquisition of the infection. Spontaneous disease has been reported in baboons and cynocephalus monkeys. Only a few hundred cases have been reported, but most cases have been reported from Uganda, Nigeria, Zaire, and Senegal. Cases in other African countries and a case in India have also been reported.[72,73]

The clinical picture associated with infection by *H. capsulatum* var. *duboisii* is distinctly different from that caused by *H. capsulatum* var. *capsulatum*.[74] Skin and skeleton are the most frequent organs affected by this pathogen. In the skin, the usual findings are ulcers, nodules, or psoriatic-like lesions that may spontaneously resolve. Involvement of the subcutaneous tissue may present with tender nodules ("cold" abscesses) in which the typical manifestations of inflammation are absent. Osteolytic bone lesions are fairly common and are noted in up to 50% of cases. The skull and ribs are the bones affected most frequently, followed by vertebrae. The organism produces granulomatous inflammation within the bone. This type of inflammation can lead to sinus formation and cystic bone lesions. In a high proportion of patients, multiple bones may be infected. Even in the presence of overt skin or bone lesions, chest radiographs are often free of evidence of previous exposure to *H. capsulatum*. Draining lymph nodes also may become inflamed.

A progressive disseminated disease has been recognized. Patients are febrile, with hematologic abnormalities. There is multiorgan involvement, including liver, spleen, kidney, and lung, and miliary lesions are observed in the lungs. The histopathology resembles that induced by *Blastomyces dermatitidis* or *Coccidioides immitis*—that is, a pyogranulomatous reaction in which there is a combination of granulomas and suppuration. One likely reason for this pathologic reaction is the large size of *H. capsulatum* var. *duboisii,* which prevents avid ingestion by macrophages. Thus, neutrophils may ingress to assist in the clearance of the fungus.

Reports of African histoplasmosis in HIV-infected individuals are emerging. A variety of manifestations in individual patients has been observed. Disseminated infection with fever, cutaneous infection, and bone infection have been recognized.[75] The outcome has been favorable in only a minority of patients.

DIAGNOSIS

Fungal Culture

The use of diagnostics for histoplasmosis is encapsulated in Table 263.2. Histoplasmosis can be definitively established only by means of isolation from body fluids or tissues. The typical medium that is used to recover the fungus from sterile fluids includes brain heart infusion agar, with the addition of antibiotics and cycloheximide when nonsterile fluids such as sputum are cultured. Cultures are incubated at 30°C for up to 6 weeks. Often, growth is noted within 3 weeks, and more than 90% of cultures exhibit fungus within 7 days. Previously, confirmation that the fungus was *H. capsulatum* required exoantigen testing or conversion of the mycelial form to the yeast form, but this step is no longer necessary. All mycelial isolates are confirmed with a DNA probe that recognizes ribosomal RNA. Manipulation of the mycelial phase in the laboratory is a potential biohazard and requires Biosafety Level 3 precautions.

The incidence of positive cultures varies considerably and often is correlated with the number of specimens collected, source of the specimen, and burden of infection. Recovery of *H. capsulatum* from sputa of patients with acute pulmonary histoplasmosis ranges from 10% to 15%, whereas in cavitary histoplasmosis, cultures are positive in up to 60% of patients.[76] The yield of positive cultures increases with the number of specimens collected. Three or more specimens are more likely to be positive for *H. capsulatum.* In AIDS patients with pulmonary manifestations, up to 90% of cultures from the lungs obtained from bronchoscopic samples will grow *H. capsulatum.* Bone marrow and blood cultures are positive in up to 50%.[76] Yields for blood cultures are considerably higher if the lysis centrifugation technique is used. The organism can be frequently isolated from oropharyngeal ulcers in patients with chronic PDH. In endocarditis, valve cultures are positive in a high percentage but blood cultures often are negative. However, much of the evidence concerning blood cultures used the biphasic medium, which may not be as sensitive as lysis centrifugation. In meningitis, the organism is recovered from CSF in 25% to 65% of patients,[66] and the yield is improved

TABLE 263.2 Diagnostics for Histoplasmosis

INFECTION SITE	DISEASE SEVERITY	DIAGNOSTIC
Lung		
Acute	Mild-moderate	H and M bands and complement fixation.
	Moderate to severe	As above. Serum or urine antigen, or both, also may be positive in up to 70%. Bronchoalveolar fluid antigen may be useful. Culture of bronchoalveolar lavage fluid and silver stain of concentrated lavage fluid. Sputum culture.
Chronic cavitary		H and M bands and complement fixation. Culture of bronchoalveolar lavage fluid and silver stain of concentrated lavage fluid. Sputum cultures.
Disseminated		
	Acute	Serum or urine antigen, or both. H and M bands and complement fixation. These are not useful in AIDS patients. Examination of the buffy coat for yeast cells in phagocytes. Biopsy of bone marrow or liver with silver stain and culture. Blood culture.
	Chronic	Serum or urine antigen, or both. H and M bands and complement fixation. Biopsy of tissue with silver stain and culture.
Central nervous system		Serum or urine antigen, or both. CSF antigen. Culture of CSF. H and M bands and complement fixation are not as useful.
Mediastinal		
	Lymphadenitis	H and M bands and complement fixation.
	Granuloma	H and M bands and complement fixation.
	Fibrosis	H and M bands and complement fixation.
Rheumatologic	Arthralgias	H and M bands and complement fixation.
Pericarditis		H and M bands and complement fixation.
Endocarditis or endovascular		H and M bands and complement fixation. Serum or urine antigen, or both. Culture and silver stain of valve.

AIDS, Acquired immunodeficiency syndrome; *CSF,* cerebrospinal fluid.

if a large volume (20 mL or more) is cultured. The organism is unlikely to be isolated from pericardial or pleural fluid, but more likely from their respective serosal tissues. Similarly, *H. capsulatum* rarely is isolated from mediastinal tissues in patients with mediastinal fibrosis.

Antigen Detection

The antigen assay, which detects polysaccharide antigen in serum or urine by means of enzyme-linked immunosorbent assay (ELISA), is the mainstay of diagnosis, especially in those with PDH. Several laboratories now offer the test. Antigen is detected in up to 90% of patients with acute PDH and 40% with cavitary disease.[75] In patients with moderate-to-severe acute pulmonary histoplasmosis, antigen testing of urine and serum yielded a detection rate of approximately 80%. If only single specimens were obtained, the values approximated 65% to 70%.[77] The test is also useful for monitoring relapses of acute PDH, especially in immunosuppressed patients. Failure of antigenemia to fall after a month of therapy should raise concern. Antigen detection is much more sensitive than serology for identifying relapsing cases, and it has been applied successfully to CSF in patients with meningitis. The test was positive in 12 of 14 cases. Thus, it has a high degree of sensitivity. Cross-reactivity in the urine test has been found for patients infected with *B. dermatitidis, C. immitis, Paracoccidioides brasiliensis,* or *Talaromyces (Penicillium) marneffei,* but with improvements in the test, cross-reactions are becoming less frequent. The galactomannan test, often used for detection of invasive aspergillosis, has been reported to be positive in patients with histoplasmosis. The 1,3 β-D-glucan assay also can be positive in histoplasmosis, especially disseminated disease. In a small study, serum IL-2 receptor levels have been reported to be elevated.

Several reports of the usefulness of the polymerase chain reaction have been published, and although such assays are not in clinical use yet, they show promise.

Serology

Since the late 1940s, serology has been a vital instrument in the diagnosis of infection with *H. capsulatum.* CF antibodies and precipitin bands have been the most common tests used in the clinical laboratory. The greatest usefulness has been in the retrospective diagnosis of acute histoplasmosis, using a fourfold or greater rise in CF titer between acute and convalescent serum. This has been particularly helpful in outbreaks that are recognized in time to collect acute sera, but the antibody titer rise occurs too late to be of value in patient management. For chronic pulmonary histoplasmosis or PDH, fourfold rises are not observed and antibody tests have insufficient sensitivity and specificity to be of clinical value. For CF antibodies, a titer of 1:8 to yeast or mycelial antigen is considered positive, and a titer of 1:32 indicates the need to pursue a possible diagnosis of histoplasmosis. Titers that fall between these two values neither exclude nor suggest the diagnosis. On occasion, a result is returned that states that the test is anticomplementary. This result signifies that the serum contained a substance or substances that interfered with the CF test. Repeat of the test with a new serum specimen frequently yields a result.

Low levels of CF antibodies are detected in approximately 10% of healthy individuals who reside in an endemic region. A low percentage of individuals with acute pulmonary histoplasmosis will develop CF antibodies within the first 3 weeks of infection, but by 6 weeks at least 75% of patients manifest a positive CF antibody titer or a fourfold rise. Over the course of months, the antibody titer will decline, although it may remain serofast for years, especially in those with cavitary pulmonary disease or with chronic PDH. The false-positive rate is estimated to be 15% and is most commonly observed in those with coccidioidomycosis or with blastomycosis.[78] The reason for the cross-reactivity is the presence of a carbohydrate antigen common to the three fungi.

Another test is the detection of H and M bands during the illness. On the agar gel precipitin test, these bands are identified by lines of identity with bands formed by control sera known to have precipitating antibody to H or M antigens. These two precipitin bands were originally identified using immunodiffusion as specific to sera from patients with histoplasmosis. The H and M antigens are glycoproteins released by mycelial and yeast phase cultures. The H antigen has been cloned and sequenced, and it demonstrates homology to β-glucosidases. Antibody to H antigen is infrequently detected (<10%) in the sera of patients but, when present, signifies active infection. The M antigen has also been cloned and sequenced, and it has a high degree of homology to catalase. Unlike the H antigen, antibody to M antigen is detected in up to 80% of individuals after exposure to the fungus. However, it is present in patients who have recovered from infection or who have active disease; therefore it is not useful in discriminating remote from current infection. A major limitation of the serologic tests is that even in the presence of active infection, their results are negative in up to 50% of immunosuppressed patients, especially those with AIDS. One explanation for the poor anti-*Histoplasma* antibody response is that the immunosuppressive agents or HIV induces dysfunctional B cells or $CD4^+$ T cells, or both, thus rendering serologic assays almost useless.

Histochemical Identification

Stains for the presence of *H. capsulatum* can be extremely useful for rapid identification of the fungus in various tissues or body fluids. The yeast is visualized poorly with hematoxylin-eosin stain but is more apparent with the periodic acid–Schiff (PAS) stain. The most useful stain is either the Gomori methenamine silver (GMS) or Grocott silver stain. The organism can be detected in peripheral blood smears stained



FIG. 263.10 Circulating neutrophil with intracellular yeast cells. (Wright-Giemsa stain, ×400.)

with Wright-Giemsa in up to 40% of cases of acute PDH (Fig. 263.10). This percentage is much lower if the reader of the blood smear is scrutinizing the slide only to determine a differential, but it is useful if the clinician suspects PDH as a cause of a patient's illness. The yeast must be discriminated from *Pneumocystis jirovecii* in the lung. The latter is larger, nonbudding, and usually extracellular. Moreover, it is exceedingly rare to find *P. jirovecii* outside the lung or in an area of caseous necrosis. Although *Leishmania* spp. and *Toxoplasma gondii* may on occasion be confused morphologically with *H. capsulatum,* neither stains with silver.

Miscellaneous Laboratory Tests

One retrospective study has suggested that patients with AIDS who are admitted to the hospital with pulmonary infiltrates and fever higher than 38°C and serum lactate dehydrogenase (LDH) levels higher than 600 IU/mL are highly likely to have disseminated histoplasmosis.[79] Other studies have suggested that elevated serum ferritin levels are strongly suggestive of histoplasmosis.[80]

THERAPY

The introduction of azoles has moved much of the treatment of histoplasmosis from an inpatient to outpatient setting. Several azoles and polyenes are now available for the treatment of this fungal disease. Updated practice guidelines for the treatment of various forms of histoplasmosis have been published.[81] Table 263.3 summarizes the treatment options.

Acute Pulmonary Histoplasmosis

The vast majority of cases of acute pulmonary histoplasmosis do not require therapeutic intervention. Bed rest and antipyretics suffice for these individuals. Treatment should be instituted for those who have not improved after 1 month of illness or who exhibit hypoxemia. In those in whom illness has not spontaneously resolved after 1 month, oral itraconazole, 200 mg three times daily for 3 days, followed by 200 mg once or twice daily for 6 to 12 weeks will be sufficient. Fluconazole is not as active as itraconazole and should be avoided. If the patient cannot ingest an oral medication or tolerate an azole, liposomal amphotericin B is the preferred agent. A dose of 3 to 5 mg/kg IV can be given every day until the symptoms subside, often within 2 weeks. The dose for the lipid complex formulations of amphotericin B (ABLC) is 5 mg/kg. The deoxycholate formulation can be used at 0.7 to 1 mg/kg daily if there is a low risk of serious nephrotoxicity. No comparative trials of amphotericin B and azoles have been performed, but clinical experience suggests that resolution of symptoms is faster with the former.

If the patient is hypoxemic and requires mechanical ventilation, liposomal amphotericin B, 3 to 5 mg/kg/day, is recommended until improvement is achieved. When patients improve to the point that they can ingest food and medications, itraconazole, 200 mg three times daily for 3 days, followed by once or twice daily should be used to complete 12 weeks of therapy. If the patient is at low risk for renal dysfunction, the deoxycholate preparation of amphotericin B may be substituted at a dose of 0.7 to 1 mg/kg/day. The inflammatory response may be responsible in part for the respiratory compromise. Although ample evidence does not exist, corticosteroids may be used to mitigate inflammation. Intravenous methylprednisolone can be used at a dosage of 0.5 to 1 mg/kg/day for up to 14 days.

TABLE 263.3 Treatment of Histoplasmosis

INFECTION SITE	DISEASE SEVERITY	TREATMENT
Lung		
Acute	Mild-moderate	None or itraconazole 200 mg 3 times daily for 3 days followed by 200 mg twice a day for 6–12 wk.
	Moderate-severe	Lipid-formulated amphotericin B, 3–5 mg/kg, or deoxycholate amphotericin B, 0.7–1 mg/kg, daily for 1–2 wk followed by itraconazole 200 mg 3 times a day for 3 days followed by 200 mg twice a day for a total duration of 12 wk. For children, itraconazole 5–10 mg/kg or deoxycholate amphotericin B, 1 mg/kg daily.
Chronic cavitary		Itraconazole 200 mg 3 times a day for 3 days followed by twice daily for at least 1 yr and as long as 2 yr.
Disseminated[a]		
	Acute	Lipid-formulated amphotericin B, 3–5 mg/kg, or deoxycholate amphotericin B, 0.7–1 mg/kg, daily for 1–2 wk followed by itraconazole 200 mg 3 times a day for 3 days followed by 200 mg twice a day for at least 12 mo. For children, deoxycholate amphotericin B (1 mg/kg) daily for 4–6 wk or 2–4 wk followed by itraconazole 5–10 mg/kg daily. Total duration = 3 mo.
	Chronic	Itraconazole 200 mg 3 times a day for 3 days followed by 200 mg twice a day for at least 1 yr. Serum levels should be monitored to ensure adequate concentrations.
Central nervous system		Liposomal amphotericin B, 5 mg/kg daily for 4–6 wk followed by itraconazole administered as above for at least 1 yr and resolution of symptoms and negative cerebrospinal fluid antigen.
Mediastinal		
	Lymphadenitis	No treatment. If symptomatic (e.g., dysphagia), itraconazole 200 mg twice daily for 12 wk. Corticosteroids (60 mg with a rapid taper) may be used to diminish lymph node size.
	Granuloma	Same as lymphadenitis. Corticosteroids are not necessary.
	Fibrosis	Surgical intervention with stents. Antifungals are not useful.
Rheumatologic	Arthralgias, etc.	Nonsteroidals
Pericarditis		Nonsteroidals or corticosteroids. If the latter, treat with itraconazole (200 mg × 3 for 3 days and then once a day) until corticosteroids have been discontinued.
Endocarditis/ Endovascular		Surgical removal of the valve combined with lipid-formulated amphotericin B, 5 mg/kg daily for 6 wk. Lifelong suppression may be considered in some who are not surgical candidates with itraconazole 200 mg once or twice a day.

[a]See text for duration of treatment in acquired immunodeficiency syndrome (AIDS) patients.

Data from Wheat LJ, Freifeld AG, Kleiman MB, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. Clin Infect Dis. *2007;45:807–825.*

Itraconazole is a lipophilic agent that inhibits the cytochrome P-450 system (see drug interactions listed in Chapter 39). The cyclodextrin oral liquid formulation of itraconazole increases absorption by 50% and makes administration to young children much easier.

Mediastinal Granuloma, Mediastinal Fibrosis, and Histoplasmoma

Hilar and mediastinal lymphadenopathy from acute pulmonary histoplasmosis is usually asymptomatic but can cause a brassy cough or compress the middle lobe bronchus, leading to temporary atelectasis. Although no therapy is usually necessary, persistent symptoms could be treated with itraconazole, 200 mg three times daily for 3 days, followed by 200 mg once or twice daily for 6 to 12 weeks. Rarely, large caseous mediastinal nodes will compress the esophagus or erode into both the esophagus and bronchus, causing a bronchoesophageal fistula. Surgical resection of the nodes may be indicated, although the nodes may be densely adherent to the pulmonary veins and other surrounding structures. Corticosteroids may be used if the enlarged nodes cause significant compression of surrounding structures. Prednisone at a dose not to exceed 80 mg may be tried, with a rapid taper over 1 to 2 weeks.

Mediastinal fibrosis is an exceptionally difficult clinical problem for which there is no consensus on optimal management. Surgery, corticosteroids, and antifungal agents have been used in the treatment of this condition, with minimal success. Surgery to remove the fibrosis area and placement of intravascular stents can alleviate the life-threatening situation, but the fibrosis often progresses. Moreover, the surgery may jeopardize essential venous collaterals, such as the hemiazygos or azygos veins. Addition of azoles after surgery has been proposed, but the usefulness of this approach is debatable.[81]

A histoplasmoma of the lung, which is a fibrocaseous nodule resulting from healed acute pulmonary histoplasmosis, does not require any therapy. Surgical resection or biopsy may be needed to exclude malignancy in a solitary pulmonary nodule if no central calcification is evident. Serology is of no value in proving the nodule is a histoplasmoma.

Cavitary Pulmonary Histoplasmosis

Although in some patients fibrocavitary disease will eventually stabilize without treatment, the inability to predict which patients will eventually develop disease progression has led to the recommendation that all patients should be treated, even those who are currently asymptomatic. Treatment does not improve pulmonary function already lost and, in fact, healing may lead to some further loss of function because of fibrosis. Discontinuing cigarette smoking is an important adjunct in preventing further loss of pulmonary capacity. Many patients with only thin-walled cavities spontaneously resolve infection without therapeutic intervention. Such patients, if untreated, should be followed with serial chest radiographs every 2 to 3 months. Those who have thick-walled cavities, progressive pulmonary infiltrates, or persistent cavities associated with declining respiratory function should be treated. Oral itraconazole, 200 mg three times daily for 3 days, followed by once or twice daily, should be given for 12 to 24 months.[81] This regimen will arrest progression in 75% to 85% of patients. Relapse may be difficult to detect radiologically in patients with extensive prior lung damage. Sputum culture is the best means for detecting relapse, although *Aspergillus* and other rapidly growing molds may overgrow the culture plate. Fluconazole is not recommended. Itraconazole levels should be determined after 2 weeks of therapy to determine if adequate levels have been achieved. A trough blood level of 2 μg/mL by bioassay or the sum of native itraconazole and its hydroxymetabolite by high-performance liquid chromatography (HPLC) has been proposed (see Chapter 39). If there is progression of infection while the patient is on azoles or if relapse has occurred after azole therapy, amphotericin B is preferable. If deoxycholate amphotericin B is used, the total dose is 30 to 35 mg/kg and can be given as 0.7 mg/kg/day or approximately 50 mg daily.[81] If renal dysfunction is a consideration, the liposomal formulation may be used at 3 to 5 mg/kg/day.

The relapse rates for cavitary pulmonary histoplasmosis are as high as 20%, with the highest relapse rates seen in patients with thick-walled cavities. If there is a failure of antifungal therapy, surgical resection may be indicated if the patient has sufficient pulmonary reserve.

Acute Progressive Disseminated Histoplasmosis

Prompt institution of amphotericin B therapy is necessary for treatment of patients with acute, life-threatening PDH. Patients should be begun on liposomal amphotericin B at a dose of 3 mg/kg/day. If ABLC is used, the dose should be 5 mg/kg/day. Within 1 to 2 weeks, most patients are symptomatically improved, and laboratory abnormalities begin to return to baseline values. If deoxycholate amphotericin B is used, the starting dose should be 25 mg followed by a rapid escalation to 1 mg/kg. Once the patient has become afebrile and clinically stable, amphotericin B can be administered at a lower dose of 0.4 to 0.5 mg/kg daily. Patients who demonstrate resolution of symptoms while on amphotericin B may be switched to itraconazole 200 mg three times daily for 3 days, followed by 200 mg twice daily for a total duration of 12 months. In acute PDH that is not associated with hemodynamic instability or severe illness, itraconazole may be used initially. Therapy should begin with 200 mg three times daily for 3 days, followed by 200 mg twice daily for at least 12 months. Itraconazole interacts with many antiretrovirals, including elevating serum concentrations of several protease inhibitors.

In patients with AIDS, lifelong suppressive therapy with itraconazole 200 mg daily is recommended for most patients. Although there are no reliable data to make this decision, it may be reasonable to discontinue maintenance therapy in patients receiving highly active antiretroviral therapy who have a CD4 count higher than 150 cells/μL for 6 months, a nondetectable viral load, at least 12 months of antifungal therapy, and a negative test for *Histoplasma* antigen in urine. If relapse occurs while the patient is receiving azole maintenance therapy, amphotericin B should be given.[81] After treatment for the relapse, the patient should receive amphotericin B as maintenance therapy with 0.7 to 1 mg/kg once or twice weekly. A self-limiting immune reconstitution syndrome (IRS) has been recognized occasionally in HIV patients being treated for PDH who have had an effective response to highly active antiretroviral therapy. The syndrome manifests as fever, with or without an elevated alkaline phosphatase level. Management is supportive. Relapse of PDH is common in other persistently immunosuppressed patients and may be difficult to detect until far advanced. Indefinite suppressive therapy with itraconazole may be a useful option.

Subacute and Chronic Progressive Disseminated Histoplasmosis

Because many of these cases develop in patients whose immune system is intact, itraconazole 200 mg three times daily, followed by 200 mg twice daily is highly efficacious. The success rate in these individuals approaches 90%. If the patient requires hospitalization, does not improve on azole therapy, is immunosuppressed, or demonstrates intolerance to azoles, lipid-based preparations of amphotericin B, 3 to 5 mg/kg/day, should be given. When the infection is controlled with this drug, it is possible to switch to itraconazole to complete a total of 12 months of therapy.

Meningitis

Patients with meningitis should be given liposomal amphotericin B, 3 to 5 mg/kg/day for 4 to 6 weeks, followed by itraconazole, 200 mg two or three times daily, for at least 1 year. Blood levels of itraconazole should be determined. CSF should have a normal glucose level and no detectable CSF *Histoplasma* antigen at the end of therapy. Repeat lumbar punctures should be performed approximately every week for the first 6 weeks and every 2 weeks thereafter to assess therapy. Although a high percentage of patients may respond initially to therapy, relapse frequently occurs. Overall cure rates are no better than 50%, and immunocompetent patients respond much better to treatment than immunosuppressed persons.

Endocarditis

As with bacterial causes of endocarditis, a microbicidal agent should be used. Therefore, liposomal amphotericin B, 3 to 5 mg/kg/day, should be given. If ABLC is used, 5 mg/kg should be administered. Alternatively, if there is a low risk of nephrotoxicity, deoxycholate amphotericin B, 0.7 to 1 mg/kg, may be used. Administration of an antifungal agent alone is not sufficient and must be used in combination with surgical

removal of the affected valve(s). One issue is how long to treat after the valve has been removed. If there are other foci of active histoplasmosis, then treatment guidelines for PDH should be used. However, if the valve was the only site involved, treatment with a lipid formulation or the deoxycholate preparation for 2 weeks after surgical extraction may be sufficient. If the patient cannot undergo surgery, an amphotericin B preparation in the highest tolerated dose should be given daily.[81]

Pericarditis

Pericarditis following acute pulmonary histoplasmosis does not require antifungal therapy. Most patients can be treated symptomatically with nonsteroidal antiinflammatory drugs (NSAIDs) for 2 to 12 weeks.[74] If patients do not respond to these agents, or if the patient manifests hemodynamic instability, corticosteroids are indicated for 1 to 2 weeks, followed by nonsteroidal agents. One must be cautious because if there are active lesions of histoplasmosis, the infection may become more aggressive during corticosteroid therapy. Itraconazole, 200 mg three times daily for 3 days, followed by 200 mg once or twice daily for 6 to 12 weeks, should be included if corticosteroids are necessary. The rationale for the antifungal is to prevent reactivation, although there is little information regarding the frequency with which this occurs in short-term use of corticosteroids. Cardiac tamponade associated with *H. capsulatum* pericarditis is uncommon, but when it occurs, it must be treated as a medical emergency with pericardiocentesis. Despite the severity of illness, antifungal therapy is not indicated. Unlike tuberculous pericarditis, constrictive pericarditis rarely develops, but patients should be monitored for several years after the acute attack. In the uncommon situation in which the pericardium is infected as a manifestation of PDH, antifungal therapy with amphotericin B or azoles is indicated, depending on the severity of illness.

Arthropathies

Nonsteroidals should be continued until resolution of symptoms. If corticosteroids are used to alleviate symptoms, itraconazole should be used concomitantly, as noted earlier.

Presumed Ocular Histoplasmosis

Presumed ocular histoplasmosis does not require antifungal therapy. Laser therapy is used to prevent additional neovascularization within the choroid, but lesions that abut the fovea cannot be subjected to this treatment.[71] Photodynamic therapy for this region of the eye appears promising, as does the implementation of intravitreous antiangiogenic agents. The role of retrobulbar local injection of corticosteroids is unclear.

Other Considerations

Histoplasmosis in pregnancy should be treated with amphotericin B, preferably a lipid formulation, but the deoxycholate preparation can be used if nephrotoxicity is of low concern. The dose of liposomal preparation should be 3 to 5 mg/kg/day for 4 to 6 weeks, and that of the deoxycholate formulation 0.7 to 1 mg/kg/day. If ABLC is used, 5 mg/kg is the preferred dose.

No clear guidelines exist for patients receiving TNF-α antagonists. Those from the endemic area are at the highest risk for reactivation or reinfection. If a patient develops histoplasmosis while on therapy, the biologic agent should be stopped and therapy for disseminated histoplasmosis completed. A study of 98 cases provided a guide to managing these patients.[82] In the vast majority of patients, the TNF-α antagonist was discontinued, and the duration of therapy was approximately 12 months on cessation of the biologic agent. The TNF-α antagonist may be restarted after cessation of antifungal therapy based on results in a small number of cases. Candidates for resumption are those in whom there is no evidence of disease and the antigen is undetectable. However, patients should be monitored closely for clinical symptoms and an antigen every 3 months. Prophylaxis with an antifungal in those who are resuming therapy appears to be unnecessary.

Immune Reconstitution Syndrome and *Histoplasma*

IRS after initiation of treatment for *H. capsulatum* and many other pathogens has been reported. The constellation of symptoms poses a conundrum for clinicians because they have to discriminate between inadequately treated infection and exaggerated host response. The development of IRS is not restricted to those who start antiretroviral therapy; it also has been observed in those in whom biologics have been discontinued or even when corticosteroids have been tapered or discontinued. If the diagnosis of IRS is established, the use of corticosteroids should be considered. TNF-α antagonists have also been recommended because of their ability to enhance the numbers of regulatory T cells. However, one must be cautious in using these agents for histoplasmosis because they are known to exacerbate disease.[83]

PREVENTION

Prophylaxis of Immunocompromised Persons

For immunosuppressed patients who have a high risk of acquiring histoplasmosis from the environment because of their work or their residence, itraconazole, 200 mg/day, is useful. Such patients would include those with AIDS whose CD4 cell count is less than 150/μL or those who require potent immunosuppressive therapy. In the former group, prophylaxis with itraconazole reduced the incidence of infection by more than twofold. Another indication for prophylaxis in immunosuppressed patients would be residence in an area that has a high incidence of infection, as defined by at least 10 cases per 100 patient-years.[84]

Other Considerations

Educational efforts must be ongoing to alert those who work in areas in which a substantial risk of infection exists. Dust control and the use of N95 masks should be considered. For example, construction workers who are restoring buildings that have served as homes for starlings and bats must be warned about the possibility of exposure, and steps taken to remove the guano safely. Spraying 3% formalin on guano deposits will kill the fungus within several days, and the material can then be removed. However, formaldehyde decontamination is rarely used because the vapor is toxic, it can seep into groundwater (thus posing an environmental hazard), and it does not penetrate dried guano uniformly.

There is a continuing effort to develop a vaccine preventive against *H. capsulatum* pathogenic fungi because of their escalating incidence. Among those in which animal studies have defined a vaccine is *H. capsulatum*. Vaccine candidates containing heat shock protein 60 and H antigen from *H. capsulatum* have been demonstrated to confer protection to mice given a pulmonary challenge. A region of heat shock protein 60 that spans amino acids 174 to 445 appears to contain the protective activity of the entire protein. In mice, a monoclonal antibody to the cell surface has been shown to augment the efficacy of antifungal therapy.[85]

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264 Blastomycosis

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SHORT VIEW SUMMARY

Definition
- Infection by *Blastomyces* species, most commonly *B. dermatitidis* and *B. gilchristii*

Microbiology
- Thermal dimorphism with growth as hyphae at ambient temperature and yeast at 37°C
- Morphologic switch from hyphae and conidia to yeast form is essential for virulence
- *Blastomyces* yeast actively subverts and evades host immune defenses

Epidemiology
- Primarily found in North America—Midwest, Central, and Southeastern United States as well as regions along the Great Lakes and St. Lawrence River
- Outbreaks and sporadic disease
- Infection often associated with recreational or occupational activities that disrupt soil
- Most patients have intact immune defenses; however, *Blastomyces* can infect people with impaired immunity
- Cell-mediated immunity required to control infection

Clinical Manifestations
- Blastomycosis can mimic other entities such as community-acquired pneumonia, tuberculosis, and malignancy
- Pneumonia is most common clinical manifestation
- Dissemination to other organs such as skin and bone is common; however, any organ can become infected

Diagnosis
- Fungal staining of clinical specimens provides rapid, presumptive diagnosis
- Broad-based budding yeast with doubly refractile cell wall
- Culture provides definitive diagnosis
- Complement fixation and immunodiffusion serologic tests have poor sensitivity
- Antigen testing of urine useful for diagnosis, but cross-reactions can occur with other dimorphic fungi

Treatment
- Itraconazole: drug of choice for mild-to-moderate pulmonary or non–central nervous system disseminated disease
- Amphotericin B (AmB; deoxycholate or lipid): for patients with moderately severe to severe blastomycosis and immunocompromised patients until improvement and then itraconazole
- Lipid AmB: for initial treatment of central nervous system blastomycosis, followed by azole antifungal therapy for at least 12 months
- Lipid AmB: for blastomycosis in pregnancy

The etiologic agents of blastomycosis belong to a group of fungi that are characterized by thermal dimorphism.[1] Advances in genomics and phylogenetics have expanded the number of *Blastomyces* species to include *B. dermatitidis, B. gilchristii, B. percursus, B. helicus, B. parvus,* and *B. silverae*.[2–6] Infection is primarily acquired through the inhalation of infectious conidia and hyphal fragments following the disruption of soil. Once inside the lungs, the infectious particles convert into pathogenic yeast, which causes pneumonia and can disseminate to other organs. Blastomycosis can mimic other diseases, which often delays diagnosis and initiation of therapy. Thus diagnosis requires a high degree of clinical suspicion. Antifungals with activity against *Blastomyces* yeast include polyenes and azoles.

HISTORY

Blastomycosis was first described in 1894 by Gilchrist[7] as a protozoan infection. Subsequent research by Gilchrist and Stokes[8–10] at Johns Hopkins University correctly identified the organism as a fungus, which they named *Blastomyces dermatitidis*. In 1907 Hamburger[11] at Rush Medical College described thermal dimorphism in *B. dermatitidis* with budding yeast at 37°C and hyphal growth at ambient temperature. Colloquial names for blastomycosis coined early in the 20th century included *Gilchrist disease, Chicago disease,* and *North American blastomycosis.*

In 1951 Schwarz and Baum[12] at the Jewish and General Hospitals in Cincinnati determined that the lung was the primary portal of entry, and involvement of other organs such as the skin was due to lympho-hematogenous dissemination. In 1955 Smith and colleagues[13] at Duke Hospital described the first recognized outbreak of blastomycosis (1953–1954) in Grifton, North Carolina. During the 1960s and 1970s the epidemiology and geographic distribution of blastomycosis were refined. In the mid-1980s *B. dermatitidis* was successfully cultured from soil at the site of an outbreak in Eagle River, Wisconsin.[14] In the 1990s clinical trials under the auspices of the National Institutes of Allergy and Infectious Diseases Mycoses Study Group were conducted to assess the efficacy of fluconazole and itraconazole.[15–17] The first practice guideline for treatment of blastomycosis was published by the Infectious Diseases Society of America (IDSA) in 2000 and updated in 2008.[18,19] The American Thoracic Society (ATS) Fungal Working Group published their guidelines in 2011.[20]

There have been substantial advances in the past 2 decades in understanding the molecular pathogenesis including identification and characterization of genes critical for immune evasion and morphogenesis. In addition, the *Blastomyces* genus has undergone taxonomic revision. In 2013 Brown and colleagues[2,21] discovered a new species in North America, which they named *Blastomyces gilchristii* in honor of Dr. Gilchrist. *B. dermatitidis* and *B. gilchristii* are estimated to have diverged 1.9 million years ago during the Pleistocene epoch.[21] In 2017 Dukik and colleagues[4] identified another new species, *Blastomyces percursus,* when analyzing human clinical isolates from South Africa and Israel. Several *Emmonsia* species have been reclassified into the *Blastomyces* genus including *Blastomyces helicus* (formerly *E. helicus*) and *Blastomyces parvus* (formerly *E. parvus*).[5,6] *B. helicus* causes blastomycosis in North America, but outside the traditional endemic region. This species chiefly infects patients with impaired immunity, and results in fungemia and high mortality.[5] *B. parvus* predominantly causes infection in rodents.[6] *Blastomyces silverae* is a new species named after Eleanor Silver Keeping who was a distinguished mycologist at the University of Alberta.[6] *B. silverae* has been misidentified as *E. parvus* and can cause infection in humans.[6] The genomic sequences of *B. dermatitidis, B. gilchristii,* and *B. percursus* are publicly available.[22]

MYCOLOGY

Blastomyces spp. belong to the Ascomycota phylum, which includes other dimorphic fungi such as *Histoplasma, Coccidioides, Sporothrix,*

Paracoccidioides, Emmonsia, and *Talaromyces marneffei* (formerly *Penicillium marneffei*).[23] The biology of these fungi is characterized by the ability to reversibly convert between hyphal and yeast forms in response to temperature.[23]

At environmental temperature (22°C–25°C), *Blastomyces* grows as septated hyphae (1–2 µm in diameter) that produce small conidia (4–5 µm) attached to a short conidiophore, which resembles a lollipop (Fig. 264.1A).[24,25] *B. percursus* has a slightly different morphologic appearance than *B. dermatitidis* or *B. gilchristii* at ambient temperature, with conidiophores bearing single or multiple conidia.[4] The appearance of *B. helicus* is unique because the hyphae can coil into a helical morphology and fail to produce conidia under most in vitro conditions.[5] In the laboratory, *Blastomyces* conidia are difficult to liberate from hyphae and require wetting the culture plate with water or phosphate-buffered saline solution followed by mechanical disruption.[26] The morphologic appearance of mycelia is not distinctive, and definitive identification requires conversion to yeast or molecular methods.[27]

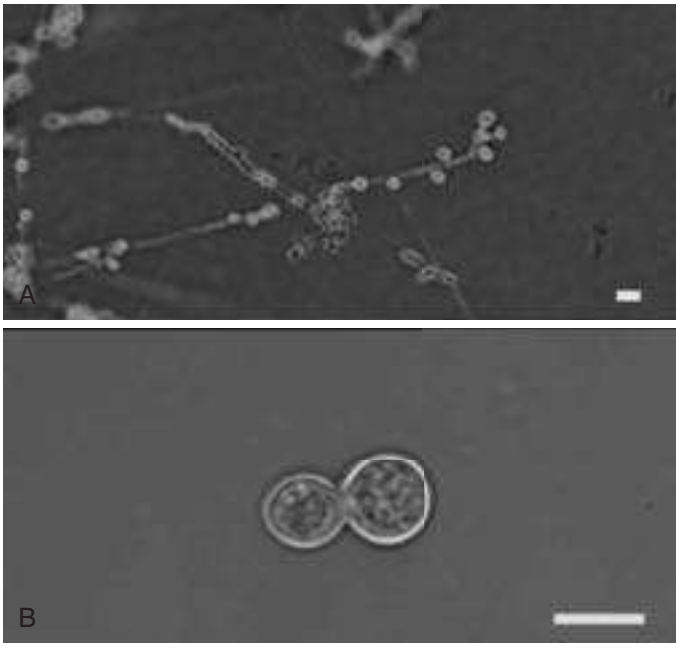

FIG. 264.1 ***Blastomyces*** **hyphae and yeast.** (A) Yeast growth at 37°C characterized by broad-based budding. (B) Hyphae with conidia at 22°C. Scale bar = 10 µm.

At 37°C, *B. dermatitidis, B. gilchristii,* and *B. percursus* yeast (8–20 µm) is characterized by a doubly refractile cell wall, multiple nuclei (typically three or four per cell), and a broad-based budding pattern between mother and daughter cells (Fig. 264.1B).[4,24,27,28,35] *B. dermatitidis* and *B. gilchristii* have similar morphology and cannot be distinguished using microscopy. Differentiation requires DNA sequencing to assess for a single nucleotide polymorphism (C for *B. gilchristii* and T for *B. dermatitidis*) at base-pair 19 in the untranslated region of *ITS2*.[2,29] *B. helicus* yeast (4–9 µm) also exhibit broad-based budding but can form short chains of yeast cells.[5] Definitive identification of *B. helicus* requires sequencing of the ITS (internal transcribed spacer) or D1/D2 domains of the long subunit of ribosomal RNA.[5]

Blastomyces refers to the asexual form, whereas the sexual form is known as *Ajellomyces*.[30,31] Asexual (or clonal) reproduction involves the production of conidia by hyphae or yeast cell division by budding. In contrast, sexual reproduction occurs only in the hyphal phase. *Ajellomyces* undergoes heterothallic mating in which the hyphae of opposite mating types (+ and −) join together to form special structures known as cleistothecia that exchange genetic material to produce ascospores.[30,32] The + mating type contains the α-box gene, whereas the − mating type contains the *HMG* gene.[30] The mating type ratio (+:−) for *B. dermatitidis* is 1:1, whereas it ranges from 1:1 to 1:2 for *B. gilchristii*.[21] Mating typically occurs within a single species (e.g., *B. dermatitidis* mating with *B. dermatitidis*); however, genotyping has suggested the potential for recombination between *B. dermatitidis* and *B. gilchristii*.[29] Sexual ascospores and asexual conidia can germinate as hyphae at 22°C to 25°C or yeast at 37°C.[30,33]

The genomes of *B. dermatitidis* and *B. gilchristii* range from 66.6 to 75.4 MB and contain 9180 to 10,187 genes.[3] Although *B. dermatitidis* and *B. gilchristii* contain a similar number of genes as other thermally dimorphic fungi, the genome size is twofold larger than other closely related fungi such as *Histoplasma capsulatum* and, surprisingly, *B. percursus* (32.3 MB).[3,4]

GEOGRAPHIC DISTRIBUTION AND ECOLOGY

Blastomycosis is a disease primarily localized to the United States and Canada. The endemic region for *Blastomyces* includes the Midwest, South Central, and Southeastern United States and Canadian providences including Quebec, Ontario, Manitoba, and Saskatchewan (Fig. 264.2A). *Blastomyces* is not uniformly distributed throughout the endemic region.



FIG. 264.2 (A) Endemic region of blastomycosis in North America. (B) African countries with case reports of blastomycosis. *(From Gauthier GM, Klein BS. Blastomycosis. In: Cherry JD, Harrison GJ, Kaplan SL, Steinbach WJ, Hotez PJ, eds.* Feigin and Cherry's Textbook of Pediatric Infectious Diseases. 7th ed. *Philadelphia: Saunders; 2014.)*

Rather, it is found in a specific ecologic niche characterized by sandy soil with an acidic pH and decaying vegetation that is located in forested areas near fresh water.[14,21,34,35] This includes watershed areas for Mississippi, Ohio, Savannah, Saint Lawrence, and Nelson rivers as well as areas adjacent to the Great Lakes. Ecologic niche modeling incorporating vegetation indices, soil characteristics, hydrologic features, and geocoded cases of blastomycosis in Wisconsin suggests that land near waterways is favorable for *Blastomyces*.[36]

Within the ecologic niche, *B. dermatitidis* and *B. gilchristii* exhibit geographic restriction and overlap.[21] On the basis of phylogeographic mapping of soil and species determination of clinical isolates, *B. gilchristii* appears to be restricted to Canada, Minnesota, Wisconsin, and New York.[21] In contrast, *B. dermatitidis* has a wider geographic distribution including Canada and Midwest, South Central, and Southeastern United States along with states along the St. Lawrence River.[21] *B. dermatitidis* and *B. gilchristii* overlap in Canada, Minnesota, and Wisconsin.[21] Genetic diversity within *B. dermatitidis* and *B. gilchristii* is influenced by geography that corresponds to freshwater drainage basins (e.g., Nelson River, Mississippi River).[21] McTaggart and colleagues[21] hypothesized that glaciation during the Pleistocene epoch, when *B. dermatitidis* and *B. gilchristii* diverged 1.9 million years ago, may have influenced the geographic distribution of *B. gilchristii* because it is restricted to formerly glaciated areas.

Human and veterinary cases of *B. helicus* infection occur outside the endemic region of *B. dermatitidis* and *B. gilchristii* in North America.[5] All clinical cases occurred in the western United States and Canada.[5] The majority of *B. silverae* isolates have been from the United States or Canada; however, the geographic range of this species has yet to be fully elucidated.[6] Outside of North America, blastomycosis is a rare cause of invasive fungal infection. Autochthonous culture-proven cases of blastomycosis have been reported from Africa and India (Fig. 264.2B).[37–39] African *Blastomyces* strains have a decreased ability to convert to yeast at 37°C in vitro, unique media requirements for growth in the laboratory, and a cell surface that is less antigenically complex.[40] *B. percursus* has been isolated from patients living in Israel and South Africa.[4]

EPIDEMIOLOGY

The epidemiology of blastomycosis is largely based on passive surveillance data, small-scale retrospective studies, and analysis of outbreaks. Mandatory reporting of blastomycosis is required in Minnesota, Wisconsin, Michigan, Arkansas, Louisiana, and Manitoba. In Ontario, laboratory-confirmed cases are tracked by the Northwestern Health Unit jurisdiction. The lack of reliable skin and serologic tests for assessing exposure to *Blastomyces* has hindered the epidemiologic understanding of blastomycosis. Thus epidemiologic data are limited to reports of patients with clinically apparent infection. However, an estimated 50% of patients with blastomycosis may have asymptomatic or subclinical infection.[14] Collectively, these limitations result in underestimation of the true incidence of blastomycosis.

Within the endemic region, the annual incidence of blastomycosis ranges from 0.11 to 1.94 cases per 100,000 persons (Table 264.1), and the overall mortality rate is 0.21 per 1 million person-years.[41–61] A study analyzing a sample of Medicare beneficiaries (1999–2008) reported a nationwide incidence of 0.7 per 100,000 with the highest rates in the Midwest and Southern United States.[62] Within the endemic region, certain areas are considered hyperendemic including Kenora (Ontario), Eagle River (Wisconsin), Vilas County (Wisconsin), and south-central Mississippi (see Table 264.1).[43,53,59] In the mid-1980s, Washington Parish in Louisiana was recognized as a hyperendemic area with 6.8 cases per 100,000 persons (vs. a statewide incidence of 0.23/100,000).[63] Subsequent analyses demonstrated that the incidence in Washington Parish is lower at 1.29 per 100,000 but remains higher than the statewide incidence of 0.11 per 100,000.[55]

Although most cases of blastomycosis are sporadic, outbreaks have been reported from seven states, with multiple outbreaks occurring in Wisconsin, North Carolina, and Minnesota (Table 264.2).[13,14,34,63–79] These outbreaks have often been associated with activities that disrupt soil, such as construction or recreation along riverbanks (see Table 264.2). Although exposure to *Blastomyces* most commonly occurs in rural areas, soil disruption in urban areas can lead to infection (sporadic cases and outbreaks) (see Table 264.2). Similar to rural areas, urban blastomycosis

TABLE 264.1 Epidemiology of Blastomycosis in North America

STATE OR PROVINCE	YEARS	INCIDENCE PER 100,000 PERSONS	AGE (Y)	MALE:FEMALE	REFERENCE
Wisconsin	1973–1982	0.5 (0.32–0.72)	43.7[a]	1.8:1	41
	1986–1995	1.4	46[b]	1.5:1	42
	1984–1990	40.4 (Vilas County); 101.3 (Eagle River)	42[b]	1.6:1	43
	2002–2006	2.0	—	—	44
	2011–2015	1.61 (sporadic only)	48[b]	2.1:1	45
Minnesota	2002–2006	0.5	—	—	44
	2012–2016	0.6	46[b]	2.9:1	46
Illinois	1981–1989	1.94 (Rockford)	40[b]	2.2:1	47
	2001–2007	0.4–1.1[c]	41–47[b]	1.4–2.4:1	48
Michigan	2012–2016	0.14 (0.11–0.20)	—	4.4:1	49
Arkansas	2007–2016	0.39 (0.2–0.74)	—	1.97:1	49, 50
Missouri	1992–1999	0.2[d]	46.7[a]	2.1:1	51
Alabama	1992–1994	0.6	—	—	52
Mississippi	1979–1988	1.3[e]	46[a]	1.7:1	53
Louisiana	1987–2016	0.11	48[b]	1.4:1	54, 55
Tennessee	1988–1995	1.23[f]	52[a]	2.3:1	56, 57
	1996–2005	1.29[f]	59[a]	2.5:1	56, 57
Northwest Ontario	1989–2005	17.0	—	—	58
Kenora, Ontario	1997–1999	117.2	41.9[a]	1.1:1	59
Manitoba	1988–1999	0.62	38[a]	1.9:1	60
Ontario, Quebec, Manitoba, Saskatchewan	—	0.62	—	—	58

[a]Mean age.
[b]Median age.
[c]Highest incidence of infection occurred in northeastern Illinois (Cook, Lake, Kane, and Will counties).
[d]Highest incidence was in southeastern Missouri with the highest incidence in Mississippi County (12/100,000).
[e]Highest incidence was in central and south-central Mississippi (>5/100,000) and accounted for 63% of blastomycosis from 1979–1988.
[f]For Carter, Cocke, Greene, Hawkins, Johnson, Sullivan, Unicoi, and Washington counties, which are located in northeastern Tennessee.

TABLE 264.2 Outbreaks of Blastomycosis in the United States

YEAR	STATE	LOCATION	NO. CASES	RURAL OR URBAN	SOURCE OF OUTBREAK	REFERENCE
1953–1954	North Carolina	Pitt County	11	Rural	Not identified	13
1972	Minnesota	Big Fork	12	Rural	Cabin construction	64
1975–1976	North Carolina	Enfield	5	Rural	Peanut harvest	65
1974–1975	Illinois	Westmont	5	Urban	Construction	66
1979	Wisconsin	Namekegon River	8	Rural	Canoeing	67
1984	Wisconsin	Eagle River	48	Rural	Beaver lodge	14
1984	Virginia	Southampton County	4	Rural	Raccoon hunting	68
1985	Wisconsin	Tomorrow River	7	Rural	Fishing on river bank	34
1985	Wisconsin	Crystal River	7	Rural	Underground fort	34
1988	Wisconsin	Watersmeet Lake	32[a]	Rural	Hotel construction	69
1989	Tennessee	Elizabethton	3	Urban	Factory construction	70
1989–1990	Wisconsin	Oconto County	8	Rural	Not identified	71
1998	Colorado	Boulder	2	Rural	Prairie dog relocation	72
1998–2000	Wisconsin	Indian reservation	9	Rural	Likely excavation	73
1999	Minnesota	Mountain Iron	18	Urban	Excavation	74
2001–2002	North Carolina	Duplin County	8	Rural	Likely construction or excavation	75
2005–2008	Indiana	Indianapolis and surrounding counties	59	Urban	Highway construction	76
2006	Wisconsin	Lincoln County	21	Urban	Pine needle yard waste	77
2009–2010	Wisconsin	Marathon County	55	Urban	Not identified	78
2015	Wisconsin	Little Wolf River	90	Rural	Tubing on the river	79

[a]3 confirmed, 18 suspected, and 11 probable cases.

has been associated with waterways such as lakes, rivers, and streams; use of a community compost pile; and highway construction.[76,77,80,81]

Outbreak investigations have advanced the clinical understanding of blastomycosis and uncovered novel areas for further study. Analysis of outbreaks along the Eagle River, Tomorrow River, and Crystal River in Wisconsin helped define the ecologic niche and established that the incubation period of blastomycosis ranges from 3 weeks to 3 months.[14,34] Moreover, these investigations demonstrated that approximately 50% of people exposed to blastomycosis develop symptomatic disease, whereas the remaining 50% have asymptomatic or subclinical disease.[14] Analysis of blastomycosis outbreaks in Marathon County, Wisconsin, in 2006 and 2009–2010 found a high rate of blastomycosis in people of Hmong ethnicity (adjusted odds ratio of 12.1 [95% confidence interval 1.3–611.9; $P = .019$] by multivariable logistic regression).[78] The high rate of blastomycosis did not appear to be related to environmental exposure.[78] This led to the hypothesis that increased susceptibility to blastomycosis in people of Hmong ethnicity may be related to genetic predisposition.[78] In Ontario and Manitoba, people of Aboriginal ethnicity have an increased incidence of blastomycosis.[60,82,83]

PATHOGENESIS, VIRULENCE, AND HOST DEFENSE

Fungal traits that contribute to invasive disease include thermotolerance (e.g., growth at 37°C), intracellular survival, and upregulation of yeast phase–specific virulence factors that subvert host defenses.[84] Hyphal growth at ambient temperature promotes environment survival, genetic diversity through mating, and transmission to mammalian hosts via spores and hyphal fragments.[1,84] Temperature is the major stimulus that drives the reversible conversion between hyphae (22°C–25°C) and yeast (37°C).[84] The uptake of exogenous cysteine, which restarts mitochondrial respiration during the morphologic switch, is required to complete the conversion to yeast.[85,86] Compared with *Coccidioides* and *Paracoccidioides*, carbon dioxide tension and estradiol do not appear to affect the morphologic switch or growth of *Blastomyces*.[87–89] Following inhalation of conidia into the lungs, resident pulmonary macrophages engulf these infectious particles, of which a subpopulation will survive and convert to yeast. Within macrophage, the morphologic switch from conidia to yeast is accelerated.[90] The capacity for intracellular survival as well as extracellular replication is a shared biologic feature among the endemic dimorphic fungi.[84]

Once in the yeast phase, *B. dermatitidis* and *B. gilchristii* upregulate *Blastomyces*-adhesin-1 (BAD1, formerly known as WI-1), an essential virulence factor that is specific to the yeast phase.[91–96] In a murine model of pulmonary infection, *BAD1* was the most upregulated gene in the *Blastomyces* transcriptome.[3,97] BAD1 is secreted by yeast and can either bind back to the yeast cell surface via interactions with chitin in the cell wall or remain soluble in the extracellular environment.[92–94] BAD1 functions as an adhesin and an immune evasin.[91–96] It facilitates binding of yeast cells to host tissue via interactions with heparan sulfate and adherence to host immune cells via interactions with complement receptors (CR3) and CD14.[92,95,96] BAD1 on the yeast cell wall inhibits the production of tumor necrosis factor-α (TNF-α) by macrophages and neutrophils in a transforming growth factor-β–dependent manner.[95,96] Soluble BAD1 also inhibits TNF-α production, but independent of transforming growth factor-β.[95] In addition to blocking TNF-α, BAD1 inhibits $CD4^+$ T-lymphocyte activation, which results in reduced production of interleukin-17 (IL-17) and interferon-γ.[92] Importantly, deletion of *BAD1* severely attenuated the pathogenicity of *Blastomyces* yeast in a murine model of pulmonary infection, which indicates that this gene is essential for virulence.[91]

Other mechanisms that contribute to virulence include secretion of dipeptidyl peptidase IVA (DppIVA), relative resistance to oxidative and nitrosative killing, and alteration of yeast cell wall carbohydrates. DppIVA is a serine protease that inactivates granulocyte-macrophage colony-stimulating factor, which in turn impairs recruitment of innate immune cells into the lungs and inhibits macrophage and neutrophil activation.[98] Silencing of DppIVA using RNA interference results in yeast cells with attenuated virulence in vivo and reduced survival when cocultivated with activated immune cells.[98] Compared with conidia, yeast is more difficult for macrophages to kill and is less susceptible to reactive oxygen species.[99] The resistance to oxidative damage may be due to upregulation of superoxide dismutases and catalase in yeast during infection.[3,97]

Blastomyces yeast suppresses the production of nitric oxide by macrophages; however, the mechanism underlying this defense strategy remains to be elucidated.[100] During the temperature-dependent morphologic switch, the amount of β-(1,3)-glucan in the cell wall decreases from 40% in hyphae to less than 5% in yeast.[101] This reduction has the potential to limit recognition of yeast by the dectin-1 and mannose receptors on host immune cells.[102] In addition, the reduction of β-(1,3)-glucan impairs the ability of echinocandin antifungals to effectively kill *Blastomyces* yeast.[103]

To uncover novel virulence factors, in vivo transcriptional profiling using RNA sequencing has been performed on RNA isolated from *Blastomyces* yeast during murine pulmonary infection.[3,97] Compared with controls (yeast and mycelia grown in medium alone or yeast cocultivated with macrophages in vitro), 72 genes were significantly upregulated more than twofold during pulmonary infection, independent of temperature, culture media, and interaction with macrophages.[3] A subset of these 72 genes contributed metal uptake (zinc, nickel) and amino acid metabolism (tyrosine, tryptophan, cysteine).[3] Exogenous uptake of zinc and nickel contributes to the virulence of *Candida albicans* and *Cryptococcus neoformans,* respectively.[104,105] The catabolism of L-cysteine influenced virulence of *C. albicans* in a murine model of infection, and sulfite, a breakdown product of L-cysteine, promoted keratin breakdown by dermatophytes such as *Arthroderma benhamiae*.[106,107] Ongoing research is deciphering the role of these processes in the pathogenesis of *Blastomyces* infection.

The application of molecular techniques to genetically manipulate fungi has led to the identification of genes that govern the temperature-dependent morphologic switch. The discovery of *DRK1* (dimorphism regulating kinase-1), which encodes a group III hybrid histidine kinase, provided the first genetic proof that the temperature-dependent morphologic switch from hyphae or conidia to yeast is essential for virulence.[108,109] Homologues of *DRK1* participate in the high-osmolarity glycerol signaling pathway, which promotes adaptation to osmotic stress, oxidative stress, and temperature. Silencing or deleting *DRK1* in *Blastomyces* or *Histoplasma* prevents the conversion of mycelia to yeast in response to an increase in temperature.[108] Thus *Blastomyces* and *Histoplasma* cells remain locked in the mold form at 22°C and 37°C, respectively.[108] Moreover, they are unable to upregulate yeast phase–specific virulence genes (*BAD1* in *Blastomyces, CBP1* in *Histoplasma*) and exhibit defects in the cell wall.[108] *DRK1*-silenced strains of *Blastomyces* and *Histoplasma* are avirulent in murine models of pulmonary infection.[108] Subsequent work in *T. marneffei* highlighted the conserved nature of *DRK1* on the phase transition. Deletion of the *DRK1* homologue in *T. marneffei* hindered the ability of conidia to germinate to yeast in macrophages.[110]

The phase transition in the other direction, from yeast to hyphae, following a drop in temperature is regulated in part by a GATA transcription factor encoded by *SREB* (siderophore biosynthesis repressor in *Blastomyces*).[111,112] Deletion of *SREB* results in (1) the failure of yeast to convert to hyphae at ambient temperature without affecting viability and (2) the inability to suppress the biosynthesis and uptake of iron-gathering molecules known as siderophores.[111,112] The defect in the morphologic switch is associated with a quantitative reduction in lipid droplets and decreased neutral lipid biosynthesis (triacylglycerol, ergosterol).[112] The addition of exogenous saturated fatty acids to *SREB* null mutants partially corrects the defect in lipid droplets and hyphal development.[112] The function of *SREB* appears to be conserved in other endemic dimorphic fungi. Targeted gene knockdown of *SRE1,* an *SREB* homologue in *H. capsulatum,* impairs hyphal conversion and siderophore biosynthesis.[113] In addition to *SREB* and *SRE1, N*-acetylglucosamine (GlcNAc) transporters NGT1 and NGT2 promote hyphal development in *Blastomyces* and *Histoplasma* spp.[114]

Although *Blastomyces* uses multiple strategies to subvert and evade the immune system, the host can mount a successful defense with innate and adaptive immune cells. Macrophages and neutrophils are capable of killing a large percentage of conidia inhaled into the lungs.[115] T lymphocytes coordinate the adaptive immune response to control infection by activating macrophages to become fungicidal against yeast.[116,117] This requires Th1 cytokines such as TNF-α and interferon-γ as well as the production of IL-17 by Th17 T lymphocytes.[116,117] Cell-mediated immunity following recovery from blastomycosis can last for at least 2 years.[118]

Cell-mediated immune defenses have the potential to be exploited to prevent invasive fungal infections. When injected subcutaneously, *BAD1* null (*BAD1*Δ) yeast induces sterilizing immunity in mice, which provides 100% protection against experimental pulmonary infection.[119] Thus *BAD1*Δ yeast can serve as a live, attenuated fungal vaccine. Following subcutaneous injection, *BAD1*Δ yeast is transported intracellularly by inflammatory monocytes to draining lymph nodes for antigen presentation to lymph node resident dendritic cells.[120] This results in priming of naïve $CD4^+$ T lymphocytes to differentiate into Th17 cells via dectin-2/FcRγ/Syk/Card9, dectin-3 (also known as MCL), and mannan receptor signaling pathways.[120–123] Once differentiated, Th17 cells migrate to the lungs in response to infection to mediate vaccine immunity by secreting IL-17 to recruit and activate phagocytes (neutrophils, macrophages).[117] In the absence of $CD4^+$ T cells, *BAD1*Δ-vaccinated mice use IL-17 $CD8^+$ T lymphocytes (Tc17 cells) to mediate protective vaccine immunity against lethal pulmonary infection.[124,125] Proliferation and activation of IL-17 $CD8^+$ T cells occurs through Myd88-Akt1-mTOR signaling.[126] The antigenic component of the *BAD1*Δ vaccine that induces T cell–based immunity is a 13–amino acid sequence in calnexin that is conserved among ascomycetes including *H. capsulatum, Coccidioides posadasii, Aspergillus fumigatus, Fonsecaea pedrosoi* (chromoblastomycosis), and *Pseudogymnoascus destructans* (white nose syndrome in bats).[127] This has led to the development of a calnexin-based vaccine. The addition of endoglucanase II as an adjuvant enhances the immunogenicity of the calnexin vaccine and protects mice against pneumonia.[128]

CLINICAL MANIFESTATIONS

Clinical recognition and diagnosis of blastomycosis are often delayed because the clinical manifestations are wide-ranging and often resemble other diseases.[129] Thus blastomycosis has been referred to as a "great pretender."[129] Pulmonary blastomycosis can mimic bacterial pneumonia, tuberculosis, and lung cancer with respect to symptoms and radiographic findings. Extrapulmonary dissemination, which can occur with or without pulmonary symptoms, can result in misdiagnosis. Skin lesions can clinically and histologically appear similar to pyoderma gangrenosum, sarcoidosis, basal cell carcinoma, and squamous cell carcinoma in the absence of fungal staining.[130,131] Similarly, laryngeal lesions can resemble malignancy.[130] Moreover, the incubation period of blastomycosis, which lasts 3 weeks to 3 months, results in clinical presentation throughout the year including winter. Many studies have demonstrated no seasonal peak with respect to onset of symptoms and diagnosis; however, one study suggested that localized pneumonia was more common in the autumn and winter, whereas diffuse pulmonary and extrapulmonary disease was more common in the spring.[43,53,132,133]

Blastomyces infection is most commonly acquired through inhalation of aerosolized conidia or mycelial fragments following disruption of soil related to recreational or occupational activities. Following evasion of host immune defenses, *Blastomyces* can remain in the lung or disseminate to other organs such as the skin, bone, brain, or prostate. Although the lung is the primary portal of entry, uncommon mechanisms of acquisition include direct inoculation of the skin from environmental trauma or laboratory accident (e.g., needlestick, injury during autopsy).[134,135] Rarely, cutaneous blastomycosis can develop through direct inoculation from infected oral secretions following the bite of an animal with severe pulmonary blastomycosis (e.g., dog, kinkajou)[134,136,137]; however, *Blastomyces* yeast is *not* transmitted from animal to human via the respiratory route. Direct cutaneous inoculation results in a localized cutaneous lesion with or without regional lymphadenopathy or lymphangitis.[134] Blastomycosis during pregnancy has the potential to result in transmission to the newborn, possibly via aspiration of infected secretions during birth or transplacentally.[138]

Pulmonary Blastomycosis

In patients with symptomatic blastomycosis, pulmonary infection is the most common clinical manifestation, occurring in 69% to 93% of patients.[a] The spectrum of pulmonary blastomycosis is heterogeneous

[a]References 53, 56, 57, 60, 82, 129, 133, 139, 140.

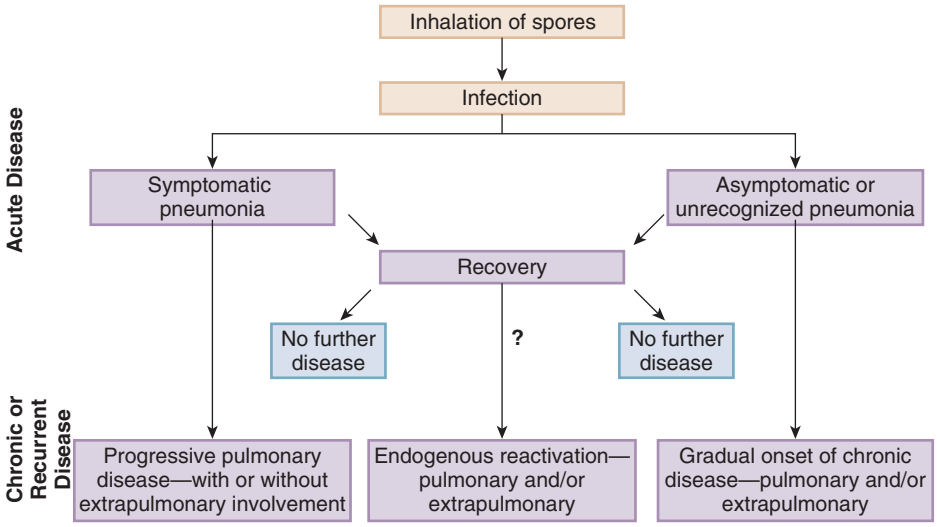

FIG. 264.3 Clinical classification of blastomycosis. *(Modified from Sarosi GA, Davies SF. Blastomycosis.* Am Rev Respir Dis. *1979;120:911–938.)*



FIG. 264.4 Left lung pneumonia. This patient had symptoms resembling acute bacterial pneumonia. Fungal smear of sputum using potassium hydroxide preparation demonstrated broad-based budding yeast. Sputum culture confirmed the diagnosis.

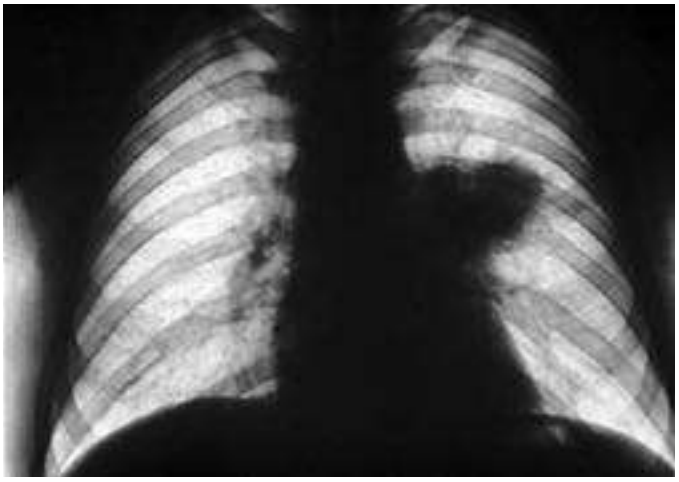

FIG. 264.5 Left lung mass. Radiographic appearance mimics that of carcinoma of the lung. *(Courtesy Dr. Stan Chapman, Jackson, MS.)*

and includes asymptomatic infection, brief influenza-like illness, acute pneumonia with or without respiratory failure, acute respiratory distress syndrome (ARDS), and chronic pneumonia (Fig. 264.3).[141] Acute pulmonary blastomycosis results in symptoms similar to community-acquired pneumonia (CAP) such as fevers, chills, cough, chest pain, malaise, decreased appetite, and hemoptysis.[b] There are no pathognomonic features on chest imaging to distinguish blastomycosis from other pulmonary diseases. Lobar consolidation, either unilobar or multilobar, is the most common finding and is indistinguishable from CAP (Fig. 264.4).[142] The next most frequent radiographic manifestations are masses, interstitial infiltrates, and nodules (Fig. 264.5).[142] Pleural effusion and hilar adenopathy are relatively uncommon.[142] Empyema is a rare manifestation.[143] Miliary blastomycosis is uncommon but is associated with severe infection and disseminated disease.[142] The clinical and radiographic features of acute pulmonary blastomycosis often result in an initial misdiagnosis of bacterial CAP and initiation of antibiotics, which fails to achieve clinical improvement.[129,144] In one study, a median of 2.5 antibiotic courses was administered before the diagnosis of blastomycosis was established.[144]

Patients with unrecognized or untreated pulmonary blastomycosis are at risk for progressive infection resulting in respiratory failure, ARDS, chronic infection, and extrapulmonary dissemination. Risk factors for severe pulmonary blastomycosis requiring admission to the intensive care unit include multilobar pneumonia, diabetes, and immunosuppression.[82,140,145] Retrospective studies from Tennessee, Indiana, Mississippi, Ontario, and Manitoba found that 4% to 15% of patients with blastomycosis met clinical criteria for ARDS.[c] Blastomycosis-induced ARDS is characterized by a fulminant clinical course and high mortality (40%–89%).[56,140,145–147] Patients with chronic pulmonary blastomycosis typically experience progressive symptoms of fever, chills, night sweats, weight loss, and hemoptysis over of a period of several months. Consolidation and cavitary lesions are usually associated with chronic infection.[142] Other manifestations include masslike lesions that resemble malignancy (see Fig. 264.5).[142]

Disseminated Blastomycosis

Once pulmonary infection is established, *Blastomyces* yeast can disseminate to nearly every organ in the body. On the basis of retrospective case studies, extrapulmonary spread occurs in 15% to 48% of sporadic cases.[d] An increased risk for extrapulmonary disease has been associated with prolonged duration of pulmonary symptoms and some forms of immunosuppression (e.g., increased risk of central nervous system [CNS] disease in human immunodeficiency virus (HIV)–seropositive patients with $CD4^+$ T-cell count <200 cells/mm^3).[60,149] In solid-organ transplant

[b]References 43, 53, 56, 60, 82, 139, 140.

[c]References 56, 57, 60, 76, 82, 140, 146.
[d]References 43, 53, 56, 57, 60, 76, 82, 133, 140, 148.

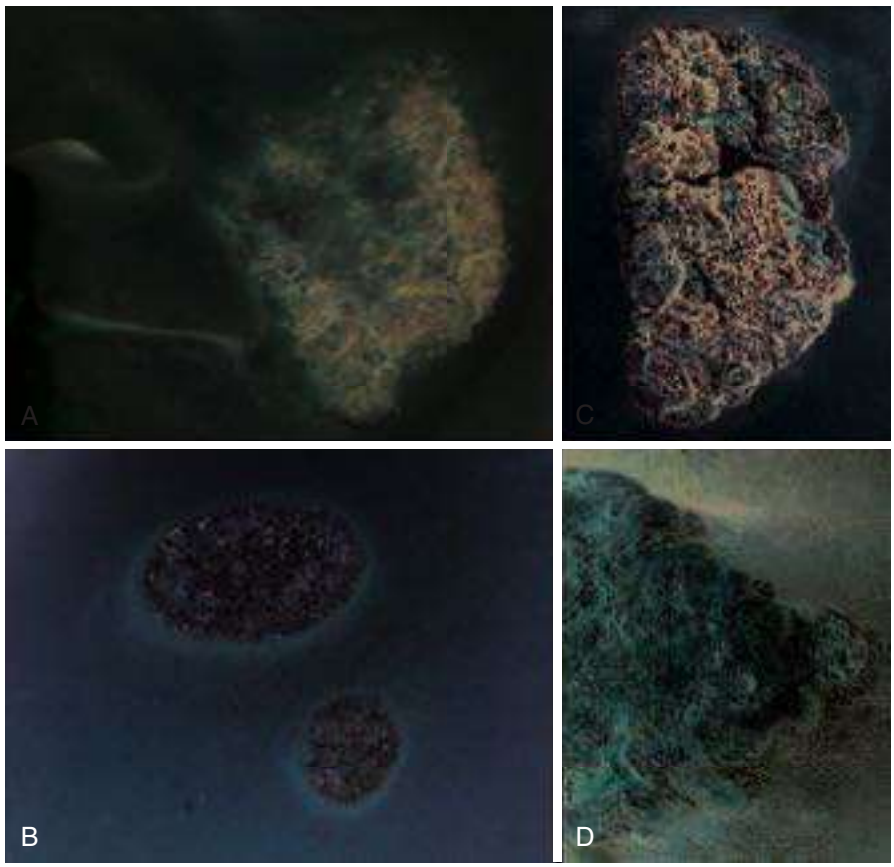

FIG. 264.6 Verrucous lesions secondary to blastomycosis. (A) Facial lesion. (B) Verrucous lesions with heaped-up margin with a satellite lesion on the shoulder. (C) Verrucous lesion on the back. (D) Verrucous lesions with beginning of ulcerations on the back. *(A courtesy Dr. Ryan Bariola, Little Rock, AR; B courtesy Dr. Dan Young, Rogers, AR, and Dr. Chris Schach, Fayetteville, AR.)*

(SOT) recipients, the rate of dissemination is 33% to 50%.[150,151] Evidence suggests that the infecting *Blastomyces* spp. may influence dissemination. An analysis of 240 human clinical isolates found that individuals infected with *B. dermatitidis* were more likely to develop disseminated disease (34.8%; 31 of 89 patients) than individuals infected with *B. gilchristii* (9.3%; 14 of 151 patients).[29] The mechanism for the differences in dissemination between *B. dermatitidis* and *B. gilchristii* is unknown.

The skin is the most common site for extrapulmonary infection, occurring in 40% to 80% of patients with disseminated disease.[19] Classically, cutaneous blastomycosis manifests as a papule that enlarges to form a verrucous or ulcerative lesion, which can involve the scalp, face, trunk, or extremities. Verrucous lesions are characterized by a raised, well-demarcated border with crusting (Fig. 264.6). Ulcerative lesions typically have an exudative base and heaped-up edges (Fig. 264.7). Ulcers can also develop from an underlying subcutaneous abscess or osteomyelitis.[130] Less common manifestations include widespread pustular lesions and subcutaneous nodules mimicking panniculitis.[152,153] Erythema nodosum, which is associated with histoplasmosis and coccidioidomycosis, is rare in blastomycosis. The severity of cutaneous involvement ranges from subtle, solitary lesions to large, destructive lesions that heal by scarring (see Fig. 264.7).[154] Skin biopsy usually demonstrates epidermal hyperplasia, papillomatosis, microabscess formation, neutrophilic infiltration, and noncaseating granulomas.[154,155] Because *Blastomyces* yeast can be difficult to visualize under light microscopy with hematoxylin and eosin staining, special fungal stains are required for optimal identification.

The bone is the second most common site of extrapulmonary blastomycosis, occurring in 15% to 44% of patients with disseminated infection.[e] Concomitant pulmonary or cutaneous disease is common.[156] However, the absence of lung infection does not exclude the diagnosis; slightly more than one-third of patients with a presumed pulmonary portal of entry for osteoarticular blastomycosis had no evidence of pulmonary blastomycosis at the time of diagnosis.[156] Bone involvement is characterized by pain, localized swelling, and sinus tract formation.[156] Soft tissue abscess and deep cutaneous ulceration can be associated with underlying osteomyelitis.[156] Most patients have a single site of bone involvement; however, a subset can have multifocal osteomyelitis or extension into the joint resulting in septic arthritis.[156,157] The most common bones affected include vertebral bodies, long bones (e.g., femur, tibia, humerus), and ribs; however, any bone can be involved including the sternum, scapula, pelvis, skull, and small bones of the hands or feet.[156–158] Vertebral osteomyelitis manifests with back pain and can be complicated by diskitis, epidural abscess, paraspinal abscess, psoas abscess, and vertebral body collapse (Fig. 264.8).[158,159] There are no radiographic features that can accurately distinguish blastomycosis from bacterial osteomyelitis, tuberculosis, or tumor. Imaging findings include periosteal reaction, sclerosis, lytic destruction, and skip lesions involving the vertebral bodies.[158] Bone biopsy with fungal culture and stains is needed for diagnosis. Histopathologic features include granulomatous inflammation, neutrophilic infiltrate, microabscesses, and necrosis.[157]

Genitourinary (GU) blastomycosis is considered the third most frequent site of dissemination. Early literature estimated that GU involvement occurred in 20% to 30% of cases; however, more recent case series suggest a lower rate (<10%).[f] Concomitant pulmonary or cutaneous blastomycosis is common.[160] The vast majority of GU blastomycosis affects men and results in prostatitis or epididymo-orchitis. Symptoms of prostatitis include dysuria, nocturia, suprapubic or perineal discomfort, and obstruction.[161,162] Urinalysis can show sterile pyuria or hematuria.[160–162] On examination, the prostate can be tender, enlarged, or indurated.[160] Epididymo-orchitis is characterized by pain, scrotal or testicular swelling, and, rarely, a draining sinus.[161] Diagnosis of GU blastomycosis can be facilitated by urine culture, collection of prostatic

[e]References 53, 56, 57, 82, 133, 140, 148.

[f]References 56, 57, 60, 82, 133, 140, 148.

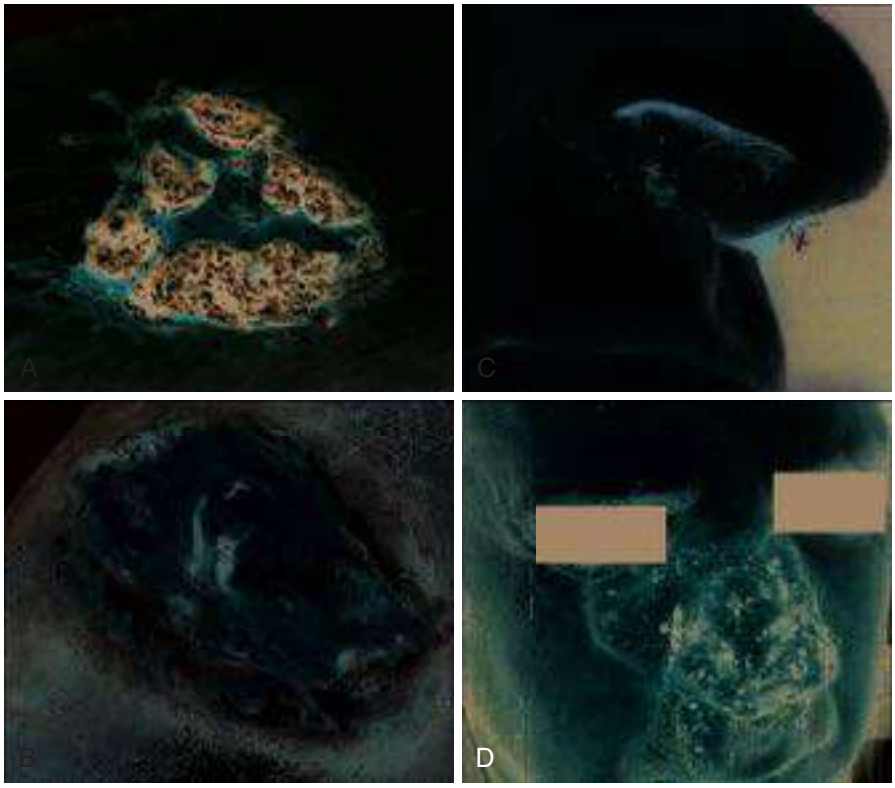

FIG. 264.7 Heterogeneity of cutaneous blastomycosis. (A) Ulcerative lesion on the leg in a patient with acquired immunodeficiency syndrome. (B) Large ulcer on the leg. (C) Small verrucous lesion on nares in a patient without other symptoms. (D) Extensive ulceration and cutaneous destruction from blastomycosis initially misdiagnosed as keratoacanthoma. When a new lesion appeared on the thigh, biopsy of both sites demonstrated *Blastomyces dermatitidis*.

secretions after massage, or needle biopsy.[161,162] Prostatic secretions and semen are potentially infectious with one reported case of male-to-female sexual transmission resulting in endometritis, salpingitis, and peritonitis.[163] GU involvement in women is rare and can include tubo-ovarian abscess with or without peritoneal involvement.[164]

Dissemination of *Blastomyces* to the CNS, which occurs in ≤10% of patients, can result in meningitis, brain abscess, or intracranial mass.[148,165] A subset of patients can have abscess or mass with concomitant meningitis.[165,166] Blastomycosis of the spinal cord (e.g., epidural abscess or granuloma) is rare.[166] Most cases of CNS blastomycosis are due to dissemination from the lungs; however, direct extension from overlying cranial osteomyelitis has been reported.[167] Most patients with CNS involvement (77%–81%) have evidence of extracranial blastomycosis involving organs such as the lung, skin, or bone.[165–168] Isolated CNS blastomycosis is uncommon.[165–168] Clinical features include headache, alteration in mental status, focal neurologic deficit, and seizures.[165–168] Symptoms are often progressive and are present for weeks to months before diagnosis is established.[165–168] Abscesses and mass lesions can involve the brainstem, cerebellum, or cerebrum.[167]

CNS blastomycosis can easily be misdiagnosed as tuberculosis or malignancy.[166,168,169] In patients with meningitis, cerebrospinal fluid (CSF) can have a neutrophilic or lymphocytic pleocytosis, elevated protein, and hypoglycorrhachia.[165] CSF cultures are positive in ≤45%.[165,166] In patients with brain masses, histopathology demonstrates granulomatous inflammation and necrosis.[167] In one study, cultures from biopsied tissue were positive in 71%.[167] Magnetic resonance imaging is the most sensitive imaging modality for CNS blastomycosis and can show meningeal or parenchymal enhancement.[167] Complications of CNS infection include mass effect from edema, cerebral herniation, and hydrocephalus.

Unusual manifestations of blastomycosis include ocular invasion, otitis media, sinusitis, lesions of the oral cavity (papular, verrucous, ulcerative) with extension to the mandible, endocarditis, splenic abscess, biliary obstruction leading to cholangitis, pancreatic mass, adrenal insufficiency from granulomatous destruction, thyroid mass, breast mass or abscess, renal abscess, and lymphadenopathy.[130,164,170,171] Laryngeal blastomycosis is uncommon but well described. Patients typically present with progressive hoarseness with variable degrees of cough, dyspnea, sore throat, and dysphagia.[171–173] Laryngoscopic examination can demonstrate polypoid masses and vocal cord paralysis.[171–173] Histopathology of vocal cord lesions is characterized by pseudoepitheliomatous hyperplasia, which can mimic malignancy if fungal staining is not performed.[171–173] Misdiagnosis as laryngeal cancer has led to patients undergoing laryngectomy and radiation therapy.[171–173] Dissemination of *Blastomyces* to the peritoneum is rare and associated with underlying tubo-ovarian abscess or abdominal organ infection.[164] Symptoms include fever, night sweats, weight loss, and abdominal discomfort related to new-onset ascites resulting in increased abdominal girth.[164] Most patients have antecedent or concomitant pulmonary blastomycosis.[164] During laparoscopy or laparotomy, nodular studding of the peritoneum, omentum, and bowel is frequently observed.[164] Ascitic fluid is characterized by pleocytosis with a lymphocytic (or rarely neutrophilic) predominance.[164] In patients with peritoneal blastomycosis, tuberculosis has been initially misdiagnosed.

Blastomycosis in Immunocompromised Patients

Although most patients with blastomycosis have intact immune defenses, *Blastomyces* is capable of infecting individuals immunocompromised by SOT, acquired immunodeficiency syndrome (AIDS), TNF-α antagonists, high-dose steroids, and malignancy.

The incidence of blastomycosis in SOT recipients in the endemic region is 0.13% to 0.14%, which is lower than the incidence of posttransplant histoplasmosis or coccidioidomycosis.[150,151,174] The onset of blastomycosis is highly variable, ranging from 0.4 to 250 months, which likely reflects the variability in disease acquisition including primary infection after transplant, reactivation of latent disease related to immunosuppression, and conversion of subclinical pretransplant infection to symptomatic posttransplant disease.[150] Donor-derived blastomycosis has not yet been reported. There is near-equal distribution of patients who develop blastomycosis before or after the first year of SOT.[150,151]



FIG. 264.8 CT imaging demonstrating vertebral osteomyelitis.

The most common manifestation is pneumonia, which is frequently complicated by respiratory failure or ARDS.[150,151] Symptomatic coinfection with opportunistic pathogens such as cytomegalovirus, varicella-zoster virus, and *Aspergillus* can occur in a subset of patients.[150] Extrapulmonary dissemination occurs in 33% to 50%, which is a rate similar to the general population analyzed in large case series (15%–44%).[150,151,174] Mortality ranges from 33% to 38% but increases to 67% in patients with ARDS.[150,151,175]

Within the endemic area, blastomycosis in patients with HIV is relatively rare. In the largest case series, which was published before the implementation of highly active antiretroviral therapy, the majority of patients had pulmonary disease (87%) with dissemination to other organs (53%) and $CD4^+$ T-cell counts <200/mm^3.[149] Multiple organ involvement was common with 33% having three or more sites of infection. Surprisingly, 40% of patients developed CNS blastomycosis, which is uncommon in other forms of immunosuppression including SOT.[149–151] A review of clinical cases published before 1994 had similar findings with 85% of patients having $CD4^+$ T-cell counts <200/mm^3 and high rates of extrapulmonary (67%) and CNS (46%) blastomycosis.[176] Since the mid-1990s, there has been a paucity of published clinical data regarding blastomycosis in patients with HIV infection.

In 2008, the US Food and Drug Administration issued a warning regarding TNF-α inhibitors increasing the risk for blastomycosis, histoplasmosis, and coccidioidomycosis.[177] A retrospective study analyzing patient health claims data from the years 2007–2009 identified 158 patients with mycobacterial or fungal infection in the setting of TNF-α inhibitor therapy.[178] *Mycobacterium tuberculosis* (61%) and *H. capsulatum* (60%) were the two most common infections (28.5% of persons had more than one infection; range, 2–8 infections per patient).[178] Less common infections were coccidioidomycosis (10%), blastomycosis (4%), *Cryptococcus* (3%) and *Pneumocystis* (2%).[178] There is very limited clinical information regarding clinical presentation and risk for dissemination of blastomycosis in individuals receiving TNF-α antagonists.[177–179]

Blastomycosis has been reported in patients with malignancies including head and neck cancer, breast cancer, gastrointestinal cancer, acute myelogenous leukemia, chronic lymphocytic leukemia, multiple myeloma, and Hodgkin disease.[175,180] Most patients have pneumonia, and extrapulmonary dissemination is common (50%).[175]

Blastomycosis is rare in patients with primary immunodeficiency disorders. Case reports include disseminated blastomycosis in a 59-year-old man with idiopathic $CD4^+$ lymphocytopenia and a 12-year-old girl with necrotizing pulmonary blastomycosis complicated by lung abscess in the setting of GATA2 deficiency.[181,182] In contrast, histoplasmosis and coccidioidomycosis has been described in individuals with CD40 ligand deficiency, interferon-γ deficiency, *STAT1* defect, and hyperimmunoglobulinemia E syndrome (Job syndrome).[183] Dimorphic fungal infections have not been associated with lymphocyte adhesion disorders or chronic granulomatous disease.[183]

Pregnancy

Blastomycosis in pregnancy is uncommon with clinical descriptions limited to 23 well-reported cases.[138] The majority of patients (91%) present during the second or third trimester with pneumonia (74%) or disseminated infection (48%).[138] Despite the high rate of disseminated disease, placental involvement appears to be uncommon.[138] Most newborns were delivered vaginally and were healthy; however, two neonates died of pulmonary blastomycosis at 18 and 21 days of life.[138,184,185] There is potential for mother-to-infant transmission; however, the route is undefined. Hypotheses include intrauterine transmission and aspiration of infected vaginal secretions during birth.[184,185] The optimal timing and route of delivery in pregnant patients with untreated blastomycosis in the third trimester is unknown.[138] Blastomycosis during pregnancy does not appear to increase the risk for congenital malformations.

DIAGNOSIS

The diagnosis of blastomycosis can be challenging; it requires a high degree of suspicion because the diverse clinical and radiographic presentations mimic more common entities such as CAP, tuberculosis, and malignancy. Even within the endemic area, the diagnosis of blastomycosis is frequently delayed longer than 1 to 2 months.[133,144] For individuals who reside in or visit endemic areas, clinical manifestations that should prompt evaluation for blastomycosis include pneumonia with cutaneous lesions; CNS disease (abscess, mass, meningitis) with concomitant pneumonia or skin lesions; new-onset ascites with antecedent or concomitant pneumonia; and the failure of lung, bone, or skin infections to respond to antibiotic therapy. A detailed exposure history has the potential to facilitate a timely diagnosis. Occupational and recreational activities associated with blastomycosis include construction (road, home, commercial), canoeing, tubing, hiking, fishing by riverbanks, logging, beaver dam exploration, and use of a community compost pile. Workers in microbiology laboratories or the veterinary field are at risk for primary cutaneous blastomycosis from direct inoculation by laboratory or medical instruments. The concurrent diagnosis of blastomycosis in a companion animal such as a dog or cat suggests a common exposure. The incidence of canine blastomycosis is 10- to 13-fold higher than human blastomycosis.[186] Thus canine blastomycosis can be a harbinger for human blastomycosis.[187]

Once suspected, establishing the diagnosis of blastomycosis is usually straightforward. Staining of clinical specimens is the most expeditious method to identify *Blastomyces* yeast but is often underused.[188] Calcofluor, 10% potassium hydroxide, or Papanicolaou is used to stain respiratory specimens (sputum, tracheal aspirate, bronchoalveolar fluid) or purulent drainage (Fig. 264.9). Sensitivity of these stains is 50% to 90% for respiratory secretions.[27] For tissue specimens, Gomori methenamine silver or periodic acid–Schiff are used because *Blastomyces* yeast is poorly visualized by Gram or hematoxylin and eosin stains (see Fig. 264.9).[27] Moreover, if *Blastomyces* is in the differential diagnosis, the microbiology laboratorian or pathologist should be notified to perform fungal stains. A presumptive diagnosis is established when broad-based budding yeast (8–20 μm) with a doubly refractile cell wall is visualized.[27,141] Delayed

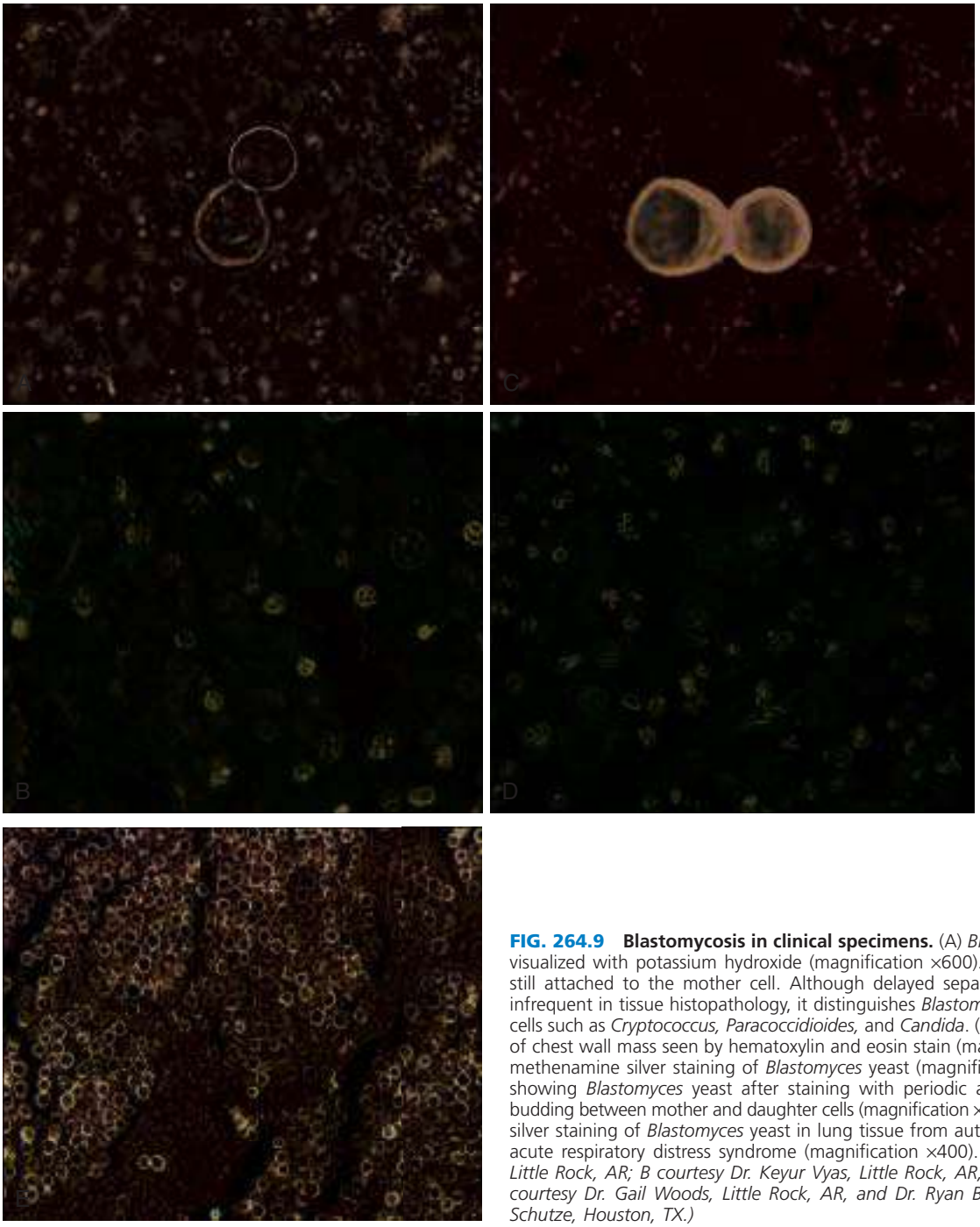

FIG. 264.9 Blastomycosis in clinical specimens. (A) *Blastomyces* yeast from exudate visualized with potassium hydroxide (magnification ×600). Note the large daughter cell still attached to the mother cell. Although delayed separation of the daughter cell is infrequent in tissue histopathology, it distinguishes *Blastomyces* from similarly sized yeast cells such as *Cryptococcus, Paracoccidioides,* and *Candida*. (B) *Blastomyces* yeast on biopsy of chest wall mass seen by hematoxylin and eosin stain (magnification ×600). (C) Gomori methenamine silver staining of *Blastomyces* yeast (magnification ×600). (D) Lung biopsy showing *Blastomyces* yeast after staining with periodic acid–Schiff. Note broad-based budding between mother and daughter cells (magnification ×400). (E) Gomori methenamine silver staining of *Blastomyces* yeast in lung tissue from autopsy of a patient who died of acute respiratory distress syndrome (magnification ×400). *(A courtesy Dr. Ryan Bariola, Little Rock, AR; B courtesy Dr. Keyur Vyas, Little Rock, AR; C courtesy Dr. Keyur Vyas; D courtesy Dr. Gail Woods, Little Rock, AR, and Dr. Ryan Bariola; E courtesy Dr. Gordon Schutze, Houston, TX.)*

detachment of the daughter cell is also a useful sign. The size of the yeast and budding pattern allow *Blastomyces* to be distinguished from other endemic fungi including *H. capsulatum* (small, narrow-based budding), *Coccidioides* spp. (spherules with endospores), *Paracoccidioides* ("pilot wheel" multiple budding yeast), *Sporothrix schenckii* (round or cigar-shaped with a narrow bud), and *T. marneffei* (oblong with central septum between mother and daughter cell). The presence of granulomatous inflammation with neutrophils (e.g., pyogranulomas) on a tissue biopsy specimen should prompt examination for yeast using special stains such as Gomori methenamine silver (see Fig. 264.9). Morphologic identification of *Blastomyces* yeast correlates well with culture results.[189] Moreover, the results of stained clinical specimens are known within 24 to 48 hours, which is often before culture and nonculture diagnostic tests results are available.

Identification of *Blastomyces* on specialized culture media such as brain-heart infusion, potato dextrose agar, or Sabouraud dextrose agar provides definitive diagnosis. Growth in culture is slow and requires 5 days to 4 weeks of incubation.[27,141] Most laboratories use 25°C to 30°C incubator temperatures, which promotes mycelial growth.[27] Because the mycelial form can appear similar to other fungal species, confirmation is required. This can include chemiluminescent DNA probes, polymerase chain reaction (PCR) amplification of specific target sequences, and conversion to yeast.[27] In several case series, 72% to 87% of patients with blastomycosis had positive cultures[53,56,76,82,140]; however, in one study the rate was only 64%.[148] The diagnostic yield for sputum cultures obtained by noninvasive means is 75% and for bronchoscopy is 92%.[188]

Nonculture diagnostics include serologic and antigen testing. Most studies have demonstrated that serologic methods such as complement fixation (CF) and immunodiffusion (ID) have poor sensitivity (≤25% for CF and ≤40% for ID).[14,56,188,190–192] Moreover, the sensitivity of ID appears to be influenced by burden of infection with reduced sensitivity in patients with localized infection.[190] An enzyme immunoassay that

detects immunoglobulin G antibodies against the *Blastomyces* A antigen has demonstrated improved sensitivity of 77% to 83%.[191,193]

Serologic testing has largely been supplanted by a quantitative antigen assay that detects a galactomannan component shed from the *Blastomyces* cell wall.[194–197] Specimens collected for antigen analysis can include urine, blood, bronchoalveolar lavage (BAL), and CSF. In patients with culture-proven blastomycosis, the sensitivity of the urine assay is 76.3% to 92.9%.[195–197] Similar to serologic testing, the sensitivity of the antigen assay can be influenced by the burden of infection.[195] Although the *Blastomyces* antigen test can be performed on BAL samples, robust data exist only for *H. capsulatum*.[198,199] In one study, five of eight patients (62.5%) with pulmonary blastomycosis had a positive BAL antigen.[197] Data regarding sensitivity of the assay in CSF for patients with CNS blastomycosis are limited.[195,200,201] The antigen assay cross reacts with other endemic dimorphic fungi including *H. capsulatum* (96%–100%), *Paracoccidioides* (100%), and *T. marneffei* (70%).[194] This cross-reactivity lowers the specificity to 76.9% to 79%.[194,195] The impact of a false-positive result is limited because histoplasmosis and blastomycosis are treated similarly, and the geographic distribution of *Paracoccidioides* spp. (Southern Mexico, Central America, South America) and *T. marneffei* (southeast Asia and China) does not overlap with *Blastomyces*. Rarely, the antigen assay cross-reacts with *Cryptococcus* (2.9%) or *Aspergillus* (1.1%).[194] The mechanism underlying these cross-reactions is likely related to varying degrees of similarity of the galactofuranose side chains in galactomannan among these different fungal species.[202] Moreover, cross-reaction of the Platelia *Aspergillus* galactomannan assay from BAL samples has been reported in patients with pulmonary blastomycosis.[202] Serial measurement of the urine antigen can be used to monitor response to antifungal therapy where a decline in antigen levels is associated with successful treatment.[197]

The serum β-(1,3)-glucan test is unreliable for diagnosis of blastomycosis because the yeast cell wall contains very little of this carbohydrate.[101,203] In the hyphal phase, β-(1,3)-glucan accounts for 40% to 50% of cell wall glucan, but in the yeast phase, it represents less than 5%.[101,203] In contrast, the serum β-(1,3)-glucan test can be positive in patients with histoplasmosis and coccidioidomycosis.[203,204]

Discoveries in the laboratory are beginning to influence the clinical diagnosis of blastomycosis. A serologic assay that detects antibodies against BAD1 rather than the A antigen has been developed. Compared with CF and ID, the BAD1 antibody assay has a sensitivity of 87% and specificity of 94% to 99%.[192] When this assay is combined with the *Blastomyces* antigen test, the sensitivity increases to 97%.[192] Moreover, this assay does not cross-react with other thermally dimorphic fungi because BAD1 is unique to *Blastomyces*. A real-time PCR assay that amplifies the *BAD1* promoter from *Blastomyces* in culture or paraffin-embedded tissues has been developed.[205] Similarly, a PCR assay that uses primers against *BAD1* has been developed to identify *Blastomyces* in the soil.[206] This assay has been used in epidemiologic investigations of human and canine outbreaks of blastomycosis.[77,206]

TREATMENT

The IDSA and ATS have published similar guidelines for the treatment of blastomycosis.[19,20] Antifungal therapy for blastomycosis is based on the severity of infection, presence or absence of CNS involvement, underlying immunodeficiency, and pregnancy status. All patients with a diagnosis of blastomycosis should be offered antifungal therapy. Although there are scattered reports of self-limited, acute pulmonary blastomycosis, most experts agree that antifungal therapy should be initiated to minimize the risk of relapse or dissemination. Moreover, there are no clinical or laboratory features that can accurately determine if blastomycosis will be self-limited and not disseminate. If a patient is not treated, careful follow-up for several years is required.

Before initiation of antifungal therapy, baseline evaluation of hematologic, hepatic, and renal function should be performed. A detailed review of all medications is required to avoid harmful drug-drug interactions. Fluconazole, voriconazole, and posaconazole can lengthen the QT interval, which can lead to fatal cardiac arrhythmias. In contrast, isavuconazonium sulfate shortens the QT interval and is contraindicated in patients with congenital short QT syndrome.[207] Azole antifungals elevate serum concentrations of statins metabolized by cytochrome P-450 3A4, which can increase the risk for rhabdomyolysis. Pravastatin is metabolized through a different pathway and can be safely used. Azole antifungals can also interact with immunosuppressive medications, sulfonylureas, antiarrhythmics, and anticonvulsants. Itraconazole has negative inotropic effects that can precipitate congestive heart failure and should be used with caution in patients with ventricular dysfunction.[208] The negative inotropic effect is unique to itraconazole and has not been observed with other azoles. For women of childbearing age, a pregnancy test must be performed because azole antifungals increase the risk for spontaneous abortion and are teratogenic.[209,210] Periodic laboratory monitoring is recommended in patients on azole therapy.[19]

Recommendations for antifungal therapy are based on prospective trials, case series, and expert opinion. Similar to other uncommon fungi, large clinical trials are challenging to conduct due to relatively low numbers of patients with a diagnosis of blastomycosis. The first clinical trials involving azole antifungals assessed the efficacy of ketoconazole 400 mg daily, which cured 79% to 81% of patients.[211,212] However, owing to endocrine side effects and increased risk of relapse (10%–14%), ketoconazole is no longer recommended.[19] An open-label, multicenter randomized controlled trial found that fluconazole 400 to 800 mg daily for a median of 8 months (range, 2–24 months) resulted in cure in 64% and improvement in 23% of patients with pulmonary and non-CNS disseminated blastomycosis.[16] The most common side effects were gastrointestinal effects, alopecia, and chapped lips.[16] The role of fluconazole is limited because it is not as efficacious as itraconazole. In addition, fluconazole minimal inhibitory concentrations (MICs) are higher than MICs of other azoles.[213] A prospective, open-label, nonrandomized trial demonstrated that itraconazole 200 to 400 mg daily for a median of 6 months (range, 3–24.4 months) cured 95% of patients with pulmonary or non-CNS extrapulmonary blastomycosis.[17] At the present time, itraconazole is considered the drug of choice for patients with mild-to-moderate non-CNS blastomycosis.[19,20] Voriconazole, posaconazole, and isavuconazonium sulfate have in vitro activity against *Blastomyces,* and case reports suggest that these agents can be effective.[150,167,213–215]

Among polyene antifungals, amphotericin B (AmB) deoxycholate results in cure in 77% to 91% when total cumulative dosage exceeds 1 g and in 97% when cumulative dosage is >2 g.[19] A cumulative dose of 2 g is recommended by the IDSA, and 1.5 to 2.5 g is recommended by the ATS if azole step-down therapy is not done.[19,20] Although no clinical trials have compared lipid and deoxycholate formulations for blastomycosis, animal studies and clinical experience suggest they should be equally efficacious.[19] In adults, lipid formulations are preferred over AmB deoxycholate because the former have lower rates of nephrotoxicity and infusion reactions.

For patients with pulmonary or disseminated blastomycosis that is mild to moderate in severity, itraconazole is considered the drug of choice for initial treatment (Table 264.3).[19,20] In general, diagnosis and treatment of patients with mild-to-moderate blastomycosis occur in the outpatient setting. Duration of itraconazole treatment is 6 to 12 months, with osteoarticular infection requiring at least 12 months of therapy.[19,20] For patients with pulmonary or disseminated disease that is moderately severe or severe, AmB (deoxycholate or lipid) is recommended for induction therapy of 1 to 2 weeks (or until clinical improvement) followed by a 6- to 12-month course of itraconazole (see Table 264.3).[19] Patients with moderately severe or severe blastomycosis usually require admission to the hospital (general ward, intermediate care unit, or intensive care unit).

All immunosuppressed patients (e.g., SOT recipients) should be treated with 1 to 2 weeks of AmB followed by 1 year of itraconazole (see Table 264.3).[19,20] To minimize nephrotoxicity, lipid AmB is preferred over AmB deoxycholate. The IDSA guidelines state that for patients with irreversible immunosuppression, lifelong suppressive therapy may be required, but specific criteria for selection of these patients is not provided due to the heterogeneity of this patient population.[19] For SOT recipients who have received the recommended duration of therapy, relapse is uncommon, and lifelong therapy is usually not required.[150] However, in patients with profound cell-mediated immunosuppression that is anticipated to be prolonged, suppressive antifungal therapy may need to be considered. For AIDS-associated blastomycosis, itraconazole suppression is recommended and should be continued until the patient

TABLE 264.3 Treatment of Blastomycosis in Adults

INDICATION	THERAPY
Mild-to-moderate blastomycosis	Itraconazole 200 mg once or twice a day for 6–12 months[a]
Moderately severe–to–severe blastomycosis	Liposomal AmB 3–5 mg/kg daily for 1–2 weeks,[b] then itraconazole 200 mg PO once or twice daily for 6–12 months[a]
Immunocompromised state	Liposomal AmB 3–5 mg/kg daily for 1–2 weeks, then itraconazole 200 mg PO once or twice daily for 12 months[a]
CNS blastomycosis	Liposomal AmB 5 mg/kg daily for 4–6 weeks, then fluconazole 800 mg daily, itraconazole 200 mg bid to tid, or voriconazole 200–400 mg bid for at least 12 months
Pregnancy	Liposomal AmB 3–5 mg/kg daily for 6–8 weeks[c]

[a]Itraconazole should be loaded as 200 mg PO tid for 3 days, then 200 mg PO daily once or twice a day with dosing frequency based on serum itraconazole levels (itraconazole plus hydroxyitraconazole).
[b]Can substitute with AmB deoxycholate 0.7–1 mg/kg.
[c]Azoles should be avoided in pregnancy.
AmB, Amphotericin B; *bid,* twice daily; *CNS,* central nervous system; *PO,* per os (orally); *tid,* three times a day.

is on antiretroviral therapy, has received ≥1 year of antifungal therapy, and has a $CD4^+$ T-cell count that is >150 cells/μL.[19,20]

For CNS blastomycosis, liposomal AmB 5 mg/kg/day is recommended for 4 to 6 weeks followed by itraconazole 200 mg two to three times a day, fluconazole 800 mg daily, or voriconazole 200 to 400 mg twice a day for at least 12 months of therapy (see Table 264.3).[19] Liposomal AmB is the preferred formulation because it penetrates the CSF better than amphotericin B lipid complex or AmB deoxycholate.[19,20,216] Although fluconazole has higher MICs than other azoles against *Blastomyces,* it has excellent CSF penetration.[217] The ATS guidelines state that high-dose fluconazole can be used as add-on therapy to liposomal AmB in patients who have severe or refractory CNS disease or are immunocompromised.[20] However, clinical data regarding combination antifungal therapy for blastomycosis are very sparse. Despite limited penetration into the CSF (<10% of serum), itraconazole has been used to successfully treat CNS blastomycosis.[167] Voriconazole has very good penetration into the CSF and has been used to successfully treat CNS blastomycosis.[167,217]

For pregnant women, liposomal AmB 3 to 5 mg/kg/day is considered the antifungal of choice because it is not teratogenic and is better tolerated than AmB deoxycholate.[19] Azole antifungal therapy is not recommended. Fluconazole during pregnancy in animals and probably in humans is associated with an Antley-Bixler–like syndrome, which is characterized by craniofacial, skeletal, and cardiac deficits.[209] Itraconazole increases the risk for spontaneous abortion.[210] Voriconazole and posaconazole cause skeletal abnormalities in animal models.[210] Echinocandins are not an option for treatment of blastomycosis because these agents have poor activity against *Blastomyces* yeast and are teratogenic in animal models.[19,20,210]

Effective management of blastomycosis requires minimizing drug toxicities, optimizing bioavailability of oral azole antifungals, and therapeutic drug monitoring. AmB deoxycholate and lipid formations require frequent monitoring of serum creatinine, potassium, magnesium, and bicarbonate. Salt loading using intravenous normal saline before and after amphotericin infusion can reduce nephrotoxicity.[218] Most patients receiving AmB require potassium and magnesium supplementation to prevent or treat hypokalemia and hypomagnesemia from urinary wasting of these electrolytes. In contrast, bicarbonate replacement is rarely required. Itraconazole can be administered as a capsule or oral solution (100 mg/10 mL). Initial therapy with either formulation should be administered as 200 mg three times a day for 3 days followed by 200 mg once or twice a day thereafter with dosing based on serum drug levels.[19] To optimize the bioavailability of itraconazole capsules, they need to be taken with an acidic beverage and food. In contrast, itraconazole solution is better absorbed and should be administered without food. Moreover, gastric acidity is not required for absorption of the liquid solution, and it can be used in patients on H2 blockers or proton pump inhibitors. The capsule formulation should not be used in individuals taking medications that reduce gastric acidity. If SUBA-itraconazole is used, the dose is 130 to 260 mg daily. With any itraconazole formulation, serum itraconazole levels should be measured at 14 days of therapy, and due to its long half-life, drug levels can be obtained independent of when the drug was administered. Goal serum itraconazole level is 1 to 5 μg/mL when itraconazole and hydroxyitraconazole concentrations are added together.[219] Serum concentrations >10 μg/mL are not needed and can be associated with significant side effects.[19] In addition to its negative inotropic effects, itraconazole can result in hypokalemia. Fluconazole has excellent bioavailability, it can be administered regardless of gastric acidity or food, and therapeutic drug monitoring is not required. Oral voriconazole should be taken without food, and goal serum trough concentrations are 1 to 5.5 μg/mL.[219] Delayed-release posaconazole tablets and isavuconazonium sulfate capsules are well absorbed and can be administered regardless of food or gastric acidity. Therapeutic drug monitoring is currently recommended for posaconazole. Voriconazole, posaconazole, and isavuconazonium sulfate can also be administered intravenously.

Despite aggressive antifungal therapy with AmB, mortality due to blastomycosis-induced ARDS remains high. The role of adjunctive steroids to decrease pulmonary inflammation remains unclear. A few case reports have suggested that intravenous steroids can result in improved survival.[220,221] A retrospective review of 43 patients with blastomycosis-induced ARDS did not find a survival benefit among patients who received steroids (odds ratio, 0.52; 95% confidence interval, 0.11–2.34).[145] In this study, patients with severe ARDS (Pao_2:Fio_2 ≤100) were more likely to receive steroids than patients with less severe ARDS.[145] Nevertheless, the authors of the study stated that definitive conclusions could not be drawn to recommend for or against adjuvant steroids, and administration should be based on clinical judgment.[145] If adjuvant steroids are used, the ATS guidelines recommend prednisone 40 to 60 mg daily (or equivalent) for 1 to 2 weeks.[20] Although combination therapy is not well studied, the ATS guidelines suggest considering AmB plus itraconazole in patients with severe pulmonary blastomycosis.[18]

Surgical intervention should be considered in patients with osteomyelitis, intracranial abscess or mass, epidural abscess, and vertebral osteomyelitis with spinal instability. Surgical débridement is not curative, and antifungal therapy is still required.

ACKNOWLEDGMENT

Dr. Robert W. Bradsher, Jr., authored this chapter in previous editions, and we acknowledge use of select portions of the 8th edition chapter.

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265 Coccidioidomycosis (*Coccidioides* Species)

John N. Galgiani

SHORT VIEW SUMMARY

Definition

- The dimorphic fungi *Coccidioides immitis* and *Coccidioides posadasii* cause a systemic fungal infection, coccidioidomycosis, also known as *San Joaquin Valley fever* or *Valley fever.*

Epidemiology

- Coccidioidomycosis is endemic to arid regions of the Western Hemisphere.
- Approximately 150,3192 new US infections occur annually, of which 50,000 produce significant illness; of those reported to the Centers for Disease Control and Prevention, 66% are from Arizona and 31% from California.
- Infections most frequently occur during dry seasons, and the incubation period until first symptoms ranges from 1 to 3 weeks.
- The most common illness is a community-acquired pneumonia, lasting weeks to months whether treated with antifungal agents or not. Progressive pneumonia or hematogenous dissemination to other organs is a serious complication that requires treatment.
- Patients with diabetes are more likely to suffer pulmonary complications.
- The risk of dissemination is much more frequent in patients with impaired cellular immunity.

Microbiology

- *Coccidioides* has been found in desert soil and associated with animal burrows but is sparsely distributed, even within the most highly endemic regions.
- Throughout the 20th century, coccidioidomycosis was recognized as caused by a single fungal species, *C. immitis.* This population contains two genetically and geographically distinct clades, now recognized as separate species: *C. immitis* predominantly found in California and *C. posadasii* in all other endemic regions.
- On most laboratory media, growth by apical mycelial elongation is visible within a week.
- Alternate hyphal cells autolyze, leaving behind single 3- to 5-μm cells (arthroconidia) that can become airborne and are capable of being inhaled deep into airways.
- In mammalian tissue, an arthroconidium remodels into a spherical cell that enlarges isotropically to form large mature spherules with scores of endospores developing within.
- Endospores, when released during spherule rupture, can develop into a new spherule within host tissue or revert to mycelial growth if removed from the infection.
- A sexual phase has not been observed, but population genetics suggest that one exists. Sequence analysis indicates that *Coccidioides* is an ascomycete.

Diagnosis

- Isolation of *Coccidioides* in culture from a clinical specimen is diagnostic of infection.
- Recognizing endospore-containing spherules in wet mounts or histologic sections is also definitive.
- Diagnosis in most patients is made presumptively by detecting anticoccidioidal antibodies in serum or cerebrospinal fluid.
- Complement-fixing anticoccidioidal antibodies are quantitated by serial dilution titration. More extensive infections are frequently associated with higher titers, and clinical improvement is associated with decreasing titers.
- Immunodiffusion techniques are routinely used to provide a qualitative mimic of the complement-fixing antibody test and also to detect other *Coccidioides*-specific antibodies, often immunoglobulin M (IgM), which occur earlier.
- Proprietary enzyme immunoassay kits that measure anticoccidioidal IgM and IgG antibodies are in wide use. They are more sensitive in detecting early coccidioidal infections but may not be as specific.
- A test for coccidioidal antigen is commercially available and is most frequently positive in patients with extensive infection.
- A skin test that measures dermal hypersensitivity in patients with prior coccidioidal infection is again commercially available.

Therapy

- Healthy patients with uncomplicated coccidioidal pneumonia usually improve with general supportive management whether or not antifungal drugs are used. If antifungal treatment is initiated for such patients, it usually consists of fluconazole given orally at a dose of 400 mg/day for periods ranging from 3 to 6 months.
- Patients with severe early pneumonia sufficient to require intensive care hospitalization are often treated with intravenous amphotericin B initially until the respiratory status stabilizes or improves.
- When infection results in symptomatic chronic fibrocavitary pneumonia or extrapulmonary dissemination, antifungal therapy involves oral fluconazole (400 mg daily or higher) or itraconazole (200 mg twice or three times daily). Treatment would normally be continued for at least 1 year. In such patients, it is not infrequent for treatment to continue for several years because relapse off treatment is common.
- In some patients, surgery in addition to antifungal drugs is essential to control infection.
- Treatment of coccidioidal meningitis is most frequently managed with oral fluconazole in doses of 400 mg or more daily. This treatment is lifelong for all patients. Patients who develop hydrocephalus usually require the placement of an internal ventricular shunt.

Prevention

- A preventive vaccine does not exist. A vaccine candidate is under development for dogs but not yet for humans.

Although the systemic fungal infection now known as coccidioidomycosis has been recognized for more than a century,[1] its endemic domains continue to be expanded.[2,3] A medical intern is credited with first identifying in 1892 a patient who had widespread disease.[4] Organisms seen microscopically were mistakenly thought to be parasites, and only several years later was the true mycotic etiology determined and the agent given the name *Coccidioides immitis.*[5] For 3 decades, coccidioidomycosis was thought to be a rare and nearly always fatal infection. In 1929, an accidental laboratory exposure of a medical student at Stanford University resulted in only a transient respiratory infection. His unexpected survival stimulated a reassessment of the natural history of coccidioidal infections, which soon led to the recognition that a common respiratory condition in the San Joaquin Valley of California (Valley fever) was the more usual result of infection.[6] This conclusion

was corroborated with the development by Smith and colleagues of a specific skin test[7] and serologic assays[8] for coccidioidomycosis. With these tools, the clinical spectrum became well described by the mid-1950s; an excellent monograph published by Fiese[9] remains a valuable contemporary reference on the disease.

The growing impact of coccidioidomycosis on public health can be attributed to changes in demography and in contemporary medicine.[10] First, the populations at risk of exposure are greatly expanded. Regions in which *Coccidioides* spp. are endemic, which previously were sparsely populated, now encompass major metropolitan centers such as Phoenix, Arizona. Many of those relocating to the Southwest are retirees, and case rates in older persons are higher than in young adults.[11–13] With this population growth has come greatly increased tourism and commerce-related movement of people into and out of endemic areas. As a result, increased numbers of people are acquiring coccidioidal infections both within and beyond endemic regions.[14,15] Second, a major segment of the population has emerged with compromised cellular immunity because of either underlying diseases or immunosuppressive therapies to control other diseases.[16–24] These patients are unusually susceptible to serious coccidioidal infections, and as a result, the severity of coccidioidal infections as a public health problem has increased. Third, advances in prevention and treatment of fungal infections offer new opportunities for management. These trends have made coccidioidomycosis more relevant to physicians everywhere.[25] Finally, the emergence of *Coccidioides* spp. as potential agents of bioterrorism was identified by the Centers for Disease Control and Prevention (CDC) in 1997.[26,27] Although *Coccidioides* spp. have since been removed from the CDC list of select agents, awareness of their potential remains in light of continued technical advances in genetic transformation.[28]

MYCOLOGY

Coccidioides spp. are dimorphic fungi that exist either as mycelia or as unique structures known as spherules.[29] Both forms of growth are asexual, and it is not possible to classify *Coccidioides* spp. in relation to other fungi by classic taxonomy. By molecular analysis, however, *Coccidioides* spp. appear related most to other ascomycetes, most closely to the medically important organisms *Blastomyces dermatitidis* and *Histoplasma capsulatum*.[30] Although a sexual phase has not been found, population genetics studies suggest that one does exist.[31,32] Two genetically distinct populations have been identified among the etiologic agents of coccidioidomycosis. The occurrence of two populations was correlated with separate endemic regions where patients resided. This finding prompted classification of the previously known single species, *C. immitis,* into two species: *C. immitis* and *Coccidioides posadasii*. Most of the *C. immitis* isolates have been obtained from California, whereas *C. posadasii* isolates have been obtained from patients in other states and from countries other than the United States.[33,34] DNA sequence analysis of *C. posadasii* strains enabled investigators to deduce the approximate geographic origin of some infections.[34,35] The two species have shown few phenotypic differences; the clinical manifestations resulting from infection with either species appear the same, and in vitro susceptibility to antifungals is similar.[36,37] Molecular identification methods for differentiation of *C. immitis* from *C. posadasii* have been described[38] but are not yet routinely employed. Thus references in the literature to *C. immitis* may actually be referring to either species. Isolates for which the species has not been determined are best designated as simply *Coccidioides* spp., which is the convention followed in this chapter.

Mycelial (Saprobic) Growth

On routine microbiologic nutrient agar media and presumably in the soil, *Coccidioides* spp. grow as mycelia by apical extension, and true septa form along their course. Maturation within 1 week of growth results in alternating mycelial cells undergoing a process of autolysis and thinning of the cell walls. The remaining intact cells become barrel-shaped arthroconidia approximately 5 µm in length, develop a hydrophobic outer layer, and are capable of remaining viable for years. Because the attachments of arthroconidia to adjacent cell remnants are fragile, they are prone to separation by physical disruption or mild air turbulence. As a result, arthroconidia readily become airborne in a form capable of deposition in the lungs if inhaled.

Spherule (Parasitic) Growth

In the lungs, arthroconidia remodel into spherical cells, shedding their hydrophobic outer wall.[39,40] During this phase, nuclear division and cell multiplication occur, and septa extend from the internal surface of the wall to transect the growing spherule into scores of subcompartments, each containing viable daughter cells or endospores. In tissue, spherules can become 75 µm in diameter (Fig. 265.1). Spherules grown in vitro demonstrate nuclear division throughout maturation, although their size is smaller and the number of endospores is fewer.[41] As a spherule matures, its outer wall thins and eventually ruptures. Early in the course of experimental infections, this rupture occurs in approximately 4 days,[42] and with the release of endospores, the number of viable fungal units is amplified by approximately 100-fold, each of which may continue to propagate in tissue or revert to mycelial growth if removed from the site of an infection.

EPIDEMIOLOGY

Geographic Range

Coccidioides spp. are endemic to the soils of certain regions of only the Western Hemisphere, nearly all of which are within the north and south 40-degree latitudes. Well-described transport of arthroconidia, either in soil on fomites[43–45] or as the result of unusually severe dust storms,[46] has produced infections in persons without endemic exposure, but this generally has not led to the establishment of new areas of endemicity. Regions of the United States in which *Coccidioides* spp. are endemic are shown in Fig. 265.2. Noncontiguous foci of endemicity also exist, such as those studied at Dinosaur National Monument, Utah[47] and in eastern Washington.[2] These regions generally have the characteristics of the "lower Sonoran life zone," which include an arid climate, yearly rainfall of 5 to 20 inches, hot summers, winters with little freezing weather, and alkaline soil. Other areas where *Coccidioides* spp. have been identified include Mexico (adjacent to the US border; western portions of the states of Sonora, Nayarit, Jalisco, and Michoacan; central regions, including the states of Coahuila, Durango, and San Luis Potosi); Central America (Guatemala, Honduras, Nicaragua); and South America (Argentina, Paraguay, Venezuela, Colombia, Brazil).[10] An archeological investigation has provided evidence that *Coccidioides* spp. infected bison 8500 years ago in what is now Nebraska, far beyond the current endemic regions.[48] This raises the possibility that climatic change could potentially affect the geographic distribution of *Coccidioides* spp.[49]

Within the endemic regions, the likelihood of finding *Coccidioides* spp. in soil samples varies considerably among different locations[32,50,51] and different seasons. The fungus is recovered most easily toward the end of winter rains.[52] This is opposite to the seasonal relationship for acquisition of new infections, which in California and Arizona occur most frequently during the summer months, after the soil has become dry. In Arizona, there is a second peak of new clinical infections from October until the winter rains, which corresponds to a similar dry period after the late summer rains in that region.[53]

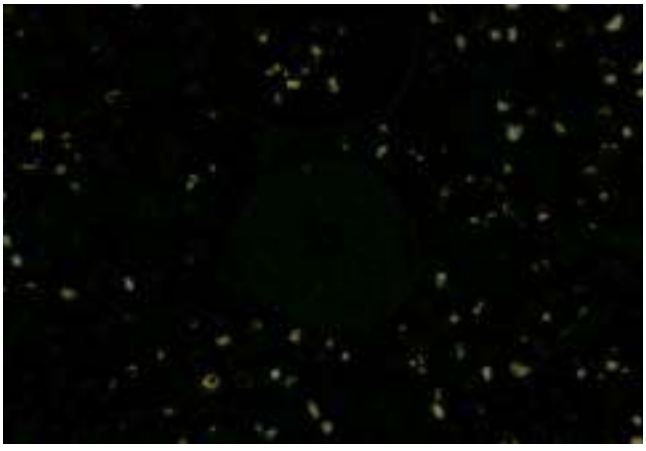

FIG. 265.1 Photomicrograph of a spherule in a tissue. Hematoxylin and eosin staining. *(Courtesy Richard Sobonya, MD, University of Arizona.)*

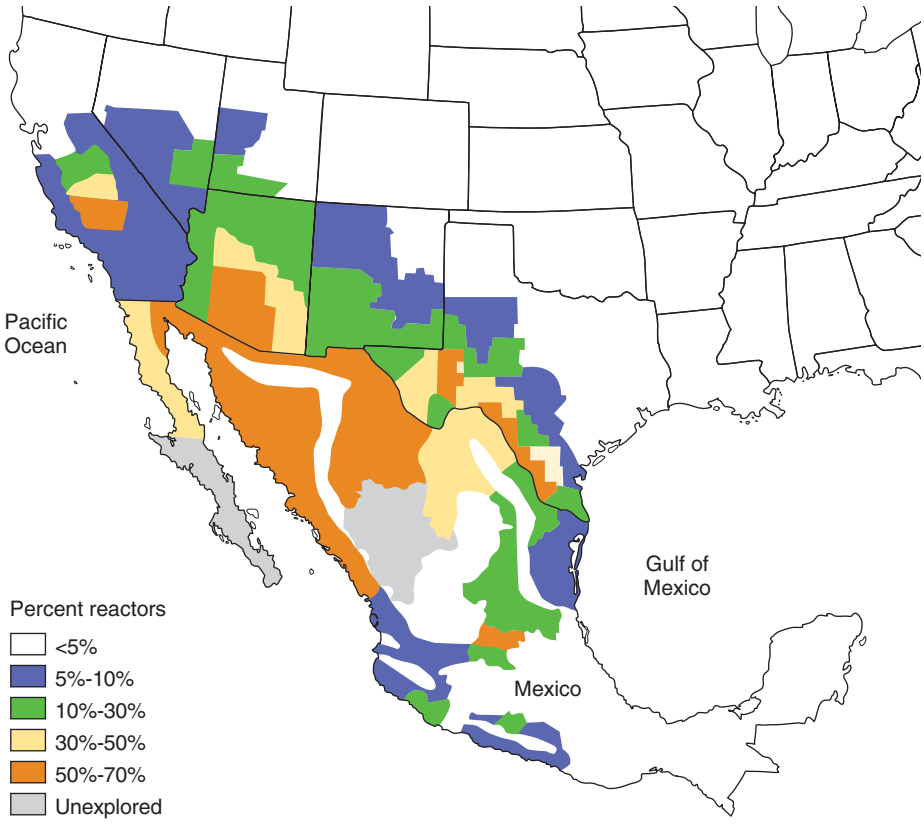

FIG. 265.2 Dermal hypersensitivity mapping of the endemic intensity of coccidioidomycosis. (*From Nguyen C, Barker BM, Hoover S, et al. Recent advances in our understanding of the environmental, epidemiological, immunological, and clinical dimensions of coccidioidomycosis.* Clin Microbiol Rev. *2013;26:505–525.*)

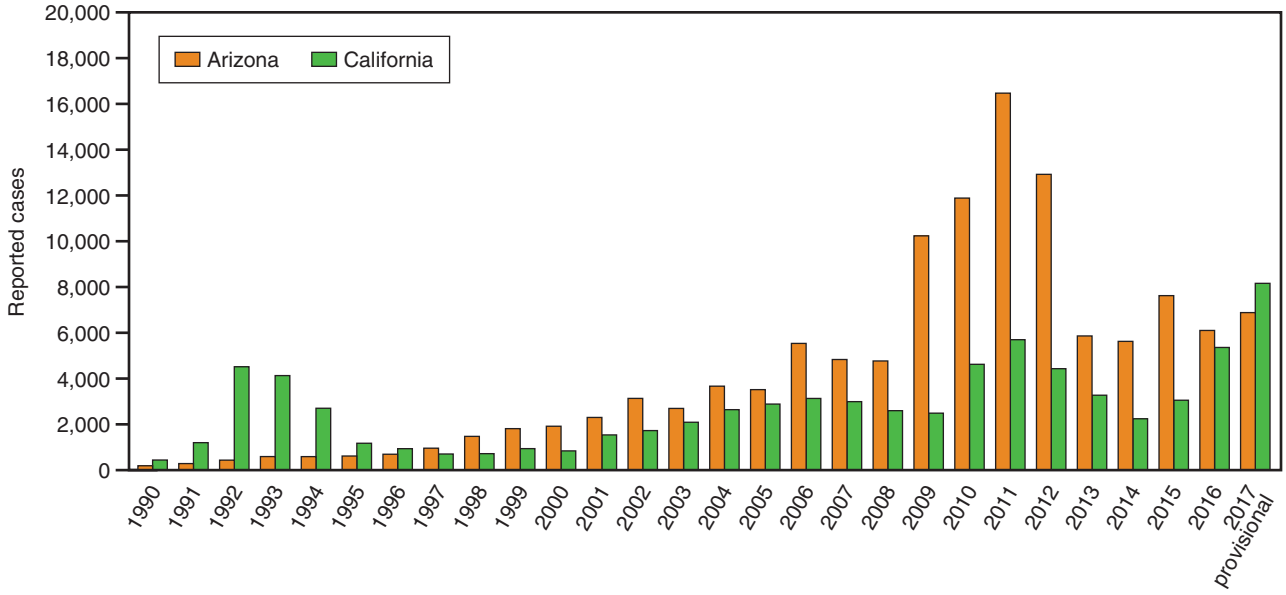

FIG. 265.3 New cases of coccidioidomycosis reported to the Arizona and California Departments of Public Health. *(Data source: Centers for Disease Control and Prevention.)*

Rates of Coccidioidal Infection

Prevalence surveys in the 1950s of skin test reactivity to coccidioidal antigens in school-age children of California's Central Valley suggested that the annual risk of infection was approximately 15%.[54] Smith and colleagues[55] showed that in 25% to 50% of military personnel in the San Joaquin Valley, skin test results converted to positive during a single year. More contemporary estimates from the same areas in California and from Tucson, Arizona, indicate that the risk has declined to approximately 3% per year.[54,56] Because of these lower rates and because of the large influx of new residents to the endemic regions from non-endemic locales (in the 2010 census, estimated to total >7 million persons for southern Arizona and south central California), the proportion of persons within the endemic region with prior infection is approximately 30%. Based upon these estimates, the expected number of infections is on the order of 150,000 annually, resulting in approximately 50,000 persons sick enough to seek medical attention.

The numbers of infections reported to state departments of public health differ significantly from year to year (Fig. 265.3). Some variation

has been associated with total winter rainfall; more cases occur in the summers after wetter winters.[53] On occasion, epidemics also have been associated with disruption of infected soil by human intent, such as with excavation; after natural events, such as severe dust storms or earthquakes; or during military maneuvers.[57–59] Some fluctuations in rates of infections are not explained, however. Such is the case for an exceptionally large epidemic in California's Central Valley in the period from 1992 to 1995, in which the incidence of infection at times was more than 10 times that normally reported, and in 2010 to 2011, in which the total number of reported cases was the highest since coccidioidomycosis was made a reportable disease.[11,60] An analysis of death certificates indicated that approximately 160 persons die each year from coccidioidomycosis,[61] although these statistics may be an underestimate.[62]

PATHOGENESIS AND CONTROL

Nearly all infections are the result of inhaling arthroconidia. Cutaneous inoculations have been reported, producing lymphatic extension to regional lymph nodes and resolving without treatment. These occurrences are exceedingly rare, however.[63]

A single arthroconidium may be sufficient to produce a naturally acquired respiratory infection. This is the case for experimental infections in mice,[64] and air sampling within coccidioidal endemic regions suggests that the ambient density of arthroconidia in the air is low.[65,66] The size of the arthroconidium would allow its deposition within the terminal bronchiole but probably not as deeply as the alveolar space. With spherule rupture, inflammation ensues,[67,68] forming a local pulmonary lesion. Extracts of *Coccidioides* spp. have been shown to react with complement, releasing mediators of chemotaxis for neutrophils.[69] In some infections, *Coccidioides* spp. leave the lungs to establish disseminated lesions in other parts of the body. In this sequence of events, fungal elements must move from the distal bronchiole into the lung parenchyma, gain entry into the vascular space, and leave the vascular space to create extrapulmonary sites of infection. It is possible that endospores within macrophages travel through lymphatic vessels to the bloodstream, as has been described for dissemination of tuberculosis and histoplasmosis. This possibility is also compatible with the common finding of infected hilar, peritracheal, supraclavicular, and cervical lymph nodes in patients with extrapulmonary coccidioidal infections.[67]

Histopathology

Microscopic examination of tissue infected with *Coccidioides* spp. shows elements of acute and chronic inflammation. Acute inflammation, including neutrophils and eosinophils, is associated with active infections and rupturing spherules.[67,68] Granulomatous lesions that include lymphocytes, histiocytes, and multinucleated giant cells are associated with chronic or arrested infections and with mature unruptured spherules.[70] In patients with widespread infections, it is common to find both inflammatory responses represented concurrently at different anatomic sites.

Host Defenses

Control of coccidioidomycosis depends on T lymphocytes. This conclusion is supported by studies of experimentally produced infections in mice[71–75] and by the increased severity of naturally acquired infections in T-cell–deficient patients.[21,23,76–79] Peripheral mononuclear blood cells from patients with active disseminated coccidioidomycosis have virtually no interferon-γ response to coccidioidal antigens.[80] This is in contrast to the brisk stimulation of similar leukocyte preparations from patients in whom coccidioidal infections are competently controlled and who have delayed-type dermal hypersensitivity to coccidioidal skin-testing antigens.[81] These findings are consistent with an absent type 1 helper T cell (Th1) response described in some experimental animals[82,83] and human infectious diseases, in which cellular immunity plays a role. In humans, however, despite the observed depression of interferon-γ levels, interleukin-4 and interleukin-10 levels were not reciprocally elevated,[81] which would be indicative of a type 2 helper T cell (Th2) response. Recently, specific mutations in Th1 pathway genes have been associated with disseminated infection.[84–86]

In addition to T-cell–mediated control of infection, innate cellular responses may contribute to host defense.[87,88] Inhibition of growth of *Coccidioides* spp. can be shown in vitro by human neutrophils and mononuclear cells from persons with or without prior coccidioidal infection as judged by skin test reactivity to coccidioidal antigens.[89] Although neutrophils do not seem to be fungicidal against *Coccidioides* spp., mononuclear cells or natural killer cells have been shown to reduce fungal viability.[90,91] These innate cellular inhibitory effects are most evident against arthroconidia or endospores and are lost as spherules increase in size and mature.[92] These in vitro observations can be extrapolated to indicate that innate defenses may serve primarily to slow fungal proliferation after infection, transforming what otherwise might be a more fulminant infection to a more subacute or chronic process.

Coccidioidal infections engender a variety of humoral responses to several different antigens in patients, and, as discussed subsequently, several are diagnostically useful. *Coccidioides*-infected B-cell–deficient mice are not as protected by vaccination as are normal mice.[93] However, a specific defensive role for immunoglobulins has thus far not been defined.

CLINICAL MANIFESTATIONS

At least one-half to two-thirds of all infections caused by *Coccidioides* spp. are either inapparent or sufficiently mild not to prompt medical evaluation.[55] Of those that do become medically significant, a large majority result in a respiratory illness that is indistinguishable without specific testing from community-acquired pneumonia caused by other entities.[94–96] In two observational studies in southern Arizona, coccidioidomycosis was estimated to be responsible for approximately one-quarter to one-third of all cases of community-acquired pneumonia in that endemic area.[95,96] Nonetheless, misunderstandings of the manifestations of coccidioidomycosis or the perceived unimportance of diagnosis of early infections have led to significant delays in diagnosis[96a,96b] and gross disparities between the numbers of expected and reported coccidioidal infections.[97–99] For example, the number of coccidioidal infections reported to Arizona's Department of Health Services (see Fig. 265.3) represent only a fraction of the expected 30,000 new illnesses. Underdiagnosis may be even more likely for patients with coccidioidomycosis evaluated outside the endemic region.[57,100] Most coccidioidal infections, whether detected or not, follow a self-limited course; only a few produce residual sequelae or chronic progressive infections. Although complications of untreated coccidioidal infections are typically manifested within weeks, months, or rarely up to 2 years after the original infection, the severity of the initial respiratory infection is not correlated with the likelihood of complications. In this context, the identification of even mild primary infections takes on added significance and clinical relevance.

Early Respiratory Infection

The first symptoms of the primary infection usually appear 7 to 21 days after exposure. Most infections seem to develop as a result of exposure to one or a small numbers of arthroconidia. However, when exposure is unusually intense, symptoms are more likely to appear early.[101] In an epidemic of coccidioidomycosis that occurred in the San Joaquin Valley of California between 1991 and 1994,[102] the findings in 536 patients with new infections included cough (73%), chest pain (44%), shortness of breath (32%), fever (76%), and fatigue (39%). These findings are typical of earlier reports. Although the infection is often subacute in development, patients occasionally report abrupt onset of symptoms, especially that of pleurisy. Weight loss is also a common sign, and headache has been noted in 21% of patients in the absence of meningeal infection.[103] Skin manifestations develop as part of the primary illness. Most frequent and easily missed is a nonpruritic fine papular rash that occurs early and transiently during the illness. More striking are erythema nodosum and erythema multiforme, which occur predominantly in women. Migratory arthralgias are also common complaints, and the triad of fever, erythema nodosum, and arthralgias (especially symmetrically of the knees and ankles) has been termed *desert rheumatism*. Routine laboratory findings, including serum procalcitonin levels,[104] are usually normal except for slightly increased peripheral blood leukocytosis and an increase in the erythrocyte sedimentation rate. Peripheral blood eosinophilia may be present, occasionally accounting for two-thirds of the circulating leukocytes. Chest radiograph results

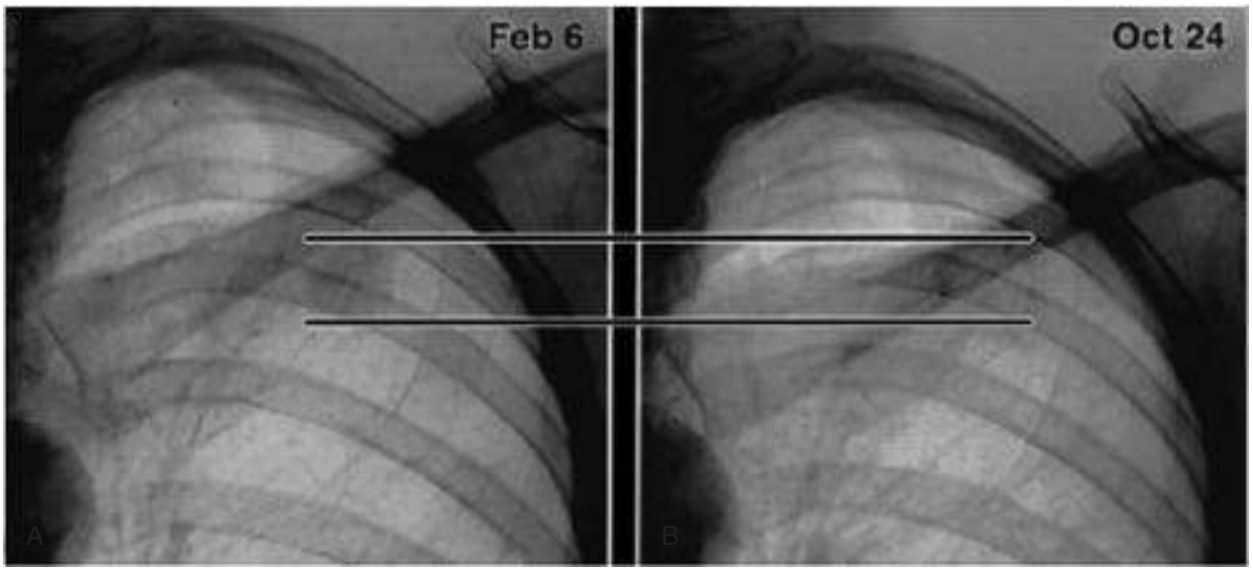

FIG. 265.4 Cavitation of a coccidioidal nodule. (A) A 1.8-cm nodule can be seen. (B) Eight months later, this lesion has become a thin-walled cavity.

are abnormal in more than half of patients. This partially accounts for different estimates in two Arizona studies of coccidioidomycosis as a cause of community-acquired pneumonia. A case definition that required an abnormal chest radiograph in one study[96] was lower than one that did not.[95] Common radiographic findings include unilateral infiltrates, hilar adenopathy, and peripneumonic pleural effusions. Persistent hilar or peritracheal adenopathy may be associated with extrathoracic spread of infection.[105] Lung cavities are present initially in approximately 8% of infections recognized in adults but are less frequent in children.[106]

Uncommonly, coccidioidal pneumonia manifests as a diffuse process leading to respiratory failure, either because of high-inoculum exposure[107,108] or because of fungi in the bloodstream that seed the lung in many sites.[22,109] The manifestation is often fulminant, mimicking that of septic shock or a bacterial infection, and despite treatment, the rate of mortality is high. Approximately one-third of human immunodeficiency virus (HIV)–infected patients with clinically acquired immunodeficiency syndrome (AIDS) exhibit this radiographic appearance. Although fungemia associated with diffuse pulmonary infiltrates may occur in immunologically intact patients,[110] it is nearly always attributable to a recognizable cellular immunodeficiency state.[111] In HIV-infected patients with fungemia, the $CD4^+$ counts are typically less than 100 cells/mm^3 and the viral load is high.[112]

Although some of the presenting signs, symptoms, and routine laboratory studies are statistically more likely to occur with coccidioidal infections than with respiratory illness of other causes, the overlap of clinical syndromes is substantial.[94–96] For most patients, specific testing is necessary to secure a definitive diagnosis of coccidioidomycosis.

Most coccidioidal respiratory infections resolve without complications but often take several weeks to many months to do so. When resolution of the self-limited illness is protracted, the symptom of fatigue is frequently the last to resolve. This fatigue syndrome, disproportionate to other evidence of infection syndrome, may strikingly interfere with normal daily activities or the ability to return to work, and it can be a source of considerable distress. A recent small study of such subjects demonstrated a striking oxygen utilization deficit,[113] but further studies will be needed to determine how extensively this mechanism accounts for this very common complaint. A few patients with infections develop various pulmonary sequelae, and even fewer patients manifest disseminated infection outside the lungs. Despite their relative infrequency, these complications pose significant difficulties in diagnosis and management (discussed later).

Pulmonary Nodules and Cavities

Approximately 4% of pulmonary infections result in a nodule, ranging up to 5 cm in diameter. A nodule typically causes no symptoms but may be indistinguishable from a neoplasm without histologic examination.[114–116] On occasion, nodules liquefy and drain into a bronchus to form a cavity (Fig. 265.4).

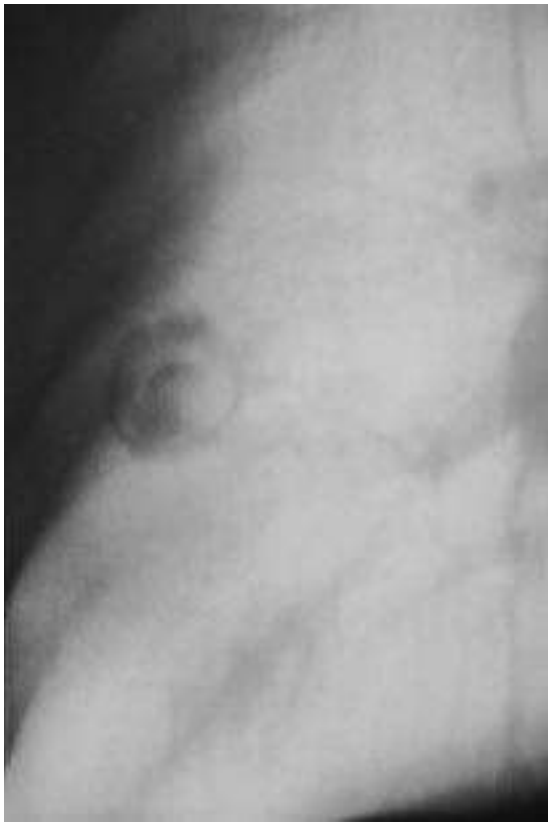

FIG. 265.5 Fungus ball in the right lung of a coccidioidal cavity. Bronchoscopy specimens yielded *Coccidioides* spp. in culture. *(From Winn RE, Johnson R, Galgiani JN, et al. Cavitary coccidioidomycosis with fungus ball formation.* Chest. *1994;105:412–416.)*

Pulmonary cavities may be present initially or in the later stages of the primary infection. They are usually peripheral and solitary, and with time, most develop a distinctive thin wall.[106] Cavities may not cause any symptoms, and half close within 2 years. Others are associated with local symptoms of pleuritic pain, cough, or hemoptysis. A fungus ball may develop within cavities, either from mycelia of *Coccidioides* spp.[117,118] or with other species of fungi (Fig. 265.5). Another infrequent but well-recognized complication is rupture of a peripheral coccidioidal

cavity into the pleural space and its manifestation as a pneumothorax. Ruptures commonly occur in athletic young men and are not associated with underlying immunodeficiency. Because the fungal walls of *Coccidioides* spp. are inflammatory, ruptured coccidioidal cavities often produce fluid in the pleural space, and the presence of an air-fluid level within the pleural space is a clue that the process is not a spontaneous pneumothorax or a ruptured pulmonary bleb (Fig. 265.6). If the cavity is diagnosed early, surgical resection of the cavity and closure of the pulmonary leak is the preferred treatment.[119,120] Less commonly, pleural disease can occur without rupture of a cavity. In a study of pleural coccidioidomycosis, 10 of 36 (28%) patients had pleural-predominant disease without cavity or cavity rupture.[121]

Chronic Fibrocavitary Pneumonia

In contrast to thin-walled coccidioidal cavities, a chronic fibrotic pneumonic process that develops in some patients is characterized by pulmonary infiltrates and pulmonary cavitation (Figs. 265.7 and 265.8).[122] This form of infection is not common among patients with T-cell deficiencies but seems to be associated with diabetes or preexisting pulmonary fibrosis related to smoking or other causes.[123] Involvement of more than one lobe is more common, and these lesions may cause systemic symptoms, such as night sweats and weight loss, and local symptoms. Recently, two patients with a mutation of *STAT1* have been described with a chronic consumptive coccidioidal pneumonia, which is strikingly devoid of cavitation in contrast to what occurs more commonly.[86]



FIG. 265.6 Chest computed tomography scan of ruptured coccidioidal cavity with pneumothorax. White star points to air in the pleural space.

Extrapulmonary Dissemination

Coccidioides spp. spread beyond the lungs in approximately 0.5% of all infections in the general population. Several factors dramatically increase the risk of dissemination, however: immunodeficiency conditions, such as the later stages of HIV infection[112] and Hodgkin lymphoma[23]; and therapies that suppress immune function, such as therapy to prevent solid-organ rejection,[124] high-dose corticosteroid therapy (equivalent to long-term prednisone doses >20 mg/day),[78] and therapeutic inhibitors of tumor necrosis factor.[18,125] In two-thirds of renal-transplant recipients who developed coccidioidal infection, the infection progressed to dissemination.[76] With transplantation, the risk is heightened mostly by either newly acquired disease or reactivation of prior infection. Transmission by the engrafted organ has also been reported.[126–128] Dissemination is more likely to develop in men than in women.[129–131] Dissemination is also more likely, however, if infection is diagnosed during pregnancy, especially during the third trimester or in the immediate postpartum period.[132] The risk of dissemination also appears to be increased among persons of African or Filipino ancestry, although the exact magnitude of the risk is controversial.[129,133,134] Extrapulmonary dissemination is not associated often with pulmonary complications. Many patients with disseminated coccidioidal infection have entirely normal chest radiographs.

The most common site of dissemination is the skin. The range of lesions includes superficial maculopapular lesions, keratotic and verrucose ulcers, and subcutaneous fluctuant abscesses. There is a predilection for lesions at the nasolabial fold (Fig. 265.9). Although most extrapulmonary dissemination is the result of hematogenous spread, supraclavicular and cervical lymphadenopathy is also a frequent manifestation and probably represents lymphatic drainage from the primary pulmonary infection. A rare manifestation is peritoneal coccidioidomycosis, which clinically resembles tuberculous peritonitis.[135]

Joints and bones are common sites of dissemination. Joint infections differ from the self-limited joint complaints of desert rheumatism in that infections are typically asymmetrically distributed and are associated with a prominent synovitis and effusion. Although any joint can become infected, the knee is involved most frequently; other common locations include the joints of the hands and wrists, feet and ankles, vertebrae, and pelvis.[136–140] Infection may be limited to the synovium or may erode to involve the underlying bone. Alternatively, bones may be involved first with secondary extension into the joint.[141,142] Although long bones

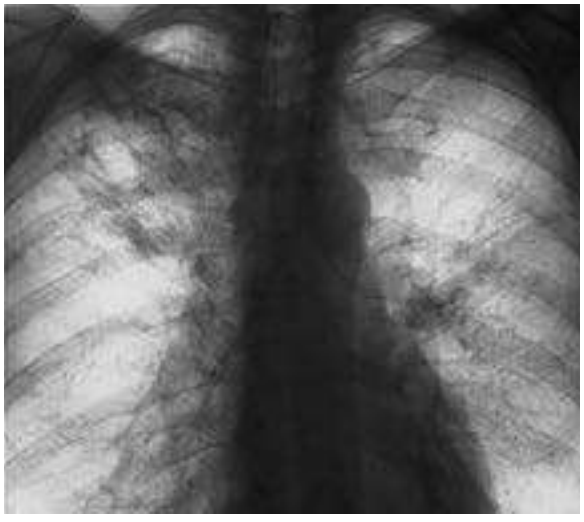

FIG. 265.7 Pulmonary cavity in the right upper lobe with surrounding fibrosis.

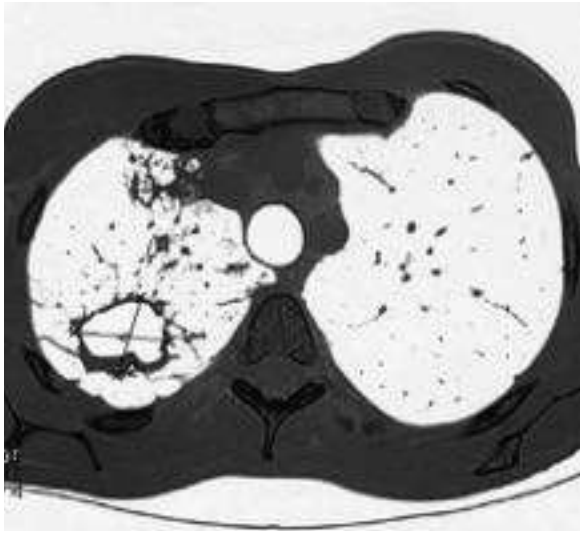

FIG. 265.8 Computed tomography scan of the fibrocavitary process shown in Fig. 265.7.



FIG. 265.9 Ulcerative lesion of disseminated coccidioidal infection. *(From Galgiani JN. Coccidioidomycosis.* West J Med. *1993;159:153–171.)*

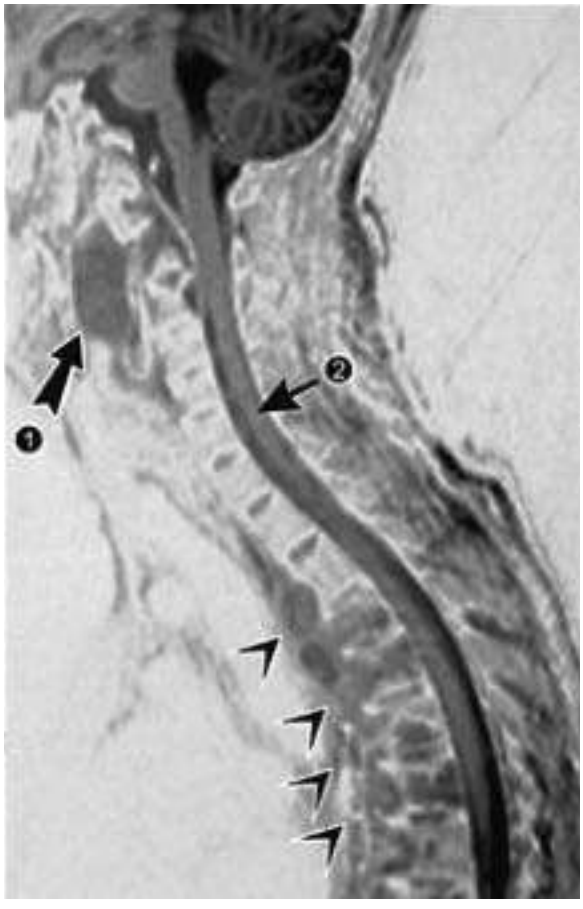

FIG. 265.10 Sagittal magnetic resonance imaging shows an anterior paraspinous abscess extending from the base of the skull to the midthoracic vertebrae. Arrow 1 points to an abscess that originated in a cervical vertebra and dissected anteriorly. Arrow 2 identifies a normal spinal cord. The arrowheads indicate abscesses anterior to the thoracic vertebrae. Multiple surgical procedures were necessary to control this infection.

may be affected, vertebral infection is much more common. Involvement of multiple vertebrae is typical.[143] These may coalesce to produce anterior or posterior paraspinous soft tissue abscesses or an epidural abscess (Fig. 265.10). Magnetic resonance imaging (MRI) is often helpful in defining the exact location of these lesions.[144]

Coccidioidal meningitis is the most serious form of disseminated infection. Untreated, it is nearly always fatal within 2 years of diagnosis.[145,146] Like most other complications of coccidioidomycosis, meningitis usually develops relatively soon after the initial infection. In one study of 22 patients who developed meningitis after a large dust storm, central nervous system symptoms developed on average after 5.4 weeks of illness.[147] Similarly, a review of cases from the Department of Veterans Affairs and military records showed that, of 25 patients who developed meningitis, 20 did so within 6 months of their first symptoms of infection.[146] Common presenting symptoms are headache, vomiting, and altered mental status.[148–150] In addition to cerebrospinal fluid (CSF) findings of an elevated white blood cell count, elevated protein levels, and a depressed glucose level, CSF eosinophils are occasionally prominent.[151] The main areas of involvement are the basilar meninges. Hydrocephalus is a common complication, especially early in children.[152] Attention has been drawn to vasculitis and focal intracerebral coccidioidal abscesses as less frequent complications.[153–155]

DIAGNOSIS

The manifestations of most early coccidioidal infections overlap substantially with those of other respiratory infections.[94–96] Specific laboratory testing is usually necessary to establish a diagnosis of coccidioidomycosis. In regions where *Coccidioides* spp. are endemic, this testing is commonplace. In most of the rest of the United States, the possibility of coccidioidomycosis is unlikely to be considered unless a geographic exposure is elicited in the patient's history. Because the incubation period is usually 1 to 3 weeks, endemic exposure within this period should raise the possibility of coccidioidomycosis to account for a respiratory condition of new onset. Exposure need not be extensive. Infections have occurred in patients whose only exposure occurred while changing airplanes at the Phoenix airport or during a single drive across California's Central Valley.

Complications of the initial infection, such as chronic pneumonia or extrathoracic dissemination, may take longer to become apparent but nearly always emerge within 2 years after exposure. One exception to this rule is the detection of a pulmonary nodule or a solitary pulmonary cavity, which may persist without symptoms for many years after the original infection. Another special case is the setting of waning immunity, such as after the development of AIDS or with immunosuppressive therapy associated with solid-organ transplantation. In such circumstances, exposure to *Coccidioides* spp. in the distant past may be sufficient to account for the current clinical illness.[156]

When the possibility of coccidioidomycosis has been raised, diagnosis is usually established in two ways: (1) identifying spherules in, or recovering *Coccidioides* spp. from, a clinical specimen; or (2) detecting specific anticoccidioidal antibodies in serum, CSF, or other body fluid.

Direct Examination and Culture

Isolating *Coccidioides* organisms from a patient is definitive evidence of a coccidioidal infection, and this diagnostic approach is used most frequently for patients with complicated pulmonary or disseminated syndromes. Sputum or other clinical specimens can be collected at no risk to personnel because the infection is not transmitted from the primary specimen. Direct microscopic examination of secretions can be performed immediately or after the addition of potassium hydroxide. Calcofluor staining of the cell wall in a wet mount may also help to distinguish spherules from leukocytes. *Coccidioides* spp. cannot be detected on Gram stains. Spherules also can be detected by cytology stains (e.g., in bronchoscopy specimens)[157]; hematoxylin and eosin stains; and other specialized procedures, such as silver or periodic acid–Schiff staining. Hematoxylin and eosin staining of spherules produces a distinctive autofluorescence that may help to identify a few organisms in tissues.[158] Using species-specific probes, researchers found that in situ hybridization was not as sensitive as silver staining but was more specific.[159] Although culture results are more sensitive, identification of spherules by direct examination is more rapid and may speed diagnosis. Direct detection of *Coccidioides* in sputum by polymerase chain reaction has been reported and may become clinically available soon.[38,160–162]

Coccidioides spp. grow well on most mycologic or bacteriologic media after 5 or 7 days of incubation. Aerobic conditions are required. When

growth occurs, it is typically as a white (nonpigmented) mold. There are many exceptions to this general appearance, however, and the morphologic appearance is not reliable in determining whether the fungus is *Coccidioides* spp.[163] When growth is evident on culture medium, care should be taken not to open the culture container except in an appropriate biocontainment cabinet. Cultures at this stage are highly infectious, and infections have occurred in laboratory personnel when cultures have not been handled properly.[164]

The mycelial form of growth is not specific for *Coccidioides* spp., and further testing is required for species identification. The most common way for microbiologists to test this is to detect a specific ribosomal RNA sequence using a commercially available DNA probe (Accuprobe, Hologic, San Diego).[165] At present, molecular methods to differentiate between *C. immitis* and *C. posadasii* are available only at a limited number of reference laboratories. Genus identification can also be done by detection of an exoantigen in an extract of fungal growth.[166,167] Until December of 2012, fungal isolates identified as *Coccidioides* spp. were subject to strict federal security regulations developed for all select agents of bioterrorism. However, *Coccidioides* spp. have been delisted, and CDC oversight is no longer in effect.

Serologic Testing

Serologic testing is the most frequent means of diagnosing primary coccidioidal infections because the patients may not be able to produce a sputum specimen, and fungal cultures often are not practical in an ambulatory setting. It may also be indispensable in establishing the cause of chronic meningitis because cultures of CSF are commonly negative in coccidioidal meningitis. Of the variety of tests available, most are highly specific for an active infection.[168,169] Minimally reactive test results are often diagnostically important and should not be dismissed as insignificant. A negative serologic test result never excludes the presence of a coccidioidal infection, however. Performing one or more repeated serologic tests over the course of 2 months increases the sensitivity of serologic diagnosis, especially for recently acquired infections.

Tube Precipitin Antibodies

Tube precipitin antibodies stimulated by a coccidioidal infection were originally detected by the presence of a precipitin button that formed at the bottom of a test tube after overnight incubation of the patient's serum mixed with coccidioidal antigen.[7] Because immunoglobulin M (IgM) is most avid at forming immune precipitins, and because these reactions were detected early after the onset of infection, this test is sometimes referred to as the *IgM test.* The antigen responsible for this reaction is a polysaccharide from the fungal cell wall. At some time within the first 3 weeks of symptoms, tube precipitin antibodies are detected in 90% of patients; this prevalence declines to less than 5% more than 7 months after the onset of a self-limited illness.

Complement-Fixing Antibodies

When the patient's serum is mixed with coccidioidal antigen, an immune complex forms that consumes complement.[170] This event is detected by the subsequent addition of sensitized red blood cells, which normally lyse in the presence of complement but remain intact if the complement is depleted. Because immunoglobulin G (IgG) is the immunoglobulin class usually involved in these immune complexes, this test is sometimes referred to as the *IgG test.* Although this test originally was developed through the use of various complex extracts of *Coccidioides* spp., it is now known that the antigen involved in this reaction is a chitinase.[171–173] Subsequent work demonstrated that a recombinant truncation of amino acids 20 to 310 eliminated serologic cross-reactivity with antibodies stimulated during histoplasmosis,[174] and that all of the coccidioidal antibody reactivity was located from amino acids 111 to 310.[175] In early coccidioidal infections, complement-fixing antibodies are detected later and for longer periods than are tube precipitin antibodies.[8] Complement-fixing antibodies can be detected in other body fluids, and their detection in CSF is an especially important aid to the diagnosis of coccidioidal meningitis. Complement-fixing antibody concentration is expressed as a titer, such as 1:4 or 1:64, indicating the greatest dilution of serum at which complement consumption is still detected. Traditionally, a titer of 1:16 or greater has been associated frequently with extrathoracic dissemination. Because of technical factors, however, end point results for the same serum samples may vary considerably on testing by different laboratories. More useful are serial determinations of complement-fixing antibody concentrations performed by the same laboratory. In general, higher titers reflect more extensive coccidioidal infection, increasing complement-fixing antibody concentrations are associated with worsening disease, and decreasing titers are useful in monitoring response to therapy. These are general relationships that are occasionally not borne out by the course of individual patients.[175a]

Immunodiffusion Tests

Antibodies that were detected by the original tube precipitin or complement-fixing tests can be detected by alternative procedures known as the *immunodiffusion tube precipitin* and *immunodiffusion complement-fixing* tests. Although these tests are conducted similarly, different antigens are used to measure different types of antibodies. As with the original tests, the immunodiffusion tube precipitin test result is reported by some laboratories as the IgM test result, and the immunodiffusion complement-fixing result is reported as the IgG test result. Both tests have been found to be at least as sensitive as their original counterparts.[176,177] The quantitative immunodiffusion procedure closely correlates with the quantitative complement-fixing antibody test.[178] Immunodiffusion tests are more amenable to being manufactured and distributed in commercially prepared kits, which allow them to be performed in laboratories not fully dedicated to a mycology specialty.

Enzyme-Linked Immunoassays

Enzyme immunoassays for coccidioidal antibodies are available commercially (Meridian Bioscience, Inc., Cincinnati, OH; IMMY, Norman OK; Mira Vista Diagnostics, Indianapolis, IN). The test kits allow the specific detection of IgM or IgG antibodies; however, these results are not interchangeable with the complement fixation or immunodiffusion test results. Positive results with this commercial kit are highly sensitive for coccidioidal infection. On occasion, false-positive results are noted, especially with the IgM enzyme immunoassay.[179–181] At present, enzyme immunoassay results should ordinarily be confirmed with immunodiffusion tube precipitin, immunodiffusion complement-fixing, or complement-fixing test results. When the more established tests fail to corroborate the enzyme immunoassay, a coccidioidal infection may in fact exist, but the diagnosis is less firmly established.[182–184]

Latex Tests

Latex tests for coccidioidal antibodies are also available commercially. They are attractive to clinical laboratories because they are easy to use, and results are obtained rapidly. There are significant numbers of false-positive reactions, however, and the latex test is not as reliable as the other tests described in this section.[168]

Skin Testing

Dermal delayed-type hypersensitivity to coccidioidal antigens is highly specific for coccidioidal infection.[185] In clinical practice, especially within endemic regions for coccidioidomycosis, perhaps the best use of coccidioidal skin testing in patients when they are not ill is to determine if they are immune from future disease as a result of past infection. Because skin test results remain positive after infection in most people for life, however, a result may not be related to the current illness. In addition, some of the most serious infections may be associated with selective anergy, and the skin test may not reveal reactivity. As useful as skin test results are for epidemiologic studies, the tests have important limitations as screening procedures for recent infection. For patients in whom coccidioidomycosis has been diagnosed by other means, skin testing may have prognostic significance.[186] Coccidioidal skin testing reagents ceased being available commercially in the 1990s. However, a reformulation of the spherule-derived skin test antigen was reintroduced in 2015 as Spherusol (Nielsen BioSciences, Inc., San Diego, CA), and is approved to test for immune responsiveness in patients previously diagnosed with coccidioidomycosis.[187–189]

Coccidioidal Antigen Detection

Antigenemia may occur with either early or chronic coccidioidal infections and could be the basis of a diagnostic test.[190–193] A commercial

test is available (MiraVista Diagnostics, Indianapolis, IN) for detecting coccidioidal antigens.[194] This test, applied to CSF specimens, may be particularly useful for diagnosing meningitis.[195,196]

MANAGEMENT

General Approaches

The three components of managing coccidioidal infections are (1) assessment of the need for intervention, (2) selection of antifungal agents for patients who would benefit from treatment, and (3) choice of surgical procedures for débridement and reconstruction of destructive lesions. A revised practice guideline has been published[25] and is available online from the Infectious Diseases Society of America (IDSA; www.idsociety.org). A training manual for nonspecialists is also available (vfce.arizona.edu).

In patients with newly diagnosed coccidioidal infections, it is crucial to assess the extent of disease at present and the factors that increase the risk of future complications. The current extent of disease can usually be assessed with a careful review of systems, physical examination, and chest radiographs. When new focal complaints of discomfort or swelling are identified, these should be evaluated further with appropriate imaging or, if necessary, biopsy. Pain referable to bones might be assessed with a radionuclide bone scan or an MRI.[197,198] Recently, positron emission tomography/computed tomography scans have been used to also detect extrapulmonary disease activity, although to what extent the metabolic activity represents areas of tissue destruction is uncertain.[199,200] An effusion that develops in a joint could be aspirated for cell count and culture, a progressively severe headache may necessitate MRI and especially lumbar puncture to evaluate the possibility of meningitis, and a nonhealing skin lesion may necessitate biopsy.

In the general population, pulmonary or extrapulmonary complications are uncommon. There is a special risk of disseminated infection, however, with conditions that prominently suppress T-cell immunity as detailed earlier. Patients with active infection and these risk factors nearly always should be treated with antifungal therapy even if there is no evidence of extrapulmonary spread. Patients with diabetes mellitus are not prone to extrapulmonary dissemination. They are more likely to develop pulmonary cavitation or chronic pneumonia, however, and may be more likely to require treatment.[123]

Therapy

Available antifungal agents include amphotericin B and the azole antifungal agents. Their pharmacology is described in detail in Chapters 40A to 40D. In coccidioidomycosis, selecting between amphotericin B and azole antifungals is based primarily on the degree of respiratory compromise in pulmonary infections or the rate of progression of disseminated infections. Amphotericin B is perceived to have a more rapid onset of action; therefore despite its well-known toxic effects, it is the preferred initial therapy for patients who have developed serious respiratory compromise or who are deteriorating rapidly. There is no evidence that a lipid formulation of amphotericin B improves on the efficacy of colloidal (conventional) amphotericin B, but liposomal or lipid complex amphotericin B is often used because of less toxicity. Azole antifungals are often selected for patients with chronic processes because possible differences in rate of response to azole antifungals would be outweighed by their ease of administration and lack of toxicity. Ketoconazole was the only orally available azole antifungal approved by the US Food and Drug Administration for the treatment of coccidioidomycosis but is no longer the preferred drug for this disease because of hepatotoxicity. Several clinical trials have indicated that fluconazole and itraconazole are efficacious.[201–207] No studies have demonstrated general superiority of one azole antifungal over another. In a comparison of fluconazole (400 mg once a day) and itraconazole (200 mg twice daily), the primary analysis showed that the two drugs were within 20% of each other in producing responses.[204] In a secondary analysis of skeletal lesions, response was obtained in twice as many subjects treated with itraconazole as those treated with fluconazole. The newer azoles, voriconazole and posaconazole, have also been used to treat coccidioidomycosis. In one study, 17 of 20 patients treated with 400 mg/day of posaconazole suspension improved.[208] However, of 9 patients in whom treatment was discontinued, 3 suffered relapse. In another study of 15 patients in whom previous treatments had failed, 11 treated with 800 mg/day of posaconazole improved.[209] In a report of 3 patients with coccidioidal meningitis, 2 improved with posaconazole.[210] For voriconazole, there is less published literature, but case reports suggest that it may also be effective.[211–214] There is also very little published experience with isavuconazole.[214a]

Because the manifestations, locations, and severity of progressive forms of coccidioidomycosis vary among patients, the need for surgery is determined by the nature of specific lesions on a case-by-case basis. In some patients, especially in whom skeletal involvement is extensive, débridement and drainage of infected sites may be essential to achieving control of the infection. Even if therapy is effective in arresting fungal proliferation, fungal debris already present may continue to produce tissue destruction until it is surgically removed. Patients with persistent fever and malaise may benefit from drainage of large collections of pus. Also, surgery may be needed to stabilize bones that are structurally unsound or when the spinal cord is at risk of compression. Advances in imaging with computed tomography or MRI have aided greatly in the evaluation of specific lesions.[215] Repeated use of these modalities often helps to identify lesions that are progressing despite the current management strategy and patients who may benefit from additional surgical intervention or other changes in management.

Early Uncomplicated Infections

For patients with neither risk factors for nor evidence of extrapulmonary spread, treatment is of unproven benefit. To date, no placebo-controlled trials concerning this self-limited form of infection have determined whether treatment hastens the resolution of symptoms or prevents the risk of complications. Experts familiar with coccidioidomycosis have widely varying recommendations for management of specific patients in this category. Although some physicians recommend treatment for all patients, others recommend treating only patients with more severe manifestations. Even with selective therapy, its benefit is uncertain.[216,217] A nonrandomized, uncontrolled, single-site study found no statistical difference in symptom resolution in patients with primary mild-to-moderate pulmonary infection treated or not treated with antifungals.[218] In that study, 20 of 36 patients received therapy, 18 of whom received fluconazole at 400 mg daily for a median of 8.5 weeks. Evidence that is often considered to indicate more severe infection includes loss of more than 10% of body weight, intense night sweats for more than 3 weeks, infiltrates involving more than half of one lung or portions of both lungs, prominent or persistent hilar or peritracheal adenopathy, anticoccidioidal complement-fixing antibody titer greater than 1:16, failure to develop dermal hypersensitivity to coccidioidal antigens, inability to work, or symptoms that persist for more than 2 months.[25] Because persons of African or Filipino descent seem to have some increased risk of dissemination, this factor sometimes also weighs in the decision for treatment. If treatment is recommended, commonly prescribed therapies include currently available oral azole antifungal agents, usually fluconazole, for courses ranging from 3 to 6 months.

Although extrapulmonary dissemination usually occurs in the first several months of untreated coccidioidal infections, it is now clear that early treatment of the pulmonary syndrome does not prevent but may delay dissemination in some patients. In fact, late dissemination may not be recognized until years after initial azole treatment is stopped.[216,218] Also, it has been noted that persons treated for early pneumonia are less likely to develop a complement-fixing antibody response.[219] These considerations should be taken into account when deciding whether to treat the early otherwise uncomplicated coccidioidal pneumonia with antifungals.

Diffuse Pneumonia

Diffuse bilateral infiltrates represent either hematogenous infection of the lungs or multiple foci of infection resulting from exposure to a high inoculum of arthroconidia. In either case, even early infections are regarded as serious and warranting therapy. Initial therapy in such cases is usually with amphotericin B, at least for the first several weeks and until the illness seems to be improving. Concomitant use of a brief course of corticosteroids in this situation is controversial but advocated by some authorities.[220] After this time, therapy is often switched to an antifungal azole agent for at least 1 year. Fungemia resulting in diffuse pulmonary infiltrates is often the consequence of severe immunodeficiency, and in such patients, treatment may need to be continued indefinitely to prevent relapse.

Pulmonary Cavity

Cavitation as a sequela of coccidioidal pneumonia is often asymptomatic and may not necessitate treatment. With the passage of time, some cavities disappear. Cavities that do not close spontaneously over 1 to several years are sometimes resected to prevent future complications, especially if the cavity shows progressive enlargement or is immediately adjacent to the pleura and may cause pneumothorax. This potential benefit must be weighed against the risks of the surgical procedure, which vary according to the general health of the patient and the skill of the surgeon.

Pulmonary cavities occasionally produce symptoms, such as local pain, superinfection, or hemoptysis. When this occurs, treatment is usually instituted with oral antifungal azole therapy. This therapy often is accompanied by a diminution of symptoms, but recurrences are frequent if therapy is stopped. Antifungal treatment does not influence the size or disappearance of the cavity. For these patients, resection is a reasonable alternative to long-term suppressive medical therapy.[120] Extrapulmonary dissemination from a solitary pulmonary cavity is very uncommon.

Chronic Fibrocavitary Pneumonia

Persistent coccidioidal pneumonia is ordinarily treated with oral azole antifungal agents. Responses to these agents are approximately 55% to 60% as judged by improved symptoms and radiographic appearance. Treatment options for patients who do not respond include surgical resection of infection localized to a single lobe; switching to an alternative antifungal azole; for fluconazole, raising the dosage; or instituting amphotericin B therapy.

Extrapulmonary Dissemination

For most patients with nonmeningeal dissemination, initial therapy is with an oral antifungal azole. Exceptional patients with rapidly progressive infection or infection in critical locations, such as vertebrae, may respond faster to initial therapy with amphotericin B, but this has not been proved. As discussed previously, surgical débridement or drainage of selected lesions may be an important component of controlling infection.[221,222] As in chronic coccidioidal pneumonia, treatment is continued for at least 1 year and for 6 months past the point at which all evidence of further improvement has ceased. Even so, relapses occur in approximately one-third of patients when therapy is stopped, and some patients may require suppressive therapy indefinitely.

In the management of coccidioidal meningitis, most patients now are treated initially with fluconazole. This is a major departure from therapy with intrathecal amphotericin B, which until the early 1990s was still standard treatment.[223] Although there have been no comparative trials of intrathecal amphotericin B and fluconazole, the response rate of approximately 70% with fluconazole at 400 mg/day is probably at least as good as that achieved with intrathecal amphotericin B, and use of fluconazole avoids most of the toxicity associated with amphotericin B. Higher doses of fluconazole have produced responses in some patients who did not respond initially to 400 mg/day.[150] Similar results have been obtained in patients treated with itraconazole, although there is less clinical experience with this drug than with fluconazole.[202] Both posaconazole and voriconazole have been used as salvage therapy but offer no benefit to patients who have responded to fluconazole. The effect of azole treatment is to arrest fungal proliferation within the meninges and, as a result, inflammation resolves, as indicated by normalization of spinal fluid cell count and hypoglycemia, and this may produce a complete and lifelong remission of symptoms. However, azole therapy is not curative and dormant spherules persist, presumably indefinitely. It is a consensus opinion that patients who respond to azole therapy should continue suppressive treatment for life.[25] In one series in which 18 patients who had achieved an azole remission discontinued azole therapy, 14 relapsed and 2 died.[224] Moreover, it is recommended to use at least 400 mg/day of fluconazole as maintenance therapy, even in patients who are completely controlled on this dose.

Patients who do not respond to oral azole therapy may benefit from intrathecal amphotericin B.[225] Routes of administration include repeated percutaneous intracisternal injection, injection into Ommaya reservoirs that drain to either the cistern or a ventricle, lumbar puncture with medication in a hyperbaric glucose solution, and lateral cervical injection. The technique, frequency, and dosage of intrathecal amphotericin B vary widely among practitioners.

In addition to antifungal therapy to control the meningeal inflammation, surgical interventions are required for two other manifestations. One is hydrocephalus, a common complication of coccidioidal meningitis. Hydrocephalus ordinarily does not respond to antifungal therapy, and a shunting procedure is required. Ventriculoperitoneal shunts could become a conduit for *Coccidioides* spp. from the cerebrospinal space to the peritoneum, but this usually does not result in clinically apparent abdominal complications in patients on azole treatment. Fluid from a ventriculoperitoneal shunt is unreliable for assessing therapy because the whole blood cell count, protein, and glucose measurements are less abnormal during infection than is found in lumbar CSF.[226] A second and uncommon complication is intracerebral abscesses.[154] These lesions may necessitate drainage or resection, in addition to systemic antifungal drug therapy.

Another complication of coccidioidal meningitis is vasculitis.[155] In a retrospective series of 221 patients with meningitis, 18 (8.1%) had cerebrovascular accidents (CVAs), presumed to be a result of the infection.[227] Administering corticosteroids for this complication is controversial. In this series, 14 of the patients received dexamethasone at doses between 8 and 40 mg/day for 10 to 21 days, with the majority of these patients (9 of 14) receiving dexamethasone 10 mg intravenously once, followed by 4 mg four times daily. Steroid tapering ranged from 2 to 6 weeks. One patient received hydrocortisone (50 mg every 6 hours for 10 days). None of these 15 patients had subsequent CVAs, whereas the three patient who did not receive corticosteroids all had additional CVAs. These courses of steroids did not appear to interfere with the effectiveness of the antifungal therapy.

New Therapies

Because the fungal wall of *Coccidioides* organisms contains (1,3)-β-D-glucan and chitin,[39] antifungals that interfere with synthesis of these polysaccharides potentially could be therapeutic for coccidioidomycosis. Caspofungin (Cancidas; Merck & Co., Inc., Kenilworth, NJ) has been effective in treatment of experimental coccidioidal infections.[228,229] However, clinical experience is limited to combination therapy including an azole.[230,231]

Nikkomycin Z, a chitin synthase inhibitor discovered in the 1970s, was subsequently shown to be effective as a therapy against experimental murine coccidioidal infection.[232–234] Clinical trials were initiated in the 1990s but were soon interrupted because the sponsoring pharmaceutical company went out of business.[235] In 2005 the inactive project was transferred to the University of Arizona, which has reactivated the clinical studies.[236,237] A multidose phase I trial has been completed without identifying any safety concerns. Currently, new supplies of nikkomycin Z need to be manufactured to support phase II trials.

Olorofim is an inhibitor of dihydroorotate dehydrogenase that has recently been shown to have antifungal activity.[237a] Recently it has shown activity against an experimental central nervous system infection in mice[237b] and is in clinical trials as treatment for other fungal infections.

PREVENTION

Developing a vaccine as a means of preventing coccidioidomycosis has been an attractive goal for many years. This strategy might be useful because immunity develops in most persons who are infected naturally. A formalin-killed, whole-cell spherule vaccine was found to be exceptionally protective against lethal intranasal infections in mice.[238–243] The whole-cell vaccine also induced a great deal of local inflammation at the injection site, however, and thus the dose in humans is limited to 1.84 mg.[244] For an average human, this is approximately 1/1000 of the vaccine dose (milligrams per kilogram) required for protection in mice. When this dose of formalin-killed spherule vaccine was used in a human field trial, vaccination failed to result in significantly fewer symptomatic cases of coccidioidal pneumonia than were detected in placebo recipients.[245] One plausible explanation for the failure is that the inflammatory reactions to the whole-cell vaccine prevented use of a sufficient dose of the antigens responsible for protection. If this is the case, use of a purified or recombinant antigen might circumvent this limitation.

Several antigens have been expressed as recombinant proteins and, when used with Th1-based adjuvants, have evoked protection against

experimental coccidioidal infections in mice.[246] Unfortunately, the costs of developing a formulation of these vaccine candidates have been prohibitive, and this has prevented their further evaluation. Alternative approaches involve the development of a live-attenuated vaccine[247] or a vaccine with a live nonpathogenic fungus that engenders cross-species protection.[248] Recently, a heterologue of a gene identified to be involved in virulence for a corn pathogen, *Cochliobolus heterostrophus,* was deleted from *C. posadasii.*[28] This mutant causes no disease either in mice without an immune system or in those infected with millions of arthroconidia. Moreover, when this mutant is used as a live vaccine, it is very protective.[248a] This vaccine is now in active development as a candidate to prevent coccidioidomycosis in dogs. If safe and effective as a canine vaccine, this would provide further evidence that it might also be useful for humans.

The public health benefits from an effective preventive vaccine for coccidioidomycosis for the population at risk are roughly equivalent to that for the polio vaccine[246] and, if available, would be cost-effective.[249] That said, the population that would benefit from a Valley fever vaccine is relatively small and may not support commercial incentive to develop the product. Thus it is likely that other sources of support may be needed if a coccidioidal vaccine development program is to be successful.

Regarding preemptive or prophylactic use of prophylactic antifungal agents for visitors or residents of endemic regions, there is no evidence that this would be of benefit, even for highly immunosuppressed persons. A special case exists where an accidental laboratory exposure is identified. A detailed approach to managing such an occurrence has been published.[164] The recommended adult prophylactic dose of fluconazole or itraconazole is 400 mg daily. Drug interactions and pregnancy must be taken into account. There are specific recommendations for prophylactic antifungals for organ transplant recipients as outlined in the current IDSA practice guidelines.[25]

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The complete reference list is available online at Expert Consult.

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266 Dermatophytosis (Ringworm) and Other Superficial Mycoses

Roderick J. Hay

SHORT VIEW SUMMARY

Definition
- Dermatophyte infections are superficial infections of the stratum corneum of the skin or of keratinized appendages, such as hair or nails, arising from it. Other superficial infections are caused by *Candida* and *Malassezia* spp. or less common organisms (e.g., *Piedraia*).

Epidemiology
- Globally, the superficial fungal infections of the skin affect more than 900 million individuals. Most have no underlying abnormality, although individual conditions such as seborrheic dermatitis and oropharyngeal candidiasis may be early signs of human immunodeficiency virus infection.

Microbiology
- The main fungi involved are dermatophyte species of the genera *Trichophyton, Microsporum,* and *Epidermophyton,* and yeasts such as *Candida* or *Malassezia* spp. Less commonly, other mold fungi (e.g., *Neoscytalidium*) are implicated.

Diagnosis
- Direct microscopy of the skin and culture is diagnostic. Molecular diagnostic techniques are in development.

Therapy
- Topical treatments include azole antifungal agents and terbinafine. Selenium sulfide and zinc pyrithione have specific activity against *Malassezia.* Oral therapies include itraconazole and fluconazole; oral terbinafine is the preferred drug for many dermatophyte infections (see Table 266.3).

Prevention
- There are no major preventive programs, although early identification of infection limits spread in communities, such as schools.

The superficial fungal infections include some of the most common infectious conditions, such as ringworm, tinea corporis, and pityriasis versicolor, and rare disorders such as tinea nigra. Their prevalence varies in different parts of the world, but in many tropical countries they are the most common causes of skin disease. Dermatophyte infections and other superficial mycoses are described in this chapter. Superficial candidiasis is discussed in Chapter 256.

DERMATOPHYTOSIS

The dermatophytes are molds that can invade the stratum corneum of the skin or other keratinized tissues derived from epidermis, such as hair and nails. They may cause infections (dermatophytoses) at most skin sites, although the feet, groin, scalp, and nails are most commonly affected.[1] The dermatophytes are among the earliest microorganisms that were found to cause infections in humans. *Trichophyton schoenleinii,* the cause of the scalp infection favus, was isolated from a patient and the culture was shown to reproduce the typical lesions after inoculation onto human skin as early as 1841. Dermatophyte infections had been described many years before this, although the identity of the cause had not been recognized. The ancient Greek physicians knew about ringworm, and there are descriptions of the manifestations of dermatophytosis in more unlikely sources, such as the records of the early explorers of the 16th century who reported a strange disease of the skin, subsequently known as *tinea imbricata,* caused by *Trichophyton concentricum,* in the islanders of the western Pacific.

Dermatophytes

There are four main genera of dermatophyte fungi pathogenic in humans: *Trichophyton, Microsporum, Nannizzia,* and *Epidermophyton.* The last genus is represented by only a single species, *Epidermophyton floccosum.* These keratinophilic organisms probably arose as saprophytic soil fungi, and some dermatophytes, which have been isolated only from soil, have not been shown to cause disease in either animals or humans. Most of the dermatophyte species, however, are parasitic and can cause disease in either humans or animals, often being adapted to a single or narrow range of host species. The dermatophytes are referred to as *zoophilic, anthropophilic,* or *geophilic,* depending on whether their primary source is an animal, human, or soil, respectively. The most common geophilic species found in human infections is *Nannizzia* (previously *Microsporum*) *gypseum.* Other soil genera such as *Paraphyton* do not appear to cause human infections

The exact taxonomic status of dermatophytes remains a subject of debate, although the wider use of molecular tools to determine species has enabled a scientifically based classification and consensus to evolve.[2] The most recent version has the merit of restricting changes to established species names to a minimum.[2]

The relationships among different dermatophytes are not simply a subject for intellectual dispute. To understand the spread of infections, for instance, it is important to attempt to differentiate strains of the same species. Significant advances have been made both in the molecular taxonomy of these organisms and in the development of schemes for strain differentiation through the use of molecular tools.[2] Attempts have also been made to classify the dermatophytes according to their protein spectra, most recently using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) techniques [3] Another new technique for identification is surface-enhanced Raman spectroscopy (SERS) coupled with principal component analysis (PCA).[4]

Such techniques have also contributed to our understanding of key issues in pathogenesis, such as spread of infection in populations and relapse after apparently successful treatment. Proteinases produced by dermatophytes are inducible by, for instance, amino acids.[5] Dermatophytes secrete a number of enzymes with different protein affinities, including keratin, the largest of which is a 200-kDa glycosylated metalloprotease, and the genes encoding subtilisin proteinases in *Microsporum canis* (*Sub1, Sub2,* and so on) have been identified.[6] Other significant gene products are heat shock proteins, thiol carbonases, and transporters. These have been shown to be expressed in vivo.[7]

Epidemiology

The factors affecting the distribution and transmission of dermatophytosis are largely dependent on the source of the infection[8]: animal, soil, or human.

Zoophilic Dermatophyte Infections

The main zoophilic dermatophyte fungi are listed in Table 266.1. Each organism is primarily an animal pathogen that sometimes causes human infection. In each case there is usually a range of host specificities, from organisms such as *Microsporum nanum,* whose natural host is the pig and which does not infect other animals, to *Trichophyton mentagrophytes,* which affects a range of different rodent species or, rarely, cats, dogs, and horses.

The host preferences of *T. mentagrophytes,* coupled with small clinical and cultural differences, have led many mycologists to subdivide this group. Under this classification, *T. mentagrophytes quinckeanum (T. quinckeanum)* is used to describe the fungus that causes the clinical pattern of favus in mice, an infection associated with the formation of epithelial crusts. However, the organisms are difficult to distinguish genetically. In most temperate countries, *Trichophyton verrucosum,* the cause of cattle ringworm, and *M. canis,* a dermatophyte that causes infections in cats or dogs, are the most common zoophilic dermatophytes that cause human infections, such as tinea capitis.

Of all the zoophilic dermatophytes, *M. canis* is the most prevalent throughout the world, in both temperate regions and some tropical regions.[8] On occasion, the distribution of zoophilic dermatophytes may appear to be difficult to explain, but usually it reflects the distribution of the animal host. For instance, *Trichophyton erinacei* (part of the *Trichophyton benhamiae* series) is confined mainly to Europe and New Zealand. It is carried by hedgehogs, which were introduced into New Zealand in the 19th century from England. *T. benhamiae* (previously *Arthoderma benhamiae*) is increasingly recognized as a cause of scalp and skin infection acquired from guinea pigs, *Trichophyton simii* is associated with monkeys in India and the Far East, and infections in humans are observed only in these areas.[8]

Geophilic Dermatophyte Infections

Dermatophytes originating from soil, such as *N. gypseum,* are infrequent causes of human disease, although they may be seen more commonly in certain parts of the tropics such as the western Pacific and Central America. In other areas, they usually cause sporadic infections, although on occasion they are responsible for outbreaks of disease among humans in appropriately exposed occupational groups, such as gardeners or farmworkers.[8]

TABLE 266.1 Classification of the Main Dermatophytes (*Trichophyton, Microsporum, Nannizzia,* and *Epidermophyton*)

ORGANISM(S) AND THEIR SOURCES			
HUMAN ORIGIN	**SOIL ORIGIN**	**ANIMAL ORIGIN**	**MAIN ANIMAL SOURCE**
Trichophyton concentricum	*Nannizzia gypseum*	*Trichophyton benhamiae*	Guinea pigs
Trichophyton interdigitale		*Trichophyton erinacei*	Hedgehogs
Trichophyton rubrum		*Trichophyton equinum*	Horses
Trichophyton schoenleinii		*Trichophyton mentagrophytes*	Rodents
Trichophyton soudanense		*Trichophyton quinckeanum*	Mice
Trichophyton tonsurans		*Trichophyton simii*	Monkeys
Trichophyton violaceum		*Trichophyton verrucosum*	Cattle
Microsporum audouinii		*Microsporum canis*	Cats, dogs
Microsporum ferrugineum		*Nannizzia nana*	Pigs
Epidermophyton floccosum		*Nannizzia persicolor*	Bank voles

Anthropophilic Dermatophyte Infections

Dermatophytes that are natural pathogens of humans are the most common cause of human dermatophytosis. They include organisms that mainly cause infections of glabrous skin of the feet or hands and a range of pathogens whose invasion may involve penetration of the hair shaft. The most common of these organisms in most parts of the world is *Trichophyton rubrum,* which causes tinea pedis or tinea cruris in temperate climates and, particularly in the tropics, tinea corporis. Cases of infection that are caused by *T. rubrum* were once rare in the Western Hemisphere, but the infection has spread rapidly since the 1960s. In the feet, among other sites, this dermatophyte can cause noninflammatory chronic infections that are easily transmitted; this is probably an important factor that has determined its spread.[8] The large population movements during World War II are also thought to have contributed to the spread of the disease. Despite this, a variant with distinct morphologic appearances may be isolated from patients with tinea corporis,[9] particularly in the tropics, which suggests that although endemic disease caused by this species has been present for a considerable time, the key adaptation leading to spread was the appearance of strains capable of causing indolent and noninflammatory infections of peripheral skin sites.

The organisms that infect glabrous skin spread largely through contact with infected desquamated skin scales. Classically, this occurs in bathing areas or shower rooms where large numbers of individuals share common facilities—for instance, in military camps or factories.[10] Workers in heavy industries such as mines or nuclear fuels may have a high frequency of foot infection, mainly caused by *T. rubrum,* although *Trichophyton interdigitale* (part of the *T. mentagrophytes* complex) may also be isolated.[11] Changing rooms used by the police and armed forces, schools, and public swimming pools are also sites for infection. In contrast, transmission within the home as a reflection of conjugal or familial cases is not common, although it has been suggested that some patients show immunologic and genetic susceptibility.[12] *E. floccosum* may also cause foot infections, although it is particularly associated with tinea cruris either as a sporadic disease or in institutions, such as prisons and military barracks. These infections are not geographically restricted, even though there are variations in different countries. In many tropical areas, particularly the Far East, *T. mentagrophytes* is less commonly a cause of interdigital foot disease, and patients are infected by the zoophilic variety of this species on sites other than the feet.[13]

Tinea imbricata (a variant of tinea corporis), caused by the anthropophilic dermatophyte *T. concentricum,* has an unusual distribution confined to remote parts of the humid tropics.[14] The main endemic areas are the western Pacific islands, Malaysia, Northeast India, and parts of the Amazon Basin in Brazil. Infants may be affected shortly after birth, and spontaneous recovery is unusual. Large numbers of viable organisms can be cultured from the houses of infected families. Visitors to areas in which the condition is endemic are rarely infected.

The distribution of some of the other anthropophilic dermatophytes that cause tinea capitis in children, and other clinical forms of disease such as tinea corporis or onychomycosis, may be more restricted. The reasons for this are not entirely clear, except that because these infections are prevalent in children, who form a relatively stable population with little opportunity for travel, the spread of the disease within the continent may be limited to certain localities. Whatever the reason, these scalp infections are often found in defined endemic areas (Table 266.2). The situation is best illustrated by the distribution of *Trichophyton* spp. that cause tinea capitis in West Africa, where the endemic areas for *Trichophyton soudanense* and *Trichophyton yaoundei,* members of the *T. rubrum* complex, are distinct, although there is some overlap.[15] However, this pattern is changing as *Trichophyton tonsurans* is now found increasingly in the region. The predominant cause of scalp infection is *T. tonsurans* in the United Kingdom, United States, and Mexico and *Trichophyton violaceum* in India, East Africa, and the Middle East. The situation does not always remain stable, and the slow increase in numbers of *T. tonsurans* in the United States was followed by spread to the United Kingdom and some parts of Europe, Latin America, and Africa.[8] In addition,

TABLE 266.2 Distribution of *Trichophyton* and *Microsporum* Species That Cause Tinea Capitis

DERMATOPHYTE	DISTRIBUTION
Trichophyton tonsurans	North, Central, and South America, Europe, Africa
Trichophyton soudanense	West and Central Africa
Trichophyton schoenleinii	North Africa United States, Middle East, South Africa, South America (sporadic)
Trichophyton verrucosum	Europe
Trichophyton violaceum	Indian subcontinent, Middle East, North and East Africa
Microsporum audouinii	Central America, West Africa Europe (uncommon)
Microsporum canis	Worldwide but uncommon in India and Far East
Microsporum ferrugineum	Central Africa, Far East

other anthropophilic *Trichophyton* infections such as *T. violaceum* and *T. soudanense* are seen in immigrants in Europe and elsewhere. Endemic anthropophilic scalp infections that are caused by *Microsporum* spp. are less common. For instance, *Microsporum ferrugineum* is now seldom found. The most widely distributed of this genus is *Microsporum audouinii.* Once common throughout Europe, it almost disappeared but has been reintroduced by immigration from regions in which it remained endemic, such as West Africa, and it is still a major cause of tinea capitis in Africa.

Favus—the infection caused by *T. schoenleinii*—has characteristic clinical manifestations. It was once common in Europe but has now largely disappeared from many areas, although pockets of infection still exist in parts of sub-Saharan Africa. One of the features of this disease is the development of crusts, or scutula, on the scalp. Hairs are invaded, but shedding is delayed because they are not structurally damaged until late in the course of the infection. Although tinea capitis is normally a disease of children, occasionally women have favus.

Dermatophytes causing scalp disease may be carried on the skin surface without invading the skin or hair. A small proportion of carriers develop infections within 6 months; in others the fungus disappears. Members of an infected individual's family can also become carriers.[16] It is likely, however, that some carriers have limited but undetected infections.

Age Incidence

Tinea capitis is mainly a disease of childhood, and cases rarely occur after puberty. However, this infection may occur in adults and may also be associated with scarring alopecia. The reason for the preponderance of the disease in children is thought to be the presence of medium-chain fatty acids (C_8 to C_{12}) in sebum that inhibit the growth of dermatophytes in postpubertal individuals. In contrast, tinea pedis is usually seen in older children or young adults.[17] Foot infections occasionally occur in young children, but in this age group the nails may be invaded without concomitant skin infection.

Pathogenesis

Transfer of infecting organisms from soil, animals, or humans is accomplished by means of arthrospores, which are vegetative cells with thickened cell walls formed by dermatophyte hyphae in vitro and in vivo. These structures are probably shed by the primary host with skin scales or hair. It has been shown that dermatophyte arthrospores can survive for considerable periods outside the host, in some cases for more than 15 months. Direct contact between the infected individual and another individual is not necessary for the development of dermatophytosis in the latter. The process of transfer itself is little understood, but invasion of the skin appears to follow adherence of fungal cells to keratinocytes in vitro, a process that is maximal after about 2 or 3 hours. Keratinocytes from different sites do not appear to differ in their binding capacity for arthrospores. Subsequent germination leads to invasion.[18]

Susceptibility to infection is not universal. In humans it has been suggested that susceptibility to tinea imbricata is mediated through an autosomal recessive gene. Mutations in genes that convey increased susceptibility to unusual forms of dermatophytosis such as those associated with chronic mucocutaneous candidiasis *(STAT1)*[19] or deep dermatophytosis[20] *(CARD9)* are being identified and are associated with reduced interleukin (IL)-17 production.

However, at the point of contact between fungus and stratum corneum there are endogenous epidermal cell defense mechanisms, such as antimicrobial peptides, including human β-defensin, that are inhibitory to dermatophytes.[21] After contact with the skin, virulence genes that aid penetration, such as the subtilisins or proteinase genes, are expressed.[22] In experimentally infected mice and guinea pigs, the inflammatory response to dermatophytosis is maximal after 9 to 16 days, and after this stage there is resolution of the infection. The main efferent limb of immunologic resistance is the T lymphocyte. Studies of mice with *T. quinckeanum* infections have shown that resistance can be transferred to sublethally irradiated mice with T cells bearing the phenotype of helper-inducer T cells.[23] Suppressor lymphocyte activity can be detected in cells from the draining lymph nodes at the peak of infection. Immunity cannot be transferred with antibody to uninfected animals. Although it is difficult to extrapolate these data to infected humans, there is evidence that the kinetics of the immune response in humans is similar. For instance, the development of delayed-type hypersensitivity in children with naturally acquired scalp ringworm caused by *T. tonsurans* is correlated with recovery. Experimentally infected humans develop both delayed-type skin reactions to trichophytin and T-lymphocyte blastogenic responses at the time of recovery.[23] Patients with chronic *T. rubrum* or *T. concentricum* infections appear to have defective T-lymphocyte–mediated responses, and patients with persistent dermatophyte infections elicit a cytokine profile suggestive of a helper T-cell type 2 response.[24] These observations suggest that appropriate T-lymphocyte activation is crucial for recovery in dermatophytosis.

The afferent limb of the immune response is provided by epidermal Langerhans cells, which have been shown to act as antigen-presenting cells in mixed cultures with human lymphocytes. The mechanisms by which T lymphocytes affect recovery are less well understood. Phagocytes—mainly neutrophils and, to a lesser extent, macrophages—can kill dermatophytes both intracellularly and extracellularly, mainly through oxidative pathways.[19] Dermatophyte antigens have been shown to be chemotactic to human leukocytes and may activate the alternative pathway of complement activation. However, other mechanisms of fungal clearance must also be involved. It has been shown that increased epidermal turnover occurs during infection. Although this also occurs in heterologous skin grafted onto T-cell–deficient *(nunu)* mice, which suggests that an intrinsic response is involved, it is maximal at the time of development of the maximal immune responses.[25] It is possible that elimination of dermatophytes is also accomplished by means of increased shedding of the stratum corneum and that the immune system amplifies an endogenous epidermal response to infection.

Different dermatophyte species vary in their ability to elicit an immune response; some organisms, such as *T. rubrum,* cause chronic or relapsing infections, and others, including *T. verrucosum,* lead to long-term resistance to reinfection. Some dermatophytes produce glycopeptides, which are capable of reversibly inhibiting T-lymphocyte blastogenesis in vitro.[5] There is variation in the ability of different dermatophyte species to stimulate release of cytokines such as IL-12 from keratinocytes in vitro, which may provide an explanation of the differences in inflammatory responses in the skin.[26]

Clinical Manifestations

The archetypal lesion of dermatophytosis is an annular scaling patch with a raised margin that exhibits a variable degree of inflammation; the center is usually less inflamed than the edge. The word *tinea* is used to refer to dermatophyte infections, and it is usually followed by the Latin description of the appropriate site. Hence, tinea pedis is an infection of the feet and tinea capitis is an infection of the scalp. The term *tinea incognito* is used to describe infections that do not have any of the usual characteristic features of dermatophytosis, often because of inappropriate application of corticosteroid creams. Disease associated with immunosuppression,

including infection with the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS), affects the clinical expression of dermatophytosis; the result is often diminished scaling but prominent folliculitis and the formation of pustules.

The clinical appearances of the infection vary with the site, the fungal species involved, and the host's immune response. Zoophilic fungi often cause inflammatory lesions, and in some cases large pustular lesions (kerions) may develop. In contrast, lesions caused by anthropophilic dermatophytes often exhibit little inflammation and may become chronic (see "Pathogenesis").

Tinea Pedis

Tinea pedis is usually caused by infection with either *T. rubrum* or *T. interdigitale* (part of the *T. mentagrophytes* complex) or, less commonly, with *E. floccosum.* The infection usually starts in the lateral interdigital spaces of the foot or on the undersurface of the lateral aspects of the toes. The main symptom is itching, although the severity is variable. The skin usually cracks and may become severely macerated. In some cases, often when *T. mentagrophytes* is the causative organism, bullae are formed and itching is severe. The infection may also spread onto the dorsum of the feet, usually on the lateral side of the foot. Involvement of the sole is common in *T. rubrum* infections, and part of or the entire sole becomes erythematous and covered with dry scales. This is most noticeable along the lateral borders of the sole, where the appearance is often characterized as "moccasin" or "dry-type" infection. Blisters may also be formed in small clusters on the sole. The course of infection is variable. In noninflammatory forms, the interdigital scaling is often chronic or intermittent, whereas if blisters are formed, the infection usually resolves but may recur several months later. The main complications of tinea pedis are bacterial cellulitis and fungal invasion of the toenails (onychomycosis) or the skin of the dorsum of the foot and leg.

Tinea pedis usually occurs in young adults or teenagers. It is particularly common in institutions or places where common bathing facilities are used. The clinical manifestations of infection are altered in patients with T-lymphocyte abnormalities, in whom there is often extensive spread of the lesions onto the dorsal surface of the foot.

Scaling between the toes is often referred to as *athlete's foot,* but similar clinical signs may be produced by a variety of organisms. Erythrasma that is due to *Corynebacterium minutissimum* may manifest as scaling and, in particular, maceration of the toe webs. Gram-negative bacteria such as *Pseudomonas* and *Proteus* spp. may contribute to interdigital disease in patients with closely apposed web spaces or whose work involves immersion in water. These organisms may replace the original dermatophytes in this site, an infection known as *dermatophytosis complex.*[27] *Staphylococcus aureus* may cause secondary infections of the foot, but this characteristically starts on the dorsum of the foot over the first two digits. The mold fungi *Neoscytalidium dimidiatum* (formerly known as *Hendersonula toruloidea*) and *Neoscytalidium hyalinum* may cause interdigital scaling, nail disease, and sole involvement that is indistinguishable from dry-type infections caused by dermatophytes. Cracking between the toes is conducive to cellulitis in predisposed patients, such as those with chronic lymphedema.

Tinea Cruris

The most common dermatophytes associated with groin infections are *T. rubrum* and *E. floccosum.* This infection is also called *jock itch.* The infection starts with scaling and irritation in the groin. The rash usually involves the anterior aspect of the thighs, less commonly the scrotum. The leading edge extending onto the thighs is prominent and may contain follicular papules and pustules. The infection may also spread to the anal cleft. Although tinea cruris is mainly a disease of young men, it may affect women, particularly in the tropics, where the infection may spread in a band around the waist area.

As with tinea pedis, there may be clustering of cases of tinea cruris in institutionalized groups, such as those in military camps. The toe webs are also often infected in patients with tinea cruris.

Erythrasma of the groin may also cause a localized rash with itching. However, here the leading edge is less prominent than in tinea cruris and the rash is covered with fine wrinkles. Erythrasma fluoresces pink under Wood light. Candidiasis of the groin may also mimic tinea cruris, but an important clue to the presence of *Candida* is the appearance of small satellite pustules beyond the free margin of the rash. Flexural psoriasis causes a vivid red and uniformly scaling rash in the groin, and there is usually at least one other site with typical psoriatic plaques.

Tinea Corporis

Tinea corporis is one of the most commonly misdiagnosed skin diseases. Cases of this infection are not common in temperate climates, although it is seen more frequently in the tropics. This form of dermatophytosis has various clinical manifestations. Most lesions have a prominent edge that may contain pustules or follicular papules, and the center of the lesion is often less inflamed and scaly (Fig. 266.1). Sites commonly involved are the trunk and legs. Itching is variable, and lesions may be single or multiple. In general, infections caused by anthropophilic dermatophytes such as *T. rubrum* are less inflammatory and less clearly demarcated, and in some patients it is necessary to search for the margin carefully to delineate the rash. Lesions are usually hyperpigmented in pigmented skins. An outbreak of very extensive tinea corporis (and tinea cruris) infection, refractory to treatment, has been highlighted in many areas of India. An association with the use of high-strength topical steroid antifungal-antibacterial combinations has been identified. Zoophilic infections such as those caused by *M. canis* and *T. verrucosum* are more inflammatory, and lesions may become elevated and contain pustules. Infections caused by *N. gypseum* are also usually inflammatory and may have a brick-red appearance.

These clinical patterns vary with the site of infection. In patients with defective T-lymphocyte function, scaling is often minimal, and the rash of tinea corporis consists of grouped papules or pustules without significant erythema. *T. rubrum* infections on the lower parts of the legs may lead to the formation of single or multiple deep nodules that may mimic erythema nodosum.[28] The overlying skin is dry, red, and scaly, which is a useful clue to the correct diagnosis. This form of infection, *nodular folliculitis,* follows follicular penetration of the hair follicles of the lower portions of the legs by the fungus. It occurs mainly in women.

Tinea corporis can occur at any age.

A number of different conditions should be considered in the differential diagnosis of tinea corporis, including eczema, psoriasis, and annular erythema. The important points to look for are the annular scaling margin of lesions and follicular prominence, which are features of dermatophytosis. However, it may be necessary to take scrapings for laboratory culture when there is doubt.

Tinea Imbricata

Tinea imbricata is a variant of tinea corporis that is caused by *T. concentricum.* The geographic distribution of the disease is shown in Table 266.2. Patients may be infected at any age, although infants and young children are most frequently affected. The main characteristic of the rash is the formation of concentric rings of scales (Fig. 266.2) that amalgamate to form waves of scaling over large parts of the body.[14] Other clinical varieties of tinea imbricata include the diffuse scaling variety, in which large flakes of skin are prominent. The disease gets its



FIG. 266.1 Inflammatory tinea corporis caused by *Trichophyton erinacei* (part of the *Trichophyton mentagrophytes* complex).



FIG. 266.2 **Early lesions of tinea imbricata, showing the first signs of concentric rings.**



FIG. 266.3 **Tinea faciei caused by *Trichophyton rubrum.***

name *imbricata* (Latin, "tiled") from this clinical pattern. Other patients may have itchy lichenified lesions on the forearms. The face may be affected, as may the sides of the fingers, but the feet, scalp, axillae, and groin are usually spared. Tinea imbricata is seldom mistaken for other diseases, and the inhabitants of areas in which the condition is endemic easily recognize the appearance of the infection and have specific names for it. In Papua New Guinea it is called *sipoma* or *grille.*

Tinea Manuum

The term *tinea manuum* is used for dermatophyte infections involving the hand. In some patients, the dorsum of the hand may be affected, but most commonly the disease occurs on the palmar surface. A characteristic of dry-type infections at this site is involvement of only one palm, although in some patients both may be affected. The clinical manifestations are identical to those seen with dry-type infections of the sole. The usual cause is *T. rubrum,* and the feet are often involved, in addition to the hands.

Dermatophytosis affecting the palm may be confused with eczema, but the unilateral distribution of the infection and the common accompanying findings of onychomycosis and tinea pedis are helpful clues. Patients with palmoplantar keratoderma (tylosis) are particularly susceptible to superinfection of the palms and soles with dermatophytes.[29] This complication may be difficult to identify, but the skin may blister and the hand usually itches. In such patients, fungi other than *T. rubrum* may be implicated.

Tinea Faciei

Dermatophyte infections of the face are usually caused by the same organisms associated with tinea corporis. Infections with *T. rubrum* at this site are often particularly difficult to recognize. The facial skin becomes itchy and red, but the margin of the rash may be difficult to discern (Fig. 266.3). Some patients report that the facial rash is exacerbated by sun exposure. In other instances, lesions are more readily noticeable and affect the ears. Tinea faciei has been reported more frequently in patients with AIDS.

Tinea barbae (infection of the neck and beard area) may be pustular and inflamed because it is often caused by zoophilic organisms such as *T. verrucosum.* It is more localized than sycosis barbae, which is caused by *S. aureus*; this difference is helpful in distinguishing the two conditions.



FIG. 266.4 **Scalp ringworm in which an ectothrix infection of the hair is caused by *Microsporum canis.***

Tinea Capitis

Scalp ringworm, or tinea capitis, is a disease of childhood. Its prevalence varies considerably in different parts of the world. The disease is widespread in some urban areas in the United States, Africa, and Europe. Tinea capitis is also common in parts of India. In northern Europe the disease is sporadic. The main reasons for these differences in the prevalence of infection in different localities are the nature of the infecting organisms and the availability of control measures. Endemic infections affecting large numbers of children are associated with anthropophilic organisms; sporadic disease is associated with zoophilic fungi. Tinea capitis is usually classified by the pattern of hair shaft invasion. Dermatophyte infections in which arthrospores are formed on the outside of the hair shaft are known as *ectothrix* infections; those in which the spores develop within the hair itself are known as *endothrix* infections. In *T. schoenleinii* infections, the fungi invade the hair medulla but then regress and leave tunnels containing air within the hair shaft (the "favic" pattern). Although it is identified as a childhood disease, adults exposed to *T. tonsurans* infections and patients with AIDS may develop tinea capitis.

The main clinical feature of dermatophyte scalp infections is the appearance of scaling of the scalp skin that is associated with a variable degree of erythema, inflammation, and alopecia. In some cases, the infection closely resembles seborrheic dermatitis or dandruff of the scalp. The infection is often accompanied by itching. A pathognomonic feature is hair loss. In ectothrix infections, hairs often break a few millimeters or more above the skin surface (Fig. 266.4). Broken or infected hairs are also slightly swollen and have a dull appearance. In endothrix infections, parasitized hairs break at the skin level. In some endothrix infections, scattered stumps can be seen within areas of hair loss (black-dot ringworm). In such cases, inflammation may be minimal. A further element in tinea capitis is the variable amount of inflammation, but in some cases the whole area becomes pustular and covered with a thick scale or exudative crust. Often one of these elements dominates the clinical pattern. For instance, in some children there is little overt hair loss; the whole infection resembles seborrheic dermatitis. Likewise, in some ectothrix infections, a pustular form of dermatophytosis (kerion) develops. This is less common in endothrix infections. In most kerions, the pustules are not a sign of secondary bacterial infection,[30] although this may occur under adherent crusts.

Tinea capitis is uncommon in adults, although it has been reported with a variety of fungi such as *T. tonsurans* or *T. violaceum.* It has been associated with scarring alopecia of unknown etiology (pseudopelade) in adults.

In favus the same processes occur, but an important clinical characteristic is the formation of an inflammatory crust, or scutulum, composed of neutrophils and serous exudate around individual hair shafts. With time, these amalgamate over the surface of the scalp, so that the hair appears to be matted together with a thick crust that is said to have a musty odor. In many patients, the signs are indistinguishable from those seen with other forms of scalp ringworm. Two other characteristics of favus are late shedding of hairs and a tendency to develop scarring alopecia. The infection may persist into adulthood.

Untreated scalp ringworm usually remits spontaneously after puberty. Permanent hair loss is uncommon unless the response has been severely inflammatory or the patient has favus. A surprising degree of recovery of hair growth occurs, even in children with severe kerions.

Tinea capitis must be distinguished from seborrheic dermatitis, which usually occurs in older children and does not cause hair loss. Alopecia areata also causes circumscribed areas of hair loss but does not scale, and the "exclamation mark" hairs seen in this condition—broken hairs tapering from the fractured end toward the skin surface—are pathognomonic.

Onychomycosis Caused by Dermatophytes

Onychomycosis, or fungal infection of the nails, usually occurs in individuals with infections of adjacent toe or palmar skin, except in rare cases of childhood nail infection in which nail plate invasion may develop without skin involvement. There are several different patterns of nail plate invasion.[31]

The most common clinical pattern of onychomycosis is distal and subungual onychomycosis, in which the nail plate is invaded from the distal and lateral borders. There is usually associated thickening of the nail, which becomes white, yellow, or brown. Occasionally, *T. rubrum* results in fungal melanonychia or black pigmentation of the nail. In onychomycosis caused by endothrix scalp fungi such as *T. soudanense,* the thickening may be minimal and the nail surface is pitted with small fissures.[32] The most common cause of onychomycosis is *T. rubrum,* which often accompanies long-standing disease, and the infection involves the entire nail plate.

Superficial white onychomycosis occurs when the nail plate is invaded from the top surface, which is eventually covered with white crumbly plaques. Other fungi, such as *Fusarium* spp., more commonly cause this pattern of nail invasion, but this may be followed by deeper penetration. However, in its pure form, superficial white onychomycosis can occur with *T. interdigitale,* and it may accompany distal and subungual onychomycosis in some *T. rubrum* infections. It may also accompany proximal subungual onychomycosis (described later).

In rare cases, invasion appears to originate from the proximal nail plate as patchy or linear discoloration. This is usually a feature of relapse of treated nails, but rapidly spreading proximal nail plate invasion or full-thickness nail penetration has also been described in patients with AIDS.[33] Patterns of infection differ with striate or continuous areas of infection. They may be caused by dermatophytes and by nondermatophyte fungi such as *Fusarium* spp.

Onychomycosis can occur at any age, although it is more common with increasing age. Men and women are affected in equal numbers.

Onychomycosis caused by dermatophytes must be distinguished from that caused by *Candida,* in which there is little nail plate thickening but toenail infection is rare. *Neoscytalidium* infections may also lead to nail plate invasion. These are similar to infections caused by dermatophytes, but the nail plate is often not grossly thickened and may be severely undermined, and invasion affects predominantly the lateral border of the plate in the early stages of disease. Psoriasis of the nail also causes onycholysis, but the nail plate is typically covered with fine pits.

Deep Dermatophyte Infections

On rare occasion, patients known to be immunocompromised or otherwise healthy individuals develop dermatophyte infections in which the fungi invade deep dermal structures or produce a disseminated infection. In the first and more localized form, dermatophytes invade subcutaneous tissues after hair shaft penetration, producing a deep nodule described previously as nodular folliculitis or a granuloma (Majocchi granuloma), or it may spread via the lymphatic vessels, causing clusters of granulomas, lymphedema (Fig. 266.5), and draining sinuses. Sometimes aggregates of fungal hyphae resembling those found in eumycetomas may be observed in histologic sections. These dermatophyte "pseudomycetoma" grains may be surrounded by neutrophil abscesses, but the fungal hyphae are often engulfed by giant cells in tissue sections. Deep dermatophyte infections may extend farther and result in widespread cutaneous lesions; these may progress to involve draining lymph nodes or disseminate to other sites, including the liver and brain, and they may be fatal. Individual patients and members of families with some forms of deep dermatophytosis have been found to have mutations within the *CARD9* gene.[20] Patients homozygous for mutations in this gene are also more predisposed to deeply invasive candidiasis, phaeohyphomycosis, and aspergillosis. Case reports of deep infection caused by dermatophytes are rare, but, in addition to the *CARD9* cases described previously, isolated examples are regularly described, often in immunodeficient patients.[34]

Dermatophyte "Id" Reactions

The immune mechanisms in dermatophytosis may lead to the appearance of secondary rashes called *id reactions.* The most common of these is acute vesicular eczema or pompholyx that occurs on the hands and feet in patients with inflammatory ringworm of the feet, mainly caused by *T. mentagrophytes.* These events are thought to be causally linked if the original dermatophyte infection becomes inflamed before the appearance of the secondary rash, if the latter is maximal on the affected foot. A second form of id reaction, seen in patients with inflammatory tinea capitis or tinea corporis and usually caused by zoophilic organisms, consists of small follicular papules, some of which appear necrotic. This is a form of cutaneous vasculitis that usually subsides spontaneously. Both reactions may be triggered by antifungal therapy. Less common types of id reactions include annular erythema and erythema nodosum.

Point of Care and Laboratory Diagnosis

In some cases it is possible to screen patients with scalp infections by using a filtered ultraviolet light source (Wood light). Infections caused by some *Microsporum* spp. fluoresce green. However, *Trichophyton* infections do not fluoresce, apart from favus, in which the hairs appear yellowish. Because fluorescent hairs are infected, Wood light examination may be helpful as a method of selecting hairs for microscopy and culture. The dermatoscope, a skin surface handheld microscope, is very useful because it may show specific patterns indicating fungal invasion in patients with tinea capitis or nail infections.[35]

The laboratory diagnosis of dermatophytosis depends on the examination and culture of scrapings or clippings from lesions.[1] It is important



FIG. 266.5 Deep dermatophytosis in which *Trichophyton rubrum* infection is causing unilateral lymphedema after invasion of the lymphatic vessels.

to sample the edge of skin lesions and infected nails. In the case of infected hairs, it is best to select broken stubs, which can be removed with forceps without undue trauma. Material should be allowed to soften in 10% potassium hydroxide before being examined under the microscope. Nails often take up to 2 hours to soften, although the process can be hastened by gentle warming. Fungal hyphae can be seen as chains of arthrospores in cleared scales or clippings. The fluorescent whitener calcofluor may also be used to stain fungi, but preparations have to be viewed with fluorescence microscopy; however, it may enhance the yield of positive samples.

Dermatophytes infecting hair have characteristic direct microscopic appearances that are helpful in recognition. Some form arthrospores on the outside of the hair shaft (ectothrix infections). The small spores can be seen by focusing the microscope on the edge of the epilated hair shaft. With most of the pathogenic *Microsporum* spp. that cause tinea capitis, small arthrospores are clustered around the outside of hair. By contrast, few *Trichophyton* spp. form ectothrix spores, but those that do, such as *T. verrucosum,* produce large arthrospores. The majority of *Trichophyton* spp. causing scalp ringworm form arthrospores within the hair shaft (endothrix infection). With some practice, it is possible to make a preliminary identification of the likely genus of invading fungus from the findings of the microscopic study of infected hair. *T. schoenleinii* invades hair, but hyphae regress and leave airspaces within the hair shaft.

Skin scrapings or nail clippings may also be cultured. Primary isolation is carried out at room temperature, usually on Sabouraud agar containing antibiotics (penicillin-streptomycin or chloramphenicol) and cycloheximide (Acti-Dione), an antifungal agent that suppresses the growth of environmental contaminant fungi. In the case of nail disease, it is important to use media without cycloheximide because certain fungi, such as *Neoscytalidium,* that may infect nails are sensitive to the latter. Most dermatophytes can be identified within 2 weeks, although *T. verrucosum* grows best at 37°C and may have formed into only small and granular colonies at this stage. Identification depends on the gross colonial and microscopic morphologic features. In some cases, other tests involving nutritional requirements and hair penetration in vitro are necessary to confirm the identification. Molecular diagnosis of dermatophytosis using defined polymerase chain reaction (PCR) assay probes are also used in dermatophytosis,[36] although at present there are few commercially available molecular test kits. Direct microscopy is still a necessary part of the diagnosis. Likewise MALDI-TOF is an alternative method of diagnosis.

Therapy

Ringworm of the Glabrous Skin (Tinea Pedis, Corporis, Manuum)

Topical Therapy

The usual approach to the management of these dermatophyte infections is topical therapy if possible, although most nail and all hair infections and widespread dermatophytosis are best treated with oral drugs (Table 266.3).[37] The main topical agents used for dermatophytosis are compounds with specific antifungal activity. The keratolytic agents such as Whitfield ointment (salicylic and benzoic acid compound) are now rarely used. Currently, a large group of specific antifungal agents are used in treatment of dermatophytosis, although some of these are largely confined to the treatment of tinea pedis. The most frequently used medications are the topical azole antifungal agents, which include miconazole, clotrimazole, econazole, tioconazole, ketoconazole, luliconazole, efinaconazole, bifonazole, and isoconazole.[37] These are active against all the common skin fungi, and many can be used once daily. Other potent topical antifungal agents used in the treatment of dermatophytosis are ciclopirox olamine, tavaborole, terbinafine, and naftifine. It is difficult to choose among the different groups of these agents on the basis of well-constructed comparative studies. A Cochrane Review of topical treatments for tinea cruris and corporis (126 studies with 18,086 participants) found many biases and poor-quality evidence but concluded that data suggested that topical terbinafine and naftifine were effective.[38] In addition, older preparations, such as chlorphenesin, undecylenate, and tolnaftate, are available in cream or, in some cases, powder form and are effective in uncomplicated cases.

TABLE 266.3 Treatment of Dermatophytosis

DERMATOPHYTOSIS, CLINICAL DISEASE PATTERN	TREATMENT
Tinea pedis	
Interdigital	*Topical cream/ointment:* terbinafine, imidazoles (e.g., miconazole, econazole, clotrimazole), undecenoic acid, tolnaftate
"Dry type"	*Oral:* terbinafine, 250 mg/day for 2–4 wk; itraconazole, 400 mg/day for 1 wk/mo (repeated if necessary); fluconazole 200 mg weekly for 4–8 wk
Tinea corporis	
Small, well-defined lesions	*Topical cream/ointment:* terbinafine, imidazoles (e.g., miconazole, econazole, clotrimazole)
Larger lesions	*Oral:* terbinafine, 250 mg/day for 2 wk; itraconazole, 200 mg/day for 1 wk; fluconazole, 250 mg weekly for 2–4 wk
Tinea capitis	Griseofulvin, 10–20 mg/kg/day for minimum 6 wk Terbinafine <20 kg: 62.5 mg/day 20–40 kg: 125 mg/day >40 kg: 250 mg/day Itraconazole, 4–6 mg/kg pulsed dose weekly Fluconazole, 3–8 mg/kg pulsed dose weekly
Onychomycosis	
Fingernails	Terbinafine, 250 mg daily for 6 wk Itraconazole, 400 mg/day for 1 wk each month, repeated for 2–3 mo Fluconazole, 200 mg weekly for 8–16 wk
Toenails	Terbinafine, 250 mg daily for 12 wk Itraconazole, 400 mg/day for 1 wk each month, repeated for 2–4 mo Fluconazole, 200 mg weekly for 12–24 wk

In general, topical therapy for tinea pedis has to be continued for at least 2 and possibly 4 weeks. Topical terbinafine can be used to clear lesions of tinea pedis in 7 days and is also available as a single-dose film-forming solution applied to the soles of the feet. Tinea cruris usually responds within 2 or 3 weeks of the outset of treatment. Some of the azole agents can be used only once daily.

Systemic Therapy

The main oral antifungal agents used for dermatophytosis are terbinafine, itraconazole, and fluconazole. Griseofulvin is an older alternative treatment but is still used in tinea capitis caused by *Microsporum* species.[39] Adverse effects include headache, nausea, and abdominal discomfort. Less common reactions are urticaria, diarrhea, and photosensitivity. Newer triazoles, such as posaconazole and voriconazole, are expensive and not widely used. Terbinafine is given in dosages of 250 mg daily for 2 weeks for tinea cruris or corporis. It produces rapid and long-lasting remissions for dry-type dermatophytosis and other skin infections. Itraconazole can be given continuously in doses of 200 mg daily and is curative for tinea cruris or corporis after 1 week. Fluconazole can also be used as a treatment for dermatophytosis, but current regimens entail 150 to 300 mg weekly for infections of the skin. All three drugs are well tolerated and have evidence of efficacy in ringworm of the glabrous skin.[40,41] Drug interactions in patients with comorbidities can limit use of itraconazole or terbinafine. With terbinafine, interactions with antidepressants and β-blockers may contraindicate use. With itraconazole, drug interactions are even more numerous. Risk of hepatic injury is very low (<1 per 70,000) with all three antifungals.

Tina Capitis

Oral therapy is used for scalp ringworm. Itraconazole and terbinafine are effective in treating scalp disease, and pediatric formulations are available in some countries.[42] Terbinafine is effective against *Trichophyton* scalp infections and usually requires only 2 to 4 weeks of therapy. Terbinafine is less active against tinea capitis caused by *Microsporum* species, for which griseofulvin is preferred and used for 8 to 12 weeks. Griseofulvin is given in doses of 10 to 20 mg/kg daily for the microsize

formulation in either tablet or syrup form. It is often useful to use a topical azole cream or shampoo in addition to systemic therapy for tinea capitis and, if crusts are present, to remove these with saline soaks.

It is important to attempt to identify the organism causing scalp infection because if the infection is of human origin, it can spread to other contacts, and it may be necessary to screen classmates or members of the families of children with anthropophilic infections. Zoophilic infections do not usually spread from child to child, although several family members exposed to the same source of infection may develop scalp disease.

Onychomycosis

Topical treatment of onychomycosis or nail mycosis is generally ineffective. Nevertheless, several topical preparations are of potential value in the management of nail disease. One, a topically applied nail solution containing 28% tioconazole, has been found to produce some mycologic and clinical remissions. Another preparation is a combination of 40% urea and bifonazole. Urea is a potent hydrating agent and softens nails after application under occlusion. The 40% urea paste may be used to remove residual areas of infection after oral therapy for onychomycosis.[43] This combination may also prove useful in addition to oral therapy for nail disease. Solutions with penetration enhancers, such as ciclopirox olamine and amorolfine, are widely used as nail lacquers. They are effective in a proportion of early cases of dermatophyte and *Candida* nail infection. They are applied once or twice weekly and are increasingly being used in combination therapy with oral drugs in severe infections.[44]

Three new topical antifungal agents have been used for the treatment of onychomycosis. Luliconazole 5%, efinaconazole 10% solution, and tavaborole 5% solution are applied to the nail daily for 48 weeks. They are effective in some patients but have not greatly improved on the cure rates seen with other topical nail agents,.[45]

Several pulsed laser treatment regimens using intermittent pulses have been applied to the treatment of onychomycosis. Treatment is expensive, and efficacy when used alone appears to be modest, usually leading to only temporary improvement.[39,46] Other new treatment approaches include the use of photodynamic therapy, iontophoresis of terbinafine, and application of nail antifungal solutions after drilling multiple holes on the nail.[47]

When the entire nail matrix is involved, atraumatic removal of infected nails using 20% to 40% urea, applied under occlusion, can significantly improve results of topical or systemic therapy.[39]

Systemic treatment of onychomycosis is preferred. Terbinafine and itraconazole have replaced griseofulvin for this indication. Treatment is given for 6 weeks for fingernail infections and 12 weeks for toenail infections. In 70% to 80% of patients, terbinafine 250 mg once daily produces cure in 6 weeks for fingernails and 12 weeks for toenails.[48] Itraconazole is also effective against toenail infections and can be given at a dosage of 200 mg daily for 3 months. However, for nail infections, it is usually administered as a "pulsed" treatment given for 1 week of each month at a dosage of 400 mg daily, the week's course being repeated once more for fingernail infections (two pulses) and two or three times for toenail disease (three or four pulses).[49] Reported remission rates are above 60%. Intermittent regimens with fluconazole (300 and 450 mg weekly) are also used in the treatment of onychomycosis.[50] There have been no large comparative studies of fluconazole versus the other two treatments for nail disease. However, in one large double-blind study of patients with toenail onychomycosis, terbinafine, given continuously at 250 mg daily for 12 or 16 weeks, was compared with pulsed itraconazole, at 200 mg twice a day for 1 week each month repeated three or four times. The results revealed significantly better responses for both terbinafine groups than for itraconazole in both mycologic and clinical remission rates.[51] To date, in vitro resistance to drugs used in dermatophytosis has not proved to be a significant problem.

NEOSCYTALIDIUM INFECTIONS

Infections caused by the pigmented fungus *N. dimidiatum* closely resemble dry-type dermatophytosis caused by *T. rubrum*. *N. dimidiatum* was originally described as a pathogen in plants, but it appears to be a genuine cause of human infection. A similar infection has been ascribed to the related nonpigmented mold *N. hyalinum*. In both cases, the affected patients come from the tropics.[52]

The precise mechanisms of infection with either organism are unknown. *N. hyalinum* has never been isolated from the environment, and although *N. dimidiatum* is a pathogen of certain plants such as fruit trees, patients do not usually give a specific history of exposure. It has been found that healthy individuals in some tropical areas carry these organisms on the feet but do not have overt disease, which suggests that asymptomatic carriage may, under the appropriate conditions, be followed by infection. Infections have been described in immigrants from tropical areas to the United Kingdom, Canada, and France. They have also been identified in the southern United States, Trinidad, West Africa, Colombia, Ecuador, and India, and it is likely that it is more widespread. On occasion, it may be seen in patients who have paid short visits to the tropics.

Clinical Manifestations

The clinical signs of skin infection with both *Neoscytalidium* species are identical to those associated with dry-type *T. rubrum* infections.[52] There is scaling of the lateral interdigital spaces, over the soles, and on one or both palms. Itching is usually minimal. Onychomycosis may also develop. Often there is early invasion of the lateral border of the nail without significant thickening of the nail plate (Fig. 266.6), but eventually the whole nail plate may be undermined and onycholysis may lead to shedding of the complete nail. Hyperpigmented streaks may occur in the nails, although these are not pathognomonic for these infections and can be seen with other forms of inflammatory nail dystrophy. Deep infections such as sporotrichoid *Neoscytalidium* infections and a brain abscess have rarely been reported, mainly in immunocompromised patients.[53]

Diagnosis

Scrapings or nail clippings examined after treatment with potassium hydroxide contain sinuous fungal hyphae. Close inspection reveals that the structure is different from that normally seen with dermatophyte hyphae, but accurate discrimination requires experience. Both organisms grow on Sabouraud agar but are inhibited if cycloheximide (Acti-Dione) is incorporated in the medium.

Therapy

There is no satisfactory therapy for either infection. Whitfield ointment (6% benzoic acid and 3% salicylic acid) may be used to treat *Neoscytalidium* infections of the sole or the palm. However, none of the specific antifungal drugs currently available produces consistent results.

OTHER FORMS OF ONYCHOMYCOSIS

A number of other fungi may cause onychomycosis. The most common of these is *Scopulariopsis brevicaulis,* which usually causes infection of the great toenails. Some patients with this form of infection have previously abnormal toenails (e.g., onychogryphosis). *Scopulariopsis* infections of the nails have a typical cinnamon color that is caused by the presence



FIG. 266.6 Onychomycosis caused by *Neoscytalidium dimidiatum*.

of fungal spores seen on direct microscopic views of the nail. The fungus is easy to isolate in culture. Treatment may be difficult, but chemical nail removal with 40% urea may be useful.

Superficial white onychomycosis may be caused by *Fusarium* species. These infections are similar to those caused by *T. mentagrophytes,* and the identity of the causative organisms should be confirmed with culture.

On occasion, other fungi are isolated from nail material. In many cases they appear to be colonizing the undersurface of dystrophic nail plate. On rare occasions, however, they may contribute to the nail disease by invasion. This is best established through repeated attempts at culture; if the organism is isolated on numerous occasions and if hyphae are present in the nail, it is likely that the organism is involved in the nail disease. Examples of infections caused by a range of different organisms, such as *Aspergillus* and *Fusarium* spp., have been recorded. *Fusarium* is now known to cause a range of fungal nail infections from superficial white onychomycosis to paronychia and proximal subungual disease. In severely neutropenic patients, nail infection may be followed by systemic dissemination of this fungus. It may also cause toe web infection. Oral antifungal therapy is unpredictable against these infections,[53] and nail removal with 40% urea is often used as an adjunctive treatment.

PITYRIASIS VERSICOLOR

Pityriasis versicolor, or tinea versicolor, is a superficial infection caused by *Malassezia* species, which are lipophilic yeasts that are normal commensals on the skin surface.[54] The infection is confined to the trunk or proximal aspects of the limbs. It does not cause hair and nail plate invasions.

Organisms

The normal skin is colonized in late childhood and adult life by lipophilic yeasts. Morphologically, these are either oval (most common on the scalp) or round (mainly on the trunk), and they were previously called *Pityrosporum ovale* and *Pityrosporum orbiculare,* respectively. These organisms have now been reclassified as members of the genus *Malassezia,* among which there are seven common pathogenic species: *Malassezia furfur, Malassezia pachydermatis* (not associated with human skin infections), *Malassezia sympodialis, Malassezia globosa, Malassezia restricta, Malassezia obtusa,* and *Malassezia slooffiae.*[55] Round yeasts, usually *M. globosa,* are seen in lesions of pityriasis versicolor accompanied by short, stubby hyphae; *M. furfur* may also produce filaments but is less common in adults. *Malassezia* species dominate the skin fungal microbiome in core scalp, body, and arm sites of adults.[5]

Pathogenesis

The infection is associated with transformation of yeast-phase organisms into hyphal forms, although occasional patients with pityriasis versicolor have only oval yeasts. The stimulus for this phase change is unknown. Infections are more common in the tropics and may appear after sun exposure, which may therefore be a trigger factor. Patients with Cushing syndrome may also develop this infection, but diseases related to T-lymphocyte suppression, such as HIV infection and AIDS, are not necessarily associated with pityriasis versicolor.[56] *Malassezia* yeasts grow in the presence of medium-chain fatty acids.

Different species of *Malassezia* obviously play a role in the development of disease, although it is not known why, for instance, *M. globosa* in particular should be associated with pityriasis versicolor.

Clinical Manifestations

Pityriasis versicolor is usually seen on the trunk or proximal portions of the limbs, although more extensive infections involving the face and waist area occur in the tropics. Lesions may be hypopigmented or hyperpigmented macules that amalgamate to cover the affected area with scaling plaques. The lesions are usually not itchy. In some patients, lesions may remit spontaneously. Rare clinical variants include some infections that result in anetoderma (localized skin atrophy).

The diagnosis can be confirmed through direct microscopic study of lesions, on which the characteristic round yeast forms and short hyphae can be seen. The scrapings can be viewed after clearing with potassium hydroxide. Lesions fluoresce yellow-green under Wood light, although this may not be seen on all affected areas. *Malassezia* yeasts are difficult to culture unless oil or Tween 80 is added to the medium.

Therapy

The most appropriate therapy for pityriasis versicolor is a topical azole or terbinafine cream[55]; less expensive alternatives, now less often used, are 2% selenium sulfide lotion or 20% sodium thiosulfate applied daily for 10 to 14 days. These alternative preparations may be irritative. In some cases, intermittent applications of 50% propylene glycol in water prevent a relapse.[57] In severe cases, oral itraconazole produces remissions. Among oral treatments, itraconazole 200 mg daily for 5 or 7 days and fluconazole 300 mg weekly for 2 weeks are effective.

Patients usually have to be warned that the pigmentary changes may return to normal only after many months, even when the infection has been successfully treated.

OTHER *MALASSEZIA* INFECTIONS

Two other skin conditions are associated with *Malassezia* yeasts: *Malassezia* folliculitis and seborrheic dermatitis. In addition, this fungus has caused catheter-acquired sepsis (see Chapter 268).

Malassezia Folliculitis

There are three main forms of *Malassezia* folliculitis. The first is a folliculitis on the back or upper part of the chest that consists of scattered follicular papules or pustules. These are itchy and often appear after sun exposure. In the second form, which is seen in patients with seborrheic dermatitis, there are numerous small follicular papules over the upper and lower portions of the back and chest. Erythema and greasy perifollicular scales are often seen in these patients. In the third form, multiple pustules are seen on the trunk and face in patients with HIV infection. This type is similar to the second form, and affected patients usually have severe seborrheic dermatitis.

Scrapings or biopsy specimens from lesions show numerous yeasts occluding the mouths of follicles. Treatment with topical azole antifungal agents may be effective, but oral therapy with itraconazole is often necessary.

Seborrheic Dermatitis

In the early part of the 20th century, seborrheic dermatitis and dandruff of the scalp were thought to be caused by *Malassezia* yeasts because numerous organisms were present in skin scales. This view was subsequently superseded by the belief that the yeasts were secondary to a hyperproliferative state. However, evidence suggests that *Malassezia,* mainly *M. globosa* and *M. restricta,* is implicated in the pathogenesis of the condition.[58]

In most cases, seborrheic dermatitis responds to oral or topical azole antifungal agents. Improvement is associated with disappearance of the organisms, and relapse is associated with recolonization. Furthermore, the clinical appearances can be mimicked in animals with the application of both live and killed organisms to the skin. Some patients with seborrheic dermatitis have high titers of antibody to *Malassezia* species. Two newly described potential pathogenetic mechanisms are the production of irritants, such as oleic acid, or of *Malassezia* metabolites that are immunomodulatory, such as indolocarbazole and malassezin, on affected skin of seborrheic dermatitis patients.[59] Lipase production by *Malassezia* isolated from affected skin can be enhanced by exposure to β-endorphin.[60]

Seborrheic dermatitis can appear in any individual, although it is said to be particularly common in patients with neurologic disease, such as parkinsonism. In patients with AIDS, the onset of seborrheic dermatitis may be sudden and the rash more extensive than in other individuals.[61] The histology of seborrheic dermatitis shows acanthosis and hyperkeratosis with elongation of dermal papillae. An infiltrate of polymorphs in the epidermis above the dermal papillae is also often seen. HIV-positive patients tend to have more plasma cells in the infiltrate. These changes are similar to those seen in some forms of psoriasis.

Clinical Manifestations

The classic features of seborrheic dermatitis make up a range of different clinical appearances. These include erythema and scaling of the central part of the anterior aspect of the chest and the upper part of the back

that are accompanied by a variable degree of itching. On the face, there is erythema with greasy scales in the eyebrows, around the alae nasi, behind the ears, and in the external ears. Scaling may also appear in the presternal areas of the chest and on the back. Scaling on the scalp is accompanied by the appearance of pustules in some patients. The clinical appearances are typical, and fungal scrapings are unnecessary.

Other forms of skin disease, including severe erythroderma in infants and an intertriginous rash in adults, have also been called "seborrheic dermatitis," but these lesions do not appear to be related to the variety discussed here.

Therapy

The main therapy involves the use of topical azole creams or oral azoles such as itraconazole and weak topical corticosteroids such as 1% hydrocortisone.[54] Relapse is common, but repeated treatment when necessary is the simplest approach to management.

Malassezia is also associated with a form of atopic dermatitis affecting the face in young adults. It is believed that immunoglobulin E (IgE)–mediated sensitization by allergenic proteins produced by *Malassezia* (e.g., Mala s1 to Mala s9) may play a role in exacerbating the inflammatory responses in eczematous skin.[62]

TINEA NIGRA

Tinea nigra is a superficial form of phaeohyphomycosis caused by *Phaeoannellomyces werneckii* (also called *Hortaea werneckii* and *Exophiala werneckii*). The infection is confined to the stratum corneum of the palms or soles and occurs mainly in the tropics or subtropics in children or young adults.

The typical lesion of tinea nigra is a superficial, scaling, brown or black macule on the palms or soles. The pigmentation is irregularly distributed over larger lesions. Spread of the infection to other sites is rare, and lesions remain asymptomatic.

The main differential diagnosis is a superficial form of melanoma or a pigmented nevus. The pigmented hyphae can be seen by direct microscopic study of skin scrapings treated with potassium hydroxide. The organism can also be cultured from scrapings, but a dermatoscope can show the very typical pigmented hyphae on the skin.

The best therapy is treatment with a topical azole or a keratolytic agent such as Whitfield ointment or 5% to 10% salicylic acid ointment.

WHITE PIEDRA

White piedra is an uncommon infection caused by yeasts of the genus *Trichosporon,* namely, *Trichosporon ovoides* (scalp hair), *Trichosporon inkin* (pubic hair), and *Trichosporon asahii* (rare in piedra). The infection occurs in both the tropics and temperate zones. It is a superficial infection of the hair shafts of the scalp, body, or pubic hair. *Trichosporon* species may also cause a systemic infection in neutropenic patients (see Chapter 268).

The organisms may be carried on the skin or around the anus. In some patients, the infection appears to be sexually transmitted. White piedra is asymptomatic and manifests as small yellow concretions on the hair shafts.[63] The pathogenesis of this infection is still unclear, but it is likely to result from synergy between the fungi and coryneform bacteria.[64] The lesions are circumscribed and appear as small nodules, unlike the more diffuse coating of axillary or pubic hair[65] seen in trichomycosis axillaris, which is caused by the presence of bacteria on the hair.

The diagnosis may be confirmed by examining an epilated hair mounted in potassium hydroxide. Each nodule contains fungal hyphae, and the organisms can be cultured from infected hairs without difficulty.

Treatment is difficult. Nodules may be removed simply by shaving. Otherwise, coating the hairs with an azole such as econazole or treating the patient with oral itraconazole may cure the infection. Relapse is common after therapy.

BLACK PIEDRA

Black piedra is another infection of the hair shafts that is caused by a black yeast, *Piedraia hortae*.[66] The disease is rare and confined mainly to parts of the humid tropics. The infection manifests as small black nodules on the hairs of the scalp and less commonly elsewhere. These have to be distinguished from pediculosis, but itching is usually absent in black piedra. With direct microscopy, these nodules can be shown to be composed of hyphal elements and small ascospores of the causative agent within a dark cement-containing stroma. Treating hairs with a topical salicylic acid or an azole cream is usually sufficient, although relapse is common.

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267 Paracoccidioidomycosis

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SHORT VIEW SUMMARY

Definition
- Paracoccidioidomycosis is a Latin American endemic and systemic fungal disease characterized by two main clinical forms as follows: (1) acute/subacute observed in children, adolescents, and immunocompromised individuals and (2) chronic as seen in adults 30 years of age or older.
- The acute/subacute form is a serious disease that results in lymph node hypertrophy and hepatosplenomegaly accompanied by fever, weight loss, and generalized malaise. Multiple skin and mucosal lesions occur in half of the cases. The primary pulmonary lesions are not apparent clinically but can be demonstrated radiologically in all cases.
- The chronic form results in pulmonary infiltrates in greater than 90% of the cases, as well as skin and mucosal lesions. Adrenal gland abnormalities are common and may lead to insufficiency.

Epidemiology
- The habitat(s) of *Paracoccidioides* spp. remain unknown, but they are restricted to Latin American countries.
- The largest endemic areas are found in Brazil.
- Most patients are males who work (or have worked) in agriculture-related occupations within the endemic areas.

Microbiology
- Paracoccidioidomycosis is caused by species in the genus *Paracoccidioides,* encompassing five distinct phylogenetic species, namely, *P. brasiliensis, P. americana, P. restrepiensis,* and *P. venezueliensis* (formerly designated as S1, PS2, PS3, and PS4, respectively). A new species, significantly different from the above on genetic and molecular grounds has been found and designated *P. lutzii.*
- *Paracoccidioides* spp. are thermally dimorphic fungi, identified by mycelial growth at 22°C to 24°C and by multiple-budding yeast cells (pilot's wheel) at 36°C to 37°C.

Diagnosis
- Both direct microscopy and histopathology allow prompt diagnosis.
- Detection of antibodies and fungal antigens prove useful not only for diagnosis but also for follow-up therapy results.
- Specific diagnosis is usually late due to confusion with other more prevalent diseases (e.g., tuberculosis). Lack of physicians' awareness is a problem.
- Lung imaging studies are essential in adult patients. Healing by fibrosis creates serious complications.

Therapy (see Table 267.1)
- Itraconazole is the treatment of choice for most patients, given at 200 to 400 mg/day for 6 to 9 months, depending on the severity of the disease.
- Amphotericin B deoxycholate is reserved for the most serious cases, at 1 mg/kg/day until achieving a 2-g total dose. This should be followed by triazole oral medications.
- Alternative treatment can be given with trimethoprim-sulfamethoxazole (TMP-SMX), given as TMP, 160 to 240 mg, and SMX, 800 to 1200 mg per day for 12 to 24 months, depending on disease severity. Treatment is long lasting (close to 2 years) and can result in relapses.

Prevention
- Taken into consideration that the precise fungus habitat remains undefined, preventing contact with *P. brasiliensis* does not appear feasible.

DESCRIPTION OF THE PATHOGEN

Until 2006[1] the genus *Paracoccidioides* was thought to consist of a single species, *P. brasiliensis,* considered the sole etiologic agent of paracoccidioidomycosis.[2,3] At present however, molecular and genetic studies have revealed that this genus includes a complex of five distinct phylogenetic species (S1a, S1b, PS2, PS3, PS4)[4] that appear to be confined to distinct regions within the endemic zones.[5–7] Recently, the *P. brasiliensis* spp. complex has been formally renamed, with the proposed names *P. brasiliensis* for S1, *P. americana* for PS2, *P. restrepiensis* for PS3, and *P. venezueliensis* for PS4.[8] Additional studies suggest that yeast morphology slightly, but significantly, differs across all five *Paracoccidioides* spp., an observation that may indicate that these phylogenetic species could well be designated as formal taxonomic species.[8] In addition, genomic comparison has indicated that *P. brasiliensis* is related to the uncultivable pathogen *Lacazia loboi*.[9,10]

Of note, a highly divergent monophyletic species, *Paracoccidioides lutzii,* has been described.[11–13] Distinguishing *P. lutzii* is based on a distinctive genome and differences in the conidia, as well as on morphology and size of the daughter yeast cells.[14] So far, *P. lutzii* has only been identified in central, southwestern, and northwestern Brazil.[13,15] Species within the complex *P. brasiliensis* also appear confined to particular endemic regions.[16]

Species within the *Paracoccidioides* genus exhibit the phenomenon called dimorphism, implicating that they can give rise to colonies corresponding to a mold or to a yeast, with temperature being the only recognized factor known to date that is capable of triggering this change. The latter phenomenon takes place when the β-glucan predominating in the mold is replaced almost entirely by the yeast-phase cell wall polysaccharide α-1,3-glucan.[7,17]

As for the *Paracoccidioides* genus classification, the relevant species are assigned to the family Ajellomycetaceae, order Onygenales.[18,19] Genomic analyses support the existence of a sexual cycle in species of the *Paracoccidioides* genus, by detection of heterothallic groups (mating type 1-1 [MAT1-1] or MAT1-2), with gene expression in certain isolates but with no production of fruiting sexual bodies.[20,21]

Since 2009 the Broad Institute at the Massachusetts Institute of Technology (Cambridge, MA) has completed the whole-genome sequencing database for the *P. brasiliensis* spp. complex, which is publicly available in the Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra).

In cultures incubated at 35°C to 37°C the fungus grows in about 10 days, producing a soft, convoluted, tan-to-cream–colored colony, microscopically composed of yeast cells of varying size (4–40 μm), some adopting the characteristic multiple-budding formation (pilot's wheel). Isolated, round-to-oval yeast cells and short chains of blastoconidia are also observed, as well as large broken yeasts (Figs. 267.1 and 267.2).[2,3,14] Viable yeasts have refractive cell walls and prominent lipid vacuoles.[2,3,14]



FIG. 267.1 ***Paracoccidioides brasiliensis.*** Multiple budding and variation in cell size are apparent (potassium hydroxide preparation from pus, ×1000).

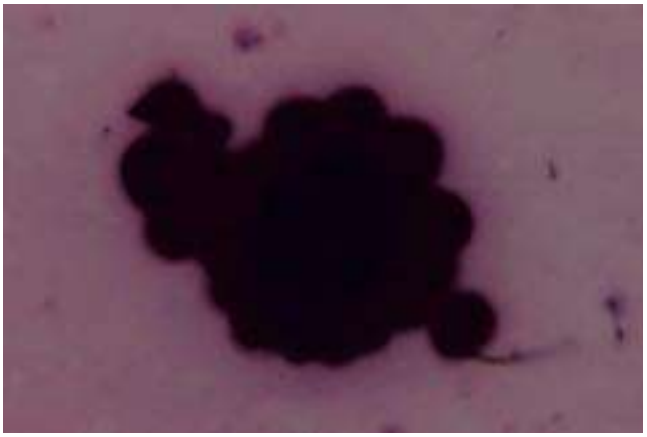

FIG. 267.2 ***Paracoccidioides brasiliensis.*** Multiple buds surrounding the mother cell can be observed in a wet mount from a 36°C culture (calcofluor white, ×1000).

P. brasiliensis has been repeatedly recovered from human clinical samples,[2,3] tissues of the nine-banded armadillo *(Dasypus novemcinctus)*[22] and, more rarely, from the northern naked-tailed armadillo *(Cabassous centralis).*[23] On occasion, *P. brasiliensis* has also been isolated from dogs and detected molecularly in other animal species.[24,25]

As indicated earlier, this fungus encompasses two morphotypes: a mold at temperatures less than 28°C and a yeast in mammalian tissues and cultures at 35°C to 37°C.[2,3] Both morphotypes require ample oxygen supply for their growth.[26] At temperatures less than 28°C, including 4°C if in liquid substrates, the fungus grows within 3 weeks and produces white, cotton-like colonies having short tufts of aerial mycelia.[2,3] Microscopically, only chlamydoconidia and thin septated hyphae are observed in media such as Sabouraud,[2,3] but in carbohydrate-reduced media, the fungus may produce conidia (size <5 μm),[2,27] especially if grown in sandy and claylike soils rich in water.[27,28] Conidia respond to temperature changes by germinating into hyphae at 20°C to 24°C or converting into yeasts, both in vitro (36°C)[27,28] and in infected mice; in the latter, conidia initiate a progressive pulmonary process ending in fibrosis and extrapulmonary dissemination.[2,3,29,30]

P. brasiliensis undergoes an intricate thermal transition process by switching from a mycelial saprobic form, probably in nature, to a virulent yeast form in infected mammal tissues.[1,3,27,29,30] This transition involves expression of an array of virulence factors (cell glucan type, proteolytic enzymes, melanins, fungal adherence factors, and others).[31–34] In addition, biofilm formation has been demonstrated and shown to correlate with increased expression of adhesins and hydrolytic and other types of enzymes.[35] All of the given factors facilitate *P. brasiliensis* interactions with its accidental mammalian host controlled by certain genes expressed during host adaptation.

ECOLOGY AND EPIDEMIOLOGY

Paracoccidioidomycosis, a noncontagious disease, is peculiarly restricted to Latin America, from Mexico (23° N) to Argentina (34° S), with Brazil accounting for the largest number of patients (>80%) and originating mainly from the States of São Paulo, Paraná, Rio Grande do Sul, Goiás, Rio de Janeiro, and Rondônia.[36,37] Venezuela, Colombia, Ecuador, Peru, Bolivia, and Argentina follow in rank with significantly fewer cases. Uruguay, Paraguay, and the Central American countries (Nicaragua, Belize) report few or no cases. The mycosis is extremely rare in the Caribbean Islands, with only single cases having been reported from Trinidad, Grenada, and Guadalupe. The mycosis is unknown in Chile, Surinam, and Guyana.[2,3,38–41] In addition, the mycosis appears to prefer a humid environment and surrounding woods.[3,38]

The main accidental hosts of *Paracoccidioides* spp. are men and armadillos, especially the northern nine-banded *Dasypus novemcinctus,* although other animals have also been implicated, presumably infected in rural or periurban environments.[13,39,42,43] As a rule, the primary infection is asymptomatic or gives rise to nonspecific symptoms that rarely progress to disease manifestations. No epidemic outbreaks or transmission between individuals have been reported.[39,44] Due to lack of knowledge on the precise habitats of the fungus, suspected to occur in rural settings,[44,45] and based on experimental animal infections,[46] it is accepted that the fungal infecting structures, the conidia (<5 μM), are inhaled and reach the lungs, where the infection is usually controlled by the immune cellular responses but may leave behind scarred focal points containing latent yeast cells that may reactivate years later, giving rise to full-blown disease.[3,38]

The disease is characterized by long periods of latency—30 or more years—as demonstrated by the nonautochthonous cases reported outside recognized endemic areas (North America, Europe, Asia).[47] In every case, however, the patient had previously resided in an endemic country, where the primary infection should have occurred, as exemplified by cases reported in Spain, France, Portugal, and Japan, among others.[48–50]

Areas of endemicity center around tropical and subtropical forests, where mild temperatures (17°C–24°C), high annual precipitations (2000–2999 mm), and abundant waterways are common, conditions that favor certain agricultural crops (e.g., coffee, tobacco).[2,3,38,51] Changes in traditional agricultural practices were implicated in the increased number of juvenile cases reported in Rio de Janeiro, Brazil.[52] Two large Brazilian studies emphasized the connection between agriculture-related jobs and paracoccidioidomycosis, pointing toward closer or repeated contacts with *P. brasiliensis* in soil.[39,53] Nonetheless, the fungus has been isolated only sporadically from the environment,[45] a fact that, together with the prolonged mycosis latency periods[47] and absence of outbreaks, has contributed to maintaining the uncertainty about the *P. brasiliensis* microniche. In an attempt at defining more precisely the fungus' environmental niche, Barrozo and coworkers[54,55] studied the role of weather conditions prevailing at the time of diagnosis of a number of juvenile patients, those with the shortest disease course. Absolute air humidity, soil, water storage, and presence of the Southern Oscillation Index influenced significantly the incidence, thereby suggesting their connection with acquisition of the infection. Imprecise knowledge of the fungal habitat has also hindered definition of the infection route, although clinical and experimental animal studies have ruled out traumatic implantation and pointed instead toward the aerosol route as the portal of entry.[2,3,44,45,56]

The incidence of paracoccidioidomycosis is difficult to estimate due to the noncompulsory report of cases and, in addition, to the influence of climate changes and also by human migration and occupation of large unexplored territories where medical care would not be available, as indicated by Martinez.[41] Nonetheless, the incidence of this mycosis was estimated for Brazil, the country with the largest number of patients—10 to 30 cases per 100,000 inhabitants per year.[40,41,57] However, variations were recorded, as indicated in the Itaipu Lake region, where 102 patients were diagnosed in a period of 18 months,[58] and in the State of Rondônia they had the closest to a "prolonged" outbreak, as 39.1 cases per 100,000 inhabitants were reported from 1997 to 2012.[59]

The age and gender distribution of clinical cases is unusual. The mycosis is rare in children (<2%) and teenagers (<9%), with the remaining patients being 30 years of age and older.[60,61,62,63] Several different series encompassing more than 5000 patients revealed that the male-to-female

ratio was 11 : 1.[64] This proportion contrasts with the infection rate as determined by a reactive paracoccidioidin skin test, which had a similar prevalence for both genders.[65] Of note, when the disease occurs in prepubertal patients, no major gender difference becomes apparent, as found with more than 100 children in whom the boy : girl ratio varied from 1.2:1.3 to 1.1:1.0.[38]

The occupational distribution reveals a predilection for individuals who have been engaged in agriculture (>60%) or have had soil-related jobs with subsequent exposure to infected soil and dust, mainly in connection with coffee, cotton, tobacco, and sugar cane plantations.[3,39,51,60,63] Increased acreage of sugar cane fields in the southwestern region of Brazil may alter these data because this crop requires pesticide application and plant burning, a combination that may affect *Paracoccidioides* spp. saprobiotic life in nature.[66] Other occupations mentioned as relatively frequent are masonry and lumberjacking.[36,38,39]

The presence of *Paracoccidioides* spp. in soils or other types of habitats in Latin America, South America in particular, indicate that a large territory is to be taken as endemic and, furthermore, considering that the most prevalent occupation for the working population of such territory would be agriculture-related, including crops manipulation, *P. brasiliensis* airborne infection could represent a problem for newcomers. According to Martinez[41] the existence of unexplored territories, such as those along the Amazon River running through various South American countries, would attract migrants pursuing occupation of promising uninhabited land open to agriculture-related endeavors. This means that a naïve population from these diverse countries may well become exposed to *P. brasiliensis,* leading to infection, which may result in continued expansion of the endemic area, as already observed in north and center-west Brazil.[41] Nonetheless, progression of the infection to the disease stage and the clinical form that may become manifested in infected people would depend on various host factors: genetic background, demography, living conditions, presence of immunosuppression, and others. New endemic areas usually have medical care deficiencies, which would cause considerable social impact. Even if the corresponding clinical manifestations would not become apparent in a short period, *P. brasiliensis* latency poses a challenge, mainly considering that the fungus may become active with time, giving rise to a chronic disease that would cause suffering, disability to the patient, and leave sequelae even in the presence of specific treatment.[44]

Deaths due to paracoccidioidomycosis resulted from extensively disseminated lesions, respiratory and adrenal gland insufficiency, and several other complications, mainly those attributed to residual fibrosis. In Brazil the death toll was estimated as 3.48 per million inhabitants, thus making paracoccidioidomycosis the fifth most common cause of death among the chronic infectious diseases of the lower respiratory tract.[41,67] During the period 1998–2006, a Brazilian study was performed covering 35% of the country and representing 27% of the 5560 Brazilian municipalities, including the larger part of the endemic paracoccidioidomycosis area, consisting of an infected population estimated at 4.3 per 1 million inhabitants. The results analyzed 6732 hospitalization events attributable to paracoccidioidomycosis (82% male). The corresponding results estimated a global mortality of 1.4 per one million inhabitants, with 60% of such deaths attributed to the mycosis itself and the remaining to the fibrotic sequelae.[67,68] In two case series from different endemic Brazilian areas, lethality reached 6.1% and 7.6%, respectively. Nonetheless, at the end of treatment, when assessing patients who did not require hospitalization, lethality was zero.[68] Considering a 5% mortality rate as a mean value, it is possible to estimate the number of new annual cases of this mycosis in Brazil as 3360.[41]

Brazilian authors studying paracoccidioidomycosis, and assessing the number of new patients, observed the large group of cases with a prolonged follow-up and the medical-social characteristics of the mycosis as mentioned earlier, and this revealed the wide dimension of this mycosis as a public health problem in Brazil and emphasized this condition as a neglected disease.[41,67,68]

PATHOGENESIS AND THE IMMUNE RESPONSE

After the initial contact with *Paracoccidioides* spp., a *subclinical infection* may develop, which, if the host is unable to control it, becomes clinically apparent.[69] There are two main clinical presentations—the acute/subacute, or juvenile, and the chronic, or adult disease.[69,70] The former is characterized by marked involvement of the reticuloendothelial system and occurs in less than 15% of cases,[3,37,52,61,71–73] whereas the chronic or adult type of disease involves the lungs and characteristically results in extrapulmonary lesions. This is the predominant form occurring in approximately 90% of cases and probably represents endogenous reactivation after years of initial contact with the fungus.[3,15,37,38,53] In addition, a *residual form* is also recognized and is represented by fibrotic scarring occurring at the sites of previously active lesions.[71,73] A recent cluster analysis done in patients with concomitant lung, mucosal, and skin lesions has strongly supported the existence of two different sets of patients.[72–134] The first analysis includes patients with mucosal damage, odynophagia, and/or dysphagia, plus alveolointerstitial infiltrates, and the second one consists of patients exhibiting dermal lesions, dyspnea, and lung fibrosis. The former conditions would represent early stages of the infection, whereas the latter would correspond to a more chronic granulomatous and fibrosing process.[133–135]

Paracoccidioides spp. infection may become dormant but may be reactivated later under the influence of ill-defined conditions prevalent in rural settings, such as chronic alcoholism, malnutrition, and smoking.[40,67]

The pathogenesis of this mycosis depends partly on *Paracoccidioides* spp. virulence factors and their antigenic composition, on environmental conditions, and also on the efficiency of the host immune response.[74] Different in vitro, in vivo, in silico, and ex vivo experiments, as well as clinical studies, have demonstrated that specific and nonspecific defense mechanisms, both innate and adaptive, are crucial in building host resistance.[39] In consequence, this response is highly complex and multifactorial, but when a particular balance of the host-fungus interaction is achieved, the infection is controlled and progression to overt disease is halted; otherwise, individuals who have lost this balance develop clinically manifested paracoccidioidomycosis.[74] Thus the initial interaction of the microorganism with the first line of host defenses would determine the type of immune response to be developed (protective, deleterious), and, accordingly, would determine the disease outcome.[74]

Paracoccidioides spp. have several virulence factors. As an example, the fungus cell wall components—glycoproteins and polysaccharides—control the fungus morphologic transition from the conidia or mycelial morphotypes (26°C) to the parasitic yeast (37°C). This form switch is obligatory if the pathogen is to adapt and thrive inside the host in whom the infection is established.[75] Furthermore, on its surface *P. brasiliensis* expresses several adhesins, such as the glycoprotein gp43 plus the 19-kilodalton (kDa), 30-kDa, and 32-kDa proteins, all fulfilling similar functions, as well as a series of enzymes (malate synthase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, enolase). The adhesins allow the fungus to recognize the host extracellular matrix protein molecules (laminin, fibronectin, fibrinogen, plasminogen) that facilitate not only fungal adherence but also invasion and dissemination to other organs and systems.[76,77] This fungal pathogen also produces melanin and exhibits the capability to form biofilms in vitro and in vivo, factors that confer resistance to both a host's microbicidal mechanisms and to antifungal therapy.[35,78,79]

Innate Immune Responses

Once fungal propagules are inhaled, they interact with pulmonary epithelial cells; such interaction induces the activation of cell signaling molecules in these epithelial cells to produce cytokines and other molecules, thus initiating the immune response.[80,81] In vitro studies have shown that *P. brasiliensis* yeasts stimulate the lung's A549 epithelial cells to produce interleukins-6 (IL-6) and IL-8 by a process dependent on activation of p38 mitogen-activated protein kinase and extracellular signal-regulated kinase.[81]

Besides the lung epithelial cells, the first line of defense is represented by neutrophils, alveolar macrophages, dendritic cells, natural killer (NK) cells, complement, peptides, proinflammatory cytokines, and chemokines, all of which hinder fungal multiplication but are unable to destroy the invading microorganism.[44,74,82] Innate immune system cells detect fungal presence through pattern recognition receptors (PRRs) that interact

with conserved molecular structures present in the microorganisms but absent in the host, structures known as *pathogen-associated molecular patterns* (PAMPs). Among the PRRs that participate in the innate immune response against *P. brasiliensis*, Toll-like receptors (TLRs), mainly TLR2, TLR4, and TLR9; dectin-1; and the mannose receptor play important roles in the recognition of this fungal pathogen and activation of innate immune responses.[74,83–85] In addition, dectin-1 in association with the nucleotide-binding oligomerization domain-like receptor P3 (NLRP3), which activates the inflammasome, leads to maturation and secretion of IL-1β and IL-18, important proinflammatory cytokines connected with resistance against *P. brasiliensis*.[86,87]

Human neutrophils activated in vitro with granulocyte-macrophage colony-stimulating factor, IL-15, tumor necrosis factor-α (TNF-α), or interferon-γ (IFN-γ), and infected with yeasts from a virulent *P. brasiliensis* strain, revealed that the indicated cytokines were able to induce increased expression of TLR2 and TLR4. On the other hand, in presence of the fungus, TLR2 expression increased, but the corresponding TLR4 decreased.[88] In addition, in vivo experimental studies using TLR2 knockout mice previously infected with *P. brasiliensis* revealed that a deficiency in this factor favored development of a less severe pulmonary infection compared with the infected TLR2-suffcient mice. This treatment increased KC (a chemokine that facilitates neutrophil chemotaxis), transforming growth factor-β (TGF-β), IL-6, IL-23, and IL-17. A Th17 cell profile was associated with decreased expansion of regulatory T cells (Tregs).[83] In addition, studies demonstrated that fungal lipid components modulated macrophage activity through TLR-dependent and -independent mechanisms.[83]

Various experimental studies have shown a dense neutrophilic infiltrate in the lungs of *P. brasiliensis*–infected animals that correlated with secretion of cytokines (TNF-α, IL-6, IL-1β) and chemokine (macrophage inhibitory protein 2 [MIP-2]), as well as with the expression of adherence molecules (intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1]), and integrins, all of which facilitated the influx of such phagocytes.[89]

In addition, human neutrophils produce extracellular traps (NETs) against both *P. brasiliensis* conidia and yeast cell morphotypes, a process that appears to be dependent or independent of reactive oxygen species production but in correlation with the fungal morphotype used.[90] However, this mechanism was ineffective in killing the fungus.[90] In addition, Della Coletta and coworkers[91] demonstrated the presence of NETs in vivo in paracoccidioidomycosis patients with extrapulmonary lesions and also that the highly virulent *P. brasiliensis* strain 18 (Pb18) and the low-virulence Pb265 strain induced different NETs patterns in vitro.

Although neutrophils are considered important players in the innate immune response against *P. brasiliensis*, recent reports using a paracoccidioidomycosis experimental model suggested that neutrophils play a dual role in this mycosis, being essential during the early infection and detrimental during the chronic course of the disease.[92,93]

NK cells also participate in the innate immune response against *P. brasiliensis*. In vitro studies demonstrated that NK cells from paracoccidioidomycosis patients exhibited a decreased cytotoxic response compared with that from healthy individuals. In addition, NK cells were capable of recognizing and killing extracellular yeasts through a mechanism dependent on granules and independent of perforins, whereas cytotoxicity against the intracellular fungus depended on the latter.[94] Granulysin seemed a possible mediator of the granules-dependent mechanism because this molecule was detected in NK cells plus *P. brasiliensis* supernatants and was capable of killing the fungus in a dose-dependent manner.[94] In addition, patients with the mycosis had a low frequency of $CD56^+$ granulysin-producing cells when compared with healthy control subjects. Reports indicated that NK cells, once stimulated with recombinant IL-15, produced proinflammatory cytokines (IFN-γ, TNF-α) that played an immunomodulatory role in paracoccidioidomycosis.[9]

Macrophages represent the major cellular defense against *P. brasiliensis* and are extremely important, not only for infection control but also for induction and regulation of the inflammatory responses. When activated by IFN-γ, they ingest and kill both conidia and yeasts through expression of inducible nitric oxide synthase.[74,89] Nitric oxide, however, plays a dual role (resistance and susceptibility), depending on its expression degree.[89] Along the same lines, IFN-γ also induced the indolamine 2,3-dioxygenase, an enzyme that catalyzes tryptophan metabolism and is associated with inhibition of *P. brasiliensis* growth.[95] In addition, it has been reported that *P. brasiliensis* induced transcription of the Notch1 receptor on macrophages, thus increasing IL-6 production, the latter favoring fungal virulence.[96]

Eosinophils are commonly connected with the immune response during allergic and parasitic infections. In the case of paracoccidioidomycosis, eosinophils appear to play a detrimental effect; thus, in these patients, the severity of the disease is associated with eosinophilia.[97] More recently, a functional evaluation of eosinophils in paracoccidioidomycosis patients and control subjects revealed that eosinophils from both groups exhibited a similar ability to kill *P. brasiliensis*, although those from patients were less responsive to IL-5 stimulation.[98]

Dendritic cells (DCs) play an important role in bridging the innate and acquired immune responses. These cells bind, capture, kill, and process pathogens and then migrate to peripheral lymphoid organs to present the processed antigens to T cells, thus initiating the acquired immune response. *P. brasiliensis* infection inhibits prostaglandin E_2 production by DCs and subsequently their maturation and migration.[99] More recently, it was shown that DCs treated with a TLR9 agonist exhibited an increased fungicidal activity against *P. brasiliensis*.[85]

As expected, the host's immune response represents the summation of a series of biologic effects resulting from fungal interaction with different cells, molecules, and receptors geared at eliminating the microorganism. If this is not achieved, the specific or acquired immune response, cellular or humoral, will then be initiated.[74]

Acquired Immune Responses

During the development of the adaptive or specific immune response, dichotomy between the humoral and cellular immune responses develops, with an increase in the production of antibodies associated with an observed hypoproliferative response of the T cells.[100]

Cell-mediated immunity is crucial to defense. It is usually depressed at the disease's peak but can be restored with successful treatment. De Castro and coworkers[101] showed that *Paracoccidioides*-infected individuals are characterized by the presence of the Th1 immune response. Patients with the acute form of the disease had a mixed Th2/Th9 response, whereas those with the chronic form had Th17/Th22 profiles, as well as a substantial Th1 cell participation. Of note, a Th1/Th17 profile is associated with resistance to disease; moreover, a balance between Th17/Treg cells is necessary to control the severity of the fungal disease.[102]

Recently, it has been reported that patients with the acute form of the mycosis, compared with those having the chronic form, expressed high serum levels of IL-1β, IL-18, IL-33, soluble IL-33 receptor (sST2), and IL-37, plus a strong expression of the given cytokines in lymph node lesions.[103] Moreover, the antifungal treatment reduced the levels of these cytokines in all patients groups, indicating that these cytokines may be also associated with the activity and severity of mycosis.[103]

The occurrence and severity of human paracoccidioidomycosis may also be associated with genetic factors, such as single nucleotide polymorphisms (SNPs) on cytokine-encoding genes. Carvalho and coworkers[104] investigated the association among these polymorphisms and the different clinical forms of the mycosis and found similar genotypic and allelic frequencies of the investigated SNPs among the clinical forms described. In another study two polymorphisms in the IL-4 gene were analyzed and showed a significant correlation between *Paracoccidioides* spp. infection and the RP2/RP2 genotype, whereas the RP1/RP1 genotype was linked to resistance.[105] Furthermore, patients with the RP2/RP2 genotype produced high levels of IL-4, whereas healthy individuals with the RP1/RP1 genotype produced low levels of this cytokine. Moreover, a higher frequency of classes I and II human leukocyte antigen alleles has been described in paracoccidioidomycosis patients.[4]

In addition, it has been indicated that *P. brasiliensis* is capable of modulating the immune response toward a permissive state, with the thymus playing a major role. In this sense, experimental studies using BALB/c mice showed that acute infection with *P. brasiliensis* yeast cells promoted thymic alterations leading to a defective repertoire of peripheral

T cells.[106] These results may represent new mechanisms by which *P. brasiliensis* subverts the host's immune response, favoring chronic infection as observed in humans.[106]

Granuloma formation, the hallmark of chronic disease, is considered the most evolved and effective biologic defense weapon against *P. brasiliensis*.[74,82,107] Granuloma formation is involved in the activation of T lymphocytes and effector cells, the latter represented mainly by macrophages and neutrophils. Several Th1 cytokines, especially IFN-γ and IL-12, are associated with resistance, whereas Th2 cytokines (IL-4, IL-10, TGF-α, TGF-β) are associated with increased host susceptibility, probably because they interfere with correct macrophage function.[107] In addition, oral paracoccidioidomycosis granulomas are predominantly populated by $CD163^+$ multinucleated giant cells,[108] with activated M2 macrophages representing the large majority among the inflammatory cells in these lesions, thus characterizing this response as Th2.[109]

Humoral Immune Responses

To date, and despite the strong polyclonal response represented by B lymphocytes associated with hypergammaglobulinemia and the elevated specific antibody titers observed in most patients, the protective nature of this particular immune response has not yet been demonstrated. In patients with the subacute form, specific antibodies of the immunoglobulin (Ig)A, IgG, and IgE subclasses are markedly increased.[74,110] In addition, these patients show eosinophilia and increased levels of TGF-β, a switching factor for IgA. Patients with severe adult-type disease also have elevated antibody titers, but those with a less severe mycosis show significantly lower antibody production.[110]

Of interest, B-1 cells appear to play a detrimental effect in *P. brasiliensis* infection as these cells migrate to the spleen and stimulate an increase in the Treg subpopulation.[111] Genetic differences in the genus *Paracoccidioides* could elicit distinct host immune responses. Lenhard-Vidal and coworkers[112] demonstrated that *P. brasiliensis* and *P. lutzii* antigens elicited different serum IgG responses in chronic paracoccidioidomycosis patients.

Immunoregulation, Immunomodulation, and Vaccines

The combination of an antifungal medication with an effective immunotherapeutic measure, such as an immunomodulator agent or a vaccine, would appear to be useful for treatment of most severe cases because reactivation of the host's immune response would permit shorter treatment periods with conventional medications and may also prevent relapses and fibrotic scarring.[113] Experimental studies are being performed along these lines with promising results when administering the gp43 protein, P10 peptide derived from gp43, and heat shock proteins (DNAhsp65), as well as other antigens (Pb27, rPb27, paracoccin, radioattenuated yeast cells).[113–122] By the same token, experimental studies in an animal model induced by inhalation of *P. brasiliensis* conidia have shown that dual treatment with the antifungal itraconazole (ITC) and the immunomodulator compound pentoxifylline significantly avoided fibrous scarring in infected versus control animals.[107] More recently, the use of a monoclonal antibody specific to neutrophils not only controlled the infection but also reduced the inflammatory response and the pulmonary fibrosis in an experimental model of paracoccidioidomycosis.[93] Moreover, the combination of the monoclonal antibody specific to neutrophils in combination with ITC reduced both disease extension and pulmonary fibrosis through downregulation of inflammatory and profibrotic genes.[123] These results open new avenues to devise therapeutic protocols that may benefit patients with this mycosis.

CLINICAL MANIFESTATIONS

Paracoccidioidomycosis has been divided into three categories—one lacking clinical manifestations and another two directly related to the disease processes and largely dependent on age and host immune status.[69]

1. Paracoccidioidomycosis infection

   This is considered to be a latent, clinically asymptomatic process lasting 1 month to years after the initial fungal contact[124] and that may later evolve to progressive, clinically manifested disease. This form is acquired when a healthy individual enters in contact with *Paracoccidioides* spp. The infection stage is diagnosed by a reactive intradermal test to specific antigens and also at necropsy by demonstrating the presence of fungal cells in tissues.[38,39,44,69]

2. Paracoccidioidomycosis disease

   A. *Acute/subacute disease (moderate or severe):* This is an overt process evidenced by involvement of multiple organs with lymph node, liver, and spleen hypertrophy and also by manifestation of skin lesions, the latter often multiple and widely distributed throughout the body. The lungs do not often reveal radiographic abnormalities, nor are clinical manifestations obvious, despite the fact that *P. brasiliensis* is frequently detected in respiratory specimens. This form is usually diagnosed in children, young adults, and acquired immunodeficiency syndrome (AIDS) patients, as well as in those with other immune alterations.[38,39,44,61,69]

   B. *Chronic progressive disease/adult form (mild, moderate, or severe):* This is the most common (90%) of the clinical presentations, diagnosed in older patients and characterized by important lung involvement and by frequent lesions in the mucosae, skin, adrenal glands, and other sites. Severe cases are defined by meeting three or more of the following criteria: (1) weight loss greater than 10% of the normal body weight; (2) intense pulmonary involvement; (3) involvement of other organs, such as adrenal glands, central nervous system (CNS), and bones; (4) presence of pseudotumoral (>2 cm diameter) lymph nodes in multiple chains, superficially or deeply located, with or without suppuration; and (5) high antibody titers.[38,39,44,69]

   Mild cases are those with weight loss less than 5% and involvement of a few organs, with no dysfunction. In both clinically manifested forms, constitutional symptoms (fever, asthenia, general malaise, weight loss) are regularly observed.[26,38,82,125]

3. Residual form

   This is characterized by the presence of sequelae originating in the previously infected fibrous tissues, mainly in the lung but also reported in adrenal glands and mucosae.[26,38,39,44]

CHARACTERISTICS OF THE LESIONS

Lungs

Lungs are the primary site of infection, but the corresponding clinical manifestations may be scarce; nonetheless, progression follows over time, and at diagnosis, symptoms (cough, expectoration, blood-tinged sputum, chest pain) and some degree of dyspnea are regularly noticed. Auscultation reveals minimal abnormalities compared with radiographic findings, with a clear dissociation between symptoms and imaging studies. The initial respiratory symptoms decrease or disappear fully after the onset of treatment, but dyspnea on heavy exertion might become worse and progress even with moderate or mild exertion. Often noticed are pulmonary function alterations and abnormalities in ventilation-perfusion.[26,38,82,124,125] Most (85%) patients exhibit the obstructive pattern that may be mild, moderate, or severe with no relation with the degree of involvement. Hypoxemia is a sequel occurring in approximately one-third of the cases.[126] These findings, however, are difficult to interpret because the coexistence with smoking habits, resulting in an obstructive pattern involving the small airways, usually accompanied by an increase in the dead space and in the alveolar-arterial oxygen gradient.[82,127]

Chest radiograph images reveal mostly interstitial infiltrates (64%), followed by mixed lesions consisting of nodular and alveolar infiltrates that are occasionally confluent, frequently bilateral, symmetrical, and located preferentially in the central and lower fields (Fig. 267.3).[3,82,127] High-resolution computed tomography demonstrates abnormal findings in greater than 90% of patients with chronic pulmonary disease, with the commonest abnormalities being interlobular septal thickening (88%), peribronchovascular interstitial thickening (78%), centrilobular opacities (63%), intralobular lines (59%), ground-glass opacities (34%), cavities (17%), and airspace consolidation (12%), with the "reversed halo sign" being observed in 10% of the cases.[82,127,128] (Fig. 267.3). Gallium-67 scans reveal pulmonary lesions in practically all patients, irrespective of the clinical forms, thus confirming the lungs as the main target.[70,82,127]

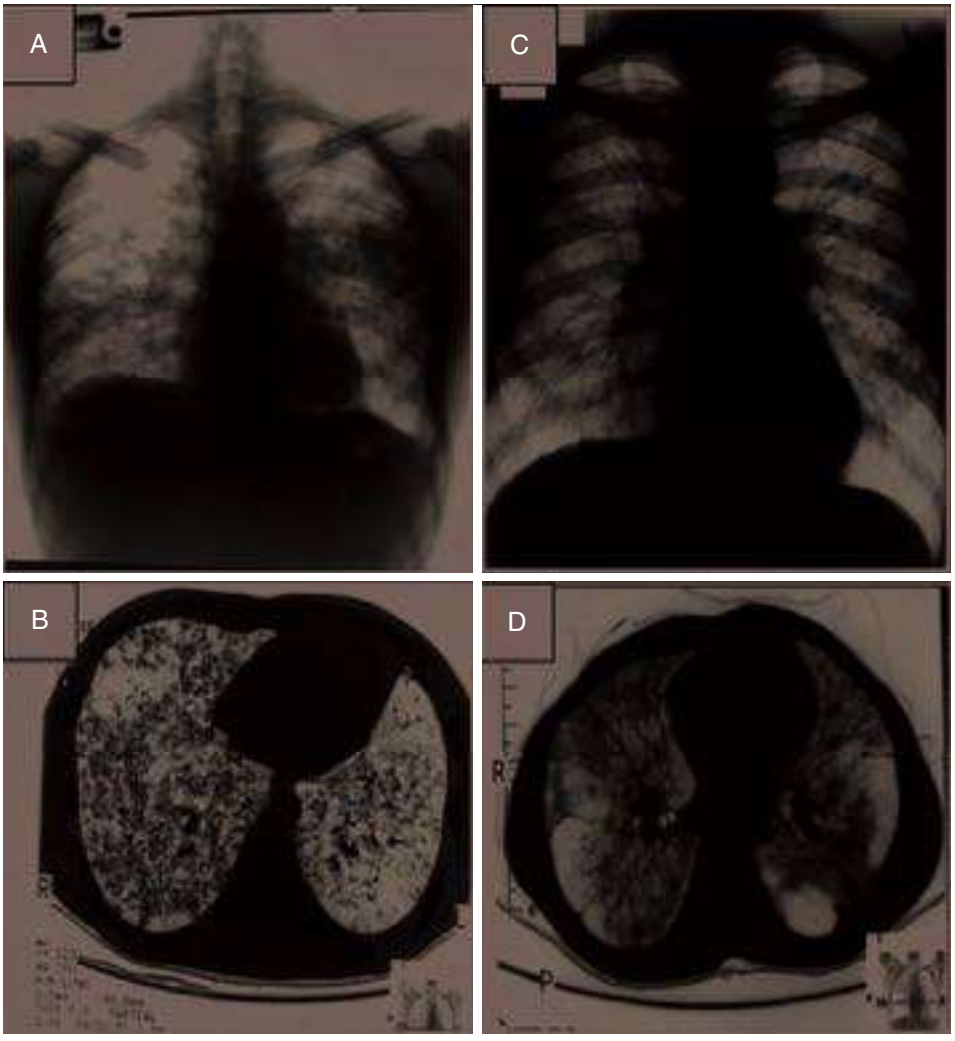

FIG. 267.3 Chest radiograph and chest high-resolution computed tomography (CT) scan in paracoccidioidomycosis patients before treatment. (A) and (C) Plain chest radiographs showing bilateral mixed alveolar and interstitial lesions in lower and central lung fields. (B) and (D) CT scan: The involved lungs show alveolar areas with the ground-glass image. Note bronchial dilatations and presence of bullae as sequelae.

At diagnosis, one-third of the patients with long-lasting disease have serious pulmonary sequelae, such as fibrosis (32%) and bullae (27%), and indirect signs of pulmonary hypertension with right ventricle enlargement and even cor pulmonale.[70,82,127]

A Brazilian study examined 1059 deaths caused by the mycosis and found that mortality could be attributed to pulmonary fibrosis, chronic lower airway diseases, and pneumonic processes, thus signaling the importance of the lung in paracoccidioidomycosis.[82,129] Awareness of the given abnormalities emphasizes early diagnosis and prompt initiation of specific treatment, thereby reducing associated morbidity and mortality.[67,82,127] Pleural involvement is detected in only 2% of cases on plain chest radiographs and is characterized by small effusions (<300 cc) and pleural thickening without effusion as observed in 60% of the autopsy cases.[130]

Mucosa

The oral mucosa is involved in 58.5% of the cases, with lesions regularly localized in the gingiva and the palate and, with extension, to the neighboring skin on the lips and nose. The larynx, pharynx, and gastrointestinal mucosa are also affected. Lesions are infiltrative, edematous, ulcerated, and have a granulomatous base, mulberry-like in appearance (Fig. 267.4). They are extremely painful and hinder the patient's food intake; upon healing, they produce fibrous scarring. Also, in the absence of proper dental care, tooth loss occurs regularly. Other symptoms, such as diarrhea, odynophagia, dysphagia, sialorrhea, and dysphonia may appear, depending on the location of the lesion.[125,131–136] Mucosal involvement is the principal difference with tuberculosis, as in the latter disease the oral presentation is rare, with a prevalence ranging from 0.1% to 0.4%.[137]

Skin

Cutaneous lesions are observed in patients with either the adult form (61.2%) or the acute/subacute presentation (50%). Skin abnormalities may represent hematogenous dissemination from the primary pulmonary focus or secondary extension from contiguous mucous membrane lesions. They tend to ulcerate and present a thick well-delimited external border with a granulomatous base, accompanied by infiltration of the neighboring tissues. In AIDS patients lesions are multiple and rather small, flat, covered by crusts, and with no inflammation in the surrounding tissues (Fig. 267.5).[131,135,136,138,139]

Lymph Nodes

All lymph node chains may be involved, with predilection for cervical, axillary, mediastinal, and mesenteric nodes. Patients with either of the two progressive forms present with clinically detectable hypertrophied lymph nodes. If mucosal or skin lesions exist, enlargement of the corresponding draining lymph nodes is noted, and they may form fistulas. Lymphatic hypertrophy can cause complications by compression of contiguous structures, such as bronchi, vessels, and the periampullary region.[a] These lesions are most easily detected by gallium-67 scans[70] and magnetic resonance imaging (MRI).[141]

[a]References 15, 36, 60, 82, 125, 133, 136, 140.

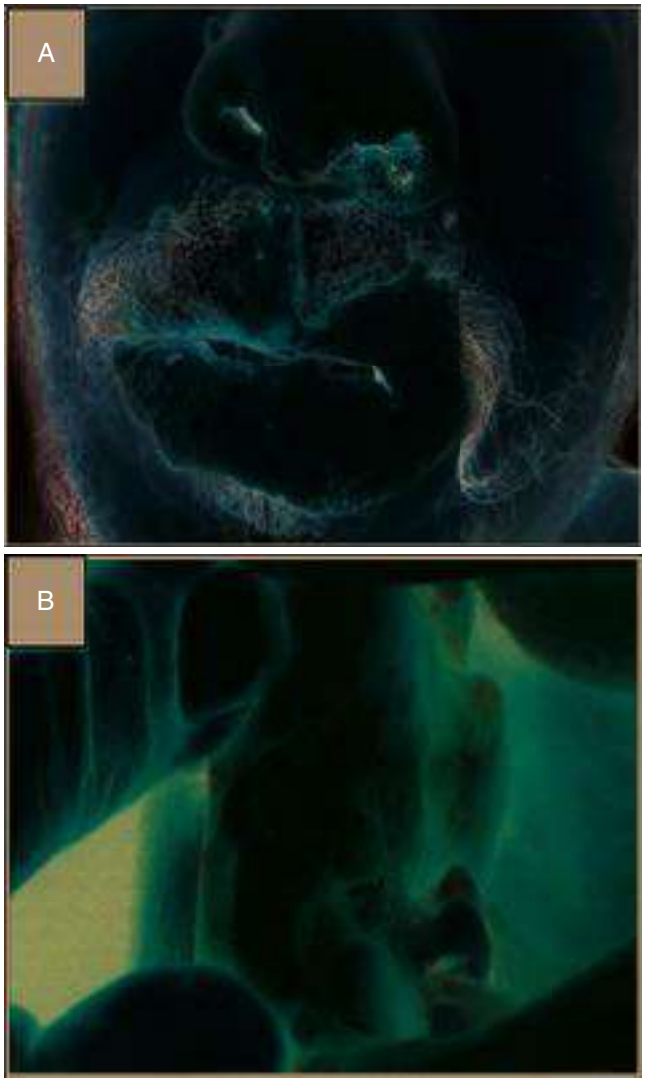

FIG. 267.4 Paracoccidioidomycosis patients. (A) Note edema, ulceration, and crust formation in lips and nostrils. This patient also had extensive lesions of the oral mucosae, as well hypertrophied lymph nodes. (B) Note typical "mulberry-like" lesions in the buccal mucosa of a patient with disseminated disease.



FIG. 267.5 Paracoccidioidomycosis and acquired immunodeficiency syndrome. Note multiple, small, polymorphic, and noninflammatory lesions disseminated throughout the back, arms, and neck areas.

Adrenal Glands

In endemic countries this mycosis is one of the main causes of adrenal gland insufficiency, with the glands considered to be the third most frequently affected organ. Involvement is evidenced by gland hypertrophy with damage to both the cortex and the medulla; in addition, 10% of the patients may present Addison syndrome. When cortisol is measured at basal and post–adrenocorticotropic hormone stimulation times, diminished adrenal glands reserve is detected in 48% of the assessed patients.[141,142] Autopsy studies, however, have shown that adrenal glands involvement may occur in up to 85% of cases. Prompt initiation of antifungal therapy may restore adrenal gland function.[26,39,70,125,143]

Other Lesions

Paracoccidioidomycosis also involves the CNS in 9.6% to 25.4% of the cases, with formation of cerebral meningeal lesions and spine involvement with spondylodiskitis. Lesions are more frequent in the cerebral hemispheres (69%), with multiple granulomas in 65% of the patients, characterized by nodular or annula, contrast-enhancing hypodense images.[144–146]

Other organs affected are liver, spleen, pancreas, and adrenal glands, thus simulating malignant neoplasia and abnormalities of vascular structures, bones, and bone marrow, each one producing its corresponding clinical manifestations.[38,125,132,134–136,141] Septic shock may present a challenging diagnosis.[147] Gallium scanning, computerized tomography, MRI, and necropsies have revealed unexpected lesions in all organs and systems, reflecting the mycosis' disseminated nature as revealed in a study of 26 patients, 69.2% of whom showed these abnormalities.[67,70,148]

PARACOCCIDIOIDOMYCOSIS IN HUMAN IMMUNODEFICIENCY VIRUS–INFECTED PATIENTS

The prevalence of paracoccidioidomycosis in immunocompromised patients with AIDS is relatively low and occurs more frequently in advanced AIDS patients. The study of Chiarella and coworkers[149] analyzed the function of CD4 and CD8 T cells in the immunity of mice infected with *P. brasiliensis* and showed that CD8 cells play an important role in pulmonary immunoprotection. In Brazil 4% to 5% of patients with paracoccidioidomycosis exhibit HIV infection as a comorbidity.[150] Use of the gp43 intradermal test has shown that 12% of HIV patients were reactive, demonstrating coinfection with *Paracoccidioides* spp. Nonetheless in rural areas of the same region, the prevalence of this coinfection was higher, at 47%.[151,152] Clinical and epidemiologic characteristics in this comorbid association differed concerning younger age at diagnosis (45.3 vs. 33.5 years), lesser involvement in agriculture-related occupations (59.4% vs. 27.5%), and a lower male : female ratio (9.6 : 1 vs. 4.3 : 1), as well as the time needed to establish the diagnosis (±30 days after initiation of symptoms).[151,153]

Skin lesions are extremely common (94%) compared with non-AIDS counterparts (38%). Other symptoms reported included cough and dyspnea in half of the patients, even in those with no radiographic abnormalities.[139,151,153] Also, these patients more often had fever, hepatosplenomegaly, and mucosal lesions in a manner resembling the subacute form of the mycosis, one that has the characteristics of an opportunistic disease.[139]

In patients with HIV, and according to the series, mortality varied between 3% and 53%, depending on the presence of alcoholism or tuberculosis. The HIV group with the stated complications had a higher relapse probability (30%) than those without these complications, both at 12 months (9.4% vs. 1.4%, respectively) and 24 months (18.5% vs. 3.3%, respectively) after treatment.[151,153,154]

At the time of diagnosis of paracoccidioidomycosis, most of the comorbid patients had overt AIDS (CD4 counts <200 cells/mL, viral copies >100,000/mL). Forty percent of them had received trimethoprim-sulfamethoxazole (TMP-SMX) treatment for pneumocystosis and toxoplasmosis prophylaxis.[151,153] This finding indicates that prevention of paracoccidioidomycosis with this medication had proved ineffective.

In these cases treatment follows the same schemes used for the non-HIV patients, except that maintenance therapy should be required up to the time when CD4 counts are consistently greater than 200 cells/mm^3 and viral copies become undetectable through administration of

TABLE 267.1 Antifungal Therapy for Paracoccidioidomycosis

ANTIFUNGAL PRESCRIBED (ADMINISTRATION ROUTE)	MINIMAL TREATMENT DURATION BASED ON ORGAN INVOLVEMENT[a]	DOSE	ADVERSE EFFECTS	RESPONSE (%)	RELAPSES (%)
TMP-SMX (PO/IV)	Minor: 12 mo Moderate: 18–24 mo	*Adults* TMP: 160–240 mg/day SMX: 800–1200 mg/day, divided into two doses per day *Children* TMP: 8–10 mg/kg/day, divided into two doses per day	Leukopenia Hypersensitivity reactions, such as rash	80	20
Amphotericin B deoxycholate (IV)[b]	Until patient improves and can be treated by the oral route and achieves a total dose of 2 g[c]	1 mg/kg/day	Nephrotoxicity Hypokalemia Nausea/vomiting Fever Anemia	70	25
Ketoconazole (PO)	9–12 mo	200–400 mg/day	Hormonal alterations Increase in hepatic enzymes Nausea/vomiting	90	11
Itraconazole (PO)	6–9 mo	*Adults* Loading dose 600 mg/day for 3 days; continue 200 mg/day *Children* (<30/kg and >5 yr) 5–10 mg/kg/day	Nausea/vomiting ↑Hepatic enzymes Drug interactions	94–98	3–5
Voriconazole (PO/IV)	6 months	Initial dose: 400 mg each 12 h for one day, then 200 mg each 12 h Diminish the dose to 50% if weight is <40 kg	Visual alterations ↑Hepatic enzymes Skin rash Photosensitivity Hallucinations Periostitis	88	No data

[a]Shown only with the aim of guiding therapy. Total duration must be defined in accordance with clinical and immune-based tests, as well with the mycosis clinical form.
[b]Other formulations can be used.
[c]Should always be continued with TMP-SMX or itraconazole oral therapy, once clinical improvement has been obtained.
IV, Intravenous; *PO,* orally; *TMP-SMX,* trimethoprim-sulfamethoxazole.

antiretroviral therapy (ART). In most cases this approach requires 18 to 24 months of treatment to curtail relapses.[151,153]

Initiation of ART may result in worsening of the mycosis' clinical symptoms because of the immune reconstitution inflammatory syndrome, one that can be treated successfully with corticosteroids.[155]

PARACOCCIDIOIDOMYCOSIS AND CANCER

Paracoccidioidomycosis can mimic neoplasia, such as colon cancer and CNS malignant tumors. Therefore, in endemic areas, the mycosis must be considered in the differential diagnosis of intraabdominal and cerebral tumors.[156] The cellular immune dysfunction known to occur in patients with active paracoccidioidomycosis may be connected to the occurrence of associated cancers. This has been postulated to be the result of the prolonged effect of fungal antigens on epithelial cells and mononuclear phagocytes, with the subsequent alterations of cellular surveillance hindering malignant transformation.[156] In 58% of the cases both diagnoses were established simultaneously.[156]

Paracoccidioidomycosis can be considered an opportunistic disease in patients undergoing chemotherapy or receiving high levels of corticosteroids. In such patients the mycosis presents in its subacute form.[156] The association between organ transplantation and paracoccidioidomycosis is rare, and in an unusual clinical presentation, a Brazilian kidney transplant recipient developed skin lesions and a cervical mass that on biopsy were indicative of paracoccidioidomycosis.[157]

DIFFERENTIAL DIAGNOSIS

The most important differential diagnosis is tuberculosis, a disease that may coexist with the mycosis in 8% to 13% of patients.[158] Other diseases that should be considered are cancer and neoplastic disorders (including lymphoma), histoplasmosis, leishmaniasis, leprosy, and syphilis. Only laboratory testing establishes the correct diagnosis.[36,38,136,156,159–161]

TREATMENT

Because of the systemic nature of the mycosis, consideration should be given to patient's general status, target organs and systems, and also to the form of presentation (subacute, chronic). Existence of comorbidities, such as tuberculosis, AIDS, and cancer, should be determined and managed accordingly. Treatment should include supportive general measures, such as bed rest, anemia correction, and suspension of tobacco use and alcohol intake, as well as balanced nutrition. Associated clinical complications should also be treated.[b]

Antifungal therapy is the mainstay for patient improvement, with three groups of medications being available: sulfonamides, amphotericin B, and azole derivatives (Table 267.1).

Sulfonamides

In some countries TMP-SMX is the preferred medication because of its low cost, facility for oral or parenteral use, plus being administered free of cost by governmental health systems. The rate of response is high, but treatment should be prolonged for 2 or more years, and the probability of relapse is high.[39,53,125,133,163]

Amphotericin B

This polyene is to be given intravenously and is reserved for patients with severe disease, for those who do not tolerate the oral route, for women in their first 12 weeks of pregnancy, and also for patients with fourfold increases in hepatic enzymes. Cumulative total dosages for the deoxycholate formulation vary from 1 to 2 g. Amphotericin B is not fungicidal in vivo, and all patients thus treated should also receive maintenance sulfonamide or azole therapy to avoid relapses. There has been little experience with the lipid formulations of amphotericin B, largely based on cost and limited availability.[39,125,133–135]

Azole Derivatives

Ketoconazole for oral administration has proven effective, but its hepatotoxicity and multiple interactions with other medications argue against its use. Among the triazoles, ITC is the preferred medication for treatment of patients with both minor and moderate clinical manifestations. It is also recommended for maintenance therapy in severe cases, once the

[b]References 39, 61, 67, 132, 133, 162.

initial amphotericin B course has been concluded. The advantages of ITC include shorter treatment periods (6 months), relapse rates lower than with other medications, and oral administration when renal function is impaired. The addition of corticosteroids has been advocated for certain patients.[164]

Voriconazole or fluconazole can be used successfully in patients with neuroparacoccidioidomycosis because both have better penetration into the CNS than other drugs.[133,162,165] Alternative triazoles have been incompletely evaluated in the treatment of paracoccidioidomycosis, although posaconazole and isavuconazole have appeared to be effective.[166]

Experimentally, the combined ITC and pentoxifylline therapy resulted in a significantly more rapid reduction of both granulomatous inflammation and pulmonary fibrosis,[167] but extrapolation to human disease has not been attempted.

Another strategy used to decrease fibrosis is by augmenting the host's cellular immune responses. DNA vaccines based on heat shock proteins, such as Hsp65 from *Mycobacterium leprae,* have shown prophylactic and therapeutic effects in experimental models of diseases, including tuberculosis, leishmaniasis, and paracoccidioidomycosis. In the last of these, decrease in fungal burden and changes in the granulomatous inflammatory reaction were noticed in vaccinated animals, with smaller and more mature granulomas and a reduction in fibrosis formation.[168,169] Until now, none of the experimental strategies just described has been evaluated in a clinical trial.

Despite efficacious antifungal therapy, latent foci with viable *Paracoccidioides* spp. fungal cells, may remain latent in tissues, requiring appropriate cellular immune responses to contain fungal proliferation.

Immune Stimulation

Patients treated with a combination of β-glucan and an antifungal agent exhibited better improvement in terms of clinical manifestations and showed, additionally, an increase in both the number of $CD4^+$ lymphocytes and TNF-α.[170]

Criteria of Recovery

1. Clinical: When the signs and symptoms of disease are no longer present and normalization of the erythrocytes' sedimentation rate is observed.[126]
2. Mycologic: There are negative results when attempts at detecting *Paracoccidioides* spp. in clinical samples are negative in those sites where it had been previously detected.[126]
3. Radiologic: Evidence is apparent when the lung's radiologic pattern remains stable after treatment, for instance, the same scarring lesions are found on five different radiographs taken every 3 months in the course of 1 year.[126]
4. Immunologic: The serum levels of specific antibodies decrease after the onset of treatment, and these levels become stabilized at a very low concentration on the complement fixation test.[126] Detection of antigens as a control measure may be required in special patients.[126,171]

Relapse is defined as the reappearance of the mycosis in patients who have remained in a state of clinical, radiologic, and serologic recovery for 2 years after discontinuation of antifungal treatment.[126]

LABORATORY DIAGNOSIS

Diagnostic tests are oriented toward visualization of the causative fungus in clinical samples or its isolation in culture.[3,15,53,143,159–161] Measurement of antibodies or detection of antigens in patients' body fluids also constitute valid indirect diagnostic criteria.[159,161,171–173]

P. brasiliensis visualization is not difficult to attain, and procedures such as fresh preparations, wet potassium hydroxide (KOH) mounts, calcofluor whitestaining, plus histopathologic studies have proven adequate.[15,38,39] It should be stated, however, that prompt diagnosis is hindered by the lack of awareness on the part of physicians.

Direct Examination

The sensitivity of direct examinations, mostly KOH preparations, varies from 85% to 100%, depending on the test, specimen, clinical manifestations, and patients' treatment status. In specimens such as sputum, exudates, bronchial washings, and pus, a simple wet mount suffices to reveal *P. brasiliensis* in greater than 85% of the cases.[c] If results are negative, repeated samples should be collected, mucous specimens digested, and samples concentrated by centrifugation. The relatively large size of the fungal yeasts, their translucent walls, and their multiple budding facilitate diagnosis.[d] On occasion, however, single buds and small yeast cells may be confused with other fungi, requiring more extensive observations. Direct *P. brasiliensis* DNA detection by the polymerase chain reaction (PCR) in exudates, tissues, and strain cultures appears promising.[174,175] On the other hand, the value of this procedure is questionable in sera.[176] Mention has been made of cytology as an appropriate diagnostic procedure.[177]

Histopathology

Biopsy is very often diagnostic, with the Grocott-Gomori silver stain being recommended. If the typical multiple-budding yeasts are not abundant, distinction from other fungi (e.g., *Blastomyces dermatitidis, Histoplasma capsulatum, Cryptococcus neoformans*) is necessary.[3,15,125,143] Frequently, with hematoxylin and eosin stains, infected tissues reveal a mixed inflammatory reaction characterized by the presence of granulomas centered on the yeasts, some of which had been phagocytized (Fig. 267.6). The granuloma is further characterized by the presence of neutrophils, mononuclear cells, and epithelioid cells, as well as multinucleated giant cells, with the latter constituting the hallmark of granulomatous inflammation. All these cells appear arranged concentrically around the yeasts in an effort to confine the infectious process.[3,26,30,38,107] Skin and oral lesions reveal pseudoepitheliomatous hyperplasia and intraepithelial microabscesses.[3,38,53,107,108] In the juvenile form, where cutaneous lesions are common and usually multiple,[61] tissue reactions are diffuse and phagocytosis is sparse, with lymph nodes exhibiting hyperplastic germinal centers and increased numbers of plasmocytes, a demonstration of the host's unsuccessful efforts to cope with the invading fungus.[61,107–109,143]

Cultures

Isolation of *P. brasiliensis* in culture proves active disease, but positive cultures are obtained only in 85% of cases and require 20 to 30 days for growth to occur.[e] Various culture media, such as Mycosel agar (BBL [Beckton Dickenson; Franklin Lakes, NJ]) and Sabouraud agar plus antibiotics and the fungal inhibitor cycloheximide, should be used and incubated preferably at room temperature (20°C–25°C).[26,38] Colonies thus obtained require temperature-mediated transformation to the yeast form for confirmation. Direct incubation of samples at 37°C carries the risk of contamination, especially in specimens rich in normal flora, for instance, respiratory secretions. Primary isolation often requires processing multiple samples (e.g., sputum) and using a variety of culture media.[26,38]

Immune-Based Diagnostic Tests

The laboratory diagnosis of paracoccidioidomycosis is achieved through several types of immune-based tests that have specific uses and limitations.[159–161] Antibody and antigen detections are valuable adjuncts to histopathology and culture. More recently, detection assays have used as antigens isolated fractions derived from purified fungal proteins, such as gp43, gp70, and the 87-kDa heat shock protein, thus improving reproducibility and facilitating monitoring antigen clearance during antifungal treatment.[171–173,178]

These tests aim to detect not only anti–*P. brasiliensis* antibodies in serum, cerebrospinal fluid (CSF), and bronchoalveolar lavage fluid[3,26,44,159–161,179] but also to determine the presence of fungal antigens in tissues and body fluids.[171–173,178] As such, they are useful not only for diagnosis but also for follow-up studies.[159,178] The simplest of the antibody detection methods, the agar gel immunodiffusion test, demonstrates circulating antibodies in greater than 90% of active patients, indicating that, if reactive, this test strongly supports the diagnosis; however, antibodies can also be detected years after apparently successful therapy.[3,26,159–161] In Brazil 15% of the healthy population residing in an endemic region was shown to have circulating anti–*P. brasiliensis* antibodies indicative of subclinical infections.[179] Another useful

[c]References 3, 15, 26, 44, 53, 159, 161.
[d]References 3, 15, 44, 47, 53, 159, 161.
[e]References 3, 15, 44, 53, 160, 161.

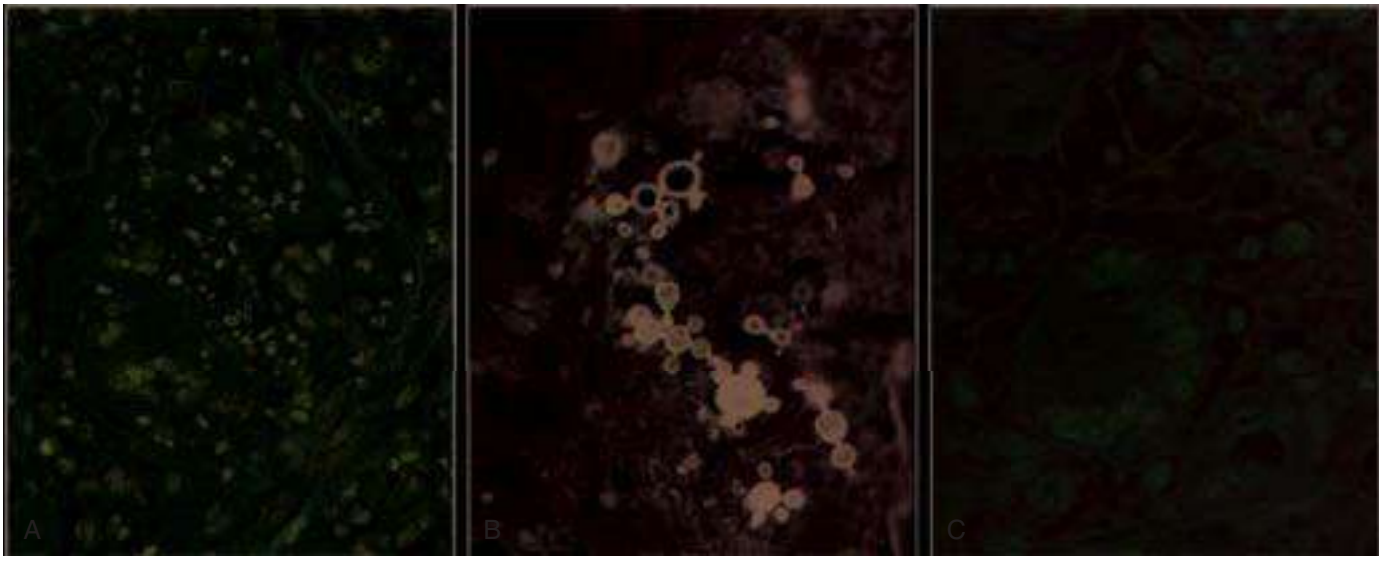

FIG. 267.6 Paracoccidioidomycosis histology. (A) Yeast cells in tissue exhibiting multiple budding and surrounding mononuclear infiltration (hemotoxylin and eosin, ×400). (B) Typical multiple-budding yeast cells (black staining) in a tissue sample (Grocott-Gomori methenamine silver, ×400). (C) Multiple-budding yeast cells inside a multinucleated giant cell in tissue (periodic acid–Schiff, ×400).

procedure, albeit a cumbersome one, is the complement fixation test because its quantitative nature allows a more precise evaluation of the patient's response to treatment. In this test, and in contrast to the immunodiffusion procedure, cross-reactions with *Histoplasma capsulatum* antigens are important.[3,39] Other tests, such as immunofluorescence, counterimmunoelectrophoresis, dot blot test, enzyme-linked immunosorbent assay, and immunoblotting, are also used.[159,161] Improvements in serodiagnosis include the detection of antibodies against chemically characterized or recombinant *P. brasiliensis* antigens, notably gp43, pb27, gp70, and the 87-kDa heat shock protein, with a combination of two recombinant products resulting in increased sensitivity (92%) and specificity (88%).[171–173] Of interest, the gp43 antigen used in diagnostic tests has excellent accuracy in patients infected with *P. brasiliensis* but has low sensitivity in diagnosing *P. lutzii* infections.[126] More recently, use of subtractive phage display with synthetic peptides was applied to the diagnosis of this mycosis with valuable results.[180]

Antigen detection in blood may be preferred for early diagnosis in immunocompromised individuals or when antibody detection appears inconclusive; greater than 60% of the active patients react in the antigenemia test.[44,171,178] Monitoring circulating antigens is also important as a criteria of recovery, whereas parallel testing for antibody levels in the same patients has revealed that titers are rather unpredictable and may persist in those who have cleared all active signs and symptoms of the mycosis.[171,178]

Molecular Testing

Several molecular diagnostic methods, such as PCR, nested PCR, PCR-enzyme immunoassay, real-time (RT) PCR, and loop-mediated isothermal amplification have been successfully used to detect specific *P. brasiliensis* DNA sequences.[172,174,175,181] The preferred target sequences have been the gp43 protein and the ribosomal RNA genes, which have been amply evaluated by means of DNA from cultures, sputum, CSF, and blood from paracoccidioidomycosis patients, as well as from tissues obtained from infected mice.[172,174,175,181] The PCR appeared to be specific and sensitive due to its low detection limit (1.1 pg/μL of DNA) in clinical specimens but not in sera.[176,181] There is no commercially available PCR kit, nor is there a molecular method established for the diagnosis of this mycosis. Nonetheless, a RT-PCR using species-specific primers and a probe that amplifies the Pb27 protein gene is under patent application.[182]

Skin Tests

Paracoccidioidin skin testing is not reliable for diagnosis, mainly because availability of standardized products that meet public health standards do not exist. In addition, it is known that at the time of diagnosis, an important number of active cases (<35%) are proven nonreactive, rendering the test unsatisfactory.[65] Cross-reactions with the histoplasmin intradermal test occur, although the use of the purified gp43 antigen appears to be more specific.[183]

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The complete reference list is available online at Expert Consult.

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268 Uncommon Fungi and Related Species

Duane R. Hospenthal

SHORT VIEW SUMMARY

SCEDOSPORIUM APIOSPERMUM (*PSEUDALLESCHERIA BOYDII*) SPECIES COMPLEX

Definition
- Infection of the lungs, bones and joints, or central nervous system (CNS); may be disseminated.
- Also causes mycetoma (see Chapter 261).

Epidemiology
- Typically occurs in the immunocompromised or following trauma.
- CNS infection in immunocompetent persons after near drowning.
- Organism can be found in soil and fresh water, especially stagnant or polluted.

Microbiology
- *Scedosporium apiospermum, Scedosporium boydii* (formerly *Pseudallescheria boydii*), and *Scedosporium aurantiacum* are the most common species infecting humans.
- Identification is typically made by DNA sequencing. Formerly, identification was based on microscopic structures of organism in culture.

Diagnosis
- Diagnosis is made by culture recovery from infected site.
- Because *S. apiospermum* complex species may colonize airways, sputum cultures may not reflect infection.

Therapy
- Voriconazole is likely the most effective agent.

LOMENTOSPORA (SCEDOSPORIUM) PROLIFICANS

Definition
- Disseminated infection and bone and joint infections are most common.
- *Lomentospora* (formerly *Scedosporium*) *prolificans* may cause onychomycosis and infections of the eye and wounds.

Epidemiology
- Disseminated infection commonly occurs in the severely immunocompromised.
- Localized infection occurs in immunocompetent individuals after trauma.
- The organism can be found in soil and colonizing the respiratory tract, especially in patients with cystic fibrosis.

Microbiology
- Identification is made by culture.

Diagnosis
- Diagnosis is made by culture recovery from the infected site.
- Because *L. prolificans* may colonize airways, sputum cultures may not reflect infection.

Therapy
- No effective therapy. Consider voriconazole with amphotericin B.

DARK-WALLED FUNGI (*BIPOLARIS, EXOPHIALA, EXSEROHILUM, PHIALOPHORA, OCHROCONIS, CURVULARIA,* OTHERS)

Definition
- This infection involves fungi that have melanin in their cell walls and may appear dark walled in tissue.
- Infection is often termed "phaeohyphomycosis" and typically presents as localized skin and soft tissue infections, CNS infections, or allergic sinusitis.
- Dark-walled fungi that cause chromoblastomycosis (see Chapter 260) and mycetoma (see Chapter 261) are not included in the agents of phaeohyphomycosis.

Epidemiology
- Infection is commonly acquired from minor trauma or inhalation.
- Fungi are found in soil, organic material, plants, and air.
- They may be spread through contaminated products (e.g., injectable steroids).

Microbiology
- The most common agents of phaeohyphomycosis are *Alternaria, Bipolaris, Cladophialophora, Curvularia, Exophiala, Exserohilum, Ochroconis,* and *Wangiella.*

Diagnosis
- Diagnosis is made by recovery of these organisms in culture from the site of infection.
- Cell walls may appear dark brown or golden on histopathology (hematoxylin and eosin). Use of a Fontana-Masson stain may allow easier identification of these fungi.

Therapy
- Surgical débridement of lesions or colonizing fungi in the case of allergic fungal sinusitis.
- Amphotericin B in life-threatening infections.
- Voriconazole and itraconazole are typically effective but prolonged therapy is needed.

FUSARIUM SPP.

Definition
- Can cause disseminated infection in immunocompromised patients.
- Common cause of keratitis and other eye infections in contact lens wearers and following trauma.
- Skin and soft tissue infection after trauma, onychomycosis; can cause mycetoma.

Epidemiology
- Common plant pathogens; found in soil and organic debris.
- Have been recovered in hospital water supplies.

Microbiology
- The most common species infecting humans belong to one of three species complexes: *Fusarium solani, Fusarium oxysporum,* or *Fusarium fujikuroi,* although the number of species identified as causing infection is increasing as molecular methods of identification have supplanted morphology.
- *Fusarium* produces banana- (or crescent)-shaped multicellular macroconidia in culture.

Diagnosis
- Recovery of the fungus from culture of an otherwise sterile site.
- One of the few molds that are commonly recovered from blood culture.

Therapy
- Optimum therapy is not known.
- Recovery from neutropenia is essential in response to therapy of disseminated infection.
- Amphotericin B or voriconazole is suggested.

TRICHOSPORON SPP.

Definition
- Most commonly presents as disseminated infection.

Epidemiology
- Typically an infection of the immunocompromised; may be associated with central venous catheter.
- Fungi found colonizing the skin, gastrointestinal, respiratory, or genital tract.
- Found in soil and water.
- Breakthrough infections in patients receiving echinocandins.

Microbiology
- Most common pathogen is *Trichosporon asahii.*

SHORT VIEW SUMMARY—cont'd

- *Trichosporon* are identified by their unique ability to produce septate hyphae, arthroconidia, and budding yeast.
- Appears yeastlike on initial culture.

Diagnosis
- By recovery of the organism from the blood or lesion biopsy.

Therapy
- Azole antifungal (voriconazole, itraconazole, isavuconazole, posaconazole, or fluconazole).

MALASSEZIA FURFUR

Definition
- Catheter-related bloodstream infection.
- Also causes pityriasis versicolor (see Chapter 266).

Epidemiology
- Typically associated with parenteral lipid infusion.
- Commonly reported in neonates.

Microbiology
- May be difficult to grow from positive blood cultures without lipid supplementation.

Diagnosis
- Recovery of fungus from blood culture.

Therapy
- Catheter removal and discontinuation of parenteral lipids.
- Susceptible to most antifungals (voriconazole, fluconazole, or amphotericin B).
- Limiting use of parenteral lipids.

OTHER UNCOMMON YEASTS

Definition
- Other less common yeasts may also cause catheter-related bloodstream infection.

Epidemiology
- Typically associated with use of central venous catheters and immunocompromise.

Microbiology
- Include *Magnusiomyces capitatus* (formerly called *Saprochaete capitata* and *Blastoschizomyces capitatus*), *Pichia anomala, Rhodotorula* spp., and *Saccharomyces cerevisiae.*

Diagnosis
- Made by recovery of the yeast in blood culture.

Therapy
- Removal of central venous catheter.
- Antifungals based on recovered yeast.

TALAROMYCES (PENICILLIUM) MARNEFFEI

Definition
- Acute disseminated infection of persons infected with human immunodeficiency virus (HIV) in Southeast Asia.
- Infection similar to acute disseminated histoplasmosis in patients with acquired immunodeficiency syndrome.
- Rarely, localized infection in apparently immunocompetent persons.

Epidemiology
- Limited to Southeast Asia. More common in rainy season, in young adult males with HIV infection, typically with low CD4 cell counts.

Microbiology
- Typical *Penicillium*-like structures on microscopic examination of culture.
- Cultures produce red pigment that diffuses into the agar.

Diagnosis
- Typically made from recovery of the organism in blood.
- May also be recovered in culture of skin lesions, lymph nodes, or bone marrow aspirates.
- Serologic testing may be available in endemic regions.

Therapy
- Amphotericin B in life-threatening presentations.
- Itraconazole or voriconazole in initial therapy (not life-threatening).
- Itraconazole secondary prophylaxis in HIV-infected patients.

LACAZIA LOBOI

Definition
- Chronic nodular or keloidal skin infection, commonly of the ears or face.

Epidemiology
- Limited to Central and South America.
- Infection also found in dolphins.

Microbiology
- Has not been recovered in culture.
- Identified as closely related to *Paracoccidioides brasiliensis* by molecular techniques.

Diagnosis
- Based on clinical presentation and finding typical structures on histopathology.
- Globose (yeast) cells end-to-end in short "strings."

Therapy
- Surgical removal.

AGENTS OF ADIASPIROMYCOSIS (*EMMONSIA* SPP.)

Definition
- Disease secondary to host immune response to nonreplicating fungal conidia, termed "adiaspores."
- Chiefly a pulmonary disease; may range from asymptomatic to rapidly progressing respiratory failure and occasionally death.
- May also present as ocular nodules.

Epidemiology
- Seen with occupational inhalation of dusts in men, average age 40.
- Outbreak in Brazilian children in association with diving and freshwater sponges.

Microbiology
- Secondary to *Emmonsia* spp., usually *Emmonsia crescens.*
- Dimorphic fungi closely related to *Blastomyces dermatitidis.*

Diagnosis
- Identification is limited to observation of typical structures on histopathology showing adiaspores, up to 500 μm in diameter, nondividing, and surrounded by granulomata composed of epithelioid and giant cells.

Therapy
- Corticosteroids appear to be useful.

EMERGOMYCES AFRICANUS

Definition
- Disseminated infection most commonly afflicting HIV-infected persons.

Epidemiology
- Severely immunocompromised persons.
- Largest report from South Africa.

Microbiology
- Thermally dimorphic fungus of soil.

Diagnosis
- Recovery of the dimorphic fungus from skin biopsy or blood culture.

Therapy
- Responses to amphotericin B and triazole antifungals have been reported.

PROTOTHECA SPP.

Definition
- Localized skin or subcutaneous infection caused by algal pathogen.
- Rare reports of disseminated or deep infection.

Epidemiology
- Likely cause infection after traumatic inoculation.
- Organisms colonize skin, gastrointestinal and respiratory tracts.

Microbiology
- Disease is typically due to *Prototheca wickerhamii* or *Prototheca zopfii.*
- Unicellular algae lack chlorophyll.
- *Prototheca* spp. grow on fungal culture media with yeastlike colonial morphology.
- Microscopic appearance in tissue is diagnostic.

Diagnosis
- Recovery of algae in culture is diagnostic.
- Yeast biochemical panels commonly identify *Prototheca.*

Therapy
- Surgical excision with amphotericin B or itraconazole.

PYTHIUM SPP.

Definition
- Vascular infections in persons with iron overload, such as thalassemia, or ocular infections following trauma.

SHORT VIEW SUMMARY—cont'd

- Skin and subcutaneous and disseminated infection possible.

Epidemiology
- Worldwide distribution, but most commonly reported from Thailand.
- Risk factors: thalassemia-hemoglobinopathy syndrome and trauma.
- Presumed association with work in swampy (e.g., rice paddy) environment.
- High morbidity (eye or limb loss) and mortality.

Microbiology
- Fungus-like protist, *Pythium* grows rapidly as mold on fungal culture.

Diagnosis
- Recovery of the organism from culture is diagnostic.
- Local serologic testing may be available.

Therapy
- Can perform surgical resection or amputation.
- No satisfactory medical therapy is known.
- Doxycycline, minocycline, tigecycline, linezolid, and macrolides have activity in vitro.

RHINOSPORIDIUM SEEBERI

Definition
- Localized polypoidal infection, chiefly of the nose, upper airway, and conjunctiva.

Epidemiology
- Worldwide distribution, but most commonly reported from southern India and Sri Lanka.

Microbiology
- Has not been grown in culture.
- Identified as a protist by molecular methods.

Diagnosis
- Made by microscopic observation of typical structures: thick-walled cysts (100–350 μm) filled with numerous spores.

Therapy
- Surgical excision with electrocautery of lesion base.

SCEDOSPORIUM APIOSPERMUM (PSEUDALLESCHERIA BOYDII) SPECIES COMPLEX

In humans, infection with *Scedosporium apiospermum, Scedosporium boydii* (formerly *Pseudallescheria boydii*) *and Scedosporium aurantiacum* (collectively, the *S. apiospermum* species complex)[1,2] can produce two distinct diseases: mycetoma and scedosporiosis (pseudallescheriasis). Mycetoma is a chronic subcutaneous infection characterized by the production of grains (see Chapter 261), whereas scedosporiosis includes all other infections caused by *Scedosporium* spp. The most common sites of *Scedosporium* infections are lung, bone, joints, and the central nervous system (CNS).[3,4] Sinusitis, keratitis, endophthalmitis, skin and soft tissue infections, prostatitis, and endocarditis have also been described. These fungi are found in soil and fresh water, especially stagnant or polluted water, throughout the world. Disease is acquired after inhalation of this organism into the lungs or paranasal sinuses or after traumatic inoculation through the skin. There are more than a dozen reported cases of *Scedosporium*-related pneumonia after near drowning in contaminated water. Although colonization is more common than infection with this organism, an invasive pulmonary disease similar to invasive pulmonary aspergillosis occurs, usually in immunocompromised patients. Local trauma is the most common cause of eye, soft tissue, and osteoarticular infections in previously healthy persons. CNS infection is seen in both immunocompromised and healthy individuals.[5] Infections in immunocompetent patients usually have subacute to chronic courses, whereas those in immunocompromised patients are frequently acute and severe.

Scedosporium can colonize bronchiectatic lungs, including those of patients with cystic fibrosis, or intermittently obstructed paranasal sinuses. Masses of hyphae (fungus balls) have been found in lung cavities.[6] *Scedosporium* has also been reported as a cause of allergic bronchopulmonary disease (similar to allergic bronchopulmonary aspergillosis),[7] pleural space infection, lung abscess, pneumonia (including aspiration pneumonia), and invasive sinusitis.[8] As with invasive pulmonary aspergillosis, invasive pulmonary scedosporiosis most commonly occurs in patients with prolonged neutropenia, those receiving prolonged high-dose corticosteroid therapy, or those who have undergone allogeneic bone marrow transplantation.[9] Invasive pulmonary disease with associated dissemination appears common[10] and has also occurred in patients with acquired immunodeficiency syndrome (AIDS) and after solid-organ transplantation.[11] Pulmonary disease in severely immunocompromised patients usually manifests with fever, cough, pleuritic pain, and often hemoptysis. Chest radiography may show areas of nodularity, alveolar infiltrates, consolidation, or cavitation.[6] The classic signs of invasive pulmonary aspergillosis, the halo or air crescent signs, may also be seen in invasive pulmonary scedosporiosis. Disseminated disease that manifests with only painful cutaneous nodules or endophthalmitis has also been described in immunocompromised patients.[12,13] Invasive pulmonary disease with extension to the vertebrae has been described in a patient without apparent immunocompromise.[14]



FIG. 268.1 *Scedosporium* (formerly *Pseudallescheria*) *boydii* olecranon bursitis in a corticosteroid-treated patient who fell on his elbow in the garden. Photograph shows the incision site over the subcutaneous abscess, which began in the bursa.

Localized disease—including infections of the eye, bone, cutaneous tissue, subcutaneous tissue (Fig. 268.1), and osteoarticular tissue—may be seen both with and without immunocompromise. Infection is commonly initiated through traumatic implantation of the fungus from soil or water. Surgery, intravenous drug injection, and repeated corticosteroid injections have less frequently been associated with localized infections.[15] Osteoarticular infection in immunocompetent patients often manifests as a painful, swollen joint with overlying erythema after penetrating injury. In occasional patients, weeks to even years may pass between antecedent trauma and the development of septic arthritis.[16,17]

Brain abscesses may result from a known or unsuspected lung lesion in immunocompromised patients, including those with AIDS.[5,18] CNS infection appears to be disproportionately prevalent among patients infected with *Scedosporium* spp., in comparison with many other mycoses. For example, of 23 solid-organ transplant recipients with *Scedosporium* spp. infections, 11 (48%) had CNS involvement.[11] Cerebral abscesses are usually multiple; in immunocompetent hosts, they are often reported in association with near drownings in polluted water, such as ponds, pig troughs, and roadside ditches.[5] CNS infection from contiguous spread of sinusitis[19] and after penetrating trauma[20] has also been described. Indolent, severe neutrophilic meningitis has been reported occasionally, usually in patients with intravenous drug abuse or human immunodeficiency virus (HIV) infection. Cerebrospinal fluid culture

and smear have yielded negative results; the diagnosis was made at autopsy. The first described human case of *Scedosporium* spp. was meningitis that was probably iatrogenic after lumbar puncture for the administration of anesthesia.[21]

Isolation of *Scedosporium* spp. from normally sterile sites is diagnostic. Only rarely are *Scedosporium* spp. cultured from blood.[4] Growth of the organism from sputum, bronchoalveolar lavage, draining wounds, or paranasal sinus aspirates is less convincing evidence of infection, unless it is accompanied by hyphae on smear or biopsy. Histologically, *Scedosporium* spp. resemble *Aspergillus* spp., with dichotomously branching septate hyphae seen in tissue, although branching off to the side at a 60- to 70-degree angle, instead of Y-shaped dichotomous branching, is more common in scedosporiosis.[22] Air-containing spaces, such as a paranasal sinus, can permit sporulation. In addition to hyphae and conidia, distinctive coremia or an ascocarp may indicate *Scedosporium* as the mold. In neutropenic patients, blood vessel invasion and thrombosis are usual. The fungus grows well in standard mycologic media. After a few days, the mold colony takes on a tan color and has sporulating structures that are quite different from those of *Aspergillus.* Cultures can produce either asexual conidia or the sexual reproductive structure, cleistothecia. Identification is usually by DNA sequencing, but matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is being used increasingly for identification of the mold.

Effective therapy of scedosporiosis remains elusive. In vitro and clinical resistance to amphotericin B, as well as breakthrough infections, have been reported repeatedly. Surgical débridement has been an important adjunct in treatment of soft tissue, bone, joint, and pleural and paranasal sinuses, although it is not curative by itself. Intraarticular instillation of amphotericin B may have contributed to success of treatment in a few patients. The rate of mortality with brain abscess has traditionally been noted to exceed 75%.[5] Susceptibility to azoles and echinocandins in vitro has proven variable.[3,23–25,26,27] Voriconazole has the best activity in vitro and is often considered the drug of choice, but reducing the level of immunosuppression is vital.[28,29,30,31] Response to posaconazole was achieved in a single reported case of brain abscess.[32] Voriconazole is approved by the US Food and Drug Administration (FDA) for patients with *Scedosporium* spp. infection refractory to other approved antifungal agents and for patients who cannot tolerate those agents. This indication was based on success reported in 15 of 24 patients treated with this agent (including 6 of 10 with CNS infection). Because of poor response to the only approved agent, amphotericin B, most experts believe that voriconazole is the drug of choice in the treatment of scedosporiosis.

LOMENTOSPORA (FORMERLY SCEDOSPORIUM) PROLIFICANS

Lomentospora prolificans (formerly *Scedosporium prolificans* or *Scedosporium inflatum*), a fungus found in soil, was first described in 1984 as an agent of human disease.[33] Since that time at least 161 additional cases have been reported. Infection can occur in both immunocompromised and immunocompetent patients. Patients with intact immunity most frequently have focal infections (usually osteoarticular) associated with trauma, whereas immunocompromised persons most frequently have disseminated disease, associated with malignancy.[34] In one review, only 34 of 162 patients (21%) were noted to have no underlying disease; 72 of the 162 patients (44%) had disseminated infection.[34]

In immunocompetent patients, infection is usually localized and associated with trauma, including surgery.[34–36] These cases have included infections of bone and joints (Fig. 268.2), eye, or wounds and onychomycosis. Immunocompromised patients, commonly those undergoing cytoreductive chemotherapy or bone marrow transplantation, present with fungemia and fever with neutropenia.[9,10,37,38] Skin lesions, myalgia, endophthalmitis, endocarditis,[39] and pulmonary infiltrates have been described in this setting.[34,40] Skin lesions have been described as a papular rash, later becoming necrotic. Disseminated disease without neutropenia has been described in patients who have undergone lung and kidney transplantation.[41] Fatal localized CNS infection was reported in a child with acute leukemia who had received six intrathecal chemotherapeutic injections.[42] *L. prolificans* has also been recovered from the external ear and sputum of patients without apparent disease. Sputum colonization has been observed in patients with AIDS and cystic fibrosis and in those who have undergone liver or lung transplantation.[36,43–46]



FIG. 268.2 *Lomentospora* (formerly *Scedosporium) prolificans* septic arthritis of the knee in an otherwise healthy 12-year-old boy. This condition developed in association with a splinter obtained during a playground fall. Magnetic resonance imaging depicts osteomyelitis of the medial condyle.

Diagnosis is most commonly established by the recovery of the organism from culture of infected sites, including skin biopsy samples. Disseminated disease in immunocompromised patients is usually diagnosed through blood culture.[34,38] Identification of *L. prolificans* is based chiefly on DNA sequencing or the morphologic characteristics of the asexual structures produced by the mold in culture.[47,48]

No antifungal therapy currently available is effective in treating these infections. *L. prolificans* appears to be intrinsically resistant to most antifungals.[2,3,27,31] Successful therapy of joint infections has, however, been reported with the use of surgical débridement with or without intraarticular amphotericin B. Disseminated infection is usually resistant to antifungal agents and carries a high mortality rate.[34,37] Survival was reported in one patient with disseminated disease and neutropenia who received granulocyte colony-stimulating factor (G-CSF) and amphotericin B, followed by itraconazole.[38] In one animal model, liposomal amphotericin B with the addition of G-CSF improved survival.[49] Of the currently available antifungal agents, voriconazole appears most promising in vitro, with better activity than amphotericin B, itraconazole, or posaconazole.[2,27] Unfortunately, current dosing regimens of voriconazole are not associated with serum concentrations at which the drug appears to be effective in vitro. One report described a 44% response rate (16 of 36 patients) with voriconazole.[28] The investigational azole, albaconazole (UR-9825), appears more active than voriconazole in vitro and has shown potential in one animal model.[27,50] Because of the in vitro and in vivo resistance of *L. prolificans* to currently available agents, the effect of combining agents has been examined. In laboratory studies, synergy has been shown through the use of combinations of amphotericin B with pentamidine[51] and of terbinafine with voriconazole, itraconazole, or miconazole.[52,53] Clinical support for this in vitro synergy is limited, although anecdotal experiences have been reported with voriconazole and terbinafine.[54,55]

DARK-WALLED FUNGI AND AGENTS OF PHAEOHYPHOMYCOSIS

Phaeohyphomycosis is a loosely defined term used to group infections caused by molds (and a few yeasts) that produce dark cell walls. Also described as *dematiaceous,* these are a diverse group of fungi found in

TABLE 268.1 Cutaneous and Subcutaneous Infections Caused by Dark-Walled Fungi

DISEASE	LESIONS	PATHOLOGIC FEATURES	ORGANISMS[a]
Chromoblastomycosis	Scaly, friable, often verrucous nodules, commonly pruritic	Muriform cells (golden brown cells with cross walls in more than one plane)	*Fonsecaea* spp., *Cladophialophora carrionii, Phialophora verrucosa, Rhinocladiella aquaspersa*
Mycetoma (eumycetoma, eumycotic mycetoma)	Nodular with draining sinuses, areas of healing	Grains composed of septate hyphae	*Madurella mycetomatis, Falciformispora* (formerly *Leptosphaeria*) *senegalensis, Trematosphaeria* (formerly *Madurella*) *grisea*
Subcutaneous phaeohyphomycosis	Painless, subcutaneous nodules	Septate hyphae (pseudohyphae or yeasts may also be apparent)	*Curvularia* (formerly *Bipolaris*) spp., *Exophiala* spp., *Exserohilum* spp., *Phaeoacremonium* spp., *Phialophora*

[a]The most common causes are listed. See individual chapters for more complete listings (Chapters 260 and 261).



FIG. 268.3 Phaeohyphomycosis manifesting as a cyst. *(From Chandler FW, Watts JC. Phaeohyphomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

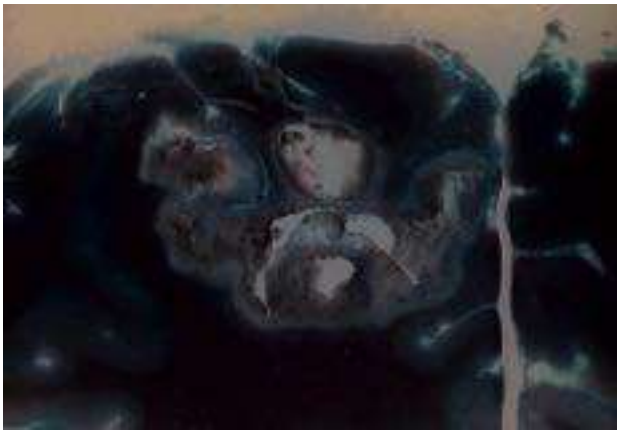

FIG. 268.4 Phaeohyphomycosis of the brain caused by *Cladophialophora* sp. *(From Chandler FW, Watts JC. Phaeohyphomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

the soil and air and growing on plants and in organic debris. The number of genera and species of fungi causing phaeohyphomycosis is quite large.[56] Frequent changes in species names have compounded the difficulty in comparing similar cases from the literature. Chromoblastomycosis (see Chapter 260) and mycetoma (see Chapter 261) are distinct infections that include dark-walled fungi as etiologic agents that are generally not included in this loose classification (Table 268.1). The syndromes most commonly produced by the dark-walled fungi include cutaneous and subcutaneous disease (other than chromomycosis or mycetoma), brain abscesses, and sinusitis. Fungemia[57] and disseminated disease[58] have more commonly been described in immunocompromised individuals. Meningitis, pneumonia, prosthetic valve endocarditis, contamination of saline-filled breast implants, infections in peritoneal dialysis and central venous catheters, osteomyelitis, and septic arthritis have also been reported. For most clinical purposes, it is preferable to describe disease by the type of infection and species name, such as "*Cladophialophora bantiana* brain abscess," and to reserve the term *phaeohyphomycosis* for cases in which no culture data exist or in which recovered fungi have not yet been identified.

Subcutaneous phaeohyphomycosis typically begins as a single red nodule, usually on the extremities. In an immunocompetent person, an indolent, painless expansion in the skin and subcutaneous tissue occurs, sometimes with cyst formation (Fig. 268.3). More rapid local progression and, in rare cases, extension to the brain can occur in immunosuppressed patients. A history of minor trauma is often present, or a splinter is found in the resected lesion. The fungi causing subcutaneous phaeohyphomycosis are extraordinarily diverse, but species of *Exophiala, Exserohilum, Phialophora,* and *Curvularia* (which includes most clinical species formerly in the genus *Bipolaris*)[59] are particularly common.

Brain abscess is one of the best-described syndromes produced by the dark-walled fungi.[60,61] Disease manifests with headache of indolent onset, low-grade or no fever, and development of focal neurologic signs. There is rarely a history of exposure to dust or mold, no obvious pulmonary portal, and no evidence of dissemination outside the CNS.



FIG. 268.5 Weakly pigmented, segmented hyphae of *Cladophialophora bantiana* in the wet mount of pus from the base of the brain.

Affected male patients have outnumbered female patients 3:1, the median age of diagnosis is 38 years, and most patients have been immunocompetent.[61,62–63] Abscesses may be single or multiple and, on computed tomography or magnetic resonance imaging, are well localized within the cerebral cortex (Fig. 268.4).[64] Purulent meningitis, with or without brain abscess, may also be observed (Fig. 268.5).[65] Hematoxylin and

eosin (H&E) staining reveals abscesses to have purulent centers with surrounding granulomatous reaction, and organisms appear as septate hyphae with golden brown cell walls. As in other forms of infections with the dark-walled fungi, hyphae are commonly irregular in diameter, and yeastlike cells are seen with some species. The species most commonly causing these infections is *C. bantiana* (previously named *Xylohypha bantiana, Cladosporium bantianum,* and *Cladosporium trichoides*), but disease is also caused by *Rhinocladiella* (formerly *Ramichloridium*) *mackenziei, Ochroconis gallopava* (formerly known as *Dactylaria constricta* var. *gallopava*), *Exophiala (Wangiella) dermatitidis, Curvularia* (formerly *Bipolaris*) *spicifera, Curvularia* (formerly *Bipolaris*) *hawaiiensis, Chaetomium* spp., and, even more rarely, other phaeohyphomycetes.[61,63,66] *R. mackenziei* infections are reported chiefly from the Middle East and India,[67,68] and *E. dermatitidis* cases predominate in the Far East. *Fonsecaea monophora* (formerly classified with *Fonsecaea pedrosoi*) has been recognized as an etiologic agent of CNS phaeohyphomycosis.[69]

Iatrogenic meningitis and other infections related to epidural injections of corticosteroids have been reported in two recent outbreaks traced to environmental contamination at compounding pharmacies. The first report that involved infection with *E. dermatitidis* resulted in four cases of meningitis and one case of sacroiliitis in 2002.[70] The second and more recent outbreak included meningitis due to *Exserohilum rostratum*. Between September and December of 2012, 590 cases of infection and 37 deaths were confirmed among the 13,534 people potentially exposed to the contaminated lots of methylprednisolone.[71] Early cases usually presented with meningitis, some with stroke from invasion of arteries in the basilar meninges.[72] Later cases more often presented as more localized disease, depending on the site injected with steroid. Lumbar injections were most common, leading to epidural abscess with increased lumbar pain and some with cauda equina syndrome. Indolent septic arthritis occurred in the sacroiliac joint or rarely in injected peripheral joints. Although hyphae were commonly found in surgical specimens, cultures were only positive for *E. rostratum* in 14% and polymerase chain reaction in 29%.[73] Amphotericin B seemed the most effective treatment but was followed by several months of voriconazole therapy. The optimal course of treatment and prevention of relapse is not yet known.

Twenty-one surgical site infections with *Bipolaris* (currently *Curvularia*) spp. were reported after cardiothoracic surgery in 10 hospitals during 2008–2013. Most infections were in the mediastinum or sternal wound. Delayed wound closure was the most frequent predisposing factor.[74]

Allergic fungal sinusitis may be caused by a wide variety of fungi, although the dark-walled fungi (usually *Bipolaris, Exserohilum, Curvularia,* or *Alternaria* spp.) and *Aspergillus* spp. are the most common causes.[75] By definition, disease is allergic and confined to the lumen of the paranasal sinuses. Patients present with an indolent onset of sinus pain or painless proptosis. A history of seasonal or allergic rhinitis is common, and there may be a history of nasal polyps. On computed tomography or magnetic resonance imaging, one or more paranasal sinuses appear full of fluid, with outward pressure on the thinner bony sinus walls, such as the lamina papyracea, medial maxillary wall, or midline sphenoidal septum. Maxillary and ethmoid sinuses are usually involved, but sphenoid and frontal sinuses may be diseased (Fig. 268.6). Surgical débridement of the paranasal sinus removes dark, inspissated mucus; histopathologic examination reveals that this mucus has eosinophils with Charcot-Leyden crystals (degenerated eosinophils) and scattered septate hyphae.[76] The walls of the hyphae may not appear as dark as those seen in brain abscess. Irregular diameter and bulbous swellings may help distinguish these hyphae from *Aspergillus,* but culture is essential for diagnosis. The most serious sequela of allergic fungal sinusitis is brain invasion, which, when it occurs, usually does so in immunocompromised hosts (Fig. 268.7). Extension from the ethmoid or frontal sinus into the frontal lobe of the brain can be clinically silent. Erosion into the frontal lobe, clivus, pterygoid space, or middle fossa occurs but is rare. Sudden blindness can result from compression of the optic nerve posterior to the orbital fissure. Compression of the orbit by lateral bulging of the lamina papyracea does not decrease visual acuity but does cause proptosis.

Diagnosis of these infections requires observation of the fungi invading tissue or recovery of the fungi in culture from an otherwise sterile site.

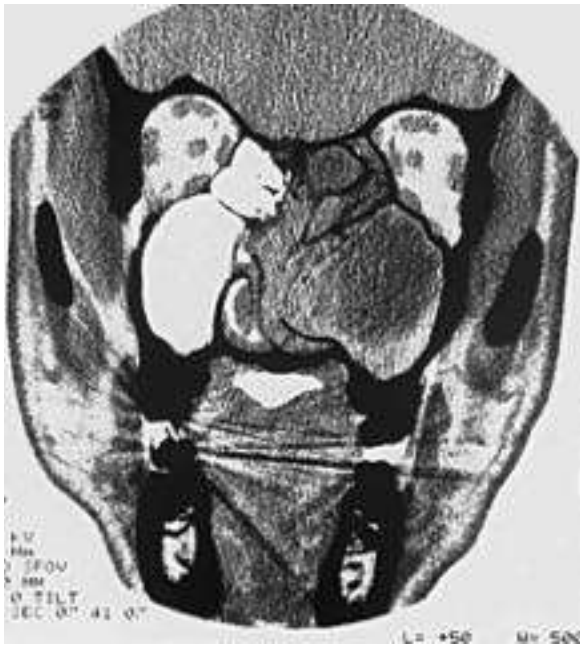

FIG. 268.6 Computed tomographic scan showing outward-bulging mass in the maxillary and ethmoid sinuses of a patient with allergic fungal sinusitis.

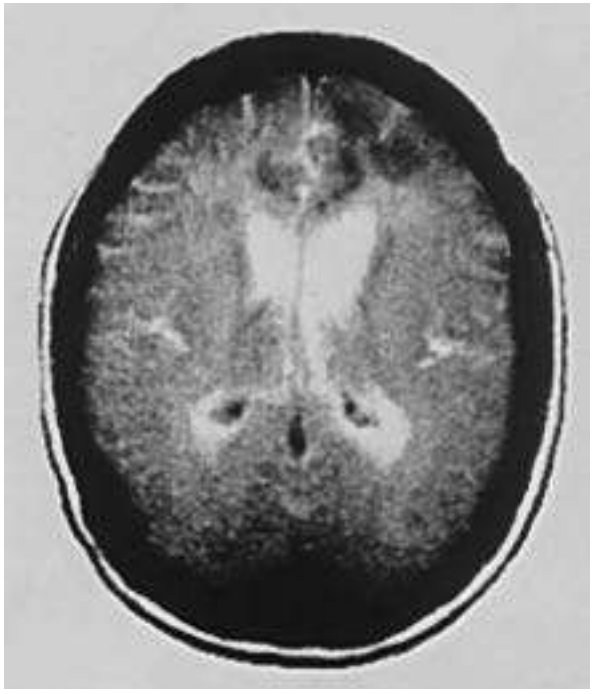

FIG. 268.7 T2-weighted magnetic resonance image depicting extension into the frontal lobe of a patient with *Curvularia* (formerly *Bipolaris*) *hawaiiensis* sinusitis.

Typical pathology is necessary for the diagnosis of allergic fungal sinusitis. In disease outside the CNS, these organisms may not always appear dark walled on standard histopathologic stains. Cell wall melanin may be visible as a brownish-yellow color on H&E stain (Fig. 268.8). If melanin is not evident on fresh preparations of H&E stain, it can be stained by the Fontana-Masson method, which better enables diagnosis, especially if culture results are negative or if culture is not performed.[77] Fontana-Masson stain is, however, not 100% specific for the dark-walled fungi because the cell walls of some *Aspergillus* and other fungi with hyaline hyphae have been shown to stain dark with this method.[78]



FIG. 268.8 Cutaneous phaeohyphomycosis caused by *Exophiala jeanselmei.* Note the brown hyphae. (Hematoxylin and eosin stain.) *(From Chandler FW, Watts JC. Phaeohyphomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

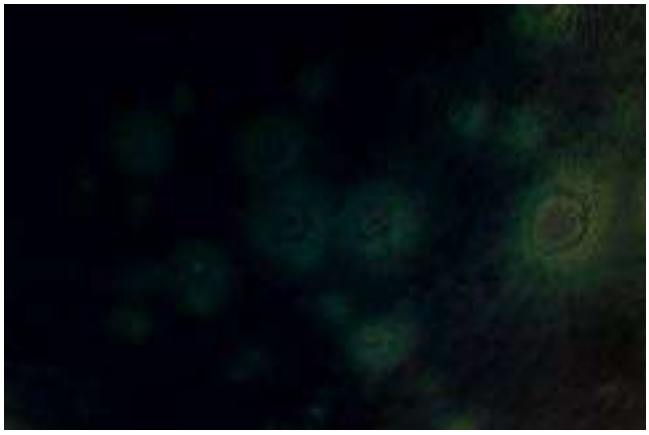

FIG. 268.9 Cutaneous lesions of disseminated infection caused by *Fusarium moniliforme.* *(From Beneke ES, Rogers AL.* Medical Mycology and Human Mycoses. *Belmont, CA: Star Publishing Company; 1996.)*

Surgical débridement is essential to the cure of most of the infections caused by the dark-walled fungi. Good surgical curettage often suffices in treating allergic sinusitis if the cranial cavity has not been invaded. Amphotericin B is probably the drug of choice for life-threatening infection, including CNS infection. Itraconazole has been used frequently with success in infections that are not life threatening.[79,80] To help prevent further recurrence in patients who have recurrent allergic fungal sinusitis, long-term itraconazole therapy may be used after repeated surgical drainage. In vitro, in response to most of these fungi, voriconazole commonly produces minimal inhibitory concentrations (MICs) that are similar to or lower than those seen with itraconazole, which makes this new drug a potentially useful therapeutic agent. Posaconazole and caspofungin have also been shown to have in vitro activity against many of these fungi. Posaconazole has been reported to produce a good clinical response in single cases of CNS and disseminated infection caused by *R. mackenziei* and *Exophiala spinifera,* respectively.[81,82] Response of a skin and soft tissue infection to terbinafine, after poor response to amphotericin B and itraconazole, has also been reported.[83]

FUSARIUM SPP.

Species in the genus *Fusarium* are common in soil and organic debris and are frequently the cause of disease in plants. Current taxonomy divides the species causing infections in humans into seven species complexes, called *Fusarium solani, Fusarium oxysporum, Fusarium fujikuroi, Fusarium dimerum, Fusarium chlamydosporum, Fusarium incarnatum-equiseti,* and the complex including *Fusarium sporotrichioides.* The first three species complexes in the list are those most commonly encountered clinically. *Acremonium falciforme* has been moved to the *F. solani* spp. complex and renamed *Fusarium falciforme.* The most common manifestations of fusariosis are keratitis[84] and onychomycosis.[85] Infection of soft tissue, including mycetoma, can occur after traumatic inoculation in the healthy host. Inhalation into the lung or paranasal sinus or minor trauma to the skin can lead to fusariosis in immunocompromised patients.[86,87]

Rare cases of dissemination have been described in the clinical setting of severe burns,[88] trauma,[89] and heat stroke.[90] Most commonly, however, fusariosis occurs in patients with acute leukemia (70%–80% of cases)[91,92] and prolonged neutropenia (>90% of cases).[91,93] In one review of 43 patients, the median duration of neutropenia was more than 3 weeks.[91] Fusariosis is also increasingly reported in patients receiving allogeneic hematopoietic stem cell transplants.[94] The portal of entry in most of these cases of disseminated infection is not known. Inhalation, ingestion, and entry through skin trauma have been suggested. Sinusitis has preceded dissemination in a few reports. Hematogenous spread has been attributed to indwelling intravascular catheters.[95,96] Onychomycosis with paronychia has appeared to be a source of disseminated infection in some patients.[91,97] Water has been suggested as a source of these infections; the fungus was found in one hospital water supply system and in several water sources at a dialysis center.[98,99]



FIG. 268.10 Multiple necrotic skin lesions in a neutropenic patient with hematogenously disseminated fusariosis.

Infection commonly manifests with fever and myalgia unresponsive to broad-spectrum antibacterial antibiotics during periods of profound neutropenia. Disseminated fusariosis has been recognized in patients who have been receiving empirical or prophylactic antifungal therapy.[92,94,100–103] Skin lesions occur in 60% to 80% of infections, usually appearing as multiple papules or deep-set, painful nodules (Fig. 268.9). They may initially be flat (macular) with a central pallor, but later they become raised, erythematous, and necrotic (Fig. 268.10).[86,91,93] Lesions are most common on the extremities but have been reported on the trunk and face as well. In profoundly neutropenic patients, this infection can progress rapidly to death, in a manner similar to that in invasive aspergillosis. Skin lesions, denoting dissemination, can occur within a day of the onset of fever. In patients whose neutrophil levels recover, the infection can progress slowly over weeks until death or can become controlled and eventually cured.

Recovery of the fungus from blood and skin lesion biopsy are the two most common and effective ways to diagnose this infection. In contrast to aspergillosis, in which blood culture results are nearly always negative, fusariosis is accompanied by positive blood culture results about 50% of the time (48 of 98 patients in one review[91]). The septate hyphae of *Fusarium* spp. have both acute and right-angle branching and usually appear similar to those of *Aspergillus* on histopathology. Hyphae are often difficult to visualize with routine H&E staining but are easily identified when tissue is stained with Gomori methenamine

silver or periodic acid–Schiff. *Fusarium,* unlike *Aspergillus,* can sporulate in deep tissue by a process called adventitious sporulation. Identification of these spores along with hyphae can suggest the diagnosis. Conidia (spores) in tissue do not resemble the banana-shaped, septate conidia in culture but are ellipsoidal, spherical, or cylindrical and not septated.[104] They may be seen free or attached to the end of a hypha. Gram stain of a blood culture may also show these spores. The spore-bearing structure, called a phialide, may appear as a flat, truncated hyphal end if the spore has become detached.[104] In culture, the characteristic feature of *Fusarium* is the production of sickle (banana)-shaped multiseptate macroconidia.[105] Identification beyond the genus level is best done by molecular methods, with multilocus sequence typing currently favored.[106] MALDI-TOF MS has the promise of rapid identification of *Fusarium* isolates, although not necessarily to the species level.[106] Patients with fusariosis may have positive beta-D-glucan tests and *Aspergillus* galactomannan assays in serum.[107]

The optimal treatment for disseminated fusariosis has not been established. Overall mortality with this infection has been reported to range from 50% to 80%.[87,94] Survival is almost always associated with the recovery from neutropenia, although corticosteroid use also impairs response to therapy.[91,93,108] In an analysis of responders versus nonresponders in one study of 43 patients, investigators noted associations with malignancy in remission (100% vs. 10%), adequate neutrophil counts (100% vs. 0%), and lack of significant (grade II or greater) graft-versus-host disease (0% vs. 66%).[91] Removal of indwelling venous catheters has been associated with improvement and thus should be considered in all cases of fungemia.[96] Surgical débridement of soft tissue infections resulting from trauma is helpful. Currently, voriconazole or a lipid formulation of amphotericin B is favored for deep infections.[31] Natamycin 5% ophthalmic suspension is used for keratitis (see Chapter 113). Echinocandins are not recommended for deep infections, although terbinafine, posaconazole, and combination regimens have been reported as useful.[109–111,112,113,114,115] Animal models have shown liposomal amphotericin B,[116] posaconazole,[117] and voriconazole[118] to be potentially useful. On the basis of successful therapy in 9 of 21 patients (43%) who received voriconazole, the FDA approved this agent for second-line use in fusariosis. Successful therapy in 10 of 21 patients (48%) with posaconazole has also been reported.[119] Unfortunately, cases of breakthrough infection in patients receiving voriconazole or posaconazole have been reported.[101–103] The addition of colony-stimulating factors (G-CSF or granulocyte-macrophage colony-stimulating factor) or granulocyte transfusions to specific antifungal therapy have also been reported,[86,91,93,120] but the benefit of these adjunctive therapies is also not proven.

OTHER OPPORTUNISTIC MOLDS

In immunocompromised hosts, virtually any of the normally nonpathogenic fungi may cause disease. In addition to more common infections caused by *Aspergillus* and *Fusarium* spp., other rare light-colored (hyaline) molds, including species of *Paecilomyces,*[121] *Acremonium,*[122] and *Trichoderma,*[123] have been described as causing clinical disease more frequently than do other rare fungi. Some authorities have grouped diseases caused by molds with light-colored cell walls into a group, termed the hyalohyphomycoses. As with the dark-walled fungi, description of these infections by the causative organisms is preferable to minimize confusion. *Paecilomyces* has been reported to cause keratitis, endophthalmitis, and cutaneous and subcutaneous infections, as well as catheter-related fungemia, sinusitis, and disseminated infection. Like *Fusarium,* both *Paecilomyces* and *Acremonium* organisms have been reported to form reproductive structures in vivo in a process called adventitious sporulation.[104] This is believed to account for the much higher frequency of recovery in blood culture observed in infections involving these three genera. Also like *Fusarium,* both are typically associated with poor response to amphotericin B and the older azoles, although resistance varies among species. *Paecilomyces varioti* is susceptible to amphotericin B, and infections have been treated successfully with this agent. *Paecilomyces lilacinus,* now named *Purpureocillium lilacinum,* responds poorly to amphotericin B and, in vitro, is resistant to this agent, caspofungin, and the older azoles.[124] In vitro testing has shown multiple strains of these fungi to be more susceptible to voriconazole and posaconazole, but clinical treatment results are limited to case reports and small case series.[26,121,125–127] *Scopulariopsis* spp. are common in the soil, air, and decaying organic debris. Isolates identified as forming ascospores are placed in the genus *Microascus,* the teleomorph of *Scopulariopsis.* Onychomycosis, keratitis, and deeply invasive or disseminated infections have been reported.[128] Hyphae are septated and branch at acute angles. Bulbous swelling of hyphal segments may be seen, a feature usually encountered in agents of phaeohyphomycosis.

TRICHOSPORON SPP.

The genus *Trichosporon* is characterized by the production of septate hyphae, arthroconidia, yeasts, and pseudohyphae and by yeastlike growth on culture media. As a result of revisions in taxonomy, there are now more than 50 species of *Trichosporon,* 16 of which are clinically relevant.[129] Invasive human infection is most commonly due to *Trichosporon asahii,* and less commonly with *Trichosporon mucoides, Trichosporon mycotoxinivorans,* or *Trichosporon faecale.*[130–134] *Trichosporon asteroides* and *Trichosporon cutaneum* cause superficial infection. White piedra of the scalp is caused by *Trichosporon ovoides,* and similar disease of the pubic hair is caused by *Trichosporon inkin* (see Chapter 266). *Trichosporon* can be found in soil and water, on plants, and colonizing the human mouth, gastrointestinal tract, respiratory tract, vagina, skin, and urine.[129,135] More than 100 patients with deep trichosporonosis have been described; approximately 60% have been severely neutropenic, usually with acute leukemia.[133,136,137] A few have had organ transplantation, HIV infection, burns, chronic ambulatory peritoneal dialysis, or catheter-acquired fungemia.[138] Seven patients had prosthetic valve infections.[135]

Trichosporonosis is an acute, febrile, often fatal infection with dissemination to multiple deep organs and is associated with a mortality rate as high as 64%. Pneumonia is not a consistent or early feature, and thus the portal of entry is often not apparent. Renal involvement is common in disseminated disease and is associated with hematuria and funguria. Multiple red papular skin lesions may occur early on and assist diagnosis.[139,140] On biopsy, *Trichosporon* is seen as a mixture of true hyphae, pseudohyphae, budding yeasts, and tubular elements with square ends, called arthroconidia. Trichosporonosis is easily mistaken for candidiasis (which does not produce arthroconidia). *Trichosporon* grows readily on most culture media, but blood cultures tend to yield positive results late in the course. In the past, therapy with amphotericin B was recommended, but poor response and failures with this drug have occurred. MICs of the echinocandins for these fungi are high, and multiple incidences of breakthrough infections in patients receiving echinocandins have been reported.[141] These fungi are typically susceptible in vitro to voriconazole, as well as fluconazole, isavuconazole, itraconazole, and posaconazole[142,143]; thus therapy should include use of one of these azole antifungals.

MALASSEZIA FURFUR

Malassezia furfur, a lipophilic yeast, commonly colonizes normal human skin and is the cause of a superficial mycosis, pityriasis (tinea) versicolor (see Chapter 266). The fungus can also cause catheter-related sepsis, almost always in patients who are receiving parenteral lipids through a central venous catheter.[144] Most reported patients have been neonates with extended stays in intensive care units, although a few have been adults with malignancy or immunosuppression.[145] Fever has been the most common finding, but bradycardia, apnea, thrombocytopenia, and catheter blockage have been observed in some infants. In the autopsy study of one case, the yeast was observed in lipid-containing areas of pulmonary vascular endothelium. *M. furfur* is rarely detected by conventional culture techniques because the yeast requires fatty acids for growth. Organisms are better recovered with culture of blood drawn back through the catheter through the use of the lysis-centrifugation technique and lipid-enriched agar.[144] Results of cultures or smears of peripheral blood are occasionally positive.[146] The yeast is identified on smear by its size and shape and the distinctive collarette between mother and daughter cells. Lipid requirement for growth also aids identification. It is likely that some cases attributed to *M. furfur* were caused by other lipid-requiring species in the *M. furfur* complex, which includes *Malassezia sympodialis, Malassezia globosa, Malassezia obtusa, Malassezia restricta,* and *Malassezia slooffiae.*[147] *Malassezia pachydermatis* has the

same appearance and has caused similar infections, including an outbreak in a neonatal intensive care unit,[148] but does not require lipids for growth. The fungus adheres to the lumen of the catheter and has not been eradicated by discontinuing lipid infusions or administering miconazole or amphotericin B through the catheter.[149] Catheter removal and discontinuing parenteral lipids have been curative. In vitro, *M. furfur* appears to be susceptible to both amphotericin B and azole antifungals, including itraconazole and voriconazole.[150,151]

OTHER UNCOMMON YEASTS

Magnusiomyces capitatus (formerly called *Saprochaete capitata*, *Blastoschizomyces capitatus, Trichosporon capitatum,* and *Geotrichum capitatum*) has been reported as the cause of severe infection in more than 75 patients, most of whom also had acute leukemia.[152,153,154,155] Blood culture findings are usually positive, and skin lesions similar to those seen in leukemic patients with disseminated candidiasis have been observed. *M. capitatus* may colonize the skin, respiratory tract, and gastrointestinal tract. Intravenous catheters are a possible portal of entry.[156] Intravenous amphotericin B with or without flucytosine[156] and voriconazole or high-dose fluconazole with amphotericin B[153] have been advocated for treatment of these infections. High-dose fluconazole was shown in an animal model to be more efficacious than amphotericin B, flucytosine, or voriconazole monotherapy.[157] Resistance to echinocandins has been reported.[154] Catheter removal was associated with improved outcome in the largest and most recent report.[153] *Saprochaete clavata,* a closely related species, is sometimes misidentified as *Magnusiomyces capitatus (Saprochaete capitata).*[158] *S. clavata* has caused an outbreak of sepsis in neutropenic patients from an unidentified source.[159]

Other noncandidal yeasts may also rarely cause infection in humans.[143,160] These include *Wickerhamomyces anomalus* (formerly *Pichia [Hansenula] anomala*),[161–163] the black yeast *Exophiala (Wangiella) jeanselmei,*[57] *Rhodotorula* spp.,[161,164,165] and *Saccharomyces cerevisiae.*[166] Infection is usually seen in immunocompromised individuals, most commonly as catheter-associated fungemia. Localized outbreaks secondary to *E. jeanselmei* and *W. anomalus* (*P. anomala*) have been described.[57,163]

TALAROMYCES (FORMERLY *PENICILLIUM*) *MARNEFFEI*

Talaromyces marneffei is a thermally dimorphic fungus that causes life-threatening disseminated infection (penicilliosis marneffei) in a geographically distinct area of the world. *T. marneffei* is a thermally dimorphic, geographically restricted, intracellular pathogen that exudes a brilliant red pigment into the agar around the colony. Endemic to Southeast Asia, *T. marneffei* infection was extremely rare before the AIDS epidemic: fewer than 30 cases had been described since the first human infections in 1959 and 1984.[167,168] Between 1984 and 2004, more than 6000 *T. marneffei* infections were diagnosed in Thailand.[169] In the mid-1990s, this was the third most common opportunistic infection seen in HIV-infected individuals in northern Thailand.[170] Infection with *T. marneffei* has a limited geographic distribution, affecting persons residing in or those who have visited Southeast Asia or southern China. Endogenous cases have been reported from Myanmar (Burma), Hong Kong, Indonesia, Laos, Malaysia, Singapore, Taiwan, Thailand, Vietnam, and the Guangxi province of China.[171–173] Although most commonly seen in young adults infected with HIV, the disease has been reported in children[174,175] and adults, both with and without detectable immunocompromise.[171,172] *T. marneffei* has been isolated from healthy bamboo rats (*Rhizomys pruinosis, Rhizomys sinensis, Rhizomys sumatrensis,* and *Cannomys badius*)[176,177] and the soil around their burrows,[178] but the role of these rats in human infection is unknown. A case-control study of the disease in 80 persons with AIDS revealed an association with recent history of occupational or other exposure to soil, but not to rats.[179] Infection occurs more commonly during the rainy season in both northern Thailand and Vietnam.[180,181] It is likely that this infection is acquired by inhalation of conidia from an environmental source such as the soil.

Patients typically present with a chronic illness averaging 4 weeks in duration associated with low-grade fever, weight loss, and one or more skin lesions.[170,182] Apart from the skin lesions, disease is quite similar in presentation, course, and treatment to that of acute disseminated histoplasmosis. The most common clinical characteristics are fever, malaise, anemia, leukocytosis, weight loss, and, in 60% to 70% of patients, skin lesions.[170,172,182] Fungemia, generalized lymphadenopathy, and cough are reported in more than half of patients. Subcutaneous and mucosal lesions, diarrhea, colonic lesions, hepatomegaly with or without splenomegaly, hemoptysis, osteoarticular lesions, and pericarditis have also been described.[171,183,184] Skin lesions commonly occur on the face, upper trunk, and extremities. They may occur as papules, pustules, nodules, ulcers, or abscesses. In HIV-infected individuals, lesions commonly become umbilicated and resemble those of molluscum contagiosum. Pharyngeal and palatal lesions are also more commonly seen in HIV-infected patients.[184] Lung lesions can appear as reticulonodular, nodular, or diffuse alveolar infiltrates, but on occasion they are cavitary and cause hemoptysis.[185] Autopsy studies have revealed involvement of lymph nodes, liver, spleen, lungs, kidneys, skin, bone, bone marrow, adrenal glands, tonsils, bowel, and meninges.[171,186] Mortality of higher than 80% has been reported in a series of 21 HIV-infected persons with *T. marneffei* meningitis.[187]

Consideration of the diagnosis of *T. marneffei* infection should be made in persons who have resided in or visited an endemic area. Laboratory exposure to the organism was causally linked to disseminated infection in one immunocompromised individual.[188] The duration of incubation is not currently known, and reactivation disease may be possible. In one report, a severely immunocompromised individual acquired disseminated infection more than 10 years after visiting an endemic area.[189] Diagnosis is based on identification of the organism on smear, histopathologic studies, or culture. Diagnosis has been made most frequently from smears of skin lesions and biopsy samples of lymph nodes and bone marrow.[170] The organism has also been noted on peripheral blood smear in at least one report.[190] Isolation of *T. marneffei* from culture of bone marrow, blood, lymph nodes, skin lesions, bronchoalveolar lavage, or sputum can be diagnostic. Microscopic examination of clinical materials reveals yeast forms (2×2 to 3×6.5 µm) both within phagocytes and extracellularly.[171] The intracellular forms are smaller, resembling *Histoplasma capsulatum,* whereas the extracellular forms are larger and often have a transverse septum (asexual fission or schizogony) (Fig. 268.11). The extracellular forms may also appear as "sausage forms," consisting of three cells (8–13 µm in length) divided by two transverse septa or, rarely, as short hyphae. Three types of histopathologic reactions have been noted in association with *T. marneffei* infection: granulomatous, suppurative, and necrotizing inflammation.[186] Granulomatous or suppurative changes are most commonly seen in patients with normal immunity. The necrotizing reaction is more commonly seen in immunocompromised patients and is characterized by focal necrosis with surrounding histiocytes and extracellular fungi. Culture at 30°C produces a mold with sporulating structures typical of *Penicillium.* Identification is aided by the formation of a soluble red

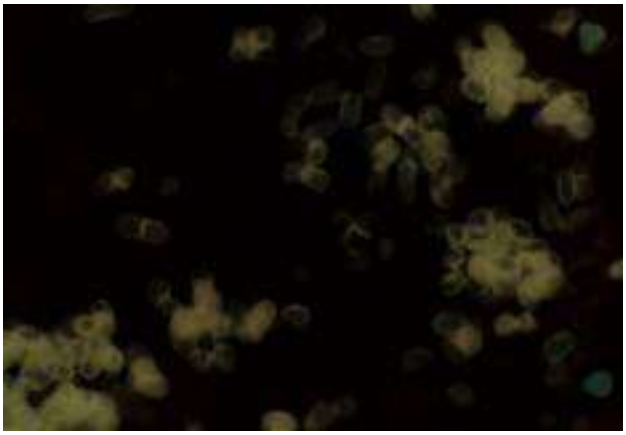

FIG. 268.11 ***Talaromyces* (formerly *Penicillium*) *marneffei* in a splenic abscess.** Note the transverse septa. (Gomori methenamine silver stain.) *(From McGinnis MR, Chandler FW. Penicilliosis marneffei. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

pigment that diffuses into the agar. The mold form can be converted to a yeast form by incubation at 37°C.[191] Because disease normally appears to result from inhalation of conidia, it seems reasonable to use Biosafety Level 2 precautions when working with the mold form. Diagnosis by specific immunologic techniques, including serum antibody and antigen tests, is still in the experimental stage.[192,193] The currently available galactomannan test for *Aspergillus* may assist in the diagnosis; two-thirds of patients with *T. marneffei* infection had positive test results in one study.[194]

Excellent response (97.3%) was achieved with a regimen of intravenous amphotericin B for 2 weeks (0.6 mg/kg/day) followed by oral itraconazole for 10 weeks (200 mg twice daily) in 74 HIV-infected patients.[195] This regimen allowed shortened hospital stays while producing a clearing of fungemia that was more rapid than that produced by itraconazole alone. An open-label, randomized trial comparing initial therapy with IV amphotericin B versus oral itraconazole in 440 HIV-infected patients found a much higher incidence of death at 24 weeks in the itraconazole arm.[196] Successful treatment of disseminated infection has been reported with amphotericin B with or without the addition of flucytosine,[171] and with oral itraconazole or voriconazole monotherapy.[197] Failure rates in a study of 86 HIV-infected patients included 8 of 35 patients (22.9%) receiving amphotericin B, 3 of 12 (25%) taking itraconazole, and 7 of 11 (63.6%) taking fluconazole.[198] Itraconazole has been shown to prevent relapse of this disease in patients with HIV infection,[199] and secondary prophylaxis of HIV-infected patients with itraconazole (200 mg once daily) is suggested to prevent relapse.[171] As with secondary prophylaxis in other fungal diseases, it appears safe to stop this therapy after response to antiretroviral therapy ($CD4^+$ cell count of ≥100 cells/μL for at least 6 months).[200] Immune reconstitution inflammatory syndrome has been reported with the initiation of antiretroviral therapy in HIV-infected persons.[201]

LACAZIA LOBOI

Lobomycosis (Lobo disease, keloidal blastomycosis) is a chronic skin infection most commonly afflicting the indigenous people of the Amazon regions of Colombia and Brazil and dolphins. The etiologic agent of lobomycosis has never been isolated in culture, but it has been shown to be closely related to *Paracoccidioides brasiliensis* by 18S ribosomal DNA sequencing.[202] This fungus has been known by many genus names; *Lacazia loboi* has been proposed to replace *Loboa loboi* and other previous designations.[203] More than 100 cases have been reported from countries in Central and South America. Lobomycosis found outside the endemic area, including one case from the United States,[204] typically occurs in persons with history of travel to an endemic country. Recently, two cases, one from South Africa and one from Greece, have been reported in people who had no history of travel to the Western Hemisphere.[205,206] Although it is difficult to confirm these cases with our inability to culture the organism (molecular techniques were also not employed), they raise the possibility that the range of lobomycosis is larger than previously believed. The fungus remains confined to the skin, progressing slowly over decades. Probably because of this slow progression, rare cases outside of the endemic area have been reported, often many years after potential exposure.[207] Lesions are typically nodules or keloidal plaques that are red, hard, and shiny, in association with fibrosis and a granulomatous reaction on histologic examination (Figs. 268.12 and 268.13). The diagnosis is made by finding the typical globose to lemon-shaped cells (about 9 μm in diameter), either singly or in short chains (Fig. 268.14). Surgical excision is the only useful therapy. One patient has been reported to respond to posaconazole.[208]

AGENTS OF ADIASPIROMYCOSIS (*EMMONSIA* SPP.)

Adiaspiromycosis is a rare disease of humans characterized by the formation of nonreplicating adiaspores, typically in the lungs. Disease severity correlates with the host's immunologic response and likely the inoculum size. Disease most commonly affects the lungs (approximately two-thirds of cases) and is often associated with occupational dust exposure. Men, average age 40, are most commonly afflicted. Disease can range from asymptomatic to severe and sometimes fatal cases.

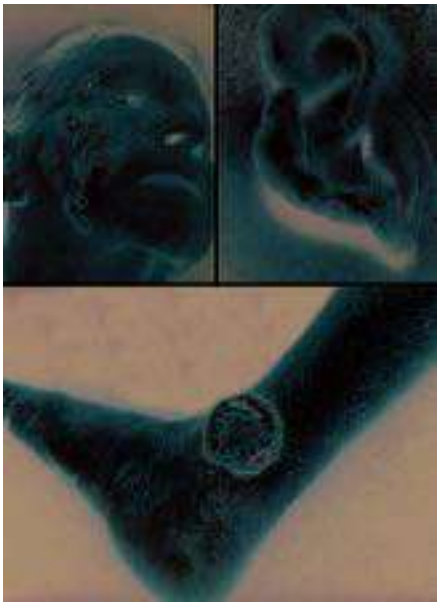

FIG. 268.12 Lobomycosis. *(From Nikolaidis G, Rosen T. Lobomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

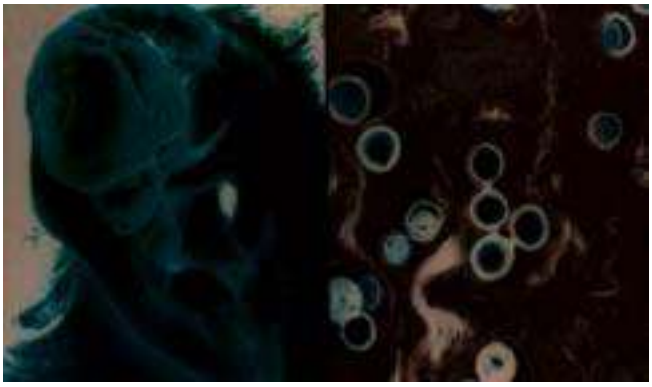

FIG. 268.13 Lobomycosis of the ear *(left)* with associated histologic findings *(right).* (Gomori methenamine silver stain.) *(From Herr RA, Herr E, Tarcha PR, et al. Phylogenetic analysis of* Lacazia loboi *places this previously uncharacterized pathogen with the dimorphic Onygenales.* J Clin Microbiol. *2001;39:309–314.)*

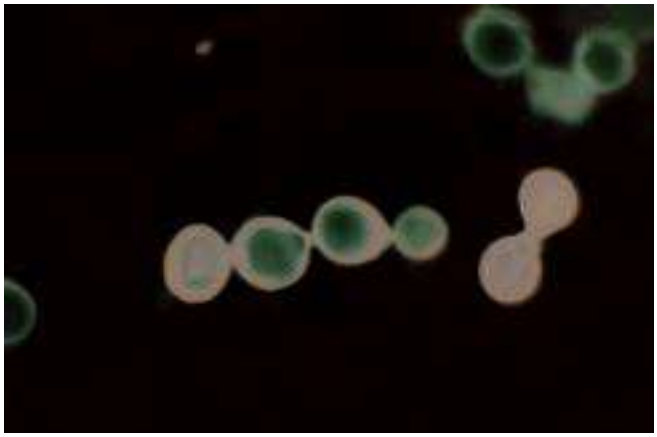

FIG. 268.14 Histologic appearance of *Lacazia loboi,* the agent of lobomycosis. (Gomori methenamine silver stain.) *(From Nikolaidis G, Rosen T. Lobomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

Adiaspiromycosis may be discovered when asymptomatic lung lesions are resected to rule out malignancy. Presentation may include fever, cough, dyspnea, hemoptysis, weight loss, fatigue, or respiratory failure. In addition to involvement of the lungs, adiaspiromycosis may present with ocular lesions and even with involvement of the gastrointestinal tract.[209] An outbreak in Brazil of ocular disease in children associated with fresh water sponges has been described.[210] Two cases of appendiceal involvement have also been reported.

Adiaspiromycosis is chiefly caused by *Emmonsia crescens* (*Ajellomyces crescens*), a fungus closely related to *Blastomyces dermatitidis,* and *Emmonsia parva.*[211] Conidia of *E. crescens* will enlarge into 25- to 400-µm adiaspores in vitro when incubated at 37°C on brain heart infusion agar. Inhaled, these 2- to 4-µm conidia can enlarge to diameters of up to 500 µm in the human lung.

Because *Emmonsia* has not been recovered from culture in human adiaspiromycosis, diagnosis is based on its unique histologic appearance, that of a single thick-walled adiaspore within a 0.5- to 3-mm granuloma composed of epithelioid and giant cells. A report of the use of polymerase chain reaction and DNA sequencing to diagnose adiaspiromycosis has been published.[212]

Corticosteroids have appeared to be beneficial in several cases of disease. It is unclear if antifungal therapy has any role in adiaspiromycosis, but low MICs have been reported with amphotericin B, itraconazole, voriconazole, and caspofungin.

EMERGOMYCES AFRICANUS AND OTHER EMERGOMYCES SPECIES

Disseminated infection in immunocompromised individuals with the thermally dimorphic fungus *Emergomyces africanus* has recently been described.[213–215] Initially, 13 cases of disseminated infection with an *Emmonsia* spp. related to *Emmonsia pasteuriana* were reported from South Africa.[214,215] Ultimately, this pathogen was named *E. africanus,* with both it and *E. pasteuriana* included in the new genus *Emergomyces.*[216] Infection with *E. africanus* was associated with skin lesions, fungemia, and high mortality, most commonly in severely immunocompromised HIV-infected (AIDS) patients.[213–215] *E. africanus* displays temperature-dependent dimorphic growth (i.e., mold at room temperature and yeast at 37°C) and can commonly be recovered from bone marrow or blood. *E. africanus* has been identified (but not cultured) from the soil in South Africa.[217] Like other dimorphic members of the family Ajellomycetaceae (including *Blastomyces* and *Histoplasma*), MICs of amphotericin B, itraconazole, posaconazole, and voriconazole for *E. africanus* are low.[218] This supports using a therapeutic approach similar to that employed with disseminated histoplasmosis (i.e., initial IV amphotericin B, followed by oral azole therapy and long-term suppression [secondary prophylaxis]). Similar cases of disseminated infection have been reported outside of Africa, caused by *E. pasteuriana, Emmonsia helica* (renamed *Blastomyces helicus*), *Emmonsia europaeus, Emergomyces canadensis,* and *Emmonsia orientalis.*[216,219–221]

PROTOTHECA SPP.

Prototheca are unicellular algae that lack chlorophyll and reproduce by endosporulation. Although not fungi, these organisms are described in this chapter because they are often preliminarily misidentified in tissue and culture as yeasts. *Prototheca* are found in a wide range of environmental sites, including tree slime, sewage, fresh and marine water, soil, pigeon droppings,[222] and foodstuffs. They may colonize human skin, the respiratory tract, and the gastrointestinal tract.[223] More than 100 cases of human infection have been reported,[223,224] almost all in adults and from widely scattered geographic areas. *Prototheca wickerhamii* is the most common cause of infection in humans; infection secondary to *Prototheca zopfii* and a single case due to *Prototheca miyajii* have also been reported.[225]

The most common manifestation of protothecosis is a single lesion of the skin or subcutaneous tissue. The typical manifestation is a painless, slowly progressive, well-circumscribed plaque or papulonodular lesion that may become eczematoid or ulcerated. Soft tissue lesions favor the olecranon bursa, sites of minor trauma or corticosteroid injection,[226] and surgical wounds exposed to soil or water, such as that of a hand tendon repair. Lesions typically enlarge gradually over weeks to months.

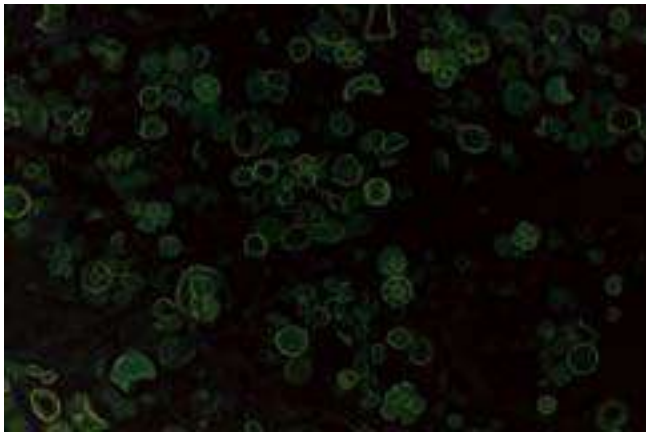

FIG. 268.15 *Prototheca wickerhamii* in an olecranon bursa biopsy sample. (Gridley stain.) *(From Ramsay E, Chandler FW, Connor DH. Prototheocosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

Skin lesions in HIV-infected patients have not differed from those seen in other patients.[227,228] Disseminated and deep-seated infections, such as peritonitis, meningitis, and endocarditis, have also been reported in rare cases.[224,229,230]

Protothecosis is best diagnosed through biopsy for histologic study and culture. The inflammatory response consists of both microabscesses and granulomas with multinucleate giant cells. *Prototheca* cells usually range from 3 to 30 µm in diameter, are well visualized with Gomori methenamine silver or periodic acid–Schiff stain, and often contain two to eight tightly packed endospores in each cell or sporangium (Fig. 268.15). White opaque colonies appear in a few days on standard mycologic media. Identification is made by gross and microscopic appearance plus biochemical testing (the algae is identified by most commercially available yeast identification systems).

Protothecosis has little, if any, tendency toward self-healing. There have been few cases of disseminated disease in the setting of solid organ or stem cell transplantation; all resulted in mortality.[229] Both surgical excision of lesions and intravenous amphotericin B have been used successfully.[227] *Prototheca* spp. are resistant to flucytosine, but prolonged therapy with ketoconazole, itraconazole, or fluconazole has been reported to benefit some patients with skin lesions.[226,231,232] Short-course itraconazole (200 mg daily for 2 months) has also been used successfully.[233] Voriconazole has proven effective in a case of cutaneous infection initially unresponsive to itraconazole.[234]

PYTHIUM SPP.

Pythium spp. are protists formerly classified as fungi (Oomycetes, or water molds), which rarely cause human infection. Disease is found worldwide, but the largest number of cases have been reported from Thailand.[235] Pythiosis is associated with environmental trauma and with underlying thalassemia or other hemoglobinopathies. The most common patient is male, age 20 to 60 years, with an agricultural occupation. In the largest reported series, vascular (typically arterial) infection was most common (59%), followed by ocular (33%), skin and subcutaneous (5%), and disseminated infection (3%).[236] Swimming in Thailand while wearing contact lenses has led to *Pythium insidiosum* keratitis.[237] Although ocular disease was usually associated with trauma, 85% of patients with vascular, skin and subcutaneous, and disseminated disease had a thalassemia-hemoglobinopathy syndrome. Disease has also been reported in association with burns and combat trauma.[238–241]

Pythiosis has been associated with poor prognosis,[236,242] due in part to difficulties in diagnosing the infection and lack of antimicrobial agents effective against this disease. In the aforementioned study, 79% of those with ocular disease required removal of the affected eye to control infection; 78% of vascular cases lost a limb (60% survived—all of whom underwent amputation). All patients with disseminated disease died.

Diagnosis is typically made by culture of the organism or by a variety of investigational serologic tests.[235] Molecular diagnosis has also been reported.[243] Delays in presenting for care may also affect outcome. In the Thai report, patients with vascular disease presented to medical care on average 3 months after developing lower extremity claudication or ulceration.

Successful therapy currently relies on surgical control of the infection.[242,244] Good response in localized skin and soft tissue infection has been reported with the use of saturated solution of potassium iodide. Commonly available systemic antifungals have been ineffective both in vitro and in clinical use. In vitro studies have identified doxycycline, minocycline, tigecycline, linezolid, azithromycin, and clarithromycin as potentially useful agents.[245–247] In combination with other agents (in vitro), terbinafine and minocycline appear to be most promising.[245,248,249] An experimental vaccine has also been used, but the results are difficult to interpret.[235,239]

RHINOSPORIDIUM SEEBERI

Rhinosporidiosis is a chronic, usually painless localized infection of the mucous membranes. Formerly believed to be a fungus, the causative agent, *Rhinosporidium seeberi*, has also never been cultured. With 18S ribosomal DNA sequencing, this organism has been shown to be a protistan parasite in the class Mesomycetozoea.[250,251,252] Rhinosporidiosis occurs worldwide, and the greatest numbers of cases are found in southern India and Sri Lanka.[253–255] Disease affects the nose and nasopharynx most commonly, the ocular structures less commonly, and the skin more rarely. Disseminated disease is rare. Lasser and Smith[256] reviewed 28 US cases, finding 19 that affected the nose and 9 the conjunctiva, with 24 occurring in men and 4 in women. Lesions increase in size over months to years to form friable pedunculated masses, typically in the nose, upper airway, or conjunctiva.[257] Nasal lesions manifest as nasal obstruction or epistaxis.[258] One or more pedunculated or verrucous skin lesions, with or without nasal or conjunctival lesions, are occasionally observed.[259] In rare cases, polyps occur in the vagina, urethra, or penis. *Rhinosporidium seeberi* forms round, thick-walled cysts (sporangia) in the submucosa, varying in diameter from 100 to 350 µm, often visible through the mucosa as white dots. Mature cysts become filled with numerous spores (endospores), which on release become new cysts (Fig. 268.16). Treatment of choice is surgery with electrocoagulation of the lesion base.[257]

SUMMARY

Key features of the uncommon fungi in this chapter are listed in Table 268.2.

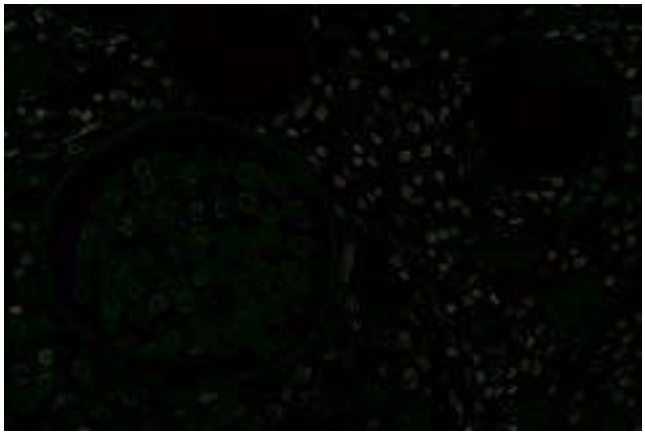

FIG. 268.16 Mature sporangium of *Rhinosporidium seeberi*. (Mayer mucicarmine stain.) *(From Watts JC, Chandler FW. Rhinosporidiosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

TABLE 268.2 Key Features of the Uncommon Fungi and Related Species

ORGANISM(S)	RISKS FACTORS/ RISK GROUPS	GEOGRAPHIC EPIDEMIOLOGIC RANGE	ECOLOGIC NICHE	CLINICAL MANIFESTATIONS	TISSUE FORMS	TREATMENT
Scedosporium apiospermum (P. boydii) species complex	Trauma, immunocompromise	Worldwide	Soil, fresh water, respiratory tract colonization	Pneumonia; osteoarticular, CNS, or disseminated infection; mycetoma	Septate hyphae	Voriconazole
Lomentospora (Scedosporium) prolificans	Severe immunocompromise, trauma	Worldwide	Soil, respiratory tract colonization	Disseminated infection, osteoarticular infection	Septate hyphae	Unknown (consider voriconazole and/ or high-dose L-AMB)
Dark-walled fungi (including *Alternaria, Bipolaris, Ochroconis, Cladophialophora, Curvularia, Exophiala, Exserohilum,* and *Wangiella* spp.)	Trauma, allergic rhinitis, immunocompromise	Worldwide	Soil, decaying organic matter, air, plants	Localized subcutaneous lesions, brain abscess, allergic sinusitis; disseminated infections (rare)	Septate hyphae; yeast or pseudohyphae (rarely)	High-dose D-AMB, L-AMB, itraconazole, or voriconazole
Fusarium spp.	Severe immunocompromise	Worldwide	Soil, plants	Disseminated infection, fungemia	Septate hyphae	High-dose D-AMB or L-AMB + voriconazole or posaconazole
Other opportunistic molds (including *Acremonium, Paecilomyces, Scopulariopsis,* and *Trichoderma*)	Severe immunocompromise	Worldwide	Soil, plants	Disseminated infection, fungemia	Septate hyphae	Based on species
Trichosporon spp.	Immunocompromise, central venous catheters	Worldwide	Skin and gastrointestinal flora	Fungemia	Yeast, hyphae, arthroconidia, pseudohyphae	Fluconazole or voriconazole or posaconazole
Malassezia furfur	Parenteral lipids	Worldwide	Skin	Catheter-related fungemia	Yeast	Catheter removal + voriconazole

Continued

TABLE 268.2 Key Features of the Uncommon Fungi and Related Species—cont'd

ORGANISM(S)	RISKS FACTORS/ RISK GROUPS	GEOGRAPHIC EPIDEMIOLOGIC RANGE	ECOLOGIC NICHE	CLINICAL MANIFESTATIONS	TISSUE FORMS	TREATMENT
Other opportunistic yeasts (including *Saprochaete/ Magnusiomyces* [*Blastoschizomyces*], *Pichia, Exophiala, Rhodotorula,* and *Saccharomyces*)	Immunocompromise, central venous catheters	Worldwide	Skin and/or environment	Catheter-related fungemia	Yeast	Catheter removal + antifungal based on species
Talaromyces (Penicillium) marneffei	AIDS	Southeastern Asia, southern China	Unknown	Disseminated infection	Yeasts or "sausage forms"	D-AMB ± 5-FC or itraconazole or voriconazole
Lacazia loboi	Rural outdoor labor, minor trauma	Central and South America	Unknown	Localized cutaneous/ subcutaneous infection	Chains of large yeasts	Surgery
Agents of adiaspiromycosis	Occupational inhalation	Worldwide	Dust	Pulmonary inflammation	Adiaspore (25–500 μm)	Corticosteroids
Emergomyces africanus	AIDS	South Africa	Soil	Disseminated infection	Yeasts	D-AMB followed by itraconazole
Prototheca spp.	Trauma	Worldwide	Water, soil, foodstuffs	Localized subcutaneous lesions	2–8 Endospores in sporangia	Surgery or itraconazole
Pythium spp.	Occupational trauma	Worldwide, Thailand	Likely aquatic	Vascular infection, ocular	Wide hyphae	Surgery
Rhinosporidium seeberi	Rural outdoor labor	India, Sri Lanka; rare worldwide	Unknown	Localized mucous membrane polypoidal lesions	Spores in large (100- to 350-μm) cysts (sporangia)	Surgery

AIDS, Acquired immunodeficiency syndrome; *CNS,* central nervous system; *D-AMB,* deoxycholate amphotericin B (1–1.5 mg/kg/day); *5-FC,* 5-fluorocytosine (should only be used when monitoring of levels is available locally); *L-AMB,* lipid preparations of amphotericin B (AmBisome, Abelcet).

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The complete reference list is available online at Expert Consult.

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269 *Pneumocystis* Species

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SHORT VIEW SUMMARY

Definition
- *Pneumocystis* spp. are genetically distinct, host-specific opportunistic fungal pathogens widely found in nature.
- *Pneumocystis jirovecii,* found in humans, causes pneumonia ("*Pneumocystis* pneumonia" or PCP") in immunocompromised patients.

Epidemiology
- Infection is acquired by inhalation of the cystic form (ascus) of the organism.
- Primary infection occurs in the first 2 years of life in most people.
- Person-to-person transmission and outbreaks of PCP in immunosuppressed patients have been reported.
- Environmental factors influence PCP hospitalizations.
- Colonization is common, associated with obstructive airway disease.

Diagnosis
- PCP in patients with human immunodeficiency virus (HIV): slow development, milder disease, 10% to 12% mortality
- PCP in non-HIV patients: rapid development, severe disease, 30% to 50% mortality
- Gold standard: microscopic demonstration of organism in respiratory tract specimens by immunofluorescence or special stains
- Other methods (polymerase chain reaction, β-1,3-D-glucan levels) are promising but considered investigational

Treatment
- Trimethoprim-sulfamethoxazole (TMP-SMX) 15 to 20 mg/kg/day (TMP) and 75 to 100 mg/kg/day (SMX) IV or PO in divided doses every 6 to 8 hours for 14 to 21 days is the treatment of choice for mild, moderate, or severe PCP.
- *Mild-to-moderate PCP*: Oral dose can be given as TMP-SMX two double-strength (DS) tablets orally three times a day.
- Several alternative regimens are available depending on the severity of PCP.
- Prednisone 40 mg PO twice daily on days 1 to 5, 40 mg PO every day on days 6 to 10, and 20 mg PO every day on days 11 to 21 are given as adjunctive therapy for moderate-to-severe PCP (PaO_2 ≤70 mm Hg, or alveolar-arterial O_2 gradient >35 mm Hg).
- Start prednisone as soon as possible or within the first 3 days after beginning antimicrobial therapy.
- Prednisone is mainly indicated for HIV-positive patients.

Prevention
- TMP-SMX one DS tablet orally every day or one single-strength tablet orally every day is the drug of choice for primary and secondary prophylaxis.
- Alternative regimens are available.
- Evidence does not support isolation of PCP patients from contact with other immunocompromised hosts.

Pneumocystis was discovered in 1909 in Brazil by Carlos Chagas, who mistakenly interpreted the organism as a trypanosome. In 1912 Pierre and Eugénie Delanöe, in Paris, identified *Pneumocystis* as a separate genus and species and named the organism in honor of Antonio Carini, another researcher. *Pneumocystis* first came to medical attention when in the 1940s–50s it was identified as the cause of interstitial plasma cell pneumonia in institutionalized and debilitated infants in central and eastern Europe. In the 1960s *Pneumocystis* became widely appreciated as an important cause of pneumonia (now termed *Pneumocystis* pneumonia, or PCP) in immunocompromised hosts; however, with the development of safe and effective antimicrobial drugs, interest in the organism waned. The dramatic rise in the incidence of PCP, associated with human immunodeficiency virus (HIV) infection in the 1980s, rekindled interest in *Pneumocystis* as a major medical and public health problem. During the 1990s advances in the treatment of HIV reduced the frequency of PCP and other complications. Nevertheless, *Pneumocystis* remains a leading cause of opportunistic infection, morbidity, and mortality in these patients.[1] It is estimated that there are more than 400,000 annual cases of PCP worldwide, with more than 52,000 deaths per year.[2] Interest in the organism has also been spurred by studies of *Pneumocystis* colonization and its association with infant sudden unexpected death,[3] chronic obstructive pulmonary disease (COPD), and asthma[4]; and also the observed increase in cases of PCP among the non–HIV-infected population; status post–organ transplantation; those with hematologic or rheumatologic conditions and in receipt of biologic disease-modifying agents[5]; continuing outbreaks of PCP in health care settings; and sequencing of the human *Pneumocystis* genome.[6,7]

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

PATHOGEN

Pneumocystis describes a genus of closely related unicellular fungi of low virulence found in the lungs of humans and a variety of mammals. The taxonomic status of the genus was resolved in the late 1980s, when analysis of the ribosomal RNA (rRNA) gene suggested that the organism is more closely related to fungi than to protozoa.[1] This conclusion has been confirmed at every molecular locus analyzed and by whole-genome sequence analysis. Phylogenetic studies place the organisms as ascomycetes on a deep basal branch among the archiascomycetes; however, *Pneumocystis* is unusual among fungi in that the organism lacks ergosterol in its plasma membranes and is insensitive to available antifungal drugs that target ergosterol biosynthesis.

Species within the genus demonstrate genotypic and phenotypic differences manifested by antigenic differences, ultrastructural morphologic differences, and host specificity.[1] Genetic studies have demonstrated differences between *Pneumocystis* spp. at a karyotypic level, in the organization and structure of gene families within specific genomes, and at a sequence level within individual genes. Whole-genome sequence analysis of *Pneumocystis carinii, Pneumocystis murina,* and *Pneumocystis jirovecii* confirm these differences and provide novel reagents to further characterize differences.[7,8] Structural genomic differences exist in the mitochondrial genomes in addition to those found in the nuclear genomes; the mitochondrial genome of *P. jirovecii* is circular, whereas those of *P. carinii* and *P. murina* are linear.[9,10] Not only are there genetic differences in *Pneumocystis* among different animal hosts, but there are also species or strain differences, or both, in organisms from the same host. Ultrastructural morphologic differences are evident only at the level of electron microscopy, whereas other phenotypic differences between species require specialized reagents to determine antigenic characterization or multilocus enzyme electrophoresis.

Experimental models have demonstrated that *Pneumocystis* taken from a given host species appears unable to proliferate in other host species. Associated with a better understanding of the host specificity and genetic differences among members of the genus *Pneumocystis,* a need has arisen to define individual species within the genus. In recognition that the organisms described by the Delanöes were isolated from infected rats, a formal taxonomic description of rat-derived species was made, retaining the name of *P. carinii.*[11,12] *Pneumocystis* isolated from humans was formally described as *P. jirovecii* in recognition of Otto Jirovec, whose group first identified *Pneumocystis* as a human pathogen and the causative agent of interstitial plasma cell pneumonia. A second species identified in rats has been named *Pneumocystis wakefieldiae,* whereas *P. murina* and *Pneumocystis oryctolagi* have been identified in mice and rabbits, respectively.[11]

Despite the strenuous efforts by many investigators, the lack of a reliable *Pneumocystis* in vitro cultivation system remains an intractable problem. Limited (up to 10-fold) replication of rat-derived organisms has been achieved in different cell lines and in axenic media.[13] A continuous culture system for rat- and human-derived *Pneumocystis* has been described but has proved difficult to reproduce and maintain. Short-term culture has been used to study *Pneumocystis* metabolism and susceptibility to antimicrobial drugs, but standardization and reproducibility among laboratories have not yet been achieved.

Studies of the life cycle of *Pneumocystis* have been based mainly on light and electron microscopic analysis of forms seen in infected lungs or in short-term culture (Fig. 269.1).[14] Three developmental stages of the organism are commonly seen in conjunction with additional intermediate forms. The trophic form is small (1–4 µm) and pleomorphic and commonly exists in clusters; this stage can be identified on Giemsa stain by its reddish nucleus and blue cytoplasm (Fig. 269.2A). In the asexual phase of the life cycle the trophic forms multiply by binary fission, although trophic binary fission is rarely visualized or documented. In the sexual phase the haploid trophic forms are postulated to conjugate to form a diploid zygote that becomes a 4- to 6-µm sporocyte (precyst); this form is difficult to distinguish from the other developmental stages at the light microscopic level. The sporocyte undergoes meiosis followed by mitosis, leading to the formation of the ascus (cyst), which contains eight haploid ascospores. The expression of meiosis-specific genes, such as the functionally conserved meiotic control kinase Ran1, the meiotic activator Mei2, and the meiosis-specific recombinase Dcm1, have been confirmed in the lungs of infected mammalian hosts, suggesting sexual replication occurs in the lungs of infected mammalian hosts.[15,16] The finding that the genomes of *P. jirovecii*, *P. murina,* and *P. carinii* all contain a single region with only three genes involved in mating-type differentiation supports the presence of a homothallic (self-fertile) sexual cycle.[8,17] The 5- to 8-µm ascus has a thick cell wall that stains well with stains such as methenamine silver or toluidine blue O (see Fig. 269.2B). The ascospores or intracystic bodies are formed by compartmentalization of nuclei and cytoplasmic organelles, exhibit different shapes, and appear to be released through a rent in the cell wall. Studies using echinocandin inhibitors of β-1,3-glucan (BG) synthase suggest that the cystic form is an integral part of the life cycle and critical for transmission.[18,19]

Biochemical and metabolic studies of *Pneumocystis* have been limited by the problems of culturing the organism.[19,20] Functional analyses of the *P. carinii* and *P. jirovecii* genomes are consistent with the lifestyles of obligate parasites with a reduced repertoire of amino-acid synthase genes and an abundance of transporter genes, suggesting limitations in the ability to synthesize essential nutrients and a necessity to scavenge these from their mammalian host.[7,21,22] The surface of *Pneumocystis* is rich in glucose and mannose, *N*-acetylglucosamine, and galactose

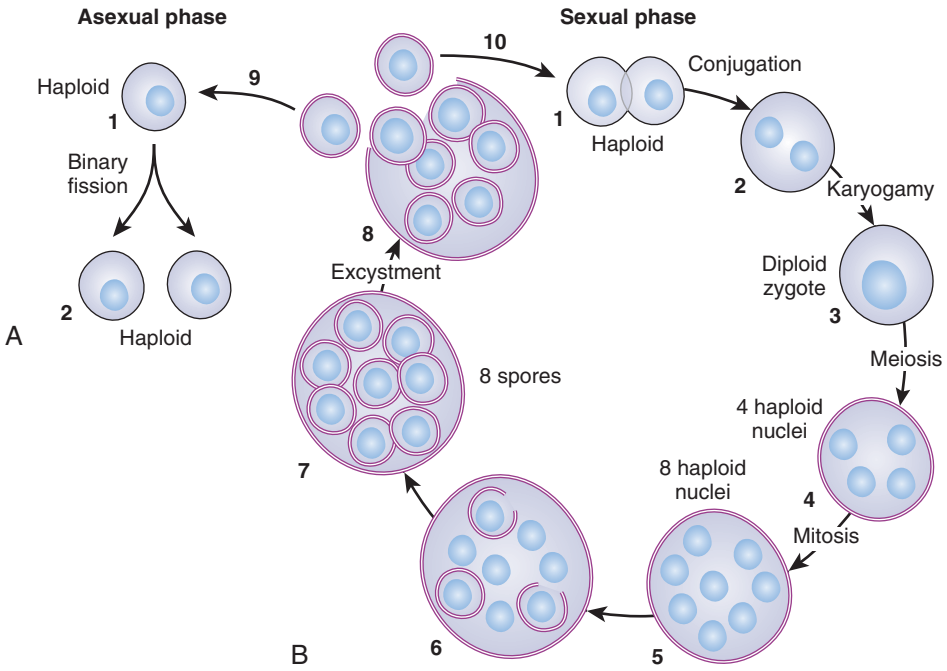

FIG. 269.1 Putative life cycle *of Pneumocystis*. The entry of *Pneumocystis* into the mammalian lung likely occurs during the first year of life. The agent of infection is suspected to be airborne spores. Recent studies suggest that the cyst/ascus (containing eight spores) may be the agent of infection. After inhalation, the spores ultimately take residence in the terminal portion of the respiratory tree, the alveoli. Neither the mechanism of migration to the alveoli nor the form in which the organism arrives in the alveoli (intact ascus or individual spores) is known. (A) Asexual phase: Haploid trophic forms are thought to replicate asexually by binary fission, whereby the nuclear content is duplicated *(1)* along with cellular contents that *(2)* divide into two haploid trophic forms. (B) Sexual phase: Two presumptive mating types conjugate *(1),* undergo karyogamy *(2),* and produce a diploid zygote *(3)* that progresses through meiosis to produce four haploid nuclei *(4),* followed by an additional mitosis to produce eight nuclei *(5).* The nuclei are packaged into spores by invagination of the ascus cell membranes *(6)* to produce eight double-membrane spores *(7).* After completion, excystment occurs via a protunicate release by unknown mechanisms *(8).* The released spores become the vegetative forms that can then undergo asexual *(9)* or sexual replication with a presumed opposite mating type *(10).* The mechanism of exit out of the lung and the life-cycle form that transits into the environment are unknown (the life cycle was composed using SmartDraw 10 (SmartDraw Software; San Diego). *(From Cushion MT. Are members of the fungal genus* Pneumocystis *(a) commensals; (b) opportunists; (c) pathogens; or (d) all of the above?* PLoS Pathog. *2010;6:e1001.)*



FIG. 269.2 Pneumocystis organisms. (A) Cluster of *Pneumocystis* trophic and cystic forms (Diff-Quik, ×1000). (B) Cluster of *Pneumocystis* cysts (Grocott-Gomori methenamine silver stain, ×1250).

N-acetylgalactosamine residues. The cell walls of cysts and trophic forms contain a number of immunogenic glycoproteins that may be part of a large complex (see Fig. 269.2).[23] BGs are a major component of the cell wall, whereas little or no chitin has been detected, although a chitin synthase has been detected, and upregulation of host chitinase activity is noted in the lung during infection.[24] Lipids have received considerable attention because of their relationship with antifungal therapy. Cholesterol is the dominant sterol present in *Pneumocystis*.[20] Instead of ergosterol, the organism synthesizes distinct Δ^7 C24 alkylated sterols. Coenzyme Q10 (CoQ10) is the major ubiquinone homologue synthesized by the organism; CoQ10 homologues, such as 8-aminoquinolones and hydroxynaphthoquinones, have shown good activity against the organism. A variety of enzymes and metabolic pathways have been characterized as potential therapeutic targets.[25,26]

Several major groups of *Pneumocystis* antigens have been identified. The 95- to 140-kilodalton (kDa) major surface glycoprotein (Msg), or gpA, is highly immunogenic, exhibits shared and species-specific antigenic determinants, and contains protective B- and T-cell epitopes.[27–31] Immunization with Msg also elicits a protective response in some, but not all, animal models.[31] Msg also facilitates interaction with host cells by adherence to the extracellular matrix proteins fibronectin, vitronectin, possibly laminin, the surfactant proteins A and D, and the mannose receptor.[32–37] Msg is composed of up to 10% N-linked carbohydrates, particularly mannose, which participate in the binding to these proteins.

Msg actually represents a complex family of proteins encoded by multiple genes that are arranged in clusters at the ends of chromosomes. Transcription of Msg genes occurs at a single expression site, termed the *upstream conserved sequence* (UCS), which is thought to result in only one Msg isoform being expressed on the surface of *Pneumocystis* at a time.[27–40] Changing the Msg gene at the UCS, by recombination and gene conversion, changes the surface Msg, resulting in antigenic variation. This ability to undergo antigenic variation may be an important mechanism whereby *Pneumocystis* evades the host immune response.[25] Studies suggest that antigenic variation and evasion of the host immune response may occur at both the cellular immune level and the humoral level.[38] Recombinant Msg preparations have overcome the previous limitations of crude *Pneumocystis* antigen preparations and offer promise to be helpful serologic tools. Variants of the recombinant Msg antigens have been used to better characterize the reactivity of the humoral responses in a number of studies.[41–44]

A second family of surface antigens was identified during studies characterizing Msg. A subtilisin-like serine protease encoded by the *PRT1* gene, also known as the Kex multigene family, was localized to the surface of rat-derived *Pneumocystis*.[45] In *P. murina* and *P. jirovecii* only a single key gene has been identified.[46] In other fungi these proteases are involved in the processing of preproteins as they make their way to the cell surface and may play a role in antigenic variation in *Pneumocystis.* There is a considerable body of evidence that Kex not only contains protective T-cell epitopes but also has protective B-cell epitopes. Immunization of $CD4^+$ cell–depleted mice with a Kex vaccine protects against the development of PCP and is protective in acquisition of *Pneumocystis* infection in immunosuppressed nonhuman primates.[6,47,48]

The third major antigen complex is a glycoprotein that migrates as a broad band of 45 to 55 and 35 to 45 kDa in rat and human *Pneumocystis,* respectively. The gene encoding a rat *Pneumocystis* 45- to 55-kDa antigen (p55) has been cloned and sequenced; the 3′ end of the molecule stimulates a host immune response.[49] Immunization with recombinant p55 antigen or DNA p55 encoding DNA afforded partial protection to subsequent infection.[50,51] Gene variation has also been demonstrated in the p55 gene, with up to five variants identified within the *Pneumocystis* genome.[52] The predicted amino-acid sequence of p55 shows a repeated motif rich in glutamic acid residues that, in other microbes, such as *Plasmodium,* has been suggested as a mechanism to divert the host immune response.[53] The 35- to 45-kDa band of human *Pneumocystis* is frequently found in respiratory tract specimens and is also recognized by serum antibodies.

EPIDEMIOLOGY

Pneumocystis infection is mainly acquired by inhalation, and recent evidence suggests that the infective form is the cystic form or ascus.[18] In addition, there are reports of *trans*-placental or vertical transmission.[52] Primary infection is acquired early in life, and infants probably serve as the natural host. This infection can either be asymptomatic or manifested by mild (usually upper) respiratory illness.[53,54] Serologic studies in different geographic locations have shown that most (≈80%) healthy children have been exposed to *Pneumocystis* by 2 to 3 years of age.[53,55] Cross-sectional studies by investigators in Chile, who used the polymerase chain reaction (PCR) amplification of the *P. jirovecii mtLSU* rRNA gene, found the prevalence of *P. jirovecii* colonization to be 52% in infants and 65% in adults who died and were autopsied in Santiago.[3,56]

Over the past decade there has been significant interest in *P. jirovecii* colonization. Risk factors for colonization include immunosuppressive conditions (HIV, low CD4 cell count, cancer, autoimmune diseases, organ transplantation); immunosuppressive drugs (corticosteroids, tumor necrosis factor-α [TNF-α] inhibitors); COPD and other chronic lung disorders; other conditions (pregnancy, cigarette smoking); treatment with ibrutinib or idelalisib; and lack of surfactant protein A in mice.[6,57,58] Colonization also varies in different geographic areas. The prevalence of colonization in healthy subjects is estimated to be about 20%.[6] The duration of colonization is variable. A study of a nonhuman primate colony revealed that all of the animals became colonized over a 2-year period, and the average duration of colonization was about 2 months.[59]

P. jirovecii colonization can have several consequences. Colonization is thought to precede the development of PCP but does not necessarily lead to PCP.[60] When PCP does develop, the *P. jirovecii* genotype associated

with pneumonia is often the same as in the genotype in colonization. Patients colonized with *P. jirovecii* can transmit the infection to others, and when recipients are immunosuppressed, PCP can result. Colonization is an independent risk factor for the development of airway obstruction, more severe PCP, or both; increased levels of matrix metalloprotease 12, an important contributor to the development of COPD; and an increased systemic inflammatory response.[6] Nonhuman primates infected with simian-human immunodeficiency virus (SHIV) that became colonized with *Pneumocystis* spontaneously developed progressive COPD, whereas SHIV-infected primates not colonized with *Pneumocystis* did not.[61]

PCP in HIV patients was first reported in 1981, and its incidence increased dramatically in high-income countries throughout the 1980s.[62] The number of cases were several orders of magnitude greater than in non-HIV patients. With widespread use of PCP chemoprophylaxis and combination antiretroviral therapy (ART), the incidence has steadily fallen since the early 1990s.[63] PCP now mainly occurs in individuals who are unaware that they are HIV positive, lack access to medical care, or who are noncompliant with medication.[64] Yet PCP still remains a leading opportunistic infection in HIV-positive patients in North America, Europe, and Australasia. PCP is increasingly recognized in low- and middle-income countries.[65,66]

Risk factors for PCP in HIV-positive patients include low absolute and percentage $CD4^+$ cell count, oral candidiasis, fevers, nonadherence with medication, polymorphism in the FcγIIIa receptor, and possibly *P. jirovecii* colonization.[6]

By contrast with HIV-positive patients, the incidence of PCP in non-HIV patients is rising.[5] The likely reasons for the increase of PCP in non-HIV patients include broader use of new and standard regimens of immunosuppressive drugs. New agents include immunomodulators, such as TNF-α inhibitors, and B-cell modifiers. Conditions requiring the use of these compounds are cancer (solid tumors, hematologic malignancies), organ transplantation, rheumatologic and connective tissue disorders, and inflammatory bowel disease.[6,67] Evidence to guide clinicians in prevention of PCP in non-HIV patients are available (Table 269.1).[68–70]

TABLE 269.1 Patients at Risk of Developing *Pneumocystis* Pneumonia, Unless Prophylaxis Is Given

	HIV/AIDS	SOLID-ORGAN TRANSPLANT	HEMATOLOGIC MALIGNANCY	CONNECTIVE TISSUE DISEASE
Risk factor for developing PCP	• CD4 count <200 cells/mm³ • CD4 <14% of total lymphocyte count • Prior episode of PCP • High plasma HIV load	• CD4 count <200 cells/mm³ • Use of: Corticosteroids Antilymphocyte therapy, (e.g., alemtuzumab, mycophenolate mofetil) Calcineurin inhibitors • CMV disease • Allograft rejection • Prolonged neutropenia • Exposure to patients with PCP	• CD4 count <200 cells/mm³ • Lymphopenia • Use of: Monoclonal antibodies (e.g., rituximab) Immunosuppression to prevent rejection of allogenic hemopoietic SCT Purine analogues or high-dose corticosteroids for autologous SCT Chemotherapy (including R-CHOP14, FCR, ABVD, gemcitabine, high-dose methotrexate) • ALL, *or* lymphoproliferative disorders (multiple myeloma, CML, NHL) • GVHD	• CD4 count <200 cells/mm³ • Lymphopenia • Use of: Anti-TNF inhibitors Azathioprine Corticosteroids (≥20 mg prednisolone OD (or equivalent) for ≥4 wk) Cyclophosphamide Methotrexate Rituximab
Prophylaxis given to patients	• CD4 count <200 cells/mm³ • CD4 <14% of lymphocyte count • CD4 200–250 cells/mm³, if regular (e.g., 3 monthly) CD4 monitoring is not possible	• All solid-organ transplants • Use of prednisolone ≥20 mg OD for ≥4 wk • Recurrent or chronic CMV disease • When immunosuppression is increased to prevent transplant rejection • Prolonged neutropenia	• ALL • Allogenic hemopoietic SCT • 20 mg prednisolone for ≥4 wk) • Use of: Alemtuzumab Fludarabine/cyclophosphamide/ rituximab Optional: • Radiotherapy and high-dose corticosteroids for primary brain tumor/metastases • Lymphoma treated with R-CHOP14 or BEACOPP • Use of: Nucleoside analogs (fludarabine, cladribine, mycophenolate mofetil)	• ANCA-positive vasculitis (especially granulomatosis with polyangiitis) • Use of: Corticosteroids with other agents, e.g., azathioprine, cyclophosphamide, methotrexate, when used to treat SLE, *or* inflammatory myopathy (dermatomyositis, polymyositis)
Duration of prophylaxis	• When CD4 increases from <200 cells/mm³ to >200 cells/mm³ for >3 mo (reintroduce if CD4 falls to <200 cells/mm³) • When CD4 is 100–200 cells/mm³, if HIV plasma RNA level is below limits of quantification for 3–6 mo	• All solid-organ transplants: for 6–12 mo posttransplant • Consider lifelong prophylaxis for: Lung/small bowel transplant • History of prior PCP • Chronic CMV disease	• ALL: From induction to end of maintenance • Allogenic hemopoietic SCT: ≥6 mo after engraftment (and for as long as immunosuppression is ongoing) • Alemtuzumab: >6 mo after completion of treatment • Fludarabine/cyclophosphamide/ rituximab: ≥6 mo after completion of treatment	
Prophylaxis regimen	First choice: TMP-SMX, 1 double-strength (DS) tablet OD *or* 1 single-strength (SS) tablet OD Alternatives: Dapsone *or* dapsone *plus* pyrimethamine *plus* leucovorin, Aerosolized pentamidine (via Respirgard II nebulizer) Atovaquone	First choice: TMP-SMX, 1 SS tablet OD *or* 1 DS tablet OD (or three times per week) Alternatives: Dapsone Aerosolized pentamidine Atovaquone Clindamycin *plus* primaquine	First choice: TMP-SMX, 1 SS tablet OD *or* 1 DS tablet OD (or three times/wk) Alternatives: Dapsone Aerosolized pentamidine	First choice: TMP-SMX, 1 DS tablet OD *or* 1 SS tablet OD Alternatives: Dapsone, or dapsone *plus* pyrimethamine *plus* leucovorin Aerosolized pentamidine Atovaquone

TABLE 269.1 Patients at Risk of Developing *Pneumocystis* Pneumonia, Unless Prophylaxis Is Given—cont'd

	HIV/AIDS	SOLID-ORGAN TRANSPLANT	HEMATOLOGIC MALIGNANCY	CONNECTIVE TISSUE DISEASE
Notes	• Prophylaxis indicated for all HIV-infected adults, including pregnant women and those on ART • Prophylaxis not needed if receiving sulfadiazine-pyrimethamine for treatment or suppression of toxoplasmosis • TMP-SMX DS tablet protects against toxoplasmosis and many respiratory infections • Atovaquone is as effective as aerosolized pentamidine (but is more expensive) • If seropositive for toxoplasmosis, and patient cannot tolerate TMP-SMX, dapsone *plus* pyrimethamine *plus* leucovorin, *or* atovaquone ± pyrimethamine *plus* leucovorin are alternatives • Insufficient data to recommend: Aerosolized pentamidine given by devices other than Respirgard II Intermittently administered IV pentamidine Oral clindamycin *plus* primaquine (could consider these regimens if recommended agents cannot be administered or are not tolerated)			

ABVD, Adriamycin (doxorubicin), bleomycin, vinblastine, dacarbazine; *ALL,* acute lymphoblastic leukemia; *ANCA,* antineutrophil cytoplasmic antibody; *ART,* antiretroviral therapy; *BEACOPP,* bleomycin, doxorubicin (adriamycin), etoposide, cyclophosphamide, vincristine (Oncovin), procarbazine, prednisolone *CML,* chronic myeloid leukemia; *CMV,* cytomegalovirus; *FCR,* fludarabine, cyclophosphamide, rituximab; *GVHD,* graft-versus-host disease; *HIV,* human immunodeficiency virus; *IV,* intravenous; *NHL,* non-Hodgkin lymphoma; *OD,* every day; *PCP, Pneumocystis jirovecii* pneumonia; *R-CHOP14,* rituximab, cyclophosphamide, doxorubicin, vincristine (Oncovin), prednisolone (given every 14 days); *SCT,* stem cell transplant; *SLE,* systemic lupus erythematosus; *TMP-SMX,* trimethoprim-sulfamethoxazole; *TNF,* tumor necrosis factor.
Data from Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed May 19, 2017; Martin SI, Fishman JA, AST Infectious Diseases Community of Practice.* Pneumocystis *pneumonia in solid organ transplantation.* Am J Transpl. *2013;13:272–279; Maertens J, Cesaro S, Maschmeyer G, et al; 5th European Conference on Infections in Leukaemia (ECIL-5); a joint venture of the European Group for Blood and Marrow Transplantation (EBMT); European Organisation for Research and Treatment of Cancer (EORTC); Immunocompromised Host Society (ICHS); European LeukemiaNet (ELN). ECIL guidelines for preventing* Pneumocystis jirovecii *pneumonia in patients with haematological malignancies and stem cell transplant recipients.* J Antimicrob Chemother. *2016;71:2397–2404; and Wolfe RM, Peacock JE.* Pneumocystis *pneumonia and the rheumatologist: which patients are at risk and how can PCP be prevented?* Curr Rheumatol Rep. *2017;19:35.*

There is an ongoing debate about the mechanisms by which PCP develops. The older theory is reactivation of latent infection. *Pneumocystis* infection is acquired in early childhood, becomes part of the host's resident microbial flora, and remains dormant for long periods of time. With a decline in the host's immune function, active replication of the organism occurs and results in PCP. The alternative view, which is held by most investigators, is that exposure to *Pneumocystis* is transient and that individuals are frequently exposed to disparate environmental sources of the organism throughout their lives. These theories have different implications when developing infection control procedures for PCP in health care facilities. If *P. jirovecii* is communicable, patients with PCP should not have direct contact with other immunocompromised hosts.[71]

Several studies have raised the possibility of environmental sources of *Pneumocystis*. *Pneumocystis* DNA has been detected in the air and in pond water.[72] Outdoor activities, such as gardening and hiking, have been shown to be independent risk factors for PCP.[73] Geographic clusters of PCP in HIV-positive patients have been found in the United States in San Francisco and Cincinnati and in the United Kingdom.[74,75] Cases of PCP were mainly found in the more affluent areas of the US cities where there was more green space. These clusters suggested the possibility of either exposure to a common environmental source of *Pneumocystis* or to a specific location where many at-risk individuals gathered and spread of *P. jirovecii* occurred.

Studies of environmental factors affecting the occurrence of PCP have mainly focused on climatologic factors. The most frequent association has been the occurrence of PCP during the warmer parts of the year.[72] Another report found a significant monthly and seasonal variation in the occurrence of specific *P. jirovecii* genotypes.[76] In addition to season, air-pollution factors are related to PCP; increased ambient sulfur dioxide is associated with risk of hospitalization with PCP, but its effects are modified by increased levels of carbon monoxide.[77]

There is considerable evidence that *Pneumocystis* is communicable. Experimental transmission of *Pneumocystis* infection has been shown by close contact or the airborne route in immunodeficient, immunosuppressed, and normal mice, rats, and other animals.[72] There have been outbreaks of PCP in immunodeficient rodent colonies, as well as *Pneumocystis* colonization and transmission of *Pneumocystis* infection in commercial rodent colonies under strict barrier conditions.[78,79]

Outbreaks of PCP in humans occurred in crowded orphanages during World War II and in immunosuppressed patients in the 1960s and 1970s.[72] PCP outbreaks in immunosuppressed patients over the past several decades have occurred mainly in renal transplant recipients. A systematic review that examined 16 outbreaks involving about 200 patients over the past 3 decades revealed common problems, such as frequent interpatient contact, a lack of chemoprophylaxis, and a lack of adherence to isolation procedures.[80] No environmental source or seasonal occurrence of PCP was found. There was considerable mortality, but it decreased over time. *Pneumocystis* genotyping showed that most outbreaks were caused by a single or dominant strain of *P. jirovecii,* and that there was person-to-person transmission. Recommendations included isolation of PCP patients and use of chemoprophylaxis. Subsequent publications have emphasized the importance of *P. jirovecii* colonization and the need for chemoprophylaxis of these patients, revised chemoprophylaxis guidelines, and the importance of PCP in other immunosuppressed patient populations.[72]

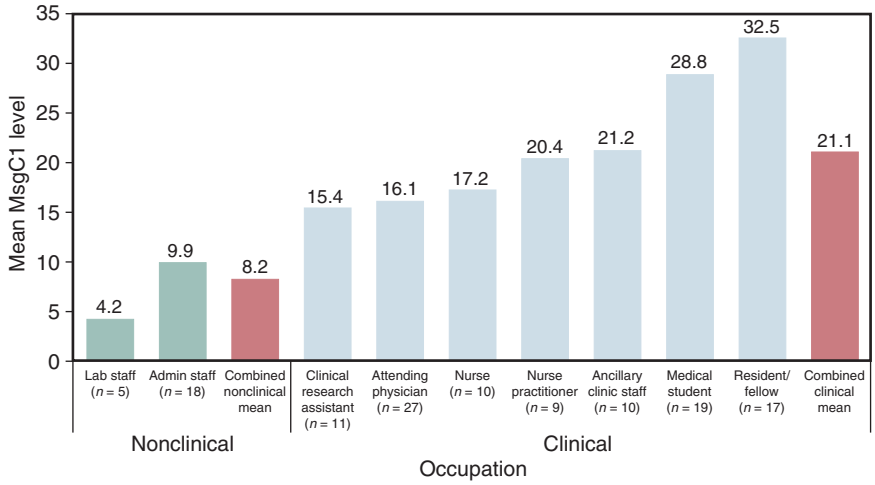

FIG. 269.3 Serologic study of San Francisco General Hospital health care workers. Major surface glycoprotein C1 (MsgC1) levels by occupation. Geometric mean MsgC1 antibody levels are shown for nonclinical and clinical staff, by job title. *(From Tipirneni R, Daly KR, Jarlsberg LG, et al. Healthcare worker occupation and immune response to* Pneumocystis jirovecii. Emerg Infect Dis. *2009;15:1590–1597.)*

Procedures to collect and quantify viable *Pneumocystis* organisms in the air of patients with PCP have been developed.[81] Viable *P. jirovecii* was detected in 80% of samples at 1 m from the bedside, in 33% of the samples at 8 m from the bedside, but not in the air at other locations inside or outside the hospital. These data support the idea that *P. jirovecii* infection can be spread from person-to-person by close contact and support the idea that patients with active PCP should be isolated from other immunocompromised (and thus at-risk) patients.

Serologic studies using crude antigen preparations have established that *P. jirovecii* infection is acquired early in life, so by age 2 or 3 years of age most (≈80%) children have been exposed to the organism[53,55]; however, these tests have lacked the sensitivity and specificity to be of help in other epidemiologic, clinical, or diagnostic studies.[51] The development of recombinant Msg fragments has helped investigators to overcome these problems. A sequential serologic study that followed up healthy infants during the first 2 years of life showed that the force or rate of infection was 8% to 10% per month, that a proportion of subjects developed recurrent episodes of *P. jirovecii* colonization, and that there were seasonal changes in these responses.[82] An analysis of health care workers showed that people who had clinical contact with patients have significantly higher antibody levels to *Pneumocystis* Msg fragments than workers who do not (Fig. 269.3).[43] This observation is important because it raises questions about whether health care workers might contribute to the spread of *P. jirovecii* in hospitals.

Other serologic studies have shown that *P. jirovecii* infection has worldwide distribution, but factors such as geography, smoking, and previous episodes of PCP independently affect antibody levels; that serology has promise as a method of diagnosing PCP; and that antibody levels to specific Msg fragments and recombinant Kex have prognostic value.[83–85]

PATHOLOGY AND PATHOGENESIS

After inhalation, the cystic stage of *Pneumocystis* releases the trophic forms, which initiate the infection by adhering to epithelial cells of the respiratory tract. Our current concepts of how this occurs are almost entirely based on studies of alveolar epithelial cells. The trophic form preferentially attaches to the alveolar type I cell, with close apposition of the cell surfaces but no fusion of the membranes.[86] Although the type I cell does not replicate, in vitro studies have shown that *Pneumocystis* attachment to cultured alveolar type II cells increased differentiation into a type I cell–like phenotype.[87] Other studies have shown that the adherence of *Pneumocystis* also occurs via extracellular matrix glycoproteins, resulting in altered gene expression, morphologic changes, and organism proliferation.

The attachment for *Pneumocystis* to lung epithelial cells requires an intact cytoskeleton and results in changes in both the organism and host.[88] *Pneumocystis* maintains an extracellular existence within alveoli and probably obtains essential nutrients from the alveolar fluid or living cells. This is supported by the finding of overexpression of multiple transporters found in the *Pneumocystis* genome and the corresponding absence of important synthetic genes.[89] In HIV-positive patients *Pneumocystis* also enhances HIV proliferation.[90] Another effect of the attachment is to suppress the growth of lung epithelial cells through cyclin-dependent kinase regulatory pathways.[91] Other reports have shown that the organism alters lung guanosine triphosphate–binding regulatory proteins and induces expression of intracellular adhesion molecule 1 and fibrinogen.[92] These properties may influence both the lung damage and host inflammatory and reparative responses in PCP.

Host defenses against *Pneumocystis* include innate immunity and adaptive or acquired immunity.[93] The innate immune system, which is the first level of defense, is composed of alveolar macrophages, surfactant protein (SP)-A, SP-D, and other factors. Because alveolar macrophages have early and late actions in the infection, they are discussed later in this chapter. SP-A is a pulmonary collectin that functions in host defense (e.g., as a nonimmune opsonin) and as an immune modulator. Immunosuppressed SP-A knockout mice develop higher infection levels of *Pneumocystis* and an increased host inflammatory response compared with wild-type mice.[94] The role of SP-D in host defenses is less clear, but it appears to modulate the inflammatory response in conditions such as the immune reconstitution inflammatory syndrome (IRIS).[95]

There is accumulating evidence that B cells and humoral immunity play an important part in host defenses against *Pneumocystis* infection. Natural immunoglobulin M (IgM) antibodies that recognize common fungal carbohydrate antigens have been discovered and may provide protection against early *Pneumocystis* infection.[96] PCP has been reported in patients and mice with B-cell defects or given medication (e.g., rituximab) that acts on B cells.[51,97] Mice depleted of CD4 T-cells after recovery from a primary infection are still protected from a secondary fungal challenge.[98,99] Depletion of CD8 T-cells or macrophages, which can mediate opsonic phagocytosis, abrogated protection in a secondary challenge model.[100] Studies in SHIV-positive primates have shown a correlation of acquired antibody levels to Kexin and susceptibility or resistance to *Pneumocystis* colonization.[101] A positive therapeutic effect has been found with the passive administration of hyperimmune serum or monoclonal antibodies to Msg and other antigens in experimental models of PCP.[29,30,101] Immunization with live *Pneumocystis* or Kex DNA with dendritic cells engineered to express CD40L, as a genetic adjuvant, protected CD4 cell–depleted mice from organism challenge and appeared to be mediated by antibodies via helper T cell (Th)1- or Th2-type responses.[102–104] A subsequent report showed that interleukin (IL)-23 produced by these dendritic cells played a critical role in eliciting recall antibody responses and that IL-23 might be of value as a potential

adjuvant.[105] Conserved antigens, such as the recently described PCA1 that is homologous between murine and human *Pneumocystis* spp., may serve as better vaccine targets.[106] B cells function as not only the source of antibodies but also as antigen-presenting cells and in helping the development of memory CD4 cells.[107] Antibodies contribute to host defenses against *Pneumocystis* by acting as opsonins.[51]

Analysis of the role of antibodies in humans has been difficult because of the high prevalence of serum antibodies in the population and the lack of information about which antigen epitopes are protective. HIV induces abnormalities in B-cell number and function, which results in impaired antibody responses to vaccines or microbial antigens.[108] In a South African cohort of children hospitalized with PCP, humoral responded to Msg antigens compared with HIV-uninfected PCP cases.[109] Recombinant Msg antigen fragments have shown promise in seroepidemiologic studies. Recent reports have suggested that low antibody levels in HIV patients to Kex are a risk factor for developing PCP,[85] and antibody levels to Msg are predictors of survival in hospitalized patients.[84] Although local bronchoalveolar lavage fluid (BALF) antibodies have received only limited attention, there is evidence of decreased antibody responses in HIV patients and in SHIV-positive nonhuman primates.[101,110–112]

Impaired cellular immunity has long been considered an important predisposing factor in the development of PCP.[51,86] Naturally occurring outbreaks of PCP have occurred in immunodeficient animals, particularly colonies of severe combined immunodeficiency disease (SCID) mice and athymic nude mice and rats.[78,113] The central role of $CD4^+$ cells in host defenses against this organism has been shown by cell depletion and reconstitution experiments and by knockout mice.[114–116] Other contributors to these defenses include T-cell costimulatory molecules, such as CD28 and CD2; the CD40 to CD40L pathway, which facilitates the interaction of T cells with B cells; and CD8 cells, which function as cytotoxic cells or by secreting cytokines.[115,117–122] The role of γδ T cells in host defenses against *Pneumocystis* is unclear, but it is thought that these cells might help recruit CD8 cells and modulate their effects.[6] Natural cells also play a role in host defenses against *Pneumocystis* but require $CD4^+$ cells to become activated.[105]

PCP can be induced in normal rodents by the administration of corticosteroids, and these models have been used for more than 3 decades.[86] Protein malnutrition and an immature immune system also impair host defenses against *Pneumocystis,* although the defect in neonatal mice is related more to factors in the lung milieu than to T cells.[123,124] The clearest evidence of the role of defective cell-mediated immunity in the development of PCP in humans comes from persons infected with HIV (see Table 269.1). The risk of developing PCP in adult HIV patients increases greatly when circulating CD4 cells fall below 200 cells/mm^3.[125] Because CD4 counts are much higher in young children than in adults, different criteria must be used. The presence of other clinical complications of HIV (e.g., fever and oral candidiasis) increases the risk of PCP independent of the CD4 count. Cases of PCP associated with CD4 counts <200 cells/mm^3 have been encountered in cancer patients receiving cytotoxic drugs, in adults with idiopathic CD4 lymphopenia, and in otherwise healthy individuals with subtle T-cell defects[126,127] (see Table 269.1).[128,129] In general, non-HIV patients have higher peripheral blood and BALF CD4 cells than HIV patients; however this patient group is often receiving corticosteroids and other immunosuppressive drugs that can lower $CD4^+$ cell counts.[130] There are clear guidelines based on $CD4^+$ counts and other risk factors for initiating PCP chemoprophylaxis in non-HIV patients.[131]

The occurrence of PCP in other patient populations with impaired cellular immunity include premature debilitated infants and children with primary immunodeficiency diseases, particularly SCID, which involves both T- and B-cell defects, and the hyper-IgM syndrome, which involves disruption of the CD40-CD40L pathway,[128] and patients receiving immunosuppressive drugs for the treatment of a variety of conditions (see Table 269.1).[132] The principal immunocompromised hosts at risk for PCP include patients with hematologic malignancies and solid tumors (e.g., brain tumors), solid-organ and bone marrow transplant recipients, and collagen vascular disorders such as granulomatosis with polyangiitis.[6,86,126,133–137] The number of these individuals has grown over the years with better survival and the more widespread use of cytotoxic and immunosuppressive therapies. Corticosteroids, used alone or in combination with other agents, such as infliximab, etanercept, and calcineurin inhibitors, remain the most common immunosuppressive drugs implicated in the development of PCP. The relationship of corticosteroids to *Pneumocystis* has been emphasized by the occurrence of PCP in patients with Cushing syndrome but also in children receiving corticosteroids for diseases such as asthma, which are not known to predispose to opportunistic infections.[138,139] Cases of PCP in patients on chemotherapy regimens without corticosteroids are described.[97] Protein malnutrition is an important risk factor for the development of PCP, both by itself and as a complication of the patient's underlying disease or chemotherapy. Lung factors (e.g., radiotherapy and fibrosis) have been suggested as additional predisposing factors in non-HIV patients.[86]

Alveolar macrophages are the first line of defense against *Pneumocystis* and the principal effector cell in clearing the organism from the lung.[140] Rodents depleted of macrophages are susceptible to *Pneumocystis* infection; however, activated macrophages in the absence of CD4 cells are unable to control *P. jirovecii* infection.[140,141] Recognition and adherence of *Pneumocystis* to macrophages occur by multiple pathways involving Msg and BG in the organism; extracellular matrix and surfactant proteins; and mannose, dectin-1, and Fc receptors.[32–37,142] Toll-like receptor 2 (TLR2) and TLR4 help regulate host cytokine responses.[143,144] Macrophages ingest, degrade, and kill *Pneumocystis,* releasing cytokines, such as TNF-α, eicosanoids, nitric acid, and reactive oxidants.[32–36,145–147] Macrophages also undergo apoptosis, which is mediated by polyamines.[148]

Both the life cycle form of *Pneumocystis* organisms and the type of macrophage response seems critical in organism clearance. The cystic form, which is rich in BG, is recognized by alveolar macrophages, dendritic cells, and lung epithelial cells and primes a robust T-cell–mediated response. Trophic forms lacking BG elicit reduced CD4 T-cell recruitment and interferon (IFN)-γ production. In addition, the trophic forms actively suppress the proinflammatory response initiated by cystic forms, in that trophic form–loaded dendritic cells had reduced capacity to stimulate CD4 T-cell proliferation and polarization, potentially promoting trophic form survival.[149] Recent studies demonstrated that classic macrophages (M1) predominate in the innate response to *Pneumocystis* infection in the absence of an intact immune system, such as seen in the immunosuppressed rat model, and were defective in clearing *P. carinii.* Immunocompetent animals had more prominent alternative macrophage (M2) responses with clearance of infection. Treatment of immunosuppressed rats with M2 cells reverted the animals to a protected phenotype.[150,151] Compared with alveolar macrophages from adult mice, macrophages from newborn mice are intrinsically unresponsive to *Pneumocystis* infection.[152] On the other hand, *Pneumocystis* infection of adult mice downregulates the transcription factor PU.1, which leads to decreased expression of dectin-1.[153] Recently, an intracellular isoform of osteopontin, a protein expressed by many cells, has been shown to play an important role in innate immunity in *Pneumocystis* infection.[154]

Alveolar macrophage function is impaired in HIV-positive patients and other immunosuppressed populations. HIV downregulates mannose receptor expression, which results in decreased binding and uptake of *Pneumocystis;* impairs MD88-associated TLR4 signaling; and changes the macrophage cytokine response.[155–157] *Pneumocystis* itself impairs phagocytosis by promoting shedding of the mannose receptor and enhances the replication of HIV.[90,158]

The BG in *Pneumocystis* cysts elicits inflammatory responses in macrophages and other types of lung cells that contribute to the lung damage in PCP. Alveolar epithelial cells secrete neutrophil chemoattractant proteins, TNF-α, IL-8 in humans and MIP-2 in rats, and other inflammatory mediators.[146,159] The process involves signal transduction and lactosylceramide, a prominent constituent of the alveolar epithelial cell microdomains.[159,160] BG also stimulates dendritic cells, which are antigen-presenting cells, to secrete IL-23 and IL-6; these latter cytokines then stimulate Th17 phenotype.[161]

Exposure to *Pneumocystis* or its antigens stimulates production of a multitude of cytokines and chemokines. Two proinflammatory cytokines, TNF-α and IL-1, have been shown to be important in host defenses against the organism, particularly in the early stages of the infections.[162,163] TNF helps recruit lymphocytes and monocytes, which

contribute to *Pneumocystis* clearance, and chemokines (e.g., IL-8) from alveolar epithelial cells as part of the inflammatory response. IL-6, another proinflammatory cytokine, has been produced in response to *Pneumocystis,* but its contributions to host resistance to the organism are unclear.[164] IFN-γ and granulocyte-macrophage colony-stimulating factor are important contributors to host defense by macrophage activation or in cooperation with TNF-α.[165,166] The role of IFN-γ is particularly complex, and the results obtained can vary, depending on the experimental design. Recent studies have shown that IL-12 and IL-23 also contribute to host defenses to *Pneumocystis*.[167,168] IL-10 has also been shown to modulate the host inflammatory response, but no role in the host defense against *Pneumocystis* has been found for IL-4 or granulocyte-colony stimulating factor.[169,170]

The pathologic changes that occur during the development of PCP in animal models and in humans are similar.[86] As the host defenses become compromised, *Pneumocystis* organisms begin to proliferate and gradually fill alveolar lumens. In the corticosteroid-treated rat model, the organism number increases from 10^5 per lung, or fewer, to 10^9 to 10^{10} per lung after 8 to 10 weeks of corticosteroid administration. The principal histologic finding is the formation of a foamy, eosinophilic alveolar exudate (Fig. 269.4); as the PCP increases in severity, there may also be hyaline membrane formation, along with interstitial fibrosis and edema. The host inflammatory response is usually inconspicuous and is characterized by type II cell proliferation (a typical reparative response) and scanty mononuclear cell infiltrate. SCID mice exhibit cytokine production only late in the course of PCP, when elevated levels of TNF-α and IL-1 are found in the lungs.[171] Several studies have shown that rats with corticosteroid-induced PCP, as well as HIV and non-HIV patients with the disease, have elevated levels of proinflammatory cytokines in their respiratory tract but not in the peripheral blood.[172–174] Some patients exhibit atypical findings, such as lack of the alveolar exudate or the development of cavitary lesions, granulomas, or microcalcifications.[175] On electron microscopy, there is increased alveolar-capillary permeability, followed by evidence of damage to the type I cell.[86] Physiologic changes include hypoxemia with an increased alveolar-arterial oxygen (PAO_2-PaO_2) gradient and respiratory alkalosis; impaired diffusing capacity, suggesting alveolar-capillary block; and alterations in lung compliance, total lung capacity, and vital capacity.[176,177] The resulting picture suggests diffuse lung damage similar to that seen in the acute respiratory distress syndrome.



FIG. 269.4 Histologic findings in pneumocystosis. *Pneumocystis* pneumonia illustrating frothy eosinophilic honeycombed material filling the alveolar space (hemotoxylin and eosin stain, ×400).

The pathophysiologic changes described are caused not only by the effects of *Pneumocystis* on the type I cell but also alterations in the surfactant system and host inflammatory response. There is a fall in surfactant phospholipids (mainly phosphatidylcholine) that is caused by inhibition of phospholipid secretion mediated by Msg and other organism constituents.[178–181] Changes in the surfactant proteins include a decline in SP-B and SP-C and rise in SP-A and SP-D levels.[182,183] Fractionation of the surfactant has shown that most of the increased SP-A and SP-D levels are localized mainly in the small aggregate compartment.[184] There is now clear evidence that the host's immune inflammatory response to *Pneumocystis* can have harmful and helpful effects on the host lung. These effects are complex and depend to some degree on the experimental model being used. Immune reconstitution and cell depletion studies using SCID mice have shown that the clearance of PCP is associated with a hyperinflammatory response composed of increased proinflammatory cytokines and chemokines and reduced oxygenation and compliance.[185–188] These effects involve not only the complex interaction of CD4 cells and CD8 cells but also subsets of CD4 cells ($CD25^+$, $CD25^-$) and CD8 cells (TCI, TC2), which have effector or immune-modulatory functions.[189–194] In contrast to humans, neutrophils are a marker of inflammation and lung damage in mice but do not cause lung damage.[195] The administration of a large number of splenocytes or CD4 cells sensitized to Msg in rats with corticosteroid-induced PCP results in clinical illness and a cytokine cascade, along with a reduction in organism burden.[196] The presence of steroids has no apparent effect on these events. The contribution of the host inflammatory response to lung damage in HIV patients with PCP has been suggested by studies that have correlated increased numbers of neutrophils and levels of IL-8 in BALF with more severe pneumonia and worse prognosis.[197,198] IL-8 functions as a potent chemoattractant, its interaction with *P. jirovecii* is mediated by Msg, and its interaction with alveolar macrophages requires the coexpression of the mannose receptor and TLR2.[199–201] Alterations in eicosanoids, TNF-α, IL-1, other cytokines, and inflammatory mediators have also been noted in these and other studies; however, the pathogenic significance of these changes is unclear. HIV patients with PCP also frequently experience a worsening of respiratory function soon after receiving antimicrobial drugs. Corticosteroids, if given promptly, can ameliorate or prevent this outcome and improve survival.[202] It is thought that the beneficial effects of corticosteroids are attributable to their antiinflammatory properties or their effects on surfactant components; however, studies examining these issues have produced inconsistent results.[203–206]

CLINICAL MANIFESTATIONS

The presenting symptoms of PCP in the immunocompromised host are nonspecific and are mimicked by a wide variety of infectious and noninfectious etiologies. Presentation is typically with progressive exertional dyspnea, fever, and a nonproductive cough,[63,207] which is often associated with an inability to make a maximal inspiration (not due to chest pain).

On occasion, sputum is produced; hemoptysis is not a feature. Patients receiving immunosuppressive drugs frequently develop these clinical manifestations after the corticosteroid/immunosuppressive therapy agent dose has been tapered and are typically increasingly symptomatic for about 1 to 2 weeks before seeking medical attention. Among HIV-infected persons, symptoms are usually of longer duration than among medically immunosuppressed patients. HIV-infected patients frequently have prolonged prodromal periods with subtle clinical manifestations developing over 3 to 8 weeks; however, some individuals present with a fulminant worsening of symptoms over 7 to 10 days, or less; the intrapulmonary organism burden is higher, but lung damage is less severe.[208,209] Studies have also compared the clinical features of PCP in adult HIV patients by age and underlying risk group.[210,211] In both HIV-infected and non–HIV-infected patients, however, the clinical picture is variable; for

example, lung allograft recipients who develop PCP are frequently asymptomatic at the time of diagnosis.

On physical examination, varying degrees of respiratory distress may be evident (manifest as tachycardia, central cyanosis, use of accessory muscles of respiration), as well as stigmata of immunodeficiency/treatment-induced immune suppression (e.g., after receipt of chemotherapy or immunosuppressive medication posttransplantation), including molluscum contagiosum, seborrheic dermatitis, cutaneous Kaposi sarcoma, oral *Candida,* and oral hairy leukoplakia. Children may additionally demonstrate central cyanosis, flaring of the nasal alae, and intercostal retractions. Auscultation of the chest is usually normal; fine end-inspiratory crackles may be heard in about one-third of adults.[216]

Other Clinical Manifestations

The spread of *P. jirovecii* beyond the lungs is rarely encountered and occurs mainly in patients with advanced HIV infection who are taking no prophylaxis or only aerosolized pentamidine. The actual incidence of extrapulmonary pneumocystosis is unclear as the diagnosis is made by histologic examination of sites where there are clinical manifestations (e.g., lymph nodes, spleen, liver, bone marrow, gastrointestinal [GI] tract, eyes, thyroid, adrenal glands, and kidneys), or at autopsy.[212] Manifestations of extrapulmonary pneumocystosis include thyroid mass, pancytopenia from bone marrow necrosis, retinal cotton wool spots, and multiple hypodense splenic lesions on ultrasound or computed tomography (CT) scan. Biopsy or fine-needle aspiration shows areas of necrosis filled with foamy material. Grocott-Gomori methenamine silver (GMS) or fluorescent monoclonal antibody stain reveals numerous organisms.

Another clinical problem is the immune reconstitution inflammatory syndrome (IRIS) that occurs in HIV-infected patients days to months after starting ART, as the patient's immune system begins to recover with a rise in $CD4^+$ cells and is manifested as worsening of a known condition or a new condition.[213–215] IRIS occurs in up to 25% of HIV patients starting ART. The inciting agent can be a viable subclinical pathogen or a residual antigen from a pathogen treated months or years previously. This distinction is important because antimicrobial drugs are administered for infection, whereas antiinflammatory agents are needed for inflammation. In the context of PCP, clinical manifestations include shortness of breath, cough, and progressive pulmonary infiltrates, often mimicking an early relapse of PCP.[216]

DIAGNOSIS

PCP should be considered in any immunocompromised patient who develops respiratory symptomatology, fever, and an abnormal chest radiograph. Because this clinical presentation is nonspecific, diagnosis of PCP must be confirmed by demonstration of the organism in a respiratory specimen.

The classic chest radiographic changes are of bilateral diffuse interstitial infiltrates extending from the perihilar region (Fig. 269.5). In early disease the chest radiograph may be normal; with rapidly progressive or late presenting disease, more confluent alveolar shadowing ("white out") with sparing of the costophrenic angles and apices may be seen. Atypical manifestations include unilateral infiltrates, nodules, cystic air spaces, pneumatoceles, mediastinal lymphadenopathy, and effusions.[86,207,217] Of note, patients with nodular granulomas on chest CT have a more indolent onset, less often have PCP seen in BALF, and may require video-assisted thoracoscopic (VATS) or open lung biopsy for diagnosis.[218] In the early years of the HIV pandemic, patients with PCP who were receiving prophylactic aerosolized pentamidine were noted to more likely develop apical infiltrates (mimicking tuberculosis) and pneumothoraces; both these presentations are now rarely encountered with the decline in use of aerosolized pentamidine.[219] Although the chest radiograph is a sensitive way of detecting PCP, it is nonspecific as these radiographic appearances can also occur in other fungal, mycobacterial, and bacterial infections, and in noninfectious conditions, such as interstitial pneumonitis and pulmonary Kaposi sarcoma.

High-resolution CT (HRCT) is important in the evaluation of symptomatic patients with normal or equivocal chest radiographs. Patches of "ground-glass" shadowing are typical for PCP but are also seen in viral (e.g., cytomegalovirus [CMV], influenza A virus) and fungal pneumonia, as well as in occult alveolar hemorrhage.[207] Nuclear medicine imaging using gallium-67 citrate, indium 111,[125] human polyclonal IgG, and technetium 113–labeled monoclonal antibody to *Pneumocystis* Msg demonstrates intrapulmonary accumulation in PCP; these techniques currently have no role in the evaluation of suspected PCP.[220,221]



FIG. 269.5 Chest radiograph. Shown are bilateral infiltrates of *Pneumocystis* pneumonia. *(Courtesy R. Miller.)*

Impaired blood oxygenation is the most frequent laboratory abnormality found in PCP; analysis of the magnitude of hypoxemia or a widened PAO_2-PaO_2 gradient can be used to evaluate disease severity and monitor progression.[202] Lack of exercise-induced arterial desaturation in a symptomatic patient with a normal PaO_2 at rest and a normal or near-normal chest radiograph virtually excludes a diagnosis of PCP.

Elevated serum lactic dehydrogenase (LDH) levels, reflecting lung injury, occur frequently in PCP and decline with successful therapy. However, the usefulness of serum LDH is limited because elevations can be produced by other pulmonary diseases, including pulmonary embolism; nonspecific interstitial pneumonitis; histoplasmosis; extrapulmonary disease, including multicentric Castleman disease; and lymphoma.[222]

Measurement of serum or plasma BG, a component of many fungal cell walls, including *Pneumocystis,* is increasingly being used as an adjunctive diagnostic tool for diagnosis of PCP. BG levels are higher among both HIV-infected and HIV-uninfected patients with PCP, when compared with symptomatic patients with confirmed alternative diagnoses, including aspergillosis and histoplasmosis. There are no clinical trials using BG for diagnosis of *Pneumocystis* pneumonia, but three meta-analyses of clinical evaluations have been reported.[223] Overall, sensitivity is between 90.8% and 94.8%. The sensitivity of BG may be better in HIV-infected patients with *Pneumocystis*, compared with non–HIV-infected patients, likely a consequence of the greater burden of organisms in HIV-infected persons with *Pneumocystis* pneumonia, compared to those with other causes of immunosuppression. Specificity is not as good, ranging between 78.1% and 86.3%, and may also be affected by noninfective factors. False-positive results have been reported in patients with bacterial pneumonia, those undergoing hemodialysis, or those who recently received intravenous (IV) Ig. Positive- and negative-predictive values (NPVs) range between 46.0% and 54.3%, and 97.2% and 99.0%, respectively.[223] These results imply that a negative BG can be used to exclude PCP; however, false-negative results have been reported. Used alone, a positive BG is not diagnostic of *Pneumocystis* pneumonia as this patient population is susceptible to a wide range of fungal pathogens. The clinician should interpret a positive BG result

in the context of compatible imaging findings and, ideally, a positive result from a *Pneumocystis*-specific assay, for instance, histochemical or molecular detection by PCR. By contrast to serum/plasma, BG has poor clinical utility when applied to respiratory samples, such as BALF.[224]

Pneumocystis is rarely identified in spontaneously expectorated sputum. Induced sputum, that is, "induced" by inhalation of an aerosol of hypertonic saline, is a useful screening technique. The diagnostic yield from induced sputum ranges from <50% to >90% at different medical centers.[222] Supervision of the procedure by an experienced respiratory therapist or nurse increases the yield; a negative result for *Pneumocystis* from sputum induction should prompt referral for bronchoscopy.

Fiberoptic bronchoscopy with BAL has a diagnostic yield of >90% for detection of PCP.[86,225] BAL specimens are usually obtained instead of washings and brushings because they have greater sensitivity and low morbidity. The diagnostic yield of BAL specimens can be increased if multiple lobes are sampled or the procedure is directed toward the sites of greatest radiographic abnormality[226]; BAL specimens also provide information that cannot be obtained from induced sputum—about *Pneumocystis* organism burden, the presence of other infectious agents, and the host inflammatory response.[227] Transbronchial biopsy is associated with complications, including pneumothorax and hemorrhage, and provides little additional diagnostic yield; thus it is not routinely performed in the diagnostic workup of suspected PCP. Treatment should not be deferred in any person with suspected PCP when results of bronchoscopy are pending because significant clinical deterioration may occur. The yield for diagnosis of PCP from BALF in HIV-infected individuals is not reduced for up to 14 days after starting treatment; in those with non–HIV-associated PCP, the diagnostic yield may be reduced within a few days of starting treatment.

Open lung biopsy was the standard procedure for the diagnosis of *Pneumocystis* before the introduction of BAL and transbronchial biopsy because it provided the greatest amount of tissue.[86] Open lung biopsy has largely been superseded by VATS lung biopsy. Lung biopsy is indicated in a patient with suspected PCP when bronchoscopy is nondiagnostic and among patients whose clinical course is at variance with laboratory-confirmed PCP, for instance, in evaluating another infection or condition complicating PCP.

One approach to the management of patients with suspected PCP is presented in Fig. 269.6. Patients with a compatible clinical picture and a chest radiograph demonstrating ground-glass opacities should undergo a diagnostic procedure (sputum induction or BAL) to collect respiratory secretions to detect the organism. In symptomatic patients with a normal chest radiograph, additional testing should be performed to assess the probability of early *Pneumocystis* infection. Either thoracic HRCT scanning or pulmonary function testing can identify patients unlikely to have PCP and who may be monitored without specific anti-*Pneumocystis* treatment. One prospective study showed that a normal, unchanged, or equivocal chest radiograph, together with a chest HRCT scan without ground-glass opacities, ruled out PCP.[228] Similarly, a normal or unchanged chest radiograph and a single-breath diffusing capacity for carbon monoxide (DL_{CO}) of >75% of predicted almost rules out the diagnosis of PCP.[229] A DL_{CO} of <75% of predicted has a low specificity, and patients with ground-glass opacities on HRCT (Fig. 269.7) or a DL_{CO} of <75% of predicted need a diagnostic workup to identify *Pneumocystis* or determine an alternative diagnosis.

A variety of stains have been used to identify *Pneumocystis* in respiratory tract secretions; in the hands of an experienced laboratory team, all have a high diagnostic yield for detection of the organism.[86,225,230] GMS stain or one of its simpler variants (e.g., toluidine blue O, cresyl violet), which selectively stain the wall of the *Pneumocystis* cystic form, are widely used because they are easy to interpret. Stains such as Wright-Giemsa or one of its more rapid variants (e.g., Diff-Quik) stain the nuclei of all *Pneumocystis* developmental stages. Calcofluor white is a chemifluorescent agent that binds to cellulose and chitin in the wall of *Pneumocystis* and other fungi. The Papanicolaou stain is a sensitive method to detect the foamy eosinophilic material surrounding *Pneumocystis,* although individual organisms do not stain well. Many laboratories use a rapid staining technique to screen for *Pneumocystis,* which is then followed by a more labor-intensive procedure for definitive identification.

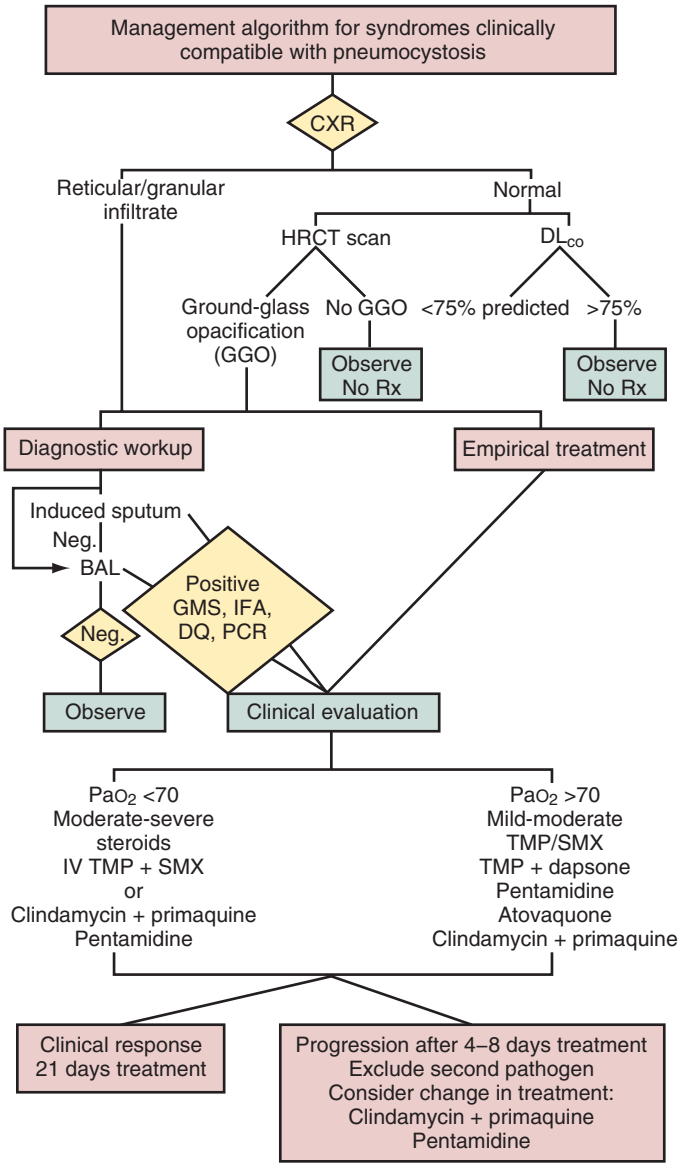

FIG. 269.6 Algorithm for the diagnostic evaluation and management of patients with suspected *Pneumocystis* pneumonia. *BAL,* Bronchoalveolar lavage; *CXR,* chest x-ray; *DL_{CO},* single-breath diffusing capacity for carbon monoxide; *DQ,* Diff-Quik (stain); *GMS,* Grocott-Gomori methenamine silver (stain); *HRCT,* high-resolution computed tomography; *IFA,* immunofluorescent antibody (stain); *IV,* intravenous; *neg.,* negative; *PaO_2,* partial pressure of arterial blood oxygen, *PCR,* polymerase chain reaction; *Rx,* treatment; *TMP/SMX,* trimethoprim-sulfamethoxazole.

Commercial kits using immunofluorescence monoclonal antibodies are more sensitive than histochemical stains in detecting *Pneumocystis*.[230,231] Soluble *Pneumocystis* antigens have been found in patients with PCP by immunoblotting.[232]

Amplification of *Pneumocystis* DNA from respiratory samples by PCR has high sensitivity for *Pneumocystis* detection. Meta-analyses show sensitivity is between 97% and 99%, and the positive likelihood ratio ≥9.9; specificity is moderate to good (between 90% and 94%). In addition, a negative result may help in excluding *Pneumocystis* pneumonia (NPV ≤99%, negative likelihood ratio ≤0.03).[223,233–235] Detection of *Pneumocystis* DNA using PCR in BALF and induced sputum is superior to histolochemical staining for detection of PCP. PCR applied to BALF or induced sputum is increasingly used in the United States and Europe for diagnosis of PCP. Currently available commercial assays (*Pneumocystis jirovecii [carinii]*–FRT PCR kit; AmpliSens), real-time PCR *Pneumocystis jirovecii* (Bio-Evolution; Bry-sur-Marne, France), and MycAssay *Pneumocystis*



FIG. 269.7 High-resolution computed tomography (HRCT) scan of patient with human immunodeficiency virus with *Pneumocystis* pneumonia. This patient had a normal chest radiograph. The HRCT scan demonstrates the characteristic ground-glass opacities. *(Courtesy R. Miller.)*

(Myconostica; South Manchester, United Kingdom), appear to have very similar sensitivity and specificity.

Pneumocystis DNA may also be detected in noninvasive samples, for instance, oropharyngeal gargle samples in individuals presenting with PCP. The specificity and clinical significance of molecular detection assays is limited by the finding of *Pneumocystis* DNA in respiratory samples (BALF, induced sputum, or gargle) from immunosuppressed/deficient patients without respiratory symptoms and in symptomatic persons without PCP (and with a confirmed alternative diagnosis), who are colonized with *Pneumocystis*. The clinical significance of detecting *Pneumocystis* DNA (representing colonization) in a respiratory sample from an immunosuppressed/immunodeficient person in the absence of respiratory symptoms or other confirmatory tests is unclear. Use of real-time quantitative PCR has been used to attempt to differentiate *Pneumocystis* pneumonia from colonization, using the fungal burden based on the PCR cycle threshold. However, this strategy is currently unable to reliably discriminate between *Pneumocystis* pneumonia and colonization. By contrast with respiratory specimens, results from PCR for detection of *Pneumocystis* in blood have been inconsistent.

Pneumocystis lacks *S*-adenosylmethionine (SAM) synthetase and is unable to metabolize SAM, or Adomet; thus it "scavenges" this enzyme from the human host. Measurement of plasma AdoMet concentrations has been proposed as a sensitive test for PCP.[236] In laboratory animal studies AdoMet is depleted in *Pneumocystis*-infected animals. One study of HIV-infected patients with PCP demonstrated lower blood AdoMet levels compared with bacterial or mycobacterial pneumonia. By contrast, overlapping AdoMet levels were described in patients with PCP and with other causes of pneumonia.[237] Currently, measurement of plasma AdoMet levels lacks clinical utility. Serologic tests using crude *Pneumocystis* antigens lack sufficient sensitivity and specificity to be of clinical value.

The collection of specimens that accurately reflect the disease process in lungs is an essential component of the diagnostic evaluation of patients with suspected PCP. The collection procedures used in adults can usually be performed in children, although infants present special problems.[238] In general, more invasive procedures have better diagnostic yields. These procedures usually have a higher diagnostic yield in HIV-infected patients than in those with other causes of immunocompromise because of the higher organism burden in the former group. Since the onset of the HIV pandemic, PCP has placed a strain on health care facilities. One way to reduce costs has been to replace invasive diagnostic procedures with algorithms and simple diagnostic techniques that are predictive of PCP (see Fig. 269.6).[207,225,229,239] A significant problem with clinicians using this approach is that no test can reliably distinguish *Pneumocystis* infection from many other infectious and noninfectious causes of pulmonary infiltrates in the immunosuppressed/deficient patient.

Another way to lower costs has been to use empirical therapy.[240,241] This approach may be pragmatic and appropriate in developing countries without ready access to expensive investigations. In the United States, historically this diagnostic approach results in higher mortality than in patients with a laboratory diagnosis of PCP. Recent studies suggest that empirical therapy may be a cost-effective approach.[242,243] Of note, use of empirical therapy may adversely impact later attempts at establishing a specific causative diagnosis.[244]

GRANULOMATOUS PNEUMOCYSTOSIS

Identifying *P. jirovecii* in GMS stains of respiratory samples is usually straightforward because the cysts collapse readily, appearing as a cluster of helmet, crescent, or spherical nonbudding cells. If lung tissue is seen, cysts are in a frothy mass of eosinophilic material in alveoli. In a small percentage of patients with HIV or hematologic malignancy, cysts are in a necrotic area surrounded by palisades of epithelioid cells and a few giant cells, very much resembling histoplasmosis. Chest CT is atypical for pneumocystosis, showing a reticulonodular pattern or one or a few rounded dense masses, also very compatible with histoplasmosis. These patients often require biopsy for diagnosis because BAL for PCP is negative. Histoplasma in necrotizing granuloma rarely are budding and *Pneumocystis* cells may be sparse and not obviously collapsed. This clinically important distinction can be aided if dark rounded structures are seen in some of the cysts or immunofluorescent stain for *Pneumocystis* is available (Fig. 269.8).

COURSE AND PROGNOSIS

Untreated, the natural history of untreated PCP is characterized by progressive respiratory insufficiency, leading to death. At the time of presentation, an assessment of PCP severity should be made using the results of arterial blood gas estimations. A PaO_2 of >70 mm Hg (9.3 KpA; while breathing room air) indicates mild PCP, and <70 mm Hg (9.3 KpA) indicates severe disease.[202] The severity of PCP can be classified as mild (<35 mm Hg), moderate (35–45 mm Hg), and severe (>45 mm Hg) when expressed as the PAO_2-PaO_2 gradient.

In the first few days of treatment of PCP, patients frequently experience a deterioration in their clinical condition, with worsening chest radiographic findings and oxygenation. This likely arises from either the host inflammatory response to dying *Pneumocystis* or because of *Pneumocystis*-induced changes in surfactant, resulting in worsening lung injury and gas exchange. This phenomenon has only been described in HIV-infected patients with PCP and as such may reflect the lower organism burden in these individuals.

Patients with mild PCP may be treated in an ambulatory care setting, under close clinical supervision. Patients with moderate-to-severe PCP should be treated with IV therapy and adjunctive corticosteroids. Any patient not responding by 4 to 8 days should be switched to alternative therapy. Before ascribing deterioration to treatment failure and considering a change in therapy, evaluation for alternative causes should be done (Table 269.2). In those with an empirical diagnosis, bronchoscopy with BAL should be performed.

All hypoxemic patients with PCP should receive supplemental oxygen delivered via a tight-fitting facemask, to maintain the PaO_2 at ≥60 mm Hg (8.0 KpA). If an inspired oxygen concentration of 60% fails to maintain the PaO_2 at ≥60 mm Hg, escalation of respiratory support should be considered and referral to the intensive care unit (ICU) for noninvasive ventilation or intubation, and mechanical ventilation should be made.

Among HIV-infected patients with severe PCP, prognosis has improved in recent years and is not due to the availability of ART. Rather, the improvements likely reflect the general improvements in ICU management of respiratory failure, including use of low tidal volume ventilation for all-cause acute lung injury, irrespective of HIV status or etiology. Recent reports describe successful outcomes from using extracorporeal membrane oxygenation in refractory hypoxemia caused by PCP.[245]

The overall outcome for HIV-infected patients with PCP has also improved over the past 30 years. Likely, this is due to a combination of factors, including earlier detection of disease, timely institution of treatment, and more effective management of complications. In studies from both the United States and the United Kingdom in the ART era, mortality from PCP is about 10% to 12%.[246,247] By contrast, mortality



FIG. 269.8 Granulomatous pneumocystosis. (A) Nodules on chest CT. (B) open lung biopsy showing necrotizing granuloma. (C) Grocott-Gomori methenamine silver stain showing cup-shaped cysts, some with a central dot-like or comma-shaped structure (see *arrow*). *(From Kumar N, Bazari J, Rhodes A, Chua F, Tinwell B. Chronic* Pneumocystis jiroveci *presenting as asymptomatic granulomatous pulmonary nodules in lymphoma.* J Infection *2011;62:484–486.)*

TABLE 269.2 Causes of Deterioration in a Patient Receiving Treatment for PCP

ETIOLOGY	EXPLANATION
Severe Progressive PCP	
Iatrogenic	Pulmonary edema due to IV fluid overload when giving TMP-SMX IRIS after early initiation of ART
Side effects of therapy	Anemia (e.g., caused by TMP-SMX), methemoglobinemia (e.g., caused by dapsone, primaquine)
Inadequate therapy	Incorrect dosage or route of administration Adjuvant glucocorticoids not given for treatment of moderate or severe PCP
Postbronchoscopy	Sedation Pneumothorax
Pneumothorax	Spontaneous Associated with intubation and positive pressure ventilation
Copathology in lung	Bacterial infection Pulmonary Kaposi sarcoma Intercurrent pulmonary embolism
Wrong diagnosis	Empirical diagnosis of PCP and correct diagnosis is another pathology (e.g., bacterial pneumonia)

ART, Antiretroviral therapy; *HIV,* human immunodeficiency virus; *IRIS,* immune reconstitution inflammatory syndrome; *IV,* intravenous; *PCP, Pneumocystis* pneumonia; *TMP-SMX,* trimethoprim-sulfamethoxazole.

of PCP among patients with non-HIV PCP remains between 30% and 50%, a figure that has not changed appreciably in more than 2 decades.[135,136,209,248,249] This likely reflects a lack of recognition among clinicians, delay in diagnosis, and thus in starting treatment.

Several clinical and laboratory factors have been shown to predict a poor outcome among HIV-infected patients with PCP at clinical presentation. These include patient age, lack of knowledge of HIV serostatus, second or third episode of PCP, poor oxygenation, marked chest radiographic abnormalities, peripheral blood leukocytosis, low hemoglobin, low serum albumin, and raised LDH enzyme levels. Other prognostic factors include identification in BALF of CMV or other copathogens, >5% neutrophilia, or elevated IL-8 levels; transbronchial biopsy evidence of fibrosis and edema; serum LDH enzyme levels that remain elevated despite treatment; the presence of pulmonary Kaposi sarcoma; previous lung damage; identification of extrapulmonary comorbidity; admission to the ICU; high Acute Physiology and Chronic Health Evaluation (APACHE) II score; the need for mechanical ventilation; and development of pneumothorax.

The value of each prognostic score has a potential to inform the clinician about the safe management of patients in ambulatory care/outpatient settings, to expedite hospitalization, and to identify individuals at greatest risk of death and who should thus be considered for transfer to the ICU. Whereas these prognostic scores are derived from large US/UK cohorts of HIV-infected patients with PCP, none have been validated among any other (US/UK) cohorts or in developing-world settings. This reduces their potential clinical utility. In addition, no validated prognostic scoring systems are available to aid decision making by clinicians in the management of non-HIV–associated PCP (either in adults or children), irrespective of the geographic or clinical setting.

Optimal management of PCP depends on prompt diagnosis and institution of therapy. Early in the HIV epidemic, survival of patients with PCP was better at hospitals with greater familiarity with the disease; however, improvement in management has occurred throughout the medical community. Patients who recover from PCP are at risk for developing recurrent episodes of the disease as long as the immunosuppressive conditions persist.[86] HIV-infected patients are much more likely to develop recurrence than non-HIV patients. It is thought that recurrent episodes occurring within 6 months of the first episode are more likely to be relapses, whereas episodes occurring at more than 6 months are more likely to represent a new episode of infection.[250] Early studies found that the prognosis of recurrent episodes of PCP is similar to that of initial episodes, although more recent analyses have shown a worse outcome with subsequent episodes.[246,251]

Pneumothorax may complicate treatment of an episode of PCP.[207,252,253] Pneumothorax is more likely in a recurrent episode of PCP, in those who have received aerosolized pentamidine, and among cigarette smokers. Respiratory support, with positive end-expiratory pressure spontaneous mechanical ventilation, is an independent risk factor. Pneumatoceles, pneumomediastinum, and subcutaneous emphysema also occur. Management is difficult and should be individualized; interventions include chest tube, surgical or chemical pleurodesis, and thoracotomy with pleural stapling or use of adhesive. Prior receipt of corticosteroids may increase morbidity.[252]

TREATMENT

Recommendations for the treatment and prevention presented here are based on the Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America (Table 269.3).[254,255] Trimethoprim-sulfamethoxazole (TMP-SMX) is the drug of choice for all forms of PCP. This agent, which acts by inhibiting folic acid synthesis, has been used for 4 decades to treat *Pneumocystis* with high success rates.[256–259] Among the attractive features of TMP-SMX are its availability in both oral and parenteral forms, well-described pharmacokinetics, antibacterial properties, and cost. The parenteral preparation should be used in

TABLE 269.3 Treatment of *Pneumocystis* Pneumonia

DRUG	DOSE	COMMENTS
Preferred		
TMP-SMX[a]	15–20 mg/kg (TMP) and 75–100 mg/kg (SMX) IV or PO divided into doses given q6–8h (AI)[b]	*Severe PCP*: IV may be switched to PO with clinical improvement *Mild-to-moderate PCP*: PO dose can be given as TMP-SMX DS 2 tablets PO tid
Alternatives		
Clindamycin *plus*	600–900 mg IV q6–8h *or* 300–450 mg PO q6–8h *plus*	*Severe PCP*: IV may be switched PO with clinical improvement *Mild-to-moderate PCP:* PO dose can be used Check for G6PD deficiency before use
Primaquine	15 or 30 mg (of the base) PO qd (BI)	
Pentamidine	4 mg/kg IV q24h (BI)	For moderate-to-severe PCP, some clinicians "dose reduce" to 3 mg/kg to reduce toxicity
TMP *plus*	15 mg/kg PO (in divided doses, q8h) *plus*	For mild-to-moderate PCP, monitor for methemoglobinemia
Dapsone	100 mg PO qd (BI)	
Atovaquone	750 mg suspension PO bid (BI)	For mild-to-moderate PCP, take with food
Adjunctive		
Prednisone	40 mg PO bid days 1–5 40 mg PO qd days 6–10 20 mg PO qd days 11–21 (AI) IV methylprednisolone can be administered as 75% of PO prednisone dose	For moderate-to-severe PCP, PaO_2 ≤70 mm Hg, or alveolar-arterial O_2 gradient >35 mm Hg Begin as soon as possible and within 72 h of starting PCP therapy

[a]DS tablet = TMP 160 mg and SMX 800 mg.
[b]AI and BI grading scores are defined as follows: *AI,* Strong recommendation for the statement: one or more randomized trials with clinical outcomes or validated laboratory end points; *BI,* moderate recommendation for the statement: one or more randomized trials with clinical outcomes or validated laboratory end points.
bid, Twice per day; *DS,* double strength; *G6PD,* glucose-6-phosphate dehydrogenase; *IV,* intravenously; *PaO_2,* partial pressure of arterial blood oxygen; *PCP, Pneumocystis* pneumonia; *PO,* orally; *qd,* every day; *SMX,* sulfamethoxazole; *tid,* three times per day; *TMP,* trimethoprim.
Duration of therapy is 21 days for patients with underlying human immunodeficiency virus infection; shorter treatment course (typically 14–17 days) for those with other causes of immunodeficiency (e.g., renal transplantation).
Modified from Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed May 19, 2017.*

patients who are seriously ill or have GI disturbances. As with all anti-*Pneumocystis* drugs, treatment should be continued for 21 days in HIV-positive patients and for at least 14 days in non-HIV patients. The reason for the longer duration in HIV-positive patients is because this patient group has a higher organism burden and responds to treatment more slowly.

TMP-SMX is well tolerated by HIV-uninfected patients, with GI symptoms and skin rashes being the most common adverse events. By contrast, HIV-positive patients experience a high frequency (up to 80% or more) of adverse reactions, which usually begin during the second week of TMP-SMX therapy and may result in discontinuation of the drug in up to 50%.[260,261] Adverse effects of therapy include rash, fever, cytopenias, nausea and vomiting, hepatitis, pancreatitis, nephritis, hyperkalemia, metabolic acidosis, central nervous system manifestations, and an anaphylactoid reaction. Most of these reactions appear to be caused by the sulfonamide component, but the mechanisms are poorly understood. Among the possible contributing factors are elevated serum drug levels, the formation of hydroxylamine metabolites, glutathione deficiency, hypersensitivity, and high CD4 counts.[261–263] Hyperkalemia has been attributed to TMP, which competitively inhibits potassium excretion by distal nephron (acting like amiloride, a potassium-sparing diuretic).[264]

Some clinicians adjust the dosage of TMP-SMX to achieve serum concentrations of 5 to 8 μg/mL TMP and 100 to 150 μg/mL SMX to achieve maximum efficacy and minimum toxicity[258,261]; however, others have not found this approach either beneficial or practical.[265] *N*-acetylcysteine and folinic acid do not prevent side effects from TMP-SMX, and folinic acid may possibly reduce efficacy. Cutaneous reactions to TMP-SMX range from mild to life threatening (e.g., toxic epidermal necrolysis, Stevens-Johnson syndrome, anaphylaxis). In some cases the rash and manifestations such as fever may resolve spontaneously or respond to conservative measures, whereas in other cases they may require discontinuation of the drug. Oral corticosteroids may also be helpful.[266] Desensitization regimens have been successful in patients who have experienced non–life-threatening reactions to TMP-SMX but should be undertaken with caution.[267]

Several alternative regimens have been developed for the treatment of mild-to-moderate PCP (see Table 269.3). Although these studies have been performed mainly in HIV-infected patients, the results should be applicable to non-HIV patients with PCP. TMP administered orally combined with dapsone orally has been shown to be as effective as TMP-SMX and is less toxic.[268] The major adverse reactions to dapsone are methemoglobinemia, rash, fever, nausea, and vomiting; hemolysis can occur in patients who have glucose-6-phosphate dehydrogenase (G6PD) deficiency. Caution is advised in administering dapsone to patients who have been intolerant of sulfonamides. The serum levels of dapsone and TMP are higher when these drugs are used together than when used alone and suggest bidirectional interference with clearance.[269]

Controlled studies have shown that the combination of clindamycin and primaquine has comparable efficacy and toxicity to TMP-SMX and TMP plus dapsone in treatment of PCP.[268,270] The mechanism of action of clindamycin and primaquine against *Pneumocystis* is not known. The usual regimen is shown in Table 269.3. Treatment may also be initiated with IV clindamycin and then switched to oral administration once recovery is evident. Adverse drug reactions to primaquine appear to be less common with use of 15 mg, rather than 30 mg, every day and include rash, fever, neutropenia, GI complaints, and methemoglobinemia.[260] Primaquine also causes hemolysis in patients with G6PD deficiency.

Atovaquone is a hydroxynaphthoquinone originally developed as an antimalarial agent. Atovaquone acts on the mitochondrial electron transport chain of plasmodia and, based on mutations found in *Pneumocystis* isolates from patients for whom atovaquone prophylaxis was not effective, drug targets are similar in *Pneumocystis*. One study compared atovaquone with TMP-SMX and another with IV pentamidine isethionate for the treatment of mild-to-moderate PCP in HIV patients.[271,272] Atovaquone was less effective than TMP-SMX and showed equivalent efficacy to IV pentamidine; however, atovaquone was better tolerated in both studies. Adverse reactions to atovaquone include skin rash, fever, GI symptoms, and abnormal liver function tests. An oral suspension is administered at a dose of 750 mg/5 mL, twice daily with food.[273]

The combination of clindamycin and primaquine is the preferred alternative regimen to TMP-SMX for moderate-to-severe PCP in HIV-positive patients, as shown by a systematic review that also included new data from the authors' patient populations, supporting a previous meta-analysis that reported clindamycin and primaquine is superior to pentamidine as salvage treatment of PCP (i.e., patients who did not respond to first-line treatment of PCP).[274,275] A dose of clindamycin (600–900 mg IV every 6–8 hours) is usually used.[276,277]

Another agent used to treat PCP is pentamidine isethionate, a drug first used to treat African trypanosomiasis.[278] Pentamidine appears to exert its antimicrobial activity by binding to DNA, but its precise mode of action against *Pneumocystis* is unknown. IV pentamidine appears to be almost as effective as TMP-SMX in treatment of PCP in HIV-positive

and non-HIV patients.[256–259] Pentamidine is administered as a single IV daily dosage of 4 mg/kg/day. The drug is diluted in 250 mL of a 5% dextrose solution and infused over a period of at least 1 hour. Aerosolized pentamidine is used in prophylaxis of PCP but has no role in treatment.

Pharmacokinetic studies have shown that IV pentamidine follows a three-compartment model with rapid passage to tissues, secondary distribution, and a long (≈12 days) elimination half-life. Only a small amount of the drug is cleared by the kidney.[261,279] Pentamidine is a toxic drug; adverse reactions occur in 80% or more of HIV-positive and non-HIV patients and are severe enough to necessitate discontinuation of the drug in approximately half of cases. Side effects include hypotension, cardiac arrhythmias (e.g., torsades de pointes), azotemia, pancreatitis, dysglycemias, hyperkalemia, hypomagnesemia, hypocalcemia, neutropenia, hepatic dysfunction, bronchospasm, and problems at intramuscular injection sites. Hypoglycemia, which develops from damage to pancreatic β cells, resulting in insulin release, occurs early in therapy; hypoglycemia may later be followed by secondary diabetes mellitus caused by necrosis of the pancreatic β cells. The frequency of hypoglycemia and azotemia has been correlated with high serum pentamidine levels, total drug dose, and duration of treatment.[278,280] The mechanism of hyperkalemia caused by pentamidine is similar to that caused by TMP.[281]

The response to anti-*Pneumocystis* drugs generally reflects other clinical features of the infection. Most patients show a clinical response after several days of treatment; if there is no response by 4 to 8 days, it is wise to consider switching to another drug. HIV-positive patients typically respond more slowly than non-HIV patients and take longer to clear *Pneumocystis* from their lungs. As noted, clindamycin with primaquine is the preferred regimen for those patients who do not respond to TMP-SMX; conversely, TMP-SMX is the preferred regimen for patients for whom other regimens are not effective.[274] Adding a second anti-*Pneumocystis* drug to the regimen is no more effective than substituting one agent for another and may increase the risk of adverse reactions.

HIV-infected patients with PCP frequently experience worsening of their blood oxygenation during the first few days of therapy; such a clinical deterioration can be particularly dangerous if the initial hypoxemia is marked. Several studies have shown that the administration of corticosteroids during the first 72 hours of treatment can lessen the decline in oxygenation and improve survival. These studies led to a recommendation by an expert panel that steroids be added to the treatment of all patients with moderate-to-severe PCP, that is, an arterial oxygen pressure ≤70 mm Hg (<9.3 kPA) or a PAO_2-PaO_2 gradient >35 mm Hg.[202] The use of "adjunctive" corticosteroids has been widely adopted by the medical community. A meta-analysis and a more recent systematic review support the use of corticosteroids both in reducing mortality and in the need for mechanical ventilation among HIV-infected adults with PCP.[275,282,283] Corticosteroids used in the manner described have generally been well tolerated.[202] The principal side effects are oral candidiasis, mucocutaneous herpes simplex, and metabolic changes such as hyperglycemia. Concerns about an increased frequency of cytomegaloviral, other fungal, and mycobacterial infections have not materialized. Nevertheless, the lack of efficacy shown in some studies,[283,284] as well as the risk of other opportunistic infections and other possible complications (e.g., increased morbidity from pneumothorax, lifetime increased risk of osteoporosis and fracture), emphasize the need for careful patient selection and follow-up.

Recommendations about the use of adjunctive corticosteroids in *Pneumocystis* among patients with other causes of immunosuppression are difficult to formulate because of the lack of data from controlled trials. Studies infer that corticosteroids may speed clinical improvement, but survival results from meta-analyses are conflicting.[285,286] Among non-HIV patients with PCP, most have received corticosteroids shortly before or at the time they developed PCP. Rapid withdrawal of steroids may have serious adverse consequences, and thus it seems prudent either to maintain or increase the steroid dose when instituting anti-*Pneumocystis* therapy. The steroid dose can then be slowly tapered. The place of adjunctive corticosteroids in non-HIV patients who have received immunosuppressive drugs other than steroids at the time of diagnosis of PCP is unknown.

Future clinical advances in the treatment of PCP might come from several current lines of investigation. Although the development of a continuous culture system remains elusive, it might be possible to use molecular techniques to identify markers of virulence or antimicrobial resistance. Sequence variation in the gene encoding dihydropteroate synthetase, the target enzyme of sulfonamides, in human *Pneumocystis* has been identified.[287] These mutations, at positions associated with sulfonamide resistance in other organisms, have been associated with failure in prophylaxis, but no clear association with treatment failure or altered outcome has been demonstrated. A systematic review has shown that patients receiving sulfa drugs for PCP prophylaxis had a significantly higher risk of developing these mutations but concluded that there was not enough evidence that the mutations adversely affected disease outcome.[288] Subsequently, a trend toward more severe disease and worse outcome associated with these mutations has been described in two of three studies.[289–291] Mutations have also been described in the gene encoding cytochrome B and have been associated with failure of atovaquone prophylaxis of PCP.[292]

New drugs with improved efficacy, less toxicity, and different mechanisms of action are needed. Animal models, which are the principal test system, have facilitated identification of several new types of drugs (e.g., echinocandins, 8-aminoquinolines, diamidines); however, data from clinical trials are lacking. A more promising approach has been to investigate drugs that are already licensed for other agents for activity against *Pneumocystis*. Several case series and additional case reports show that echinocandin monotherapy may be effective as salvage in patients with PCP who are not responding to, or intolerant of, first-line therapy. Echinocandins have not been prospectively evaluated against TMP-SMX or other regimens as first-line therapy for PCP and therefore, based on animal studies, their use should only be considered in combination with an additional anti-*Pneumocystis* agent.

Several small studies (largely retrospective case series, and thus potentially susceptible to selection bias) show low-dose TMP-SMX (doses ≤10 mg/kg/day of TMP, with ≤50 mg/kg/day SMX) have shown good treatment outcomes and reduced toxicity. In addition, several case reports describe good outcomes from use of low-dose TMP-SMX with caspofungin. These observations require evaluation in prospective clinical trials before clinicians incorporate them into treatment algorithms.

PREVENTION

There are several methods of preventing PCP: (chemo)prophylaxis, improving immune responses, and reducing exposure.

Chemoprophylaxis

The need for PCP chemoprophylaxis for PCP in HIV-positive patients is widely recognized. Prophylaxis can be considered primary, given to prevent a first episode of PCP, or secondary, given to prevent recurrent episodes. A decision to institute chemoprophylaxis depends on factors including the incidence of PCP in the target population, as well as the efficacy of specific drug regimens, their safety, ease of administration, and cost. As none of the currently available anti-*Pneumocystis* drugs used in humans has a "cidal" activity against *Pneumocystis,* chemoprophylaxis should be continued for as long as the underlying immunosuppressive condition exist.

With progression of HIV infection and decline in the CD4 count, HIV-infected patients are increasingly at risk of developing PCP. *Primary prophylaxis* is given to patients with CD4 lymphocyte counts <200 cells/mm^3, or the proportion of CD4 lymphocytes is <14%; to patients with HIV-related constitutional symptoms, such as unexplained fever (>100°F) for 2 or more weeks' duration; those with oral candidiasis (regardless of CD4 count); and to patients who have other AIDS-defining diagnoses, such as Kaposi sarcoma. *Secondary prophylaxis* is given to prevent a recurrence; indications for secondary prophylaxis are similar to those for primary prophylaxis. Indications of giving chemoprophylaxis to patients with other causes of immunosuppression are shown in Table 269.1.

TMP-SMX is the drug of choice for chemoprophylaxis. Three oral TMP-SMX regimens have similar rates of efficacy; however, single-strength TMP-SMX may have fewer adverse reactions than double-strength TMP-SMX. Rash, with or without fever, occurs in up to 20% of patients. Desensitization should be attempted (with caution) in those

TABLE 269.4 Prevention of *Pneumocystis* Pneumonia

DRUG	DOSE	COMMENTS
Preferred		
TMP-SMX[a]	1 DS tab PO qd (AI)[b] 1 SS tab PO qd (AI)	Both regimens are effective; SS tab may have fewer side effects
Alternative		
TMP-SMX	1 DS tab PO tiw (BI)	Similar to other TMP-SMX regimens
Dapsone	100 mg PO qd (BI) or 50 mg PO bid	Methemoglobinemia may occur
Dapsone *plus*	50 mg PO qd *plus*	Methemoglobinemia may occur
Pyrimethamine *plus*	50 mg PO qwk (BI) *plus*	May also prevent toxoplasmosis
Leucovorin	25 mg PO qwk	
Pentamidine	300 mg qmo aerosolized via Respirgard II nebulizer (BI)	
Atovaquone	750 mg suspension PO bid or 1500 mg qd (BI)	
Atovaquone *plus*	750 mg suspension PO bid or 1500 mg qd *plus*	
Pyrimethamine *plus*	25 mg PO qd (BII) *plus*	
Leucovorin	10 mg PO qd	

[a]SS tablet = TMP 80 mg and SMX 400 mg; DS tablet = TMP160 mg and SMX 800 mg.
[b]AI and BI grading scores are defined as follows: *AI,* Strong recommendation for the statement: one or more randomized trials with clinical outcomes/or validated laboratory end points; *BI,* moderate recommendation for the statement: one or more randomized trials with clinical outcomes/or validated laboratory end points; *BII,* moderate recommendation for the statement: one or more well-designed, nonrandomized trials, or observational cohort studies with long-term clinical outcomes.
bid, Twice per day; *DS,* double strength; *qd,* every day; *qmo,* every month; *qwk,* every week; *PO,* orally; *SS,* single strength; *tiw,* three times per week; *TMP-SMX,* trimethoprim-sulfamethoxazole.
Modified from Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_0i.pdf. Accessed May 19, 2017.*

unable to tolerate TMP-SMX when used as prophylaxis. Guidelines for desensitization or rechallenge with TMP-SMX for prophylaxis are similar to those for use of TMP-SMX in treatment.

Recommended chemoprophylaxis regimens for patients who cannot tolerate TMP-SMX include dapsone, aerosolized pentamidine, and atovaquone. Dapsone may be administered alone or in combination with pyrimethamine and leucovorin (Table 269.4). Additional dosage schedules have also been used. Overall, dapsone-containing regimens show inferior efficacy but equivalent toxicity to TMP-SMX regimens. Both dapsone and pyrimethamine additionally protect against *Toxoplasma gondii* but not against bacterial infections. Atovaquone has similar efficacy to dapsone in patients intolerant of TMP-SMX. Pentamidine is administered at a dose of 300 mg, delivered via Vital Signs Respirgard II nebulizer (Vital Signs/Vitality Medical; Salt Lake City, UT) once monthly. Other delivery systems cannot be recommended. Aerosolized pentamidine is less effective, better tolerated, and much more expensive than TMP-SMX or dapsone. Major side effects include cough and bronchospasm, which can be controlled by use of a β-agonist. Administration of aerosolized pentamidine in a health care setting requires a negative-pressure room with adequate ventilation and should not be administered to patients with suspected/proven smear-positive tuberculosis because of the risks of nosocomial transmission. A number of other drug regimens have been considered possible *Pneumocystis* prophylactic agents, but there is insufficient supportive information to recommend their use. Intermittent IV pentamidine used as prophylaxis is inferior to all other regimens and cannot be recommended for use by clinicians.

Stopping Prophylaxis

In the late 1990s the widespread availability and uptake of ART by patients in the United States, Europe, and Australasia resulted in marked reductions in the incidence of many opportunistic infections, including PCP, hospital admissions, and mortality from HIV infection. In a majority of patients, within a few weeks of starting ART rapid reductions in plasma HIV RNA occur; these parallel increases in peripheral blood CD4 counts. Primary *Pneumocystis* prophylaxis should be discontinued for patients who after starting ART have an increase in CD4 counts from <200 cells/mm^3 to >200 cells/mm^3 maintained for more than 3 months. Discontinuing primary prophylaxis in this patient group is recommended because its preventive benefits against PCP are limited, and stopping prophylaxis additionally reduces the potential for adverse drug reactions, drug-drug interactions, selection of drug-resistance, as well as reducing pill burden and health care costs. Prophylaxis should be reintroduced if the CD4 count decreases to <200 cells/mm^3.

The incidence of PCP in patients receiving ART and with CD4 counts between 100 and 200 cells/mm^3, who have HIV viral loads between <50 and 400 copies/mL, and who stop or never receive PCP prophylaxis is low.[254] This observation suggests that both primary and secondary PCP prophylaxis can be safely discontinued in patients with CD4 counts between 100 and 200 cells/mm^3 and HIV plasma RNA levels that are below limits of detection (<50 copies/mL).

Prophylaxis of PCP Among Patients With Non–HIV-Associated Immunosuppression

Recent reviews and guidelines have better defined the need and prophylactic regimens indicated to prevent PCP in non–HIV-infected individuals. The indications for prophylaxis and recommended regimens are shown in Table 269.1.

Boosting Host Immune Response

Manipulation of the host innate or adaptive immune response could improve defenses against *Pneumocystis* while lessening their deleterious effects on the host.[293] An example of this principle is the use of an anti-CD3 antibody in an animal model of PCP.[294] These studies might lead to the development of drugs with greater specificity than corticosteroids. Immunization of immunocompromised patients at an early stage of their disease, for example, HIV patients with a CD4 count >500 cells/mm^3 or newly diagnosed cancer patients, might prevent, delay, or lessen the severity of PCP. Boosting the host immune response might also lessen the need for, or lower the dosage of, antimicrobial drugs. A promising antigen with the potential to achieve these objectives is the Kex antigen, which contains protective B-cell and T-cell epitopes; immunization with Kex to CD4-depleted mice protects against PCP.[104] Clinical trials of a Kex vaccine are needed as there is no evidence of protective effect in humans.

Preventing Exposure

A third method of preventing PCP is by preventing or reducing exposure. There is accumulating evidence showing *Pneumocystis* is transmissible in both humans and experimental animal models. Although *Pneumocystis* can be quantified in the air near patients with PCP—and multiple outbreaks have been described in kidney transplant patients, each caused by distinct strains of *Pneumocystis*—data that strongly suggest high-risk immunocompromised patients without PCP may benefit from isolation from other patients with known PCP infection in health care settings nonetheless are currently insufficient to support isolation as routine practice.[254]

Little is known about the effects of environmental factors on the viability of *Pneumocystis* outside of its host. In animal models *Pneumocystis* cysts derived from mice are susceptible to most common disinfectants, except 0.5% hypochlorous acid.[295] *Pneumocystis* cystic forms obtained from rats can be stored at −80°C for several months yet maintain their viability and are able to infect other mice and rats.[296]

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The complete reference list is available online at Expert Consult.

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270 Microsporidiosis

Louis M. Weiss

SHORT VIEW SUMMARY

Definition
- Microsporidia are obligate eukaryotic intracellular pathogens related to fungi.
- Taxonomy of the phylum is based on ultrastructural descriptions of the spores and life cycle.
- Molecular phylogeny, based on ribosomal RNA sequences, is also used for classification.

Epidemiology
- The phylum Microsporidia contains at least 1500 species distributed into more than 200 genera.
- Several different genera and species of microsporidia cause disease in humans, including *Nosema, Vittaforma, Pleistophora, Encephalitozoon, Enterocytozoon, Trachipleistophora, Anncaliia, Tubulinosema, Endoreticulatus,* and *Microsporidium.*
- Microsporidiosis occurs in both immunocompromised and immune-competent hosts.
- These pathogens can be transmitted by food or water and are likely zoonotic.
- Diarrhea or keratoconjunctivitis are the most common presenting manifestations of infection, but infection can occur in any organ system.
- An increasing incidence is reported in transplant recipients. Although multiple sites may be infected, the most common manifestation of reported disease leading to diagnosis is infection of either native or transplanted kidneys. Therapy with albendazole or fumagillin has been successful.
- Infection in solid-organ transplant recipients may be derived from the organ donor.

Diagnosis
- Diagnosis can be made by finding characteristic spores in body fluids (e.g., stool, urine, conjunctival scraping, etc., using stains such as chromotrope 2R or Uvitex 2B).
- Definitive identification of the microsporidia causing an infection can be done using ultrastructural examination or molecular techniques.
- Patients with diarrhea or keratoconjunctivitis should have urine examined to look for disseminated infection.
- Species-specific diagnosis is useful for guiding treatment.

Therapy
- Systemic albendazole and fumagillin are active therapeutic agents for the treatment of microsporidiosis.
- Topical fumagillin is useful for microsporidian keratoconjunctivitis.
- Immune restoration, such as combination antiretroviral treatment in patients with acquired immunodeficiency syndrome, often results in resolution of infection.

Prevention
- There are limited data on effective preventive strategies for microsporidiosis, but the most effective prophylaxis is restoration of immune function in immunocompromised hosts.
- The usual sanitary measures that prevent contamination of food and water will decrease the chance of infection.
- Hand washing and general hygienic habits reduce the chance of contamination of the conjunctiva and cornea with microsporidian spores.

The Microsporidia are a group of obligate eukaryotic intracellular parasites that were recognized more than 150 years ago when a parasite of silkworms, *Nosema bombycis,* which causes the disease pébrine in these economically important insects, was described.[1–5] Franzen[2] has reviewed the history of research on these pathogens. The class or order Microsporidia was elevated to the phylum Microspora by Sprague in 1977.[1] In 1998 Sprague and Becnel suggested that the term "Microsporidia" instead be used for the phylum name.[3] Although the Microsporidia were historically considered "primitive" protozoa, molecular phylogenetic analysis has led to the recognition that these organisms are not primitive, but degenerate, and that they are related to the Fungi, either as a basal branch of the Fungi or as a sister group.[6,7] Molecular phylogeny has also led to the recognition that traditional phylogeny based on structural observations may not reflect the relationships among the various microsporidian species and genera.

The Microsporidia infect almost all animal phyla, including other protists. They are important agricultural parasites in insects, fish, laboratory rodents, rabbits, fur-bearing animals, and primates and are found infecting various animals kept as household pets.[4,5,8] Some species of these pathogens are used as biologic control agents for destructive species of insects.[9] In their hosts the majority of the Microsporidia infect the digestive tract, but infections of almost all organ systems have been documented.[10] Microsporidia were first recognized in mammalian tissue samples more than 75 years ago[11] and described in 1959[12] as being human pathogens when they were found in a child with encephalitis. Many of the human pathogenic Microsporidia are zoonotic or waterborne infections, or both. In the immunosuppressed host (e.g., those treated with immune-suppressive drugs or infected with human immunodeficiency virus [HIV], particularly at advanced stages of the disease), the Microsporidia can produce a wide range of clinical diseases. Reports of diarrheal syndromes associated with microsporidiosis and HIV infection were first reported in 1985,[13] and the number of articles describing human disease increased rapidly after 1990. Since the advent of antiretroviral therapy (ART) the incidence of microsporidiosis has declined in the HIV-infected population. In addition to gastrointestinal (GI) tract involvement, patients with encephalitis, ocular infection, sinusitis, myositis, and disseminated infection are well described in the literature.[4,5] These organisms are also seen in immune-competent individuals.

The phylum Microsporidia (Microspora) contains at least 1500 species distributed into approximately 200 genera, of which the following have been demonstrated in human disease (Table 270.1)[4,5,14]: *Nosema* (*Nosema corneum,* renamed *Vittaforma corneae*[15]; *Nosema algerae,* reclassified initially as *Brachiola algerae*[16] and now as *Anncaliia algerae*[17]); *Pleistophora; Encephalitozoon; Enterocytozoon*[13]; *Septata*[18] (reclassified as *Encephalitozoon*[19]); *Trachipleistophora*[20,21]; *Brachiola*[16]; *Anncaliia*[17]; *Tubulinosema*[22,23]; *Endoreticulatus*[24]; and *Microsporidium.*[4] *Encephalitozoon hellem* has been associated with superficial keratoconjunctivitis, sinusitis, respiratory disease, prostatic abscesses, and disseminated infection.[4,10,25] *Encephalitozoon cuniculi* has been associated with hepatitis, encephalitis, and disseminated disease.[26–28] *Encephalitozoon (Septata) intestinalis* is associated with diarrhea, disseminated infection, and superficial keratoconjunctivitis.[18,29,30] *Nosema, Vittaforma,* and *Microsporidium* have been associated with stromal keratitis associated with trauma in

TABLE 270.1 Microsporidia Identified as Pathogenic to Humans

GENUS AND SPECIES	REPORTED INFECTIONS	ANIMAL HOSTS[a]
Encephalitozoon		
E. cuniculi[b]	Hepatitis, peritonitis, encephalitis,[c] urethritis, prostatitis, nephritis, sinusitis, keratoconjunctivitis, cystitis, diarrhea,[c] cellulitis, disseminated infection	Mammals (rabbits, rodents, carnivores, primates)
E. hellem[b]	Keratoconjunctivitis,[c] sinusitis, pneumonitis, nephritis, prostatitis, urethritis, cystitis, diarrhea, disseminated infection	Psittacine birds (parrots, lovebirds, parakeets), birds (ostrich, hummingbirds, finches)
E. intestinalis[b]	Diarrhea,[c] intestinal perforation, cholangitis, nephritis, keratoconjunctivitis	Mammals (donkeys, dogs, pigs, cows, goats, primates)
Enterocytozoon		
E. bieneusi	Diarrhea,[c] wasting syndrome, cholangitis, rhinitis, bronchitis	Mammals (pigs, primates, cows, dogs, cats), birds (chickens)
Trachipleistophora		
T. hominis[b]	Myositis, keratoconjunctivitis,[c] sinusitis	Unknown
T. anthropopthera[b]	Encephalitis, disseminated infection, keratitis	Unknown
Pleistophora		
P. ronneafiei	Myositis	Unknown
Pleistophora sp.	Myositis[c]	Fish
Anncaliia		
A. vesicularum	Myositis	Unknown
A. algerae[d]	Keratoconjunctivitis, myositis, skin infection	Mosquitoes
A. connori	Disseminated infection	Unknown
Nosema		
N. ocularum	Keratoconjunctivitis[c]	Unknown
Vittaforma		
V. corneae[b]	Keratoconjunctivitis,[c] urinary tract infection	Unknown
Tubulinosema		
T. acridophagus (and *Tubulinosema* sp.)	Myositis, disseminated infection (skin, liver, peritoneum, lung and retinal involvement)	Insects (*Drosophila melanogaster* and grasshoppers)
Endoreticulatus		
Endoreticulatus sp.	Myositis[c]	Lepidopteran insects
Microsporidium		
M. africanus	Corneal ulcer[c]	Unknown
M. ceylonensis	Corneal ulcer[c]	Unknown

[a]Animals in which organism has been found other than humans.
[b]Organism can be grown in tissue culture.
[c]Cases reported in immune-competent hosts.
[d]Previously called *Brachiola* and *Nosema*.[16,17]

immune-competent hosts.[25,31] *Pleistophora, Anncaliia, Tubulinosema, Endoreticulatus,* and *Trachipleistophora* have been associated with myositis and sometimes with associated disseminated disease.[20,23,32–34] *Trachipleistophora* has been associated with encephalitis, keratitis, and disseminated disease.[21,35] *Enterocytozoon bieneusi,* originally described in humans,[13] is associated with malabsorption, diarrhea, and cholangitis.[36,37]

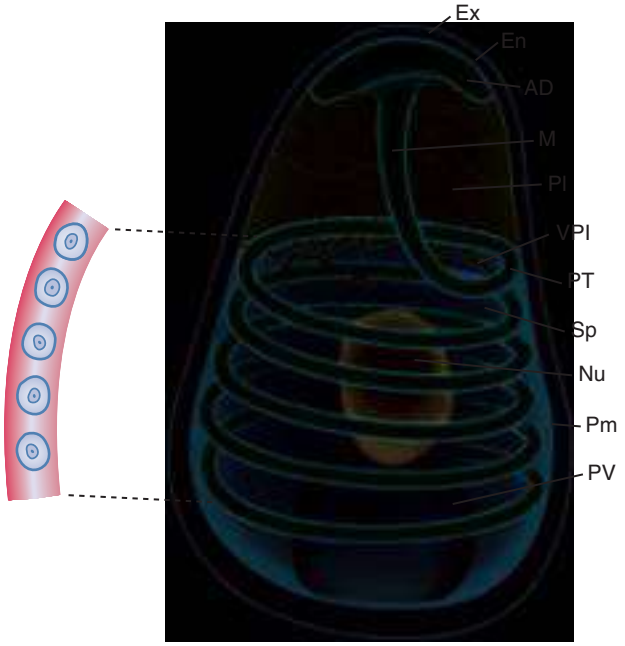

FIG. 270.1 Structure of a microsporidian spore. Depending on the species, the size of the spore can vary from 1–10 µm, and the number of polar tubule coils can vary from a few to 30 or more. Extrusion apparatus consists of the polar tube *(PT),* vesiculotubular polaroplast *(VPl),* lamellar polaroplast *(Pl),* anchoring disk *(AD),* and manubrium *(M).* This organelle is characteristic of the Microsporidia. The inset demonstrates a cross section of the polar tube coils (five coils in this spore), demonstrating the various concentric layers of different electron density and electron-dense core present in such cross sections. The nucleus *(Nu)* may be single, such as in *Encephalitozoon* spp., or a pair of abutted nuclei known as a diplokaryon, such as in *Nosema* spp. The endospore *(En)* is an inner, thicker, electron-lucent region. The exospore *(Ex)* is an outer electron-dense region. The plasma membrane *(Pm)* separates the spore coat from the sporoplasm *(Sp),* which contains ribosomes in a coiled helical array. The posterior vacuole *(PV)* is a membrane-bound structure. (Modified from Wittner M, Weiss LM, eds. *The Microsporidia and Microsporidiosis.* Washington, DC: American Society of Microbiology Press; 1999:199.)

GENERAL CHARACTERISTICS

The Microsporidia are eukaryotes containing a nucleus with a nuclear envelope, an intracytoplasmic membrane system, chromosome separation on mitotic spindles, vesicular Golgi,[38] and a mitochondrial remnant organelle called a mitosome.[39] Microsporidia are ubiquitous in the environment and infect almost all invertebrates and vertebrates.[4,10,14] They form characteristic unicellular spores (Fig. 270.1), which are environmentally resistant. Microsporidian spore size and shape vary depending on the species. Whereas microsporidian spores can be as large as 12 µm, microsporidia infecting humans have spores that range from 1 to 3 µm × 1.5 to 4 µm in size and are usually ovoid. Spore structure is characteristic of the phylum.[40] The spore coat consists of an electron-dense, proteinaceous exospore, an electron-lucent endospore composed of chitin and protein, and an inner membrane or plasmalemma.[41] Proteins making up the spore coat have adhesion domains that may facilitate the binding of microsporidian spores to either the cell surface or mucus of the GI tract before germination.[42] A defining characteristic of all microsporidia is an extrusion apparatus that consists of a polar tube attached to the inside of the anterior end of the spore by an anchoring disk and, depending on the species, forms 4 to approximately 30 coils around the sporoplasm in the spore. Proteomic and genetic studies have defined some of the proteins of the polar tube and spore wall,[43–45] the presence of *O*-mannosylation on these proteins,[46–48] and how these proteins interact.[49]

During germination the polar tube rapidly everts, forming a hollow tube that brings the sporoplasm into intimate contact with the host cell (Fig. 270.2). The polar tube provides a bridge to deliver the sporoplasm to the host cell. The mechanism whereby the polar tube

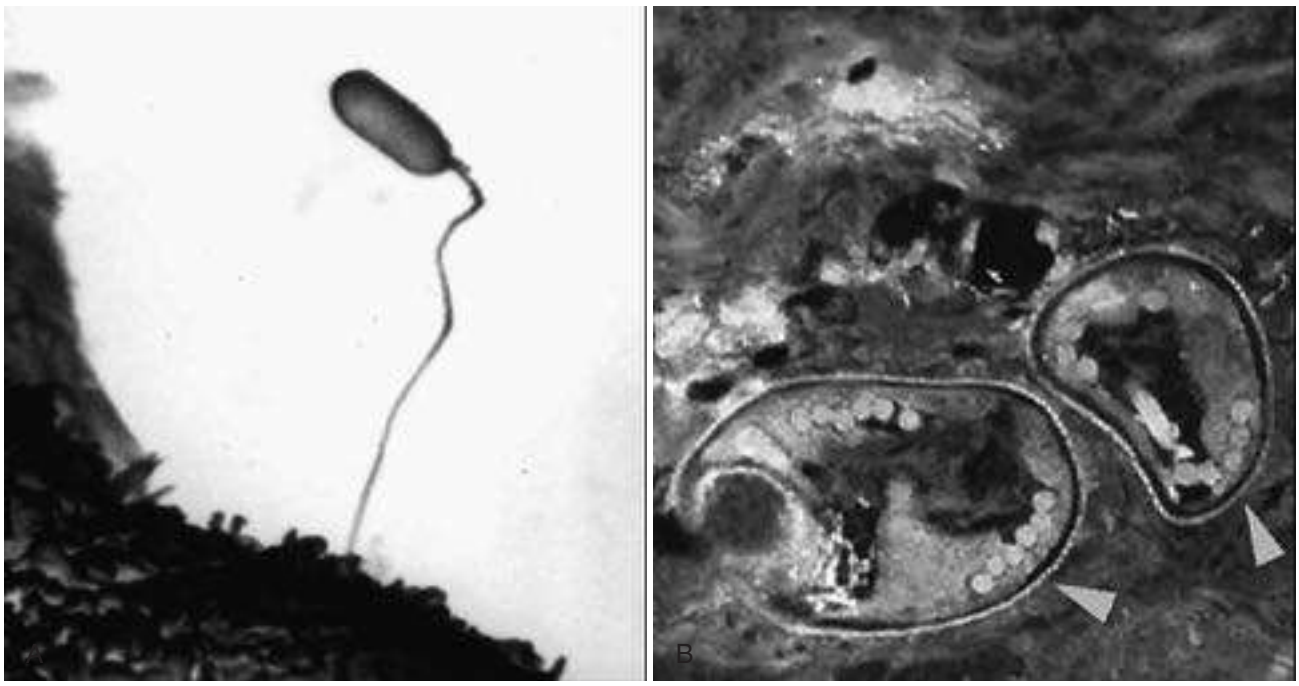

FIG. 270.2 Microsporidian polar tube. (A) Scanning electron micrograph of a tissue culture demonstrating *Encephalitozoon intestinalis* invading a Vero cell in vitro. (B) Transmission electron micrograph of conjunctival scraping demonstrating microsporidia *(Encephalitozoon hellem).* Arrowheads identify thick-walled spores with single nuclei and five or six coils of polar tubes. ([A] from Kock NP. *Diagnosis of Human Pathogenic Microsporidia.* Dissertation, Berhard Nocht Institute for Tropical Medicine, Hamburg, Germany. With permission of N.P. Kock, C. Schmetz, and J. Schottelius.)

interacts with the host cell membrane is not known, but it may require the participation of host cell proteins such as actin.[50] It is possible that the sporoplasm interacts with the host cell membrane as it emerges from the polar tube. If a spore is phagocytosed by a host cell, germination occurs, and the polar tube can pierce the phagocytic vacuole, delivering the sporoplasm into the host cell cytoplasm. The overall process of germination and formation of the polar tube delivers the sporoplasm to the host cell, functioning essentially like a hypodermic needle.[51,52]

The polar tube discharges from the anterior pole of the spore in an explosive reaction, occurring within less than 2 seconds.[51] Conditions that promote germination vary widely among species, presumably reflecting the organisms' adaptation to their host and external environment[53,54] (reviewed by Keohane and Weiss[55]). Conditions that promote spore discharge include pH shifts, dehydration followed by rehydration, various cations and anions, mucin or polyanions, hydrogen peroxide, ultraviolet irradiation, and the calcium ionophore A23187. Inhibitors of spore discharge include magnesium chloride, ammonium chloride, low salt concentrations, sodium fluoride, ultraviolet light, temperatures higher than 40°C, calcium channel antagonists, calmodulin inhibitors, cytochalasin D, demecolcine, and itraconazole. Regardless of the stimuli required for activation, most microsporidia appear to exhibit the same response to the stimuli, an increase in intrasporal osmotic pressure. This results in an influx of water into the spore accompanied by swelling of the polaroplasts and posterior vacuole before spore discharge. In *A. (Nosema) algerae,* it has been proposed that activation brings trehalose in contact with the enzyme trehalase, causing an increase in osmotic pressure.[56,57]

Microsporidia display a number of characteristics that are unusual for eukaryotic organisms. They have prokaryotic-size ribosomes[58] that do not have a 5.8S ribosome subunit but do have sequences homologous to the 5.8S region in the 23S subunit.[59] The small subunit of ribosomal (rRNA) of most of the microsporidia that have been sequenced is significantly shorter than both eukaryotic and prokaryotic small subunit rRNA.[60,61] These rRNA genes are in a subtelomeric location on each chromosome of *E. cuniculi*[62,63] and lack the paromomycin binding site seen in protozoa and animals.[64] The genome size of the Microsporidia varies from 2.3 to 19.5 megabase (Mb).[65] The genomic size of the Encephalitozoonidae is less than 3.0 Mb, making them among the smallest eukaryotic nuclear genomes so far identified. There are almost no introns in the compact genome of *E. cuniculi,* the gene density is high, and proteins are shorter than the corresponding genes in *Saccharomyces cerevisiae.* There appears to be a high degree of gene conservation among the Microsporidia.[66] Genome data for the Microsporidia are available from the Microsporidia database (http://microsporidiadb.org/micro/),[67] which is part of the EuPath database (http://eupathdb.org/eupathdb/) website. This website includes the genomes of the human pathogenic microsporidia *E. cuniculi*[62,68] (all three strains[69]), *E. hellem,*[70] *E. intestinalis,*[71] *E. bieneusi,*[72,73] *A. algerae, V. corneae*, and microsporidia found in other organisms. Chromosomal analysis of *E. cuniculi*[74] and studies on the heterogeneity of gene loci in both *E. cuniculi*[75] and *Nematocida parisii*[76] indicate that microsporidia are diploid. The karyotypes of several members of the phylum Microsporidia have been determined by pulsed-field electrophoresis. Small subunit rRNA (16S rRNA) diverges greatly from the small subunit rRNA sequences of other eukaryotes, and microsporidian rRNA genes are significantly shorter than those of eukaryotes and prokaryotes.[77] Sequence data of rRNA from the microsporidia have been used to develop diagnostic polymerase chain reaction (PCR) primers and to study phylogenetic relationships[78,79] (reviewed by Weiss and Vossbrinck[61]).

Data on rRNA sequences have proven useful in the identification of environmental reservoirs for microsporidia that infect humans. *E. cuniculi* isolates from various animal species have been identified and separated on the basis of the number of tetranucleotide repeats (5′-GTTT-3′) in the intergenic spacer region of their rRNA genes.[80] Differences have also been found in the intergenic spacer region of rRNA genes of *E. bieneusi* and have been used to identify isolates associated with particular animals or environments.[81–84] Currently, all the different isolates that have been identified within an animal species are considered to be the same microsporidian species; however, it is possible that there may be more than one species of *Enterocytozoon* in its mammalian and avian hosts.

PHYLOGENY OF THE MICROSPORIDIA

Microsporidia are currently classified based on their ultrastructural features, including the size and morphology of the spores, number of coils of the polar tube, developmental life cycle, and host-parasite

relationship (see Fig. 270.2).[5] Tuzet and colleagues,[85] Sprague,[1] Larsson,[86] Issi,[87] Weiser,[88] and Sprague and associates[14] have provided overviews of the history, ultrastructural and structural characteristics, and life-cycle differences among taxa of the Microsporidia. Microsporidia can be divided into three main groups:

1. The "primitive" (Metchnikovellidae) hyperparasites of gregarines in annelids may be distinguished from the other microsporidia by the presence of a rudimentary polar filament and a spore without a polaroplast.
2. The Chytridopsidae, Hesseidsae, and Burkeidae may be seen as "intermediates," having a short polar filament and minimal development of the polaroplast and endospore.
3. The "higher" Microsporidia have a well-developed polar filament, polaroplast, and posterior vacuole.

Differences among modern classifications of the Microsporidia focus on the characteristics used to divide the third group, the higher Microsporidia, into subgroups. Molecular analysis of rRNA genes is also useful for classification.[61,89,90] Antigenic differences between the Microsporidia demonstrable by sodium dodecylsulfate polyacrylamide gel electrophoresis and immunoblot analysis have been used as adjunctive evidence when determining phylogenetic relationships among the Microsporidia infecting humans.[19,91]

The general features of the life cycle are as follows:

1. Spores are ingested or inhaled and then germinate, resulting in extension of the polar tube, which injects the sporoplasm into the host cell.
2. Merogony follows, during which the injected sporoplasm develops into meronts (the proliferative stage), which multiply, depending on the species, by binary fission or multiple fission, forming multinucleate plasmodial forms.
3. The next step is sporogony, during which meront cell membranes thicken to form sporonts.
4. After subsequent division, the sporonts give rise to sporoblasts, which go on to form mature spores without additional multiplication. Once a host cell becomes distended with mature spores, the cell ruptures, releasing mature spores into the environment and thereby completing the life cycle.

The combination of multiplication during merogony and sporogony results in a large number of spores being produced from a single infection and illustrates the enormous reproductive potential of these organisms.

Data on rRNA gene sequences (e.g., molecular phylogenetic data) provide additional evidence for the assignment of microsporidia to specific genera and provide a method to distinguish morphologically similar microsporidia at the species level. Analysis of the rRNA genes of a variety of microsporidia highlights the polyphyletic nature of the Microsporidia and brings into doubt the use of any single character for developing higher taxonomic groupings. For example, *E. hellem* and *E. cuniculi* are indistinguishable at the ultrastructural level, and *E. intestinalis* has a distinct extracellular matrix surrounding the sporoblasts and spores; however, on the basis of rDNA analysis, *E. intestinalis* and *E. cuniculi* are more similar to each other than to *E. hellem*.[92]

Molecular phylogenetic data indicate that the Microsporidia are related to the Fungi (either as a basal branch of the Fungi or as a sister group[6,7]) and are not primitive eukaryotes.[93–95] As early as 1994, on the basis of β-tubulin sequence analysis, it was suggested that the Microsporidia were not ancient eukaryotes but instead were related to the Fungi.[96] Keeling and Doolittle[97] reported similar results for α-tubulin from three species of microsporidia *(E. hellem, Nosema locustae, Spraguea lophii)* and for β-tubulin from *E. hellem*. Analysis of the heat shock protein gene *(hsp70)* for *Nosema locustae, Vairimorpha necatrix, E. cuniculi,* and *E. hellem* provided confirmatory evidence of this relationship.[98–101] Comparative analysis of the largest subunit of the RNA polymerase II *(RPB1)* gene produced a phylogeny similar to the result obtained from an analysis of *hsp70* or the β-tubulin gene. Additional evidence for the relationship of the Microsporidia to the Fungi includes the following:

1. The *E. cuniculi* genes for thymidylate synthase and dihydrofolate reductase are separate genes.[102]
2. The small subunit rRNA gene of microsporidia lacks a paromomycin binding site, similar to the fungi.[103]
3. The elongation factor-1α sequence of the microsporidian *Glugea plecoglossi* has an insertion that is found only in fungi and animals, not in protozoa.[103,104]
4. Microsporidia display similarities to the Fungi during mitosis (e.g., closed mitosis and spindle pole bodies[105]) and meiosis.[106]
5. Microsporidia have chitin in their spore wall and store trehalose, as do fungi.
6. Analyses of many genes (e.g., glutamyl-tRNA synthetase, seryl-tRNA synthetase, vacuolar adenosine triphosphate [ATP]ase, TATA box–binding protein, transcription initiation factor IIB, subunit A of vacuolar ATPase, and guanosine triphosphate [GTP]-binding protein and transcription factor IIB sequences[68,107]) support a relationship between the Microsporidia and the Fungi.
7. Analysis of the *E. cuniculi* genome and other microsporidian genomes demonstrates that most microsporidian proteins are most similar to fungal homologues.[6,7,68]
8. The presence in *E. cuniculi* of the principal enzymes for the synthesis and degradation of trehalose confirm that this disaccharide could be the major sugar reserve in microsporidia, as is seen in many fungi.
9. Analysis of glycosylation pathways has indicated that *O*-mannosylation (e.g., *O*-linked glycosylation with mannose), as seen in fungi, occurs in microsporidia. Experimental evidence has confirmed that *O*-mannosylation occurs on the major polar tube protein PTP1.[46]
10. Keeling,[108] in an analysis of β-tubulin data that included additional species of microsporidia and more fungal phyla, found that the Microsporidia were a sister group to the Zygomycota. Comparative analysis using the complete genomes of several microsporidia also supports a similar relationship of the Microsporidia with the Fungi.[6,7]
11. Microsporidian genomes display two syntenic ribosomal genes (*RPL21* and *RPS9*) that are also syntenic throughout the Fungi but are linked in other eukaryotic lineages.
12. An identified microsporidian sex-related locus is highly similar to the sex locus of the Zygomycetes; both contain genes for a triose phosphate transporter (TPT), a high mobility group protein, and an RNA helicase.[7,109,110]

Environmental microbiome studies have identified a previously overlooked large and diverse group of organisms that have been termed Cryptomycota[111] and, although poorly described at the biologic level, are closely related to the Microsporidia. Genome-scale phylogenies place microsporidia together with Cryptomycota as the basal branch of the fungal kingdom or as a sister phylum to the Fungi. Cryptomycota that grow as intranuclear parasites of ameba have been discovered, and these parasites bear strong morphologic similarities to the Microsporidia.[112] These, and other microsporidia-like organisms, such as *Mitosporidium daphniae*, *Nucleophaga terricolae,* and *Paramicrosporidium*, demonstrate that more environmental sampling is required to resolve the relationship of fungi and microsporidia and to provide a better understanding of the Cryptomycota and the origin of microsporidia.

EPIDEMIOLOGY

Microsporidian spores are commonly found in surface water, and human pathogenic microsporidia have been found in municipal water supplies, tertiary sewage effluent, and ground water.[113–118] It is likely that many of the Microsporidia are waterborne pathogens, and they can be transmitted by food.[119] Water contact has been found to be an independent risk factor for microsporidiosis in some studies[120,121] but not in others.[122,123] Outbreaks of *V. corneae* infection have been associated with hot springs exposure[124] and exposure to soil.[125,126] *E. cuniculi* spores remain viable for 6 days when in water and 4 weeks when dry at 22°C, and *Nosema bombycis* spores may remain viable for 10 years in distilled water.[127] Spores may be killed, however, by exposure for 30 minutes to 70% ethanol, 1% formaldehyde, or 2% Lysol or by autoclaving at 120°C for 10 minutes.[128] Most microsporidian infections are transmitted by oral ingestion of spores, with the site of initial infection being the GI tract. Viable infective spores of microsporidia are present in a number of body fluids (e.g., stool, urine, respiratory secretions) during infection,

suggesting that person-to-person transmission can occur and that ocular infection may be transmitted by external autoinoculation caused by contaminated fingers.[129] It has been possible to transmit *E. cuniculi* via rectal infection in rabbits, suggesting the possibility of sexual transmission.[130] *E. hellem* has been demonstrated in the respiratory mucosa and in the prostate and urogenital tract of patients, raising the possibility of respiratory and sexual transmission in humans.[131,132] Person-to-person transmission is supported by concurrent infections in cohabiting homosexual men.[133] Although congenital transmission of *E. cuniculi* has been demonstrated in rabbits, mice, dogs, horses, alpaca, foxes, and squirrel monkeys, no such congenital transmission has been demonstrated in humans.[134]

There have been two clusters of microsporidiosis due to transplanted organs.[135] Three patients were infected by transplanted organs (kidney, kidney, and bilateral lungs) from an *E. cuniculi*–seropositive donor, and kidney biopsy provided the diagnosis in all three cases. Onset of symptoms (fever, renal dysfunction, and encephalopathy) was 7 to 10 weeks posttransplant, and death in one recipient of a kidney may have been directly related to failure of the transplanted organ. The surviving kidney recipient received 6 months of albendazole therapy and remained healthy afterward.[135] In the second cluster, which also involved three patients infected by transplanted organs (kidney, liver, heart/kidney), the patients presented with neurologic symptoms, including headache and encephalitis.[136] Kidney or urine specimens demonstrated *E. cuniculi* in these patients. The liver transplant patient responded to albendazole treatment.[136]

Many of the Microsporidia probably cause zoonotic infections in humans (see Table 270.1).[137] Microsporidia of the genus *Encephalitozoon* are widely distributed parasites of mammals and birds, and the onset of microsporidiosis has been associated with exposure to livestock, fowl, and pets.[138] *E. hellem* infections have been described in lovebirds and budgerigars (parakeets),[8] and *E. hellem* infection has been reported in a patient who had infected lovebirds.[139] Up to 30% of dogs in animal shelters may excrete microsporidia in their stools. Microsporidia of the genus *Encephalitozoon* were found in the stools of many animals in an epidemiologic survey in Mexico.[120] *Enterocytozoon bieneusi* appears to be widely distributed[82–84] and has been reported in many mammals, including pigs,[140] dogs,[141] chickens,[142] pigeons,[143] falcons, and simian immunodeficiency virus (SIV)-infected rhesus monkeys.[144] There is a documented case of transmission of *E. bieneusi* between a child and guinea pigs.[145] Enterocytozoonidae such as *Nucleospora* (previously *Enterocytozoon*) *salmonis* are pathogens found in fish. *Nosema* and *Vittaforma* infections are believed to be caused by traumatic inoculation of environmental spores of insect pathogens into the cornea.[15,31,146] *Tubulinosema* and *Anncaliia* are insect pathogens.

Although initially regarded as rare, microsporidia are now believed to be common enteric pathogens that cause self-limited or asymptomatic infections in normal hosts.[138,147] Cases of microsporidiosis have been identified from all continents except Antarctica.[133,148–154] Surveys of pathogens seen in stool samples in Africa, Asia, South America, and Central America have demonstrated that microsporidia are often found during careful stool examinations. In immune-competent hosts most reported cases of microsporidiosis manifested as self-limited diarrhea. It has been observed that latent infection with shedding of spores is common in immune-competent humans.[155] They have included cases of *E. bieneusi* infections in travelers to and residents of tropical countries[82,114,150,156–163] and of *E. intestinalis* infections in travelers to and residents of tropical countries.[164] In addition, there are several reports from India and Singapore of ocular infection with microsporidia, in particular *V. corneae,* presenting as a keratoconjunctivitis in immune-competent hosts.[165–169] In immune-deficient hosts (e.g., those with acquired immunodeficiency syndrome [AIDS] or who have undergone transplantation), most reported cases have manifested as diarrhea with wasting syndrome and disseminated infection. Infections with *E. bieneusi* have been reported in patients who have undergone kidney, liver, or heart-lung transplantation. *Encephalitozoon* spp. infections have been reported in patients who have undergone kidney, pancreas, liver, or bone marrow transplantation. *Tubulinosema acridophagus* has been reported in a patient with bone marrow transplantation, and *A. algerae* has been found in a patient with lung transplantation.[22,170–182] A case of chronic bilateral keratoconjunctivitis caused by *Encephalitozoon* sp. has been reported in a patient taking prednisone (20 mg/day).[183]

Reported prevalence rates in the 25 studies conducted on patients with HIV infection before the widespread use of combination antiretroviral therapy (cART) (1989–98) varied between 2% and 70%, depending on the symptoms of the population studied and the diagnostic techniques used.[a] These studies suggest that asymptomatic carriage can occur in immunocompromised patients. Coinfection with different microsporidia or other enteric pathogens can occur. There was no overall trend in these prevalence studies with regard to country of origin or other demographic characteristics. When combined, these studies identified 375 *E. bieneusi* infections among 2400 patients with chronic diarrhea, for a prevalence of 15% in this population. More recent studies suggest that, in areas where cART is not widely available, the epidemiology of this disease has not changed and that a similar prevalence rate is present.[82] In addition, studies of the prevalence of *E. bieneusi* in HIV-negative individuals in developing countries have also demonstrated prevalence rates of 2% to 70%, depending on the country and population studied (e.g., children or adults, rural or urban).[82] It is clear that since the institution of cART and its associated immune reconstitution, the prevalence of diarrhea among AIDS patients has decreased, as has the incidence of microsporidiosis.

The aggregate data available confirm that the Microsporidia demonstrate strength of association, coherence, and reproducibility with respect to being causative for a diarrheal syndrome. Further evidence of the association of microsporidia with diarrhea is provided by the usefulness of albendazole in the treatment of microsporidian infection. Therapy with albendazole results in cure of the diarrhea associated with elimination of *E. intestinalis* from the stool of infected patients.[190] Treatment with fumagillin has a similar effect in patients with *E. bieneusi* infection.[191,192]

Serosurveys in humans have demonstrated a high prevalence of antibodies to *E. cuniculi* and *E. hellem,* suggesting that asymptomatic infection may be common.[151,193] Serologic cross-reactivity among microsporidia has been demonstrated by both immunofluorescence[194] and immunoblot testing.[195] Singh and coworkers[196] found positive titers in 6 of 69 healthy adults in England, 38 of 89 Nigerians with tuberculosis, 13 of 70 Malaysians with filariasis, and 33 of 92 Ghanaians with malaria. In another study 14 of 115 travelers returning from the tropics and 0 of 48 nontravelers were seropositive.[197] In a study of HIV-positive men 10 of 30 were seropositive and all had traveled to the tropics.[198] Antibodies to *E. intestinalis* were found in 5% of pregnant French women and 8% of Dutch blood donors.[199] In HIV-positive Czech patients 5.3% were seropositive to *E. cuniculi* and 1.3% to *E. hellem*.[200] In Slovakia 5.1% of slaughterhouse workers were seropositive to *Encephalitozoon* spp.[201] In a survey of blood donors in the United States 5% of donors had antibodies to *E. hellem* PTP1 antigen (L. M. Weiss, unpublished observations). Overall, these studies suggest that exposure to microsporidia is common and that asymptomatic infection may be more common than originally suspected.

IMMUNOLOGY

Infection with *E. cuniculi* in many mammals results in chronic infection with persistently high antibody titers and ongoing inflammation (e.g., persistent encephalitis in rabbits and chronic renal disease and congenital transmission in foxes). In immune-competent murine models of *E. cuniculi* infection, ascites develops and then clears; however, if corticosteroids are administered, the mice redevelop ascites, consistent with latent persistence of microsporidia in these animals.[202,203] Studies on immune-deficient mice have demonstrated that latent infection occurs, allowing relapse, and that albendazole treatment cannot eliminate latency in immune-deficient mice.[204] In mice with severe combined immunodeficiency (SCID) or athymic mice, infection with *E. cuniculi* results in death, with visceral dissemination of the organism and persistent ascites.[205] Adoptive transfer of sensitized syngeneic T-enriched spleen cells protects athymic or SCID mice against lethal *E. cuniculi* infection.[206,207] Transfer of naïve lymphocytes or hyperimmune serum failed

[a]References 10,133,138,148,154,184–189.

to protect or prolong the survival of these mice. Cytokine-activated murine peritoneal macrophages can inhibit the replication of *E. cuniculi* in vitro.[208] This inhibition is probably mediated by nitric oxide; studies have demonstrated that inhibition of nitric oxide synthesis inhibits such killing.[209] Studies using macrophages have shown that reactive nitrogen and oxygen species, and iron sequestration, all contribute to the control of *E. cuniculi* in these cells.[210] Mice deficient in inducible nitric oxide synthase, however, had no change in susceptibility to *E. cuniculi* infection.[211]

Humoral immunity is not sufficient for protection against *E. cuniculi* infection because adoptive transfer of immune B lymphocytes into athymic BALB/c (nu/nu) or SCID mice or passive transfer of hyperimmune serum into athymic mice does not protect these animals from death after infection. Nonetheless, during *E. cuniculi* infection there is a strong antibody response to many components of this organism, and many of these antibodies are cross-reactive with other microsporidia. Maternal antibodies protect newborn rabbits from infection with *E. cuniculi* during the first 2 weeks of life.[212] The in vitro infectivity of microsporidia is reduced by treatment with immune serum and complement,[206] monoclonal antibody (mAb 3B6) to the spore coat,[213] or polyclonal antibodies to polar tube protein-1 (PTP1) (L.M. Weiss, unpublished data). Immunoglobulin M (IgM) antibodies against PTP1 in normal human serum may play a role in preventing infection with *E. cuniculi*.[214] Overall, it is probable that antibodies play a role in limiting infection in the host, although they are not sufficient to prevent mortality or cure infection.

Interferon-γ (IFN-γ) and interleukin-12 (IL-12) are important for protective immunity against a number of intracellular viral, bacterial, and parasitic infections.[215] Based on in vitro observations, it has been suggested that IFN-γ plays an important role in the protective immunity against *E. cuniculi* infection. Both natural killer cells and γδ T cells, which are increased at early stages of infection, are likely important sources of IFN-γ production. Studies with *E. intestinalis* and *E. cuniculi* have demonstrated that IFN-γ knockout mice cannot clear infection.[216] Treatment of *E. cuniculi*–infected mice with neutralizing antibody to IFN-γ or IL-12 results in increased mortality.[211] The importance of IL-12 is illustrated by the fact that lethal infection with *E. cuniculi* occurs in p40 knockout mice, which are unable to produce IL-12 and have impaired $CD8^+$ cell function to this pathogen.[217,218]

The role of individual T-cell subtypes during *E. cuniculi* infection has been evaluated in murine models, mostly in studies of disseminated infection using intraperitoneal infection.[217] Phenotypic analysis of the spleen cells from infected animals has revealed an increase in the $CD8^+$ T-cell population starting at day 10 after infection, with no significant increase in $CD4^+$ T cells. Mice deficient in $CD8^+$ cells succumb to the parasitic challenge, but there was no change in mortality for mice deficient in $CD4^+$ cells. In most cases $CD8^+$ T cells are primed via IL-2–producing $CD4^+$ T cells, although a normal in vivo $CD8^+$ T-cell response in the absence of $CD4^+$ T cells has been described with many viral infections and appears to occur with *E. cuniculi* infection. A normal antigen-specific $CD8^+$ T-cell response to *E. cuniculi* infection has been found to occur in CD4 knockout mice. The protective effect of $CD8^+$ T cells is mediated by their ability to produce cytokines and to reduce the parasite load by killing the infected targets in the host tissue.[219] The major killing mechanism exhibited by $CD8^+$ T cells is via the perforin pathway, and mice lacking the perforin gene die when infected with *E. cuniculi*. These observations suggest that the cytotoxic T-cell response is a key factor in the immune response to *E. cuniculi*–infected mice.

When mice are infected with microsporidia orally, instead of by peritoneal injection, studies have shown that both $CD4^+$ and $CD8^+$ T cells are involved in the protective immune response generated by the GI tract.[220,221] However, the $CD8^+$ αβ population was the most important cellular subset in providing protective immunity.[222] IFN-γ production by dendritic cells (DCs) has been demonstrated to be important for priming the gut intraepithelial lymphocyte response after oral infection with *E. cuniculi*.[222]

DCs play a key role in promoting the T-cell response via recognition of the pattern recognition receptors expressed by these pathogens (e.g., TLR4[223] and TLR2[224] have both been described to be important for the immune response to microsporidiosis). Plasmacytoid DCs have been demonstrated in older mice to downregulate $CD8^+$ T-cell responses by inhibiting the maturation of normal DCs after *E. cuniculi* infection.[225]

There are scant data to confirm the immune response to microsporidia in humans. It is clear that a strong humoral response occurs during infection and that it includes antibodies that react with the spore wall and polar tube. The immunosuppressive states associated with microsporidiosis (e.g., AIDS and transplantation) are those that inhibit cell-mediated immunity. Microsporidiosis is usually seen in HIV-infected patients when there is a profound defect in cell-mediated immunity (e.g., a $CD4^+$ cell count $<100/mm^3$); spontaneous cure of microsporidiosis can be induced by immune reconstitution with cART.[226–228] Overall, these data are consistent with observations on the immunology of the mouse model of microsporidiosis and suggest that restoration of cell-mediated immunity and possibly administration of IFN-γ or IL-12 are useful adjuncts for treating microsporidiosis.

PATHOLOGY AND PATHOGENESIS

Gastrointestinal Tract Infections

Infection of the epithelium of the GI tract (small intestine and biliary epithelium) is the most frequent presentation of microsporidiosis (Fig. 270.3). Greater than 90% of these infections are caused by *E. bieneusi,* with the remainder mostly caused by *E. intestinalis*.[37,40,133,138] Because most of these infections are acquired by ingesting spores, it is likely that other species of microsporidia also cause asymptomatic GI infections. Granulomatous hepatitis caused by *E. cuniculi* is commonly seen in mammals infected with this organism, and granulomatous hepatitis caused by *Encephalitozoon* has been reported in patients with HIV infection.[229] Hepatitis with infection of the biliary system (including the portal triad and gallbladder epithelium) caused by *E. bieneusi* has been seen in SIV-infected *Macaca mulatta* (rhesus macaques).[230] Chronic gallbladder infection with *E. bieneusi* is seen in pigs. *E. bieneusi* and *E. intestinalis* infections of the biliary tract can result in sclerosing cholangitis in AIDS patients.[231,232] These observations suggest that the biliary epithelium may be a reservoir for relapse of *E. bieneusi* and perhaps other microsporidia.

E. bieneusi infection does not produce active enteritis or ulceration, although the infection results in variable degrees of villous blunting and crypt hyperplasia. There is a gradient in parasite burden along the small intestine, with the distal duodenum and proximal jejunum having higher burdens than the proximal duodenum.[231] The organism can be found in the ileum but is rarely found in the colon. On endoscopy scalloping of the valvulae conniventes and villous fusion may be evident.[233] The organism is located on the apical surface of the enterocytes of the small intestine and epithelial cells of the biliary tract and pancreas. Spores are rarely found on the basal surface or in the lamina propria.[234,235] This organism rarely disseminates, unlike the Encephalitozoonidae, which are commonly found in the lamina propria and disseminate to visceral organs. Infection may be associated with increased intraepithelial lymphocytes and epithelial disarray. At the villous tips teardrop-shaped cells can be seen during the process of sloughing, which is characteristic of infection with *E. bieneusi.* Spores are smaller (1×1.5 μm) than those of *Encephalitozoon* spp. (1.2×2.2 μm) and more difficult to find in tissue sections (see Fig. 270.3). Infection is associated with malabsorption because of decreased mucosal surface area and functional immaturity of the villous epithelial cells.

E. bieneusi displays a unique intracellular developmental life cycle. A characteristic feature of the organism is the presence of electron-lucent inclusions with a lamellar structure. These inclusions are closely associated with the nuclear envelope, the endoplasmic reticulum, or both. The earliest intraepithelial stages of the parasite are rounded proliferative cells limited by a typical unit membrane in direct contact with the host cell cytoplasm. Nuclear division is not immediately followed by cytokinesis in these cells, resulting in the production of multinucleate proliferative plasmodia. After the production of multiple nuclei, the parasites form electron-dense disklike structures that cluster in stacks of three to six, eventually forming the coiled portion of the polar tube. When these multinucleated sporagonial plasmodia divide by invagination of the plasmalemma, multiple spores are formed. In mature spores the polar

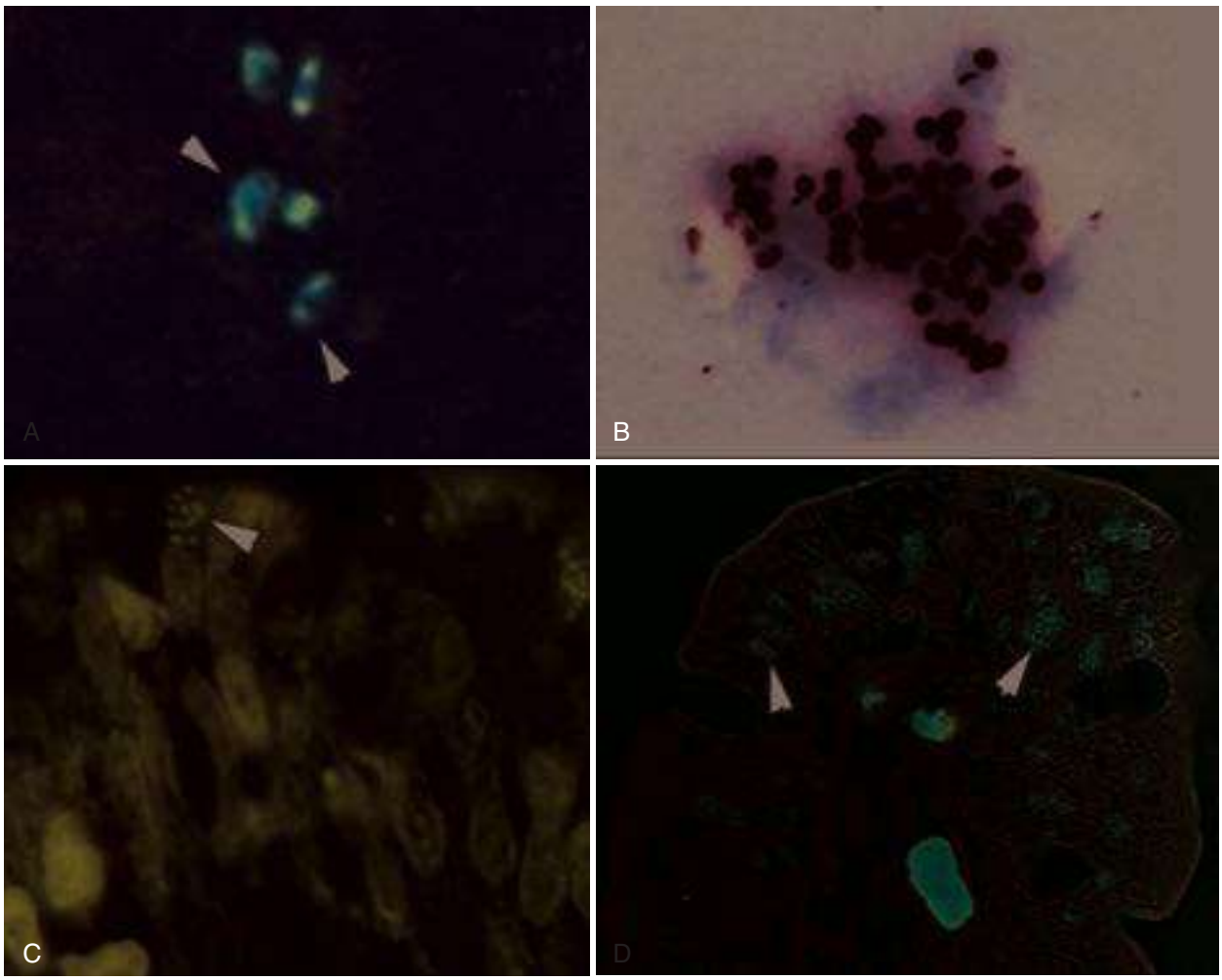

FIG. 270.3 Demonstration of Microsporidia in stool, urine, and tissue samples. (A) Chromotrope 2R stain (modified trichrome stain) of a stool sample from a patient with microsporidian enteritis due to *Enterocytozoon bieneusi.* Arrowheads point to microsporidian spores. Using chromotrope 2R–based stains, spores appear as 1- to 3-µm ovoid light pink structures with a beltlike stripe girding them diagonally and equatorially. (B) Calcofluor white stain (a chemifluorescent optical brightening agent that stains chitin) demonstrating fluorescent *Encephalitozoon hellem* spores in the urine of a patient with disseminated infection. (C) Giemsa stain of a small intestinal biopsy from a patient with electron microscopy–proven *Enterocytozoon bieneusi* enteritis. The arrowhead points to a collection of microsporidian spores at the apical end of an enterocyte. Spores are not found on the basal side or in the lamina propria with this organism. (D) Chromotrope 2R tissue stain of a small intestinal biopsy from a patient with electron microscopy–proven *Encephalitozoon intestinalis* infection. Arrowheads point to microsporidian spores. Note that spores are found in the apical and basal sides of the enterocytes, and in the lamina propria. This is consistent with the ability of this microsporidian to disseminate. *(B courtesy E. Didier, Tulane Regional Primate Research Center, Covington, LA; D courtesy D. Kotler, St. Lukes-Roosevelt Hospital Center and J. M. Orenstein, George Washington University School of Medicine, Washington DC.)*

tubule has five to seven coils that appear in two rows when seen in cross section by transmission electron microscopy.

E. intestinalis is invasive, and spores are commonly found in the apical and basal sides of infected intestinal enterocytes and in cells in the lamina propria, including fibroblasts, endothelial cells, and macrophages (see Fig. 270.3).[236] This pattern of infection is also seen with other *Encephalitozoon* spp. and probably reflects the ability of these infections to disseminate to visceral organs after ingestion. Dissemination can result in necrosis of areas of the bowel, with a presentation resembling that of an acute abdomen[26,237] and perforation of the intestine with peritonitis.[238] Histopathology can demonstrate areas of necrosis and mucosal erosion. In pathology sections the septated parasitophorous vacuole can be seen surrounding the developing spores. *E. intestinalis* spores are easier to detect than *E. bieneusi* spores because of their larger size, strong birefringence, and bluish color on hematoxylin and eosin staining. In addition, spores of *E. intestinalis* are more numerous in tissue. Sporogony is tetrasporous, and tubular appendages originate from the sporont surface and terminate in an enlarged bulblike structure. Unlike infections with other Encephalitozoonidae, *E. intestinalis*–infected cells have a unique parasite-secreted fibrillar network surrounding the developing organisms, so the parasitophorous vacuole appears septate. Mature spores in cross section have a single row of four to seven coils of polar tubules.

E. cuniculi has been described in a case of peritonitis.[239] At autopsy, a mass of omentum was found that displayed focal necrosis, nongranulomatous inflammation, and microsporidian spores. *E. hellem* and *E. cuniculi* have similar developmental life cycles.[38] The genus is characterized by the presence of a phagosome-like parasitophorous vacuole. Nuclei of all stages are unpaired. Meronts divide repeatedly by binary fission. Sporonts divide into two sporoblasts that mature into spores. No tubular appendages or fibrillar networks are produced, as is seen with *E. intestinalis.* In cross section, the mature spore has five to seven coils in single rows.

A case of peritonitis with hepatitis due to *T. acridophagus* infection has been described in a patient with an allogeneic bone marrow transplant.[22]

Genitourinary Tract Infection

Encephalitozoon spp. infect the genitourinary system in most mammals. Contamination of the environment by spores passed in urine is believed

to be the mechanism of transmission of these organisms between successive hosts, which appears to be the case in human infections,[147,190,234,240] in whom infection discovered in any organ (e.g., eye, GI tract, liver, central nervous system [CNS]) is often associated with the shedding of spores in the urine. In HIV-infected patients with keratitis there is usually asymptomatic infection of the urinary tract and bronchial tree. Granulomatous interstitial nephritis composed of plasma cells and lymphocytes is the most frequent pathologic finding. It is associated with tubular necrosis, with the lumen of the tubules containing amorphous granular material. On occasion, microabscesses and granulomas form around necrotic tubules. Spores are located in the necrotic tubes and sloughing tubular epithelial cells. Organisms may also be found in the interstitium. Glomerular involvement is rarely seen. This pattern of interstitial nephritis has been seen in AIDS patients and in those with renal transplants complicated by microsporidiosis.[b] An identical pattern has been described in rabbits with *E. cuniculi* infection.

As spores and infected tubular cells are shed into the bladder, they can infect other epithelial cells of the urogenital tract. Microsporidia have been reported to cause necrotizing ureteritis and cystitis.[234] Inflammatory cystitis has been seen during cystoscopy. These organisms can disseminate from the urogenital epithelial cells and can be found in macrophages, muscle, and supporting fibroblasts of the associated mucosa. Genital tract infection with *Encephalitozoon* has been reported to occur in association with urinary shedding of spores and has included prostatitis with abscess formation.[131] Postmortem examination of eight Cuban women who died of HIV "wasting" syndrome revealed disseminated *E. cuniculi* and *E. hellem* infection with involvement of the fallopian tubes and endometrium.[243] The frequency of microsporidian prostatitis is not known, nor is it known if sexual transmission occurs. A case of urinary tract infection and chronic prostatitis caused by *V. corneae* has also been reported in a patient with AIDS.[146]

Central Nervous System Infection

Granulomatous encephalitis is the classic presentation of *Encephalitozoon cuniculi* infection in many mammals, such as rabbits. This infection has been recognized in a few patients with HIV infection, in whom it "mimicked" toxoplasmic encephalitis.[28] At autopsy, CNS involvement was documented in a case of disseminated *E. cuniculi* (type III or dog strain) involving almost all organs.[244] Spores were present in the cerebral parenchyma, perivascular spaces, and macrophages but were not seen in oligodendrocytes, neurons, astrocytes, or meningeal cells. A second case of *E. cuniculi* encephalitis was caused by the type II or rabbit strain. There have also been case reports of encephalitis caused by *Trachipleistophora anthropopthera*.[21,35] These patients had multiple ring-enhancing lesions on computed tomography (CT) scans suggestive of CNS toxoplasmosis. On pathologic examination the 2- × 2.8-µm birefringent spores were located in the gray matter, and there was extensive necrosis. The disease also involved the heart, kidney, pancreas, thyroid, parathyroid, liver, bone marrow, lymph nodes, and spleen in addition to the brain. The most heavily infected cells were epithelial cells, cardiac myocytes, and astrocytes.

Ocular Infection

Infection with *E. cuniculi, E. hellem,* or *E. intestinalis* can result in punctate keratopathy and conjunctivitis characterized by multiple punctate corneal ulcers (e.g., superficial epithelial keratitis; Fig. 270.4). Microsporidian spores are present in corneal and conjunctival epithelium, which can be obtained by scraping or biopsying the lesions. The organisms do not invade the corneal stroma but remain limited to the epithelium. Inflammatory cells are rarely present. *Trachipleistophora anthropopthera* can also cause keratitis.[245] Ocular disease may be the presenting manifestation when there is disseminated infection with any of the *Encephalitozoon* spp.[91,129,139,229,246] These infections most often occur in the setting of HIV or other immune dysfunction, although there are case reports of superficial epithelial keratitis caused by *Encephalitozoon* spp. in immune-competent patients. Most of these immune-competent cases of *Encephalitozoon* infection have occurred in contact lens wearers. In addition, there are

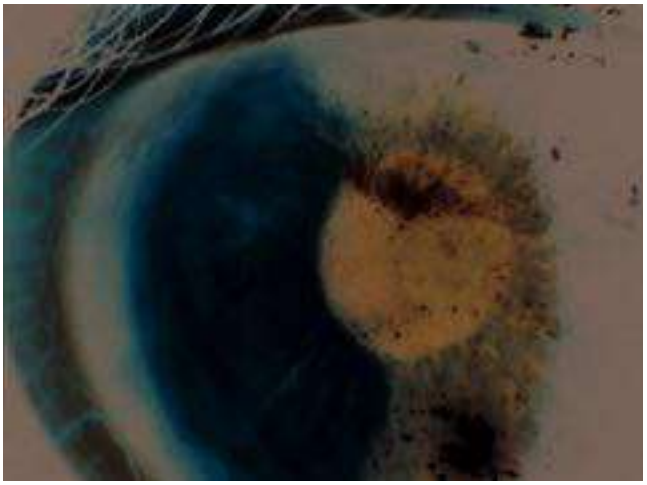

FIG. 270.4 Ocular examination of a patient with keratoconjunctivitis caused by *Encephalitozoon hellem*. Slit-lamp examination findings in a patient with punctate keratoconjunctivitis. A conjunctiva scraping from this patient demonstrated spores consistent with *E. hellem* (see Fig. 270.2B). Sequencing of the small subunit ribosomal RNA gene obtained by the polymerase chain reaction from this conjunctival biopsy confirmed that the infection was caused by *E. hellem*.

numerous reports, involving more than 300 patients from India and Singapore, of *V. corneae* infection presenting as keratoconjunctivitis in immune-competent hosts.[165–169] This microsporidia-associated illness presents as seasonal keratoconjunctivitis and may be more common than previously appreciated. *V. corneae, Microsporidium africanum, Microsporidium ceylonensis*, and *Nosema ocularum* have also been reported to cause infection in other immune-competent patients.[247] These reported infections have usually involved deeper levels of the corneal stroma and have often been associated with trauma. Biopsies have demonstrated necrosis and acute inflammatory cells with some giant cells in several cases. Clinically, these patients have a corneal stromal keratitis and occasionally uveitis. Stromal keratitis caused by a unspeciated microsporidial organism was initially mistaken for corneal graft rejection and only diagnosed in the explanted host button after regrafting.[248]

Musculoskeletal Infection

Myositis with inflammation caused by several microsporidia has been described in humans. The organisms in these case reports have included *Pleistophora ronneafiei, Pleistophora* sp., *Trachipleistophora hominis, Tubulinosema* spp., *Endoreticulatus* spp., *Anncaliia vesicularum* (formerly *Brachiola vesicularum*), and *A. algerae*.[c] A case of endocarditis due to *E. cuniculi* has been described.[249]

The prevalence or incidence of microsporidian myositis in humans is not known. Biopsies from the cases of *Pleistophora* spp. infection have demonstrated atrophic and degenerating muscle fibers infiltrated with focal clusters of large microsporidian spores that were up to 3.4 µm in length. There was a mixed inflammatory response consisting of plasma cells, lymphocytes, eosinophils, and histiocytes that was mild in the two cases occurring in AIDS patients but severe in the case involving an HIV-seronegative person.[33,252,253] *T. hominis* infection was associated with degeneration, atrophy, scarring, and intense inflammation.[20] There are several reports of *Trachipleistophora* spp. causing myositis. The *A. vesicularum* infection, which occurred in a patient with AIDS, was associated with cytolysis around the spores in the muscle fibers, but no cellular immune response was seen.[16] The *A. algerae* infection occurred in a patient with rheumatoid arthritis treated with steroids and monoclonal antibody to tumor necrosis factor-α (TNF-α).[250] In this case there was a minimal cellular response to the numerous spores present in the muscle fibers. The *Tubulinosema* spp. infection occurred in a patient with chronic lymphocytic leukemia, and a Richter transformation

[b]References 172,176,179,181,182,241,242.

[c]References 16,20,23,24,33,34,250,251.

and muscle tissue examination demonstrated abundant clusters of small, ovoid, basophilic organisms that could be stained with Warthin-Starry silver stain.[23] The *Endoreticulatus* spp. infection occurred in an otherwise healthy man who presented with difficulty swallowing and muscle pain.[24] A muscle biopsy demonstrated necrotizing granulomatous inflammation, and a bone marrow biopsy demonstrated increased plasma cells and histiocytes and focal aggregation of microsporidial spores. An examination of urine demonstrated similar microsporidian spores.

Sinus and Respiratory Infection

Respiratory tract involvement is often seen with disseminated infections caused by the Encephalitozoonidae or other microsporidia.[22,254] The pathologic features are nonspecific and may include rhinitis, sinusitis, and nasal polyposis in any combination.[255–261] A tongue ulcer containing *E. cuniculi* spores was reported in a patient with disseminated infection with this organism.[262] In a study of patients presenting with keratitis caused by *E. hellem,* spores of this organism were present in many of these patients' sputum samples in the absence of respiratory symptoms.[263] *E. hellem* infection of the entire length of the respiratory tract, including the terminal bronchioles, associated with erosive tracheitis, bronchitis, and bronchiolitis, has been described in an autopsy report. Spores were seen in the epithelial cells, neutrophils in the bronchiolar wall, cells lining the alveoli, and extracellularly in the alveolar spaces. Case reports have confirmed that *E. cuniculi, E. hellem,* and *E. intestinalis* can cause bronchiolitis with or without pneumonia. Sinus biopsies in AIDS patients with chronic sinusitis and microsporidiosis have demonstrated spores in epithelium and supporting structures.[264] The inflammatory response has been variable, with lymphocytes, neutrophils, macrophages, and occasional granuloma formation. Respiratory tract infection caused by *E. bieneusi* has been reported twice, with spores found in stool, bronchoalveolar lavage (BAL) fluid, and transbronchial biopsy specimens.[265,266] A case of rhinosinusitis caused by *E. bieneusi* has also been reported.[267] It has been suggested that these cases reflect contamination and colonization of the respiratory tract as a result of vomiting rather than dissemination of this organism from the GI tract. In a case of disseminated *T. acridophagus* infection with respiratory failure and pulmonary infiltrates, microsporidian spores were present in BAL fluid.[22]

Skin

Microsporidia infections of the skin have been described. In a child with leukemia the infection was caused by *A. algerae,* with spores infecting the cellular elements of the dermis.[268] In another case an *Encephalitozoon* sp. was reported to be the cause of nodular skin lesions.[269,270] An infection with *T. acridophagus* presented as red macules and papules in a patient with an allogeneic bone marrow transplant.[22] Biopsy demonstrated cysts containing microsporidian spores with minimal inflammatory cell infiltration.

CLINICAL MANIFESTATIONS

The clinical manifestations of microsporidiosis are shown in Table 270.1.

Microsporidian Infection in Non-AIDS Patients

Levaditi and colleagues[271] suggested that microsporidia were associated with human disease as early as 1923, but the first definitive proof of human infection was not reported for another 50 years. In 1973 a 4-month-old athymic male infant died with severe diarrhea and malabsorption. At autopsy the microsporidian *Anncaliia (Nosema) connorii* was discovered in his lungs, stomach, small and large bowel, kidneys, adrenal glands, myocardium, liver, and diaphragm.[272]

In all patients (those with or without HIV infection) the most common symptomatic microsporidian infection is probably diarrhea.[d] *E. bieneusi* has been identified as a cause of self-limited diarrhea in immune-competent hosts, including travelers.[147,161–164,273,274] In epidemiologic studies *E. bieneusi* has been identified in 1% to 10% of African children with diarrhea.[e] Clinical manifestations have included watery, nonbloody diarrhea, nausea, diffuse abdominal pain, and fever.

[d]References 120,133,149,150,156,159,164–171,275.
[e]References 82–84,148,170–182,276–279.

Numerous reports document infection in transplant recipients. The diagnosis is often delayed, perhaps because of the lack of specificity of symptoms and a low index of suspicion in transplant medicine. Kidney biopsy and examination of urine sediment is a frequent modality for diagnosis. Fever and kidney graft dysfunction led to a histologic diagnosis *(E. cuniculi)* in the transplanted organ of two pediatric renal transplant patients.[280] Concomitant urinary tract infection with *E. cuniculi* and *E. bieneusi* was thought to have occurred in a renal transplant recipient, based on PCR of urine sediment, although *E. bieneusi* is rarely found outside the GI and biliary tracts.[281] *E. bieneusi* infection with diarrhea that was successfully treated with fumagillin has been reported in patients with allogeneic hematopoietic stem cell transplantation.[282] Dissemination (spores in urine and BAL) has occurred with *E. cuniculi* in a renal transplant recipient presenting with fever, cough, and acute kidney injury. Treatment with albendazole for 6 months was needed to clear the urinary tract.[283] Disseminated infection with *E. hellem* has been seen in patients with leukemia.[284] Death occurred with *T. acridophagus* infection of liver, skin, and BAL fluid of a patient with diffuse alveolar hemorrhage after allogeneic stem cell transplant for multiple myeloma.[22] Fumagillin was used successfully to treat *E. bieneusi* intestinal disease in pediatric kidney and liver-kidney transplant recipients.[285,286] Diarrhea tends to be self-limited in immune-competent patients but is persistent in patients with immunosuppression. *E. intestinalis* was found in 7.8% of the stools of patients in a survey regarding the causes of diarrhea in Mexico[120] and has been described in travelers with chronic diarrhea. In a study of 98 patients with rheumatic disease treated with anti–TNF-α or other disease-modifying drugs, there was an increased incidence of intestinal microsporidiosis.[287]

Ocular infections with ulcer or deep cornea stroma infection associated with eye pain have also been reported in immune-competent patients. In 1973 and 1981 two cases of corneal microsporidiosis, caused by *Microsporidium africanus* in Botswana[288] and *Microsporidium ceylonensis* in Sri Lanka, were described (*Microsporidium* is used at the generic level for Microsporidia of unknown phylogenetic placement).[289] Additional cases of microsporidian keratitis have been identified in immune-competent hosts.[4,247] One of these organisms was classified as *N. ocularum,*[290] and the other, which was successfully propagated in vitro, was named *N. corneum*[31] (now *V. corneae*[15]). An outbreak of *V. corneae* has been associated with exposure during a rugby tournament in Singapore.[126] *A. algerae* infection of the cornea has also been reported,[268,291] and this infection has been associated with endophthalmitis.[291] Among these immunologically normal patients with corneal infections the outcomes included enucleation, unsuccessful penetrating keratoplasty, successful treatment with a corneal transplant,[246] and therapy with topical agents until keratoplasty.[292] Recently, it has been recognized that seasonal keratitis in both India and Singapore is due to microsporidiosis. More than 300 cases have been reported involving *V. corneae* presenting as keratoconjunctivitis.[165–169] One outbreak was associated with exposure to soil at a sporting event attended by children from several countries. These infections have been treated with various topical agents, including 1% voriconazole,[293] 0.02% polyhexamethylene biguanide,[294] albendazole,[295,296] ciprofloxacin,[295,296] fumagillin,[296] and repeated swabbing.[297] A randomized trial of 0.02% polyhexamethylene biguanide in 145 patients did not demonstrate efficacy for polyhexamethylene compared with no treatment.[294] The majority of these cases of seasonal keratitis resolved spontaneously within 5 days without specific treatment. A case of stromal keratitis due to *T. hominis* has also been reported.[298]

Cerebral infections caused by *E. cuniculi* are commonly described in many animals but have been reported only rarely in immune-competent humans. *Encephalitozoon* infection was demonstrated in a 3-year-old boy with seizures and hepatomegaly by positive IgG and IgM indirect immunofluorescence assays (using *E. cuniculi* as the antigen).[193] Recurrent fever infection with *Encephalitozoon* sp. was also reported in a 9-year-old Japanese boy with headache, vomiting, and spastic convulsions.[12] *E. cuniculi* together with *Streptococcus intermedius* was isolated from a brain abscess in a patient with diabetes.[299]

Pleistophora sp. was identified in the skeletal muscle of an HIV-negative patient and HIV-positive patients with myositis associated with normal creatine phosphokinase (CPK) levels.[33,34,252,253] *A. algerae* infection

of the skin has been seen in a patient with leukemia[268] and in another patient with myositis who had significant elevations in CPK and muscle pain; the latter patient had rheumatoid arthritis treated with steroids and antibody to TNF-α.[250] *A. algerae* has also caused myositis in a patient after a lung transplant,[276] and vocal cord lesions[277] in a patient with chronic lymphocytic leukemia. Five cases of *A. algerae* myositis (four from Australia) have been reported in patients with either rheumatoid arthritis or a lung transplant. The only surviving patient received albendazole.[300] Successful treatment with a combination of fumagillin and albendazole of a case of disseminated infection caused by *A. algerae* in a patient with chronic lymphocytic leukemia has been reported.[301] *E. cuniculi* caused a 4- × 7-cm right atrial vegetation attached to a ventricle pacing lead in an immune-competent male.[249] A recent study found *E. cuniculi* in the periprosthetic tissue of several patients in Poland who presented with hip implant loosening requiring hip replacement, despite an absence of symptoms associated with infection.[302]

Microsporidian Infection in AIDS Patients

Microsporidia were recognized as opportunistic pathogens causing diarrhea and wasting in AIDS patients in 1985.[13] Since then, although most reported cases still involved diarrhea, the spectrum of diseases caused by these organisms has expanded to include keratoconjunctivitis, disseminated disease, hepatitis, myositis, sinusitis, kidney and urogenital infection, ascites, and cholangitis, as well as no symptoms at all.[4,10,37] Postmortem examination revealed microsporidial encephalitis in a patient whose clinical and radiographic presentation had suggested progressive multifocal leukoencephalopathy.[303]

Enterocytozoonidae

GI infection is most common with *E. bieneusi,* and the presentation classically involves chronic diarrhea (which can last years[304]), anorexia, weight loss, and bloating without associated fever. It is most frequently seen in AIDS patients with $CD4^+$ counts lower than 50 cells/μL. Frequent (3–10) bowel movements occur daily, consisting of loose to watery stool that does not contain blood or fecal leukocytes.[10,40,305,306] There is no fever when infection is limited to the intestinal mucosa. Diarrhea is often associated with malabsorption and is worsened by food ingestion. Malabsorption can result in weight loss and a wasting syndrome. The mortality of patients with advanced HIV disease and chronic diarrhea with wasting has been reported to be in excess of 50%.[232] Other intestinal pathogens may occur simultaneously or sequentially with the presence of this or other microsporidia.[307]

Although originally thought to invade only enterocytes, it has been demonstrated that *E. bieneusi* can also invade cholangioepithelium.[232] When present in the cholangioepithelium, this organism has been associated with sclerosing cholangitis, AIDS cholangiopathy, and cholecystitis.[308] Presentations include abdominal pain, nausea, vomiting, and fever; jaundice is rarely seen. Fever is most likely the result of concomitant bacterial biliary infection, which produces the typical clinical manifestations of cholangitis. Imaging studies, including abdominal ultrasonography, CT, endoscopic ultrasonography, and endoscopic retrograde cholangiopancreatography, usually demonstrate dilated biliary ducts, irregularities of the bile duct wall, and gallbladder abnormalities, such as thickening, distention, or the presence of sludge. Papillary stenosis has also been seen. Bilirubin is normal, although most patients have elevated liver function tests (e.g., alkaline phosphatase, γ-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase). Of interest, an *E. bieneusi*–like organism has been identified as a causative factor for cholangitis and hepatitis in SIV-infected rhesus monkeys.[144]

Systemic dissemination is rare with *E. bieneusi.* One case report described this organism in nasal mucosa, which probably resulted from direct inoculation of spores from GI secretions.[309] There are two case reports of respiratory tract involvement with *E. bieneusi* associated with chronic diarrhea, persistent cough, dyspnea, wheezing, and chest radiographs showing interstitial infiltrates.[265,304] Spores of *E. bieneusi* were detected in these cases in stool, BAL fluid, and transbronchial biopsy specimens. *E. bieneusi* has been found to be a cause of proliferative serositis (peritonitis) in macaques *(Macaca mulatta).*

Encephalitozoonidae

Encephalitozoonidae are widely distributed among animals.[310] Three members of the family Encephalitozoonidae have been associated with disease in humans—*E. cuniculi, E. hellem,* and *E. intestinalis* (previously known as *Septata intestinalis*). It appears that these microsporidia have the capacity to disseminate widely in their hosts, and their involvement in most organs has now been documented.[4,26,37,40] The ability of these organisms to disseminate correlates with their ability to grow in many cell types in vivo and in vitro. These organisms have been associated with gastroenteritis, keratitis, sinusitis, bronchiolitis, nephritis, cystitis-ureteritis, urethritis, prostatitis, hepatitis, fulminant hepatic failure, peritonitis, cerebritis, and disseminated infection.[26–28,234,239,244,311] An *Encephalitozoon* sp. has also been reported in a case of nodular skin lesions.[269,270]

The major syndrome associated with microsporidiosis is diarrhea and wasting. This is usually caused by *E. bieneusi* (>90% of cases in the United States) and occasionally *E. intestinalis,* although in Europe this organism may be a more frequent cause of diarrhea.[312] *E. intestinalis* can also cause cholangitis,[18,313] keratoconjunctivitis, osteomyelitis of the mandible,[314] upper respiratory infections, renal failure, keratoconjunctivitis, and disseminated infection in AIDS patients.[190,231,315] Elimination of this parasite by treatment with albendazole correlates with the resolution of symptoms.[316]

E. cuniculi has been associated with hepatitis,[229] peritonitis,[239] hepatic failure,[27] disseminated disease with fever,[244] renal insufficiency, and intractable cough.[317] Granulomatous encephalitis caused by *E. cuniculi* was first described in rabbits in 1922, and cases of encephalitis and seizures caused by *E. cuniculi* have been reported in AIDS patients.[28,244] These infections have been reported to respond to albendazole.[26,317]

E. hellem has been reported to cause disseminated disease associated with renal failure, nephritis, pneumonia, bronchitis, and keratoconjunctivitis.[263,318,319] Punctate keratoconjunctivitis is the most commonly recognized clinical manifestation of infection with this organism. Most of the reports of ocular infection caused by Encephalitozoonidae in the literature have been attributed to *E. hellem,* including three cases originally classified as *E. cuniculi*.[25,320] The remaining cases were caused by *Encephalitozoon* sp. or *E. intestinalis*.[244] Patients present with bilateral coarse punctate epithelial keratopathy and conjunctival inflammation, resulting in redness, foreign-body sensation, photophobia, excessive tearing, blurred vision, and changes in visual acuity. Ocular microsporidian infection in HIV-1–infected patients has been restricted to the superficial epithelium of the cornea and conjunctiva (i.e., superficial keratoconjunctivitis), and it rarely progresses to corneal ulceration (see Fig. 270.4). Physical examination reveals conjunctival hyperemia and superficial punctate keratopathy, without deep corneal ulcers or retinal involvement. Slit-lamp examination usually demonstrates punctate epithelial opacities, granular epithelial cells with irregular fluorescein uptake, conjunctival injection, superficial corneal infiltrates, and a noninflamed anterior chamber. Infection may be bilateral or unilateral. It is often associated with disseminated disease.[262,321,322] Examination of the urine in patients with keratoconjunctivitis often reveals microsporidian spores.[132] This organism is also recognized as an important cause of infection of the nasal epithelium, which in turn causes sinusitis in AIDS patients.[264]

Other Microsporidia

Trachipleistophora hominis is a pansporoblastic microsporidian that has been described in several patients with disseminated disease in the setting of AIDS.[20] It has been reported to cause myositis, sinusitis, and keratoconjunctivitis. *T. anthropopthera* infection presents as encephalitis, myositis, and keratoconjunctivitis.[21,35,245] Several of these patients responded clinically to albendazole. *A. (Brachiola) vesicularum* caused myositis in an HIV-1–infected patient who responded to a regimen of albendazole and itraconazole.[16] Cases of myositis caused by *Pleistophora* sp. and *Pleistophora ronneafiei* have also been reported in patients with AIDS.[33,34,252,323] The myositis presentation of these microsporidia has included myalgias, weakness, elevated serum CPK and aldolase levels, and abnormal electromyograms consistent with inflammatory myopathy. A case of urinary tract infection, prostatitis, and *V. corneae* infection has been reported in an AIDS patient.[146]

DIAGNOSIS

Examination of stool specimens by light microscopy has become the standard method for diagnosing GI microsporidiosis (see Fig. 270.3). Because renal involvement with shedding of spores in the urine is common in all the species of the Microsporidia that disseminate, urine specimens should be obtained whenever the diagnosis of microsporidiosis is considered. This has therapeutic implications because the Microsporidia that disseminate (e.g., *Encephalitozoon* spp.) are usually sensitive to albendazole, whereas those that do not disseminate (e.g., *E. bieneusi*) are resistant. Definitive identification of the Microsporidia causing an infection can be done using ultrastructural examination (e.g., electron microscopy) or molecular techniques (e.g., species-specific PCR assay). If stool examination is negative in the setting of chronic diarrhea (>2 months' duration), endoscopy should be performed. A summary of the available diagnostic tests and their usefulness in patients with suspected microsporidiosis is presented in Table 270.2.

Demonstration of microsporidia by light microscopy is accomplished with staining methods that produce differential contrast between the spores of microsporidia and the cells and debris in clinical samples in which microsporidia are found. Adequate magnification using a 60× to 100× objective is required for visualization because the spores are 1 to 3 µm in size. Chromotrope 2R,[324] calcofluor white (fluorescent brightener 28),[325] and Uvitex 2B[326] are useful selective stains for microsporidia in stool specimens and other body fluids. The chromotrope 2R–based method of Weber and associates[324] is a modification of a standard trichrome stain using a 10-fold higher chromotrope 2R concentration and a longer staining time. The Weber method, modified by Ryan and coworkers[327] (which uses aniline blue in place of fast green) and by Kokoskin and colleagues[328] (which uses a higher temperature), are preferred by some laboratories. With a chromotrope 2R–based stain, the spores appear as 1- to 3-µm ovoid, light pink structures with a beltlike stripe girding them diagonally and equatorially against a green (Weber chromotrope stain) or blue (Ryan modification) background (see Fig. 270.3A). A rapid (11-minute) stain, the Gram-chromotrope stain, combines chromotrope 2R staining with a Gram-staining step and results in violet-staining spores.[329] Microsporidian spores can also be visualized by ultraviolet microscopy using chemifluorescent optical brightening agents, such as calcofluor white M2R (fluorescent brightener 28; Fungi-Fluor; Polysciences, Warrington, PA) or Uvitex 2B (Fungiqual A; Medical Diagnostics, Kandern, Germany), which stain chitin in the spore wall (endospore layer) (see Fig. 270.3B). Such stains also stain fungi and other fecal elements; however, microsporidian spores can be distinguished from yeast as they have a uniformly oval shape and are nonbudding.

In a study that examined 50 electron microscopy-proven microsporidia-positive stool specimens, both the chromotrope 2R and chemifluorescent brightening stains identified 100% of specimens if at least 50 100×-objective fields were examined.[330] With Uvitex 2B, all the 186 stool samples examined from 19 patients with biopsy-proven *E. bieneusi* infection were positive, whereas none of the 55 stool samples from 16 biopsy-negative patients were positive.[326] In another study evaluating Uvitex 2B staining, microsporidia were identified in all the samples known to be chromotrope 2R stain positive and in seven additional samples that were chromotrope negative on initial examination.[331] On reexamination, however, these 7 stool samples were found also to be positive with the chromotrope 2R stain. All patients with positive duodenal biopsies had positive stool examinations according to the chromotrope or chemifluorescent methods. The limit of detecting microsporidia by these techniques appears to be 50,000 organisms/mL. Overall, the sensitivity of the chemifluorescent brightener-based stains is slightly higher than chromotrope-based stains, especially when low numbers of spores are present in a sample; however, the specificity of the chemifluorescent stains is lower (90% vs. 100% in one study). Neither the chromotrope nor the chemifluorescent stain provides information on the species of microsporidia being identified. Although it has been reported that microsporidian spores in food can give a false-positive result, and despite the fact that microsporidia are common in the environment, it does not appear to be a common problem when using stool specimens for diagnosing these infections.

Microsporidia in body fluids other than stool (e.g., urine, cerebrospinal fluid [CSF], bile, duodenal aspirates, BAL fluid, sputum) have been visualized using chemifluorescent optical brightening agents or chromotrope 2R, Giemsa, Brown-Hopps Gram, acid-fast, or Warthin-Starry silver stain.[40,332,333] In general, it is easier to identify microsporidian spores in body fluids other than in stool because of the absence of bacteria and debris, which can be confused with microsporidian spores. Because microsporidian infections usually involve mucosa or epithelium, cytologic preparations are especially useful for diagnosis.[40] Specimens that have been useful for diagnosing microsporidian infections include intestinal and biliary epithelium, epithelium of the cornea and conjunctivae, epithelium of the sinonasal and tracheobronchial regions, renal tubular epithelium, and urothelium. Diagnosis has also been accomplished by examining touch preparations of biopsy material. Microscopic examination of corneal tissue in patients with microsporidian keratitis, obtained by gently rubbing the conjunctiva and cornea with a tissue swab, usually reveals multiple, gram-positive, oval organisms in epithelial cells.

Histologically, microsporidian spores are easily discernible with a modified tissue chromotrope 2R, tissue Gram stain (Brown-Hopps or

TABLE 270.2 Diagnostic Tests for Microsporidiosis

TEST	SPECIMENS	USEFULNESS
Chromotrope or chemifluorescent stain	Urine	This is often positive in cases of disseminated microsporidiosis (e.g., *Encephalitozoon* spp.). It should be done in all suspected microsporidia cases.
	Stool	At least three stools should be examined. The combination of chromotrope and chemifluorescence stains provides the highest sensitivity and specificity. Monoclonal antibodies for immunofluorescent antigen detection are also useful.
	Conjunctival scrapings	This is useful for the diagnosis of keratoconjunctivitis. Urine should also be examined in suspected cases to screen for disseminated microsporidiosis.
	Nasal scrapings	This can be useful for the diagnosis of microsporidian sinusitis. Because most microsporidia associated with sinusitis are present in the kidneys, examination of urine should be routine for suspected sinusitis cases. If these tests are negative, biopsy of the nasal mucosa may be useful for diagnosis.
Endoscopy	Touch preparations	Touch preparations are useful for rapid diagnosis (within 24 hours).
	Biliary fluid	Examination is useful for diagnosis of microsporidian cholangitis.
	Biopsy (small intestine)	Biopsy should be considered for all patients with chronic diarrhea longer than 2-mo duration and negative stool and urine examinations. In this group endoscopy has yielded a diagnosis of microsporidia in up to 30% of patients. Tissue can be examined with chromotrope 2R, tissue Gram, or silver stain. If microsporidia are demonstrated to invade the lamina propria, urine examination should be repeated because *Encephalitozoon* spp. are the most likely causative agents. In this setting albendazole has high treatment efficacy.
Polymerase chain reaction (PCR)	Urine, stool, or tissue	Available from reference laboratories (e.g., Centers for Disease Control and Prevention). PCR allows species identification.
Electron microscopy	Tissue	It provides species identification; it is crucial for identifying new species or for the characterization of microsporidia in unusual or new locations.
Serology	Serum	Not useful for diagnosis but may be useful for epidemiologic surveys.

Brown-Brenn), or Luna stain[334] in sections prepared from tissue fixed using routine procedures. Most microsporidian spores are gram positive in tissue sections. With experience, microsporidia can also be seen on hematoxylin and eosin–stained sections. Other stains that may be useful include periodic acid–Schiff, Giemsa, and Steiner silver stains. Some microsporidia are also acid-fast stain positive. Fresh tissue can also be examined by phase-contrast microscopy; because of their thick wall, unstained spores are retractile, appearing green, and such spores can be birefringent. If possible, biopsy or autopsy material should also be placed in electron microscopy fixative when microsporidiosis is suspected because the definitive diagnosis of species requires ultrastructural information. It is possible, however, to use formalin-fixed tissue for ultrastructural analysis. Molecular methods can also be used on formalin-fixed tissue, although unfixed tissue or tissue fixed in ethanol yields the best results with PCR techniques. The kidney is a frequent site of disseminated disease in transplant recipients, both renal and other organs. Pathologic examination of a renal biopsy in a lung transplant recipient revealed granulomatous interstitial nephritis on hematoxylin and eosin staining, associated with *E. cuniculi* infection. A "beltlike stripe" identified in tissue was accentuated in organisms seen in urine by staining with Gram-chromotrope and a modified trichrome stain. Electron microscopy and species-specific PCR were confirmatory.[335]

Polyclonal serum prepared to *E. cuniculi* has been reported to react with *E. bieneusi*.[195,336] Monoclonal antibodies to *E. hellem,*[337] *E. intestinalis,*[338] and *E. bieneusi*[339–342] have been described. These methods have been used to detect microsporidia in tissue sections using immunofluorescence techniques. Several of these antibodies have been demonstrated to be useful for the examination of stool specimens and have demonstrated good sensitivity and specificity. Detection kits for microsporidia in stool and environmental samples using antibodies to Encephalitozoonidae and *Enterocytozoon bieneusi* are now commercially available (Waterborne, New Orleans, LA).

V. corneae,[31] *E. cuniculi, E. hellem,*[91] *T. hominis,*[20] *T. anthropopthera,*[245] and *E. intestinalis*[30] have been cultivated in tissue culture systems in vitro (for a review, see Visvesvara[343]). *E. bieneusi* has not been cultivated continuously in vitro, although limited in vitro cultivation of *E. bieneusi* has been reported (Dr. S. Tzipori, personal communication, 2006).[344] Adenovirus can mimic the cytopathologic effect of microsporidia.[345] Experimental infection of SIV-infected rhesus monkeys with *E. bieneusi* from human tissue has been demonstrated,[346] and serial propagation has been described in immunocompromised rodents.[347] The isolation of microsporidia from clinical specimens is not a routine procedure and is available in only a few specialized research laboratories.

Serologic tests for diagnosing microsporidiosis have been developed and used for epidemiologic studies. Such serologic tests have, for the most part, not been proven useful for diagnosing microsporidiosis. In a study of 12 AIDS patients with *E. bieneusi,* 2 AIDS patients with *E. intestinalis,* and 2 immune-competent patients with *V. corneae,* enzyme-linked immunosorbent assay titers for *E. hellem, E. cuniculi,* or *V. corneae* were not useful for diagnosis.[348] False-negative titers were present in 7 of the patients with microsporidiosis, and half of the control patients (without clinical microsporidiosis) had positive serology to microsporidia. This is consistent with other AIDS-associated infections in which serology has not proven useful.

A number of molecular diagnostic tests have been developed for pathogenic microsporidia. Weiss and Vossbrinck[65] and Ghosh and Weiss[349] have reviewed the molecular diagnostic tests for microsporidiosis. Homology cloning of the rRNA genes of many of the Microsporidia pathogenic in humans has been accomplished using PCR techniques, and numerous microsporidian rRNA sequences are now in the GenBank database. It has been possible to design PCR primers for these small subunit rRNA genes to identify microsporidia at the species level in clinical samples without the need for ultrastructural examination. Two main approaches have been used for constructing PCR primers for the Microsporidia: the use of universal pan-Microsporidia primers and of species-specific primer pairs. These PCR techniques have been applied to biopsy specimens, urine, cultures, and, more recently, stool specimens, and should greatly facilitate diagnostic and epidemiologic studies.[79,247,349–352] Currently, these molecular tests are available in reference laboratories such as the Centers for Disease Control and Prevention.

THERAPY

Treatment for microsporidiosis is outlined in Table 270.3.

Gastrointestinal and Systemic Disease

Microsporidian infection often occurs in immunocompromised hosts, particularly in those with HIV infection and $CD4^+$ cell counts lower than 50/μL. Clinical studies have demonstrated that improved immune function can result in the clinical response of patients with GI microsporidiosis, with elimination of the organism and normalization of the intestinal architecture.[227,228,353–355] Relapse has been reported in patients who developed failure of their antiretroviral therapy associated with a decline in immune function and falling $CD4^+$ counts. In immunodeficient mice albendazole treatment has not been able to eliminate latent microsporidiosis, suggesting that persistence will occur despite drug treatment in the setting of immune suppression. Overall, these observations suggest that part of the primary treatment of microsporidiosis in the setting of AIDS is the institution of effective cART. There have been limited reports of immune reconstitution syndromes with cART and microsporidiosis.[356]

Although several species of the Microsporidia that infect humans can be grown in vitro (but not without a host cell monolayer), the most common human pathogenic microsporidian, *E. bieneusi,* has yet to be grown continuously in vitro. This has limited in vitro testing of antimicrosporidial agents to those active against *Encephalitozoon* spp. and *V. corneae*.[15,357,358] Several agents have also been evaluated in animal models of microsporidiosis, but until recently no practical animal model had been developed for in vivo studies of *E. bieneusi*.[347] See Costa and Weiss[359] for a review of drugs used against microsporidiosis in humans and animals.

Among the compounds tested in vitro and in vivo for treatment of microsporidiosis, fumagillin and albendazole have demonstrated the most consistent activity and have been demonstrated to have clinical efficacy in human infections with various microsporidia.[f] Albendazole binds to β-tubulin and is active against all the Encephalitozoonidae *(E. hellem, E. cuniculi, E. intestinalis)* in vitro at concentrations lower than 0.1 mg/mL; it is also active in animal models of microsporidiosis.[360] Data on *Encephalitozoon* β-tubulin genes have demonstrated an amino-acid sequence associated with sensitivity to benzimidazoles,[361] whereas the sequences of *Enterocytozoon*[362] and *Vittaforma*[363] demonstrate amino acids associated with albendazole resistance. This is consistent with the observed clinical responses to albendazole in these microsporidia.

Albendazole is 70% protein bound. It is distributed to blood, bile, and CSF and eliminated by the kidneys. Peak serum levels 2 hours after an oral dose are 0.20 to 0.94 μg/mL. Drug absorption is increased if albendazole is taken with food containing relatively high concentrations of fat. After oral administration the hepatic metabolism converts albendazole to albendazole sulfoxide, which is detectable in the systemic circulation. Albendazole is not carcinogenic or mutagenic, although in rats and rabbits at dosages of 30 mg/kg it is embryotoxic and teratogenic. Albendazole is therefore not recommended for use in pregnant women. Side effects are rare, although the following have been reported: hypersensitivity (rash, pruritus, fever), neutropenia (reversible), CNS effects (dizziness, headache), alopecia, GI disturbances (abdominal pain, diarrhea, nausea, vomiting), hair loss (reversible), and elevated hepatic enzyme levels (reversible). There is a report of pseudomembranous colitis after albendazole treatment.[364]

There are few placebo-controlled comparative treatment trials of microsporidiosis caused by *Encephalitozoon* spp., but there are numerous case reports demonstrating the efficacy of 2 to 4 weeks of albendazole 400 mg twice daily for these infections. In a double-blind, placebo-controlled trial of eight patients with AIDS and diarrhea caused by *E. intestinalis,* treatment with albendazole (400 mg twice daily for 3 weeks) resulted in resolution of the diarrhea and elimination of the organism in all eight patients, similar to that seen in several case reports.[g] In case reports of chronic sinusitis, respiratory infection, and disseminated infection caused by *E. hellem,* treatment with 400 mg of albendazole twice daily resulted in resolution of symptoms and clearance of the

[f]References 191,192,256,258,315,360–370.
[g]References 190,240,258,315,316,371,372.

TABLE 270.3 Current Treatment Options for Microsporidiosis

ORGANISM	DRUG	DOSAGE AND DURATION[a]
All microsporidian infections	cART with immune restoration (an increase of $CD4^+$ count to >100 cells/μL) is associated with resolution of symptoms of enteric microsporidiosis. All patients should be offered cART as part of the initial management of microsporidial infection. Severe dehydration, malnutrition, and wasting should be managed by fluid support and nutritional supplement. Antimotility agents can be used for diarrhea control if required.	
Enterocytozoon bieneusi	No effective commercial treatment. Fumagillin (oral) has been effective in a clinical trial. *Alternatives:* Albendazole[a] resulted in clinical improvement in up to 50% of patients in some studies, but was not effective in other studies. Nitazoxanide, 1000 mg bid with food for 60 days, has been used but is less effective in patients with low CD4 counts.	20 mg tid (e.g., 60 mg/day)
Encephalitozoonidae infection (e.g., systemic, sinusitis, encephalitis, hepatitis)		
E. cuniculi	Albendazole	400 mg bid[b]
E. hellem	Albendazole	400 mg bid
E. intestinalis	Albendazole	400 mg bid
Encephalitozoonidae keratoconjunctivitis	Fumagillin solution[c] (70 μg/mL). Patients may also need albendazole[a] if systemic infection is present.	2 drops every 2 hr for 4 days, then 2 drops 4 times a day[d]
Trachipleistophora hominis	Albendazole	400 mg bid
Anncaliia (formerly *Brachiola*) *vesicularum*	Albendazole	400 mg bid
	±Itraconozole	400 mg qd
Tubulinosema (*T. acridophagus* and *Tubulinosema* sp.)	There was no response to albendazole (400 mg/day) in the published cases. Oral fumagillin 20 mg tid would be a reasonable choice.	
Endoreticulatus sp.	Albendazole	400 mg bid

[a]Albendazole, 400 mg bid.
[b]The duration of treatment for microsporidiosis has not been established. Relapse of infection has occurred on stopping treatment. Patients should be maintained on treatment for at least 4 weeks, and most patients should continue on treatment until their CD4 count is higher than 200 cells/μL for at least 6 months after the initiation of cART.
[c]Fumidil B (fumagillin bicylohexylammonium; Mid-Continent Agrimarketing, Overland Park, KS) is used at 3 mg/mL in saline (final concentration of fumagillin, 70 μg/mL).
[d]Eye drops should be continued indefinitely; relapse is common on stopping treatment.
bid, Twice daily; *cART,* combination antiretroviral therapy; *tid,* three times daily; *qd,* daily.
Modified from Costa S, Weiss LM. Drug treatment of microsporidiosis. Drug Resist. *2000;3:1–16.*

organism.[365,366] Clinical improvement was demonstrated with albendazole treatment in a patient with disseminated *E. cuniculi* infection involving the CNS, conjunctiva, sinuses, kidneys, and lungs.[28] It has also been reported to be effective in cases of urethritis,[367] renal failure,[368] and disseminated infection.[317] In addition to its efficacy in *Encephalitozoon* spp., disseminated infections with other microsporidia have also been reported to respond to albendazole treatment. In patients with disseminated infection accompanied by myositis due to *T. hominis* and in a patient with myositis caused by *A. vesicularum,* albendazole (400 mg twice daily) resulted in clinical improvement.[16,20]

In contrast to its success in treating patients with the species of microsporidia that disseminate, albendazole has displayed only limited efficacy against *E. bieneusi* infection. In two studies examining 66 patients with diarrhea caused by *E. bieneusi* during the pre-ART era, symptoms were alleviated in about 50% of patients treated with albendazole, but the presence of *E. bieneusi* persisted during treatment in all patients, and there was no improvement in any patient's D-xylose absorption test.[369,370,373] The symptoms rapidly recurred on discontinuation of albendazole therapy in the patients who had reported symptom alleviation with it. Most other studies have found that albendazole has no efficacy against *E. bieneusi* infection.[374]

Despite a few case reports indicating that metronidazole was effective for *E. bieneusi* intestinal infection, most studies have demonstrated that this drug is not effective against this infection.[240,375,376] In vitro studies have also demonstrated that metronidazole has no activity against microsporidia (e.g., *E. cuniculi*).[357] Other medications used without success to treat GI microsporidiosis are azithromycin, paromomycin (microsporidia lack the rRNA binding site for this drug), and quinacrine. Atovaquone has been anecdotally reported to have limited efficacy in patients with microsporidiosis,[375,377] although it has no in vitro activity.[357] Transient clinical remission has been reported with furazolidone or nitazoxanide (1000 mg twice daily) treatment.[235,378] Anecdotal experience in other immune-suppressed hosts has demonstrated that nitazoxanide can be effective for *E. bieneusi* infection; however, it has had variable efficacy in patients with advanced AIDS and low CD4 counts. Sparfloxacin and chloroquine have demonstrated in vitro activity against microsporidia but have not been used clinically. Prophylaxis with trimethoprim-sulfamethoxazole is not effective for preventing microsporidiosis, and this drug has no in vitro or in vivo activity against these organisms.[379] Thalidomide and octreotide have both been reported to decrease diarrhea in about 50% of patients with microsporidiosis, which is probably secondary to the effect of these agents on the physiology of enterocytes.[380] Examination of the biopsies of patients treated with thalidomide (100 mg daily for 1 month) demonstrated persistence of the parasite and no change in parasite load.

Fumagillin was isolated from *Aspergillus fumigatus* in 1949 and, because of its efficacy against *Entamoeba histolytica* in vitro, it was used during the 1950s to treat amebiasis. Fumagillin is used commercially to treat honeybees infected with the microsporidian *Nosema apis* and has been used to treat infections by microsporidia and myxosporeans in various types of fish.[381,382] Fumagillin and its semisynthetic analogue TNP-470 have been found to have activity in vitro and in vivo against the Microsporidia pathogenic for humans, including *E. cuniculi, E. hellem, E. intestinalis, V. corneae,* and *E. bieneusi*.[h] A dose escalation trial of fumagillin performed on AIDS patients infected with *E. bieneusi* used dosages of 10 mg/day for 14 days, 20 mg/day for 14 days, 40 mg/day for 14 days, and 60 mg/day for 14 days. Altogether, 21 of 29 patients exhibited transient clearing of parasites from their stool; all these patients were in the first three dosage groups. In the 60-mg/day group, 8 of 11 patients did not have spores in their stools at week 6 and remained free of spores in stool specimens for a mean of 11 months. Duodenal biopsies on the same 8 patients did not demonstrate microsporidia by light or electron microscopy. A subsequent randomized trial evaluating 12 patients (with either AIDS or transplants) confirmed that 60 mg/day (given as 20 mg three times daily) effectively treated *E. bieneusi* intestinal infection.[191] Fumagillin has also been successful in treating renal transplant patients with *E. bieneusi* intestinal infection,[181] a

[h]References 191,192,360,375,384–386.

pediatric patient with liver transplantation and *E. bieneusi* infection,[286] and patients with allogenic hematopoietic stem cell transplants with *E. bieneusi* infection.[282] Treatment was associated with resolution of diarrhea, clearance of spores, improvement of Karnofsky scores, and improvement in D-xylose absorption tests. The main limiting toxicity of this treatment was thrombocytopenia, which was reversible on stopping fumagillin treatment. A case of aseptic meningitis due to fumagillin treatment has been described in a patient with a kidney transplant.[383]

Fumagillin, ovalicin, and their analogues (e.g., TNP-470) bind in a selective, covalent fashion to the metalloprotease methionine aminopeptidase type 2 (MetAP2). Methionine aminopeptidase activity is essential for eukaryotic cell survival because removal of the terminal methionine of a protein is often essential for its function and posttranslational modification. Homology PCR assay has been used to demonstrate the presence of *MetAP2* genes in several microsporidia.[387] The crystal structure of *E. cuniculi* MetAP2 has been determined (Molecular Modeling Database [MMDB] ID: 63862; Protein Data Bank [PDB] ID: 3CMK).[388] Data from the *E. cuniculi* genome project[68] indicates that *E. cuniculi* does not have a methionine aminopeptidase type 1 gene *(MetAP1),* unlike mammalian cells, which have both *MetAP1* and *MetAP2;* therefore MetAP2 is an essential enzyme in microsporidia.

Ocular Disease

Solutions of the soluble salt fumagillin bicyclohexylammonium (Fumidil B; Mid-Continent Agrimarketing, Overland Park, KS) applied topically have been demonstrated to be nontoxic to the cornea. Treatment of ocular microsporidiosis can be accomplished using a 3-mg/mL solution of Fumidil B in saline (fumagillin, 70 μg/mL)[384,389–392]; the treatment should be continued indefinitely because recurrence has been reported on stopping these drops. Although clearance of microsporidia from the eye can be demonstrated, the organism is still often present systemically and can be demonstrated in the urine or in nasal smears. In such cases the use of albendazole as a systemic agent is reasonable and effective. Topical treatment with thiabendazole (0.4% suspension), a related benzimidazole, was ineffective in one case of keratitis caused by *E. hellem.* Two patients with *Encephalitozoon*-like organisms were reported to respond to imidazole (fluconazole and itraconazole) administration.[393] Yee and associates[139] described complete improvement with oral itraconazole (200 mg twice daily) in a patient with *E. hellem* infection over a 6-week period after debulking the cornea. However, Diesenhouse and coworkers[384] observed no improvement in a patient with *E. hellem* treated with itraconazole, 100 mg three times daily. In vitro data have not confirmed antimicrosporidial activity for imidazole compounds. Sulfa drugs have had variable results in vitro and in vivo and are not recommended for treatment. Polymyxin B, propamidine isethionate 0.1% (Brolene), gramicidin, neomycin sulfate, and tetracycline appear to have limited efficacy for the treatment of microsporidian infection and should not be used except to treat secondary bacterial infections. Keratoplasty appears to provide temporary improvement in some cases, but no advantage to débridement was found in a series of 120 patients with superficial keratoconjuctivitis.[394] Steroids may be useful for decreasing the associated inflammatory response but have no direct action on microsporidia. *V. corneae* has been described to cause seasonal keratoconjunctivitis.[165–169] These infections have been treated with various topical agents, including 1% voriconazole,[293] 0.02% polyhexamethylene biguanide,[294] albendazole,[295,296] ciprofloxacin,[295,296] and fumagillin[296]; however, a randomized trial of 0.02% polyhexamethylene biguanide in 145 patients did not demonstrate efficacy for polyhexamethylene compared with no treatment.[294] A study from Thailand indicated that topical moxifloxicin was effective for microsporidian keratoconjunctivitis (due to *V. corneae*).[395]

Prevention

There are limited data on effective preventive strategies for microsporidiosis. Currently, no prophylactic agents have been identified for these organisms. Patients have developed microsporidiosis while on trimethoprim-sulfamethoxazole prophylaxis,[379] and microsporidiosis has occurred in patients receiving dapsone, pyrimethamine, itraconazole, azithromycin, and atovaquone.[226] No studies have evaluated albendazole for prophylaxis, but given its relative lack of efficacy for *E. bieneusi* infections, it is unlikely to be effective in preventing most cases of intestinal microsporidiosis. The most effective prophylaxis is the restoration of immune function in immunocompromised hosts. Several studies in AIDS patients have demonstrated that cART can produce remission of intestinal microsporidiosis.[227,228,353–355] Moreover, the declining incidence of microsporidiosis and other opportunistic infections during the cART era suggests that it also prevents symptomatic infection.

Microsporidian spores can survive and remain infective in the environment for prolonged periods. Experiments with *E. cuniculi* have demonstrated that they can survive for years in the environment with the correct humidity and temperature.[127] In the typical hospital environment, *E. cuniculi* spores can survive and remain infectious for at least 1 month. Spores can be rendered noninfectious by a 30-minute exposure to most common disinfectants, so the procedures used to clean most hospital rooms should be sufficient to limit infection. Spores are also killed by the methods commonly used for sterilization.

Although the epidemiology of the Microsporidia that infect humans has not been fully elucidated, it is likely they are foodborne or waterborne pathogens, and the usual sanitary measures that prevent contamination of food and water with animal urine and feces should decrease the chance for infection. Hand washing and general hygienic habits probably reduce the chance of contamination of the conjunctiva and cornea with microsporidian spores. It is not known whether person-to-person respiratory transmission occurs. Given the presence of microsporidian spores in respiratory secretions in cases of disseminated microsporidiosis, it may be useful to consider preventing contact of these patients with other immune-suppressed patients until the infection has been treated. Existing guidelines for the prevention of opportunistic infections that address food, water, and animal contact may be useful for preventing microsporidiosis. The presence of these organisms in genitourinary secretions raises the possibility of sexual transmission of these infections. It is reasonable to screen close contacts of patients with index cases of microsporidiosis for the presence of these organisms. Their importance and prevalence in our water supplies is an open question, but severely immunocompromised patients may wish to consider using bottled or filtered water in some settings.

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H Protozoal Diseases

271 Introduction to Protozoal Diseases

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The protozoans known to infect humans are a diverse group, as indicated by phylogeny (Table 271.1), epidemiology (Table 271.2), clinical manifestations (Table 271.3), preferred diagnostic studies (Table 271.4), and chemotherapeutic agents effective in eradicating or arresting infection (see Chapter 42).[1,2] The phylum Protozoa is composed of morphologically simple eukaryotic organisms. Protozoa may be divided, for convenience, into four distinct groups based on method of locomotion: Mastigophora (flagella), Sarcodina (pseudopodia), Apicomplexa (microtubule complex, commonly referred to as sporozoa), and Ciliophora (ciliates) (see Table 271.1). Protozoans such as *Plasmodium* spp., *Entamoeba histolytica,*

TABLE 271.1 Classification of Protozoans That Infect Humans

Phylum I. Sarcomastigophora (flagella, pseudopodia)
  Subphylum I. Mastigophora (flagella)
    Class 2. Zoomastigophorea
      Order 2. Kinetoplastida
        Suborder 2. Trypanosomatina
          Leishmania, Trypanosoma
      Order 5. Diplomonadida
        Suborder 2. Diplomonadina
          Giardia
      Order 7. Trichomonadida
        Dientamoeba, Trichomonas
  Subphylum III. Sarcodina (pseudopodia)
    Superclass 1. Rhizopoda
      Class 1. Lobosea
        Subclass 1. Gymnamoebia
          Order 1. Amoebida
            Suborder 1. Tubulina
              Entamoeba
            Suborder 5. Acanthopodina
              Acanthamoeba
          Order 2. Schizopyrenida
            Naegleria
Phylum III. Apicomplexa (apical microtubule complex)
  Class 2. Sporozoea
    Subclass 2. Coccidia
      Order 1. Piroplasmida
        Babesia
      Order 3. Eucoccidia
        Suborder 2. Eimeriina
          Cryptosporidium, Cystoisospora, Cyclospora, Sarcocystis, Toxoplasma
        Suborder 3. Haemosporina
          Plasmodium
        Suborder 3. Piroplasmia
Phylum VII. Ciliophora (ciliated)
  Class 1. Kinetofragminophorea
    Subclass 2. Vestibuliferia
      Order 1. Trichostomatida
        Suborder 1. Trichostomatina
          Neobalantidium (Balantidium)

Data from Committee on Systematics and Evolution of the Society of Protozoologists. A newly revised classification of the protozoa. J Protozool. *1980;27:37–58. For an updated phylogeny of Sarcodina, see Pawlowski J, Burki F.J. Untangling the phylogeny of amoeboid protists.* Eukaryot Microbiol. *2009;56:16–25.*

TABLE 271.2 Geographic Distribution and Mechanism of Transmission of Protozoal Infections

ORGANISM	GEOGRAPHIC DISTRIBUTION	MEANS OF TRANSMISSION
Acanthamoeba spp.	Undefined	Water
Babesia spp.	North America, Europe	Tick-borne, blood transfusions
Neobalantidium (Balantidium) coli	Worldwide	Zoonosis (pigs), water,[a] fecal-oral
Blastocystis species *(hominis)*	Unknown	Fecal-oral, water
Cryptosporidium spp.	Worldwide	Water, fecal-oral, zoonosis
Cystoisospora	Worldwide	Fecal-oral, suspected zoonosis
Entamoeba histolytica	Worldwide	Water, fecal-oral, foodborne
Giardia duodenalis (lamblia)	Worldwide	Water, fecal-oral, foodborne
Leishmania spp.[b]		Female sand fly, blood transfusion[16]
L. donovani	India, Pakistan, East Africa, China	
L. tropica	Middle East, Central Asia	
L. major	Middle East, India, Pakistan	
L. aethiopica	Ethiopia, Kenya	
L. mexicana	Central America, Texas	
L. amazonensis	South America	
L. chagasi	Latin America	
L. viannia braziliensis	Latin America	
Naegleria spp.	Worldwide	Fresh water, intranasal exposure
Plasmodium spp.	Africa, Asia, South and Central America, Oceania	Female anopheline mosquito, inoculation of infected blood
Sarcocystis spp.	Unknown	Foodborne (meat)
Toxoplasma gondii	Worldwide	Zoonosis (cats), foodborne (meat), blood or organ transplant, congenital
Trichomonas vaginalis	Worldwide	Venereal, during birth (?); nonvenereal, sexually transmitted
Trypanosoma spp.		
T. cruzi	South and Central America	Reduviid bugs
T. brucei gambiense	West Africa	Tsetse fly
T. brucei rhodesiense	East Africa	Tsetse fly

[a]Ingestion of water contaminated with fecal material.
[b]Other *Leishmania* spp. also infect humans but are less common.

TABLE 271.3 Clinical Syndromes Caused by Protozoan Infection

ORGANISM (DISEASE)	MAJOR CLINICAL SYNDROME
Acanthamoeba spp.	Keratitis, granulomatous amebic encephalitis
Babesia spp. (babesiosis)	Fever, malaise, hepatosplenomegaly, and hemolytic anemia, especially in the asplenic
Neobalantidium (Balantidium) coli (balantidiosis)	Colitis
Blastocystis species *(hominis)* (blastocystosis)	Diarrhea
Cryptosporidium spp. (cryptosporidiosis)	Self-limiting noninflammatory diarrhea, chronic severe diarrhea in children and immunocompromised adults, and cholangitis in AIDS patients[17]
Cystoisospora spp. (cystoisosporiasis)	Diarrhea
Dientamoeba fragilis	Diarrhea
Entamoeba histolytica (amebiasis)	Diarrhea, colitis, liver abscess
Giardia duodenalis (lamblia) (giardiasis)	Noninflammatory diarrhea with malabsorption
Leishmania spp. (cutaneous and visceral leishmaniasis)	Cutaneous or mucosal ulceration, visceral disease with fever, hepatosplenomegaly
Leptomyxida (Balamuthia mandrillaris)	Granulomatous amebic encephalitis
Naegleria spp.	Meningoencephalitis
Plasmodium spp. (malaria)	Paroxysmal fever, chills, headache, hepatosplenomegaly
Sarcocystis spp.	Myositis, fever
Toxoplasma gondii (toxoplasmosis)	Fever, malaise, lymphadenopathy; chorioretinitis; congenital abnormalities; in immunocompromised host, encephalitis, myocarditis, pneumonitis
Trichomonas vaginalis (trichomoniasis)	Vaginitis, urethritis
Trypanosoma spp. (African sleeping sickness and Chagas disease)	Fever, lymphadenopathy, meningoencephalitis, myocarditis; megaesophagus and megacolon; congestive cardiopathy

AIDS, Acquired immunodeficiency syndrome.

TABLE 271.4 Diagnostic Tests for Protozoal Diseases

DISEASE	PREFERRED DIAGNOSTIC TESTS
Amebiasis	
Intestinal	Stool antigen or PCR, serologic tests, colonoscopy
Liver	Ultrasonography or computed tomography, serologic tests, PCR on liver abscess aspiration
Amebic keratitis	Corneal scraping for microscopy and culture
Babesiosis	Giemsa or Wright staining of thin smear, PCR, serology[18,19]
Cryptosporidiosis	Stool antigen or PCR or acid-fast and auramine-rhodamine staining of fecal samples
Giardiasis	Stool antigen or PCR
Granulomatous amebic encephalitis	Brain biopsy
Leishmaniasis	
Cutaneous and mucocutaneous	PCR, antigen detection, biopsy, touch preparation, culture, serologic tests
Visceral	PCR, antigen detection, bone marrow or splenic aspiration, touch preparation, culture, serologic tests, lymph node biopsy
Malaria	Wright or Giemsa stain of thin and thick blood smear, antigen detection or PCR
Primary amebic meningitis	Cerebrospinal fluid examination, wet mount of CSF for amebic trophozoites, CSF PCR,[20] culture for amebas
Toxoplasmosis	Serologic tests, PCR, Wright-Giemsa stain of tissue, antigen detection
Trichomoniasis	Microscopy, culture, PCR, or antigen detection in genital secretions
Trypanosomiasis	
Chagas disease	Fresh blood or Giemsa-stained smear, PCR, xenodiagnosis; serologic tests for chronic disease
African sleeping sickness	Giemsa-stained blood smear, PCR, serologic tests, CSF examination[21]

CSF, Cerebrospinal fluid; *PCR,* polymerase chain reaction.

Trypanosoma spp., and *Leishmania* spp. are major worldwide pathogens and are among the leading causes of morbidity and mortality in areas of Africa, Asia, and Central and South America. *Giardia lamblia* and *Cryptosporidium* are frequent causes of diarrhea in developing areas and established industrialized countries. *Toxoplasma gondii, Cryptosporidium* spp., *Cyclospora, Cystoisospora belli, Trypanosoma cruzi*, and *Leishmania* spp. all have been noted to cause severe diseases in patients with acquired immunodeficiency syndrome.

Important advancements in the past several years include the first vaccine for a parasite to show some degree of effectiveness in humans, the RTS,S circumsporozoite-based vaccine for malaria; radical changes in parasite diagnostic techniques as microscopy is replaced with more accurate and sensitive techniques (e.g., antigen detection and the polymerase chain reaction assay); and improved understanding of pathogenesis as host, parasite, and environmental factors influencing disease are unraveled. Limited therapeutic options have led to a drive for novel drug discovery. Finding new uses for old drugs, that is, drug repurposing, has shown promising results for treating ulcerative leishmaniasis, second-stage trypanosomiasis, primary amebic meningoencephalitis, and enteric protozoa.[3–9]

Advances even in taxonomy of the protozoan parasites have been profound. A fifth species of malaria, *Plasmodium knowlesi,* is now known to infect humans, as well as a fourth member of the *Entamoeba histolytica-dispar* complex, *E. bangladeshi.*[10–12] Cryptosporidia that infect humans are now recognized to include not only *Cryptosporidium parvum* but also *Cryptosporidium hominis* and additional zoonotic species.[13] Within species, differentiation of genotypes is proving to be important for organisms such as *Giardia, Blastocystis,* and *Toxoplasma,* where it is becoming clear that genotypes differ in their pathogenicity for humans.[13–15]

The key to the recognition of protozoal infection is a knowledge of epidemiologic risk factors, such as the parasites' geographic distribution (see Table 271.2), and the most common modes of clinical presentation (see Table 271.3). The clinical diagnosis of protozoal infection presenting outside normal areas of high prevalence is usually dependent on physicians considering this possibility in their differential diagnosis. Given present levels of travel, changing immigration patterns, and the immunosuppressive effects of infection with human immunodeficiency virus, all clinicians need to have a heightened awareness of diseases caused by the protozoans. Diagnosis and therapy often require a specialized expertise with the use of tests (see Table 271.4) or drugs with which most physicians lack experience. Infectious disease consultants will frequently be called on to diagnose and manage protozoal infection; this requires the maintenance of an updated, in-depth database as provided by the chapters in this section.

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The complete reference list is available online at Expert Consult.

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272 *Entamoeba* Species, Including Amebic Colitis and Liver Abscess

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SHORT VIEW SUMMARY

Definition

- Amebiasis is defined as human infection by *Entamoeba* species, including *E. histolytica,* which is the cause of amebic colitis, liver abscess, and, rarely, brain abscess; *E. moshkovskii,* which causes diarrhea; and *E. bangladeshi,* and *E. dispar,* which are nonpathogenic.

Epidemiology

- Amebiasis is spread by fecal-oral transmission from person to person, and is also waterborne and foodborne spread.
- Most infections are in impoverished communities in the developing world.
- Amebic colonization, diarrhea, and colitis are most common in infants and children through the preschool years.
- Amebic liver and brain abscesses occur 90% of the time in young men.
- *E. histolytica* is a common cause of diarrhea in returning international travelers.
- Men who have sex with men (MSM) are at risk of both human immunodeficiency virus (HIV) infection and amebiasis, and one should consider the possibility of HIV in the setting of amebiasis in MSM.

Microbiology

- Cyst form is infectious, environmentally stable, and resistant to chlorination.
- Trophozoite is the tissue-invasive stage.

Diagnosis

- Stool ova and parasite examination should not be used because it is insensitive and nonspecific. Instead, diagnosis is best accomplished in the laboratory through fecal antigen detection or quantitative polymerase chain reaction, in combination with serologic tests for antiamebic antibodies (which can be negative early in illness).
- Colonoscopy and abdominal imaging techniques (ultrasound, computed tomography, and magnetic resonance imaging) are useful adjuncts for diagnosis of intestinal and extraintestinal disease, respectively.

Therapy (See Table 272.2)

- Noninvasive infection is treated with paromomycin: 30 mg/kg/day orally in three divided doses per day for 5 to 10 days.
- Invasive infection is treated with tinidazole (2 g once daily for 5 days) or metronidazole (750 mg three times daily by mouth for 10 days), followed by paromomycin (to prevent relapse from gut lumen parasites).
- Consider percutaneous drainage for liver abscesses of 5 to 10 cm or greater in diameter or if they are in the left lobe.

Prevention

- Prevention is accomplished by sanitation and clean water.
- Vaccine is in preclinical development.

Entamoeba histolytica is an invasive enteric protozoan parasite that is the cause of amebiasis.[1] *Entamoeba histolytica* is morphologically indistinguishable from three other species of human intestinal amebae, *Entamoeba dispar, Entamoeba moshkovskii,* and the more recently described *Entamoeba bangladeshi. E. dispar* and *E. bangladeshi* are nonpathogenic, while *E. moshkovskii* causes noninvasive diarrhea.[2–6] Because *E. histolytica, E. dispar, E. moshkovskii,* and *E. bangladeshi* cannot be distinguished by a stool ova and parasite (O&P) test, which has been the traditional diagnostic method, it is important clinically to use *E. histolytica*–specific diagnostic tests (stool antigen detection or polymerase chain reaction [PCR]) whenever possible, especially since *E. dispar* and *E. moshkovskii* are often as prevalent as *E. histolytica* in many regions of the world.[7–10]

Entamoeba spp. are taxonomically within the subphylum Sarcodina, class Lobosea, and family Entamoebidae.[11] Revisions of this taxonomy have been proposed.[11a] At least seven additional species of amebae (*Entamoeba coli, Entamoeba hartmanni, Entamoeba polecki, Entamoeba chattoni, Dientamoeba fragilis, Iodamoeba bütschlii,* and *Endolimax nana*) infect the human intestine.[11–19] However, these are generally accepted as commensal organisms, although *E. polecki, D. fragilis,* and *I. bütschlii* have occasionally been implicated as causes of diarrhea.[14–16]

Hippocrates (460–377 BC) wrote that "Dysentery, if it commence with black bile, is mortal," and the Old Testament and Huang Ti's *Classic of Internal Medicine* (140–87 BC) also made reference to dysentery. In 1828, James Annesley may have made the first association of dysentery to liver abscess when he wrote in *Prevalent Diseases of India* that "hepatic disease seems to be induced by the disorder of the bowels, more particularly when this disorder is of a subacute or chronic kind." Approximately 3 decades later, in 1855, Vilém Lambl described amebae in the stool of a child who had diarrhea.[20] In 1875, Fedor Lösch described ameba in the stool of a young farmer with chronic dysentery that resulted in death. He described the amebae as "round, pear shaped or irregular form and which are in a state of almost continuous motion." Autopsy studies revealed ulcerations of the colon, and Robert Koch's postulates were met when the patient's stool inoculated orally and rectally into a dog caused dysentery with amebic ulcers.[21] Stephen Kartulis is credited with first demonstrating amebae in liver and brain abscesses in the 1880s.[22,23]

The first North American case of amebiasis was reported in 1890 by Sir William Osler: "Dr. B., age 29, resident in Panama for nearly six years, where he had had several attacks of dysentery, or more correctly speaking a chronic dysentery, came north in May, 1889." Subsequently in 1890 the patient developed tenderness and hepatosplenomegaly, and amebae were observed in the stool and abscess fluid: "The general character of the amoeba [found in the stool] correspond in every particular with those found in the liver." A year later, Osler's colleagues William Councilman and Henri Lafleur proceeded through a classic investigation of 14 cases of amebic dysentery to distinguish amebiasis from bacterial dysentery, and they coined the terms *amebic dysentery* and *amebic liver* abscess.[23]

Ipecac bark was used in the treatment of dysentery for centuries in Peru. Piso introduced ipecac bark to Europe in 1658. Helvetius used ipecac to successfully treat the dysentery of King Louis XIV and subsequently sold it as a secret remedy to the French government. Not until 1858 was the use of large doses of ipecac for the treatment of dysentery promoted by the surgeon E. S. Docker in Mauritius. He demonstrated that ipecac (60 grains two to three times a day) decreased mortality from as much as 18% to only 2%. However, large doses of ipecac by mouth were complicated by severe nausea and vomiting and necessitated the coadministration of opium, chloral hydrate, or tannic acid. An alternative therapy was discovered by Leonard Rogers in India, who found that emetine, the principal alkaloid in ipecac, killed amebae

in the mucus of stools from patients with dysentery at dilutions as high as 1:100,000. In 1912 he reported successfully treating three patients in Calcutta, who had been unable to tolerate oral ipecac, by injection of emetine.[24]

The life cycle of *E. histolytica* was described by Dobell.[25] The cyst form of *E. histolytica* was implicated as the infective form of the parasite by Walker and Sellards[26] in the Philippines in 1913, and the parasite's life cycle was outlined by Dobell in 1925. Brumpt[27] proposed that *E. histolytica* and *E. dispar* were identical morphologically, but only *E. histolytica* was pathogenic for humans. Axenic culture of *E. histolytica* (free of any associated microorganisms) was accomplished by Diamond[28] at the National Institutes of Health in 1961. In 1978, Sargeaunt and colleagues[2] wrote that *E. histolytica* and *E. dispar* species could be differentiated using zymodeme analysis, and in 1989, Tannich and associates[3] demonstrated that their DNA genomes were distinct.

ORGANISM

Species of *Entamoeba*

Many *Entamoeba* species infect humans, but of greatest significance because of their prevalence are the four morphologically identical amebae *E. histolytica, E. dispar, E. bangladeshi,* and *E. moshkovskii.* Only *E. histolytica* is a cause of invasive amebiasis, whereas *E. moshkovskii* is associated with a noninvasive diarrhea and other two are nonpathogenic.[1,4–6] All of these species are in the quadrinucleated cyst clade of *Entamoeba.* The *E. histolytica* and *E. dispar* genomes share 90% identity in genic regions, and *E. moshkovskii* is also closely genetically related.[29] In most industrialized countries, *E. dispar* is 10 times more common than *E. histolytica,*[1,30–34] and *E. histolytica* and *E. dispar* can be equally prevalent even in a developing country.[18] The presence of ingested erythrocytes was the sole morphologic characteristic of some usefulness in identifying *E. histolytica,* but in one study, this characteristic was present in only 68% of cases of *E. histolytica* but also in 16% of cases of *E. dispar.*[18] *E. moshkovskii* is also prevalent and geographically widely distributed.[5,6,29,35–37] In preschool children from an urban slum, *E. moshkovskii* was present in 21%, *E. histolytica* in 16%, and *E. dispar* in 36%.[6,30] In another study in Tanzania, among approximately 100 human immunodeficiency virus (HIV)–infected individuals with diarrhea, *E. histolytica* was present in 4%, *E. moshkovskii* in 13%, and *E. dispar* in 5%.[36] In Sydney, Australia, 50% of cases of *Entamoeba* identified by stool O&P examination were *E. moshkovskii.*[37] *E. moshkovskii* has recently been shown to be a cause of diarrhea.[5]

E. hartmanni is also in the quadrinucleated cyst clade, but it is smaller than *E. histolytica,* having trophozoites of 3 to 12 µm in diameter and cysts of 10 µm in diameter, whereas *E. histolytica* trophozoites are 12 to 60 µm in diameter, and cysts are 10 to 20 µm in diameter. *E. coli* cysts have up to eight nuclei, with trophozoites of size similar to those of *E. histolytica. Entamoeba gingivalis* does not form a cyst and is not an inhabitant of the intestine; instead, it is observed in the mouth in gingival scrapings. Human infection with the uninucleated cyst amebae *E. polecki, E. chattoni,* and *E. suis* is generally rare. *E. polecki* and *E. suis* infections are linked to contact with pigs, and *E. chattoni* infection is linked to contact with monkeys.[19,38,39] Other nonpathogenic amebae include *I. bütschlii,* which has characteristic glycogen vacuoles in the cysts; *E. nana,* which has a characteristic nuclear structure that lacks peripheral chromatin; and *D. fragilis,* which is more closely related to the flagellates than to the ameba with binucleate trophozoites.[12,13]

Genotypes of *Entamoeba histolytica*

In addition to the genetic differences between the three morphologically identical amebae *E. histolytica, E. dispar,* and *E. moshkovskii,*[4,5,11] genetically distinct strains or genotypes also exist within *E. histolytica.* Genotypes have been distinguished by the use of isoenzymes, polymorphisms in protein-coding DNA, and polymorphisms in short tandem repeat loci linked to transfer RNA genes (reviewed by Ali and colleagues[30]). Important findings from genotyping include the following: *E. histolytica* contains many genotypes[40–46]; patients may be infected with more than one genotype at a time[46]; and certain genotypes are associated with diarrhea, others with colonization, and others with amebic liver abscess formation.[45,46] In contrast to this high level of diversity in repetitive noncoding DNA, individual protein-encoding genes such as the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc) lectin are conserved between genotypes.[44] An additional nuance to genotypes is that in a study of patients with amebic liver abscess, comparison of the amebae in the intestine and liver demonstrated in every case that each patient had different genotypes in the two locations. This surprising result suggested that there is a genetic bottleneck between the intestine and liver, and only a subset of intestinal isolates is capable of causing extraintestinal disease.[46]

Life Cycle

The life cycle of *E. histolytica* begins with an infectious quadrinucleated cyst and continues with an invasive uninucleated trophozoite. The cyst is ingested from fecally contaminated food or water or through oral-anal sexual practices and, in the intestine, excysts to eight trophozoites (Fig. 272.1). The environmental stability of the cyst and relative resistance to chlorine has resulted in waterborne outbreaks caused by contamination of municipal water supplies.[47] In most laboratory-based studies of the cyst, the reptilian parasite *Entamoeba invadens* has been used

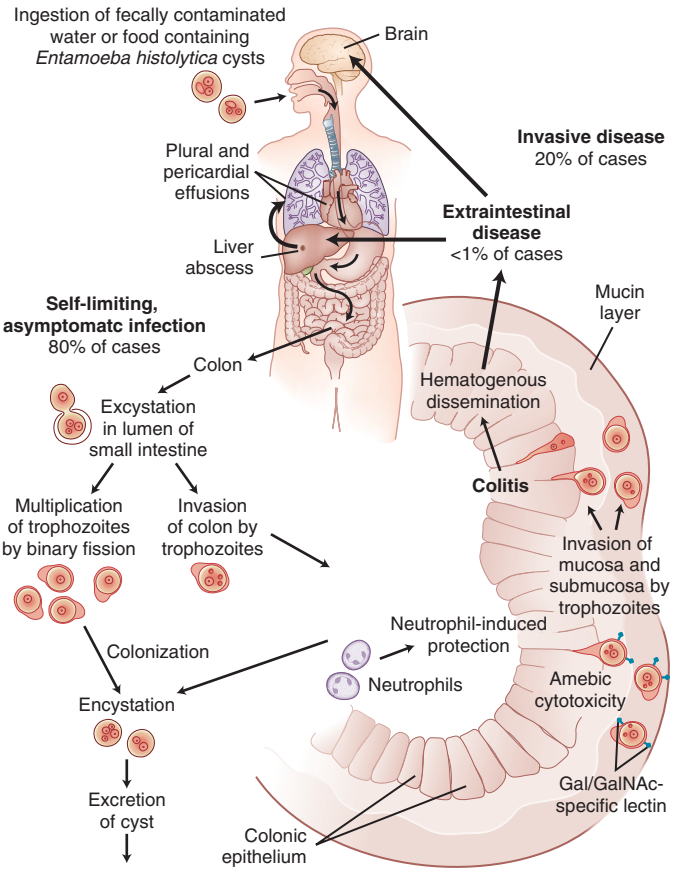

FIG. 272.1 Life cycle of *Entamoeba histolytica.* Infection is normally initiated by the ingestion of fecally contaminated water or food containing *E. histolytica* cysts. The infective cyst form of the parasite survives passage through the stomach and small intestine. Excystation occurs in the bowel lumen, where motile and potentially invasive trophozoites are formed. In most infections, the trophozoites aggregate in the intestinal mucin layer and form new cysts, which results in a self-limited and asymptomatic infection. In some cases, however, adherence to and lysis of the colonic epithelium, mediated by the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin, initiates invasion of the colon by trophozoites.[104] Neutrophils responding to the invasion contribute to cellular protection at the site of invasion. Once the intestinal epithelium is invaded, extraintestinal spread to the peritoneum, liver, and other sites may follow. Factors controlling invasion, as opposed to encystation, probably include parasite "quorum sensing" signaled by the Gal/GalNAc-specific lectin, interactions of amebae with the bacterial flora of the intestine, and innate and acquired immune responses of the host. *(From Haque R, Huston CD, Hughes M, Houpt E, et al. Current concepts: amebiasis.* N Engl J Med. *2003;348: 1565–1573.)*

because *E. histolytica* does not encyst in culture. Studies of the process of encystation in *E. invadens* have demonstrated the role of quorum sensing through a surface Gal/GalNAc lectin to initiate encystation[48] after an initial environmental signal such as osmotic shock, low glucose level, or interaction with colonic mucins. Later steps in formation of the cyst require signaling through β-adrenergic receptors and autophagy.[49,50] The cyst wall of *E. invadens* contains a chitin-binding lectin.[51] Transcriptional networks associated with encystation were identified in cultures of clinical isolates of *E. histolytica* that contain encysting organisms.[52,52a] A total of 672 cyst-specific transcripts were identified, including chitin synthetase, some of the transmembrane kinases, and cysteine proteinases, as well as genes involved in transcription, such as those for chromodomain proteins and an *myb*-like protein, EhMyb.[52]

Metabolism

No tricarboxylic acid cycle or oxidative phosphorylation exists for *E. histolytica*.[53] Many metabolic enzymes appear to have been acquired through lateral gene transfer from bacteria.[54] Functional mitochondria, or any other compartmentalized energy generation system, are lacking, although there is a remnant of the mitochondria called the *mitosome*.[55] Glycolysis is the major pathway for adenosine triphosphate generation and occurs in the cytosol.[56] Catabolism of amino acids is a second energy source.[57] Pyruvate ferredoxin oxidoreductase is essential in both glycolysis and amino acid catabolism and also serves to activate metronidazole through its reduction of ferredoxin, which suggests that metronidazole resistance would probably not develop.[53] Energy stores are predominantly in glycogen that occurs in cytoplasmic granules.[53]

Pathways for the biosynthesis of amino acids, with the exception of serine and cysteine, are lacking.[58,59] Cysteine is of special importance because it is the major thiol. Synthetic enzymes for cholesterol fatty acid and phospholipids also have been identified. In contrast, de novo purine synthesis is lacking.[60] More than 100 transporters have been identified in the genome, but their characterization is incomplete at this time.[53]

Cell Biology

Vesicular trafficking is of paramount importance to the parasite, inasmuch as endocytosis and phagocytosis serve as mechanisms of nutritional uptake. Exocytosis of the cysteine proteinases and ameba pores implicated in virulence and cyst wall components are also important functions of vesicular trafficking, in addition to the more typical roles in transport to and from the endoplasmic reticulum to the Golgi complex and cell surfaces. This complexity of function is reflected in the presence of 91 *Rab* genes (in comparison with 11 in *Saccharomyces cerevisiae*) involved in vesicle fusion.[61,62] Lysosomal acidification is key in cell amebic trogocytosis and cell killing.[63] N-linked glycosylation of proteins is unusual in that mannose-5 *N*-acetylglucosamine-2 is the most abundant N-linked glycan, whereas in other eukaryotes this would typically be processed by the addition of branching sugars.[64]

Transmembrane kinases are present and extraordinarily diverse (Fig. 272.2).[65–68] These kinases number more than 100 and are part of the family of Gal/GalNAc lectin-related proteins that share the extracellular CXXC and CXC motifs of the lectin intermediate (Igl) subunit. The kinase activity (Ser/Thr vs. Tyr) of the transmembrane kinases, as well as their substrates, ligands, and biologic functions, are all yet to be determined. The immediate downstream effectors of the transmembrane kinases are also yet to be identified, although more than 100 protein phosphatases are known to be present in the genome.[68] An unusual feature of these phosphatases is the presence of leucine-rich repeat domains implicated in protein-protein interactions. There are numerous seven-transmembrane G protein–coupled receptors and trimeric G proteins. A G protein–regulated adenylyl cyclase that functions downstream of an adrenergic ligand receptor has been biochemically identified. Cytosolic proteins involved in signal transduction include Ras, Rac, Rab, Rho, and Arf and their exchange factors: EF-hand motif calcium-binding proteins, phosphatidylinositol 3-kinase, and protein kinase C and mitogen-activated protein kinases.[68] It seems likely that this complex signaling system is required for the adaptation of the parasite to its host.

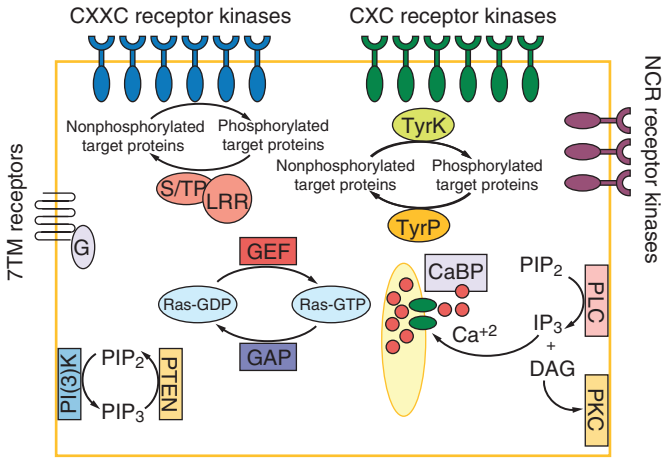

FIG. 272.2 Predicted signal transduction mechanisms of *Entamoeba histolytica*, based on analysis of the genome sequence data. *E. histolytica* possesses three types of receptor serine/threonine kinases: one group has CXXC repeats in the extracellular domain; a second has CXC repeats; and a third has non–cysteine-rich *(NCR)* repeats. *E. histolytica* has cytosolic tyrosine kinases *(TyrK),* but not receptor tyrosine kinases. Some serine/threonine phosphatases *(S/TP)* have an attached leucine-rich repeat *(LRR)* domain. *CaBP,* calcium-binding protein; *DAG,* diacylglycerol; *G,* G protein; *GAP,* guanosine triphosphatase (GTPase)–activating protein; *GEF,* guanine nucleotide exchange factor; *IP_3,* inositol-1,4,5-trisphosphate; *PI(3)K,* phosphatidylinositol 3-kinase; *PIP_2,* phosphatidylinositol-4,5-bisphosphate; *PIP_3,* phosphatidylinositol-3,4,5-trisphosphate; *PKC,* protein kinase C; *PLC,* phospholipase C; *PTEN,* phosphatase and tensin homologue; *Ras-GDP,* Ras protein–guanosine diphosphate; *Ras-GTP,* Ras protein–guanosine triphosphate; *TyrP,* tyrosine phosphatase; *7TM receptors,* seven-transmembrane receptors. *(From Loftus B, Anderson I, Davies R, et al. The genome of the protist parasite* Entamoeba histolytica. Nature. *2005;433:865–868.)*

Some of the unique aspects of the cytoskeleton include the lack of dependence on microtubules for motility, which is instead mediated by actin-myosin motors, and the lack of intermediate filament proteins such as keratins, desmin, and vimentin. Polymerization of actin into polymers of F-actin leads to microfilament assembly, which, through the myosin family of molecular motors, provides vesicular transport and motility by means of pseudopods.[68,69]

Genome Structure

The original genome sequence of *E. histolytica* was from the HM-1:IMSS strain originally isolated from the rectal biopsy sample of a Mexican man with amebic dysentery.[68] Because of a high content of repetitive DNA, the genome has not been completely assembled but instead exists in approximately 1800 fragments, with the average of 12 sequencing reads per fragment. The incomplete nature of the genome ensures that some genes will be missing and that some misassembly will occur. The *E. histolytica* genome is estimated to be 14 chromosomes, 8000 genes, and 24 million base pairs of DNA, a size that is approximately comparable with those of *Plasmodium* and *Trypanosoma* spp. The average gene length of 389 amino acids, however, is approximately half that of *Plasmodium*.[53,68] Approximately 50% of the genome is noncoding DNA, including 20% of the genome that is dedicated to ribosomal RNA genes that are encoded in extrachromosomal circles, and another 10% that encodes transfer RNA genes organized in repetitive linear arrays (probably at chromosome ends). Additional repetitive DNA in the genome includes long interspersed repeated sequence (LINE) and short interspersed repeated sequence (SINE) transposable elements.[68]

The DNA content of *Entamoeba* appears to vary under different growth conditions. The nuclear DNA content of *E. histolytica* was shown to be 10-fold higher in axenic (bacteria-free) than xenic culture. In addition, 40-fold increases in DNA content were observed as trophozoites emerged from *E. invadens* cysts. The most plausible explanation for these observations is that the ploidy of *E. histolytica* varies through a growth-dependent process of DNA replication without nuclear division.[70]

Control of messenger RNA (mRNA) expression in *E. histolytica* shares similarities with later branching eukaryotes: The parasite transcribes mRNA monocistronically by using RNA polymerase II under the control of upstream regulatory elements.[71] Thirty percent of genes are predicted to contain introns, and the pre-mRNA splicing machinery includes conserved U2, U4, and U5 small nuclear RNAs.[72] Most of the protein subunits of RNA polymerase II are also conserved, but not all general transcription factors are identified yet. Other differences from yeast and metazoans include the presence of a third core promoter regulatory element for RNA polymerase II,[73] altered histone code,[74,75] and unique aspects of mRNA silencing through small RNAs.[52,76]

PATHOGENESIS

The pathogenesis of amebiasis centers on the unique tissue-destructive properties for which the organism was named *histolytica*. Tissue invasion involves a contact-dependent process of adherence followed by cell killing that is called "trogocytosis-like" because the host cell is killed by its partial ingestion by the parasite (Fig. 272.3).[77–79]

Adherence

The initial contact of parasite to host is mediated by the parasite's Gal/GalNAc lectin, which binds to carbohydrate determinants on the host.[78–82] Adherence to human colonic mucin glycoproteins,[83] human neutrophils and erythrocytes,[84] certain bacteria,[85] and a variety of cell culture lines[86,87] is inhibited by up to 90% by Gal or GalNAc.[83] Blockade of lectin activity with Gal or GalNAc prevents contact-dependent cytolysis,[78] and glycosylation-deficient mutant cell lines lacking terminal Gal/GalNAc residues on N- and O-linked sugars are nearly totally resistant to amebic adherence and cytolytic activity.[87]

The colonic mucin layer of the large intestine is the first receptor encountered by the trophozoite lectin. Binding of the lectin to colonic mucins inhibits Gal/GalNAc and is of very high affinity (dissociation constant of 8.2×10^{-11} M^{-1}).[83] The mucin layer may protect the host from the parasite's contact-dependent cytolysis by binding to and neutralizing the lectin, while serving as a site of attachment for the parasite to colonize the large bowel. Interaction of trophozoites with colonic mucins appears to be a dynamic process, whereby trophozoites both induce the secretion of colonic mucins and degrade them.[88]

The Gal/GalNAc lectin is composed of a 260-kDa heterodimer of disulfide-linked heavy (170-kDa) and light (35- to 31-kDa) subunits that is noncovalently associated with an intermediate subunit of 150 kDa.[79–82] The 170-kDa subunit contains a carboxyl-terminal cytoplasmic and transmembrane domain adjacent to a cysteine-rich extracellular domain.[89,90] Five distinct genes (termed *hgl1* to *hgl5*) encoding the lectin's heavy subunit have been identified, sequenced, and shown to be expressed in trophozoites.[91] The sequence of the *hgl* genes is nearly completely conserved in isolates of *E. histolytica* from different continents, an important consideration for vaccine design.[44] The carbohydrate recognition domain is located within the cysteine-rich domain of the heavy subunit.[92] The lectin localizes to lipid rafts in the plasma membrane.[93] The lectin can be specifically released from the cell surface through the action of an amebic rhomboid protease, which probably explains earlier observations of both membrane-bound and soluble forms of the lectin.[79,80,94]

The Gal/GalNAc lectin appears to have other biologic functions in addition to adherence. Interference with lectin activity blocks chemotaxis in response to tumor necrosis factor-α (TNF-α).[95] The function of the light subunits may include lateral mobility of the Gal/GalNAc lectin, as evidenced by defects in capping of the lectin in amebae silenced for one of the genes that encodes the light subunits (*lgl* genes).[96] In an animal model, disruption of lectin function by expression of a dominant negative mutant blocked the ability of the parasite to cause liver abscess.[97,98] The lectin may serve as an organizing site for cytoplasmic proteins, with cytoplasmic proteins of the amebae that bind directly or indirectly to the lectin, including a thiol-specific antioxidant, spectrin, actin, myosin, talin, calreticulin, and cysteine proteinase 2.[99–101] The lectin has a role in evasion of serum lytic activity by inhibiting the formation of the complement membrane attack complex through blockade of C5b-9 assembly.[102] As mentioned previously, the lectin also appears to play an initiating role in cyst production.[48]

A recent study has shown that *E. histolytica* invades through the intestinal epithelium via a unique cell biologic process called trogocytosis.[78,103,104]

Cytolysis

Cytolysis occurs after adherence in a process that involves phagocytic ingestion of the host cells in bites, a process called "trogocytosis-like" (see Fig. 272.3). Apposition of amebic and target cell plasma membranes, as can be achieved by centrifugation of target cells and amebae into a pellet, does not lead to cytolysis if the amebic lectin is inhibited with Gal/GalNAc[77] or if the target cell lacks Gal and GalNAc on its surface.[85–87] This is consistent with the fact that lectin not only mediates adherence but also participates in the trogocytosis-like cytolytic event. Anti-lectin monoclonal antibody directed against epitope 1 of the lectin heavy subunit blocked cytotoxicity but not adherence, which implicates the lectin directly in the cytotoxic event. Killing occurs after the lectin engages GalNAc on O-linked target cell surface oligosaccharides: lectin-mediated capping of the O-linked structures (sialic acid-Gal–[sialic acid]–Gal-NAc) could be deduced to mediate killing.

Killing of host cells is not caused by an isolated toxin, inasmuch as parasite extracts have no cytotoxic activity. Cytolysis does require an intact parasite cytoskeleton, as demonstrated by inhibition of Rho,[105] by cytochalasin disruption of the cytoskeleton,[78] and by expression of dominant-negative myosin II.[106] The earliest observed event in a dying cell is a rise in intracellular calcium within seconds of direct contact by an amebic trophozoite; this event is associated with membrane blebbing.[107] Extracellular ethylenediaminetetraacetic acid and treatment of the target cells with the slow sodium-calcium channel blockers verapamil and bepridil[108] significantly reduce amebic killing of target cells in suspension. Isolation of amebic pore-forming proteins similar in function to pore-forming proteins of the immune system has been reported by a number of investigators. A purified 5-kDa amebapore and a synthetic peptide based on the sequence of its third amphipathic α-helix have cytolytic activity for nucleated cells at high concentrations (10–100 μM).[109,110] Silencing of amebapore A blocked the ability of amebae to release monolayer tissue culture cells from a plastic well, although cell death was not specifically measured.[111] The optimal pH of amebapore is 5.3, and amebapore is inactive at a pH of 7, which may be of some significance in view of the inhibition of cytotoxicity with weak base treatment of amebae.[112] Interestingly, no DNA degradation

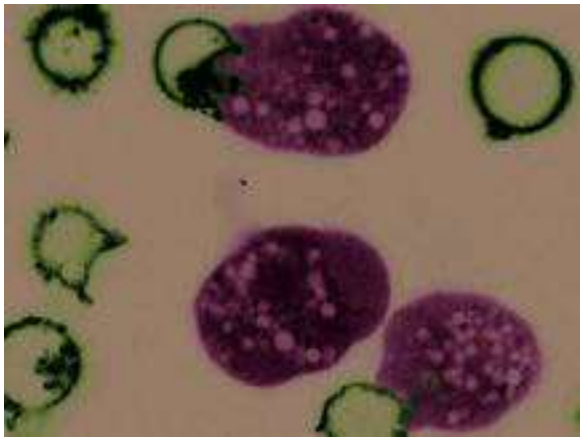

FIG. 272.3 Trogocytosis-like killing of host cells by *Entamoeba histolytica*. Trophozoites of *E. histolytica* (labeled *green* with CMFDA [5-chloromethylfluorescein diacetate]) are shown interacting with Jurkat T cells (labeled *purple* with DiD [1,1′-dioctadecyl-3,3,3′,3′-tetramethylindod icarbocyanine]). The trophozoites at the top and bottom right show partial ingestion of pieces of the Jurkat cells in a trogocytosis-like process by the amebae. Host cell death follows trogocytosis-like partial ingestion. *(From Ralston KS, Solga MD, Mackey-Lawrence NM, et al. Trogocytosis by* Entamoeba histolytica *contributes to cell killing and tissue invasion.* Nature. *2014;508:526–530.)*

was observed in cells lysed in vitro by the purified amebapore, which is suggestive of a different mechanism of cell killing by the purified amebapore than by the intact parasite.[113]

Cells killed by the parasite undergo nuclear chromatin condensation, membrane blebbing, and internucleosomal DNA fragmentation.[113] There is evidence of a nonclassical mechanism of apoptotic killing by *E. histolytica.* Overexpression of the Bcl-2 protein that inhibits apoptosis caused by a variety of cellular stresses (e.g., serum starvation, ultraviolet radiation) did not prevent murine cell DNA fragmentation after exposure to *E. histolytica.*[113] Furthermore, *E. histolytica* caused hepatocyte apoptosis in mice deficient in the Fas/Fas ligand and tumor necrosis factor receptor-1 signaling pathways.[114] Caspase 8–deficient cells, resistant to killing by Fas ligand, were readily killed by *E. histolytica.* Caspase 8–deficient cells treated with a caspase 9 inhibitor (Ac-LEHD-fmk) (at a level sufficient to inhibit apoptosis through etoposide) were readily killed as well. Together, these data suggest that *E. histolytica* initiates host cell apoptosis by directly activating the host cell's distal apoptotic machinery. Caspase 3 was activated within minutes of *E. histolytica* adherence, and the caspase 3 inhibitor Ac-DEVD-CHO at 100 µmol (sufficient to block killing through actinomycin D) blocked *E. histolytica* killing, as measured both by DNA fragmentation and by Cr51 release; this outcome indicates that both apoptotic death phenotype and necrosis were necessary.[115] In conclusion, amebic killing of the host is a result of parasite nibbling (trogocytosis) on the human cell, which results in apoptosis in the host cell at the level of caspase 3 activation.

Phagocytosis

In multicellular organisms, phagocytosis is the final step in the apoptotic pathway and serves to limit inflammation by preventing spillage of toxic intracellular contents of dead cells. Although amebic killing of cells by contrast involves phagocytosis followed by death, phagocytosis could similarly limit the host inflammatory response and enable *E. histolytica* to establish a persistent infection.[67,116,117] Inhibition of endocytosis by galactose and phosphatidylserine is additive, consistent with the Gal/GalNAc lectin and an as-yet unidentified phosphatidylserine receptor acting as coreceptors for ingestion.[118,119] Understanding the molecular mechanisms of engulfment of and subsequent death of the host promises to reveal much about pathogenesis, inasmuch as amebae that are defective in phagocytosis are also defective in virulence.[67,120]

Role of Bacteria

The effect of the gut bacteria on the biologic properties of *E. histolytica* may be profound. As mentioned previously, the genome content of *Entamoeba* is lower when the parasite is grown in the presence of bacteria.[53] In addition, amebae cultured with bacteria are better able to destroy monolayers of tissue culture cells and resist oxidative stress.[121–123] Recently it was shown that the gut microbiome is enriched in *Prevotella copri* in children with amebic diarrhea, and that antibiotic-induced alterations of the gut microbiome act via alterations in neutrophil trafficking to the gut in the mouse model of amebic colitis.[124,124a] Thus ultimate susceptibility to amebic colitis may in part be due to dysbiosis limiting neutrophil migration to the colon.

Cysteine Proteinases

E. histolytica encodes at least 44 genes that are cysteine proteinases, some of which are membrane bound and others predicted to be soluble.[53] These activities are implicated in a number of potentially important activities and include degradation of colonic mucin glycoproteins,[125] digestion of hemoglobin and villin,[126,127] inactivation of interleukin (IL)-18,[128] and digestion of extracellular matrix.[129]

Role of Leptin in Host Resistance

Amebiasis is more common in malnourished children (a physiologic state of leptin deficiency). This suggests that the nutritional hormone leptin could play a protective role in amebiasis. In fact, a mutation in the leptin receptor was discovered that was associated with amebiasis susceptibility in children. The mutation is a nonconservative substitution (Q223R) in the extracellular cytokine receptor homology domain 1. The mutation accounted for the majority of susceptibility to amebiasis in children because it conferred a 3.9-fold greater risk of intestinal amebiasis and was present in almost half the children. The purely nutritional role of leptin did not explain protection because the Q223R mutation was not associated with children's nutritional status. Experiments in mice validated the human study, with leptin-deficient (*ob*/*ob*), leptin receptor–deficient (*db*/*db*), and 223R leptin receptor knock-in mice highly susceptible to intestinal *E. histolytica* infection. The site of leptin action was localized to the gut, because an intestinal epithelial cell–specific deletion of the leptin receptor rendered mice susceptible, whereas lack of the leptin receptor in the central nervous system or bone marrow–derived cells did not. Leptin receptor signal transducer and activator of transcription 3 (STAT3) and Src homology 2 domain–containing phosphatase 2 (SHP2) signaling were required for protection in vivo because susceptibility was conferred by mutation of tyrosine 985 or 1138, which mediate leptin signaling through the SHP2/extracellular signal–regulated kinase and STAT3 pathways, respectively. The importance of leptin signaling in prevention of amebic killing of the host could even be seen in single-cell studies, where transfection of human embryonic kidney cells with the leptin receptor rendered them resistant to amebic killing.[130,131]

Macrophage Migration Inhibitory Factor and Inflammation

Colonic inflammation is a hallmark of *E. histolytica* infection, hence the term *amebic colitis.*[132] *E. histolytica,* like several pathogenic protozoans, secretes a protein homologue of the proinflammatory cytokine macrophage migration inhibitory factor (MIF).[133–138] A recent study found a positive correlation between *E. histolytica* MIF levels and intestinal inflammation in persons with amebic colitis. In the same study, the researchers demonstrated a causal relationship between *E. histolytica* MIF and gut inflammation using cellular and mouse models of amebic colitis.[139] *E. histolytica* MIF-induced mucosal inflammation resulted in increased matrix metalloproteinases (MMPs) production.[139] MMPs are enzymes that break down extracellular matrix proteins and promote cell migration. MMPs were shown recently to promote *E. histolytica* tissue invasion in a human colon explant model.[140] This potential link between *E. histolytica* MIF, inflammation, and tissue invasion might explain why a parasite would encode a proinflammatory cytokine.

IMMUNE RESPONSE AND IMMUNITY

Innate Immunity

Neutrophils and Eosinophils

Neutrophils are the earliest innate cellular immune response for both intestinal and hepatic amebiasis. They occur as a dominant infiltration of polymorphonuclear leukocytes surrounding trophozoites. Lymphocytes, macrophages, and epithelioid cells are recruited to infected tissue by day 3, in association with the formation of granulomas, which contribute to the confinement of invading trophozoites.[141–145] Neutrophils may be recruited by the chemotactic activity of an amebic membrane-bound peptide and chemokines secreted by epithelial cells exposed to *E. histolytica.*[146–149] As the consequence of interaction with trophozoites, neutrophils become activated and release reactive oxygen species and antimicrobial peptides. Many in vitro studies have reported neutrophil amebicidal activity after stimulation by interferon-γ (IFN-γ), TNF-α lipopolysaccharide, or amebic antigens.[84,150] In accordance with a protective role for neutrophils, depletion of neutrophils with anti–Gr-1 neutralizing antibodies resulted in exacerbated amebic hepatic and intestinal disease.[151–153] It is worth noting that the GR-1 antibodies also deplete other granulocytes such as eosinophils, which are also observed as part of the innate immune response and are protective in the murine model of amebic colitis.[154] Neutrophils can also be lysed by virulent amebae.[155,156] This can occur through disruption of the oxidase activities of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH)[157,158]; through protection of the amebae from oxidative damage by amebic peroxiredoxin, a 29-kDa surface protein conferring resistance to host reactive oxygen defenses[99,159,160]; or by inducing neutrophil apoptosis.[158] Neutrophil destruction in turn could result in tissue damage through the release of cytotoxic oxidase and lytic peptidases.[155]

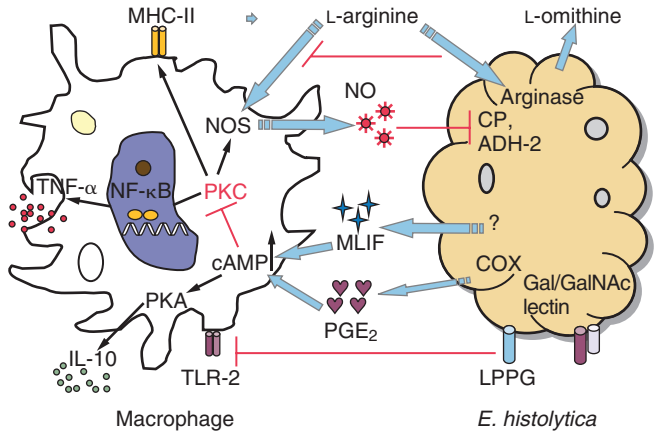

FIG. 272.4 Modulation of macrophage functions by *Entamoeba histolytica*. The killing of *E. histolytica* trophozoites by macrophages is mainly mediated by nitric oxide (NO), derived from L-arginine by nitric oxide synthase (NOS). NO could inhibit amebic cysteine proteinases (CP) and alcohol dehydrogenase 2 (ADH-2), the critical enzymes conferring virulence of *E. histolytica*. The arginase activity was detected in *E. histolytica,* which putatively converts L-arginine into L-ornithine, in turn limiting NO production by macrophage NOS. Prostaglandin E_2 (PGE_2) is an immunoregulatory molecule produced by cyclooxygenase (COX) in amebae or ameba-exposed macrophages. By activating the cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) pathway, PGE_2 suppresses macrophage effector functions by inhibiting protein kinase C (PKC)–mediated expression of major histocompatibility complex class II (MHC-II) and production of tumor necrosis factor-α (TNF-α), while favoring production of interleukin-10 (IL-10). Monocyte locomotion inhibitory factor (MLIF) produced by amebae may suppress macrophage functions in a manner similar to that of PGE_2. Amebic lipopeptidophosphoglycan (LPPG) might downregulate Toll-like receptor 2 (TLR2) expression on macrophages and thus control the effector mechanisms triggered through TLR2 signaling. *Gal/GalNAc, N*-acetyl-D-galactosamine; *NF-κB,* nuclear factor kappa B. *(From Guo X, Houpt E, Petri WA Jr. Crosstalk at the initial encounter: interplay between host defense and ameba survival strategies.* Curr Opin Immunol. *2007;19:376–384.)*

Macrophages

Macrophages acquire amebicidal activity after in vitro stimulation with IFN-γ, TNF-α, or colony-stimulating factor-1 (Fig. 272.4).[161–163] The Gal/GalNAc lectin of *E. histolytica* upregulates Toll-like receptor (TLR) 2 expression in macrophages, resulting in activation of nuclear factor kappa B and production of proinflammatory cytokines.[164] Macrophages lacking TLR2 and TLR4 showed impaired response to *E. histolytica* lipopeptidophosphoglycan, which suggests that pattern recognition is essential in the macrophage response.[164] Inducible nitric oxide synthase–deficient mice were more susceptible to amebic liver abscess and to *E. histolytica*–induced hepatocytic apoptosis,[165] which suggests that nitric oxide plays a critical role in host defense against amebiasis.

Despite the sensitivity of *E. histolytica* to nitric oxide–mediated cytotoxicity,[165,166] impaired macrophage function has been observed in human and experimental amebiasis, which suggests that amebae have developed strategies to modulate macrophage responses (see Fig. 272.4). Macrophage exposure to *E. histolytica* trophozoites or amebic components suppresses the respiratory burst and nitric oxide production.[167,168] A decrease in TNF-α secretion and IFN-γ–induced expression of major histocompatibility complex class II has also been observed.[169,170] Macrophage suppression may depend at least partially on prostaglandin E_2, an immunoregulator produced by *E. histolytica,* or on macrophages exposed to amebic proteins.[170,171] Prostaglandin E_2 elevates cyclic adenosine monophosphate levels in macrophages, triggering the protein kinase A pathway, which in turn inhibits the expression of major histocompatibility complex class Ia molecules, the release of helper T-cell type 1 cytokines, NADPH-mediated oxidative burst, and nitric oxide synthesis through the protein kinase C pathway (see Fig. 272.4). In addition, monocyte locomotion inhibitory factor, an immunosuppressor synthesized by amebae, also contributes to the modulation of host immune responses. Monocyte locomotion inhibitory factor is a soluble pentapeptide with antiinflammatory properties.[172,173]

Natural Killer Cells and Natural Killer T Cells

Natural killer cells and natural killer target cells have an innate role in host defense by production of IFN-γ and cytolytic peptides. Elevated cytotoxic activity of natural killer cells was found in mice infected with pathogenic amebae, and this may explain gender-dependent differences in the control of amebic liver abscess in C57BL/6 mice.[174,175]

Activated Mast Cells

Activated mast cells produce IL-6 and TNF-α, can recruit phagocytes, and can influence lymphocytic development and functions. Increased mast cell infiltration and upregulated mast cell protease expression have been observed in infected mouse ceca, but whether mast cells contribute to parasite clearance or play a pathologic role in tissue damage remains unanswered.[176]

Complement-Mediated Lysis of *Entamoeba histolytica*

After trophozoites penetrate the epithelial layer, the alternative complement pathway is initiated at least in part through cleavage of C3 and C5 by the amebic cysteine proteinase.[177,178] C3a and C5a act to chemoattract neutrophils to the site of infection. The Gal/GalNAc lectin heavy subunit of *E. histolytica* inhibits the assembly of C8 and C9 into the C5b-9 membrane attack complex, thereby preventing complement-mediated lysis of the parasite.[102]

Intestinal Epithelial Cells

Intestinal epithelial cells serve as the effectors of the mucosal immune system. Coculture of epithelial cell lines with *E. histolytica* trophozoites results in increased production of TNF-α, IL-1α, IL-6, IL-8, growth-regulated peptide-α, and granulocyte-macrophage colony-stimulating factor by the epithelial cells.[179–181] In the murine model of intestinal amebiasis, innate resistance is conferred by nonhematopoietic cells, as expected because leptin signaling is a critical mechanism to protect the epithelium.[130,131] Of note is that hematopoietic IL-10 is required for intestinal epithelial resistance to amebiasis.[182]

Acquired Immunity

Mucosal Immunoglobulin A Response

A mucosal immunoglobulin A (IgA) response directed at the carbohydrate recognition domain of the parasite Gal/GalNAc lectin is linked to protection from both infection and disease (Fig. 272.5).[33,183,184] IgA anti-Gal/GalNAc lectin in breast milk is also associated with passive immunity of infants to amebiasis.[185] These findings suggested that IgA antilectin antibody responses provide both passive and active immunity.

Cell-Mediated Response

Cell-mediated production of IFN-γ would be expected to provide protection from amebiasis through its ability to activate neutrophils and macrophages to kill the parasite. In a prospective study of a cohort of preschool children in Dhaka, Bangladesh, amebic antigen–stimulated IFN-γ production by peripheral blood mononuclear cells was associated with protection from future diarrhea caused by *E. histolytica* (Fig. 272.6).[186] An experimental amebic colitis model suggested that $CD4^+$ T cells that produced helper T-cell type 2 cytokines mediated pathogenesis[176]; therefore the pattern of $CD4^+$ T-cell response may be critical. Vaccine-mediated immunity to amebiasis was transferrable by CD4 or CD8 T cells producing IFN-γ and IL-17.[187] In summary, host protection from amebiasis includes innate defenses at the intestinal epithelium mediated by leptin and acquired immunity from anti-Gal/GalNAc lectin IgA and T-cell production of IFN-γ and IL-17.

EPIDEMIOLOGY

The best estimate is that *E. histolytica* infection results in 34 to 50 million symptomatic cases worldwide each year and as many as 100,000 deaths. The bulk of the morbidity and mortality from amebiasis occurs in Central and South America, Africa, and the Indian subcontinent.[188] Local prevalence rates in these regions can be astounding. A carefully conducted national serologic survey in Mexico demonstrated antibody to *E. histolytica* in 8.4% of the population.[189] In the urban slums of Fortaleza, Brazil, 25% of the population tested carried antibody to *E.*

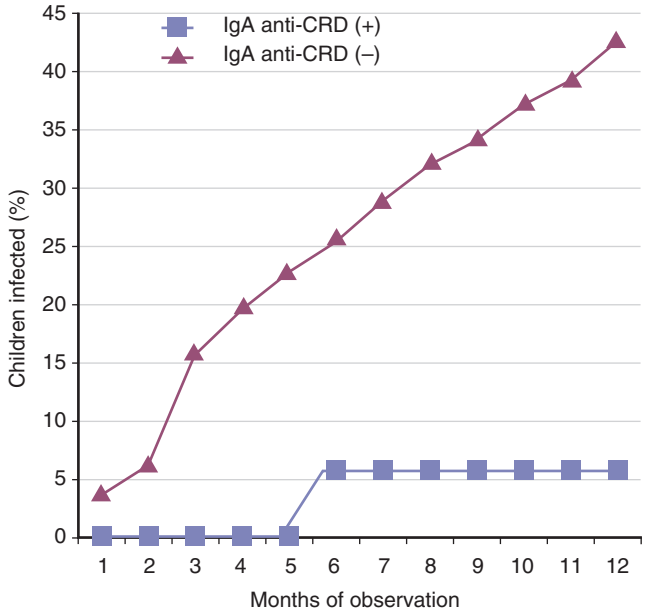

FIG. 272.5 Immunoglobulin A (IgA) anti–carbohydrate recognition domain (CRD) is associated with immunity to *Entamoeba histolytica* infection. Children with fecal IgA antibodies against the *N*-acetyl-D-galactosamine (Gal/GalNAc) lectin CRD (IgA anti-CRD$^+$; $n = 81$) had a lower incidence of new intestinal *E. histolytica* infection than did children who lacked this response (IgA anti-CRD$^-$; $n = 149$). The two groups were statistically significantly different ($P \leq .04$) at every time point. The average duration of protection was 437 days (95% confidence interval, 346–528 days). *(From Haque R, Mondal D, Duggal P, et al. Entamoeba histolytica infection in children and protection from subsequent amebiasis.* Infect Immun. *2006;74:904–909.)*

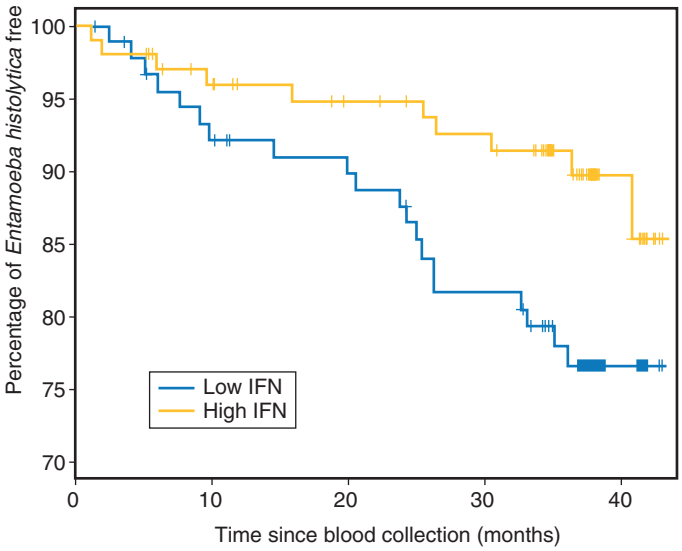

FIG. 272.6 High levels of interferon (IFN)-γ are predictive of increased survival free of *Entamoeba histolytica* diarrhea. Peripheral blood mononuclear cells were stimulated with soluble amebic extract, and children were grouped by IFN-γ production in response to stimulation with soluble amebic antigen. Children were then monitored for 44 months, and the incidence of *E. histolytica* diarrhea was measured. The upper line and lower line indicate percentages of children with and without IFN-γ response, respectively, above the median for all children (580 pg/mL). The two lines are significantly different: logrank test $P = .03$; $n = 92$ for low IFN-γ, and $n = 103$ for high IFN-γ. *(From Haque R, Mondal D, Shu J, et al. Correlation of interferon-gamma production by peripheral blood mononuclear cells with childhood malnutrition and susceptibility to amebiasis.* Am J Trop Med Hyg. *2007;76:340–344.)*

histolytica, and the prevalence in children 6 to 14 years of age was 40%.[190] In Dhaka, Bangladesh, where diarrheal diseases are the leading cause of childhood death, the annual incidence of infection in cohorts of infants and preschool children was greater than 40%.[33,185] The annual incidence of amebic liver abscess was reported to be 21 cases per 100,000 inhabitants in Hue City, Vietnam.[191] Country-specific prevalence data for amebiasis have been reviewed.[30]

Reported prevalences of *E. histolytica* can vary widely, depending on the method of diagnosis used, the patient population, and the area studied. Recent data from the large case-control Global Enteric Multicenter Study identified *E. histolytica* to be one of the top seven pathogens causing dysentery in young children (under the age of 5 years) living in developing countries throughout Africa and Asia.[192]

In the United States, amebiasis is the third most common parasitic infection, after giardiasis and cryptosporidiosis (1.2 cases per 100,000 US population). Travelers to and immigrants from endemic regions and institutionalized individuals are at increased risk of acquiring amebiasis.[193] In returning travelers, diarrhea is the predominant reason for a patient to seek medical care, and amebiasis is the second most common cause of diarrhea in returning travelers (Fig. 272.7).[193] Travel of intimate or household contacts to an endemic area may also be a risk for acquisition of infection.[194] Previously reported high rates of *E. histolytica* infection in homosexual men in the United States actually reflect a high prevalence of *E. dispar* infection in this population.[1] In contrast, in Asia, amebiasis is more frequently a presenting symptom of HIV infection and acquired immunodeficiency syndrome,[195–198] and recent studies from Taiwan and Japan have shown high rates of invasive *E. histolytica* infection in HIV-infected men who have sex with men.[199,200] Several groups are at increased risk of severe invasive amebiasis, including those with extremes of age, malnutrition, pregnancy, and receipt of corticosteroids. Fulminant amebic colitis, the most life threatening complication of amebic infection is associated with high mortality and morbidity; case fatality rates upwards of 40% have been reported.[201]

The National Institute of Allergy and Infectious Diseases also considers *E. histolytica* a Biodefense Research category B pathogen because of its low infectious dose (<100 organisms), chlorine resistance, and

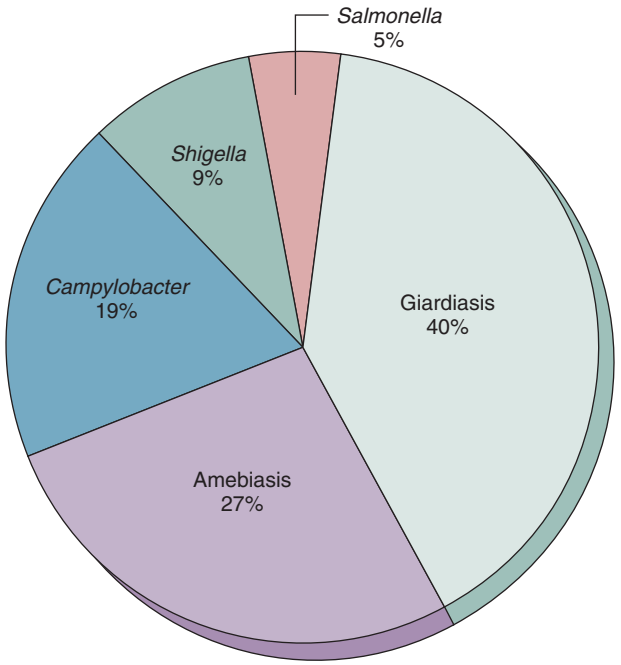

FIG. 272.7 Amebiasis as a cause of diarrhea in returning travelers. Amebiasis is the second most common cause of diarrhea in returning travelers, according to the GeoSentinel Surveillance Network of 30 travel or tropical medicine clinics on six continents. *(Data from Freedman DO, Weld LH, Kozarsky PE, et al. Spectrum of disease and relation to place of exposure among ill returned travelers.* N Engl J Med. *2006;354:119–130.)*

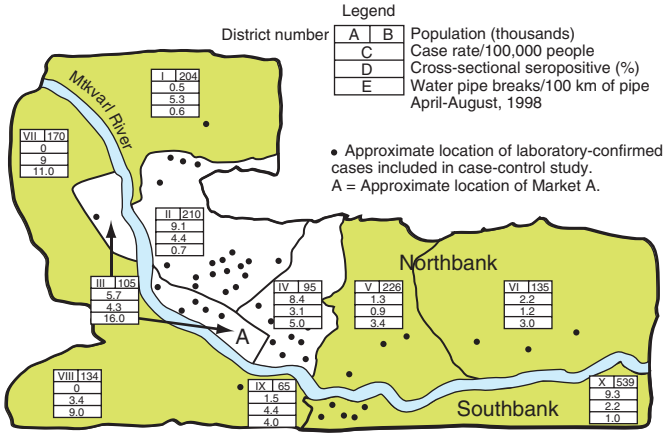

FIG. 272.8 Location of cases of amebic liver abscess in Tbilisi, Republic of Georgia. Cases were mapped by area of residence during the 1998 outbreak. Interruptions in water supply, decreases in water pressure, and increased water consumption were all significantly associated with infection. *(From Barwick R, Uzicanin A, Lareau S, et al. Outbreak of amebiasis in Tbilisi, Republic of Georgia, 1998.* Am J Trop Med Hyg. *2002;67: 623–631.)*

environmental stability. All these properties make it a threat to food and water supplies, as the municipal water outbreak of amebic liver abscess in Tbilisi, Republic of Georgia, demonstrated (Fig. 272.8).[47]

CLINICAL MANIFESTATIONS

Asymptomatic Intraluminal Amebiasis

The most common type of amebic infection is an asymptomatic cyst-passing carrier state. All *E. moshkovskii* and *E. dispar* infections and up to 80% of *E. histolytica* infections are asymptomatic. Asymptomatically infected individuals represent a risk to the community because they are a source of new infections. Asymptomatic infection with *E. histolytica* also carries a small but definite risk to the carrier for the subsequent development of invasive amebiasis. In a study in Bangladesh of children 2 to 5 years old who were colonized with *E. histolytica,* there was a small risk of developing invasive amebiasis with *E. histolytica* colonization: 2 of 17 colonized children developed dysentery during a 1-year follow-up period.[202] A subsequent study in Bangladesh revealed that 4% (25) of 651 asymptomatically infected children went on to develop amebic diarrhea or dysentery.[203] A study in South Africa revealed that of individuals colonized with *E. histolytica,* 10% developed invasive disease within 1 year.[31]

The median duration of asymptomatic colonization in children in Bangladesh was 2 months[33]; in adults in Vietnam, colonization was observed for more than 1 year.[32] In both studies, the same approach to DNA fingerprinting of *E. histolytica* was performed to distinguish continued infection with the same isolate from a second new infection; thus the differences in duration of colonization appear to be real, although currently unexplained.

The host has a bearing on whether infection is asymptomatic. In addition to the aforementioned importance of the leptin receptor mutation in susceptibility,[130,131] children heterozygous for the human leukocyte antigen class II DQB1*0601/DRB1*1501 haplotype were protected from symptomatic infection with amebiasis.[204] The genotype of *E. histolytica* also may determine whether infection is asymptomatic; certain genotypes appear to be associated with the propensity for colonization, as opposed to invasion.[46,47]

Amebic Diarrhea

Amebic diarrhea without dysentery is the most common disease manifestation of infection with *E. histolytica.* Amebic diarrhea is defined as diarrhea in an *E. histolytica*–infected individual; for the diagnosis of amebic diarrhea, mucus need not be visible and microscopic blood need not be present in the stool. In one community-based study of a cohort of preschool children in Bangladesh, the annual incidences of amebic infection, diarrhea, and dysentery were 45%, 9%, and 3%, respectively.[33] In a case-control study of diarrhea severe enough to prompt a patient to go to the hospital, approximately 2% of all such cases were caused by *E. histolytica.* This was true for all age groups. The mean duration of amebic diarrhea was 3 days.[33]

Amebic Dysentery or Colitis

Amebic dysentery, or amebic colitis, is defined as diarrhea with mucus or visible or microscopic blood in a patient with *E. histolytica* infection (Fig. 272.9). Approximately 15% to 33% of cases of *E. histolytica* diarrhea are accompanied by amebic dysentery.[33,205] Of patients with either condition, 70% have a gradual onset of symptoms over the 3 or 4 weeks after infection; increasingly severe diarrhea is the primary complaint, accompanied by general abdominal tenderness. On occasion, the onset may be acute or delayed for several months after infestation. This is different from bacterial causes of dysentery, in which patients usually have symptoms for only 1 to 2 days. The diarrhea is associated with abdominal pain and may be of such severity that an acute abdomen is suspected.[206,207] Surprisingly, fever is present in only the minority of patients with amebic colitis.[206] Abdominal distention or dehydration is unusual. In young children, intussusception, perforation, and peritonitis or necrotizing colitis may develop rapidly.[206–208] Amebic colitis may mimic inflammatory bowel disease.[201] Unusual manifestations of amebic colitis include toxic megacolon, perforation, or peritonitis (accounts for only 0.5% of cases, but is associated with a high mortality and usually necessitates surgical intervention) and ameboma (granulation tissue in colonic lumen whose appearance mimics that of colonic cancer). *Entamoeba histolytica* can also cause acute appendicitis, and was noted in up to 15% of HIV-infected patients presenting with acute appendicitis and undergoing appendectomy in a report from Japan. The only distinguishing feature was higher median white blood cell counts in those with amebic infection, compared to those without.[209] Corticosteroid use has been described as a risk factor for the development of fulminant forms of amebic colitis.[201]

Amebic Liver Abscess

Amebic liver abscess is 10 times more common in men than in women and is uncommon in children (Fig. 272.10); the reason for these differences in gender and age are not well understood. Approximately 80% of patients with amebic liver abscess have symptoms that develop relatively quickly (typically within 2–4 weeks), including fever, cough, and a constant, dull, aching abdominal pain in the right upper quadrant or epigastrium. Involvement of the diaphragmatic surface of the liver may lead to right-sided pleural pain or referred shoulder pain. Associated gastrointestinal symptoms, which occur in 10% to 35% of patients, include nausea, vomiting, abdominal cramping, abdominal distention, diarrhea, and constipation. Hepatomegaly with point tenderness over the liver, below the ribs, or in the intercostal spaces is a typical finding.[210,211] Hepatic amebiasis can develop even 20 or more years after the last visit to an endemic area.[212] The typical patient with an amebic liver abscess in the United States is an immigrant, usually a Hispanic man 20 to 40 years old, who has fever, right upper quadrant pain, leukocytosis, abnormal serum levels of transaminases and alkaline phosphatase, and a defect on hepatic imaging study. The abscess is usually single and is in the right lobe of the liver in 80% of cases.[208,210,211,213–215] Most frequently, patients present with liver abscess but without concurrent colitis, although a history of dysentery within the previous year is often present. Amebae are infrequently seen in the stool at the time of diagnosis of liver abscess. Liver abscess can manifest acutely (with fever and right upper abdominal tenderness and pain) or subacutely (with prominent weight loss and less frequent fever and abdominal pain). The peripheral white blood cell count is elevated, as is the alkaline phosphatase level, in many patients. Early evaluation of the hepatobiliary system with ultrasonography or computed tomography is essential for demonstrating the abscess in the liver.

The differential diagnosis of the lesion in the liver includes pyogenic abscess (less likely if the gallbladder and ducts appear normal), hepatoma, and echinococcal cyst. Aspiration of the abscess is occasionally necessary in order to diagnose amebiasis (although amebae are visualized in the pus in only the minority of cases; if the abscess is pyogenic, the responsible bacteria is seen or cultured, or both). If a space-filling defect in the



FIG. 272.9 Endoscopic and pathologic features of intestinal amebiasis. (A) Colonoscopic appearance of intestinal amebiasis. (B) Colonic ulcers averaging 1 to 2 mm in diameter on gross pathologic examination. (C) Cross section of a flask-shaped colonic ulcer (hematoxylin and eosin stain; magnification ×20). (D) Inflammatory response to intestinal invasion by *Entamoeba histolytica* (hematoxylin and eosin stain; magnification ×100). Arrows indicate *E. histolytica* trophozoites. (E and F) *E. histolytica* cysts in a saline preparation (magnification ×1000). (G) Iodine-stained cyst from stool (magnification ×1000). (H) *E. histolytica* trophozoite with an ingested erythrocyte, in a saline preparation from stool (magnification ×1000). (I) Trophozoite from stool stained with trichrome (magnification ×1000). *(B, C, and D courtesy the late Dr. Harrison Juniper. From Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis.* N Engl J Med. *2003;348:1565–1573.)*

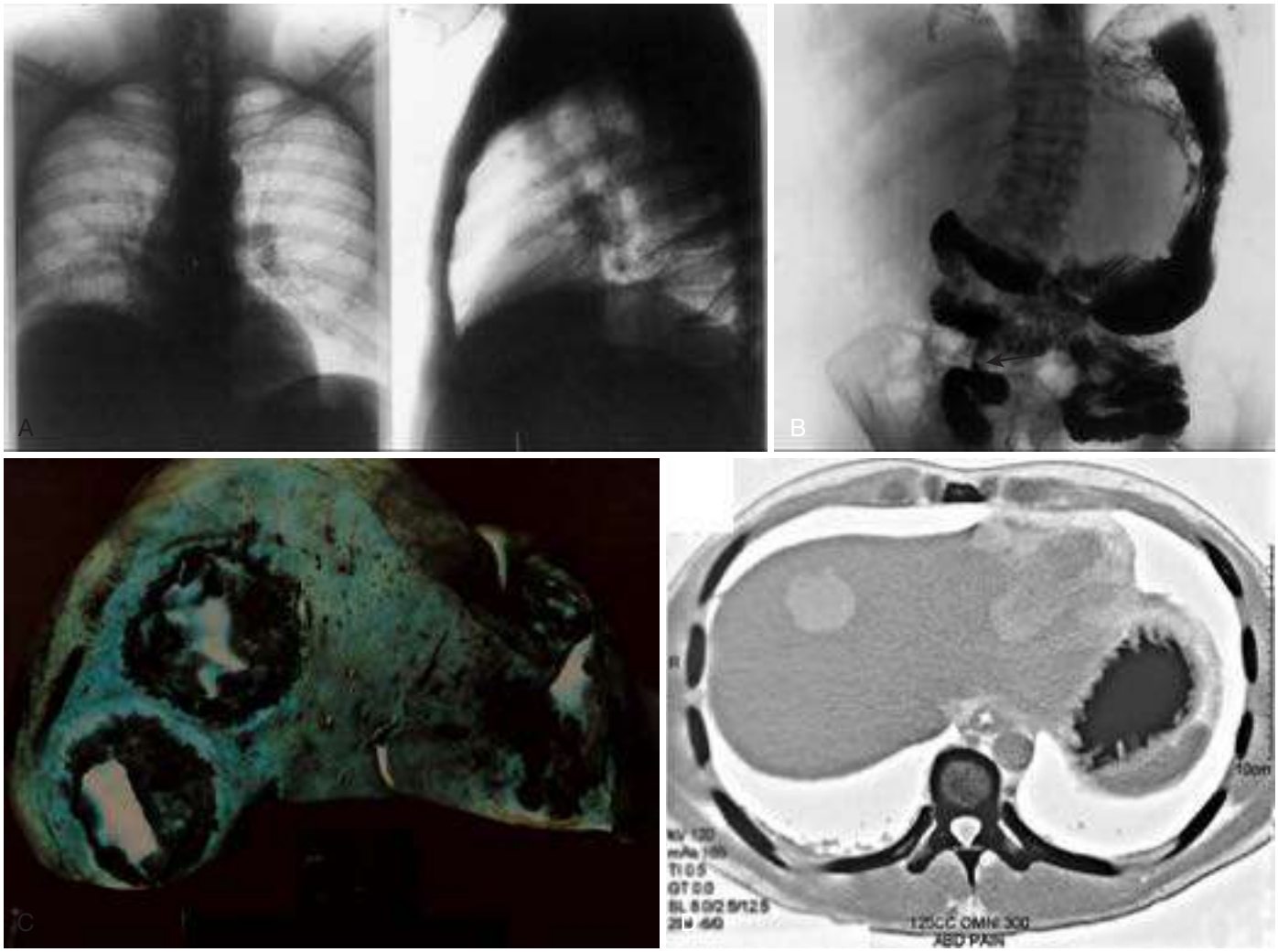

FIG. 272.10 Radiographic and pathologic features of extraintestinal amebiasis. (A) Left posteroanterior and right lateral chest radiographs in a patient with amebic liver abscess. The findings include elevated right hemidiaphragm and evidence of atelectasis. (B) Luminal narrowing *(arrow)* revealed by a barium-enema examination in a patient with ameboma. (C) Two abscesses in the right lobe and one abscess in the left lobe of a patient with amebic liver abscess. (D) One abscess in the right lobe and one abscess in the left lobe in a patient with amebic liver abscess, shown on abdominal computed tomography. *(From Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis.* N Engl J Med. *2003;348:1565–1573.)*

liver is observed, the differential diagnosis includes (1) amebiasis (most common in men with a history of travel or residence in a developing country; (2) pyogenic or bacterial abscess (suspected in women, patients with cholecystitis, elderly patients, individuals with diabetes, and patients with jaundice); (3) echinococcal abscess (an incidental finding, inasmuch as echinococcal abscess should not cause pain or fever); and (4) cancer. Most patients with amebic liver abscess have detectable circulating antigen in serum, as well as serum antiamebic antibodies.

In children, abdominal pain is reported infrequently with amebic liver abscess.[208,213,214] More commonly, high fever, abdominal distention, irritability, and tachypnea are noted. Some of these children are admitted to the hospital with a fever of unknown origin. Hepatomegaly occurs frequently, but elicitation of hepatic tenderness is not well documented. In one report, four of five children younger than 5 years of age died with amebic liver abscesses because the diagnosis was not suspected. Unusual extraintestinal manifestations of amebiasis include direct extension of the liver abscess to pleura or pericardium and brain abscess. Death usually results from rupture of the liver abscess into the peritoneum, thorax, or pericardium but may follow extensive hepatic damage and liver failure.[208,213–215]

Metastatic Amebiasis

Extraabdominal amebiasis presumably follows direct extension from liver abscesses rather than direct dissemination from the intestine.[1,211,216] Thoracic amebiasis is the most common type of extraabdominal amebiasis and occurs in about 10% of patients with amebic liver abscess.[208,211] Symptoms depend on the type of involvement. Empyema, bronchohepatic fistulas, or extension of a pleuropulmonary abscess into the pericardium may occur. Pericardial involvement is the next most common form of extraintestinal amebiasis and may result from rupture of a liver abscess in the left lobe of the liver into the pericardium or through extension of the right-sided pleural amebiasis.[208,216–218] It is estimated to occur in 3% of patients with hepatic abscesses. It manifests as acute pericarditis with tamponade and, on occasion, as pneumopericardium.[217,218] Amebic liver abscess in the left lobe also may rupture directly into the left side of the chest. Cerebral amebic abscesses were found in 0.66% to 4.7% of patients with amebic liver abscess.[219] In 18 patients with proven cerebral amebiasis, initial neurologic examination yielded normal findings in 13, and only 1 later developed seizures. Other foci of infection are rare, but amebic rectovesical fistula formation and involvement of pharynx, heart, aorta, and scapula have been reported. Cutaneous extension after the adherence of perforated, inflamed bowel to the skin is an extremely painful but rare complication.[220,221] This situation also may arise after invasion of the skin by trophozoites emerging from the rectum.

DIAGNOSIS

Diagnosis of amebiasis is best accomplished by the combination of serology and identification of the parasite in feces through antigen

TABLE 272.1 Sensitivity of Tests for Diagnosis of Amebiasis

TEST	COLITIS[a]	LIVER ABSCESS[a]	COMMENTS
Microscopy			
Microscopy: stool	25%–60%	10%–40%	Requires specialized expertise to perform, and even the most experienced observer cannot differentiate the cysts of *E. histolytica* in the stool from less pathogenic/nonpathogenic species (*E. dispar, E. moshkovskii,* and *E. bangladeshi*)
Microscopy: abscess fluid	N/A	~40%	
Antigen Detection			
Stool antigen detection	90%	~40%	Offers improved sensitivity and specificity over stool microscopy. Relatively easy to perform and rapid.
Serum antigen detection	65%	>95%	
Molecular Detection			
Real-time polymerase chain reaction (PCR)	>95%	>95%	Best performance of all diagnostic tests. Increasing availability through incorporation on multiplex PCR panels. Can be performed on stool and abscess fluid.
Serologic Testing (Indirect Hemagglutination)			
Acute	70%	70%–80%	Antibodies detectable within 5–7 days of infection but persist for years, and so does not readily distinguish between acute or past infection in endemic areas.
Convalescent	>90%	>90%	Negative result has a strong negative predictive value.

[a]Diagnostic test sensitivity reported is for before the initiation of therapy.
N/A, Not applicable.
Modified from Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis. N Engl J Med. *2003;348:1565–1573.*

detection or by molecular analysis, or at extraintestinal sites of invasion (such as liver abscess pus). The traditionally utilized microscopic examination of stool is of limited value and should not be utilized if other modalities are available for diagnosis. This section reviews the use of the O&P examination, culture, antigen detection, PCR, serologic study, colonoscopy and biopsy, and imaging for the diagnosis of amebiasis (Table 272.1).

Stool Ova and Parasite Examination

Inadequacies of the stool O&P examination have been appreciated since at least 1978, when Krogstad and colleagues[222] showed that its insensitivity and lack of specificity led to frequent misdiagnosis of amebiasis, with at times fatal results. Surprisingly, 30 years later the stool O&P examination remained the most common test ordered by US physicians when intestinal amebiasis was suspected.[223] In this survey of 2800 physicians from five US states, 97% of respondents believed that a routine O&P examination tested for *E. histolytica.*[223] The glaring problems with stool O&P examination were highlighted in a prospective study of 112 patients presenting at three Canadian centers with amebiasis symptoms (at least three loose bowel movements per day, abdominal pain, bloody stool, or weight loss) or risk factors (travel to or immigration from the tropics in the previous 2 years; men who have sex with men) or both. The specificity of stool O&P examination in community laboratories was an astoundingly low 10%.[34] The problems with stool O&P examination were magnified by the fact that only 5% (3) of the 65 positive results for *E. histolytica–E. dispar* complex were in fact *E. histolytica.*[34] This is because it is not possible with a stool O&P examination to distinguish morphologically the three closely related and common amebae: pathogenic *E. histolytica* and commensal *E. dispar* and *E. moshkovskii.*

In most industrialized countries, *E. dispar* is 10 times more common than *E. histolytica,*[1,30,34] and even in a developing country, *E. histolytica* and *E. dispar* can be equally prevalent.[18] The presence of ingested erythrocytes was the sole morphologic characteristic that was of some use in identifying *E. histolytica;* however, in one study, it was present in only 68% of cases of *E. histolytica* but also present in 16% of cases of *E. dispar.*[18] A third species of *Entamoeba* that is morphologically identical to *E. histolytica* is *E. moshkovskii.*[5,6,35–37] In a study of preschool children from an urban slum, *E. moshkovskii* was present in 21%, *E. histolytica* in 16%, and *E. dispar* in 36%[6]; in another study from Tanzania of approximately 100 HIV-infected individuals with diarrhea, *E. moshkovskii* was present in 13%, *E. histolytica* in 4%, and *E. dispar* in 5%.[36] In Sydney, Australia, 50% of *Entamoeba* organisms identified by stool O&P examination were *E. moshkovskii.*[37] Anecdotal experience confirms continued difficulties in misdiagnosis in the United States.[208,224] We conclude that the stool O&P examination suffers from insensitivity and the inability to distinguish *E. histolytica* from *E. dispar* and *E. moshkovskii.*

Culture

Culture of *E. histolytica* from stool samples is available in only a few research laboratories worldwide.[225] Culture is, in general, more sensitive than stool O&P examination but significantly less sensitive than antigen detection or PCR.[7] It is also not specific for *E. histolytica,* and thus an *E. histolytica*–specific antigen detection or PCR test must be used on the cultured material.

Antigen Testing for Amebiasis

The only fecal antigen test that distinguishes *E. histolytica* from *E. dispar* and *E. moshkovskii* is the TechLab (Blacksburg, VA) *E. HISTOLYTICA II* enzyme-linked immunosorbent assay (ELISA). This microwell ELISA, which detects the Gal/GalNAc adherence lectin of *E. histolytica,* is more sensitive than stool O&P examination or culture, and it is rapid (<2 hours).[7] A limitation is the need for fresh or frozen stool samples for the analysis by antigen detection. Other antigen detection tests are the RIDASCREEN *E. histolytica* immunoglobulin G (IgG) enzyme immunoassay for serum antibody (R-Biopharm, Darmstadt, Germany) and the Triage Micro Parasite Panel (Biosite Diagnostics, San Diego).[226] Neither of these tests can distinguish *E. histolytica* from *E. dispar,* and so they would at best serve as screening tools, with additional specific testing for *E. histolytica* required for all positive results. The TechLab *E. HISTOLYTICA II* ELISA was also found to be more sensitive than the RIDASCREEN enzyme immunoassay.[227] The Triage Micro Parasite Panel has the advantage of coupling the testing of *E. histolytica–E. dispar* with that of *Giardia lamblia* and *Cryptosporidium parvum,* covering the three most common parasites that cause diarrhea in the United States; its disadvantage is that it requires a tedious multistep process of fecal processing and filtration. The TechLab *E. HISTOLYTICA QUIK CHECK,* a rapid test for detection of *E. histolytica* in stool, has proved sensitive and specific in studies from Bangladesh,[7] Canada,[34] The Netherlands,[228] the United Kingdom,[229] and India.[230] In a single study, researchers observed a discrepancy between the results of PCR and antigen detection,[231] and it is not clear if this discrepancy was attributable to false-positive PCR results, which is a recognized issue with some PCR formats for *E. histolytica.*[232] Antigen detection is also useful in the diagnosis of amebic liver abscess. In one study, the TechLab *E. HISTOLYTICA II* assay detected Gal/GalNAc lectin in the sera of 22 of 23 patients (96%) with amebic liver abscess before they underwent treatment with the antiamebic drug metronidazole.[7] For liver abscess pus, it was 41% to 74% sensitive[7,233] for detection of the parasite. Furthermore, for stool specimens collected at the time of diagnosis of amebic liver abscess (and before metronidazole treatment), it detected the parasite in 43% (3 of 7).[7] We conclude that the TechLab *E. HISTOLYTICA II* ELISA antigen detection test has sensitivity and specificity superior to those of stool O&P examination, and its sensitivity is inferior but its specificity

is comparable to those of PCR, but it is technically simpler to perform. Recently, clinical evaluation of the *E. HISTOLYTICA QUIK CHEK* stool antigen detection test showed that the sensitivity and specificity are 98% and 100%, respectively, compared with the *E. HISTOLYTICA II* ELISA.[234] False-negative results should be rare. In one case of amebic dysentery reported from Spain, the enzyme immunoassay was initially negative, but became positive with subsequent testing. Interference of antigen detection by pH or amebic lytic activity was postulated by the authors. Repeat testing of the stool should be considered if high clinical suspicion remains, and serology is also a useful adjunct in such cases.[235]

Polymerase Chain Reaction Testing for Amebiasis

Real-time PCR is superior in sensitivity to stool antigen detection, and is considered the gold standard for diagnosis.[233] PCR tests are becoming more widely available through US Food and Drug Administration–cleared gastrointestinal panels such as the xTAG Gastrointestinal Pathogen Panel (Luminex, Austin, TX) and the BioFire FilmArrray GI Panel (BioFire Diagnostics, Salt Lake City, UT), though *E. histolytica* is not a target of the VERIGENE Enteric Pathogens Test (Luminex).[236,237] There are several real-time PCR assay formats for *E. histolytica,*[8,9,233,238] many of which are reviewed by Qvarnstrom and colleagues[232] and Fotedar and associates.[239] These tests are more sensitive than conventional PCR. Real-time PCR is also a sensitive test for detection of *E. histolytica* in liver abscess pus.[240] In one study, real-time PCR yielded positive results in 20 of 23 liver abscess pus specimens; the 3 specimens with negative findings had been collected from patients who had already received antiamebic therapy (8 days for one patient and 30 days for two patients).[233] Detection of *E. histolytica* DNA in saliva and urine could also be used as a diagnostic tool for amebic liver abscess.[241]

Serologic Tests for Amebiasis

Serologic tests are a cornerstone of the diagnosis of amebic liver abscess and are an important adjunct in the diagnosis of intestinal amebiasis. However, it is important to realize that false-negative results may be obtained early in the course of intestinal amebiasis and amebic liver abscess.[7,206,210,242] The serologic tests are therefore best used in conjunction with antigen detection or PCR for *E. histolytica.* In general, different assays for amebic antibodies behave similarly, but commercially the enzyme immunoassay is more widely available than the indirect hemagglutination assay.[243,244] In one study, the RIDASCREEN enzyme immunoassay was reported to have sensitivity of 97%.[245] The Microtiter ELISA (LMD Laboratories, Carlsbad, CA), the ImmunoTab assay (Institut Virion, Wurzburg, Germany), and an indirect hemagglutinin assay (Behring Diagnostics, Marburg, Germany) were compared in one study of amebic liver abscess patients from Kuwait.[244] All three tests had equal sensitivities of 98% to 99%. The ImmunoTab and Microtiter ELISA specificities were 95%, less than the 99.8% calculated specificity of the indirect hemagglutinin assay. The lower specificity was attributed to the high (5%) background titer of *E. histolytica* infection in individuals from endemic countries. The specificity of antibody tests for amebiasis is limited by the 5% to 10% baseline seropositivity of populations from endemic countries, because patients will remain seropositive for years after infection.

Serologic tests for *E. histolytica* intestinal infection are generally less sensitive than those for amebic liver abscess, and seropositivity is rarely detected in asymptomatic carriers.[7,31,246] In a study in Egypt, IgG antibodies to the Gal/GalNAc lectin were found in the sera of 89% of patients with amebic colitis,[247] and in a small study from the Netherlands, 86% (six) of seven patients with intestinal amebiasis were seropositive. Serologic study can be particularly helpful when *E. histolytica*–specific stool diagnostic techniques (antigen detection or PCR) are not available because infection with *E. histolytica,* and not *E. dispar* or *E. moshkovskii,* results in seroconversion.[1,242] Recently, a sensitive bead assay has been used to detect IgG responses against *E. histolytica* lectin adhesion antigen.[248] In conclusion, serologic study is an important part of the diagnosis of intestinal and extraintestinal amebiasis. A limitation of current tests is the lack of a point-of-care test for single use at the patient's bedside. False negatives have occasionally been reported.[249]

Colonoscopy and Biopsy

Colonoscopy and biopsy can be helpful in the diagnosis of intestinal amebiasis.[250–252] Amebae can be difficult to visualize in the biopsy samples, and periodic acid–Schiff stains or, ideally, immunoperoxidase with anti–*E. histolytica* antibodies, can help to identify the parasites (see Fig. 272.9).[253] A limitation of colonoscopy is that it is an invasive procedure and not widely available in developing nations.

Imaging

Ultrasound, computed tomography, and magnetic resonance imaging can all be useful imaging modalities in the diagnosis of amebic liver abscess (see Fig. 272.10). By ultrasound, a solitary cystic intrahepatic hypoechoic lesion is often seen, most typically involving the right lobe. With computed tomography scanning, an abscess with a nonenhancing center surrounded by a rim of inflammation can be seen following contrast administration.[254]

THERAPY

It is recommended that all infections with *E. histolytica* be treated; however, therapy for invasive infection differs from therapy for noninvasive infection (Table 272.2).

TABLE 272.2 Drug Therapy for Treatment of Amebiasis

DRUG	ADULT DOSAGE	SIDE EFFECTS
Amebic Liver Abscess[a]		
Metronidazole[b]	750 mg PO tid × 10 d	Primarily GI side effects: anorexia, nausea, vomiting, diarrhea, abdominal discomfort, or unpleasant metallic taste; disulfiram-like intolerance reaction to alcoholic beverages; neurotoxicity, including seizures, peripheral neuropathy, dizziness, confusion, irritability
or		
Tinidazole	2 g PO once daily × 5 d	Primarily GI side effects and disulfiram-like intolerance reaction to alcoholic beverages for 5 days
Followed by a luminal agent		
Paromomycin	30 mg/kg/d PO in three divided doses per day × 5–10 d	Primarily GI side effects: diarrhea, GI upset
or		
Diloxanide furoate	500 mg PO tid × 10 d	Primarily GI side effects: flatulence, nausea, vomiting Pruritus, urticaria
Amebic Colitis[c]		
Metronidazole	750 mg PO tid × 10 d	Same as for amebic liver abscess
Tinidazole	2 g PO once daily × 5 d	Same as for amebic liver abscess
Plus a luminal agent (same as for amebic liver abscess)		
Asymptomatic Intestinal Colonization		
Treatment with luminal agent as for amebic liver abscess		

[a]Amebic liver abscess may necessitate antiparasitic treatment plus percutaneous or surgical drainage. Nitazoxanide may be effective therapy as well, but clinical experience is limited.
[b]Drug of choice for treatment of amebic liver abscess.
[c]Amebic colitis may necessitate antiparasitic treatment plus surgical treatment if complicated by toxic megacolon, perforation, peritonitis, or other intraabdominal complication.
GI, Gastrointestinal; *PO,* by mouth; *tid,* three times daily.
Modified from Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis. N Engl J Med. *2003;348:1565–1573.*

Noninvasive Infections

Noninvasive infections, characterized by asymptomatic detection of infection with *E. histolytica,* may be treated with a luminal agent, among which the nonabsorbable aminoglycoside paromomycin is recommended. Diiodohydroxyquin and diloxanide furoate can also eliminate intestinal cysts, but safety concerns limit the use of iodoquinol, which has been associated with optic atrophy and peripheral neuropathy, in particular with long courses or high doses.

Invasive Infections

The nitroimidazoles, particularly tinidazole and metronidazole,[255] are the mainstay of therapy for invasive amebiasis, including amebic colitis and amebic liver abscess (see Table 272.2). Nitroimidazoles with longer half-lives (namely, tinidazole, secnidazole, and ornidazole) are better tolerated and allow shorter periods of treatment. Metronidazole is as effective as tinidazole at clearing parasites.[256,257] Tinidazole and metronidazole are both available in the United States. Approximately 90% of patients with mild-to-moderate amebic dysentery have a response to nitroimidazole therapy. Nitazoxanide is a relatively newer alternative therapy. In a small, single-center trial performed in Egypt, over 90% of patients treated for hepatic or intestinal amebiasis with nitazoxanide demonstrated both clinical and microbiologic response.[258] Of concern was the high response rate in the placebo group, with 40% to 50% of those receiving placebo showing clinical and microbiologic resolution, so until more is known, a nitroimidazole should be considered the preferred treatment over nitazoxanide, when available.

In the rare case of fulminant amebic colitis, it is prudent to add broad-spectrum antibiotics to treat intestinal bacteria that may spill into the peritoneum. Metronidazole is often administered intravenously in these patients, if they are unable to tolerate enteral administration, though there is limited experience with intravenous metrondiazole.[259] Surgical intervention is indicated for acute abdomen, gastrointestinal bleeding, or toxic megacolon.

Parasites persist in the intestine in up to 40% to 60% of patients who receive nitroimidazole. Therefore, tinidazole or metronidazole treatment should be followed with the nonabsorbable aminoglycoside paromomycin or the second-line agent diloxanide furoate to cure luminal infection. A nitroimidazole and paromomycin should not be given at the same time because diarrhea, a common side effect of paromomycin, may make it difficult to assess the patient's response to therapy.[260–262]

Therapeutic aspiration of an amebic liver abscess is occasionally required as an adjunct to antiparasitic therapy. Drainage of the abscess should be considered in patients who have no clinical response to drug therapy within 5 to 7 days or those with a high risk of abscess rupture, as defined by a cavity with a diameter of greater than 5 to 10 cm or by the presence of lesions in the left lobe.[254] Bacterial coinfection of amebic liver abscess has occasionally been observed (both before and as a complication of drainage), and it is reasonable to add antibiotics, drainage, or both to the treatment regimen in the absence of a prompt response to nitroimidazole therapy. Imaging-guided percutaneous treatment (needle aspiration or catheter drainage) has replaced surgical intervention as the procedure of choice for reducing the size of an abscess.[254]

PREVENTION

Prevention of fecal contamination of food and water has dramatically reduced transmission of amebiasis in industrialized nations. Transmission can be further reduced by the use of safe sexual practices in men who have sex with men and by proper maintenance of municipal water supplies to prevent access of chlorine-resistant amebic cysts to the treated water supply. In developing nations, however, more than 1 billion people still have no access to safe food and water.[263]

Travelers to endemic areas should be given advice on the avoidance of risk factors for acquisition of infection. Infection with *E. histolytica* should be considered prior to the diagnosis of inflammatory bowel disease in anyone with a relevant travel history, given the overlapping clinical presentation. Similarly, infection with *E. histolytica* should be excluded with appropriate diagnostic screening prior to the administration of corticosteroids in anyone with a relevant travel history to prevent the development of fulminant colitis in the setting of unrecognized amebiasis.[201]

An effective vaccine is a desirable and feasible goal. The high incidence of amebiasis in community-based studies suggests that an effective vaccine would improve child health in developing countries. Because humans naturally acquire partial immunity against intestinal infection, an effective acquired immune response should be achieved. Aiding vaccine design is the demonstration that several recombinant antigens, including the Gal/GalNAc-specific lectin, provide protection in animal models of amebiasis and that human immunity is linked to intestinal and breast milk IgA against the lectin.[185,264–269] The clonal-population structure of *E. histolytica* and, specifically, the high degree of sequence conservation of the Gal/GalNAc-specific lectin suggest that a vaccine could be broadly protective. Finally, the absence of epidemiologically significant animal reservoirs suggests that herd immunity could interrupt fecal-oral transmission in humans. The challenges will be to design vaccines capable of eliciting durable mucosal immunity, to understand the correlates of acquired immunity, and, most important, to enlist the continued support of industrialized nations to combat diarrheal diseases of children in developing countries.

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The complete reference list is available online at Expert Consult.

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273 Free-Living Amebae

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SHORT VIEW SUMMARY

Microbiology and Epidemiology

- Free-living amebae are protists that are common in the environment worldwide but rarely cause recognized human infections. The trophozoite stages of these organisms feed on bacteria and debris in the environment.
  - *Naegleria fowleri* is widely distributed globally and has been isolated from fresh water, most commonly in warm environments.
  - *Acanthamoeba* spp. are ubiquitous members of the environment and are found worldwide in soil and fresh water.
  - *Balamuthia mandrillaris* is also likely widely distributed and has been isolated from soil.

Clinical Manifestations and Diagnosis

- *N. fowleri* typically causes a fulminant meningoencephalitis in healthy, immunocompetent young patients, usually in association with immersion in warm fresh water. The disease is nearly always fatal, with few survivors reported to date.
  - The cerebrospinal fluid (CSF) profile of patients with N. fowleri primary amebic meningoencephalitis (PAM) is similar to that of bacterial meningitis (high white cell count and neutrophil predominance, low glucose, high protein) but with a negative Gram stain and culture. Motile trophozoites can sometimes be seen on wet mount of the CSF. Polymerase chain reaction (PCR) on CSF is the preferred diagnostic test.
  - Neuroimaging studies in patients with PAM are usually nonspecific.
- *Acanthamoeba* spp. and *B. mandrillaris* cause the subacute onset of focal neurologic deficits and mental status changes (granulomatous amebic encephalitis [GAE]) related to central nervous system mass lesions. *Acanthamoeba* is mostly seen in immunocompromised and debilitated individuals. *Balamuthia* occurs in both immunocompromised and immunocompetent patients and has been associated with both acute and subacute presentations. The case-fatality rate for these infections is also high.
  - In the United States, *Balamuthia* GAE may be more common in male Hispanic patients than in other groups.
  - CSF studies of patients with GAE are usually nonspecific, and it is rare to isolate organisms from the CSF.
  - Neuroimaging studies generally reveal multiple space-occupying lesions in the brain, with or without contrast enhancement. Hemorrhage within the lesions may suggest *Balamuthia*.
  - Biopsy of involved tissues (skin, brain, etc.) can be diagnostic, usually via histopathologic examination/immunohistochemical staining or PCR. Next-generation sequencing technology that does not require an a priori suspicion for *Acanthamoeba* or *Balamuthia* is often diagnostic in the right clinical circumstance; some institutions are now making this approach readily available for clinical samples (see http://nextgendiagnostics.ucsf.edu/our-diagnostic-lab/ and http://nextgendiagnostics.ucsf.edu/providers/).
- *Acanthamoeba* spp. and *B. mandrillaris* can involve other sites (lungs, sinuses, adrenal glands, kidneys, and skin).
- *Acanthamoeba* spp. also cause sight-threatening keratitis in otherwise healthy individuals in association with contact lens use. Diagnosis depends on a high clinical suspicion, in conjunction with in vivo confocal microscopy or demonstration of *Acanthamoeba* in corneal scrapings or biopsy specimens by histopathologic examination, culture, or PCR.

Therapy

- Therapeutic regimens for free-living amebic infections of humans are not well defined.
- Treatment for *N. fowleri* PAM should include high-dose intravenous amphotericin deoxycholate; intrathecal amphotericin may also provide some benefit, and the addition of azoles, rifampin, azithromycin, miltefosine, other antimicrobials, and dexamethasone should be strongly considered, along with aggressive management of increased intracranial pressure.
- *Acanthamoeba* keratitis should be treated with topical chlorhexidine or polyhexamethylene biguanide; adjunctive surgical therapy may also be necessary.
- *Acanthamoeba* GAE should be treated with combination antimicrobial regimens, possibly including pentamidine, an azole, a sulfonamide, miltefosine, and flucytosine. However, the most efficacious regimen is not currently known.
- *B. mandrillaris* GAE should be treated with combination antimicrobial regimens, possibly including pentamidine, flucytosine, a sulfonamide, albendazole, an azole, a macrolide, amphotericin, and/or miltefosine. However, the most efficacious regimen is not currently known.
- Surgical débridement may play an adjunctive role in the management of both forms of GAE.

Free-living amebae (FLAs) are aerobic, eukaryotic protists that comprise several genera. Clinically apparent infection of humans with FLAs is an infrequent but often fatal occurrence in both normal and immunocompromised individuals. Central nervous system (CNS) invasion by *Naegleria fowleri, Acanthamoeba* spp., and *Balamuthia mandrillaris* has been reported in hundreds of patients worldwide, with thousands of *Acanthamoeba* keratitis cases described.[1–4] Other FLA species have each been reported as the cause of disease in single human cases and are discussed briefly below.

Distinct from other pathogenic protozoa by nature of their free-living existence, these organisms have no known insect vectors, no human carrier states of epidemiologic importance, and little relationship between poor sanitation and their transmission. Four distinct clinical syndromes are caused by the FLAs that infect humans: (1) primary amebic meningoencephalitis (PAM), (2) granulomatous amebic encephalitis (GAE), (3) disseminated granulomatous amebic disease (e.g., skin, pulmonary, and sinus infection), and (4) amebic keratitis.

PAM is caused almost exclusively by *N. fowleri* and occurs most commonly in healthy children and young adults with recent recreational freshwater exposure, usually warm lakes, rivers, or streams. Increasingly, however, it is being recognized that *N. fowleri* can be found in household water systems, with some infections occurring through tap

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water exposure. *N. fowleri* gains access to the CNS by direct invasion through the nasal mucosa and the cribriform plate and causes a rapidly fatal meningoencephalitis. GAE, caused by *Acanthamoeba* spp. and *B. mandrillaris,* is a subacute infection that likely spreads hematogenously from pulmonary or skin lesions to the CNS. The resultant focal neurologic deficits progress over days to months to a diffuse meningoencephalitis and death. Disseminated granulomatous amebic disease involving the skin, lungs, or sinuses (but without CNS infection) with *Acanthamoeba* and *Balamuthia* have also been reported.[5,6] *Acanthamoeba* spp. also cause a subacute to chronic keratitis that is most often associated with contact lens use or corneal trauma, with rare reports of cases occurring after radial keratotomy and laser-assisted in situ keratomileusis (LASIK).[4,7]

ORGANISMS

Naegleria

N. fowleri was named after Malcolm Fowler of Adelaide Children's Hospital of Australia, who (with R.F. Carter) described the initial cases of PAM.[8] Of the approximately 30 species in the genus, *N. fowleri* is the only known pathogen of humans, although other species cause disease in mice (e.g., *Naegleria australiensis* and *Naegleria italica*).[9] Based on sequencing analysis, *N. fowleri* seems to have evolved from the nonpathogenic species *Naegleria lovaniensis* in North America. Globally, eight genotypes of *N. fowleri* have been identified by sequencing with types 1, 2, and 3 endemic to the United States. Type 1 is unique to the United States, and types 2 and 3 appear to be the most common worldwide.[10] Although the pathogenicity among strains appears similar, genotyping isolates can help define connections between cases within an outbreak or cluster.

Naegleria spp. have three life-cycle stages: trophozoites, flagellates, and cysts (Fig. 273.1). The trophozoites are the reproductive stage of the parasite and cause invasive human disease. Trophozoites feed predominantly on bacteria, are 10 to 25 µm in diameter, have pseudopodia, and have a clear nucleus with a prominent dense central nucleolus (Fig. 273.2). The granular cytoplasm can contain ingested red blood cells and leukocytes along with cytoplasmic organelles. On transfer to distilled water or a nonnutrient medium, trophozoites can transform rapidly to a transitory flagellate form, which does not divide or feed. The flagellate form can spontaneously revert to the trophozoite. Among the FLAs, only *Naegleria* has a flagellated life-cycle stage. When trophozoites encyst, the cyst is resistant to environmental stresses and is approximately 9 µm in diameter with a central nucleus and a single-layered wall containing an average of two pores. *N. fowleri* is thermophilic, with trophozoites growing well at temperatures as high as 45°C.[9] Organisms can be cultivated from clinical specimens and grow well in vitro on nonnutrient agar plates coated with bacteria. Like *Acanthamoeba* and *Balamuthia, N. fowleri* can be grown in a cell-free axenic medium, as well as in a chemically defined medium.

Acanthamoeba

Previously, *Acanthamoeba* were grouped by morphology and cyst size into three groups (I, II, and III), but they are now grouped by genetic similarity via newer molecular mechanisms. The genotyping is generally consistent with the morphologic grouping. Seventeen genotypes (T1–17) have been described, and groups T1, T2a, T3–6, T10–12, and T15 have been associated with human disease. Genotype T4 (correlating to the *Acanthamoeba castellanii* complex) is the most commonly identified in the environment and in human disease.[11] The life cycle of *Acanthamoeba* consists of trophozoite and cyst stages (see Fig. 273.1). Trophozoites are 15 to 50 µm in diameter, contain a single nucleus with a prominent central nucleolus, and have distinctive slender, spinelike projections of the plasma membrane (Fig. 273.3). The cysts have a double-layered wall, are less than 18 to 30 µm in diameter, and like *Naegleria* may contain pores in the cyst wall (Fig. 273.4). Trophozoites are the active form of *Acanthamoeba,* feeding on bacteria and environmental debris, whereas the cyst is the inactive but an environmentally resistant stage

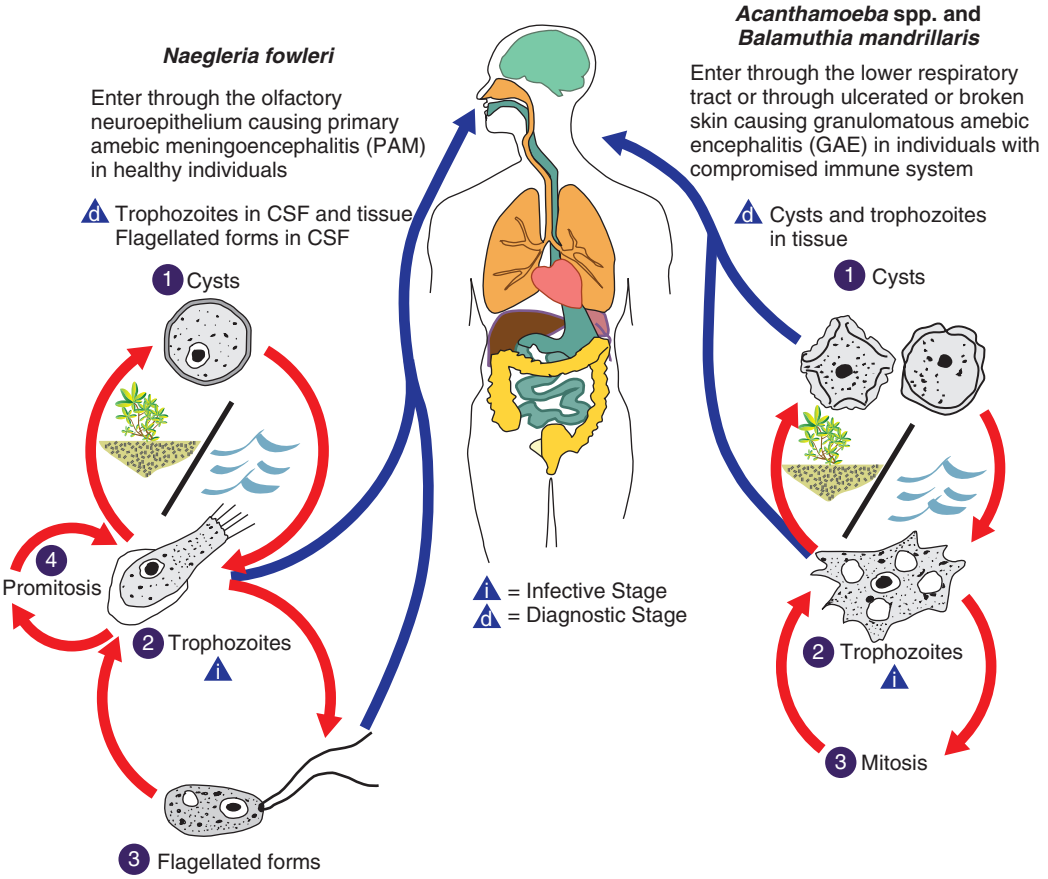

FIG. 273.1 Life cycles of *Naegleria fowleri, Acanthamoeba* spp., and *Balamuthia*, showing stages and proposed portals of entry. *CSF,* Cerebrospinal fluid. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*



FIG. 273.2 Trophozoites of *Naegleria fowleri* in brain tissue stained with hematoxylin and eosin. Note the clear nucleus with a prominent dense, central nucleolus. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

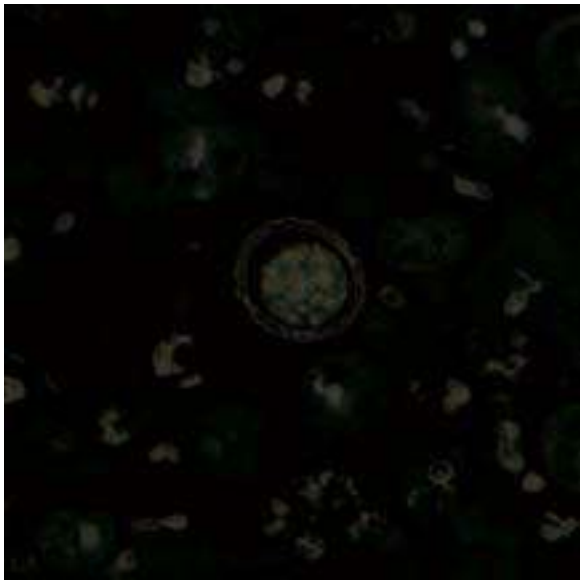

FIG. 273.4 *Acanthamoeba* cyst in brain tissue stained with hematoxylin and eosin. Note the double wall of the cyst. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

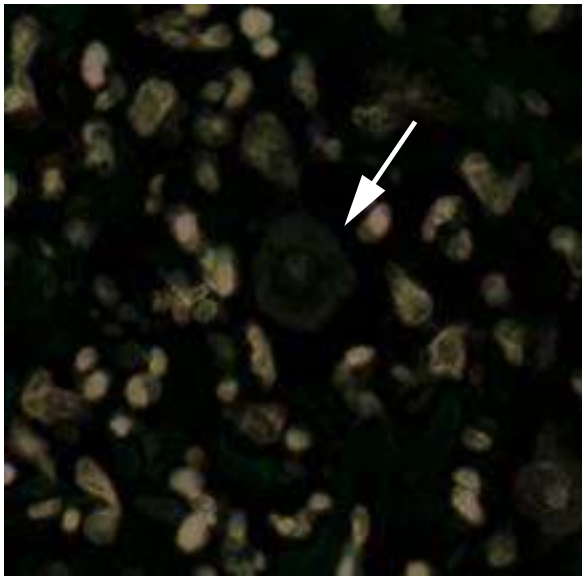

FIG. 273.3 *Acanthamoeba* trophozoite *(arrow)* in tissue stained with hematoxylin and eosin. Note the single nucleus with prominent central nucleolus. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

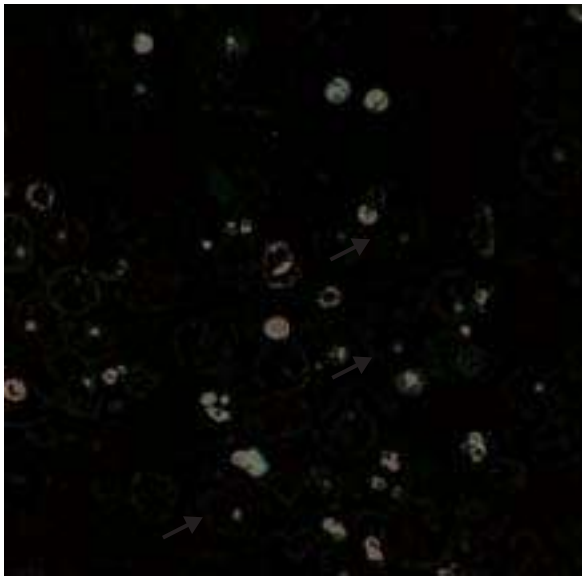

FIG. 273.5 *Balamuthia* trophozoites *(arrows)* in brain tissue stained with hematoxylin and eosin. Trophozoites are uninucleate and cannot be reliably distinguished from *Acanthamoeba* spp. trophozoites without specific immunostaining. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

and may explain the resistance of the organism to antimicrobial therapy, especially in the setting of amebic keratitis.[11] *Acanthamoeba* spp. can be easily cultivated in the laboratory; this is best accomplished by the use of tryptic soy agar with rabbit or horse blood, buffered charcoal yeast extract agar, and nonnutrient agar overlaid with live organisms, such as *Escherichia coli* or *Enterobacter aerogenes*.[1] Like *N. fowleri,* most *Acanthamoeba* spp. can be grown in both cell-free axenic and chemically defined media.[1]

Balamuthia

B. mandrillaris, formerly referred to as a *leptomyxid ameba,* is the only known species of this genus and was originally isolated from the brain of a mandrill baboon in 1986.[12] The life cycle consists of the trophozoite and cyst stages (see Fig. 273.1). *B. mandrillaris* trophozoites have a mean diameter of 30 µm (range, 12 to 60 µm) and are uninucleate (Fig. 273.5). Cysts have a mean diameter of 15 µm (range, 12–30 µm) with a wavy and irregular outer wall that is composed of three layers (Fig. 273.6).[1] On hematoxylin and eosin (H&E)-stained specimens, the organism cannot be reliably differentiated from *Acanthamoeba*. Laboratory growth of *Balamuthia* is more difficult than for *Acanthamoeba* or *Naegleria* because it does not grow on bacteria-coated agar plates and has a long doubling time. Currently, using a cell-free growth medium, axenization, and mammalian cell culture to grow *Balamuthia* in vitro, efforts are being made to better understand the components of the cyst and so offer new drug targets.[11,13] In addition, whole-genome sequencing of several *Balamuthia* strains was recently done. Functional analyses of the genome will offer new insights into *Balamuthia* as an organism and identify potential drug targets.[14]

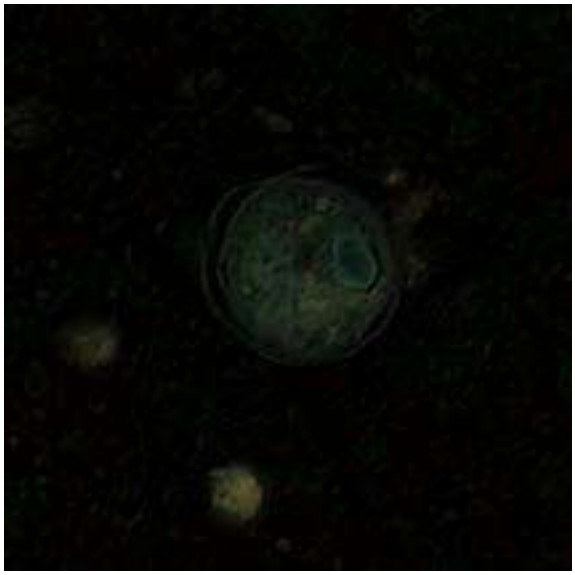

FIG. 273.6 *Balamuthia* cyst in brain tissue stained with hematoxylin and eosin. By light microscopy, only two walls can be distinguished. Specific immunostaining reliably distinguishes *Balamuthia* cysts from those of *Acanthamoeba* spp. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention and University of Kentucky Hospital, Lexington, Kentucky.)*

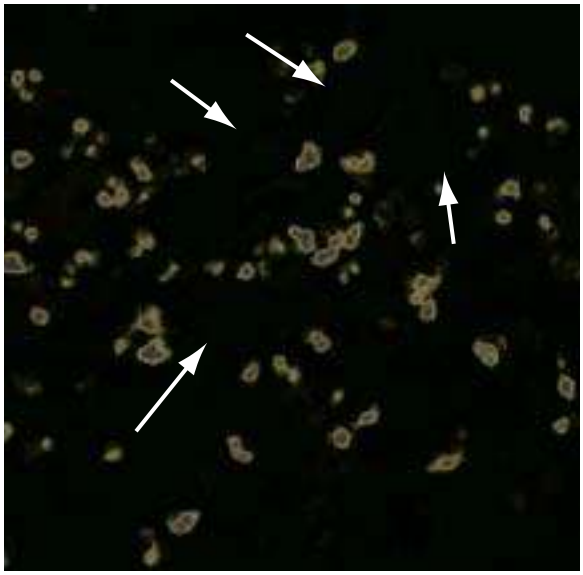

FIG. 273.7 Trophozoites of *Sappinia (arrows)* in brain tissue stained with hematoxylin and eosin. Note the distinctive double nucleus that defines this species. *(Courtesy Division of Parasitic Diseases/Centers for Disease Control and Prevention.)*

Other Free-Living Amebae Reported in Human Disease

Although *Acanthamoeba* spp. are the most common cause of amebic keratitis, *Allovahlkampfia* spp., *Vahlkampfia* spp., *Hartmannella* spp., and *Paravahlkamfia* spp. have each caused at least one human case of subacute keratitis, often in association with contact lens use or corneal trauma.[15–18]

In 2009 a previously healthy man originally diagnosed with viral meningitis was subsequently determined to have PAM caused by *Paravahlkamfia* spp. A newly designed real-time polymerase chain reaction (PCR) assay contributed to this diagnosis and suggests that this is a new species of FLA, termed *Paravahlkamfia francinae*.[19] Of note, he did not receive antimicrobial therapy active against FLAs but spontaneously recovered. This suggests the possibility that some previous survivors of *Naegleria* PAM, which is almost always fatal, may actually have been infected with *Paravahlkamfia* spp. instead. In addition, this case raises the possibility that some previously undiagnosed cases of "aseptic meningitis" may have been unrecognized PAM caused by a mildly virulent FLA.

In 2001 a report described *Sappinia diploidea* as the cause of GAE in a previously healthy 38-year-old man who survived the infection.[20] The identification of this FLA as *Sappinia diploidea* was done by H&E staining and transmission electron microscopy of the patient's excised brain lesion. Two subsequent reports that used molecular diagnostics suggest that this *Sappinia* sp. was most likely either *Sappinia pedata*[21] or a novel species of *Sappinia* that is molecularly closer to *S. pedata* than *S. diploidea*.[22] This is the only case of *Sappinia*-associated human disease reported to date (Fig. 273.7).

Overall, these single-case reports highlight the constant interaction between humans and FLAs and the need for a high index of suspicion for such infections to arrive at an accurate diagnosis.

EPIDEMIOLOGY

Naegleria

N. fowleri has been found worldwide in river and lake water samples and in soil.[23] Importantly, *N. fowleri* is not found in seawater. *N. fowleri* has also been found in thermal springs, thermal saline baths, and mud springs. Pathogenic *N. fowleri* are thermophilic and proliferate at temperatures up to 45°C.[9] The presence of *N. fowleri* in fresh water is directly related to water temperature,[24] and *N. fowleri* has been frequently isolated from thermally polluted waters in temperate climates, including as far north in the United States as Minnesota.[25]

In semitropical locations such as Florida, it is not uncommon to isolate at least one *N. fowleri* organism per 25 mL of water.[24] As water temperatures drop in winter, *Naegleria* is typically isolated from lake-bottom sediments; *Naegleria* cysts are stable for up to 8 months at 4°C. Although there have probably been billions of people exposed to *Naegleria*-contaminated fresh water, few develop PAM.[24] The factors that protect most individuals from invasive *Naegleria* infection are not understood. In the southern United States the presence of serum-agglutinating activity against *N. fowleri* in many young adults (but not infants) indicates that subclinical infection or exposure to *Naegleria* is common.[26] PAM has been reported throughout much of the world.[27] Although the true incidence of this entity is unknown, hundreds of cases of PAM have been reported worldwide, with most having a recent history of recreational freshwater exposure. In the United States more than 100 cases of PAM were reported from 1962–2008. Annual reported case numbers ranged from 0 to 8 per year but do not appear to be increasing over time. The median age of patients was 12 years, 79% were male, and only one patient survived. Eighty-five percent of cases occurred in the summer months, and all were in southern-tier states, with more than half in Florida or Texas[28] (Fig. 273.8). Similar findings were noted in a review of 142 US cases from 1937–2013, including three well-documented survivors.[29] In August 2010 a 7-year-old girl died of PAM due to *Naegleria* acquired in Minnesota, expanding the range of this organism over 500 miles north of the previously northernmost known human case in the United States. She had swum in two lakes in the area during the third-hottest summer on record in Minneapolis, possibly an unfortunate consequence of climate change.[25] Several additional cases have been reported recently in non–Southern-tier states, including Kansas, Indiana, and another case in Minnesota.[29]

New reports of PAM continue to appear in the literature, and the current incidence is undoubtedly higher, with some estimates placing the incidence at about 16 deaths annually in the United States.[30] Clusters of PAM cases with common environmental exposures have occurred, including 16 deaths over a 3-year period that were retrospectively traced to a swimming pool in Czechoslovakia with a low chlorine concentration.[31] A young woman died of PAM after swimming in a pool in California that was served by water that was piped in from a mountain hot spring.[32] Recently, a young woman died of PAM after falling out of a raft and being submerged underwater at an artificial whitewater river facility in North Carolina; *N. fowleri* was detected in all of the water

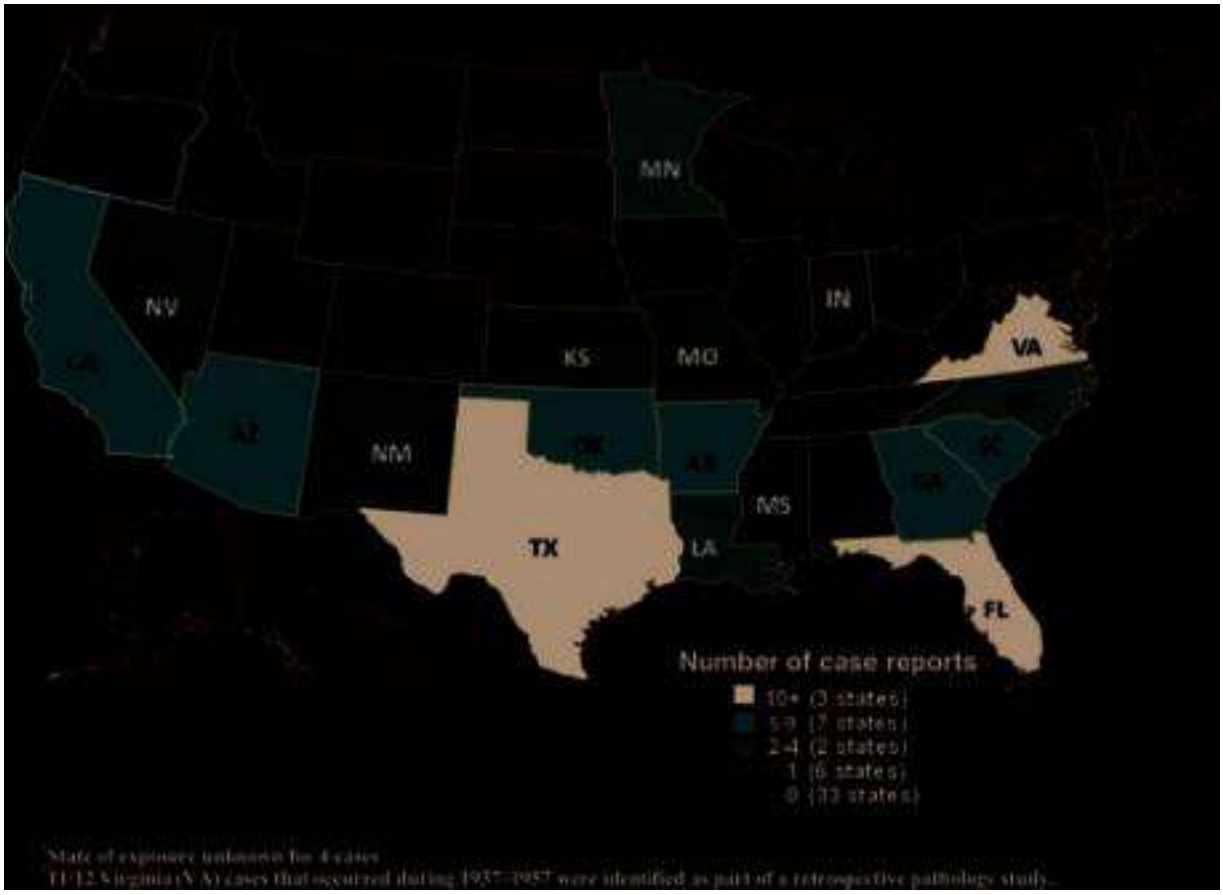

FIG. 273.8 **Number of case reports of *Naegleria fowleri* meningitis (*N* = 142) by state of exposure.**[29]

samples subsequently tested from the facility.[33] In 2002 two previously healthy children from the same neighborhood in Arizona died of PAM due to *N. fowleri* within 1 day of each other, apparently acquired through exposure to household bathwater.[34] Drinking water for the respective families came from the same untreated community well-water system, and PCR testing revealed *N. fowleri* in the water system.[35] These cases were the first association of a drinking water system with *N. fowleri* infection in the United States.[34] A subsequent study demonstrated *N. fowleri* (detected by PCR) in 16% of 185 wells sampled in Arizona, indicating that transmission in this manner does pose a potential risk.[36] In 2011 two patients in Louisiana died of PAM due to *Naegleria,* both likely secondary to acquisition through sinus irrigation with contaminated tap water. *N. fowleri* was detected in water samples from both households; these were the first US cases reportedly due to exposure to contaminated treated municipal water.[37] PAM deaths related to sinus irrigation or nasal ablution with contaminated tap water have been reported elsewhere, including recently in the US Virgin Islands.[38] In 2013 a young boy died of PAM after likely acquiring the infection through exposure to tap water that was used on a backyard lawn water slide. This occurred at a house that was served by the same water system implicated in the 2011 Louisiana cases previously noted and represented the first time *N. fowleri* was cultured from a US municipal water system (not just the house at which the patients lived); the presence of *N. fowleri* in this water seems to have been related to undetectable chorine levels and high temperatures in the water.[39] Two other recent clusters of PAM due to *N. fowleri* occurred in Pakistan, one a cluster of 13 cases likely due to ablution with contaminated tap water[40] and another cluster of 19 cases exposed only to public water in Karachi.[41] *Naegleria* has recently been found in other drinking water systems worldwide (including in Spain, Australia, Canada, the United Kingdom, and other parts of the United States).[42] No known cases of *N. fowleri* transmission have occurred to date through organ transplantation; although it appears that *N. fowleri* can disseminate outside the CNS and that at least five organ donors had PAM as the cause of their death just before donation, none of the organ recipients in these cases have developed clinically apparent disease due to *N. fowleri*.[43]

Acanthamoeba

Acanthamoeba spp. have been isolated from soil, water, and air in diverse geographic locations.[11,27] In contrast to *Naegleria, Acanthamoeba* growth is inhibited by temperatures above 35°C to 39°C, although recent evidence suggests that *Acanthamoeba* may be able to grow at least inefficiently at higher temperatures.[44] Human exposure to *Acanthamoeba* spp. is likely a common event, as evidenced by the relatively frequent finding of the organisms in pharyngeal swab cultures of healthy individuals.[1] In addition, serologic surveys have demonstrated serum anti-*Acanthamoeba* antibodies in 50% to 100% of some cohorts of healthy people. Persons of Hispanic descent may be less likely to develop antibodies to *Acanthamoeba,* especially *Acanthamoeba polyphaga,* than white persons; the clinical significance of this finding is currently unclear.[45,46] Although exposure of the general population to *Acanthamoeba* appears to be common, GAE caused by *Acanthamoeba* spp. is mostly limited to debilitated or immunosuppressed individuals, although a number of recent cases in immunocompetent individuals have been reported.[1,47–50] Underlying conditions reported in patients with GAE have included acquired immunodeficiency syndrome (AIDS),[47] liver disease, diabetes mellitus, organ transplantation, steroid therapy, chemotherapy, and exposure to rituximab.[1,51,52] Recently, chronic meningitis and meningoencephalitis secondary to *Acanthamoeba* spp. have been reported in several Indian children with no clear immunosuppression, other than malnourishment.[53–55] Whether this represents a syndrome specific to this region or is due to better reporting is unclear at present. Disseminated *Acanthamoeba* infection without overt CNS manifestations has been increasingly recognized, most commonly in AIDS patients but also in transplant patients and those with long-term corticosteroid use.[11,51,56,57] Most commonly, these patients have cutaneous disease, although involvement of the liver, lungs, and bones has been

reported.[6,56] *Acanthamoeba* infection associated with a total artificial heart has also been reported.[58]

Unlike GAE or meningitis, which occurs most commonly in the immunosuppressed, amebic keratitis occurs predominantly in healthy people who wear contact lenses or have had corneal trauma. Worldwide the annual incidence ranges from 0.15 per million to 1.4 per million.[3] In countries with high contact lens usage rates, greater than 80% of cases are likely related to this risk factor. The annual incidence among contact lens wearers is estimated at 1.65 per million to 19.50 per million contact lens wearers with significant associated health care expenditures.[3,59] Amebic keratitis incidence increases during the warmer months, presumptively because of *Acanthamoeba* persistence in warmer temperatures and increased recreational water activity.[3,22] Before 2005 poor contact lens hygiene practices, such as improper lens cleaning, swimming or showering with lenses, or rinsing the lens case with tap water, were associated with this disease, although it had also occurred in persons who reported none of these behaviors.[4] In the United States, from 2005–07 a multistate outbreak involving at least 170 patients was associated with use of Advanced Medical Optics Complete Moisture Plus (AMOCMP), although no specific contamination of the solution was found.[60] After the voluntary removal of AMOCMP the number of cases declined, but not to preoutbreak levels; this new increase in baseline amebic keratitis cases in the outbreak area is currently not understood.[61,62] In addition, an increase of amebic keratitis in other parts of the world, not all of which can be accounted for by AMOCMP use, suggests that amebic keratitis will continue to become more common.[11,63] These examples highlight the fact that commercially available disinfectant solutions are often ineffective against *Acanthamoeba* spp. and that high-risk behavior associated with contact lens use is highly prevalent.[64] The cysts of *Acanthamoeba* spp. in particular may play a critical role in the establishment and persistence of amebic keratitis.[65,66]

Balamuthia

B. mandrillaris infects animals and humans worldwide. Until recently this organism had only rarely been isolated, mostly from soil and water. Now, new PCR methods and cell-free growth media/axenization have made identification and isolation of *Balamuthia* from the environment easier and more common.[17,67] Studies using these methodologies suggest that *Balamuthia* is found worldwide (with regional variability) and that this ameba is more commonly associated with soil than water.[2,67] Like *Naegleria* and *Acanthamoeba,* serologic studies of healthy populations in the United States, Africa, and Australia suggest that many people are exposed to *Balamuthia,* but few develop clinical disease.[2] The first human cases of infection with this organism were reported in 1990.[12] The isolation of *Balamuthia* from potting soil in the home of a fatally infected 3-year-old child that matched the clinical isolate from the patient confirmed the free-living and pathogenic status of *B. mandrillaris*.[68] Worldwide there have been more than 200 cases of human disease reported to date, with a very high mortality rate.[69] Across the world only a small number of patients (12) have survived *Balamuthia* encephalitis in response to a variety of antimicrobial treatments.[69–71] Analysis of US cases suggests that risk factors for balamuthiasis include male sex, Hispanic race, and residence in the most southern states of the United States; infections are more commonly seen in immunocompetent patients than in the immunocompromised. Soil exposure appears to be a common risk factor (85%), though water exposure was also common (66%).[70,72] Whether this epidemiologic pattern is a result of common environmental exposures or a genetic predisposition is currently unknown.

There have now been three documented clusters of transmission of *Balamuthia* through organ transplantation.[73,74] All three donors were young, immunocompetent males. Although one donor was reported to have died of a stroke and the other of acute disseminated encephalomyelitis, the latter donor was subsequently determined to have died of *Balamuthia* GAE. The third donor died from trauma. His postmortem brain and lung examination, done after the liver recipient died of GAE, showed no evidence of *Balamuthia*, although the donor was found to be seropositive for *Balamuthia*. This third case suggests that *Balamuthia* can be transmitted from donors who are asymptomatic. Although 13 patients received organs from these three donors, only 5 (2 liver, 1 kidney-pancreas, and 2 kidney recipients) became symptomatic and were confirmed to have acquired *Balamuthia* from the transplant. In 4 of these patients, illness developed 17 to 24 days posttransplant.[74] Four of these 5 symptomatic patients died despite treatment; the lone survivor had a poor neurologic outcome.[74] Of the remaining 8 patients, 6 were *Balamuthia* seropositive (titer 1:64 or greater). All 8 were empirically treated with a variety of regimens, with the seropositive patients showing declines in antibody titer after treatment. Given the very low rate of GAE among the seropositive patients, it is unclear if any of them would have developed GAE had they not received preemptive therapy.[73,74]

PATHOGENESIS AND PATHOLOGIC FINDINGS

Naegleria

PAM produces a diffuse meningoencephalitis, which affects the cortical gray matter most severely. Animal models[75,76] and autopsy studies[23,27] indicate that CNS invasion by *N. fowleri* occurs after nasal inoculation with amebae by disruption of the olfactory mucosa. The amebae penetrate the respiratory epithelium,[77] as well as the submucosal nervous plexus and the cribriform plate, and gain access to the CNS. In the CNS cortical hemorrhage and edema are seen, and the olfactory bulbs are hemorrhagic and necrotic. *Naegleria* trophozoites are found in the olfactory nerves and the adventitia and perivascular spaces of small to midsize arteries and arterioles.[1] They can be identified in wet mounts of cerebrospinal fluid (CSF) in patients with acute meningoencephalitis.[27,78] No cysts are seen in the brain.[1] A prominent fibrinopurulent leptomeningeal inflammatory infiltrate is usually seen. In addition, in a series of 16 autopsies of patients with PAM, myocarditis was present in 7, although no congestive heart failures or arrhythmias were noted. The inflammatory infiltrate was predominantly neutrophilic, and no amebae were seen in the myocardium.[79] The tissue necrosis elicited by *Naegleria* is likely mediated in part by secreted cysteine proteases and direct phagocytosis by feeding cups found on the trophozoites.[80] The protein Nfa1, which mediates contact between *N. fowleri* pseudopods and target cells, is an important virulence factor for this organism, and mice immunized against this protein exhibit increased survival in experimentally induced PAM.[81–83]

Other potential virulence determinants include nitric oxide production, pore-forming proteins, low-molecular-mass thiol compounds, and calcium-mediated complement resistance. Virulence appears to be mediated by an interaction between the environment and *N. fowleri,* resulting in alterations of the expression of virulence factors by the organism.[84]

Acanthamoeba

Both *Acanthamoeba* spp. and *B. madrillaris* likely gain entry to the body via the skin or the respiratory tract. Although initially contained at the site of entry by the immune system in immunocompromised/debilitated individuals, the amebae can enter the circulation and disseminate to the brain and other organs. The histologic appearance of GAE from *Acanthamoeba* spp. is of parenchymal necrosis and granulomas, although granulomatous tissue reaction may not be present in immunocompromised individuals.[1] When the leptomeninges are involved, two patterns are seen: infiltrates containing equal numbers of polymorphonuclear cells, lymphocytes, and macrophages or infiltrates that are predominantly of lymphocytes and macrophages.[85] Moderate-to-severe cerebral edema occurs, and thus bilateral uncal or cerebellar tonsillar herniations can occur. Necrotizing granulomatous lesions containing perivascular trophozoites and cysts are most frequently located in the cerebellum, midbrain, and brainstem. Multinucleated giant cells are occasionally present within the granulomas.[27] The perivascular location of amebic trophozoites and cysts suggests a hematogenous dissemination of *Acanthamoeba* to the CNS; this is also supported by identification of *Acanthamoeba* in the skin, lung, adrenal glands, and lymph nodes. Amebic skin lesions, sinusitis,[5] and pneumonitis[86] may be sites of primary human infection that lead to hematogenous dissemination.[27]

The histologic appearance of amebic keratitis is similar to that of *Acanthamoeba* infections of other organs. Both amebic cysts and trophozoites are found within the cornea. There is an acute or mixed inflammatory infiltrate that may contain epithelial and giant cells. However, amebae have also been found in tissue in the absence of an inflammatory infiltrate. Corneal neovascularization occurs to a variable extent.[87,88] Involvement

of the posterior chamber of the eye is a rare complication of amebic keratitis. Sterile inflammation of the posterior segment occurs without isolation or visualization of amebic cysts or trophozoites.[87] The corneal ringlike infiltrate caused by *Acanthamoeba* appears to be caused by the neutrophil chemoattractant effect of antigen-antibody complexes in the cornea.[87,88] Amebae adhere to corneal epithelial cells and secrete proteinases that facilitate invasion of the cornea and stroma. Trophozoites and cysts are seen between the lamellae of the cornea, and inflammatory infiltrates in the superficial and middle layers of the corneal stroma are common. Infiltration of nerves causes radial keratoneuritis, and later a characteristic stromal ring infiltrate develops. Anterior uveitis is common. In late stages amebic keratitis is characterized by necrosis, ulceration, descemetocele formation, and perforation of the cornea.[89]

Significant progress has been made toward understanding the molecular basis of pathogenesis in *Acanthamoeba* infections, especially keratitis. The pathology induced by *Acanthamoeba* can be classified into contact-dependent mechanisms, which require the ameba to physically contact the host cell, and contact-independent mechanisms.[11,90] Keratitis begins with the contact-dependent action of trophozoites using a mannose-binding protein to adhere to mannose glycoproteins on the corneal epithelium. The mannose glycoproteins also stimulate the release of cytopathic factors from the parasites, leading to the killing of corneal cells and destruction of the extracellular matrix.[90] In addition, amebic ecto–adenosine triphosphatases (ATPases), neuraminidases, elastases, phospholipases, glycosidases, and proteases, some of which act in a contact-independent manner, potentially play a role in corneal destruction.[11,90] *Acanthamoeba* also evade the immune response by degrading human immunoglobulin A (IgA) antibodies with secreted proteases, switching from trophozoites to cysts, and infecting the cornea, a site that lacks resident antigen-presenting cells, thus avoiding a delayed-type hypersensitivity response or a serum IgG response.[91] Finally, a recent study has shown that *Acanthamoeba* express a pore-forming protein, which can kill both bacteria and mammalian host cells, although the role of this acanthaporin in vivo is unknown.[92]

An ongoing development in the understanding of *Acanthamoeba* pathogenesis at the cellular level is the observation that many bacteria (including *Legionella* spp., some *Mycobacteria, Shigella* spp., *Chlamydia* spp., *Pseudomonas aeruginosa, Vibrio cholerae,* and *Burkholderia* spp.), viruses, and even yeast and protozoa survive within the ameba.[11] This endosymbiosis has several potential sequelae. First, it may render the intracellular microbe more pathogenic for the human host; second, it may facilitate gene transfer between the ameba and bacteria[93]; third, it may allow the microbe to survive an otherwise inhospitable environment; and fourth, in coinfections, *Acanthamoeba* may shield the intracellular microbe from the immune response and antibiotics, thus leading to more fulminant infections with these bacterial species. Although the endosymbiotic relationship of intracellular microbes and ameba continues to be explored, the actual impact of this relationship on human disease remains unclear.[94]

Balamuthia

B. mandrillaris causes a subacute or chronic meningoencephalitis clinically similar to that caused by *Acanthamoeba* spp. However, the histopathologic changes in *Balamuthia* GAE are broader than what is observed in *Acanthamoeba* GAE. In balamuthiasis the histopathology of lesions in the brain parenchyma or the meninges can range from an acute or neutrophilic immune response to a primarily chronic or granulomatous response.[85] Cysts and trophozoites of *B. mandrillaris* are seen in a perivascular pattern and are associated with angiitis and hemorrhagic necrosis of the underlying meninges and brain tissue. The angiotropic location, as well as the fact that organisms have been isolated and transmitted from other tissues (skin,[51] adrenal glands,[2] kidney,[95,96] and liver[96]), suggests that it may be spread hematogenously. An animal model of encephalitis with *B. mandrillaris* has been developed[97] and may be useful in studying the disease pathogenesis. Both immunocompetent and immunocompromised mice are susceptible to intranasal and oral challenge with *B. mandrillaris;* the most likely portal of entry for human disease remains to be determined.[97,98] These mouse models have also shown that CD4 T lymphocytes appear to be more important for resistance to *Balamuthia* than CD8 T lymphocytes.[99] Some progress has been made in understanding how *Balamuthia* crosses the blood-brain barrier (BBB). Several mechanisms likely allow *Balamuthia* to enter the CNS, including amebic surface-expressed galactose-binding proteins and ecto-ATPases, secreted amebic proteases, and phospholipases. In addition, the host response can induce interleukin-6 production and secretion, which by itself can increase BBB permeability by upregulating expression of adhesion molecules on endothelial cells and potentially increasing host immune cell recruitment to the BBB.[100] Despite these advances, much work remains in determining the mechanisms by which *Balamuthia* causes GAE.

CLINICAL MANIFESTATIONS

Table 273.1 lists comparisons of the epidemiologic, clinical, diagnostic, and therapeutic characteristics of *Naegleria, Acanthamoeba,* and *Balamuthia*.

Naegleria

PAM usually occurs in otherwise healthy children and young adults who have had recreational exposure to warm fresh water.[101] The onset of symptoms usually occurs 2 to 5 days after exposure, but apparent incubation periods of up to 2 weeks have been reported. Very early in the illness and consistent with the involvement of the olfactory nerves, the patient may notice changes in taste or smell, followed by an abrupt onset of fever, anorexia, nausea, and vomiting. On initial presentation, headache and meningismus are noted in 86% to 100% of patients, and mental status changes in 66%. Patients rapidly progress to coma and death a median of 5 days after the onset of illness, usually without developing focal neurologic signs.[27] Spinal cord involvement has been reported, and one AIDS patient with *Naegleria* CNS infection has been reported.[98,102]

Acanthamoeba

In contrast to PAM, *Acanthamoeba* GAE is usually an illness of immunocompromised and debilitated individuals, has an insidious onset, and presents with focal neurologic deficits.[1,47] Signs and symptoms in a series of 15 patients with GAE included mental status abnormalities (86%); seizures (66%); fever, headache, and hemiparesis (53%); meningismus (40%); visual disturbances (26%); and ataxia (20%).[103] The duration of illness from presentation until death was 7 to 120 days (mean, 39 days). The incubation period of GAE is difficult to determine, given the ubiquitous environmental presence of these organisms, but *Acanthamoeba* skin ulcers and lesions have been documented for months before the onset of CNS disease.[27,51,104] The skin lesions can be ulcerative, nodular, or subcutaneous abscesses and on biopsy demonstrate amebic granulomas.[51,104,105] Other clinical syndromes that have been described with *Acanthamoeba* infection include pneumonitis,[86] adrenalitis,[86] leukocytoclastic vasculitis,[106] osteomyelitis,[56] sinusitis,[5,104] and infection of a peptic ulcer[107] and an ependymal cyst.[108]

Amebic keratitis is generally a unilateral, sight-threatening infection that leads to corneal ulceration, blindness, and enucleation if not treated promptly.[109] It is frequently misdiagnosed as herpetic, bacterial, or fungal keratitis, resulting in a delay to definitive treatment. The common symptoms are a foreign-body sensation in the affected eye, photophobia, tearing, and pain. Pain out of proportion to the signs of inflammation in early amebic keratitis has been noted, but amebic keratitis can present as a painless keratitis, so the lack of pain should not be used to rule out amebic keratitis.[3] Periods of temporary remission are common, which lead to further delays in diagnosis because they are incorrectly interpreted as responses to antibacterial or antiviral therapy. Signs of amebic keratitis include dendriform epitheliopathy, subepithelial infiltrates and neovascularization, a characteristic corneal ring infiltrate (Fig. 273.9), stromal infiltrates, radial keratoneuritis, hypopyon, and late in the disease, corneal perforation and melt.[3,110] The dendriform epithelial pattern has been described as an early sign of amebic keratitis before stromal involvement. Recognition of this manifestation and high clinical suspicion are important because early treatment increases the chance of a good outcome and more rapid cure.[63] A nonsuppurative keratitis with recurrent ulceration and a waxing and waning clinical course is a typical history, which in part is why amebic keratitis is often misdiagnosed as dendritic keratitis due to herpes simplex virus.[89]

TABLE 273.1 Microbiology, Clinical Characteristics, Diagnosis, and Treatment of Free-Living Amebae Known to Cause Human Disease

	NAEGLERIA FOWLERI	*ACANTHAMOEBA* SPP. (NONKERATITIS DISEASE)	*ACANTHAMOEBA* SPP. (KERATITIS)	*BALAMUTHIA MANDRILLARIS*
Disease	Primary amebic meningoencephalitis (PAM)	Granulomatous amebic encephalitis (GAE); cutaneous lesions; sinus infections	Amebic keratitis	GAE; cutaneous lesions; sinus infections
Epidemiology	Most human cases associated with exposure to recreational warm fresh water	Can acquire from soil, water, air	Corneal trauma; poor contact lens hygiene; association with Advanced Medical Optics Complete Moisture Plus	Can acquire from soil, water, air
Groups at risk	Healthy children and young adults, usually male	Immunocompromised individuals	Contact lens wearers (>80% of cases)	Immunocompromised individuals; healthy children and adults; Hispanic persons
Signs and symptoms at presentation	Headache, neck stiffness; seizures; coma	Headache, neck stiffness; behavioral changes; coma; sinus disease; skin ulcers	Intense pain, photophobia, tearing; dendriform epitheliopathy (early); stromal ring	Headache, fever, cognitive/behavior changes, seizures, hydrocephalus, sinus infection, skin nodules
Clinical course	Prodrome of few days; fulminant disease; without treatment, death usually within 1 week	Prodrome of weeks to months; subacute course; acute stage fatal in weeks	Prodrome of days; subacute to chronic keratitis	Prodrome of weeks to months; subacute course; acute stage fatal in weeks
Laboratory diagnosis	CSF wet mount positive for motile amebae; CSF with polymorphonuclear pleocytosis; no cysts seen in brain tissue; PCR from CSF	Amebae rarely seen in CSF wet mount; cysts seen in brain tissue—test by IFA, IIF, or PCR for definitive identification	Corneal scraping or biopsy to find trophozoites or cysts; confocal microscopy	Amebae rarely isolated from CSF, CSF pleocytosis with mild lymphocytic predominance and protein elevation; cysts seen in brain tissue—test by IFA, IIF, and PCR
Distinct morphologic features	Vesicular nucleus; limacine movement of amebae; flagellate stage; cysts with pores flush at surface	Vesicular nucleus; finger-like pseudopodia projecting from surface; cyst wall with two layers and with pores		Vesicular nucleus with single or multiple nucleoli; ameboid and "spider-like" movements in culture; cyst wall with three layers
In vitro cultivation	Axenic, bacterized, and defined media; tissue culture cells; optimal growth at ≥37°C	Axenic, bacterized, and defined media; tissue culture cells; optimal growth at 37°C (CNS isolates) or optimal growth at 30°C (corneal isolates)		Axenic medium; tissue culture cells; optimal growth at 37°C (bacterized medium not useful)
CT/MRI of head	Nonspecific	Space-occupying or ring-enhancing lesion	Not applicable	Space-occupying or ring-enhancing lesions
Antimicrobial therapy	Intrathecal and IV amphotericin B deoxycholate, fluconazole, rifampin, azithromycin, miltefosine; consider dexamethasone and other adjuncts to manage increased ICP	Pentamidine, azoles, flucytosine, sulfadiazine, miltefosine, amikacin IV and IT, voriconazole	PHMB, chlorhexidine, propamidine, hexamidine, topical and oral voriconazole	Pentamidine, azithromycin or clarithomycin, fluconazole, sulfadiazine, flucytosine, miltefosine

CNS, Central nervous system; *CSF,* cerebrospinal fluid; *CT,* computed tomography; *ICP,* intracranial pressure; *IFA,* immunofluorescent antibody staining; *IIF,* indirect immunofluorescent staining; *IT,* intrathecal; *IV,* intravenous; *MRI,* magnetic resonance imaging; *PCR,* polymerase chain reaction; *PHMB,* polyhexamethylene biguanide. *Modified from Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae:* Acanthamoeba *spp.*, Balamuthia mandrillaris, Naegleria fowleri, *and* Sappinia diploidea. FEMS Immunol Med Microbiol. *2007;50:1–26.*



FIG. 273.9 *Acanthamoeba* keratitis with the characteristic ring infiltrate. *(From Garg P, Rao GN. Corneal ulcer: diagnosis and management.* J Comm Eye Health. *1999;12:21.)*

Balamuthia

Unlike *Acanthamoeba, Balamuthia* can cause disease in both immunocompetent (especially in children) and immunocompromised hosts.[70] Subacute or chronic granulomatous meningoencephalitis is the most common clinical presentation, resulting in death 1 week to several months after the onset of neurologic symptoms. Important signs and symptoms include fever, headache, nausea, vomiting, seizure, and focal neurologic signs.[2]

In South America it appears common for patients to present with recognized *Balamuthia* skin lesions preceding GAE, while preceding skin lesions rarely occur in US *Balamuthia* patients.[2,70,111] The skin lesions are usually solitary, poorly defined, and firm or rubbery; have a normal to violaceous coloring; and have preserved sensation.[2] The lesions are usually several centimeters in size, although if untreated they can enlarge and ulcerate; biopsy can show granulomatous inflammation without organisms. The skin lesions can rapidly expand if the patient is treated with systemic corticosteroids.[2,111,112] The lesions most commonly appear on the central face, although other locations such as the lower face, abdomen, and extremities have been reported.[2,111,112] As a rule, it is thought that *Balamuthia* skin lesions herald an eventual progression to CNS involvement in untreated patients, although in a Peruvian cohort, four patients with isolated skin disease were identified, with one patient

spontaneously recovering without treatment and three others improving on systemic antimicrobial therapy directed against *Balamuthia*.[2,113] Recently, a patient with isolated cutaneous *Balamuthia* disease was also identified in the United States.[114]

LABORATORY DIAGNOSIS

Naegleria

PAM should be included in the differential diagnosis for children and young adults with meningoencephalitis or suspected bacterial meningitis, especially if there is a history of recent exposure to fresh water. The peripheral white blood cell (WBC) count is usually elevated, with a predominance of neutrophils. Neuroradiologic imaging ranges from unremarkable to showing diffuse contrast enhancement of the gray matter, cisternal exudates and obliteration, and infarction.[27,115,116] The CSF pressure is often elevated, and the CSF is often hemorrhagic, especially late in the course of the disease. CSF WBC counts may be low early in disease but later range from 400 to 26,000 (median, 2400) WBCs/μL, with neutrophils predominating. The CSF glucose level is low to normal, and the protein level is elevated.

It is important to examine a wet mount of CSF for motile amebic trophozoites. *Naegleria* trophozoites are generally destroyed by the fixation procedure for Gram stain and missed if not looked for on a wet mount. In one series, motile trophozoites in the CSF were seen in 14 of 16 patients with PAM in which a wet mount of CSF was made.[1] However, an antemortem diagnosis was made in only 27% of the 142 patients with PAM in the United States from 1937–2013, highlighting the nonspecific nature of these findings and need for a high index of suspicion for this difficult diagnosis.[29] Wright-Giemsa stain of the CSF can also highlight *N. fowleri* trophozoites.

Serologic diagnosis of PAM has limited utility in the clinical setting because most patients with PAM die soon after infection, which leaves insufficient time to mount a detectable immune response. Nevertheless, a specific antibody response to *N. fowleri* was detected in a California patient who recovered from this disease. In addition, sera collected from several individuals with a history of extensive swimming in freshwater lakes in both the southeastern United States and California had detectable antibodies to *N. fowleri*. Whether these antibodies have protective activity is not clear. More recent diagnostic tools for *Naegleria* infections include molecular methods, such as monoclonal antibodies and PCR for detecting organisms in the CSF; isoenzyme profile analysis from clinically isolated, cultured organisms; and DNA probes.[1] A real-time PCR test that is highly sensitive has recently been developed and is now considered the gold standard for the diagnosis of PAM due to *N. fowleri*.[117,118]

Acanthamoeba

In the past GAE due to *Acanthamoeba* was usually diagnosed only at autopsy. Brain biopsy is the most reliable diagnostic approach because *Acanthamoeba* spp. have only rarely been isolated from the CSF or seen on wet mount; both an immunohistochemical stain and PCR testing are available to aid in the analysis of brain tissue.[53,54,119] Exposure to *Acanthamoeba* results in detectable serum antibodies, but whether this leads to protective immunity is unknown. Serologic studies are generally not useful for diagnosis, although high titers to *Acanthamoeba* were recently used as evidence of *Acanthamoeba* GAE in an immunocompetent patient.[48] Previous studies have shown that antibodies to *Acanthamoeba* exist both in sera of persons without symptoms of disease and in patients who developed GAE or skin infections or both.[45,46]

Neuroimaging generally shows single or multiple space-occupying lesions in the brain, with or without contrast enhancement.[1,27,120] The differential diagnosis in these patients is extensive and includes toxoplasmosis, primary CNS lymphoma, tuberculosis, neurocysticercosis, nocardiosis, aspergillosis, bacterial brain abscess, and GAE secondary to *Balamuthia*. Radiographically, these entities cannot be differentiated reliably, and tissue stained with *Acanthamoeba*-specific antibodies is required for definitive diagnosis. Given the mass lesions, lumbar puncture may be contraindicated because of the risk of herniation. When performed, CSF results have been nondiagnostic, with intermediate elevations in the WBC count, elevated protein, and decreased glucose levels.[1,27] As some GAE cases with wet mounts positive for *Acanthamoeba* have been reported, CSF wet mount is recommended if lumbar puncture is performed.

Acanthamoeba skin infections are often present in patients with GAE. Thus skin nodules or ulcers should be biopsied and examined for *Acanthamoeba* in patients suspected of having GAE. *Acanthamoeba* have been successfully cultured from brain and cutaneous biopsy specimens. PCR of brain tissue has also demonstrated *Acanthamoeba* spp., including on formaldehyde-fixed or paraffin-embedded tissue.[52,120]

The successful treatment of amebic keratitis depends on early diagnosis and treatment. Diagnosis still rests on a high clinical suspicion and demonstrating *Acanthamoeba* in corneal scrapings or biopsy specimens by histopathologic examination, culture, or molecular mechanisms such as PCR or DNA probes, although in vivo confocal microscopy for visualization of cysts is being more commonly used for the diagnosis of amebic keratitis.[3,4,11,63,121] Initial corneal scrapings, Gram stains, and cultures may be misleading because many amebic keratitis cases are associated with coinfection with *Staphylococcus epidermidis, Staphylococcus aureus,* β-hemolytic streptococcus, or *Propionibacterium* spp. Culture of contact lenses and contact lens saline solution has also yielded *Acanthamoeba*.[4,11] Corneal scrapings should be examined under wet mount for motile trophozoites. Spray fixatives may best preserve the morphology of the trophozoites before air drying occurs.[122] The cysts and trophozoites can be visualized with a number of different stains, including H&E, Wright, Giemsa, periodic acid–Schiff, calcofluor white, and acridine orange.[4]

Balamuthia

Like *Acanthamoeba, Balamuthia* GAE is often diagnosed at autopsy, and several of the reported cases of *B. mandrillaris* had initially been ascribed to *Acanthamoeba* because of the difficulty in distinguishing the two morphologically. Given the lack of laboratory or clinical findings specific for *Balamuthia* GAE, tissue stained with *Balamuthia*-specific antibodies or *Balamuthia*-specific PCR from tissue or CSF is required for definitive diagnosis.[2] In brain biopsy specimens of patients with *B. mandrillaris* infection, both the cysts and trophozoites of the organism have been identified, and both an immunohistochemical stain and PCR test are available to aid in the analysis of brain tissue. Isolation of *B. mandrillaris* from CSF has only been reported once; nevertheless, in patients for whom GAE is suspected, wet mount and PCR of the CSF should be done if lumbar puncture is performed.[74,123] The CSF findings usually include a moderate lymphocytic pleocytosis, mild hypoglycorrachia, and an elevated protein level, which occasionally can exceed 1000 mg/dL.[2,70] Neuroimaging results can be quite varied. Many reports describe findings similar to *Acanthamoeba* GAE, such as multifocal lesions ranging from nonenhancing to ring-enhancing or homogeneously enhancing.[2,70] Solitary lesions have also been described,[112] as have mycotic aneurysms.[124,125] Hemorrhage in the ring-enhancing lesion, unusual for most infectious causes of such lesions, has been described in cases of *Balamuthia* and should prompt consideration of *Balamuthia*.[126,127] Although a *Balamuthia* serologic test is available, given the prevalence in apparently healthy seropositive individuals, it is unclear how useful this test is for diagnosis.[2,72]

A multiplex real-time PCR assay, used primarily on CSF or brain tissue, can simultaneously detect *N. fowleri, Acanthamoeba* spp., and *Balamuthia*. This assay targets the 18S ribosomal RNA gene and appears to be both specific and sensitive.[128] Detection of *Sappinia* has now been added to this assay.[54] Another multiplex PCR assay can simultaneously detect *Acanthamoeba, Hartmannella, Vahlkampfia,* and *Naegleria*.[129] An up-and-coming technology is next-generation sequencing in which RNA or DNA is extracted from tissue or CSF, sequenced, and the resulting sequences are queried against databases of known expression or DNA libraries. The advantage of such testing is that it should be highly sensitive and specific and does not require a priori concern for FLAs. The downside of such testing is the cost and relatively slow turnaround.[130,131]

TREATMENT

Naegleria

The optimal treatment for PAM has not been well defined, and few patients have survived this fulminant disease.[1,132]

Clinical Evidence

Amphotericin products form a cornerstone of therapy for PAM due to *Naegleria*. Most survivors of well-documented PAM have received high-dose systemic and intrathecal amphotericin B.[1,132] In vitro data suggest that amphotericin deoxycholate has greater activity against *N. fowleri* than lipid formulations of amphotericin and may therefore be preferred.[29,133] Additional drugs used in patients who have recovered from PAM include fluconazole, rifampin, sulfonamides, and azithromycin.[29,133,134] Increased intracranial pressure has been treated with ventricular drainage, hyperosmolar therapy, dexamethasone, and occasionally hypothermia.[1,133,135] Oral miltefosine, now commercially available in the United States, might be a useful adjunct.[29,133,135] Since 2013 all three surviving US patients with PAM were treated with miltefosine as part of their antimicrobial regimen, whereas among fatal US PAM cases, only about a third received this drug.[133] The three recent US PAM survivors, two children in 2013 and one adolescent in 2016, received a treatment regimen that included intravenous (IV) and intrathecal amphotericin deoxycholate, fluconazole, rifampin, azithromycin, miltefosine, and dexamethasone; two have had good functional outcomes, whereas one was left with profound neurologic deficits.[135] Adjuncts in some of these cases included CSF drainage, hyperosmolar therapy, hyperventilation, and hypothermia.[133] However, several other recent patients treated with similar regimes have not survived. The survival of patients with PAM, especially with a good neurologic outcome, likely depends on several factors, including early diagnosis and treatment, the use of appropriate combination drug therapy, and aggressive management of elevated intracranial pressure.

Animal Models and In Vitro Assays

Azithromycin was shown to be active against *N. fowleri* in a mouse model of primary amebic encephalitis.[136] The authors report that azithromycin had less activity against *N. fowleri* in vitro but nonetheless protected 100% of mice challenged in a PAM model; other macrolides appear to be less effective. Another report describes the synergistic activity of azithromycin plus amphotericin, both in vitro and in a mouse model.[137] Artemisinin derivatives were investigated in an animal model, but these agents were all inferior to amphotericin B.[138] Corifungin, a polyene macrolide (chemically related to amphotericin) was shown to have better activity against *N. fowleri* than amphotericin, both in vitro and in mouse models. This experimental drug has been granted orphan drug designation for the treatment of PAM by the US Food and Drug Administration (FDA).[139] Passive immunotherapy in animal models has been attempted, and intrathecal administration of anti-*Naegleria* immune serum or monoclonal antibody prolonged the survival of rabbits inoculated intracisternally with *N. fowleri*.[140,141]

In vitro and mouse studies suggest that that both miltefosine and voriconazole have good activity against *N. fowleri*.[142] Phenothiazines (chlorpromazine and trifluoperazine) have also demonstrated inhibitory effects against *N. fowleri* in vitro and in mouse models.[132,141]

Recommendations

Patients with suspected PAM should immediately receive high-dose IV amphotericin (preferably the deoxycholate formulation of amphotericin, although if unavailable, lipid formulations of amphotericin could be given instead) as part of their empirical antiinfective regimen; intrathecal amphotericin may have an additional role in confirmed or highly suspect cases. In the absence of clinical trials to guide therapeutic decisions for this rapidly fatal infection, combination antimicrobial therapy seems warranted and should be administered promptly, given that most survivors received multiple drugs. The addition of azoles, rifampin, azithromycin, miltefosine, or other antimicrobials should be strongly considered. All three recent US survivors of PAM received a treatment regimen that included IV and intrathecal amphotericin deoxycholate, fluconazole, rifampin, azithromycin, miltefosine, and dexamethasone, and some authors now feel this is the preferred first-line treatment regimen for this infection.[135] Aggressive management of increased ICP also seems to be an important therapeutic adjunct. For diagnostic assistance and treatment recommendations, clinicians should contact the Centers for Disease Control and Prevention (CDC) Emergency Operations Center at 770-488-7100.

Acanthamoeba Granulomatous Amebic Encephalitis and Disseminated Disease

Data are limited regarding the treatment of *Acanthamoeba* GAE. Most cases have been diagnosed postmortem, and premortem diagnosis has generally preceded death by only a few days, making evaluation of therapy difficult.

Clinical Evidence

Among the successfully treated patients with GAE, meningitis, and disseminated *Acanthamoeba* disease, all except two were given a combination of antimicrobials. These combination regimens included trimethoprim-sulfamethoxazole (TMP-SMX), flucytosine, and sulfadiazine[132]; penicillin G and chloramphenicol[132]; sulfadiazine, pyrimethamine, and fluconazole[132]; pentamidine, levofloxacin, amphotericin B, flucytosine, rifampin, and itraconazole[132]; pentamidine, flucytosine, itraconazole, topical chlorhexidine, and ketoconazole[132]; IV pentamidine and oral itraconazole[51]; fluconazole, sulfadiazine, and surgical debulking[51]; ketoconazole, rifampin, and TMP-SMX[53,54]; TMP-SMX, rifampin, and surgical debulking[143]; and oral and topical miltefosine with intrathecal and systemic amikacin.[144] The two successfully treated cases in which only a single antimicrobial was used received sulfamethazine or TMP-SMX.[132,145] In one patient with disseminated *Acanthamoeba* and AIDS, the initiation of antiretroviral therapy was felt to be a critical component of the patient's survival, in addition to treatment with itraconazole, flucytosine, rifampin, and topical chlorhexidine.[104] A recent report of an apparently immunocompetent person with *Acanthamoeba* GAE was successfully treated with surgical debulking, miltefosine, and voriconazole for 3 months.[48] Whether this combination would be effective in an immunocompromised patient is unknown. In a recent retrospective review of patients who had nonkeratitis *Acanthamoeba* infections, mortality appeared to be significantly lower among patients who had been treated with miltefosine as a component of their antimicrobial regimens than among patients who did not receive miltefosine, although this study has a number of caveats that make it suggestive but not conclusive.[146]

Animal Models and In Vitro Assays

In mice *Acanthamoeba* infections can be treated with sulfadiazine, rifampin, and flucytosine; however, this requires treatment either before or within 24 hours of infection, an unlikely clinical scenario.[11] In general, the diamidine derivatives (including pentamidine) have the greatest activity against *Acanthamoeba*. Other drugs with in vitro activity include ketoconazole, miconazole, paromomycin, polymyxin, sulfadiazine, TMP-SMX, azithromycin, neomycin, flucytosine, voriconazole, miltefosine, and, to a lesser extent, amphotericin B.[11,87]

Recommendations

The mainstay of successful treatment appears to be early diagnosis and multidrug therapy. On the basis of the experience gained from successfully treated patients, combination regimens might include pentamidine, an azole, a sulfonamide, and possibly flucytosine. Emerging evidence also indicates that miltefosine may be an important component of multidrug therapeutic regimens. However, given the wide range of anecdotally successful regimens previously listed, it is difficult to determine the most efficacious regimen. Because it is now possible to test clinical isolates in vitro for drug susceptibilities, this testing offers one potential way to guide definitive therapy.[144,147]

Acanthamoeba Keratitis

Treatment of amebic keratitis has been notably more successful than that of GAE or PAM, with medical success rates ranging from 75% to 84%.[4,60] Successful treatment requires early diagnosis and aggressive medical management, although surgical débridement and corneal transplantation are sometimes necessary. Recognition of the dendriform pattern on the corneal epithelium, the earliest recognized sign of amebic keratitis should be followed immediately with institution of antiamebic medical therapy.[4] In vitro susceptibility testing for *Acanthamoeba* spp. is available and may prove useful to guide medical therapy; however, there are reports of a poor correlation between in vitro drug susceptibilities and in vivo response of patients with amebic keratitis.[148]

Topical chlorhexidine (0.02%) and polyhexamethylene biguanide (PHMB, 0.02%) are effective against both the trophozoites and the cysts and have become the mainstay of therapy for amebic keratitis.[3,4] Using chlorhexidine or PHMB with a diamidine, propamidine (Brolene) or hexamidine (Desmodine) is common, but neither of the latter medications is available in the United States. Hexamidine monotherapy has been used in France.[4] Other agents that have been used in conjunction with chlorhexidine or PHMB include oral ketoconazole, itraconazole, voriconazole, and topical imidazoles (1%).[4] Miltefosine has excellent activity against trophozoites and is partially active against cysts,[142] but its use in combination with the cationic antiseptics is still investigational.[4] Propamidine isethionate has caused a reversible epithelial keratopathy after prolonged treatment that can be confused with recurrent amebic keratitis.[149] Topical voriconazole by itself and in combination with systemic and intrastromal injections has now been used as an adjunctive therapy to treat several patients who failed chlorhexidine and hexamidine combination therapy.[150] In addition, a recent study suggests that hydroxymethylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors, which can block the *Acanthamoeba* HMG-CoA enzyme, may soon be added to armamentarium to treat amebic keratitis.[13]

The use of corticosteroids in amebic keratitis is controversial. Crystalline keratopathy caused by viridans streptococci has occurred after topical corticosteroid therapy of amebic keratitis in two patients.[14] In animal models and in vitro studies, steroid treatment made the disease worse. However, recent studies revealed no increased risk of treatment failure in patients treated with adjuvant steroids and improved graft survival in patients who did not have inflammation at the time of graft placement.[4]

Recommendations

Topical chlorhexidine or PHMB with or without an adjuvant diamidine or other agent should be applied every hour for the first several days. Treatment is then tapered on the basis of the clinical response. Medical therapy may fail or require adjunctive surgical therapy.[3,4] Neomycin should not be used because it is ineffective against cysts, has limited clinical efficacy data, and can cause hypersensitivity.[4,148] The role of steroids remains unclear.

Balamuthia

Therapy for *B. mandrillaris* infections is not well defined. Systemic infections are associated with a high mortality rate.

Clinical Evidence

Only 12 survivors have been documented among patients in whom brain involvement has occurred. The majority of the survivors were immunocompetent and had early diagnoses of their CNS balamuthiasis, allowing antiamebic treatments to be started relatively early. Survivors were treated with various combinations that included pentamidine, flucytosine, sulfadiazine, and fluconazole or itraconazole, plus one or more of the following drugs: a macrolide, thioridazine/trifluoperazine, liposomal amphotericin, and/or miltefosine.[69,71] In four of eight cases where pentamidine was used and the two cases where thioridazine was used, these drugs were stopped relatively quickly. In Peru three patients who survived CNS balamuthiasis were treated with albendazole, fluconazole, and the addition of TMP-SMX in two cases and miltefosine in one case.[130] Three patients with isolated cutaneous balamuthiasis in Peru were treated with albendazole, itraconazole, and miltefosine; one received this regimen for 6 months and was disease free after 3 years. The US patient with isolated cutaneous disease was treated with azithromycin, fluconazole, sulfadiazine, and briefly miltefosine. Both sulfadiazine and miltefosine were stopped secondary to side effects.[114] There is limited evidence that miltefosine use may be associated with survival, but these data are not conclusive, and fatalities have occurred in patients treated with miltefosine early in their course.[146,151]

Two of these 12 survivors had poor neurologic outcomes, and both cases were associated with an increase in intracranial pressure (ICP) that was only treated after precipitous declines to a comatose state.[125,152] The increased ICP was thought to have been due to cerebral edema (not obstructive hydrocephalus), which may have led to a delay in the recognition of the elevated ICP. Thus, during the management of *Balamuthia* GAE, clinicians should be vigilant about diagnosing and treating elevated ICP.[2,125,152]

One patient with cutaneous disease reportedly noted spontaneous resolution without therapy.[153] Surgical débridement has been used in one patient with isolated cutaneous disease case and one patient who had both cutaneous and CNS disease, although it is unclear if the débridement impacted the outcomes.[2,112]

In Vitro Assays

Prior work suggested that pentamidine, azithromycin, and clarithromycin appear to have some anti-*Balamuthia* activity[154,155]; fluconazole and ketoconazole were poor inhibitors of *Balamuthia* growth; amphotericin B was marginally inhibitory; and TMP-SMX had minimal effect.[154] In vitro data suggest that miltefosine may inhibit *Balamuthia,* but voriconazole had virtually no effect on this organism.[142] A more recent study that interrogated the minimal amebicidal effect of drugs on cysts and trophozoites suggests that amphotericin, miltefosine, and pentamidine have limited efficacy in vitro.[156] A recent in vitro screen using FDA-approved drugs found that amlodipine, apomorphine, demethoxycurcumin, haloperidol, loperamide, prochlorperazine, procyclidine, and resveratrol all had amebicidal activity in two in vitro assays and at concentrations readily achieved in patients.[157]

Recommendations

The mainstay of successful regimens is multidrug therapy, but given the various combinations used, it is difficult to determine the most effective regimen. *Balamuthia* GAE should probably be treated with multidrug antimicrobial regimens that include some combination of pentamidine, flucytosine, a sulfonamide, albendazole, an azole, a macrolide, amphotericin, and miltefosine. Given the poor tolerance of some of these medications (e.g., pentamidine, amphotericin), a reasonable choice is to pursue a regimen that has limited side effects or consider an early change to other antiamebic agents in light of severe side effects. Given the current rarity of survival, it is likely that immune status and early diagnosis and medical therapy are key determinants of prognosis.

PREVENTION

Given the ubiquitous nature of *Acanthamoeba* and *B. mandrillaris,* primary prevention efforts will likely prove difficult. PAM occurs so rarely that active surveillance for *N. fowleri* in public swimming areas is probably not justified as a public health measure. However, a single source of infection, such as a popular swimming area, can cause an outbreak of PAM. Thus, because of the occurrence of clusters of patients with PAM with common environmental exposures,[8,31] health officials should consider closing implicated lakes, rivers, or streams to swimming. Swimmers can decrease their risk by avoiding activities in warm fresh water, especially if the water temperature is particularly high and the water level low, and by keeping their nose shut and avoiding sediment disturbance if they do swim in warm fresh water.[89] Domestic water supplies have recently been implicated as the source of *N. fowleri* in the United States,[28,34] and new water treatment measures are being developed to eliminate FLAs.[158] *N. fowleri* is susceptible to chlorine and can be controlled by adequate chlorination. Given cases recently associated with nasal irrigation with tap water (both for sinus irrigation and ritual ablution), water used for these practices should ideally be sterile. Preliminary experimental work indicates that vaccination using the *nfa1* gene of *Naegleria* may result in an effective immune response, but no clinical data are yet available regarding this approach.[141]

Amebic keratitis associated with contact lens use is generally preventable. Contact lenses should not be worn while swimming or showering, new contact lens cleaning solution should be used nightly, homemade saline solutions should be avoided, contact lens cases should be allowed to air dry each day, and orthokeratology should be avoided. Some authors advocate daily disposable lenses, disposing of the contact lens case after 3 months of use, or microwaving the lens case for 3 minutes on high power. Finally, commercially available contact lens disinfectant solutions that are specifically effective against *Acanthamoeba* are needed.[4,60,159]

RESOURCES

In the United States, the CDC reference laboratory in Atlanta, Georgia currently performs diagnostic testing for all FLAs. Information regarding *N. fowleri* can be found at www.cdc.gov/parasites/naegleria/, for *Acanthamoeba* at www.cdc.gov/parasites/acanthamoeba/, and for *Balamuthia* at www.cdc.gov/parasites/balamuthia/. For round-the-clock diagnostic assistance, specimen collection guidance, shipping instructions, and treatment recommendations regarding all of the FLAs, the CDC Emergency Operations Center can be contacted at 770-488-7100.

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The complete reference list is available online at Expert Consult.

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274 Malaria (*Plasmodium* Species)

Rick M. Fairhurst[a] *and Thomas E. Wellems*

SHORT VIEW SUMMARY

Definition
- A history of travel and exposure in a malaria-endemic area is typical.
- Uncomplicated malaria can easily be confused with the "flu": fever, accompanied by headaches, body aches, and malaise.
- Severe malaria can present with any one of the following: diminished consciousness, convulsions, respiratory distress, prostration, hyperparasitemia, severe anemia, hypoglycemia, jaundice, renal insufficiency, hemoglobinuria, shock, cessation of eating and drinking, repetitive vomiting, or hyperpyrexia.

Epidemiology
- Malaria is endemic in tropical and subtropical areas of Africa, South America, Asia, and Oceania.
- Malaria is transmitted by the bite of a female *Anopheles* mosquito. Falciparum malaria usually occurs within 6 months of exposure, but other malarias can occur more than a year after exposure.
- Transmission can also occur congenitally and by blood transfusion, imported mosquitoes, or autochthonous mosquitoes infected by immigrants.

Microbiology
- Plasmodia are parasitic protozoa of the Apicomplexa phylum.
- *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi* are the major human malaria parasites. Mixed infections can occur.

Laboratory Diagnosis
- Identification of parasites is by expert examination of Giemsa-stained thick and thin blood smears.
- Rapid diagnostic tests are available (e.g., BinaxNOW in the United States [US]) and are accurate for *P. falciparum* and *P. vivax* but less so for other species. Polymerase chain reaction is the most sensitive test but is not rapidly available.

Therapy (See Tables 274.3 and 274.4 for Dosages)
- Treatment depends primarily on *Plasmodium* species, disease severity, drug-resistance epidemiology, patient age, and pregnancy status.
- Uncomplicated malaria
  - Chloroquine-sensitive *P. falciparum* (Mexico, Central America west of the Panama Canal, Haiti, the Dominican Republic, and most areas of the Middle East), *P. vivax, P. ovale, P. malariae,* and *P. knowlesi* can be treated with oral chloroquine phosphate.
  - Chloroquine-resistant *P. falciparum* (most areas of the world) or chloroquine-resistant *P. vivax* (Papua New Guinea and Indonesia) can be treated with oral artemether-lumefantrine (Coartem, the preferred treatment), or with atovaquone-proguanil (Malarone), quinine sulfate *plus* doxycycline, or mefloquine (dihydroartemisinin-piperaquine is registered and available in some countries outside the US).
  - Relapses of *P. vivax* and *P. ovale* malaria can be prevented with oral primaquine phosphate (contraindicated in pregnant or breastfeeding women and glucose-6-phosphate dehydrogenase [G6PD]–deficient persons).
- Severe malaria
  - Intravenous artesunate (preferred but available only from the Centers for Disease Control and Prevention in the US), quinidine gluconate (not available in the US), or quinine dihydrochloride (not available in the US).

Prevention
- Chemoprophylaxis decisions depend primarily on drug-resistance epidemiology, patient age, and pregnancy status.
  - Areas with chloroquine-sensitive malaria: chloroquine phosphate
  - Areas with mefloquine-sensitive malaria: mefloquine
  - All areas: atovaquone-proguanil or doxycycline
  - Areas with mostly *P. vivax:* primaquine (contraindicated in pregnant or breastfeeding women and G6PD-deficient persons)
- Mosquito repellents and avoidance measures

THE MALARIA PROBLEM

Malaria remains an overwhelming problem in tropical developing countries, accounting for an estimated 216 million cases and 445,000 deaths in 2016.[1–3] Nearly 40% of the world's population is at risk for acquiring malaria.[4–7] In sub-Saharan Africa, most severe cases and deaths occur in children younger than 5 years and in pregnant women.

The introduction of chloroquine and dichlorodiphenyltrichloroethane (DDT) at the end of World War II brought dramatic new power to malaria control efforts worldwide. With postwar economic recovery and a renewed spirit of international cooperation, optimism ran high that the widespread use of these new compounds would eliminate malaria, and in 1955, the World Health Organization (WHO) launched its campaign to eradicate the disease. This goal proved overly optimistic, and the centrally organized DDT spraying programs at the core of the campaign were discontinued in 1967. The campaign, nevertheless, brought regional successes that coincided with other factors to reduce malaria incidence in many areas of the world (e.g., in Asia) (Fig. 274.1A).[8]

A stark exception to this general progress has been sub-Saharan Africa, where malaria remains deeply entrenched. Even the most committed spraying and eradication programs in endemic areas of this region could not defeat malaria's efficient transmission by the *Anopheles gambiae* mosquito.[9] The wide availability and use of chloroquine did, however, boost the health of young African children who suffer most from *Plasmodium falciparum,* the species responsible for the deadliest forms of malaria. As chloroquine became increasingly available in the 1950s to 1970s, death rates from malaria in Africa began to drop, approaching half the level of the prechloroquine years.[10]

Unfortunately, the massive use of chloroquine (hundreds of tons sufficient for hundreds of millions of treatments annually) in the 1980s[11] selected for chloroquine-resistant *P. falciparum* strains in Southeast Asia that entered and spread across Africa. In the 1980s and 1990s,

[a]All material in this chapter is in the public domain, except any borrowed figures or tables.

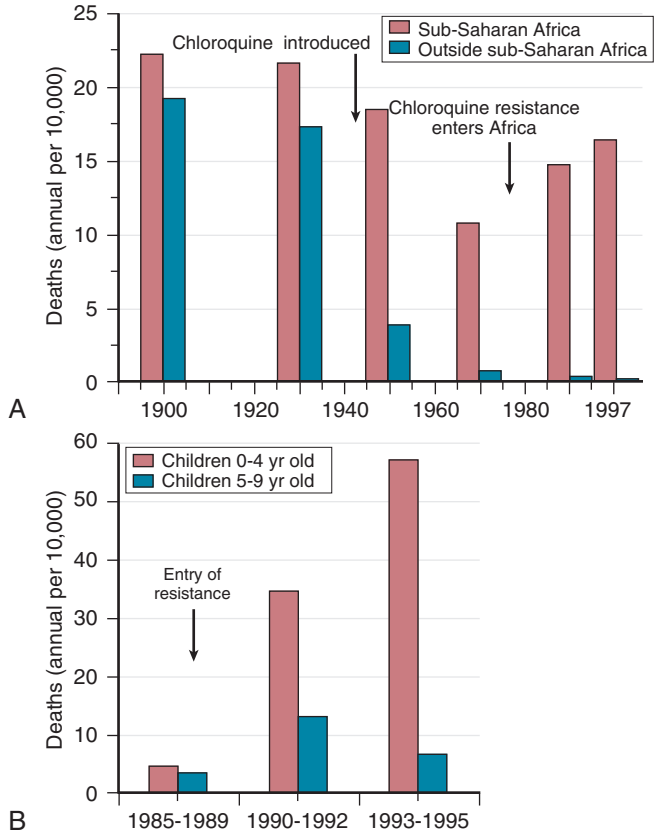

FIG. 274.1 Malaria death rates after the introduction of chloroquine and subsequent evolution of chloroquine-resistant *Plasmodium falciparum*. (A) Malaria death rates in the 20th century. Dramatic reductions in mortality were achieved outside sub-Saharan Africa. In Africa, mortality rates declined after the introduction of chloroquine but rose again after the spread of chloroquine resistance across the sub-Saharan region. (B) Rise in mortality among children in the village of Mlomp, Senegal. Increased death rates were observed after chloroquine resistance entered the village, chiefly among children younger than 5 years, the most susceptible age group in highly endemic areas. *(A modified from Carter R, Mendis KN. Evolutionary and historical aspects of the burden of malaria.* Clin Microbiol Rev. *2002;15:564–594. B modified from Trape JF, Pison G, Preziosi MP, et al. Impact of chloroquine resistance on malaria mortality.* C R Acad Sci III. *1998;321:689–697.)*

malaria resurged and death rates increased. The impact of chloroquine resistance was especially evident in young children, who do not have the partially protective immunity to malaria that usually develops after repeated episodes of the illness (Fig. 274.1B).[12,13] In the absence of a fully effective vaccine, success against malaria in Africa will continue to depend on effective drugs, such as artemisinin-based combination therapies (ACTs), that are reliable, affordable, and readily available. Although increased international support and funding for prevention, control, and elimination reinvigorated the efforts of malaria control programs, decreasing the incidence of malaria by an estimated 18% globally between 2010 and 2016, the number of cases and deaths have plateaued in the past several years.[2] Continued progress against the disease will require strengthened community commitments and health infrastructures, supported by new and improved drugs, advances in diagnostics, more effective vaccines, and better insecticides and vector control measures.[14]

PLASMODIUM AND ITS LIFE CYCLE

Plasmodium parasites belong to the Apicomplexa group of protozoa, which includes other pathogens, such as *Babesia, Toxoplasma,* and *Cryptosporidium* species. Apicomplexa are distinguished morphologically by the presence of a specialized complex of apical organelles (i.e., micronemes, rhoptries, and dense granules) involved in host cell invasion (see Fig. 274.4A).[15] Four *Plasmodium* species are classified as human malaria parasites: *P. falciparum, P. vivax, P. ovale,* and *P. malariae.* Some malaria parasites of other primates (e.g., *P. knowlesi, P. cynomolgi,* and *P. simium*) can also infect humans under natural conditions.[16] Indeed, with the recently appreciated extent of human infections from *P. knowlesi,* a natural pathogen of macaque monkeys, this parasite has been proposed to be a "fifth human malaria parasite" responsible for significant morbidity and mortality in Malaysia.[17–21]

In 1880, Alphonse Laveran[22] first observed malaria parasites in a human blood sample, witnessing the exflagellation of microgametes that usually emerge in the mosquito. It was eventually established that parasites in the bloodstream reproduce asexually in the haploid state (Fig. 274.2). During erythrocytic development, a small minority of parasites undergo a switch to sexual-stage development.[23] The resulting male and female gametocytes are the forms that are taken up by and infect anopheline mosquitoes, as proved by Ronald Ross and Battista Grassi[24,25] in the 1890s. Gametocytes emerge from erythrocytes in the mosquito midgut as male and female gametes that cross-fertilize to form diploid zygotes, which in turn differentiate into ookinetes that burrow through the midgut wall. Each ookinete develops into an oocyst containing up to 1000 sporozoites, which emerge and are then carried by the insect hemolymph to invade the salivary glands. These processes in the mosquito require an incubation period of about 1 to 2 weeks.

Female mosquitoes inject sporozoites into humans while probing the dermis in preparation for taking a blood meal. Shortt and Garnham[26] demonstrated in 1948 that sporozoites must first invade and replicate in hepatocytes before they can differentiate into merozoites capable of entering the intraerythrocytic cycle. The injected sporozoites typically take several hours to travel through dermal tissues and migrate across host cell barriers before they enter blood and lymphatic systems and are carried to the liver.[27] A molecular motor installed between the sporozoite plasma membrane and a double, inner membrane complex powers motility in this journey, whereas sporozoite surface proteins that are linked to this motor provide traction for gliding and crossing cellular barriers in tissue transit and invasion.[28,29]

Invasion of sporozoites into hepatocytes takes place by a coordinated series of steps, including host cell contact, signaling events with discharge of calcium, release of ligands and processing molecules from apical organelles, and active entry of the sporozoite into an induced parasitophorous vacuole in the hepatocyte cytoplasm. The host tetraspanin molecule CD81 is important for sporozoite entry into hepatocytes.[30,31] Two studies have shown that the class B type 1 scavenger receptor SR-B1, a known coreceptor with CD81 for invasion of hepatocytes by hepatitis C virus, also promotes efficient *Plasmodium* infection of hepatocytes.[32,33] A likely role for SR-B1 is the organization of CD81 into tetraspanin-enriched microdomains that are preferred membrane areas for sporozoite entry.[32] Because SR-B1 is vital in providing cholesterol to the hepatocyte by high-density lipoprotein (HDL)–cholesteryl ester uptake, its exploitation by *Plasmodium* and hepatitis C virus represents the evolutionary selection of a dependable invasion pathway by these pathogens. SR-B1's role in HDL–cholesteryl ester uptake and activation of the liver fatty acid–binding protein (L-FABP) carrier also supports the transformation and massive growth requirements of a parasite inside its host hepatocyte. Individual infected hepatocytes support the development of 10,000 to 30,000 merozoites, a process that is not associated with symptoms. All *P. falciparum* and *P. malariae* parasites complete their liver-stage development in about 1 to 2 weeks.[34] *P. vivax* and *P. ovale* liver stages also can develop promptly or can remain latent as hypnozoites in the liver for months to years before emerging to produce relapses of malaria (see Fig. 274.2).

Once a merozoite[35] egresses by protease activity from its host hepatocyte (or from its host erythrocyte in the bloodstream cycle),[36] it engages loosely with an uninfected erythrocyte and then reorients so that its apical end faces the cell surface.[37] The merozoite then drives itself into the erythrocyte through a ring-shaped, electron-dense junction that moves from the front to the back end of the merozoite by the power of an actin-myosin motor.[37] An envelope of invaginated membrane surrounds the merozoite as it enters, forming the parasitophorous vacuole once invasion is complete.[38] These steps of invasion are supported by cell-signaling events, energy-dependent migration, and discharge of contents from the rhoptries, micronemes, dense granules, and perhaps

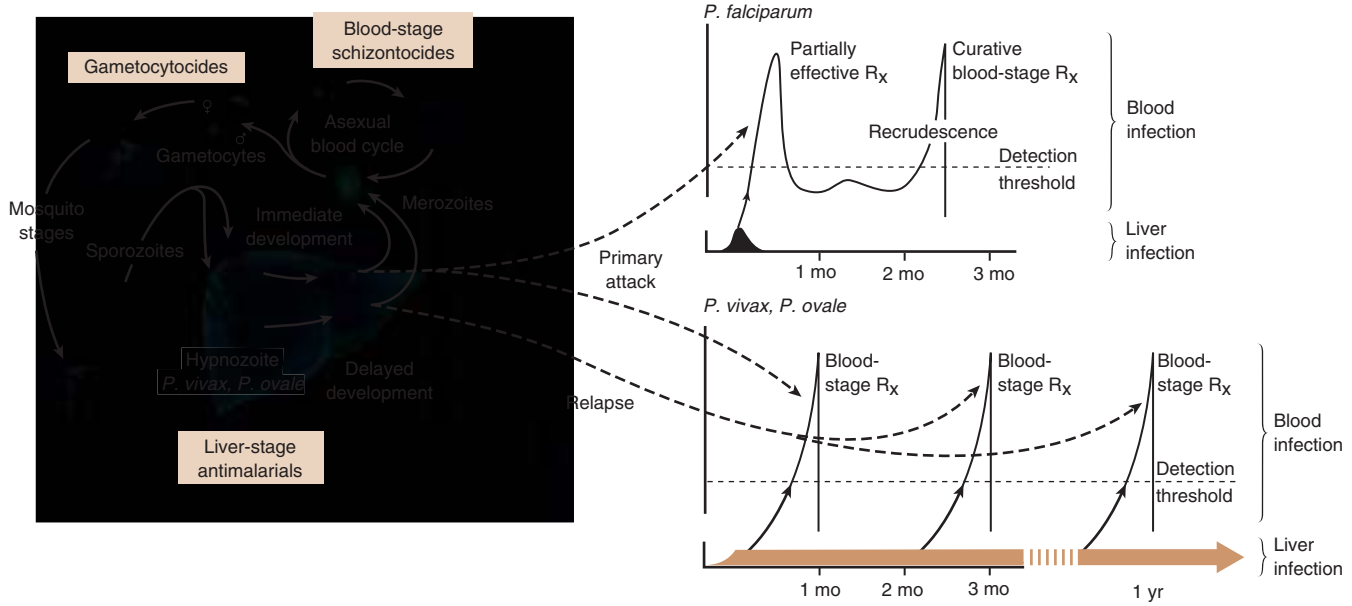

FIG. 274.2 The *Plasmodium* life cycle and disease patterns of recrudescence and relapse. Anopheline mosquitoes transmit malaria by injecting sporozoites into the human host. The sporozoites then invade hepatocytes, in which they develop into schizonts. Each infected hepatocyte ruptures to liberate 10,000 to 30,000 merozoites that invade circulating erythrocytes. Growth and development of the parasites in red cells result in subsequent waves of merozoite invasion. This asexual blood cycle repeats every 24 (*Plasmodium knowlesi*), 48 (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*), or 72 (*Plasmodium malariae*) hours, leading to amplification of parasite density; paroxysms of chills, fevers, and sweats; and other manifestations of disease. Malaria symptoms are typically experienced 2 to 4 weeks after the mosquito bite. If the parasites are not cleared (e.g., patient receives partially effective therapy), recrudescence of parasitemia and malaria symptoms can occur. Eradicating parasites with an effective drug regimen cures malaria. Some *P. vivax* and *P. ovale* parasites can postpone their development in the liver, persisting as latent forms called hypnozoites. Hypnozoites are not eradicated by standard therapy (e.g., chloroquine) directed against blood stages. Resumption of hypnozoite development months to years after initial infection can lead to malaria relapse that requires an additional round of drug therapy to treat recurrent symptoms and eradicate blood-stage parasites. A course of primaquine can prevent relapses of malaria, but this treatment may not always be successful because of inadequate patient compliance, interindividual variations of drug metabolism, or variable parasite responses to low or high drug doses.

other apical compartments.[39] In *P. falciparum,* invasion can be supported by multiple different interactions between parasite molecules and erythrocyte surface molecules, including glycophorins.[40,41] Recent work has demonstrated the essential role of a ligand-receptor interaction between the *P. falciparum* RH5 (PfRH5) protein and the Ok blood group antigen, basigin.[42] A number of studies have also established a dependence of *P. vivax* merozoites on interaction with erythrocyte Duffy antigen receptor for chemokines (DARC),[43,44] but this may not be an absolute requirement considering that *P. vivax* infections occur in DARC-negative populations of Africa.[45–47]

Within erythrocytes, merozoites develop from ring forms into trophozoites and then schizonts over 24 hours (*P. knowlesi*), 48 hours (*P. falciparum, P. vivax,* and *P. ovale*), or 72 hours (*P. malariae*). After breaking down their host cell membrane by enzymatic digestion, 24 to 32 merozoites enter the bloodstream and are each capable of infecting a new erythrocyte. Cycles of invasion and growth in erythrocytes produce a parasite biomass that enlarges rapidly, causing fever and leading to pathologic processes, such as erythrocyte loss (anemia), sequestration of infected erythrocytes in microvascular beds (cerebral malaria), and adverse sequelae of inflammatory cascades and cytokine release.

PATHOPHYSIOLOGY

The Malaria Paroxysm and General Considerations

Malaria presents as an acute febrile illness that is often but not always characterized by the classic malaria paroxysm: chills and rigors, followed by fever spikes up to 40°C (104°F), and then profuse sweating that can ultimately give way to extreme fatigue and sleep. Paroxysms last several hours, can occur with a regular periodicity coinciding with the synchronous rupture of blood schizonts, may alternate with relatively asymptomatic periods, and are associated with high levels of tumor necrosis factor (TNF).[48] Paroxysms can occur in 24-hour, tertian 48-hour, or quartan 72-hour cycles, or in other more complicated patterns.[49] TNF may originate from monocytes stimulated by glycosylphosphatidylinositol moieties or other substances released on schizont rupture.[50,51]

Malaria can be acutely malignant and painful or more indolent and undermining. It predisposes African children to bacteremia[52] and increases the morbidity and mortality associated with other diseases by stressing host systems and producing effects such as dehydration, anemia, and some degree of immune suppression. Malaria is tremendously debilitating to health and impedes economic development through its adverse effects on fertility, population growth, saving and investment, worker productivity, absenteeism, premature mortality, and medical costs.[53,54] A single episode of malaria has been estimated to result in a loss of 5 to 20 working days, and an agricultural family afflicted by malaria may be up to 60% less productive than a family without malaria.[55]

Plasmodium falciparum

P. falciparum malaria can be much more acute and severe than malaria caused by other *Plasmodium* species (Fig. 274.3). Although *P. vivax* can cause serious and fatal illness,[56,57] by far the largest fraction of deaths directly attributable to malaria are caused by severe complications of *P. falciparum* infection, including cerebral malaria, severe anemia, respiratory distress, renal failure, and severe malaria of pregnancy.[58–63] Important contributory factors include metabolic acidosis, hypoglycemia, and superimposed bacterial infections. Fatal *P. falciparum* infections are often associated with the failure of multiple organ systems.

An important feature of the pathogenesis of *P. falciparum* is the ability of its mature trophozoite and schizont forms to sequester in the deep venous microvasculature. This sequestration is promoted by a number of processes: the adherence of infected erythrocytes to endothelial cells[64–66] (Fig. 274.4F and G); rosetting—the binding of infected erythrocytes to uninfected erythrocytes[66–68] (see Fig. 274.4H); reduced erythrocyte deformability[69,70] (see Fig. 274.4C and D); and platelet-mediated clumping of infected erythrocytes.[71,72] In malaria of pregnancy, infected erythrocytes accumulate within the proteoglycan matrix of placental intervillous spaces.[73] *P. falciparum*–infected erythrocytes can thus accumulate throughout the body, including the heart,[74] lung,[75] liver,[76] brain,[75,77–80] kidney,[81] intestine,[75,77] dermis,[77] bone marrow,[82] and placenta.[83] Uninfected erythrocytes, monocytes, platelets, and deposits

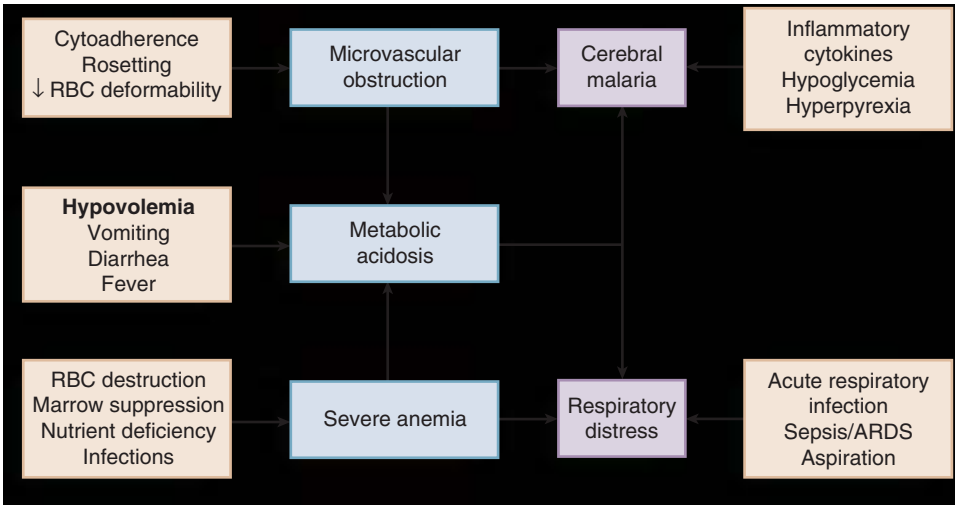

FIG. 274.3 Pathogenesis of severe *Plasmodium falciparum* malaria. Deaths from severe *P. falciparum* malaria are commonly attributable to the effects of severe anemia, cerebral malaria, and respiratory distress in young children. This schematic illustrates how multiple pathogenic events such as cytoadherence, destruction of uninfected erythrocytes, and production of inflammatory cytokines combine to produce the microvascular sequestration and metabolic acidosis that are central to the development of severe disease. *ARDS,* Acute respiratory distress syndrome; *RBC,* red blood cell.

of thrombin and fibrin are often found in association with these infected erythrocytes.[75,78,80,81]

By sequestering in microvessels, *P. falciparum* may avoid filtration and destruction by the spleen and thus multiply to high densities.[84] The survival and propagation of parasites may be enhanced when they sequester in the low-oxygen-tension environment of postcapillary venules. Attachment points to endothelium have been shown by electron microscopy to be dense protrusions, termed *knobs,* on the surface of infected erythrocytes (see Fig. 274.4D and E), where antigenically variant cytoadherence proteins (*P. falciparum* erythrocyte membrane protein 1 [PfEMP-1] variants) are anchored. Attachment at knobs (see Fig. 274.4F) supports cytoadherence in vitro and sequestration in vivo (see Fig. 274.4G).[85,86] Under flow conditions, cytoadherence events are reminiscent of leukocyte adhesion, involving distinct phases of tethering, rolling, and stable adhesion.[87]

PfEMP-1 is central to malaria pathogenesis.[88] PfEMP-1 is a family of antigenically variant proteins encoded by the multicopy *var* gene family.[89] Approximately 60 different *var* genes are present in the haploid genome of each parasite, encoding PfEMP-1 variants with unique antigenic and cytoadherence properties.[90,91] A single PfEMP-1 variant is thought to be predominantly expressed on the surface of an individual infected erythrocyte,[92] whereas all others are silenced.[93] Switches in expression between individual members of the *var* gene family occur at an estimated rate of 2% to 18% per cell per generation[94,95] and produce the antigenic variation in *P. falciparum* populations during the course of an infection. These switches may be structured to safeguard sequential expression among variant antigenic genes and thereby promote longevity of infection.[96]

The various PfEMP-1 proteins exposed on knobs have binding domains that adhere to host molecules, including CD36, intercellular adhesion molecule 1 (ICAM-1), thrombospondin, platelet endothelial cell adhesion molecule (PECAM/CD31),[87,97–100] chondroitin sulfate A (CSA),[73,101] and endothelial protein C receptor, which is involved in the deadly sequestration of parasitized erythrocytes that leads to cerebral malaria.[102–104] In addition to the association of cerebral malaria with subsets of PfEMP-1 variants that adhere to brain endothelium, other pathologic conditions have also been linked to particular PfEMP-1 variants and host receptors.[64,105–110] CD36 is an important cytoadherence receptor expressed on microvascular endothelium, as well as on monocytes and platelets, and is thought to mediate the sequestration of parasites as well as the host immune response to infection.[111] Infected erythrocytes that bind CSA expressed by syncytiotrophoblasts sequester selectively in placental tissue and are responsible for malaria of pregnancy.[73]

Some PfEMP-1 variants are also important parasite ligands in rosetting[112] because they can adhere to complement receptor 1 (CR1)[113] and blood group A antigen[114] on uninfected erythrocytes. A human CR1 polymorphism that reduces *P. falciparum* rosetting was found in one study to protect against severe malaria[115]; data from other studies of rosetting and disease severity have, in some cases, shown an association[116–120] and in others have not.[121,122] PfEMP-1 variants that bind to glycoprotein CD36 on the surface of platelets are also thought to play an important role in the platelet-mediated clumping of infected erythrocytes.[71]

P. falciparum can infect erythrocytes of all ages,[123] promoting heavy parasite burdens. High parasite densities,[124] increased parasite multiplication rates,[125] and evidence of high parasite biomass (e.g., intraleukocytic pigment, mature trophozoites, and schizonts) on peripheral blood smears are associated with increased severity of malaria and death.[126–128] Such high peripheral blood parasitemias are not observed with the severe pathology of vivax malaria. *P. vivax* parasites selectively infect reticulocytes[129,130] and accumulate as developing sexual stages (gametocytes) and mature replicative stages (schizonts) in the bone marrow and liver.

Cerebral Malaria

The classic histopathologic finding of fatal cerebral falciparum malaria is the intense sequestration of infected erythrocytes in cerebral microvessels (see Fig. 274.4G), often accompanied by "ring" hemorrhages, perivascular leukocyte infiltrates, thrombin deposition, activated platelets,[79,131–135] and immunohistochemical evidence for endothelial cell activation.[78,136,137] In one autopsy series of patients who died from cerebral malaria, 94% of brain microvessels contained adherent infected erythrocytes, compared with 13% of patients who died from noncerebral malaria.[138] In another study, 7 of 31 (24%) African children who received a clinical diagnosis of cerebral malaria were found at autopsy to have nonmalarial causes of coma, underscoring the possibility that other illnesses may mimic the cerebral malaria presentation in areas where incidental parasitemia is common.[133]

Sequestration of *P. falciparum*–infected erythrocytes stimulates the local production of inflammatory cytokines, such as TNF, elevated levels of which may correlate with disease severity.[139–141] These cytokines and other inflammatory mediators also upregulate adhesion molecules, such as ICAM-1, in the cerebral microvasculature,[142,143] which may lead to further sequestration of infected and uninfected erythrocytes, leukocytes, and activated platelets.[131] Impaired nitric oxide bioavailability may also contribute to endothelial dysfunction[144] by increasing microvascular tone, endothelial cell adhesion molecule expression, cytokine production, and infected erythrocyte sequestration.[145] These processes may cause

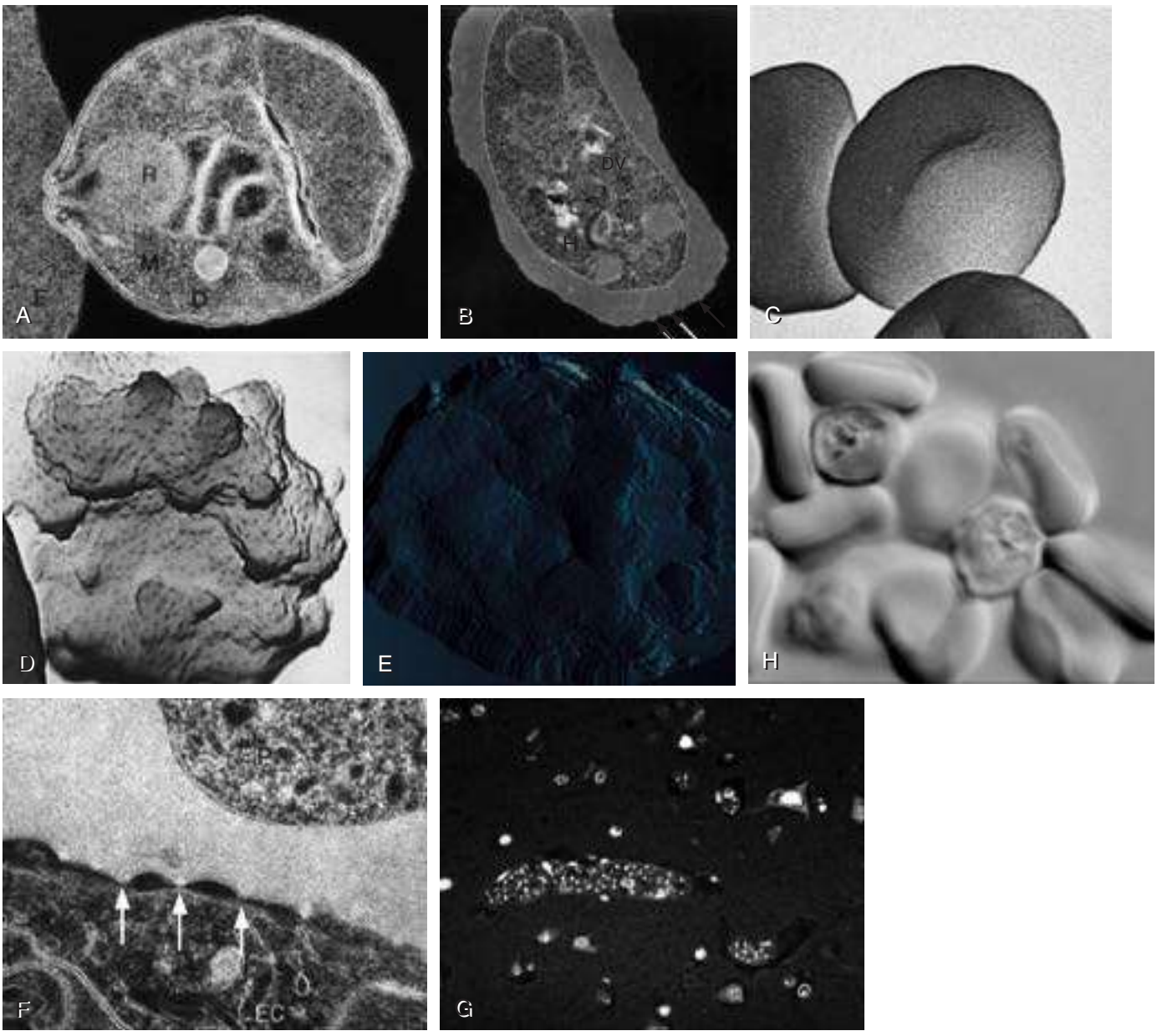

FIG. 274.4 Morphologic features of *Plasmodium* parasites. (A) Transmission electron micrograph of a *P. knowlesi* merozoite invading an erythrocyte (*E*) via its apical end, which contains rhoptries (*R*), micronemes (*M*), and dense granules (*D*). (B) Transmission electron micrograph of an intraerythrocytic *P. falciparum* trophozoite containing cytosomes, digestive vacuole (*DV*), and crystalline hemazoin (*H*). Arrows identify numerous small electron-dense protrusions, termed *knobs,* on the surface of the host erythrocyte. (C and D) Scanning electron micrographs demonstrate the effects of the malaria parasite on its host erythrocyte. Distention from the growth of the parasite *P. falciparum* converts the erythrocyte from a deformable biconcave disk (C) to a nondeformable cell (D) displaying knobs over its surface. (E) Atomic force microscopic image of the surface of a *P. falciparum*–infected erythrocyte showing numerous knob structures. (F) Transmission electron micrograph showing adherence via knobs *(arrows)* between a *P. falciparum*–infected erythrocyte (*P*) and a host endothelial cell (*EC*) of a cerebral microvessel. (G) Histologic section of brain tissue showing pigmented mature *P. falciparum* parasites sequestered in microvessels. (H) Light microscopic image of rosetting, the binding of a *P. falciparum*–infected erythrocyte to multiple uninfected erythrocytes. *(A from Fujioka H, Aikawa M. The malaria parasite and its life cycle. In: Wahlgren M, Perlmann P, eds.* Malaria: Molecular and Clinical Aspects. *Harwood Academic; 1999:19–55. B courtesy Hisashi Fujioka, Cleveland, OH. C and D from Aikawa M, Rabbege JR, Udeinya IJ, et al. Electron microscopy of knobs in* Plasmodium falciparum*–infected erythrocytes.* J Parasitol. *1983;69:435–437. E courtesy James Dvorak and Takayuki Arie, Bethesda, MD. [From Atkinson CT, Aikawa M. Ultrastructure of malaria-infected erythrocytes.* Blood Cells. *1990;16:351–368]. G courtesy Hisashi Fujioka, Cleveland, Ohio. H courtesy James Dvorak, Bethesda, MD.)*

varying degrees of functional obstruction and consequently impair local delivery of oxygen and glucose. However, obstruction by infected erythrocytes and other blood elements does not generally produce neurologic sequelae akin to those that follow the physical occlusion in thrombotic stroke, because most patients with cerebral malaria who recover can do so rapidly within 48 hours and without such consequences. Systemic sequestration of metabolically active parasites, blood cells, and platelets likely contributes to the metabolic acidosis and thrombocytopenia commonly seen in severe malaria. Metabolic acidosis, hypoglycemia, hyperpyrexia, and nonconvulsive status epilepticus can contribute significantly to the cerebral malaria presentation, as suggested by the rapid clinical improvement of some patients after fluid resuscitation, blood transfusion, dextrose infusion, fever reduction, and anticonvulsant therapy in addition to antimalarial treatment.[146–149]

Hypoglycemia

Hypoglycemia in malaria can cause coma and convulsions and thus may contribute substantially to the morbidity and mortality associated with cerebral malaria.[58] The pathophysiologic mechanisms of hypoglycemia in children and adults seem to be different. In children, insulin

levels are appropriate and hypoglycemia is associated with impaired hepatic gluconeogenesis and increased consumption of glucose by hypermetabolic peripheral tissues.[148,150–153] Large amounts of glucose are also consumed by intraerythrocytic parasites.[154] In adults, hypoglycemia is often associated with hyperinsulinemia,[155] which may result from pancreatic islet cell stimulation by parasite-derived factors or parenteral quinine or quinidine therapy, or both.[156] Depletion of liver glycogen stores after decreased food intake during the prodromal period may also contribute to hypoglycemia.

Anemia

The pathophysiology of malarial anemia is multifactorial and complex.[157–159] The intravascular lysis and phagocytic removal of infected erythrocytes[160] contribute to anemia but do not account for the dramatic reductions in erythrocyte mass that can accompany acute *P. falciparum* malaria episodes. Additional processes have therefore been implicated in the pathogenesis of malarial anemia. Excess removal of uninfected erythrocytes may account for up to 90% of erythrocyte loss[161] and may be mediated by processes (e.g., oxidative stress) that accelerate the senescence and reduce the deformability of erythrocytes. The contribution of impaired erythropoietic responses to malarial anemia is significant and probably involves general processes also found in other diseases. Release of inflammatory cytokines (e.g., TNF) is associated with impaired production of erythropoietin,[162,163] decreased responsiveness of erythroid progenitor cells to adequate levels of erythropoietin,[164,165] and increased erythrophagocytic activity.[166] These pathogenic processes account for the normochromic normocytic anemia seen in malaria and explain the notable absence of a robust reticulocyte response. Although microcytosis and hypochromia are seen in malaria, these findings are often attributable to thalassemias and iron deficiency in endemic areas. Bacteremia, nutritional deficits (e.g., vitamin A and B_{12} deficiencies), concomitant infections (e.g., hookworm and *Schistosoma*), and genetic polymorphisms (glucose-6-phosphate dehydrogenase [G6PD] deficiency) have also been associated with greater degrees of anemia in malaria episodes,[167] presumably by lowering the baseline from which hemoglobin levels acutely decline. In endemic areas where chloroquine resistance is prevalent, the inability of young children to clear their parasitemias with chloroquine contributes to their higher baseline prevalence of anemia when compared with children treated with more effective drugs.[168]

Pulmonary Edema and Respiratory Distress

The most significant pulmonary manifestation directly attributable to *P. falciparum* is noncardiogenic pulmonary edema.[169,170] Sequestration of infected erythrocytes in the lungs is thought to initiate regional production of inflammatory cytokines that increase capillary permeability, leading sequentially to pulmonary edema, dyspnea, hypoxia, acute lung injury, and acute respiratory distress syndrome.[171,172] Pulmonary edema is common with severe malaria in adults but infrequent in children, and it is not associated with pleural effusion. Iatrogenic fluid overload and acute renal failure may contribute to the development or worsening of pulmonary edema. Although pulmonary edema usually occurs after other features of severe disease (e.g., coma, acute renal failure) become manifest, it may occur at any time during the clinical course, even when the patient appears to be recovering on antimalarial therapy. Dyspnea and increased respiratory rate are features of impending pulmonary edema and often precede other clinical (e.g., use of accessory muscles of respiration) and radiologic (e.g., generalized increase in interstitial markings) signs.

Pulmonary manifestations of deep breathing and respiratory distress associated with severe malaria may also arise from metabolic acidosis,[59] severe acute respiratory infections,[152] sepsis-related acute respiratory distress syndrome, aspiration (especially with diminished consciousness or convulsions), and nosocomial pneumonia. Cerebral pathologic processes may result in abnormal breathing patterns, including Cheyne-Stokes respirations and respiratory failure.[173]

Metabolic (Lactic) Acidosis

Metabolic acidosis is a common feature of severe malaria and is associated with significant lactic acidemia in up to 85% of cases. Metabolic acidosis is principally caused by reduced delivery of oxygen to tissues, from the combined effects of anemia (decreased oxygen-carrying capacity), sequestration (microvascular obstruction), and hypovolemia (reduced perfusion) resulting from fluid losses caused by fever, decreased oral intake, vomiting, and diarrhea.[174] These effects produce a shift from aerobic to anaerobic metabolism and cause lactate levels to increase.[175] The following factors may also contribute to metabolic acidosis: production of lactate by anaerobic glycolysis in sequestered parasites[176]; reduction of hepatic blood flow, leading to diminished lactate clearance[177,178]; induction of lactate production by TNF and other proinflammatory cytokines[179]; impairment of renal function[177]; and ingestion of exogenous acids (e.g., salicylate) or unknown constituents of traditional herbal remedies for fever.[180]

Malaria of Pregnancy

Placental malaria results in maternal morbidity and mortality, intrauterine growth retardation, premature delivery, low birth weight, and increased newborn mortality.[181–183] Selective accumulation of infected erythrocytes in the placenta involves their interaction with syncytiotrophoblastic CSA,[73] which is possibly complemented by interactions with other molecules, such as hyaluronic acid and immunoglobulins.[184–186] This is in contrast to the sequestration of infected erythrocytes in the systemic microvasculature, where CD36 is the major endothelial receptor. Parasites that accumulate in the placenta express PfEMP-1 variants that bind CSA[187,188] but not CD36.[189] Evidence suggests that women experiencing a malaria episode from CSA-binding parasites during their first pregnancy lack immunity to the PfEMP-1 antigenic variants presented by these strains and, despite immunity to CD36-binding variants from previous infections, are highly susceptible to the new placental infection. Placental malaria in subsequent pregnancies is typically less severe than in the first pregnancy,[190] presumably because of a woman's previous exposure to CSA-binding parasites.

Plasmodium vivax and *Plasmodium ovale*

Infections with *P. vivax* and *P. ovale* can be considered similar to each other from a clinical perspective. *P. vivax* infections can be tremendously debilitating and are sometimes associated with serious complications, including acute lung injury[191,192] and splenic pathology.[193,194] Splenic rupture has been associated with acute and chronic infections and can occur spontaneously or with minor trauma, including manual examination of the spleen. Although more commonly associated with *P. vivax* malaria, splenic rupture has been associated with all four human malaria parasites. Anemia is frequently observed as a consequence of acute or chronic infections, or as a result of repeated acute infections.[160] Suppressed erythrocyte production and hemolysis of both infected and uninfected erythrocytes have been implicated in the pathogenesis of *P. vivax* malarial anemia. Although not often fatal, *P. vivax* infections have recently been associated in Papua New Guinea and Indonesia with severe disease manifestations, including cerebral malaria, severe anemia, and respiratory distress.[56,57,195]

P. vivax merozoites selectively invade reticulocytes.[130] Because these cells account for only a small proportion of the total erythrocyte mass, parasitemias in *P. vivax* infections are usually less than 1% even when the pathology of vivax malaria is severe. *P. vivax* parasites can accumulate as developing sexual stages (gametocytes) and mature replicative stages (schizonts) in the bone marrow and liver.[196] Recent evidence suggests that *P. vivax* can cytoadhere to lung endothelial cells[197] and can cause inflammation and injury by sequestering in the pulmonary microvasculature.[198] *P. vivax* parasites may avoid splenic entrapment by increasing rather than decreasing erythrocyte deformability.[199]

Plasmodium malariae

The quartan malaria of *P. malariae* usually presents with fever and paroxysms similar to those of *P. vivax* but with a 3-day rather than 2-day periodicity. *P. malariae* often establishes parasitemias that are below the level of detection by microscopy. Patients can remain infected and asymptomatic for many years before presenting with fevers, malaise, and splenomegaly decades after they have left an endemic area.[200] Chronic *P. malariae* infection can lead to nephrotic syndrome in young children living in endemic areas.[201,202] This complication has features of an immune complex–mediated glomerulonephritis.[203,204]

Plasmodium knowlesi

A large focus of human malaria caused by *P. knowlesi* occurs in Malaysia, where high case-fatality rates have been reported.[17,205] *P. knowlesi* is indistinguishable from *P. malariae* on blood smear examination, showing both immature and mature forms in the circulation. Unlike *P. malariae,* however, *P. knowlesi* replicates every 24 hours and can cause daily fever spikes and hyperparasitemias that are life threatening. In addition to hyperparasitemia, severe *P. knowlesi* malaria cases have been associated with metabolic acidosis, hepatorenal dysfunction, respiratory distress, severe anemia, and refractory hypotension.[18,205]

GENETIC RESISTANCE TO MALARIA

Fatal *P. falciparum* malaria has been a potent evolutionary force in shaping the human genome. Evidence for the natural selection of genetic polymorphisms can be found in the ethnic and geographic distributions of hemoglobin variants, thalassemias, G6PD deficiencies, erythrocyte membrane proteins, and cytokines and other mediators of inflammation and immunity.[206–215]

Hemoglobins S, C, and E

The geographic distributions of hemoglobin variants overlap considerably with those of *P. falciparum* malaria.[206,208] Case-control and longitudinal cohort studies have associated malaria protection with hemoglobin S (HbS) heterozygosity (HbAS; sickle cell trait), in which the sixth amino acid of one β-globin chain is changed from glutamate to valine.[216–220] The genetic fitness of HbS homozygosity (HbSS; sickle cell disease) is very poor in sub-Saharan Africa,[221] whereas the prevalence of HbAS individuals can be more than 25% in some areas.[222] The HbS mutation thus exists as a balanced polymorphism: the malaria protective benefit afforded to HbAS heterozygotes offsets the childhood deaths of HbSS homozygotes. The hemoglobin C (HbC) mutation is in the same sixth position as the HbS mutation but differs in that the amino acid is changed from glutamate to lysine. A number of case-control studies in West Africa have associated HbC with malaria protection.[216,223–227]

Most studies have associated HbAS protection against malaria with reductions in parasite density.[216] However, some epidemiologic studies have found similar parasite densities in HbAA and HbAS children, very high parasite densities in some HbAS children,[228] and occasional cases of severe malaria in HbAS children.[223,229] These observations are not fully explained by proposals that infected HbAS erythrocytes are more likely to sickle or support reduced parasite growth rates under conditions of low oxygen tension in microvessels,[230–233] by enhanced phagocytosis of infected HbAS erythrocytes by macrophages,[234,235] or by translocation of sickle cell erythrocyte microRNAs that may negatively regulate parasite growth in the infected host cell.[236] Other mechanisms of protection that include additional genetic or environmental factors are likely operating in HbAS children. Unlike for HbAS, malaria protection by HbC has not been associated with reduced parasite densities in vivo or significant impairment of parasite multiplication in vitro,[223,224,227,237] suggesting that HbC erythrocytes support normal invasion and development of *P. falciparum.*

In other studies of possible mechanisms of protection, freshly drawn and infected HbAS and HbAC erythrocytes were found to be impaired in their adherence to microvascular endothelial cells and monocytes,[237,238] two interactions critical to the development of severe malaria. Abnormal display of the parasite's main virulence factor and antigenically variable cytoadherence ligand, PfEMP-1, on the surface of infected erythrocytes offers one explanation for these findings. In this model of protection, reduced cytoadherence of infected HbAS and HbAC erythrocytes enables them to sequester in microvessels and achieve high parasite densities while lessening the inflammatory consequences of cytoadherence and thus reducing the chances of progressing to severe disease.[239] Evidence indicates that aberrant remodeling of host cell actin, compromised formation of knobs, and reduced PfEMP-1–mediated cytoadherence are the result of a redox imbalance inherent to hemoglobinopathic and fetal erythrocytes.[240,241] PfEMP-1–specific antibodies, which are acquired rapidly in endemic areas, may synergize with HbS and HbC to further weaken cytoadherence interactions and ameliorate disease severity.[238]

Hemoglobin E (HbE) is another hemoglobin variant characterized by the substitution of lysine for glutamate at the 26th amino acid of β-globin. This mutation also introduces an alternative splice site that reduces the amount of β-globin produced,[242] thereby conferring a β-thalassemia phenotype to HbE erythrocytes. Unlike HbS and HbC, HbE is found predominantly in Cambodia and neighboring countries in Southeast Asia. Some epidemiologic studies have found HbE to protect against malaria,[243,244] whereas others have not.[245,246] Although HbE has been suggested to decrease the multiplication rate of *P. falciparum,*[247] parasite densities do not differ between HbAA and HbE patients with malaria.[244,246]

Thalassemias

The thalassemias arise from deletion of one or more of the four genes encoding the α-globin chains or mutations or deletions in one of the two genes encoding the β-globin chains of hemoglobin. These hemoglobinopathies are generally benign in the heterozygous state and are associated with varying degrees of hypochromic microcytic anemia. Further loss of expression in the homozygous state causes severe disease and can be incompatible with life. Mutations associated with thalassemias are thus believed to exist as balanced polymorphisms in human populations; indeed, some of them (e.g., α-thalassemia) have been associated with protection from severe malaria.[216,248–250] Although *P. falciparum* development can be supported by thalassemic erythrocytes, some studies have demonstrated impaired growth, especially under conditions of oxidative stress.[235,251–253] Other studies have demonstrated that infected thalassemic erythrocytes bind increased amounts of antibody from both nonimmune and immune sera, which suggests the possibility of enhanced opsonization in vivo.[254,255] These mechanisms are difficult to reconcile with several more recent studies from Africa, which show that both heterozygous and homozygous α-thalassemias protect against severe malaria without reducing parasite densities in vivo.[225,256–258] Other evidence suggests that α-thalassemia may protect against severe malaria in Africa by the same mechanism proposed for HbS and HbC: namely, abnormal PfEMP-1/knob display, decreased cytoadherence, and reduced activation of endothelium.[259] Increased microerythrocyte counts in α-thalassemia homozygotes have been proposed to contribute to protection against severe malarial anemia in Papua New Guinea by a mechanism that reduces loss of erythrocytes and hemoglobin during *P. falciparum* infection.[260]

Hemoglobin F

Hemoglobin F (HbF, α_2/γ_2) is a normal hemoglobin variant expressed by the fetus in utero and by the infant during the first few months of life. The expression of HbF dramatically declines after the third month of life as adult hemoglobin A (HbA, α_2/β_2) replaces it. The uncommon presentation of malaria in neonates younger than 6 months led to the hypothesis that HbF contributes to malaria protection, along with maternal antibody. Proteases responsible for digesting host cell hemoglobin in the food vacuole of the parasite may work less efficiently on HbF than HbA.[261] Impaired antioxidant capacity of HbF-containing erythrocytes has also been proposed to contribute to malaria protection.[262] More recently, abnormal PfEMP-1/knob display has been associated with HbF.[263] In a new model of infant protection, the combined effects of HbF and maternal PfEMP-1–specific immunoglobulin G substantially diminish the ability of infected erythrocytes to bind and activate microvascular endothelium, thus impeding the development of malaria in infants.[239]

Glucose-6-Phosphate Dehydrogenase Deficiency

G6PD is a cytoplasmic enzyme that is essential for an erythrocyte's capacity to withstand oxidant stress, such as that exerted by the developing malaria parasite. The *G6PD* gene is located on the X chromosome and is therefore present in only one copy in males. In heterozygous females carrying a mutant gene, mosaic populations of G6PD-deficient and G6PD-normal erythrocytes are produced from hematopoietic cells that have one or the other X chromosome inactivated. G6PD deficiency is the most common enzymopathy in humans, with more than 300 allelic polymorphisms identified to date. The most common polymorphism in Africa (the A^- allele, 10%–50% enzyme activity) has been associated with malaria protection in children[264,265] and pregnant women.[266]

In a large case-control study performed in populations of West and East Africa, male hemizygotes and female heterozygotes carrying the A^- allele were reported to be 58% and 46% protected against severe malaria, respectively.[264] However, a more recent and larger study,[265] which included a reanalysis of data from the earlier study,[264] found that male hemizygotes, but not female heterozygotes, carrying the A^- allele are protected against severe malaria. These findings agree with the facts that male hemizygotes carry erythrocytes uniformly deficient in G6PD, whereas female heterozygotes carry mosaic populations of G6PD-normal and G6PD-deficient erythrocytes. Enhanced phagocytosis of infected G6PD-deficient erythrocytes has been proposed to play a role in malaria protection.[267] Although such phagocytosis is consistent with greater protection of males than females by G6PD deficiency, it does not account for the presence of similar parasite densities in G6PD-deficient males and females.[264,265] Alternative mechanisms of protection may therefore operate in vivo.

Southeast Asian Ovalocytosis and Hereditary Xerocytosis

A 27-base pair deletion in band 3 (the major anion transporter in erythrocytes) causes Southeast Asian ovalocytosis and leads to reduced membrane deformability.[268–270] These properties may be associated with reduced parasite invasion rates of ovalocytes in vitro and reduced parasitemias in heterozygous individuals.[271–274] How these findings relate mechanistically to the dramatic reduction in cerebral malaria episodes among Southeast Asian ovalocytosis heterozygotes[275] has not yet been established.

In studies of *PIEZO1* mutations and hereditary xerocytosis, cerebral malaria from *Plasmodium* infection was prevented in an engineered mouse model. Prevalence of the *PIEZO1* allele, E756del, in about one-third of Africans may reflect an association of this allele with malaria resistance.[276]

ABO Blood Groups

Blood group O antigen has been associated with protection against severe malaria[277,278] and rosetting, a phenotype of infected erythrocytes that correlates with severe disease in Africa.[116–120] A mechanism of reduced rosetting in type O erythrocytes, compared with type A, B, or AB erythrocytes, has been associated with this protective effect.[277]

Duffy Antigen Receptor for Chemokines Negativity

DARC is an erythrocyte receptor for *P. vivax* merozoite invasion.[129,130] Erythrocytes lacking DARC are resistant to invasion, but *P. vivax* infections nevertheless occur among DARC-negative individuals.[279,280] These facts are consistent with the low observed incidence of *P. vivax* malaria in regions of sub-Saharan Africa, where erythrocyte DARC negativity is highly prevalent.[43,44,281,282] The mechanism for *P. vivax* infection of DARC-negative individuals is unknown. Reduced DARC expression on erythrocytes has been identified as a protective polymorphism against *P. vivax* malaria in Papua New Guinea.[283] DARC negativity does not protect against malaria from *P. falciparum*.

ACQUIRED IMMUNITY AND ANTIGENIC VARIATION

Acquired immunity against malaria (premunition) is not a sterilizing immunity against parasitemia. Instead, it is an immunity that protects against symptomatic and severe disease without necessarily preventing the continued presence of malaria parasites in the bloodstream. It is an immunity that increases with age, cumulative number of episodes of malaria, and time spent living in an endemic area.[284] Parasitemia in individuals with premunition is usually low, whereas nonimmune individuals may develop high parasitemias (up to 80%) after infection.[285] As individuals gain the experience of numerous infections in their lifetime, they can be chronically infected yet have only mild symptoms or none at all; that is, they develop "disease-controlling" immunity. In highly endemic areas, children have multiple bouts of malaria each year and suffer greatly under its morbidity and mortality when they are young, generally less than 5 to 10 years old, depending on the transmission level. Pregnant women, especially primigravidae, are an important exception to this general rule of disease-controlling immunity from previous malaria episodes and thus can be highly susceptible to antigenically new CSA-binding parasites that sequester in the placenta (see "Malaria of Pregnancy" earlier).

Acquired immunity to malaria is believed to be short-lived without the continual stimulation of various antigenic exposures from different *P. falciparum* strains. Although individuals who reside outside an endemic area for more than a year or two can develop symptomatic or severe malaria, or both, after their return,[286] one study found evidence that acquired immunity to *P. falciparum* malaria persists after several years of nonexposure in African immigrants living in France.[287]

Neonates appear to be resistant to malaria during the first few months of life. This immunity may be conferred by transplacentally acquired maternal immunoglobulin G, although the presence of fetal hemoglobin within erythrocytes likely plays a role as well (see "Hemoglobin F" earlier).

Splenomegaly often accompanies malaria and is thought to indicate an important role of the spleen in parasite clearance.[288,289] Removal of uninfected erythrocytes by the stimulated spleen, however, may contribute to anemia. In asplenic individuals, *P. falciparum* malaria can progress extremely rapidly to high parasitemias that include mature forms not usually found circulating in the bloodstream.[84,290,291]

The immunity of endemic human populations to severe malaria is complex and not well understood.[292] Although antibodies and T-cell responses develop against a number of parasite antigens during natural infection, none of them has been found to be superior to age or parasite exposure as correlates of protective immunity.[293] Studies in which humans were infected with a single inoculum of *P. falciparum* in the use of malariotherapy[294] for tertiary syphilis showed that erythrocyte infection rose and fell in successive waves of decreasing peak parasitemia[295] (Fig. 274.5). Individuals who eventually cleared their infection were protected against subsequent reinfection by the same parasite strain but not protected against reinfection with a different *P. falciparum* strain.[296,297]

Antigen switching results in new waves of parasitemias that escape the antibody response already produced against previous waves, manifest clinically as recurrent or relapsing fevers reminiscent of those seen in *Borrelia recurrentis* relapsing fever or *Trypanosoma brucei rhodesiense* African sleeping sickness, and may not be cleared for months to years.[298,299] An important component of the eventual acquisition of disease-controlling premunition after repeated episodes of malaria is the development of an antibody repertoire that can recognize a full spectrum of PfEMP-1 variant antigens. Studies in endemic areas have shown that the ability of serum to recognize diverse heterologous parasite strains increases with age and that children tend to be infected with parasites against which they have no preexisting antibody.[300–303] The mechanisms by which variant-specific antibodies act in acquired immunity may include antibody-dependent cellular cytotoxicity, opsonization for uptake and destruction by splenic macrophages, and interference with PfEMP-1–mediated cytoadherence interactions.

EPIDEMIOLOGY OF MALARIA

Malaria occurs mostly in tropical regions of sub-Saharan Africa, Asia, Oceania, and Latin America (Fig. 274.6), but its distribution is continually changing.[304,305] The Malaria Atlas Project (https://www.map.ox.ac.uk) and the Centers for Disease Control and Prevention (CDC; https://www.cdc.gov/malaria/travelers) provide up-to-date information on the geographic distribution of malaria. Information on drug-resistant malaria is also available from the publication CDC Health Information for International Travel 2018 (https://wwwnc.cdc.gov/travel/page/yellowbook-home).

P. falciparum and *P. vivax* infections account for most cases of human malaria. *P. falciparum* and *P. malariae* are found worldwide. *P. vivax* is infrequent in most of sub-Saharan Africa but is common elsewhere. *P. ovale* occurs in Africa and in foci within Asia and Oceania and is often present with other *Plasmodium* species as a mixed infection.[306,307] Although most *P. knowlesi* infections of humans have been reported from Borneo and peninsular Malaysia,[17,18] cases have also been reported from other areas of Southeast Asia, including the Philippines, Singapore, Thailand, and Myanmar.[308]

Malaria is transmitted person to person by anopheline mosquitoes. Its endemicity depends upon competent mosquito vectors, a reservoir

of infected humans, and conditions that bring them into proximity. The *Anopheles gambiae* complex of species and *Anopheles funestus* transmit malaria with notoriously high efficiency and are the predominant vectors in sub-Saharan Africa, where environmental conditions favor their robust reproduction and transmission of parasites to large numbers of people. Highest levels of transmission typically occur during the wet season in endemic areas.

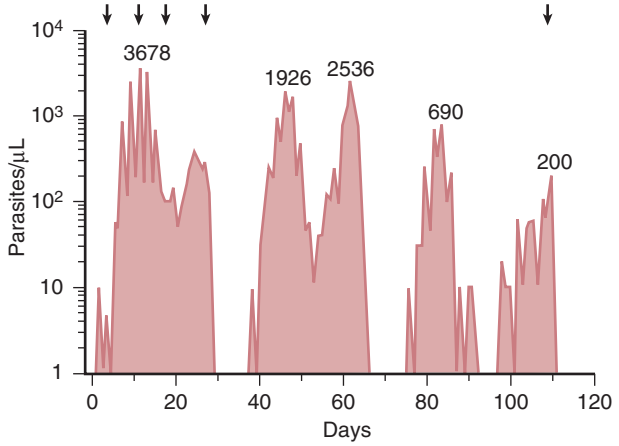

FIG. 274.5 Premunition and antigenic variation. Parasitemia waves during recrudescence in a patient infected with *Plasmodium falciparum* as malariotherapy for tertiary syphilis. Although the individual had previously been infected with *P. falciparum,* drug treatments *(arrows)* were necessary to modify the primary attack and later for radical cure. After subcurative drug treatments, four recrudescences occurred with parasite density peaks ultimately declining 10-fold. *(Modified from Collins WE, Jeffery GM. A retrospective examination of sporozoite- and trophozoite-induced infections with* Plasmodium falciparum: *development of parasitologic and clinical immunity during primary infection.* Am J Trop Med Hyg. *1999;611[suppl]:4–19.)*

Malaria epidemics can result from the movement of people with no immunity into an endemic area (e.g., nomadic traders, seasonal forest laborers, and military personnel), the breakdown of control measures in areas under previously successful management programs, or unusually heavy rainfalls that can place indigenous populations at risk for higher-than-normal transmission.[309–311] Man-made environmental alterations (e.g., agricultural and waterworks projects, damming of rivers, and deforestation) can lead to increases in malaria transmission by creating new mosquito habitats. Malaria may be imported into areas previously free of the disease as a result of immigration of populations from endemic areas (e.g., migrating workers, persons displaced by natural disasters or civil strife, and resettlement of refugees).[312–315]

In the United States, changes that include new agricultural and animal husbandry practices, improved housing with screens, water management with swamp drainage, and a radically altered landscape with urban development led to a steady decline in malaria after the mid-19th century.[316] Final pockets of transmission were removed by the mid-20th century with the help of focused water management and insecticide spraying. Malaria diagnosed in the United States today is therefore almost always acquired in a malaria-endemic country by a returning traveler or immigrant. Because of parasite or host factors, immigrants may harbor parasites for months to years and not be recognized as possible sources of transmittable infection. In 2015, the CDC received reports of 1517 confirmed cases of imported malaria.[317] *P. falciparum, P. vivax, P. ovale,* and *P. malariae* species were identified in 67%, 12%, 4%, and 3% of cases, respectively. Less than 1% of cases were infected by two species, and the infecting species was unreported or undetermined in 13% of cases.

Autochthonous transmission, although infrequent, typically occurs when parasitized individuals infect competent vectors (*Anopheles*

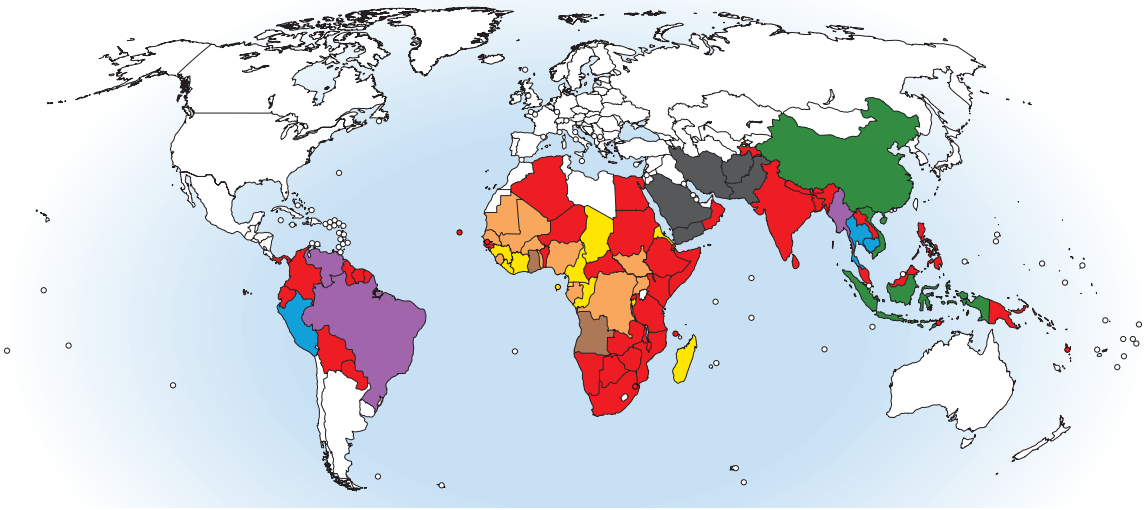

FIG. 274.6 Artemisinin-based combination therapies and the threat of artemisinin resistance. Artemisinin-based combination therapy (ACT)—the combination of an artemisinin derivative and a partner drug—is now recommended for the treatment of *Plasmodium falciparum* malaria in virtually all endemic areas. Selection of an appropriate ACT depends on the regional profile of parasite susceptibility to various partner drugs. Countries in which artemether-lumefantrine (*red*), artesunate-amodiaquine (*yellow*), artesunate-mefloquine (*blue*), dihydroartemisinin (DHA)-piperaquine (*green*), artesunate-sulfadoxine-pyrimethamine (*gray*), or some combination thereof (*orange*, *purple*, *brown*) are the first-line treatments are shown. Because of the widespread prevalence of resistance to chloroquine and sulfadoxine-pyrimethamine, these drugs are not commonly recommended as ACT partner drugs. In areas of Southeast Asia (Cambodia, Vietnam, Laos, Thailand, Myanmar, and southern China), the emergence and spread of artemisinin resistance now threatens the efficacy of all ACTs. The Centers for Disease Control and Prevention provides up-to-date information on the distribution of malaria and drug resistance at http://www.cdc.gov/malaria/travelers. *(Modified from 2018 World Health Organization listings provided by Peter Olumese.)*

albimanus, Anopheles quadrimaculatus, and *Anopheles freeborni*) that remain common in the United States.[318,319] "Airport malaria" occurs when infected mosquitoes arrive from an endemic country on an aircraft from which they escape to bite local residents.[320] Because mosquitoes travel short distances, infections of local residents tend to occur near airports.[321] The spraying of insecticide within aircraft leaving endemic areas reduces the incidence of airport malaria.[322] Malaria may also be acquired from needles shared among drug users, blood transfusion,[323,324] or solid-organ transplantation.[325] These blood and organ donors are usually asymptomatic persons from endemic areas with low-level parasitemia. The incidence of transfusion-acquired malaria is reduced when returned travelers and former residents of malaria-endemic areas are required to wait 1 and 3 years, respectively, before donating blood.[323] Malaria may also be acquired congenitally.[326]

DISTRIBUTION OF DRUG RESISTANCE

The widespread and complicated patterns of *P. falciparum* resistance to antimalarials has resulted in various ACT regimens becoming the first- or second-line drug regimens in most of the world's malaria-endemic countries (see Fig. 274.6).

Chloroquine-resistant *P. falciparum* malaria is widespread in sub-Saharan Africa, Asia, and Latin America. It has also been reported in areas of the Middle East, including Iran, Yemen, Oman, and Saudi Arabia,[327–331] but not in Mexico, other regions of Central America west of the Panama Canal, Haiti, or the Dominican Republic. High-grade resistance of *P. vivax* malaria to chloroquine has been reported in Papua New Guinea and Indonesia.[332–334] Case reports of *P. vivax* malaria not responsive to chloroquine treatment have also been reported from India, Myanmar, and Central and South America.[335–340] Chloroquine-resistant *P. malariae* has been reported in Sumatra, Indonesia.[341] In some regions of Africa and China where chloroquine availability has ceased, chloroquine-sensitive *P. falciparum* strains have gradually returned to a greater prevalence.[342–347]

Amodiaquine-resistant *P. falciparum* has been reported in several regions of Africa[348–352] and Asia.[353,354] Mefloquine-resistant *P. falciparum* malaria now occurs along the various border regions of Thailand, Myanmar, southern China, Cambodia, Laos, and Vietnam,[355–357] with scattered cases reported in the Amazon Basin[358] and Africa.[359]

Resistance to sulfadoxine-pyrimethamine (SP) is widespread through much of Southeast Asia,[360–362] the Amazon Basin,[363,364] and Africa.[365–367] The prevalence of SP resistance varies among African regions, with some areas of West Africa showing relatively low rates of resistance.[368] Malaria strains resistant to cycloguanil (the active metabolite of proguanil) have been reported since the late 1940s[369–371] and can exhibit different degrees of cross-resistance to pyrimethamine.[372,373] Interindividual variations of conversion to cycloguanil may also contribute to clinical success or failure of proguanil treatment.[374–376]

Resistance to atovaquone-proguanil was previously reported only with its use in prophylaxis, but with increasing use for the treatment of uncomplicated malaria, frank treatment failures have been reported from several countries.[377–381] Reduced susceptibility to quinine has been reported mostly in Southeast Asia[382] but also in sub-Saharan Africa and South America.[383,384]

Concerns about the emergence of resistance to artemisinin derivatives were first raised with reports of treatment failures with artesunate-mefloquine and artemether-lumefantrine in Thai and Cambodian malaria control programs.[385,392] These failures may be associated with slow parasite clearance rates in response to artemisinin derivatives in vivo, which have now been carefully documented in Cambodia,[386–388] Thailand,[389] Vietnam,[390] Myanmar, Laos, and southern China (see Fig. 274.6).[391–393] These findings have invoked the specter of current ACT regimens becoming less effective throughout Southeast Asia, prompting WHO to launch a "Global Plan for Artemisinin Resistance Containment" in 2011 and an "Emergency Response to Artemisinin Resistance in the Greater Mekong Subregion" in 2013.[394,395] These observations have also raised the possibility that rapid parasite clearance, a hallmark benefit of artemisinins in the treatment of severe malaria, may become less dependable after artemisinin dosing in this region.[396]

ANTIMALARIAL DRUGS: MECHANISMS OF ACTION AND RESISTANCE

Chloroquine

Intraerythrocytic parasites consume the hemoglobin of their host cells, breaking it down within a large digestive vacuole (see Fig. 274.4B) and releasing heme molecules that are poisonous if not detoxified. Malaria parasites normally allow these heme molecules to polymerize into inert crystals called hemozoin, which can be visualized by light microscopy as intraerythrocytic pigment in thin blood smears (Fig. 274.7E and H). Chloroquine acts by forming toxic complexes with heme molecules and interfering with their crystallization.[397] This mechanism of action explains why chloroquine is effective against developing intraerythrocytic trophozoites but ineffective against other parasite stages (i.e., mature gametocytes, liver schizonts) that do not actively consume hemoglobin.

Chloroquine-resistant *P. falciparum* parasites reduce the amount of drug that accumulates in their digestive vacuoles.[398] The mechanism involves mutations in a conserved transport molecule of the digestive vacuole membrane known as *P. falciparum* chloroquine resistance transporter (PfCRT).[399–401] Evidence indicates that the mutated PfCRT molecule functions as a saturable simple carrier under the influence of an electrochemical gradient[402,403] and that a specific interaction between chloroquine and the modified form of PfCRT[400] is involved in the facilitated diffusion of chloroquine from the digestive vacuole.[404–406] The *pfcrt* mutations responsible for chloroquine resistance include a key change from lysine to threonine in the 76th amino acid (K76T) plus additional mutations that depend on their geographic origin.[399,407–409] Drug selection for mutant PfCRT is evident in the association of the K76T marker with increased plasma chloroquine levels[410] and with treatment failures in children receiving the drug.[411]

Although PfCRT is the central determinant of chloroquine resistance, other host and parasite factors also influence treatment outcomes. For example, clearance of phenotypically chloroquine-resistant parasites can occur after chloroquine treatment and becomes increasingly prevalent in children as they grow older, presumably because of the immunity that develops from repeated episodes of malaria.[411,412] Parasite transport molecules in addition to PfCRT have also been proposed to modulate or contribute to the ability of chloroquine-resistant parasites to cope with the drug.[413,414]

Amodiaquine

Amodiaquine, a 4-aminoquinoline compound related to chloroquine, is often used successfully against chloroquine-resistant strains of *P. falciparum* in Africa, but it is largely ineffective in South America. The resistance profiles responsible for these different responses are attributable to the particular histories of drug selection pressure in Africa or South America, and the evolution of distinct suites of mutations in *pfcrt* and *pfmdr1,* the *P. falciparum* multidrug resistance gene 1 encoding P-glycoprotein homologue-1.[415]

Piperaquine

Piperaquine, a *bis*-aminoquinoline compound related to chloroquine, is used widely as a partner drug in artemisinin-based combination therapy in Southeast Asia and Africa. Resistance to piperaquine has recently emerged in Cambodia,[416] where it has been associated with emerging mutations of the *pfcrt* gene[417] as well as amplifications of the *plasmepsin 2* and *plasmepsin 3* genes[418,419] that encode aspartic proteases involved in hemoglobin digestion. The role of these enzymes in conferring piperaquine resistance is under investigation.

Sulfadoxine-Pyrimethamine

Dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) are sequentially involved in the folate pathway of nucleic acid synthesis. Pyrimethamine inhibits parasite DHFR activity and the production of tetrahydrofolate, an essential cofactor for one-carbon metabolism required for the synthesis of nucleic acids and certain amino acids. Point mutations in DHFR reduce its affinity for pyrimethamine. The substitution of asparagine for serine at position 108 in DHFR is critical for the initial development of pyrimethamine resistance, with additional mutations (Ile51, Arg59, Leu164) increasing the degree of pyrimethamine

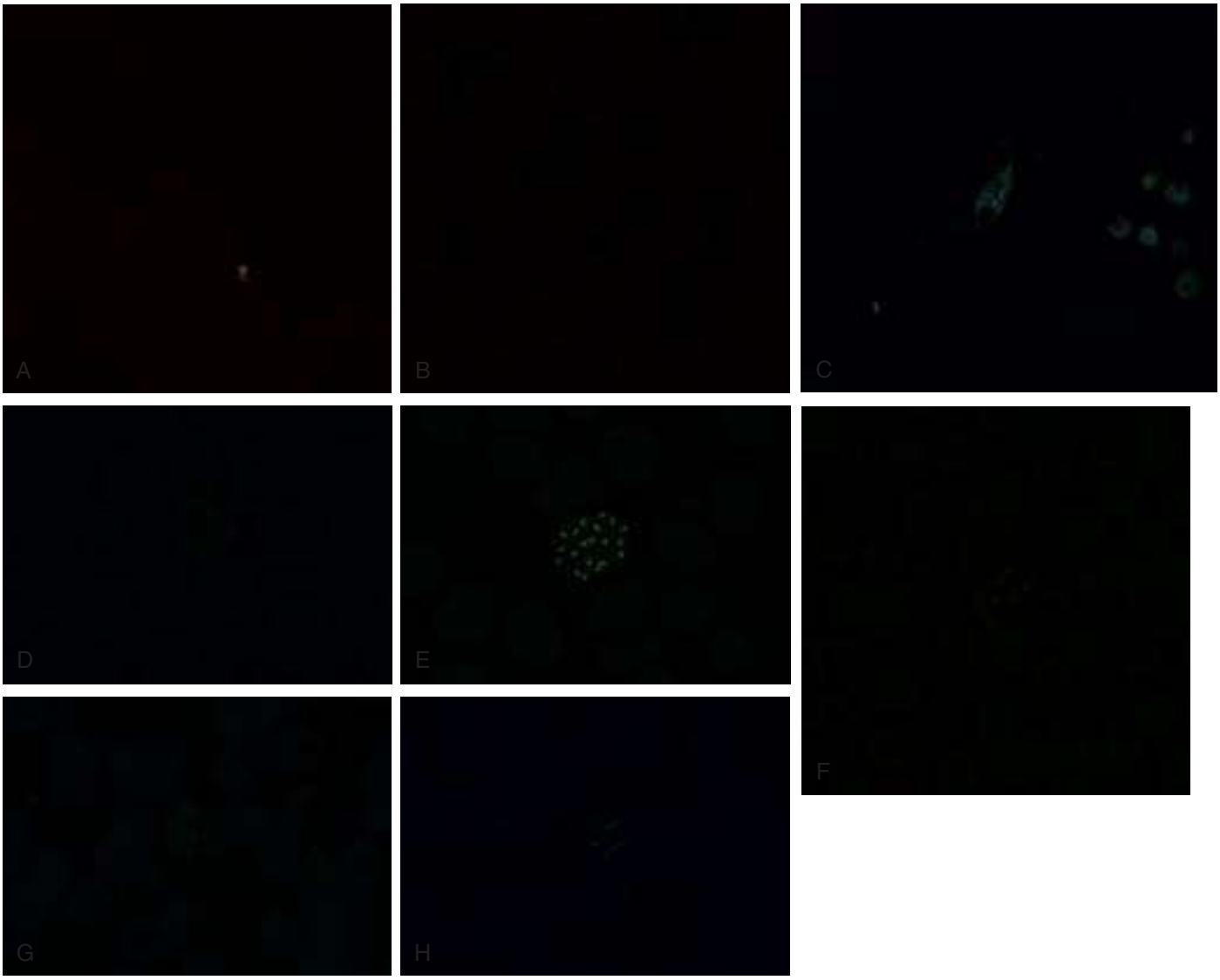

FIG. 274.7 Giemsa-stained thick (A) and thin (B–H) smears used for the diagnosis of malaria and the speciation of *Plasmodium* parasites. (A) Multiple signet-ring *P. falciparum* trophozoites, which are visualized outside erythrocytes in thick blood smear preparations. (B) A multiply infected erythrocyte containing signet-ring *P. falciparum* trophozoites, including an accolade form positioned up against the inner surface of the erythrocyte membrane in fixed thin blood smear preparations. (C) Banana-shaped gametocyte unique to *P. falciparum.* (D) Ameboid trophozoite characteristic of *P. vivax.* Both *P. vivax*– and *P. ovale*–infected erythrocytes exhibit Schuffner dots and tend to be enlarged compared with uninfected erythrocytes. (E) *P. vivax* schizont. Mature *P. falciparum* parasites, by contrast, are rarely seen on blood smears because they sequester in the systemic microvasculature. (F) *P. vivax* spherical gametocyte. (G) *P. ovale* trophozoite. Note Schuffner dots and ovoid shape of the infected erythrocyte. (H) Characteristic band form trophozoite of *P. malariae* containing intracellular pigment hemozoin. *(A, B, and F courtesy DPDx - Laboratory Identification of Parasites of Public Health Concern [the CDC's website for parasitology identification] at* https://www.cdc.gov/dpdx/index.html. *C, D, E, G, and H courtesy David Wyler, Newton, MA.)*

resistance.[420,421] Part of sulfadoxine's action is thought to be inhibition of parasite DHPS, and point mutations in DHPS reduce its affinity for sulfadoxine.[422,423] Analysis of the mutant *dhfr* and *dhps* alleles in field studies supports conclusions that clinically significant resistance to pyrimethamine arises from multiple mutations in *dhfr* and *dhps* and that *dhps* mutations are likely selected after mutations in *dhfr* are already present.[424]

Atovaquone-Proguanil (Malarone)

Atovaquone binds cytochrome *b* and inhibits parasite mitochondrial proton transport, leading to collapse of the mitochondrial membrane potential.[425,426] This effect is potentiated by proguanil.[427] The substitution of serine for tyrosine at codon 268 of the cytochrome *b* gene is associated with resistance to atovaquone and the atovaquone-proguanil (Malarone) combination.[378,379,428,429] Cycloguanil, the active metabolite of proguanil, inhibits DHFR.[430] Point mutations in *dhfr* confer resistance to cycloguanil.[374,375,431,432]

Doxycycline

Doxycycline inhibits protein synthesis elongation by preventing binding of aminoacyl–transfer RNA to the ribosome 30S subunit. Resistance of human malaria parasites to this drug has not been described.

Mefloquine, Quinidine, Quinine

Mefloquine, quinidine, and quinine are thought to form complexes toxic to the parasite by binding to heme and inhibiting its polymerization.[433,434] Mefloquine resistance may be associated in part with increases in expression and mutations in *pfmdr1*.[413,435,436] Decreased quinine susceptibility is associated with resistance to other structurally related drugs such as mefloquine and halofantrine, suggesting that drug resistance mechanisms may share various genetic determinants.[437–439] Some studies have implicated *pfmdr1* mutations in mefloquine, quinine, and halofantrine resistance and *pfcrt* mutations in quinine and quinidine responses.[400,401,413,440,441] The different levels of quinine susceptibility among parasites and the relatively slow rate at which quinine resistance

has spread throughout the world indicate that quinine resistance is a complex phenotype and is probably affected by other genes in addition to *pfmdr1* and *pfcrt*. The results of a linkage analysis and surveys of parasites from Southeast Asia, Africa, and South America support a model in which multiple genes can combine in different ways to produce similar phenotypes of reduced quinine response.[414,441]

Artemisinin Derivatives

In original studies of artemisinin treatment responses in humans, frequent recrudescences were observed after 3-, 5-, and 7-day regimens of monotherapy.[442,443] This inherent resistance of *P. falciparum* (Rl level by WHO definition)[444] led to an early recommendation that artemisinin always be used with partner drugs in combination therapy.[443] Although high-level resistance to artemisinin derivatives has not been found in clinical samples, successful selection of rodent malaria parasite strains with reduced susceptibility[445,446] and reports of *P. falciparum* strains with slow clearance rates in vivo[396] initially raised concerns that strains of human malaria parasites with significant clinical resistance may evolve and spread. Indeed, artemisinin resistance,[447,448] defined as a slow parasite clearance half-life in patients receiving an artemisinin derivative, has been reported extensively from Cambodia,[386–388] Thailand,[389] Vietnam,[390] Myanmar, Laos, and southern China.[386–391,449–451] Multiple single nucleotide polymorphisms in the propeller domain of the *P. falciparum* kelch13 (K13) protein have been associated with slow clearance half-life in Cambodia,[452–454] although exceptions are reported[455,456] and immunity has been shown to have an important effect on the clearance phenotype.[457] These single nucleotide polymorphisms have arisen independently and are linked to decreased susceptibility of young (0–3 hours old) ring-stage parasites to a pharmacologically relevant exposure to artemisinin in vitro.[452,458] Genome-wide association studies of parasite subpopulations in Western Cambodia[459,460] have suggested that the genetic determinants of artemisinin response may involve multiple genes, including *pfcrt, pfmdr2, arps10,* and *fd*.[460,461]

Artemisinin-resistant ring-stage parasites were recently found to have increased expression of the unfolded protein response pathway, which is proposed to mitigate the protein damage caused by artemisinin.[462] Apart from this ring-stage phenotype, a dormancy phenotype has been proposed for the clinical recrudescences inherent to *P. falciparum* infection.[463,464] Recent studies suggest that parasite dormancy may involve integrated stress response pathways and global inhibition of protein synthesis through phosphorylation of *Plasmodium* eukaryotic initiation factor 2α, leading to a protective latent state.[465]

In sub-Saharan Africa fast parasite clearance half-lives predominate after ACT treatment, despite the finding of K13 propeller mutations at low prevalence.[466–469] This difference may be attributed to the absence of the "genetic background" mutations found in Southeast Asia.[468] Natural factors of resistance and acquired immunity from greater exposure to malaria could also be responsible for major differences in clearance rates.[469]

CLINICAL PRESENTATION AND DIAGNOSIS OF MALARIA

The malaria incubation period after an infective mosquito bite includes the time required for the parasites to progress through liver schizogony and produce symptoms by their propagation in the bloodstream. For primary attacks, this period is typically about 8 to 25 days but may be much longer, depending on the immune status of the infected person, the strain as well as the species of *Plasmodium,* the dose of sporozoites, and the possible effects of partially effective chemoprophylaxis. Relapses from latent hypnozoites (*P. vivax, P. ovale*) may develop months or years after mosquito bites. Late-onset or recrudescent *P. falciparum* malaria may also occur in individuals who have suppressed the parasitemia of drug-resistant parasites with chemoprophylactic drugs[470] (see Fig. 274.2). Febrile patients presenting within 7 days of entering an endemic area are unlikely to have malaria, unless there has been earlier exposure to infective mosquito bites. As a general rule, and because of the dangers of acute *P. falciparum* infection, all travelers who have visited a malaria-endemic area in the 3 months before onset of fever or other suggestive symptoms should be considered to have malaria until proven otherwise. Even in patients beyond this time frame, it is wise to consider *P. falciparum* malaria, as illustrated, for example, in the report of a symptomatic presentation in an 18-year-old patient with sickle cell disease 4 years after visiting an endemic area.[299] Latent attacks from the reactivation of *P. vivax* or *P. ovale* hypnozoites usually occur within 3 years and are rare more than 5 years after exposure. Recrudescence of *P. malariae* symptoms in individuals with subclinical parasitemia has been reported decades after initial infection.[200,471,472]

History and Physical Examination

Uncomplicated malaria typically presents as an undifferentiated febrile illness.[473] A series of 160 German nationals or residents with imported malaria presented to a travel clinic with the following symptoms: fever (100%); headache (100%); weakness (94%); profuse night sweats (91%); insomnia (69%); arthralgias (59%); myalgias (56%); diarrhea (13%); and abdominal cramps (8%).[474] The bloodstream parasites of *P. falciparum* infections are often asynchronous and may produce continuous fever. In other infections, fever can recur in a pattern that depends on the synchrony of the replicating parasites, and whether mixed subpopulations of parasites (possibly including mixed *Plasmodium* species) are on different cycles in the bloodstream. Patients with cyclic fevers may be relatively asymptomatic during afebrile periods.

Signal elements from the history and physical examination, when considered together, may be suggestive of the diagnosis of malaria.[475–477] Cyclic paroxysms of chills and rigors, fever, and drenching sweats are characteristic although not necessarily specific for malaria. A travel history that reveals risk of exposure months to years before in an endemic region is an alert for malaria and should always be sought in presentations of fever. Findings on physical examination may include pallor and hepatosplenomegaly. Rarely, acute *Plasmodium* infections present with splenic rupture requiring surgery or conservative management.[478,479] Findings such as jaundice, diminished consciousness, or convulsions indicate severe malaria (see "Severe *P. falciparum* Malaria" later). Rash, lymphadenopathy, and signs of pulmonary consolidation are distinctly uncommon.

Thick and Thin Blood Smears

Light microscopy of Giemsa-stained blood smears is the accepted standard for malaria diagnosis. Thick and thin diagnostic blood smears should be *prepared and read immediately by experienced personnel* when the clinical presentation and travel history are compatible with malaria.[480] Preparation instructions and representative images from thick and thin smears are available from the CDC's website for parasitologic diagnosis: DPDx - Laboratory Identification of Parasites of Public Health Concern (https://www.cdc.gov/dpdx/index.html).

Thick smears concentrate red cell layers approximately 40-fold and are used to screen a relatively large amount of blood for the presence of parasites. Because erythrocytes are lysed in the process of staining in the thick smear technique, parasites are visualized *outside* red cells (see Fig. 274.7A). Assuming an average white cell count of 8000/μL, parasitemia can be estimated from a thick smear by counting the number of parasites until 200 white cells have also been counted. This count, when multiplied by 40, gives an indication of the number of parasites per microliter of blood. The parasitemia percentage can then be calculated by dividing the parasite density by 4,000,000 (the average number of erythrocytes per microliter in blood) and multiplying by 100.

Parasite density can be associated with disease severity and must be monitored during and after treatment to ensure adequate resolution of infection. Detectable parasitemia may lag behind aches, fevers, and chills, sometimes for many days; individuals with no antimalarial immunity may have severe manifestations of malaria even though parasites are very difficult to detect on blood smear. A *P. falciparum* infection also may not be apparent on an initial blood smear if the parasites are predominantly mature cytoadherent forms (i.e., trophozoite- and schizont-infected erythrocytes) and are sequestered in the microvasculature. Therefore if the initial blood smear is negative and malaria remains possible, the smear should be repeated every 12 hours until a diagnosis of malaria is made or ruled out. Before reading a thick blood smear as negative, at least 200 to 500 fields should be examined at a magnification of 1000× with an oil immersion objective lens; some experts recommend examining a thick smear for 20 minutes. Blood smear–confirmed (or polymerase chain reaction–confirmed) malaria

is a nationally notifiable disease in the United States; typically, such cases are reported by health care providers or laboratories to their local or state health departments and then to the CDC. The CDC has requested that a pretreatment whole blood sample (in ethylenediaminetetraacetic acid anticoagulant) be submitted for all cases diagnosed in the United States for species confirmation and drug resistance surveillance. Details on how to report a case using the CDC's Malaria Case Surveillance Form and how to ship blood specimens using the CDC's Specimen Submission Form are provided at the CDC's malaria website (https://www.cdc.gov/malaria/report.html).

Giemsa-stained thin smears are prepared from a much smaller amount of blood than thick smears and are used to determine the *Plasmodium* species (see Fig. 274.7B to H). Speciation of malaria parasites has important implications for treatment. *P. falciparum* infections are characterized by thin delicate rings that may be positioned at the membrane of the red cell (so-called accolade forms) (see Fig. 274.7B), multiply infected cells containing signet-ring forms (see Fig. 274.7B), *absence* of trophozoites and schizonts because they are sequestered in the microvasculature, and banana-shaped gametocytes (see Fig. 274.7C). *P. vivax* and *P. ovale* are characterized by relatively thicker rings, ameboid trophozoites (see Fig. 274.7D), schizonts (see Fig. 274.7E) in the peripheral blood, and spherically shaped gametocytes (see Fig. 274.7F). Erythrocyte enlargement and Schuffner dots (see Fig. 274.7D and G) are common features of *P. vivax* and *P. ovale,* but not *P. falciparum* or *P. malariae. P. malariae* can be distinguished by its band forms, if present (see Fig. 274.7H). On blood smear examination, ring forms of *P. knowlesi* are indistinguishable from those of *P. falciparum,* and mature forms and gametocytes are indistinguishable from those of *P. malariae.*[17,308,481] Thin-smear examination can yield additional useful information, such as the presence of intraleukocytic pigment (a poor prognostic sign)[482] or other blood pathogens (e.g., filaria, *Borrelia recurrentis*). *Babesia* species produce intraerythrocytic forms that may be confused with *Plasmodium* species,[483] but experienced personnel are able to distinguish between them.

Rapid Diagnostic Tests

Although evaluation of Giemsa-stained thick and thin smears remains the accepted standard for malaria diagnosis, rapid diagnostic tests (RDTs) have become increasingly useful.[484–490] In situations in which expert microscopic examination is delayed or difficult to obtain, medical decisions and management of malaria cases can benefit greatly from the appropriate use of RDTs. In US health care settings, the CDC recommends that all positive and negative RDT results be confirmed by microscopy, which can provide additional information on species, life-cycle stages, and level of parasitemia that may be useful in clinical management. Two types of RDTs based on different detection schemes are presently available and are becoming more frequently used.

The first type is based on the detection of *Plasmodium* histidine-rich protein-2 (HRP-2).[491] In 556 travelers returning to France with suspected malaria, a US Food and Drug Administration (FDA)–approved commercial test based on HRP-2 had 96% sensitivity and 99% specificity for *Plasmodium* infection when compared with microscopy.[492] In 32 US marines returning from Liberia with febrile illness, this test had a 100% sensitivity and a 100% specificity for *P. falciparum* infection when compared with microscopy.[493] Although HRP-2 tests are highly sensitive and specific for malaria diagnosis, they have certain limitations. First, HRP-2–based detection is limited to *P. falciparum* and may yield negative results in cases in which the *P. falciparum* strain either does not express HRP-2 antigen[494–497] or produces a test-interfering prozone effect.[498,499] Other antigen detection schemes are required for *P. vivax, P. ovale,* and *P. malariae.* These are generally less sensitive than is HRP-2 detection for *P. falciparum*,[492,500] making them less useful for the diagnosis of malaria in returned travelers, who typically are infected with *P. vivax* at least as often as with *P. falciparum.* Second, HRP-2 tests are of limited use in monitoring therapeutic responses because the tests are persistently positive up to 28 days after treatment. Third, the sensitivity of many rapid detection tests for *P. falciparum* and *P. vivax* infections drops at parasite densities of less than 100 to 1000/μL,[500] making them less useful for the diagnosis of malaria in nonimmune returned travelers, who may experience symptoms of malaria at low parasite densities. A recently improved HRP-2–based RDT format may improve the detection of *P. falciparum* infection as much as 10-fold.[501,502] WHO provides a list of prequalified malaria RDT products and manufacturers (https://www.who.int/diagnostics_laboratory/evaluations/190527_prequalified_product_list.pdf?ua/=1). In the United States, the BinaxNOW Malaria test (Abbott, Abbott Park, IL) is FDA approved for use by hospital and commercial laboratories, but not clinicians or patients (https://www.cdc.gov/malaria/diagnosis_treatment/diagnostic_tools.html).

The second type of RDT is based on detection of *P. falciparum*–specific lactate dehydrogenase (LDH) and pan-*Plasmodium* LDH.[503] In 556 travelers returning to France with suspected malaria, a parasite LDH test had 80% sensitivity and 98% specificity for *Plasmodium* infection when compared with microscopy.[492] Parasite LDH tests commonly detect *P. vivax, P. ovale,* and *P. malariae* with less sensitivity than *P. falciparum* relative to expert microscopy.[492,504,505] For example, only 3 of 12 (25%) *P. ovale* infections tested positive in French peacekeepers returning from Côte d'Ivoire with malaria.[506] A parasite LDH RDT has a lower sensitivity for *P. falciparum* and *P. vivax* infections when parasite densities are less than 100 to 1000/μL.[507] An advantage to parasite LDH detection is that the signal is proportional to *P. falciparum* parasitemia,[508] allowing for monitoring of therapeutic responses. Although this test is commercially available worldwide, it is currently provided only for research purposes in the United States.

A combination *P. falciparum* HRP-2/LDH test, which takes advantage of the lower limit of HRP-2 detection relative to LDH and can still detect blood parasitemia in the event of no HRP-2 expression or a prozone effect, is under evaluation.[509]

Other Laboratory Tests

The results of routine clinical laboratory tests are not specific for malaria but may support its diagnosis. Some degree of anemia may be seen with malaria from all *Plasmodium* species. Decreases in hemoglobin, hematocrit, and haptoglobin and increases in lactic acid dehydrogenase may be marked with large *P. falciparum* parasite burdens. Microcytosis may be seen in patients from malaria-endemic areas but is often due to iron deficiency or thalassemia. Leukocyte counts may be high, normal, or low.[510] Platelet counts may be normal or slightly low[511] but have been observed to be less than 70,000/μL in *P. falciparum* infection[512] and occasionally in *P. vivax* infection.[513] Sodium may be slightly low, possibly because of the syndrome of inappropriate antidiuretic hormone, excessive vomiting, or urinary losses.[514] Acidemia (pH <7.35), acidosis (bicarbonate <15 mmol/L), and lactate levels greater than 5 mmol/L can be seen in severe *P. falciparum* malaria. Some degree of renal impairment is common in falciparum malaria and may be associated with increased creatinine, proteinuria, and hemoglobinuria.[515] Serum glucose is often low in children with *P. falciparum* malaria, but it is commonly normal in adults. In African children with severe *P. falciparum* malaria, bacteremia/sepsis may be present at the time of initial clinical evaluation and blood cultures may be positive.[52,516] This finding is much less common in adults with imported severe malaria.[517]

Severe *P. falciparum* Malaria

WHO has established clinical and laboratory criteria for severe *P. falciparum* malaria, which must be treated as an emergency requiring intensive medical care.[518] Severe malaria is established by any of the criteria in Table 274.1 in the presence of *P. falciparum* parasitemia and with reasonable exclusion of an alternative diagnosis. Although these WHO criteria are largely based on the clinical presentation of severe malaria among young African children living in endemic areas,[62] they are consistent with the clinical spectrum of severe malaria in travelers who might be encountered in nonendemic developed countries.[516,519] Nonimmune individuals who do not meet these criteria for severe malaria should be treated initially with antimalarial drugs as if they have it. In practice, because of the ability of *P. falciparum* infection to progress in just a few hours to severe and life-threatening complications, it is advisable to hospitalize all nonimmune individuals during their initial period of treatment. If blood smears or RDTs are negative but severe malaria is strongly suspected, patients should be treated for severe malaria, while repeated thick smears and tests for other possible diseases should be pursued for a definitive diagnosis.

TABLE 274.1 Diagnostic Features of Severe Malaria

Cerebral malaria (diminished consciousness, seizures)
Respiratory distress
Prostration
Hyperparasitemia
Severe anemia
Hypoglycemia
Jaundice/icterus
Renal insufficiency
Hemoglobinuria
Shock
Cessation of eating and drinking
Repetitive vomiting
Hyperpyrexia

TABLE 274.2 Differential Diagnosis of the Malaria Presentation: Selected Examples

Influenza
Enteric fever
Bacteremia/sepsis
Classic dengue fever
Acute schistosomiasis (Katayama fever)
Leptospirosis
African tick fever
East African trypanosomiasis (sleeping sickness)
Yellow fever

Cerebral malaria is a syndrome characterized by diminished consciousness, seizures, or both. In endemic areas, it typically occurs in young children and manifests clinically as coma with or without convulsions. The Blantyre coma scale is frequently used to measure the level of consciousness in children.[520] Seizures may be clinically inapparent, subtle motor, partial motor, generalized tonic-clonic, or partial motor with secondary generalization.[521] Multiple factors can contribute to cerebral malaria: hypoglycemia, acidosis, hyperpyrexia, the postictal state, and effects of anticonvulsant medication. In endemic areas, there may also be considerable overlap in the clinical presentations of cerebral malaria and other syndromes, such as bacterial or viral meningitis, subdural hematoma, or sepsis.[133] Although cerebral malaria commonly resolves without neurologic sequelae,[522] some children (especially those with status epilepticus) may develop psychosis, cerebellar ataxia, extrapyramidal rigidity, hemiplegia, or long-term cognitive and language impairment.[523–527]

Respiratory distress is characterized by dyspnea or deep breathing (Kussmaul respirations), which may be accompanied by nasal flaring or intercostal retraction. In one study, deep breathing was 91% sensitive and 83% specific for the presence of severe metabolic acidosis (base excess ≤ −12), the underlying cause of respiratory distress.[59] *Prostration* from fluid and electrolyte depletion is determined by clinical judgment. If a child is 7 months of age or older, prostration may be defined as inability to sit unassisted. *Hyperparasitemia* is defined in endemic areas by WHO as parasite density of 500,000/μL or greater (approximately 10% parasitemia) and is associated with severe anemia, hypoglycemia, cerebral malaria, and renal failure. Although the severity of disease is generally thought to correlate with parasite density, nonimmune individuals may present with severe malaria at any parasitemia, even at levels that may be difficult to detect by microscopy. *Severe anemia* is defined in endemic areas as hemoglobin of 5 g/dL or less. Nonimmune individuals can present with signs and symptoms of severe anemia at hemoglobin levels significantly higher than 5 g/dL because of dehydration; with fluid repletion, rapid reductions from baseline hemoglobin levels can aggravate the symptoms of severe anemia. *Hypoglycemia* is defined as blood glucose of 40 mg/dL or less and may contribute to diminished consciousness and seizures.

Hyperbilirubinemia (manifesting as icterus or jaundice) is also an indicator of severe malaria and may reflect underlying liver compromise. *Renal insufficiency* of severe malaria is defined in endemic areas as anuria for at least 24 hours. However, an infected nonimmune patient with any evidence of renal insufficiency, even that caused by hypovolemia and improved with fluid replacement, should be considered to have severe malaria. *Hemoglobinuria* manifests as dark (cola) colored urine ("blackwater fever"), distinct from the red appearance of hematuria. *Shock* of malaria is clinically indistinguishable from that of sepsis caused by gram-negative bacteria. Special caution needs to be taken in the evaluation of shock because concurrent sepsis is frequently present with parasitemia in severe malaria. In addition to potentiating hypoglycemia, *cessation of eating and drinking* contributes to hypovolemia and consequently to severe acidosis and respiratory distress. *Repetitive vomiting* also contributes to hypovolemia and may complicate oral treatment of severe malaria in resource-poor countries, where parenteral therapy is not readily available. Medications used to treat malaria (e.g., mefloquine) may also cause vomiting, often warranting directly observed therapy. *Hyperpyrexia* is defined as axillary temperature of 40°C or greater and likely contributes to the severity of malaria through its association with febrile seizures.

DISTINGUISHING MALARIA FROM OTHER ILLNESSES WITH SIMILAR CLINICAL PRESENTATIONS

The differential diagnosis of the malaria presentation is broad and includes many febrile illnesses (Table 274.2). However, malaria should always lead the list in the differential diagnosis of fever in travelers or immigrants who have been in an endemic area within the previous 3 months and remain in consideration for years afterward. Travelers and immigrants often present with common ailments, and physicians must be alert to recognize and treat malaria to avoid a morbid or fatal outcome,[528–531] especially when they are working in temperate zones and do not often see malaria and other tropical diseases. It is estimated that 30 million travelers visit malaria-endemic regions each year. In 2003, cases of malaria acquired by international travelers were estimated to number 25,000 annually, of which 10,000 were reported and 150 were fatal.[532]

Clinical criteria to distinguish malaria from other illnesses are critical because RDTs for those illnesses are often limited or not available, and definitive diagnoses often require nonroutine methods of pathogen isolation or serologic testing that rely on comparisons of acute and convalescent antibody titers 2 to 4 weeks later. The probabilities of specific diseases are affected by geographic area visited (e.g., yellow fever is not prevalent in Asia or India), type of travel (e.g., adventure travelers to Lake Malawi are more likely to acquire schistosomiasis or leptospirosis from fresh water contact than are visitors to Nairobi, Kenya), time of travel (e.g., dengue fever is much less likely to be acquired during the dry season, when mosquito transmission is markedly reduced), type of food and water ingested (e.g., enteric fever is relatively unlikely in persons consuming only cooked food and bottled water), and vaccination history (e.g., the efficacy of yellow fever, hepatitis A, and hepatitis B vaccines makes these diseases unlikely if the patient is vaccinated). Self-reported compliance with mosquito repellents and malaria chemoprophylactic drugs, especially during the posttravel period, should *not* be used to rule out malaria because these reports are often inaccurate, and no preventive regimen is 100% effective. Features of selected infectious diseases that may occur as for malaria are briefly summarized in the following paragraphs.

Influenza

Like malaria, influenza may present with fever, headache, myalgias, and malaise. Prominent upper respiratory symptoms (rhinorrhea, sore throat, or dry cough) may help to distinguish influenza from malaria. The symptoms of many cases of malaria that were ultimately fatal to returned travelers in North America have been initially and mistakenly attributed to influenza.

Enteric Fever

Salmonella typhi and *Salmonella paratyphi* can be acquired in developing countries worldwide. As for malaria, patients with enteric fever may present with fever, headache, nausea, malaise, anorexia, and myalgias. Prominent gastrointestinal symptoms (abdominal pain, constipation, or diarrhea), the findings of rose spots or relative bradycardia, and a history of unsanitary food or water consumption may help to support

a diagnosis of enteric fever. A history of prior vaccination against *S. typhi* may not be useful in ruling out enteric fever because it is only 50% to 80% effective and does not protect against paratyphoidal illness.

Bacteremia/Sepsis

The fever, hypotension, evidence of poor peripheral perfusion, altered mental status, and multiorgan dysfunction that characterize bacteremia and sepsis can mimic severe malaria. One study found that bacteremia accompanied *P. falciparum* infection in 12% of young children who were admitted to a hospital with the primary diagnosis of severe malaria.[533]

Dengue Fever

Patients with classic nonhemorrhagic dengue fever may present with fever, headache, nausea, malaise, or anorexia. Myalgias tend to be much more severe than those experienced during malaria episodes. Dengue fever may be distinguished from malaria by its centrifugal rash, petechiae, lymphadenopathy, conjunctival injection, pharyngeal erythema, and relative bradycardia. Although dengue virus is also transmitted by mosquitoes during the rainy season in tropical regions worldwide, its incubation period of 4 to 7 days is not at all typical of malaria.

Acute Schistosomiasis (Katayama Fever)

Schistosoma trematodes are acquired from freshwater exposure (wading, swimming) in tropical regions worldwide. Patients with acute schistosomiasis may present 4 to 8 weeks after exposure with fever, headache, myalgias, malaise, and anorexia. Acute schistosomiasis can be distinguished from malaria by generalized urticaria and the findings of a pruritic rash at the site of cercarial penetration (usually on the legs), lymphadenopathy, and blood eosinophilia. Patients may present initially with focal neurologic signs as a result of egg dissemination to the central nervous system.

Leptospirosis

Leptospira interrogans spirochetes are acquired from freshwater or soil exposure in tropical and temperate regions worldwide. Patients with leptospirosis usually present within 7 to 12 days of exposure with fever, headache, nausea, and myalgias. Leptospirosis can be distinguished from malaria by the findings of conjunctival suffusion or rash but may progress to hepatic and renal insufficiency marked by hemorrhagic manifestations and pronounced hyperbilirubinemia (Weil syndrome). This complication is similar to a severe malaria presentation, but extremely high bilirubin levels are more characteristic of Weil syndrome.

African Tick Fever

Rickettsia africae is transmitted by tick bites, usually acquired during game hunting or safari travel to southern Africa between April and November. Presenting symptoms of African tick fever may be fever, headache, and myalgias and can be differentiated from malaria by the findings of lymphadenitis or multiple inoculation eschars.

East African Trypanosomiasis (Sleeping Sickness)

Trypanosoma brucei rhodesiense causes the acute form of African sleeping sickness and is acquired from tsetse fly bites, typically in association with game and brush in eastern and southern Africa. Trypanosomiasis may present with fever, headache, myalgias, malaise, and anorexia, yet it may be differentiated from malaria by a red chancre at the bite site, posterior cervical lymphadenopathy, or rash. Like malaria, it may progress to involve multiple organs, including the central nervous system.

Yellow Fever

Yellow fever virus is acquired from mosquito bites in tropical regions worldwide. It is characterized by fever, headache, myalgias, nausea, anorexia, and jaundice. Yellow fever may be differentiated from malaria by the presence of conjunctival suffusion or relative bradycardia and by its short incubation period (average, 3–6 days). Yellow fever is extremely unlikely in patients who have been vaccinated against it within the previous 10 years. As with severe malaria, patients may appear acutely ill and progress to liver failure and hemorrhagic manifestations, multiorgan system failure, and death.

THERAPY (see also Chapter 41)

General Principles

P. falciparum malaria can be fatal if not diagnosed and treated promptly and appropriately. This is especially true of nonimmune travelers returning from visits to malaria-endemic areas.[519,534,535] Malaria is a disease of protean manifestations.[536] Its diagnosis can be delayed by the nonspecificity of the clinical presentation and routine laboratory tests, especially if blood smears (and, if available, RDTs) are not promptly examined. Life-threatening manifestations of malaria, such as convulsions, hypoglycemia, and pulmonary edema, may develop rapidly in patients who appear relatively well at presentation or appear to respond initially to antimalarial drugs.[172]

Although some patients with uncomplicated *P. falciparum* malaria can be treated successfully in an outpatient setting,[537] patients with no immunity against the disease are at increased risk for sudden development of severe complications and should be hospitalized for at least 48 hours to ensure adequate response to therapy, regardless of how well they appear at presentation. Hospitalization is likewise recommended for individuals from malaria-endemic countries but whose immune status nevertheless cannot be known with certainty.[538] Acute *P. falciparum* malaria in a nonimmune individual can be dangerous and unpredictable; even without warning signs at presentation, a patient's condition may deteriorate dramatically after prompt hospitalization and apparently adequate treatment.[538] Contributing factors in such cases may include (1) replication of the parasites and their synchronous development into mature forms that sequester in the brain, leading to cerebral malaria, and (2) complications from the infection that lead to acute renal failure, lung injury, or hepatic insufficiency, even when the parasitemia is decreasing or the patient appears to be improving in other ways. Pregnant women, young children, and the elderly are at increased risk of morbidity and mortality and should be hospitalized regardless of their clinical state.[539–542]

Patients with *P. vivax, P. ovale,* or *P. malariae* malaria infrequently require hospitalization, although it is certainly necessary in cases with severe manifestations.[56,57] Patients with *P. knowlesi* malaria may require hospitalization because life-threatening manifestations of disease can develop rapidly.[18]

Uncomplicated Malaria

Treatment of uncomplicated *P. falciparum* malaria should always be initiated emergently because the risk of its morbidity and mortality increases with even short delays in medical care.[543] Uncomplicated malaria can be treated with oral medication if the patient is able to retain the drug; directly observed therapy may be appropriate in some cases to ensure adequate treatment. Drugs currently recommended for treating uncomplicated malaria are listed in Table 274.3. Most antimalarials, *except* for halofantrine (not available in the United States), doxycycline or tetracycline, and primaquine, can be safely used in pregnant or breastfeeding women.[544,545]

Because of widespread and ever-changing patterns of drug resistance in the world today, there is no blanket recommendation for antimalarial use, and treatment options must be carefully considered. Chloroquine can be used to treat uncomplicated *P. falciparum* malaria acquired from areas where chloroquine resistance has *not* been reported: Mexico, Central America west of the Panama Canal, Haiti, the Dominican Republic, and most areas of the Middle East. Chloroquine can also be used to treat uncomplicated malaria caused by any of the other four human malaria parasites, except for *P. vivax* malaria acquired in Papua New Guinea and Indonesia.

The blood-stage parasites of chloroquine-resistant *P. falciparum* or *P. vivax* uncomplicated malaria can be treated with the combination drugs atovaquone-proguanil[546] or artemether-lumefantrine,[547] both of which are highly effective in nonimmune individuals[548,549] (treatment to prevent relapse from *P. vivax* hypnozoites is discussed later). An alternative regimen is quinine plus doxycycline (or tetracycline or

TABLE 274.3 Malaria Treatment

DRUG	ADULT DOSE	PEDIATRIC DOSE[b]	PRECAUTIONS
Uncomplicated Malaria: Chloroquine-Resistant *Plasmodium falciparum*[c] or Chloroquine-Resistant *Plasmodium vivax*[d]			
***Atovaquone-Proguanil (Malarone)*[e]**			
Supplied in fixed-dose combination tablets containing 250 mg atovaquone and 100 mg proguanil (*adult* tablets) or 62.5 mg atovaquone and 25 mg proguanil (*pediatric* tablets)	Four adult tablets daily for 3 d (may be administered as 2 tablets 2× daily)	The number of *pediatric* or *adult* tablets taken daily for 3 d depends on patient's weight: 5–8 kg: 2 pediatric tablets 9–10 kg: 3 pediatric tablets 11–20 kg: 1 adult tablet 21–30 kg: 2 adult tablets 31–40 kg: 3 adult tablets >40 kg: 4 adult tablets	Not recommended in pregnant or breastfeeding women or infants weighing <5 kg. Take with food or whole milk. If patient vomits within 30 min of taking a dose, the dose should be repeated.
Artemether-Lumefantrine (Coartem)			
Supplied in fixed-dose combination tablets containing 20 mg artemether and 120 mg lumefantrine	Six-dose regimen: first day: 4 tablets initially, then 4 tablets 8 h later second day: 4 tablets 2× daily third day: 4 tablets 2× daily	Number of tablets per dose taken according to adult schedule depends on patient's weight: 5–14 kg: 1 tablet 15–24 kg: 2 tablets 25–34 kg: 3 tablets ≥35 kg: 4 tablets	Can be used in second and third trimesters of pregnancy and, if no other option is available, in first trimester of pregnancy. Not recommended in infants weighing <5 kg.[f] Take with food or whole milk. If patient vomits within 30 min of taking a dose, the dose should be repeated.
***Quinine Sulfate Plus One of the Following: Doxycycline, Tetracycline, or Clindamycin*[g]**			
Quinine sulfate supplied in 325-mg salt tablets. Doxycycline supplied in 100-mg tablets. Tetracycline supplied in 250-mg tablets. Clindamycin supplied in 300-mg capsules.	Quinine sulfate, 650 mg salt 3× daily for 3 d[h] *plus* either doxycycline, 100 mg 2× daily for 7 d, *or* tetracycline, 250 mg 4× daily for 7 d, *or* clindamycin, 20 mg/kg/d divided 3× daily for 7 d	Children ≥8 yr old: quinine sulfate, 10 mg salt/kg 3× daily for 3 days[h] *plus*[i] either doxycycline, 3 mg/kg daily for 7 d, *or* tetracycline, 25 mg/kg/d divided 4× daily for 7 d, *or* clindamycin, 20 mg/kg/d divided 3× daily for 7 d	Do not use doxycycline or tetracycline in children <8 yr old; use Malarone or Coartem for chloroquine-resistant *P. falciparum* or Malarone or mefloquine for chloroquine-resistant *P. vivax* instead. Do not use doxycycline or tetracycline in pregnant or breastfeeding women; use clindamycin instead.
***Mefloquine*[j] (Lariam, Generics)**			
Supplied in 250-mg salt tablets	750 mg salt, then 500 mg salt 6–12 h later	15 mg salt/kg, then 10 mg salt/kg 6–12 h later	Do not administer to individuals with cardiac conduction abnormalities, history of seizures, or serious psychiatric illnesses (e.g., psychosis, major depression). Do not use concomitantly with quinidine, quinine, or halofantrine.
Uncomplicated Malaria: Chloroquine-Sensitive *P. falciparum*; Chloroquine-Sensitive *P. vivax*,[k] *P. ovale*, *P. malariae*, or *P. knowlesi*			
Chloroquine Phosphate (Aralen, Generics)			
Supplied in 300-mg base tablets	600 mg base, then 300 mg base at 6, 24, and 48 h	10 mg base/kg (max. 600 mg base), then 5 mg base/kg at 6, 24, and 48 h	None. Safe in pregnant women.
Severe Malaria			
***Quinidine gluconate*[l]**			
Not available in the United States	Intravenous: 10 mg salt/kg loading dose (max. 600 mg) in normal saline infused slowly at a constant rate over 1–2 h, then continuous infusion of 0.02 mg salt/kg/min for at least 24 h until parasitemia <1% and patient can take oral therapy[m]	Intravenous: same dosing as for adults[m]	Do not administer as a bolus. Check blood glucose every 4–6 h during first 24 h of therapy. Administer 5%–10% dextrose along with quinidine to reduce risk of hypoglycemia. Monitor drug levels to keep between 3 and 8 μg/mL.
Artesunate			
Available in the United States from the CDC through an IND protocol	Intravenous: artesunate, 2.4 mg/kg, then 2.4 mg/kg at 12, 24, and 48 h[n]	Intravenous: for children >20 kg, same dosing as adults; children <20 kg use 3.0 mg/kg at 0, 12, 24, and 48 h[n]	None.
Quinine dihydrochloride			
Not available in the United States	Intravenous: 20 mg/kg loading dose in 5% dextrose infused slowly at a constant rate over 4 h, followed by maintenance dose 10 mg/kg over 3–4 h at 8-h intervals (max. 1800 mg/d) until oral therapy can be started[m]	Intravenous: same dosing as for adults[m]	Do not administer as a bolus. Check blood glucose every 4–6 h during first 24 h of therapy. Administer 5%–10% dextrose along with quinine to reduce risk of hypoglycemia.
Artemether			
Not available in the United States	Intramuscular: artemether, 3.2 mg/kg on first day, then 1.6 mg/kg daily for 4 d[n]	Intramuscular: same dosing as for adults[n]	None.

TABLE 274.3 Malaria Treatment—cont'd

DRUG	ADULT DOSE	PEDIATRIC DOSE[b]	PRECAUTIONS
Prevention (Terminal Prophylaxis) of Relapsing Malaria: *P. vivax* or *P. ovale*			
Primaquine Phosphate			
Supplied in 15-mg base tablets	30 mg base once daily for 14 d after departure from malaria-endemic area	0.5 mg base/kg once daily for 14 d (max. 30 mg base/d) after departure from malaria-endemic area	Test patient for G6PD deficiency and administer *only* if enzyme activity is normal.[o] Do *not* administer to pregnant or breastfeeding women.[p] Take with food.

[a]If a person develops malaria while taking a drug as prophylaxis, that drug should not be used as part of his or her treatment regimen. Use another option instead.
[b]Pediatric doses should *never* exceed the adult dose.
[c]Suspect chloroquine-resistant *P. falciparum* malaria in all areas *except* Mexico, Central America west of the Panama Canal, Haiti, the Dominican Republic, and most areas of the Middle East.
[d]Suspect chloroquine-resistant *P. vivax* malaria in Papua New Guinea and Indonesia.
[e]This regimen can also serve as presumptive self-treatment in travelers who have febrile illness but no immediate access to medical care. This regimen is not recommended for self-treatment of individuals on atovaquone-proguanil prophylaxis.
[f]Data from Ballard S-B, Salinger A, Arguin PM, et al. Updated CDC recommendations for using artemether-lumefantrine for the treatment of uncomplicated malaria in pregnant women in the United States. *MMWR.* 2018;67:424–431. https://www.cdc.gov/mmwr/volumes/67/wr/mm6714a4.htm?s_cid=mm6714a4_w.
[g]Because there are more data on the efficacy of quinine *plus* doxycycline or tetracycline, these combinations are preferred to quinine *plus* clindamycin.
[h]For infections acquired in Southeast Asia, where reduced susceptibility to quinine has been reported, treat with quinine sulfate for 7 days.
[i]Pediatric dosing may be difficult due to the unavailability of noncapsule forms of quinine.
[j]Mefloquine is recommended only if Malarone, Coartem, or quinine sulfate *plus* doxycycline (or tetracycline or clindamycin) cannot be used because of the higher rates of severe neuropsychiatric reactions at treatment doses. Mefloquine is *not* recommended for treatment of *P. falciparum* infections acquired in Southeast Asia because of drug resistance.
[k]If a patient does not respond to chloroquine, switch to a regimen for chloroquine-resistant *P. vivax* and notify the CDC via its Malaria Hotline.
[l]No longer available in the United States.
[m]Complete therapy with a quinine sulfate *plus* doxycycline (or tetracycline) regimen to complete a 7-day total course of therapy. In pregnant or breastfeeding women or children younger than 8 years, substitute clindamycin for doxycycline (or tetracycline).
[n]To prevent parasite recrudescence after artemisinin monotherapy, complete treatment with a standard course of atovaquone-proguanil, doxycycline (or tetracycline), clindamycin (in pregnant women), or mefloquine given orally.
[o]If G6PD activity is borderline, or as an alternative, administer primaquine, 45 mg base weekly for 8 consecutive weeks. If G6PD activity is deficient, contact the CDC or an infectious diseases/tropical medicine specialist for advice.
[p]Pregnant women requiring terminal prophylaxis should receive chloroquine 300 mg base weekly until delivery, and then primaquine after delivery if G6PD activity is normal and there is no breastfeeding.
CDC, Centers for Disease Control and Prevention; *G6PD,* glucose-6-phosphate dehydrogenase; *IND,* Investigational New Drug.
Drug regimens modified from CDC. Guidelines for Treatment of Malaria in the United States. *https://www.cdc.gov/malaria/resources/pdf/TreatmentTable_2018.pdf.*

clindamycin). Mefloquine is recommended only if atovaquone-proguanil, artemether-lumefantrine, or quinine plus doxycycline cannot be used because of the higher rates of severe neuropsychiatric reactions at treatment doses.[550] Because of drug resistance, however, mefloquine is *not* recommended for *P. falciparum* malaria acquired in Southeast Asia.

If a patient cannot tolerate oral therapy, parenteral formulations of antimalarial drugs must be administered (see later). Up-to-date information on malaria treatment and assistance with the management of suspected cases of malaria are available from the CDC Malaria Hotline (770-488-7788 or 855-856-4713 toll-free, Monday through Friday, 9:00 AM to 5:00 PM EST; 770-488-7100 after hours, weekends, and holidays). Current guidelines for malaria treatment in the United States are also available from the CDC (https://www.cdc.gov/malaria/resources/pdf/TreatmentTable_2018.pdf).

Chloroquine Phosphate

Chloroquine is considered safe in pregnant women (all trimesters)[551,552] and in children of all ages, including newborns. It has a bitter taste and may cause nausea or vomiting (take with food to ameliorate gastrointestinal symptoms), headache, dizziness, blurred vision, or dysphoria. Chloroquine commonly produces a nonallergic pruritus in dark-skinned persons and may exacerbate psoriasis. It is associated with retinal toxicity at high doses taken for chronic illnesses[553] but not at doses taken for malaria. Acute chloroquine toxicity can produce convulsions, hypotension and shock, and cardiorespiratory arrest.[554]

Atovaquone-Proguanil (Malarone)

Atovaquone-proguanil is used to treat uncomplicated malaria caused by multidrug-resistant *P. falciparum*[356,381,555] and is relatively well tolerated, although almost one-fourth of patients have gastrointestinal side effects.[556–558] Only rarely has atovaquone-proguanil been associated with severe adverse reactions.[559,560] Clearance of parasites is relatively slow: only 66% at 3 days.[556] Atovaquone-proguanil resistance is rare, being confined to case reports in mostly nonimmune individuals in whom recrudescence and initial treatment failure have been documented.[378–380,428,429,561–565]

Artemisinin-Based Combination Therapies

ACTs[566] are the first-line recommended treatments for uncomplicated *P. falciparum* malaria,[556,567] including in children and pregnant women during the second and third trimesters.[568] An ACT is a 3-day regimen of artemisinin or one of its derivatives (i.e., artesunate, artemether, or dihydroartemisinin) in combination with a partner drug (i.e., amodiaquine, mefloquine, lumefantrine, piperaquine, or pyronaridine). Although artemisinins are potent and fast acting, recrudescences are frequent after their use as monotherapy, and long-acting partner drugs are therefore needed to ensure cure.[569] Patterns of resistance to partner drugs determine which ACT should be used in particular geographic locations. Because of widespread resistance to chloroquine, ACTs that contain amodiaquine or lumefantrine are commonly used in Africa.[570] After a period of declining efficacy of mefloquine in Southeast Asia, ACTs containing piperaquine were increasingly used in this region.[571–579] Piperaquine, however, is now threatened by the rapid rise of resistance,[580,581] but with some return of mefloquine susceptibility; artesunate-mefloquine may therefore be useful in rotation against *P. falciparum* strains that are resistant to dihydroartemisinin-piperaquine.[582] Artesunate-pyronaridine has been shown to be an effective treatment in Western Cambodia, where multidrug-resistant *P. falciparum* is highly prevalent.[583,584]

A fixed-dose combination of artemether-lumefantrine (Coartem)[585] is the only ACT that is FDA-approved for use in the United States. For adults, four tablets are given initially and repeated at 8, 24, 36, 48, and 60 hours.[556] This ACT should be taken if possible with a high-fat snack to maximize absorption of lumefantrine. Combinations of dihydroartemisinin-piperaquine (Eurartesim)[586] and artesunate-pyronaridine (Pyramax)[587] are licensed for use in Europe.

Quinine Plus Doxycycline

The combination of quinine plus doxycycline is effective against multidrug-resistant parasites. To prevent parasite recrudescence after quinine monotherapy, doxycycline (or tetracycline or clindamycin) is given as a partner drug. Quinine has a bitter taste and may cause gastrointestinal upset and cinchonism[588] (nausea, vomiting, dysphoria,

tinnitus, and high-tone deafness). Hypoglycemia from quinine-induced insulin secretion can be an adverse event, particularly in pregnancy.[554] Quinine is considered safe in pregnant women (all trimesters).[552] Doxycycline also causes gastrointestinal upset and commonly results in vaginal candidiasis, requiring concomitant use of antifungal suppositories. The requirement for multiple doses over 7 days and the gastrointestinal upset caused by both drugs may reduce the compliance and hence effectiveness of this regimen.[589] The use of all tetracyclines is contraindicated in children younger than 8 years or in pregnant women because of adverse effects on tooth and bone development. In these cases, clindamycin may be a safe and effective substitute for doxycycline.[590–593]

Mefloquine

Mefloquine can be used to treat most chloroquine-resistant parasites except for strains in areas such as Cambodia, Thailand, Myanmar, and Vietnam, where resistance against this drug is present.[557] It may cause gastrointestinal upset, vomiting, dysphoria, dreams, mood changes, and neuropsychiatric reactions in a significant proportion of patients,[594–596] although there is no evidence for increased first-time diagnosis of depression with mefloquine relative to other commonly used antimalarials.[597] Mefloquine is cleared slowly because its elimination half-life is 2 to 3 weeks. Because mefloquine may prolong the corrected QT (QTc) interval, it cannot be administered concurrently with quinine or quinine-like drugs (e.g., quinidine, halofantrine). Mefloquine should not be used in persons with cardiac conduction diseases because it can aggravate conduction abnormalities.

Standby Emergency Treatment of Uncomplicated Malaria in Travelers

Because treatment delay increases morbidity and mortality associated with malaria, travelers to isolated areas may benefit from standby emergency treatment while they actively seek medical care.[598] Standby antimalarials may be advised to travelers for emergency self-treatment of fever or other symptoms compatible with malaria that occur at least 1 week after entering an endemic area. Drugs used for this purpose include atovaquone-proguanil, artemether-lumefantrine, artesunate-pyronaridine (available in Europe), and dihydroartemisinin-piperaquine (available in Europe), all of which are likely to be effective in all malaria-endemic areas because of lack of significant drug resistance. Although standby emergency treatment can be safe, effective, and potentially lifesaving, no regimen is currently registered for this use in any country. No randomized controlled clinical trials have been or are likely to be performed because of the high morbidity and mortality of untreated or inappropriately treated malaria in nonimmune individuals. Travelers should be discouraged from self-treatment with the same drug they are using for chemoprophylaxis or with locally acquired products that may be of poor quality or outright fake.[599–604]

Intermittent Presumptive Treatment in Infants, Children, and Pregnant Women

In areas with intense transmission, infants 6 to 12 months of age suffer multiple episodes of malaria and are therefore at risk for life-threatening severe anemia.[158,605] Weekly chemoprophylaxis of infants protects against malarial fevers as well as anemia but may compromise development of natural immunity.[606,607] Intermittent presumptive treatment (IPT, e.g., amodiaquine every 2 months for a total of 6 months; also known as intermittent preventative treatment) of infants can reduce malaria morbidity by 50% to 65% during the first year of life while still allowing sufficient exposure to parasites and development of immunity.[608,609] In areas with seasonal malaria transmission, IPT in children younger than 5 or 6 years reduces the incidence of uncomplicated and severe malaria.[158,610] In areas of endemic transmission, malaria of pregnancy is associated with severe maternal anemia and low birth weight in newborns. IPT has been shown to reduce the risk of severe anemia in women who received one to three doses of SP over the duration of their first pregnancy.[611] A systematic review and meta-analysis of seven trials in sub-Saharan Africa showed that IPT with three or more doses of SP was associated with a lower risk of low birth weight than two-dose regimens.[612] A meta-analysis of 32 national cross-sectional data sets in Africa showed that preventing malaria with IPT and insecticide-treated nets in pregnant women substantially reduces neonatal mortality and low birth weight.[613] These and other studies support the WHO recommendation to provide at least three doses of IPT during pregnancy at each antenatal visit.

Seasonal Malaria Chemoprevention

Seasonal malaria chemoprevention (SMC) is defined as the intermittent administration of full treatment courses of antimalarial drugs to children in areas with highly seasonal transmission during the malaria season. SMC aims to reduce the incidence of falciparum malaria in areas where the youngest children are most vulnerable to severe disease and death, and where *P. falciparum* remains largely susceptible to appropriate regimens. For example, WHO recommends[614] monthly doses of amodiaquine *plus* SP in children ages 6 months to 5 years in areas with highly seasonal malaria transmission (typically for 3–4 months) in the Sahelian region of Africa. This intervention, which is safe and cost-effective and can be administered by community health workers, has been shown to be 75% protective against uncomplicated and severe malaria in this setting.

Mass Drug Administration

In contrast to SMC, which treats a subpopulation of young children but does not address the reservoir of transmittable parasites from older children and adults, mass drug administration (MDA) aims to clear parasites from all individuals. MDA is of particular interest for its use in control programs that aim to dramatically reduce and eventually eliminate malaria.[615] Island regions with monitored migration have used MDA to move toward elimination; sustainable successes in these settings recognize the critical role of high-level community engagement and unwavering commitments to permanent surveillance and outbreak control.[616,617]

Severe Malaria

Successful treatment of patients with severe malaria requires frequent clinical monitoring and intensive nursing care and may demand sophisticated interventions, such as continuous electrocardiography (ECG), hemodynamic monitoring, mechanical ventilation, or hemodialysis. Replacement of blood and fluids can lead to rapid reductions in lactate, resolution of metabolic acidosis, improvement in renal function, and clinical improvement of critically ill children.[618] On the other hand, liberal fluid resuscitation in adults may *not* improve acid-base status or renal function and may promote the development of pulmonary edema.[619]

Quinidine gluconate is the only FDA-approved parenteral treatment for severe malaria in the United States, but the intravenous formulation is no longer available in the United States as of this writing.[620,621] Intravenous artesunate is now the preferred treatment for severe malaria in the United States. Artesunate is much safer than intravenous quinidine and is readily available from the CDC through its Malaria Hotline (see hours and phone numbers under "Uncomplicated Malaria" earlier).[620]

Outside the United States, parenteral formulations of artemisinin derivatives and quinine are widely used to treat severe malaria, and rectal suppositories of artesunate are available for the emergent treatment of severe malaria if patients cannot be treated orally and access to injections will take several hours.[534,623] In Canada, intravenous formulations of artesunate and quinine are available through the Canadian Malaria Network (https://www.canada.ca/en/public-health/services/travel-health/medical-access-artesunate-quinine-malaria-treatment.html). Antimalarial drug regimens used to treat severe malaria are presented in Table 274.3.

Quinidine Gluconate

Many physicians will be unfamiliar with quinidine because this drug has been replaced by newer antiarrhythmic agents. Quinidine has a narrow therapeutic window and must be used with extreme care in an intensive care unit.[620] Its use in consultation with a cardiologist or a physician with experience in treating malaria is advised. It is administered intravenously as an infusion until the patient improves clinically and can complete antimalarial treatment orally. Quinidine levels should be monitored throughout the period of its administration (see Table 274.3).

Quinidine is *never* administered as a bolus injection, which can cause fatal hypotension. Potentially fatal adverse reactions can occur even at treatment doses. It can cause postural hypotension, so frequent blood pressure measurements should be made. Quinidine may prolong the QTc interval, putting the patient at risk for ventricular tachycardias (e.g., torsades de pointes), and therefore should be administered with continuous ECG monitoring.[624] If QTc prolongation greater than 25% of baseline or hypotension unresponsive to fluid challenge develops, the infusion rate should be reduced or the infusion stopped. Because quinidine may cause hyperinsulinemic hypoglycemia, serum glucose must be monitored every 4 to 6 hours and with any acute neurologic change (e.g., diminished consciousness, convulsions) that may arise from severe hypoglycemia. Administration of 5% or 10% dextrose while infusing quinidine can reduce the incidence of hypoglycemia.

In patients taking medications that also prolong the QT interval, particularly when coadministered with drugs that suppress hepatic metabolism, the use of quinidine can be problematic. Although the initial loading dose of quinidine is not reduced in renal insufficiency, patients with malaria and acute renal failure may not clear quinidine effectively. Case reports illustrating the use of quinidine in the treatment of severe malaria have highlighted common clinical scenarios, such as adjustment of infusion rates associated with elevated quinidine blood levels; prolonged QTc intervals; and arrhythmias as well as hypoglycemia, hypotension, and vomiting.[624] Quinidine levels should be maintained below 8 µg/mL; this may require 30% to 50% dose reductions to prevent drug accumulation in patients who remain seriously ill after 3 days of treatment. The response to quinidine is assessed by frequent blood smears every 6 to 8 hours to ensure rapid decrease in parasitemia. Once the patient improves and can take oral medications without vomiting, quinidine can be discontinued, and a 7-day total course of treatment completed with a combination of quinine tablets and doxycycline.

Artemisinin and Its Derivatives

Artemisinin is derived from *Artemisia annua* (qing hao), an herbal plant used in China for 2000 years as therapy for fevers.[625] Artemisinin and its derivatives (artesunate, artemether, and dihydroartemisinin) are highly effective against multidrug-resistant parasites and result in rapid clearance of parasitemia and clinical improvement, usually within 24 to 36 hours. They are well tolerated and safe in adults, children, and pregnant women.[626,627] Millions of people have taken artemisinins with no significant adverse or treatment-limiting effects being reported.[628] Two large, multicenter, randomized trials conducted in Southeast Asian adults[629] and sub-Saharan African children[630] concluded that parenteral treatment with artesunate reduced mortality from severe falciparum malaria 22% to 35% more than did treatment with parenteral quinine. A review of eight randomized controlled trials found that parenteral or rectal artesunate was superior to parenteral quinine for treating severe malaria in both adults and children in different regions of the world.[631] Post-artesunate hemolysis occurs in 10% to 15% of patients receiving intravenous artesunate, particularly those with high parasite counts, usually beginning after the seventh day from the start of therapy. A mechanism that removes pitted, previously infected erythrocytes is thought to contribute to this phenomenon.[632–634] Patients should be informed of this and have their hemoglobin level checked around day 14.[556,635] The course is self-limited but may require red blood cell transfusion.

In June 2007, the Walter Reed Army Institute for Research and the CDC received FDA approval for a collaborative IND protocol for intravenous artesunate to treat severe malaria (https://www.cdc.gov/malaria/diagnosis_treatment/artesunate.html). Artesunate is provided by the CDC to hospitalized malaria patients on an emergency basis to treat those who need intravenous treatment for severe disease, who have high parasitemia, or who are unable to take oral medications.

Quinine Dihydrochloride

In some endemic areas, quinine is the only readily available drug for the parenteral treatment of patients who have severe malaria or patients with uncomplicated malaria who cannot take oral medication for reasons including vomiting. Quinine commonly causes hypoglycemia and the unpleasant side effects of cinchonism. Administration of quinine in a dextrose infusion and frequent (every 4–6 hours) glucose checks help to avoid hypoglycemia, which in some cases is life threatening. Quinine is much less cardiotoxic than quinidine, requiring no continuous ECG monitoring during its administration. Intravenous formulations of quinine are not commercially available in the United States, nor can they be obtained from the CDC on an emergency basis.

Bacteremia and Sepsis in Severe Malaria

In patients who present with a clinical picture consistent with sepsis syndrome, broad-spectrum antibiotics should be administered while awaiting blood culture results. Bacteremia complicating severe malaria is not uncommon in infants and children and may cause any patient's clinical status to deteriorate abruptly.[533,636,637]

Exchange Transfusion in Severe Malaria

High parasitemia has been correlated with mortality in falciparum malaria, leading to the use of exchange transfusion (ET) as an adjunct therapeutic measure.[638] ET may reduce parasite load, remove toxic substances, reduce microcirculatory sludging, and rapidly correct anemia. Although case reports and series claim beneficial effects of ET (including partial ET in young children),[639–644] a meta-analysis concluded that a randomized controlled trial is necessary to determine whether ET is beneficial.[645] ET may be harmful and is associated with fluid overload, risk of transfusion reactions and related infections, and line sepsis. In addition, ET does not remove infected erythrocytes that are sequestered in deep tissue capillary beds, including those in the brain. Exchange transfusion is no longer recommended by the CDC.

Nonfalciparum Malaria

All cases of malaria should be treated as falciparum malaria until proved otherwise because *P. falciparum* infections can rapidly become life threatening. Uncomplicated malaria caused by *P. vivax, P. ovale, P. malariae,* or *P. knowlesi* is treated with chloroquine, unless (1) it is acquired in geographic regions where these species are known or suspected to be chloroquine resistant (e.g., *P. vivax* in Papua New Guinea and Indonesia) or (2) any doubt exists as to the parasite species or if there is a mixed infection. Mixed infections consisting of two or more *Plasmodium* species may sometimes mask a chloroquine-resistant *P. falciparum* subpopulation that can emerge during or after treatment. The blood stages of *P. vivax* infections that are likely to be chloroquine resistant can be treated with quinine plus doxycycline, atovaquone-proguanil,[646] or mefloquine at doses listed in Table 274.3. The CDC does not currently recommend artemether-lumefantrine for treatment of nonfalciparum malaria, although significant clinical data support its use for this indication.[547,647,648] Infections with *P. knowlesi* can be treated with chloroquine unless severe manifestations are present,[649] in which case parenteral treatment with quinidine, an artemisinin derivative, or quinine would be indicated.

Persistent liver stages (hypnozoites) of *P. vivax* and *P. ovale* may be treated with primaquine (see Table 274.3) if there is no contraindication, but this treatment frequently fails because of noncompliance with the recommended 14-day regimen. After blood-stage *P. vivax* and *P. ovale* infections are treated with chloroquine (a drug *not* effective against hypnozoites), primaquine must be administered to prevent relapse.[650–652] Even when taken appropriately, however, this treatment may not always be successful because of interindividual variations of drug metabolism or variable parasite responses to low versus high drug doses.[653,654] Dihydroartemisinin-piperaquine *plus* primaquine has also been shown to be effective in treating and preventing relapses of chloroquine-resistant *P. vivax* malaria in Indonesia.[655] Taking primaquine with food ameliorates gastrointestinal side effects and improves compliance. Patients should be advised to discontinue this drug and seek medical evaluation if their urine becomes dark because primaquine occasionally can cause some hemolysis in persons with mildly deficient or even normal G6PD activity. Primaquine causes methemoglobinemia in nearly all persons treated, but this is rarely clinically significant (bluish discoloration of mucous membranes may be observed).

Primaquine is contraindicated in persons with severe forms of G6PD deficiency (e.g., Mediterranean type) or methemoglobin reductase deficiency because of the danger of massive and potentially fatal

hemolysis.[210] Persons with less severe forms of G6PD deficiency have been treated with standard primaquine doses, but significant decreases in hematocrit levels were observed in some cases.[656] In such individuals, weekly dosing of primaquine for longer periods (e.g., 8 weeks) has been recommended.[651,657] Primaquine should not be administered to pregnant women because of the risk of hemolytic disease in the fetus. Individuals who do not receive primaquine (including pregnant women) should be monitored for relapses, and if these occur, should be treated with blood-stage antimalarials. Primaquine should be administered to individuals who reside permanently in areas endemic for *P. vivax* or *P. ovale,* even if reinfection is likely. Primaquine is not administered to persons who acquire infection by transfusion or transplantation because hypnozoites develop only from mosquito-inoculated sporozoites. Tafenoquine[658] has been developed as a potential replacement for primaquine and continues to be evaluated for safety and efficacy in clinical trials (see Chapter 41).[659] Based on data from the recent GATHER and DETECTIVE[660] studies, the FDA in July 2018 approved single-dose tafenoquine 300 mg to prevent relapse of *P. vivax* malaria in patients aged 16 years and older. Pregnancy and G6PD deficiency are contraindications for tafenoquine use.

PREVENTION

Risk Assessment

The risk of acquiring malaria varies according to the geographic region visited,[661] travel destination within geographic areas (e.g., urban versus rural setting), type of accommodations (e.g., camping tent vs. air-conditioned hotel), duration of stay (e.g., a less than 1-week business trip vs. a 3-month travel adventure), time of travel (high- vs. low-transmission season), altitude of destination (<2000 meters vs. higher), and efficacy of and compliance with malaria prophylaxis measures.[662–664] First- and second-generation immigrants and their children living in nonendemic countries who return home to visit friends and relatives (so-called VFR travelers)[665,666] are at high risk for acquiring malaria[317] because they often do not take prophylaxis (thinking that malaria is not a serious threat because of their previous experience with it), are unaware they have "lost" immunity to malaria and are now at risk for severe disease, do not realize the risks of traveling when pregnant or when taking young nonimmune children to malarious regions, or are medically underserved and therefore less likely to seek pretravel advice.[667]

Chemoprophylaxis

Lack of proper chemoprophylaxis continues to be associated with severe complications from malaria and death.[317,543] Malaria is effectively prevented in travelers and in pregnant women in endemic areas by using antimalarial drugs when prescribed and taken appropriately (Table 274.4). In 2015, only 36 of 766 (4.7%) US residents who acquired malaria in an endemic area reported they had been compliant with a chemoprophylaxis regimen recommended by the CDC for their regions of travel.[317]

Selection of an effective prophylactic regimen depends on geographic patterns of drug resistance, medical conditions, concomitant medications, and other factors that may affect compliance: number of pills, dosing interval (i.e., daily vs. weekly), duration of travel, duration of pretravel and posttravel medication, cost, and the reputation as well as the actual tolerability of side effects.[663,668,669] Chemoprophylaxis recommendations change frequently because of regional and temporal variability in malaria risk, even within countries; the ongoing spread and independent emergence of drug-resistant parasites; and resurgence of malaria in areas previously free of the disease. Updates on outbreaks are reported by the CDC at https://www.cdc.gov/parasites/malaria/index.html. In 2012, for example, the CDC reported a series of updates on locally transmitted *P. vivax* malaria cases in Greece but did not recommend chemoprophylaxis for travelers to this country. Information on the geographic risk of acquiring malaria can be found by using the CDC's Infectious Diseases Related to Travel website (https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/malaria#5210).

Areas With Chloroquine-Sensitive Malaria

Currently, chloroquine (or hydroxychloroquine) can be used in travelers to those limited areas where chloroquine-resistant *P. falciparum* has *not* been reported, such as some areas of Central America and the Caribbean. Chloroquine is very well tolerated but may cause gastrointestinal disturbance, headache, dizziness, and pruritis, and may exacerbate psoriasis. Chloroquine may also cause retinopathy and arrhythmias when it accumulates because of excessive or prolonged dosing. Periodic funduscopic examination is therefore recommended with long-term use. Chloroquine prophylaxis has been used extensively and safely in pregnant and breastfeeding women[670,671] and in children of all ages, including newborns. Travelers who are exposed to *P. vivax* or *P. ovale* and have normal G6PD activity can receive terminal prophylaxis with primaquine on their return, as discussed earlier.

Areas With Mefloquine-Sensitive Malaria

Mefloquine can be used in travelers to areas where chloroquine-resistant *P. falciparum* has been reported,[672,673] *except* in areas where mefloquine resistance has also been reported: Thailand, Myanmar, southern China, Cambodia, Laos, and Vietnam. Mefloquine may prolong the QTc interval and so cannot be given to individuals with cardiac conduction abnormalities and should also not be administered concurrently with quinine or quinine-like drugs (e.g., quinidine, halofantrine), which can lead to sudden cardiac death. Mefloquine is also contraindicated in individuals with serious neuropsychiatric disorders (e.g., psychosis, major depression, or history of seizures). An estimated 5% of individuals report neuropsychological events, such as sleep disturbances, insomnia, nightmares, cognitive changes, anxiety, or depression, that led to drug discontinuation.[674,675] A systematic review of chemoprophylaxis studies identified eight randomized controlled trials meeting inclusion criteria and found that mefloquine use was associated with more reports of adverse neuropsychiatric events than with doxycycline or atovaquone-proguanil use.[550] In 2013, the FDA updated its label for mefloquine, warning that this drug may cause dizziness, balance problems, and ringing in the ears, which can occur at any time during use and can last for months to years after the drug is stopped or can become permanent. Although not approved for use during pregnancy (FDA pregnancy category B), mefloquine prophylaxis has been reported safe and effective during the second and third trimesters[676–678] and possibly during the first trimester.[679] Because most adverse effects are noted within the first three doses, starting prophylaxis 3 weeks before travel enables individuals to test their tolerance of the drug before they depart.

All Areas

Atovaquone-proguanil or doxycycline can be used in travelers to all areas, including where mefloquine-resistant *P. falciparum* has been reported, or in those travelers who are at risk of acquiring chloroquine-resistant malaria but who cannot take mefloquine.[672,680–683] Atovaquone-proguanil is safe, effective, and well tolerated in both short-term and long-term nonimmune travelers, and there are no contraindications to its use.[673,681,682,684–690] However, it is not recommended for children weighing less than 5 kg, individuals with severe renal impairment, or pregnant women. Although its safety profile in pregnancy has not been established, one study suggests that atovaquone-proguanil use in early pregnancy is not associated with the risk of major birth defects.[691] Atovaquone-proguanil failures have been reported from infections with *P. falciparum* strains that carry mutations in cytochrome *b*.[692]

Doxycycline frequently causes gastrointestinal upset (take with food) and may cause esophageal irritation and ulceration (take with water, sit up for 30 minutes), photosensitivity (use sunscreen), vaginal candidiasis (carry over-the-counter antifungal suppositories), and decreased effectiveness of hormonal contraceptive agents (use backup method). Doxycycline should not be used by pregnant or breastfeeding women and children less than 8 years of age because of deleterious effects on bone and tooth development. In addition, doxycycline should not be taken with metal-containing antacids, which can decrease its absorption. Actual effectiveness of doxycycline may be slightly lower than that reported from some studies because of frequent noncompliance with its daily dosing requirement.[693] Failure of properly taken doxycycline prophylaxis is unlikely because *Plasmodium* resistance has not been reported against this drug.

TABLE 274.4 Malaria Chemoprophylaxis

DRUG	ADULT DOSE	PEDIATRIC DOSE	PRECAUTIONS
Areas With Chloroquine-Sensitive Malaria ***Chloroquine Phosphate (Aralen, Generics)***			
Supplied in 300-mg base tablets	300 mg base once weekly[b]	5 mg/kg base once weekly, up to the maximum adult dose of 300 mg base[b]	Drug accumulation from prolonged use or inadvertent daily dosing may cause retinopathy. May exacerbate psoriasis.
Areas With Mefloquine-Sensitive Malaria ***Mefloquine (Lariam, Generics)***			
Supplied in 250-mg salt tablets	250 mg salt once weekly[c]	Dosed according to body weight: ≤9 kg: 5 mg/kg salt once weekly 10–19 kg: ¼ tablet once weekly 20–30 kg: ½ tablet once weekly 31–45 kg: ¾ tablet once weekly >45 kg: 1 tablet once weekly[c]	Do not use in individuals with cardiac conduction abnormalities, history of seizures, or serious psychiatric illnesses (e.g., psychosis, major depression). Do not use concomitantly with quinidine, quinine, or halofantrine. Do not use in first trimester of pregnancy.
All Areas ***Atovaquone-Proguanil (Malarone)***			
Supplied in fixed-dose combination tablets containing 250 mg atovaquone and 100 mg proguanil (adult tablet) or 62.5 mg atovaquone and 25 mg proguanil (pediatric tablet)	1 adult tablet daily[d]	Dose per body weight: 5–8 kg: ½ pediatric tablet daily 9–10 kg: ¾ pediatric tablet daily 11–20 kg: 1 pediatric tablet daily 21–30 kg: 2 pediatric tablets daily 31–40 kg: 3 pediatric tablets daily >40 kg: 1 adult tablet daily[d]	Take with food or whole milk. Not recommended for children weighing <5 kg, pregnant women, or women who breastfeed a child weighing <5 kg. Prophylactic dosing in children <11 kg constitutes off-label use in the United States. Contraindicated in individuals with severe renal impairment.
Doxycycline			
Supplied in 100-mg tablets	100 mg daily[e]	Children ≥8 yr old: 2.2 mg/kg up to adult dose of 100 mg daily[e]	Do not use in children <8 yr old or in pregnant women. May increase risk of sun sensitivity and vaginal yeast infection.
Areas With Mostly *P. vivax* ***Primaquine Phosphate***			
Supplied in 15-mg base tablets	30 mg base daily[f]	0.5 mg/kg base up to adult dose of 30 mg base daily[f]	Recommended for short-duration travel only. Contraindicated in individuals with G6PD deficiency and pregnant women. Also contraindicated in women who breastfeed, unless the infant being breastfed has a normal G6PD activity level.

[a]When several different drugs are recommended for an area, this table might help in the decision-making process (see https://www.cdc.gov/malaria/travelers/drugs.html).
[b]Beginning 1–2 weeks before travel and continuing *weekly* for 4 weeks after leaving a malarious area.
[c]Beginning 2–3 weeks before travel and continuing *weekly* for 4 weeks after leaving a malarious area.
[d]Beginning 1–2 days before travel and continuing *daily* for 7 days after leaving a malarious area.
[e]Beginning 1–2 days before travel and continuing *daily* for 4 weeks after leaving a malarious area.
[f]Beginning 1–2 days before travel and continuing *daily* for 7 days after leaving a malarious area.
G6PD, Glucose-6-phospate dehydrogenase.
Drug regimens modified from CDC. Drugs Used in the Prophylaxis of Malaria. *https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/malaria.*

Most antimalarial drugs (chloroquine, mefloquine, and doxycycline) are taken for 4 weeks after a person leaves a malarious area. This is to ensure that all liver-stage *P. falciparum* parasites, against which these drugs have no or questionable activity, have entirely completed their development into merozoites and passed into the bloodstream, where they can be killed by these drugs. Atovaquone-proguanil eradicates the liver stages of *P. falciparum,* although not those of *P. vivax.* The current recommendation is to take this drug for only 7 days after leaving a malarious area.

In some areas, multiple species of *Plasmodium* are transmitted to travelers by mosquitoes and can include *P. falciparum, P. vivax, P. ovale, P. malariae,* and even *P. knowlesi*[481,694,695] in various combinations of risk. Although the presence of chloroquine- or mefloquine-resistant *P. falciparum* might be used as the primary reason for selecting mefloquine, atovaquone-proguanil, or doxycycline prophylaxis against malaria, these drugs are also effective in preventing *P. vivax* malaria.[335,696] However, these drugs will not kill *P. vivax* (or *P. ovale*) hypnozoites in the liver to prevent malaria relapse,[697–699] so terminal prophylaxis with primaquine is necessary. For this reason, primaquine and tafenoquine[700] have been evaluated for primary prophylaxis of *P. falciparum* and *P. vivax* malaria in travelers to areas with primarily *P. vivax* who are not pregnant and have normal G6PD activity levels.[697,701,702] Based in part on a recent analysis of five clinical trials,[703] the FDA in August 2018 approved weekly dosing of tafenoquine for this indication in adult travelers.

Mosquito Repellent and Avoidance Measures

No chemoprophylactic regimen is 100% effective. Despite adequate serum mefloquine levels and other laboratory evidence of compliance, mefloquine prophylaxis has failed where mefloquine resistance is not thought to be common.[704] This example, as well as others, highlights the recommendation that measures to reduce mosquito bites should always accompany chemoprophylaxis.[705,706] Travelers can reduce mosquito bites by using *N, N*-diethyl-meta-toluamide (DEET)–containing insect repellents on exposed skin (applied after sunscreen),[707–709] wearing permethrin-treated clothing, wearing clothes and footwear that cover as much skin as possible,[704] sleeping under permethrin-treated bed nets,[710] staying in housing with air-conditioning and well-screened areas cleared of mosquitoes, and refraining from outdoor activity during peak *Anopheles* biting hours (from dusk to dawn).[711] For information on ordering insecticide-treated bed nets, see www.travmed.com.

Vaccination

Volunteers immunized under chloroquine prophylaxis with live *P. falciparum* sporozoites have been shown to develop long-lasting pre-erythrocytic immunity.[712] RTS,S, a vaccine based on a protein component (circumsporozoite protein) from the surface of *P. falciparum* sporozoites, proved safe, well tolerated, and immunogenic in initial clinical trials.[713] During an initial 12-month follow-up period in efficacy trials, the RTS,S vaccine conferred approximately 50% and 30% protection against *P.*

falciparum malaria in African children ages 5 to 17 months[714] and 6 to 12 weeks,[715] respectively. Over a 4-year follow-up period, however, the efficacy of the RTS,S vaccine declined from 44% to −0.4% in African children ages 5 to 17 months.[716] In July 2015, European regulators found the risk-benefit profile of RTS,S/AS01 (Mosquirix) to be favorable from a regulatory perspective for use as the world's first malaria vaccine.[717] Some recent publications review important concepts in malaria vaccinology and discuss the state of research in the field.[718–721] Progress has been made with vaccine candidates such as the Duffy-binding protein of *P. vivax*,[722,723] a PfEMP-1 molecule involved in *P. falciparum* malaria of pregnancy,[724] live or radiation-attenuated *P. falciparum* sporozoites,[725,726] and molecules critical to the invasion of erythrocytes by *P. falciparum* merozoites.[727]

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The complete reference list is available online at Expert Consult.

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275 *Leishmania* Species: Visceral (Kala-Azar), Cutaneous, and Mucosal Leishmaniasis

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SHORT VIEW SUMMARY

OVERVIEW AND GENERAL PRINCIPLES

- Leishmaniasis is an infection by the protozoan parasites in the genus *Leishmania* and causes three primary clinical syndromes: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucosal leishmaniasis (ML).
- *Leishmania* parasites exist as flagellated promastigotes in sand flies and transform into oval amastigotes after tissue infection in mammalian hosts.
- *Leishmania* organisms are transmitted by the bites of female sand flies of the *Lutzomyia* and *Phlebotomus* genera. Sand fly saliva and inoculum size influence human infection.
- Leishmaniasis is an intracellular infection, and as such adequate control requires a robust cell-mediated immune response. Parasite and host factors influence the tropism of infection.

VISCERAL LEISHMANIASIS

- VL (or *kala-azar*) is endemic in 75 countries worldwide; global incidence has significantly declined since 2010. *Leishmania donovani* occurs primarily in South Asia and East Africa, and *Leishmania infantum* (synonym *Leishmania chagasi*) predominantly occurs in Brazil and the Mediterranean littoral region.
- Polymerase chain reaction testing, parasite culture, or microscopic visualization of amastigotes on bone marrow or liver biopsy confirms diagnosis. Serology (rK39) may be helpful, although it is less sensitive in East African VL.
- Most infected individuals become latently infected and do not manifest symptoms, although they remain infected and at risk for developing symptomatic disease. Classic kala-azar can begin acutely or with a subacute/chronic picture.
- Fever, progressive weight loss, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia are the classic pentad of symptoms. Symptoms can vary, and multiple organ systems can be affected.
- Persons with human immunodeficiency virus/acquired immunodeficiency syndrome or other immunosuppressing conditions can present atypically and are at higher risk of relapse or death.
- Liposomal amphotericin B is generally the treatment of choice where available. In East Africa, combinations of pentavalent antimony with paromomycin are often used as first-line treatment. Immunocompromised patients require higher doses and longer courses of therapy.

CUTANEOUS LEISHMANIASIS

- CL is a neglected tropical disease with an estimated 12 million people affected. Global prevalence has been greatly increasing in recent decades.
- Providers should think of CL after exposure in endemic regions when one or more chronic, generally painless skin lesions develop. There are a wide variety of clinical manifestations, but typically induration exists. Diagnosis requires sampling of a skin lesion and testing using several techniques including stained tissue smears, polymerase chain reaction, and parasite culture for species identification (requires a reference laboratory).
- Metastatic infection (e.g., ML) is a concerning complication, mostly associated with New World CL species such as *Leishmania (Viannia) braziliensis* and less commonly *Leishmania (Viannia) guyanensis* and *Leishmania (Viannia) panamensis*. In addition, cellular immunocompromised hosts are at risk for disseminated infection.
- Although healing may occur spontaneously after months, treatment is recommended for ML-associated species, immunocompromised hosts, and more extensive or severe infection (see Table 275.2). Treatment must be individualized; there is no treatment of choice. The objectives of treatment are to expedite healing of the skin lesion, to limit scarring, and to prevent metastatic infection. As CL ulcers are chronic wounds, wound care should be part of management.

PREVENTION AND CONTROL

- There is no chemoprophylaxis, immunoprophylaxis, or vaccine available for human use to prevent leishmaniasis. Standard personal protective measures against arthropod bites should be used.

OVERVIEW AND GENERAL PRINCIPLES

Leishmaniasis refers to a diverse spectrum of clinical syndromes caused by infection with protozoan parasites of the genus *Leishmania* transmitted by the bite of a phlebotomine sand fly. Leishmaniasis can be separated geographically into Old World and New World disease, referring to the Eastern and Western Hemispheres, respectively. The clinical manifestations of leishmaniasis vary widely but are often divided into three clinically distinct syndromes: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucosal leishmaniasis (ML). A single *Leishmania* species can produce more than one clinical syndrome, and each of the syndromes is caused by more than one species. The outcome in any one patient is a result of the interplay of parasite factors (e.g., invasiveness, tropism, pathogenicity) and host factors (e.g., genetic predisposition, immune status, medication exposure). It is useful to view infection as potentially leading to leishmaniasis, a heterogeneous collection of clinical diseases, each with its own relatively unique geographic distribution, biology, ecology, local mammalian reservoir, and sand fly insect vector. In this chapter, shared aspects will be discussed initially, followed by syndrome-specific information.

[†]Deceased.

Life Cycle and Morphology

Leishmania organisms are dimorphic protozoa existing in two distinct morphologies within sand fly vectors and mammalian hosts, respectively. Although no clear sexual form has been identified, there is evidence that sexual recombination does occur.[1] Additionally, whereas generally diploid, *Leishmania* organisms use aneuploidy at various points in their life cycle, likely as a mechanism of gene regulation.[2] Mammalian infection begins when a female sand fly, taking a blood meal, regurgitates promastigotes into the bite site. Promastigotes (1.5–3.5 μm × 15–200 μm) are elongate, flagellated, and motile forms found in the digestive tract and proboscis of the sand fly. Promastigotes avoid the innate immune response and are phagocytized by neutrophils, macrophages, and dendritic cells. Within 24 to 48 hours after infection, intracellular promastigotes transform into oval or round amastigotes (1.5–3 μm × 3–5 μm) that lack a visible flagellum on light microscopy. Amastigotes have a distinct, rod-shaped structure called a



FIG. 275.1 Tissue smear demonstrating intracellular amastigotes from a *Leishmania major* infection. Note the rod-shaped kinetoplast *(red arrow)* seen next to the nucleus *(green arrow).* (Giemsa stain, magnification ×1000). *(Courtesy Mr. Juan Méndez, Silver Spring, MD.)*



FIG. 275.2 *Phlebotomus papatasi,* the sand fly vector most commonly associated with transmission of zoonotic *Leishmania major* in the Old World. Note the feathered edges (fine hairs), characteristic "V" shape of wings at rest, and parallel venation on wings. *(Courtesy Dr. Ed Rowton, Silver Spring, MD.)*

kinetoplast, which is a specialized mitochondrial structure. Visualization of a kinetoplast, as seen in Fig. 275.1, confirms the diagnosis of leishmaniasis.

Amastigotes multiply by simple binary division, eventually rupturing the cell and invading other mononuclear phagocytes. Depending on the species and host factors, amastigotes may spread regionally or systemically from the bite site through lymphatics and/or the vascular system to infect mononuclear phagocytes elsewhere. This variable tropism is important in the clinical manifestations characteristic for a given species. The cycle is completed when female sand flies ingest parasitized cells. When in the digestive tract of the sand flies, *Leishmania* parasites develop through a series of flagellated intermediate stages to become infectious metacyclic promastigotes over the course of approximately 1 week.

Taxonomy and Classification

The taxonomy of *Leishmania* parasites can be confusing and is evolving. Traditional classification schemes using geographic, clinical, isozyme, or vectorial criteria are useful and widespread in the literature.[3] However, molecular techniques such as sequencing and multilocus sequence typing are influencing taxonomy, as exemplified by the recognition of the synonymous nature of *Leishmania donovani* and *Leishmania archibaldi*. The division of the genus *Leishmania* into the subgenera *Leishmania* and *Viannia* was originally based on the location of promastigote development within sand flies, and this has generally been confirmed by molecular techniques.[4] The advancement of molecular techniques, including rapid whole-genome sequencing, transcriptomics, and proteomics, will continue to refine the taxonomy of *Leishmania*.[5,6,7]

From the clinician's perspective, a useful classification accurately predicts the natural history of infection and response to treatment. However, different definitions, standards, and technical methods have been used over the past several decades to determine the species-level characterization of *Leishmania* parasites, making the correlation of species with treatment outcomes and prognosis challenging. The *Leishmania* species that infect humans and the clinical syndromes they produce are summarized in Table 275.1.

Transmission Principles and Sand Fly Biology

Transmission depends on the sand fly vector, the presence of a suitable reservoir, and susceptible humans. Female sand flies of the genera *Lutzomyia* and *Phlebotomus* transmit *Leishmania* species in the Americas and elsewhere, respectively.[5] As seen in Fig. 275.2, phlebotomine sand flies are small, delicate insects ranging from about 1.5 to 3.5 mm in length. Sand flies breed in the cracks of dwelling walls, rubbish, rubble, or rodent burrows. They are weak fliers and tend to remain close to the ground near their breeding sites and feed on nearby hosts. Sand flies make small cuts in host skin and feed on pooled blood. Saliva from the sand fly enhances the infectivity of promastigotes through its antihemostatic and immunomodulatory properties. Factors in the saliva such as adenosine or maxadilan influence the host immune response, favoring a Th2-type response along with decreased macrophage activation and diminished nitric oxide (NO) production.[8]

Transmission back to the sand fly occurs when a susceptible sand fly feeds on an infected reservoir host. It is unclear whether host parasitemia or parasites within the skin are responsible for this transmission. Sand fly saliva has been shown to increase host parasitemia after repeated feedings,[9] but the variability of sand fly infection seen in xenodiagnostic studies may be better accounted for by patchy skin infection.[10]

Depending on the *Leishmania* species, the sand fly genus, and the geographic location, the major reservoirs for leishmaniasis may be canines, rodents, or humans.

Pathogenesis and Immunology

Leishmaniasis can be thought of as a polar disorder similar to other intracellular infections such as leprosy (Fig. 275.3). On one end of the spectrum are polyparasitic infections (e.g., diffuse CL or VL) characterized by a predominantly Th2-type immune response with relative anergy, akin to lepromatous leprosy. Heavily parasitized macrophages are abundant, and diagnosis is readily made by smear. The oligoparasitic end of the spectrum includes ML and latent VL infection. Amastigotes are sparse, and a mononuclear cell infiltrate predominates in a Th1-type immune response, analogous to tuberculoid leprosy. In reality, the interaction between Th1 and Th2 is much more complex and includes variable modulation by Th17-type $CD4^+$ T cells among others.[11,12]

Why some species spread systemically causing visceral disease and others remain relatively confined to the skin is not fully understood. The A2 gene family, found as a functional gene among species that cause VL, is likely important in visceralization, protecting the parasite from heat shock and oxidative stress.[13] This is further supported by the observation that subspecies of *L. donovani* isolated from patients with CL have much less A2 production, but when A2 production is increased, the capacity to visceralize is restored.[14] Comparing the sequences of different CL and VL species shows that only 19 of >8000 total genes are *L. donovani*–specific genes.[15] Insertion of some of these *L. donovani*–specific genes into *Leishmania major* promotes the survival of *L. major* in the viscera of mice in murine models.[13]

TABLE 275.1 Major Clinical Syndromes, *Leishmania* Parasites, Key Details, and Treatment Overview

CLINICAL SYNDROMES	*LEISHMANIA* SPECIES	KEY DETAILS	TREATMENT OVERVIEW[a]
VL	*Leishmania donovani*	Classic kala-azar in South Asia and East Africa	Regimen may vary by region in which infection is acquired, but for providers in North America the general preference is L-AmB (various regimens, but FDA-approved regimen is 3 mg/kg/d on days 1–5, 14, and 21, with higher doses for immunocompromised hosts)[1]; other options include SbV (20 mg/kg/d for 28 days) alone or in combination with PM (preferred in East Africa VL over L-AmB),[2,3] MIL (2.5 mg/kg/d for 28 days, FDA-approved regimen for *L. donovani* infection), PM, or combination therapy
	Leishmania infantum (syn *Leishmania chagasi*)	Major species in Americas; in Mediterranean causes infantile splenomegaly or, in adults, is often immunocompromise-related	
	Leishmania amazonensis, Leishmania martiniquensis, Leishmania tropica, and others	Rare causes of atypical, viscerotropic leishmaniasis	
Post–kala-azar dermal leishmaniasis	*L. donovani*	Generally macular in South Asia and nodular in East Africa	MIL for at least 12 weeks (India)[4] or SbV for 8–12 weeks (East Africa; treatment reserved for severe or persistent disease),[5] potentially amphotericin B formulations or adjunctive immunotherapy[6]
	L. infantum (syn *L. chagasi*)	Rare and often immunocompromise-related	Not well established[7]
CL	Various species	Chronic, often ulcerating, skin lesions with some characteristic features by region and species	See Table 275.2; for simple CL, options include watchful waiting, local therapy, and azoles; for complex CL, systemic therapy with amphotericin B formulations, SbV, MIL, or pentamidine is generally indicated
Old World CL	*Leishmania major*	So-called wet, rural oriental sore; widespread including Africa, Mediterranean littoral region, and Southwest and Central Asia	See Table 275.2
	L. tropica	So-called dry, urban oriental sore with predilection for the face	See Table 275.2
	Leishmania aethiopica	Major cause of CL in Ethiopia	Limited data; cryotherapy, SbV, and pentamidine reported[8]
	L. donovani and *L. infantum* (syn *L. chagasi*)	Less common causes of CL; often genetically distinct from strains causing VL	Can often be treated as simple CL in immunocompetent hosts
New World CL	*Leishmania mexicana*	Involves exposed skin including ear (chiclero's ulcer); seen from Texas to South America	Ketoconazole[9] or local therapies are first-line options
	Leishmania (Viannia) braziliensis	Widespread in Central and South America; challenging to treat; associated with ML and disseminated leishmaniasis	Treat as complex CL, usually systemically; some believe that *L. (V.) braziliensis* north of Costa Rica has less risk of ML and can be treated as simple CL; see IDSA-ASTMH guidelines[1]
	Leishmania (Viannia) guyanensis	Bush yaws or pian bois in northern Amazon basin; may be associated with ML	May be role for systemic pentamidine; recently intravenous therapy reported better than intramuscular[10]; treat systemically due to risk of ML
	Leishmania (Viannia) panamensis	Causes high rates of CL in Panama, Costa Rica, and Colombia; may be associated with ML	See Table 275.2
	Leishmania (Viannia) peruviana	La uta in Peru	Typically treated with systemic SbV, but resistance noted (efficacy 75.5%)[11]
	L. infantum (syn *L. chagasi*)	Nodular CL in scattered areas of Central and South America	Can often be treated as simple CL in immunocompetent hosts
	L. amazonensis, Leishmania (Viannia) colombiensis, Leishmania venezuelensis, Leishmania naiffi Leishmania pifanoi, Leishmania garnhami, and others	Other agents of New World CL spread across Central and South America	See Table 275.2
Leishmaniasis recidivans	*L. tropica*	Relapsing, satellite lesions around scar of primary CL lesion	Challenging; amphotericin formulations may have effect[12,13]; many relapse, retreatment depends on what was used in initial therapy
Diffuse CL	*L. mexicana, L. amazonensis,* and *L. aethiopica*	Anergic CL with spreading nodular disease progressing out from primary lesion	Resistant to treatment; may respond to MIL, but relapse is expected; immunotherapy has also been used[14–18]; amphotericin formulations seem to have effect[19–22]; WHO recommends SbV and MIL; if due to *L. aethiopica,* SbV and allopurinol[6]
	Other *Leishmania* spp.	Less common and generally in immunocompromised hosts	
Disseminated leishmaniasis	*L. (V.) braziliensis* and *L. amazonensis*; less commonly other *Viannia* spp.	Widespread, noncontiguous, pleomorphic lesions in immunocompetent hosts; particularly noted in Bahia, Brazil; oligoparasitic	Not well established; options include L-AmB with total doses of 17–37 mg/kg,[23] combination of L-AmB and MIL,[24] or amphotericin B deoxycholate[25]

Continued

TABLE 275.1 Major Clinical Syndromes, *Leishmania* Parasites, Key Details, and Treatment Overview—cont'd

CLINICAL SYNDROMES	*LEISHMANIA* SPECIES	KEY DETAILS	TREATMENT OVERVIEW[a]
ML	*L. (V.) braziliensis*; less commonly *L. (V.) guyanensis and L. (V.) panamensis*	Metastatic involvement of mucous membranes of upper airways in 2%–10% of CL cases caused by these species; often seen months to years after primary lesion	Regimen is tailored to individual with few randomized trials to guide therapy; options include SbV (20 mg/kg/d for 28 days), amphotericin B formulations (total dosage 20–40 mg/kg), or MIL (2.5 mg/kg/d for 28 days); adjunctive pentoxifylline and/or plastic surgery may be indicated
	L. tropica, L. major, L. donovani, L. infantum (syn *L. chagasi*), and others	Rare mucosal involvement either as a primary focus or contiguous spread from CL; may be associated with immunocompromise	Not well established; L-AmB and MIL have been used with success in *L. infantum* (syn *L. chagasi*)[26]

[a]The treatment of leishmaniasis must be individualized. Please see the clinical practice guidelines for additional details.[1,27] In general, treatment studies are not robust, and many comments included here are based on data from small observational series or individual case reports. The nuances of *Leishmania* species, geographical variability in drug resistance, and host factors are often ignored.

CL, Cutaneous leishmaniasis; *FDA,* US Food and Drug Administration; *IDSA-ASTMH,* Infectious Diseases Society of America–American Society of Tropical Medicine and Hygiene; *L-AmB,* liposomal amphotericin B; *MIL,* miltefosine; *ML,* mucosal leishmaniasis; *PM,* paromomycin; *SbV,* pentavalent antimony; *syn,* synonym; *VL,* visceral leishmaniasis; *WHO,* World Health Organization.

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Regardless of tropism, intracellular survival is essential to the pathogenesis of all forms of leishmaniasis. Multiple virulence factors enable *Leishmania* organisms to survive intracellularly, including lipophosphoglycan and leishmanolysin. The former aids in the transformation of phagosomes into parasitophorous vacuoles by promastigotes. Leishmanolysin, or GP63, is expressed on the parasite cell surface and secreted in exosomes; it facilitates complement and natural killer cell inactivation, prevents antigen cross-presentation to T cells, and modulates macrophage signaling and transcription pathways.[16,17]

Diagnostic Principles

There are three ways to approach the diagnosis of all suspected *Leishmania* infections: clinical, parasitologic, and immunologic. Clinical diagnosis combines epidemiology with clinical manifestations and serves as a useful guide but is rarely adequate in and of itself due to the cost and toxicity of available therapies as well as the prognostic importance of species identification, particularly in CL.

A parasitologic diagnosis is confirmed by visualizing amastigotes in a tissue biopsy specimen or smear, visualizing promastigotes in culture,

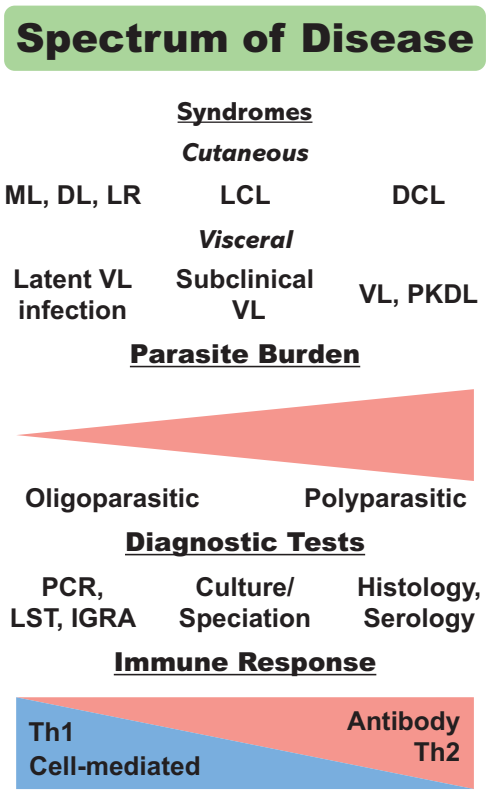

FIG. 275.3 Spectrum of *Leishmania* infection and disease. *DCL,* Diffuse cutaneous leishmaniasis; *DL,* disseminated leishmaniasis; *IGRA,* interferon-γ release assay; *LCL,* localized cutaneous leishmaniasis; *LR,* leishmaniasis recidivans; *LST,* leishmanin skin test; *ML,* mucosal leishmaniasis; *PCR,* polymerase chain reaction; *PKDL,* post–kala-azar dermal leishmaniasis; *Th1,* Th1-predominant response; *Th2,* Th2-predominant response; *VL,* visceral leishmaniasis.



FIG. 275.4 Children with visceral leishmaniasis in Kenya. Note signs of malnourishment and protruding abdomen with massive splenomegaly. *(Courtesy Dr. Charles Oster, Washington, DC.)*

or amplifying *Leishmania*-specific nucleic acids by polymerase chain reaction (PCR). Optimally, parasites should be speciated using either isoenzyme techniques from cultured parasites or molecular methods such as multilocus sequence typing or PCR from a clinical sample, although molecular methods lack standardization. Specimens for culture can be inoculated into one of several media (Schneider's modified medium, Novy-MacNeal-Nicolle medium, and others) and maintained at 22°C to 26°C. In vitro, amastigotes transform into motile promastigotes and multiply, taking a few days to several weeks to reach detectable levels depending on the inoculum size. Parasite culture and *Leishmania* molecular assays are generally relegated to reference laboratories, and it is helpful to contact the laboratories in advance to ensure appropriate collection methods. In the United States, culture media and expert assistance are available from the Centers for Disease Control and Prevention (http://www.cdc.gov/parasites/leishmaniasis/health_professionals/index.html#dx). Protocols using matrix-assisted laser desorption/ionization may allow more widespread future availability of species identification.[18–20]

Immunologic diagnosis is an adjunct in most cases, with various antibody tests, cytokine release assays, and the leishmanin (Montenegro) skin test. The leishmanin skin test and cytokine release assays such as the interferon (IFN)-γ release assay (IGRA), evaluate for cell-mediated immune responses, but neither the skin test nor cytokine release assays are standardized or commercially available.

The choice of the optimal diagnostic test or procedure depends on the parasite burden of the leishmaniasis syndrome (see Fig. 275.3). The guidelines from the Infectious Disease Society of America (IDSA) and the American Society for Tropical Medicine and Hygiene (ASTMH) recommend a multipronged approach in most cases, with the use of molecular methods whenever possible.[21]

Treatment Principles

The diversity of *Leishmania* infections makes standard treatment recommendations impossible. Each region has different species complexes with a greater or lesser degree of genetic heterogeneity within the complex.[22,23] These genetic differences may be reflected in a variable natural history of infection and response to treatment. In addition, each geographic region has its own unique combination of sand fly vectors, mammalian reservoirs, and human hosts with different genetic backgrounds in varying zoonotic or anthroponotic cycles, leading to different outcomes and treatment responses. Optimal drug treatment regimens for each geographic region and major syndrome are best defined in consideration of demonstrated regional efficacy, available resources, and risk-benefit assessments. In resource-rich countries, the efficacy, safety, availability, and tolerability of drug regimens can be the primary factors regarding choice, whereas in low-resource endemic areas, cost and availability are crucial.

In addition to regional differences, variations in trial design, particularly treatment end points, make definitive recommendations difficult. The end points used in clinical trials include clinical, parasitologic, and immunologic cure. These refer to the resolution of clinical symptoms and signs within a defined time period; the absence of parasites by smear, culture, or PCR; and a falling antibody titer or conversion of a skin test from negative to positive. Although current chemotherapy options result in a clinical cure, they may seldom lead to true parasitologic cure, with persistence of *Leishmania* parasites in host tissue being the rule, not the exception.[24–27]

The IDSA-ASTMH guidelines summarize the treatment recommendations along with approved or recommended doses and side-effect profiles for providers practicing in North America.[21]

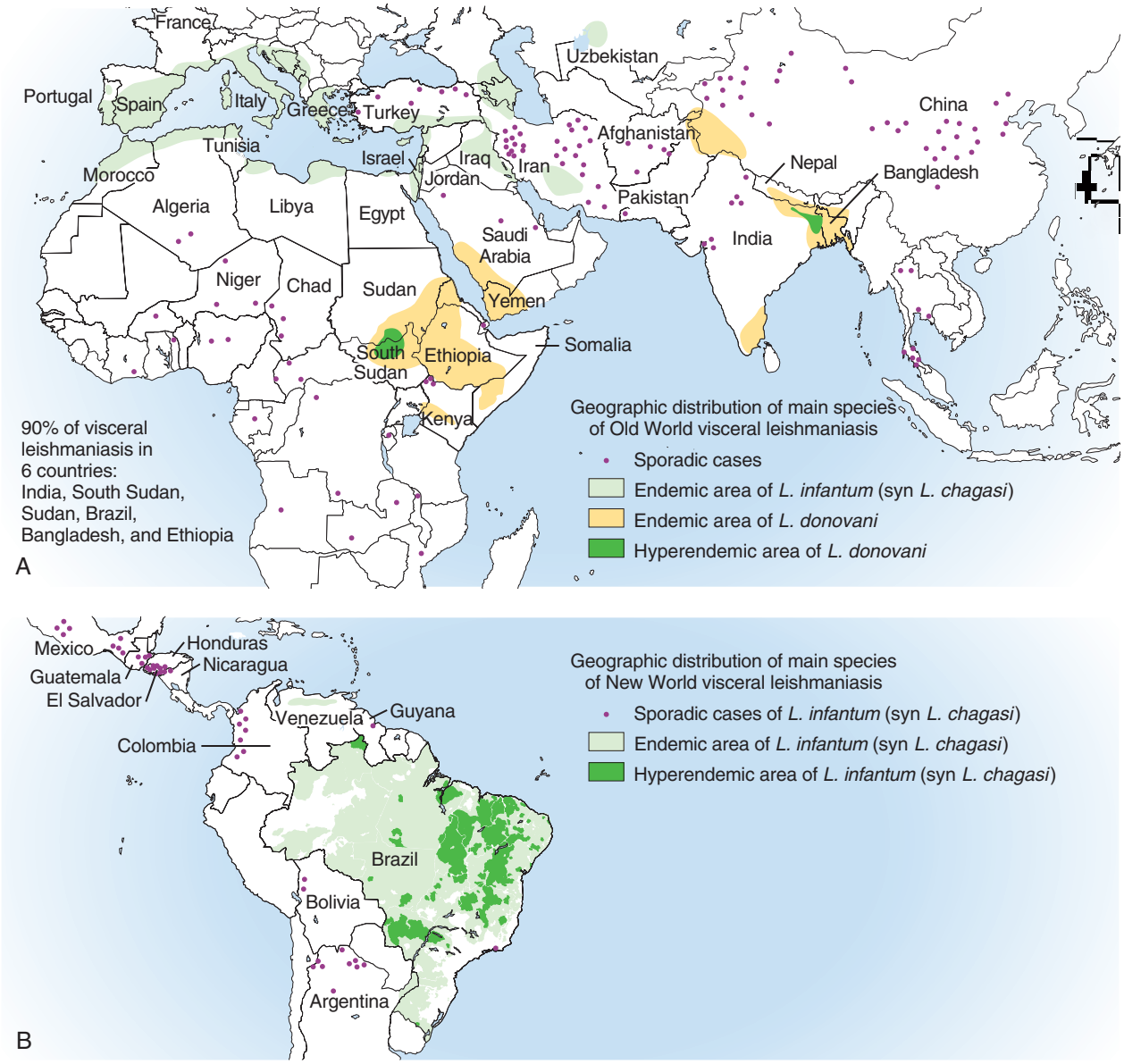

FIG. 275.5 Distribution of visceral leishmaniasis. (A) Old World (Eastern Hemisphere). (B) New World (Western Hemisphere).

VISCERAL LEISHMANIASIS

VL is a spectrum of symptoms and findings ranging from latent infection to classic VL, with the characteristic pentad of prolonged fever, weight loss, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia (Fig. 275.4). Parasites in the *L. donovani* complex are responsible for most cases of VL (see Table 275.1). The complex is made up of two principal species, *L. donovani* and *Leishmania infantum* (synonym [syn] *Leishmania chagasi*). The latter was previously considered two species, but it appears that early explorers from the Old World introduced it into the New World.[28] In the Indian subcontinent, VL is also known as *kala-azar* (Hindi for black or fatal sickness), *Dum Dum fever,* and *Assam fever,* among others. Mediterranean VL caused by *L. infantum* (syn *L. chagasi*) is also known as *infantile splenomegaly*.

Epidemiology

VL is endemic in 75 countries across the tropics and subtropics, predominantly in low-income or low-middle–income countries (Fig. 275.5). More than 90% of reported cases from the last 10 years were in Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan. The World Health Organization (WHO) estimated the incidence of VL to be 200,000 to 400,000 clinical cases/year in 2010[29]; as of 2015, the number of reported cases had fallen by nearly 60% corresponding to an estimated 82,000 to 164,000 cases/year globally (assuming similar rates of underreporting).[30] The global decline has largely been driven by declines seen in the Indian subcontinent. VL is often clustered locally and regionally and may undergo epidemic cycles in some regions.[31] VL is generally either an anthroponosis (sand fly–human–sand fly) or zoonosis depending on the region and parasite species. In anthroponotic disease, clinically ill patients with VL have the greatest potential of transmitting the parasite, although persons with post–kala-azar dermal leishmaniasis (PKDL) or latent infection also play a role in maintaining the disease.[32,33]

There is an increasing recognition of asymptomatic or latent infection occurring in endemic areas.[34] Depending on the region, testing modality, and definition of asymptomatic infection, the prevalence is 0.3% to 37%,[33,35–38] with the ratio of latent to active VL in the range of 4:1 to 50:1.[33,39]

Both *L. donovani* and *L. infantum* (syn *L. chagasi*) cause VL in the Old World (see Fig. 275.5A). In the Indian subcontinent, humans serve as the reservoir for *L. donovani,* and transmission is by *Phlebotomus argentipes* and other anthropophilic species. As a result of a multinational elimination effort in South Asia, reported cases and deaths in India have decreased by greater than 70% and 95%, respectively, from 2010 to 2015.[40]

In East Africa, VL caused by *L. donovani* is endemic in Eritrea, Ethiopia, Kenya, Somalia, South Sudan, Sudan, and Uganda and sporadic in other countries of the region. Conflict-related instability and resulting

displaced people have produced multiple waves of epidemic VL.[41] The exact reservoir in the region is unclear, but putative reservoirs include humans (especially during epidemics), rats, gerbils, other rodents, small carnivores, and potentially dogs.[42] Also, VL caused by *L. donovani* exists in southwestern Saudi Arabia, western Yemen, northeast Afghanistan, Pakistan, and western China, with humans and black rats serving as potential reservoirs, depending on the region.[43–46]

In Europe and the Mediterranean littoral region, VL caused by *L. infantum* (syn *L. chagasi*) is a zoonosis with dogs being the principal reservoir. Clinically apparent cases are typically encountered among infants, young children, and immunocompromised persons. In the 1990s VL emerged as an important opportunistic disease among patients with acquired immunodeficiency syndrome (AIDS) in southern Europe (Spain, France, and Italy).[47,48] The rates of coinfection with human immunodeficiency virus (HIV) and VL have declined significantly with the advent of antiretroviral therapy (ART). Highlighting the clustered, cyclic nature of VL, a large outbreak occurred near several parks in southwestern Madrid, Spain, in the years 2009–12.[49,50] VL caused by *L. infantum* (syn *L. chagasi*) also extends eastward out of the Mediterranean into Southwest and Central Asia as well as northwestern and central China, where dogs, other canines, cats, and hares are reservoirs.

In Latin America, *L. infantum* (syn *L. chagasi*) is endemic and broadly distributed (see Fig. 275.5B). Most areas have focal disease risk with a background of latent or subclinical infection with sporadic clinical cases in rural areas. The clustering of cases in households suggests that humans may also be reservoirs in these settings. Since the 1950s disease epidemiology has shifted from rural to predominantly urban, and major periurban outbreaks of VL have been reported from cities in northeastern Brazil.[51] Children are most frequently affected. *Lutzomyia longipalpis* is the major vector. Domestic dogs and wild foxes are reservoirs of infection.

Although sand fly bites account for most VL transmission, the parasite can be transmitted by blood transfusion, needle sharing, occupational exposure, and congenital exposure.[52–54] Clear evidence of sexual transmission exists in canines, but only a single case of human sexual transmission has been reported.[55,56]

Leishmania species that are typically associated with cutaneous syndromes—such as *Leishmania amazonensis* in Latin America[57,58] and *Leishmania tropica* in Kenya, India, Iran, and Saudi Arabia—are isolated in rare instances from patients with oligosymptomatic visceral syndromes.[59,60] For example, a small group of American military personnel who served in the Persian Gulf War acquired a viscerotropic form of *L. tropica* infection.[61] Additionally, *Leishmania martiniquensis* in the Caribbean[62] and related species in the *Leishmania enrietti* complex in Thailand[63] can cause visceral disease.

Pathogenesis and Immunology

After infection the parasites of the *L. donovani* complex are phagocytized and spread systemically. From a pathologic perspective, VL is characterized by inadequately controlled parasite replication leading to increasing numbers of amastigote-infected mononuclear phagocytes in the liver and spleen with progressive hypertrophy and clinically apparent hepatosplenomegaly. The splenic lymphoid follicles are replaced by parasitized mononuclear cells. There is a marked increase in the number and size of Kupffer cells in the liver, many of which contain amastigotes. Autopsy studies reveal wide dissemination of the parasite with infected mononuclear phagocytes observed in the bone marrow, lymph nodes, skin, and other organs.[64,65]

The immune response is complex and modulated by multiple factors.[13,66] A protective immune response involves the production and interaction of interleukin (IL)-12, IFN-γ, and tumor necrosis factor (TNF)-α, among others. IL-12 is produced by macrophages and dendritic cells in response to intracellular parasites and costimulation by T cells.[67,68] IL-12 not only promotes differentiation of Th1 $CD4^+$ T cells but also stimulates the production of IFN-γ by multiple cell types. IFN-γ stimulates the secretion of TNF-α, and together these cytokines activate macrophages to kill intracellular amastigotes through the production of NO and reactive oxygen species. In the liver, granuloma formation seems to be important to the control of VL, although perhaps not essential, and is influenced by TNF-α, various chemokines, and T-cell granzyme production.[69,70]

An ineffectual immune response is characterized by the production of IL-10 and transforming growth factor (TGF)-β. IL-10 inhibits IFN-γ–mediated macrophage activation, suppresses NO production, and inhibits the production of IL-12 and TNF-α. TGF-β interferes with macrophage activation and suppresses expression of major histocompatibility complex type II on monocytes. Although IFN-γ, TNF-α, and other Th1-type cytokines are often elevated in active VL, the influence of IL-10 and the Th2 environment results in a pathologic response.[71]

Multiple other cell types contribute to an ineffectual immune response including macrophages,[72–75] neutrophils,[76,77] and B cells.[78] B-cell proliferation and antibody production is generally regarded as nonspecific and ineffectual, but *N*-glycosylation patterns may be involved in the pathophysiology.[79]

The reason some people develop a protective response when others develop disease is not fully understood. The sequence of early cytokine responses and the manner in which *Leishmania* antigens are presented by macrophages and dendritic cells—influenced by vector, parasite, and host factors—may be important variables determining the response to an individual infection.[13]

Vector determinants associated with progressive disease include the vasodilatory effects of sand fly saliva, a smaller size of the parasite inoculum, and an absence of preexisting immune responses to sand fly salivary proteins. Parasite factors associated with visceralization were discussed previously. It is likely that there are subspecies differences among VL-causing *Leishmania* species that influence immune response and parasite virulence, but further genomic, transcriptomic, and proteomic comparisons are needed.[80]

Host determinants associated with the outcome of infection and the spectrum of clinical manifestations include genetically determined human immune responses, host nutritional status, and immunocompetence. Genetic risk factors include loci on chromosomes 22q12 and 2q22-23 in Sudan, the HLA-CDR-HLA-DQA1 locus in Brazil and India, and various cytokine pathways (including IL-12, IL-10, TNF-α, and mannose-binding lectin).[13,81–92] Additionally, risk for disease relapse has been seen in families from Sudan with mutations involving the alkylglycerol monooxygenase gene.[93] Malnutrition is a risk factor for progression of infection to disease and results in lower concentrations of leptin, which influences IFN-γ and IL-12 production.[94] As discussed subsequently, various forms of immunosuppression predispose infected individuals to develop symptomatic VL. Additionally, the extremes of age and in utero exposure to the parasite are associated with disease.

Natural History and Latent Visceral Leishmaniasis Infection

The natural history of leishmaniasis generally progresses along two pathways: latent VL infection or progression to disease. There is not a consensus in the literature regarding the definition of asymptomatic VL, with patients with latent infection being identified variably by skin test, IGRA, serology, or PCR.[33,39] When multiple tests are performed on the same asymptomatic population, they may not show agreement.[34] Latent infection progresses to active VL in 2% to 29% of individuals at up to 3 years.[33,95] Most individuals with latent infection remain asymptomatic, but such individuals may develop subclinical symptoms or splenomegaly before progressing to frank disease or resolving spontaneously. Activation of latent infection is more common in patients who are antibody positive or immunosuppressed and can occur many years after exposure.[96]

For patients progressing more directly to symptomatic disease, the incubation time has generally been regarded as 2 to 8 months but can range from 10 days to >1 year. However, incubation periods are often extrapolated from travelers with known exposure periods, and the latent infection paradigm adds further uncertainty. Individuals may have a subclinical course with spontaneous resolution or progression to overt disease, or they may immediately develop classic kala-azar. Although most patients with fully symptomatic kala-azar will die without intervention, there are reports of spontaneous disease resolution.[97] Various studies have shown that risk of death is increased with delayed diagnosis, incomplete treatment, lower social status, age <2 years or >40 to 45 years, HIV or tuberculosis coinfection, low body mass index, jaundice, ascites or edema, bleeding, severe anemia, thrombocytopenia, hypoalbuminemia, or hyponatremia.[98–101]

Persons with self-resolving infection and persons who have undergone successful chemotherapy develop protective immune responses. However, the parasite is not eradicated, and disease can redevelop years later if the infected person becomes immunocompromised.

Clinical Manifestations

The fully developed clinical manifestations of VL are similar in all endemic areas. Skin lesions at the site of inoculation are usually not apparent in persons with VL. In cases with a subacute or chronic course, there is an insidious onset of fever, weakness, loss of appetite, weight loss, failure to thrive, and abdominal enlargement caused by hepatosplenomegaly. In endemic areas, low-grade symptoms may persist for weeks to months but may not be sufficiently severe to warrant medical attention in low-resource areas, and the condition of such patients may be called subclinical when oligosymptomatic would be more appropriate. Fever may be intermittent, remittent with twice-daily temperature spikes (double quotidian), or, less commonly, continuous. Fever is relatively well tolerated, and older clinical references routinely noted that patients were not acutely ill or toxic in appearance.

Acute presentation in nonimmune persons comprises abrupt onset with high fever and chills, sometimes with a periodicity that suggests malaria.[102] Chills, but seldom rigors, accompany the temperature spikes. As time passes, the spleen can become massively enlarged. It is usually soft to firm and nontender. The presence of a hard spleen suggests a hematologic disorder or another diagnosis such as schistosomiasis. The liver also enlarges; it usually has a sharp edge, soft consistency, and a smooth surface. Lymphadenopathy is common in patients in Sudan[103] but uncommon in other geographic areas. Elevated liver enzymes and bilirubin may be observed. Peripheral edema may be seen late in disease, particularly in malnourished children. Hemorrhage can occur from one or more sites; epistaxis and gingival bleeding may be noted as well as petechiae and ecchymoses on the extremities in late-stage disease. Many patients with VL become cachectic, mediated in part by TNF-α and other cytokines known to have catabolic and anorectic effects.[104]

Secondary bacterial infections are common in persons with advanced VL. Patients can present with coinfection or acquire secondary bacterial infections during hospitalizations. It is important to recognize and treat clinically significant bacterial coinfections.[105–107] Death may result from pneumonia, septicemia, tuberculosis, dysentery, or measles, or it may be the consequence of malnutrition, severe anemia, or hemorrhage.

The laboratory findings include anemia, leukopenia, thrombocytopenia, and hypergammaglobulinemia. Anemia is almost always present and may be severe. It is usually normocytic and normochromic and appears to be due to a combination of factors including hemolysis, marrow replacement with *Leishmania*-infected macrophages, hemorrhage, splenic sequestration of erythrocytes, hemodilution, and marrow suppressive effects of cytokines such as TNF-α. Secondary hemophagocytic lymphohistiocytosis is increasingly reported, frequently in children.[108,109] Leukopenia is also prominent, with white blood cell counts occasionally as low as 1000/mm^3. Severe eosinopenia is frequently observed. Of note, anemia and neutropenia have not been prominent in patients with VL who have undergone splenectomy. Hypergammaglobulinemia, circulating immune complexes, and rheumatoid factors are present in the sera of most patients with VL.[110,111] The globulin level may be as high as 9 g/dL; the ratio of globulin to albumin is typically high. The erythrocyte sedimentation rate is usually elevated.[112]

Renal involvement in VL is common and includes proteinuria, acute renal failure, nephrotic syndrome, glomerulonephritis, acute interstitial nephritis, tubular necrosis, and tubulitis.[113–118] Elevated serum creatinine has been observed both retrospectively and prospectively in 26% to 28% of patients with VL in Brazil, but abnormalities in urinary concentration and acidification were seen in >60%.[119,120]

Viscerotropic disease caused by atypical species is characterized by chronic low-grade fever, malaise, fatigue, and in some cases diarrhea. Mild splenomegaly can occur, but generally patients do not develop classic kala-azar or progressive VL.[61]

Diagnosis

The positive predictive value of a clinical diagnosis of VL in patients with the pentad of prolonged fever, progressive weight loss, pronounced hepatosplenomegaly (especially splenomegaly), pancytopenia, and hypergammaglobulinemia from a known endemic area is very high. However, clinical diagnosis can be challenging in patients with atypical presentations such as oligosymptomatic or immunosuppressed patients or in returned travelers presenting to physicians in nonendemic regions months to years after an exposure.

Samples for parasitologic diagnosis can be obtained by splenic aspiration, bone marrow aspiration, liver biopsy, and lymph node aspiration. Splenic aspiration to obtain a few drops of fluid for Wright-Giemsa–stained smears, culture, or PCR assay is the most sensitive method for parasite identification.[121] Splenic aspirations have sensitivities of 93% to 99%, and the parasite burden can be quantified to stage patients and monitor response to treatment.[122] However, splenic aspiration carries the risk of life-threatening hemorrhage, particularly in advanced disease, and is not recommended in North America.[21] Bone marrow aspiration is safer and preferred in nonendemic settings but is less sensitive, ranging from 60% to 85%, but improving to as high as 95% when microscopists review smears for an hour.[123,124] Liver biopsy is even less likely to yield the diagnosis but occasionally is positive when bone marrow is negative.[125] Lymph node aspiration or biopsy may be diagnostic when enlarged nodes are present, as is often the case in East Africa. Rarely, amastigotes may also be seen on blood smears or cultured from the buffy coat or blood.[126] When the diagnosis of VL is suspected, parasitologic confirmation may require more than one technique and repeated procedures.[102] Whenever possible, aspirates should also be cultured.

Molecular approaches have the potential to replace traditional parasitologic methods due to their diagnostic accuracy even when using blood samples rather than more invasive splenic or bone marrow aspirates. A meta-analysis found a pooled sensitivity and specificity from whole blood of 93% and 96%, respectively.[127] Certain molecular assays are more sensitive than microscopic methods for the detection of parasites and can detect parasites in asymptomatic individuals with exposure risks. Whereas various techniques exist, quantitative PCR assay has the advantage of being able to assess parasite load in patients with VL, which is highly correlated to splenic score.[128] Also, quantitative PCR may provide a threshold for distinguishing latent infection from active disease and is useful in monitoring response to treatment.[129,130] There is a lack of standardization in methodology and target sequence, and larger studies are needed to compare different methods, both within and between VL regions. Assays targeting kinetoplast DNA minicircles have been shown to be the most sensitive target, likely as a result of each parasite containing approximately 10^4 minicircles, although this number is variable, making quantification less reliable.[131] Replacing traditional parasitologic methods with PCR has also been shown to be potentially cost saving.[132] However, most molecular methods require well-equipped laboratory facilities, minimizing their applicability to resource-limited settings. Techniques such as loop-mediated isothermal amplification or recombinase polymerase amplification may offer reliable alternatives for field application but require further standardization and development.[133–135]

Immunologic tests are widely used in the diagnosis of VL worldwide. High-titer antileishmanial antibodies are typically present in immunocompetent persons with symptomatic VL and are variably present in individuals with latent infection. A number of serologic tests using different antigens and assays are available; the most widely used include the recombinant K39 (rK39) (a kinesin-related protein) test and direct agglutination test (DAT). Both tests perform well with pooled sensitivities and specificities >90%, but sensitivity is diminished in some regions, particularly East Africa—potentially related to host differences or, for rK39, the extensive diversity of kinesin-related proteins found in East African isolates.[136,137] Performance of DAT may be improved by using locally derived antigens instead of commercial preparations.[138] Both rK39 and DAT are suited for use in resource-limited settings, with the former using rapid immunochromatographic test strips. Antibody tests are limited by remaining detectable for months to years after successful treatment and by the potential to cross-react with antibodies from past infections such as leprosy, Chagas disease, CL,[139] and others. Although antigen-based tests have the potential for overcoming many of these shortcomings, the commercially available KAtex antigen test has poor

sensitivity in clinical specimens, and other potential alternatives are still early in development.[137,140,141]

Other immunologic tests measuring cell-mediated immunity such as the IGRA or leishmanin skin test are often negative in patients with active VL, potentially becoming positive after spontaneous or treatment-related disease resolution.[142] Individuals with latent infection can be positive as well, making these tests epidemiologically useful. In transplant patients, cytokine release assays can be positive in asymptomatic patients but cannot distinguish between latent infection and successfully treated cases.[143]

A reasonable approach to the diagnosis of VL in immunocompetent hosts in North America consists of screening clinically suspect cases with serologic tests and confirming the diagnosis via bone marrow aspirate/biopsy for histopathology, parasite culture, and PCR.

The differential diagnosis of late-stage VL with the full complement of symptoms and signs is limited to hematologic and lymphatic malignancies and, occasionally, disseminated histoplasmosis and tropical splenomegaly syndrome. Acute VL has a much broader differential diagnosis including malaria, miliary tuberculosis, hemophagocytic syndromes, enteric fevers, HIV, bacterial endocarditis, sarcoidosis, acute Chagas disease, acute schistosomiasis, typhus, and amebic liver abscess. Subacute or chronic VL may be confused with brucellosis, prolonged *Salmonella* bacteremia, infectious mononucleosis, myeloproliferative disease, hepatosplenic schistosomiasis, and chronic malaria.

Visceral Leishmaniasis in Immunocompromised Patients

Immunocompromised patients represent a unique spectrum of VL with multiple differences across the clinical picture from presentation to management. Immunosuppressing conditions that have been shown to influence VL include HIV/AIDS, transplant-related immunosuppression, exposure to immunosuppressive drugs such as TNF-α inhibitors or steroids, malignancies or related chemotherapy, and other rare conditions such as genetic disorders in the IL-12 pathway.[91,144,145] Most data in this context are from patients with HIV/AIDS, but there is a growing body of evidence from other etiologies of immunosuppression.

The prevalence of VL in immunosuppression is not fully known. In the Mediterranean, HIV previously accounted for most VL cases, but with the use of ART it is much less common, and other etiologies of immunosuppression have become increasingly recognized.[146–148] In an outbreak of VL in Spain, HIV coinfection was seen in 10% of patients, whereas 13% had another form of immunosuppression.[49] Elsewhere in the world, HIV clearly predominates, with estimated coinfection rates highest in East Africa (20%–40%), followed by Brazil (4%) and India (<1%).[149–151] VL is seen in <1% of transplant patients globally, but increasing rates of transplantation in VL endemic regions such as Brazil and India may result in more cases.[144] Immunosuppressed individuals are often older at diagnosis than immunocompetent patients.

Immunosuppressed patients with VL often have a condition impairing cell-medicated immunity making it harder, if not impossible, for the immune system to control parasite replication, resulting in a wider dissemination and greater burden of mononuclear cells infected with amastigotes. VL also impacts the pathophysiology of HIV infection, causing increased immune activation, which leads to immunosenescence, progression of HIV disease, and poorer $CD4^+$ T-cell recovery with ART.[152–154]

The natural history of VL in immunosuppressed patients differs in several aspects. All immunosuppressed persons are at higher risk of progressing to clinical VL (4-fold in transplant patients and 100- to 2300-fold in HIV-infected patients).[153] In endemic regions, it is often impossible to distinguish activation of latent infection versus an incident case in immunocompromised patients, although patients have been observed to experience reactivation on becoming immunosuppressed years after their last potential VL exposure. Anyone with a history of birth, residence, or travel in a *Leishmania*-endemic area is at risk of late reactivation if he or she becomes immunocompromised. The role of primary preventive chemotherapy among latently infected immunocompromised persons has not been studied. Regardless of etiology, spontaneous resolution is unlikely to occur in immunosuppressed individuals.

Immunosuppressed individuals often present similarly to patients with classic VL, although atypical presentations are more common, correlating with the degree of immunosuppression. In general, HIV-infected patients have more weight loss, weakness, bleeding, and secondary infections but relatively less fever, pallor, and organomegaly.[150,155] When $CD4^+$ counts fall below 50 cells/mm^3, atypical presentations are much more common and include atypical skin lesions similar to PKDL; extensive gastrointestinal tract involvement, which may manifest with chronic diarrhea and malabsorption; intraabdominal lymphadenopathy; airway, pleural, or pericardial involvement; aplastic anemia; and anterior uveitis.[156–159] Compared with patients with HIV coinfection, patients with other types of immunosuppression tend to have more fever, lower leukocyte counts, and less hepatomegaly.[160]

Immunosuppression, particularly due to HIV, influences the diagnosis of VL by increasing the burden of parasites but simultaneously impairing specific antibody production. Parasitologic diagnosis is easier with parasites being found in a wide variety of samples including bronchoalveolar lavage fluid, pleural effusions, or biopsy specimens of the oropharynx, stomach, or intestine. Patients with HIV infection are also more likely to have circulating parasites found in their blood compared with immunocompetent patients. PCR remains highly sensitive in a wide variety of samples. Serologic tests are variable in immunosuppressed individuals, with less sensitivity overall.[144,145,160–162] In immunocompromised patients, the optimal testing regimen is not clear and should involve a multipronged approach, ideally including quantitative PCR of the blood for its utility in monitoring response to therapy.[21]

Treatment and secondary prophylaxis are discussed later in this chapter. Posttreatment relapse is problematic, especially in patients with HIV infection. Rates of relapse vary significantly in heterogeneously designed studies, but more recent rates range from 26% to 37%.[155,163,164,165] Relapse is more common in HIV-coinfected patients not on ART or with a history of prior relapse, high parasite loads at diagnosis, baseline $CD4^+$ counts <100 cells/mm^3, or persistence of low $CD4^+$ counts.[154,163,165] Although relapses can occur at higher $CD4^+$ counts, consideration can be given to stopping secondary prophylaxis in patients on ART if the $CD4^+$ count is >200 cells/mm^3 for at least 6 months, especially in the setting of negative blood PCR.[166] Continued monitoring by PCR could be useful to predict relapse in this context as well. Although ART is essential for sustained prevention of relapse, timing of ART initiation is not clear.[167,168] Whereas relapse is less common in patients without HIV/AIDS, transplant patients still have a rate of approximately 25%.[144]

Finally, death is more common in all immunosuppressed patients, even with treatment. Mortality rates of 25% to 46% are reported in patients with HIV (the higher end of range in patients not on secondary prophylaxis)[150,155,162] and 22% in transplant patients.[144]

Post–Kala-Azar Dermal Leishmaniasis

PKDL follows after active VL caused by *L. donovani* in 5% to 10% of patients within 2 to 4 years after treatment in India and approximately 50% of patients within 0 to 6 months after treatment in Sudan (Fig. 275.6).[169–172] In Bangladesh, active surveillance resulted in higher than previously observed rates, with >25% developing PKDL after therapy.[173] PKDL is rarely seen in areas where *L. infantum* (syn *L. chagasi*) predominates and, when reported, has been seen in patients with concurrent AIDS.[174] Additionally, studies in India have shown that 4% to 29% of patients with PKDL have no history of clinical VL.[33,175,176]

The pathophysiology of PKDL is not conclusively known, but suboptimal therapy, host genetic predisposition, parasite strain difference, and environmental factors such as ultraviolet light exposure or water arsenic levels likely combine to create a Th2-type response in the skin with alternative macrophage activation despite a Th1 response predominating in the viscera.[177,178] This is even more pronounced in nodular lesions, resulting in a higher parasite burden relative to macular lesions.

Skin lesions of PKDL are macular, papular, nodular, or verrucous.[176,179] The lesions are more often macular and chronic in India, whereas in Sudan, they are more frequently papulonodular but generally resolve within 12 months without therapy. Lesions are more typical on sun-exposed skin. Patients generally feel well. The diagnosis is mainly clinical, but amastigotes can be detected in the skin microscopically or with PCR; the latter has high sensitivity even in macular lesions.[173,176] PKDL must be differentiated from syphilis, leprosy, and yaws.

Patients with PKDL have been shown to be infectious to sand flies with no difference in infectiveness seen between nodular and macular

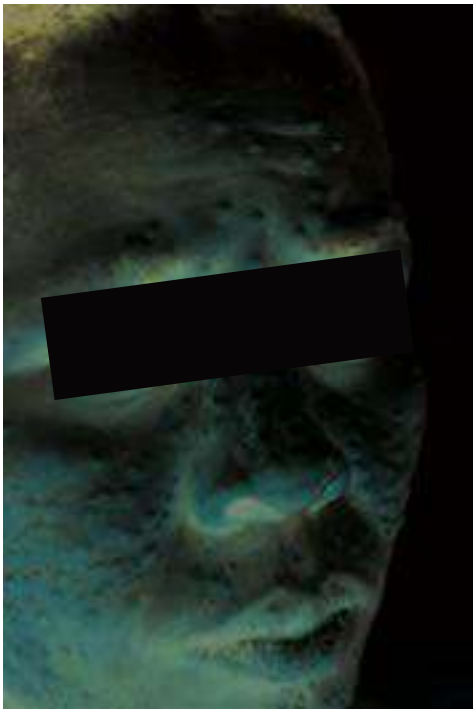

FIG. 275.6 Post–kala-azar dermal leishmaniasis. Papular lesions after treatment of visceral leishmaniasis in a patient in Kenya. *(Courtesy Dr. Tom Simpson, Baltimore, MD.)*

lesions.[33,180] Because of this, patients with PKDL are thought to be reservoirs for anthroponotic infection and may become increasingly important to elimination programs. However, further study is needed regarding the true potential of PKDL in disease transmission, as no VL seroconversion was seen in households with patients with PKDL over a 2-year period.[32] Treatment, discussed next, speeds resolution and is thought to decrease the risk of transmission, but this also remains to be demonstrated.

Treatment

Treatment options for VL and PKDL include amphotericin B formulations, pentavalent antimony (SbV) compounds such as sodium stibogluconate (SSG) or meglumine antimoniate, miltefosine, and paromomycin. Pentamidine is no longer recommended for treatment of VL but is an option for use in secondary prophylaxis and salvage therapy. All are administered parenterally with the exception of oral miltefosine. The precise mechanism of action is not known for any of these therapies; however, they frequently influence NO production in macrophages, and liposomal amphotericin B (L-AmB) and miltefosine promote a Th1-type immune response.[181] Treatment is recommended only for symptomatic individuals at this time. Studies are needed to determine if treatment of latent infection, although theoretically beneficial, impacts disease transmission or prevents disease activation. Patients with VL often have serious bacterial coinfections or tuberculosis that must be treated as well.

There are no standard parasitologic or immunologic tests of cure. Patients respond quickly to efficacious treatment, with resolution of fever, improved sense of well-being, and return of appetite within a week and improved anemia and leukopenia seen in the second week, with normalization by 6 months. Resolution of splenomegaly may take 3 to 6 months. Delayed responses to therapy can be seen, but lack of a rapid clinical response suggests treatment failure. Relapses usually occur within 6 months of initial treatment success.

Liposomal Amphotericin B and Other Formulations

L-AmB (AmBisome; Astellas, Northbrook, IL) is the drug of choice for treatment of VL outside of East Africa. It is licensed for the treatment of VL in the United States, and the approval was based on studies conducted in the Mediterranean in the 1990s.[182,183] The US Food and Drug Administration (FDA)–approved regimen for immunocompetent patients is 3.0 mg/kg/day intravenously (IV) on days 1 to 5, 14, and 21 (total dosage 21 mg/kg).

Cost, availability, and the requirement for a cold chain have limited its use in developing areas, although the WHO has negotiated preferred pricing for developing nations, making it more affordable. In South Asia, L-AmB or an Indian formulation (Fungisome) at doses of 10 to 15 mg/kg, either as a single dose or split over two or three doses, has been shown to be highly effective in treating primary VL in children and adults.[156,184–187] Success with L-AmB has been more difficult to achieve in East Africa, typically requiring significantly higher doses (totaling 24–50 mg/kg) to achieve >90% cure rates.[188,189] In *L. infantum* (syn *L. chagasi*) endemic regions, total doses of approximately 20 mg/kg are generally effective and well tolerated, and a randomized, open-label trial in Brazil demonstrated equivalent cure rates to the prior SbV-based standard-of-care regimen with fewer side effects.[190] L-AmB is generally well tolerated, but side effects include fevers, rigors, nausea, vomiting, hypokalemia, and some nephrotoxicity.

Amphotericin B deoxycholate, a nonlipid formulation, has similar efficacy to L-AmB but must be given over prolonged periods of time and is associated with increased risk of nephrotoxicity and other side effects compared with L-AmB. The recommended dosage is 1 mg/kg/dose every 1 to 2 days for 15 to 20 total doses. Other lipid-associated amphotericin B preparations have been used in the treatment of VL, but the supporting data are much less extensive, and animal studies suggest the liposomal preparation is superior.[191]

Pentavalent Antimony

Pentavalent antimony is still used for the treatment of VL in areas where *Leishmania* isolates remain susceptible and the cost of L-AmB is prohibitive. The dosage regimen recommended for treatment of VL is SbV at 20 mg/kg/day intramuscularly (IM) or IV for 28 days. Intramuscular injections are large volume and painful, and intravenous infusion requires dilution to decrease the risk of local thrombosis. Patients who respond slowly or relapse may respond to a second course.

The use of SbV is second-line treatment in many regions of the world due to poor tolerability or lack of efficacy, with up to 40% resistance seen in parts of India.[192] However, SbV, alone or in combination therapy, continues to be used extensively in East Africa, where they often remain effective for treatment of VL in patients without HIV coinfection.[193,194]

There are multiple preparations of SbV manufactured and sold worldwide. SSG (Pentostam; GlaxoSmithKline, London, UK) and meglumine antimoniate (Glucantime; Sanofi-Aventis, Paris, France) are the two best known commercial products and are considered equivalent in efficacy and tolerability. One SSG generic formulation has been shown to be equivalent to brand-name products,[195,196] but numerous other SbV products vary in content and toxicity profile, even between lots by the same manufacturer. Cardiac deaths have been associated with generic SbV with high osmolarity.[197,198]

Side effects are common and often increase during treatment with SbV. Side effects include anorexia, nausea, vomiting, midepigastric pain, myalgia, large joint arthralgia, headache, asthenia, and malaise. Common laboratory abnormalities include cytopenias and elevations in amylase, lipase, and liver associated enzymes. Although chemical pancreatitis is common, a full treatment course can generally be completed, although we favor pausing therapy until high levels decline (over several days) or stopping therapy for symptomatic pancreatitis.[199] Electrocardiographic changes, including QT prolongation, are common, and arrhythmias, sudden death, and torsades de pointes can occur. Clearance is primarily by the kidneys, and toxicity increases when drug accumulates during renal failure. Patients receiving SbV should be closely monitored clinically along with periodic complete blood counts, serum amylase and lipase, liver enzymes, renal function tests, and serial electrocardiograms.[200] SbV should not be given during pregnancy because a 57% spontaneous abortion rate was observed in one study.[201]

Miltefosine

Miltefosine (Impavido; Profounda, Orlando, FL) is the only oral agent available for the treatment of VL and was licensed by the FDA in 2014

for the treatment of VL caused by *L. donovani*. Initially developed as an antineoplastic agent, miltefosine is associated with nausea, vomiting, and abdominal pain. Whereas early use was promising,[202] prompting use in South Asia's elimination program, a subsequent trial in Nepal showed 86% efficacy, with 13% relapse among 853 patients.[203] Experience in other regions is limited.

Adverse effects include significant nausea, vomiting, dizziness, scrotal pain, nephrotoxicity, and hepatotoxicity. Miltefosine is teratogenic and therefore contraindicated in pregnancy. Adherence to the 28-day oral regimen is challenging due to gastrointestinal distress.[204] Additionally, the recommended weight-based dosing may be inadequate for children[205] and is challenging in persons weighing >60 kg, as doses >150 mg daily are not well tolerated. Resistant parasites have mutations leading to diminished drug accumulation and resistance to the oxidative stress of miltefosine.[206,207]

Paromomycin

Paromomycin (parenteral formulation not commercially available in the United States) is an aminoglycoside with activity against *Leishmania* species. For VL it is administered as 11 mg/kg of base (equivalent to 15 mg/kg paromomycin sulfate) IM for 21 days. Randomized studies in Bangladesh and India have shown 94% to 95% cure rates, with the Indian study showing noninferiority to amphotericin B.[208,209] Results from East Africa were less promising, with efficacy of <85% even when paromomycin was administered longer or at higher doses.[210,211]

Adverse events are more common than with amphotericin and include injection site pain, elevations of aspartate aminotransferase levels, reversible ototoxicity, and nephrotoxicity. Whereas the low cost makes paromomycin attractive, there are concerns about the development of resistance. Use is generally reserved for combination with other agents.

Combination Therapy for Visceral Leishmaniasis

Many experts now advocate combination chemotherapy regimens for VL, as it is a polyparasitic disease syndrome, and using two or more drugs may shorten therapy, decrease toxicity, and prevent the emergence of resistance. In East Africa, SSG with paromomycin is the WHO recommended first-line therapy for uncomplicated VL.[210,212] A multisite, prospective pharmacovigilance study in Ethiopia, Kenya, Sudan, and Uganda demonstrated 95% initial cure, low mortality, and high therapy completion rates with this combination.[213] A phase 2 study in Kenya and Sudan evaluated the combination of L-AmB at 10 mg/kg in a single dose, with either SSG or miltefosine for 10 days versus miltefosine monotherapy for 28 days, but none of the regimens reached >90% efficacy in the formulations tested.[214] Conversely, in Bangladesh and India, combinations of single-dose L-AmB (5 mg/kg) with a short course of miltefosine or paromomycin as well as miltefosine and paromomycin together demonstrated excellent efficacy and safety profiles.[187,215] Further studies are needed with long-term follow-up to assess rates of relapse and PKDL.

Treatment and Secondary Prophylaxis of Visceral Leishmaniasis in Immunocompromised Hosts

Treatment in immunocompromised patients is challenging, and most data come from patients with HIV/AIDS. Cure can be very difficult to achieve and may require multiple courses of therapy. Data are limited on the optimal therapy in this setting.[144,216] A systematic review among patients with HIV coinfection suggests that L-AmB is superior to SbV, with higher rates of clinical improvement and lower mortality.[217] In North America the recommended treatment for immunosuppressed individuals is L-AmB 4.0 mg/kg/day IV on days 1 to 5, 10, 17, 24, 31, and 38 (total dosage 40 mg/kg).[21] Longer courses and higher total doses along with combination therapy are considerations in this population, especially with treatment failure or relapse.

There is evidence that protease inhibitors and efavirenz have in vitro activity against *Leishmania,* but in vivo efficacy is unknown.[218,219] If VL manifests as part of an immune reconstitution inflammatory syndrome, corticosteroids may be necessary in addition to continued ART and pathogen-directed therapy.

Secondary prophylaxis is recommended for the prevention of relapse.[21] Amphotericin B lipid complex and intravenous pentamidine have been studied prospectively for use in secondary prophylaxis, and both decrease relapse substantially compared with no prophylaxis or historical control subjects, respectively.[165,220] Other medications have been looked at retrospectively, including L-AmB and SbV, with the former showing 100% prevention of relapse and death at 6 months in Indian patients.[162] When indicated, options include amphotericin B lipid complex (3 mg/kg IV every 3 weeks), pentamidine (4 mg/kg IV every 2–4 weeks), L-AmB (3–5 mg/kg IV every 3–4 weeks), or SbV (20 mg/kg IM or IV every 3–4 weeks).[21,165,220]

Treatment of Post–Kala-Azar Dermal Leishmaniasis

Chemotherapy in patients with PKDL is frequently unsatisfactory, and robust trials are lacking. Clinical and parasitologic end points in PKDL are poorly defined, although PCR may have an emerging role in future trials.[173,221] Severe PKDL in Sudan is treated with SSG at 20 mg/kg/day for up to 3 months.[222] First-line therapy in the Indian subcontinent is miltefosine for 12 weeks, although concerning rates of relapse have been seen, and a longer course may be required, which presents a significant challenge regarding patient compliance.[221,223] Formulations of amphotericin B have been very promising when used in small numbers of patients, including patients with nodular disease,[224–226] but additional studies are needed to define its role in the treatment of PKDL.

CUTANEOUS LEISHMANIASIS

CL is endemic in widely scattered areas throughout the world (Fig. 275.7). The classic form of Old World CL (OWCL) is the *oriental sore,* also known by a variety of colorful local expressions such as *bouton d'orient, bouton de Crete, bouton de Biskra, Aleppo evil, Baghdad boil,* and *Delhi boil,* in various regions of Africa, India, the Mediterranean littoral region, and Southwest/Central Asia. The spectrum of OWCL disease includes single or multiple, localized, cutaneous ulcers (Fig. 275.8); diffuse CL; uncommon mucosal involvement; and leishmaniasis recidivans (Fig. 275.9). The resulting skin lesions range from troublesome and unsightly to severe and complicated, healing with scarring. Severe cases and facial lesions are very stigmatizing.

The spectrum of New World CL (NWCL) disease includes single or multiple, localized, cutaneous ulcers (Figs. 275.10 to 275.13); diffuse CL; disseminated CL (Fig. 275.14); and mucosal disease (Fig. 275.15) caused by *Leishmania (Viannia) braziliensis* or, less commonly, other related *Leishmania* species.

Epidemiology

CL is a severely neglected tropical disease whose incidence is increasing worldwide. An estimated 12 million people have leishmaniasis; 1 to 1.5 million new cases of CL occur per year, with a mean CL disability-adjusted life-year estimate of 0.6 per 100,000 persons (not including the social stigma associated with scarring of healed CL).[227–229] Approximately 90% of the world's CL cases occur in the high-burden countries of Afghanistan, Algeria, Brazil, Colombia, Iran, Morocco, Pakistan, Saudi Arabia, Syrian Arab Republic, Tunisia, and Turkey; CL cases in these countries tripled between 1998 and 2005.[230] In the Global Burden of Disease study, a 174% change in CL prevalence was seen between 1990 and 2013.[231] CL is an important problem for residents, refugees, settlers, travelers, and military personnel visiting endemic areas. More than 2000 cases of CL have been reported among American troops serving in Iraq and Afghanistan since 2001.[232] Other cases occur among North American travelers after exposure in endemic regions.[233,234] Finally, 90% of the cases of ML occur in three Latin American countries: Bolivia, Brazil, and Peru.

In the Old World, CL is usually a sporadic disease in endemic areas, but it occasionally occurs in an epidemic pattern, particularly when large groups of susceptible people are exposed during road construction, refugee movements, or military activities.[235–237] OWCL is most frequently caused by *L. major, L. tropica,* or *Leishmania aethiopica,* but *L. donovani* and *L. infantum* (syn *L. chagasi*) can also cause simple CL.

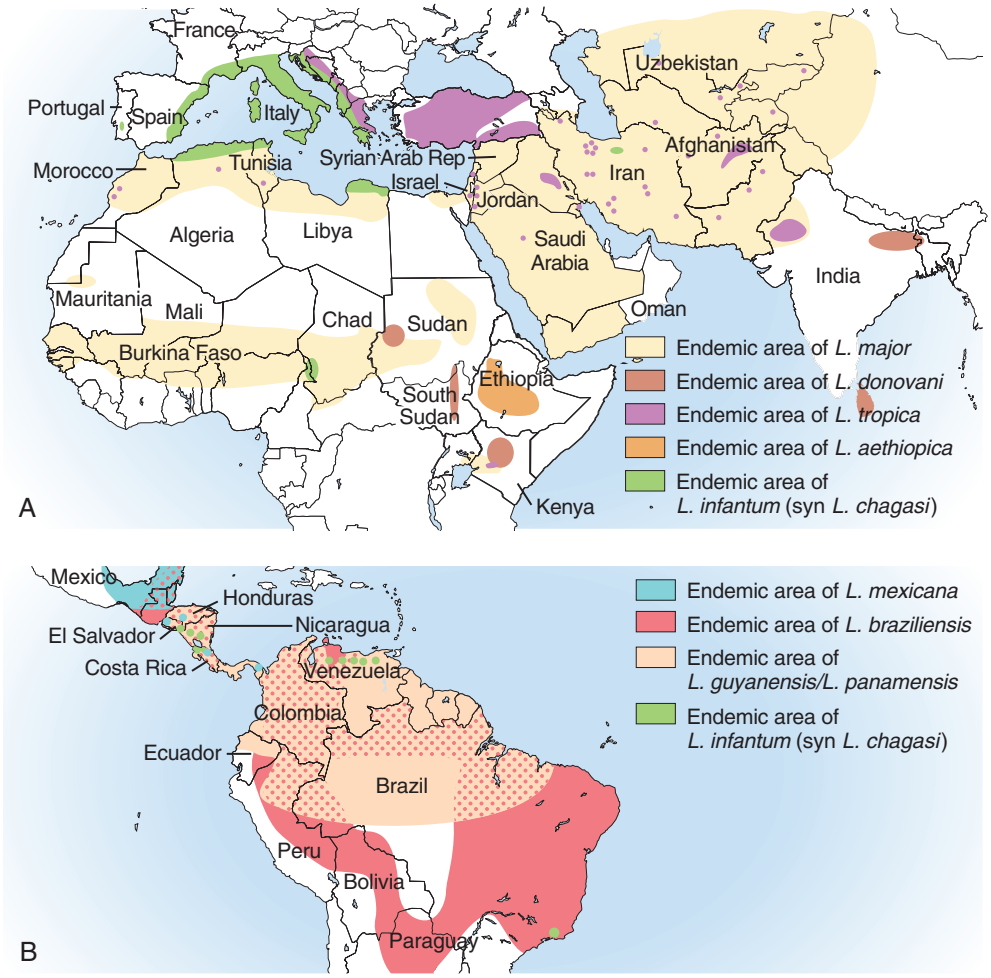

FIG. 275.7 Distribution of cutaneous leishmaniasis (CL). (A) Old World (Eastern Hemisphere). (B) New World (Western Hemisphere). Other species causing CL that are not shown may be found in Table 275.1.



FIG. 275.8 Old World cutaneous leishmaniasis caused by *Leishmania major*. Note the thick crust over the ulcers, alignment with skin creases, and indurated rim.

FIG. 275.9 Leishmaniasis recidivans due to *Leishmania tropica*. Note the central scar from a previous primary ulcer now surrounded by small papules with few parasites present.



FIG. 275.10 New World cutaneous leishmaniasis caused by *Leishmania (Viannia) panamensis*. Note the large painless ulcer with indurated border; a skin biopsy site is seen on the right side of the lesion.

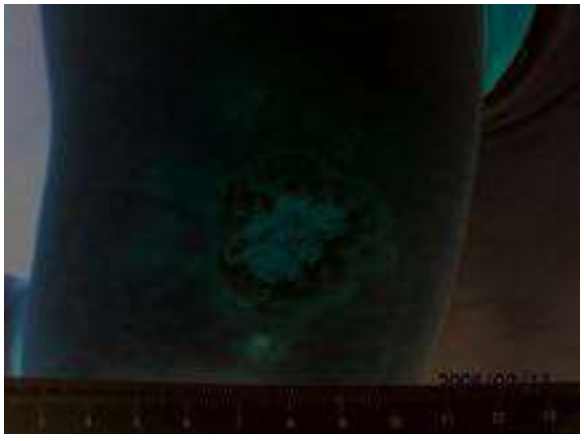

FIG. 275.11 New World cutaneous leishmaniasis caused by *Leishmania (Viannia) guyanensis*. *(Courtesy Dr. Glenn Wortmann, Washington, DC.)*



FIG. 275.12 New World cutaneous leishmaniasis caused by *Leishmania (Viannia) peruviana*. *(Courtesy Dr. Alejandro Llanos-Cuentas, Lima, Peru.)*



FIG. 275.13 New World cutaneous leishmaniasis caused by *Leishmania mexicana*. Note the clean-based ulcer with a rolled, indurated margin. *(Courtesy Dr. Byron Arana, Guatemala City, Guatemala.)*



FIG. 275.14 Disseminated cutaneous leishmaniasis due to *Leishmania (Viannia) braziliensis*. Note the many pleomorphic lesions, both ulcers and nodules; this patient had 162 total skin lesions of about 4 months' duration. *(Courtesy Dr. Anastacio Q. Sousa, Ceara, Brazil.)*



FIG. 275.15 Mucosal leishmaniasis. Note the absence of the nasal septum and full upper lip.

L. major is an infection of desert rodents and affects humans in arid and rural regions of North Africa and Southwest/Central Asia (see Fig. 275.8). *Phlebotomus papatasi* and other *Phlebotomus* species are the vectors.

L. tropica infects dogs and humans in urban areas of Southwest Asia such as Baghdad, Teheran, Kabul, and Damascus, as well as cities in the Mediterranean littoral region, India, and Pakistan. The lesions tend to be crusted and dry, with a predilection for a centrofacial location compared with *L. major*. The vectors include *Phlebotomus sergenti* and *P. papatasi*.

L. aethiopica is endemic in Ethiopia, Kenya, and less commonly southwest Africa, including Namibia. The primary reservoir is the rock hyrax, with rodents as secondary reservoirs. *Phlebotomus longipes* and *Phlebotomus pedifer* are the vectors. Diffuse CL caused by *L. aethiopica* infection is reported from Ethiopia and adjacent areas of Africa.

NWCL is usually a rural zoonosis.[238] The causative species include *L. (V.) braziliensis, Leishmania mexicana, Leishmania (Viannia) panamensis, Leishmania (Viannia) guyanensis, L. amazonensis,* and others (see Table 275.1). *L. infantum* (syn *L. chagasi*) is associated with simple nodular CL in Latin America. The main NWCL reservoirs are forest rodents except in the case of *Leishmania (Viannia) peruviana,* for which dogs are the primary reservoir. The vectors are ground-dwelling or arboreal *Lutzomyia* species. NWCL is an occupational hazard of people living, working, or touring in endemic areas; disease is common in people working at the edge of the forest and among rural settlers. Outbreaks occur when areas of forest are cleared for roads, villages, or farms or when military personnel or tourists enter endemic regions.

L. mexicana is responsible for NWCL from northern Argentina to Texas and Oklahoma, where a small number of autochthonous cases have been reported.[239–241] Lesions typically appear on exposed areas of the extremities (see Fig. 275.13), face, or ears (termed *chiclero's ulcer* when lesions involve the ear). *L. mexicana* is also associated with diffuse CL, an anergic form.

L. amazonensis produces a spectrum of disease in South America that includes simple CL, diffuse CL, ML, and VL.[58]

L. (V.) braziliensis is found in widely scattered areas of Latin America. It is responsible for CL as well as the metastatic complication, ML (see Fig. 275.15). ML caused by *L. (V.) braziliensis* has been diagnosed among American tourists returning from Latin American areas. *L. (V.) panamensis* is found in Panama and adjacent countries (see Fig. 275.10). Of note, Panama and Nicaragua had the highest incidence of CL in the Americas in a more recent study.[242] *L. (V.) guyanensis* is responsible for pian bois or bush yaws in the northern Amazon basin (see Figs. 275.7B and 275.11). *L. (V.) peruviana* is the cause of CL, called *la uta,* in Peru. It typically causes dry lesions (see Fig. 275.12).

New *L. enrietti* species complex parasites, which rarely cause VL as discussed earlier, were identified as causing CL in Ghana's Volta region, Martinique, and Thailand. Lastly, in Australia, *L. enrietti* complex parasites infected red kangaroos, with a proposed vector of midges rather than sand flies.[63]

Pathogenesis and Immunology

The clinical manifestations of CL depend on the virulence factors of the infecting *Leishmania* species and the genetically determined immune responses of their human hosts. At the initial bite site, a papule forms, enlarges to a papulonodule, usually develops central ulceration, and slowly enlarges. Surrounding induration and raised borders are typical.

CL is characterized by a mixed acute and chronic inflammatory infiltrate with infected and uninfected mononuclear phagocytes, lymphocytes, and plasma cells. There are areas of focal necrosis.[243] Early in infection, amastigote-containing macrophages dominate. Eventually, parasitized mononuclear phagocytes are eliminated, lymphocytes become prevalent, and a granulomatous response containing epithelioid cells and giant cells evolves. Lesions heal slowly leaving a flat, atrophic scar. Recovery is associated with a high level of resistance to reinfection by the homologous *Leishmania* species.

Infection occurs when the balance between parasite virulence factors and host cellular immune responses tilts to the parasite, the outcome of proinflammatory and antiinflammatory factors. In localized CL a *Leishmania*-specific Th1 lymphocyte response including IFN-γ, IL-2, and IL-12 is typical.[244] Infected individuals exhibit cutaneous delayed hypersensitivity responses, as evidenced by positive leishmanin skin tests. For durable immunity, memory T cells and $CD8^+$ T lymphocytes have a role.[245,246] Lack of immune control of *Leishmania* is associated with IL-10 and TGF-β. Transcripts from *L. (V.) braziliensis* CL lesions that were upregulated compared with control skin were arginine, C-X-C motif chemokine ligand 9 (CXCL9), and CXCL10.[247,248] Interestingly, RNA-sequencing investigation of CL lesions found many transcripts encoding inhibitory molecules and B-cell functions.[249]

Recently about one-quarter of 52 *Leishmania* (and related *Crithidia* and *Leptomonas*) cultures of multiple species were found to have associated RNA viruses; *Leishmania* RNA virus (LRV) 1 and LRV2 seem to have been acquired about the time that *Leishmania* demonstrated vertebrate parasitism.[250] Varying percentages of *L. (V.) braziliensis, L. (V.) guyanensis,* and *L. amazonensis* isolates from South America have been described as containing a cytoplasmic LRV1. LRV1-infected parasites promoted high levels of inflammatory cytokines through a Toll-like receptor 3–dependent pathway in mice.[251] Among 156 patients, including 109 with CL, 38 with ML, and 5 with mucocutaneous leishmaniasis (78% isolates were *L. [V.] braziliensis*), 71% of patients with ML were LRV1 positive versus 37% patients with CL, suggesting an association of ML and LRV1 in humans. Increased expression of IL-1β, TNF-α, CXCL10, and IL-6 was seen in LRV1-positive *Leishmania* strains compared with LRV1-negative strains.[252] The presence of LRV1-infected *Leishmania* has been associated with an increased rate of SbV or pentamidine failure in the treatment of CL and ML, despite a lack of demonstrable drug resistance.[253,254] Current knowledge suggests some utility of the knowledge of this pathogenic factor in establishing prognosis and stratification for new treatment strategies.

Clinical Manifestations of New World and Old World Cutaneous Leishmaniasis

The incubation period of CL typically varies from 2 weeks to several months but can be as long as several years. A wide variety of skin manifestations, ranging from small papules to papulonodules to dry crusted lesions to large, deep, mutilating ulcers, may be seen. Plaques, satellite lesions, psoriasiform, or verrucous lesions all have been described. The characteristics vary among *Leishmania* species and from one geographic area to another, but overlap occurs. There may be a single lesion, multiple lesions, or widely disseminated disease with hundreds of smaller acneiform lesions. They are usually found on exposed areas of the body. No characteristic of the skin lesion is pathognomonic for CL or can be used to identify the causative *Leishmania* species.

Ulcerative lesions are usually painless, shallow, and circular with well-defined, indurated borders and a central bed of granulation tissue. They gradually increase in size and may develop a "pizza-like" appearance with a raised, circular outer margin, beefy red granulating base, and yellowish exudate on the surface or a thick crusted eschar (see Figs. 275.8 and 275.10). Satellite lesions occur, and they may fuse with the original ulcer. A sporotrichoid chain of lymphatic nodules may be palpated proximal to the lesions, and regional lymphadenopathy may be present. Secondary staphylococcal or streptococcal skin and soft tissue infections can occur. After a variable period, ranging from several months to longer than a year, ulcers heal and leave flat or depressed, atrophic, and depigmented or hyperpigmented scars. The natural history of this healing without intervention ranges from 2 to 6 months for *L. major,* 3 to 9 months for *L. mexicana,* and 6 to 24 months for *L. tropica* and is only 6% at 9 months for *L. (V.) braziliensis*.[255–257]

Several skin manifestations of leishmaniasis are uncommon but should be reviewed. One is PKDL, which is related to VL and was discussed previously. The others are diffuse CL, disseminated CL, and leishmaniasis recidivans.

Diffuse Cutaneous Leishmaniasis

Diffuse CL (anergic CL) is uncommon and is usually associated with infection with *L. mexicana, L. amazonensis,* and sometimes *L. aethiopica*. It is also associated with other *Leishmania* species in hosts with cellular immunocompromise. The initial lesion starts as a localized papule or nodule that does not ulcerate but may coalesce into plaques. Satellite lesions develop around the initial papule, and organisms gradually spread in the skin, resulting in disseminated nodules primarily on the face and

extremities, although eventually all skin can be involved. Diffuse CL has a protracted course and may last for the patient's lifetime, being resistant to treatment.[258–260] Miltefosine leads to an initial favorable response, but relapse follows. Immunotherapy with a combination of heat-killed *L. mexicana* and viable bacillus Calmette-Guérin has been successful in Venezuela with minimal toxicity.[261]

Leishmaniasis Recidivans

Leishmaniasis recidivans is usually associated with *L. tropica* infection. It has been described across North Africa and Southwest Asia.[262] The lesions are often on the face and consist of small papules that surround a scar after initial resolution of the primary CL lesion (see Fig. 275.9). Leishmaniasis recidivans is a relapsing, tuberculoid form of CL characterized by very few parasites, with an intense cell-mediated immune response. In nonendemic areas, it is most often seen in refugees or immigrants from endemic areas.

Disseminated Leishmaniasis

Disseminated leishmaniasis is a syndrome characterized by hundreds of pleomorphic, acneiform, papular, nodular, or ulcerated lesions in multiple noncontiguous sites (see Fig. 275.14) in otherwise immunocompetent patients infected with *L. (V.) braziliensis,* occasionally other species of the *Viannia* subgenera, and *L. amazonensis,* particularly noted in the state of Bahia, Northeast Brazil.[263–267] Parasites are very few, lesion histopathology shows lymphocytes and plasma cell infiltration, and mucosal involvement is seen in up to 44%.[268] This syndrome may be associated with unique strains of *L. (V.) braziliensis* that produce high levels of proinflammatory cytokines such as TNF-α and IFN-γ.[266,269] Disseminated cutaneous lesions have also been observed in a limited number of patients with AIDS or solid-organ transplants.[270,271] Treatment with antimony is unsatisfactory in most cases. An open-label trial of L-AmB in 20 patients with disseminated CL (total dosages 17–37 mg/kg, divided into 7–14 daily doses) demonstrated a cure rate of 65% at 4 months, which increased to 75% among 8 patients receiving ≥30 mg/kg.[268]

Diagnosis

CL is suggested by one or more chronic skin lesions with the appropriate characteristics and a history of exposure in an endemic area. The lesion should be sampled for diagnosis, and generally several diagnostic tests are performed to maximize the likelihood of confirmation of leishmaniasis and to limit the number of procedures.[21] Knowing the infecting species has relevance for the choice of therapeutic agent and regimen; species identification usually requires successful parasite culture; additionally, molecular assays such as PCR in combination with restriction fragment length polymorphism analysis or sequencing can identify subgenera and species.[272]

Obtaining a specimen from an ulcer base requires thorough cleaning and removal of exudate before sampling. Several techniques can yield an adequate sample, including scraping the base or ulcer center, brushing (i.e., cytology brush) the ulcer, obtaining touch impressions (such as using tape or glass slide) from ulcer base, and aspirating the indurated margin.[56,273] Skin biopsy may be useful to allow assessment of diagnoses other than leishmaniasis; in this case the indurated edge of the lesion is preferred for sampling (see biopsy site in Fig. 275.10). Material obtained by scrapings, brushings, and touch preparations made from biopsy samples are stained with a Wright-Giemsa or Giemsa preparation and examined under oil-immersion microscopy for amastigotes. Portions of the lesion biopsy may also be used for histologic examination using hematoxylin and eosin staining along with standard mycobacterial and fungal culture as clinically indicated.

The sensitivity of direct parasite identification and culture varies with the type and duration of the lesion and the infecting *Leishmania* species. The combined sensitivity of these methods ranges from 50% to 90%.[274] Molecular assays performed on lesion samples have better sensitivity and are faster tests; however, there is no single standard assay, and the performance characteristics vary.[21]

Current immunologic tests for CL diagnosis are generally not recommended. Antileishmanial antibodies are detectable in the serum of only a few patients with CL, but actual sensitivity depends on the assay used, and the titers are usually low. The leishmanin skin test is usually positive in patients with localized CL but is not available in North America.

The differential diagnosis includes sporotrichosis, blastomycosis, chromoblastomycosis, mycobacterial infection, cutaneous myiasis, syphilis, yaws, leprosy, sarcoidosis, lupus vulgaris, pyoderma gangrenosum, vasculitis, fixed drug eruption, and neoplasms of the skin. Rarely, lesions may assume a keloidal form and give the appearance of lobomycosis. In returning travelers the competing diagnosis is tropical pyoderma, a chronic ulcerative lesion caused by typical gram-positive bacteria often at sites of minor trauma (i.e., excoriated insect bites).

Treatment

The treatment of CL syndromes is best approached by considering the extent and severity of lesions in each individual, the confirmed or most likely infecting parasite strain, the likelihood of natural healing, and the probability of late manifestations of ML. Because most cases of CL will eventually resolve, and treatment, especially systemic, is not without risk, the first management decision in a patient with CL is whether to recommend specific treatment or not.[275] Table 275.2 lists criteria that should be considered in making the initial treatment decision and the relative need for a systemic versus local treatment option. Clinical trials in OWCL and NWCL have been reviewed, and North American clinical practice guidelines have been published.[21,276,277–279] Miltefosine is the only drug approved by the FDA for the treatment of CL due to *L. (V.)*

TABLE 275.2 Continuum of Cutaneous Leishmaniasis Management

SIMPLE CL	COMPLEX CL
Species unlikely associated with ML	New World CL species associated with ML, particularly *Viannia* subgenera in mucosal belt of Bolivia, Brazil, Peru
No mucosal involvement	Local subcutaneous nodules Large regional adenopathy
Single or few lesions	>4 lesions of significant size (generally >1 cm)
Small size (<1 cm)	Individual lesion ≥5 cm
Location feasible for local treatment	Size or location of lesion for which local treatment is not feasible
Nonexposed skin	Lesions on face, including ears, eyelids, lips; fingers, toes, genitalia
Immunocompetent host	Suppressed cell-mediated immune response
Lesions resolve without prior therapy, not ML-associated species	Clinical failure of local therapy (2–3 months posttreatment)
	Unusual syndromes of leishmaniasis recidivans, diffuse cutaneous leishmaniasis, disseminated cutaneous leishmaniasis
MANAGEMENT OPTIONS: SIMPLE CL[a]	**MANAGEMENT OPTIONS: COMPLEX CL[a]**
Watchful waiting (wound care) Local therapy Cryotherapy Thermotherapy Topical paromomycin Intralesional SbV or pentamidine Photodynamic therapy Systemic therapy—azoles	Systemic therapy Miltefosine SbV Liposomal amphotericin B Amphotericin B deoxycholate Pentamidine Adjuvant immunomodulatory therapies, including imiquimod, pentoxifylline, CpG DNA

[a]Not all inclusive.

CL, Cutaneous leishmaniasis; *ML,* mucosal leishmaniasis; *SbV,* pentavalent antimony

Modified from Aronson N, Herwaldt BL, Libman M, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. *2016;63:e202–e264.*

braziliensis, L. (V.) guyanensis, and *L. (V.) panamensis*. The ThermoMed device (Thermosurgery Technologies, Inc., Phoenix, AZ) is cleared by the FDA for treatment of CL. In addition, several other treatments have been used successfully, albeit off-label, for the treatment of CL.

In all forms of CL, attention should be directed to local wound care. CL is both a parasitic infection and an open wound. Ulcers should be washed daily in soap and water, and then a moisturizing ointment (i.e., white petrolatum) may be applied. Antibiotics should be administered if there is evidence of cellulitis, painful or tender areas, or purulence.

Systemic treatment is recommended for immunocompromised patients with CL due to concerns about further dissemination (mainly skin). Data to determine the best treatment strategy in this situation are very limited, and further research is encouraged.

One of the challenges of CL treatment recommendations is that they are based on many poor quality studies, including diverse treatment courses and outcome measures, and the results are not necessarily generalizable between various species or in the same species between geographic areas. This has spurred many systematic reviews and meta-analyses of CL treatment modalities, some of which are mentioned here, as well as clinical practice guidelines.

Old World Cutaneous Leishmaniasis

Treatment of CL acquired in the Old World has been reviewed more recently; additionally see the IDSA-ASTMH guidelines supplemental material, which includes a searchable spreadsheet of treatment trials for OWCL.[21,277,279] Key factors affecting treatment decisions are host factors, patient preferences, location and number of lesions, spontaneous healing status, metastatic potential of a given species, and availability of and technical experience in delivering local therapies. Local therapies for OWCL including intralesional SbV (available in Canada, but not in the United States), paromomycin-based topical creams and ointments (generally studied for treatment of ulcerative lesions), and thermotherapy and cryotherapy have long been favored in endemic regions, although they are less commonly used in nonendemic countries.[280]

The interpretation of clinical trial results with topical paromomycin-based products is confused by the variety of formulations used. However, the combination of 15% paromomycin plus 12% methylbenzethonium chloride in soft white paraffin, administered twice daily for 28 days, is better than placebo in *L. major* infections but has less benefit in *L. tropica* infections.[279,281,282] WR 279,396 (15% paromomycin, 0.5% gentamicin) cream has been associated with 81% to 94% response rates in phase 2 and 3 clinical trials (for *L. major* and *L. [V.] panamensis*) but is no longer available; currently 15% paromomycin cream can be used in military beneficiaries under an expanded access protocol.[283–285] A meta-analysis of 14 studies comprising 279 patients treated with topical paromomycin found that clinical cure was 2.6 times more likely in patients treated with paromomycin compared with placebo; in OWCL, efficacy was similar to that of intralesional SbV, whereas in NWCL, topical paromomycin was inferior to parenteral SbV.[281]

Locally applied heat therapy administered with the ThermoMed device is generally applied in one treatment session and results in an initial second-degree burn that heals with good cosmetic effect. Heat is delivered to the skin lesion using localized radiofrequency waves, and the 50°C temperature requires advance application of local anesthesia. In a meta-analysis including eight trials (622 thermotherapy-treated subjects), efficacy was estimated as 73%.[286] The practical limitation to a single or a few lesions and access to the expensive delivery modality limit its impact.

Cryotherapy, delivering liquid nitrogen treatment to the CL lesion and surrounding 1 to 2 mm of skin with a freeze-thaw-freeze cycle, is a reasonable choice for lesions <1 cm, few in number and early in development (<3 months). It may leave permanent hypopigmentation at the site in dark-skinned individuals. Treatment is repeated multiple times, usually at 2- to 3-week intervals. In a meta-analysis of eight studies the per-lesion efficacy of cryotherapy was estimated as 67% including both OWCL and NWCL.[287] Combining cryotherapy with intralesional SbV may result in higher cure rates.[288,289] Other local treatment modalities include intralesional injection of SbV (efficacy was 77% in a systematic review),[290] intralesional amphotericin,[291,292] photodynamic therapy,[293] and laser ablation. Further details about local therapy can be found in the IDSA-ASTMH leishmaniasis clinical practice guidelines.[21]

When systemic treatment is indicated (complex CL) (see Table 275.2), options include oral off-label treatment with azoles or miltefosine and parenteral treatment with SbV (either SSG or meglumine antimoniate, both investigational new drugs). Although case series of off-label use of L-AmB suggest efficacy, the evidence base is poorly developed.[21,294,295,296] Healing of cutaneous lesions occurs slowly over a period of weeks and is often incomplete at the end of the treatment course; 3 months is the target to healing—with no relapses over the next 6 to 12 months.

Oral systemic treatment in OWCL with several agents has been studied. Miltefosine given at 2.5 mg/kg/day for 28 days was shown to be as efficacious (81% in 32 patients) as parenteral SbV at 20 mg/kg/day for 14 days for the treatment of *L. major*.[297] In a case series of 34 Dutch military soldiers with *L. major,* 88% were considered healed, although molecular testing showed persistent parasites, making the point that the goal of therapy is clinical healing and not necessarily parasitologic cure.[236] In two small case series (21 subjects) with likely *L. tropica* infections, efficacy with miltefosine treatment was >80%.[298,299]

Azoles in general have modest efficacy in the treatment of OWCL, and the evidence base is characterized by few controlled trials, small numbers of subjects, and various doses and durations. In a systematic review, among 24 OWCL studies a pooled azole efficacy of 62%, including a 53% efficacy in *L. major* and 15% in *L. tropica* was observed.[300] Fluconazole, 200 mg daily for 6 weeks, cured 59% after 3 months compared with 21% in a no-treatment control arm in patients with CL caused by *L. major* in Saudi Arabia.[301] *L. major* in other locations (e.g., Iran or Iraq) seems to be less sensitive. Fluconazole 400 mg daily for 6 weeks was nearly five times better than fluconazole 200 mg for treatment of *L. major* CL in Iran.[302] Ketoconazole (which carries a black box warning from the FDA) at 600 mg daily for 1 month cured 89% of 64 patients with *L. major* CL[303] but none of 32 patients with *L. tropica* CL.[304] A Cochrane systematic review of OWCL suggested, albeit with low confidence due to poor study design, that itraconazole 200 mg/day orally for 6 to 8 weeks versus placebo has a relative risk for healing of 3.7; itraconazole was associated in some studies with a better outcome in *L. major* than *L. tropica*.[277,305–307]

New World Cutaneous Leishmaniasis

Treatment of NWCL has also been reviewed.[276,278,308] In NWCL, species identification may allow a more diverse consideration of treatment options when *L. (V.) braziliensis* is not identified. Generally current practice is to treat systemically for *Viannia* species that are associated with ML (complicated CL) (see Table 275.2). Additionally, NWCL due to *L. (V.) braziliensis* is less likely to heal spontaneously, with rates of 6% at 9 months.[255] The dosing in NWCL is the same as OWCL, and in general local therapy is not used south of Nicaragua, although studies are assessing this further.[309–311]

Pentavalent Antimony

Parenteral SbV, usually given at 20 mg/kg/day for 20 days, is the most common medical treatment in the Americas and produced an overall cure rate of 76% (range, 58%–100%) in >1000 patients entered into selected controlled trials.[308] Various clinical, epidemiologic, and laboratory criteria have been evaluated to find correlates of therapeutic success.[312–315] Young age (presumably reflecting a nonimmune or less immune status), other nonimmune status (e.g., tourists and new immigrants to endemic locations), intrinsic SbV susceptibility, and geographic location (presumably reflecting *Leishmania* species or strain variations) all have been reported to correlate with therapeutic failure. Reported SbV efficacy tended to be lower in Brazil and higher in Colombia, and *L. (V.) braziliensis* and *L. (V.) peruviana* respond less well than *L. (V.) guyanensis*.

Liposomal Amphotericin B

The efficacy of L-AmB for NWCL has not been systematically assessed in a controlled study, but efficacy in the 84% to 85% range was initially reported in small series of nonimmune travelers.[296,316,317] In a European case series, the rate of CL response was 46%, primarily due to lower response rates in *Viannia* species.[295] The optimal L-AmB dose and regimen is not defined for CL.[318] Use of the FDA-approved regimen for

VL of 3.0 mg/kg/day for days 1 to 5 and subsequent doses on days 14 and 21 (21 mg/kg total dosage) is reasonable pending more data but inconvenient. Collapsing the VL regimen to 3.0 mg/kg/day for days 1 to 5, boosting on days 8 and 9[296] or solely on day 10,[316] has been reported for CL treatment. Where resources are less limited, off-label use of L-AmB for CL is appealing because the drug is available, it is approved by the FDA for VL, and clinicians are familiar with its use. L-AmB is expensive and less used in endemic countries; amphotericin B deoxycholate is often used with good results in patients who fail an initial course of SbV.

Pentamidine

Pentamidine has a unique niche in the treatment of CL caused by *L. (V.) guyanensis* in French Guiana and Surinam. Cure rates as high as 90% are reported with just two intramuscular injections of 4 mg/kg, although a trial in Surinam suggested a lower healing rate.[319–321] Pentamidine has not been considered as efficacious in other geographic locations or in other *Leishmania* parasites.[308] However, a comparison of intralesional antimony (five injections) and intralesional pentamidine (120 μg/mm^2 lesion area) for patients with single-lesion Bolivian *L. (V.) braziliensis* showed equivalent cure rates.[310]

Azoles

In the New World, ketoconazole (black box warning), 600 mg daily for 28 days, cured 89% (8 of 9) of patients with *L. mexicana* in Guatemala[322] and 76% (16 of 21) of patients with *L. (V.) panamensis* in Panama.[323] Ketoconazole is not efficacious for *L. (V.) braziliensis* in Guatemala.[322] The treatment of *L. (V.) braziliensis* CL in Brazil with up to 8 mg/kg/day fluconazole for 28 days was not effective, with only 22% healing at 2 months.[324] In a systematic review of azole therapy for CL, the NWCL pooled azole efficacy was 66%, with *L. mexicana* response of 89%.[300]

Miltefosine

The efficacy of miltefosine in treatment of NWCL differs from region to region and must be evaluated in different settings to determine utility.[325] The FDA approved miltefosine for treatment of CL in adults and adolescents at least 12 years of age who weigh at least 30 kg. The FDA-approved regimen for persons who weigh at least 45 kg is one 50-mg capsule three times a day (total 150 mg/day) for 28 consecutive days. Increasing beyond this 150-mg daily dose is poorly tolerated, and efficacy may correlate with doses that are ≥2.5 mg/kg/day, which limits it to persons weighing ≤75 kg.[205] The FDA-approved indication is CL caused by three New World species in the *Viannia* subgenus—*L. (V.) braziliensis, L. (V.) panamensis,* and *L. (V.) guyanensis*. Even for these species, the effectiveness of miltefosine has been variable in different geographic regions. For example, in regions of Guatemala, the per-protocol cure rates were 53% (20 of 38) for miltefosine (and only 33% in *L. [V.] braziliensis*) and 21% (4 of 19) for placebo; yet for *L. (V.) braziliensis* CL in Colombia, miltefosine group cure rates were 80% (32 of 40).[326] In Bahia, Brazil, 75% (49 of 60) of patients with presumed *L. (V.) braziliensis* treated with miltefosine were cured at 6 months versus 53% (18 of 30) of patients treated with parenteral SbV 20 mg/kg/day for 20 days.[327]

MUCOSAL LEISHMANIASIS

Epidemiology

ML is a metastatic complication usually associated with NWCL and is the primary reason that NWCL is treated systemically. About 2% to 10% of persons infected with *L. (V.) braziliensis,* or, less commonly, *L. (V.) panamensis, L. (V.) guyanensis,* or *L. amazonensis*[58,328] develop mucous membrane involvement of the nose, oral cavity, pharynx, or larynx during or months to years after their skin lesions have healed.[329,330,331] A study following >3000 subjects with CL found a lifetime risk for ML of 13%.[332] Mucosal involvement usually occurs after a resolved primary ulcer but can be concurrent; additionally up to 21% of persons never recall a CL lesion.[333] The time between the primary lesion and mucosal involvement may be as short as 1 month or as long as 2 decades.[329,334] Although it is well known that the risk of ML associated with *L. (V.) braziliensis* is much higher in Bolivia, Brazil, and Peru, the distribution of the parasite extends from Mexico to Argentina.[335] ML is relatively uncommon north of Costa Rica. Whether this difference is related to different parasite strains, host immune response differences, or even differences in sand fly vectors such as components of sand fly saliva awaits clarification.

Mucosal involvement is seen occasionally because of the contiguous spread of cutaneous lesions caused by *L. tropica* or other *Leishmania* species. ML has also been described in Sudan associated with both *L. major* and *L. donovani*. In Sudan, ML is sporadic, involves the upper respiratory tract and oral mucosa, is usually a primary disease (but has been seen concurrently with or after VL), and may be caused by parasites that are genetically different from parasites associated with VL.[336–338] Mucosal involvement with lesions described as nodular or tumor-like masses has also been described with *L. infantum* (syn *L. chagasi*),[339] which is usually not associated with prior cutaneous lesions and is often associated with immunocompromised patients.[340,341]

Pathogenesis and Immunology

Various theories have been advanced to explain mucosal involvement including lower temperature, which favors parasite growth; failure of cell-mediated immune responses to be effective in mucosal tissue; local trauma; and capillary plexus trapping of amastigotes in the nose. Spontaneous cure of ML has been reported, but it is thought to be rare.[342] Risk factors associated with the development of ML are male sex, young adult age, severe malnutrition, and duration of primary ulcer greater than 4 months.[343] Host immune response,[344] racial background,[345] parasite strain differences,[346] and familial clustering[347] are associated with increased risk of ML, but the associations are not seen in all studies.

Histopathologically, chronic mucosal lesions are characterized by an intense mononuclear cell infiltrate with few parasites. In patients with ML, peripheral blood mononuclear cells proliferate and produce IFN-γ in response to leishmanial antigens in vitro, and their leishmanin skin tests are usually strongly positive.[348] CD8$^+$ T cells increase in the lesions and express high levels of perforin and granzyme suggesting cytolytic activity.[349] Th17$^+$ cells are increased in ML lesions and associated with areas of neutrophils.[350]

Clinical Manifestations

The initial symptoms are often nasal stuffiness, discharge, discomfort, blockage, or epistaxis. Over time a small nodule develops on the inferior turbinate or septum. Anterior septal ulceration appears early and may progress to perforation and eventual destruction of the septum, resulting in nasal collapse, sometimes called a *tapir nose*. Perforation can also occur through the soft palate. The upper lip may be involved in addition to the buccal, pharyngeal, or laryngeal mucosa.[342] When hypertrophy predominates, resulting in a protuberant nose and lips, the condition is called *espundia,* a term likely derived from the Spanish word for sponge (see Fig. 275.15).[351] If ulceration predominates, there is severe mutilation. A clinical grading system for ML lesions has been published.[352] A series of 327 persons with ML in Brazil found that 97% had involvement of the nasal cavity, 8% had involvement of pharynx, 7% had involvement of oral cavity, and 1% had involvement of larynx.[333] Any patient with possible ML who complains of a change in voice or is observed to have dysphonia requires a thorough examination of the epiglottis, vocal cords, and surrounding laryngeal tissue. Occasionally, severe inflammation can involve the epiglottis and threaten the airway. In this setting, high-dose intravenous steroids must be given 1 or 2 days before the initiation of chemotherapy for ML. Acute airway obstruction can occur with the increase in inflammation that usually follows treatment of ML.[353] On rare occasions, mucosal lesions or fibrosis or both are so extensive that the individual is unable to eat or experiences fatal aspiration pneumonia.

The IDSA-ASTMH clinical practice guidelines recommend that all persons at risk for ML should have a thorough naso-oropharyngeal examination, even if no symptoms are present; if symptoms are present, referral to an otolaryngologist should be done to permit fiberoptic endoscopy and biopsy of any visualized lesions.[21] Additionally, periodic careful ML examinations should be done up to 2 years after CL treatment for ML-risk species and for any later consistent symptoms. Computed tomography with or without fluorodeoxyglucose positron emission

tomography to follow lesions during and after treatment may provide additional assessment techniques.[354,355]

Diagnosis

The clinical suspicion of ML is raised by visualizing abnormal mucosal lesions or when any nasal, oral, pharyngeal, or laryngeal symptoms are vocalized or elicited in a patient with a past history of CL, a scar consistent with CL, or residence or travel to known endemic areas, especially in the highest-risk countries of Bolivia, Brazil, and Peru. Most of the approach to the diagnosis of CL applies here, specifically sample abnormal-appearing tissue, with the recognition that ML is pauciparasitic, which necessitates using molecular assays (see Fig. 275.3).

A confirmed parasitologic diagnosis of ML is preferred. A clinical diagnosis of ML can have a high pretest positive predictive value in an endemic area on the basis of typical clinical findings, a characteristic scar representing previous cutaneous infection, a positive leishmanin skin test result, or the presence of antileishmanial antibodies in serum.

The differential diagnosis of ML includes paracoccidioidomycosis, syphilis, tertiary yaws, histoplasmosis, sarcoidosis, leprosy, tuberculosis, granulomatosis with polyangiitis, basal cell carcinoma, nasal cocaine use, lethal midline granuloma, and T-cell lymphoma. The polyp-like nasal lesions that occur in some patients with ML may occasionally mimic rhinosporidiosis.

Treatment

Randomized trials to assess optimal treatment for ML are lacking.[356] All persons with New World ML should receive treatment, and the selection of agent, duration, and dose should be tailored to the individual patient.[21] SbV has been used for the treatment of New World ML for decades, but the response is variable (51%–88%), and relapses are common.[333,357] Early disease responds better than late-stage disease. Treatment regimens have varied, but a dose of SbV of 20 mg/kg/day for 28 days is typically administered, with some patients requiring longer courses. Patients who do not clinically respond and patients who relapse (most in the first year) can be treated with amphotericin B deoxycholate.[358,359] Depending on the patient's tolerance, 1.0 mg/kg is given IV daily or every other day for 30 doses. L-AmB has been used successfully in a small number of cases.[360,361] A retrospective series of 29 cases (predominantly *L. [V.] braziliensis*) with contraindications to the use of antimony reported an L-AmB–associated cure rate of 93%.[362] The total dosage is not well established, but ranges of 20 to 40 mg/kg have been reported.

Miltefosine given in a 28-day course is approved in the United States for the treatment of ML secondary to *L. (V.) braziliensis*. A nonrandomized trial of Bolivian ML caused by *L. (V.) braziliensis* treated with miltefosine 2.5 mg/kg/day for 28 days cured 62% (49 of 79) of patients at 12 months of follow-up in an intention-to-treat analysis, including 58% (21 of 36) with more extensive disease (palate, pharynx, and larynx).[363] In a follow-up study of 6 weeks of miltefosine with an extended follow-up of 21 patients for 24 months, 71% receiving primary treatment with miltefosine were cured, and 7% had definite or probable relapse.[364] In a case series of 7 *L. infantum* (syn *L. chagasi*) ML infections, miltefosine was uniformly effective.[365]

Other adjunctive approaches have been used with success. Oral pentoxifylline, an inhibitor of TNF-α, combined with SbV resulted in 100% cure (11 of 11) compared with a 58% cure (7 of 12) in the SbV-only trial arm of Brazilian ML. Time to healing was shorter in the combination group as well.[366] Plastic surgery may be necessary to ameliorate the sequelae of ML. It should be delayed for a year after successful therapy.

PREVENTION

For anthroponotic leishmaniasis (*L. donovani* or *L. tropica*) where humans are thought to be the reservoir, differences in time to diagnosis factor into transmission rates. Improved basic health system functioning combined with improved testing (better specificity in early infection) could decrease incidence.[367] There are several approaches to prevention for individuals and communities as public health interventions. For the individual, there is no form of chemoprophylaxis or active (vaccine) or passive (immunoglobulin) immunoprophylaxis for travelers. Standard personal protective measures such as *N,N*-diethyl-meta-toluamide–based insect repellents and permethrin or other insecticides applied to clothing and insecticide-impregnated bed nets provide protection against sand flies if used correctly.[368] Sand flies are one-third the size of a mosquito and can travel up to 960 m in 36 hours, although 200 m is more common.[369] Mosquito protecting bed nets with 156 holes/in^2 treated with insecticide were better than untreated nets[370]; they were effective in preventing CL in Afghanistan and the Syrian Arab Republic and were associated with decreased rates of VL in Bangladesh, Nepal, and Syria.[371] However, results of insecticide-treated bed net studies are variable, as in Morocco, long-lasting insecticide-treated nets were not superior to standard-of-care environmental management.[372–374] Insecticide-impregnated fabrics used as curtains, uniforms, and bedsheets were associated with reduced burden of leishmaniasis.[375,376] It has been suggested that there is not enough evidence to determine if indoor residual spraying is advantageous.[375]

For community-based efforts in endemic areas, vector control and reservoir control are effective, depending on local sand fly behavior and transmission dynamics. Residual insecticides applied in houses and other buildings have yielded good results in sites where peridomestic transmission occurs. However, spraying is necessary at intervals, sand flies may become resistant, and there is concern about the environmental impact. Of note, the cessation of dichlorodiphenyltrichloroethane spraying for malaria in Bangladesh, India, southern Iran, and Peru was followed by major epidemics and a resurgence of leishmaniasis.[377–381] In areas where transmission occurs away from dwellings, residual insecticides are of no benefit.

Reservoir control is another option in areas with domestic animal or human reservoirs. In *L. infantum* (syn *L. chagasi*) areas, domestic and wild dogs are thought to be a primary reservoir. Immunizing dogs, preventing infection through insecticide dog collars, and culling feral dogs should interrupt transmission and reduce human disease. In northeastern Brazil, infected domestic dogs have been identified by mass serologic testing and exterminated, but the efficacy of the program has not been proven, and it is poorly accepted.[382] Other studies suggest that insecticide-impregnated collars may protect dogs from sand fly bites and reduce the risk of human disease.[383,384] In sites where leishmaniasis is a zoonosis involving sylvatic mammals, reservoir control is rarely possible. In areas of anthroponotic transmission, case identification and treatment is important in control. Persons living in the same household as active cases and asymptomatic human infections in the community are a transmission risk.[385,386] In countries where VL has been spread among intravenous drug users, needle exchange programs might limit transmission. Also, for transfusion-associated infection, leukodepletion effectively reduces transmission risk.[387]

Although no commercial vaccine is currently available, there is a rationale to expect one in the future. The spontaneous resolution of human infection is associated with high-level immunity against the homologous infecting *Leishmania* species.[388] Mothers living in endemic areas have exposed the buttocks of their children to sand flies to ensure that leishmanial infection occurs at an inconspicuous site, thus protecting them from later disfiguring facial lesions. In Iran, Israel, and Uzbekistan, leishmanization has been performed by inoculating live *L. major* promastigotes taken from culture. Although this practice was effective in preventing naturally acquired disease, it was discontinued because some of the resulting lesions were slow to heal, others became secondarily infected, and parasites persisted at the site of inoculation even after the lesions healed.[389,390] Leishmanization cannot be used on a large scale or in HIV-endemic areas. Because *Leishmania* organisms lose their infectivity when subcultured and cannot be lyophilized, they must be kept cryopreserved; hence their delivery is impractical. The first generation of *Leishmania* vaccines included whole killed parasites with or without bacillus Calmette-Guérin as adjuvant.[391] Second-generation efforts have focused on identifying protective leishmanial immunogens, polyprotein vaccines, recombinant antigens, and effective adjuvants. Third-generation efforts are focused on the development of genetically engineered, live, avirulent parasites.[21,392–396] More recently interest has increased in the use of sand fly salivary molecules in leishmaniasis vaccines.[397,398]

In 2005 the governments of Bangladesh, India, and Nepal signed a memorandum of understanding to eliminate VL from the region. This ambitious elimination goal has five pillars: early diagnosis and complete

treatment, integrated vector management and vector surveillance, effective disease surveillance through passive and active case detection, social mobilization and building partnerships, and clinical and operational research.[399,400] This effort has led to significant declines in VL incidence but with lower rates faces challenges and will require sustained program implementation, coordination, and political support.

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276 *Trypanosoma* Species (American Trypanosomiasis, Chagas Disease): Biology of Trypanosomes

Louis V. Kirchhoff[a]

SHORT VIEW SUMMARY

Definition
- Chagas disease, or American trypanosomiasis, is an infection caused by the single-cell protozoan parasite *Trypanosoma cruzi*.

Microbiology
- *T. cruzi* is spread among its various mammalian hosts, including domestic and wild animals as well as humans, by blood-sucking triatomine insects, also called cone-nosed or kissing bugs.
- The parasites multiply in the gut of these insects, and their feces contain forms that can infect mammals. When contaminated feces touch vulnerable mammalian tissues such as the conjunctivae, oral and nasal mucosal surfaces, or skin abrasions, transmission can take place.
- Once the parasites gain a foothold in a mammalian host, they alternate between multiplying intracellular forms and free-swimming forms in the bloodstream that spread the infection internally or get swept up by feeding vectors, thus completing the cycle.
- *T. cruzi* can also be transmitted from mother to fetus, by ingestion of contaminated food and drink, through blood products and organs obtained from infected donors, and in laboratory accidents.
- Infection with *T. cruzi* in humans is lifelong.

Epidemiology
- Chagas disease is endemic in Mexico, as well as all of Central and South America.
- The infection is not endemic in any of the Caribbean islands.
- About 8 million persons are chronically infected with *T. cruzi*, roughly 56,000 new infections occur each year, and about 12,000 persons die of the illness annually. An estimated 238,000 immigrants with Chagas disease currently live in the United States.
- During recent decades, large numbers of people in the endemic countries have been migrating from rural areas to cities, and at the same time many millions of people have emigrated from endemic countries to industrialized regions, particularly the United States and the European Union, thus urbanizing and globalizing the disease.

Clinical Manifestations
- Between 10% and 30% of persons who are chronically infected with *T. cruzi* ultimately develop cardiac or gastrointestinal symptoms caused by pathologic processes related to the persistent presence of the parasite.
- Chronic cardiac Chagas disease typically involves rhythm disturbances and cardiomyopathy.
- Gastrointestinal problems can include megaesophagus and megacolon.
- Immunosuppression of persons who harbor *T. cruzi* chronically can result in life-threatening reactivation of the infection.

Diagnosis
- The diagnosis of acute or congenital Chagas disease is made by parasitologic methods, typically direct microscopic examination of blood or polymerase chain reaction–based assays.
- Chronic *T. cruzi* infection is diagnosed serologically, and many accurate enzyme-linked immunosorbent assays, immunofluorescence assays, and chemiluminescent tests are available commercially for this purpose.

Therapy
- Benznidazole and nifurtimox (Lampit; Bayer, Berlin, Germany) are the only two drugs available for treating *T. cruzi* infections. Benznidazole is approved by the US Food and Drug Administration for treatment of children 2 to 12 years of age and can be obtained from Exeltis (Florham Park, NJ; 877-303-7181, fastaccess@exeltis.com). Nifurtimox is available from the CDC Drug Service (404-639-3670, drugservice@cdc.gov).
- Parasitologic cure rates for these drugs are high for acute and congenital infections but unfortunately very low in persons with long-standing infections.
- There are no convincing data from randomized controlled trials indicating that treatment of chronically infected adults with either drug significantly delays pathogenesis or affects long-term outcomes.

Prevention
- No vaccine or prophylactic drugs are available for reducing transmission of *T. cruzi*.
- In endemic countries, reducing vector-borne transmission depends primarily on educating at-risk populations, housing improvement, and spraying insecticides to eliminate vectors in dwellings.
- Serologic screening of blood and organ donors is also a key element in the control of Chagas disease.
- In the majority of endemic countries, enormous progress has been made in reducing transmission through vector control and blood screening, and effective programs for the latter have been implemented in the United States, Canada, and several countries in the European Union.

The protozoan genus *Trypanosoma* consists of several dozen species.[1,2] Two of the three species that infect humans are pathogenic, and several other species cause severe and economically important diseases in domestic mammals. Broadly defined, the organisms belonging to this genus are protozoan flagellates of the family Trypanosomatidae, order Kinetoplastida, that pass through different morphologic stages (epimastigote, amastigote, and trypomastigote) in their vertebrate and invertebrate hosts. The criterion of three morphologic stages, however, is not fulfilled by each species in the genus. For example, only *Trypanosoma cruzi*, the etiologic agent of American trypanosomiasis, or Chagas disease, and one other species multiply in mammalian hosts as intracellular amastigotes similar to those seen in infections caused by organisms belonging to the genus *Leishmania*. In contrast, African trypanosomes, which cause sleeping sickness in humans and varying degrees of morbidity in wild and domestic mammals, do not have an intracellular form and multiply as trypomastigotes that circulate in the mammalian bloodstream and other extracellular spaces.

The trypomastigote form has a single flagellum originating near the kinetoplast, which is a DNA-containing structure located in the parasite's single, complex mitochondrion. The flagellum runs alongside the body of the parasite and is enveloped in an undulating membrane. It extends beyond the body as a free, threadlike structure. The undulating membrane and the free portion of the flagellum give the organism considerable motility.

[a]All material in this chapter is in the public domain, with the exception of borrowed figures.

According to their course of development in the vector, trypanosomes have been classified into two major groups:

1. *Stercoraria:* Multiplication in the mammalian host is discontinuous, taking place in the amastigote stage. Development in the vector (triatomines, or kissing bugs) is completed in the hindgut (posterior station), and mammalian hosts become infected by contaminative transmission. The subgenus *Schizotrypanum* belongs to this group and includes *T. cruzi.*
2. *Salivaria:* Multiplication in the mammalian host is continuous, taking place in the trypomastigote stage. Development in the vector (*Glossina,* or tsetse fly) is completed in the salivary glands (anterior station), and inoculative transmission to mammalian hosts occurs. The subgenus *Trypanozoon* belongs to this group and includes, among others, the subspecies *Trypanosoma brucei brucei,* which causes disease in animals but does not infect humans. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense,* the two causative agents of African sleeping sickness, or human African trypanosomiasis, are also found in this subgenus. As a group, these three subspecies are often referred to as the *T. brucei* complex. Endemic areas of Chagas disease and African sleeping sickness do not overlap (Fig. 276.1). Moreover, there are such important differences in the transmission, pathogenesis, and clinical course of the two diseases that they have little in common except the genetic and morphologic similarities of the causative agents.

CHAGAS DISEASE

Life Cycle and Transmission

T. cruzi, the causative agent of American trypanosomiasis, is transmitted by various species of blood-sucking triatomine insects, or kissing bugs (Fig. 276.2).[3,4]

These vectors are found in large numbers in the wild, where they transmit the parasite among many mammalian species that constitute the natural reservoir; in endemic areas, they live in the nooks and crannies of substandard dwellings. The insects become infected by sucking blood from humans or other mammals that have circulating trypomastigotes (Fig. 276.3). The ingested parasites multiply in the midgut of the insects as epimastigotes, which are flagellates of a distinct morphologic type, and in the hindgut transform into infective metacyclic trypomastigotes that are discharged with the feces at the time of subsequent blood meals. Transmission to another vertebrate host occurs when mucous membranes, conjunctivae, or breaks in the skin are contaminated with bug feces containing the infective forms. The parasites then enter a variety of host cell types and multiply in the cytoplasm after transformation into amastigotes. When multiplying amastigotes fill the host cell, they differentiate into trypomastigotes and the cell ruptures. The parasites released invade local tissues or spread hematogenously to distant sites, thus initiating further cycles of multiplication, largely in muscle cells, and maintaining a parasitemia infective for vectors.

Transmission of *T. cruzi* also occurs through blood transfusions,[5,6] and historically this typically took place in cities when infected but asymptomatic migrants from endemic rural areas donated blood. Serologic screening of donated blood essentially has eliminated transmission by this route in most endemic countries. *T. cruzi* can also be transmitted by transplantation of organs obtained from chronically infected persons.[7,8,9] Roughly 5% to 10% of infants born to *T. cruzi*–infected women have congenital Chagas disease. Although some of these infants have severe problems as a result of the infection, most are completely asymptomatic.[10,11,12,13,14] Numerous laboratory accidents resulting in acute Chagas disease have occurred as a consequence of the ease with which highly infective parasite forms can be produced in the laboratory, but no reports of such events have appeared in recent years.[15,16] Transmission of *T. cruzi* by ingestion of food and drink contaminated by infected vectors has also been reported.[17,18]



FIG. 276.2 ***Rhodnius prolixus,*** **a common vector of** ***Trypanosoma cruzi.*** First- and second-stage nymphs, eggs, and adult.

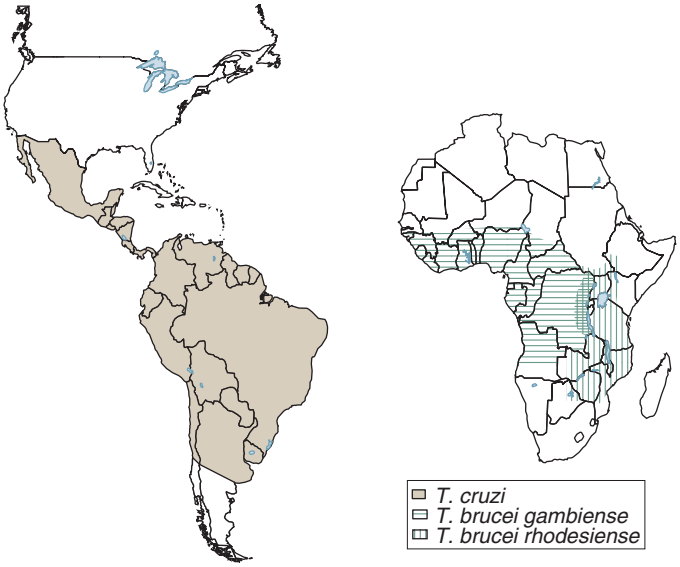

FIG. 276.1 **Distribution of human trypanosomiasis.**



FIG. 276.3 ***Trypanosoma cruzi*** **trypomastigotes in a smear of mouse blood. (Giemsa stain; magnification ×625).** *(Courtesy Dr. Herbert B. Tanowitz, New York, NY.)*

Pathology

In acute Chagas disease, the inflammatory lesion caused by *T. cruzi* at the site of entry is called a *chagoma.*[19] Local histologic changes include intracellular parasitism of muscle and other subcutaneous tissues, interstitial edema, lymphocytic infiltration, and reactive hyperplasia of adjacent lymph nodes. Trypomastigotes released when infected host cells rupture can often be detected by microscopic examination of fresh blood. Muscles, including the myocardium, are the most heavily parasitized tissues. Myocarditis may develop in association with patchy areas of infected cells and necrosis.[20–22]

The characteristic "pseudocysts" seen in sections of infected tissues are aggregates of intracellular amastigotes (Fig. 276.4). Lymphocytosis accompanies the high parasitemias of the acute illness, and mild elevation of transaminase levels is occasionally seen. In some patients, parasites can be found in the cerebrospinal fluid.[23,24]

The heart is the organ most commonly affected in chronic Chagas disease. Gross examination of the hearts of chronic chagasic patients who died of heart failure reveals marked bilateral ventricular enlargement, often involving the right side of the heart more than the left. Thinning of the ventricular walls is common, as are apical aneurysms and mural thrombi. Widespread lymphocytic infiltration is present, accompanied by diffuse interstitial fibrosis and atrophy of myocardial cells. Parasites are rarely seen in stained sections of myocardial tissue, but studies using polymerase chain reaction (PCR) assays have demonstrated the presence of parasites in areas of focal inflammation.[25–27]

Pathologic changes are also common in the conduction system of chronic chagasic hearts. Dense fibrosis and chronic inflammatory lesions most frequently involve the right branch and the left anterior branch of the bundle of His, but lesions of this type are found in other parts of the conduction system as well.[28]

The striking features apparent on gross examination of the esophagus or colon of a patient with chronic Chagas disease of the digestive tract (megadisease) are the enormous dilatation and muscular hypertrophy of the affected organs.[29,30] On microscopic examination, focal inflammatory lesions with lymphocytic infiltration are seen. A marked reduction in the number of neurons in the myenteric plexus is also apparent, and periganglionic and intraganglionic fibrosis in the presence of Schwann cell proliferation and lymphocytosis is found. In most patients the clinical effects of this parasympathetic denervation are confined to the esophagus or the colon, or both, but similar lesions have been observed in the biliary tree, the ureters, and other hollow viscera.

The pathogenesis of the cardiac and gastrointestinal lesions of chronic Chagas disease was debated for many years. Starting in the early 1990s, however, convincing evidence has accumulated indicating that the persistence of parasites in heart muscle stimulates a chronic inflammatory process that often results in rhythm disturbances and cardiomyopathy.[31,32]



FIG. 276.4 ***Trypanosoma cruzi*** **in the cardiac muscle of a child who died of acute Chagas disease in Texas (see reference 20). (Hematoxylin & eosin stain; magnification ×900).**

Epidemiology

T. cruzi infection is a zoonosis, and humans are merely unfortunate hosts whose involvement in the cycle of transmission is not necessary for the perpetuation of the parasite in nature. The triatomine vectors necessary for natural transmission of *T. cruzi* are found in the Americas from the southern half of the United States to southern Argentina.[3] Infected insects have been found in uneven distributions throughout this range. Burrows, hollow trees, palm trees, and other animal shelters are sites where transmission of *T. cruzi* occurs among infected insects and nonhuman mammalian hosts. The ingestion of infected insects likely plays a major role in *T. cruzi* transmission among the latter, and experimental data relating to oral transmission support this concept.[33–35] Interestingly, outbreaks of acute Chagas disease in humans have been reported in several endemic countries and attributed to oral transmission through ingestion of food or drink contaminated with *T. cruzi*–infected vectors or their excreta.[18,36,37] In these incidents, the first of which was reported in Brazil in 1968, many dozens of people became infected and some even died of acute Chagas disease. The occurrence of these outbreaks also underscores the ease with which *T. cruzi* can be transmitted orally[16] and the need to keep vectors out of areas where food and drink are prepared and consumed. The marked reduction of vector-borne and transfusion transmission of *T. cruzi* in much of the endemic range has made oral and particularly congenital transmission proportionally more important.

T. cruzi has been isolated from more than 150 species of wild and domestic mammals. The ability of the parasite to adapt to such a wide variety of hosts, coupled with the long-term parasitemias in infected mammals, results in the presence of an enormous sylvatic and domestic reservoir in enzootic areas. Infected mammals are widely distributed in the southern United States[38–40] and from there southward to central Argentina and Chile.[34,41,42]

Typically, humans become involved in the cycle of transmission while living in rural enzootic areas where vector species adaptable to living in human dwellings, such as *Rhodnius prolixus* and *Triatoma infestans,* are prevalent. The insects live in niches in the settlers' primitive wood, mud, and stone houses, and emerge at night to take blood meals from both domestic animals and humans.[43–45] Thus human *T. cruzi* infection in endemic countries historically has been primarily a public health problem among poor persons who live in rural areas. This pattern has changed dramatically during the past few decades, however, as many millions of at-risk persons have migrated to cities in endemic regions as well as to countries outside the endemic range, thus urbanizing and globalizing the problem of Chagas disease.[46–51] Most new vector-borne infections occur in children younger than 10 years old. In one early study of selected patients the case-fatality rate for untreated acute Chagas disease was 12%,[52] but such a high rate likely reflects the fact that only seriously ill patients came to medical attention. The rate for all new infections is probably less than 1%.

The Pan American Health Organization currently estimates that 8 million people are infected with *T. cruzi,* 56,000 new cases occur each year, and annually 12,000 persons die of Chagas disease.[53–55] The endemic range of Chagas disease includes Mexico as well as all countries in Central and South America. Chagas disease is not endemic in any of the Caribbean nations. A recent estimate has put the global annual cost of Chagas disease at $7.19 billion US dollars.[56] Despite this bleak picture, the current situation relating to the transmission of *T. cruzi* is much brighter. A major international control program in the Southern Cone nations of South America (Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay), initiated in 1991, has achieved a marked reduction in transmission rates through education of at-risk populations, vector control, and serologic screening of donated blood. Gradual reduction in prevalence rates in younger age groups and progressive reduction in the percentage of blood donors infected with *T. cruzi* stand as clear evidence of the success of the program.[57–59,60,61] Uruguay was certified as free of vector-borne transmission in 1997, and Chile followed in 1999. Brazil was declared transmission-free in 2006 and Paraguay in 2018, and it is likely that Argentina will be added to the list within a few years. Similar control programs in the Andean nations and in Central America have achieved considerable success. Historically, relatively less attention has been paid to Chagas disease by public health authorities

in Mexico, where the overall prevalence is likely to be 0.5% to 1.0%. Vector control programs have made progress in limited areas, but despite the 2012 federal mandate that all donations be tested for Chagas disease, a substantial proportion of donated blood is still not screened,[62] and testing for congenital transmission of *T. cruzi* is rarely done.[63,64] Taking a broad view of the problem of Chagas disease, it is fair to say that the barriers hindering the elimination of *T. cruzi* transmission to humans throughout the endemic range and elsewhere are economic and political, and no technical breakthroughs are necessary for its completion.

Only 10% to 30% of persons with chronic *T. cruzi* infections develop symptomatic Chagas disease.[65] The age distribution of the onset of the two types of chronic disease is broad. The relatively high frequency of sudden death in young adults observed in some regions in the past was attributed to the disturbances of cardiac rhythm associated with Chagas disease; decades ago in one highly endemic area in Brazil, chagasic cardiac disease was found to be the leading cause of death in young adults.[66] There is considerable geographic variation in the prevalence of symptomatic chronic Chagas disease among infected persons. The prevalence of cardiac disease among persons who harbor the parasite chronically is lower in Venezuela, Colombia, Central America, and Mexico than in the rest of the endemic range. Moreover, megaesophagus and megacolon associated with *T. cruzi* infection are virtually unknown in the northern endemic range, whereas their prevalence reaches 15% to 20% in the southern endemic regions. It is not known what roles are played by host genetic factors or parasite strain differences in the geographic variation in the patterns of disease.[67,68]

Despite the presence of *T. cruzi*–infected triatomine vectors in many parts of the southern and western United States, only seven autochthonous cases of acute Chagas disease have been reported: four in Texas and one each in California, Tennessee, and Louisiana.[20,69–71] Moreover, screening of US blood donors for Chagas disease, which began in January 2007 and during the first 3 years involved the testing of more than 30 million units, turned up only 17 donors who appeared to have acquired *T. cruzi* infection from vectors in the United States.[72] These data suggest that autochthonous transmission here might be slightly more common than previously thought. It is important to recognize, however, that these 17 donors, who appear to have autochthonously acquired chronic *T. cruzi* infection, came to light through what basically is a cross-sectional prevalence study that looked back over their entire lives. In view of this, then, their identification presents an extremely limited perspective on the overall incidence of vector-borne transmission and certainly says absolutely nothing regarding the secular trend of such transmission.

In a recent review focused solely on studies of Chagas disease in Texas, the authors tabulated the results of earlier heterogeneous prevalence studies published over several decades.[72a] The aggregated data suggest that autochthonous transmission may be a bit more common than previously thought. Nonetheless, in my view the potential pitfalls inherent in aggregating data from such methodologically heterogeneous and chronologically isolated studies cast a long shadow over the authors' conclusion in this regard. In any event, as in the case of the 17 chronically infected donors, no inferences can be drawn from the aggregated data in terms of a secular trend of the incidence of vector-borne *T. cruzi* in Texas. Taking a broad perspective, then, it is clear that vector-borne transmission of *T. cruzi* is a rare event in the enzootic regions of the United States, and there is no clear evidence that the incidence is increasing. Importantly, the last reported acute case of autochthonous transmission of *T. cruzi* in the United States occurred more than a decade ago.[70]

The rarity of transmission of *T. cruzi* to humans in the United States probably results from our relatively high housing standards and the low overall vector density. In the past 30 years, about 15 laboratory-acquired and imported cases of acute Chagas disease have been reported to the Centers for Disease Control and Prevention (CDC), but only one in the latter group occurred in a returning tourist.[73] However, three instances of tourists returning to Europe from endemic countries with acute *T. cruzi* infections have been reported, as well as one similar case in Canada.[74–76]

In contrast, in recent decades the number of persons in the United States with chronic *T. cruzi* infections has grown considerably. It is estimated that 23 million persons born in countries in which Chagas disease is endemic currently reside in the United States. Roughly 17 million of these immigrants are from Mexico,[77,78] where, as noted, the overall prevalence of Chagas disease likely is 0.5% to 1.0%. Moreover, a sizable proportion of these immigrants have come from Central America and Bolivia, areas in which the prevalence of *T. cruzi* infection is substantially higher than in Mexico. A study of Salvadoran and Nicaraguan immigrants done 30 years ago in Washington, DC, found a 5% prevalence of *T. cruzi* infection.[79] Prior to the implementation of donor screening, *T. cruzi* infection rates of 1 in 3285 and 1 in 5995 were found in blood donors in Los Angeles, California, and Tucson, Arizona, respectively.[80] More recently, in Los Angeles a cross-sectional serologic study in immigrants from endemic countries showed a prevalence of 1.24%,[81] which merely confirmed an earlier estimate,[47] and a prospective cohort study of immigrants from endemic countries with nonischemic cardiomyopathy found that Chagas disease was present in 19%.[82]

A recent estimate based on country-specific census data for immigrants from Chagas-endemic countries and the prevalence rates of Chagas disease in those countries suggests that 238,000 persons with Chagas disease currently reside in the United States.[51]

Before the implementation of donor screening, the presence of infected immigrants posed a risk for transfusion-associated transmission of *T. cruzi;* seven such cases had been reported in the United States and Canada[83] and two additional instances were found in trace-back studies done after screening started.[84,85] The confirmed rate of *T. cruzi* infection found in blood donors since the implementation of screening in January 2007 has been about 1 in 13,300.[86] The transplantation of organs obtained from three chronically infected immigrants resulted in five cases of acute Chagas disease, one of which was fatal.[8,87] Regarding congenital transmission, although a reasonable estimate would put the number of infants born in the United States each year with congenital Chagas disease at 63 to 315,[47,88,89] only 2 such cases have been reported to date.[12,90] Several factors likely underlie the lack of reported US cases of congenital transmission, but the low level of knowledge among caregivers about Chagas disease and the risk for congenital transmission certainly play major roles.[91,92]

Clinical Manifestations

The clinical syndromes of acute *T. cruzi* infection and chronic Chagas disease are quite different. Acute illness results from the first encounter of the host with the parasite, and chronic disease involves persistent infection and, in many infected persons, late sequelae.

Acute Chagas disease[19] is usually an illness of children, but can occur at any age. Only a small portion of acute infections caused by *T. cruzi* are recognized as such because of the mild and nonspecific nature of the symptoms in most patients and the lack of access to medical care. The first signs of illness occur a week or so after invasion by the parasites. When the parasites have entered through a break in the skin, a chagoma may appear, consisting of an indurated area of erythema and swelling accompanied by local lymph node involvement. The Romaña sign (Fig. 276.5), the classic sign of acute Chagas disease, consists of painless edema of the palpebrae and periocular tissues and may appear when the conjunctiva is the portal of entry. These initial local signs can be followed by fever, malaise, anorexia, and edema of the face and lower extremities. Generalized lymphadenopathy and hepatosplenomegaly may also appear.

Overt central nervous system signs are not common, but meningoencephalitis develops in some patients and is associated with a poor prognosis.[93] Severe myocarditis also develops in a small proportion of patients with acute disease, and most deaths are due to resulting congestive heart failure.[20,21] Nonspecific electrocardiographic changes are seen, but the life-threatening arrhythmias that are frequent in chronic Chagas disease generally do not occur. In untreated patients, symptoms resolve gradually over a period of weeks to months. Areas of local reaction around the eye or other sites of parasite entry can persist for several weeks, as can the lymphadenopathy and splenomegaly. After spontaneous resolution of the acute illness, the patient enters what is called the indeterminate phase of Chagas disease, which is characterized by asymptomatic and subpatent parasitemia and antibodies to a variety of *T. cruzi* antigens.



FIG. 276.5 Romaña sign in an Argentinean child with acute chagas disease. *(Courtesy Dr. Humberto Lugones, Santiago del Estero, Argentina.)*



FIG. 276.6 Chest radiograph of a Bolivian patient with chronic *Trypanosoma cruzi* infection, congestive heart failure, and rhythm disturbances (see reference 96). Pacemaker wires are present in the area of the left ventricle.



FIG. 276.7 Barium swallow radiographic study of a Brazilian patient with chronic *Trypanosoma cruzi* infection and megaesophagus. The markedly increased diameter of the esophagus as well as its failure to empty are typical findings in chagasic patients with megaesophagus. *(Courtesy Dr. Franklin A. Neva, Bethesda, MD.)*

Chronic symptomatic Chagas disease becomes apparent years or even decades after initial infection. The heart is the organ most commonly involved, and symptoms reflect the rhythm disturbances, congestive heart failure, and thromboembolism that are characteristic of the chronic illness (Fig. 276.6).[94–98] Dizziness, syncope, and, less commonly, seizures result from a wide variety of arrhythmias. The cardiomyopathy that develops insidiously often primarily affects the right ventricle, and the classic signs of right-sided heart failure are frequently present. As in patients with arrhythmias, the progression of symptoms related to the cardiomyopathy may be gradual, and a validated risk-score tool for following progression has been developed.[99] The clinical course is frequently complicated by emboli to the brain or other areas.

In patients with megaesophagus, symptoms are similar to those of idiopathic achalasia and may include dysphagia, odynophagia, chest pain, cough, and regurgitation (Fig. 276.7).[100,101] Hypersalivation and salivary gland hypertrophy have been observed. Aspiration can occur, especially during sleep, and in untreated patients repeated episodes of aspiration pneumonitis are common. Weight loss and even cachexia in patients with megaesophagus can combine with pulmonary infection to result in death. As in idiopathic achalasia, an increased incidence of cancer of the esophagus has been reported in patients with chagasic esophageal disease.

Patients with chagasic megacolon are plagued by chronic constipation and abdominal pain (Fig. 276.8).[102,103] Individuals with advanced disease can go for several weeks between bowel movements, and acute obstruction, occasionally with volvulus, can lead to perforation, septicemia, and death.

Immunosuppression and Transplantation in *T. Cruzi*–Infected Patients

When persons who harbor *T. cruzi* chronically become immunosuppressed, reactivation of the infection can occur, sometimes with a severity greater than is typical of acute Chagas disease in immunocompetent patients.[104–107] The incidence of reactivation in *T. cruzi*–infected patients who become immunosuppressed is not known. Immunosuppressed patients in whom chronic *T. cruzi* infection reactivates often develop skin lesions and cerebral mass lesions, neither of which occurs in immunocompetent persons infected with the parasite.[108–110] There have been several reports of reactivations of chronic *T. cruzi* infections after renal transplantation, and in two of these instances the central nervous system was involved. Nonetheless, *T. cruzi* infection should not be a contraindication for kidney transplantation. Infected patients who do undergo the procedure, however, should be monitored periodically for



FIG. 276.8 Air-contrast barium enema of a constipated Bolivian patient with megacolon and chronic Chagas disease (see reference 96). The markedly increased diameters of the ascending, transverse, and sigmoid segments of the colon are readily apparent.

signs and symptoms of acute Chagas disease, and a specific search for *T. cruzi,* including careful neurologic evaluation, should be performed when acute illnesses occur postoperatively.

Immunosuppression caused by the human immunodeficiency virus (HIV) can also lead to recrudescence of chronic *T. cruzi* infection. To date, dozens of such patients have been described, the first of whom was a Central American immigrant living in the United States.[107,111–113,114] As with other immunosuppressed patients, many of these coinfected patients developed *T. cruzi* brain abscesses. Calculations based on the epidemiologies of HIV and *T. cruzi* infections in endemic countries suggest that the incidence of brain abscesses caused by the latter in coinfected persons is low. Moreover, it is likely that the incidence of *T. cruzi* brain abscesses in HIV-infected patients has decreased markedly in the era of highly active antiretroviral therapy.

Heart transplantation is an option in patients with end-stage Chagas cardiac disease, and several hundred *T. cruzi*–infected patients have undergone the procedure, mostly in Brazil.[115,116] Because *T. cruzi* cannot reliably be eliminated by antiparasitic treatment before heart transplantation, reactivation of the infection is a frequent problem and is complicated by the fact that the parasitologic approaches usually used to detect acute *T. cruzi* infections often are not sensitive detectors of reactivation.[117] Moreover, a higher than expected incidence of malignancies was observed in the Brazilian patients.[118,119] It is also noteworthy that patients who have received transplants for Chagas heart disease occasionally develop cutaneous lesions containing large numbers of parasites,[120] as has been reported in Chagas disease patients who received renal transplants[121] and in coinfected persons with HIV/acquired immunodeficiency syndrome.[122] Despite these problems, the long-term survival of Chagas disease patients with heart transplants is greater than that of persons who receive transplants for other reasons, probably because the pathology of chronic *T. cruzi* infection is generally limited to the heart.[115]

Diagnosis

The first consideration in the diagnosis of acute Chagas disease is a history consistent with exposure to *T. cruzi.* This includes residence in an environment in which vector-borne transmission is known to occur, a recent transfusion in an endemic area where effective blood screening programs are not in place, birth of an infant to a *T. cruzi*–infected mother, risk of involvement in a foodborne outbreak, or a laboratory accident involving the parasite. It is important to keep in mind, moreover, that autochthonous transmission of *T. cruzi* in the United States is extremely rare and that only one imported case among tourists returning to the United States has been reported,[73] even though many millions of people travel from the United States to endemic countries each year.

The diagnosis of acute Chagas disease is made by detecting parasites, and testing for anti–*T. cruzi* IgM is not useful. Circulating parasites are motile and can often be seen in wet preparations of anticoagulated blood or buffy coat viewed under a coverslip or in microhematocrit tubes. In many cases the parasites can also be seen in Giemsa-stained smears. In acutely infected immunocompetent patients, examination of blood preparations is the cornerstone of detecting *T. cruzi.* In immunocompromised patients suspected of having acute Chagas disease, however, other specimens such as lymph node and bone marrow aspirates, pericardial fluid, and cerebrospinal fluid should be examined microscopically. When these methods fail to detect *T. cruzi* in a patient whose clinical and epidemiologic histories suggest that the parasite is present, as is often the case, efforts to grow the organism can be undertaken. This can be attempted by culturing blood (hemoculture) or other specimens in liquid medium.[123] Major problems with hemoculture are that its sensitivity may be no greater than 50% even in acutely infected patients and that it takes at least several weeks to complete, which is beyond the time at which a decision regarding drug treatment must be made. When available, PCR assays are a better alternative because the sensitivity is higher and completion time is relatively short (see later). The diagnosis of congenital Chagas disease also must be parasitologic (microscopic examination of cord blood or PCR assay) when performed right after birth because of the presence of maternal anti–*T. cruzi* antibodies. Serologic testing for specific IgG should be performed at least 9 months later if the initial parasitologic studies are negative.[10,124]

Chronic *T. cruzi* infection is usually diagnosed by detecting IgG antibodies that bind specifically to parasite antigens, and detecting the presence of the organism is not of primary importance. Currently, more than 30 assays for serologic diagnosis of *T. cruzi* infection are available commercially. The majority are based on enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination, and chemiluminescent and indirect immunofluorescence formats, and are used widely in the endemic countries for clinical testing and for screening blood and organ donors.[125,126] In recent decades enormous efforts have been invested in the development and validation of these tests, and their sensitivities and specificities are generally at the level of the best assays for other infectious diseases. Nonetheless occasional false-positive reactions do occur, typically with specimens from patients having leishmaniasis, malaria, syphilis, and other parasitic and nonparasitic diseases. Because of this issue, the World Health Organization recommends that samples be tested in two assays based on different formats before diagnostic decisions are made.[127]

The results of a recent evaluation of 11 commercially available rapid diagnostic tests indicate that their sensitivities and specificities generally are not high enough to warrant their use for making treatment decisions, even when they are used in highly controlled laboratory settings.[128]

Three serologic assays have been cleared by the US Food and Drug Administration (FDA) for clinical testing: the Hemagen Chagas Kit (Hemagen Diagnostics, Inc., Columbia, MD),[126] the Chagatest ELISA recombinante (Laboratorios Wiener, Rosario, Argentina),[126] and the Chagas Detect *Plus* Rapid Test (Inbios International, Inc., Seattle, WA).[129] The Abbott Architect Chagas Assay (Abbott Laboratories, Chicago, IL),[130,131] which is an accurate chemiluminescence test based on a mixture of four chimeric recombinant proteins, has not been cleared yet for use in the United States but is approved and used widely for both clinical and donor testing in many endemic countries.

The Ortho *T. cruzi* ELISA Test System (Ortho Clinical Diagnostics, Raritan, NJ), an ELISA based on a parasite lysate,[125] and the Abbott Prism Chagas Assay,[132] a highly automated chemiluminescent microparticle immunoassay based on the same mixture of recombinant proteins used in the Abbott Architect Chagas Assay, have been approved by the FDA for donor testing. These latter two assays are currently being used to screen the US blood supply in a selective testing protocol in which an initial donation at a given donor center is tested for Chagas disease, and if the result is negative, subsequent donations by the same person at that site are not tested. The Abbott ESA (Enzyme Strip Assay) Chagas, also based on the group of recombinant antigens used in the Architect Chagas Assay, has been approved by the FDA for confirmatory testing of screen-positive donor samples (i.e., positive in the Abbott Prism

Chagas Assay or the Ortho ELISA).[133] In addition, a Clinical Laboratory Improvement Amendments–approved radioimmunoprecipitation assay (Chagas RIPA)[126,134] was used from 2007 to 2014 for confirmatory testing of donated units that were positive in the Abbott Prism Chagas Assay or the Ortho ELISA, and is currently available in my laboratory for testing clinical and research specimens.

The possibility of using PCR assays for detecting *T. cruzi* infection has been studied extensively. The number of parasites in the blood of patients with chronic *T. cruzi* infection is extremely low, but PCR assays have the potential for detecting such low numbers because the organisms have highly repetitive nuclear and kinetoplast DNA sequences that can be amplified. Three decades ago, two of my colleagues and I described a PCR test in which a 188–base pair nuclear repetitive DNA sequence is amplified (TCZ1-TCZ2 primers).[135] Each parasite contains approximately 100,000 copies of this sequence, and in contrived experiments as little as 0.5% of the genome of a single parasite gave a positive result. Studies in mice with acute and chronic *T. cruzi* infections indicated clearly that this PCR assay is much more sensitive than microscopic examination of blood.[136] In a second PCR test, described at the same time by Sturm and coworkers, a 330–base pair segment of the *T. cruzi* kinetoplast mini-circle is amplified (S35-S36 primers).[137] Each parasite is believed to have approximately 120,000 copies of this sequence, and in mixing experiments the authors were able to detect 0.1% of one parasite genome. The results of the five head-to-head comparisons of PCR assays based on these two primer pairs published to date suggest that the TCZ1-TCZ2 assay has an edge in terms of sensitivity.[136,138–140]

Since the publication of these two original reports in 1989,[135,137] hundreds of articles focused on PCR detection of *T. cruzi* have been published. Importantly, in a group of nine key human studies published in the 1990s, the sensitivities of the PCR assays ranged from 44.7% to 100%.[123,141,142] Three more recent major studies showed sensitivities of 60% to 75% in persons chronically infected with *T. cruzi,* thus confirming that the lack of sensitivity of PCR assays continues to be a major issue.[139,140,143] Clearly this level of sensitivity is not high enough to allow use of these assays for confirmatory testing of screen-positive blood donations. Nonetheless, PCR assays are useful for detecting *T. cruzi* in persons with borderline serologic results, in infected individuals who have received specific treatment, and in patients suspected of having acute or congenital Chagas disease in whom parasites are not detected microscopically. In all such persons, only positive results can be taken as truly indicative of their infection status. At the present time no PCR test for the detection of *T. cruzi* is available commercially, but detailed guidance for optimization of assays has been published.[140]

Therapy

Current therapy for persons infected with *T. cruzi* is unsatisfactory from a variety of perspectives. Two drugs are currently used to treat patients infected with *T. cruzi.*[144] The first is the nitroimidazole derivative benznidazole (Exeltis), which is viewed as the drug of choice by most specialists and is used widely in the endemic countries.[144] In acute and congenital Chagas disease, benznidazole markedly reduces the duration and severity of the illness and decreases mortality. However, it results in parasitologic cure in only about 70% of acute patients, can cause severe side effects, and must be taken for prolonged periods. Therapy with benznidazole should be initiated as early as possible in cases of acute or congenital Chagas disease. Moreover, when a laboratory accident occurs in which there is a reasonable likelihood that *T. cruzi* infection will become established, therapy should be started without waiting for clinical or parasitologic indications of infection.

Side effects of benznidazole are common and include peripheral neuropathy, rash, and granulocytopenia.[145,146–149] High rates of discontinuation due to adverse reactions have been observed recently in studies done in nonendemic areas (20% in the United States; 32% in Milan, Italy).[150,151] The rash may associated with a Th2 response in patients who carry an HLA-B*3505 allele.[152] To address the high rate of discontinuation due to rash, a progressive regimen was proposed that increases the daily dose by one pill until the daily weight-based recommended dose is reached, and this led to a discontinuation rate of only 1 of 30 patients.[153] Experimental data indicate that benznidazole crosses the placenta and has teratogenic effects, and thus it should not be given to pregnant women. The recommended oral dosage of benznidazole is 5 mg/kg/day for 60 days.

The second drug used to treat Chagas disease is the nitrofuran derivative nifurtimox, which has been in use for more than 4 decades, and extensive clinical experience has accumulated.[154] The efficacy of nifurtimox is similar to that of benznidazole. A large proportion of patients treated with nifurtimox experience adverse side effects. Gastrointestinal complaints include abdominal pain, nausea, vomiting, anorexia, and weight loss. Possible neurologic symptoms include restlessness, insomnia, twitching, paresthesias, and seizures. These symptoms generally resolve when the dosage is reduced or therapy is discontinued. Nifurtimox should not be given to pregnant women.

Nifurtimox is supplied as 30- and 120-mg tablets. The recommended oral dosage for adults is 8 to 10 mg/kg/day. The dosage for adolescents is 12.5 to 15 mg/kg/day, and for children 1 to 10 years of age it is 15 to 20 mg/kg/day. The drug should be given in four divided doses each day, and therapy should be continued for 90 to 120 days. Nifurtimox is available from the Parasitic Disease Drug Service of the CDC.

In terms of which groups of *T. cruzi*–infected persons should be given specific treatment, there is broad consensus that all persons with acute *T. cruzi* infection, including infants with congenital Chagas disease, should be treated. In addition, all chronically infected persons up to 18 years old should be treated. This latter perspective is supported by data from older clinical trials in which reduced anti–*T. cruzi* antibody levels were observed in a substantial proportion of treated patients in this age group. Nonetheless, data regarding parasitologic cure in treated patients up to 18 years of age are not available, in large measure because of a lack of a workable diagnostic tool to assess cure. It merits mention, moreover, that the recent FDA approval of benznidazole for treating chronically infected 2- to -12-year-old children was based on surrogate antibody level reduction data rather than evidence of parasitologic cure.[155–157,158]

The question of whether adults in the indeterminate or chronic symptomatic phases of *T. cruzi* infection should be treated with benznidazole or nifurtimox has been debated for many years. This is a thorny issue because these two drugs have to be taken for 2 to 4 months, frequently cause bothersome side effects, and result in parasitologic cure in less than 10% of persons with long-standing *T. cruzi* infections.[159,160–162] Moreover, there is no convincing evidence from properly controlled trials that treatment with either of the drugs improves long-term outcomes in adults with chronic *T. cruzi* infection.[163] The issue is complicated further by the lack of sensitivity of parasitologic assays, which ideally would be used in trials to look for treatment failure because antibody titers can remain positive for years even when parasitologic cure has occurred. A panel of experts convened by the CDC in 2007 suggested that treatment be offered to adults with presumably long-standing indeterminate phase infections.[157] A major trial involving approximately 5000 study subjects in Argentina, Bolivia, Brazil, Colombia, and El Salvador (the BENEFIT Multicenter Trial) analyzed the effects of benznidazole therapy in seropositive patients with incipient Chagas cardiomyopathy. The outcomes of death, resuscitated cardiac arrest, sustained ventricular tachycardia, insertion of a pacemaker or implantable cardioverter-defibrillator, cardiac transplantation, new heart failure, stroke, or other thromboembolic events were not significantly different between benznidazole and placebo recipients, with a mean follow-up of 5.4 years.[164]

The usefulness of fluconazole, ketoconazole, itraconazole, posaconazole,[165,166] and several other azoles, as well as allopurinol, has been studied extensively in *T. cruzi*–infected laboratory animals and to a lesser extent in persons with Chagas disease. None of these drugs should be used to treat patients with acute or chronic Chagas disease.

Most patients with acute Chagas disease require no therapy other than benznidazole or nifurtimox because symptoms are generally self-limited even in the absence of drug treatment. The management of the occasional severely ill acute-phase patient with myocarditis or meningoencephalitis is largely supportive. The treatment of patients with chronic chagasic heart disease is also supportive. Chronically infected persons should have electrocardiograms performed every 6 months or so because pacemakers have been shown to be useful in the management of arrhythmias seen in chronic Chagas disease. The congestive heart failure

of cardiomyopathic Chagas disease is generally treated with measures used in patients with cardiomyopathies resulting from other causes.[30,167–169] The usefulness of implantable cardioverter-defibrillators in patients with advanced chagasic cardiac disease is controversial.[170,171–173]

Megaesophagus associated with Chagas disease generally should be managed as for idiopathic achalasia.[30,101,174] The first approach to relieving symptoms is balloon dilation of the lower esophageal sphincter. Patients who fail to respond to repeated attempts at this approach are treated surgically. The procedure used most frequently is wide esophagocardiomyectomy of the anterior gastroesophageal junction, combined with valvuloplasty to reduce reflux. Patients with extreme megaesophagus are often treated with esophageal resection with reconstruction using an esophagogastroplasty. In industrialized countries, laparoscopic myotomy is often used to treat idiopathic achalasia. This relatively simple procedure may become the approach of choice for both idiopathic achalasia and Chagas megaesophagus. Patients in the early stages of colonic dysfunction associated with chronic Chagas disease can be managed with a high-fiber diet and occasional laxatives and enemas. Fecal impaction necessitating manual disimpaction may occur, as can toxic megacolon, which requires surgical treatment. Another complication of chagasic megacolon that requires immediate attention is volvulus. This usually occurs when the lengthened and enlarged sigmoid colon twists and folds on itself, causing a constellation of symptoms resulting from obstruction and vascular compromise. Endoscopic emptying can be performed initially in patients without radiographic, clinical, or endoscopic signs of ischemia in the affected area. Complicated cases should be treated with surgical decompression. In either event, however, surgical treatment of the megacolon is eventually necessary because of the high probability of recurrence of the volvulus. A number of surgical procedures have been used to treat advanced chagasic megacolon, and all include resection of the sigmoid colon as well as removal of part of the rectum.[175] The latter is performed to avoid recurrence of megacolon in the segment of the colon that is anastomosed to the rectum.

Prevention

In view of the possible serious consequences of chronic *T. cruzi* infection, all immigrants from endemic regions and children of immigrant mothers with geographic risk for Chagas disease who are not known to be seronegative should be screened serologically. Identification of infected persons is important so that they can be monitored for rhythm disturbances and other signs of cardiac disease, as well as gastrointestinal dysfunction, and treated appropriately when indicated. The possibility of congenital transmission is another justification for screening. Over the past decade, substantial evidence has accumulated indicating that treating chronically infected girls and women of childbearing age markedly reduces the probability of congenital transmission to their babies conceived after treatment.[176,177,178–180] Summarizing the data presented in these five somewhat heterogeneous studies, 171 girls and women had received specific treatment for chronic *T. cruzi* infection prior to pregnancies that all told resulted in 296 evaluable babies. None of these babies had any evidence of *T. cruzi* infection, which is surprising given that the historical rate of congenital Chagas disease in infants born to infected mothers is 5% to 10%. Moreover, it is noteworthy that many if not most of the girls and women so treated likely were not cured parasitologically, and in some instances many years had elapsed between treatment and the pregnancies. These data suggest strongly that specific prepregnancy treatment of chronic *T. cruzi* infection has a marked suppressive effect on the likelihood of subsequent congenital transmission, and it seems likely that treating all infected women of childbearing age will become the standard of care (the current consensus foresees that all persons of both sexes 18 years old or less will be treated). It merits mention, moreover, that given the potential fetal toxicity of benznidazole and nifurtimox, care must be taken to ensure that chronically infected women of childbearing age are not pregnant when treatment is initiated and that they do not become pregnant during treatment.

As noted earlier, to date nine cases of transfusion-associated transmission of *T. cruzi* have been reported in the United States and Canada. The courses of acute Chagas disease in seven of these patients were particularly fulminant because of immunosuppressive therapy, which certainly contributed to the definitive diagnoses. The question as to how best to avoid transmission of the parasite via transfusion in the United States was debated for more than a decade before December 2006, when an FDA-approved screening test first became available. Common sense suggested that if serologic screening was warranted in endemic countries from which the 23 million at-risk immigrants living in the United States had come, they should be screened when they presented for donation here. In the end, governmental and blood industry authorities embraced this view, and, as noted earlier, an FDA-approved selective screening protocol is currently implemented and essentially eliminates the risk of *T. cruzi* transmission by transfusion in the United States.[86,181]

A panel of experts recently reviewed the limited data available relating to the transplantation of organs obtained from *T. cruzi*–infected donors.[7] They and others[182] have concluded that, with informed consent, transplantation of livers and kidneys from infected donors is reasonable but cautioned that careful postoperative and long-term monitoring for *T. cruzi* infection in the recipients should be performed. Hearts from *T. cruzi*–infected donors should not be transplanted.[183]

T. cruzi is a Risk Group 2 organism, and laboratorians working with it should use personal protective measures consistent with this classification.[184,185] Persons traveling in endemic areas should avoid sleeping in dilapidated dwellings and should use insect repellent and bed nets to reduce exposure to vectors.[186,187] No vaccine is available for the prevention of transmission of *T. cruzi*.[188,189] Special precautions beyond the usual measures for reducing the insect exposure of campers, hunters, and others engaging in outdoor activities in the United States are not warranted.

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The complete reference list is available online at Expert Consult.

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277 Agents of African Trypanosomiasis (Sleeping Sickness)

Louis V. Kirchhoff[a]

SHORT VIEW SUMMARY

Definition
- Human African trypanosomiasis (HAT), also known as sleeping sickness, is caused by two protozoan parasite subspecies: *Trypanosoma brucei gambiense* (West African trypanosomiasis) and *Trypanosoma brucei rhodesiense* (East African trypanosomiasis).

Microbiology
- The trypanosomes that cause HAT are transmitted among their mammalian hosts by tsetse flies. The flies become infected when they take a blood meal from an infected mammal. There is a developmental cycle in the flies, after which infective parasites migrate to the salivary glands and are injected into a new host when the flies take subsequent blood meals. In their mammalian hosts African trypanosomes are found primarily in the bloodstream and, to a lesser extent, in perivascular areas of the brain and other tissues. In contrast to *Trypanosoma cruzi,* the parasite that causes Chagas disease, African trypanosomes do not have an intracellular phase. They avoid immune destruction by antibodies by periodically changing their glycoprotein coats through a molecular process called *antigenic variation.*

Epidemiology
- HAT occurs only in sub-Saharan Africa, where endemic foci are found in about two dozen countries.
- HAT is much less of a problem today than in the past. Only 2184 new cases were reported to the World Health Organization in 2016. Although this number reflects substantial underreporting, there is no doubt that control efforts implemented in many endemic countries during the past 25 years have achieved considerable success. Each year in industrialized countries HAT is diagnosed in a sprinkling of persons who have traveled to endemic areas, but given the large numbers of persons who make such trips, the incidence of such cases is extremely low.

Clinical Manifestations
- Two clinical stages exist. Stage 1 (hemolymphatic disease) is characterized by fever, adenopathy, and headache. Stage 2 (central nervous system or encephalitic disease) involves mainly neuropsychiatric signs and symptoms.
- The primary difference between the clinical patterns of *gambiense* and *rhodesiense* HAT is that the latter follows a much more rapid course and can lead to death in a matter of months, whereas *gambiense* HAT typically develops a chronic pattern that can last for years.
- Untreated HAT almost inevitably ends in death.
- It is important for caregivers attending to travelers with fever and other nonspecific symptoms who have been in endemic areas to include HAT in the differential diagnosis.

Diagnosis
- Given the life-threatening nature of untreated HAT, a high index of suspicion should be maintained with persons who have been in areas endemic for HAT.
- Numerous other illnesses common in the tropics cause symptoms similar to those seen in both the early and late stages of sleeping sickness.
- A definitive diagnosis of African trypanosomiasis requires demonstration of the parasite. Examination of blood smears and cerebrospinal fluid for parasites is the cornerstone of HAT diagnosis.
- There is a growing role for polymerase chain reaction and related molecular methods in the diagnosis of HAT.
- Diagnostic approaches and treatment should be discussed in detail with appropriate staff at the Centers for Disease Control and Prevention.

Therapy
- Treatment is complicated because it varies according to the clinical stage of the disease and the trypanosome subspecies causing the infection.
- Toxicity of the drugs used to treat HAT is a major problem.

Prevention
- Individuals can reduce their risk of acquiring infections with African trypanosomes by avoiding areas known to harbor infected insects, wearing clothing that reduces the biting of the flies, and using insect repellent.
- Chemoprophylaxis is not recommended because of the high toxicity of the drugs that are active against African trypanosomes, and no vaccine is available to prevent transmission of the parasites.

PARASITES AND THEIR TRANSMISSION

The agents of human African trypanosomiasis (HAT, sleeping sickness) are flagellated protozoan parasites that belong to the genus *Trypanosoma,* subgenus *Trypanozoon.*[1,2] A general description of the members of this genus and specific characteristics of the subgenus are presented in the introduction to Chapter 276. Three trypanosome subspecies, *Trypanosoma brucei brucei, T. brucei rhodesiense,* and *T. brucei gambiense,* are considered here. They are indistinguishable morphologically, and as a group they are often referred to as the *T. brucei* complex. *T. b. brucei* is a parasite of wild and domestic animals that is not infectious to humans. In contrast, *T. b. rhodesiense,* which is primarily a parasite of wild game, can infect humans, and this difference in host specificity forms the primary basis of the distinction between the two subspecies. *T. b. gambiense* primarily infects humans, and infections of wild and domestic animals are of limited importance.

The members of the *T. brucei* complex are transmitted by various species of tsetse flies that belong to the genus *Glossina.*[3,4] These bloodsucking insects are found only in Africa, where their range covers millions of square kilometers of rain forest and savanna. The parasites undergo a developmental cycle in the insect vectors. Tsetse flies of both sexes become infected with trypanosomes when they ingest blood from infected mammalian hosts that contains trypomastigotes, the form of the parasite that circulates in the bloodstream. There are two forms of circulating trypomastigotes: long, slender organisms that are capable

[a]All material in this chapter is in the public domain, with the exception of borrowed figures.

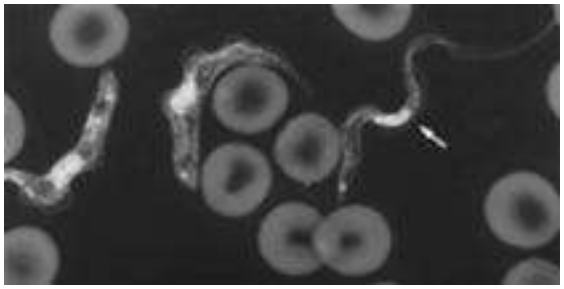

FIG. 277.1 ***Trypanosoma brucei rhodesiense*** **trypomastigotes in rat blood.** The parasite indicated by the arrow is typical of the long, slender forms capable of multiplying in the mammalian host. The other two organisms represent the stumpy, nondividing forms infective for the insect vector (Giemsa stain, ×1250). *(Courtesy Dr. G.A. Cook, Madison, WI.)*

of dividing and short, stumpy forms thought to be nondividing parasites that are infective for the insect vectors (Fig. 277.1). Once in the midgut of the tsetse flies, stumpy trypomastigotes transform into relatively long, slender procyclic trypomastigotes. After many cycles of multiplication the procyclic forms migrate to the salivary glands, where they differentiate into epimastigotes and continue to multiply. A final transformation occurs as the epimastigotes become nondividing metacyclic trypomastigotes. Transmission takes place when these infective forms are inoculated during a subsequent blood meal. The cycle is completed when the injected metacyclic forms become bloodstream trypomastigotes and begin to multiply in the blood or other extracellular spaces. The capacity of African trypanosomes to multiply in the bloodstream of their mammalian hosts, where they are continually exposed to humoral defenses, constitutes a fundamental difference between the agents of sleeping sickness and *Trypanosoma cruzi,* the cause of Chagas disease in the Americas. The African trypanosomes are able to evade immune destruction indefinitely because they undergo antigenic variation, a process in which they periodically change the antigenic structure of the coat of glycoproteins that covers the surface of the parasite. The molecular mechanisms that control this complex process have been studied intensively.[5–7] When epimastigotes transform into metacyclic trypomastigotes in the salivary glands of the tsetse fly, each parasite synthesizes a surface coat made up of one of about a dozen types of antigenic glycoproteins, called variant antigen types (VATs). Presumably, this occurs as a preadaptation to the relatively hostile environment of the mammalian host into which the metacyclics must be inoculated if they are to survive. After injection into a mammalian host, the parasites express metacyclic VATs for approximately 5 days, after which they switch to the expression of bloodstream VATs. Over time, the host sequentially mounts specific humoral responses directed against the predominantly expressed VATs. The population of parasites survives because an intrinsic rate of VAT switching provides an apparently endless supply of parasites that have surface glycoprotein coats to which the host has not been exposed previously.

Virtually all transmission of African trypanosomes to both wild and domestic animals, as well as to humans, takes place in the cyclic fashion just described. There is no evidence that these parasites can be transmitted by insects other than tsetse flies, and mechanical transmission by vectors is not important, although it may occur occasionally. Congenital transmission can occur, but in humans it is extremely rare,[8,9] as is transmission by blood transfusion. A small number of laboratory accidents resulting in infection with African trypanosomes have been reported.[10]

PATHOGENESIS AND PATHOLOGY

The pathogenesis of African sleeping sickness is complex, and many aspects of the process are poorly understood.[11–13] The first sign of infection with African trypanosomes can be the acute inflammatory lesion (trypanosomal chancre) that appears a week or so after the bite of an infected tsetse fly and resolves spontaneously over several weeks. Interstitial multiplication of the trypanosomes takes place within the chancre, and there is an intense mononuclear cell reaction to the parasites, as well as edema and local tissue destruction.

After this initial local response the infection evolves over weeks and months into a systemic hemolymphatic illness as the parasites disseminate widely through the lymphatic system and the bloodstream. Systemic African trypanosomiasis without central nervous system (CNS) involvement is generally referred to as stage 1 disease. The parasites first travel from the site of inoculation to regional lymph nodes, where they proliferate and cause an inflammatory response. They then move through the lymphatics into the bloodstream, where multiplication continues. Egress of trypanosomes from vessels into interstitial spaces, where multiplication also takes place, is thought to be facilitated by increased vascular permeability.

In stage 1 trypanosomiasis there is widespread lymphadenopathy and histiocytic proliferation, which may be followed by fibrosis. Morular cells (Mott cells) are also often present in tissue. These cells are plasmacytes with vacuolated cytoplasm and pyknotic nuclei that are thought to play a role in the production of immunoglobulin M (IgM).[14] The spleen may be enlarged, with generalized cellular proliferation, congestion, and focal necrosis. As the disease evolves, an endarteritis with perivascular infiltration of both parasites and lymphocytes may develop in lymph nodes and the spleen.

The heart is frequently involved in this stage of the disease, especially with *T. b. rhodesiense* infections. A pancarditis may develop involving all layers of the heart, including the mural and valvular endocardia.[15] The conduction system may also be affected, and involvement of the autonomic innervation of the heart has also been reported.[16] At the cellular level, pathologic changes include intense mononuclear infiltration consisting of lymphocytes, plasmacytes, and morular cells. As the infection progresses, myocytolysis and fibrosis may develop.

A number of hematologic manifestations accompany the development of stage 1 disease. Normocytic anemia is a regular feature in this phase of the illness and is usually accompanied by a brisk reticulocytosis. Several factors are thought to contribute to the anemia, and immune-mediated hemolysis may be important. Platelet counts are often reduced, especially in infections with *T. b. rhodesiense,* and disseminated intravascular coagulation before and during therapy has also been described. A moderate degree of leukocytosis is usually present, especially in the early months of the infection, and this is accompanied by polyclonal B-cell activation. High titers of immunoglobulins are a striking and constant feature of the illness. They consist primarily of polyclonal IgM that, for the most part, is not directed against specific parasite antigens. A number of other factors, including heterophile antibodies, rheumatoid factor, and anti-DNA antibodies, are often detectable. In addition, high levels of circulating antigen-antibody complexes are uniformly present, and these may play a role in the anemia, tissue damage, and increased vascular permeability that facilitate the dissemination of the parasites. Erythrocyte sedimentation rates are elevated, and hypocomplementemia has also been noted.

Stage 2 African trypanosomiasis involves invasion of the CNS. Parasites reach the brain and meninges via the bloodstream and cause meningoencephalitis or meningomyelitis, or both.[17–19] In the brain they are found mainly in the frontal lobes, pons, and medulla, but other areas may be parasitized as well. Edema and hemorrhages may be evident on gross examination of affected areas at autopsy. Trypanosomes are present in perivascular areas, and nests of organisms can be found without apparent relation to blood vessels. The presence of parasites in the CNS is associated with infiltration of mononuclear cells that are predominantly lymphocytes, plasmacytes, and morular cells. The presence of parasites in the CNS is heralded by abnormal findings in the cerebrospinal fluid (CSF). The CSF may be under increased pressure, and the total protein concentration is elevated, with mononuclear cells predominating in addition to small numbers of morular cells and eosinophils. Trypanosomes are frequently present in the CSF as well. A model of *T. brucei* infection in rats has been developed that differs from a standard view of disease and highlights the prominent role of penetration and residence of parasites in the pia mater, an immune-privileged site.[20] According to this model, the production of prostaglandin D_2 may influence the development of sleeping sickness, and brain parenchymal disease proceeds from this chronic infection. The authors suggest that

a better understanding of the physiology of disease might influence assumptions about the pharmacokinetic characteristics of drugs in development.

EPIDEMIOLOGY

HAT, which is limited to sub-Saharan Africa, was a much greater problem in the past than it is at present.[21] Hundreds of thousands of people died in major epidemics around the beginning of the 20th century and between 1920 and 1948. Between World War II and independence, the colonial powers invested heavily in controlling HAT and succeeded to the point where the disease was almost eliminated. In the decades after independence, however, in the context of continuing poverty and civil strife in many areas, control programs were neglected and HAT underwent a resurgence that reached a peak in the late 1990s. Angola, Uganda, South Sudan, and the Democratic Republic of Congo were particularly affected, and even today 80% of reported cases occur in the latter. Since then, efforts of the World Health Organization (WHO), governments of the endemic countries, and nongovernmental organizations have resulted in effective control in many affected areas, so much so that the annual number of reported cases decreased to 9878 in 2009 and to 2184 by 2016, which is roughly equivalent to the low point reached in the early 1960s. Although underreporting is a persistent problem, there is no doubt, to the credit of the many organizations involved in these efforts, that the level of control reached to date is impressive.[22] In 2012 a panel of experts convened by WHO to review the then-current situation and developed a vision for the elimination of HAT.[23,24] The current view at WHO is that efforts are on track for achieving, by 2020, the elimination of HAT as a public health problem, which is defined as fewer than 1000 reported cases per year.[25]

West African *(gambiense)* trypanosomiasis and East African *(rhodesiense)* trypanosomiasis are epidemiologically distinct diseases. The general geographic distributions of these two illnesses are presented in Fig. 276.1 in Chapter 276, and the remaining foci where transmission is known to occur are found in the indicated areas. Distinguishing epidemiologic and clinical features of the two diseases are presented in Table 277.1.

West African Trypanosomiasis

West African trypanosomiasis is caused by *T. b. gambiense,* which is transmitted primarily by tsetse flies belonging to the *palpalis* group: *Glossina palpalis, Glossina tachinoides,* and *Glossina fuscipes*. These vectors inhabit forests and wooded areas along rivers, where conditions of temperature, moisture, and darkness favorable for them are combined with the availability of mammalian blood. This distribution of the vectors restricts the occurrence of human infection to the tropical rain forests of Central and West Africa. Despite the facts that these tsetse flies adapt to feeding on a variety of mammals and *T. b. gambiense* has been found in several wild animal species, infected humans apparently constitute the only epidemiologically important reservoir of this subspecies. The primary determinant of the risk of acquiring the infection is the frequency of contact with the vector. This risk increases during the dry season, when the density of both vectors and humans increases around limited numbers of water holes. Because of this pattern of transmission, West African trypanosomiasis is primarily a problem in rural populations, and tourists rarely become infected with *T. b. gambiense.* The course of the illness caused by *T. b. gambiense* is less severe than that caused by *T. b. rhodesiense,* although both forms almost always lead to death if not treated.[26,27] In fact, many persons infected with *T. b. gambiense* are asymptomatic for long periods and continue to have contact with the vectors. This may be an important element in the persistence of the infection in the reservoir between epidemics.

TABLE 277.1 Comparisons of West African and East African Trypanosomiasis

	WEST AFRICAN *(GAMBIENSE)*	EAST AFRICAN *(RHODESIENSE)*
Organism	*Trypanosoma brucei gambiense*	*Trypanosoma brucei rhodesiense*
Vectors	Tsetse flies (*palpalis* group)	Tsetse flies (*morsitans* group)
Primary reservoir	Humans	Antelope and cattle
Human illness	Chronic (late CNS disease)	Acute (early CNS disease)
Duration of illness	Months to years	<9 mo
Lymphadenopathy	Prominent	Minimal
Parasitemia	Low	High
Epidemiology	Rural populations	Tourists in game parks; workers in wild areas; rural populations

CNS, Central nervous system.

East African Trypanosomiasis

The etiologic agent of East African trypanosomiasis is *T. b. rhodesiense.* This subspecies is transmitted by tsetse flies of the *morsitans* group, principally *Glossina morsitans, Glossina pallidipes,* and *Glossina swynnertoni.* These vectors are widely distributed in savanna and woodland areas of Central and East Africa. Wild animals are the reservoir of this organism, principally antelope such as the bushbuck and hartebeest. These animals are trypanotolerant and generally do not suffer significant morbidity unless weakened by other illnesses. Cattle are the only domestic animals that can serve as a reservoir of *T. b. rhodesiense,* and infection with the parasite usually causes death if left untreated. Many other wild and domestic animals can be infected with these parasites, but their importance as reservoirs is minimal because parasitemias are either low or they succumb quickly to the infection. The presence of the reservoir of *T. b. rhodesiense* and other trypanosome species in wild game in vast areas of Africa makes opening these lands for livestock difficult. Humans become infected with *T. b. rhodesiense* only incidentally because for the most part risk results from contact with tsetse flies that principally feed on wild animals. Thus the illness is an occupational hazard for persons such as game wardens who work in areas where infected wild animals and vectors are present. In addition, sporadic cases of *T. b. rhodesiense* infection occur among non-African tourists who visit game parks in East Africa.

The natural cycle of the African trypanosomes does not exist outside Africa, and HAT in the United States and other nonendemic countries is limited to occasional imported cases, most of which are caused by *T. b. rhodesiense.*[28,29] During the past 25 years, roughly two dozen cases of imported African trypanosomiasis have been reported to the Centers for Disease Control and Prevention (CDC), several of whom had CNS involvement.

CLINICAL COURSE

West African Trypanosomiasis

An indurated, painful trypanosomal chancre may develop at the site where parasites were inoculated by an infected tsetse fly. This lesion usually appears 1 to 2 weeks after the bite of the infected fly and resolves spontaneously over several weeks. The chancre may ulcerate and reach a diameter of several centimeters; regional lymphadenopathy may also develop. However, the trypanosomal chancre is seldom seen in clinical practice. Thus most patients develop systemic trypanosomiasis without experiencing the symptoms of localized disease.

The development of stage 1 (hemolymphatic) disease with dissemination of the parasites is marked by fever, which may appear weeks or months after the acquisition of the infection. The fever is characterized by intermittent bouts of high temperatures lasting for several days, and extended periods may intervene during which the patient is afebrile. As the chronic illness evolves, a wide variety of other signs and symptoms develop. Lymphadenopathy is a fairly constant feature of *gambiense* trypanosomiasis. The nodes are typically discrete, movable, rubbery, and nontender. With time they frequently become indurated as fibrosis develops. Supraclavicular and cervical nodes are often visibly discernible, and enlargement of the nodes of the posterior cervical triangle, or Winterbottom sign, is a classic finding in persons infected with *T. b. gambiense*. Hepatosplenomegaly may be present as well.

Transient edema is a frequent sign during the hemolymphatic phase of the illness and can occur in the face, as well as in the hands, feet,

and other periarticular areas. Pruritus is common, and an irregular circinate rash is often present. The rash is typically located on the trunk, shoulders, buttocks, and thighs and consists of erythematous areas 5 to 10 cm in diameter with clear centers.[30] Other inconstant findings include malaise, headache, weakness, weight loss, arthralgias, and tachycardia.

Stage 2 (meningoencephalitic) disease is characterized by the insidious development of protean neurologic manifestations, accompanied by progressive alterations in the composition of the CSF.[17,18] In *gambiense* trypanosomiasis CNS findings may develop months or even years after the initiation of the infection. Irritability, personality change, and loss of the ability to concentrate may develop before changes in the CSF become evident, and this underscores the somewhat arbitrary nature of the distinction between the hemolymphatic and CNS stages of the illness. A picture of progressive indifference develops, associated with daytime somnolence, sometimes alternating with restlessness and insomnia at night. Severe headache is common. The frequency and progressive nature of the somnolence result in the use of the term *sleeping sickness*. A listless gaze reflects a loss of spontaneity, and speech may become indistinct. Extrapyramidal signs often develop and may include choreiform movements of the trunk, neck, and extremities; tremors of the tongue and fingers; and fasciculations of a variety of muscle groups. Ataxia is a frequent sign, and the patient may appear to have Parkinson disease, as a shuffling gait, hypertonia, tremors, and slurred speech develop. The final phase of the CNS disease is one of progressive neurologic impairment ending in coma and death. During the weeks and months of stage 2 disease, patients often develop signs and symptoms of hypothyroidism and adrenal insufficiency, but in general these diagnoses are not supported by laboratory data.[31,32]

Trypanosomiasis in children, which is relatively uncommon because they have less exposure to the vectors, does not differ greatly from the clinical illness seen in adults. However, the illness tends to run a more acute course, and the distinction between the hemolymphatic and CNS stages may be difficult to make.[17,33,34] Moreover, due to the protean nature of the symptoms and the lack of pathognomonic signs, the diagnosis is often missed in the early stages of the infection and is made only after neurologic impairment has developed.[35]

East African Trypanosomiasis

The most striking general difference between West African and East African trypanosomiases is that the latter illness tends to follow a more acute course, presumably reflecting a relatively less effective adaptation of *T. b. rhodesiense* to humans.[17,36,37] The onset of symptoms usually occurs a few days after the patient has been bitten by an infected tsetse fly, but the incubation period may be as long as several weeks. Typically in tourists, systemic signs of infection such as fever, malaise, and headache appear before the end of the trip or shortly after their return home. As the illness progresses, the pattern of intermittent fever develops, and rash is a nearly constant feature of the early weeks of the illness. Lymph node swelling is not prominent in *rhodesiense* trypanosomiasis, and thus the Winterbottom sign is generally absent. Persistent tachycardia unrelated to the fevers is frequently present early in the course of the illness, and in some patients death may result from arrhythmias and congestive heart failure due to pancarditis even before CNS disease develops. In general, untreated *rhodesiense* trypanosomiasis usually leads to death in a matter of weeks to months, often without a clear distinction between the hemolymphatic and CNS stages.

DIAGNOSIS

Epidemiologic information and clinical findings often combine to suggest the diagnosis of African trypanosomiasis, and a high index of suspicion should be maintained with persons who have been in endemic areas. However, there are numerous other illnesses common in the tropics that cause symptoms similar to those seen in both the early and late stages of sleeping sickness, and a definitive diagnosis of African trypanosomiasis requires demonstration of the parasite.[18,38,39]

If a chancre is present, fluid should be expressed and examined directly under light microscopy for the highly motile trypanosomes. Part of the specimen should be fixed and stained with Giemsa. Aspiration of soft lymph nodes early in the course of the infection can also be used to demonstrate the presence of parasites. This method is more effective in patients with West African trypanosomiasis because of the prominence of lymphadenopathy, but even in such patients, multiple aspirates are sometimes necessary before parasites are found. An enlarged node should be punctured and kneaded gently during aspiration, and the sample obtained should be examined directly and also after staining.

Examination of wet preparations and Giemsa-stained thin and thick smears of peripheral blood is also a sensitive method for detection of infection with African trypanosomes (see Fig. 277.1). This approach is more likely to be successful in the hemolymphatic stage of the illness, and it is much more useful in patients infected with *T. b. rhodesiense* because of the relatively high parasitemias. Because parasitemias may vary considerably from one day to the next, serial specimens should be examined. If parasites are not seen in blood from a patient whose history and clinical findings point to African trypanosomiasis as a possible diagnosis, efforts should be made to concentrate the organisms. This can be done by microscopic examination of buffy coat obtained by centrifuging 10 to 15 mL of anticoagulated blood as a wet preparation and after Giemsa staining. Miniature anion exchange columns, which retain blood cells but not trypanosomes, can also be useful in detecting parasites,[40] and refinements of these approaches are being developed.[41]

Considerable work has been done on the development of polymerase chain reaction (PCR)-based assays for the detection of African trypanosomes, and more than a dozen articles have been published on the subject.[42] Although these studies have shown clearly that PCR-based assays can have high levels of sensitivity and specificity, the lack of technical facilities in many of the areas of active transmission of the parasites makes implementation in the field difficult. Loop-mediated isothermal amplification of DNA (LAMP) methodology has also been applied to the development of assays for HAT diagnosis, but it too will require further work before it can be applied broadly in field conditions.[43,44]

Examination of the CSF is mandatory in all patients suspected of having African trypanosomiasis.[12] An increase in the CSF cell count is the first abnormality to be detected. Increased opening pressure of the fluid develops later, as do an elevated IgM level and total protein concentration. Examination of CSF processed by single or double centrifugation methods often reveals trypanosomes in patients with CNS involvement.[45] Any CSF abnormality in a patient in whom trypanosomes have been found in specimens from other sites must be viewed as indicative of CNS involvement, and this has implications for treatment that are discussed later. CSF cell counts and IgM levels may remain elevated for long periods after curative therapy.

Accurate diagnostic staging of HAT is crucial because treatment for stage 2 disease can be far more toxic than that given for stage 1 illness. WHO criteria for stage 2 disease are the presence of trypanosomes in the CSF or a whole blood cell count (WBC) greater than 5 cells/μL. The latter criterion is controversial, however, and some experts have recommended that a WBC greater than 10 cells/μL be taken as indicative of stage 2 disease.[18] This controversy has driven a search for other biomarkers that would be more accurate indicators of stage 2 disease, but to date none of the parameters investigated have found their way into use, in large measure because there is no gold standard for stage 2 disease that can serve as a benchmark for determining the accuracy of new methods.[46–48]

An additional approach to patients in whom parasites cannot be demonstrated by the previously described methods is bone marrow aspiration. Trypanosomes may be found by careful examination of Giemsa-stained specimens. Moreover, material aspirated from the bone marrow can be inoculated into special liquid culture medium, as can blood, CSF, or lymph node aspirates obtained from the patient in whom trypanosomiasis is suspected.[49] Simple agglutination tests for trypanosomes performed on cards (card agglutination trypanosomosis test [CATT])[38,39] are available commercially and are easy to use under field conditions. These card assays are the cornerstone for screening populations at risk, after which parasitologic studies are done on persons having positive results. Immune trypanolysis, a test with high positive and negative predictive values for infection with *T. b. gambiense*, detects specific antibodies by measuring the lysis of trypanosomes mixed with patient serum, but it is performed only in reference centers. Commercial rapid diagnostic tests (RDTs) are now used for the diagnosis of *T. b. gambiense,* and have the advantage of not requiring a cold chain, but

they are not appropriate for large-scale screening. HAT Sero-K-Set (Coris BioConcept; Gembloux, Belgium) and SD Bioline HAT (SD Diagnostics, Giheung-gu, South Korea) have been compared, with immune trypanolysis as a gold standard. No significant difference between RDTs was found, and both of the tests have acceptable sensitivity, but specificity has not been sufficient.[50] Thus the RDTs performed in fixed health centers require confirmation, either parasitologically or with trypanolysis. Cost concerns with RDTs remain paramount, as they would presumably increase health care costs in areas of high prevalence.

THERAPY

The treatment of HAT is complex, and currently recommended protocols have been reviewed in detail elsewhere.[18,51] Assessment of the efficacy of treatment of HAT can be difficult and prolonged, and this complicates the development of new drugs.[52] Suramin, pentamidine, and organic arsenicals have been the mainstay for treating African trypanosomiasis for more than 50 years. Eflornithine (difluoromethylornithine [DFMO], Ornidyl; Sanofi, Bridgewater, NJ), which is effective in both the hemolymphatic and CNS stages of West African trypanosomiasis, was added to the group in 1990. Therapy of *gambiense* and *rhodesiense* trypanosomiases must be individualized based on the absence or presence of CNS disease, side effects, and occasionally drug resistance[53,54] of the infecting organisms. Suramin and pentamidine do not penetrate the CNS adequately. Thus eflornithine should be used in patients with *gambiense* trypanosomiasis who have CNS disease, but because of its relative lack of activity against *T. b. rhodesiense,*[55,56] patients with stage 2 *rhodesiense* trypanosomiasis must be treated with the arsenical melarsoprol, which is highly toxic (Table 277.2). In the United States these drugs can be obtained from the Drug Service of the CDC.

Patients with the hemolymphatic stage of *gambiense* trypanosomiasis and normal CSF (stage 1 disease) should be treated with pentamidine isethionate (Lomidine).[57] The dosage for both adults and children is 4 mg/kg, up to 300 mg/day, intravenous (IV) or intramuscular (IM) for 10 days. IM injections of pentamidine are painful and may cause sterile abscesses. Immediate side effects of pentamidine can include nausea, vomiting, hypotension, and tachycardia. These reactions are generally transient and do not warrant discontinuation of therapy. Other side effects include nephrotoxicity, elevation of hepatic transaminases, neutropenia, rashes, and hypoglycemia.

Eflornithine is highly effective in both the hemolymphatic and CNS stages of *gambiense* trypanosomiasis.[58] This drug produces a dramatic reduction of symptoms and rapid clearing of parasites from blood and CSF. For adults the recommended dosage is 400 mg/kg per day IV in four divided doses for 14 days, and for children it is 500 to 600 mg/kg per day on the same schedule. Anemia, leukopenia, and thrombocytopenia are frequent in patients treated with eflornithine, but generally they are not clinically significant. Seizures and hearing loss have been reported rarely. Major disadvantages of eflornithine are the requirement that it be given IV, the amount of drug that must be given, and the duration of therapy. These factors make widespread use difficult, leaving pentamidine as the better choice for stage 1 *gambiense* disease. For patients with *gambiense* disease and CNS involvement, until recently eflornithine has been the drug of choice because it is as effective as melarsoprol but has far fewer side effects. However, recent field trials of nifurtimox (Bay 2502 and Lampit; Bayer, Leverkusen, Germany) in combination with eflornithine (nifurtimox-eflornithine combination treatment, or NECT) for stage 2 *T. b. gambiense* disease have been carried out.[59,60] NECT is given as eflornithine 200 mg/kg every 12 hours IV for 7 days plus nifurtimox 15 mg/kg/day orally in three divided doses for 10 days.

TABLE 277.2 Drugs Recommended for Treatment of the African Trypanosomiases

	CLINICAL STAGE	
CAUSATIVE AGENT	**1**	**2**
Trypanosoma brucei gambiense	Pentamidine Alternative: suramin	Eflornithine Alternatives: eflornithine/nifurtimox; melarsoprol
Trypanosoma brucei rhodesiense	Suramin	Melarsoprol

The safety and efficacy of the combined regimen have been amply demonstrated,[61–63] and in comparison with eflornithine monotherapy, NECT is markedly less complicated to administer and costs less overall. Thus, if available, NECT is the recommended treatment for stage 2 *T. b. gambiense* disease. The extent to which human immunodeficiency virus coinfection affects the course of African trypanosomiasis and the efficacy of treatment is not clear and merits further investigation.[64,65]

Suramin (Bayer 205, Naphuride, Antrypol; Bayer, Leverkusen, Germany) is the first-choice drug for stage 1 *rhodesiense* trypanosomiasis. A 100- to 200-mg test dose is recommended, although anaphylactic reactions are rare. The treatment regimen for both adults and children, beginning 24 hours after the test dose, is 5 mg/kg on day 1, 10 mg/kg on day 3, and 20 mg/kg on days 5, 11, 17, 23, and 30 (1 g/day max). The exact spacing of the doses most likely is not important because suramin has a long half-life. The drug is administered by slow IV infusion of a freshly prepared 10% aqueous solution. Suramin causes a number of side effects and must be administered under the supervision of a physician knowledgeable on its use. Approximately 1 patient in 20,000 has an immediate, severe, and potentially fatal reaction to the drug consisting of nausea, vomiting, seizures, and shock. A number of less severe reactions can also occur, including fever, pruritus, photophobia, arthralgias, and skin eruptions. The most important side effect of suramin is renal damage. Transient proteinuria is often seen during treatment. Urinalysis should be done before each dose, and if proteinuria increases or casts and red cells appear in the urine sediment, the drug should be discontinued. Suramin should not be used in patients with renal insufficiency.

The drug of choice for *rhodesiense* trypanosomiasis with CNS involvement is the arsenical melarsoprol (mel B, Melarsen-BAL, Arsobal).[66–69] Melarsoprol cures both stages of the disease. Thus it is also indicated for treatment of the hemolymphatic stage in patients in whom suramin or pentamidine has failed or could not be tolerated. However, it should never be the first choice for therapy of stage 1 trypanosomiasis because of its relatively high toxicity. The "short course" of melarsoprol currently recommended has been shown to be noninferior to the previous treatment course for *T. b. rhodesiense,*[70,71] which was administered over several weeks and was more toxic. The short-course regimen is 10 daily doses of 2.2 mg/kg IV given with prednisolone 1 mg/kg. Melarsoprol is a highly toxic drug and should be administered with great care. The most important side effects involve the CNS. A substantial percentage of patients treated with melarsoprol develop reactive encephalopathy. The risk of this immune-mediated phenomenon and its associated mortality is reduced significantly by concomitant administration of prednisolone. Thus all patients treated with melarsoprol should be given prednisolone at a dose of 1 mg/kg up to 40 mg per day, starting a day or two before initiation of melarsoprol therapy, continued through the period of treatment, and then tapered over several days.[72] Clinical indications of reactive encephalopathy include high fever, headache, tremor, impaired speech, seizures, and finally coma and death. Melarsoprol should be discontinued at the first sign of encephalopathy. It may be restarted cautiously with smaller doses a few days after the signs have resolved.

A number of other side effects are associated with melarsoprol therapy. Extravasation of the drug results in intense local reactions and, as with administration of other heavy metals, abdominal pain and vomiting are commonly observed. Jarisch-Herxheimer-type reactions have been reported, as have nephrotoxicity, abnormal liver function tests, and myocardial damage.

If a patient with *gambiense* CNS disease cannot tolerate eflornithine, or if the latter is not available, melarsoprol should be given at the dose listed earlier. This compressed regimen for stage 2 *gambiense* disease has been shown in a randomized trial to be equally effective and no more toxic than the traditional regimen outlined previously.[70]

Finally, the oral drug fexinidazole was recently shown in a randomized phase 2/3 trial to be noninferior to NECT for the treatment of patients with stage 2 gambiense HAT.[73] If fexinidazole is approved by regulatory authorities and becomes available commercially, the fact that it is an oral drug may ultimately result in its becoming the definitive tool in

the elimination of West African HAT. Fexinidazole is discussed at some length in Chapter 42.

PREVENTION

The trypanosomiases constitute complex public health and epizootic problems in many developing countries in Africa. Control programs that focus on eradication of vectors and drug treatment of infected people have been in operation in most endemic regions for decades. As noted, enormous progress has been made to the extent that a vision for the elimination of HAT as a public health problem by 2020 has been developed. Individuals can reduce their risk of acquiring infections with trypanosomes by avoiding areas known to harbor infected insects, wearing clothing that reduces the biting of the flies, and using insect repellent. Chemoprophylaxis is not recommended because of the high toxicity of the drugs that are active against African trypanosomes, and no vaccine is available to prevent transmission of the parasites.

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278 *Toxoplasma gondii*

José G. Montoya, John C. Boothroyd, and Joseph A. Kovacs[a]

SHORT VIEW SUMMARY

Definition

- *Toxoplasma gondii* is a ubiquitous coccidian protozoan that usually causes asymptomatic infection in humans but can cause significant disease in congenitally infected infants and immunodeficient patients and occasionally in immunocompetent individuals.

Epidemiology

- Toxoplasmosis is a worldwide zoonosis that can infect a wide range of animals and birds.
- Transmission to humans is mainly by ingestion of viable tissue cysts in meat or of oocysts in food or water. Transmission can also occur via the placenta or a transplanted organ.
- Positive immunoglobulin G (IgG) representing prior infection increases with age; seroprevalence is ≈11% in the United States and up to ≈78% in other parts of the world.
- Congenital transmission occurs almost exclusively when a seronegative mother becomes infected during pregnancy but can also occur among immunosuppressed mothers who reactivate toxoplasmosis during gestation. Retinochoroiditis can occur after congenital infection or recently acquired infection. Infection in human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome patients almost always results from reactivation of latent infection. Among organ transplant patients, disease can result from either newly acquired infection from the transplanted organ or from reactivation of latent infection in the setting of hematopoietic cell transplantation. Reactivation has also been observed among patients who are immunosuppressed with the expanding armamentarium of biologics that significantly depress adaptive immunity.

Microbiology

- *Toxoplasma* organisms are exclusively intracellular. The sexual phase occurs in felines. Excreted oocysts require 1 to 5 days to become infectious. Tachyzoites actively replicate in essentially all cell types. Tissue cysts with intracystic bradyzoites maintain organism viability during latent infection.
- Tachyzoites replicate well in tissue culture and are responsible for clinical manifestations during primary infection or reactivation of latent infection.
- Multiple strains are identified by genotyping. Strains differ in virulence, with the most virulent strains so far reported being found in Latin America.

Diagnosis

- Direct detection of the organism is by polymerase chain reaction (PCR) assays, histopathology with immunoperoxidase staining, or, rarely, by tissue culture or mouse inoculation.
- Serologic assays can help distinguish acute from chronic infection and can identify patients at risk for reactivation. Immunoglobulin M (IgM)-positive test results should be confirmed at a reference laboratory (e.g., the Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory [PAMF-TSL]; www.pamf.org/serology/; 650-853-4828).
- Maternal infection is often asymptomatic; serology shows acute infection, and IgM-positive test results must be confirmed at a reference laboratory. Congenital infection may be asymptomatic or appear with neurologic or ocular manifestations; this form is diagnosed in utero by PCR of amniotic fluid or after birth by serology or PCR.
- Chorioretinitis may be asymptomatic or show visual loss; ophthalmologic examination can reveal typical lesions, and PCR of vitreous or aqueous fluid should be considered in cases with atypical lesions or inadequate response to therapy. The Goldmann-Witmer coefficient (anti-*Toxoplasma* IgG/total IgG in aqueous fluid divided by anti-*Toxoplasma* IgG/total IgG in serum) can be helpful.
- HIV-infected patients usually present with focal neurologic symptoms. Patients are usually IgG positive and IgM negative, computed tomography or magnetic resonance imaging may show one or more contrast-enhancing lesions, cerebrospinal fluid PCR is specific but not sensitive, brain biopsy sensitivity is improved by immunoperoxidase staining, and diagnosis is often presumptive and extends to a response to empirical therapy.
- Immunodeficient patients present with encephalopathy, seizures, pneumonia, and fever. Diagnosis is based on positive PCR or histopathology. Hematopoietic stem cell transplantation (HSCT) requires pretransplant serology in the recipient; solid-organ transplantation requires pretransplant serology in the donor and recipient.

Therapy

- Immunocompetent patients: These patients usually require no therapy if asymptomatic; they may benefit from treatment if symptoms are severe or persist.
- Immunocompromised patients: The therapy of choice is pyrimethamine (200-mg load, then 50–75 mg/day) plus sulfadiazine (1000–1500 mg every 6 hours) plus leucovorin (10–20 mg/day). The dosage is then decreased to maintenance dosing of pyrimethamine (25–50 mg/day) plus sulfadiazine (500–1000 mg every 6 hours) plus leucovorin (10–20 mg/day) after 3 to 6 weeks if a clinical response occurs. Alternatives include pyrimethamine, as above, plus leucovorin plus either clindamycin (intravenous [IV] or oral [PO]; 600 mg every 6 hours) or atovaquone (1500 mg every 12 hours). Other alternatives include trimethoprim-sulfamethoxazole (TMP-SMX) (IV or PO; 5 mg/kg of TMP every 12 hours) or sulfadiazine plus atovaquone. Corticosteroids are given only for clinically significant edema or mass effect, and anticonvulsants are given only after a seizure.
- HIV patients: Start antiretroviral therapy (ART) after 2 to 3 weeks. Consider stopping anti-*Toxoplasma* medications if the CD4 count is >200 cells/mm^3 for more than 6 months. High relapse rate occurs without ART and maintenance therapy.
- Acute infection in pregnant women less than 14 weeks of gestation: Give spiramycin (1 g every 8 hours; available at no cost through the PAMF-TSL and the US Food and Drug Administration) until delivery. If infection in the fetus is documented or suspected, or if maternal infection at 14 weeks of gestation or later, give pyrimethamine (50 mg every 12 hours for 2 days, then 50 mg/day) plus sulfadiazine (initial dose 75 mg/kg, followed by 50 mg/kg every 12 hours; maximum, 4 g/day), plus folinic acid (10–20 mg/day). Before 14 weeks of gestation, give no pyrimethamine or leucovorin.
- Congenitally infected infant: Give pyrimethamine (1 mg/kg every 12 hours for

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SHORT VIEW SUMMARY—cont'd

2 days, then 1 mg/kg/day for 2 or 6 months); then this dose is given every Monday, Wednesday, and Friday; plus sulfadiazine (50 mg/kg every 12 hours) plus folinic acid (10 mg three times weekly) for at least 12 months.

- Chorioretinitis patients: If therapy is clinically indicated, give pyrimethamine (100-mg loading dose over 24 hours, then 25–50 mg/day) plus sulfadiazine (1 g every 6 hours) plus leucovorin (10–20 mg/day) for 4 to 6 weeks. TMP-SMX, one single-strength tablet every day, can prevent relapse.

Prevention and Prophylaxis

- Avoid undercooked meat and potentially contaminated food or water; clean cat litter daily.
- Immunocompromised patients: Give TMP-SMX, one double-strength or single-strength tablet daily. An alternative is dapsone (50 mg/day) plus pyrimethamine (50 mg/wk) plus leucovorin (25 mg/wk). If the patient has HIV, start if the CD4 count is less than 100 to 200 cells/mm^3; discontinue if the patient is on ART and the CD4 count is greater than 200 cells/mm^3 for at least 3 months (primary prophylaxis) or 6 months (chronic maintenance). For *Toxoplasma* seropositive HSCT recipients, start TMP-SMX after engraftment and/or consider preemptive strategy with weekly peripheral blood *Toxoplasma* PCR until posttransplantion day +100. If the patient is a solid-organ transplant recipient, start at transplantation if classified as seropositive donor (D^+) or seropositive recipient (R^+).

Although *Toxoplasma gondii* infects a large proportion of the world's human population, it is an uncommon cause of disease. Certain individuals, however, are at high risk for severe or life-threatening disease because of this parasite. These include congenitally infected fetuses and newborns and immunologically impaired individuals. Congenital toxoplasmosis is the result of maternal infection acquired during gestation, an infection that is most often clinically inapparent. In immunodeficient patients toxoplasmosis most often occurs in persons with significant defects in T-cell and/or B-cell–mediated immunity, such as those receiving corticosteroids, anti–tumor necrosis factor (TNF) therapies, certain monoclonal antibodies, or cytotoxic drugs, and in those with hematologic malignancies, organ transplants, or acquired immunodeficiency syndrome (AIDS). In the vast majority of otherwise immunocompetent individuals, primary or chronic (latent) infection with *T. gondii* is asymptomatic; after the acute infection a small percentage suffer chorioretinitis, lymphadenitis, mononucleosis-like syndrome, flulike symptoms, or, even more rarely, hepatitis, pneumonia, brain abscesses, myocarditis, and polymyositis.[2]

T. gondii was first observed in the North African rodent *Ctenodactylus gundi* by Nicolle and Manceaux in 1908[2] and was recognized as a cause of human disease in an 11-month-old congenitally infected child by Janku[3] in 1923. It was reported as a cause of encephalitis by Wolf, Cowen, and Paige,[4] who in 1939 observed it in a newborn who presented with seizures, intracranial calcifications, hydrocephalus, and chorioretinitis.

Although relatively few cases of severe toxoplasmosis in adults were reported during the ensuing years, the remarkable report in 1968 by Vietzke and his colleagues,[5] from the National Cancer Institute of the National Institutes of Health, highlighted *T. gondii* as a cause of life-threatening infection in patients with malignancy, predominantly in those with hematologic malignancies. Brain involvement with focal areas of encephalitis was the primary finding at autopsy in these patients. Since that time, several hundred cases in non-AIDS immunodeficient patients have been recorded in the literature.[6] In 1983 the first report of toxoplasmosis in AIDS patients appeared.[7] Toxoplasmic encephalitis (TE) subsequently was recognized as the major cause of space-occupying lesions in the brains of these patients, almost all of whom had serologic evidence of prior exposure to the parasite.[7] Between 2003–12 more than 6000 estimated cases of toxoplasmosis occurred in the United States, based on International Classification of Disease(s)–9 coding of privately insured patients: 38% with eye disease, 5% with meningoencephalitis, and 45% with unspecified toxoplasmosis.[8] Despite the significant advances that have been achieved in the recent past, major challenges remain in the areas of prevention and management of the acute infection in pregnancy, the fetus, and the newborn[9]; in understanding the correlation between parasite strains and geographic origin versus disease outcomes[10]; immune responses in humans[11]; contribution of the chronic infection to human behavior and psychiatric and other disorders[12,13]; and in the understanding and treatment of toxoplasmic chorioretinitis[14] and infection in immunocompromised individuals.[1,6,15]

ETIOLOGY

T. gondii is a coccidian parasite of felids, with humans and other warm-blooded animals serving as intermediate hosts. It belongs to the subphylum Apicomplexa, class Sporozoa, and can exist in many forms: macrogametes and microgametes, the oocyst (which releases sporozoites), the tissue cyst (which contains and may release bradyzoites), and the tachyzoite (Fig. 278.1).[16]

Population genetic analysis has demonstrated that, at least within Europe and North America, most organisms isolated from both domesticated animals and humans can be grouped into one of three clonal genotypes—types I to III—that may identify clinically relevant biologic differences.[17] Clear differences have been observed in the frequency of parasite genotypes when *T. gondii* isolates from animals were compared with those of humans. Type III strains are common in animals but observed significantly less often in cases of human toxoplasmosis; most cases in humans in Europe and North America are caused by type II strains. Type II strains are significantly more often associated with reactivation of chronic infections and accounted for 65% of strains isolated from AIDS patients.[18] Both type I and type II strains have been associated with human congenital toxoplasmosis.[18–21] Multiple genotypes have been associated with ocular disease; in Germany an unusual nonreactive serotype was associated with ocular disease and with more frequent recurrences.[22–24] Atypical and recombinant strains have been identified with increasing frequency in regions other than the United States and Europe and from animals other than humans and domestic animals; some of these strains appear to be associated with more severe disease, suggesting greater virulence, even in immunocompetent individuals.[25,26] The most exhaustive studies have now identified six major population clades,[27] although detailed sequence analysis indicates there is a varied amount of genetic exchange within and between these clades.[28,29] Hence, although the "three-dominant-strain" paradigm is holding for humans in Europe and North America, the situation appears much more complex in other regions and in other animal hosts.

ORGANISM STAGES

Oocyst

Cats eventually shed oocysts after they ingest any of the forms of the parasite, at which time an enteroepithelial cycle begins. This sexual form of reproduction begins when the parasites penetrate the epithelial cells of the small intestine and initiate development of asexual and sexual (gametogony) forms of the parasite. Oocyst wall formation begins around the fertilized gamete, and when still immature, oocysts are discharged into the intestinal lumen by rupture of intestinal epithelial cells.[16] Unsporulated oocysts are subspherical to spherical and measure 10 × 12 µm in diameter (see Fig. 278.1A). Oocysts are formed in the small intestine only in felids and are excreted in the feces for periods varying from 7 to 20 days. More than 100 million oocysts may be shed in the feces in a single day.[16] Sporulation, required for oocysts to become infectious, occurs outside the cat within 1 to 5 days, depending on temperature and the availability of oxygen. Sporulated oocysts contain two sporocysts (see Fig. 278.1A), each of which contains four sporozoites. Maturation is more rapid at warm temperatures (2–3 days at 24°C compared with 14–21 days at 11°C).[16] Oocysts may remain viable for as long as 18 months in moist soil; this results in an environmental reservoir from which incidental hosts may be infected. Recent clues to

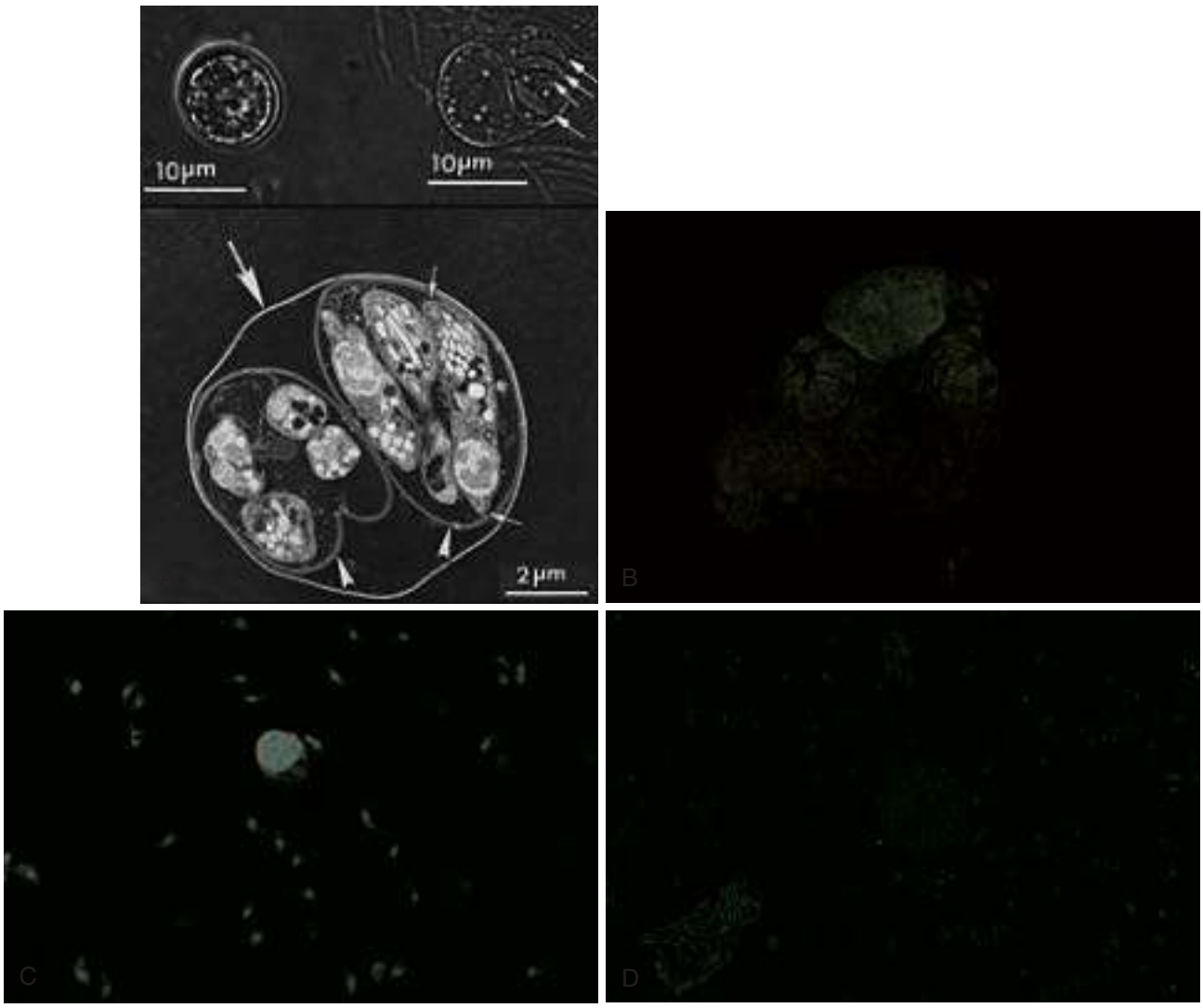

FIG. 278.1 The three forms of *Toxoplasma gondii* observed in nature. (A) Oocysts. Unsporulated oocyst *(top left).* Sporulated oocyst with two sporocysts *(top right).* Four sporozoites *(arrows)* are visible in one of the sporocysts. Transmission electron micrograph of a sporulated oocyst *(bottom).* Note the thin oocyst wall *(large arrow)*, two sporocysts *(arrowheads)*, and sporozoites, one of which is cut longitudinally. (B) Giemsa stain demonstrating two rosettes of intracellular tachyzoites in a mouse bone marrow macrophage. (C), Giemsa-stained smear of mouse peritoneal fluid demonstrating the tachyzoite form. (D) Hematoxylin and eosin stain of the cyst form in brain. *(A courtesy Dr. J.P. Dubey, U.S. Department of Agriculture. Beltsville, MD. B to D courtesy Dr. Jack S. Remington, Stanford University and Palo Alto Medical Foundation.)*

some of the features that make oocysts so robust have come from proteomic and transcriptomic analyses.[30,31] Among other findings, these studies have revealed an abundance of small tyrosine-rich proteins in the oocyst wall. Cross-linking of the tyrosines in these proteins could confer a natural "sun-screen" for the sporozoites within because they are strong absorbers of ultraviolet light.

Tachyzoite

The tachyzoite form (see Fig. 278.1B) is oval to crescentic and measures 2 to 3 μm wide and 5 to 7 μm long; it requires an intracellular habitat to multiply despite having all of the usual eukaryotic machinery necessary for reproduction. Tachyzoites are responsible for triggering immune responses leading to clinical manifestations seen in both primary and reactivated infection; presence of tachyzoites is the hallmark of active infection requiring treatment. They reside and multiply within vacuoles in their host's cells, can infect virtually all phagocytic and nonphagocytic cell types,[16] and multiply approximately every 6 to 8 hours to form rosettes.[32] Continuous multiplication leads to cell disruption and release of organisms that go on to invade nearby cells or are transported to other areas of the body by blood and lymph.[33] Tachyzoites appear to actively and rapidly migrate across epithelial cells and may traffic to distant sites while extracellular.[34] Recent evidence suggests they might also use the infected host cell as a "Trojan horse" to traffic and gain access to tissues that might not otherwise be easily accessed.[35]

At the anterior end of the tachyzoite there is a cone-shaped structure termed the *conoid*. It is protruded during the parasite's entry into host cells. *Rhoptries*, numbering 4 to 12, are club-shaped organelles that terminate within the conoid. The rhoptries, together with surrounding small, rod-shaped organelles (micronemes), have important secretory functions for parasitic invasion. *Dense granules* are organelles distributed throughout the cytoplasm. Their contents are released into a vacuole, termed the *parasitophorous vacuole*, that is formed around the parasite during entry into the cell and also into the external environment as excreted-secreted antigens.[16] Some dense granule proteins transit across the membrane surrounding the parasitophorous vacuole, eventually reaching the host nucleus, as described further.[36]

The rhoptries and micronemes produce a collection of proteins that are crucial for the invasion process.[37] These appear to mediate the attachment to the host cell, including the moving junction, a ringlike point of contact between the parasite and host cell surface that migrates down the length of the parasite during invasion. Like the dense granules, the rhoptries also introduce proteins into the host cell that are critical in manipulating the cell, presumably to the advantage of the parasite.[38] These rhoptry and dense granule proteins can be very different (polymorphic)

between different strains of *T. gondii* and appear responsible for many of the differences in virulence seen for types I, II, and III in mice, as described earlier.

A fourth organelle that is restricted to *Toxoplasma* and its Apicomplexa cousins is the apicoplast.[39] This is akin to chloroplasts of plants, with a similar evolutionary origin involving endosymbiotic algae, but photosynthetic functions have been completely lost. It has its own DNA, RNA, and protein translation, the latter of which is prokaryotic in nature, making for a very attractive target for drug therapies. Indeed, one of the currently used drugs for treatment of human infection, clindamycin, targets the ribosomes of the apicoplast.[40] A major role of this organelle is fatty-acid biosynthesis.[39] Recent work with *Plasmodium,* which also has this organelle, has demonstrated that the apicoplast is crucial to the synthesis of isoprenoids,[41] making further work on attacking this Achilles heel an attractive prospect.

Tachyzoites cannot survive desiccation, freezing and thawing, or extended exposure to gastric digestive juices.[33] They are propagated in the laboratory in the peritoneum of mice and in cultured cells. Tachyzoites can be visualized in sections stained with hematoxylin and eosin (H&E) but are better visualized with Wright-Giemsa and immunoperoxidase stains.[42] They are used as the killing target in the gold standard test for detection of *Toxoplasma* IgG.

Tissue Cyst

Once the tachyzoite has invaded the target cell, it can undergo stage conversion into the bradyzoite form.[16] Tachyzoites and bradyzoites are structurally and phenotypically different. Tachyzoites multiply rapidly and synchronously, forming rosettes and lysing the cell, whereas the more slowly replicating bradyzoites form tissue cysts.[43] Molecules are expressed in a stage-specific manner and are responsible for certain of the phenotypic differences between tachyzoites and bradyzoites. Interferon-γ (IFN-γ), nitric oxide (NO), heat shock proteins, and pH and temperature manipulations can trigger conversion of tachyzoites to bradyzoites in vitro and perhaps in vivo as well.[16]

Tissue cysts grow and remain within the host cell cytoplasm, wherein the intracystic bradyzoites continue to divide. Tissue cysts vary in size from younger ones that contain only a few bradyzoites to older tissue cysts that may contain several thousand bradyzoites and may reach more than 100 μm in size (see Fig. 278.1D). They appear spherical in the brain and conform to the shape of muscle fibers in heart and skeletal muscles. The central nervous system (CNS); eye; and skeletal, smooth, and heart muscles appear to be the most common sites of latent infection.[44] Because of their persistence in tissues in asymptomatic individuals, demonstration of tissue cysts in histologic sections does not necessarily mean that the infection was recently acquired or that it is clinically relevant. Tissue cysts stain well with periodic acid–Schiff, Wright-Giemsa, Gomori methenamine silver, and immunoperoxidase stains. Tissue cysts in meat are rendered nonviable by γ-irradiation (0.4 kGy),[45] heating meat throughout to 67°C, or freezing to –20°C for 24 hours and then thawing,[46,47] but not by gentle heating in a microwave.[48]

Although the tachyzoite form appears to be indiscriminate in the type of host cell parasitized, it has been suggested that, in brain tissue, there is a predilection for tissue cyst formation to occur predominantly within neurons.[49,50] However, it has been shown that tissue cysts can form within astrocytes cultured in vitro.[51] In an electron microscopic study of the pathologic changes in brains of infected mice, tissue cysts were observed to remain intracellular throughout the period of study (22 months).[49] There is compelling evidence to suggest that bradyzoites can exit from intact tissue cysts and invade contiguous cells, where they convert to the tachyzoite form.[52] This is the likely explanation for the appearance of "daughter" cysts or clumps of cysts in the brain. Recent evidence suggests that neurons are capable of destroying the invaded parasite and/or that rhoptry proteins are injected into cells they do not infect.[53] These results have implications for how the parasite commandeers host functions.

TRANSMISSION AND EPIDEMIOLOGY

T. gondii infection is a worldwide zoonosis. The organism infects herbivorous, omnivorous, and carnivorous animals, including birds.[54] Infection in humans most commonly occurs through the ingestion of raw or undercooked meat that contains tissue cysts, through the ingestion of water or food contaminated with oocysts, or congenitally through transplacental transmission from a mother who acquired her infection during gestation (Fig. 278.2). Less common are transmission by transplantation of an infected organ or transfusion of contaminated blood cells. Transmission has also occurred by accidental sticks with contaminated needles[55] or through exposing open lesions or mucosal surfaces to the parasite.[56] Because the sexual cycle of the parasite takes place in the small bowel of members of the cat family, cats play a significant role as powerful amplifiers of the infection in nature (see "Oocyst").[16] Epidemiologic surveys have revealed that in most areas of the world, the presence of cats is of primary importance for the transmission of the parasite. Excretion of oocysts has been reported to occur in approximately 1% of cats in diverse areas of the world.[56] Wild felines, and especially bobcats in the United States, may be among the most important sources of oocysts.[57]

Although ingestion of raw or undercooked meat that contains viable *T. gondii* tissue cysts will result in infection, the relative frequency with which this occurs in relation to the frequency of infection caused by ingestion of oocysts is unclear. For instance, in countries such as France, where eating undercooked meat is common and the prevalence of the infection is high, meat may be an important cause of the infection. (It was in Paris that the meat-to-human hypothesis of spread of *T. gondii* was proved.[58]) In contrast are countries such as those in Central and South America, where the prevalence of the infection in humans is high but the ingestion of undercooked meat is relatively less common. In these areas, oocysts may be the more important source of human infection.[59] Until a newly described method for distinguishing bradyzoite- versus oocyst-initiated infection is validated in these regions,[60] their respective contributions to human infection will largely remain a matter of speculation.

Ingestion of tissue cysts in infected meat (primarily pork and lamb) is a major source of the infection in humans in the United States.[61,62] *T. gondii* infection is common in many animals used for food, especially sheep and pigs, with a lower prevalence in cattle, horses, and water buffaloes. Organisms may survive in tissue cysts in these animals for years and can be found in nearly all edible portions of an animal.[63] A seminal study on the prevalence of *T. gondii* in samples of meat used for human consumption (obtained from grocery stores) was performed in the United States in the 1960s.[64] The parasite was isolated from 32% of pork chops and 4% of lamb chops; there were no isolations from beef, and indeed, cattle appear to be generally not an important intermediate host for this parasite.[65] A recent polymerase chain reaction (PCR)-based study in England found 33% (19/57) of pork and 67% (6/9) of lamb samples positive for *T. gondii* DNA.[66]

Serologic surveys conducted in the past 20 years in the United States indicate that the prevalence of *T. gondii* in pigs is declining, with an overall prevalence of 2.6% in a recent National Animal Health Monitoring Survey,[67] presumably due to changing management practices and consolidation of pig production into large-scale operations. However, there are still many isolated small swine farms, including those that raise organic pigs, and the prevalence of *T. gondii* in these animals can be greater than 90%.[68] Of note, meat for human consumption is not routinely inspected for *T. gondii* infection in the United States or elsewhere in the world.[69] Seroprevalence of *T. gondii* infection in a study of lambs in the mid-Atlantic region was recently reported to be 27%.[70] Although *T. gondii* infection of sheep is widely prevalent, in the United States meat from adult sheep is not usually used for human consumption.[56] Reports of suspect transmission by unpasteurized goat's milk have appeared.[71,72] In addition to differences in how meat is cooked, the tendency for beef, compared with lamb and pork, to harbor few, if any, cysts may partly explain the differences in seroprevalence in the United States versus Europe; beef accounts for a much greater fraction of meat consumed in the United States compared with Europe, where lamb and pork are more popular.

Acute infection appears to exhibit seasonality patterns in studies reported from Europe and the United States.[73] In a study from the Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory (PAMF-TSL; www.pamf.org/serology/; 650-853-4828) of 112 consecutive cases of

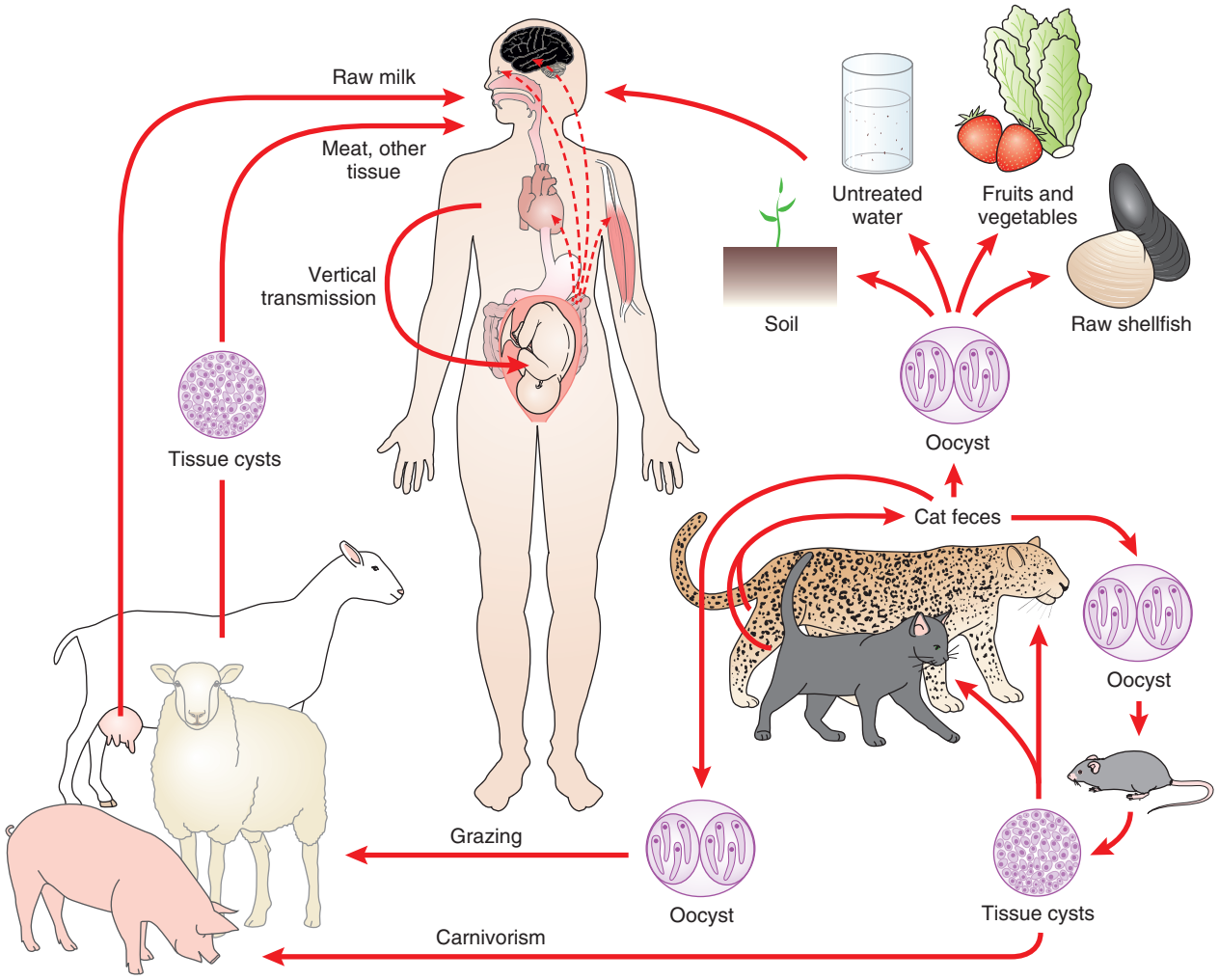

FIG. 278.2 **Transmission and life cycle of *Toxoplasma gondii*.**

acute toxoplasmic lymphadenitis (TL) in the United States, the distribution of cases was not uniform across the 12 calendar months. The highest peak of cases was in December, followed by a peak in September. Similar months were identified in patients with acute toxoplasmosis in rural areas in France.[74]

T. gondii infection is prevalent in game animals, especially black bears (80% infected) and white-tailed deer, as well as in raccoons (60% infected).[63] Thus wild animal meat can serve as a source of the infection for hunters and their families, especially when care is not taken while eviscerating and handling the game or when meat and other organs from these animals is served undercooked or uncooked.[63]

Although *T. gondii* tissue cysts may be found in edible tissues of chickens,[75] poultry products are probably not important in the transmission of *T. gondii* to humans in urban areas because they are usually frozen for storage and thoroughly cooked to avoid diseases that could be caused by contamination by other organisms.[56]

The ingestion of vegetables and other food products contaminated with oocysts probably accounts for infection in seropositive vegetarians. Although isolation of tachyzoites from secretions of people with the acute infection has been claimed, human-to-human transmission of infection by this route has not been established. Outbreaks within families and other groups are common,[76–79] but there is no evidence of natural human-to-human transmission other than from mother to fetus.

Several epidemiologic studies have identified water as a potential source for *T. gondii* infection both in humans and animals.[76,80–83] In vitro studies have demonstrated that oocysts can sporulate in seawater within 1 to 3 days, can survive in seawater for up to 6 months, and can survive in water treated with sodium hypochlorite or ozone, but not ultraviolet radiation.[84–86] Population mapping studies of acutely infected individuals as well as case-control studies linked drinking unfiltered water (presumably contaminated with oocysts) to an outbreak of toxoplasmosis in a municipality in the Western Canadian province of British Columbia[76] and to high endemic rates of toxoplasmosis in Rio de Janeiro State, Brazil.[81] In another Brazilian outbreak, *T. gondii* organisms were detected in water by a variety of methods from an implicated reservoir.[87] Coastal freshwater runoff was observed to be a risk factor for *T. gondii* infection among southern sea otters along the California coast.[88]

In humans the incidence of *T. gondii* antibodies increases with increasing age; the incidence does not vary significantly between sexes. The incidence tends to be less in cold regions, in hot and arid areas, and at high elevations. Slaughterhouse workers may have an increased risk for infection. The prevalence of antibody titers to *T. gondii* varies considerably among different geographic areas and also among individuals within a given population. These differences depend on a variety of factors, including culinary habits and cleanliness of surroundings. A decrease in antibody prevalence over the past few decades has been observed in many countries. In the United States the seroprevalence in US military recruits decreased by one-third between 1965 and 1989; the crude seropositivity rate among recruits from 49 states was 9.5% in 1989 compared with 14.4% in 1965.[89] As another example, in the 1970s, 24% of women in the childbearing age group in Palo Alto, California were seropositive, whereas the rate in 2008 was 10%. Seroprevalence rates in the United States among such women range from 3% to greater than 35%, whereas rates greater than 50% are present in women of childbearing age in much of Western Europe, Africa, and South and

Central America.[90] The recent (2011–14) overall age-adjusted seroprevalence of *T. gondii* infection in persons 6 years or older in the United States was reported to be 10.4%, with a seroprevalence among women age 15 to 44 years at 7.5%. This represented a continuous decline in seroprevalence compared with a similar survey from 15 to 20 years earlier. In multivariable analysis, *Toxoplasma* seroprevalence increased with age and was higher in males; persons living below the poverty level; persons with a high school or less education; and non-Hispanic black, Mexican American, and foreign-born non-Hispanic white persons compared with US-born non-Hispanic white persons.[91]

Although the prevalence of the infection appears to be declining in certain areas of the world, such as Europe and the United States, this has not been the case, or there has been a documented increase, in other geographic locales.[92] A meta-analysis of blood donors worldwide found an overall seroprevalence of 33%, with a wide range among countries.[93]

T. gondii may survive in citrated blood at 4°C for as long as 50 days, and infection has been transmitted through transfusion of whole blood or white blood cells. Leukocyte transfusions may pose a special risk.[94] The transmission of infection by organ transplantation has been documented and may result from the transplantation of an organ (e.g., heart) from a seropositive donor to a seronegative recipient.[95] In bone marrow transplant (BMT) recipients, toxoplasmosis almost always is a result of recrudescence of a latent infection rather than from the transplant.[96,97]

The incidence of TE among HIV-infected individuals directly correlates with the prevalence of *T. gondii* antibodies among the general HIV-infected population, the degree of immunosuppression (best measured by the CD4 cell count),[98] the use of effective prophylactic treatment regimens against development of TE, and the immunologic response to antiretroviral therapy (ART).[99] AIDS-associated TE and toxoplasmosis involving other organs are almost always due to reactivation of a chronic (latent) infection that results from the progressive immune dysfunction that develops in these patients.[100] It is estimated that 20% to 47% of AIDS patients who are infected with *T. gondii* but are not taking anti-*Toxoplasma* prophylaxis or antiretroviral drugs will ultimately develop TE.[98,100] This makes TE a major concern in areas where the use of antiretrovirals is still a treatment relatively few HIV-positive individuals receive.

A substantial decline in the incidence of TE[101] and toxoplasmosis-associated hospitalizations[102] and deaths[103] has been seen in HIV-infected patients who adhere to effective anti-*Toxoplasma* prophylactic regimens and to ART.

In the United States *T. gondii* seropositivity among HIV-infected patients has been reported to range from 10% to 45%[86] and directly correlates with the seropositivity in the general non–HIV-infected population. In contrast, the seroprevalence from a similar period was approximately 50% to 78% in certain areas of Western Europe and Africa.[104,105] In a study in France, 1215 (72.2%) of 1683 HIV-infected patients had serologic evidence of exposure to *T. gondii*.[106] During the study period (1988–95), the overall incidence of toxoplasmosis in this population was estimated to be 1.53 per 100 patient-years, with an increase from 0.68 per 100 patient-years in 1988 to 2.1 per 100 patient-years in 1992, and a subsequent decline to 0.19 per 100 patient-years in 1995 that was likely related to the widespread use of anti-*Toxoplasma* prophylaxis. Toxoplasmosis is rare in the HIV-infected pediatric population: 0.06 cases per 100 patient-years were reported among more than 3000 patients participating in clinical trials in the pre-ART era but during a time when *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis was recommended.[107]

Of interest is the low reported incidence of TE in Africa, despite *T. gondii* seroprevalence rates of 32% to 78%. Lack of autopsy data and a lack of neuroimaging studies likely contribute to the low reported incidence. It has also been suggested that because of poor access to medical care, many HIV-infected patients in Africa succumb to infection with organisms such as *Mycobacterium tuberculosis* before they develop the opportunistic infections associated with the advanced stage of HIV infection, including toxoplasmosis. However, in one autopsy series from the Ivory Coast, of 175 patients with AIDS-defining abnormalities, the prevalence of TE was 21%,[108] and in a recent report of 547 HIV-infected patients from Ghana, TE accounted for 29% of admissions and 28% of deaths.[109]

T. gondii infection may be acquired after the acquisition of HIV infection. Seroconversion rates between 2% and 5.5% have been reported in patients followed for periods up to 28 months.[110]

Even before the emergence of AIDS, TE had been recognized as a cause of incapacitating disease and death among HIV-negative immunosuppressed patients,[6,111] especially in those whose underlying disease or therapy caused a deficiency in cell-mediated immunity. Patients with hematologic malignancies are at a particularly higher risk to develop recrudescence of the infection.[112] Among organ transplantation patients, those with heart, lung, kidney, and BMTs develop toxoplasmosis at a higher rate.

PATHOGENESIS AND IMMUNITY

T. gondii multiplies intracellularly at the site of invasion, with the gastrointestinal [GI] tract as the major route for and the initial site of infection in nature; bradyzoites released from tissue cysts or sporozoites released from oocysts invade, differentiate to tachyzoites, and then rapidly multiply within intestinal epithelial cells. Organisms may spread first to the mesenteric lymph nodes and then to distant organs by invasion of lymphatics and blood. *T. gondii* tachyzoites infect virtually all cell types, and cell invasion occurs as an active process. Survival of tachyzoites is due to the formation of a parasitophorous vacuole that lacks host proteins necessary for fusion with lysosomes,[113] and consequently acidification does not occur. Active invasion of macrophages by tachyzoites does not trigger oxidative killing mechanisms. With the appearance of humoral and cellular immunity, only those parasites protected by an intracellular habitat or within tissue cysts survive. An effective immune response significantly reduces the number of tachyzoites in all tissues, and after the initial acute stages, tachyzoites are rarely demonstrable histologically in tissues of infected immunocompetent humans. Tachyzoites are killed by reactive oxygen intermediates,[114] acidification,[115] osmotic fluctuations, reactive nitrogen intermediates,[116] intracellular tryptophan depletion,[117] and specific antibody combined with complement.[118] In rodents two classes of immune-stimulated guanosine triphosphate (GTP)ases play a crucial role in destruction of tachyzoites within the parasitophorous vacuole: the p47 immunity-related GTPases (IRGs)[119] and the larger GTP-binding proteins (GBPs).[120]

Tissue cyst formation takes place in multiple organs and tissues during the first week of infection. Despite the ability to isolate *T. gondii* from normal brains of chronically infected humans, the tissue cyst form is rarely observed in histologic preparations; it has been isolated from both brain and skeletal muscle in 10% of 52 *T. gondii*–seropositive patients who, at autopsy, had no clinical or pathologic evidence of the infection.[44] The tissue cyst form is responsible for residual (chronic or latent) infection and persists primarily in the brain, skeletal and heart muscle, and the eye.[44,121]

In immunocompetent individuals the initial infection and the resultant seeding of different organs leads to a chronic or latent infection with little, if any, clinical significance. This chronic stage of the infection corresponds to the asymptomatic persistence of the tissue cyst form in multiple tissues. It is believed that periodically bradyzoites are released from tissue cysts or that cysts "rupture"; cyst disruption in this setting appears to be a clinically silent process effectively contained by the immune system and in the CNS likely results in small inflammatory nodules, with a limited degree of neuronal cell death and architectural damage.[52] However, several investigators have suggested that chronic infection may not be completely asymptomatic and may result in important behavioral changes and neuropsychiatric disorders,[122–124] although other studies do not have similar findings, and definitive research to support such associations has not yet been reported.

Toxoplasmosis in severely immunodeficient individuals may be caused by primary infection or be the result of recrudescence of a latent infection. It is widely held that reactivation is the result of disruption of the tissue cyst form, followed by differentiation to and uncontrolled proliferation of tachyzoites and tissue destruction. In individuals with deficient cell-mediated immunity, rapid, uncontrolled proliferation of *T. gondii* results in progressively enlarging necrotic lesions. It has been postulated that damage to any organ in these patients, including the

brain, eye, heart, lung, skeletal muscle, GI tract, and pancreas, can result directly from tissue cyst disruption in the parenchyma of the organ itself or from tissue cyst disruption elsewhere in the body, followed by subsequent spread to that organ.[125] Hematogenous spread is supported by the observation of the development of simultaneous lesions in the brain and the presence of parasitemia in 14% to 38% of AIDS patients with TE.[126,127]

Infection with *T. gondii* induces both humoral and cell-mediated immune responses. A well-orchestrated and effective systemic immune response, combining both innate and adaptive mechanisms, is responsible for the early disappearance of *T. gondii* from peripheral blood during the acute infection and limits the parasite burden in other organs. Immunity in the immunocompetent host is lifelong. Exogenous reinfection, which has been demonstrated in laboratory animals, likely also occurs in humans but does not appear to result in clinically apparent disease, although in one case report a chronically infected pregnant woman was infected with a highly virulent strain that resulted in infection of the fetus.[128]

Because *T. gondii* is a natural parasite of rodents, inbred mice have been used extensively as an animal model for studies of both immunity and immunopathology in this protozoan infection and have yielded a remarkably detailed picture of its host interaction.[129–131] When tachyzoites invade, they inject the contents of their rhoptries into the host cell cytosol. This delivers not only some of the machinery needed for invasion (contained within the rhoptry necks and known as RON proteins) but also a collection of "effectors" that intercept or co-opt host immune pathways, presumably to the parasite's advantage. These effectors include the rhoptry protein ROP16, which functions as a mimic of host Janus kinases (JAKs), phosphorylating critical tyrosines on host STATs (signal transducers and activators of transcription). Depending on the particular flavor of ROP16 that a given strain carries, the host immune response can be driven in differing directions, either more or less inflammatory, and this can have a profound effect on the host's response and ultimate outcome of the infection. In the case of another set of injected ROPs—ROP5, ROP17, and ROP18—the target is murine IRGs, a key part of a mouse cell's defense machinery.[132] Normally, IRGs attack the membrane of the vacuole in which a pathogen resides, disrupting it and leading to death of the organisms within. ROP5, ROP17, and ROP18, however, collaborate to phosphorylate and thereby inactivate IRGs, although again, the effectiveness of this depends on the specific alleles of ROP5 and ROP18 that a given strain of *Toxoplasma* carries. Last, dense granules can also introduce polymorphic effectors into the host cell; one such effector, granule 15 (GRA15), has been shown to be crucial to the activation of one of the most central transcription factors in mammalian immune response, nuclear factor kappa B.[133]

Another, GRA6, activates nuclear factor of activated T cells (NFAT) activity.[134] Some GRA proteins transit across the parasitophorous vacuole membrane (PVM) that separates the growing parasites from the host cytosol, eventually reaching the host nucleus. These include GRA16,[135] GRA24[136] and TgIST (*T. gondii* inhibitor of STAT1 transcriptional activity),[137,138] all of which have profound effects on how the host cell responds to infection. In the case of TgIST, for example, the secreted protein suppresses the immune response by blocking the activity of a key host factor STAT1. These proteins transit across the PVM by an as yet uncharacterized machinery, although one component was recently described (myc regulation 1 [MYR1]).[139] It is important to recognize that these findings do not necessarily represent the mechanisms underlying the immune response to *T. gondii* in humans. For example, although the effect of ROP16 on STATs may well have a parallel in human cells, IRGs are not part of the human immune response, and so ROP5, ROP17, and ROP18, if they are impacting the outcome of human infection, must be doing so by acting on other targets, such as activating transcription factor-6β.[140] Similarly, Toll-like receptors TLR11 and TLR12 play a major role in the induction of interleukin-12 (IL-12) and host resistance in the mouse, but neither receptor exists in humans, indicating that innate recognition of the parasite in humans must involve distinct mechanisms.[141] This might involve another branch of the innate immune system, NLRs (nucleotide oligomerization domain [NOD]-like receptors), that detect molecular signatures or patterns specific to various pathogens. Recent work has suggested that susceptibility to *Toxoplasma* infection in humans is associated with a polymorphism in a human NLR known as NALP1 (NACHT-LRR-PYD domains–containing protein 1).[142] The overall question of how *Toxoplasma* is sensed by the innate arm of the human immune system and how different strains of the parasite do this with differing degrees of success is just beginning to be explored. Of interest, in direct contrast to murine innate cells, human monocytes and dendritic cells do not produce cytokines in response to soluble tachyzoite antigens due to the absence of TLR11 and TLR12. Instead, in human cells phagocytosis of live parasites appears to be a major stimulus for proinflammatory cytokine production.[143]

In murine models T cells, macrophages, and type 1 cytokines (IFN-γ, IL-12) are crucial for control of *T. gondii* infection. Adoptive transfer and depletion experiments not only confirmed that T cells are essential for control of *T. gondii* infection but also demonstrated an interplay between $CD4^+$ and $CD8^+$ T cells in both the induction of resistance and the maintenance of latency. Expansion of both natural killer (NK) and γδ T cells early in infection provides innate resistance while the adaptive response mediated through αβ CD4 and CD8 T cells develops. These different subsets of T cells and NK cells are likely to protect the host by secreting cytokines, such as IFN-γ, IL-2, and TNF-α, and apparently not by lysing *T. gondii*–infected cells.[144–147] Dendritic cells and inflammatory monocytes also play an important role in control of acute infection and the early production of IL-12, with dendritic cells critically dependent on the Fms-like tyrosine kinase 3 (Flt3) ligand for this response.[147–149] NK-cell–derived IFN-γ appears critical to the differentiation of IL-12–producing dendritic cells.[147] Early studies suggested that neutrophils were an important component of the early response, but more recent studies suggest that they are not and may, in fact, contribute to the pathology.[148]

The costimulatory molecules CD28 and CD40 ligand are pivotal for the regulation of IL-12 and IFN-γ production in response to the parasite.[150] *T. gondii* infection of antigen-presenting cells, such as dendritic cells and macrophages, causes upregulation of the counterreceptors for CD28 and CD40L, CD80/CD86, and CD40.[150] Binding of CD80/CD86 to CD28 enhances production of IFN-γ by $CD4^+$ T cells. In addition, binding of CD40L to CD40 triggers IL-12 secretion, which in turn enhances production of IFN-γ. The relevance of CD40L in the immune response to *T. gondii* is supported by reports of TE and disseminated toxoplasmosis in children with congenital defects in CD40L signaling (hyper-IgM syndrome).[151] Moreover, recent studies have demonstrated that expression of CD40L is defective on $CD4^+$ T cells from HIV-infected patients.[152] This deficiency may play a role in defective IL-12/IFN-γ production associated with HIV infection.

Cytokines play a critical role in defense against the infection and are important in the pathogenesis of toxoplasmosis and TE.[153] IL-12 enhances survival of T-cell–deficient mice during *T. gondii* infection, by stimulating the production of IFN-γ by NK cells,[154] and is thought to also regulate the expression of the latter cytokine by T cells in immunocompetent mice.[155] IFN-γ has been shown to play a significant role in the prevention or development of TE in mice.[156] The administration to chronically infected mice of a monoclonal antibody against IFN-γ resulted in a dramatic worsening in the degree of encephalitis.[157] In mice with active TE, treatment with IFN-γ significantly reduced the inflammatory response and numbers of tachyzoites.[158]

Differences in IL-12 levels elicited during infection by different strains of the parasite may be responsible for some of the strain-specific differences in virulence in mice.[159] These differences appear to be related to the activation (phosphorylation) of the transcription factor STAT3, which in turn is dependent on the particular allele of ROP16 injected by a given strain, as detailed earlier.[160]

TNF-α is another cytokine pivotal for control of *T. gondii* infection. TNF-α is required for triggering of IFN-γ–mediated activation of macrophages for *T. gondii* killing activity[161] and for nitric oxide (NO, an inhibitor of *T. gondii* replication) production by macrophages.[162] The administration of TNF-α neutralizing antibody to infected mice caused the death of the mice and an increase in the number of *T. gondii* tissue cysts in the brains of survivors.[163]

IL-10 has been shown to deactivate macrophages and result in reduced in vitro killing of *T. gondii.* Nevertheless, infected IL-10–deficient mice rapidly succumb to proinflammatory tissue damage, indicating an

important protective role for this cytokine.[164] Similarly, IL-4 and IL-6, which are usually considered downregulatory cytokines, have been shown to be important in resistance against TE in the murine model.[165,166] IL-7 has also been shown to have a protective role against *T. gondii* in mice.[167] During the early stages of the infection, IL-12, IL-1, and TNF act in concert with IL-15 to stimulate NK cells to produce IFN-γ.[168]

IL-17[169] and IL-23[170,171] have also been implicated in the generation of a potent immune response but are not thought be essential for host resistance.

Several hypotheses have been proposed to explain the role of IFN-γ in host resistance to *T. gondii*. Involvement of reactive nitrogen intermediates (including NO) is suggested by the observation that L-NG-monomethyl-L-arginine acetate (L-NMMA), a competitive analogue of L-arginine, simultaneously inhibits NO synthesis and intracellular tachyzoite killing by cytokine-activated peritoneal macrophages and microglial cells.[116,172,173] In addition, mice in which NO synthesis is impaired as a result of genetic disruptions of the *IFN-γ* or *IFN-1* genes succumb to the acute infection.[174,175] Similar enhanced susceptibility was observed in mice treated with the reactive nitrogen intermediate inhibitor aminoguanidine[176] and in NO synthase–deficient mice.[177] The protective role of NO appears to be tissue specific rather than systemic.[177] Because control of the acute infection in vivo was unaffected by NO synthase deficiency, the major role of reactive nitrogen intermediates appears to be to maintain control of established infections in this mouse model.[177]

The IFN-γ–inducible p47 GTPases IRGM3 (IGTP) and IRGM1 (LRG47) have been shown to be required for host control of *T. gondii* infection in the mouse,[178] and recent studies have linked IRGM3 with the autophagic destruction of *Toxoplasma*-containing vacuoles in IFN-γ–activated macrophages.[179,180]

In a recent cytokine study using a high-throughput and multiplex assay, C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10 levels were higher ($P < .05$) and resistin levels were lower ($P < .05$) in pregnant women with TL compared with those without TL. Among patients with TL, levels of vascular cell adhesion molecule 1 (VCAM-1) and C-C motif chemokine ligand 2 (CCL2) were lower ($P < .05$) in pregnant women than in nonpregnant women.[181] These findings likely illustrate human immune responses and parasite subversion efforts to strike a balance among immune protection, pathology, and evasion.

Immunoglobulin G (IgG), IgM, IgA, and IgE antibodies are produced in response to the infection. Extracellular tachyzoites are lysed by specific antibody when combined with complement; this is the basis for the detection of *Toxoplasma* IgG in what is considered to be the gold standard test for this Ig. In mice, humoral immunity results in limited protection against less virulent strains of *T. gondii* but not against virulent strains.[182]

Both astrocytes and microglia likely play important roles in the immune response against *T. gondii* within the CNS. In the early stages of TE in both humans and mice there is a remarkable and widespread astrocytosis restricted to areas in which the parasite is detected.[153] Whereas *T. gondii* can invade, survive, and multiply within astrocytes, they are killed by activated microglia.[183]

GENETIC SUSCEPTIBILITY

The observations in mice that genetic factors in the host contribute to the development and severity of TE[184–186] and the fact that not all HIV-infected patients with positive *T. gondii* serologic findings develop TE suggested the possibility that genetic factors may also play a role in the predisposition of AIDS patients for this disease.[187] The major histocompatibility complex (MHC) class II gene *DQ3 (HLA-DQ3)* has been significantly associated with the development of TE in North American white AIDS patients, whereas the *HLA-DQ1* genotype was marginally protective.[187] The *HLA-DQ3* genotype was also significantly associated with the development of hydrocephalus in children with congenital toxoplasmosis.[188] In the latter study a mouse model transgenic for human class II MHC found higher organism burden with the *HLA-DQ3* than *HLA-DQ1* genotype. In a South American white population a study using a higher-resolution typing method identified *HLA-DQB*0402* and *HLA-DRB1*08* genes, which were in linkage disequilibrium, as risk factors for TE, whereas alleles of *HLA-DQB3* were not.[189] Certain *HLA-DQA1* and *HLA-DQB1* alleles were associated with congenital infection in one recent Brazilian study.[190] Another Brazilian study identified an association between the presence of certain KIR receptors and their class I HLA ligands with the development of ocular lesions in *Toxoplasma*-seropositive individuals.[191] Thus further studies are needed to better define the contribution of various HLA alleles to susceptibility to TE. In single studies polymorphisms in other genes, including those for IL-6, IL-10, TLR9, NLR family, pyrin domain–containing protein 1 (NALP1, also known as NLRP1), and purinergic receptor P2X(7) (P2RX7) have been associated with congenital toxoplasmosis or retinochoroiditis.[142,192–195]

Of interest, although humans with genetic defects in IFN-γ and IL-12 signaling are highly susceptible to mycobacterial and other bacterial infections, there are no reports of toxoplasmosis in these patients, again suggesting important differences in how *T. gondii* is controlled in humans versus mice.[196]

PATHOLOGY

Our knowledge of the pathology of infection in humans has come largely from autopsy studies in severely infected infants and immunodeficient patients. Data from immunocompetent adults are limited almost entirely to results obtained from lymph node biopsy specimens[197] and occasionally from myocardial or skeletal muscle tissue specimens.[198] In addition to the direct demonstration of parasites in tissue and associated pathology, recent studies have revealed that the impact of the parasite may extend to many more cells than are actively infected; that is, mouse studies have shown evidence of injected rhoptry proteins in upward of 50-fold more neurons in a chronically infected brain than actually harbor parasites at that time.[53] The impact of these "injected-uninfected" cells on pathogenesis and other interactions with the host has yet to be determined.

Lymph Node

The histopathologic changes in TL in immunocompetent individuals are frequently distinctive and often diagnostic (Fig. 278.3A).[197] There is a typical triad of findings: a reactive follicular hyperplasia, irregular clusters of epithelioid histiocytes encroaching on and blurring the margins of the germinal centers, and focal distention of sinuses with monocytoid cells (see Fig. 278.3A).[199] Langerhans giant cells, granulomas, microabscesses, and foci of necrosis are not typically seen. Rarely, tachyzoites or tissue cysts are demonstrable. *T. gondii* DNA has infrequently been amplified from lymph node tissue.[200]

Central Nervous System

Damage to the CNS by *T. gondii* is characterized by multiple foci of enlarging necrosis and microglial nodules.[201] Necrosis is the most prominent feature of the disease because of vascular involvement by the lesions. In cases of congenital toxoplasmosis, necrosis of the brain is most intense in the cortex and basal ganglia and at times in the periventricular areas.[9,202] The necrotic areas may calcify and lead to striking radiographic findings suggestive but not pathognomonic of toxoplasmosis. Hydrocephalus may result from obstruction of the aqueduct of Sylvius or foramen of Monro. Tachyzoites and tissue cysts may be seen in and adjacent to necrotic foci, near or in glial nodules, in perivascular regions, and in cerebral tissue uninvolved by inflammatory change.[203] The necrotic brain tissue autolyzes and is gradually shed into the ventricles. The protein content of such ventricular fluid may be in the range of grams per deciliter and has been shown to contain significant amounts of *T. gondii* antigens.

The presence of multiple brain abscesses is the most characteristic feature of TE in severely immunodeficient patients and is particularly characteristic in patients with AIDS.[6,204] Brain abscesses in AIDS patients are characterized by three histologic zones. The central area is avascular. Surrounding this is an intermediate hyperemic area with a prominent inflammatory infiltrate and perivascular cuffing by lymphocytes, plasma cells, and macrophages. Many tachyzoites and, at times, tissue cysts as well, appear at the margins of necrotic areas. An outer peripheral zone contains *T. gondii* tissue cysts.[205] In the areas around the abscesses, edema, vasculitis, hemorrhage, and cerebral infarction secondary to vascular involvement may also be present.[206] Important associated features in TE are the presence of arteritis, perivascular cuffing, and astrocytosis.

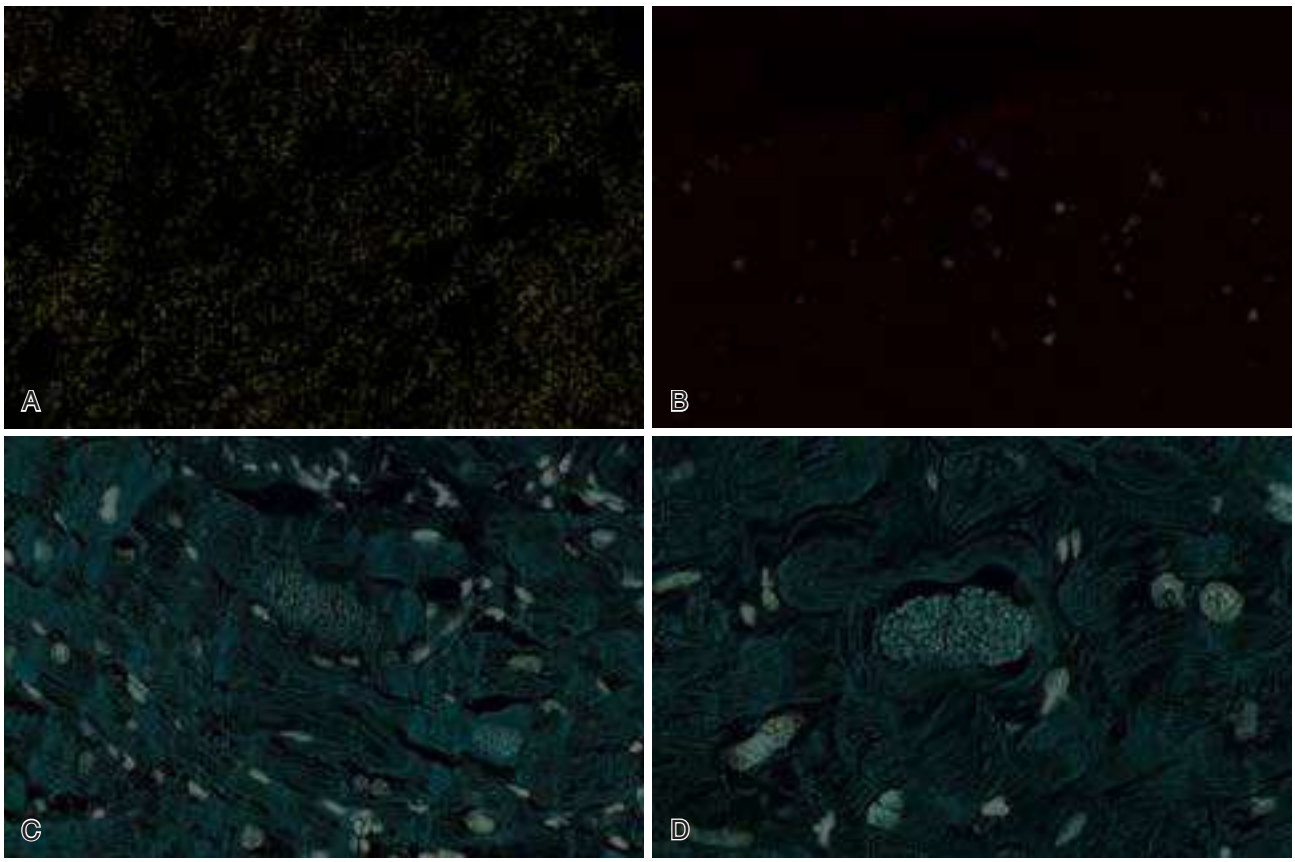

FIG. 278.3 Histologic features of *Toxoplasma gondii* in humans. (A) Hematoxylin and eosin (H&E) stain of a lymph node biopsy specimen from an immunocompetent patient with toxoplasmic lymphadenitis. (B) Positive immunoperoxidase stain of a brain biopsy specimen in a patient with acquired immunodeficiency syndrome and toxoplasmic encephalitis. (C) H&E stain of a right ventricle endomyocardial biopsy specimen from a patient with toxoplasmic myocarditis. Organisms are seen within myocytes (see text under "Clinical Manifestations"). (D) H&E stain of a right quadriceps muscle biopsy specimen depicting tissue cyst from the same patient as shown in C. She also developed toxoplasmic polymyositis. *(A courtesy Dr. Henry Masur, Critical Care Medicine Department, National Institutes of Health, Bethesda, MD. B to D courtesy Dr. Jack S. Remington, Stanford University and Palo Alto Medical Foundation.)*

Because these findings may also be present in patients with viral encephalitis, immunoperoxidase staining is important for differentiating these pathologic processes. Widespread, poorly demarcated, and confluent areas of necrosis with minimal inflammatory response are seen in some patients.[206] Identification of tachyzoites is pathognomonic of active infection, but their visualization may be difficult in H&E-stained sections. The use of immunoperoxidase staining markedly improves the identification of both tissue cyst and tachyzoite forms and highlights the presence of *T. gondii* antigens (see Fig. 278.3B).[42] *T. gondii* DNA can be amplified from cerebrospinal fluid (CSF) or brain biopsy specimens of patients with TE.[207] Of note, PCR-positive results in brain biopsy specimens need to be interpreted with caution. These results may be positive in patients chronically infected with the parasite whose CNS pathology can be explained by a diagnosis other than TE.

At autopsy in AIDS patients with TE, there is almost universal involvement of the cerebral hemispheres and a remarkable predilection for the basal ganglia.[100] In a consecutive autopsy study of 204 patients who died of AIDS, 46 (23%) had morphologic evidence of cerebral toxoplasmosis.[206] In 38 (83%) of the 46 cases, histologic evidence of toxoplasmosis was restricted to the CNS. The cerebral hemispheres were affected in 91% of cases and the rostral basal ganglia in 78%.

A "diffuse form" of TE has been described with histopathologic findings of widespread microglial nodules without abscess formation in the gray matter of the cerebrum, cerebellum, and brainstem.[208] In these patients involvement by *T. gondii* was confirmed by immunoperoxidase stains that demonstrated tissue cysts and tachyzoites. In diffuse TE the clinical course progresses rapidly to death. It has been postulated that in such cases the lack of characteristic findings on computed tomography (CT) or magnetic resonance imaging (MRI) studies is due to insufficient time for abscesses to form before death occurs.

Leptomeningitis is infrequent and, when present, occurs over adjacent areas of encephalitis. Spinal cord necrotizing lesions are seen at autopsy in approximately 6% of patients with TE. The differential diagnosis of TE lesions includes CNS lymphoma, progressive multifocal leukoencephalopathy, pyogenic brain abscesses, and infection caused by organisms such as *Nocardia* spp., *Aspergillus* spp. and other molds, *Trypanosoma cruzi*, *Cryptococcus neoformans, M. tuberculosis, Balamuthia* spp., and cytomegalovirus (CMV). More than one agent may be present.

Lung

Pulmonary toxoplasmosis in the immunodeficient patient may appear in the form of interstitial pneumonitis, necrotizing pneumonitis, consolidation, pleural effusion, empyema, or all of these.[209] The pneumonitis is associated with the development of fibrinous or fibrinopurulent exudate. Tachyzoites may be found in alveolocytes, alveolar macrophages, or pleural fluid, or extracellularly within alveolar exudate. *T. gondii* DNA may be demonstrated in bronchoalveolar lavage (BAL) fluid by the PCR.[210]

Eye

Chorioretinitis in AIDS patients is characterized by segmental panophthalmitis and areas of coagulative necrosis associated with tissue cysts and tachyzoites.[211] Numerous organisms in the absence of remarkable inflammation may be seen around thrombosed retinal vessels adjacent to necrotic areas. Multiple and bilateral lesions may occur.[211] Amplification of parasite DNA in both aqueous humor and vitreous fluid has confirmed or supported the diagnosis of toxoplasmic chorioretinitis in patients

with atypical retinal findings for ocular toxoplasmosis or who are immunocompromised.[212,213]

Eye infection in immunocompetent patients produces acute chorioretinitis characterized by severe inflammation and necrosis.[211] Granulomatous inflammation of the choroid is secondary to the necrotizing retinitis. There may be exudation into the vitreous or invasion of the vitreous by a budding mass of capillaries. Although rare, tachyzoites and tissue cysts may be demonstrated in the retina. The pathogenesis of recurrent chorioretinitis is controversial. One school proposes that rupture of tissue cysts releases viable organisms that induce necrosis and inflammation, whereas another school contends that chorioretinitis results from a hypersensitivity reaction triggered by unknown causes.[211] A study demonstrating efficacy of trimethoprim-sulfamethoxazole (TMP-SMX) in preventing recurrences of chorioretinitis is consistent with the hypothesis that active organism replication is necessary for recurrence.[214]

Recent studies have revealed a much higher incidence of ocular disease, which is often severe, among infected immunocompetent persons in South America than in North America or Europe.[215–218] This difference seems most likely to be due to differences in the strains of *Toxoplasma* that predominate in these different regions.[25] This is consistent with observations within the United States, where specific strains appeared to be associated with severe disease, although these studies involved relatively few patients and cannot be considered definitive.[219]

Skeletal and Heart Muscle

Myositis caused by *T. gondii* has been reported in as high as 4% of HIV-infected patients who present with neuromuscular symptoms, and the same percentage has been observed in autopsy series of AIDS patients in whom a systematic histologic evaluation of the skeletal muscle was performed.[220] Successful isolation from skeletal muscle biopsies has been reported.[221] Microscopy has revealed necrotic muscle fibers with a variable inflammatory reaction. Skeletal muscle involvement has also been reported in the non-AIDS immunodeficient patient.[6,198]

Toxoplasmic myocarditis is frequently noted at autopsy in AIDS patients but is usually clinically inapparent,[222] with CNS manifestations predominating.[223] Focal necrosis with edema and an inflammatory infiltrate is typical,[222] although abscesses may also be noted.[222,223] Similar histologic findings are seen in the non-AIDS immunodeficient population,[6] and in both groups cardiac myocytes may be packed with tachyzoites (to produce pseudocysts) in the absence of an inflammatory response.

Biopsy-proven toxoplasmic myocarditis and polymyositis in the setting of acute toxoplasmosis have been reported in otherwise immunocompetent individuals and in patients on corticosteroids (see Fig. 278.3C and D).[198]

Other Organ Systems

Extensive involvement of the GI tract in AIDS patients may occur with tremendous variation in the inflammatory response.[224,225] Hemorrhagic gastritis and colitis have been described.[226] Other organs reported to be involved during toxoplasmosis include the liver,[227] pancreas,[228] seminiferous tubules,[229] prostate,[229] adrenal glands,[230] kidneys,[231] and bone marrow.[232]

CLINICAL MANIFESTATIONS

Toxoplasmosis describes the clinical or pathologic disease caused by *T. gondii* and is distinct from *T. gondii* infection, which is asymptomatic in the vast majority of immunocompetent patients.

Toxoplasmosis is conveniently classified into five clinical categories: (1) acquired in the immunocompetent patient, (2) acquired or reactivated in the immunodeficient patient, (3) ocular, (4) in pregnancy, and (5) congenital. In any category the clinical presentations are not specific for toxoplasmosis, and a wide differential diagnosis must be considered for each clinical syndrome. Furthermore, methods of diagnosis and interpretation of test results may differ according to the patient's specific clinical category. For instance, whereas serologic test results consistent with an infection acquired in the distant past for a nonimmunocompromised pregnant woman in her first half of pregnancy are interpreted as no risk for congenital toxoplasmosis, the same results for a patient about to undergo an allogeneic hematopoietic stem cell transplantation (HSCT) are interpreted as high risk for life-threatening toxoplasmosis in the posttransplantation period.

Toxoplasmosis in the Immunocompetent Patient

In the United States and Europe only 10% to 20% of cases of *T. gondii* infection in immunocompetent adults and children are symptomatic.[233] Recent reports suggest that this proportion may be higher in other areas of the world, such as Brazil and other countries in Latin America.[215,218] In addition, it appears that disease severity can also be greater in countries outside Europe and the United States. For instance, community outbreaks of acute toxoplasmosis with an unusually severe clinical presentation have been recently reported from Suriname and French Guiana.[234,235] In two reports immunocompetent patients presented with severe disseminated disease, including pneumonia and hepatitis, that resulted in four deaths, including one newborn and one fetus, among 22 patients. Based on genotype analysis with microsatellite markers, "atypical" strains (i.e., not one of the three major strains seen in Europe and North America) were responsible for these outbreaks. The remarkable differences in clinical presentation of toxoplasmosis among patients from various regions of the world have significant implications when generating a differential diagnosis in travelers who become ill and are returning from highly endemic areas (e.g., Latin America).[236]

When clinical manifestations are present, toxoplasmosis most often manifests as painless cervical lymphadenopathy, but any or all lymph node groups may be enlarged. On palpation the nodes are usually discrete and nontender, rarely more than 3 cm in diameter, may vary in firmness, and do not suppurate.[237] However, the nodes may be occasionally tender or matted. Fever, malaise, night sweats, myalgias, sore throat, arthralgias, maculopapular rash, hepatosplenomegaly, or small numbers of atypical lymphocytes (<10%) may be present. The clinical picture may resemble infectious mononucleosis or CMV infection, but toxoplasmosis probably causes no more than 1% of "mononucleosis" syndromes.[238] Retroperitoneal or mesenteric lymphadenopathy may produce abdominal pain accompanied by diarrhea. Neurocognitive abnormalities appear to be common during the acute infection among immunocompetent patients.[239]

Toxoplasmic chorioretinitis as a manifestation of acute acquired infection is more common than previously recognized.[240–242] Chorioretinitis in the setting of acute acquired toxoplasmosis can occur either sporadically or in the context of an epidemic of acute toxoplasmosis.[241,243] For further discussion of this clinical entity, see "Ocular Toxoplasmosis in Immunocompetent Patients."

In most cases the clinical course of toxoplasmosis in the immunocompetent patient is benign and self-limited. Symptoms, if present, usually resolve within a few months and rarely persist beyond 12 months. Lymphadenopathy may wax and wane for months and in unusual cases for 1 year or longer. Rarely, an apparently healthy person develops clinically overt disease, for instance, fever of unknown origin or potentially fatal disseminated disease, with myocarditis, pneumonitis, hepatitis, or encephalitis. In children nephrotic syndrome has been reported in association with acute toxoplasmosis.[244] These more aggressive forms of the disease have been more commonly reported from South America.[234,235] None of the clinical presentations of acquired toxoplasmosis is distinctive; the differential diagnosis of TL includes bacterial lymphadenitis, lymphoma, infectious mononucleosis, CMV or human herpesvirus 6 (HHV-6) "mononucleosis," cat-scratch disease, sarcoidosis, tuberculosis, tularemia, metastatic carcinoma, endemic fungi (e.g., coccidioidomycosis), and leukemia. Acute acquired toxoplasmosis associated with multiorgan involvement has been reported to mimic other causes of pneumonitis, hepatitis, myocarditis, polymyositis, or fever of unknown origin in apparently immunocompetent patients.[233]

T. gondii has been estimated to cause 3% to 7% of clinically significant lymphadenopathy.[237] The major diagnostic confusion with toxoplasmic lymphadenopathy occurs with Hodgkin disease and the lymphomas. The diagnosis of recently acquired toxoplasmic lymphadenopathy is easily made serologically, but unfortunately, physicians often do not consider this diagnosis in patients with lymphadenopathy. Serologic test titers diagnostic of acute *T. gondii* infection are often obtained after histologic examination of a biopsied node has suggested the possibility of toxoplasmosis.[245]

Myocarditis as a manifestation of acute toxoplasmosis has been reported in relatively few patients.[198,246,247] It may occur clinically as an isolated disease process or as part of a variety of manifestations of disseminated infection. Manifestations include arrhythmias, pericarditis, and heart failure.[198,248]

Myositis resembling polymyositis as a manifestation of acute toxoplasmosis has also been reported infrequently.[198,249,250] Dermatomyositis has been associated with toxoplasmosis, although a cause-and-effect relationship has not been proved.[251,252]

The clinical features of toxoplasmic myocarditis and polymyositis are illustrated by a case in which both were present in the same individual.[198] A 43-year-old woman presented with cardiogenic pulmonary edema, followed by progressive sinus bradycardia and subsequent complete heart block; viral myocarditis was considered the most likely diagnosis. During the ensuing months, she developed proximal muscle weakness while being treated with corticosteroids; an endomyocardial biopsy (see Fig. 278.3C) and a quadriceps muscle biopsy (see Fig. 278.3D) revealed *T. gondii*.[198] Her symptoms improved on pyrimethamine-sulfadiazine. One year after her initial presentation with myocarditis, retinal lesions characteristic of toxoplasmic chorioretinitis were observed in her right eye. Serologic test results and follow-up were consistent with recently acquired toxoplasmosis.[198]

Several epidemiologic studies have suggested an association between infection with *T. gondii* and schizophrenia and other mental illnesses, but a definitive etiologic role of the parasite in such disorders has not been established.[123,124,253–255] Population-based studies that include following large cohorts of patients following gestation will be necessary to clarify this potential association.

Toxoplasmosis in the Immunodeficient Patient

In immunocompromised patients toxoplasmosis can present with a wide spectrum of clinical manifestations. Disseminated disease has a 100% case-fatality rate if untreated. Early diagnosis requires a high index of suspicion because routine laboratory tests aimed at detecting bacterial, viral, or fungal infections will not alert clinical laboratory personnel or the clinician to the presence of *T. gondii* as the etiologic agent. T-cell– and/or B-cell–mediated immunity defects appear to confer the highest risk for toxoplasmosis as observed in patients with hematologic malignancies (especially Hodgkin disease and other lymphomas), organ transplant recipients, or those with AIDS and those receiving immunosuppressive therapy with high doses of corticosteroids or immunomodulators, such as anti–TNF-α agents, for instance rituximab,[256] natalizumab,[257] or alemtuzumab.[258] In immunodeficient patients encephalitis, pneumonitis, and myocarditis reflect active replication and infection in the most commonly involved organs.[6] Pneumonitis is a common and underrecognized manifestation of toxoplasmosis in these patients. Fever of unknown origin may be the sole manifestation of toxoplasmosis in the early stages of the disease. Disseminated infection with multiorgan involvement is not unusual; clinical manifestations may not necessarily reflect the extent and severity of the disseminated infection. Mortality approaches 100% if the infection is not treated or is treated only late in its course. Whereas serious toxoplasmosis in these patients often reflects recrudescence of a latent infection acquired in the distant past (as observed in the setting of AIDS or HSCT), it may also result from recently acquired acute infection (as observed in solid-organ transplants through the transplanted organ) or, more rarely, through the oral route. Although clinical manifestations are similar in patients with different causes for their immunosuppression, additional considerations are provided here for the organ transplant recipient and patient with AIDS.

Toxoplasmosis in the Solid-Organ Transplant Patient

Patients with solid-organ transplants will develop toxoplasmosis most commonly as a result of acquiring *T. gondii* infection through the transplanted organ, when the allograft of a seropositive donor (D^+) is given to a seronegative recipient (R^-), resulting in a D^+/R^- mismatch (Table 278.1).[259] Toxoplasmosis can also be the result of reactivation of a previously acquired infection in the recipient, regardless of the serologic status of the donor (D^-/R^+ or D^+/R^+; see Table 278.1). Fever is often the first manifestation in transplant recipients, followed by signs referable to the brain and lungs.

TABLE 278.1 Source of Toxoplasmosis in the Organ Transplant Patient

Transplant of an Infected Organ to a Seronegative Recipient (D^+R^-)
Heart
Heart-lung
Kidney
Liver and liver/pancreas
Bone marrow (rare)
Reactivation of Latent Infection in a Seropositive Recipient (D^-R^+ and D^+R^+)
Bone marrow
Hematopoietic stem cell
Liver
Kidney (rare)

D, Donor; *R,* recipient.

Knowledge of the overall prevalence of *Toxoplasma* antibodies in a population does not accurately predict the percentage of D^+/R^- *T. gondii* mismatches. Rather, this will depend on the prevalence of *T. gondii* antibodies in the age groups of the donor and recipient populations. For example, in a given geographic area, the prevalence of antibodies in young heart donors may be 3% to 10%, whereas in the older population of individuals, who would more likely be recipients, it may be 15% to 30%. Testing for *Toxoplasma* IgG antibodies should be performed in every solid-organ transplant candidate before transplantation and on serum from every organ donor.

Since April 6, 2017, organ procurement organizations in the United States have made it mandatory to test all potential organ donors for toxoplasmosis.[260] This allows identification of those recipients at greatest risk of developing toxoplasmosis either because they were seronegative before transplantation and received an organ from a seropositive individual or because they were seropositive before transplantation and thus are at risk for reactivation of their latent (chronic) *Toxoplasma* infection. *Toxoplasma* serologies obtained in the posttransplantation period, unfortunately, are frequently not helpful, even in the presence of serious toxoplasmosis. *T. gondii* antibodies that were demonstrable before transplantation might become negative, rise, or show no change posttransplantation despite life-threatening toxoplasmosis.[261] Transfusion may further compound the difficulties encountered in serodiagnosis during the posttransplantation period.

The incidence of toxoplasmosis among various organ transplant recipients managed at 11 tertiary care hospitals in Spain between 2000 and 2009 was 0.14% (22/15,800).[262] The incidence was significantly greater in heart compared with kidney and liver recipients. The only independent risk factor identified in multivariate analysis was negative serostatus before transplant. Overall mortality was 14%, although two of the three deaths occurred in untreated patients who were diagnosed at autopsy.

At autopsy, histopathologic evidence of multiorgan involvement has been observed, most commonly of the brain, heart, and lungs but also including the eyes, liver, pancreas, adrenal, and kidney.

Heart Transplantation

In a review of infections in cardiac transplant recipients at Stanford Medical Center from 1980–96, results of serologic testing for *Toxoplasma* were available for 582 donors (35 [6%] had *T. gondii*–specific IgG antibodies) and 607 recipients (98 [16%] were positive).[263] Results of serologic testing for *Toxoplasma* were available for 575 D/R pairs; of these, 454 (79%) were D^-/R^-, 84 (14.6%) D^-/R^+, 32 (5.6%) D^+/R^-, and 5 (0.8%) D^+/R^+. Of the 32 D^+/R^- patients, 16 were receiving TMP-SMX and/or pyrimethamine prophylaxis, and none developed toxoplasmosis; however, 4 (25%) of the 16 D^+/R^- patients who were not taking either TMP-SMX or pyrimethamine developed toxoplasmosis, and all died of the infection. None of the 98 patients who were seropositive for *T. gondii*

preoperatively developed clinical evidence of reactivation of the infection. The importance of prophylaxis is further evidenced from an earlier study at Papworth Hospital in England. Fatal or severe toxoplasmosis developed in 57% (4/7) of D^+R^- mismatched heart transplant patients not receiving prophylaxis. Use of pyrimethamine, 25 mg/day for 6 weeks, reduced the transmission rate to 14% (5/37). In those patients who received pyrimethamine and were infected by the donor heart, only 1 (20%) developed symptoms of the infection in contrast to 4 of 4 (100%) who did not receive pyrimethamine prophylaxis. Subsequently, prophylaxis with TMP-SMX (80/400 mg twice daily orally for 1 year posttransplantation and when on oral prednisolone) was used in heart and lung transplant patients. Of those who were alive at 3 months post-transplantation, 28 (8.75%) were *T. gondii* mismatches; none had evidence of having acquired *Toxoplasma* infection. These investigators observed that use of prophylaxis might prolong the period before observation of seroconversion of donor-acquired infection in heart transplant patients for as long as 14 months posttransplantation.[264,265] Anti-*Toxoplasma* prophylaxis may not always work, and toxoplasmosis should still be entertained in D^+/R^- patients who present with unexplained syndromes even when there is a history of taking their prophylactic drugs.[266]

Toxoplasmosis in heart transplant recipients may simulate organ rejection. In such cases toxoplasmosis has frequently been diagnosed by endomyocardial biopsy.

It is important to recognize that many heart transplant recipients with *T. gondii* antibodies before transplantation may show increases in *T. gondii*–specific antibodies (IgG and IgM). These patients have not necessarily developed a clinical illness that can be attributed to toxoplasmosis.

Kidney Transplantation

In a review of 31 cases of toxoplasmosis in renal transplant patients the majority occurred within the first 3 months after transplantation; 3 cases occurred more than 1 year after transplantation, and 9 occurred during or immediately after a rejection episode.[267] The greatest risk was in D^+/R^- mismatches. Fever, CNS symptoms and signs, and pneumonia were the main clinical features. Chest radiographs showed bilateral pneumonia in most cases. The most common organs involved in the 15 cases diagnosed at autopsy were brain, heart, and lungs. *T. gondii* was not demonstrable in the kidneys. Whereas the overall mortality rate was 64%, 10 of 11 treated patients survived, emphasizing the importance of early diagnosis and treatment. Fatal cases of disseminated toxoplasmosis in kidney transplant recipients have been, unfortunately, unexpectedly diagnosed postmortem.[268] Acute toxoplasmosis in two recipients of renal allografts from the same donor has occurred. Chorioretinitis has been reported as the presenting manifestation of toxoplasmosis in a kidney transplant patient.[269]

Liver Transplantation

After orthotopic liver transplantation, toxoplasmosis most often results from activation of a latent infection in the transplanted allograft,[270,271] but it occurs as well from activation of a quiescent pretransplantation infection in the recipient. In most published cases clinical manifestations of toxoplasmosis appeared within the first 3 months posttransplantation. Fever was usually the first manifestation, and pneumonia, meningitis/encephalitis, and multiorgan failure were frequently observed. Retinochoroiditis requiring enucleation was observed in one patient.[272] Although it is a rare event, toxoplasmosis in this population is most often fatal.[273]

Toxoplasmosis in the Bone Marrow Transplant and Hematopoietic Stem Cell Transplant Recipient

Donor bone marrow from the patient (autologous) or a matched related or unrelated donor (allogeneic) used to be the only source of cells for BMT recipients. Stem cell transplantation is now also done using stem cells harvested from the peripheral blood (HSCT) of the patient (autologous), matched related or unrelated donor (allogeneic), matched parent (haploid), or umbilical cord blood. To keep this literature separate, HSCT and BMT are presented separately in this section. A review of 41 cases of toxoplasmosis in patients who had undergone HSCT in 15 European transplantation centers, from 1994–98, found no cases among 6787 autologous HSCTs, whereas it occurred in 0.97% of 4231 allogeneic transplants.[274] The relatively low number of cases in their large survey is likely due to the use of TMP-SMX prophylaxis after engraftment in all allogeneic BMTs in 91% of the institutions. Of the patients who develop toxoplasmosis and who had available serologies before transplantation, 94% were seropositive for *T. gondii*. Graft-versus-host disease (GVHD) had developed before toxoplasmosis in 73%. Thirty patients (73%) had not received prophylaxis for toxoplasmosis, and only 3 were receiving it at the time of disease onset. Median time of onset was day 64 (range, 4–516 days). Fever with neurologic or pulmonary symptoms was seen most commonly; myocarditis was frequently seen at autopsy. Six patients had fever without evidence of organ involvement. Twenty-two (63%) died of toxoplasmosis. It is noteworthy that of the 23 patients who received specific therapy for toxoplasmosis for greater than or equal to 6 days, 11 (48%) had a complete response, and 3 others (13%) improved. More recently, among 444 HCT patients (87% HSCT) followed prospectively in the United States between 2006 and 2011, most of whom received effective prophylaxis, no cases of toxoplasmosis were seen.[275] During a similar time period (2006–15), 3.9% of 588 transplant patients at a single center in France were diagnosed with toxoplasmosis ($n = 20$), primarily pulmonary ($n = 14$) or CNS ($n = 10$) disease, or *T. gondii* infection ($n = 3$). Effective prophylaxis was used less routinely (22% of cases, 70% of control subjects).[276] Toxoplasmosis was also seen in 2.1% (4/187) of Italian pediatric HSCT patients.[277]

In 106 *T. gondii*–seropositive adult recipients of HSCTs the incidence of reactivation of toxoplasmosis in the first 6 months after transplantation was prospectively studied.[278] *Toxoplasma* serologies had been obtained pretransplantation. The incidence of reactivation within 6 months after transplantation, as established by a positive PCR test result in peripheral blood, was observed in 16 of the 106 patients (16%). Of the 16 patients with positive PCR test results, 6 (38%) developed disease (toxoplasmosis). Thus PCR may be useful in monitoring at-risk patients (i.e., seropositive patients identified in the pretransplantation period) (see "Diagnosis").

Survival in this setting is highest in patients with ocular and/or isolated cerebral toxoplasmosis, primarily when treatment is begun as soon as the diagnosis is suspected.[96,274] Survival in the presence of disseminated toxoplasmosis is rare in these HSCT patients.[96,274] Although reactivation of latent *Toxoplasma* infection in allogeneic HSCT recipients most often occurs in the first 6 months posttransplantation (the majority occur in the first 30–90 days), late reactivation has been observed and must be considered in patients in whom late-onset (beyond 6 months posttransplantation) GVHD occurs.[278,279]

The incidence of toxoplasmosis in BMT has been reported to range from 0.3% to 5% and is influenced by the prevalence of pretransplantation antibodies and whether toxoplasmosis prophylaxis was used. Major risk factors for toxoplasmosis included the presence of pretransplantation *T. gondii* antibodies in recipients and the occurrence of GVHD. In a review of 110 published cases of toxoplasmosis after BMT,[96] 96% occurred after allogeneic BMT. Onset of infection posttransplantation occurred on days 1 to 30 in 13%, on days 31 to 100 in 64%, and on days past 100 in 23%. The infection occurred primarily in recipients who were seropositive pretransplantation (88%), meaning infection was more often reactivation in the recipient, not transplanted from the donor. The diagnosis was made antemortem in only 47% of the cases. Overall mortality was 80% (median, 87 days posttransplantation), and in 66% it was attributed to toxoplasmosis (median, 74 days posttransplantation). Patients with isolated cerebral involvement had a better outcome (58% survival) than patients with disseminated toxoplasmosis (20% survival); underlying disease was the only factor associated with clinical presentation, with acute leukemia being more common in patients with disseminated disease.

TMP-SMX prophylaxis, primarily used by transplantation teams to prevent *Pneumocystis* pneumonia, has been successful in prevention of toxoplasmosis. In HSCT patients, this is usually begun after engraftment because of the potential of the drug combination for bone marrow suppression. The delay in instituting prophylaxis likely results in many more cases of toxoplasmosis than would be expected to occur if adequate prophylaxis was begun early after transplantation. This problem highlights the importance of PCR monitoring of at-risk patients and identifying additional drugs for prophylaxis in these patients.[280,281]

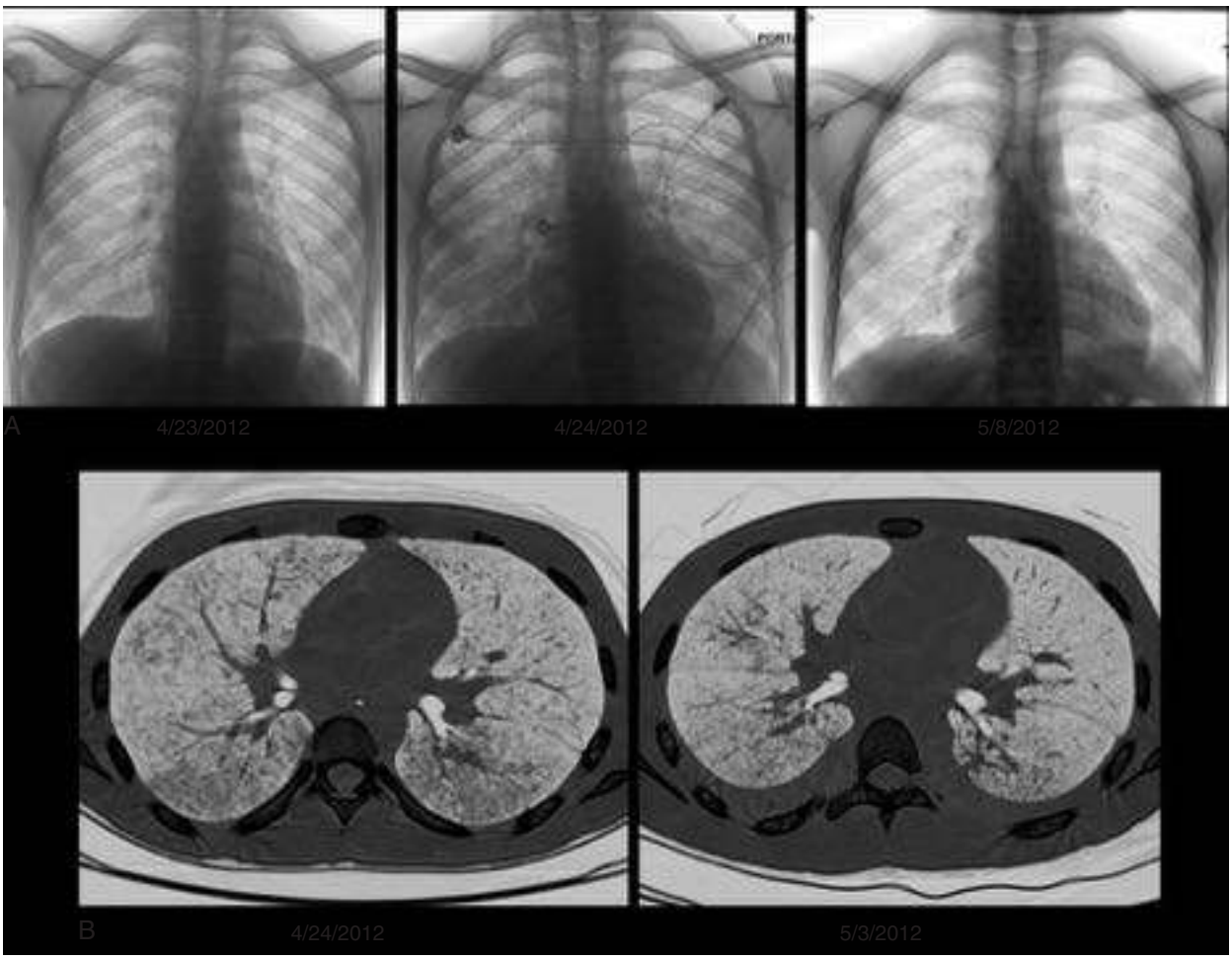

FIG. 278.4 Pulmonary toxoplasmosis. Serial chest radiographs (A) and computed tomography scans of the chest (B) of a patient who had received a haplocord transplant as treatment for myelodysplastic syndrome complicating aplastic anemia and subsequently presented with pulmonary symptoms. Pulmonary toxoplasmosis was diagnosed by polymerase chain reaction (PCR) of a bronchoalveolar lavage sample obtained April 24, 2012. The patient also had a positive PCR for toxoplasmosis in blood and cerebrospinal fluid. The patient responded to treatment with intravenous trimethoprim-sulfamethoxazole and subsequently oral pyrimethamine-sulfadiazine-leucovorin, with resolution of pneumonia and conversion of PCR to negative. *(Courtesy Dr. Richard Childs, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD.)*

Toxoplasmosis in BMT patients frequently involves the lung, usually in the setting of multiorgan disease, with a high associated mortality (>90%) (Fig. 278.4). Most patients die within 7 days of onset of pulmonary symptoms and often suffer acute respiratory distress syndrome. In a review of 25 cases of pulmonary toxoplasmosis in BMT, onset of symptoms referable to the lungs occurred from 7 days to 1 year post-BMT; the majority occurred in the first 6 weeks.[282] Fever may be the first sign; if pulmonary infiltrates are observed, especially in the setting of rapid deterioration, immediate attempts at diagnosis must be made and empirical treatment begun. On CT scan of the lungs, most patients present with bilateral ground-glass opacities.[283] In HSCT patients with this radiologic pattern, the differential diagnosis should include *Pneumocystis* pneumonia (PCP), atypical pneumonia (e.g., *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*), viral pneumonia (e.g., CMV), strongyloidiasis, diffuse alveolar hemorrhage, pulmonary edema, and drug hypersensitivity. However, toxoplasmosis is often overlooked by those generating differential diagnoses in this setting, including radiologists, cancer and organ transplant providers, and infectious diseases specialists. Undoubtedly, the high mortality in many instances has been due to lack of early diagnosis and treatment. The organism can be observed on microscopic examination of BAL material. PCR on material obtained at BAL is the diagnostic procedure of choice.[282] PCR also can be performed on blood, serum, CSF, and bone marrow aspirates.

Ocular toxoplasmosis has been reported after allogeneic and autologous BMT and HSCT. In some cases the ocular disease was due to reactivation of a previously observed toxoplasmic chorioretinitis, whereas in most it has been associated with disseminated infection. Definitive diagnosis has been by direct observation of the parasite in histopathology sections, culture of tissue samples, or by PCR on vitreous fluid.

Toxoplasmosis in the AIDS Patient

Clinical manifestations of toxoplasmosis in AIDS patients commonly reflect encephalitis (i.e., TE), or infection of the lung (pneumonitis), and the eye (chorioretinitis).[284] Toxoplasmosis with multiorgan involvement manifesting with acute respiratory failure and hemodynamic abnormalities similar to septic shock has been reported, although septic shock has not been definitely proved to be due to *T. gondii*.[285] TE is the most common presentation of toxoplasmosis in AIDS patients and is a frequent cause of focal CNS lesions in these patients.[100] A wide range of clinical findings, including altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar signs, meningismus, movement disorders, and neuropsychiatric manifestations are seen in TE. The characteristic presentation usually has a subacute onset with focal neurologic abnormalities in 58% to 89% of patients. However, in 15% to 25% of cases the clinical presentation may be more abrupt, with seizures or cerebral hemorrhage. Most common, hemiparesis or abnormalities of speech, or both, are the major initial manifestations. Brainstem involvement often produces cranial nerve lesions, and many patients exhibit cerebral dysfunction with disorientation, altered mental state, lethargy, and coma. Less common, parkinsonism, focal dystonia,

rubral tremor, hemichorea-hemiballismus, panhypopituitarism, diabetes insipidus, or the syndrome of inappropriate antidiuretic hormone secretion may dominate the clinical picture. In some patients neuropsychiatric symptoms, such as paranoid psychosis, dementia, anxiety, and agitation, may be the major manifestations.

Diffuse TE[208] has been reported in relatively few AIDS patients; its actual incidence is unknown. This form of TE may manifest acutely and can be rapidly fatal; generalized cerebral dysfunction without focal signs is the most common manifestation, and CT scans may be within normal limits or reveal cerebral atrophy.

Spinal cord involvement by *T. gondii* in AIDS patients manifests as motor or sensory disturbances of single or multiple limbs, bladder or bowel dysfunctions (or both), and local pain. Patients may present with a clinical syndrome resembling a spinal cord tumor. Reports of cervical myelopathy,[286] thoracic myelopathy,[287] and conus medullaris syndrome[288] have been published.

Pulmonary disease caused by toxoplasmosis has been reported in patients with AIDS, and the diagnosis may be made by demonstration of the parasite in BAL fluid.[289] In France, before ART and routine use of prophylaxis, the prevalence of pulmonary toxoplasmosis in patients dually infected with HIV and *T. gondii* was estimated to be approximately 5%.[290] Pulmonary toxoplasmosis occurs mainly in patients with advanced AIDS (mean CD4 count, 40 cells/mm^3 ± 75 standard deviation) and primarily presents as a prolonged febrile illness with cough and dyspnea,[289] which may be clinically indistinguishable from *Pneumocystis* pneumonia. Mortality, even when treated appropriately, may be as high as 35%. Extrapulmonary disease may be present in about 50% of cases with toxoplasmic pneumonitis.[285] Often, pulmonary toxoplasmosis is not associated with TE; however, TE may develop after successful treatment of pulmonary toxoplasmosis when therapy is discontinued. The differential diagnosis of toxoplasmic pneumonitis in AIDS patients includes *Pneumocystis* pneumonia, viral pneumonia (e.g., caused by CMV or community-acquired respiratory viruses), pneumonia caused by atypical pathogens (e.g., *Chlamydia* spp., *Mycoplasma pneumoniae*), and infection with *M. tuberculosis, Cryptococcus neoformans*, *Coccidioides* spp., and *Histoplasma capsulatum*.

Toxoplasmic chorioretinitis is seen relatively infrequently in AIDS patients[291]; it commonly manifests with ocular pain and loss of visual acuity (Fig. 278.5). Funduscopic examination usually demonstrates necrotizing lesions that may be multifocal or bilateral.[291] Overlying vitreal inflammation is often present and may be extensive. The optic nerve may be involved in as many as 10% of cases. Toxoplasmic chorioretinitis in AIDS patients has been associated with concurrent TE in up to 63% of patients. The differential diagnosis of toxoplasmic chorioretinitis in AIDS patients includes CMV retinitis, syphilis, herpes simplex, varicella zoster, and candidiasis. The diagnosis relies primarily on clinical findings and the response to anti–*T. gondii* therapy, although definitive diagnosis may be made by demonstration of the organism in retinal biopsies,[291] isolation of the parasite from vitreous aspirates,[292] or amplification of the parasite DNA by PCR.[212]

GI involvement may result in abdominal pain, ascites (resulting from involvement of the stomach, peritoneum, or pancreas), or diarrhea. Acute hepatic failure caused by *T. gondii* has been reported,[227] as has musculoskeletal involvement.[220]

Although the incidence of toxoplasmosis in HIV-infected patients began to decrease after the broad use of TMP-SMX for PCP prophylaxis, the incidence decreased dramatically as a result of the immune reconstitution associated with ART regimens. Approximately fourfold decreases in both incidence and death have been reported after the wide availability of ART regimens.[101,103,293] Estimated hospitalization rates for HIV-related toxoplasmosis similarly decreased from a peak of ≈10,500 to ≈3,000 in the early 2000s but subsequently stabilized at that level.[102] Yet despite this, TE remains one of the most common HIV-related neurologic disorders, accounting for 26% of such cases in one recent study, with a 1-year estimated survival of 77%.[294] In contrast to mycobacterial and cryptococcal infections, immune reconstitution inflammatory syndrome (IRIS) after the initiation of ART has been only rarely reported in association with toxoplasmosis.[295–298] A recent retrospective review of patients followed at six Dutch hospitals identified paradoxical IRIS at a median of 49 days after starting combination antiretroviral therapy (cART) in 3.5% of 143 patients with previously diagnosed TE, and unmasking IRIS in 0.36% of 2228 patients with no prior diagnosis at a median of 35 days after starting cART.[299] In patients with CD4 counts that are sustained to levels greater than 200 cells/mm^3 for 3 to 6 months while receiving ART, primary prophylaxis and chronic maintenance regimens can be safely discontinued, although rare cases of disease can occur even in patients with well-controlled HIV infection.[300–303] Primary

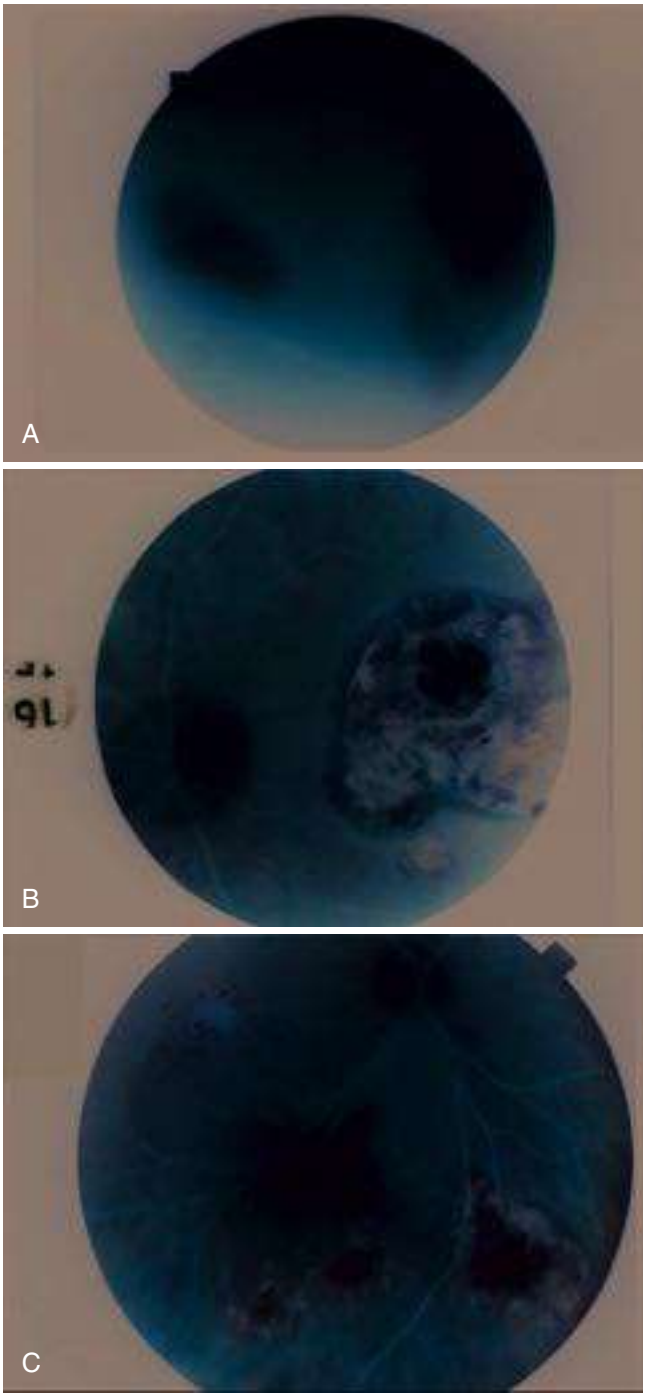

FIG. 278.5 Toxoplasmic chorioretinitis. (A) Active chorioretinitis with two lesions and vitreous haze in a human immunodeficiency virus–infected patient. (B) A large inactive macular lesion typical of congenital disease. (C) Active chorioretinitis in an immunocompetent patient from an endemic region in Brazil. There is an inactive lesion on the right. Note the macular star, which is due to an exudate around the macula. *(Courtesy Dr. Robert Nussenblatt, National Eye Institute, National Institutes of Health, Bethesda, MD; Dr. Claudio Silveira, Erechim, Brazil; and Dr. Rubens Belfort, São Paulo, Brazil.)*

prophylaxis can also be discontinued in those with CD4 counts of 100 to 200 cells/μL in the setting of controlled viremia (below detection limits for at least 3–6 months).[304]

There are no data on whether the strain of *T. gondii* affects the clinical outcome of infection in AIDS patients, and there are conflicting reports on whether a particular strain is more likely to cause infection in such patients.[18,305]

Ocular Toxoplasmosis in Immunocompetent Patients

T. gondii is one of the most frequently identified etiologies of uveitis and the most commonly identified pathogen to infect the retina of otherwise immunocompetent individuals.[306] It accounted for 8.4% of cases of uveitis seen in a clinic in San Francisco over a 24-year period.[307] Toxoplasmosis is responsible for more than 85% of posterior uveitis cases in southern Brazil, where in one study 9.5% of 21 seroconverters and 8.3% of 131 seropositive patients without ocular involvement developed typical lesions during 7 years of follow-up.[308–310] Chorioretinal lesions may result from congenital or postnatally acquired infection. In both these situations lesions may occur during the acute or latent (chronic) stage of the infection.[241,311] Recurrences are frequent, occurring in 79% of patients followed for more than 5 years in one study, with a median time to recurrence of 2 years; they tend to occur in clusters, that is, immediately after an episode rather than randomly, are more common in patients older than 40 years, are more common in the eye originally involved, and may be more common after cataract extraction.[312–314]

It is frequently difficult to determine whether the original infection was congenital or acquired in patients who suffer recurrences of chorioretinitis.[242] Although previously it was thought that less than 1% of cases were acute infections, the remainder being reactivations, recent evidence indicates that 11.7% of US ocular infections are acute.[315] Patients who present with chorioretinitis as a late sequela of the infection acquired in utero tend to have more severe disease and are more frequently in the second and third decades of life (it is rare after the age of 40 years); bilateral disease, old retinal scars, and involvement of the macula are hallmarks of the retinal disease in these cases, as are recurrences (see Fig. 278.5).[9] By contrast, patients who present with toxoplasmic chorioretinitis in the setting of acute toxoplasmosis are more often between the fourth and sixth decades of life, most often have unilateral involvement, and have eye lesions that usually spare the macula and do not present with associated old scars.[241,242]

Whereas acquired *T. gondii* infection in otherwise healthy adults is most often subclinical, toxoplasmic chorioretinitis in these individuals may result in retinal detachment, complete or partial loss of vision, or glaucoma, and it may necessitate enucleation.[9,316–318] Acute chorioretinitis may produce symptoms of blurred vision, scotoma, pain, photophobia, and epiphora. Impairment or a loss of central vision occurs when the macula is involved. As inflammation resolves, vision improves, frequently without complete recovery of visual acuity, caused in large part by macular scar formation.[307] In a cohort of 230 patients recently followed prospectively in Brazil for a mean of nearly 3 years, 45% experienced recurrent episodes, 4% developed a lesion in the previously unaffected eye, and 53% had decreased visual acuity (<20/30) at the end of follow-up.[317] In most cases toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empirical therapy directed against the organism is often instituted based on clinical findings and serologic test results. Typical features of toxoplasmic chorioretinitis include intensely white focal lesions with an overlying, intense, vitreous inflammatory reaction (see Fig. 278.5). After acute infection, lesions may present with focal retinal whitening only; such patients are at higher risk for developing chorioretinitis than patients with no lesions.[319] Focal necrotizing retinitis initially appears in the fundus as a yellowish-white, elevated cotton patch with indistinct margins, usually on the posterior pole. The lesions are often in small clusters, and individual lesions in the cluster may be of varied ages. With healing, the lesions pale, atrophy, and develop black pigment (see Fig. 278.5). There can also be an associated, secondary iridocyclitis and increased intraocular pressure.[316] The classic "headlight in the fog" appearance is due to the presence of active retinal lesions with severe vitreous inflammatory reaction. The choroid is secondarily inflamed. Recurrent lesions tend to occur at the borders of chorioretinal scars, and scars are often found in clusters. Panuveitis may accompany chorioretinitis, but isolated anterior uveitis has never been proved to occur.

Although the morphology of the lesions of acute toxoplasmic chorioretinitis in the setting of postnatally acquired disease may be indistinguishable from those observed in patients who suffer acute eye disease in later life caused by a congenitally acquired infection, it is important to attempt to establish which type of the infection (postnatally acquired or congenital) is occurring in a given patient.[241] It appears that the congenitally acquired disease has a more guarded prognosis. From the public health perspective, it is important epidemiologically to establish whether the patient has acute acquired infection, to initiate efforts to identify the possible source of *T. gondii* infection, and to determine whether other individuals who may be at high risk for developing severe, life-threatening disease (i.e., fetuses of serologically negative pregnant women or immunodeficient individuals) shared the same exposure as the individual with acute acquired toxoplasmic chorioretinitis. Serologic tests have been useful in establishing whether such patients have been infected recently.[240,241] In patients with chorioretinitis and IgG antibodies, additional serologic tests should be performed to determine whether the patient's infection is recently acquired.[241]

T. gondii chorioretinitis may resemble the posterior uveitis of tuberculosis, syphilis, leprosy, or presumed ocular histoplasmosis syndrome.

Atypical clinical and serologic manifestations of toxoplasmic chorioretinitis have been reported most commonly in elderly and in immunodeficient individuals.[212,320] Patients are considered to have atypical-appearing lesions when one or more of the following features are present: multiple foci of active retinitis, acute retinal necrosis syndrome (vitritis, peripheral retinitis, retinal vasculitis), significant intraretinal hemorrhage, an absence of ophthalmoscopically visible chorioretinal scarring. In patients with atypical lesions or an inadequate clinical response to anti-*Toxoplasma* therapy or in whom other diagnostic procedures have not proved helpful, obtaining vitreous or aqueous fluid (in some cases indicated for therapeutic reasons as well) for PCR should be considered early in the workup (see "Diagnosis").[213] In the future it may be possible to routinely determine which strain of the parasite is responsible for the infection and make a more accurate prognosis. Preliminary indications are that strain type may play an important part in determining the course of the infection,[218,219] and methods to distinguish strain type by looking for strain-specific antibodies using polymorphic peptides have shown promise.[23,87,321]

Toxoplasmosis During Pregnancy

As in other immunocompetent individuals, acute *Toxoplasma* infection is asymptomatic in the majority of pregnant women. Based on newly developed serologic assays that can detect antibodies to sporozoites,[60] the majority of women in one US study appeared to have been initially infected with oocysts, and a high proportion were unable to identify the potential source of such infection.[322] Given these factors, women should be universally screened by serology to maximize diagnosis of toxoplasmosis during pregnancy. The most commonly recognized clinical manifestation of recent infection is regional lymphadenopathy. The primary concern is transmission of infection to the fetus. The risk to the fetus does not correlate with whether the infection in the mother was symptomatic or asymptomatic during gestation. Transmission to the fetus has been limited almost solely to those women who acquire the infection during gestation. Otherwise healthy women with prior *Toxoplasma* infection are protected from transmitting the infection to their fetuses. Rare exceptions to this dictum have been observed. In immunocompetent women infected with *T. gondii* shortly before conception, transmission to the fetus has occurred; in these very rare instances, the acute infection was acquired within 3 months of conception.[323–325] Transmission to the fetus has been rarely recognized as a consequence of reactivation of latent *T. gondii* infection in immunocompromised women infected with *T. gondii* before conception (chronic infection) (e.g., pregnant women coinfected with HIV and *T. gondii*,[326] patients with systemic lupus erythematosus who are being treated with corticosteroids). In addition, reinfection with a second, more aggressive strain of *T. gondii* of otherwise healthy pregnant women who are already

chronically infected with the parasite has been proposed and documented as a possible mechanism of transmission to the fetus.[128,323]

Congenital Toxoplasmosis

Congenital infection may present in one of five forms: (1) ultrasound abnormalities consistent with toxoplasmosis or positive amniotic fluid PCR test results in the fetus; (2) neonatal disease; (3) disease (mild or severe) occurring in the first months of life; (4) sequelae or relapse of a previously undiagnosed infection during infancy, childhood, or adolescence; and (5) subclinical infection.

Data accumulated from prospective studies indicate that the incidence and severity of congenital toxoplasmosis vary with the trimester during which the infection was acquired by the mother.[9] Moreover, there is an inverse relationship between the frequency of transmission and the severity of disease. Infants born of mothers who acquire their infection in the first and second trimester more frequently show severe congenital toxoplasmosis.[327] In contrast, the majority of children born of women who acquire their infection during the third trimester are born with the subclinical form of the infection. However, if left untreated, as many as 85% of these latter children develop signs and symptoms of the disease, in most cases chorioretinitis or delays in development.[328,329] Although the majority of neonates born to mothers infected later in gestation have subclinical infection, recent reports suggest that when infected with a more virulent or atypical strain, severe congenital disease may occur despite having acquired their infection late in gestation.[330]

Infection acquired in the first trimester by women who were not treated with anti–*T. gondii* drugs resulted in congenital infection in 25% of cases in one report.[327] For second- and third-trimester infections, the incidences of fetal infection were 54% and 65%, respectively.[327] The overall rate of vertical transmission in this study of untreated women was 50%.[327] Early treatment of the mother with spiramycin appears to reduce the incidence of congenital infection by about 60%.[327,331–334] Maternal infection acquired around the time of conception and within the first 2 weeks of gestation and treated with spiramycin usually does not result in transmission.[333] Because of the high transmission rates observed in the late second trimester and during the third trimester, it is recommended that pyrimethamine-sulfadiazine be used in patients in whom acute infection is highly suspected or confirmed as having occurred at 14 weeks of gestation or later.[334a,334b]

Frequency of transmission to the fetus and severity of disease in the offspring appear to be significantly affected by drug treatment with spiramycin and pyrimethamine-sulfadiazine as well. In cohorts where most of the mothers have been treated during gestation, transmission rates were low in the first trimester. (i.e., 6% [95% confidence interval [CI], 3% to 9%)] at 13 weeks) and increased as expected with advancing gestational age (i.e., 40% [95% CI, 33% to 47%] at 26 weeks and 72% [95% CI, 60% to 81%] at 36 weeks).[335] The overall rate of vertical transmission in this study of treated women was 29% (95% CI, 25% to 33%).[335] Clinical signs in the infected infant were more likely observed in offspring of women whose infection was acquired early in gestation. Depending on when during gestation the mother acquired her infection, the risk for severity of clinical manifestations in an infected fetus was 61% (95% CI, 34% to 85%) at 13 weeks, 25% (95% CI, 18% to 33%) at 26 weeks, and 9% (95% CI, 4% to 17%) at 36 weeks.[335] A recent study reported the lowest rates of overall transmission (4.8%) and clinical manifestations in the newborn (1.6%) published to date.[336] In this cohort pregnant women with *T. gondii* infection acquired during gestation received spiramycin until the 16th week of gestation, followed by at least 4 weeks of a pyrimethamine-sulfadiazine–folinic acid combination independent of the infection status of the fetus. If fetal infection was suspected (e.g., ultrasound abnormalities suggestive of congenital infection are observed) or confirmed (e.g., positive amniotic fluid PCR test), combination treatment was continued until delivery. In their cohort their rates of transmission for the first, second, and third trimester were 1.3%, 10.6%, and 21.7%, respectively. Another study reported that prenatal treatment with spiramycin and/or pyrimethamine-sulfadiazine resulted in a significantly reduced risk of severe neurologic sequelae or death in the infected offspring.[337] Of note, recently severe congenital toxoplasmosis has only been reported from countries such as the United States and Brazil, where universal screening and treatment during gestation have not been implemented.[338,339]

In addition to gestational age and treatment, transmission rates and severity of congenital disease are also likely to be affected by the strain of the parasite,[340,341] infecting form of the parasite (cyst vs. oocyst), parasite load,[342,343] and immune status and genetics of the host.

Clinical manifestations of congenital toxoplasmosis vary. Most signs and clinical presentations are nonspecific and may mimic disease caused by organisms such as herpes simplex virus (HSV), CMV, Zika virus, and rubella virus. Signs include chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, anemia, jaundice, rash, petechiae resulting from thrombocytopenia, encephalitis, pneumonitis, microcephaly, intracranial calcification, hydrocephalus, diarrhea, hypothermia, and nonspecific illness.[9] There may be no sequelae, or sequelae may develop or be evident at various times after birth.

A detailed examination by an experienced clinician may be necessary to detect signs of the infection.[9] In one prospective study[344] 210 congenitally infected infants were identified: 2 patients (0.9%) died, 21 (10.9%) had severe disease, 71 (33.8%) were mildly afflicted, and 116 (54.4%) were without signs of the infection. More intensive examination of the latter 116 infants revealed abnormalities in 39; abnormal CSF was detected in 22 infants, chorioretinitis was seen in 17, and intracranial calcifications were found in 10. Premature infants often suffer CNS disease and ocular disease in the first 3 months of life. Full-term infants frequently develop a milder disease manifested by hepatosplenomegaly and lymphadenopathy that usually appear in the first 2 months of life. In these infants, disease reflecting damage to the CNS may occur later, and eye disease may occur months to years after birth.

Most untreated infants with subclinical infection at birth subsequently develop signs or symptoms of congenital toxoplasmosis, including chorioretinitis, which may lead to blindness, neurologic manifestations such as seizures, and sensorineural hearing loss.[329,345] Prospective observational studies suggest that early initiation of specific therapy (prenatally and postnatally) in infants with congenital infection but without clinical signs will markedly reduce untoward sequelae.[346,347] In one cohort, among children who were diagnosed with toxoplasmic chorioretinitis only after their first year of life (thus who were not treated during their first year of life), new chorioretinal lesions were detected in more than 70%,[348] whereas among 108 congenitally infected children treated during their first year of life, only 31% developed at least one new chorioretinal lesion. Thirteen percent had occurrences when they were 10 years or older, indicating that long-term follow-up is important.[346,349] Similar results were seen in a prospective French study of 477 treated patients followed for up to 22 years.[350] It appears that, when treated, initially asymptomatic congenital toxoplasmosis has an overall good prognosis.[346]

Uncommonly, latent *T. gondii* infection may reactivate in HIV-infected women and result in congenital transmission of the parasite. Congenital toxoplasmosis appears to occur more frequently in the offspring of women infected with both HIV and *T. gondii* than in those of women who are infected with *T. gondii* but not with HIV.[351] Infants with congenital toxoplasmosis born to HIV-infected mothers are also infected with HIV, suggesting that factors predisposing to the vertical transmission of HIV also favor the transmission of *T. gondii*, or vice versa. Congenital toxoplasmosis in the HIV-infected infant appears to run a more rapid course than that in the non–HIV-infected infant, with the development of failure to thrive, fever, hepatosplenomegaly, chorioretinitis, and seizures. Most children have multiorgan involvement, including CNS, cardiac, and pulmonary disease.

It has been recently reported that *T. gondii* causes more severe ocular disease in congenitally infected children in Brazil when compared with those in Europe.[216] As stated earlier, this may be explained by the fact that pregnant women in Brazil are not routinely screened and treated for toxoplasmosis during gestation and possibly that more virulent genotypes of the parasite predominate in Brazil but are rarely found in Europe.

Congenital toxoplasmosis must be differentiated from rubella virus, CMV, Zika virus, HSV, HHV-6, parvovirus B19, and lymphocytic choriomeningitis virus infections; syphilis, listeriosis, and other bacterial infections; other infectious encephalopathies; erythroblastosis fetalis; and sepsis. HSV, CMV, Zika virus, rubella virus, and syphilis may cause

chorioretinitis; both CMV and rubella have been associated with hydrocephalus, microcephaly, and cerebral calcification. Zika virus particularly has been associated with microcephaly.[352] A markedly elevated CSF protein concentration is a hallmark of congenital toxoplasmosis.

T. gondii infection acquired during pregnancy has been implicated in spontaneous abortion, stillbirth, and premature births. On rare occasions *T. gondii* has been isolated from the abortuses of women with chronic infection, but the frequency of *T. gondii* infection as a cause of abortion is unknown and controversial.

As with ocular disease the strain of *T. gondii* may have an impact on the outcome of congenital infection. Studies in Europe have revealed conflicting data on whether type I strains, in particular, might be more likely to be responsible for congenital infection and/or serious disease.[19,20,353] Non–type II strains were associated with prematurity and severity of disease at birth in a study from the United States that used serologic tests as a method to determine the strain type.[354] A recent meta-analysis found that type I and atypical strains were associated with clinical complications.[340] As more accurate and sensitive tests for determining the genotype of an infecting strain are developed, this picture could change significantly.

DIAGNOSIS

When considering toxoplasmosis in the differential diagnosis of a patient's illness, emphasis should not be placed on whether the patient has been exposed to cats. Transmission of oocysts virtually always occurs without the knowledge of the patient and may be unrelated to direct exposure to a cat (e.g., transmission by contaminated vegetables, other foods, or water). Patients with an indoor cat that is fed only cooked food are not at risk of acquiring the infection from that cat. Serologic investigation of a cat to establish whether it is a potential source of the infection is useless and should be discouraged; the prevalence of *T. gondii* antibodies among cats in a given locale is usually similar to their prevalence in humans. Seropositivity does not predict shedding of oocysts. In several epidemiologic studies in Europe and the United States, acute *Toxoplasma* infection has been documented in patients without symptoms and without known epidemiologic risk factors for acute infection.[355,356]

Because the clinical manifestations of *T. gondii* infection may be protean and nonspecific, toxoplasmosis must be carefully considered in the differential diagnosis of a large variety of clinical presentations. The correct diagnostic tests must be performed and appropriately interpreted in light of the patient's clinical presentation. The usefulness of a given diagnostic method may differ considerably with the clinical category, which can be toxoplasmosis in the immunocompetent or immunodeficient patient, ocular toxoplasmosis, toxoplasmosis in pregnancy, or congenital toxoplasmosis.[357]

Acute infection is diagnosed by characteristic serologic test results; demonstration of tachyzoites in tissue or body fluids; amplification of *T. gondii* DNA or isolation of the parasite in body fluids; the demonstration of a characteristic lymph node histologic appearance; or demonstration of *T. gondii* tissue cysts in the placenta, fetus, or neonate.[9] Rarely, asymptomatic patients with latent infection have recurrent parasitemia.[358] Amplification of parasite DNA or isolation of *T. gondii* from the tissues of older children or adults may only reflect the presence of tissue cysts. Finding numerous tissue cysts in tissue sections, especially associated with inflammation, suggests but does not prove the presence of active infection.

Serologic Tests for Demonstration of Antibody

The use of serologic tests for the demonstration of specific antibody to *T. gondii* tachyzoites or antigens is the primary method of diagnosis. A large number of tests have been described, some of which are available only in highly specialized laboratories. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with the time after infection.[357] However, initial serologic testing can be accomplished by simultaneously requesting IgG and IgM antibody tests. This task can be easily achieved by hospital-based, commercial, or nonreference laboratories. Only positive results in IgM antibody tests need to be sent for confirmatory testing to reference laboratories (see later).[359]

There is no single serologic test that can be used to support the diagnosis of acute or chronic infection by *T. gondii*. In most cases a battery of tests is required to enable the distinction between acute and chronic infection. Which particular combination of tests is used depends on the specific clinical category of the patient (i.e., pregnant vs. immunodeficient patient; see "Clinical Manifestations"), the interval between acquisition of infection and sampling of sera,[245] and the question posed by the practitioner. The clinician must be familiar with these problems and consult reference laboratories if the need arises. A panel of tests consisting of the Sabin-Feldman dye test (DT; detecting IgG), the IgM-, IgA-, and IgE–enzyme-linked immunosorbent assays (ELISAs), IgM immunosorbent agglutination assay (IgM-ISAGA) (bioMérieux; Marcy-l'Étoile, France), differential agglutination test (measures IgG antibody and is also known as the AC/HS test), and the IgG avidity test is used successfully by the PAMF-TSL to determine whether serologic test results are more likely consistent with infection acquired in the recent or more distant past.[198,241,245,360–362] It is also important to note that in 2011 a test was reported that showed great promise for discriminating between an infection derived from ingestion of oocysts versus tissue cysts.[60] It relies on detection of antibodies to a protein that is abundantly expressed in sporozoites but not in tachyzoites or bradyzoites, and it has the potential to address both the epidemiologic origins of infection in different cohorts/regions and could also allow a determination of whether disease initiated by one or other means leads to different clinical outcomes. What follows concerns only the generic detection of a *Toxoplasma* "infection," that is, tachyzoites, without regard to the original source of that infection, but such refinement is something anxiously anticipated for the near future.

Immunoglobulin G Antibodies

The most widely used tests for the measurement of IgG antibody are the Sabin-Feldman DT,[363] ELISA,[364,365] the indirect fluorescent antibody (IFA) test,[366] and the modified direct agglutination test.[367] In these tests IgG antibodies usually appear within 1 to 2 weeks of acquisition of the infection, peak within 1 to 2 months, fall at variable rates, and usually persist for life at relatively low titers.

Reports have shown that detecting antibodies to strain-specific peptides can reveal the genotype of the infecting strain; although this approach has been helpful in epidemiologic studies, it has not yet been developed into a reliable, clinically useful diagnostic method.[321,354]

Sabin-Feldman Dye Test

The Sabin-Feldman DT is the reference serologic test against which other methods have been evaluated.[363] It is a sensitive and specific neutralization test. It measures primarily IgG antibodies that usually appear 1 to 2 weeks after the initiation of infection, reach peak titers in 6 to 8 weeks, and then gradually decline over 6 to 12 months.[9] Titers, usually at low levels, probably persist for life. This test is available in only a few reference laboratories, primarily because live organisms are required. A negative Sabin-Feldman DT practically rules out prior exposure to *T. gondii*, except in patients who have been infected very recently (e.g., within 1–2 weeks of exposure), are significantly immunocompromised (e.g., allogeneic BMT patients), or who have a primary immunodeficiency (e.g., congenital agammaglobulinemia).

Recently, patients with established *T. gondii* infection have been documented to have lost their *Toxoplasma* IgG (and evidence of T-cell mediated immunity as well), likely indicating that lifelong persistence may not be universal in humans.[368] Early in infection (e.g., within 4 weeks of infection), patients may be negative in IgG-ELISA, agglutination, or IFA kits but positive in the DT. However, although rare, cases of documented TE and chorioretinitis have been reported in DT-negative patients.

Indirect Fluorescent Antibody Test

The IFA test appears to measure the same antibodies as the DT, and its titers tend to parallel DT titers.[9] False-positive results may occur with sera that contain antinuclear antibodies,[369] and false-negative results may occur in sera with low IgG antibody titers. Use of the *Toxoplasma* IFA test should be discouraged because of the relative high frequency of false-negative and false-positive results.

Agglutination and AC/HS Tests

The agglutination test using formalin-preserved whole tachyzoites is available commercially (bioMérieux) and detects IgG antibody. The test is very sensitive to IgM antibody, and "natural" IgM antibody causes nonspecific agglutination in sera that yield negative results when tested in the DT and the IFA test. This problem is avoided by including 2-mercaptoethanol in the test. The method is accurate, simple to perform, inexpensive, and excellent for screening purposes.[370] This method, that is, with 2-mercaptoethanol, should not be used for the measurement of IgM antibodies.

When two different compounds (i.e., methanol and formalin) are used to fix parasites for use in the agglutination test, a "differential" agglutination test (AC/HS test) results because the different antigenic preparations vary in their ability to recognize sera obtained during the acute and chronic stages of the infection.[371] This test has proved useful in helping differentiate acute from chronic infections and is best used in combination with a battery of other tests. When the AC/HS yields a "nonacute" pattern, the infection has been present for at least 12 months from the time of serum sampling.[372]

Immunoglobulin G Enzyme-Linked Immunosorbent Assay

The IgG-ELISA method is now the most widely used for the demonstration of IgG antibodies to *T. gondii*. Most commercial IgG antibody test kits are accurate for demonstration of IgG antibodies; however, it is important to recognize that for patient care one cannot use a single IgG titer, no matter what its level, to predict whether the infection was recently acquired or acquired in the distant past.

Immunoglobulin G Avidity Test

A number of tests for avidity of *Toxoplasma* IgG antibodies have been introduced to help differentiate between recently acquired and distant infection.[373] This method is based on the observation that during acute *T. gondii* infection, IgG antibodies bind antigen weakly (i.e., have low avidity), whereas chronically infected patients have more strongly binding (high avidity) antibodies.[373] Protein-denaturing reagents, including urea, are used to dissociate the antibody-antigen complex. Low or equivocal avidity test results can persist for months to years after the primary infection,[373] and for this reason a low or equivocal avidity test result must not be used to determine whether the infection was acquired recently. The time of conversion from low or equivocal to high avidity is highly variable among different individuals, including pregnant women.[360,374,375] However, it has been demonstrated that once the avidity test result is high, the patient was infected at least 3 to 5 months earlier.[375] This timing depends on the method used. High-avidity test results by the IgG-VIDAS avidity test (or VIDAS [Vitek Immuno-Diagnostic Assay System] *Toxoplasma* IgG Avidity, bioMérieux), for example, have been essentially found only in pregnant women who have been infected for at least 4 months.[362,376,377]

The avidity test should only be used as an additional confirmatory diagnostic method in patients with positive and/or equivocal IgM test results or when the results of a battery of tests are equivocal or interpreted as consistent with the possibility of a recently acquired infection. Health care providers involved in the care of pregnant women should be aware that avidity testing is only a confirmatory test. It should not be used alone as a definitive test for decision making.

Immunoglobulin M Antibodies

IgM antibodies may appear earlier and decline more rapidly than IgG antibodies. IgM antibody tests have been widely used for the diagnosis of acute infection and to determine whether a pregnant woman has been infected during gestation or before conception. There has been a heightened awareness of the fact that titers in tests for IgM antibodies may persist for years after the acute infection and that the reliability of commercially available assays varies considerably.[378–380] Both the laboratory performing the test and the physician requesting the test should be aware of this problem. In 1997 the US Food and Drug Administration (FDA) issued a health advisory warning about the use of *T. gondii* IgM commercial test kits as the sole determinant of recent infection in pregnant women.[381] At present, the decision to treat or undertake other medical interventions, including the termination of pregnancy, should be based on clinical evaluation and additional testing performed in reference or research laboratories with experience in the diagnosis of toxoplasmosis. In support of this recommendation, a recent retrospective study of samples from 451 patients with positive IgM at nonreference laboratories that were analyzed at a reference laboratory between 2003 and 2013 found that only 22% had an acute infection; of the remainder, 36% were IgM positive in the context of chronic disease.[359] For further discussion, see "*Toxoplasma gondii* Infection in Pregnancy."[382] The persistence of IgM antibodies for several years appears not to have clinical significance.[9]

Immunoglobulin M Indirect Fluorescent Antibody Test

IgM-IFA antibody appears within the first week of infection; titers rise rapidly and then fall to low titers and usually disappear within a few months. Low titers may persist 1 year or longer.[383] Antinuclear antibodies and rheumatoid factor may cause false-positive results.[384] IgG-blocking antibodies can cause false-negative results in this test when IgG is not removed.[385]

Immunoglobulin M Enzyme-Linked Immunosorbent Assay

The double-sandwich IgM-ELISA for detection of IgM-specific antibodies to *T. gondii*[386–388] is currently the most widely used method for demonstration of IgM antibodies to *T. gondii* in adults, the fetus, and newborns.[9] In contrast to the conventional method, in which the wells of microtiter plates are coated with antigen, the wells are coated with specific antibody to IgM. The double-sandwich IgM-ELISA is more sensitive than the IgM-IFA test for diagnosis of recently acquired infection, and serum samples that are negative in the DT, but that contain either antinuclear antibodies or rheumatoid factor and thus cause false-positive results in the IgM-IFA test, are negative in the double-sandwich IgM-ELISA. This latter observation is attributed to the fact that serum IgM fractions are separated from IgG fractions during the initial step in the double-sandwich IgM-ELISA procedure.[9]

Despite the wide distribution of commercial test kits to measure IgM antibodies, these kits often have low specificity, and the reported results are frequently misinterpreted. False-positive results and the problems associated with the persistence of positive titers, even years after the initial infection, remain major obstacles to correct interpretation of the results obtained in these tests.[359,379] Moreover, heat inactivation of serum (56°C, 30 minutes) can lead to decreases in IgM titers and false-negative results.[389]

Immunoglobulin M Immunosorbent Agglutination Assay

The IgM-ISAGA, which binds the patient's IgM to a solid surface and uses intact, killed tachyzoites to detect IgM antibodies, is highly sensitive.[390] The test is simple to perform, does not require the use of enzyme conjugate, and is read in the same manner as the agglutination test. Overall, it is more sensitive and specific than the IgM-IFA test. The presence of rheumatoid factor or antinuclear antibodies does not cause false-positive results in the IgM-ISAGA. In adults it is more sensitive but much less specific than the double-sandwich IgM-ELISA method. In infants the IgM-ISAGA is the most sensitive method and is used effectively for diagnosis of congenital infection in infants 6 months of age or younger.[391] A positive IgM-ISAGA test result in the first 10 days of life should be repeated after 10 days to rule out the possibility of maternal contamination of IgM antibodies.[392] The ISAGA method has also been used to detect IgA and IgE antibodies.

Immunoglobulin A Antibodies

IgA antibodies may be detected in sera of acutely infected adults and congenitally infected infants using ELISA or ISAGA.[393–395] As is true for IgM antibodies to the parasite, IgA antibodies may persist for many months or more than 1 year. However, IgA antibodies tend to disappear earlier than the IgM, and their presence at high titers is usually associated with early infections (e.g., within 3 months of the date of serum sampling). The increased sensitivity of IgA assays over IgM assays for the diagnosis

of congenital toxoplasmosis represents a major advance in the serologic diagnosis of the infection in the fetus and newborn.[395] A recent retrospective study confirmed the specificity of the ISAGA in diagnosing congenital infection.[396] IgA antibodies are rarely detectable by ELISA in sera of AIDS patients with TE.[395] If IgA antibodies are detected in the newborn during the first 10 days of life, the test should be repeated 10 days after birth to make certain that what is being measured is not contaminating maternal IgA antibodies. The possibility that such contamination might occur is the reason that, under most circumstances, peripheral blood rather than cord serum should be used to measure IgM, IgA, or IgE antibodies in the newborn.

Immunoglobulin E Antibodies

IgE antibodies are detectable by ELISA in sera of acutely infected adults,[397,398] congenitally infected infants,[397,398] and children with congenital toxoplasmic chorioretinitis.[399] The duration of IgE seropositivity is briefer than that with IgM or IgA antibodies and hence appears useful for identifying recently acquired infections.[245,398] *T. gondii*–specific IgE antibody has been detected in patients with TE and may be useful as a marker for TE in this population of patients.[398] IgE antibodies have been reported to be present in 85.7% of asymptomatic seroconverters and in 100% of seroconverters with overt toxoplasmosis.[400] For neonatal diagnosis of congenital toxoplasmosis, IgE was less sensitive than IgM and IgA, but simultaneous measurement of the three immunoglobulins at birth improved the diagnostic yield to 81%.[400] Emergence of specific IgE during postnatal treatment for congenital toxoplasmosis may indicate poor adherence or inadequate dosing.

Polymerase Chain Reaction

PCR amplification for the detection of *T. gondii* DNA in body fluids and tissues has successfully diagnosed congenital,[333,401] ocular,[212,213] pulmonary,[210] cerebral, and disseminated toxoplasmosis.[402,403] PCR of amniotic fluid has revolutionized the diagnosis of intrauterine *T. gondii* infection by enabling an early diagnosis to be made, thereby avoiding the use of invasive procedures on the fetus.[9,401] PCR has also been successfully used on samples of CSF, blood, urine, and placental and fetal tissues for diagnosis of congenital infection.[9,404–407] The sensitivity of PCR in CSF varies between 11% and 77%, whereas the specificity is close to 100%.[402] PCR may also detect the parasite in buffy coat specimens of AIDS patients with TE.[403] The sensitivity of PCR on whole blood or buffy coat ranges from 15% to 85%. PCR on blood appears to be a valuable tool primarily in patients with disseminated disease; it is less sensitive in the detection of TE because a relatively low percentage of AIDS patients with TE have parasitemia.[408,409] Prophylaxis or therapy for toxoplasmosis adversely impacts the sensitivity of the method; sensitivity is higher in CSF or blood samples collected before or within the first week of therapy.[403] In a recent case report, quantitative real-time PCR proved useful for the diagnosis and monitoring of *Toxoplasma* infection in a heart transplant recipient who developed toxoplasmosis after TMP-SMX for PCP prophylaxis had been stopped. Decreasing parasitic burdens in sequential samples of CSF, blood, and BAL fluid correlated with a favorable outcome and allowed modulation of the immunosuppressive drug regimen in this patient.[410] Peripheral blood PCR has also been found to be positive in immunocompetent patients in Brazil who present with symptomatic ocular toxoplasmosis as a result of reactivation of an infection acquired in the distant past.[411,412] It is unclear whether these findings apply only to patients in this area of the world, where atypical and more virulent strains are common in contrast to Europe and the United States.

Because there is no standardized PCR assay, performance characteristics will vary widely depending on the laboratory, gene target, primers, and sample preparation.[413] Primers targeting multicopy *B1* or *AF146527* (also known as the 529–base pair [bp] repeat element) genes appear to be the most sensitive and are the most broadly used.[413–415] A number of investigators have reported a higher sensitivity of the 529-bp target for the prenatal diagnosis of congenital toxoplasmosis when compared with the *B1* gene.[416,417] For maximal reliability, clinical samples should be sent to reference laboratories experienced in performing this assay.[406,418]

A retrospective analysis of French laboratories performing routine surveillance on blood from HSCT patients using PCR for the *AF146527* gene found that 23 of 1220 blood samples (1.9%) were positive, a lower figure than other series.[419] Fifteen patients were not receiving TMP-SMX prophylaxis and were treated for toxoplasmosis. The other eight were simply continued on TMP-SMX prophylaxis. No clinical signs of toxoplasmosis resulted. This and other studies suggest that a PCR-based preemptive strategy is useful in the earlier diagnosis and treatment of *Toxoplasma* infection, before patients develop organ involvement.[278,281,420]

Histologic Diagnosis

Demonstration of tachyzoites in tissue sections or body fluids (e.g., CSF, amniotic fluid, or BAL) establishes the diagnosis of acute infection or reactivation of a latent infection.[9] It is often difficult to demonstrate tachyzoites in stained tissue sections. Multiple tissue cysts near an inflammatory necrotic lesion can be considered as diagnostic of acute infection or reactivation of a latent infection.[421] Fluorescent antibody staining may be useful, but this method often yields nonspecific results.[422] The immunoperoxidase technique, which uses antisera to *T. gondii*, has proved both sensitive and specific; it has been used successfully in clinical settings to demonstrate the organisms in the CNS of patients with TE.[42] Both the fluorescent antibody and immunoperoxidase methods are applicable to unfixed or formalin-fixed paraffin-embedded tissue sections.[42] Fluorescein-labeled monoclonal antibodies to *T. gondii* for staining touch preparations of specimens[423] and rapid electron microscopy[424] have been used successfully to diagnose TE.

A rapid, technically simple but underused method is the detection of *T. gondii* in air-dried, Wright-Giemsa–stained slides of centrifuged (e.g., cytocentrifuged) sediment of CSF or of brain aspirate or in impression smears of biopsy tissue.

Endomyocardial biopsy has been used successfully to diagnose toxoplasmosis in heart transplant recipients.[425] Characteristic histologic criteria alone are probably sufficient to establish the diagnosis of TL in older children and adults (see "Lymph Node" under "Pathology").[197]

Isolation of *Toxoplasma gondii*

Although largely replaced by use of PCR, isolation of *T. gondii* from blood or body fluids can also be used to establish that the infection is acute. In neonates isolation of the organism from the placenta is highly suggestive of fetal involvement, and isolation from fetal tissues is diagnostic of congenital infection.[9] Attempts at isolation of the parasite can be performed by mouse inoculation or inoculation of tissue cell cultures.[9] In tissue cell cultures parasite-laden cells can be demonstrated with appropriate staining, and plaques are formed in which tachyzoites are easily recognized. Tissue cell culture has the advantage of widespread availability (e.g., virology laboratories) and yields results more rapidly (within 3–6 days) than does mouse inoculation.

Radiologic Methods

Radiologic studies are of particular help in patients with toxoplasmosis of the CNS. The presence of calcifications in the brain of a newborn, detected by radiography, ultrasonography, or CT, should heighten the suspicion of *T. gondii* as the cause of the disease (Fig. 278.6A). In severely affected infants with congenital toxoplasmosis, unilateral or, more often, bilateral and symmetrical dilatation of the ventricles is a common finding.[9]

In the majority of immunodeficient patients with TE, CT scans show multiple bilateral cerebral lesions.[426] Although multiple lesions are more common in toxoplasmosis, they also may be solitary; a single lesion should not exclude TE as a diagnostic possibility. Clinicians should be aware that toxoplasmosis may manifest as an encephalitis that at autopsy is "diffuse," in which case the neuroimaging study results may appear normal or reveal findings suggestive of HIV encephalopathy.[208]

CT scans in AIDS patients with TE reveal multiple ring-enhancing lesions in 70% to 80% of the cases.[284] In AIDS patients who are not receiving appropriate ART or anti-*Toxoplasma* prophylaxis but have detectable *Toxoplasma* IgG and multiple ring-enhancing lesions seen on CT or MRI, the predictive value for TE is approximately 80%.[427] Lesions tend to occur at the corticomedullary junction, frequently involving the basal ganglia, and are characteristically hypodense.[428,429] The number of lesions is frequently underestimated by CT, although delayed imaging after a double dose of intravenous (IV) contrast

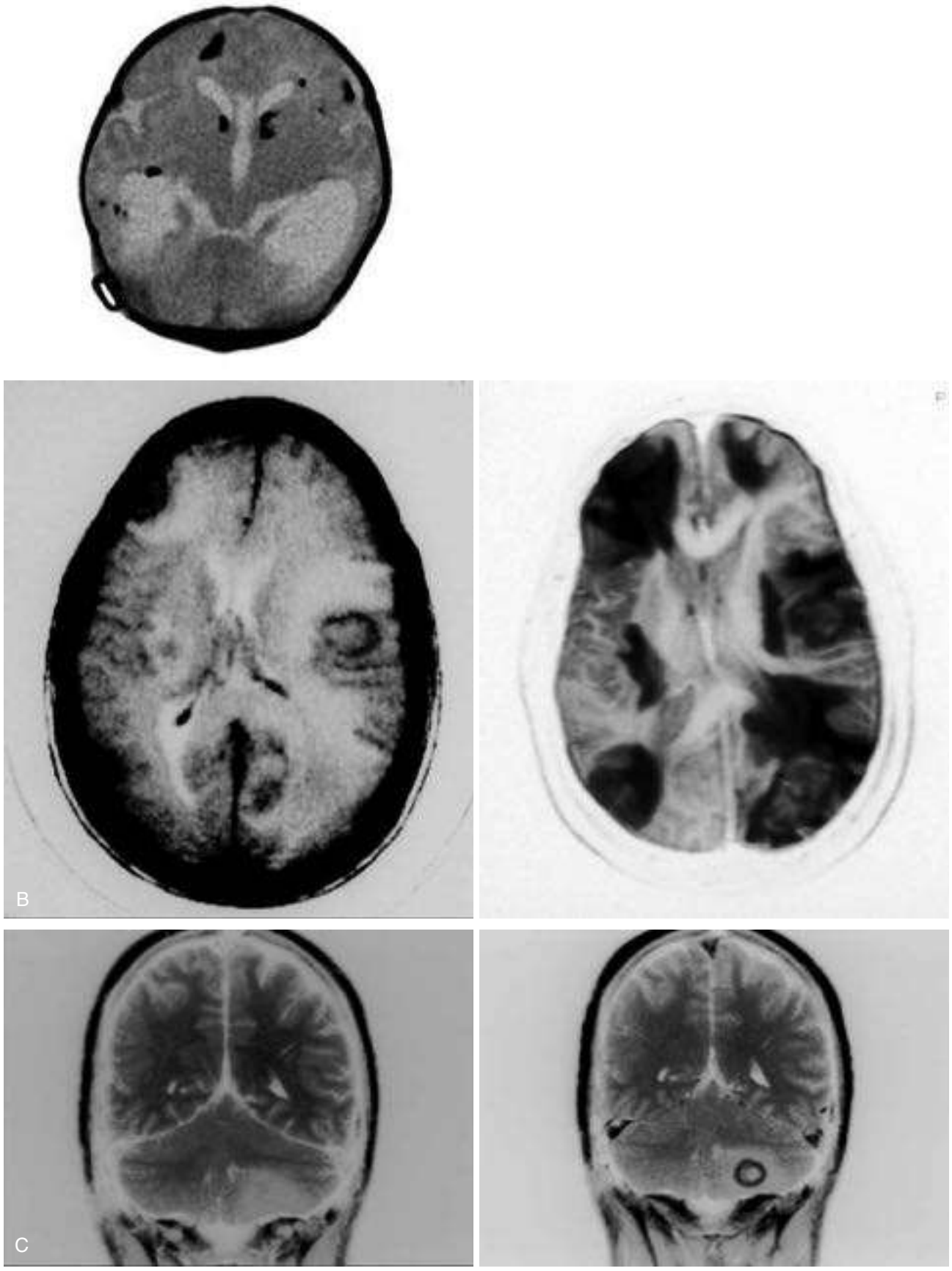

FIG. 278.6 Imaging studies of central nervous system toxoplasmosis. (A) Computed tomography (CT) scan of an infant born with congenital toxoplasmosis, illustrating the calcifications and hydrocephalus that are typically seen in the brain. (B) CT scan with contrast enhancement *(left)* and T2-weighted magnetic resonance imaging (MRI) scan *(right)* of an acquired immunodeficiency syndrome (AIDS) patient with toxoplasmic encephalitis, demonstrating multiple lesions, which are more easily identified in the MRI scan. (C) T1-weighted MRI scan without *(left)* and with *(right)* contrast enhancement of an AIDS patient with toxoplasmic encephalitis. Note the ring-enhancing lesion on the right.

material may improve the sensitivity of this modality.[428–430] An enlarging hypodense lesion that does not enhance is a poor prognostic sign.[431] TE lesions on MRI studies appear as high-signal abnormalities on T2-weighted studies and reveal a rim of enhancement surrounding the edema on T1-weighted contrast-enhanced images (see Fig. 278.6B and C). MRI has superior sensitivity compared with CT, particularly if gadolinium is used for contrast, and often demonstrates a lesion or lesions or more extensive disease not seen by CT.[430,432] Hence MRI should be used as the initial procedure when feasible or if a single lesion is demonstrated by CT. The eccentric target sign in a T1 postcontrast image is particularly suggestive of toxoplasmosis. Nevertheless, even characteristic lesions on CT or MRI studies are not pathognomonic of TE. The major differential diagnosis of focal CNS lesions in AIDS patients is CNS lymphoma, which may manifest with multiple enhancing lesions in 40% of cases. The probability of TE falls and the probability of lymphoma rises in the presence of single lesions on MRI.[426] A brain biopsy may therefore be required in the patient with a solitary lesion, especially if confirmed by MRI, to obtain a definitive diagnosis.[433] In non-AIDS immunocompromised patients, TE can also present as single or multiple ring-enhancing lesions. However, additional etiologies, including invasive fungal, nocardial, and mycobacterial infections, are more common in these patients with this radiologic presentation than in AIDS patients. Early brain biopsy and empirical treatment with a wider antimicrobial spectrum is usually required in non-AIDS immunocompromised patients with multiple space-occupying brain lesions.

In AIDS patients with TE, CT-scan improvement is seen in up to 90% of patients after 2 to 3 weeks of treatment.[426,428] Complete resolution can take from 6 weeks to 12 months; peripheral lesions resolve more rapidly than deeper ones. Smaller lesions usually resolve completely on MRI studies within 3 to 5 weeks, but lesions with a mass effect tend to resolve more slowly and leave a small residual lesion.[434] In patients receiving combination ART, lesions may persist with contrast enhancement for months to years despite adequate treatment for TE. A radiologic response to therapy lags behind the clinical response, with better correlation between them observed by the end of acute therapy.[435]

CT and MRI in toxoplasmic myelopathy usually demonstrates localized enlargement of the spinal cord,[287,436] which may result in obstruction to dye flow on myelography.[287] Gadolinium enhancement of MRI studies usually highlights as an intramedullary lesion at the site of spinal cord enlargement.[436,437]

A variety of positron-emission tomography (PET) scanning,[438] radionuclide scanning,[439] and MRI techniques[440] have been used to evaluate AIDS patients with focal CNS lesions, specifically to differentiate between toxoplasmosis and primary CNS lymphoma.[440] ^{18}F-fluorodeoxyglucose (FDG)-PET scanning is now widely used in the evaluation of patients with tumors. There is a significantly higher uptake of ^{18}F-FDG in patients with cerebral lymphoma than in patients with TE.[441] Radionuclide scanning has also been used to differentiate between CNS toxoplasmosis and lymphoma. Neoplasms usually demonstrate increased uptake of thallium 201 on both early and late scanning.[440] Although published studies suggest a high sensitivity and specificity of these studies, in practice they often are not helpful, in part because of variability in uptake and in part because they are often used after an empirical trial of anti-*Toxoplasma* therapy. Thallium scans may have decreased diagnostic utility in the setting of ART.[442]

Proton magnetic resonance (MR) spectroscopy to evaluate brain lesions has been used in a few patients. MR spectroscopy in patients with TE reveals an elevation in the lactate and lipid contents[440] and a decrease in the levels of choline. In contrast, MR spectroscopy in patients with CNS lymphoma reveals mildly elevated levels of choline.[440]

Cerebrospinal Fluid Abnormalities

CSF abnormalities in patients with TE are nonspecific; mild mononuclear pleocytosis and mild-to-moderate elevations in CSF protein are often observed, and hypoglycorrhachia is uncommon.[232,443] Almost unique to infants with neonatal toxoplasmosis, however, is the very high protein content of the ventricular fluid and eosinophilia. Although in some infants the protein level is just slightly above normal, in others it can be measured in grams per deciliter rather than in milligrams per deciliter.[9] Demonstration of intrathecal production of *T. gondii*–specific IgG or IgM in the absence of CSF contamination with blood is diagnostic of TE.[444,445]

Diagnosis of Specific Clinical Entities

The initial step in pursuing the diagnosis of *T. gondii* infection or toxoplasmosis is to determine whether the patient has been exposed to the parasite. In virtually all cases, tests for IgG antibodies reliably establish the presence or absence of the infection; a negative IgG test essentially rules out prior or recent exposure to the parasite. However, clinicians should be aware that IgG antibodies may be absent in immunocompetent patients who have been tested within the first 2 weeks of the acute infection, in patients with severe immunodeficiencies involving defective production of IgG antibodies, and in BMT patients despite having had detectable IgG antibodies in the pretransplantation period. Because of the higher sensitivity of the IgG DT, on occasion, IgG antibodies are initially negative in commercially available kits but positive in the IgG DT (see "Serologic Tests for Demonstration of Antibody"). In addition, cases of documented toxoplasmic chorioretinitis and TE in adult patients have been observed in which IgG antibodies were not demonstrable; such cases are very uncommon.

In the presence of clinical illness it is important to establish whether the patient's symptoms are due to a recently acquired infection or to recrudescence of latent infection (chronic infection) or are unrelated to the infection. A true negative IgM test in an otherwise immunologically normal individual essentially rules out that the infection has been acquired in recent months. A positive IgM test is more difficult to interpret correctly. One must not assume that a positive IgM test result is diagnostic of recently acquired infection. The presence of *T. gondii*–specific IgM antibodies can be interpreted as a true-positive result consistent with recently acquired infection, a true-positive result consistent with a chronic infection (IgM antibodies have been shown to persist in some individuals for as long as several years after the acute infection), or a false-positive result. To establish which of these is most likely in a given case, confirmatory testing in a reference laboratory should be performed whenever feasible.[359,379,381]

The use of a combination or battery of tests has been useful for determining whether the patient has been recently infected or infected in the more distant past.[245,361] If the patient has received a blood transfusion or intravenous immunoglobulin (IVIG), serologic tests may measure exogenously administered rather than endogenous antibody. The use of serologic tests to evaluate the response to therapy should be discouraged. The PAMF-TSL currently serves as a reference laboratory. Physicians in the PAMF-TSL also offer interpretation of test results and consultation on treatment and patient management to clinicians in the United States and worldwide.

Toxoplasmosis in the Immunocompetent Patient

Tests for IgG and IgM antibodies should be used for initial evaluation of immunocompetent patients. Testing of serial specimens obtained 3 weeks apart (in parallel) provides the best discriminatory power if the results in the initial specimen are equivocal. Negative results in both of these tests virtually rule out the diagnosis of toxoplasmosis. Early in infection, IgG antibodies may not be detectable, whereas IgM antibodies are present, hence the need for both tests to be performed. Acute infection is supported by documented seroconversion of IgG or IgM antibodies or a greater-than–two-tube rise in antibody titer in sera run in parallel. A single high titer of any Ig antibodies is insufficient to make the diagnosis; IgG antibodies may persist at high titers for many years,[9] and IgM antibodies may be detectable for more than 12 months. When only a single serum sample is available, a battery or combination of tests is usually required in determining the likelihood that the infection is acute.

Toxoplasmosis should be considered in the differential diagnosis of lymphadenopathy, whether or not symptoms are present and especially in those without symptoms. Confirmatory serologic tests should be obtained in such patients. The interval between the clinical onset of lymphadenopathy and the date that the specimen is drawn is critical for interpretation of the test results.[245] In patients whose serum is available during the first 3 months after the clinical onset, at least the IgG test

and the IgM-ELISA are positive, IgG avidity will be low, AC/HS will exhibit an acute pattern, and IgA and IgE may be positive. In those patients in whom sera are obtained more than 6 months after the clinical onset, the IgG will be positive, the IgM-ELISA is most likely to be negative, and the IgA-ELISA and IgE-ELISA are likely to be negative and the avidity and AC/HS maybe high and exhibit a chronic pattern, respectively.[245] A high IgG avidity test result in an individual who has recent onset of lymphadenopathy (e.g., within 2–3 months of sera sampling) suggests a cause other than toxoplasmosis,[372] and further workup is warranted. A nonacute AC/HS pattern earlier than 12 months after clinical onset of lymphadenopathy should suggest an etiology other than TL. In such cases investigation for alternative causes, including malignancy, should be undertaken.[372]

Histologic diagnosis can be useful in some cases of suspected toxoplasmosis in the immunocompetent patient. The histologic criteria for the diagnosis of TL have been well established (see Fig. 278.3A; see "Histologic Diagnosis").[197] In this setting there is no need to visualize the parasite. Endomyocardial biopsy and biopsy of skeletal muscle have been successfully used to establish *T. gondii* as the etiologic agent of myocarditis and polymyositis in rare cases in immunocompetent patients.[198] Isolation studies and PCR have rarely proved useful in immunocompetent patients.

Toxoplasmosis in the Immunodeficient Patient

Because reactivation of chronic infection is the most common cause of toxoplasmosis in patients with AIDS, malignancies, or organ transplants, initial assessment of these patients should routinely include an assay for *T. gondii* IgG antibodies. IgG antibody testing should be ideally performed as soon as it is established that the patient is immunocompromised or is about to be immunosuppressed. Those with a positive result are at risk of reactivation of the infection; those with a negative result should be instructed on how they can prevent becoming infected (see "Prevention"). Those at highest risk (e.g., HIV-infected patients not receiving appropriate ART or anti-*Toxoplasma* prophylaxis) who are initially seronegative should be retested on an annual basis to determine whether they have seroconverted. Seronegative organ transplant recipients should be identified before transplantation because they will be at risk for infection if a seropositive donor who can potentially transmit the parasite via the allograft is selected. In this setting administration of anti-*Toxoplasma* prophylaxis in the posttransplantation period can avoid unnecessary morbidity and mortality.[95]

In patients with AIDS and toxoplasmosis, the IgG titer may be relatively low, and tests for IgM, IgA, and IgE antibodies are usually negative.[284]

In the early postoperative period in heart transplant recipients with pretransplant *Toxoplasma* antibodies who present with a clinical illness, serologic test results may be misleading.[261,425] In these patients results indicating apparent reactivation (rising IgG and IgM titer) may be present in the absence of clinically apparent infection. In addition, serologic test results consistent with chronic infection may be seen in the presence of toxoplasmosis.[261,425] In heart transplant recipients in whom toxoplasmosis is suspected as a cause of altered myocardial function, endomyocardial biopsy has proved useful.[425] The parasite has been demonstrated in the myocardium of patients in whom the biopsy was performed because of a suspicion of rejection.[425] PCR testing of peripheral blood, BAL, CSF, vitreous fluid, and other body fluids or tissues may prove to be useful in patients with solid-organ transplants who are suspected to have toxoplasmosis.

Diagnosis in the BMT recipient often requires special consideration. It is critical in all BMT patients that a serum IgG titer be performed before the transplantation. Toxoplasmosis in these patients is almost always due to recrudescence of a latent infection. After BMT, the preexisting IgG antibody titer may rise, remain stable, decrease, or become negative. Thus posttransplantation serology frequently is not helpful in this group of patients and emphasizes the need for knowing the patients' pretransplantation serologic status. Clinical evidence of encephalitis, pneumonia, fever, brain lesions, or any other unexplained syndrome in BMT patients with preexisting *T. gondii* IgG antibodies must include toxoplasmosis in the differential diagnosis. The ultimate diagnosis of toxoplasmosis in these patients requires the use of histologic, cytologic, PCR, or isolation methods to detect direct or indirect evidence of the actively replicating parasite. In patients with allogeneic HSCT who are IgG antibody test positive for *T. gondii* before transplantation, routine PCR testing of peripheral blood specimens in the posttransplantation period has been proposed as an appropriate tool for guiding preemptive therapy.[281] In one report of 106 patients toxoplasmosis developed in 38% of those who were PCR positive versus 0% in those who were PCR negative.[278] In later studies, however, a first positive PCR was seen only at the time of presentation with clinical symptoms or subsequent to that.[420,446]

Serologic tests in patients with hypoglobulinemia or agammaglobulinemia may not be useful to diagnose toxoplasmosis; active infection can occur in these patients in the setting of negative IgG titers. Serologic testing in patients receiving IVIG is also potentially misleading because positive titers may reflect those of the individuals in the donor pool.

A definitive diagnosis of toxoplasmosis in the immunodeficient patient relies on histologic demonstration of the parasite (usually in association with an inflammatory process), on detection of *T. gondii* DNA by PCR, or on isolation of the parasite. PCR or attempts to isolate the parasite can be performed in essentially any body fluid or tissue that is clinically affected. Peripheral blood testing by PCR (and by isolation) should be considered in immunocompromised patients as diagnostic of disseminated toxoplasmosis. The visualization of tachyzoites is diagnostic of active infection regardless of tissue or body fluid. The presence of a solitary *T. gondii* tissue cyst may only reflect chronic infection unless it is associated with an area of inflammation (e.g., as seen in myocardial biopsy); however, visualization of several tissue cysts virtually always means that active infection is present.

When clinical signs suggest involvement of the CNS or spinal cord, the workup should include CT or MRI (see "Radiologic Methods") of the brain. These studies should be performed even if the neurologic examination does not reveal focal deficits.

A lumbar puncture should be performed if it can be done safely, ideally before or soon after initiation of therapy; PCR can then be performed on the CSF specimen. A positive *Toxoplasma* PCR essentially confirms the diagnosis of TE. CSF can also be sent for isolation studies if available. PCR examination of the CSF also can be used for the detection of Epstein-Barr virus, JC virus, or CMV DNA in patients in whom primary CNS lymphoma, progressive multifocal leukoencephalopathy, or CMV ventriculitis, respectively, have been entertained in the differential diagnosis. Especially in HIV-infected patients, a positive Epstein-Barr virus PCR in the setting of a focal contrast-enhancing CNS lesion is strongly suggestive of CNS lymphoma.[447] Demonstration of the intrathecal production of *T. gondii*–specific antibody within the CSF may help confirm the diagnosis of TE.[445] Unless sufficient CSF is available, we suggest that the highest priority be for PCR and an attempt at isolation of the parasite. Detection of *T. gondii*–specific IgM has a very low yield and is not recommended.

Empirical anti–*T. gondii* therapy for patients with multiple ring-enhancing brain lesions (usually established by MRI), positive IgG antibody titers against *T. gondii,* and AIDS (e.g., patients with a CD4 count of <200 cells/mm^3) is accepted practice; a clinical and radiologic response to specific anti–*T. gondii* therapy essentially confirms the diagnosis of TE (Fig. 278.7). Use of adjunctive corticosteroids to decrease cerebral edema can cause temporary clinical improvement that is incorrectly attributed to the pyrimethamine and sulfa. This empirical approach is not recommended for non-AIDS immunocompromised patients with this presentation because the differential diagnosis is wider and often includes other etiologic agents, such as invasive molds, nocardiae, bacterial brain abscess, and mycobacteria. In these patients an early brain biopsy is recommended. Brain biopsy should be considered in AIDS patients with presumed TE if there is a single lesion on MRI, a negative IgG antibody test, an inadequate clinical response (within a 2- to 3-week period) or progression during optimal therapy, or in patients the physician considers to have adhered to an effective prophylactic regimen against *T. gondii* (e.g., TMP-SMX). An impression smear of the brain biopsy specimen can be made and immediately examined for the presence of tachyzoites with the conventional Wright-Giemsa stain for blood smears as used in most laboratories. In addition to H&E staining, *T. gondii*–specific immunoperoxidase staining should be performed. Because the amount of brain tissue obtained at aspiration

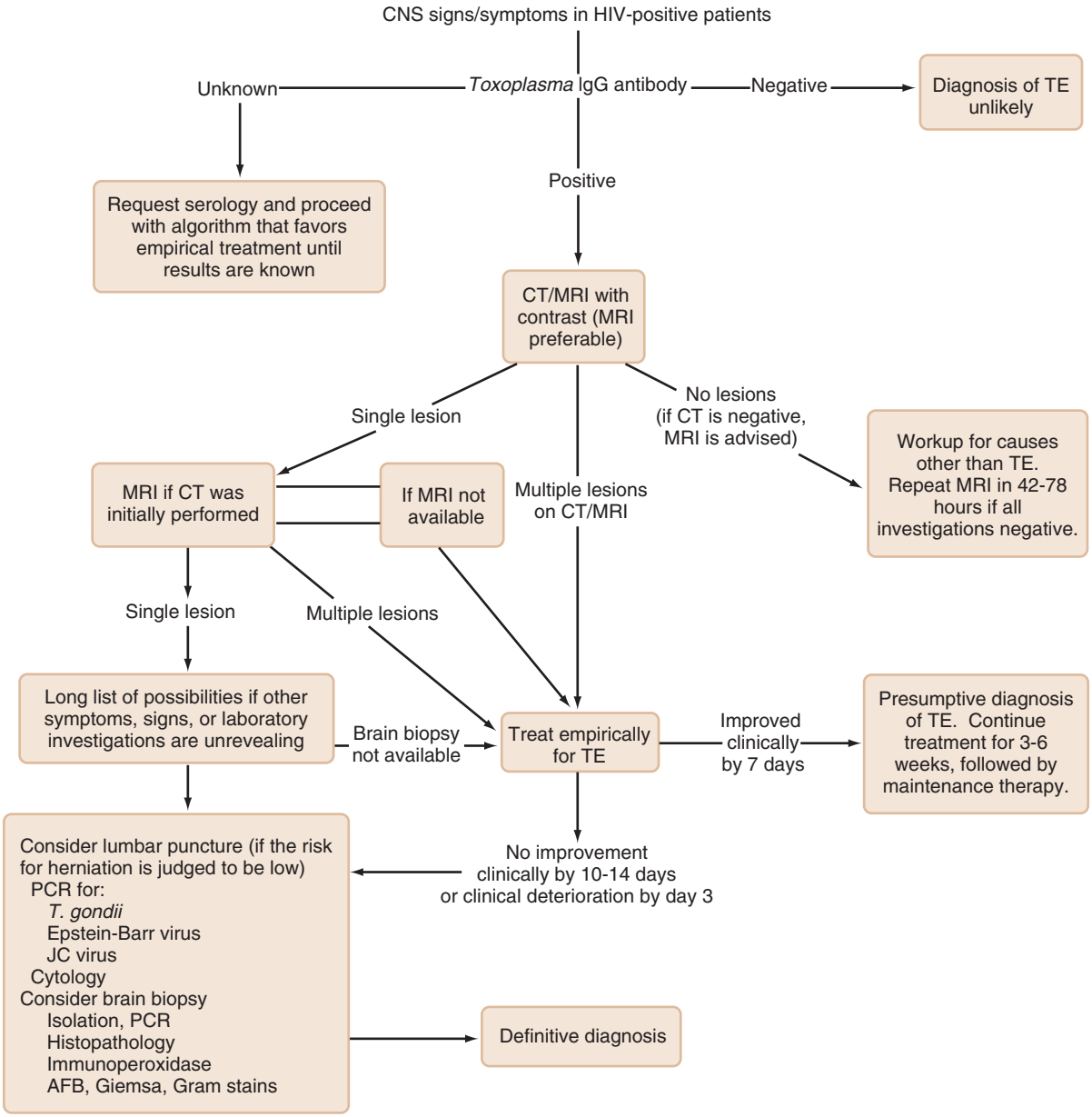

FIG. 278.7 Diagnostic approach and management algorithm for human immunodeficiency virus *(HIV)*-infected patients with central nervous system *(CNS)* symptoms or signs that might potentially be toxoplasmic encephalitis *(TE).* *AFB,* Acid-fast bacilli; *CT,* computed tomography; *IgG,* immunoglobulin G; *MRI,* magnetic resonance imaging; *PCR,* polymerase chain reaction.

or biopsy is usually small, sufficient tissue for mouse inoculation may not be available; however, this should be performed whenever feasible. A positive result may often be obtained with far less than 1 g of brain tissue. PCR has been used successfully in brain tissue to diagnose TE,[448] but a positive result should be interpreted with caution because it may not distinguish between the patient with TE from one with latent infection (asymptomatic carrier of brain tissue cysts) who has CNS pathology resulting from a process other than toxoplasmosis.

In the appropriate clinical setting it is important to include toxoplasmosis in the differential diagnosis of pulmonary symptoms, particularly in those individuals with interstitial infiltrates or ground-glass opacities. Wright-Giemsa staining and PCR of BAL specimens are useful for the diagnosis of pulmonary toxoplasmosis.[210,449]

In patients with visual symptoms in whom toxoplasmic chorioretinitis is a possibility, PCR examination of vitreous or aqueous fluid can be considered and is particularly helpful in patients with atypical clinical features of toxoplasmic chorioretinitis and in immunocompromised patients.[213,320,450]

The intraocular production of *T. gondii*–specific IgG antibodies has also been reported to be diagnostically useful.[451] Local antibody production in aqueous humor can be quantified by calculating the Goldmann-Witmer (GW) coefficient. The GW coefficient expresses the level of *Toxoplasma*-specific IgG relative to the level of total IgG in the aqueous humor as a fraction of the level of *Toxoplasma*-specific IgG relative to the level of the total IgG in the serum.[452] The GW coefficient = *Toxoplasma* IgG/total IgG in aqueous humor ÷ *Toxoplasma* IgG/total IgG in serum. A coefficient greater than 2 is considered positive and diagnostic of ocular toxoplasmosis. It has been well established that, in contrast to ocular viral infections, the GW coefficient is a more sensitive method than PCR for the diagnosis of ocular toxoplasmosis. However, PCR in vitreous fluid appears to be superior to the GW coefficient in immunocompromised patients, the elderly, and those with atypical lesions and extensive retinochoroiditis.

PCR and isolation studies in peripheral blood can help establish *T. gondii* as the etiologic agent of a febrile syndrome or systemic symptoms of unclear cause.[403,453] These studies tend to have a higher yield early in

the disease and before or shortly after specific anti–*T. gondii* therapy is initiated.[403]

Ocular Toxoplasmosis

In patients with active chorioretinitis caused by reactivation of congenital *T. gondii* infection, low titers of IgG antibody are usual, and IgM antibodies are not usually detected. In contrast, in patients with chorioretinitis as a result of an acute infection, IgG and IgM antibodies will be detected.

In most cases toxoplasmic chorioretinitis is diagnosed by ophthalmologic examination, and empirical therapy directed against the organism is often instituted based on clinical findings and serologic test results. In a number of patients the morphology of the retinal lesion or lesions may be nondiagnostic, or the response to treatment may be suboptimal, or both. In such cases (unclear clinical diagnosis or inadequate clinical response, or both) the detection of an abnormal *T. gondii*–antibody response in ocular fluids (GW coefficient),[450,454] or demonstration of the parasite by isolation, histopathologic examination, or PCR have been used successfully to establish the diagnosis.[452,455] PCR has been used in both vitreous and aqueous fluids in an attempt to support or confirm the diagnosis of *T. gondii* as the cause of the retinal lesions.[212,213,320,450]

Toxoplasma gondii Infection in Pregnancy

Acute acquired *T. gondii* infection is diagnosed serologically by the same methods used for immunocompetent adults discussed earlier (Fig. 278.8).[382] Special care is taken to determine whether the infection was acquired before or after conception. This determination is frequently difficult because routine serologic screening is not conducted in pregnant women in the United States.

The diagnosis of acute *T. gondii* infection or toxoplasmosis ideally requires demonstration of a rise in titers in serial serum samples, either conversion from a negative to a positive titer or a fourfold rise from a low to a higher titer.[9] These specimens should be obtained at least 3 weeks apart and be tested in parallel. Because the diagnosis is frequently considered relatively late in the course of the patient's pregnancy, serologic test titers may already have reached their peak at the time the first serum is obtained for testing. Therefore it is often difficult to discriminate between infections acquired recently (possibly during pregnancy) and those acquired in the more distant past, when the only available serum sample has been obtained in the third trimester of the pregnancy. Thus the initial serum should be obtained as early as possible during gestation.

Initial screening of maternal serum involves testing for IgG and IgM antibodies; a lack of both Ig antibodies essentially excludes active infection but identifies the patient as being at risk for acquisition of the infection and hence in need of instruction about primary prevention. The presence of IgG antibodies in the absence of IgM antibodies in the first two trimesters almost always indicates chronic maternal infection with essentially no risk to the fetus; the exceptions are severely immunodeficient patients. In the third trimester a negative IgM test titer is most likely consistent with a chronic maternal infection but does not exclude the possibility of an acute infection acquired early in pregnancy; this is especially true in those patients who exhibit a rapid decline in their IgM titers during the acute infection. In these cases the use of other serologic tests (e.g., IgA, IgE, AC/HS, avidity) may be of particular help.

A positive IgM test result requires further assessment with confirmatory testing at a reference laboratory (see also "Diagnosis of Specific Clinical Entities").[361,379,381,382] The use of confirmatory testing with a combination of serologic tests in a reference laboratory has proved helpful in discriminating between recently and more distantly acquired infections, and having an expert interpret the results to the patient's physician has been shown to reduce unnecessary induced abortions among pregnant women reported to have IgM antibodies.[361] Women who are informed that they have a positive IgM test titer and that it signifies that their offspring will or might be infected often choose abortion. Unfortunately, a positive IgM test may not necessarily indicate infection acquired during gestation (a false-positive result or persistence of a IgM-positive result in the chronic stage of the infection), and thus the abortion may not be indicated.[389] It is for this reason that confirmatory testing in a reference laboratory has been recommended by many experts and by the FDA.[381]

A number of tests for avidity of *Toxoplasma* IgG antibodies have been introduced to help differentiate between recently acquired and distant infection.[373,374,456] Studies of the kinetics of the avidity of IgG in pregnant women who have seroconverted during gestation have shown that women with high-avidity test results have been infected with *T. gondii* for at least 3 to 5 months. Recently, it has been demonstrated that, when used as a confirmatory test, along with a battery of other tests, in women in their first 16 weeks of gestation, detection of high-avidity IgG antibodies by the IgG-VIDAS avidity test, for example, can be a useful addition to the discriminatory power of a combination of tests in distinguishing recently acquired from chronic infection.[357,362,377] It is critical to recognize that the value of the avidity test is in the first 3 to 5 months of gestation, that is, the fetus of a woman in the 14th week of gestation with a positive IgM test and a high-avidity result is not at risk for congenital toxoplasmosis.[362] Because low or equivocal avidity test results may persist for many months, their presence does not necessarily indicate recently acquired infection.

Confirmatory testing, using a battery of tests and the VIDAS avidity method in pregnant women during their first 16 weeks of gestation, has the potential to decrease the need for follow-up sera and thereby reduce costs, to make the need for PCR on amniotic fluid and for treatment of the mother with spiramycin unnecessary, to remove the pregnant woman's anxiety associated with further testing, and to decrease unnecessary abortions.[360–362] Although the avidity test represents an additional confirmatory method (most useful if high-avidity antibodies are detected within the first 16 weeks of gestation), it should not be used as the only confirmatory test for pregnant women with positive IgG and/or IgM antibodies because of the potential to misinterpret low- or borderline-avidity antibody results.

Once the diagnosis of acute acquired infection during pregnancy has been presumptively established, diagnostic efforts should focus on determining whether the fetus has been infected.

Congenital Infection in the Fetus and Newborn

Prenatal diagnosis of fetal infection is advised when a diagnosis of acute infection is established or highly suspected in a pregnant woman. Methods to obtain fetal blood, such as periumbilical fetal blood sampling, have been largely abandoned because of the rate of false-negative prenatal diagnoses, the risk involved for the fetus, and the delay in obtaining definitive results with conventional parasitologic tests.[333]

Prenatal diagnosis of congenital toxoplasmosis is presently based on ultrasonography and amniocentesis. PCR on amniotic fluid for the detection of *T. gondii*–specific DNA performed at 18 weeks of gestation or later is more sensitive, more rapid, and safer than conventional diagnostic procedures involving fetal blood sampling.[333,457] Amniotic fluid should be tested by PCR in all cases, with serologic test results diagnostic of, or highly suggestive of, acute acquired infection during pregnancy, and also if there is evidence of fetal damage by ultrasound examination (e.g., hydrocephalus and/or calcifications). In a prospective cohort study conducted in France, amniotic fluid PCR was performed in 261 women who had been identified as having an acute infection during gestation through routine prenatal screening for toxoplasmosis.[457] Overall sensitivity and negative predictive value of the amniotic fluid PCR for the diagnosis of congenital infection were 92.2% (95% CI, 81% to 98%) and 98.1% (95% CI, 95% to 99.5%), respectively. Specificity and the positive predictive value were 100%.[457] Overall sensitivity has been reported by several groups as varying from 65% to 92.2%; specificity and the positive predictive value have been reported to be 100% by most groups.[413,458] Recently, one group proposed a novel and more realistic interpretation of amniotic fluid PCR test results according to the pretest probability of infection and gestational age at the time of maternal infection[458]; their analysis revealed that negative test results are more significant than previously thought, given that transmission occurs only in a percentage of women at various stages of gestation and that a negative amniotic fluid PCR further reduces this percentage. Of note, the vast majority of their pregnant women, as is the case for all studies reported from Western Europe, received the benefit of prenatal treatment. Thus caution should be exercised when attempting to apply risks of congenital infection to infants born to women who were not treated during gestation. The preferable time for amniocentesis is at 18

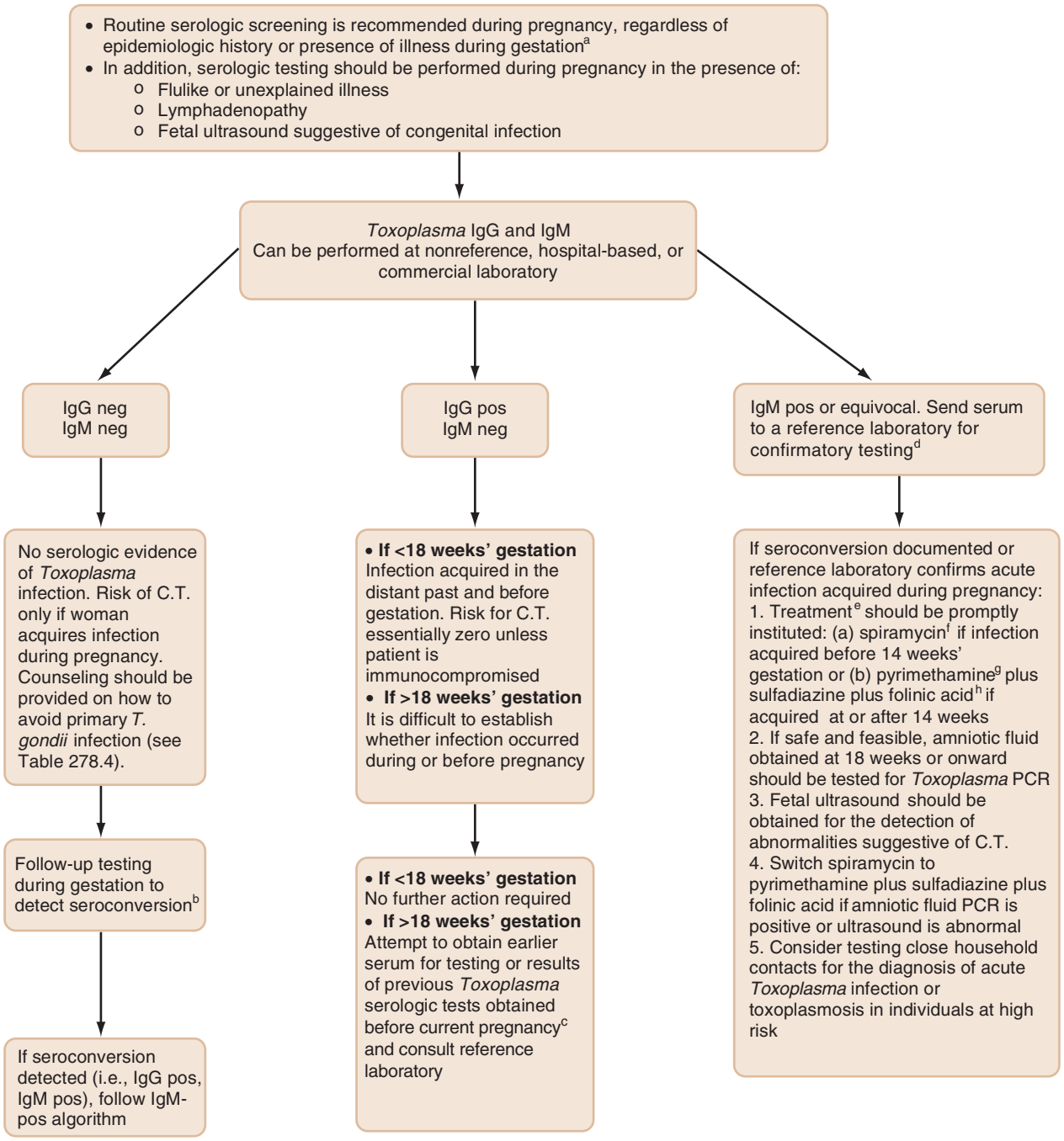

FIG. 278.8 Diagnostic approach and management algorithm of toxoplasmosis during pregnancy. Most of the initial serologic screening can be accomplished by nonreference or commercial laboratories. Only positive immunoglobulin M results should be considered for additional testing and consultation with medical experts at a reference laboratory. *C.T.,* Congenital toxoplasmosis; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *neg,* negative test result; *pos,* positive test result. [a]Up to 50% of women who acquire *Toxoplasma* infection during gestation do not have a known risk factor for acute infection or an illness suggestive of toxoplasmosis. Thus, to identify all women at risk, serologic screening should be performed in all pregnant women, along with other routine screening tests. [b]In a recent study from Lyon, France monthly screening of seronegative pregnant women was reported to significantly decrease the risk of vertical transmission and of clinical signs at 3 years of age.[558] [c]Consider consultation with a physician expert in management of toxoplasmosis during pregnancy (e.g., in the United States, Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory [PAMF-TSL], www.pamf.org/serology/; 650-853-4828; e-mail, toxolab@pamf.org or US [Chicago] National Collaborative Treatment Trial Study [NCCTS]; 773-834-4152). [d]Consider sending serum sample to a reference laboratory (e.g., PAMF-TSL). [e]Treatment regimens vary by country. The pyrimethamine-sulfadiazine–folinic acid regimen should not be offered to any pregnant women before 12 weeks of gestation because of potential teratogenicity. In some centers in Europe this regimen is offered at 14 weeks of gestation or later; in the United States it is recommended at 14 weeks or later. [f]Spiramycin is not commercially available in the United States. It can be obtained at no cost and after consultation with PAMF-TSL or the NCCTS through the US Food and Drug Administration. [g]When using pyrimethamine, folic acid should be discontinued from the prenatal multivitamins. Folic acid can potentially counteract the antiparasitic effect of the drug. [h]Folic acid should not be erroneously used instead of folinic acid.

weeks of gestation or later if acute infection was acquired after 18 weeks. The sensitivity of the amniotic fluid PCR test appears to be lower when the amniocentesis is performed before 18 weeks of gestation[333] and should be avoided before 18 weeks for the purpose of diagnosing congenital toxoplasmosis.

Maternal IgG antibodies present in the newborn may reflect either past or recent infection in the mother. For this reason, tests for the detection of IgA and IgM antibodies are commonly used for the diagnosis of infection in the newborn (Fig. 278.9).[392] It is essential that maternal contamination of blood obtained at birth be excluded; serum samples

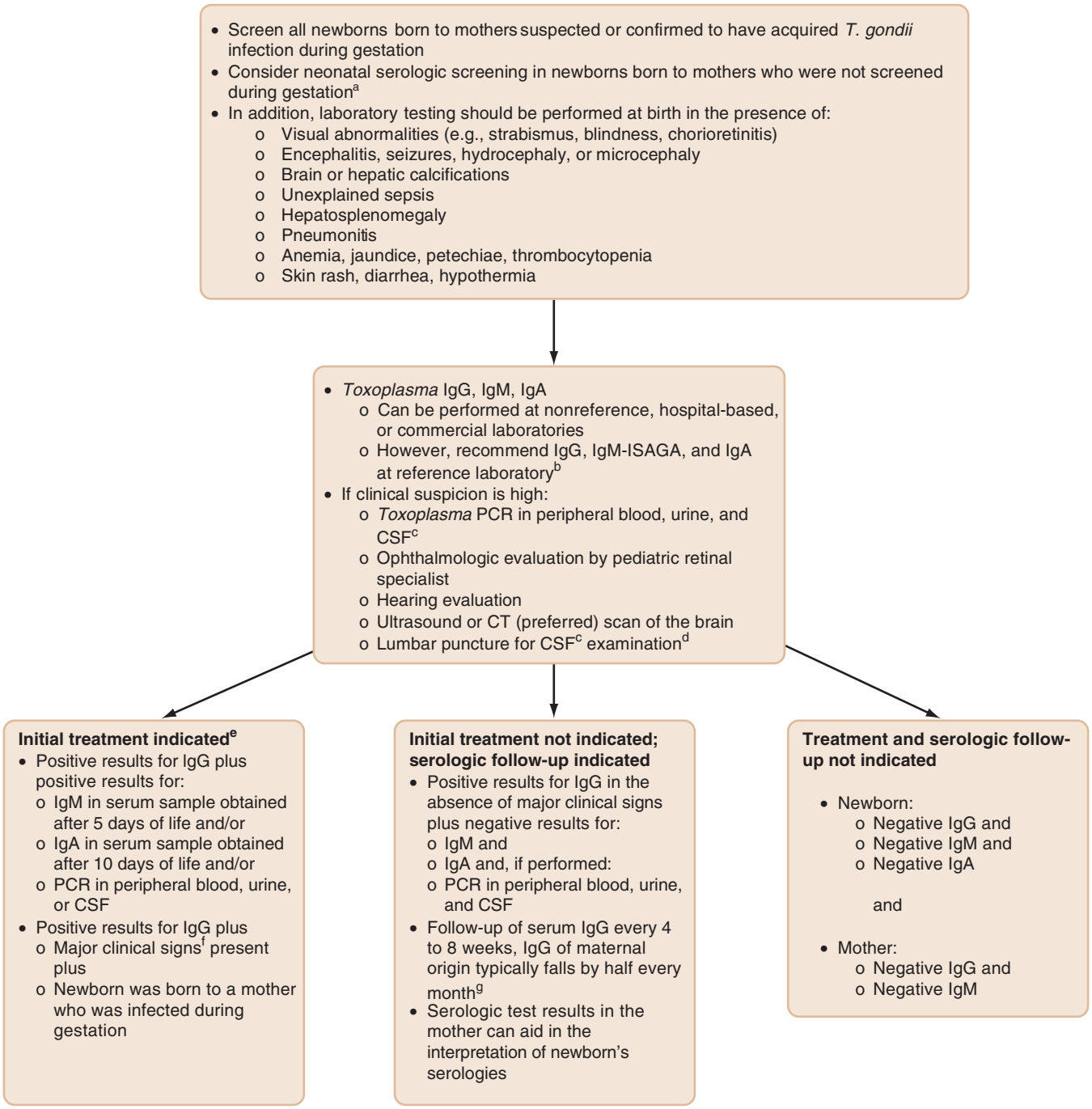

FIG. 278.9 Diagnostic approach and management algorithm of the newborn whose mother has been suspected or confirmed to have acquired toxoplasmosis during gestation. *CSF,* Cerebrospinal fluid; *CT,* computed tomography; *IgG, IgM,* and *IgA,* immunoglobulins G, M, and A, respectively; *ISAGA,* immunosorbent agglutination assay; *PCR,* polymerase chain reaction. [a]Consider consultation with a physician expert in management of toxoplasmosis during pregnancy (e.g., in the United States, Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory [PAMF-TSL], www.pamf.org/serology/; 650-853-4828; e-mail, toxolab@pamf.org or US [Chicago] National Collaborative Treatment Trial Study, 773-834-4152). [b]Consider sending serum sample to a reference laboratory (e.g., PAMF-TSL). [c]Testing CSF assumes that lumbar puncture is clinically indicated, deemed safe, and feasible. [d]In an attempt to confirm the diagnosis of congenital toxoplasmosis, CSF should be sent for cell count and differential (congenital toxoplasmosis is one of the few causes of eosinophilic meningitis), protein (congenital toxoplasmosis is one of the few causes of extreme elevation of CSF protein), glucose, and *T. gondii* PCR. [e]The recommended regimen is pyrimethamine plus sulfadiazine plus folinic acid (see text). [f]Major clinical signs are referred here: chorioretinitis, brain calcifications, and hydrocephalus. [g]Maternally transferred IgG antibodies usually decline and disappear within 6 to 12 months of life.

obtained from peripheral blood (and not from the umbilical cords) are preferred. In a retrospective study of 164 untreated infants age 0 to 180 days, in the United States, *T. gondii*–specific IgM, IgA, and IgE antibodies were demonstrable in 86.6%, 77.4%, and 40.2% of the infants, respectively.[338] Testing for IgM and IgA antibodies increased the sensitivity of making the diagnosis of congenital toxoplasmosis to 93%, compared with testing for IgM or IgA alone. The sensitivity of the IgM and IgA has been reported to be somewhat lower in cohorts of treated (prenatal treatment) infants: 64% to 70% for IgM, 53% to 65% for IgA, and 66% to 81% for both.[458]

If IgG antibodies are detected but serologic tests for IgM and IgA antibodies are negative and *T. gondii* is not isolated, follow-up serologic

testing in suspect cases is indicated to attempt to establish the diagnosis. Maternally transferred antibodies usually decline and disappear within 6 to 9 months.

Studies using the Western blot technique have shown that maternal and infant sera may recognize different *T. gondii* antigens when the infant is congenitally infected.[459,460] Combining Western blot with conventional serologic analysis (i.e., IgG, IgM, and IgA tests) has been reported to be more sensitive for the diagnosis of congenital toxoplasmosis at birth and within the first 3 months of life than either test alone.[460–462] Recently, three IgM-bands at 75, 90, and 100 kilodaltons (also known as the "IgM triplet") have been reported to increase the sensitivity of the diagnosis of congenital toxoplasmosis to 95.8% when combined with prenatal and serologic neonatal tests.[463]

Additional diagnostic methods that have been used successfully to diagnose the infection in infants are direct demonstration of the organism by isolation in mice or cell culture (e.g., placental tissue, body fluid) and PCR in body fluids (e.g., CSF, blood, and urine).[407,464–468] In a study of 161 consecutive infants, CSF PCR was positive in 27 of the 58 (46.5%) congenitally infected infants and was negative in each of the 103 infants without congenital toxoplasmosis.[468] Evaluation of infants with suspected congenital toxoplasmosis should always include ophthalmologic examination, radiologic studies (particularly to detect the presence of cerebral calcifications; if feasible, CT is preferred over ultrasound), and examination of CSF. A more detailed discussion of diagnostic procedures in congenitally infected infants is available from Peyron and colleagues.[9]

In the absence of a systematic screening program for pregnant women, a secondary prevention program that consists of serologic testing of all newborns for IgM antibodies against *T. gondii* has been implemented in Massachusetts.[469,470] Using routine screening of all newborns, congenital infection was confirmed in approximately 1 in 12,000 infants. Greater than 90% of these were identified only through neonatal screening and not through initial clinical examination.

In infants with congenital toxoplasmosis or congenital infection, a rebound in IgG and IgM antibody titers is frequently observed after discontinuation of therapy. In our experience such a serologic rebound has not been shown to be clinically significant.[9]

THERAPY

Currently recommended drugs against *T. gondii* act primarily against the tachyzoite form and thus do not eradicate the encysted form (bradyzoite). Pyrimethamine is considered to be the most effective anti-*Toxoplasma* agent, and it should, if possible, always be included in a combination drug regimen used against the parasite. Pyrimethamine is a folic acid antagonist. The most common side effect is dose-related suppression of the bone marrow, which may be decreased by concomitant administration of folinic acid (calcium leucovorin). It is not well established how often a blood count should be obtained; a reasonable strategy would be to check a peripheral blood cell and platelet count twice weekly until hematologic parameters have stabilized in a nontoxic range, and then every 2 to 4 weeks. Folinic acid should be administered concomitantly to avoid bone marrow suppression. The parenteral form of folinic acid is well absorbed orally, and 10 to 20 mg/day of folinic acid (up to 50 mg/day is used in AIDS patients) may be given orally (e.g., with orange juice at the same time as the pyrimethamine). Whereas folinic acid does not inhibit the action of pyrimethamine on tachyzoites, folic acid does and should not be used in patients being treated with pyrimethamine. Less serious side effects of pyrimethamine include GI distress, rash, headaches, and a bad taste in the mouth.

Unless there are circumstances that preclude the use of more than one drug, there is no role for monotherapy in the treatment of toxoplasmosis. A second drug, such as sulfadiazine or clindamycin, should be added. Sulfadiazine acts synergistically with pyrimethamine; most other sulfonamides have inferior activity. The patient must maintain a good urine output to prevent crystalluria and oliguria. The most common side effects associated with sulfadiazine are skin rashes, which may be life threatening,[471] and crystal-induced nephrotoxicity.[472] Worsening encephalopathy, hallucinations, or a new onset of psychiatric symptoms in patients with AIDS may be sulfadiazine induced and must be considered in the patient who is nonresponsive to otherwise appropriate anti-*Toxoplasma* treatment.[473] A drug rash with sulfonamide therapy does not necessarily preclude its use because successful desensitization protocols have been reported.[474,475] Clindamycin appears to act by targeting translation in the apicoplast of *T. gondii*.[40] Adverse reactions to clindamycin include rash, nausea, vomiting, and diarrhea, which may be associated with *Clostridioides difficile* (formerly *Clostridium difficile*) infection. Myopathy with electromyographic abnormalities and elevated serum creatine phosphokinase levels have been described.[476]

Although clinical experience with TMP-SMX is more limited, this drug combination, which targets folate metabolism in a manner similar to pyrimethamine plus sulfadiazine, has documented activity and can be used in patients requiring parenteral therapy or those in whom cost of pyrimethamine becomes prohibitive.[477] Atovaquone combined with pyrimethamine or sulfadiazine, or in unusual circumstances as a single agent, is another less-well-studied alternative for patients who can be treated with oral therapy. The role of other drugs, including azithromycin, clarithromycin, and dapsone, is less clear; they should only be used as alternatives when the regimens described earlier cannot be used and should be used in combination with pyrimethamine whenever possible.

Spiramycin has been used in pregnant women to reduce transplacental transmission to the fetus; it has not been shown to be effective for acute therapy, maintenance therapy, or primary prophylaxis of TE in AIDS patients. There is no evidence that spiramycin is teratogenic.

Although drugs used to treat toxoplasmosis in the setting of different clinical entities are basically the same, careful attention should be given to the dose and dosing regimen. Recommended doses in immunocompromised patients are usually higher than those in immunocompetent patients. For instance, the recommended dose of pyrimethamine for patients with TE is 50 to 75 mg/day after a loading dose of 200 mg, whereas the dose to treat fetal infection during pregnancy is 25 to 50 mg/day after a loading dose of 100 mg/day for 2 days in the mother.

Therapy Regimens in Specific Clinical Entities

Toxoplasmosis in the Immunocompetent Patient

Treatment of immunocompetent adults with the lymphadenopathic form is rarely indicated; this form is usually self-limited. One small randomized trial demonstrated efficacy of a 1-month course of TMP-SMX in reducing lymphadenopathy (65% response vs. 22% for placebo).[478] If visceral disease is clinically overt or symptoms are severe or persistent, treatment may be indicated for 2 to 4 weeks, followed by reassessment of the patient's condition (Table 278.2). Infections acquired by laboratory accident or transfusion of blood products are potentially more severe, and patients who have been infected in these ways probably should be treated (see Table 278.2).

Toxoplasmosis in the Immunodeficient Patient

Because experience with treatment of toxoplasmosis in immunodeficient patients has been most extensively studied in patients with AIDS, this section focuses primarily on this group of patients. However, information on treatment in AIDS patients likely can, in large part and in the absence of data, be extrapolated directly to other immunodeficient patients (see Table 278.2).

If left untreated, toxoplasmosis in immunodeficient patients is often lethal. Treatment is recommended for 4 to 6 weeks after the resolution of all signs and symptoms (often for 6 months or longer). At one medical center, 80% of non-AIDS immunodeficient patients with toxoplasmosis improved with specific therapy.[479] This rate of improvement is similar to that observed in appropriately treated AIDS patients with TE.[435,480] Chronic (latent) asymptomatic infection in immunodeficient patients is not treated.

The exact dosing schedule for the treatment of toxoplasmosis in non-AIDS immunocompromised patients has not been defined. However, useful information in this regard has resulted from studies performed in AIDS patients with toxoplasmosis.

Therapy for toxoplasmosis in AIDS patients includes acute (primary or induction) treatment, chronic maintenance treatment (secondary

TABLE 278.2 Treatment Regimens for Toxoplasmosis in Immunocompetent and Immunocompromised Patients

	IMMUNOCOMPETENT PATIENTS[b]	IMMUNOCOMPROMISED PATIENTS, INCLUDING THOSE WITH AIDS, TRANSPLANTS, CANCER, OR TAKING IMMUNOSUPPRESSOR DRUGS[c]
Preferred Regimen		
Pyrimethamine (PO)	50 mg q12h for 2 days, followed by 25–50 mg daily	200-mg loading dose, followed by 50 (<60 kg) –75 mg/day (≥60 kg)
plus		
Folinic acid[d] (PO)	10–20 mg daily (during and 1 wk after therapy with pyrimethamine)	10–20 mg daily (up to 50 mg/day) (during and 1 wk after therapy with pyrimethamine)
plus		
Sulfadiazine (PO)	1 g q6h	1000 (<60 kg)–1500 mg (≥60 kg) q6h
Preferred Alternative Regimens		
Pyrimethamine–folinic acid	Same doses as above	Same doses as above
plus		
Clindamycin (PO or IV)	300 mg q6h	600 mg q6h (up to 1200 mg q6h)
or		
Atovaquone (PO)	1500 mg PO twice daily	1500 mg PO twice daily
Trimethoprim-sulfamethoxazole (PO or IV)	10 mg/kg/day (trimethoprim component) divided in two to three doses	10 mg/kg/day (trimethoprim component) divided in two to three doses (doses as high as 15–20 mg/kg/day have been used)
Alternative Regimens With Limited Supportive Data[e]		
Pyrimethamine–folinic acid	Same doses as above	Same doses as above
plus		
Clarithromycin (PO)	500 mg q12h	500 mg q12h
or		
Dapsone (PO)	100 mg/day	100 mg/day
or		
Azithromycin (PO)	900–1200 mg/day	900–1200 mg/day
Sulfadiazine (PO)	Same doses as above	Same doses as above
plus		
Atovaquone (PO)	Same doses as above	Same doses as above

[a]Assistance is available for the diagnosis and management of toxoplasmosis at the Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory; Palo Alto, CA; www.pamf.org/serology/; 650-853-4828; toxolab@pamf.org.
[b]Indicated in patients with (1) ocular disease associated with primary infection or reactivation of latent infection; (2) severe disease, including myocarditis, myositis, hepatitis, pneumonia, brain, or skin lesions and lymphadenopathy; (3) persisting symptoms; and (4) laboratory accidents.
[c]After the successful use of a combination regimen during the acute/primary therapy phase, same agents at half doses are usually used for maintenance or secondary prophylaxis; for pyrimethamine, use 25 mg (<60 kg) to 50 mg/day (>60 kg); for clindamycin, use 600 mg PO every 8 hours.
[d]Folinic acid (leucovorin); should always be given with pyrimethamine to minimize toxicity; folic acid must not be used as a substitute for folinic acid.
[e]These agents have been used in clinical studies with small numbers of patients and have response rates lower than the standard regimens (see text for references). They should be used only in patients who are intolerant of the standard regimens.
AIDS, Acquired immunodeficiency syndrome; *IV,* intravenously; *PO,* orally.

prophylaxis), and primary prophylaxis. There are no convincing data from prospective, carefully designed trials to allow the recommendation of monotherapy for induction, maintenance, or primary prophylaxis. Because relapse occurs in up to 80% of cases[471] after the discontinuation of primary therapy, maintenance therapy is recommended for all patients until the CD4 count rises above 200 cells/mm^3 for at least 6 months in response to ART (plasma HIV viral load will often be below detection limits during this period).

Acute therapy should be for at least 3 weeks,[100] and up to 6 weeks or more may be required for more severely ill patients who have not achieved a complete response. Pyrimethamine combined with sulfadiazine and folinic acid is the therapy of choice for AIDS patients with toxoplasmosis and is the standard to which experimental regimens should be compared. This regimen is associated with clinical response in 68% to 95% of patients with TE.[480,481] Unfortunately, up to 40% of patients develop side effects from one or more of the drugs, often requiring discontinuation of one or both agents.[232,426] Pyrimethamine-clindamycin and folinic acid appear comparable in efficacy to pyrimethamine-sulfadiazine,[480,482] but this combination also has substantial toxicity. TMP-SMX[483–485] (at 10 mg/kg/day of the TMP component, divided in two doses) has shown efficacy similar to the pyrimethamine-sulfadiazine regimen (with a more rapid radiologic response in the TMP-SMX group) in a randomized pilot trial in 77 patients with AIDS[477]; this provides an alternative regimen for situations in which parenteral therapy is required or when pyrimethamine is unavailable or unafordable. An international, noncomparative study of atovaquone[486,487] (administered orally as a suspension), combined with either pyrimethamine or sulfadiazine as treatment for acute disease, demonstrated 6-week response rates of 75% (21/28 patients) for atovaquone-pyrimethamine and 82% (9/11) for atovaquone-sulfadiazine.[488] Serum levels of atovaquone may predict response but are not commercially available.[487] Doses of atovaquone lower than currently recommended for treatment (750 mg twice daily vs. 1500 mg twice daily) can often achieve therapeutic levels if not coadministered with efavirenz, which can reduce atovaquone levels by approximately 45%.[489] TMP-SMX and atovaquone both are active against *Pneumocystis,* and thus anti-*Pneumocystis* prophylaxis does not need to be administered when these drugs are used. Because clindamycin-pyrimethamine has no demonstrated anti-*Pneumocystis*

activity, additional prophylaxis (e.g., aerosolized pentamidine) needs to be coadministered if patients are at risk for developing *Pneumocystis* pneumonia.

In a 13-patient pilot study of TE using the combination of pyrimethamine, 75 mg/day, and clarithromycin, 1 g every 12 hours, 62% of patients had a complete and 23% a partial clinical response; 15% of patients died by week 3 of therapy,[490] and adverse events resulted in discontinuation of therapy in 27%. No long-term follow-up data are available, and no additional studies with this drug combination have been published. Doses of clarithromycin higher than 500 mg twice daily have been associated with increased mortality in HIV-infected patients receiving therapy for *Mycobacterium avium* infection and should not be used.[491,492]

Dapsone in combination with pyrimethamine has been reported anecdotally to be effective for the treatment of TE when used in an oral dose of 100 mg/day with 25 mg/day of oral pyrimethamine.[493] Doxycycline has had success in the treatment of TE in a few patients when used at 300 mg/day IV in three divided doses.[494] A dosage of 100 mg twice a day was given to six patients intolerant to pyrimethamine-sulfadiazine, but five had associated neurologic and radiologic recurrences while receiving the drug.[495] Further studies are needed to compare the relative efficacy and toxicity of these alternative regimens. Although azithromycin plus pyrimethamine is effective for the treatment of some cases of TE in AIDS patients, its use should be limited, based on results of a recent study demonstrating an inferior response rate, especially during maintenance therapy.[496,497] Alternative regimens used for acute therapy and their dosage schedules are listed in Table 278.2.

Corticosteroids are often given to patients with TE for the reduction of cerebral edema and raised intracranial pressure. The clinical response and survival in patients with TE who received corticosteroids in addition to antimicrobial therapy have been reported to be no different from that of those who received antimicrobial agents alone.[435] The use of these agents may complicate the interpretation of empirical therapy of TE because partial clinical and radiologic improvement may be seen solely resulting from a reduction in cerebral edema and inflammation or a response of CNS lymphoma; moreover, they may further compromise the immune systems of these already very immunodeficient patients. Their use thus should be limited to situations where clinically significant edema or a mass effect is present.

Seizures occur in up to 35% of patients with TE.[426] One retrospective study demonstrated a poorer outcome in those patients who received anticonvulsant therapy, compared with those who did not.[471] Whether this result represents a true drug effect or a selection bias, given that those receiving anticonvulsant therapy are likely to be more severely ill, is unclear. Anticonvulsant agents may be responsible for numerous side effects and drug interactions; for instance, potentially serious interactions can occur between agents such as carbamazepine, phenobarbital, or phenytoin and other drugs used to treat HIV infection, such as protease inhibitors. Anticonvulsant therapy is probably best administered only when seizures have occurred.[471]

The time to clinical response in AIDS patients with TE who were receiving appropriate anti-*Toxoplasma* therapy has been evaluated in a study that included an objective, graded neurologic examination.[435] Of those with a response, 91% improved with respect to at least half of their baseline abnormalities by day 14.[435] AIDS patients with presumed TE had some degree of improvement within 7 to 10 days of the initiation of appropriate anti-*Toxoplasma* therapy. By contrast, a significant number of patients with an alternative diagnosis, including lymphoma, exhibited signs of clinical deterioration as early as 3 to 5 days after the initiation of the empirical regimen for presumed TE.[435] Headaches and seizures were insensitive indicators for a response to therapy. In some cases toxoplasmosis has progressed to death despite the use of appropriate drug regimens.[480,482]

After successful primary therapy, drug dosages are generally decreased for maintenance therapy. No single maintenance regimen that is efficacious with an acceptable adverse reaction profile has yet been identified. Relapse of TE occurs in approximately 20% to 30% of patients who are receiving maintenance therapy, in part because of nonadherence to and patient intolerance of the prescribed regimen.[232] Pyrimethamine, 25 mg/day, plus sulfadiazine, 500 mg four times daily, has been associated with the lowest relapse rate[480] and is recommended unless there are contraindications or cost considerations preventing its use. When daily therapy with pyrimethamine-sulfadiazine was compared with a twice-weekly regimen for the prevention of recurrence of TE, the latter was found to be less effective.[498,499] Although a subsequent trial by the same group found that three-times weekly therapy was equivalent to daily therapy, the relapse rates for both groups (≈14.5/100 patient-years) was higher than was seen with daily therapy (4.4/100 patient-years) in the earlier study.[499,500] Patients receiving the pyrimethamine-sulfadiazine combination do not require another regimen for PCP prophylaxis. Whereas 25% of patients receiving pyrimethamine-clindamycin subsequently developed PCP,[501] no patient receiving pyrimethamine-sulfadiazine developed PCP.[499,501] One 17-patient study demonstrated that TMP-SMX could be safely substituted for pyrimethamine-sulfadiazine after a median of 24-months' maintenance therapy in patients also receiving ART; the major benefit was in decreasing pill burden.[502]

Because of drug toxicity, many patients are unable to continue taking the pyrimethamine-sulfadiazine combination for maintenance therapy. A higher relapse rate has been reported with the use of pyrimethamine-clindamycin compared with pyrimethamine-sulfadiazine for chronic maintenance of TE; hence it is recommended that the clindamycin dose be 1800 mg/day if tolerated.[480,503,504] Encouraging results have been reported with other drug combinations. These include Fansidar (pyrimethamine-sulfadoxine), which has been used in a dose of one tablet twice weekly,[505] and pyrimethamine-dapsone administered on an intermittent schedule (two to three times a week).[506–508] The long half-life of these agents allowed the longer dosing interval, but Fansidar (currently not available in the United States) may have an increased risk of severe cutaneous reactions, and the longer half-life results in slower drug clearance after discontinuation of therapy.[509] When pyrimethamine was used alone as maintenance therapy at 50 mg/day[510,511] and 100 mg/day,[510] the relapse rates were 10% to 28% and 5%, respectively. Atovaquone alone or in combination regimens also appears to have activity based on uncontrolled trials; combination therapy (with sulfadiazine or pyrimethamine) should be used whenever possible.[486–488,512]

The optimal time to initiate ART in HIV-infected patients with TE has not been well defined. In one randomized trial of 282 patients with opportunistic infections other than tuberculosis (only 5% with TE), which compared early (median, 12 days after initiation of opportunistic infection therapy) to deferred (median, 45 days) initiation of ART, the secondary end point of AIDS progression or death occurred significantly less frequently in the early initiation group.[513] Thus, in the absence of contraindications, ART can reasonably be started 2 to 3 weeks after diagnosis of TE.

Primary prophylaxis against *T. gondii* in patients with AIDS has been shown to be effective in preventing acute TE.[514–517] In addition, the use of combination ART in HIV-infected patients has had a profound effect in decreasing the incidence of TE in these patients.[101,103] Primary prophylaxis is recommended for patients who have detectable *Toxoplasma* IgG antibodies and whose lowest $CD4^+$ count has been less than 100/mm^3 (many experts use <200/mm^3 as the cutoff rather than 100/mm^3), regardless of the HIV RNA viral load.[504] TMP-SMX (1 double-strength or single-strength tablet/day), dapsone (50 mg/day) plus pyrimethamine (50 mg/wk), and Fansidar (twice weekly; not available in the United States) have been reported to be effective in preventing the first episode of TE.[504,514,517,518] Atovaquone (1500 mg daily) alone or combined with pyrimethamine (25 mg daily) can also be used, although failures in HSCT patients have been reported.[519]

Studies have demonstrated that it is safe to discontinue primary anti-*Toxoplasma* prophylaxis or chronic maintenance therapy when the recovery of a $CD4^+$ count greater than 200 cells/mm^3 is achieved and sustained in patients on ART.[300–302,520,521] It is important to recognize that in most of these studies, the median $CD4^+$ count was greater than 300 cells/mm^3 at enrollment, and viral loads were below detection limits or reasonably controlled in most patients. More recent data from a European collaboration after multiple cohorts demonstrated a low risk of developing TE in seropositive patients with $CD4^+$ counts of 100 to 200 cells/mm^3 and HIV plasma viral loads below detection limits even if they are not receiving primary prophylaxis; similar benefit was not, however, seen in patients receiving chronic maintenance therapy for

prior TE.[522] Current recommendations are to discontinue prophylaxis in patients receiving cART when the CD4 count has risen above 200 cells/mm^3 for at least 3 months (primary prophylaxis) or 6 months (chronic maintenance), and to consider discontinuing primary prophylaxis in patients with CD4 counts of 100 to 200 cells/mm^3 if HIV plasma viral loads are below detection limits for 3 to 6 months.[504]

At present, TMP-SMX is used by most transplant teams as prophylaxis against *Pneumocystis* pneumonia. Its use has also been shown to protect against toxoplasmosis. However, TMP-SMX is not protective in every case, and some patients are not able to tolerate the drug combination. In addition, in some patients sulfonamides may be contraindicated. Thus use of TMP-SMX alone may be sufficient for prevention of toxoplasmosis in patients who are seronegative for *T. gondii* antibodies and who receive heart transplants from seropositive donors (i.e., D^+/R^- patients).[523] The optimal schedule for administration of TMP-SMX in heart transplant patients has not been defined. Physicians must decide whether a schedule of daily administration or administration three times each week is to be used. We routinely recommend daily use of single-strength TMP-SMX whenever feasible.[15]

Roxithromycin, administered at 900 mg once a week and may be given in three divided doses,[524] has been reported in a small randomized trial to be effective for primary prophylaxis; roxithromycin is unavailable in the United States. Based on a number of clinical trials, pyrimethamine alone cannot be recommended for primary prophylaxis.[98,525,526] Clarithromycin[527] and spiramycin[528] have been ineffective for primary prophylaxis when they were used alone. In a randomized, placebo-controlled, primary prophylaxis trial, clindamycin (600 mg/day) was associated with an unacceptably high rate of associated GI disease, in particular diarrhea.[529]

Data on the outcome of treatment of AIDS patients with toxoplasmosis outside the CNS are limited; available information on the therapy of ocular[291,530,531] and pulmonary involvement[285,532] indicates that these forms of toxoplasmosis are also responsive to treatment. Therapy was successful in 50% to 77% of patients with pulmonary toxoplasmosis.[285,532]

Ocular Toxoplasmosis

A recent report from the American Academy of Ophthalmology highlighted the limited data that are available from randomized controlled trials with well-defined end points demonstrating the benefits of therapy.[533] Treatment is most likely indicated in the following settings: any decrease in visual acuity, macular or peripapillary lesions, lesions greater than one optic disk diameter, lesions associated with a moderate-to-severe vitreous inflammatory reaction, the presence of multiple active lesions, the persistence of active disease for more than 1 month, and any ocular lesions associated with recently acquired infection. Because the disease can be self-limited in immunocompetent patients, many clinicians may not treat small, peripheral retinal lesions that are not immediately vision threatening.[14,316,534–536]

The reported benefits of medical therapy are related primarily to the clinical presentation.[316,534] Because there is so much variation in the clinical manifestations of the retinal disease, and because the disease may be self-limited even without treatment, the response to therapy is difficult to interpret. The combination of pyrimethamine (100-mg loading dose given over 24 hours for 2 days, followed by 25–50 mg daily) and sulfadiazine (1 g given four times daily for 4–6 weeks), depending on the clinical response, which is considered "classic" therapy for ocular toxoplasmosis, is the most common drug combination used (see Table 278.2).[534] TMP-SMX showed responses similar to pyrimethamine-sulfadiazine in a recent randomized, single-blind trial, although the latter regimen was used at lower-than-standard doses.[537] Two recent, open, randomized trials found no differences in response rates to intravitreal clindamycin plus dexamethasone compared with an oral regimen combining pyrimethamine, sulfadiazine, leucovorin, and prednisone/prednisolone.[538,539]

Clindamycin (300 mg orally every 6 hours for a minimum of 3 weeks) has also been used with favorable clinical results.[534] Intravitreal clindamycin combined with dexamethasone has been shown to have similar benefits to the oral combination regimen of pyrimethamine and sulfadiazine.[464] Intravitreal TMP-SMX combined with dexamethasone has shown benefit in small uncontrolled studies.[540,541] Other drugs that may have activity but have been inadequately studied include atovaquone and pyrimethamine plus azithromycin.[542,543] A small randomized trial comparing oral azithromycin to TMP-SMX found no difference in clinical responses.[544]

Systemic corticosteroids are indicated when lesions involve the macula, optic nerve head, or papillomacular bundle, although no randomized trials have demonstrated their benefit compared with antimicrobial therapy alone.[545] Corticosteroid therapy alone may be associated with progressive disease.[546] Photocoagulation has been used both for the treatment of active lesions and for prophylaxis against the spread of lesions because new lesions appear contiguous to old lesions.[534] In some patients vitrectomy and lens removal may be necessary.

Given the high relapse rate seen in some patients with ocular toxoplasmosis, prevention of recurrences would be highly desirable. A randomized, open-label trial of 124 patients in Brazil found that TMP-SMX (1 double-strength tablet every 3 days) was effective in decreasing the frequency of recurrences from 24% to 7% in a population at high risk for recurrences.[214] Although a 10-year follow-up report suggested that efficacy was lost once drug was stopped,[547] a subsequent randomized, placebo-controlled Brazilian trial confirmed the efficacy of prophylactic TMP-SMX (1 double-strength tablet every 2 days) and suggested sustained benefit for 2 years after discontinuing therapy.[548] Such a regimen should be used in patients with frequent or severe recurrences.[549]

For the approach to ocular toxoplasmosis during pregnancy see "Acute Acquired *Toxoplasma* Infection in Pregnant Women."

Acute Acquired *Toxoplasma* Infection in Pregnant Women

Treatment of the acutely infected pregnant woman does not completely eliminate but does decrease the incidence and severity of fetal infection. Because there is usually a delay between the acquisition of acute maternal infection, infection of the placenta, and subsequent infection of the fetus, identification of acute maternal infection necessitates immediate institution of treatment of the mother. Most experience of maternal treatment to prevent transmission to the fetus has been with spiramycin (3 g/day, obtainable in the United States from the FDA, 301-796-1400; after hours, 301-796-8210) (Table 278.3). Spiramycin has been accepted by most investigators as being effective in reducing the frequency of maternal transmission of *T. gondii* to the fetus by approximately 60%.[331,382] Spiramycin is indicated for patients confirmed or suspected to have been infected before 14 weeks of gestation. It appears that its maximal efficacy is best achieved when given within 8 weeks of seroconversion.[334] Spiramycin should be continued until delivery, even if results of the amniotic fluid PCR are negative and ultrasound examinations are normal. A retrospective study suggested that the combination of spiramycin and TMP-SMX (after the 14th week) is more effective than spiramycin alone in reducing transmission during the second trimester.[550] If spiramycin cannot be used or is not available, it may be replaced by sulfadiazine (with appropriate precautions at term) or clindamycin alone. However, there are no data on the efficacy of sulfonamides, including sulfadiazine, or clindamycin when these drugs are used for this purpose.

Because spiramycin does not reliably cross the placenta,[551] if fetal infection is documented or highly suspected or maternal infection is confirmed or highly suspected of having been acquired at 14 weeks of gestation or later, the recommended therapeutic regimen is the combination of sulfadiazine (initial dose 75 mg/kg, followed by 50 mg/kg every 12 hours; maximum, 4 g/day), pyrimethamine (50 mg every 12 hours for 2 days, followed by 50 mg daily), and folinic acid (10–20 mg daily, during, and 1 week after completion of pyrimethamine therapy) (see Table 278.3).[334a,334b] Such treatment might be an alternative to the termination of pregnancy when abortion is not allowed by law or for women who desire to continue their pregnancy. Pyrimethamine should not be used in the first 14 weeks of pregnancy because of a concern for teratogenicity. In this circumstance, if indicated, sulfadiazine plus clindamycin can be considered, although there are no data on its efficacy in this situation.[9] In addition, pyrimethamine-sulfadiazine is also recommended for pregnant women in whom a recently acquired acute infection is highly suspected or confirmed during the late second or third trimesters; this is due to the high rates of vertical transmission observed in those

TABLE 278.3 Treatment Regimens for Toxoplasmosis During Pregnancy and for Congenital Disease

INDICATION	DRUG REGIMEN
Pregnant Women	
Pregnant women suspected or confirmed of having acquired infection before 14 weeks of gestation. Not recommended during pregnancy if the fetus has documented or suspected infection (see below)	**Spiramycin (oral)**[b]**:** 1 g (3 million units) q8h (for a total of 3 g or 9 million units/day) Spiramycin should be continued until delivery, even if fetal infection is not detected (e.g., negative amniotic fluid PCR test and negative follow-up ultrasound)
Pregnant women at ≥14 weeks of gestation and (1) in whom it is suspected or confirmed that acute infection was acquired at or after 14 weeks of gestation or (2) who have a positive amniotic fluid PCR test or an abnormal ultrasound suggestive of congenital toxoplasmosis	**Pyrimethamine:** 50 mg q12h for 2 days, followed by 50 mg daily *plus* **Sulfadiazine:** 75 mg/kg (first dose), followed by 50 mg/kg q12h (max., 4 g/day) *plus* **Folinic acid**[b]**:** 10–20 mg daily during and for 1 wk after pyrimethamine therapy Pyrimethamine is teratogenic and should not be used during pregnancy before week 18 (in some centers in Europe, it is used as early as week 14). Sulfadiazine should not be used alone; consider clindamycin plus sulfadiazine
Infants	
Congenital infection; treatment regimen is usually recommended for 1 yr	**Pyrimethamine:** 1 mg/kg q12h for 2 days, followed by 1 mg/kg/day for 2 or 6 mo, followed by 1 mg/kg/day every Monday, Wednesday, and Friday *plus* **Sulfadiazine:** 50 mg/kg q12h *plus* **Folinic acid**[c]**:** 10 mg three times weekly **Prednisone (if CSF protein ≥1 g/dL or severe chorioretinitis):** 0.5 mg/kg q12h (until CSF protein <1 g/dL or resolution of severe chorioretinitis)
Older Children	
Active disease; treatment is usually continued for 1–2 wk beyond resolution of clinical manifestations	**Pyrimethamine:** 1 mg/kg q12h (max., 50 mg) for 2 days, followed by 1 mg/kg/day (max., 25 mg) *plus* **Sulfadiazine:** 75 mg/kg (first dose), followed by 50 mg/kg q12h (max., 4 g/day) *plus* **Folinic acid**[a]**:** 10–20 mg three times weekly **Prednisone (severe chorioretinitis):** 1 mg/kg/day in two divided doses; max., 40 mg/day, rapid taper

[a]Assistance is available for the diagnosis and management of toxoplasmosis at the Palo Alto Medical Foundation–*Toxoplasma* Serology Laboratory; Palo Alto, CA; www.pamf.org/serology/; 650-853-4828; toxolab@pamf.org; or US (Chicago) National Collaborative Treatment Trial Study; 773-834-4152.
[b]Spiramycin is not teratogenic, and it is available in the United States through the investigational new drug process at the US Food and Drug Administration (301-796-1600). Prior consultation with medical consultants is required.
[c]Folinic acid (leucovorin) should always be given with pyrimethamine to minimize toxicity; folic acid must not be used as a substitute for folinic acid.
CSF, Cerebrospinal fluid; *max.,* maximum; *PCR,* polymerase chain reaction.

stages of gestation and should be recommended even though fetal infection may not yet have been confirmed.

A group of European investigators reported between 1999 and 2007 that, in their studies, a significant effect of prenatal treatment on the risk of vertical transmission and clinical signs of congenital toxoplasmosis was not detected.[552–555] These results are not surprising because the studies included very few untreated women in their analysis, most untreated women were infected during the third trimester, and severe cases were excluded.[556,557] More recently, several studies from Europe have consistently reported evidence for an association between early treatment and reduced risk in the incidence and severity of congenital toxoplasmosis.[334,336,337,558,559] Given that recent studies have consistently reported a clinically significant benefit[556] and that a significant benefit was never ruled out in previous studies, most authorities continue to recommend spiramycin or pyrimethamine-sulfadiazine for women with suspected or confirmed acute *T. gondii* infection acquired during gestation.

Pregnant women with toxoplasmic chorioretinitis as a result of reactivation of chronic disease do not have a higher risk to transmit the parasite to their offspring than do pregnant women who have been infected before pregnancy and do not have ocular disease.[560] Their eye disease should be treated according to the indications discussed in the section "Ocular Toxoplasmosis." Pregnant women with toxoplasmic chorioretinitis thought to be a manifestation of recently acquired infection should be treated because of both the eye disease and the risk of transmission of the infection to their fetus.

Congenital Infection

Detailed information on and recommendations for the postnatal treatment of congenital toxoplasmosis are reviewed elsewhere,[9,561] but we favor continuous sulfadiazine (50 mg/kg every 12 hours), pyrimethamine (loading dose, 1 mg/kg every 12 hours for 2 days; then beginning on day 3, 1 mg/kg per day for 2 or 6 months; then this dose every Monday, Wednesday, and Friday), and folinic acid (10 mg three times weekly during and for 1 week after pyrimethamine therapy) for a minimum of 12 months (see Table 278.3).[557,562] Serial follow-up to gauge the response of the infant to therapy should include neuroradiology, ophthalmologic examinations, and CSF analysis if indicated.[9] Fansidar (unavailable in the United States) has also been used and reported to be well tolerated,[563,564] although safety concerns and availability of alternative regimens preclude recommending its use.

Studies have shown that outcomes are substantially better for most, but not all, infants treated from the neonatal period for 12 months with pyrimethamine-sulfadiazine and leucovorin, compared with historical control subjects receiving no or short-course therapy.[562,565–567] Improvement in intellectual function, regression of retinal lesions, reduction in anticonvulsant drug requirements, and prevention of auditory sequelae appear to be the major benefits of such treatment, which was combined with CSF shunting if required.[562] Signs of active infection resolved within weeks of initiation of treatment. In a significant number of treated children, cerebral calcifications diminished in size or resolved.[567] They also reported that new central chorioretinal lesions were uncommon (14%) in children with congenital toxoplasmosis who have been treated during their first year of life, compared with historical reports of much higher rates (≥82%) for untreated children or those treated for only 1 month near birth.[349]

PREVENTION AND PROPHYLAXIS

General Methods

Prevention is most important in seronegative pregnant women and immunodeficient patients. It is most readily accomplished through education of these patients by their personal physicians (Table 278.4). The goal is to avoid the ingestion of and contact with tissue cysts or sporulated oocysts. Tissue cysts in meat are made noninfectious by heating the meat to 67C°/153°F (i.e., should be cooked to "well done" with no pink meat visible in the center) or by freezing it to –20°C/–4°F (which is not attainable in most home freezers) for at least 48 hours. Meat that is smoked, cured in brine, or dried may still be infectious. Oysters, clams, or mussels should not be eaten raw. Hands should be washed thoroughly after handling raw meat or vegetables, eggs should not be eaten raw, and unpasteurized milk (particularly milk from goats) should be avoided. Vectors such as flies and cockroaches should be controlled. Areas contaminated with cat feces should be avoided altogether. Disposable gloves should be worn while disposing of cat litter material, working in the garden, or cleaning a child's sandbox. Oocysts are killed if the cat litter pan is soaked in nearly boiling water for 5 minutes. If the litter pan is cleaned every day, oocysts will not have a chance to sporulate. Serologic testing of cats is unwarranted because testing does not demonstrate whether the infected cat is excreting oocysts. Untreated water has been shown to be an effective vehicle for the transmission of the parasite, and drinking water sources potentially contaminated with oocytes should be avoided.

Serologic Screening and Prophylaxis Acute *Toxoplasma gondii* Infection and Toxoplasmosis in the Immunodeficient Patient

Transmission of *T. gondii* and death caused by the infection have resulted from the transfusion of leukocyte-rich blood products and by organ transplantation in immunodeficient patients. Transmission of infection by these routes may occur frequently enough to warrant screening for antibody to *T. gondii* in leukocyte-rich blood product donors and possibly to exclude seropositive people as organ donors to seronegative potential recipients whenever feasible.

Primary prophylaxis can prevent toxoplasmosis in patients dually infected with HIV and *T. gondii* (see "Toxoplasmosis in the Immunodeficient Patient" under "Therapy"). Prophylactic treatment (pyrimethamine, 25 mg orally every day for 6 weeks after transplantation) has been used with apparent success in seronegative recipients of hearts transplanted from seropositive donors.[568] However, TMP-SMX used for PCP prophylaxis in solid-organ transplant patients is also effective as primary prophylaxis against *T. gondii* and can be used without addition of pyrimethamine. Primary prophylaxis in BMT and HSCT patients is particularly challenging because TMP-SMX cannot safely be used early (i.e., before engraftment), whereas in patients with all other transplant organs, it can be used immediately after transplantation. Atovaquone is an alternative, but its efficacy has not been evaluated in controlled trials, and breakthroughs have been reported.[569] A preemptive approach using *Toxoplasma* PCR in whole blood, similar to the strategy for preventing CMV disease using serum PCR, should be entertained for immunocompromised patients in whom prophylaxis is not feasible during periods of high risk for development of toxoplasmosis.[281]

TABLE 278.4 Measures to Prevent Primary *Toxoplasma gondii* Infection

- Avoid contact with materials potentially contaminated with cat feces, especially handling of cat litter and gardening. Gloves are advised when these activities are necessary. Because oocysts require 1 to 2 days to mature, dispose of all cat feces daily.
- Disinfect cat litter box with near-boiling water for 5 minutes before handling.
- Avoid mucous membrane contact when handling raw meat.
- Wash hands thoroughly after contact with raw meat.
- Kitchen surfaces and utensils that have come in contact with raw meat should be washed.
- Freeze meat to –20°C (–4°F) for at least 48 hours.
- Cook meat to 67°C (153°F) or "well done" (meat should not be "pink" in the center).
- Avoid ingestion of dried, smoked, or cured meat because they may be infectious.
- Wash fruits and vegetables before consumption.
- Refrain from skinning animals.
- Avoid drinking untreated water potentially contaminated with oocysts.
- Avoid drinking unpasteurized goat's milk.
- Avoid eating raw oysters, clams, and mussels.

Congenital *Toxoplasma gondii* Infection and Toxoplasmosis

Congenital toxoplasmosis is a preventable disease. It is therefore the responsibility of physicians who care for pregnant women to educate them on how they can prevent themselves from becoming infected and thereby not place their fetus at risk. A lack of adoption of a systematic serologic screening program in the United States leaves education as the principal means of preventing this tragic disease. If physicians choose to screen their patients serologically, the appropriate tests must be used, the laboratory performing the tests must be competent, and the test results must be interpreted correctly. Nonreference laboratories can effectively accomplish the initial screening testing by simultaneously performing IgG and IgM antibody tests. Women with positive results in the initial IgG antibody test should have a test for IgM antibody performed on the same serum. Only IgM-positive test results need to be sent to a reference laboratory for confirmatory testing (e.g., PAMF-TSL).[359,379,381] Clinical decisions should not be made based on positive IgM test results alone. In patients with IgG antibodies at any titer, a negative IgM antibody test result in the first trimester, and no clinical signs of acute toxoplasmosis, no further testing would be necessary because the probability of acute acquired infection in these women is extremely low. Given the same circumstances in the second trimester of pregnancy, a negative IgM test result rules out, for practical purposes, recent acquisition of acute infection. A negative IgM test in the third trimester may occur in a patient who acquired the infection earlier in gestation.

In some countries (e.g., France, Austria, Italy, Slovenia, Lithuania, and Uruguay), initially seronegative pregnant women are retested at various intervals during gestation to detect seroconversion and institute treatment.[570] Monthly screening of seronegative pregnant women was recently reported to significantly decrease the risk of vertical transmission and of clinical signs at 3 years of age.[558] The appropriate use of prenatal diagnosis, followed by the optimal use of spiramycin and/or pyrimethamine-sulfadiazine–folinic acid, can markedly reduce the incidence of clinically significant congenital toxoplasmosis.[a] Its absence is often associated with poor outcomes.[338] Results of a study in France[333] on the incidence of *T. gondii* infection in fetuses of women whose date of acquiring the infection during the gestation was known and who were treated with spiramycin are shown in Table 278.5.

[a]References 333, 336, 337, 558, 571, 572.

TABLE 278.5 Rates of Congenital Transmission in 270 Women and the Sensitivity and Negative Predictive Value of Amniotic Fluid PCR for Prenatal Diagnosis of Congenital Toxoplasmosis, According to Gestational Age at Which Maternal Infection Was Acquired

GESTATIONAL AGE AT MATERNAL INFECTION[a] (wk)	NO. OF INFECTED FETUSES[b]/TOTAL (%)	AMNIOTIC FLUID PCR	
		Sensitivity (CI) (%)	NPV (CI) (%)
±6	0/14 (0)	N/A	100 (78–100)
7–11	7/50 (14)	29 (8–65)	90 (81–98)
12–16	7/61 (11.5)	57 (21–94)	95 (86–98)
17–21	14/66 (21.2)	93 (68–99)	98 (90–99.7)
22–26	16/36 (44.4)	63 (39–86)	77 (61–93)
27–31	19/30 (63.3)	68 (48–89)	65 (42–87)
≥32	12/13 (92.3)	50 (22–78)	14 (3–52)
TOTAL	75/270 (28)	N/A	N/A

[a]Maternal infection was diagnosed by seroconversion in the 270 women; 261 (97%) were given treatment with spiramycin.
[b]Congenital infection was diagnosed by the persistence of *Toxoplasma* immunoglobulin antibodies after 1 year of life.
CI, Confidence interval; *N/A,* not applicable; *NPV,* negative predictive value; *PCR,* polymerase chain reaction.
Note: The positive predictive value was 100%, regardless of gestational age.
Modified from Montoya JG, Remington JS. Management of Toxoplasma gondii *infection during pregnancy.* Clin Infect Dis. *2008;47:554–566.*

In pregnant women infected with both HIV and *T. gondii*, we recommend that primary prophylaxis against *T. gondii* be introduced when their CD4 T-cell count falls below 200/mm^3. If the patient is receiving TMP-SMX, additional prophylaxis is probably not necessary. Otherwise, spiramycin can be used at a dose of 3 g/day.

ACKNOWLEDGMENTS

We gratefully acknowledge and thank Dr. Jack S. Remington, whose lifelong study of toxoplasmosis and authorship of previous editions of this chapter provided the foundation upon which the current chapter is based. We would also like to thank Dr. Alan Sher for his review of the manuscript and helpful suggestions.

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279 *Giardia lamblia*

Theodore E. Nash and Luther A. Bartelt[a]

SHORT VIEW SUMMARY

Definition

- Giardiasis, caused by the protozoan *Giardia lamblia*, is a common cause of sporadic, endemic, and epidemic diarrhea throughout the world.
- Infected persons can have acute diarrhea with malaise, cramping and bloating, chronic diarrhea with malabsorption, or asymptomatic infection. Asymptomatic infection is most common in children, particularly in low-income settings, and may contribute to poor nutrition.

Epidemiology

- *Giardia* is one of the most widely distributed enteric parasites.
- In the United States there are approximately 17,000 documented infections annually, with estimated infections of more than 1.2 million; it is most frequently reported in children aged 1 to 9 years and adults aged 40 to 54 years.
- In low-income countries, *Giardia* infects nearly all children by the age of 10 years.
- Transmission of this fecal-oral parasite is most common through person-to-person transmission and through untreated or inadequately treated surface water, and occasionally via contaminated food.
- Although specific human genotypes (assemblages A and B) are found in some animals, epidemics of giardiasis caused by animals have not been reliably observed. Animals typically harbor other assemblages.

Diagnosis

- A clinical syndrome of diarrhea and or other gastrointestinal symptoms lasting 7 to 10 days, and often associated with weight loss without fever, is helpful in distinguishing giardiasis from other enteric infections.
- Useful epidemiologic risk factors are a history of travel, drinking untreated surface water, having young children in daycare, or engaging in sexual practices with the potential for fecal-oral transmission.
- Stool examination for ova and parasites is the traditional method of diagnosis.
- Antigen detection is widely available and is more sensitive and specific than stool examination for ova and parasites. Polymerase chain reaction assays are becoming more widely used and can identify the assemblage.

Therapy

- Tinidazole is the drug of choice, taken in a single dose (see Table 279.3).
- Alternatives are metronidazole, nitazoxanide, and albendazole.
- Potential adverse events associated with treatment should be discussed with the patient.
- Treatment during pregnancy requires special considerations (see "Therapy").
- Post-*Giardia* irritable bowel syndrome, lactose intolerance, or another organism or cause should be considered if treatment appears to fail. If parasites are present, then re-treatment with a drug of a different class is usually successful.

Prevention

- Prevention of giardiasis requires the proper handling and treatment of drinking and recreational water supplies, good personal hygiene, and protection of food supplies from contamination.
- Bringing water to a boil, filtration, and careful halogenation can be effective measures to treat small volumes of water.

DESCRIPTION OF THE PATHOGEN

Giardia was probably described in the late 1600s, when van Leeuwenhoek likely discovered it in his own stool. It was in the early 1900s that the parasite received the genus name *Giardia*. The designated species name for the human parasite has been *lamblia*; *intestinalis* and *duodenalis* are also used. The genus *Giardia* is in the category of intestinal flagellates in the division Protozoa. The life cycle of *G. lamblia* has two stages: the motile dividing trophozoite present in the intestine of the host, and the environmentally resistant infectious cyst excreted in the feces. The trophozoite is 9 to 21 µm long and 5 to 15 µm wide (Fig. 279.1A). It has a convex dorsal surface and a flat ventral surface containing a disk with contractile proteins used for attachment (sucking or adhesive disk). There are four pairs of posteriorly directed flagella that are involved in locomotion and perhaps attachment.[1–3] The protozoan has two anteriorly placed diploid nuclei, each with a prominent central karyosome and a complete copy of the genome.[4] In stained preparations, the nuclei create the characteristic facelike image. Each of the two *Giardia* nuclei maintains a high degree of homozygosity, most likely through fusion and exchange of DNA during encystation.[5,6] Median bodies—tight collections of microtubules—are visualized transversely in a clawlike manner in *G. lamblia* and have been used to identify morphologically distinct types of *Giardia*. Of the *Giardia* spp., only *G. lamblia* has been successfully cultured in vitro.[7] Growth[8] is enhanced by the presence of biliary lipids and a high concentration of cysteine, consistent with the predilection of *Giardia* for colonizing the upper small bowel. The trophozoite divides by longitudinal binary fission and has a doubling time in culture of 6 to 12 hours. It is an aerotolerant anaerobe that scavenges oxygen to enhance survival.[9] *Giardia* uses glucose as the major source of carbohydrate energy, metabolizing it to the end products of acetate, ethanol, alanine, and carbon dioxide; adenosine triphosphate (ATP) is generated during this process.[8] Metabolism of arginine via the arginine dihydrolase pathway is another mechanism for ATP generation.

The differentiation of *Giardia* into species traditionally depended on morphology and the host of origin, with a number of species described: *G. lamblia* in humans, *Giardia muris* in mice, *Giardia agilis* in amphibians, *Giardia psittaci* in parakeets, and *Giardia microti* in voles and muskrats (Table 279.1) However, molecular studies later revealed morphologically identical *Giardia* that were found in both humans and animals. The earliest molecular typing of these *Giardia* isolates divided them into at least three groups,[10] which were subsequently renamed and expanded into eight genotypes or referred to as assemblages A to H based on sequence differences in housekeeping genes[11,12] (see Table 279.1).[12,13,14] Assemblages A (often subtype AII) and B are associated with most but not all[15] human infections and a number of nonhuman mammalian hosts.[11,13,14] Their biology, biochemistry, and molecular mechanisms differ and fit criteria to be separate species.[16,17] Occasional infections with non-A, non-B assemblages, including assemblage E, have been reported in humans.[15] Improved sequencing methods using a broader sampling of isolates will better define genetic diversity and range of *Giardia* genotypes.[12,15,18]

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.



FIG. 279.1 *Giardia lamblia* trophozoite (A) and cyst (B) are demonstrated in a trichrome stain of fecal material. Note the prominent nuclei in the trophozoite. In the cyst, centrally located axonemes, a clawlike median body, and two nuclei can be seen.

TABLE 279.1 Typing of *Giardia* Species

NAME	GENOTYPE	HOST RANGE
Giardia agilis		Amphibians
Giardia ardeae		Birds
Giardia microti		Muskrats and voles
Giardia muris		Rodents
Giardia psittaci		Birds
Giardia lamblia	Assemblage A	Humans, primates, dogs, cats, cattle, sheep, deer, rodents
	Assemblage B	Humans, primates, dogs, cattle, horses, beaver
	Assemblage C	Dogs
	Assemblage D	Dogs
	Assemblage E	Cattle, goats, sheep, pig
	Assemblage F	Cats
	Assemblage G Assemblage H	Rodents Marine vertebrates

Modified from Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. Clin Microbiol Rev. *2011;24:110–140.*

Different *G. lamblia* genotypes have unique capacities to establish infection and to cause disease in animal models and experimental human infections, but whether specific genotypes differ in virulence in human natural infections[8,12,19–22] is unclear.

Some Unique Biologic Aspects

The genome of the prototypic assemblage A isolate, WB (http://giardiadb.org),[23] is approximately 11.7 Mb, distributed over five chromosomes. Analysis of its small subunit ribosomal RNA[8,24] sequence and genes of certain pathways[25] indicate that it is one of the earliest-branching eukaryotes.[23] The genome is compact and simplified, including DNA synthesis, transcription, RNA processing, and cell-cycle machinery. The parasite contains only a few introns, extremely short promoters, sometimes 60 bp or less, overlapping transcriptional controlling elements, and mitosomes, remnants of earlier mitochondria.[26,27] Pathways to synthesize phospholipids, fatty acids, cholesterol, and purine and pyrimidine nucleosides are reduced or lacking, so these essential metabolic building blocks are largely scavenged from the small intestine milieu. There are only enzymes sufficient for remodeling but not synthesis of lipid membrane components.[8,23] Except for alanine, amino acids are taken up from the environment.

Because *Giardia* is an early-branching, highly adapted organism and an important disease-causing parasite, many aspects of its unique biology have been extensively studied and reviewed elsewhere.[8] However, a few important examples of its particularities include surface antigenic variation, a unique vesicular transport system devoid of a Golgi apparatus, and the processes of encystation and excystation.

Giardia is the only known intestinal dwelling organism to undergo frequent surface antigenic variation.[28,29] A single variant-specific surface protein (VSP) is expressed on the surface of *Giardia* and, after several generations, is replaced by another VSP.[29–32] VSPs are a family of about 250 cysteine-rich proteins with similar motifs and general structure and a conserved transmembrane carboxyl-terminal sequence.[30,33] Although all VSPs are transcribed, only one is expressed. The others VSPs are possibly eliminated by interference RNA mechanisms.[32,34] Antigenic variation occurs during infection of humans and animals, in vitro in culture, and during encystation.[8,30] Although it is important in evading the host immune response, certain VSPs are favored or disallowed even in the absence of an adaptive immune response.[35] Specific VSPs seem to have unique physical-chemical characteristics that allow them to be biologically selected and expressed or disfavored and not expressed, depending on the intestinal environment and host.[35]

G. lamblia trophozoites encyst to form fibrillary, oval, thin-walled cysts 8 to 12 µm long and 7 to 10 µm wide (see Fig. 279.1B). *Giardia* encystation is a model system for vesicular transport and developmental biology because *Giardia* lacks a typical Golgi apparatus that is an essential component of vesicular transport in most higher organisms.[5,36–38] Encystation induced in vitro[39] is a complex process involving many of *Giardia's* basic processes[5,40,41,42] The early phase is characterized by the formation of highly characteristic encystment vesicles (ESVs), in which cyst wall proteins (CWPs) 1 through 3 and other molecules are processed.[36,43,44] The late phase of encystation consists of transport of the CWPs to the cell surface, their assembly with *N*-acetylgalactosamine (GalNAc) into the cyst wall, and then nuclear division and DNA replication without cell division so that cysts are generated with a ploidy of 16N.[5,38,39,41,45] At ultrastructural analysis, the cyst wall contains CWPs and GalNAc polymer in insoluble fibrils.[46]

Excystation is a highly coordinated process that is initiated when cysts are exposed to environmental stimuli, such as gastric acid and pancreatic enzymes.[5,47] The process is complex and well described morphologically but not well understood on a molecular level.[48–50] An excyzoite containing four nuclei (4N each) is released; this divides twice without further DNA replication, resulting in four daughter trophozoites.[5,51]

EPIDEMIOLOGY

Giardia is widely distributed. In the United States, *G. lamblia* has been demonstrated in 4% to 7% of submitted stool specimens, making it the most commonly identified intestinal parasite. US. epidemiologic surveys of giardiasis from 2011 to 2012 found that 15,223 to 16,868 cases were reported annually, with the case rates in different states varying from 1.7 to 29.2 cases per 100,000 persons and an overall mean of 5.8.[52] Because of substantial underreporting, a current estimate is that more than 1.2 million cases may occur annually, accounting for an estimated

$34 million dollars in annual hospitalization costs.[53,54] Only 1.3% of cases were associated with a detected outbreak.[52] *Giardia* was most frequently reported in children 1 to 9 years old and to a lesser extent in adults 40 to 54 years old, and during the early summer through early fall months[52]; these findings are similar to those in Canada and other high-income countries.[55] In the United States, coenteric infections with *Giardia* are uncommon (2.2%).[56]

In low-income regions of the world, *Giardia* is one of the first enteric pathogens to infect infants.[57–60,61] In these settings, incidence ranges from 37.7% to 96.4% in the first 2 years of life, and 40% of these children have persistent (>8 weeks) infection. Peak prevalence rates of 15% to 30% occur in children younger than 10 years.[62,63,64,65,66] Nearly all children in these settings become infected, and *Giardia* is frequently detected together with other viral, bacterial, and parasitic pathogens.[60,67]

Natural acquisition of the parasite occurs through fecal-oral ingestion of *Giardia* cysts through contaminated water, food, or person-to-person transmission. *Giardia* cysts survive well in the environment, particularly in cold water. *Giardia* was the most frequent cause of outbreaks of diarrhea resulting from drinking water in the United States from 1971 to 2006[68]; however, the risk from drinking water dropped substantially in the 1980s and continues to steadily decline[69] as improved measures for water treatment have been implemented. Recreational water,[70] primarily in treated swimming venues and lakes, is also a risk for *Giardia*. Surface water can become contaminated by human or animal sources[69,71] so backpackers who do not adequately treat water can become infected. Most waterborne outbreaks can be traced to untreated or improperly treated water or faulty purification systems.[68,72,73] Food handler contamination at restaurants due to poor hand hygiene, particularly with produce, has been linked to the majority of foodborne outbreaks.[69,74,75]

Person-to-person transmission occurs in groups with close contact and an increase in fecal contamination, especially between young children and their parents and siblings, in men who have sex with men, and between young children in daycare centers. Historically, the prevalence of *Giardia* in children in daycare has been as high as 20% to 50%,[76] which seems to have decreased in the United States lately. Many young children are asymptomatic, but they can spread the infection within their homes and contribute to secondary spread within their communities.[77,78]

Giardia was the pathogen identified most frequently in returning travelers and immigrants seeking medical care and diagnosed with infectious gastrointestinal disease—diagnosed at a rate of 31 per 1000 ill returned adult travelers.[79] Travel to the Indian subcontinent was the highest-risk destination.

Many mammalian hosts such as gerbils, mice, beavers, sheep, cattle, dogs, and cats (see Table 279.1)[11,12,14,80,81] have harbored or been experimentally infected with *Giardia* morphologically similar to, and in some instances identical to, the genotypes infecting humans. Genetic subtyping indicates the low zoonotic potential for most animal isolates.[12,18] The strongest epidemiologic link for animal isolates associated with outbreaks of human infection were Canadian waterborne outbreaks of giardiasis linked to beavers.[82,83]

PATHOGENESIS AND IMMUNE RESPONSE

Giardia was once thought to be a harmless commensal, but its association with symptomatic diarrhea, malabsorption in children, disease after waterborne outbreaks, travel, and experimental human infections[19] has clearly established its pathogenicity. Pathophysiologic mechanisms need to explain the variety of symptoms and clinical outcomes that occur in exposed and infected populations, the high level of asymptomatic infections in endemic regions compared with common symptomatic epidemic disease in primarily nonendemic intermittently exposed populations, and the variety of infection rates and symptoms in singular populations. Although there is a large body of experimental data, as a group the studies are particularly difficult to interpret because of the variability of the models and methods employed. Most nonhuman experimental infections have used a variety of rodent and small mammal models with either *G. muris* in mice or rats or different human *Giardia* isolates in mice, gerbils, or rats. Frequently, the isolates used were not well characterized. Many of the findings, particularly those from studies of immunity, are based on murine host responses and extrapolated to human infections. The emerging picture reveals a complex interaction between a genetically diverse, adaptable group of closely related, morphologically similar organisms and their hosts.

Infection occurs after oral ingestion of as few as 10 to 25 cysts.[84] The ability to establish infection and to cause diarrhea varies among isolates, assemblages, and possibly VSP expression patterns.[8,19–21,35,85] After excystation, trophozoites adhere to the brush border of the epithelium via their ventral disk[1] and multiply in the upper small bowel. Adherence can be modified or prevented by means of a number of mechanisms.[86] The parasite may avoid being expelled by mechanical means.[87] Small bowel biopsies in most cases show little inflammation. However, some may demonstrate villous shortening and atrophy[23,88]; and the degree of histologic abnormality correlates with severity of clinical manifestations.[89–91] Among the postulated mechanisms of trophozoite pathogenesis in the small intestine—mucosal invasion, elaboration of an enterotoxin, and disruption of the intestinal epithelial brush border and function—there is little reproducible evidence that mucosal invasion or production of an enterotoxin occurs. *G. lamblia* does disrupt brush border functional enzymes, including disaccharidases in vitro in human small intestinal epithelial cell monolayers.[92] Trophozoites also disrupt tight junctions, increase permeability, and induce apoptosis.[93–96,97] Many of these effects are caspase-3 dependent.[93,94] Epithelial barrier dysfunction and apoptosis have also been seen in duodenal biopsy specimens from persons with giardiasis and some murine models of persistent infection.[98,99]

The effects of *Giardia* on epithelial cells in vitro appear nutrient dependent. High glucose concentrations can counteract caspase-3–dependent epithelial cell apoptosis through upregulation of the sodium-dependent glucose cotransporter in response to *Giardia* proteases.[100] *Giardia*-mediated arginine consumption reduces epithelial cell proliferation.[101] Arginine consumption may favor parasite colonization by suppressing arginine-dependent nitric oxide synthetase in the host, thus limiting the protective effect of nitric oxide[102,103] These interactions may partially explain dietary influences on epithelial cell responses and *G. lamblia* persistence in mice.[99,104]

Host immunity plays a role in clearance of the parasite,[51] protection against reinfection, and pathogenesis.[51] In part, the findings are dependent on the model system that is used. Immune responses in humans, especially during acute illness, are not well studied, and much of the information is extrapolated from mouse and gerbil models of infection with *G. muris* or *G. lamblia.* There are differences in the immune responses of mice to infection with a rodent *G. muris* compared with infections with *G. lamblia* isolated from humans.[99] Immune responses and course of infection also differ among *G. lamblia* isolates of different assemblages.[105,106] Time-dependent clearance of *G. lamblia* in murine models coincides with upregulation of intestinal genes related to α-defensin function, mast cell proteases, and antibody production.[107] Early mechanisms of control of *G. lamblia*–infected mice include interleukin-6 (IL-6).[108,109] This effect may be mediated via IL-6–producing mast cells and subsequent increased intestinal motility.[110–112] In addition, dendritic cells producing IL-6 can reverse the inability of IL-6–deficient mice to clear *G. lamblia.*[113] Innate immune-mediated signaling through TLR2 may counteract parasite clearance and in turn promote infection.[114] T-cell responses control infection, whereas B-cell responses appear to be more important at later time points.[115]

Several observations indicate that partial protective immunity can develop to *Giardia* infection. Protective immunity to reinfection develops in most rodent and gerbil infection models; partial immunity also develops in some situations in humans.[19,35,105] Lower rates of symptomatic disease were found in long-term residents of endemic areas of North America than in visitors or short-term residents.[116,117] In contrast, reinfection is common, particularly in children, in endemic settings.[59,60,64,118] Previously infected volunteers can be reinfected with the identical isolate after treatment but at a lower intensity and without symptoms.[19] Possible reasons for the ability to become reinfected are exposure to a different genotype, expression of a different VSP by an individual isolate, age and nutritional status of the host, and inability to develop intestinal immunity after a single infection.

Both B-cell and T-cell immunity appear to play essential roles in protective immunity,[110] but which arm of the immune system is dominant

depends on the infection model studied. A systemic antibody response occurs in individuals with *Giardia*. Although serum immunoglobulin M (IgM) and IgG antibodies develop and with complement can be lethal to *Giardia* trophozoites,[29] it is likely that gastrointestinal IgA antibodies secreted into the lumen where trophozoites are located play a more important role.[119–121] Failure to develop IgA against specific *Giardia* antigens has been suggested to correlate with chronic giardiasis in humans.[122] Evidence supporting a role for humoral antibodies. specifically IgA responses, in the control of *Giardia* infection initially has come from the increase in chronic giardiasis in patients with common variable immunodeficiency (CVID).[123] Because these patients may also have cellular immune defects, this association neither supports nor refutes humoral mechanisms. Patients living with acquired immunodeficiency syndrome (AIDS) do not have an increased susceptibility to *Giardia,* although they uncommonly develop severe disease that is difficult to treat.[124,125] More recent studies have demonstrated that natural exposure in immunocompetent adults leads to development of $CD4^+$ memory T-cell responses.[126,127] These cells produce greater IL-17A after stimulation with *Giardia* antigens in exposed compared with unexposed individuals.[126]

In mice, immunization with the full repertoire of VSPs expressed by a single isolate can lead to protection against homologous *Giardia* challenge.[128] This effect coincides with the development of broad anti-VSP antibodies, but the mechanisms are likely multifactorial. Either complete absence of IgA or defective transcellular transport due to deletion of the polymeric immunoglobulin receptor (pIgR) is associated with inability to resolve infection in humans.[110,119,121,129] The mechanism by which IgA prevents or helps to clear infection is probably by binding to trophozoites and preventing a critical adherence step. However, there is no evidence that IgA can kill trophozoites, and studies comparing IgA-expressing or total B-cell–deficient mice have demonstrated that B-cell–mediated clearance of *Giardia* is not limited to IgA production.[121]

More recently, IL17A has been identified as a key regulator of host defense responses against *Giardia* in animal models. Consequences of impaired IL-17A signaling through the cognate IL17RA receptor include defective transcellular IgA transport through pIgR together with reductions in several antimicrobial peptides.[130,131] Similar to humans, IL17A-producing $CD4^+$ T cells increase in mice following *Giardia* challenge. However, $CD4^+$ T-cell–depleted mice are unable to clear parasites despite robust IL17A upregulation, likely from innate sources.[130]

The role for T-cell responses in parasite control and pathogenesis is substantial. Athymic, T-cell–deficient mice are unable to clear infection with *G. muris* or *G. lamblia* until the mice are reconstituted with lymphoid cells, particularly the $CD4^+$ helper T lymphocyte.[115,132,133] After reconstitution, animals develop an abnormal intestinal histologic appearance that parallels the changes seen in some humans with giardiasis: flattening of villi, crypt hypertrophy, and a mononuclear cell infiltration of the submucosa.[89,134] Similarly, $CD4^+$ T-cell neutralization prolongs infection but also attenuates brush border defects in *Giardia*-infected mice.[106]

The complex host immune responses to *Giardia,* and immunomodulatory properties of the parasite itself, influence mucosal responses and disease manifestations of other pathogens in vitro and in animal models. The cathepsin B–like cysteine protease produced by some *Giardia* strains is capable of cleaving mucosal-derived IL-8, limiting neutrophil chemotaxis, and attenuating proinflammatory intestinal responses to other stimuli.[104,135,136] In a murine malnutrition model, sequential coinfection with *Giardia* and then enteroaggregative *Escherichia coli* enhanced weight loss and partially altered the mucosal responses observed during enteroaggregative *E. coli* infection alone.[137]

There is increasing recognition that resident microbiota influence both *Giardia* susceptibility and pathogenesis. Singer and Nash made the first sentinel observation that murine microbiota are capable of protecting against infection even in severely immunocompromised mice.[138] In addition, some *Lactobacillus* strains limit growth of *Giardia* through bile-salt hydrolases.[139] Microbiota, however, also contribute to *Giardia* pathogenesis. Jejunal aspirate cultures from patients with symptomatic giardiasis are more pathogenic to germ-free mice than axenic trophozoites (assemblage A).[140] These manifestations may be the result of direct microbial interactions. *Giardia* alters the intestinal bacterial composition throughout the gut of mice[141] and perturbs gut microbial amino acid metabolism in vivo.[137] Culturing otherwise commensal bacteria in the presence of *Giardia* or its products functionally promotes their pathogenic potential.[142,143] In some models, increases in mucosal-associated bacteria and myeloid inflammation persist beyond parasite clearance.[97,144] In contrast, antibiotics that reduce intestinal bacteria result in diminished $CD8^+$ T-cell activity and disaccharidase deficiency and prevent host growth impairment after *Giardia* challenge, even during malnutrition.[137,145] Whether and to what extent these clearly relevant interactions between *Giardia* and other microbes influence outcomes in humans, including post-*Giardia* infection sequelae, require further investigation.

CLINICAL MANIFESTATIONS

The clinical manifestations of giardiasis are broad and range from asymptomatic cyst passage to acute diarrhea and dehydration necessitating hospitalization. Infections may be short or prolonged, resulting in spruelike symptoms of chronic diarrhea with malabsorption. The proportion of individuals manifesting symptoms in a population appears to be influenced by *Giardia* prevalence and possibly age at first exposure, with significant variability in nonendemic versus endemic populations, the host nutritional and immune status and genetic makeup, coinfection with other enteric organisms, and the assemblage or strain of the prevalent *Giardia*. The natural history of *Giardia* infections in nonendemic populations is uncertain because most patients have not been rigorously followed without treatment and because of the demonstrated variability of different *Giardia* genotypes to cause disease. Patients with or without symptoms may self-cure or become chronically infected, sometimes lasting years.[146]

After the ingestion of *G. lamblia* cysts, there is an incubation period of 1 to 2 weeks before the onset of symptoms. The prepatent and incubation periods after instillation of 50,000 axenically grown trophozoites into the small intestine can be as short as 5 and 6 days, respectively.[19] Symptoms may occur before detection of cysts.[19] Symptomatic giardiasis is characterized by the acute onset of diarrhea, abdominal cramps, bloating, and flatulence (Table 279.2). The patient usually has malaise, nausea, and anorexia and may report sulfuric belching. Foul-smelling flatus and stools are common complaints, as is weight loss. Vomiting and upper gastrointestinal symptoms occur less frequently but may sometimes be a dominant symptom, leading to a delay in diagnosis. Fever and tenesmus are atypical and should raise suspicions for an alternative diagnosis. Initially, stools may be profuse and watery; the median number of stools per day has been as high as nine.[147] In short-term travelers, giardiasis is an infrequent cause of diarrhea compared with bacterial causes; it is more common in long-term travelers. Gross blood, pus, mucus, and

TABLE 279.2 Symptoms of Giardiasis

	PERCENTAGE	RANGE
Diarrhea	89	64–100
Malaise	84	72–97
Flatulence	74	35–97
Foul-smelling, greasy stools	72	57–79
Abdominal cramps	70	44–85
Bloating	69	42–97
Nausea	68	59–79
Anorexia	64	41–82
Weight loss	64	56–73
Vomiting	27	17–36
Fever	13	0–21
Urticaria	9	4–14
Constipation	9	0–17

Data from Hill DR. Giardiasis. Issues in diagnosis and management. Infect Dis Clin North Am. *1993;7:503–525.*

microscopic polymorphonuclear white blood cells (WBCs) are absent, and, if present, suggest another or concomitant diagnosis.

One of the most important distinguishing features of giardiasis is the prolonged duration of diarrhea, which is commonly intermittent with waxing and waning symptoms and usually is accompanied by weight loss. At the time of presentation, most patients have been symptomatic for more than 1 week to 10 days.[147] Weight loss of about 10 pounds occurs in more than 50% of patients with prolonged symptoms.[148] Unusual reported associations include urticaria, other skin rashes, reactive arthritis,[149] eye complaints, biliary tract disease, and gastric infection.[147] These are uncommon, and it is unclear whether a casual relationship exists. Gastric infection occurs in the presence of achlorhydria and has been seen in conjunction with *Helicobacter pylori* in both adult and child populations.[150,151]

Although most persons with giardiasis have a relatively benign course, some can have an acute severe illness necessitating hospitalization. Children and pregnant women were particularly affected in the United States,[152] but not in a similar study of those hospitalized in Scotland.[153]

Patients who develop chronic diarrhea may report weight loss, profound malaise, lassitude, occasional headache, and diffuse abdominal and epigastric discomfort, often exacerbated by eating. Stools, passed frequently in small volume, commonly because of malabsorption, may be greasy and foul smelling or frothy and yellowish. Waxing and waning symptoms over months is characteristic. Infected children can present with spruelike disease and failure to thrive.[91,154] Consequently, malabsorption of vitamins A and B_{12}, protein, D-xylose, and iron develops.[155,156] Lactase is the most common disaccharidase deficiency, occurring in 20% to 40% of cases,[157] with post-*Giardia* lactose intolerance sometimes persisting for several weeks after treatment. This is often confused with relapse or reinfection. Some individuals may be intolerant to specific fermentable oligosaccharides, disaccharides, monosaccharides, and polyols.[158]

Post-*Giardia* irritable bowel syndrome (IBS) is increasingly recognized in nonendemic settings. Both IBS-like symptoms and chronic fatigue have reportedly lasted for years after primary infection in a previously unexposed nonendemic population in Norway.[159] These *Giardia*-exposed patients with persisting symptoms have developed elevated serum sCD40L, a finding that warrants confirmation in other populations.[160] Within a US insurance database, a single episode of giardiasis increased the likelihood of a new IBS diagnosis within the next year (hazard ratio [HR], 4.8; 95% confidence interval [CI], 3.6–6.4) that was only slightly attenuated after adjustment for anxiety, depression, and health care utilization (HR, 3.9; 95% CI. 2.9–5.4).[161]

Giardia is a nearly universal infection in children residing in low-income regions of the world. Despite the high prevalence of *Giardia* infection in these children, the vast majority of infections are asymptomatic; the variability in manifestations has been perplexing.[162] Although many *Giardia* infections in this setting do not result in acute diarrhea, there is evidence that the parasite is associated with persistent diarrhea and nutritional abnormalities. In a review and meta-analysis of studies describing *Giardia* in children younger than 5 years, *Giardia* was not seen to cause acute diarrhea (odds ratio [OR], 0.60; 95% CI, 0.38–0.94; $P < .03$) but was associated with diarrhea lasting at least 14 days (persistent diarrhea; OR, 3.2; 95% CI, 1.50–6.76; $P < .001$).[163] In several subsequent case-controls studies of pathogen-attributable causes of pediatric diarrhea in these settings, *Giardia* was detected more often in controls than cases of moderate-to-severe acute diarrhea,[164,165] a finding that has not been universally observed[60] and merits more analysis. Unlike viruses, *Cryptosporidium,* and other gastrointestinal pathogens associated with acute diarrhea,[166] there is no clear relationship between quantitative *Giardia* burden or stool inflammatory markers and symptomatic infection in these children.[60,167]

Persistent *Giardia* infection, either with or without diarrhea, could be part of the larger condition of childhood environmental enteric dysfunction in low-income regions. Studies over the last 20 years have documented stunting in Brazilian and Ecuadorian children infected with *Giardia*,[59,168] poor intestinal permeability in Nepali children,[169] low weight-for-age and height-for-age in Brazilian children with persistent symptomatic giardiasis,[63,170,171] underweight status in Rwandan children,[172] significant wasting in Malaysian and Indian children,[62,173,174] and decreased cognitive function in Peruvian children with multiple episodes of giardiasis.[58] In a prospective study of 629 children in Bangladesh where 74% of children had had a *Giardia* exposure by 2 years of life, *Giardia* in the first 6 months of life, regardless of symptoms, was associated with decreased length-for-age scores.[61] In a larger multicenter study including >2000 children, early *Giardia* exposures and persistent infections were also associated with impaired growth attainment by 2 years of life.[60] The findings of these studies suggest that treatment of infection might help to reverse some of the deleterious effects.[59,175–177] However, persistent infections and reinfections occur so rapidly that repeated therapy is currently impractical and not indicated.[60,64,178,179–180]

In contrast, longitudinal studies in Bangladeshi children[181,182] and Peruvian children[64] did not demonstrate effects on well-being or nutrition, and in a setting where children were well-nourished, asymptomatic infection did not have apparent deleterious effects.[183,184] Furthermore, many children and adults will be simultaneously infected with other bacterial, viral, and parasitic infections, and there is the potential for adverse synergy.[185,186]

DIAGNOSIS

The diagnosis of giardiasis should be considered in all patients with prolonged diarrhea, particularly if it is associated with malabsorption or weight loss, a history of recent travel to an endemic area, ingestion or exposure to untreated water sources, the presence of small children in the home who attend daycare centers, or sexual risk factors. In the usual patient with uncomplicated giardiasis, the WBC count is normal without eosinophilia, and gastrointestinal imaging is nondiagnostic. The diagnosis depends on detection of the parasite or its specific antigen or DNA in the feces.

Microscopic detection of cysts or trophozoites in feces for ova and parasites (O&P) is the traditional way to diagnose most gastrointestinal parasites. The O&P test is time-consuming and costly; the sensitivity is dependent on the number of organisms, the skill of the microscopist, the amount of feces examined, and the time taken to examine the stool. A 90% chance or greater of detecting a true *Giardia* infection requires three stool examinations over multiple days.[148,187,188] Tests requiring little training with high sensitivity and specificity and ease of performance such as assays to detect the *Giardia* soluble specific cyst wall antigen(s) of *Giardia* or cyst detection with immunofluorescence are widely available,[189,190] reproducible, and rapidly performed.[191] Polymerase chain reaction (PCR)–based diagnostic testing,[12,192,193,194,195] with *Giardia* usually included as one target in a larger array of gastrointestinal pathogens, has become commercially available in the United States. Comparisons between the *Giardia* antigen tests and PCR testing indicate that PCR testing is at least as sensitive and in some formats twice as sensitive[166] as antigen detection tests.

The detection of *Giardia* antigen in stools has proved to be extremely useful in diagnosing clinically significant infections. These assays are most helpful when giardiasis is the leading consideration. They are often less expensive than an O&P examination and are 85% to 98% sensitive and 90% to 100% specific.[189,190,196–200] Various methods to detect *Giardia* trophozoites in the small intestine were previously used to establish or confirm the diagnosis. Because of the availability of highly sensitive noninvasive tests, small intestinal sampling is rarely required.

Testing for systemic anti-*Giardia* antibody is neither generally available nor useful in the individual case because of persistence of antibodies from prior infections. Detection of IgG antibodies has been useful in seroepidemiologic studies.[201–204]

THERAPY

Susceptibility testing of *Giardia* is not performed because of the difficulty in establishing cultures from clinical specimens and the lack of standardization of sensitivity testing.[205] Drug resistance can be induced in vitro[206]; however, the significance of this is unclear because some isolates that appear clinically resistant are susceptible in vitro, and vice versa. Most information on drug efficacy, therefore, is provided by clinical trials and cumulative experience.[205,207,208,209] The drug of choice for the therapy of giardiasis in the United States is tinidazole (Tindamax; Mission Pharmacal, San Antonio, TX) (Table 279.3). Like metronidazole, it is a member of the nitroimidazole family. It has high efficacy (approximately

TABLE 279.3 Treatment of Giardiasis

DRUG (FDA PREGNANCY CATEGORY)[a]	DOSAGE	
	ADULT	PEDIATRIC
Tinidazole (C)	2 g, single dose	50 mg/kg, single dose (maximum, 2 g)
Metronidazole[b] (B)	250 mg tid × 5–7 days	5 mg/kg tid × 7 days
Nitazoxanide (B)	500 mg bid × 3 days	Age 12–47 mo: 100 mg bid × 3 days Age 4–11 yr: 200 mg bid × 3 days
Albendazole[b] (C)	400 mg qd × 5 days	15 mg/kg/day × 5–7 days (maximum, 400 mg)
Paromomycin[b] (NC)	500 mg tid × 5–10 days	30 mg/kg/day in 3 doses × 5–10 days
Quinacrine[c] (C)	100 mg tid × 5–7 days	2 mg/kg tid × 7 days
Furazolidone[c] (C)	100 mg qid × 7–10 days	1.25–2 mg/kg qid × 10 days

[a]FDA pregnancy categories: B, Animal reproduction studies have failed to demonstrate a risk to the fetus, and there are no adequate and well-controlled studies in pregnant women. C, Animal reproduction studies have shown an adverse effect on the fetus, and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.
[b]Not an FDA-approved indication. Maximum of 8.8 mg/kg/day and not recommended for <1 month of age.
[c]No longer produced in the United States; may be obtained from some compounding pharmacies.
FDA, US Food and Drug Administration; *NC,* not categorized.

90%) and a favorable side effect profile, and because of a longer half-life than metronidazole, it can be given once daily.[205] Tinidazole is effective as a single dose for both children and adults and was approved in 2004.[210] Although the US Food and Drug Administration (FDA) has never approved metronidazole for giardiasis, there are over 50 years of experience in using it.

Metronidazole is given in divided doses for 5 to 7 days, with an efficacy of 80% to 95%. Adverse effects for tinidazole and metronidazole are similar: a metallic taste; nausea; dizziness; headache; and, rarely, reversible neutropenia, peripheral neuropathy, or seizures. When taken with alcohol, they can produce a disulfiram-like effect. High-dose, short-course regimens of metronidazole have lower efficacy rates and are sometimes poorly tolerated. There have been concerns about potential mutagenicity of metronidazole; however, this has not been documented in humans.[211] There have been reports of increasing nitroimidazole treatment failures.[207]

The mechanism of action of metronidazole and presumably tinidazole is reductive activation of the nitro group by acceptance of electrons from parasite ferredoxins, although other pathways to activate the nitro group may be involved.[212–214] Nitazoxanide (Alinia; Romark Laboratories, Tampa, FL) was approved in 2003 for use in pediatric giardiasis and cryptosporidiosis and, more recently, for giardiasis treatment in adults.[215] There is limited clinical experience with this drug compared with the nitroimidazoles; efficacy is in the range of 65% to 85% with a 3-day treatment course.[216,217] Its mechanism of action is inhibition of pyruvate-ferredoxin oxidoreductases; however, there does not appear to be cross-resistance with metronidazole-resistant parasites.[218] Its main side effect is gastrointestinal upset.

Meta-analyses of a limited number of trials of albendazole (400 mg for 5 days), a benzimidazole, compared with standard regimens of metronidazole have shown comparable results.[208,219] Albendazole is attractive because it is usually better tolerated than the nitroimidazoles, has efficacy against many intestinal helminths, and is used in low-income regions to decrease intestinal parasitism. However, more clinical experience will be needed to determine its place in therapy.

Quinacrine and furazolidone are no longer manufactured in the United States. Quinacrine has an efficacy of more than 90%, and can be obtained through compounding pharmacies; it is given in divided doses for 5 to 7 days (see Table 279.3). The most common side effects are bitter taste, nausea, vomiting, and abdominal cramping. Yellow discoloration of the skin, urine, and sclerae can occasionally occur. Exfoliative dermatitis is rare. Psychosis occurs uncommonly, and we avoid its use in patients with a significant psychiatric history. Furazolidone has a success rate of about 80% to 85% but may cause gastrointestinal side effects and brown urine and may cause mild hemolysis in glucose-6-phosphate dehydrogenase–deficient individuals.

For patients in whom one drug course fails or who infrequently relapse, a switch to a drug from a different class is generally effective.[205,220] For the unusual patient who is not cured with single-drug therapies, combination treatment, often with metronidazole and quinacrine, can be effective.[125,221,222] A randomized trial comparing metronidazole alone versus metronidazole plus the probiotic *Saccharomyces boulardii* demonstrated higher cure rates in the combination group.[223] When refractory infection is suspected, it is important to establish true persistence by obtaining a repeat stool examination to distinguish it from post-*Giardia* IBS or lactose intolerance. For pregnant women with giardiasis, there is no consistently recommended therapy because of the theoretical adverse effects of anti-*Giardia* drugs on the fetus.[205] When disease is mild and hydration and nutrition can be maintained, therapy can be delayed until after delivery, or at least until after the first trimester. If treatment is necessary, paromomycin, an oral nonabsorbable aminoglycoside,[224] can be tried. In limited clinical experience, this approach has an efficacy rate of 60% to 70%.[224] Paromomycin is given in divided doses for 5 to 10 days (see Table 279.3). Metronidazole has been used extensively in pregnancy for the treatment of trichomoniasis. The teratogenic effect appears to be minimal and, if present, is greatest during the first trimester, when both metronidazole and tinidazole should not be used.[205,225] If therapy cannot be avoided, then metronidazole, tinidazole, and nitazoxanide can probably be used safely in the last two trimesters of pregnancy. Table 279.3 lists the FDA pregnancy categories for each of the agents.

There is no established role for nonpharmaceutical therapies. A number of studies have found various effects on *Giardia* infections, disease, or interactions with other gut pathogens as a result of micronutrient administration[167,226–228] The interpretation and generality of the effects are unclear and require confirmation. However, the findings of these studies suggest a complex interaction of micronutrients with the pathogenicity of enteric organisms.

A defined management approach to chronic post-*Giardia* infection chronic sequelae in nonendemic populations has yet to be established.[229]

PREVENTION

The prevention of giardiasis requires proper handling and treatment of water used for communities and good personal hygiene. Although halogenation (e.g., chlorine and iodine) alone is sufficient to kill *G. lamblia* cysts, important variables, such as water temperature, clarity, pH, and contact time, alter the efficacy of chlorine, and higher chlorine levels (4–6 mg/L) may be required.[72,230] Thus, in addition to chlorination, public water supplies intended for drinking should also be subjected to coagulation-flocculation, sedimentation, and filtration.[69]

Travelers to low-income regions of the world or to wilderness areas should consider all water potentially contaminated because of the wide array of animal and human reservoirs of giardiasis. Bringing water to a boil is sufficient to kill all protozoal cysts; at high altitudes, boiling for a minute is reasonable. If boiling is impossible, halogenation is generally effective for *Giardia,* but *Cryptosporidium* is resistant.[72,230] There are many chlorine-based (e.g., chlorine bleach: 5% to 6%, 2 to 4 drops/L) or iodine-based (e.g., tincture of iodine: 2%, 5 drops/L) preparations in liquid or tablet form.[230] Contact time should be increased for water that is cold, and the concentration of halogen should be increased for turbid water. Personal water filters with pores of an "absolute" micron size of 0.2 to 1.0 µm can be used.[230] Filtered water should also be halogenated to kill enteric viruses if they are considered a risk. Uncooked foods that may have been washed or prepared in contaminated water should be avoided.

Giardiasis outbreaks in daycare centers can be difficult to resolve. Some recommend that only symptomatic children be treated; however, infected children transmit *Giardia* to parents and family members, with further potential spread to the community. Therefore, each situation requires an individual decision. If strict hand washing and treatment of

symptomatic children fail to control an outbreak of diarrhea, consideration can be given to treating all infected children.[231] Treatment-based strategies for transmission prevention in endemic settings, however, have not proven beneficial. Although metronidazole exposure within the previous 7 days decreased the likelihood of *Giardia* detection[60] in separate community-based studies of children in high-transmission endemic settings, community-based administration of metronidazole, albendazole, or nitazoxanide did not diminish *Giardia* prevalence.[118,195,232]

Human milk can be protective against *Giardia*. Both human and animal breast milk can contain anti-*Giardia* antibodies; studies have demonstrated protection of breastfeeding infants from symptomatic infection.[114,233,234]

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The complete reference list is available online at Expert Consult.

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280 *Trichomonas vaginalis*

Jane R. Schwebke and Laura Hinkle Bachmann

SHORT VIEW SUMMARY

Microbiology and Epidemiology
- *Trichomonas vaginalis* is a flagellated parasite.
- Infection is through sexual transmission.
- Susceptibility to human immunodeficiency virus (HIV) infection may be increased in infected women.

Diagnosis
- Infection causes vaginitis in women and may cause urethritis in men; it is often asymptomatic.
- In women, most cases are diagnosed using wet prep microscopy of vaginal fluid as the point-of-care test, but this lacks sensitivity.
- The gold standard for diagnosis is nucleic acid amplification testing.

Therapy
- Metronidazole is the usual therapy.
- Resistance to metronidazole may occur.
- Tinidazole may be more effective.

Prevention
- Sexual partners should be treated.
- Condoms prevent infection.

Trichomonas vaginalis is the causative agent of trichomoniasis, a common cause of vaginitis. In men, it may cause urethritis but is more commonly asymptomatic. Despite being a readily diagnosed and treatable sexually transmitted disease (STD), trichomoniasis is not a reportable infection and its control has received relatively little emphasis from public health STD control programs. More recently, however, recognition of the high rates of disease and of associations of trichomoniasis in women with adverse outcomes of pregnancy and increased risk for human immunodeficiency virus (HIV) infection suggest a need for increased control efforts.

TAXONOMY

Trichomonas vaginalis is a parasitic pear-shaped protozoan, with an average size of 10 × 7 µm (Fig. 280.1). It has four free flagella and one recurrent flagellum, along the outer margin of the undulating membrane; a costa at the base of the undulating membrane; and an axostyle extending through the cell.[1] Unlike most eukaryotes, *T. vaginalis* lacks mitochondria and instead uses the hydrogenosome to accomplish fermentative carbohydrate metabolism, with hydrogen as the electron acceptor. The hydrogenosome appears to have a common ancestry with mitochondria based on similarities in protein import.[2] However, major differences exist between hydrogenosomes and mitochondria in that hydrogenosomes lack cytochromes, mitochondrial respiratory chain enzymes, and their own DNA.

Trichomonas tenax, found in oral gingival and tracheobronchial sites, and *Pentatrichomonas hominis,* isolated from the intestinal tract, are considered nonpathogenic. Each human species has specific tropism for its site of infection. *Tritrichomonas foetus,* which is found exclusively in cattle, is perhaps the nonhuman trichomonad most similar to *T. vaginalis.* Aside from *T. foetus* having three anterior flagella (vs. four in *T. vaginalis*), there are few morphologic differences between the parasites, and *T. foetus* causes the STD known as bovine trichomoniasis. *Tritrichomonas foetus* can be invasive to the fetus, having been demonstrated in the placenta and the fetal lung, gut, and lymph nodes, and is a known cause of abortion in infected cattle.[3]

The life cycle of *T. vaginalis* is simple in that the trophozoite is transmitted through coitus and no cyst form is known. The trophozoite divides by binary fission and, in natural infections, gives rise to a population in the lumen and on the mucosal surfaces of the urogenital tracts of humans. In an infected person there are estimated to be 10^1 to 10^5 protozoa/mL of vaginal fluid.[4] The organisms create microulcerations in the genital mucosa by direct contact, mediated by surface proteins.[5]

Our current understanding of immunity to *T. vaginalis* has come largely from observations of responses in human patients and experimentation using in vitro and animal models of the related species *T. foetus.* Natural infection seems to produce immunity that is only partially protective, because reinfection of patients can be as high as 30% on follow-up.[6] The presence of *T. vaginalis* in the reproductive tract results in parasite-specific antibodies in the reproductive tract and, in most cases, circulating antibodies in the serum[7]; there is also evidence of lymphocyte priming, as detected by antigen-specific proliferation of peripheral blood mononuclear cells.[8] Thus natural infection with *T. vaginalis* results in priming of acquired immune responses. A study of patients infected with *T. vaginalis* and HIV indicated no evidence of increased levels or duration of parasite infection in these patients compared with those in patients infected with *T. vaginalis* but not HIV.[9] These observations may indicate that innate immunity involving chemotaxis and subsequent influx of neutrophils is much more important than acquired immunity in controlling infections with *T. vaginalis,* because neutrophils are often the most numerous leukocytes present in response to infection.[10]

The genome of *T. vaginalis* has been sequenced and provides considerable resources that may be used to define genes important in the pathogenesis of human trichomoniasis. The release of the 5X genome sequence data makes the most comprehensive genomic sequence of this parasite available to date.[11]

EPIDEMIOLOGY

Humans are the only natural host of *T. vaginalis.* Trichomoniasis is an extremely common infection in the United States and worldwide. The prevalence of trichomoniasis in inner-city US STD clinics typically approaches 25% and may be higher in certain populations. In Los Angeles, for example, the prevalence among African-American patients at a public clinic was 38%.[12] Among women ages 14 to 19 years, the overall prevalence of trichomoniasis in the United States was recently found to be 3.1%, but among African Americans the prevalence was 13.3%.[13] An estimate of the incidence of STDs in the United States yielded an annual incidence of trichomoniasis of 7.4 million new cases.[14] The World Health Organization has estimated that this infection accounts for almost 50% of all curable infections worldwide.[15] Studies of African

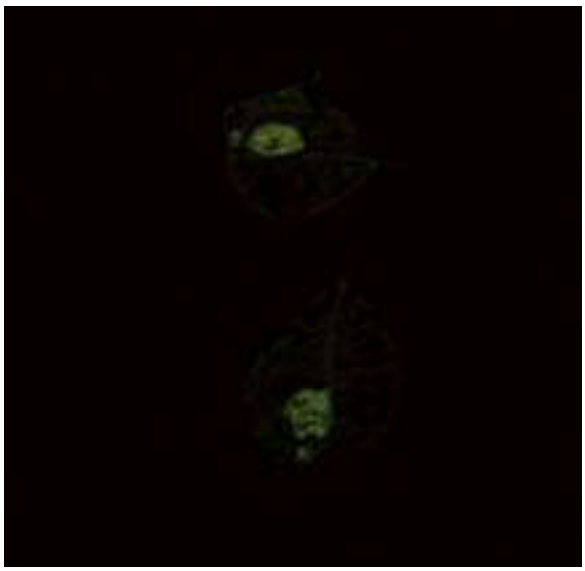

FIG. 280.1 ***Trichomonas vaginalis.*** *(From Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Parasitic Diseases.)*



FIG. 280.2 **Vaginal discharge in a patient with trichomoniasis.** Note the bubbles, which give the discharge a "frothy" appearance.

populations have reported the prevalence of vaginal trichomoniasis to be between 11% and 25%. Laga and colleagues reported an incidence of 38% during a 4-month exposure interval among HIV-infected women in Zaire.[16]

Epidemiologically, *T. vaginalis* infections are commonly associated with other STDs and are a marker of high-risk sexual behavior. Trichomoniasis is frequently seen concomitantly with other STDs, particularly gonorrhea. Unlike other STDs, which have a higher prevalence among adolescents and young adults, the rates of trichomoniasis are more evenly distributed among sexually active women of all age groups, probably as a result of a lack of an organized disease control effort for this infection.[17] Although survival on fomites is documented, the organism is thought to be transmitted almost exclusively by sexual activity.[18] Vertical transmission from mother to infant can occur rarely and cause genital infection or respiratory distress.

The reported prevalence of urethral infection with *T. vaginalis* in males has varied, depending on the population studied and the diagnostic techniques used. In a series of sentinel studies using cultures of urine, urethra, coronal sulcus, and semen, Krieger and associates reported a prevalence of 11% in men attending an STD clinic.[19] Urethritis was present in half of the men with *Trichomonas* as the sole urethral pathogen. In a similar study conducted at an STD clinic in Denver, investigators found a prevalence rate of 2.8% by using urine sediment culture.[20] At an STD clinic in Birmingham, Alabama, *T. vaginalis* was detected by polymerase chain reaction assay in 17% of men attending for a new-problem visit or screening. There was no significant difference in the detection of the organism between men with and without urethral symptoms (20% and 14.5%, respectively). Of men with nongonococcal urethritis (NGU), nearly as many had *T. vaginalis* detected as had *Chlamydia* (19.9% and 25.2%, respectively).[21] Detection of *T. vaginalis* in men is increased if semen is tested in addition to urine and urethral specimens.[22]

CLINICAL MANIFESTATIONS

The organisms create microulcerations in the genital mucosa by direct contact, mediated by surface proteins.[5] In women, it is the squamous epithelium of the vagina that is infected. Interestingly, although 2% to 17% of female neonates may have vaginal colonization if the mother is infected, the infection may not be sustained once the maternal effects of estrogen wear off and the vaginal pH becomes neutral.[23] Neonatal respiratory tract infections with *T. vaginalis* have also been reported.[24] Detection of *Trichomonas* infection in older children should raise the suspicion of child abuse.[25]

TABLE 280.1 Sensitivity of Clinical and Laboratory Findings in Vaginal Trichomoniasis

	CLINICAL MANIFESTATION	PERCENT POSITIVE
Symptoms	None	9–56
	Discharge	50–75
	Malodorous	~10
	Irritating, pruritic	23–82
	Dyspareunia	10–50
	Dysuria	30–50
	Lower abdominal discomfort	5–12
Signs	None	~15
	Vulvar erythema	10–20
	Excessive discharge	50–75
	Yellow, green	5–20
	Frothy	10–50
	Vaginal wall inflammation	40–75
	Strawberry cervix (direct visualization)	1–2
	Colpitis macularis (colposcope)	45
Laboratory findings	pH >4.5	66–91
	Positive whiff test	~75
	Excess polymorphonuclear neutrophils on wet mount	~75

Data from Honigberg BM, ed. Trichomonads Parasitic in Humans. *New York: Springer-Verlag; 1990; Bickley LS, Krisher KK, Punsalang A Jr, et al. Comparison of direct fluorescent antibody, acridine orange, wet mount, and culture for detection of Trichomonas vaginalis in women attending a public sexually transmitted disease clinic.* Sex Transm Dis. *1989;16:127–131; and Rein MF. Uncertainties and controversies in trichomoniasis. In: Sobel JD, ed.* Vulvovaginal Infections: Current Concepts in Diagnosis and Therapy. *New York: Academy Professional Information Services; 1990:73–85.*

The incubation period of this infection is unknown; however, human challenge studies done in the 1940s suggested an incubation period of 4 to 28 days in women.[26] The clinical manifestations of trichomoniasis in the female are summarized in Table 280.1. Symptoms in women include vaginal discharge, pruritus, and irritation. Signs of infection include vaginal discharge (42%), odor (50%), and edema or erythema (22% to 37%). The discharge is classically described as frothy, but it is actually frothy in only about 10% of patients (Fig. 280.2). The color of the discharge may vary. Colpitis macularis (strawberry cervix) is a specific clinical sign for this infection but is detected with reliability only by colposcopy and rarely during routine examination.[10] Other complaints may include dysuria and lower abdominal pain; the cause

of the latter is unclear. The urethra is also infected in most women.[27] Almost 50% of all women with *T. vaginalis* are asymptomatic[28]; therefore if these women are not screened, the diagnosis will be missed. The extent of the inflammatory response to the parasite may determine the severity of the symptoms. Factors that influence the host inflammatory response are not well understood but may include hormonal levels, coexisting vaginal flora, and strain and relative concentration of the organisms present in the vagina. In men the infection is usually asymptomatic, but some studies have suggested that it is a more common cause of NGU than was previously recognized[19,21] and should certainly be considered as a diagnostic possibility in a man who does not respond to initial therapy for NGU. Trichomoniasis in men may also rarely cause epididymitis, prostatitis, and superficial penile ulcerations.[29] Spontaneous resolution of trichomoniasis in men has been reported.[30]

DIAGNOSIS

Direct microscopic examination of vaginal fluid for the presence of motile trichomonads is the most common diagnostic method employed for the detection of trichomoniasis. However, even for skilled diagnosticians, the sensitivity of this test varies from 40 to 60%, and may be less in asymptomatic women.[31] Sensitivity decreases dramatically if specimen transport time is longer than 10 minutes.[32] The pH of the vaginal fluid will usually be higher than 4.5 in women with trichomoniasis but can be normal (≤4.5). In the latter cases, the trichomonads are often sparser because they prefer a more alkaline pH. Neutrophils as well as altered vaginal bacterial microbiota are often seen. Trichomoniasis is most often associated with intermediate vaginal microbiota as defined by Nugent criteria, raising the possibility that *T. vaginalis* may in some way be able to alter the vaginal microbiota composition to enhance its survival.[33] Culture media are commercially available and until recently have been the gold standard for diagnosis, but the cultured specimen requires incubation for up to 7 days prior to finalization.[34] Though not recommended routinely, culture is currently the only acceptable specimen type for susceptibility testing. Additional tests include the Affirm VPIII (Becton Dickenson, Franklin Lakes, NJ), a nucleic acid probe hybridization semiautomated system that also detects candidiasis and bacterial vaginosis and has results available in less than an hour. The OSOM Trichomonas Rapid Test (Sekisui, Lexington, MA), an antigen detection test using immunochromatographic capillary flow dipstick technology, is Clinical Laboratories Improvement Amendments–waived with results available in 10 minutes and sensitivity estimates from 77% to 98%, with specificities of 99% to 100%. Finally, AmpliVue Trichomonas assay and Solana Trichomonas assay (Quidel, San Diego, CA) use an isothermal helicase-dependent amplification-based method to detect trichomonas DNA, with results available within an hour and performance characteristics comparable to culture. All of these tests are currently licensed only for vaginal specimens, can be used as point of care tests, and have sensitivities of about 80%.[35,36] They may be a good choice if microscopy is not available.

Nucleic acid amplification tests (NAATs) are now commercially available for the diagnosis of trichomoniasis and have replaced culture as the gold standard diagnostic test. Genital (endocervical, provider and patient-collected vaginal swabs), urine, and liquid PAP-based specimens are acceptable, with some variation depending on the specific NAAT test being utilized.[37] With sensitivities greater than 90% and specificities from 95% to 100%, current US Food and Drug Association (FDA)–approved NAATs include the Aptima *Trichomonas vaginalis* assay (Hologic, San Diego, CA); the ProbeTec *Trichomonas vaginalis* Q^x assay (BD Diagnostics, Franklin Lakes, NJ); and the Xpert TV test (Cepheid, Sunnyvale, CA),[38] which was recently approved by the FDA for use with male urine. Diagnosis in general is much more difficult for males, with the best culture results obtained by combining urethral swabs and urine sediment into one specimen. However, compared with NAATs, this method is highly insensitive for use in men.[39] Although most are not approved for men by the FDA, several studies have found NAATs to be highly sensitive and specific in this population and these tests are available from laboratories that have performed the necessary validation studies.[39]

Lack of suitable screening and diagnostic tests for males has hampered public health control efforts of the disease. The increasing use of NAATs testing in women and men should be of great importance in this regard.

THERAPY

In most cases, the infection is easily treated with a single 2-g oral dose of metronidazole or tinidazole, and because it is an STD, sexual partners should be routinely treated.[40] The reported cure rates are 90% to 100%. Failure to treat the male sexual partner is likely the most common cause of recurrent disease in women and should be explored before assuming that the woman has a strain of *T. vaginalis* that is resistant to metronidazole. Metronidazole intravaginal gel has limited efficacy and should not be used. Although there continues to be some controversy about the safety of metronidazole in pregnancy, there has never been a documented case of fetal malformation attributed to its use, even when it is used in the first trimester.[41] Tinidazole has a plasma half-life twice that of metronidazole (12–14 hours for tinidazole vs. 6–7 hours for metronidazole)[42] and appears to be better tolerated, with fewer gastrointestinal side effects.

Resistance has been reported, estimated to be from 2.5% to 10% for metronidazole and less than 1% for tinidazole.[43] One mechanism of development of anaerobic resistance to metronidazole is controlled by hydrogenosomes, in that metronidazole competes for hydrogen as an electron acceptor. In metronidazole-resistant *T. vaginalis,* the expression levels of the hydrogenosomal enzymes pyruvate ferredoxin oxidoreductase, ferredoxin, malic enzyme, and hydrogenase are reduced dramatically, which probably eliminates the ability of the parasite to activate metronidazole.[44] Resistance has also been associated with mutations in nitroreductase genes.[45] Resistance is relative and can usually be overcome with higher doses of oral metronidazole. However, there does not appear to be a definitive correlation between in vitro and in vivo resistance. Intravenous formulations offer no advantage over the oral drug. Some authorities have recommended higher doses of oral medication in combination with pharmacy-prepared intravaginal preparations.

Tinidazole, with its more favorable pharmacokinetics, may be the drug of choice when resistance is encountered. The Centers for Disease Control and Prevention (CDC) tested 195 metronidazole-resistant *T. vaginalis* clinical isolates submitted for minimal lethal concentration (MLC) testing for both metronidazole and tinidazole. The mean aerobic metronidazole MLC was 400 μg/mL compared with an aerobic tinidazole MLC of 100 μg/mL.[46] Several clinical studies have evaluated various doses of tinidazole for treatment of metronidazole-resistant trichomoniasis. The largest series of patients was reported by Sobel and associates.[47] In this study, 20 patients with clinically refractory trichomoniasis (failure to respond to therapy with oral metronidazole, at least 500 mg twice daily for 7 days) were treated with high doses of oral and vaginal tinidazole (2–3 g PO, plus 1–1.5 g intravaginally for 14 days). The cure rate was 92% (22 of 24 patients); no patients discontinued therapy because of side effects. Current CDC recommendations for patients failing a single 2-g dose of metronidazole are to treat next with metronidazole, 500 mg PO twice daily for 7 days, or a 2-g dose of tinidazole immediately. Failing that, treatment with tinidazole or metronidazole at 2 g PO for 5 days is suggested.[40]

There are limited anecdotal reports of success with paromomycin cream; however, this therapy may also be associated with a high incidence of local side effects.[48] A case report of two women with resistant trichomoniasis reported cure with a combination of intravaginal paromomycin cream and high-dose oral tinidazole.[49] There is also a report of cure with prolonged intravaginal boric acid suppositories.[50] Women with asymptomatic infection should be treated. If their infection is left untreated, they may later become symptomatic and may continue to transmit the infection while untreated. Occasionally, patients manifest a true allergy to metronidazole. Because there is no effective alternative, desensitization is the only option.[51]

COMPLICATIONS

Long considered a minor STD, with few associated complications, infection with *T. vaginalis* has more recently been implicated as a cause of preterm delivery in several studies. In a large multicenter study, after adjusting for demographic, behavioral, and microbiologic variables, *T. vaginalis* was significantly associated with low birth weight, premature rupture of membranes, and preterm delivery (relative risk, 1.4).[52] Minkoff and coworkers[53] also documented a significant correlation between trichomoniasis and premature rupture of membranes. In that study,

the incidence of this complication at term was 27.5% in women with *T. vaginalis* infection versus 12.8% in those without ($P = .03$).

Prospective studies of treatment of trichomoniasis during pregnancy for the prevention of preterm birth have yielded disappointing results. Among women with asymptomatic infection treated with metronidazole during the second and third trimesters of pregnancy, a trend toward increased preterm delivery was seen compared with the placebo group. However, the dose of metronidazole used was four times the recommended dose. In addition, the study was stopped prematurely because of a slow accrual of subjects and the trend for increased risk for preterm delivery in the treatment group.[54] A second study, conducted in Uganda, also found that treatment of trichomoniasis during pregnancy resulted in an increase in the incidence of preterm birth. However, this study was actually a subgroup analysis of a larger trial and was not properly designed to determine the effect of treatment of *T. vaginalis* during pregnancy on preterm birth[55]; therefore the question remains unanswered. Since the publication of these reports, the CDC has not revised recommendations for treatment during pregnancy. Trichomoniasis has also been associated with vaginal cuff cellulitis after abdominal hysterectomy.[56]

Acquisition of HIV has been associated with trichomoniasis in several African studies, possibly as a result of the local inflammation often caused by the parasite. Leroy and colleagues[57] found a significant difference between the prevalence of trichomoniasis in a cohort of HIV-infected and uninfected pregnant women in Rwanda (20.2% and 10.9%, respectively; $P = 0.0007$). In a prospective study by Laga and associates,[16] trichomoniasis positivity was significantly associated with HIV seroconversion (odds ratio, 1.9) in a multivariate analysis of a cohort of women in Zaire. Similar findings were reported from a cohort of women in Kenya.[58] The associations between HIV and trichomoniasis, as well as other STDs, may relate to the following: (1) increased shedding of HIV as a result of the local inflammation produced by the STD; (2) increased susceptibility to HIV as a result of the macroscopic or microscopic breaks in mucosal barriers caused by the STD; (3) a higher prevalence of STDs among HIV-infected individuals as a result of common risk factors for both infections; and (4) an increased susceptibility to STDs as a result of the immunosuppression associated with HIV infection. Given the higher prevalence and incidence of trichomoniasis than most other treatable STDs in most studies to date, the attributable fraction of HIV acquisitions caused by trichomoniasis may eclipse the relative contribution of other STDs.[12] The RNA concentration of HIV in the seminal fluid of men with urethritis was significantly higher in men with trichomoniasis than in those with symptomatic urethritis with an unidentified cause.[59] In addition, successful treatment of trichomonal urethritis reduced the levels of HIV RNA so that they were similar to those seen in uninfected control subjects.[60]

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281 *Babesia* Species

Edouard Vannier and Jeffrey A. Gelfand

SHORT VIEW SUMMARY

Definition

- Babesiosis is an emerging infectious disease caused by hemoprotozoan parasites of the genus *Babesia*.

Epidemiology and Microbiology

- Most cases of human babesiosis occur in the United States, particularly the Northeast and the upper Midwest, and are caused by *Babesia microti*. *B. microti* is primarily transmitted during the bite of an *Ixodes scapularis* nymphal tick but also by transfusion of contaminated blood products, predominantly packed red blood cells. *B. microti* has been the pathogen most frequently implicated by investigations of transfused-related illnesses in the United States and accounted for one-fourth of transfusion-related deaths caused by microbial infection from fiscal year 2010 to fiscal year 2016 (see Chapter 304).
- Human babesiosis also occurs in Europe and East Asia, where the major etiologic agents are *Babesia divergens* and *Babesia venatorum,* respectively.
- Risk factors for severe babesiosis include anatomic or functional asplenia, low $CD4^+$ T-cell counts associated with human immunodeficiency virus/acquired immunodeficiency syndrome or therapy for transplantation, and impaired antibody production associated with chronic immunosuppressive therapy.

Diagnosis

- Fever is the salient symptom, and often is accompanied by chills, sweats, headache, anorexia, and/or myalgia.
- Anemia is the major laboratory finding. Severe anemia often is preceded by severe thrombocytopenia. Elevated levels of lactate dehydrogenase and total bilirubin are consistent with hemolytic anemia.
- The definitive diagnosis is made on Giemsa-stained thin blood smears. Trophozoites often appear as rings. Tetrads of merozoites are pathognomonic.
- If parasites are not visualized on smear but babesiosis remains suspected, polymerase chain reaction assay–based amplification of parasite DNA should be performed.

Therapy (See Table 281.1)

- Mild *B. microti* illness should be treated with a single 7- to 10-day course of oral atovaquone (750 mg every 12 hours) *plus* oral azithromycin (500 mg on day 1, then 250 mg from day 2 onward). Symptoms typically abate within 48 hours and resolve within 2 weeks.
- Severe *B. microti* illness requires hospital admission, and can be complicated by pulmonary edema and respiratory distress. Other complications include congestive heart failure, renal failure, disseminated intravascular coagulation, shock, and splenic rupture. Initial therapy should consist of a 7- to 10-day course of oral atovaquone (750 mg every 12 hours) *plus* intravenous azithromycin (500 mg daily). If symptoms do not abate, therapy should be extended as needed. If microbial resistance is suspected, intravenous clindamycin (600 mg every 6 hours) *plus* oral quinine (650 mg every 8 hours) or oral atovaquone is an alternative.
- If the patient is severely immunocompromised, antimicrobial therapy should be administered for at least 6 consecutive weeks, including 2 final weeks during which parasites are no longer detected on blood smear.
- Partial or complete red blood cell exchange transfusion is recommended for cases with high-grade parasitemia (≥10%), severe anemia (hemoglobin <10 g/dL) in the context of parasitemia, or organ (pulmonary or renal) compromise.

Prevention

- No vaccine is available. Individuals at risk of severe babesiosis should avoid tick exposure in endemic areas. Protective measures include covering exposed skin, applying an acaracide to skin, using permethrin-impregnated clothes, and thoroughly examining the skin for ticks.

The first recorded mention of babesiosis is believed to date to biblical times. In the Book of Exodus, the fifth plague is described as "very grievous murrain" that fell "upon thy cattle which is in the field, upon the horses, upon the asses, upon the camels, upon the oxen and upon the sheep." Bovine babesiosis is still referred to as murrain in the Irish countryside. In 1888, the microbiologist and pathologist Victor Babes attributed the febrile hemoglobinuria and death of cattle in Romania to an intraerythrocytic microorganism.[1] Soon after, Theobald Smith and Frederick Kilborne described a similar piroplasm (*L. pirum,* "pear") in the erythrocytes of Texas cattle with fever. Initially named *Pyrosoma,* this organism became known as *Babesia bigemina.* Smith and Kilborne also identified the tick *Rhipicephalus annulatus* as its vector, establishing for the first time that hematophagous arthropods can transmit an infectious agent to a vertebrate host. The first well-documented case of human babesiosis was reported in 1957 in a 33-year-old splenectomized herdsman who had been grazing cattle on tick-infested pastures near Zagreb, Croatia. The infection was fulminant and fatal. Originally identified as *Babesia bovis,* the causative agent was later reported to be *Babesia divergens,* a parasite of cattle. In 1969, a 59-year-old woman who had summered on Nantucket Island off the coast of Massachusetts presented with a history of fever, headache, and crampy abdominal pain. This patient had an intact spleen, and the causative agent was *Babesia microti,* a parasite of white-footed mice. Andrew Spielman and colleagues identified the vector as the deer tick *Ixodes dammini* (now recognized as *Ixodes scapularis*). As the number of cases on the island grew, the disease became known as "Nantucket fever." Babesiosis caused by *B. microti* is now recognized as endemic on the mainland of the United States, particularly in the Northeast and the upper Midwest.

EPIDEMIOLOGY

United States: *Babesia microti*

Geographic Distribution

Human babesiosis became a nationally notifiable disease in January 2011.[2] Notification is made via the National Notifiable Diseases Surveillance System using the standard case definition developed by the Centers for Disease Control and Prevention (CDC) and the Council of State and Territorial Epidemiologists. In 2014, babesiosis was reportable from 31 states, of which 22 notified the CDC of at least one case.[3] Most cases (>90%) are reported from the seven states with well-established foci of zoonotic transmission (Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, Wisconsin). In the Northeast, areas of high endemicity include islands off the southern coast of New England.[4] On the mainland, babesiosis is highly endemic in southeastern Massachusetts, including Cape Cod, the coastal counties of western

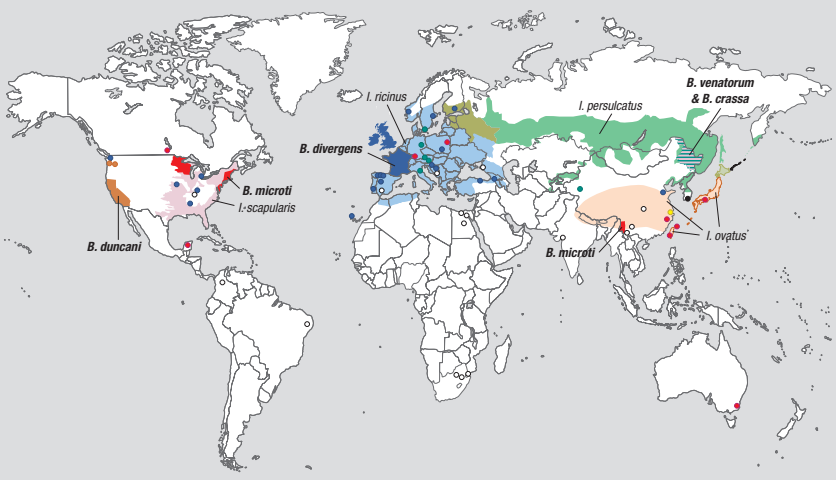

FIG. 281.1 Geographic distribution of human babesiosis and its tick vectors. Dark colors indicate states or provinces where human babesiosis is endemic or sporadic (defined by ≥5 cases), whereas light colors indicate areas where tick vectors are present but human babesiosis is rare (<5 cases), undocumented, or absent. Circles depict single cases, except in three locations (Mexico, Montenegro, and eastern Poland) where all patients were diagnosed at the same hospital. Colors distinguish the etiologic agents: red for *Babesia microti,* orange for *Babesia duncani,* blue for *Babesia divergens,* green for *Babesia venatorum,* pink for *Babesia crassa,* black for KO1, and yellow for *Babesia* sp. XXB/HangZhou. White circles depict cases caused by *Babesia* isolates that were not characterized at the molecular level. Asymptomatic infections and cases of travel-associated babesiosis are omitted. *Ixodes ricinus* and *Ixodes persulcatus* are sympatric in southern Finland, Estonia, Latvia, and northwestern Russia. The island of Hokkaido in northern Japan is enzootic for both *I. persulcatus* and *Ixodes ovatus. (From Vannier E, Krause X. In: Ryan ET, Hill DR, Solomon T, et al, eds.* Hunter's Tropical Medicine and Emerging Infectious Diseases*. 10th ed. Oxford, UK: Elsevier; 2019.)*

Rhode Island and eastern Connecticut, the counties east of the Hudson River in the Lower Hudson Valley (New York), and the south central counties of New Jersey. In the upper Midwest, babesiosis caused by *B. microti* is moderately endemic in Minnesota and Wisconsin (Fig. 281.1).

Incidence and Prevalence

The incidence of babesiosis has steadily increased over the past 30 years. In New York State, the first state to mandate the reporting of babesiosis, more than 5800 cases have been notified to the health authorities. About 1100 cases were reported from 1986 to 2005, and more than 3500 cases in the following decade. The sharp increase noted between 2006 and 2015 resulted from the emergence of babesiosis in the Lower Hudson Valley combined with the maintenance of highly endemic foci on eastern Long Island.[5,6] Along the northeastern seaboard, babesiosis has emerged in areas that were once at the periphery of the "historical heartland," namely southern Maine and southern New Hampshire to the north and Pennsylvania, Delaware, and Maryland to the south.[3,4] In the upper Midwest, the number of annual cases has risen steadily since 2005.[7] A few cases have been reported in Illinois, Indiana, and Michigan.[3] Babesiosis caused by *B. microti* remains an emerging infectious disease in the United States.[1,3,4]

The seroprevalence of antibodies against *B. microti* antigen varies by year, study site, and sample population but has consistently been high in endemic areas, such as Nantucket (7% among blood donors),[8] Block Island (9% among residents),[9] southern Connecticut (0.5%–9% among blood donors),[9,10] Shelter Island (4% in blood donors; 4%–7% among residents),[11] and eastern Long Island (16% among residents).[12] In Minnesota, seroprevalence among blood donors has been reported at 2%.[13] Given that seroprevalence is much higher than prevalence of clinical babesiosis, asymptomatic infection is more common than recognized or reported.

Modes of Transmission

Tick Bite

B. microti is primarily acquired during the blood meal of the tick *I. scapularis,*[14] but only one-half of patients recall a tick bite in the 8 weeks prior to symptom onset.[3] The nymphal stage, the primary vector, is most active from late spring to early summer (see "Microbiology" later).[14] Because the latency period typically lasts from 1 to 4 weeks, one-fifth of cases are diagnosed in June, one-half in July, and another one-fifth in August.[3] Some cases present as early as late spring. Patients who present in late summer or early fall likely acquired the infection from an adult female tick.

Blood Transfusion

B. microti can be transmitted via transfusion of blood components prepared from contaminated blood donated by asymptomatic carriers or symptomatic individuals who did not suspect the infection.[15] Implicated blood donors typically have engaged in outdoor activities.[16] Less than one-fifth have experienced fatigue or fever within 24 months prior to index donation.[16] Even fewer (8%) have experienced chills. Consistent with the seasonality of tick-borne babesiosis, three-fourths of transfusion-transmitted babesiosis (TTB) cases are diagnosed from June through November, with one-fifth in August alone.[17] Given that asymptomatic infection can persist in untreated individuals for longer than a year,[16,18] TTB is diagnosed year-round.[17] Most TTB cases (87%) occur in residents of highly endemic states (see earlier list).[17] Outside endemic areas, TTB typically involves contaminated blood products that are imported from endemic areas or are derived from blood donations by individuals following their return from endemic areas.

More than 250 TTB cases caused by *B. microti* have been identified since the first case was reported in 1979; three-fourths have occurred since 2005.[16,17,19] Most cases involve red blood cell (RBC) units. A few have been attributed to whole-blood–derived platelets contaminated with residual RBCs. At time of transfusion, the age of liquid-stored RBC units has ranged from 4 to 42 days; one platelet unit was 5 days old.[16,17,20] In March 2018, when the US Food and Drug Administration (FDA) approved two tests for donor blood screening (see next paragraph), *B. microti* was still the pathogen most frequently implicated by investigations of transfusion-related illnesses.[19] From fiscal year 2010 to fiscal year 2016, *B. microti* accounted for one-fourth of transfusion-related deaths caused by microbial infection.[21]

Using an arrayed fluorescence immunoassay for detection of *B. microti* immunoglobulin G (IgG) and a real-time polymerase chain reaction (PCR) for detection of *B. microti* DNA, the risk posed by blood donations in some highly endemic areas (Massachusetts and Connecticut) has been carefully assessed.[16] Among donations collected from June 2012 through September 2014, 0.3% reacted in one test or both. Three-quarters of the donations were positive for *B. microti* IgG but negative for *B. microti* DNA, whereas one-fifth were positive for both. Antibody-positive donations were obtained throughout the year. Consistent with the seasonality of tick-borne babesiosis, donations that were DNA positive but IgG

negative were obtained from June through September. Among donors who tested positive for *B. microti* DNA at time of index donation, most (86%) were no longer infected a year later. The median time to parasite DNA clearance was 4.7 months. Among those who tested positive for *B. microti* IgG, only 8% had seroreverted a year later. The median time to seroreversion was 17.1 months. None of the 75,000+ donations screened with the two FDA-approved tests were implicated in a TTB case. In contrast, 1 in 18,000 unscreened donations obtained from the same area was implicated in a TTB case. When five highly endemic states (Connecticut, Massachusetts, Minnesota, New Jersey, New York) were analyzed along with two states that recently emerged as endemic (Maine and New Hampshire), the risk for TTB was estimated at 1 case per 100,000 donations for the period from January 1, 2010 to August 31, 2016.

Transplacental Passage

Unequivocal evidence of transplacental transmission came from a case reported in 2012.[22] A splenectomized mother was admitted after a 10-day history of fever and rigors. Intraerythrocytic *B. microti* ring forms were identified on a blood smear and in the amniotic fluid. On the second day of hospitalization, the mother underwent cesarean section. Immediately after delivery, blood was obtained from the neonate and intraerythrocytic parasites were noted on a thin blood film.

Probable congenital babesiosis has been reported for at least nine other cases.[23–26] For seven cases, mothers were asymptomatic throughout pregnancy. In one such case, *B. microti* likely was acquired during the first trimester of pregnancy.[25] Two symptomatic mothers received a diagnosis of Lyme disease during the last trimester of pregnancy, but *B. microti* infection was not suspected.[24] Symptoms of babesiosis developed in the neonates during the third to the sixth week of life and often consisted of fever accompanied by pallor.[24] Laboratory tests revealed hemolytic anemia, thrombocytopenia, and neutropenia. In all cases but one, anemia was so severe that a blood transfusion was required. At diagnosis, parasitemia ranged from 2% to 40%. When tested for *B. microti* antibody, neonates and their mothers were seropositive. One neonate who presented with tachycardia and respiratory distress required an exchange transfusion, which was performed manually.[26] This neonate had a twin who was seropositive for *B. microti* but remained healthy. In another case, *B. microti* antibody was detected in a heel stick blood sample obtained on the third day of life, raising the possibility that maternal IgG directed against *B. microti* protects newborns from congenital babesiosis very early in life.[23] In addition to vertical transmission, babesiosis has been diagnosed in neonates following blood transfusion.

Solid Organ Transplantation

Two cases of transplantation-associated babesiosis have been described.[27] Both men had received a kidney allograft obtained from the same deceased donor and began experiencing symptoms 5 weeks posttransplantation. The diagnosis of babesiosis was made 8 weeks posttransplantation. Despite immunosuppressive therapy, both men tested seropositive for *B. microti.* Pretransplantation serum samples did not react with *B. microti* antigen, an observation consistent with the denial by these allograft recipients of tick exposure and blood transfusion prior to transplantation. The organ donor, however, had received multiple blood transfusions, including one from a blood donor who tested positive for *B. microti* antibody during the retrospective investigation. It is presumed that some *B. microti* parasites from the blood donor had remained in the vasculature/fluids of the renal allografts at time of transplantation. In support of this explanation, corneas from the deceased organ donor were transplanted but neither recipient presented clinical or serologic evidence of *B. microti* infection.

Risk Factors

Babesiosis caused by *B. microti* often is severe in individuals with one or several of the following risk factors: asplenia,[28–30] X-linked agammaglobulinemia,[29] malignancy,[31] low $CD4^+$ T-cell counts associated with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS),[32,33] blockade of tumor necrosis factor-α (TNF-α) for chronic inflammatory disorders such as inflammatory bowel disease,[34] and immunosuppressive therapy for transplantation,[35,36] B-cell lymphoma, or an autoimmune disorder.[29,32] These risk factors also pertain to the transmission of *B. microti* by blood transfusion. In this setting, given that most cases have significant comorbidity, risk factors for TTB include conditions that require blood transfusion, such as hematologic disorders, cardiovascular surgery or procedure, gastrointestinal disease, bleeding and surgery, and anemia of prematurity.[17] Two-thirds of TTB patients are older than age 50 years, but two-thirds of those younger than age 40 years have a hereditary RBC disorder.[17]

United States: Other *Babesia* Species

Babesia duncani and *B. duncani*–type organisms are the etiologic agents of human babesiosis along the Pacific Coast.[1] The index case (WA1) occurred in a 41-year-old normosplenic man from a forested area of south central Washington State.[37] The four cases in California (CA1–CA4) developed in splenectomized men in their 20s to 40s who lived or had outdoor exposure in areas ranging from Sonoma County to San Bernardino County.[38] The first case of TTB caused by *B. duncani* involved a 76-year-old normosplenic man who had received RBCs donated by a 34-year-old Washington State resident.[39] The second case was diagnosed in a premature male infant who had received two blood transfusions.[40] The implicated donor lived in the San Francisco Bay area and had vacationed in a rural area of central Oregon 3 months prior to donating. The third case involved a 59-year-old patient who had sickle cell disease.[41] The 67-year-old donor reported a possible tick bite during a hike in the San Francisco Bay area. The winter tick *Dermacentor albipictus* presumably is the vector for transmission of *B. duncani*.[41a] The prevalence of *B. duncani* infection remains unclear because clinical cases have been too few to validate the serologic test.

B. divergens–like organisms have caused disease in Arkansas, Kentucky, Michigan, Missouri, and Washington State.[42–45] All six patients were older than 50 years of age, and asplenic. In one case, transfusion is the suspected mode of transmission.[43] The patient lived in Arkansas but blood donated in Missouri was the likely culprit, thereby bringing to three the number of *B. divergens*–like infections that originated in Missouri. Isolates from the five cases that originated in the Midwest were identical to piroplasms found in eastern cottontail rabbits, implying that *Ixodes dentatus* may be a vector for zoonotic transmission of *B. divergens*–like organisms. In Tennessee, three cases were uncovered by querying claims data from a managed care organization.[46] One such case may have been caused by a *B. divergens*–like organism.

Europe: *Babesia divergens*

Approximately 40 cases of babesiosis have been attributed to *B. divergens,* a parasite of cattle that is transmitted by *Ixodes ricinus*.[47] Most cases occurred in France and Ireland, particularly in regions with extensive cattle farming. Isolated cases have been reported from Portugal, Spain, Norway, Sweden, Finland, Georgia (ex-USSR), Turkey, and Croatia (including the index case). Nearly all patients experienced a severe illness and had been splenectomized,[47] although one had a rudimentary spleen and another had functional hyposplenism caused by celiac disease.[48,49] TTB caused by *B. divergens* has not been reported from Europe, although asymptomatic *B. divergens* infection is not uncommon, as indicated by seroprevalence in Slovenia (4.1% of forestry workers), western Austria (2.1% of blood donors), midwestern Germany (4.2% of individuals with clinical or serologic evidence of Lyme borreliosis), and southern Sweden (7.0% of individuals seropositive for *Borrelia burgdorferi* s.l.).[50–51a]

Europe: Other *Babesia* Species

Cases of *B. venatorum* infection have been reported in Italy, Austria, Germany, and Sweden.[52–54] The five patients were splenectomized men older than 50 years of age. Roe deer are a reservoir host for *B. venatorum* in Europe, and the bite of an *I. ricinus* tick is the suspected mode of transmission. The prevalence of asymptomatic infection remains unknown because *B. venatorum* sera cross-react with *B. divergens* antigen. Attributed to *B. divergens* based on morphology and seroreactivity, some early cases of babesiosis in Europe may have been caused by *B. venatorum.*

The first case of *B. microti* infection native to Europe was reported in 2007 from Germany.[55] A 42-year-old woman presented with fever and chest pain 1 week after chemotherapy for acute myeloid leukemia. Intraerythrocytic parasites were observed on Giemsa-stained blood smears and identified as US-type *B. microti.* The platelet concentrate transfused 6 days before the diagnosis of babesiosis had been prepared

from blood donated by an individual who subsequently tested seropositive for *B. microti.* Additional cases were reported in 2016 from northeastern Poland.[56] All six patients were immunocompetent and had been bitten by a tick within 8 weeks prior to presentation. No parasites were seen on blood smear, but a fragment of the *B. microti* 18S ribosomal RNA (rRNA) gene was amplified and sequenced, establishing for the first time that *B. microti* organisms of the Munich lineage can cause disease in humans. Cases of travel-associated babesiosis have been reported throughout Europe and explained by a recent stay in the northeastern United States.[47] Asymptomatic *B. microti* infection is prevalent in residents of rural, forested areas across Europe as indicated by serosurveys conducted in northeastern Poland (4.4% of foresters), eastern Switzerland (1.3% of blood donors), midwestern Germany (8.3% of individuals with clinical or serologic evidence of Lyme borreliosis), eastern France (2.% of foresters who are seropositive for other tick-borne pathogens), and Belgium (9% of patients presenting within 1 month after a tick bite).[50,57–60]

Asia: *Babesia venatorum*

The first case of *B. venatorum* infection in Asia was documented in northwestern China.[61] A series of 48 cases was subsequently reported from northeastern China.[62] All 48 cases reported a tick bite within 2 months prior to seeking medical care; none had received a blood transfusion within 2 years. All had a spleen, but only one-third were older than 50 years. A survey of ticks revealed that the taiga tick *Ixodes persulcatus* is the likely vector for *B. venatorum* in northeastern China.

Asia: Other *Babesia* Species

The first two cases of *B. microti* infection in Asia were documented in Taiwan.[63] A case of TTB was subsequently reported from Kobe, Japan.[64] The isolate has defined the Kobe lineage, one of the four lineages within the *B. microti* species complex.[65] The asymptomatic blood donor resided on the nearby Awaji Island, where the field mouse *Apodemus speciosus* was identified as a reservoir host for Kobe-type organisms.[66] The vector is unknown. *B. microti* organisms of the Hobetsu lineage are found in *A. speciosus* mice throughout Japan, and can be transmitted by *Ixodes ovatus* ticks.[67] Hobetsu-type organisms have infected humans, as revealed by a serosurvey, but no cases of babesiosis have been reported. In mainland China, *B. microti* has caused disease in the southern provinces, particularly in Yunnan along the border with Myanmar.[68,69] At least two cases were caused by US-type organisms. One case was coinfected with *Plasmodium falciparum* and another with *Plasmodium vivax.*[68]

In South Korea, a 75-year-old splenectomized woman who presented with fever and severe anemia was infected with a large *Babesia* species (KO1) that is closely related to organisms found in sheep. A series of 31 cases caused by *Babesia crassa,* a species found in sheep, has been documented in northeastern China.[70] All cases were mild to moderate; none were asplenic.

Rest of the World

A case of US-type *B. microti* has been documented in Australia.[1] On the American continent, a case of *B. microti* infection was identified in Manitoba, Canada, whereas three cases were diagnosed on the Yucatan peninsula, Mexico. Asymptomatic *B. microti* infection has been documented in Bolivia and Brazil. Cases caused by *Babesia* spp. that infect cattle (*B. bigemina*, *B. bovis*) have been reported from Colombia. Asymptomatic infections with these two *Babesia* spp. have been reported from Brazil, Colombia, and Cuba. On the African continent, three cases have been reported from Egypt, of which one was acquired from a pet dog. A case of *B. divergens*–like infection was diagnosed in a splenectomized resident of the Canary Islands. In South Africa, babesiosis was diagnosed in two patients upon their return from Namibia and Zimbabwe. Isolated cases of *B. bovis* infection, of which two were fatal, have been documented in Mozambique.

MICROBIOLOGY

Babesia spp. are protozoa parasites of the phylum Apicomplexa, class Aconoidasida, and order Piroplasmida. More than 100 *Babesia* spp. are known to infect vertebrates, including mammals and birds. In the northern hemisphere, most *Babesia* spp. are maintained in their enzootic cycle by hard-bodied ticks of the genus *Ixodes.* In the United States, *B. microti* is maintained by *I. scapularis* ticks.

The Enzootic Cycle of *Babesia microti*

Ticks and Reservoir Hosts

The life cycle of *I. scapularis* has three active stages (larva, nymph, adult) and requires 2 years for completion.[1,14] In the fall, adult ticks feed primarily on white-tailed deer (*Odocoileus virginianus*). Deer are incompetent hosts for *B. microti* but are essential for the mating of *I. scapularis* and therefore its maintenance in the life cycle. Adult female ticks overwinter in an engorged state and lay eggs in the spring. Because *B. microti* does not reach the ovaries of adult ticks, eggs are free of *B. microti* (no transovarial transmission). Larvae hatch in late July and become infected as they feed in late summer on a wide range of small mammals. The primary reservoir host for *B. microti* is the white-footed mouse (*Peromyscus leucopus*).[14] Other competent reservoirs include short-tailed shrews, eastern chipmunks, and raccoons.[71] Passerines, raptorial birds, and ground-dwelling birds (e.g., wood thrush, veery) are reservoirs but of lesser competence. Fed to repletion, larvae overwinter and molt into nymphs in late spring. If the larva is infected, so can be the nymph (transstadial transmission). Nymphs feed on *P. leucopus* and other vertebrates from May through July. Feeding of nymphs in early summer ensures that *P. leucopus* mice are reservoirs of *B. microti* when larvae feed in late summer. In the fall, nymphs molt into adults.

Parasite Acquisition by Ticks

Soon after attachment of a larva or a nymph, host erythrocytes accumulate in its gut.[1,14] *Babesia microti* pregametocytes mature into gametocytes. Microtubules accumulate at the anterior end of the parasite to form a raylike structure that contributes to the fusion of gametocytes into a zygote. Using an arrowhead structure, zygotes penetrate the tick gut epithelium. The arrowhead structure dissociates, and zygotes translocate to the basal lamina. In the hemolymph, zygotes become ookinetes that travel to the salivary acini. There, ookinetes hypertrophy into sporoblasts, which remain dormant while the larva or nymph overwinters and, until the next tick stage, feeds on a reservoir host or, incidentally, on a human.

Parasite Transmission to Hosts

Larvae, nymphs, and adult ticks all feed on humans, but the nymph is the primary vector for *B. microti* transmission.[1,14] The size of the nymph (<2.5 mm; as small as a poppy seed), its gray ground color, and the inconspicuous feeding site explain why only one-half of patients recall a tick bite.[3] As nymphs attach to the host, their temperatures rise. Sporogony is initiated, and the sporoblast membrane folds. Micronemes and rhoptries appear. The cytoplasm starts to bud from the parent sporoblast, and nuclear division ensues. During the final hours of feeding, which typically last 72 hours, as many as 100,000 sporozoites are deposited in the dermis of the host. Events that lead sporozoites toward the bloodstream are not well understood. *Babesia* sporozoites do not undergo merogony in an exoerythrocytic compartment but readily invade erythrocytes. Surface antigens, particularly glycosylphosphatidylinositol-anchored proteins encoded by genes of the *bmn* family, are thought to mediate initial attachment.[72,73] Micronemes also contribute to host cell invasion, as evidenced by the role of the apical membrane antigen 1 (AMA1),[74] a micronemal protein that anchors in the plasma membrane of the parasite at its apex. A role for AMA1 is consistent with a role for the rhoptry neck protein (RON) macromolecular complex that is projected onto the erythrocyte and anchors in its plasma membrane.[75] As with *Plasmodium* species, the erythrocyte membrane is thought to invaginate as the parasite glides using the moving junction formed by nascent AMA1-RON2 interactions. Once the invasion process is completed, the parasitophorous vacuole gradually disintegrates. The organism differentiates into a trophozoite that moves freely in the RBC cytoplasm and eventually undergoes binary fission, resulting in two to four merozoites (Fig. 281.2). Merozoites egress the erythrocyte, thereby triggering its lysis, and soon invade other erythrocytes.

Classification of *Babesia* Species

Single-Gene Analysis

Using full-length 18S rRNA gene sequences, phylogenetic analysis of the order Piroplasmida has revealed five clades.[76] The first clade contains the *B. microti* species complex as well as *Babesia rodhaini,* a parasite of rodents. The second clade consists of piroplasms found in the western United States and may constitute a species complex because *B. duncani*

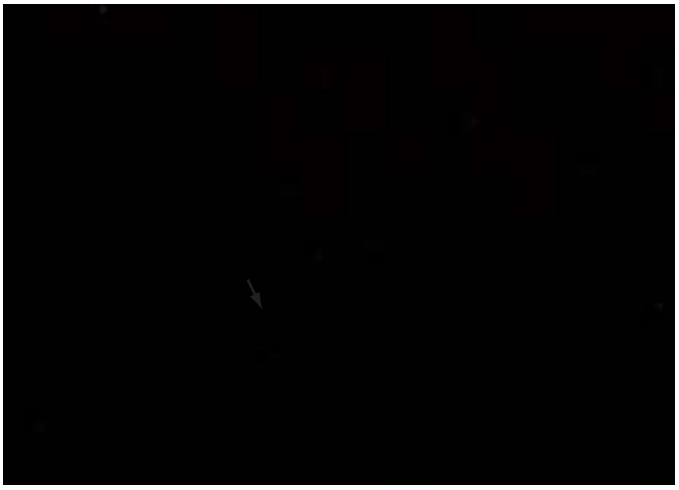

FIG. 281.2 Giemsa stain of a thin blood film. The patient was diagnosed with babesiosis caused by *Babesia microti.* Trophozoites often are seen as ring forms, some with two chromatin dots. Ring forms have a peripheral location, as with *Plasmodium falciparum,* but their large clear central vacuole and the absence of brown pigment (hemozoin) are characteristic of *B. microti.* A single trophozoite can divide by merogony to generate four merozoites arranged in a tetrad, also known as Maltese cross *(arrow).* Tetrads are rare but pathognomonic of small *Babesia* spp. such as *B. microti* and *B. duncani.* Tetrads also can be seen in human red blood cells infected with *B. divergens* or *B. venatorum. (Courtesy Dr. J. Vyas.)*

(WA1, CA5) and *B. duncani*–type (WA2, CA6) organisms cluster separately from other patient isolates (CA1–CA4) and those of wildlife roaming in the same habitat.[77] The third clade contains *Theileria* spp. only. The fourth clade contains *B. divergens* isolates from cattle in Europe, but also *B. divergens*–like organisms from patients in the United States; *B. odocoilei,* which infects white-tailed deer in the United States; and *B. venatorum* isolates from patients in Europe and China.[43,54,62] The fifth clade contains *Babesia* spp. that infect ungulates but rarely humans. These include *B. bovis* and *B. bigemina,* parasites of cattle, as well as *B. crassa* and *B. caballi,* which infect sheep and horses, respectively. Piroplasms of the fourth and fifth clades are *Babesia* spp. sensu stricto.

Long regarded as a single species, *B. microti* is a species complex. The internal topology of this complex was refined by analysis of the gene encoding the η subunit of the chaperonin-containing *t*-complex polypeptide 1 (CCTη).[65] The number, position, and length of introns, as well as the nucleotide-based and amino-acid–based phylogenies, all concur to indicate that the *B. microti* species complex consists of four lineages (US-type, Munich, Kobe, and Hobetsu) and that these four lineages correspond to four separate species.

Whole-Genome Analysis

The genomes of *B. microti*, *B. divergens,* and *B. bovis* have been fully sequenced.[72,78,79] Each genome consists of four nuclear chromosomes, a linear mitochondrial genome, and a circular apicoplast genome. The nuclear genome of *B. microti* is the smallest (6.7 Mb vs. approximately 8 Mb for *B. bovis* and approximately 10 Mb for *B. divergens*). It is notable for the absence of a large family of *ves*-like genes that, in *B. bovis,* encode variant erythrocyte surface antigens (VESAs). VESAs mediate the sequestration of infected erythrocytes in the vasculature. The four *ves*-like genes present in the *B. microti* genome are not arranged in tandem and most likely are not expressed, hence the lack of cerebral manifestations in babesiosis caused by *B. microti. Babesia divergens,* a species with numerous *ves*-like genes, does not evoke cerebral manifestations because the encoded VESA proteins lack the cysteine-rich domains critical for cytoadhesion of infected erythrocytes.

Genome-wide analysis of single nucleotide polymorphisms among *B. microti* isolates obtained from ticks and humans in the United States revealed strong population structure with deep divergence between the Midwest and the Northeast.[80,81] Within the Northeast, three clusters were identified. The first cluster is found on Nantucket, an island 30 miles off Cape Cod. The second cluster encompasses the Northeast mainland except Cape Cod. The third cluster stretches from Cape Cod to the nearby Martha's Vineyard island. The first and second clusters are closely related, whereas the third cluster emerged earlier. Despite the close geographic proximity of these clusters, there is little evidence of genetic recombination.[80] This strong population structure is consistent with the low dispersal capability of *B. microti* that is imposed by the short-range migration of *P. leucopus,* the primary reservoir host. Unlike the Nantucket cluster, the Northeast mainland population presents genetic evidence of recent demographic expansion, possibly from west to east.[80,81] The cluster that spans from Martha's Vineyard to Cape Cod may correspond to a relictual (surviving) population that was maintained in its enzootic cycle by *Ixodes muris* or *Ixodes angustus* prior to the arrival of *I. scapularis.*[80] Whether these distinct *B. microti* populations differ by the number or severity of symptoms they evoke, or both, is unknown.

CLINICAL MANIFESTATIONS

Babesia microti Infection

Symptoms typically appear 1 to 4 weeks after the bite of an infected tick, but 3 to 7 weeks when the infection is transmitted through blood transfusion (median interval, 37 days; range, 11–176 days).[1,17] Most patients experience a gradual onset of fatigue, weakness, or malaise or a combination of these, followed within days by fever and one or more of the following: chills, sweats, headache, myalgia, and anorexia.[1] Fever is intermittent or persistent and has reached 40.9°C (105.6°F). Less frequent symptoms include arthralgia, neck stiffness, nonproductive cough, sore throat, nausea/vomiting, weight loss, and emotional lability. Photophobia, conjunctivitis, joint swelling, diarrhea, and crampy abdominal pain are rare. Dark urine raises the suspicion of severe hemolytic anemia, and may be accompanied by shortness of breath.

On physical examination, fever is the salient feature.[1] The skin may be pale or yellowish. A local red rash can mark the tick bite site; an erythema migrans rash is diagnostic of intercurrent Lyme disease.[4] Ecchymoses and petechiae are rare. Examination of the mouth is unremarkable except when a slight pharyngeal erythema is noted. Scleral icterus is consistent with severe hemolytic anemia. Retinopathy with splinter hemorrhages and retinal infarcts have been reported. Tenderness of the upper left quadrant suggests splenomegaly and may be accompanied by hepatomegaly. Abdominal pain or unexplained hypotension with increased heart rate raises the suspicion of splenic rupture and hemoperitoneum. Splenic rupture with or without palpable splenomegaly has been reported.[82,83] Splenic infarction and subcapsular hematoma may develop in the absence of splenic rupture. Splenic infarction and splenic rupture are confirmed by computed tomography.

Severe babesiosis requires hospital admission.[5,28–30] Of the cases reported to the CDC from 2011 to 2014, one-half were admitted at least overnight.[3] Of those for which the length of hospitalization was documented, one-half had a hospital stay of 3 to 5 days (range, 1–39 days). In smaller case series, the median length of stay typically has ranged from 5 to 9 days, with one patient remaining in the hospital for 100 days.[5,7,28–30,32] Two-thirds of cases are diagnosed in persons 50 to 79 years of age, with the largest number occurring in people 60 to 69 years of age.[3] Patients who are admitted to the hospital are older (median age, 68 years) than those who are not admitted (median age, 58 years).[2] Although age is a risk factor for hospitalization, it is not a risk factor for severe babesiosis.[9,29,30] This finding is best explained by the fact that comorbidities and immunosenescence are not strict correlates of chronologic age. Asplenia and autoimmune disorders predispose to severe babesiosis and therefore hospital admission.[29] Among clinical features, nausea/vomiting and diarrhea are strong predictors of hospitalization.[29]

Severe babesiosis can lead to complications. Pulmonary edema and acute respiratory distress syndrome are most common, followed by congestive heart failure and renal failure.[5,7,28–30,32] Disseminated intravascular coagulation, shock, and splenic rupture are rare. In earlier case series, when clindamycin plus quinine was the mainstay of therapy, death occurred in 6% to 9% of hospitalized patients.[28,30] Of the 10,305 Medicare recipients who received a diagnosis of babesiosis between 2006 and 2013, 1% died within 30 days.[84] Among inpatients, the 30-day mortality rate was 3%. In this series, atovaquone plus azithromycin was

the mainstay of therapy, whereas clindamycin plus quinine was used to treat one-fourth of patients. Death is more frequent (approximately 20%) among immunocompromised patients.[32] Of the TTB cases caused by *B. microti* until fiscal year 2016, one-tenth ended in death.[16,17,21]

Babesia duncani Infection

The eight documented cases caused by *B. duncani* were moderate to severe.[37–41] All but one had one or several comorbidities. Clinical manifestations were similar to those of *B. microti.* Of the five patients who presumably acquired the infection via tick bite, two had a complicated course.[38] One died of cardiopulmonary arrest despite antimicrobial therapy, transfusion, and hemodialysis. The other experienced disseminated intravascular coagulation, pulmonary edema, and renal insufficiency but recovered following antimicrobial therapy and exchange transfusion. The three cases acquired via blood transfusion were severe. One was so anemic that packed RBCs were transfused before diagnosis.[39] Another, a neonate, developed severe respiratory distress.[40] The third developed acute renal failure requiring hemodialysis.[41] All three patients recovered following standard antimicrobial therapy. The implicated blood donors were unaware of their babesial infection but recalled experiencing nausea, fatigue, or a mild flulike illness.

Babesia divergens Infection

Most cases caused by *B. divergens* infection occur in asplenic patients, and are severe.[47] Symptoms tend to appear abruptly 1 to 3 weeks following a tick bite, and consist of persistent high fever (40°C–41°C [104°F–105.8°F]), headache, shaking chills, drenching sweats, myalgia, and lumbar and abdominal pain. Dark urine and jaundice are consistent with severe hemolytic anemia. Without immediate treatment, a shocklike syndrome can develop, with renal failure and pulmonary edema. Once a fatal disease, symptomatic *B. divergens* infection is now successfully managed with exchange transfusion as an adjunct to antimicrobial therapy. *B. divergens* rarely causes disease in spleen-intact individuals. One such case was mild and resolved without adequate antimicrobial therapy.[85] Another case was severe and relapsed despite standard antimicrobial therapy, but eventually resolved after extended therapy with an alternative regimen.[86] Cases of *B. divergens*–like infection in the United States have been severe. All six patients had been splenectomized and three died despite medical intervention.[42–45]

Babesia venatorum Infection

B. venatorum causes mild to severe illness. All five cases in Europe occurred in splenectomized patients.[52–54,86a] One patient was markedly fatigued and produced dark urine.[54] Another patient experienced fever (39°C), chills, headache, jaundice, and dark urine. He recently had begun chemotherapy for diffuse large B-cell lymphoma. The third patient had been treated with rituximab for relapsing Hodgkin disease, and presented with weakness, shortness of breath, and lethargy.[53] He was hospitalized for severe anemia and tested positive in the direct Coombs test, as sometimes seen in babesiosis. He was initiated on prednisolone for presumptive autoimmune hemolytic anemia, which likely worsened the illness. The fourth patient was admitted for recurrent fever, hemolysis, and acute renal failure.[52] He too had been treated with prednisolone for suspected autoimmune hemolytic anemia. The fifth patient was admitted for fever, myalgia, and dark urine. His history included idiopathic thrombocytopenic purpura, bouts of hemolytic anemia, and administration of rituximab 2 years earlier. He was treated with cyclosporine, prednisolone, human immunoglobulin, and a series of antimicrobial agents, including co-trimoxazole (trimethoprim-sulfamethoxazole). The diagnosis of babesiosis was made 2 months later, during a third hospital stay for fever and rigors. Co-trimoxazole, which was curative in a mild case of *B. divergens* infection, may have prevented the progression into severe disease. Despite a large spectrum of clinical severity, symptoms in the five European patients resolved following standard or extended antimicrobial therapy without exchange transfusion. A report from northeastern China indicated that *B. venatorum* causes disease in two-thirds of spleen-intact individuals.[62] Symptoms were those evoked by *B. microti,* although fever, fatigue, chills, and myalgia were experienced by fewer patients. Of the 32 symptomatic individuals, 7 were admitted for irregular fever as high as 40°C. The median length of stay was 16 days (range, 11–20 days). Only 4 patients received appropriate antibiotic therapy (although clindamycin monotherapy), but all 32 patients recovered.

PATHOGENESIS

Erythrocyte Clearance and Rupture

Continuous clearance of *Babesia*-infected RBCs and uninfected RBCs by phagocytes that reside in the red pulp of the spleen contributes to splenomegaly and splenic rupture,[87] and can lead to hemophagocytic lymphohistiocytosis, a fatal condition.[88] As the immune response unfolds, inflammatory cytokines are produced. By suppressing erythropoiesis, interferon-γ (IFN-γ) and interleukin-6 likely contribute to severe anemia. Persistent anemia, despite parasite clearance, has been attributed to autoantibodies that target RBCs.[89] Egress of merozoites is accompanied by erythrocyte rupture and hemoglobin release into the bloodstream. Hemoglobin is rapidly complexed by haptoglobin; minute amounts of free hemoglobin, however, are sufficient to promote systemic inflammation.[90]

Inflammatory and Immune Response

The spleen is the immunodominant organ in babesiosis.[1] This feature, along with the clearance of parasitized RBCs, explains why asplenia is a major risk factor for severe babesiosis.[29] Another key risk factor for severe babesiosis is a low $CD4^+$ T-cell count, as seen in HIV/AIDS patients and allograft recipients.[32,33,35,36] In mice infected with *B. microti,* depletion of $CD4^+$ T cells, a major source of IFN-γ, or neutralization of IFN-γ is sufficient to break host resistance and prevent parasite clearance.[91,92] While central to host immunity, cytokines likely contribute to clinical manifestations. For example, circulating TNF-α and interleukin-6 are elevated during acute illness with *B. microti,*[93] and likely fuel the triad of symptoms consisting of fever, chills, and sweats. Cytokines may also contribute to complications. Mice infected with *B. duncani* die from pulmonary edema associated with intravascular margination of leukocytes. TNF-α is localized to the alveolar septa, whereas IFN-γ is expressed by cells around and within pulmonary vessels.[94] In this model, TNF-α blockade promotes survival, whereas IFN-γ receptor blockade promotes death.[95,96] Although nearly every immunocompetent patient infected with *B. microti* has a positive serology at diagnosis, a role for antibodies is uncertain, as demonstrated in mice.[91] Patients who are or were recently treated with rituximab are prone to persistent or relapsing babesiosis or both,[32,97–99] indicating that B cells, and most likely antibodies, are key to clearing *B. microti* parasites in some immunocompromised hosts.

DIAGNOSIS

A diagnosis of babesiosis should be considered for patients who experience typical symptoms of babesiosis and may have been exposed to ticks in an endemic area between late spring and early fall or who have received blood components, particularly packed RBCs, in the past 6 months.[1] Given that *I. scapularis* transmits *B. microti, B. burgdorferi,* and *Anaplasma phagocytophilum* in the Northeast and upper Midwest of the United States, babesiosis should always be suspected in patients diagnosed with Lyme disease or human granulocytic anaplasmosis in these regions, particularly if symptoms worsen or do not abate within days to weeks of appropriate antibiotic therapy.[4,100]

Routine Laboratory Findings

Routine laboratory tests provide clues. Anemia is evident on a complete blood count (low RBC count, low hematocrit, and low hemoglobin), and often accompanied by an elevated reticulocyte count.[1] The white blood cell (WBC) count is usually normal to slightly decreased, but sometimes elevated.[29,30] A differential may reveal neutropenia as seen in one-third of adults and three-quarters of neonates.[101] A severe thrombocytopenia often precedes severe anemia. Elevated blood levels of lactate dehydrogenase and total bilirubin are consistent with hemolytic anemia, findings that are substantiated by a depressed haptoglobin level. Elevated levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase indicate hepatocyte injury, whereas elevated

aspartate aminotransferase levels without elevated alanine aminotransferase levels are consistent with hemolysis. Severe hemolysis is associated with excess urobilinogen, but rarely with hemoglobinuria. Elevated levels of blood urea nitrogen and serum creatinine denote renal compromise. The direct Coombs test may be positive on admission or in follow-up or both,[52,53,89] implicating autoantibodies as contributors to the anemia. Autoantibodies against platelets can be induced, leading to immune thrombocytopenia.[102] In asplenic patients, babesiosis has reactivated or provoked an Evans syndrome.[103]

A study of 34 patients admitted to a hospital on Long Island, NY, reported that severe anemia (hemoglobin <10 g/dL) is a strong risk factor for complications as a whole, and that high-grade parasitemia (≥10%) is marginally associated with disseminated intravascular coagulation.[28] A review of 139 cases that occurred in New York State between 1982 and 1993 indicated that elevated alkaline phosphatase levels (>125 U/L) and elevated WBC counts ($>5 \times 10^3/\mu L$) are strong predictors of severe outcome, defined as hospitalization longer than 2 weeks, stay in an intensive care unit longer than 2 days, or death.[30] A review of 128 cases diagnosed at Marshfield Clinic in Wisconsin between 1999 and 2015 identified total bilirubin levels greater than 1.9 mg/dL as a strong predictor of severe disease, defined as admission to the intensive care unit; an illness complicated by heart failure, shock, or splenic rupture; or the need for intubation or exchange transfusion.[29] In this study, high-grade parasitemia (>10%), elevated creatinine levels (>1.2 mg/dL), and elevated WBC counts ($>10 \times 10^3/\mu L$) were moderate predictors of severe illness, whereas low WBC counts ($<5 \times 10^3/\mu L$) were highly predictive of severe disease.[29]

Microscopy

Microscopic examination of Giemsa-stained thin blood smears has long been used to make a definitive diagnosis of babesiosis.[1,47,54,77] *B. microti* trophozoites appear round, oval, or ameboid (see Fig. 281.2). The ring form is most common, with one or two red chromatic dots and a light blue cytoplasm. Tetrads of merozoites arranged in a Maltese cross are rare, but pathognomonic of *B. microti* and *B. duncani*. *B. divergens* and *B. venatorum* merozoites appear as paired piriforms but may form tetrads in human RBCs. The analytic sensitivity of microscopy (approximately 0.01% parasitemia as evaluated by highly experienced laboratory personnel) is sufficient in most cases because initial parasitemia typically ranges from 0.05% to 10% in spleen-intact patients, and even higher in asplenic patients (30%–50%, with one case at 85%). When parasitemia is high, extracellular *B. microti* merozoites, either single or in clumps, can be observed. *Babesia* rings resemble early-stage *P. falciparum* trophozoites but lack the brownish deposit (hemozoin) typical of older rings of *P. falciparum*. Gametocytes and schizonts are not observed in babesiosis.

Nucleic Acid Amplification

When *Babesia* organisms are not identified on smear but babesiosis is suspected, amplification of the parasite 18S rRNA gene is recommended. PCR assays that use a fluorescent probe can detect as few as 1 to 5 parasites per microliter of blood, that is, as low as 0.0001% parasitemia.[104–106] Often qualitative, PCR testing provides little help in predicting the course of disease. By use of species-specific primers or probes, real-time PCR assays are well-suited for speciation of the causative organism. The CDC now uses a real-time PCR assay to identify *B. microti* in human blood.[107] This assay, although less sensitive than others, can detect *B. microti* isolates from all four lineages (US-type, Munich, Kobe, and Hobetsu) of the species complex. In patients who experience acute babesiosis caused by *B. microti* in the United States, the persistence of babesial DNA correlates with the persistence of symptoms and is shortened by antimicrobial therapy.[31]

Serology

The caveat of serologic testing is that IgG titers can be low or negative at time of presentation, and typically persist beyond parasite clearance.[1] An indirect fluorescent antibody (IFA) test to detect *B. microti*–specific IgG in serum or plasma can be ordered from commercial laboratories. A titer of 1:64 or greater is considered positive. Titers of 1:1024 or greater indicate active or recent infection. Following acute illness, titers slowly wane over 6 to 12 months.[31] Serology is not practical for *B. divergens* infection because the incubation period is short and the infection fulminant.[47] Sera that react with *B. microti* antigen do not cross react with *B. divergens* or *B. duncani* antigen and vice versa.

THERAPY

Asymptomatic *Babesia microti* Infection

Asymptomatic carriers need not be treated because they typically are immunocompetent and clear the infection within 1 year.[16] Should they become at risk of severe babesiosis during this period (see "Risk Factors" earlier), treatment is recommended. At present, asymptomatic carriers often are identified during investigational product-release testing at selected blood drives. Their numbers will significantly increase once a systematic screening of the blood supply is instituted.

Mild *Babesia microti* Infection

Mild babesiosis caused by *B. microti* is treated with oral atovaquone plus oral azithromycin for 7 to 10 days.[108] Clindamycin plus quinine was the first regimen to demonstrate efficacy against *B. microti*; quinine, however, is poorly tolerated and often interrupted. A prospective, nonblinded, randomized clinical trial demonstrated that atovaquone plus azithromycin is as effective as clindamycin plus quinine in resolving symptoms of babesiosis and clearing *B. microti* parasites.[109] Importantly, untoward drug reactions were noted in fewer of the patients treated with atovaquone plus azithromycin (15% vs. 72% for clindamycin plus quinine).[109] Regimens for adults and children are provided in Table 281.1. Symptoms usually abate within 48 hours following initiation of antimicrobial therapy, and resolve within 1 to 2 weeks. Fatigue may persist for a few months. Low-grade parasitemia can persist for as long as 3 months, but follow-up PCR testing is not recommended because immunocompetent patients eventually clear the infection. Symptoms can recrudesce should a malignancy develop or an immunosuppressive regimen be administered before complete parasite clearance.[31]

Severe *Babesia microti* Infection

First-Line Antimicrobial Therapy

Severe babesiosis caused by *B. microti* should be treated with oral atovaquone plus intravenous azithromycin. Based on cumulative clinical experience, it has long been recommended that severe babesiosis be treated with intravenous clindamycin plus oral quinine.[108] Quinine, however, has frequent and often severe side effects (cinchonism) and cardiotoxicity. No prospective study has compared the two regimens for severe *B. microti* illness, but a retrospective study of 40 patients admitted for babesiosis, including 11 admitted to intensive care, found that all patients but one resolved the infection while on atovaquone plus azithromycin.[110] Regimens for adults and children are provided in Table 281.1. Intravenous azithromycin should be initiated at 500 mg/day.[100] Hematocrit, platelet count, parasitemia, liver enzymes, and renal function should be monitored daily until symptoms abate and parasitemia is reduced (<4%). Thereafter, for patients who are immunocompetent and have a functional spleen, azithromycin can be prescribed orally and the dosage reduced to 250 mg/day.[100] In such patients, atovaquone plus azithromycin typically is administered for a total of 7 to 10 days. Duration, however, should be extended if symptoms persist. The approach to severely immunocompromised patients and asplenic patients is discussed separately (see later).

Adjunctive Exchange Transfusion

Partial or complete RBC exchange transfusion should be considered for patients with high-grade parasitemia (≥10%) or severe anemia (hemoglobin <10 g/dL) in the context of parasitemia.[108] RBC exchange transfusion reduces parasitemia, corrects anemia, and removes inflammatory mediators and toxic by-products of RBC lysis (e.g., hemoglobin). Prompt use of RBC exchange transfusion is associated with favorable outcome.[111] A 90% reduction in parasitemia has been recommended as a target of apheresis and can be achieved by transfusion of a volume of allogeneic RBCs that amounts to 2.5 times the estimated volume of recipient RBCs.[111] When parasitemia is less than 10% and hemoglobin is 10 g/dL or greater, one should take into account the degree of systemic illness; acute respiratory distress syndrome and a systemic inflammatory

TABLE 281.1 Treatment of Human Babesiosis

SPECIES	ILLNESS	HOST[a]	FIRST-LINE REGIMEN[b]	ALTERNATIVE REGIMEN[b]
Babesia microti	Mild		Atovaquone 750 mg q12h PO plus azithromycin 500 mg PO on day 1 and 250 mg/d PO from day 2 on; for 7–10 d[d]	
	Severe[c]	Immunocompetent	Atovaquone 750 mg q12h PO plus azithromycin 500 mg/d IV[e,f]; for 7–10 d[d]	Clindamycin 600 mg q6h IV plus quinine 650 mg q6–8h PO[f,g,h,i]; for same duration
		Immunocompromised and/or asplenic	Atovaquone 750 mg q12h PO plus azithromycin 500 mg/d IV[j]; for at least 6 consecutive wk, including 2 final wk during which parasites are no longer detected on blood smear[k,l]	Atovaquone 750 mg q12h PO plus clindamycin 600 mg q6h IV with or without azithromycin 500 mg/d IV[m,n]; for same duration
Babesia divergens	Mild	Immunocompetent	Clindamycin 600 mg q8h PO plus quinine 650 mg q6–8h PO; for 7–10 d[d,h]	Atovaquone 750 mg q12h PO plus azithromycin 500 mg PO on day 1 and 250 mg/d PO from day 2 on; for 7–10 d[d,n]
	Severe	Immunocompromised and/or asplenic	Immediate complete RBC exchange transfusion combined with clindamycin 600 mg q6h IV plus quinine 650 mg q6–8h PO; for 7–10 d[d,h]	

[a]Dosages are provided for treatment of adults. For pediatric cases, consider using atovaquone 20 mg/kg q12h PO (up to 750 mg/dose) plus azithromycin 5–10 mg/kg PO (up to 250–500 mg/dose), *or* clindamycin 7–10 mg/kg q6–8h IV or PO (up to 600 mg/dose) plus quinine 8 mg/kg q8h PO (up to 650 mg/dose).
[b]Atovaquone is available in a suspension form, and should be taken with dietary fat to increase absorption.
[c]Consider partial or complete RBC exchange transfusion in cases of high-grade parasitemia (≥10%), severe anemia (hemoglobin <10 g/dL), or organ (pulmonary or renal) compromise.
[d]Duration may be extended if symptoms other than fatigue linger.
[e]Intravenous azithromycin may be replaced with oral azithromycin (250–500 mg/day) once the patient has improved.
[f]Recommended for the treatment of severe babesiosis in hospitalized patients.
[g]Intravenous clindamycin may be replaced with oral clindamycin (600 mg q8h) once the patient has improved.
[h]Because quinine carries a risk of QT segment prolongation and torsade de pointes, patients should be monitored by electrocardiography. In the setting of hepatic or renal disease, quinine serum levels should be monitored.
[i]If cardiac toxicity or untoward adverse effects occur, quinine can be replaced with oral atovaquone (750 mg q12h).
[j]Intravenous azithromycin can be replaced with oral azithromycin (500 mg/d) once the patient has improved. Higher doses of oral azithromycin (600–1000 mg/d) accelerate symptom resolution and parasite clearance.
[k]Blood smear is recommended to monitor resolution of infection in severely immunocompromised patients, but recent case reports support the use of a real-time polymerase chain reaction assay to ensure complete parasite clearance.
[l]Duration may vary. In a patient who had been treated with rituximab, parasite clearance was achieved after 27 months of uninterrupted antimicrobial therapy. If asplenia is the only comorbidity, antimicrobial therapy may be shortened as long as clinical cure and parasite clearance are achieved in less than 4 weeks.
[m]Prolonged administration of clindamycin places the patient at risk of *Clostridioides difficile* (formerly *Clostridium difficile*) infection.
[n]Atovaquone can be replaced with atovaquone-proguanil. Atovaquone-proguanil should be taken with dietary fat, but should not be given to patients with severe renal impairment.
IV, Intravenously; *PO,* orally; *RBC,* red blood cell.

response–like syndrome are indications for apheresis.[112] RBC exchange transfusion also is recommended for patients with renal compromise.[108]

Alternative Antimicrobial Regimens

In patients who do not improve clinically within 48 hours of treatment with atovaquone plus azithromycin, intravenous clindamycin plus oral quinine should be considered.[100,108] An alternative regimen can consist of intravenous clindamycin plus oral atovaquone with or without intravenous azithromycin, a regimen favored by the authors.[112] Once the patient has improved, antibiotics can be administered orally.[5,97] Atovaquone-proguanil has been added to azithromycin or various two- or three-drug regimens, particularly when symptoms persisted or relapsed.[36,97–99,112] In an AIDS patient, high-grade parasitemia resolved following addition of atovaquone-proguanil to a four-drug regimen that consisted of azithromycin, atovaquone, clindamycin, and quinine; an asymptomatic recurrence was treated with atovaquone-proguanil monotherapy.[33] Doxycycline is prescribed for intercurrent Lyme disease but has yet to be shown effective against *B. microti.*

Severely Immunocompromised Patients

A retrospective case-control study conducted more than 10 years ago provided the first evidence that severely immunocompromised patients, particularly those who are or were recently treated with rituximab for B-cell lymphoma, are at risk of relapsing babesiosis and should be treated with antimicrobial therapy for at least 6 consecutive weeks, including 2 final weeks during which parasites are no longer seen on blood smear.[32] Given that no particular drug regimen is superior to another,[32] the first-line regimen can consist of oral atovaquone plus intravenous azithromycin. Regimens for adults and children are provided in Table 281.1. Intravenous azithromycin should be initiated at 500 mg/day.[100] Hematocrit, platelet count, parasitemia, liver enzymes, and renal function should be monitored daily until symptoms abate and parasitemia is reduced (<4%). Thereafter, azithromycin can be administered orally but the dosage should be no less than 500 mg/day because lower dosages may promote antibiotic resistance.[99,100] Higher dosages of oral azithromycin (600–1000 mg/day) can be considered because they accelerate resolution of fever and clearance of parasites,[113] thereby minimizing the risk of microbial relapse. Once the patient is no longer critically ill, laboratory testing can be performed every 2 to 3 days until antimicrobial therapy is completed. Close clinical follow-up is recommended.

The approach to treatment outlined for B-cell lymphoma patients extends to any patient who is or was recently treated with rituximab for autoimmune disorders, including rheumatoid arthritis, Evans syndrome, and confirmed or presumptive autoimmune hemolytic anemia.[29,32,97,98] Time from last dose of rituximab to seroconversion and parasite clearance appears to be approximately 18 months.[97] This finding is consistent with the observation that patients who ended rituximab therapy as far as 18 months prior to a diagnosis of babesiosis are at risk of relapsing babesiosis should a standard course of antimicrobial therapy be administered.[32] A prolonged antimicrobial therapy is also recommended for patients with X-linked agammaglobulinemia or low $CD4^+$ T-cell counts due to HIV/AIDS, as well as patients who receive chronic immunosuppressive medication for malignancy, stem cell or solid organ transplantation, or autoimmune disorders.[29,32,100] Reduction or interruption of such immunosuppressive regimens, when possible, is desirable.

Blood smear is recommended to monitor parasite clearance in severely immunocompromised patients.[32] Recent case reports, however, stress the benefit of using real-time PCR to detect low-grade parasitemia.[97,98] A premature interruption of antimicrobial therapy due to apparent cure has led to the emergence of microbial resistance to atovaquone and azithromycin.[99] Such resistance has been associated with missense mutations in the mitochondrial cytochrome b gene (*cytb*) and the apicoplast-encoded ribosomal protein subunit L4 gene (*rpl4*), respectively.[81]

Asplenic Patients

Asplenic patients account for at least one-half of reported cases of relapsing babesiosis.[29,32] Asplenic patients, particularly when presenting with comorbidities that impair the immune response to *B. microti*

parasites, should be treated with prolonged antimicrobial therapy.[32] If asplenia is the only comorbidity, therapy may be shortened to less than 6 weeks as long as clinical cure and parasite clearance are achieved in less than 4 weeks. Given the importance of the spleen for host resistance to *Babesia* parasites, a nonsurgical management of splenic rupture is preferred when the patient is at risk of tick exposure in areas endemic for babesiosis.[82,87]

Infection With Other *Babesia* Species

Symptomatic *B. duncani* infection typically has been treated with intravenous clindamycin plus oral quinine.[37–41] One patient developed a diffuse urticarial rash attributed to quinine, and was cured while receiving intravenous clindamycin monotherapy.[37]

In Europe, symptomatic *B. divergens* infection in asplenic patients is managed with immediate, complete RBC exchange transfusion followed by intravenous clindamycin plus oral quinine.[47] Although used empirically, quinine may provide little to no benefit as demonstrated by its lack of activity in vitro against *B. divergens*[114] and the full recovery by two asplenic patients despite premature interruption of quinine. A splenectomized individual who had a low $CD4^+$ T-cell count due to HIV infection resolved a severe *B. divergens* infection while treated with atovaquone plus azithromycin.[115] A relapse of babesiosis in a spleen-intact individual was treated successfully with atovaquone-proguanil plus azithromycin.[86] In the United States, cases of *B. divergens*–like infections typically have been treated with intravenous clindamycin plus oral quinine or quinidine.[42–45] Quinidine is no longer available in the United States.

Two of the European cases of *B. venatorum* infection were successfully treated with intravenous clindamycin, alone or in combination with oral quinine.[54] A third case was treated with oral clindamycin plus oral quinine but the latter had to be interrupted.[53] Four weeks after clindamycin monotherapy ended, symptoms and parasitemia relapsed. Symptoms quickly resolved following initiation of oral atovaquone plus oral azithromycin, but low-level parasitemia persisted despite 11 weeks of therapy. To avoid relapse, atovaquone monotherapy was prescribed for 6 months until seroconversion and parasite clearance were noted. A pediatric case of mild *B. venatorum* infection in China was successfully treated with oral atovaquone plus oral azithromycin.[61] Symptoms resolved in 3 days, and parasites cleared within 30 days.

PREVENTION

Despite the rise of transfusion-transmitted babesiosis, protection of the blood supply has relied unsatisfactorily on the permanent deferral of blood donors with a history of symptomatic babesiosis.[15,19,21] In March 2018, the FDA approved a nucleic acid test and an arrayed fluorescent immunoassay for the detection of *B. microti* DNA and *B. microti* antibody in human whole blood and human plasma, respectively. These two tests, when combined, hold promise of dramatically reducing the incidence of TTB caused by *B. microti* in endemic areas, and possibly across the United States.[16] In January 2019, the FDA approved the use of another nucleic acid test that is highly sensitive as it detects transcripts of the *B. microti* 18S rRNA gene (rather the gene itself).

Individuals at risk of severe babesiosis (e.g., asplenic and immunocompromised individuals) should avoid areas endemic for *Babesia* spp. from May through September when ticks seek blood.[1] Strategies for tick avoidance and tick removal, and a review of various acaricides are found at the CDC website (http://www.cdc.gov/ticks/avoid/index.html). No vaccine is available to protect humans from any of the zoonotic *Babesia* spp.

Given the increasing confluence of *Ixodes* ticks, *Babesia* spp., and individuals at risk of severe babesiosis in ever-enlarging areas, clinicians should be increasingly alert to the possibility of this previously arcane disease.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to Dr. Tristram C. Dammin for inspiring discussions and unwavering support. They also acknowledge the support of the Dorothy Harrison Egan Foundation (EV) and the Edmund C. Lynch Jr. Research Fund (JAG).

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282 Cryptosporidiosis (*Cryptosporidium* Species)

A. Clinton White, Jr.

SHORT VIEW SUMMARY

Definition
- Cryptosporidiosis is caused by ingestion of oocysts of *Cryptosporidium* species.

Epidemiology
- Cryptosporidiosis has a global distribution with a higher prevalence in resource-poor countries.
- Cryptosporidiosis is a major cause of prolonged diarrhea and malnutrition in children in resource-poor countries.
- Cryptosporidiosis is a major cause of diarrhea in adults infected with human immunodeficiency virus (HIV).
- Cryptosporidiosis is associated with person-to-person and waterborne transmission in wealthy countries.
- The major species, *Cryptosporidium hominis,* primarily infects humans.
- Zoonotic species, including *Cryptosporidium parvum,* are also common in humans.

Microbiology
- *Cryptosporidium* species are apicomplexan protozoan parasites.

Clinical Manifestations
- Most patients present with diarrhea that is frequently prolonged.

Diagnosis
- Diagnosis depends on demonstration of the organism in stool through antigen detection, nucleic acid amplification, or microscopy with acid-fast or fluorescent stains.

Management
- For immunocompromised hosts, reversal of immune defects is critical (e.g., treating underlying HIV with antiretroviral therapy or decreased immunosuppression in transplant recipients).
- Nitazoxanide alone is effective in immunocompetent hosts but not alone in severely immunocompromised patients.

Prevention
- Water treatment and hand hygiene are key measures to prevent infection.

Protozoan parasites of the genus *Cryptosporidium* were first identified in the stomach of mice in 1907.[1] The species name *Cryptosporidium parvum* was proposed in 1912 to describe parasites identified in murine intestines.[2] Although *Cryptosporidium* was linked to gastrointestinal disease in turkeys in 1955 and to bovine diarrhea in 1971, the first human cases were described only in 1976.[3–6] Only a handful of cases had been reported before 1982. In the early 1980s, large numbers of cases were noted to be associated with the emerging epidemic of acquired immunodeficiency syndrome (AIDS).[7] Soon studies identified cases among animal handlers and children.[8] Shortly thereafter, *Cryptosporidium* was associated with waterborne outbreaks of diarrhea, including an outbreak in Milwaukee, Wisconsin in 1993 that affected an estimated 403,000 persons.[9] Studies have now demonstrated that *Cryptosporidium* is an important cause of diarrhea in normal hosts worldwide and one of the main causes of childhood diarrhea in resource-poor countries, including causing prolonged diarrhea and malnutrition, and of chronic diarrhea in immunocompromised hosts, including patients with AIDS. Cryptosporidiosis is now recognized as a major cause of childhood diarrhea morbidity and mortality.[10–12]

THE PARASITES

The genus *Cryptosporidium* consists of a group of protozoan parasites within the phylum Apicomplexa, which also includes *Plasmodium* species. *Cryptosporidium* has been reclassified from the subclass Coccidiasina (coccidia, along with *Toxoplasma, Cyclospora,* and *Cystoisospora*) into the class Gregarinomorphea, subclass Cryptogregaria.[13–15] The genomes of a number of *Cryptosporidium* species have been sequenced, including *Cryptosporidium hominis*, *C. parvum*, an anthroponotic strain of *C. parvum* subtype IIc, and others.[16–23] Compared with other apicomplexan parasites, the genomes are relatively compact (approximately 9.1 Mb), with the loss of approximately 1400 genes, compared with the *Plasmodium* parasites. Many of these gene deletions may be due to loss of the mitochondria and apicoplast, organelles found in most other apicomplexans but not found in *Cryptosporidium* spp. *Cryptosporidium* spp. also lack the genes for variable surface proteins contained in the *Plasmodium falciparum* genome (e.g., *var*, *rif*, and *stevor* genes). The metabolic pathways are also simplified (e.g., no Krebs cycle), but a number of transporters are present to scavenge molecules from the host. Important metabolic pathways do exist, however, such as fatty acyl-coenzyme A synthetase, and their inhibition in a mouse model reduced parasite oocyst production.[24]

Species were initially named based on the host species. In the late 20th century, human isolates were thought to belong to a single species, *C. parvum*. Molecular studies subsequently demonstrated that parasites previously termed *C. parvum* include a number of genotypes and occult species.[15,25] As of 2018, there were at least 35 named *Cryptosporidium* spp. thought to be valid, based on host specificity, morphology, and molecular biology studies, and numerous other genotypes that may emerge as separate species. Among the isolates speciated as *C. parvum,* however, there is also a subtype, IIc, that mainly infects people and shares sequence homology in some regions with *C. hominis* instead of other *C. parvum* isolates.[18,25,26] Molecular biology studies have demonstrated that humans can also be infected with *Cryptosporidium meleagridis, Cryptosporidium cuniculus, Cryptosporidium ubiquitum, Cryptosporidium viatorum, Cryptosporidium canis, Cryptosporidium felis, Cryptosporidium muris,* and others.[27–29] No specific clinical characteristics of rare species have been reported, but volunteer studies have demonstrated mild diarrhea with *C. meleagridis* and *C. muris.*[30,31] *C. meleagridis,* formerly thought to mainly infect birds, has been identified in most large series and appears to cause approximately 1% of human cryptosporidiosis overall and more in some series from Asia.[29,32] Other species are either rarely noted to cause human infection or have been noted to infect only reptiles, fish, birds, or nonhuman mammals.[31]

Cryptosporidium spp. can complete their entire life cycle within a single host, including both asexual (merogony) and sexual (sporogony) reproductive cycles (Fig. 282.1).[10,33] In the stomach and upper intestines, the oocysts are activated, producing serine and cysteine proteases and aminopeptidases, which allow the organisms to excyst, releasing four infective sporozoites.[33,34] Contact with the sialylated carbohydrate surface of the epithelial cells may be an important trigger for excystation.[35]

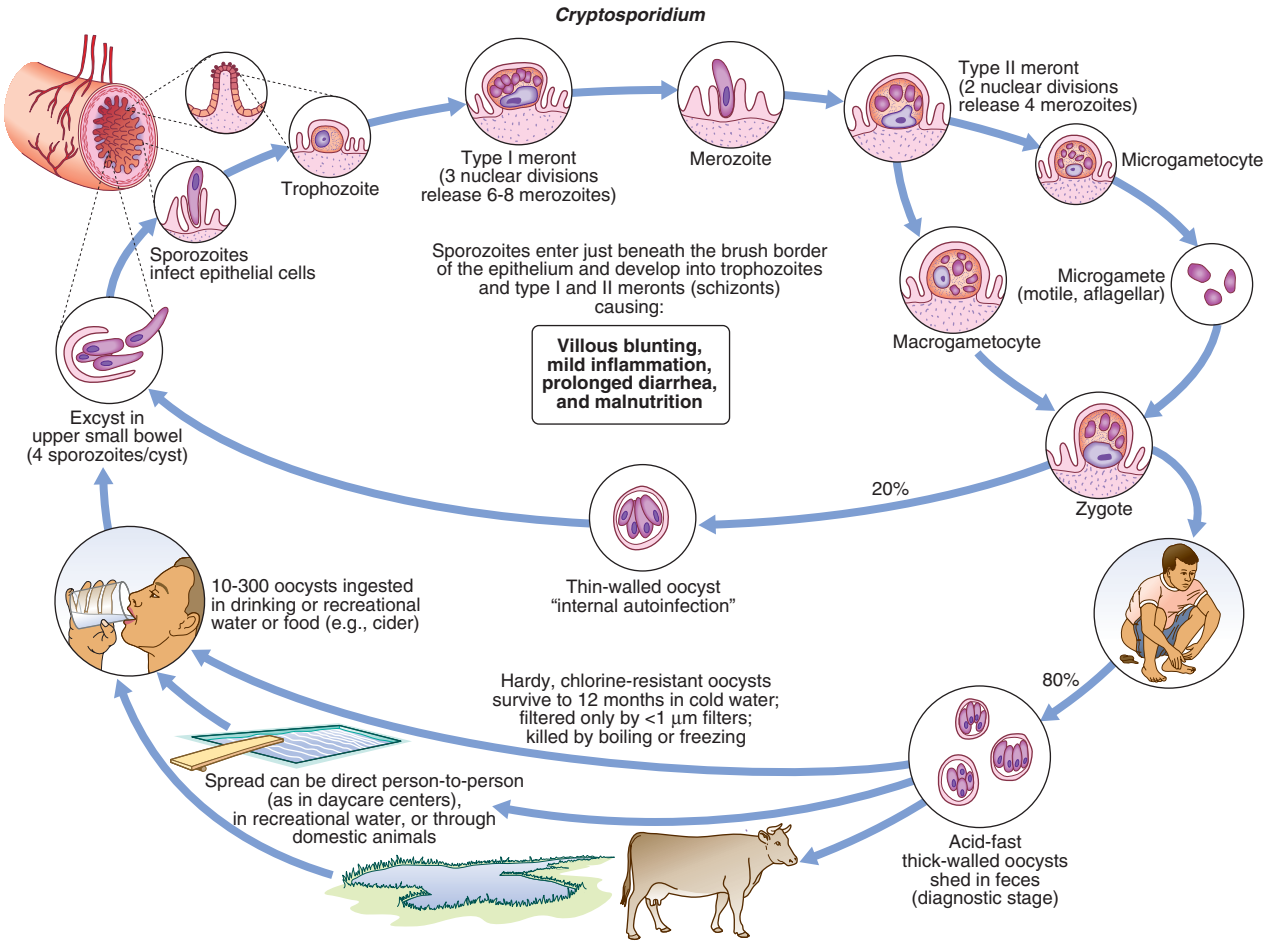

FIG. 282.1 Life cycle of *Cryptosporidium*. Oocysts are excreted in the feces. After ingestion, the sporozoites are released from the oocysts and attach to and invade intestinal epithelial cells. The cells engulf the parasites into a parasitophorous vacuole, where they enlarge to form the trophozoites; undergo asexual multiplication, forming type I meronts; and release the motile merozoites. The merozoites reattach to epithelial cells. They may continue the asexual cycle or may undergo sexual multiplication, producing type II meronts. The type II meronts differentiate into microgamonts and macrogamonts. The microgametes fertilize the macrogametes to form the zygote. The zygotes develop into the oocysts. Two different types of oocysts are produced: the thick-walled oocyst, which is commonly excreted from the host, and the thin-walled oocyst, which is primarily involved in autoinfection. Oocysts are infective at excretion, thus permitting direct and immediate fecal-oral transmission. *(From Lima AM, Samie A, Guerrant RL. Cryptosporidiosis. In: Guerrant RL, Walker DH, Weller PF.* Tropical Infectious Diseases. *3rd ed. Philadelphia: Saunders; 2011.)*

Other factors in excystation may include temperature, stomach acid, bicarbonate pancreatic enzymes, and bile.[36] Each sporozoite contains an apical complex with specialized organelles involved in invasion, including rhoptries, micronemes, and dense granules.[36] The motile sporozoites bind to receptors on the surface of the intestinal epithelial cells. Several parasite ligands (including p30, the galactose-*N*-acetylgalactosamine lectin; p23, the 1300-kDa circumsporozoite-like antigen; gp900, the thrombospondin-related adhesive protein of *Cryptosporidium*-1, Cpgp40/15, and CP47) have been implicated in parasite attachment to the intestinal epithelium.[33,34] The parasites then induce actin polymerization and protrusion of the intestinal epithelial cell membrane, which is mediated by tyrosine kinase growth factor receptor (TKGFR), phosphatidylinositol 3 (PI3) kinase, and CDC42.[37–39] The membrane surrounds the sporozoite and fuses to form the parasitophorous vacuole, which remains in the microvillus layer on the surface of the epithelium.[40] A band of dense cytoskeletal elements separates the parasite from the host cytoplasm.[40] This band prevents free flow of materials between parasite and the host cell cytoplasm.[41] It also contains an adenosine triphosphate–binding cassette, which likely functions as an efflux pump, contributing to the resistance of the organisms to chemotherapy.[42] Inside the parasitophorous vacuole, the parasites undergo asexual reproduction (merogony). They enlarge into trophozoite forms and divide to form type I meronts, which mature and rupture to release the motile merozoites. The merozoites bind to receptors on the epithelial cells and are engulfed by the cells. They then either repeat the process of merogony or undergo sexual differentiation. In that case, the merozoites differentiate into the microgamonts and macrogamonts. The microgamont releases the microgametes, which penetrate the cells infected with a macrogamont. The macrogamont and microgametocytes fuse to form the zygote form, which then undergoes meiosis to form the oocyst, containing four sporozoites. Two morphologic forms of the oocyst have been described. Thin-walled oocysts are thought to excyst within the same host in a process of self-infection. The thick-walled oocysts are shed into the environment.

EPIDEMIOLOGY

Several factors define the epidemiology of cryptosporidiosis.[33,43–46] First, the oocysts are infectious when shed. Thus, parasites are readily transmitted directly from person to person. Second, although *Cryptosporidium* does not multiply outside of the host, the infectious dose is low, facilitating transmission from sources with low-grade contamination, such as recreational water. Third, the oocyst stage can survive for prolonged periods in the environment and resists disinfection, including chlorination.[47,48] Its small size and resistance to chlorination facilitate waterborne transmission. Fourth, the host immune response limits the duration and severity of infection such that disease is more commonly recognized in children (preimmune) or in compromised hosts, especially patients with AIDS. Although some genotypes have important animal reservoirs, molecular studies demonstrate that most human infection is caused by species and subtypes that mainly affect humans. Still, transmission of some species (e.g., *C. parvum*) is associated with animal contact, rural

areas, and exposure to surface water.[44] In contrast to zoonotic species, *C. hominis* is more common in urban settings and is associated with higher population density (especially the number of children).[44] In some studies, household income is not protective against cryptosporidiosis and may pose an increased risk of *C. hominis* infection.[49]

Cryptosporidium parasites have been found in every region of the world except Antarctica. Infection is generally more common in warm or moist months.[50–52] For example, cases in the United States peak in late summer and early autumn.[53] Studies from England found that *C. parvum* peaks in the spring, whereas *C. hominis* peaks in autumn.[44] The peak that occurs in spring is thought to be associated with runoff from pastures, and the autumn peak follows the season for swimming in recreational water. Most studies on the prevalence of infection have relied on detection of oocysts in fecal specimens submitted for parasitologic examination. Fewer than 3800 cases per year were reported in the United States from 1995 through 2002, but the number of reported cases increased to more than 7600 per year after then, without other evidence suggesting more illness.[53,54] This increase coincided with the marketing of nitazoxanide and was thought to be the result of increased awareness and improved diagnosis. Inconsistencies exist in these data, perhaps driven by changes in diagnostic criteria. By contrast, estimates based on antibody prevalence suggest that more than 750,000 persons in the United States are affected each year.[55] The main reason for this difference stems from both insensitivity and underuse of diagnostic tests for *Cryptosporidium*.[56–58] With improving diagnostic techniques, more cases are also being identified in high-income countries. Similarly, studies from England and the Netherlands showed that use of polymerase chain reaction (PCR) assays led to a doubling of the number of *Cryptosporidium* cases identified.[59,60]

The prevalence of cryptosporidiosis in low- and middle-income countries has been characterized in three large multicenter studies of diarrhea in resource-poor countries; these studies used sensitive techniques to study all of the major pathogens.[11,50,61–63] The Global Enteric Multicenter Study (GEMS) was a case-control study of moderate-to-severe diarrhea conducted at seven centers in sub-Saharan Africa and South Asia. *Cryptosporidium* was second to rotavirus as a cause of diarrhea in children younger than 2 and was associated with an increased fatality rate in infants and toddlers.[11] Based on these data and a subsequent study of milder diarrhea, the authors estimated that 7.6 million children in South Asia and sub-Saharan Africa develop diarrhea due to cryptosporidiosis annually and that those infections are associated with over 200,000 deaths.[61] The Malnutrition and Enteric Disease Study prospectively studied birth cohorts from India, Bangladesh, Nepal, Tanzania, South Africa, Brazil, and Peru. *Cryptosporidium* was among the top four causes of diarrheal disease.[50,64] Among 1486 individuals for whom full data through 2 years were available, 962 (65%) developed a *Cryptosporidium* infection. The World Health Organization (WHO) Global Rotavirus Surveillance Network collects stool specimens from children hospitalized with acute watery diarrhea from 178 sentinel surveillance sites in 60 countries. A recent study tested 878 stool samples from children with watery diarrhea in sub-Saharan Africa, India, Myanmar, Philippines, and Brazil for enteric pathogens by PCR card.[62] The fraction of diarrheal episodes attributable to *Cryptosporidium* was 5.8%, with slightly higher rates in children younger than 2. Rates were less than for rotavirus but similar to those of norovirus.[62] A recent report on global burden of diarrheal disease estimated that there are 48,000 child deaths each year from cryptosporidiosis and more than 4.2 million disability-adjusted life-years lost.[65]

Prospective studies of birth cohorts in South Asia have noted that virtually all children are infected by age 2.[66,67] Summary data from published and unpublished sources in India suggest that that country may see 3.9 to 7.1 million diarrheal episodes, 66,400 to 249,000 hospitalizations, and 5800 to 14,600 deaths each year in children younger than 2 years.[68] Older studies from sub-Saharan Africa have identified *Cryptosporidium* in 7.5% to 22.2% of cases with use of microscopy.[69,70] However, studies using PCR or antigen-detection methods have documented *Cryptosporidium* in up to 30% of acute diarrhea cases.[70–73] Studies using PCR assays on stool samples from AIDS patients with diarrhea have identified *Cryptosporidium* DNA in 18% to 77% of cases, significantly higher than with staining alone.[74–80] Thus, the prevalence is likely higher than suggested by earlier stool studies.

In a systematic review of cryptosporidiosis in low- and middle-income countries, overcrowding, diarrhea in the household, and animal contact were the major risk factors for infection.[81] Breastfeeding was protective. Surprisingly, there was no increased risk associated with water source.

A series of human challenge experiments was performed to determine the infectious dose of *Cryptosporidium* spp. The initial studies were performed in seronegative volunteers with different strains of *C. parvum* parasites (maintained in calves). The studies discovered a low infectious dose but considerable variability among isolates, with the dose infecting half of subjects ranging from approximately 1000 oocysts (*C. parvum* UCP strain), to approximately 100 oocysts (*C. parvum* Iowa strain), to approximately 10 oocysts (*C. parvum* TAMU strain, *C. hominis* TU502 strain).[82–84] Based on these data, even a single oocyst should result in infection in a portion of those exposed.[85] When the volunteers were rechallenged, they had a higher infectious dose.[86] They also developed less severe manifestations in that they were less likely to shed organisms but frequently developed symptomatic illness, with oocyst shedding detectable only with flow cytometry. When volunteers who were seropositive were challenged, the infectious dose was 20- to 50-fold higher.[87] Because the infectious dose is low, transmission can readily occur from exposure to low doses, such as might be found in waterborne outbreaks or in person-to-person spread.

Oocysts of *Cryptosporidium* are relatively resistant to environmental conditions. Oocysts can remain infectious for at least 6 months if kept moist, but viability decreases rapidly with desiccation.[48,88,89] Oocyst viability does not decrease with storage at temperatures between 0°C and 20°C. Viability decreases over a few hours with freezing (−20°C or lower). The oocysts can also be killed by heat, including pasteurization and microwave heating.[90] Oocysts are highly resistant to chlorination. For example, 80 ppm chlorine inactivated only 90% of oocysts after 90 minutes of incubation,[91] and the concentrations in tap water (e.g., 5 ppm) had no effect.[92] The sensitivity of oocysts to chlorine is further decreased in the presence of fecal contamination.[93] Even incubation of oocysts for up to 2 hours in household bleach failed to decrease infectivity.[94] By contrast, oocysts are sensitive to hydrogen peroxide, ozone, and ultraviolet radiation. Sunlight decreased oocyst viability up to 90%, but the effects vary with water turbidity, radiation intensity, the presence of biofilms, and time.[95–98]

Surveys have demonstrated that many sources of drinking water were contaminated with oocysts before treatment.[43,99–104] Oocysts are more frequent and at higher densities in water contaminated with agricultural runoff, sewage, urban runoff, or recreational use. However, up to 39% of apparently pristine sources were contaminated. Organisms found in the water included both *C. hominis* and *C. parvum* genotypes, in addition to animal species.[102,103] Even groundwater can be contaminated. Low-grade contamination has also been documented in samples of treated water, but this has been decreasing with improvements in water standards.[105]

Numerous outbreaks of cryptosporidiosis have been linked to contaminated drinking water.[43,44,104,106,107] The largest documented waterborne outbreak of diarrhea occurred in Milwaukee in 1993.[9] One of two city water treatment plants was contaminated. More than 600 cases of *Cryptosporidium* infection were confirmed by parasitologic examination. Based on telephone surveys, diarrhea episodes were more widespread, with an estimated 403,000 people developing a diarrheal illness. Of interest, water quality never failed to meet the standards current at the time for turbidity and fecal coliform counts. Many of the waterborne outbreaks, including the outbreak in Milwaukee, have been caused by *C. hominis*.[44,107,108] Thus, the outbreaks are thought to result from fecal contamination of drinking water. Other outbreaks are associated with *C. parvum*.[104] Most outbreaks of *C. parvum* can be tied to contamination of the watershed with agricultural runoff.[44,104] Over the past decades, there has been a marked reduction in the number of outbreaks and cases associated with drinking water in the United States, but *Cryptosporidium* remains one of the more common causes of waterborne outbreaks of disease.[109] Similarly, there has been a marked decrease in cryptosporidiosis in England and Wales associated with improved water standards.[110]

Outbreaks of cryptosporidiosis associated with contaminated recreational water are common.[43,45,106] The number of outbreaks increased throughout the 1990s.[53,54,111] In 2007 approximately 5700 people in Utah developed cryptosporidiosis associated with contamination of 450 swimming pools with an epidemic strain of *C. hominis*.[112] *Cryptosporidium* is now the most common organism associated with waterborne-disease outbreaks associated with recreational water in the United States.[54] In fact, small outbreaks appear to be an important source of endemic cryptosporidiosis.[45] Swimming is an important risk factor for cryptosporidiosis, and public swimming pools are frequently contaminated with *Cryptosporidium*.[43,44,113–116] Outbreaks have also involved lakes, rivers, beaches, and fountains.[43,117,118] The concentration of chlorine in pool water and limited filtration are often insufficient to disinfect the water. Not surprisingly, most outbreaks associated with fecal accidents are attributable to *C. hominis*.[54]

Foodborne infection occurs less frequently.[119] Well-documented outbreaks have been tied to contaminated apple cider, unpasteurized milk (cow and goat), salads, raw produce, and shellfish.[119–123] In resource-poor countries, oocysts are commonly found on vegetables.[124] Oocysts have been frequently identified in shellfish and in flies, but their role in transmission to humans is not clear.[125,126]

Oocysts of *Cryptosporidium* are immediately infectious when shed. Thus, *Cryptosporidium* is associated with direct person-to-person spread. Person-to-person transmission was initially recognized in outbreaks associated with contact with daycare centers.[127,128] There are also documented cases of nosocomial transmission.[129–132] The risk of transmission from adult patients is small with standard precautions.[133] However, contact with a person ill with diarrhea or with children in diapers remains a major risk factor for cryptosporidiosis.[44,117]

Secondary transmission within households is also common.[44,134,135] For example, in a study of household contacts of children with cryptosporidiosis in Brazil, Newman and coworkers noted secondary cases in 18 of 31 households (58%) and involving 19% of household members.[134] In daycare-associated outbreaks, secondary transmission is common.[127,128] By contrast, few cases in adults or school-aged children were associated with secondary transmission.[135,136]

Cryptosporidiosis in high-income countries is also associated with travel to resource-poor countries.[44,117,137,138] This was first recognized in Finnish travelers to Russia and was thought to reflect contamination of drinking water.[139] *Cryptosporidium* was thought to rarely cause traveler's diarrhea. However, in studies using molecular detection methods, the actual rates were up to 6% of cases.[140–142] Most travel-associated cases are caused by *C. hominis*.[44,138] However, a number of cases of *C. viatorum* have been reported in travelers returning to England from India.[143]

Sexual transmission has been postulated to occur. Among human immunodeficiency virus (HIV) patients, men who have sex with men are more likely to develop cryptosporidiosis.[144–146] Transmission is associated with anal-genital sex and the number of sex partners.[144] *Cryptosporidium* transmission among men who have sex with men shares risk factors and may occur concurrently with other fecal-oral pathogens, such as *Shigella*.[147]

C. parvum was thought to infect primarily domestic animals, with zoonotic transmission to humans. However, molecular studies have demonstrated that many of the human *C. parvum* infections are caused by subtype IIc, which typically only infects humans.[18,26,148] Animal contact is also associated with acquisition of *C parvum* in sporadic cryptosporidiosis and occasional outbreaks.[33,44,117,149–151] Cryptosporidiosis is common among veterinary students.[152–154] In addition to cattle, sheep, goats, pigs, and pets have also been implicated in zoonotic infection.[150,155,156]

The host immune response plays a key role in susceptibility. In studies from resource-poor countries where there is heavy exposure, most cases of cryptosporidiosis develop in young children, presumably because of rapid development of immunity.[134,157] Human challenge studies document resistance to infection associated with previous challenge or prechallenge immunity, as documented by anti-*Cryptosporidium* antibodies.[86,87] There is also strong evidence of an increased frequency of infection in patients with altered cellular immunity.[158] Among AIDS patients with diarrhea, *Cryptosporidium* was found in up to three-fourths of patients with chronic diarrhea from resource-poor countries.[77,159] In a waterborne outbreak affecting a drug treatment center, only 190 of 1392 (13.6%) HIV-negative patients developed cryptosporidiosis, compared with 104 of 339 (30.7%) infected with HIV.[160] Among those with HIV, the infection rate varied with the CD4 cell count, ranging from 23% of those with CD4 cell counts greater than 1000 cells/μL to 46% of those with CD4 cell counts less than 100 cells/μL. However, infection was not more frequent in HIV patients during the Milwaukee outbreak.[161] Cryptosporidiosis has also been noted in other immunodeficient hosts, including patients with primary immunodeficiencies, organ transplants, cancer, and diabetes.



FIG. 282.2 Intestinal biopsy specimen showing *Cryptosporidium* intracellular forms (e.g., trophozoites and merozoites) (arrows) inside the surface of the intestinal epithelial cells. *(From Petri WA. Therapy of intestinal protozoa.* Trends Parasitol. *2003;19:523–526.)*

PATHOLOGY AND PATHOGENESIS

Cryptosporidium organisms are found within parasitophorous vacuoles in the microvillus layer of the epithelial cells (Fig. 282.2). In immunocompetent individuals, the organisms are localized primarily to the distal small intestines (e.g., terminal ileum) and proximal colon. However, studies from Uganda documented frequent infection of the respiratory tract in apparently immunocompetent children and adults.[162–164] In immunodeficient hosts, the parasites have been identified throughout the gut, in the biliary tract, and even in the respiratory tract.[165,166] Children with persistent cryptosporidiosis may have villous atrophy and a mild increase in lamina propria lymphocytes.[167] The distribution of parasites is limited and often spares the proximal small intestines. Heavier infection is associated with villous atrophy, crypt hyperplasia, and marked infiltration with lymphocytes, plasma cells, and even neutrophils,[168–172] and is also associated with extraintestinal involvement.

Villous atrophy and crypt hyperplasia are thought to reflect epithelial cell turnover.[171,173,174] Although infection of epithelial cells stimulates antiapoptotic mechanisms in infected cells, there is increased apoptosis in adjacent cells that is likely mediated by the interaction of Fas and Fas ligand.[175–178] Increased epithelial cell apoptosis has been demonstrated in biopsy specimens from infected intestines.[169] In vitro infection models show that infection initially stimulates antiapoptotic signals, but after 24 hours of infection, proapoptotic molecules dominate.[179,180] Furthermore, as the organisms complete their cycle, they cause necrotic death of the infected cells.[181,182] The resultant loss of villous surface was, in turn, associated with decreased expression of glucose-stimulated sodium pumps.[174] Similarly, loss of villous surface area has been demonstrated in human infection as D-xylose malabsorption.[171,183] Studies of AIDS patients with severe cryptosporidiosis have also demonstrated malabsorption of bile acids, vitamin B_{12}, and fatty acids.[171,184,185] Metabolic studies of AIDS patients with chronic cryptosporidiosis demonstrate fat wasting with a decreased metabolic rate, consistent with decreased absorption.[186]

Cryptosporidiosis is characterized clinically by watery diarrhea and malabsorption. The physiologic processes that are thought to account for these symptoms include sodium malabsorption, electrogenic chloride secretion, and increased intestinal permeability. Increased permeability may result in decreased absorption of fluids and electrolytes and solute fluxes into the gut. Studies of AIDS patients with cryptosporidiosis have

demonstrated a direct correlation between the severity of disease and altered intestinal permeability, as measured by ratios of excretion in the urine of lactulose and mannitol.[171,183,187] Similar defects have been noted in children with cryptosporidiosis.[188,189] *Cryptosporidium*-induced defects in intestinal epithelial cell barrier function can be reversed by antiinflammatory cytokines, such as transforming growth factor-β.[190]

The voluminous, watery diarrhea resembles that of toxin-mediated illnesses, but no secretory activity was detected in formal studies. Prostaglandins, induced by tumor necrosis factor-α (TNF-α), mediate decreased sodium absorption and cause diarrhea in porcine and bovine cryptosporidiosis.[191–193] However, studies in volunteers and AIDS patients with chronic cryptosporidiosis did not demonstrate any correlation between expression or level of proinflammatory cytokines and symptoms.[194–197] Furthermore, prostaglandin inhibitors have not proven to be effective symptomatic therapy in human cryptosporidiosis.

Robinson and colleagues demonstrated a correlation between expression of the neuropeptide substance P and the presence and severity of diarrhea in volunteers challenged with *C. parvum* and AIDS patients with chronic cryptosporidiosis.[198] In monkey models, *Cryptosporidium* infection is associated with increased expression of substance P and its receptor, and substance P inhibitors blocked *Cryptosporidium*-induced intestinal permeability, glucose malabsorption, and chloride secretion.[199,200] Similarly, mice were protected against *C. parvum*–induced intestinal inflammation and illness by a substance P receptor antagonist.[201,202]

Infection of intestinal epithelial cells leads to activation of nuclear factor kappa B (NF-κB).[175,176] Upstream signals may include Toll-like receptor 2 (TLR2) and TLR4 signaling via MyD88 (myeloid differentiation primary response 88).[203–205] NF-κB then activates several hundred target genes, including genes for antiapoptotic molecules such as osteoprotegerin.[34,206,207] These antiapoptotic molecules allow the parasite to form and release merozoites before cell death.[176,179] However, NF-κB activation also leads to upregulation of a proinflammatory cascade, including expression of chemokines, chemokine receptors, and cytokines. Many of these effects are mediated by upregulation or downregulation of micro-RNAs, including let-7, miR-27b, and miR-98.[204,208–210] These mediate secretion of exosomes, containing defensins (LL-37 and β-defensin 2), which may limit infection. The parasite also secretes noncoding RNAs into the host cytoplasm, which are transported to the nucleus by host HSP70. This in turn leads to activation of host histone methylation, increased expression of proinflammatory molecules (e.g., interleukin [IL]-8, IL-6, and CXCL2), and decreased expression of other host molecules, including the alarmin IL-33.[211,212] Both biopsy specimens and stool studies from human infection also demonstrate increased expression of proinflammatory cytokines and markers of inflammation, including TNF-α, IL-1β, IL-8, and lactoferrin.[194,213–216] Chemokines, including IL-8 and CXCL10, are produced by the infected epithelial cells.[216–219] Among the chemokines, CCL20 actually is microbicidal to extracellular forms of *Cryptosporidium*.[220] It is downregulated by the parasite via upregulation of MiR21. Studies of infected human tissues noted that *Cryptosporidium* infection leads to upregulation of the TNF receptor family decoy receptor osteoprotegerin.[207] It was also detected in intestinal tissues after experimental infection. Its ligand, TRAIL (TNF-related apoptosis-inducing ligand), was able to eliminate infected cells in vitro, but this effect was reversed by osteoprotegerin, suggesting that TRAIL may be a key mediator of parasite clearance.[207]

HOST RESPONSE AND IMMUNITY

Both innate and adaptive immune responses are critical for control of cryptosporidiosis.[10,216,221–223] $CD4^+$ T cells play a key role in the adaptive immune response to cryptosporidiosis. In patients with HIV infection, cryptosporidiosis is usually self-limited in individuals with CD4 cell counts higher than 150/μL, is often chronic in patients with CD4 cell depletion to less than 100/μL, and can be fulminant in some of those with counts less than 50/μL.[80,160,224–226] Similarly, infection is chronic in mice without functional CD4 cells (e.g., nude mice, SCID mice, or RAG knockout mice).[221,223] These defects can be reversed by infusion of $CD4^+$ cells, particularly $CD4^+$ intraepithelial lymphocytes. Kirkpatrick and colleagues[227] noted an association between DQ alleles, which present antigen to $CD4^+$ T cells, and susceptibility to infection. Furthermore, resolution of cryptosporidiosis among AIDS patients in response to effective antiretroviral therapy (ART) is associated with an influx of CD4 cells into the intestines.[228] The role of other cell populations has been less clear. Although major histocompatibility complex (MHC) class I deficiency and CD8 depletion have little effect on murine cryptosporidiosis, there are associations of MHC class I types and human infection.[227] CD8 cells produce interferon-γ (IFN-γ) in response to *Cryptosporidium* antigens, and sensitized human CD8 T cells can clear parasites from infected cells in vitro.[229,230]

The role of antibody in the immune response to cryptosporidiosis is controversial.[231] Early studies noted cases of chronic cryptosporidiosis in patients with low antibody levels, but these studies did not exclude coexisting T-cell dysfunction. In animal models, inactivation of B cells by inactivation of the *muMT* gene did not affect clearance of cryptosporidiosis.[232] Treatment with high concentrations of anti-*Cryptosporidium* antibody did facilitate clearance in mice.[233] Anecdotes suggested that hyperimmune bovine colostrums might improve cryptosporidiosis in AIDS, but a large randomized, controlled trial demonstrated no clinical benefit and decreases in oocyst shedding only at very high doses.[234] High levels of serum and fecal antibodies to *C. parvum* have been found in AIDS patients with chronic cryptosporidiosis.[235,236] Studies of the fecal antibody response in volunteers challenged with *C. parvum* demonstrated specific fecal antibody in most volunteers.[237] However, the presence and timing of antibody correlated with oocyst shedding rather than clearance or resistance to infection. Similarly, cytokines such as TGF-β that stimulate immunoglobulin A (IgA) production often develop only after resolution of illness.[238] Studies have noted higher antibody responses to specific antigens in patients with acute versus persistent diarrhea, but this may have been a marker for prior exposure.[80,239,240] Also, specific antibody in breast milk was associated with decreased risk of infection in infants.[241] Thus the importance of antibody in cryptosporidiosis remains unclear.

Production of IFN-γ is a key mediator of the adaptive immune response to *Cryptosporidium*. In murine models, IFN-γ knockout mice develop chronic infection.[242,243] Furthermore, inactivation or depletion of IFN-γ causes further exacerbation of infection even beyond that noted with CD4 depletion.[244] Lymphocytes from persons who have recovered from cryptosporidiosis produced IFN-γ after antigen stimulation in vitro, including HIV patients.[230,245,246] Approximately half of volunteers challenged with *C. parvum* express IFN-γ in the intestinal mucosa.[247] Treatment with IFN-γ can directly activate intestinal epithelial cell lines to prevent *C. parvum* infection, but this effect was not confirmed with primary cells.[248] Similarly, inactivation of IL-12, the major factor stimulating production of IFN-γ, causes chronic infection.[249]

Surprisingly, IFN-γ expression in normal volunteers was limited to the subset with evidence of previous exposure (either seropositive before challenge or demonstrating resistance to infection).[247] Similarly, IFN-γ production by cells from HIV patients during active cryptosporidiosis and from Haitian children with active cryptosporidiosis was very low despite the fact that they had self-limited disease.[214,246] Thus, other factors appear to be involved in limiting human infection after initial exposure. In murine models, inactivation of IFN-γ expression resulted in only a mild chronic infection in BALB/c mice but fatal infection in C57BL/6 mice. Mild disease in BALB/c mice was associated with expression of IL-12, IL-4, and TNF-α.[243,250] In the absence of IFN-γ, IL-12 treatment only worsened cryptosporidiosis.[251] Similarly, IL-12 treatment of AIDS patients with chronic cryptosporidiosis was associated with gastrointestinal side effects.[252] IL-4 synergizes with IFN-γ in preventing infection of epithelial cells, and IL-4 knockout mice displayed prolonged oocyst shedding.[251,253,254] IL-4 treatment did not modulate infection in IFN-γ knockout mice and IL-4 expression did not correlate with protection in human volunteers.[251,255] By contrast, TNF-α limited infection in IFN-γ knockout mice, activated human epithelial cells to limit infection, but did not correlate with resolution in human infection.[194,248,256]

Studies have increasingly focused on the innate immune response to cryptosporidiosis.[223] Mononuclear phagocytes likely play a key role in control of cryptosporidiosis. Takeuchi and colleagues demonstrated that mice can be rescued from a fatal infection by type I macrophages.[257] Macrophages can be a source of IL-15 and IL-18, which have been implicated in control of infection. IL-18 stimulates IFN-γ production, natural killer (NK) cell activation, and secretion of defensins.[258–260]

Depletion of dendritic cells ($CD11c^+$) increased susceptibility to *C. parvum* infection in a mouse model, and dendritic cells stimulated with sporozoites and cocultured with $CD4^+$ and $CD8^+$ lymphocytes produce higher levels of IFN-γ, and the subsequent control of infection is sustained.[261] Dendritic cells were also important in a neonatal mouse system in which they limited infection in part via stimulation of Th1 responses.[261–263] NK cells have a role in parasite clearance in some animal models, and human NK cells can clear infection of cell lines in vitro.[223,264–266] In seronegative normal volunteers experimentally infected and AIDS patients recovering from cryptosporidiosis in response to ART, control of infection was associated with expression of IL-15.[197,255] This effect is likely mediated by activation of NK cells.[264] Mannose-binding lectin levels have been shown to be low in AIDS patients and children with cryptosporidiosis, and mice with the gene inactivated are more susceptible to infection.[267–271] Antimicrobial peptides seem to be a key host defense mechanism against the luminal forms of the parasite. β-Defensins and CCL20 are induced in response to *Cryptosporidium* infection and can kill the parasites in vitro.[220,259,260,272–274] More frequent or severe cryptosporidiosis has been noted in a number of primary immunodeficiencies.[158,275,276] Some cases have primary T-cell defects. However, recent descriptions primarily involve innate responses. For example, hyper-IgM syndrome, caused by a defect in CD40 ligand (also termed CD154), is associated with increased frequency and severity of *Cryptosporidium* infection.[276–279] This syndrome is associated with profound defects in the ability of antigen-presenting cells to produce IL-12 and TNF-α and to stimulate production of IFN-γ.[280] In murine models, recovery required expression of CD40 on donor spleen cells but not recipient epithelial cells.[281] Similarly, treatment of patients with hyper-IgM syndrome with CD40 agonist antibody can lead to resolution of severe forms of cryptosporidiosis in patients with CD40L defects.[282] The prevalence of cryptosporidiosis ranges from 6% to over half of cases. Furthermore, patients often developed biliary disease, primarily sclerosing cholangitis, which is usually due to *Cryptosporidium.* Studies using PCR assays have demonstrated that most biliary tract infections were caused by *Cryptosporidium.*[275] An association with sclerosing cholangitis and cryptosporidial infection has also been reported with other primary immunodeficiencies affecting T cells, including dedicator of cytokinesis 8 (DOCK 8) deficiency, and NIK (nuclear factor kappa B–inducing kinase) loss-of-function mutations.[283,284]

CLINICAL MANIFESTATIONS

Symptoms of cryptosporidiosis develop after a prepatent period, during which the parasites invade the intestinal epithelium and proliferate. Studies of immunocompetent individuals with discrete exposures (e.g., travelers, point-source outbreaks, or experimental infection) demonstrate a prepatent period of approximately 1 week.[9,83,84] There is, however, considerable variability, with a range of 1 to 30 days. This variability reflects, in part, strain differences among the organisms rather than dose.[83,285] Cryptosporidiosis is noted in both males and females. The age distribution varies considerably with the epidemiology of exposure. In low- and middle-income countries, most cases occur among children younger than 2 years.[10,11] This is thought to reflect both high rates of fecal-oral exposure in children and the development of immunity in older children and adults. In higher income countries, the prevalence is higher in children younger than 5 years, but the condition occurs in all age groups.[53] Waterborne epidemics in high-income countries affect people of all ages.[9,43,107,286] Because *Cryptosporidium* infects primarily intestinal epithelial cells, it is not surprising that diarrhea is the most common clinical presentation.[287] However, there are significant differences in the clinical presentation, depending on the host and parasite population. The major groups include immunocompetent individuals in high-income countries, children in low- and middle-income countries, and immunocompromised hosts (e.g., patients with AIDS or after organ transplantation).

Immunocompetent Individuals in High-Income Countries

Most case series of immunocompetent individuals from high-income countries have been associated with waterborne outbreaks, infection in travelers, animal contact, or infections of children in daycare and their contacts.[114,117,138,288,289] Immunocompetent adults most commonly present with diarrhea.[9,107,287] The diarrhea is usually described as watery but may also be described as mucoid. The median duration of illness in most case series is approximately 5 to 14 days but may range from 1 to 100 days. Data from Sweden noted a duration of less than 4 days from a survey after a waterborne outbreak, but more than 10 days for cases identified from laboratory records.[107,138] Accompanying symptoms are similar to those noted with other diarrheal illnesses, including abdominal cramps, nausea, vomiting, and fever. The frequency of abdominal pain varies among reports. In some studies, cryptosporidiosis was more frequently associated with respiratory symptoms.[163] Nearly half of cases develop recurrent symptoms after initial resolution.[107,135,290,291] Relapses may follow a diarrhea-free period of several days to weeks. The presence of chronic gastrointestinal symptoms similar to irritable bowel syndrome is not uncommon, including diarrhea, abdominal pain, and weight loss.[291–294] Other chronic sequelae include arthralgias, fatigue, and eye pain. Clinical illness can be severe among the elderly.[114,295,296]

Milder or even asymptomatic infection may also be very common. For example, seroconversion is more common than is clinically diagnosed disease.[297] Similarly, in waterborne outbreaks, only a minority of affected individuals presented for clinical care.[107,298] Patients identified by active case-finding or children with negative stool studies were less severely ill and had a shorter duration of diarrhea than laboratory-confirmed cases.

Childhood Diarrhea in Low- and Middle-Income Countries

Childhood diarrhea is the most common clinical manifestation of cryptosporidiosis in resource-poor countries. Investigators in Asia, Africa, and Latin America have carefully studied cryptosporidial diarrheal illness among children.[a]

These studies have demonstrated that cryptosporidiosis is common, with most children infected by age 2 or 3.[66,67] In a prospective, multicenter birth cohort study from Asia, sub-Saharan Africa, and Latin America, 65% of infants were infected by age 2.[64] In birth cohort studies that used PCR to detect organisms, most infections were subclinical.[66,67] The main clinical presentation is with an acute diarrheal syndrome similar to that seen with other enteric pathogens—with watery diarrhea, cramps, nausea, and vomiting. Signs of dehydration are common. Less common features may include abdominal pain, fever, shortness of breath, cough, and foul stools. Although most cases resolve within a few days, many affected individuals go on to develop prolonged, recurrent, or persistent diarrhea.[50,71,299,301–304] Thus, *Cryptosporidium* is among the more common causes of persistent diarrhea in resource-poor countries, causing approximately one-third of cases. It can also cause chronic diarrhea and malabsorption.[305,306] Furthermore, an episode of prolonged or persistent diarrhea, especially if caused by *Cryptosporidium,* is a marker for the onset of increased risk of recurrent episodes of diarrhea, weight loss, and premature death.[11,301,303,304,307] A long-term follow-up study of children with onset of cryptosporidiosis before age 1 year suggested an association with poorer physical fitness and poorer cognitive development that persists for years.[308,309] Molecular studies have demonstrated clinical differences between *Cryptosporidium* species and subtypes. In some studies, *C. hominis* is associated with more severe disease (more dehydration, longer duration, more oocysts shed) and *C. meleagridis* with milder disease.[26,32,310,311]

Cryptosporidium and Malnutrition

Studies of childhood diarrhea in resource-poor countries have demonstrated an association between cryptosporidiosis and malnutrition. Cryptosporidiosis is more severe in children with malnutrition.[10] For example, most deaths occur in malnourished children.[71,312,313] Early studies did not clearly distinguish the effects of cryptosporidiosis on nutritional status from the effects of malnutrition on cryptosporidiosis. Studies prospectively examining both nutritional status and *Cryptosporidium* infection in cohorts of children demonstrated significant differences in nutritional status before *Cryptosporidium* infection.[64,301] Onset of cryptosporidiosis was associated with growth faltering, with a decrease of 300 to 400 g.[64,67,301,314,315] Older children or children in sites with better

[a]References 26, 51, **61**, **64**, **66**, **67**, 299, 300.

nutrition eventually recovered and experienced catch-up growth. In contrast, children infected before 1 year of age or those in South Asia often never recovered. Furthermore, even subclinical infection (i.e., no diarrhea) was associated with growth faltering. Animal models have confirmed the bidirectional interaction between malnutrition and cryptosporidiosis.[316] The burden of disease from the nutritional impact of cryptosporidiosis is thought to be greater than that of diarrhea.[65] Thus, *Cryptosporidium* infection is a cause of acute malnutrition, and the long-term consequences of this interaction are likely to be worse in those infected in infancy or with previous malnutrition.

Cryptosporidiosis in HIV Infection

HIV infection has been the most common host defense defect associated with cryptosporidiosis. Before the advent of effective antiretroviral combinations, most patients diagnosed with cryptosporidiosis had underlying HIV infection.[145] However, the incidence of cryptosporidiosis in HIV has dramatically decreased with improvements in combination antiretroviral therapy (cART).[317,318] However, *Cryptosporidium* remains the most prevalent enteric pathogen among those with poor access or adherence to ART.[76–78,80,319,320] For example, in Kenya, more than a third of HIV patients with or without diarrhea shed *Cryptosporidium* oocysts.[80] The clinical manifestations of cryptosporidiosis in HIV patients are variable. Among patients with CD4 cell counts greater than 150 cells/μL, most cases of cryptosporidiosis are self-limited, similar to those in normal hosts.[169,321–324] However, even these cases are more likely to relapse if the cellular immune response deteriorates. Surprisingly, in population-based studies, a substantial portion of *Cryptosporidium* infections in HIV patients are asymptomatic, and some cases are mild and self-limited even in patients with advanced HIV infection.[320,325,326] Other patients develop a chronic diarrheal illness. The chronic diarrhea is associated with frequent, foul-smelling, bulky stools. Most patients experience weight loss. Not surprisingly, studies have demonstrated nutrient malabsorption. Voluminous watery diarrhea or cholera-like illness develops in a minority of patients. The clinical picture is often confused by other concomitant opportunistic infections, including microsporidiosis, disseminated *Mycobacterium* infection, or cytomegaloviral colitis.[145,169] There may be slight differences in the clinical manifestations depending on the parasite species, with milder disease caused by *C. meleagridis* and some *C. hominis* subtypes.[325]

Cryptosporidiosis in AIDS is also associated with extraintestinal disease, including involvement of the biliary and respiratory tract.[145,327–331] Respiratory tract involvement is often asymptomatic but may also manifest as pulmonary infiltrates with dyspnea.[163,164,327,328] Biliary tract involvement in cryptosporidiosis has been limited to patients with profound immunodeficiency. Biliary involvement correlated with a low CD4 cell count and a markedly shortened survival.[145,329] Patients may present with acalculous cholecystitis, sclerosing cholangitis, papillary stenosis, or pancreatitis.[145,329,330,332] Most patients present with right upper quadrant abdominal pain, which may be intermittent and colicky. Laboratory studies characteristically reveal elevated levels of alkaline phosphatase. Levels of bilirubin and transaminases are often elevated. In patients with associated pancreatitis, amylase and lipase are increased. Ultrasound examination may reveal dilatation of the biliary duct and/or signs of gallbladder inflammation. Magnetic resonance cholangiopancreatography can usually confirm the diagnosis.[331] However, in many patients an endoscopic retrograde cholangiopancreatographic evaluation is required in order to make the anatomic diagnosis. Biopsy specimens of the biliary ducts, staining of the bile, or stool studies may demonstrate the parasites. Many cases of biliary disease will reveal evidence of coinfection with cytomegalovirus or microsporidia.

Transplant Patients

Cryptosporidiosis is being increasingly recognized in organ transplant patients.[333–337] Cases typically manifest a few years after initial transplantation with persistent diarrhea, usually lasting about 10 days before diagnosis. Cryptosporidiosis appears to be more frequent in patients on tacrolimus than on cyclosporine, and the former typically have elevated tacrolimus levels.[333,335] Renal transplant recipients often demonstrate evidence of acute kidney injury, thought to be due to a combination of volume depletion and tacrolimus toxicity. Cryptosporidiosis is particularly problematic among transplant patients in areas highly endemic for cryptosporidiosis.[335,338] For example, a study in Pakistan demonstrated *Cryptosporidium* oocysts with acid-fast staining in 343 of 644 (53%) renal transplant patients with diarrhea.[338] Extraintestinal disease, including biliary and pulmonary disease, is noted in about 10% of transplant patients with cryptosporidiosis.[337]

DIAGNOSIS

Parasites were first demonstrated by means of histologic staining of intestinal tissues. The organisms are found along the surface of the epithelial cells and may appear to be in the lumen (see Fig. 282.2). The intracellular forms stain purple with hematoxylin. Tissues are available only after invasive procedures, and the organisms are not consistently identified in biopsy specimens. *Cryptosporidium* infection was traditionally diagnosed through microscopic examination of stool. In general, stools are preserved in 10% buffered formalin.[27,339,340] Fresh stools can also be tested but are infectious to laboratory personnel. Polyvinyl alcohol interferes with staining techniques and is not recommended. Frozen stools can be used for some immunoassays and nucleic acid tests. Potassium dichromate (2.5%) can also be used to preserve organisms without decreasing oocyst viability.

A number of concentration methods have been attempted. The formalin–ethyl acetate method is commonly used in clinical laboratories. However, oocysts may fail to sediment if centrifugation speed or time is not increased.[27,339,340] Immunomagnetic beads can be used to isolate and concentrate organisms and improve sensitivity of stool examination.[341]

On wet mounts oocysts are small, 4 to 6 μm in diameter, and similar in size and shape to yeast forms normally found in stool. They do not stain well with iodine or trichrome and cannot be differentiated from yeast forms with Giemsa staining. Thus, traditional approaches to stool examinations usually cause the organism to be missed. Many laboratories still do not routinely test all stools for *Cryptosporidium*.[27,57,58]

Differential staining was first noted with acid-fast stains. Oocysts stain pink or red, whereas yeast cells and fecal debris stain green or blue (Fig. 282.3). The most commonly used stain is a modification of the Ziehl-Neelsen stain.[27,339,340] The sensitivity of stool examination with acid-fast staining remains poor, with fewer cases detected than with antigen-detection, fluorescent, or molecular methods.[27,340,342,343] Fluorescent stains (e.g., auramine-rhodamine) can be read more quickly than other acid-fast stains and may have improved sensitivity (see Fig. 282.3). However, these assays are plagued by false-positive results. All of the acid-fast stains detect other parasites that may cause similar illnesses (e.g., *Cystoisospora* and *Cyclospora*).

Immunofluorescence assays (IFAs) using oocyst-specific monoclonal antibodies can be used to test for cryptosporidiosis.[27,339,340,342] IFA is more sensitive than acid-fast staining. Direct immunofluorescence using monoclonal antibodies is now a gold standard for stool examination. Some of the commercial IFAs (Merifluor *Cryptosporidium/Giardia,* Meridian Bioscience, Cincinnati, OH) also include antibodies to *Giardia* in addition to *Cryptosporidium.*

Antigen detection assays are being increasingly used for stool diagnosis.[58,339,340,344] Commercial kits for *Cryptosporidium* are available in enzyme-linked immunosorbent assay (ELISA) and immunochromatographic formats.[340,341,345] The ELISA kits for *Cryptosporidium* have generally performed well for diagnosis of cryptosporidiosis, with sensitivities ranging from 66% to 100% and excellent specificity.[27,340,341,345] Most studies suggest improved sensitivity compared with microscopic methods, and these assays are being increasingly used by clinical laboratories.[57,58,339] Quality control, however, may be an issue. For example, antigen detection kits have been associated with pseudo-outbreaks stemming from false-positive results, and the results may be less reliable in community hospitals.[345–347] Some commercial ELISA kits also test for *Giardia* and *Entamoeba* antigens. The immunochromatographic tests are rapid tests for *Cryptosporidium* and *Giardia* antigen. Antigen assays as a group have the advantage of not requiring skills in microscopic identification of organisms.

Molecular methods for *C. parvum* DNA are increasingly used in diagnostic laboratories.[339,344] They also have increased sensitivity compared with microscopic studies of stool, and nearly double the number of cases diagnosed compared with stool assays.[348] Several multiplex

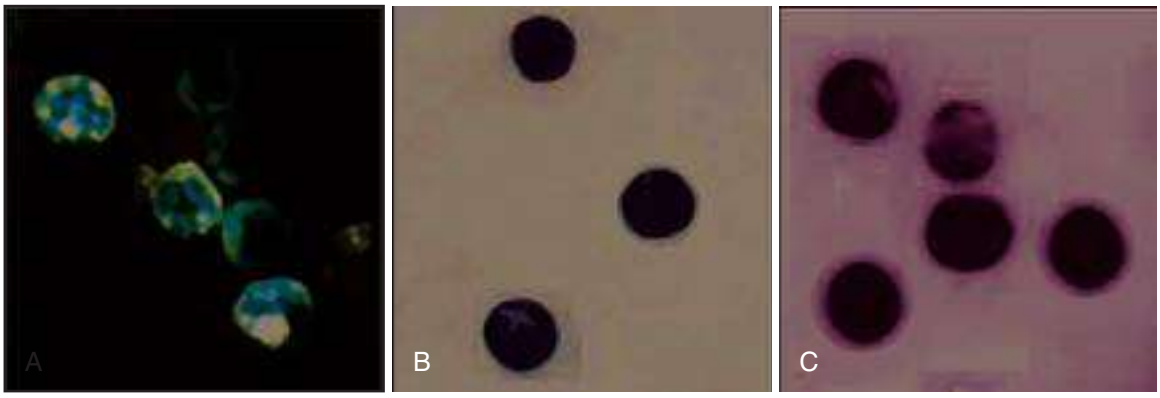

FIG. 282.3 Cryptosporidiosis is diagnosed through demonstration of organisms in stool samples. This can be done with immunoassays for antigen, with microscopic demonstration of the organisms, or through nucleic acid amplification. Because the organisms are similar in size and shape to yeast normally found in stool, differential stains are required to identify the organism. These may include modified acid-fast staining (A) showing that the oocysts are red organisms; fluorescent stains, such as auramine-rhodamine (B); or immunofluorescence (C) (green organisms). *(From the Centers for Disease Control and Prevention. DPDx. Cryptosporidiosis.* http://www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.htm.*)*

molecular assays have been approved by the US Food and Drug Administration (FDA), including Luminex xTAG gastrointestinal pathogen panel, BioFire FilmArray gastrointestinal panel, NanoCHIP gastrointestinal panel, and BD Max parasitic panel.[349–352] EasyScreen is available in Australia. All detect multiple parasites. The Luminex, NanoCHIP, and BioFire assays also detect a range of other bacterial and viral pathogens. In general, these assays are quite sensitive and specific.[339,344,353] However, they are costly and may generate positive results that are not clinically relevant.

MANAGEMENT

Supportive therapy is a key component in the management of cryptosporidiosis. As is the case for all causes of diarrhea, replacement of fluids and electrolytes is a critically important first step in management. Oral rehydration is preferred, but severely ill patients may require parenteral fluids. Fluids should include sodium, potassium, bicarbonate, and glucose. Cryptosporidiosis is characterized by preferential loss of mature epithelial cells at the tips of the villi, and enzymes expressed on these cells, including lactase, are lost. Thus, supportive care should include a lactose-free diet. In contrast to glucose-stimulated sodium pumps, which are expressed on the villus tips, glutamine-stimulated sodium absorption is not affected.[354] Glutamine supplementation, usually given in the form of alanyl-glutamine, may improve fluid absorption.[316,355,356] Nutrition remains important. As is the case for other forms of childhood diarrhea, breastfeeding should be continued and zinc supplementation provided for children in low-resource settings.[357,358] Although some patients have received parenteral nutrition, oral feeding is as effective as parenteral nutrition in those who can tolerate it.[359]

Cryptosporidiosis is associated with increased intestinal transit, which could interfere with absorption of fluids, electrolytes, and drugs.[183,360] Thus, antimotility agents play a key role in therapy. Opiates, such as loperamide and diphenoxylate/atropine combination, may ameliorate mild symptoms, but their efficacy is limited in severe disease. More potent opiates, including tincture of opium, may work in patients who have not responded. Several trials have examined octreotide therapy in AIDS patients with diarrhea. Overall, octreotide was effective but not consistently more effective than other oral antidiarrheal agents.[361,362] Because of its high cost, its use is generally limited to refractory cases. The enkephalinase inhibitor racecadotril (acetorphan) has been widely used as an antisecretory drug in watery diarrhea in Europe and many other countries but is not approved in the United States.[358] The results of a single study suggested that racecadotril is more effective than somatostatin in AIDS-associated diarrhea.[363] However, none of the studies of racecadotril specifically addressed cryptosporidiosis.

For immunodeficient patients, restoration of the immune response should be pursued. For AIDS patients with chronic cryptosporidiosis, effective ART can result in dramatic improvement in diarrhea.[197,364–368] This should take the form of recommended combinations of potent antiretroviral drugs. The HIV protease inhibitors have anticryptosporidial activity in vitro and reduced infection by up to 90% in an animal model.[369,370] However, they are no longer recommended as first-line HIV therapy. Despite improved immune response, one study documented continued early mortality in patients treated with just antiretrovirals.[368] Several studies have combined ART with antiparasitic agents.[197,371,372] Although unproven, this approach could in theory improve the response to both treatments.

Solid-organ and stem cell transplant patients also present with severe disease similar to that seen in patients with AIDS, including sclerosing cholangitis.[333–337] When possible, immunosuppression should be minimized. Cryptosporidiosis has been associated with elevated levels of tacrolimus, which may worsen the course of cryptosporidiosis.[333,335] Some patients have improved when switched from tacrolimus to cyclosporine. There are anecdotes of responses to antiparasitic drugs, including nitazoxanide, paromomycin, and/or azithromycin.[334,335,337] Most patients required a prolonged regimen and/or combinations of drugs.

Biliary involvement in cryptosporidiosis is a severe complication seen in immune-compromised hosts including patients with AIDS, transplant patients, and some patients with primary immunodeficiencies (e.g., hyper-IgM syndrome). Initial therapy should focus on improving immune function with cART in patients with AIDS, moderating immunosuppression in transplant patients, or agonist antibody to CD40 in patients with hyper-IgM syndrome.[282,330,337,373] Optimal treatment often also requires invasive approaches. Acalculous cholecystitis should be treated with cholecystectomy.[374] Patients with sclerosing cholangitis or papillary stenosis may require endoscopic retrograde cholangiopancreatography. Sphincterotomy often results in temporary improvement. However, symptoms often recur unless a stent is placed.[145,375]

Antiparasitic Drugs

The role of antiparasitic therapy in cryptosporidiosis has been difficult to demonstrate.[10,376] Initial attempts to screen drugs for anticryptosporidial activity in vitro and in animal models have met with limited success. For some drugs, resistance is attributable to target insensitivity. For example, the *Cryptosporidium* dihydrofolate reductase–thymidylate synthetase contains novel amino acids at sites associated with antifolate resistance in other species.[377] A second reason for drug resistance stems from the unique location of the parasite within the host cell, but segregated in the parasitophorous vacuole, which does not communicate with the epithelial cell cytoplasm.[41]

No antiparasitic drug has proved reliably curative in severely immunocompromised patients.[378] Because cryptosporidiosis is eventually self-limited in immunocompetent hosts and can be variable in immunocompromised hosts, controlled trials are critically important. In compromised hosts, however, most trials have not been designed to detect partially active agents. Few of the studies of AIDS patients have rigorously excluded common coinfection with *Mycobacteria,*

microsporidia, or cytomegalovirus, all of which may mask the effects of anticryptosporidial treatments.[379] Although a meta-analysis of seven randomized, controlled clinical trials found no clear evidence for efficacy of antiparasitic agents in the management of cryptosporidiosis in compromised hosts,[380] partially active drugs (which might prove useful in combination or in situations in which the patient's immune response can be boosted) may have been labeled as ineffective.

Nitazoxanide is a nitrothiazolyl-salicylamide, broad-spectrum antiparasitic drug.[381] Initial studies noted efficacy versus *Taenia saginata* and *Hymenolepis nana.* However, clinical development progressed only after antiprotozoal activity was demonstrated in the early 1990s. Nitazoxanide suspension was approved in the United States for treatment of cryptosporidiosis and giardiasis in children in 2002. Nitazoxanide inhibits growth in vitro at concentrations of less than 10 μg/mL.[382,383] The metabolite tizoxanide is less active, but tizoxanide glucuronide is nearly as active as the parent compound. Nitazoxanide decreased parasite numbers but was not curative in gnotobiotic pigs and was not effective in SCID mice depleted of IFN-γ.[382]

A randomized, controlled study of nitazoxanide in HIV patients with cryptosporidiosis was performed in Mexico; it compared doses of 500 mg twice daily, 1 g twice daily, or placebo for 2 weeks.[384] Among HIV patients with CD4 cell counts greater than 50 cells/μL, 10 of 14 (71%) responded to 1 g/day and 9 of 10 (90%) to 2 g/day, compared with 3 of 15 (20%) treated with placebo. By contrast, the response was no better than placebo in patients with CD4 cell counts of 50 cells/μL or lower.

Three randomized trials were performed in patients with cryptosporidiosis who were not infected with HIV. An outpatient study was performed in Egypt in adults and children with cryptosporidiosis and prolonged diarrhea (mean duration, 13 days).[385] Adults, children aged 4 to 11 years, or children aged 1 to 3 years received nitazoxanide in doses of 500 mg, 200 mg, or 100 mg twice daily or matching placebo for 3 days. Diarrhea resolved by day 7 in 39 of 49 (80%) in the nitazoxanide group, compared with 20 of 49 (41%) in the placebo group. Oocysts were no longer detected in 33 of 49 (67%) treated with nitazoxanide, compared with 11 of 50 (22%) treated with placebo. A second trial of outpatients in Egypt compared nitazoxanide tablets, suspension, and placebo. The response rate was 26 of 28 (93%) in the nitazoxanide arms, compared with 10 of 27 (37%) in the placebo arm.[386] Parallel randomized trials of HIV-infected and HIV-negative children hospitalized with chronic cryptosporidiosis were performed in Zambia.[305] Nearly all the participants had moderate-to-severe malnutrition and persistent diarrhea or chronic diarrhea. All children were treated with nitazoxanide suspension (100 mg twice daily for 3 days) or matching placebo. In the trial of HIV-negative children, diarrhea had resolved by day 7 in 14 of 25 (56%) of those treated with nitazoxanide, compared with 5 of 22 (23%) for those treated with placebo. Follow-up stool studies were free of oocysts in 13 of 25 (52%) children in the treatment group, compared with 3 of 22 (14%) in the placebo group. Most of those who did not respond became well after a second course, given on an open-label basis. Significantly more HIV-negative children died in the placebo group. A fourth trial in hospitalized children in Egypt noted an 87% cure rate with nitazoxanide compared with 69% with paromomycin.[387]

Among HIV-infected children, Amadi and colleagues noted no significant differences in clinical and parasitologic responses or in mortality rate with nitazoxanide treatment.[305] A subsequent placebo-controlled trial in children with AIDS and cryptosporidiosis did not show significant improvement with nitazoxanide compared with placebo, despite use of higher doses and continuation of treatment for 2 weeks.[388] A compassionate use program noted that AIDS patients could be treated safely with nitazoxanide at doses of 1 g twice daily for at least 2 weeks.[389]

Paromomycin is an orally administered nonabsorbable aminoglycoside originally approved in the 1960s as a luminal amebicide. Initial in vitro studies noted poor activity against *C. parvum* with inhibitory concentrations in the range of 100 to 500 μg/mL. However, when AIDS patients with cryptosporidiosis were treated with available antiparasitic drugs, some improved when treated with paromomycin. The first 12 published case series of AIDS patients treated with paromomycin included more than 300 patients, with a response rate of 67%.[145] In many of those with initial improvement, relapse occurred later. Two randomized, controlled trials have examined the effects of paromomycin in AIDS patients with cryptosporidiosis. In a small placebo-controlled trial incorporating quantitation of oocyst excretion, the paromomycin arm demonstrated a significant reduction in oocyst shedding (about 70%), decreased stool frequency in those treated with paromomycin, but no cures.[390] Biliary tract involvement and *Mycobacterium* coinfection were common in those not responding. Hewitt and colleagues[391] compared paromomycin with placebo in a trial including 35 AIDS patients. There was no difference between groups when analysis of those on treatment was performed, but dropouts occurred only in the placebo arm. By intent-to-treat analysis with dropouts grouped with failures, the response rate was similar to that in the previous trials, with a trend favoring paromomycin over placebo.[392] The trial was prematurely terminated because of poor enrollment and was not powered to detect limited response rates. Limited efforts were made to exclude coinfections. Dose escalation demonstrated no further improvement with higher doses, and higher doses have been associated with gastrointestinal toxicity and ototoxicity.[392]

Macrolide antibiotics, including spiramycin, azithromycin, roxithromycin, and clarithromycin, have some activity against *Cryptosporidium.*[376] Sáez-Llorens and colleagues[393] reported shorter duration of symptoms and oocyst shedding when children were treated with 100 mg/kg/day of spiramycin, but a second trial showed no effect.[394] In a randomized placebo-controlled trial among intravenous drug users, Huang and colleagues reported a higher cure rate with acetyl-spiramycin, especially when combined with garlicin.[395] However, no information is provided on HIV status despite this being a high-risk population. In an unpublished study, the AIDS Clinical Trials Group (ACTG) conducted a randomized, controlled trial in 75 AIDS patients, comparing spiramycin with placebo, but noted that spiramycin was not significantly better than placebo.[376] In a second unpublished trial, intravenous spiramycin was associated with significantly decreased oocyst shedding and a partial response in 75% of participants.[376] However, there were high rates of adverse events, including drug-associated intestinal injury.[396]

Azithromycin has some activity against *Cryptosporidium* in vitro and in animal studies. Uncontrolled reports have noted improvement in cryptosporidiosis among HIV and transplant patients treated with azithromycin.[334,337,397] In a placebo-controlled, multicenter trial, AIDS patients with cryptosporidiosis were randomly assigned to receive azithromycin, 900 mg orally daily, or placebo. Overall, oocyst shedding, stool frequency, and weight loss were not significantly different.[398] A subsequent pilot trial of intravenous azithromycin also did not demonstrate changes in stool frequency or oocyst shedding.[399] A pilot study of Egyptian schoolchildren suggested more rapid resolution with azithromycin treatment.[400]

Rifamycin antibiotics have some activity against *Cryptosporidium* in vitro.[376] Rifaximin, a nonabsorbable rifamycin, is approved by the FDA for the treatment of traveler's diarrhea. Although it has some activity against cryptosporidiosis in HIV patients, its efficacy has never been assessed in controlled studies.[401,402] Rifabutin is active against *Cryptosporidium* in vitro, and use of rifabutin for prophylaxis of *Mycobacterium avium* infection in AIDS patients was associated with a decreased incidence of cryptosporidiosis.[403–405]

Anecdotal reports noted improvement in chronic cryptosporidiosis in patients treated with oral anti-*Cryptosporidium* immunoglobulin preparations. However, three controlled trials have examined oral bovine anti-*Cryptosporidium* immunoglobulin preparations in cryptosporidiosis. Bovine anti-*Cryptosporidium* immunoglobulin did not significantly decrease symptoms or oocyst shedding in experimental infection of volunteers.[406] In a large trial of bovine anti-*Cryptosporidium* immunoglobulin for cryptosporidiosis in AIDS patients, there was no effect on symptoms, and oocyst shedding decreased only slightly at a dose of 20 g/day.[234] At higher doses, oocyst excretion decreased, but the immunoglobulin preparation caused diarrhea. A third trial was stopped with only five participants after demonstrating similar improvement in hyperimmune and control colostrum.[407]

Because individual drugs have limited activity, studies have investigated the effects of combinations. In vitro studies have suggested that azithromycin and rifabutin may enhance the parasiticidal activity of nitazoxanide.[404] Lee and colleagues[408] studied azithromycin and nitazoxanide in gnotobiotic pigs infected with *C. hominis.* They noted optimal parasitologic responses with just nitazoxanide, but combination

therapy with azithromycin improved the clinical response. There are a number of anecdotes of transplant and AIDS patients being treated with antiparasitic combinations.[337] Smith and colleagues[409] conducted a prospective pilot study of the combination of paromomycin combined with azithromycin in AIDS patients with chronic cryptosporidiosis. Overall, there was a 2-log decrease in oocyst shedding, but few patients were cured. Clinical failures were associated with biliary disease, coinfection with other enteric pathogens (especially cytomegalovirus), or side effects of the medications. Combination therapy therefore warrants further study.

In recent years, the increased recognition of cryptosporidiosis as a cause of childhood morbidity has led to renewed efforts at developing drugs for cryptosporidiosis.[10,410–412] Several groups have pursued high-throughput screening of pharmaceutical compound libraries, including some with licensed drugs and the Medicines for Malaria Venture library.[412] A piperaquine compound from the Medicines for Malaria Venture library (MMV665917) was highly effective in a calf model.[413,414] Malaria lipid kinase (MI4K8) was identified as a target for chemotherapy from a high-throughput screen. An inhibitor of MI4K8, KDU731, has proven effective in vitro, in small animal models, and in cattle.[415] The leprosy drug clofazimine was identified as active against *Cryptosporidium* by high-throughput screening.[416] A phase II study in AIDS patients with cryptosporidiosis is currently enrolling. A drug repurposing screen identified 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors as potential treatments, and atorvastatin significantly reduced oocyst shedding in immunosuppressed mice.[417,418]

Other groups have targeted specific parasite molecules.[10,412] For example, an inhibitor of cryptopain (a parasite cysteine protease) was able to control infection in an IFN-γ knockout mouse model.[10,419] Calcium-dependent protein kinases (CDPKs) of apicomplexan parasites have an active site that is more open than in mammalian enzymes.[420–422] A number of bumped kinase inhibitors have also demonstrated efficacy in vitro without inhibiting mammalian kinases. Some have efficacy in small animal models and in cattle. However, so far all have stopped short of clinical trials due to unanticipated adverse effects. Although some of these compounds have worked in large animal models, only clofazimine has entered clinical trials, but so far there is no proof of efficacy in people.

PREVENTION

Cryptosporidiosis is transmitted from person to person and via contaminated water and food. Water purification is an important public health measure. Because chlorination has little effect on the oocysts, water purification should generally involve flocculation and filtration.[46,97,423] As noted, enhanced screening and improved water treatment have dramatically decreased the number of outbreaks associated with drinking water in the United States and United Kingdom.[43,46,110] Ultraviolet radiation or ozonation can disinfect contaminated water, but these methods are rarely used. Recreational waters, such as lakes, may pose a danger for compromised hosts, who should avoid untreated water.[424] Swimming pools and other recreational aquatic facilities are now an important source of infection, and anyone with diarrhea should not swim in public facilities.[116,286,425,426] Contamination of treated recreational water, such as a fecal accident in a swimming pool, should prompt aggressive measures, including closing the pool temporarily.

Personal measures can be used to decontaminate infected or potentially infected water, such as during travel to resource-poor countries when the public water supply is contaminated, or as a routine practice in compromised hosts. Water can be decontaminated by bringing it to a boil or by using a filter with a pore size of 1 μm or smaller.[427] Studies in resource-limited settings have demonstrated inconsistent effects of water treatment. Use of ceramic filters has decreased diarrheal diseases in some settings, but had mixed results in cryptosporidiosis.[428,429] Even providing bottled water did not decrease cryptosporidiosis in India.[430]

Although cryptosporidiosis can be transmitted within health care facilities, risk is minimal with standard precautions.[133] Gloves should be worn and hands washed after handling material contaminated with fecal material. Instruments such as endoscopes need to be carefully disinfected between uses. Wearing gloves and hand washing can also prevent infection in daycare centers. However, one outbreak did not abate until all of the infected children were treated with nitazoxanide.[128]

For patients with AIDS (CD4 cell counts <200 cells/μL), water should be boiled or filtered when water is contaminated.[424] If HIV-infected persons travel in resource-poor countries, they should be warned to meticulously avoid drinking tap water. Rifaximin is approved for prevention of traveler's diarrhea and has been used to treat some cryptosporidiosis cases.[431] However, there are no data on use of rifaximin to prevent cryptosporidiosis. These individuals should avoid obvious sources of *Cryptosporidium* oocysts, such as persons with diarrhea (particularly avoiding sexual practices that might involve exposure to feces), farm animals (particularly cattle), and domestic pets that have diarrhea. Chemoprophylaxis may also be considered but is generally not recommended. Two retrospective studies examined data from trials of prophylaxis of *M. avium* for their effects on cryptosporidiosis. Both noted a lower incidence of cryptosporidiosis in groups treated with rifabutin.[403,405] However, the incidence of cryptosporidiosis was low in both studies, such that chemoprophylaxis is not routinely recommended.

Experimental studies have suggested that it may be possible to develop a vaccine to prevent cryptosporidiosis.[10,222,432] However, current studies with recombinant vaccines have been less effective than priming by infection.[433] Vaccination would likely have to involve both human and animal hosts and would need to work against a number of species of parasites. Studies have so far not even demonstrated the feasibility of this approach.

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283 *Cyclospora cayetanensis, Cystoisospora belli,* Species, *Balantidium coli,* and *Blastocystis* Species

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SHORT VIEW SUMMARY

CYCLOSPORA AND *CYSTOISOSPORA*

Epidemiology
- Both are opportunistic pathogens in immunocompromised hosts but also infect immunocompetent patients.
- *Cyclospora:* distribution is worldwide and is endemic in developing areas, with outbreaks in developed areas.
- *Cystoisospora:* occurrence is primarily in tropical and subtropical climes, especially South America, Africa, and Southeast Asia.
- Contaminated food and water are primary sources.
- Oocysts can survive in the environment for months but must sporulate to become infective.

Diagnosis
- Acute or chronic diarrhea occurs with other constitutional and gastrointestinal symptoms.
- Oocysts in stool may be visualized using modified acid-fast stain.
- Multiple stool examinations may be required.

Therapy
- Trimethoprim-sulfamethoxazole (1 double-strength tablet twice daily for 7 to 10 days) or ciprofloxacin (500 mg orally twice daily for 7 days) is effective (see Tables 283.1 and 283.2).

SARCOCYSTIS SPECIES

Epidemiology
- Distribution is mainly tropical and subtropical, especially in Southeast Asia and Malaysia.
- Widely distributed in various animals; human disease is very rare and occurs after consumption of infected raw or undercooked animal flesh.
- *Sarcocystis suihominis* and *Sarcocystis hominis* are the main causes of human disease.
- *Sarcocystis nesbitti* has caused outbreaks of disease and may be associated with ingestion of water contaminated by snake feces.

Diagnosis
- Infection is generally asymptomatic.
- Rarely, self-limited gastrointestinal illness or myositis (fever, myalgias) occurs after exposure in endemic areas.
- Sporocysts or oocysts may be visible in microscopic examination of stool; muscle biopsy may be required.

Therapy
- None; albendazole has been used.

BALANTIDIUM COLI

Epidemiology
- Distribution is worldwide, with infections in Latin America, Southeast Asia, Papua New Guinea, and the Middle East most common.
- Pigs are primary reservoirs and shed cysts in stool.
- Cysts in contaminated food or water are infectious.

Diagnosis
- The infection is generally asymptomatic; occasionally a diarrheal illness is reported.
- Trophozoites are visible in stool.

Therapy
- Tetracycline, metronidazole, or iodoquinol is used (see Table 283.3).

BLASTOCYSTIS SPECIES

Epidemiology
- Distribution is worldwide, primarily in developing countries, but prevalence varies markedly.

Diagnosis
- Role in human disease is unclear; diarrhea, flatulence, and abdominal discomfort are the most commonly reported symptoms.
- Microscopic diagnosis is challenging; trichrome stain is the most sensitive.
- Polymerase chain reaction assay is more sensitive and specific, and is becoming more widely available.

Therapy
- Therapy is often unsatisfactory.
- Trimethoprim-sulfamethoxazole, metronidazole, or iodoquinol is used (see Table 283.4).

COCCIDIA OTHER THAN CRYPTOSPORIDIA

Cyclospora, Cystoisospora, and *Sarcocystis* are coccidian parasites belonging to the phylum Apicomplexa, family Eimeriidae. Coccidian protozoan infections are well recognized but still relatively uncommon causes of diarrheal disease. *Cryptosporidium parvum* and *Cyclospora cayetanensis,* two of the more commonly identified coccidian pathogens in diarrheal illness, account for a small but increasing proportion of laboratory-confirmed diarrheal disease reported to the Foodborne Diseases Active Surveillance Network (FoodNet) of the US Centers for Disease Control and Prevention. In 2011, the reported incidences of cryptosporidiosis and cyclosporiasis were 2.85 and 0.05 per 100,000 population, respectively[1]; in 2017, these rates increased to 3.7 and 0.3 per 100,000 population (1836 and 163 cases), respectively.[2] The almost fivefold increase in cyclosporiasis incidence may reflect, at least in part, changes in testing practices as well as increased use of culture-independent tests (e.g., molecular tests). However, these data need to be put into perspective when statistics show that *Campylobacter* and *Salmonella* infections occurred at rates of 16.0 and 19.1 per 100,000, respectively, during the same year.[2]

Cyclospora species are genetically closely related to *Eimeria* and are more distantly related to *Cystoisospora, Sarcocystis,* and *Toxoplasma.*[3] Although several species of *Cyclospora* have been identified, humans are the only known hosts for *C. cayetanensis.* Humans are also the only recognized hosts for *Cystoisospora belli,* and no other *Cystoisospora* species has been confirmed to infect humans.

Clinical signs and symptoms do not distinguish disease caused by *Cyclospora, Cryptosporidium,* microsporidia, *Cystoisospora,* or other noninflammatory causes of diarrhea. However, knowledge of endemic regions, the occurrence of global outbreaks, and the seasonal and geographic variation of diseases such as cyclosporiasis can help point to a particular pathogen. Infection with any of these agents can cause protracted and severe illness in immunocompromised hosts, in particular those with human immunodeficiency virus (HIV) infection, although

the incidence of many coccidian infections has decreased since the introduction of highly active antiretroviral therapy.[4,5] *Cyclospora* continues to be implicated in foodborne and waterborne outbreaks of diarrheal disease. In contrast, *Sarcocystis* infection is typically asymptomatic and rarely causes gastrointestinal symptoms.

Cyclospora

Cyclosporiasis was first described in humans in Papua New Guinea in 1977. The organism was considered to be a blue-green alga but eluded accurate taxonomic classification until 1993, when Ortega and colleagues[6] in Peru succeeded in inducing sporulation and thus confirmed its genus, *Cyclospora.* It was named *C. cayetanensis* after the Universidad Peruana Cayetano Heredia in Lima, Peru, a major site of research on the infection.

Life Cycle

Cyclospora oocysts are spherical, measuring 8 to 10 µm in diameter. Ultrastructural studies of the unsporulated oocyst reveal an outer fibrillar coat and a cell wall and membrane. Unsporulated oocysts are excreted in the stool of infected individuals. Oocysts are quite resistant and can survive under diverse environmental conditions, including freezing, 2% formalin, 2% potassium dichromate, and chlorination. Sporulation is required for infectivity and requires at least 7 days of maturation outside the human host; experimentally, in moderate temperatures, sporulation occurs within 7 to 13 days.[7] Each sporulated oocyst contains two sporocysts that each hold two sporozoites.

After ingestion of sporulated oocysts, excystation occurs in the proximal small bowel. Sporozoites penetrate the epithelial cells of the small intestine, where both asexual and sexual reproduction take place. Although the asexual life cycle can continue endogenously within the intestinal epithelium, sexual reproduction leads to the development of zygotes. Zygotes mature into oocysts within the intestinal epithelium, which in turn are released into the stool after causing rupture of the host cells.

Epidemiology

Cyclospora infections occur worldwide, sporadically and in clusters, with a major increase in reported cases after its widespread recognition in the mid-1990s.[8] Cases have been reported from all regions of the world. The majority have been described in developing countries of the tropics and subtropics, where the disease seems to be endemic; sporadic cases of disease occur commonly in underdeveloped areas. Prevalence studies in stool samples from developed countries have identified *Cyclospora* in no more than 0.5% of samples.[8] Cases in developed nations tend to be more frequently associated with recognized waterborne and foodborne outbreaks. Outbreaks in North America in the early and mid-1990s—notably, one outbreak among employees of a Chicago hospital that was attributed to ingestion of water from a contaminated water storage tank[9] and a more widespread outbreak throughout the United States and Canada associated with consumption of contaminated raspberries imported from Guatemala[10]—brought considerable attention to this organism. Other produce, including lettuce, basil, cilantro, watercress, and sugar snap and snow peas, has been implicated in North American foodborne outbreaks since 2000.[11–14] Produce is presumably contaminated by being washed or sprayed with contaminated surface water.[8,15] *Cyclospora* can also cause traveler's diarrhea, although it is not one of the major causes of this illness. Travelers accounted for 37% of all cyclosporiasis cases reported to FoodNet in 2015, but only 1.3% of all travel-related enteric infections were attributed to *Cyclospora* in the same year.[16] Cyclosporiasis is a recognized opportunistic infection in those with HIV infection and other immunosuppressed conditions.

Humans are the only known hosts for *C. cayetanensis.* Transmission occurs via the fecal-oral route. The risk for transmission and infection depends on the level of sanitation, as well as the availability of water and food that are at risk of being contaminated. Direct person-to-person spread is unlikely, owing to the need for oocysts to sporulate to become infectious. The infectious dose has not been determined but is presumed to be low. In developing countries infection is more common in children younger than 10 years of age,[17–19] with the risk for infection decreasing with increasing age. Infants may be somewhat protected through breastfeeding and the absence of exposure to environmental sources of the parasite. Infection occurs seasonally but varies according to geography, with the highest incidence in spring and summer (May through July) in Canada and the United States, in the warm season (April through June) in Peru,[17] before and during the monsoon season (May through October) in Nepal,[20] during drier months (January through March) in Haiti,[21] and during the rainy season (May to August) in Honduras.[22] Factors affecting seasonality and possible reservoirs during the off-season have not yet been defined. Multilocus sequence (genetic) typing suggests that there are geographic differences in circulating subpopulations of *C. cayetanensis,*[23] which could potentially benefit outbreak investigations by facilitating identification of the source(s) of an outbreak.

Cyclospora cayetanensis–like oocysts have been recovered from a variety of other animals, including mice, rats, dogs, chickens, ducks, and nonhuman primates. Attempts to infect mammals and birds in the laboratory setting have been largely unsuccessful. It is unclear what if any role animals play in the spread of infection and whether oocysts recovered from animal feces represent coprophagy or other zoonotic organisms that resemble *Cyclospora.* Oocysts have also been identified in sewage and vegetable washings.[15]

Clinical Manifestations

The clinical manifestations of *Cyclospora* infection are varied and differ according to age as well as the degree of endemnicity of the region in which infected individuals live. Asymptomatic infection is more common in the indigenous populations of developing countries, particularly in adults but also in children, suggesting that previous exposure may induce some degree of protective immunity among residents of these regions.[17,24] However, asymptomatic infection may also occur in others, including those with HIV infection.

Symptomatic disease occurs in both endemic and nonendemic regions. In developing countries, symptomatic disease is more likely to develop in the absence of previous exposure and is thus more common in children. After an incubation of 1 to 11 days (mean, 7 days), illness begins abruptly. A flulike illness may precede the onset of diarrhea, which is invariably present with a median of 6 (range, 5 to 15) watery stools per day.[25] Fatigue, anorexia, myalgia, abdominal cramps, flatus, and nausea occur frequently. Fever is present in approximately 25% of cases. Illness generally lasts from 1 to 7 weeks or longer and may result in dehydration and significant weight loss. Diarrhea can be cyclic or relapsing, especially in the absence of therapy. Disease may be severe in the elderly and life threatening in the immunocompromised; diarrhea and weight loss tend to be more severe, and illness can be prolonged, in individuals with acquired immunodeficiency syndrome (AIDS). Postinfectious fatigue can be profound in some individuals and may persist long after the resolution of other clinical symptoms. It is unknown whether the pathogenesis of disease is due to enterocyte dysfunction or whether toxins are secreted.

Extraintestinal complications of *Cyclospora* infection are exceedingly uncommon. Reactive arthritis[26] and Guillain-Barré syndrome[27] have both been reported after infection with *Cyclospora.* Biliary tract disease has been described in patients with AIDS.[28]

Diagnosis

The diagnosis of cyclosporiasis generally relies on the microscopic identification of oocysts in stool samples (Fig. 283.1A). Shedding of oocysts in stool can precede the onset of clinical illness, but the disappearance of symptoms and oocysts usually occurs simultaneously. Oocysts may be shed in low numbers during infection, and both concentration of stool specimens and collection of multiple specimens may be required to make the diagnosis.[8]

Although *Cyclospora* oocysts are approximately twice the size of *Cryptosporidium* oocysts, the two may be confused if oocysts are not measured. The organism is variably acid-fast on modified Ziehl-Neelsen or Kinyoun stain (see Fig. 283.1B), and such techniques are superior to the examination of routine wet mounts, which require a trained eye for identification of the organism. Therefore if cyclosporiasis is suspected, notification of the laboratory is prudent so that appropriate tests can be performed. Demonstration of blue autofluorescence of the oocysts

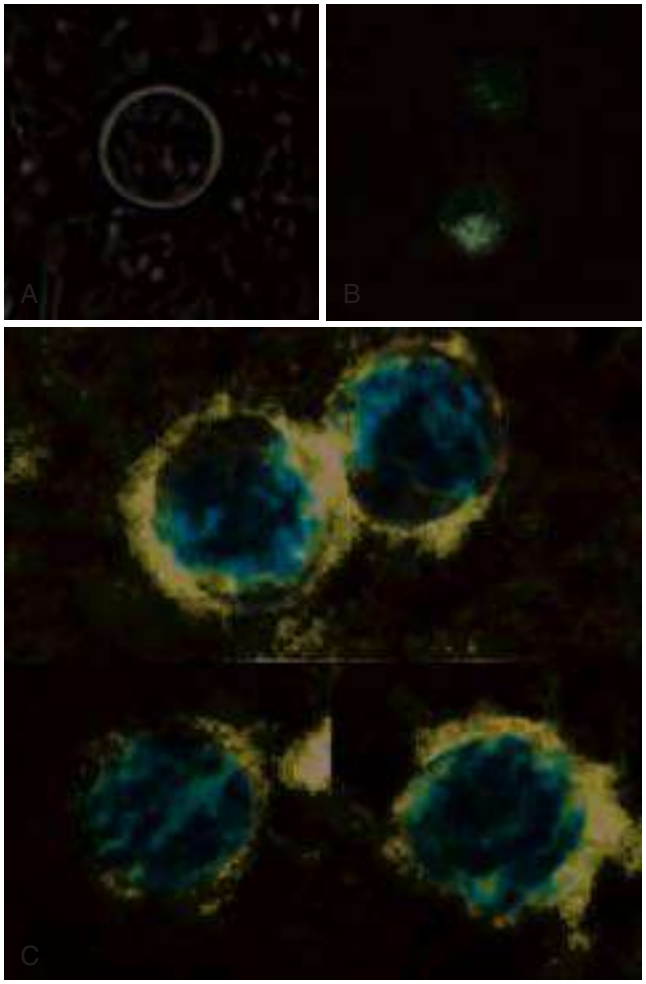

FIG. 283.1 *Cyclospora* oocysts visualized with different staining methods. (A) Wet mount. (B) Variable staining with modified acid-fast stain. (C) Uniform staining with modified safranin stain. Modification consists in heating in a microwave during staining. *(From DPDx Image Library, Centers for Disease Control and Prevention, Atlanta, GA.)*

under ultraviolet epifluorescence microscopy is both rapid and sensitive, although not specific. Additional stains, including auramine, safranin (see Fig. 283.1C), and lactophenol cotton blue, can also be used. Oocysts cannot be identified using Giemsa and trichrome stains.

Species-specific real-time polymerase chain reaction (PCR) assays have been developed that are capable of detecting low concentrations of oocysts in stool. Newer multiplex PCR-based diagnostic panels include *Cyclospora.* Although PCR assays may be more sensitive than conventional diagnostic methods,[29] they are costly and may lead to an increased number of diagnosed cases that in turn may require more expert decision making to interpret the results, particularly in endemic settings.[30] Flow cytometry has been proposed as an alternate method of diagnosis.[31] Antibodies to *Cyclospora* can be detected, but serologic tests are not commercially available.

The diagnosis may also be made by histopathologic or electron microscope examination of jejunal aspirates or biopsy specimens. Endoscopic findings may be normal or may demonstrate inflammation. Microscopic examination of tissue reveals altered histologic architecture of the small bowel, and loss of the brush border and altered epithelial cell morphology may be noted.[32] Villous atrophy, acute and chronic inflammation in the lamina propria, and vascular dilation may be seen. Routine hematoxylin and eosin staining of biopsy material may not permit adequate visualization of the organisms. Tissue sections may reveal *Cyclospora* in supranuclear locations within the cytoplasm, distinguishing them from *Cryptosporidium,* which are on the surface of enterocytes.

TABLE 283.1 Therapy (Adult) for Cyclosporiasis

DRUG[a]	THERAPEUTIC DOSAGE	PROPHYLACTIC DOSAGE
Trimethoprim-sulfamethoxazole	1 DS tablet[b] PO bid for 7–10 days	1 DS tablet PO 3 times weekly
Ciprofloxacin	500 mg PO bid for 7 days	500 mg PO 3 times weekly
Nitazoxanide	500 mg PO bid for 7 days	

[a]Drugs are listed in order of preference.
[b]One DS tablet contains 160 mg trimethoprim/800 mg sulfamethoxazole.
DS, Double strength; *PO,* orally.

Therapy

Trimethoprim-sulfamethoxazole (TMP-SMX) is the recommended therapy for cyclosporiasis (Table 283.1). One double-strength tablet (160 mg TMP/800 mg SMX) given twice daily is the usual dose for adults with normal renal function; for children, weight-based dosage (TMP 5 mg/kg twice daily) should be used. Treatment is continued for 7 days in immunocompetent hosts[17,33] and for 7 to 10 days in patients with HIV infection.[33,34] Eradication of oocysts correlates with treatment success. Suppressive therapy (TMP-SMX 160/800 mg three times weekly for 4 weeks) may be required in HIV-infected patients because of the historically high relapse rate (almost 50%) in this population.[34]

Individuals who cannot tolerate TMP-SMX may be treated with ciprofloxacin 500 mg twice daily for 7 days, based on a study conducted in HIV-infected patients.[35] If suppressive therapy is indicated, a dose of 500 mg three times weekly may be used. Ciprofloxacin was, however, slightly less efficacious than TMP-SMX for both treatment and prophylaxis.[35] The thiazolide agent nitazoxanide (500 mg twice daily for 7 days) has also been used successfully to treat cyclosporiasis in an immunocompetent adult[36] and was efficacious in the treatment of mixed parasitic infections (including cyclosporiasis) in children.[37] However, studies demonstrating its efficacy in the treatment of cyclosporiasis in HIV-infected patients are lacking.

Cystoisospora belli

Cystoisospora belli (formerly *Isospora belli*) was first described in 1915. It is the only one of more than 200 identified *Cystoisospora* species that is known to cause human infection. Human infections previously attributed to *Cystoisospora hominis* are more likely to have been caused either by *Sarcocystis* species or by misidentified *C. belli.*

Life Cycle

Immature *Cystoisospora* oocysts, each containing a single sporoblast, are excreted in the stool of infected hosts. Oocysts can remain viable in the environment for months. Sporulation in the environment is required before oocysts become infectious. Sporulation generally requires 24 to 48 hours but can occur within 16 hours in ideal conditions (30°C–37°C) and is hindered at temperatures below 20°C or above 40°C.[38] The single sporoblast divides in two, and each newly formed sporoblast subsequently matures into a sporocyst. The resulting infective elliptical oocyst (22–33 × 12–15 µm) contains two sporocysts, each with four sporozoites.

Ingestion of sporulated oocysts results in the release of sporozoites in the proximal small intestine. Sporozoites may develop into merozoites, with subsequent asexual reproduction occurring within enterocytes; over time, sexual reproduction follows, resulting in the development and passage of immature, unsporulated oocysts in feces. Rarely, sporozoites can migrate out of the intestine to various tissues, where they may remain dormant as cysts and later give rise to extraintestinal disease.

Epidemiology

Cystoisospora species are found worldwide but predominantly in tropical and subtropical climates, especially in South America, Africa, and Southeast Asia. *Cystoisospora* occasionally cause traveler's diarrhea. In the United

States, cystoisosporiasis has been more commonly associated with HIV infection and other immunosuppressed conditions, immigration from Latin America, daycare centers, and psychiatric institutions. In patients with AIDS in the United States, *C. belli* infection accounted for 2% to 3% of AIDS-defining illnesses in the 1980s, but this decreased to less than 0.1% in the late 1990s,[39] largely because of the widespread use of TMP-SMX to prevent *Pneumocystis jirovecii* pneumonia. A recent systematic review of *Cystoisospora* in HIV infection identified a global pooled prevalence of 2.5%, with a higher prevalence in Africa and southeast Asia.[40]

Cystoisospora species other than *C. belli* have been found in a wide variety of animals, including cats and dogs. It is unclear whether most animals develop clinical disease or whether they merely act as paratenic hosts. Pigs are notable exceptions; *Cystoisospora suis* can cause severe diarrheal disease and death in piglets and has been implicated in outbreaks of disease among nursing piglets.[38]

Clinical Manifestations

The pathogenesis of cystoisosporiasis has not been determined but may be the result of cell damage from direct consequences of parasite invasion, cell-mediated inflammation, or proteins and oxidants released from mast cells.

In immunocompetent hosts, *Cystoisospora* infection is indistinguishable from other noninflammatory intestinal infections. After an incubation period of approximately 1 week, a self-limited diarrheal illness usually develops that lasts 2 to 3 weeks and is characterized by malaise, anorexia, weight loss, abdominal cramps, and profuse watery diarrhea without blood. Fever is uncommon and if present is usually low grade. Oocyst shedding may persist for several weeks after recovery. Rarely, chronic persistent or intermittent symptoms may continue for many years. Biliary tract disease due to *Cystoisospora* has been described in immunocompetent hosts.[41]

In immunocompromised hosts, including those with HIV infection or malignancy and those receiving cytotoxic therapy, infection may result in protracted, severe diarrheal illness. Cystoisosporiasis in HIV-infected patients generally occurs with CD4 T-cell counts less than 200 cells/mm.[3] Hemorrhagic colitis,[42] biliary tract disease,[43] disseminated disease,[44] and reactive arthritis[45] have been reported in the literature.

Diagnosis

Typically, *C. belli* infection is diagnosed by identification of oocysts in stool in wet mounts (Fig. 283.2A) or modified acid-fast–stained fecal smears (see Fig. 283.2B). Because *C. belli* parasites are shed intermittently in low numbers, multiple stool examinations may be required for diagnosis, and stool concentration (using flotation or sedimentation methods) may be required. Direct or concentrated wet mounts are preferable to permanent stain smears because oocysts are difficult to detect in preserved stool specimens. Auramine-rhodamine, lactophenol cotton blue, and safranin (see Fig. 283.2C) may also be used. Ultraviolet autofluorescence microscopy (see Fig. 283.2D) is a simple, rapid, and sensitive diagnostic method that is based on the detection of oocysts that autofluoresce (blue) when a 330- to 380-nm ultraviolet filter is used. Examination of small bowel specimens (e.g., duodenal aspirates) may be helpful if stool examination is negative. Peripheral blood eosinophilia and Charcot-Leyden crystals in stool, both unusual in other protozoan infections, have been reported.[46] PCR assay can identify *C. belli* with high sensitivity and specificity.

Histologic examination of the small bowel of infected patients is relatively nonspecific and reveals villous atrophy, crypt hyperplasia, and lamina propria infiltration with inflammatory cells, particularly eosinophils. Asexual and sexual stages of the parasite can be identified within parasitophorous vacuoles of enterocytes. In case reports of extraintestinal disease, intracellular cysts containing one to three trophozoites were identified in lymph nodes, liver, and spleen.[44] Gallbladder pathology in biliary disease reveals eosinophilic intraepithelial parasites within parasitophorous vacuoles, epithelial disarray, and intraepithelial lymphocytosis.[41]

Therapy

Drug therapy (Table 283.2) has been studied predominantly in HIV-infected patients. TMP-SMX (160 mg TMP/800 mg SMX), the treatment of choice, is administered two times daily for 10 days, although HIV patients may need 1 double-strength tablet four times daily for up to 4 weeks.[47] Patients with HIV infection usually respond to antimicrobial therapy within several days but have a 50% chance of relapse within 6 to 8 weeks if suppressive therapy is not administered.[48] TMP-SMX for this purpose is beneficial when taken either daily or three times weekly.[49]

Alternative antibiotics include ciprofloxacin (500 mg twice daily for 7 days followed by suppressive therapy three times weekly),[35] or

TABLE 283.2 Therapy (Adult) for Cystoisosporiasis

DRUG[a]	THERAPEUTIC DOSAGE	PROPHYLACTIC DOSAGE
Trimethoprim-sulfamethoxazole	1 DS tablet[b] PO bid for 10 days	1 DS tablet PO daily or 3 times weekly
Ciprofloxacin	500 mg PO bid for 7 days	500 mg PO daily or 3 times weekly

[a]Drugs are listed in order of preference.
[b]One DS tablet contains 160 mg trimethoprim/800 mg sulfamethoxazole.
DS, Double strength; *PO,* orally.

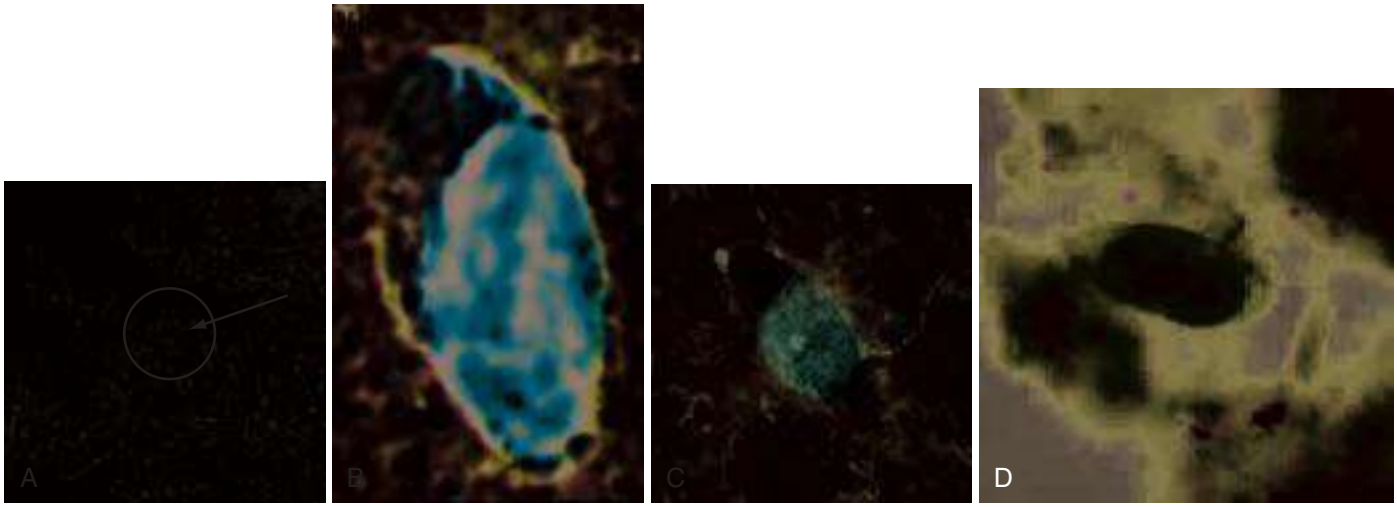

FIG. 283.2 Immature oocysts of *Cystoisospora belli,* each containing a single sporoblast. (A) Wet mount. (B) Modified acid-fast stain. (C) Stained with safranin. (D) Viewed under ultraviolet autofluorescence microscopy. *(From DPDx Image Library, Centers for Disease Control and Prevention, Atlanta, GA.)*

pyrimethamine (75 mg/day) together with folinic acid (10 to 25 mg/day).[50] Other drugs, including nitazoxanide, diclazuril, roxithromycin, a combination of albendazole and ornidazole, metronidazole, quinacrine, and furazolidone, may also be effective.

Sarcocystis Species

Sarcocystis species, previously known as *Sarcosporidia,* are zoonotic protozoan parasites. Since the first report of sarcocystosis in mice in 1843, more than 130 species of *Sarcocystis* have been reported from a wide range of domestic and wild animals. Unlike many other coccidian parasites, *Sarcocystis* has an obligatory two-host cycle. Definitive and intermediate hosts are generally species specific but have been identified for only half of all *Sarcocystis* species. Human intestinal sarcocystosis, in which humans are the definitive hosts, is caused by one of two species, *Sarcocystis hominis* or *Sarcocystis suihominis.* Humans may also be accidental intermediate hosts for other *Sarcocystis* species, leading to human muscular sarcocystosis.

Life Cycle

Through the ingestion of poorly cooked or raw meat containing tissue cysts, humans may serve as definitive hosts for *Sarcocystis* in pigs (*S. suihominis*) and cattle (*S. hominis*). Humans may also be incidental intermediate hosts when food or water contaminated with fecal sporocysts is ingested.

After consumption of tissue cysts by the definitive host (usually a carnivore), motile bradyzoites emerge from sarcocysts and enter the intestinal lamina propria. Bradyzoites mature into male and female forms, and sexual reproduction follows in the intestinal mucosa; mature oocysts, each of which contains two sporocysts, are formed. The thin walls of oocysts are readily disrupted, leading to shedding of both oocysts and infectious sporocysts in the feces. In contrast, sporocysts are hardy, resisting treatment with bleach, chlorhexidine, and iodophors. Each sporocyst measures approximately 10 by 15 μm and contains four sporozoites.

After ingestion of sporocysts by the intermediate host (usually an herbivore), sporozoites are released, penetrate the intestinal epithelium, and migrate to vascular endothelium, where they undergo cycles of asexual multiplication. The resulting merozoites are then hematogenously disseminated and invade cardiac or striated muscle cells. Within muscle, the characteristic septate cysts (sarcocysts) containing bradyzoites develop. Sarcocysts become infectious only after they have matured, a process that may take 2 months or more depending on the species. The cycle is complete when mature muscle cysts are eaten by an appropriate definitive host.

Epidemiology

Although worldwide in distribution, most human cases of *Sarcocystis* infection have been reported from tropical and subtropical climes, mainly Southeast Asia and, in particular, Malaysia. Cases have been less commonly reported from other continents. Identification of *Sarcocystis* in stool or muscle, or of antibodies in serum, is most often an incidental finding. As many as 20% of residents in some endemic areas are seropositive, reflecting the sanitary conditions and dietary habits in these regions.[51] In 2011, an outbreak of suspected symptomatic muscular sarcocystosis was first reported among travelers to Tioman Island, Malaysia,[52] with the outbreak continuing into 2012.[53] To date, over 100 travelers are infected, and no definite source has been identified. Studies after an outbreak of 89 cases associated with a trip to Pangkor Island, Malaysia,[54] demonstrated molecular identity of the disease isolates with *Sarcocystis nesbitti* found in the stool of snakes (*Naja naja*), with the route of transmission presumably being the consumption of water or food contaminated with snake feces.[55]

Sarcocystis infections are common in many other animals, including nonhuman primates, cattle, dogs, cats, rodents, and reptiles, among others.

Clinical Manifestations

Most individuals with either intestinal or muscular sarcocystosis have mild symptoms or are asymptomatic. Development of symptoms may depend in part on the species of sarcocysts that are ingested.[56] In human volunteers who have ingested pork or beef containing *Sarcocystis* species, a mild self-limited gastrointestinal illness developed in some, whereas others passed sarcocysts but remained asymptomatic. Symptoms induced by experimental challenge with infected meat include nausea, abdominal discomfort, and self-limited nonbloody diarrhea, with symptom severity dependent on the amount of meat consumed.[56] Onset of diarrhea is generally rapid, within 48 hours of ingestion, and illness is typically brief and self-limited. Segmental eosinophilic and necrotizing enteritis attributed to sexual forms of *Sarcocystis* has been reported; however, causation in these cases was not definitely established.[57]

Muscular sarcocystosis is thought to be typically asymptomatic. Muscle cysts vary greatly in size, ranging from 50 μm to 5 cm. Eosinophilic myositis has been reported, occurring in association with other symptoms, including fever, bronchospasm, transient pruritic rashes, lymphadenopathy, subcutaneous nodules, and arthralgias.[58] In the first Malaysian outbreak, travelers had myalgias and most had fever, in conjunction with eosinophilia. The hallmark of the illness was clinically significant myositis that occurred 30 days or more after travel.[53] A second outbreak in persons attending a retreat on Pangkor Island was attributed to *S. nesbitti.*[54] The onset of symptoms occurred between days 1 and 26 after the retreat, with the majority (70.7%) beginning between days 9 and 11. The median duration of symptoms was 17 days. The disease was characterized as an acute, febrile, relapsing myositis. Myocarditis seems to be rare. Other reports include systemic disease in one patient with AIDS, with organisms recovered from the stool, small bowel, liver, and blood[59]; acalculous cholecystitis requiring cholecystectomy in a second patient with AIDS[60]; and laryngeal sarcocystosis.[61]

Diagnosis

Sarcocystosis can be suspected when compatible symptoms are present in conjunction with a history of recent consumption of raw or undercooked meat in an endemic area. Sporocysts may be identified in the stool of symptomatic (and asymptomatic) individuals with intestinal sarcocystosis. Oocysts (Fig. 283.3) are less commonly visualized in stool samples owing to the fragility of their cell walls. Brightfield microscopy and fecal flotation wet mounts are optimal, using density gradient media rather than other sedimentation methods.[56] The Kato thick smear technique also has excellent sensitivity for diagnosis.[62] Speciation cannot be determined by microscopy.

For patients with symptoms suggestive of acute muscular disease, muscle biopsy using conventional histologic staining can demonstrate sarcocysts, although staining may be variable. Myositis and myonecrosis associated with mixed inflammatory infiltrates and sometimes tissue eosinophilia may be seen in cases of acute muscular sarcocystosis.[58]

Serology using Western blot assay is highly suggestive of previous exposure but is not diagnostic for acute disease and is not believed to be able to distinguish between intestinal and muscular disease.

Therapy

No specific treatment for *Sarcocystis* infection is known. Infection, if symptomatic, is generally self-limited. Albendazole suppressed symptoms in one human case but was not curative.[58] TMP-SMX has been used



FIG. 283.3 Mature oocyst of *Sarcocystis*, containing two readily visualized sporocysts. *(From DPDx Image Library, Centers for Disease Control and Prevention, Atlanta, GA.)*

with apparent clinical effect in several cases.[63] The efficacy of other antiparasitic agents in treating human disease is unknown. Corticosteroids may provide symptomatic relief in cases of acute myositis.

Prevention

Intestinal sarcocystosis can be prevented by ensuring meat is cooked or frozen such that bradyzoites are killed. Muscular sarcocystosis can be prevented by reducing individual exposures to sporocyst ingestion. This may be challenging given potential environmental seeding and contamination of food and water; however, drinking filtered, boiled, or bottled water and thorough cooking of food that may be contaminated with sporocysts can mitigate the risk of infection.

BALANTIDIUM COLI

Balantidium coli was identified in 1857. It is the largest protozoan and the only ciliate pathogen of humans.

Life Cycle

After ingestion of cysts via contaminated food or water, trophozoites are released in the small intestine. The oval trophozoite usually measures 30 to 150 µm in length and 25 to 120 µm in width but may reach 200 µm in length. Its surface is covered with tiny cilia that propel it through the intestinal lumen. Trophozoites migrate to the large intestine, where they multiply in the colon wall and formation of cysts occurs. Cysts, which measure 40 to 60 µm in diameter, are the infective forms of the organism and survive well in the external environment.

Epidemiology

B. coli has a worldwide distribution but is most frequently reported from Latin America, Southeast Asia, Papua New Guinea, and parts of the Middle East. Although *B. coli* is found in many mammals, domestic and wild pigs are considered to be the main reservoir for human infection, with prevalence rates of 40% to 100%.[64] In humans, the prevalence is usually less than 1%; higher rates have been reported among individuals in hyperendemic areas and residential institutions. Human infection most often results from the ingestion of produce or water contaminated with pig excrement or from handling of the animal. An increase in the population of feral pigs in Australia was associated with the first reported case of balantidiasis on that continent.[65] Person-to-person transmission can also occur. However, humans are generally resistant to infection, and poor nutrition and underlying debility seem to be risk factors for disease.[64]

Clinical Manifestations

Most infections are asymptomatic. Clinical manifestations may include a chronic course characterized by intermittent diarrhea, abdominal pain, and weight loss. Rarely, a more fulminant colitis with blood and mucus in stools may occur; this may lead to intestinal perforation with subsequent peritonitis or extraintestinal disease.[66–68] Rare presentations include a fatal case of pneumonia in a patient with cancer, pulmonary hemorrhage, symptomatic (hematuria) and asymptomatic urinary infection, vertebral osteomyelitis, liver abscess, and keratitis.[69–75]

Diagnosis and Therapy

Balantidiasis can be diagnosed by finding rapidly motile trophozoites (Fig. 283.4A) in fresh or preserved stool; cysts (Fig. 283.4B) are infrequently detected. In invasive intestinal disease, endoscopic findings include necrosis and ulceration similar to those found in invasive amebiasis, bacterial dysentery, and inflammatory bowel disease; trophozoites may be recovered from scrapings of the periphery of ulcers detected on endoscopic examination. Pulmonary disease may be diagnosed by the identification of trophozoites in bronchoalveolar lavage specimens.

Tetracycline is the treatment of choice; metronidazole and iodoquinol are alternatives (Table 283.3).[47] Nitazoxanide has also been used effectively. Courses of therapy for as long as 20 days may be required for cure in persons with HIV infection.[76]

BLASTOCYSTIS SPECIES

Despite its high prevalence throughout the world, major issues about *Blastocystis* remain unresolved, including its taxonomy and pathogenicity.[77,78] Uncertainties about the role of *Blastocystis* in causing human disease are due to challenges in diagnosis and increasing recognition of varying pathogenicities of different *Blastocystis* subtypes.

TABLE 283.3 Therapy (Adult) for Balantidiasis

DRUG[a]	DOSAGE
Tetracycline	500 mg PO qid for 10 days
Metronidazole	750 mg PO tid for 5 days
Iodoquinol	650 mg PO tid for 20 days

[a]Drugs are listed in order of preference.
PO, Orally.

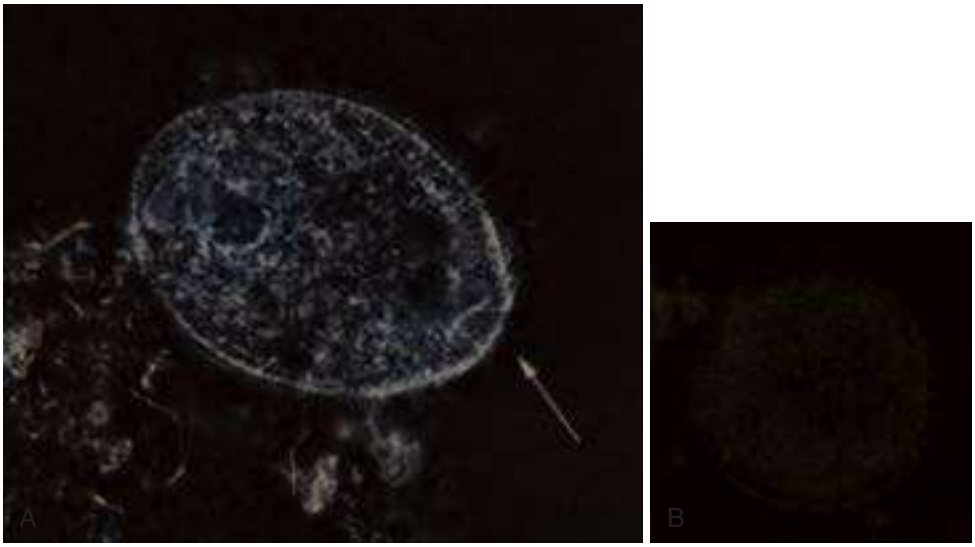

FIG. 283.4 *Balantidium coli* trophozoite and cyst. (A) *B. coli* trophozoite demonstrating cilia on the cell surface *(arrow).* A bean-shaped macronucleus is visible toward the left of the trophozoite. (B) Wet mount of an unstained *B. coli* cyst. Cysts are infrequently visualized in stool. *(From DPDx Image Library, Centers for Disease Control and Prevention, Atlanta, GA.)*

Sequences of the small subunit ribosomal RNA gene place *Blastocystis* in the stramenopiles, a diverse group of protist organisms that includes brown algae and diatoms[79]; under the six-kingdom classification, it is placed in the kingdom Chromista. Analysis of the elongation factor-1α gene, however, has indicated relatedness to *Entamoeba histolytica.*[80] *Blastocystis* lacks a cell wall, contains organelles with features of a protozoan, and reproduces asexually, usually by binary fission. The organism exhibits considerable morphologic variability and karyotype diversity. At least 17 species from humans and animals have been identified, 9 of which have been identified in humans.[81] As a result, *Blastocystis* in humans is referred to as *Blastocystis* spp. rather than *Blastocystis hominis.* Subtypes 1 to 4, and particularly subtype 3, are most frequently found in humans. Humans may also be infected by *Blastocystis* species of other primates, mammals, rodents, and birds.[82]

Life Cycle

Four major forms of *Blastocystis* predominate: vacuolar, granular, ameboid, and cystic forms. All four forms contain cytoplasm and organelles. The vacuolar form, with a large central vacuole, usually measures between 4 and 15 µm but is highly variable in size and is the most frequently detected form in fecal specimens. The granular form is similar to the vacuolar form but is characterized by intracytoplasmic or intravacuolar granules. The ameboid form, rarely visualized, has been considered to be important in pathogenesis.[83] More recently, cysts measuring 2 to 5 µm have been identified; they may be readily misidentified as fecal debris owing to their small size. Multivacuolar and avacuolar forms have also been described.

Various life cycles have been proposed for *B. hominis.* Currently, it is believed that after ingestion of infective cysts, excystation occurs in the large intestine. Cysts then develop into vacuolar forms and undergo encystation before being excreted in stool.[82]

Epidemiology

Whereas *Blastocystis* is found worldwide, differences in the geographic distribution of specific subtypes have been reported, although definitive conclusions may be difficult to make given the varying diagnostic techniques used in different studies. For example, subtype 3 appears to be present globally and is the most commonly identified subtype among human isolates,[84] whereas subtype 4 is prevalent in Europe[85] but is uncommon in Asia.[86] The significance of these observations is unclear. Regardless, the prevalence of blastocystosis in humans seems to be higher in developing countries (30%–50%) than in developed countries (1.5%–10%).[87] However, even within a given country, reported prevalence rates vary greatly; in China, for example, the prevalence in four communities ranged from 1.9% to 32.6%.[78] With the advent of PCR, previous epidemiologic study results may be found to be significantly underestimated, as noted by a recent study from Senegal showing a 100% infection rate.[88] Such variability may reflect differences not only in socioeconomic status or hygienic conditions but also in local customs and living conditions.

Risk factors associated with infection include immune compromise, travel to and immigration from developing countries, and exposure to contaminated food and water.[82] Among Polish soldiers involved in peacekeeping missions to Iraq and Afghanistan, *B. hominis* was identified in 15.3%.[89]

Pathogenicity

The role of *Blastocystis* in causing disease remains controversial. It has been hypothesized that there may be virulent and avirulent strains of *Blastocystis,* which could account for the variability in symptoms. However, most studies fail to show a definite correlation between pathogenicity and subtypes or genotypes.[90,91] Evidence that *Blastocystis* is causally linked to intestinal disease is based on numerous case reports and uncontrolled or retrospective series in which infection was associated with nonspecific gastrointestinal symptoms. In many studies that have included asymptomatic controls, neither the identification of *Blastocystis* in stool samples nor the concentration of organisms in stool has correlated with the presence of gastrointestinal symptoms. However, observational studies of TMP-SMX therapy[92] and small placebo-controlled studies with metronidazole[93] and nitazoxanide[94] have demonstrated symptomatic improvement coincident with reduction or elimination of the organism in stool and suggest that *Blastocystis* may be pathogenic. The pathogenicity of the organism remains unclear because of the uncertainty that other causes of symptoms, including other pathogens, have been eliminated in case series and treatment trials, and the inability of case-control studies to determine the association of a low-grade pathogen with disease.

Although some authors have suggested that *Blastocystis* is most likely to be pathogenic when large numbers of organisms are present (>5/oil immersion field), no association has been noted between number of organisms and symptoms.[95] In addition, since the parasite is intermittently excreted, there is often no consistency in organism count.

Clinical Manifestations

Clinical signs and symptoms attributed to *Blastocystis* typically include acute or chronic diarrhea, bloating, flatulence, abdominal cramps, and fatigue. Irritable bowel syndrome (IBS) has been associated with *Blastocystis* in some studies. A meta-analysis of epidemiologic studies of *Blastocystis* species showed that there was a 2.34-fold relative risk of *Blastocystis* infection in those with IBS.[96] Cases of refractory ulcerative colitis[97] recurrent megacolon,[98] and invasive colonic disease[99] caused by *Blastocystis* have been reported. Extraintestinal manifestations attributed to *Blastocystis* infection, including generalized urticaria and iron-deficiency anemia during pregnancy, have also been described.

Diagnosis

Until the recent availability of PCR assays, diagnosis relied primarily on the demonstration of parasites in stool. Microscopic diagnosis is challenging owing to the variable forms and sizes of *Blastocystis,* and sensitivity is poor compared with PCR assay.[100,101] *Blastocystis* may be visualized in wet mounts (Fig. 283.5A), but permanent smears are preferred for microscopic diagnosis. The trichrome stain (see Fig. 283.5B) seems to be the most sensitive stain for detection. Concentration methods may be less sensitive than other microscopic techniques. Use of electron microscopy or immunofluorescence assay for diagnosis is neither practical nor readily available. In vitro cell cultivation requires 48 to 72 hours for incubation but is more sensitive than microscopy and concentration methods,[100] especially if small numbers of organisms are present. PCR assay is not only highly sensitive and specific but also facilitates subtyping[100–102]; this technique is becoming widely available for routine diagnosis. Serology is not useful in diagnosing infection.

Therapy

Treatment of asymptomatic infections is unnecessary.[103] Symptoms attributed to *Blastocystis* infection are often self-limited, and therapy for "symptomatic" infections should be withheld until other causes of intestinal symptoms have been ruled out. Treatment of blastocystosis is often unsatisfactory; metronidazole, TMP-SMX, iodoquinol, and paromomycin are the most commonly recommended therapies[47] (Table 283.4) but are variably successful.[92,93,104] Nitazoxanide[94] and *Saccharomyces boulardii*[105] have been used with good effect in limited clinical studies. In one small study of 12 isolates, in vitro susceptibility tests did not support the efficacy of many commonly used agents, such as metronidazole, paromomycin, or triple therapy with furazolidone,

TABLE 283.4 Therapy (Adult) for Blastocystosis

DRUG[a]	DOSAGE
Metronidazole	750 mg PO tid or 1.5 g daily, for 10 days
Trimethoprim-sulfamethoxazole	1 DS tablet[b] bid or 2 DS tablets daily for 7 days
Iodoquinol	650 mg PO tid for 20 days
Nitazoxanide	500 mg bid for 3 days
Paromomycin	25–35 mg/kg divided tid for 7 days

[a]Drugs are listed in order of preference.
[b]One DS tablet contains 160 mg trimethoprim/800 mg sulfamethoxazole.
DS, Double strength; *PO,* orally.

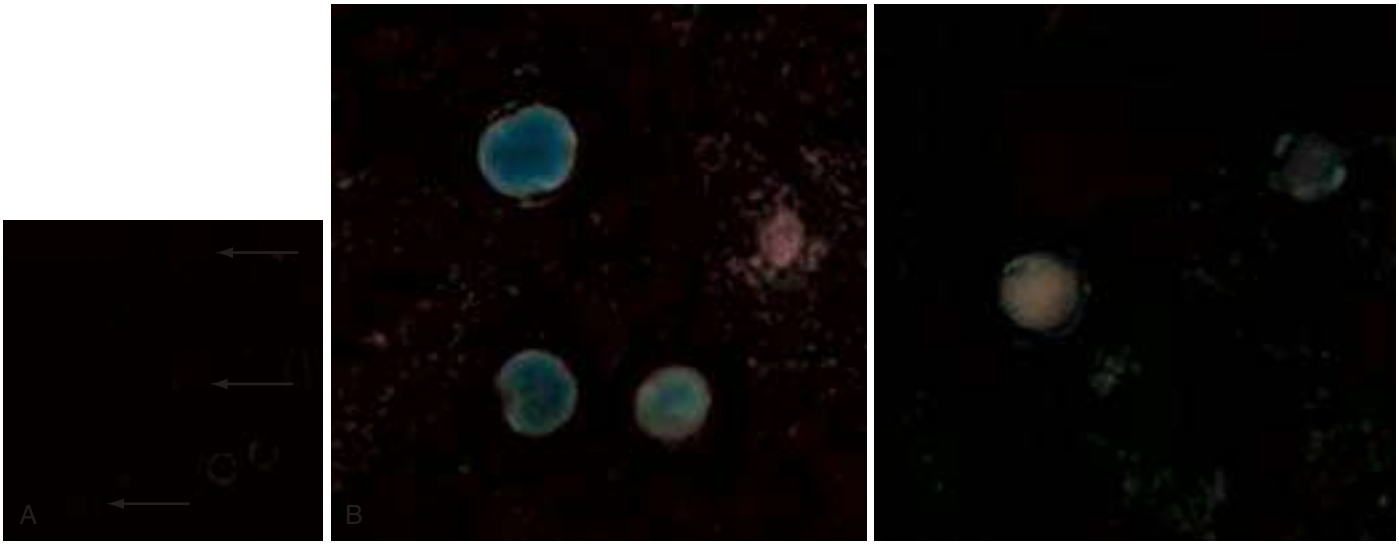

FIG. 283.5 ***Blastocystis hominis* cysts.** (A) Unstained wet mount. Arrows indicate cysts. (B) Stained with trichrome. Vacuoles vary from red to blue. *(From DPDx Image Library, Centers for Disease Control and Prevention, Atlanta, GA.)*

nitazoxanide, and secnidazole, but consideration was offered on the possible utility of TMP-SMX or ivermectin.[106] However, in vitro data do not always correlate with clinical studies that do show therapeutic efficacy, for example, in studies of metronidazole, paromomycin, and nitazoxanide.[93,94,107] It is important to remember that symptomatic improvement in those who are successfully treated for blastocystosis may be due to other drug effects on the microbiome or on organisms that were not detected prior to treatment.

ACKNOWLEDGMENT

The authors acknowledge Dr. Doug Esposito for his review of the *Sarcocystis* section in the previous edition of this chapter.

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284 Human Illness Associated With Harmful Algal Blooms

J. Glenn Morris, Jr.

SHORT VIEW SUMMARY

Definition
- Illnesses of primary clinical concern related to preformed toxins produced by algal species include ciguatera fish poisoning, paralytic shellfish poisoning, and amnesic shellfish poisoning.

Epidemiology and Microbiology
- Depending on the agent, illness is caused by ingestion of toxins in seafood or by inhalation or skin contact.
- Occurrence of illness is usually linked with blooms of specific toxic algal species (see Table 284.1). Harmful algal bloom–related illnesses in the United States have been reported from the Atlantic, Pacific, and Gulf Coasts; Hawaii; Alaska; and the Caribbean (see Fig. 284.1).

Diagnosis
- Diagnosis is based on clinical presentation (see Table 284.1).

Therapy
- Therapy is supportive. The syndrome of hypotension/bradycardia seen in severe cases of ciguatera fish poisoning may require atropine; respiratory support may be necessary in severe cases of paralytic shellfish poisoning.

Prevention
- Prevention is based on avoidance of fish or shellfish that contain toxins. Environmental monitoring of shellfish for toxins responsible for paralytic, amnesic, and neurotoxic shellfish poisoning is conducted by state and local health departments.

During the past several decades, the association of human health and environmental problems with harmful and toxic algae has been increasingly recognized,[1,2] as has awareness of the complex range of natural toxins (and toxin congeners) that can be produced by these microorganisms. Toxic species constitute a small percentage of the thousands of species of microscopic algae at the base of the marine food chain. However, when these species proliferate, they may cause massive killing of fish and shellfish, the death of marine mammals and seabirds, alterations in marine habitats, and, with specific exposure, human illness and death. Although blooms of certain species such as *Karenia brevis* (formerly known as *Gymnodinium breve*) may be manifested as red tides, adverse events often occur in the absence of visible discoloration of water.

Harmful algal blooms appear to be increasing in frequency; in the United States, problems that in the past were confined to a few geographic locations are now being seen at multiple sites along the US coastline (Fig. 284.1). The factors leading to this apparent increase in incidence are not well understood, although it has been postulated that human-related phenomena such as nutrient enrichment of waterways, climatic change, and disruption of ecosystems play a role.[1,3–9] These are, however, complex systems, and there may be wide variability in incidence rates, with a number of factors influencing risk for disease.[10]

Eight clinical syndromes or illnesses are currently linked with harmful algal blooms (Table 284.1).[1] As more research is done in this area, it is possible that other syndromes will be identified. Ciguatera fish poisoning, paralytic shellfish poisoning, and neurotoxic shellfish poisoning are also described in Chapter 101.

CIGUATERA FISH POISONING

Worldwide, ciguatera fish poisoning is the most clinical syndrome associated with marine biotoxins, with estimates that global case numbers range from 50,000 to 500,000 per year. It is a major public health problem in the Caribbean and South Pacific regions, particularly in areas with tropical reefs.[8,10–14] Illness is caused by ciguatoxins that are passed up the marine food chain, with large predatory reef fish (e.g., barracuda, jacks, snappers, moray eels) posing the greatest risk for toxicity.[14,15] A 2013 study in the US Virgin Islands found that 12% of lionfish (a highly invasive species being found with increasing frequency on tropical reefs in Florida and the Caribbean) had potentially toxic levels of ciguatoxin,[16] suggesting that recommendations to "eat more lionfish" to save reefs may be ill-advised.

Toxins are produced by dinoflagellates within at least eight species in the genera *Gambierdiscus* and *Fukuyoa,*[14,17,18] with toxicity varying by species. Increases in numbers of these toxic dinoflagellates have been associated with disruption of normal reef ecology and reef communities, including disruption by storms, human activity, and climate change.[3,14] Multiple ciguatoxin conjoiners have been identified, with ciguatoxins from the Pacific having slight structural differences from ciguatoxins found in the Caribbean. The toxin acts by stimulation of mucosal ion transport in the gastrointestinal tract and interaction with voltage-gated sodium channels along the peripheral nerves; in animal studies, it has been shown to cross the blood-brain barrier.

Among patients with ciguatera fish poisoning, gastrointestinal symptoms—nausea, vomiting, and diarrhea—are usually the presenting symptoms, occurring within 6 to 24 hours of eating a toxic fish. Patients typically present to emergency departments in the early hours of the morning after unknowingly eating a toxic fish for dinner. Within 12 to 48 hours of onset of illness, most patients also begin to experience neurologic symptoms, including headache; pain and weakness in the legs; and dysesthesias such as tingling sensations in the extremities and around the mouth, cold allodynia (cold objects feeling burning hot), burning sensation in the mouth, and aching pain around the teeth. These neurologic symptoms may persist for weeks to months[13,19] and may be linked with clinical depression. In a small percentage of cases, this can lead to a chronic/recurring syndrome that has many of the manifestations of chronic fatigue syndrome.

In severe cases, patients may be acutely bradycardic and hypotensive; in the Pacific, respiratory difficulties have also been reported. Restlessness and confusion may occur, and seizures and coma have rarely been reported. Deaths are exceedingly rare and are generally associated with high toxin exposure (consumption of viscera of a highly toxic fish), often in the setting of underlying cardiac or respiratory illness.[15] The diagnosis of ciguatera fish poisoning is clinical, based on the combination

TABLE 284.1 Human Illness Associated With Harmful Algal Blooms

SYNDROME	CAUSATIVE ORGANISMS	TOXIN PRODUCED	CLINICAL MANIFESTATIONS
Ciguatera fish poisoning	*Gambierdiscus* spp. and others	Ciguatoxin	Acute gastroenteritis followed by paresthesias and other neurologic symptoms
Paralytic shellfish poisoning	*Alexandrium* spp. and others	Saxitoxins	Acute paresthesias and other neurologic manifestations; may progress rapidly to respiratory paralysis
Neurotoxic shellfish poisoning	*Karenia brevis*	Brevetoxins	Gastrointestinal and neurologic symptoms; formation of toxic aerosols by wave action can produce respiratory irritation and asthma-like symptoms
Diarrhetic shellfish poisoning	*Dinophysis* spp.	Okadaic acid and others	Acute gastroenteritis, abdominal pain
Amnesic shellfish poisoning	*Pseudo-nitzschia* spp.	Domoic acid	In acute cases, gastroenteritis followed by memory loss, neurologic manifestations; may progress to amnesia, coma, and death; chronic, low-level exposure may result in mild memory loss
Azaspiracid shellfish poisoning	*Azadinium* spp. and others	Azaspiracid	Acute gastroenteritis, abdominal pain
Cyanobacteria exposure syndromes, *Lyngbya*	*Lyngbya* spp. *Microcystis* spp.	Lyngbyatoxin A, debromaplysiatoxin Microcystins ? BMAA	Swimmers' itch, particularly in inguinal area; sore eyes, ears; headache; possibly gastrointestinal symptoms Possible hepatotoxicity, neurotoxicity
Pfiesteria-associated syndrome	*Pfiesteria* spp. (?)	Unidentified to date	Deficiencies in learning and memory; acute respiratory and eye irritation; acute confusional syndrome

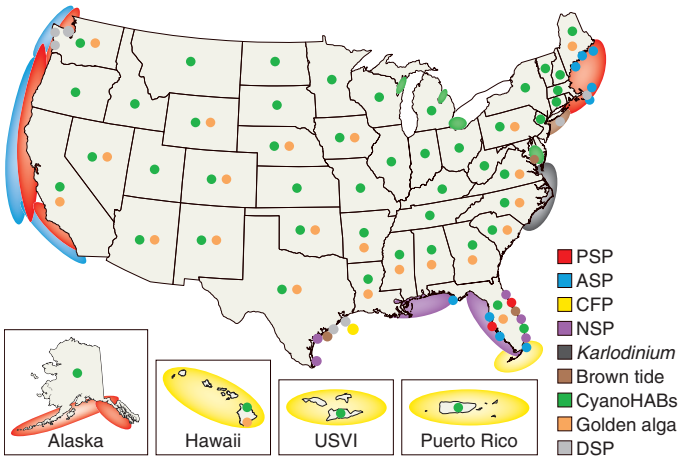

FIG. 284.1 US map depicting the various harmful algal bloom poisoning syndromes and other impacts that occur in specific areas. Colored dots or ovals indicate locations where the incidence of a particular syndrome has been reported or where toxins have been detected in tissue extracts or plankton. Ovals are used to indicate regional phenomena that occur at multiple locations along a coastline. All 50 states are impacted by cyanobacterial harmful algal blooms *(cyanoHABs)*, typically in many different rivers, streams, reservoirs, and so forth. The same is true for 23 states impacted by golden algal blooms caused by *Prymnesium parvum*. It is not practical to indicate the location of each cyanoHAB or golden algal bloom, so each state experiencing these blooms is indicated using a single green or gold dot. Larger green ovals denote widespread cyanoHAB problems in those areas. *ASP,* Amnesic shellfish poisoning; *CFP,* ciguatera fish poisoning; *DSP,* diarrhetic shellfish poisoning; *NSP,* neurotoxic shellfish poisoning; *PSP,* paralytic shellfish poisoning; *USVI,* US Virgin Islands. *(From National Office for Harmful Algal Blooms at Woods Hole Oceanographic Institution. Distributions of HABs in the U.S. Updated 2016.* http://www.whoi.edu/redtide/regions/us-distribution.*)*

of gastrointestinal and characteristic neurologic symptoms occurring after eating a reef fish that carries a risk for toxicity. Other individuals who have eaten the same fish may also be ill, although not everyone who eats a toxic fish will manifest symptoms. Individuals who have had prior episodes of ciguatera fish poisoning are more likely to be symptomatic, and there is a suggestion that alcohol consumption increases symptom risk. There are no confirmatory laboratory tests for illness. Identification of toxin in fish is possible but technically difficult. In the United States, testing is available only through the US Food and Drug Administration laboratories as part of an outbreak investigation.

Treatment is symptomatic and includes maintenance of adequate hydration, use of atropine for bradycardia/hypotension, and administration of analgesics and antidepressants as appropriate. For severe cases in the Pacific, monitoring of respiratory status is indicated. Some literature suggests that intravenous mannitol alleviates acute symptoms, and use of mannitol is common in emergency departments in endemic areas; however, no benefit with use of mannitol was seen in one double-blind randomized clinical trial.[20] At an anecdotal level, case reports suggest that neurologic manifestations, including pain syndromes, can be reduced by drugs such as amitriptyline, nifedipine, gabapentin, or pregabalin.[21]

Prevention is difficult because the toxin is not inactivated by cooking, and toxic fish have a normal appearance and taste. For native populations in endemic areas, prevention requires avoidance of high-risk fish from reef areas known to be toxic. Families concerned about the toxicity of a specific fish often report the use of crude bioassays, including feeding of suspect fish to the family cat.

In endemic areas in the Pacific and Caribbean, ciguatera fish poisoning can have major economic and nutritional impact, as local populations are often reluctant to eat locally caught fish because of the risk for illness. Reflecting these concerns, tourist hotels and restaurants in endemic areas such as the Caribbean tend to import all of their seafood from nonendemic regions. Cases are common in local populations in Puerto Rico and the US Virgin Islands, where the annual incidence in one study was estimated at 1200 cases per 100,000 population.[10] Cases seen in South Florida and Hawaii are generally associated with recreational fishing in reef areas. In Florida, data from a population survey are consistent with an incidence of between 5 and 6 cases per 100,000 population per year; rates are highest in Hispanic populations (relative risk, 3.4), possibly due to differences in patterns of fish consumption, including increased consumption of high-risk fish such as barracuda.[22]

PARALYTIC SHELLFISH POISONING

Based on reported cases, paralytic shellfish poisoning is one of the most common causes of marine biotoxin–associated illness in the continental United States and Alaska.[23,24] Illness has traditionally been associated with eating clams and mussels that contain saxitoxins produced by *Alexandrium* spp. and related dinoflagellates, although a variety of other vehicles have been reported.[25] Saxitoxins exert their effect by binding directly to the voltage-dependent sodium channels in nerve and muscle cell membranes, interrupting nerve signal transmission and leading to paralysis.

In contrast to ciguatera poisoning, gastrointestinal symptoms are less prominent than neurologic manifestations. Circumoral paresthesias and paresthesias of the extremities usually appear within 1 hour of

ingesting toxic shellfish, and they may be accompanied by ataxia, dysphagia, and changes in mental status. Hypertension may occur and corresponds to the extent of the ingested dose; in the most severe cases, patients may proceed to respiratory paralysis, usually within the first 24 hours of illness. Assays are available for identification of saxitoxins in serum and urine samples from affected patients, albeit at an experimental level.[26,27] Treatment is symptomatic; respiratory support may be necessary in the most severe cases.

Prevention is achieved through regular monitoring of shellfish populations for saxitoxin by public health authorities, with sampling data available on state health department web pages (e.g., the biotoxin web page of the Washington State Department of Health, www.doh.wa.gov/CommunityandEnvironment/Shellfish.aspx). Blooms of toxic *Alexandrium* spp. occur primarily between April and October along cold water marine coasts. In North America, this includes Alaska, the Pacific Northwest, and the St. Lawrence Seaway region of Canada. Toxic shellfish have also been found in cold water regions of southern Chile, England, Japan, and the North Sea. Most shellfish remain toxic for several weeks after a bloom subsides, although some shellfish species including butter clams may retain toxicity for more than a year.

NEUROTOXIC SHELLFISH POISONING

Illness is caused by brevetoxins produced by *Karenia brevis,* a major cause of red tides along the Florida coast; other *Karenia* spp. have been implicated in illness in other parts of the world. Ingestion of shellfish containing the toxin causes nausea and vomiting and circumoral paresthesias and paresthesias of the extremities. In more severe cases, patients may report ataxia, slurred speech, dizziness, and, in rare cases, mild respiratory distress.[28] Aerosolization of toxins by heavy wave action on the Atlantic coast of Florida can result in respiratory irritation and asthma-like symptoms in people walking along affected beaches.[29] Brevetoxin metabolites have been identified in urine samples from affected patients.[30] Treatment is symptomatic. The Florida Department of Health and other health authorities regularly monitor coastal areas for the presence of *K. brevis,* and they notify consumers accordingly. Data on occurrence of the organism in Florida waters are posted on the website of the Florida Department of Health (http://www.floridahealth.gov/environmental-health/aquatic-toxins/red-tide.html).

DIARRHETIC SHELLFISH POISONING

Diarrhetic shellfish poisoning results from eating mussels, scallops, or clams that have been feeding on toxic *Dinophysis* spp. or *Prorocentrum* spp. Symptoms include diarrhea, nausea, vomiting, and abdominal pain (which may be quite severe). Although okadaic acid appears to be the primary toxin responsible for the observed clinical syndrome, other toxic compounds have been isolated from these species. Although case reports came initially from Japan, diarrhetic shellfish poisoning has now been reported from multiple locations in Europe, Asia, South America, South Africa, and North America.

AMNESIC SHELLFISH POISONING

Amnesic shellfish poisoning results from the ingestion of shellfish containing domoic acid, which is produced by the diatom *Pseudo-nitzschia.*[1] An outbreak of illnesses caused by this toxin was reported in the Atlantic provinces of Canada in 1987.[31] Symptoms included vomiting, abdominal cramps, diarrhea, headache, and loss of short-term memory. On neuropsychological testing several months after the acute intoxication, patients were found to have severe antegrade memory deficits with relative preservation of other cognitive functions; patients also had clinical and electromyographic evidence of pure motor or sensorimotor neuropathy or axonopathy. In four patients who died, neuropathologic studies demonstrated neuronal necrosis and loss, predominantly in the hippocampus and amygdala.[32] Canadian authorities now analyze mussels and clams for domoic acid, and they close shellfish beds to harvesting when levels in shellfish exceed 20 mg/g.

Domoic acid has been identified in the marine food web in multiple locations in the United States including the Monterey Bay and Puget Sound areas. Elevated levels of domoic acid have been linked to neurologic illness and death in seabirds and sea lions[33] in these areas, possibly in relation to consumption of shellfish or anchovies; the toxin itself has been identified in 13 species of marine mammals in Alaska, in both adult and fetal tissue.[25] No cases of acute, severe amnesic shellfish poisoning comparable to the cases initially reported from Canada in 1987 have been confirmed in humans in the United States. However, a study of subsistence shellfish eating in Native American tribes in the Puget Sound area found that during years when high domoic acid levels were present in shellfish, infants born to shellfish-eating mothers had a significantly lower score on the Mental Developmental Index compared with infants born in other years.[34] An analysis of 4 years of data from this same cohort demonstrated a slight, albeit statistically significant, reduction in memory functioning in adults who consumed 15 or more razor clams per month (razor clams are a major source of domoic acid exposure in this population).[35] These data suggest that exposure to elevated domoic acid levels represents a health risk to infants and children and raise questions about the effect of chronic, low-level exposure to the toxin. In response to these data, the Washington State Department of Health in 2017 released an advisory recommending that people limit razor clam consumption to fewer than 15 clams per month. Further studies are needed to define the levels of domoic acid that may represent a risk in the setting of chronic exposure; it is likely that this level will be substantially below the current 20 mg/g limit currently used as the basis for closure of shellfish harvesting areas.

AZASPIRACID SHELLFISH POISONING

In 1995 an outbreak of shellfish-associated illness that very closely resembled diarrhetic shellfish poisoning was reported in The Netherlands. Subsequent studies demonstrated that the implicated shellfish were contaminated not with okadaic acid (the cause of diarrhetic shellfish poisoning) but with what is now known as azaspiracid, a class of toxins linked with dinoflagellates in the genus *Azadinium* (and possibly other related dinoflagellates in Amphidomataceae).[36] Toxic shellfish have subsequently been identified in multiple outbreaks of azaspiracid shellfish poisoning in western Europe and in Morocco.[37] Although no cases of azaspiracid shellfish poisoning have been reported to date in the United States, *Azadinium* spp. producing a new azaspiracid toxin have been identified in Puget Sound.[38]

CYANOBACTERIA EXPOSURE SYNDROMES, INCLUDING *LYNGBYA*

Cyanobacterial species can produce thick, foul-smelling, high-biomass blooms (triggered in many instances by increasing nutrient flows) that have been linked to human illness, animal mortality, and adverse ecosystem and economic effects in the United States and worldwide. Cyanobacterial species can produce microcystins, which are well recognized as hepatotoxins. While their role in causing liver disease in human populations remains controversial, there are increasing data that suggest a link between exposure to blooms and occurrence of illness.[39] There has also been recent interest in the cyanobacterial toxin beta-N-methylamino-L-alanine (BMAA), which has been implicated as a possible cause of neurodegenerative disease, including amyotrophic lateral sclerosis (AML).[39a] *Lyngbya* spp. have been implicated in a series of studies as a cause of skin itching (swimmers' itch), particularly in the inguinal region; sore eyes and ears; headache; and, in a small percentage of patients, abdominal pain and vomiting.[40,41]

PFIESTERIA-ASSOCIATED SYNDROME

Pfiesteria was first isolated during the early 1990s as a suspected cause of massive fish killings in the New River and Albemarle-Pamlico estuarine system of North Carolina, with respiratory symptoms, rashes, and problems with cognition being reported among laboratory personnel working with the microorganism. In subsequent studies conducted in the Chesapeake Bay region,[42] a significant association was found between the degree of exposure to waterways where *Pfiesteria* was known to be present and short-term deficiencies (<6 months' duration) in learning, memory, and higher-order cognitive function. No further outbreaks or cases have been reported, and, in the absence of identification of a toxin or other laboratory confirmation, questions remain about the role of *Pfiesteria* in the observed symptoms.

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J Diseases Due to Helminths

285 Introduction to Helminth Infections

James H. Maguire[a]

The helminthiases are among the most prevalent infections in the world and a leading cause of morbidity, particularly in low-income and resource-constrained regions. An estimated 1.5 billion persons harbor at least one species of parasitic worm.[1–3] The helminths that parasitize humans include the nematodes (roundworms) and platyhelminths (flatworms); the latter group consists of cestodes (tapeworms) and trematodes (schistosomes and other flukes). Leeches, ectoparasites belonging to the phylum Annelida (segmented worms), are not discussed here (see Chapter 291). Some helminths are exclusively or primarily human parasites, whereas others parasitize both humans and various other mammals, and others are parasites of lower mammals and infect human beings incidentally.

BIOLOGY OF HELMINTHS

Helminths are multicellular organisms that range from less than 1 cm to more than 10 m in length. They are covered by a cuticle or tegument that protects them from digestion and environmental stresses. Reproductive organs take up a large part of the body regardless of whether the sexes are separate or the species is hermaphroditic, as is the case with cestodes and nonschistosomal trematodes. Neuromuscular, digestive, excretory, and secretory systems typically are smaller and less complex, in keeping with the parasitic state.

The life cycle of all worms includes an egg, one or more larval stages, and the adult. Transmission to humans occurs by ingestion of helminth eggs or larvae, penetration of intact skin by larvae, or inoculation of larvae by biting insects. Depending on the species, humans are the only host; the intermediate host, in which asexual reproduction takes place; or, when there are one or two intermediate hosts, the definitive host in which sexual reproduction occurs.

Most helminths are unable to complete their life cycle within the human host, and development of eggs or larvae on soil, in water, within a plant, arthropod, or other animal intermediate host is necessary. Hence the geographic distribution of these parasites reflects the environmental conditions necessary for development of eggs or larvae or for survival of intermediate hosts and vectors. The only way for the intensity of infection in a person to increase is by further exposure to the infective stage; in the absence of continued exposure the infection lasts only as long as the life span of the adult worm.

In contrast, a few species, most notably *Strongyloides stercoralis*, are able to reproduce and multiply in numbers within the definitive human host.[4] In the case of *Strongyloides*, infectious larvae can be passed directly from one person to another, and transmission is possible in all geographic areas. Infection can persist for the life span of the host, and in the setting of immunosuppression, accelerated autoinfection can lead to overwhelming numbers of organisms even after a distant and light exposure.

[a]This chapter is based in part on the chapter by Adel A.F. Mahmoud in the fifth edition. All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

EPIDEMIOLOGY

The prevalence of helminth disease is highest in warm, developing areas, where climate, environment, and an abundance of vectors favor completion of the life cycle and where poverty leads to increased exposure to parasites because of poor sanitation, lack of clean water, and inadequate housing. Human activity can facilitate transmission, as seen in the huge numbers of new cases of schistosomiasis and foodborne trematode infections resulting from water resource development projects for hydroelectric power, irrigation, and aquaculture.[5,6] Conversely, in some endemic areas, large-scale control programs have led to interruption or dramatically decreased transmission of dracunculiasis (guinea worm disease), filariasis, onchocerciasis, and other parasitic worms.[7] Helminth infections are less common in temperate and industrialized areas, where they have been imported after travel or residence in tropical areas or acquired locally from domestic or wild animals via improperly prepared meat, fish, or vegetables, or from close personal contact, as in the case of pinworm infections.

Helminths produce large numbers of eggs or larvae and have a high reproductive capacity, which can lead to an extremely high prevalence of human infection when conditions are conducive to transmission, such as in rural areas in the tropics. Helminths are not uniformly distributed in human populations but are overdispersed, with most infected individuals harboring low worm burdens and only a small number harboring heavy infections.[8] The basis for aggregation of helminths in human populations may be related to the intrinsic biology of the parasites and density-dependent constraints on parasites, such as competition for nutrients, parasite-induced pathology, and host factors, including genetic susceptibility to infection, immunity, nutrition, and behavioral factors.

PATHOGENESIS AND HOST-PARASITE RELATIONSHIP

Most infected persons harbor few worms and have few or no signs or symptoms of disease, whereas a small proportion of persons with large numbers of worms are at risk of severe disease. Children with even moderate numbers of worms appear to be at risk of malnutrition, impaired growth, and impaired intellectual development.[9,10] Polyparasitism is widespread throughout the tropics and subtropics, and infection with multiple species of helminths seems to have an additive or multiplicative effect on nutrition and pathology.[11] Although mortality rates attributable to helminth infections are low, rates of chronic morbidity and debilitation are substantial.

Helminths produce disease by a variety of mechanisms, including mechanical effects such as intestinal obstruction (e.g., ascariasis), invasion of host cells or tissues with damage or loss of function (e.g., trichinellosis), or competition for nutrients (e.g., vitamin B_{12} deficiency from fish tapeworm infection). The host responses may lead to immunopathologic lesions, such as schistosome egg granulomas, which contribute significantly to disease. Interactions with other pathogens or potential carcinogens may contribute to chronic sequelae, such as advanced liver disease associated with coinfection of hepatitis B or C with *Schistosoma mansoni* or bladder cancer associated with *Schistosoma haematobium*.[12]

Basic to understanding the pathogenesis of helminthiasis is an appreciation of the size of the organisms, the multiplicity of their antigens, and the chronicity of the infection. Host responses are composed of myriad immunologic and nonimmunologic factors, some of which contribute to disease. Sterilizing immunity to helminth infections does not develop, and the extent to which previous infections with helminths lead to resistance to subsequent reinfection is not well defined. A degree of acquired immunity has been shown in infected individuals who were cured chemotherapeutically and then continued to live under the same conditions of exposure to infection.[13] These findings suggest that induction of resistance by vaccines may be a viable control strategy.

Eosinophilia is a characteristic of many helminth infections.[14] Peripheral blood, bone marrow, and tissue eosinophilia is associated with the migration or presence of worms in tissues. Eosinophilia is not observed in infections with helminths that reside in the lumen of the human gut (e.g., tapeworms) or are contained in cystic structures (e.g., echinococcal cysts). Eosinophils seem to play a significant role in the killing of helminths and host resistance to helminth infections and are responsible for a considerable amount of inflammatory pathology.[15] Chronic infection with worms typically leads to a constant state of immune activation characterized by a dominant Th2 type of cytokine profile, high immunoglobulin E levels, and proliferation and activation of eosinophils that acts to reduce parasite burden and reduce host tissue damage.[16]

Worms have successfully developed multiple strategies to evade host protective responses. Mechanisms include encapsulation within a host fibrous reaction (hydatid cyst), intraluminal location (e.g., *Ascaris*), acquisition of host antigens (schistosomes), and inhibition and downregulation of the host's immune response (filariae, cysticerci). Immune modulation by helminths can dampen the host response to other pathogens, allergens, and autoantigens and may have an adverse impact on the efficacy of vaccines against other organisms, promote progression of infections, such as tuberculosis and human immunodeficiency virus/acquired immunodeficiency syndrome, or affect the expression of allergic and autoimmune disease.[17–21] Helminth infections have been associated with modulation of inflammatory bowel disease, arthritis and diabetes, and, in different studies, increased or decreased risk of asthma and other atopic conditions.[17,22]

DIAGNOSIS OF HELMINTH INFECTIONS

Recognition of helminth infections requires knowledge of their clinical presentation, geographic distribution, and epidemiologic risk factors. Persons with light infections may be asymptomatic, and the only clues to diagnosis may be a history of travel and potential exposure to the parasite, as well as peripheral blood eosinophilia. It should be kept in mind, however, that eosinophilia may be absent, even in persons with invasive infections. The diagnosis of helminth infections rests heavily on microscopic examination of stool, urine, blood, other body fluids, and tissue (Table 285.1).[23,24] When eggs or larvae are produced in abundance, microscopy can be extremely sensitive, but multiple examinations or concentration procedures may be necessary to detect light infections or infections with organisms such as *Strongyloides*, which often shed low numbers of larvae in the stool. Serologic tests offer greater sensitivity than microscopic examination and may be the only way to avoid invasive diagnostic procedures for infections with tissue-invading helminths. Some serologic tests for helminths are available only from reference laboratories, and they may lack sensitivity or specificity and not distinguish between past and present infections. Assays to detect helminth antigens and molecular diagnostic techniques have been used mostly for research purposes and monitoring and evaluating control programs rather than diagnosis and management of individual patients.[25] Detailed information on laboratory procedures for diagnosis of parasitic infections, an image library, and instructions for obtaining prompt assistance in diagnosis is available on *DPDx*, the parasitology diagnostic website of the Centers for Disease Control and Prevention (http://dpd.cdc.gov/dpdx).

TREATMENT OF HELMINTH INFECTIONS

Excellent drugs are available for treating most helminth infections. Because agents such as albendazole, mebendazole, praziquantel, and ivermectin are highly effective in single or a few orally administered doses, as well as being safe and inexpensive, they are suitable for mass drug administration as well as individual treatment.[26,27] Heavy use of these drugs in control programs raises concerns for the emergence of resistance and the need for new drugs. A novel approach to treatment of individual persons with onchocerciasis and filariasis is the use of doxycycline to eliminate the endosymbiotic bacteria *Wolbachia* and thereby sterilize or kill the adult worms.[28,29] For infections such as cysticercosis, toxocariasis, and trichinellosis, antiinflammatory agents are used to minimize tissue damage and symptoms caused by the host's response to the parasite.

PREVENTION AND CONTROL

Helminth infections are prevented by (1) avoiding ingestion of infective eggs, larvae, or intermediate hosts infected with larvae; (2) preventing contact of bare skin with infective larvae; and (3) avoiding bites of infected vectors. At the personal level, these measures entail drinking safe water; properly cleaning, cooking, and otherwise preparing food; adequate hand washing and general hygiene; and using measures to avoid insect bites, among others. Communities can be protected by interventions such as provision of clean water and sanitation; enforcement of appropriate food-producing practices to prevent infection of fish, meat, and vegetables; vector control; and prevention or treatment of infections in domestic animals. Chemoprophylaxis with diethylcarbamazine can prevent infection with *Loa loa,* and artemisinin derivatives have prophylactic activity against *Schistosoma japonicum.*[30,31]

Global efforts to eradicate, eliminate, or reduce transmission of helminth diseases are advancing as a result of partnerships among governments, international agencies, nongovernmental organizations, philanthropic institutions, and industry.[27,32] The guinea worm eradication program, which relies on community education and participation, provision of filters for drinking water, clean water sources, and case containment, is nearing its goal of zero cases globally.[33] The cornerstone of global programs to eliminate onchocerciasis and lymphatic filariasis and reduce disease caused by schistosomiasis and intestinal helminths is periodic mass administration of anthelmintic drugs to populations at risk.[34] Pharmaceutical companies have donated and will continue to donate hundreds of millions of tablets of anthelmintic medications in support of these programs, and integration of different large-scale programs is lowering costs and improving efficiency.[34,35] The World Health Organization has set ambitious but realistic targets for the year 2020 as part of its Neglected Tropical Diseases program: global eradication of dracunculiasis, global elimination of lymphatic filariasis; elimination of onchocerciasis from the Americas and elimination as a public health problem elsewhere; and elimination of schistosomiasis from the Americas, Western Pacific region, and selected countries in Africa.[7,35] Ongoing research in support of these programs and to expand efforts to include other helminth diseases focuses on the development of new drugs, diagnostic methods, control strategies, and novel tools such as anthelmintic vaccines.[36,37]

TABLE 285.1 Diagnosis of Major Helminth Infections

PARASITE	STAGE	MICROSCOPIC DIAGNOSIS: SPECIMEN	OTHER METHODS
Roundworms (Nematodes)			
Intestinal Roundworms			
Ascaris lumbricoides (large intestinal roundworm)	Eggs	Feces	Identification of passed worm
Trichuris trichiura (whipworm)	Eggs	Feces	
Ancylostoma duodenale, Necator americanus (hookworm)	Eggs, larvae	Feces	
Strongyloides stercoralis (threadworm)	Larvae	Feces, duodenal fluid, sputum	Serology[a]
Enterobius vermicularis (pinworm)	Eggs	Swab of perianal skin; occasionally in feces	Cellophane tape test; identification of adult worms on skin
Tissue Roundworms			
Trichinella spiralis (trichinellosis)	Larvae	Muscle biopsy	Serology[a]
Dracunculus medinensis (guinea worm)			Identification of emergent adult worm
Wuchereria bancrofti, Brugia malayi (lymphatic filariasis)	Microfilariae	Blood, urine (in setting of chyluria)	Serology,[a] antigen test (blood)
Loa loa (African eye worm)	Microfilariae	Blood	Identification of adult worm in eye, serology
Onchocerca volvulus (river blindness)	Microfilariae	Skin snip	Identification of adult worm in resected nodules
Ancylostoma braziliense, other species (cutaneous larva migrans, creeping eruption)			Inspection of rash
Toxocara canis, Toxocara cati (visceral larva migrans), *Baylisascaris procyonis*	Larvae	Biopsy of liver, other tissues (usually not necessary)	Serology[a] (preferred)
Flukes (Trematodes)			
Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Schistosoma mekongi	Eggs	Feces, rectal snips, urine (S. haematobium)	Serology,[a] antigen test (serum and urine)
Fasciolopsis buski (intestinal fluke)	Eggs	Feces	
Heterophyes heterophyes (intestinal fluke)	Eggs	Feces	
Metagonimus yokogawai (intestinal fluke)	Eggs	Feces	
Clonorchis sinensis, Opisthorchis spp. (liver fluke)	Eggs	Feces, bile	Serology
Fasciola hepatica (liver fluke)	Eggs	Feces, bile	Serology[a]
Paragonimus spp. (lung fluke)	Eggs	Sputum, feces	Serology[a]
Tapeworms (Cestodes)			
Intestinal Tapeworms			
Taenia saginata (beef tapeworm)	Eggs	Stool	Identification of passed proglottid (segment)
Hymenolepis nana (dwarf tapeworm)	Eggs	Stool	
Diphyllobothrium latum (fish tapeworm)	Eggs	Stool	Identification of passed proglottid
Taenia solium (pork tapeworm)	Eggs	Stool	Identification of passed proglottid; stool antigen test; serology
Larval Tapeworms			
Echinococcus granulosus (cystic hydatid disease)	Protoscolices, hooklets	Fluid from cyst	Serology,[a] CT, MRI, or ultrasonography can be diagnostic
Echinococcus multilocularis (alveolar hydatid disease)	Larvae	Liver biopsy	Serology
Cysticercus (larval *Taenia solium*)	Larvae	Brain biopsy	Serology,[a] CT, or MRI of head can be diagnostic

[a]Serologic test is available through Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.
CT, Computed tomography; *MRI,* magnetic resonance imaging.

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286 Intestinal Nematodes (Roundworms)

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SHORT VIEW SUMMARY

Definition
- Soil-transmitted helminths (STHs) are parasitic worms of the human gastrointestinal tract (see Table 286.1).
- *Ascaris, Ancylostoma duodenale,* and *Trichuris* have a fecal-oral route in the consumption of contaminated soil.
- *Strongyloides* and the hookworms have dermal penetration when human are exposed to soil.
- *Enterobius* can be transmitted person to person via consumption of eggs.

Epidemiology
- More than 1 billion persons are infected with an intestinal parasite. Poverty and poor sanitation are associated with STH infections.
- Some resource-rich countries also have disparate populations in poverty and are at risk for parasite infections.

Microbiology
- Intestinal parasites have a limited life span inside the host, and their life cycle requires development in soil.
- *Strongyloides* is the exception and can continuously reinfect the host via autoinfection. This can last for decades and can lead to hyperinfection or dissemination if the host is treated with immunosuppressants including corticosteroids.
- *Ascaris,* hookworms, and *Strongyloides* all migrate through the vasculature and have a lung stage before residing in the gastrointestinal tract.
- *Trichuris* and *Enterobius* have only a fecal-oral route and do not migrate internally to other organ systems.

Diagnosis
- The majority of symptoms are subclinical (especially for light infections), but symptoms can include abdominal pain and diarrhea for some species, and malnutrition and physical growth and cognitive deficits when they occur in children and adolescents. In addition to these effects, each of the STHs can cause unique symptoms (especially during moderate and heavy infections):
  - *Ascaris:* intestinal obstruction
  - Hookworms: anemia
  - *Strongyloides:* hyperinfection and disseminated infection
  - *Trichuris:* colitis, rectal prolapse
  - *Enterobius:* anal itching
- Eosinophilia occurs during tissue migration and is persistent only in strongyloidiasis.
- All STHs can be diagnosed by looking for eggs or through larvae stool microscopy.
- Only *Strongyloides* infections can reliably be detected with serologic assessment.
- Pinworm can be detected with the cellophane or paddle method.

Therapy (see Table 286.1)
- Albendazole or mebendazole in a single dose is used for *Ascaris* and pinworm.
- Albendazole in a single dose (although sometimes additional doses are required) or mebendazole in multiple doses is used for hookworm
- Albendazole is given in three doses or in combination with oxantel pamoate or ivermectin for *Trichuris.*
- Daily ivermectin is given for *Strongyloides,* in an extended course for hyperinfection or dissemination.

Prevention
- Proper sanitation and waste management, including hand washing, are important.
- Mass drug administration may be used in schools or community-level programs.
- Empirical therapy for strongyloidiasis is administered to avoid dissemination before the patient receives immunosuppressants.

INTESTINAL NEMATODES

More than 1 billion people are infected with intestinal nematodes worldwide.[1] Also referred to as soil-transmitted helminths (STHs), intestinal nematodes are complex, nonsegmented, multicellular worms. Each organism is surrounded by a species-specific acellular cuticle composed of structural proteins, enzymes, and lipids that permit tissue migration through the host and evasion of the host immune response. Under the cuticle lies a muscular ring of tissue and entire gastrointestinal and reproductive tracts. There are more than a dozen species of intestinal nematodes that cause human disease, led by the roundworm *(Ascaris lumbricoides),* whipworm *(Trichuris trichiura),* hookworms (*Necator americanus, Ancylostoma duodenale,* and *Ancylostoma ceylanicum*) and threadworm *(Strongyloides stercoralis).*[2] Intestinal nematode infections are found commonly in people living in extreme poverty in tropic and subtropic regions of the Americas, sub-Saharan Africa, and Asia. Although these infections most commonly occur in low- and middle-income countries, vulnerable populations living in high-income countries are also at risk, with evidence showing that hookworm and other STH infections are present among the poor in the southern United States.[3,4] Persons living in conditions with poor sanitation, overcrowding, unsafe water supply, poor health care infrastructure, and low income potential (earning less than $2 per day) are at the highest risk of repetitive exposure and infection.[3] In addition, because these organisms have overlapping geographic endemicity and affect similar at-risk groups, polyparasitism is common (e.g., a single individual may be simultaneously infected with *Ascaris* roundworms, *Trichuris* whipworms, and hookworms),[5] as are STH coinfections with malaria and other tropical diseases.[6]

Intestinal nematodes infect all age groups from neonates to adults; however, only a small proportion of the community will harbor the greatest disease intensity.[2] The age of peak prevalence and peak worm intensity varies based on the specific organism, development of partial immunity after repeated infection, and changes in behavior-associated risk factors.[7] Intestinal nematodes are not a major cause of death, but instead result in severe chronic disability, causing more than 3 million disability-adjusted life years (DALYs) worldwide.[5] However, alternative estimates indicate that the global disability from STH infections may be much higher than previously realized.[8] Malnutrition; weight loss; gastrointestinal symptoms; increased bacterial, viral, and parasitic infections such as human immunodeficiency virus (HIV), malaria, and tuberculosis; and cognitive and growth stunting are well-recognized features of intestinal nematode infection.[7] These organisms impair physical and mental growth, impede educational advancement, decrease workforce productivity, and suppress economic development, creating a sustained cycle of poverty in endemic regions.[8]

High prevalence and heavy worm burden can be devastating to a community. Annual or biannual mass preventive chemotherapy programs have been established in endemic areas worldwide.[9] Administration of a single dose of albendazole or mebendazole is recommended for all

TABLE 286.1 Features of Intestinal Nematodes

MAJOR HUMAN SPECIES	COMMON NAME	ESTIMATED NO. OF CASES (MILLIONS)	DURATION OF INFECTION	MAJOR CLINICAL SYNDROME	TREATMENT
Ascaris lumbricoides	Roundworm	800	1–2 years	Vitamin A malabsorption Intestinal obstruction Asthma	Albendazole 400 mg PO once or mebendazole 500 mg PO once
Trichuris trichiura	Whipworm	435	1–3 years	Colitis Trichuris dysentery syndrome Rectal prolapse	Albendazole 400 mg PO × 3–7 days or mebendazole 500 mg daily or 100 mg PO twice × 3–7 days Also combined with ivermectin (200 µg/kg daily)
Necator americanus *Ancylostoma duodenale/ ceylanicum*	Hookworm	450	3–5 years 1 year	Anemia Ground itch Abdominal pain	Albendazole 400 mg PO once or mebendazole 100 mg twice daily x 3 days
Strongyloides stercoralis	Threadworm	100	Lifelong	Larva currens Eosinophilia Abdominal pain Dissemination to other organs including skin and brain	Ivermectin (200 µg/kg daily ×2) Disseminated: ivermectin (200 µg/kg daily ×14)
Enterobius vermicularis	Pinworm	Estimated at 30% world population	1 month	Pruritus ani Vulvovaginitis	Pyrantel pamoate (11 mg/kg ×1) Albendazole (400 mg ×1)

preschool and school-aged children, nonpregnant adolescent girls (10–19 years old), and nonpregnant women of reproductive age (15–49 years old) living in areas with disease prevalence >20% to reduce worm burden and to reduce morbidity. In addition, pregnant women in their second or third trimester living in areas with hookworm or *Trichuris* prevalence >20% and in areas where >40% of pregnant women have anemia should also be routinely treated.[5] The major goal of these programs is improvements in STH-associated morbidities including growth stunting, cognitive disabilities, and pregnancy outcome, although some investigators have questioned the actual and sustainable benefits of global deworming programs.[10] Interruption of intestinal nematode transmission within a community using mass preventive chemotherapy programs is also feasible in some areas with low worm prevalence, a robust health system, and in-country financial support to maintain control programs.[10] However, global elimination of these diseases will not be feasible without prioritizing reduction in poverty, making changes in infrastructure, investing in health education, and improving hygiene and sanitation, alongside the expansion of preventive chemotherapy programs across broader age classes[11] (Table 286.1). There is also a need for improvements in the efficacy of single-dose anthelmintic drugs, especially for trichuriasis and hookworm, prompting the search for new-generation anthelmintic drugs and vaccines.[12,13]

ASCARIS LUMBRICOIDES

Epidemiology

A. lumbricoides, human roundworm, is the most common helminthic infection in the world, chronically infecting 800 million individuals.[14] Year-round transmission is standard in humid tropical and subtropical regions of the Americas, sub-Saharan Africa, and Asia.[3] In more arid environments, where eggs can remain viable in the soil for months, seasonal transmission occurs. *Ascaris* prevalence and worm intensity increase from infancy to preschool and school-aged children. Young children disproportionately harbor the highest amount of worms within a community, leading to the most significant morbidity.[3] Mortality secondary to ascariasis is low, accounting for only 2700 deaths per year, but ascariasis is a significant cause of severe morbidity, causing 1.27 million DALYs owing to the effects of intestinal parasitism on child growth and development.[15] In addition, the larval stages of *Ascaris* worms migrating through the lungs have been shown to cause asthma and other lung disorders.[16]

Ascaris suum, swine roundworm, is morphologically indistinguishable from *A. lumbricoides* and can cause human disease.[17] *A. suum* and *A. lumbricoides* have minimal genetic differences, with greater than 98% similarity of the mitochondrial genome, with known nucleotide differences commonly resulting in synonymous mutations.[18] In addition, the life cycles of both species are identical and can be completed in both humans and pigs, causing similar clinical manifestations.[19] As with *A. lumbricoides, A. suum* infection has a high prevalence in pigs worldwide, including in the United States, with few farms free of infection.[20] Humans become infected by ingesting pig manure or soil contaminated with *A. suum* eggs.[17]

Life Cycle

Adult *Ascaris* worms live in the small intestine of the host and can survive in the presence of host digestive hydrolases owing to the presence of a resistant outer cuticle. Adult worms are large and can grow up to 30 cm in length and live up to 1 to 2 years within the host.[3] Female adult worms release over 200,000 eggs per day, or 3540 eggs per gram of stool[21] (Table 268.2). *Ascaris* eggs are durable; they are resistant to extreme environmental conditions and survive up to 10 years in ideal soil conditions and up to 2 months without soil. The ability to survive in extreme conditions promotes transmission of ascariasis in both moist and arid environments and in rural and urban communities. Infection occurs by fecal-oral transmission when embryonated eggs from the environment are ingested.[3] After ingestion, stage 2 larvae (L2) hatch in the host small intestines, penetrate the intestinal wall, and are carried by the portal vasculature system to the liver. From the liver, the L2 larvae are transported in the blood to the lung parenchyma, where the larvae molt from stage L2 to L3 larvae. The L3 larvae migrate up the bronchotracheal tree, across the epiglottis, and are swallowed back into the intestinal lumen of the small intestine, where they develop into adult worms (Fig. 286.1). Adult worms generally live from 12 to 24 months. During the migratory process, *Ascaris* larvae release excretory/secretory products (ES) that elicit a strong host inflammatory response represented by visceral eosinophilic infiltrates, peripheral eosinophilia, and high levels of immunoglobulin E (IgE).[22] Egg production can be detected in the host stool for 2 to 3 months after embryonated eggs are ingested[3] (Fig. 286.2).

Clinical Syndromes

The symptoms of ascariasis vary based on the phase of the disease—that is, acute migratory phase versus chronic intestinal phase. Symptoms associated with the acute migratory phase are a result of the intense host type 2 immune response, with very high levels of IgE and eosinophilia, that occurs as the larvae migrate through visceral organs.[22] Infected people may have abdominal pain, nausea, and vomiting as the larvae migrate through the intestines and liver, and symptoms of acute lung inflammation known as Loeffler syndrome, dyspnea, bronchohyperreactivity, and eosinophilic infiltrate as the larvae migrate through the lung parenchyma.[23] Chronic intestinal infection is commonly asymptomatic or associated with mild symptoms of abdominal distention, pain, and nausea.[20] However, people harboring a moderate-to-heavy

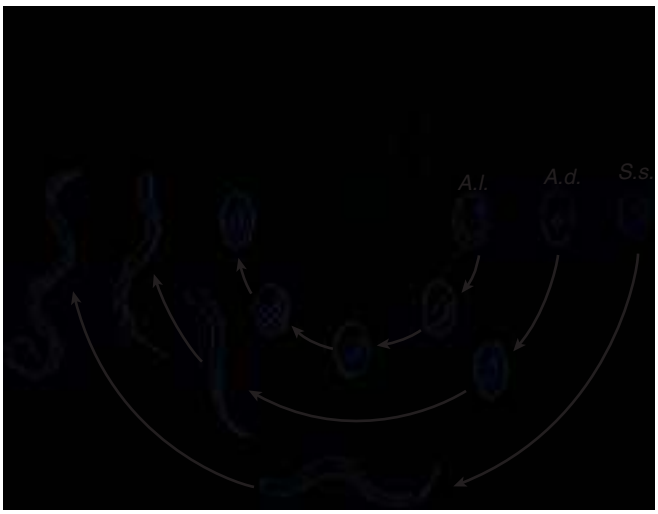

FIG. 286.1 Life cycle of intestinal nematodes that travel through the lungs. Eggs of *Ascaris lumbricoides (A.l.), Ancylostoma duodenale (A.d.),* and *Strongyloides stercoralis (S.s.)* are consumed and migrate from the gut to the lungs, undergoing maturation. *Necator americanus, A. duodenale,* and *S. stercoralis* penetrate the dermis and migrate to the lungs. These worms migrate from the trachea down the esophagus and mature in the gastrointestinal tract, releasing eggs to compete the cycle.

worm burden can have significant morbidity. Ascariasis can have a severe impact on the host nutritional status, specifically protein loss and vitamin A deficiency, leading to impairments in physical and cognitive development. Reduction in cognitive abilities leads to decreased school participation and future economic productivity.[20] Because of the large size of the adult *Ascaris* worm, small bowel obstruction, typically in the terminal ileum, is common in hosts (especially young children) with high worm burden and may necessitate surgical removal. Less commonly, volvulus, intussusception, perforation, and peritonitis can occur. Chronic *Ascaris* infection can also increase the risk of developing wheezing and bronchohyperreactivity, possibly because of the high levels of IgE and cross-reactivity with aeroantigens or because of parenchymal lung damage during acute larvae migration.[23] This syndrome produces a Loeffler pneumonitis, which clinically resembles asthma.

Despite living in the small intestines, adult worms are motile. Under stress, adult worms will migrate into the appendix, stomach, oropharynx, or biliary and pancreatic ducts, causing mechanical obstruction, appendicitis, cholecystitis, cholangitis, pancreatitis, and biliary strictures. Surgical intervention through an endoscopic removal of common bile duct worms or open surgery may be required in circumstances of worsening intestinal obstruction, persistent biliary impaction, or hepatic disease with an abscess.[24] Dead adult *Ascaris* worms within the hepatobiliary system can become a nidus of pigmented hepatic stone formation within the common bile duct. Biliary migration of adult *Ascaris* worms is enhanced in patients with previous sphincterotomy or sphincter ablation, cholecystectomy, or bilioenteric anastomosis. Infected pregnant



FIG. 286.2 Eggs and larvae of intestinal nematodes. (A) *Ascaris lumbricoides,* (B) *Enterobius vermicularis,* (C) hookworm egg, (D) *Strongyloides stercoralis* larvae, and (E) *Trichuris trichiura*. *(Courtesy Rojelio Mejia.)*

women are also at high risk of *Ascaris* migration into the hepatobiliary system secondary to relaxation of the sphincter of Oddi due to high progesterone levels.[25]

Diagnosis

Detection of *Ascaris* eggs in stool through use of the Kato-Katz thick smear method is the gold standard for diagnosis of *Ascaris* infection[26] (see Fig. 286.2). Kato-Katz is a standardized microscopy technique that measures worm burden by quantitating the number of eggs per gram of stool in two separate stool samples.[26] The technique is cheap and has few false-positive test results but is labor-intensive. Daily fluctuation in egg excretion and dependence on trained microscopists can cause highly variable results, particularly in areas of low disease burden, leading to a false-negative test result.[26] To overcome the variability in areas with low worm burden, concentration techniques such as FLOTAC can increase the sensitivity by using larger volumes of stool. Use of concentration techniques in combination with Kato-Katz is critical for increasing the diagnostic accuracy in epidemiologic mapping and for monitoring mass preventive chemotherapy programs. However, the use of concentration techniques remains labor-intensive and increases the cost of the test by requiring additional equipment.[26]

Highly sensitive, specific, and rapid diagnostic methods are required for eradication efforts.[27] Polymerase chain reaction (PCR) methods using multiplex, multiparallel real-time technology improve accuracy in estimating *Ascaris* egg burden and increase sensitivity to detect infection in areas with low worm burden. Although more expensive in terms of upfront costs, PCR methods are less labor-intensive, use a single stool sample, and can be used to identify a higher distribution of polyparasitism compared with standard microscopy, making such methods ideal for monitoring both organism elimination and organism resurgence.[27] False-negative and false-positive results can occur with PCR owing to variation in DNA extraction and target amplification.[28]

Management

Use of an oral benzimidazole, albendazole 400 mg daily or mebendazole 500 mg daily, is efficient to treat *Ascaris* infection and reduce morbidity in persons with high worm intensity. Single-dose oral albendazole, mebendazole, and pyrantel pamoate, used in mass preventive chemotherapy programs, have cure rates of 88%, 95%, and 88%, respectively.[29] However, mass preventive chemotherapy programs targeting high-risk groups are not sufficient to lead to elimination because rapid reinfection is common.[29] More frequent anthelmintic therapy applied to a broader age group within a community is required to overcome high reinfection rates.[9] Improvements in water, sanitation, and hygiene (WASH), including proper disposal of human and animal feces, and increased access to potable water and health infrastructure will be essential to interrupt transmission and prevent reinfection.[20]

ENTEROBIUS VERMICULARIS

Epidemiology

Also known as pinworm, *E. vermicularis* is endemic in Western countries, including the United States. Updated studies in the United States are lacking; the last prevalence study estimated 40 million infections in the 1980s.[30] Worldwide, the prevalence can be as high as 30%.[31] Pinworm infections are not limited to lower socioeconomic groups and can affect families and people living in close quarters.[32]

Life Cycle

The gravid female measures approximately 1 cm; the organism is limited to fecal-oral transmission, and the main gastrointestinal location is the cecum and appendix.[33] The lifespan of a worm is less than 3 months; it spends 1 month as an adult and releases over 10,000 eggs (see Fig. 286.2). The females migrate to the perianal area at night, where they release their eggs and die (Fig. 286.3). Eggs will embryonate and become infectious at 6 hours. These activities create a nidus of pruritus, leading to scratching and contamination, especially underneath the fingernails. The infectious eggs can then be transferred via the fecal-oral route to other individuals or through autoinfection to the host. Bedding, clothing, or surrounding surfaces can be contaminated with eggs. Eggs will become noninfectious after a few days unless ingested, continuing the life cycle. Sexual transmission can occur between partners engaging in oral-anal sex.

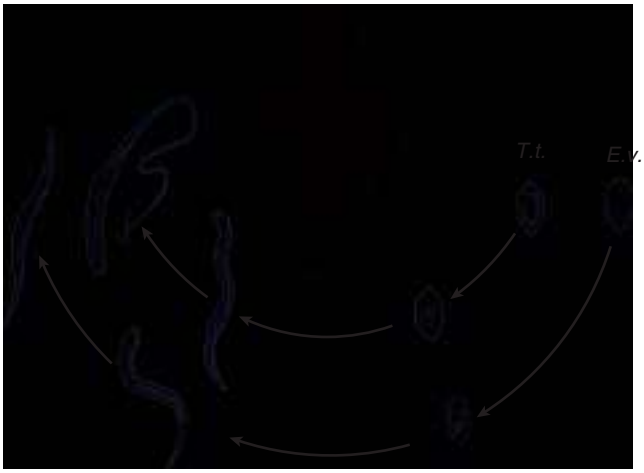

FIG. 286.3 Fecal-oral life cycle of intestinal nematodes. *Trichuris trichiura (T.t.)* and *Enterobius vermicularis (E.v.)* eggs are ingested and mature into larvae in the intestines. *T. trichiura* releases eggs in the stool, and *E. vermicularis* releases at the perianal site.

Clinical Syndromes

The majority of pinworm infections cause only pruritus ani. This perianal itching is due to inflammation caused by the larvae and eggs during the night. Pruritus ani, if severe, can lead to secondary bacterial infections resulting from aggressive scratching, and also difficulty sleeping.[34] Ectopic diseases are rare but have been reported in vulvovaginal regions.[31] Such disease occurs via direct migration of worms to the perineal region and has been associated with vulvovaginitis and urinary tract infections in women.[35] Because the worms are found in the appendix, there are controversial debates regarding whether pinworm can cause appendicitis, with inflamed appendixes containing *E. vermicularis* worms.[36] Although eosinophilic enterocolitis has been reported, there is generally no peripheral eosinophilia and likely no hematogenous spread to other occult body sites.[37]

Diagnosis

Adult worms can be seen, especially at night at the perianal site. The standard of diagnosis involves use of a clear paddle or adhesive tape that is pressed on the anus early in the morning to capture eggs for visualization with a microscope.[33] Stool should not contain eggs, and there are currently no PCR or serologic assays for pinworms.

Management

Pyrantel pamoate (11 mg/kg, maximum of 1 g) is available without a prescription in many locations. Albendazole (400 mg once) also has high efficacy. Both medications should be given 2 weeks apart and have almost 100% posttreatment cure rates.[38] Treatment includes washing all bedding and clothes to remove eggs. Hand washing will also decrease household contact exposure. All household contacts should be treated, given that transmission is so high between family members.[39]

HOOKWORMS

Epidemiology

Hookworm is a major cause of anemia in the world's resource-poor nations.[40,41] Three main hookworm species infect humans and have complete life cycles. These include *N. americanus, A. duodenale,* and *A. ceylanicum*. Although other hookworm species can cause cutaneous larva migrans (especially the dog and cat hookworm, *Ancylostoma braziliense*), they do not complete their life cycle in the human host and do not typically induce intestinal or pulmonary pathologic conditions. There are approximately 450 million people chronically infected with hookworm worldwide.[8] *N. americanus* is the predominant species globally, and the major species found in sub-Saharan Africa, Southeast Asia, and the

Americas, whereas *A. duodenale* is more focally endemic in parts of Africa, India, China, and elsewhere.[3,40,42] *N. americanus* also still occurs in areas of extreme poverty in the southern United States.[4] *A. ceylanicum*, previously considered a nonhuman parasite, has been identified as a zoonotic infection in East Asia, the South Pacific, and Australia.[43,44] There is considerable overlap of these species throughout the world.[45]

Life Cycle

The eggs of all three species are thinly shelled ova with multinucleated centers, and they are indistinguishable via microscopy (see Fig. 286.2). The worm will bind to the small intestinal mucosal lining, using cutting plates to consume host blood. The hookworm larvae range in length from 7 to 13 mm, with *Ancylostoma* being generally larger; the size allows it to take in up to 10 times more human blood than *Necator* (0.30 mL/day vs. 0.03 mL/day per worm).[46] *Necator* can survive 3 to 5 years in the host, while *Ancylostoma* live about a year. The initial infection is through dermal penetration by the hookworm, although *A. duodenale* infection has been shown to occur through oral consumption of contaminated foods or soil. After dermal penetration, the larvae will produce a local pruritic maculopapular rash that will track as the worm begins deeper tissue invasion. The larvae will travel via vasculature to the lungs. The majority of people will be asymptomatic, but some may exhibit a transient pneumonitis.[47] As with *Ascaris* and *Strongyloides,* the hookworms migrate up the trachea and are swallowed, then enter the gastrointestinal tract and reside in the small intestine (see Fig. 286.1).

Clinical Syndromes

Clinical symptoms are based on the location of the larvae in the host. The rash can form a serpiginous or vermicular pattern, usually on the feet (known as "ground itch"), but also on the ankles because the infectious larvae can elevate on blades of grass and ground vegetation. Typical gastrointestinal symptoms include abdominal pain and diarrhea; these symptoms are most severe with initial infections and decrease with chronic exposures.[48] Symptom severity is dependent on worm burden, with massive infections producing increased morbidity[49] (see Table 286.2).

The term *hookworm* comes from the attachment of the buccal cutting plates to the intestinal mucosa in the small intestine. This unique pathophysiologic feature of the hookworms enables its consumption of red blood cells from the intestinal vasculature. The worms produce anticoagulants at the biting site, which allow continuation of bleeding after detachment.[50,51] After hookworms digest blood, they use parasite-derived hemolysins to open the red blood cells, and then digest host hemoglobin through a cascade of hemoglobin-digesting proteases, after which the released heme is detoxified through a modified glutathione *S*-transferase.[52,53] The resulting blood loss leads to both iron and protein deficiencies. Iron-deficiency anemia is a chronic problem and correlates with worm burden, duration, and recurrence of infection.[54] Symptoms include fatigue, weakness, and edema due to hypoproteinemia. Growth and cognitive delays are associated in children with moderate and heavy infections (see Table 286.2).[55] In severe infections, the chronic blood loss can lead to congestive heart failure and eventually death of the host.[7] Today, hookworm infection is a leading global cause of iron deficiency anemia.[41,55]

Increasingly it is recognized that hookworm anemia represents a major complication of pregnancy,[56] especially in resource-poor areas of Africa, Asia, and Latin America, where it leads to increased maternal morbidity and mortality and decreased neonatal survival. Hookworm and malaria coinfections in pregnancy can lead to especially profound anemia.[6,57]

Diagnosis

Fecal microscopy is the standard of diagnosis throughout the world. Direct smears and concentration techniques such as Kato-Katz and McMaster's are semiquantitative.[58] Hookworm egg morphologic features are indistinguishable with microscopy (see Fig. 286.2), and quantitative PCR-based methods are species specific and can correlate with the burden of infection[27,45] (see Table 286.2).

Management

There is a high degree of variability in the efficacy of oral albendazole (400 mg orally) and mebendazole (500 mg orally). For example, a systematic review found that single-dose mebendazole results in only 15% cure rates, and although single-dose albendazole appears to be a better drug for hookworm, there are highly variable effects of single-dose albendazole when used in deworming programs.[29,59] Whether these low cure rates result from emerging drug resistance is under investigation. In some cases, repeat treatment may improve cure rates, although it has been noted that efficacy can also decrease with repetition.[60] Although benzimidazole anthelmintics such as albendazole and mebendazole are embryotoxic in laboratory animals, it is increasingly recognized that pregnant women are at high risk of complications due to severe hookworm anemia, so in resource-poor settings the benefits of deworming with mebendazole or albendazole during the second and third trimesters may outweigh the risks.[55] According to the World Health Organization, "in endemic areas preventive anthelminthic treatment is recommended for pregnant women after the first trimester as part of worm infection reduction programmes."[61] Alternatively, pregnant women can be treated with pyrantel pamoate, an intraluminal drug with little systemic absorption, orally at a dosage of 11 mg/kg daily for 3 days. As with the other STHs, reinfection is common in high-prevalence areas.[62] Retreatment at 6 months is acceptable if the risk of reinfection is high, especially in areas with greater than 50% percent prevalence.[63] A vaccine that targets hookworm hemoglobin degrading and detoxifying enzymes is in development.[64–66]

STRONGYLOIDES STERCORALIS

Epidemiology

The epidemiology of *S. stercoralis* infection differs from that of most other helminth infections in large part because of the parasite's ability to reinfect its human host, a process known as autoinfection.[67] *S. stercoralis* is a parasitic nematode that can infect humans. It is found throughout most tropical and temperate climate zones of the world including North America and parts of Europe. Approximately 100 million people are infected with *S. stercoralis* worldwide,[68] with billions at risk. The incidence is dependent on poor sanitation, often seen in resource-limited regions of the world. The improper management of human waste and runoff from sewage systems allow for continued transmission.[69]

Life Cycle

Whereas most other STH infections are self-limited, *Strongyloides* larvae in the stool can penetrate the perianal skin or the intestinal wall and continue the life cycle. The majority of primary infections occur through contact with soil in which filariform larvae (derived from the eggs of free-living adult worms) (see Fig. 286.2) can penetrate the skin. These larvae then migrate through the venous system to the lungs. In the lungs, the larvae continue to mature in the alveoli and are eventually coughed up and swallowed. The parasitic females lodge into the small intestine surface (lamina propria) and release eggs that hatch into the rhabditiform larvae. These larvae either are excreted during defecation or can mature into the infectious filariform larvae that pass through

TABLE 286.2 World Health Organization Intensity of Infection for Soil-Transmitted Helminths

ORGANISM	LIGHT-INTENSITY INFECTIONS	MODERATE-INTENSITY INFECTIONS	HEAVY-INTENSITY INFECTIONS
Ascaris lumbricoides	1–4999 epg	5000–49,999 epg	>50,000 epg
Trichuris trichiura	1–999 epg	1000–9999 epg	>10,000 epg
Hookworms	1–1999 epg	2000–3999 epg	>4000 epg

epg, Eggs per gram of feces.
From World Health Organization. Eliminating Soil-Transmitted Helminthiases as a Public Health Problem in Children: Progress Report 2001–2010 and Strategic Plan 2011–2020. *Geneva, Switzerland: World Health Organization Press; 2012.*

the colonic walls or perianal skin, again entering the venous system and restarting the cycle.[70] Because of this unique autoinfection capability, a host can remain infected lifelong[71] (see Fig. 286.1). Another mode of infection is the fecal-oral route, in which unwashed fruits or vegetables exposed to night soil (human feces used as a fertilizer) that contain the eggs or larvae are consumed.[72]

Clinical Syndromes

Strongyloidiasis can manifest in many forms. The majority of infections are clinically asymptomatic, although some can manifest with gastrointestinal symptoms including bloating and abdominal discomfort after eating. A prevalent symptom is a new onset of heartburn that is not related to being supine. Often patients are diagnosed with gastroesophageal reflux disease (GERD) and prescribed a proton pump inhibitor with little resolution of symptoms. Episodic diarrhea is common, although this may alternate with constipation and can mimic irritable bowel syndrome.[73] Surprisingly, there are very few symptoms during the pulmonary migration of the *Strongyloides* parasite. It has been postulated that the worms are sufficiently small that despite their migration through lung tissue, there is no significant tissue damage or inflammatory response.[74] Skin manifestations are seen quite commonly. Early in the infection as the worms travel through the skin, they may leave a serpiginous trail of dermatitis known as *larva currens*. In chronic infections, urticaria (hives) or pustular eruptions may occur, which is a sign presumably related to allergic sensitization to parasite antigens. These occur most commonly on the extremities and over the abdomen.

Because chronic infections are often clinically asymptomatic, it is not uncommon for the infection to be uncovered after an incidental finding of peripheral blood eosinophilia.[75] Because *S. stercoralis* is an invasive tissue parasite, it is felt that the organisms induce a Th2-type $CD4^+$ T-cell response dominated by interleukin (IL)-4 and IL-5, (the latter cytokine being responsible for eosinophilia[76]).

Accelerated autoinfection leading to hyperinfection syndrome with an accumulation in the numbers of larvae penetrating the gut and an increase in adult worms in the gut, or even disseminated infection, in which adult worms are found at ectopic extraintestinal sites, is the most dangerous and often fatal condition associated with *S. stercoralis* infection. With respect to hyperinfection, for reasons that are not entirely clear, although often related to corticosteroid administration or underlying human T-cell leukemia virus 1 (HTLV-1) infection, larvae are present in the gastrointestinal tract in large numbers. Some of these invade the gastrointestinal mucosa, often carrying enteric bacteria into the bloodstream. The gram-negative (or polymicrobial) bacteremia can lead to meningitis and septic shock. The parasites can cause devastating pulmonary inflammation with widespread pneumonitis and bleeding. Periumbilical purpura is also common. Untreated, this process can lead to almost certain death, with mortality estimates ranging from 35% to 100% depending on the study. Hyperinfection rarely occurs in the healthy host, but does occur when the patient is immunosuppressed. Oral corticosteroids have been associated with *Strongyloides* hyperinfection most commonly.[77] Although other immunosuppressive agents—chemotherapeutics, biologics—have also been associated with disseminated strongyloidiasis,[77] steroids remain the common link, and dissemination can occur within as few as 6 days of therapy.[78] Surprisingly, HIV/AIDS does not show a significant risk for hyperinfection and dissemination, but HTLV-1 has a substantial risk of dissemination.[76]

Diagnosis

It can take several weeks after infection for larvae to be detected in the stool.[79] Stool microscopy techniques such as Baermann concentration and the agar plate method have improved sensitivity to standard stool examination, but because *Strongyloides* has intermittent egg excretion, almost seven different stool examinations are needed to diagnose strongyloidiasis[80] (see Fig. 286.2). Serologic assays are useful for diagnosing chronic strongyloidiasis; these include crude-antigen enzyme-linked immunosorbent assays (ELISAs) that cross-react with other IgG antibodies against helminths and give a false-positive result.[81,82] Recombinant *Strongyloides* antigens (NIE and SsIR) ELISA and newer bead-based assays approach 100% sensitivity and specificity.[81,83,84] Real-time PCR has been used to detect small amounts of parasite DNA without cross-reactivity to other helminths.[45,85] Hyperinfection and dissemination syndromes are diagnosed by means of finding larvae in body fluids outside the gastrointestinal tract.[77]

Management

Ivermectin (200 µg/kg daily) is the cornerstone of treatment; a 2-day dose has been shown to cure 93% of patients[86] (see Table 286.1). For disseminated strongyloidiasis, daily ivermectin for 14 (or more) days is the standard treatment. In those unable to take oral medication, there are rectal and injectable routes of administration, including subcutaneous routes, that have been used successfully.[87–90] Parenteral ivermectin is a veterinary formulation that has not been approved by the US Food and Drug Administration (FDA), and requires approval under a compassionate use protocol to be administered to severely ill patients.

Although cure is commonplace in acute and chronic strongyloidiasis, treated patients are still at risk for reinfection, because immunity to *S. stercoralis* infection has not been formally demonstrated. As for other parasitic infections, there is active research to develop vaccines and prevent reinfection,[91] and for *S. stercoralis,* there have been some vaccine candidates that have undergone preclinical testing.[92]

Thus, infection with *S. stercoralis,* a parasitic nematode (worm) with a relatively cosmopolitan global distribution, can lead to lifelong infection because of its unique life cycle. Its ability to cause hyperinfection or disseminated infection in the face of immunosuppression makes its diagnosis, treatment, and ultimate prevention through public health measures or vaccines of paramount importance.

TRICHURIS TRICHIURA

Epidemiology

Over 430 million people are infected with *T. trichiura,* human whipworm, worldwide.[16] Similar to other intestinal nematodes, trichuriasis is most common in poverty-stricken areas of the Americas, sub-Saharan Africa, and Asia,[3] where it is a major cause of colitis and inflammatory bowel disease. Disease prevalence and worm intensity are highest in preschool- and school-aged children, with a decline in disease intensity as children age into early adulthood.[3] Coinfection with other intestinal nematodes is common.

Life Cycle

Adult whipworms live and mature for approximately 1 to 2 years in the colon, preferentially residing in the cecum.[3] Female worms can grow up to 30 to 50 mm and male worms up to 30 to 56 mm in length.[3] After male and female copulation in the colon, female worms excrete 3000 to 5000 eggs per day in host feces, which are released into the soil. *Trichuris* eggs require high humidity, sandy soil, and warm temperatures to embryonate.[3] After 3 weeks in the environment, the eggs are infective.[1] Infection occurs by fecal-oral transmission as the host ingests embryonated eggs from contaminated soil. L1 larvae hatch in the small intestines, penetrate the columnar epithelium, molt, and emerge as immature adult worms. The immature adult worms are then carried to the colon, where they develop into adults. Adult worms secrete natural products, including parasite macromolecules, that reduce the barrier function of the intestinal epithelium, leading to increased mucosal permeability.[3] The adult worm can embed the anterior portion, the stichosome, into the mucosal epithelium of the colon, while the posterior portion protrudes into the lumen (see Fig. 286.3). Adults worms generally live 1 to 3 years in the host. Egg production can be detected in the stool 3 months after ingestion of embryonated eggs by the host.[3]

Clinical Syndromes

Heavy-intensity infection can be associated with severe gastrointestinal disease (see Table 286.2). Colitis extending from the ileum to the rectum can resemble inflammatory bowel disease with chronic abdominal pain and diarrhea.[7] *Trichuris*-induced dysentery is a clinical syndrome consisting of mucoid diarrhea and rectal bleeding. Severe chronic colitis and *Trichuris*-induced dysentery have been associated with significant weight loss, iron deficiency anemia, and tenesmus leading to rectal prolapse. Chronic infection in children has also been shown to cause severe malnutrition, growth stunting, and cognitive suppression.[10]

Low-intensity trichuriasis may be asymptomatic or associated with mild symptoms. Despite the lack of symptoms, low-intensity trichuriasis

is associated with chronic growth restriction and should be treated to prevent long-term morbidity.[93] Mild *Trichuris* infection may also have immunomodulatory effects that suppress colonic inflammation.[3] Infection with nonhuman whipworm species, including *Trichuris suis,* has been evaluated as potential immunomodulatory therapy for the treatment of human allergic and autoimmune diseases.[94] However, iatrogenic treatment with *T. suis* eggs has not shown essential effects over placebo for treatment of allergic rhinitis or inflammatory bowel disease.[95] Not only does *T. suis* lack efficacy, but infection is also associated with increased risk of adverse gastrointestinal reactions including moderate-to-severe flatulence, diarrhea, and upper abdominal pain.[96] Ultimately, human trichuriasis represents a major global cause of, and not a cure for, human colitis and inflammatory bowel disease.

Diagnosis

Similar to other STHs, microscopy is the primary form of diagnosis, with the characteristic taper ends and terminal plugs of the ova making visualization easier. There are several molecular methods used for detection in research, but none are available commercially.[27,97]

Management

Unlike ascariasis, trichuriasis can be a challenge to treat with single-dose anthelmintic therapy. A single dose of oral albendazole or mebendazole leads to a 25% to 33% cure rate.[29] Prolonged therapy with albendazole 400 mg for 3 to 7 days or mebendazole 500 mg daily or 100 mg twice a day by mouth for 3 to 7 days[98] may be more successful[29] in heavy infections of at least 1000 eggs per gram of stool (see Table 286.1). For preventive chemotherapy programs, the use of albendazole plus oxantel pamoate in combination has shown higher cure rates and egg reduction against *T. trichiura* compared with a single dose of benzimidazoles.[9] Albendazole can also be combined with ivermectin for improved treatment compared with monotherapy.[9] People living in highly endemic regions are also at high risk for reinfection and benefit from regular mass drug administration programs to reduce morbidity.

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287 Tissue Nematodes, Including Trichinellosis, Dracunculiasis, Filariasis, Loiasis, and Onchocerciasis

James W. Kazura

SHORT VIEW SUMMARY

Definition
- Tissue nematode infections cause a spectrum of disease manifestations ranging from asymptomatic cases to chronic pathologic processes and, occasionally, severe illness and death.

Epidemiology
- Infections with tissue nematodes occur throughout the world and have the highest prevalence in tropical regions where obligatory insect vectors are present.
- Trichinellosis
- Dracunculiasis
- Filariases

Microbiology
- The life cycles of these parasites are complex, with five distinct stages that involve their human host and, where applicable, insect vectors.
- Trichinellosis
- Dracunculiasis
- Filariases

Diagnosis
- Parasites are identified microscopically in body fluids, or diagnostic assays are used to detect parasite antigens.

Therapy
- Anthelmintic drugs are available for many infections.
- For trichinellosis, no drugs are available for treatment of newborn larvae or maturing first-stage larvae. Corticosteroids and mebendazole are sometimes utilized in severe disease. During the enteral stage of infection (1–2 weeks after eating contaminated meat), mebendazole or albendazole can be used to eliminate adult worms from the small intestine.
- For lymphatic filariasis, diethylcarbamazine is more active against *Wuchereria bancrofti* than *Brugia malayi*.
- Diethylcarbamazine is also used to treat tropical pulmonary eosinophilia and loiasis.
- Ivermectin is used to treat onchocerciasis.

Prevention
- Controlling insect vectors and mass administration of anthelmintic drugs to endemic populations reduces and may potentially eliminate transmission of medically significant tissue nematode infections.

Tissue-dwelling nematode (roundworm) infections are widely distributed throughout the world. The health and socioeconomic impacts of these infections are greatest in resource-poor settings in the tropics and subtropics, although populations in temperate and industrialized regions of the world continue to be at risk for infection and morbidity. Like all parasitic nematodes, the life cycle of these multicellular organisms includes five distinct stages: adult male or female worms and four larval stages distinguished from each other by a molting process that involves shedding of the parasite's surface cuticle and by organ system differentiation dictated by developmentally regulated changes in gene expression. With the exception of *Trichinella* spp., which are transmitted directly to humans by ingestion of contaminated meat, infection involves obligatory development in and subsequent transmission by blood-feeding invertebrate arthropods (the filariases) or swallowing of small freshwater crustaceans (copepods). Adult worms do not multiply in the human host and, therefore, the likelihood of developing a high enough worm burden to cause morbidity is directly related to the duration and intensity of exposure to vectors harboring infective larvae. Definitive diagnosis is made by microscopic visualization of parasites isolated from or present in host tissue, although this may be difficult or not indicated in all cases. Simple and inexpensive measures can be taken to avoid infection. Global efforts are underway to eliminate some tissue nematode infections as public health problems or even eradicate them by permanently stopping transmission. Effective and safe anthelmintic drugs are available for the majority of these infections.

Infections acquired by eating contaminated meat and swallowing copepods harboring infective larvae are considered first. Filarial infections transmitted by blood-feeding insects are described next.

TRICHINELLOSIS

Trichinellosis is acquired when undercooked meat of domestic pigs, horses, or game containing infective larvae of various *Trichinella* spp. is consumed. Symptomatic infections characterized by diarrhea, myositis, fever, and periorbital edema develop when large numbers of larvae are ingested.

Life Cycle of *Trichinella*

Members of the *Trichinella* genus are zoonotic nematodes that infect carnivorous and omnivorous mammals in various ecologic and climatic settings.[1] Recent genomic and phylogenetic analyses indicate that, of the 11 known species, there are two clades distinguished by the presence or absence of encapsulated third-stage larvae that have parasitized striated muscle cells (Table 287.1).[2,3] The two clades are estimated to have diverged from a common ancestor 15 to 20 million years ago. *Trichinella spiralis,* a species distributed throughout the world that infects pigs, rodents, and horses, has historically been the most common cause of human infection. *Trichinella* spp. of wild game are of increasing importance in areas where food safety measures and livestock practices have led to a reduction in *T. spiralis*.[4]

The parasite life cycle begins with the enteral phase when meat containing infective third-stage larvae is eaten. Larvae of approximately 1 mm in length are liberated after digestion of the encapsulated cyst wall in the acid-pepsin environment of the stomach. These larvae pass rapidly to the lumen of the small intestine, where they parasitize cells of the columnar epithelium. Four molts occur over 10 to 28 hours, culminating in the development of mature adult female and male worms in the epithelium of the small intestine. Fecund female worms produce and release first-stage (newborn) larvae that initiate the systemic phase of infection when they penetrate the gut wall and enter the lymphatics and eventually the blood circulation via the thoracic duct. Newborn larvae are dispersed in capillary beds throughout the body and ultimately parasitize striated muscle cells. After entering muscle, the larvae molt, encyst, and undergo development to become infective third-stage larvae within 15 days.[5] *T. spiralis* infective larvae encapsulated in collagenous cysts may remain viable for months to years. The cysts may calcify and be visible on radiographs.

Epidemiology

Trichinellosis historically has been associated with the consumption of undercooked pork products prepared from domestic swine. The epidemiologic significance of this source of infection has diminished over

TABLE 287.1 Hosts and Geographic Distribution of Species of the Genus *Trichinella*

SPECIES	CODE	COMMON HOSTS	GEOGRAPHIC DISTRIBUTION	ENCAPSULATED
Trichinella spiralis	T1	Pigs, rodents, horses, bears, foxes	Worldwide	Yes
Trichinella nativa	T2	Bears, foxes, dogs	Arctic, sub-Arctic	Yes
Trichinella britovi	T3	Dogs, cats, bears	Temperate areas, sub-Arctic	Yes
Trichinella pseudospiralis	T4	Birds, omnivorous mammals	Arctic, Tasmania	No
Trichinella murrelli	T5	Bears	North America	Yes
Not yet defined	T6	Bears	Sub-Arctic	Yes
Trichinella nelsoni	T7	Hyenas, cats	Tropical Africa	Yes
Not yet defined	T8	Lions, panthers	Southern Africa	Yes
Not yet defined	T9	Sylvatic carnivores	Japan	No



FIG. 287.1 Coiled *Trichinella spiralis* larvae within a skeletal muscle cell. *(From McAdam AJ, Sharpe AH. Infectious diseases. In: Kumar V, ed.* Robbins and Cotran Pathologic Basis of Disease. *8th ed. Philadelphia: Saunders; 2009.)*

the latter part of the 20th century as the practice to feed pigs garbage containing *Trichinella*-infested meat scraps or rodents has been eliminated. Notably, outbreaks of *T. spiralis* infection associated with the consumption of pork from domestic swine have recently been observed in several countries in eastern Europe and in western China.[6,7] Consumption of meat from horses and wild game (boar, deer, bear, cougar, and walrus in areas where sylvatic trichinellosis is endemic) may also be the source of human infection, and has been reported in the United States.[8–10] *T. spiralis* is the most common cause of human infection; *Trichinella britovi, Trichinella pseudospiralis,* and *Trichinella murrelli* often underlie cases associated with consumption of wild game.

Pathogenesis and Pathology

Pathologic manifestations of infection first appear in the gastrointestinal tract. Two to 3 weeks after ingestion of contaminated meat and establishment of adult worms in the upper small intestine, local villous atrophy and mucosal and submucosal infiltration with neutrophils, eosinophils, and macrophages develop. However, the most characteristic pathologic change induced by the parasite is evident in skeletal muscle fibers. Histologically, edema and basophilic degeneration are present. Coiled worms, cyst walls resulting from parasitization of muscle cells, and infiltrates consisting of eosinophils and lymphocytes may be observed (Fig. 287.1). Although nonstriated muscle and other host tissues do not support the complete development of *Trichinella* to infective third-stage larvae, newborn larvae that disseminate to the myocardium, lung, and central nervous system in heavily infected individuals may cause local inflammation and tissue damage that have serious pathologic consequences (e.g., myocarditis, encephalitis, meningitis).

Clinical Manifestations

Based on observations made under circumstances in which a common source of infested meat has been identified, the majority of infected people are asymptomatic. Morbidity is most likely to develop in individuals who have ingested the highest parasite inocula. Watery diarrhea is the most common manifestation during the enteral phase of infection. Vomiting, abdominal discomfort, and nausea may also be observed. Prolonged diarrhea, sometimes lasting for weeks, and other gastrointestinal symptoms in Native American adults who traditionally consume polar bear or walrus meat infested with *Trichinella nativa* have been suggested to reflect immunity acquired as a result of earlier infections. Facial and periorbital edema, fever, weakness, malaise, myalgia, urticarial rash, conjunctivitis, and conjunctival and subungual hemorrhages appear during the systemic phase when newborn larvae disseminate. These signs and symptoms are most severe and peak 2 to 4 weeks after ingestion of contaminated meat.[11–14] Weakness and myalgia appear first and are most severe in the extraocular, masseter, and neck muscles. Patients with high infection burdens may die of myocarditis, encephalitis, or pneumonia that becomes progressively severe after 4 to 8 weeks. It has been suggested that trichinellosis can lead to chronic muscle pain and weakness.

Diagnosis

Trichinellosis should be considered in the differential diagnosis of patients presenting with myositis, eosinophilia, fever, elevated creatine phosphokinase and lactate dehydrogenase levels, and signs consistent with systemic dissemination of newborn larvae as described previously. Questioning regarding a history of consumption of undercooked meat from wild or farmed game, such as bear and boar or pigs raised in noncommercial and unregulated farms, is informative. It is also helpful to determine whether a similar illness has developed in others who have consumed the same food. Antibodies to *Trichinella* spp. can be detected by a variety of techniques[15] (enzyme-linked immunosorbent assay is now used by the Centers for Disease Control and Prevention). Seroconversion usually occurs by approximately 3 weeks after ingestion of infective larvae. A biopsy specimen of painful muscle may reveal the presence of *Trichinella* spp., although this procedure is not recommended in most situations. It is useful to obtain a sample of the meat suspected to harbor the parasite because this can be used to confirm the origin of the infection. Various *Trichinella* spp. can be differentiated by DNA-based technologies such as polymerase chain reaction assay.[16]

The differential diagnosis of trichinellosis observed during the enteral phase of infection includes a wide variety of causes of gastroenteritis and diarrheal illnesses. During the systemic phase of infection, febrile illnesses including influenza and typhoid fever, connective tissue diseases such as dermatomyositis, and angioneurotic edema should be considered.

Therapy

Currently, no anthelmintic drug has proved effective against newborn larvae or maturing first-stage larvae that cause myositis and other signs

and symptoms that appear during the systemic phase of infection. Systemic corticosteroids in conjunction with mebendazole may be used in patients with severe illness, although proven benefit for this approach is lacking.

If the diagnosis is made during the enteral phase of infection (e.g., within 1–2 weeks of eating contaminated meat or when gastrointestinal signs and symptoms are present), mebendazole (200–400 mg orally three times daily for 3 days, then 400–500 mg orally three times daily for 10 days for all ages) may be used to eliminate adult worms from the small intestine.[17] Mebendazole is available in the United States only through compounding pharmacies. Albendazole (400 mg orally twice daily for 8–14 days) may also be used.

Prevention

Awareness of and compliance with safety regulations prohibiting the use of garbage as a source of food for domestic animals, control of rodents, segregation of livestock from wild animals, and proper preparation of meat from wild animals will reduce the risk for infection with *Trichinella* spp. Inspection of meat for *Trichinella* is ideally done by direct dissection and visualization of encysted larvae.

Cooking meat to a temperature of 55°C or higher (until pink fluid or flesh is not visible) kills *Trichinella* spp. Storage in a freezer (–15°C) for 3 or more weeks kills *T. spiralis* in pork, but this is not effective for *Trichinella* spp. larvae in horse and game meat. Drying, smoking as in the preparation of jerkies, and salting of meat should not be relied on to kill *Trichinella*.

HAYCOCKNEMA PERPLEXUM INFECTION

Haycocknema perplexum is a species of Muspiceoidia nematodes that has been observed in the tissues of Australian mammals and marsupials. Adult and larval stages of the nematode have been identified in muscle biopsy specimens of Australian patients from Tasmania and tropical northern Australia who present with chronic myositis, peripheral muscle weakness, and dysphagia accompanied by eosinophilia and elevated creatine kinase level.[18,19] Muscle weakness improved, and killing of parasites was observed, after administration of multiple doses of albendazole. Corticosteroids are not indicated because this class of drugs may exacerbate muscle weakness and lead to a reduction of eosinophilia that misleads the clinician to conclude that a beneficial effect on a connective tissue disease such as polymyositis has occurred.

DRACUNCULIASIS

Dracunculiasis, or guinea worm disease, is caused by the parasitic nematode *Dracunculus medinensis*. Once prevalent throughout southern Asia and parts of the Middle East, as of 2015–2016 indigenous infections were limited to Chad, Ethiopia, Mali, and South Sudan, with the majority in Chad.[20] Between 2016 and 2017, the number of reported cases worldwide fell from 10 to 8, a 20% decline in 1 year. The parasite causes debilitating skin lesions and secondary bacterial infections. Dracunculiasis has had great socioeconomic impact in affected rural communities.

Life Cycle of the Parasite

Humans are infected by swallowing fresh water from stagnant pools containing minute freshwater crustaceans (copepods) harboring infective larvae of *D. medinensis*. When the copepods are digested in the acid-pepsin environment of the stomach, larval forms are released from the body of the crustacean, after which they penetrate the wall of the small intestine and migrate through the thoracic musculature. Sexually mature male and female worms develop over 2 to 3 months. Gravid female worms mature over 10 to 14 months, migrate throughout the body, and ultimately reach the skin, particularly over the lower legs, ankles, and feet (Fig. 287.2). When skin comes into contact with water, the female worm (which may reach a length of 1 m) induces a local blister that eventually ruptures. Large numbers of larvae are released into the water when prolapsed loops of the uterine cavity contract. The motile free-swimming larvae infect copepods. *D. medinensis* larvae become infective for humans after further development in the intermediate copepod host over a 2-week period.

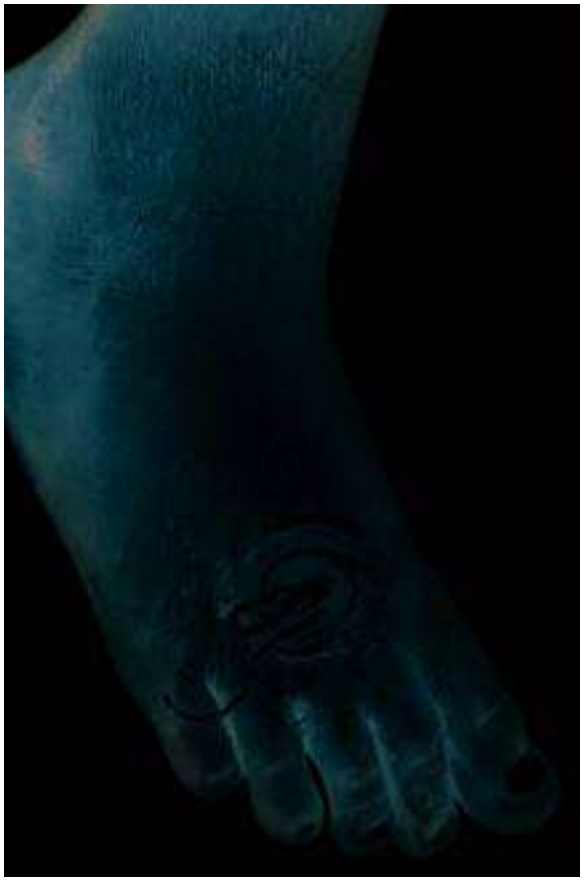

FIG. 287.2 ***Dracunculus medinensis*** **emerging from the skin over the foot.**

Epidemiology

Clinical manifestations of *D. medinensis* infection appear approximately 1 year after ingestion of contaminated water and are highly seasonal because they coincide with the appearance of stagnant pools of surface water that vary according to local climatic conditions. The prevalence of dracunculiasis is a strong indicator of socioeconomic development because communities are at highest risk where treatment of contaminated water, access to safe drinking water, and separation of bathing and drinking facilities are inadequate.

Clinical Manifestations

Signs and symptoms of dracunculiasis appear approximately 1 year after infection when fecund adult female worms appear near the surface of the skin. The initial presentation is a painful papule that enlarges over hours to days to form a blister that allows a portion of the worm to emerge from the skin. The blister may be accompanied by local erythema, urticaria, fever, nausea, and pruritus. The entire worm may emerge over a period of several weeks. Complications include secondary bacterial infections that may lead to sepsis, local abscesses, and pyogenic arthritis. Affected individuals are incapacitated for approximately 8 weeks. The vast majority of worms emerge from the lower leg, ankle, and foot, although aberrant sites of emergence have been reported (e.g., head, neck, genitalia).

Diagnosis and Therapy

Dracunculiasis is diagnosed by the appearance of the skin blister and adult worm (see Fig. 287.2). No anthelmintic drugs are known to be effective against *D. medinensis*. Application of wet compresses to the affected skin, administration of analgesics, and prevention of secondary bacterial infection by the use of topical antibiotics are recommended. Worms should be slowly and gently extracted over a period of several

TABLE 287.2 Filarial Diseases and Infections of Humans

DISEASE	GENUS AND SPECIES OF FILARIAL PATHOGEN	MAJOR CLINICOPATHOLOGIC MANIFESTATIONS	AREAS OF ENDEMICITY	INSECT VECTORS
Lymphatic filariasis	*Wuchereria bancrofti, Brugia malayi, Brugia timori*	Lymphedema of extremities and breasts, hydrocele, funiculitis, orchitis, tropical (pulmonary) eosinophilia	Sub-Saharan Africa, Southeast Asia, Oceania and western Pacific, Caribbean, limited areas of South America	*Anopheles, Culex, Aedes* spp. mosquitoes
Loiasis	*Loa loa*	Subcutaneous and conjunctival swelling	West and central Africa	*Chrysops* spp. tabanid flies
Onchocerciasis	*Onchocerca volvulus*	Dermatitis, keratitis, chorioretinitis	Sub-Saharan Africa, Arabian peninsula, parts of Central and South America	*Simulium* spp. black flies
Mansonellosis	*Mansonella ozzardi*	Rarely symptomatic	Caribbean, Central and South America	*Culicoides* spp. midges, *Simulium amazonicum* black flies
	Mansonella perstans	Rarely symptomatic	Sub-Saharan Africa, northern coast of South America, Tunisia, Algeria	*Culicoides* spp.
	Mansonella streptocerca	Dermatitis, inguinal lymphadenopathy	Central and West Africa	*Culicoides grahamii*

days using a small stick because breaking the worm can lead to allergic reactions and secondary bacterial infection.

Control and Eradication

The global effort to eradicate dracunculiasis began in 1986 when it was estimated that approximately 3.5 million cases occurred annually in 17 countries in Africa and in 3 countries in Asia. The program was closely linked with efforts to improve the safety of drinking water. Eradication is based on simple and cost-effective measures that include (1) filtration of drinking water through fine-meshed cloth to remove copepods, (2) killing copepods in sources of drinking water by application of temephos (Abate; BASF, Research Triangle Park, NC) larvicide, (3) provision of clean drinking water from boreholes or wells, and (4) educating residents of communities to avoid entering sources of drinking water if worms are emerging. The eradication effort has been largely successful, but a zoonotic reservoir in dogs and baboons along with civil unrest have been associated with incident cases. The total number of new cases in the world was estimated to be 25 in 2016.[20]

FILARIASES

Filarial parasites are threadlike nematodes transmitted to humans by obligatory blood-feeding insect vectors. The lymphatic system, skin, and eyes are the main areas of involvement (Table 287.2). Repeated and long-duration exposure to insect vectors harboring infective larvae is generally necessary for humans to acquire these infections, although travelers to endemic areas occasionally become infected.[21] All filariae of medical significance except for *Loa loa* harbor *Wolbachia* bacterial endosymbionts that are important in maintaining the reproductive capacity of adult female worms.

Lymphatic Filariasis

Wuchereria bancrofti, Brugia malayi, and *Brugia timori* are the causative agents of bancroftian and brugian (sometimes referred to as Malayan) filariasis. Approximately 1 billion persons worldwide are at risk; 120 million residents of developing countries were estimated to be infected before the initiation of a global elimination effort in the late 1990s.[22] In contrast to many other mosquito-borne infections of medical significance, lymphatic filariae are transmitted by not just one but several genera of mosquitoes. These include *Anopheles, Culex,* and *Aedes* spp., which differ greatly in their efficiency of transmission (*Anopheles* and *Culex* spp. being the least and most efficient, respectively).[23] The major pathologic manifestations are acute transient episodes of fever accompanied by painful inflammation of the lymphatics of the extremities and male genitalia and chronic lymphatic dysfunction that leads to gross disfigurement of the male genitalia and progressive lymphedema and swelling of the legs, arms, or breasts.

Life Cycle of the Parasite

Infection is initiated when female mosquitoes release infective third-stage larvae into the puncture site of the skin created during blood-feeding. These larvae pass rapidly through the dermis and enter local lymphatic vessels, where they molt to form fourth-stage larvae. Over 6 to 9 months, the parasites undergo another molt in afferent lymphatic vessels and eventually develop into sexually mature adult male and female worms. Adult worms largely reside in afferent lymphatic vessels of the upper and lower extremities and the lymphatics of the male genitalia, such as those draining the epididymis, testes, and spermatic cord. Other areas of the body, such as skin, may also harbor adult worms. Fecund female worms release as many as 10,000 first-stage larvae (commonly referred to as microfilariae) per day, which migrate from the lymphatics and enter the bloodstream. Microfilariae in peripheral blood are ingested by mosquitoes and undergo development to infective third-stage larvae after completing two molts in the mosquito over a period of 14 days. Adult worms are larger than microfilariae (100 × 0.25 mm vs. 150 × 7 μm, respectively; Fig. 287.3) and have a reproductive life span of 5 to 7 years. A characteristic feature of lymphatic filariasis in most endemic areas is the nocturnal periodicity of microfilaremia, whereby peak levels of parasites appear in peripheral blood at night when the mosquito vectors are seeking a blood meal. During the day, microfilariae are sequestered in deep vascular beds and may not be detectable in peripheral blood.

Epidemiology

Lymphatic filariasis is endemic in South Asia, sub-Saharan Africa, and Pacific regions. Countries with the highest prevalence include India, Indonesia, Papua New Guinea, Nigeria, Ghana, Kenya, and Tanzania. *W. bancrofti* is transmitted in nearly all endemic areas and constitutes 90% of cases worldwide. There is no animal reservoir for *W. bancrofti*. *B. malayi* is limited to southern and Southeast Asia and parts of the Pacific, and may infect cats and primates as well as humans. *B. timori* is found only in the islands of eastern Indonesia. The distribution of infection is heterogeneous within a given geographic region because the local spatial ecology is important in determining transmission and exposure to infective larvae (i.e., the proximity of mosquito breeding sites to human dwellings).[24] Because less than 1% of competent mosquito vectors contain infective larvae, intense exposure to mosquitoes is necessary to develop patent infection. Lymphatic filariasis has a profound detrimental effect on the economy and psychosexual health in societies where manual labor and subsistence agriculture are important in daily life.

Clinical Manifestations

The vast majority of individuals with patent infections documented by the presence of microfilaremia do not have clinically overt manifestations

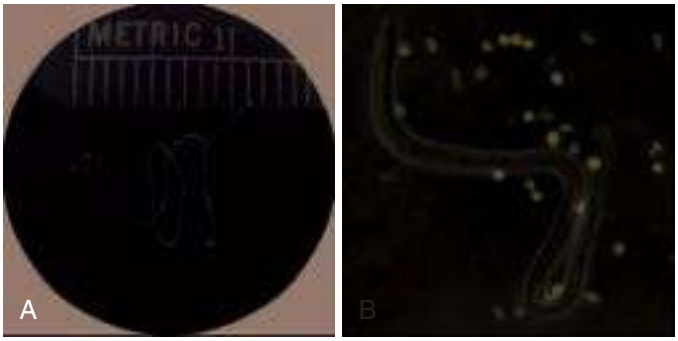

FIG. 287.3 Adult worms and microfilaria of *Wuchereria bancrofti.* (A) Male adult worm *(left)* and female adult worm *(right).* (B) Giemsa-stained microfilaria in a thick blood smear. *(From Lymphatic filariasis image gallery. In DPDx Laboratory Identification of Parasites of Public Health Concern.* https://www.cdc.gov/dpdx/lymphaticfilariasis/index.html.*)*

of a lymphatic pathologic process. Nevertheless, imaging studies indicate that asymptomatic infected adults and children may have compromised lymphatic function.[25,26] Overt pathologic sequelae first become apparent during adolescence and early adulthood, often as acute adenolymphangitis with fever and swelling of the leg, the arm, or the male genitalia. These episodes may last for 4 to 7 days in persons who have previously been asymptomatic or longer in persons who have experienced repeated attacks. The lymphatic inflammation typically progresses retrograde from axillary or inguinal lymph nodes, a feature that distinguishes acute adenolymphangitis from the more recently described entity of acute dermatolymphangioadenitis. The latter progresses toward the lymph node from a peripheral site and is believed to be due to secondary bacterial infection and not inflammation elicited by filarial worms.[27] A cut or entry wound of the skin may be observed in an interdigital area. So-called filarial fever in the absence of lymphatic inflammation may also be observed. In endemic populations, filarial fevers may be difficult to distinguish from other common causes of acute febrile illness.

Repeated episodes of acute filarial disease often precede the development of chronic lymphatic pathology that includes lymphedema of the legs, arms, and breasts and chronic disfigurement of the male genitalia (Fig. 287.4). Male genital involvement is very common in *W. bancrofti* infection and uncommon in *Brugia* spp. infection. Chronic swelling of the legs and compromised lymphatic drainage may result in secondary bacterial infections and sclerosis and verrucous changes of the overlying skin. The most severe cases are sometimes referred to as elephantiasis. Male genital involvement includes hydrocele, funiculitis, epididymitis, and orchitis. There is a poor understanding of the pathogenesis of the various genital manifestations of lymphatic filariasis.[28] Chyluria precipitated by the rupture of dilated lymphatic vessels into the genitourinary tract is an uncommon manifestation. Tropical pulmonary eosinophilia is described separately at the end of this section.

Pathogenesis and Pathology

Adult worms residing in afferent lymphatic vessels and lymph nodes draining the legs, arms, male genitalia, and occasionally other anatomic sites presumably initiate the disease process when poorly characterized parasite mediators produce local lymphatic dilation. Live motile worms exhibiting the "filaria dance" sign and nearby dilated lymphatic vessels can be detected by ultrasonography in the scrotum, inguinal lymph node, and breast[29,29a] (videos can be viewed at https://filariajournal.biomedcentral.com/articles/10.1186/1475-2883-2-3).

It is not clear why the majority of infected individuals remain asymptomatic, whereas others develop acute or chronic manifestations of lymphatic filariasis. With respect to acute filarial pathologic processes, the intensity of transmission and secondary bacterial infections may both be important in determining susceptibility.[30,31] The importance of worm burden as a determinant of a chronic lymphatic pathologic process is controversial. Many persons with chronic lymphedema of the extremities have no evidence of active infection (particularly in India), whereas



FIG. 287.4 Disfigurement of male genitalia with repeated episodes of acute filarial disease. Man with inguinal lymphadenopathy, large bilateral hydroceles, marked edema of the lower left limb (particularly the leg and foot), and early signs of elephantiasis in the foot.

this is not the case in other *W. bancrofti* endemic areas such as Papua New Guinea. Adaptive T-cell responses, genetic susceptibility, worm burden, and systemic inflammatory responses have been suggested to contribute to the complex clinical phenotypes of lymphedema and hydrocele, but a single unifying mechanism has not been defined.[32,33] An increased incidence of human immunodeficiency virus infection has been reported in individuals with lymphatic filariasis and may be due to the systemic immune activation induced by this disease.[34]

Diagnosis

Lymphatic filariasis is diagnosed by obtaining an appropriate history of exposure to mosquitoes in areas where the infection is endemic, observing pathologic signs, and performing a variety of laboratory tests. As described previously, a chronic lymphatic pathologic process such as lymphedema of an extremity or hydrocele primarily affects adults who are residents of endemic areas. A definitive diagnosis is made by microscopic detection of microfilariae in the blood or occasionally from other sites such as fluid aspirated from hydroceles. Blood to detect microfilaremia should generally be obtained at night when the peak density of parasitemia occurs. This is the case for most endemic areas except for *W. bancrofti* in some areas of Oceania. The sensitivity of detecting microfilaremia is enhanced by concentrating parasites by filtration of blood through polycarbonate (Nuclepore; Whatman/GE Healthcare Life Sciences, Pittsburgh, PA) filters that retain microfilariae. Enzyme-linked immunosorbent assay–format antigen capture assays that detect antigen secreted by *W. bancrofti* adult worms have been an important diagnostic advance because these tests allow point-of-care documentation of active infection in the absence of microfilaremia.[35,36] An analogous test for *Brugia* spp. is not yet available. Biopsy of lymphatic tissues to identify adult worms is not justified. As described earlier, adult worms can be imaged in vivo by the use of Doppler ultrasonography.

Therapy

Persons with asymptomatic parasitemia should be treated with diethylcarbamazine (6 mg/kg/day divided into two or three doses over 12 days to a total of 72 mg/kg). The drug is highly effective at eliminating microfilariae but has modest activity against adult worms. The filaricidal activity of diethylcarbamazine is greater against *W. bancrofti* than *B. malayi*. Side effects include fever and occasionally asthma-like symptoms in persons with high-level microfilaremia, and painful nodules may appear in the lymphatics, lymph nodes, skin, and male genitalia. The development of these nodules, usually less than 1 cm in diameter, is an inflammatory reaction to the death of adult worms or migrating larvae. They may appear days to weeks after taking antifilarial drugs, particularly diethylcarbamazine, because it has greater macrofilaricidal activity than albendazole or ivermectin. Systemic posttreatment reactions are likely related to innate immune reactions to *Wolbachia* endosymbionts released by dying microfilariae. The severity of acute side effects may be reduced by initiating treatment with a lower dose of diethylcarbamazine (50 mg on day 1, followed by 50 mg three times on day 2 and 100 mg three times on day 3). Hydroceles may be repaired surgically, but prevention of recurrence is contingent on drug treatment.

It is not known whether diethylcarbamazine or other antifilarial drugs such as ivermectin and albendazole ameliorate a preexisting lymphatic pathologic process because randomized clinical trials have not been performed. Some studies of mass drug treatment to control filariasis in endemic populations suggest that reducing transmission may decrease the incidence of chronic lymphatic pathologic processes.[37]

Prevention, Control, and Eradication

Lymphatic filariasis due to *W. bancrofti* has been targeted for elimination as a public health problem and for worldwide eradication by annual mass treatment with single-dose diethylcarbamazine combined with albendazole (in areas of the world where onchocerciasis and loiasis are not endemic) or ivermectin combined with albendazole (in areas where onchocerciasis and loiasis are endemic). By 2014, mass drug administration programs had been implemented in more than 60 countries, and it has been estimated that more than 5 billion doses of antifilarial medications have been administered.[38,39] *Brugia* spp. are less amenable to eradication because the parasite has an animal reservoir. It is currently believed that at least 5 years of annual mass treatment is necessary to stop transmission because this is the estimated reproductive life span of adult worms. Results of studies in Egypt indicate that this strategy will likely be successful in endemic areas where a good public health infrastructure exists.[40] It is not yet known whether similar success will be achieved in rural areas where population coverage with antifilarial drugs is suboptimal or vectors with greater transmission efficiency exist. The use of drugs that target the *Wolbachia* endosymbiont of filarial parasites (e.g., doxycycline) may provide added benefit because killing of these *Rickettsia*-like bacteria reduces the fecundity and shortens the reproductive life span of adult worms.[41] The use of insecticide-treated bed nets has been shown to significantly reduce the number of mosquitoes harboring infective larvae.[42]

Tropical Pulmonary Eosinophilia

This syndrome is associated with *W. bancrofti* and *B. malayi* infection. It is most commonly observed in South and Southeast Asia and endemic areas of Brazil and Guyana. Patients are typically middle-aged men (male-to-female ratio, 4 : 1) who present with nocturnal asthma, cough, fever, and weight loss. Microfilariae are not detectable in peripheral blood. High-level eosinophilia (>3000/μL blood) with elevated antifilarial antibodies and polyclonal immunoglobulin E are present. Chest radiographs typically show increased bronchovascular markings with a mottled appearance in the middle and lower lungs. Treatment with diethylcarbamazine leads to symptomatic improvement and reduces eosinophilia and immunoglobulin E levels. Re-treatment may be necessary in some cases. If the infection is not treated, progressive interstitial fibrosis and restrictive lung disease may develop.[43]

Loiasis

Adult worms of these nematodes migrate in subcutaneous tissue and occasionally the conjunctiva, where they elicit transient painful swelling. The infection is endemic in central and West Africa.

Life Cycle of the Parasite

Loa loa infective larvae are transmitted by female tabanid (red) flies (*Chrysops* spp.) that seek blood meals during the day. Adult worms measuring 30 to 70 × 0.3 mm develop over 6 to 12 months and migrate in subcutaneous tissues. Microfilariae released from fecund female worms migrate to the blood and have a diurnal periodicity. In contrast to other filarial pathogens of medical significance, *L. loa* do not harbor *Wolbachia* endosymbionts.

Epidemiology

Loiasis is endemic in coastal and rain forest regions of central and West Africa. The infection has been observed as far east as Uganda and Ethiopia. Infected individuals usually have a long history of residence in endemic areas with prolonged exposure to infected vectors; however, repeated short durations of intense exposure can also result in infection and morbidity, including in expatriate nonresidents who travel to endemic areas.[44]

Clinical Manifestations

Most infected individuals are asymptomatic. The main clinical presentation is a Calabar swelling, which represents an angioedematous response to adult worms migrating through subcutaneous tissue. The 10- to 20-cm swelling lesions most commonly appear on the face and extremities and are preceded by itching and pain. Calabar swellings are transient and persist from several days to weeks. Adult worms may also migrate to the conjunctiva and produce an "eye worm" (Fig. 287.5). Other manifestations include renal complications (hematuria and proteinuria) and encephalitis, which are usually precipitated by treatment with the antifilarial drug diethylcarbamazine. In the case of encephalitis, persons with high-level microfilaremia (>2500 microfilariae/mL) are at high risk.[45] Precautions for treating such individuals are described later under "Therapy."

Diagnosis

The diagnosis should be considered in persons who have resided in an endemic area and present with urticaria, an eye worm, or eosinophilia. Identification of microfilariae in the blood (which should be obtained during the day) or visualization of an adult worm in the conjunctiva confirms loiasis. Standardized and specific serologic tests for *L. loa* are not widely available outside the research laboratory setting. Molecular assays with high specificity and the ability to quantify *L. loa* microfilaremia are under development to address this gap.[46] The differential diagnosis includes other causes of angioedema such as C1 inhibitor deficiency, worm infections such as onchocerciasis and mansonellosis (both of which may overlap in endemicity with loiasis), and the various causes of high-level eosinophilia.

Therapy

Diethylcarbamazine at a dose of 8 to 10 mg/kg/day for 21 days should be given to persons who do not have microfilaremia. Repeated



FIG. 287.5 Serpentine adult *Loa loa* passing through the subconjunctiva.

treatment courses with diethylcarbamazine may be necessary in 40% to 50% of patients. Calabar swelling, pruritus, and eye worms may be precipitated by treatment. Side effects can be minimized by concurrent administration of antihistamines or corticosteroids. In persons with high-level microfilaremia (>2500/mL), there is a significant risk for renal and central nervous system complications due to the rapid destruction of large numbers of microfilariae. Options include withholding anthelmintic drugs, cytapheresis to remove microfilariae before administration of diethylcarbamazine, and administration of a single dose of ivermectin. In the context of mass drug administration programs in West and central Africa aimed at elimination of onchocerciasis or lymphatic filariasis, it is important that prior screening for coendemic, high-level *L. loa* microfilaremia be performed to exclude treatment in order to avoid serious adverse events such as encephalitis.

Prevention and Control

Travelers who plan prolonged residence in endemic areas should take 300 mg diethylcarbamazine weekly as chemoprophylaxis.[47] There are currently no effective means to control the tabanid flies that transmit *L. loa.*

Onchocerciasis

Onchocerca volvulus is transmitted by blood-feeding *Simulium* spp. black flies. Infection can cause dermatitis, subcutaneous nodules, keratitis, and chorioretinitis. The disease has a great socioeconomic impact because it causes reduced vision ("river blindness") and chronic skin disease in adults.

Life Cycle of the Parasite

Infection is acquired when female *Simulium damnosum* sibling species (in Africa) and several other *Simulium* spp. (in the Americas and Arabian peninsula) take blood meals. Infective third-stage larvae migrate to subcutaneous tissue, undergo two molts, and eventually over 6 to 12 months develop into threadlike sexually mature adult male (300 × 0.3 mm) or female (400 × 0.3 mm) worms. Adult worms aggregate in nodules in subcutaneous tissue and muscle. Fecund female worms release unsheathed microfilariae (200 × 8 µm) that migrate to the dermis and eye.

Epidemiology

Onchocerciasis is endemic in West Africa, limited areas of East Africa, and the Arabian peninsula. Mass administration of ivermectin in six endemic countries in the Americas has resulted in interruption of transmission in all geographic regions with the exception of remote border areas of southern Venezuela and northern Brazil.[48] The greatest number of infected individuals live in West Africa, particularly Nigeria and the Democratic Republic of the Congo.[49,50] Although there is great variability in the frequency of disease manifestations in various endemic areas, the majority of infected individuals are asymptomatic. Onchocerciasis in savanna regions of West Africa is characterized by a greater likelihood of reduced vision and blindness compared with rain forest regions. Dermatologic manifestations of infection are common in areas of Africa outside either the rain forest or savanna.

Pathology and Clinical Manifestations

Adult worms of both sexes reside in nonpainful vascularized fibrous nodules (0.5 × 3.0 cm) in a subcutaneous or intramuscular site. The lesions may be palpable. The major pathologic sequelae of clinical significance are due to inflammatory responses to microfilariae. In the skin, these include acute and chronic papular dermatitis, lichenified dermatitis, atrophy, and depigmentation.[51] Pruritus is usually the presenting symptom. In the eye, microfilariae that have migrated to the cornea initially elicit punctate keratitis. This can progress to a sclerosing keratitis that causes blindness. As described previously, this outcome is most common in savanna forms of onchocerciasis in West Africa. Iridocyclitis and chorioretinitis are less common. *Onchocerca volvulus* infection may also cause inguinal lymph node fibrosis and atrophy of the overlying skin that leads to the appearance of hanging groin.

Diagnosis

The diagnosis should be considered when the previously described signs are present in an individual who has resided in an endemic area. Because transmission is inefficient, residence of months to years is usually (but not always) required to acquire *O. volvulus* infection. Identification of microfilariae in the skin or eye or adult worms in nodules is required for definitive diagnosis. Microfilariae in the skin are accessed by using a corneoscleral instrument to obtain a 1- to 2-mg biopsy specimen of the dermis overlying both scapulae, iliac crests, and calves. The skin snips are placed in microtiter plates containing saline held at 37°C for 60 minutes to 24 hours (depending on the microfilarial burden) to allow the parasites to emerge. The parasites are identified under low-power microscopy. Microfilariae in the eye can be identified by slit-lamp examination. In the research setting, polymerase chain reaction assay can be used to identify persons with light infections that are not detectable by microscopy.[52] The differential diagnosis of onchocercal dermatitis includes scabies and the many other infectious and noninfectious causes of dermatitis. Eye disease may be mimicked by other infectious and noninfectious causes of chorioretinitis. Eye worms similar to those observed for loiasis do not occur because adult *O. volvulus* parasites do not migrate to the conjunctiva.

Therapy

Ivermectin, which is effective in killing microfilariae but not adult *O. volvulus* worms, is the drug of choice. The dose is 150 to 200 µg/kg given as a single dose. A reduction in skin microfilariae is detectable within 2 weeks but may be incomplete. For expatriates who are more prone than lifelong residents of endemic areas to develop severe pruritus, more frequent treatment with ivermectin (e.g., every 3 months) may be necessary to control symptoms. Doxycycline, which kills the obligatory *Wolbachia* endosymbiont of *O. volvulus,* is highly effective in reducing microfilarial production by adult worms and may represent a novel strategy for reducing transmission and disease pathogenesis.[53,54] However, there is no indication that this approach offers benefit for the treatment of the individual patient. In cases in which loiasis coexists with onchocerciasis (both infections are endemic in some areas of West Africa), the use of ivermectin carries a risk for precipitating encephalopathy in persons with high levels of *L. loa* microfilaremia. Cytapheresis may be used to reduce the level of *L. loa* microfilariae before treatment, but this is usually not an option in resource-poor settings.

Prevention and Control

Control of the *Simulium* vector by aerial dispersion of larvicides from 1974 to 2002 was highly successful in reducing blindness in 11 countries in West Africa where the savanna form of onchocerciasis was common. The African Program for Onchocerciasis Control is based on mass distribution of ivermectin to reduce morbidity and possibly stop transmission. The latter goal has recently been emphasized as feasible with programmatic changes that include improved mapping of endemic foci, better monitoring tools, and use of drug combinations that kill adult worms.[55]

Mansonellosis

Mansonellosis is a poorly understood infection of humans that is asymptomatic in most individuals. Transmission is by blood-feeding midges (*Culicoides* spp. for *Mansonella ozzardi, Mansonella perstans,* and *Mansonella streptocerca*) or the black fly (*Simulium amazonicum* for *M. ozzardi*).

M. ozzardi infection is endemic in northern South America, Central America, and some Caribbean islands. Unsheathed microfilariae circulate in the blood. Most infections are asymptomatic. Lymphadenopathy, urticaria, pruritus, pulmonary symptoms, and keratitis have been described. Ivermectin reduces microfilariae, but optimal treatment has not been defined.[56]

M. perstans infection is endemic in parts of central and northern Africa, South America, and the Caribbean. Adult worms reside in serous cavities of the body and sheathed microfilariae circulate in the blood. Angioedema, urticaria, and pruritus have been attributed to the infection. Diethylcarbamazine lowers the level of microfilaremia. Doxycycline has been shown to be effective in reducing microfilaremia, likely through its ability to eliminate adult worms by killing *Wolbachia* bacterial endosymbionts.[57]

M. streptocerca is endemic in rain forest regions of central Africa and parts of Uganda.[58,59] Many infected individuals are asymptomatic,

although some people develop inguinal lymphadenopathy and a chronic dermatitis with pruritus. The latter may be similar to that seen in persons with onchocerciasis. Microfilariae reside in the dermis and can be identified by obtaining a skin snip and identifying the parasite microscopically as described for onchocerciasis. *M. streptocerca* microfilariae are distinguished from those of *O. volvulus* by the hook-shaped tail of the former.[60] Treatment is with diethylcarbamazine at a dose similar to that described for lymphatic filariasis.

Zoonotic Filariae Reported to Cause Human Disease

Dirofilaria repens is a parasite of felids and canids transmitted by mosquitoes. Humans are nonpermissive hosts, but the parasite may undergo incomplete development in the eye. Subconjunctival, intraocular, and orbital lesions have been described. Treatment is by excision.[61] Subconjunctival infections with the canine parasite *Onchocerca lupi* and with *Setaria labiatopapillosa* have been reported.

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288 Trematodes (Schistosomes and Liver, Intestinal, and Lung Flukes)

James H. Maguire[a]

SHORT VIEW SUMMARY

Definition
- Trematodes or flukes are flatworms that live in blood vessels, biliary tract, intestines, and lungs of humans and lower animals.
- Included are the schistosomes and the foodborne trematodes: liver flukes *(Fasciola, Clonorchis, Opisthorchis),* intestinal flukes (various species), and lung fluke *(Paragonimus).*

Epidemiology
- Schistosomes are prevalent in tropical and subtropical Africa, the Middle East, Southeast Asia, East Asia, the Philippines, and limited areas in the Caribbean and South America in areas with inadequate sanitation or access to clean water.
- Foodborne trematodes are most prevalent in Southeast and East Asia but also in other parts of the world where persons ingest raw or undercooked fish, crayfish, or plants produced in fresh water contaminated with human or animal feces or, for *Paragonimus*, sputum.

Microbiology
- Persons become infected with schistosomes when the larval parasites (cercariae) shed by freshwater snails (intermediate hosts) penetrate bare skin.
- Persons become infected with foodborne trematodes by ingesting the larval parasites (metacercariae) encysted in fish, aquatic vegetation, or crustaceans (second intermediate host) that were infected with cercariae shed from snails (first intermediate host).

Diagnosis
- Trematode infections are frequently light and may cause few or no symptoms but often elicit peripheral blood eosinophilia.
- Suggestive clinical syndromes during the first weeks after initial infection include acute illness with fever, urticaria, eosinophilia, and other symptoms when larval flukes are migrating through the body and maturing; ectopic migration can lead to disease of the central nervous system, the skin, and other parts of the body.
- Suggestive clinical syndromes during chronic infections include: intestinal schistosomes (diarrhea, intestinal polyps, portal hypertension), urinary schistosomes (hematuria, bladder cancer), liver flukes (biliary obstruction), intestinal flukes (diarrhea, abdominal discomfort), and lung flukes (cough, hemoptysis, cavitary lung lesions).
- Diagnosis is made by microscopic identification of characteristic eggs in stool, urine, or sputum or by identification of larval or adult worms in tissue.
- Serology, available for some trematode infections, may be more sensitive than microscopic examination, especially during acute infections.

Therapy
- Praziquantel is the drug of choice for all trematode infections except fascioliasis, for which triclabendazole is preferred (see Table 288.1).

Prevention
- For schistosomiasis, preventive methods include sanitation, provision of clean water, snail control, and avoidance of contact with contaminated fresh water.
- For foodborne trematodes, prevention of infection includes maintaining freshwater bodies free of contamination by humans and lower animals, snail control, and proper cooking of aquatic fish, plants, and crustaceans.
- Periodic screening and treatment or mass drug administration for populations at risk for infection are essential.

The trematode flatworms that infect humans include the schistosomes, which live in venules of the gastrointestinal (GI) or genitourinary tract, and other flukes, which inhabit the bile ducts, intestines, or lungs.[1] The geographic distribution of each species of trematode parallels the distribution of the specific freshwater snail that serves as its intermediate host (Table 288.1).[1a] Six species of schistosomes infect more than 230 million persons, and more than 100 species of other trematodes infect at least 65 million persons.[2–6]

Trematodes vary in length from 1 mm to more than 10 cm, are flattened dorsoventrally, and have an anterior and ventral sucker and a blind bifurcate intestinal tract. Schistosomes differ from other trematodes in several ways: The sexes of adult worms are separate, transmission is through penetration of skin by larvae, and there is only a single intermediate host, whereas other trematodes are hermaphroditic and are transmitted through ingestion of infected fish, crustaceans, or aquatic plants that serve as second intermediate hosts. Unusually, free-swimming larvae of nonschistosomal trematodes directly invade the human conjunctiva or anterior chamber and cause ocular granulomata, as in the case of *Procerovum varium*, a parasite of fish-eating birds in Asia and the western Pacific.[6a] Only a small proportion of persons with trematode infections harbor large numbers of worms and are at risk for severe disease; most are asymptomatic or experience subtle morbidity, such as fatigue and cognitive or physical impairment.[3,4,7–10]

[a]All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

SCHISTOSOMES

Of more than 230 million persons infected with schistosomes in 78 countries and territories, about 120 million have symptoms, 20 million have severe disease, and as many as 200,000 or more die each year.[1,1a,2,4,6–8] Although control programs and socioeconomic development have eliminated or nearly eliminated transmission of schistosomiasis in more than a dozen countries, progress has been slow elsewhere, especially in sub-Saharan Africa, where greater than 90% of cases occur.[2,4,8] Moreover, water resource development projects and population movements continue to spread the disease into regions where it was not previously endemic.[5a] Three main species, namely, *Schistosoma mansoni, Schistosoma japonicum,* and *Schistosoma haematobium,* and three species with narrow geographic distribution, namely, *Schistosoma mekongi, Schistosoma intercalatum,* and *Schistosoma guineensis,* cause human schistosomiasis or bilharziasis, named after Theodor Bilharz who first identified the parasite in 1852.[8] A dozen or more other species of animal schistosomes can cause human infection, including schistosomes of birds and small mammals that cannot mature in the human host but die

TABLE 288.1 Features of Schistosomes and Other Important Trematodes

PARASITE	SNAIL INTERMEDIATE HOST (GENUS)	SECOND INTERMEDIATE HOST	GEOGRAPHIC DISTRIBUTION	LOCATION OF ADULT WORMS	TREATMENT
Schistosomes[a]					
Schistosoma mansoni	*Biomphalaria*	None	Brazil, Venezuela, Suriname, Sub-Saharan Africa, Caribbean, Middle East	Mesenteric venules	Praziquantel, 40 mg/kg/day in 1 or 2 doses × 1 day Oxamniquine[b]
Schistosoma japonicum	*Onchomelania*	None	China, Philippines, Indonesia	Mesenteric venules	Praziquantel, 60 mg/kg/day in 3 doses × 1 day
Schistosoma mekongi	*Neotricula*	None	Cambodia, Laos	Mesenteric venules	Praziquantel, 60 mg/kg/day in 3 doses × 1 day
Schistosoma intercalatum, Schistosoma guineensis	*Bulinus*	None	Central and West Africa	Mesenteric venules	Praziquantel, 40 mg/kg/day in 1 or 2 doses × 1 day
Schistosoma haematobium	*Bulinus*	None	Africa, Middle East, Corsica (France)	Venules of lower urinary tract	Praziquantel, 40 mg/kg/day in 1 or 2 doses × 1 day Metriphonate[b]
Liver Flukes					
Clonorchis sinensis	*Bithynia, Parafossarulus*	Freshwater fish	China, Taiwan, Korea, Japan, Vietnam	Bile, pancreatic ducts	Praziquantel, 75 mg/kg/day in 3 doses × 1–2 days Albendazole,[c] 10 mg/kg/day × 10 days
Opisthorchis viverrini	*Bithynia*	Freshwater fish	Thailand, Laos, Cambodia	Bile, pancreatic ducts	Praziquantel, 75 mg/kg/day in 3 doses × 1–2 days Albendazole,[c] 10 mg/kg/day × 10 days
Opisthorchis felineus	*Bithynia*	Freshwater fish	Eastern Europe, former Soviet Union	Bile, pancreatic ducts	Praziquantel, 75 mg/kg/day in 3 doses × 1–2 days Albendazole,[c] 10 mg/kg/day × 10 days
Fasciola hepatica	*Lymnaea*	Watercress, other aquatic plants	Americas, Europe, Asia, western Pacific, North Africa	Bile ducts	Triclabendazole,[d] × 10 mg/kg × 1 day Nitazoxanide[e]
Intestinal Flukes					
Fasciolopsis buski	*Segmentina*	Aquatic plants	Far East, India	Small intestine	Praziquantel, 25 mg/kg/day × 1 day Niclosamide,[c] 1 g × 1 day Triclabendazole[e]
Heterophyes heterophyes	*Pirenella, Cerithidea*	Freshwater fish	Far East, Egypt, Middle East, southern Europe	Small intestine	Praziquantel, 25 mg/kg/day × 1 day Triclabendazole[e]
Metagonimus yokogawai	*Semisulcospira*	Freshwater fish	Far East, Russia, southern Europe	Small intestine	Praziquantel, 25 mg/kg/day × 1 day Triclabendazole[e]
Lung Flukes					
Paragonimus westermani; other species	*Semisulcospira, Onchomelania, Thiara*	Freshwater crabs, crayfish	Far East, South Asia, Philippines, West Africa, South and Central America, USA	Lungs	Praziquantel, 75 mg/kg/day in 3 doses × 2 days Triclabendazole,[c] 10 mg/kg day × 3 days

[a]Some experts recommend higher doses (e.g., praziquantel 60 mg/kg/day in divided doses for all species), multiple doses (e.g., for 2 or 3 days), or a repeated dose at 4 to 6 weeks to achieve higher rates of cure in persons not exposed to reinfection.[9]
[b]Not available or limited availability.
[c]Alternative drug.
[d]In the United States it is available from the Centers for Disease Control and Prevention Drug Service after approval by the US Food and Drug Administration.
[e]Limited data.

in the skin, where they cause a dermatitis.[7,9,11,11a,12] Hybrid schistosomes infective for human beings result from crossbreeding of different species of human schistosomes or human and zoonotic schistosomes.[5a]

Life Cycle

Adult worms, measuring 1 to 2 cm in length and 0.3 to 0.6 mm in width live, mate, and feed on blood in the portal and mesenteric vessels (*S. japonicum, S. mekongi, S. mansoni, S. intercalatum,* and *S. guineensis*) or vesical plexus *(S. haematobium).*[8,11] The male worm folds around and encloses the female in its gynecophoral canal. Egg production varies from about 300 eggs per day for female *S. mansoni* and *S. haematobium* and 3000 eggs daily for *S. japonicum* (Fig. 288.1). Eggs, measuring 145 × 55 µm for *S. mansoni* and *S. haematobium* and 85 × 60 µm for *S. japonicum* (Fig. 288.2), are deposited in the venules and make their way into the urine or feces and hatch in fresh water, where the miracidium, a 0.1-mm ciliated larva, emerges. The miracidium penetrates the body of the appropriate snail intermediate host and multiplies asexually. Within 4 to 6 weeks, thousands of motile, forked-tail cercariae, 0.1 to 0.2 mm long, emerge. On encountering human skin, the cercariae penetrate with the help of their glandular secretions, and within minutes they lose their tails and change into schistosomula. This transformation from a freshwater environment to parasitic existence in the human host

is associated with the unique formation of a heptalaminate membrane and other dramatic changes in morphology, metabolism, and physiology.[13] The schistosomula migrate to the lungs and liver and, in about 6 weeks, they mature to adult worms and descend through the venous system to their final habitat. Eggs appear in the feces or urine 4 to 6 weeks after cercariae penetrate the skin. Adult schistosomes live an average of 3 to 5 years but can survive for 30 years or more.[7,8,11]

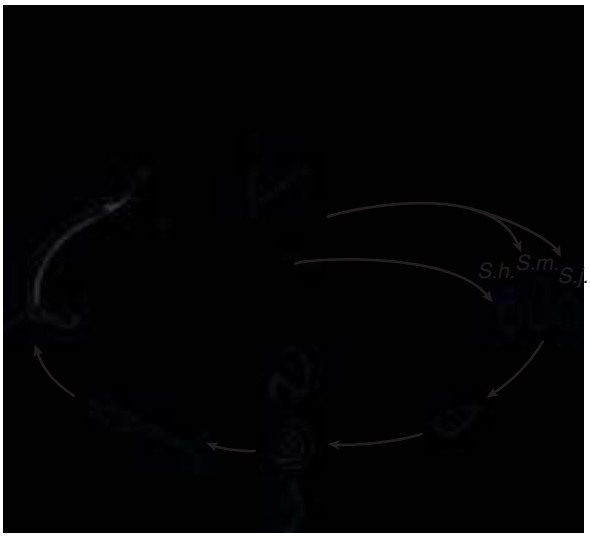

FIG. 288.1 Life cycle of schistosomes. Eggs are passed in stools for *Schistosoma mansoni (S.m.)* and *Schistosoma japonicum (S.j.)* and in urine for *Schistosoma haematobium (S.h.).* The eggs hatch in fresh water, miracidia invade specific snail intermediate hosts, and, in a few weeks, forked-tail cercariae are liberated. These infective forms penetrate human skin, pass through a migratory phase in the lung and the liver, and then pass to their final habitat in the portal venous system (*S.m.* and *S.j.*) or the urinary bladder venous plexus *(S.h.).* Three other species infect humans, although less frequently. *Schistosoma intercalatum* and *Schistosoma guineensis* produce terminal spined eggs that may be found in feces, whereas *Schistosoma mekongi* produces eggs similar to, but smaller than, those of *S. japonicum,* which may also be found in stools. These two species of schistosomes have characteristic snail intermediate hosts.

Epidemiology

Transmission of schistosomiasis requires an appropriate snail intermediate host; fecal or urinary contamination of warm, slowly moving fresh water; and human entry into the snail-infested water. The snail host is specific for each species and strain of schistosome, which have a specific geographic distribution (Table 288.1).[1a,11] *S. mansoni* occurs in three South American countries, several Caribbean islands, and, along with *S. haematobium,* in Africa and the Middle East, often in areas where the two species overlap.[8] *S. intercalatum* and *S. guineensis* also can overlap with *S. haematobium* in parts of West and Central Africa but are less common. *S. japonicum* is found in China, the Philippines, and Indonesia; and *S. mekongi* is found in Cambodia and Laos.[8] Cattle, water buffaloes, pigs, dogs, and other mammals are naturally infected with *S. japonicum* and *S. mekongi* and act as reservoir hosts, with a major role in transmission. Infections of rodents, sheep, primates, and other animals occur with *S. mansoni* and, rarely, *S. haematobium;* although they seem to contribute little to maintenance of the life cycle, they may jeopardize public health efforts to eliminate transmission and lead to the emergence of zoonotic parasitic hybrids infective to humans.[7,8,11,13a]

Transmission is focal in endemic countries and most intense in poor rural areas with inadequate sanitation and water supplies. The distribution of schistosomiasis is changing in many areas. The risk for infection is now nonexistent or negligible in previously highly endemic countries, including Japan, Morocco, Tunisia, Algeria, Oman, Jordan, Turkey, Iran, Mauritius, and the Caribbean countries.[2,13b,13c] Control programs have significantly reduced the incidence of infection and morbidity in Brazil, Venezuela, China, Saudi Arabia, Egypt, Cambodia, and the Philippines. Since 2000 strategies to reduce morbidity from schistosomiasis have been successfully implemented in several African countries, but in most of sub-Saharan Africa, high levels of endemicity persist, including in areas where dams and irrigation projects have led to major increases in the prevalence and extension of transmission to new areas.[4,7,8] An outbreak of urogenital schistosomiasis, likely related to arrival of infected individuals from endemic regions of Africa, was detected in Corsica, France, which marked the first instance of European transmission since the 1960s.[5a] New foci of schistosomiasis have been



FIG. 288.2 Life cycle of important parasitic flukes. Eggs are passed in stools for *Fasciola hepatica (F.h.), Clonorchis sinensis (C.s.),* and *Fasciolopsis buski (F.b.)* or in sputum for *Paragonimus westermani (P.w.)* infections. The next stage of multiplication occurs in specific snail intermediate hosts, followed by liberation of cercariae, which encyst on the second intermediate hosts (aquatic plants, fish, or crabs). These metacercariae represent the infective stage, and humans develop the infection after consumption of the second intermediate hosts. The final habitats of these flukes are the liver (*F.h.* and *C.s.*), intestines *(F.b.),* or lungs *(P.w.).*

identified in Myanmar.[13d] Urban transmission occurs in some large cities of Brazil and Africa, and global climate change is predicted to increase transmission in many areas.[13e]

In endemic communities the distribution of the infection fits a negative binomial curve, with most infected persons harboring low worm burdens and only a small proportion, usually children ages 8 to 12 years, having heavy infections.[4,7,8] Aggregation of the worm burden in a small proportion of infected individuals probably reflects a combination of factors, including the amount of water exposure, partial acquired immunity, age, and genetic susceptibility.[4,7,8,14,15] Typically, the intensity of infection declines after the early teenage years as partial immunity to new infections builds up, older worms die, and contact with fresh water decreases. Because worms do not multiply in the host, the intensity of the infection depends on the number of cercariae encountered. Persons with heavy infections are at most risk for developing severe disease.

In the United States and other temperate areas, infection cannot be transmitted because of the absence of the appropriate snail intermediate host. Schistosomiasis is seen among immigrants from endemic areas and returning travelers, and sometimes it appears in small epidemics among travelers engaging in adventure and nature tourism.[16–19]

Pathogenesis

The disease associated with schistosomiasis is largely due to the host's immune response to the larvae and eggs.[14,20,21] Mature adult worms evade the host's immune defenses by binding host antigens to their tegument and regenerating their tegument, along with other mechanisms, thus contributing little to the immunopathology of the disease.[22] Different mechanisms are responsible for tissue injury during the stage of larval penetration, the acute stage, and the chronic infection.

In previously exposed persons a protective response consisting primarily of specific immunoglobulin E (IgE) antibodies, eosinophils, and macrophages is directed against the schistosomula after cercarial penetration of the skin.[14,21] As a result, some organisms die in the skin and are surrounded by edema and cellular infiltrates in the dermis and epidermis that can give rise to a papular dermatitis.

The syndrome of acute schistosomiasis occurs 2 to 12 weeks after a heavy first exposure to larvae during worm maturation and the initiation of egg deposition.[12,19,23,24] A febrile illness with features of serum sickness results from formation of circulating immune complexes in response to larval and egg antigens and production of high levels of proinflammatory cytokines. Acute disease develops primarily in previously unexposed persons and less commonly in heavy reinfections, usually due to *S. japonicum*. Symptomatic acute infections are rarely reported among persons who grow up in an endemic area, perhaps because of sensitization in utero as a result of maternal infection.[12]

Disease during the chronic stage of infection is due to the presence of eggs in host tissues and the immune response directed against the egg antigens.[14,20,21] Miracidia in the eggs secrete antigenic glycoproteins through pores in the eggshell that induce an inflammatory response that facilitates passage of the eggs through blood vessel walls and tissues en route to the lumen of the intestinal or urinary tract. One-third to one-half of eggs reach the environment, with the remainder trapped in tissues or embolized to a distant site. The host response to toxic egg antigens and eggs retained in the tissues includes acute eosinophilic inflammation, followed by granuloma formation, initially consisting of neutrophils, eosinophils, and mononuclear cells and, later, mostly lymphocytes, macrophages, multinucleated giant cells, and fibroblasts.[14,25,26] In *S. mansoni* and *S. haematobium* infections the granulomatous response has been shown to be orchestrated by $CD4^+$ T lymphocytes and is tightly regulated by various immunologic mechanisms.[21,23,27,28] They involve balanced helper T cell type 1 and 2 responses and the production of cytokines locally in the granulomas and systemically; these responses in turn are regulated by numerous other mediators and mechanisms.[14,27–29] In addition to destroying eggs, granulomas may mediate their passage into the lumen of the bowel or urinary tract. This hypothesis is supported by the finding of reduced egg output in persons with advanced human immunodeficiency virus (HIV) disease.[30]

Granulomas initiate tissue injury first through the inflammatory infiltrate and replacement of normal tissue and later through extensive collagen deposition and scarring.[14,21,31,31a] Large granulomas and fibrosis cause the major pathologic lesions in chronic schistosomiasis. In the case of schistosomes that inhabit the mesenteric vessels, the pathologic process is greatest in the intestines and the liver, the major site of egg embolism. With *S. haematobium* infection, the main system involved is the urinary tract. The result is inflammatory lesions and ulcerations of the mucosa; fibrotic scarring of the bowel, bladder, and lower ureteral walls; and obstruction of portal blood flow in the liver and urine flow through the ureters and bladder. During the early stages of schistosome infection, the granulomatous response is exuberant. Later, modulation of granulomatous hypersensitivity results in smaller granulomas and less fibrosis, which in turn probably play a significant role in limiting progression of the disease.[21,27] Treatment of children during the early stage can completely resolve the granulomatous inflammation.[8] A subset of untreated persons immunoregulate their response to egg antigens poorly and develop extensive fibrosis and severe disease.[4,14]

The severity of disease in schistosomiasis is determined in part by the duration and intensity of the infection.[32–34] This relationship is not exact, however, and other variables, such as genetic susceptibility to disease, parasite strain, and coinfections with malaria, hepatitis viruses, HIV, and other infectious agents, may be important.[4,30,35–37] Chronic schistosomiasis appears to be associated with a partial degree of resistance to reinfection directed against invading immature worms and mediated by IgE antibodies, eosinophils, and cytokines, such as interleukin-4 (IL-4) and IL-5.[14,21,38]

Clinical Syndromes

Infections with human schistosomes are frequently asymptomatic. Illness, when present, is strongly influenced by location in endemic versus nonendemic sites and related to factors such as prevalence of immunity and intensity of infection.[16,39–40] Acute symptomatology is more frequent in previously uninfected individuals, and chronic illness, which is associated with a higher burden of infection, occurs most often in endemic areas.

Schistosome Dermatitis ("Swimmer's Itch")

During penetration of cercariae, some previously exposed and unexposed persons experience a prickling sensation and may note urticaria, followed by a macular rash several hours later.[8,11a,12,19,41] In persons exposed for the first time, this rash disappears quickly, but in previously sensitized persons, it may persist and progress to a pruritic maculopapular rash that lasts for days. The rash is most severe in persons infected with schistosomes of birds or aquatic mammals, which die in the skin. This "swimmer's itch" is common in the Great Lakes region, New England, and other parts of the United States, Canada, Europe, and elsewhere in the world (see Chapter 290).

Acute Schistosomiasis (Katayama Fever)

Previously uninfected persons from nonendemic areas may have no symptoms after first exposure or may develop symptoms of acute schistosomiasis (Katayama syndrome) 2 to 12 weeks after exposure, particularly to *S. japonicum* or *S. mansoni*.[8,12,16,19,23,24] The Katayama fever syndrome is unusual with *S. haematobium* and most severe with heavy infections, especially with *S. japonicum*. It may occur in persons previously infected with *S. japonicum*. Onset of fever is often acute and accompanied by chills, fatigue, headache, myalgia, abdominal pain, diarrhea, and occasionally bloody stools. As many as 70% of patients develop nonproductive cough, dyspnea, chest pain, and diffuse and nodular infiltrates seen on chest radiography.[42] In a few cases in which lung nodules were biopsied, granulomas around eggs were seen.[43] The liver, spleen, and lymph nodes are often enlarged. Urticaria is common, and eosinophilia occurs in nearly all cases, but eggs may not be seen in the stools until late in the illness. Symptoms and signs usually disappear after 2 to 10 weeks, but persistent and more serious disease and even death may occur with heavy infections. Lesions of the central nervous system (CNS), genital tract, and skin are due to aberrant migration of adult worms and ectopic deposition of eggs, which occurs in a small number of cases of acute infection, including in persons without systemic manifestations.[12] In some cases eosinophil-mediated toxicity leading to vasculitis and small vessel thrombosis may be responsible for neurologic disease during acute infection.[44] Patients with acute schistosomal

encephalopathy present with headache, altered mental status, and often seizures and focal deficits. Computed tomography (CT) and magnetic resonance imaging (MRI) show edema and multifocal enhancing lesions and occasionally border zone infarctions.[45]

Chronic Schistosomiasis

Symptoms may be absent or mild in many patients who have light or moderate worm burdens. However, careful analysis of clinical and epidemiologic data has demonstrated subtle but important morbidity in persons with even light infections.[46] Chronic granulomatous inflammation and elevated levels of proinflammatory cytokines are believed to contribute to poor caloric intake, undernutrition, anemia of chronic inflammation, stunting, and impairment of work capacity and cognitive development in persons with chronic schistosomiasis.[46,46a] Similar mechanisms, along with placental infection and inflammation, may be responsible for decreased birth weight and poor birth outcomes in infants born to mothers with chronic schistosomiasis.[47] When these previously underappreciated sequelae of infection have been taken into account, estimates of the disability associated with schistosomiasis suggest a burden of disease as much as 50 times greater than previously reported.[48] Regardless of symptoms, eosinophilia is often present in persons with chronic schistosomiasis.

Intestinal and Hepatosplenic Schistosomiasis

Patients with light infections caused by the intestinal schistosomes *S. mansoni, S. japonicum,* and *S. mekongi* may complain of fatigue, intermittent abdominal pain, and diarrhea.[8,49,50] In heavy infections blood loss from ulcerations or dysentery may lead to a moderate degree of anemia. Intestinal polyps have been observed, most commonly in Egypt, and strictures or large inflammatory masses may cause obstruction or mimic carcinoma.[51] An association between intestinal schistosomiasis and cancer of the bowel has been suggested but not yet confirmed.[52,52a]

An early sign of chronic schistosomiasis caused by *S. mansoni, S. japonica,* or *S. mekongi* is hepatomegaly from granulomas around embolized eggs that become trapped in small portal venules. Such inflammatory hepatomegaly is common during childhood and diminishes or resolves with time; it should be distinguished from hepatomegaly due to periportal, or Symmers "pipestem," fibrosis that is seen after years of infection in up to 5% to 10% of infected young and middle-aged adults.[8,31] Granulomas and fibrosis cause a presinusoidal block to portal blood flow and eventually portal hypertension, splenomegaly, hypersplenism, and development of portosystemic collateral blood vessels. In most cases of hepatosplenic schistosomiasis, liver cell perfusion is not reduced, hepatic function is preserved, and levels of hepatic aminotransferases remain normal.[53] Persons with coexisting alcoholic cirrhosis, chronic hepatitis B, or hepatitis C may develop jaundice and ascites.[54] Natural progression of schistosomal disease occurs more rapidly in persons with schistosomiasis from *S. japonicum* than from *S. mansoni* and occasionally leads to decompensated liver disease as well. Repeated episodes of hematemesis from bleeding esophageal varices occur, which usually are associated with low mortality in persons with compensated disease but may lead to hepatic failure and death in persons with decompensated disease. Persons with both schistosomiasis mansoni and chronic hepatitis B or C are at higher risk for cirrhosis and perhaps hepatocellular carcinoma than persons infected with hepatitis viruses alone.[54]

Infections with *S. intercalatum* and *S. guineensis* tend to be lighter and produce less pathology than infections due to *S. mansoni* or *S. japonicum*.[55] Egg deposition occurs primarily in the colon, and patients may present with blood and mucus in the stool; in these cases endoscopy shows polyps and inflamed rectal mucosa. More severe disease can be seen in persons with *S. mekongi* infections, with advanced hepatosplenic disease similar to that seen in *S. mansoni* and *S. japonicum* infections.[56]

Genitourinary Schistosomiasis

In infection with *S. haematobium,* hematuria and dysuria from inflammation and small ulcerations in the bladder mucosa may appear within 3 to 4 months of infection.[57,58] In endemic areas most children have microhematuria by age 10 years, and gross hematuria is common as well. Later, polyps, hypertrophic nodules, and "sandy patches" around egg deposits may be visible on cystoscopy. Granulomas, fibrosis, and, ultimately, calcification of the bladder wall cause reflux and obstruction of urine flow, with hydroureter, hydronephrosis, chronic bacteriuria, bladder cancer, and, in a small percentage of cases, renal failure. Deposition of *S. haematobium* eggs in the genital tract occurs in up to 75% of women and causes sandy patches with mucosal bleeding, abnormal blood vessels, and occasionally ulcerative, nodular, or papillomatous lesions of the vulva, perineum, and cervix.[59–61] These lesions, which are illustrated in WHO's pocket atlas for health care professionals (available at https://www.who.int/schistosomiasis/genital_schistosomiasis), increase the patient's susceptibility to HIV infection and other sexually transmitted diseases, and lesions of internal pelvic organs may cause bleeding and infertility. Antischistosomal treatment of infected girls can cause regression of lesions before they become irreversible. Hematospermia results from involvement of the prostate and seminal vesicles with schistosomiasis haematobia, which has been implicated in male infertility as well.[62,63] Associations have been demonstrated between *S. haematobium* infection and squamous cell carcinoma of the bladder; the latter has especially affected young and middle-aged men in Egypt.[57,63–66]

Pulmonary Hypertension and Glomerulopathy

With infection with *S. mansoni* and *S. japonica,* eggs may bypass the liver through portosystemic collateral vessels and cause pulmonary disease; in infection with *S. haematobium,* eggs escape the vesical plexus and reach the lungs directly.[65,66] Pulmonary hypertension develops in 10% to 20% of persons with hepatosplenic schistosomiasis, but only in the most severe cases does obstruction to pulmonary blood flow due to granulomatous inflammation and arteritis of small pulmonary arteries lead to cor pulmonale. Subclinical glomerulonephritis is not uncommon in persons with chronic schistosomiasis; kidney biopsy has shown deposits of immune complexes containing schistosomal antigens in the glomerular basement membrane.[58,67] Clinically important glomerulopathy occurs most commonly in persons with hepatosplenic disease; its prevalence has decreased in areas where cases of severe schistosomiasis have become uncommon.[68]

Ectopic Egg Deposition and Central Nervous System Schistosomiasis

Ectopic egg deposition from aberrant migration of adult worms and embolization of eggs from distant sites are common with all species of schistosomes and can involve almost any organ. In most cases the resulting lesions do not produce symptoms, but involvement of the CNS can cause serious cerebral and spinal cord disease.[45,69] CNS schistosomiasis is most common with *S. japonicum* infection, occurring in as many as 2% to 5% of infections and accounting for high rates of epilepsy in endemic areas. The brain is the usual site of CNS disease with *S. japonicum* infection, only occasionally with *S. mansoni* infection, and virtually never with *S. haematobium* infection. Patients present with focal or generalized seizures, focal neurologic deficits, signs of increased intracranial pressure due to the mass effect, and diffuse encephalitis. CT and MRI of the head show nodular and ring-enhancing lesions with surrounding edema. With *S. haematobium* and *S. mansoni* infections, eggs reach the lower spinal cord through the Batson plexus and produce either transverse myelitis with back pain and paraplegia or myeloradiculopathy from granulomatous lesions of the conus medullaris and cauda equina.[69]

Coinfections

Concurrent infection with other organisms may affect the clinical course of schistosomiasis. Prolonged bacteremia with *Salmonella typhi* and other *Salmonella* species has been reported in persons chronically infected with *S. mansoni, S. japonicum, S. intercalatum,* and *S. haematobium*.[70,71] Unlike typhoid fever, the illness is indolent, with persistent fever, weight loss, and continuous bacteremia, and it can last months. Treatment of the bacterial infection without treating the schistosomiasis may result in relapse of bacteremia and symptoms. *Salmonella* can attach to the tegument and gut of schistosomes, and there is evidence that schistosomes and *Salmonella* share antigens that elicit immunologic tolerance to the bacterial infection.[70,71] Chronic *Salmonella* bacteriuria can complicate infection with *S. haematobium*. *Salmonella* bacteremia and bacteriuria have been associated with glomerulonephritis and nephrotic syndrome in persons infected with either *S. haematobium* or *S. mansoni*.[58,68]

Chronic coinfection with hepatitis B or C worsens the prognosis of persons with hepatosplenic schistosomiasis, as already described.[54,72] The high rate of hepatitis C coinfection noted in Egypt probably reflects widespread transmission associated with parenteral antischistosomal treatment that was practiced until the 1980s.[72] HIV coinfection has been associated with decreased egg excretion in persons with *S. mansoni* and *S. haematobium* infections and decreased hematuria with *S. haematobium* infections because of impaired granuloma formation and increased trapping of eggs in tissue.[30] Schistosomiasis appears to increase susceptibility to HIV infection by as much as threefold to fourfold in women with genital lesions.[61,73] Infection with schistosomes may increase HIV viral load, accelerate progression of HIV disease, and increase HIV transmission by the vertical and horizontal routes.[73] Treatment of schistosomiasis in persons with HIV coinfection has led to a decrease in HIV viral load, an increase in $CD4^+$ T-cell counts, and a decrease in schistosomal fecundity and egg excretion but poor killing of adult worms.[73–75] An immune reconstitution syndrome after initiation of antiretroviral therapy among persons previously treated for schistosomiasis may present with worsening manifestations of schistosomiasis, such as new or increasing bloody diarrhea or hematuria, increasing hepatomegaly or splenomegaly, rash, or constitutional symptoms.[76]

Diagnosis

Schistosomiasis should be suspected in persons who have a history of freshwater exposure in endemic areas even in the absence of suggestive clinical findings or eosinophilia.[9,12,77] Hematuria is common with *S. haematobium* infections, and screening children for blood in their urine by using dipsticks has provided reliable estimates of the prevalence of infection in areas of high endemicity.[57] Commonly used diagnostic tests for schistosomiasis include serologic tests and microscopic examination of stool, urine, or tissue for eggs.[77]

Parasitologic Tests

Microscopy on a simple smear of feces or a drop of urine can detect heavy infections, but because eggs may be passed intermittently or in small numbers, concentration procedures and repeated examinations are needed to detect lighter infections. Several concentration procedures also allow quantification of egg output, such as the Kato-Katz technique, which uses 20 to 50 mg of fecal material, or filtration of a standard volume of urine through a Nuclepore membrane.[77] Counts higher than 400 eggs per gram of feces or 10 mL of urine are considered heavy and are associated with an increased risk for complications. Even repeated concentration procedures may miss low-intensity infections, which are common in low-prevalence areas or in travelers or expatriates with chronic infections. Microscopic examination of snips of rectal or bladder mucosa obtained at proctoscopy or cystoscopy may reveal eggs when the stool or urine examination is negative. Because persons with inactive infections may continue to shed dead eggs into stool or urine for months, tests for egg viability, such as egg hatching or microscopic examination of eggs for movement of flame cells, should be performed

Antigens shed by adult worms into the blood can be detected by enzyme-linked immunosorbent assay (ELISA) or monoclonal lateral flow assays in serum and urine.[8,77] A point-of-care lateral flow assay that detects *S. mansoni* antigens in urine is available commercially in some countries outside of the United States; its sensitivity is equal or superior to that of duplicate Kato-Katz smears for light infections, and it can detect acute infections several weeks before eggs are shed in the stool.[77–79] Polymerase chain reaction (PCR)-based assays that detect schistosome DNA in stool, urine, serum, or plasma have sensitivities greater than that of microscopy of duplicate samples in chronic infections and microscopy or serology in acute infections.[80,81]

Serologic Tests

Serologic tests for antibodies to schistosomes are available at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, and some commercial laboratories.[82] The CDC uses a combination of tests with purified adult worm antigens. Its Falcon assay screening test–ELISA (FAST-ELISA) is 99% specific for all species and has a sensitivity of 99% for *S. mansoni* infection, 95% for *S. haematobium,* but less than 50% for *S. japonicum.* Because the FAST-ELISA may miss some *S. haematobium* and *S. japonicum* infections, immunoblots using species-specific antigens are performed in cases of potential exposure to these parasites. Serologic tests cannot distinguish active from past infections but are useful for screening previously unexposed travelers and expatriates.[19] In such persons a positive serologic test is presumptive evidence of infection even if microscopic examination of stool or urine fails to reveal schistosome eggs. In acute schistosomiasis eggs do not appear in the feces or stool for 30 to 50 days (or longer after treatment).[19] Serologic tests and PCR-based assays typically turn positive sooner than microscopic studies but may not be readily available, and seroconversion may not occur for 2 months or longer after exposure.[82a] In such cases the diagnosis rests on the epidemiologic history and clinical picture.[19,82a]

Evaluation of Persons With Confirmed Schistosomiasis

Persons with confirmed schistosomiasis should be evaluated for evidence of disease. Urinalysis, urine culture, and serum creatinine determination are indicated for persons with *S. haematobium* infection. Any abnormality should prompt an ultrasound or CT scan to detect complications, such as thickening or calcification of the bladder wall, hydroureter, hydronephrosis, polyps, stones, or carcinoma of the bladder.[57,83] Evaluation of infections due to the intestinal schistosomes includes liver function tests, tests for chronic hepatitis B and C, and imaging to document periportal fibrosis and signs of portal hypertension.[49,50,84] Contrast studies, CT, MRI, and ultrasonography are sensitive means of detecting and evaluating urinary tract and hepatic pathologic processes, although ultrasonography has become the preferred modality in endemic countries after standardized protocols developed by World Health Organization (WHO) for use in hospitals and the field.[83,84] Biologic markers, such as hyaluronic acid, procollagen peptides, and others, can identify persons with hepatic fibrosis but do not replace ultrasonography for assessing portal hypertension.[85] Liver biopsy may be necessary to identify any pathologic process in addition to schistosomiasis in persons with decompensated hepatosplenic disease. Esophageal varices are visualized by barium swallow or endoscopy.[8]

Therapy

Treatment is indicated for all persons with schistosomiasis. Cure of infection is desirable because even a single pair of worms may be responsible for a catastrophic neurologic complication, such as transverse myelitis. Successful deployment of antischistosomal medication at the individual level not only prevents the development of complications, but, if given early and reinfection is avoided, it also can cause partial or complete regression of intestinal lesions, urinary bladder wall thickening, bladder polyps, urinary obstruction, genital sandy patches, and periportal fibrosis.[86–88] For control of schistosomiasis at the population level in endemic areas where reinfection is inevitable, the goal is to reduce worm burdens to levels that are unlikely to produce disease, decrease prevalence of infection, and, if feasible, eliminate transmission.[5,8,89,89a]

Praziquantel for Treatment of Schistosomiasis

The drug of choice for treating all species of schistosomes is praziquantel (see Table 288.1), which affects membrane permeability to calcium ions in the parasite.[90] Paralysis and vacuolation of the tegument immobilize the worm and expose it to attack by the host immune system. After a single treatment, cure rates of chronic infections range from 65% to 95%, and in persons not cured, egg excretion is reduced by greater than 90%.[8,91] A second dose several weeks later may increase the rate of cure in persons not exposed to reinfection.[12] Praziquantel does not affect developing schistosomula or eggs and may not abort an early infection.[90,92] Treatment with praziquantel has beneficial effects on host immunity: Damage to the tegument exposes parasite antigens to the immune system and thus accelerates the development of protective acquired immunity that otherwise would take years to acquire naturally.[92a] Resistance to praziquantel has been documented in the laboratory, and there have been reports of decreased responsiveness in the field and anecdotal reports of returned travelers who required multiple courses of praziquantel to clear their infections.[91,93–95]

Adverse effects, which are usually mild and last less than 24 hours, are largely due to reactions to dying worms rather than drug toxicity.

Patients may report headache, dizziness, or abdominal discomfort and, less commonly, nausea, vomiting, diarrhea, bloody stools, fever, and urticaria. Pediatric formulations of praziquantel in a smaller, orally dispersible tablet that masks the drug's bitter taste appear promising in clinical trials.[96]

Pregnant women were formerly restricted from receiving praziquantel, but WHO now recommends that praziquantel be given to pregnant and lactating women with schistosomiasis during mass treatment campaigns.[97] Two randomized controlled trials of pregnant women who received praziquantel after 12 weeks of gestation did not detect an increased risk of adverse maternal or neonatal outcomes.[98,99] The safety of praziquantel during the first trimester has not been well assessed, but animal data, observations on treatments inadvertently given during the first trimester, and decades of postmarket surveillance suggest that it is safe.[97] Praziquantel is excreted in breast milk in minute amounts. Suspending breast feeding for 3 days after praziquantel therapy and avoiding praziquantel during the first trimester seems advisable for individual patients.

Persons with known or suspected cysticercosis should remain under observation during praziquantel therapy because of the risk for seizures or other neurologic consequences of dying cysticerci; treatment should be withheld in persons with intraocular cysticerci. Persons with schistosomal disease of the CNS should also receive corticosteroids to reduce the inflammation and edema around eggs because miracidia in eggs in tissues remain viable and secrete antigenic proteins for several weeks.[44,45]

Other Antischistosomal Drugs: Oxamniquine and Artemisinin Derivatives

Oxamniquine, an alternative for treatment of *S. mansoni* infections but with limited availability, is as effective as praziquantel for infections acquired in the Western Hemisphere.[4,100] Higher doses are needed for strains of the parasite from Africa, and drug resistance has been documented. Metrifonate is effective only against urinary schistosomes; it requires three doses 2 weeks apart and is currently not available.[57]

The antimalarial drugs artemether and artesunate are active against all species of schistosomes, can kill schistosomula during the first 3 weeks of infection, and are synergistic with praziquantel in killing adult worms.[101,102] They are effective as prophylactic agents against *S. japonicum* when given every 2 weeks and have been used for chemoprophylaxis in China.[101–103] Artesunate combined with pyrimethamine-sulfadoxine or mefloquine can cure chronic *S. haematobium* or *S. mansoni* infections but is less effective than praziquantel alone.[102,104] Most trials of artesunate in combination with praziquantel have shown superiority of the combination over praziquantel alone for treatment of chronic infections.[102,103] Widespread use of artemisinin derivatives for treatment or prophylaxis in malaria-endemic areas should be avoided because of the risk for promoting artemisinin-resistant plasmodia, but the deployment of praziquantel with an artemisinin derivative might hasten elimination of transmission of schistosomiasis in nonmalarious areas.[102–105]

Management of Acute Schistosomiasis

The symptoms of Katayama syndrome often require administration of corticosteroids to suppress the inflammatory process (e.g., prednisone 40 mg/day orally for 5 days), but there is no consensus about proper anthelmintic treatment.[8,12,19,23,24] Most authorities administer praziquantel shortly after administration of corticosteroids and treat *S. mansoni* and *S. haematobium* infections for up to 3 days and *S. japonicum* infections for up to 6 days in the doses listed in Table 288.1.[8,12] Other authors believe that praziquantel should not be used during the acute phase because of its limited effectiveness against schistosomula, the lowering of serum levels by concomitant corticosteroid therapy, and the risk for reactions that develop in response to killing of parasities.[93,16,106] Data on effectiveness of artemisinin compounds alone or with praziquantel for treatment of acute schistosomiasis are limited, and in one study the efficacy of the combination of artemether and praziquantel was not superior to that of praziquantel alone.[16,19,105] In all cases of acute schistosomiasis a second course of therapy is recommended 4 to 6 weeks after the onset of symptoms. Treatment of asymptomatic persons with praziquantel 28 to 40 days after exposure seems to prevent acute illness but not chronic infection, whereas treatment of persons within 10 to 15 days of exposure has precipitated an acute illness, including severe complications, such as bronchospasm and cerebral microinfarcts.[44,93,106]

Evaluation of Therapy

Because antischistosomal drugs may temporarily inhibit egg laying by adult worms, stool and urine should be examined 6 to 8 weeks after completion of therapy and again 3 to 6 months later for both acute and chronic infections; persons who remain infected should receive a single dose of praziquantel, 40 to 60 mg/kg.[8,12,44] Eosinophilia, hematuria, or persistence of symptoms should prompt repeat parasitologic studies. Serologic tests may remain positive for several years after successful treatment.

New Medications for Schistosomiasis

Development of new chemotherapeutic agents for treatment of schistosomiasis is essential because of the incomplete efficacy of praziquantel and the threat of emerging resistance.[8,107] Current efforts include investigating existing drugs (such as antimalarials, anthelmintics, and anticancer drugs), combinations of existing drugs, and natural products, as well as exploring differences between host and parasitic metabolic pathways and analyzing genomes and transcriptomes to identify new drug targets.[107] The publication of genomic sequences of the three major human schistosome species is stimulating the search for new drug targets.[8] Unfortunately, at this time no new antischistosomal candidate is undergoing human trials, and it is unlikely that an alternative to praziquantel will be available in the next decade.[8,107]

Prevention and Control of Schistosomiasis

Travelers to areas where schistosomiasis is endemic should avoid contact with fresh water that may be infested with cercariae.[18] If contact is unavoidable, vigorous toweling of the skin may limit cercarial penetration after leaving the water, and medical follow-up should be sought after return from travel. With the possible exception of 50% DEET (*N,N*-diethyl-*m*-toluamide), topical lotions and soaps do not reliably prevent infection after contact with cercariae.[19] Further data are needed before recommending artemether for prophylaxis, which at any rate should not be used in malarious areas.

In theory transmission of schistosomiasis in endemic communities can be interrupted by provision of sanitation and safe water supplies and elimination of snail intermediate hosts or their habitats, and indeed persons with safe water and adequate sanitation are at lower risk of infection.[108,109] However, the scale and cost of these interventions are beyond the reach of many countries, and the cornerstone of schistosomiasis control programs has been the WHO-recommended strategy of regular administration of antischistosomal drugs (along with drugs for soil-transmitted helminthiases, onchocerciasis, and lymphatic filariasis in areas where these infections are endemic) to populations at risk, primarily to maintain individual worm burdens at levels below those that cause morbidity and mortality and secondarily to decrease transmission and prevalence of infection.[2,5,92a] More than 180 million tablets of praziquantel were donated in 2016, and almost 90 million persons received treatment, representing approximately 36% of the persons thought to be in need of treatment.[5] Although mass drug administration has achieved reduction in intensity of infection and infection-related morbidities, it is not sufficient to interrupt transmission in most endemic areas.[89a,110] Moreover, difficulties in sustaining large-scale drug administration programs and the potential emergence of praziquantel resistance may make this strategy merely a temporary solution.

Interruption of transmission in many regions will require integrated and intensive strategies, as demonstrated by a highly successful community-based trial in China that used regular chemotherapy, removing cattle from snail-infested grasslands, providing farmers with mechanized farm equipment, supplying tap water, building lavatories and latrines, providing boats with fecal-matter containers, and implementing an intensive health-education program.[111] These strategies have markedly reduced transmission in China, but factors such as population movements, flooding, and socioeconomic changes pose difficult challenges that require intensified efforts and new tools.[13c,112] Snail control

can increase the impact of mass drug administration, for instance, in the Senegal River Basin, where the reintroduction of the river prawn, a natural predator of the intermediate snail host, significantly decreased schistosomiasis prevalence and egg burden.[112a,112b] Development of a vaccine to prevent heavy infections seems possible based on experimental studies in animals and evidence of acquired immunity in human populations.[113,114] Numerous vaccine candidates have been identified, and several antigens have entered clinical trials.[113,114] Vaccines and other new tools and scaled-up efforts will be necessary to achieve WHO's goals of eliminating schistosomiasis as a public health problem by 2025 and interrupting transmission in all endemic countries (except for a number of countries in the African region, where a marked reduction in prevalence is the goal).[2]

LIVER FLUKES

The major liver flukes of humans are *Clonorchis sinensis* and several species of *Opisthorchis* (largely found in the Far East, Southeast Asia, and Russia) and *Fasciola hepatica,* which is widely distributed throughout the world (see Table 288.1).[1a,3,115,116,116a] Less common liver flukes include *Fasciola gigantica* in South America, Africa, and Southeast Asia; *Metorchis conjunctus* in North America and *Metorchis bilis* in Russia; and *Opisthorchis guayaquilensis* in the Americas.[116,117] All are infections of various lower animals, particularly cats and dogs, and are transmitted through food. The lancet fluke, *Dicrocoelium dendriticum,* which inhabits the biliary tree of ruminants and humans, is the exception: It is transmitted by accidental ingestion of infected ants, the second intermediate host.[118,119] Most commonly found in sheep-raising countries of the Mediterranean, it produces mild right upper quadrant pain and loose stools. Ingestion of raw or undercooked liver of an infected animal can lead to an allergic pharyngitis (halzoun), with adult worms present in expectorated secretions, or, more commonly, to false or spurious infection from swallowed eggs found in the stool.[120]

Clonorchiasis and Opisthorchiasis

Clonorchis sinensis, Opisthorchis viverrini, and *Opisthorchis felineus* have similar life cycles, eggs, and capacity to produce disease; they differ primarily in their geographic distribution, the morphology of the adult worms, and the frequency with which the clinical syndromes occur.[3,10,121–123] *C. sinensis* is found primarily in East and Southeast Asia, with *O. viverrini* in Southeast Asia and *O. felineus* in the regions of the former Soviet Union and western Europe.[1a,122–124] Millions of persons are infected, and in some endemic areas it is not uncommon to find prevalence rates of 20% to 80%.

The hermaphroditic adult flukes are flat, elongated worms measuring 5 to 25 mm × 2 to 5 mm that inhabit the intrahepatic bile ducts (see Fig. 288.2). Their yellow-brown operculated eggs (30 × 12 µm) are fully embryonated when they pass out of the body in feces into fresh water (Fig. 288.3). The eggs are ingested by specific snails, inside of which they hatch into miracidia that develop and replicate to produce large numbers of cercariae. Cercariae are released into the water and penetrate susceptible freshwater fish, primarily carp, and then encyst as metacercariae. Several species of freshwater shrimp can also serve as the second intermediate host of *C. sinensis.*[122,123,125] Humans, cats, dogs, and other fish-eating mammals become infected by ingesting the metacercariae in raw or inadequately cooked fish; through the action of trypsin and cysteine proteases the metacercariae excyst in the duodenum and pass through the ampulla of Vater to the bile ducts, where adult worms mature and begin egg laying in about 3 to 4 weeks and live as long as 30 years, feeding on lipids in the bile. An adult worm can produce 4000 eggs or more per day.

Clinical Manifestations

Most patients are asymptomatic but may have eosinophilia; symptoms are related to worm burden. An acute illness resembling acute schistosomiasis with fever, abdominal pain, hepatomegaly, urticaria, and

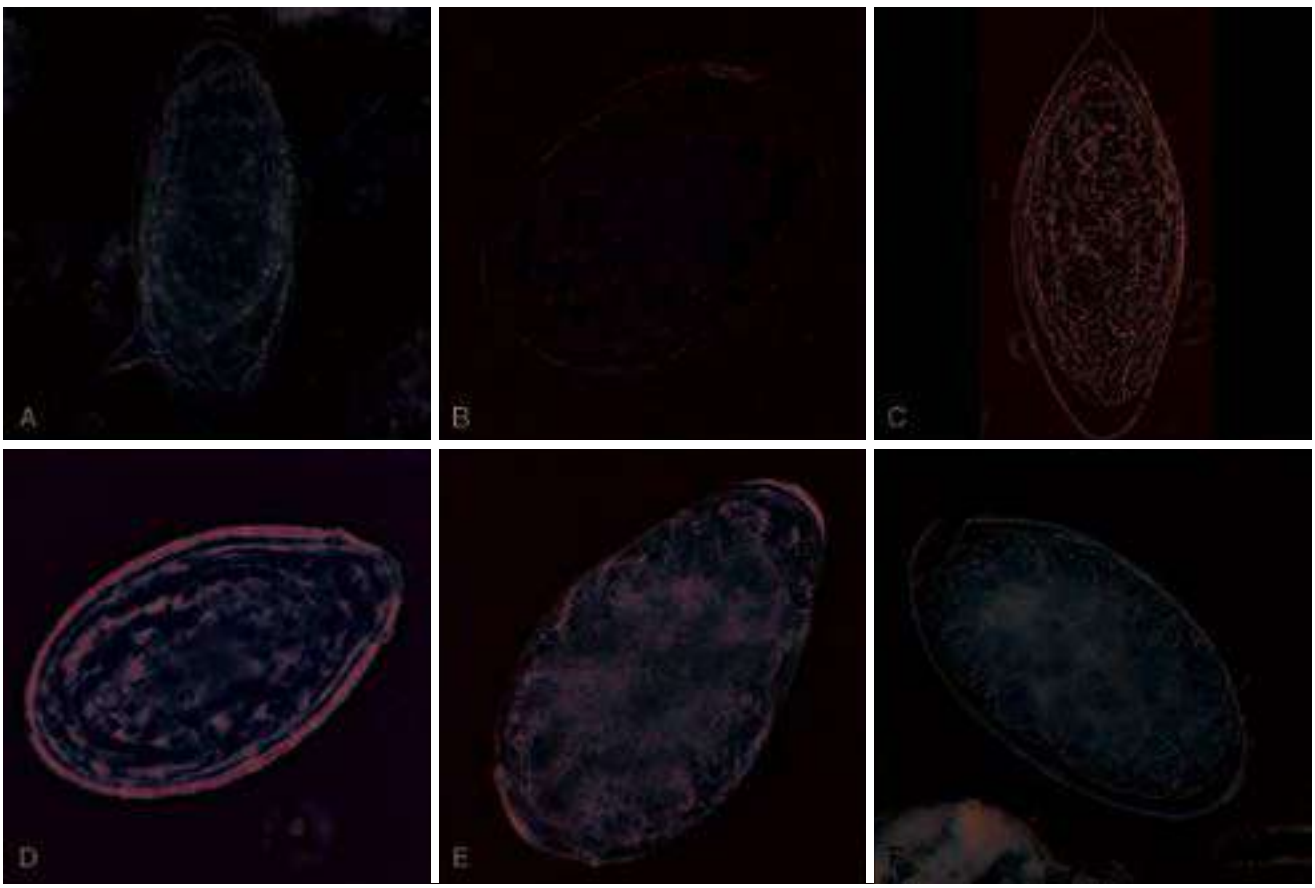

FIG. 288.3 Eggs of common human trematodes. (A) *Schistosoma mansoni.* (B) *Schistosoma japonicum.* (C) *Schistosoma haematobium.* (D) *Clonorchis sinensis.* (E) *Paragonimus westermani.* (F) *Fasciola hepatica* (note the partially open operculum). *(From Centers for Disease Control and Prevention. DPDx—Laboratory Identification of Parasitic Diseases of Public Health Concern.* http://www.dpd.cdc.gov/DPDx/*.)*

eosinophilia occasionally develops 2 to 4 weeks after the initial exposure.[126] In acute *O. felineus* infection, increased liver enzymes and enhancing hypodense nodules on CT have been reported.[124] Irritation of bile duct walls by the suckers of the flukes and secreted metabolic products leads to inflammation and thickening of bile duct walls and localized obstruction in about 10% of persons with heavy chronic infections (1000–25,000 or more eggs per gram of stool). Patients complain of right upper quadrant discomfort, anorexia, and weight loss. On physical examination the liver is palpable and firm. Periductal fibrosis, pigment stones, recurring episodes of cholangitis with bacterial sepsis, cholecystitis, liver abscess, and occasionally pancreatitis occur in the most heavily infected persons.[122,127,128] Developmental retardation has been reported in children with heavy infections.[122] Immune-complex glomerulonephritis may account for the frequent finding of high levels of IgG antibodies to *O. viverrini* in the urine of infected persons in Thailand.[128] An increased incidence of cholangiocarcinoma, an adenocarcinoma originating from hyperplastic biliary epithelium, has been associated strongly with *O. viverrini* infection in Thailand and also with *C. sinensis* infections in China and elsewhere.[116,122,129,130] The pathogenesis is thought to be related to injury and inflammation from feeding activities of worms, obstruction of bile ducts, bacterial infection, and toxicity of worms' excreted products.[122]

Diagnosis and Therapy

Liver fluke infection is diagnosed by finding eggs in the stool or by identifying adult worms during surgery or endoscopic retrograde cholangiopancreatography.[115,126,131] Eggs may not be found in the stool in the presence of biliary obstruction or in infections with fewer than 20 adult flukes; multiple examinations of concentrated specimens may be necessary unless the highly sensitive multivalent flotation method (FLOTAC) is used.[115,131] Serologic tests, antigen detection tests, and PCR-based assays are not widely available outside endemic areas or research laboratories.[131a,131b,132] Molecular techniques can discriminate species of *Clonorchis, Opisthorchis*, and several intestinal flukes that produce small morphologically similar eggs that are difficult to distinguish microscopically.[133] Ultrasonography, CT, or MRI can demonstrate dilation and stricture of bile ducts, thickening of the gallbladder wall, stones, and cholangiocarcinoma.[131] Flukes within bile ducts are difficult to visualize and require high-resolution equipment, but flukes in the gallbladder are more readily seen.[131] M-mode ultrasonography may demonstrate moving worms, which appear as thin linear echoes in ducts or can be seen floating in the gallbladder.

A single course of praziquantel (75 mg/kg in three doses for 1–2 days) eradicates the infection in more than 85% of cases.[132] A single dose regimen of 30 to 40 mg/kg was as effective for treatment of light and moderate infections with *O. viverinni* in Thailand.[134] Tribendimidine (available in China, 400 mg/day orally for 1 to 3 days) results in cure rates of 58% for *C. sinensis*.[135] Albendazole in a dose of 10 mg/kg for 7 days may also be used.[132] Rarely, surgery is needed to relieve biliary tract obstruction. Prevention of infection can be achieved by freezing fish or cooking fish adequately. In areas where opisthorchiasis is endemic, mass treatment with praziquantel has been effective.[136,137]

Fascioliasis

Infection with the sheep liver fluke *Fasciola hepatica* results from ingesting uncooked watercress or other fresh aquatic vegetation in 61 countries worldwide, especially in sheep- and cattle-raising areas.[3,138–140] Estimates of the global prevalence of human infection range from 2.4 to 17 million.[6,10] Infections have been reported from all continents except Antarctica, with the highest rates of infection in Bolivia, Peru, Egypt, Iran, Portugal, and France. The ability of *F. hepatica* to adapt to new definitive hosts and new environments, such as the Bolivian Altiplano at 3800 to 4100 meters, accounts for its continued spread from its Near Eastern and European origins.[115,138,139] A small number of cases have been reported in the United States; most have been imported, but fascioliasis occurs in domesticated herbivores in the United States, and several cases of domestically acquired infection have been reported.[141–143] The closely related but larger *F. gigantica* has a more limited distribution, and its distribution often overlaps that of *F. hepatica* in parts of Africa and Asia.[144]

The hermaphroditic adult fluke is flat, brown, and leaf shaped, and it measures about 3 × 1.5 cm. Mature worms in their natural hosts (mainly sheep and cattle) live in the common and hepatic bile ducts, where they deposit their eggs (see Fig. 288.2). The large oval, yellowish brown, operculate ova measuring 140 × 75 µm (see Fig. 288.3) pass to the intestines, are evacuated in the feces, and complete their development in fresh water. Within a few days miracidia hatch and invade their specific snail intermediate host, within which they undergo asexual replication, resulting in the release of cercariae that encyst as metacercariae on aquatic plants, including watercress, water caltrops, water lettuce, mint, and parsley. Whether embedded in plants or free floating in contaminated water or juice made from plants, the infective metacercariae excyst after being swallowed, and the larvae penetrate the intestinal wall into the peritoneum, from whence they migrate through the liver capsule and feed on liver parenchyma before entering the biliary tract.[3,115,116,144a] Three to 4 months are needed from infection to oviposition in humans. Adult flukes can live as long as 10 years.

Clinical Manifestations

Infection with *F. hepatica* has two distinct clinical phases corresponding to (1) the hepatic migratory phase of its life cycle and (2) the presence of the worms in their final habitat in the bile ducts.[10,115] Symptoms corresponding to the initial acute migration of the larval fluke appear within 6 to 12 weeks of ingestion of metacercariae and can last 4 months or longer. Marked eosinophilia is seen in most infected persons, and abdominal pain, intermittent high fever, weight loss, and urticaria are common.[145] There may be tender hepatomegaly, jaundice, anemia, and elevated results of liver function tests. Cough and chest pain occur in 10% to 15% of infected persons, sometimes accompanied by an eosinophilic pleural effusion. Aberrant migration may produce migratory nodules in the skin, painful inflammation of the intestinal wall, and lesions in the lung, brain, genitourinary tract, or elsewhere. CT or MRI shows hypodense lesions, measuring 1 cm or more in diameter, that move to various parts of the liver over the course of several weeks.[131,146,147] Hypodense tortuous and branching linear tracks under the capsule correspond to necrosis, and eosinophilic inflammatory infiltrates are seen along the path of larval migration.[148,149]

Within several weeks to months the symptoms and signs of the acute phase subside as the worms enter the bile ducts. The chronic phase of fascioliasis is usually subclinical, although eosinophilia is common, and anemia may develop in children.[150] Some persons have symptoms of biliary colic and cholecystitis due to inflammation and intermittent obstruction of bile ducts; on occasion, there is ascending cholangitis.[151] Ultrasonography or cholangiography may show masses in the common bile duct corresponding to adult worms, and worms have been visualized directly by cholangioscopy and removed after sphincterotomy for identification.[152] Pancreatitis may also occur and has been reported in up to 30% of cases.[153]

Ectopic fascioliasis may also occur, either bloodborne or from migration through soft tissues. It consists of tender, erythematous nodules or occasionally localized abscesses. Most commonly, they involve the subcutaneous tissue of the abdominal wall, but they have also been reported to be in muscle, heart, lung, GI tract, eye, and brain.[154,155]

Diagnosis

The diagnosis during the acute stage is based on epidemiology, the clinical picture, and, often, characteristic lesions seen on CT or MRI. Biopsy of nodules on the surface of the liver, in the skin, or elsewhere may show inflammatory tracts or immature worms. Serologic tests are useful during acute infection because symptoms develop 1 to 2 months before eggs are detectable in the stool. Whole-worm antigens, coproantigens, and excretory-secretory proteins have been used in enzyme immunoassays and immunoblots; sensitivities of greater than 90% are reported, but the specificity may be less, owing to cross-reactivity with other helminths.[156] CDC offers an enzyme immunoassay combined with a confirmatory immunoblot based on a recombinant *F. hepatica* antigen (FhSAP2) that has a specificity of greater than 98% for chronic fascioliasis.[157] The definitive diagnosis is made by demonstrating eggs in samples of stool, bile, or duodenal aspirates or by recovering worms at surgery. Repeat examinations and concentration procedures may be

necessary to detect eggs in the stool. A duplex PCR-based method can identify and discriminate *F. hepatica* and *F. gigantica* in stool, and a recombinase polymerase amplification method has potential for deployment in resource-limited settings.[158]

Therapy

Unlike infections with other flukes, fascioliasis responds poorly to praziquantel. First-line treatment is with a single oral dose of triclabendazole, a well-tolerated benzimidazole used in veterinary practice that is highly effective against mature and immature flukes.[115,159,160] Rates of cure of 78% to 99% have been reported, and persons not cured with a single dose usually respond to a second dose. Treatment should be repeated if radiographic findings or eosinophilia fail to resolve or the titers of serologic tests do not decrease.[161] Resistance of *F. hepatica* to triclabendazole is widespread in cattle and has been observed in human fascioliasis.[161a,161b] Triclabendazole is not available commercially in the United States but can be obtained through the CDC Drug Service under a special protocol requiring CDC and FDA approval.

Rates of cure with an alternative regimen, nitazoxanide, 500 mg twice daily for 6 to 7 days, have been as high as 95% in several small trials but lower in others.[162,163] Another alternative, bithionol, requires 10 to 15 doses, causes frequent side effects, and is no longer available from the CDC Drug Service. Artesunate has activity against *F. hepatica,* but cure rates are less than those with triclabendazole, and artemether had little effect in a trial in Egypt.[164,165]

INTESTINAL FLUKES

More than 70 flukes are known to infect the human GI tract.[3,6,116,166,167] Most are found in parts of Asia where the appropriate intermediate hosts and animal reservoirs are found.[1a] Cases in the United States occur primarily in immigrants from endemic areas, and less commonly among persons who ingested imported food, such as sushi made from infected fish.[141] Consumption of raw or undercooked local freshwater fish has led to *Heterophyes* infection in Hawaii.[141] *Nanophyetus salmincola,* which is transmitted in the Pacific Northwest of the United States, is discussed in Chapter 290. The best-known intestinal flukes include *Fasciolopsis buski;* the heterophytid flukes *Heterophyes, Metagonimus,* and *Haplorchis;* and *Echinostoma* spp.

Fasciolopsiasis

Human infection with the large intestinal fluke *Fasciolopsis buski* occurs in the Far East, Southeast Asia, and southern Asia, where pigs are the major reservoir of infection.[139,167,168] The thick, fleshy adult worms range in length from 2 to 7.5 cm and in breadth from 0.8 to 2 cm, making them the largest intestinal flukes that parasitize humans. They inhabit the duodenum and jejunum, where they produce large operculated eggs (135 × 80 μm) (see Fig. 288.2). On reaching fresh water the eggs hatch, releasing miracidia that penetrate a specific snail intermediate host in which they multiply and develop into free-living cercariae. The cercariae encyst into metacercariae on almost any aquatic plant. The metacercariae survive in most environments for up to 1 year. When raw or poorly cooked infected plants, frequently water chestnut, bamboo, caltrop, and hyacinth and roots of the lotus, are ingested by humans, the metacercariae excyst in the intestines, and, within 3 months, the parasites develop into mature worms that survive 6 months or more in the human host.

Adult flukes live in the upper portion of the small intestine, where they attach to the mucosa and produce local inflammation, ulceration, and abscesses. Fasciolopsiasis is usually asymptomatic, but anemia and eosinophilia are common.[10,18,116,168a] In some cases epigastric pain and diarrhea develop 1 or 2 months after exposure; rare cases of upper GI bleeding and appendicitis have been reported.[168b,168c] With heavy infections, flukes may cause transient obstruction and ileus. Edema of the face and extremities may result from hypersensitivity to worm metabolites or from hypoalbuminemia due to malabsorption or protein-losing enteropathy.

Heterophyiasis, Metagonimiasis, and Haplorchiasis

There are at least 22 species of flukes in the family Heterophyidae, of which *Heterophyes heterophyes* and *Metagonimus yokogawai* are the most common. *H. heterophyes* is found primarily in the Nile delta region, Tunisia, Turkey, and Iran, whereas *M. yokogawai* is most prevalent in the Far East.[4,138,169] *Haplorchis taichui* infection is common in parts of Southeast Asia, where its eggs are often confused with those of *O. viverrini.*[170,171] The adult flukes of all species measure less than 2 mm in length and inhabit the small intestine of human, animal, or avian hosts, where they produce small operculate eggs (30 × 15 μm). The life cycle is similar to that of other trematodes and involves snails and fish living in fresh or brackish water. The metacercariae encyst under the scales of the fish. Infection is acquired by consumption of undercooked or salted fish. Adults begin producing eggs in about 9 days and live only a few months to less than 1 year.

Flukes attach to the small intestine wall, where they produce focal inflammation and ulcerations. Asymptomatic eosinophilia occurs with light infections, whereas moderate infections have been associated with irritable bowel syndrome–like symptoms, and heavy infections may lead to colicky abdominal pain, dyspepsia, and diarrhea.[10,166,172] Flukes may penetrate the mucosa and deposit eggs that pass through lymphatics into the bloodstream. Eggs may embolize to the heart or CNS, where they can cause myocarditis or focal lesions of the brain or spinal cord and occasionally death.

Echinostomiasis

At least 20 species of *Echinostoma* infect humans, primarily in Southeast and East Asia, where infection is acquired by ingestion of raw or undercooked clams, frogs, snakes, snails, and fish, which can serve as the second intermediate host.[116,167,173,173a] Rare cases have been reported in India and Nepal, and a group of six travelers became infected after eating raw fish caught in Lake Tanganyika in Tanzania.[173b,173c] Illness in heavy infections tends to be more severe than that observed in other intestinal trematode infections and includes abdominal pain, diarrhea, malnutrition, anemia, intestinal ulceration, and perforation.

Diagnosis and Therapy

A definitive diagnosis of intestinal trematode infection depends on demonstration of eggs or adult worms in stools. Proper identification of the species by examining eggs is difficult because the morphology and size of eggs of different species are similar.[115] Eggs of *F. buski* can be confused with those of *F. hepatica,* and eggs of *Heterophyes, Metagonimus,* and *Haplorchis* can be confused with each other and with the eggs of *C. sinensis* and *Opisthorchis.* Species identification is best made by examining adult worms expelled after treatment or by PCR-based methods applied to stool specimens.[132,170,174]

The treatment of choice of all intestinal trematodes infections is praziquantel. Niclosamide has been used to treat *Fasciolopsis* and *Heterophyes* infections, and triclabendazole may be effective, but experience using this drug is limited.[115,132]

LUNG FLUKES

Paragonimiasis

More than 40 species of *Paragonimus* are recognized as parasites of mammals, and of these about 15 cause significant infections in humans.[10,116,175–177] *Paragonimus westermani,* the most important species infecting humans, is found in the Far East, principally in Korea, Japan, Taiwan, China, and the Philippines. Other important species are *Paragonimus miyazaki* (Japan), *Paragonimus skrjabini* and *Paragonimus hueitungensis* (China), *Paragonimus heterotrema* (China, Southeast Asia), *Paragonimus uterobilateralis* and *Paragonimus africanus* (Central and West Africa), *Paragonimus mexicanus* (Central and South America), and *Paragonimus kellicotti* (North America).[176–178] More than a dozen cases of locally acquired paragonimiasis have been reported in the United States among persons who ate raw or undercooked crayfish, most often while canoeing or camping in the Mississippi River drainage basin.[141,176–178]

Reddish brown adult worms measuring 7 to 16 mm × 4 to 7 mm live in encapsulated cystic cavities in the parenchyma of the lung, usually close to the bronchioles. Worms produce golden-brown operculate eggs (80–120 μm × 50–65 μm) that pass into the bronchioles and are coughed up; they are then either passed in sputum or swallowed and passed in feces (see Figs. 288.2 and 288.3). On reaching fresh water they require 2 to 3 weeks to develop before miracidia hatch at 29°to 32°C. After 3

to 5 months of development and reproduction in the snail, stumpy-tailed cercariae emerge. They encyst in the muscles and viscera of crayfish and freshwater crabs. Human infection is initiated by consumption of these freshwater crustaceans if they are uncooked, partially cooked, salted, or pickled. Cases in Japan have been reported from consumption of wild boar and deer, which can serve as paratenic hosts.[178a] Metacercariae excyst in the duodenum, penetrate the intestinal wall, and enter the peritoneal cavity. After several days they migrate through the diaphragm to the pleural cavities and then into the lungs. A fibrous cyst wall develops around them, and egg deposition starts 5 to 6 weeks after infection. Worms may develop also in extrapulmonary sites, including the liver, lymph nodes, skin, spinal cord, and brain. Adults may live as long as 20 to 25 years but generally have much shorter lives.

Clinical Manifestations

At 2 to 15 days after ingestion of metacercariae, some persons develop abdominal pain and diarrhea, followed by fever, chest pain, cough, urticaria, and eosinophilia. However, these initial symptoms are often absent, and light or moderate infections may remain undetected until first seen on a radiograph obtained for other reasons. In cases of North American paragonimiasis diagnosed in Missouri, cough, fever, eosinophilia, and pleural effusions developed within 2 to 12 weeks of ingesting raw crayfish.[178]

The inflammatory reaction to adults encapsulated in the lungs and the shedding of eggs into the bronchial tree are responsible for chronic symptoms. Cough productive of brownish sputum with intermittent hemoptysis are the initial manifestations of chronic infection.[179] Later the clinical picture resembles chronic bronchitis or bronchiectasis with profuse expectoration and pleuritic chest pain, dyspnea, chronic cough, chest pains, and occasional hemoptysis.[176] Radiographs may be negative or show diffuse infiltration, cysts measuring about 4 cm in diameter, nodules, calcifications, pleural effusion, and pneumothorax.[180] In North American cases, CT has shown nodules, internal mammary and cardiophrenic lymphadenopathy, pericardial thickening or effusion, linear tracks between nodules and pleura, and inflammation of the omentum, corresponding to the worm's migratory path.[181] Pleural and pericardial fluid is exudative and may contain large numbers of eosinophils. The illness is often confused with pulmonary tuberculosis, but eosinophilia and the lack of fever suggest the true diagnosis.[176] Lung cancer may be suspected in patients with nodular or mass lesions, especially in the absence of other symptoms.[182]

The most commonly recognized form of extrapulmonary disease is involvement of the CNS, which is seen in as many as 25% of hospitalized cases.[175,176,183] Parasite-induced meningitis may be the first manifestation, usually occurring within a year of pulmonary infection; eosinophilic meningitis has been seen in US cases of *P. kellicotti* infection with migration of parasites in the brain.[183a] Cerebral infections present as space-occupying tumors, with seizures, headaches, visual disturbances, and motor or sensory deficits. Radiographs of the skull may show clusters of calcified cysts that resemble soap bubbles, and MRI and CT show aggregates of ring-enhancing lesions with surrounding edema.[180,183b]

Other extrapulmonary infections result in migratory allergic skin lesions similar to those seen with cutaneous larva migrans; these lesions are most common during infections with *P. skrjabini* but also occur with infections with *P. westermani* and other species. Flukes can migrate through the liver and enter the biliary tree and have been found in the liver, spleen, peritoneum, intestinal wall, intraabdominal lymph nodes, and elsewhere.[176,183,184,184a]

Diagnosis and Therapy

The diagnosis of paragonimiasis is established by identifying expectorated eggs in the sputum, swallowed eggs in the feces, or worms and eggs in biopsy specimens. Multiple examinations of stool and sputum may be necessary. Serologic tests are useful for diagnosing early, light, or extrapulmonary infections.[185] Enzyme immunoassay tests are available, and an immunoblot assay performed at the CDC that uses a crude antigen extract of *P. westermani* has a sensitivity of 96% and a specificity of almost 100%.[186,187] A Western blot that uses *P. kellicotti* antigens recognized all 11 cases of North American paragonimiasis examined, including 2 cases negative by *P. westermani* immunoblot.[188]

The treatment of choice for paragonimiasis is praziquantel. The alternative, triclabendazole, also has a high rate of cure.[132,176] A patient with *P. westermani* infection and a severe allergy to praziquantel failed to respond to several courses of triclabendazole but was successfully treated after desensitization to praziquantel.[189] Bithionol is no longer used because of more frequent side effects. Because an inflammatory reaction to dying worms may precipitate seizures or other neurologic complications, corticosteroids should be used simultaneously with praziquantel for cerebral paragonimiasis.

PREVENTION OF FOODBORNE FLUKE INFECTIONS

As aquaculture for production of freshwater fish, crustaceans, and aquatic vegetables continues to expand and provide a rapidly growing percentage of the supply for local and international markets, efforts to prevent and control nonschistosomal trematode infections are critical at all stages of the food chain. The focus is on food production and preparation, because elimination of snail and animal hosts is neither desirable nor feasible. Educational interventions focus on preventing contamination of water sources with human and animal feces, especially ponds used for the cultivation of fish and aquatic plants. Effective education about proper preservation, cooking, or other preparation of food takes cultural practices and beliefs into account. WHO's current strategy to control foodborne trematodiases also includes control of morbidity and mortality by widespread screening of populations and anthelmintic treatment of confirmed cases or, in areas of high prevalence, mass treatment of persons at risk for infection. National programs in Southeast Asia have delivered hundreds of thousands of doses of praziquantel for control of clonorchiasis and opisthorchiasis, and in Egypt, Bolivia, and Peru, triclabendazole has been administered to populations with high rates of fascioliasis.[2,190]

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289 Tapeworms (Cestodes)

Jessica K. Fairley and Charles H. King

SHORT VIEW SUMMARY

Definition
- Cestodes are flatworms (platyhelminths) that cause human parasitic infection in the form of intestinal tapeworms or invasive larval cysts (i.e., neurocysticercosis or echinococcosis) (see Table 289.1).

Epidemiology
- *Diphyllobothrium latum,* fish tapeworm, is associated with eating undercooked freshwater fish and is endemic worldwide.
- *Hymenolepis nana* is the most common tapeworm worldwide and is transmitted person to person.
- *Taenia saginata* (beef tapeworm) occurs in cattle-breeding areas of the world.
- *Taenia solium* (pork tapeworm), and consequently neurocysticercosis, is endemic to resource-limited areas including Mexico, Central and South America, Asia, and Africa.
- *Echinococcus granulosus* infection occurs worldwide in livestock-raising areas and primarily is associated with domestic dogs.
- *Echinococcus multilocularis* has a life cycle that involves wild canines and is endemic only in the Northern Hemisphere.

Microbiology
- Mature tapeworms reside in intestines of carnivorous animals.
- Eggs are shed and then pass to an intermediate host, causing cystic infection via dissemination of oncospheres into host tissues.
- A definitive carnivore host ingests cyst-infected tissue, and a tapeworm then develops in intestines.

Diagnosis
- Diagnosis of intestinal tapeworms relies on examination of stool for evidence of eggs or tapeworm segments (proglottids).
- Neurocysticercosis is diagnosed by typical appearance on imaging scans, and diagnosis is supported by clinical symptoms and serologic testing.
- Diagnosis of echinococcosis is made by typical imaging appearance and supported by serology.

Therapy
- The mainstay of therapy for intestinal tapeworms is praziquantel or niclosamide.
- Neurocysticercosis therapy depends on the location and stage of the cyst.
- Parenchymal neurocysticercosis is generally treated with a combination of anthelmintic agents and corticosteroids (albendazole or praziquantel or both).
- Echinococcosis, depending on the stage and species, often requires surgery in combination with anthelmintic agents.

Prevention
- Improved sanitation is essential.
- Education programs on symptoms of infection and keeping livestock in corrals away from humans should be implemented.
- Livestock at market or slaughterhouse should be screened for cysts.
- Dogs should be screened and treated to eliminate *E. granulosus.*
- Prolonged freezing or thorough cooking kills cysts in tissue.

In humans, parasitic cestode infections occur in either of two forms: as mature tapeworms residing in the gastrointestinal tract or as one or more larval cysts (variously called hydatidosis, cysticercosis, coenurosis, or sparganosis) embedded in liver, lung, muscle, brain, eye, or other tissues.[1,1a,1b] The form taken by the infecting parasite depends on which cestode species causes the infection and, to a lesser extent, on the route by which the infection was acquired. Table 289.1 summarizes the common cestode parasites of humans, their typical vectors, and their usual symptoms.

In this chapter, parasite biology and the immunology of cestode infection are discussed, followed by descriptions of individual parasite species: intestinal tapeworms (e.g., *Diphyllobothrium latum, Hymenolepis nana, Taenia saginata,* and *Taenia solium*) and invasive cestode parasites (cysticercosis *[T. solium],* hydatid and alveolar cyst disease [*Echinococcus* spp.], sparganosis, and coenurosis *[Taenia multiceps]*). Diagnosis and therapy, outlined briefly under the individual parasite headings, are discussed in greater detail at the end of each section.

CESTODE BIOLOGY

Parasite Life Cycle

The parasitic cestodes discussed in this chapter are flatworms (platyhelminths) of the orders Pseudophyllidea (*Diphyllobothrium* and *Spirometra*) and Cyclophyllidea (other species), which divide their life cycle between two animal hosts (Fig. 289.1).[1,1a,1b] As mature tapeworms, these parasites reside in the intestinal tract of a definitive host, a carnivorous mammal. Depending on the parasite species, mature tapeworms vary in length from several millimeters (*Echinococcus* spp.) to 25 m *(Diphyllobothrium).*[1,1a,1b]

The tapeworm consists of a head *(scolex),* a neck, and a tail. The head has two or more suckers and in some cases a *rostellum,* or knob of small hooks, used to attach to the wall of the host's intestine (Fig. 289.2A).[2] The scolex is connected by a short neck to the lower portion of the tapeworm, the *strobila,* which is a ribbon-like chain of independent but connected segments called *proglottids* (Fig. 289.2B). Each proglottid has both male and female sexual organs and is responsible for producing the parasite's eggs. Proglottids begin to develop in the neck region of the parasite and then mature and move downward in the strobila as new segments are added from above. The hermaphroditic proglottids become gravid and eventually break free of the tapeworm. Proglottids may degenerate in the stool, releasing eggs (thousands to millions per day) into the feces. Alternatively, intact proglottids may be passed in the stool, with egg release occurring outside the body. In some cases, a section of strobila may be passed in a single day, with no further release of proglottids for several days thereafter. In practical terms, this means that although the number of tapeworm eggs in the stool is usually high, detection of parasite eggs by standard stool examination may be sporadic. Therefore multiple stool samples, rectal swabs, and visual examination of stool and perineum for proglottids may be required to detect a tapeworm infection. For some species of tapeworm (e.g., *T. saginata*), the proglottids are motile. They may migrate within the gastrointestinal tract, causing biliary or appendiceal obstruction, or out of the body, to be found in the perineum.

At the point at which eggs are released, two effective biotypes of parasite can be defined. If the eggs released from the parasite are partially developed, they are called *embryonated.* If the egg embryo has not yet begun its differentiation, the egg is referred to as *nonembryonated.* In

TABLE 289.1 Common Cestode Parasites of Humans, Their Typical Vectors, and Their Usual Symptoms

PARASITE SPECIES	DEVELOPMENTAL STAGE FOUND IN HUMANS	COMMON NAME	TRANSMISSION SOURCE	SYMPTOMS ASSOCIATED WITH INFECTION
Diphyllobothrium latum	Tapeworm	Fish tapeworm	Plerocercoid cysts in freshwater fish	Usually minimal; with prolonged or heavy infection, vitamin B_{12} deficiency
Hymenolepis nana	Tapeworm, cysticercoids	Dwarf tapeworm	Infected humans	Mild abdominal discomfort
Taenia saginata	Tapeworm	Beef tapeworm	Cysts in beef	Abdominal discomfort, proglottid migration
Taenia solium	Tapeworm	Pork tapeworm	Cysticerci in pork	Minimal
Taenia solium (Cysticercus cellulosae)	Cysticerci	Cysticercosis	Eggs from infected humans	Local inflammation, mass effect; if in CNS, seizures, hydrocephalus, arachnoiditis
Echinococcus granulosus	Larval cysts	Hydatid cyst disease	Eggs from infected dogs	Mass effect leading to pain, obstruction of adjacent organs; less commonly, secondary bacterial infection, distal spread of daughter cysts
Echinococcus multilocularis	Larval cysts	Alveolar cyst disease	Eggs from infected canines	Local invasion and mass effect leading to organ dysfunction; distal metastasis possible
Taenia multiceps	Larval cysts	Coenurosis, bladder worm	Eggs from infected dogs	Local inflammation and mass effect
Spirometra mansonoides	Larval cysts	Sparganosis	Cysts from infected copepods, frogs, snakes	Local inflammation and mass effect

CNS, Central nervous system.

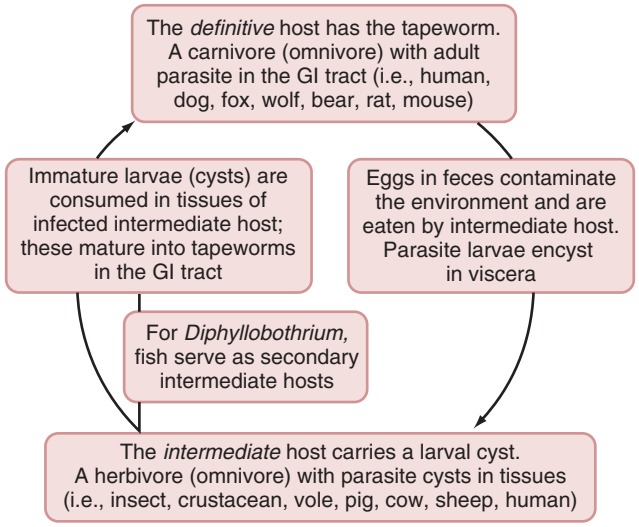

FIG. 289.1 Cestode parasites alternate larval and adult stages in two different hosts. *GI,* Gastrointestinal.

biologic terms, the embryonated egg can immediately infect the next intermediate mammalian or insect host, typically a herbivore or omnivore, through ingestion of food containing the egg.[1,1a,1b] Such eggs, typical of *Echinococcus* spp., *Taenia* spp., and *H. nana,* may lie dormant in grazing areas or become scattered in the home environment and remain infectious for several months to years.[1,1a,1b,3] Once ingested, the egg hatches in the intermediate host's intestine, releasing an *oncosphere,* which penetrates the gut mucosa to reach the circulation. The oncosphere passes to any of several organs to form a parasite cyst, which is variously called a cysticercoid, cysticercus, alveolar cyst, or hydatid cyst, depending on its morphology. The life cycle of these parasites is completed when the carnivorous definitive host consumes the cyst-infected tissues of the intermediate host and the cyst develops into a mature tapeworm in the lumen of the definitive host's intestine.

For nonembryonated eggs, such as those of the fish tapeworm *D. latum,* initial development takes place outside the body in water, after which the eggs hatch to release a free-swimming *coracidium* larva.[2] In time, the coracidium is ingested by a small crustacean called a copepod and then develops into a procercoid larva within the copepod's tissues. When the copepod is ingested by a fish or other intermediate host, the procercoid infects its musculature, developing into the next larval stage, the *plerocercoid* cyst, or *sparganum.* If an uncooked plerocercoid of *D. latum* is ingested by a human, its definitive host, it develops into a mature, intraluminal fish tapeworm. However, if the fish containing the plerocercoid is ingested by another, larger fish, it does not become a tapeworm. It reencysts instead as a plerocercoid in the muscles of the second, larger fish.

Plerocercoid encystment or reencystment is significant in terms of human disease (as *sparganosis*) for cestode species for which humans cannot serve as the definitive host (e.g., *Spirometra mansonoides,* a tapeworm of dogs and felines). Plerocercoids can develop in human tissues if *Spirometra*-infested copepods are ingested in drinking water. Alternatively, human plerocercoid cysts may be acquired via the intestine from another intermediate host (e.g., tadpole, frog, snake) if the meat of that aquatic host is eaten uncooked. Migrating plerocercoid cysts can also transfer directly into the skin or the eye if raw flesh of an aquatic intermediate host is used as a poultice in traditional healing.[2]

As a rule, humans are either definitive or intermediate hosts for a given cestode parasite, but not both. For example, humans are solely definitive hosts (i.e., with tapeworms) for *D. latum* (fish tapeworm) and *T. saginata* (beef tapeworm) and are solely intermediate hosts for *Echinococcus* spp. (hydatid cysts and alveolar cysts), *Spirometra* (sparganosis), and *T. multiceps* (coenurosis). There are two exceptions to this rule. The first is *T. solium,* which develops in humans as a cysticercus if ingested as an egg or as a tapeworm if ingested as a cysticercus in infected pork. It is thus possible for one patient to harbor both cyst and tapeworm forms of *T. solium.* Such dual infection is seen in approximately 25% of cysticercosis cases. The second exception is the dwarf tapeworm, *H. nana,* whose eggs, after ingestion, hatch in the gut and encyst within the wall of the human intestine. After 5 to 7 days, the cyst breaks open, and the larva develops (within the same host) to become a mature tapeworm. The fertile eggs of this tapeworm may directly infect the mucosa, permitting a continued increase in the number of tapeworms in the affected host, without further exposure to environmental egg contamination. In this manner, humans serve as both intermediate and definitive hosts for *H. nana.* Single-host proliferation such as that of *H. nana* is highly unusual among human cestode infections and helminth infections in general. Normally, heavy cestode infections can be acquired only by repeated environmental exposure to eggs or infectious parasite cysts.

Disease Pathogenesis and Immunology

Adult tapeworms in the intestinal tract generally cause minimal local pathology. Reduced nutrient absorption and alteration of gut motility have been described, but there is no firm association of adult tapeworm infection with specific bowel symptoms. An immune response to adult tapeworms provokes eosinophilia and immunoglobulin E (IgE) elevation in some patients, but the immune response does not appear to alter the course of an intraluminal tapeworm infection. In light of the limited

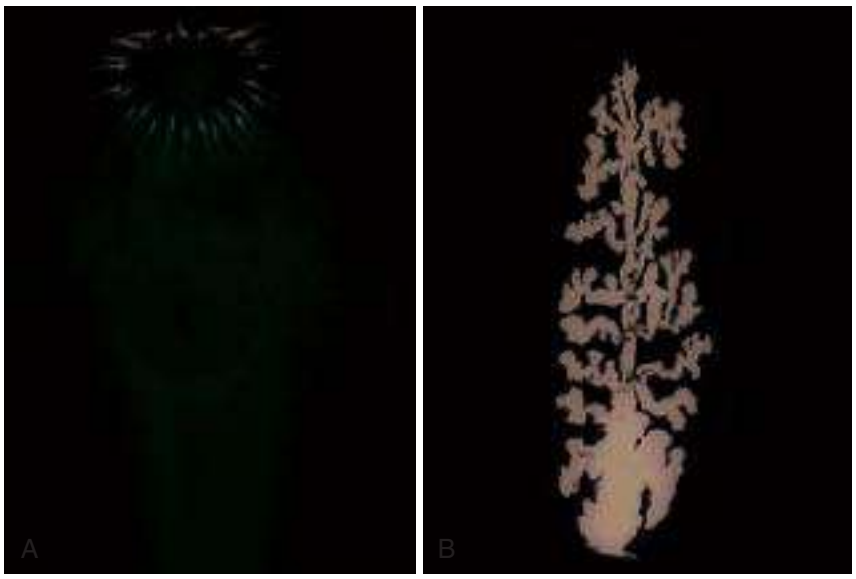

FIG. 289.2 (A) The scolex and (B) a proglottid of the cestode *Taenia solium. (From Ash LR, Orihel TC.* Atlas of Human Parasitology. *3rd ed. Chicago: American Society for Clinical Pathology; 1990.)*

range of potential hosts observed for most adult tapeworms, it has been suggested that host factors, including the presence or absence of specific immunoreactivity, may determine the success of parasite infections in various potential host species.[4]

The immune response to invasive cyst infection is more pronounced but is often unsuccessful in eradicating the cyst in susceptible hosts. Infiltration with neutrophils and eosinophils is followed by local fibrosis, leading to cyst encapsulation and macrophage infiltration. Once the cyst is encapsulated, antigen release may be limited, leading to a reduction in the local inflammatory response. Specific antibody production remains detectable in the serum, however, and delayed-type hypersensitivity may be detected on skin testing. The immune response often increases later as the cyst begins to die and leak antigen or as it erodes into a body cavity, duct, or vessel, increasing local or systemic exposure to antigen. Experimentally, the anticyst immune response appears to limit dissemination of *Echinococcus* spp. after an initial infection.[4,5] Anticyst immunity is also likely to contribute to the spontaneous clearance of *H. nana* infection in older children. There is evidence of increased amounts of IgG subclasses as well as proinflammatory cytokines within the cerebrospinal fluid (CSF) of patients having severe neurocysticercosis.[6] It is unclear if this response contributes to the severity of the disease or is a consequence of severe infection; however, it does not effectively control the infection. Furthermore, symptomatic cysticercosis appears to be related to a depressed cellular immune response compared with the immune response of asymptomatic individuals.[6]

INTESTINAL TAPEWORMS

Diphyllobothrium latum

D. latum, or fish tapeworm, is one of the pseudophyllidean cestodes transmitted via aquatic species.[2] Human infection with *D. latum* is acquired by eating uncooked freshwater fish containing the parasite's plerocercoid cysts. Traditional modes of infection include consumption of dried or smoked fish, which may contain viable cysts if not further cooked, or tasting flavored freshwater fish (e.g., gefilte fish) before cooking. The enthusiasm for raw bar foods such as ceviche, sushi, and sashimi prepared from freshwater fish, especially salmon, has increased the transmission potential for *D. latum* in developed areas of North America.[7,8] Areas of the world in which *D. latum* is highly endemic (>2% prevalence) include specific lake and delta areas of Siberia, Europe (especially Scandinavia and other Baltic countries), North America, Japan, and Chile. In 2006 there was an outbreak in Lake Geneva, Switzerland, caused by freshly caught raw perch served at a wedding.[9] Plerocercoids were also found in 6% to 25% of fish caught in four Italian lakes.[10] Endemicity in rural areas is favored by stable zoonotic transmission through alternative nonhuman definitive hosts including seals, cats, bears, minks, foxes, and wolves.

Human *D. latum* tapeworms are large, reaching up to 25 m (3000–4000 proglottids) in length. It takes 3 to 6 weeks after exposure for the tapeworm to mature. Once established, a *D. latum* parasite may survive 30 years or more. Multiple tapeworms in the same patient are common. Normally, infection is asymptomatic, but a proportion of infected individuals report nonspecific symptoms of weakness (66%), dizziness (53%), salt craving (62%), diarrhea (22%), and intermittent abdominal discomfort.[2]

Prolonged (more than 3 or 4 years) or heavy *D. latum* infection may lead to megaloblastic anemia caused by vitamin B_{12} deficiency. The vitamin B_{12} deficiency is a consequence of two factors: parasite-mediated dissociation of the vitamin B_{12} intrinsic factor complex in the gut lumen (making vitamin B_{12} unavailable to the host) and heavy vitamin uptake and use by the parasite. Megaloblastic anemia may be worsened by concurrent folate deficiency, which also occurs as a consequence of *D. latum* infection. Vitamin B_{12} deficiency may be sufficiently severe to cause injury to the nervous system including peripheral neuropathy and severe combined degeneration of the central nervous system (CNS).

Tapeworm infection may first be suspected based on the patient's history or when contrast studies of the intestine show an intraluminal, ribbon-like filling defect. Definitive diagnosis of *D. latum* infection is made by detection of 45- × 65-mm operculated parasite eggs on stool examination (Fig. 289.3A–B). Recovery of proglottids (with a characteristic central uterus) also establishes the diagnosis.

Treatment is with a single course of niclosamide or praziquantel (see "Therapy").[11] Mild vitamin B_{12} deficiency is reversed by eradicating the tapeworm. Severe vitamin B_{12} deficiency should be treated with parenteral vitamin injections. If a patient presents with vitamin B_{12} deficiency and epidemiologic risk factors for fish tapeworm infection, one should maintain a high index of suspicion for possible infection.

Hymenolepis nana

H. nana, also known as dwarf tapeworm, is a cyclophyllidean tapeworm with embryonated eggs.[2] It is probably the most prevalent tapeworm worldwide, and it is the only tapeworm that can be transmitted directly from human to human. Areas of endemicity (1%–30% prevalence) include Asia, southern and eastern Europe, Central and South America, and Africa.[12] In North America, infection is most frequently found among institutionalized populations (up to 8% prevalence reported in the past)[13] and among malnourished or immunocompromised patients.

Ingestion of parasite eggs on fecally contaminated food or fomites allows the initial infection. Once in the intestine, the eggs hatch to form oncospheres, which penetrate the mucosa to encyst as cysticercoid larvae. The larval cyst ruptures 4 or 5 days later into the lumen to form the relatively small, adult *H. nana* tapeworm (15–50 mm in length). Internal autoinfection may occur as parasite eggs are released from gravid

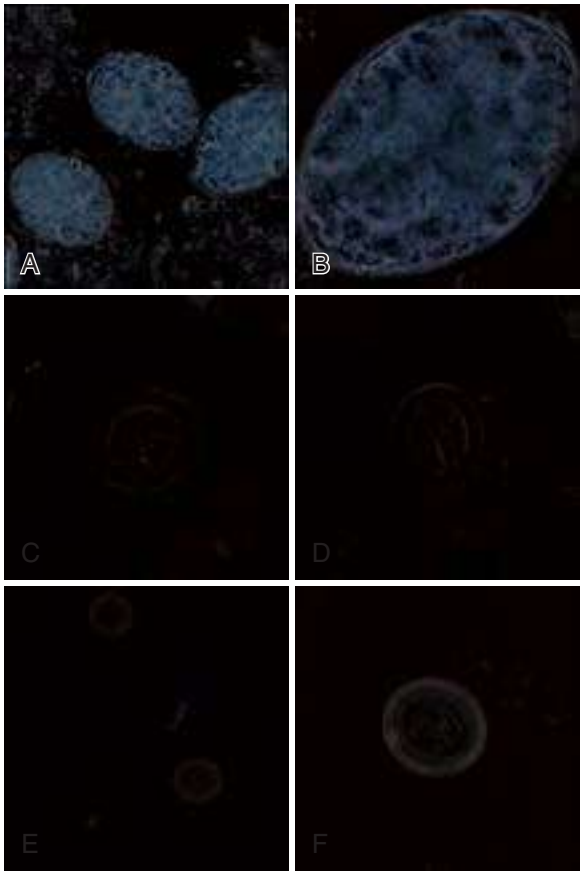

FIG. 289.3 (A–B) Eggs of *Diphyllobothrium latum* (55–75 µm × 40–50 µm) in an iodine-stained wet mount. Note the knob at the abopercular end in (B). (C–D) Eggs of *Hymenolepis nana* (30–50 µm) in an unstained wet mount. Note the presence of hooks in the oncosphere and polar filaments within the space between the oncosphere and outer shell. (E–F) *Taenia* spp. eggs (30–35 µm) in unstained wet mounts. The eggs of *T. solium* and *T. saginata* are indistinguishable from each other as well as from other members of the Taeniidae family.

proglottids in the ileum. In addition, poor sanitary practices promote external (fecal-oral) autoinfection as well as transmission to others sharing the same living quarters. Heavy infection is common among children and may be associated with abdominal cramps, anorexia, dizziness, and diarrhea. In areas of mass drug administration for other soil-transmitted helminth infections, *H. nana* has emerged as a prevalent pathogen because it does not respond to albendazole or mebendazole therapy given for intestinal nematodes.[14] *H. nana* has been associated with anemia and weight loss among affected children.[15]

H. nana infection is diagnosed by identifying the 30- × 47-µm parasite eggs (with their characteristic double membrane) in the stool (Fig. 289.3C–D). Treatment is with praziquantel or niclosamide (see "Therapy").[11] Developing *H. nana* cysticercoids are not as susceptible to drug therapy as adult tapeworms. Because these cysts can emerge several days later to form new tapeworms, effective therapy for *H. nana* requires either higher than usual doses of praziquantel to reach cysticidal levels or more prolonged therapy with niclosamide (to eliminate emerging tapeworms) for 5 to 7 days. Nitazoxanide has also been used to treat infection; it appears to be highly effective and has been used as an alternative therapy after treatment failures with niclosamide.[16]

Taenia saginata

T. saginata, known as the beef tapeworm, is transmitted to humans in the form of infectious larval cysts found in the meat of cattle, which serve as the parasite's usual intermediate host.[1,1a,1b,2] The *T. saginata* tapeworm is common in cattle-breeding areas of the world. Areas with the highest prevalence (up to 27%) are in central Asia, the Near East, and central and East Africa. Areas of lower prevalence (<1%) are found in Europe, Southeast Asia, Central America, and South America. Consumption of measly (i.e., cyst-infected) uncooked or undercooked beef is the usual means of transmission. Rare steak or kebabs and steak tartare are dishes typically associated with *T. saginata* infection. In the definitive human host, adult *T. saginata* tapeworms are long (10 m) and can contain more than 1000 proglottids, each capable of producing thousands of eggs. If, through poor sanitary practices, eggs released in the feces are allowed to reach grazing areas, cattle are subsequently infected with *T. saginata* cysticerci. Alternative intermediate hosts include llamas, buffalo, and giraffes. Humans are the only definitive host.

Symptoms are absent in most patients with *T. saginata* infection. A small number report mild abdominal cramps or malaise. The proglottids of *T. saginata* are motile and occasionally migrate out of the anus, to be found in the perineum or on clothing. The patient may report seeing moving segments in the feces or passing several feet of strobila at one time. These events are often psychologically distressing and are associated with significant anxiety-associated symptoms.

Specific diagnosis of *T. saginata* infection can be established by recovery of parasite proglottids.[1,1a,1b,2] If only eggs are found in the stool, it is important to note that *T. saginata* eggs are morphologically indistinguishable from *T. solium* eggs (Fig. 289.3E–F). With *T. solium* tapeworms there is potential for autoinfection causing cysticercosis; therefore if any *Taenia* spp. eggs are detected, treatment should be given without delay for further speciation. Effective oral treatment for either *T. saginata* or *T. solium* is obtained with praziquantel or niclosamide (see "Therapy").[11]

Taenia solium

Humans can serve as either intermediate or definitive hosts for *T. solium.* Individuals who ingest *T. solium* eggs develop tissue infection with parasite cysts, a condition known as cysticercosis (see "Cysticercosis" for details of this illness). Patients who consume raw or undercooked pork containing infectious larval cysts (cysticerci) acquire the pork tapeworm—that is, the adult form of *T. solium,* which resides in the intestinal tract.[1,1a,1b,2] These tapeworms develop to 2 to 8 m in length and may survive for 10 to 20 years. Some patients harbor both cysticerci and *T. solium* tapeworms, and it is possible for a tapeworm-carrying individual to develop cysticercosis by autoinfection. Areas in which *T. solium* infection is endemic include Mexico, Central America, South America, Africa, Southeast Asia, India, the Philippines, and southern Europe.

Infection with *T. solium* tapeworms is generally asymptomatic unless cysticercosis, caused by autoinfection with parasite eggs, supervenes. If tapeworm infection is diagnosed, one should also have a high index of suspicion for concomitant cysticercosis. The proglottids of *T. solium* are not motile (in contrast to those of *T. saginata*) and do not migrate. Infection with *Taenia* spp. is readily diagnosed by detecting eggs during stool examination, but *T. solium* eggs are indistinguishable from *T. saginata* eggs (see Fig. 289.3E–F). If a proglottid is recovered, the species can be identified based on the characteristic features of the uterine canals in the segment.[1,1a,1b] Species identification is not required for therapy, which can be with either praziquantel or niclosamide.[11]

OTHER SPECIES CAUSING TAPEWORM INFECTION IN HUMANS

Human tapeworm infection also may be caused by *Dipylidium caninum,* a more frequent parasite of dogs and cats, or by *Hymenolepis diminuta,* a tapeworm that usually infects rats.[1,1a,1b,2] Such infections are acquired by consumption of insects (fleas or beetles) containing the larval cysticercoids of these species and are most commonly seen among children. Human infection with tapeworm species related to *D. latum—Diphyllobothrium klebanovskii, Diphyllobothrium dendriticum, Diphyllobothrium ursi,* and *Diphyllobothrium dalliae*—has been described in the Arctic and areas of Siberia. *Diphyllobothrium nihonkaiense,* endemic to Japan, is another related fish tapeworm that can cause infections in humans, and it has been reported in isolated cases in Europe after ingestion of imported fish.[17] *Taenia asiatica,* described in 1993 and confused with *T. saginata* in the past, is found in some countries of Southeast Asia and India. In contrast to *T. saginata* and *T. solium,* this parasite is viscerotropic and is transmissible through ingestion of visceral organs from pigs. It has been found in the muscle of pigs, but, in contrast to *T. solium,* it

does not cause cysticercosis.[15,18] Rarely, other tapeworm species infect humans, particularly individuals with unusual dietary habits such as the consumption of uncooked animal viscera. Infection is diagnosed by identifying characteristic parasite eggs in the stool. Effective treatment is obtained with praziquantel or niclosamide.

DIAGNOSIS

Because mature tapeworm infection is strictly an intraluminal intestinal infection, the most practical approach to diagnosis is examination of the feces for parasite eggs or proglottids. As discussed under "Cestode Biology," egg release in the stool may be variable because of an irregular rate of proglottid detachment and degeneration. Thus examination of stool samples taken on several different days may be required to establish a diagnosis. Sensitivity for egg detection may be improved by formyl ethyl acetate or other concentration techniques. Because cestode eggs are relatively heavy, sedimentation procedures (not flotation) provide a more efficient means to isolate tapeworm eggs. When handling specimens, it is important to remember that *T. solium* eggs are infective for humans and cause cysticercosis. For this reason, precautions should be taken to avoid any potential contamination of fingers or clothing with parasite eggs.

In some cases, intact proglottids are passed in the stool. This is most common with *D. latum, T. saginata, T. solium,* and *D. caninum.* Expelled proglottids tend to degenerate over time, so fixation and staining of specimens are recommended to allow effective microscopic speciation. Although species identification is not essential for treatment, identification of *T. solium* infection is significant and should prompt consideration of possible cysticercosis in the index patient or among his or her household contacts.[19] Proglottids of *D. latum* (fish tapeworm) often pass as short chains of grayish white connected segments, each 11 × 3 mm, with a central uterine structure.[2] Proglottids of the pork tapeworm, *T. solium,* are 11 × 5 mm, with a lateral genital pore and 7 to 13 branches on either side of the central uterine canal. Proglottids of *T. saginata,* the beef tapeworm, have a similar appearance but may be distinguished by the larger number of lateral uterine branches (15–20) in the proglottid. *T. saginata* proglottids are motile and may emerge spontaneously from the anus to be found on the perineum, on the legs, or on clothing. Proglottids of *D. caninum* (23 × 8 mm) are also motile and may be described by the patient (or parent) as whitish, moving cucumber seed–like objects in the stool. *D. caninum* proglottids may also become adherent to perianal hairs and then dry to form a whitish yellow object resembling a small grain of rice. Detection of specific coproantigens in the stool has improved diagnosis of *Taenia* infection and is genus specific. It carries a specificity of up to 95% to 98%. Newer polymerase chain reaction–based tests also show potential to identify infection at a species level.[15]

THERAPY

Tapeworm infection should be treated whenever diagnosed. Safe, effective treatment of intestinal tapeworm infection may be achieved with either praziquantel or niclosamide. Both are well-tolerated oral agents that have direct parasiticidal effects on intraluminal cestode parasites. Although both agents are usually effective and considered first-line therapy, there have been reports of treatment failure, likely secondary to resistance. In some cases, nitazoxanide has been used as an alternative drug and has been found to be safe and effective.[20]

Niclosamide

Niclosamide is a poorly absorbed, narrow-spectrum anthelmintic that is available as 500-mg chewable tablets (e.g., Yomesan; Bayer).[11] However, it is no longer commercially available in the United States. The drug is normally taken as a 2-g (four-tablet) single dose for adults, as a 1.5-g (three-tablet) dose for children weighing more than 34 kg (75 lb), or as a 1-g (two-tablet) dose for children weighing 11 to 34 kg (25–75 lb). A single treatment is effective for *D. latum, T. saginata, D. caninum,* and *T. solium* tapeworms.

Eradication of *H. nana* tapeworm infection requires a more prolonged course of therapy (repeat daily doses for 1 week) because of concomitant infection with maturing *H. nana* cysts, which are not affected by the drug. It is normally recommended that after the first dose, subsequent doses for *H. nana* (i.e., days 2–7) be reduced to 1 g daily for adults and large children (>34 kg) and to 0.5 g daily for small children. These follow-up doses are intended to kill any newly emerging *H. nana* tapeworms and should completely eliminate the infection. Nevertheless, it is appropriate to rescreen the patient's stool for parasite eggs 1 and 3 months after therapy to ensure cure. A repeated cycle of standard dosing (as just outlined) is usually sufficient to eliminate persistent infection.

Niclosamide must be thoroughly chewed before swallowing to obtain the maximal anthelmintic effect. Because the drug is poorly absorbed, the typical side effects of niclosamide are mild, occurring at a rate of approximately 10%.[11] Side effects include malaise, mild abdominal pain, and nausea on the day of administration. High doses of niclosamide have not been shown to have mutagenic effects in animals, and the drug has been placed in US Food and Drug Administration (FDA) pregnancy category B. Normally, treatment should be delayed until after pregnancy, but with *T. solium* there is concern that patients may develop cysticercosis through autoinfection with parasite eggs. Considering the relative risks and benefits, it may be appropriate to treat pregnant women who have *T. solium* tapeworms at the time of diagnosis and not delay therapy. Concern has also been raised about the possibility of internal autoinfection during *T. solium* therapy because of the release and possible retrograde intestinal movement of eggs during therapy. Although such autoinfection has not been documented, some experts recommend a mild laxative 1 or 2 hours after niclosamide treatment to avoid this possibility in *T. solium*–infected patients.

Praziquantel

Praziquantel is a broad-spectrum anthelmintic used to treat both trematode and cestode infections.[11] It is available as a scored, 600-mg coated tablet (e.g., Biltricide, Bayer); it has excellent activity against all tapeworms and is given as a single dose of 5 to 10 mg/kg for both children and adults. The exception to this regimen is *H. nana* infection, for which a higher dose of 25 mg/kg is recommended. If the *H. nana* infection is heavy, it is recommended that the dose be repeated 1 week after the initial therapy. Follow-up stool screening is recommended at 1 and 3 months to ensure eradication of infection.

Mild side effects occur in 10% to 50% of patients treated, depending on the population. Side effects include transient dizziness, headache, malaise, abdominal pain, and nausea. Moderate side effects, including sedation, vomiting, diarrhea, urticaria, rash, fever, and mild transaminitis, are not as common (<10%) and are also transient. Similar to niclosamide, praziquantel is classified in FDA pregnancy category B. However, two well-powered, placebo-controlled randomized trials of praziquantel treatment during pregnancy showed no harm to the fetus and no adverse birth outcomes.[21,22] Potential risks and benefits of treatment should be weighed before use; however, praziquantel appears safe for use in later pregnancy and during lactation.[23]

INVASIVE CESTODE INFECTIONS

Cysticercosis

Cysticercosis is a tissue infection with larval cysts of the cestode *T. solium* in which the patient serves as an intermediate host for the parasite. Infection is acquired by consumption of *T. solium* eggs by fecal-oral transmission from a *T. solium* tapeworm carrier. Prevalence is high wherever *T. solium* tapeworms are common (i.e., Mexico, Central America, South America, Southeast Asia, Africa, China, India, and Nepal),[24,25] and the World Health Organization (WHO) has estimated that there are more than 50,000 deaths per year from neurocysticercosis.[26] In recent years, cases in "nonendemic" countries, in particular the United States, have increased due to both imported cases and locally acquired disease,[27] whereas numbers seem to be decreasing in some highly endemic areas, possibly from better sanitation and public awareness of the disease.[27] A survey of hospitalizations for neurocysticercosis in the United States for the years 2003–2012 found 18,584 hospitalizations, with an estimated cost of greater than $908 million.[28] Nearly three-quarters of the cases were in Hispanic patients. Infected subjects normally harbor multiple cysts in many parts of the body. In areas of endemicity, the cumulative infection risk increases with age, frequent consumption of pork, and poor household hygiene.[29] Symptoms may develop because of local inflammation at the site of involvement; however, apart from CNS and cardiac involvement, serious disease is rare.

Clinical Presentation

Neurocysticercosis, which refers to the most common presentation of cysticercosis, is the term used for human CNS involvement with *T. solium* cysts.[30] Infection may involve any part of the CNS, but symptomatic disease is most often related to intracerebral lesions (causing mass effects, seizures, or both) (Fig. 289.4), intraventricular cysts (causing hydrocephalus), subarachnoid lesions (causing chronic meningitis), and spinal cord lesions (causing cord compression syndrome or meningitis). Seizures, occurring in up to 70% of patients with neurocysticercosis, and intracranial hypertension are the most common clinical manifestations.[26,30] Intraparenchymal cerebral cysts typically enlarge slowly, causing minimal symptoms until years or decades after the onset of infection, when the cysts begin to die. At this point, cysts may lose osmoregulation and begin to swell. They may also leak antigenic material that provokes a severe inflammatory response (cerebritis and meningitis). Both processes contribute to symptoms of focal or generalized seizures, sensorimotor deficits, intellectual impairment, psychiatric disorders, and symptoms of hydrocephalus. The peak incidence of onset of neurologic symptoms is 3 to 5 years after infection but may not occur until 30 years later or even longer. In regions where *T. solium* is endemic, 30% to 50% of patients with seizures have antibodies to *T. solium*, compared with the 2% to 11% prevalence in the general population, suggesting the likelihood that neurocysticercosis is an underlying cause of their disease.[30,31] Further studies using computed tomography (CT) in areas of Guatemala, Mexico, Ecuador, and Honduras have shown that up to 20% of the general population have asymptomatic brain calcifications.[32] However, the strength of the association varies across studies, and good-quality epidemiologic data are often lacking.[33]

Because of their critical location, intraventricular cysts and basilar cysts tend to cause symptoms early during the course of the infection. The symptoms are due to obstruction of CSF flow or local meningeal irritation, which leads to injury to local blood vessels, cranial nerves, or the brainstem.[30] An aggressive form of basilar neurocysticercosis, called *racemose cysticercosis,* has been described in which cysts proliferate at the base of the brain, resulting in mental deterioration, coma, and death. Another severe form of neurocysticercosis, cysticercotic encephalitis, represents a massive infection of the brain parenchyma and induces a significant immune response of the host that complicates treatment regimens. Intraparenchymal spinal cord lesions are often symptomatic early because of direct local pressure effects. When spinal column cysts develop outside the cord itself, the onset of symptoms may be more gradual. The slow onset of external cord compression, arachnoiditis, or radiculopathy may result in a confusing progression of symptoms. In the CNS, multiple cysticerci are the rule, and active symptoms may refer to one or several anatomic locations.

Cysts outside the CNS tend not to be symptomatic. These cysts eventually die and calcify, to be detected incidentally on plain radiographs of the limbs. Although symptomatic cardiac cysticercosis is rarely reported, autopsy reports of patients with cysticercosis have shown cysts involving the heart in up to 23% of cases. Symptoms of cardiac cysticercosis can include heart failure or conduction abnormalities.[34]

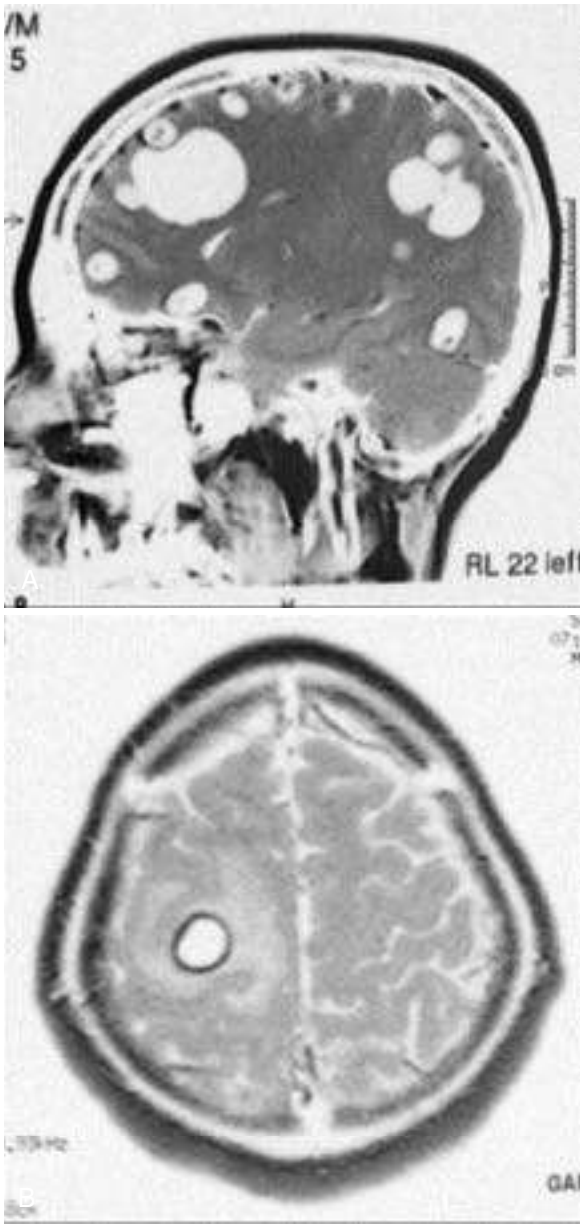

FIG. 289.4 Magnetic resonance images of (A) massive cysticercosis and (B) colloidal cysticercus defined by early signs of inflammation around the cysticercus. *(From Garcia HH, Del Brutto OH. Imaging findings in neurocysticercosis.* Acta Trop. *2003;87:71–78.)*

Diagnosis

The site of involvement and the symptoms experienced determine the best mode of diagnosis and eventual treatment. Travel to or residence in an endemic area significantly increases the likelihood of the diagnosis, although transmission has been documented to occur within the United States in people living in the same household as *T. solium*–infected individuals.[27] Presenting symptoms often suggest a tumor, and a specific diagnosis of cysticercosis is often first suspected on the basis of imaging studies (CT or magnetic resonance imaging [MRI]), which show multiple enhancing and nonenhancing unilocular cysts.[30] Cerebral cysts are usually multiple (an average of 7–10 per patient). Typical radiographic signs on MRI depend on the stage of cyst development. Vesicular cysticerci (small, rounded cysts without edema) often have the pathognomonic eccentric hyperdense nodule representing the scolex. Subsequent stages include colloidal and granular cysticerci, which appear ill-defined with surrounding edema. Lastly, calcified cysts can be detected on CT as hyperdense nodules without contrast enhancement.[35] Multiple cysts can be present in different stages; this pattern is often described as a "starry sky."[36] Ventricular and subarachnoid cysts are less distinct on MRI because CSF and cyst fluid have similar densities, which can present a diagnostic challenge.[36] CSF examination may show lymphocytic or eosinophilic pleocytosis, hypoglycorrhachia, and elevated protein levels. Newer CSF tests using lentil lectin glycoproteins Western blot have shown promising sensitivity (>90%) and specificity (100%) when multiple cysts are present, but decreased sensitivity in the case of single lesions.[30,37] A suspected diagnosis may also be strengthened by serology through enzyme-linked immunotransfer blot that is available commercially or through the US Centers for Disease Control and Prevention (CDC). The serology indicates prior exposure to *T. solium* antigens. Patients infected with other helminths, particularly other cestodes, may have circulating antibodies that cross react with antigens of *T. solium* in some assays.[34,38] However, immunoblotting techniques with the purified glycoprotein fraction of cyst fluid appear to offer a sensitive and specific diagnosis.[32,34,38,39] The sensitivity of antibody testing tends to be high for patients with multiple cysts (94%) but substantially lower for patients with single cysts or calcified cysts (as low as 28%).[40] CT and MRI remain the most effective means of diagnosis, however, and negative serology in the presence of a characteristic scan should not exclude the diagnosis of

cysticercosis.[30] Because the diagnosis can often be challenging, guidelines to assist with diagnosing neurocysticercosis have been proposed based on radiographic appearance, immunoassays, clinical manifestations, and epidemiologic characteristics.[35,41] Newly proposed diagnostic criteria were found to have a sensitivity of 93.2% and a specificity of 81.4% for the diagnosis of neurocysticercosis in a multicenter retrospective cohort.[42] Last, basal subarachnoid cysticercosis has been associated with a higher incidence of concomitant spinal lesions; therefore spinal MRI is recommended in patients with a diagnosis of subarachnoid disease.[43]

Treatment

Although older nonrandomized studies showed mixed results, current general consensus and more recent studies now support anthelmintic and corticosteroid therapy for viable parenchymal lesions.[30,44] Two randomized controlled trials showed benefit of anthelmintic therapy, with reductions in generalized seizures and faster resolution of cysts in one study and faster resolution of active cysts in the other study.[45,46] Despite prior concerning case reports describing increased adverse neurologic events with anthelmintic therapy, neither study showed significant increases in adverse effects with antiparasitic therapy, apart from a slight increase in seizures early in treatment. In addition, the second trial confirmed previous studies that indicated treatment of purely calcified cysts did not provide benefit. Another study of treatment of single active cysts and a meta-analysis provide supporting data for the use of cysticidal agents in the treatment of parenchymal disease.[47,48]

Because, despite antiinflammatory therapy, pericystic inflammation can cause permanent damage to the eye when there is an intraocular cyst, a careful eye examination should always precede treatment to exclude cysticercosis of the eye.[44] Because pharmacologic evidence suggests that concurrent corticosteroid therapy lowers serum praziquantel levels while increasing the circulating levels of albendazole (and its active metabolites) in some patients, many experts now favor the use of albendazole as the drug of first choice when treating a patient with just one or two viable cysts.[49,50] However, albendazole monotherapy has been shown to be only 60% to 70% effective at killing parasites.[51] If repeat MRI scans show persistence of cysts at 6 months following initiation of therapy, a repeat course of drug treatment should be given.[44] Double-blind, randomized, controlled trials have shown the superiority of a combined regimen of albendazole plus praziquantel over albendazole alone at killing parasites and resolving cysts. There were no increased adverse events in these studies.[52,53]

Preferred regimens for multicystic parenchymal disease (more than two viable cysts) involve treatment with albendazole (15 mg/kg/day divided in two doses, with maximum of 1200 mg per day, for 10 days to 2 weeks)[13,44,54] plus a high-dose regimen of praziquantel (50 mg/kg/day divided in three doses per day for 10–14 days). Before, during, and after drug therapy, seizures should be controlled with appropriate antiepileptic medications, and symptomatic hydrocephalus should be relieved by shunting before initiation of anthelmintic treatment.[44] Shunt complications caused by blockage and bacterial infection are common in patients with neurocysticercosis.[55] CNS inflammation should be reduced by concurrent administration of pharmacologic doses of corticosteroid (dexamethasone, 6–8 mg/day divided into three daily doses for 28 days, then a slow tapering off of corticosteroids over 2–8 weeks to avoid rebound symptoms)[56,57]; however, corticosteroids do not necessarily eliminate the risk for serious complications such as infarction[58,59] or intracranial pressure elevation.[49] Anthelmintic treatment is contraindicated in cases of encephalitic or disseminated neurocysticercosis and for ocular disease owing to the intensity of the inflammatory response provoked by drug therapy. For subarachnoid disease, expert opinion supports the use of prolonged courses of anthelmintic agents (2–12 months) owing to variable responses and more frequent relapses than for parenchymal disease. Anthelmintic agents have no role in the treatment of calcified cysts.[54]

Formal, evidence-based consensus guidelines for neurocysticercosis management have been developed by experts from the Infectious Diseases Society of America and the American Society of Tropical Medicine and Hygiene, and these were published by both societies in 2018.[44] Nonetheless, guideline recommendations and the most recent literature should be reviewed in deciding intervention for complex individual patient presentations.

Well-controlled, randomized trials have not yet evaluated the treatment of less common forms of neurocysticercosis (i.e., intraventricular, subarachnoid/basilar, and spinal cysticercosis), and the true value of drug treatment for these forms of the disease is not known.[30] Surgical removal of intraventricular cysts is recommended, where possible, preferably via endoscopic techniques performed by experienced neurosurgeons. Preoperative anthelmintics are not advised in this case, given the fact that the inflammation induced by treatment could prevent adequate removal of the cysts. However, when surgical removal is not possible, drug treatment of intraventricular cysts may prove efficacious. Likewise, owing to the progression and degree of complications of disease with subarachnoid or sylvian fissure cysts, treatment with cysticidal medications is generally recommended, with close attention to the management of intracranial hypertension.[26] Anthelmintic treatment for extensive infections such as diffuse encephalitis is generally not recommended, given the potential adverse effects due to treatment-induced inflammation.

A poor response to either surgical or drug therapy is more common with intraventricular or cisternal cysts and with racemose neurocysticercosis.[44] For these lesions the drug levels achieved in the CSF and cyst during medical therapy are likely to be lower than for parenchymal CNS cysts, making drug failure more likely and often necessitating a longer duration of cysticidal treatment and multiple courses of treatment. Surgical approaches to these areas are difficult, and local inflammation may prevent easy removal of the cyst, although endoscopic removal of ventricular cysts has shown promising results and is recommended if possible.[26] Retained cyst material may result in postoperative recurrence. Nevertheless, successful therapy for ventricular and basilar cysts has been achieved in a small number of patients by either medical or surgical means.[60,61] Individualized therapy, possibly including a combined surgical-medical approach, is recommended in such cases.[30,54]

Spinal cysticercosis presents a challenging scenario as well, due to its sensitive location. Data are limited on the best management of spinal and perispinal disease, and cases should be approached on an individual basis, with consideration of medical or surgical therapy or both. For ocular cysticerci, surgical removal without anthelmintic treatment is recommended.

For symptomatic cysts outside the CNS, the optimal approach is surgical resection. In some cases, lesions may involve critical organs, making surgical removal technically not feasible. In such cases, medical therapy with praziquantel or albendazole may be employed.[11,30]

Echinococcosis (Hydatid and Alveolar Cyst Disease)

When humans serve as inadvertent intermediate hosts for cestodes of *Echinococcus* spp., which are carried as tapeworms by canines such as dogs, wolves, and foxes, disease may result from the development of expanding parasite cysts in visceral organs.[62] This condition, termed *echinococcosis,* has two forms: hydatid or unilocular cyst disease, caused by *Echinococcus granulosus,* and alveolar cyst disease, caused by *Echinococcus multilocularis.* In addition, polycystic or neotropical echinococcosis is caused by either *Echinococcus vogeli* or the much less common *Echinococcus oligarthrus,* both found in Central or South America. *E. vogeli* infections are similar to alveolar cyst disease, whereas *E. oligarthrus* infections appear less aggressive.[63] Sheep, goats, camels, and horses are among the usual intermediate hosts for *E. granulosus;* however, because *E. granulosus* is transmitted by domestic dogs in livestock-raising areas, hydatid disease is prevalent worldwide (in Africa, Middle East, southern Europe, Latin America, and southwestern United States). *E. multilocularis* infections (found in northern areas of Europe, Asia, and North America and in the Arctic regions)[64] and *E. vogeli* infections (found in South American highlands) are transmitted by wild canines and are less common.

Humans acquire echinococcosis by ingesting viable parasite eggs with their food.[62] The parasite eggs are distributed via local environmental contamination by the feces of tapeworm-infected canines. Eggs are partially resistant to desiccation and remain viable for many weeks,[3] allowing delayed transmission to individuals with no direct contact with vector animals. Once in the intestinal tract, the eggs hatch to form oncospheres that penetrate the mucosa and enter the circulation. Oncospheres then encyst in host viscera, developing over time to form mature larval cysts (Fig. 289.5).

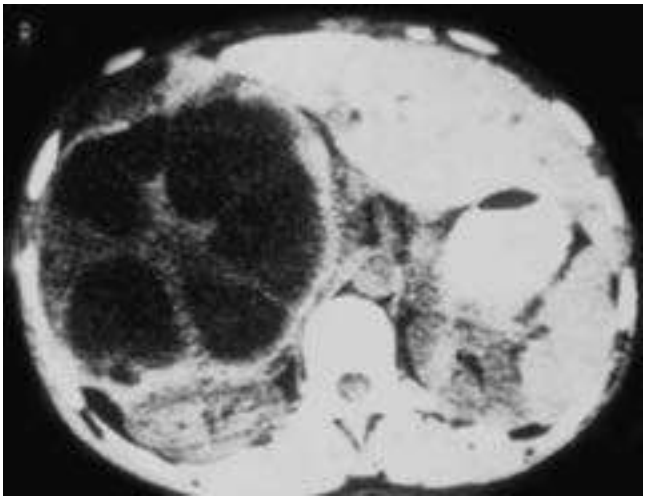

FIG. 289.5 Hydatid cysts of the liver detected on computed tomography scan. Note the well-demarcated wall and characteristic septate internal structures (daughter cysts).



FIG. 289.6 Daughter cyst formation from the germinal membrane of a hydatid cyst. *(From Ash LR, Orihel TC.* Atlas of Human Parasitology. *3rd ed. Chicago: American Society for Clinical Pathology; 1990.)*

Infection with *E. granulosus,* cystic echinococcosis, is estimated to occur in 2% to 6% of endemic populations. Risk factors include unsanitary living conditions, slaughter of livestock in close proximity to humans and dogs, and uncontrolled dog populations. Sheep raising, in particular, is associated with a high prevalence of disease.[65]

Cystic Echinococcosis

Clinical Presentation

The hydatid cysts of *E. granulosus* tend to form in the liver (50%–70% of patients) or lung (20%–30% of patients) but may be found in any organ of the body, including brain, heart, and bones (<10%). They grow to 5 to 10 cm within the first year and can survive for years or even decades. Symptoms are often absent, and infection is detected only incidentally by imaging studies in many cases. When symptoms do occur, they are usually due to the mass effect of the enlarging cyst in a confined space. Hydatid cysts contain a germinal layer that allows asexual budding to form daughter cysts within the primary cyst (Fig. 289.6). If a cyst erodes into the biliary tree or a bronchus, the cyst contents, including daughter cysts, may enter the lumen and cause obstruction or postobstructive bacterial infection. Bacteria may enter the cyst, causing pyogenic abscess formation in the cyst. Cyst leakage or rupture may be associated with a severe allergic reaction to parasite antigens; in the most extreme cases, patients may have anaphylactoid reactions after cyst rupture, including hypotension, syncope, and fever. A dangerous complication of cyst rupture is secondary seeding of daughter cysts into other areas of the body. Their subsequent enlargement may be associated with critical failure of one or more organs, which is associated with significant morbidity and mortality. Fewer than 10% of patients develop such complications, and because the infection is normally self-limited, it is likely that most infections never come to medical attention.[66] Nevertheless, symptomatic cysts should be treated, and asymptomatic cysts should be carefully observed for a number of years to avert complications of infection.

Diagnosis

Infection that is suspected based on imaging studies (ultrasound, CT, and MRI) may be confirmed by a specific enzyme-linked immunosorbent assay and Western blot serology (available in the United States through the CDC) confirming exposure to the parasite.[67,68] Serology is 80% to 100% sensitive and 88% to 96% specific for liver cyst infection but less sensitive for lung (50%–56%) or other organ (25%–56%) involvement. Additional assays continue to be developed using recombinant *Echinococcus* antigens and may provide better diagnostic sensitivity and specificity.[67–69] Eosinophilia is not a consistent or reliable finding. Imaging remains more sensitive (90% with ultrasound and higher with CT and MRI) than serodiagnostic techniques, and a characteristic scan in the presence of negative serologic results still suggests the diagnosis of echinococcosis.[66,70] The WHO has classified the typical imaging patterns on ultrasound to guide treatment decisions (Fig. 289.7). The stages range from undifferentiated cystic lesions (CL) to active (CE1 and CE2), transitional (CE3), and inactive (CE4 and CE5). Transitional cysts are divided into CE3a (detached endocyst present) and CE3b (predominantly solid with daughter vesicles).[71] A WHO working group has provided guidelines for diagnosis based on clinical and diagnostic criteria that define possible, probable, and confirmed cases.[71]

Treatment

There is no one optimal treatment for cystic echinococcosis, and no clinical trials have evaluated and compared all the different options. Expert clinical opinion from the WHO Informal Working Group on Echinococcosis recommends a stage-specific approach.[71] Optimal treatment of symptomatic, complicated cysts (large CE2 to CE3b cysts with multiple daughter vesicles) is surgical resection to remove the cyst in toto. Therapy is also indicated when cysts exert pressure on vital organs or if percutaneous therapy is not available. Traditionally, because of the risk for spreading infection due to cyst rupture, the recommended approach has been to visualize the cyst, remove a fraction of the fluid, and instill a cysticidal agent (hypertonic [30%] saline, cetrimide, or 70%–95% ethanol) to kill the germinal layer and daughter cysts before resection.[1a] The cyst is totally removed 30 minutes after instillation. A number of drains are left in the cyst bed to limit the risk for secondary bacterial infection.

Although time honored, the efficacy of this open surgical approach has not been validated in clinical trials, and given the availability of effective perioperative drug therapy to limit spread, some experts question the need to instill potentially tissue-damaging cysticidal agents during surgery.[1a] Laparoscopic surgery for cyst removal has been performed in less advanced cases (in which spillage of contents is not as likely to occur). In this type of case, the minimally invasive approach may have fewer complications with approximately equal efficacy.[70] Cysts communicating with the biliary tree should not have a cysticidal agent instilled because of the risk for postoperative sclerosing cholangitis,[62,72] and cysticidal agents should not be instilled in lung cysts.

An intermediate intervention for inoperable cysts has been developed, known as the *PAIR* (*p*uncture, *a*spiration, *i*njection, *r*easpiration) procedure.[62,73–75] It has also been recommended as first-line therapy by some experts in cases of CE1 and CE3a cysts.[71] While the patient is receiving anthelmintics to reduce the risk for cyst dissemination, the hydatid cyst may be aspirated with a thin needle under CT guidance.

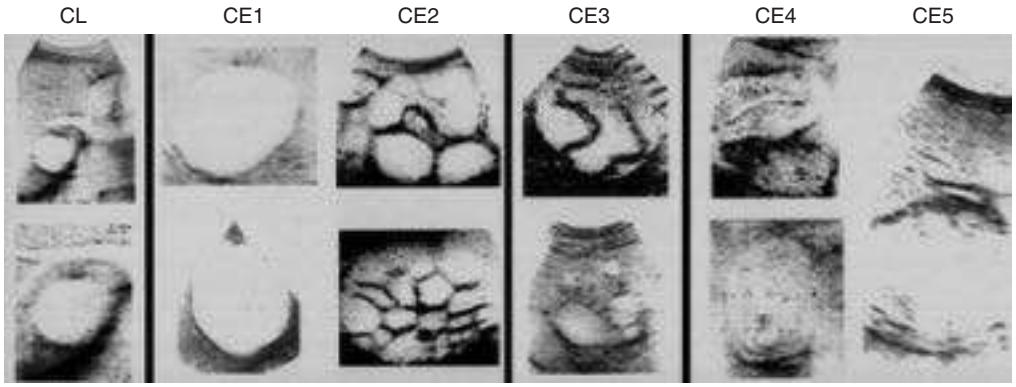

FIG. 289.7 World Health Organization Informal Working Group on Echinococcosis classification of echinococcal cystic lesions as they appear on ultrasound. Lesions are divided into three relevant groups: active (CE1 and CE2), transitional (CE3), and inactive (CE4 and CE5). CL represents an early, undifferentiated cystic lesion. *(From Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans.* Acta Trop. *2010;114:1–16.)*

Approximately 30% of the cyst fluid volume is removed. Detection of protoscolices in the cyst fluid allows confirmation of cyst viability. An equal volume of 95% ethanol or other scolicidal agent (e.g., 0.5% cetrimide) is then instilled into the cyst cavity and allowed to react for 30 minutes before removing the needle. However, to prevent postintervention sclerosis, care must be taken to ensure that the cyst does not communicate with the bile ducts before introducing cysticidal chemicals. Results indicate arrest or involution of cysts after treatment. A modified technique uses a special catheter system to simultaneously evacuate the cyst contents while infusing the scolicidal agent. The PAIR procedure has several specific indications, and the overall experience with PAIR is relatively limited compared with traditional treatment methods. However, there is some evidence, based on meta-analysis, that there are fewer complications and lower recurrence rates with PAIR compared with standard surgery. More studies are needed to confirm this finding.[70]

Most surgeons treat patients perioperatively with an anthelmintic agent active against *Echinococcus* cysts (albendazole and mebendazole)[71,76,77] to limit the risk for intraoperative dissemination of daughter cysts. Preoperative treatment with albendazole for 1 to 3 months has been shown to significantly reduce the number of viable cysts found at surgery.[63] Medical therapy for inoperable cysts with albendazole or mebendazole has provided improvement in most patients (55%–79%) and cure in a smaller percentage (29%).[76,77] Young cysts (CE1) can sometimes be treated with anthelmintics alone.[78] The preferred agent is albendazole because of its greater absorption from the gastrointestinal tract and higher plasma levels. Optimal therapy has not been adequately studied but may need to be prolonged in some cases. Typically, it is given at a dose of 400 mg twice a day (for patients weighing <60 kg, 15 mg/kg/day divided into two doses) for 3 to 6 months. It is no longer recommended to interrupt treatment for 2 weeks every month of treatment.[71] The alternative agent, mebendazole, is poorly absorbed and must be taken at higher doses (50–70 mg/kg/day) for several months to achieve a therapeutic effect.[77]

The response to drug therapy depends on the cyst size and location.[73,77] Bone cysts, which are frequently not amenable to surgery, respond less well to drug treatment than other cysts. The response to drug therapy is best monitored by serial imaging studies; cyst disappearance or shrinkage along with increasing cyst density is thought to indicate a positive response. In cases of uncomplicated CE4 and CE5 cysts, some experts advocate the "watch and wait" approach, although this has not been formally studied.[71]

Alveolar Echinococcosis

Infection with *E. multilocularis* (alveolar cyst disease) is relatively more aggressive. It is much less common in humans, with an incidence in endemic countries ranging from 0.02 to 1.4 per 100,000. However, increasing urban fox populations, with a noted increase in prevalence of parasite infection among foxes, may pose an increasing health concern for humans.[64] For example, in Europe, with the development of an effective rabies vaccine program, fox populations began to increase after the 1980s. In concert, the incidence of alveolar echinococcosis doubled from 2001 to 2005 compared with previous years.[79] *E. multilocularis* cysts reproduce asexually by lateral budding. Their gradual invasion of adjacent tissue is tumor-like, and sections of the parasite may "metastasize" to distal parts of the body.[62] Symptoms are usually of gradual onset, referring to the organ involved, which is most commonly the liver. Complications include biliary tract disease, portal hypertension, and Budd-Chiari syndrome. Consequently, morbidity and mortality are higher than for cystic (hydatid) echinococcosis. Findings of initial imaging studies are usually highly suggestive of carcinoma or sarcoma, and biopsy may provide the first indication of infection. A serologic test, available from the CDC, is highly sensitive and specific and, when combined with characteristic imaging studies, offers an alternative means of establishing the diagnosis.[67,78] The WHO Informal Working Group on Echinococcosis developed a classification similar to cancer staging using the acronym *PMN*: *P* for location of parasite in the liver; *N* for neighboring organs involved, and *M* for metastatic spread.[78] For operable cases, wide surgical resection (e.g., hepatic lobectomy or liver transplantation) is recommended to ensure total removal of the cyst.[71,80] Adjuvant albendazole therapy to reduce cyst size before surgery or limit intraoperative spread has been reported to be beneficial in case series.[81] For inoperable cases, drug therapy with mebendazole or albendazole (lifelong) has provided arrest or cure of disease in some patients.[71] The efficacy of surgical or drug therapy may be monitored by serial imaging and serology.

Advances in the understanding of the molecular biology of *Echinococcus* have led to active investigation of new targets and new drugs for therapy.[78]

Other Invasive Cestodes

Human tissue infection with plerocercoid cysts of several cestode species is referred to collectively as *sparganosis.* These parasites, such as *D. latum,* pass through several developmental stages in copepods and vertebrates.[1,1a,1b] The definitive hosts for tapeworms of these species are usually canines or felines. Humans acquire inadvertent parasite infection by ingestion of copepods (in water) or by consumption of or prolonged exposure to uncooked meat of plerocercoid-infected animals.[82–84]

Sparganosis has been reported in South America, Japan, China, and other areas of Asia in association with traditional use of frog-meat or snake-meat poultices. Infection has rarely been reported from Europe and North America. Sparganosis may be the proliferating or nonproliferating type. Infection acquired in the United States is usually due to *S. mansonoides,* which is nonproliferating. In other areas of the world, proliferating forms are more common. These forms branch by lateral division and may detach to spread to other, distal areas of the body.[82] Clinical presentation typically involves local inflammation at the site of invasion (skin and eye are the most common sites for poultice application). Cerebral sparganosis is a rare and severe complication. There is local lymphocytic and eosinophilic inflammation surrounding the parasites. Tissue injury may be particularly severe in the eye. Diagnosis is usually by biopsy, although serologic testing has been

used in some areas.[2] Treatment is by injection with ethanol, surgical resection, or both. Medical therapy with various anthelmintics has not produced a beneficial effect. *Coenurosis* is a human cyst infection with the cestodes *T. multiceps, Taenia crassiceps,* and *Taenia serialis,* which cause tapeworms in dogs.[85] The cysts are unilocular, with multiple protoscolices, but do not contain daughter cysts. Symptomatic disease is usually associated with involvement of the eye or the CNS. Clinically the cysts may be difficult to distinguish from cysticercosis or hydatid disease. Basal arachnoiditis and hydrocephalus are common. There is no reliable serologic test. Surgical resection is the recommended therapy.[85]

PREVENTION

Prevention of cestode infection depends on interrupting the parasite life cycle. Transmission of human tapeworm infection can be reduced or eliminated by the following sanitary measures: (1) careful disposal of human sewage to limit environmental spread of parasite eggs; (2) limitation of forage areas and use of safe feed for vector animals such as cattle or swine that serve as common intermediate hosts; (3) meat inspection before marketing to exclude meat from cyst-infested carcasses; and (4) prolonged freezing (at <–18°C) or thorough cooking of meat (at >50°C), or both, to kill any cysts in the tissues. Control of fish tapeworm is more difficult to achieve because the infected fish can range freely and there are nonhuman reservoirs for the tapeworm (e.g., bear and seals) that can continue to infect fish despite the presence of good human sanitation.

Prevention of invasive cestode infection is more complex. Because this form of human infection results from egg ingestion and the eggs may have been spread throughout an area by free-ranging definitive hosts such as dogs or humans, infection may be difficult to avoid. It is significant that half of patients with hydatid cysts do not recall specific exposure to dogs, although they may have resided in or visited an area of endemicity. Successful control of *E. granulosus* transmission has been achieved by regular screening and treatment of dogs in areas of endemicity in New Zealand, Tasmania, and the British Isles to eliminate adult tapeworm carriage and local release of eggs.[86] Similar programs to treat rural dogs and foxes have been proposed for control of *E. multilocularis* transmission.[87] Treatment of human carriers in areas in which *T. solium* is endemic, as well as health education campaigns focusing on self-recognition of human tapeworm carriers and the corralling of pigs, has proved effective in controlling transmission of cysticercosis.[32,88,89] However, many barriers, particularly involving socioeconomic factors, still exist. Field trials also indicate that anti-*Echinococcus* and anticysticercus vaccines can significantly reduce infection in farm animals (sheep and pigs), which may further reduce the level of peridomestic transmission.[90]

In areas of good sanitation (e.g., the United States), autochthonous transmission of cysticercosis is rare but can occur in settings in which a person with a *T. solium* tapeworm shares living or cooking quarters with susceptible individuals.[85] Screening of immigrants from *T. solium*–endemic areas and treatment of identified tapeworm infections would eliminate the risk for cysticercosis transmission to nontravelers.

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290 Visceral Larva Migrans and Other Uncommon Helminth Infections

Theodore E. Nash[a]

SHORT VIEW SUMMARY

VISCERAL LARVA MIGRANS

Definition

- Visceral larva migrans (toxocariasis) is caused by *Toxocara canis* and less frequently by *Toxocara cati* and other helminths. It commonly occurs in children and may be manifested by fever, wheezing, hepatomegaly, and other generalized symptoms.

Diagnosis

- Diagnosis is based on finding larvae in affected tissues and the presence of eosinophilia.

Treatment

- Most patients recover without therapy.
- Antiinflammatory drugs may be considered, and albendazole, mebendazole, and diethylcarbamazine have been tried but are of uncertain efficacy.

OCULAR LARVA MIGRANS

Definition

- Ocular larva migrans is caused by *T. canis* larvae in the eye and results in a chorioretinal granuloma or occasionally in panuveitis. Retinal detachment may also occur. There is no specific therapy.

OTHER UNCOMMON HELMINTHS

- Other helminthic infections are anisakiasis, cutaneous larva migrans, eosinophilic meningitis, gnathostomiasis, angiostrongyliasis, eosinophilic gastroenteritis, dirofilariasis, capillariasis, nanophyetiasis, and swimmer's itch.

Most helminths that infect humans are relatively host specific to humans, undergo characteristic migration and development, and are found in typical anatomic locations. However, these helminths sometimes undergo atypical or aborted migrations and cause symptoms or signs because of their unusual or ectopic location. A good example of this is the deposition of schistosomal ova and the subsequent granulomatous inflammatory lesions in the spinal cord or brain. In addition, some helminths of animals can also infect humans. Examples are *Echinococcus granulosus* and *Trichinella spiralis,* which commonly infect humans, migrate and develop normally, and reside in locations similar to those in the animal host. In contrast, other helminths of animals are unable to develop or migrate normally. Commonly, they undergo prolonged aberrant migrations or locate abnormally in the tissues as underdeveloped larvae and incite an eosinophilic inflammation that is responsible for many of the symptoms and signs of these infections. Although a large number of animal parasites may infect humans, most do so rarely. In contrast, some helminths of animals infect humans more commonly and cause distinctive clinical syndromes (Table 290.1), sometimes associated with characteristic epidemiology, exposure history, and geographic locations. More often than not, similar clinical syndromes are caused by a group of related parasites. The diagnosis is suggested on clinical and epidemiologic grounds. Although pathologic examination of tissue can sometimes establish the diagnosis, the detection of larvae is commonly unrewarding. Serologic tests are sometimes helpful (see "Visceral Larva Migrans [Toxocariasis]") but usually are not fully evaluated, are experimental, or are unavailable.

The diagnostic procedures used to detect infections differ for each parasite, so a clear idea of the potential causes is essential. The physician must understand the sensitivity of the diagnostic procedures and the abilities of the laboratory personnel performing them.

VISCERAL LARVA MIGRANS (TOXOCARIASIS)

Visceral larva migrans (VLM) is a syndrome characterized in its most florid state by eosinophilia, fever, and hepatomegaly. It is caused primarily by infection with *Toxocara canis* but also, less frequently, infection with *Toxocara cati* and other helminths.[1,2,3]

Life Cycle in the Dog

T. canis infects dogs and related mammals by a number of mechanisms.[1] Most commonly, ingested eggs hatch in the small intestine, and the resulting larvae migrate to the liver, lung, and eventually the mucus layer of trachea. They are then swallowed and mature in the lumen of the small intestine, where eggs are shed. Other larvae migrate to and remain dormant in the muscles but are capable of development years after the primary infection, particularly in pregnant bitches. During pregnancy, larvae again develop and infect the puppies transplacentally and transmammarily. Infective larvae are commonly found in the feces of the puppies. Eggs are not infectious when passed in the feces and take 3 to 4 weeks to develop. They are hardy and often remain viable for months. Large numbers of viable eggs contaminate the environment because of the high prevalence of infection in dogs and the ability of eggs to survive relatively harsh environmental conditions. Humans become infected mostly from ingesting viable ova that contaminate the soil where dogs defecate. Ingestion of raw organs containing larvae, which is common in some regions, is another means of infection.[4]

Infection in Humans

Prevalence

Toxocariasis is prevalent wherever dogs or cats are found, and *Toxocara* eggs are able to survive. The prevalence of infection or disease in humans is not known, but seroepidemiologic studies show wide differences in prevalence, depending on the population tested. In the United States, seropositivity ranged from 2.8% in an unselected population,[5] to 23.1% in a kindergarten population in the southern United States,[6] to 54% in a selected rural community.[7] None of the seropositive persons had recognizable disease. A review of reported seroprevalence of toxocariasis in North America reported an overall prevalence rate of 13.9% in the United States, with higher rates in Mexico.[8]

Clinical Manifestations

VLM occurs most commonly in children younger than 6 years of age, frequently after ingestion of contaminated soil.[3,5] Disease manifestations vary

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TABLE 290.1 Clinical Syndromes Associated With Unusual Helminth Infections in Humans

CLINICAL SYNDROME	PARASITE	USUAL HOST
Visceral larva migrans	*Toxocara canis* *Toxocara cati* *Baylisascaris procyonis*	Canines Felines Raccoons
Eosinophilic gastroenteritis	*Anisakis* spp. *Phocanema* spp. *Ancylostoma caninum*	Sea mammals Sea mammals Canines
Cutaneous larva migrans	*Ancylostoma braziliense* *Ancylostoma caninum* *Uncinaria stenocephala*	Canines, felines Canines, felines Canines, felines
Eosinophilic meningitis	*Angiostrongylus cantonensis* *Gnathostoma spinigerum*	Rats Felines, other mammals
Pulmonary or cutaneous nodules	*Dirofilaria* spp.	Canines, other mammals
Abdominal angiostrongyliasis	*Angiostrongylus costaricensis*	Cotton rats
Capillariasis	*Capillaria philippinensis*	Birds
Swimmer's itch	*Trichobilharzia* spp.	Birds

and range from asymptomatic infection to fulminant disease and death, but it is increasingly appreciated that most infections are asymptomatic. Individuals who come to medical attention most commonly present with cough, fever, wheezing, and other generalized symptoms.[6,7,9] The liver is the organ most frequently involved, and hepatomegaly is a common finding, although almost any organ can be affected. Splenomegaly occurs in a small number of patients, and lymphadenopathy has been noted. Lung involvement with radiologic findings has been documented in 32% to 44% of patients, but respiratory distress occurs rarely. Skin lesions such as urticaria and nodules have also been described. Seizures have been noted to occur with increased frequency in VLM, but severe neurologic involvement is infrequent.[10] Eye involvement in VLM is unusual but has been documented (see "Ocular Larva Migrans"). Eosinophilia, usually accompanied by leukocytosis, is the hallmark of VLM. Laboratory findings include hypergammaglobulinemia and elevated isohemagglutinin titers to A and B blood group antigens, which are caused by the host's immune response to cross-reacting antigens on the surface of *T. canis* or *T. cati* larvae. Serologically positive populations may be at increased risk for seizures.[11]

Diagnosis

The diagnosis of VLM is usually suggested clinically by the presence of eosinophilia, leukocytosis, or both in a young child also presenting with hepatomegaly or signs and symptoms of other organ involvement. A history of pica and exposure to puppies is common. In the United States, patients are more commonly black and from rural areas.

The diagnosis is definitively confirmed by finding larvae in the affected tissues by histologic examination or by digestion of tissue; however, larvae are frequently not found. Computed tomography scans have demonstrated ill-defined hypodense round lesions in the liver of patients with VLM, the basis of which is unclear.[4] Standard assays including enzyme-linked immunosorbent assay or Western blot using excretory-secretory products of T. canis larvae have been used to confirm the clinical diagnosis for decades.[12] Newer assays using recombinant antigens in a Luminex Bead format have obviated the need for collection of infectious larvae and have led to assays with high sensitivity and less cross-reactivity with other helminths. *Toxocara* antibody titers in populations without clinically apparent VLM vary dramatically, and elevated titers cannot definitively establish the diagnosis.

Differential Diagnosis

Eosinophilia, fever, and hepatomegaly are frequently caused by helminths that migrate through the body. *Baylisascaris procyonis* (an ascarid of raccoons) is a recognized cause of larval migrans in the United States.[13] Other helminth infections include acute schistosomiasis, *Fasciola hepatica* infections, *Ascaris lumbricoides* abscess of the liver, acute liver fluke infections *(Clonorchis sinensis, Opisthorchis viverrini, Fasciola hepatica)*, complications from *Echinococcus* infection of the liver, and *Capillaria hepatica*. Diseases not caused by parasitic infections should also be considered. Children with mild disease may manifest only eosinophilia.

Treatment and Management

Most patients recover without specific therapy. Treatment with antiinflammatory or anthelmintic drugs may be considered for patients with severe complications usually caused by involvement of the brain, lungs, or heart. There is no proven effective therapy, although albendazole, mebendazole, diethylcarbamazine, and other anthelmintics have been used. Injury to the parasite may provoke an intense inflammatory response, leading to worsening of the clinical picture. Corticosteroids are frequently used to control symptoms of infection or to suppress possible potentiation of symptoms caused by the host's immune responses to killed or injured larvae as a consequence of anthelminthic treatment.

Prevention

VLM can be easily prevented by a number of simple but effective measures that prevent *T. canis* or *T. cati* eggs from contaminating the environment and children from ingesting eggs and pica. Dogs, particularly puppies, should be periodically tested and treated for *Toxocara* and other worms. To prevent defecation by dogs and cats in sandboxes where young children play, they should be covered when not in use.

OCULAR LARVA MIGRANS

Ocular larva migrans is caused primarily by an infection of the eye with *T. canis* larvae.[14,15] Although a present or past history of clinically recognized VLM has occasionally been noted, almost all patients present with unilateral eye visual loss without a past history or present systemic symptoms or signs. Presumably, a larva becomes entrapped in the eye by chance, resulting in an eosinophilic inflammatory mass. Children are most commonly affected and, on average, are older (mean, 8.6 years old in one study) than children with VLM. Although the most common lesion is a chorioretinal granuloma in the posterior pole, or occasionally more peripherally, diffuse panuveitis may also be seen. Retinal detachment may occur. This entity was first recognized in the examination of eyes enucleated for the treatment of presumed retinoblastoma, and it remains the most important distinction that ophthalmologists must make in children with subretinal lesions.[14]

Eosinophilia, hepatomegaly, and other signs and symptoms of VLM are usually lacking. The diagnosis is established clinically. Although the serum titers to *Toxocara* larvae are higher than those of a control population,[16] many patients with ocular larva migrans have low or negative titers. However, elevated vitreous[17] and aqueous fluid titers[18] to *Toxocara* larvae, compared with serum levels, appear useful for establishing the diagnosis. There is no specific therapy.

A characteristic clinically recognizable syndrome, diffuse unilateral subacute neuroretinitis is caused by infection with helminth larvae of *B. procyonis* and *Toxocara* spp.[19] and other unidentified nematodes.[20,21] *Angiostrongylus cantonensis* larvae can also involve the eye.[22] A motile larva is commonly found in or below the retina. Photocoagulation is curative. Anthelmintic therapy such as albendazole may be effective.

BAYLISASCARIASIS

B. procyonis, an ascarid of raccoons, is a recognized cause of VLM in humans and many other animals.[23,24,25] The life cycle is similar to that of dog and cat ascarids, and infection occurs after ingestion of ova excreted in raccoon feces that subsequently contaminate soil and the environment. Although the clinical manifestations are similar to those caused by dog and cat ascarids, severe and commonly fatal eosinophilic meningoencephalitis occurs in more than half the cases. Eye involvement is common and is one of the known causes of diffuse unilateral subacute neuroretinitis.[19,20] The diagnosis is established by detecting typical larvae in tissues; an experimental serologic examination is useful.[26] There is no proven therapy. Of the available drugs, albendazole and corticosteroids are most commonly tried.[20]

ANISAKIASIS

Anisakiasis is caused by infection of humans by larvae found in saltwater fish and squid. The definitive hosts are marine mammals. The clinical syndrome is caused by penetration of larvae into the stomach or small intestine. It is characterized by upper or lower abdominal symptoms or both. The diagnosis is suggested by a history of ingesting raw, salted, pickled, smoked, or poorly cooked fish.[27]

Life Cycle in Marine Mammals

Larvae of the family Anisakidae, including *Anisakis, Pseudoterranova,* and occasionally other genera, can accidentally infect humans.[1,28,29,30] The adults are found in the stomach of marine mammals. The eggs, passed in the feces, hatch as free-swimming larvae, are ingested by certain crustaceans, and are eaten by fish and squid. When ingested by appropriate marine mammals such as dolphins, seals, and whales, the larvae burrow head first into the stomach. When consumed by humans, the larvae attempt, many times successfully, to burrow into the stomach or intestine, resulting in typical symptoms.

Clinical Syndrome

Anisakiasis occurs after ingestion of raw or improperly cooked marine fish. The disease, initially recognized in the Netherlands after the ingestion of raw herring, is most frequently reported from Japan, where raw fish is commonly eaten. In the United States, infection is still uncommon but is now more frequently recognized because of increased ingestion of raw fish, particularly Pacific salmon. Cod, halibut, pollack, greenling, herring, anchovies, hake, tuna, sardines, and mackerel are other fish that have been implicated.

Clinical manifestations are caused by penetration of worms into the gastrointestinal tract, usually the stomach but also the lower small intestine, most commonly the ileum.[29,30,31] Occasionally, throat irritation is followed by coughing up the characteristic worm. Initial invasion is associated with acute symptoms, whereas the presence of worms for longer periods causes chronic symptoms. The symptoms and the location of the worms depend on the genus, with *Pseudoterranova* commonly associated with infection of the stomach and *Anisakis* associated with infection of the intestine. Symptoms usually occur within 48 hours after ingestion, but this pattern is variable. With gastric anisakiasis, patients complain of intense abdominal pain, nausea, and vomiting. Small intestinal involvement results in lower abdominal pain and signs of obstruction mimicking those of appendicitis. The incubation period is shorter with gastric involvement, in one series peaking at 6 hours usually without significant eosinophilia. In contrast, small bowel infection symptoms peaked at 48 hours and more commonly caused eosinophilia.[31] Symptoms may be chronic, sometimes lasting for months and, rarely, years. These symptoms are associated with intestinal masses containing the parasite and are sometimes confused with a tumor, regional enteritis, or diverticulitis. Worms are occasionally located ectopically outside the gastrointestinal tract. *Anisakis* larvae in seafood have been implicated as a cause of acute allergic manifestations such as urticaria, pruritus, angioedema, and anaphylaxis, with or without accompanying abdominal gastrointestinal symptoms in patients who ingest raw fish.[32,33,34,35,36] In vitro studies and skin tests indicate that sensitization to *Anisakis* antigen is common in this population, whereas sensitization to fish is uncommon.[30,34,35,36]

Laboratory Findings

Eosinophilia is usually absent in patients with gastric anisakiasis[31] but is usually present in small bowel involvement. Leukocytosis is not consistently present with acute anisakiasis but has been noted in almost two-thirds of patients with intestinal involvement in one series.

Diagnosis

Anisakiasis should be considered in anyone with a history of ingesting raw marine fish and suggestive abdominal symptoms. A definitive diagnosis can be established by endoscopy, radiographic studies, or pathologic examination of tissue. In the upper gastrointestinal tract, worms are found partially embedded in any area of the stomach and may be associated with localized mucosal edema, erosions, or mass lesions.[37,38] Upper gastrointestinal radiographic studies may reveal the outline of a worm associated with mucosal edema or tumor formation. Removing the worm during endoscopy definitively establishes the diagnosis and is curative.[31] Intestinal anisakiasis is diagnosed clinically and suggested radiologically.[31,37] Varied degrees of thickening of the walls and narrowing of the lumen of the ileum or jejunum are found on radiographic studies. High-resolution ultrasonography has demonstrated small intestinal wall thickening and localized ascites around the involved section of bowel. Examination of aspirated ascites has revealed a preponderance of eosinophils.[39] Lesions resolve within 2 to 3 weeks. Removal of the intestinal mass is occasionally required to establish the diagnosis and effectively treat the patient. Most, but not all, resolve spontaneously with conservative management and close observation.[31] Tissues show inflammatory masses, many eosinophils, and the characteristic helminth. Serologic tests are not generally available but may be useful, particularly in patients whose symptoms have lasted longer than 1 week.[40]

Treatment

Symptoms diminish spontaneously in most patients without specific therapy, although the process is hastened by removing worms lodged in the stomach during endoscopy. In one series of intestinal anisakiasis, all 12 patients became asymptomatic by 2 weeks.[37] A report of treatment with albendazole in a case diagnosed only by serology is, at best, suggestive of efficacy.[41] One Japanese investigator commonly prescribed antacids after removing the stomach worms.

Prevention

Larvae resist heating up to 50°C (122°F) as well as pickling, salting, and some methods of smoking. Infection can be prevented by cooking or freezing fish for 24 hours before ingestion.

Cutaneous Larva Migrans (Creeping Eruption)

Cutaneous larva migrans is characterized as serpiginous, reddened, elevated, pruritic skin lesions usually caused by the dog or cat hookworms *Ancylostoma braziliense, Ancylostoma caninum,* or *Uncinaria stenocephala*.[42] Other animal hookworms (including *Bunostomum phlebotomum*), the human hookworms *Strongyloides stercoralis* and *Gnathostoma spinigerum,* and, rarely, insect larvae can cause a similar picture. Patients may also present with folliculitis consisting of follicular papules and pustules in the company of serpiginous tracks.

Similar to human hookworms, *A. braziliense* larvae infect dogs and cats by burrowing through the skin. The adults reside in the intestine and shed eggs, which undergo development into infectious larvae outside the body in places protected from desiccation and temperature extremes, such as sandy, shady areas around beaches or under houses. Infections are most common in warm climates, such as the southeastern United States, and occur in children more commonly than in adults. Larvae penetrate the skin, causing tingling followed by itching; vesicle formation; and typically raised, reddened, serpiginous tracks that mark the route of the parasite.[1,43] With severe infections, individuals may have hundreds of tracks. Little further development of the parasite occurs. Usually there are few, if any, systemic symptoms, although some reports have documented lung infiltrates and, rarely, severe lung dysfunction and recovery of parasites in the sputum. Eosinophilia has been noted with some infections. The skin lesions are readily recognized, and the diagnosis is made clinically. Biopsy specimens usually show an eosinophilic inflammatory infiltrate, but the migrating parasite is usually not identified. Therefore biopsies are usually not indicated to establish the diagnosis.

Without treatment, skin lesions gradually disappear[44] but commonly cause severe itching. Ivermectin given once or twice, if needed, at 200 μg/kg orally is the treatment of choice.[45] Albendazole, 400 to 800 mg/day orally for 3 to 5 days in two divided doses with food, is an effective alternative treatment.[46,47]

EOSINOPHILIC MENINGITIS

Infection of humans with larvae of *A. cantonensis,* the rat lung worm, is characterized by invasion of the brain leading to signs and symptoms of meningitis and encephalitis associated with an eosinophilic pleocytosis in the cerebrospinal fluid (CSF) and peripheral eosinophilia. A number

of other helminths cause eosinophilic meningitis; the most common are *Gnathostoma* spp. and *Taenia solium*. The adults of *A. cantonensis* reside in the lungs of rats[1,22] and produce eggs that hatch into larvae in the lungs. These are swallowed, expelled in the feces, and then either invade or are ingested by susceptible slugs, land snails, or a planarian in which they develop into infectious third-stage larvae. The range of animals carrying infectious larvae is substantially increased because infectious larvae can be transferred unchanged to other animals such as freshwater prawns, land and coconut crabs, and frogs through predation (paretenic hosts). After ingestion by rats, the larvae migrate to the pulmonary arteries and then the lung, enter the blood vessels, and thereby migrate to the brain. Eventually they return to the lungs via the vasculature. In humans, the ingested larvae attempt to recapitulate the migration in the rat and migrate to the brain, leading to eosinophilic meningitis or encephalitis.

Epidemics and sporadic infections occur most commonly in the South Pacific,[48] Southeast Asia,[49] and Taiwan[50] but more recently have been recognized in an increasing number of regions including, but not limited to, Jamaica,[51] Cuba,[52] Egypt,[53] Hawaii, China, Brazil, Ecuador, and the United States.[54,55] The most commonly recognized sources of human infection are raw or undercooked snails, prawns, and crabs. Foods such as leafy vegetables may be contaminated by larvae deposited by slugs or snails. Caesar salad was implicated in one epidemic.[51]

Clinical manifestations vary, and although fatalities occur, particularly with massive infections, most patients have a relatively uncomplicated course.[22,48,49,50,56] During one well-characterized epidemic, the incubation period ranged from 1 to 6 days after ingestion of infected snails.[48] Symptoms include headache, stiff neck, fever, rash, pruritus, abdominal pain, constitutional complaints, nausea, and vomiting. Neurologic involvement varies from no complaint to paresthesias and pain, weakness, various focal neurologic findings (sixth and fourth cranial nerve palsies are frequently noted), coma, and death. Eye involvement has been documented uncommonly and is important to rule out.[22] In general, patients do not appear to be as ill as patients who have bacterial meningitis. Signs of meningitis are frequent but nonspecific. CSF leukocytosis with more than 10% eosinophils is frequent. CSF glucose levels are usually normal, but depressed values have been noted.

The diagnosis is suggested by the clinical presentation, presence of increased eosinophils in the CSF, appropriate travel history, and a history of ingestion of raw or partially cooked implicated foods. PCR detected parasite DNA in randomly collected CSF samples of 78% of patients with positive serology.[57] A number of serologic tests for antibody in serum or CSF appear to be useful in later collected serum but are likely of limited value at presentation.[57] In severe cases, magnetic resonance imaging (MRI) shows meningeal enhancement, tracts in the brain or spinal cord, increased abnormal subcortical and periventricular T2-weighted MRI signals, and enhancing subcortical lesions.[58,59] A heavy worm burden increases the probability of brain involvement.

Symptomatic therapy includes corticosteroids, which have been shown to decrease the duration of headaches in a randomized trial.[60] Evidence for the efficacy of albendazole is controversial.[61–63] Repeated CSF lumbar punctures appear to be helpful for treating associated headaches, presumably by decreasing CSF pressure. Recovery usually occurs within 2 months, although prolonged symptoms and signs are occasionally noted.

GNATHOSTOMIASIS

A characteristic syndrome of intermittent, nonpitting edematous swellings of subcutaneous tissues associated with eosinophilia is caused by migration throughout the body of larvae from a number of species of helminths of the genus *Gnathostoma* (most commonly, *G. spinigerum* in Southeast Asia and *G. binucelatum* in Latin America).[64,65,66] Although cutaneous symptoms are frequent, any organ may be involved, and the most serious manifestations involve migration of larvae into the spinal cord and brain. Most of these infections occur in Southeast Asia, but large numbers have been recognized in Mexico.[67] Endogenous human infections have also been documented in Spain, Peru, Ecuador, India, China, Japan, Bangladesh, Botswana, Zambia, the United States, and other regions.

Life Cycle

The adult worms reside in tumorous burrows in the stomachs of a large number of mammals including cats of various types, dogs, opossums, and raccoons.[1,68] Eggs are shed in the feces, hatch after about 1 week, and are ingested by small crustaceans called *Cyclops*. These crustaceans are subsequently ingested by a variety of other animals, including fish, frogs, and snakes, where they encyst in the muscles as infectious larvae. When eaten by the appropriate definitive host, larvae migrate through the body and eventually invade the stomach, where they mature, mate, and release eggs in the feces. The spectrum of animals that spread infections to humans has broadened considerably because infectious larvae can be passed unaltered from animal to animal after ingestion (paratenic carriage). Most infections occur after eating undercooked freshwater fish, chicken, or pork. However, infectious larvae can also burrow through the skin, or infections may occur after ingestion of *Cyclops* in contaminated water. Rarely, prenatal transmission has been documented.

Clinical Manifestations

Acute signs and symptoms such as nausea, vomiting, gastrointestinal pain, and fever may occur shortly after ingestion and are most likely caused by the initial invasion of the infecting larva into and out of the intestines.[66,69] The most prominent signs, which occur 3 to 4 weeks after ingestion, are intermittent migratory subcutaneous swellings. They may occur anywhere. They are usually nonpitting, often erythematous, and occasionally pruritic and painful. They may also occur as nodules or abscesses or resemble classic cutaneous larva migrans. Eosinophilia is usually present and may be extreme. Migrating larvae may invade any tissue and give rise to symptoms related to specific organs such as the eye, intestines, spinal cord, and brain; involvement of the brain is commonly heralded by radicular nerve signs and symptoms as the worm gains entrance into the central nervous system. Brain involvement results in the most serious complication, eosinophilic encephalomyelitis.[64,65] Although gnathostomiasis is a less frequent cause of encephalomyelitis compared with *A. cantonensis,* it tends to result in permanent neurologic deficits and death because there is more invasion of the brain substance, commonly reflected by hemorrhagic lesions or tracts on computed tomography and MRI.[70] Consistent with this, the CSF has an increased number of red blood cells.

Diagnosis

Although gnathostomiasis was previously a rare diagnosis in nonendemic regions, it is more frequently recognized because of travel and ingestion of undercooked freshwater fishes, as well as by increased recognition of endogenous infections. In some areas of Southeast Asia, gnathostomiasis is a relatively common illness. The diagnosis is suggested when there is a history of intermittent subcutaneous swelling in the presence of eosinophilia and a history of ingesting raw fish or other implicated foods from endemic areas. A positive serology establishes the diagnosis in most cases but is not readily available in nonendemic regions. Usefulness between different geographic regions where infecting species differ has not been proven with all antibody tests.[71] Occasionally, the worms can be isolated from the migratory swellings or from lesions that develop after treatment, but attempts at parasite recovery are mostly unsuccessful.

Differential Diagnosis

The syndrome is relatively distinctive. *Loa loa* may manifest with Calabar swellings and eosinophilia, but the epidemiology is distinctive, and the swellings are not erythematous and do not resemble larval migrans. Cutaneous larval migrans is usually not accompanied by eosinophilia, and there is a distinctive epidemiology. Strongyloidiasis is not commonly associated with swellings and eosinophilia and can be distinguished by a serologic test or stool examination positive for *Strongyloides*.

Treatment

Both ivermectin, 200 μg/kg for two doses, and albendazole, 800 mg/day in two divided doses for 21 days, have resulted in high cure rates, but a high number of relapses over the long-term have been reported.[72,73]

Prevention

Avoiding uncooked or pickled implicated foods prevents infection in most individuals.

ABDOMINAL ANGIOSTRONGYLIASIS

Clinical manifestations of human infections of *Angiostrongylus costaricensis* are caused by penetration and development of the parasite in the lower small bowel and adjacent colon. They are characterized by abdominal pain, vomiting, and a right lower quadrant mass.[74] In the normal host, the rat, adult parasites reside in the arteries and arterioles of the ileocecal area of the intestine. Eggs deposited in the tissue hatch, and the larvae migrate through the intestinal wall into the lumen and are excreted in the feces. Larvae are then ingested by the intermediate host (the slug) and, after further development, are infectious for rats. After ingestion by rats, the larvae most likely undergo a systemic migration, mature in the intestinal lymphatics, and penetrate the arterioles and arteries in the ileocecal area, where they reside as adults. In humans, the parasite follows a similar pattern of migration except that eggs are retained in the tissues, and larvae do not appear in the feces. Adult parasites are found most commonly in the arteries and arterioles around the ileocecum and deposit eggs there. Both the eggs and the worms provoke an inflammatory response, which results in occluded vessels; an accompanying vasculitis; and an eosinophilic, granulomatous, edematous mass.

Infection of humans, most commonly children, has been recognized in Central and South America, occasionally the Caribbean, and rarely Africa and other areas. The manner of human infection is not usually known, but it may occur after accidental ingestion of infected slugs or of foods contaminated with larvae deposited in the mucous slime trail of slugs. Mint was implicated in one small epidemic.[75]

Patients are mildly to moderately ill and complain of abdominal pain and tenderness, vomiting, and fever; a right lower quadrant mass is noted in about 50% of cases.[76] Surgery reveals that the cecum, ascending colon, ileum, and appendix are involved to varied degrees. The syndrome resembles appendicitis except for the usual presence of eosinophilia and leukocytosis in angiostrongyliasis. Perforation occurs uncommonly, and the worms may be found ectopically in extraintestinal sites.[76]

The diagnosis is suspected clinically and confirmed by examination of biopsy or excised specimens. Radiographic findings are nonspecific and show filling defects and spasticity of the ileum, cecum, or colon. Relatively sensitive and specific serologic tests have been described, but they are not widely available.[77]

Most patients undergo laparotomy with removal of the inflamed areas; the natural history of infected children is unclear. It is not known if specific anthelmintic therapy is effective. An alternative treatment is mebendazole, 200 to 400 mg twice a day for 10 days.

Massive *Ascaris* infections may manifest with intestinal masses and may be confused with infections caused by *A. costaricensis*. Infections can be prevented by treating potentially contaminated vegetables with 1.5% bleach at room temperature for 15 minutes.[78]

EOSINOPHILIC GASTROENTERITIS

One cause of eosinophilic gastroenteritis is infection of humans with *Ancylostoma caninum,* a hookworm of dogs.[79,80,81] This syndrome is apparently limited to Northern Australia, although the conditions for human infection are likely present in many regions. After invading the skin, the usually single larva migrates to the ileum or to the colon to a lesser degree, where there is an intense focal response to the worm, including ulceration, inflammatory nodules, inflammation, thickening, and stricture formation. Gastrointestinal pain, nausea, vomiting, diarrhea, and bowel obstruction are common and are almost always accompanied by eosinophilia and leukocytosis. Ova are not produced, but the adult worm can sometimes be seen by endoscopy. Patients respond to a standard course of mebendazole, 100 mg twice a day for 3 days.

DIROFILARIASIS

A number of species of *Dirofilaria,* a filarial nematode that infects animals, accidentally infect humans through the bite of mosquitoes or black flies, most commonly resulting in lung nodules or subcutaneous masses, depending on the species. Two groups of parasites of the genus *Dirofilaria* accidentally infect humans.[13,82] The clinical presentations are generally different, which reflects the final location of the adults in the usual animal host. The adult worms of *Dirofilaria immitis,* the dog heartworm and the only important parasite in the first group, reside in the right side of the heart and the right pulmonary vessels; they are most commonly located in the lungs in humans but may also occur in other areas, mostly the subcutaneous tissues.

D. immitis is transmitted by a mosquito to its most common host, the domesticated dog, and other related mammals. After development in subcutaneous tissues, the parasites migrate as young adults to the right side of the heart and the right pulmonary vessels. In humans, the immature filariae migrate similarly but do not fully develop and die, which causes a local vasculitis leading to pulmonary infarcts. Histologic examination usually reveals a dead worm in an infarct with vasculitis and with granulomatous and occasionally eosinophilic inflammation.

Most infections occur in the southeastern United States through infections and transmission to dogs and by accidental transmission to humans. Persons are asymptomatic in more than 50% of the infections and show a coin lesion on a routine chest radiograph.[83,84] Others complain of cough, chest pain, or hemoptysis, most likely caused by pulmonary infarction. In some cases, lung infiltrates are noted that resolve into nodules.[85] Eosinophilia occurs in less than 15% of cases.

The diagnosis is made with certainty only by biopsy. Although serologic tests are available, their sensitivity and specificity are not adequate to rule out other potential life-threatening conditions such as a tumor.

Adults of the second group of filariae (subgenus *Nochtiella*) reside in the subcutaneous tissues of various mammals and usually cause inflammatory subcutaneous masses in humans.[86,87] These parasites include *D. tenuis* (raccoon), *D. ursi* (bear), *D. subdermata* (porcupine), and *D. repens* (dogs and cats in Europe and Asia). Ocular, primarily conjunctival, infection caused by *D. repens* has been increasingly reported.[82] Patients present with inflammatory subcutaneous masses containing increased numbers of eosinophils. As in infections with *D. immitis,* there are few, if any, systemic symptoms, and eosinophilia is not usually present. The diagnosis is established by biopsy. However, careful inspection of the entire tissue may be needed to find the parasite. Rarely, patent infections in humans occur.[82] Other filariae of animals, such as *Brugia* spp., have also been found in lymphoid or other tissues of humans.

CAPILLARIASIS

Capillaria philippinensis inhabits the small bowel of humans, causing diarrhea and malabsorption.[88,89,90] Infections have been recognized mostly in the Philippines but also in Thailand, Taiwan, Japan, Korea, Egypt, China, Indonesia, and Iran. The life cycle is surmised as follows: water birds harbor the adult worms and excrete ova, which are ingested by freshwater fish that become infected and produce larvae. Humans and birds become infected after eating infected fish possessing infective larvae. These larvae invade the jejunum and ileum, and the resulting adults produce both eggs and larvae. In contrast to almost all helminths that infect humans, with the exception of *S. stercoralis,* the parasite multiplies in the gut. This process is termed *autoinfection* and results in an overwhelming infection. In fulminant cases, autopsies reveal a thickened, edematous small bowel with a flattened mucosa containing a mononuclear infiltrate. Numerous adults, larvae, and eggs are present in both the lumen and the mucosa. Larvae infectious for birds, humans, and other mammals develop in certain freshwater fish after ingestion of eggs. Almost all signs and symptoms are related to progressive diarrhea and malabsorption. Patients complain of borborygmi, abdominal pain, vomiting, weight loss, and malaise, resulting in wasting, abdominal distention, and edema. Fever and eosinophilia are uncommon, although eosinophilia has been noted after therapy. Laboratory examinations document the typical findings of protein-losing enteropathy; fat, mineral, and vitamin malabsorption; and electrolyte loss. The diagnosis is established by detecting the characteristic *Trichuris trichiura*–like ova or larvae in the stool. A serologic test has been developed.

In untreated patients, mortality rates of 33% have been documented, but specific anthelmintic therapy is effective and lifesaving. Therapy with mebendazole, 200 mg orally twice daily for 20 days, or albendazole, 400 mg/day orally for 10 days,[89] is effective. Relapses are treated with prolonged courses of therapy. Infection is prevented by eating only properly cooked freshwater fish.

SWIMMER'S ITCH

Swimmer's itch is also known as *schistosomal dermatitis, cercarial dermatitis,* and *clam digger's itch*. Cercariae, the infective form of a large number of blood flukes of birds such as avian schistosomes (commonly *Trichobilharzia*), those in nonhuman mammals, and less commonly human schistosomes, can cause a characteristic dermatitis in humans that is associated with penetration of the cercariae into the skin.[1] They are produced and then released by various species of mollusks, which are the intermediate hosts. The clinical manifestations in humans are almost always limited to the skin.[91] Infections are frequent in many areas of the world but are particularly common in persons exposed to the freshwater lakes of the northern United States. However, infections also occur after exposure to salt water (clam digger's itch).

Although the clinical manifestations vary after the initial exposure, symptoms are typically mild and sometimes go unnoticed.[92,93] The patient complains of itching, followed by the appearance of macules at the site of penetration of the cercariae. By 24 hours, the macules have disappeared and begin to be replaced by papules. After repeated exposures, reactions occur earlier than 24 hours after exposure and are more severe. Papules are larger and associated with erythema, itching, and edema. The symptoms subside by 4 to 7 days, but in severe cases, they may last weeks.

Infection can be controlled by ridding bathing areas of the molluscan intermediate host or the definitive host or by avoiding infected bodies of water. Treatment is symptomatic. There is no specific anthelmintic therapy.

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The complete reference list is available online at Expert Consult.

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K Ectoparasitic Diseases

291 Introduction to Ectoparasitic Diseases

James H. Diaz

Ectoparasites infest the skin and its appendages, such as the hair and sebaceous glands, and most external orifices, especially the ears, nares, and orbits. Like endoparasites, ectoparasites may be obligatory parasites, programmed to feed on human hosts to complete their life cycles, or facultative parasites, preferring to feed on nonhuman hosts, infesting humans only as accidental or dead-end hosts. Over the past 2 decades, there have been several reports of significant outbreaks of ectoparasitic diseases, principally myiasis, scabies, and tungiasis, both in indigenous populations and in travelers returning from developing nations and even exclusive tropical beach resorts.[1,2] Many common ectoparasites, such as head lice and scabies mites, are also developing increasing resistance to medical therapies, including the safest topical insecticides.[3–6] Other ectoparasites, such as the New World human botfly, *Dermatobia hominis,* and the jigger or chigoe flea, *Tunga penetrans,* are resistant to systemic and topical antiparasitics and can be treated only surgically.

Ectoparasitic diseases have reemerged as unusual, but not uncommon, infectious diseases worldwide, especially in high-risk populations. Indigenous populations of ectoparasite-endemic tropical nations often have recurrent infestations and superinfestations that can result in severe disfigurement from facial cavitary myiasis or permanent disability from tungiasis-associated autoamputations.

TAXONOMY OF ECTOPARASITES

The phylum Arthropoda is the largest phylum of the animal kingdom and includes the subphylum Crustacea and the classes Insecta and Arachnida. All the medically important ectoparasites, including fleas, flies, lice, mites, and ticks, are members of the phylum Arthropoda and have chitinous exoskeletons, segmented bodies, and jointed appendages. Fleas, flies, and lice are six-legged members of the class Insecta, which also includes the mosquitoes and true bugs (order Hemiptera). Mites, including chigger and scabies mites, and ticks are the eight-legged members of the class Arachnida, subclass Acari. The arthropod ectoparasites of medical importance are stratified by taxonomic classes and distinguishing external anatomic characteristics in Table 291.1.

EPIDEMIOLOGY OF ECTOPARASITIC DISEASES

Ectoparasitic diseases share many of the general characteristics of emerging infectious diseases. Commonly shared characteristics of ectoparasitoses and emerging infectious diseases include the following: (1) origination as zoonoses, with disease establishment dependent on arthropod vector competency; (2) introduction into new, susceptible host populations; (3) infection by endemic agents given selective advantages by changing ecologic or socioeconomic conditions; and (4) recent movement from rural to urban endemic areas, often following migrating human host populations seeking better economic opportunities.[7–9]

To assess the potential combined impact of increasing international travel and the relaxation of quarantine regulations for imported animals in the United Kingdom on arthropod-induced ectoparasitic dermatoses, McGarry and colleagues analyzed 73 insect specimens removed from symptomatic patients and submitted to their laboratory for identification at the Liverpool School of Tropical Medicine during the years 1994 to 2000.[10] Of the 73 specimens identified, there were 27 ticks, 24 flies, 15 miscellaneous insects, and 7 mites. Most of the arthropod dermatoses originated in the United Kingdom ($n = 46$; 63%), and were caused by tick bites ($n = 18$), principally *Ixodes ricinus* (the common sheep tick), an important European vector of Lyme disease and neuroborreliosis. Myiasis cases predominated in returning travelers ($n = 18$; 67%), principally furuncular myiasis from larval infestation by *Cordylobia anthropophaga* (n = 9), the tumbu fly, or *Dermatobia hominis* ($n = 4$), the human botfly. Among the arthropod dermatoses caused by miscellaneous arthropods, most were pediculosis pubis caused by infestation with *Phthirus pubis,* the pubic louse ($n = 7$), or hemorrhagic, bullous bite groupings caused by *Cimex lectularius,* the common bedbug ($n = 3$). The authors concluded that exotic ectoparasitic infestations, particularly myiasis, predominated in returning travelers from Africa and Latin America; pubic lice were domestic, likely sexually transmitted, infestations; and bedbug infestations were domestically and internationally acquired, often from exposure to fomites, including bedding and luggage.

MECHANISMS OF ECTOPARASITE-BORNE DISEASES AND INJURIES

The arthropod ectoparasites can threaten human health directly by burrowing into and feeding, dwelling, and reproducing in human skin and orifices (mites, fleas, flies), or by blood or tissue juice sucking (fleas, lice, mites, ticks). The arthropod ectoparasites can also threaten human health indirectly by infectious disease transmission (fleas, mites, ticks). Ticks are the most versatile ectoparasitic arthropods and can transmit a variety of infectious diseases (viral, bacterial, and protozoan) and even inject paralytic toxins (tick paralysis) during their prolonged blood meals. Unlike other ectoparasites, ticks can be infective as males and females at birth (by transovarial pathogen transmission) and throughout all stages of their development (by transstadial pathogen transmission). The most commonly encountered arthropod ectoparasites, excluding ticks, and the major clinical manifestations of their infestations are featured in Table 291.2. The tick-borne pathogens and the clinical manifestations of their infections are featured in Chapter 296, Tables 296.1 through 296.8.

CONCLUSIONS

Recent epidemiologic evidence now supports the endemicity of several ectoparasitic diseases and their arthropod vectors (Table 291.3) and human and animal reservoir hosts throughout the developing world and in many parts of the developed world, including Europe and the United States. Ectoparasitic diseases have also reemerged in regions where they were once effectively controlled. Ectoparasitic diseases will continue to reemerge in the developed world for several reasons, including the following: (1) the globalization of trade and commerce with ectoparasites and their human and animal hosts traveling worldwide on airplanes and container ships; (2) mass movements of populations from rural to urban areas and from developing to developed nations; (3) the worldwide legitimate and illegal trade of exotic animals and animal hides and skins; (3) the accidental and intentional introduction of exotic animal species into new regions with welcoming ecosystems; (4) the

TABLE 291.1 Taxonomy of Arthropods (Phylum Arthropoda) of Major Medical Importance

	COMMON NAMES	NO. OF LEGS, NO. OF BODY SEGMENTS, OTHER IDENTIFYING ANATOMIC FEATURES
Phylum Arthopoda, Class Insecta		
Order Diptera, family Culicidae	Mosquitoes	Six, three, wings
Order Diptera	Flies[a]	Six, three, wings
Order Hemiptera	True bugs (e.g., bedbugs, reduviid bugs)	Six, three, ± wings
Order Hymenoptera	Ants, bees, wasps	Six, three, ± wings
Order Phthiraptera	Lice[a]	Six, three, no wings
Order Siphonaptera	Fleas[a]	Six, three, no wings
Phylum Arthropoda, Class Arachnida		
Subclass Acari	Mites and ticks[a]	Eight, one globose body, no distinct heads[b], no wings
Order Araneae	Spiders	Eight, two, no wings
Order Scorpiones	Scorpions	Eight, two, abdomens with terminal stingers

[a]The arthropod ectoparasites of major medical importance by taxonomic order and distinctive anatomic features.
[b]Mouthparts visible dorsally only in ixodid (hard) ticks.

TABLE 291.2 Common Arthropod Ectoparasites (Excluding Ticks) and Clinical Manifestations of Ectoparasitoses

REPRESENTATIVE SPECIES OF INFESTING ARTHROPOD ECTOPARASITES	COMMON NAMES OF INFESTING ARTHROPOD ECTOPARASITE	GEOGRAPHIC DISTRIBUTION	MAJOR CLINICAL MANIFESTATIONS OF ECTOPARASITOSES
Class Insecta, Order Phthiraptera, Suborder Anoplura	Lice		
Pediculus humanus corporis	Body louse	Worldwide	Pediculosis corporis
Pediculus humanus capitis	Head louse	Worldwide	Pediculosis capitis, trench fever (Bartonella quintana)
Phthirus pubis	Crab (pubic) louse	Worldwide	Pediculosis pubis (phthiriasis)
Order Diptera	Flies		
Family Calliphoridae	Screwworms		
Auchmeromyia senegalensis	Congo floor-maggot fly	Sub-Saharan Africa, Cape Verde Islands	Larvae are nocturnal blood feeders, no myiasis (tissue invasion); wound (cutaneous) myiasis
Callitroga americana	American screwworm	North and Central America	Cavitary (invasive) myiasis
Chrysomyia bezziana	Old World screwworm	Tropical Africa, Asia, Indonesia	Cavitary (invasive) myiasis
Cochliomyia hominivorax	New World screwworm	Central and South America	Furuncular myiasis
Cordylobia anthropophaga	Tumbu (mango) fly	Africa	Furuncular myiasis
Family Oestridae	Botflies		
Cuterebra spp.	Rodent botfly	North and Central America	Furuncular myiasis
Dermatobia hominis	Human botfly	Central and South America	Furuncular myiasis
Order Siphonaptera	Fleas		
Ctenocephalides spp.	Cat *(C. felis)* and dog fleas *(C. canis)*	Worldwide	Bite groupings (mechanical vectors of dog and rat tapeworms, less efficient bubonic plague vectors)
Pulex irritans	Human flea	Worldwide	Bite groupings (efficient plague vector in Chilean Andes)
Tunga penetrans	Chigoe (jigger) flea	Central and South America, Africa Europe, Asia	Tungiasis
Xenopsylla cheopis	Oriental rat flea	Africa, Americas	Most efficient bubonic plague vector
Class Arachnida	Spiders, mites, ticks	Worldwide	
Subclass Acari	Mites and ticks		
Sarcoptes scabiei	Itch (scabies) mite	Worldwide	Scabies, crusted (Norwegian) scabies
Eutrombicula alfreddugesi	Common chigger (redbug chigger)	Worldwide	Chiggers
Leptotrombidium akamushi	Japanese-Asian rodent chigger	Japan, India, Australia	Potential scrub typhus (Tsutsugamushi disease) vector
Leptotrombidium deliensis	Indian-Asian rodent chigger	Eurasia-Eastern Asia, Southeast Asia, India, Australia, Indo-Pacific Islands	Potential scrub typhus (Tsutsugamushi disease) vector

increasing frequency of pyrethroid-resistant strains of ectoparasites, especially head lice and scabies mites; and (5) the growing populations of susceptible, and often immunocompromised, human hosts living in long-term care facilities and in crowded and impoverished periurban communities.[11,12]

The isolation of the trench-fever pathogen, *Bartonella quintana,* in head lice from homeless persons in the United States illustrates how well socioeconomic factors, human behavioral trends, and vector adaptations can support ectoparasite persistence with significant public health consequences.[13] Formerly felt to be susceptible to the safest pyrethroid pesticides and incapable of transmitting infectious diseases, head lice have acquired the capability to harbor *B. quintana* like body lice and potentially to transmit trench fever to new naïve host populations. The eradication of pyrethroid-resistant head lice infestations in homeless persons in crowded shelters and children in schools will require the use of more powerful pesticides with potential for adverse effects, such as carbaryl, lindane, and malathion; or the use of new, safer alternatives, such as oral and topical ivermectin-containing pediculicides.[11]

TABLE 291.3 Selected Infectious Diseases Transmitted by Arthropods

Infectious Disease	Vector
Anaplasmosis (human granulocytotropic)	Hard ticks
Arbovirus diseases (including yellow fever, dengue fever, encephalitis)	Mosquitoes and ticks
Babesiosis	Hard ticks
Boutonneuse fever (tick bite fever; *Rickettsia conorii*)	Hard ticks
Cat scratch disease, cat scratch fever *(Bartonella henselae)*	Cat fleas
Chagas disease (American trypanosomiasis)	Triatomine (kissing) bugs
Colorado tick fever	Hard ticks
Ehrlichiosis, monocytotropic *(Ehrlichia chaffeensis)* and granulocytic *(Ehrlichia ewingii)*	Hard ticks
Endemic relapsing fever *(Borrelia duttonii)*	Soft ticks
Epidemic relapsing fever *(Borrelia recurrentis)*	Human body lice
Epidemic typhus *(Rickettsia prowazekii)*	Human body lice
Filariasis *(Wuchereria bancrofti, Brugia malayi)*	Mosquitoes
Leishmaniasis (*Leishmania* spp.)	*Lutzomyia* sand fly in the Americas, phlebotomid flies elsewhere
Loiasis *(Loa loa)*	Tabanid flies
Lyme disease *(Borrelia burgdorferi)*	Hard ticks
Malaria (*Plasmodium* spp.)	Mosquitoes
Murine typhus *(Rickettsia mooseri)*	Rat fleas, lice
Onchocerciasis *(Onchocerca volvulus)*	Black flies
Plague *(Yersinia pestis)*	Rat fleas
Q fever *(Coxiella burnetii)*	Hard ticks, fleas
Rickettsialpox *(Rickettsia akari)*	Mouse mites
Rocky Mountain spotted fever *(Rickettsia rickettsii)*	Hard ticks
Scrub typhus *(Rickettsia tsutsugamushi)*	Mites (chiggers)
Trench fever *(Bartonella quintana)*	Body lice, potentially head lice
Trypanosomiasis, African sleeping sickness	*Glossina* (tsetse) flies
West Nile fever	Mosquitoes

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The complete reference list is available online at Expert Consult.

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292 Lice (Pediculosis)

James H. Diaz

SHORT VIEW SUMMARY

Definition
- Pediculosis is a complex of three different human infestations (head lice, body lice, and pubic lice) with two species of blood-sucking lice: *Pediculus humanus* var. *capitis* or *corporis* and *Phthirus pubis*.

Epidemiology
- Head lice infestations, or pediculosis capitis, are transmitted primarily by head-to-head contact and less commonly by fomites.
- Head lice afflict millions of people annually, mostly school-aged children.
- Body lice infestations, or pediculosis corporis, are transmitted primarily by bodily contact and less commonly by fomites.
- Body lice are associated with poor hygiene and primarily infest the indigent, institutionalized, homeless, refugees, and immunocompromised.
- Pubic lice infestations (phthiriasis) are transmitted primarily during sexual contacts and often coexist with other sexually transmitted diseases.

Microbiology
- Body lice can transmit several bacterial diseases, including (1) relapsing fever caused by *Borrelia recurrentis,* (2) trench fever caused by *Bartonella quintana,* and (3) epidemic typhus caused by *Rickettsia prowazekii. B. quintana* has also been isolated in head lice from homeless persons in the United States, establishing the potential for transmission of trench fever by head lice in addition to body lice.

Diagnosis
- Lice infestations are diagnosed by clinical inspection demonstrating live adult lice, nymphs, and viable eggs, or nits, in their precise human anatomic niches.
- Recently, dermoscopy has been used to immediately distinguish viable nits from hatched, empty nits.
- Dermoscopy may provide a more sensitive screening tool for head lice infestations than inspection alone.

Therapy
- A combination of pharmacologic therapy with topical or oral (ivermectin) pediculicides and physical removal of viable nits by wet combing is required.
- Pharmacotherapy should begin with the least toxic pediculicides, such as pyrethrins.
- Topical or oral ivermectin preparations should be reserved for pyrethrin-resistant cases.

Prevention
- Combinations of sanitizing the environment and eliminating all human reservoirs of head lice in households, apartments, housing complexes, homeless shelters, classrooms, and schools are recommended.
- Prevention strategies for pubic lice are similar to the prevention strategies for body lice and should include hot-cycle washing and drying of all clothing and bedding; institution of basic personal hygiene and sanitation measures; and treatment of sexual contacts with active body or pubic lice infestations.

Pediculosis is a complex of three different human infestations with two species of blood-sucking lice of the insect order Phthiraptera, suborder Anoplura: *Pediculus humanus* and *Phthirus pubis.* Sometime after early humans began to wear clothes, *P. humanus* evolved into two clinically distinct ectoparasitic variants, *P. humanus* var. *corporis,* the body louse (Fig. 292.1), and *P. humanus* var. *capitis,* the head louse (Fig. 292.2). Although morphologically indistinct, these human louse variants do not interbreed, prefer unique anatomic niches on human hosts, and are now considered distinct species. Specifically, head lice *(P. humanus capitis)* leave their hair shaft nests for blood meals on the scalp, and body lice *(P. humanus corporis)* leave their clothes seams nests for blood meals on the body. *Phthirus pubis,* the crab or pubic louse (Fig. 292.3), is morphologically distinct from the two *P. humanus* species, has a crab-shaped body, and prefers to dwell in the hair-bearing areas of the pubic and inguinal areas but may also infest the hairy areas of the axillae, chest, and abdomen and even the eyelashes (phthiriasis palpebrum). Unless mating or egg-laying, pubic lice remain relatively stationary, anchored to the bases of hair shafts while blood-feeding.

Lice are among the oldest ectoparasites of humans and are distributed into three separate mitochondrial clades (A, B, and C) based on their continents of origin.[1] Recently, a new clade of head and body lice (mitochondrial clade D) that were infected by *Bartonella quintana* and *Yersinia pestis* has been discovered in lice-infected persons living in plague-endemic areas of the Democratic Republic of the Congo.[2] Since the analysis of ancient DNA from Old World archeological sites is often compromised by low concentrations in poor specimens, the DNA from ancient head louse eggs or nits detected by reverse-transcriptase polymerase chain reaction (PCR) has recently proven to be valuable in mapping the migration patterns of ancient humans and the spread of their louse-borne infectious diseases such as epidemic typhus, relapsing fever, and trench fever.[1]

EPIDEMIOLOGY

Pediculosis capitis, or head lice infestation, is the most common of the three types of human pediculoses, afflicting millions of people annually, mostly school-aged children, in both developing and industrialized nations. Body lice infestations, or pediculosis corporis, are associated with poor hygiene and low socioeconomic status and occur primarily in the indigent, the institutionalized, the homeless, refugees from civil unrest, and the immunocompromised. Body and head lice are usually transmitted by direct body or head-to-head contact or sharing headgear between infested individuals and, much less commonly, by indirect contact with fomites, such as bedding, clothing, towels, headgear, combs, and brushes. Pubic lice infestations, or phthiriasis, are caused by *P. pubis,* the pubic or crab louse. Pubic lice are more often transmitted during sexual rather than fomite contacts and often coexist with crusted (Norwegian) genital scabies and other sexually transmitted diseases.

Unlike pubic lice, body lice can transmit several bacterial diseases. Homeless, immunocompromised, and refugee populations are at greatest risk for body lice infestations and epidemics of body louse–borne bacterial diseases, including the following: (1) relapsing fever caused by *Borrelia recurrentis,* (2) trench fever caused by *Bartonella quintana,* and (3) epidemic typhus caused by *Rickettsia prowazekii.* Body lice have been recognized as infectious organisms of high importance, not only in displaced populations in evacuee and refugee camps but also in immunocompromised subjects, particularly homeless individuals with acquired immunodeficiency syndrome (AIDS).[3–5]



FIG. 292.1 Dorsal view of a female body louse, *Pediculus humanus* var. *corporis.* *(From Public Health Image Library. Image 9204. Atlanta, GA: Centers for Disease Control and Prevention.)*



FIG. 292.3 Enlarged ventral image of a pubic or crab louse, *Phthirus pubis.* *(From Public Health Image Library. Image 4077. Atlanta, GA: Centers for Disease Control and Prevention.)*

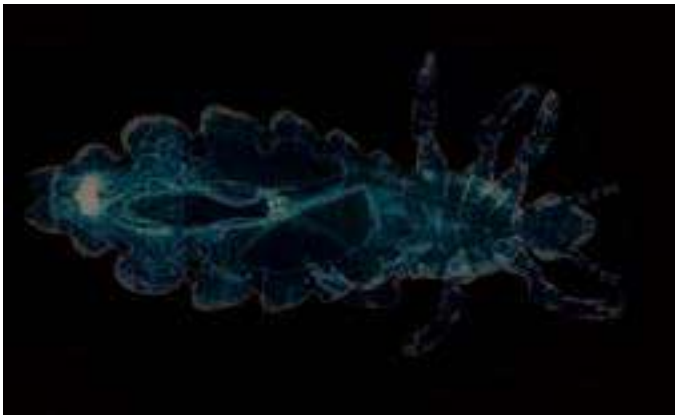

FIG. 292.2 Dorsal view of a female head louse, *Pediculus humanus* var. *capitis,* containing eggs or nits. *(From Public Health Image Library. Image 377. Atlanta, GA: Centers for Disease Control and Prevention.)*



FIG. 292.4 The unhatched nit or egg of the head louse, *Pediculus humanus* var. *capitis,* attached to a hair shaft. Note the red eye spots of the developing nymph embryo. *(From Public Health Image Library. Image 378. Atlanta, GA: Centers for Disease Control and Prevention.)*

B. quintana has now been isolated in head lice as well as body lice from homeless persons in the United States, establishing the potential for transmission of trench fever by blood-feeding head lice in addition to body lice.[6] During a 2011 outbreak of relapsing fever in Ethiopia, investigators demonstrated *B. recurrentis* DNA by quantitative real-time PCR in 23% of head lice from patients with positive blood smears, establishing the potential for transmission of relapsing fever by head lice in addition to body lice.[7]

In addition to serving as vectors for epidemic typhus (*R. prowazekii*), trench fever (*B. quintana*), and relapsing fever (*B. recurrentis*), body lice have been suspected in the transmission of a fourth pathogen, *Yersinia pestis,* the causative agent of plague. Collectively, these pathogens have killed millions of persons, especially homeless people and displaced war refugees without access to proper sanitation, personal hygiene, and clean clothing. In 2010, Drali and coinvestigators collected body and head lice from 37 infected individuals in plague-hyperendemic areas of the Democratic Republic of the Congo. Using multiplex, real-time PCR to rapidly differentiate head and body lice, the investigators detected evidence of *B. quintana* in 33.5% of body lice and 19% of head lice and *Y. pestis* in one head louse PCR-negative for other pathogens and two body lice PCR-positive for *B. quintana*.[2] Although these results will require independent confirmation in larger numbers of study subject samples, plague should probably be added to the list of dangerous pathogens that potentially can be transmitted by body lice and head lice.

DIAGNOSIS

Lice infestations are diagnosed by demonstrating live adult lice, nymphs, and viable eggs, or nits, in their precise human ecologic niches. Adult lice are flattened dorsoventrally and are 1 mm (pubic lice) to 3 mm (head and body lice) in length, have three pairs of legs ending in powerful claws that can grip hair shafts, and exhibit a reddish-brown hue after blood-feeding (see Figs. 292.2 and 292.3). Females can live on their hosts for up to 3 months, lay up to 300 nits in a lifetime (see Fig. 292.2), and die within 24 hours when separated from hosts. Nits are oval, less than 1 mm in diameter, and grayish white (Fig. 292.4); they fluoresce in ultraviolet or Wood light when viable. Nits are deposited on hair shafts at the skin surface and hatch nymphs within 6 to 10 days (see Fig. 292.4). Nymphs resemble miniature adults and grow to adulthood within 10 days. Empty egg cases remain attached to hair shafts after hatching and are not diagnostic of active infection.[8] Head lice and their viable nits are often attached to hairs close to the scalp, especially in occipital and postauricular locations. Because body lice visit their human hosts only to feed on blood, adults, nymphs, and nits are found in clothing, usually aligned along inner seams (Fig. 292.5). Pubic lice and their nits may be found in the pubic, perianal, and inguinal areas; in axillary and chest hair; and even in the eyelashes (phthiriasis palpebrum), especially in children who acquire pubic lice infestations from their parents (Fig. 292.6).[9]

CLINICAL MANIFESTATIONS AND DIFFERENTIAL DIAGNOSIS

Head Lice

The clinical manifestations of pediculosis capitis range from asymptomatic infestations to severe pruritus, with self-inflicted, often secondarily infected, excoriations with impetigo and postoccipital lymphadenopathy. The differential diagnosis of pediculosis capitis includes eczema, lichen



FIG. 292.5 Live eggs from the body louse, *Pediculus humanus* var. *corporis,* lining the seams of clothing. *(From Public Health Image Library. Image 5270. Atlanta, GA: Centers for Disease Control and Prevention.)*

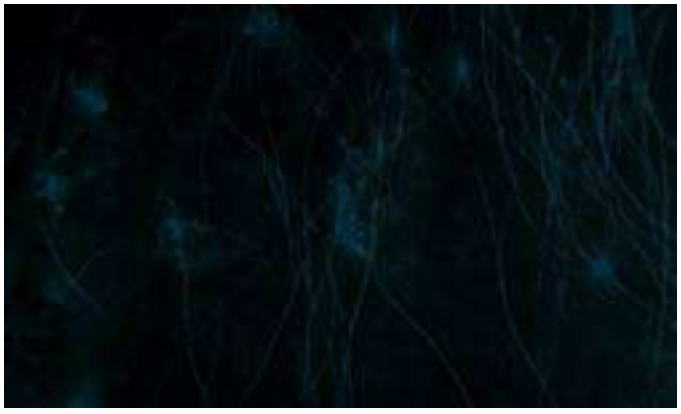

FIG. 292.6 Erythematous lesions seen in the pubic region of a patient in response to the bites of the blood-feeding crab or pubic louse, *Phthirus pubis.* *(From Public Health Image Library. Image 4078. Atlanta, GA: Centers for Disease Control and Prevention.)*

simplex chronicus, dandruff, seborrheic dermatitis, and bacterial impetigo.

Body Lice

Despite the fact that body lice reside in clothing, often along seams and not on the body, like head lice infestation, pediculosis corporis is often much more symptomatic than pediculosis capitis and causes severe pruritus, with extensive self-inflicted excoriations (see Fig. 292.5). The sites of blood-feeding are often present as erythematous macules, papules, or areas of papular urticaria with a central hemorrhagic punctum. The differential diagnosis includes eczematous dermatitis, lichen simplex chronicus, and scabies.

Crab Lice (Pediculosis Pubis)

Infestation with crab lice, or pediculosis pubis (phthiriasis), is less symptomatic compared with pediculosis capitis. These organisms affect all hair-bearing regions, most commonly the pubic and perianal areas, but also the upper eyelashes and the hairy areas of the axillae, chest, and abdomen. More extensive infestations usually occur in males with more body hair–bearing regions than females. Pubic lice may appear as 1- to 2-mm brownish-gray specks in infested hairy areas, where they remain stationary for days with claws grasping hair shafts and mouth parts embedded in the skin (see Fig. 292.3). The average life span for *Phthirus pubis* is 17 days for females and 22 days for males. Females deposit grayish-white eggs or nits at the skin-hair junctions. The egg incubation period is 7 to 8 days, and the life cycle from egg to adult is 22 to 27 days. Clinical manifestations include papular urticaria and self-inflicted, often infected, excoriations at blood-feeding sites, as well as regional, usually inguinal, lymphadenopathy (see Fig. 292.6). Pathognomonic findings may include maculae ceruleae *(taches bleues),* bluish-gray irregularly shaped macules, 0.5 to 1 cm in diameter, scattered over the lower abdominal wall, buttocks, and upper thighs. Maculae ceruleae may be caused by subcutaneous tissue staining from heme pigments altered by louse saliva and digestion. The differential diagnosis of crab lice includes eczematous dermatitis, seborrheic dermatitis, tinea cruris, folliculitis, molluscum contagiosum, and scabies, which frequently coexists with phthiriasis. Management includes initial bathing with soap and water, followed by two topical or systemic treatments with pediculicides, 7 to 10 days apart (Table 292.1).

THERAPY

Both *Pediculus humanus* species (head and body lice) and *Phthirus pubis* (the crab or pubic louse) have now demonstrated high levels of resistance worldwide to the safest topical pediculicides, specifically the natural pyrethrins and synthetic pyrethroids (permethrin, phenothrin).[8,10–14] In addition, resistance to lindane, an organochlorine insecticide, and malathion, an organophosphate insecticide, both alone and combined with pyrethroids, has been reported in the United Kingdom and elsewhere.[8,10] In a randomized comparison of wet combing versus 0.5% malathion shampoos for head lice in the United Kingdom, Roberts and colleagues[10] reported a 78% cure rate for malathion shampoo versus 38% for wet combing. In an in vitro pediculicidal efficacy comparison of five pediculicides available in the United States, Meinking and associates[11] reported the following results:

1. There were significant differences in the pediculicidal efficacies of the five pesticides tested.
2. Malathion was the only tested pesticide in their study that had not become less effective as a pediculicide.
3. The ranked order of therapeutic effectiveness from most to least effective was 0.5% malathion, undiluted natural pyrethrins with piperonyl butoxide, 1% permethrin, diluted natural pyrethrins with piperonyl butoxide, and 1% lindane.
4. Some head lice in the United States had become resistant to most pediculicides.

The increasing resistance of head lice to the pyrethrins and pyrethroids has led to the increasing use of more toxic pesticides, specifically lindane, malathion, and carbaryl (not approved by the US Food and Drug Administration [FDA] in the United States) in treating pyrethroid-resistant pediculosis capitis worldwide.[10,13,14] Lindane is being inappropriately overprescribed, especially for recurrent infestations with lindane-resistant head lice.[8,15] Lindane is an organochlorine insecticide that bioaccumulates in adipose and nerve tissue with overapplication or, if ingested, can cause seizures, especially in children.[8,11] Although malathion, an organophosphate pesticide, has demonstrated the greatest therapeutic efficacy against head lice in the United States, it is an irreversible acetylcholinesterase inhibitor that can cause a cholinergic toxidrome and fatal neuromuscular paralysis after overapplication or ingestion.[13] Carbaryl, a carbamate pesticide highly effective against both head lice and scabies, is being increasingly prescribed for pediculosis capitis outside the United States, especially in the United Kingdom and Europe.[14] Carbaryl is a reversible (nonaging) acetylcholinesterase inhibitor that is closely related to the organophosphate pesticides and can also cause a cholinergic (muscarinic and nicotinic) toxidrome after overapplication or ingestion.

Unfortunately, all the topical pesticides used to treat ectoparasitic infections share the same three characteristics as the three most commonly ingested childhood poisons: (1) prescribed, or often over-the-counter, medications; (2) household products; and (3) pesticides.[14] As the prevalence of ectoparasitic infections with pesticide-resistant ectoparasites increases, alternative pesticides, more toxic than pyrethrins and pyrethroids, will be prescribed for ectoparasitic infestations. In addition, medications will continue to be administered in households, and household accidental overapplication or ingestion of more toxic pesticide formulations for pediculosis may increase without enhanced public health education measures.[14,16]

TABLE 292.1 Recommended Pediculicide Treatments for Pediculosis Capitis

PEDICULICIDE	TRADE NAMES	THERAPEUTIC EFFICACY (OVICIDAL, PEDICULICIDAL)	SAFETY PROFILE	CONTRAINDICATIONS
0.33% Pyrethrins + 4% piperonyl butoxide shampoo	A-200 (OTC) RID (OTC)	95% ovicidal; no residual activity; increasing drug resistance	Excellent	Chrysanthemum and daisy (plant family Compositae) allergies possible contraindications
1%–5% Permethrin cream rinse	Acticin (OTC, Rx) Nix (OTC)	2-wk residual activity; increasing drug resistance	Excellent	Prior allergic reactions
0.5% Malathion lotion, 1% malathion shampoo	Ovide (Rx)	95% ovicidal; rapid (5-min) killing; good residual activity; increasing drug resistance, but not in the United States	Flammable 78% isopropyl alcohol vehicle stings eyes, skin, mucosa; increasing drug resistance; organophosphate poisoning risks with overapplication and ingestion	Infants and children <6 mo of age; pregnancy; breastfeeding
1% Lindane lotion and shampoo	Generic (Rx)	95% ovicidal; no residual activity; increasing drug resistance	Potential for CNS toxicity from organochlorine poisoning, usually manifesting as seizures, with overapplication and ingestion	Preexisting seizure disorder; infants and children <6 mo of age; pregnancy; breastfeeding; not recommended for use due to toxicity
0.9% Spinosad suspension	Natroba (Rx)	New to market; no reports of resistance; not ovicidal	Excellent	Infants and children age 4 yr and younger; presumed safe in pregnancy based on animal studies
5% Benzyl alcohol lotion	Ulesfia (Rx)	No resistance reported; not ovicidal	Excellent	Infants and children age 6 mo and younger; presumed safe in pregnancy based on animal studies
0.5% Ivermectin lotion	Sklice (Rx)	No resistance; single 10-min application; not ovicidal but nymphs die when they emerge from nits	Excellent	Infants and children age 6 mo and younger; safety in pregnancy uncertain
Ivermectin, 200–400 µg/kg tablet	Stromectol (Rx)	Excellent; not ovicidal; single PO dose, second dose in 7–10 days recommended	Excellent, but not in widespread use; nausea and vomiting possible; take on empty stomach with water only	Safety in pregnancy uncertain; not recommended for children weighing <5 kg; not FDA approved for pediculosis in United States

[a]Carbaryl (Sevin), a carbamate pesticide, is not currently approved or available as a human topical preparation for use for pediculosis in the United States. Carbaryl is, however, prescribed for pediculosis in Europe and elsewhere. Ectoparasite resistance to carbaryl has not been reported.
CNS, Central nervous system; *FDA,* US Food and Drug Administration; *OTC,* over-the-counter availability; *Rx,* available by prescription only.

A descriptive meta-analysis of pesticide poisonings in children in the United States over the period 1966–2008 demonstrated that malathion pediculicide ingestions were increasing, possibly as a result of increasing pyrethroid-resistant head lice infestations.[14] Subsequently, a state health department analysis of pesticide exposures in children younger than 7 years of age over the period 2003–2007 reported that lice shampoo exposures by ingestions, intraocular instillations, and prolonged topical applications were second only to mosquito-repellent exposures and resulted in more medical visits than all other pesticide exposures.[16]

Therapy for Pediculosis Capitis

Management of pediculosis capitis includes two topical or systemic treatments with pediculicides, 7 to 10 days apart, and removal of all viable nits by carefully combing wet hair. Olive oil, petroleum jelly, and HairClean 1-2-3 are preferred hair-wetting agents, and plastic combs are preferred over metal combs. Unfortunately, the ideal pediculicide with 100% killing activity against lice and nits does not exist. Table 292.1 presents the most commonly used pediculicides for lice infestations. As noted, drug resistance is increasing against the safest pediculicides, the pyrethrins and synthetic pyrethroids, and even against lindane and malathion, an effective ovicidal insecticide with 95% efficacy against viable nits.[8,10,11]

A randomized controlled trial has now demonstrated that a single oral dose of ivermectin, 400 µg/kg of body weight, repeated at 7 days, established higher louse-free rates by day 15 than two applications of 0.5% malathion lotion in patients with pyrethroid-resistant head lice infestations.[17] In 2012, the FDA approved the use of topical 0.5% ivermectin lotion for head lice infestations after two multisite, randomized, double-blind studies comparing single applications of 0.5% ivermectin lotion with vehicle control that demonstrated significantly greater louse-free days at 1, 7, and 14 days in the ivermectin group than in the vehicle control group.[18] Today, both oral ivermectin (although not FDA approved) and topical ivermectin lotion for head lice offer convenient, single-dose treatments that kill nymphs when they emerge from nits and can be reserved for drug-resistant head lice cases to limit the potential for ivermectin resistance.

Therapy for Body Lice

Management includes initial bathing with soap and water, followed by two topical or systemic treatments with pediculicides, 7 to 10 days apart (see Table 292.1). Topical medications should be applied to clean affected areas, allowed to dry, and not rinsed for 8 (malathion) to 24 (pyrethrins, pyrethroids) hours.

PREVENTION

Prevention strategies for head lice include combinations of sanitizing the environment and, more important, eliminating all human reservoirs of carriage of head lice in households, apartments, housing complexes, homeless shelters, classrooms, and schools. Some common preventive interventions include the following: (1) avoiding contact with potentially contaminated items, such as hats, head sets, clothing, towels, combs, brushes, bedding, and upholstery; (2) soaking all combs and brushes in isopropyl alcohol or 2% Lysol solution; (3) sanitizing the household environment by hot-cycle washing and drying of all bedding, clothing, and headgear; and (4) inspecting high-risk schoolchildren for active head lice, viable nits, and nymphs. Lebwohl and colleagues[8] have recommended that "no-nit policies" in schools be abandoned. As noted, nonviable nits on hair shafts are simply empty egg cases and do not indicate active louse infestation. Dermoscopy can now clearly distinguish viable nits from hatched, empty nits and pseudonits immediately and reliably and offers a more sensitive screening tool for head lice infestations than inspection alone.[19]

Prevention and control strategies for pediculosis corporis should include the following:

1. Hot-cycle washing and drying of all clothing and bedding
2. Clothing and body delousing with 1% permethrin dusting powder, especially in outbreak situations with potential for bacterial disease transmission

3. Institution of basic personal hygiene and sanitation measures, including showering, body washing, and clean clothing changes

Prevention strategies for pubic lice are similar to the prevention strategies for body lice and should include the following:

1. Hot-cycle washing and drying of all clothing and bedding
2. Institution of basic personal hygiene and sanitation measures
3. Treatment of sexual contacts with active infestations
4. Examination and laboratory testing of patients and their sexual contacts for other sexually transmitted diseases, especially crusted scabies and AIDS.

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The complete reference list is available online at Expert Consult.

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293 Scabies

James H. Diaz

SHORT VIEW SUMMARY

Definition
- Scabies is an infestation by the itch or scabies mite *Sarcoptes scabiei* var. *hominis* and has become a significant reemerging ectoparasitosis in its most severe form, as crusted or Norwegian scabies, among homeless people, institutionalized older adults, individuals with intellectual disability, and immunocompromised individuals.

Epidemiology
- The worldwide annual prevalence of scabies has been estimated to be about 300 million cases.
- Scabies occurs worldwide in both sexes, at all ages, and among all ethnic and socioeconomic groups. Scabies is hyperendemic throughout the developing world, especially in sub-Saharan Africa, India, the Aboriginal regions of northern Australia, and the South Pacific Islands, especially the Solomon Islands.
- Crusted or Norwegian scabies is highly transmissible in the hospital environment.

Microbiology
- The human scabies mite is an obligate parasite and completes its entire life cycle on its human hosts, as females burrow intradermally to lay eggs and larvae emerge and mature to reinfest the same or new hosts. The entire incubation period from eggs to full-grown mites lasts 14 to 15 days.
- The human incubation period from initial infestation to symptom development is 3 to 6 weeks in initial infestations and 1 to 3 days in reinfestations as a result of prior sensitization to mite antigens.

Diagnosis
- The diagnosis of scabies is made by epidemiologic considerations and clinical observations.
- A clinical diagnosis may be confirmed by low-power microscopic examination of a burrow skin scraping that excavates the female mite.
- Skin biopsy may help confirm the diagnosis in atypical cases.
- Newer diagnostic methods for scabies include enhanced microscopic techniques (dermoscopy), immunologic detection of specific scabies antibodies, and molecular identification of scabies DNA.

Therapy
- Both topical 5% permethrin and oral ivermectin appear most effective for individual classic scabies.
- The management of crusted (Norwegian) scabies may require combined, intense scabicidal therapies with both topical 5% permethrin and oral ivermectin, especially in high-risk community and institutional outbreaks.

Prevention
- Aggressive treatment of infested patients and all close household, institutional, and sexual contacts, especially in cases of highly infectious crusted (Norwegian) scabies, is required.
- Disposal or hot wash-dry sterilization (by machine washing and drying at 60°C [140°F] or higher) of all contaminated clothing and bedding of index cases is essential.
- Provision of improved access for personal hygiene and health care for all displaced, homeless, or institutionalized people should be implemented.

Scabies, an infection by the itch or scabies mite, *Sarcoptes scabiei* var. *hominis,* remains a major public health problem throughout the developing world (Fig. 293.1). Scabies in its most severe form, crusted or Norwegian scabies (Fig. 293.2), has now become a significant reemerging ectoparasitosis in the developed world, especially among homeless people, institutionalized older adults, individuals with intellectual disability, and immunocompromised individuals.[1]

EPIDEMIOLOGY

The worldwide annual prevalence of scabies has been estimated to be about 300 million cases.[2] Although more often associated with crowding, homelessness, and institutionalization, scabies occurs worldwide in both sexes, at all ages, and among all ethnic and socioeconomic groups. In the United Kingdom, scabies is more prevalent in women and children living in urban areas and occurs more often in winter than summer.[3] In a prospective survey in Belgium, Lapeere and colleagues[4] reported a crude incidence for scabies of 28 cases/100,000 inhabitants per year. The highest annual incidence of scabies was noted in immigrants (88/100,000) and in people older than age 75 years (51/100,000). Scabies is hyperendemic throughout the developing world, especially in sub-Saharan Africa (13% annual prevalence rate), India, the Aboriginal regions of northern Australia, and the South Pacific Islands, especially the Solomon Islands.[2,5,6]

Scabies infections with crusted (Norwegian) scabies are more prevalent among several specific high-risk groups including men who have sex with men, patients treated in sexually transmitted disease clinics, homeless individuals with the acquired immunodeficiency syndrome (AIDS), and patients with human T-cell lymphotropic virus type 1 (HTLV-1) infection.[1,7,8] Many experts now recommend evaluating all high-risk patients with crusted scabies for human immunodeficiency virus (HIV) and HTLV-1 infection.[9] In a prospective study of 23 patients with crusted scabies in Peru, HTLV-1 infection was diagnosed in 16 patients (69.6%) by enzyme-linked immunosorbent assay and confirmed by Western immunoblot analysis.[8] In addition to HTLV-1 infection, other significant comorbid features for crusted scabies in the Peruvian study included corticosteroid therapy (8.6%), malnutrition (8.6%), and Down syndrome (4.3%).

TRANSMISSION

In contrast to ectoparasitic fleas and flies, scabies mites cannot jump or fly, but they can crawl at a rate of 2.5 cm/min on warm, moist skin (see Fig. 293.1).[2] They can survive for 24 to 36 hours at room temperature and average humidity and remain capable of infesting humans.[10] Scabies is most easily transmitted by skin-to-skin contact, as with sex partners and children playing, as well as health care providers examining highly infectious patients with crusted scabies. High-risk sexual behaviors for contracting scabies include sporadic sexual contacts and men who have sex with men.[4] Scabies mites have not been demonstrated to transmit HIV, HTLV-1, or any other infectious agent. The more mites there are on a human host, the greater is the risk for transmission by close direct contact, more so than by indirect contact with fomites, such as shared bedding and clothing. Although rare, indirect transmission of scabies



FIG. 293.1 Micrograph of a cleared and slide-mounted scabies mite, *Sarcoptes scabiei* (ventral view). *(From Centers for Disease Control and Prevention [CDC], Atlanta, GA. CDC Public Health Image Library, image 6301.)*



FIG. 293.2 Crusted (Norwegian) scabies on the extensor surface of the elbow secondarily infected with *Staphylococcus aureus.* Note the confluence of the crusts and pustules and the similarity of the lesion to psoriasis, both in its hyperkeratotic appearance and in its location on an extensor surface. Risk factors for crusted scabies include immunocompromise secondary to advanced age, prolonged glucocorticoid therapy, cancer chemotherapy, and human immunodeficiency virus or human T-cell lymphotropic virus type 1 infection. *(From Fitzpatrick TB, Johnson RA, Wolff K, Suurmond D.* Color Atlas and Synopsis of Clinical Dermatology. *4th ed. New York: McGraw-Hill; 2001:841.)*

occurs and is more common in immunocompromised hosts with AIDS, in family members of an index atypical (crusted) case, and within the institutional settings described.[1]

Several nonhuman species of sarcoptic mites can cause animal scabies with itching, inflammation, and hair loss. Animal scabies occurs commonly in domestic pets and animals, especially in cats, dogs, pigs, horses, and camels. Immunocompromised individuals may also contract animal scabies from domestic animals, usually dogs, with sarcoptic mange. Animal scabies mites are facultative ectoparasites in humans and cannot effectively complete their life cycles in human (dead-end) hosts. Infections are usually self-limited in humans but can be treated successfully, if indicated, with 5% permethrin lotion, 10% crotamiton cream or lotion, or oral ivermectin.

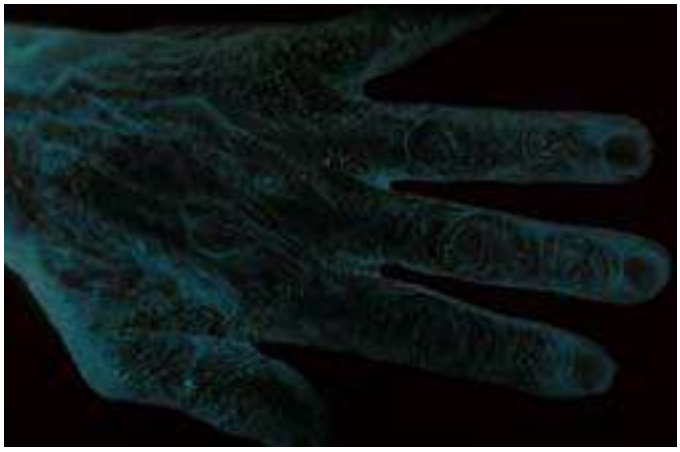

FIG. 293.3 Dorsal view of an older patient's hand demonstrating a crusted scabies infestation by the scabies mite, *Sarcoptes scabiei.* Note the localized crusting in the interdigital web spaces. *(From Centers for Disease Control and Prevention [CDC], Atlanta, GA. CDC Public Health Image Library, image 4800.)*

CLINICAL MANIFESTATIONS

The human scabies mite is an obligate parasite and completes its entire life cycle on its human hosts, as females burrow intradermally to lay eggs and larvae emerge and mature to reinfest the same or new hosts. Female mites burrow preferentially into the thinner areas of the epidermis by dissolving the stratum corneum with proteolytic secretions. Burrows are usually no deeper than the stratum granulosum. Female mites then lay their eggs at the end of tunneled burrows 5 to 10 mm long, and larvae hatch 2 to 3 days after eggs are laid. The entire incubation period from eggs to full-grown mites lasts 14 to 15 days.[11] The human incubation period from initial infection to symptom development is 3 to 6 weeks in initial infections and 1 to 3 days in reinfections as a result of prior sensitization to mite antigens.[2]

Classic or typical scabies manifests as generalized, intense nocturnal itching in a characteristic topographic distribution as 10 to 15 fertile female mites are transferred from infected patients to new hosts. The skin eruptions in reinfections and atypical forms of scabies are considered consequences of infection and hypersensitivity reactions to mite antigens.[2]

In classic scabies, the preferred distribution of skin eruptions includes hairless areas with a thin stratum corneum, such as the sides and interdigital web spaces of fingers and toes, popliteal fossae, flexor surfaces of the wrists, buttocks, and female breasts (Fig. 293.3).[2] Although inflammatory pruritic papules are present at most infested sites, the pathognomonic linear to serpiginous intradermal burrows, 5 to 10 mm long, dotted with fecal lithes (pellets) or scybala, and terminating in raised papules hiding ovipositing females, may be absent. Nonspecific secondary lesions occur commonly as the result of scratching and secondary infection and include self-inflicted excoriations, eczematization, lichenification, and impetigo.

Bullous scabies is a rare subtype of classic scabies, with only 44 cases reported to date.[12] The highly pruritic bullous lesions resemble bullous pemphigoid and most commonly cluster on the trunk and extremities but may involve genitals, feet, thighs, inguinal folds, and neck.[12] Facial and mucosal involvement has not been reported.[12] Risk factors for bullous scabies include male sex and advanced age.[12] To date, all cases have been successfully treated with available antiscabietic therapies.[12]

In addition to classic and bullous scabies, scabies may also manifest in three atypical forms, especially in high-risk institutionalized or immunocompromised individuals with HIV or HTLV-1 infections. These atypical forms of scabies include scalp scabies in infants, crusted (Norwegian) scabies in institutionalized and immunocompromised individuals and in high-risk populations such as Aboriginal populations in remote areas of tropical northern Australia, and sexually transmitted nodular scabies. Scabietic nodules develop in 7% to 10% of patients with scabies infections, usually in men on the penis and scrotum, and

appear as darkened, tender nodules 5 to 20 mm in diameter, often with a raised female mite burrow on top (Fig. 293.4). The atypical forms of scabies are compared with classic scabies and stratified by high-risk human host populations, clinical manifestations, and differential diagnoses in Table 293.1.



FIG. 293.4 Sexually transmitted nodular scabies infestation in a man caused by the scabies mite, *Sarcoptes scabiei.* Note the nodular pustular lesions clustered around the umbilicus and inner thighs. *(From Centers for Disease Control and Prevention [CDC], Atlanta, GA. CDC Public Health Image Library, image 6538.)*

DIAGNOSIS

Although newer diagnostic methods are under investigation, the diagnosis of scabies is made predominantly by epidemiologic considerations and clinical observations. A clinical diagnosis may be confirmed by low-power microscopic examination of a burrow skin scraping that excavates the female mite, 0.2 to 0.5 mm in length, translucent with brown legs, and too small to be seen with the unassisted eye. Eggs (0.02–0.03 mm in diameter), smaller eggshell fragments, and fecal lithes or pellets may also be identified in microscopic specimens of burrow scrapings (see Fig. 293.1).[2] Potassium hydroxide should not be used to mount the burrow scrapings because it can dissolve mite fecal lithes. As noted, failure to identify pathognomonic burrows and to find mites is common, particularly in initial cases with low ectoparasite burdens, and does not rule out scabies. One of the simplest and most often overlooked bedside tests to identify scabies mite burrows is the burrow ink test (BIT), which relies on ink to highlight burrows. In the BIT, fountain pen ink or any liquid ink is gently rubbed on skin surfaces with suspected scabies and then wiped off with an alcohol pad to reveal the ink-highlighted wavy tunnels made by the burrowing female mite in the stratum corneum.

In atypical scabies cases, skin biopsy may help confirm the diagnosis.[2] Although untested in controlled trials in large study populations, newer diagnostic methods for scabies now under investigation include enhanced microscopic techniques (e.g., epiluminescence microscopy, computed or digital dermoscopy, or videodermoscopy), immunologic detection of specific scabies antibodies by enzyme-linked immunosorbent assay, and molecular identification of scabies DNA by polymerase chain reaction assay.[13,14-16] Handheld dermoscopy (also known as dermatoscopy) is an accurate test that requires a dermatoscope and a trained microscopist.[16] The simplest dermatoscopes consist of a low-power (10×) magnifier, a nonpolarized light source, and a liquid medium between the light source and a transparent lens to limit skin reflections. More sophisticated dermatoscopes, such as digital epiluminescence dermatoscopes, rely on polarized light rather than a liquid medium to cancel out skin surface reflections and can be wirelessly linked to computers for videodermoscopy and display screens for real-time imaging and video capturing of scabies lesions. Low-cost videomicroscopy was compared with videodermoscopy in a small trial that showed noninferiority of videomicroscopy, which may have implications in diagnostic testing in settings with scarce resources.[17] Serologic and molecular methods for diagnosing scabies are under development and testing and are not universally available.[16] At the present time, epidemiologic considerations and clinical observations,

TABLE 293.1 Different Presenting Forms of Scabies

PRESENTING FORMS OF SCABIES	SPECIFIC HIGH-RISK POPULATIONS	CLINICAL MANIFESTATIONS	LIMITED DIFFERENTIAL DIAGNOSES
Classic scabies (scabies vulgaris)	Infants and children; sexually active adults; men who have sex with men	Intense generalized pruritus, worse at night; inflammatory pruritic papules localized to finger webs, flexor aspects of wrists, elbows, axillae, buttocks, genitalia, female breasts; lesions and pruritus spare the face, head, and neck; secondary lesions include eczematization, excoriation, impetigo	Dermatitis herpetiformis, drug reactions, eczema, pediculosis corporis, lichen planus, pityriasis rosea
Scalp scabies	Infants and children; institutionalized older adults; AIDS patients; patients with preexisting crusted scabies	Atypical crusted papular lesions of the scalp, face, palms, and soles	Dermatomyositis, ringworm, seborrheic dermatitis
Crusted scabies (Norwegian scabies, scabies norvegica, scabies crustosa)	Institutionalized older adults; institutionalized developmentally disabled (Down syndrome) individuals; homeless people, especially HIV-positive; all immunocompromised patients, particularly those with AIDS or positive for HIV or HTLV-1; transplant recipients; patients on prolonged systemic corticosteroid therapy and chemotherapy	Psoriasiform hyperkeratotic papular lesions of the scalp, face, neck, hands, feet, with extensive nail involvement; eczematization and impetigo common	Contact dermatitis, drug reactions, eczema, erythroderma, ichthyosis, psoriasis
Nodular scabies	Sexually active adults; men who have sex with men; HIV-positive men > HIV-positive women	Violaceous pruritic nodules localized to male genitalia, groin, axillae, representing hypersensitivity reaction to mite antigens	Acropustulosis, atopic dermatitis, Darier disease, lupus erythematosus, lymphomatoid papulosis, papular urticaria, necrotizing vasculitis, secondary syphilis

AIDS, Acquired immunodeficiency syndrome; *HIV,* human immunodeficiency virus; *HTLV-1,* human T-cell lymphotropic virus type 1.

TABLE 293.2 Currently Recommended Treatment for Scabies

SCABICIDES	FDA APPROVED?	PREGNANCY CATEGORY[A]	DOSING SCHEDULE	SAFETY PROFILE	CONTRAINDICATIONS
5% Permethrin cream (Actin, Nix, Elimite)	Yes	B	Apply from neck down; wash off after 8–14 h; good residual activity, but second application recommended after 1 wk	Excellent; itching and stinging on application	Prior allergic reactions; infants <2 mo of age; breastfeeding
1% Lindane lotion or cream	Yes	B	Apply 30–60 mL from neck down; wash off after 8–12 h; no residual activity; increasing drug resistance	Potential for central nervous system toxicity from organochloride poisoning, usually manifesting as seizures, with overapplication and ingestions	Preexisting seizure disorder; infants and children <6 mo of age; pregnancy; breastfeeding
10% Crotamiton cream or lotion (Eurax)	Yes	C	Apply from neck down on 2 consecutive nights; wash off 24 h after second application	Excellent; not very effective; exacerbates pruritus	None
2%–10% Sulfur in petrolatum ointments	No	C	Apply for 2–3 days, then wash	Excellent; not very effective	Preexisting sulfur allergy
10%–25% Benzoyl benzoate lotion	No	None	Two applications for 24 h with 1-day to 1-wk interval	Irritant; exacerbates pruritus; can induce contact irritant dermatitis and pruritic cutaneous xerosis	Preexisting eczema
0.5% Malathion lotion (Ovide), 1% malathion shampoo (unavailable in United States)	No	B	95% Ovicidal; rapid (5 min) killing; good residual activity; increasing drug resistance	Flammable 78% isopropyl alcohol vehicle stings eyes, skin, mucosa; increasing drug resistance; organophosphate poisoning risk with overapplication and ingestions	Infants and children <6 mo of age; pregnancy; breastfeeding
Ivermectin (Stromectol)	Yes	C	200-μg/kg single PO dose, may be repeated in 14–15 days; not ovicidal, second dose on day 14 or 15 highly recommended; recommended for endemic or epidemic scabies in institutions and refugee camps	Excellent; may cause nausea and vomiting; take on empty stomach with water	Safety in pregnancy uncertain; probably safe during breastfeeding; not recommended for children <5 yr of age or weighing <15 kg

[a]US Food and Drug Administration safety in pregnancy categories: A, safety established; B, presumed safe; C, uncertain safety; D, unsafe; X, highly unsafe.
PO, Per os (orally).

often aided by bedside techniques such as the BIT and dermoscopy, remain the most rapid and practical methods for diagnosing scabies.[16]

THERAPY

Topical or oral scabicides should be used to treat infested persons and their close personal contacts simultaneously, regardless of the presence of symptoms.[2] Currently recommended treatment options for scabies are listed in Table 293.2. In a review on the treatment of scabies, Strong and Johnstone[18] noted that both topical 5% permethrin and oral ivermectin appear most effective for individual infections, more research would be needed to compare the effectiveness of malathion with permethrin for individual infections, and there was insufficient evidence at the present time to recommend specific miticides to control community and institutional outbreaks of scabies. The most effective topical agents for scabies are 5% permethrin cream and 1% lindane cream or lotion, with permethrin safer and slightly more effective than lindane, which is an organochlorine pesticide capable of causing seizures and sudden death caused by overapplication or accidental ingestions.[19,20] The other topical agents for scabies include 10% to 25% benzoyl benzoate lotions (not available in the United States), 10% crotamiton cream or lotion, 2% to 10% sulfur in petrolatum ointments, and 0.8% ivermectin lotion (not available in the United States) (see Table 293.2).

The topical agents for scabies may not be well accepted or tolerated by some patients for many reasons, including severe burning and stinging (with 25% benzyl benzoate and 5% permethrin) in cases of secondarily excoriated or eczematous infections and inability of demented or disabled patients to comply with application regimens. In such cases, two doses of oral ivermectin, 200 μg/kg/dose, taken with food, one dose on day 1 and a second dose between days 8 and 15, may offer a more acceptable and equally effective alternative. In a 2007 prospective trial, Sule and Thacher[21] compared the effectiveness of oral ivermectin, 200 μg/kg/dose, with topical 25% benzoyl benzoate and monosulfiram soap in 210 Nigerian patients 5 to 65 years of age with scabies. Subjects with persistent lesions received a second course of therapy after 2 weeks. The investigators observed resolution of all lesions in 77 of 98 subjects (79%) treated with ivermectin and in 60 of 102 subjects (59%) treated topically ($P = .003$). The scabies cure rate at 4 weeks was 95% in the ivermectin group and 86% in the topical treatment group ($P = .04$). It was concluded that oral ivermectin is as effective as topical treatment with benzyl benzoate and monosulfiram in scabies and leads to more rapid improvement. Ivermectin, however, is not ovicidal, and a second course of oral treatment at adult mite maturation time of 14 to 15 days is now recommended.[2,22]

In a prospective trial comparing oral ivermectin with topical 5% permethrin in scabies, Usha and Gopalakrishnan Nair[22] reported a 70% cure rate with a single dose of ivermectin, compared with a 95% cure rate with topical 5% permethrin ($P < .003$), but a second dose of ivermectin, 200 μg/kg, taken 2 weeks later, increased the cure rate to 95%. Nevertheless, the US Centers for Disease Control and Prevention (CDC) recommends topical 5% permethrin cream or lotion as first-line therapy for scabies, especially in initial classic infections.[23]

Scabies mite drug resistance to both topical 5% permethrin preparations and oral ivermectin has emerged in severe outbreaks of crusted scabies in nursing homes and in the hyperendemic regions noted.[24,25] As a result, the management of crusted scabies may require combined, intense scabicidal therapies in high-risk community and institutional outbreaks. Currie and McCarthy[26] have recommended both 5% topical permethrin every 2 to 3 days for 1 to 2 weeks and oral ivermectin, 200 μg/kg/dose, taken with food and administered as three doses (days 1, 2, and 8), five doses (days 1, 2, 8, 9, and 15), or seven doses, depending on the severity of the infection. For refractory institutional and community outbreaks, the authors recommended combined therapy with topical permethrin and oral ivermectin for all symptomatic cases with classic or crusted scabies and a single oral dose of ivermectin, 200 μg/kg, for all exposed, asymptomatic residents, visitors, and staff.[26]

The emerging resistance of the scabies mite to commonly used standard treatments, specifically topical permethrin and oral ivermectin, will limit the future therapeutic usefulness of these agents and encourage

the development of new and effective acaricidal agents.[27] Tea tree oil (TTO) has already demonstrated acaricidal effects against scabies mites in vitro and has been used as an effective topical adjunct for treating crusted scabies not responding to standard therapies.[28] Thomas and coworkers[28] reviewed the therapeutic potential of TTO for scabies and recommended larger scale randomized controlled trials comparing the therapeutic efficacy of TTO with standard treatments for scabies, especially crusted scabies and resistant scabies.

PREVENTION

Prevention and control strategies for scabies include the following: (1) aggressive treatment of infested patients and all close household, institutional, and sexual contacts, especially in cases of highly infectious crusted scabies; (2) disposal or hot wash-dry sterilization (by machine washing and drying at 60°C [140°F] or higher) of all contaminated clothing and bedding of index cases; (3) provision of improved access for personal hygiene and health care for all displaced, homeless, or institutionalized persons; and (4) aggressive control of outbreaks of zoonotic scabies with the potential for human transmission caused by the sarcoptic mites of various domestic animals, especially cats, dogs, camels, pigs, and horses.[2,25]

As noted, epidemic outbreaks of scabies that are often associated with impetigo and are difficult to control frequently plague isolated, impoverished communities in the Aboriginal regions of northwestern Australia and throughout the South Pacific islands. In a prospective study designed to control endemic scabies in isolated island communities, Romani and coworkers[28] randomly assigned three island communities in Fiji to one of three different drug treatment interventions for mass scabies control: (1) topical permethrin to affected persons and their contacts (standard care group, $n = 803$); (2) mass topical permethrin administration (permethrin group, $n = 532$); and (3) mass administration of oral ivermectin (ivermectin group, $n = 716$).[28] Although the prevalence of scabies declined significantly in all three groups, the greatest decline was observed in the ivermectin group (relative reduction 94% from a prevalence rate of 32.1% to 1.9%). The prevalence of impetigo also declined significantly in all three groups, with the greatest decline again in the ivermectin group (relative reduction 67%).[28] The authors concluded that in addition to improvements in living standards, the mass administration of scabicides, especially oral ivermectin, was efficacious for the population-based control of endemic scabies and associated impetigo.[28]

CONCLUSION

All patients with scabies and their close household, institutional, and sexual contacts should be informed that scabies is a transmissible ectoparasitic infection and that several topical treatments and an effective oral treatment are now readily available and highly effective (see Table 293.2). Precise diagnosis should be confirmed, if possible, by microscopic, immunologic, or molecular methods. Topical 5% permethrin preparations remain reasonable first-line therapies for classic scabies and are recommended by the CDC. Topical 25% benzoyl benzoate preparations are equally effective alternative choices, especially for crusted scabies.[26] Oral ivermectin, 200 µg/kg initially and repeated in 2 weeks, may be preferred for patients who cannot tolerate topical therapies, who are unable to adhere to topical application schedules, or who have atypical or topical drug–resistant scabies. Sexually active patients with nodular scabies should be screened for other sexually transmitted diseases. Serious consideration should be given to screening patients with crusted scabies for HIV and HTLV-1 infections, particularly in scabies and HTLV-1 hyperendemic regions of the world and in homeless shelter outbreaks of crusted scabies. Future molecular investigations of scabies mite biology and genetic drug resistance are needed to permit the development of better diagnostic tools and treatment strategies for human scabies, especially atypical scabies.

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294 Myiasis and Tungiasis

James H. Diaz

SHORT VIEW SUMMARY

MYIASIS

Definition
- Myiasis is an ectoparasitic infestation of viable or necrotic tissues by the dipterous larvae of higher flies. Furuncular myiasis is the most common clinical manifestation of myiasis and occurs when one or more fly larvae penetrate the skin, causing pustular lesions that resemble boils or furuncles.

Epidemiology
- Myiasis is an opportunistic infestation that occurs in travelers returning from tropical jungles and in vulnerable populations living in endemic areas of the tropics.

Microbiology
- In human botfly furuncular myiasis, botfly larvae rapidly burrow into the skin with sharp mandibles to begin their developmental instar stages, which can last 6 to 12 weeks and cause draining, boil-like lesions, or furuncles.

Diagnosis
- The diagnosis of myiasis is usually by clinical inspection and examination, often with microscopy. Some immunodiagnostic tests have been developed to detect the antibodies to the antigens of specific fly species causing myiasis.

Therapy
- Furuncular myiasis may be treated conservatively by coaxing embedded larvae from furuncles by smothering their respiratory spiracles with occlusive coatings of petrolatum (Vaseline), clear fingernail polish, tobacco tar, pork fat, raw beefsteak, or bacon strips. However, unsuccessful occlusive therapy may asphyxiate larvae and necessitate their surgical or vacuum extraction. Along with larval removal, myiasis wounds should be cleansed and conservatively débrided, tetanus prophylaxis administered, and bacterial secondary infections treated with antibiotics.

Prevention
- Methods include control of domestic and livestock animal larval infestations; sanitary disposal of animal carcasses and offal to deny flies their preferred breeding grounds; proper management of any open human wounds or cutaneous infections; cementing floors to deny floor maggot flies their preferred egg-laying surfaces; sleeping on raised beds or cots in screened huts or tents; wearing long-sleeved shirts and pants, which can be pyrethrin- or pyrethroid-impregnated; spraying exposed skin with diethyl toluamide (*N,N*-diethyl-meta-toluamide [DEET])–containing repellents; and ironing both sides of all clothes and diapers left outside to dry in tumbu fly habitats.

TUNGIASIS

Definition
- Tungiasis is a painful, cutaneous infestation with the gravid female jigger flea that usually occurs on the feet but may occur anywhere where bare skin touches soil containing gravid female fleas.

Epidemiology
- In travelers returning to accessible health care infrastructures in developed nations, tungiasis is an exotic infestation with a minimal parasite burden and a simple surgical cure, but in the impoverished and underserved communities of developing tropical nations, tungiasis is a recurrent infestation of the feet with a high parasite burden, causing significant morbidity, including autoamputation.

Microbiology
- In tungiasis the gravid female jigger (chigoe) flea penetrates bare skin, usually on the feet (or heels), under or near the toenails or in the interdigital web spaces, to feed on blood and tissue juices and to incubate hundreds of developing eggs within days; it swells to 2000 times its size and then expels eggs over a period of 3 weeks or less before dying and leaving its shriveled carcass in a contaminated wound tract.

Diagnosis
- The diagnosis of tungiasis is usually by clinical inspection and examination, often with microscopy.

Therapy
- Tungiasis is treated by extracting all embedded fleas immediately with sterile needles or curets, administering tetanus prophylaxis, and treating secondary wound infections with appropriate topical or oral antibiotics.

Prevention
- Methods include wearing shoes, which can be sprayed with pyrethroid or DEET-containing solutions; not sitting naked on bare ground; insecticide treatment of flea-infested domestic and stray animals and pets with 10% pyrethrin or pyrethroid sprays, or 1% to 4% malathion powder; bathing the feet of domestic and stray dogs and pigs with insecticide solutions, such as 2% trichlorfon (Neguvon); and spraying or dusting households, especially those with dirt floors, with 1% to 4% malathion.

Flies and fleas are mostly bothersome biting nuisances of humans and animals that can also transmit infectious diseases and deeply invade living tissues, causing amputation, disfigurement, and, rarely, death. Flies can serve as mechanical vectors of shigellosis, and rat fleas can transmit bubonic plague and murine typhus. Flies may lay their eggs on human flesh, and their developing larvae, or maggots, can invade subcutaneous tissues and penetrate external body cavities, such as the orbits, ears, and nares. Flea larvae can also burrow into subcutaneous tissues to feed, complete their developmental stages or instars, and promote secondary infections with incapacitating sequelae, including autoamputation of toes and fingers, especially in impoverished tropical communities plagued by endemic jigger fleas *(Tunga penetrans)*.

MYIASIS

Myiasis is an ectoparasitic infestation of viable or necrotic tissues by the dipterous larvae of higher flies and may be broadly classified as obligatory or facultative myiasis. In obligatory myiasis, maggots must live and feed on human or animal hosts as part of their life cycle. In facultative myiasis, normally free-living maggots that preferentially feed on carrion and decaying matter attack and feed on the necrotic sores and wounds of living human and animal hosts. Maggot therapy with blowfly larvae is still used today to débride necrotic wounds.

Myiasis may be further stratified clinically as furuncular (subcutaneous) myiasis, wound (superficial cutaneous) myiasis, cavitary (atrial or invasive) myiasis, intestinal myiasis, urinary myiasis, and vaginal myiasis.

Furuncular myiasis is the most common clinical manifestation of myiasis and occurs when one or more larvae penetrate the skin, causing pustular lesions that resemble boils or furuncles. Larval maggots can also infest external orifices, sores, or open wounds, causing cavitary and wound myiasis. Cavitary myiasis is usually caused by screwworm larvae that can penetrate festering wounds or invade the orbits, nostrils, or external ear canals (Figs. 294.1 and 294.2). Intestinal myiasis is uncommon, usually caused by the accidental ingestion of maggot-contaminated food, and characterized by self-limited nausea, vomiting, and diarrhea. Genitourinary myiasis is also uncommon and may present as dysuria, hematuria, and pyuria, after larval invasion of the urethra (urinary myiasis) or vagina (vaginal myiasis).

Although there are many families of dipterous flies (order Diptera), flies from three families cause most human and animal myiasis: Oestridae, or botflies; Calliphoridae, screwworms and blowflies; and Sarcophagidae, carrion-feeding flies. The most common myiasis-causing fly species are classified taxonomically and stratified by clinical type of myiasis infestation in Table 294.1.

Epidemiology

In a retrospective epidemiologic study in Rio de Janeiro, Marquez and colleagues[1] described 71 patients with furuncular and cavitary myiasis during the period 1999–2003. Myiasis was more prevalent among adults older than 51 years (42%) and children younger than 10 years (34%). Most of the population was male (61%) and impoverished (62%). The predominant causative agent of furuncular myiasis was *Dermatobia hominis,* the New World human botfly, and the predominant causative agent of cavitary myiasis was *Cochliomyia macellaria,* an indigenous species of New World screwworm. The authors concluded that myiasis is an opportunistic infestation of disadvantaged vulnerable populations living in nonhygienic conditions. In a similar retrospective collective analysis, Jiang[2] described 54 cases of human myiasis in China from 1995–2001. Although the Chinese cases were equally distributed between genders, most cases occurred in infants and children (72%) and were described as either hypodermic-invasive ($n = 31$) or ocular ($n = 12$). In another collective review, Schwartz and Gur[3] reported 12 cases of furuncular myiasis caused by *D. hominis,* the human botfly, in 12 Israeli travelers returning from four South American countries in the Amazon Basin. Clyti and coworkers[4] described an epidemic of human botfly furuncular myiasis in 30 patients living in coastal urban communities of French Guiana between January and March 2000 after heavy rainfall that accelerated soil maturation of pupae into adults.

Tamir and associates[5] reported two cases of furuncular myiasis caused by *Cordylobia rodhaini,* the Lund fly, in Israeli travelers returning from Ghana. In addition to *D. hominis* and *Cordylobia* spp., *Cuterebra* spp. of botflies can also cause furuncular myiasis in North America and throughout Africa and Asia (Fig. 294.3). Shorter and coworkers[6] reported two cases of *Cuterebra* spp. botfly-induced furuncular myiasis in children in New England (see Fig. 294.3). *Cuterebra* or rabbit botflies are endemic in the northeastern United States and southeastern Canada, where they have established a zoonotic reservoir in lagomorphs (rabbits and hares) and cause periodic cluster outbreaks of furuncular myiasis in domestic animals and humans.[7]



FIG. 294.1 Forearm exit site wound of a third-stage (instar) New World screwworm fly larva or maggot, *Cochliomyia hominivorax.* *((From Seppänen M, Virolainen-Julkunen A, Kakko I, et al. Myiasis during adventure sports race.* Emerg Infect Dis. *2004;10:137–139.)*



FIG. 294.2 16-mm–long third-stage (instar) New World screwworm fly larva or maggot, *Cochliomyia hominivorax.* This had just emerged from a forearm exit wound (see Fig. 294.1) after tissue-feeding in a traumatic wound for 9 days. *(From Seppänen M, Virolainen-Julkunen A, Kakko I, et al. Myiasis during adventure sports race.* Emerg Infect Dis. *2004;10: 137–139.)*

TABLE 294.1 Myiasis-Causing Flies

FAMILY (COMMON FAMILY NAME)	TAXONOMIC CLASSIFICATION	COMMON NAME	GEOGRAPHIC DISTRIBUTION	TYPE OF MYIASIS INFESTATION
Oestridae (botflies)	*Dermatobia hominis*	New World botfly	Caribbean, Central and South America	Furuncular
	Cuterebra spp.	Rodent and rabbit botflies	North America, northern Central America	Furuncular
	Hypoderma sinense	Indian botfly	India	Furuncular
Calliphoridae (screwworm flies, blowflies)	*Cordylobia anthropophaga*	Tumbu fly	Africa	Furuncular
	Cordylobia rodhaini	Lund fly	Africa	Furuncular
	Auchmeromyia senegalensis	Congo floor mat fly	Africa	Superficial cutaneous (no tissue invasion)
	Cochliomyia hominivorax	New World screwworm	Southern North America, Central and South America	Wound, cavitary
	Chrysomyia bezziana			Wound, cavitary
	Lucilia spp.	Old World screwworm	Africa, Asia	Wound, cavitary
	Calliphora spp.	Greenbottle blowflies	Worldwide	Wound (used for maggot therapy)
		Bluebottle blowflies	Worldwide	Wound (used for maggot therapy)
Sarcophagidae (carrion flies)	*Sarcophaga carnaria*		Africa	Wound, cavitary, gastrointestinal
	Wohlfahrtia magnifica		Africa	Wound, cavitary, gastrointestinal



FIG. 294.3 First-stage (instar) larva of a *Cuterebra* spp. botfly native to North America. Note the rows of anterior hooklets that can anchor the feeding larva to the dermis. *(From Centers for Disease Control and Prevention. Public health image library: ID #1427; page last reviewed December 20, 2017. https://phil.cdc.gov/details_linked.aspx?pid=1427. Accessed June 4, 2019.)*

Although dermatoses are among the leading medical complaints in returning travelers, myiasis and tungiasis remain among the least frequently diagnosed dermatologic conditions. However, recently reported case series of myiasis and tungiasis in returning travelers have provided new epidemiologic analyses of the frequencies, distributions, and risk factors for these infections.[8,9] In a retrospective observational study of 90 returning Israeli travelers with myiasis over the reporting period 1999–2014, most cases were caused by *D. hominis* and acquired in Latin America ($n = 72$, 80%), and fewer cases were caused by *Cordylobia* spp. and acquired in Africa ($n = 18$, 20%).[8] Manual extraction was sufficient to remove the embedded larvae in most cases (76%), with fewer cases (24%) requiring surgical extraction.[8] Most patients did not receive antibiotic prophylaxis, and only one patient developed a secondary infection after partial removal of the larva.[8] The authors concluded that myiasis is not a rare dermatoses in returning travelers, with most cases acquired in Latin America not Africa, and treatment should include full larval extraction without antibiotic prophylaxis.[8]

In an outbreak of tungiasis in 13 travelers returning from Madagascar, walking barefoot (100% of cases) and/or wearing open sandals (62% of cases) were the highest risk factors for tungiasis, with a mean clinical incubation period of 15 days or longer than observed in prior case series (7–12 days).[9] Observations such as these in returning travelers have provided clinicians with a better understanding of the causes, clinical incubation periods, and treatments of these infections, which are less rare than generally suspected.[10]

Clinical Manifestations

The most common forms of human myiasis worldwide are furuncular myiasis and cavitary (invasive) myiasis. Furuncular myiasis is most often caused by subcutaneous larval invasion by the tumbu fly, *Cordylobia anthropophaga,* in Africa, and the New World human botfly, *D. hominis,* in the subtropical and tropical areas of the Americas (see Table 294.1). Cavitary myiasis is usually caused by zoonotic screwworm larval deposition in open wounds or external orifices, such as the nares, ears, and orbits, and may be characterized by deep tissue larval invasion, with secondary infection and extensive tissue necrosis. *Cochliomyia hominivorax,* the New World screwworm, is a common cause of cavitary myiasis in the Americas, and *Chrysomyia bezziana,* the Old World screwworm, is a common cause of cavitary myiasis in Africa, Asia, and Indonesia.[11] Cavitary myiasis must be managed aggressively with surgical débridement and antibiotic therapy for secondary infections to limit tissue damage and disfigurement (see Figs. 294.1 and 294.2).[11]

Although the clinical manifestations, treatments, and prevention strategies are similar in furuncular myiasis, the mechanisms of larval fly invasion are often different. The gravid female tumbu fly deposits its eggs on moist sandy soil or on wet clothing (e.g., cloth diapers) hung outside to dry. When the human victim dons egg-infested clothing, larvae emerge and rapidly burrow into the skin with sharp mandibles for further development. On the other hand, the female botfly captures blood-feeding insects, usually mosquitoes, in midflight and attaches her eggs to the undersurface of the insect.[12] The intermediate biting vector then delivers the botfly eggs to its blood meal victims, where the eggs hatch immediately and release their larvae to feed on warm-blooded hosts. Human botfly larvae then rapidly burrow into the skin with sharp mandibles to begin their developmental instar stages, which can last 6 to 12 weeks.[12]

In addition to travel history in endemic regions, the mechanisms of larval fly invasion assist in differentiating the cause of furuncular myiasis. In tumbu fly (*C. anthropophaga)* myiasis, lesions are usually located on body regions covered by clothing, such as the buttocks and trunk. In New World human botfly *(D. hominis)* myiasis, lesions are usually located on exposed areas, such as the scalp, face, and extremities.

After completing three instar stages, the final larval forms of the tumbu fly and human botfly wriggle out of their draining, boil-like, 1- to 2-cm furuncular swellings; drop to the ground; and pupate in warm, moist soil into adult flies within 9 to 14 days. Victims may recall a flying insect bite that preceded human botfly-induced furuncular myiasis. While developing in their furuncles, larvae are active, protrude intermittently through draining wounds, and maintain surface contact for respiration with their posterior, paired spiracles.[13] Anterior hooklets anchor the maggots in place subcutaneously, making manual removal, even with forceps, difficult (see Fig. 294.3).[13]

Therapy

Management strategies for furuncular myiasis include coaxing embedded larvae from furuncles by smothering their respiratory spiracles, often visible in lesions, with occlusive coatings of petrolatum (Vaseline), clear fingernail polish, tobacco tar, pork fat, raw beefsteak, or bacon strips.[13] The injection of lidocaine into draining lesions has also been recommended as a successful extraction technique.[14] Nevertheless, unsuccessful occlusive therapy may asphyxiate larvae and necessitate their surgical or vacuum extraction.[15] Along with larval removal, myiasis wounds should be cleansed and conservatively débrided, tetanus prophylaxis administered, and bacterial secondary infections treated with antibiotics. Although *Clostridium tetani* infection of penetrating wounds does occur, tetanus has not been reported in myiasis but has been reported after ectoparasitic infestations with *T. penetrans,* the chigoe (jigger) flea, in Africa and South America.[16]

Prevention and Control

Prevention and control strategies for myiasis include the following: (1) control of domestic and livestock animal larval infestations; (2) sanitary disposal of animal carcasses and offal to deny flies their preferred breeding grounds; (3) proper management of any open human wounds or cutaneous infections; (4) cementing floors to deny floor maggot flies their preferred egg-laying surfaces; (5) sleeping on raised beds or cots in screened huts or tents; (6) wearing long-sleeved shirts and pants, which can be pyrethrin or pyrethroid impregnated; (7) spraying exposed skin with diethyl toluamide (*N,N*-diethyl-meta-toluamide [DEET])–containing repellents; and (8) ironing both sides of all clothes and diapers left outside to dry in tumbu fly habitats.

FLEA INFESTATIONS

Fleas of the insect order Siphonaptera are a small group of morphologically similar wingless ectoparasites of warm-blooded animals, including humans. They are not only biting nuisances but also competent vectors of infectious diseases, most notably *Yersinia pestis* and murine typhus (Table 294.2). Although fleas are often classified by host specificity (or presence of head combs), all fleas can rapidly adapt from animal to nearby human hosts, especially if preferred hosts are exterminated by disease or pesticides. Fleas undergo complete metamorphosis from egg to adult stages, with larvae, pupae, and adults exhibiting different morphologies and preferred habitats. Signaled by vibrations and locally rising carbon dioxide levels, adult fleas emerge from egg cases within weeks, leap onto the closest mammalian hosts, and begin blood-feeding and reproducing.

TABLE 294.2 Flea-Transmitted Infectious Diseases

INFECTIOUS DISEASE	CAUSATIVE AGENT	FLEA VECTOR (COMMON NAME)	ANIMAL RESERVOIR	MAJOR CLINICAL MANIFESTATIONS	THERAPY
Bubonic plague	*Yersinia pestis*	*Xenopsylla cheopis* (rat flea); *Oropsylla montana* (squirrel flea)	Rodents (rats, prairie dogs, squirrels); domestic animals (cats > dogs)	Headache, fever, chills, regional lymphadenopathy—draining buboes, pneumonitis (secondary plague pneumonia), septicemia, meningitis; case-fatality rate, 14%	Antibiotic therapy recommended within 24 h with any of the following effective antibiotics: tetracyclines, gentamicin, streptomycin, chloramphenicol
Murine typhus	*Rickettsia typhi*	*Xenopsylla cheopis* (rat flea); *Nosopsyllus fasciatus* (northern rat flea); *Oropsylla montana* (squirrel flea)	Rodents (rats and mice)	Fever, headache, maculopapular rash, thrombocytopenia, rarely pneumonitis and encephalitis	Doxycycline, 100 mg bid × 7–10 days
Flea-borne spotted fever	*Rickettsia felis*	*Ctenocephalides felis* (cat flea)	Rodents (rats, mice, opossums)	Nonspecific fever, headache, maculopapular rash	Tetracyclines (doxycycline)
Cat-scratch fever (disease)	*Bartonella henselae*	*Ctenocephalides felis* (cat flea)	Feral cats (kittens)	Low-grade fever, malaise, regional and rarely multifocal lymphadenopathy, endocarditis; complications more common in HIV infection and include bacillary angiomatosis, peliosis hepatis, neuroretinitis, and encephalopathy	Doxycycline and macrolides (azithromycin) effective; add rifampin for complications
Tungiasis—portal of entry for *Clostridium tetani*	Ectoparasite	*Tunga penetrans* (chigoe or jigger flea)	Humans; domestic animals (dogs > cats and pigs)	Painful white papules with central black pits discharging eggs and feces with lateral pressure, especially on dorsal aspects of toes under toenails and on heels	Surgical extraction of gravid female fleas; ivermectin ineffective in humans and only partially effective in dogs for jigger flea management
		Tunga trimamillata (only in Ecuador and Peru)		Same	Same

HIV, Human immunodeficiency virus.

TUNGIASIS

A currently reemerging, combless, ectoparasitic flea, *T. penetrans,* the chigoe or jigger sand flea, is endemic in the Caribbean and South America, where it originated, and in sub-Saharan Africa, where it was introduced. Tungiasis, a painful, cutaneous infestation with the gravid female jigger flea, is now hyperendemic in underprivileged communities in Africa, South America, and the Caribbean; has successfully reemerged in Mexico and Central America; and has been increasingly reported in travelers returning from subtropical and tropical areas worldwide.[17–23]

Epidemiology

In travelers returning to accessible health care infrastructures in developed nations, tungiasis is an exotic infestation, with a minimal parasite burden and a simple surgical cure. However, in the impoverished and underserved communities of developing tropical nations, tungiasis is a recurrent infestation with a high parasite burden, causing significant morbidity. In a descriptive study of greater than 90% of a population of a poor fishing village in Brazil, Muehlen and colleagues[20] found a 51.3% point prevalence of tungiasis, with more males (54%) infested than females, and prevalence peaks in children aged 5 to 9 years and adults aged 60 years and older. In a point prevalence study in another poor community in northeastern Brazil, Ariza and associates[21] examined 142 persons with jigger flea superinfestations and counted a total of 3445 lesions on feet (median, 17 lesions; maximum, 18 lesions). Greater than 70% of patients presented with foot pain, 59% complained of difficulty walking, 46% had toenail loss, 42% had foot abscesses, and 25% had deformed toes. In a regional prevalence study of five towns in southwestern Trinidad, Chadee[22] found the prevalence of tungiasis to range from 15.7% to 17.9%, with feet more often infected than other anatomic regions, males more likely infested than females, and higher parasite burdens in males (5.44 ± 2.54 fleas) than females (2.38 ± 2.00 fleas). In a 2007 cross-sectional study of 142 households in a rural community in western Nigeria, Ugbomoiko and associates[23] reported a 45.2% point prevalence of tungiasis, with 95% of the lesions on the feet, no gender difference in prevalence, but prevalence peaks between ages 6 and 14 years and at age 60 years and older. Ectopic lesions of the elbows and hands occurred in 10% of the population. In a study of the ectopic localization of tungiasis among 1184 residents of a poor community in northeastern Brazil with a 33.6% point prevalence of tungiasis, Heukelbach and coworkers[24] reported that 6% of all lesions presented at sites other than feet, most commonly on the hands (5.5%) but also on the buttocks, elbows, and thighs (Fig. 294.4).



FIG. 294.4 Three periungual lesions of tungiasis on the index finger of a 6-year-old girl. These were caused by tissue-feeding gravid female jigger fleas, *Tunga penetrans. (From Feldmeier H, Eisele M, Sabóia-Moura RC, et al. Severe tungiasis in underprivileged communities: case series from Brazil.* Emerg Infect Dis. *2003;9:949–955.)*



FIG. 294.5 Periungual lesion of tungiasis on the fourth toe of a 50-year-old woman. This was caused by a tissue-feeding gravid female jigger flea, *Tunga penetrans*. Note the elevation of the nail bed by the lesion. *(From Feldmeier H, Eisele M, Sabóia-Moura RC, et al. Severe tungiasis in underprivileged communities: case series from Brazil.* Emerg Infect Dis. *2003;9:949–955.)*



FIG. 294.6 The right foot of a 50-year-old man suffering from recurrent tungiasis. Note that all nails have been lost in the recurrent infestations and nonhealing wounds remain on all toes. The patient had chronic pain and could only wear slippers and walk with difficulty. *(From Feldmeier H, Eisele M, Sabóia-Moura RC, et al. Severe tungiasis in underprivileged communities: case series from Brazil.* Emerg Infect Dis. *2003;9:949–955.)*

In French Guiana and Brazil the most important zoonotic reservoir of flea-transmitted tungiasis was in domestic and stray dogs.[25,26] In a comparison of the prevalence of tungiasis in animals and humans in an impoverished Brazilian community, Pilger and colleagues[26] reported a human prevalence of 39%, a combined domestic cat and dog prevalence of 59%, and a higher prevalence (42%) of tungiasis in households with infested dogs and cats. By comparison, Sanushi and associates,[27] in a review of 14 cases of tungiasis in travelers returning to the United States, reported that patients manifested at most two lesions and complained only of local pain and itching.

Clinical Manifestations

Tungiasis is caused by the dermal penetration of the gravid female jigger (chigoe) flea to feed on blood and tissue juices, usually on the feet (or heels), under or near the toenails, or in the interdigital web spaces (Fig. 294.5).[17] Although the smallest of flea species (1 mm long or shorter), within days the gravid female swells with hundreds of developing eggs to 2000 times its size, expelling eggs over a period of 3 weeks or less, and then dying and leaving its shriveled carcass in a contaminated wound tract.[17] Initially, the embedded jigger flea produces a subcutaneous papule or vesicle 6 to 8 mm in diameter, with a central black dot pinpointing the exteriorized segments, including the anus, genital opening, and breathing spiracles (see Figs. 294.4 and 294.5). The papule darkens with intralesional hemorrhage and, if squeezed, extrudes eggs, feces, and internal organs through exteriorized posterior abdominal segments. The differential diagnosis of tungiasis includes bacterial skin infections (impetigo), bacterial and fungal paronychia, cercarial dermatitis, fire ant bites, folliculitis, and scabies. The complications of tungiasis include septicemia, abscesses, fissures, toenail (fingernail) loss, necrotic ulcers, osteomyelitis, and eventual autoamputation of toes and, less often, fingers (Fig. 294.6).[17] Tungiasis has been associated with lethal tetanus in nonvaccinated individuals and was identified as the place of entry for 10% of tetanus cases in São Paulo in a 1991 study.[16,28]

Therapy

Management strategies for tungiasis include extracting all embedded fleas immediately with sterile needles or curets, administering tetanus prophylaxis, and treating secondary wound infections with appropriate topical or oral antibiotics. Other than surgical extraction, there are no therapeutic options for tungiasis. There remains a definite need for an effective antiparasitic drug treatment option for tungiasis, especially in superinfestations.[29] In a double-blinded, randomized, placebo-controlled trial, Heukelbach and coworkers[29] have shown that a single dose of oral ivermectin, 300 µg/kg repeated at 24 hours, has no clinical efficacy compared with placebo as measured by parasite signs of viability or death.

Prevention and Control

In addition to wearing shoes, which can be sprayed with pyrethroid or DEET-containing solutions, and not sitting naked on bare ground, preventive strategies for tungiasis include the following: (1) insecticide treatment of flea-infested domestic and stray animals and pets with 10% pyrethrin or pyrethroid sprays or 1% to 4% malathion powder; (2) bathing the feet of domestic and stray dogs and pigs with insecticide solutions, such as 2% trichlorfon; and (3) spraying or dusting households, especially those with dirt floors, with 1% to 4% malathion. Other strategies for the environmental control of jigger fleas include improved stray animal control, especially for cats and dogs; providing cement foundation or slab flooring for dirt-floored homes or building raised homes with solid floors; discouraging stray dogs and cats and other domestic animals, especially pigs, as indoor pets; and spraying rodent and stray animal runways and paths, household unpaved walkways, and dirt floors with solutions containing kerosene, fuel oil, 1% lindane, 1% to 4% malathion, or 2% trichlorfon.

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The complete reference list is available online at Expert Consult.

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295 Mites, Including Chiggers

James H. Diaz

SHORT VIEW SUMMARY

Definition
- Mites are among the smallest arthropods, with more than 3000 species, only about 25 of which, mostly chigger, animal, plant, and scabies mites, are of any medical importance.

Epidemiology
- Most mites are simply biting nuisances that can cause highly symptomatic maculopapular eruptions and do not transmit infectious diseases.

Microbiology
- Only biting larvae of Asian scrub typhus chiggers (*Leptotrombidium* spp.) can transmit scrub typhus caused by *Orientia tsutsugamushi* (formerly *Rickettsia tsutsugamushi*), and only biting house mouse mites *(Liponyssoides sanguineus)* can transmit rickettsialpox caused by *Rickettsia akari.*

Diagnosis
- Clinical diagnoses are highly sensitive and specific in the presence of a mite-bite outbreak.
- Mite-transmitted scrub typhus and rickettsialpox manifest clinically in a similar fashion with fever, bite eschar, regional lymphadenopathy, conjunctival injection, central nervous system symptoms in severe scrub typhus (e.g., confusion, delirium, coma, or transient hearing loss), and centrifugal rash in scrub typhus (see Chapter 193).

Therapy
- Treatment of chigger bites is supportive with soap and water cleansing, warm water soaks, and topical and local anesthetics and antihistamines. Impetigo and secondary infections are potential complications that would necessitate antibiotic treatment.
- Both scrub typhus and rickettsialpox respond to treatment with oral doxycycline.

Prevention
- Chigger bites can be prevented with campsite spraying of pyrethrin or pyrethroid-containing insecticides; by spraying or impregnating pyrethrin and pyrethroid-containing repellents on clothing and sleeping bags; and by applying diethyltoluamide–containing insect repellents (*N,N*-diethyl-meta-toluamide [DEET]) to exposed skin.
- Rickettsialpox can be prevented by improving rodent reservoir control in campgrounds, homes, apartments, barns, sheds, and, especially, crowded public housing.
- There are no vaccines for scrub typhus or rickettsialpox.
- Weekly doses of 200 mg of doxycycline can prevent *O. tsutsugamushi* infections in endemic regions.

Mites, including chigger and scabies mites, are among the smallest arthropods, with most barely visible without magnification. Only about 20 species of the more than 3000 species of chigger, animal, plant, and scabies mites are of any medical importance, and most of these are simply biting nuisances and do not transmit infectious diseases.[1] Mites are closely related to ticks but not as prodigious at blood-feeding. They also do not transmit as broad a range of infectious microbial diseases as ticks. The most serious diseases transmitted by mites are scrub typhus and rickettsialpox.

Only biting larvae of Asian scrub typhus chiggers (*Leptotrombidium* spp.) can transmit scrub typhus caused by *Orientia tsutsugamushi* (formerly *Rickettsia tsutsugamushi*), and only biting house mouse mites *(Liponyssoides sanguineus)* can transmit rickettsialpox caused by *Rickettsia akari*. Both scrub typhus mites and house mouse mites are, like ticks, capable of inheriting bacterial infections by transovarial transmission and maintaining infections in several mite generations as bacteria are passed from adult to juvenile stages (nymphs and larvae) by transstadial transmission. Originally considered vectors of a rodent zoonosis, scrub typhus chiggers are the main environmental reservoirs of *O. tsutsugamushi* in endemic regions, with much smaller secondary reservoirs in wild rodents.[1] Common house mice are the zoonotic reservoirs of *R. akari,* not only in crowded urban apartment buildings in the United States but also in mice-infested buildings, such as sheds and barns, in more rural locations worldwide.[2]

MITE TAXONOMY AND ECOLOGY

Mites may be commonly classified as scabies mites (see Chapter 293), trombiculid or chigger mites (also called chiggers, red bugs, and itch mites), human follicle mites, dust mites, and a variety of animal, plant, and wood mites (Table 295.1). All mite species develop close generational associations with their ecosystems and zoonotic reservoirs, often referred to as mite islands.[1] Mite islands usually border cleared land and scrub bush and have several habitat requirements, including grassy vegetation with warm soil temperatures and high humidity, frequently visiting rodent hosts to feed larvae, and sufficient small insect fauna to feed nymphs and reproducing adults. Humans stumbling onto mite islands are at significantly higher risk for multiple larval chigger bites or trombidiosis worldwide or scrub typhus in the endemic regions of Eurasia and Asia.

EPIDEMIOLOGY AND OUTCOMES OF MITE INFESTATIONS

Among the trombiculid chiggers (the Trombiculidae), including the scrub typhus–transmitting *Leptotrombidium* spp., only the larvae are human and animal ectoparasites. The larger chigger nymphs and adults are free living and feed on small insects and their eggs. All trombiculid larvae exhibit a unique method of feeding on their human hosts and transmitting salivary secretions, which may contain *O. tsutsugamushi* in endemic regions. When larval mites have selected a human host, they will congregate where the skin is soft, warm, and moist, particularly where clothing is tight against the skin, such as under waistbands, undergarment elastic bands, and socks. Initially painless, chigger bites cluster in these regions on the genitalia, perineum, thighs, buttocks, waist, and ankles and become symptomatic in 3 to 6 hours (Fig. 295.1). Larvae pierce the skin with sharp mouthparts and inject tissue-dissolving saliva to create a pool of lymph, other body fluids, and dissolved epithelial cells to drink from (see Fig. 295.1). Unlike ticks, mites are not blood feeders, but tissue juice feeders. The repeated injection of saliva into the bite wound induces a host reaction that forms a strawlike hollow tube, known as a hypostome or stylostome, which extends downward into the host's skin, anchoring the mite firmly.[1,3] Some trombiculid larvae remain attached to and feeding on human hosts for up to a month, but the larval vectors of scrub typhus feed only for 2 to 10 days before dropping to the ground engorged and ready to mature into free-ranging nymphs.

TABLE 295.1 Mites of Medical Importance

FAMILY, GENUS, SPECIES	COMMON NAMES (PLANT OR ANIMAL MITE)	GEOGRAPHIC DISTRIBUTION	MAINTENANCE IN NATURE	CLINICAL MANIFESTATIONS	INFECTIOUS DISEASE TRANSMISSION
Sarcoptidae					
Sarcoptes scabiei var. *hominis*	Scabies (itch) mite (human mite)	Worldwide	Obligate ectoparasite of humans, human reservoir	Classic scabies Atypical scabies	None
Trombiculidae					
Neotrombicula autumnalis	European harvest mite (animal mite)	Europe	Free-living ectoparasites of small mammals and birds	Scrub itch (trombidiosis)	None
Eutrombicula alfreddugesi	American chigger mite (animal mite)	Western Hemisphere	Free-living ectoparasites of small mammals and birds	Scrub itch (trombidiosis)	None
Eutrombicula sarcina	Asian chigger mite (animal mite)	Asia, Australia	Free-living ectoparasites of small mammals and birds	Scrub itch (trombidiosis)	None
Leptotrombidium deliense	Asian rodent chigger (animal mite)	Southeast Asia, Japan, Philippines, South Pacific, Australia	Free-living ectoparasites of rodents and insectivores, transovarial/transstadial passage of infectious disease agent	Scrub typhus (tsutsugamushi disease)	Orientia tsutsugamushi (formerly Rickettsia tsutsugamushi), causative agent of scrub typhus
Leptotrombidium akamushi, *Leptotrombidium pallidum*, and *Leptotrombidium scutellaris*	Japanese rodent chiggers (animal mites)	Japan	Same	Same	Same
Leptotrombidium arenicola and *Leptotrombidium fletcheri*	Malaysian rodent chiggers (animal mites)	Malaysia	Same	Same	Same
Leptotrombidium pavlovskyi	Russian rodent chigger (animal mite)	Far east of former Soviet Union	Same	Same	Same
Demodicidae					
Demodex folliculorum	Hair follicle mite	Worldwide	Obligate ectoparasite of man, human host reservoir in hair follicles	Benign follicular (scaling) dermatitis Chronic blepharitis (demodicidosis)	None
Demodex brevis	Sebaceous gland mite	Worldwide	Obligate ectoparasite of man, human host reservoir in sebaceous glands	May potentiate granulomatous acne	None
Pyroglyphidae					
Dermatophagoides pteronyssinus	European house dust mite (human mite)	Worldwide	Free-living ectoparasites of man; live in human bedrooms, especially in mattresses; feed on human skin detritus	House dust mite allergies and asthma	None
Dermatophagoides farinae	American house dust mite (human mite)	Worldwide	Same	Same	None
Dermanyssidae					
Liponyssoides sanguineus (formerly *Allodermanyssus sanguineus*)	House mouse mite	North America, Northern Europe, Asia, and Africa	Free-living ectoparasites of field mice, transovarial/transstadial passage of infectious disease agent	Rickettsialpox	Yes (Rickettsia akari)
Dermanyssus gallinae	Red poultry (chicken) mite	Worldwide	Free-living ectoparasites of domestic and wild birds	Poultry workers' dermatitis of hands	None
Macronyssidae					
Ornithonyssus bacoti	Tropical rat mite	Temperate and tropical regions worldwide	Free-living ectoparasites of large rodents: *Rattus rattus, Rattus norvegicus*	Urticarial papulovesicular to pustular dermatitis	None
Ornithonyssus bursa	Tropical fowl mite	Same	Free-living ectoparasites of domestic and wild birds	Pruritic papules in a scabietic distribution: finger webs, axillae, groin, buttocks	None
Laelapidae					
Laelaps echidnina	Spiny rat mite	Worldwide, the most prevalent rodent mite species in the United States	Free-living ectoparasites of large rodents: *Rattus rattus, Rattus norvegicus*	Nonspecific mite-bite dermatitis	None

TABLE 295.1 Mites of Medical Importance—cont'd

FAMILY, GENUS, SPECIES	COMMON NAMES (PLANT OR ANIMAL MITE)	GEOGRAPHIC DISTRIBUTION	MAINTENANCE IN NATURE	CLINICAL MANIFESTATIONS	INFECTIOUS DISEASE TRANSMISSION
Pyemotidae					
Pyemotes tritici ventricosus	Grain (hay) itch mite	Worldwide	Free-living ectoparasites of straw-, hay-, grain-, and rice-eating moths, beetles, weevils	Grain workers' pruritic vesicular eruption	None
Pediculoides ventricosus Newport	European wood beetle itch mite	Worldwide	Free-living ectoparasites of straw-, hay-, grain-, and rice-eating moths, beetles, weevils	Solitary to multiple highly erythematous pruritic macules, some of which have attached macular tracts resembling comet tails	None
Pyemotes herfsi	Oak leaf gall mite	Europe, introduced into the United States	Free-living ectoparasites of gall-making larvae of oak trees	Pruritic, erythematous, vesicular eruptions of limbs, face, and neck	None
Acaridae[a]					
Carpoglyphus lactis	Cheese and dried fruit mites	Worldwide	Free-living ectoparasites of cheeses and dried fruits	Cheese and fruit workers' dermatitis	None
Tyrophagus putrescentiae	Copra (dried coconut meat or kernel) mite	Copra (dried coconut meat or kernel) growing areas	Free-living ectoparasites of coconut copra	Copra itch	None
Glycyphagidae[a]					
Glycyphagus domesticus	Grocer's mite	Worldwide	Free-living ectoparasites of fruits and vegetables	Grocer's itch	None
Glycyphagus destructor	Hay mite	Worldwide	Free-living ectoparasites of cut hay	Hay workers' and hay wagon riders' allergy, asthma, rhinitis, conjunctivitis	None

[a]The acarid and glycyphagid plant mites may rarely enter the gastrointestinal tract if swallowed with food and cause intestinal distress (gastrointestinal acariasis). They may also be inhaled in aerosols and cause bronchial irritation and respiratory distress (respiratory acariasis). They may crawl into the urethra to cause dysuria (urinary acariasis). The mites can be recovered from feces, sputum, and urine. Treatment is supportive. Although they may rarely infest humans, plant mites are all free living and do not reproduce in human dead-end hosts.



FIG. 295.1 An intensely pruritic red bleb 24 hours after a bite inflicted by a larval trombiculid species chigger mite. Although these species of lymph-sucking mites do not transmit infectious diseases in the United States, many species of larval trombiculids transmit scrub typhus or tsutsugamushi disease, caused by the rickettsial microorganism *Orientia* (formerly *Rickettsia*) *tsutsugamushi,* throughout Southeast Asia and the western Pacific region. *(From Centers for Disease Control and Prevention [CDC], Atlanta, GA. CDC Public Health Image Library, image 3806.)*

All of the noninfectious chigger larvae can cause trombidiosis or trombiculiasis (trombiculidiasis), with the American chigger mite *(Eutrombicula alfreddugesi)* being the most common culprit in the United States; the European harvest mite, *Neotrombicula autumnalis,* the most common culprit in Europe; and the Asian chigger, *Eutrombicula sarcina,* the most common culprit in Asia. In the United States, clusters of trombiculid larval bites are often referred to as "chiggers," a colloquial term for the intensely pruritic groupings of erythematous welts inflicted by American chigger mites. In Asia and Australia, similar clusters of larval mite bites may be referred to as "scrub itch," especially in Australia, where scrub itch mites, *Trombicula hirsti,* commonly cause trombidiosis. Among the scrub typhus–carrying *Leptotrombidium* larval chigger mites, *Leptotrombidium deliense,* the Asian rodent chigger, is a principal vector throughout eastern Asia and Eurasia.

Unlike North American "chiggers," scrub typhus is a serious and potentially fatal zoonotic infectious disease caused by *O. tsutsugamushi* organisms transmitted by bites from infected *Leptotrombidium* species chigger larvae. As a result of experiences with scrub typhus among US troops in the Pacific during World War II and later during wars in Korea, Vietnam, and Afghanistan, scrub typhus was originally considered a regional zoonosis confined to the "tsutsugamushi triangle," which extended from Pakistan in the west to the Pacific coast of Russia in the east to northern Australia in the south.[4] In 2006, additional cases were detected in the Middle East caused by a new species, *Orientia chuto*, and in Chile caused by *O. tsutsugamushi.*[4] In 2016, three additional cases of scrub typhus caused by *O. tsutsugamushi* were confirmed serologically (with enzyme-linked immunosorbent assay [ELISA] and indirect immunofluorescent antibody assay [IFA]) and molecularly (with polymerase chain reaction [PCR] detection of DNA in the 47-kD and 56-kD genes) in Chile, which is 12,000 km away from the tsutsugamushi triangle.[4] Although the zoonotic reservoirs and larval mite vectors of scrub typhus outside of the tsutsugamushi triangle remain unidentified, there is now a wider global distribution of the disease than initially assumed.[4]

After *O. tsutsugamushi*–infected *Leptotrombidium* chigger bites, an 8- to 10-day incubation period precedes the onset of classic clinical manifestations of scrub typhus with bite eschar, regional lymphadenopathy, conjunctival injection, central nervous system (CNS) manifestations (e.g., headache, confusion, delirium, transient hearing loss), and centrifugal rash (Fig. 295.2).[5] Many cases are nonclassic with nonspecific

clinical manifestations and go undiagnosed, especially when serologic tests are unavailable. In the temperate regions of Eurasia, there is a definite scrub typhus seasonal transmission cycle determined by peaking temperatures and humidity during weeks of marked seasonal change in late spring to early summer and again in late fall to early winter. In the tropics, scrub typhus transmission occurs year-round. Fatal complications may include adult respiratory distress syndrome (especially in older patients), hypotensive shock, acute renal failure, encephalomyelitis, and disseminated intravascular coagulation.[5]



FIG. 295.2 A transmission electron micrograph of an extracellular *Orientia tsutsugamushi* rickettsial microorganism, the causative agent of scrub typhus or tsutsugamushi disease throughout Southeast Asia and the Western Pacific region. During World War II, scrub typhus was second only to malaria as a cause of hospitalization among US troops in the Pacific theater of operations.[1] *(From Centers for Disease Control and Prevention [CDC], Atlanta, GA. CDC Public Health Image Library, image 8730.)*

Although the most common CNS manifestations of scrub typhus are meningitis or meningoencephalitis, Yun and coauthors recently reported a case of acute transverse myelitis in a 67-year-old man with dysuria and lower-leg weakness associated with serologically confirmed scrub typhus in Korea and successfully treated with doxycycline and corticosteroids.[5]

In 2016, Thipmontree and coauthors reported another very rare complication of scrub typhus in a 74-year-old Thai woman with acute abdominal symptoms requiring surgery, due to a ruptured spleen.[6] In this case, scrub typhus was confirmed by a fourfold increase in immunoglobulin M (IgM) titer by ELISA and positive PCR targeting the 47- and 56-kD genes of *O. tsutsugamushi*.[6] The patient underwent splenectomy and was treated successfully with intravenous chloramphenicol and oral doxycycline over 10 days.[6]

The house mouse mite, *Liponyssoides sanguineus,* maintains a rickettsial zoonosis in its preferred house mouse *(Mus musculus)* reservoir and can transmit rickettsialpox caused by *R. akari* through its bites.[1,3] Although initially described in clusters in crowded apartment complexes in large US cities, including New York, Boston, Cleveland, Philadelphia, and Pittsburgh, rickettsialpox has now been reported in rural areas of the eastern United States and eastern Europe Many experts believe that rickettsialpox is underreported and more widely distributed in silent sylvan cycles worldwide. The incubation period and initial clinical manifestations of rickettsialpox mirror those of scrub typhus, with eschar formation at the bite site within 10 to 12 days followed by fever, chills, severe headache, conjunctival injection, and truncal maculopapular then vesicular rash.[2,7,8] Illness is typically mild, and regional lymphadenopathy is uncommon (Table 295.2). Unlike with scrub typhus, complications are rare, but they may include thrombocytopenia and interstitial pneumonia.[2,8]

TABLE 295.2 Presentation, Diagnosis, Differential Diagnosis, and Management of Mite-Transmitted Scrub Typhus Versus Rickettsialpox

	SCRUB TYPHUS	RICKETTSIALPOX
Bacterial agent	*Orientia* (formerly *Rickettsia*) *tsutsugamushi*[a]	*Rickettsia akari*
Mite vector	Larvae of *Leptotrombidium* species of Asian rodent chigger mites	Common house mouse mites, *Liponyssoides sanguineus*
Incubation period	8–10 days (range, 8–20 days)	10–12 days
Mite-bite site	Painless initial bite, eschar at bite site (50%)	Painless initial bite, eschar at bite site
Presenting constitutional symptoms	Fever, chills, headaches, myalgia, pathognomonic hearing loss (30%)	Fever, chills, severe headache, myalgia
Conjunctival injection	Present	May be present
Regional lymphadenopathy	Present regionally and tender	Usually absent
Associated rash exanthema	Delayed truncal onset, erythematous macules, then maculopapules that spread peripherally	Abrupt truncal onset, erythematous macules that develop central vesicles in crops
Pulmonary findings	Cough, tachypnea, dyspnea, bibasilar rales	Asymptomatic bibasilar rales
Radiographic findings	Infiltrates common	Usually normal
Potential complications	Adult respiratory distress syndromes, acute renal failure, disseminated intravascular coagulation, encephalomyelitis	Thrombocytopenia
Differential diagnosis	Infectious mononucleosis, leptospirosis, tularemia, anthrax, spotted fever rickettsioses	Chickenpox, tick-bite eschar
Diagnostic methods	*Screening:* rapid dipstick recombinant 56-kDa protein antigen test *Serodiagnostic:* indirect immunofluorescent antibody tests, immunoperoxidase assays *Confirmatory:* microscopic isolation of agent from blood or tissues, polymerase chain reaction for agent DNA (or RNA)	*Serodiagnostic:* immunofluorescent antibody assay for IgG to both *R. akari* and *R. rickettsii,* with follow-up cross-adsorption testing for predominant antibodies *Confirmatory:* isolation from skin biopsy specimen
Recommended treatment	Tetracycline 500 mg orally 4 × daily × 1 wk, or doxycycline 100 mg twice daily × 1 wk, or IV chloramphenicol, 50–75 mg/kg/day × 1 wk (only for complicated cases) In childhood and pregnancy, consider the macrolides: azithromycin, clarithromycin, or roxithromycin	Doxycycline 100 mg orally twice daily × 7–10 days
Outcomes/case-fatality rates (%)	1%–15%	<1%

[a]Although initially classified among the *Rickettsia,* the causative agent of scrub typhus, *Orientia tsutsugamushi* (originally *Rickettsia tsutsugamushi*) has now been reclassified into a separate genus, *Orientia,* based on molecular evidence that its cell wall differs significantly from that of *Rickettsia* both ultrastructurally and in its component proteins.[4]

Dermatophagoides spp. dust mites have highly allergenic exoskeletons, body fragments, and feces, all of which can be easily aerosolized during bed making and pillow fluffing. Allergens from living and dead dust mites frequently cause allergic rhinitis and asthmatic bronchitis in predisposed, atopic persons. The American house dust mite, *Dermatophagoides farinae,* is now distributed worldwide, as is the European house dust mite, *Dermatophagoides pteronyssinus.*[3] House dust mites prefer to live in bedrooms year-round, especially in mattresses and carpets in warm, humid homes. They exhibit maximum growth and reproduction during seasonal warming cycles at ambient temperatures at or above 25°C and relative humidity at or above 75%.[3]

Scabies and follicle mites are the only exclusively human ectoparasitic mites and cannot transmit infectious diseases. Less serious but more common than scabies is infection with the human follicle mites: *Demodex folliculorum* inhabits hair follicles, and *Demodex brevis* inhabits sebaceous glands. These diminutive (0.1–0.4 mm) human mites feed on sebum and exfoliated skin while lodged in human hair follicles or sebaceous gland pores.

Demodex mites or face mites are commensal ectoparasites that cluster in hair follicles and sebaceous glands on the nose, eyelids, and nasolabial folds and have even been found living in earwax *(D. folliculorum).* All of the developmental stages of *Demodex* mites occur over an egg-to-egg cycle of 13 to 15 days entirely within hair follicles or sebaceous glands, especially in females overusing cream-based facial cosmetics and adolescents with increased sebaceous gland activity. Other than causing comedones or "blackheads," *Demodex* infections cause few adverse symptoms and rarely need treatment, unless infections are associated with acne, blepharitis, impetigo, rosacea, or seborrheic dermatitis.[1,3] In some cases, infection or demodicosis may be precipitated by immunosuppression from stress, advancing age, or coexisting illness.

Although of limited clinical significance, a number of animal, plant, and wood mite species can cause bothersome erythematous papulovesicular eruptions if encountered. Bites from the red chicken or poultry mite, *Dermanyssus gallinae,* can cause a pruritic dermatitis, usually on the backs of the hands and forearms in poultry workers (Fig. 295.3).[9] Both St. Louis encephalitis virus and western equine encephalitis virus have been isolated from naturally infected red chicken mites, but they are not preferred vectors for these mosquito-borne arboviruses (see Fig. 295.3).[9] Bites from the rat mite *Ornithonyssus bacoti,* which is ubiquitous in the temperate areas of Europe and the Americas, can cause a papulovesicular dermatitis in stockyard and warehouse workers.[9] The rat mite can also transmit *Rickettsia typhi,* the agent of murine typhus from rat to rat, maintaining the rodent zoonosis, but is incapable of human transmission.[9] The bird mite, *Ornithonyssus bursa,* is a common ectoparasite of pigeons worldwide and a frequent cause of mite infections with maculopapular dermatitis of the finger webs and axillae in pigeon breeders and fanciers.[10]

Like chigger mites, the plant and wood mites do not transmit infectious diseases but are common causes of annoying infections with pruritic, erythematous maculopapular rashes on the limbs and face of arborists, landscapers, and campers.

The North American hay itch mite, *Pyemotes tritici ventricosus,* feeds preferentially on the larvae of insects that infest cane, hay, straw, and some grains, especially rice (Fig. 295.4).[11] In 1965, Fine and Scott[11] were the first investigators to describe *P. tritici ventricosus* dermatitis in the southern United States, where hayrides, cane furniture, and straw rugs are popular.[11] Such exposures frequently place patients in skin contact with infested hay, straw, or cane furniture during peak mite feeding and breeding seasons in the spring and summer.[11] Hay itch mite dermatitis is characterized by pruritic, maculopapulovesicular eruptions on the limbs and trunk, which resolve rapidly with topical corticosteroid therapy.[11]

A close relative of the North American hay itch mite, *P. tritici ventricosus,* the oak leaf gall mite *(Pyemotes herfsi),* which preferentially feeds on insect larvae in oak trees, caused an outbreak of plant insect mite dermatitis in the United States in 2004.[12] Over 300 residents of Pittsburg, Kansas, sought immediate medical attention for an intensely pruritic, erythematous maculopapular rash clustering on the face, neck, and limbs (Fig. 295.5).[12] All lesions healed within days after topical treatment with antihistamines and corticosteroids.[12]

Three separate outbreaks of dermatitis affecting over 100 patients caused by the European hay itch mite *(Pyemotes ventricosus)* were reported from Spain in 2000.[13] A similar outbreak, also suggestive of mite bite–induced dermatitis, was reported in southeastern France in 2006.[14] The dermatitis was characterized by solitary to multiple, highly erythematous pruritic macules; some were accompanied by contiguous, linear erythematous macular tracts that resembled comet tails (Fig. 295.6).[14] In a 2007 outbreak investigation of an additional 42 cases of dermatitis with comet tail signs in the same region, Del Giudice and coworkers[15] identified *P. ventricosus* mites as causative agents and described the epidemiology and outcomes of *P. ventricosus* infections in homes and humans.

Most residences of index case patients with *P. ventricosus* dermatitis were infested with live furniture beetles, *Anobium punctatum,* which do not bite or infest humans.[15] However, adult *P. ventricosus* mites, common ectoparasites of furniture beetles, were present in stereomicroscopic examination of wood dust beneath beetle-infested furniture.[15] Confocal laser scanning microscopy (CLSM) of a central microvesicle in a maculopapular lesion on an experimentally infested coinvestigator demonstrated an ovoid foreign body consistent with a *P. ventricosus* mite.[15] Both naturally occurring and experimental infections caused the characteristic maculopapular rash of *P. ventricosus* dermatitis, again associated with comet signs (see Fig. 295.6).[15] Although oral prednisone

ACARINA > DERMANYSSIDAE

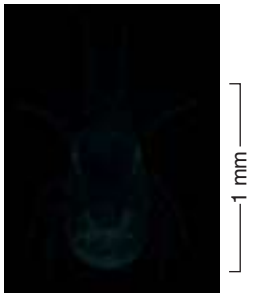

Dermanyssus gallinae (DeGeer)
(female, dorsal aspect)

FIG. 295.3 An illustration of the dorsal aspect of the female red chicken (poultry) mite, *Dermanyssus gallinae,* which is a common cause of poultry workers' and pigeon breeders' dermatitis worldwide. *(From Centers for Disease Control and Prevention [CDC], Atlanta, GA. CDC Public Health Image Library, image 5482.)*

ACARINA > PEDICULOIDIDAE



Pyemotes ventricosus
(Newport)
(female, dorsal aspect)

FIG. 295.4 An illustration of the dorsal aspect of the female grain (hay) itch mite, *Pyemotes tritici ventricosus* (formerly *Pyemotes ventricosus*), which is a common cause of highly pruritic and papulovesicular rashes among hay threshers, hay wagon riders, outdoor yard workers, and campers during late summers worldwide. A similar plant mite, *Pyemotes herfsi,* the European oak leaf gall mite, caused an outbreak of pruritic, erythematous papulovesicular rashes, inducing 300 residents of Pittsburg, Kansas, to seek medical care in late August 2004.[8] *(From Centers for Disease Control and Prevention [CDC], Atlanta, GA. CDC Public Health Image Library, image 5482.)*

(0.5 mg/kg) rapidly relieved pruritus, *P. ventricosus* dermatitis would persist or recur in index case patients until beetle-infested furniture was removed from households or patients permanently vacated their infested residences, which were often in resort regions.[15]

DIAGNOSIS AND MANAGEMENT OF MITE INFECTIONS

All larval chigger bites cluster where clothing is tight against the skin, especially the genitalia, thighs, buttocks, waist, and ankles, and generally do not create itching and discomfort until the larvae have withdrawn their mouthparts and departed. Forcibly removing feeding chiggers often decapitates larvae, leaving mouthparts embedded to cause further inflammation.[1] Untested strategies for removing feeding, engorged chiggers intact have included painting chigger bite sites with collodion, clear fingernail polish, or Liquid Skin and then drying the sites with a hair dryer and peeling the coated and dried chiggers off the skin intact. Intense itching commences within 3 to 6 hours after bites, which is then followed by intensely pruritic, erythematous papules (10–12 hours) and crusting and healing (24–48 hours; see Fig. 295.1).[1,3]

Several noninvasive techniques, such as surface microscopy, dermoscopy or dermatoscopy, epiluminescence microscopy, and videodermatoscopy, can provide rapid high-power magnification (10× to 1000×) and in vivo observation of the skin, and these techniques have been used to differentially diagnose several chronic ectoparasitic disorders, including scabies, pediculosis, phthiriasis, tungiasis, and myiasis.[16] In 2014, Italian investigators used videodermatoscopy to diagnose a case of chronic, intensely pruritic trombiculosis caused by the larval European harvest or autumn mite, *N. autumnalis*, in a man with multiple self-induced excoriations on the extremities previously treated as scabies (Fig. 295.7).[16]

Treatment is supportive with soap and water cleansing, warm water soaks, and topical local anesthetics and antihistamines. Impetigo and secondary infections are potential complications that would necessitate antibiotic treatment.

Follicle mite infections on the face usually require no treatment other than soap and water washing to reduce infections.[1,3,9] Scalp and eyelash infections respond to washes with 0.5% selenium or 10% sulfur-containing creams, lotions, or shampoos, with care to avoid ocular instillation. Chronic follicle mite infections (demodicidosis) with blepharitis and rosacea-like dermatitis may require treatment with a single oral dose of ivermectin, 200 µg/kg, especially in immunocompromised patients. Most animal and plant mite bites also can be managed symptomatically with topical agents unless active infections are present, which can be controlled with topical 10% crotamiton, 25% benzyl benzoate, or topical 1% lindane or 1% malathion preparations. House dust mite allergies may be managed with immunotherapy with house dust mite extracts.

Although initially classified among the *Rickettsia,* the causative agent of scrub typhus, *O. tsutsugamushi* (originally *R. tsutsugamushi*), has now been reclassified into a separate genus, *Orientia,* based on molecular evidence that its cell wall differs significantly from that of *Rickettsia*



FIG. 295.5 Close-up photograph of the pruritic, erythematous papulovesicular bite lesions inflicted by the European oak leaf gall mite on a resident of Pittsburg, Kansas, in late August 2004.[12] *(From Centers for Disease Control and Prevention [CDC]. Outbreak of pruritic rashes associated with mites—Kansas, 2004.* MMWR Morb Mortal Wkly Rep. *2005;54;952–955. Photograph courtesy A. Broce, L. Zurek, Kansas State University.* http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5438a3.htm.*)*



FIG. 295.6 (A–F) Photographs of six persons with skin lesions of *Pyemotes ventricosus* dermatitis. Note the central microvesicles, ulcerations or crusts, and many lesions with the pathognomonic comet signs. (D) Lymphangitis-like dermatitis. (E–F) Lesions resulting from natural infections of two of the investigators. *(From Del Giudice P, Blanc-Amrane V, Bahadoran P, et al. Pyemotes ventricosus dermatitis, southeastern France.* Emerg Infect Dis. *2008;14:1759–1761.* http://www.cdc.gov/Ncidod/eid/vol14no11/.htm.*)*



FIG. 295.7 (A) Visual observation of a nonspecific, slightly raised and red pruritic lesion *(circle).* (B) Videodermatoscopic observation of a skin-attached and feeding larval *Neotrombicula autumnalis* mite (magnification ×150). *(From Nasca MR, Lacarrubba F, Micali G. Diagnosis of trombiculosis by video-dermatoscopy.* Emerg Infect Dis. *2014;20:1059-1060. Courtesy Centers for Disease Control and Prevention, Atlanta, GA.)*

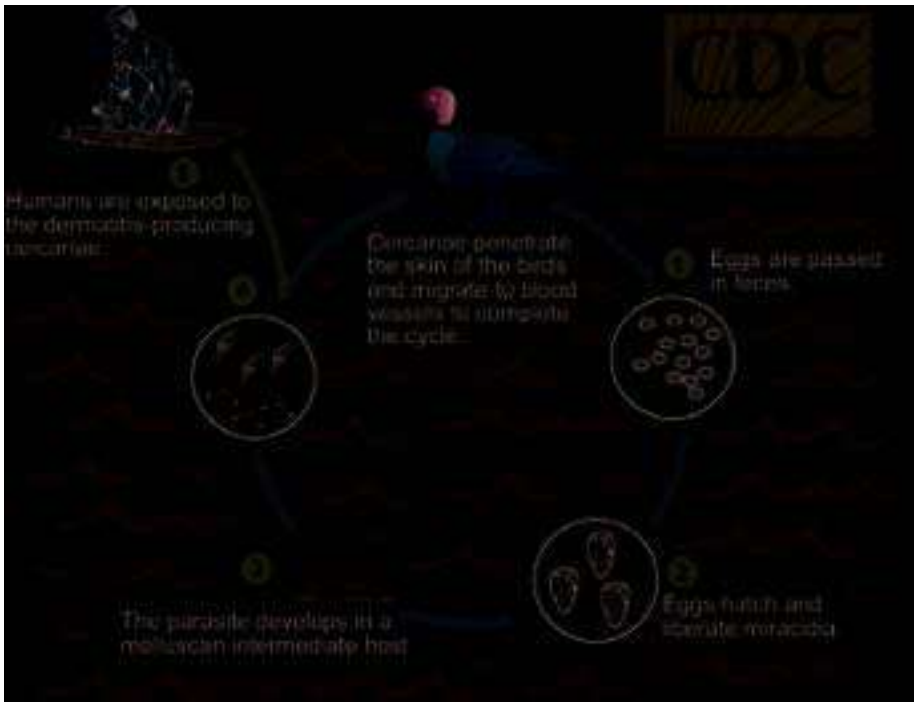

FIG. 295.8 The life cycle of several species of avian schistosomes of resident and migratory waterfowl whose eggs are passed in bird feces and hatch into ciliated miracidia that penetrate aquatic snail intermediate hosts. Infected snails later release infective cercariae into freshwater sources to penetrate the skin of definitive bird hosts or inadvertent or dead-end hosts, such as humans, causing self-limited, intensely pruritic cercarial dermatitis (commonly known as "swimmer's itch." *(From Centers for Disease Control and Prevention. DpDx Image Library.* http://www.dpd.cdc.gov/dpdx/HTML/CercarialDermatitis.html.*)*

both ultrastructurally and in its component proteins (see Fig. 295.2). Nevertheless, mite-transmitted scrub typhus and rickettsialpox have similar clinical presentations; both infections respond to treatment with oral tetracycline, oral doxycycline, or intravenous chloramphenicol, which is not recommended owing to its bone marrow toxicity (see Table 295.2 for comparison of the clinical manifestations, diagnostic methods, differential diagnosis, treatment strategies, and outcomes for scrub typhus and rickettsialpox).

Delusional Mite and Other Ectoparasitic Infestations

In 2014, Diaz and Nesbitt reported their case series and review of delusional infestations in very distraught patients who believed that they were infested with external or internal parasites and described crawling sensations of mites or worms on or under their skin.[17] Some synonyms for delusional infestations have included Ekbom syndrome, acarophobia, and Morgellons disease.[17] These patients should never be dismissed by clinicians without careful environmental exposure histories and physical examinations.[17]

Several true ectoparasitoses have been incorrectly described as delusional infestations, including cutaneous larva migrans, cercarial dermatitis, and *Pyemotes* mite infestations (see Fig. 295.4).[17] Cercarial dermatitis is a very common condition among aquatic gardeners, birdwatchers, fishermen, wildlife photographers, and wildfowl hunters.[17]

Cercarial dermatitis is characterized by pruritic rashes caused by aquatic exposures to the infective-stage cercariae of several avian schistosomes or flatworms that are released into freshwater lakes and rivers in the tens of thousands by infected aquatic snail intermediate hosts in complicated life cycles (Fig. 295.8).[17] Cercarial dermatitis, also known as duck hunter's or swimmer's itch, has occurred in massive seasonal outbreaks in swimmers in freshwater lakes and rivers whose resident or migratory waterfowl are infected with avian schistosomes (Fig. 295.9).[17]



FIG. 295.9 The typical self-limited, intensely pruritic, papulovesicular rash of cercarial dermatitis or swimmer's (duck hunter's) itch caused by penetration of the skin by the cercariae of avian (waterfowl) schistosomes. *(From Centers for Disease Control and Prevention. DpDx Image Library.* http://www.dpd.cdc.gov/dpdx/HTML/CercarialDermatitis.html.*)*

The demographic and behavioral features of true delusional infestations have remained constant over the years and have included: (1) onset in well-educated, middle-aged adults who are pet owners; (2) production of purported specimens of causative parasites; (3) pesticide overtreatment of themselves, their households, and their domestic pets; (4) excessive cleaning or vacuuming of households; (5) intense anger and resentment directed at physicians failing to confirm their self-diagnoses; and (6) sharing of delusional symptoms with spouses or relatives.[17] The most effective management strategies for true delusional infestations include empathetic history taking and active listening to patients; careful exclusion of the most commonly underdiagnosed true ectoparasitoses; and therapeutic regimens in consultation with mental health providers.[17]

PREVENTION OF MITE INFECTIONS AND MITE-TRANSMITTED INFECTIOUS DISEASES

Prevention and control strategies for mite ectoparasites include (1) household and campsite spraying of pyrethrin and pyrethroid-containing insecticides; (2) spraying or impregnating pyrethrin and pyrethroid-containing repellents on clothing; (3) applying diethyltoluamide–containing insect repellents (*N,N*-diethyl-meta-toluamide [DEET]) to exposed skin; (4) gently washing exposed or infested areas of the body with soap and water to remove stylostome-attached mites without decapitating them; (5) improving rodent reservoir control in campgrounds, homes, apartments, barns, sheds, and, especially, crowded public housing; (6) treating straw beds, straw mattresses, and hayride wagons with pyrethrin- and pyrethroid-containing insecticides; and (7) vacuuming bedrooms and mattresses and washing bed linens regularly, and covering mattresses and pillows with plastic covers to minimize house dust mite levels. There are no vaccines for scrub typhus or rickettsialpox. Weekly doses of 200 mg of doxycycline can prevent *O. tsutsugamushi* infections.

CONCLUSION

In summary, mites are ubiquitous and bothersome pests, and of these, most are trombiculid larvae (chiggers) and animal and plant mites that do not transmit infectious diseases. Only about 20 species can cause dermatitis or transmit infectious diseases.[3] The Asian and Eurasian *Leptotrombidium* species of trombiculid larvae (chiggers) can transmit scrub typhus in endemic regions, and the house mouse mite can transmit rickettsialpox from a mouse zoonosis in urban and rural settings, which is more widespread worldwide than initially thought.

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296 Ticks, Including Tick Paralysis

James H. Diaz

SHORT VIEW SUMMARY

Definition
- Ticks can transmit the broadest range of infectious microbes among all arthropods, including bacteria, viruses, and parasites.
- Gravid ticks may also transmit paralytic salivary toxins during blood-feeding.

Epidemiology
- Ticks are among the most competent and versatile of all arthropod vectors of infectious diseases.
- Tick-transmitted Lyme borreliosis, or Lyme disease, is now the most common arthropod-borne infectious disease in the United States and Europe.
- Most tick-borne infectious diseases can also be transmitted to humans by blood transfusions and organ transplants, and babesiosis can be transmitted congenitally.

Microbiology
- Ticks of all ages and both genders may remain infectious for generations without having to reacquire infections from host reservoirs.
- New tick-transmitted pathogenic species are constantly being described in the United States.

Diagnosis
- Ticks can transmit several pathogens during one blood-feeding, resulting in coinfections that can complicate differential diagnosis and treatment.
- The diagnosis of tick-transmitted infectious diseases is based on combinations of tick-bite history and characteristic lesions (e.g., erythema migrans and eschars), microscopic identification of pathogens in blood and tissue biopsy specimens, serologic and immunocytologic tests, and nucleic acid serotyping.

Therapy
- Most tick-transmitted bacterial diseases remain sensitive to doxycycline, amoxicillin, and chloramphenicol.
- The tick-transmitted viral diseases can be managed only supportively.
- Babesiosis is caused by a malaria-like parasite and must be treated with combinations of antimalarial agents and azithromycin or clindamycin.

Prevention
- Combinations of immunization, prophylactic antibiotics, personal protective measures, landscape management, and wildlife management are all effective strategies for the prevention and control of tick-borne infectious diseases.
- A single 200-mg dose of doxycycline administered within 72 hours of a tick bite is more than 80% effective in preventing Lyme disease.

Ticks are the most competent and versatile of all arthropod vectors of zoonotic infectious diseases for several reasons. First, ticks are not afflicted by most of the microorganisms that they may transmit or the paralytic salivary toxins that they may transfer during blood-feeding. Second, and unlike mosquitoes, ticks can transmit the broadest range of infectious microbes among all arthropods, including bacteria, viruses, and parasites. In addition, tick-transmitted coinfections appear to be increasing and complicate differential diagnosis and antimicrobial treatment. Third, ticks can vertically transmit infectious microorganisms congenitally to their offspring of both genders (transovarian transmission) and then disseminate carrier state infections among all generational growth stages (transstadial transmission). Tick-borne infectious diseases can also be transmitted to humans by blood transfusions and organ transplants, and babesiosis, a tick-borne infection caused by malaria-related parasites, can be transmitted congenitally. Fourth, ticks have capitalized on many competitive advantages afforded them by evolving changes in climate and human lifestyle, including the following: (1) wider geographic distributions and longer active breeding and blood-feeding seasons as a result of increases in global mean temperatures and humidity; (2) greater abundance of wild animal reservoir hosts no longer effectively controlled, especially deer, rabbits, and rodents; (3) greater residential construction in recently cleared woodlands adjacent to pastures and yards frequented by wildlife, domestic animals, and humans; and (4) more vacation and leisure-time activities enjoyed by humans and their pets during prolonged tick host-questing and blood-feeding seasons from earlier springs through later falls and milder winters.[1] In short, ticks of all ages and both genders may remain infectious for generations without having to reacquire infections from host reservoirs, and environmental and behavioral changes now place humans and ticks together outdoors for longer periods for tick breeding, blood-feeding, and infectious disease transmission.

TICK BIOLOGY, BEHAVIOR, AND TAXONOMY

With the exception of toothed hypostomes for blood-feeding and clawless palps, adult ticks resemble large mites with eight legs and disk-shaped bodies.[2] There are four stages in the tick life cycle: egg, six-legged larva, nymph, and adult. Ticks are classified into three families: the Ixodidae, or hard ticks; the Argasidae, or soft ticks; and the Nuttalliellidae, a much lesser known family with characteristics of both hard and soft ticks.[2] Ixodid ticks have a hard dorsal plate or scutum, which is absent in the soft-bodied, argasid ticks. Ixodid ticks also exhibit more sexual dimorphism than argasid ticks, with both genders looking alike. However, all blood-fed ticks, especially females, are capable of enormous expansion, and engorged ixodid females are often confused with engorged argasid females. Although ticks from all families may serve as disease vectors, the ixodid or hard ticks are responsible for most tick-borne diseases in the United States.

Ixodid ticks have mouth parts that are attached anteriorly and visible dorsally. They live in open exposed environments, such as woodlands, grasslands, meadows, and scrub brush areas. Argasid ticks are leathery and have subterminally attached mouthparts that are not visible dorsally. Argasid ticks prefer to live in more sheltered environments, including animal nests, caves, crevices, woodpiles, and uninhabited rural cabins. All ticks feed by cutting a small hole in the host's epidermis with their chelicerae and then inserting their hypostomes into the cut, with blood flow maintained by salivary anticoagulants.[2] Ticks are attracted to warm-blooded hosts by vibration and exhaled carbon dioxide. Ixodid ticks actually "quest" for hosts by climbing onto vegetation with their forelegs outstretched, waiting to embrace passing hosts (Fig. 296.1). Ticks spend relatively short periods of their lives mating and blood-feeding on hosts. Soft ticks feed rapidly for hours and then drop off, whereas hard ticks blood-feed for days (6–12) before dropping off for egg laying.



FIG. 296.1 Female *Dermacentor andersoni*, the Rocky Mountain wood tick, questing for a host. The Rocky Mountain wood tick is the preferred tick vector for *Rickettsia rickettsii,* the causative riskettsial agent of Rocky Mountain spotted fever in the US Rocky Mountains and the Canadian southwest. *(From Biggs HM, Behravesh CB, Bradley KK, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States.* MMWR Recomm Rep. *2016;65:1–44.)*

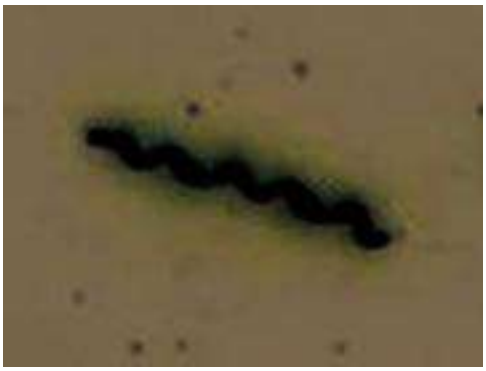

FIG. 296.2 *Borrelia burgdorferi,* the causative bacterium of Lyme disease. Note the characteristic coiled spring appearance of a spirochete (peripheral blood smear, immunofluorescence stain under darkfield microscopy, ×1000). *(From Public Health Image Library. Atlanta, GA: Centers for Disease Control and Prevention. Courtesy Dr. Robert D. Gilmore.)*

EPIDEMIOLOGY OF TICK-BORNE INFECTIOUS DISEASES

Tick-borne infectious diseases have challenged researchers and physicians since Dr. Howard T. Ricketts identified the wood tick, *Dermacentor andersoni,* as the vector of Rocky Mountain spotted fever (RMSF) in 1906 and firmly established the insect vector theory of infectious disease transmission (see Fig. 296.1).[3] The emergence and recognition of Lyme disease in the early 1970s in the United States, whose causative agent, the spirochete *Borrelia burgdorferi,* was not identified until 1982, sparked renewed interest in tick-borne diseases in the United States and Europe (Fig. 296.2).[4]

By the early 1990s, Lyme borreliosis (LB) had become the most common arthropod-borne infectious disease in the United States and Europe.[5] Since the 1970s, every decade now describes emerging or rediscovered tick-borne infectious disease and new vectors for previously described tick-borne diseases, such as RMSF.[6] These latest discoveries have been spawned by new immunodiagnostic technologies, especially by nucleic acid identification technologies, particularly the polymerase chain reaction (PCR) assay.

By the 1980s and 1990s, the causative agents of the ehrlichioses were stratified as newly emerging, *Rickettsia*-like species, and later (2001) they were completely reorganized into separate genera, *Ehrlichia* and *Anaplasma.*[7,8] In 1997, Kirkland and colleagues[9] described a new erythema migrans–like rash illness in North Carolina, a nonendemic region for Lyme disease, transmitted by the Lone Star tick, *Amblyomma americanum* (Fig. 296.3). This new borreliosis would soon be named the southern tick-associated rash illness (STARI) or Masters disease, but its causative agent, *Borrelia lonestari,* a new *Borrelia* species, would not be identified until 2004.[10,11]

By 2004, ticks were recognized as the most common vectors of all arthropod-borne infectious diseases in Europe, five new spotted fever (SF)–causing rickettsiae were described, four new subspecies of the Lyme disease–causing *B. burgdorferi* complex were identified, a new relapsing fever *Borrelia* species was isolated, and anaplasmosis was exported to Europe from the United States.[12] In a seemingly unending era of new discoveries in tick-transmitted diseases, another new and unanticipated vector for RMSF—*Rhipicephalus sanguineus,* the brown dog tick—was identified in the United States in 2005 (Fig. 296.4).[13]

In 2011, the first human cases of relapsing fever caused by tick-transmitted *Borrelia miyamotoi* were reported from Russia.[14] In 2013, 1% to 3% of surveyed residents of New England states where Lyme disease is endemic were seropositive for prior *B. miyamotoi* infection.[15] Since 2011, more than 50 patients with acute febrile illnesses resembling Lyme disease without erythema migrans have been described as having *B. miyamotoi* infections.[16] In most cases, patients present with fever, malaise, fatigue, headache, myalgias, and arthralgias, and they do not have recurrent fevers. This initial clinical presentation more closely resembles other hard tick–transmitted diseases, especially anaplasmosis and Lyme disease without a significant rash, rather than soft tick–transmitted classic relapsing fever. In two cases, immunocompromised patients developed meningoencephalitis.[15] *B. miyamotoi* infections are transmitted by the same ixodid ticks that carry Lyme disease: *Ixodes scapularis,* the eastern black-legged tick (Fig. 296.5), and *Ixodes pacificus,* the western black-legged tick (Fig. 296.6).[15,16] Uncomplicated cases may be effectively treated with oral doxycycline, and the cases complicated by meningoencephalitis responded to intravenous therapy with ceftriaxone or penicillin G.

In 2009, a new pathogenic *Ehrlichia* species in addition to endemic *Ehrlichia chaffeensis* and *Ehrlichia ewingii* was identified in four febrile patients in Minnesota and Wisconsin and presumed to be related to *Ehrlichia muris.*[17]

In July 2008, an 80-year-old man with a tick-bite–appearing eschar, lymphadenitis, and regional lymphadenopathy, and three additional patients with similar presenting manifestations, were reported in northern California.[18] An afebrile, "spotless" rickettsial disease was suspected initially in all cases.[18] Convalescent sera from all four patients exhibited cross-reacting immunoglobulin antibody titers to the RMSF agent *Rickettsia rickettsii,* and also to a newly recognized SF group agent, *Rickettsia* 364D (*R. philipii* [proposed]), later detected in the Pacific Coast tick *Dermacentor occidentalis* (Figs. 296.7 and 296.8).[18] All patients were effectively treated without fatalities with oral doxycycline over a 2-week course.[18]

The Heartland virus, a novel phlebovirus (family Bunyaviridae) transmitted by the Lone Star tick *A. americanum* (see Fig. 296.3), was first described in 2009 in two elderly Missouri farmers believed to have ehrlichiosis and who presented with fever, fatigue, headache, confusion, myalgia, and laboratory evidence of leukopenia and thrombocytopenia.[19] In 2013, a fatal case of Heartland virus disease was reported in an 80-year-old man in Tennessee who presented with similar clinical and laboratory findings, but he could not recall a tick bite.[20] Other than supportive care, there are no specific drug treatments for Heartland virus disease.[19,20]

In June 2014, a previously healthy man in eastern Kansas with a history of tick bite, fever, and fatigue was treated with doxycycline for a presumed tick-borne illness.[21] He later died from a critical illness characterized by high fever, thrombocytopenia, leukopenia, and multiorgan failure.[21] Given the proximity to the Heartland virus cases in adjacent Missouri, blood specimens were analyzed for antibodies against Heartland virus.[21] Subsequently, a series of serologic, culture-based, and molecular tests demonstrated the presence of a novel thogotovirus (the first representative of the family was isolated in the Thogoto forest

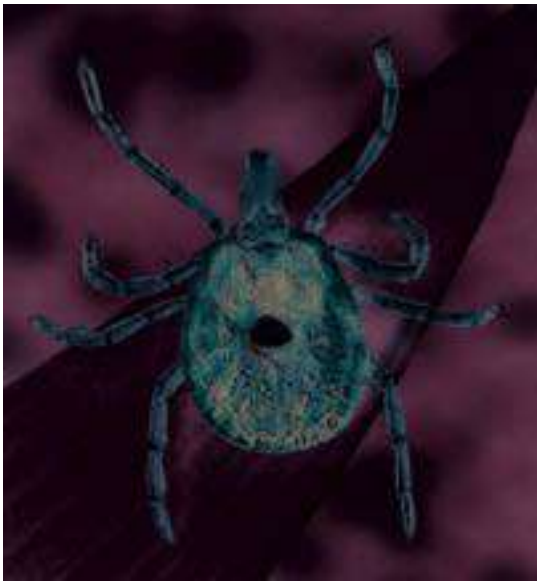

FIG. 296.3 ***Amblyomma americanum,*** **the Lone Star tick, questing for a host.** Shown is the dorsal view of a female Lone Star tick, the vector of southern tick-associated rash illness (STARI) caused by the spirochete *Borrelia lonestari.* Note the "lone star" mark in the center of the dorsal surface. *(From Public Health Image Library. Image 8683. Atlanta, GA: Centers for Disease Control and Prevention.)*



FIG. 296.5 ***Ixodes scapularis,*** **the eastern black-legged tick.** Shown are the adult female and two nymphs. These are arthropod vectors of babesiosis and Lyme disease, especially nymphs, whose bites are most often unnoticed. *(From Public Health Image Library. Image 1205. Atlanta, GA: Centers for Disease Control and Prevention.)*



FIG. 296.4 ***Rhipicephalus sanguineus,*** **the brown dog tick, questing for a host.** This is a dorsal view of a male tick, a new and unanticipated vector for Rocky Mountain spotted fever in addition to the historical vectors *Dermacentor andersoni,* the Rocky Mountain wood tick, and *Dermacentor variabilis,* the American dog tick. *(From Public Health Image Library. Image 7646. Atlanta, GA: Centers for Disease Control and Prevention.)*



FIG. 296.6 ***Ixodes pacificus,*** **the western black-legged tick, questing for a host.** *Ixodes pacificus* is the preferred tick vector for the transmission of *Borrelia* spirochetal infections, including *B. burgdorferi,* the causative agent of Lyme borreliosis, and *B. miyamotoi,* the causative agent of a Lyme disease–like borreliosis without the characteristic erythema migrans rash. *(From Biggs HM, Behravesh CB, Bradley KK, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States.* MMWR Recomm Rep. *2016; 65:1–44.)*

of Kenya) phylogenetically distinct from Heartland virus and named Bourbon virus after the patient's county of residence.[21]

Another novel, tick-transmitted phlebovirus (family Bunyaviridae), the severe fever with thrombocytopenia syndrome virus (SFTSV), was first described in 2009 in China and has now been detected in *Haemaphysalis* species ticks in Korea and Japan.[22] SFTSV is transmitted by at least two species of *Haemaphysalis* ticks, *H. longicornis* and *H. concinna,* and is characterized by the abrupt onset of high fever, abdominal pain, nausea, vomiting, leukocytopenia, and thrombocytopenia with hemorrhage.[22] The zoonotic reservoir for SFTSV is in domestic animals, especially goats.[22] Since 2010, about 2500 cases of severe fever with thrombocytopenia syndrome (SFTS) have been reported from central and eastern China, mostly in hospitalized patients, with a median case-fatality rate of 7.3%.[22] Other than supportive care, there are no specific drug treatments for SFTS.[22]

In each case of these newly described tick-transmitted diseases, another tick-borne disease was initially suspected. Ehrlichiosis was suspected in the Heartland virus disease cases, Lyme disease in the *B. miyamotoi* cases, and "spotless" RMSF with eschar in the *Rickettsia* 364D cases.

Because most tick-borne diseases are caused by obligate intracellular organisms, many of which infect erythrocytes, monocytes, granulocytes, or vascular endothelial lining cells, many tick-borne infections may also be transmitted congenitally (e.g., babesiosis) and by blood product transfusions and organ transplants. Blood product–transmitted infections have now been described for the tick-borne rickettsial diseases (including Q fever), babesiosis, and ehrlichiosis. In 2008, the Centers for Disease Control and Prevention (CDC) reported the first case in which transfusion transmission of *Anaplasma phagocytophilum,* the tick-borne causative agent of anaplasmosis (formerly, human granulocytic ehrlichiosis) was

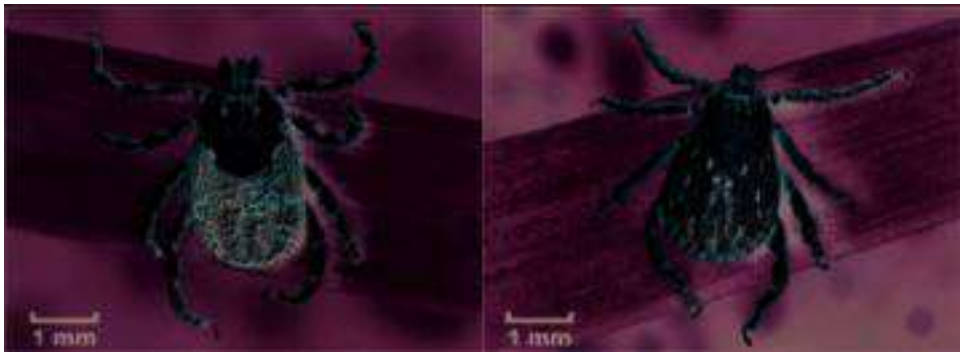

FIG. 296.7 ***Dermacentor occidentalis,*** **the Pacific Coast tick, adult female** ***(left)*** **and adult male** ***(right).*** *Dermacentor occidentalis,* the Pacific Coast tick, has now been identified as the tick vector of *Rickettsia* 364D (*Rickettsia philipii* [proposed]) rickettsiosis in California, which has been described as a "spotless" fever with a tick-bite eschar. *(From Shapiro MR, Fritz CL, Tait K, et al.* Rickettsia *364D: a newly recognized cause of eschar-associated illness in California.* Clin Infect Dis. *2010;50:541–548; and Centers for Disease Control and Prevention, Atlanta, GA. Photographs courtesy James Gathany.)*



FIG. 296.8 **Rickettsia 364D eschar.** A pediatric patient in California with a tick-bite eschar on the left brow from the newly discovered rickettsial species *Rickettsia* 364D, transmitted by the bite of an infected Pacific Coast tick (*Dermacentor occidentalis*). *(From Biggs HM, Behravesh CB, Bradley KK, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States.* MMWR Recomm Rep. *2016;65:1–44. Photo courtesy Samantha H. Johnson, MD, Oakland Children's Hospital and Research Center, Oakland, CA.)*

confirmed microscopically and serologically by testing of both the recipient and the donor.[23]

In 2016, Moritz and coinvestigators from the American Red Cross reported the results of their screening intervention designed to reduce the risk of blood transfusion-transmitted babesiosis caused by *Babesia microti* in highly endemic regions of the United States, including Connecticut, Massachusetts, Minnesota, and Wisconsin.[24] *Babesia microti* is an intraerythrocytic parasite, related to the malaria parasite, that, like malaria, can be transmitted by blood transfusion. Babesiosis is a potentially fatal disease as a result of splenic rupture. The investigators screened over 89,000 blood donation samples for *B. microti* antibodies (with arrayed fluorescence immunoassays) and for *B. microti* DNA (with PCR assays) and confirmed 335 samples (0.38%) as serologically positive and 67 (20%) of these as PCR-positive.[24] In Connecticut and Massachusetts from June 2012 through September 2014, no reported cases of transfusion-transmitted babesiosis were associated with the screened donations (0 cases/75,331 screened blood donations), compared with 14 cases per 253,031 unscreened donations (1 case/18,074 donations; odds ratio, = 8.6; 95% confidence interval, 0.51–144; $P = .05$).[24] Over the reporting period, 29 cases of transfusion-transmitted babesiosis were linked to unscreened blood from infected donors.[24] Although no currently licensed tests exist to screen for *B. microti* in donated blood, the authors concluded that screening for antibodies to and DNA from *B. microti* could significantly decrease the rate of transfusion-transmitted babesiosis in highly endemic US states.[24]

Today, the seroprevalence of tick-borne diseases is increasing significantly among blood and organ donors in the United States, combined tick-transmitted coinfections have been described in regional US populations, and an unexplained increase in the virulence of tick-borne infectious diseases has been described in the United States (RMSF), Europe, and North Africa (Mediterranean spotted fever [MSF]) and Australia (Queensland tick typhus [QTT]). Several tick-borne infectious diseases have now been reclassified by the CDC as potential biological terrorism agents, including the following: *Francisella tularensis* (tularemia), a category A agent (highly likely microorganism to be weaponized); *Coxiella burnetii* (Q fever), a category B agent (less likely to be weaponized); and the tick-borne encephalitis and hemorrhagic fever viruses, category C agents (least likely to be weaponized). In the future, the tick-transmitted infectious diseases will increase in prevalence over wider distributions at higher altitudes in a warmer world. Unexpected tick vectors of emerging infections caused by obligate intracellular microorganisms will continue to be discovered as people spend more leisure time outdoors in temperate climates in tick-preferred ecosystems.

TICK-BORNE BACTERIAL INFECTIONS

Spirochetal Infections (Borrelioses)

The borrelioses are a large group of tick-borne spirochetal diseases caused by several species of *Borrelia,* with unique geographic distributions, tick vectors, and host animal reservoirs (Table 296.1). The borrelioses are stratified into three separate epidemiologic and clinical presentations: LB, STARI, and the tick-borne relapsing fevers (TBRFs) (Table 296.2).

Lyme Borreliosis

LB, or Lyme disease (see Chapter 241), is now the most common tick-borne infectious disease in the Northern Hemisphere and the most common arthropod-borne infectious disease in the United States. In the United States, LB is caused by *Borrelia burgdorferi* (sensu stricto), first identified as a novel bacterial spirochete in 1982, and transmitted to humans by *Ixodes* spp. hard ticks in US regional pockets, specifically the Northeast *(I. scapularis),* upper Midwest *(I. scapularis),* and Pacific Coast (*I. pacificus;* see Figs. 296.2, 296.5, and 296.6). Although *B. burgdorferi* is the sole agent of LB in the United States, most cases of LB in Europe and northern Asia are caused by *Borrelia afzelii* and *Borrelia garinii* (see Table 296.1). Collectively, the three *Borrelia* species are often referred to as *B. burgdorferi* (sensu lato). Ticks usually acquire *Borrelia* infections as larvae or nymphs by blood-feeding on small reservoir hosts, most commonly birds and rodents, and may transmit LB to humans during blood-feeding, which may

TABLE 296.1 Tick-Borne Spirochetal Borrelioses

BORRELIA SPECIES	TICKBORNE DISEASES	GEOGRAPHIC DISTRIBUTION	TICK VECTORS	WILD ANIMAL RESERVOIRS
B. afzelii	European Lyme borreliosis (LB) or Lyme disease	Europe, Scandinavia	*Ixodes ricinus*	Mammals: deer, rodents
B. burgdorferi	American LB or Lyme disease	North America, specifically US Northeast, Midwest, Pacific Northwest; Europe	*Ixodes scapularis* (eastern United States), *Ixodes pacificus* (western United States)	Mammals: deer, rodents (preferred by nymphs)
B. crocidurae	North African tick-borne relapsing fever (TBRF)	North Africa, Mediterranean Basin	*Carios erraticus*	Mammals: rodents, birds
B. duttonii	East African TBRF	East, central, and South Africa	*Ornithodoros moubata*	Humans (main reservoir)
B. garinii	European LB	Northern Europe, Russia, Asia	*I. ricinus* (Europe), *Ixodes persulcatus* (Asia)	Mammals: rodents, birds
B. hermsii	American TBRF	Western United States and Canada	*Ornithodoros hermsii*	Mammals: rodents, chipmunks, squirrels
B. hispanica	Hispano-African TBRF	Iberian peninsula: Spain, Portugal; northwestern Africa: Algeria, Morocco, Tunisia	*Ornithodoros marocanus*	Mammals: rodents
B. latyschewii	White TBRF	Russian Caucasus regions (Tajikistan, Uzbekistan), Central Asia	*Ornithodoros tartakovskyi*	Mammals: rodents
B. lonestari	Southern tick-associated rash illness (STARI) or Masters disease	Southeastern United States from southeastern Atlantic Coast west to Central Texas, Oklahoma, Missouri	*Amblyomma americanum*	Mammals: rodents, cattle, other domestic animals; some reptiles, especially lizards
B. mazzottii	Southern TBRF	Southern United States, Mexico, Central America, South America	*Ornithodoros talaje*	Mammals: rodents
B. miyamotoi	Russian TBRF	Japan, Eastern Europe, US Northeast	*Ixodes scapularis*	Mammals: rodents
B. parkeri	Western TBRF	Southwest and south central United States, Mexico	*Ornithodoros parkeri*	Mammals: rodents
B. persica	Asiatic-African TBRF	Middle East (Egypt, Iran), central Asia, western China, northern India	*Ornithodoros tholozani*	Mammals: rodents
B. turicatae	American Southwestern TBRF	Southwest and south central United States, Mexico, Central America	*Ornithodoros turicata*	Mammals: rodents, armadillos, opossums, pigs, and monkeys (Panama)
B. venezuelensis	Venezuelan TBRF	Central and South America	*Ornithodoros rudis*	Mammals: rodents, opossums, armadillos, monkeys (Panama, Colombia)

go unnoticed (see Fig. 296.2). *Borrelia* organisms are further maintained in nature as infected adult *Ixodes* ticks blood-feed on larger mammals, especially deer.

Unlike argasid or soft ticks, *Ixodes* ticks prefer temperate ecotonal zones of canopied forests abutting cleared scrub or grasslands and transmit *B. burgdorferi* to humans during outdoor exposures in such habitats. Because *Borrelia* spirochetes must migrate from the tick's midgut to the salivary gland during blood-feeding, tick attachments for less than 24 hours rarely result in LB in humans.[25,26] After an incubation period of 1 to 2 weeks, the hallmark of spirochete transmission manifests as solitary erythema migrans, a maculopapular erythematous rash with a bull's-eye pattern, at the site of tick attachment (Fig. 296.9).[25–29] Erythema migrans in LB results from the subcutaneous centrifugal movement of the spirochetes from the bite sites to the central circulation and target organs (see Figs. 296.3 and 296.9).[27] An erythema migrans -like rash also occurs in STARI at the site of *A. americanum* (Lone Star tick) attachment, but the underlying mechanisms responsible for the rash in STARI are unclear.

In a meta-analysis of 53 longitudinal studies of LB in the United States and Europe, Tibbles and Edlow[26] reported that many patients did not recall a tick bite (74% in the United States, 36% in Europe), constitutional symptoms of low-grade fever (<39°C [102.2°F]) and headache were common but nausea and vomiting were rare, and a solitary erythema migrans lesion was the most common initial presentation of LB (81% in the United States, 88% in Europe). If LB is recognized and treated early in the erythema migrans stage, cure rates will exceed 90%, and outcomes will be excellent (see Table 296.2).[28–31] Although deaths from LB are rare, 5% to 8% of patients may develop cardiac manifestations, and 15% to 20% of patients may develop neurologic manifestations.[28,29]

In patients who previously had antibiotic-treated erythema migrans, reinfections with *B. burgdorferi* may occur in subsequent summers and will be again heralded by another erythema migrans lesion.[29] Recent investigations by Nadelman and coworkers demonstrated that repeated episodes of pathognomonic erythema migrans in patients with previous appropriately treated early LB were due to reinfections and not to recurrences.[29–31] However, persistent arthritis despite adequate antibiotic treatment of LB (now called post-infectious Lyme arthritis) may occur and was formerly referred to as chronic Lyme arthritis.[31a] Despite spirochetal elimination with antibiotic therapy, the proliferative synovitis of Lyme arthritis may extend for months to years and has been attributed to retained spirochetal fragments (such as *B. burgdorferi* peptoglycan) and/or autoimmune responses to these antigens and not to recurrent LB.[31a]

Unfortunately, some practitioners continue to diagnose patients who are suffering from a variety of generalized myofascial, arthritic, and neurologic pain conditions as having "chronic Lyme disease" and have prescribed long courses of oral and intravenous antibiotics despite evidence demonstrating that such therapies provide no improvement and can cause harm. In 2017, Marzec and coauthors reported a case series of five patients diagnosed with "chronic Lyme disease" who were then treated with protracted courses of antibiotics.[32] The resulting complications from this unnecessary therapy included (1) septic shock from central venous catheter–associated bacteremia in two patients, with one fatality; (2) vertebral osteomyelitis in one patient; (3) intractable *Clostridioides difficile* (formerly *Clostridium difficile*) colitis in one patient who later

TABLE 296.2 Clinicopathophysiologic Comparison of Lyme Borreliosis, Southern Tick-Associated Rash Illness (STARI), and Tick-Borne Relapsing Fever

INFECTIOUS DISEASE CHARACTERISTICS	LYME BORRELIOSIS	SOUTHERN TICK-ASSOCIATED RASH ILLNESS	TICK-BORNE RELAPSING FEVER
Microbial agents	*Borrelia burgdorferi* (United States, Europe), *B. afzelii* (Europe, Asia), *B. garinii* (Europe, Asia)	*Borrelia lonestari* has now been isolated from a skin biopsy of a patient with STARI and cultured in vitro from infected *Amblyomma americanum* ticks	Many *Ornithodoros* species of soft ticks (see Table 296.1)
Preferred tick vectors	*Ixodes* spp. hard ticks	*A. americanum*	*Ornithodoros* spp. soft ticks
Preferred animal reservoirs	Rodents (nymphs); deer, birds (adults)	Lizards	Rodents (nymphs); humans (*B. duttonii* only); deer, birds (adults)
Endemicity	Highly endemic in United States and Europe	Southeastern United States	Highly endemic among vector-populated regions worldwide
Fever ≤39°C (102.2°F)	Very uncommon	Absent; low-grade fever may occur rarely	Present in relapsing episodes 1–3 days each; may reach 43°C (109.4°F)
Relapsing fevers	Not present	Not present	Present
Erythema migrans, or other rash	Present as annular or target-like maculopapular rash (mean diameter, 7 cm); more common on extremities	Present and mimics that of Lyme disease but with a smaller mean diameter of 4.5 cm; more common on trunk	Absent
Arthritis	May be present in untreated (up to 60%) late, or "chronic" infections, manifesting as oligoarthritis	Arthralgias, myalgias, and neck stiffness may occur less commonly than with Lyme disease; no chronic arthritic complications	Neck stiffness, arthralgias, myalgias common, not arthritis
Neurologic manifestations	May be present in up to 15% of cases; include headache, neuritis of cranial nerve (CN) VII (Bell palsy)	Dizziness, headache, memory loss, concentration difficulty may occur; no chronic neurologic complications	Common: meningitis, meningoencephalitis; neuritis of CN VII (Bell palsy); neuritis of CN VIII (deafness, myelitis, radiculopathy)
Other presenting clinical manifestations	Myocarditis, conduction defects in up to 8% of late-onset and "chronic" cases	Regional lymphadenopathy may occur; chronic complications have not been described	Splenomegaly in most, hepatomegaly in 10% of cases; myocarditis manifesting as prolonged QTc interval
Best screening serodiagnostics	Giemsa- or Wright-stained peripheral smear, phase-contrast, or darkfield microscopy for spirochetes; ELISA, IFA	Epidemiologic and clinical presentation; no screening serodiagnostics available at present; Lyme disease ruled out by ELISA, IFA, Western immunoblot	Giemsa- or Wright-stained peripheral smear, phase-contrast, or darkfield microscopy for spirochetes; ELISA, IFA
Best confirmatory diagnostics	In vitro cultivation, Western immunoblot, PCR assay	PCR assay on skin biopsy; in vitro cultivation	In vitro cultivation (not recommended; Biosafety Level 3 laboratory required), rodent inoculation, PCR assay
Recommended antibiotic therapy	Doxycycline, 100 mg PO bid, or amoxicillin, 500 mg PO tid, for 14–21 d; parenteral therapy for CNS involvement	Doxycycline, 100 mg PO bid, or amoxicillin, 500 mg PO tid, for 14–21 d	Tetracycline, 500 mg or 12.5 mg/kg PO qid, or doxycycline, 100 mg PO bid, or erythromycin, 500 mg or 12.5 mg/kg PO qid for 10 d; parenteral therapy with penicillin G or ceftriaxone recommended for CNS involvement

CNS, Central nervous system; *ELISA,* enzyme-linked immunosorbent assay; *IFA,* immunofluorescence assay; *PCR,* polymerase chain reaction.

died from complications of amyotrophic lateral sclerosis (ALS); and (4) a paraspinal abscess that required surgical drainage in the last case.[32]

Although the concept of chronic LB has been dispelled by carefully conducted clinical studies, lingering and unconfirmed associations continue to be made between LB cases and subsequent deaths due to four neurodegenerative disorders: ALS, Alzheimer disease (AD), multiple sclerosis (MS), and Parkinson disease (PD). If such associations truly existed, then the geographic distributions of LB cases and deaths from these four neurodegenerative disorders would be anticipated to significantly overlap. Forrester and coinvestigators compared LB incidence rates in each state from the National Notifiable Diseases Surveillance System during 2001–2010 with age-adjusted death rates for AD, ALS, MS, and PD obtained from the CDC Wide-ranging Online Data for Epidemiologic Research (CDC WONDER) database over the same reporting period.[33] LB incidence per US state was not correlated with rates of death due to ALS, MS, or PD. However, an inverse correlation was detected between LB and AD.[33] The authors concluded that their failure to accurately confirm any positive correlations between the geographic distribution of LB and the geographic distribution of deaths due to AD, ALS, MS, and PD provided further evidence that LB was not associated with the development of these common neurodegenerative conditions in an aging population.[33]

The Jarisch-Herxheimer reaction (JHR), an inflammatory cytokine-mediated reaction to dying spirochetes with a worsening of presenting symptoms, vasodilation, and myocardial dysfunction, may occur during antibiotic treatment for LB but is more common after antibiotic therapy forTBRFs.[25,34] There have been no reported deaths from JHR during antibiotic therapy for LB, and the very rare case fatalities from LB have been attributed to cardiac conduction abnormalities from myocarditis in untreated cases.[25]

Southern Tick-Associated Rash Illness (STARI)

First recognized in 1998, STARI manifests initially as erythema migrans, as in LB, but occurs in regions in which *B. burgdorferi* is not endemic and follows the prolonged attachment of blood-feeding Lone Star ticks *(A. americanum),* more abundant in the southeastern and south central United States (see Fig. 296.3).[3,25–27] Patients who are bitten by Lone Star ticks may develop LB-like erythema migrans rashes and occasionally develop milder constitutional symptoms than in LB, including fever, headache, fatigue, and generalized myalgias.[27] However, unlike LB, STARI is not a reportable infectious disease and has no diagnostic serologic tests, such as enzyme-linked immunosorbent assays (ELISAs), immunofluorescence assays, and Western immunoblot assays. In addition, a microbiologic analysis of skin biopsy specimens obtained from the rashes of 30 patients in Missouri with clinical diagnoses of STARI failed to detect *B. lonestari,* suggesting that STARI could be caused by other pathogens.[35] Because some patients have recovered from STARI without antibiotic treatment and there have been no long-term sequelae reported



FIG. 296.9 Erythema migrans. Shown is the pathognomonic "bull's-eye" rash at the bite sites of *Borrelia burgdorferi*– or *B. lonestari*–infected ixodid ticks, tick vectors of Lyme disease and southern tick-associated rash illness (STARI), respectively, in endemic regions of the United States. *(From Public Health Image Library. Image 9875. Atlanta, GA: Centers for Disease Control and Prevention.)*

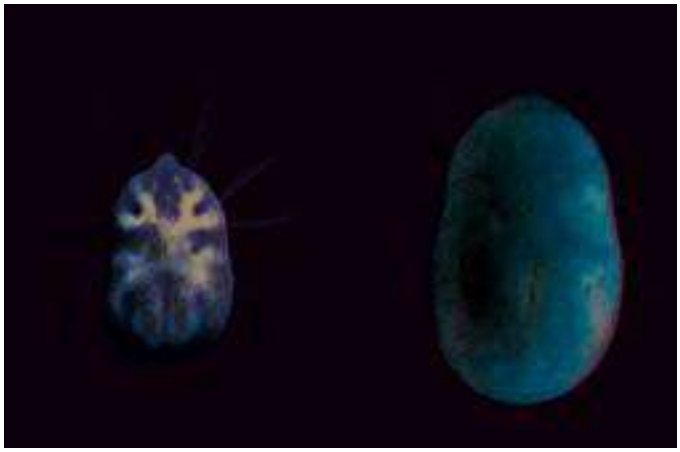

FIG. 296.10 *Ornithodoros* species soft tick, before *(left)* and after *(right)* a blood meal. The *Ornithodoros* species of soft ticks include *Ornithodoros hermsii, O. parkeri,* and *O. turicatae.* They are widely distributed throughout the mountainous regions of the western half of the United States and southwestern Canada at elevations above 1500 m and transmit the spirochetes that cause tick-borne relapsing fever (*Borrelia hermsii, Borrelia parkeri,* and *Borrelia turicatae*). *(From Tickborne Diseases of the United States. Soft tick. Atlanta, GA: Centers for Disease Control and Prevention.* https://www.cdc.gov/ticks/tickbornediseases/tickID.html.*)*

in STARI cases, some have questioned whether antibiotic therapy is indicated in STARI. Because distinguishing STARI from LB may be difficult, Wormser and coworkers[35] have recommended that the differential diagnosis rely on a combination of regional exposures, clinical presentations, serologic results, and potential for long-term sequelae based on their comparison of LB cases from New York and STARI cases from Missouri. The investigators noted that the timing of rash onset was shorter (6 days) in STARI compared with LB (10 days) and that STARI patients were less likely to be symptomatic than LB patients.[35] In addition, the STARI rash was more often circular with central clearing than the LB rash.[35] Most authorities recommend antibiotic therapy for STARI with oral doxycycline or amoxicillin, following the same regimen as for LB to cover any missed diagnoses of LB with the potential for chronic arthritic and cardiac sequelae (see Table 296.2).[35]

Tick-Borne Relapsing Fevers

The TBRFs (see Chapter 240) comprise a worldwide group of serious bacterial infections by *Borrelia* spirochetes after brief, painless, and usually unnoticed bites by *Ornithodoros* spp. argasid or soft ticks (Fig. 296.10). *Ornithodoros* ticks prefer indoor living—in cabins, caves, and crevices—and quickly abandon warm-blooded rodent hosts for egg laying (see Table 296.1).[34,36] Transovarian transmission of the TBRF spirochetes occurs commonly among all species and, unlike LB-causing *Borrelia* species, TBRF spirochetes are already present in the salivary glands at the onset of blood-feeding and do not need time to migrate from the gut to the mouthparts. The wild animal host reservoirs of TBRF are maintained in birds and several mammals, most commonly rodents. Adult ticks can live for as long as 15 to 20 years and survive without blood meals for several years.

The *Ornithodoros* species of soft ticks in the United States include *Ornithodoros hermsii, Ornithodoros parkeri,* and *Ornithodoros turicatae* (see Fig. 296.10). These soft ticks are widely distributed throughout the mountainous regions of the western half of the United States and southwestern Canada at elevations above 1500 m and transmit the spirochetes that cause TBRF (*Borrelia hermsii, Borrelia parkeri,* and *Borrelia turicatae*). Humans typically come into contact with *Ornithodoros* soft ticks when they stay in frequently unoccupied and rodent-infested mountain cabins. The ticks emerge at night and feed briefly and unnoticed while the person is sleeping. Unlike the ixodid ticks, *Ornithodoros* ticks feed very rapidly, usually for less than 30 minutes, and always at night.[34,36,37] The bites are painless as a result of local anesthetic-like chemicals in the tick saliva. The bite site is marked after a few days by a small red to violaceous papule with a central eschar. One spirochete is sufficient to initiate TBRF, and the infection rate after a single bite by an infected tick is more than 50%. The incubation period from tick bite to onset of the first febrile episode is 3 to 12 days.[36] Since most people are unaware that they have been bitten, the history of potential regional exposures in high-altitude mountainous regions is essential to the correct diagnosis.

TBRF is defined clinically by the sudden onset of two or more episodes of high fever (>39°C [102.2°F]) spaced by afebrile periods of 4 to 14 days, with the first febrile episode lasting 3 to 6 days and the relapsing episodes lasting 1 to 3 days each.[25,36,37] The first episode ends with a 15- to 30-minute "crisis" with tachycardia, hypertension, hyperpyrexia (as high as 43°C [109.4°F]), and rigors, followed by diaphoresis and defervescence.[25,36,37] All febrile episodes are accompanied by nausea, headache, neck stiffness, myalgia, and arthralgia. The relapsing febrile episodes result from the growth of new spirochete populations in the blood to replace those killed by macrophages and cytokines. Most patients have splenomegaly, and 10% will have hepatomegaly. Direct neurologic involvement is more common than in LB and may include cranial nerve neuritis (especially cranial nerves VII and VIII), radiculopathy, and myelopathy. Myocarditis is also more common than in LB; may be complicated by adult respiratory distress syndrome, pulmonary edema, and cardiomegaly; and is often fatal.[37]

Laboratory diagnostics include identifying the spirochetes microscopically on peripheral blood smears and detecting their nucleic acids using PCR assays. Thrombocytopenia (platelets <150 μL) is a common finding on the complete blood count. Most patients will have elevated aminotransferase levels, unconjugated bilirubin, and prolonged prothrombin and partial thromboplastin times.[34,37]

Treatment strategies include tetracycline (500 mg or 12.5 mg/kg PO four times daily), or doxycycline (100 mg PO twice daily), or erythromycin (500 mg or 12.5 mg/kg PO four times daily for 10 days). Parenteral therapy with penicillin G or ceftriaxone is recommended for central nervous system (CNS) involvement.



FIG. 296.11 *Rickettsia rickettsii* tissue biopsy specimen, with immunohistochemical stain. Immunohistochemical stain of a tissue biopsy specimen demonstrating intracellular gram-negative *Rickettsia rickettsii* bacteria *(red)* within vascular endothelial lining cells. Infection with *R. rickettsia* causes the systemic vasculitis of Rocky Mountain spotted fever that manifests initially as petechial skin lesions and may progress without antirickettsial therapy to microvascular leakage and ischemic vasculopathy with gangrene and autoamputation of digits and limbs (see Fig. 296.14). *(From Biggs HM, Behravesh CB, Bradley KK, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States.* MMWR Recomm Rep. *2016;65:1–44.)*

The JHR is much more common, although rarely fatal, during treatment of TBRF than during treatment of LB and occurs in 30% to 40% of patients with TBRF.[25] At present, no prophylactic strategies to reduce the severity of the JHR have proved beneficial or have been adequately tested in multiple clinical trials, including therapy with antipyretics, corticosteroids, or naloxone. Antispirochetal therapy for TBRF with penicillin instead of tetracycline has a slightly lower risk of associated JHR.[25]

Spotted Fever Group Rickettsial Infections

The family Rickettsiaceae contains two genera: the SF–causing genus *Rickettsia* and the scrub typhus–causing genus *Orientia* (see Chapters 186 and 191). The rickettsiae may be further stratified clinically into the tick-borne SF group and mouse mite–transmitted rickettsialpox caused by *Rickettsia akari* (see Chapter 187). The rickettsiae are obligate intracellular, gram-negative bacteria that thrive in ixodid tick salivary glands and are transmitted during blood-feeding. Once injected into the host, rickettsiae are initially distributed regionally via lymphatics, with some species causing marked regional lymphadenopathy (e.g., *Rickettsia slovaca*). Within 2 to 14 days (mean, 7 days), rickettsiae are disseminated hematogenously to vascular endothelial lining cells of target organs, including the CNS, lungs, and myocardium (Fig. 296.11).

Rickettsiae gain entry into host endothelial cells in a Trojan horse–like manner by using their outer membrane proteins (OmpA and OmpB) to stimulate endocytosis. Once within phagosomes, rickettsiae escape to enter the cytosol or nucleus for rapid replication by binary fission, safe from host immune attack. The tick-borne rickettsial diseases that cause SFs are compared in a descending order of clinical severity of infection by preferred tick vectors and wild animal reservoirs in Table 296.3.

The global epidemiology of the tick-borne SF–causing rickettsiae has dramatically evolved since the transmission cycle of RMSF was first described by Ricketts in 1906 with the following: emerging new strains and diseases (*R. slovaca*–associated lymphadenopathy); greater understanding of the highly conserved genome of several related species (*Rickettsia africae–Rickettsia parkeri* and the *Rickettsia conorii* subspecies); wider geographic distribution and greater virulence of existing strains (*R. rickettsii, R. conorii* subspecies, *Rickettsia australis*); unanticipated new tick vectors for some SFs (*Rhipicephalus sanguineus* for RMSF in the United States); cluster outbreaks of tick-borne rickettsioses in returning travelers (*R. africae* causing African tick-bite fever); and regional clusters and epidemic cycles of more severe SFs worldwide (RMSF in the United States, MSF in Europe, and QTT in Australia).[38–43] The reasons for such changes in rickettsial SF epidemiology are unclear and may include warming temperatures and increasing humidity, more frequent drought-rain cycles, residential development in preferred tick ecosystems, more competent tick vectors given competitive advantages by environmental and genetic changes, more frequent contact between ticks and humans outdoors, and international trade and travel distributing tick vectors and their preferred animal hosts quickly and widely.

In the United States, the SF group rickettsioses are nationally notifiable infectious diseases that are primarily caused by the highly pathogenic *R. rickettsii* and less pathogenic rickettsial species such as *R. parkeri* and *Rickettsia* 364D (Fig. 296.12; see also Fig. 296.8). In 2016, Drexler and coinvestigators reported the results of their summary analysis of all passive surveillance data regarding SF group rickettsioses reported to the CDC during the period 2008–2012 in order to reassess the epidemiology of reported cases and to analyze any significant trends in incidence rates.[44] The incidence rate for SF group rickettsioses in the United States increased from 1.7 cases per million person-years in 2000 to 14.3 cases per million person-years in 2012.[44] SF group rickettsiosis cases were more frequently reported among males and persons of white race and non-Hispanic ethnicity.[44] Although the case-fatality rate (CFR) was low (0.4%), it was significantly higher for American Indian/Alaska Natives (relative risk = 5.4) and Asian/Pacific Islanders (relative risk = 5.7) and was highest overall in children under 10 years of age (CFR = 1.6%).[44] The authors recommended the increased use of PCR and the improved reporting of clinical signs, such as eschars, to better delineate the risk factors, incidence rates, and outcomes of SF group rickettsioses.[44] Following these recommendations, Herrick and coauthors utilized improved clinical reporting and molecular confirmation of *R. parkeri* infections by real-time PCR to establish a new geographic distribution and tick vector for *R. parkeri* infections in the United States (see Fig. 296.12).[45] Although all prior reported cases of *R. parkeri* rickettsiosis were described in the coastal Southeast and linked to transmission by the Gulf Coast tick (*Amblyomma maculatum*), Herrick and coauthors described one confirmed and one probable case of *R. parkeri* rickettsiosis acquired in a mountainous region of Arizona, well outside of the distribution range of the Gulf Coast tick and likely transmitted by a newly recognized tick vector, *Amblyomma triste* (see Fig. 296.12).[45]

The tick-borne SF rickettsioses share many common presenting features, including: (1) incubation periods of about 1 week; (2) flulike prodromes of fever, headache, myalgia, nausea, vomiting, and abdominal pain (that may mimic acute appendicitis in RMSF); (3) spotty rashes within 3 to 5 days of fever onset (Fig. 296.13); and (4) necrotic eschars at tick-bite sites (see Fig. 296.12B). Some SF rickettsial diseases may be "spotless," including RMSF in 10% to 15% of cases, complicating early differential diagnosis. The tick-borne rickettsial infections that can cause spotty rashes include *R. rickettsii* (RMSF), *R. conorii* (MSF), *R. australis* (QTT), and *R. africae–R. parkeri* (African–North American tick-bite fever) in about 50% of cases. The tick-borne rickettsial infections that are associated with one or more necrotic eschars at tick-bite sites include *R. conorii, R. australis, R. africae–R. parkeri, R. helvetica, R. japonica, R. slovaca, R. aeschlimannii, R. honei,* and *R.* 364D (*R. philipii* [proposed]). The SF rickettsioses may vary in severity from causing multisystem organ failure (RMSF, MSF) to painful lymphadenopathy (*R. africae–R. parkeri, R. slovaca*) to mild to subclinical disease (*R. aeschlimannii*).

After an average incubation period of 1 week, RMSF starts with a flulike, febrile prodrome followed by a characteristic maculopapular evolving to petechial rash in 85% to 90% of cases in 3 to 5 days. The pathognomonic rash starts distally on the wrists and ankles and then spreads centripetally up the limbs (see Fig. 296.13). The pathophysiologic mechanisms of petechial rashes and target organ system damage (CNS, lungs, heart) in the SF rickettsioses include vascular endothelial cell damage by microbial replication, vascular inflammation (vasculitis), and increased widespread vascular permeability, which may result in hypovolemic shock, oliguric prerenal failure from acute tubular necrosis, cerebral edema, and noncardiogenic pulmonary edema (see Fig. 296.11).

TABLE 296.3 Spotted Fever Group of Tick-Borne Rickettsioses

RICKETTSIA SPECIES	TICK-BORNE DISEASES	GEOGRAPHIC DISTRIBUTION	TICK VECTORS	WILD ANIMAL RESERVOIRS (MAMMALS)
R. rickettsii	Rocky Mountain spotted fever (SF), Brazilian SF	Continental United States, Central America (Costa Rica, Mexico, Panama), South America (Argentina, Brazil)	*Amblyomma, Dermacentor, Rhipicephalus* spp.	Ungulates, rodents
R. conorii	Boutonneuse fever: Mediterranean SF, Israeli SF, Astrakhan SF, Indian tick typhus, Kenyan tick typhus	Mediterranean Basin, Africa, Middle East, Asia	*Rhipicephalus* spp.	Ungulates, rodents
R. sibirica	North Asian tick typhus (Siberian tick typhus)	Africa (Niger, Mali, South Africa), Asia (Russia, China, Mongolia, Pakistan, Kazakhstan, Kirgizia, Tajikistan), Europe (France)	*Dermacentor, Haemaphysalis, Hyalomma* spp.	Ungulates, rodents
R. japonica	Japanese SF	Japan and China	*Haemaphysalis* spp., *Ixodes ovatus*	Ungulates, rodents
R. australis	Queensland tick typhus	Eastern Australian seaboard from Cairns, Queensland, to Gipps Island, Victoria	*Ixodes* spp., especially *I. holocyclus* and *I. tasmania*	Rodents, bandicoots, wombats, cattle, domestic dogs
R. honei	Flinders Island SF	Southern Australia, Thailand	*Aponomma* spp.	Rodents
R. honei subspecies *marmionii*	Australian SF	Cape York, Queensland	*Haemaphysalis novaeguineae*	Similar to *R. australis:* Rodents, bandicoots, wombats, cattle, domestic dogs
R. africae and *R. parkeri*	African tick-bite fever	Sub-Saharan Africa, North America, South America, Caribbean	*Amblyomma* spp.	Rodents
R. 364D (*R. philipii* [proposed])	*R.* 364D "spotless" rickettsiosis with tick-bite eschar	California	*Dermacentor occidentalis*	Rodents, other small mammals
R. slovaca	Tick-borne lymphadenopathy; *Dermacentor*-borne eschar, lymphadenopathy, or necrosis	Europe	*Dermacentor* spp.	Ungulates, rodents
R. aeschlimannii	Not named at present	Southern Europe, Africa	*Hyalomma* spp.	Ungulates, rodents



FIG. 296.12 *Rickettsia parkeri* rickettsiosis, preferred tick vectors, eschar, and immunohistochemical stain of tissue biopsy. *Rickettsia parkeri* rickettsiosis is transmitted by female (A) and male (C) Gulf Coast ticks (*Amblyomma maculatum*). Rickettsiosis is characterized by initial erythema, swelling, and itching at the bite site that ulcerates and then scabs over as an inoculation eschar (B). Patients often report fever, rash, and regional lymphadenopathy. Additional symptoms may include chills, myalgia, arthralgia, malaise, and headache. Laboratory diagnostics include immunohistochemical staining of eschar biopsy sites to demonstrate intracellular, gram-negative spotted fever group *Rickettsia* (D); growth of the organism in cell cultures from specimens; and nucleic acid speciation by quantitative polymerase chain reaction assays. Treatment is with oral doxycycline, 100 mg twice a day for a minimum of 10 days. *(From Straily A, Feldpausch A, Ulbrich C, et al. Notes form the field:* Rickettsia parkeri *rickettsiosis—Georgia, 2012–2014.* MMWR Morb Mortal Wkly Rep. *2016;65:718–719.)*



FIG. 296.13 Characteristic initial distal maculopapular-petechial rash of Rocky Mountain spotted fever. This rash is shown on the dorsal aspect of a child's right hand and wrist. *(From Public Health Image Library. Image 1962. Atlanta, GA: Centers for Disease Control and Prevention.)*



FIG. 296.14 Digital ischemia with gangrene resulting from Rocky Mountain spotted fever. Gangrene of the digits in a patient with ischemic vasculopathy from late-stage Rocky Mountain spotted fever caused by tick-transmitted infection with *Rickettsia rickettsii. (From Biggs HM, Behravesh CB, Bradley KK, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States.* MMWR Recomm Rep. *2016;65:1–44.)*

Distal, digital skin necrosis and gangrene of the digits and limbs may occur in severe cases of RMSF and QTT from hypoperfusion (Fig. 296.14).

Cardiac vasculitis may manifest as myocarditis with intraventricular conduction blocks. Aside from petechial rash and thrombocytopenia, other hemorrhagic manifestations in RMSF and other SFs are rare. CNS complications in RMSF and other severe SF infections may include ataxia, photophobia, transient deafness, focal neurologic deficits, meningismus, meningoencephalitis, seizures, and coma. Pulmonary complications may include cough, alveolar infiltrates, interstitial pneumonitis, pleural effusions, pulmonary edema, and adult respiratory distress syndrome.

Initially, MSF caused by *R. conorii* was thought to be a more benign disease than RMSF. Severe cases of MSF with multiple eschars and multisystem disease similar to RMSF with CNS, renal, and pulmonary complications were first reported in 1981, and now appear to be increasing across Europe.[44] In a 1997 outbreak of MSF in Portugal, CFRs of 32% were recorded and exceeded those of untreated RMSF (CFR of 23%).[44] QTT, African tick-bite fever, and *R. slovaca*–associated lymphadenopathy are generally milder diseases than RMSF and MSF.[43] However, severe cases of QTT with RMSF-like complications, including renal insufficiency and pulmonary infiltrates, were recently reported from Australia.[43]

Although African tick-bite fever caused by *R. africae,* a similar tick-bite fever in North America caused by *R. parkeri,* and *R. slovaca* infections may all cause multiple necrotic eschars and painful regional lymphadenopathy, these SF infections are often spotless (≤50%) and follow typical rickettsial SF prodromes.[42,43] A history of tick bites, eschars, and painful regional lymphadenopathy helps to establish the correct diagnosis, especially in the absence of adequate diagnostic laboratory services. The precise laboratory diagnosis of tick-borne rickettsial SFs may be established by microbiologic isolation of the causative organisms from skin biopsy specimens or blood cultures, nonspecific immunofluorescence antibody tests that cross react with many SF antigens, other immunocytologic techniques to demonstrate intracellular rickettsiae, and PCR assay to identify and speciate rickettsial DNA or RNA.

Antibiotic treatment mainstays for the tick-borne rickettsial SFs remain the tetracyclines for most cases and chloramphenicol for severe multisystem disease and during pregnancy.[39] Although the quinolones, azithromycin, and clarithromycin may be as effective as tetracyclines and chloramphenicol for rapidly managing some SFs, they are not recommended for initial therapy at this time. Although short (1- to 2-day) courses of doxycycline have been reported to be as successful as 10-day courses in some SF infections (e.g., MSF), such treatment strategies have not been tested in randomized controlled trials in other SF infections and are also not recommended at this time. Most authorities now recommend that tetracycline, chloramphenicol, or ciprofloxacin for tetracycline-allergic patients be continued for a minimum of 7 days or until the patient has been afebrile for at least 48 hours and is improving clinically.

Q (Query) Fever

Q (query) fever (see Chapter 188) was first described in Australia in 1935, and its causative organism, *C. burnetii,* was isolated shortly thereafter.[46] *C. burnetii* is a gram-negative, intracellular, spore-forming bacterium that is the sole species of its genus. *C. burnetii* is genetically related to *Legionella pneumophila* and, like *L. pneumophila, C. burnetii* is usually transmitted to humans by inhalation of contaminated aerosols.[46,47] Q fever is a zoonosis with worldwide distribution and extensive domestic animal (cattle, sheep, goats, cats, dogs), wild animal (birds, rabbits, reptiles), and arthropod (ticks) reservoirs.[47] In most cases, humans are infected not by tick bites but by inhaling spores or bacteria in aerosols contaminated with infectious particles in dried animal feces, milk, or products of conception.[46–48] Q fever may also be transmitted by ingestion of contaminated milk, by vertical transmission from mother to fetus, by contaminated blood product transfusion, and even percutaneously by crushing infected ticks near breaks in the skin barrier.[46]

C. burnetii is reactivated during pregnancy and multiplies extensively in the placenta, exposing abattoir workers, veterinarians, researchers (especially those working with parturient sheep), and domestic pet owners (especially of cats) to highly infectious aerosols during delivery.[46,47] Several cases of Q fever were reported among US military personnel deployed to Iraq and Afghanistan and in travelers returning from Asia, Latin America, and sub-Saharan Africa.[48,49] *C. burnetii* has long been considered a potential bioterrorism weapon for several reasons, including its environmental stability, spore-forming capability, ease of aerosolized dispersal, and high pathogenicity, with an ability to initiate infection with a single microorganism.

Over 3000 cases of acute Q fever presenting as pneumonia in most cases (62%) were reported from the Netherlands during the period 2007–2009. Most of the patients were males, smokers, and adults 40 to 60 years old. Very few patients worked in agriculture (3.2%) or in meat-processing plants, including abattoirs (0.5%). Public health investigators determined that the Q fever epidemic was caused by the aerosolization of contaminated dust particles from commercial dairy goat farms located in densely populated areas that were experiencing waves of Q fever–induced abortions in infected goats. Strict veterinary infectious disease control measures on dairy goat and sheep farms halted the epidemic in 2010, but left many infected patients at increased risk of developing chronic Q fever endocarditis years later.[50]

After an average 2-week incubation period (range, 2–29 days), Q fever may manifest as a wide variety of illnesses in humans, including the following: acute Q fever, a self-limited febrile illness with severe headache, retro-orbital pain, and nonproductive cough; Q fever pneumonia with consolidated opacities, pleural effusions, and hilar lymphadenopathy on chest radiographs; Q fever granulomatous hepatitis, usually after ingestion of unpasteurized contaminated milk; CNS Q

fever with protean manifestations ranging from aseptic meningoencephalitis and transient behavioral and sensory disturbances to cranial nerve palsies and hemifacial pain mimicking trigeminal neuralgia; and chronic Q fever endocarditis, especially in predisposed patients with congenital valvulopathies, prosthetic heart valves, aortic aneurysms, or vascular grafts.[49,51] Patients who are immunocompromised by pregnancy, congenital immunodeficiency disorders, cancer, human immunodeficiency virus infection/acquired immunodeficiency syndrome, organ transplant antirejection therapy, renal dialysis, or prolonged corticosteroid therapy are at greater risk for acquiring more severe and chronic Q fever infections.[47,51]

Recent reports have recommended that clinicians should consider a diagnosis of Q fever in the absence of infected livestock exposure and should preoperatively screen patients undergoing elective cardiac valve surgery for Q fever.[49,51] In an analysis of two national surveillance systems for Q fever cases in the United States during the reporting period 2000–2012, CDC investigators noted that most cases (61%) did not report any livestock exposures, but a substantial proportion reported drinking raw milk (prevalence rate, 8.4%).[49] The overall incidence rate of Q fever during the reporting period was 0.38 cases per million person-years, with a hospitalization rate of 62% and a CFR of 2.0%.[49] In 2015, Dutch investigators reported three cases of postoperatively diagnosed chronic Q fever endocarditis in patients requiring cardiac valve surgery.[51] Earlier diagnosis and antimicrobial therapy might have prevented progressive valve dysfunction from chronic Q fever endocarditis in these cases and eliminated the need for cardiac valvuloplasty or replacement.[51]

Because the isolation of *C. burnetii* requires Biosafety Level 3, most diagnostic laboratory strategies for Q fever rely on microscopic detection on Giemsa-stained smears of blood or sputum or tissue biopsies (liver, excised heart valves), on antibody detection by immunofluorescence assays, or on DNA detection by PCR assay.[46,48] The prognosis is usually excellent in the acute Q fever illnesses, and mortality is rare after appropriate antibiotic therapy with tetracyclines (doxycycline [100 mg PO twice daily for 14 days] is preferred) or fluoroquinolones. Chronic Q fever endocarditis will require prolonged treatment with two antibiotics, either rifampin (300 mg PO twice daily) and ciprofloxacin (750 mg PO twice daily) for 3 years or doxycycline (100 mg PO twice daily) and hydroxychloroquine (200 mg PO three times daily) for at least 18 months. Such combined therapies will require close monitoring for drug toxicities, especially hepatotoxicity from rifampin and oculotoxicity from hydroxychloroquine. In addition, all patients with Q fever endocarditis should undergo screening transesophageal echocardiography for underlying valvulopathies and/or aortic aneurysms.[51] Chronically infected heart valves and vascular grafts will require surgical replacement.

Tularemia

Tularemia, also known as rabbit fever or deer fly fever (see Chapter 227), was first described as a zoonosis in squirrels in Tulare County, California, in 1911. Its causative agent, *F. tularensis,* was later identified as a gram-negative coccobacillus by Dr. Edward Francis during an investigation of deer fly fever in Utah in 1921.[52] Tularemia occurs in regional pockets worldwide, has a very large wild and domestic animal reservoir, and is seasonally transmitted to humans by ixodid tick and deer fly bites and by contact with infected animals, especially rabbits and muskrats. The primary tick vector of tularemia in the United States is the American dog tick, *Dermacentor variabilis* (Fig. 296.15). Tick-transmitted tularemia is most commonly reported during the spring and summer (May to August) worldwide.[53] Tularemia transmitted through contact with an infected animal occurs more often during the fall through hunting and trapping seasons, especially among male hunters who field-clean infected animal carcasses.[53] *F. tularensis* is an extremely stable microorganism in nature, surviving in soil, water, and animal carcasses for months to years. In addition to fecal or vomit contamination of tick bites and direct inoculation of intact skin or mucosal surfaces when crushing ticks or skinning animals, tularemia may be transmitted by ingesting raw or undercooked infected game or bush meats, drinking contaminated water, or inhaling aerosolized microorganisms.[53–55]

In 2000, a cluster outbreak of primary pneumonic tularemia in 11 patients (with one fatality) was reported from Martha's Vineyard, Massachusetts.[56] A case-control study of the outbreak implicated aerosolized exposure to *F. tularensis* during summertime brush cutting and lawn mowing as significant (odds ratio, 9.2; 95% confidence interval, 1.6–68.0) risk factors for pneumonic tularemia.[56] Concerns about inhalational transmission and potential biological weaponization of *F. tularensis* led to the reinstatement of tularemia as a nationally notifiable infectious disease in 2000.[53,54] The CDC reported a total of 1368 cases of tularemia from 44 states from 1990 to 2000 (period prevalence, 124 cases/year; range, 86–93 cases/year), with most cases occurring in males during May to August in regional pockets, including Arkansas and Missouri, eastern Oklahoma and Kansas, southern Montana and South Dakota, and Martha's Vineyard.[53]



FIG. 296.15 *Dermacentor variabilis,* the American dog tick, questing for a host. This female American dog tick is a vector of tick paralysis in the southeastern United States and Pacific Northwest and is a vector of Rocky Mountain spotted fever in addition to the Rocky Mountain wood tick (*D. andersoni*) in the western United States. *(From Public Health Image Library. Image 170. Atlanta, GA: Centers for Disease Control and Prevention.)*

There are two biovars of *F. tularensis,* with biovar A (*F. tularensis* biogroup *tularensis*) causing 60% to 90% of tularemia cases in North America and biovar B (*F. tularensis* biogroup *palearctica*) causing a milder disease throughout Europe and Asia.[54,55,57] The presenting clinical manifestations of infection depend on the virulence of the biovars (A > B), route of entry of microorganisms, multisystem infections, and immunocompetence of infected hosts.

The portal of entry of *F. tularensis* has historically been used to classify the clinical manifestations of tularemia, with untreated pneumonic tularemia having the highest CFRs of 30% to 60% (Table 296.4).[53,55] The differential diagnosis of ulceroglandular tularemia, the most common presentation, is extensive and includes other arthropod bites, bacterial and viral infections, and fungal diseases capable of causing skin ulcers with painful regional lymphadenopathy.[54] Diagnostic strategies for tularemia include the following: microscopic identification or culture in Biosafety Level 3 facilities of microorganisms from blood, sputum, gastric lavage fluid, lung biopsy, or lymph node aspirates (sensitivity, 10%–25%); acute and convalescent serology comparing antibody titers (sensitivity, >85%); direct immunofluorescence antibody testing; and antigen detection by PCR assay (sensitivity, 50%–73%). Frequently accompanying laboratory abnormalities in tularemia include significant elevations in the erythrocyte sedimentation rate; significant leukocytosis (>10,000/μL), often with normal differential counts; and thrombocytosis.[55] The recommended treatment strategies for tularemia have evolved considerably from historical treatments with painful intramuscular injections of streptomycin to oral therapy with the aminoglycosides and fluoroquinolones, which are effective in 86% of cases and may result in resolution of ulcers within 72 hours.[55] Most cases in adults, including pneumonic tularemia, may be managed with fluoroquinolones alone (ciprofloxacin, 400 mg IV or 500 mg PO twice daily for 7–14 days, or levofloxacin, 500 mg IV or PO twice daily for 7–14 days), with aminoglycosides (gentamicin or amikacin, 3–5 mg/kg/day for 10–14 days) reserved for pediatric infections and

TABLE 296.4 Clinical Classification of Tularemia Based on the Portal of Entry

CLINICAL CLASSIFICATION OF TULAREMIA CASES	CASE DEFINITION BY CLINICAL PRESENTATION	PORTALS OF ENTRY OF *FRANCISELLA TULARENSIS*	CASE FREQUENCY, UNITED STATES (%)
Ulceroglandular	Malaise, fever, bite eschars or ulcers, painful regional lymphadenopathy	Tick or deer fly bite, or direct inoculation across intact dermis	80
Glandular	Malaise, fever, suppurative lymphadenopathy	Direct inoculation across intact dermis	15
Oropharyngeal	Malaise, fever, sore throat, dysphagia, painful cervical lymphadenopathy	Ingestion of raw or undercooked infected game or bush meats	<5
Oculoglandular	Malaise, fever, ocular infection, regional facial lymphadenopathy	Ocular inoculation of infectious fluids or animal danders or autoinoculation from bite eschar or ulcers	1
Typhoidal	Malaise, fever, abdominal pain, mesenteric lymphadenopathy; mimics typhoid fever	Ingestion of contaminated water	Rare
Pneumonic	Malaise, fever, pneumonia with multiple ill-defined infiltrates, hilar lymphadenopathy; mimics inhalational anthrax	Inhalation of contaminated aerosols, aerosolized bioweapon exposures, or hematogenous spreading from glandular or typhoidal infections	Rare, except on Martha's Vineyard after aerosolized exposures during mechanized bush trimming and lawn mowing

widely disseminated systemic infections. Relapse rates are highest with oral tetracyclines, including doxycycline, and chloramphenicol, which may still be indicated for cases with CNS dissemination despite its potential for bone marrow toxicity.

Tick-Borne Ehrlichioses and Anaplasmosis

The human ehrlichioses and anaplasmosis (formerly known as human monocytic and human granulocytic ehrlichiosis, respectively) are classic examples of emerging tick-borne infectious diseases (see Chapter 192). Since 1986, four new tick-borne bacterial species have been identified and classified into a new family, Anaplasmataceae. The four genera of Anaplasmataceae comprise obligate, intracellular, gram-negative bacteria closely related genetically to the family Rickettsiaceae. The Anaplasmataceae include two genera that are synergistic parasites of flatworms *(Neorickettsia sennetsu)* and filarial worms (*Wolbachia* spp.) and two genera that are tick-borne bacterial infections of many mammals, including humans: *Ehrlichia* and *Anaplasma*.[58,59] Like rickettsiae, the Anaplasmataceae attach to molecular ligands on phagocytic cells to gain Trojan horse–like entry into leukocytes and then trick intracellular phagosomes into releasing them into the cytosol for replication (Figs. 296.16 and 296.17).[58,59]

The human ehrlichioses are potentially fatal tick-borne infectious diseases caused by either *E. chaffeensis,* first identified in 1991, or *E. ewingii,* first identified in 1992.[60] Although the ehrlichioses became nationally notifiable infectious diseases in the United States in 1999, the case definitions did not differentiate between cases of ehrlichiosis and anaplasmosis or between *E. chaffeensis* and *E. ewingii* infections until 2008.[60] In 2016, Heitman and coworkers reported a summary analysis of ehrlichiosis cases in the United States reported to two national passive surveillance systems, the National Notifiable Diseases Surveillance System and the CDC's Case Report Forms.[60] During the reporting period 2008–2012, 4613 cases of *E. chaffeensis* infections were reported, for an incidence rate (IR) of 3.2 cases per million person-years.[60] The hospitalization rate was 57%; the CFR was 1%, and children less than 5 years of age had the highest CFRs (4%).[60] During the same period, 55 cases of *E. ewingii* infection were reported, for a national IR of 0.04 cases per million person-years with a hospitalization rate of 77% and no deaths.[60] Although prior reports had linked ehrlichiosis cases to concurrent immunocompromise, immunosuppressive conditions were only reported in 26% of cases.[60] Using improved surveillance of newly reportable diseases since 1999, the authors observed that the overall IR for ehrlichiosis infections had increased fourfold in the United States between 2000 and 2012; most cases occurred in immunocompetent persons; and children under 5 years of age had increased CFRs relative to older patients.[60]

The tick-borne Anaplasmataceae are endemic in the United States and have preferred geographic distributions, tick vectors, and wild and domestic animal reservoirs (Table 296.5). They spread from the infected



FIG. 296.16 Intracellular morulae in ehrlichiosis and anaplasmosis. Wright-stained peripheral blood smears that demonstrate an intramonocytic morula characteristic of ehrlichiosis caused by *Ehrlichia chaffeensis (left)* and an intragranulocytic morula associated with either ehrlichiosis caused by *Ehrlichia ewingii* or anaplasmosis caused by *Anaplasma phagocytophillum (right). (From Biggs HM, Behravesh CB, Bradley KK, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States.* MMWR Recomm Rep. *2016;65:1–44. Photos courtesy J. Stephen Dumler, MD, University of Maryland, and Bobbi S. Pritt, MD, Mayo Clinic.)*

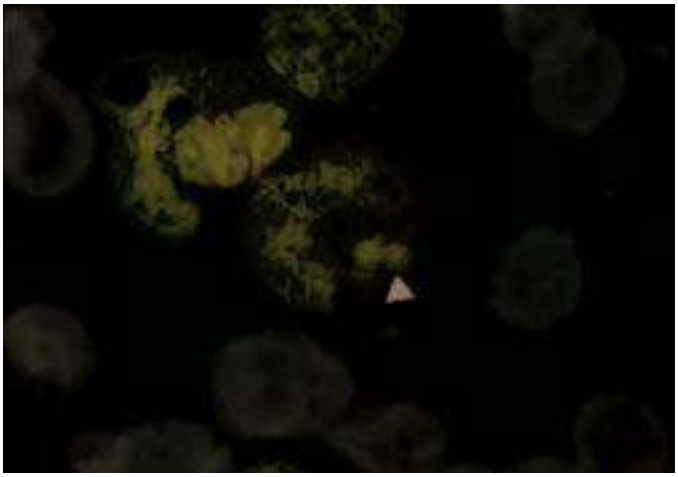

FIG. 296.17 *Ehrlichia chaffeensis* morula *(arrowhead)* within a monocyte (peripheral blood smear, Wright stain, ×1000.) *(From Safdar N, Love RB, Maki DG. Severe* Ehrlichia chaffeensis *infection in a lung transplant recipient: a review of ehrlichiosis in the immunocompromised patient.* Emerg Infect Dis. *2002;8:320–323.)*

TABLE 296.5 Human Ehrlichioses and Anaplasmosis

	HUMAN MONOCYTOTROPIC EHRLICHIOSIS	HUMAN GRANULOCYTOTROPIC EHRLICHIOSIS	HUMAN GRANULOCYTOTROPIC ANAPLASMOSIS
Former disease nomenclature	Human monocytic ehrlichiosis	Human granulocytic ehrlichiosis	Human granulocytic ehrlichiosis
Causative agent(s)	*Ehrlichia chaffeensis*	*Ehrlichia ewingii, Ehrlichia canis*—one asymptomatic human case reported in Venezuela	*Anaplasma phagocytophilum*
Leukocyte targets	Monocytic cell phagosomes	Neutrophil phagosomes	Granulocyte-neutrophil phagosomes
Tick vectors	*Amblyomma americanum* (Lone Star tick)	*Amblyomma americanum* (Lone Star tick), *Dermacentor variabilis* (American dog tick)	*Ixodes persulcatus* complex (American deer tick): *I. scapularis, I. ricinus, I. pacificus*
Animal reservoirs	White-tailed deer, coyotes, dogs	White-tailed deer, dogs	Rodents, deer, ruminants, horses
US regional distribution	Southeastern and south central United States	South central United States	Northeast US, upper Midwest, northern California
US regional prevalence	2–5 cases/100,000	Up to 10% of presumed cases have *E. ewingii* infections in south central United States	50–60 cases/100,000; high seroprevalence rates in children (>20%) who have had subclinical infections
Seasonal occurrences	April–September, peaking in July	Spring–fall	May–July
Incubation periods (wk)	1–4	1–4	1–4
Modes of transmission	Tick bite, blood product transfusion	Tick bite, blood product transfusion	Tick bite, blood product transfusion, nosocomial
Frequently presenting clinical manifestations	Fever, malaise, headache, myalgias, rash in <40%	Same initial manifestations, but much milder, except in immunocompromised individuals	Fever, malaise, headache, myalgias; rarely rash
Laboratory abnormalities	Leukopenia, thrombocytopenia, transaminitis	Leukopenia, thrombocytopenia, transaminitis	More pronounced and prolonged leukopenia, thrombocytopenia, transaminitis
Potential complications, especially in immunocompromised individuals	Meningoencephalitis, acute renal and respiratory failure, hepatitis, myocarditis	Milder and less likely, except in patients immunocompromised by HIV/AIDS, organ transplantation, prolonged corticosteroid therapy	May be significant in immunocompromised patients with high fevers, seizures, confusion, hemorrhagic diathesis, rhabdomyolysis, shock, acute tubular necrosis, adult respiratory distress syndrome; some specific CNS complications may include eighth nerve palsy, brachial plexopathy, demyelinating polyneuropathy
Case-fatality rate (CFR)	3%, higher in immunocompromised individuals	No deaths reported	0.5%, higher CFR in immunocompromised individuals
Recommended confirmatory diagnostic tests	Wright-stained peripheral blood smears with characteristic intracytoplasmic morulae in monocytes, DNA detection by PCR assay, culture	Wright-stained peripheral blood smears with characteristic intracytoplasmic morulae in neutrophils, DNA detection by PCR assay	Wright-stained peripheral blood smears with characteristic intracytoplasmic aggregates in neutrophils, DNA detection by PCR assay, increased immunofluorescence antibodies in initial and paired serum samples
Current antibiotic resistance	Fluoroquinolones	Fluoroquinolones	Fluoroquinolones
Currently recommended antibiotic therapy, adults	Doxycycline, 100 mg PO bid, or tetracycline, 250–500 mg PO qid, for minimum of 3 days after defervescence to maximum of 14–21 days	Doxycycline, 100 mg PO bid, or tetracycline, 250–500 mg PO qid, for minimum of 3 days after defervescence to maximum of 14–21 days	Doxycycline, 100 mg PO bid, or tetracycline, 250–500 mg PO qid, for minimum of 3 days after defervescence to maximum of 14–21 days
Currently recommended antibiotic therapy, children	Doxycycline, 4.4 mg/kg PO bid, or tetracycline, 25–50 mg/kg PO qid, for minimum of 3 days after defervescence to maximum of 14–21 days	Doxycycline, 4.4 mg/kg PO bid, or tetracycline, 25–50 mg/kg PO qid, for minimum of 3 days after defervescence to maximum of 14–21 days	Doxycycline, 4.4 mg/kg PO bid, or tetracycline, 25–50 mg/kg PO qid, for minimum of 3 days after defervescence to maximum of 14–21 days

CNS, Central nervous system; *HIV/AIDS,* human immunodeficiency virus infection/acquired immunodeficiency syndrome; *PCR,* polymerase chain reaction.

tick's gut to its salivary gland, are inoculated over 24 to 36 hours into the host's dermis, and cause subclinical (especially in children) to severe and potentially fatal infections (especially in immunocompromised adults) within 1 to 4 weeks. Because transovarian transmission in ticks has not been observed, the major reservoirs of the Anaplasmataceae in nature are wild and domestic animals.[58,59] Although the presenting clinical manifestations are similar among Anaplasmataceae infections, the potential multisystem complications and resulting CFRs from these diseases are ultimately determined by the immunocompetence of human hosts (see Table 296.5). The human Anaplasmataceae are resistant to fluoroquinolones but remain susceptible to tetracyclines, which are now recommended for children and adults. Because there are no vaccines for the tick-borne ehrlichioses and anaplasmosis, the best preventive measures are tick avoidance and control and rapid removal of blood-feeding ticks within 36 hours from the exposure.[58,59]

TICK-BORNE PROTOZOAL INFECTIONS

Babesial Infections

Babesiosis is a tick-borne, malaria-like zoonosis (see Chapter 281) that usually causes subclinical infections with prolonged parasitemias in humans and can be transmitted vertically in utero and horizontally by blood product transfusion.[61–65] Babesiosis was initially described in cattle with red water (hemoglobinuric) fever in 1888, when Victor Babes

observed inclusions within bovine erythrocytes. Theobald Smith later identified the causative agent of bovine red water fever in 1893 as *Babesia bigemina,* accurately described the parasite's life cycle, and demonstrated for the first time the arthropod-borne transmission of an infectious disease to a mammal. Although more than 100 species of *Babesia* have now been identified as zoonoses in domestic and wild mammals, only a few species can cause babesiosis in humans, a disease characterized by fever, intravascular hemolysis, and hemoglobinuria (Table 296.6). In severe cases, usually in elderly, immunocompromised, or splenectomized human hosts, massive hemoglobinuria may be associated with severe nonimmune hemolytic anemia, jaundice, acute renal failure, and increased CFRs. Babesiosis is now reemerging as an arthropod-borne parasitic disease, as confirmed by increasing numbers of reported cases in the northeastern United States and Wisconsin and better laboratory detection of increasing seroprevalence rates there and in California.[62]

Human babesiosis may be divided into two epidemiologic and clinical patterns based on the causative *Babesia* species, their regional endemicity, and the immunocompetence of their human dead-end hosts (see Table 296.6). The first pattern is caused by *Babesia divergens* and related species or subspecies and occurs in immunocompromised, and often splenectomized, human hosts. It includes *B. divergens* babesiosis, first recognized in Eastern and now in Western Europe, a *B. divergens*–like babesiosis in the US Midwest caused by a *Babesia* species designated MO-1, and a babesiosis along the US Pacific Coast caused by *B. divergens*–like species designated as WA-1 and as CA types (e.g., CA-1, CA-2).[61–65] The *B. divergens*–related species are maintained in tick vectors by transovarian and transstadial transmission of the parasites, and most infections are transmitted by diminutive and usually unidentified and unnoticed nymphal ticks.[66] The human *B. divergens*–like cases occur primarily in cattle-ranching regions during the summer months, when tick vectors are most active and the incidence of bovine red water fever is greatest. These are the more severe cases of babesiosis, with hemolytic anemia, hemoglobinuria, and renal failure, usually in splenectomized persons.

The second and more common pattern of babesiosis in the United States occurs in regional pockets on the Northeast Coast (New York, Massachusetts, Rhode Island, Connecticut, New Jersey, and offshore islands [Block Island, Long Island, Nantucket]) and in the upper Midwest (Minnesota, Wisconsin) and is caused by *B. microti,* a rodent *Babesia* species transmitted to humans by the same ixodid ticks (black-legged or deer ticks) that transmit Lyme disease (see Fig. 296.5).[61] Thus *B. microti* babesiosis in the United States parallels the distribution of Lyme disease, occurs in clusters in the same regional pockets as Lyme disease, and may coexist with Lyme disease in an increasing number of recognized cases.[61,62,67,68] *B. microti*–induced babesiosis occurs during the warmest months (April through October), with 80% of cases reported between May and August, when black-legged ticks are most active. White-tailed deer serve as the primary hosts for adult black-legged ticks, but white-footed mice (*Peromyscus leucopus*) and other small mammals serve as the zoonotic reservoir for *B. microti*. Humans are usually infected by unnoticed bites by nymphal deer ticks.

Diagnostic strategies for babesiosis include the demonstration of characteristic intraerythrocytic and extraerythrocytic organisms on Giemsa-stained thin smears and subinoculation of human blood samples into hamsters for suspected *B. microti* infections or into gerbils for

TABLE 296.6 Causal Agents and Clinical Manifestations of Babesiosis

BABESIA SPECIES	GEOGRAPHIC DISTRIBUTION	TICK VECTORS	ANIMAL RESERVOIRS	EPIDEMIOLOGY	CLINICAL MANIFESTATIONS
B. divergens	United Kingdom, Western Europe, Eastern Europe, Sweden, Russia; not reported in United States	*Ixodes ricinus*	Cattle, reindeer	Incubation 1–4 wk Occurs during summer months in cattle-raising regions Targets splenectomized or immunocompromised patients primarily	Fulminant course with high case-fatality rate Fever, rigors, headache, myalgia, jaundice, hemoglobinuria, hemolytic anemia, acute renal failure, multiorgan failure
B. microti	Parallels the US Northeast endemic regions for *Borrelia burgdorferi,* especially the islands off New York, Massachusetts, Connecticut, and Rhode Island and focal areas in Connecticut, New Jersey, Wisconsin, and Minnesota	Deer ticks: *Ixodes scapularis*	White-footed mouse *(Peromyscus leucopus)*	Incubation 1–4 wk after tick bites or 4–9 wk after blood transfusions Transmission primarily by nymphal ticks Targets older, not necessarily immunocompromised patients; particularly severe in those immunocompromised by HIV infection, advanced age, coinfections with *Borrelia burgdorferi* Seasonality parallels tick nymph activity; 80% of cases occur May–August	Often asymptomatic in young, healthy patients Self-limited influenza-like febrile illness with onset of anorexia, malaise, lethargy, followed in 1 wk by high fever, diaphoresis, myalgias; mild splenomegaly, rarely hepatomegaly Later hemolysis, hemolytic anemia, thrombocytopenia, jaundice, acute renal failure, especially in the splenectomized, elderly, or immunocompromised Complications include ARDS and DIC Case-fatality rate: 5%
MO-1 (a relative or subspecies of *B. divergens*)	Rural Missouri and Kentucky	*Ixodes dentatus* (rabbit tick)	Rabbits, birds	Incubation 1–4 wk after tick bites Spring to autumn seasonality Targets splenectomized patients, like *B. divergens*	Same as above: often asymptomatic, except in the splenectomized, who will develop high parasitemias and multiorgan failure
WA-1 (a relative or subspecies of *B. gibsoni*)	Rural Washington State	Ixodid ticks, including *Ixodes dentatus*	Unknown; wild canids and ungulates suspected	Incubation 1–4 wk Targets the splenectomized, elderly, immunocompromised, premature infants May be transmitted by blood transfusion	Same as above: often asymptomatic, except in the splenectomized, who will develop high parasitemias and multiorgan failure
CA-1, CA-2, etc. (relatives or subspecies of mule deer and bighorn sheep *Babesia* species)	US Pacific Coast, primarily rural and semirural areas of California	Ixodid ticks	Unknown; mule deer and bighorn sheep suspected	Incubation 1–4 wk Targets the splenectomized, elderly, immunocompromised, and premature infants	Same as above: often asymptomatic, except in the splenectomized, who will develop high parasitemias and multiorgan failure

ARDS, Acute respiratory distress syndrome; *DIC,* disseminated intravascular coagulation; *HIV,* human immunodeficiency virus.

suspected *B. divergens*–related infections (Fig. 296.18). The serologic methods, especially useful when microscopic methods fail in low parasitemias, include indirect immunofluorescence antibody testing for specific immunoglobulin M antibodies in acute infections and PCR-based assays to detect *Babesia* DNA and species-specific DNA sequences.

Quinine (650 mg PO three times daily) and clindamycin (1.2 g IV twice daily or 600 mg PO three times daily), continued for 1 week or until parasitemias are in remission, can be used to treat babesiosis caused by all species.[61] Quinine and clindamycin are preferred therapies for WA-1 babesiosis and for severe *B. microti* infections, especially in older adults and splenectomized or immunosuppressed individuals.[61,65] For non–life-threatening *B. microti* infections, a 2-week course of oral atovaquone (750 mg twice daily) and azithromycin (500 mg on day 1, followed by 250 to 600 mg/day for 1 week) cleared parasitemias as effectively as quinine and clindamycin, with fewer side effects. For coinfections with *B. burgdorferi,* patients should be treated specifically for Lyme disease with doxycycline (200 mg PO twice daily for 2 weeks) and with antimalarial agents for babesiosis.[61,68]

Although the most common clinical presentation of babesiosis is a febrile viral-like illness with laboratory evidence of nonimmune hemolytic anemia and thrombocytopenia, Woolley and coinvestigators recently reported 6 cases of warm autoimmune hemolytic anemia among 86 patients treated for babesiosis at their institution over a 7.5-year period.[69] All 6 patients were asplenic ($P < .0001$) and demonstrated positive direct antiglobulin tests for immunoglobulin G and complement component 3, which confirmed production of autoantibodies against erythrocytes.[69] Unlike the non–immune-mediated hemolysis which characterizes most cases of babesiosis and resolves after appropriate antimicrobial therapy, autoimmune hemolysis continues to cause hemolysis in babesiosis patients posttreatment and requires immunosuppressive therapy with corticosteroids and/or monoclonal antibodies, such as rituximab (anti-CD20 monoclonal antibody).[69] The investigators recommended that asplenic and immunosuppressed patients with worsening or recrudescent hemolytic anemia after treatment for babesiosis be evaluated for warm autoimmune hemolytic anemia and potential immunosuppressive therapy.[69]

TICK-BORNE VIRAL INFECTIONS

Tick-Borne Viral Encephalitides

The tick-borne viral infections are caused primarily by flaviviruses (family Flaviviridae) and may be divided into two separate clinical presentations, each with preferred tick vectors and wild animal reservoirs: the viral encephalitides (Table 296.7) and the viral hemorrhagic fevers (Table 296.8). The tick-borne viral infections share several common clinical and epidemiologic characteristics, including the following: incubation periods of approximately 1 week; biphasic illnesses separated by symptom-free periods, beginning with flulike viremic stages and ending with CNS or hemorrhagic manifestations with increased CFRs;



FIG. 296.18 ***Babesia microti.*** Note the vacuolated intraerythrocytic ring forms and the clumped extraerythrocytic forms (thin blood smear, Giemsa stain, ×1000). *(From DPDx Image Library. Babesiosis Image Library: Babesia sp. in thin blood smears stained with Giemsa, Fig. A. Atlanta, GA: Centers for Disease Control and Prevention.* http://www.cdc.gov/dpdx/babesiosis/index.html.*)*

TABLE 296.7 Representative Tick-Borne Encephalitis Viruses

VIRUS NAME	FAMILY TAXONOMY	GEOGRAPHIC DISTRIBUTION	TICK VECTORS	WILD ANIMAL RESERVOIRS
Central European tick-borne encephalitis virus (TBEV-Eu)	Flaviviridae	Europe, except Iberian Peninsula	Ixodid ticks, especially *Dermacentor marginatus, Ixodes persulcatus,* and *Ixodes ricinus*	Mammals: especially rodents, including hedgehogs, wood mice, and voles; also deer and other ungulates, birds, and domestic livestock, especially goats
Deer tick virus	Flaviviridae	US New England states (Connecticut, Massachusetts, New York)	*Ixodes scapularis*	Deer
Far Eastern TBEV (TBEV-FE)	Flaviviridae	Eastern Russia, China to far eastern Japan	*I. persulcatus*	Mammals: rodents, including hedgehogs, wood mice, voles; also birds, deer, other ungulates, and domestic livestock, especially goats
Langat virus	Flaviviridae	Malaysia	Ixodid ticks	Mammals: monkeys, rodents
Louping ill virus	Flaviviridae	United States, Scotland	Ixodid ticks	Sheep
Powassan encephalitis virus	Flaviviridae	Canada, US Northeast, far eastern Russia	*Ixodes* spp., particularly *I. scapularis, I. cookei; Dermacentor andersoni*	Mammals: rodents, skunks, and other medium-sized mammals, especially woodchucks
Siberian (Russian) spring-summer TBEV (TBEV-Sib)	Flaviviridae	Russia	*Ixodes* spp., particularly *I. persulcatus, I. ricinus*	Mammals: rodents, including hedgehogs, wood mice, voles; also birds, deer, other ungulates, and domestic livestock, especially goats
Turkish sheep encephalitis virus	Flaviviridae	Turkey	Ixodid ticks	Sheep
Bhanja virus	Bunyaviridae	Eastern Europe, Russia, central and West Africa	*Dermacentor* spp.; *Haemaphysalis intermedia*	Cattle, sheep, goats, hedgehogs
Crimean-Congo hemorrhagic fever virus	Bunyaviridae	Asia, Eastern Europe, Africa, Middle East	*Hyalomma marginatum, Hyalomma anatolicum*	Mammals: many domestic animals (buffalo, camels, cattle, goats, sheep), rabbits, rodents (hedgehogs), birds

TABLE 296.8 Representative Tick-Borne Hemorrhagic Fever Viruses

VIRUS NAME	FAMILY TAXONOMY	GEOGRAPHIC DISTRIBUTION	TICK VECTORS	WILD ANIMAL RESERVOIRS
Alkhurma hemorrhagic fever virus	Flaviviridae	Saudi Arabia	*Ornithodoros savignyi* (suspected)	Camels, sheep
Omsk hemorrhagic fever virus	Flaviviridae	Western Siberia	*Dermacentor reticulatus, Ixodes apronophorus*	Mammals: rodents, especially muskrats, water voles
Kyasanur Forest disease	Flaviviridae	Western India	*Haemaphysalis spinigera*	Mammals: especially monkeys, domestic livestock (cattle, goats, sheep), rodents, insectivores
Heartland virus	Bunyaviridae	Missouri, Kansas, Tennessee	*Amblyomma americanum*	Mammals: rodents, skunks, other medium-sized mammals
Crimean-Congo hemorrhagic fever virus	Bunyaviridae	Asia, Eastern Europe, Africa, Middle East	*Hyalomma marginatum, Hyalomma anatolicum*	Mammals: many domestic animals (buffalo, camels, cattle, goats, sheep), rabbits, rodents (hedgehogs), birds
Severe fever with thrombocytopenia virus	Bunyaviridae	China (mostly), Japan, Korea	*Haemaphysalis* spp., especially *H. longicornis* > *H. concinna*	Primarily domestic animals, especially goats
Bourbon virus	Orthomyxoviridae	Missouri, Kansas, Oklahoma	Unknown	Unknown

nonspecific serodiagnosis by comparing acute and convalescent sera for increased antibody titers or by hemagglutination inhibition; specific serodiagnosis by ELISA and antigen detection from blood or cerebrospinal fluid (CSF) by reverse-transcriptase PCR; no specific treatments other than supportive therapy; and significantly increased postinfection morbidity.[70]

From a global distribution perspective, the tick-borne encephalitis viruses (TBEVs) are separated into the Old World (Eastern Hemisphere) and New World (Western Hemisphere) strains, with the Old World strains having significantly higher CFRs (20%–40%) and permanent neurologic morbidity rates (28%–30%) than the New World strains (CFR, 10%–15%; morbidity rate, <10%).[70] Although additional Old World flaviviral strains have now been discovered in sheep reservoirs, the most common Old World TBEVs have been further stratified regionally into three major subtypes: European or central European (TBEV-Eu), Siberian or Russian spring-summer (TBEV-Sib), and Far Eastern (TBEV-FE; see Table 296.7). Except for the Old World TBEVs with sheep reservoirs, all the TBEVs are transmitted by the injection of infected saliva from viremic ixodid ticks. During blood-feeding, viruses in tick saliva increase up to 10-fold and render early removal of the feeding tick ineffective in preventing disease.[70] The preferred wild animal reservoirs for TBEVs include rodents, insectivores, medium-sized mammals, deer and other ungulates, birds, and, less often, domestic animals (see Table 296.7).[70]

New World TBEVs: Powassan Encephalitis

Powassan encephalitis, first isolated in 1958, typifies a New World TBEV with a confined regional distribution in the New England states (especially Connecticut, Massachusetts, and New York) and Eastern Canada; several ixodid tick vectors, primarily *Ixodes* spp.; an extensive wild animal reservoir in rodents and medium-sized mammals, especially woodchucks and skunks; and a seasonal occurrence.[71] Cases occur from May to December and peak during June to September, when ticks are most active.[71] Patients with Powassan encephalitis present with somnolence, headache, confusion, high fever, weakness, ataxia, and CSF lymphocytosis.[71] Transient improvement may be followed by neurologic deterioration, evidence of ischemia or demyelination on magnetic resonance imaging (MRI), and slow recovery, often with permanent deficits including memory loss, weakness, ophthalmoplegia, and lower extremity paraparesis.[71–73] Unlike the Old World TBEVs, Powassan encephalitis is uncommon, with only 31 confirmed cases reported by the CDC from 1958 to 2001.[71] Because there is no vaccine or specific therapy for Powassan encephalitis, the best means of prevention is protection from tick bites. Since 2008, Powassan encephalitis cases historically confined to the Northeastern United States and Canada have been increasingly confirmed farther westward in Minnesota and Wisconsin, with fatal cases reported in the elderly.[72]

Although uncommon, Powassan virus (POWV) disease can be transmitted to humans by blood-feeding ixodid ticks in as little as 15 minutes and in clinical presentation mimics other arthropod-borne viral encephalitidies, such as La Crosse encephalitis and West Nile virus neuroinvasive disease, in clinical presentation.[73] The CFR can be as high as 10%, and permanent neurologic sequelae are common.[73] Therefore clinicians should include POWV in their differential diagnosis of encephalitis in endemic regions, such as New England and Minnesota, during the mosquito-borne encephalitis season and also obtain serologic tests for POWV on CSF samples (POWV-specific neutralizing immunoglobulin M antibody titers).[72,73] Unlike the prolonged attachment time required for the transmission of LB, the short attachment time required for transmission of POWV underscores the critical importance of personal protective measures, such as repellents and clothing, in preventing tick-transmitted infectious diseases.[72,73]

The deer tick virus is closely related to Powassan virus and is also transmitted by ixodid ticks in the same endemic regions of New England.[74] It also causes a meningoencephalitis syndrome with a high CFR.[74]

Old World TBEVs

The Old World TBEVs remain common causes of permanent neurologic morbidity from Scandinavia to eastern Japan, with more than 10,000 cases reported per year, a third of which result in permanent neurologic deficits.[70] In addition to tick bites, the Old World TBEVs may occasionally be transmitted by ingestion of unpasteurized milk products from viremic livestock (especially goats), breastfeeding, and slaughter of viremic animals.[70] Old World TBEV is typically biphasic in over 70% of cases, with an initial febrile flulike presentation followed by a 1-week (range, 1–21 days) symptom-free interval.[70] This honeymoon or recovery period is followed by meningoencephalitis with CSF pleocytosis, with or without myelitis, and a poliomyelitis-like flaccid paralysis that targets the arms, neck, and shoulders.[70] MRI and electroencephalographic abnormalities are common but nonspecific. Other acute neurologic complications may include altered consciousness, seizure activity, cranial nerve palsies, and an often-fatal bulbar syndrome with cardiorespiratory failure. Because no specific treatments other than supportive therapy exist, tick avoidance and immunization remain the best preventive measures. Effective vaccines have now been developed for the three subtypes of Old World TBEVs, and some have been shown to even provide cross-protection among the subtypes in experimentally infected animals.[70]

Tick-Borne Hemorrhagic Fever Viruses

The tick-borne hemorrhagic fever (TBHF) viruses (see Chapter 166) are maintained in nature in extensive wild and domestic animal reservoirs and are transmitted by infected ixodid tick bites, squashing infected ticks, creating infective aerosols, direct contact with blood or tissues

from infected animals or humans, or nosocomial spread among medical personnel.[75] TBHFs are usually caused by either flaviviruses or bunyaviruses, which are distributed throughout eastern Europe, Africa, and Asia. As noted earlier, a previously unidentified tick-borne thogotovirus (family Orthomyxoviridae) was described in the United States in 2014 in a healthy man in eastern Kansas and later named Bourbon virus after the patient's county of residence.[21]

The TBHFs are characterized clinically by biphasic illnesses that present as febrile flulike symptoms and end as hepatomegaly with liver failure and hemorrhagic manifestations (petechiae, purpura, subconjunctival and pharyngeal hemorrhage, thrombocytopenia, cerebral hemorrhage, severe intrapulmonary hemorrhage, disseminated intravascular coagulation) separated by a few afebrile days. CFRs range from 10% to over 50%, with most deaths occurring within 5 to 14 days of symptom onset during hemorrhagic stages. Diagnoses may be confirmed by immunologic techniques, such as antibody increases in paired sera and ELISA, and by molecular techniques, such as real-time PCR. Although ribavirin can inhibit Crimean-Congo hemorrhagic fever (CCHF) virus replication in animal models, it has not been tested in clinical trials in humans with CCHF. Nevertheless, if TBHF is suspected in the tropics and laboratory confirmation is unavailable, intravenously administered ribavirin (30 mg/kg initially, followed by 16 mg/kg four times daily for 4 days, and then 8 mg/kg three times daily for 6 days) is recommended for severe cases, and oral ribavirin is recommended for high-risk contacts.[75,76] All patients with TBHFs should be placed in isolation, and strict universal precautions, including personal protective equipment, should be practiced by all medical personnel because nosocomial transmission has been reported, most likely by the generation of infectious aerosols during bag-valve-mask ventilation and bronchoscopy.[76] A mouse brain–derived CCHF vaccine has been developed in Bulgaria but is not available elsewhere. In the absence of a universal vaccine, the best preventive measures for the TBHFs are tick avoidance and control, rapid burial of dead animals, and personal protective equipment for abattoir workers and medical personnel.

Tick-Borne Coltiviruses

The tick-borne coltiviruses of the family Reoviridae (see Chapter 149) are all double-stranded RNA viruses of the genus *Coltivirus* and include Colorado tick fever virus (CTFV), which is endemic in the United States and Canadian Rocky Mountain regions; the California tick fever virus of rabbits (CTFV-Ca) and the Salmon River virus (SRV) of Idaho, serotypes of the CTFVs; and the European Eyach virus (EYAV).[77] The ixodid or hard ticks are the only vectors of the coltiviruses, with *Dermacentor* ticks (mainly *D. andersoni*) being the principal vectors of CTFV and SRV in the Rocky Mountains and *Ixodes* ticks (*I. ricinus, I. ventalloi*) being the only vectors of EYAV throughout Europe.[77] Among the coltiviruses, CTFV has the widest host range, which includes squirrels, other rodents, rabbits, porcupines, marmots, deer, elk, sheep, and coyotes.[77] The remaining coltiviruses have fewer, more specific wild animal hosts, including the black-tailed jackrabbit (*Lepus californicus*) for CTFV-Ca and primarily the European rabbit (*Oryctolagus cunniculus*) but also rodents, deer, domestic goats, and sheep for EYAV.[77] The coltiviruses are maintained in nature by ixodid ticks that blood-feed on wild animal hosts with prolonged viremias and then transmit coltiviruses transstadially but not transovarially.[77] Infected nymphs hibernate over winter, and previously infected nymphs and newly infected adults then transmit coltiviruses to human dead-end hosts during spring-summer blood-feeding.[77] CTFV has also been transmitted by blood transfusion and congenitally.[77]

Both CTFV and SRV can cause biphasic to triphasic febrile illnesses that mimic mild cases of RMSF without rash. Leukopenia and thrombocytopenia are common laboratory manifestations of coltivirus infections and are strong indicators for the diagnosis.[77] Complications are rare but may include meningoencephalitis, orchitis, hemorrhagic fever, pericarditis, and myocarditis. EYAV infections are more often complicated by CNS manifestations than American strain coltivirus infections.[77] The most common differential diagnoses for the tick-borne coltiviruses are other tick-borne febrile diseases, most commonly RMSF in North America, which may be distinguished from CTFV and SRV infections by its characteristic rash and leukocytosis.[77] Serologic diagnostic methods to detect anticoltivirus antibodies include complement fixation test, seroneutralization assay, immunofluorescence assay, ELISA, and Western immunoblot assay.[77] The most specific and confirmatory laboratory diagnostic methods include reverse-transcriptase PCR assays to identify CTFV RNA (or the RNA of its cross-reacting serotypes, CTFV-Ca and SRV) or the isolation of coltiviruses after intracerebral inoculation of infected human blood into suckling mice.[77] Treatment of all tick-borne coltivirus infections is entirely supportive, and long-term complications are rare in uncomplicated cases.

TICK PARALYSIS

First described in 1912 in Australia, Canada, and the United States, tick paralysis is a rare, regional, and seasonal cause of acute ataxia and ascending paralysis with an incubation period of 4 to 7 days after female tick attachment, mating, and blood-feeding.[78–80] Although 43 species of ticks have been implicated in tick paralysis cases worldwide, most cases occur in the United States and Canadian Pacific Northwest (Washington State and British Columbia) and in Australia.[81–83] In the US Pacific Northwest, tick paralysis is caused by the American dog tick (*D. variabilis*) or the Rocky Mountain wood tick (*D. andersoni*) during April through June, when *Dermacentor* ticks emerge from hibernation to mate and to seek blood meals (see Figs. 296.1 and 296.15).[81–83] The mechanism of neurotoxic paralysis in *Dermacentor* tick paralysis is unknown, but neuroelectrophysiologic studies have suggested that sodium flux across axonal membranes is blocked at the nodes of Ranvier, leaving neuromuscular transmission unimpeded.[82,84] In Australia, the marsupial ixodid tick, *Ixodes holocyclus,* can cause a more severe form of ascending neuromuscular paralysis by producing a botulinum-like neurotoxin that blocks neuromuscular transmission by inhibiting the presynaptic release of acetylcholine.[84]

Most cases of tick paralysis in North America have occurred sporadically in young girls with long hair concealing ticks feeding on the scalp or neck.[83] However, a four-patient cluster of *Dermacentor* tick paralysis, including a 6-year-old girl with a tick on her hairline and three adults with ticks on the neck ($n = 1$) and back ($n = 2$), was reported from Colorado in 2006.[81]

Although botulism causes a descending neuromuscular paralysis with a preserved sensorium, tick paralysis, Guillain-Barré syndrome, acute poliomyelitis, and spinal cord tumors may all cause acute ascending paralysis with preserved mental status and must be differentiated from each other (Table 296.9).[84–87] Because poliomyelitis has been nearly eradicated by vaccination worldwide, tick paralysis is frequently misdiagnosed as Guillain-Barré syndrome, and the correct diagnosis is made accidentally by finding an engorged, usually female, tick on the scalp, head, or neck during hair combing or when applying electroencephalographic electrodes (see Table 296.9).[83,87]

Before 1954, postmortem examinations of persons who died suddenly of unexplained paralytic illnesses demonstrated attached ticks on their heads and necks.[78–80] In a review of Canadian tick paralysis cases in the 1950s before the widespread availability of mechanical ventilation in intensive care units, Rose[88] reported a CFR of 10% to 12% without tick removal. In a review of 33 tick paralysis cases in Washington State over the period 1946–1966, Dworkin and coworkers[86] reported a CFR of up to 10%, with most deaths occurring in the 1940s. In a 60-year meta-analysis of confirmed tick paralysis cases in the United States, Diaz[87] reported a CFR of 6% in the first 30 years, a seasonal pattern of case clusters in children and adults in both urban and rural locations, and a significant increase in initial misdiagnoses of tick paralysis as Guillain-Barré syndrome in more recently reported cases. In addition, the misdiagnoses of tick paralysis cases as Guillain-Barré syndrome often directed unnecessary therapies, such as central venous plasmapheresis with immunoglobulin G, and delayed correct diagnosis and treatment by tick removal.[87] In all cases, the diagnosis of tick paralysis was later established when attached ticks were discovered either by caregivers or by cranial neuroimaging studies.[87] The CFR from tick paralysis has steadily declined over the past 60 years, with almost all deaths in Canada and the United States reported in the 1940s and 1950s.[78,79,86,87]

The emergence of a new form of acute paralysis in children is relevant to the clinical recognition and diagnosis of tick paralysis. In 2014, 120 cases of acute-onset flaccid limb weakness in children were reported in

TABLE 296.9 Clinical Differential Diagnosis of Tick Paralysis Versus Ascending Neuromuscular Paralysis With Preserved Sensorium

PRESENTING CLINICAL FEATURES	TICK PARALYSIS	GUILLAIN-BARRÉ SYNDROME	CERVICAL SPINAL CORD LESION	POLIOMYELITIS
Onset of ascending paralysis	Acute, rapid, within 24–48 h	Slower onset, days to weeks	Abrupt to gradual	Days to weeks
Ataxia	Present	Absent	Absent	Absent
Deep tendon reflexes	Hyporeflexia progressing to areflexia	Hyporeflexia progressing to areflexia	Variable	Hyporeflexia progressing to areflexia
Babinski sign	Absent	Absent	Present	Absent
Sensory loss	None	Mild	Present	None
Meningeal signs	Absent	Rarely present	Absent	Present
Fever	Absent	Rarely present	Absent	Present
CSF findings Protein levels (mg/dL) White cells (per mm^3) Differential counts	 Normal <10 Normal	 High (≥40) <10 <10 mononuclear cells/mm^3	 Normal to high Variable	 High >10 Lymphocytosis
Nerve conduction studies	↑ Latency in distal motor nerves ↓ Nerve conduction velocity ↓ Amplitude of motor and sensory nerve action potentials	Similar	Similar	Similar
Time to neurologic recovery	Rapid, ≤24 h after tick removal	Weeks to months	Variable	Months to years
Permanent neurologic deficits	None after tick removal	Permanent paresis possible	Permanent paresis possible	Permanent paresis possible

CSF, Cerebrospinal fluid.

clusters of two or more cases from states throughout the continental United States.[89] These cases all shared similar presentations and diagnostic features, including short prodromes of fever and respiratory or gastrointestinal symptoms before onset of focal limb weakness in one or more limbs and scattered lesions in the gray matter of the spinal cord on MRI. The condition was later described as acute flaccid myelitis (AFM) based on its diagnostic neuroimaging findings. Temporal associations between AFM and enterovirus D68 (EV-D68) infections were reported in some cases.[89]

By 2015, the CDC established a case definition for AFM that required the following criteria: (1) acute onset of flaccid limb weakness in one or more limbs; (2) CSF leukocytosis (white blood cell count >5/mm^3, corrected for red blood cells); and (3) MRI-documented lesions confined to the gray matter of the spinal cord (later lesions were detected throughout the neuraxis).[90] By 2016, the CDC reported an increase in cases of AFM, with 144 cases confirmed by the CDC case definition in 37 states.[90]

In an outbreak in Washington State during September–November, 2016, 10 confirmed cases of AFM were reported with EV-D68 detected by PCR in nasopharyngeal swabs from two patients and with enterovirus A71 (EV-A71) detected by PCR in a stool specimen from a third patient.[91] In all cases, poliovirus and West Nile virus were ruled our as infectious causes of acute flaccid paralysis. Since both EV-D68 and EV-D71 have been associated with cluster outbreaks of acute flaccid paralysis in children in the past, an infectious or postinfectious autoimmune etiology of AFM was suspected.[92,93]

Although no deaths from AFM have been reported, several cases have required mechanical ventilatory support, and others have been treated by intravenous immunoglobulin G therapy as recommended for Guillain-Barré syndrome.[91] Some of the largest cluster outbreaks of AFM have now been reported from states also reporting the highest prevalence rates for tick paralysis in children (e.g., Colorado and Washington). Clinicians should therefore include AFM in their differential diagnosis of acute flaccid paralysis in children and should collect specimens for laboratory testing (blood, nasopharyngeal swabs, CSF, and stool); order appropriate neuroimaging studies (MRI of the entire neuraxis from the cerebrum to the cauda equina); and exclude or confirm any infectious or noninfectious etiologies, including tick paralysis.[89–91]

The treatment of *Dermacentor* tick paralysis simply requires removing the tick with forceps (or tweezers) to restore neuromuscular function within 24 hours. Although *I. holocyclus* tick paralysis is also treated by tick removal, transient neuromuscular deterioration may occur for 24 to 48 hours after tick removal.[84] The administration of *I. holocyclus* antitoxin before tick removal and prolonged observation for hypoventilation have been recommended.[84]

RED MEAT ALLERGY AFTER TICK BITES

Immunoglobulin E (IgE)-mediated urticarial and anaphylactic reactions to tick bite–injected salivary proteins were initially described in Australia in the 1980s following paralysis tick *(I. holocyclus)* bites.[93a] Between 2003 and 2007, 25 patients who lived in a paralysis tick–endemic area of Sydney, northern New South Wales, developed red meat allergies after extensive local reactions to paralysis tick bites, suggesting an immunologic cross-reaction between arthropod-injected and foodborne proteins.[93b] Although red meat allergic reactions are rare and responsible for only 3% of food allergies, red meat allergies have been reported following antigenic sensitization by ingestion of cow's milk, by exposures to bovine and porcine albumin, by intravenous chemotherapy with the chimeric mouse-human monoclonal antibody, cetuximab, and by prior allergic reactions to foods containing bovine and porcine-derived gelatin.[93c] The sensitizing foodborne allergen in all of these exposures was determined to be glalactose-alpha-1,3-galactose (α-gal), an oligosaccharide constituent of all red meats (beef, lamb, pork, horse, and game meats) that structurally resembles the human blood group antigen B.[93d] The association between tick bites and red meat allergies, however, remained uncertain until recently.

In 2018, Carter and colleagues at the US National Institute of Allergy and Infectious Diseases (NIAID) identified IgE antibodies to α-gal in 6 of 70 patients with idiopathic anaphylaxis, all of whom reported prior tick bites and lived in Lone Star tick (*A. americanum*)-endemic US states (see Fig. 296.3).[93e] Unlike typical foodborne allergies, which occur within minutes of ingestion, α-gal allergies following tick bites are delayed by 3 to 6 hours and present with combinations of urticarial reactions, angioedema, and anaphylaxis.[93e] In addition to having tick bite histories in Lone Star tick–endemic regions, all 6 patients in that study were males and had non-B blood types.[93e] Two of the 6 patients also had indolent systemic mastocytosis, a condition known to predispose patients to venom allergies, and suffered more severe anaphylactic reactions.[93e] After adopting red meat–free diets that included chicken, turkey, and seafood, all 6 patients experienced no further episodes of unexplained anaphylaxis for follow-up periods ranging from 18 months to 3 years.[93e] The authors concluded that α-gal allergy should be excluded as a cause of unexplained delayed anaphylaxis following red meat

ingestion, especially in patients residing in Lone Star tick–endemic regions of the United States. In addition, the NIAID study identified several potential risk factors for red meat allergy after tick bites including (1) male gender due to increased outdoor exposures to repeated tick bites, (2) non-B blood type as the B-blood group antigen resembles α-gal and probably provides cross-protection, and (3) a history of indolent systemic mastocytosis.[93e]

Like tick paralysis, tick-bite induced red meat allergy is a rare, noninfectious phenomenon. It results from prior sensitization to α-gal following specific tick bites and may occur in a wide geographic distribution. All life stages of ticks feed on warm-blooded mammals and absorb α-gal sugars from their hosts. During human blood-feeding, ticks inject small amounts of α-gal into bite wounds in addition to other salivary chemicals, such as anticoagulants and local anesthetics. Repeated tick bites over time may predispose certain individuals to IgE-mediated allergic responses including anaphylaxis on reexposures to α-gal antigens in red meats, dairy products, drugs and foods containing gelatin, and during cetuximab chemotherapy.[93e,93f]

TICK-TRANSMITTED COINFECTIONS

Ticks may be asymptomatically coinfected with several pathogens that can be simultaneously transmitted to human hosts during blood feeding. In 1996, Krause and coworkers initially reported increased duration and severity of illness in patients coinfected with the Lyme disease spirochete, *B. burgdorferi,* and *B. microti,* the protozoan agent of babesiosis.[94] In 1997, Schwartz and coinvestigators in the eastern United States first identified coinfections in ixodid ticks with *B. burgdorferi* and *Anaplasma phagocytophila*, the bacterial agent of anaplasmosis.[95] In 1997, Nadelman and coauthors reported the first US case of concurrent Lyme borreliosis and anaplasmosis.[96]

In 2002, Belongia reviewed the epidemiology and impact of human coinfections from ixodid tick bites in the United States and reached the following conclusions[68]:

1. Ixodid tick coinfections with *B. burgdorferi* and *B. microti* ranged regionally from 2% in New Jersey to 19% on Nantucket Island, Massachusetts.[68]
2. Ixodid tick coinfections with *B. burgdorferi* and *A. phagocytophila* were consistently less common regionally, ranging from 1% to 6% in six selected regions, with occasional regional pockets of high prevalence (26% in Westchester County, New York).[68]
3. Among patients with confirmed tick-borne coinfections, the highest regional coinfection rates reached 39%; and the most commonly diagnosed human tick-borne coinfections in the Eastern United States were Lyme borreliosis and babesiosis, which accounted for 80% of coinfections.[68]
4. Human coinfections with Lyme borreliosis and anaplasmosis were less common and accounted for 3% to 15% of coinfections in Connecticut and Wisconsin.[68]
5. As initially described by Krause and coinvestigators in 1996, coinfections with Lyme borreliosis and babesiosis were more severe, with a longer duration of illness, than Lyme borreliosis alone.[67,94]
6. Lastly, clinicians should always consider the possibility of tick-borne coinfections in overlapping endemic regions; order appropriate laboratory tests for the molecular or serologic detection of multiple tick-borne pathogens; and prescribe the most appropriate antimicrobials for each infecting agent.[67,94–97]

To date, most tick-borne coinfections have been reported from geographic areas with overlapping endemic ranges of infected ticks capable of transmitting multiple pathogens during blood-feeding. The eastern black-legged or deer tick, *I. scapularis* (see Fig. 296.5), has been implicated most often by transmitting Lyme disease and babesiosis in a northeastern coastal band extending from New Jersey north to New Hampshire. *Ixodes scapularis* has also cotransmitted Lyme disease and anaplasmosis in an upper Midwest geographic band extending from Wisconsin across Minnesota to Canada, another endemic region of overlapping tick-transmitted infectious diseases (Fig. 296.19).

Although unusual, ticks have also transmitted pathogens and paralytic toxins. In 2003, Inokuma and coauthors reported a case of tick paralysis in a 59-year-old male traveler returning to Japan from a vacation to the Gold Coast of Queensland, Australia.[98] The authors noted that the patient demonstrated both the characteristic features of Australian tick paralysis and other unusual features, suggestive of a tick-borne rickettsial coinfection.[98] The characteristic features of Australian tick paralysis included a prolonged prodrome of lethargy and weakness, ascending paralysis beginning in the right leg, external ophthalmoplegia, and constricted visual fields.[98] The more unusual features of the case included febrile prodromal and incubation periods, a tick-bite eschar with surrounding anesthesia on the scalp, and regional submandibular lymphadenopathy.[98] The results of all initial laboratory studies were within normal limits, including CSF analysis.[98] Following the self-removal of a semiengorged gravid female *I. holocyclus* tick from his scalp, the patient's paralysis and visual findings gradually resolved, but the anesthesia surrounding the tick-bite eschar persisted for over 2 weeks.[98]

Subsequent serologic comparisons of rising antibody titers to other tick-borne diseases in paired serum samples suggested that the more unusual features of the case could have been consistent with coinfection by *R. helvetica,* a common cause of tick-bite eschar with regional lymphadenopathy in Europe.[22] Additional Western blot analysis demonstrated cross-reactivity of the patient's serum against several high-molecular-weight outer membrane proteins, all species-specific for *R. helvetica.*[10] The authors concluded that the patient may have been coinfected with *I. holocyclus*–transmitted tick paralysis and *R. helvetica* tick-bite fever with eschar and regional lymphadenopathy. In addition, the authors noted that a rickettsial species closely related to *R. helvetica* had been isolated from ticks in Japan in 2002 and that further investigations of the distributions of *R. helvetica*–like rickettsia in Australia and Japan should be conducted.[98]

In summary, the ability of ixodid ticks to be coinfected with multiple pathogens and to transmit coinfections to humans during prolonged blood-feeding has now been demonstrated in the United States. The immunologic confirmation of tick-borne coinfections by rising antibody titers in paired serum samples and/or by nucleic acid determinations using PCR will take a significant period of time and may only be available in state and federal reference laboratories. Some critical tick-borne infections, such as RMSF, Lyme disease meningoencephalitis, and babesiosis with splenomegaly may require immediate, empirical intravenous antibiotic management prior to confirmatory laboratory diagnoses. In such cases, Diaz has recommended the generation of probability-based decision tree models to expose potential coinfections based on: (1) the regional distributions and prevalence rates of potentially coinfected tick vectors; (2) tick identification by experts; and (3) any cutaneous presenting manifestations of tick bites, such as eschars or erythema migrans, if present (Fig. 296.20).[99] Clinicians should suspect tick-borne coinfections in returning travelers and all patients with clinical and immunologic evidence of multiple infecting agents, especially in cases of unusual presentation or severity, prolonged duration, or nonresponse to single (typically doxycycline) antibiotic therapy.[99]

PREVENTION AND CONTROL OF TICK-BORNE INFECTIOUS DISEASES AND PARALYTIC POISONINGS

A number of strategies can be used in the prevention and control of tick-borne infectious diseases and paralytic poisonings, including immunization, personal protective measures, landscape management, and wildlife management. In the 1990s, a Lyme disease vaccine was developed for the United States, but it was withdrawn from the market in 2002 because of poor sales. Immunization strategies to prevent tick-borne infectious diseases have proved far more effective in Europe and Asia than in the United States, where neurologic complications from TBEVs are second only to Japanese encephalitis virus as causes of permanent paraparesis. Current immunization programs for tick-borne viral diseases now provide primary prevention of TBEV-Eu in Europe, TBEV-Sib in Russia and the Middle East, TBEV-FE in China and the Far East, and CCHF in Bulgaria. A canine antitoxin for *I. holocyclus*–induced tick paralysis has been used to reverse tick paralysis in animals and humans in Australia.[84]

In addition to immunization, available only for Old World TBEVs, antibiotic therapy after presumed ixodid tick bites with erythema migrans has been recommended as a prophylactic therapeutic strategy for the

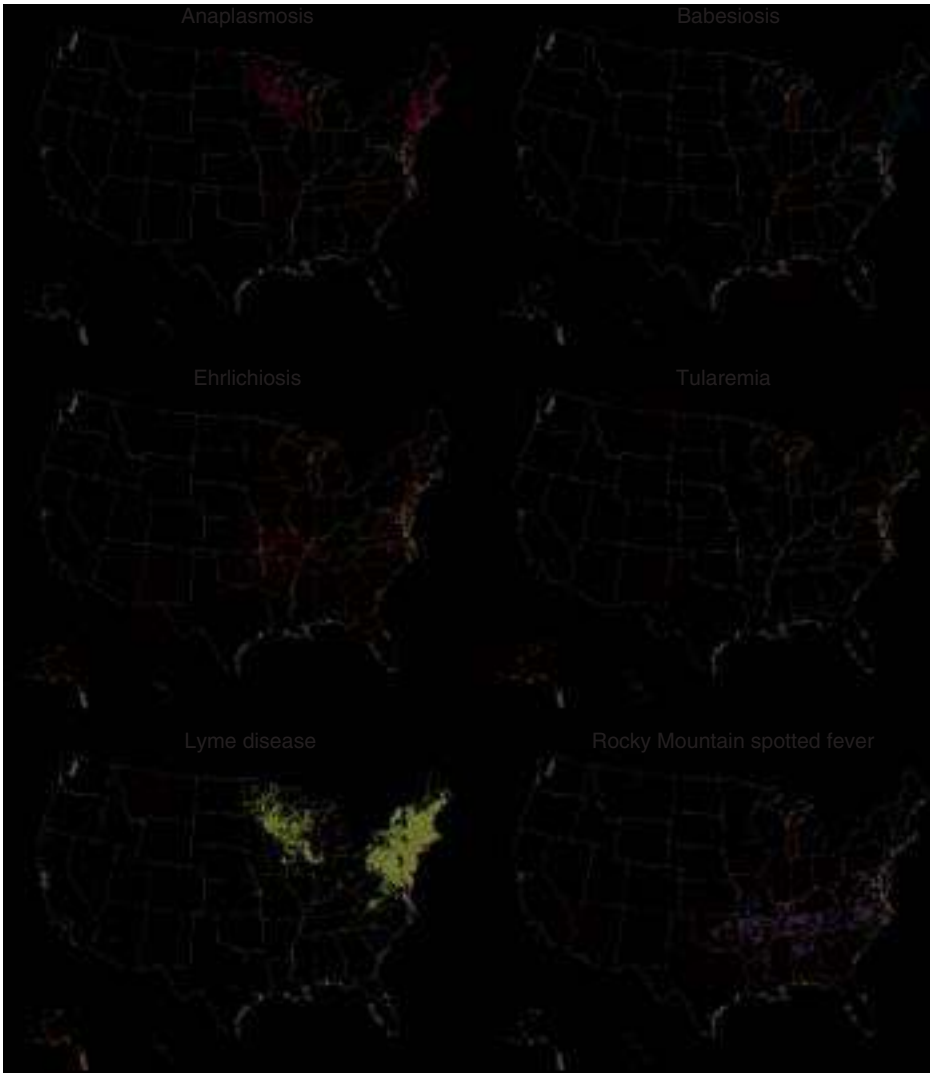

FIG. 296.19 Regional US maps of the most common tick-transmitted infectious diseases. One colored dot is one reported case in 2015. The eastern black-legged or deer tick, *Ixodes scapularis* (see Fig. 296.5), has been implicated most often in coinfections by transmitting Lyme disease and babesiosis in a northeastern coastal band extending from New Jersey north to New Hampshire. *Ixodes scapularis* has also cotransmitted Lyme disease and anaplasmosis in an upper Midwest geographic band extending from Wisconsin across Minnesota to Canada, another endemic region of overlapping tick-transmitted infectious diseases. *(From* Tickborne Diseases of the United States: A Reference Manual for Health Care Providers. *4th ed. Atlanta, GA: Centers for Disease Control and Prevention; 2017.)*

primary prevention of some tick-borne infections.[100] A randomized clinical trial found that a single 200-mg dose of doxycycline administered within 72 hours of a tick bite was 87% effective in preventing Lyme disease.[100]

Finally, because most tick-borne infectious diseases may also be transmitted by blood product transfusions, screening blood product donors in high seroprevalence areas for Lyme disease and other borrelioses, babesiosis, ehrlichioses, and anaplasmosis would eliminate transfusion-transmitted cases.[64,65,69] Physicians are encouraged to order leukocyte-reduced blood components for blood product transfusions to potentially reduce the risks for ehrlichiosis and anaplasmosis, especially in regions that are highly endemic for leukocytotropic tick-borne infectious diseases.

Personal protective measures to prevent tick-transmitted diseases include wearing appropriate clothing, using insect repellents, and performing regular tick checks. Wearing long pants tucked into socks, long-sleeved shirts, and light-colored clothing can help keep ticks off the skin and make them easier to spot on clothing. Impregnating clothing with permethrin, routinely performed by the military on maneuvers, is a highly effective repellent against ticks and other insects. The topical application of insect repellents containing 20% to 50% formulations of *N,N*-diethyl-meta-toluamide (DEET) directly on the skin is another effective and recommended measure.

Most patients with Lyme disease, TBRF, babesiosis, ehrlichioses, and anaplasmosis will not recall tick bites because these diseases are often transmitted by diminutive nymphal ticks. Nevertheless, tick localization and removal as soon as possible, preferably within 36 hours, remain recommended strategies to prevent the rickettsial and viral ixodid tick-borne diseases and to reverse tick paralysis. Ticks should always be removed with forceps (or tweezers), not fingers (because squashing ticks can transmit several tick-borne diseases across dermal barriers or create infectious aerosols), and in contiguity with their feeding mouthparts, rather than burning ticks with spent matches or painting embedded ticks with adhesives or nail polishes.

Landscape management strategies to prevent tick-borne diseases include widespread application of acaricides over tick-preferred ecosystems, removal of vegetation and leaf litter near homes and recreation sites, and creation of dry barriers of gravel, stone, or wood chips between forested areas and yards or playgrounds. Wildlife management strategies to prevent tick-borne diseases include encouraging the development of better veterinary vaccines for tick-borne diseases with large domestic animal reservoirs, applying acaricides actively to domestic animals and

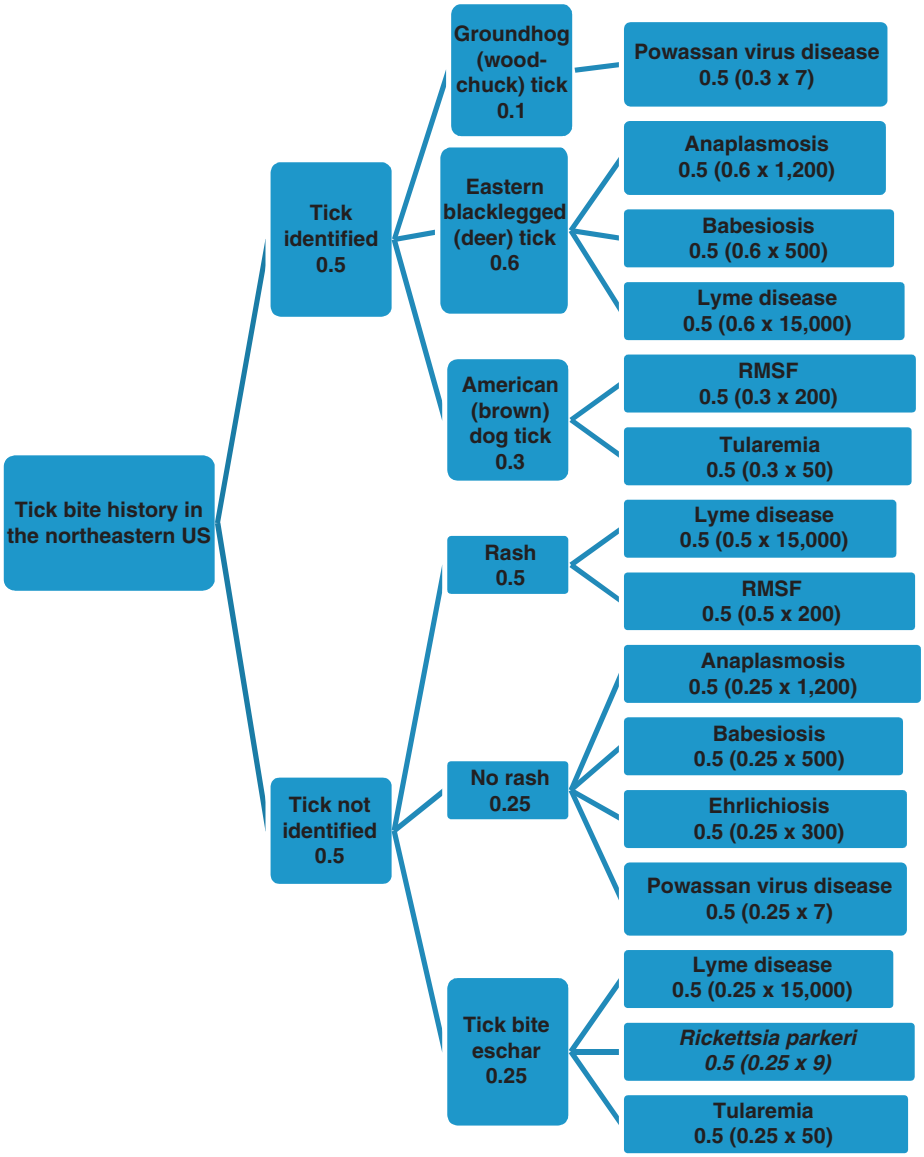

FIG. 296.20 A hypothetical probability-based decision tree model for predicting tick-borne infections and coinfections in an endemic region. This sample hypothetical decision tree illustrates the method for analyzing decision tree models of potential tick-borne infections and coinfections in the northeastern United States. Decision trees are solved from right to left. (1) The first and largest entry on the far right is the outcome of confirmed number of cases by the CDC for the given year (2015 was used here). (2) Distributions of tick vectors are expressed as a percent or as probabilities. (3) The sum of probabilities for any set of possibilities must equal 1.0. (4) Multiply from right to left to solve for the greatest expected values. The calculations for expected values are shown in the outcomes boxes on the right.*Predictions:* Based on solution of the decision tree, the most common tick-borne infections in this region will be Lyme disease transmitted by an Eastern black-legged (deer) tick, with an expected incidence rate of 21% (10,125/48,143); followed by anaplasmosis transmitted by the same tick, with an incidence rate of less than 1% (510/48,143); and followed by babesiosis transmitted by the same tick, with an incidence rate of less than 1% (213/48,143). The remaining 79% of tick-borne diseases in the northeastern United States will be caused by other pathogens and other tick vectors, including the groundhog (woodchuck) tick and the American (brown) dog tick. Coinfections, although rare, may occur more commonly with Lyme disease and babesiosis than with Lyme disease and anaplasmosis. Triple infections with Lyme disease, anaplasmosis, and babesiosis are possible but unlikely, and will be transmitted by the Eastern black-legged (deer) tick. *RMSF,* Rocky Mountain spotted fever.

passively to deer and cattle at baited feeding and watering stations or salt licks, and setting out acaricide-baited rodent houses for rodents to occupy or acaricide-baited cotton balls for rodents to adopt as nesting materials, especially in crawl spaces under homes and near playgrounds.

CONCLUSIONS

Most emerging infectious diseases today, such as West Nile virus and severe acute respiratory syndrome, arise from zoonotic reservoirs, and many are transmitted by arthropod vectors. Because ticks are the most common insect vectors of zoonotic diseases, ticks have become common arthropod vectors of emerging zoonotic diseases, including Lyme disease, ehrlichiosis, and anaplasmosis. Ticks are highly competent and versatile vectors of infectious diseases because ticks of all ages and both genders may remain infectious for generations, without having to reacquire infections from host reservoirs. Recent environmental changes and human behaviors now place humans and ticks together outdoors for longer periods in welcoming ecosystems for breeding, blood-feeding, and infectious disease transmission. Better prevention and treatment strategies for tick-borne diseases are indicated, before the highly conserved genomes of tick-transmitted microorganisms reassort their nucleic acids with their hosts and develop antimicrobial resistance (especially to doxycycline) or superpathogen capabilities, either by nature's own design or human terrorist intent.

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297 Kawasaki Disease

Jane C. Burns

SHORT VIEW SUMMARY

Definition
- Kawasaki disease (KD) is an acute, self-limited pediatric vasculitis that is the most common cause of acquired heart disease in children. Coronary artery aneurysms develop in 25% of untreated patients.

Epidemiology
- KD affects children of all ethnicities worldwide. Children of Asian and African-American descent are disproportionately affected. There is no evidence for person-to-person transmission. Temporospatial clustering of cases has been observed.

Etiology
- The cause of KD is unknown. According to the current paradigm, KD is triggered in genetically susceptible children after exposure to a widely dispersed agent.

Diagnosis
- The diagnosis is established by fulfillment of four of five clinical criteria in a child with fever and no other apparent cause. Clinical criteria include bilateral conjunctival injection; a polymorphous rash; oropharyngeal changes including erythematous, fissured lips, erythematous oropharynx without discrete lesions, and strawberry tongue; extremity changes including edema of the dorsa of the hands and feet, palm and sole erythema, and periungual desquamation during the convalescent phase; and a cervical lymph node mass measuring at least 1.5 cm. Coronary artery abnormalities are detected by transthoracic echocardiography.

Therapy
- High-dose intravenous immune globulin (IVIG) (2 g/kg) plus aspirin is the standard therapy for KD. If administered within the first 10 days after fever onset, the incidence of coronary artery aneurysms is reduced from 25% to 5%. A subset of patients (10%–20%) may be relatively resistant to IVIG and require additional antiinflammatory therapy.

Prevention
- There is no known intervention that can prevent KD.

Kawasaki disease (KD) is a self-limited vasculitis of infants and children that is now the most common cause of acquired heart disease in developed countries.[1,2] Although the disease was first described by Tomisaku Kawasaki in Japan in 1967, the cause remains elusive decades later. The current paradigm is that an environmental agent triggers a severe immune response in genetically susceptible children. Historical evidence suggests that KD was newly emergent in Japan in the 1950s and 1960s.[3] However, in the West, cases of infantile periarteritis nodosa, a fatal vasculitis of infants that is histologically indistinguishable from fatal KD, were reported in the 19th and early 20th centuries.[4,5]

EPIDEMIOLOGY

Although KD has been reported from all inhabited continents, the disease is most prevalent in Japan, where 1% of Japanese children develop the disease by age 10 years.[6] For every 100,000 children younger than 5 years of age, the incidence of KD ranges from 308 in Japan to 20 to 30 in the continental United States.[7–9] The incidence of KD among Japanese living in Hawaii is similar to rates in Japan, thus pointing to an important influence of host genetics on disease susceptibility.[10] In the United States, both Asians and African Americans are overrepresented among patients with KD.[11] The male-to-female ratio is 1.5:1, and 85% of patients with KD are younger than 5 years of age.

KD occurs with a distinct seasonality in the Northern Hemisphere with increased cases in winter and spring and midsummer and a nadir in the fall.[12] Temporospatial clustering of cases has also been documented, but person-to-person spread has not been observed.[8] Seasonality of KD cases has been linked to tropospheric wind currents from central Asia, although whether the association is causal has not been determined.[13,14]

ETIOLOGY

Despite 3 decades of investigation, the cause of KD remains unknown. Current understanding of the immune response that downregulates the acute inflammation through interleukin-10 suggests a response to a classic antigen that is protective against future exposure in most patients.[15,16] Previous theories postulating a response to a superantigen have been largely abandoned. One line of investigation suggests infection with a novel RNA virus that enters through the upper respiratory tract.[17] Analysis of coronary arteries obtained at autopsy has revealed upregulated expression of genes related to antigen presentation, myeloid dendritic cell function, and induction of type I interferons.[18] These findings were confirmed by immunohistochemistry and outline the complexity of the immune response in the arterial wall.[19]

CLINICAL MANIFESTATIONS

KD is recognized by a constellation of clinical signs and supportive laboratory data in the absence of other identifiable cause (Table 297.1). KD can mimic other rash/fever syndromes of children, and cases may be missed or misdiagnosed without a diagnostic test. Not all patients manifest the classic four of five criteria, and clinical presentation may be more subtle in infants younger than 6 months of age.[20] Clinicians must have a high index of suspicion for KD in any child presenting with the triad of fever lasting at least 5 days, rash, and bilateral conjunctival injection with sparing of the limbus and without exudate. Fever is essentially invariant, and no one particular pattern is characteristic. The fever is often high (>39°C [102.2°F]) and accompanied by extreme irritability. Description of each of the classic clinical signs follows.

TABLE 297.1 Diagnostic Criteria for Kawasaki Disease

A. Fever of at least 4 days' duration[a]
B. Presence of at least four of the following five conditions[b]
   1. Bilateral conjunctival injection
   2. Changes in lips and oral mucosa
      a. Dry, red, fissured lips
      b. "Strawberry tongue"
      c. Oropharyngeal erythema
   3. Changes in extremities
      a. Erythema of palms and soles
      b. Edema of hands and feet
      c. Periungual desquamation
   4. Polymorphous rash
   5. Cervical lymph node >1.5 cm
C. Illness not explained by other known disease processes

[a]Diagnosis may be made earlier in the course of fever by experienced clinicians.
[b]Kawasaki disease may be diagnosed in patients with fever and with fewer than four of five criteria in the presence of coronary artery abnormalities.

Exanthem

The exanthem of KD can take many different forms and is often polymorphous in the same patient. In its maculopapular presentation on the trunk and extremities, it can be easily confused with a drug reaction because many of these patients have been treated with antibiotics for erroneous diagnoses. Another common form of the rash is raised papules coalescing into plaques. This may be associated with target-like lesions with central clearing and can be confused with erythema multiforme. A pure erythroderma with no palpable component as in staphylococcal toxin–mediated disease is not associated with KD. Similarly, a confluent, fine maculopapular ("sandpaper") rash extending onto the face as in scarlet fever is unlikely and should prompt consideration of scarlet fever. A fine micropustular eruption on the buttocks, thighs, and extensor surfaces can sometimes be seen and is perhaps pathognomonic of KD.[21] Accentuation of the rash with confluent erythema in the groin that peels during the acute, febrile phase is seen in up to 50% of patients.[22,23] The histology of the exanthema is nonspecific and includes marked edema of dermal papillae, focal intercellular edema of the basal cell layer, and very slight perivascular infiltration of mononuclear cells in the papillary dermis, with dilation of small vessels.[24] Thus, skin biopsy is helpful only in excluding other diagnoses.

In the subacute phase, patients may develop an acute flare or first episode of eczema or psoriasis.[25] The lesions associated with both these rashes may be extremely severe and require aggressive, specific therapy in consultation with a pediatric dermatologist. The psoriasis may manifest as the pustular form with severe lesions on the cheeks.[26]

Conjunctival Injection

Prominent dilated vessels within the bulbar conjunctivae without edema or infiltration of inflammatory cells on conjunctival biopsy is the hallmark of KD (Fig. 297.1).[27,28] The lack of edema in the conjunctiva allows the limbus to be easily seen, giving the appearance of a white halo around the iris. The lack of edema and exudate are important features in distinguishing KD from other acute infections associated with fever and conjunctivitis, most notably adenovirus, enterovirus, and measles infection. The presence of exudative conjunctivitis should prompt a search for a different diagnosis.[29] Careful slit-lamp examination within the first week after fever onset reveals mild anterior uveitis with a cellular infiltrate in most patients.[30] Keratitis is less often seen, and photophobia is usually mild or absent. Uveitis usually resolves without treatment and without subsequent scarring.

Oropharyngeal Changes

Erythema of the lips with fissuring and erythema of the posterior pharynx without tonsillar exudate or discrete intraoral lesions are the most common mucocutaneous findings in acute KD. Sloughing of the filiform papillae of the tongue, leaving a glossy red surface punctuated by the fungiform papillae, creates the classic "strawberry tongue" appearance associated with both KD and streptococcal and staphylococcal toxin-mediated diseases (Fig. 297.2). The presence of a true strawberry tongue essentially eliminates common viral infections in the differential diagnosis of a patient because these agents are not associated with this pattern of injury. The presence of exudative pharyngitis or discrete intraoral lesions should prompt search for an alternative diagnosis.[29]



FIG. 297.1 Bilateral conjunctival injection with perilimbal sparing and without exudate. *(From the KD Foundation [www.kdfoundation.org].)*



FIG. 297.2 Dry, erythematous, fissured lips with "strawberry" tongue. The filiform papillae that give the tongue its characteristic gray appearance have sloughed off, leaving a denuded, red surface. The prominent fungiform papillae appear as the "seeds of the strawberry." *(From the KD Foundation [www.kdfoundation.org].)*



FIG. 297.3 Symmetrical, diffuse vasodilation of the soles of the feet with associated edema. *(From the KD Foundation [www.kdfoundation.org].)*

Peripheral Extremity Changes

Edema of the dorsa of the hands and feet with or without erythema of the palms and soles often develops after several days of fever (Fig. 297.3). The fusiform swelling of the digits accompanied by swelling and purple discoloration of the proximal interphalangeal joints is a classic finding. The painful arthritis may limit the use of the hands, and

the swelling of the feet often discourages ambulation. The vasodilation of the palms and soles is distinct from the exanthem on the rest of body and is a diffuse, blanching erythema that may be fluctuating.

Cervical Lymphadenopathy

Cervical lymphadenopathy is the least reliable feature and is seen in only 30% to 50% of patients. It is most commonly unilateral, and inflammation in the sternocleidomastoid muscle often limits rotation of the head. The cervical mass should be at least 1.5 cm in diameter to qualify for this criterion.[29] Studies have shown that correct diagnosis of KD is often delayed in patients who initially present with the cervical mass and fever (node-first presentation of KD), and bacterial lymphadenitis is diagnosed in these patients.[31] The appearance of the rash can further delay diagnosis when it is misinterpreted as a drug eruption, prompting a change in antibiotics.[32] In distinguishing node-first KD from bacterial lymphadenitis, helpful features include a higher erythrocyte sedimentation rate, C-reactive protein level, absolute band count, ultrasound or computed tomography that demonstrates clusters of nodes (rather than one dominant node), and retropharyngeal edema. Histopathology of the nodes reveals sinus expansion and paracortical zone enlargement, sometimes in the presence of subcapsular necrosis.[33] However, these findings are nonspecific, and the diagnosis of KD cannot be confirmed by lymph node biopsy.

Associated Clinical Features

Nonspecific respiratory, gastrointestinal, and rheumatologic complaints can be associated with KD and often cloud the diagnosis (Table 297.2). In a study of associated symptoms in the first 10 days after fever onset in 198 patients with KD, irritability was reported in 98 (50%), vomiting in 88 (44%), cough in 55 (28%), diarrhea in 52 (26%), rhinorrhea in 37 (19%), abdominal pain in 35 (18%), and joint pain (arthralgia or arthritis) in 29 (15%). One or more gastrointestinal symptoms (vomiting, diarrhea, or abdominal pain) were present in 120 patients (61%), and 69 patients (35%) had at least one respiratory symptom.[34]

The most important associated clinical feature of KD is myocarditis, which is present in virtually all patients. Overt signs of congestive heart failure are rare, but biopsy-proven myocardial inflammation is universal.[35,36] Diastolic dysfunction may be apparent clinically with a gallop rhythm on cardiac examination. Although coronary artery aneurysms are the most severe complication of KD, signs of myocardial ischemia during the acute phase of the illness are rare. A presentation with shock has been described and is most commonly associated with peripheral vascular dilation (warm shock) with or without compromise in ventricular function.[37,38] Other inflammatory complications of KD may include hoarseness,[39] sensorineural hearing loss,[40] aseptic meningitis,[41] anterior uveitis,[28] cranial nerve palsies,[42,43] arthritis, urethritis, pancreatitis, and hydrops of the gallbladder.

Arthritis

Both large and small joint arthritis have been described in the acute and subacute stages of KD. Small joint arthritis frequently involves the proximal interphalangeal joints of the hands and can be extremely painful. The original series describing arthritis associated with KD found small joint arthritis in 15% of patients and axial arthropathy in 35%.[44] In a more recent series, the prevalence of arthritis was 7.5% (31 of 414 patients). In the 31 children with arthritis, 55% had oligoarticular involvement, and 45% had polyarticular involvement.[45] Limited magnetic resonance imaging studies suggest a nonerosive synovitis that resolves with nonsteroidal antiinflammatory therapy.[46] Naproxen (Naprosyn) (15 mg/kg/day divided into two equal doses for 1 week) is usually effective therapy.

TABLE 297.2 Associated Features of Kawasaki Disease

Cardiac disease
Coronary artery aneurysms
Myocarditis
Pericarditis
Mitral or aortic regurgitation
Arrhythmias
Irritability
Arthralgia, arthritis
Aseptic meningitis
Urethritis with sterile pyuria
Hepatitis
Hydrops of the gallbladder
Pancreatitis
Pneumonitis
Anterior uveitis
Sensorineural hearing loss
Peripheral ischemia

Gastrointestinal Features

Noncalculous cholecystitis or hydrops of the gallbladder with or without bilirubinuria and elevated levels of γ-glutamyltransferase is seen in up to 20% of patients with KD.[47–49] This unusual feature is also associated with streptococcal infection and toxin-mediated disease, thus complicating the differential diagnosis.

Acute pancreatitis can also be associated with the acute phase of the illness.[50] Prominent complaints of abdominal pain should prompt laboratory testing for elevated levels of serum lipase and amylase. Inflammation can rarely lead to common bile duct stenosis.[51] Successful treatment of pancreatitis associated with KD with a single infusion of infliximab (anti–tumor necrosis factor agent) has been reported.[52]

DIAGNOSIS

The laboratory evaluation of patients with suspected KD should include markers of inflammation and tests to exclude other competing diagnoses (see Table 297.2). The acute phase is associated with an elevated white blood cell count with neutrophil predominance and elevated acute-phase reactants including fibrinogen, α_1-antitrypsin, C-reactive protein, C3, C4, and haptoglobin. The platelet count usually rises during the second week after illness onset, whereas the acute-phase reactants normalize over 4 to 6 weeks after treatment.[53]

The American Heart Association has established criteria for the diagnosis of complete and incomplete KD.[54] The diagnosis of KD may be made on or before the fifth day of fever in the presence of four of the five classic criteria or in the presence of fewer criteria with a coronary artery Z-score (internal diameter of the left anterior descending artery or right coronary artery expressed as standard deviation units from the mean normalized for body surface area) greater than or equal to 2.5. Diagnosis may be made in patients who present with fewer than four of the five classic criteria according to the American Heart Association algorithm, which was validated in a retrospective study to identify greater than 97% of patients at risk for developing coronary artery aneurysms (Fig. 297.4).[55] A computer-based decision support tool is being tested to aid in the diagnosis of KD.[56] Until the etiologic agent of KD is established, both underdiagnosis and overdiagnosis will continue.

DIFFERENTIAL DIAGNOSIS

KD is most commonly confused with viral infections associated with rash and conjunctivitis, including infections caused by adenovirus, enterovirus, measles, and parvovirus (Table 297.3). In KD, the lack of tearing or conjunctival exudate may be helpful in differentiating it from these common viral infections. Laboratory testing for these specific agents, coupled with the standard laboratory evaluation for KD, is usually sufficient to differentiate KD from these other infections.[56] However, sensitive methods such as polymerase chain reaction assays may detect viruses that are present concomitant with KD.[57] Such results must be interpreted cautiously, and given the seriousness of the potential sequelae of this vasculitis, treatment with intravenous immune globulin (IVIG) may be warranted in patients for whom the diagnosis is unclear. Bacterial toxin–mediated disease caused by staphylococci and streptococci should also be considered in the differential diagnosis. Bilateral conjunctival injection is specifically not a feature of scarlet fever, and this physical finding may be helpful in distinguishing this form of streptococcal infection from KD. In patients presenting with the triad of fever, conjunctival injection, and rash, leptospirosis and tularemia may also be considered. Noninfectious conditions that can be confused with KD include systemic drug reactions such as Stevens-Johnson syndrome and systemic-onset juvenile idiopathic arthritis.[58]

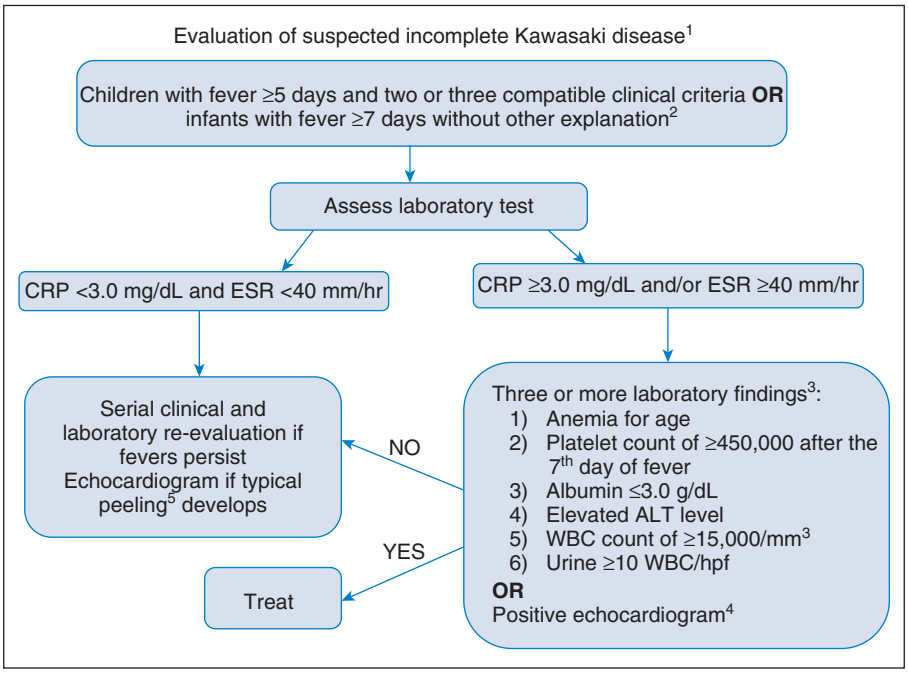

FIG. 297.4 Evaluation of suspected incomplete Kawasaki disease (KD). [1]In the absence of a gold standard for diagnosis, this algorithm cannot be evidence-based, but rather represents the informed opinion of the expert committee of the American Heart Association. Consultation with an expert should be sought anytime assistance is needed. [2]Infants ≤6 months old on day ≥7 of fever without other explanation should undergo laboratory testing and, if evidence of systemic inflammation is found, an echocardiogram, even in the absence of clinical criteria. Patient characteristics suggesting KD are listed in Table 297.1. Characteristics suggesting diseases other than KD include exudative conjunctivitis, exudative pharyngitis, discrete intraoral lesions, bullous or vesicular rash, or generalized adenopathy. Consider alternative diagnoses (see Table 297.3). [3]Supplemental laboratory criteria include albumin ≤3.0 g/dL, anemia for age, elevation of alanine aminotransferase level, platelets after 7 days ≥450,000/mm^3, white blood cell count ≥15,000/mm^3, and urine ≥10 white blood cells/high-power field. Can treat before performing echocardiogram. [4]Echocardiogram is considered positive for purposes of this algorithm if any of three conditions are met: Z-score of left anterior descending artery or right coronary artery ≥2.5; coronary arteries meet Japanese Ministry of Health criteria for aneurysms; or three or more other suggestive features exist, including perivascular brightness, lack of tapering, decreased left ventricular function, mitral regurgitation, pericardial effusion, or Z-scores in left anterior descending artery or right coronary artery of 2 to 2.5. If echocardiogram is positive, treatment should be given to children within 10 days of fever onset and to children beyond day 10 with clinical and laboratory signs (C-reactive protein, erythrocyte sedimentation rate) of ongoing inflammation. [5]Typical peeling begins under nail bed of fingers and then toes. *ALT,* Alanine aminotransferase; *CRP,* C-reactive protein; *ESR,* erythrocyte sedimentation rate; *hpf,* high-power field; *WBC,* white blood cell. *(From McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association.* Circulation. *2017;135:e927-e999.)*

TABLE 297.3 Differential Diagnosis of Kawasaki Disease

Infections
Adenovirus infection
Enterovirus infection
Measles
Scarlet fever
Staphylococcal or streptococcal toxic shock syndrome
Rocky Mountain spotted fever
Parvovirus B19 infection
Leptospirosis
Other Conditions
Rheumatic fever
Stevens-Johnson syndrome
Systemic-onset juvenile idiopathic arthritis
Polyarteritis nodosa
Reactive arthritis
Drug reaction

PATHOLOGY

KD is a systemic vasculitis that primarily affects medium-sized, extraparenchymal musculoelastic arteries with a predilection for the coronary arteries. Myofibroblast infiltration with secretion of proinflammatory cytokines, matrix metalloproteinases, and elastases destroys the structural integrity of the vessel wall and can result in aneurysm formation.[59] Three linked vasculopathic processes characterize the acute and chronic pathologic changes in the vessel wall and include an acute, self-limited necrotizing arteritis, a subacute or chronic vasculitis, and luminal myofibroblastic proliferation.[60]

The necrotizing arteritis that occurs during the first 2 weeks after onset of fever is characterized by infiltration with neutrophils from the lumen of the vessel. Myocarditis is universal at this stage and can progress to fibrosis and scarring in the later stages.[35] During the subacute phase, which may begin within the first 2 weeks after fever onset, there is an infiltration of lymphocytes, plasma cells, and eosinophils from the adventitial vasa vasorum that progresses toward the lumen. Aneurysms of the coronary arteries with or without thrombosis can occur. Immunohistochemistry shows a predominance of CD8$^+$ T cells and macrophages infiltrating the vessel wall with secretion of matrix metalloproteinases during this stage.[61,62] Infiltration of immunoglobulin A–secreting plasma cells is also noted.[63]

A different vasculopathic process involves a progressive, asynchronous intraluminal stenosis mediated by myofibroblasts. This process is associated with intimal thickening, remodeling of aneurysms, thrombotic occlusion, and stenosis in the medium-sized arteries.[60]

GENETICS

Evidence for a genetic component to KD susceptibility includes the increased incidence among Japanese children and among children of Japanese descent residing outside Japan, the occurrence of KD within extended pedigrees, the increased incidence of a history of KD in the parents of a patient with KD, and the increased incidence among siblings of an index case.[10,64,65] Twin studies have yielded conflicting results, and numbers of monozygotic twins concordant for KD are

small, owing in part to the rarity of monozygotic twins in the Japanese population.[66] Family linkage studies and genome-wide association studies have implicated single nucleotide polymorphisms in the transforming growth factor-β and calcineurin-NFAT (nuclear factor of activated T cells) pathways as well as variants in the Fcγ receptor genes.[67–73] Whole-genome sequencing of a family with two affected siblings revealed compound heterozygous mutations in TLR6.[70] Clearly the genetics of KD susceptibility is multifactorial, and further studies will be needed to capture this complexity. Whether additional genetic determinants predispose to aneurysm formation is currently under study.

THERAPY

The standard therapy for KD is infusion of IVIG (2 g/kg) in combination with aspirin. If administered within the first 10 days after fever onset, this regimen reduces the risk for coronary artery aneurysms from 25% to 5%.[74] Although this regimen rapidly reduces markers of inflammation, fever, and clinical signs in most patients, 10% to 20% of patients will have persistent or recrudescent fever at least 36 hours after the end of the IVIG infusion and are classified as IVIG resistant. Different strategies are used to treat this subset of patients, including a second infusion of IVIG, infliximab, and methylprednisolone.[75–78] However, the requirement for a large sample size and associated costs of a prospective, randomized clinical trial have prevented the generation of evidence-based guidelines for treatment of IVIG-resistant patients. At the present time, a comparative effectiveness clinical trial (KIDCARE) is randomly assigning IVIG-resistant subjects with KD to receive either a second IVIG infusion (2 g/kg) or infliximab (10 mg/kg) (Kawasaki Disease Comparative Effectiveness Trial [KIDCARE]; ClinicalTrials.gov Identifier: NCT03065244; https://clinicaltrials.gov/ct2/show/NCT03065244).[79]

In an attempt to prevent IVIG resistance, investigators have tested different strategies for intensification of initial therapy in three different randomized, placebo-controlled clinical trials. The addition of single-dose intravenous methylprednisolone to standard therapy did not reduce IVIG resistance or median coronary artery Z-score.[78] However, standard therapy with the addition of intravenous followed by oral methylprednisolone for up to 4 weeks in Japanese patients selected by a scoring system to be at increased risk for IVIG resistance resulted in a significant decrease in both treatment failures and coronary artery Z-score in the corticosteroid-treated group.[80] The validated scoring system for Japanese patients has had low sensitivity when applied to patients with KD of other ethnic backgrounds, and attempts to create alternative scoring systems have failed.[81,82] In the third clinical trial, the addition of infliximab (5 mg/kg) to standard therapy resulted in a more rapid decrease in inflammatory markers, fever, and coronary artery Z-scores but did not prevent IVIG resistance.[83]

Therapy for patients with highly resistant KD may include steroids, cyclosporine, low-dose methotrexate, plasmapheresis, and cytotoxic agents such as cyclophosphamide. There are no evidence-based recommendations to guide therapy in this subset of patients with KD.

CARDIOVASCULAR INVOLVEMENT

In his initial description of KD, Kawasaki was unaware of the cardiovascular complications and described KD as a benign, self-limited illness with no sequelae. In 1970, after a survey commissioned by the Japanese Ministry of Health, it became clear that KD could cause coronary artery aneurysms and lead to myocardial infarction and death.[1] Coronary artery aneurysms develop in 25% of untreated children, although the lesions may remain silent until adulthood, when sudden thrombosis can lead to myocardial infarction and death.[84] Transthoracic echocardiography is the method of choice for measuring the internal diameter of the proximal coronary arteries in children, and detailed guidelines are available for the method and timing of these studies during the acute and subacute illness.[54] Arteries with a Z-score less than 2.0 are considered normal. In patients treated within the first 10 days after fever onset, 20% to 30% will have transiently dilated arteries, whereas 5% to 7% will develop permanent damage to the coronary artery wall with aneurysm formation. Although most of these aneurysms will remodel over time, the vessel wall is never again normal, and the risk for subsequent stenosis and thrombosis is still being defined.[85]

Patients with aneurysms require lifelong follow-up regardless of internal dimension or subsequent remodeling. Acute therapy may include aspirin at antiplatelet doses with or without clopidogrel for small to medium-sized aneurysms (Z-score <10). Patients with larger aneurysms (>8 mm or Z-score ≥10) require systemic anticoagulation to prevent thrombosis and may be treated with low-molecular-weight heparin or warfarin.[86]

LONG-TERM OUTCOME

Patients with large coronary aneurysms after KD are at high risk for subsequent cardiovascular events and will require lifelong surveillance by cardiologists knowledgeable about management issues in this patient population.[87,88] Patients with regressed aneurysms after KD remain at increased risk for thrombosis and other adverse outcomes and should be followed closely. Whether KD is associated with accelerated development of atherosclerosis remains controversial, but the vasculopathy of KD appears to be distinct from that of atherosclerosis.[89]

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Special Problems

IV

A Nosocomial Infections

298 Infection Prevention and Control in the Health Care Setting

Tara N. Palmore

SHORT VIEW SUMMARY

- Infection-prevention and infection-control programs aim to reduce the risk of health care–associated infections in institutions that care for an increasingly vulnerable, elderly, and often immunosuppressed patient population. The goal is to make the hospital a safe place for patients and staff.
- Health care epidemiologists use an expanding repertoire of evidence-based techniques and technology to identify and mitigate risks from an ever-changing array of invasive devices and procedures. Hand hygiene remains the staple infection-prevention measure; although hospitals have attempted many methods for improving adherence, an improving array of electronic compliance monitoring systems may increasingly perform that function.
- The built environment of the hospital is a well-documented reservoir for pathogens that can infect susceptible patients. Environmental disinfection depends on manual cleaning with effective disinfectant solutions. Microbial killing on surfaces can be augmented with use of adjunctive ultraviolet C light, which reduced acquisition of nosocomial pathogens in a randomized trial, or with hydrogen peroxide vapor or mist. Hospital water systems can harbor *Legionella* or other waterborne pathogens and must be managed attentively in order to minimize the risk to patients. Engineering, including tailored air handling, is used to help protect patients from airborne pathogens such as *Mycobacterium tuberculosis* and mold spores.
- Multidrug-resistant organisms are an evolving challenge for infection-control personnel, who must monitor and attempt to control the spread of resistant organisms within the institution, including emerging pathogens such as *Candida auris*. Newer, more rapid tools for surveillance aid in decisions to isolate carriers. Old measures have found new evidence; in a randomized trial chlorhexidine baths reduced transmission of methicillin-resistant *Staphylococcus aureus* and in other studies prevented central line–associated bloodstream infections. Because antimicrobial stewardship, dealt with in Chapter 51, can affect antimicrobial resistance, some hospitals assign this responsibility to hospital infection-control staff.
- Outbreak investigation involves increasingly sophisticated tools, such as microbial genome sequencing, to confirm clonality. Shoe-leather epidemiology remains a critical means of collecting exposure data that can be combined with genomic data to elucidate routes of transmission. Emergence and rapid global spread of new pathogens requires hospital epidemiologists to be nimble. The 2014–15 Ebola outbreak required rapid initiation of drastically new screening and isolation measures in hospitals, despite the low risk of identifying an Ebola-infected patient in any given facility.
- Collaboration of infection-control and occupational medicine staff helps reduce risk of occupational exposures and infection. Plans need to be in place for immediate management of exposures to varicella and bloodborne pathogens. Rapid investigation of health care personnel and patient exposures to these communicable pathogens allows prompt initiation of postexposure prophylaxis. Preexposure immunization of health care personnel to influenza and other vaccine-preventable diseases makes the hospital a safer place for both staff and patients.

Hospital infection control traces its roots to the mid-19th century, when medical and scientific investigators noted the preventive benefits of hand hygiene, surgical antisepsis, and hospital hygiene. These measures remain at the core of infection prevention, with an expanding base of scientific research to guide their application in the setting of increasingly complex medical care and increasing antibiotic resistance.

Infection-prevention programs are charged with a mission that involves conducting surveillance; implementing isolation; investigating and rapidly intervening in suspected nosocomial transmission; educating health care personnel, patients, and visitors; reporting infections to public health authorities; participating in antimicrobial stewardship; and working with occupational health specialists to anticipate and avert

preventable exposures and infections and to manage those that occur despite those efforts.[1] This chapter aims to address important aspects of some of these infection-prevention and infection-control activities. Antimicrobial stewardship is discussed separately, in Chapter 51.

PATHOGEN TRANSMISSION IN THE HOSPITAL

Bacterial pathogens of epidemiologic significance typically inhabit specific niches on or in the human body, or in the hospital environment, that can serve as reservoirs for transmission. Patients' skin, intestinal, and respiratory microbiota are distorted within a few days in the hospital, and their flora in turn colonize their inanimate environment within the hospital.[2] Patients' flora can also be deranged by antibiotic therapy or chemotherapy, lowering resistance to colonization with antibiotic-resistant nosocomial pathogens.[3]

Patients who are colonized with resistant bacteria serve as accidental reservoirs for spread to other patients. Colonization pressure, the proportion of patients in a given ward who are carriers, is an independent risk factor for transmission of resistant bacteria.[4,5] Most nosocomial pathogens are thought to be transmitted from person to person on the hands of health care personnel, from contaminated surfaces in the hospital environment, or from contaminated patient care equipment. Hand hygiene and correct disinfection of equipment and hospital surfaces are thus important means of preventing spread.

Hand Hygiene

Hand hygiene is a critical infection-control measure[6,7] yet one of the most challenging to follow consistently. The World Health Organization's "Five Moments for Hand Hygiene" is a simple representation of transitions in patient care at which hand hygiene should be done in order to prevent cross-transmission: before touching a patient, before clean or aseptic procedures, after body fluid exposure or risk, after touching a patient, and after touching patient surroundings.[6] Monitoring hand hygiene compliance is required of hospitals in many countries. Despite evidence that hand hygiene prevents nosocomial infections,[8] adherence remains as low as 40% to 60%.[9,10]

Researchers have attempted to understand the basis for inadequate hand hygiene compliance in order to identify opportunities for improvement. A study found that full compliance with World Health Organization guidelines would cost each intensive care unit (ICU) nurse 58 to 70 minutes spent on hand hygiene per patient during a 12-hour day shift.[11] The report illustrates the tension between patient care workload and commitment of time to meticulous hand hygiene. Studies aimed at improving hand hygiene compliance have focused on improving compliance monitoring and addressing behavioral and psychological barriers to consistent hand hygiene. One hospital system addressed both goals by broadening compliance monitoring to all frontline health care personnel, producing more robust monitoring data, engaging staff more deeply in the effort, and perhaps instilling an interest in improving hand hygiene.[12] Many studies have explored ways to improve hand hygiene and (perhaps more challenging) sustain the improvement. Some reports describe engagement of patients in the effort. Electronic hand hygiene monitoring systems hold promise, but results from early-generation systems have been mixed,[13,14] demonstrating that the technology cannot yet accurately measure adherence to a simple manual task. As treatment options for some classes of multidrug-resistant bacteria diminish, hand hygiene remains the single most important intervention to limit morbidity and mortality due to these organisms. Achieving better hand hygiene compliance is a major goal of infection-control programs.

Disinfection and Sterilization (See Chapter 299)

Disinfection and sterilization of patient care equipment are a fundamental aspect of infection prevention in a hospital. The Spaulding classification for disinfection and sterilization is outlined in Table 298.1. Patients and health care personnel generally take for granted that medical instruments used for invasive procedures are appropriately disinfected or sterilized and represent the least of the patient's risk for postprocedure complications. Yet hospital disinfection and sterilization programs present many opportunities for errors and mishaps. For example, disinfection or sterilization can fail if visible organic or inorganic material is not first cleaned from instruments, reagents are expired and have lost potency, or biologic indicators are not used properly. Sizeable clonal outbreaks of multidrug-resistant bacteria in recent years have brought to light the frailties of high-level disinfection procedures for gastrointestinal endoscopes, particularly those used for biliary procedures. Some large outbreaks were traced to endoscopes that retained infectious material even after cleaning and disinfection according to published guidelines.[15,16]

Environmental Cleaning and Disinfection

Environmental cleaning in the health care environment is aimed at removing visible soiling, and disinfection is aimed at reducing the burden of microorganisms on surfaces in the health care setting.[17,18] A patient who occupies a room that has been vacated by a person infected or colonized with *Clostridioides difficile* (formerly *Clostridium difficile),* vancomycin-resistant enterococci (VRE), or methicillin-resistant *Staphylococcus aureus* (MRSA) has elevated risk of developing *C. difficile* infection or becoming colonized with the respective organism.[19–22] In a separate transmission scenario, health care personnel can transfer viable organisms through contact with surfaces in the patient room, even if they do not touch the patient, and, in the absence of proper hand hygiene, carry them outside the room to the common hospital environment from which they can be spread to other patients.[17,18] This chain of transmission can be mitigated by meticulous hand hygiene and thorough disinfection of the patient care environment, but neither activity is performed with consistently high quality in many hospitals.[23,24]

Methods of Environmental Cleaning and Disinfection

Liquid chemicals used for environmental cleaning are also low-level disinfectants, and, if applied correctly, can serve both purposes. Environmental cleaning and disinfection focus on surfaces that are repeatedly soiled, such as bathrooms and surfaces that are "high touch." Even floors should be considered as potential sources of transmission to patients; guidelines recommend use of disinfectant floor cleaners in

TABLE 298.1 Indications for Sterilization, High-Level Disinfection, and Low-Level Disinfection

TECHNIQUE	DESCRIPTION	INDICATION
Sterilization	Elimination of all microbial life, including spores,[a] using pressurized steam, gas, or liquid chemicals	Critical medical and surgical devices and instruments that enter normally sterile tissue or the vascular system or through which blood or other sterile body fluids flow
High-level disinfection	Elimination of all microbial life, except bacterial spores, using liquid chemicals	Semicritical patient care equipment (e.g., gastrointestinal endoscopes, bronchoscopes, respiratory therapy equipment) that touches either mucous membranes or nonintact skin
Low-level disinfection	Removal of most microorganisms, except bacterial spores, using liquid chemicals	Noncritical patient-care surfaces (e.g., bed rails, over-the-bed table) and equipment (e.g., blood pressure cuff) that touch intact skin.

[a]Standard sterilization techniques are not considered sufficient to destroy prions.
Data from Rutala WA, Weber DJ; Healthcare, Infection Control Practices Advisory Committee (HICPAC). Guideline for Disinfection and Sterilization in Healthcare Facilities. *Atlanta: Centers for Disease Control and Prevention; 2008.*

wards with high endemic rates of multidrug-resistant organisms (MDROs).[25,26] Cleaning solutions that contain quaternary ammonium compounds are relatively ineffective at killing *C. difficile* spores and inactivating nonenveloped viruses such as norovirus. Hydrogen peroxide and bleach solutions have activity against these pathogens, in addition to standard viruses and vegetative bacteria, and may be preferable for routine environmental cleaning and disinfection.[27]

C. difficile spores and MDROs, particularly those that are hardy (e.g., resist desiccation, tolerate a range of temperatures) such as VRE, *Acinetobacter baumannii,* and *Candida auris,* contaminate the environment in the rooms of patients who are colonized with these bacteria.[28] Several techniques developed to enhance environmental disinfection have been tested in clinical trials, most notably machines emitting hydrogen peroxide and ultraviolet light.

Hydrogen peroxide vapor treatment requires rooms, or entire wards, to be sealed, disconnected from air handling systems, and then filled with vapor at a concentration that is monitored and adjusted throughout the treatment. The treatment, which must be administered by specially trained technicians, inactivates heavy concentrations of vegetative bacteria and spores, even in the presence of proteinaceous material.[29] Its potent inactivation of viruses made it the decontamination method of choice in Europe, the United Kingdom, and the United States during the 2014–15 Ebola virus outbreak (see later). Another form of hydrogen peroxide, an aerosolized mist, is aimed more directly on targets of disinfection and has less impact on microbial contamination in vitro.[30,31]

Ultraviolet light–emitting devices for environmental disinfection use low-pressure mercury vapor lamps to emit germicidal ultraviolet C (UV-C) wavelengths or use pulsed xenon-generating broad-spectrum ultraviolet light that includes UV-C wavelengths. Prospective clinical trials have evaluated the ability of hydrogen peroxide vapor and UV-C disinfection, as adjuncts to terminal room cleaning, to reduce the risk that newly admitted patients would acquire MDROs from prior room occupants.[32] A single-center, prospective cohort study found that standard terminal room cleaning followed by hydrogen peroxide vapor treatment reduced the risk of MDRO acquisition by more than 60% compared with standard cleaning.[32] A large cluster-randomized crossover trial compared four arms (standard cleaning, standard cleaning followed by UV-C, bleach cleaning, and bleach cleaning followed by UV-C), and found that the addition of UV-C to standard cleaning reduced subsequent acquisition of pathogens by 30%.[33] Oddly, standard cleaning followed by UV-C had a greater impact than bleach cleaning followed by UV-C, and the addition of UV-C to bleach cleaning had no impact on acquisition of *C. difficile* infection. Clinical outcome data from these trials suggest that these adjunctive modalities do reduce nosocomial transmission of pathogens above and beyond what is accomplished with manual cleaning. Ultraviolet devices can be used by trained housekeeping staff, and may be worthwhile for targeted use if financially feasible.

Water Management

Hospital water safety is a priority and a challenge for health care epidemiologists, hospital safety officers, and facility engineers. As with other health care–associated infections, waterborne infections can cause significant morbidity and mortality, and some are preventable. Pathogens such as *Legionella* and nontuberculous mycobacteria can colonize the central pipes or outlets of potable water distribution systems in hospitals, and other gram-negative bacteria reside in biofilms near the points of use. Because so many outbreaks of nosocomial waterborne infections are preventable,[34] hospitals are required to have proactive water management programs.

Although municipal and hospital tap water is not expected to be free of potential pathogens, municipal water is tested routinely to ensure safe levels of important pathogens such as coliform bacteria. Contaminated municipal water can cause outbreaks that affect immunocompromised patients in health care settings,[35] but contamination of hospital water usually occurs within the plumbing of the health care facility.[36] Waterborne bacteria that would not affect community dwellers or even the majority of hospitalized patients may infect the most highly vulnerable because of underlying diseases, immunosuppression, and the presence of invasive devices that provide a route of entry, bypassing the normal defenses.

Transmission of Waterborne Pathogens

Waterborne pathogens can be transmitted to patients via aerosols, such as through a shower or room humidifier,[37–40] cooling tower,[41] or aspiration while drinking water. Recent outbreaks of deep-seated nontuberculous mycobacterial infections among cardiac surgery patients around the globe were traced to heater-cooler units that aerosolized the bacteria from contaminated water tanks in the direction of the surgical field.[42] Some waterborne bacteria may be transmitted indirectly from hands[43] or objects that had contact with contaminated water, such as bath supplies and linens[44] or by means of tap water used inappropriately for respiratory care of ventilated patents[45,46] or rinsing of respiratory therapy or endoscopic equipment in tap water.[47,48] As in most nosocomial infections, the precise route of transmission is often unknown, even when the link to a water source is apparent.[49]

Biofilms in Hospital Plumbing

Biofilms in the pipes and outlets of water distribution systems provide the environment for growth of *Legionella,* mycobacteria, *Pseudomonas*, *Sphingomonas,* and other waterborne organisms. Eliminating bacteria that dwell in biofilms is difficult because they are somewhat impervious to disinfectants.[50,51] Stagnation of water, due to pipe design or low flow, creates optimal conditions for biofilm formation and colonization. Certain water pipe formations, such as dead legs and long horizontal runs of pipe,[52,53] contribute to stagnation and are discouraged in hospital construction guidelines. Many older facilities have convoluted plumbing that has, over time, developed low-flow zones and may be more vulnerable to water system colonization with *Legionella,* mycobacteria, and others. Cooling towers have been implicated in nosocomial and community outbreaks.[41,54] Biofilms can also form at the points of use; electronic eye faucets,[55–58] aerators, ice machines, decorative fountains,[52,59] and other items that are at the interface between water and patients can become colonized and have been implicated in nosocomial transmission.

Legionnaires' Disease

Legionnaires' disease, a potentially severe pneumonia caused by *Legionella* species, is an important health care–associated infection. *Legionella pneumophila,* the species responsible for the vast majority of infections, is one of the most dreaded nosocomial waterborne pathogens. The mortality rate of hospital-acquired legionnaires' disease is approximately 32%, more than three times higher than that of community-acquired infection, likely because of the underlying comorbidities of hospitalized patients.[60] Although most legionnaires' disease is acquired in the community, approximately 33% of outbreaks occur in health care facilities, accounting for 85% of deaths due to legionnaires' disease outbreaks.[61] As described elsewhere (see Chapter 232), the most immunologically vulnerable patients in the hospital and those with advanced age and chronic lung disease are at highest risk for nosocomial legionnaires' disease.

Legionella experts are divided regarding proper primary prevention of nosocomial *Legionella* outbreaks and whether hospital water should be cultured prospectively for *Legionella* spp. The Centers for Disease Control and Prevention (CDC) recommends close clinical surveillance for *Legionella* infection and a low threshold for investigation when a suspected nosocomial case occurs.[62] Other sources, including published guidelines in much of Europe, recommend routine sampling and testing of hospital water even when no nosocomial infections have occurred. Most would agree with testing water regularly for the presence of *Legionella* after an outbreak has already occurred, for secondary prevention.

Disinfection of Hospital Water

Water circulating in health care facilities should have adequate concentrations of disinfectant at the point of use. Although most municipal water may be adequately chlorinated, free chlorine, the component of total chlorine that has antimicrobial activity, can dissipate as water travels through the water distribution system, leaving low residual levels at the patient room faucet.[63] If hospital chlorine measurements are performed at a central location, the concentrations that directly affect patient safety will not be apparent. Supplemental disinfection systems add chlorine (in the form of sodium hypochlorite, or bleach), monochloramine, chlorine dioxide, ozone, or copper-silver ions to the water supply. Even

when seemingly optimal supplemental disinfection is used, clinicians must be vigilant for breakthrough contamination, which generally manifests earliest as infections among the most immunocompromised or critically ill patients.[64]

Wastewater Plumbing Contamination

A more recently recognized aqueous threat to hospitalized patients is contamination of sink drains and other drains in rooms of patients with MDROs. Although the drains are not in direct contact with patients, experimental studies have demonstrated the dispersal of organisms from the sink drain when water from the faucet splashes back from the drain or its sieve.[65] Resistant organisms may also disseminate in an aerosol when hospital toilets are flushed.[66] In one quasiexperimental study, addition of a lid to ICU hoppers (toilet-like waste disposal fixtures) and biofilm-inhibiting devices to sink drains reduced by half the nosocomial acquisition of carbapenemase-producing organisms.[67]

Air Handling

Hospital ventilation is a critical feature of the building infrastructure that must be engineered to meet a range of infection-control requirements in different areas of the hospital. Notable issues include negative pressure isolation rooms for patients who have suspected or confirmed airborne infections; positive pressure protective environment rooms for patients who are undergoing treatment for leukemia or stem cell transplantation; and laminar flow in operating rooms.

Air Handling for Airborne Infections

Patients infected with airborne infections, such as tuberculosis, measles, and chickenpox, cough or exhale pathogens in tiny droplets that travel and remain viable in air currents, and can infect susceptible persons who are in the path of airflow from the affected patient. Health care personnel and other patients outside the room of a patient with an airborne infection may be exposed when the door is opened, and such exposures have been documented in health care outbreaks.[68,69] Therefore, hospitalized patients who have suspected or known airborne infection should be housed in airborne isolation rooms—private rooms that have monitored negative airflow with respect to the anteroom or hallway, and 6 to 12 air changes per hour, with the exhausted air filtered through a high-efficiency particulate air (HEPA) filter or released to the outside.[70] Negative airflow draws infectious particles toward the patient and prevents their dispersal out of the room when the door is opened.

Air Handling to Prevent Nosocomial Mold Infections

Patients who have prolonged neutropenia during treatment of leukemia, stem cell transplantation, and some immunotherapies are highly vulnerable to nosocomial mold infections (see Chapter 306). Mold spores, which are as small as 2 to 4 μM, are ubiquitous and easily disseminate in the hospital via air currents, dust, and other particulate matter. Protective isolation rooms are equipped with HEPA-filtered air intake, use positive pressure airflow with at least 12 air changes per hour to repel air currents that carry mold spores, and have seams and cracks sealed to prevent incursion of mold spores through tiny breaches in the envelope of the room.[71] When vulnerable patients leave their rooms and their wards for tests or procedures, they are usually entering areas of the hospital that do not have special environmental precautions, and where they may be exposed to mold spores. Directional airflow and HEPA filtration are also used to minimize escape of fungal spores from construction sites within the hospital. Exposure to construction is a well-described risk factor for invasive mold infections among neutropenic patients, and planned engineering measures, in addition to traffic control and other dust containment measures, can significantly reduce the mold spore content of air near an indoor construction zone.[72–74] Neutropenic patients who leave their rooms are advised to don surgical masks, which offer little protection against mold spores, or less commonly N95 respirators, which have not been tested for protection against mold infections.

Air Handling in the Operating Room

Operating rooms require fresh, filtered air in order to prevent surgical site infections. Operating rooms should have positive pressure airflow with respect to the surrounding rooms and hallway, with at least 20 filtered air changes per hour, including fresh (outside) air. Airflow can be laminar or directional within the room, such that it enters at the ceiling and exhausts near the floor. Operating room air must be maintained at a moderate range of humidity, 20% to 60%, in order to minimize risk of both static electricity (low humidity) and conditions that promote microbial growth (high humidity).[75]

Air Quality

Routine air samples for fungal culture are not recommended. Air samples may be collected as part of an investigation of a suspected cluster or outbreak of nosocomial mold infections. Even then, they are rarely helpful because there is not a clear correlation between air sample mold growth and risk to patients.[76,77] In addition, mold outbreaks among immunocompromised patients are typically polyclonal, and air samples often capture different species or strains than those that are relevant in the outbreak. Use of enhanced clinical monitoring and surveillance for possible nosocomial mold infections is recommended in order to detect possible acquisition from the hospital environment.[71] An exception is the required microbiologic air sampling that must be conducted in hospital pharmacies and clean-room laboratories in which medications or cell products are prepared for intravenous infusion. In such facilities, routine air sampling is a required part of an environmental monitoring program to ensure sterility of parenteral solutions.

TRANSMISSION-BASED INFECTION-CONTROL PRECAUTIONS

Colonization

Colonization refers to the peaceable presence of bacteria or fungi on or in a person, including organisms that are part of the normal human microbiota. Patients develop infection if the organisms with which they are colonized subsequently invade, stimulate a symptomatic immune response, or both. Colonized patients can serve as silent reservoirs for transmission. The routes by which pathogens are thought to spread determine the isolation precautions used to limit their transmission.[78] In a more conservative approach, patient isolation is implemented not only when a patient has a confirmed communicable infection or colonization, but also when a transmissible pathogen is suspected, in order to prevent transmission during the diagnostic interval. Such empirical isolation is used until the results of testing confirm or refute the need for ongoing isolation.

Organisms Transmitted by Contact

Most epidemiologically important bacteria, including MDROs and many viruses, are spread directly by means of person-to-person contact, or indirectly via contact with contaminated patient care equipment or surfaces. Intestinal pathogens (e.g., *C. difficile, Salmonella, Shigella, Escherichia coli,* norovirus, astrovirus, and adenovirus 40/41) are shed in high concentrations, and therefore are highly transmissible, when patients are symptomatic. Of note, some are also transmissible when shed by asymptomatic persons.[79,80] Contact precautions with use of barriers such as gowns and gloves are intended to interrupt transmission of MDROs and other pathogens, although their effectiveness can be undermined by contaminated equipment such as stethoscopes or portable radiography film cartridges that are not properly disinfected.

Despite established recommendations to use contact isolation to care for patients who are colonized or infected with MDROs,[78] the measures are controversial in part because of a paucity of evidence to support their use. In a cluster-randomized trial by Harris and colleagues, ICUs were randomized to universal gowns and gloves for all patient care or contact precautions for standard indications.[81] Universal contact precautions reduced acquisition of MRSA but not VRE, demonstrating that barrier precautions do not thwart all the nosocomial routes by which patients become colonized. Implementation of universal gowning and gloving may be a useful measure during an ICU-based outbreak.

Organisms Transmitted by Droplet

Pathogens that infect or colonize the upper respiratory tract, such as respiratory viruses, staphylococci, *Bordetella pertussis,* and group A streptococci, can spread via droplet routes. These pathogens are shed

from the upper respiratory tract in droplets that are typically greater than 5 μM in size and fall within 1 to 2 meters of the patient. Pathogens within those droplets can spread to health care personnel when they land on mucous membranes or may be transmitted indirectly via hands or fomites. Thus a face mask and gloves are components of droplet isolation.[78]

Organisms Transmitted by the Airborne Route

Infections known to spread via the airborne route include tuberculosis, varicella, and measles. Pathogens that are shed in droplet nuclei, which are viable particles 2 to 5 μM in size, can remain airborne for prolonged periods, traveling in air currents. Isolation rooms for airborne infections were described earlier. The room door must remain closed in order for these measures to contain infectious particles, and an anteroom provides an additional layer of protection between the patient room and the hallway. Health care personnel wear fitted particulate respirators, referred to as N95 respirators because they filter 95% of airborne particles, or powered air-purifying respirators that draw air into a hood through a HEPA filter and provide better protection.[82]

Organisms Transmitted by Aerosol

Some pathogens may be shed by forceful coughing, sneezing, or vomiting, in aerosols that include droplets of varying sizes, some of which may travel farther than 2 meters before falling as a result of gravity. Aerosols are thought to play a role in spread of influenza, among other pathogens.[83,84] Aerosol-producing procedures, such as endotracheal intubation, bronchoscopy, sputum induction, and pentamidine administration, merit use of respirators to protect health care personnel from the temporarily airborne particles.[70]

DEVICE-RELATED INFECTIONS

Invasive procedures and indwelling devices, often essential to providing lifesaving supportive care, can serve as portals of entry for pathogens. Each major category of device has distinct, evidence-based preventive measures that can reduce the associated risk of infection.

Ventilator-Associated Pneumonia (See Chapter 301)

Ventilator-associated pneumonia (VAP) is a largely preventable complication of mechanical ventilation in the ICU. Although the incidence of VAP varies by definition,[85] VAP is diagnosed in approximately 10% of ventilated patients,[86] leading to prolonged length of ICU stay, greater duration of mechanical ventilation, and an attributable mortality of about 13%.[87]

Measures to Prevent Ventilator-Associated Pneumonia

Avoiding intubation is the most obvious method of preventing VAP and may be feasible in some clinical scenarios with use of noninvasive positive pressure ventilation. In patients who are already intubated, national consensus guidelines recommend minimizing sedation, conducting spontaneous awakening trials, and assessing patients' readiness for extubation daily in an effort to reduce the duration of mechanical ventilation.[88] In addition, patients should have the head of the bed elevated to 30 to 45 degrees to reduce reflux of gastric contents and should undergo regular subglottic secretion suctioning to avoid pooling of secretions above the endotracheal tube cuff.[88]

Additional methods to prevent VAP that are less uniformly accepted include oral care with antiseptics and silver-coated endotracheal tubes.[88] A Cochrane review evaluated 18 randomized controlled trials of oral hygiene care, including those employing chlorhexidine mouthwash or gel, and concluded that oral hygiene, compared with placebo or usual care, reduced the risk of VAP from 25% to 19% but had no impact on mortality, ventilation days, or duration of ICU stay.[89] A meta-analysis of randomized trials comparing chlorhexidine oral care with placebo concluded that use of chlorhexidine prevented nosocomial pneumonia in cardiac surgery patients but did not prevent VAP or significantly affect mortality, duration of ventilation, or ICU length of stay in other ventilated patients.[90] Another meta-analysis reviewed randomized controlled clinical trials of silver-coated endotracheal tubes compared with noncoated tubes. Although the tubes led to a significant reduction in VAP rates in one well-designed trial,[91] the Cochrane review authors concluded that overall evidence that the tubes prevent VAP or have other beneficial clinical impact is weak.[92]

Selective Oral and Digestive Decontamination

Selective oral decontamination and selective digestive decontamination use antibiotic combinations to reduce the microbial burden in the oral cavity and gastrointestinal tract, respectively, and have shown promising results in clinical trials conducted in Europe. Selective oral and digestive decontamination reduced mortality by 3.5% and 2.9%, respectively, in a cluster-randomized trial of 13 ICUs in the Netherlands.[93] The concern that they could increase antibiotic resistance has not been borne out conclusively by clinical trial data. Studies conducted in ICU settings with low prevalence of multidrug resistance showed low impact on prevalence of resistance.[93–95] The practices have not been widely adopted in the United States in part because of unresolved concerns about antimicrobial stewardship, *C. difficile* infection, and long-term exacerbation of antimicrobial resistance, and the paucity of data on comparative effectiveness of these and other strategies for preventing VAP.[96]

Ventilator-Associated Events

A recent development in the surveillance and measurement of ventilator-associated complications in the United States is the use of ventilator-associated events, an umbrella category that developed owing to the ambiguity of VAP definitions and the difficulty of differentiating infectious and noninfectious conditions in complex intubated patients. Among ventilator-associated events, a minority of events have been classified as infections.[97] Because this surveillance definition is relatively new, there are not yet evidence-based estimates on the overall preventability of ventilator-associated events.

"Bundled" Ventilator-Associated Pneumonia Prevention Strategies

Although most of the individual prevention strategies discussed earlier have an evidence base for their use, they are often implemented in various combinations as VAP-prevention bundles, in an effort to amplify effect and ensure consistency. The prototypical VAP bundle was published by the Institute for Healthcare Improvement, which recommends the following collective interventions: head-of-bed elevation, daily sedative interruption and daily assessment of readiness to extubate, peptic ulcer prophylaxis, deep vein thrombosis prophylaxis, and daily oral care with chlorhexidine.[98] Bundles have been controversial in part because, despite comprising individual evidence-based elements, the composite interventions are inadequately studied in clinical trials to determine their overall effect on patient outcomes. Whether prevention methods are introduced individually or as bundles, educating and engaging the multidisciplinary ICU staff are essential to implementing and improving compliance with VAP-prevention measures and decreasing the burden of VAP on mechanically ventilated patients.[88,99]

Central Venous Catheter–Associated Infections (See Chapter 300)

Central venous catheters (CVCs) are often essential to the care of hospitalized patients. Their use is associated with complications, including bloodstream infections introduced by contamination of the foreign material that is penetrating the skin and residing in a large vein. Patients who develop catheter-associated bloodstream infections have increased length of stay and approximately triple the risk of in-hospital death.[100] Although evidence-based measures and concerted efforts have led to a 50% reduction in catheter-associated bloodstream infections across the United States since 2008,[101] they remain an important health care–associated infection that requires continual efforts toward prevention.

Preventive Measures for Catheter Insertion

Prevention of catheter-associated bloodstream infections begins with evidence-based preventive strategies during catheter insertion, including checklists providing reminders of appropriate insertion practices, nursing observation of insertion procedures, hand hygiene, all-inclusive catheter kits, maximum sterile barrier precautions for the operator and for the patient, and chlorhexidine-alcohol antiseptic skin preparation.[102]

Catheter placement into the femoral or internal jugular vein poses an increased risk of infection.[103] Infections related to infection-prevention breaches during catheter insertion typically manifest within 5 days of insertion.[104] Studies using antiseptic- or antibiotic-impregnated catheters have shown variable benefits for catheter-associated bloodstream infection prevention and have not identified the ideal antiseptic agent or antibiotic combination.[105–109]

Preventive Measures for Catheter Maintenance

Although measures related to CVC insertion significantly reduce infection rates,[110,111] practices related to maintenance of the catheters are also vital to preventing infections.[102,112] Evidence-based measures include nurse-to-patient ratios of at least 1 to 2, scrubbing of the catheter hub with disinfectants each time it is accessed, and cleansing of catheter sites with chlorhexidine-based antiseptic every 5 to 7 days along with dressing changes, or sooner if soiled.[102] Disinfectant caps and chlorhexidine-impregnated dressings have been shown to reduce catheter colonization and catheter-associated bloodstream infection rates.[113,114] Disruption of catheter dressings poses risk for infection,[103] but the impact of that accidental occurrence may be mitigated by the vigilance that is possible with a high nurse-to-patient ratio. The longer a CVC remains in place, the greater the risk of infection[102]; however, routine line changes based on time since insertion are not recommended.[115] Once the catheter has been inserted, the medical team must make efforts to reduce the number of catheter-days for each patient by reviewing the indications for maintenance of the catheter daily on rounds, with a goal of removing the device as soon as possible.

Chlorhexidine Gluconate Daily Baths

Daily rinses with 2% chlorhexidine gluconate are an effective preventive public health measure in the ICU. The antiseptic solution is applied on each patient from jaw to toes and left on to dry, leaving residual antimicrobial that reduces, for approximately a day, skin colonization with some antibiotic-resistant organisms, including MRSA, VRE, and possibly carbapenemase-producing Enterobacteriaceae.[116–118] Chlorhexidine baths can replace or follow regular showers or baths. Occlusive dressings, the perineum, and the 6 inches of lines, tubes, and drains closest to the skin can all be wiped with 2% chlorhexidine as part of the daily baths in order to minimize skin recontamination.[119] Because of strong (although not unanimous[120]) evidence from clinical trials demonstrating reduced rates of catheter-associated bloodstream infections, chlorhexidine daily baths have become standard of care in ICUs.[102] Few studies have been conducted outside the ICU, and existing studies of chlorhexidine baths in other hospital wards have shown mixed results for catheter-associated bloodstream infections rates, but very rare adverse effects.[121–123]

"Bundled" Catheter-Associated Bloodstream Infection–Prevention Strategies

As with other device-related infections, catheter-associated bloodstream infection–prevention measures, many of which have been tested individually in clinical trials, are often collected into bundles for insertion[110] and maintenance.[124] A systematic review and meta-analysis evaluating the effectiveness of insertion and maintenance bundles to prevent central line–associated bloodstream infections across adult, pediatric, and neonatal ICUs found that implementation resulted in a 60% decrease in such infections.[125,126] Evidence-based preventive measures that are recommended are clearly effective, assuming that they are implemented with sustained effort.

Catheter-Associated Urinary Tract Infections (See Chapter 302)

Catheter-associated urinary tract infections are among the most common health care–associated infections,[127,128] confounding efforts to limit antimicrobial use, prolonging hospital stay, and contributing cumulatively to nosocomial morbidity and mortality.[129] Risk factors for catheter-associated urinary tract infections were established long ago and include duration of catheterization, female sex, critical illness, and older age.[130–132]

Measures to Prevent Catheter-Associated Urinary Tract Infections

Avoiding catheter placement and limiting the duration of each patient's catheterization are key preventive measures because they reduce the days at risk of infection.[130,133] Current guidelines have outlined appropriate indications for placement of indwelling catheters, including acute urinary retention or obstruction, urinary incontinence in the presence of perineal or sacral wounds, precise monitoring of urine output, improved comfort for end-of-life care, and perioperative management of selected surgical procedures (e.g., urologic procedures or surgeries with long duration or need for intraoperative urinary monitoring).[129,134] Daily reevaluation of indications for urinary catheters identifies unnecessary catheter use and significantly reduces catheter use, duration of catheter use, and the rate of catheter-associated urinary tract infections.[135,136] Use of alternatives to indwelling catheters, such as condom catheters and intermittent straight catheterization, also reduces risk of urinary tract infection.[128] Other strategies that prevent catheter-associated urinary tract infections include education, sterile and atraumatic insertion, and maintenance of a sterile, closed drainage system.[128,137]

A national program implementing these measures demonstrated a one-third reduction in catheter-associated urinary tract infection rates outside ICUs, and no change in infection rates in ICUs.[128] One possible reason for the lack of progress is a plateau in catheter use within ICUs.[128] In addition, urine cultures are often reflexively collected during the course of evaluations for fever; positive cultures may meet the CDC criteria for catheter-associated urinary tract infections, but these fevers are often found to have plausible alternative explanations.[138] Treatment of asymptomatic bacteriuria is a major driver of overdiagnosis and overtreatment of catheter-associated urinary tract infections. A reduction in kneejerk culturing of urine combined with evidence-based preventive measures can result in fewer infections and improved patient outcomes.[139]

SURVEILLANCE

Hospital epidemiologists conduct two forms of surveillance for nosocomial infection: passive surveillance and active surveillance. Passive surveillance (known simply as "surveillance") involves collection of existing clinical data in order to track rates of infection or colonization, and to detect unusual infections or clusters that merit further investigation (discussed subsequently). Active microbial surveillance ("active surveillance") is testing specifically to screen patients for colonization with epidemiologically significant organisms.

Active Microbial Surveillance

Identification and isolation of colonized patients are standard measures to reduce the risk of transmission from otherwise silent reservoirs. The knowledge that a patient is colonized with an antibiotic-resistant organism provides an opportunity to interrupt nosocomial spread from that patient by using isolation precautions or, in limited circumstances, decolonization. Health care facilities transferring patients must notify receiving facilities of a patient's colonization status in order to prevent nosocomial spread within the institution accepting the transfer.[78]

Although targeted screening and isolation have been highly effective in some clinical settings,[140–143] various ICU-based strategies have shown no significant reduction in acquisition of resistant organisms in cluster-randomized clinical trials.[144,145] Screening tests have imperfect sensitivity, likely owing to variations in prevalence of the target organism in the screened population, variations in specimen collection, and the limits of detection of molecular and culture-based assays.

Widespread implementation of microbial screening and isolation is highly resource intensive and often does not show clear benefit in single centers that have low endemic rates of resistant organisms.[146] Universal screening for MRSA, implemented in 150 Veterans Affairs hospitals in combination with other measures, reduced MRSA infections by more than 40% among nearly 2 million admissions.[147] Using a centralized infection-control program involving active surveillance and isolation in all health care facilities (including long-term care facilities), the government of Israel, combatting a nationwide nosocomial outbreak of carbapenemase-producing Enterobacteriaceae, achieved sustained declines in transmission.[148] As gram-negative bacterial resistance rises in prevalence with limited treatment options, screening patients on transfer

TABLE 298.2 Sites for Microbial Surveillance to Detect Colonization With Select Multidrug-Resistant Organisms

RESISTANT ORGANISM	HIGHEST-YIELD SITES
Methicillin-resistant *Staphylococcus aureus*	Anterior nares[187]
Vancomycin-resistant *Enterococcus faecium/faecalis*	Rectal/perirectal[188]
Multidrug-resistant *Acinetobacter baumannii*	Groin and throat[189,190]
Multidrug-resistant Enterobacteriaceae	Rectal/perirectal[189]
Candida auris	Groin and axillae[28]

from other health care facilities and those who have been hospitalized in the past 6 months abroad for carbapenem-resistant gram-negative organisms has become a stronger imperative than screening for MRSA, for which there are usually multiple treatment options (Table 298.2).

Decolonization and Skin Antisepsis

Decolonization aims to eliminate carriage of potential pathogens in order to reduce the patient's risk of developing invasive infections and transmitting those pathogens to others. Despite clinical trials of decolonization for a number of nosocomial pathogens, the strongest evidence exists for *S. aureus* decolonization.[149]

Although no-rinse 2% chlorhexidine gluconate daily baths reduce skin colonization with a range of gram-positive and gram-negative nosocomial organisms, the treatments have shown more convincing benefit for preventing gram-positive infections than gram-negative infections. Some, but not all, clinical trials of their use in ICUs have demonstrated reduced rates of MRSA and VRE acquisition.[120,121,150,151] Data from single-center studies suggest a possible effect on infection with and transmission of *A. baumannii*.[152,153]

Management of Nosocomial Transmission of Multidrug-Resistant Organisms

When a nosocomial outbreak is suspected, hospital epidemiologists simultaneously escalate infection-control measures and initiate an investigation. Outbreak-control measures may involve isolating and cohorting infected or exposed patients and encouraging heightened adherence to hand hygiene, disinfection of equipment, enhanced environmental cleaning and disinfection, and other rigorous interventions. The use of dedicated adherence monitors may help ensure appropriate infection-control behaviors among health care staff, patients, and visitors.[154,155]

Outbreak investigations generally involve identifying cases, determining uniformity or clonality of the infecting pathogen, cataloguing detailed exposure histories to identify possible sources of infection or routes of transmission, and conducting surveillance to identify additional patients who are colonized or have subclinical infection. Surveillance should target the highest-risk patients first. Because of low sensitivity of surveillance swabs, variable intervals between exposure and detectable colonization, and the possibility of ongoing transmission, surveillance should be conducted repeatedly on at-risk populations.[156] If colonized or infected patients are identified in multiple wards, signaling a broader at-risk patient population, a surveillance sweep of the entire inpatient population, or large sections thereof, could identify isolated colonized outliers who could serve as reservoirs for new phases of transmission.[155]

Typing of Isolates

Spread of epidemiologically significant pathogens in a health care facility may manifest initially as an increase in clinical infections or an increase in positive results of surveillance tests (cultures or polymerase chain reaction [PCR] assays). Epidemiologic data are used to identify potential sources and routes of transmission, and molecular or genomic testing can determine relatedness of isolates. When clusters are detected via PCR testing, infection-control personnel may need to collect additional specimens for culture, in order to have isolates for comparison.

Older typing methods used for decades in health care epidemiology and public health include bacteriophage typing, ribotyping, and pulsed-field gel electrophoresis. Pulsed-field gel electrophoresis, in which bacterial DNA fragments are separated by size through electrical pulses, and repetitive extragenic palindromic PCR (rep-PCR), which compares the pattern of repetitive DNA sequences, remain in use. The techniques have quickly become outdated with the advent of fast and inexpensive microbial genome sequencing that can compare strains with high resolution to detect minor differences.

Genome sequencing provides information at a level of detail and precision previously unattainable with other typing methods. Hospital epidemiologists can use the unambiguous results of sequencing combined with epidemiologic data to elucidate an outbreak. Sequencing has become rapid and inexpensive, with bioinformatic expertise a limiting factor in its widespread adoption as a typing method.

Surveillance and Public Health Reporting

Infection-control departments conduct surveillance for incident infections, syndromes, and colonization, focusing on the most critical patient populations and the most concerning pathogens.[1] Surveillance results are used to detect clusters and trends that may require intervention, and to track the success of performance improvement initiatives, such as introduction of checklists for indwelling urinary catheters or new environmental cleaning procedures.

US states and territories publish criteria for reporting communicable diseases. Hospitals and laboratories must report these conditions to local health departments for the purposes of public health surveillance. In many states, nosocomial outbreaks must also be reported to public health departments. Highly contagious conditions, such as measles, or clusters of infection, such as legionnaires' disease or Shiga toxin–producing *E. coli,* may trigger urgent public health investigations.

A majority of US states and territories require hospitals to report data on nosocomial infections to the CDC's National Healthcare Safety Network. In 2008, Centers for Medicare and Medicaid Services instituted nonpayment of health care–associated conditions, including infections, as an incentive for hospitals to implement preventive measures; the policy has been associated with an 11% reduction in targeted health care–associated conditions.[157] The specter of nonreimbursement has further complicated the role of the hospital epidemiologist.

OUTBREAK INVESTIGATION

Investigation of outbreaks and exposures to prevent spread of a communicable disease is an integral part of hospital infection control. Outbreaks are detected through surveillance, notification by the microbiology laboratory, and reports from alert clinicians, among other sources. A cluster of infections, or even one case of a high-concern infection (such as chickenpox or nosocomial legionnaires' disease) may prompt an investigation. The goals of investigation are to identify the source of the outbreak and inform strategies for control.

Special Situation: Varicella-Zoster Virus Exposure Investigation

When a diagnosis of primary varicella-zoster virus (VZV) infection (chickenpox) or disseminated zoster is confirmed, the investigation is divided into two parallel lines of inquiry: the patient-related epidemiologic investigation, and the staff-related epidemiologic investigation. Because of the urgency of administering passive immunoglobulin and antiviral prophylaxis to exposed, susceptible, immunocompromised persons, the initial focus of the investigation is on patients.

Management of Exposed Patients

An in-hospital "travel history" is first obtained from the index case. Departmental records from any area in the patient's travels should be examined. Other patients documented to be in an area at the same time as the index patient should be included in the roster of patients at risk. The second component of the patient-related investigation is the rapid identification of exposed patients at high risk for severe complications of primary VZV infection, including pediatric, oncology, and transplant patients who are hospitalized as well as those who are outpatients. Interviewing pediatrics, oncology, and transplant

staff in addition to the staff along the index patient's travel route may yield additional contacts or other crucial information that was not documented.

Once the population at risk has been identified, the immunologic status of all patients on the list is assessed. If a patient is found to be potentially immunosuppressed, the patient, patient's family, and medical staff are questioned regarding the potential exposure and prior history of chickenpox or varicella immunization. Immunosuppressed patients who were potentially exposed and have a negative or an equivocal history of VZV infection have baseline serologies ordered, and prophylactic acyclovir and VariZIG are administered. VariZIG can be given up to 10 days after exposure.[158]

Exposure and VZV histories are obtained from immunocompetent patients after evaluation of immunocompromised patients. Exposed, susceptible immunocompetent patients have serologies drawn and, when possible, are discharged from the hospital. In general, those with negative exposure histories or positive histories of prior VZV infection need no further follow-up. In immunocompetent patients up to 120 hours from exposure, varicella vaccination should be considered as postexposure prophylaxis.

Exposed, susceptible, immunocompetent patients should be placed in strict isolation from 9 days after the first possible exposure until 21 days after the last possible exposure to the index patient. Exposed, susceptible, immunosuppressed patients (even those receiving VariZIG or prophylactic acyclovir) should be placed in strict isolation at the time the patient is identified as being at risk until at least 21 days after the last possible exposure. The incubation period in immunosuppressed patients can be shorter than 10 days, and, based on case reports, prolonged to 28 days in those who receive VariZIG.[159] Although there are no studies of acyclovir prophylaxis in immunocompromised patients, experts recommend that acyclovir be administered to exposed immunosuppressed patients prophylactically from days 3 to 22, or, if the patient has received VariZIG, days 3 to 28.[160–163]

Management of Exposed Personnel

After exposure and VZV histories have been taken from all potentially exposed staff, those who relate negative or equivocal histories of VZV infection should undergo serologic assessment of immunity. There are conflicting data on the reliability of a recalled history of chickenpox among health care personnel.[164,165] Even naturally immune, immunocompetent medical staff can rarely become reinfected with VZV.[166] Immunocompromised employees should be managed with the same sense of urgency that is used for immunosuppressed patients.

Susceptible employees should be immunized within 3 to 5 days of exposure for postexposure prophylaxis; some may develop chickenpox, but the disease may have milder manifestations.[167] Susceptible staff, including those who have received postexposure prophylaxis, should be reassigned to a low-risk area or placed on administrative leave from 8 days after the first possible exposure until 21 days after the last possible exposure,[168,169] although some have advocated a more liberal approach.[170–172] Employees who develop primary VZV infection should not return to work until the last lesion is crusted over (usually 7 or 8 days after the appearance of the last lesion).

OCCUPATIONAL MEDICINE

Hospital epidemiology services work closely with occupational medicine programs to safeguard the health and safety of patients and staff.

Preexposure Screening

Hospital personnel who may have patient contact or work in patient care areas undergo preplacement screening before entry into the workplace, including personal communicable disease and immunization histories and the presence of underlying medical conditions that may place them at higher risk for occupational infection. Clinical personnel should undergo preexposure screening—with serologies in combination with documented vaccine history—for immunity to vaccine-preventable infections, including hepatitis B, measles, mumps, rubella, pertussis, and varicella.[173] Surgeons who are infected with bloodborne pathogens, namely hepatitis B, hepatitis C, or human immunodeficiency virus (HIV), can be managed according to published guidelines that consider providers' individual risk for transmission to patients.[174]

Health care personnel should also undergo preexployment screening for tuberculosis with two-step tuberculin skin testing or, if immunized in the past with bacillus Calmette-Guérin (BCG), an interferon-γ release assay (IGRA) such as T-SPOT.TB or QuantiFERON-TB Gold. The IGRAs do not cross-react with BCG, but they do cross-react with *Mycobacterium marinum, Mycobacterium szulgai,* and *Mycobacterium kansasii*.[175] Discordance between tuberculin skin test and IGRA results is common, and low-positive IGRA results frequently revert to negative when the test is repeated.[176] Health care personnel who have positive test results should be managed according to CDC guidelines.[70] Subsequent testing is part of a collaborative tuberculosis prevention program among the occupational health, hospital biosafety, and hospital epidemiology services. The frequency of testing for personnel in various job categories in a given facility is based on an institutional risk assessment that takes into consideration the hospital size, annual tuberculosis case load, history of tuberculosis transmission, and the prevalence of tuberculosis in the local community.[70] Other components of the tuberculosis-prevention program include medical evaluations for N95 respirator fit testing (mandated by the Occupational Safety and Health Administration), tuberculosis surveillance among patients and employees, and exposure management. When a patient is found to have previously unrecognized pulmonary tuberculosis, occupational health and hospital epidemiology staff launch a contact investigation to identify and gather exposed personnel for repeat testing to detect skin test or IGRA conversions.

Preexposure Immunizations

Preexposure screening and review of vaccine history provide an opportunity for catch-up and booster immunization against hepatitis B, measles, mumps, rubella, and pertussis. VZV-susceptible health care personnel should be strongly encouraged to be immunized with the VZV vaccine. Immunocompromised persons should not receive live vaccines, but some vaccines can be given to health care personnel who have contact with immunocompromised patients. The measles, mumps, and rubella vaccine has no reported secondary transmission from recipients.[177] The Oka strain of varicella-zoster, on the other hand, has rarely spread to contacts of vaccinees who developed postvaccination rashes; health care personnel who develop a rash should avoid patient contact until the rash has crusted or ceased to progress.[178]

Influenza Immunization

Influenza immunization of clinical staff is an important patient safety measure, particularly because many patients cannot mount effective immune responses after vaccination and are at high risk for complications if they develop influenza. National guidelines recommend that staff who have patient contact or work in patient care areas be required to undergo annual influenza immunization.[178,179] Most health care facilities offer clinical staff the inactivated influenza vaccine, although some allow (or offer) immunization with live-attenuated intranasal vaccine. Although no secondary transmission of the vaccine strain from health care personnel to patients has been reported, hospitals serving highly immunocompromised patients may choose to interdict the live-attenuated vaccine on the basis of rare documented cases.[180] Egg allergies, which have previously led to exemption of some health care personnel from immunization with egg-based vaccines, are no longer a barrier with the advent of recombinant influenza vaccines, which are more expensive than egg-based vaccines but are increasingly available.

Preventing Transmission of Respiratory Viruses to Patients

Because the inactivated influenza vaccine is 40% to 80% effective at preventing influenza infections among healthy persons, influenza viruses may still circulate among a fully immunized health care staff. Symptoms of mild influenza may be indistinguishable from those of other community respiratory viruses, such as parainfluenza, respiratory syncytial virus, and adenovirus, which can all cause severe, even life-threatening infection in vulnerable hosts, or from rhinovirus, which rarely causes severe illness. Health care personnel must therefore take personal measures to avoid transmitting their upper respiratory infections to patients, ideally remaining out of the workplace during the peak of illness and for at least 24 hours after a fever (in the absence of antipyretics) to minimize the risk of spread to patients or to other health care personnel. The

work ethic of many health care staff, especially medical trainees, may lead to "presenteeism," or coming to work despite illness; occupational medicine and infection-control staff must educate all personnel that doing so may directly harm patients. Donning a mask during patient care after return to work is a common practice when milder respiratory symptoms remain present. There are no clinical data to support this practice, but one experimental study found that surgical masks reduce exhaled influenza viral copies by more than 70%.[181] In combination with meticulous hand hygiene, surgical masks used while lower levels of virus are shed as the infection resolves may reduce the risk of transmission. Apart from respiratory symptoms, fever, diarrhea, and vomiting should prompt immediate removal from the workplace to avoid spread of communicable diseases.

Universal and Standard Precautions

Universal precautions are a basic set of occupational safety measures designed to prevent transmission of bloodborne pathogens (such as HIV, hepatitis B, and hepatitis C) in the health care workplace. Because patients may have undiagnosed infection with a bloodborne pathogen, all patient interactions and handling of patient specimens should be performed in a manner that prevents the transmission of bloodborne pathogens; these precautions are applied universally, disregarding perception of a patient's level of risk. Standard precautions encompass all aspects of universal precautions but apply to contact with additional body fluids that do not necessarily transmit bloodborne pathogens, such as urine, sputum, spinal fluid, and others. These precautions differ from isolation precautions, which are designed to contain known or suspected communicable diseases.

Postexposure Prophylaxis

Personnel who experience a potential blood or body fluid exposure should wash the wound or area of nonintact skin with soap and water or flush the exposed mucous membrane, and seek prompt evaluation by an occupational medicine specialist.[182] The risk for infection with a bloodborne pathogen following an exposure depends in part on the route of inoculation, inoculum size, exposure severity, and magnitude of viremia.[183] The occupational health provider will assess the route and extent of the exposure, the condition of the source patient (if known), and the employee's own underlying health conditions in making a decision about postexposure prophylaxis.[183]

The literature suggests that the average risk of HIV infection after a percutaneous exposure or mucous membrane exposure to HIV-infected blood is approximately 0.3% and 0.09%, respectively.[183] In contrast, the average risk of acquiring hepatitis C after a sharps or needlestick injury is approximately 1.8%.[184] In a nonimmune worker, serologic evidence of hepatitis B infection following parenteral exposure to blood from a hepatitis B surface antigen–positive and e antigen–positive patient develops in 37% to 62%, and clinical infection in 22% to 31%. The risk of transmission is lower if the source patient is e antigen negative.[184]

Postexposure prophylaxis for hepatitis B is discussed in Chapter 303. Occupational exposures to pathogens for which there is no recommended postexposure prophylaxis, such as hepatitis C, should nonetheless be documented and followed. Institutional surveillance for needlestick injuries and exposures is used to identify opportunities for performance improvement initiatives that can reduce the risk of these events.[182]

Management of Occupational Exposure to Human Immunodeficiency Virus

Occupational exposures that put health care personnel at risk for HIV infection are percutaneous injuries or mucous membrane or nonintact skin contact with blood or other body fluids that potentially contain infectious virus. Injuries that are deep, made by hollow-bore needles, or involve blood or other fluids containing high titers of virus increase risk of transmission.[185] Exposed personnel should be evaluated immediately or within hours of exposure and should be managed according to published guidelines for postexposure prophylaxis (Table 298.3). When the source patient's HIV status is unknown, prophylaxis should be started immediately if there is likely to be a delay in acquiring medical records or laboratory results.

TABLE 298.3 Treatment Regimens (Recommended Duration 28 Days)

RECOMMENDED REGIMEN	ALTERNATIVE REGIMENS
Raltegravir 400 mg twice daily or, if not pregnant,[a] dolutegravir 50 mg once daily *PLUS* Tenofovir 300 mg coformulated with emtricitabine 200 mg once daily	Tenofovir plus emtricitabine or tenofovir plus lamivudine *or* Zidovudine plus lamivudine or zidovudine plus emtricitabine *PLUS one of the following:* Raltegravir or, if not pregnant,[a] dolutegravir *or* Ritonavir-boosted protease inhibitor *or* Rilpivirine or etravirine

[a]Recent reports have raised concerns about neural tube defects potentially associated with use of dolutegravir during pregnancy.[191]
Data from references 183 and 192.

Postexposure Testing and Follow-Up

In addition to baseline HIV testing, serologic testing for a documented occupational HIV exposure is usually performed 6 weeks, 3 months, and 6 months after exposure. If a more sensitive fourth-generation combination antibody-antigen test is used, testing may conclude earlier and is typically performed 6 weeks and 4 months after exposure.[183] CDC guidelines recommend a prolonged HIV follow-up period (e.g., 12 months) for health care personnel who develop hepatitis C virus infection after an exposure to a source who is coinfected with hepatitis C virus and HIV.[183] Close medical follow-up is recommended during postexposure treatment with antiretroviral drugs in order to help manage side effects, encourage adherence, and provide support.[183]

EMERGING INFECTIOUS DISEASES

Hospitals must consider and plan for exceptional infectious diseases events, such as influenza pandemics and the emergence or reemergence of highly contagious diseases. As the 2003–04 severe acute respiratory syndrome epidemic, 2014–15 Ebola epidemic, and 2015 Middle East coronavirus outbreaks demonstrated vividly, the regularity of international travel allows importation of infectious agents to distant regions with secondary spread in health care facilities. Hospitals must be prepared for such possibilities, with procedures and infrastructure poised to contain severe infections that may be highly contagious. Patients infected with emerging pathogens do not typically present to health care with a diagnosis, and the transmission dynamics of the pathogen may be unknown, warranting enhanced infection-control measures for suspected cases. Clinicians can remain vigilant for possible cases, taking careful travel histories and maintaining awareness of current international infectious diseases epidemiology. The lessons of past epidemics have been codified into guidelines and training courses that can inform and steer the process of maintaining preparedness.

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299 Disinfection, Sterilization, and Control of Hospital Waste

William A. Rutala and David J. Weber

Each year in the United States there are approximately 53,000,000 outpatient surgical procedures and 46,000,000 inpatient surgical procedures.[1] For example, there are at least 18 million gastrointestinal endoscopies per year.[2] Each of these procedures involves contact by a medical device or surgical instrument with a patient's sterile tissue and/or mucous membranes. A major risk of all such procedures is the introduction of infection. Failure to properly disinfect or sterilize medical devices and surgical instruments may lead to transmission via these devices (e.g., endoscopes contaminated with carbapenem-resistant Enterobacteriaceae [CRE]).[3]

Achieving disinfection and sterilization through the use of disinfectants and sterilization practices is essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients. Because it is unnecessary to sterilize all patient care items, health care policies must identify whether cleaning, disinfection, or sterilization is indicated, based primarily on the items' intended use.

Multiple studies in many countries have documented lack of compliance with established guidelines for disinfection and sterilization.[4–6] Failure to comply with scientifically based guidelines has led to numerous outbreaks.[4] In this chapter, which is an update of previous chapters,[7–13] a pragmatic approach to the judicious selection and proper use of disinfection and sterilization processes is presented, based on well-designed studies assessing the efficacy (via laboratory investigations) and effectiveness (via clinical studies) of disinfection and sterilization procedures. In addition, we briefly review the management of medical waste in health care facilities.

DEFINITION OF TERMS

Sterilization is defined as the complete elimination or destruction of all forms of microbial life and is accomplished in health care facilities through either physical or chemical processes. Steam under pressure, dry heat, ethylene oxide (ETO) gas, hydrogen peroxide gas plasma, vaporized hydrogen peroxide, hydrogen peroxide with ozone, and liquid chemicals are the principal sterilizing agents used in health care facilities. Sterilization is intended to convey an absolute meaning, not a relative one. Unfortunately, some health professionals and the technical and commercial literature refer to "disinfection" as "sterilization" and items as "partially sterile." When chemicals are used for the purposes of destroying all forms of microbiologic life, including fungal and bacterial spores, they may be called chemical sterilants. These same germicides used for shorter exposure periods may also be part of the disinfection process (i.e., high-level disinfection).

Disinfection describes a process that eliminates many or all pathogenic microorganisms on inanimate objects, with the exception of bacterial spores. Disinfection is usually accomplished with the use of liquid chemicals or wet pasteurization in health care settings. The efficacy of disinfection is affected by a number of factors, each of which may nullify or limit the efficacy of the process. Some of the factors that affect both disinfection and sterilization efficacy are the prior cleaning of the object; the organic and inorganic load present; the type and level of microbial contamination; the concentration of and time of exposure to the germicide; the nature of the object (e.g., crevices, hinges, and lumens); the presence of biofilms; the temperature and pH during the disinfection process; and in some cases the relative humidity of the sterilization process (e.g., ETO).

By definition, then, disinfection differs from sterilization by its lack of sporicidal property, but this is an oversimplification. A few disinfectants will kill spores with prolonged exposure times (e.g., 3–12 hours) and are called *chemical sterilants.* At similar concentrations but with shorter exposure periods (e.g., 12 minutes for 0.55% ortho-phthalaldehyde [OPA]) these same disinfectants will kill all microorganisms with the exception of large numbers of bacterial spores and are called *high-level disinfectants*. *Low-level disinfectants* may kill most vegetative bacteria, some fungi, and some viruses in a practical period of time (<10 minutes), whereas *intermediate-level disinfectants* may be cidal for mycobacteria, vegetative bacteria, most viruses, and most fungi but do not necessarily kill bacterial spores. The germicides differ markedly among themselves primarily in their antimicrobial spectrum and rapidity of action. Table 299.1 will be discussed later and consulted in this context.

Cleaning, on the other hand, is the removal of visible soil (e.g., organic and inorganic material) and microbial contaminants from objects and surfaces, and it normally is accomplished by manual or mechanical means using water with detergents or enzymatic products. Thorough cleaning is essential before high-level disinfection and sterilization because inorganic and organic materials that remain on the surfaces of instruments interfere with the effectiveness of these processes. Also, if the soiled materials become dried or baked onto the instruments, the removal process becomes more difficult and the disinfection or sterilization process less effective or ineffective. Surgical instruments should be pretreated or rinsed to prevent drying of blood and to soften or remove blood from the instruments immediately or as soon as feasible after use. Treating contaminated instruments with alcohol, allowing instruments to soak in water for prolonged periods, or drying increases cleaning difficulty and should be discouraged.[14] *Decontamination* is a procedure that removes pathogenic microorganisms from objects so they are safe to handle, use, or discard.

Terms with a suffix "cide" or "cidal" for killing action also are commonly used. For example, a germicide is an agent that can kill microorganisms, particularly pathogenic organisms ("germs"). The term *germicide* includes both antiseptics and disinfectants. *Antiseptics* are germicides applied to living tissue and skin, whereas *disinfectants* are antimicrobials applied only to inanimate objects. Preservatives are agents that inhibit the growth of microorganisms capable of causing biologic deterioration of substances and materials. In general, antiseptics are used only on the skin and not for surface disinfection, and disinfectants are rarely used for skin antisepsis because they may cause injury to skin and other tissues. Other words with the suffix "cide" (e.g., virucide, fungicide, bactericide, sporicide, and tuberculocide) can kill the type of microorganism identified by the prefix. For example, a bactericide is an agent that kills bacteria.[15–18]

RATIONAL APPROACH TO DISINFECTION AND STERILIZATION

About 50 years ago, Earle H. Spaulding[16] devised a rational approach to disinfection and sterilization of patient care items or equipment. This classification scheme is so clear and logical that it has been retained, refined, and successfully used by infection-control professionals and others when planning methods for disinfection or sterilization.[8–13,15–17] Spaulding believed that the nature of disinfection could be understood more readily if instruments and items for patient care were divided

TABLE 299.1 Methods for Disinfection and Sterilization of Patient Care Items and Environmental Surfaces

PROCESS	LEVEL OF MICROBIAL INACTIVATION	METHOD	EXAMPLES (WITH PROCESSING TIMES)	HEALTH CARE APPLICATION (EXAMPLES)
Sterilization[a]	Destroys all microorganisms, including bacterial spores	High temperature Low temperature Liquid immersion	Steam (approximately 40 min), dry heat (1–6 h depending on temperature) Ethylene oxide gas (approximately 15 h), hydrogen peroxide gas plasma (24–60 min, 100 NX), hydrogen peroxide and ozone (46–60 min, VP4), hydrogen peroxide vapor (28–35 min, V-Pro MAX) Chemical sterilants[b]: >2% glut (approximately 10 h at 20°C–25°C); 1.12% glut with 1.93% phenol (12 h at 25°C); 7.35% HP with 0.23% PA (3 h at 20°C); 7.5% HP (6 h at 20°C); 1.0% HP with 0.08% PA (8 h at 20°C); approximately 0.2% PA (12 min at 50°C–56°C)	Heat-tolerant critical (surgical instruments) and semicritical patient care items Heat-sensitive critical and semicritical patient care items Heat-sensitive critical and semicritical patient care items that can be immersed
High-level disinfection	Destroys all microorganisms except some bacterial spores	Heat automated Liquid immersion	Pasteurization (65°C–77°C, 30 min) Chemical sterilants or high-level disinfectants[b]: >2% glut (20–90 min at 20°C–25°C); >2% glut (5 min at 35°C–37.8°C); 0.55% OPA (12 min at 20°C); 1.12% glut with 1.93% phenol (20 min at 25°C); 7.35% HP with 0.23% PA (15 min at 20°C); 7.5% HP (30 min at 20°C); 1.0% HP with 0.08% PA (25 min at 20°C); 650–675 free chlorine (10 min at 30°C); 2.0% HP (8 min at 20°C); 3.4% glut with 20.1% isopropanol (5 min at 25°C)	Heat-sensitive semicritical items (e.g., respiratory therapy equipment) Heat-sensitive semicritical items (e.g., GI endoscopes, bronchoscopes, endocavitary probes)
Low-level disinfection	Destroys vegetative bacteria, some fungi and viruses, but not mycobacteria or spores	Liquid contact	EPA-registered hospital disinfectant with no tuberculocidal claim (e.g., chlorine-based products, phenolics, improved hydrogen peroxide, hydrogen peroxide plus peracetic acid, quaternary ammonium compounds ["quats"], quats plus alcohol—exposure times approximately 1 min) or 70–90% alcohol	Noncritical patient care item (blood pressure cuff) or surface (bedside table) with no visible blood

[a]Prions (as in Creutzfeldt-Jakob disease) exhibit an unusual resistance to conventional chemical and physical decontamination methods and are not readily inactivated through conventional sterilization procedures.[238,239]

[b]Consult the FDA cleared package insert for information about the cleared contact time and temperature, and see references 8, 25, and 31 for discussion of why >2% glutaraldehyde products are used at a reduced exposure time (2% glutaraldehyde at 20 min, 20°C). Increasing the temperature through use of an automated endoscope reprocess (AER) will reduce the contact time (e.g., OPA 12 min at 20°C but 5 min at 25°C in AER). Exposure temperatures for some high-level disinfectants listed in the table varies from 20°C to 25°C; check FDA-cleared temperature conditions.[28] Tubing must be completely filled for high-level disinfection and liquid chemical sterilization. Material compatibility should be investigated when appropriate (e.g., HP and HP with PA may cause functional damage to endoscopes). Intermediate-level disinfectants destroy vegetative bacteria, mycobacteria, most viruses, and most fungi but not spores and may include chlorine-based products, phenolics, and improved hydrogen peroxide). Intermediate-level disinfectants are not included in Table 299.1 because there is no device or surface for which intermediate-level disinfection is specifically recommended over low-level disinfection.

EPA, US Environmental Protection Agency; *FDA,* US Food and Drug Administration; *GI,* gastrointestinal; *glut,* glutaraldehyde; *HP,* hydrogen peroxide; *OPA,* ortho-phthalaldehyde; *PA,* peracetic acid; *ppm,* parts per million.

Modified from Rutala and Weber,[7] Rutala and Weber,[8] Rutala and Weber,[9] Rutala and Weber,[10] Rutala and Weber,[11] Rutala and Weber,[333] and Kohn et al.[334]

into three categories based on the degree of risk of infection involved in the use of the items. Although the scheme remains valid, there are some examples of disinfection studies with viruses, mycobacteria, and protozoa and of disinfectants that challenge the current definitions and expectations of high- and low-level disinfection.[19] The three categories Spaulding described were critical, semicritical, and noncritical.

Critical Items

Critical items are so called because of the high risk of infection if such an item is contaminated with any microorganism, including bacterial spores. It is critical that objects that enter sterile tissue or the vascular system be sterile because any microbial contamination could result in disease transmission. This category includes surgical instruments, cardiac and urinary catheters, implants, arthroscopes, laparoscopes, and ultrasound probes used in sterile body cavities. Most of the items in this category should be purchased as sterile or be sterilized with steam sterilization if possible. If heat sensitive, the object may be treated with ETO, hydrogen peroxide gas plasma, vaporized hydrogen peroxide vapor, hydrogen peroxide vapor plus ozone, or liquid chemical sterilants if other methods are unsuitable. Tables 299.1, 299.2, and 299.3 summarize sterilization processes and liquid chemical sterilants and the advantages and disadvantages of each. Sterilization technologies can be relied on to produce sterility only if cleaning—to eliminate organic and inorganic material and microbial load—precedes treatment.[20–22] Other issues that sterile reprocessing and operating room professionals must deal with when reprocessing instruments include weight limits for instrument trays, wet packs, packaging, loaned instruments, cleaning monitoring, and water quality.[22,23]

Semicritical Items

Semicritical items are those that come in contact with intact mucous membranes or nonintact skin. Respiratory therapy and anesthesia equipment, some endoscopes, laryngoscope blades and handles,[24,25] esophageal manometry probes, endocavitary probes, nasopharyngoscopes, prostate biopsy probes,[26] infrared coagulation devices,[27] anorectal manometry catheters, cystoscopes, and diaphragm fitting rings are included in this category.[24] These medical devices should be free of all microorganisms, although small numbers of bacterial spores may be present. Intact mucous membranes, such as those of the lungs or the gastrointestinal tract, generally are resistant to infection by common bacterial spores but susceptible to other organisms such as bacteria, mycobacteria, and viruses. Semicritical items minimally require high-level disinfection with chemical disinfectants. Glutaraldehyde, hydrogen peroxide, OPA, peracetic acid, hypochorite (via superoxidized water) and peracetic acid with hydrogen peroxide are cleared by the Food and Drug Administration (FDA)[28] and are dependable high-level disinfectants, provided that the factors influencing germicidal procedures are met (see Tables 299.1 and 299.2). When a disinfectant is selected for use with certain patient care items, the chemical compatibility after extended use with the items to be disinfected also must be considered.

TABLE 299.2 Summary of Advantages and Disadvantages of Chemical Agents Used as Chemical Sterilants or as High-Level Disinfectants

STERILIZATION METHOD	ADVANTAGES	DISADVANTAGES
Peracetic acid and hydrogen peroxide	No activation required	Material compatibility concerns (lead, brass, copper, zinc), both cosmetic and functional Limited clinical experience Mucous membrane and respiratory health effects[165] Potential for eye and skin damage
Glutaraldehyde	Numerous use studies published Relatively inexpensive Excellent material compatibility	Respiratory irritation from glutaraldehyde vapor Pungent and irritating odor Relatively slow mycobactericidal activity (unless other disinfectants added, such as phenolic, alcohol) Coagulates blood and fixes tissue to surfaces Allergic contact dermatitis
Hydrogen peroxide (standard)	No activation required May enhance removal of organic matter and organisms No disposal issues No odor or irritation issues Does not coagulate blood or fix tissues to surfaces Inactivates *Cryptosporidium* at 6%–7.5% Use studies published	Material compatibility concerns (brass, zinc, copper, and nickel/silver plating), both cosmetic and functional Serious eye damage with contact
Ortho-phthalaldehyde (OPA)	Fast acting high-level disinfectant No activation required Odor not significant Excellent materials compatibility claimed Does not coagulate blood or fix tissues to surfaces claimed	Stains protein gray (e.g., skin, mucous membranes, clothing, and environmental surfaces) More expensive than glutaraldehyde Eye irritation with contact Slow sporicidal activity Anaphylactic reactions to OPA in bladder cancer patients with repeated exposure to OPA through cystoscopy
Peracetic acid	Standardized cycle (e.g., liquid chemical sterilant processing system using peracetic acid, rinsed with extensively treated potable water) Low temperature (50°C–55°C) liquid immersion sterilization Environmental friendly by-products (acetic acid, O_2, H_2O) Fully automated Single-use system eliminates need for concentration testing May enhance removal of organic material and endotoxin No adverse health effects to operators under normal operating conditions Compatible with many materials and instruments Does not coagulate blood or fix tissues to surfaces Sterilant flows through scope, facilitating salt, protein, and microbe removal Rapidly sporicidal Provides procedure standardization (constant dilution, perfusion of channel, temperatures, exposure)	Potential material incompatibility (e.g., aluminum anodized coating becomes dull) Used for immersible instruments only Biologic indicator may not be suitable for routine monitoring One scope or a small number of instruments can be processed in a cycle More expensive (endoscope repairs, operating costs, purchase costs) than high-level disinfection Serious eye and skin damage (concentrated solution) with contact Point-of-use system, no sterile storage An AER using 0.2% peracetic acid has not been cleared by FDA as sterilization process but for high-level disinfection
Improved hydrogen peroxide (2.0%); high-level disinfectant	No activation required No odor Nonstaining No special venting requirements Manual or automated applications 12-mo shelf life, 14-day reuse 8 min at 20°C high-level disinfectant claim	Material compatibility concerns owing to limited clinical experience Organic material resistance concerns owing to limited data

[a]All products are effective in presence of organic soil, are relatively easy to use, and have a broad spectrum of antimicrobial activity (bacteria, fungi, viruses, bacterial spores, and mycobacteria). The listed characteristics are documented in the literature; contact the manufacturer of the instrument and high-level disinfectant or chemical sterilant for additional information. All products listed here are FDA cleared as chemical sterilants except OPA, which is an FDA-cleared high-level disinfectant.
AER, Automated endoscope reprocessor; *FDA,* US Food and Drug Administration.
Modified from Rutala and Weber,[7] Rutala and Weber,[8] Rutala and Weber,[9] Rutala and Weber,[10] Rutala and Weber,[11] Rutala and Weber.[333]

The complete elimination of all microorganisms in or on an instrument with the exception of small numbers of bacterial spores is the traditional definition of high-level disinfection. The FDA's definition of high-level disinfection is a sterilant used for a shorter contact time to achieve at least a 6-$\log_{10}$ kill of an appropriate *Mycobacterium* species. Cleaning followed by high-level disinfection should eliminate all pathogens capable of causing infection.

Ideally, semicritical items should be rinsed with sterile water after high-level disinfection to prevent their contamination with organisms that may be present in tap water, such as nontuberculous mycobacteria, *Legionella,* or gram-negative bacilli such as *Pseudomonas.*[29] In circumstances in which rinsing with sterile water rinse is not feasible, a tap water or filtered water (0.2-μ filter) rinse should be followed by an alcohol rinse and forced-air drying.[8,30,31] Forced-air drying markedly reduces bacterial contamination of stored endoscopes, most likely by removing the wet environment favorable for bacterial growth.[30] After rinsing, items should be dried and stored (e.g., packaged or hung) in a manner that protects them from recontamination.

Some items that may come in contact with nonintact skin for a brief period of time (i.e., hydrotherapy tanks, ultrasound probes on intact skin [includes central line puncture site]) are usually considered noncritical surfaces and are disinfected with low or intermediate-level disinfectants.[8,32] Because hydrotherapy tanks have been associated with spread of infection, some facilities have chosen to disinfect them with recommended levels of chlorine.[8,32]

Noncritical Items

Noncritical items are those that come in contact with intact skin but not mucous membranes. Intact skin acts as an effective barrier to most microorganisms; therefore the sterility of items coming in contact with intact skin is "not critical." Examples of noncritical items are bedpans, blood pressure cuffs, crutches, bed rails, bedside tables, patient furniture,

TABLE 299.3 Summary of Advantages and Disadvantages of Commonly Used Sterilization Methods

STERILIZATION METHOD	ADVANTAGES	DISADVANTAGES
Steam	Nontoxic to patient, staff, environment Cycle easy to control and monitor Rapidly microbicidal Least affected by organic and inorganic soils among sterilization processes listed Rapid cycle time Penetrates medical packing, device lumens	Deleterious for heat-sensitive instruments Microsurgical instruments damaged by repeated exposure May leave instruments wet, causing them to rust Potential for burns
Hydrogen peroxide gas plasma	Safe for the environment and health care personnel Leaves no toxic residuals Cycle time is 24–60 min, and no aeration necessary Used for heat- and moisture-sensitive items; process temperature <50°C Simple to operate, install (208-V outlet), and monitor Compatible with most medical devices Requires only electrical outlet	Cellulose (paper), linens, and liquids cannot be processed Endoscope or medical device restrictions based on lumen internal diameter and length (see manufacturer's recommendations) Requires synthetic packaging (polypropylene wraps, polyolefin pouches) and special container tray Hydrogen peroxide may be toxic at levels greater than 1 ppm TWA
100% Ethylene oxide (ETO)	Penetrates packaging materials, device lumens Single-dose cartridge and negative-pressure chamber minimize the potential for gas leak and ETO exposure Simple to operate and monitor Compatible with most medical materials	Requires aeration time to remove ETO residue ETO is toxic, a probable carcinogen, and flammable ETO emission regulated by states, but catalytic cell removes 99.9% of ETO and converts it to CO_2 and H_2O ETO cartridges should be stored in flammable-liquid storage cabinet Lengthy cycle and aeration time
Vaporized hydrogen peroxide	Safe for the environment and health care personnel It leaves no toxic residue; no aeration necessary Cycle time, 28–35 min Used for heat- and moisture-sensitive items (metal and nonmetal devices)	Medical device restrictions based on lumen internal diameter and length; see manufacturer's recommendations (e.g., stainless steel lumen 1 mm in diameter, 125 mm long) Not used for liquid, linens, powders, or any cellulose materials Requires synthetic packaging (polypropylene) Limited materials compatibility data Limited clinical use and comparative microbicidal efficacy data
Hydrogen peroxide and ozone	Safe for the environment and health care personnel Uses dual sterilants, hydrogen peroxide and ozone No aeration needed owing to absence of toxic by-products Compatible with common medical devices Cycle time, 46–60 min FDA cleared for general instruments and multichannel flexible endoscopes (see manufacturer's instructions)	Endoscope or medical device restrictions based on lumen internal diameter and length (see manufacturer's recommendations) Limited clinical use (no published data on material compatibility, penetrability, organic material resistance) and limited microbicidal efficacy data Requires synthetic packaging (polypropylene wraps, polyolefin pouches) and special container tray

FDA, US Food and Drug Administration; *TWA,* time-weighted average.
Modified from Rutala and Weber,[7] Rutala and Weber,[8] Rutala and Weber,[9] Rutala and Weber,[10] Rutala and Weber,[11] Rutala and Weber.[333]

toys,[33] portable equipment (e.g., wheelchairs, infusion pumps, pulse oximeters, medication carts),[34,35] and floors.[36,37] The five most commonly touched noncritical items in the patient environment have been quantitatively shown to be bed rails, bed surface, supply cart, overbed table, and intravenous pump.[38] In contrast to critical and some semicritical items, most noncritical reusable items may be decontaminated where they are used and do not need to be transported to a central processing area. There is virtually no documented risk of transmitting infectious agents to patients via noncritical items[39] when they are used as noncritical items and do not contact nonintact skin and/or mucous membranes. However, these items (e.g., bedside tables, bed rails) could potentially contribute to secondary transmission by contaminating hands of health care providers or by contact with medical equipment that will subsequently come in contact with patients.[40] Table 299.4 lists several low-level disinfectants that may be used for noncritical items. Many Environmental Protection Agency (EPA)–registered liquid disinfectants have a 10-minute label claim. However, multiple investigators have demonstrated the effectiveness of these disinfectants against vegetative bacteria (e.g., *Listeria, Escherichia coli, Salmonella,* vancomycin-resistant enterococci [VRE], methicillin-resistant *Staphylococcus aureus* [MRSA]), yeasts (e.g., *Candida*), mycobacteria (e.g., *Mycobacterium tuberculosis*), and viruses (e.g., poliovirus) at exposure times of 30 to 60 seconds.[8,41–45] Accordingly, it is acceptable to disinfect noncritical medical equipment (e.g., blood pressure cuff) and noncritical surfaces (e.g., bedside table) with an EPA-registered disinfectant or disinfectant-detergent at the proper use-dilution and a contact time of approximately 1 minute.[8,46,47] Because the typical drying time for a liquid disinfectant on a surface is 1 to 2 minutes (unless the product contains alcohol [e.g., a 60%–70% alcohol product will dry in about 30 seconds]), one application of the germicide on all hand contact or touchable surfaces to be disinfected is recommended.

Mops (microfiber and cotton-string), reusable cleaning cloths, disposable wipes, and sprays are regularly used to achieve low-level disinfection.[48] Disinfectant cleaning wipes and sprays (e.g., quaternary ammonium compounds ["quats"] and alcohol, chlorine) have been found to be highly effective (>4-$\log_{10}$ reduction) in removing or inactivating epidemiologically important pathogens.[49,50] Hospital laundering practices may not be sufficient to remove microbial contaminants of reusable cleaning towels.[51,52] Microfiber mops have demonstrated superior microbial removal compared with cotton string mops when used with detergent cleaner (95% vs. 68%, respectively). Use of a disinfectant did significantly improve microbial removal when a cotton string mop was used when compared with the detergent cleaner (95% vs. 68%, respectively).[53] Mops (especially cotton-string mops) are commonly not kept adequately cleaned and disinfected, and if the water-disinfectant mixture is not changed regularly (e.g., after every three to four rooms, no longer than 60-minute intervals), the mopping procedure may actually spread heavy microbial contamination throughout the health care facility.[54] In one study, standard laundering provided acceptable decontamination of heavily contaminated mopheads, but chemical disinfection with a phenolic was less effective.[54] The frequent laundering of cotton-string mops (e.g., daily) is therefore recommended.

Hospital cleanliness continues to attract patient attention, and in the United States it is still primarily assessed via visual appearance, which is not a reliable indicator of surface cleanliness.[55] Three other methods have been offered for monitoring patient room hygiene: adenosine triphosphate (ATP) bioluminescence[56,57]; fluorescent markers[58–60]; and microbiologic sampling. Studies have demonstrated suboptimal cleaning through use of aerobic colony counts, ATP bioluminescence, and fluorescent markers.[56,60] ATP bioluminescence and fluorescent markers are preferred to aerobic plate counts because they provide an immediate assessment of cleaning effectiveness. When the four major

TABLE 299.4 Summary of Advantages and Disadvantages of Disinfectants Used as Low-Level Disinfectants

DISINFECTANT ACTIVE	ADVANTAGES	DISADVANTAGES
Alcohol	Bactericidal, tuberculocidal, fungicidal, virucidal Fast acting Noncorrosive Nonstaining Used to disinfect small surfaces such as rubber stoppers on medication vials No toxic residue	Not sporicidal Affected by organic matter Slow acting against nonenveloped viruses (e.g., norovirus) No detergent or cleaning properties Not EPA registered Damages some instruments (e.g., hardens rubber, deteriorates glue) Flammable (large amounts require special storage) Evaporates rapidly, making contact-time compliance difficult Not recommended for use on large surfaces Outbreaks ascribed to contaminated alcohol[134]
Sodium hypochlorite	Bactericidal, tuberculocidal, fungicidal, virucidal Sporicidal Fast acting Inexpensive (in dilutable form) Not flammable Unaffected by water hardness Reduces biofilms on surfaces Relatively stable (e.g., 50% reduction in chlorine concentration in 30 days)[79] Used as the disinfectant in water treatment EPA registered	Reaction hazard with acids and ammonias Leaves salt residue Corrosive to metals (some ready-to-use products may be formulated with corrosion inhibitors) Unstable active (some ready-to-use products may be formulated with stabilizers to achieve longer shelf life) Affected by organic matter Discolors or stains fabrics Potential hazard is production of trihalomethane Odor (some ready-to-use products may be formulated with odor inhibitors); irritating at high concentrations
Improved hydrogen peroxide	Bactericidal, tuberculocidal, fungicidal, virucidal Fast efficacy Easy compliance with wet-contact times Safe for workers (lowest EPA toxicity category, IV) Benign for the environment Surface compatible Nonstaining EPA registered Not flammable	More expensive than most other disinfecting actives Not sporicidal at low concentrations
Iodophors	Bactericidal, mycobactericidal, virucidal Not flammable Used for disinfecting blood culture bottles	Not sporicidal Shown to degrade silicone catheters Requires prolonged contact to kill fungi Stains surfaces Used mainly as an antiseptic rather than disinfectant
Phenolics	Bactericidal, tuberculocidal, fungicidal, virucidal Inexpensive (in dilutable form) Nonstaining Not flammable EPA registered	Not sporicidal Absorbed by porous materials and can irritate tissue Depigmentation of skin caused by certain phenolics Hyperbilirubinemia in infants when phenolic not prepared as recommended
Quaternary ammonium compounds[a] (e.g., didecyl dimethyl ammonium bromide, dioctyl dimethyl ammonium bromide)	Bactericidal, fungicidal, virucidal against enveloped viruses (e.g., HIV) Good cleaning agents EPA registered Surface compatible Persistent antimicrobial activity when undisturbed Inexpensive (in dilutable form)	Not sporicidal In general, not tuberculocidal and not virucidal against nonenveloped viruses High water hardness and cotton or gauze can make less microbicidal A few reports documented asthma as result of exposure to benzalkonium chloride Affected by organic matter Absorption by cotton, some wipes Multiple outbreaks ascribed to contaminated benzalkonium chloride[134]
Peracetic acid and hydrogen peroxide	Bactericidal, fungicidal, virucidal and sporicidal (e.g., *Clostridioides difficile* [formerly *Clostridium difficile*]) Active in the presence of organic material Environmental friendly by-products (acetic acid, O_2, H_2O) EPA registered Surface compatible	Lack of stability Potential for material incompatibility (e.g., brass, copper) More expensive than most other disinfecting actives Odor may be irritating Can cause mucous membrane and respiratory health effects

[a]If low-level disinfectant is prepared on-site (not ready-to-use product), document correct concentration at a routine frequency.
EPA, Environmental Protection Agency; *HIV,* human immunodeficiency virus.
Modified from Rutala and Weber,[9] *Rutala and Weber.*[46]

hospital cleaning validation methods (i.e., visual, microbiologic, ATP, and fluorescence) were compared, the fluorescent marker was the most useful tool in determining how thoroughly a surface was cleaned, and mimicked the microbiologic data better than ATP (<500 relative light units). There was no statistical correlation between ATP levels and standard aerobic plate counts.[61]

DISINFECTION OF HEALTH CARE EQUIPMENT AND SURFACES

Disinfectants are used alone or in combinations in the health care setting. These include alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, OPA, standard and improved (or accelerated) hydrogen peroxide, hydrogen peroxide plus peracetic acid, iodophors, peracetic acid, phenolics, quats, and quats and alcohol. Key considerations for selecting the optimal disinfectant include kill claims; treatment time or contact time; safety; and ease of use.[46] With some exceptions (e.g., ethanol or bleach), commercial formulations based on these chemicals are considered unique products and must be registered with the EPA or cleared by the FDA. In most instances, a given product is designed for a specific purpose and is to be used in a certain manner. Therefore the label should be read carefully to ensure that the right product is selected for the intended use and applied in an efficient manner. In addition, caution must be exercised to avoid hazards with use of cleaners and disinfectants on electronic medical equipment. Problems associated

with inappropriate use of liquids on electronic medical equipment have included equipment fires, equipment malfunctions, and health care worker burns.

Disinfectants are not interchangeable, and an overview of the performance characteristics of each is provided in the following sections so the user has sufficient information to select an appropriate disinfectant for any item and use it in the most efficient way. It should be recognized that excessive costs and infection risks may be attributed to incorrect concentrations and inappropriate disinfectants.[62] Finally, occupational diseases among cleaning personnel have been associated with the use of several disinfectants such as formaldehyde, glutaraldehyde, and chlorine, and precautions (e.g., gloves, proper ventilation) should be used to minimize exposure.[63,64] Asthma and reactive airway disease may occur in sensitized individuals exposed to any airborne chemical including germicides. Clinically important asthma may occur at levels below ceiling levels regulated by the Occupational Health and Safety Administration (OSHA) or recommended by the National Institute for Occupational Safety and Health (NIOSH). The preferred method of control is to eliminate the chemical (by means of engineering controls or substitution) or relocate the worker.

Chemical Disinfectants

Alcohol

In the health care setting, "alcohol" refers to two water-soluble chemical compounds whose germicidal characteristics are generally underrated: ethyl alcohol and isopropyl alcohol.[65] These alcohols are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria; they also are tuberculocidal, fungicidal, and virucidal but do not destroy bacterial spores. Their cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is in the range of 60% to 90% solutions in water (volume/volume).[66,67]

Alcohols are not recommended for sterilizing medical and surgical materials, principally because of their lack of sporicidal action and their inability to penetrate protein-rich materials. Fatal postoperative wound infections with *Clostridium* have occurred when alcohols were used to sterilize surgical instruments contaminated with bacterial spores.[68] Alcohols have been used effectively to disinfect oral and rectal thermometers, computers,[69] hospital pagers, scissors, cardiopulmonary resuscitation (CPR) manikins, applanation tonometers,[70] external surfaces of equipment (e.g., ventilators), and stethoscopes.[8] Alcohol towelettes have been used for years to disinfect small surfaces such as rubber stoppers of multiple-dose medication vials or vaccine bottles.

Alcohols are flammable and consequently must be stored in a cool, well-ventilated area. Large volumes of alcohol products need to be stored in rooms meeting special fire department regulations. They also evaporate rapidly, and this makes extended exposure time difficult to achieve unless the items are immersed.

Chlorine and Chlorine Compounds

Hypochlorites are the most widely used of the chlorine disinfectants and are available in a liquid (e.g., sodium hypochlorite) or solid (e.g., calcium hypochlorite) form. The most prevalent chlorine products in the United States are aqueous solutions of 5.25% to 6.15% sodium hypochlorite, which usually are called household bleach. They have a broad spectrum of antimicrobial activity (i.e., bactericidal, virucidal, fungicidal, mycobactericidal, sporicidal), do not leave toxic residues, are unaffected by water hardness, are inexpensive and fast acting,[70,71] remove dried or fixed organisms and biofilms from surfaces,[72] and have a low incidence of serious toxicity.[73,74] Sodium hypochlorite at the concentration used in domestic bleach (5.25%–6.15%) may produce ocular irritation or oropharyngeal, esophageal, and gastric burns,[63,75,76] but the frequency is rare.[77] Other disadvantages of hypochlorites include corrosiveness to metals at high concentrations (>500 ppm), inactivation by organic matter, discoloring or "bleaching" of fabrics, release of toxic chlorine gas when mixed with ammonia or acid (e.g., household cleaning agents),[78] and relative stability.[79]

Reports have examined the microbicidal activity of a new disinfectant, "superoxidized water." The concept of electrolyzing saline to create a disinfectant or antiseptics is appealing because the basic materials of saline and electricity are inexpensive and the end product (i.e., water) is not damaging to the environment. The main products of this water are hypochlorous acid (e.g., at a concentration of about 144 mg/L) and chlorine. This is also known as electrolyzed water, and as with any germicide, the antimicrobial activity of superoxidized water is strongly affected by the concentration of the active ingredient (available free chlorine).[80] The free available chlorine concentrations of different superoxidized solutions reported in the literature range from 7 to 180 ppm.[80] Data have shown that freshly generated superoxidized water is rapidly effective (<2 minutes) in achieving a 5-$\log_{10}$ reduction of pathogenic microorganisms (i.e., *M. tuberculosis, Mycobacterium chelonae,* poliovirus, human immunodeficiency virus [HIV], MRSA, *E. coli, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa*) in the absence of organic loading. However, the biocidal activity of this disinfectant was substantially reduced in the presence of organic material (5% horse serum).[81,82]

Hypochlorites are widely used in health care facilities in a variety of settings.[71] Inorganic chlorine solution is used for disinfecting tonometer heads[83] and for disinfection of noncritical surfaces and equipment. A 1:10 to 1:100 dilution of 5.25% to 6.15% sodium hypochlorite (i.e., household bleach)[8] or an EPA-registered tuberculocidal disinfectant[8] has been recommended for decontaminating blood spills. For small spills of blood (i.e., drops of blood) on noncritical surfaces, the area can be disinfected with a 1:100 dilution of 5.25% to 6.15% sodium hypochlorite or an EPA-registered tuberculocidal disinfectant. Because hypochlorites and other germicides are substantially inactivated in the presence of blood,[84,85] large spills of blood require that the surface be cleaned before an EPA-registered disinfectant or a 1:10 (final concentration) solution of household bleach is applied. If there is a possibility of a sharps injury, there should be an initial decontamination, followed by cleaning and terminal disinfection (1:10 final concentration).[85] Extreme care should always be employed to prevent percutaneous injury. At least 500 ppm available chlorine for 10 minutes is recommended for decontamination of CPR training manikins.[8] Other uses in health care include as an irrigating agent in endodontic treatment and for disinfecting laundry, dental appliances, hydrotherapy tanks,[32] regulated medical waste before disposal,[71] applanation tonometers,[70] and the water distribution system in hemodialysis centers and hemodialysis machines.[8] Disinfection with a 1:10 dilution of concentrated sodium hypochlorite (i.e., bleach) has been shown to be effective in reducing environmental contamination in patient rooms and in reducing *Clostridioides difficile* (formerly *Clostridium difficile*) infection rates in hospital units where there is a high endemic *C. difficile* infection rate or in an outbreak setting.[86] At the University of North Carolina (UNC) Hospitals, we use a sporicidal solution (1:10 dilution of household bleach or approximately 5000 ppm chlorine) in all *C. difficile*–infected patient rooms for routine daily and terminal cleaning. This is done by means of one application of the sporicide, covering all hand contact surfaces to allow sufficient wetness for an approximately 1-minute contact time.

Chlorine has long been favored as the preferred disinfectant in water treatment. Hyperchlorination of a *Legionella*-contaminated hospital water system[32] resulted in a dramatic decrease (30% to 1.5%) in the isolation of *Legionella pneumophila* from water outlets and a cessation of health care–associated legionnaires' disease in the affected unit.[87,88] Chloramine T and hypochlorites have been used in disinfecting hydrotherapy equipment.[71]

Hypochlorite solutions in tap water at a pH greater than 8 stored at room temperature (20°C) in closed, opaque plastic containers may lose up to 40% to 50% of their free available chlorine level over a period of 1 month. Thus, if a user wished to have a solution containing 500 ppm of available chlorine at day 30, a solution containing 1000 ppm of chlorine should be prepared at time 0. There is no decomposition of sodium hypochlorite solution after 30 days when stored in a closed brown bottle.[79]

Glutaraldehyde

Glutaraldehyde is a saturated dialdehyde that has gained wide acceptance as a high-level disinfectant and chemical sterilant.[89–93] Aqueous solutions of glutaraldehyde are acidic and generally in this state are not sporicidal. Only when the solution is "activated" (made alkaline) by use of alkalinizing agents to pH 7.5 to 8.5 does the solution become sporicidal. Once activated, these solutions have a shelf-life of minimally 14 days

because of the polymerization of the glutaraldehyde molecules at alkaline pH levels. This polymerization blocks the active sites (aldehyde groups) of the glutaraldehyde molecules that are responsible for its biocidal activity.

Novel glutaraldehyde formulations (e.g., glutaraldehyde-phenol-sodium phenate, potentiated acid glutaraldehyde, stabilized alkaline glutaraldehyde) produced in the past 50 years have overcome the problem of rapid loss of activity (e.g., current use life, 28–30 days) while generally maintaining excellent microbicidal activity.[90,91] However, it should be recognized that antimicrobial activity is dependent not only on age but also on use conditions such as dilution and organic stress. The use of glutaraldehyde-based solutions in health care facilities is common because of their advantages, including excellent biocidal properties; activity in the presence of organic matter (20% bovine serum); and noncorrosive action when used on endoscopic equipment, thermometers, or rubber or plastic equipment. The advantages, disadvantages, and characteristics of glutaraldehyde are listed in Table 299.2.

The in vitro inactivation of microorganisms by glutaraldehydes has been extensively investigated and reviewed.[92] Several investigators showed that ≥2% aqueous solutions of glutaraldehyde, buffered to pH 7.5 to 8.5 with sodium bicarbonate, were effective in killing vegetative bacteria in <2 minutes; *M. tuberculosis,* fungi, and viruses in <10 minutes; and spores of *Bacillus* and *Clostridium* species in 3 hours.[70,92,93] Spores of *Clostridioides difficile* are more rapidly killed by 2% glutaraldehyde than are spores of species of *Clostridium* and *Bacillus*;[94,95] this includes the hypervirulent binary toxin strains of *C. difficile* spores (WA Rutala, unpublished data, 2017). There have been reports of microorganisms with relative resistance to glutaraldehyde, including some mycobacteria (*M. chelonae, Mycobacterium avium-intracellulare, Mycobacterium xenopi*),[96–98] *Methylobacterium mesophilicum,*[99] *Trichosporon,* fungal ascospores (e.g., *Microascus cinereus*, *Chaetomium globosum*), and *Cryptosporidium.*[100] *M. chelonae* persisted in a 0.2% glutaraldehyde solution used to store porcine prosthetic heart valves.[101] In a large outbreak of *Mycobacterium massiliense* infections in Brazil after videolaparoscopic equipment was used for different elective cosmetic procedures (e.g., liposuction), the organism was highly tolerant to 2% glutaraldehyde.[102] Porins may have a role in the resistance of mycobacteria to glutaraldehyde and OPA.[103]

Dilution of glutaraldehyde during use commonly occurs, and studies have shown a glutaraldehyde concentration decline after a few days of use in an automatic endoscope washer.[104] This occurs because instruments are not thoroughly dried and water is carried in with the instrument, which increases the solution's volume and dilutes its effective concentration. This emphasizes the need to ensure that semicritical equipment is disinfected with an acceptable concentration of glutaraldehyde. Data suggest that 1.0% to 1.5% glutaraldehyde is the minimum effective concentration (MEC) for >2% glutaraldehyde solutions when used as a high-level disinfectant.[104–106] Chemical test strips or liquid chemical monitors are available for determining whether an effective concentration of glutaraldehyde is present despite repeated use and dilution. The frequency of testing should be based on how frequently the solutions are used (e.g., used daily, test daily; used weekly, test before use), or the manufacturer's recommendation should be followed. The strips should not be used to extend the use life beyond the expiration date. Data suggest that the chemicals in the test strip deteriorate with time,[107] and a manufacturer's expiration date should be placed on the bottles. The bottle of test strips should be dated when opened and used for the period of time indicated on the bottle (e.g., 120 days). The results of test strip monitoring should be documented. The glutaraldehyde test kits have been preliminarily evaluated for accuracy and range,[107] but the reliability has been questioned.[108] The concentration should be considered unacceptable or unsafe when the test indicates a dilution below the product's MEC (generally to 1.0%–1.5% glutaraldehyde or lower) by the indicator not changing color.

Glutaraldehyde is used most commonly as a high-level disinfectant for medical equipment such as endoscopes, endocavitary probes, spirometry tubing, dialyzers, transducers, anesthesia and respiratory therapy equipment, hemodialysis proportioning, and dialysate delivery systems.[8] Glutaraldehyde is noncorrosive to metal and does not damage lensed instruments, rubber, or plastics. The FDA-cleared labels for high-level disinfection with >2% glutaraldehyde at 20°C to 25°C range from 20 to 90 minutes depending on the product. However, multiple scientific studies and professional organizations support the efficacy of >2% glutaraldehyde for 20 minutes at 20°C.[8,31,109] Minimally, one should follow this latter recommendation. Glutaraldehyde should not be used for cleaning noncritical surfaces, because it is too toxic and expensive.

Colitis believed to be due to glutaraldehyde exposure from residual disinfecting solution in the endoscope solution channels has been reported and is preventable through careful endoscope rinsing.[63] One study found that residual glutaraldehyde levels were higher and more variable after manual disinfection (<0.2–159.5 mg/L) than after automatic disinfection (0.2–6.3 mg/L).[110] Similarly, keratopathy and corneal decompensation were caused by ophthalmic instruments that were inadequately rinsed after soaking in 2% glutaraldehyde.[111]

Glutaraldehyde exposure should be monitored to ensure a safe work environment. In the absence of an OSHA permissible exposure limit (PEL), if the glutaraldehyde level is higher than the American Conference of Governmental Industrial Hygienists (ACGIH) ceiling limit of 0.05 ppm, it would be prudent to take corrective action and repeat monitoring.[112]

Hydrogen Peroxide

The literature contains several accounts of the properties, germicidal effectiveness, and potential uses for stabilized hydrogen peroxide in the health care setting. Reports ascribing good germicidal activity to hydrogen peroxide have been published and attest to its bactericidal, virucidal, sporicidal, and fungicidal properties.[113–116] Some other studies have shown limited bactericidal and virucidal activity of standard 3% hydrogen peroxide.[45,70] The advantages, disadvantages, and characteristics of hydrogen peroxide are listed in Tables 299.2 and 299.4. As with other chemical sterilants, dilution of the hydrogen peroxide must be monitored through regular testing of the MEC (i.e., 7.5%–6.0%). Compatibility testing by Olympus America of 7.5% hydrogen peroxide found both cosmetic changes (e.g., discoloration of black anodized metal finishes)[117] and functional changes with the tested endoscopes (Olympus, written communication, October 15, 1999).

Commercially available 3% hydrogen peroxide is a stable and effective disinfectant when used on some inanimate surfaces. It has been used in concentrations from 3% to 6% for the disinfection of soft contact lenses (e.g., 3% for 2–3 hours),[113,118] tonometer biprisms, ventilators, fabrics,[119] and endoscopes.[120] Hydrogen peroxide was effective in spot-disinfecting fabrics in patients' rooms.[119] Corneal damage from a hydrogen peroxide–soaked tonometer tip that was not properly rinsed has been reported.[121]

Improved Hydrogen Peroxide

An improved hydrogen peroxide–based technology has been introduced into health care for disinfection of noncritical environmental surfaces and patient equipment[122–124] and high-level disinfection of semicritical equipment such as endoscopes.[122,125–127] Improved hydrogen peroxide contains very low levels of anionic and/or nonionic surfactants in an acidic product that act with hydrogen peroxide to produce microbicidal activity. This combination of ingredients speeds the antimicrobial activity of hydrogen peroxide and cleaning efficiency.[122,125] Improved hydrogen peroxide is considered safe for humans and equipment, and benign for the environment. Improved hydrogen peroxide has the lowest EPA toxicity category (i.e., category IV) based on its oral, inhalation, and dermal toxicity, which means it is practically nontoxic and not an irritant.[122,127,128] It is prepared and marketed by several companies in various concentrations (e.g., 0.5%–2%), and different companies may use different terminology for these products, such as "accelerated" or "activated." Lower concentrations (i.e., 0.5%, 1.4%) are designed for the low-level disinfection of noncritical environmental surfaces and patient care objects; higher concentrations (i.e., 2.0%) can be used as high-level disinfectants for semicritical medical devices (e.g., endoscopes).

When a study compared the bactericidal activity of two improved hydrogen peroxide products versus standard 0.5%, 1.4%, and 3% hydrogen peroxide formulations, the improved hydrogen peroxide–based environmental surface disinfectants proved to be more effective (>6-$\log_{10}$ reduction) and fast-acting (30–60 seconds) microbicides in the presence

of a soil load (to simulate the presence of body fluids) than commercially available hydrogen peroxide. Only 30- to 60-second contact times were studied because longer contact times (e.g., 10 minutes) are not achievable in clinical practice.[45] In 2017, Boyce and colleagues compared a quat disinfectant and an improved hydrogen peroxide and found the improved hydrogen peroxide reduced surface contamination and reduced a composite colonization or infection outcome.[129] Another study demonstrated that 1.4% activated hydrogen peroxide is very effective in reducing microbial contamination of hospital privacy curtains. In fact, the activated hydrogen peroxide completely eliminated contamination with MRSA and VRE and resulted in a 98.5% reduction in microbes (only *Bacillus* sp. was recoverable).[130] Thus, at UNC Hospitals, privacy curtains are being disinfected at the grab area by means of spraying the grab area of the curtain three times with activated hydrogen peroxide at discharge cleaning.

Iodophors

Iodine solutions or tinctures have long been used by health professionals, primarily as antiseptics on skin or tissue. The FDA has not cleared any liquid chemical sterilant or high-level disinfectant with iodophors as the main active ingredient. However, iodophors have been used both as antiseptics and as disinfectants. An iodophor is a combination of iodine and a solubilizing agent or carrier; the resulting complex provides a sustained-release reservoir of iodine and releases small amounts of free iodine in aqueous solution. The best known and most widely used iodophor is povidone-iodine, a compound of polyvinylpyrrolidone with iodine. This product and other iodophors retain the germicidal efficacy of iodine but, unlike iodine, are generally nonstaining and are relatively free of toxicity and irritancy.[131]

There are several reports that document intrinsic microbial contamination of antiseptic formulations of povidone-iodine and poloxamer-iodine.[132–134] It was found that "free" iodine (I_2) contributes to the bactericidal activity of iodophors and that dilutions of iodophors demonstrate more rapid bactericidal action than does a full-strength povidone-iodine solution. Therefore iodophors must be diluted according to the manufacturers' directions in order to achieve antimicrobial activity.

Published reports on the in vitro antimicrobial efficacy of iodophors demonstrate that iodophors are bactericidal, mycobactericidal, and virucidal but may require prolonged contact times to kill certain fungi and bacterial spores.[16,135–138]

Besides their use as an antiseptic, iodophors have been used for the disinfection of blood culture bottles and medical equipment such as hydrotherapy tanks and thermometers. Antiseptic iodophors are not suitable for use as hard-surface disinfectants because of concentration differences. Iodophors formulated as antiseptics contain less free iodine than those formulated as disinfectants.[139]

Ortho-phthalaldehyde

OPA is a high-level disinfectant that received FDA clearance in October 1999. The solution contains at least 0.55% 1,2-benzenedicarboxaldehyde or OPA, and it has supplanted glutaraldehyde as the most commonly used "aldehyde" for high-level disinfection in the United States. OPA solution is a clear, pale-blue liquid with a pH of 7.5. The advantages, disadvantages, and characteristics of OPA are listed in Table 299.2.

In vitro studies have demonstrated excellent microbicidal activity,[70,140–144] including superior mycobactericidal activity (5-$\log_{10}$ reduction in 5 minutes) compared with glutaraldehyde. Walsh and colleagues also found OPA effective (>5-$\log_{10}$ reduction) against a wide range of microorganisms, including glutaraldehyde-resistant mycobacteria and *Bacillus atrophaeus* spores.[142]

OPA has several potential advantages compared with glutaraldehyde. It has excellent stability over a wide pH range (pH 3–9), is not a known irritant to the eyes and nasal passages, does not require exposure monitoring, has a barely perceptible odor, and requires no activation. OPA, like glutaraldehyde, has excellent material compatibility. A potential disadvantage of OPA is that it stains proteins gray (including unprotected skin) and thus must be handled with caution.[117] However, skin staining would indicate improper handling and indicates a need for additional training and/or personal protective equipment (PPE) (gloves, eye and mouth protection, fluid-resistant gowns). OPA residues remaining on inadequately water-rinsed transesophageal echocardiography probes may leave stains on the patient's mouth. Meticulous cleaning, use of the correct OPA exposure time (e.g., 12 minutes), and copious rinsing of the probe with water should eliminate this problem. Because OPA has been associated with several episodes of anaphylaxis after cystoscopy,[145] the manufacturer has modified its instructions for use of OPA and contraindicates the use of OPA as a disinfectant for reprocessing all urologic instruments for patients with a history of bladder cancer. PPE should be worn for handling of contaminated instruments, equipment, and chemicals.[146] In addition, equipment must be thoroughly rinsed to prevent discoloration of a patient's skin or mucous membrane. The MEC of OPA is 0.3%, and that concentration is monitored with test strips designed specifically for the OPA solution. Monitoring of OPA exposure level revealed that the concentration during the disinfection process was significantly higher in the manual group (median, 1.43 ppb) than in the automatic group (median, 0.35 ppb). These findings corroborate other findings that show that it is desirable to introduce automatic endoscope reprocessors to decrease disinfectant exposure levels among scope-reprocessing technicians.[147]

Peracetic Acid

Peracetic, or peroxyacetic acid, is characterized by a very rapid action against all microorganisms. Special advantages of peracetic acid include its lack of harmful decomposition products (i.e., acetic acid, water, oxygen, hydrogen peroxide); it enhances removal of organic material[148] and leaves no residue. It remains effective in the presence of organic matter and is sporicidal even at low temperatures. Peracetic acid can corrode copper, brass, bronze, plain steel, and galvanized iron, but these effects can be reduced with additives and pH modifications. The advantages, disadvantages, and characteristics of peracetic acid are listed in Table 299.2.

Peracetic acid will inactivate gram-positive and gram-negative bacteria, fungi, and yeasts in less than 5 minutes at less than 100 ppm. In the presence of organic matter, 200 to 500 ppm is required. For viruses the dosage range is wide (12–2250 ppm), with poliovirus inactivated in yeast extract in 15 minutes with 1500 to 2250 ppm. A processing system using peracetic acid at a temperature of 50°C to 56°C can be used for processing heat-sensitive semicritical and critical devices that are compatible with the peracetic acid and processing system and that cannot be sterilized by other legally marketed traditional sterilization methods validated for that type of device (e.g., steam, hydrogen peroxide gas plasma, vaporized hydrogen peroxide). After processing, the devices should be used immediately or stored in a manner similar to that of a high-level disinfected endoscope.[149,150] The sterilant, 35% peracetic acid, is diluted to 0.2% with tap water that has been filtered and exposed to ultraviolet (UV) light. Some data demonstrate the effectiveness of the liquid chemical sterilant processing system for reprocessing duodenoscopes.[151] Simulated-use trials with the earlier version of this processing system have demonstrated excellent microbicidal activity,[70,149,152–154] and three clinical trials have demonstrated both excellent microbial killing and no clinical failures leading to infection.[155–157] Three clusters of infection with use of the earlier version of the peracetic acid automated endoscope reprocessor were linked to inadequately processed bronchoscopes when inappropriate channel connectors were used with the system.[158] These clusters highlight the importance of training, proper model-specific endoscope connector systems, and quality control procedures to ensure compliance with endoscope manufacturer's recommendations and professional organization guidelines. An alternative high-level disinfectant available in the United Kingdom contains 0.35% peracetic acid. Although this product is rapidly effective against a broad range of microorganisms,[159,160] it tarnishes the metal of endoscopes and is unstable, resulting in only a 24-hour use life.[160]

Peracetic Acid With Hydrogen Peroxide

Three chemical sterilants that contain peracetic acid plus hydrogen peroxide (e.g., 0.08% peracetic acid plus 1.0% hydrogen peroxide, 0.23% peracetic acid plus 7.35% hydrogen peroxide) have been cleared by the FDA. The advantages, disadvantages, and characteristics of peracetic acid with hydrogen peroxide are listed in Table 299.2.

The bactericidal and sporicidal properties of peracetic acid plus hydrogen peroxide have been demonstrated.[161,162] Manufacturer's data demonstrated that this combination of peracetic acid plus hydrogen peroxide inactivated all microorganisms with the exception of bacterial spores within 20 minutes. The 0.08% peracetic acid plus 1.0% hydrogen peroxide product was effective in inactivating a glutaraldehyde-resistant *Mycobacterium*.[163]

The combination of peracetic acid and hydrogen peroxide has been used for disinfecting hemodialyzers[164] and environmental surfaces.[165] This disinfectant product was associated with mucous membrane and respiratory health effects when used by hospital staff for surface disinfection.[165] A study used a peracetic acid and hydrogen peroxide solution for all daily, discharge, and common area cleaning and found a significant decrease in hospital-onset *C. difficile* rates.[166] The percentage of dialysis centers using a peracetic acid with hydrogen peroxide–based disinfectant for reprocessing dialyzers increased from 5% in 1983 to 72% in 1997.[167]

Phenolics

Phenol has occupied a prominent place in the field of hospital disinfection since its initial use as a germicide by Lister in his pioneering work on antiseptic surgery. In the past 40 years, however, work has been concentrated on the numerous phenol derivatives or phenolics and their antimicrobial properties. Phenol derivatives originate when a functional group (e.g., alkyl, phenyl, benzyl, halogen) replaces one of the hydrogen atoms on the aromatic ring. Two phenol derivatives commonly found as constituents of hospital disinfectants are ortho-phenylphenol and ortho-benzyl-para-chlorophenol.

Published reports on the antimicrobial efficacy of commonly used phenolics showed that they were bactericidal, fungicidal, virucidal, and tuberculocidal.[16,70,85,135,168–172]

Many phenolic germicides are EPA registered as disinfectants for use on environmental surfaces (e.g., bedside tables, bed rails, laboratory surfaces) and noncritical medical devices. Phenolics are not FDA cleared as high-level disinfectants for use with semicritical items.

The use of phenolics in nurseries has been questioned because of the occurrence of hyperbilirubinemia in infants placed in bassinets where phenolic detergents were used.[173] In addition, Doan and coworkers demonstrated bilirubin level increases in phenolic-exposed infants compared with nonphenolic-exposed infants when the phenolic was prepared according to the manufacturers' recommended dilution.[174] If phenolics (or other disinfectants) are used to clean nursery floors, they must be diluted according to the recommendation on the product label. Phenolics (and other disinfectants) should not be used to clean infant bassinets and incubators while occupied. If phenolics are used to terminally clean infant bassinets and incubators, the surfaces should be rinsed thoroughly with water and dried before the infant bassinets and incubators are reused.[8,175]

Quaternary Ammonium Compounds

The quaternary ammonium compounds are widely used as surface disinfectants. There have been some reports of health care–associated infections associated with contaminated quaternary ammonium compounds used to disinfect semicritical or critical patient care equipment such as cystoscopes or cardiac catheters.[176,177] As with several other disinfectants (e.g., phenolics, iodophors), gram-negative bacteria have been found to survive or grow in them.[134]

Results from manufacturers' data sheets and from published scientific literature indicate that the quaternaries sold as hospital disinfectants are generally fungicidal, bactericidal, and virucidal against lipophilic (enveloped) viruses; they are not sporicidal and generally not tuberculocidal or virucidal against hydrophilic (nonenveloped) viruses.[a] Best and colleagues and Rutala and colleagues demonstrated the poor mycobactericidal activities of quaternary ammonium compounds.[41,135]

The quats are commonly used in ordinary environmental sanitation of noncritical environmental surfaces such as floors, bed rails, bedside tables, and furniture. EPA-registered quaternary ammonium compounds are appropriate to use when disinfecting medical equipment that comes into contact with intact skin (e.g., blood pressure cuffs). Several factors can affect the use of quats, including the types of wipes and towels used[182] and the concentration of quat delivered by automated disinfectant dispensers (approximately 70% disinfectant solution had quat concentrations below 50% of expected concentration).[183] Microfiber wipes, cotton towels, and one of two types of disposable wipes soaked in a quat disinfectant revealed significant binding of the disinfectant (≤50% of the original quat in parts per million—for example, from 800 ppm to 400 ppm or less).[183] Quats are commonly combined with alcohol, which significantly improves their speed of action and antimicrobial spectrum. Disinfectant wipes with alcohol and quaternary ammonium compounds are effective in killing health care pathogens and are commonly used for surface disinfection.[70] For example, studies showed that quats alone are not effective against *Candida auris*, whereas quats combined with alcohol have excellent activity against *C. auris*.[62,184]

Pasteurization

Pasteurization is not a sterilization process; its purpose is to destroy all pathogenic microorganisms with the exception of bacterial spores. The time-temperature relation for hot-water pasteurization is generally greater than 70°C (158°F) for 30 minutes. The water temperature and time should be monitored as part of a quality assurance program.[185] Pasteurization of respiratory therapy[186,187] and anesthesia[188] equipment is a recognized alternative to chemical disinfection.

Ultraviolet Light

UV light has been recognized as an effective method for killing microorganisms. It has been suggested for use in health care for several purposes, including air disinfection, room decontamination (see "'No Touch' Methods for Room Decontamination"), surface disinfection, biofilm disinfection,[189] and ultrasound probe disinfection.[190] Contaminated ultrasound probes can potentially transmit pathogens. When the probe is in contact with only the patient's skin, there is a low risk of infection, and low-level disinfection is recommended.

STERILIZATION

Most medical and surgical devices used in health care facilities are made of materials that are heat stable and thus are sterilized by heat, primarily steam sterilization. However, since 1950, there has been an increase in medical devices and instruments made of materials (e.g., plastics) that require low-temperature sterilization. ETO gas has been used since the 1950s for heat- and moisture-sensitive medical devices. Within the past 25 years, a number of new, low-temperature sterilization systems (e.g., hydrogen peroxide gas plasma, vaporized hydrogen peroxide, hydrogen peroxide plus ozone) have been developed and are being used to sterilize medical devices. This section reviews sterilization technologies used in health care and makes recommendations for their optimum performance in the processing of medical devices.[8]

Sterilization destroys all microorganisms on the surface of an article or in a fluid to prevent disease transmission associated with the use of that item. Although the use of inadequately sterilized critical items represents a high risk of transmitting pathogens, documented transmission of pathogens associated with an inadequately sterilized critical item is exceedingly rare.[191–193] This is likely due to the wide margin of safety associated with the sterilization processes used in health care facilities. The concept of what constitutes "sterile" is measured as a probability of sterility for each item to be sterilized. This probability is commonly referred to as the sterility assurance level (SAL) of the product and is defined as the probability of a single viable microorganism occurring on a product after sterilization. SAL is normally expressed as 10^{-n}. For example, if the probability of a spore surviving were 1 in 1 million, the SAL would be 10^{-6}.[194,195] Dual SALs (e.g., 10^{-3} SAL for blood culture tubes, drainage bags; 10^{-6} SAL for scalpels, implants) have been used in the United States for many years and the choice of a 10^{-6} SAL was strictly arbitrary and not associated with any adverse outcomes (e.g., patient infections).[194]

Medical devices that have contact with sterile body tissues or fluids are considered critical items. These items should be sterile when used because any microbial contamination could result in disease transmission. Such items include surgical instruments, biopsy forceps, and implanted

[a]References 16, 41, 42, 135, 168, 178–181.

medical devices. If these items are heat resistant, the recommended sterilization process is steam sterilization, because it has the largest margin of safety owing to its reliability, consistency, and lethality and is least affected by organic and inorganic soils. However, reprocessing heat- and moisture-sensitive items requires use of a low-temperature sterilization technology (e.g., ETO, hydrogen peroxide gas plasma, vaporized hydrogen peroxide, hydrogen peroxide plus ozone).[196] A summary of the advantages and disadvantages of commonly used sterilization technologies is presented in Table 299.3.

Health care facilities must have a quality assurance process in place to ensure the adequacy of staff training, competency testing (at employment and at least annually), compliance with evidence-based guidelines and/or manufacturer's instructions for use, appropriate and adequate space, and appropriate process monitoring (e.g., cleaning monitors) with documentation.[197] All areas that reprocess semicritical or critical patient care equipment should be audited to ensure that they comply with evidence-based guidelines and/or manufacturer's instruction for use, and if deficiencies are identified, they should be corrected.[198] If noncompliance with evidence-based guidelines occurs during reprocessing of critical and semicritical equipment, a patient risk assessment using a 14-step protocol can guide an institution in determining if and how patients should be notified of the potential adverse event.[199]

Steam Sterilization

Of all the methods available for sterilization, moist heat in the form of saturated steam under pressure is the most widely used and the most dependable. Steam sterilization is nontoxic, inexpensive,[200] rapidly microbicidal, sporicidal, and rapidly heats and penetrates fabrics (see Table 299.3).[201] Like all sterilization processes, steam sterilization has some deleterious effects on some materials, including corrosion and combustion of lubricants associated with dental handpieces,[202] reduction in ability to transmit light associated with laryngoscopes,[203] and increased hardening time (5- to 6-fold) with plaster cast.[204]

The basic principle of steam sterilization, as accomplished in an autoclave, is to expose each item to direct steam contact at the required temperature and pressure for the specified time. Thus, there are four parameters of steam sterilization: steam, pressure, temperature, and time. The ideal steam for sterilization is dry saturated steam and entrained water (dryness fraction ≥97%).[205] Pressure serves as a means to obtain the high temperatures necessary to quickly kill microorganisms. Specific temperatures must be obtained to ensure the microbicidal activity. The two common steam sterilizing temperatures are 121°C (250°F) and 132°C (270°F). These temperatures (and other high temperatures) must be maintained for a minimal time to kill microorganisms. Recognized minimum exposure periods for sterilization of wrapped health care supplies are 30 minutes at 121°C (250°F) in a gravity displacement sterilizer or 4 minutes at 132°C (270°F) in a prevacuum sterilizer. At constant temperatures, sterilization times vary depending on the type of item (e.g., metal versus rubber, plastic, items with lumens), whether the item is wrapped or unwrapped, and the sterilizer type.

The two basic types of steam sterilizers (autoclaves) are the gravity displacement autoclave and the high-speed prevacuum sterilizer. In the former, steam is admitted at the top or the sides of the sterilizing chamber and, because the steam is lighter than air, forces air out the bottom of the chamber through the drain vent. Gravity displacement autoclaves are primarily used to process laboratory media, water, pharmaceutical products, regulated medical waste, and nonporous articles whose surfaces have direct steam contact. With gravity displacement sterilizers the penetration time into porous items is prolonged because of incomplete air elimination. High-speed prevacuum sterilizers are similar to gravity displacement sterilizers except that they are fitted with a vacuum pump (or ejector) to ensure air removal from the sterilizing chamber and load before the steam is admitted. The advantage of using a vacuum pump is that there is nearly instantaneous steam penetration even into porous loads.

Like other sterilization systems, the steam cycle is monitored with physical, chemical, and biologic monitors.[8] Steam sterilizers usually are monitored with a printout (or graphically) that shows temperature, the time at the temperature, and pressure. Typically, chemical indicators are affixed to the outside and incorporated into the pack to monitor the temperature or time and temperature. The effectiveness of steam sterilization is monitored with a biologic indicator containing spores of *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*). Positive spore test results are a relatively rare event and can be attributed to operator error, inadequate steam delivery,[206] or equipment malfunction.

Portable steam sterilizers are used in outpatient, dental, and rural clinics. These sterilizers are designed for small instruments, such as hypodermic syringes and needles and dental instruments. The ability of the sterilizer to reach physical parameters necessary to achieve sterilization should be monitored with physical, chemical, and biologic indicators.[8]

Steam sterilization should be used whenever possible on all critical and semicritical items that are heat and moisture resistant (e.g., respiratory therapy and anesthesia equipment), even when not essential to prevent pathogen transmission. Steam sterilizers also are used in health care facilities to decontaminate microbiologic waste[207] and sharps containers, but additional exposure time is required in the gravity displacement sterilizer for these items.

Immediate-Use Steam Sterilization

"Flash" steam sterilization was originally defined by Underwood and Perkins as sterilization of an unwrapped object at 132°C for 3 minutes at 27 to 28 pounds of pressure in a gravity displacement sterilizer.[208] It was intended for instruments (e.g., dropped instruments) when there is insufficient time to sterilize an item by means of the preferred package method. The term "flash" arose out of the abbreviated time of exposure of the unwrapped instrument. "Flash sterilization" is an antiquated term that does not fully describe the various steam sterilization cycles now used to process items not intended to be stored for later use. "Immediate use" is defined as the shortest possible time between a sterilized item's removal from the sterilizer and its aseptic transfer to the sterile field. This implies that the sterilized item is used during the procedure for which it was sterilized and in a manner that minimizes its exposure to air and other environmental contaminants. The same critical reprocessing steps (such as cleaning, decontamination, rinsing, and aseptic transfer from the sterilizer to the point of use) must be followed. Immediate-use steam sterilization (IUSS) should not be used for convenience, as an alternative to purchasing sufficient instrument sets, or as a time saver.[209,210] Some hospitals have reduced or eliminated the need for IUSS by means of various practices such as a multidisciplinary collaboration to analyze the reason for each IUSS event.[211,212]

Ethylene Oxide "Gas" Sterilization

ETO is a colorless gas that is flammable and explosive. The four essential parameters (operational ranges) are gas concentration (450–1200 mg/L); temperature (37 °C–63°C); relative humidity (40%–80%; water molecules carry ETO to reactive sites); and exposure time (1–6 hours). These influence the effectiveness of ETO sterilization.[213–215] Within certain limitations, an increase in gas concentration and temperature may shorten the time necessary for achieving sterilization.

The main disadvantages associated with ETO are the lengthy cycle time and its potential hazards to patients and staff; the main advantages are that it is highly penetrating and can sterilize occluded locations in medical items and can sterilize heat- or moisture-sensitive medical equipment without deleterious effects on the material used in the medical devices (see Table 299.3).[214] Acute exposure to ETO may result in irritation (e.g., to skin, eyes, gastrointestinal or respiratory tract) and central nervous system depression.[63] Chronic inhalation has been linked to the formation of cataracts, cognitive impairment, neurologic dysfunction, and disabling polyneuropathies.[63] Occupational exposure in health care facilities has been linked to hematologic changes and an increased risk of spontaneous abortions and various cancers.[63] ETO should be considered a probable human carcinogen.[216]

The use of ETO evolved when few alternatives existed for sterilizing heat- and moisture-sensitive medical devices; now there are several low-temperature sterilization technologies (see Table 299.3). However, favorable properties account for its continued use.[217] Two ETO gas mixtures replaced ETO-chlorofluorocarbon (CFC) mixtures for large-capacity, tank-supplied sterilizers. The ETO–carbon dioxide (CO_2)

mixture consists of 8.5% ETO and 91.5% CO_2. This mixture has limited use in US health care facilities but is sometimes used in hospitals in India and China. It is less expensive than ETO-hydrochlorofluorocarbons (HCFCs), but a disadvantage is the need for pressure vessels rated for steam sterilization, because higher pressures (28-psi gauge) are required. The other mixture, which is a drop-in CFC replacement, is ETO mixed with HCFC. HCFCs are approximately 50-fold less damaging to the earth's ozone layer than are CFCs. The EPA began regulations of HCFC in the year 2015 and will terminate production in the year 2030. An alternative to the pressurized mixed-gas ETO systems is 100% ETO. In part because of the aforementioned events, the 100% ETO sterilizers using unit-dose cartridges are the system for ETO use in US health care facilities.

The excellent microbicidal activity of ETO has been demonstrated in several studies[20,154,217–220] and summarized in published reports.[221] ETO inactivates all microorganisms, although bacterial spores (especially *B. atrophaeus*) are more resistant than other microorganisms. For this reason, *B. atrophaeus* is the recommended biologic indicator organism.

As with all sterilization processes, the effectiveness of ETO sterilization can be altered by lumen length, lumen diameter, inorganic salts, and organic materials.[20,154,218–220,222] For example, although ETO is not used commonly for reprocessing endoscopes,[223] several studies have shown failure of ETO in inactivating contaminating spores in endoscope channels [224] or lumen test units.[20,154,219,220] Residual ETO levels averaging 66.2 ppm have been found even after the standard degassing time.[120] Failure of ETO also has been observed when dental handpieces were contaminated with *Streptococcus mutans* and exposed to ETO.[225] It is recommended that dental handpieces be steam sterilized.

ETO is used in health care facilities to sterilize critical items (and sometimes semicritical items) that are moisture or heat sensitive and cannot be sterilized with steam sterilization.

Hydrogen Peroxide Gas Plasma

New low-temperature sterilization technology based on hydrogen peroxide and plasma was patented in 1987 and marketed in the United States in 1993. Gas plasmas have been referred to as the fourth state of matter (i.e., liquids, solids, gases, and gas plasmas). Gas plasmas are generated in an enclosed chamber under deep vacuum using radiofrequency or microwave energy to excite the gas (i.e., hydrogen peroxide) molecules and produce charged particles, many of which are in the form of free radicals (e.g., hydroxyl and hydroperoxyl). This system works by diffusing hydrogen peroxide into the chamber and then "exciting" the hydrogen peroxide into a plasma state. The combined use of hydrogen peroxide vapor and plasma safely and rapidly sterilizes instruments without leaving toxic residues. The biologic indicator used with this system is *G. stearothermophilus* spores.

This process has the ability to inactivate a broad range of microorganisms, including resistant bacterial spores. Studies have been conducted against vegetative bacteria (including mycobacteria), yeasts, fungi, viruses, and bacterial spores.[20,154,219,226–232] Like all low-temperature sterilization processes, the effectiveness can be altered by lumen length, lumen diameter, inorganic salts, and organic materials.[20,154,222,226–228]

Materials and devices that cannot tolerate high temperatures and humidity, such as some plastics, electrical devices, and corrosion-susceptible metal alloys, can be sterilized with hydrogen peroxide gas plasma. This method has been compatible with most (>95%) medical devices and materials tested.[233,234]

Vaporized Hydrogen Peroxide

Another low-temperature sterilization system used in health care facilities to sterilize reusable metal and nonmetal devices is vaporized hydrogen peroxide.[235] The system is compatible with a wide range of medical instruments and materials (e.g., polypropylene, brass, polyethylene). There are no toxic by-products; only water vapor and oxygen are produced. The system is not intended to process liquids, linens, powders, or any cellulose materials. The system can sterilize instruments with diffusion-restricted spaces (e.g., scissors) and medical devices with a single stainless steel lumen based on lumen internal diameter and length (e.g., an inside diameter of 1 mm or larger and a length of 125 mm or shorter; see manufacturer's recommendations). Thus, gastrointestinal endoscopes and bronchoscopes cannot be sterilized in this system at the current time. Although this system has not been comparatively evaluated with other sterilization processes, vaporized hydrogen peroxide has been shown to be effective in killing spores, viruses, mycobacteria, fungi, and bacteria.[235] Table 299.3 lists the advantages and disadvantages of this and other processes.

Hydrogen Peroxide Plus Ozone

A new low-temperature sterilizer is marketed as a combination of hydrogen peroxide plus ozone. This sterilizer is primarily a hydrogen peroxide sterilizer similar to the vaporized hydrogen peroxide and hydrogen peroxide gas plasma sterilizers discussed earlier. The sterilizer uses ozone to lower the hydrogen peroxide concentration.[236] In July 2016, the sterilizer was cleared by the FDA for colonoscopes, gastroscopes, and other multichannel flexible endoscopes of up to four channels having internal lumens of ≥1.45 mm in inner diameter and ≤3.5 meters in length.[236,237] Although this system has not been comparatively evaluated with other sterilization processes, vaporized hydrogen peroxide has been shown to be effective in killing spores, viruses, mycobacteria, fungi, and bacteria.[236] Table 299.3 lists the advantages and disadvantages of this and other sterilization processes.

Current Issues in Sterilization

Inactivation of Creutzfeldt-Jakob Disease Agent

Creutzfeldt-Jakob disease (CJD) is a degenerative neurologic disorder of humans with an incidence in the United States of approximately 1 case per million population per year.[238,239] CJD is thought to be caused by a proteinaceous infectious agent or prion. CJD is related to other human transmissible spongiform encephalopathies (TSEs), which include kuru (incidence rate of zero; now eradicated), Gerstmann-Sträussler-Scheinker (GSS) syndrome (1 case per 40 million), and fatal insomnia syndrome (FFI) (<1 case per 40 million). The agents of CJD and other TSEs exhibit an unusual resistance to conventional chemical and physical decontamination methods. A new practical diagnostic test for detecting the pathogenetic prion protein in spinal fluid has been reported and is called the real-time quaking-induced conversion test.[240] Because the CJD agent is not readily inactivated with conventional disinfection and sterilization procedures and because of the invariably fatal outcome of CJD, the procedures for disinfection and sterilization of the CJD prion have been both conservative and controversial for many years.[238,239,241–243]

The current recommendations consider inactivation data but also use epidemiologic studies of prion transmission, infectivity of human tissues, and efficacy of removing proteins by cleaning.[238,239,241,242] On the basis of scientific data, only critical (e.g., surgical instruments) and semicritical devices contaminated with high-risk tissue (i.e., brain, spinal cord, and eye tissue) from high-risk patients (e.g., known or suspected infection with CJD or other prion disease) require special prion reprocessing. A moist environment after contamination reduces the attachment of both protein and prion amyloid to the stainless steel surface, so moist conditions should be maintained.[244] After the device is clean, it should be sterilized by means of either autoclaving (i.e., steam sterilization) or a combination of sodium hydroxide and autoclaving using one of the following options: (1) autoclave at 134°C for 18 minutes in a prevacuum sterilizer; (2) autoclave at 132°C for 1 hour in a gravity displacement sterilizer; (3) immerse in 1N NaOH for 1 hour, remove and rinse in water, then transfer to an open pan and autoclave [121°C gravity displacement or 134°C porous or prevacuum sterilizer] for 1 hour; or (4) immerse in 1N NaOH for 1 hour and heat in a gravity displacement sterilizer at 121°C for 30 minutes, then clean and subject to routine sterilization.[238,239,243,245] Some data suggest that the temperature should not exceed 134°C because the effectiveness of autoclaving may decline as the temperature is increased (e.g., 136°C, 138°C).[246] Prion-contaminated medical devices that are impossible or difficult to clean should be discarded. To minimize environmental contamination, noncritical environmental surfaces should be covered with plastic-backed paper, and when contaminated with high-risk tissues, the paper should be properly discarded. Noncritical environmental surfaces (e.g., laboratory surfaces) contaminated with high-risk tissues should be cleaned and then spot decontaminated with a 1:10 dilution of hypochlorite solutions.[238,245]

DISINFECTION

High-Level Disinfection of Semicritical Items

Semicritical items are those that come in contact with intact mucous membranes or nonintact skin. Respiratory therapy and anesthesia equipment, gastrointestinal endoscopes, bronchoscopes, laryngoscopes, transesophageal echocardiogram probes, tonometers, endocavitary probes, prostate biopsy probes, cystoscopes, hysteroscopes, infrared coagulation devices, and diaphragm fitting rings are included in this category. These medical devices should be free of all microorganisms (i.e., mycobacteria, fungi, viruses, bacteria), although small numbers of bacterial spores may be present.[3,24,247]

Because semicritical equipment has been associated with reprocessing errors that result in patient lookback and patient notifications, it is essential that control measures be instituted to prevent patient exposures.[199] Before new equipment (especially semicritical equipment, for which the margin of safety is less than that for sterilization)[248] is used for patient care on more than one patient, reprocessing procedures for that equipment should be developed. Staff should receive training on the safe use and reprocessing of the equipment and should be competency tested. At UNC Hospitals, to ensure patient-safe instruments, all staff members who reprocess semicritical instruments (e.g., instruments that come into contact with a mucous membrane, such as vaginal probes, endoscopes, prostate probes) are required to attend a 3-hour class on high-level disinfection of semicritical instruments. This includes health care providers reprocessing instruments in outpatient care facilities.[249] The class includes the rationale for and importance of high-level disinfection, discussion of high-level disinfectants and exposure times, reprocessing steps, monitoring for MEC, use of PPE, and the reprocessing environment (e.g., establish "dirty-to-clean" flow). Infection-control rounds or audits should be conducted annually in all clinical areas in which critical and semicritical devices are reprocessed, in order to ensure adherence to the reprocessing guidelines, manufacturers' instructions for use, and institutional policies.[198]

Current Issues With High-Level Disinfection of Semicritical Items

Reprocessing Endoscopes: A Shift From Disinfection to Sterilization

Physicians use endoscopes to diagnose and treat numerous medical disorders. Although endoscopes represent a valuable diagnostic and therapeutic tool in modern medicine and the incidence of infection associated with use has been reported as low, more health care–associated outbreaks have been linked to contaminated endoscopes than to any other medical device. In fact, over the past few years alone there have been more than 25 outbreaks of multidrug-resistant organisms (MDROs; e.g., CRE) in major hospitals in the United States and the world that have killed dozens of patients and caused morbidity in hundreds more.[6] These outbreaks have been linked primarily to contaminated duodenoscopes that are used to diagnose and treat disease of the liver, bile ducts, and pancreas. However, other gastrointestinal endoscopes (e.g., colonoscopes, gastroscopes) and bronchoscopes have been associated with about 100 more outbreaks causing additional death and illness.[4] The key concern raised by these outbreaks is whether current endoscope reprocessing guidelines are adequate to ensure a patient-safe endoscope (i.e., one devoid of potential pathogens) or whether the features of endoscopes—their long, narrow channels; right-angle turns; components that are difficult to clean and disinfect; heavy microbial contamination; and biofilm development (and required removal)[14]—make it impossible to achieve high-level disinfection of these instruments.[250] Because gastrointestinal endoscopes and bronchoscopes come into contact with intact mucous membranes but frequently have contact with nonintact mucous membranes and sterile tissue, there is a risk of patient-to-patient transmission of potential pathogens, with a subsequent risk of infection. The critical need and rationale for this transition from disinfection to sterilization for endoscopes have been discussed in various peer-reviewed publications including *JAMA, American Journal of Infection Control,* and *Infection Control and Hospital Epidemiology*.[3,248,251]

Recommendations for the cleaning and disinfection of endoscopic equipment have been published and should be strictly followed.[8,31] Unfortunately, audits have shown that personnel do not adhere to guidelines on reprocessing,[5] and outbreaks of infection continue to occur.[6] To ensure that reprocessing personnel are properly trained, initial and annual competency testing should be conducted for each individual who is involved in reprocessing endoscopic instruments.

In general, endoscope disinfection or sterilization with a liquid chemical sterilant or high-level disinfectant involves five steps after leak testing: (1) clean—mechanically clean internal and external surfaces, including brushing internal channels and flushing each internal channel with water and an enzymatic cleaner; (2) disinfect—immerse endoscope in high-level disinfectant (or chemical sterilant) and perfuse (eliminates air pockets and ensures contact of the germicide with the internal channels) disinfectant into all accessible channels such as the suction/biopsy channel and air/water channel, and expose for a time recommended for specific products; (3) rinse—rinse the endoscope and all channels with sterile water, filtered water (commonly used with automated endoscope reprocessors), or tap water; (4) dry—rinse the insertion tube and inner channels with alcohol and dry with forced air after disinfection and before storage; and (5) store—store the endoscope in a way that prevents recontamination and promotes drying (e.g., hung vertically).[8]

Inactivation of Human Papilloma Virus

Human papilloma virus (HPV) is an extremely common sexually acquired pathogen and is considered the cause of cervical cancer. A 2014 paper demonstrated that the FDA-cleared high-level disinfectants (i.e., glutaraldehyde, OPA) tested did not inactivate HPV, a nonenveloped virus.[252] These findings are inconsistent with many papers in the peer-reviewed literature that demonstrate that high-level disinfectants such as OPA and glutaraldehyde inactivate nonenveloped viruses such as hepatitis A virus, polio, adenovirus, and norovirus.[8] Because the high-level disinfectants are commonly used to disinfect endocavitary probes (e.g., vaginal probes, rectal probes) and this equipment and associated surfaces are contaminated,[253] there is an urgency to corroborate these data. At present, hospitals should continue to use the FDA-cleared high-level disinfectants consistent with the manufacturers' instructions until the data can be corroborated. Studies are being done to validate these findings. Data have demonstrated the activity of a hydrogen peroxide mist device to inactivate HPV.[254]

Immersion Versus Perfusion of Channel Scopes Such as Cystoscopes

In the United States, it is estimated that over 4 million cystoscopies are performed each year. Cystoscopy is a diagnostic procedure in which the examiner uses a specially designed endoscope to examine the bladder, lower urinary tract, and prostate gland or to collect urine samples, perform biopsies, or remove small stones. A flexible or rigid scope can be used to carry out the procedure. Because the endoscope and other channeled scopes (e.g., hysteroscopes, some nasopharyngoscopes) come into contact with the patient's mucous membranes, such instruments are considered semicritical devices that must at a minimum undergo high-level disinfection.

A study evaluated the disinfection of cystoscopes, and the results demonstrated that disinfection (i.e., a reduction in bacterial load of greater than 7-$\log_{10}$ colony-forming units [CFUs]) did not occur unless the channel was actively perfused with the glutaraldehyde disinfectant. Failure to perfuse the channel led to only minimal, if any, reduction in bacterial contamination. However, complete inactivation of 10^8 CFUs of both VRE and CRE was achieved when the channel was actively perfused. It appears that no high-level disinfectant entered the channel unless it was actively perfused, because the level of microbial contamination was not reduced with immersion.[255] This occurs because the air pressure in the channel is stronger than the fluid pressure at the fluid-air interface. Recommendations are provided for high-level disinfection of cystoscopes and include actively perfusing the device while it is immersed in the high-level disinfectant.[255] Unfortunately, some cystoscope reprocessing recommendations published in the literature are incorrect. For example, authors recommended complete immersion of the cystoscope into the high-level disinfectant but did not mention perfusion of the high-level disinfectant into the channel.[256]

Ultrasound Probe Disinfection With Vaporized Hydrogen Peroxide

Although the most common method of performing high-level disinfection of contaminated endocavitary probes is by immersion in an FDA-cleared high-level disinfectant (e.g., glutaraldehyde), an alternative procedure for disinfecting the endocavitary and surface probes is a proprietary hydrogen peroxide vapor system, which uses 35% hydrogen peroxide at 56°C with the probe reaching no more than 40°C (i.e., Trophon). The efficacy of this technology, which has been cleared by the FDA for high-level disinfection, has been described in recent publications.[257,258] The results demonstrated complete inactivation (>6-$\log_{10}$ reduction) of VRE and a carbapenem-resistant *Klebsiella pneumoniae* strain, in both the presence and the absence of 5% fetal calf serum (FCS). The Trophon EPR system showed good, but not complete, inactivation of *Mycobacterium terrae* (5.2-$\log_{10}$ reduction for *M. terrae* with FCS, 4.6-$\log_{10}$ reduction for *M. terrae* without FCS) and *C. difficile* spores.[257] Another study showed a 4-$\log_{10}$ reduction of virus titer with various test methods and murine norovirus, adenovirus, and parvovirus.[258]

Low-Level Disinfection of Noncritical Environmental Surfaces and Patient Care Equipment

Over the past decade, excellent evidence in the scientific literature has indicated that contaminated environmental surfaces and noncritical patient care items play an important role in the transmission of several key health care–associated pathogens including MRSA, VRE, *Acinetobacter,* norovirus, and *C. difficile*.[34,40,259–263] All these pathogens have been demonstrated to persist in the environment for days (in some cases months), frequently contaminate the environmental surfaces in rooms of colonized or infected patients,[264] transiently colonize the hands of health care personnel,[265] can be transmitted by health care personnel, and have caused outbreaks in which environmental transmission was deemed to play a role. Important to note, a study by Stiefel and colleagues demonstrated that contact with the environment was just as likely to contaminate the hands of health care providers as was direct contact with the patient.[265] Furthermore, admission to a room in which the previous patient had been colonized or infected with MRSA, VRE, *Acinetobacter,* or *C. difficile* was shown to be a risk factor for the newly admitted patient to develop colonization or infection.[59,266]

Adequacy of Room Cleaning and Disinfection With Chemical Germicides

It has long been recommended in the United States that environmental surfaces in patient rooms be cleaned and disinfected on a regular basis (e.g., daily, three times per week), when surfaces are visibly soiled, and after patient discharge (terminal cleaning).[8] In the past 10 years there have been several studies that have affected the implementation and effectiveness of cleaning of hospital room surfaces.[263] A randomized trial demonstrated that daily disinfection in rooms of patients with *C. difficile* and MRSA reduced acquisition of MRSA and *C. difficile* on hands after contact with surfaces and reduced contamination of hands of health care providers caring for the patients.[267] In 2015 a study demonstrated that use of a daily disinfectant cleaner instead of a daily cleaner reduced hospital-acquired infection (HAI) rates.[268] These studies and others[86] support the use of hospital disinfectants, which are generally EPA registered. Unfortunately, studies have demonstrated that adequate environment cleaning is frequently lacking.[269] For example, Carling and coworkers assessed the thoroughness of terminal cleaning in the patient's immediate environment in 23 acute-care hospitals (1119 patient rooms) by using a transparent, easily cleaned, stable solution that fluoresces when exposed to hand-held UV light.[60] The overall thoroughness of cleaning, expressed as a percent of surfaces evaluated, was 49% (range for all hospitals, 35%–81%). A 2017 study of hospitals that implemented a program to improve the thoroughness of disinfection of near-patient surfaces found that thoroughness of cleaning scores improved from 61% to >80% and that the increase could be maintained over time by means of repeated cycles of assessment paired with repeated feedback and education sessions.[270] An ATP detection device combined with educational feedback for environmental service workers also resulted in significant improvement in cleaning efficacy of the hospital room environment.[271] Preliminary results suggest that a novel chemical additive that colorizes chlorine-based disinfectants to visualize surface coverage improves sites of application, which may improve cleaning by environmental services personnel and may reduce microbial contamination of surfaces.[272]

Improving Room Cleaning and Disinfection, and Demonstrating the Effectiveness of Surface Decontamination in Reducing Health Care–Associated Infections

Investigators have reported that intervention programs aimed at environmental service workers resulted in significant improvement in cleaning practices.[60] Such interventions have generally included multiple activities: improved education, monitoring of the thoroughness of cleaning (e.g., through use of ATP assays or fluorescent dyes) with feedback regarding performance given to the environmental service workers,[270] and/or use of cleaning checklists. We have found that assignment of cleaning responsibility (e.g., medical equipment to be cleaned by nursing; environmental surfaces to be cleaned by environmental service) is also important to ensure that all objects and surfaces are decontaminated, especially the surfaces of medical equipment (e.g., cardiac monitors). Improved environmental cleaning has been demonstrated to reduce the environmental contamination with VRE,[273,274] MRSA,[274] and *C. difficile.*[275] Important to note, no study has reported proper cleaning of more than 85% of objects in the postintervention period. Furthermore, all studies have focused on improvement in only a limited number of "high-risk" objects. Therefore a concern regarding published studies is that they have demonstrated improved cleaning of only this limited number of "high-risk" objects (or "targeted" objects) rather than an improvement in the overall thoroughness of room decontamination.

"No-Touch" Methods for Room Decontamination

As noted earlier, multiple studies have demonstrated that environmental surfaces and objects in rooms are frequently not properly cleaned and that these surfaces may be important in transmission of health care–associated pathogens. Furthermore, whereas interventions aimed at improving cleaning thoroughness have demonstrated effectiveness, many surfaces remain inadequately cleaned and therefore potentially contaminated. For this reason, several manufacturers have developed room disinfection units that can effectively decontaminate environmental surfaces and objects and/or inoculated test surfaces.[276–296] The two systems that have been studied comprehensively and are discussed here are UV light and hydrogen peroxide.[259,297,298] These technologies supplement, but do not replace, standard cleaning and disinfection because surfaces must be physically cleaned of dirt and debris.

Ultraviolet Light for Room Decontamination

UV irradiation has been used for the control of pathogenic microorganisms in a variety of applications, such as control of legionellosis, and for disinfection of air, surfaces, and instruments.[297,299] At certain wavelengths, UV light will break the molecular bonds in DNA, thereby destroying the organism. UV-C has a characteristic wavelength of 200 to 270 nm (e.g., 254 nm), which lies in the germicidal active portion of the electromagnetic spectrum of 200 to 320 nm. Another UV device uses pulsed xenon radiation, which produces UV light in the 200- to 320-nm range. The efficacy of UV irradiation is a function of many different parameters such as organic load, distance from the UV device, pathogen, dose, exposure time, lamp placement, direct or indirect line of sight from the device, room size and shape, and air movement patterns. Studies have systematically investigated how these parameters affect the effectiveness of UV irradiation.[300,301]

Weber and colleagues summarized multiple studies that assessed the effectiveness of UV devices to inactivate microbes inoculated onto test surfaces that were then placed in a typical room.[300] The most commonly tested organisms were epidemiologically important health care–associated pathogens and included MRSA, VRE, *C. difficile*, and *Acinetobacter* spp. In general, the studies showed that >3-$\log_{10}$ vegetative bacteria can be killed in 5 to 25 minutes by UV-C and that UV-C

requires more time and energy to kill spore-forming organisms—for example, *C. difficile* spores.[276–284]

Hydrogen Peroxide Systems for Room Decontamination

Several systems that produce hydrogen peroxide (e.g., hydrogen peroxide vapor, aerosolized dry mist hydrogen peroxide) have been studied for their ability to decontaminate environmental surfaces, objects in hospital rooms, and unused medical supplies.[302] Hydrogen peroxide vapor has been used increasingly for the decontamination of rooms in health care settings.[281,285–294] These investigators found that hydrogen peroxide systems are a highly effective method for eradicating various pathogens (e.g., MRSA, *M. tuberculosis, Serratia, C. difficile* spores, *Clostridium botulinum* spores) from rooms, furniture, and equipment.

Comparison of Ultraviolet Irradiation Versus Hydrogen Peroxide for Room Decontamination

The UV-C device studied and the systems that use hydrogen peroxide have their own advantages and disadvantages,[303] and there is now ample evidence that these "no-touch" systems can reduce environmental contamination with health care–associated pathogens. However, each specific system should be studied and its efficacy demonstrated before being introduced into health care facilities. The main advantage of both types of units is their ability to achieve substantial reductions in vegetative bacteria. As noted earlier, manual cleaning has been demonstrated to be suboptimal because many environmental surfaces are not cleaned. Another advantage is their ability to substantially reduce *C. difficile,* given that low-level disinfectants (such as quaternary ammonium compounds) have only limited or no measurable activity against spore-forming bacteria.[303] Both systems are residual free and they decontaminate all exposed surfaces and equipment in the room.

The major disadvantages of both decontamination systems are the substantial capital equipment costs; the need to remove personnel and patients from the room, thus limiting their use to terminal room disinfection (must prevent or minimize exposure to UV light and hydrogen peroxide); the staff time needed to transport the system to rooms to be decontaminated and monitor its use; the need to physically clean the room of dust and debris; and the sensitivity to use parameters. There are several important differences between the two systems. The UV-C system offers faster decontamination, which reduces the downtime of the room before another patient can be admitted. The hydrogen peroxide systems have been demonstrated to be more effective in eliminating spore-forming organisms. Whether this improved sporicidal activity is clinically important is unclear; studies have demonstrated that although environmental contamination is common in the rooms of patients with *C. difficile* infection, the level of contamination is relatively low (also true for MRSA and VRE).

In the past 5 years, multiple trials have assessed the efficacy of UV light and hydrogen peroxide room decontamination units for reducing HAIs. Currently, six clinical trials have demonstrated a reduction of HAIs with the use of hydrogen peroxide systems, and seven clinical trials have demonstrated a reduction in HAIs with UV light.[259,298] However, 11 of these studies used a before-after design, which is more likely subject to bias than crossover studies or randomized clinical trials. Two studies used a stronger epidemiologic design—a prospective cohort study[304] or a randomized clinical trial.[279] Specifically, the later study in nine hospitals was a prospective, multicenter, cluster-randomized, crossover trial that evaluated three strategies for enhanced room disinfection: quat plus UV-C; bleach alone, and bleach plus UV-C. The study showed that enhanced room decontamination strategies (i.e., bleach and/or UV-C decontamination) decreased the clinical incidence of acquisition of target MDROs (i.e., MRSA, VRE, *C. difficile*) by approximately 10% to 30% ($P = .036$). Comparing the best strategy with the worst strategy (i.e., quat vs. quat plus UV) revealed that a reduction of 94% in epidemiologically important pathogens (i.e., 60.8 vs. 3.4) led to a 35% decrease in colonization or infection (2.3% vs. 1.5%). These data demonstrate that a decrease in room contamination was associated with a decrease in patient colonization or infection. To our knowledge, this was the first study that quantitatively described the entire pathway whereby improved disinfection decreased microbial contamination, which in turn reduced patient colonization or infection.[279,305]

Based on these data, hospitals should use a "no-touch" device for terminal room decontamination after discharge of patients on contact precautions. However, the multitude of commercially available devices makes choosing a device difficult. UV devices may vary because of differences in UV wavelength, dose, ability to measure dose, and cost. Similarly, hydrogen peroxide devices may differ with regard to concentration, method of injecting hydrogen peroxide into the room, dissemination of the hydrogen peroxide in the room, and cost. For these reasons, infection preventionists should review the peer-reviewed literature and choose for purchase only devices with demonstrated bactericidal capability as assessed by carrier test method and/or ability to disinfect actual rooms. Ultimately, one should choose only "no-touch" devices that have demonstrated the ability to reduce HAIs.[259,298]

Continuous Room Decontamination

Even after cleaning and disinfection, surfaces can rapidly become recontaminated. Thus, hands of health care providers can become colonized by touching contaminated environmental surfaces and patient care equipment, and inadequate hand hygiene or inappropriate glove use can then result in the transfer of health care pathogens from patient to patient. Because routine cleaning of room surfaces by environmental services is frequently inadequate, continuous room decontamination methods are being evaluated. These include visible light disinfection (high-intensity narrow-spectrum light)[306]; low-concentration hydrogen peroxide; persistent disinfectants[69,146,307,308]; and self-disinfection surfaces (e.g., copper).[309] These methods are under active investigation but to date have not been assessed for their ability to reduce HAIs.[309]

Surface Disinfection: Treatment Time (Wipes/Sprays) Versus Contact Time (Liquids)

Another current issue in disinfection is the correct interpretation, based on testing methodology, of the contact time (wet time) for liquid disinfectants used for surface disinfection versus the treatment time (undisturbed time, duration of wet time not relevant) for wipes and sprays used in surface disinfection. The issue of "contact time" and "treatment time" is complex because it is based on different EPA test methods used for liquid disinfectants versus a disinfectant "towelette" or "spray," respectively. The registration test for liquid disinfectants is the Association of Official Analytical Chemists (AOAC) use-dilution test, and the contact time should be the "wet" time. This simulates the contact time in the test tube with the inoculated carrier. The registration tests for a disinfectant wipe and the spray are the EPA disinfectant towelette test and germicidal spray test, respectively, and the label should be interpreted as the "treatment time." For the wipe, the "treatment time" is the kill time and is equal to the combination of the physical removal and inactivation caused by the disinfectant regardless of the surface appearance (e.g., wet vs. dry). For the spray, the "treatment time" is the kill time or the time for complete inactivation of the test bacteria caused by the disinfectant regardless of the surface appearance (e.g., wet or dry). So if a product is a liquid disinfectant (e.g., dilutable quaternary ammonium compound) and the label indicates an EPA registration label based on the use-dilution test of 2 minutes, then the treated surface should remain wet for 2 minutes. In contrast, if a disinfectant wipe or a spray has an EPA registration time of 2 minutes, then the surface (i.e., wiped or sprayed) should be allowed to remain undisturbed for the EPA registration time of 2 minutes (i.e., duration of wet time is not relevant). Infection preventionists, environmental service workers, nurses, regulators (e.g., state and federal), and accrediting agencies (e.g., The Joint Commission) surveying health care facilities should be aware of the different requirements for EPA registration of surface disinfectants registered by the Use Dilution Method (liquids have a contact time and wet time) and those tested by the towelette and spray tests (wipes have a treatment time and no wet time).[47]

OSHA Bloodborne Pathogen Standard

In December 1991, OSHA promulgated a standard entitled "Occupational Exposure to Bloodborne Pathogens" to eliminate or minimize occupational exposure to bloodborne pathogens.[310] One component of this

requirement is that all equipment and environmental and working surfaces be cleaned and decontaminated with an appropriate disinfectant after contact with blood or other potentially infectious materials. In February 1997, OSHA amended its policy that allowed the use of EPA-registered tuberculocidal disinfectants and/or diluted bleach solution and expanded it to include EPA-registered disinfectants that are labeled as effective against HIV and hepatitis B virus (HBV). These disinfectants would be considered as appropriate disinfectants "provided such surfaces have not become contaminated with agent(s) or volumes of or concentrations of agent(s) for which higher level disinfection is recommended."[311] When bloodborne pathogens other than HBV or HIV are of concern, OSHA continues to require the use of EPA-registered tuberculocidal disinfectants or hypochlorite solution (diluted 1:10 or 1:100 with water).[311] Studies have demonstrated that in the presence of large blood spills, a 1:10 final dilution of EPA-registered hypochlorite solution initially should be used to inactivate bloodborne viruses[85,312] in order to minimize risk of disease to health care workers from percutaneous injury during the cleanup process.

Emerging Pathogens, Antibiotic-Resistant Bacteria, and Bioterrorism Agents

Emerging pathogens are of growing concern to the general public and infection-control professionals. Relevant pathogens include *Cryptosporidium parvum, C. difficile,* severe acute respiratory syndrome (SARS) coronavirus, *Helicobacter pylori, E. coli* O157:H7, HIV, hepatitis C virus, rotavirus, multidrug-resistant *M. tuberculosis*, HPV, Middle East respiratory syndrome (MERS) coronavirus, hemorrhagic fever viruses (Lassa and Ebola), highly pathogenic avian influenza viruses (e.g., H5N1, H7N9), *C. auris,* norovirus, and nontuberculosis mycobacteria (e.g., *M. chelonae*).[313] Similarly, publications have highlighted the concern about the potential for biologic terrorism.[314] The Centers for Disease Control and Prevention (CDC) has categorized several agents as "high priority" because they can be easily disseminated or transmitted person to person, cause high mortality, and are likely to cause public panic and social disruption.[315] These agents include *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), variola major (smallpox), *Francisella tularensis* (tularemia), filoviruses (Ebola hemorrhagic fever, Marburg hemorrhagic fever), arenaviruses (Lassa [Lassa fever], Junin [Argentine hemorrhagic fever]), and related viruses.[315] *C. auris* is an emerging multidrug-resistant yeast that can cause invasive infections. *C. auris* can persist on surfaces in health care environments, and the CDC's interim disinfection recommendation for *C. auris* patient rooms is a hospital-grade disinfectant effective against *C. difficile* spores.[316] Studies have demonstrated that *C. auris* is inactivated (>4-$\log_{10}$ reduction in 1 minute) by numerous disinfectants (e.g., chlorine, accelerated hydrogen peroxide, quat-alcohol).[184,317]

With rare exceptions, the susceptibility of each of these pathogens to chemical disinfectants or sterilants has been studied, and all of these pathogens (or surrogate microbes such as feline calicivirus for norovirus, vaccinia for variola,[136] and *B. atrophaeus* [formerly *Bacillus subtilis*] for *B. anthracis*) are susceptible to currently available chemical disinfectants or sterilants.[94,229,318] Standard sterilization and high-level disinfection procedures for patient care equipment (as recommended in this chapter) are adequate to sterilize or disinfect instruments or devices contaminated with blood or other body fluids from persons infected with bloodborne pathogens, emerging pathogens, and bioterrorism agents, with the exception of prions (see CJD above). No changes in procedures for cleaning, disinfecting, or sterilizing need to be made.[8]

In addition, there are no data to show that antibiotic-resistant bacteria (MRSA, VRE, multidrug- resistant *M. tuberculosis*) or CRE are less sensitive to the liquid chemical germicides than antibiotic-sensitive bacteria at currently used germicide contact conditions and concentrations.[319–321]

CONTROL OF HOSPITAL WASTE

Health care facilities that generate medical, chemical, or radiologic waste have a moral and legal obligation to dispose of these wastes in a manner that poses minimal potential hazard to the environment or public health. The proper disposal of these wastes requires a dynamic waste management plan that conforms to federal, state, and local regulations and provides adequate personnel and financial resources to ensure implementation.

Medical waste disposal has been as a major problem in the United States for the past 40 years. The problem has developed as a result of medical waste washing ashore in some coastal states in 1987 and 1988 and the perceived threat of acquiring HIV infection via this waste. This has led to restrictive rules governing the disposal of medical waste in many states and an increase in the volume of waste defined as regulated medical waste. Coincidentally, with an increase in volume of regulated medical waste (formerly called "infectious waste"), the options for medical waste treatment and disposal are diminishing because of space and environmental concerns. This section reviews some of the principles associated with medical waste management; more detailed descriptions of collection, storage, processing, transporting, treatment, and public health implications of medical waste may be found elsewhere.[322–329]

Despite the attention given to medical waste by the public, the media, and all levels of government, the terms "hospital waste," "medical waste," "regulated medical waste," and "infectious waste" are often used as synonymous. Hospital waste refers to all waste, biologic or nonbiologic, that is discarded and not intended for further use. Medical waste refers to materials generated as a result of patient diagnosis, immunization, or treatment, such as soiled dressings or intravenous tubing. Infectious waste refers to that portion of medical waste that could potentially transmit an infectious disease. Congress and the EPA used the term "regulated medical waste" rather than "infectious waste" in the Medical Waste Tracking Act (MWTA) of 1988 in deference to the remote possibility of disease transmission associated with this waste. Thus, "medical waste" is a subset of "hospital waste," and "regulated medical waste" (which is synonymous with "infectious waste" from a regulatory perspective) is a subset of "medical waste."[323,327]

As stated, regulated medical waste (or infectious waste) is capable of producing an infectious disease. This definition requires a consideration of the factors necessary for disease induction that include dose, host susceptibility, presence of a pathogen, virulence of a pathogen, and the most commonly absent factor, a portal of entry. For waste to be infectious, therefore, it must contain pathogens with sufficient virulence and quantity that exposure to the waste by a susceptible host could result in an infectious disease. Because there are no tests that allow infectious waste to be identified objectively, responsible agencies (such as the CDC, the EPA, or state agencies) define waste as infectious when it is suspected to contain pathogens in sufficient number to cause disease. Not only does this subjective definition result in conflicting opinions from the CDC, the EPA, and state agencies regarding what constitutes infectious waste and how it should be treated, but it also gives undue emphasis to the mere presence of pathogens.

Guidelines produced by the CDC have designated five types of hospital waste as regulated medical waste (i.e., microbiology laboratory waste, pathology and anatomy waste, contaminated animal carcasses, blood, and sharps).[32] The EPA guidelines (not regulations) from 1986 consider the same types of waste as infectious or regulated medical waste but also designate communicable disease isolation waste.[328] In the MWTA, the EPA modified its position on "communicable disease isolation waste" by including only certain "highly" communicable disease waste such as waste associated with Biosafety Level 4 organisms (e.g., Marburg, Ebola, and Lassa viruses) as regulated medical waste (Table 299.5).[328] In a systematic random survey of all US hospitals conducted in July 1987 and January 1988, the overall compliance rates with the CDC and EPA recommendations were 82% and 75%, respectively. Not only were the majority of hospitals in compliance, but the hospitals frequently treated other hospital waste as infectious, including contaminated laboratory waste (87%), surgery waste (78%), dialysis waste (69%), items contacting secretions (63%), intensive care unit waste (37%), and emergency room waste (41%).[323]

A key component in evaluating the impact of a medical waste management program is the quantity of waste produced per patient. Hospitalized patients generate about 15 pounds of hospital waste per day. The amount of hospital waste generated by US hospitals is approximately 6700 tons per day. US hospitals designate approximately 15%

TABLE 299.5 Types of Medical Waste Designated as Infectious (or Regulated Medical Waste) and Disposal and Treatment Methods Recommended by the CDC and EPA[a]

	CDC		EPA		MWTA
SOURCE OR TYPE OF MEDICAL WASTE	**REGULATED MEDICAL WASTE METHODS**	**DISPOSAL AND TREATMENT**	**INFECTIOUS WASTE METHODS**	**DISPOSAL AND TREATMENT**	**REGULATED MEDICAL WASTE[b]**
Microbiologic (e.g., stocks and cultures of infectious agents)	Yes[c]	S, I	Yes	S, I, TI, C	Yes
Blood and blood products	Yes	S, I, Sew	Yes	S, I, Sew, C	Yes
Pathologic (e.g., tissue, organs)	Yes	I	Yes	I, SW, CB	Yes
Sharps (e.g., needles)	Yes	S, I	Yes	S, I	Yes[d]
Communicable disease isolation	No	—	Yes	S, I	Yes[d]
Contaminated animal carcasses, body parts, and bedding	Yes	S, I (carcasses)	Yes	I, SW (not bedding)	Yes
Contaminated laboratory wastes	No	—	Optional[e]	If considered IW, use S or I	No
Surgery and autopsy wastes	No	—	Optional	If considered IW, use S or I	No
Dialysis unit	No	—	Optional	If considered IW, use S or I	No
Contaminated equipment	No	—	Optional	If considered IW, use S or I	No

[a]The Joint Commission requires that there be a hazardous waste system designed and operated in accordance with applicable law and regulations.
[b]The Act went into effect on June 22, 1989 and expired June 22, 1991. It affected only four states (New Jersey, New York, Connecticut, and Rhode Island). The Act required both treatment (any method, technique, or process designed to change the biologic character or composition of medical waste so as to eliminate or reduce its potential for causing disease) and destruction (waste is ruined, torn apart, or mutilated so that it is no longer generally recognizable as medical waste).
[c]The CDC guidelines specify "microbiology laboratory waste" as an infectious waste. This term includes stocks and cultures of etiologic agents and microbiology laboratory waste contaminated with etiologic agents (e.g., centrifuge tubes, pipettes, tissue culture bottles).
[d]MWTA specified used and unused sharps. The Act regulated wastes from persons with highly communicable diseases such as class 4 etiologic agents (e.g., Marburg, Ebola, Lassa).
[e]Optional infectious waste: EPA states that the decision to handle these wastes as infectious should be made by a responsible, authorized person or committee at the individual facility.
C, Chemical disinfection for liquids only; *CB,* cremation or burial by mortician; *CDC,* Centers for Disease Control and Prevention[32]; *EPA,* Environmental Protection Agency[328]; *I,* incineration; *IW,* infectious waste; *MWTA,* Medical Waste Tracking Act[329]; *S,* steam sterilization; *Sew,* sanitary sewer (EPA requires secondary treatment); *SW,* steam sterilization with incineration or grinding; *TI,* thermal inactivation.
Modified from Rutala WA, Mayhall CG, Society of Hospital Epidemiology of America[327]; and Rutala and Weber.[9]

of the total hospital waste by weight as infectious (about 1000 tons of infectious waste per day).[323] Not surprisingly, the percentage of medical waste treated as infectious increases with the number and types of medical waste classified as infectious. For example, about 6% of hospital waste would be treated as infectious waste if the CDC guidelines are followed, but 45% of hospital waste could be considered infectious waste under the MWTA.[323,330]

The vast majority of US hospitals designate and treat microbiologic, pathologic, isolation, blood, and sharp waste as infectious.[323] In the late 1980s, treatment of infectious waste by US hospitals was most commonly accomplished by means of incineration (range, 64%–93% depending on type of waste), but emission regulations that limit air pollutants have reduced the number of incinerators and use of incineration for medical waste. For example, in September 1997 there were an estimated 2373 medical waste incinerators in the United States, but based on the EPA's 2010 inventory there were 54 infectious waste incinerators at that time.[331] Autoclaves or steam sterilizers have become the primary nonincineration technology used by hospital to process their regulated medical waste (except pathology waste) (E. Krisiunas, written communication, 2008). Several other nonincineration alternatives have been proposed for treating regulated medical waste (e.g., mechanical and chemical disinfection, microwave decontamination). Nonregulated medical waste is generally discarded in a properly sited and operated sanitary landfill because this is a safe and inexpensive disposal method (e.g., landfill disposal, $0.02–$0.05 per pound for nonregulated medical waste versus contract incineration [i.e., off-site], $0.20–$0.60 per pound for regulated medical waste).

The conflicting information in state and federal regulations is related to the paucity of microbiologic and epidemiologic evidence that medical waste represents a threat to public health. First, with the exception of "sharps" such as needles, which have caused disease only in an occupational setting, there is no scientific evidence that medical waste has caused disease in the hospital or the community. Second, data demonstrate that household waste contains on average of 100 times as many microorganisms with pathogenic potential for humans than medical waste.[327] Third, detailed reports of the beach washups found that the vast majority of waste on beaches was debris (about 99%) such as wood, plastic, and paper, not medical waste. EPA documents acknowledge that much of the medical waste that washed ashore in the summer of 1988 was syringe related (65%) and came from home health care and illegal drug use. Fourth, studies have shown that most US hospitals are in compliance with the CDC infectious waste guidelines. Fifth, although the principal purpose of the MWTA was to reduce medical waste on beaches, it has not demonstrated its intended benefit. The relative number of syringes on the beaches in the MWTA states was significantly greater during implementation of the Act (17.23%) than before the Act went into effect (3.2%).[330] If regulatory controls were based on epidemiologic, microbiologic, and environmental data, only two types of medical waste would require special handling and treatment—sharps and microbiologic waste.

Federal medical waste regulations have been promulgated by the Department of Transportation (DOT) and OSHA. The DOT regulation involves the transport of infectious substances and medical waste and went into effect January 1996.[332] The OSHA Bloodborne Pathogen Standard requires labeling to designate waste that poses a health threat in the workplace. The OSHA definition of regulated waste is not intended to designate waste that must be treated. Generators that apply the OSHA definition of regulated waste (rather than state regulations) to designate infectious waste for treatment by incineration or other means may unintentionally incur additional expenses.[310]

CONCLUSION

When properly used, disinfection and sterilization can ensure the safe use of invasive and noninvasive medical devices. However, current disinfection and sterilization guidelines must be strictly followed.

ACKNOWLEDGMENTS

We thank Maria F. Gergen and Amy W. Powell for recovering papers on disinfection and sterilization in the peer-reviewed literature from 2012 through 2017.

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The complete reference list is available online at Expert Consult.

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300 Infections Caused by Percutaneous Intravascular Devices

Susan E. Beekmann and David K. Henderson[a]

SHORT VIEW SUMMARY

Definition
- Infections caused by peripheral and central intravenous catheters, including nontunneled central catheters and tunneled catheters, peripherally inserted central venous catheters (PICCs), totally implanted intravascular access devices (ports), pulmonary artery catheters, and arterial lines

Epidemiology
- Rates of central line–associated bloodstream infections (CLABSIs) in 2009 ranged from 1.05 (pooled mean hemodialysis rate) to 1.14 (pooled mean inpatient ward rate) to 1.65 (pooled mean intensive care unit rate) bacteremias per 1000 central venous catheter (CVC) days.
- These rates reflect a 58% reduction in total estimated CLABSIs from 2001 to 2009; the reduction in incidence of *Staphylococcus aureus* CLABSIs was greater than for any other pathogen.
- The majority of CLABSIs now occur in inpatient wards and outpatient hemodialysis centers.

Microbiology
- Staphylococci predominate as the most frequently encountered pathogens in device-related infections; coagulase-negative staphylococci are the single most common cause of these infections, whereas *S. aureus* bacteremias have decreased.
- CLABSIs increasingly are caused by multidrug-resistant gram-negative rods.

Diagnosis
- Clinical markers show a poor correlation with intravenous device–related bacteremia.
- Blood culture results positive for coagulase-negative staphylococci, *S. aureus,* or *Candida* spp., in the absence of any other identifiable source of infection, increase the possibility of intravenous device–related bacteremia.
- Quantitative or semiquantitative culture of the catheter combined with two blood cultures (one peripheral and one through the catheter) is the most accurate diagnostic method for short-term central catheters.
- Paired quantitative blood culture was determined to be the most accurate diagnostic method for long-term devices, including tunneled and totally implanted catheters.
- Differential time to positivity (between cultures drawn through the catheter and peripherally) compares favorably with quantitative blood cultures for the diagnosis of CLABSIs.

Prevention
- Many CLABSIs can be prevented using simultaneous implementation of an array of practice improvements (i.e., "bundle").
- All health care personnel involved in catheter insertion and maintenance should complete an educational program regarding catheter-associated infections.
- Chlorhexidine solutions should be used for skin preparation before catheter insertion.
- Maximal sterile barriers should be used during insertion of CVCs.
- Do not routinely replace CVCs, PICCs, hemodialysis catheters, peripheral arterial catheters, or pulmonary artery catheters to prevent infection.
- Antimicrobial-impregnated catheters, if used, should only be used as part of a comprehensive nosocomial bacteremia prevention strategy.

The relentless progress of medical science and technology has been accompanied by the development of a host of new diagnostic and therapeutic medical devices, each of which is associated with its own complications. Included in the list of devices and the complications of their use to be discussed in this chapter are peripheral and central intravenous catheters, including nontunneled central catheters and tunneled catheters, peripherally inserted central venous catheters (PICCs), totally implanted intravascular access devices (ports), pulmonary artery catheters, and arterial lines.

As early as 1977, Maki[1] suggested that more than 25,000 patients develop device-related bacteremia in the United States each year. The burgeoning use of an ever-expanding array of vascular access devices in medicine has resulted in even more complications associated with their use. Rates of bacteremia associated with the use of intravascular devices increased significantly into the early 2000s. More recently, data indicate that the rates of central line–associated bloodstream infections (CLABSIs) are decreasing, at least partly as a result of prevention programs implemented in many hospitals in 2001. In 2016, the Centers for Disease Control and Prevention (CDC) estimated that CLABSIs decreased by 50% between 2008 and 2014.[2] An intense focus on prevention of health care–associated infections, including CLABSIs, and requirements for the incorporation of performance measures into regulatory and financial reimbursement systems is believed to have had a significant impact on these infections,[3] although some have suggested that linking infection rates directly to financial reimbursement may result in underreporting.[4–6] CLABSIs have been estimated to be the most costly health care–associated infection, increasing costs by $45,814 per case and thereby providing a clear incentive to invest in additional prevention measures.[7]

Such device-associated infections occur as sporadic cases, as well as in case clusters caused by the same organism. Vascular catheters have become an increasingly important source of bacteremias, increasing from 3% in the mid-1970s to 19% in the early 1990s.[8] Primary bacteremias (i.e., no apparent local infection elsewhere caused by the same organism) account for approximately one-half of all intensive care unit (ICU)–related bacteremias.[9–11] In cancer patients, 56% of all bloodstream infections from 1999 to 2000 were CLABSIs.[10] The problem of iatrogenic, device-associated bacteremia is not unique to the United States; in one prospective study of bacteremia from Australia, nosocomial bacteremias accounted for 40% of all cases of bacteremia, and half of the nosocomial cases were device associated.[12]

Both local and systemic infection may result from contamination of intravascular devices. Local cellulitis, abscess formation, septic thrombophlebitis, device-associated bacteremia, and endocarditis all occur as complications of intravascular therapy and monitoring.

PATHOGENESIS

For intravascular device–related bacteremia to occur, microorganisms must gain access to the extraluminal or intraluminal surface of the

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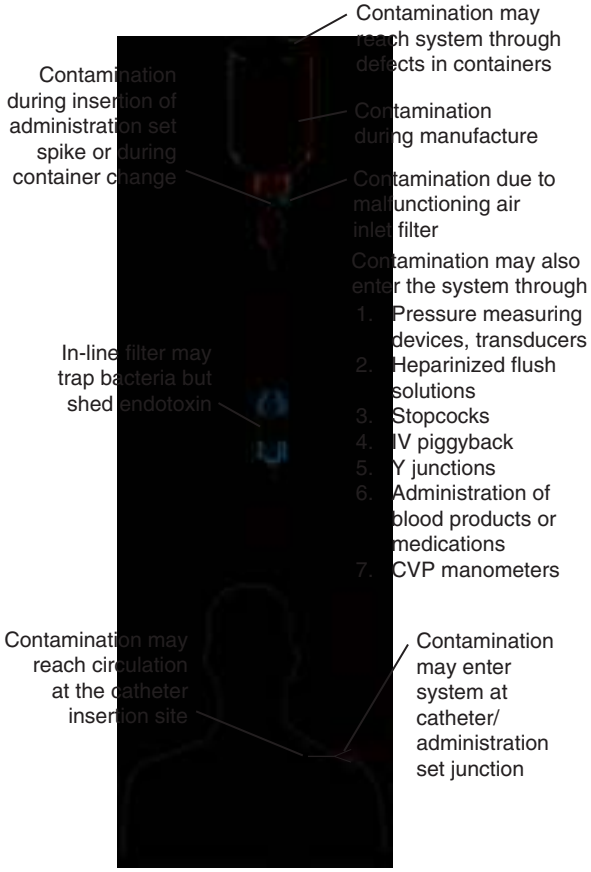

FIG. 300.1 Points of access for microbial contamination in infusion therapy. *CVP,* Central venous pressure; *IV,* intravenous.

device. Microbial adherence and incorporation into biofilms then occurs, resulting first in infection and then, in some instances, in hematogenous dissemination.[13] Fig. 300.1 illustrates the potential points of access to an intravascular device, each of which has been associated with both sporadic cases and case clusters of nosocomial bacteremia. Whereas the skin entry site has long been thought to be the most important portal of entry for invading microorganisms, the catheter hub–lumen has also been shown to be a major contributor to catheter-related bacteremia.[14,15] The most common point of access appears to vary, depending on the duration of time the catheter has been in place. Each of the three major sources of intravenous device–related bacteremia is discussed in the following sections.

Contamination of the Infusate

Contamination of the fluid administered through the device is a major cause of epidemic intravenous device–related bacteremias. Nonetheless, infusate contamination is a rare cause of bacteremia. Infusion-related sepsis has been reviewed in detail,[16] and both manufacture-related[17] and in-use[18] contamination of infusate have been documented as causes of device-associated sepsis.

Another factor influencing the pathogenesis of infusate-associated infection is the composition of the fluid. Different infusion fluids support the growth of differing pathogens. The microbiology of outbreaks of infusate-related sepsis is somewhat monotonous; pathogens such as *Enterobacter, Citrobacter,* and *Serratia* predominate. No infusate is entirely free of risk; even sterile water for injection can support the growth of *Burkholderia cepacia.*[19] Parenteral nutrition solutions are superb substrates for the growth of certain microorganisms.[20] Lipid emulsions support bacterial growth extremely well,[21] and their use has also been associated with a risk for fungemia caused by the lipid-dependent yeast *Malassezia furfur,* although not with contaminated infusates.[22] This risk has been primarily identified in the neonatal intensive care setting and has been less commonly seen in adults.[22] Several additional outbreaks of bacteremias have been linked to compounding pharmacies, some of which adhere to suboptimal quality control standards.[23,24] One national outbreak of *Serratia marcescens* bacteremias occurred as a result of contaminated magnesium sulfate solution,[24] and two more recently reported outbreaks were associated with contaminated heparin solutions.[23,25]

Parenteral nutrition solutions may also become contaminated during compounding in the hospital pharmacy.[26,27] Two similar outbreaks of *Candida parapsilosis* infections were linked to the backflow of yeasts into parenteral nutrition solution because vacuum pumps were used improperly.[26,27]

The composition of the infusate also influences the degree of irritation of the vascular intima at the site of infusion. Fluids that are not isotonic, those at nonphysiologic pH, and those containing particulates all may irritate the vascular wall, thus provoking thrombus formation. Such thrombi may be seeded with microbes—either hematogenously or by direct extension.

Contamination of the Catheter Hub and Lumen (Intraluminal Source)

Currently, bacteremias associated with long-term catheters appear to arise most frequently from an intraluminal source, perhaps after intraluminal colonization with biofilm-producing microbes.[28] Contamination of the catheter hub–infusion tubing junction is a significant contributor to device-associated infection.[15,29,30] Endemic coagulase-negative staphylococcal bacteremias often arise as a result of contamination of the catheter hub with these organisms. A randomized study examining the effects of a redesigned protective hub found these hubs to be associated with a significantly lower rate of catheter sepsis and culture-positive catheter hubs,[31] suggesting that the hub is a common portal of entry for bacteria. Other investigators have incriminated the hub-tubing junction (particularly when it does not allow a good fit) in the pathogenesis of epidemics of coagulase-negative staphylococcal infection.[32,33] Maki and Ringer[34] found hub contamination to be the second most heavily weighted risk factor for catheter-associated infection in a large, prospective study. Salzman and colleagues[35] noted that greater than 50% of episodes of central venous catheter (CVC)–related sepsis occurring in a neonatal ICU were preceded by colonization of the catheter hub with the infecting organism. In a subsequent experimental study, these investigators found that swabbing the catheter hub with disinfectant substantially reduced the hub's microbial burden and that preparations containing 70% ethanol were both more effective and more likely to be safer for the patient than preparations containing chlorhexidine. Sherertz[36] estimated that the hub, lumen, or both contributed two-thirds of the microorganisms that infected long-term catheters and that one-fourth of the microorganisms were from the skin.

Conversely, some newer technologies may be associated with increased risks for catheter-associated infection. Needleless intravenous systems were developed to provide a safer workplace environment for health care providers, and needle-free devices now constitute more than 80% of access devices and are recommended for all tubing/catheter access.[37] However, use of these devices has been associated with increased risk for device-associated infection.[38,39] Multiple investigations of bacteremia outbreaks associated with needleless devices have suggested that the mechanism for bacteremia may involve contamination from the end cap.[40–43] Disinfection is required before needleless connector access, with a minimum of 5 seconds of scrubbing,[44] but compliance has been poor in multiple studies.[45] Products to reduce contamination of needleless connectors have been developed, including silver-coated needleless connectors and disinfection caps.[46,47] A recent meta-analysis reported that needleless connectors with improved engineering design were associated with a lower central line–associated bloodstream infection risk.[48] Different studies have paradoxically found either increased or decreased risk with the same needleless system (e.g., the Interlink IV Access System [Baxter, Deerfield, IL]),[41,42] supporting the conclusion that the primary risk associated with these devices is related to how the systems are used (e.g., frequency of changing end caps and adherence to recommended infection control procedures) rather than factors intrinsic to the system.[42] Appropriate staff education regarding use of these devices, ensuring compliance with the manufacturer's recommendations, and consideration of needleless connectors with improved

engineering design when CLABSI rates remain stubbornly high is recommended to prevent device-related bacteremia.

Contamination of Skin at the Device Insertion Site (Extraluminal Source)

Many authorities believe that the catheter insertion tract provides the major avenue for the ingress of microbial invaders.[a] Several studies have focused on microbial colonization around the catheter insertion site as a significant risk factor for catheter-associated infection.[52] Supporting this contention are the studies of Cooper and Hopkins that demonstrated organisms on the exterior surface of catheters rather than within the catheter lumen.[53] In the prospective study of Maki and colleagues,[54] colonization around the catheter insertion site was the most strongly associated risk factor for local catheter infection. Similarly, Safdar and Maki[55] determined that most catheter-related bacteremias occurring with short-term noncuffed central catheters were extraluminally acquired and derived from the cutaneous microflora. Skin appears to be the primary source of intravenous device–related bacteremia for short-term catheters placed for an average duration of less than 8 days.[56,57]

Skin colonization is a dynamic process, and the dynamics of the cutaneous microbiota are only now being explored aggressively using genomic technology. Atela and coworkers[49] conducted a prospective study to assess the turnover of superficial skin colonization by performing serial quantitative cultures of skin and the catheter hub. Strains recovered from the targeted superficial skin sites demonstrated a poor correlation both with strains from previous skin cultures and with catheter tip isolates. Herwaldt and colleagues examined the source of coagulase-negative staphylococcal bacteremias in hematology-oncology patients and found that the same strain was identified in both skin and blood cultures in only 6 of 20 episodes.[58] The matching strain was isolated only from other sites (primarily nares) in the remaining 70% of episodes, leading these investigators to the conclusion that mucous membranes might be a reservoir for strains of coagulase-negative staphylococci causing bacteremia in immunocompromised patients. Of importance, these investigators were unable to identify colonization with the same strain for the majority of bacteremias; only 4 of the 21 nosocomial bloodstream infections were preceded by colonization with the same strain. Most nosocomial coagulase-negative staphylococcal bacteremias in this study appeared to result from extrinsic introduction of the organism.

EPIDEMIOLOGY

CLABSI Rates

Rates of CLABSIs in the United States have decreased substantially over the last two decades.[59–62] The introduction of widely adopted guidelines for the prevention of CLABSI in 2002,[37] followed by additional guidelines and central line care maintenance "bundles" (see later in this chapter), have altered patient care practices and contributed significantly to the overall decrease in these infections.[59,63–68] Other factors that have impacted CLABSI rates include the requirement for mandatory reporting of CLABSI rates in some states,[69] Medicare reimbursement penalties for health care–associated conditions imposed beginning in October 2008 and other pay-for-performance programs,[70–72] changes in surveillance definitions,[73,74] universal decolonization,[75] attention to decreasing blood culture contamination rates,[76,77] and new technology-based interventions (discussed later in this chapter). Some data suggest that institutions may also attempt to "game" the system to artificially decrease CLABSIs, either by decreasing the number of blood cultures drawn or by attributing bacteremias to sources other than central lines.[4–6]

Patient-Related Issues

Intravenous device–related bacteremia rates are influenced by patient-related parameters, catheter-related parameters, and hospital-related parameters (Table 300.1). Because of methodologic difficulties in performing appropriate scientific studies to characterize relative risk, many of these risk factors have been identified either retrospectively or in the epidemic setting. Still, each of the patient-related factors identified in Table 300.1 has been associated with an increased risk of device-associated infection.[78] Alteration of the patient's skin microbiota, either as a result of antimicrobial therapy or by colonization with an epidemic strain carried on the hands of hospital personnel, is a common event preceding catheter site infection. Failure of hospital personnel to perform appropriate hand hygiene procedures, particularly in the ICU setting, has been well documented.[79–81] Numerous epidemics of device-associated bacteremia have been linked to hospital personnel carrying an epidemic strain on their hands. Manipulating the system for repositioning, for obtaining a sample, or for any other reason increases the likelihood that the catheter may become contaminated.[82] The increasing prevalence of multiple drug resistance among health care–associated isolates causing these infections (e.g., vancomycin-resistant enterococci, multidrug-resistant *Acinetobacter baumannii,* carbapenem-resistant Enterobacteriaceae) has magnified this problem in the past decade.

The risk of developing catheter-associated bacteremia is related to the patient and his or her intrinsic host defense mechanisms, as well as to factors related to the patient's hospital environment and therapy (see Table 300.1). The physician cannot alter most such patient-related factors; however, these data can be used when evaluating the risks associated with, the necessity for, and the duration of intravenous therapy.

Catheter-Related Issues

Several catheter characteristics or properties have been suggested to be associated with an increased risk for catheter-associated infection. Catheters that irritate the vascular intima and provoke thrombogenesis and catheters that are made of intrinsically thrombogenic materials are likely to be associated with an increased risk for device-associated infection.[83] Older studies suggest that stiff catheters were associated with higher infection rates.[84] Such catheters are likely to be more mobile in the insertion tract and are thought to be more thrombogenic. A clear association has been established between the thrombogenicity of a catheter and the risk for device-associated infection.[85,86] Despite differences in thrombogenicity, some authorities believe that all catheters become coated with a fibrin sheath soon after placement.[87] Currently, the majority of catheters are manufactured with antithrombogenic polymers, such as polyurethane, Teflon, or others.

Catheter composition may influence the risk for infection in another way. Sheth and coworkers[88] have shown that certain microorganisms,

TABLE 300.1 Risk Factors for Device-Associated Bacteremia

Granulocytopenia
Immunosuppressive chemotherapy
Hematologic malignancy (versus solid tumor)[440]
Loss of skin integrity (e.g., burns, psoriasis)
Severity of underlying illness
Active infection at other site
Alteration in patient's cutaneous microflora
Failure of health care provider to wash hands
Contaminated ointment or cream
Catheter composition/construction:
Flexibility/stiffness
Thrombogenicity[83]
Microbial adherence properties and biofilm production[87]
Size of catheter
Number of catheter lumens[91]
Catheter function/use
Catheter management strategies—number of entries into the system
Type of catheter: plastic > steel
Location of catheter:
Central > peripheral
Jugular > femoral > subclavian[57,112,441]
Lower extremity sites > upper extremity sites
Type of placement: cutdown > percutaneous
Duration of placement[a] (at least 72 hours > less than 72 hours)[16,34]
Emergent placement > elective placement
Skill of venipuncturist (others > intravenous team)[16,106]
Type/use of catheter[262]:
Balloon-tipped, flow-directed > percutaneously placed
Central venous > implanted central venous
Nursing staffing variables[114,435,437]:
Nurse-to-patient ratio[434]
Lower regular registered nurse-to-patient ratio
Higher float pool registered nurse-to-patient ratio

[a]Although several studies support this precept, another has questioned it.

[a]References 1, 12, 15, 16, 34, 49–51.

most notably staphylococci, are able to adhere better to a catheter made from polyvinyl chloride than to a Teflon catheter. Rotrosen and colleagues[89] demonstrated increased adherence of *Candida* spp. to polyvinyl chloride catheters compared with Teflon catheters. In a rabbit model, silicon catheters are easier to infect with *Staphylococcus aureus* than are those made of polyurethane, Teflon, or polyvinyl chloride.[90] One might hypothesize that materials that facilitate microbial adherence may be associated with an increased risk for device-associated infection. Newer therapeutic interventions have focused on diminishing adherence to the catheter through use of chelating agents, such as ethylenediaminetetraacetic acid (EDTA) or sodium citrate, or ethanol locks.

The physical size of the catheter (and therefore the size of the defect in the skin's intrinsic host defenses) is also likely to be correlated with increased risk. Similarly, increasing the number of lumens in a catheter has been suggested to increase the risk for catheter-associated infections. Several studies have suggested that the use of multiple-lumen catheters is associated with an increased risk for catheter-associated infection compared with the use of single-lumen catheters,[42,91,92] although not all studies have found this difference.[93,94]

The presence of distant infection resulting in hematogenous seeding of the intravascular device has been incriminated in the pathogenesis of device-associated infection in some series.[52,95] Several factors may influence the risk for catheter seeding, including catheter composition, local thrombus formation at the catheter insertion site, intensity of bacteremia, the infecting pathogen, duration of catheterization, duration of bacteremia, and the patient's ability to mount an immunologic response to the infection.

Formation of a bacterial biofilm is now thought to be a virtually universal phenomenon that begins within 3 days after insertion of intravascular devices.[87] Biofilm formation is more predominant on the external surface of catheters in place fewer than 10 days, and then predominates intraluminally in catheters in place for at least 30 days.[96] Microorganisms embed themselves in and under the biofilm layer and become the source of intraluminal colonization and, eventually, the sources of CLABSIs.[96] Certain chelators, including EDTA and sodium citrate, inhibit bacterial growth and fibrin formation.[97] Ethanol lock therapy, in which a high concentration of ethanol is instilled within a catheter and left to dwell, also results in biofilm reduction or eradication.[96] The presence of fibrin deposits may, at least in part, explain the difficulty in treating totally implanted venous access port bacteremias without removal of the device.[98,99] Antibiotic concentrations should be 100 to 1000 times greater to kill sessile bacteria within a biofilm than for planktonic bacteria.[100–102]

Finally, the manner in which the catheter is used may influence risk. For example, risks for infection with pulmonary artery catheters may be higher because of the manner in which they are used.[103] In critically ill patients, these catheters are used intensively (although now somewhat less frequently than in the recent past). They are frequently repositioned to obtain accurate readings, they are used to obtain samples for the measurement of cardiac output, and they can be used to obtain mixed venous blood to measure oxygen and carbon dioxide tensions.

Catheter management, including both insertion and maintenance, also may influence risk for infection. Several studies have shown that catheters placed by less experienced personnel are at increased risk for infection.[104,105] Another study analyzed the efficacy of using a skilled team for the placement of peripheral intravenous catheters.[106] In this study, an intravenous therapy team significantly reduced both local and bacteremic complications associated with the placement of peripheral intravenous catheters, in part related to the timely replacement of the catheters. Two studies suggest that insertion of CVCs with less than maximal sterile barriers increases the risk of catheter-related infection.[57,107] Several studies have suggested that the number of times the system is entered also influences the risk for infection.[82,108,109] More than a single attempt to insert the catheter has also been found to be a risk factor for bacteremia,[94] as has the number of venous catheters in place and the intensity of their use.[110] Insertion at a subclavian rather than a femoral site is clearly associated with a lesser risk of both infectious and thrombotic complications.[91,111,112] Ultrasound-guided insertion of nontunneled CVCs has been found to reduce mechanical complications and decrease time to insert these catheters, but was found to have no effect on incidence of CLABSI or on mortality.[113]

Hospital-Related Issues

In addition to patient-related risk factors, several hospital-related risk factors for CLABSIs have been either identified or proposed (see Table 300.1). In contradistinction to the patient-related factors, such hospital-related factors can often be altered for patient benefit. Nurse staffing variables, including nurse-to-patient ratio, level of training, and permanent assignment to the unit ("float" nurse vs. regular unit staff nurse), have been shown to affect bacteremia rates.[114,115] As noted previously, the level of experience of the individual inserting the catheter (i.e., the number of previous catheter insertions) was also found to affect catheter-related bacteremia rates.[116] In addition, a number of studies have found that an education program focusing on risk factors and practice modifications is associated with decreased rates of CLABSIs.[117,118]

MICROBIOLOGY

Staphylococci continue to predominate as the most frequently encountered pathogens in device-related infections. Although *S. aureus* is a frequent cause of device-associated infection, the coagulase-negative staphylococci are the most common causes of these infections, especially in immunocompromised patients and those in whom long-term central venous access is required.[119] Multiple reports indicate that CLABSIs caused by *S. aureus* have decreased more than other pathogens over the last decade.[120]

Although there are some minor microbiologic differences among the devices or therapies under discussion, as a genus, *Staphylococcus* accounts for the majority of the episodes of bacteremia associated with these devices.[8,121] Studies suggest that coagulase-negative staphylococci may be able to adhere to plastic catheters more aggressively than can other organisms.[122] This property would result in a selective advantage for coagulase-negative staphylococci in causing device-associated infections.

Other commonly encountered isolates are listed in Table 300.2. One study of patients with hematologic malignancies and having Hickman catheters inserted identified a predominance of gram-negative organisms (68%) causing catheter-related bacteremias in this nonneutropenic population.[123] Raad and colleagues[10] reported that gram-negative bloodstream infections in cancer patients with solid tumors were most likely catheter related. National Healthcare Safety Network (NHSN) data from 2009 to 2012 from adult oncology units still revealed a dominance of coagulase-negative staphylococci, but increased rates of CLABSI in this population with fluoroquinolone-resistant *Escherichia*

TABLE 300.2 Microbiology of Device-Associated Bacteremia

Coagulase-negative staphylococci, including *Staphylococcus epidermidis*[a]
Staphylococcus aureus
Enterococcus spp.
Serratia marcescens[b]
Candida albicans[c]
Candida tropicalis[c]
Pseudomonas aeruginosa[d]
Klebsiella spp.[b]
Enterobacter spp.[b]
Citrobacter freundii[b]
Corynebacterium (especially *C. jeikeium*)[e]
Acinetobacter (especially *A. baumannii*)[f]
Burkholderia cepacia complex[d]

[a]Most common pathogen for long-term lines; also associated with lipid infusions in neonates.
[b]Frequently associated with contaminated infusate.
[c]Most often associated with total parenteral nutrition; usually along the catheter path but occasionally as a result of contaminated infusate.
[d]May arise from a water source (e.g., infusate) or may reflect cutaneous colonization.
[e]*C. jeikeium* bacteremia occurs almost exclusively in severely immunosuppressed patients who are or have been receiving broad-spectrum antibiotics and who have indwelling intravascular devices.
[f]*A. baumannii* (often multiply drug-resistant) is becoming increasingly prevalent as a pathogen in intensive care units, especially among critically ill patients who require life support interventions (e.g., ventilator support) and those who have received multiple courses of antimicrobials.

coli and vancomycin-resistant *Enterococcus faecium* were also noted.[124] Our own experience would suggest that gram-negative bacilli may predominate among stem cell transplant recipients, particularly those who have gastrointestinal involvement with graft-versus-host disease. The past decade has witnessed an increasing occurrence of CLABSIs caused by multidrug-resistant gram-negative rods, most notably *A. baumannii.*[125] CLABSIs caused by *A. baumannii* often occur in critically ill, immunosuppressed, highly antimicrobial agent–experienced patients who can ill afford any bacteremia, let alone one caused by multidrug-resistant bacteria.[126]

Patients with femoral catheters in one study had higher rates of gram-negative bacteremias and yeast-related fungemias.[127] The occurrence of some of the more unusual isolates (e.g., *Enterobacter* spp., *B. cepacia,* and *Citrobacter freundii*) as a clear cause of the device-associated infection should at least suggest the possibility of a contaminated infusion product or an aqueous environmental reservoir for these pathogens.[128–130]

Other organisms may cause such infections (e.g., *Flavobacterium* and *Acinetobacter* spp.); however, such organisms have been infrequently associated with either infusion-related or cannula-related infections.[18] Concomitant with the increasing empirical use of broad-spectrum antimicrobials in severely immunosuppressed patients, cases of device-associated bloodstream infection caused by a variety of unusual bacterial, mycobacterial, and fungal pathogens have been reported with increasing frequency[12,131–138]

DIAGNOSIS

Clinical distinction between catheter-associated septicemia and sepsis from another site is sometimes difficult. Clinical markers show a poor correlation with intravenous device–related bacteremia.[139] Serum procalcitonin has been studied extensively as a marker of sepsis, and one analysis concluded that it was the best predictor of CLABSI of any available test.[140] Nonetheless, currently available assays often do not provide definitive results; many patients with bacteremia have indeterminate levels of procalcitonin.[141] The presence of fever has a high sensitivity for bacteremia but poor specificity, and local inflammation has better specificity but poor sensitivity. In addition to the presence of an indwelling intravascular device, several clinical features should alert the physician to the possibility of device-associated bacteremia. Salient features of device-associated sepsis that help distinguish it from other bacteremic syndromes are listed in Table 300.3. In general, blood culture results positive for coagulase-negative staphylococci, *S. aureus,* or *Candida* spp., in the absence of any other identifiable source of infection, increase the possibility of intravenous device–related bacteremia.[139] The 2009 Infectious Diseases Society of America (IDSA) guidelines add to this definition one of the following: (1) the same organism growing from a percutaneous blood culture and a catheter tip culture; (2) a catheter draw-back culture and a peripheral culture that have the same organism, with the catheter blood culture becoming positive 2 hours earlier or having a quantitative blood culture that is threefold higher; or (3) blood cultures drawn through different catheter lumens that differ threefold in quantitative cultures.[139] These definitions were intended to apply to treatment of catheter-acquired infections, not for surveillance purposes.

TABLE 300.3 Factors Differentiating Device-Associated Bacteremia From Other Septic Syndromes

Local phlebitis, inflammation, or both at catheter insertion site
Lack of other source for bacteremia
Sepsis occurring in a patient not otherwise at high risk for bacteremia
Localized embolic disease distal to cannulated artery[146]
Hematogenous *Candida* endophthalmitis in patients receiving total parenteral nutrition[230,231]
Presence of ≥15 colonies of bacteria on semiquantitative culture of the catheter tip[144,145]
Sepsis apparently refractory to "appropriate" antimicrobial therapy
Resolution of febrile syndrome after device removal
Typical (*Staphylococcus aureus, Staphylococcus epidermidis,* or other coagulase-negative staphylococci) or unusual (*Burkholderia cepacia* complex, *Pantoea [Enterobacter] agglomerans, Enterobacter cloacae*) microbiology
Clustered infections caused by infusion-related organisms

Although none of these criteria specifically identifies the intravascular device as the source of sepsis, the presence of these clinical findings should at least raise the possibility of device-associated bacteremia.

Cultures of the catheter tip have been reported to be of variable and limited value. Catheter tip cultures should not be performed for diagnosis of bacteremia related to subcutaneous venous ports; instead, culture of the material inside the port reservoir is more sensitive.[99,142,143]

Using the semiquantitative culture technique, which defines a positive catheter tip culture as yielding 15 or more colonies,[144,145] in combination with a relatively strict definition of catheter-associated sepsis, Maki and colleagues reported a specificity in short peripheral catheters ranging between 76% and 96% and a positive predictive value of a positive catheter tip culture ranging between 16%[145] and 31%[144] in four studies.[144–147] Data regarding the sensitivity of the semiquantitative culture technique are not available because the authors incorporated having a positive catheter tip culture as part of their definition of both local catheter infection and catheter-acquired bacteremia.[144–147] The cutoff point of 15 colonies per catheter as a definition for "infection" appears to have been somewhat arbitrary in these studies. The authors noted that most infected catheters yield confluent growth when using the semiquantitative technique. These original studies from the late 1970s found that *S. aureus,* rather than coagulase-negative staphylococci, *Candida* spp., and *Enterococcus* spp., were the predominant microorganisms causing bacteremia, and short peripheral catheters (5.7 cm) or steel needles comprised most of the catheters studied. Given the current differences in microbiology and in intravascular devices now in use, these studies are less relevant in the 21st century than they were 40 years ago.[148]

Several investigators have tried to modify Maki's technique to improve the predictability of the procedure. Cleri and coworkers[149] reported a technique for quantitatively culturing catheters in broth. This system, which is slightly more cumbersome for the laboratory, was considered by these authors to have three advantages over the system described by Maki and colleagues: (1) the ability to detect organisms within the lumen, (2) the ability to evaluate relative numbers of organisms from different catheter segments, and (3) the ability to compare relative numbers of organisms present in mixed infections.[149] Brun-Buisson and colleagues[150] used a simplified quantitative broth dilution tip culture to evaluate catheters as potential sources for infection and found this technique to be 97.5% sensitive and 88% specific for the diagnosis of device-associated bacteremia, using a strict clinical definition.[150] Use of this technique was not found to be predictive of subsequent bacteremia when all catheter tips were cultured upon removal.[151] Subsequently, Gutierrez and associates[152] found a modified broth dilution technique to be only slightly more sensitive, but substantially more labor intensive, than the semiquantitative technique. The latter authors advocated the use of semiquantitative cultures because of the ease with which this test is performed. Hnatiuk and coauthors[153] demonstrated a substantial increase in sensitivity for the semiquantitative technique when the catheter tip cultures are plated at the patient's bedside rather than cutting the tip into a sterile tube and sending the tip to the laboratory for culture.

Siegman-Igra and colleagues[154] conducted a meta-analysis of catheter culturing techniques and suggested that the accuracy increases for catheter segment cultures with increasing quantitation (i.e., qualitative < semiquantitative < quantitative). The increase in accuracy is primarily due to the increased specificity of the more quantitative tests. They found that quantitative catheter segment culture was the only method associated with sensitivity and specificity greater than 90%.[154] Similarly, Sherertz and associates[155] suggested that the common practice of culturing a single segment of a central vascular catheter is inadequate. Safdar and colleagues[156] also found that qualitative culture of the catheter segment was the least accurate of the tests they studied, primarily because of poor specificity.

Although the relative merits of these various procedures remain to be definitively delineated, the ease of performing the semiquantitative technique described by Maki and coworkers[144,145] has kept this procedure in widespread clinical use. Attempts to culture newer catheters with antimicrobial coating used for prevention of CLABSIs may lead to false-negative results.[157] Specific inhibitors are available to inhibit the effect for chlorhexidine–silver sulfadiazine–coated catheters,[157] but no inhibitors have been identified for minocycline-rifampin–coated catheters.

Other investigators have suggested alternative techniques for diagnosing catheter-associated infections[154,158–160] Acridine orange leukocyte cytospin testing of blood drawn through the catheter has been studied as a method of diagnosing infection while maintaining the catheter.[161,162] A meta-analysis concluded that this test offers rapid diagnosis of catheter-related bacteremia, with good accuracy but lower sensitivity.[156] Although some experience is being gained with the direct Gram stain, none of these newer techniques appears to be effective enough to supplant the semiquantitative method. Kite and colleagues[159] have published a description of an endoluminal brush method, suggesting that this procedure can be used without sacrificing the intravascular line. Although these authors suggest that the procedure is substantially more sensitive and more specific than the semiquantitative technique, only 1% of 692 infectious disease physician survey respondents recently reported using this method.[4] Others have reported that endoluminal catheter colonization is invariably present in cases of catheter-related bacteremia.[32] Some authors have recommended a combination of direct and microbiologic techniques.[154,158]

Mosca and associates[163] emphasized the benefits of obtaining blood cultures by using the Isolator system (Wampole Laboratories, Cranbury, NJ), which allows a quantitative estimate of microbial burden in the specimen. Whereas several additional studies have underscored the usefulness of the Isolator system, one study has suggested that results obtained with traditional blood cultures may be complementary to those obtained with the Isolator system and that, whenever feasible, both approaches should be used.[164] A survey of infectious diseases physicians published in 2012 found that quantitative blood cultures were unavailable in 75% of their institutions.[4] Paired quantitative blood cultures also have been studied.[142] This technique involves drawing one set of cultures through the device and another set percutaneously; catheter-related infection is diagnosed when cultures are positive from both sites and the concentration of microorganisms in the culture from the device is three- to fivefold higher than in the peripheral culture.

Safdar and colleagues[156] performed a meta-analysis to determine the most accurate diagnostic methods for intravenous device–related bacteremia. They determined that quantitative or semiquantitative culture of the catheter, combined with two blood cultures (one peripheral and one through the catheter), is most accurate for short-term central catheters.[156] Paired quantitative blood culture was determined to be the most accurate diagnostic method for long-term devices, including tunneled and totally implanted catheters. These authors specifically recommend, as does the IDSA,[68,139] that vascular catheters not be cultured if no signs of infection are present. As noted previously, several studies have recommended a differential comparison of quantitative cultures obtained peripherally and quantitative cultures obtained by drawing blood back through the suspected catheter to document the occurrence of catheter-acquired sepsis.[10,165,166] Some authors have suggested that paired quantitative blood cultures (as described previously) obviate the need to remove the catheter and include culture of the tip in the diagnostic algorithm.[167] Because of the complexity of the epidemiology of device-associated bacteremia and the increased technical complexity of the differential blood culturing procedure, the broad applicability of these and the earlier findings nonetheless remains unclear. In addition, despite the evidence that quantitative blood cultures are useful in diagnosing intravascular catheter bacteremias, many clinical microbiology laboratories do not offer this service because of the cost and complexity.[4]

Recently, interest has focused on a method that is based on differential time to positivity of qualitative blood cultures drawn simultaneously both from a catheter and peripherally.[168–172] Blot and coworkers[169] suggested that the speed with which bacterial isolates can be detected in the microbiology laboratory may distinguish catheter-associated from non–catheter-associated infection. Presumably because of a higher bacterial concentration, the blood cultures drawn through an infected CVC often demonstrate growth much more rapidly than those drawn peripherally.[171] Other groups have determined that this differential time to positivity (between cultures drawn through the catheter and peripherally) compares favorably with quantitative blood cultures for the diagnosis of CLABSIs.[168,172,173] This same differential time to positivity was examined in a group of cancer patients[173–175] and in critically ill patients[176] and was found not to be useful for many clinical presentations, leaving the validity of this factor undetermined for clinical use. Safdar and coauthors[156] examined this methodology in a meta-analysis and concluded that although paired quantitative blood cultures were the most accurate diagnostic method, using the differential time to positivity provides comparable sensitivity and acceptable specificity. This technique is available in most, but not all, clinical laboratories.[4] The recorded time is when the bottle is logged into the laboratory, not when the blood was placed in the bottle, assuming that growth was minimal before incubation.

The usefulness of "through-the-line" cultures has been questioned repeatedly because these cultures may become contaminated easily while being drawn from the catheter hub.[177,178] Other data suggested that rates of contamination for venipuncture versus cultures drawn through the catheter were not significantly different.[179] The incidence of false-positive draw-back cultures may greatly depend on the type of intravenous device used to draw the cultures and the care taken in obtaining the specimen. If the details of the method used to obtain the culture are unknown, relying entirely on cultures obtained by drawing blood through an indwelling catheter may be imprudent.[180] In well-defined circumstances in which device-associated bacteremia is an important consideration in the patient's differential diagnosis, these cultures may provide valuable information.[149–152,163–166,181] As reported by DesJardin and colleagues,[182] culture of blood drawn through either a central catheter or a peripheral vein shows excellent negative predictive value. Blood cultures drawn through a central catheter have a lower positive predictive value than do blood cultures drawn percutaneously, but drawing blood through a catheter may be an acceptable method for ruling out bacteremia. We recommend drawing at least two sets of blood cultures when device-related bacteremia is suspected, with at least one set drawn percutaneously.

CDC surveillance definitions for CLABSIs have become increasingly important over the last several years because the Centers for Medicare and Medicaid Services (CMS) now requires hospitals that receive reimbursement from Medicare to report CLABSIs acquired in their ICUs to the NHSN, and also now is using CLABSI rates to qualify hospitals for their annual payment update. In the CDC's 2013 update to the NHSN definitions and surveillance protocols, hospitals were then allowed to differentiate bloodstream infections associated with mucosal barrier injuries–laboratory-confirmed bloodstream infections (MBI-LCBIs) in their reports to the NHSN, although these infections are still considered to be CLABSIs for reporting purposes if central line requirements are met.[183] MBI-LCBIs are identified based on either a patient's white blood cell count or history of allogeneic stem cell transplant or both, combined with gastrointestinal graft-versus-host disease or a minimum amount of diarrhea in a 24-hour period. Inclusion of an NHSN definition for bloodstream infections associated with mucosal barrier injuries should assist with informing preventive efforts because this specific subset of infections is not amenable to bundled strategies to prevent CLABSIs (see later) and may otherwise prevent hospitals from "getting to zero."[184] Differences between the NHSN and IDSA definitions of CLABSI and MBI-LCBI may account for important differences in attributing infection to a catheter source. This difference in attribution has been assessed retrospectively at the MD Anderson Cancer Center. The distinction in attribution of source is largely based on the IDSA inclusion of quantitative blood cultures drawn simultaneously from the catheter and peripheral blood. In this cancer population, NHSN definitions overestimate the catheter as the source of bloodstream infection. However, the authors emphasized that patients with MBI should not just be excluded from assessments of CLABSI, which would lead to underestimation in cancer patients.[185] The CDC reported that CLABSI rates decreased significantly in location types after moving MBI-LCBI events.[74]

During the past 15 years, non–culture-based diagnostic methods have begun to play an increasingly prominent role in the diagnostic microbiology laboratory.[186,187] Examples of the usefulness of these techniques include the use of a variety of polymerase chain reaction–based diagnostics to more rapidly detect pathogens, compared with conventional blood culture,[188] and the molecular identification of antimicrobial resistance even before speciation can be completed.

These molecular techniques are particularly valuable in epidemiologic investigations.

Finally, avoiding contaminating blood cultures is vitally important to accurate diagnosis of bloodstream infection.[188] Blood culture contamination can result in unnecessary antibiotic treatment, laboratory expense, and longer hospitalization.[177,189,190] A number of approaches can decrease contamination rates, including drawing blood through peripheral venipuncture rather than via an intravascular catheter, use of sterile gloves, cleaning tops of blood culture bottles with antiseptics, inoculating blood culture bottles before other blood tubes, and using a phlebotomy team to draw blood cultures.[190] Several technologic approaches to reducing blood culture contamination have been suggested. For cultures drawn through existing lines, one study recommended changing the needleless cap before drawing blood cultures as a way to obtain more specific and reliable results.[191] A needleless device is used to inject blood into the blood culture bottles. More recently, Rupp and colleagues studied a specimen diversion device that diverts and sequesters the initial 1.5- to 2-mL portion of blood, which presumably carries contaminating skin cells, microbes, and infusate.[192]

DEVICE-SPECIFIC ISSUES

Peripheral Intravenous Cannulization

In general, peripheral catheters are associated with much lower infection risks than are central catheters. The incidence of bacteremia associated with peripheral catheters was recently estimated to be 0.18% (range 0%–2.2%), with a mean of 22% (range 7%–60%) of nosocomial catheter-related bloodstream infections due to peripheral catheters.[193] The CDC recommends that peripheral catheters be replaced every 72 to 96 hours in adults to prevent phlebitis, whereas catheters should not routinely be replaced in children.[68] A large, multisite, randomized trial of peripheral catheter replacement found no difference in the primary outcome of phlebitis (7%) when catheters were replaced every 3 days versus when clinically indicated.[194] A recent meta-analysis found that a dwell time beyond 3 days independently increases risk of colonization and independently increases risk of bloodstream infection in adult patients.[193]

The location of catheter placement also may influence subsequent infection rates. Catheters placed in the lower extremities, particularly those placed in the femoral veins, are associated with increased risk for many complications, including infection.[195–197] One in three health care–associated *S. aureus* catheter-related bloodstream infections is due to peripheral catheters, and these infections remain a cause of significant morbidity and mortality.[193,198,199]

Catheters placed percutaneously are associated with lower infection rates than are those placed by cutdown.[200] Catheters placed emergently are also at higher risk for infection, presumably as a result of breaks in technique at the time of placement.[201] Several authors have suggested that catheters placed by members of an intravenous therapy team are associated with lower complication rates than are those placed by other health care professionals.[106,202]

Finally, two studies have demonstrated that routinely changing intravenous administration sets at 48 rather than 24 hours was not associated with a significant increase in the infusion-related bacteremia rate.[203,204] Snydman and associates[205] and Maki and colleagues[206] compared the relative safety of changing administration sets at 72-hour intervals with changing them at 48 hours. Neither study identified an increased risk with the 72-hour interval.

Thus inserting a peripheral catheter, dressing it, hooking up the administration set, and changing all three at 72- to 96-hour intervals seems safe, practical, and reasonable.

Central Venous Catheters

Short-Term Issues

Because CVCs frequently remain in place longer than peripheral catheters, certain problems either occur with more frequency or are unique to these catheters. In addition, because of the placement of these catheters in the great veins, complications of placement, such as infective endocarditis and suppurative thrombophlebitis of the great veins, represent life-threatening events.

Safdar and colleagues,[78] in a review of risk factors for CVC-related bloodstream infections, found that heavy colonization of the insertion site or contamination of a catheter hub, as well as duration of catheter placement for more than 7 days, significantly increased the risk of catheter-related bloodstream infection. Insertion technique and follow-up care were also reported as significant risks.[78] A multicenter trial with 3027 adult ICU patients randomly assigned subclavian, jugular, or femoral vein nontunneled central vein catheters. Subclavian lines were associated with less infection and symptomatic thrombosis but held an increased risk of pneumothorax.[207]

The presence of either intraluminal or extraluminal fibrin has been proposed as predisposing to the development of catheter-associated infection.[85,86,208,209] Contaminated fibrin sheaths have also been implicated in infection of right heart structures, producing an endocarditis-like infection associated with vegetations near the location where the tip of a central catheter had previously been placed.[210] Stillman and colleagues[86] studied 94 central catheters and found that all 11 catheters categorized as infected in their study and 30 of 83 not found to be either infected or colonized had gross visible evidence of either intraluminal or surface thrombin at the time of removal. Lloyd and coworkers[211] failed to find a deleterious effect associated with the so-called fibrin sheath when evaluating a rat model of device-associated bacteremia. Another rat model found that catheter colonization decreases with the decrease in fibrin within the pericatheter sheath.[212]

Routine replacement of CVCs is not necessary and exposes the patient to the increased risks of catheter manipulation and mechanical complications with new sites.[213] The CDC has recommended against routine replacement of CVCs, PICCs, hemodialysis catheters, or pulmonary artery catheters to prevent catheter-related infections.[68] The issue of whether central catheters should be changed over a guidewire remains controversial. Use of a guidewire obviates the need for a second percutaneous puncture of the great veins and may be preferable for catheter exchanges judged routine or mandated by some reason other than suspected infection. Little scientific evidence supports guidewire use if catheter-associated infection is suspected. Maher and colleagues[214] used this technique successfully for catheter exchange in situations assessed as "low risk for infection." Two additional studies suggest that guidewire exchange may not be particularly useful if device-associated infection is suspected.[215,216] In one of these two studies, guidewire catheter exchange was associated with an increased risk of bloodstream infection but a lower risk of mechanical complications.[216] In one experimental study, replacement of a biofilm-colonized CVC over a guidewire was associated with an increased risk for colonization of the new catheter, as well as an increased risk for production of detached, slime-enclosed, antibiotic-resistant aggregates that may disseminate the infection to other sites.[166]

Whereas guidewire exchange of CVCs may be associated with a greater risk for catheter-related infection, this technique may result in many fewer mechanical complications than would be the case for repuncture.[217] Specifically, exchange over a guidewire is associated with lower risks for some complications (e.g., bleeding and pneumothorax).[215,216] If a guidewire is to be used for catheter exchange, culture of the removed or "old" catheter tip should be performed. In addition, blood cultures should be drawn through the old line before removal. If either of these cultures becomes positive, the most conservative approach would be to remove the "replacement" catheter and perform appropriate cultures. If central access is still desired, a third catheter should be placed at a new puncture site. In situations in which the catheter is being removed for suspected sepsis, in our opinion, exchange over a guidewire should not be attempted. Conversely, one study suggested a possible benefit of guidewire exchange for patients who have catheter-associated candidemia.[218]

Thrombosis of the great veins, with the attendant risk for suppurative thrombophlebitis, is a major complication of central catheter placement.[197] Thrombosis occurs with increased frequency in patients with malignancies[219] (particularly those who have mediastinal lymphadenopathy) and in patients with sickle cell disease.[220] In instances in which central catheters are placed in patients with these underlying illnesses, thrombotic and infectious complications should be anticipated, prevented (when possible), diagnosed early, and treated aggressively.

Additional issues relating to catheter composition and effectiveness of subcutaneous tunneling of catheters, and risks associated with electronic monitoring devices, are discussed elsewhere in this chapter.

Parenteral Nutrition Issues

Several aspects of the delivery of parenteral nutrition separate this mode of intravascular therapy from others. First, the composition of the infusate supports the growth of different microorganisms, most notably certain of the *Candida* spp.[20,221] Patients receiving parenteral nutrition in one study had an increased incidence of polymicrobial and multidrug-resistant bacteremias.[222] Second, catheters used to deliver parenteral nutrition are often required to remain in place much longer than either peripheral or other central venous cannulas. For this reason, problems with catheter contamination become much more of a concern. Third, the hypertonicity of the solution causes irritation of the vascular intima, which in turn may cause thrombosis. Thrombosis provides a nidus for, and an increased risk for, infection. Fourth, because patients who require parenteral nutrition are frequently severely ill as a result of neoplasms, trauma, or inflammatory bowel disease, the risk for bacteremia is higher. Therefore the potential for hematogenous seeding of the catheter is high. Several studies have found an association between use of parenteral nutrition and an increased risk of death.[223,224] One study found that use of total parenteral nutrition declined significantly from 2000 to 2005 in critical care trauma patients, and this decrease was associated with fewer complications, including bacteremia and sepsis.[224]

For these and other reasons, the placement, management, and care of catheters used for parenteral nutrition have received a great deal of attention. Ryan and colleagues,[108] in a prospective study of 200 catheters, documented that the risk of catheter-associated infection increased significantly when the integrity of the delivery system was interrupted. Snydman and coworkers[109] subsequently found that the occurrence of so-called line violations was highly associated with the development of catheter-associated sepsis. For these reasons, administration of parenteral nutrition should be supervised and conducted by members of a team (Table 300.4).[225] In their study, Snydman and coworkers also attempted to correlate the results of twice-weekly 1-mL pour-plate blood cultures with the subsequent development of catheter colonization and sepsis. Although concordance was high among blood cultures, catheter tip cultures, and peripheral blood cultures, cultures obtained through the catheter demonstrated a reasonably high incidence of false positivity, primarily as a result of *Staphylococcus epidermidis* contamination.[109] One study found that increased parenteral caloric intake is an independent risk factor for bacteremia and that this association is unrelated to hyperglycemia.[226] A recent study attempted to ameliorate the infectious complications of parenteral nutrition by prospectively maintaining tight glucose control.[227] Tight glucose control in these patients was still associated with a greater than fourfold increase in the odds of having a catheter-related bloodstream infection.

Candida infection has been a particular problem in patients receiving parenteral nutrition.[228–231] Curry and Quie[229] reported a 16% incidence of candidemia among patients receiving parenteral nutrition in a prospective study in a hospital that did not have a dedicated team. In another prospective study of 131 postoperative patients who were receiving parenteral nutrition, 13 patients were detected as developing chorioretinal lesions consistent with hematogenous *Candida* endophthalmitis; 7 of the patients had positive blood cultures for *Candida*.[230] Although most of these infections are presumed to arise as a result of yeast contamination at the catheter entry site, occasional outbreaks of *Candida* infection resulting from parenteral nutrition solution that was intrinsically contaminated have been reported.[26,27]

Because the catheter frequently must be left in place for an extended period of time and because of the increased thrombogenicity of parenteral nutrition fluid, several modifications of the delivery system of the catheter have been advocated. Among the suggested mechanisms for decreasing infections in this situation are (1) either bonding heparin or a heparin-like substance to the catheter or infusing heparin with the infusate in an attempt to minimize fibrin sheath formation[232,233]; (2) constructing the catheter of a more flexible substance, thereby producing less trauma to the vascular endothelium[234]; and (3) tunneling the catheter under the skin in an attempt to decrease access of pathogens to the circulation.[234]

A final and often difficult issue is deciding when to remove a parenteral nutrition catheter for suspected sepsis. In the past, most authorities recommended the removal of a catheter whenever infection was suspected. In separate studies, Ryan and coworkers[108] and Maher and associates[214] suggested that nearly 70% of catheters removed for suspected sepsis are apparently removed unnecessarily. Thus the parenteral nutrition team is often faced with the dilemma of whether or not to remove the catheter from a patient in whom the evidence for infection is equivocal. Such a patient may have many reasons for fever; therefore the diagnosis of infection may be difficult. Often patients are severely immunosuppressed, thrombocytopenic, or both, and the risks associated with catheter replacement may be quite high. Several clinical features may help the physician decide how to manage the catheter.[208,235] The presence of positive blood cultures (particularly for *Candida* or coagulase-negative staphylococci) in the absence of another source for the infection or in the presence of hemodynamic instability, embolic phenomena, leukocytosis, or profound leukopenia may herald the onset of catheter-associated sepsis. In addition, the development of new glucose intolerance in a parenteral nutrition patient whose carbohydrate metabolism had been previously well regulated may be an early subtle sign of bacteremia.[208,230]

TABLE 300.4 Prevention of Infusion-Related Infection in Total Parenteral Nutrition (TPN)

- Administration of TPN should be under the supervision of a team of health care professionals (usually a nurse, pharmacist, and physician). Both the decision regarding appropriateness of TPN therapy and protocols for insertions, maintenance, and delivery of TPN should be under the responsibility of this team.
- TPN solution should be prepared using sterile or aseptic technique, when possible in a laminar flow hood. Once prepared, the solution should be infused immediately or stored at 4°C.
- Placement of the catheter should be performed by using maximal sterile barrier precautions, including mask, sterile gown and gloves, large sterile drape, and appropriate skin preparation preferably with chlorhexidine.[349–351]
- Once placed, the catheter should be anchored to avoid movement, which may result in local irritation of the insertion site or transport of organisms along the insertion path.
- If possible, the system should be kept closed, avoiding unnecessary entry for blood drawing and administration of other fluids or blood products via the TPN line.
- If multiple lumens/ports are present, one lumen/port should be designated as the parenteral nutrition site.
- Other aspects of TPN administration are either of empirical or theoretical value, have shown equivocal or borderline results in studies, have shown conflicting results in studies, or have been demonstrated to be of value in small studies. Definitive studies to document the merit of these techniques are needed before they are routinely implemented; these include the following:
  - Routine application of antiseptic cream at the site of catheter insertion (either at the time of venipuncture or at routine dressing change)
  - Routine dressing changes and skin defatting with acetone
  - Routine use of semipermeable dressing materials. A meta-analysis of data from several studies suggests that these dressings may actually play a detrimental role in catheter infections.[368]
  - Routine use of in-line membrane filters (no benefit demonstrated)
  - Use of silicone or other less traumatic, nonthrombogenic catheters; use of heparin-bonded catheters; use of low-dose heparin infusions
  - Tunneling the catheter subcutaneously to increase the anatomic distance between catheter insertion site and the point at which the catheter enters the vessel (appears to be useful in several small studies)
  - Routine use of antibiotic lock prophylaxis with heparin plus vancomycin

Modified from Goldmann D, Maki D. Infection control in total parenteral nutrition. JAMA. *1973;223:1360–1364.*

Long-Term Issues

In 1973, Broviac and colleagues[236] reported their initial experience with the use of a chronic indwelling right atrial catheter for the delivery of long-term parenteral nutrition. Venous access also has long been a problem for patients receiving chemotherapy for malignancy. Hickman and coworkers[237] modified the Broviac catheter, which has a smaller lumen, for use in patients undergoing bone marrow transplantation. This modified catheter can be used for the administration of intensive chemotherapy, the administration of other medicines and fluids, transfusion, and phlebotomy. These catheters spare the patient both physical and psychological trauma. In the ensuing years, a number of additional modifications of these catheters have been devised, including the Groshong valve.[238] These modified catheters and implanted infusion

TABLE 300.5 Infectious Complications Associated With Implanted, Long-Term Catheters

AUTHORS AND YEAR PUBLISHED	PATIENT NUMBER, TYPE	TYPE OF CATHETER	CLABSIs (%)	CATHETERIZATION DURATION (RANGE) OR TOTAL CATHETER DAYS	CLABSIs PER 100 CATHETER DAYS
Blacklock et al. (1980)[239]	25, oncology	T	2 (8)	70 (5–256)	0.11
Larson et al. (1981)[250]	34, oncology	T	4 (11.8)	110.3 (3–355)	0.12
Wade et al. (1982)[442]	51, oncology	T	3 (5.9)	91 (4–457)	0.06
Lokich et al. (1985)[251]	92, oncology	T	2 (2.2)	127 (7–450)	0.02
Cairo et al. (1986)[242]	46, pediatric oncology	T	23 (50)	163 (9–365)	0.30
Johnson et al. (1986)[248]	64, pediatric oncology	T	33 (51.6)	251 (NG)	0.19
Shulman et al. (1987)[254]	31, pediatric oncology	P	4 (12.9)	232 (14–607)	0.05
Ross et al. (1988)[253]	39, pediatric oncology 49, pediatric oncology	T P	4 (10) 0 (0)	365 (30–426) 350 (7–395)	0.03 0.00
Viscoli et al. (1988)[256]	145, pediatric oncology	T	57 (39)	171 (2–647)	0.19
Hockenberry et al. (1989)[245]	82, pediatric oncology	P	4 (4.9)	168 (7–1030)	0.02
Kappers-Klunne et al. (1989)[249]	23, oncology 20, oncology	T P	19 (83) 9 (45)	166 (1–605) 164 (1–971)	0.50 0.27
van Hoff et al. (1990)[443]	59, pediatric oncology	T	30 (51)	220 (NG)	0.23
Ulz et al. (1990)[255]	111, oncology	T	63 (57)	81 (1–167)	0.66
Rizzari et al. (1992)[252]	125, pediatric oncology	T	106 (85)	134 (6–488)	0.51
Hogan and Pulito (1992)[246]	84, NICU	T	9 (10.7)	33.4 (2–119)	0.29
Alurkar et al. (1992)[241]	91, oncology	T	31 (34)	74.6 (NG)	0.41
Wacker et al. (1992)[257]	44, pediatric oncology 33, pediatric oncology	T P	15 (34) 6 (18)	236 (15–806) 316 (12–1294)	0.06 0.10
Biffi et al. (1997)[444]	175, oncology	P	4 (2.2)	180 (4–559)	0.003
Schwarz et al. (1997)[445]	680, oncology	P	31	310 (2–1960)	0.01
Elishoov et al. (1998)[243]	242, oncology	T	46 (19)	40 (7–187)	0.52
Jean et al. (2002)[446]	89, hemodialysis	T	56 (63)	52,030 total days	0.11
Chang et al. (2003)[228]	572, oncology	P	21 (3.7)	358 (1–1742)	0.01
Hung et al. (2009)[447]	209, pediatric oncology	P	18 (8.6)	597 (15–1962); 137,924 total days	0.013
Martin-Pena et al. (2012)[448]	123, hemodialysis	T	11 (8.9)	284 (NG)	0.034
Toure et al. (2012)[449]	315, oncology	P	41 (13)	170.5±117	0.076
Moore et al. (2014)[450]	555, hemodialysis[a]	T	174 (31)	155,518 total days	0.112
Nurse et al. (2014)[451]	191, LTAC[b]	P	15 (7.9)	183,183 total days	0.008
Hord et al. (2016)[452]	99, pediatric oncology 190, pediatric oncology	P T	144 (100) 193 (100)	97,212 total days 55,048 total days	0.148 0.351
Wildgruber et al. (2016)[453]	681, oncology[c]	P	41 (6)	299 (4–1228); 208,740 total days	0.02
Viana Taveira et al. (2017)[454]	188, pediatric oncology	P	94 (50)	NG (4–778)	0.121
Subtotal (by catheter type)	**2232** **3325**	**T (20 studies)** **P (14 studies)**	**881 (39.5)** **432 (13.0)**	— —	**0.240 (range 0.02–0.52)** **0.061 (range 0.0–0.27)**
Total	**5557**	—	**1313 (23.6)**	—	**0.15**

[a]Trial of gentamicin/citrate versus heparin catheter locking solutions.
[b]Medically complex patients at long-term acute care hospital.
[c]Trial of polyurethane versus silicone catheters.
CLABSIs, Central line–associated bloodstream infections; *LTAC,* long-term acute care hospital; *NG,* not given; *NICU,* neonatal intensive care unit; *P,* totally implantable port; *T,* tunneled central venous catheter.

ports, which were introduced in 1982, represent a major step forward in the management of all patients who require long-term central venous access but have been especially useful in the management of immunosuppressed patients (and particularly in immunosuppressed children) in whom venous access is frequently problematic. Use of these devices in a variety of clinical settings is currently the standard of care.

Several centers have reported their experiences using these catheters, and many series report remarkably low rates of infection. Initial reports suggested that the rate of catheter infection in nonneutropenic patients was approximately one infection per 5.5 patient-years.[239] Press and colleagues[240] summarized 1088 catheter placements from 18 studies in their literature review. In their summary data, these authors reported approximately 0.14 infections per 100 catheter days. Table 300.5 presents a similar summary, including several published studies evaluating infection and bacteremia risks associated with the use of tunneled catheters and infusion ports.[241–257] The elevated risk for bacteremia (0.15 infections for each 100 catheter days), may be more of a reflection of the severity of the patients' illnesses, the increasing immunosuppression associated with their therapies, and the invasive care provided to critically ill patients.

Several centers have reported remarkable success—few infections and few other complications as well—with totally implantable access

ports.[258–261] In one of the earliest of these studies, the rate of infections for each 100 catheter days was 0.43—comparable to rates of other implantable catheters.[258] Subsequent studies have demonstrated even lower infection risks.[b] One epidemiologic study concluded that infections are the primary reason for late-term complications and port removal.[263] This group determined that patients with recurrent device implantation and with ongoing chemotherapy are significantly more likely to need device removal.[263]

Pocket infections, defined as the spread of infection into the subcutaneous pocket of a totally implanted intravascular device,[139] are considered an absolute reason for device removal because of the poor response to antimicrobial therapy by bacteria inside the biofilms on the port inner surface.[139,264] Pocket infections remain among the most difficult infectious complications associated with implanted ports. Evidence from studies of intravascular cardiac device infections suggests that risk of local device infections, including pocket infections, in these devices is increasing at a faster rate than the corresponding increase in device implantations.[265,266]

Peripherally Inserted Central Venous Catheters

The use of PICC lines for intermediate and long-term access has increased dramatically in both home care and hospital settings.[267–270] These catheters have several advantages over some of the other long-term access devices: PICCs may be inserted at the bedside[267] by nonphysician providers[271,272]; they are placed into children and neonates under fluoroscopy or ultrasound guidance with relative ease[94,268]; and they are useful for administration of chemotherapy, antimicrobial agents, and parenteral nutrition,[268,270,273] particularly in pediatric patients requiring long-term nutritional support.[270,273] Skilled interventional radiology is needed for difficult insertions, for catheter salvage, and to ensure that the catheter is not misplaced or misdirected.[267]

A major problem is device failure, with as many as 10%[273] to 27%[274] of these catheters developing mechanical malfunction. Another group determined that PICC lines are more likely to develop mechanical complications and have a shorter survival time than CVCs.[274] Many institutions have elected to develop a skilled team approach to the insertion and management of these catheters. Nurse PICC teams inserted these devices in at least 60% of US hospitals in 2013 in one survey, and were more likely to use a variety of best-practice prevention approaches.[275] Placement of PICCs in our hospital requires scheduling in a procedure room with a trained team, ultrasound guidance, and follow-up chest x-ray to assure tip placement in the superior vena cava. PICC lines may be less cost-effective than currently believed because of the more difficult insertion into some patients and higher thrombophlebitis rates.[271,276] The extra expense and procedural requirements may be warranted when more than a week of intravenous therapy is anticipated. Placement complications are still less than with implanted CVCs, and removal is easy.

Improved insertion and maintenance practices have resulted in longer durations of PICC catheterization, as reviewed by Milstone and Sengupta.[277] Studies that evaluated routine catheter replacement as a strategy to prevent CLABSIs compared catheter replacements at up to 7 days; no studies have addressed whether catheter replacement beyond 7 days might reduce CLABSI. Milstone and Sengupta[277] therefore suggested that, in some instances, PICC replacement may reduce the incidence of CLABSI. One risk factor for CLABSI found in two recent studies is the number of lumens in the PICC catheter, with a higher odds ratio for infection with more lumens.[278,279]

Care and Maintenance of Implanted Central Venous Catheters

Intraluminal contamination may represent the most important route of infection for implanted catheters and ports, further suggesting that strategies aimed at decreasing the risks for intraluminal contamination could substantially lower infection rates with these types of intravascular devices. Several issues regarding the care and maintenance of these catheters remain unsettled. Among these issues are the following:

1. Should a dressing be placed over the exit site, and if so, what dressing materials should be used and how frequently should the dressing be changed?
2. Should the system be routinely flushed, and if so, how frequently and with what?
3. Are blood cultures obtained through the catheter reliable indicators of catheter contamination?
4. What are the indications for catheter removal, and can either or both local infection and bacteremia be treated with the catheter in place?

Although definitive answers to these questions remain elusive, at the National Institutes of Health Clinical Center, a semipermeable membrane is kept in place over the exit site. These dressings are changed at least weekly. For the few remaining catheters that require it, we also use an every-other-day heparin flush (5 mL of 100 units/mL) to attempt to keep the catheters patent. Higher heparin concentrations are associated with an increased risk for anticoagulating the patient. Newer, valved catheters do not require heparin and are locked with saline after use. One recent study suggested that disruption of catheter dressings was common and was an important risk factor for catheter-related infections.[280] The CDC guidelines suggest that well-healed exit sites might not require dressings.[68]

Repeated isolation of the same organism from cultures drawn through the catheter indicates a need for therapy. Individual positive cultures and sporadic positive cultures are difficult to interpret in the absence of clinical or laboratory correlates. As noted previously, some groups believe that quantitative cultures may prove particularly helpful in establishing a diagnosis in this setting.

Pulmonary Artery Catheters

The use of indwelling, balloon-tipped pulmonary artery catheters[281] revolutionized the management of hemodynamically unstable, critically ill patients. Despite an ongoing controversy about the safety of and benefit associated with the use of these catheters,[282,283] approximately 1.5 million are used each year in the United States alone.[284] The placement of such a catheter in one of the great veins, across the tricuspid and pulmonic valves, and into the pulmonary vasculature is not without complications, however. Michel and coworkers[285] demonstrated that 29 of 153 pulmonary artery catheter tips produced microbial growth in thioglycolate broth. Although no patient in this study was considered to develop sepsis secondary to the contaminated catheter, other studies have suggested a reasonably high rate of contamination, with occasional episodes of catheter-related sepsis and nosocomial endocarditis.[286] The majority of these catheters are heparin bonded, which reduces catheter thrombosis and microbial adherence to the catheter.

Katz and colleagues[286] retrospectively studied complications associated with the placement of 392 balloon-tipped catheters; of these, 17 (4.2%) were assessed to be associated with bacteremia. Maki[235] estimated that 3% to 5% of these catheters kept in place for more than 72 hours will result in CLABSIs, and concluded that efforts should be made to limit the duration of pulmonary artery catheters to no more than 4 days.[287] A study of 1000 patients randomized to receive either pulmonary artery catheters or CVCs found similar rates of infection in both groups (five CLABSIs in the pulmonary artery catheter group vs. three CLABSIs in the CVC group).[283] Another group found that 4 of 215 (1.9%) patients undergoing pulmonary artery catheterization developed infection, compared with no infections in the control group.[288] One study evaluated complications associated with the use of a "hands-off" pulmonary artery catheter that is completely shielded during balloon testing, preparation, and insertion[289]; use of this catheter was associated with a substantial reduction in systemic infections related to the catheter.[289]

Another problem relatively unique to the flow-directed, balloon-tipped pulmonary artery catheter is that such catheters may traumatize the right-sided heart valves and the right-sided endocardium. In a study of 102 consecutive autopsies of patients who died in the hospital, 26 (25.5%) had had an indwelling intracardiac catheter inserted before death.[290] Six of these patients were excluded from analysis (4 patients died 48 hours or less after catheter placement; 2 patients had permanent transvenous pacemakers in place for many years, with the anticipated endocardial fibrosis). Of the remaining 20 patients, 6 had vegetations

[b]References 246, 249, 251, 253, 254, 257, and 260–262.

present, and 88% of the patients had some evidence of intracardiac damage.[290] One patient had infective endocarditis on the tricuspid valve. Other studies have reported slightly lower but significant incidences of right-sided heart vegetations among monitored patients coming to autopsy.[291] Greene and coworkers[291] noted a 10-fold increase in the incidence of valvular vegetations when they compared a period of time before the introduction of balloon-tipped pulmonary artery catheters with a time in which the catheters were in wide use. Nosocomial endocarditis is increasing in frequency. In one study, 9.3% of cases of endocarditis diagnosed in a referral hospital were both nosocomial in origin and unrelated to prior cardiac surgery.[292] Two studies found that 28%[293] to 33%[294] of cases of endocarditis were nosocomial in origin, with the majority from line-related sepsis. Hemodialysis patients, in particular, are at increased risk of nosocomial or health care–associated endocarditis.[295,296]

No prospective study has addressed risk factors associated with infection of these catheters, nor have studies assessed the efficacy of devices designed to decrease the risk of catheter-associated infection (e.g., leaving the introducer sheath in the vein to protect the catheter).[285] Changing these catheters over a guidewire may present a major risk factor for infection. A study in cardiac surgery patients found that more than 4 days of catheterization was the single variable associated with increased risk of pulmonary artery catheter colonization.[297] CDC guidelines currently recommend that pulmonary artery catheters need not be changed more frequently than every 7 days.[68] Infection risks associated with other aspects of monitoring equipment (e.g., transducer domes and heparin flush solution) are discussed next.

Arterial Lines, Transducers, and Transducer Domes

The widespread use of arterial lines for blood pressure monitoring or for obtaining arterial samples for blood gas determinations yields yet another source of device-associated infection. In addition, the technical electronic equipment used for hemodynamic monitoring—transducers and their associated paraphernalia—has also been cited as a source of device-associated infection.

Stamm and colleagues[18] reported an outbreak of *Flavobacterium* bacteremia among monitored patients in an ICU. Ultimately, these organisms were cultured from in-use radial artery catheters, from stopcocks, and from ice used to cool syringes for blood gas determinations.[18] Band and Maki[146] used the semiquantitative catheter tip culture technique to study 130 arterial catheters in 95 patients. In their series, 23 catheters were classified as showing "local infection" (e.g., >15 colonies per semiquantitative culture), and there were five episodes of sepsis.[146] Factors associated with increased risk for infection were (1) duration of catheterization (especially longer than 96 hours); (2) placement by cutdown rather than percutaneously; and (3) clinical signs of local inflammation. Femoral placement of arterial catheters also has been associated with higher rates of colonization and catheter infection than other placement sites.[298,299] Two studies published in 2011 reported differing results with regard to length of arterial catheterization.[300,301] The French study found a lower rate of catheter-related bloodstream infections with nonscheduled replacement (0.6% with an average duration of 5 days vs. 1.4% with an average duration of 4 days).[301] In contrast, a Spanish group reported that both days of insertion and length of ICU stay increased the risk of catheter-associated bloodstream infection.[300] Recent CDC guidelines recommend against routine replacement of arterial catheters and for replacement only with clinical indication.[68]

Maki and colleagues[262] reviewed 14 studies of arterial catheters used for hemodynamic monitoring, and they determined that the pooled mean rate of arterial catheter–related bacteremia per 100 catheters was 0.8 (95% confidence interval [CI], 0.6–1.1) and the pooled mean rate of arterial catheter–related bacteremia per 1000 catheter-days was 1.7 (95% CI, 1.2–2.3), a rate approaching that of short-term CVCs. Koh and associates[298] found similar incidence densities of arterial and CVC colonization but more than twofold increased rates of bacteremic infection with CVCs compared with arterial catheters. Mermel (as cited in Maki and colleagues[262]) noted in an editorial that if 6 million arterial catheters are used each year in the United States and the risk of bacteremia is 0.8, then there are approximately 48,000 arterial catheter–related bloodstream infections each year.[302] Others have noted similar infectious risks with arterial catheters and short-term CVCs.[303]

Several epidemics of infection resulting from improper sterilization of reusable transducer domes have been reported.[304,305] However, with the introduction of disposable transducer domes, one might assume that these problems would be overcome. Buxton and colleagues[304] reported an epidemic of *Enterobacter* infections that was associated with the contamination of disposable transducer domes during their initial setup. The chambers and domes were apparently contaminated by the hands of hospital personnel who had handled heavily contaminated transducer heads.[304] West and coworkers[306] also reported *Serratia* sepsis resulting from transducer dome cracks. In this study, supposedly disposable transducer domes were being resterilized, with resultant cracks or breaks in the dome membrane.

Another potential reservoir for nosocomial bacteremia is the heparin flush solution used to irrigate certain intravascular devices continually. This fluid has been implicated as a reservoir for outbreaks of device-associated bacteremia in several instances.

Several authors have made recommendations regarding the prevention of infection associated with intravascular monitoring devices.[68,146,305] A summary of these recommendations is presented in Table 300.6.

Treatment of CLABSIs

In this section we discuss the therapeutic interventions associated only with the management of CLABSIs. Treatment strategies for other device-associated intravascular infections is beyond the scope of this chapter. A summary of these recommendations is presented in Table 300.7.

The antibiotic choice for empirical therapy for suspected CLABSI depends on an individual patient's risk factors for infection, underlying

TABLE 300.6 Prevention of Infection Associated With Hemodynamic Monitoring

- Place arterial lines, central venous lines, and flow-directed, balloon-tipped catheters using sterile technique. Maximal sterile barrier precautions should be used. The skin should be prepared with an effective antiseptic solution (e.g., chlorhexidine).[68,349,352,354]
- Place the catheters percutaneously and anchor well to avoid catheter movement. Dress the insertion site appropriately.
- Use heparinized saline (not dextrose-containing solutions or parenteral nutrition) for continuous-flush solutions.
- Do not reuse transducer domes; sterilize reusable part of transducer setup according to manufacturer's instructions between patients.
- Replace transducers, tubing, and continuous-flow devices every 96 hours[68,455]; do not routinely replace peripheral arterial catheters to prevent infection.
- Use sterile fluid to fill the chamber dome; use aseptic technique in the assembly.
- Avoid placing unnecessary junctions or stopcocks into the apparatus; minimize manipulation of the system.
- Whenever possible, avoid exchanging arterial catheters over guidewires; use of guidewire exchange technique for arterial catheters is associated with increased infection risk.

TABLE 300.7 Management of CLABSI in Hospitalized Patients

Catheter removal advised at diagnosis:
- Patients in septic shock or unstable vital signs
- Tunnel infection of tunneled catheter
- Pocket infection of subcutaneous port
- Yeast, *S. aureus,* gram-negative bacilli, or mycobacteria
- Endocarditis or suppurative thrombophlebitis

Trial of antibiotic infusion through suspected catheter ports:
- Coagulase-negative staphylococcal CLABSI treated with vancomycin is most likely to succeed.
- All ports should be infused with antibiotic daily by splitting or rotating doses.
- Repeat blood cultures through catheter every day until negative.

Antibiotic lock therapy plus catheter infusion:
- Lock solution is prepared separately by pharmacy.
- Dwell time often interferes with catheter use.
- Advantages for treatment remain controversial.

Bacteremia/fungemia persisting after 72 hours of appropriate antibiotic therapy requires catheter removal.

diseases, known allergies, documented past colonization or infection with resistant organisms, and likely pathogens associated with the specific CVC type. Coagulase-negative staphylococci remain the single most common cause of CLABSI, making vancomycin the drug of choice for empirical therapy with daptomycin the preferred alternative. Empirical coverage for gram-negative bacilli should be based on local antimicrobial susceptibility data and the severity of disease (e.g., a fourth-generation cephalosporin, carbapenem, or β-lactam–β-lactamase inhibitor combination, with or without an aminoglycoside).[139] Empirical coverage for multidrug-resistant gram-negative bacilli, including *Pseudomonas aeruginosa,* should be added for patients who are neutropenic, septic, or known to be colonized with such pathogens. Empirical coverage for suspected catheter-related candidemia should be used for septic patients who receive parenteral nutrition or have had prolonged broad-spectrum antibiotics, a hematologic malignancy, any transplant, or colonization due to *Candida* species at multiple sites.

While catheter salvage is an appealing concept because of risks and costs associated with replacement, patients in whom retention is considered should be carefully selected. Catheter salvage should not be attempted for patients with *S. aureus* CLABSI because early removal of the line has been associated with improved outcome.[307] Catheter salvage also should not be attempted for catheter-related candidemia or mycobacteremia. The gram-negative bacilli most associated with CLABSI are those that form biofilms, including *Klebsiella, Pseudomonas,* and *Acinetobacter* species; catheters should be removed for infections with these organisms.[308] Catheter salvage also should not be attempted for long-term catheters when there are signs or symptoms of exit site or tunnel infections, or a pocket infection. If salvage is attempted, the patient should be closely observed for the development of acute complications, persistent infection, or sepsis. The catheter should be removed if complications develop or if bacteremia or fungemia persists after 72 hours of appropriate antibiotic therapy.[139] Although evidence is limited, intravenous antibiotics should be divided through all lumens of the CVC by splitting or rotating doses. Antibiotic lock treatment should be used for catheter salvage where possible, and is discussed below.

Antimicrobial Lock Therapy

A number of studies have shown that instilling antimicrobials into a catheter and leaving the solution to dwell (i.e., antimicrobial catheter lock) is effective in treating some catheter-related bacteremias.[309–312] Benoit and colleagues[309] suggested in the 1990s that intraluminal antibiotic treatment was effective in treatment of bacteremia in a small uncontrolled study. These investigators also found that intraluminal therapy with amphotericin B also may suppress, but not eradicate, *Candida* infections in tunneled catheters.[309] Three more recent studies suggested that antibiotic lock therapy in combination with appropriate systemic antimicrobials was effective in clearing the bacteremia and allowing retention of the catheter in 84%,[310] 67%,[311] and 93%[312] of patients. A retrospective study reported successful salvage in 74% of catheters, with an average duration of treatment of 13.4 days.[313] Despite data indicating that coagulase-negative staphylococci are better at adhering to foreign material with biofilm production than is *S. aureus,* coagulase-negative staphylococci apparently are more responsive to antibiotic lock therapy,[139,310,313] and routine vancomycin antibiotic lock therapy may not be appropriate for patients with *S. aureus* catheter-related bacteremia.[313,314] Success rates in one study did not differ by type of catheter[313] but, rather, were dependent on the infecting organism, with higher rates of successful catheter retention for gram-negative bacilli[313,315] and lower rates for coagulase-negative staphylococci,[316] and lowest rates for *S. aureus* and yeasts. A review found that a combination of systemic antibiotics and culture-guided lock therapy was superior to systemic antibiotics alone, with 10% of the locked catheters requiring replacement compared with 33% of catheters not locked.[317]

A large variety of antibiotic lock solutions and dosing regimens have been proposed, more recently including agents that are not usually used to treat serious infections because of concern about development of antibacterial resistance. Daptomycin, minocycline, and tigecycline have been used,[318] as well as minocycline-EDTA (with or without) 25% ethanol.[319,320] Use of an ethanol catheter lock in combination with systemic antimicrobials may be relatively effective in treatment of CLABSIs caused by a variety of organisms.[321–323] Ethanol is an antiseptic that demonstrates bactericidal and fungicidal activity against a broad range of gram-positive and gram-negative bacteria and fungi.[324] A study of pediatric patients found that the combination of a 70% ethanol solution with standard antibiotic therapy resulted in a 77% catheter salvage rate.[325] Nonetheless, a systematic review concluded that use of ethanol locks has been associated with structural changes in catheters, as well as the elution of molecules from the catheter polymers.[326] These authors recommended that if ethanol locks are used, the shortest dwell time and lowest concentration needed to eradicate microbes in biofilm might reduce these risks.

Thus additional experience has suggested that, perhaps with the exception of true tunnel and pocket infections, many infections of indwelling central catheters may be amenable to lock therapy if the device must be left in place. Of interest, despite support for use of lock therapy in some circumstances by current guidelines,[139] a 2007 survey of infectious diseases physicians found that fewer than half used antimicrobial lock therapy for any organism causing CLABSI.[327] One limitation of antibiotic locks is that dwell time is limited when the catheter is needed for frequent infusion. Patients should be carefully evaluated for evidence of complicated device-related infections, including tunnel infection, pocket infections of ports, bloodstream infection that continues despite 72 hours or more of antimicrobial therapy to which the infecting organisms are susceptible, septic emboli, septic thrombosis, endocarditis, and osteomyelitis. The device/catheter should be removed for any of these conditions.

PREVENTION OF DEVICE-ASSOCIATED BACTEREMIA

Avoiding the unnecessary use of CVCs should be a high priority. Criteria for the use of PICCs include proposed duration of use of more than 5 days and irritant or vesicant infusions.[328] A number of reports have suggested that CLABSIs can be prevented by using simultaneous implementation of an array of practice improvements (i.e., "bundle").[59,64,329–331] A summary of practice improvement recommendations is presented in Table 300.8. Evidence of improved outcomes after multifaceted interventions, along with the emergence of prevention of health care–associated infections as a national priority,[3,332] resulted in the publication of a compendium

TABLE 300.8 Summary of Practice Improvement Recommendations ("Bundle" Components) for CLABSI Prevention

Before and At Insertion
• Educational programs for all personnel involved in catheter insertion and maintenance
• Hand hygiene
• Catheter insertion checklists
• Standardized catheter insertion kits/carts
• Maximal sterile barrier precautions
• Site disinfection with chlorhexidine gluconate–alcohol solution
• Avoid femoral sites when possible
Maintenance
• Daily assessment of need for catheter; remove all nonessential catheters promptly
• Disinfect catheter hubs/injection ports with mechanical friction for ≥5 seconds
• Use chlorhexidine-impregnated dressing for short-term nontunneled CVCs
• Attempt to decrease dressing disruptions; replace dressing if it becomes damp, loose, or visibly soiled
• Do not routinely replace CVCs, PICCs, hemodialysis catheters, arterial catheters, or pulmonary artery catheters
• Chlorhexidine bathing daily for intensive care unit patients
Special Approaches
• Antibiotic lock prophylaxis in select patient populations
• Central catheters impregnated with chlorhexidine–silver sulfadiazine or minocycline-rifampin
• Use of needleless connectors with improved engineering design, including silver-coated needleless connectors and disinfection caps
• Adequate nurse staffing

of strategies to prevent CLABSIs by the Society for Healthcare Epidemiology of America and the IDSA Standards and Practice Guidelines Committee[67,333] that supplement existing, more detailed guidelines.[68] The limitation of additional reimbursement by the CMS for a diagnosis of vascular catheter–associated infection will provide additional financial incentives for health care facilities to prevent these infections.

Before Insertion of Vascular Catheters

Education of health care personnel regarding standardized catheter insertion, catheter care, and prevention of infection has been shown to reduce the incidence of catheter-associated infections.[105,118,334–336] All health care personnel involved in catheter insertion and maintenance should complete an educational program regarding catheter-associated infections before performing these duties.[67] Engaging hospital leadership and allowing for flexibility in implementation have been suggested as key components to maximize the benefits of these programs.[337] In a meta-analysis, Safdar and Abad[338] concurred that educational interventions can reduce rates of health care–associated infections. They also concluded that additional studies with clearly described, easily reproducible, and widely generalizable educational tools that had been validated were needed, particularly in general hospital settings rather than ICUs.[338]

Catheter insertion checklists to ensure adherence to infection control practices have been used as part of the practice improvement bundle in a number of studies.[59,330] These recommendations have recently been summarized.[339] Use of these checklists should be accompanied by standardized catheter insertion kits/carts containing all necessary items for insertion.[59,67]

Whereas the implementation of bundles of interventions has had a clear and measurable impact on rates of device-associated bacteremias in many centers,[340] the relative importance (or unimportance) of each of the various components of the bundles in varied clinical settings (e.g., oncology units, trauma units, burn units) remains unclear.[341] Nonetheless, while a recent study found that compliance with all bundle elements was associated with the lowest CLABSI rates, excellent compliance with even one bundle element was associated with lower CLABSI rates in adult ICUs.[64] Conversely, for many of the individual components of such bundles, we do not have scientific evidence for independent efficacy—most available data are for implementation of the entire bundle. Therefore it remains possible that some of the interventions may be superfluous—or perhaps actually even harmful.

At Insertion

Hand hygiene before catheter insertion or manipulation is an absolute requirement[342] and has been shown to be successful in reducing health care–associated infections.[336,343–345] Maximal sterile barrier precautions, including a mask, a cap, sterile gown and gloves, and a large sterile drape to cover the patient, have also been shown to reduce CVC-associated infections.[107,346,347] This tenet was confirmed by a prospective study that demonstrated that use of maximal sterile barrier precautions had an independent and significant association with a decrease in the risk of catheter infection of more than fivefold.[348] This same group performed a multivariate logistic regression analysis to determine that wearing a mask alone had a statistically significant association with a reduced infection rate.[348]

Techniques used for skin preparation before catheter insertion also have been shown to influence the risk for infection. In one study, skin preparation and decontamination with 0.5% chlorhexidine gluconate in 70% isopropyl alcohol was more effective than 10% povidone-iodine in preventing colonization of peripheral catheters in neonates.[349] Chlorhexidine-containing antiseptics have been shown to be effective in diminishing rates of catheter colonization, and they have shown varying efficacy in reducing intravenous device–related bacteremias.[350] A 2002 meta-analysis determined that chlorhexidine gluconate significantly reduces the incidence of bacteremia in patients with CVCs compared with povidone-iodine for insertion-site skin disinfection.[351] A comparison of 10% aqueous povidone-iodine, 2% aqueous chlorhexidine gluconate, and 0.5% alcoholic chlorhexidine gluconate determined that both chlorhexidine solutions were similarly effective in preventing colonization of central venous and arterial catheters.[352] Small and colleagues[353] found that 2% chlorhexidine gluconate in 70% isopropyl alcohol was more effective in reducing the number of peripheral venous catheters that were colonized or contaminated than 70% isopropyl alcohol alone. Likewise, Mimoz and coworkers[354] recommended use of chlorhexidine-based solutions rather than povidone-iodine. An open-label randomized trial enrolling 2546 patients assessed catheter insertion with 2% chlorhexidine–70% isopropyl alcohol or 5% povidone iodine–69% ethanol, each with or without scrubbing. Scrubbing provided no benefit, but chlorhexidine-alcohol provided significantly greater protection for the primary outcome of catheter-related infections per 1000 catheter-days.[355] However, hypersensitivity reactions to chlorhexidine have been reported.[356,357] The CDC guidelines recommend disinfecting skin before CVC and peripheral arterial catheter insertion by using greater than 0.5% chlorhexidine preparation with alcohol.[68] The antiseptic solution must be allowed to dry before making the skin puncture. Finally, chlorhexidine tolerance has increased, according to data from Texas Children's Hospital. *Staphylococcus aureus* isolates with the *qacA/B* and *smr* genes were associated with elevated minimal inhibitory concentrations to chlorhexidine and were often associated with CVC use, elevated vancomycin minimal inhibitory concentrations, and increased rates of bloodstream infection.[358] We agree that skin should be disinfected with an alcoholic chlorhexidine solution containing more than 0.5% chlorhexidine for patients without chlorhexidine allergy and allowed to dry before catheter insertion.

After Insertion

Even with the most stringent application of strategies to decrease catheter-associated infections, the presence of a vascular catheter remains a clear risk factor for infection. Thus the need for continued vascular access should be assessed at least daily,[359] and all nonessential catheters should be removed immediately.[360]

Contamination of catheter hubs, needleless connectors, and injection ports is a major risk factor for catheter-associated infection, and disinfection has been determined to reduce microbial burden.[35,40,361,362] Recent guidelines recommend cleaning all catheter hubs and injection ports with an alcoholic chlorhexidine preparation or 70% alcohol to reduce contamination[67]; access ports for needleless intravascular devices should be scrubbed with an appropriate antiseptic for a minimum of 5 seconds.[68] Because monitoring compliance with this vital step is difficult, passive port protectors have been developed. Caps with passive port protection have alcohol in the cap that scrubs and remains in contact with the port. While passive port disinfection may decrease the risk of CLABSI, randomized controlled trials in hospitalized adults are needed because these devices potentially represent an additional component of a comprehensive CLABSI reduction strategy.[363–367] We agree that, at a minimum, all catheter hubs and injection ports should be disinfected thoroughly, with mechanical friction applied for a minimum of 5 seconds, and allowed to dry before access.

Authorities have recommended placing a sterile dressing over the catheter entry site, using either sterile gauze or sterile, transparent, semipermeable dressings.[68] Maki and colleagues[54] studied standard tape and gauze dressings in comparison with two transparent polyurethane dressings. They concluded that polyurethane dressings appear to be safe and may be left on for up to 5 days.[54] Hoffmann and associates[368] performed a meta-analysis of studies in an attempt to assess the use of semipermeable membrane dressing materials at catheter insertion sites. These investigators found a significantly increased risk of catheter-tip colonization when transparent compared with gauze dressings were used to dress either central or peripheral catheter insertion sites. In addition, they found a trend (although not statistically significant in their meta-analysis) toward an increased risk for bacteremia and catheter sepsis associated with the use of semipermeable dressings as insertion-site dressings for CVCs.[368] The CDC guidelines suggest that because the risk for catheter-related bacteremias did not differ between the groups,[368] choice of dressings can be a matter of preference.[68]

Chlorhexidine gluconate–impregnated dressings have been studied extensively, and there are substantial supporting data for the use of both chlorhexidine sponge dressings and gel dressings.[369–374] The CDC recommended in 2017 that chlorhexidine-impregnated dressings with a US Food and Drug Administration–cleared label that specifies a clinical

indication for reducing catheter-related bloodstream infection are recommended to protect the insertion site of short-term, nontunneled CVCs.[375] We agree that chlorhexidine-impregnated dressings are appropriate in adult patients with short-term nontunneled CVCs, although the relative contribution of these dressings in addition to chlorhexidine bathing is unknown. In addition to the type of dressing, the frequency of dressing disruption may also affect catheter-related infections.[280] Efforts to decrease dressing disruptions should be included in postinsertion bundles of care.

The data on frequency of dressing changes are not conclusive. Randomized clinical trials have most often concluded that increasing time to dressing changes with transparent dressings does not affect site colonization.[376–378] Anecdotal institutional data suggest that increased time intervals between dressing changes and perhaps transparent dressings themselves are associated with increased bacteremia rates.[379] The increased ease of visual inspection and savings on nursing personnel time related to less frequent dressing changes likely does affect dressing choices in at least some institutions. One study of 55 hospitals' practices found that two-thirds used transparent, semipermeable dressings.[380] Nonetheless, no definitive data exist to determine the best dressing methodology. Failure of dressing integrity is a risk factor for catheter-related infection,[280] so dressings should be changed if they become soiled, damp, or loosened. The CDC has recommended changing the site dressing at least every 7 days for transparent dressings for adults. When gauze dressings are used for short-term CVCs, those dressings should be replaced every 2 days.[68]

Use of topical antibiotic or antiseptic ointments at the insertion site of catheters is currently not recommended by US authorities[68] except in the case of hemodialysis catheter insertion sites in patients with a history of recurrent *S. aureus* CLABSIs.[67] Several studies examining the use of povidone-iodine ointment have produced conflicting results. Results of topical mupirocin ointment application have been more promising,[381] but mupirocin resistance has been documented.[382] In addition, ointments without fungicidal activity have been associated with increased rates of catheter colonization with *Candida* spp.[383] The clinical utility of these ointments is questionable, and we do not recommend their routine use except as an adjunct for extremely high-risk hemodialysis patients with recurrent CLABSIs. Maki and Band[384] prospectively studied the following three regimens of catheter care: (1) application of polymyxin-neomycin-bacitracin ointment at insertion and every 48 hours, (2) application of iodophor ointment at insertion and every 48 hours, or (3) no ointment. In their study of 827 random catheter insertions, there were no differences in either catheter-acquired sepsis (two cases in each group) or local inflammation among the regimens (38.9% vs. 41.9% vs. 41.7%, respectively). The only difference noted was in semiquantitative cultures of catheter tips.[384] In the polymyxin-neomycin-bacitracin ointment group, there were 6 positive cultures, in the iodophor group there were 10, and in the control group there were 18. This difference was greatest in catheters that were left in place for more than 4 days. Thus information regarding the efficacy of these antimicrobial ointments or creams for intravascular cannulas is contradictory and confusing, and the clinical utility of these compounds remains questionable.

CVCs, PICCs, hemodialysis catheters, peripheral arterial catheters, and pulmonary artery catheters should not be routinely replaced to prevent catheter-related infections.[68] Guidewire exchanges should not routinely be used to prevent infection.

Another tool in the armamentarium of preventive measures is chlorhexidine gluconate bathing. Three cluster-randomized trials examining the efficacy of this measure were recently published: one evaluating patients in a crossover trial in adult ICUs[385]; one evaluating universal decolonization for methicillin-resistant *Staphylococcus aureus*, including chlorhexidine bathing in ICU patients[386]; and a crossover trial in adult ICU patients.[387] A reduction in blood cultures with skin-colonizing organisms was observed in two of these studies, although the overall benefit of chlorhexidine bathing was less clear.[388] Reduced chlorhexidine susceptibility has been reported,[389] though the clinical significance of this resistance is unclear. Intuitively, such resistance would seem foreboding.[390] Although these and some other studies have shown a reduction in hospital-acquired bloodstream infections with daily chlorhexidine bathing, the largest benefit appears to be in settings where the baseline prevalence of multidrug-resistant organisms is high. Improved adherence to hand hygiene may have a similar effect, with less risk of allergy, higher costs, and resistance to antiseptics and disinfectants.[391] Nevertheless, the 2011 CDC guidelines for prevention of intravascular catheter–related infections recommend using a 2% chlorhexidine wash for daily skin cleansing to reduce catheter-related bloodstream infections in ICU patients.[68]

Special Approaches for the Prevention of CLABSIs

Antimicrobial Prophylaxis

Some investigators have advocated using prophylactic antimicrobials in specific, defined circumstances to prevent catheter-associated infection. For example, Baier and colleagues[392] found that prophylactic treatment of neonates with central catheters with vancomycin effectively prevented coagulase-negative staphylococcal bacteremia associated with the use of these catheters. The use of continuous-infusion vancomycin for low-birth-weight infants has been shown to decrease rates of coagulase-negative staphylococcal bacteremia.[393] Unfortunately, prolonged low levels of vancomycin, such as result from this form of prophylaxis, could predispose to vancomycin resistance. Rates of early infection after implantation of totally implantable venous access devices were recently reported as 0.6%.[394] These authors concluded that the rate of early infection without antibiotic prophylaxis was so low that use of prophylactic antibiotics for totally implantable venous access devices is not recommended. Systemic antimicrobial prophylaxis is strongly discouraged.[67,68]

Antimicrobial Lock Prophylaxis

The use of antimicrobial lock solutions, in which an antibiotic or other antimicrobial is injected into the catheter lumen and the solution is left to dwell within the lumen for periods of some hours or days, has been examined as a method of preventing catheter infection during the past several years.[395–399] Currently, antimicrobial locks for prevention of CLABSIs or other catheter-related infections are not recommended for use in all patients with CVCs.[67,68] This technique has been shown in four meta-analyses to help prevent CLABSIs among hemodialysis patients.[396,397,400] Similar results have been found in patients with malignancy,[399] although the American Society of Clinical Oncology, in recent clinical practice guidelines, indicated that data are not sufficient to recommend for or against this prophylaxis modality.[401] Four of five studies in hematology-oncology patient populations demonstrated efficacy of vancomycin plus heparin as antibiotic lock prophylaxis[398,402–404]; the fifth study found no significant difference in bacteremia rates or time to the first episode of bacteremia when heparin flush was compared with a heparin plus vancomycin flush solution.[405] Although neither vancomycin[404] nor ciprofloxacin[403] could be detected in the blood after flushing with either antibiotic, concern about development of vancomycin-resistant organisms after widespread use of small amounts of vancomycin has limited use of this prophylactic technique. Safdar and Maki[406] performed a meta-analysis examining the use of vancomycin-containing locks in preventing bloodstream infection in patients with long-term central venous access devices. They concluded that use of vancomycin lock solution in high-risk patient populations, including patients with malignancy, with long-term central catheters reduces the risk of bloodstream infection. These authors also acknowledged the concern regarding promotion of antimicrobial resistance with use of antibiotic lock solutions, and they suggested that antiinfective solutions should be studied that have broad-spectrum antiinfective activity but that do not select for resistance.[406] Another group used a gentamicin and heparin lock, and within 6 months gentamicin-resistant catheter-related bloodstream infections had emerged.[407] Alternative agents include taurolidine,[408–410] minocycline-EDTA,[319,411] and ethanol.[412,413] Antimicrobial locks are currently recommended for use in preventing CLABSIs for two groups of patients: (1) those with limited venous access and a history of recurrent CLABSIs[68] and (2) those with heightened risk for severe sequelae from CLABSIs.[67] We believe that, in general, vancomycin use in antimicrobial locks should be avoided.

Antimicrobial Catheters

Another approach that has been advocated to reduce the risk for CVC-associated infection is bonding of an antimicrobial agent or antiseptic to the device or the addition of a subcutaneous catheter cuff impregnated with an antiseptic or antimicrobial, most commonly including chlorhexidine–silver sulfadiazine and minocycline-rifampin. A 2005 review of these catheters concluded that 40% of intravascular device–related bloodstream infections are preventable with the use of antimicrobial-impregnated CVCs.[414] These authors also emphasized that, based on the results of 19 randomized controlled trials, three meta-analyses, and two cost-benefit analyses, the overwhelming preponderance of evidence concerning the use of antimicrobial catheters suggests efficacy.[414] Another systematic review examined 32 trials of various types of antimicrobial CVCs and concluded that the pooled results for all catheters suggest a statistically significant advantage in reducing CLABSIs, with an odds ratio of 0.45.[415] This review also found that the pooled results of nine studies examining catheters treated only externally showed a nonsignificant effect on decreasing CLABSIs.[415]

Minocycline-rifampin coating appears to have superior and more prolonged activity against staphylococci.[416] Several subsequent studies have shown that these minocycline-rifampin catheters significantly decrease the risk of CLABSIs.[417–421] In vitro and in vivo data suggest that the efficacy of the minocycline-rifampin catheters may be prolonged beyond that of chlorhexidine–silver sulfadiazine catheters.[419,422] The efficacy of the minocycline-rifampin catheters, when kept in situ for longer periods of time, has been assessed in one study.[420] In this study, the minocycline-rifampin silicone catheters had a mean dwell time of 68.2 days and were associated with a significant decrease in CLABSIs.[420] These antibiotic-impregnated catheters are associated with a theoretical risk of increased antimicrobial resistance,[423] and one in vitro study found that second-generation chlorhexidine–silver sulfadiazine catheters appear to be more resistant to colonization with rifampin-resistant *S. epidermidis* than minocycline-rifampin catheters.[424] Another study of minocycline-rifampin catheters over 7 years reported no increased resistance of staphylococcal isolates to tetracycline or rifampin.[421]

Chlorhexidine–silver sulfadiazine catheters have been shown to be cost-effective in patients at high risk of intravenous device–related bacteremia. Another cost-effectiveness analysis suggested that the clinical and economic benefits of minocycline-rifampin catheters increase with days of catheterization.[425] For patients with CVCs in situ for 8 days, minocycline-rifampin catheters were more beneficial than chlorhexidine–silver sulfadiazine catheters, and cost savings accrued in patients catheterized for at least 13 days. Another review examined 11 randomized studies comparing patients with CVCs impregnated with antimicrobial agents with control patients receiving nonimpregnated CVCs.[426] These authors concluded that the efficacy of antimicrobial-impregnated CVCs in preventing catheter-related bacteremias is questionable, and that routine use of these catheters must be reevaluated. Halton and Graves[427] reviewed a series of cost-effectiveness studies in 2007 and concluded that use of antibiotic-coated catheters, compared with use of either antiseptic-coated or standard catheters, was both clinically effective and cost-saving. Another study suggested that only patients receiving parenteral nutrition may benefit from antiseptic-impregnated CVCs.[114] The most recent meta-analysis suggested that minocycline-rifampin catheters appear to be the most effective in preventing catheter-related bacteremia, although overall benefits in reducing clinical sepsis and mortality remain uncertain.[428] These authors also suggested that surveillance for antibiotic resistance attributed to use of these catheters should be emphasized in future trials.[428]

Recent guidelines suggest that use of antimicrobial-impregnated catheters should be reserved for locations or patient populations, or both, that have unacceptably high CLABSI rates despite implementation of the basic prevention strategies, including education, use of maximal sterile barriers, and use of chlorhexidine antisepsis of skin before catheter insertion.[67,68] None of these antimicrobial catheters were, alone, capable of reducing CLABSI rates to zero. We believe that antiinfective catheters should be implemented only as part of a comprehensive nosocomial bacteremia prevention strategy, which also includes education of staff and adequate skin antisepsis. Institutions may choose to implement one of these catheters after reviewing both their current and their goal intravenous device–associated bacteremia rates. Further research is needed to define the actual effect of these catheters on bacteremia rates, as well as the most efficacious catheters for different durations of catheterization and different subpopulations of patients.

Use of heparin or other anticoagulants has also been advocated as a method for reducing both thrombotic and infectious complications of central venous catheterization. Randolph and coworkers[429] concluded that heparin administration effectively reduces thrombus formation and may reduce catheter-related infections in patients who have CVCs and pulmonary artery catheters in place. Several anticoagulants have been suggested for use in this setting, and Randolph and coworkers[429] noted that cost-effectiveness comparisons of these several preparations (e.g., unfractionated heparin, low-molecular-weight heparin, and warfarin) are needed. Recombinant tissue plasminogen activator used once weekly instead of heparin three times per week significantly reduced the incidence of both catheter malfunction and bacteremia in another study.[430] Several reviews of various anticoagulants used in the prevention of CLABSIs all concluded that these agents, including urokinase,[431] tissue plasminogen activator,[432] and heparin-bonded catheters,[433] may be effective, but they underscored the fact that more adequately powered clinical trials are needed. Currently, we do not recommend routine use of urokinase or other thrombolytic agents as adjunctive therapy in patients with catheter-related bacteremia.

Other Prevention Issues

The role of appropriate nurse staffing in preventing catheter-associated infection deserves attention. In one study, nursing staff reductions during a period of increased use of parenteral nutrition were directly associated with an increase in catheter-associated bacteremias in a surgical ICU.[434] Robert and colleagues[435] reported in 2000 that bacteremias in surgical ICU patients increased when nurse staffing changed to include fewer "regular" registered nurses and more pool/agency nurses. "Float" nurses (usually assigned elsewhere in the hospital or from an agency) were associated with an increased risk of CLABSIs in ICU patients in another study,[114] suggesting that an increased proportion of nurses with less than 1 year of experience in the ICU can lead to increased risk of CLABSIs.[436] Adequate nurse staffing has been identified as one factor integral to preventing bacteremia.[437] In this era of increased focus on preventing these infections, the impact of staffing reductions on untoward outcomes is deserving of careful scrutiny.[434]

New scientific approaches are needed to help establish optimal techniques for catheter management, and further technologic advances, such as bonding antimicrobial and antiseptic agents to the intravascular device, may also reduce risks for device-associated infection. A 2005 survey of use of CLABSI prevention practices in 516 US hospitals found that fewer than half of non–Veterans Affairs hospitals reported concurrent use of maximal sterile barrier precautions, chlorhexidine gluconate, and avoidance of routine central line changes.[438] Although recommended prevention practices, including the bundling of complementary strategies, is increasing both in hospitals subject to the CMS no-payment rule as well as in Department of Veterans Affairs hospitals,[439] additional effort is needed to reach the desired outcome of zero CLABSIs. Increased attention to such details can significantly lower the endemic rate of device-associated infection as well as decrease the number of epidemics of such infections.

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The complete reference list is available online at Expert Consult.

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301 Nosocomial Pneumonia

Michael Klompas

SHORT VIEW SUMMARY

Diagnosis

- Classic clinical signs of ventilator-assisted pneumonia (VAP) include fever, leukocytosis, purulent secretions, worsening oxygenation, infiltrates, and pathogenic cultures. These signs are neither sensitive nor specific. Paucity of neutrophils or lack of organisms on Gram stain makes VAP unlikely, but their presence is not specific. Stable oxygenation/ventilator settings argue against clinically significant disease.
- Quantitative bronchoalveolar lavage (BAL) cultures are 57% sensitive, are 80% specific, and have a positive predictive value of 77% for histologically confirmed VAP.
- Randomized controlled trials of quantitative BAL cultures versus endotracheal aspirates for diagnosis have found no difference between these sampling strategies on duration of mechanical ventilation, length of stay, mortality, superinfection, or acquired resistance rates. Endotracheal aspirates are therefore preferred.
- Diagnostic studies are performed to guide therapy: blood and endotracheal aspirate cultures, urine pneumococcal and *Legionella* antigens, with or without viral studies are recommended.

Microbiology

- The most common pathogens are *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* spp., and *Acinetobacter* spp. Drug resistance in VAP is common; 25% to 40% of *S. aureus* isolates are methicillin resistant, 20% to 30% of *Pseudomonas* and *Klebsiella* isolates are ceftazidime and cefepime resistant, and 55% to 70% of *Acinetobacter* isolates are carbapenem resistant. Drug resistance is less common in early hospital-acquired pneumonia (HAP), particularly in patients who have not spent time in an intensive care unit. Risk factors for methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant gram-negative pathogens include recent broad-spectrum antibiotic use, prolonged hospitalization, poor functional status, hemodialysis, and severe illness.

Treatment

- Initiate broad-spectrum antibiotics as soon as pneumonia is suspected. Tailor coverage to the local distribution of pathogens and susceptibilities associated with HAP and VAP. In the absence of local data, use vancomycin or linezolid plus two antipseudomonal agents for empirical therapy. Narrower-spectrum regimens are appropriate if local antibiotic resistance rates are low and there are no patient-level risk factors for MRSA or other antibiotic resistant pathogens. Consider a loading dose of vancomycin 25 to 30 mg/kg for seriously ill patients. Include anaerobic coverage only if there is frank aspiration.
- Reassess the likelihood of pneumonia daily; if the diagnosis no longer seems likely, then stop antibiotics.
- Narrow treatment as soon as susceptibilities are available. Avoid double coverage once pathogens and susceptibilities are known. If cultures are negative and the patient's condition is improving, trim or stop antibiotics.
- Vancomycin and linezolid are similarly effective for MRSA. Vancomycin is administered at 15 to 20 mg/kg every 8 to 12 hours. The goal trough is 15 to 20 mg/L. There is an increased risk of clinical failure with underdosage and increased risk of nephrotoxicity with supratherapeutic doses.
- Treat patients for 7 to 8 days, or for a shorter duration in those with rapid clinical improvement, and longer in those who are slow to improve or who have complications such as abscess or empyema. Daily procalcitonin monitoring can be used to safely shorten the duration of antibiotics.
- Adjunctive aerosolized antibiotics in addition to intravenous therapy enhance clearance of pulmonary cultures and may improve clinical cure rates, but they do not affect more objective patient outcomes such as duration of mechanical ventilation, nephrotoxicity, or mortality. Vibrating mesh plate is preferred for delivery. Data are sparse.

Prevention

- VAP rates are subjective and nonspecific. Therefore preferentially select interventions proven to improve concrete outcomes such as duration of mechanical ventilation, mortality, and/or antibiotic utilization.
- Noninvasive positive pressure ventilation, minimizing sedation, and ventilator-weaning protocols (especially paired daily spontaneous awakening and breathing trials) shorten the average duration of mechanical ventilation.
- Digestive decontamination decreases mortality, but there are ongoing concerns about the potential impact on antibiotic resistance rates, especially in units with high baseline resistance rates.
- Oral care with chlorhexidine reduces the frequency of positive pulmonary cultures but has no effect on objective outcomes. Some studies have found an association between oral care with chlorhexidine and higher mortality rates.

Nosocomial pneumonia refers to pneumonia acquired while in a hospital. It is classically divided into hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) but has also been applied to the concept of health care–associated pneumonia (HCAP). The majority of studies on nosocomial pneumonias focus on VAP; hence, this chapter also focuses primarily on VAP, but the principles developed from VAP are thought to be generally applicable.

HEALTH CARE–ASSOCIATED PNEUMONIA

Pneumonias acquired outside the hospital by patients with significant contact with the health care system (HCAP) have historically been conflated with HAP because some early case series found that their pathogen distribution, resistance profiles, and mortality rates were similar to pneumonias acquired by patients inside hospitals.[1–6] The equilibration of HCAP with HAP has been called into question, however, owing to the fear that the HCAP category is unnecessarily broad and drives substantial overuse of broad-spectrum antimicrobials.[7,8] Prospective case series do document higher rates of methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas, Acinetobacter,* and enteric gram-negative pathogens in patients with HCAP, but the overall prevalence of these pathogens is still low. The pooled prevalences of MRSA and *Pseudomonas* in HCAP patients across eight prospective studies, for example, were only 2.2% and 2.1%, respectively.[9] Investigators in some case series do report much higher prevalences, but this typically occurs when the analysis is restricted to patients with positive cultures, typically a sicker subset of the total pneumonia population.[10,11] Pathogen prevalences can also vary substantially by geography, hospital type, and population

characteristics. The local prevalence of drug-resistant pathogens, recent exposure to broad-spectrum antibiotics, hemodialysis, poor functional status, and severity of illness appear to be better predictors of drug-resistant pathogens and morbidity than patients' outpatient health care contacts.[6,10,12,13]

EPIDEMIOLOGY

"Official" VAP rates reported by infection control programs have fallen to extremely low levels over the past 15 years. According to the Centers for Disease Control and Prevention (CDC), the mean VAP rate for medical intensive care units (ICUs) fell from 4.9 to 0.9 VAPs per 1000 ventilator-days between 2002 and 2012.[14,15] During the same period, VAP rates in surgical ICUs fell from 9.3 to 2.2 events per 1000 ventilator days. Remarkably, the median VAP rate for US medical ICUs in 2012 (the last year for which data are available) was zero.[15] These incidence densities correspond to VAP incidence rates of less than 1 VAP per 100 ventilated patients.

The credibility of these rates has been questioned. They were generated by infection preventionists using the CDC's traditional surveillance definitions for nosocomial pneumonia, but the CDC's old definitions included many subjective criteria such as "new or progressive infiltrates," "change in the character of sputum," and "worsening oxygenation."[16–19,20] Many observers were therefore concerned that increasing pressures on US hospitals to report low VAP rates might have influenced surveyors to interpret these very subjective clinical signs more strictly over time.[21,22] An audit of chart diagnoses of VAP in ventilated patients conducted by the US Centers for Medicare and Medicaid Services found that the rate of clinically diagnosed and treated VAP was in fact stable at approximately 10 cases per 100 ventilated patients between 2005 and 2013.[23] Likewise, cross-sectional surveys of ICUs continue to suggest that about 15% of patients are on antibiotics for nosocomial respiratory infections at any given time.[19,20,22,24,25]

Fewer data are available on the incidence of nosocomial pneumonia outside ICUs. A 1-day point-prevalence survey of health care–associated infections in 183 hospitals in 10 US states identified 67 nosocomial pneumonias among 10,748 nonventilated patients (0.6% incidence).[26] A second study using discharge diagnosis codes from a 20% sample of US hospitals reported that 1.6% of hospitalized patients had secondary diagnosis codes for pneumonia.[27] A third set of investigators reviewed the charts of all patients with discharge diagnosis codes for pneumonia in 21 US hospitals. Nosocomial pneumonia rates in nonventilated patients ranged from 0.12 to 2.28 cases per 1000 patient-days.[28]

MORBIDITY AND MORTALITY

VAP is estimated to extend the duration of mechanical ventilation and intensive care by 4 to 6 days.[29,30] Crude mortality rates for VAP range between 16% and 78%.[31] Crude mortality rates for non-ICU nosocomial pneumonia range from 15% to 53%.[28,32–34] Identifying the fraction of these deaths attributable to pneumonia itself rather than to patients' underlying frailties, however, is controversial. Estimates have ranged from 0% to 50%.[35–40] More recent studies using multistate models to account for the time-dependent nature of VAP (the longer a patient remains on a ventilator, the higher his or her risk of both VAP and death) have found attributable mortality rates of 8% to 10%.[41–43] Dutch researchers calculated almost identical figures (9%–13%) using an entirely different method. They estimated attributable mortality as the ratio of the pooled mortality risk reduction to the pooled VAP risk reduction in the treatment versus control arms of randomized controlled trials of VAP prevention interventions.[44,45] Estimates using marginal structural models are even lower: 6% of ICU deaths are due to VAP, corresponding to a net-attributable mortality rate of 1.5%.[36]

PATHOPHYSIOLOGY

The histologic hallmark of VAP is heterogeneity. Autopsies of ventilated patients' lungs are often notable for widely scattered, patchy areas of inflammation.[46] The lesions vary significantly in age and severity, ranging from bronchiolitis to bronchopneumonia to frank abscess, often within the same lung. Dependent areas tend to be affected more often than nondependent areas. Different organisms can be cultured from different lung segments of the same patient in 25% to 37% of cases.[46,47] Cultures of histologically benign–appearing lung segments are often positive.[48,49] Taken together, these findings suggest that ventilated patients are prone to repeated microaspirations around the endotracheal tube cuff, some of which progress to clinically manifest pneumonia, whereas others resolve spontaneously.

Microbiologic, structural, humoral, and pharmacologic factors combine to increase the risk of pneumonia in critically ill patients. The flora of the oral tract rapidly shifts on admission from typical community respiratory organisms (streptococci, *Haemophilus*) toward "hospital-associated" pathogens such as *S. aureus,* Enterobacteriaceae, *Pseudomonas,* and *Acinetobacter* species. The likelihood of these organisms being drug resistant steadily increases with time in the hospital, exposure to antimicrobials, and severity of illness. Orogastric and nasogastric feeding tubes disrupt the lower esophageal sphincter and increase the risk of aspiration of gastric contents.[50] The presence of an endotracheal tube disrupts normal ciliary clearance of constitutive bronchial secretions and impairs patients' capacity to cough. Secretions therefore pool above the endotracheal tube cuff and intermittently seep around folds in the cuff, particularly if the cuff is underinflated or if it shifts during patient movement or repositioning.[51] Biofilm begins to form both inside and outside the endotracheal tube within a day of placement and serves as a bacterial reservoir within the trachea and oropharynx. Suctioning or instillation of aerosols through the endotracheal tube can mobilize and embolize bacteria from the biofilm into the lungs.[49] Critical illness, poor nutrition, sedation, and immobilization may increase patients' susceptibility to infection.[52,53] These factors interact with and reinforce one another to enhance the risk of microaspiration and the likelihood that pulmonary parenchymal colonization will lead to invasive infection.

RISK FACTORS

Understanding the pathophysiology of pneumonia helps predict HAP and VAP risk factors. Factors that enhance the risk of aspiration increase the likelihood of infection. These include mechanical factors such as emergency intubation, reintubation, duration of intubation, supine positioning, enteral feeding through use of orogastric or nasogastric tubes, use of paralytic agents, and underinflation of the endotracheal tube cuff[51,54–62]; factors that affect mental status such as central nervous system disease, impaired consciousness, and depth of sedation[55,59,60]; factors that increase bacterial bioburden in the upper respiratory and orogastric tracts, such as duration of hospitalization, nasogastric intubation, prolonged antibiotic exposures, and the use of proton pump inhibitors or other gastric acid suppressants[60,63,64]; factors that increase handling or breaking of the ventilator circuit, such as inhaled β-agonist therapy[65]; and patient factors such as age, preexisting lung disease, and severity of illness.[54,60,66] Intensive care staffing levels and transportation out of the ICU for diagnostic imaging or procedures are additional factors that can increase risk.[67–69] Surgical patients in general, and burn and trauma patients in particular, have higher VAP rates than medical patients.[55,70]

DIAGNOSIS

More than 100 years ago, Sir William Osler noted a striking discrepancy in pneumonia rates between the wards and the autopsy suite.[71] Contemporary autopsy series continue to confirm the limitations of bedside diagnosis for nosocomial pneumonia.[72] Tejerina and colleagues,[73] for example, evaluated common clinical definitions for VAP in a population of 253 patients, with histologic findings as the reference standard. Defining VAP as a new radiographic infiltrate and two additional signs—leukocytosis, fever, or purulent respiratory secretions—was 65% sensitive and 36% specific. Defining VAP more strictly by requiring radiographic infiltrates and all three of leukocytosis, fever, and purulent respiratory secretions increased specificity to 91% but dropped sensitivity to 16%. Further requiring positive cultures (tracheal aspirate culture with pathogenic organisms, no minimum growth threshold) raised the specificity of both the loose and the strict definitions to 93% and 99%, respectively, but with further cost in sensitivity.[73]

HAP and VAP diagnosis are challenging because the cardinal clinical signs of pneumonia are neither sensitive nor specific in hospitalized patients. Hospitalized patients in general, and critically ill ventilated patients in particular, are at risk for a host of pulmonary complications

including pulmonary edema, atelectasis, thromboembolic disease, acute respiratory distress syndrome (ARDS), hypersensitivity reactions, and hemorrhage, in addition to pneumonia. All of these complications tend to present with some combination of a common core set of clinical signs: fever, leukocytosis, impaired oxygenation, changes in the character and quantity of sputum production, and radiographic infiltrates. The problem is compounded by the fact that many hospitalized patients have abnormal lung conditions at admission that further complicate the interpretation of radiographs and result in impaired oxygenation and abnormal sputum production. These include cancers, scars from prior surgeries, bronchiectasis, obstructive lung disease, pulmonary fibrosis, and, in the case of trauma patients, contusions, lacerations, pulmonary hemorrhage, inhalation injuries, or combinations of these insults. Most patients are found to have two or more conditions contributing to their "pneumonia-like" syndrome.[74–76] Sometimes fever, leukocytosis, and inflammatory syndromes caused by extrapulmonary disease (e.g., bloodstream infections, surgical site infections, pancreatitis) can occur concurrently with noninfectious pulmonary conditions (e.g., atelectasis, pulmonary edema, ARDS) to render a net clinical picture that looks like pneumonia even though the contributing conditions in isolation would rarely be confused with pneumonia.[77] All told, VAP is confirmed at autopsy in only two-thirds of patients with clinically suspected pneumonia.[48,73,78–81]

Alveolar infiltrates and air bronchograms are the most sensitive radiographic signs for autopsy-proven VAP, but neither is specific (sensitivity 83% and 88%, specificity 58% and 26%, respectively).[75] Focal, unilateral infiltrates are more specific than bilateral infiltrates (specificities of 80% and 47%, respectively) but are present in only 21% of cases.[75] Radiographic progression alone is a poor predictor of the presence or absence of histologic pneumonia.[82] Accurate radiographic diagnosis is even more challenging in patients with ARDS.[75,83] In general, there is no correlation between the presence or absence of radiographic infiltrates and positive cultures on quantitative bronchoalveolar lavage (BAL) or autopsy.[47,82,84] Computed tomography (CT) is more accurate than portable radiographs to diagnose pneumonia,[85,86] but few studies have been published on the use of CT specifically for nosocomial pneumonias. On blinded comparison of immediate postmortem CT versus autopsy in 182 patients, radiologists missed 32% of pneumonias and overcalled 14%.[87]

Quantitative BAL cultures have been advocated to increase the accuracy of VAP diagnosis, but studies comparing quantitative bronchoscopic cultures and histologic findings only partially confirm this. Specimens are prone to contamination with organisms residing in the mouth and on the endotracheal tube; sampling the incorrect lung segment can generate both false positives (subclinical bronchiolitis rather than frank pneumonia) and false negatives (an uninfected lung segment instead of the infected segment); and recent antibiotic exposure can misleadingly suppress growth.[46,88] Bronchoscopy studies bear witness to the microbial complexity and diversity of intubated patients. There is little or no correlation between positive quantitative cultures and the presence of infiltrates or leukocytosis.[47,84] Cultures taken from different lung segments are discrepant in more than one-third of patients with suspected VAP.[47] There is little correlation between Gram stain characteristics (gram positive vs. gram negative) and final culture results.[89] In addition, VAP exists on a microbiologic and histologic spectrum that may not cross arbitrary quantitative thresholds for positivity.[90] Studies evaluating the accuracy of quantitative BAL for VAP relative to histologic findings have found sensitivities of 14% to 90% (weighted average, 57%), specificities of 42% to 100% (weighted average, 80%), and positive predictive values of 29% to 100% (weighted average, 77%).[79,82,91–94] Of note, negative cultures are not protective: Patients with culture-negative VAP have outcomes similar to or worse than those of patients with culture-positive VAP.[95–97]

Clinical Pulmonary Infection Score

Some workers have proposed scoring systems to aid diagnosis. The most common of these is the Clinical Pulmonary Infection Score (CPIS).[98] The original score was calculated by assigning 0 to 2 points for each of six clinical criteria, including temperature, white blood cell count, quantity and quality of respiratory secretions, Gram stain and culture findings, ratio of Pao_2 to Fio_2, and radiographic results. A score of more than 6 was considered diagnostic for pneumonia. Many workers have proposed modified versions of the CPIS to simplify application or make it more suitable for early diagnosis of VAP by deemphasizing cultures and increasing emphasis on dynamic changes in radiographs (Table 301.1).[99,100] Unfortunately, validation studies do not bear out the value of the CPIS.[92,99–104] In the autopsy series by Tejerina and colleagues,[73] for example, the sensitivity and specificity of a CPIS score of greater than 6 were 46% and 60%, respectively.

TABLE 301.1 Clinical Pulmonary Infection Score (CPIS) as Modified by Schurink and Colleagues

CPIS POINTS	0	1	2
Temperature, °C	≥36.1 and ≤38.4	≥38.5 and ≤38.9	≤36 or ≥39
White blood cell count, per 10^9/L	≥4 and ≤11	≤3.9 or ≥11.1	≥11.1 with band forms or ≥17.1
Tracheal secretions	Absent	Present but nonpurulent (white or light yellow)	Purulent (yellow, green, or brown)
Oxygenation (Pao_2 to Fio_2 ratio)	>240		<240 and no adult respiratory distress syndrome
Chest radiograph	No infiltrate	Diffuse or patchy infiltrate	Localized infiltrate
Tracheal aspirate culture result (semiquantitative growth amount)	None (0)	Moderate (1+ or 2+)	Heavy (3+)

From Schurink CA, Van Nieuwenhoven CA, Jacobs JA, et al. Clinical pulmonary infection score for ventilator-associated pneumonia: accuracy and inter-observer variability. Intensive Care Med. *2004;30:217–224.*

SUMMARY AND RECOMMENDATIONS FOR CLINICAL DIAGNOSIS

The best available evidence suggests that clinical examination can alert physicians to the possibility of VAP but that it is insufficient to definitely rule in or rule out pneumonia.[105] Examination of sputum or BAL specimens can help refine clinical suspicions. The absence of organisms on Gram stain or less than 50% neutrophils in BAL fluid help exclude the diagnosis.[79,81,82,89] The presence of organisms or neutrophils, however, is not specific. Stable ventilator settings argue against a clinically impactful pneumonia.[106,107] The absence of new infiltrates makes pneumonia unlikely, but their presence is not specific. None of these findings are sufficiently powerful to establish or refute a diagnosis of VAP definitively. Clinicians caring for ventilated patients with a clinical syndrome consistent with VAP should therefore have a low threshold to consider additional diagnoses and further investigations, particularly if an empirical trial of antibiotics does not lead to improvement within 48 to 72 hours.

Microbiologic Evaluation

It is important to try to establish a microbiologic diagnosis in order to ensure coverage of the active pathogen(s), to minimize exposure to unnecessary agents, and to help define the distribution of pathogens and resistance profiles in one's practice setting with a view to informing local empiric treatment guidelines. Inappropriate and delayed therapy are associated with increased mortality risk.[108–111] Blood and respiratory cultures are indicated in all patients. Pneumococcal and *Legionella* urine antigen testing increase diagnostic yield compared with respiratory sampling alone.[32] Viruses account for up to one-quarter of nosocomial pneumonia cases.[34,112–116] Viral studies are therefore indicated during periods of endemicity, in immunocompromised patients, and in patients with diffuse ground-glass opacities on CT.[112–114]

Invasive Versus Noninvasive Respiratory Tract Sampling

A number of options for respiratory tract sampling are available, including endotracheal aspirates, BAL, and protected specimen brush. These in

turn can be analyzed qualitatively or quantitatively. There has been a great deal of controversy over whether clinicians should routinely acquire specimens by using invasive techniques (BAL or protected specimen brushes) or noninvasive techniques (endotracheal aspiration). This question has now been evaluated in five randomized trials.[117–121] Route of sampling made no difference in average duration of mechanical ventilation or intensive care length of stay. All but one trial[120] found no difference in mortality rates. A meta-analysis of all five trials also found no difference in any patient outcome, including the frequency of antibiotic changes.[122] Invasive sampling should consequently be reserved for patients with negative endotracheal aspirate cultures whose condition worsens despite empirical coverage or patients with positive endotracheal aspirate specimens whose condition worsens despite targeted antibiotics. Repeat sampling in patients in whom empirical treatment fails can lead to antibiotic switches or stops in 30% to 50% of patients.[3,123,124] Sampling is still worthwhile in patients on antibiotics. In one study, for example, BAL yield was 72% despite a mean 11.4 days of antibiotics before sample acquisition.[123] Not surprisingly, these patients are often found to be harboring multidrug-resistant pathogens.

MICROBIOLOGY

The microbiology of nosocomial pneumonia varies considerably depending on the duration of hospitalization before pneumonia, the severity of illness, comorbid conditions, the reason for admission, and prior antibiotic exposures. *Streptococcus pneumoniae,* β-hemolytic streptococci, and *Haemophilus influenzae* are the most frequent organisms in patients just admitted to the hospital (≤2 days).[125] Thereafter, *S. aureus, Pseudomonas aeruginosa,* Enterobacteriaceae, and *Acinetobacter* species begin to predominate. The risk of *S. aureus* being methicillin resistant rises progressively with duration of hospitalization.[126] Patients hospitalized for extended periods or exposed to prolonged courses of antibiotics, or both, are also susceptible to *Stenotrophomonas maltophilia, Burkholderia cepacia,* and other difficult-to-treat organisms. Some VAP cases are polymicrobial, especially in aspiration pneumonia and ARDS.[127–129] *Candida* species, enterococci, and coagulase-negative staphylococci are almost always colonizers rather than invaders.[130–132]

Although clinicians have long recognized that the distribution of causative pathogens evolves over the course of hospitalization, the breakpoint at which microbial ecology shifts has been subject to debate.[133] Different investigators have used different cutoffs to define early versus late pneumonia (4, 5, and 7 days) and have variously counted these days from hospital admission, ICU admission, or initiation of mechanical ventilation. A number of studies note little or no differences in pathogen mix between early- versus late-onset pneumonias.[134,135] It is clear, however, that the ecology of respiratory flora can shift within 1 to 2 days of hospital admission, so even studies using breakpoints of 4 days or less to define early-onset pneumonia may still be lumping together patients who have their native flora with those who have begun to acquire hospital spectrum flora.[22,136] Duration of hospitalization before pneumonia is probably a better predictor of microbiology than duration of mechanical ventilation.[125] However, it is clear from studies comparing early- versus late-onset pneumonia that just as so-called late pathogens can be isolated early in the course of hospitalization, so too is it possible to isolate so-called early pathogens late in the course of hospitalization.[22,128] The median numbers of ventilator days and hospital days until the appearance of various pathogens at bronchoscopy in a series of 346 patients in a Swedish ICU are summarized in Table 301.2.

Certain comorbidities predispose to particular pathogens.[127] Patients with cystic fibrosis are prone to *S. aureus* and *P. aeruginosa*. Patients with chronic obstructive pulmonary disease have higher rates of *S. pneumoniae, H. influenzae,* and *Moraxella catarrhalis.* Diabetes and head trauma are risk factors for *S. aureus.*

The prevalence of nosocomial pneumonias attributable to resistant pathogens has risen over the past 2 decades.[137–139] The overall distribution of pathogens among VAP cases reported to the CDC's National Healthcare Safety Network in the years 2011 and 2012 is shown in Table 301.3, along with their antibiotic resistance patterns.[139] Risk factors for drug-resistant bacteria include severe illness, prolonged mechanical ventilation, recent broad-spectrum antibiotic exposure, recent hospitalization or residence in an extended-care facility, and immunosuppression.[94,128,140,141] The findings

TABLE 301.2 Median Number of Hospital Days and Ventilator Days Until First Isolation of Different Pathogens With Bronchoscopy

PATHOGEN	VENTILATOR DAYS MEDIAN (IQR)	HOSPITAL DAYS MEDIAN (IQR)
β-Hemolytic streptococci	0.26 (0.01–1.91)	1.15 (0.38–2.24)
Streptococcus pneumoniae	1.20 (0.45–1.79)	1.33 (0.78–2.03)
Haemophilus influenzae	1.94 (1.20–2.88)	2.12 (1.44–3.12)
Staphylococcus aureus	0.96 (1.15–2.24)	2.70 (1.68–5.36)
Escherichia coli	2.01 (0.45–5.11)	4.26 (2.18–6.85)
Klebsiella species	2.10 (0.53–5.65)	4.83 (2.43–10.94)
Other Enterobacteriaceae	2.55 (0.39–5.76)	4.40 (2.27–13.32)
Acinetobacter species	2.86 (0.24–5.90)	4.25 (1.61–5.90)
Pseudomonas aeruginosa	3.79 (1.09–10.48)	5.29 (2.85–18.66)
Stenotrophomonas maltophilia	5.73 (2.70–15.72)	11.09 (5.75–34.35)

IQR, Interquartile range.
From Hyllienmark P, Martling CR, Struwe J, et al. Pathogens in the lower respiratory tract of intensive care unit patients: impact of duration of hospital care and mechanical ventilation. Scand J Infect Dis. *2012;44:444–452.*

of studies on whether or not drug-resistant pathogens increase attributable morbidity and mortality are inconsistent.[126,140,142–146]

There is a correlation between severity of pneumonia and culprit organisms. Patients who require intubation are more likely to harbor MRSA and resistant gram-negative pathogens than patients who do not (Table 301.4).[6,10,147] Likewise, among patients with ICU-acquired pneumonia, there is a linear trend between severity of illness score and progressive likelihood of MRSA, extended-spectrum β-lactamase producers, and gram-negative pathogens resistant to third-generation cephalosporins.[140] This parallels the early work of Johanson and colleagues,[136] who compared the frequency of gram-negative pathogens in oropharyngeal cultures from healthy community dwellers, healthy clinicians working in a hospital setting, psychiatric inpatients, "moderately ill" orthopedic patients, and moribund medical patients. The prevalence of gram-negative pathogens was less than 5% in the first three groups, 22% in the moderately ill orthopedic patients, and 66% in the moribund patients.[136]

Viruses can account for up to one-third of severe pneumonias, including HAP, particularly in immunocompromised hosts.[112–116] There are reports of influenza and other respiratory virus outbreaks in hospitalized patients, including patients on mechanical ventilation.[148–150] Reactivation disease secondary to cytomegalovirus (CMV) can also manifest well after admission. The mortality rate for viral pneumonias that merit intensive care is similar to that for bacterial pneumonias.[113]

The causative pathogens for non-ICU pneumonia are less often identified, presumably because of prior antibiotic exposures, the difficulty of obtaining adequate sputum specimens in nonintubated patients, and some rate of misdiagnosis due to aspiration pneumonitis and other pneumonia mimickers. For example, a causative pathogen was identified in only one-third of patients with 165 cases of HAP of any severity versus two-thirds of 96 cases of HAP that necessitated ICU admission (see Table 301.4).[32,33] The microbiology of severe HAP necessitating ICU admission is similar to that of VAP.[32] Severe HAP necessitating ICU admission is more likely due to "nosocomial" pathogens such as MRSA and *P. aeruginosa,* whereas patients with less severe HAP not necessitating ICU care are more likely to have *S. pneumoniae* and *H. influenzae* pneumonia (see Table 301.4).[32,33] There is considerable overlap, however, between these two populations. Anaerobic bacteria are common in patients with frank aspiration but are otherwise rarely implicated.[151,152]

Knowledge of the microbiology of nosocomial pneumonia is limited by the sensitivity of conventional culture methods. Molecular diagnostic studies that use 16S and 18S polymerase chain reaction (PCR)

TABLE 301.3 Distribution of Pathogens and Their Antibiotic Resistance Patterns Among VAP Cases Reported to the CDC's National Healthcare Safety Network, 2011–2012, and ICU-Acquired Pneumonias Reported to the European Center for Disease Prevention and Control, 2015

PATHOGEN	FREQUENCY USA	FREQUENCY Europe	ANTIBIOTIC-RESISTANCE PROFILE[a]
Staphylococcus aureus	24.7%	17.0%	Methicillin/oxacillin resistant—USA 42%, Europe 23%
Pseudomonas aeruginosa	16.5%	20.1%	Ciprofloxacin/levofloxacin resistant—USA 32% Imipenem/meropenem resistant—USA 28%, Europe 24% Cefepime/ceftazidime resistant—USA 26%, Europe 24% Piperacillin-tazobactam resistant—USA 19% Aminoglycoside resistant—USA 18% Resistant to ≥3 of the above classes—USA 20%
Klebsiella species	10.2%	14.9%	Cefepime/ceftazidime/ceftriaxone resistant—USA 21%, Europe 43% Imipenem/meropenem resistant—USA 10%, Europe 11% Resistant to ≥3 classes—USA 13%
Enterobacter species	8.3%	10.5%	Cefepime/ceftazidime/ceftriaxone resistant—USA 27%, Europe 42% Imipenem/meropenem resistant—USA 3%, Europe 2.2% Resistant to ≥3 classes—USA 3%
Acinetobacter baumannii	6.1%	5.2%	Imipenem/meropenem resistant—USA 55%, Europe 69% Resistant to ≥3 classes—USA 54%
Escherichia coli	5.4%	13.2%	Ciprofloxacin/levofloxacin resistant—USA 31% Cefepime/ceftazidime/ceftriaxone resistant— SA 17%, Europe 20% Imipenem/meropenem resistant—USA 2%, Europe 0.5% Resistant to ≥3 classes—USA 10%

[a]Resistance rates for USA specifically for VAP, resistance rates for Europe for all health care–associated infections in ICUs.
CDC, Centers for Disease Control and Prevention; *ICU,* intensive care unit; *VAP,* ventilator-assisted pneumonia.
From Weiner LM, Webb AK, Limbago B, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol. *2016;37:1288–1301 and European Center for Disease Prevention and Control (ECDC). Healthcare-associated infections acquired in intensive care units. In: ECDC. Annual epidemiological report for 2015. Stockholm: ECDC; 2017.*

TABLE 301.4 Causative Pathogens of Nosocomial Pneumonia Acquired in Non-ICU Settings as a Function of Severity of Illness

	165 HAP CASES OF VARIABLE SEVERITY[31]	96 CASES OF SEVERE HAP NECESSITATING ICU ADMISSION[36]
No pathogen identified	105 (64%)	32 (33%)
Pathogen identified	60 (36%)	64 (67%)
Gram-positive pathogens:		
Streptococcus pneumoniae	16 (27%)	11 (17%)
Staphylococcus aureus	4 (6.7%)	9 (14%)
methicillin susceptible	3 (5.0%)	8 (13%)
methicillin resistant	1 (1.7%)	1 (1.6%)
Gram-negative pathogens:		
Pseudomonas aeruginosa	7 (12%)	18 (28%)
Enterobacteriaceae	8 (13%)	6 (9.4%)
Haemophilus influenzae	2 (3.3%)	2 (3.1%)
Acinetobacter species	5 (8.3%)	1 (1.6%)
Legionella pneumophila	7 (12%)	12 (19%)
Aspergillus species	7 (12%)	13 (20%)

HAP, Hospital-acquired pneumonia; *ICU,* intensive care unit.

amplification suggest that the microbiota of nosocomial pneumonia is far broader than current conventional wisdom. One evaluation of BAL fluid from 185 patients with ICU pneumonia, for example, identified 160 different organisms.[153] Almost half of these organisms had not been previously associated with pneumonia. The extent to which these agents are colonizers versus invaders, however, remains to be determined.

TREATMENT

Treating nosocomial pneumonia is a delicate balancing act between factors that favor more antibiotics and those that favor less. Concern that therapeutic delay is associated with worse outcomes, recognition of the limited sensitivity of clinical examination for VAP, and awareness of the frequent presence of multidrug-resistant pathogens favor early, aggressive treatment with broad-spectrum agents for most patients.[124,154,155] On the other hand, recognition that one-third to one-half of patients labeled as having VAP have been misdiagnosed, fear of catalyzing *Clostridioides difficile* (formerly *Clostridium difficile*) infection and other adverse effects of antibiotics, and hesitancy to drive further patient- and unit-level antibiotic resistance warrant restraint in prescribing.[73,156–162] The current solution to this dilemma is diagnostic and therapeutic humility. In practice, this means early coverage with broad-spectrum agents as soon as pneumonia seems likely, active microbiologic investigation to facilitate antibiotic narrowing, and continual reevaluation of the likelihood of pneumonia with a view to rapidly withdrawing antibiotics if the patient's clinical evolution is not consistent with pneumonia. If the evidence for pneumonia is equivocal and the patient is not in septic shock, observational data suggest that it is reasonable to forestall treatment while obtaining more diagnostic data.[163,164]

Empirical Therapy

Empirical therapy for VAP should include both gram-positive and gram-negative coverage. Specific antibiotic choices should be informed by the local distribution of pathogens and their drug resistance profiles.[165–167] US guidelines recommend designing a regimen with a 95% or greater probability of covering the patient's likely pathogens based on the patient's specific risk factors for resistant organisms and the prevailing organism and resistance profiles in one's ICU.[94] The US guidelines list the following risk factors for drug-resistant pathogens:

- Prior intravenous antibiotic use within 90 days
- Septic shock at the time of VAP
- ARDS preceding VAP
- ≥5 days since admission to hospital
- Acute renal replacement therapy before VAP onset

In the absence of local data, national surveillance data can guide antibiotic choices. In both Europe and the United States, the most common VAP pathogens are *S. aureus, P. aeruginosa,* and Enterobacteriaceae.[22,139,168] Approximately 25% to 40% of *S. aureus* isolates are resistant to methicillin, 20% to 30% of *P. aeruginosa* isolates are resistant to at least one class of antipseudomonals, and 20% to 40% of *Klebsiella pneumoniae* isolates are resistant to ceftazidime and cefepime (see Table 301.2).[139,168] Other gram-negative organisms are similarly likely to be resistant to at least one class of gram-negative agents. An ideal empirical regimen should therefore include an agent active against MRSA and two antipseudomonal agents from two different classes in order to increase the likelihood that at least one antibiotic will be active. Reasonable empirical choices are shown in Table 301.5.

The choices presented in Table 301.5 should be modified or supplemented depending on specific patient or hospital circumstances. For

TABLE 301.5 Empirical Treatment Options for Ventilator-Associated Pneumonia

GRAM-POSITIVE AGENTS (CHOOSE ONE)	ANTIPSEUDOMONAL AGENTS (CHOOSE ONE AGENT FROM COLUMN 1; IF PATIENT HAS RISK FACTORS FOR MULTIDRUG-RESISTANT PATHOGENS, INCLUDING HIGH LOCAL PREVALENCE OF MULTIDRUG PATHOGENS, THEN ALSO CHOOSE AN AGENT FROM COLUMN 2)	
Vancomycin *or* Linezolid	Antipseudomonal cephalosporin (ceftazidime or cefepime) Antipseudomonal carbapenem (imipenem or meropenem) Antipseudomonal β-lactam (piperacillin-tazobactam) Monobactam (aztreonam)	Antipseudomonal quinolone (ciprofloxacin or levofloxacin) Antipseudomonal aminoglycoside (amikacin, tobramycin, or gentamicin) Polymyxin (colistin, polymyxin B)

[a]Select one gram-positive agent and two gram-negative agents.

example, if a patient previously colonized with a resistant organism develops pneumonia, then empirical treatment should include coverage for that pathogen. Similar special circumstances might include local outbreaks or high local prevalence of multidrug-resistant *Acinetobacter,* multidrug-resistant *Pseudomonas,* or carbapenem-resistant Enterobacteriaceae.[165] Empirical treatment choices in these cases should be informed by the antibiograms of previously isolated pathogens and might include ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, colistin, tigecycline, or ampicillin-sulbactam if needed to ensure coverage. Some units customize this approach on a patient-by-patient basis by obtaining regular surveillance cultures.[169–171] Clinicians should also consider covering for *Legionella* or influenza if the clinical presentation or local epidemiologic findings suggest that one of these pathogens is possible. Anaerobic coverage is generally not required except after an episode of frank aspiration.[129,151,152,172] Empirical antifungal coverage is not typically indicated for immunocompetent individuals. New oral ulcers or vesicles consistent with herpes simplex virus merit empirical addition of acyclovir.

Treatment for HAP outside the ICU should also be informed by patient risk factors and the local distribution of pathogens and resistance rates. The major risk factor for multidrug-resistant pathogens for nonventilator HAP per US guidelines is intravenous antibiotic use in the preceding 90 days.[94] Other potential risk factors mentioned in the literature include prolonged hospitalization, hemodialysis, poor functional status, and severe pneumonia.[94,128,140,141] It may be possible to treat patients with none of these risk factors more narrowly. US and European guidelines both suggest that monotherapy may be adequate for patients without septic shock who do not need ventilator support and do not have any risk factors for MRSA.[94,173] As with VAP, continual reevaluation of the decision to treat and expeditious antibiotic narrowing or withdrawal in patients whose condition rapidly improves can help minimize superfluous antibiotic exposures.

Vancomycin Versus Linezolid for MRSA Coverage

Vancomycin and linezolid are both reasonable choices for empirical or directed treatment of MRSA nosocomial pneumonia. There are theoretical and/or logistic grounds in favor of each. Linezolid has superior lung penetration, may reduce biofilm formation on endotracheal tubes, does not need dose adjustment based on body mass or renal function, is less nephrotoxic, and is available in an oral formulation for patients with limited intravenous access in the hospital or at discharge.[174,175] On the other hand, the threshold for linezolid resistance appears lower, patients are more likely to develop thrombocytopenia and gastrointestinal symptoms, and it interacts with serotonin reuptake inhibitor medications.[176,177]

There appears to be little difference in clinical effectiveness between vancomycin and linezolid.[178] Early work suggested a possible advantage to linezolid on the basis of retrospective subgroup analysis of the MRSA population in two trials of empirical therapy for nosocomial pneumonia.[179,180] Prospective randomized trials, however, have not confirmed this initial signal.[178] Wunderink and colleagues[181] randomized 448 patients to linezolid versus dose-optimized vancomycin (15 mg/kg every 12 hours). The authors concluded that linezolid was superior to vancomycin on the basis of clinical cure rates (58% vs. 47%, $P = .04$); however, this result was based on a *per-protocol* analysis that only included 339 out of the 448 study patients.[182] Broadening the analysis to include all 448 patients eliminated the apparent difference in clinical response rates (46% vs. 41%, $P = .34$) and there was no difference in 60-day mortality rates between study arms.

Vancomycin has a narrow therapeutic window. Underdosage increases the probability of clinical failure, and overdosage increases the risk of nephrotoxicity.[183] Initial vancomycin trough levels less than 15 mg/L increase the risk for clinical failure, relapse, or both.[166–168,184,185] Conversely, higher vancomycin trough levels and longer treatment courses increase the risk of nephrotoxicity.[186] Concurrent treatment with aminoglycosides or piperacillin-tazobactam further potentiates the risk of acute kidney injury.[186,187] The net incidence of nephrotoxicity in critically ill patients treated with vancomycin for pneumonia is approximately 15%, although the extent to which this risk is due to vancomycin versus underlying illness and concomitant therapies is unclear.[176,186]

The current recommended dosage strategy for vancomycin is 15 to 20 mg/kg every 8 to 12 hours, with a goal of achieving serum trough concentrations of 15 to 20 mg/L.[188] In seriously ill patients, clinicians can also consider a loading dose of 25 to 30 mg/kg to speed attainment of target trough concentrations.[188]

The results of observational studies differ on whether elevated MICs are associated with worse outcomes or not.[189–191] Prospective randomized trials comparing vancomycin and linezolid for treatment of MRSA pneumonia due to isolates with elevated vancomycin MICs have not been conducted.

Ceftaroline is the only FDA-approved cephalosporin with activity against MRSA. It is approved for the treatment of MRSA soft tissue infections and methicillin-susceptible *S. aureus* (MSSA) pneumonia. Whether the drug is effective in MRSA pneumonia remains to be determined.[192] Ceftobiprole is also active against MRSA but is not currently approved in the United States. A randomized controlled trial comparing ceftobiprole versus linezolid plus ceftazidime for the treatment of nosocomial pneumonia reported comparable clinical cure rates for patients with non–ventilator-associated HAP but significantly lower cure rates with ceftobiprole for patients with VAP.[193]

Combination Versus Monotherapy for Gram-Negative Pathogens

Three reasons have been proposed for double covering gram-negative bacteria: (1) to increase the probability that an empirical regimen includes an agent active against the infecting pathogen, (2) to achieve synergy between agents and thereby enhance treatment effectiveness, and (3) to decrease the probability that the organisms will acquire resistance during treatment.[194] An increasing body of literature speaks to these claims.

Multiple studies have suggested that inappropriate initial therapy (defined as failure to prescribe an antibiotic active against the causative pathogen) increases mortality risk.[110,111] Initiating two gram-negative agents rather than one increases the likelihood that initial therapy will include an active agent, which in turn is associated with lower mortality risk.[195–199] There are no differences in clinical outcomes, however, between patients receiving definitive therapy with one versus two active agents once susceptibilities are available.[196,198,200,201] Indeed, with some regimens, particularly β-lactam and aminoglycoside combinations, double coverage is associated with more clinical failure, more nephrotoxicity, and greater risk of nurturing antimicrobial resistance and superinfection.[201–203] Adding an aminoglycoside even for a brief period may increase the risk of clinical failure and nephrotoxicity.[203] Definitive treatment with a carbapenem and fluoroquinolone may mitigate the short-term risk of acquired resistance in some patients,[200] but this risk is generally small[200,204] and likely outweighed by greater long-term risk for multidrug-resistant pathogens at the population level.[157–161]

These findings were borne out in the largest randomized trial to date of empirical monotherapy versus combination therapy for suspected VAP. Heyland and colleagues[204] randomized 740 patients with suspected VAP to meropenem alone versus meropenem plus ciprofloxacin. Combination therapy was more likely to be active than monotherapy (93% vs. 85%, $P = .01$), but there were no differences in patients' outcomes, including duration of mechanical ventilation, intensive care length of stay, hospital length of stay, or mortality. Similarly, there were no differences in rates of acquired antimicrobial resistance, sputum colonization, or *C. difficile* infections. This study specifically excluded patients at high risk for multidrug-resistant pathogens (including patients known to be colonized with MRSA or *Pseudomonas* or those recently treated with a carbapenem or ciprofloxacin), however, so the majority of patients were infected with pathogens susceptible to both the monotherapy and combination therapy agents. This study design limits the generalizability of this study to routine practice. The authors did, however, conduct a subgroup analysis among the subset of patients infected with *Pseudomonas, Acinetobacter,* or multidrug-resistant gram-negative bacilli. There were only 56 patients in this subset (8% of this study population vs. 30% or more in an unselected VAP population). Among these patients, empirical double coverage was much more likely to include an active agent (84% vs. 19%, $P < .001$) and to achieve microbiologic cure (64% vs. 29%, $P = .05$) compared with monotherapy. There were also trends toward shorter duration of mechanical ventilation and intensive care length of stay and decreased mortality with combination therapy.

Taken together, these studies suggest that combination therapy is appropriate for empirical treatment of severe pneumonia, particularly in patients at high risk for multidrug-resistant pathogens. Once the culprit pathogen's antibiotic susceptibilities are known, however, coverage should rapidly be tapered to a single agent.

Aerosolized Versus Intravenous Drug Delivery

Aerosolizing antibiotics is theoretically attractive to improve pulmonary penetration while limiting systemic toxicities, particularly for nephrotoxic agents with poor lung penetration such as colistin and the aminoglycosides. Optimal delivery of aerosolized agents requires vibrating mesh plate nebulizers rather than ultrasonic or jet nebulizers and careful coordination of nebulization, ventilator settings, and sedation.[205,206] Aerosolized antibiotics have been studied both as an adjunct and as an alternative to intravenous treatment. There is a paucity of randomized controlled trials evaluating these strategies, but available data suggest that adjunctive aerosolized antibiotics may enhance microbiologic clearance but have little or no impact on patients' duration of mechanical ventilation, length of stay, or mortality.[207–209] Less is known about aerosolization as an alternative to intravenous therapy. Lu and colleagues[210] randomized 40 patients with *P. aeruginosa* VAP to intravenous amikacin and ceftazidime versus aerosolized amikacin and ceftazidime. They found no difference in clinical response or superinfection rates but less acquired resistance in the aerosolized antibiotic group. The same investigators also followed 165 patients with VAP caused by *P. aeruginosa* or *Acinetobacter baumannii*.[211] Patients with isolates sensitive to β-lactams, aminoglycosides, or quinolones were treated with intravenous antibiotics for 14 days. Patients with multidrug-resistant strains were treated with aerosolized colistin. Clinical response, mortality, and nephrotoxicity rates were similar between the two groups. Of note, there is risk associated with aerosolizing antibiotics. Up to 10% of patients treated with nebulized antibiotics develop cardiorespiratory complications, some of which can be life-threatening.[209] Nebulized antibiotics should therefore be reserved for the treatment of patients with multidrug-resistant pathogens for whom other options are limited.[212]

Tailoring and Deescalating Therapy

Tailoring treatment to the single narrowest-spectrum agent available is critical as soon as antimicrobial susceptibility data are available, not only because sustained double coverage is unnecessary but because continuing treatment with superfluous agents is expensive, liable to drive population-level antibiotic resistance, and potentially harmful.[157–161,202,213–215]

There appears to be little difference in clinical effectiveness among most available agents. A meta-analysis of 41 treatment trials of 29 different treatment regimens found no difference in mortality rates among the regimens studied.[201] There is a possible signal toward lower short-term mortality rates when VAP is treated with carbapenems versus other classes, but the impact of this strategy on long-term mortality and population-level resistance rates has not been adequately characterized.[94] Clinicians can therefore select the narrowest-spectrum agent available for definitive treatment. Non-aminoglycosides should be preferentially selected if possible, given the poor lung penetration and nephrotoxicity of aminoglycosides.[94]

Narrowing treatment for patients with negative cultures requires careful clinical judgment. Some proportion of patients with negative cultures probably do not have pneumonia at all but rather mimicking conditions such as mucous plugging, pulmonary edema, atelectasis, thromboembolic disease, ARDS, and others. If an alternative diagnosis is established or if the patient's clinical course does not seem consistent with pneumonia, then antibiotics should be stopped.[216–218] There are fewer data on how to manage culture-negative patients whose clinical course does still seem consistent with pneumonia. The findings of observational studies suggest that antibiotics can be safely stopped as soon as the patient's condition clinically improves, but more data are necessary.[97,219–222]

Duration of Therapy

Chastre and colleagues[223] randomized 401 patients with culture-positive VAP to 8 versus 15 days of antibiotics. They found no difference in ventilator-free days, intensive care length of stay, mortality, or recurrent infection between short-course and long-course therapy. The one exception was pneumonia due to nonfermenting gram-negative bacilli, including *P. aeruginosa*. Patients with these pathogens had higher rates of microbiologic recurrence when randomized to 8-day therapy (41% vs. 25%). There was no difference, however, in ventilator-free days, length of stay, or mortality rates in patients with nonfermenting gram-negative bacilli randomized to 8 days versus 15 days. Other studies mirror these findings.[224,225] The default treatment course for HAP and VAP for all pathogens is therefore 7 to 8 days.[94] This treatment regimen can be modified, however, depending on clinical evolution and complicating factors such as bacteremia, empyema, or lung abscess.

Clinicians can also use clinical and laboratory data to shorten treatment duration. Antibiotics can be safely stopped even before 8 days of treatment if all clinical signs and symptoms completely resolve (defervescence, improvement in white blood cell count, resolution of purulent secretions, normalization of oxygenation, and stabilization or regression of infiltrates).[107,217,219] Multiple randomized controlled trials have suggested that measuring procalcitonin levels daily or every other day can shorten the average duration of antimicrobial therapy without adversely affecting pneumonia patients' ventilator-free days, intensive care length of stay, or mortality.[226–229] Indeed, a patient-level meta-analysis of randomized controlled trials suggested that measuring procalcitonin levels daily to guide antibiotic discontinuations may lower mortality rates.[229] CT or ultrasound quantification of reaeration after several days of treatment can help measure antibiotic response, but this strategy has not yet been studied as a means of guiding antibiotic deescalation.[230]

PREVENTION

Interpreting the literature on nosocomial pneumonia prevention is challenging because of circularity between clinical definitions for pneumonia and the mechanisms by which most prevention measures work.[231] Most pneumonia definitions incorporate some assessment of the quality or quantity of pulmonary secretions or the results of pulmonary cultures acquired via the oropharynx or endotracheal tube (expectorated sputum, endotracheal aspirates, or BAL), or both. Most prevention strategies in turn are designed to decrease the volume of regurgitant secretions or decrease the bacterial burden in and around the oropharynx and endotracheal tube, or both. There is consequently a risk that some observed decreases in "pneumonia" better reflect fewer secretions or less colonization of the oral-respiratory tract, rather than a decline in true invasive infections. Evidence for this effect is apparent in a striking paradox of the VAP prevention literature: Many interventions lower VAP rates, but very few improve concrete outcomes such

TABLE 301.6 Interventions Proposed to Lower Ventilator-Assisted Pneumonia Rates

MECHANISM	INTERVENTIONS AND SELECTED REFERENCES	IMPACT ON DURATION OF MECHANICAL VENTILATION AND/OR MORTALITY[a]
Avoid intubation	Noninvasive positive pressure ventilation[266]	Lower mortality rates
Minimize duration of intubation[b]	Ventilator weaning protocols[267–270]	Less time to extubation
	Daily spontaneous breathing trials[271,272]	Less time to extubation, possibly lower mortality rates
	Daily spontaneous awakening trials[271,273,274]	Less time to extubation, possibly lower mortality rates
	Early mobility[275–278]	Conflicting data
Decrease pathogen burden in the upper gastrointestinal and respiratory tract	Regular oral care with antiseptics, especially chlorhexidine[241,242]	May increase mortality rates
	Tooth brushing[279]	Insufficient data
	Selective digestive decontamination with topical and parenteral antibiotics[235,280,281]	Lower mortality rates
	Probiotics[282,283]	Insufficient data
	Saline instillation before tracheal suctioning[284]	Insufficient data
Decrease pathogen burden on endotracheal tubes	Silver-coated endotracheal tubes[285]	No impact
	Mucous shaving of the endotracheal tube[286]	Insufficient data
Decrease pooling, seepage, and aspiration of secretions	Elevation of the head of the bed[287]	Insufficient data
	Optimization of endotracheal tube cuff pressures[51]	Insufficient data
	Endotracheal tubes with ultrathin polyurethane cuff membranes[288–291]	Insufficient data
	Tapered endotracheal tube cuffs[291,292]	No impact
	Subglottic secretion drainage[236]	No impact

[a]Conclusions regarding impact on duration of mechanical ventilation and mortality drawn from Klompas M, Branson R, Eichenwald EC, et al. Strategies to prevent ventilator-associated pneumonia in acute care hospitals: 2014 update. *Infect Control Hosp Epidemiol.* 2014;35:915–936.
[b]These interventions have been shown to decrease average duration of mechanical ventilation, but their impact on ventilator-assisted pneumonia rates has not yet been adequately studied.

as duration of mechanical ventilation, intensive care length of stay, or hospital mortality.[231–234]

Given the risk of misclassification bias favoring the intervention arm in VAP prevention trials, studies reporting lower VAP rates should be interpreted with caution. Objective outcomes such as duration of mechanical ventilation, intensive care or hospital length of stay, mortality, and antibiotic dispensing are more credible and reliable metrics to measure the impact of prevention interventions. Table 301.6 lists selected interventions proposed to lower VAP rates. The only interventions that have been shown to affect the concrete outcomes listed earlier, however, are noninvasive positive pressure ventilation, ventilator weaning protocols (especially paired daily sedative interruptions and spontaneous breathing trials), and selective oral and digestive decontamination.[234–236] Digestive decontamination has not been widely adopted owing to concern that the intervention will increase *C. difficile*–associated diarrhea and antibiotic resistance rates; the latter does not appear to be a concern in ICUs with low baseline resistance rates but has not yet been adequately studied in ICUs with high baseline resistance rates.[237–239] Additional interventions, such as elevation of the head of the bed in patients receiving enteral nutrition and optimizing endotracheal tube cuff pressures, appear promising but have not yet been sufficiently studied to affirm an impact on objective patient outcomes.[240] Emerging evidence suggests the possibility that routine oral care with chlorhexidine may be harmful in ventilated patients. Two meta-analyses of randomized, controlled trials and one large observational study have reported a possible association with increased mortality rates.[240–242]

Ventilator-Associated Pneumonia Prevention Bundles

Grouping multiple interventions (see Table 301.6) into VAP prevention "bundles" may enhance their effectiveness by exploiting synergies between interventions (e.g., conducting spontaneous breathing trials during spontaneous awakening trials to maximize patients' capacity for participation) or by enhancing their visibility, immediacy, and hence performance by frontline providers.[243] VAP prevention bundles have become a standard of care in most hospitals, but there is wide variability in their components and definitions for adherence.[244] Nonetheless, many hospitals have reported substantial and sustained drops in VAP rates after bundle implementations.[245–247] Very few hospitals have reported improvements in ventilator days, hospital length of stay, or mortality rates, however, raising concern that some of the apparent decline in VAP may be due to surveillance bias.[21] Bundles have mostly been studied in unblinded, longitudinal observational studies that compare rates before and after implementation. Lack of blinding and surveyors' expectations of what effect bundles should be having on VAP rates may have influenced their interpretations of ambiguous clinical signs.[21]

VENTILATOR-ASSISTED PNEUMONIA AS A QUALITY METRIC

The complexity, subjectivity, and questionable accuracy of VAP surveillance definitions have limited the utility of VAP as a metric to measure and compare quality of care across hospitals. These concerns prompted the CDC to develop a new framework called ventilator-associated events (VAEs) to measure quality of care for ventilated patients.[248,249] VAEs broaden the focus of surveillance from pneumonia alone to complications of mechanical ventilation in general. The new system includes a hierarchy of definitions that use quantitative criteria to make surveillance more objective, reproducible, and potentially electronic. The first tier of the VAE definition set flags episodes of nosocomial respiratory deterioration ("ventilator-associated conditions" [VACs]) on the basis of sustained increases in ventilator settings. The second tier seeks to identify the subset of VACs that may be infection related ("infection-related ventilator-associated complications" [IVACs]) on the basis of concurrent abnormalities in temperature or white blood cell count and sustained new antibiotic starts. The third tier flags IVACs that might be possible VAPs on the basis of culture results and Gram stain neutrophil counts.[106,250,251] VAEs are strongly associated with increased morbidity, mortality, and antibiotic utilization.[69,106,250–253] The crude mortality rate in patients with VAEs is 31%.[254]

There is limited overlap between VAEs and traditionally defined VAP; by design, VAEs focus on an expanded set of ICU complications and include only pneumonias that required a sustained increase in ventilator support.[255,256] The most common clinical events that trigger VAEs include pneumonia, volume overload, atelectasis, and ARDS.[251,257,258] Risk factors for VAEs include prolonged mechanical ventilation; deep sedation, particularly with benzodiazepines; positive fluid balance; red blood cell transfusions; and high tidal volume ventilation.[250,259–262] Strategies to prevent VAEs therefore include avoiding intubation, minimizing sedation, pairing daily spontaneous awakening with spontaneous breathing trials, using intravenous fluids conservatively to maintain euvolemia, ventilating with low tidal volumes, and setting conservative thresholds for blood transfusions.[262–265]

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The complete reference list is available online at Expert Consult.

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302 Health Care–Associated Urinary Tract Infections

Barbara W. Trautner and Thomas M. Hooton

SHORT VIEW SUMMARY

Definitions
- Health care–associated urinary tract infection (UTI) refers to symptomatic UTI acquired in any institutional setting providing health care.
- Catheter-associated UTI (CAUTI) refers to symptomatic UTI in a patient currently or recently catheterized with an indwelling transurethral, suprapubic, intermittent, or external catheter.
- Catheter-associated bacteriuria is composed mostly of asymptomatic bacteriuria (ASB) but also includes CAUTI.
- In this chapter, "bacteriuria" encompasses both ASB and CAUTI. The terms *catheter-associated urinary tract infection* and *asymptomatic bacteriuria* are used when referring specifically to one of these conditions.

Epidemiology
- UTIs are the most commonly reported health care–associated infections in the United States. However, many reported CAUTIs are actually catheter-associated ASBs.
- 75% of UTIs acquired in the hospital are associated with urinary catheters.
- The incidence of bacteriuria associated with indwelling urethral catheterization with a closed drainage system is approximately 3% to 8% per day.
- The duration of catheterization is the most important risk factor for catheter-associated bacteriuria.
- Although bacteremia is a rare complication of catheter-associated bacteriuria, catheter-associated bacteriuria is so common that it is the most common source of gram-negative bacteremia in hospitalized patients.
- Catheter-associated bacteriuria is a frequent target for inappropriate antimicrobial therapy.

Microbiology
- Catheter-associated bacteriuria is caused by a broad range of bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Klebsiella* spp., and *Enterococcus* spp.
- Many of these organisms are resistant to multiple antimicrobial agents.
- *Candida albicans* is also a common cause of catheter-associated funguria.

Diagnosis
- Significant bacteriuria: $\geq 10^3$ colony-forming units per milliliter (CFU/mL) in a symptomatic person is an indicator of CAUTI, whereas $\geq 10^5$ CFU/mL in an asymptomatic person is an indicator of ASB.
- The majority of patients with catheter-associated bacteriuria are asymptomatic, and signs and symptoms commonly associated with UTI, such as fever, dysuria, urgency, flank pain, or leukocytosis, are either nonspecific or not apparent in catheterized patients.
- In the catheterized patient, pyuria does not differentiate ASB from CAUTI, but its absence suggests that CAUTI is not the cause of symptoms.

Prevention
- Reducing exposure to urinary catheterization is the most effective way to prevent catheter-associated bacteriuria.
- A closed catheter drainage system is indicated in all catheterized patients who have an indwelling transurethral catheter.
- Routine use of antimicrobial-coated urinary catheters is not supported by available data.
- Routine use of systemic antimicrobial agents to prevent catheter-associated bacteriuria should be discouraged.
- Use of multiple infection control techniques and strategies simultaneously (*bundling*) is recommended.

Health Care–Associated Asymptomatic Bacteriuria
- Routine screening for and treatment of ASB in health care settings is discouraged, other than for pregnant women and patients undergoing urologic procedures that cause mucosal bleeding.
- Overtreatment of ASB is a major contributor to unnecessary antimicrobial use in acute and long-term care.

Management (See Table 302.7)
- Urine cultures should be obtained before treatment of CAUTI, ideally after catheter change.
- Recommended treatment duration for CAUTI ranges from 5 to 14 days, depending on the severity and choice of drug.

Fungal Urinary Tract Infection
- Asymptomatic health care–associated candiduria rarely requires treatment.

DEFINITIONS

Health care–associated urinary tract infection (UTI) refers to UTI that is acquired while a patient is receiving medical treatment in a health care setting.[1] The majority of health care–associated UTIs occur in patients whose urinary tracts are currently or were recently catheterized. All types of urinary catheters (indwelling, suprapubic, intermittent, and external or condom catheters) increase the risk of acquisition of bacteriuria and thus UTI. The focus of this chapter is on catheter-associated bacterial UTIs occurring in adults in hospitals and long-term care facilities (LTCFs). Candiduria, the most common type of health care–associated fungal UTI, is discussed in a separate section of the chapter.

There are major differences in the epidemiology, pathogenesis, treatment, and prevention of health care–associated UTI and acute uncomplicated cystitis, which are shown in Table 302.1. *Urinary tract infection* is a nonspecific term that generally refers to symptomatic bacterial or fungal infection of the bladder or kidney, or both, in a patient. Although this term is often used without regard to the presence or absence of urinary symptoms, a strict definition of UTI requires the presence of symptoms related to the urinary tract (Table 302.2). Unfortunately, *catheter-associated bacteriuria* is a nonspecific term frequently used in the urinary catheter literature that encompasses two more specific terms, *catheter-associated asymptomatic bacteriuria* (ASB) and *catheter-associated urinary tract infection* (CAUTI).[2] Catheter-associated bacteriuria is composed mostly of ASB, but in most publications one cannot discern what proportion of patients have CAUTI.

The relationship between ASB and CAUTI and other clinical outcomes remains unclear. Most patients with ASB do not progress to CAUTI, and factors that trigger a symptomatic event in patients with ASB are not known. Thus even though the presence of bacteriuria is presumably necessary for the development of CAUTI, the development of urinary

TABLE 302.1 Comparison of Uncomplicated Cystitis/Pyelonephritis and Health Care–Associated Urinary Tract Infections

	UNCOMPLICATED CYSTITIS AND PYELONEPHRITIS	HEALTH CARE–ASSOCIATED UTI
Age	Younger	Older
Sex	Female, rare in males	Male and female, female predominance
Main risk factor	Intercourse	Urinary catheter
Pathogenesis	Fecal organisms ascend urethra to bladder	Extraluminal: fecal organisms ascend catheter-urethra interface to bladder Intraluminal: fecal or exogenous (cross-infection) organisms enter drainage system that has been disconnected, ascend through catheter to bladder
Uropathogen virulence	More virulent: pyelonephritis > cystitis > ASB or fecal	Generally less virulent than in uncomplicated UTI
Microbiology	Single pathogen, usually *Escherichia coli;* yeast rare	Single (short-term catheter) to multiple (long-term catheter) organisms; diverse flora with gram-negatives, gram-positives, *Candida* spp.
Clinical	ASB in about 5% of women, but prevalence a function of culture frequency; transient, benign Cystitis: dysuria, frequency, or urgency Pyelonephritis: fever, back pain/tenderness	Catheter-associated bacteriuria in about 5% per day of catheterization; >90% is ASB, usually persistent, most do not progress to CAUTI CAUTI: fever, delirium, other nonspecific signs/symptoms, may have no lower tract symptoms
Diagnosis	ASB: $\geq 10^5$ CFU/mL Cystitis/pyelonephritis: $\geq 10^3$ CFU/mL	Catheter-associated ASB: $\geq 10^5$ CFU/mL CAUTI: $\geq 10^3$ CFU/mL
Resistance	Common but predictable; fluoroquinolone and trimethoprim/sulfamethoxazole resistance increasing; ESBL uropathogens more common	Multidrug resistance common and less predictable; ESBL uropathogens and fluoroquinolone resistance common; CRE uropathogens still uncommon in most places.
Treatment	Short-course (single-dose to 5-day regimen, depending on drug)	5- to 10-day regimen, depending on severity
Prevention	Education about risk factors; abstinence; antimicrobial prophylaxis	Reduce urinary catheterization; use condom or intermittent vs. indwelling urethral catheter; strict closed system with indwelling urethral catheter
Public health	Strains spread via food chain and within family units	Large reservoir of multidrug-resistant uropathogens; cross-infection a concern

ASB, Asymptomatic bacteriuria; *CAUTI,* catheter-associated urinary tract infection; *CRE,* carbapenem-resistant Enterobacteriaceae; *ESBL,* extended-spectrum β-lactamase; *UTI,* urinary tract infection.

TABLE 302.2 Definitions for Urinary Tract Infection Terminology

TERM	DEFINITION
Urinary tract infection (UTI)	Nonspecific term that generally refers to symptomatic bacterial or fungal infection of the urinary tract
Acute, uncomplicated cystitis	Symptomatic cystitis in a person with no signs or symptoms suggestive of infection outside the bladder
Catheter-associated bacteriuria	Presence of significant bacteriuria[a] in a catheterized or recently catheterized patient without regard to the presence or absence of urinary symptoms
Catheter-associated asymptomatic bacteriuria (ASB)	Presence of significant bacteriuria[a] in a catheterized or recently catheterized patient without symptoms referable to the urinary tract
Catheter-associated UTI (CAUTI)	Presence of significant bacteriuria[a] in a catheterized or recently catheterized patient with symptoms or signs referable to the urinary tract
Catheter-associated funguria	Presence of funguria in a catheterized or recently catheterized patient. Fungal colony counts have not been shown to be meaningful in interpreting the significance of funguria. Catheter-associated funguria should be distinguished as asymptomatic or symptomatic.

[a]Quantity of bacteria in the urine suggestive of infection rather than contamination (see text).

symptoms must require some facilitating event(s), such as tissue invasion, that we do not yet understand. On the other hand, even if ASB itself is benign, there are several reasons that may justify efforts to prevent it. For example, it may predispose the person to CAUTI through a common pathogenic pathway, in which case interventions that prevent ASB would be expected to prevent CAUTI. Additionally, catheter-associated bacteriuria (mostly ASB) is the source of many episodes of health care–associated bacteremia and may be associated with increased mortality,[3,4] although this latter point is controversial. In hospitals and LTCFs, catheter-associated bacteriuria represents a large reservoir of antimicrobial-resistant urinary pathogens in patients that increases the risk of cross-infection among catheterized patients. ASB also provides a scapegoat infection for physicians who have a low threshold for using antimicrobial therapy (inappropriately), and detection of ASB can terminate the diagnostic evaluation prematurely. The relationships between ASB and clinical outcomes, including CAUTI, are difficult to demonstrate in most studies, given the large sample sizes needed to demonstrate such a benefit.

Treatment of ASB is not recommended except in certain circumstances, such as pregnancy and urologic procedures that cause mucosal bleeding.[5] Antimicrobial treatment of ASB, particularly in catheterized persons, typically leads only to short-term suppression of bacteriuria, and clearly increases the likelihood of emergence of resistant urinary organisms.[6,7] Antimicrobial exposure also increases the patient's risk for developing *Clostridioides difficile* (formerly *Clostridium difficile*) colitis.[8] Bacteremia can arise from bacteriuria in patients with both ASB and CAUTI,[9] and at this point it is not possible to predict which patients with bacteriuria will develop bacteremia. Therefore a policy of widespread treatment of ASB is likely to be detrimental rather than beneficial. On the other hand, prevention of ASB might lead to fewer episodes of CAUTI, bacteremia, fever episodes, cross-infection, and inappropriate antimicrobial use. In fact, the greatest impact of effective CAUTI prevention interventions may be that removing unnecessary urinary catheters will also prevent episodes of ASB, and their sequelae, rather than the few episodes of CAUTI that occur in these patients. The subset of patients who have an ongoing need for urinary catheters for bladder drainage will be persistently bacteriuric. In these patients, CAUTI prevention efforts should focus on avoiding overdiagnosing CAUTI in patients who are asymptomatic and thus have ASB.

EPIDEMIOLOGY

Incidence and Prevalence

Approximately 75% of UTIs acquired in the hospital are associated with urinary catheters. In one study of 1453 health care–associated, symptomatic UTIs, 72% were CAUTIs and 28% were not catheter associated.[10] Almost half of the CAUTIs (45%) occurred among intensive care unit (ICU) patients. The National Healthcare Safety Network (NHSN) is the United States' national health care infection surveillance system, maintained by the Centers for Disease Control and Prevention (CDC). Of note, the current NHSN surveillance and reporting for UTI includes only CAUTI, and the only catheter type that meets surveillance definitions is the indwelling urethral (Foley) catheter; UTIs not associated with indwelling urinary catheters and episodes of ASB are not reported. Despite national emphasis on decreasing CAUTI, CAUTI rates did not

decrease between 2009 and 2014.[11] Whether the lack of change relates to changes in the surveillance definitions, which have changed often, or to an overall decrease in use of urinary catheters, thus shrinking the denominator, is unclear.[12] Per the 2015 NHSN report on device-related infections, which included data from calendar year 2013, the ratio of urinary catheter-days to patient-days decreased from the prior report in 2010.[13]

Urinary catheters are placed for different reasons in acute-care facilities and LTCFs. Common indications for urinary catheter use in acute care are discussed later under "Reduction of Unnecessary Catheterization" (see Table 302.6). Most of these patients are catheterized for only 2 to 4 days.[14] The most common appropriate reason for urinary catheterization in LTCFs is to relieve bladder outlet obstruction, and these catheters are often in place for years. Management of incontinence is no longer considered an appropriate justification for urinary catheterization.[15]

The incidence of bacteriuria associated with indwelling urethral catheterization with a closed drainage system is approximately 3% to 8% per day[16–18] and, thus, many patients catheterized for short periods of time and almost all those catheterized for a month or more will have catheter-associated bacteriuria. One month, or 30 days, is a convenient dividing line between short-term and long-term catheterization,[14] and it is used as such in this chapter, except where stated otherwise.

NHSN data from 2013–2014 showed the mean incidence of CAUTI per 1000 catheterized days was 1.3% to 4.8% in adult critical care units, 1.3% in inpatient medical-surgical wards, 2.0% to 2.5% in long-term acute care hospitals, and 2.6% in inpatient rehabilitation facilities.[13] Compared to data from 2012, CAUTI rates increased in the majority of critical care locations while decreasing in most non–critical care locations. Organisms causing CAUTI and reported to the NSHN are often resistant, with *Escherichia coli* fluoroquinolone resistance reported at 34.8% in 2014.[19]

CAUTI Risk Factors

The duration of catheterization is the most important risk factor for the development of catheter-associated bacteriuria.[14,20,21] Other risk factors for catheter-associated bacteriuria include the lack of systemic antimicrobial therapy, female sex, meatal colonization with uropathogens, microbial colonization of the drainage bag, catheter insertion outside the operating room, catheter care violations, absence of use of a drip chamber, rapidly fatal underlying illness, older age, diabetes mellitus, and elevated serum creatinine at the time of catheterization.[16,21–23] Risk factors in patients with health care–associated UTIs not associated with catheterization include other forms of instrumentation of the urinary tract. Many noncatheterized patients with health care–associated UTI are probably also at increased risk for UTI in the community, due to host behavioral or genetic factors associated with increased risk for UTI.[24]

Complications

Most episodes of catheter-associated bacteriuria occur in asymptomatic patients, with studies showing that less than one-fourth of patients with catheter-associated bacteriuria develop UTI symptoms.[25–28] In one study of 235 new cases of catheter-associated bacteriuria, more than 90% of the patients were asymptomatic and afebrile.[28] In another study of catheterized and bacteriuric female patients in an LTCF, the incidence of febrile episodes of possible urinary origin was only 1.1 episodes per 100 catheterized patient-days, and most of these episodes were low grade, lasted for less than a day, and resolved without antimicrobial treatment.[29]

However, as noted previously, there are significant consequences of catheter-associated bacteriuria. Catheter-associated bacteriuria is the most common source of gram-negative bacteremia in hospitalized patients.[30] Of 7217 episodes of bloodstream infection in acute-care hospitals across Canada, 21% were associated with the urinary tract; 71% of these episodes were associated with the presence of a urinary device.[31] However, one study of 1497 newly catheterized hospitalized patients found that only 1 of the 235 episodes of catheter-associated bacteriuria was unequivocally associated with secondary bloodstream infection.[28] Similarly, a retrospective cohort study of 444 episodes of catheter-associated bacteriuria in 308 patients found that only 3 episodes of bacteremia (0.7% of bacteriuric subjects) were directly attributed to bacteriuria.[32] Both of these studies demonstrated that bacteremia in catheterized patients can arise from either CAUTI or ASB (i.e., with or without urinary tract symptoms). Risk factors for urinary catheter–associated bacteremia include male sex, immunosuppression, and urinary tract procedures.[33] Urinary tract organisms are also the most common source of bacteremia in LTCFs, accounting for 40% to 55% of bacteremias,[34,35] and bacteremia is often polymicrobial in these patients.

Patients undergoing long-term indwelling catheterization, in addition to almost universal polymicrobial bacteriuria, may develop symptomatic lower and upper UTI, bacteremia, frequent febrile episodes, catheter obstruction, renal and bladder stone formation associated with urease-producing uropathogens, local genitourinary infections, fistula formation, incontinence, and bladder cancer.[14] Chronic renal inflammation and pyelonephritis are often found at autopsy in patients who had been on long-term urinary catheterization, many of whom were afebrile at the time of death.[36] Catheter blockage can be a recurrent problem in long-term catheterized patients and results from encrustation formed by urease-producing organisms, especially *Proteus mirabilis,* which hydrolyze urea to ammonia with formation of struvite and apatite crystals in the catheter lumen.

The effect of catheter-associated bacteriuria, either CAUTI or ASB, on mortality remains controversial. Inability to fully adjust for confounding variables probably explains some of the association, because patients who require an indwelling catheter tend to be sicker or have comorbidities. A review and meta-analysis of the relationship of CAUTI (defined as bacteriuria) to mortality and length of stay in ICU patients found that CAUTI was associated with increases in both mortality and length of stay, but the significant association disappeared when the analysis was restricted to studies that adjusted for other predictors of mortality.[37] Increased mortality has also been reported in residents of LTCFs with chronic indwelling catheters,[38] but catheterized patients in long-term care also tend to be sicker and more functionally impaired.[39,40]

One of the most serious adverse effects of catheter-associated bacteriuria is that bacteriuria is a frequent target for unnecessary antimicrobial therapy, which contributes to the problem of antimicrobial resistance in hospitals and LTCFs. Inappropriate treatment of ASB in hospitalized patients is well documented. For example, in a prospective, multicenter study of inpatients, 72% of 961 patients with ASB were treated with antimicrobial therapy unnecessarily.[41] Of those treated inappropriately, 14% received over 14 days of antimicrobials. Inappropriate treatment is associated with older age, predominantly gram-negative bacteriuria, and pyuria.[41,42] Inappropriate antimicrobial use also unnecessarily exacerbates the growing problem of health care–associated *C. difficile* colitis,[43] by 8.5-fold in a study of ASB treatment in nursing homes.[44] Catheter-associated bacteriuria is also harmful in that it comprises a large reservoir of antimicrobial-resistant organisms that may be transmitted between patients who have urinary catheters or other invasive devices.[21,45–48]

MICROBIOLOGY

Unlike the narrow and predictable spectrum of causative agents in uncomplicated UTI,[49] a broad range of bacteria can cause health care–associated UTI, and many are resistant to multiple antimicrobial agents.[40] Most episodes of bacteriuria in short-term catheterized patients are caused by single organisms, mostly gram-negative bacilli and enterococci.[28] NSHN surveillance data from 2011 to 2014 provide the most comprehensive picture of the organisms causing CAUTI in acute-care, long-term acute care, and inpatient rehabilitation facilities.[19] *E. coli* was the most common pathogen, accounting for 23% of 153,805 pathogens from CAUTIs reported in the surveillance network, although it is not as dominant as in uncomplicated UTI. The second-ranked pathogen was *Candida albicans* (11.7%). However, all species of *Candida* and unidentified yeast isolates from CAUTIs combined account for 23.9% of CAUTI pathogens. After 2015, CAUTI surveillance definitions changed to exclude yeast, reflecting the clinical uncertainty about whether *Candida* in the urine of catheterized patients represents infection or colonization. After *Candida,* the most commonly isolated bacterial pathogens, in order of relative rank, were *Pseudomonas aeruginosa, Klebsiella pneumoniae/oxytoca,* and *Enterococcus faecalis.* Other organisms reported include *Proteus, Enterobacter,* coagulase-negative *Staphylococci,* and *Staphylococcus aureus.* New gram-positive uropathogens that are

rarely reported, yet have been identified through improved culture techniques or newly recognized as pathogens through microbiome studies of the bladder, include *Actinotignum schaalii* and *Aerococcus urinae*.[50] Health care–associated UTIs arising from external catheters (condom drainage systems) are not reported to the NHSN, so less is known about their epidemiology. A single-center study of urine cultures collected from both indwelling and condom catheters in men found equivalent prevalence of Enterobacteriaceae and enterococci in both catheter types.[32] However, *Pseudomonas* and *Candida* were more prevalent in cultures from indwelling catheters.

Bacteriuria in long-term catheterized patients is usually polymicrobial and, in addition to the pathogens commonly seen in short-term catheterized patients, commonly includes less familiar species such as *P. mirabilis, Providencia* spp., and *Morganella morganii*.[40] In these patients, new episodes of infection often occur periodically in the presence of existing infection with organisms that may persist for months.[51,52] A urine culture obtained from a patient whose catheter has a biofilm may not accurately reflect the status of bacteriuria in the bladder,[53,54] and it is recommended that urine cultures from chronically catheterized patients be obtained from a freshly placed catheter.

PATHOGENESIS

In noncatheterized patients the usual origin of uropathogens is their own fecal microflora, which colonize the periurethral area and ascend to the bladder, resulting in bacteriuria with or without symptoms. In the mouse model of UTI, inoculation of *E. coli* into the bladder is followed by invasion of the superficial bladder cells and the formation of large intracellular bacterial colonies that, in response to infection, exfoliate and are removed with the flow of urine.[55] To avoid clearance by exfoliation, these intracellular uropathogens can reemerge and eventually establish a persistent, quiescent bacterial reservoir within the bladder mucosa that may serve as a source for recurrent acute infections.[55] Although internalization of uropathogenic *E. coli* into bladder and renal epithelial cells has been observed in vitro and in vivo,[56] there is only sparse evidence that this phenomenon occurs in humans[57,58] and only indirect evidence that the intracellular bacterial colonies observed in the mouse occur in humans.[59] Specifically, intracellular bacterial clusters have been seen in desquamated bladder epithelial cells shed from children and adults with UTIs.[59,60] It is possible that invasion of uropathogens into uroepithelial cells is the trigger for urinary symptoms, but an inflammatory response is not sufficient to cause urinary symptoms because pyuria often accompanies ASB in both catheterized and noncatheterized patients.

Strains of *E. coli* associated with lower or upper tract UTI in healthy hosts are more likely to have certain putative virulence determinants, such as P fimbriae, compared with colonic strains and those causing ASB.[61,62] However, many UTIs are caused by *E. coli* with a virulence profile similar to that in strains causing ASB, and these putative virulence factors can be found in strains causing ASB or in colonic flora.

The most important predisposing factor for nosocomial UTI is urinary catheterization, which perturbs host defense mechanisms and provides easier access of uropathogens to the bladder. The indwelling urethral catheter introduces an inoculum of bacteria into the bladder at the time of insertion; facilitates ascension of uropathogens from the meatus to the bladder via the catheter-mucosa interface; provides a pool of organisms in the drainage bag, if the closed system is not maintained, which can ascend intraluminally to the bladder; compromises complete voiding; and constitutes a frequently manipulated foreign body on which pathogens are deposited via the hands of personnel. Indwelling urinary catheters provide a surface for the attachment of host cell binding receptors that are recognized by bacterial adhesins, thus enhancing microbial adhesion, as well as disrupting the uroepithelial mucosa to expose new binding sites for bacterial adhesins.[56] Bacteria attached to the catheter surface form exopolysaccharides that entrap bacteria, which replicate and form microcolonies that mature into biofilms on the inner and outer surfaces of the catheter.[22,56] These biofilms protect uropathogens from antimicrobials and the host immune response and facilitate transfer of antimicrobial resistance genes.[56] Some uropathogens in biofilms, such as *Proteus* spp., have the ability to hydrolyze urea to free ammonia and raise the urinary pH, which facilitates precipitation of minerals such as hydroxyapatite or struvite, creating encrustations that can block catheter flow.[22,56]

Whether external (condom) catheters also contribute to an increased risk of health care–associated UTI is unclear, because NHSN surveillance omits catheter types other than indwelling, transurethral catheters. A single center study in both acute and long-term care wards of 1009 sequential positive urine cultures collected from male veterans with either an indwelling (transurethral), external, suprapubic, or intermittent urinary catheter found that external catheters accounted for 37.4% of positive cultures.[63] Indwelling catheters accounted for 57.8% of these cultures, and the other two catheter types combined accounted for the remaining 4.9%.

The source of uropathogens in catheterized patients includes patients' endogenous flora, health care personnel, or inanimate objects.[22,45,64,65] Not unexpectedly, uropathogen virulence determinants such as P fimbriae appear to be of much less importance in the pathogenesis of health care–associated UTIs compared with uncomplicated UTIs.[61,62,66] Microbial pathogens can enter the catheterized bladder extraluminally (ascending outside the catheter along the urethral mucosa interface) or intraluminally (through the internal lumen from a contaminated drainage bag or break in the closed drainage system).[67] Rectal and periurethral colonization with the infecting strain often precedes catheter-associated bacteriuria, especially in women.[65] The negative impact of the catheter is demonstrated by the finding that, despite the continuous drainage of urine through the catheter, in patients with catheter urine colony counts as low as 3 to 4 colony-forming units per milliliter (CFU/mL) who are not given antimicrobials, the level of bacteriuria or candiduria uniformly rises to greater than 10^5 CFU/mL within 24 to 48 hours in those who remain catheterized.[68]

DIAGNOSIS

The clinical diagnosis of CAUTI is based on the presence of significant bacteriuria in a catheterized or recently catheterized person who has signs or symptoms of UTI not explainable by another condition after a thorough evaluation. Bacteriuria, urinary signs and symptoms, and pyuria in a catheterized patient are all nonspecific, and thus the clinician must exercise clinical judgment as to whether treatment is warranted. The latest NSHN definition (from January 2019) does not permit attribution of symptoms such as fever to another cause—if the patient has a urinary catheter and a positive urine culture that meets NHSN criteria for a CAUTI on the same day as the fever, a CAUTI must be reported. Mixed bacterial cultures and funguria in the absence of a bacterial organism do not meet the NHSN CAUTI definition.

Significant Bacteriuria

Significant bacteriuria is the level of bacteriuria that suggests bladder bacteriuria rather than contamination and is based on growth from a urine specimen collected in a manner to minimize contamination and transported to the laboratory in a timely fashion to limit bacterial growth. Definitions for significant bacteriuria are summarized in Table 302.3. The preferred method of obtaining a urine culture in patients with short-term catheterization is by sampling through a needleless catheter port in catheter tubing that is well cleaned with a disinfectant before accessing. If a port is not present, puncturing the catheter tubing with a needle and syringe is satisfactory.[40] In those patients with long-term indwelling catheters, the catheter urine may be unreliable,[53,54] so a urine specimen should be obtained from a freshly placed catheter. Cultures should not be obtained from the drainage bag. Although data are lacking, placing a fresh condom catheter prior to urine specimen collection makes intrinsic sense.

The level of bacteriuria considered significant in an asymptomatic noncatheterized woman is derived from studies in which colony counts in voided urine specimens were compared with paired catheter or suprapubic aspirate specimens.[69] In these studies, a bacterial count of 10^5 CFU/mL or greater in a catheterized specimen was confirmed by a repeat catheterized specimen in more than 95% of cases. On the other hand, 10^5 CFU/mL or greater in a voided urine specimen was confirmed in a second voided specimen in only 80% of cases. However, two consecutive positive voided urine cultures predicted a third positive voided urine culture with 95% confidence. Therefore two consecutive voided

TABLE 302.3 Definitions for Significant Bacteriuria

Noncatheterized, Clean-Catch Voided Specimen
Symptomatic Female or Male
$\geq 10^3$ CFU/mL (based on data with coliforms; sparse data on gram-positive organisms)
Asymptomatic
Female: $\geq 10^5$ CFU/mL of same species in two consecutive voided specimens Male: $\geq 10^5$ CFU/mL in single voided specimen
Catheterized: Urine From Freshly Placed Catheter Preferable
Symptomatic Female or Male
$\geq 10^3$ CFU/mL
Asymptomatic Female or Male
$\geq 10^5$ CFU/mL

[a]See text for details.

TABLE 302.4 Signs and Symptoms Compatible With Catheter-Associated Urinary Tract Infection (CAUTI)

For all symptoms attributed to CAUTI, other causes should be considered and ruled out:
- New onset or worsening of fever
- Altered mental status
- Flank pain
- Costovertebral angle tenderness
- Rigors
- Pelvic discomfort
- Malaise or lethargy
- Suprapubic pain or tenderness
- Dysuria[a]
- Urgent or frequent urination[a]

Patients with spinal cord injury may, in addition, demonstrate the following:
- Increased spasticity
- Autonomic dysreflexia
- Sense of unease

[a]In patients whose catheters have been removed.

specimens with 10^5 CFU/mL or greater of the same uropathogen predict bladder bacteriuria with the same degree of accuracy as a single urine specimen obtained through a catheter. Nevertheless, for practical purposes and cost containment, a single urine specimen with 10^5 CFU/mL or greater is often used to define significant bacteriuria in clinical practice and many studies.[5] The finding of a single voided urine specimen with 10^5 CFU/mL or greater of an Enterobacteriaceae was reproducible in 98% of asymptomatic ambulatory noncatheterized men when the culture was repeated within 1 week.[70] Thus a single, clean-catch voided urine specimen with 10^5 CFU/mL or greater of a uropathogen identifies ASB in men.[5] Based on a comparison of voided urine specimens (from freshly applied condom catheters) and paired urethral catheter specimens, 10^5 CFU/mL or greater is also the appropriate quantitative criterion for ASB in a man with a condom catheter.[71]

In symptomatic, noncatheterized men and women, lower colony counts of coliforms (e.g., *E. coli, K. pneumoniae*) have been shown to be significant.[72] A recent study of 236 symptomatic episodes of acute, uncomplicated cystitis in 226 women confirmed that the presence of even 10^2 *E. coli* in a midstream urine specimen was highly predictive of bladder bacteriuria (as determined by in-and-out catheterization).[73] This study also found that enterococci and group B streptococci growing in voided urine generally were not found in the bladder urine, suggesting that they were usually contaminants—in fact, when these organisms were found in the midstream urine specimen, *E. coli* was present in the bladder urine in 61% of these episodes. In men with urinary symptoms, a quantitative count of 10^3 CFU/mL or greater in a voided specimen best defines UTI.[74]

In urine specimens obtained by urethral catheterization from symptomatic or asymptomatic men and women, periurethral contamination is less of a problem, and lower quantitative counts of 10^2 CFU/mL or greater are considered to be significant in both men and women.[5,75] However, most clinical laboratories do not routinely quantify urine cultures to 10^2 CFU/mL, so it is reasonable to use a quantitative count of 10^3 CFU/mL or greater in a symptomatic person, whether catheterized or not, as an indicator of CAUTI, because this threshold is a reasonable compromise between sensitivity in detecting bladder bacteriuria and feasibility for the microbiology laboratory in quantifying organisms. Of note, the level of bacteriuria or candiduria rapidly increases from small quantities to greater than 10^5 CFU/mL in catheterized individuals.[68] In asymptomatic men and women, a colony count of 10^5 CFU/mL or greater is a reasonable criterion for the diagnosis of ASB, even though lower counts probably represent true bladder bacteriuria in catheter specimens, because increased specificity is desirable.

Symptoms and Signs

The majority of patients with catheter-associated bacteriuria lack symptoms, as demonstrated in a study of 1497 newly catheterized patients who were followed prospectively with daily urine cultures, urine leukocyte counts, and symptom assessment.[28] Only 8% of 194 patients with catheter-associated bacteriuria (defined as $>10^3$ CFU/mL; 85% of patients had $>10^5$ CFU/mL in at least one culture) who could respond to symptom assessment reported symptoms referable to the urinary tract, although bacteriuria and pyuria had been present in most for many days. Additionally, there were no significant differences between patients with and without bacteriuria in signs or symptoms commonly associated with UTI—fever, dysuria, urgency, or flank pain—or in leukocytosis. The lack of an association between fever and catheter-associated bacteriuria has also been convincingly demonstrated in other studies.[29,39] An ICU study found no relationship between fever and a UTI (defined as $\geq 10^5$ CFU/mL of urine).[76] Thus in the presence of an indwelling urinary catheter, symptoms referable to the urinary tract are unreliable, and fever or peripheral leukocytosis have little predictive value for the diagnosis of CAUTI. Likewise, no studies have demonstrated that malodorous or cloudy urine in a catheterized individual has clinical significance.

Nevertheless, catheterized patients with symptoms or signs compatible with UTI that are not explainable by another condition after a thorough evaluation warrant treatment. Signs and symptoms compatible with CAUTI are listed in Table 302.4. Patients with CAUTI who are currently catheterized usually do not manifest the classic symptoms of dysuria, frequency, and urgency.

Noncatheterized adult residents of LTCFs are also at risk for health care–associated UTI. Most available evidence about what constitutes symptoms and signs of UTI in nursing home residents is from studies performed in women. Symptoms in older women that should prompt further urinary testing are fever, acute dysuria (<1 week in duration), new or worsening urinary urgency, frequency, new urinary incontinence, gross hematuria, suprapubic pain or tenderness, and costovertebral angle pain or tenderness.[77] In a patient with cognitive impairment who is unable to express symptoms, a persistent change in mental status plus change in character of the urine that is not responsive to other interventions (i.e., hydration) may suggest a need for urine testing, among other evaluations.[77]

Pyuria

Pyuria is evidence of inflammation in the genitourinary tract and is present in almost all persons with symptomatic UTIs. It is also common in persons with ASB,[4] including 30% to 75% of bacteriuric patients with short-term indwelling urethral catheters and 50% to 100% of individuals with long-term indwelling catheters. In 761 newly catheterized patients in a university hospital, the specificity of pyuria for catheter-associated bacteriuria ($>10^5$ CFU/mL—almost all were asymptomatic) was 90%, but the sensitivity was only 47%.[78] In a longitudinal study of patients with long-term urinary catheters, bacteriuria and pyuria were common, even during asymptomatic periods, and did not change during symptomatic UTI episodes.[79] Thus in the catheterized patient, the presence

or absence or degree of pyuria alone does not, by itself, differentiate catheter-associated ASB from CAUTI, but in a symptomatic patient its absence suggests that CAUTI is not the cause of the symptoms.

PREVENTION

Prevention of symptomatic CAUTI is the main objective of prevention strategies in patients for whom urinary catheterization is being considered or has been performed. However, there may also be benefits to preventing catheter-associated ASB in such patients, although such benefits have usually not been evaluated as end points in clinical trials. In the discussion that follows, the impact of interventions on catheter-associated ASB and CAUTI are mentioned when data are available, but most studies use catheter-associated bacteriuria (composed mostly of ASB) as the outcome of interest. Of note, interventions that reduce the risk of catheter-associated ASB are likely to also reduce the risk of CAUTI.

The following text discusses in detail those practices for which published data suggest that they should be implemented, those for which there are some positive data but not enough to warrant routine implementation, and those that do not warrant routine implementation on the basis of interpretation of currently available data. Prevention of one of the most significant harms of catheter-associated bacteriuria, inappropriate antimicrobial use to treat ASB, is addressed under "Health Care–Associated Asymptomatic Bacteriuria" later.

Prevention Strategies That Should Be Routine

Prevention Components of Infection Control Programs

Intensive infection surveillance and control programs in US hospitals are strongly associated with reductions in rates of nosocomial UTI.[80] The decision in 2008 by the Centers for Medicare and Medicaid Services to stop reimbursing US hospitals for CAUTIs that develop during hospitalization[81,82] has made CAUTI reduction a key component of many quality improvement programs, both state-wide and nationwide.[83–85] Several evidence-based comprehensive guidelines have been published for prevention of CAUTIs, with an emphasis on infection prevention in hospitals.[86,87] CAUTI prevention strategies that are strongly recommended for hospitals to incorporate into their infection control programs are shown in Table 302.5. UTI bundles that combine several prevention techniques to accomplish reduction in CAUTI have been described.[88–91]

It should be noted that our ability to prevent catheter-associated ASB or CAUTI in patients who have appropriate indications for catheterization is quite limited, especially in those patients requiring long-term bladder drainage.[92] Many of the key evidence-based prevention strategies are being used more widely, although significant gaps in implementation exist. A national study of US hospitals in 2009 to ascertain the practices used to prevent CAUTI showed that although 79% of hospitals performed CAUTI surveillance, only 39% monitored duration or catheter removal or both.[93] Another survey in 2009 in acute-care hospitals that participate in a geriatric nursing quality program showed that only 89% of respondents routinely wash hands before urinary catheter placement.[94] In contrast, a survey in acute-care hospitals in 2013 found significant increases from 2005 to 2013 in use of catheter stop orders, use of bladder ultrasound to assess for urinary retention, and facility-wide monitoring of CAUTI rates ($P \leq .001$ for all comparisons).[95]

Interestingly, a study of 398 health systems found no evidence that the 2008 Centers for Medicare and Medicaid Services policy to reduce payments for CAUTI has had any measurable effect on infection rates in US hospitals from 2006 to 2011 (incidence-rate ratio [IRR] in the postimplementation vs. preimplementation period, 1.03; $P = .08$).[96] These findings align with those of the CDC summary report of NHSN surveillance in acute-care hospitals, which likewise found no change overall in CAUTI from 2009 to 2014.[11]

Residents of LTCFs have a risk of developing health care–associated infections similar to that in acute-care hospital patients, and in the United States almost as many such infections occur annually in LTCFs as in hospitals.[97] To address this problem, guidelines for infection prevention and control in LTCFs have been published.[97] There has been less research on the efficacy of infection control strategies in LTCFs compared with hospitals, but guidelines for prevention of CAUTIs in hospitalized patients are thought to be generally applicable to catheterized residents in LTCFs.[97] A systematic review of strategies to reduce CAUTI in nursing home residents found a variety of effective interventions, often implemented in bundles, including strategies to minimize catheter use, strategies to improve catheter care, and also general infection prevention strategies (such as hand hygiene).[98] NHSN surveillance for health care–associated infections, including CAUTI, is currently optional for LTCFs.

TABLE 302.5 Catheter-Associated Urinary Tract Infection (CAUTI) Prevention and Monitoring Strategies Strongly Recommended for Acute-Care Hospitals

- Develop and implement written guidelines for insertion, use, maintenance, and removal of urinary catheters.
- Ensure that only trained personnel insert urinary catheters.
- Ensure that supplies necessary for aseptic-technique catheter insertion are available.
- Implement a medical record documentation system for catheter use.
- Ensure sufficient trained personnel and resources for surveillance of catheter use and outcomes.
- Perform surveillance for CAUTI using standardized criteria (NHSN definitions).
- Educate health care personnel about CAUTI prevention techniques.
- Monitor health care professional competency in catheter use, catheter care, and catheter maintenance.
- Insert urinary catheters only when necessary and remove them when appropriate.
- Consider alternatives, such as condom catheters or intermittent catheterization, when appropriate.
- Practice hand hygiene on insertion and any manipulation of the catheter and site.
- Insert catheters using aseptic technique and sterile equipment.
- Use sterile gloves, a drape, and sponges; a sterile or antiseptic solution for cleaning the urethral meatus; and a single-use packet of sterile lubricant jelly for insertion.
- Use as small a catheter as appropriate to reduce urethral trauma.
- Properly secure catheter to prevent movement.
- Maintain a sterile, continuously closed drainage system.
- Replace the catheter and the collecting system when breaks in the drainage system occur.
- To examine urine, aspirate a small sample from the sampling port with a sterile syringe.
- Collect larger volumes of urine for special analyses aseptically from the drainage bag.
- Maintain unobstructed urine flow.
- Empty collecting bag regularly; use separate collecting container for each patient.
- Keep the collecting bag and tubing below the level of the bladder at all times.
- Keep catheter and collecting tube free from kinking.
- Routine hygiene is appropriate for cleaning the meatal area.
- In locations or populations with unacceptably high CAUTI rates despite the above:
  - Implement a program to systematically identify and remove catheters that are no longer necessary.
  - Develop a protocol for management of postoperative urinary retention.
  - Establish a system for analyzing and reporting data on catheter use and adverse events.

NHSN, National Healthcare Safety Network.
Modified from Yokoe DS, Anderson DJ, Berenholtz SM, et al. A compendium of strategies to prevent healthcare-associated infections in acute care hospitals: 2014 updates. Infect Control Hosp Epidemiol. *2014;35 Suppl 2:S21–31.*

Reduction of Unnecessary Catheterization

Reducing unnecessary catheterization is the most effective way to prevent catheter-associated ASB and CAUTI. Other reasons to reduce urinary catheterization are that catheters cause discomfort, restrict mobility, and delay hospital discharges.[99] Catheter-related trauma can occur on both insertion and removal, may necessitate urologic intervention, and is probably underappreciated, because CAUTI surveillance does not include noninfectious harms of catheterization.[100]

Studies have repeatedly documented that urinary catheters are often inserted for inappropriate reasons or remain in place longer than necessary. In studies of hospitalized patients with urinary catheters, the initial indication for catheter use was judged inappropriate in up to 50% of

cases, and continued catheterization was judged inappropriate for almost half of catheter-days.[27,101–103] Additionally, the reason for catheter placement is often not stated and orders for catheterization are often not written.[104] In the medical ICU, many unjustified catheter-days are due to monitoring of urine output when it is no longer indicated, and on medical wards, urinary incontinence is a major reason for unjustified initial and continued urinary catheterization. One problem is that clinicians are often unaware that their patients are catheterized. In one study, providers were unaware of patient catheterization for 28% of the 319 provider-patient observations—21% for students, 22% for interns, 27% for residents, and 38% for attending physicians.[105] For patients who were inappropriately catheterized, providers were unaware of catheter use for 41% of the 108 provider-patient observations.

Several strategies have been demonstrated to be effective in reducing inappropriate insertion of catheters and duration of catheterization. Strategies shown to reduce inappropriate insertion of catheters include education and use of a catheter indication sheet in the emergency department,[106] use of a multifaceted intervention restricting urinary catheterization in the operating room and postanesthesia care unit and expedited catheter removal on the postoperative surgical ward,[107] use of an ultrasound bladder scanner to assess bladder volumes following surgery,[108] and use of in-and-out catheterization rather than short-term indwelling catheterization in postoperative patients with urinary retention.[109] Use of portable bladder ultrasound devices to reduce unnecessary catheterization warrants further study in the care of oliguric patients.[110,111] Operating room and emergency department personnel are appropriate targets for educational efforts to enhance use of appropriate indications for catheter insertion given that approximately three-fourths of catheters are inserted there.[112]

A systematic review of interventions to minimize the initial use of indwelling urinary catheters in acute care concluded that there was insufficient evidence to support or rule out the effectiveness of interventions due to the small number of studies, limitations in study design, and variation in clinical environments.[113] Moreover, the reviewed studies also showed a lack of consensus as to the appropriate indications for placing an indwelling catheter.

Strategies shown to reduce the duration of indwelling urethral catheterization, some of which were associated with a reduction in CAUTI in clinical trials, include physician reminders by the nursing staff, reminder stickers on medical charts or computer prompts to remove unnecessary catheters after admission, and prewritten orders for the removal of urinary catheters if predetermined criteria are not met.[99,114–123] A systematic review found that interventions to routinely prompt physicians or nurses to remove unnecessary urinary catheters reduced the rate of CAUTI (not clearly defined as symptomatic or not) by 52% ($P < .001$) and reduced the mean duration of catheterization by 1.06 days overall, although the significant decrease in duration was seen only in studies that used stop orders, rather than reminders.[124] Hospitals have responded to the evidence supporting stop orders, in that a national survey in 2013 found that 53% of hospitals surveyed were using catheter reminders or stop orders, up from 9% in 2005 ($P < .001$).[95]

Hospitals and LTCFs should develop institution-specific indications for inserting indwelling urinary catheters, monitor adherence to use of such indications in catheterizing patients, implement reminder systems for nurses and physicians as to which patients are catheterized, and consider implementing automatic catheter stop orders. Generally accepted indications for use of indwelling urinary catheters are shown in Table 302.6.

TABLE 302.6 Indications for Use of Indwelling Urinary Catheters

Perioperative use for selected surgical procedures
Use during prolonged surgical procedures with general or spinal anesthesia
Urine output monitoring in critically ill patients
Management of urinary retention and urinary obstruction
Assistance in pressure ulcer healing for incontinent patients
Prolonged immobilization
Improve comfort for end-of-life care or patient preference

Modified from Gould et al.[86] and Lo et al.[87]

Alternatives to Indwelling Urethral Catheterization

Although comparative studies are sparse, the consensus is that indwelling urethral catheterization places the patient at the greatest risk for catheter-associated bacteriuria and traumatic complications, and that alternative bladder drainage modalities should be used when appropriate.[86,87] Alternatives to indwelling urethral catheterization include the use of external collection devices, including condom catheters, intermittent catheterization, and suprapubic catheterization. Each of these alternative catheterization methods has been shown in selected populations to reduce the risk of catheter-associated bacteriuria, compared with indwelling catheterization, over the short term.[125,126] For patients requiring long-term catheterization, there are no trials comparing indwelling urethral catheterization, suprapubic catheterization, or intermittent catheterization.[127]

Condom Catheterization

In nonrandomized studies, use of condom catheters has been shown to result in a lower incidence of catheter-associated bacteriuria compared with indwelling urethral catheters.[20] In a prospective, randomized trial of 75 men at a Veterans Administration hospital, patients without dementia who had an indwelling catheter were about five times as likely to develop catheter-associated bacteriuria, develop CAUTI, or die (catheter-associated bacteriuria was the predominant outcome in this combined outcome variable) compared with those who had appropriately sized condom catheters.[128] No difference was seen in those patients with dementia, which may have to do with the increased risk of catheter-associated bacteriuria in patients who manipulate their condom catheters.[20] Thus in men with low postvoid residual volume who are not cognitively impaired, condom catheters are preferable to indwelling urethral catheters for short-term catheterization and probably for long-term catheterization. Although condom catheter–associated bacteriuria is not included in NSHN CAUTI surveillance and reporting, the positive urine cultures from condom catheters still trigger inappropriate antimicrobial use.[63] Some men may not be able to wear a condom catheter, such as those with a small or ulcerated penis. External catheters suitable for use in women are under development and testing.

Intermittent Catheterization

Intermittent catheterization is a technique in which the bladder is drained of urine by catheterization usually every 4 to 6 hours, so the amount of urine obtained with each collection is generally no more than 500 mL.[129] The schedule of intermittent catheterization is tailored for each individual to minimize the number of catheterizations while not allowing the bladder to become overdistended. Guttman and Frankel[130] in 1966 described intermittent catheterization using sterile technique, and Lapides and colleagues[131] later demonstrated that the clean (nonsterile) technique was safe and associated with a low incidence of complications in patients with neurogenic bladders. Intermittent catheterization is widely viewed to be associated with fewer complications than indwelling catheterization, including catheter-associated bacteriuria, hydronephrosis, bladder and renal calculi, bladder cancer, and autonomic dysreflexia,[132,133] and it has become the standard of care for appropriate women and men with spinal cord injury (SCI). It is also a commonly used alternative in patients without SCI who need long-term assistance with voiding.[40,134] No randomized controlled trials have compared intermittent urethral, indwelling urethral, and suprapubic or condom catheterization in patients on long-term catheterization, including those with neurogenic bladders.[132,135] On the other hand, a meta-analysis of trials comparing catheterization methods in patients (mostly postsurgical) undergoing short-term catheterization found no difference in ASB between indwelling and intermittent catheterization (20% vs. 22%; risk ratio [RR], 1.04; 95% confidence interval [CI], 0.85–1.28), although urinary retention was lower in patients with indwelling compared with intermittent catheterization (RR, 0.45; 95% CI, 0.22–0.91).[125] Nevertheless, intermittent catheterization is not commonly used for short-term catheterization because of the educational, motivational, and staff-time requirements necessary for its implementation.

Among patients undergoing long-term intermittent catheterization, randomized controlled studies in adults in hospitals, LTCFs, and outpatient settings with or without neurogenic bladders have shown no

difference in risk of catheter-associated ASB or CAUTI with use of sterile technique compared with clean (nonsterile) technique, with use of sterile catheters versus multiple-use catheters with the clean technique, or with daily or weekly replacement of multiple-use catheters.[134,136] Different techniques have been studied to reduce the microbial contamination of multiple-use catheters, including rinsing with tap water, air-drying, keeping dry until reuse, and microwaving or soaking the catheter in disinfectants, but there are no published trials evaluating the effectiveness of these methods in preventing catheter-associated bacteriuria. Although there are no data that reuse of catheters increases infection risk, catheter reuse is inconvenient for many patients who find it difficult to clean their catheters away from home, and others find it nonaesthetic.

Complications associated with long-term intermittent catheterization, although apparently less common than with indwelling urethral catheterization, include catheter-associated bacteriuria, prostatitis, epididymitis, urethritis, urethral trauma with bleeding, urethral strictures, and false passages.[129,133] Hydrophilic catheters, compared with standard catheters, reduce the friction of catheter insertion and urethral inflammation and are associated with improved patient satisfaction. These catheters have been widely used in Europe for many years in patients on intermittent catheterization. Systematic reviews and meta-analyses have come to different conclusions on whether hydrophilic catheters confer an advantage over standard catheters plus lubricant gel. A recent meta-analysis of hydrophilic versus standard catheters for intermittent catheterization included both children and adults with voiding difficulties, mostly from neurogenic bladders. This meta-analysis found a decreased risk ratio of UTIs associated with hydrophilic catheters in comparison with nonhydrophilic ones (RR, 0.84; 95% CI, 0.75–0.94; $P = .003$).[137] However, this analysis used an "antimicrobial-treatment given" definition of UTI, and the largest study included was in persons with traumatic SCI of less than 3 months' duration.[138]

Limitations to intermittent catheterization include limited availability of staff to perform the procedure or educate patients, inability or unwillingness of patients to perform frequent catheterizations, or abnormal urethral anatomy such as stricture, false passages, or bladder neck obstruction. UTIs and urethral trauma are the main complications.[137] Upper extremity impairment due to cervical SCI or other abnormality, obesity, and spasticity also make intermittent catheterization challenging for both males and females.

Suprapubic Catheterization

Potential advantages of suprapubic catheters in patients who need bladder drainage, compared with indwelling urethral catheters, include lower risk of catheter-associated bacteriuria because abdominal skin is less likely to be colonized with uropathogens compared with the urethra, reduced risk of urethral trauma and stricture, less interference with sexual activity, and, in those undergoing short-term catheterization, ability to more easily assess the appropriate time for catheter removal. A meta-analysis of randomized trials in primarily surgical patients undergoing short-term catheterization found insufficient evidence to determine whether indwelling urethral catheterization, compared with suprapubic catheterization, was associated with higher risk of UTI.[125] On the other hand, fewer patients with suprapubic catheters developed ASB (RR, 2.25; 95% CI, 1.63–3.10). Recatheterization was more common with indwelling urethral catheters (RR, 2.21; 95% CI, 1.19–4.09). Pain and discomfort were not included in this meta-analysis because the measurements were too heterogeneous.[125] Suprapubic catheters appear to be commonly used in gynecologic surgery in some centers, but their use is limited, presumably because their insertion is an invasive procedure, and they are harder to change when necessary. Potential complications include visceral injury (rare) and less serious complications, such as leakage, catheter blockage, and hematuria.[139] As mentioned previously, different catheterization methods have not been compared in patients with neurogenic bladders[132,135] or other patient groups on long-term catheterization.

Techniques for Catheter Insertion and Maintenance

Although use of aseptic technique for inserting indwelling urethral catheters is widely recommended,[140] few data exist to support such a recommendation. Some studies have shown that catheter insertion outside the operating room (where adherence to aseptic technique may be less than optimal) is associated with a higher risk of catheter-associated bacteriuria.[67] However, no significant difference in risk of catheter-associated bacteriuria was found in a study of 156 patients undergoing preoperative urethral catheterization who were randomized to sterile versus clean technique for catheter insertion.[141] Moreover, in patients managed with intermittent catheterization who are catheterized multiple times daily, there appears to be no difference in infection risk with nonsterile compared with sterile technique. Nevertheless, given the ubiquity of multidrug-resistant pathogens in the health care environment, it seems prudent to use aseptic technique for inserting indwelling urethral catheters in patients in the hospital or LTCF.[140]

Introduction of the closed catheter drainage system, in which the collecting bag is attached to the distal end of the collecting tube, was perhaps the most important advance in prevention of catheter-associated bacteriuria.[14,17,20] In patients managed with catheter drainage into open containers, 95% of patients develop catheter-associated bacteriuria by 96 hours.[142] By comparison, about 50% of patients managed with closed drainage systems develop catheter-associated bacteriuria by 14 days of continuous catheterization.[17] Based on such historical comparisons, closed systems have become the standard for bladder drainage. Disconnections at the catheter–collecting tube junctions have been shown to increase the risk of catheter-associated bacteriuria,[4,16,143] so many hospitals use preconnected urinary drainage systems in which the catheter, tubing, and drainage bag are supplied as a single connected unit. Diagnostic urine samples should be aspirated using aseptic technique through ports in the distal catheter, and larger volumes of urine for special analyses (not microbiologic studies) should be collected aseptically from the drainage bag with care not to contaminate the end of the drainage tube from potentially contaminated measuring containers.[64] The catheter should be properly anchored to minimize movement because movement of urethral catheters may cause urethral trauma and may facilitate the ascension of organisms up the urethra-catheter interface. Importantly, the drainage tube should not be allowed to move above the level of the bladder or below the level of the collection bag.[21]

Bundled CAUTI Prevention Programs

Two widespread, successful CAUTI prevention programs sponsored by the Agency for Healthcare Research and Quality have led to a decrease in CAUTI rates among participating facilities despite the overall lack of change in CAUTI rates per NSHN reports to the CDC over the same time period. One was performed in 926 units in 603 acute-care hospitals, representing more than 10% of acute-care hospitals in the United States,[84] while the other reported results from 404 nursing homes.[85] Both programs explicitly focused on both technical and socioadaptive components of CAUTI prevention. Technical components involved education in proper catheter insertion and maintenance techniques, and avoiding unnecessary catheter use, for example. The socioadaptive components focused on improving attitudes and behaviors related to infection prevention and patient safety. Facilities were also provided regular feedback on their rates of CAUTI and urinary catheter utilization. In acute-care units, CAUTI rates decreased from 2.4 to 2.05 per 1000 catheter-days, in adjusted analysis (IRR, 0.86; 95% CI, 0.76–0.96; $P = .009$).[84] In nursing homes, CAUTI rates in the adjusted analysis decreased from 6.42 to 3.33 per 1000 catheter-days (IRR, 0.46; 95% CI, 0.36–0.58; $P < .001$).[85] The effort from both the project team and the participants at the health care facilities was considerable and included in-person and virtual teaching sessions, coaching calls, webinars, educational materials, manuals, tools, and checklists. Of note, changes in the safety culture as measured by the Hospital Survey on Patient Safety Culture did not show any association with improvements in CAUTI rates at participating acute-care facilities.[144] Which aspects of these interventions are necessary for success and whether these interventions would succeed in facilities that did not volunteer to be part of this type of project is not known. However, these interventions are presented here as evidence that change in CAUTI rates is possible through national collaboratives.

Prevention Strategies With Possible Benefit

Although these practices might have benefit, they are not recommended for routine use.

Antiseptic- and Antimicrobial-Coated Catheters

In vitro studies have shown antiadherence or antimicrobial activity associated with silver-, minocycline- and rifampin-, and nitrofurazone-coated catheters,[145–147] although nitrofurazone appears to be the most inhibitory.[148,149] Currently two types of coated or impregnated catheters are available in the United States: catheters with an antiseptic silver alloy coating, and catheters impregnated with the antibiotic nitrofurazone (related to nitrofurantoin). Both are intended for short-term (<14 days) use. Silver oxide urinary catheters were inferior to silver alloy catheters at preventing bacteriuria in meta-analysis and are no longer manufactured in the United States.[150–152] There are no data to show that antimicrobial-coated catheters are beneficial in patients managed with long-term catheterization (>30 days).[153]

The largest and most clinically relevant trial of these coated catheters was a multicenter trial by Pickard and colleagues, in which 7102 patients undergoing short-term (1–14 days) catheterization were randomized to indwelling urethral catheterization with a silver alloy–coated catheter, a nitrofurazone-impregnated catheter, or a standard latex catheter.[154,155] In this trial 95% of the patients were catheterized for perioperative monitoring. The primary outcome was symptomatic UTI, defined as UTI symptoms and signs plus prescription of an antimicrobial for UTI, and the time frame for CAUTI was up to 6 weeks after catheter removal (unusually long for catheter trials). Compared to standard catheters, silver alloy–coated catheters showed no reduction in CAUTI, while nitrofurazone-coated catheters showed a minimal reduction in CAUTI (−2.1%; 95% CI, −4.2 to −0.1%). This reduction was less than the 3.3% reduction established a priori to be of clinical significance.[154]

In a meta-analysis of randomized trials comparing types of indwelling urinary catheters in hospitalized adults undergoing short-term catheterization, only the large, randomized, controlled trial by Pickard and colleagues measured symptomatic CAUTI as the primary outcome.[154,155] In a meta-analysis by Lam and coworkers, silver alloy catheters were associated with a slightly reduced risk of bacteriuria in comparison to standard catheters (RR, 0.82: 95% CI, 0.73–0.92).[156] However, the trial by Pickard and colleagues, judged to have low risk of bias in subject allocation, did not find that silver catheters significantly decreased ASB.[155] In the Lam and coworkers meta-analysis, nitrofurazone-impregnated catheters were associated with a lower risk of bacteriuria in comparison to standard catheters (RR, 0.73; 95% CI, 0.62–0.85) and in comparison to silver alloy catheters (RR, 0.78; 95% CI, 0.67–0.91).[156]

In summary, nitrofurazone-coated urinary catheters appear to have some benefit in the prevention of catheter-associated ASB in some trials of short-term catheterized patients, while silver alloy catheters have minimal effect. Whether the reduction in bacteriuria translates into reduction of secondary bloodstream infection or other health care–associated infections is unclear. Thus available data do not support routine use of antiseptic or antimicrobial urinary catheters to prevent CAUTI. Resistance to catheter-impregnated antimicrobial agents or antibiotics has not been demonstrated in published clinical trials, but this remains a concern.

Prophylaxis With Antimicrobial Agents

Systemic Antimicrobials

Systemic antimicrobial drug therapy has been shown repeatedly in prospective and retrospective studies to transiently prevent or delay the onset of catheter-associated bacteriuria, although development of antimicrobial resistance has been noted in some studies.[14,20,65] A meta-analysis of randomized controlled trials in surgical and nonsurgical patients undergoing short-term catheterization found limited evidence that postoperative antimicrobial prophylaxis for catheterized patients could reduce the risk of bacteriuria and other signs of infection, and limited evidence suggested that prophylactic antimicrobials reduce bacteriuria in catheterized nonsurgical patients.[157] However, resistance to the antibiotics used for prophylaxis (such as trimethoprim-sulfamethoxazole and ciprofloxacin) was observed in several trials. Meta-analysis of antimicrobial prophylaxis with long-term urinary catheter use only found one randomized, controlled trial in patients with indwelling catheters, and in this study, only 23 patients completed the 6-month study of norfloxacin suppression versus placebo.[132,158] Although this study demonstrated a significant decrease in symptomatic CAUTI, 25% of strains in placebo patients versus 90% of strains in norfloxacin patients were resistant to norfloxacin at the end of the prophylaxis period.[158] The other studies addressed in this meta-analysis were of patients performing intermittent catheterization, and results were inconsistent about whether prophylactic antimicrobials reduced UTIs.[132] Clinical trials in adults with neurogenic bladders secondary to SCI, using either indwelling[159] or intermittent catheterization,[160] and those in older adults with indwelling catheters,[161] have found that recurrent bacteriuria, after a course of targeted antimicrobials intended to sterilize the urine, is the rule rather than the exception.

Because of the potential for the development of antimicrobial resistance and adverse effects and cost, routine use of systemic antimicrobial agents to prevent catheter-associated bacteriuria or CAUTI should be discouraged. Unfortunately, up to 60% to 80% of hospitalized, catheterized patients receive antimicrobial therapy for a variety of reasons,[17,162] and not controlling for this important variable in the analyses of many intervention trials may explain why some interventions have not been shown to be effective in preventing catheter-associated bacteriuria.

Methenamine Salts

Methenamine salts (methenamine mandelate and methenamine hippurate) are hydrolyzed to ammonia and formaldehyde, which is responsible for the antibacterial activity of methenamine. Antimicrobial activity in urine is correlated with urinary concentrations of formaldehyde, and the urinary concentration of formaldehyde is dependent on the concentration of methenamine in the urine and the urine pH.[163] Maintaining a low urinary pH (below 6) is necessary to achieve bactericidal concentrations of formaldehyde, but the association between therapeutic efficacy and low urinary pH has not been confirmed consistently.[163] In addition, the optimal method to acidify the urine in a patient on methenamine is not known. Ascorbic acid is often used to acidify the urine, but up to 4 g/day have shown no significant effect on mean urinary pH, and doses as high as 12 g/day may be required to adequately acidify the urine.[163]

Methenamine salts have been shown to prevent uncomplicated cystitis in young women[163–165] but are not as effective or convenient as currently available regimens for prevention of UTI. In addition, methenamine salts are generally considered to have limited effectiveness in preventing CAUTI in patients with indwelling catheters for whom the time for hydrolysis to formaldehyde is limited.[20,163] A meta-analysis of 13 randomized or quasi-randomized controlled studies of methenamine hippurate for the prevention of UTIs suggested that methenamine hippurate may have some benefit in patients without renal tract abnormalities (symptomatic UTI: RR, 0.24; 95% CI, 0.07–0.89; bacteriuria: RR, 0.56; 95% CI, 0.37–0.83), but not in patients with known renal tract abnormalities (symptomatic UTI: RR, 1.54; 95% CI, 0.38–6.20; bacteriuria: RR, 1.29; 95% CI, 0.54–3.07).[166] The benefit in one study of women undergoing short-term catheterization following gynecologic surgery may have been due in part to the administration of methenamine for several days after the catheters had been removed in some cases.[167] Methenamine does not appear to prevent catheter-associated bacteriuria or CAUTI in SCI patients with neurogenic bladders irrespective of the method of bladder management.[166,168,169] There are no published data on the use of methenamine in patients using condom catheterization.

Overall, the data are unconvincing that methenamine is effective in reducing the risk of catheter-associated bacteriuria or CAUTI in patients managed with long-term indwelling catheterization—patients most in need of an effective agent that does not select for antimicrobial resistance. Methenamine does appear to be effective in post–gynecologic surgery patients undergoing short-term catheterization, and its use in this situation may be considered, although this group has little morbidity from catheter-associated bacteriuria. It may also be reasonable to consider a trial use of methenamine in patients on intermittent catheterization who have recurrent episodes of CAUTI, even though its benefit in such patients is unproven. If used, the recommended dose of methenamine hippurate is 1 g twice daily and that for methenamine mandelate is 1 g four times daily, and the urinary pH should be maintained below 6.[163]

Prevention Strategies With Little Benefit

These strategies have little or no demonstrated benefit and are not recommended for routine use.

Enhanced Meatal Care

Reducing meatal colonization would seem to be a reasonable measure to reduce the risk of CAUTI, given that ascension of uropathogens along the catheter-urethra interface is the predominant source of catheter-associated bacteriuria.[67] However, periurethral cleansing with chlorhexidine solution versus water before insertion of an indwelling urinary catheter did not reduce the rate of catheter-associated bacteriuria in two different trials.[170,171] Moreover, results of large, randomized trials have shown no significant reduction in catheter-associated bacteriuria in men or women, compared with usual care (debris removal at daily baths), with one or more times daily meatal cleansing using nonantiseptic green soap or applications of povidone-iodine solution and ointment, polyantibiotic ointment or cream, or silver sulfadiazine 1% cream.[14,18,20,65] Likewise, simultaneous interventions to block bacterial entry at the urethral insertion site, catheter–drainage tube junction, and outflow tube of the drainage bag were not effective in preventing catheter-associated bacteriuria.[18] Possible reasons why enhanced meatal care has not been effective in reducing catheter-associated bacteriuria include the negative effect of increased catheter manipulation associated with the interventions, inadequate residual antiseptic activity of the topical agent, and lack of effect on the intraluminal route of infection.

Cranberry Products

Although there is conflicting evidence from randomized controlled trials about whether cranberry products are effective in reducing the risk of symptomatic UTIs in women with recurrent UTI, a Cochrane review concluded that cranberry did not significantly reduce the occurrence of symptomatic UTI in any subgroup, including people requiring catheterization.[172] Randomized, placebo-controlled studies of cranberry in doses up to 2 g daily to prevent catheter-associated bacteriuria or CAUTI in patients with neurogenic bladders are mostly negative.[168,172] The routine use of cranberry products for prevention of health care–associated UTI should be discouraged due to lack of clearly demonstrated efficacy in preventing catheter-associated ASB or CAUTI, problems of tolerance with long-term use, and cost. There are no published data on the use of cranberry products for prevention of ASB or CAUTI in catheterized adults without neurogenic bladder. More recently a double-blind, randomized, placebo-controlled trial of cranberry capsules in 185 noncatheterized women in nursing homes did not find any difference in the primary outcome of bacteriuria plus pyuria in the two arms of the study.[173]

Bladder Irrigation With Antimicrobial Therapy or Saline

Bladder irrigation with antimicrobial agents such as povidone-iodine, chlorhexidine, neomycin, or polymyxin B sulfate has shown little overall benefit in the era of closed urinary drainage.[14,20] In one demonstrative study, 187 adult patients who required short-term urinary catheterization were randomized to closed drainage with a triple-lumen, neomycin-polymyxin irrigated system, or with a double-lumen nonirrigated catheter system.[143] There was no significant difference in the rates of catheter-associated bacteriuria between the two groups, but uropathogens isolated from irrigated patients were significantly more resistant to the irrigating antimicrobial than those in the nonirrigated group. Bladder irrigation with saline or acidic solutions also does not have any clear benefit in reducing the risk of catheter blockage resulting from encrustation or in reducing symptomatic UTIs.[174] In summary, bladder irrigation does not appear to be effective in preventing or eradicating catheter-associated bacteriuria in the majority of patients with short-term or long-term indwelling catheterization, is time consuming, may damage the bladder mucosa, and may select for antimicrobial-resistant organisms. Catheter irrigation may have a role in reducing blood clots after urologic surgery, but this topic is outside the scope of this chapter.

Antimicrobial Drugs in the Drainage Bag

Once the drainage bag becomes contaminated, subsequent catheter-associated bacteriuria occurs in almost all patients who remain catheterized,[16] and positioning of the drainage tube above the level of the bladder or below the level of the collection bag is a predictor for an increased risk of catheter-associated bacteriuria.[21] Investigators have attempted to prevent catheter-associated bacteriuria by sterilizing the drainage bag, but results of randomized trials evaluating the addition of chlorhexidine, hydrogen peroxide, or povidone-iodine to the bag suggest that this intervention is not effective.[14,20,65,175] Such a strategy would not be expected to be effective if the integrity of the closed drainage system is carefully maintained because that should minimize bacterial entry into the drainage bag.

Routine Catheter Change

Urinary catheters readily develop biofilms on their inner and outer surfaces once they are inserted, and these biofilms protect uropathogens from antimicrobials and the host immune response.[56] In long-term catheterized patients, catheters are often changed routinely at periodic intervals (e.g., monthly) to reduce the risk of catheter-associated bacteriuria, as well as for aesthetic reasons. A meta-analysis found only one randomized, controlled trial of this practice; 17 men in a nursing home were randomized to catheter change only when obstructed/infected versus monthly scheduled changes as well as when necessary for obstruction/infection.[176] The trial was too small to provide reliable evidence about CAUTI or blockage. Moreover, it has been recommended that patients who experience repeated early catheter blockage should have their catheters changed every 7 to 10 days to avoid obstruction,[177] but this practice has not been evaluated in clinical trials. The practice of routine catheter change with the purpose of preventing infection or blockage, or both, is unlikely to change in the absence of data to address this issue.

Prophylactic Antimicrobial Therapy at Catheter Removal or Replacement

Fever and bacteremia can occur at the time of removal or replacement of a urethral catheter, and prophylactic antimicrobials are sometimes used to prevent such events. Among catheterized and bacteriuric women in LTCFs, transient fever is twice as common within 24 hours of catheter replacement, compared with other days.[29] Studies in chronically catheterized and bacteriuric men and women have shown that bacteremia occurs in 4% to 10% of patients after urethral catheter removal or replacement, but episodes are transient and asymptomatic.[178–180] The effect of antimicrobial prophylaxis on prevention of bacteremia is unknown. A prospective, randomized, placebo-controlled trial of antimicrobial treatment of catheter-associated ASB persisting 48 hours after removal of short-term catheters in hospitalized women reported significantly improved microbiologic and clinical outcomes at 14 days in treated women.[181] Seven (17%) of 42 women randomized to receive no therapy developed symptomatic UTI by 14 days, whereas no women in the treatment group became symptomatic. Whether these results are generalizable to current medical practice is unclear, because all had persistent bacteriuria documented at 48 hours after catheter removal; checking for bacteriuria postcatheterization is not a standard practice or even feasible in discharged patients. Screening for and eradicating postcatheterization catheter-associated ASB to prevent CAUTI is currently not recommended.

A meta-analysis of whether prophylactic antimicrobials at the time of removal of a short-term urinary catheter reduced the risk of symptomatic UTI found seven controlled studies on this topic, of which five were in surgical patients and six were randomized controlled trials.[182] Overall, a benefit was seen with prophylactic antimicrobials, with a 5.8% absolute reduction in symptomatic UTI and a risk ratio of 0.45 (95% CI, 0.28–0.72). Nevertheless, prophylactic antimicrobial agents are not routinely recommended for catheter removal or replacement because it is unclear whether these findings apply to the more general medical inpatient population, and widespread implementation of prophylactic antimicrobials at the time of catheter removal has potential to cause harm in terms of cost, side effects, and resistance.

Preclinical Strategies for CAUTI Prevention

Many novel approaches have been studied in vitro, with the goal of prevention or eradication of the catheter-associated biofilm of uropathogens.[183] Of these, mannosides—small molecule inhibitors of the type 1 pilus adhesin, FimH—seem likely to move to clinical trials in

humans and could potentially impact prevention of UTI more generally. In a study in mice, mannosides provided significant protection against uropathogenic *E. coli* catheter-associated bacteriuria by preventing bacterial invasion and shifting the *E. coli* niche primarily to the extracellular milieu.[184] Mannosides can be targeted to the fimbrial adhesins on uropathogenic *E. coli* while having less affinity for the adhesins of commensal gut *E. coli*; thus mannosides were able to deplete the mouse gut of uropathogenic *E. coli* without perturbing the commensal *E. coli*.[185] A selective agent that could reduce colonization by urinary pathogens without disrupting the gut microbiota would have obvious advantages over prophylactic antimicrobials for UTI prevention.

HEALTH CARE–ASSOCIATED ASYMPTOMATIC BACTERIURIA

Routine Screening and Treatment

Screening and treatment of ASB have not been shown to be beneficial, will select for antimicrobial resistance, and are not recommended, except in pregnant women and patients who undergo traumatic genitourinary procedures associated with mucosal bleeding.[5,186] Populations that have been extensively studied and for whom these recommendations apply include premenopausal, nonpregnant women; diabetic women; older people living in the community; elderly, institutionalized subjects; persons with SCI; and catheterized patients.[5] For example, in 35 patients undergoing long-term catheterization, a prospective randomized trial of cephalexin or no antibiotic therapy for episodes of catheter-associated ASB caused by susceptible organisms reported no differences between the two groups in incidence and prevalence of catheter-associated bacteriuria, CAUTI, or catheter obstruction in patients followed up to 44 weeks.[187] In addition, 47% of reinfecting organisms in the cephalexin group compared with 26% in the control group were highly resistant to cephalexin. Even if treatment of catheter-associated ASB was found to be useful, one study in which daily catheter urine cultures were obtained found that 60% of 25 episodes of CAUTI occurred on the same day that catheter-associated bacteriuria was first detected,[26] complicating attempts at preemptive therapy. It is not known whether eradication of catheter-associated ASB might be beneficial in reducing cross-infection or inappropriate antimicrobial usage.

Prevention of Overtreatment of Asymptomatic Bacteriuria in Health Care Settings

The greatest impact that ASB has on clinical care is probably the excessive testing and treatment done for this condition in health care settings.[188] Prevention of inappropriate treatment of ASB is probably the second most beneficial strategy for patients related to health care–associated UTI, secondary only to avoidance of unnecessary urinary catheterization. Estimates vary a bit by site of care delivery, but studies in acute-care hospitals, LTCFs, SCI clinics, and emergency departments have documented that 20% to 83% of episodes of detected ASB are treated with antimicrobials.[189–191] Thus prevention of inappropriate treatment of ASB is essential to antimicrobial stewardship in acute and long-term care settings, as endorsed by the American Board of Internal Medicine's Choosing Wisely campaign.[192,193] A positive urine culture is a powerful stimulus for subsequent use of antimicrobials, as demonstrated by surveys of practitioners and also by an innovative study in which urine culture results from the microbiology laboratory were suppressed.[194,195] A stewardship intervention that focused on the "culture of culturing,"[196] discouraging unnecessary cultures in patients without urinary-specific symptoms, successfully reduced both urine cultures and antimicrobial treatment of ASB in acute and long-term care wards of a large medical facility.[197] Unnecessary urine cultures in patients with ASB can lead to false elevation of the reported CAUTI rates, because a positive urine culture in a febrile patient must be reported to the NSHN as a CAUTI, regardless of whether the fever can be attributed to another cause.[198] The co-occurrence of fever and bacteriuria is particularly common in ICU and long-term care patients. A tertiary care academic medical center reduced CAUTI rates significantly in its ICUs through a multifaceted program that centered on the stewardship of urine culturing.[199] Likewise, emphasis on judicious use of urine cultures in a nationwide project in nursing homes was associated with a decrease in both urine cultures and CAUTI.[85] Removing unnecessary urinary catheters through CAUTI prevention efforts may have the side benefit of also reducing testing and treatment for ASB, because cloudy urine or visible sediment in the urine is a stimulus for urine cultures among nursing personnel.[194,200] A recent national study of over 4 million hospital admissions documented that 47% had a urinalysis and 27% had a urine culture during hospitalization[201]; thus there is considerable room for improvement in the stewardship of urine testing as well as stewardship of antimicrobial use for ASB.

MANAGEMENT OF HEALTH CARE–ASSOCIATED URINARY TRACT INFECTION

The issues relevant to clinical management of health care–associated UTI, particularly CAUTI, include whether the catheter should be changed prior to urine collection for culture, whether the catheter should be changed during the course of treatment, choice of drugs, duration of therapy, and when to consider complicating factors that may impair response to antimicrobials. The wide variety of underlying conditions, diverse spectrum of possible etiologic agents, and paucity of controlled clinical trials with stratification according to specific complicating factors make generalizing about clinical management of CAUTI difficult. Antimicrobials alone may not be successful, if underlying anatomic, functional, or metabolic defects are not corrected.

Urine Culture and Catheter Replacement Before Treatment

CAUTIs, especially in patients with long-term catheterization, are often polymicrobial and caused by multidrug-resistant uropathogens, so urine cultures should be obtained prior to treatment to confirm that the empirical regimen provides appropriate coverage and to allow tailoring of the regimen based on antimicrobial susceptibility data.[40,202] The culture should be obtained from a freshly placed catheter if the catheter has been in place for a few days because the catheter biofilm may result in spurious culture results.[53,54] Moreover, clinical outcomes are improved if the catheter is replaced, as shown in a prospective, randomized, controlled trial in elderly nursing home residents with long-term indwelling catheters and CAUTI. This study demonstrated that patients whose catheters had been in place for longer than 2 weeks and who underwent catheter replacement before antimicrobial treatment had significantly shorter time to improved clinical status and significantly lower rates of polymicrobial catheter-associated bacteriuria and CAUTI after therapy, compared with those who did not undergo catheter replacement.[203] These study findings support replacing the catheter and obtaining a urine culture from a freshly placed catheter before antimicrobial treatment for CAUTI if the catheter has been in place for at least 2 weeks and cannot be permanently removed.

Choice of Antimicrobial Agent

The choice of antimicrobial for empirical treatment should take into account the facility antibiogram, the patient's specific risk factors for having a multidrug-resistant urinary pathogen, the severity of illness, and the patient's underlying comorbidities.[204] Risk factors for colonization with a multidrug-resistant organism, particularly an extended-spectrum β-lactamase–producing Enterobacteriaceae, include international travel to an area endemic for resistant Enterobacteriaceae, such as southern Asia, within the prior 12 months, and receipt of antibiotics to treat traveler's diarrhea during a prior international trip.[205,206] Microbiologic data from urine cultures within the prior 2 years can be used to guide empirical antibiotic choice and improve the likelihood of choosing an antimicrobial agent effective against the current urinary pathogen.[207] Health care exposure (such as hospitalization or stay in an LTCF) and receipt of antibiotics within the prior 6 months are also risk factors for colonization with resistant organisms.[208] In nursing home residents, indwelling devices such as urinary catheters or feeding tubes are risk factors for colonization with, and thus infection by, multidrug-resistant organisms.[209] Whether the patient is able to take oral agents is also a consideration in antimicrobial choice.

A general perspective on resistance trends in CAUTI-causing organisms can also be useful, particularly if a facility-specific antibiogram is not available. Data from the NHSN between 2011 and 2014 showed a

high prevalence of multidrug-resistant phenotypes among strains causing CAUTI.[19] Thus in 2014, 34.8% of *E. coli* strains tested were resistant to fluoroquinolones and 15.5% to extended-spectrum cephalosporins (e.g., cefepime); 9.5% of *Klebsiella* spp. were resistant to carbapenems and 22.5% to extended-spectrum cephalosporins. Global studies from 2003 to 2010 of urology patients with health care–associated bacteriuria, which included ASB, found that slightly over 50% of urinary isolates were resistant to fluoroquinolones (excluding *Candida* from the possible pathogens).[210]

Appropriate empirical treatment choices require that patients be categorized on a spectrum from mild illness and ability to take oral agents to severe illness and inability to take oral agents. In all patients a urine culture should be collected before starting antimicrobials so that the antimicrobial can be changed to a more appropriate agent if necessary. Outpatients who are not severely ill can be empirically treated with oral fluoroquinolones, cephalosporins, trimethoprim-sulfamethoxazole, or amoxicillin-clavulanate (Table 302.7). Consideration should be given to providing an initial dose(s) of a broader-spectrum parenteral agent, such as ceftriaxone, a carbapenem, or an aminoglycoside, if there is concern about antimicrobial resistance, while waiting for urine culture results. Patients at low risk for multidrug resistance and who are not critically ill but who require inpatient therapy can be treated with ceftriaxone or piperacillin-tazobactam. If a patient who is not critically ill has risk factors for an extended-spectrum β-lactamase–producing organism and no prior cultures with carbapenem-resistant Enterobacteriaceae, a carbapenem should be considered for empirical therapy. Patients who are critically ill should be empirically treated with an antipseudomonal carbapenem (meropenem, imipenem, or doripenem) and vancomycin or daptomycin. Patients with prior carbapenem-resistant Enterobacteriaceae cultures who are critically ill or unable to take an oral agent, may warrant, in consultation with an infectious diseases expert, empirical use of some of the newer, expensive, and very broad-spectrum agents such as ceftolozane-tazobactam, ceftazidime-avibactam, meropenem-vaborbactam, or plazomicin. The antimicrobial regimen should be tailored as appropriate when the infecting strain has been identified and antimicrobial susceptibilities are known.

TABLE 302.7 Empirical Management of Catheter-Associated Urinary Tract Infection

Antimicrobial Agent and Dosing
Mild to Moderate Illness, Can Tolerate Oral Therapy (Dosage Duration: 5–10 Days)[a]
Ciprofloxacin 500 mg PO twice daily or 1 g (extended release) PO once daily[c]
Levofloxacin 750 mg PO once daily[c]
Cefpodoxime 200 mg PO q12h[d] (or other third-generation cephalosporin)
Trimethoprim-sulfamethoxazole (TMP-SMX) 160/800 mg PO q8h[e]
Amoxicillin-clavulanate immediate release 875 mg orally twice daily[d]
Severe Illness or Unable to Tolerate Oral Therapy (Dosage Duration: 5–14 Days)[b]
Ciprofloxacin 400 mg IV twice daily[c]
Levofloxacin 750 mg IV once daily[c]
Ceftriaxone 1–2 g IV once daily[d]
Cefepime 1 g IV twice daily[d]
Piperacillin-tazobactam 3.375 g IV (as piperacillin) q6h[d]
Meropenem 1 g IV q8h[d]
Imipenem-cilastatin 500 mg IV q6h[c]
Doripenem 500 mg IV q8h[d]
Ertapenem 1 g IV once daily[d]
Gentamicin 5–7 mg/kg IV once daily[e] ± ampicillin 1–2 g IV q6h[d]
Ceftolozane-tazobactam 1.5 g IV (as both drugs) q8h[d]
Ceftazidime-avibactam 2.5 g IV (as both drugs) q8h[d]
Meropenem-vaborbactam 4 g IV (as both drugs) q8h (pregnancy category unknown)
Plazomicin 15 mg/kg IV once daily[e]
In Choosing an Empirical Agent, Consider the Following:
Collect a urine culture before beginning treatment to guide subsequent therapy
Severity of illness and comorbidities—choose a broader agent for more severely ill patients or those who are immunocompromised
Antimicrobial susceptibility of bacteriuria strains within past 2 years
Antibiotic exposure within prior 6 months
Local resistance data—in some locations, fluoroquinolones and/or TMP-SMX are no longer reasonable empirical choices given high resistance levels among *E. coli*
Hospitalization or stay in a long-term care facility within past 6 months
Travel to an area with high prevalence of drug resistance in prior 12 months
Consider adding vancomycin if gram-positive organism suspected
Use a carbapenem (meropenem, imipenem, doripenem, or ertapenem) if an extended-spectrum β-lactamase strain is known or suspected
Tailor regimen based on susceptibility data, and transition to oral medications as soon as condition allows

[a]Consider a concomitant single initial dose of ceftriaxone 1 mg IV/IM, ertapenem 1 g IV/IM, meropenem 1 g IV, gentamicin 5 mg/kg IV/IM, amikacin 15 mg/kg IV/IM, or tobramycin 5 mg/kg IV/IM, particularly in febrile patients or patients at risk for being unable to follow up. Duration of therapy can be 5 days in patients receiving fluoroquinolones. Other agents should be used for 7–10 days.
[b]Consider an initial intravenous dose of a broader-spectrum agent, such as meropenem, particularly if using fluoroquinolones in a severely ill patient. Duration of therapy can be shortened to 5 days in patients receiving fluoroquinolones who have a rapid response. Other agents should be used for 7–14 days, with longer courses for patients with a slow response to therapy or with complications, such as an abscess. Doses in the table are for adults with normal renal function.
[c]Pregnancy category C—animal studies have shown an adverse effect on the fetus; use only if potential benefit justifies the potential risk to the fetus.
[d]Pregnancy category B—no clear risk to fetus based on animal or human studies, or both.
[e]Pregnancy category D—associated with human fetal risk; use only if the potential benefit justifies the potential risk to the fetus.
Modified from Hooton et al.[2] and Johnson and Russo.[204]

Duration

Few studies have been performed that evaluate duration of treatment in populations with CAUTI or other complicated UTIs. Reviews of complicated UTI have recommended treatment durations from 7 to 10 days,[14] 7 to 14 days,[40] and 7 to 21 days,[202] depending on the severity of the infection. However, it is desirable to limit the duration of treatment, especially for milder infections, to reduce the selection pressure for drug-resistant flora, especially in patients on long-term catheterization. In one small trial of women with lower urinary tract CAUTI, cure rates were comparable with a single-dose and a 10-day regimen of trimethoprim-sulfamethoxazole.[181] However, single-dose therapy was ineffective in eradicating bacteriuria in institutionalized elderly men.[211] In a randomized, double-blind, placebo-controlled trial comparing 3-day and 14-day regimens of ciprofloxacin for the treatment of mild CAUTI in 60 patients with SCI, there was no difference in clinical outcomes at long-term follow-up.[212] Most recently, clinical and microbiologic success rates following treatment were almost identical in a noninferiority study of 619 patients with acute pyelonephritis or complicated UTI treated with a 5-day course of levofloxacin or a 10-day course of ciprofloxacin.[213] These data suggest that a 7-day regimen is reasonable for most patients with CAUTI, depending on their clinical response, and that shorter regimens, such as a 5-day regimen of a urinary fluoroquinolone, are likely to be sufficient in those patients who are less severely ill, are infected with uropathogens susceptible to the antimicrobial used, and have a rapid response to treatment.

Nephrostomy Tubes and Ureteral Stents

Patients with obstruction of the ureter or ureteropelvic junction, often due to stones, malignancy, or benign strictures, may have a ureteral catheter (stent) or a percutaneous nephrostomy tube inserted. A ureteral catheter may be inserted from the bladder during cystoscopy or through a percutaneous nephrostomy into the renal pelvis and further down into the ureter, with a drainage hole at the level of the renal pelvis. J-shaped curls at catheter ends are used to keep the catheter in place. Nephrolithiasis at the at the time of tube placement can trigger either systemic inflammatory response syndrome or sepsis; the risk of both is low but higher in patients who have a preexisting UTI or infected stone.[214] Intraoperative cultures of urine from the renal pelvis and stone material can be useful to guide postprocedure antibiotics.[215] Once a percutaneous nephrostomy tube is in place, colonization of the tube and urine with bacteria and/or *Candida* occurs commonly, often with more than one organism. Usually this colonization is asymptomatic, but it can lead to fever, pyelonephritis, renal abscess, or bacteremia if drainage from the renal pelvis becomes obstructed or, less often, if instrumentation of an infected urinary tract introduces organisms into the blood stream.[216] A clinical definition of nephrostomy-associated pyelonephritis, to help distinguish an actual infection from the more

common asymptomatic colonization, is the presence of fever, costovertebral angle tenderness, or flank pain associated with a positive urine culture, or a combination of these.[217] An obstructed, infected renal pelvis can rapidly evolve into a pyonephrosis (infection of the kidney with pus in the upper collecting system), which can be complicated by renal papillary necrosis or perinephric abscess. Infected urine in the pelvis can track along the nephrostomy tube into the perinephric space and even extend down the iliacus muscle. Treatment involves immediate drainage and appropriate antimicrobial drugs. Consideration should be given to exchange of the nephrostomy tube during treatment,[218] analogous to the recommendation to change an indwelling urethral catheter during treatment of a CAUTI. Penetration of the drug into the urine is not imperative in treating pyonephrosis or perinephric abscess but is relevant to treating bacteriuria or candiduria prior to a planned urologic procedure.[219] Fluconazole and flucytosine obtain high urine concentrations, while intravenous conventional amphotericin B achieves low concentrations. Other antifungals, such as the echinocandins, may have insufficient urine concentrations to affect candiduria but are active within the renal parenchyma. Amphotericin B irrigation of the renal pelvis as 50 mg/L in sterile water has been used for prophylaxis.[219] Toxicity is minimal but efficacy is unclear. When used as prophylaxis, an antifungal or antibacterial should be begun prior to the procedure and discontinued shortly after an uncomplicated procedure (duration of 24 hours or less).[222]

FUNGAL URINARY TRACT INFECTION

Yeasts, mostly *Candida* species, are commonly isolated from the urine of catheterized patients.[223] NHSN data from 2011 to 2014 reported that *C. albicans* was the second most common organism identified in CAUTI, second only to *E. coli*; other *Candida* species (including all except *C. albicans*) ranked 10th, and *Candida glabrata* alone ranked 14th.[19] In January 2015 the NHSN surveillance definitions for CAUTI changed to exclude *Candida* species and other fungal organisms as causative agents of CAUTI, so nationwide estimates on the prevalence of fungal CAUTI will be difficult to obtain after this date.

Among patients with nosocomial funguria enrolled in a multicenter prospective surveillance study, 85% had concomitant nonfungal infections (urinary and nonurinary); 90%, previous exposure to antimicrobial agents; 83%, urinary tract drainage devices; 39%, diabetes; 38%, urinary tract abnormalities; and 22%, a malignancy—only 11% had no obvious underlying illness.[224] Although only 1.3% of the 530 patients with candiduria followed for 12 weeks had documented candidemia, the importance of comorbid conditions was reflected in the 20% mortality rate. Candiduria in itself may be a marker for severity of underlying illness, because multiple studies have shown that patients with candiduria have higher mortality rates, although not typically from *Candida* infection.[225]

Determination of the clinical significance of candiduria can be problematic because it can represent contamination of a voided specimen, colonization of catheters or stents, bladder infection, ascending kidney infection, or kidney infection associated with candidemia. Hematogenous dissemination is relatively much more likely to be the source of candiduria than is the case with bacteriuria.[226] Neither colony count thresholds nor the presence of pseudohyphae in the urine are helpful to distinguish contamination from bladder infection. In noncatheterized women with candiduria, a catheter specimen may be indicated to rule out contamination with perineal flora.[226] In the catheterized patient, pyuria is a nonspecific finding, but its absence suggests that candiduria is not causing tissue invasion.

Most health care–associated candiduria occurs in catheterized patients, and most episodes are asymptomatic. In the large prospective study of nosocomial funguria mentioned earlier, only 2% to 4% of patients had urinary symptoms,[224] although comorbidities and urinary catheterization may complicate assessment of symptoms. However, catheterized patients with candiduria may have symptoms or signs such as suprapubic or flank pain, lower abdominal discomfort associated with prostatitis, or an enlarged and tender scrotum associated with epididymo-orchitis.

Ascending infection of the kidney and disseminated candidiasis are rarely associated with candiduria and usually occur in the setting of obstruction of the urinary tract. In a retrospective study of 26 cases of candidemia associated with a well-defined urinary tract source, urinary tract abnormalities (mostly obstruction) were present in 88%, and 73% had undergone urinary tract procedures before the onset of candidemia.[227] Episodes of candidemia were brief and low grade in intensity, although two of five in-hospital deaths were attributable to candidiasis. Paired urine and blood strains of *Candida,* however, may not be the same strain, as demonstrated in a case-control study in which 52% of paired strains were different by molecular typing.[228] Other complications associated with fungal infections of the genitourinary tract include fever, fungus balls in the bladder or renal pelvis, renal and perirenal abscesses, emphysematous pyelitis or pyelonephritis, and papillary necrosis—many of these complications are more likely to occur in diabetics.

Few treatment studies have been performed in patients with candiduria,[229] and there remain questions as to whom to treat, when to treat, and how long to treat. Asymptomatic candiduria rarely requires treatment because it often resolves spontaneously, morbidity is low, and treatment is often followed by rapid recurrence and may select out for resistant organisms.[225,230] In the large prospective observational study mentioned previously, funguria cleared in 76% of 155 patients who had no specific therapy for funguria and in 35% of 116 patients who had their catheter removed as the only treatment.[224] Other studies have shown spontaneous clearance rates of candiduria ranging from 29% to 62%.[224] In a randomized, placebo-controlled trial of 316 hospitalized patients with asymptomatic or minimally symptomatic candiduria, a 2-week course of fluconazole resulted in significantly higher eradication rates than placebo (50% vs. 29%), but there was no significant difference in candiduria rates 2 weeks after completion of treatment.[230] In placebo recipients who had their catheters removed, 41% had eradication of candiduria, compared with only 20% who had their catheters replaced. Pyelonephritis, candidemia, and fungus-related death were not observed in this study. The possibility of disseminated candidiasis should be considered in all hospitalized patients with candiduria, especially in the ICU, where candidemia is common and up to 80% of persons with candidemia will have accompanying candiduria.[231] However, candidemia occurs in less than 5% of ICU patients and thus most such patients with candiduria do not have candidemia.[231] If candiduria fails to resolve despite changing the catheter or stopping antibiotics (if feasible), a more deep-seated infection should be suspected, especially in critically ill patients, and imaging of the kidneys and collecting system is indicated to look for a renal abscess, fungus ball, or other urologic abnormality that may require treatment.[231] Antifungal treatment of candiduria should be reserved for those patients who have solid evidence of infection of the kidney or collecting system or disseminated candidiasis.[231] Treatment is also indicated in asymptomatic patients with neutropenia, infants with low birth weight, and patients who will undergo urologic manipulations because these conditions have a higher association with upper tract infection or dissemination.[225,226] Recent data have raised questions as to whether asymptomatic candiduria in patients with renal transplants warrants treatment.[232] Of note, in 2000, a multicenter study in the United States reported that 43% of instances of candiduria were treated, likely reflecting overtreatment of funguria.[224]

Candiduria should be treated in symptomatic patients, and those with systemic signs or symptoms should be evaluated for disseminated infection with imaging and blood cultures. Oral fluconazole is the drug of choice for cystitis and pyelonephritis due to most species of *Candida* (but not resistant *C. glabrata, Candida krusei,* or other resistant yeasts); of note, doses adjusted for renal insufficiency may result in subtherapeutic concentrations.[229] Amphotericin B and flucytosine can be used for *C. glabrata* resistant to fluconazole, and amphotericin B is the recommended agent for *C. krusei.*[225] Other azoles that could serve as alternative choices for resistant isolates, including itraconazole, voriconazole, and posaconazole, are not recommended for *Candida* UTI because of minimal excretion into the urine, even though they achieve antifungal activity in renal parenchyma.[231] Systemic amphotericin B deoxycholate is no more effective than fluconazole for susceptible strains, and bladder irrigation with amphotericin B deoxycholate is rarely used. Lipid formulations of amphotericin B may not be the first choice for *Candida* UTI because of presumed low concentrations of the drug in renal tissue. Failure of the lipid complex formulation of amphotericin B therapy has

been described in the treatment of *Candida* pyelonephritis in experimental animals and patients.[225] Echinocandins are minimally excreted and should not be considered first-line agents for candiduria. However, case reports suggest that caspofungin and micafungin may be effective in patients with *C. glabrata*–associated UTI.[233,234] Even with apparently successful local or systemic antifungal therapy for candiduria, relapse is frequent, and this likelihood is increased by continued use of a urinary catheter. Persistent candiduria, especially in immunocompromised patients, warrants radiologic imaging of the kidneys to evaluate for hydronephrosis, fungal bezoars (common in critically ill neonates[224]), or perinephric abscesses associated with ascending infection.

SUMMARY

Health care–associated bacteriuria and candiduria are common, mostly associated with urinary catheterization, and usually asymptomatic. Whereas any symptomatic UTI should be treated, published data do not support routine screening to detect health care–associated bacteriuria or candiduria in asymptomatic patients because treatment does not appear to alter the natural course of infection and an increase in antimicrobial resistance often results. However, among asymptomatic patients with bacteriuria or funguria, we need a better understanding as to who has upper tract involvement or tissue invasion, how these affect the clinical course, and thus which patients, if any, might benefit from screening and treatment. The quality of clinical trials needs to be improved because many are nonrandomized and underpowered and use nonspecific terminology and poorly described methodology. Studies of interventions to prevent catheter-associated bacteriuria need to address whether they prevent not only CAUTI (which many have not), but also whether prevention of catheter-associated ASB reduces inappropriate antimicrobial use, bacteremia, and cross-infection.

Strategies that are effective for prevention of catheter-associated bacteriuria are likely to also prevent catheter-associated candiduria. The most effective way to reduce health care–associated UTIs is to reduce urinary catheterization by restricting use to patients who have clear indications and by removing the catheter as soon as it is no longer needed. Implementation of strategies to reduce catheterization is likely to have more impact on catheter-associated bacteriuria than implementation of other strategies addressed in this chapter. However, use of multiple infection control techniques and strategies simultaneously (called *bundling*) likely offers the best opportunity to reduce the morbidity and mortality related to health care–associated infections.[235] In those patients who do require urinary catheterization, an effective stewardship program for both urine testing and antimicrobial treatment in asymptomatic patients offers potential benefits to patients and the health care system. More research is needed in terms of how to best implement effective stewardship programs for bacteriuria.

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The complete reference list is available online at Expert Consult.

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303 Health Care–Acquired Hepatitis

Mini Kamboj and Kent A. Sepkowitz

SHORT VIEW SUMMARY

Definition
- Viral hepatitis can be acquired by patients or health care workers (HCWs) during the process of health care delivery.

Epidemiology
- Transmission between patients and from patient to HCW or HCW to patient is rare, but the risk remains. Dialysis patients and workers are at particular risk, especially for hepatitis C virus (HCV) and, if unvaccinated, for hepatitis B virus (HBV).
- Increasingly, transmissions to patients are seen in long-term care settings and can be traced to inappropriate use of blood glucose monitoring devices.
- Transfusion-based risk of hepatitis E varies by geographic region. Implementation of universal screening of blood products varies accordingly.

Diagnosis
- Identification of transmission is difficult unless a cluster of cases occurs.
- Most investigations are conducted in conjunction with public health authorities.
- Use of a genotype to determine homology is useful in investigation.

Postexposure Treatment
- Hepatitis A: Vaccine is the preferred approach for postexposure prophylaxis (PEP) of otherwise healthy individuals. Vaccine and immunoglobulin are recommended for those with immunocompromising or chronic liver conditions.
- Hepatitis B: Give hepatitis B immune globulin (HBIG) to all exposed nonimmune persons (patients or HCWs) with or without initiation of vaccine series. The role of antivirals has not been determined.
- Hepatitis C: Monitor and treat only if the exposed person develops evidence of acute infection. Use of direct-acting antiviral (DAA) therapy has not been studied in this context.

Vaccination
- Hepatitis A vaccine is not routinely recommended for HCWs.
- Hepatitis B vaccine or formal declination is mandated for HCWs in the United States. Rapid decrease in measured antibody levels within months of immunization can occur despite persistence of immune protection. Booster doses are not recommended.
- No vaccine is available for HCV.

HISTORICAL BACKGROUND

The potential for bloodborne transmission of hepatitis B first was noted in 1885, when Lurman described jaundice in factory workers who had received smallpox vaccine prepared from "human lymph."[1] More reports appeared in the subsequent decades as use of vaccines derived from human serum became more common.[2] In addition, more frequent use of phlebotomy equipment,[3] insulin therapy,[4] and intramuscular injection of antibiotics all led to small outbreaks of jaundice, which were ascribed to a transmissible "icterogenic" agent.

By the late 1940s, studies to clarify the modes of transmission were undertaken. Central to these was the use of human volunteers, who were given putatively infectious material intradermally, intranasally, or by ingestion of feces, and then observed for development of jaundice.[2,5–7] From these landmark reports arose our current understanding of the basic principles of transmission of infectious hepatitis (hepatitis A) and serum hepatitis (hepatitis B).

The first report of occupational disease in health care workers (HCWs) was provided by Leibowitz and colleagues,[8] who described jaundice in a blood bank nurse with numerous needle pricks on her hands and fingers. A spate of similar reports followed, describing occupationally acquired hepatitis among nurses, blood bank workers, phlebotomists, house staff, and others.[9,10] Soon, the workers' compensation boards of certain states ruled that viral hepatitis was a compensable occupational hazard. Improved understanding of routes of transmission, more comprehensive and rigorous infection control including needle disposal, and, for hepatitis B, vaccination of workers at risk have helped to decrease, but not eliminate, this occupational risk. A corollary risk, that of transmission of infection from infected HCWs, particularly surgeons, to nonimmune patients, has been described for hepatitis B[11,12] and hepatitis C.[13]

CURRENT EPIDEMIOLOGY OF HEALTH CARE–ASSOCIATED HEPATITIS B AND C OUTBREAKS IN THE UNITED STATES

A health care–associated outbreak of hepatitis is defined as two or more cases that are epidemiologically and/or genetically linked.[14,15] Since 2008, The Centers for Disease Control and Prevention (CDC) has maintained a Web-based inventory of health care–associated cases and outbreaks of hepatitis B and C.[16] This source described 59 outbreaks affecting more than 450 people that were investigated between 2008 and 2016 (Fig. 303.1). In addition, 18 (15 hepatitis C and 3 hepatitis B) sporadic cases of patient-to-patient health care–associated transmission of hepatitis B and C were recognized during this time.

Health care–associated outbreaks of hepatitis mostly have been recognized in nonhospital settings, with a growing trend in long-term care facilities[17] and ambulatory areas,[18] especially outpatient hemodialysis units.[16] Practices associated with transmission of hepatitis reveal a shift in etiology: syringe reuse and needlestick injuries have less commonly been the cause, and more cases have been traced to other health care exposures.[19] These include improper use of blood glucose monitoring fingerstick devices on multiple patients,[20,21] contamination of saline flush and multidose vials,[18] and breaches in disinfection and sterilization of medical equipment.[19,22]

Hemodialysis-related outbreaks of hepatitis C continue to occur, with most being attributed to environmental contamination or unsafe practices during vascular access or medication preparation or administration.[16,23] Diversion of medication, wherein a practitioner personally administers an intravenous narcotic and then reuses the remainder of the vial of medication or the needle in a patient or patients, is also increasingly recognized as a source of transmission.[24] At least five

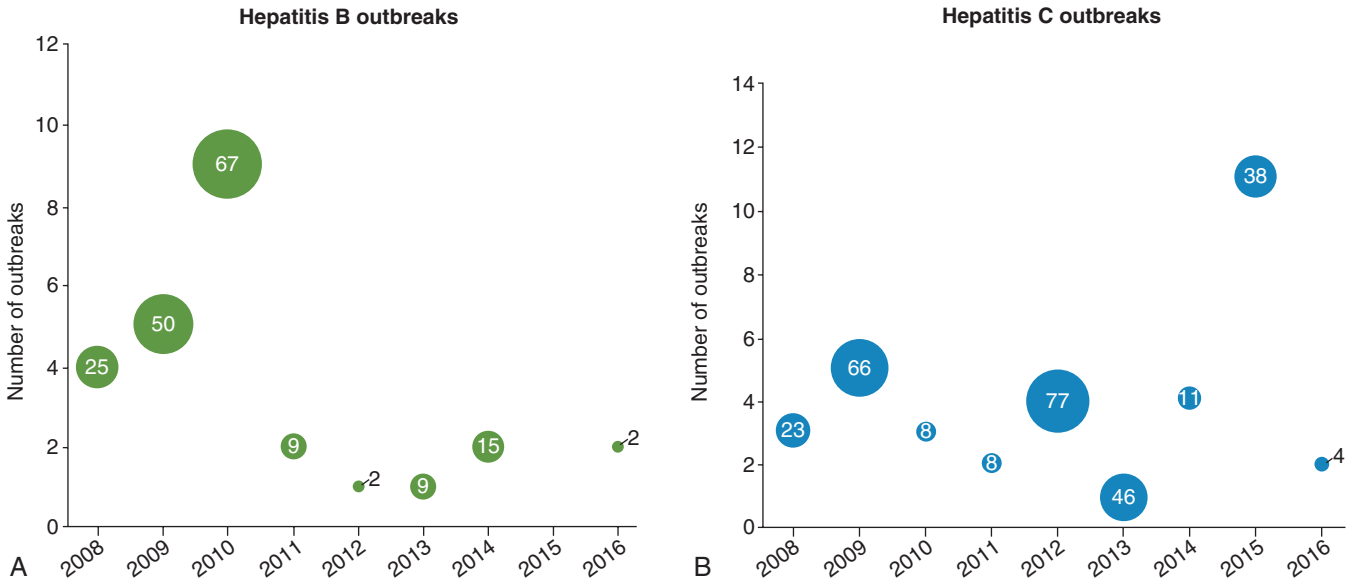

FIG. 303.1 (A and B) Hepatitis B and C outbreaks investigated between 2008 and 2016.

TABLE 303.1 Risk of Transmission, Prevention, and Postexposure Management of Health Care Workers With Occupational Exposure to Hepatitis Viruses

HEPATITIS	RATE[a]	PREVENTION	POSTEXPOSURE MANAGEMENT
A	10%–30%	Vaccine not recommended routinely for HCWs except in outbreak setting	Vaccine within 2 weeks of exposure. Immunoglobulin plus vaccine may be considered for HCWs with chronic liver or immunocompromising conditions.
B	HBeAg⁻ source: 3% HBeAg⁺ source: 22%–31%	HBV vaccination	HCWs with previous response to vaccine need no additional postexposure treatment regardless of level of exposure and time since vaccination. HCWs with no history of vaccination or nonresponders should receive HBIG and vaccine (for those with no history) as soon as possible after the exposure. HCWs with unknown response after primary series should have anti-HBs titers checked. • Levels <10 mIU/mL: Give HBIG × 1 and revaccinate • No further treatment for immune titers If anti HBs titers cannot be readily obtained, first dose of HBIG should be administered. The role of antivirals is not defined.
C	3%	Immune globulin not recommended; see Chapter 154 for treatment	No role for direct-acting antivirals. Immediate baseline testing for hepatitis C antibody, then HCV RNA if antibody positive. If source patient confirmed with HCV infection, or with unknown status, repeat HCV RNA three weeks after exposure.
Delta	Unknown; outbreaks described only in dialysis units	HBV vaccination	Segregate HBsAg⁺ dialysis patients by delta antibody status.
E	Unknown	Standard precautions	

[a]Rate of transmission from outbreak or needlestick exposure.
HBeAg, hepatitis B e antigen; *HBIG,* hepatitis B immune globulin; *HBsAg,* hepatitis B surface antigen; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HCWs,* health care workers.

diversion-related outbreaks have been recognized in the United States since 2009,[16] including one from a traveling medical technician that spread across eight states, resulting in over 11,000 exposed individuals with 45 confirmed infections. This outbreak led to a criminal conviction of the source HCW.[25]

Fecal-Oral Transmission

Hepatitis A

HCWs are not considered to be at a higher occupational risk for hepatitis A, because nosocomial outbreaks and transmission from patient to care provider have been infrequent. The Advisory Committee on Immunization Practices (ACIP) does not recommend routine vaccination of HCWs to protect against hepatitis A.[26] The rare reports of nosocomial transmission have largely occurred in pediatric units and nurseries.[27,28] The preponderance of hepatitis A outbreak reports in neonatal settings is due to the asymptomatic nature of infection in infants and young children and prolonged shedding[29]; the disease in index cases often goes unrecognized until secondary cases in adult contacts have occurred.[30] Rare outbreaks in adults have been related to high physical dependency or diarrhea or fecal incontinence.[31–35]

Although dominantly an enterically transmitted infection, hepatitis A can rarely be acquired via blood and platelet transfusion from donors with incubating infection[36]; in addition, a single transplant-associated case has also been described. Tertiary transmission of hepatitis A from a child who had initially contracted hepatitis A via multi–visceral organ transplant eventually led to infection in 2 of 42 home health nurses who provided direct care to the child.[37,38]

Postexposure prophylaxis (PEP) by administration of intramuscular immune globulin to contacts has been used effectively for many years (Table 303.1).[39] Although not specifically assessed in health care–related exposures, vaccination within 2 weeks has been shown to be 80% to 90% effective in preventing clinical hepatitis A among immunocompetent

TABLE 303.2 Country-Specific Rates of Hepatitis E RNA Prevalence Among Screened Blood Products and Associated Risk of Transmission

COUNTRY (YEAR)	UNIT SCREENED	SAMPLES SCREENED	HEV RNA PREVALENCE	POSTTRANSFUSION HEV IN RECIPIENT	COMMENT
Netherlands (2013)	Mini-pools	59,474	0.076%	NA	Estimated duration of viremia in donors = 68 days
Germany (2012)	Mini-pools	16,125	0.08%	NA	
Spain (2015)	Individual	9998	0.03%	NA	
Denmark (2016)	Individual	25,637	0.04%	7/11 recipients without detectable viremia	Low copy numbers in donors (13 IU/mL)
Ireland (2016)	Individual	24,985	0.02%	NA	Universal screening implemented
France (2014)	Mini-pools	53,234	0.045%	NA	
United Kingdom (2014)	Mini-pools	225,000	0.035%	42% attack rate	Universal screening implemented
Australia (2017)	Pools	74,131	0.001%		
United States (2013)	Individual	1930	0%	No transmission	
United States (2016)	Individual	18,829	0.01%	NA	Low copy numbers in positive donors
United States (2017)	Mini-pools	128,028	0.002%	NA	Type 3a infections with low copy numbers

HEV, Hepatitis E virus; *NA,* not applicable.

adults younger than 40 years.[40] Vaccine was successfully used in two eligible nurses in the transplant recipient–related outbreak.[37] Since 2000, Canadian guidelines have routinely recommended vaccine as the preferred PEP for nonimmunocompromised patients older than 1 year. In United Kingdom, immune globulin is recommended to be given in conjunction with vaccine only for healthy adults older than 50 years, and those with chronic liver disease or an immunocompromising condition, regardless of age.[41]

Hepatitis E

First identified as a cause of acute hepatitis in Kashmir, India, hepatitis E virus (HEV) is primarily transmitted via contaminated water. The virus has caused several large-scale outbreaks among refugees and other displaced populations. Person-to-person spread among household contacts has been demonstrated only in resource-constrained settings, where transmission is attributed to poor sanitation and to communal hand-washing and eating practices.[42–44]

In Europe, indigenous infection from zoonotic contact or acquisition via meat products (undercooked pork, pork liver sausage, game meat, and shellfish) is on the rise, with predominantly genotype 3a infections.[45] Currently in Europe, infections due to HEV are at least as common as hepatitis A among patients hospitalized with acute hepatitis.[46] Approximately half of all HEV cases are autochthonous in origin[45–47] because risk of acquisition is strongly influenced by regional farming and dietary practices. Certain areas in eastern Japan, southwestern France, and the Netherlands have exceedingly high seropositivity rates as a result of these factors. A detailed description of the epidemiology of hepatitis E can be found in Chapter 178.

Seroprevalence of Hepatitis E in Developed Countries and Risk of Bloodborne Transmission

Seroprevalence of hepatitis E in the United States is estimated to be around 6.25%, with the highest rates in the Midwest.[48] Genotypes 3 and 4 prevail among the sporadic locally acquired infections. Based on population estimates from the National Health and Nutrition Examination Survey (NHANES), seroprevalence rates in US adults older than 30 years declined from 20% (1988–1994) to 8.4% (2009–2010); a decrease in seropositivity was seen among all age groups within this time period. Overall, anti-HEV immunoglobulin G (IgG) prevalence decreased from 9.6% to 5.2%.[49,50]

Several assessments of blood donors in the United States have concluded that the transmission risk of HEV is minimal.[48,51,52] Screening of blood products for HEV is not currently recommended in the United States. Blood donor screening data from several European countries have also demonstrated variable rates of hepatitis E viremia, ranging from 0.02% to 0.08% with an associated transmission risk of 0.04% (Table 303.2).[53–56] Some European countries have adopted universal screening of blood products for hepatitis E. A detailed discussion on this topic can be found in Chapter 304.

Other Nosocomial Transmission

Non–transfusion-related nosocomial HEV transmission is rare; an outbreak in Pakistan attributed to sharing of parenteral equipment affected 18 individuals, with 7 confirmed cases.[57] A single case of patient-to-patient spread of hepatitis E via contaminated hospital environment was suspected in a French hospital in which the donor patient with detectable virus in blood and stool had an overlapping stay with the case patient, who developed acute infection 3 weeks later. The strains were closely related by genotypic analysis.[58]

Hepatitis B

Epidemiology

Hepatitis B was the first bloodborne disease recognized to pose an occupational hazard.[8–10] In the prevaccine era, the prevalence of hepatitis B virus (HBV) among HCWs was 10-fold greater than in the general population. An early review found a preponderance of cases among pathologists, laboratory workers, and blood bank workers.[10] Later studies added dentists, physicians, nurses, and laboratory and dialysis staff to the list of HCWs with increased risk.[9,10,59–62]

Vaccine to prevent HBV infection became available in the United States in 1982. Introduction of the currently used recombinant HBV vaccine improved vaccine acceptance. Since 1991, it has been recommended as a universal childhood vaccination. In 1987, concern about occupational acquisition of human immunodeficiency virus (HIV) led the US Department of Labor, in conjunction with the Department of Health and Human Services, to recommend universal precautions to protect against exposure to body fluids.[63] Four years later, the Occupational Safety and Health Administration published the federal Bloodborne Pathogens Standard, which went into effect in early 1992.[64] This document mandated that HCWs with potential exposure to blood or other potentially infectious materials should be offered the hepatitis B vaccine series free of charge, should demonstrate immunity to hepatitis B, or should formally decline vaccination. In order to identify nonresponders and to intervene early, postvaccination serologic testing is recommended for HCWs prone to blood and body fluid exposures. Compliance with these recommendations has resulted in a 98% reduction in occupationally acquired hepatitis B,[65,66] although rare cases continue to occur. Based on a survey conducted by the CDC between 2005 and 2010, 203 cases of hepatitis B were reported among HCWs; among these, only 40% were possibly related to occupational exposures. Complete vaccination

with three or more doses could be confirmed for 35 HCWs infected with hepatitis B, 1 had immune titers, and others were nonresponders or without additional information.[66]

Incidence After Exposure

The risk of transmission from a single needlestick exposure varies according to the hepatitis B e antigen (HBeAg) status of the source case. The risk of developing clinical hepatitis ranges from 1% to 6% for HBeAg-negative blood to 22% to 31% for HBeAg-positive blood (see Table 303.1).[66,67] In addition, transmission from a viremic hepatitis B surface antigen (HBsAg)–negative patient, even with low viral burden, may occur.[68]

Reported Transmissions

Worker-to-patient transmission. Over the past several decades, dozens of episodes involving HCW-to-patient transmission of hepatitis B have been described, resulting in hundreds of secondary cases (range, 1–55 secondary cases per source case).[11,12,66,68–73] In a series of 10 clusters reported from the United Kingdom, the transmission rate ranged from 0.3% to 9%.[12,69] At least 42 of the 47 HCWs involved were dentists or surgeons. In a majority of the cases, no obvious infection-control breaches or overt percutaneous exposures were recognized. Lack of glove use during oral surgery and chronic skin conditions on the surgeon's exposed hands have been proposed as contributing factors in some of the older reports. No cases of dentist-to-patient transmission have been reported since 1987, which suggests the effectiveness of standard precautions and routine vaccination of HCWs,[69] although lack of case clustering may prevent recognition of sporadic transmission events.[73]

In the United States, the last documented HBV transmission from a surgeon to patients occurred in 2009.[74] The source was an orthopedic surgeon with previously unrecognized infection and an HBV DNA level of almost 18 million IU/mL. Of note, he had twice received a three-dose HBV vaccination series but had not been evaluated for chronic HBV infection, despite failure to respond to the vaccine and recent immigration from a country with high rates of HBV. The last reported transmission during a surgical procedure occurred in Japan from an HBeAg-positive gynecologic surgeon with high level of virus in serum, sweat, and saliva.[75] In a classic outbreak from the 1990s,[11] 19 (13%) of 144 susceptible patients operated on by an HBeAg-positive thoracic surgery resident developed acute hepatitis B infection despite appropriate infection-control measures. Thirteen available isolates, including the surgeon's, were identical when compared by means of genetic analysis. Examination of the resident's surgical technique suggested that small cuts in his fingers, sustained while he tied sutures, resulted in entry of his blood into patients' wounds. Of note, the surgeon had declined hepatitis B vaccine 2 years earlier and then had become infected as the result of an occupational exposure.

In most but not all of the reported outbreaks, the source worker was HBeAg positive. However, in one series, four HBeAg-negative surgeons all transmitted disease.[12] The cases occurred in England, where restriction of HBeAg-positive surgeons (principally cardiothoracic, gynecologic, orthopedic, and abdominal) is strictly enforced. Confirmation of transmission was aided by HBV DNA sequencing of both the putative source and secondary cases.

In response to concerns around HCW-to-patient transmission, in 1991, the CDC promulgated recommendations for preventing transmission of HIV and HBV to patients during exposure-prone invasive procedures, which were modified by non-CDC experts in 2012 and are currently in revision.[66,68,76] These and other guidelines addressing infected HCWs have attempted to balance patient safety against a worker's right to privacy. Most US and European guidelines now recommend that workers performing exposure-prone procedures maintain a low HBV DNA level, usually less than 1000 IU/mL.[76]

Patient-to-worker transmission. Widespread transmission from a single patient to several HCWs is rare. In one instance, a patient in the preclinical window period for hepatitis B sustained severe trauma and underwent several operations.[77] At least four HCWs, including nurses and physicians, developed acute hepatitis temporally consistent with transmission from this patient. As with worker-to-patient transmission, clustered cases lead to the identification of transmission more readily than individual transmissions. Other instances of transmission may go undetected and unreported.

Dialysis setting. For many years, dialysis patients and staff were at high risk for occupational acquisition of hepatitis B, given their high frequency of sharps injury or mucocutaneous exposure (5 instances per 10,000 dialysis procedures[78]), the high titers of HBV in blood (≥10^9/mL), and the ability of HBV to survive well in the environment[16,79] However, with segregation of patients by room, staff, and machine according to surface antigen (HBsAg) status; institution of active vaccination programs; monthly serologic testing of susceptible patients; and attention to disinfection, equipment, and cleaning procedures, this rate has decreased sharply.[16,59,79–81] When rates were compared from a classic study conducted before the availability of vaccine,[59,81] the incidence of new HBsAg among patients was found to have decreased from 3% to 0.05%, and the prevalence from 7.8% to 0.9%. During the same period, vaccination coverage of staff increased to 90% and HCW HBV incidence decreased from 2.6% to less than 0.5%. In US dialysis centers in 2002, the prevalence and incidence of HBV among dialysis patients had remained steady at 1% and 0.12%, respectively; 56% of patients and 90% of staff had been vaccinated.[59]

Despite these gains, transmission continues in centers that fail to identify HBV-infected patients, share staff and equipment, or fail to vaccinate susceptible patients.[16,79] Medication preparation close to treatment areas, infection-control lapses during vascular access, and inability to separate clean and contaminated environments continue to provide reservoirs of health care–based transmission of HBV.[16,59,79] Nonimmune persons undergoing hemodialysis should be vaccinated with high-dose or double-dose vaccine formulations.[80] Serologic assessment of postvaccination titers 1 to 2 months after completion of the series is recommended. The determination for booster doses is made on an annual basis, after anti-HBs level assessment, and with the goal of maintaining titers over 10 mIU/mL. Clinically significant hepatitis has been reported in patients on dialysis who achieve a minimum protective antibody level but with subsequent decline.[82] The approach for nonresponders is similar to others (refer to section "Postexposure Management").

Other nosocomial transmissions. In some countries, nosocomial transmission due to unsafe practices continues to account for a substantial proportion of overall hepatitis B cases.[83,84] In US and UK health care settings, transmission related to blood glucose monitoring devices,[20,85] endoscopy equipment, reuse of single-dose vials in podiatry care, and jet injections has been increasingly reported,[a] especially in long-term care.[15,16] This group of exposures now account for up to 37% of all health care–associated acquisition of HBV among older adults in the United States.[86] In addition, patients (so-called medical tourists) who for financial or other reasons receive care in countries with a high prevalence of HBV may unknowingly invite the additional risk of virus acquisition.[88] Hepatitis B was transmitted to a patient operated on by the same oral surgeon and assistants who had performed tooth extraction in an HBeAg-positive patient 2 hours before. Thorough review of the case failed to disclose any overt infection-control breach.[14]

Vaccine Acceptance and Response Among Health Care Workers, and Duration of Immunity

The National Health Interview Survey (NHIS) estimated vaccination coverage of 63.8% for health care personnel in 2011.[89] Three out of every four HCWs with direct patient contact are vaccinated against hepatitis B. Approximately 88% of individuals achieve seroprotective titers after vaccination; suboptimal responses are related to older age, obesity, and smoking.[90] Among those who do not develop protective titers after the primary series (<10 mIU/mL), cumulative response on revaccination with additional three doses approaches 70%.[66] Preemployment serologic testing and HBsAg testing are recommended for HCWs from geographic regions with prevalence rates of ≥2% regardless of vaccination status, and for those with known nonresponse to vaccine previously.[66]

Knowledge of serostatus aids in decisions to revaccinate and postexposure management. Therefore, serologic testing is routinely recommended for those with an anticipated risk of occupational exposure.

[a]References 15, 16, 20, 21, 86, 87.

If protective levels are achieved 1 to 2 months after immunization, no further testing is necessary regardless of future exposures. For vaccinated HCWs with anti-HBs <10 mIU/mL immediately after completion, an additional dose or revaccination with the three-dose series is recommended. Any additional immunization beyond the sixth dose is not recommended for those without a response to two complete series. These individuals should be considered susceptible in the event of a significant exposure, and hepatitis B immune globulin (HBIG) administered promptly.[91,92]

It is well recognized that antibody response wanes after completion of primary immunization series, regardless of initial response.[93,94] The most precipitous drop in titers occurs in the years immediately after vaccination. In longitudinal reports spanning across at least 2 decades, persons with declining antibody level (to <10 mIU/mL) remain protected.[95,96] Age at the time of vaccination and higher anti-HBs titers after vaccination correlate with duration of measurable immune titers.[93] In the longest cohort of vaccinees, including children and adults from 12 Alaskan communities, followed over a period of 30 years after primary hepatitis B vaccination series, approximately half of the individuals showed loss of measured protective antibody. Despite this, anamnestic response after booster challenge could be elicited in the vast majority (89%), confirming a protective response on exposure to HBV.[95] For the vaccinees without demonstrable anamnestic response, future susceptibility to acute or chronic HBV disease remains unclear.

Other studies conducted in areas of high endemicity have demonstrated breakthrough infection among vaccine responders marked by the development of antibodies against hepatitis B core protein.[93,97] HCWs vaccinated as adults also demonstrate durable antibody response for up to 28 years after primary immunization.[98] Loss of protective titers was detected in 22% and did not correlate with interval since vaccination. Among those given a booster dose, 94% developed adequate levels. Immunized HCWs with occupational exposure to HBV have been shown to develop core and polymerase-specific CD8 (+) T-cell responses akin to acquired immunity after HBV infection.[99] These data collectively suggest that newly infected vaccinees typically develop subclinical disease and that vaccine-induced immunity confers protection from chronic HBsAg carriage and clinical hepatitis, but may not entirely prevent infection. Based on this, the CDC does not recommend routine revaccination of otherwise healthy HCWs.[66]

In November 2017 a recombinant HPV vaccine with a novel CpG adjuvant was marketed for persons at least 18 years of age and is given as two doses 1 month apart. Immunogenicity is reported at least as good as the earlier two recombinant HBV vaccines. The vaccine, Heplisav-B, was recommended as an option in 2018 by the ACIP and may offer convenience for HCWs over the prior three-dose 16 weeks schedule.[99a]

Postexposure Management

Management in exposed or susceptible workers has been well summarized elsewhere[26,66] (see Table 303.1 and Chapter 145). Treatment should ideally be administered within 24 hours after exposure. Intramuscular HBIG was the original intervention for PEP.[66,100] It is still used, in conjunction with initiation of a vaccine series, for management after exposure in unvaccinated persons and vaccine nonresponders. For vaccine responders, no postexposure intervention is required, regardless of the HBsAg or HBeAg status of the source. Vaccinated persons with an unknown response to vaccine should have their antibody titer checked immediately after exposure and should be treated according to the result (see Table 303.1). If HBIG and vaccine are indicated, titers should be checked at least 3 months after HBIG after antibody levels from passive immunization have waned, in order to accurately assess response to the vaccine. Nonresponders to hepatitis B vaccine should receive a second dose of HBIG 1 month later (HBIG dose, 0.06 mL/kg or 5.0 mL for adults).[66,100] Vaccines and HBIG may be given at the same time but should be administered with separate needles and syringes and at different anatomic sites. Plain immune globulin does not contain sufficient titers of HBIG and should not be considered an acceptable alternative. The role of antiviral agents such as lamivudine, entecavir, and tenofovir in the management of nosocomial exposure has not been determined.

The best long-term management for the hepatitis B vaccine nonresponder is not known. All nonresponders to the first three-dose series should be tested for evidence of HBsAg if not already done at the time of hire.

Hepatitis C

Seroprevalence

Most[78,101] series suggest that many HCWs with increased risk for hepatitis B, including dialysis workers, laboratory workers, surgeons, nurses, and workers with the mentally impaired, have no increase in hepatitis C virus (HCV) seroprevalence. The overall prevalence of HCV among persons receiving dialysis is approximately 8% (nearly fivefold higher than the general population) and among dialysis center staff is around 1.7% (same as US general population).[59] Some studies, including a recent meta-analysis, have suggested that medical and laboratory staff and dental health workers may have an elevated risk.[102,103]

Incidence After Occupational Exposure

Percutaneous exposure is the most common mode of health care–associated transmission. Risk is greatest with deep injuries from a hollow-bore needle that has been used to access the source patient's artery or vein.[104] Rare cases of acquisition from blood splash on the conjunctiva have been reported. HCV after exposure to body fluids other than blood or via contaminated environment has not been conclusively documented. Seroconversion occurs in 0% to 10% of nonimmune HCWs who sustain needlesticks from a source patient with hepatitis C.[26,78,105–108] Maternal-fetal transmission rates are similar (5%–9%). Rates may vary because of differences in the diagnostic test used (antibody or HCV RNA) and nature of evaluated exposures. The highest transmission rate (10%) was from a study in which HCV RNA was used to detect infection in exposed workers.[106,108] This high rate has not been duplicated, and most studies have placed the transmission rate at less than 3%. In a large case series from the United Kingdom, 626 percutaneous HCV exposures to blood or bloodstained fluids occurred from 1997 to 2007, resulting in a seroconversion rate of 2.2%.[104] Existing evidence supports a correlation between HCV RNA level in the source patient's blood and higher risk of transmission.[109] This association has not been established for other body fluids. A report of simultaneous transmission of HCV and HIV from a single needlestick was remarkable for the delayed time to seroconversion against each virus and the fulminant, fatal course of the HCV infection.[110] This phenomenon appears to be extremely rare.

Reported Transmissions

Worker-to-patient transmission. HCWs have transmitted HCV to patients on several occasions, sometimes after acquiring their own infection from a different patient.[111–113] A cardiac surgeon transmitted HCV to at least 5 of 222 patients; all infected patients underwent valve replacement.[13] Molecular analysis showed significant homology between the surgeon's and the patients' viruses. The surgeon was treated until his HCV RNA level became undetectable. At that point, he was allowed to resume performing surgery.

Additional isolated cases of HCV transmission during thoracic, cardiac, and orthopedic surgery have been reported.[114] In the UK experience, look-back investigations covering exposure-prone procedures from an HCV-infected source have yielded a transmission rate of 0.13% among susceptible patients. In Germany, an anesthesiologist at an outpatient gynecology clinic transmitted HCV to three patients during minor procedures on the same day; the precise cause of this cluster could not be determined. Additional testing did not reveal transmission events originating from the anesthesiologist.[115]

HCV infection in two Scottish tourists was traced back to an HCW; the two patients had received hemodialysis during a holiday in Spain.[116] Most other transmissions involving nonsurgical personnel have occurred from drug diversion practices through cross-contamination of equipment and supplies by an HCV-infected HCW.[117] In the last decade, three separate outbreaks in the United States, two from different radiology technicians and one from a nurse anesthetist, led to spread of HCV infection in 68 individuals.[24,25,118] Ensuring secure access, tracking mechanisms, and safe administration of narcotics are

essential to eliminate HCV and other infection risks associated with drug diversion.

Transmission in dialysis setting. Despite improvements, transmission of HCV remains a substantial problem for dialysis patients throughout the world. In most series, HCV seroprevalence among dialysis patients is 2- to 10-fold higher than in the general population. The CDC reported regularly on dialysis-associated diseases in the United States through 2002. In their last survey, which included 96% of all US centers, representing more than 260,000 patients and 58,000 staff members, 63% of the institutions routinely tested patients for HCV, an increase from 39% in 1995.[59] HCV seroprevalence is even higher in South American, African, and Asian dialysis centers, exceeding 40% in some areas. Prevalence estimates have ranged from 5% to 10% in Europe and the United States.[59,119] The prevalence of HCV among peritoneal dialysis patients, in contrast, is not elevated.[120]

Because of their compromised immune response, dialysis patients may not mount a significant antibody response to HCV; therefore, concurrent testing with HCV RNA is recommended. Studies done among hemodialysis patients from Germany and France with an overall HCV prevalence of 7% found that 0.6% to 12.3% of HCV-infected patients were viremic without detectable antibody with third-generation assays.[121,122] Cost remains a prohibiting factor for the routine use of HCV RNA in resource-limited settings. Select hepatitis C core antigen assays have demonstrated comparable diagnostic performance to nucleic acid amplification testing (NAAT) at HCV RNA levels >3000 IU/mL and may become an affordable option for broader screening in these areas.[123] The current recommendation by the CDC, endorsed by the National Kidney Foundation, is monthly measurement of alanine aminotransferase (ALT) levels combined with semiannual anti-HCV testing for susceptible dialysis patients as a more cost-effective approach.

Early reports demonstrated that nosocomial spread was caused by overt interruptions in infection control,[124] and one more recent report suggested that HCV was transmitted by contaminated hands of HCWs, demonstrating that the problem persists.[125,126] In most newer reports, however, obvious breaches of infection control in dialysis centers and elsewhere have not been identified, suggesting that either subtle interruptions are responsible for spread or that HCV transmission is incompletely understood.[16] Supporting the latter interpretation, some studies using genotypic analysis have not demonstrated homology among persons treated in the same or adjacent beds; rather, linked cases have been located throughout the dialysis center, suggesting widely dispersed transmission by means of an uncertain mechanism.[127]

Recognized risk factors for acquisition of HCV include blood transfusion and the number of months for which a patient has required dialysis,[128] with the latter being more significant. The risk of blood transfusion has been reduced by the use of erythropoietin. Possible explanations for the association of acquisition of HCV with duration of dialysis include sharing of dialysis machines by HCV-infected and uninfected patients and reprocessing of dialyzers from HCV-infected patients.[129,130] However, the current cleaning standards are extremely high, and the CDC therefore does not recommend against sharing machines or against reusing dialysis filters.[59]

Some researchers have advocated keeping HCV-infected dialysis patients together, similar to the successful approach taken with HBV-infected patients. The CDC, however, does not advocate this approach because of the lack of sensitivity of the anti-HCV test, which means that not all infectious persons would be isolated, and because of the risk of superinfection or reinfection for those already infected.[59] Refer to Chapter 154 for further discussion.

Solid tumor transplantation. Transplantation of HCV-infected organs into HCV-infected or HCV-uninfected hosts is sometimes necessary.[131] However, almost all susceptible recipients of HCV-positive organs eventually develop HCV infection,[132] which may be severe.[132,133] In most series, however, no adverse effect on overall survival of patients or grafts has been found, although recipients of HCV-positive organs had a higher rate of liver-related morbidity and mortality.[132,133] Many organ banks now avoid using organs from HCV-positive donors except for lifesaving procedures such as heart, lung, and liver transplantation.[131] Transmission of HCV to eight organ or tissue recipients from a single, seronegative, and NAAT-positive organ donor has been described.[134] The donor died early in the HCV "window period." Introduction of tissue donor NAAT screening in conjunction with serologic testing has reduced the likelihood of undetected hepatitis C transmission; despite this, human error in interpreting results led to transmission in two kidney and a cardiopulmonary patch recipient from an antibody-negative, NAAT-positive donor.[135] In other transplant-related incidents, potential transmission via blood vessel conduit from a seropositive donor and through common use of perfusion machines has also been reported.[136] The use of active antivirals for organ recipients in these settings has not been studied.

Other nosocomial transmissions. As previously noted, in recent years more reports have described clustering of hepatitis C cases around various health care–related exposures, including transmissions related to endoscopy[137,138]; myocardial perfusion[139]; computed tomography scanning[140]; reuse of saline flushes,[141] needles and syringes,[16,142] and multidose vials[16,22,86,87,143]; and use of a spring-loaded fingerstick device.[144]

In a highly publicized report from Las Vegas, several patients seen at one endoscopy clinic were found to have hepatitis C.[137] The infection was thought to be related to inappropriate reuse of syringes and the use in multiple persons of medication vials intended only for single use. In other reports from endoscopy clinics, the transmission may have occurred as a result of inadequate cleaning of the biopsy suction channel of the colonoscope or failure to autoclave some equipment, such as biopsy forceps.[22,145] Hepatitis C transmission during a uterine dilation procedure was genotypically determined to have originated from a source patient who had undergone an operatation earlier on the same day. The only common link was an anesthesiologist who prepared medications on a cart that was rolled between operating rooms throughout the day without cleaning and disinfection in between.[146]

Transmission of hepatitis C between patients in a dental office has been documented.[147] The mechanism of transmission is unclear but might involve improperly sterilized instruments.

Several reports have suggested an association between development of HCV infection and hospitalization on an oncology or bone marrow transplant floor[18,148,149] or on a urology,[150] liver,[151] or general ward.[152] Although some studies are from relatively resource-poor countries, where reuse of needles may be the cause, the source of spread in other centers remains puzzling. Prolonged hospitalization is often a risk in these reports, and, in one large US outbreak, transmission was thought to be related to shared saline bags contaminated through syringe reuse.[148] Persistence of live HCV for several weeks on dried surfaces has been shown with use of a genetically engineered reporter strain.[153] Whether contact with contaminated surfaces can transmit hepatitis C is plausible but unproven.

Management

The CDC recommends determining the HCV serostatus of the source patient after any exposure.[154] For the exposed HCW, immediate anti-HCV testing within 48 hours is recommended. HCV RNA testing should be done if the initial testing result is positive. For those who are anti-HCV negative, an assessment for HCV RNA should be done at least 3 weeks after exposure to a source patient with HCV or unknown status. HCWs who test positive for HCV RNA at symptom onset or at postexposure testing at ≥3 weeks should be retested at 6 months for spontaneous clearance of infection.[154]

Optimal management of a needlestick exposure is unknown, but each exposure need not be managed with antivirals. For the small subset of HCWs who seroconvert and remain with detectable RNA, newer direct-acting antivirals (DAAs) are highly efficacious at achieving a sustained virologic response. The role of DAAs has not been evaluated in the context of PEP, and they are not currently recommended for this purpose (see Chapter 154). Interferon-α (IFN-α) remains the only drug that has been studied in early infection; in a single report, HCV resolved in all 14 HCWs who were treated with IFN soon after acute infection.[155]

Hepatitis D

Delta virus is a defective RNA virus that requires the presence of active HBV infection (acute HBV or HBsAg carrier state) to infect the liver. In a report from the 1980s,[72] an HBV- and delta-infected source patient

regularly shared a dialysis machine with an asymptomatic HBsAg carrier. The latter patient subsequently developed acute delta hepatitis. A surgeon may also have become dually infected after a deep needlestick sustained while operating on the same source patient. Review identified several additional possible instances of delta hepatitis transmission in dialysis centers. This review led to the current recommendation that patients and staff be vaccinated against HBV and that dialysis patients be separated according to delta virus status. An additional benefit of hepatitis B vaccination is that the incidence of hepatitis D has also substantially declined. Refer to Chapter 146.

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304 Transfusion- and Transplantation-Transmitted Infections

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SHORT VIEW SUMMARY

Definition
- Transfusion- and transplant-transmitted infections occur when a pathogen is spread from the donor to recipients through blood, organs, or other tissue.

Epidemiology
- Transfusion- and transplant-transmitted infections are unusual but occur when screening does not prevent transmission, or when a donation is not screened for a pathogen. The risk of infection transmitted through organ transplantation is likely greater than through blood or tissue owing to the lack of exclusion criteria for organ donors.

Microbiology
- Transfusion of blood product has transmitted infections caused by bacteria, viruses, parasites, and prions.
- Solid-organ transplantation has transmitted infections caused by viruses, bacteria, fungi, mycobacteria, and parasites.
- Encephalitis after solid-organ transplantation has resulted from transmission of West Nile virus, lymphocytic choriomeningitis virus, rabies, and *Balamuthia mandrillaris*.

Diagnosis
- Diagnosis is dependent on clinical recognition that a recipient has an infection that could have been acquired from a transfusion or transplant.

Therapy and Prevention
- Better donor screening, or prompt recognition when transfusion- or transplant-transmitted infection occurs, can improve recipient outcomes.

TRANSFUSION-ASSOCIATED INFECTIONS

Beeson reported the first cases of transfusion-associated hepatitis in 1943, describing seven patients who developed illness 1 to 4 months after having received a red blood cell (RBC) or plasma transfusion.[1] Later identified as transfusion-transmitted hepatitis B virus (HBV), the advent of the acquired immunodeficiency syndrome (AIDS) epidemic in the 1980s followed by recognition of transfusion transmission of hepatitis C virus (HCV) in the 1990s and, most recently, reports of transfusion-transmitted West Nile virus (WNV) and Zika virus (ZIKV) infections in the 21st century have continued to draw attention to the safety of the blood supply, particularly the potential risk from emerging infectious agents.[2–5]

Although infections with the human immunodeficiency virus (HIV), HBV, HCV, WNV, and ZIKV have been the most publicized, a wide spectrum of other organisms, including viruses, bacteria, parasites, and prions, have been transmitted by transfusion (Table 304.1).[6] Of these, transfusion transmission of bacterial infections is most common and earliest recognized in the history of transfusion, now is most often associated with platelet transfusions, and infrequently results in sepsis and death.[7] Recognition of the threat of *Trypanosoma cruzi* transmission via transfusion and the availability of suitable testing have led to screening for this pathogen, which is the cause of Chagas disease. In addition to WNV, mosquito-borne pathogens, such as dengue virus, chikungunya virus, and most recently, ZIKV, have shown potential for transfusion transmission, although the burden of disease is unknown. Tick-borne agents also are recognized to pose an increasing risk to transfusion safety, including transmission of babesiosis and, most recently, anaplasmosis and ehrlichiosis. Transmission of variant Creutzfeldt-Jakob disease (vCJD) via transfusion has occurred in the United Kingdom.

An ongoing dilemma of the blood-banking community is a mandate to ensure a maximally safe blood supply while giving consideration to the cost of such measures. The current questions for screening donors that have been adopted by most blood banks are presented in Fig. 304.1, and the laboratory screening measures currently in place are listed in Table 304.2. As of 2014, pathogens or diseases screened solely through donor interview include malaria and vCJD. Real or perceived threats to the blood supply occur regularly, as emerging or reemerging infectious diseases raise concern over potential transmission. A previously published review summarizes potential blood supply risks from pathogens, the majority of which are not screened for with laboratory testing.[8]

Pathogen reduction technology (PRT) is now available to reduce the need to develop screening measures for new infectious threats. In December 2014, the US Food and Drug Administration (FDA) approved a PRT method for plasma and apheresis platelets that uses chemicals activated by ultraviolet light to inactivate nucleic acid material comprising DNA and RNA.[9,10] However, there is no approved method for treatment of RBCs. Although this technology is likely to reduce the risk of transmitted infections of viruses, bacteria, and parasites, it will not likely eliminate it, because there are organisms occurring in high titers or that have structures (e.g., non–lipid-enveloped viruses, bacterial spores, prions) that resist or are not affected by the inactivation mechanism.[9,10]

SCOPE OF BLOOD TRANSFUSION

According to estimates from the World Health Organization (WHO), more than 112 million units of blood were collected in 2013.[11] Less than half of donated blood is collected in developing and transitional countries, which are home to about 80% of the world's population. Of the 156 countries providing data to WHO for 2013, 13 were not able to screen all of their donated blood for one or more of the four infections (HIV, HBV, HCV, and syphilis) that are most widely recognized to be transmitted through blood and are recommended by WHO to be screened at donation.[11] A total of 126 countries have national guidelines on the appropriate clinical use of blood, and 70 countries have a national hemovigilance system to monitor adverse events associated with transfusion.[11]

Surveys to determine blood product use in the United States, led by the National Heart and Lung Institute (now called the National Heart, Lung, and Blood Institute), began in 1971.[12] In the United States, surveys have reported the frequency of blood collection and utilization

[a]The findings and conclusions in this chapter are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

TABLE 304.1 List of Notable Infections Transmitted Through Blood Transfusion

Viruses

Colorado tick fever virus
Cytomegalovirus
Dengue virus
Epstein-Barr virus
Hepatitis A virus
Hepatitis B virus
Hepatitis C virus
Hepatitis E virus
Human herpesvirus 8
Human immunodeficiency virus 1 and 2
Human T-lymphotropic virus 1 and 2
Parvovirus B19
Tick-borne encephalitis virus
West Nile virus
Zika virus

Bacteria

Anaplasma phagocytophilum
Brucella spp.
Coxiella burnetii
Ehrlichia
Gram-positive organisms[a]
Gram-negative organisms[b]
Rickettsia rickettsii
Treponema pallidum[c]

Parasites

Babesia spp.
Leishmania spp.
Trypanosoma cruzi
Plasmodium spp.

Prions

Variant Creutzfeldt-Jakob disease

[a]Higher risk in platelets (see Table 304.3); in addition, *Streptococcus gallolyticus (bovis)* is notable for being associated with colon cancer in the donor.
[b]Noted in red blood cell units associated with cryophilic organisms, including *Pseudomonas fluorescens, Serratia liquefaciens,* and *Yersinia enterocolitica.*
[c]Not thought a current risk; last reported transfusion transmission of syphilis in the United States was in 1966.
Modified from Perkins HA, Busch MP. Transfusion-associated infections: 50 years of relentless challenges and remarkable progress. Transfusion. 2010;50:2080–2099; and Stramer SL, Hollinger FB, Katz LM, et al. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion. *2009;49:1S–29S.*

TABLE 304.2 Laboratory Screening Performed for Pathogens or Diseases by US Blood Collection Centers

Required or Recommended by US Food and Drug Administration

Hepatitis B surface antigen (HBsAg)
Hepatitis B core antibody (anti-HBcAb)
Hepatitis C virus antibody (anti-HCV)
HIV-1 and HIV-2 antibody (anti-HIV-1 and anti-HIV-2)
HIV p24 antigen
HTLV-1 and HTLV-2 antibody (anti-HTLV-1 and anti-HTLV-2)
Serologic test for syphilis
NAAT for HIV-1, HBV, and HCV
NAAT for West Nile virus
Serologic test for *Trypanosoma cruzi* (one-time donor testing)

Additionally Required by Accrediting Organizations[a]

Platelet screening for bacterial contamination[b]

Performed Voluntarily

Serologic test for cytomegalovirus (on request)

Performed Under Investigational Protocols

PCR and serologic test for *Babesia microti*
NAAT for Zika virus

[a]Including AABB (formerly known as the American Association of Blood Banks) and the College of American Pathologists.
[b]In general, liquid culture media for apheresis units and pH/glucose indicators or bacterial antigen point-of-use tests for whole-blood–derived pooled units.
HBV, Hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *HTLV,* human T-cell lymphotropic virus; *NAAT,* nucleic acid amplification testing; *PCR,* polymerase chain reaction.

since the late 1980s, most recently through the National Blood Collection and Utilization Survey (NBCUS), which has been conducted biannually since 1998.[12–14] In the 2017 NBCUS report, reflecting data collected in 2015, there were nearly 11 million whole-blood and 3.7 million apheresis collections, with approximately 19 million blood components transfused.[14] In 2015, 11.33 million whole-blood and RBC units, 2.7 million units of plasma, 1.8 million units of apheresis platelets, 0.17 million units of whole-blood–derived platelets (measured in apheresis equivalent units), and 1.2 million units of cryoprecipitate were given.[14] The number of RBC transfusions has continued to decline since 2008, perhaps reflecting the growing adoption of more aggressive hospital blood management practices. The number of transfused platelets has remained approximately the same, but transfusion of apheresis platelets has continued to increase, while the amount of whole-blood–derived or pooled platelets has continued to decline. In addition to contributions from the voluntary donor pool, plasma units also are collected annually from paid donors and are used to prepare immune globulin, albumin, and various other plasma-derived products. By 1987, the cost of collecting, processing, and transfusing patients exceeded $3 billion; since then, costs have increased steadily with the addition of new screening tests and the implementation of leukoreduction.[12] In 2015, the mean price paid by a hospital was $217 for a unit of leukocyte-reduced RBCs, $60 for fresh-frozen plasma, and $537 for apheresis platelets, so current costs are likely to exceed $4 billion annually.[14,15]

It has been estimated that the annual likelihood of an individual's receiving a transfusion increases dramatically with age; in 2015, the transfusion rate in the US population was 35.3 units per 1000 people.[14] Because of concern about contracting an infectious disease, there has been historic interest in autologous and donor-directed blood donation. However, donor-directed units, usually given by family members for a specific patient, have been shown to have higher rates of various infectious agents. There has been movement away from donor-directed donation and toward building a dedicated, voluntary repeat donor population.[14,15] Viral infections are much less common among repeat donors compared with first-time whole-blood donors, and they may be even less common among donors associated with apheresis collection.[16,17]

A majority of, but not all, blood components transfused in the United States are leukoreduced, a modification that is performed to filter out the majority of white blood cells. Leukoreduction is performed to reduce nonhemolytic transfusion reactions, but it may also reduce the risk for infectious disease transmission through removal of infected white blood cells, particularly for cell-associated agents.[18]

In 2015, only about 0.42% of the donated allogeneic blood supply was discarded on testing, usually because of detection of a potentially transmissible infection.[14] This reflects a continued steady decline in units discarded owing to testing, perhaps indicating improved accuracy of screening tests and retention of repeat donors. The incidence of viral markers in donated units was increased among volunteers who donated after September 11, 2001, primarily owing to a large cohort of first-time and infrequent repeat donors.[19]

BLOODBORNE PATHOGENS

Transfusion-associated transmission risk has persisted for HIV, HBV, and HCV for two distinct reasons: (1) the incomplete sensitivity of the available screening tests, and (2) the "window" period, which is defined as the period between acute infection (and potential infectivity) and the point at which serologic tests can reliably detect infection.

The development and implementation of routine nucleic acid amplification testing (NAAT) has transformed blood bank screening for viral pathogens.[3,20–22] NAAT can detect viral RNA after the first 10 to 14 days of infection, narrowing the window period by 7 to 10 days for HIV and by 50 to 60 days for HCV compared with serology. NAAT for blood donor screening, in addition to HIV, HBV, and HCV, is now

additionally required for WNV and ZIKV, and is voluntarily performed by plasma manufacturers in pools for parvovirus B19.[23–26]

With introduction of NAAT, the risk of acquiring HIV or HCV per unit of blood transfused has plummeted from approximately 1 per 500,000 for HIV and 1 per 100,000 for HCV before NAAT to a current rate of 1 per 2 million for both viruses. Detected cases are so unusual that the incidence can only be estimated statistically.[27] As remarkable as this advance is, a small window period remains, meaning that the risk for receiving one of these viruses in a unit of blood is not zero. Four cases of HIV transmission and many more cases of HCV transmission through transfusion have been recognized and reported to public health authorities since NAAT was implemented; the modeling data estimate more cases than the number reported, so some cases likely go unrecognized.[28]

The advent of NAAT also has had a substantial impact on the choice of serologic tests. In addition to sensitivity, blood bankers seek optimal specificity: False-positive or indeterminate test results exclude otherwise appropriate donors from subsequent donations and create anxiety due to incorrect diagnosis of infection. The introduction of an extremely sensitive test such as NAAT allows blood banks to focus on the specificity of other screening tests, thereby limiting the number of persons potentially excluded from the donor pool because of misleading test results. Although sensitivity varies little between the second- and third-generation antibody tests, the third-generation assay has superior

Full-Length Donor History Questionnaire

	Yes	No	
Are you			
1. Feeling healthy and well today?	❑	❑	
2. Currently taking an antibiotic?	❑	❑	
3. Currently taking any other medication for an infection?	❑	❑	
Please read the Medication Deferral List.			
4. Are you now taking or have you ever taken any medications on the Medication Deferral List?	❑	❑	
5. Have you read the educational materials?	❑	❑	
In the past **48 hours**			
6. Have you taken aspirin or anything that has aspirin in it?	❑	❑	
In the past **6 weeks**			
7. Female donors: Have you been pregnant or are you pregnant now? (Males: check "I am male.")	❑	❑	❑ I am male
In the past **8 weeks have you**			
8. Donated blood, platelets or plasma?	❑	❑	
9. Had any vaccinations or other shots?	❑	❑	
10. Had contact with someone who had a smallpox vaccination?	❑	❑	
In the past **16 weeks**			
11. Have you donated a double unit of red cells using an apheresis machine?	❑	❑	
In the past **12 months have you**			
12. Had a blood transfusion?	❑	❑	
13. Had a transplant such as organ, tissue, or bone marrow?	❑	❑	
14. Had a graft such as bone or skin?	❑	❑	
15. Come into contact with someone else's blood?	❑	❑	
16. Had an accidental needle-stick?	❑	❑	
17. Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?	❑	❑	
18. Had sexual contact with a prostitute or anyone else who takes money or drugs or other payment for sex?	❑	❑	
19. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything not prescribed by their doctor?	❑	❑	
20. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates?	❑	❑	
21. Female donors: Had sexual contact with a male who has ever had sexual contact with another male? (Males: check "I am male.")	❑	❑	❑ I am male
22. Had sexual contact with a person who has hepatitis?	❑	❑	
23. Lived with a person who has hepatitis?	❑	❑	
24. Had a tattoo?	❑	❑	
25. Had ear or body piercing?	❑	❑	

v.1.3 eff May 2008

FIG. 304.1 AABB (formerly American Association of Blood Banks) Blood Donor History Questionnaire, Version 1.3, May 2008. *(From AABB. Full-Length Donor History Questionnaire.* http://www.aabb.org/tm/questionnaires/Documents/dhq/v2/DHQ%20v2.0.pdf. *Accessed May 29, 2019.)*

Full-Length Donor History Questionnaire—cont'd

	Yes	No	
26. Had or been treated for syphilis or gonorrhea?	❑	❑	
27. Been in juvenile detention, lockup, jail, or prison for more than 72 hours?	❑	❑	
In the past **three years** have you			
28. Been outside the United States or Canada?	❑	❑	
From **1980 through 1996,**			
29. Did you spend time that adds up to three (3) months or more in the United Kingdom? (Review list of countries in the UK.)	❑	❑	
30. Were you a member of the U.S. military, a civilian military employee, or a dependent of a member of the U.S. military?	❑	❑	
From **1980 to the present,** did you			
31. Spend time that adds up to five (5) years or more in Europe? (Review list of countries in Europe.)	❑	❑	
32. Receive a blood transfusion in the United Kingdom or France? (Review list of countries in the UK.)	❑	❑	
From **1977 to the present,** have you			
33. Received money, drugs, or other payment for sex?	❑	❑	
34. Male donors: had sexual contact with another male, even once? (Females: check "I am female.")	❑	❑	❑ I am female
Have you **EVER**			
35. Had a positive test for the HIV/AIDS virus?	❑	❑	
36. Used needles to take drugs, steroids, or anything not prescribed by your doctor?	❑	❑	
37. Used clotting factor concentrates?	❑	❑	
38. Had hepatitis?	❑	❑	
39. Had malaria?	❑	❑	
40. Had Chagas disease?	❑	❑	
41. Had babesiosis?	❑	❑	
42. Received a dura mater (or brain covering) graft?	❑	❑	
43. Had any type of cancer, including leukemia?	❑	❑	
44. Had any problems with your heart or lungs?	❑	❑	
45. Had a bleeding condition or a blood disease?	❑	❑	
46. Had sexual contact with anyone who was born in or lived in Africa?	❑	❑	
47. Been in Africa?	❑	❑	
48. Have any of your relatives had Creutzfeldt-Jakob disease?	❑	❑	

v.1.3 eff May 2008

FIG. 304.1, cont'd

specificity, decreasing the proportion of persons with indeterminate antibody tests for HCV.[29] HBV NAAT has been adopted for blood donor screening.

Human Immunodeficiency Virus Type 1

More than 8000 persons in the United States have developed AIDS from receipt of blood or tissue.[30] In addition, at least 50% of all hemophiliacs in the United States and Europe became infected with HIV from 1978 to 1985, most from receipt of infected plasma factors, with the highest incidence in 1982, when there were 22 infections per 100 person-years.[31] The first HIV screening test was introduced in 1985, and since 1987 few new infections among hemophiliacs have been reported.[31] As noted, the introduction of NAAT screening has substantially lowered the risk for HIV transmission, with the last reported case occurring in 2008.[28]

Redundant methods of testing also have been useful in confirming the status of infection for blood donor follow-up. NAAT has been useful in clarifying the HIV serostatus of persons with indeterminate results of Western blot tests.[32] These reactions occur in about 1 of every 5000 donations and usually represent false-positive test results.[33]

Human Immunodeficiency Virus Type 2

In June 1992, the FDA mandated screening for HIV-2.[34] Since then, few HIV-2–positive donors have been identified, and no cases of transmission have occurred in the United States, although transfusion-related HIV-2 cases have occurred elsewhere.[35,36]

Human T-Cell Lymphotropic Virus Types 1 and 2

HTLV-1 and HTLV-2, unlike HIV-1 and HIV-2, are cell associated and therefore predominantly transmissible only with blood component transfusions.[37] Screening for both types is done with a single test. The rate of transmission of HTLV-1 decreased almost 10-fold (from 1 per 8,500 to 1 per 69,000) after introduction of the screening test.[38] Transplantation of solid organs from an HTLV-1–infected donor resulted in rapid progression to subacute myelopathy in three recipients; this phenomenon has not been described in transfusion recipients.[39]

Other Retroviruses

A short-lived perceived threat to the blood supply was raised when xenotropic murine leukemia virus–related virus (XMRV) was linked to prostate cancer and then chronic fatigue syndrome. Furthermore,

evidence of viral infection, either by serology or NAAT, was found in controls, including in over 5% of healthy blood donors. XMRV later was proved to be a laboratory contaminant and is not thought to be associated with pathogenic human infection.[40]

Hepatitis B and D Viruses

HBV remains the virus with the highest residual risk of transfusion transmission despite screening; HBV is transmitted in approximately 1 in every 200,000 to 300,000 transfusions.[41,42] Risk persists both because the screening test is incompletely sensitive and because of the prolonged window period of about 2 months. In countries that screen for hepatitis B core antibody (HBcAb), most transmissions derive from the window period, whereas in those that do not check HBcAb status, half derive from the window period and the others are due to the insensitivity of the hepatitis B surface antigen (HBsAg) test as a screening test.[43]

NAAT for HBV recently has been implemented for blood screening using minipool testing. With current sensitivity of minipool testing, NAAT shortens the window period by about 7 days, leaving a residual risk for HBV of about 1 per 250,000 to 350,000 transfusions.[24,42] Sensitive screening tests are particularly needed in HBV-endemic areas, such as Taiwan, where HBcAb testing is not routinely performed and transfusion-acquired HBV is a relatively frequent event.[44] Testing for HBcAb, in addition to HBsAg, remains important; in some cases, it is more sensitive than NAAT, so implementation of new testing does not obviate the use of these other tests for donor HBV infection.[45] Increasing use of hepatitis B vaccination may further reduce the risk for transmission.

Screening of donors for HBsAg excludes most, but not all, carriers of hepatitis D, also known as delta virus.[46] Identification of donors with antibody to the delta agent is not done routinely, so there is a residual risk for transmission of delta virus to HBsAg-positive recipients.

Hepatitis C Virus

Hepatitis C, previously known as non-A, non-B hepatitis, was associated with a lifetime risk of 7% to 10% in transfusion recipients in the late 1970s and early 1980s.[47] The transmission rate of HCV has decreased with improved screening tests to identify the virus, providing an excellent example of how implementation of each new generation of screening technology has improved blood safety. More than 90% of recipients of HCV-contaminated blood products develop HCV infection.[48] An older survey using the second-generation test found that 3.6 per 1000 US donors were positive for HCV,[49] a prevalence lower than that in the US population. HCV infection in blood donors usually is the result of intravenous drug use, although other health care–associated risks have been identified, including questionable injection practices.

Before any testing, 0.45% of all transfusions transmitted HCV. With the introduction in 1986 of surrogate marker testing (alanine aminotransferase and HBcAb), the rate decreased to 0.19%, and with the introduction of the first-generation antibody test for HCV in 1990, the rate fell to 0.03%. The second-generation test lowered the rate even further, mostly by identifying chronic infections more accurately (i.e., increasing sensitivity) rather than by shortening the window period. Despite the continuing relatively high prevalence of HCV infection in the US population, the introduction of NAAT has reduced the transmission risk to about 1 in 1.5 million transfusions.[20,21,27]

Hepatitis A Virus

Transmission of hepatitis A virus (HAV) via transfusion has been described with RBCs, particularly in infants,[50,51] and with factor VIII concentrate.[52] Donors with early, asymptomatic infection may transmit HAV infection.[53] Factor VIII–associated transmission has occurred despite appropriate use of organic solvent and detergent to inactivate the virus. The lack of a lipid envelope around HAV may have contributed to incomplete virus killing during preparation of the concentrate. Because of this small but persistent risk, vaccination for HAV has been recommended for chronic recipients of products made from pooled plasma.[54] Review of all cases of hepatitis from 1998 to 2002 in the United States among persons with bleeding disorders revealed no acquisition of HAV via factor concentrates, although as the incidence of HAV infection increases in the US population, some plasma manufacturers voluntarily screen donation pools using NAAT.[55,55a]

Hepatitis E Virus

Hepatitis E virus (HEV) is endemic in many developing countries and is believed to have similar epidemiology to HAV with transmission via the fecal-oral route. HEV has been implicated in transfusion transmission worldwide but has not yet been reported in the United States.[56–58] However, some studies have documented high seroprevalence rates nationally among blood donors.[59,60] One US study included NAAT testing of blood donations and noted a frequency of 1 in 9500 donations with evidence of HEV RNA.[60] These findings have raised the concern about an underappreciated blood safety risk. Furthermore, because it is nonenveloped, HEV is not effectively inactivated by current approved PRT methods, with documented transfusion-transmission among psoralen-treated blood products.[61]

West Nile Virus and Other Arboviruses

WNV has been transmitted by blood transfusion to at least 32 persons since this route of transmission was first recognized during the summer of 2002.[4] In addition, it has been transmitted by organ transplantation.[62,63] In response to this emerging threat, NAAT for WNV was quickly developed and implemented by 2003 and is now in routine use for blood donor screening; thousands of WNV-infected donations have been interdicted.[22,23] Because of the logistic and financial burden of individual testing, WNV screening assays test pools of 6 or 16 donations (i.e., minipools), with follow-up screening of positive minipools by individual sample NAAT. However, "breakthrough" cases of WNV transfusion transmission still occur, because minipool testing is not as sensitive as individual sample NAAT. In response, blood banks are using strategies to "trigger" a switch from minipool to individual NAAT in areas with high epidemic activity during seasonal peaks.[64] Twelve transfusion transmissions have occurred despite use of minipool NAAT.[65] Five cases occurred after blood collection agencies voluntarily put in place processes to trigger more sensitive individual donation testing based on local WNV activity. There have been no recognized cases of transfusion transmission when individual donation testing has been used, although one transmission involved a positive minipool with a negative confirmatory individual test.[66] WNV has also been transmitted through granulocyte transfusion.[67] Clinicians should suspect WNV in individuals who develop unexplained encephalitis or flaccid paralysis and who have recently undergone transfusion or transplant procedures. Immunosuppressed patients, including solid-organ and stem cell recipients, are at greatest risk for developing complications.

ZIKV, a flavivirus transmitted primarily by *Aedes aegypti* mosquitoes, rapidly spread in the Americas in 2015 and 2016.[68] Although most infections are asymptomatic, ZIKV has been identified as a cause of adverse pregnancy outcomes, including microcephaly and other congenital brain defects,[69] and has been linked to Guillain-Barré syndrome[70] and severe thrombocytopenia.[71–73] Retrospective nucleic acid testing (NAAT) of blood donations after a large ZIKV outbreak in French Polynesia in 2013 and 2014 found detectable ZIKV RNA in 2.8% of blood donations,[74] and ZIKV infections that were likely transmitted by transfusion (via whole blood–derived platelets) have been documented in Brazil.[5,75]

To reduce the risk for transfusion-transmitted ZIKV infection in the United States, in February 2016 the FDA recommended that all US areas with active ZIKV transmission cease blood collections unless donations are screened with NAAT or treated with approved PRT.[76] Blood safety interventions in Puerto Rico, which experienced a significant ZIKV epidemic in 2016, included importation of blood units from unaffected US areas and treatment of plasma and apheresis platelets with PRT until early April 2016, when the FDA authorized the use of an individual donation NAAT test under an investigational new drug (IND) protocol.[76] Currently, the US blood supply is subjected to universal ZIKV individual donation NAAT under investigational protocols, and no transfusion-transmitted cases have been identified in the US states or Puerto Rico.[77,78]

Other arboviruses, including chikungunya virus, have been associated with widespread epidemics and theoretically can be transmitted through transfusion, although no cases have been recognized and documented to date. Transfusion-transmitted dengue has been reported, but published cases are far less frequent than would be expected, given the worldwide

burden of disease, perhaps because symptoms are mild in recipients who are immune or who receive donor antibodies with infected units.[79,80]

Herpesviruses

Human herpesvirus 8 (HHV-8), the cause of Kaposi sarcoma, is believed to be primarily white cell associated, suggesting a low risk for transmission via leukoreduced blood transfusion, and few cases of transmission have been documented. The low rate of recognized transmission may reflect, in part, the difficulty in test performance and the high positive background rate, but studies have suggested a link.[81,82] One epidemiologic study completed in Uganda estimated the rate of transmission to be 2.8% for nonleukoreduced RBC units, with excess risk associated with storage times shorter than 4 days.[83] Further studies are needed to determine whether leukoreduction can completely mitigate this risk, but it is more likely partially effective, because viral loads in free plasma have been found in infected individuals with Kaposi sarcoma.[84] Transmission of HHV-8 resulting in Kaposi sarcoma has been described more frequently in recipients of solid-organ transplants.[85]

The transmission risk for cytomegalovirus (CMV) has been well summarized.[86] CMV, similar to HHV-8, is highly cell associated and can be transmitted with white blood cells that persist in RBC or platelet transfusions. Transmission of CMV from fresh-frozen plasma or cryoprecipitate has not been reported. The insensitivity of the serologic test has resulted in a residual risk for transmission of 0% to 6%, even when CMV-seronegative donors are used.[86] Because the demand for CMV-seronegative blood outstrips the supply, approaches such as leukocyte filtration of CMV-seropositive blood are often used, although the two approaches may not be equivalent in reducing CMV transmission risk.[18]

Screening for Epstein-Barr virus and other herpesviruses is not performed routinely, despite sporadic reports of transfusion-associated transmission.

Parvovirus B19

Parvovirus B19 has been transmitted in coagulation factor concentrates, resulting in NAAT screening by some plasma manufacturers. The risk for transmission to cryoprecipitate recipients may persist despite treating with solvent and detergent and heating to 100°C after lyophilization.[87] An epidemiologic study showed a significant correlation between receipt of treatment products, B19 seropositivity, and joint range-of-motion limitations.[88]

Non–A-E Hepatitis

The search for the cause of hepatitis not caused by hepatitis A, B, C, D, or E virus has yielded many contenders but no indisputable etiology to date. Hepatitis G virus (HGV) has been found in 1% to 7% of donors and can be transmitted by transfusion.[89] The risk for transmission is in the range of 5.3 per 10,000 units.[90] However, HGV has been shown not to be a cause of non–A-E hepatitis, and the clinical implications of HGV infection remain undetermined.[89]

Two somewhat related, single-stranded, unencapsulated DNA viruses, called transfusion-transmitted virus (TTV) and SEN virus (designated by the initials of the first patient investigated) have been considered as causes of non–A-E hepatitis.[91,92] Both viruses are present worldwide and are often found in the blood of persons with post-transfusion non–A-E hepatitis. However, after initial interest, no causal association was established.

BACTERIAL PATHOGENS

Infusion of blood products contaminated by bacteria is a relatively frequent and potentially lethal risk of blood transfusion.[6,93–94a] As the risk for transfusion-transmitted viral infection has decreased dramatically, the frequency of transfusion-transmitted bacterial infection has remained unchanged. A possible exception to this persistent risk is transfusion transmission of *Treponema pallidum,* the etiologic agent of syphilis, which has not been reported to have occurred for decades, presumably because of poor spirochete survival under current storage conditions.[95–97]

Bacterial contamination may arise from donation, processing, storage, or transfusion and can result in transfusion-transmitted sepsis and death. Because platelets are stored at room temperature, they are at higher risk for bacterial growth than RBCs, which are stored at refrigerated temperatures. Bacterial contamination of blood components was the cause of approximately 10% of transfusion-related fatalities reported to the FDA between 2011 and 2015.[98] Although visual inspection can sometimes reveal a significantly contaminated unit, this is not always the case (Fig. 304.2).



FIG. 304.2 Pooled platelet unit containing *Klebsiella pneumoniae* (quantitative culture, 6.5 colony-forming units per milliliter), showing fibrinous coagulation 4 days after laboratory inoculation. *(From Hay S, Brecher M. Egg drop soup platelets: gross bacterial contamination of a platelet product. Transfusion. 2007;47:1335–1336.)*

Based on culturing, estimates have placed the rate of bacterial contamination at 1 per 2000 to 3000 units for platelets and at 1 per 30,000 units for RBCs.[94,99–101] Estimates of the rate of clinically evident reactions after transfusion of bacterially contaminated blood products vary more widely, from 1 in 5000 to 1 in 100,000, although it is generally agreed that pooled platelets confer a greater risk per therapeutic dose than do platelets collected by apheresis.[102] A national study, Assessment of the Frequency of Blood Component Bacterial Contamination Associated With Transfusion (BaCon), was designed to identify severe reactions that resulted in confirmed transfusion-transmitted sepsis through passive reporting. Over a 3-year period, there were 56 reports and 34 confirmed cases, principally from contaminated platelets. Although *Staphylococcus* and other gram-positive organisms were most frequently associated with transfusion-transmitted sepsis, gram-negative organisms, such as *Escherichia coli* and *Serratia* species, were statistically associated with fatal outcome. Nine persons (26.5%) died of the transmitted infection. The BaCon study estimates of transfusion-transmitted sepsis were 1 in 100,000 units for platelets and 1 in 5 million units for RBCs, reflecting the difficulty of recognition and confirmation of these poorly appreciated clinical events.[7,103]

For both RBCs and platelets, longer storage time is well established as a risk factor for bacterial contamination. However, gram-negative organisms can be fast growing and can result in lethal endotoxin levels in 3-day-old platelets. Further shortening of the current 5-day platelet storage times is not feasible, because it would probably result in discarding too many uninfected units, exacerbating the nation's intermittent blood supply shortage of platelets. In addition, contamination may occur during production and packaging of blood bags.[104] The AABB (formerly known as the American Association of Blood Banks) requires members to use a method to detect bacterial contamination in platelets;

in apheresis platelets, liquid culture media methods are typically used by the blood collection centers, whereas for pooled platelets the methods are varied. This has resulted in a risk that is significantly reduced but still present. The American Red Cross has described a decrease in risk for septic reactions due to apheresis platelets from 1 in 33,000 to 1 in 75,000.[105] Other approaches to risk reduction are being actively pursued in the United States and have already been implemented in other countries. These include avoidance through improved skin preparation and diversion of the initial 15 to 30 mL of the blood draw; optimization of volume and storage time before culture; development of point-of-use tests at the time of transfusion; and pathogen reduction methods, including inactivation by means of photochemical treatment of units.[6] Routine culture of apheresis platelets has led to the discovery of unusual pathogens in asymptomatic donors, including *Listeria* species.[106]

In December 2014, the FDA published a draft guidance on bacterial detection testing. This document provides recommendations for testing for bacterial contamination of platelets by blood collection centers and transfusion services, but it is not yet a regulatory requirement. Although the AABB standard results in most apheresis platelets being tested, most pooled platelets remain untested, with presumably higher risk compared with units collected by means of apheresis.

Red Blood Cells

Gram-negative bacteria, including *Yersinia enterocolitica, Pseudomonas fluorescens, Serratia marcescens,* and *Serratia liquefaciens,* accounted for most of the reported cases of transfusion-transmitted infection caused by contaminated RBCs historically (Table 304.3).[99,107] Transmission of bacterial infection through RBCs is less common than through platelets, because RBCs are stored at refrigerated temperatures, allowing a longer shelf life. Storage of RBCs may extend 35 to 42 days depending on the type of additive used. Almost all instances of infection are associated with erythrocytes stored longer than 9 days and appear to derive from enhanced growth of certain organisms at cold storage (4°C) temperatures.[108,109] This storage growth is best exemplified by multiple cases of transfusion-associated *Y. enterocolitica* in autologous blood donors, who became bacteremic after receiving their own stored blood.[110–113]

Donors with *Y. enterocolitica* infection are typically symptom free at the time of blood donation, although many recall having had a diarrheal illness about 1 month before donation. Although diarrhea may indicate exposure to contaminated food, a donor question regarding diarrheal illness has not been adopted into the standard questionnaire because of lack of specificity of the complaint. If it were used as a screening question, up to 10% of donors would be excluded.[99] Despite its suggestion of an association with *Y. enterocolitica*–contaminated products, the BaCon study detected only one case of transfusion-transmitted sepsis caused by *Y. enterocolitica* and heralded replacement of this organism in RBC contamination with other gram-negative organisms, such as *E. coli* and unusual *Pseudomonas* and *Serratia* species.[107] Patient bacteremia with *P. fluorescens* or *S. liquefaciens* should prompt a thorough search for a potential health care–associated source, such as contaminated transfusion or infusion products. Unlike platelets, there are no current laboratory-based screening measures in place to reduce the risk for bacterial contamination in RBCs, although development of a rapid endotoxin test is underway.

Platelets

A different spectrum of bacteria is associated with transfusion of platelets.[114,115] The most commonly recovered organisms are staphylococcal and streptococcal species. Gram-negative bacteria, including the Enterobacteriaceae (*E. coli, S. marcescens, Enterobacter* spp.) are less common but may be associated with rapid growth, production of endotoxin, and death (see Table 304.3).[7]

As with RBCs, contaminated platelets tend to be older (e.g., 4 or 5 days of storage).[116] Because of this, the FDA in 1985 shortened the acceptable storage time for platelets from 7 days to the current 5 days. Recently, the FDA has recommended strategies to extend platelet storage time to 7 days. These strategies can include primary and secondary culture (no earlier than day 4), primary culture and secondary rapid testing, or large volume delayed sampling.[117]

TABLE 304.3 Organisms Implicated in Fatal Blood Transfusion–Transmitted Bacteremia, Including Association With Endotoxin (Results From BaCon Study)

Platelets
Group B streptococci
Escherichia coli
Providencia rettgeri[a]
Serratia marcescens[a]
Red Blood Cell Units
Staphylococcus epidermidis
Serratia liquefaciens[a]

[a]Associated with 9090 to 273,500 endotoxin-forming units per milliliter.
From Kuehnert MJ, Roth VR, Haley NR, et al. Transfusion-transmitted bacterial infection in the United States, 1998 through 2000. Transfusion. *2001;41:1493–1499.*

OTHER AGENTS TRANSMITTED THROUGH BLOOD COMPONENTS

Tick-borne Bacteria

Anaplasmosis and other tick-borne diseases, including human ehrlichiosis and Rocky Mountain spotted fever, represent a potential risk for transmission via blood transfusion in the United States.[118] Screening for a recent history of tick bite is unlikely to identify high-risk donors, because this exposure is unlikely to be recalled and may result in needless loss of uninfected blood donors.[118] As the incidence of tick-borne diseases increases, physician vigilance for possible transmission of these agents via transfusion also should increase. *Anaplasma phagocytophilum,* the causative agent of human granulocytic anaplasmosis, has been reported to have been transmitted by transfusion of RBCs.[119] Rickettsiae also may rarely be transmitted by blood transfusion.[118] Because *Rickettsia, Ehrlichia,* and *Anaplasma* infect white blood cells, leukoreduction techniques would be expected to reduce the risk for transmission through cellular components, although transfusion-transmitted *Ehrlichia ewingii* through a leukoreduced platelet unit has been reported.[120] Lyme disease has long been postulated to be transmitted by transfusion, but no single case of transmission through blood components has yet been recognized. Possible infection with tick-borne agents should be suspected if transfusion recipients develop unexplained thrombocytopenia or hemolytic anemia after transfusion, especially if accompanied by fever. Simultaneous infection with multiple tick-borne agents (e.g., *Babesia* and *Anaplasma* spp.) also should be considered.[119]

Parasites

T. cruzi, which causes Chagas disease, is endemic in certain areas of Mexico, South America, and Central America. Rare cases of *T. cruzi* transmitted by blood transfusion have been reported by US investigators.[121] More than 300,000 persons with *T. cruzi* infection reside in the United States. In Los Angeles and Miami, where immigration from endemic areas is high, a study found that 7.3% and 14.3% of all blood donors, respectively, had a risk for *T. cruzi* by history. Of these, 1 in 7500 to 9000 had detectable antibody. In a lookback study, no seropositivity or disease was found in 18 recipients of the units from seropositive donors, but the data were limited.[122] Although prevalence of the disease among donors is low overall, the availability of a blood antibody test resulted in voluntary adoption of screening by multiple blood centers in 2007, followed by an FDA recommendation for one-time screening for all donors.[123] An estimated 1 in 30,000 donors had evidence of *T. cruzi* infection at initial screening and confirmatory testing; the most frequent positive results were reported from California and Florida, although there was a nationwide distribution, reflecting immigration broadly from endemic countries.[123] Lookback studies in progress indicate that the transmission rate may be very low, and treatment of the hundreds of infected donors recognized at screening is a difficult issue.[124,125] The number of donors with evidence of infection who have no clear risk factors has also raised the possibility that there may be more autochthonous cases of Chagas disease than currently appreciated, with an estimate of 1 in 354,000 donors.[126]

Babesiosis is the most common cause of RBC transfusion–associated infections reported in the United States and accounted for 3.7% of transfusion-associated deaths between 2005 and 2010.[127,128] Most investigated cases have been found to be due to *Babesia microti,* found mostly in the Northeast and upper Midwest states. However, other species, such as *Babesia duncani* (previously known as WA-1), have also been implicated. In one series in which data from 84 patients with transfusion-associated *B. microti* infection between 1979 and 2009 were available, the interval from transfusion to clinical manifestations ranged from 11 to 176 days, with a median onset of 37 days.[129]

Because of donor travel and because blood products are transported outside endemic areas, transfusion-transmitted babesiosis can occur anywhere in the United States.[130] Patients who are asplenic or otherwise immunocompromised are at particular risk for increased disease severity. Patients with more than 4% parasitemia are at increased risk for clinically manifest infection, including severe, potentially life-threatening *Babesia* infections. Since 2011, laboratory screening of blood donations is conducted by blood collection centers in some endemic areas of the United States under investigational protocols.[131,132] Elsewhere in the United States, blood donor screening for babesiosis is required by questionnaire only, a method shown to be ineffective.[133] Boundaries such as geography and seasonality would not exclude asymptomatic donors, who can remain parasitemic for more than a year.[134] Because the number of recognized cases of transfusion transmission of babesiosis appears to be increasing, the need for more widespread screening through laboratory testing of donors is more urgent.

In May 2015, the FDA's Blood Products Advisory Committee recommended that available scientific data support the concept of nationwide, year-round testing of blood donors for *Babesia* risk with an antibody-based test, and that nucleic acid amplification–based testing should be performed in blood donations in certain high-risk states, including Connecticut, Massachusetts, Rhode Island, New York, New Jersey, New Hampshire, Maine, Minnesota, and Wisconsin.[76]

Transfusion-related transmission of malaria has long been recognized. In the United States between 1963 and 1999, 93 cases of transfusion-associated malaria were diagnosed and 10 patients died.[135] The majority of the infected donors would have been excluded had current deferral criteria been in place, or fully implemented, at the time of donation. Transfusion transmission of malaria has continued to decline, with the few cases recognized being associated with a history of malaria or with residence in a malarious area, rather than an isolated history of brief travel.[136]

Prions

The risk for transmission of sporadic Creutzfeldt-Jakob disease via transfusion is unknown, although no cases have been recognized.[137] In contrast, vCJD, which is acquired from eating beef and beef products contaminated with the agent that causes bovine spongiform encephalopathy, has been documented to be transmitted by transfusion. Between 2003 and 2007, there were four reports, all in the United Kingdom, of individuals who acquired the vCJD agent through RBC transfusions. Two of the RBC recipients developed vCJD and died of the disease; the third died of an unrelated illness but had evidence of infection, and the fourth affected individual developed symptoms of vCJD 8½ years after having received a transfusion of RBCs.[138,139] These cases were unlikely to have been detected without a robust system operating in the United Kingdom for follow-up of health care outcomes in transfusion recipients. Before cases were reported, considerable concern in the United States led to exclusion of blood donors from Great Britain and of those with prolonged stays in western Europe. Although significant donor deferrals resulted, the precautionary measures appeared to be justified.[140]

INFECTIOUS DISEASES TRANSMITTED THROUGH TRANSPLANTATION OF SOLID ORGANS AND OTHER TISSUES

Advances in health care technology have led to a proliferation in biologic products collected to sustain and improve the quality of life; over 35,000 solid organs and 2 million tissue allografts are distributed each year for transplantation. However, these advances also have increased the opportunities for transmission of infectious pathogens, including viruses, bacteria, and parasites, particularly for solid-organ transplant recipients.[141] One organ and tissue donor potentially can transmit disease, including infections and malignancies, to as many as 100 recipients. Data from mandated reporting have led to estimates that unexpected infectious diseases or malignancies may be suspected to be transmitted to as many as 1% of all solid-organ transplant recipients, and to many more in whom transmission goes unrecognized.[142,143] Transmission of disease through solid-organ and tissue transplantation is both a public health and patient safety issue, because events can result in serious illness and death in transplant recipients over a wide geographic area, making recognition difficult. For organs, there is a delicate balance between safety and availability, given the number of patients on the transplant wait list. There is no behavioral risk factor or test result that uniformly disqualifies an organ donor, unlike extensive deferral criteria for donors of blood and tissue. For instance, organs from donors who test positive for active hepatitis B or C virus are often accepted for transplantation and are most often matched to individuals already infected with these diseases, particularly with the increasing prospect for successful therapies.[144,145]

Progress has been made in increasing the availability of organs through the passage of a new law to also allow the use of organs donated from HIV-positive individuals. Given progress in treatment of HIV, increasing numbers of infected patients are on the transplant wait list. Entitled the HIV Organ Policy Equity (HOPE) Act, the law removes the prohibition against use of organs from HIV-positive donors for HIV-positive patients contingent on research showing beneficial outcomes. The US Health Resources and Services Administration has published rules allowing transplantation of organs from HIV-infected donors under research protocols, and results will be reviewed to determine whether these transplants can be done in the future without required participation in clinical research trials.[146]

Organ Transplant–Transmitted Bloodborne Pathogens

Examples of organisms transmitted through organs or other tissues include HIV, HAV, HBV, and HCV. HAV transmission has been reported in the United States in a multi–visceral organ transplant recipient.[147] In this case, the donor was unvaccinated and transmission occurred to the organ recipient and multiple health care workers, underscoring the increase in incidence of this infection and importance of achieving higher hepatitis A vaccination coverage in the US population.[147] Previously, HIV and HCV were transmitted to four organ recipients, and HIV was transmitted from a living donor to a kidney transplant patient. In both cases, only serology was performed for screening, and NAAT performed at the time of donation could have prevented transmission.[148,149] In 2013, a Public Health Service guideline for the reduction of HIV, HBV, and HCV transmission through organ transplantation was published and provides recommendations for donor screening, recipient testing, informed consent, and disease tracking and reporting; it recommends the use of NAAT in donors at increased risk for HIV and among all donors for HCV.[150] Despite NAAT, HCV transmission through organ transplantation has still occurred owing to window period infection in donors with nonmedical intravenous drug use.[151] The risk of undetected window period HIV or HCV infection among increased-risk donors in the setting of negative NAAT results is highest at 1 day after exposure, but is negligible at 1 month.[152] Through modeling, it has been estimated that the risk for transplant-transmitted HIV is approximately 1 in 50,000 and the risk of transplant-transmitted HCV is about 1 in 5000. The risk is higher for those donors with risk factors for bloodborne pathogens; the rate is highest for HCV associated with increased-risk donors and is estimated at 1 in 1000.[153]

Pathogens Causing Encephalitis

In addition to major bloodborne pathogens, other pathogens also can be transmitted through organ transplantation and are of concern (Table 304.4). Over the past decade, several emerging pathogens causing encephalitis among organ transplant recipients have been described and include WNV, rabies, lymphocytic choriomeningitis virus (LCMV), and *Balamuthia mandrillaris.* Each of these pathogens have been

TABLE 304.4 List of Notable Infections Transmitted by Solid-Organ Transplantation

Viruses
Cytomegalovirus
Eastern equine encephalitis virus
Epstein-Barr virus
Human immunodeficiency virus
Hepatitis A virus
Hepatitis B virus
Hepatitis C virus
Human herpesvirus 8
Herpes simplex virus
Lymphocytic choriomeningitis virus
Rabies virus
West Nile virus
Bacteria
Multiple species[a]
Mycobacteria
Mycobacterium tuberculosis
Fungi
Apophysomyces elegans
Coccidioides immitis
Cryptococcus neoformans
Histoplasma capsulatum
Encephalitozoon cuniculi
Parasites
Balamuthia mandrillaris
Leishmania spp.
Strongyloides stercoralis
Toxoplasma gondii
Trypanosoma cruzi

[a]Morbidity associated with antimicrobial-resistant strains (e.g., methicillin-resistant *Staphylococcus aureus*) and gram-negative organisms (e.g., *Francisella tularensis,*[177] *Pseudomonas* spp.).

Modified from Machado CM, Levi JE. Transplant-associated and blood transfusion–associated tropical and parasitic infections. Infect Dis Clin North Am. *2012;26:225–231; and unpublished data from the Centers for Disease Control and Prevention.*

described to have been transmitted from donor to recipient on multiple occasions. More recently, eastern equine encephalitis virus has also been reported to have been transmitted from donor to recipients.[153a] It is critical for health care providers to recognize unusual outcomes in these patients and to communicate information about suspected transmission in such cases as quickly as possible, so that public health authorities can investigate and help initiate prevention measures in other recipients of organs from the same donor.

The natural mode of transmission of WNV is through infected mosquitoes, primarily of the *Culex* species, with birds serving as amplifying hosts.[154] In 2002, several cases of serologically confirmed WNV infection occurred in persons with little or no known mosquito exposure, and epidemiologic evidence suggested transmission of the virus through blood transfusion. Later in 2002, the first recognized cases of organ transplant–transmitted WNV infection were described in the United States. In fact, the two mechanisms of transmission (transfusion and solid-organ transplant) were linked early in the epidemic, when an organ donor who had been transfused with WNV-contaminated blood was infected. The occurrence of WNV encephalitis in two recipients of kidneys and the recipient of a heart and of febrile illness in a fourth recipient of a liver, all from the common donor, raised the concern of transmission through transplantation. Laboratory and epidemiologic data substantiated this mode of transmission and documented that the organ donor had likely acquired WNV infection through blood transfusion.

The 2002 WNV cluster resulted in heightened awareness about the potential increased severity of WNV infection in transplant recipients, and subsequent studies have substantiated a higher risk for severe manifestations, particularly neuroinvasive disease, which includes meningitis, encephalitis, and myelitis, among immunocompromised populations.[155] In contrast to transfusion transmission, which has become uncommon since initiation of blood donor screening with NAAT, this may not be the case in transplant recipients because no organ donor screening has been mandated. There have been seven organ-transmission WNV clusters reported in the United States, in which 16 of 20 organ recipients were infected; 12 of 16 infected organ recipients developed encephalitis, and 6 of these patients died.[63,156] In these clusters, onset of neuroinvasive disease among recipients likely led to recognition.[63,156] In the setting of blood transfusion, experience has suggested that transmission is unusual from an immunoglobulin M (IgM)–positive donor, presumably because a WNV-specific neutralizing antibody response results in clearing of viremia, rendering the blood as no longer infectious and possibly safe for transfusion.[157] However, in two of the reported transplant transmission clusters, there were detectable WNV IgM antibodies but no detectable RNA in donor serum specimens collected shortly before organ recovery. In one cluster, donor serum contained neither detectable RNA or WNV IgM antibodies.[63,156]

In July 2004, three solid-organ recipients and one additional recipient of an iliac artery segment from a common donor all developed encephalitis, eventually found to be due to rabies virus infection.[158] All four recipients died. Although several cases of rabies transmission through corneal transplants had been previously described,[159–162] solid-organ transplant transmission of rabies was not previously recognized. The common-source outbreak of transplant-associated rabies infection highlighted the difficulties inherent in the evaluation of encephalopathy in a potential organ donor. Prior to death, the organ donor had been twice evaluated in an emergency department after complaining of nausea, vomiting, and dysphagia. He eventually presented again with a subarachnoid hemorrhage and died. In retrospect, after determination of transmission of rabies infection through organ transplantation in multiple recipients, it was noted that the donor had additional symptoms consistent with encephalitis and had been bitten by a bat. Although this transmission was initially thought to be a unique event, another transplant cluster of rabies infection was reported from Germany soon after, demonstrating the potential importance of increased awareness from known transmission events.[163]

In the two previously recognized clusters of rabies virus transmission through solid-organ transplantation, infection was attributed to bat or canine virus variants, respectively. All recipients, except one in the German cluster who was previously vaccinated, experienced rabies symptoms within 6 weeks of transplantation and died.[164] These observations suggested a high infectivity rate and incubation periods of approximately 6 weeks in unvaccinated recipients of solid organs from donors with rabies. In 2013, another case of transplant-transmitted rabies virus infection was identified in the United States.[165] A raccoon rabies virus variant was identified in both the organ donor and infected recipient. In contrast to previous transplant rabies transmissions, the infected recipient developed symptoms approximately 17 months after transplant and three unvaccinated recipients remained asymptomatic. Postexposure prophylaxis was initiated in the three asymptomatic recipients, and all developed an appropriate protective neutralizing antibody response, suggesting that rabies infection can be prevented in organ transplant recipients if timely preventive measures are implemented.

In 2003 and 2005, two clusters of meningoencephalitis caused by LCMV occurred among solid-organ transplant recipients in the United States.[166] The first was reported among organ recipients from a common donor from Wisconsin. In this cluster, however, there was no serologic evidence of LCMV infection or rodent exposure in the donor. Eventual identification of LCMV through cell culture and electron microscopy of specimens from all four organ recipients led to pathogen identification and attribution of the source infection to the organ donor.[166] A second cluster occurred in 2005 among four organ recipients from a common donor who had died from an ischemic stroke.[167] Pathologic study and immunohistochemical staining of specimens led to LCMV diagnosis in this cluster. Investigation of this cluster revealed that the donor had had exposure to a pet hamster.[167] Although the donor had no evidence of active LCMV infection, the pet hamster was infected with a strain of LMCV that was genetically similar to those infecting the recipients. In 2008, hepatic insufficiency, multiorgan system failure, and death were reported in two kidney recipients with a common organ donor.[168] Although neither recipient exhibited signs of encephalitis, the donor had

had aseptic meningitis at death and retrospectively was found to have serum antibodies to LCMV. The donor was homeless and likely had had multiple opportunities for rodent exposure in his living environment.[168] Another cluster in which three organ transplant recipients died of encephalitis within 6 weeks of transplant was reported from Australia and was found to be caused by a novel arenavirus classified as LCMV.[169] This virus was identified through unbiased high-throughput sequencing.[169] The donor had died of a hemorrhagic stroke but had serologic evidence of recent infection and travel history to rural southern Europe, where he may have had rodent exposure.[169] It appears that transplant-associated LCMV infection may be an uncommon, but quantifiable, infectious risk in organ transplantation. Immunosuppression among these patients predisposes them to development of severe disease, including encephalitis, even though infected donors do not have central nervous system abnormalities, apart from the observation that the majority of those recognized die of cerebrovascular accident. Recognition of the potential for donor-derived LCMV, however, can result in reduced risk of mortality among recipients through prompt reduction of immunosuppression and perhaps implementation of antiviral therapy.[170]

Transplant-transmitted infection caused by *B. mandrillaris,* a free-living ameba described as causing a granulomatous amebic encephalitis, was first identified in 2009 after reports from clinicians in Mississippi noting a cluster of encephalitis in two kidney recipients from a common donor.[171] The organ donor, aged 2 years, reportedly had copious soil and water exposure before dying of a subarachnoid hemorrhage that was deemed secondary to seizures in the setting of what was initially diagnosed as an immune-mediated encephalitis but was later shown to be *Balamuthia* infection.[171] A second cluster was reported in 2010 when two organ transplant recipients in Arizona developed encephalitis with multiple ring-enhancing lesions revealed by cerebral magnetic resonance imaging.[171] The organ donor was presumed to have died of a stroke. Both patients ultimately died, and diagnosis was confirmed by immunohistochemical staining and reverse-transcriptase polymerase chain reaction detection of *Balamuthia* DNA in brain biopsy specimens.[171]

Transplant-transmitted infections resulting in encephalitis highlight several important diagnostic and clinical challenges related to recognition and treatment of certain emerging infections. Encephalitis resulting from WNV, rabies, LCMV, and *Balamuthia* among transplant recipients is quite rare and may not be immediately recognized by clinicians. Diagnosis is further complicated by limitations with laboratory screening for several of these diseases. Clinically, few effective treatment options are available once patients exhibit symptoms. However, there is some limited evidence that prophylaxis or treatment, even among asymptomatic transplant recipients, may be effective after exposure to these diseases. As a result, identification of possible infectious encephalitis among organ donors and establishment of proactive notification systems for transplant centers is crucial. It is critical for health care providers to recognize unusual outcomes in these patients and to communicate information about suspected transmission in such cases as quickly as possible, so that public health authorities can investigate and help initiate prevention measures.[172]

Strongyloidiasis

In a review of 21 symptomatic cases of *Strongyloides stercoralis* transmitted through solid-organ transplantation, symptom onset averaged 82 days after transplantation (range, 33–270 days), reflecting time for the worm burden to increase in the recipient by means of autoinfection.[173] Symptoms included gastroenteritis and, with larval migration through the lung, diffuse pneumonia. Most organ donors were from Central America, South America, or South Asia.[173,174] Diagnosis has been made by finding larvae in bronchoalveolar lavage fluid, cerebrospinal fluid, stool, or urine.[173] Screening of high-risk donors for antibody to *Strongyloides* and empirical use of ivermectin in at-risk recipients can be considered.

Microsporidiosis

Three organ recipients acquired *Encephalitozoon cuniculi* infection after transplantation of organs from a woman who had emigrated from Mexico a year earlier.[175] Infection manifested as unexplained fever beginning 7 to 10 weeks after transplantation.

Ehrlichiosis

Life-threatening transplant-associated ehrlichiosis occurred in both recipients of kidneys from the same deceased donor.[176]

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The complete reference list is available online at Expert Consult.

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305 Infections in the Immunocompromised Host: General Principles

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COMPONENTS OF HOST DEFENSE

Appreciation of the predisposing risk factors is an essential but perplexing exercise because it suggests that each individual component plays an independent role. Certain organisms infect patients with specific defects, and these associations should be taken into account when selecting therapy. However, this is by no means always predictable. Theoretically, a specific deficiency increases the patient's susceptibility to the very pathogens that are eradicated by that particular host defense mechanism (Table 305.1; see Chapters 4 through 9). Although a basic pattern is recognizable, the types and severity of infectious complications are often unpredictable. Single, isolated deficiencies are infrequently encountered, and malfunction of one part of the system often influences several other components. Moreover, therapeutic interventions and the underlying disease will disturb a range of defense mechanisms. Although the risks associated with neutropenia are well known, other toxicities, especially those affecting the mucosal barrier, are considered to be of greater importance than was previously the case. The earlier advent of aggressive treatments and, recently, targeted therapy has altered the concept of specific defects of host defense mechanisms in the various types of diseases because the effects of these drugs and irradiation are now seen as the primary factors determining the nature and extent of the defect. Also, transplantation (especially of hematopoietic stem cells) can cause defects in host immunity as a result of graft-versus-host disease (GVHD), as well as of immunosuppressive therapy. Patients with impaired humoral immunity as manifested by defective opsonization and phagocytosis of bacteria will also be exposed to therapy-induced neutropenia or deficient cellular immunity as a result of treatment with purine analogues, monoclonal antibodies, or targeted therapies (e.g., tyrosine kinase inhibitors [TKIs] and inhibitors of phosphatidylinositol 3 kinase [PI3K]) for treating malignancies.

The genetic makeup of the host also has an impact on the risk for infection because functional changes due to polymorphisms in immune genes influence individual susceptibility for infection.[1,2] The specific context of an immune deficiency determines which of the remaining components of the immune system will be most important. Therefore, variation in the genes that correspond to these components may become clinically important. Components of the innate immune system are believed to survive chemotherapy and contribute to immune defenses. Consequently, in the setting of cancer therapy and hematopoietic stem cell transplantation (HSCT), most gene polymorphisms associated with the risk for infection involve innate immune genes, especially those coding for pattern recognition receptors and cytokines.[3,4]

Cellular and Humoral Immunity

The host defense against pathogenic microorganisms encompasses innate and acquired immunity. The innate immune system comprises both cellular components, including monocytes, neutrophils, natural killer (NK) cells, and innate lymphoid cells, and humoral components, including complement, some antibodies ("natural" antibodies, perhaps directed against normal microbiota), antimicrobial peptides, and lysozyme. This mechanism is very effective in dealing with the vast majority of infectious agents. It has become clear that the innate immune system not only specifically recognizes various classes of microorganisms via pattern recognition receptors (microbe-associated molecular patterns) that sense conserved structures of the invading microorganisms, but also initiates and modulates the subsequent adaptive responses delivered by T cells and B cells through their interaction with antigen-presenting cells (especially dendritic cells).

Innate Immunity

Granulocytes

In normal circumstances, neutrophils, sometimes accompanied by eosinophils, congregate at the site of inflammation and are followed by macrophages. Formation of this inflammatory exudate is the result of activation of humoral factors and normal function of the vascular endothelium (see Chapter 8). Meanwhile, in the peripheral blood, granulocytosis evolves as a consequence of release of the marrow reserve and increased granulocytopoiesis, which is regulated by hematopoietic growth factors such as interleukin (IL)-3, granulocyte-macrophage colony-stimulating factor, and granulocyte colony-stimulating factor.[5]

Virtually all cytotoxic drugs used in the treatment of malignant diseases have a deleterious effect on the proliferation of normal hematopoietic progenitor cells. Therefore, after obliteration of the mitotic pool and depletion of the marrow pool reserve, neutropenia ensues. Likewise, therapeutic radiation can induce clinically important neutropenia, depending on the dose rate, total dose given, and irradiated area of the body. Total-body irradiation, as used to prepare for HSCT, is the most obvious illustration of the possible negative impact of irradiation. Thus, profound neutropenia is an unavoidable consequence of the treatment of malignancy and may persist for 3 or 4 weeks or even longer. Neutropenia or a treatment-related decrease in the granulocyte count is probably the most important primary risk factor for infection. Fever develops in nearly all cases of profound neutropenia (i.e., a granulocyte count $<100/mm^3$ for more than 2–3 weeks), whereas only one-fifth of the febrile episodes in cancer patients occur when granulocyte counts are normal.[6] Moreover, during iatrogenic neutropenia, the risk for infection and infection-related mortality increases proportionally with time.

Granulocytes that accumulate at the site of infection are of little use if they are unable to function normally. Antineoplastic drugs and irradiation interfere with these nonproliferating cells and their function, resulting in decreased chemotaxis, diminished phagocytic capacity, and defective intracellular killing by granulocytes. Glucocorticosteroids seem to enhance granulocytopoiesis and mobilize the marginal and the marrow pool reserve, but these putative positive effects on neutrophilic granulocytes are offset by numerous disadvantages. These drugs curb the accumulation of neutrophils at the site of inflammation by reducing their adherent capacity and diminishing their chemotactic activity. Furthermore, they decrease phagocytosis and intracellular killing of microorganisms. The lack of functioning neutrophils deprives the host

TABLE 305.1 Immunodeficiencies and Associated Prevalent Pathogens

DEFECT	PATHOGEN
Neutropenia	Gram-positive cocci *Staphylococcus aureus* Coagulase-negative staphylococci *(S. epidermidis, S. haemolyticus, S. hominis)* Viridans-group streptococci *(S. mitis, S. oralis)* *Granulicatella* and *Abiotrophia* spp. (formerly nutritionally variant streptococci) Enterococci *(E. faecalis, E. faecium)* Gram-negative bacilli *Escherichia coli* *Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Enterobacter* and *Citrobacter* spp.
Damaged integument	
Skin and central venous catheter related	Coagulase-negative staphylococci *(S. epidermidis, S. haemolyticus, S. hominis)* *Staphylococcus aureus* *Stenotrophomonas maltophilia* *Pseudomonas aeruginosa* *Acinetobacter* spp. Corynebacteria *Candida* spp. *(C. albicans, C. parapsilosis)* *Rhizopus* spp.
Oral mucositis	Viridans-group streptococci *(S. mitis, S. oralis)* *Abiotrophia* and *Granulicatella* spp. (nutritionally variant streptococci) *Capnocytophaga* spp. *Fusobacterium* spp. *Rothia mucilaginosa* *Candida* spp. *(C. albicans, C. tropicalis, C. glabrata)* Herpes simplex virus
Gut mucosal barrier injury	*Escherichia coli* *Pseudomonas aeruginosa* Coagulase-negative staphylococci Enterococci *(E. faecalis, E. faecium)* *Candida* spp. Viridans-group streptococci *(S. oralis, mitis)*
Neutropenic enterocolitis	*Clostridium* spp. *(C. septicum, C. tertium)* *Staphylococcus aureus* *Pseudomonas aeruginosa*
Impaired cellular immunity	Herpesviruses Cytomegalovirus Respiratory viruses *Listeria monocytogenes* *Nocardia* spp. *Mycobacterium tuberculosis* Nontuberculous mycobacteria *Pneumocystis jirovecii* *Aspergillus* spp. *Cryptococcus* spp. *Histoplasma capsulatum* *Coccidioides* spp. *Talaromyces marneffei* *Toxoplasma gondii* Human papillomavirus Polyomavirus (BK, JC, and others)
Impaired humoral immunity	*Streptococcus pneumoniae* *Haemophilus influenzae* Norovirus Hepatitis B virus Polyoma virus (JC) Hepatitis B virus *Campylobacter/Helicobacter*
Compromised organ function	
Splenectomy	*Streptococcus pneumoniae* *Haemophilus influenzae* *Neisseria meningitidis*
Deferoxamine for iron overload	*Rhizopus* spp.
New targeted drugs	
Bruton tyrosine kinase inhibitor (ibrutinib); a specific inhibitor of B-lymphocyte signaling	*Aspergillus* spp. *Pneumocystis jirovecii*
JAK-STAT inhibitor (ruxolitinib); downregulates proinflammatory cytokines, impairs dendritic cell, NK cell, and $CD4^+$ T-cell function	*Mycobacterium tuberculosis* *Hepatitis B reactivation*
PI3K inhibitor (idelalisib); specific inhibitor of phosphatidylinositol-3-kinase delta-Akt pathway B-lymphocyte signaling	*Pneumocystis jirovecii*

of a primary defense mechanism against invading microorganisms, which are consequently able to readily establish themselves, initiate local infection, disseminate unhindered, and eventually lead to fulminant sepsis and death unless managed promptly and effectively.

Monocytes and Macrophages

Monocytes reside in the bloodstream and contribute to rapid responses against bacteria and fungi that gain access to the bloodstream. Monocytopenia occurs in parallel with neutropenia after cytotoxic therapy and contributes to the susceptibility to bacteremia and fungemia in cancer patients.

The descendants of monocytes, the tissue-residing type l macrophages, have a limited capacity for killing. Various intracellular microorganisms are able to survive and replicate inside the cell, unless the macrophage becomes activated. Activation of macrophages is a complex process, primarily under the control of cytokines (e.g., interferon-γ) provided by T lymphocytes. This explains the prominent susceptibility of patients with hairy cell leukemia to opportunistic infections, including those with filamentous fungi, because there exist synergistic intrinsic (monocytopenia and decreased interferon-γ release) and extrinsic (therapy-related, including purine analogues) immune defects.

With the use of the Bruton tyrosine kinase (BTK) inhibitor ibrutinib, there has been an increase in pulmonary and cerebral aspergillosis in patients with lymphoid malignancies, in the context of corticosteroids use. Because ibrutinib was considered to be a specific inhibitor of B-lymphocyte signaling, this was unanticipated. Further analysis, however, revealed a role for BTK in monocytes and granulocytes and additional inhibition of inducible tyrosine kinase by ibrutinib in lymphocytes that explain at least partially this unexpected event. This highlights the complexity of approaching patients with cancer and choosing therapies and illustrates the challenges with presumably specific and less toxic targeted therapies.

Natural Killer Cells

NK cells are cytotoxic lymphocytes belonging to the pool of innate lymphoid cells. Although thought to mainly contribute to responses against viral infections and antitumor immunity, NK cells have been identified as important effectors in bacterial and fungal infections. Depletion of NK cells by monoclonal antibodies and during HSCT contributes to the overall susceptibility to viral and probably fungal infections. It turns out that the surface receptor CD56 of NK cells is a pattern recognition receptor of *Aspergillus fumigatus* and plays a role in fungus-mediated NK cell activation.

Impact of Treatment on Cellular Immunity

Both antigen-specific and antigen-nonspecific cells contribute to the development of cellular immunity. The antigen-specific branch of cell-mediated immunity can be divided into two major categories. One category involves cytotoxic effector cells, which are able to lyse virus-infected or foreign cells, including malignant cells. The second category involves subpopulations of helper T cells that mediate differentiated cytokine reactions (e.g., Th1, Th2, Th17) after antigen recognition.

This fine-tuned system can easily be deregulated by congenital defects or defects acquired as a result of a disease or its treatment. Long-term cytotoxic therapy, extensive irradiation, and immunosuppressive drugs such as corticosteroids, azathioprine, cyclosporine, tacrolimus, and mTOR inhibitors (sirolimus, and everolimus) suppress cellular immunity. Some monoclonal antibodies, such as alemtuzumab, are being used as

antitumor and immunosuppressive agents and can exert profound and prolonged effects on cellular immunity. Purine analogues, including fludarabine and cladribine, are particularly detrimental to cellular immunity and create a situation similar to acquired immunodeficiency syndrome. Likewise, lymphatic malignancies, particularly Hodgkin lymphoma and chronic lymphocytic leukemia (CLL), are associated with impaired cellular immunity. Emerging targeted therapies—including the TKIs imatinib, dasatinib, and bosutinib; small molecules such as BCL2 inhibitors (venetoclax); JAK-STAT inhibitors; and PI3K inhibitors, often used in heavily pretreated patients—have an impact on specific cellular immunity (see Chapter 6). This is exemplified by the occurrence of opportunistic infections with the use of ruxolitinib (*Mycobacterium tuberculosis* and hepatitis B reactivation), ibrutinib (aspergillosis), and idelalisib (*Pneumocystis jirovecii*). On the contrary, the use of immunomodulatory drugs (lenalidomide) or proteasome inhibitor drugs (bortezomib) increases the risk of severe nonspecific infections, especially in the relapsed/refractory phase of multiple myeloma, and mostly as a result of therapy-related neutropenia.

Allogeneic stem cell transplantation brings about a long-lasting dysfunction of T and B cells, especially in association with GVHD and its treatment (Table 305.2). The coordination of cellular immunity is often lost, and when aided and abetted by suppressed humoral immunity, the paracrine mediators that are released go on to induce the sepsis cascade, which may culminate in multiorgan failure instead of arresting infection.[7]

TABLE 305.2 Sequence of Infective Events in Relation to the Phases of Allogeneic Stem Cell Transplantation

	EARLY PHASE	MID-RECOVERY PHASE	LATE PHASE
Host Defense Mechanisms Without Graft-Versus-Host Disease			
Phagocytes	Absent	Deficient	Normal
Integument Skin Mucous membranes	 Damaged Severely damaged	 Damaged Damaged	 Intact Intact
Cellular immunity	Slightly impaired	Impaired	Impaired
Humoral immunity	Normal	Impaired	Severely impaired
Host Defense Mechanisms With Graft-Versus-Host Disease			
Phagocytes	Absent	Deficient	Normal
Integument Skin Mucous membranes	 Damaged Damaged	 Damaged Severely damaged	 Damaged Damaged
Cellular immunity	Slightly impaired	Severely impaired	Severely impaired
Humoral immunity	Normal	Impaired	Severely impaired
Prevalent Infections			
Mucosa	Herpes simplex virus Viridans streptococci Coagulase-negative staphylococci	Herpes simplex virus *Candida* spp.	Herpes simplex virus *Candida* spp.
Lung	Gram-negative bacilli *Aspergillus* spp.	Cytomegalovirus *Aspergillus* spp.	*Streptococcus pneumoniae* Viruses, including cytomegalovirus *Pneumocystis jirovecii*
Blood	Viridans streptococci Staphylococci Gram-negative bacilli *Candida* spp.	Staphylococci *Streptococcus pneumoniae* *Candida* spp.	*Neisseria meningitidis*

Impact of Treatment on Humoral Immunity

The humoral branch of the immune system, which is primarily responsible for clearing extracellular bacteria, involves the interaction of B cells with antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells (see Chapter 5). An important difference in antigen recognition by T cells and B cells is that the latter can recognize some antigens without the help of an antigen-presenting cell. The humoral system can identify a plethora of bacterial or viral microorganisms, in addition to the soluble proteins that they release. When challenged by an antigen, immunoglobulins are produced that bind to the antigen. The specific functions of immunoglobulin G (IgG) and immunoglobulin M (IgM) include neutralization of the antigen, and complement activation and opsonization—that is, enhancement of phagocytosis of the antigen by neutrophils and macrophages. Secretory immunoglobulin A (IgA), which is found on mucosal surfaces, is not an opsonin but nonetheless inhibits the motility of bacteria and prevents them from adhering to epithelial cells. The production of immunoglobulins is decreased in lymphoproliferative disorders such as CLL and multiple myeloma, whereas humoral immunity is generally well preserved in patients with acute leukemia. However, intensive irradiation and chemotherapy will lead not only to neutropenia but also, ultimately, to hypogammaglobulinemia. In particular, monoclonal antibodies such as rituximab and blinatumomab and CD19- and CD22-targeted chimeric antigen receptor (CAR) T cells deplete B lymphocytes, inducing profound and long-lasting hypogammaglobulinemia and, consequently, infections.[8] The impact of new therapies such as BTK and PI3K inhibitors is currently less clear, but considering the fact that they target molecules with vital roles in B-cell maturation and function, disrupted antibody responses can be anticipated. At the least, reduced serologic responses to vaccination indicate impaired humoral immunity.

Cytokines and chemokines are indispensable for communication between innate and acquired immune and nonimmune cells in shaping effective antimicrobial immune reactions. Hence, interference by anticytokine antibodies and cytokine scavengers (e.g., infliximab, anakinra, tocilizumab) results in increased risk for infection in autoimmune diseases, transplantation, and cancer therapy.[9]

Humoral Immunity and the Spleen

The primary immunoglobulin response of spleen-produced specific opsonizing antibodies is necessary for efficient phagocytosis of encapsulated bacteria. Macrophages that occupy strategic positions within the organ are subsequently able to remove them. Splenectomy may result in a reduced level of the complement factor properdin and thereby lead to suboptimal opsonization, a decrease in functional tuftsin, and low levels of circulating IgM.[10] The lack of opsonizing antibodies in serum against common encapsulated bacteria impairs the activity of all phagocytic cells, including granulocytes, monocytes, and macrophages. As a consequence, infections with *Streptococcus pneumoniae* and *Haemophilus influenzae* are often more severe in splenectomized patients and in those who have undergone HSCT and are functionally asplenic. Opsonizing antibodies are also important for effective antibody-dependent cell-mediated cytotoxicity of NK cells.

Platelets

The protective role of platelets in healthy individuals is often underestimated but becomes obvious during treatment of patients with malignant disease. Thrombocytopenia is an almost inevitable repercussion of intensive chemotherapy and irradiation, but decreased thrombocyte function is also a matter of concern. Thrombocytopathy is either disease related or caused by concurrent medication. The consequences of both increased susceptibility to infection and a decreased capacity to repair damaged tissues can be considerable and may have an impact on the eventual outcome of a treatment episode. Thrombocytopenia also appears to be an independent risk factor for bacteremia,[11] and the incidence of major hemorrhage at autopsy of patients who die with or of an infection is striking.[12]

The Integument as Host Defense

The skin, the respiratory tract (including the nasal cavity), the ears and conjunctiva, the alimentary tract, and the genitourinary tract are in contact with the environment and provide a first line of defense against microbial invasion. The skin and the mucosal surfaces of the alimentary and respiratory tracts form principal barriers against microbial invasion. These surfaces are normally colonized with a variety of microorganisms, including many different genera of bacteria, viruses, and yeast that have an intimate association with a particular ecologic niche and help to maintain the function and integrity of this first line of defense. When intact and healthy, both the mucosa and the skin are capable of resisting colonization with the allochthonous organisms found in the immediate environment, as long as an ecologic balance is maintained within the indigenous microbial microbiota. Acidity plays a crucial role both in disinfecting the stomach and in regulating the microbial milieu of the vagina. The integrity of the mucosa, production of saliva and mucus, peristalsis, bile acids, digestive enzymes, and levels of defensins, trefoil factors, and secretory IgA also play an important role in maintaining a favorable microecology. Elimination of an inoculum is achieved by sneezing and coughing of microbes trapped in mucus, whereas flushing of the mouth and esophagus with saliva, micturition, and peristalsis inhibit continuous intimate contact between a given surface area and unattached invasive microorganisms.

Skin

Healthy skin provides an effective barrier against invasion by microorganisms, mainly by remaining intact. Desquamation helps limit the opportunities for transient organisms to establish residence. Normally, very little water is present on the skin surface. Colonization with organisms sensitive to desiccation, such as gram-negative bacilli, is not favored. The skin also forms an acid mantle with a pH of 5.0 to 6.0, and its surface temperature is on average approximately 5°C lower than the core body temperature.[13] Besides containing secretory IgA, sweat also possesses sufficient salt to create a high osmotic pressure. Organisms that can withstand these conditions and compete successfully for binding sites and nutrients include staphylococci, corynebacteria, and the lipophilic yeast *Malassezia furfur.* These organisms further modulate the microecology of the skin by releasing fatty acids from sebaceous secretions to produce a hydrophobic milieu, and lactic and propionic acids, which help maintain a low pH. Many of the bacteria also elaborate bacteriocins that inhibit other microorganisms, and induce antimicrobial peptides and heterologous immune responses that protect the skin barrier.

The composition of the skin microflora is influenced by general factors including climate, body location, age, sex, race, occupation, and the use of soaps, detergents, and disinfectants. Antibiotics secreted in sweat disturb the balance within the commensal microbiota and leave the surface vulnerable to colonization by exogenous gram-negative bacilli. Antibiotics also exert selective pressure on the skin microbiota and cause resistance to emerge, as has been observed during treatment with ciprofloxacin. Moreover, ciprofloxacin is excreted in sweat and induces resistance among skin staphylococci within a few days of exposure.[14] β-Lactam antibiotics, including ceftazidime, ceftriaxone, cefuroxime, benzyl penicillin, and phenoxymethylpenicillin (penicillin V), can also be found in sweat, which might explain the ready selection of resistant staphylococci. Chemotherapy and irradiation can cause radical changes in healthy skin that cause hair loss, dryness, and loss of sweat production.

Needle punctures and catheters provide a ready means of access for microorganisms through the stratum corneum and into the bloodstream. When the skin is broken, the release of fibronectin is thought to assist colonization with *Staphylococcus aureus,* whereas other changes facilitate colonization with gram-negative bacilli such as *Acinetobacter baumannii* and enteric bacteria. Abraded skin can lead to local infection, which can be a reservoir that promotes further spread to entry sites of intravenous catheters. When the balance is lost between host defenses and commensal microbiota around hair follicles, the follicles can become inflamed and necrotic and form a potential nidus of infection. Clinical infection therefore results from breaks in the skin, loss of local immunity, and disturbances within the resident microbiota. Some common environmental organisms, mycobacteria among them, are prominent skin pathogens because of their preferential growth at lower temperatures.

Vascular devices have gained widespread acceptance as a relatively safe form of long-term venous access, but regular use is associated with a marked increase in the incidence of bacteremia with coagulase-negative staphylococci, which frequently colonize the catheter lumen (see Chapter 300). These staphylococci are commonly resistant to aminoglycosides, trimethoprim-sulfamethoxazole, and penicillinase-resistant penicillins and may also be resistant to fluoroquinolones. Unless the catheter ends in an implanted port, skin commensal microbiota have potential access into the bloodstream. The hub is the most likely source of contamination, leading to catheter colonization,[15] and the risk increases with use.[16] Infections related to the external surface of the catheter (exit site infections and tunnel infections) can result in serious soft tissue infection, most notably with *S. aureus.* Exit site infections occur much less frequently than does intraluminal contamination. The latter may be caused by a variety of bacteria, many of which have relatively low virulence. Once established, these infections can be very difficult to treat without removing the device, particularly those caused by *Bacillus* spp., *Candida* spp., and *Pseudomonas aeruginosa.*[17,18]

Respiratory Tract

The lung appears to be very vulnerable to damage by cytostatic chemotherapy and irradiation and is exquisitely susceptible to infection. Immunopathologic reactions mediated by pulmonary macrophages that survive chemotherapy can lead to various other syndromes, including respiratory distress. Lung hemorrhage as a result of profound thrombocytopenia further imperils the lung and thereby increases the risk for infection. Inhalation of spores of *Aspergillus* spp. and other molds may lead to infection of the sinuses, bronchi, and lungs.

Alimentary Tract and Normal Microbiota as Host Defense

Anaerobic bacteria predominate among the resident microbiota of the oral cavity and large intestine population and play a crucial role in maintaining a healthy commensal microbiota by providing the facility to withstand the establishment of exogenous organisms, which is known as *colonization resistance.*[19] However, the microbiota is not the only participant in the establishment and maintenance of colonization resistance. There has been renewed interest in the human commensal microbiota, which can be divided into the microbiome, mycobiome, and virome, all of which are associated with health and disease. The various species also participate in training and shaping the immune response, maintaining it, and keeping it healthy. In fact, the host-microbial interaction is far more complex than hitherto imagined, involving host pattern recognition receptors, bacteriocins, and lactic acid, to name but a few, in addition to competitors for available nutrients and the release of host antimicrobial peptides.[20,21,22]

Dysbiosis

Many antibiotics also exert a negative influence on the commensal microbiota. Very susceptible bacteria such as the oral *Neisseria* spp. are suppressed by a wide range of antimicrobial agents, whereas oral viridans-group streptococci, such as *Streptococcus mitis* and *Streptococcus oralis,* and other unusual oral commensal microbiota, such as *Rothia mucilaginosa* and *Capnocytophaga* spp., are likely to be selected by antimicrobial agents, to which the bacteria are only marginally susceptible, if at all. In particular, penicillins, rifampin, clindamycin, macrolides, bacitracin, and vancomycin significantly impair colonization resistance, probably because they inhibit the gram-positive nonsporulating, lactic acid–producing bacilli such as bifidobacteria. Certain cephalosporins are also detrimental, whereas trimethoprim-sulfamethoxazole and the quinolones have been declared "friendly," hence their frequent use as prophylaxis.[23] Unexpectedly, imipenem used at higher doses has led to an increase in cases of diarrhea caused by *Clostridioides difficile* (formerly *Clostridium difficile*).[24] Newer antibiotics such as tigecycline, with activity against anaerobic gram-positive bacteria, may lead to increases in the numbers of patients with *Candida.* This change usually reflects marked perturbations of the gut ecology.[25] However, this effect does not increase

the risk for infection due to *C. difficile*.[26] Chlorhexidine mouthwashes used to minimize plaque and gingivitis also influence the microflora.[27]

Because the normal commensal microbiota attach to the surfaces of the epithelium, their loss creates an ecologic vacuum that allows other organisms to establish colonization by occupying the vacant cell surfaces or by taking advantage of the surfeit of nutrients. Collapse of the ecology invariably manifests with yeast overgrowth and colonization with nosocomial bacteria such as *Klebsiella pneumoniae* and *P. aeruginosa* and failure to detect viable anaerobes directly or indirectly.[28–30] Examples of infectious complications associated with disturbance of the normal microbial equilibrium include the selection of previously uncommon species such as *Enterococcus faecium* and *Clostridium septicum*.

Dyspepsia is sufficiently commonplace for antacids such as histamine-2 (H2) blockers and proton pump inhibitors to be regularly prescribed. Reduced gastric acidity inadvertently destroys the natural barrier that prevents gastric and intestinal colonization by oral commensal microbiota, many of which are resistant to most of the antimicrobial agents used for prophylaxis in impaired hosts. When patients swallow large amounts of mucus as a result of severe mucositis, any oral commensal microbiota may survive passage to the bowel. Loss of the gastric barrier therefore effectively extends the area of potential sites for colonization to the full length of the alimentary tract, which may explain the pathogenesis of viridans-group streptococcal bacteremia.[31]

Finally, the ecology of the gut microbiota is markedly altered by damage induced by treatment with certain cytostatic agents such as cytarabine,[32] by GVHD,[33] and by total-body irradiation.[34] The direct impact of enterocyte and Paneth cell damage on the microbiota seems mostly related to changes in production and release of antimicrobial peptides, normally involved in controlling microbial abundance and composition. Nevertheless, the strongest impact remains the detrimental effect of antimicrobial agents used in immunocompromised hosts.

Mucosal Barrier Injury

Injury to the mucosal barrier induced by chemotherapy and radiation therapy is probably the most substantial and earliest breach in the host defenses against infecting microorganisms. The process is more complex than just the direct effect of cytotoxic therapy on cells with a high mitotic index, such as epithelial cells of the mouth and gastrointestinal tract, and the indirect effect of local infections associated with evolving neutropenia. Sonis[35] postulated that the pathobiology of mucositis involves five sequential but overlapping phases (Fig. 305.1), culminating in tissue damage that manifests clinically as mucositis. The initiation phase involves free-radical generation and induction of apoptotic cell death induced by both DNA and non-DNA damage. The primary damage response phase occurs next, during which the master transcription factor, nuclear factor kappa B (NF-κB), leads to the upregulation of several genes, resulting in the production of the proinflammatory cytokines (tumor necrosis factor-α [TNF-α], IL-1β, and IL-6) and then signal amplification. The net result is the ulceration phase, in which microorganisms and microbe-associated molecular patterns such as peptidoglycan and lipopolysaccharide can translocate the damaged physical barrier more easily and are able to activate tissue macrophages to produce more proinflammatory cytokines, thereby exacerbating the damage.[36,37] In addition, damage-associated molecular patterns, including endogenous ligands, the alarmin, high-mobility box group-1, ATP, and heat shock proteins released as a result of tissue damage, all contribute to inflammation and tissue damage.[35] Last, the healing phase occurs and is to a certain extent dependent on the rate of recovery of stem cells that are capable of repopulating the epithelium. Cytostatic chemotherapy regimens and irradiation injury induce varying degrees of mucosal barrier injury with differing dynamics, although the progression and decline of signs may be similar.[38] Individual patients are also likely to vary in their susceptibility to mucosal damage, suggesting that there are genetic differences in the expression of proinflammatory cytokines or proteins that control stem cell apoptosis (e.g., TP53 and BCL2 along the gastrointestinal tract).

There is extensive gastrointestinal-associated lymphoid tissue in which lymphocytes and macrophages are located and that responds to irradiation and chemotherapy with inflammation.[39,40] Moreover, gut epithelial cells actively participate in the innate and adaptive immune

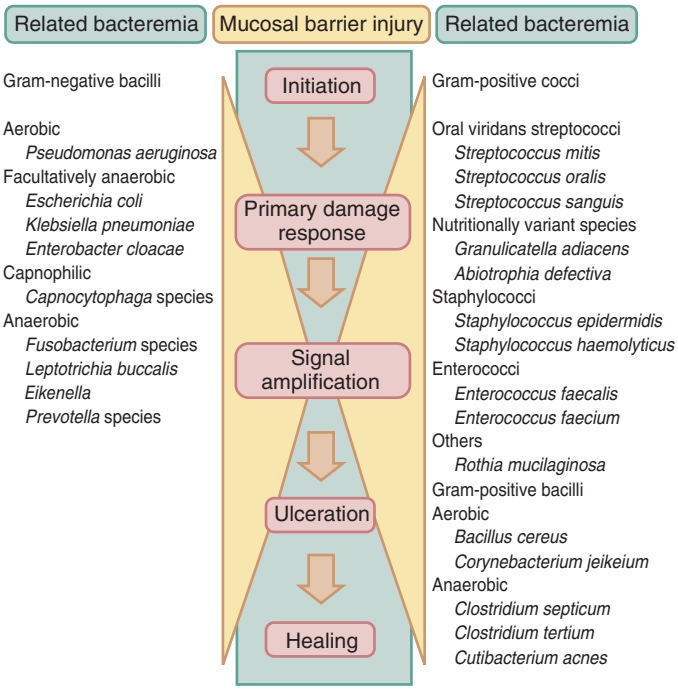

FIG. 305.1 Mucosal barrier injury induced by cytostatic chemotherapy and irradiation is believed to occur in five more or less sequential phases. The process elicits a local and systemic inflammatory response, which may be accompanied by signs and symptoms of oral mucositis and gut dysfunction and even sepsis. Infection may be local but often involves dissemination via the bloodstream to distant sites. Bacteremia can be caused by a variety of normally commensal resident flora found on the skin and mucous membranes of the alimentary tract and by potential pathogens acquired exogenously.

response. They are capable of producing and secreting proinflammatory cytokines and upregulating the production of major histocompatibility complex class II molecules and other adhesion molecules after sensing specific microbe-associated molecular patterns, such as lipopolysaccharide (endotoxin), muramyl dipeptide, and other bacterial antigens.[39] In mucosal barrier injury, the innate immune response is dysfunctional and host microbe interactions that are normally beneficial now result in detrimental inflammation and perpetuation of mucosal damage. Uncontrolled activation of pattern recognition receptors appears to play a key role. Muramyl dipeptide is sensed by NOD2, an intracytosolic pattern-recognition receptor expressed in Paneth cells, dendritic cells, neutrophils, and monocytes. *NOD2/CARD15* polymorphisms result in uncontrolled inflammation of gut mucosa in Crohn disease and gut GVHD after allogeneic stem cell transplantation.[41]

Oral and gastrointestinal mucositides are the clinical manifestations of mucosal barrier injury, and the duration of mucositis may define the period of risk for infection more strongly than even neutropenia in some contexts (HSCT).[42] Oral mucositis is characterized by functional complaints, such as pain and difficulty in swallowing (dysphagia), and by anatomic changes, such as edema, erythema, ulceration, pseudomembrane formation, and alterations in mucus consistency with changes in saliva production (xerostomia). Gastrointestinal mucositis is accompanied by nausea and vomiting, diarrhea, intolerance for enteral nutrition, and abdominal pain. Mucositis results in significant morbidity and markedly lowers the quality of life for several weeks after cytotoxic chemotherapy and irradiation. Modern remission induction cytostatic chemotherapy and conditioning regimens for HSCT and GVHD regimens containing methotrexate often induce substantial injury to the mucosa, and reduced intensity conditioning may not lessen this risk.[43] Combinations containing melphalan, etoposide, methotrexate, cytarabine, and idarubicin have all been shown to induce mucositis, which can be very severe when anthracyclines are combined with total-body irradiation and cyclophosphamide to condition patients for HSCT or reinfusion.[44] Systemic drug exposure is the key determinant driving severe mucositis risk in

high-dose chemotherapy-treated recipients of autologous transplants.[45] Patients receiving a melphalan dose of 70 mg/m^2 or greater had a 23-fold increased risk for developing mucositis compared with those receiving lower doses.[46] The duration and incidence of fever, parenteral narcotic use, total parenteral nutrition, antibiotic therapy, and the length of stay in a hospital are all correlated with the severity of mucositis, as is the risk for significant infections and mortality.[45,47,48] It is likely that severe mucositis leads to a commensurate increase in the number of unusual bacteria causing infection by providing them with a portal of entry, which may explain the increase in bacteremia caused by oral commensal gram-positive cocci such as the oral viridans streptococci, *R. mucilaginosa,* and *Capnocytophaga* spp.[31,49,50] The consequences of probable dietary exposure in neuroinvasive *Bacillus cereus* infections in leukemic patients are supported by a case-control analysis that identified food contamination in all cases.[51] Besides damage to the oropharyngeal, esophageal, and gastric mucosa, chemotherapy and irradiation impair gut function and lead to rapid alterations in permeability. The increased absorption of sugars such as rhamnose, mannose, and lactulose and the decreased uptake of xylose after chemotherapy, irradiation, or a combination of both indicate a loss of integrity and damage to tight junctions.[52] Perturbed gut function has been shown to be one of the factors that, together with antibiotic use and colonization with *Candida* spp., predispose patients with leukemia to invasive candidiasis, and it also appears to be a risk factor for neutropenic enterocolitis. Impaired gut function and integrity may also facilitate translocation, particularly translocation of gram-negative bacilli such as *P. aeruginosa,* into the bloodstream of patients colonized with the organism.

Gut toxicity has also been shown to be responsible for the reduced absorption of quinolones[53] and has been implicated in the erratic bioavailability of the antifungal agents itraconazole and older formulations of posaconazole.[54] Finally, a dysfunctional gut will have a marked effect on the nutritional status of the patient, not least because of the lower release of citrulline, the nitrogen transporter of the human body, as a result of the reduced number of functioning enterocytes.[55]

Nutritional Status

Patients who weigh less than 75% of their ideal body weight or who have experienced rapid weight loss and have hypoalbuminemia are severely nutritionally deficient, which correlates inversely with survival. Poor nutritional status endangers the integrity of host defenses because of the catabolic state induced by cachexia and the malnutrition that results from anorexia, therapy-induced nausea and vomiting, gastrointestinal obstruction, altered permeability, mucositis, and metabolic derangements. A state of iron deficiency reduces the microbicidal capacity of neutrophils and T-lymphocyte function in vitro. Nutrition may be given parenterally or via a nasogastric tube to redress the balance, but neither is entirely without risk because each introduces yet another breach in the normal barriers and increases the risk for aspiration, particularly when consciousness is impaired.

Comorbidity and Host Defense

Psychological stress is thought perhaps to suppress host defense mechanisms. This possibility has been corroborated by observations that psychological stress has a negative influence on the function of T cells and NK cells. Indeed, stress appears to be connected with an increased risk for acute viral respiratory illness, a risk that was related to the amount of stress, most likely mediated by endogenous opioids, hormones from the hypothalamic-pituitary-adrenal axis, catecholamines, and cytokines. The gut microbiota also influence the brain and vice versa.[56]

Concomitant chronic illnesses enhance the risk for infection. Patients with a preexistent immune disturbance, such as a congenital immunodeficiency syndrome, are doubly jeopardized. The negative impact of smoking on patients with primary lung tumors is obvious and due to colonization of their airways with virulent encapsulated microorganisms and impaired clearance of secretions. Patients with diabetes mellitus are prone to genitourinary tract and wound infections, and they frequently have concurrent vascular disease and neuropathy. The proclivity to infection in patients with poorly controlled diabetes mellitus is not difficult to explain in view of aberrations such as impaired opsonization and decreased chemotactic activity of granulocytes and monocytes. Opportunistic bacteria and fungi depend on free iron for a growth advantage, and iron overload has been associated with infections with *P. aeruginosa,* staphylococci, *Vibrio vulnificus, Listeria monocytogenes, Yersinia enterocolitica,* and members of the order Mucorales.[57] In HSCT the risk for invasive mold disease has been related to iron overload.[58] Treatment of iron overload with deferoxamine predisposes to rhinocerebral and pulmonary mucormycosis by providing the fungus with an available source of iron.[59,60] The mechanisms of increased susceptibility to infection with iron overload are related to impairment of phagocytosis, chemotaxis, and cellular immunity.

Tumors themselves may predispose to infection through local organ dysfunction. In patients with solid tumors, obstruction of natural passages can lead to inadequate drainage of secretory or excretory fluids from nasal sinuses, bronchi, bile ducts, and so forth. Furthermore, tissue invasion may create connections between normally sterile spaces and the outside world through disruption of epithelial surfaces. Examples include perforation of the esophagus by mediastinal tumors, invasive gynecologic malignancies with local pelvic abscesses caused by gram-negative bacilli and anaerobes, skin ulceration with cellulitis and even deep soft tissue infections, and invasion of the bowel wall by tumors of the lower gastrointestinal tract with seeding of bacteria into the bloodstream. Central nervous system (CNS) tumors, spinal cord compression, and paraneoplastic neuropathy are associated with an increased risk for infection because of a diminished ability to cough and swallow or vomit and incomplete emptying of the bladder.

Treatment of malignancy inevitably damages healthy tissue. Even when the tumor is localized in a single area, relatively superficial, and readily removed, any surgery and local irradiation will nonetheless extend impairment of the normal defenses.

FEVER AND THE INFLAMMATORY RESPONSE

An infectious origin of a fever can be confirmed microbiologically or clinically in only 30% to 50% of all febrile neutropenic patients (Fig. 305.2). Infectious complications usually arise insidiously in these patients because of a muted inflammatory response and the lack of pus, which can be attributed to the absence of granulocytes; even the presence of chills or rigors does not always correspond with bacteremia.[61] However, foci of infections can also remain undetected because the physical examination

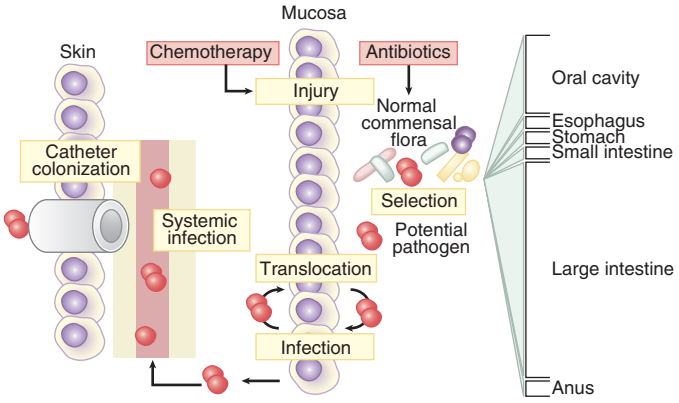

FIG. 305.2 Model to explain the origins of infection in neutropenic patients. Chemotherapy induces injury to the mucosal barrier. At the same time, antimicrobial agents used for prophylaxis (e.g., fluoroquinolones) exert selective pressure on the ecology, whereby more resistant members of the resident flora (e.g., viridans streptococci, staphylococci, gram-negative bacilli, and yeasts) increase in number. The gastric acid barrier of the stomach is usually breached by the use of drugs such as the histamine type 2 antagonists (e.g., ranitidine) and proton pump inhibitors (e.g., omeprazole) so that microorganisms that are ingested pass onward to the small intestine and beyond. Any colonization occurring on damaged mucosa would allow translocation from the alimentary tract or local infection, either of which can lead to bloodstream invasion and ultimately to systemic infection. In the case of the coagulase-negative staphylococci (e.g., *Staphylococcus epidermidis*), the alimentary tract might be the original source of bacteremia leading to colonization of the lumen of a vascular catheter.

was too cursory, specimens were either inappropriate or not collected at all, or the microbiologic investigations were incomplete or too insensitive. Obtaining a careful recent medical history and examining the patient thoroughly for any evidence of inflammation or infection should be considered obligatory, but getting a proper specimen is much more difficult because aspiration or biopsy is usually required, which is ill advised for thrombocytopenic patients. Besides, even when a specimen is obtained from a normally sterile site, the yield is generally low. Consequently, failure to undertake an adequate physical examination and to obtain appropriate samples will result in fever being unexplained. Yet the fact that most patients without any proven infection improve clinically after treatment with broad-spectrum antibacterial agents suggests the presence of an occult bacterial infection in many cases. However, noninfectious inflammatory complications of cancer therapy and transplantation contribute to episodes of unexplained fever because mucositis and GVHD are known to be inflammatory complications.[62,63]

Systemic Inflammatory Response Syndrome, Immune Reconstitution Inflammatory Syndrome, and Host-Directed Therapy (See Chapters 49 and 73)

Although neutropenic patients cannot mount a normal host response to tissue injury, the systemic inflammatory response syndrome and sepsis can and do develop when inflammation is uncontrolled and proinflammatory substances spread to other distant sites, ultimately leading to multiple organ dysfunction. Oral viridans streptococci elicit a proinflammatory response that may play a role in the pathogenesis of shock[64] but is associated with life-threatening complications, including septic shock and acute respiratory distress syndrome (ARDS). High-dose cytarabine in particular appears to predispose to these conditions.[65,66] Systemic inflammatory response syndromes are not always the result of endotoxemia or even infection and can be brought about by noninfective tissue injury, such as severe burns, acute pancreatitis, elective surgery, and trauma. They also result from extensive mucositis because the levels of some proinflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α, are elevated and parallel the course of mucosal damage after chemotherapy and conditioning therapy for HSCT. The degree of gastrointestinal mucositis after conditioning therapy as measured by low citrulline levels strongly correlates with the inflammatory response seen after HSCT and the occurrence of ARDS induced by viridans streptococci.[63] Determination of C-reactive protein and cytokines such as IL-6 is recommended by some for diagnosing bacterial infection,[67,68] whereas others remain unconvinced.[69,70] Fever as a symptom of a systemic inflammatory response can be measured, as can C-reactive protein, lipopolysaccharide-binding protein, and IL-8. The inflammatory response might precede bacteremia by several days.[71] Earlier observations indicated that ARDS associated with *S. mitis* bacteremia could be prevented through preemptive use of corticosteroids, pointing toward an imbalance in host-related inflammatory response.[72] Among Spanish patients admitted with severe community-acquired pneumonia and a high initial inflammatory response, the acute use of methylprednisolone compared with placebo decreased treatment failure.[73] Treatment failure was defined as development of shock, need for invasive mechanical ventilation, or early death. The proper and timely use of corticosteroids is still debated, as blocking the production of potent inflammatory cytokines activating local and systemic inflammatory processes that are involved in protective immune responses against infections could be detrimental instead. However, their dysregulated production and signaling can aggravate tissue damage during infection, inflammatory diseases, and chemotherapy-induced mucositis.

Corticosteroids are also used in the advent of an immune reconstitution inflammatory syndrome (IRIS). Increasing evidence suggests that chronic disseminated candidiasis is a form of IRIS.[74] A retrospective multicenter study of patients with symptomatic chronic disseminated candidiasis with fever unresponsive to antifungal therapy suggested that systemic corticosteroids for at least 3 weeks can result in rapid clinical improvement. The duration of antifungal therapy and corticosteroids in symptomatic chronic disseminated candidiasis patients has been reported to be as long as several months. The hallmark of IRIS is an increasing number of cytokine-activated neutrophils and monocytes producing myeloperoxidase and oxygen radicals that might promote clinical deterioration. But normalization of $CD4^+$ helper T cells (Th1 and Th17) and $CD8^+$ cytotoxic T cells after successful cancer therapy in general, and more specifically after HSCT, can occur. It is worth noting that new therapeutic modalities, such as bidirectional T-cell contacts and CAR T cells, may induce noninfectious inflammatory states—that is, the cytokine release syndrome.[75] In some cases, the pathophysiology will include defined roles of cytokines, such as IL-6, which limits their usefulness in the diagnosis of an infectious episode, and excessive cytokine release syndrome sometimes requires anti-IL-6 directed therapy (tocilizumab).[76] Nowadays the use of immune checkpoint inhibitor therapy of cancer (PD-1/PD-L1 antibodies) can induce severe immune-related organ toxicities that are difficult to distinguish from real infections and necessitate immediate immunosuppressive therapy. Of course, this latter use will increase the likelihood of opportunistic infections.[77]

On the other hand, host-directed therapeutic strategies are now becoming viable adjuncts to standard antimicrobial treatment. Host-directed therapies include commonly used drugs for noncommunicable diseases with good safety profiles, immunomodulatory agents, biologics (e.g., monoclonal antibodies), nutritional products, and cellular therapy using the patient's own immune or bone marrow mesenchymal stromal cells.[78]

INFECTIONS BY SITE

Bacteremia

Blood cultures are usually the most productive microbiologic investigation and help explain 10% to 40% of fevers, but the sensitivity of blood cultures is crucially dependent on the volume of blood cultured, with 30 to 40 mL per session being recommended for optimal results.,[79–83] It has become common practice to draw at least 10 mL of blood for culture by venipuncture when investigating fever immediately before starting empirical therapy, together with at least 10 mL through each lumen of a central vascular catheter when fever is present. During the past decade a change from gram-negative to gram-positive bacteria has occurred,[84] but virtually any microorganism can cause infection in severely immunosuppressed patients, depending on a variety of factors, including the type of cancer therapy, the use of antimicrobial agents, and whether the patient is at home or in the hospital. Infusion of the appropriate antibiotic through catheter ports that are culture positive may be helpful in clearing colonized catheters. Once therapy is started, the value of further blood cultures is small if the patient is responding clinically. Patients with catheter-acquired sepsis who remain febrile with positive blood cultures usually need their catheters removed. In the past, at least in clinical trials, blood cultures have been repeated 3 or 4 days after the start of empirical therapy, as a means of detecting persistent bacteremia.

Bacteremia Related to Intravascular Catheters

Coagulase-negative staphylococci are still the most common cause of catheter-acquired sepsis,[85] but they are also recovered from catheter-drawn blood under circumstances that suggest they are not causing the fever that prompted the blood culture. It is always easier to interpret the results of culturing of blood drawn from a peripheral vein. Simultaneous quantitative blood cultures from the catheter and a peripheral vein have been advocated but have not convincingly enabled discrimination between catheter-acquired sepsis, sepsis from another source, asymptomatic intraluminal colonization of the catheter, and accidental contamination that occurred while blood was being drawn from the catheter hub. The usefulness of differential time to positivity of blood cultures drawn through a catheter lumen in relation to those drawn simultaneously by venipuncture to distinguish "true bacteremia" from catheter-related bacteremia is debated.[86,87] In all cases, the decision to treat is based not just on the blood culture but also on the clinical findings. Infections associated with intravenous catheters are assumed to mostly originate from contamination of the catheter hub. The contaminated catheter may have already been removed since the culture was obtained, or the culture may have been contaminated at the time that blood was drawn. In afebrile patients, repeating blood cultures may be all that is indicated. In other cases, the organism can be cultured repeatedly from blood drawn through the same catheter port but not from blood drawn

peripherally, thus suggesting that the organism has not yet been able to cause sepsis but has colonized the catheter. Removing the device or administering antibiotics through the same port may be indicated to circumvent future sepsis[88] (see Chapter 300).

Bacteremia From the Gastrointestinal Tract

Oral viridans streptococcal bacteremia is related to mucosal barrier injury of the upper part of the digestive tract, particularly the oral cavity, whereas enteric gram-negative bacillary bacteremia and neutropenic enterocolitis are related to the lower part of the digestive tract. The digestive tract has long been implicated as the principal origin of infections caused by the enteric gram-negative bacilli, including *Escherichia coli, K. pneumoniae,* and *Enterobacter* spp., providing the motivation for adopting prophylaxis with fluoroquinolones.[89]. Especially in patients who prophylactically receive oral quinolones, oral viridans streptococci, usually *S. mitis,* will survive on damaged mucous membranes.[31,72] The signs and symptoms of viridans streptococcal infection might be inconspicuous to completely absent. In view of the direct correlation between the rate of positive blood cultures and the severity of damage to the mucosal surface, the normal habitat for these organisms, it is questionable whether viridans streptococci are true pathogens in all cases or whether they represent only an epiphenomenon.[63] In fact, during the course of a normal day, the acts of chewing and tooth brushing lead to transient bacteremia caused mainly by viridans streptococci. However, significant gastrointestinal mucositis has also been shown to predispose to bacteremia with coagulase-negative staphylococci and viridans streptococci.[90] Various coagulase-negative staphylococci, including *Staphylococcus epidermidis,* are also present in the endogenous microbiota of the digestive tract of neutropenic patients.[91] Plasmid pattern analysis of coagulase-negative staphylococcal bloodstream isolates showed that the mucosa was the origin of bacteremia in 70% of patients managed in a hematology ward of one center.[92] However, there is clear evidence that the coagulase-negative staphylococci responsible for bacteremia can originate from both the mucosa of the oral cavity and the gut, as well as from the catheter.[93] The use of new definitions of bloodstream infections (BSIs) such as CLA-BSI (central line–associated BSI, stratified as mucosal barrier injury–related, laboratory-confirmed BSI [MBI-LCBI] versus non–MBI-LCBI) and secondary BSI, using National Healthcare Safety Network (NHSN) definitions, could help in a patient-centered, clinically relevant approach to better assess across-center and within-center differences in infection rates, including CLA-BSI.

Oral Cavity Infections

The oral cavity is a complex region providing other likely portals of entry besides mucositis, which essentially affects the nonkeratinous areas of the inner lips, buccal mucosa, underside of the tongue, and roof of the mouth. Moreover, mucosal changes normally progress to a peak severity, coinciding independently with the nadir of bone marrow aplasia, and then begin to recover as hematopoiesis returns.[62] Extensive mucosal damage is often accompanied by a decline in saliva production, leading to dry mouth. Any mucus produced may be extremely viscous and difficult to either swallow or cough up. Periodontal disease may be exacerbated, and minor oral cuts and abrasions may become inflamed and ulcerated. The health of the periodontium probably also plays a role. For instance, organisms found in periodontal pockets, including viridans streptococci, appeared in the bloodstream of 13 (65%) of 20 patients soon after probing.[94] Tooth brushing[95,96] and more invasive procedures, including tooth extraction and periodontal and endodontic treatment, can also lead to transient bacteremia. Important to note, a recent prospective study supports the hypothesis that chronic oral foci of infection can be left untreated because this does not increase infectious complications during intensive chemotherapy.[97,98] Not surprisingly, the origin of several different species of bacteria-causing bacteremia has been traced to the oral cavity. These include, in addition to the oral viridans-group streptococci, *Fusobacterium* spp. and the related *Leptotrichia* spp.,[99] and even *S. epidermidis*.[92,100] Oral infections are difficult to diagnose by appearance in patients with mucositis. Polymerase chain reaction (PCR) assay (culture if drug resistance is suspected) of oral lesions for herpes simplex virus (HSV), even in the presence of prophylaxis, and smears of scrapings for *Candida* pseudohyphae can be helpful.

Respiratory Tract Infections

The proliferation of diagnostic assays, usually molecular, often available as multiplex panels, has increased the ability to detect organisms. The ability to clearly associate the findings from such assays with clinical disease remains a challenge. Nevertheless, previously innocuous pathogens such as rhinoviruses or even viruses (human herpesvirus 6 [HHV-6]) not considered respiratory pathogens are now detected in the lower respiratory tract of severely compromised patients and are associated with disease, although causality, as so often is the case in complex settings, has not been established.[101,102]

Apparently trivial complaints such as a persistent dry cough may prove to be early signs of impending pneumonia from *Aspergillus* spp., respiratory syncytial virus, or influenza virus. Thoracic computed tomographic scans are more sensitive in detecting pulmonary infiltrates compatible with aspergillosis than are plain chest radiographs.[103,104] Bronchoalveolar lavage specimens from patients with pulmonary infiltrates should undergo a battery of tests that usually includes smears for *P. jirovecii,* acid-fast bacilli, and *Nocardia* spp., bacteria, and molds, and culture for fungi and bacteria, including *Legionella* spp., *Mycobacterium* spp., *Nocardia* spp., and respiratory viruses (influenza and parainfluenza viruses, adenovirus, respiratory syncytial virus, and cytomegalovirus [CMV]). *Aspergillus* galactomannan antigen can also be detected in bronchoalveolar specimens, although the sensitivity and specificity of the test on respiratory specimens are unclear.[105,106,107] Changing patterns in prophylaxis in transplantation may explain the need to prolong the period of suspicion for CMV disease after lung transplantation.[108] Rapid assays by enzyme-linked immunosorbent assay, direct fluorescent antibody, or dot blot are also available for influenza virus, respiratory syncytial virus, and adenovirus but are often replaced by PCR now. Nasopharyngeal swabs in children and nasopharyngeal washes in adults, formerly used in culturing respiratory viruses in patients with upper respiratory symptoms, are now being tested with molecular panels, with results available within hours. These panels are also used on lower respiratory tract specimens. Molecular assays for *Pneumocystis* pneumonia await an international standard and further work to establish the appropriate thresholds for organism burden sufficient to diagnose disease, as reported in a multiinstitutional study from France.[109] Testing for *Legionella* antigen in the urine of patients with pneumonia is also useful when local epidemiology suggests that *Legionella pneumophila* type 1 is the predominant clinical isolate. *Toxoplasma gondii,* most commonly associated with CNS (including the eye) disease, may be an agent of aggressive pulmonary or disseminated disease, and PCR as a diagnostic tool with both cerebrospinal fluid (CSF; for CNS disease) and blood may allow early detection and treatment.[110]

Skin Infections

Identifying the cause of skin and underlying soft tissue infections is equally difficult because culturing swabs of lesions rarely discriminates between pathogens and commensal microbiota. As an exception, Gram stain and culture from pus expressed from a catheter exit site can be useful. Culture and histologic examination of skin punch biopsy specimens is very helpful in the diagnosis of isolated maculopapular or ulcerated lesions. Disseminated infections by *Candida* spp., *Trichosporon* spp., and *Fusarium* spp. in a neutropenic patient may manifest with skin lesions, while blood cultures remain negative. Skin lesions may be the source of *Fusarium* fungemia.[111] Ecthyma gangrenosum from *P. aeruginosa* may be accompanied by positive blood cultures, but *Aspergillus* spp. or the agents of mucormycosis can cause lesions with the same appearance in a neutropenic patient with negative blood cultures. These fungal lesions require biopsy for diagnosis. Aspiration of skin lesions is seldom successful unless pus is present. Cutaneous *L. pneumophila* and *Legionella longbeachae* (usually associated with potting compost), may occur in the context of transplant or chronic corticosteroid use. Biopsy is necessary to make the diagnosis.[112,113] New associations with specific immunosuppression may aid in the understanding of the physiology of some cutaneous infestations, as demonstrated by a nosocomial outbreak of Norwegian scabies in patients under TNF-α inhibitor treatment.[114] *Demodex* mites may proliferate in conditions that are associated with sebaceous gland dysfunction and cause a pruritic rash that resembles rosacea but may also be mistaken for GVHD.[115] Firm

diagnostic criteria to distinguish colonization of the skin from disease caused by the mites is lacking, although a heavy burden of infestation may suggest that a trial of ivermectin is appropriate.

The increasing breadth of defined immunocompromising conditions has also increased the understanding of the role of human papillomaviruses (HPVs) in skin disease, both warts and cancer. Careful screening for HPV and aggressive medical and surgical follow-up and vaccination are now commonly implemented.[116]

Gastrointestinal Tract Infections

It is difficult to diagnose enteric infection, especially when nausea, vomiting, diarrhea, bowel cramps, and melena can all be due to toxicity related to chemotherapy, irradiation, GVHD, or primary immunodeficiency. The role of norovirus continues to expand as a pathogen in patients with immunodeficiencies such as X-linked severe combined immunodeficiency who lack adequate humoral reconstitution after transplantation, in common variable immunodeficiency, and occasionally after solid-organ or hematologic malignancy transplantation.[117,118] Endoscopy can be critical in the diagnosis of herpes simplex, *Candida* spp., and CMV mucosal lesions. Molecular diagnostic panels that identify multiple targets retain the same caution as noted earlier; the virtue of high analytical sensitivity may be offset by the identification of infectious agents that are not the cause of disease, and there is insufficient guidance in current clinical experience to solve this problem. Detection of HSV and CMV in intestinal biopsy specimens can be enhanced if both culture and immunoperoxidase staining of the tissue are done. The gut microbiota can become the major reservoir of vancomycin-resistant enterococci, *Candida albicans, K. pneumoniae*, and *P. aeruginosa.* Organisms can be spread from the bowel onto the skin and, by the fecal-oral route, into the mouth. Skin organisms can be spread to other patients by the hands of health care workers and, through intravenous catheters, into a patient's bloodstream. Oral microbiota can be aspirated into the airway, particularly when the patient is intubated. Passage of bowel organisms into the bloodstream can occur through chemotherapy-induced ulcers. Fever in the presence of diarrhea or abdominal pain should prompt a PCR assay on stool for the *C. difficile* toxin gene. That test has largely replaced the more cumbersome cytotoxicity test. The enzyme immunoassay for toxin is less sensitive. Neutropenic patients with right lower quadrant pain may have developed neutropenic enterocolitis, which predominantly localizes in the cecum, the diagnosis of which can be supported by an edematous colonic wall on abdominal contrast-enhanced computed tomography. Recovery of *C. septicum* from the bloodstream usually portends neutropenic enterocolitis. In view of the extent of the damage in many cases, it is not surprising that *C. septicum* and *Clostridium perfringens* septicemia, classically with massive hemolysis and diffuse intravascular coagulation, can arise,[119] but also various toxin-producing bacteria, including *S. aureus, P. aeruginosa,* and even *B. cereus.*[120–123] Neutropenic enterocolitis is also an independent risk factor for invasive candidiasis. Colonization by *Candida* spp. of the mucosal surfaces appears to be a prerequisite for local mucosal infection and subsequent invasive disease.[124] Mucosal barrier injury and exposure to antimicrobial agents may explain the emergence of most infections arising in neutropenic patients (Fig. 305.3).

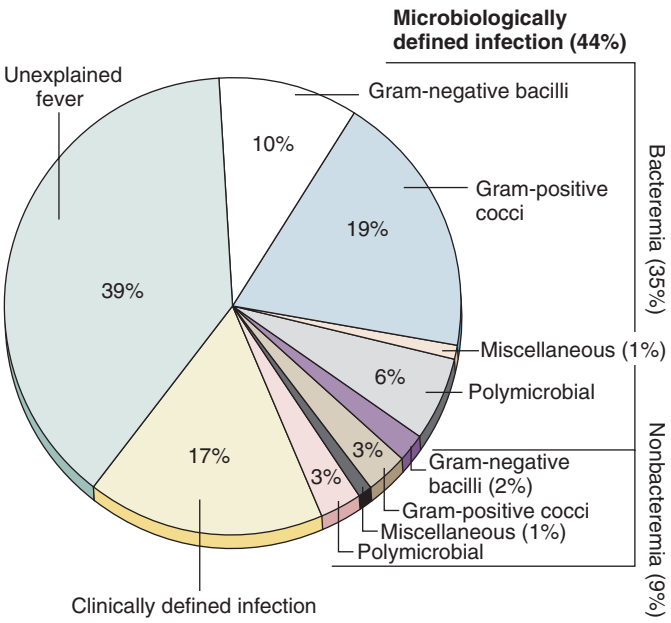

FIG. 305.3 Causes of infection in 968 episodes of fever and neutropenia. *(Unpublished data derived from the study of De Pauw BE, Deresinski SC, Feld R, et al. Ceftazidime compared with piperacillin and tobramycin for the empiric treatment of fever in neutropenic patients with cancer: a multicenter randomized trial.* Ann Intern Med. *1994;120:834–844.)*

Urinary Tract Infections

Urine should be obtained for standard culture when there are signs or symptoms of a urinary tract infection, but not otherwise. Urine from patients after HSCT who have hemorrhagic cystitis should be tested for adenovirus by culture, and BK virus quantity by PCR assay. Although classically a cause of nephritis in the transplanted kidney, native organ disease caused by BK virus after solid-organ and hematopoietic transplantation has been reported.[125,126] Urinary tract infections, although rare in the absence of a Foley catheter, can be initiated through hematogenous spread from the gut and by means of perianal or vaginal contamination.

Central Nervous System Infections

Infections of the neuraxis can be difficult to diagnose in patients whose thrombocytopenia is not sufficiently correctable by platelet transfusion to permit lumbar puncture. CSF is useful not only for microbial culture but also for testing of cryptococcal polysaccharide, *Aspergillus* galactomannan, flow cytometry or cytology for malignant cells, and PCR for JC virus, CMV, Epstein-Barr virus (EBV), and HHV-6. CNS infection is usually hematogenous, although direct infection can occur as a rare complication of neurosurgery, intrathecal cancer chemotherapy, or extension of invasive mold sinusitis. Magnetic resonance imaging is the imaging procedure of choice, particularly with fluid-attenuated inversion recovery (FLAIR) imaging. Positron-emission tomography (PET) scan with ^{18}F-fluorodeoxyglucose is of limited usefulness in distinguishing malignant from infectious brain lesions in cancer patients, in that both can be PET avid.

Diagnosis can be challenging in the absence of CSF or brain biopsy, but diagnosing a brain abscess in an immunosuppressed patient based on diagnosis of a lung lesion with a probable agent, such as *Nocardia*, *Aspergillus*, or *Mucorales,* may be sufficient. Clinical syndromes are rarely diagnostic, although the posterior reversible encephalopathy syndrome (PRES) is distinctive. Limbic encephalitis caused by HHV-6 should be considered in allogeneic stem cell transplant patients, particularly those receiving cord blood cells, who have memory loss, decreased consciousness, and seizures.[128] The use of OKT-3 or alemtuzumab for in vivo T-cell depletion is associated with an increased risk of viral encephalitis after allogeneic stem cell transplantation. Different viruses (HHV-6 [28%], EBV [19%], HSV [13%], JC virus [9%], varicella-zoster virus [VZV; 6%], CMV [6%], and adenovirus [3%] in 32 patients with a viral encephalitis out of 2628 HSCT patients) are frequently associated with distinct characteristics, such as onset time, response to treatment, and outcome. Patients with HSV encephalitis had the most favorable outcome, with no encephalitis-related deaths.

Liver Infections

The presence of markers of hepatic inflammation or biliary tract obstruction should initiate a search for infectious etiologies. Serologic screening for acute disease in this context is insufficient, and PCR for hepatotropic viruses should include hepatitis B, C, and E (if available), in addition to organ-specific or disseminated infections with adenovirus and CMV.

Two considerations related to hepatotropic viruses deserve emphasis. First, the course of infection with a virus in the immunocompromised patient can be aggressive (hepatitis A and E) or lead to chronicity (hepatitis E).[129,130] Although the incidence of development of acute

hepatitis E after allogeneic HSCT is relatively low, the probability of development of chronic hepatitis with genotype 3 in severely immunocompromised patients is high. Therefore, before transplantation, allogeneic HSCT recipients from countries where genotype 3 is prevalent should be screened with hepatitis E virus (HEV) serology and RNA. Furthermore, a differential diagnosis, including hepatitis E, is mandatory in all allogeneic HSCT patients with severe liver enzyme abnormalities. Second, immunodeficiencies and chemotherapy (especially B-cell–depleting agents) may allow the recrudescence or progression from asymptomatic chronicity or latency of hepatitis B virus. Thus, screening for hepatitis B antecedent to iatrogenic immunosuppression is recommended, and prophylactic strategies include anti–hepatitis B immunoglobulin (usually after orthotopic liver transplant) and antiviral agents such as entecavir, which may be preferred over older agents such as lamivudine.[131,132]

SEQUENCE OF INFECTIONS

The sequence of risk factors (Fig. 305.4) determines to a large extent the order of infectious events in granulocytopenic cancer patients, which means that the types of infection occurring early in the granulocytopenic period differ from late infectious complications. Profound neutropenia and mucosal damage usually ensue approximately 10 days after initiation of a course of chemotherapy and are followed by fever and bacteremia within a few days, when toxicity is at its most pronounced. Clinically defined infections usually lag behind by a few days. Invasive pulmonary aspergillosis develops in a few patients early in the course of neutropenia.[133] Infections related to central venous catheters have to be considered approximately 10 days after their insertion, with the risk increasing with the length of time that the catheter is left in place.[85]

The initial risk period resolves with recovery of the granulocyte count. Very intensively treated patients are still at risk because of granulocytopathy, deficient cell-mediated immunity, and hypogammaglobulinemia. The kind of infectious complications for such patients is determined by the pace of reconstitution of these other components of the immune system (Fig. 305.5).[134] The major factor that influences immunologic reconstitution after allogeneic stem cell transplantation is acute GVHD and its treatment. CMV, adenovirus, and fungi, including *P. jirovecii,* constitute the major pathogens during this episode. A third major risk period in these patients begins approximately 3 months after allogeneic stem cell transplantation, when chronic GVHD develops. Sinopulmonary infections and cutaneous infections are common and probably related to the IgA deficiency, with or without sicca syndrome and severely impaired cellular immunity. Varicella-zoster is probably the most frequent cutaneous infection, and pulmonary infections caused by CMV and *P. jirovecii* are regularly encountered. Months, if not years, after successful engraftment or recovery from other very aggressive treatment, encapsulated organisms can cause rapidly fatal bacteremia and severe respiratory tract infections because of the lack of opsonizing antibodies.

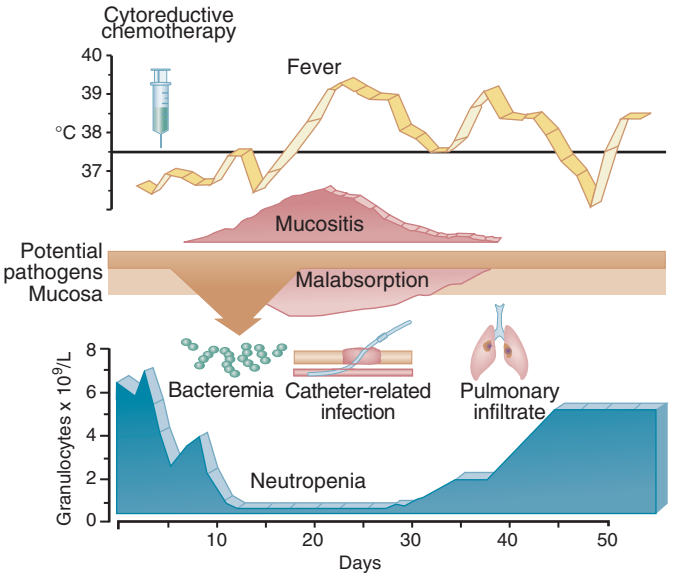

FIG. 305.4 Sequence of events during neutropenia. Profound neutropenia and mucosal damage usually develop approximately 1 week after the start of cytoreductive chemotherapy. Thereafter, infectious and other complications tend to coincide with one another, placing the patient at greatest risk. Fever develops approximately 1 week later, and if there is bacteremia, it mostly occurs at this time. The risk for infections related to the central venous catheter increases with the length of time that the catheter is left in place, but signs and symptoms usually manifest during the first few days of fever—that is, during the third week after the start of chemotherapy. Infectious complications related to the lung tend to occur a few days later and often are recognized only after 5 or 6 days of fever. The period of risk for bacterial and fungal infection diminishes with recovery of the granulocytes, when the clinical manifestations of tissue infections may be temporarily exacerbated before finally resolving.

ROLE OF MOLECULAR DIAGNOSTICS IN INFECTIONS IN THE IMMUNOCOMPROMISED HOST

Molecular diagnosis, most often PCR based, continues to increase in frequency and breadth. The labor and material burdens of traditional culture-based diagnostics limit their use, and the appearance of multiplex panels with little need for operator training explains the proliferation of this technology. Panels for the detection of viral, bacterial, and fungal pathogens are in use for respiratory, gastrointestinal, blood, and CSF specimens, with the determination of drug susceptibility targets not far behind. In considering molecular diagnostics in the immunocompromised host, several considerations should be taken. First, a broad literature that assesses potential differences in sensitivity and specificity by host predisposition is generally lacking. Second, the immunocompromised host may provide a substrate with competing diagnostic considerations (e.g., GVHD in hematologic transplant) that make the issue of specificity crucial. Third, drug susceptibility testing tends to still be culture derived, and so limiting diagnosis to molecular assays, until such assays include well-defined approaches to resistance determinants, has flaws. Finally, a lack of specificity may be an important flaw when assessing results as positive for rhinovirus or enterovirus, because specific viruses or strains may be important in different contexts (enterovirus D68 in recent outbreaks of severe respiratory tract disease in hematologic or transplant patients or coxsackievirus in CNS disease in patients with rituximab-induced loss of humoral immunity).[135,136]

Nevertheless, these sensitive assays improve our understanding of the range of pathogens whose role in disease processes must be understood. Furthermore, the sensitivity of molecular diagnostics for rapid detection of pathogens to prevent nosocomial spread is a major advance. This is demonstrated for pathogens newly discovered (a novel poxvirus associated with equine exposure) or not previously commonly diagnosed (human metapneumovirus). These sensitive tests may help to define possible transmission patterns *(P. jirovecii)*[137–139] and understand persistence of infection with agents such as influenza virus, norovirus, or varicella virus (vaccine strain), whose course may be atypical and prolonged in some patients.[140–142] Finally, new pathogens such as astrovirus VA1/HMO-C, reported as a cause of encephalitis, are now identified through use of deep sequencing, a technology whose general application is yet to be determined.[143]

CONCLUSION

Bacterial infections were once a major obstacle to the treatment of patients with acute leukemia. Improvement in management strategies, including the use of broad-spectrum antibacterial drugs targeting gram-negative bacteria, has reduced the mortality in neutropenic patients who develop bacteremia and other severe infections. In many countries these achievements are threatened by development of multidrug-resistant bacteria, such as *K. pneumoniae, P. aeruginosa,* and methicillin-resistant *S. aureus,* but also triazole-resistant *Aspergillus* spp. New approaches are required in order to overcome "old" infectious complications in immunocompromised hosts.[144]

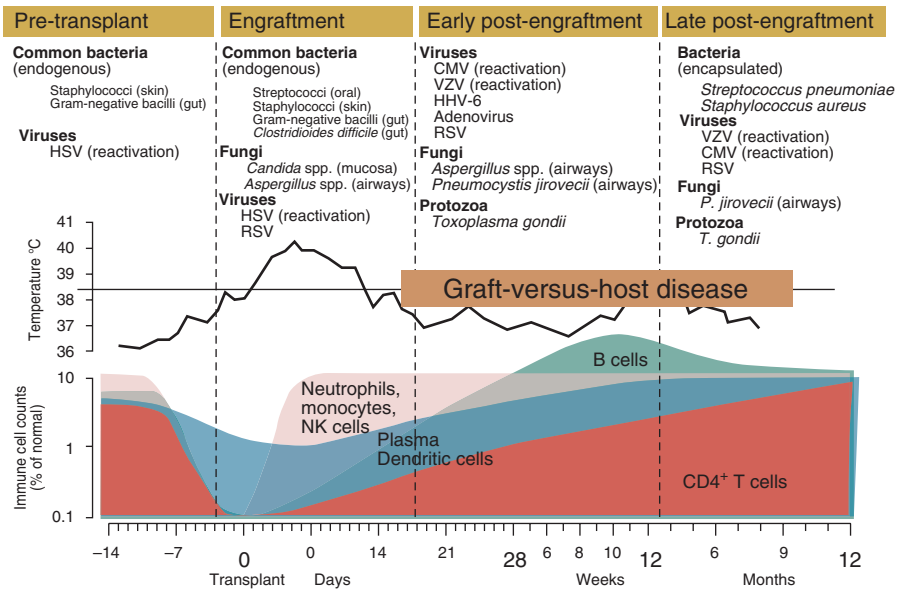

FIG. 305.5 Infectious phases after transplant. The infectious complications that occur most commonly are set against the phases before and after an allogeneic hematopoietic stem cell transplant. Note that the time scale progresses from days to weeks, then to months. The fluctuations in the different components of the immune system are represented at the bottom of the figure and show when severe deficiency coincides with the time of engraftment and begins to recover thereafter. The various classes of microorganism change during the different phases, but note that viruses and fungi can cause infections throughout the entire period. *CMV,* Cytomegalovirus; *HHV-6,* human herpesvirus 6; *HSV,* herpes simplex virus; *NK,* natural killer; *RSV,* respiratory syncytial virus; *VZV,* varicella-zoster virus.

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306 Prophylaxis and Empirical Therapy of Infection in Cancer Patients

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SHORT VIEW SUMMARY

RISK FACTORS FOR INFECTIONS IN CANCER PATIENTS

- Neutropenia is the most important, particularly if severe (<100 polymorphonuclear neutrophils) and prolonged (7–10 days).
- Other risk factors include mucositis; underlying disease and its status; intensity of chemotherapy; the use of targeted and biologic therapies, especially monoclonal antibodies, such as alemtuzumab or rituximab; surgical procedures; devices such as central venous catheters; neoplastic obstruction; and genetic factors.

EPIDEMIOLOGY AND ETIOLOGY

- Epidemiology of bloodstream infections in neutropenia is constantly changing, and after years of predominance of gram-positive cocci, gram-negative rods have been emerging in many centers as the most frequent pathogens.
- This shift has been accompanied by an increasing rate of resistant pathogens, such as extended-spectrum β-lactamase–producing Enterobacteriaceae, carbapenem-resistant gram-negative pathogens, methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci, and vancomycin-resistant enterococci.
- The main challenge is the management of multidrug-resistant (MDR) gram-negative bacteria for which few therapeutic options exist.
- Invasive fungal diseases (IFDs) in hematology patients are caused mainly by *Aspergillus* species. Probably due to the widespread use of fluconazole prophylaxis, *Candida* is less frequent in hematology, but fluconazole-resistant strains are emerging; patients with solid tumors remain at risk for candidemia; and resistant or new *Candida* strains, or both, have been reported (echinocandin-resistant *Candida glabrata* or *Candida auris*).

PROPHYLAXIS (see Table 306.4)

- The role of fluoroquinolone antibacterial prophylaxis during neutropenia in an era of widespread fluoroquinolone resistance and increasing multidrug resistance among gram-negatives is controversial.
- In general, antifungal prophylaxis is indicated in patients receiving induction chemotherapy for acute myelogenous leukemia (AML), in patients with myelodysplastic syndromes undergoing high-intensity treatment, and in selected groups of cancer patients, if the incidence of IFD is approximately 8% to 10% or higher.
  - Posaconazole as mold-active prophylaxis is recommended for patients receiving induction chemotherapy for AML or myelodysplastic syndrome. Voriconazole is an alternative.
  - Fluconazole is recommended as yeast-active prophylaxis in AML patients receiving anthracycline regimens. It should be combined with an intense mold-directed diagnostic approach in centers with high baseline incidence of mold infections.
- Secondary antifungal prophylaxis should be administered to patients with previous IFD who receive high-intensity chemotherapy or a transplant.
- Prophylaxis against *Pneumocystis jirovecii* (usually with trimethoprim-sulfamethoxazole three times per week) is beneficial in patients with deficits of T-cellular immunity, particularly with chronic lymphocytic leukemia, or in those receiving high-dose corticosteroids, fludarabine, or alemtuzumab. Anti-*Pneumocystis* prophylaxis is also warranted in case of certain novel targeted therapies or novel combinations, such as idelalisib or bendamustine plus rituximab.
- Antiherpes prophylaxis with acyclovir or valacyclovir is recommended in patients with acute leukemia or receiving drugs such as alemtuzumab and bortezomib.
- Varicella postexposure prophylaxis with specific immunoglobulins and/or acyclovir is recommended for high-risk varicella-zoster virus–susceptible patients.
- Antiviral prophylaxis of hepatitis B virus (HBV) reactivation is recommended for cancer patients with chronic inactive hepatitis B (hepatitis B surface antigen [HBsAg] positive, HBV DNA <2000 IU/mL, normal aminotransferases) and for selected patients with a resolved HBV infection (HBsAg negative/hepatitis B core antibody [HBcAb] positive), particularly if receiving anti-CD20 therapies (see Chapter 145). Frequent HBV DNA testing and antiviral therapy in case HBV DNA becomes positive is the alternative for those with a resolved HBV infection.

Immunization

- Influenza and pneumococcal vaccination of patients, especially during less aggressive treatment phases, is recommended.
- Influenza and varicella vaccination of household contacts and health care workers is recommended.

MANAGEMENT OF FEBRILE NEUTROPENIA (see Figs. 306.2 and 306.3)

Initial Approach

- Blood cultures, including at least one set from the central venous catheter, if present
- Assessment of the risk of severe infection (e.g., Multinational Association for Supportive Care in Cancer score; see Table 306.5)
- Assessment of the risk of infection caused by resistant pathogens; risk is high in case of:
  - Colonization or previous infection caused by resistant bacteria
  - Local epidemiology with high incidence of infections caused by resistant pathogens

Choice of Appropriate Therapy: Antibacterial

- Oral versus intravenous depending on inpatient or outpatient setting and the risk of complication (e.g., Multinational Association for Supportive Care in Cancer score; see Table 306.5)
- Escalation versus deescalation strategy
  - Escalation strategy: start with anti-*Pseudomonas* β-lactam monotherapy, usually other than a carbapenem.
  - Deescalation strategy: Usually start with a carbapenem in combination with vancomycin or an aminoglycoside or both, and then deescalate to piperacillin-tazobactam or a third-generation cephalosporin depending on clinical outcome and microbiologic results. In patients colonized with MDR gram-negative organisms, start with appropriate agents targeting the colonizing pathogen (e.g., novel anti–gram-negative agents or combinations of antibiotics) and then deescalate as noted previously if no MDR pathogen is isolated.

Choice of Appropriate Therapy: Antifungal

- Empirical antifungal therapy (adding antifungal agent in patients persistently febrile despite broad-spectrum antibiotics) could be replaced by diagnostic-driven strategy based on the use of diagnostic tools, such as computed tomography scan and fungal serum markers (galactomannan and β-D-glucan).

SHORT VIEW SUMMARY—cont'd

OTHER THERAPEUTIC RECOMMENDATIONS

- In the era of increasing antibiotic resistance and shortage of agents active against MDR pathogens, antimicrobial stewardship in cancer centers is mandatory and should include:
  - Infection-control practices
  - Local surveillance of antibiotic resistance, antibiotic consumption, and patient outcomes
  - Promoting appropriate antibiotic use (e.g. timely de-escalation or discontinuation, appropriate dosing)
  - Establishing antibiotic regiments for empirical therapy according to local epidemiology

Cancer patients probably represent the best example of how both a disease and its treatment can impair the complex immunologic network aimed at maintaining the integrity of our body and defending it against infections from both the external and the internal environment. For decades we have known that a granulocyte count of less than 500 cells/mm^3 (and especially <100 cells/mm^3) is associated with an increased risk of severe bacterial and fungal infections.[1,2] There is also evidence that patients with a granulocyte count between 500 and 1000 cells/mm^3, especially if rapidly decreasing, are also at high risk of infectious complications, because neutropenia is not a static but a dynamic concept. Indeed, a survey on fever during neutropenia in children with cancer showed the presence of severe infectious complications (e.g., bacteremia or invasive mycosis) in patients with a granulocyte count that never dropped below 500 cells/mm^3, suggesting the presence of a "gray zone" that should be carefully monitored.[3] The other three main factors that impact the risk of infectious complications in these patients are the damage of anatomic barriers, such as skin or mucosal membranes; the alteration of the microbiota diversity; and the presence of indwelling devices. Indeed, mucositis itself might result in severe infections due to microbial translocation, even in the absence of neutropenia, and a central venous catheter (CVC) may facilitate the entrance of endogenous and exogenous bacteria and fungi in the bloodstream or in subcutaneous tissues. The alteration of the microbiota diversity has been recently shown to affect the risk of infection and other important outcomes in hematology, in a way that is still not properly understood. In patients with solid tumors, cancer-related obstructions and surgical procedures, together with prolonged hospital admission, are additional conditions able to increase the infection risk. Neutropenia and mucositis are usually related to the type and intensity of traditional chemotherapy, while the microbiome alteration is multifactorial in origin, although the improper and excessive use of antibiotics and other drugs (e.g., proton pump inhibitors) is pivotal in this sense. In addition to the appreciation of the role of a damaged microbiota in affecting the risk of infection, in recent years, the widespread use of the novel targeted and biological agents, which have become part of many chemotherapeutic regimens, is posing new challenges that might change substantially what we know about the pathogenesis of infections in cancer patients, as well as their type and incidence.

Finally, the most important issue now challenging physicians and health care workers, as well as the scientific and nonscientific community, is the phenomenon of the growing antibiotic bacterial resistance worldwide, which has the potential to change substantially prophylactic and treatment issues. Antibiotic-resistant pathogens such as Enterobacteriaceae resistant to third-generation cephalosporins (producers of extended-spectrum β-lactamases [ESBLs]) or even carbapenems, multidrug-resistant (MDR) *Pseudomonas aeruginosa* or *Acinetobacter baumannii,* methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis,* vancomycin-intermediate *S. aureus,* and vancomycin-resistant enterococci (VRE) are spreading in cancer patients, with increasing endemicity and sporadic cases or outbreaks occurring almost everywhere in the world. For example in Italy, where carbapenemase-producing *Klebsiella pneumoniae* is currently endemic, a study of bloodstream infections (BSIs) in hematologic cancer patients found that 35% of *K. pneumoniae* strains were resistant to carbapenems and 70% of *P. aeruginosa* strains were MDR.[4] An increase in colonization and infection by carbapenem-resistant Enterobacteriaceae has been reported in the pediatric cancer population as well.[5] Although there are important geographical differences in the prevalence of carbapenem-resistant Enterobacteriaceae, no country can be considered untouched by the global spread of MDR gram-negatives.[6] National systems for surveillance, with obligation for notification and recommendations for containment and infection control measures, should be put in place.[7] This increase in MDR strains is radically changing our ability to prevent and cure infections in immunocompromised patients. The feasibility of the more aggressive medical interventions in hematologic cancer patients might be put under discussion if the actual trend toward more resistance is not reversed, since the rate of untreatable infections might become unacceptable. In addition, very few new antibiotics active against gram-negative rods have been marketed or are on the horizon, and the challenge is to ensure that an increasing antimicrobial resistance does not reverse the gains that have been made in improving survival and quality of life in patients with cancer through novel therapies or better surgical techniques.

As shown in Table 306.1, the clinical approach to a cancer patient with signs and symptoms of infection is multifactorial. Before planning a rational management strategy, physicians should answer several crucial questions about the type and stage of the underlying disease and the clinical presentation in order to provide an effective intervention. In addition, factors potentially associated with the presence of MDR bacteria, such as local epidemiology and the patient's colonization or previous infection with MDR strains, should currently be very carefully considered.

In the following sections, the epidemiology and management principles of infections in cancer patients are described. Risk factors and clinical presentations of specific infections, along with their treatment, are not discussed here but are dealt with in chapters focused on individual infectious agents. Similarly, infections in recipients of allogeneic hematopoietic stem cell transplants (HSCTs) are discussed elsewhere (see Chapter 307), and we will only briefly address these patients, particularly in regard to what concerns early infections during the preengraftment, neutropenic phase.

EPIDEMIOLOGY AND RISK FACTORS FOR INFECTIONS IN CANCER PATIENTS

The knowledge of the incidence of fever and documented infections in cancer patients according to the type of the underlying disease and related chemotherapy is mandatory for the implementation of effective management strategies, especially prophylaxis. However, the vast majority of epidemiologic data about these patients come from studies on empirical antibiotic therapy or prophylaxis, in which patients were selected according to inclusion and exclusion criteria. Thus this approach might be inadequate to describe the epidemiologic situation in real-life settings. In addition, little information is available about nonneutropenic patients.

Epidemiologic data on the incidence of infections are usually reported as percentages of events over a given number of patients or treatment courses, without adjusting for the duration of the period at risk. This is probably incorrect because the duration of exposure is crucial to understand the clinical impact of a given phenomenon. It is probably more appropriate to speak of incidence rates, that is, the number of events during a given risk period (usually 1000 days). For example, data on the rates of infectious complications that account for the number of days at risk allow for correct evaluation of the feasibility of antimicrobial prophylaxis and for comparing infectious risk among different cohorts or different treatment regimens. Unfortunately, such studies have rarely been done.

Pediatric studies reported rates of any infection or fever episode ranging from 12 to 31.1 per 1000 days at risk, being higher in case of aggressive treatment for acute leukemia and in neutropenic phases of

TABLE 306.1 What Should a Clinician Wonder About and Look for When Approaching a Cancer Patient With a Suspected Infection?

QUESTIONS	RATIONALE FOR THE QUESTION
The underlying disease: 1. Acute leukemia? Solid tumor? Lymphoma? Other? 2. Active disease? In remission? Not evaluable?	The incidence of infectious complications is different according to the underlying disease and consequent intensity of chemotherapy. The stage of disease may influence type, risk, and outcome of infection.
Recent treatments: 1. Did the patient recently (within 1 month) receive chemotherapy? 2. Which drugs and which schedule? How long ago? 3. Did the patient receive autologous or allogeneic HSCT? 4. If allogeneic HSCT, what donor type? 5. Did the patient receive monoclonal antibodies (anti-CD20, anti-CD52, etc.) in the past 6 months?	Different drugs may give different type of immunosuppression and favor different infectious complications. Previous transplantation might result in long-term immunodeficiency, particularly if immunosuppressive treatment is continued. Immune reconstitution depends on the type of donor and conditioning regimens used in allogeneic HSCT.
White blood cell count: 1. Is the patient neutropenic (PMNs <500/mm^3 or <1000/mm^3 but rapidly decreasing)? 2. Was the patient neutropenic in the previous 30 days?	The presence of neutropenia increases significantly the risk of infection. The knowledge of local epidemiologic data on antimicrobial susceptibility is mandatory for a correct choice of empirical therapy.
Risk of infection caused by resistant bacteria: 1. Is the patient colonized with resistant bacteria, particularly gram-negatives? 2. Is the hospital or the country endemic for resistant organisms? 3. Any previous infections caused by resistant pathogens?	In patients colonized by resistant bacteria, particularly if neutropenic, initial empirical therapy should cover these pathogens. If not colonized, but cared for in a setting where resistance is an issue, then consider the possibility of deescalation strategy.
Central venous catheter: 1. Yes or no? 2. Has the catheter been manipulated (including infusions) within a few hours before the onset of fever?	The central venous access may be an important source of infection.
Past history of infections (both before and after the diagnosis of cancer)	It may suggest the etiology and drive the therapeutic choice (e.g., tuberculosis, toxoplasmosis, multidrug-resistant bacteria, or opportunistic fungal infections).
Country of origin	Specific endemic infections can reactivate (Chagas' disease, strongyloidiasis, tuberculosis, endemic mycoses). Epidemiology of antibacterial resistance varies worldwide; thus, patients coming from areas endemic for resistant bacteria should be treated accordingly.
The clinical picture: 1. Presence of (severe) mucositis? 2. New onset of pain (perianal, chest, everywhere)?	It may suggest the etiology and drive the therapeutic choice. The presence of mucositis is suggestive of infection with pathogens from oral flora or gastrointestinal tract. The pain may help to locate formation of abscesses or indicate presence of a locally invasive process, such as pulmonary aspergillosis.
Administration of prophylaxis (no, yes, which drugs): 1. Antibacterial? 2. Antifungal, including *Pneumocystis jirovecii*? 3. Antiviral? 4. Was the patient compliant? 5. Is there the possibility of inadequate blood levels due to lack of absorption or PK/PD problems?	Breakthrough infections are possible, and fever during prophylaxis should be considered as failure of prophylaxis, unless proven otherwise. The occurrence of a bacterial/fungal/viral infection during specific prophylaxis may influence the choice of empirical therapy, depending on the drug used for prophylaxis. A resistant pathogen should be suspected in every case, unless the patient was clearly noncompliant or there is the possibility of low drug levels caused by poor absorption, increased metabolism, or drug interaction (e.g., azoles such as itraconazole, voriconazole, or posaconazole), or both. Knowledge of local epidemiology, including susceptibility pattern, is mandatory for correct diagnostic and therapeutic management.

HSCT, Hematopoietic stem cell transplantation; *PK/PD,* pharmacokinetic/pharmacodynamic; *PMNs,* polymorphonuclear neutrophils.

autologous transplantations.[3,8,9] The rate of bacteremia ranged, respectively, from 3.2 to 18.9 in adults and from 0.9 to 5.1 in children.[3,8–15] The rate of invasive fungal disease in pediatric studies ranged from 0.1 to 0.84, and was 2.4 in a study that included both children and adults.[3,9–12] Table 306.2 reports the epidemiology of febrile episodes, bacteremia, and invasive mycoses in cancer patients.[3,10–13,16–24]

These data clearly show that the incidence rate and proportion of infectious complications are mainly related to the intensity of antineoplastic chemotherapy. Additional factors are represented by the phase of chemotherapy and the status of the neoplastic disease, with higher incidence of infectious complications in patients receiving remission-induction and rescue chemotherapy, compared with maintenance or consolidation treatments.[11,18] The state of the underlying disease in terms of remission or relapse and progression is also an important factor for the occurrence and prognosis of infectious complications, as shown by studies in patients with invasive aspergillosis.[25–27] Patients with acute myeloid leukemia, both adults and children, have the highest frequency of fevers, bacteremia, and invasive fungal diseases, especially during the first induction of remission and in relapsing leukemia, when the intensity of chemotherapy is higher. Lower frequencies have been observed in lymphoblastic leukemia, chronic lymphatic disorders, multiple myeloma, and non-Hodgkin lymphomas, whereas the lowest rates are observed in solid tumors clearly depending on the lower intensity of antineoplastic treatment strategies. A new and rapidly increasing problem is represented by patients (usually with chronic leukemia or multiple myeloma) who are treated with novel targeted or biologic therapies, such as imatinib, dasatinib, ibrutinib, rituximab, ruxolitinib, and others. These new drugs have the potential to modify the infection risk profiles, with an increasing risk of infections caused by previously rare pathogens, such as *Pneumocystis jirovecii, Mycobacterium tuberculosis,* fungal pathogens, or many viruses.[28,29] On the other hand, their use instead of traditional chemotherapeutic agents might result in lower traditional toxicity, such as neutropenia.

Neutropenia

After the pivotal studies performed by Bodey and coworkers in 1966,[1] many others confirmed the strict relationship between neutropenia and infection and some of them tried to estimate an infection rate correlated with neutropenia. For example, in 1993 Carlisle and colleagues showed that the rate of infections in neutropenic cancer patients was 46.3 episodes per 1000 days of neutropenia, with rates of 12.9 for bacteremia and 2.9 for invasive mycoses.[30] More recent data from a prospective study in children with neutropenia showed a median incidence of infectious complications of 43% and a rate of 22.8 episodes per 1000 neutropenic days; bacteremia was diagnosed in 21% of the episodes and mold infections in 5%, with rates of 10.2 and 2.4 for 1000 neutropenic days,

TABLE 306.2 Incidence of Main Infectious Complications in Cancer Patients

		PERCENTAGES OF PATIENTS OR PERIODS WITH INFECTIOUS COMPLICATIONS[a]					
				INVASIVE FUNGAL DISEASE			
PATIENT POPULATION	TYPE OF DISEASE	ANY TYPE OF INFECTIOUS OR FEBRILE EPISODE	BACTEREMIA	Any (Proven, Probable, and Possible)	Proven and Probable		REFERENCE
					Yeasts	*Molds*	
Adults and children	Malignancies, not analyzed in detail	62	35	—	—	—	Gafter-Gvili et al., 2005[16]
Adults	Malignancy or autologous HSCT, not analyzed in detail	—	18	0	—	—	Dettenkofer et al., 2005[13]
Adults	High-risk acute leukemia	78	30	—	—	—	Bucaneve et al., 2005[17]
	High-risk NHL or HSCT in solid tumors	73	23	—	—	—	
Adults	Acute promyelocytic leukemia	59	22	7.3	3	0.3	Girmenia et al., 2003[18]
	Other AnLL	—	37	4.2	4	0.2	
Children	AnLL	94	25	4.2	0.6	1.6	Lehrnbecher et al., 2004[19]
Children	ALL, aggressive treatment	—	30	10	—	—	Castagnola et al., 2005[10]
	ALL, less aggressive treatment	—	17	3	—	—	
	AnLL, aggressive treatment	—	34	9	—	—	
Adults	Low-risk solid tumors, not analyzed in detail, including lymphomas	12	0.4	—	—	—	Cullen et al., 2005[20]
Adults	AnLL	—	—	—	6	11	Caira et al., 2008[21]
Adults	New-onset hematologic malignancies	27	10	4	0.5	1.3	Pagano et al., 2012[22]
Adults and children	Hematologic malignancies	—	21	5	—	—	Orasch et al., 2010[12]
Children	AnLL:	—	51	16	—	—	Castagnola et al., 2010[11]
	Incidence per patient						
	Incidence per treatment course		32	10			
Children	Aggressive treatment for solid tumor, including autologous HSCT	—	24	1.6	1	0.5	Haupt et al., 2001[23]
	Less aggressive treatment for solid tumors, not analyzed in detail		3	0			
Children	Neutropenic AL/NHL, aggressive treatment	48	25	10	1.6	1.2	Castagnola et al., 2007[3]
	Neutropenic AL/NHL, not aggressive treatment	21	8	1	0.8	0	
	Neutropenic ST, aggressive treatment	32	6	0.4	0.1	0.1	
	Neutropenic ST, not aggressive treatment	22	7	4	0.5	0.5	
	Neutropenic postautologous HSCT	58	14	2	0.6	0.6	
Children	Neutropenic with AML, receiving G-CSF	61	14	2	1.8	0.6	Lehrnbecher et al., 2007[24]
	Neutropenic with AML, not receiving G-CSF	56	11	0	—	—	

[a]Numbers are percentages of event over enrolled patients.
—, Data not reported; *AL,* acute leukemia; *ALL,* acute lymphocytic leukemia; *AML,* acute myelocytic leukemia; *AnLL,* acute nonlymphocytic leukemia; *G-CSF,* granulocyte colony-stimulating factor; *NHL,* non-Hodgkin lymphoma; *HSCT,* hematopoietic stem cell transplant; *ST,* solid tumor.

respectively.[12] The rate of infections is higher after high-intensity chemotherapies and lower after maintenance treatment. The majority of primary febrile episodes usually occur soon (a few days) after the onset of neutropenia.

Mucositis and Microbiota Alterations

As already mentioned, in addition to neutropenia, the severity of mucosal barrier injury may have an impact on infection rates (for major details, see Chapter 305). Mucositis is one of the most important factors predisposing to fever and BSIs, caused both by bacteria and by *Candida*.[31] The ulcerative phase induced by antineoplastic drugs with increased permeability and damage to the intestinal mucosal barrier promotes bacterial translocation, but the potential role of alterations in diversity of the intestinal microbiome in the development of mucositis and subsequent BSIs is increasingly recognized.[32] Quantitative and qualitative alterations of the normal oral and intestinal microflora have been described in cancer patients and depend on many factors, such as underlying disease, chemotherapy (drugs and dosages) and radiotherapy, mucosal disruption, bowel motility disturbance, enteral/parenteral nutrition, and broad-spectrum antibiotic administration.[33–35]

Microbiologic analyses of fecal samples of children aggressively treated for acute leukemia showed a decreased amount of microbial flora, and in particular of bifidobacteria, *Lactobacillus,* and *Escherichia coli,* and a 100-fold reduction of the total number of bacteria, mainly for anaerobes, with a concomitant increase of potentially pathogenic enterococci.[33,36] Moreover, in patients receiving HSCT, occupation of at least 30% of the microbiota by a single predominating bacterial taxon (intestinal domination) was associated with an increased risk for bacteremia due to specific pathogens.[37,38] Finally, *non-albicans Candida* strains, mainly *C. glabrata* and *C. krusei*, also seem to be increased in stool from children receiving chemotherapy or HSCT, especially in the case of prolonged hospitalization.[39,40]

Central Venous Catheters

The presence of a CVC is another well-known factor facilitating infections in cancer patients and influencing their etiology. A detailed description of problems related to infections associated with CVCs is beyond the scope of this chapter and can be found elsewhere in this text (see Chapter 300). Long-term vascular access devices adopted during antineoplastic chemotherapy or HSCT are represented by partially implanted silicone devices (Hickman or Broviac catheter), with or without valves, single or double lumen; peripherally or centrally totally implanted catheters (Port-A-Caths); and peripherally inserted central catheters.[41] Patients with less frequent need of venous access are usually fitted with totally implanted catheters, while more intensive supportive care generally requires partially implanted single- or double-lumen devices, which allow rapid infusion of large amounts of liquids. Risk factors generally associated with the development of CVC-related infections are represented by the type of CVC, the number of lumens, and the number and characteristics of CVC manipulations, all mainly related to the intensity, duration, and schedule of antineoplastic chemotherapy, the type of supportive care, and the severity of clinical conditions. Bacteremia represents the most frequent infectious complication, whereas exit site, tunnel, and pocket CVC-related infections are less frequently reported. Partially implanted catheters have been associated with a three- to fourfold increase in the risk of infectious complications compared with totally implanted devices, and in a study in patients with a totally implanted device, younger age was the only identified risk factor.[42,43] The risk of infection is generally higher in adults compared with children, and lower in the outpatient setting.[42,44,45]

A recent review reported infection rates varying from a mean of 22.5 (95% confidence interval [CI], 21.2–23.7) per 100 devices for Hickman and Broviac catheters, 3.5 to 4 (95% CI, 2.4–5.6) for Port-A-Caths, and 3.1 (95% CI, 2.6–3.7) for peripherally inserted central catheters, with mean rates per 1000 catheter-days of 1.6 (95% CI, 1.5–1.7), 0.1 (95% CI, 0.01–0.2), and 1.1 (95% CI, 0.9–1.3), respectively.[41] Patients with hematologic malignancies, and especially those with acute leukemia, have a higher incidence of catheter-related infections compared with patients with lymphoma, myeloma, or solid tumors, probably due to different type of catheters and frequency of catheter use.[41] It is important to be very balanced when discussing the role of central catheters in predisposing to infection. Indeed, the role might be overestimated, because the catheter might just be the site of a secondary localization of pathogens actually coming from the intestinal flora.[46] New definitions now suggest that 40% to 50% of BSIs in oncologic settings are actually endogenous and associated with mucosal barrier injury.[41,47–49] This has an impact on the expectations regarding strategies that should prevent infections by improving catheter management.[47–49]

Gram-positive bacteria represent the pathogens most frequently associated with CVC-related infections, but gram-negatives are reported with increasing frequency, especially in nonbacteremic episodes.[41] Finally, some age-related differences in the proportions of pathogens causing CVC-related bacteremia have been reported, with gram-negatives being observed more frequently in adults and gram-positives and fungi in children.[47–50]

Genetic Factors

The existence of genetic factors able to increase or decrease the susceptibility to infection in immunocompromised patients underlines an apparently trivial but important aspect: cancer patients are not the same, and every single patient might deserve an individualized approach. For example, in nonleukemic patients receiving less intensive chemotherapy, decreased levels of mannose-binding protein were associated with an increased risk of infection (49.9 vs. 29.6 per 1000 days at risk, $P = .01$).[15] In a recent study of 269 children with cancer, mannose-binding lectin deficiency influenced both the incidence and the severity of febrile neutropenia.[51] In breast cancer patients, genetic factors also were found to influence the risk of febrile neutropenia.[52] Polymorphisms of Toll-like receptors and other components of innate immunity have been associated with an increased risk of invasive aspergillosis, both in cancer patients (including recipients of HSCT) and in other immunocompromised patients.[53] The future will tell us whether genetic polymorphisms, alone or in combination, have a clinically significant impact on infection risk and might dictate prophylactic or therapeutic approaches.

New Therapies

Biologic Agents and Other New Drugs

As already mentioned, in recent years, monoclonal antibodies and other pharmaceutical compounds that are specifically engineered for targeting enzymes, cytokines, or receptors involved in the pathogenesis of specific types of cancer have been introduced into the armamentarium of antineoplastic chemotherapy. Main infectious complications associated with their use are reported in Table 306.3.[54–59]

Several issues impair our ability to understand the exact role of these new compounds on the risk of infection in cancer patients. First, even in randomized, placebo-controlled trials and in large open-label studies, it is difficult to establish the rate of infectious complications, because these trials were powered to measure efficacy in relation to their primary objective, but not safety. If the risk of infection is low, it might go undetected. Second, there are many confounding factors because new drugs are usually used together or in sequence with old therapies, making it difficult, if not impossible, to evaluate their respective role. Third, very often, because of the lack of infectious disease expertise, registration studies did not use the same definitions of infectious complications or simply did not pay enough attention to them. Sadly, in some cases there was a tendency toward minimization and covering. There is at least one example showing that the infection risk might have been forecast. This is the case of eculizumab used for paroxysmal nocturnal hemoglobinuria, which targets the C5 complement component. As should be widely known, the inherited deficiency of the C5 complement component is associated with repeated episodes of invasive meningococcal disease. Thus this risk might have been forecast before starting large trials or before marketing this new drug, so appropriate preventive strategies could have been put in place.

There is hope that multicenter projects concentrating on infectious complications might help in determining their true risk and possible early preventive or diagnostic measures. For example, the Southern Network on Adverse Reactions (SONAR) project conducts safety initiatives focusing on monoclonal antibody–associated progressive multifocal leukoencephalopathy (PML).[60] The field of biologic agents and anticancer molecules is rapidly changing, with a constant influx of new drugs and with new indications for the older ones. Therefore the data on their safety profile is also in constant evolution. As already mentioned, novel drugs are frequently administered in combination with "classic" antineoplastic agents and therefore it is not easy to disentangle their role in the development of infectious diseases. For example, a recent meta-analysis that investigated the impact of immunomodulatory drugs (e.g., thalidomide and derivatives) and proteasome inhibitors (e.g., bortezomib), variously associated with "classic" antineoplastic drugs in different phases of treatment for multiple myeloma, showed that the rate of severe infections in protocols including immunomodulatory drugs seemed generally lower than that observed with conventional therapy.[61] Unfortunately, despite the great number of patients and clinical trials analyzed, the authors repeatedly commented on insufficient detail about methods for detecting and diagnosing infections and limited generalizability to broader nontrial populations.[61]

For the compounds that have been in use for many years, we obviously have more information. For example, the monoclonal antibody anti-CD52 has been associated with a wide spectrum of infectious complications (mainly viral and fungal), and a low $CD4^+$ T-lymphocyte count (with

TABLE 306.3 Selected Biologic Drugs for Treatment of Solid Tumors and Hematologic Malignancies and Possible Infectious Complications

CLASS/SITE AND MECHANISM OF ACTION	DRUG	POSSIBLE INFECTIOUS COMPLICATIONS
Monoclonal Antibodies (mAbs) Targeting Surface Antigens on Lymphoid Cells		
Bispecific T-Cell Engager (BiTE): anti-CD19 mAb conjugated to anti-CD3 mAb leading to T-mediated lysis of CD19+ cells	Blinatumomab	CVC-associated infections due to continuous prolonged intravenous infusion. Severe and prolonged hypogammaglobulinemia and possible neutropenia, with consequent infectious risk. VZV and HSV infections, pneumocystosis, and HBV reactivation.
Anti-CD20 mAbs	Rituximab	Possible hypogammaglobulinemia of variable duration, not associated with an increased risk of bacterial infections. HBV reactivation (both of chronic inactive and resolved infection); exacerbation of HCV infection. Possible neutropenia; severe viral respiratory tract infection. Cases of severe enteroviral infections, PML, VZV, CMV parvovirus infections. Vaccine response is almost absent in the first 6 months after rituximab administration.
	Obinutuzumab	Similar to rituximab. Neutropenia and severe enteroviral infections have been reported.
	Ofatumumab	Probably similar to rituximab. Reported neutropenia, HSV, viral respiratory infection, fatal case of HBV reactivation.
	Ocrelizumab	Risk of infections associated with increasing doses, PML. Potentially increased risk of neutropenia and HBV reactivation.
	Veltuzumab Ublituximab	No data available for evaluation of the risk of severe infectious complications. Probably similar to rituximab.
Anti-CD20 mAbs conjugated with a radioactive isotope	^{90}Y-Ibritumomab tiuxetan ^{131}I-tositumomab	Higher rate of cytopenias, such as lymphopenia or neutropenia, due to combination with radioactive isotope. Cytopenia risk is higher if bone marrow is infiltrated by CD20+ hematologic malignancy. General safety profile probably similar to rituximab.
Anti-CD22 mAb Anti-CD22 mAb conjugated with the DNA-damaging agent calicheamicin Anti-CD22 mAb conjugated with *Pseudomonas* exotoxin A	Epratuzumab Inotuzumab ozogamicin Moxetumomab pasudotox	No significant increase in infections in placebo-controlled trials. Profile of infections similar to anti-CD20 mAbs: respiratory tract infections (viral and bacterial), pneumonia.
Anti-CD30 mAb conjugated with the microtubule disrupting agent monomethyl auristatin E	Brentuximab vedotin	Herpetic infections, including CMV; cases of PML and pneumocystosis.
Anti-CD33 mAb conjugated with a calicheamicin agent	Gemtuzumab ozogamicin	No significant increase in infections.
Anti-CD38 mAb	Daratumumab	Increased risk of VZV (prophylaxis warranted for 3 months after the end of treatment). Slightly higher risk of pneumonia and upper respiratory tract infections.
Anti-CD40 mAb	Dacetuzumab	Higher rate of neutropenia with dacetuzumab vs. placebo. Opportunistic infections similar to those observed in hyper-IgM syndrome (pneumocystosis, CMV reactivation, etc.) might be theoretically expected.
Anti-CD52 mAb	Alemtuzumab	Severe viral infections (particularly HSV, VZV and CMV, but also HHV6, BKV, parvovirus, adenovirus). Pneumocystosis and other fungal infections, toxoplasmosis, mycobacteriosis, reactivation or exacerbation of HBV infection, tuberculosis, listeriosis, infections due to acanthamoebae, *Balamuthia mandrillaris,* reactivation or worsening of chronic HBV infection have also been described.
Anti-CD139 (signaling lymphocytic activation molecule F7 [SLAMF7]) mAb	Elotuzumab	Lymphopenia and increased risk of opportunistic infections, particularly due to VZV.
Anti–C-C motif of chemokine receptor 4 (CCR4) mAb	Mogamulizumab	Infectious risk difficult to distinguish from the intrinsic effect of underlying diseases and concomitant lymphotoxic therapies. Pneumocystosis, HSV, VZV, HBV reactivation (risk of severe hepatitis in patients with high-level HBV DNA due to T-regulatory cell downregulation).
Tyrosine Kinase Inhibitors ***Breakpoint Cluster Region–Abelson Murine Leukemia (BCR-ABL) Signaling Pathway***		
Inhibitor of tyrosine kinase BCR-ABL, c-KIT, and platelet-derived growth factor (PDGF)-receptor	Imatinib	Febrile neutropenia has been reported. Cases of pneumocystosis and viral diseases, including reactivation of hepatitis.
Inhibitor of tyrosine kinase BCR-ABL	Nilotinib	Cases of pneumocystosis and viral diseases, including reactivation of hepatitis.
Inhibitor of tyrosine kinase BCR-ABL, SRC family, c-KIT, and others	Dasatinib	Cases of pneumocystosis and viral diseases, including reactivation of HBV. Noninfectious pleural effusions, less often pneumonitis (in differential diagnosis with infections).

Continued

TABLE 306.3 Selected Biologic Drugs for Treatment of Solid Tumors and Hematologic Malignancies and Possible Infectious Complications—cont'd

CLASS/SITE AND MECHANISM OF ACTION	DRUG	POSSIBLE INFECTIOUS COMPLICATIONS
Bruton Tyrosine Kinase Signaling Pathway		
Bruton tyrosine kinase inhibitor	Ibrutinib	Bacterial pneumonia, urinary tract infection and cellulitis, noninfectious pneumonitis, invasive fungal infections.
Janus Kinase–Signal Transducers and Activators of Transcription (JAK-STAT) Signaling Pathway		
Inhibitor of the JAK pathway (kinases 1 and 2)	Ruxolitinib	VZV reactivation, cases of tuberculosis, PML, pneumocystosis, cryptococcosis, and other opportunistic infections associated with T-cell dysfunction.
Phosphatidylinositol 3-Kinase (PI3K) Delta Isoform Signaling Pathways		
Inhibitor of PI3Kδ	Idelalisib	Pneumonia, sepsis (20%), opportunistic infections, along with noninfectious pulmonary and gastrointestinal inflammation. Prophylaxis of HBV reactivation and pneumocystosis and preemptive management strategy for CMV are warranted.
Others		
Multikinase inhibitors	Lapatinib Pazopanib Regorafenib	Possible skin infections because of skin toxicity. No significant data available for evaluation of the risk of severe infectious complications.
Vascular Endothelial Growth Factor (VEGF) Signaling Pathway		
Anti–human VEGF mAb	Bevacizumab	Infections in cases of chemotherapy-induced neutropenia: sepsis and pneumonia. Cases of intestinal perforation (with or without abscess formation), probably from the inhibition of endothelial cell proliferation and new blood vessel formation. Possible endophthalmitis due to intravitreal injections, but an increased risk of this type of infection has not been clearly demonstrated.
Recombinant protein circulating antagonist that prevents VEGF receptor binding	Aflibercept	Severe infections: incidence 7.3% (95% CI, 4.3–12.0%) with increased risk (RR, 1.87; 95% CI, 1.52–2.30). Mortality: 2.2% (95% CI, 1.5–3.1%), with increased risk (OR, 2.16; 95% CI, 1.14–4.11). The risk of infections with aflibercept substantially higher than with bevacizumab.
Inhibitor of tyrosine kinases on receptors for vascular endothelial growth factor (VEGFR), platelet-derived growth factor (PDGFR), and isoform B of tyrosine kinase (known as rapidly accelerated fibrosarcoma [B-RAF])	Sorafenib	Infections, as well as gastrointestinal perforations, are reported in less than 1% of treated patients.
Inhibitors of tyrosine kinases on PDGFRs and VEGFRs	Sunitinib	Cases of necrotizing fasciitis, respiratory infections, and sepsis.
Inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2), epidermal growth factor receptor (EGFR), and RET (rearranged during transfection) tyrosine kinase	Vandetanib	No significant increase in infections.
Tyrosine kinase inhibitor that inhibits angiogenesis blocking VEGFR-1– VEGFR-3, c-KIT, and PDGFR	Axitinib	No significant data available for evaluation of the risk of severe infectious complications.
Inhibitor of tyrosine kinases c-Met and VEGFR-2, and also AXL and RET	Cabozanitinib	No significant data available for evaluation of the risk of severe infectious complications.
Inhibitor of tyrosine kinases VEGFR-1–VEGFR-3	Lenvatinib	No significant data available for evaluation of the risk of severe infectious complications.
Epidermal Growth Factor (EGF) Signaling Pathway		
Anti–EGF receptor (ErbB1 or EGFR) mAb	Panitumumab (IgG2) Cetuximab (IgG1, chimeric)	Cases of necrotizing fasciitis, abscesses sepsis caused by *Staphylococcus aureus.* Important dermatologic toxicity, such as rash, skin drying and fissuring, or paronychial inflammation, with infectious complications (abscess, bacteremia) in up to 30% of patients, including sepsis caused by *S. aureus.* More severe infections have been described when combined with neutropenia-inducing chemotherapy.
	Nimotuzumab (humanized)	No significant data available for evaluation of the risk of severe infectious complications.
mAb against human EGF receptor 2 (human ErbB-2 or HER2)	Trastuzumab	Infections are generally mild and reported as URTIs or UTIs. Increased risk of infections has been described in combination with chemotherapy. Exacerbation of chemotherapy-induced neutropenia. Infection ≥ grade 3: 8.5% of patients (95% CI, 4.5–15.4), RR vs. control, 1.21 (95% CI, 1.07–1.37). Febrile neutropenia in the neoadjuvant/adjuvant and combination therapies: 12.0% (95% CI, 8.1–17.4), RR vs. control, 1.28 (95% CI, 1.08–1.52)
	Pertuzumab	>10% of febrile neutropenia, URTIs (associated with chemotherapy).

TABLE 306.3 Selected Biologic Drugs for Treatment of Solid Tumors and Hematologic Malignancies and Possible Infectious Complications—cont'd

CLASS/SITE AND MECHANISM OF ACTION	DRUG	POSSIBLE INFECTIOUS COMPLICATIONS
Inhibitor of epidermal growth factor receptor–tyrosine kinase inhibitor (EGFR-TKI), possibly also of mutated JAK2	Erlotinib	Approximately 9% higher rate of infections compared to control arms for erlotinib.
Inhibitor of EGFR-TKI	Gefitinib	In a meta-analysis of over 6000 patients with NSCLC: any infection, 7% (95% CI, 4.7–10.3), severe infection, 2.1% (95% CI, 1.7–2.8), and fatal, 0.7% (95% CI, 0.4–1.0). There is a trend toward a higher risk with longer treatment.
Immune Checkpoints Inhibitors		
mAbs inhibiting programmed cell death protein 1 (PD-1)	Nivolumab	No significant increase in infectious complications but immune-mediated complications are frequent and should be considered in differential diagnosis (pneumonitis, colitis, hepatitis).
	Pembrolizumab	No significant data available for evaluation of the risk of severe infectious complications.
mAbs inhibiting programmed cell death ligand 1 (PD-L1)	Atezolizumab Avelumab Durvalumab	Cases of fever and UTIs. No significant data available for evaluation of the risk of severe infectious complications.
mAb inhibiting cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4)	Ipilimumab Tremelimumab	No significant data available for evaluation of the risk of severe infectious complications.
Immunomodulating Drugs		
Angiogenesis inhibitor	Thalidomide	Neutropenia and lymphopenia described, reported increased risk of infections (RR, 1.64; 95% CI, 1.40–1.92).
Apoptosis inducer in vivo, with antiangiogenic and osteoclastogenic effects	Lenalidomide	Described a 14% (95% CI, 12.08–16.90) incidence of severe infections, with RR 2.23 (95% CI, 1-71–2.91), with possible fatal events. May increase risk and duration of cytopenia if combined with chemotherapy.
Immunomodulatory, like thalidomide and lenalidomide, based on its use in multiple myeloma	Pomalidomide	Severe infectious complications in 23%, frequently in the absence of neutropenia. Pneumonia is the most frequently reported localization. Antibacterial prophylaxis has been recommended for patients receiving this drug, but fluoroquinolones could be contraindicated for the risk of drug interactions.
Activator of monocytes and macrophages	Mifamurtide	Fever frequently described, but no specific data on severe infectious complications.
Proteasome inhibitors	Bortezomib Carfilzomib	Increased risk of herpes zoster. Low risk of febrile neutropenia. Pneumonia (13%) and URTIs (28%) observed in patients receiving single-drug therapy, but approximately 2%–6% had severe infections.
	Ixazomib	Increased risk of herpes zoster. No significant data available for evaluation of the risk of severe infectious complications.
Others mAb against disialoganglioside (expressed on a variety of embryonal cancers (e.g., neuroblastoma, retinoblastoma, osteosarcoma, Ewing sarcoma, rhabdomyosarcoma)	Dinutuximab (anti-GD2)	Fever is frequently observed, but there are data available for significant infectious complications.
Cyclopamine-competitive antagonist of the smoothened (a transmembrane protein) receptor that is a part of the hedgehog signaling pathway that is involved in proper cell differentiation	Vismodegib	No significant data available for evaluation of the risk of severe infectious complications.
Inhibitors of Histone Deacetylases		
Histone deacetylase (HDAC) inhibitor, induces intracellular increase of transcription factors important for the expression of genes needed to induce cell differentiation	Vorinostat	Neutropenia reported, but no data available for significant infectious complications.
Nonselective HDAC (pan-HDAC inhibitor).	Panobinostat	Pneumonia described in 13% of treated patients.
Selective inhibitor of HDACs	Romidepsin	No significant data available for evaluation of the risk of severe infectious complications.
Mammalian target of rapamycin (mTOR) inhibitors	Everolimus Temsirolimus	Infections attributable to mTOR inhibitors (mainly everolimus): any infection 9.3% (95% CI, 5.8–14.6%), severe infections 2.3% (95% CI, 1.2–4.4%). Important difference in the incidence depending on the type of cancer treated.

BKV, BK virus; *95% CI,* 95% confidence interval; *CMV,* cytomegalovirus; *CVC,* central venous catheter; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HHV6,* human herpesvirus 6; *HSV,* herpes simplex virus; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M; *NSCLC,* non–small cell lung cancer; *OR,* odds ratio; *PML,* progressive multifocal leukoencephalopathy; *RR,* relative risk; *URTI,* upper respiratory tract infections; *UTIs,* urinary tract infections; *VZV,* varicella-zoster virus.

a possible cutoff of 200 $CD4^+$ T-lymphocytes/mm^3) has been indicated as one of the most important factors related to the development of infections. Anti-CD20 compounds, the most widely studied being rituximab, cause a prolonged (2 to 6 months in median, but sometimes longer) suppression of immunoglobulin production.[62] Reactivation of hepatitis B virus (HBV) has been observed in 85% of hepatitis B surface antigen (HBsAg)–positive and in 41.5% of HBsAg-negative/hepatitis B core antibody (HBcAb)–positive lymphoma patients treated with chemotherapy that included rituximab.[63] More rarely, other severe viral infections (enteroviral infections, PML, parvovirus infections) have been reported.[62] Brentuximab, another monoclonal antibody targeting lymphocytes through CD30, has also been associated with severe infections. In particular, PML after brentuximab was found to develop earlier than with rituximab or natalizumab (2–3 months vs. 63 weeks vs. 26 months, respectively).[29,60,62,64,65] Therefore a low threshold for brain magnetic resonance imaging should be applied to patients treated with monoclonal antibodies who develop neurologic symptoms. Ruxolitinib was associated with varicella-zoster virus (VZV) reactivation and tuberculosis (TB), ibrutinib with invasive fungal diseases (IFDs), and idelalisib with herpes and cytomegalovirus (CMV) reactivations and with pneumocystosis. Last but not least, checkpoint inhibitors such as ipilimumab, which inhibits the cytotoxic T-lymphocyte–associated antigen 4 checkpoint, and nivolumab or pembrolizumab, which inhibit the programmed cell death protein 1 (PD1) checkpoint, are associated with severe immune-related adverse events, such as diarrhea, pneumonitis, or hepatitis. In these cases, differential diagnosis might be difficult and patients should always be tested for infectious etiologies, such as *Clostridioides difficile* (formerly *Clostridium difficile*) colitis, pneumocystosis, or viral hepatitis. Tyrosine kinase inhibitors seem to be associated with infectious complications similar to those observed with other immunosuppressive drugs affecting mechanisms of cell-mediated immunity. For these drugs, among the most frequently reported infections are pneumocystosis and reactivation of HBV (observed also in HBsAg-negative/HBcAb-positive patients). Finally, high-dose retinoic acid has been used for treatment of acute promyelocytic leukemia and some pediatric solid tumors (neuroblastoma). The dosages adopted in acute leukemia can induce fever and hepatotoxicity (vitamin A "intoxication"), while in patients with solid tumors, cases of skin and soft tissue infections, especially due to *S. aureus,* can be observed. Intravesical administration of Calmette-Guérin bacillus, a live strain of *Mycobacterium bovis,* is adopted as a treatment of non–muscle-invasive bladder cancer. Disseminated genitourinary or osteomuscular infections have been reported.[66]

Chimeric Antigen Receptor T-Lymphocyte Therapy

Originally designed to treat lymphocyte-derived malignancies, chimeric antigen receptor (CAR) T-cell therapy is now being used in a wide range of solid tumors.[67] CAR T cells are derived from the patient's peripheral blood lymphocytes, which are transformed in vitro using a DNA construct that causes the lymphocyte to produce on its surface receptors that recognize a specific, selected tumor antigen. After CAR T cells are infused into the patient and encounter a cell with that antigen on its surface, the CAR T cells activate and become cytotoxic, thus killing the neoplastic cell. Potential adverse effects include killing of normal cells bearing the same antigen, as well as a cytokine release syndrome, which can cause high fever and multiorgan failure.[68] Little is known about infectious complications associated with CAR T-cell therapy. In cases of therapy of leukemia or lymphoma with CAR T cells targeting CD19, the incidence of infections was comparable to observations from clinical trials of salvage chemoimmunotherapies in similar patients.[69]

Surgery

Bacteremia, usually associated with surgical site infection and deep organ abscess, is not uncommon in urologic, gynecologic, and abdominal surgery in cancer patients, but it is difficult to say with certainty if this happens significantly more often in oncologic versus nononcologic patients.[70,71] Several studies reported the rates of postsurgery infections in different cancer populations. For example, among patients with peritoneal carcinomatosis undergoing peritonectomy and intraperitoneal hyperthermic chemotherapy, the proportion of infectious complications was rather high, varying from 24% to 36%, with more than two infectious episodes per patient.[70–72] The rate of infectious complications is lower in other oncologic surgeries. In breast cancer, surgical site infection is a complication in 4% to 8% of cases, depending whether breast reconstruction is performed in one or two steps and whether surgery follows previous chemotherapy cycles.[73,74] In case of malignant biliary obstruction, early infectious complications after percutaneous biliary stent insertion were present in 6.5% of patients.[75] Similar incidence was reported in patients undergoing surgery for hepatocellular or metastatic carcinoma (3%–11%), and this incidence was apparently lower than in surgery for nonmalignant conditions such as hepatolithiasis (24%).[76] The rate of infectious complications after hepatectomy for hepatocarcinoma was associated with surgical risk factors such as bile leakage and blood loss.[77] A similar incidence of surgical site infections was reported after elective colon and rectal surgery (9% and 18%, respectively), and after orthopedic surgery (9.5%).[78,79] Of interest, in the latter study the use of an implant or allograft did not represent a risk factor for infectious complications.[79] Finally, postoperative respiratory infections have been reported in nearly 4% of patients undergoing surgery for lung cancer, and they occurred more frequently in the presence of advanced age, impaired respiratory function, advanced pathologic stage, and induction chemotherapy.[80]

In conclusion, although not many data are available on infectious complications after surgery in solid tumors, it seems that surgical and intensive care unit–related factors are more important than previous antineoplastic chemotherapy in determining the risk of infection. Finally, in patients with solid tumors, surgery, together with long hospital stay and use of third-generation cephalosporins and glycopeptides, have been associated with an increased risk of infections caused by MDR pathogens.[81]

ETIOLOGY

Surveillance studies on pathogens causing infections in cancer patients are of the utmost importance for the implementation of management strategies. Large-scale studies are obviously crucial because they can provide information about worldwide trends, but single-center surveillance reports may be even more important, because every geographic region, country, or single center may have peculiarities related to the type of patient, type of care, and local previous antibiotic policies. Most of the available information concerns bacterial and fungal pathogens isolated in the bloodstream, whereas the role of deep-seated infections, as well as the impact of viral infections, are less known.

Bacterial Infections

Over the last 30 years, gram-positive bacteria were the most frequent pathogens causing bloodstream infections in cancer patients. However, more recently, an increase in the frequency of bacteremias caused by gram-negative rods has been reported, with gram-negative pathogens becoming either predominant or at least as frequently isolated as the gram-positives.[14,82,83] This trend has also been observed in a retrospective literature review and contemporary surveillance study performed in 2011 in 39 European hematology centers from 18 countries belonging to the network of the European Conference of Infections in Leukemia (ECIL).[84] As shown in Fig. 306.1, this study found that gram-negative pathogens were almost as frequently isolated as the gram-positives, with gram-positive/gram-negative ratios in bloodstream infections of 60%/40% and 55%/45%, in the literature review and the ECIL-4 surveillance, respectively. The detailed etiology was similar (see Fig. 306.1) in the literature review and the surveillance study, with a slightly higher rate of enterococci and Enterobacteriaceae and a decreased rate of *P. aeruginosa* in the surveillance study.[84] These changes in etiology seemed to be associated with an important and alarming increase in the proportion of resistant pathogens, such as ESBL-producing Enterobacteriaceae, VRE, MDR *P. aeruginosa,* and the most worrisome, carbapenem-resistant gram-negative pathogens. Last but not least, in leukemic patients, most staphylococci are resistant to methicillin, whereas most gram-negative pathogens are resistant to fluoroquinolones.[84]

Of note, the rates of resistance were generally higher in southern and eastern than in northern and western Europe, and this trend is also evident in the non–hematologic cancer population.[84,85] A recent

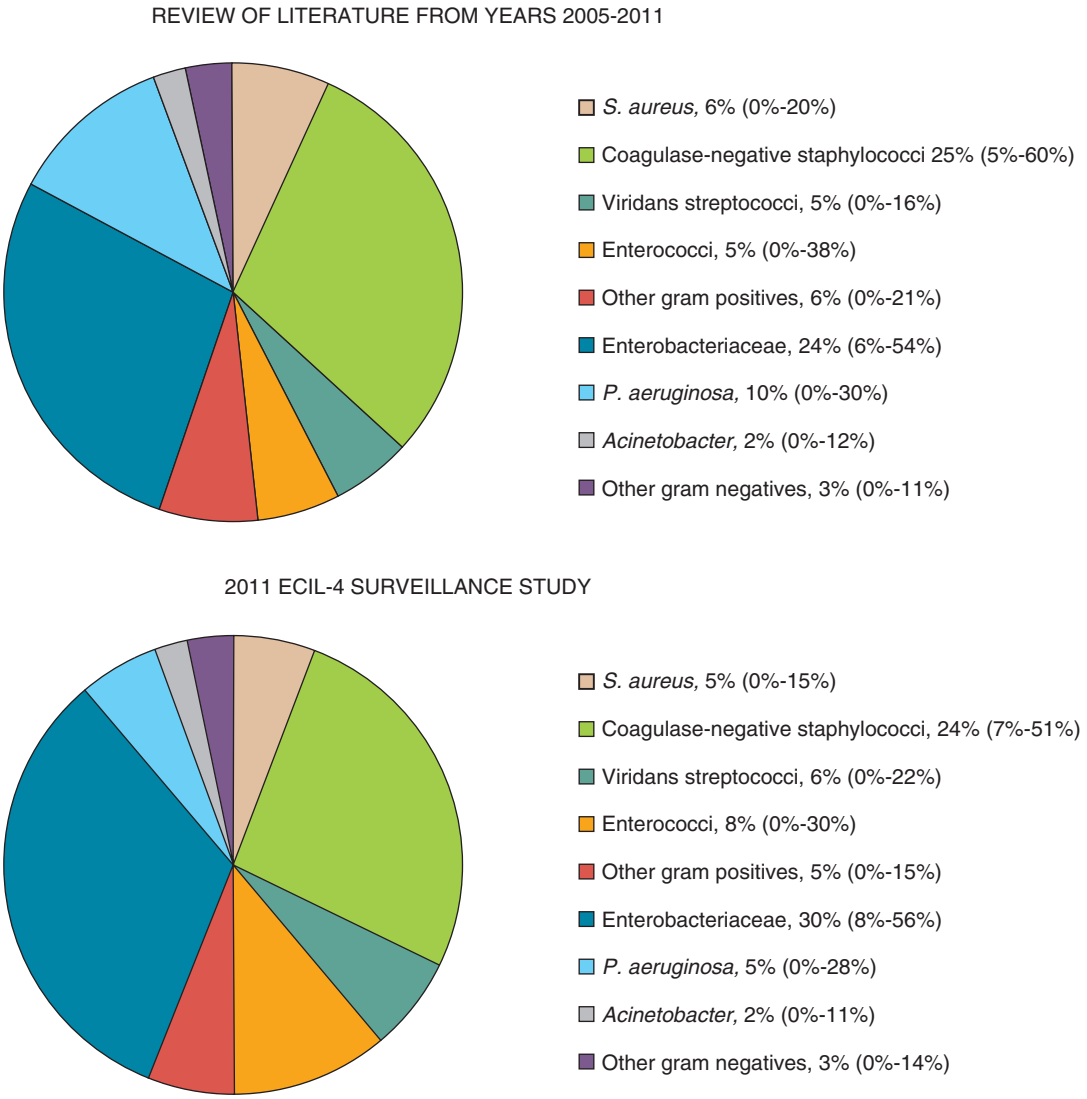

FIG. 306.1 Etiology of bloodstream infections in cancer patients. *Top,* Data from recent literature review. *Bottom,* Data from surveillance study for the Fourth European Conference of Infections in Leukemia (ECIL-4) in 2011. *(Modified from Mikulska M, Viscoli C, Orasch C, et al. Fourth European Conference on Infections in Leukemia Group (ECIL-4), a joint venture of EBMT, EORTC, ICHS, ELN and ESGICH/ESCMID. Aetiology and resistance in bacteraemias among adult and paediatric haematology and cancer patients.* J Infect. *2014;68:321–331.)*

study in HSCT recipients has confirmed the alarming increase in resistance in this population as well, with increasing mortality rates.[86] The increase in infections caused by resistant strains usually follows an increase in colonization with these pathogens, because cancer patients typically first get colonized and then develop endogenous infection. Indeed, colonization with resistant bacteria is one of the most important risk factors for infection with resistant bacteria. The association between colonization and subsequent infection has been reported for VRE, ESBL-producing Enterobacteriaceae, *P. aeruginosa, Stenotrophomonas maltophilia,* and carbapenem- or colistin-resistant *K. pneumoniae.*[87–89] Obviously, the negative and positive predictive values of colonization are not 100% because not all the cases of MDR-BSI are preceded by documented colonization, whereas some colonized patients will not develop MDR-BSI. However, colonization may be the most easily identifiable risk factor, and screening protocols for drug-resistant bacteria should probably be implemented everywhere.

Anaerobic bacteria are isolated in less than 1% of positive blood cultures in cancer patients, but the proportion may increase to 3% among those undergoing abdominal surgery.[70] Anaerobes are usually isolated in polymicrobial bacteremias, especially together with gram-negative rods, with a rate that seems to be higher than that observed in nononcology patients undergoing similar surgery (0.597 vs. 0.033 per 1000 hospital days, respectively).[90] Differences in the etiology of bacterial infection between neutropenic and nonneutropenic cancer patients have been reported.[14]

CVC-related bacteremias are generally caused by gram-positive cocci (especially coagulase-negative staphylococci), which are isolated in more than 50% of the episodes compared with the rate of 25% to 40% for gram-negative rods.[9,91–98] As mentioned previously, the source of infection is likely to be partially different in cases of gram-positive and gram-negative CVC-related bacteremias. Infusate contamination is a rare but possible event, and in this case gram-negative rods, such as *Klebsiella, Enterobacter, Citrobacter, Achromobacter, Serratia, Ralstonia,* and *Pseudomonas* (other than *P. aeruginosa*), are more likely involved. Polymicrobial infections are not rare with a predominance of gram-negative bacteria, whereas fungi (mainly *Candida* spp.) are usually monomicrobial and infrequent.

Bacterial gastroenteritis caused by classic enteric pathogens (*Salmonella* and *Shigella*) is a rare event in patients with acute leukemia, involving less than 1% of acute enteritis after chemotherapy.[99] On the contrary, *C. difficile* is not unusual in cancer patients, with an incidence that is twofold higher than in the noncancer population.[100] *Helicobacter pylori* has also been described as a possible cause of gastrointestinal disease in cancer patients.

Legionellosis and nocardiosis are rare but potentially life-threatening infections, with *Legionella* pneumonia sometimes presenting with unusual

radiologic patterns. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have been rarely described as a cause of pneumonia in cancer patients, but it is possible that their incidence is underreported. Similarly, TB is probably underestimated and underdiagnosed in cancer populations, although there are data showing that the rate approximates 90 cases per 100,000 persons (i.e., ninefold higher than in the general population in developed countries).[101] Patients from high-endemicity countries account for most of the cases. Infections caused by nontuberculous mycobacteria are rare, outside of the already mentioned rare occurrence of local or even disseminated *M. bovis* infections in patients receiving Calmette-Guérin bacillus immunotherapy for bladder cancer.

Fungal Infections

Aspergillus spp. and *Candida* spp. are the most common fungal pathogens in cancer patients, with the former now seen more frequently than the latter. Other fungal pathogens include *P. jirovecii,* cryptococci, and molds such as *Mucorales* or *Fusarium.*

Among yeasts, *Candida* is the most frequently isolated organism, usually in BSIs, with an increasing proportion of non-*albicans* strains, probably due to the extensive use of prophylactic fluconazole. This phenomenon, known since 1999, was confirmed in a more recent European Organization for Research and Treatment of Cancer (EORTC) study, which reported that in cancer patients the overall incidence of fungemia was 2.3%, with *C. albicans* responsible for 72% of candidemia cases in the whole study population, but only 27% in patients with hematologic malignancies.[102,103] Of note, in this study only 38% of fungemias occurred during neutropenia. In general, yeasts belonging to the *Candida parapsilosis* complex are usually associated with CVC contamination, whereas other *Candida* species are supposed to come from the gastrointestinal tract after selection and translocation. *Candida glabrata* is the species increasing in frequency and resistance, not only to fluconazole but also to echinocandins.[104] Additionally, *Candida auris* is a recently identified species that caused outbreaks worldwide.[105] It is characterized by resistance to numerous antifungals, mainly fluconazole, less frequently voriconazole and amphotericin B. Therefore echinocandins are the mainstay of treatment. *Candida auris* has a potential for clonal outbreaks, and is associated with 40% to 50% mortality due to patients' poor general conditions, antifungal resistance, and its ability to form biofilm and cause persistent infection.[105]

Among molds, *Aspergillus* represents the most frequently isolated or suspected organism. The majority of episodes are caused by *Aspergillus fumigatus,* although some centers report a predominance of infections caused by *Aspergillus flavus* and *Aspergillus terreus.*[106,107] *Aspergillus* species are ubiquitous molds whose primary ecologic niche is represented by decomposing vegetable material, including potted plants, soil, flowers, and carpets. In healthy individuals, *Aspergillus* conidia are trapped in the upper respiratory tract, and only a small proportion of them enter the lower airways where *Aspergillus* may become an allergen. In immunocompromised patients, especially those with hematologic malignancies or after allogeneic HSCT, spores can germinate and cause an invasive disease. Thus invasive aspergillosis in patients with malignancy or receiving HSCT is an endemic disease, which is usually community acquired and endogenous, although epidemic outbreaks of exogenous infection associated with massive environmental exposures (in and outside the hospital) can occur. The incidence of invasive aspergillosis depends on the patient's age (lower in those younger than 10 years), the underlying malignancy, and its treatment, being the highest in patients with prolonged neutropenia, followed by those receiving high doses of steroid therapy. In a multicenter Italian study, the incidence of aspergillosis among hematologic cancer patients varied from 7.9% in acute nonlymphoblastic leukemia to 4.3% in acute lymphoblastic leukemia, 2.3% in chronic myelogenous leukemia, and less than 1% in chronic lymphocytic leukemia, Hodgkin and non-Hodgkin lymphoma, and multiple myeloma.[106] A recently described risk group is represented by patients with chronic lymphoproliferative disorders, probably as a result of introducing more intensive treatment protocols.[107] Additionally, the risk of aspergillosis in patients with acute lymphoblastic leukemia was reported as high as 11.7%.[108] The incidence of invasive aspergillosis after autologous HSCT is low (0.3%–2%), and it occurs during preengraftment neutropenia.[109]

The main challenge in the current management of invasive aspergillosis, which consists of voriconazole or isavuconazole as first-line therapy and prophylaxis with posaconazole in high-risk patients, is the increasing problem of primary resistance to azoles in *A. fumigatus.*[110] Azole-resistant isolates harboring the *TR34/L98H* or the *TR46/Y121F/T289A* mutations have been found in the environment due to a widespread use of azoles in the agriculture industry in some countries. Patients may inhale spores of these strains and develop primary azole-resistant disease despite no previous antifungal therapy. The prevalence of resistant strains is only about 3% among 3788 isolates screened in Europe and differs highly between regions.[111] Data on high prevalence of resistant strains in patients with hematologic malignancies in the Netherlands and Germany have been reported, but methodologic issues, including the choice of denominator to evaluate resistance rate, are crucial.[112] Despite the fact that the phenomenon is so far relatively limited in most of the settings, knowledge of the frequency of azole resistance at the country and hospital level and within different patient groups is warranted. Secondary azole resistance, with different genetic patterns, has been reported in patients with chronic fungal infection after prolonged treatment, particularly in the case of suboptimal blood levels or high fungal burden.[113]

Mucormycosis is being increasingly reported by some centers, especially in the US. It is unclear whether this represents a general trend, if it is influenced by local factors, or if these infections are simply diagnosed more often because of an increased clinical awareness or improved patient survival. Other fungi, such as *Cryptococcus, Fusarium*, *Trichosporon, Saprochaete (Magnusiomyces/Blastoschizomyces),* and *Scedosporium,* have been reported sporadically, but it is likely that previously uncommon organisms may appear more frequently because they are being selected by the widespread use of mold-active antifungal prophylaxis.[114]

Pneumocystis jirovecii is a well-known cause of pneumonia in cancer patients not receiving trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis, especially if treated with high-dose and prolonged steroid therapy or certain antineoplastic drugs such as fludarabine or temozolomide. Attack rates vary from 6.5% to 43% in acute lymphoblastic leukemia, 4% to 25% in rhabdomyosarcomas, and nearly 1% in Hodgkin lymphoma and primary or metastatic central nervous system tumors.[115,116]

Finally, infections or reactivations of dimorphic fungi, such as *Histoplasma* or *Coccidioides,* are possible in patients who live in or used to live in endemic areas.

Viral Infections

Apart from herpes simplex virus (HSV) reactivation, which occurs in up to 60% of HSV-seropositive patients with acute leukemia, other viral infections are rather infrequently reported outside the setting of allogeneic HSCT.[3] For example, a positive pp65 antigenemia for CMV has been reported in 9% of non-HSCT recipients and in 12% of patients undergoing autologous HSCT, without necessarily being accompanied by CMV disease.[117] For this reason, routine monitoring of CMV reactivation and preemptive therapy were not considered necessary in cancer patients other than HSCT recipients. However, the risk of viral reactivation might change significantly with the increasing use of novel T-cell–suppressing agents or drug combinations, such as alemtuzumab or idelalisib or a combination of bendamustine and rituximab.[62,118–120]

Community-acquired respiratory viruses, such as influenza, parainfluenza, respiratory syncytial virus (RSV), metapneumovirus, adenoviruses, rhinoviruses, and coronaviruses, are a frequent cause of respiratory disease in cancer patients and are probably underestimated as a cause of fever. Whereas most cancer patients would experience self-limited upper respiratory illness, those with a severe immune deficit, such as those treated for leukemia, are at increased risk for progression from upper respiratory tract infection to pneumonia, with possible respiratory failure and fatal outcome.[121] The incidence rate of viral respiratory infections in patients with acute lymphocytic and acute myelogenous leukemia is estimated to be 68 and 31 infections per 1000 new admissions, respectively.[122] Almost half of these patients had pneumonia, and the mortality was 14%.[122] In cancer patients with a viral respiratory disease, deferral of chemotherapy could be considered. Specific treatment is warranted for influenza and in some cases of RSV infection (e.g., in leukemic patients with risk factors for RSV-related mortality) (see Chapter 158).[121]

Viral gastroenteritis, mainly caused by rotavirus but also norovirus or sapovirus, may be a frequent complication in pediatric oncology, with a potential to cause outbreaks in cancer centers because of persistent gastrointestinal shedding in immunocompromised hosts. Both adenoviruses and parvovirus B19 have been reported as rare causes of severe gastrointestinal disease in cancer patients.

Finally, the reactivation/exacerbation of infections due to hepatotropic viruses (HBV and hepatitis C virus) represents an important problem in areas of high endemicity. HBV reactivation is frequent in cancer patients with chronic inactive HBV infection (HBsAg positive, with negative or low-level serum HBV DNA), but it can occur also in patients with an occult HBV infection (HBsAg negative, HBcAb positive, or with low-level serum HBV DNA), particularly in association with the use of rituximab (up to 40% of patients).[63] The possibility of chronic hepatitis E virus infection due to genotype 3, which is endemic in certain industrialized regions, has been recently reported in patients with hematologic malignancies and transplant recipients.[123,124] Repeated transfusions might be a risk factor for hepatitis E virus infection.[125]

Other Pathogens

The risk of rare infections or reactivations caused by protozoa (leishmaniasis, South American trypanosomiasis, and malaria), helminths (strongyloidiasis), endemic fungi and other tropical diseases should be considered in patients who lived in endemic areas. Obtaining a history to identify potential exposure is the most important screening, with additional serology to document past exposure. However, in the case of strongyloidiasis, for example, the suboptimal performance of stool examination or serologic screening may warrant empirical treatment with ivermectin in patients who present with unexplained eosinophilia and who lived in endemic areas, such as the tropics, the subtropics, or the southeastern United States and Europe.

PREVENTION OF INFECTIONS IN CANCER PATIENTS

Prevention is obviously a desirable goal, given the remarkable mortality and morbidity associated with infections in cancer patients. Table 306.4 summarizes different regimens for primary chemoprophylaxis and other approaches that have been considered appropriate in cancer patients based on clinical trials and guidelines. In the following sections, advantages and disadvantages of different procedures are discussed.

Prevention of Bacterial Infections

Antibacterial Chemoprophylaxis

The use of antibiotics to prevent bacterial infections should be weighed against their efficacy, their toxicity, and especially their impact on the development of resistance. In general, to evaluate the cost-effectiveness

TABLE 306.4 Suggested Prophylaxis for Infections in Cancer Patients

	DRUG	SCHEDULE	COMMENTS
Antibacterial	Ciprofloxacin Levofloxacin	500 mg bid 500 mg once daily	Use of FQs should be based on local epidemiology and careful evaluation of potential drawbacks of FQ prophylaxis. Probably not active anymore in many sites. Some studies showed possible effects on increasing resistance. Traditionally administered to adults receiving chemotherapy for acute leukemia with expected neutropenia >7–10 d; starting with chemotherapy and continuing until resolution of neutropenia or initiation of empirical antibacterial therapy for febrile neutropenia.
Antifungal	Posaconazole	100-mg tablets: 3 tablets twice daily on the first day, then 3 tablets daily As alternative, oral solution 200 mg tid orally with a (fatty) meal or acidic drink	Patients receiving chemotherapy for acute myelogenous leukemia or myelodysplastic syndrome. Therapeutic drug monitoring is warranted.
	Fluconazole	400 mg once daily	Patients receiving chemotherapy for acute myelogenous leukemia with cytarabine plus anthracycline regimens (administered for 7 and 3 d, respectively) and high-dose cytarabine-containing regimens.
	Other		Secondary prophylaxis according to isolated pathogen, clinical presentation, or both.
Anti–*Pneumocystis jirovecii*	Trimethoprim-sulfamethoxazole (TMP-SMX)	One double-strength tablet (160/800 mg) three times weekly, *or* 25 mg/kg TMP-SMX (5 mg/kg of TMP), max. 1920 mg (2 double-strength capsules) in 2 divided doses for 3 consecutive days/wk	All patients receiving chemotherapy with steroids, including those with solid tumors (e.g., brain cancer).
	Dapsone	2 mg/kg/d (max. 100 mg), on alternate days three times/wk	In patients who cannot tolerate TMP-SMX.
	Aerosolized pentamidine	300 mg once a month with nebulizer	In patients who cannot tolerate TMP-SMX; effective, but it is more difficult to administer.
	Atovaquone	750 mg twice daily or 1500 mg once daily	In patients who cannot tolerate TMP-SMX.
Antiviral	Acyclovir *or*	40 mg/kg in children in 2 divided doses In adults >40 kg: 800 mg twice daily	Patients with positive anti-HSV antibodies and severe mucositis or receiving treatment for acute leukemia.
	Valacyclovir	For HSV or VZV prophylaxis: 500 mg twice daily or 1000 mg daily For VZV exposure: 1 g three times daily (see text)	VZV-susceptible patients exposed to chickenpox who did not receive prompt administration of specific immunoglobulins.
	Lamivudine	100 mg once daily	Patients with chronic inactive HBV infection (HBsAg positive, HBV DNA low level or negative). Patients with resolved HBV infection (HBsAg negative and HBcAb positive), particularly if receiving rituximab or allogeneic HSCT.
	Entecavir	0.5 mg daily	HBsAg-positive patients treated with rituximab with HBV DNA <2000 IU/mL.

Continued

TABLE 306.4 Suggested Prophylaxis for Infections in Cancer Patients—cont'd

	DRUG	SCHEDULE	COMMENTS
Antituberculosis	Isoniazid	5 mg/kg (max. 300 mg) in adults, 10 mg/kg (max. 300 mg) once daily in children once daily for 6–9 mo	Patients with latent tuberculosis. Efficacy not specifically evaluated in cancer patients. See Chapter 249 and text for other regimens to treat latent tuberculosis.
Anti–CVC-associated infection	None	Good skin preparation and the use of sterile technique at time of device insertion. Good maintenance procedures.	All patients with indwelling central venous catheter.
Others	Growth factors	Filgrastim either subcutaneously or as an intravenous infusion over at least 1 h, *or* pegylated filgrastim	For the prevention of febrile neutropenia in patients who have a high risk of this complication based on age, medical history, disease characteristics, and myelotoxicity of the chemotherapy regimen. Secondary prophylaxis with G-CSFs recommended for patients who experienced a neutropenic complication from a prior cycle of chemotherapy (for which primary prophylaxis was not received), in whom a reduced dose of chemotherapy may compromise disease-free or overall survival or treatment outcome. Efficacy not fully demonstrated for pegylated filgrastim.
	Immunoglobulins	Polyclonal immunoglobulins: 400 mg/kg every 21–28 d	Patients with chronic lymphocytic leukemia after the second episode of severe bacterial infection. Patients with leukemia or lymphoma with hypogammaglobulinemia (<400g/dL) and severe bacterial infections (reasonable, but not proved).
		Specific anti-VZV (VariZIG): 125 IU for every 10 kg of body weight (max., 625 IU)	In high-risk contact with a negative history of varicella preferably within 96 h after exposure to chickenpox.
	Vaccines	Influenza	Influenza vaccination of patients, especially during less aggressive treatment phases. Vaccination of household contact and health care workers.
		Varicella	VZV-seronegative household contacts and health care workers.
		Pneumococcus	Conjugated 13-valent antipneumococcal vaccine.
	Isolation procedures	Perform hand hygiene with an alcohol-based hand rub or by washing hands with soap and water if soiled, before and after all patient contacts or contact with the patients' potentially contaminated equipment or environment. Use contact precautions (gowns and gloves). Ensure adherence to standard environmental cleaning with an effective disinfectant.	Patients colonized or infected with multidrug-resistant pathogens (such as VRE, CRE, etc.) or infected with other pathogens for which contact isolation precautions are advisable (*C. difficile,* norovirus, etc.); of note, alcohol-based hand rubs are not cidal against *C. difficile* or norovirus.

CRE, Carbapenem-resistant Enterobacteriaceae; *CVC,* central venous catheter; *FQ,* fluoroquinolone; *G-CSFs,* granulocyte colony-stimulating factors; *HBcAb,* hepatitis B core antibody; *HBsAg,* hepatitis B surface antigen; *HBV,* hepatitis B virus; *HSCT,* hematopoietic stem cell transplantation; *HSV,* herpes simplex virus; *IU,* international unit; *max.,* maximum; *VRE,* vancomycin-resistant enterococci; *VZV,* varicella-zoster virus.

of a prophylactic protocol, one should know, in every center, the rate of the complication to prevent and, consequently, the number of patients needed to treat to prevent the occurrence of a single infectious episode and attributable death. The safety—that is, the number needed to harm—should be also taken into consideration. Chemoprophylaxis for the prevention of bacterial infections was first proposed in clinical practice based on the discovery that 80% of the bacterial pathogens causing infection in neutropenic cancer patients were originating from the endogenous flora, but that approximately half of the pathogens were acquired during the hospital stay. The first approach relied on the oral administration of nonabsorbable antibiotics (gentamicin, vancomycin, and nystatin) aimed at totally (including anaerobes) or partially (excluding anaerobes) suppressing the intestinal bacterial flora and preventing the acquisition of exogenous organisms (gut decontamination). Subsequently, absorbable drugs such as TMP-SMX, usually given in combination with oral nystatin and then fluoroquinolones (FQs), were introduced. Several studies and meta-analyses were performed on the effectiveness of quinolone prophylaxis in cancer patients. The last and the largest one was published in 2012 and showed that antibacterial prophylaxis, especially with quinolones, was able to reduce in a statistically significant way both overall and infection-related mortality, in addition to preventing bacterial infections.[126] The number needed to treat (NNT) to prevent one death was around 50 and was higher than in a previous meta-analysis (NNT = 24), and there was apparently no untoward effect in terms of induction of resistance, although the issue was only partially studied because of undeniable difficulties.[127] Therefore most of the guidelines recommended FQ prophylaxis for adult patients with expected neutropenia longer than 7 days. It is important to note that these results were based on studies published between 1973 and 2011, thus before the spread of bacterial resistance.[128]

Following an alarming increase in bacterial resistance worldwide, two issues need to be addressed: (1) whether the benefit of FQ prophylaxis can still be expected, and (2) what is the contemporary impact of routine prolonged administration of broad-spectrum agents such as FQ on the selection of resistant strains and local epidemiology. A recent literature review on studies of FQ prophylaxis published in the last decade, in an era of increased resistance, did not confirm any effect on mortality, although prophylaxis was still associated with lower rates of BSI and febrile episodes.[128] The reason for that might be that the sample size could be insufficient to show a difference in mortality. However, a more

clinical explanation could be proposed: that the infections that quinolones were still able to prevent were those caused by susceptible organisms, in which mortality was very low in any case, because of the effective and timely empirical treatment.[129] Indeed, as in other settings, the mortality in neutropenic patients with BSI is most importantly influenced by the time between the onset of infection and the administration of appropriate antibiotic therapy.[130] As demonstrated recently by several studies, BSI-associated mortality is mainly driven by MDR pathogens, which are unlikely to be prevented by FQs.[86,131,132] This might explain the fact that FQ prophylaxis may no longer have any effect on overall mortality, despite their ability to prevent some infections (i.e., those due to organisms that are effectively treated with classic empirical therapy).[128,129]

As far as the impact of FQ prophylaxis on antibiotic resistance is concerned, data were scarce and discordant, but some studies reported an increase in ESBL-producing or MDR bacteria. There was no association with the rate of background resistance to FQs and the efficacy of FQ prophylaxis, but the NNT to prevent one BSI in the last decade was twice as high as reported in the meta-analysis from 2005.[128] For all previously mentioned reasons, some recent guidelines, considering the limited clinical benefit (mortality rates low and not improved by FQs, high NNT) and potential negative impact of FQs (toxicity and selection of resistant strains), do not support the use of FQ prophylaxis.[133,134] In patients with solid tumors, who usually experience low rates of BSI and shorter (<7 days) neutropenia, FQ prophylaxis is not recommended given no difference in the incidence of severe infections or both infection-related and overall mortality.[20]

In conclusion, FQ prophylaxis of febrile neutropenia has probably lost part of its efficacy because of the decreasing quinolone activity related to increasing resistance rates. Countries and centers with no or negligible resistance might still benefit from prophylaxis, while in others the advantage is likely to be minimal. The global situation of antibiotic usage has changed substantially in recent years, thus calling for a change in the way we look at indications that were apparently consolidated. Every center should review its prophylactic approach according to its own data. The world is facing a global crisis in terms of increased antibiotic resistance and spread of MDR pathogens, which are resistant to almost all available therapeutic options. This calls for new responsibilities of every specialist concerning proper use of antibiotics. Fluoroquinolones should be used cautiously in patients receiving cyclophosphamide since they were reported to cause significantly lower exposure to 4-hydroxycyclophosphamide (the active metabolite of cyclophosphamide) in patients with non-Hodgkin lymphoma.[135]

In children with acute leukemia, single studies on the use of amoxicillin/clavulanate, ciprofloxacin, or levofloxacin showed a reduction of fever episodes.[136–138]

Decolonization for Prevention of Infections Due to Resistant Bacteria, Including Fecal Microbiota Transplantation

Colonization with an MDR pathogen is a well-established risk for BSI due to the same strain, and the rate of BSI due to carbapenem-resistant *K. pneumoniae* was over 30% in allogeneic HSCT recipients colonized with such a pathogen.[131,139] Therefore attempts at decolonization by oral administration of nonabsorbable molecules such as gentamicin or colistin have been carried out and were reviewed in 2014.[139] Briefly, the decolonization rate varied from 37% to 68%, at the expense of a risk of developing resistance to the used drug (frequently the only therapeutic option in cases of subsequent MDR infection) as high as 45%. However, the applicability of this strategy before prolonged periods of neutropenia might be reevaluated in light of the availability of new antibiotics that might be used if an MDR breakthrough infection occurs.

Stemming from observations that alterations of intestinal microbiota can represent an important risk factor for development of bacteremia in cancer patients, fecal microbiota transplantation (FMT) could represent a strategy for prevention of infections due to antibiotic resistant pathogens. Indeed, a recent prospective trial in 20 patients with hematologic malignancies (40% of whom were neutropenic) colonized with MDR bacteria showed that 25 intraduodenal FMT procedures resulted in complete and partial decolonization in 60% and 80%, respectively.[140] The FMT procedure was more effective in the absence of concomitant antibiotic administration (79% vs. 36%), and no severe adverse events were observed.[140] Further studies are warranted to determine all the potential benefits and real-life applications of manipulation of gut microbiota.

Prophylaxis of Tuberculosis

Latent TB may reactivate during immunosuppression after antineoplastic chemotherapy. No specific study on latent TB treatment in cancer patients has been performed. The data on efficacy come from other immunocompromised patients, mostly human immunodeficiency virus (HIV) positive, and show that daily isoniazid monotherapy for 6 to 9 months, or rifapentine and isoniazid weekly for 3 months, or rifampin daily for 4 months are effective in treating a latent TB infection and preventing TB.[141,142] This approach should be considered in cancer patients with a positive TB skin test or a positive interferon-γ release assay (T-SPOT TB test [Oxford Immunotech, Marlborough, MA] or QuantiFERON-TB Gold In-Tube test [Qiagen, Valencia, CA]) and in cases of household exposure to TB or history of inadequately treated TB. Attention should be paid to potential interactions between rifampin and antineoplastic drugs and possible hepatotoxicity associated with all the regimens.

Prevention of CVC-Related Infections (See Chapter 300)

Prevention of CVC-related infections lies mainly in the correct insertion and manipulation of the devices, not only by health care workers but also by caregivers who perform CVC-maintenance procedures (and sometimes drug infusions) at home. Aseptic bundles during insertion and postinsertion care, bundles that include assessment of need for CVC, daily assessment of the site of insertion, change of dressing at least weekly or whenever wet or soiled, use of chlorhexidine gluconate–impregnated sponges, application of alcohol scrub for 15 seconds before each access of the line, and hand hygiene are effective measures to prevent infectious complications.[41] Continuous education and regular audits of bundle implementation represent other helpful activities.[143] Antibiotic catheter flushing has also been suggested to reduce the risk of infections, but this could represent a risk for selection of resistant bacteria. On the contrary, taurolidine lock has been associated with a significant reduction of CVC-related BSI, even if the susceptibility of gram-positives and gram-negatives to taurolidine are indeterminate due to limited data.[144]

Prevention of Fungal Infections

Primary Antifungal Chemoprophylaxis

Although fungal infections usually represent no more than 10% of all infections (see Table 306.2), their associated mortality is very high. Therefore preventing invasive fungal infections has always been considered a desirable approach. Two main drugs have been used in recent years for antifungal prophylaxis in acute leukemia patients: fluconazole (active against yeasts such as *Candida* but not molds) and posaconazole (mold-active prophylaxis). Meta-analyses showed that the use of fluconazole is effective in preventing *Candida* infections in allogeneic HSCT and in acute myeloid leukemia patients and suggest some possible benefit in other populations, if the incidence of invasive candidemia is higher than 7% to 10%.

As far as mold-active prophylaxis is concerned, in 2007 a study of oral posaconazole (oral solution) in adults receiving multiple cycles of chemotherapy for acute myeloid leukemia or myelodysplastic syndrome showed a statistically significant advantage in terms of mortality and a 6% absolute reduction in the relative risk of invasive mycosis, from 8% to 2% (primary end point), with a significant reduction also in the cumulative risk of infection compared with standard prophylaxis (fluconazole or itraconazole).[145] In this study, it was impossible to confirm the difference in mortality in a multivariate analysis including all baseline and time-dependent factors potentially able to affect survival, thus substantially limiting the likelihood of the survival benefit. Given the reduction in the incidence of fungal infections and the incidence observed in the control group, the NNT to prevent one case of proven/probable invasive mycosis was 16, and the NNT to prevent one fungal infection–related death was 27.[146] However, if the of incidence of invasive mycoses is lower than 8%, the NNT to prevent

one infection would be higher, and this observation underlines the need for every center to obtain accurate information on the local epidemiology of invasive mycosis and to tailor the results of clinical trials to the local situation. Until recently, posaconazole was only available as an oral solution and absorption was highly variable, depending heavily on ingestion of a fatty meal or an acidic drink, on subdividing the daily dose (three to four times a day), and on maintaining gastric acidity (no proton pump inhibitors). Thus therapeutic drug monitoring of serum levels has been recommended.[147] The recent introduction of an intravenous formulation and a new oral formulation (oral delayed-release tablets whose absorption is not influenced by gastric pH) resolved the problem of the low bioavailability, resulting in adequate plasma levels in most patients. Indeed, blood levels were sometimes too high, with some cases of toxicity.[148] As shown in Tables 306.4 and 306.5, particular attention is warranted if oral formulations are interchanged, since the dosing is different (200 mg tid for oral solution vs. 300 mg bid on the first day and then 300 mg daily for tablets). Of note, posaconazole has also been effective in reducing the incidence of invasive fungal infections in allogeneic HSCT recipients with graft-versus-host disease.[149]

Voriconazole, another mold-active azole, has never been studied for and is not approved for prophylaxis in acute leukemia. It has been tested in two trials in the setting of allogeneic HSCT, including the neutropenic preengraftment phase.[150,151] In the first study, the primary end point (survival) was not met, but voriconazole prophylaxis reduced the incidence of invasive aspergillosis, although in a non–statistically significant way.[150] Of note, efficacy was more evident among patients undergoing transplantation for acute myeloid leukemia as an underlying disease. In the second study, voriconazole was superior to itraconazole in a composite end point, in which the main driver of success was better tolerability and not efficacy. Unfortunately, the design of the voriconazole studies was suboptimal because, in both cases, both low- and high-risk patients were included, with consequent dilution of the possible benefit of prophylaxis. Issues related to therapeutic drug monitoring are also relevant for voriconazole, because of its erratic metabolism. In addition, triazoles (especially voriconazole) have many pharmacokinetic interactions with other drugs metabolized via the cytochrome P-450 system, with the risk of reducing the efficacy of treatments and increasing the incidence of (severe) adverse events.[152,153]

Finally, prophylaxis with nebulized liposomal amphotericin B plus systemic fluconazole, compared with fluconazole only, was demonstrated effective in reducing proven/probable IFD during repeated periods at risk after chemotherapy for acute leukemia in adults (a 10% reduction in IFD events).[154] A systemic antifungal prophylaxis with liposomal amphotericin B at 2.5 mg/kg twice weekly was found feasible and safe in high-risk pediatric cancer patients, compared with a historical control

TABLE 306.5 Antibacterial and Antifungal Agents Usually Used in Cancer Patients

PATIENTS' TYPE OF THERAPY	DRUG	ROUTE OF ADMINISTRATION	DAILY PEDIATRIC DOSAGE	USUAL DAILY ADULT DOSAGE	NO. OF DAILY DIVIDED DOSES
Antibacterial, intravenous	Piperacillin-tazobactam	IV	400 mg/kg (as piperacillin)	13.5–18 g (as piperacillin)	3–4 in adults, 4 in children; preferably in prolonged or continuous infusion with a loading dose
	Ceftazidime	IV	100 mg/kg	6000 mg	3
	Cefepime	IV	100 mg/kg	6000 mg	3
	Meropenem	IV	60 mg/kg	3000–4000 mg	3, infuse over 3–6 h
	Ceftolozane-tazobactam	IV	ND	4500 mg (as both drugs) 9000 mg in studies on nosocomial pneumonia (off-label dosage)	3
	Ceftazidime-avibactam	IV	ND	7500 mg (as both drugs)	3, infuse over 2 h
	Ceftaroline	IV	2 mo to <2 yr: 8 mg/kg every 8 h ≥2 yr to <18 yr (≤33 kg): 12 mg/kg every 8 h ≥2 yr to <18 yr (>33 kg): 400 mg every 8 h or 600 mg every 12 h	1200 mg	2, infuse over 5–60 min
	Ceftobiprole	IV	ND	1500 mg	3
	Imipenem-cilastatin	IV	60–100 mg/kg (as imipenem)	2000–4000 mg (as imipenem)	3–4
	Meropenem-vaborbactam	IV	ND	12 (as both drugs)	3, infuse over 3 h
	Ciprofloxacin	IV	15–30 mg/kg	800–1200 mg	2–3
	Ceftriaxone	IV	80 mg/kg	2000 mg	1
	Amikacin	IV	20 mg/kg	1000–1500 mg	1
	Vancomycin	IV	40–60 mg/kg	2000 mg	2 or as continuous infusion after a loading dose
	Teicoplanin	IV; not available in United States	10 mg/kg (loading dose of 10 mg/kg bid on first day of treatment)	600–1200 mg (loading dose of 600 mg bid on first day of treatment)	1 (2 on first day of treatment)
	Daptomycin	IV	10 mg/kg	6–8 mg/kg	1
	Linezolid	IV (also available oral)	30 mg/kg in three doses if <12 yr, then 20 mg/kg	1200 mg	2
	Tedizolid	Oral/IV	ND	200 mg	1
	Tigecycline	IV	2.4 mg/kg loading dose, then 2.4 mg/kg (proposed)	100 mg loading dose, then 100 mg	2
	Colistimethate sodium (colistin)	IV	150,000–200,000 IU/kg loading dose, then 150,000–200,000 IU/kg[a]	9,000,000 IU loading dose, then 9,000,000 IU[a]	2
	Polymyxin B	IV	Infants: up to 40,000 U/kg Children: 15,000–25,000 U/kg	15,000–25,000 U/kg (= 1.25–2.5 mg/kg, 1 mg = 10,000 U; a loading dose might be indicated)	
	Fosfomycin	IV; not available in United States	300 mg/kg	15–24 g	3–4

TABLE 306.5 Antibacterial and Antifungal Agents Usually Used in Cancer Patients—cont'd

PATIENTS' TYPE OF THERAPY	DRUG	ROUTE OF ADMINISTRATION	DAILY PEDIATRIC DOSAGE	USUAL DAILY ADULT DOSAGE	NO. OF DAILY DIVIDED DOSES
Antibacterial, oral	Amoxicillin-clavulanate	Oral	60–80 mg/kg (as amoxicillin)	2–3 g (as amoxicillin)	2–3
	Ciprofloxacin	Oral	30 mg/kg	1000–1500 mg	2
	Cefixime	Oral	6–8 mg/kg	400 mg	2–3
	Moxifloxacin	Oral, IV	Based on age, 4–6 mg/kg twice daily[225]	400 mg	1
Antifungal	Amphotericin B deoxycholate[b]	IV	0.5–1 mg/kg	0.5–1 mg/kg	1
	Liposomal amphotericin B	IV	3 mg/kg	3–5 mg/kg	1
	Amphotericin B lipid complex	IV	5 mg/kg	5 mg/kg	1
	Caspofungin	IV	50 mg/m^2 for age <17 yr	70 mg the first day, then 50 mg the following days	1
	Micafungin	IV	2–4 mg/kg	100 mg	1
	Anidulafungin	IV	3 mg/kg q24h the first day, then 1.5 mg/kg q24h	200 mg the first day, then 100 mg the following days	1
	Voriconazole	IV, oral	9 mg/kg bid the first day, then 8 mg/kg bid[c]	6 mg/kg bid the first day, then 4 mg/kg bid	2
	Fluconazole	IV, oral	10 mg/kg	400 mg	1
	Posaconazole[d]	Oral solution (doses are different for tablets and IV)	If >12 yr, dose as adults	800 mg (with a fatty meal or acidic carbonated drink)	4
		Oral delayed-release tablets	Not approved for <18 yr but PK/PD data available for per-kg dosing[226,227]	600 mg/d divided in 2 doses, then 300 mg daily	1
	Isavuconazole	IV/oral	ND	200 mg q8h for 6 doses, then 200 mg daily (372 mg if ordered as isavuconazonium sulfate)	3 during the first 48 h, 1 thereafter

[a]An important and potentially confusing characteristic is colistin's dosage expression. Colistin is available in two salt forms, colistin sulfate and colistimethate sodium. In Europe, colistimethate sodium (salt) is available, and dosing is expressed usually in international units (IU), and sometimes in milligrams of colistimethate sodium, whereas in the United States, the dosage of US Food and Drug Administration–approved colistimethate sodium is defined in milligrams of colistin base activity. Thus particular attention should be paid to avoid dosage errors. Approximate dose conversion: 1,000,000 IU = 80 mg of colistimethate sodium = 30 mg colistin base.[228] The optimal dosing of colistin remains to be established. Recent studies in adults with normal renal function reported the use of 6,000,000 to 9,000,000 IU daily, with pharmacologic analyses supporting the use of loading dose of 9,000,000 IU, followed by 4,500,000 IU every 12 hr.[229–231] See Chapter 32 for recommended doses in the United States, which are 5 mg colistin base/kg loading and then 5 mg colistin base/kg/d in two or three divided doses. If ordered as colistimethate, the dose per day would be 13.3 mg/kg, divided into two or three doses.

[b]Contraindicated in the presence of risk factors for renal toxicity (e.g., impaired renal function at baseline; nephrotoxic comedication, including aminoglycoside antibiotics; and history of previous toxicity).

[c]In patients 2 to 12 yr of age, and 12 to 14 yr if weighing less than 50 kg; otherwise, dose as for adults if 12 yr of age and older. Therapeutic drug monitoring is highly recommended. Children metabolize voriconazole more rapidly than adults.

[d]Therapeutic drug monitoring might be useful to assess if trough levels are in the range for efficacy in case of oral solution.

ND, No established dosing for children; *PK/PD,* pharmacokinetic/pharmacodynamic.

group.[155] On the other hand, a randomized controlled trial that included 355 adult patients undergoing remission-induction chemotherapy for newly diagnosed acute lymphoblastic leukemia demonstrated a high incidence of IFD in this population, but failed to demonstrate the efficacy of liposomal amphotericin B at 5 mg/kg twice weekly (IFD 7.9% in prophylaxis arm vs. 11.7% in placebo arm).[156]

Secondary Antifungal Prophylaxis

Patients with a history of invasive mycosis are at high risk of reactivation when undergoing further chemotherapy. The recurrence of invasive aspergillosis after HSCT has been associated with less than 1 month of antifungal therapy and with persistence of radiologic abnormalities after treatment.[157] Therefore secondary antifungal prophylaxis is recommended during HSCT or high-intensity chemotherapies for patients with previous IFD. The drug for secondary prophylaxis should be chosen according to the etiology of the original IFD, the localization, the drugs available and their formulations, and risks of interactions with other therapies, especially those for the treatment of the underlying disease.

Prophylaxis Against *Pneumocystis jirovecii*

In recent years, *P. jirovecii* pneumonia has been described with increasing frequency in non-HIV patients.[158] The risk is particularly high in patients with acute (especially lymphoblastic) leukemia, non-Hodgkin lymphoma, Waldenström macroglobulinemia, multiple myeloma, and chronic lymphocytic leukemia treated with standard chemotherapy or undergoing autologous transplantation.[116] Other patients at risk for this complication are those with central nervous system solid tumors, in correlation with the prolonged use of high-dose steroids and the alkylating agent temozolomide, or those receiving bendamustine for breast cancer.[158] Several other drugs affecting cell-mediated immunity have also been associated with the risk of *P. jirovecii* pneumonia, including fludarabine, ara-C (cytarabine), methotrexate, D-actinomycin, bleomycin, and L-asparaginase. In addition, pneumocystosis has been associated with administration of alemtuzumab, which causes profound depletion of T lymphocytes; with rituximab if used in combination with bendamustine or CHOP (cyclophosphamide, doxorubicin [hydroxydaunomycin], vincristine [Oncovin], and prednisolone) chemotherapy every 14 days; and with idelalisib, ibrutinib, and, in two cases, with the tyrosine kinase inhibitor dasatinib.[62,116] It remains to be determined whether novel biologic drugs are responsible for an increased infection risk or whether other concomitant or previous treatments (purine analogues, alkylating agents, or steroids) contribute significantly to the risk of pneumocystosis, and the duration of risk after treatment discontinuation is not known. Indications for prophylaxis of pneumocystosis include certain diseases (e.g., acute lymphoblastic leukemia) and treatments (e.g., fludarabine, alemtuzumab, or corticosteroid at the dose of >20 mg of prednisone daily for at least 4 weeks).[159] An absolute CD4$^+$ lymphocyte count of 200/mm^3 or less, or a proportion of 15% or less (or similar age-related values for children), has been suggested as an indication for prophylaxis, at least after HSCT. Because the efficacy and tolerability of 160/800 mg of TMP-SMX given three or seven times a week are comparable, one 160/800 mg TMP-SMX tablet three times weekly remains the best prophylactic option, provided the patient is strictly compliant with the prescription. Other drugs, such as daily oral dapsone

or atovaquone or monthly aerosolized pentamidine, have demonstrated efficacy and are alternative options in patients who cannot tolerate TMP-SMX (see Chapter 269).[159]

Prevention of Viral Infections

For the prevention of HSV or VZV reactivation, antiviral prophylaxis with acyclovir or valacyclovir is recommended for seropositive patients undergoing HSCT, therapy for acute leukemia, or treatment with certain new agents such as bortezomib, alemtuzumab, idelalisib, or daratumumab. In patients not on active prophylaxis who are exposed to varicella, intravenous administration of anti-VZV immunoglobulins (VariZIG) within 72 to 96 hours of the exposure is recommended, although protection is not guaranteed (see Chapter 136). If anti-VZV immunoglobulins are not available or in case of delayed notification of the risk contact, prophylaxis with valacyclovir (1 g three times daily for adults weighing more than 40 kg) or acyclovir (in children per os 20 mg/kg per dose, administered four times per day, with a maximum daily dose of 3200 mg; in adults 800 mg five times a day) is an option, although the efficacy has not been established. Prophylactic chemotherapy should be started on day 7 after exposure and continued for 7 days thereafter.

Routine CMV prophylaxis is not recommended outside of the transplantation setting. Despite that, it seems worthwhile to report that letermovir, a new antiviral with significantly fewer side effects than (val)ganciclovir or foscarnet, has been recently shown to reduce the clinically significant CMV infection rate in adult allogeneic HSCT recipients from 61% to 38%.[160] CMV DNA testing should be performed in all cancer patients with signs or symptoms compatible with CMV reactivation (cytopenias, unexplained fever) or disease (gastroenteritis, pneumonia, retinitis, hepatitis, encephalitis) (see Chapter 137).[119] In certain settings, such as patients receiving alemtuzumab for hematologic malignancies, idelalisib, rituximab and bendamustine, and possibly also brentuximab, the risk of CMV reactivation and subsequent disease may warrant routine, usually weekly, CMV monitoring.[119]

All cancer patients should be screened for an active or past HBV infection (with at least HBsAg and HBcAb) before starting chemotherapy because viral reactivation and acute hepatitis, with consequences ranging from delaying chemotherapy to fatal fulminant infection, can occur in patients receiving chemotherapy. Patients with chronic hepatitis should receive antiviral treatment, while those with inactive infection (i.e., HBsAg-positive, HBV DNA negative) should receive prophylaxis to prevent reactivation if they are scheduled to receive intensive chemotherapy, particularly including rituximab. Lamivudine (100 mg/day) used to be the mainstay of antiviral prophylaxis. Very limited data on new antivirals are available in this setting, with only one recent randomized, open-label trial showing entecavir (0.5 mg/day) to be superior to lamivudine in preventing clinical hepatitis in Chinese patients with chronic inactive HBV infection undergoing chemotherapy, including rituximab with CHOP (R-CHOP), for lymphoma (rate of reactivation, respectively, 4 of 61 [7%] vs. 18 of 60 [30%]).[161] Nevertheless, based on data from immunocompromised patients treated for a chronic HBV infection, most guidelines recommend agents with a high genetic barrier (such entecavir or tenofovir in any of the two formulations) for prophylaxis in HBsAg-positive patients.[63] Prophylaxis should be started before chemotherapy if possible, but chemotherapy should not be delayed because of it, and prophylaxis should be continued for at least 6 to 12 months after the end of chemotherapy (18 months for rituximab), depending on the predicted duration and intensity of immunosuppression.

HBV reactivation is also possible, although less likely, in patients with resolved HBV infection (HBsAg negative, HBcAb positive). The risk of reactivation can be as high as 42% in patients with lymphoma treated with rituximab, and 20% in chronic lymphocytic leukemia, 13% in T-cell leukemia, and 5% in multiple myeloma patients.[63] The options are either to provide lamivudine prophylaxis in those with high risk (i.e. >10%) of reactivation (e.g., patients receiving anti-CD20 agents) or to monitor them closely for HBV DNA appearance and treat at the first sign of reactivation.

Vaccination against influenza is the most effective preventive measure globally. Additionally, during community or nosocomial outbreaks of influenza infection, prophylaxis with oseltamivir 75 mg once daily for 10 days should be considered, especially in severely immunocompromised patients, because of the risk of severe infection and its complications.

Other Prophylactic Measures

Role of Colony-Stimulating Factors in Prophylaxis

Colony-stimulating factors are used with the aim of facilitating dose-intense treatments and decreasing infectious complications by preventing the development of neutropenia or reducing its duration. In solid tumors, studies using prophylactic granulocyte colony-stimulating factor (G-CSF) have consistently demonstrated a decrease in the length and severity of neutropenia and a decrease in the incidence of febrile neutropenia.[162] On the contrary, in patients with hematologic malignancies, G-CSF significantly reduced the duration of severe neutropenia, but without any significant reduction in febrile complications, duration of hospitalization, or survival.[163] Only two studies have compared antibiotics and G-CSF in the prevention of febrile neutropenia. When assessed together in a meta-analysis, a non–statistically significant difference favoring antibiotics was demonstrated.[164] Several guidelines consistently recommend the prophylactic use of G-CSF in adult cancer patients receiving a chemotherapy regimen associated with more than a 20% risk of febrile neutropenia or in patients at lower risk but with relevant comorbidities (older age, advanced underlying disease).[134] Finally, patients with solid tumors who experienced neutropenia and fever during previous cycles may benefit from prophylactic administration of G-CSF during subsequent cycles of chemotherapy.[134]

Role of Immunoglobulins in Prophylaxis

Based on the experience in patients with primary immunodeficiency syndromes, the administration of immunoglobulins has been recommended in those with secondary, iatrogenic immunoglobulin deficiencies, such as in patients with acute and chronic lymphocytic leukemia or non-Hodgkin lymphoma and in those receiving rituximab. Indeed, keeping the immunoglobulin G (IgG) level higher than 600 mg/dL has been associated with a reduction of infectious episodes due to bacteria, provided the treatment could be given for at least 6 months, but had no effect on viral and fungal diseases.[165] Similarly, a meta-analysis of studies in patients with chronic lymphocytic leukemia or multiple myeloma receiving immunoglobulins reported a reduction in the occurrence of infections, but without any impact on mortality.[166] Patients considered to deserve regular IgG replacement therapy are those with chronic lymphocytic leukemia and recurrent infections (mainly pneumonia or sinusitis) and an IgG level less than 500 mg/dL.[167]

Infection Control: Isolation and Antimicrobial Stewardship

In consideration of the growing antimicrobial resistance and shortage of new antibiotics, infection control and antimicrobial stewardship programs should be implemented in all cancer centers.[168] Infection control practices include surveillance, promoting and auditing the use of standard and transmission mode–based precautions, appropriate screening for colonization with resistant pathogens, and preventive measures, such as proper hand hygiene and contact precautions for patients colonized or infected with resistant bacteria (see Chapter 298). Active surveillance—for example with rectal swabs for colonization with carbapenemase-producing *K. pneumoniae* or VRE—should be performed in institutions where these pathogens are regularly encountered. Additional transmission mode–based precautions include the use of high-level masks/respirators for health care personnel, such as N95 or higher level or free-flow pressure 3 (FFP3) masks. These *airborne precautions* should be applied, for example, in cases of active TB or measles. *Contact precautions* (hand hygiene, use of disposable gowns and gloves) are indispensable when caring for a patient colonized or infected with MDR bacteria. In these cases, patients should be isolated in single rooms, possibly with staff cohorting. If single rooms are not available, then patient cohorting is recommended. In addition, in cases of patient transfer to another ward or hospital, it is of utmost importance to notify promptly and completely the receiving unit about the carrier status and the need for precautions. *Droplet precautions,* including disposable gown and gloves and a surgical mask, should also be applied

in cases of infection with respiratory viruses such as influenza. For all precaution measures, regular monitoring of adherence is warranted and the facilities should ensure access to adequate hand hygiene stations (sinks, alcohol-based rubs, etc.) and personal protective equipment.

For neutropenic patients, it is not clear whether staying at home or in a hospital may have an impact on the development of chemotherapy-related complications. In a hospital, keeping the patient in a reverse isolation/protective environment is believed to reduce colonization or infection with various pathogens. Unlike for transmission-based precautions, there is no universal consensus on the appropriate protective environment for neutropenic patients, and standards may vary between institutions. However, they usually include single rooms and, for people entering the room, surgical masks (to prevent mainly respiratory viruses) and in some institutions also disposable gloves and gowns (to prevent contamination with contact-transmitted pathogens). General guidelines for preventing the diffusion of infectious diseases in health care facilities should be followed in any ward where cancer patients are admitted.[169] Additionally, since aspergillosis and other mold infections are acquired via the respiratory route, the use of high-efficiency particulate air (HEPA) filters in rooms or wards where leukemic or transplant patients are hospitalized is recommended.[170] The use of masks with adequate filtration power (FFP3 or free-flow pressure 2 [FFP2] or N95 respirators) could reduce *Aspergillus* colonization and infection when the patient is moved from the protective environment. This approach has been demonstrated effective, together with other physical barriers, during building renovations.[171] The use of laminar airflow rooms is not deemed necessary and does not impact substantially on the rate of infection. On the contrary, it impacts negatively on patients' quality of life and on the possibility for the health care providers to care for them properly.

Antimicrobial stewardship (see Chapter 51) should include four main aspects: (1) local surveillance of antibiotic resistance, antibiotic consumption, and patient outcomes; (2) development and regular update of protocols and algorithms for the diagnosis, prevention, and treatment of infections; (3) prompt reporting of microbiologic results by the laboratory, allowing timely deescalation of broad-spectrum empirical regimens and shortening of antibiotic therapy; and (4) optimization of dosing regimens. All these aspects call for a multidisciplinary approach and close collaboration between the treating oncologist and hematologists; the microbiology laboratory; the infectious diseases consultation service, including infection control unit; and the hospital pharmacy.

Food and Lifestyle

A low-bacterial-count diet seems not to offer any benefit compared with a normal diet.[172,173] However, some precautions should be recommended, such as avoiding unpasteurized milk; unpasteurized or mold cheese products; raw or undercooked (including insufficiently reheated) meat, fish, shellfish, tofu, or eggs; and unpeeled fruits and salad ingredients, unless properly washed at home. *Listeria* colonization and infection has been described in association with unpasteurized dairy products and ready-to-eat deli meats, whereas raw vegetable sprouts have been associated with outbreaks of *E. coli, Salmonella,* and *Listeria* infection. Transmission of toxoplasmosis can also occur with raw meat and vegetables contaminated with cat feces. Although probiotics (foods with live yeast cultures) are advertised as useful in reducing the risk of antibiotic-associated diarrhea, bloodstream infections from probiotic administration have been reported. Food safety practices for food handling should be followed, and specific information for cancer patients is available online.[174] Diet recommendations that are too restrictive may have a negative impact on the patient's nutritional status or quality of life, or both.

Cancer patients, especially during less intensive treatment, may seek information about the safety of traveling or other recreational activities. Although few data exist that quantify the risk of travel or recreational activities, some considerations can be made. First, the assessment of the underlying conditions should be made, with particular attention to the stability of the patient's clinical condition and his or her potential need for rapid access to health care facilities (in that case, remote destinations or cruises are not recommended). In addition, evaluation of any ongoing treatment that might constitute a contraindication to the disease prevention measures recommended for the proposed destination, such as vaccines or antimalaria prophylaxis, is necessary. In immunocompromised hosts, live-attenuated vaccines (such as those against yellow fever or *Salmonella typhi*) might be contraindicated, whereas effectiveness of other vaccines, such as that against hepatitis A, might be reduced.

Cancer patients should not be advised to part with their pets, although some precautions are necessary; for example, a different household member should be assigned to scoop cat litter, because of potential *Toxoplasma* cyst exposure. Aquariums should not be touched or maintained by patients because water in fish tanks may be contaminated with atypical mycobacteria, whereas *Salmonella* can be acquired directly from reptiles or from fomites; thus patients should avoid contact with a reptile's food or aquarium. Finally, large pet birds should be avoided because they may transmit *Chlamydia psittaci.* In general, the potential benefit of recommendations on safety of food, pet care, travel, and other lifestyle measures should be weighed against the unclear value of such recommendations and their potential to have a negative impact on patients' nutritional intake and quality of life.

Vaccination

The use of inactivated vaccines in cancer patients has been demonstrated to be safe and effective, especially during nonaggressive/maintenance treatment phases, with the only pitfall that the immune response can often be poor, especially with certain drugs. International recommendations are available for vaccination of patients with hematologic malignancies and HSCT recipients.[175,176] Influenza vaccination with inactivated vaccines is strongly recommended for all immunocompromised patients, with the possible exception of those treated with intensive chemotherapy (e.g., a remission induction regimen for acute leukemia) and those who received rituximab within the previous 6 months, not because of safety concerns but because the probability of responding is very low. Both unvaccinated and vaccinated but severely immunocompromised patients should be offered empirical antiviral treatment in cases of clinical signs or symptoms of influenza during the influenza season. Influenza vaccination is also recommended for household contacts and health care personnel caring for cancer patients.

Pneumococcal vaccination is also strongly recommended for immunocompromised patients, although, again, response to vaccine may be suboptimal. Pneumococcal conjugate vaccine (PCV13) is thought to elicit better response than the pneumococcal polysaccharide vaccine (PPSV23).

In some cases—for example, for varicella—vaccination is recommended for seronegative household contacts. However, it has been suggested that prejudice about safety in those to be vaccinated is the major difficulty for implementing a varicella vaccination program targeted at seronegative household contacts to prevent varicella in immunocompromised children.[177]

TREATMENT OF COMPLICATIONS IN NEUTROPENIC CANCER PATIENTS

Empirical Antibacterial Therapy of Fever During Neutropenia

Fever during neutropenia has always been considered a medical emergency and should always be considered as due to infection, unless proven otherwise. Febrile episodes during the course of neutropenia are classified according to the presence or absence of a microbiologic or clinical documentation of infection, as (1) microbiologically documented infections with bacteremia (isolation of a significant pathogen from one or more blood cultures); (2) microbiologically documented infections without bacteremia (isolation of a significant pathogen from a well-defined site of infection, usually urine or respiratory secretions obtained with sterile procedures or fluid aspiration); (3) clinically documented infections, in the presence of a clinical picture clearly and objectively infectious in nature but without any microbiologic proof; and (4) unexplained fever or fever of unknown origin, when both clinical and microbiologic proof is lacking, but the clinical course is compatible with an infection. For the purpose of starting empirical antibiotic therapy, fever is usually defined as an axillary temperature greater than 38°C at three different times within a 12-hour period or as a temperature greater than 38.5°C in a single measurement. In a

neutropenic cancer patient, the development of fever or other signs of infection (e.g., altered mental status, hypotension, skin or mucosal lesions, respiratory failure) without fever or even with hypothermia should always raise the suspicion of an infection and must receive prompt attention, which involves diagnostic procedures (at least blood cultures) and empirical antibacterial treatment.

The effectiveness of the empirical therapy approach has been clearly demonstrated, and empirical antibiotic therapy has certainly contributed substantially to the impressive reduction in mortality from infectious complications observed during the last decades.[178] Unfortunately, some recent studies on infection in acute leukemia and HSCT patients seem to show that the mortality rate associated with infections due to gram-negative rods, and possibly also VRE, may be increasing, mainly driven by MDR isolates with peaks as high as 70% in BSI due to carbapenem-resistant *K. pneumoniae* in allogeneic HSCT recipients.[130,139,179,180] Indeed, in the era of bacterial resistance and shortage of new antibiotics, especially those active against gram-negative rods, infectious complications might once again significantly compromise the success of cancer treatment. Bacterial epidemiology and patterns of resistance may vary from patient to patient (in cases of individual colonization with resistant pathogens), from center to center (in cases of environmental colonization), and from country to country (different endemicity of resistant strains). Therefore for each patient, the risk of a severe and complicated clinical course and the risk of infection caused by resistant pathogens should be evaluated individually and the treatment chosen accordingly.[181] Fig. 306.2 summarizes a possible initial approach to a patient with febrile neutropenia. Table 306.5 summarizes the major antibiotics used for empirical or targeted therapy in febrile neutropenia.

Patients at Low Risk

An important change in the natural history of infections in cancer patients has been the increasing number of patients with solid tumors and nonleukemic hematologic diseases who are treated with high-dose chemotherapy and therefore may develop neutropenia and fever. However, patients with solid tumors rarely receive regimens that make them neutropenic for more than 7 to 8 days, and neutropenia is seldom

TABLE 306.6 Factors Associated With Low Risk of Severe Infection or an Uncomplicated Clinical Course in Febrile Neutropenic Cancer Patients

	MASCC SCORE	
	Clinical Parameters	**Score[a]**
Clinical data available at onset of febrile neutropenia or soon after admission	1. Burden of illness: no or mild symptoms[b]	0, 3, or 5
	2. No hypotension	5
	3. No chronic obstructive pulmonary disease	4
	4. Solid tumor or hematologic malignancy with no prior fungal infection	4
	5. No dehydration	3
	6. Outpatient status	3
	7. Patient's age <60 yr	2

[a]The maximal theoretical score is 26. Low-risk patient: score ≥21.
[b]No or mild symptoms = 5, moderate = 3, severe or morbid = 0.
MASCC, Multinational Association for Supportive Care in Cancer.

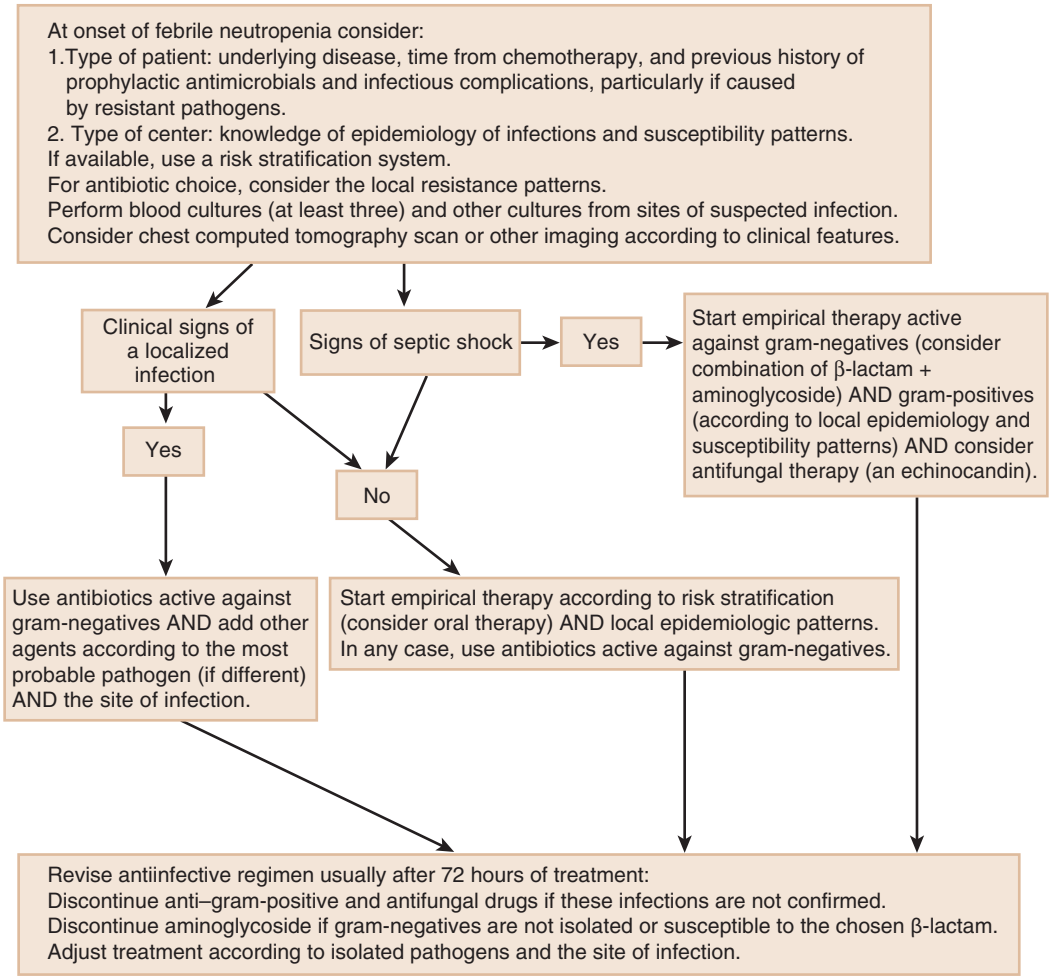

FIG. 306.2 Possible initial approach to a patient with febrile neutropenia.

severe. In most cases, these patients are clinically stable within 48 hours after the appearance of fever and are without fever within 3 to 4 days. On the basis of this concept, the Multinational Association for Supportive Care in Cancer (MASCC) score has been shown to be an acceptable tool to identify patients with low probability of complicated febrile neutropenia (Table 306.6). A risk-index score greater than 21 identified low-risk patients, with positive and negative predictive values of 91% and 36%, respectively. At this threshold, sensitivity and specificity were 71% and 68%, respectively, with a 30% misclassification rate. Studies in children evaluating the risk of complicated outcome were less successful. Although six pediatric stratification systems for identifying low-risk patients were established in a retrospective analysis, none of them could be validated in separate data sets.[182]

If low-risk patients can be reliably identified, the next logical step would be to try to discharge these patients early or even to treat them as outpatients or at home. The mandatory conditions for home or outpatient treatment include the presence of a reliable caregiver at home, a stable intravenous access, hospital proximity and adequate transportation, and the necessary facilities (telephone, running water, heating, and refrigeration). Antibiotic choices for oral therapy in the absence of risk factors for resistant pathogens include ciprofloxacin plus amoxicillin-clavulanate combination therapy, moxifloxacin monotherapy, or intravenous antibiotics such as ceftriaxone plus amikacin once daily.[183,184] In neutropenic patients with fever of unknown origin, switching therapy from intravenous to oral (e.g., ciprofloxacin or cefixime) has been also demonstrated to be a safe practice. Recent developments in antibiotic therapy suggest that skin and soft tissue infections due to gram-positive cocci might be safely treated with a single administration of 1.5 g of dalbavancin, thus allowing outpatient treatment of infections due to resistant gram-positive bacteria, although there is no experience in neutropenic patients.[185]

Despite still being only partially valid, these data and recommendations should be put in a more modern context. First of all, as previously mentioned, targeted therapies and biologic response modifiers have partially changed the spectrum of infecting pathogens and put patients at a risk that is higher than and different from what was previously realized. Second, all these studies were performed in times when antibiotic resistance was not a major problem and the risk for a bacterial infection caused by an ESBL-producing and/or quinolone-resistant gram-negative rod was low or absent. The current situation might be different, because the increasing resistance phenomenon does not necessarily affect only high-risk patients but can be present in specific settings, hospitals, or countries in low-risk patients as well, although the shorter duration and severity of neutropenia should still mitigate the clinical course. Therefore once again, every center should make its own choices based on its local epidemiology and every patient should be evaluated separately.

Patients at High Risk

As already mentioned, the practice of administering empirical antimicrobial therapy very early at the onset of fever during neutropenia has probably been the main driver of the undeniable progress obtained in this field. However, in recent years, because of the decreasing activity of the classic antibiotics used for fever during neutropenia and the shortage of new molecules, choices and strategies have been rediscussed. Indeed, physicians have been forced to diversify empirical regimens based on colonization, local epidemiology, antibiotic policies, and patient-related factors such as clinical presentation, organ failure, and status of the underlying disease. Currently, two strategies for empirical therapy have been envisaged: the escalation approach and the deescalation approach, as first suggested by us in the 7th edition of the present text in 2010 and then expanded in the ECIL guidelines, published in 2013.[181,186] In both strategies, there are two key time points: the day of onset of fever or suspected infection (day 0), when the patient is initially evaluated, cultures are drawn, and antibiotic therapy is started; and the day in which everything should be reevaluated, based on clinical conditions and availability of microbiologic results (days 2–4, depending on the performance of local laboratories). Being ready to review the composition of empirical therapy is mandatory in both cases, in light of the possible need to widen or restrict the antibiotic coverage or focus on a specific pathogen.

The escalation approach consists of starting with piperacillin-tazobactam or a third-generation cephalosporin (a choice that used to be broad spectrum and active against *P. aeruginosa* 20 years ago, but it is not so any more in many places) and then adjusting therapy if necessary. This approach seems to be still appropriate in many countries and centers, especially when resistance rates are low among pathogens commonly causing infections in neutropenia. With this approach, the carbapenems are used as second-line therapy either in patients failing the initial therapy or in case of a documented infection.[181,187] In this clinical situation, combining an aminoglycoside with a β-lactam is not deemed necessary because of more toxicity and no clinical advantage. This was clearly shown by a double-blind, placebo-controlled clinical trial comparing piperacillin-tazobactam with placebo versus the same drug with amikacin, and was confirmed in subsequent meta-analyses.[188] The empirical use of an anti–gram-positive antibiotic such as vancomycin, teicoplanin, linezolid, or daptomycin is not recommended by any guideline, either as initial therapy or in persistently febrile patients, unless a MRSA or a penicillin-resistant pneumococcus or enterococcus is isolated, or the patient has signs and symptoms suggesting a gram-positive etiology (e.g., CVC involvement or pneumonia).[183] The escalation approach avoids universal, and usually unnecessary, up-front use of antibiotics with the broadest spectrum, such as carbapenems or combinations with aminoglycosides or glycopeptides, and consequently minimizes potential disadvantages, such as selection of resistant pathogens or toxicity. However, in light of increasing resistance, there is a growing concern with this approach, because it has been shown that if the initial regimen fails to cover the pathogen responsible for infection, the outcome can be fatal.[189] For this reason, a new approach has been suggested, which has been called "deescalation empirical therapy."

The deescalation approach consists of starting with a very broad initial empirical regimen, chosen based of the severity of the patient's clinical presentation, the presence of colonization with MDR bacteria, risk factors for harboring MDR bacteria, and the local bacterial epidemiology. Examples of deescalation empirical therapy include:

1. Starting with carbapenem or ceftolozane-tazobactam (no experience in neutropenic patients for the latter at the time of this writing), to cover ESBL-producers and MDR *P. aeruginosa*;
2. Up-front use of vancomycin in patients colonized with MRSA, methicillin-resistant *Staphylococcus epidermidis,* or penicillin-resistant gram-positive cocci or in centers with high incidence of infections due to these pathogens;
3. Up-front use of ceftazidime-avibactam, meropenem-vaborbactam, or a combination including colistin, if a patient is colonized with carbapenem-resistant Enterobacteriaceae;
4. Addition of daptomycin, tigecycline, or linezolid in the presence of colonization with MDR gram-positive pathogens (Table 306.7).

In the deescalation approach, the key issues are (1) to safeguard the patient and to avoid the risk of undertreating his or her infection, which has the potential to be overwhelming; and (2) to reduce as much as possible the unnecessary use of precious drugs (e.g., carbapenems

TABLE 306.7 Risk Factors for Infections Caused by Resistant Bacteria in Cancer Patients

1. Previous infection or previous or current colonization by resistant bacteria, in particular:
   - Enterobacteriaceae resistant to third-generation cephalosporins or carbapenems
   - Multidrug-resistant, nonfermenting, gram-negative rods: *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia*
   - Vancomycin-resistant enterococci
   - Methicillin-resistant *Staphylococcus aureus*
2. Previous exposure to broad-spectrum antibiotics, in particular to third-generation cephalosporins
3. Health care–related infection
4. Prolonged hospital stay and/or repeated hospitalizations
5. Presence of urinary catheter
6. Older age
7. Intensive care unit stay

or vancomycin) by reassessing the antibiotic treatment after 48 to 96 hours and by streamlining therapy, based on microbiologic results, whenever possible. For example, in our center, where an increase in the incidence of BSIs caused by ampicillin-resistant enterococci and ciprofloxacin-resistant/ESBL-producing gram-negative rods (around 60% of gram-negative pathogens) was noted, piperacillin-tazobactam was the standard therapy when the clinical presentation allowed watchful waiting. However, patients presenting with severe sepsis, septic shock, or suspected bacterial pneumonia and those known to be colonized by resistant pathogens may be started with meropenem plus vancomycin and then deescalated to piperacillin-tazobactam or ceftazidime with vancomycin stopped, if a susceptible pathogen is isolated. In patients with colonization or previous infection with an MDR gram-negative pathogen, empirical antibiotic therapy targeting this strain is decided upon and noted in the chart at hospital admission to be started at the onset of fever. It frequently contains a combination regimen of ceftazidime-avibactam or colistin, plus an aminoglycoside, tigecycline, or other suitable molecule.[83]

The bottom line is that knowledge of local epidemiologic data represents pivotal information for deciding whether to start with an escalation or a deescalation approach and deciding on the type of drugs to be administered, as recently suggested in a pediatric study, as well.[190] Data from neutropenic cancer patients in an intensive care unit, and more recently from neutropenic HSCT recipients, showed that the deescalation approach is safe and feasible.[191–193]

Duration of Antibacterial Treatment

Another traditional practice that has been challenged by the ECIL guidelines is the need for empirical antibiotics to be continued until neutrophil recovery, with the idea that this is the way to avoid infection relapse and superinfections and to reduce mortality. This recommendation was based on a very small, open-label, randomized trial published in 1979.[194] The ECIL position was that antibiotics can be safely discontinued after 72 or more hours of intravenous therapy in patients who are and have been hemodynamically stable since the onset of fever, and are without fever for 48 or more hours, irrespective of the granulocyte count and the expected duration of neutropenia. Obviously, very careful clinical monitoring was recommended in order to immediately restart antibiotics at first signs of infection relapse or novel infection. In microbiologically documented infection, the recommendation was to treat for at least 7 days. The rationale for this revolutionary recommendation was based on acknowledging that, as already forecast a long time ago, alteration of a patient's microbiota leads to an increased risk of intestinal colonization by resistant pathogens, which, in turn, increases the risk of infection.[195,196] Thus antibiotics, which are the main drivers of dysbiosis, may be not protective but in fact harmful if used unnecessarily. Decreasing the antibiotic selective pressure is crucial for future generations and should be pivotal in modern medicine. Contrary to what is commonly believed, controversies about the dogma that antibiotic therapy should be continued until the end of neutropenia can be traced back to 1988, and the 2008 Infectious Diseases Society of America guidelines left some room for the possibility of stopping empirical therapy before recovery of neutropenia in a clinically stable patient, although providing the shortcut of resuming quinolone prophylaxis.[183,197] Was there any validation of the discontinuation recommendation? Actually not much. Only two prospective randomized studies in low-risk children found that discontinuation of antibiotics before marrow recovery did not result in deaths caused by bacterial infections or in an increased rate of the recurrence of fever.[198,199] Other observational studies performed in high-risk patients with prolonged neutropenia had confirmed that discontinuation of antibiotics was associated with relapse of fever in a few patients but without an increase in mortality, providing that antibacterial treatment was restarted immediately.[200,201]

Since going against a dogma is always controversial, the ECIL approach was promptly criticized. In 2014, Micol and colleagues prospectively assessed their version of a discontinuation approach (different from the ECIL one) in high-risk acute myeloid leukemia patients with neutropenic fever of unknown origin.[202] According to their criteria, treatment was discontinued in only 7 of 59 episodes of fever during neutropenia in 38 acute myeloid leukemia patients. In this observational study, 3 of 7 patients had relapsing fever due to severe infections. The conclusion was that discontinuing therapy was probably unethical and, in any case, allowed to spare only 3 days of antibiotics. The ECIL answer was that these results were based on too low numbers to draw any conclusion and that, more importantly, the criteria for discontinuation were different from those recommended by the ECIL group.[203]

More recently, in the first and so far only open-label, multicenter, randomized trial, a Spanish group addressed the issue of whether or not a clinically oriented approach was superior to a standard end-of-neutropenia approach for discontinuing empirical antibacterial therapy in patients with fever and neutropenia, according to the ECIL guidelines.[204] The hypothesis was that the experimental approach would have been able to increase the number of antibiotic-free days with respect to the standard approach during a 28-day period. Mortality and days of fever during the follow-up were secondary end points. The results of the study in the intention-to-treat analysis show that the clinically oriented approach significantly increased the number of antibiotic-free days from 13.6 to 16.1, without impacting on mortality and days of fever. Indeed, the rate of fever recurrences and fungal or bacterial superinfections in the two groups was basically the same and so was the outcome, likely due to timely and adequate interventions.

In conclusion, in the face of the antibiotic crisis and the increasing recognition of the importance of maintaining as much as possible the integrity and diversity of the human microbiota, we are forced to rationalize antibiotic intervention in hematologic patients. This rationalization includes four main aspects:

1. To review antibiotic prophylaxis measures
2. To adopt stringent criteria for driving empirical choices at the onset of fever and neutropenia and on day 3 to 4 after starting treatment
3. To be acquainted with the concept that a planned progressive succession of antibiotics in fever and neutropenia does not exist, and every patient and every episode should be evaluated separately
4. To accept the idea that in neutropenic patients with fever of unknown origin, empirical antibiotics could be discontinued even before neutropenia recovery, provided close clinical observation as inpatients is maintained with a high level of suspicion for restarting treatment.

Empirical and Preemptive (Diagnostic-Oriented) Antifungal Therapy

The empirical antifungal therapy approach consists of administering an antifungal drug in a persistently febrile neutropenic cancer patient after a variable period of empirical antibacterial therapy (usually 4–7 days), in the absence of any clinical, microbiologic, or radiologic documentation of a fungal infection. This practice is based on autopsy studies showing fungal infections undetected during life and on two randomized studies that enrolled, in total, less than 200 patients.[205–208] These studies were not double blind or placebo controlled and did not actually conclude that there was an unequivocal advantage of the empirical antifungal approach. In both randomized studies, the statistical power of the observed results was very small. Nevertheless, empirical antifungal therapy in persistently febrile neutropenic patients without a documented infection has become common practice in many cancer centers worldwide, and numerous drugs have been tested for this indication. Except for the first studies, which used persistence of fever and survival as the main end points, almost all other studies used a composite end point that included five criteria: defervescence, no discontinuation for toxicity, treatment of baseline fungal infections, prevention of breakthrough fungal infections, and survival. In general, no drug has been demonstrated significantly more effective than others, and differences were only based on lower toxicity. Of interest, a meta-analysis of the six trials in which empirical treatment was compared with no treatment or preemptive therapy confirmed that empirical antifungal treatment was associated with a lower rate of (diagnosed) invasive fungal diseases, but gave no significant advantage in terms of overall mortality.[205] The aim of empirical therapy was to treat as early as possible both candidiasis and aspergillosis. However, when fluconazole prophylaxis became widely used and reduced

the incidence of *Candida* infections, it became evident that empirical therapy was mainly directed against *Aspergillus*.

In recent years, awareness has grown that the empirical approach results in a tremendous overtreatment of just one symptom (fever) and has encouraged development of a preemptive or, maybe better named, diagnostic-driven approach aimed at treating a fungal disease when highly suggestive, although not conclusive, diagnostic criteria are present (not just fever). In the diagnostic-driven strategy, clinical considerations (fever, thoracic pain, cough), biologic markers (e.g., *Aspergillus* galactomannan in serum or bronchoalveolar lavage fluid, cytologic detection of fungal hyphae, or positive culture of sputum or bronchoalveolar lavage fluid), and imaging data (e.g., chest computed tomography [CT], with whole-volume scanning with thin-slice reconstruction preferable to high-resolution CT scanning) are combined together to obtain the highest possible diagnostic likelihood of aspergillosis and consequently to start therapy. Whether or not any pulmonary infiltrate is enough or typical radiologic signs of invasive aspergillosis are required to start antifungal therapy is a matter of debate.

Some studies analyzed the feasibility of this approach in adult patients. The first one concluded that the diagnostic-driven approach was feasible, associated with less use of antifungal therapy, and without increased mortality with respect to historical controls. Of particular interest, in this study, 10 patients who were diagnosed with positive galactomannan and CT scanning would have not received any antifungal therapy with the classic empirical approach because of absence of fever.[209] In the first randomized noninferiority trial that compared the empirical and preemptive approaches (defined differently from the previous study), no difference was found in the primary end point (survival). As expected, in the arm of preemptive therapy, in which an active diagnostic workup was performed, there were more fungal infections than in the other arm. In this study, patients were stratified by status of underlying disease, and the lower limit of the confidence interval of the difference in survival between the two strategies, among patients in first remission-induction therapy (the highest risk period), was exactly at the 8% predefined delta limit, thus leading the investigators to conclude that noninferiority was not demonstrated in this subgroup.[210] Other studies compared the two strategies, with results that were consistently in favor of the diagnostic-driven approach.[211]

There are some pitfalls in using the diagnostic-driven approach. The first is the need for a readily available CT scan with skillful radiologists who are willing to collaborate. The second is the availability of different antigen detection assays, of which galactomannan is the most important. The third is the turnaround time, which should be no more than 2 to 3 days to allow timely intervention. Finally, it had been demonstrated that a mold-active prophylaxis might lead to a reduction in the sensitivity and specificity of the galactomannan test, therefore lowering its reliability. Indeed, due to low pretest probability, screening with galactomannan in this setting is unhelpful, but targeted testing in case of suspicion of breakthrough invasive aspergillosis has been shown effective.[212]

In conclusion, in our opinion, the empirical and diagnostic-driven strategies are not mutually exclusive. Some combination of the two (e.g., lung CT scanning combined with a fever-driven approach) is probably the wisest integrated clinical approach to mold infections in cancer patients. For example, empirical antifungal therapy could be started at clinical suspicion while awaiting the results of diagnostic procedures but then discontinued if the results are not confirmatory. Fig. 306.3 summarizes the possible approaches to a patient with persistent febrile neutropenia. Table 306.5 lists drugs indicated for empirical or targeted antifungal therapy. Drugs approved for empirical therapy include liposomal amphotericin B, caspofungin, and itraconazole, whereas there is no drug approved specifically for preemptive treatment. In cases of preemptive treatment, the indirect diagnostic tests, such as galactomannan or (1→3)-β-D-glucan, or radiologic imaging may suggest a possible etiology. Therefore the preemptive antifungal treatment is the same as that recommended for first-line therapy. For example, for aspergillosis, it usually has been voriconazole or liposomal amphotericin B. Recently, isavuconazole has been approved for treating invasive aspergillosis and mucormycosis, following the results of a large randomized study that demonstrated noninferior efficacy and better tolerability compared to voriconazole for treatment of aspergillosis, and data of similar efficacy (survival) in 21 patients with mucormycosis compared to 33 treated with amphotericin B.[213,214] The management of specific fungal infections is beyond the purposes of this chapter.

Finally, a new issue of the choice of an antifungal treatment in case of failing mold-active prophylaxis warrants some consideration. Failure of mold-active prophylaxis is suspected when a patient develops signs and symptoms suggestive of a fungal infection without microbiologic documentation (e.g., a lung infiltrate unlikely caused by bacterial superinfection, with negative galactomannan, or the appearance of liver/spleen nodules) while receiving posaconazole or voriconazole prophylaxis. Four possible explanations include (1) the patient was not taking prophylaxis (lack of compliance); (2) the drug was not absorbed (posaconazole) or was metabolized too fast (voriconazole), as shown by inadequate blood levels; (3) the "new" fungal infection is due to a non-*Candida*/non-*Aspergillus* fungus intrinsically resistant to azoles; or (4) the "new" fungal infection is due to an azole-resistant *Candida* or *Aspergillus* species. In the first two cases, adjusting dosages without changing therapy seems an adequate option, whereas in the third and fourth cases, shifting to another drug family (caspofungin for *Candida* and lipid amphotericin B for *Aspergillus*) seems the only possible option.

It has been proposed that in centers with resistance to azoles among *A. fumigatus* exceeding 10%, the first-line therapy could consist of a combination of an azole and echinocandin or liposomal amphotericin B.[215]

Management of a Neutropenic Patient With a Localized Infection

Catheter-Related Infection

It is likely that the role of indwelling catheters in causing fever and infection in neutropenic patients might have been overestimated. The suspicion that the catheter is actually involved should only be raised in case of septic shock, endocarditis, rapidly progressive bacterial infection, fever with concomitant signs of infection at the catheter site (including the subcutaneous tunnel), persistence of positive blood cultures in the absence of any other detectable site of infection (although this might have explanations as well), and fever developing concomitantly with catheter flushing. In addition to clinical criteria, there are some microbiologic criteria (time to blood culture turning positive, differential colony count between peripheral and catheter-drawn blood culture, and absolute very high colony count in a catheter-drawn blood culture) that could be used, although most of them require quantitative evaluation systems. When a catheter-related infection is proven or suspected, the first thing to do is to establish whether or not the catheter is still indispensable. If not, the catheter should be removed and treatment administered, if possible, through a peripheral line.

In terms of therapy, the choice of the antibiotic regimen should be based on the epidemiology of CVC-related infections in every individual center and on the pharmacokinetic/pharmacodynamic characteristics of the available antibiotics. As a general rule, an anti–gram-positive drug should always be included in the initial regimen, although the choice should not necessarily be vancomycin, except for centers with a high rate of methicillin-resistant staphylococci. On the other hand, in centers where staphylococci with high minimal inhibitory concentration values for vancomycin (≥2 μg/mL) have been isolated, daptomycin or linezolid might be considered. Moreover, because gram-negative organisms are not infrequent in single-agent or polymicrobial CVC bacteremias, an anti–gram-negative coverage is recommended in all cases. In contrast, the empirical inclusion of an antifungal drug seems not to be appropriate, considering the relatively low incidence of fungal infections in this clinical setting. Once the causative pathogen is identified, treatment should be tailored according to its susceptibility pattern.

Treatment of CVC-related bacteremias requires the use of antibiotics that can be administered systemically (possibly through the infected line to improve the possibility of its "sterilization") but that also can (and sometimes must) be administered "locally", as catheter lock, in order to increase "local" (i.e., within the device) antibiotic concentrations to overcome resistances and penetrate into the biofilm.[41] Many drugs have been proposed for catheter lock, and their use depends on both the antimicrobial spectrum and chemical stability.[216,217]

Duration of treatment varies according to etiology, presence or absence of complications, and whether the catheter has been removed

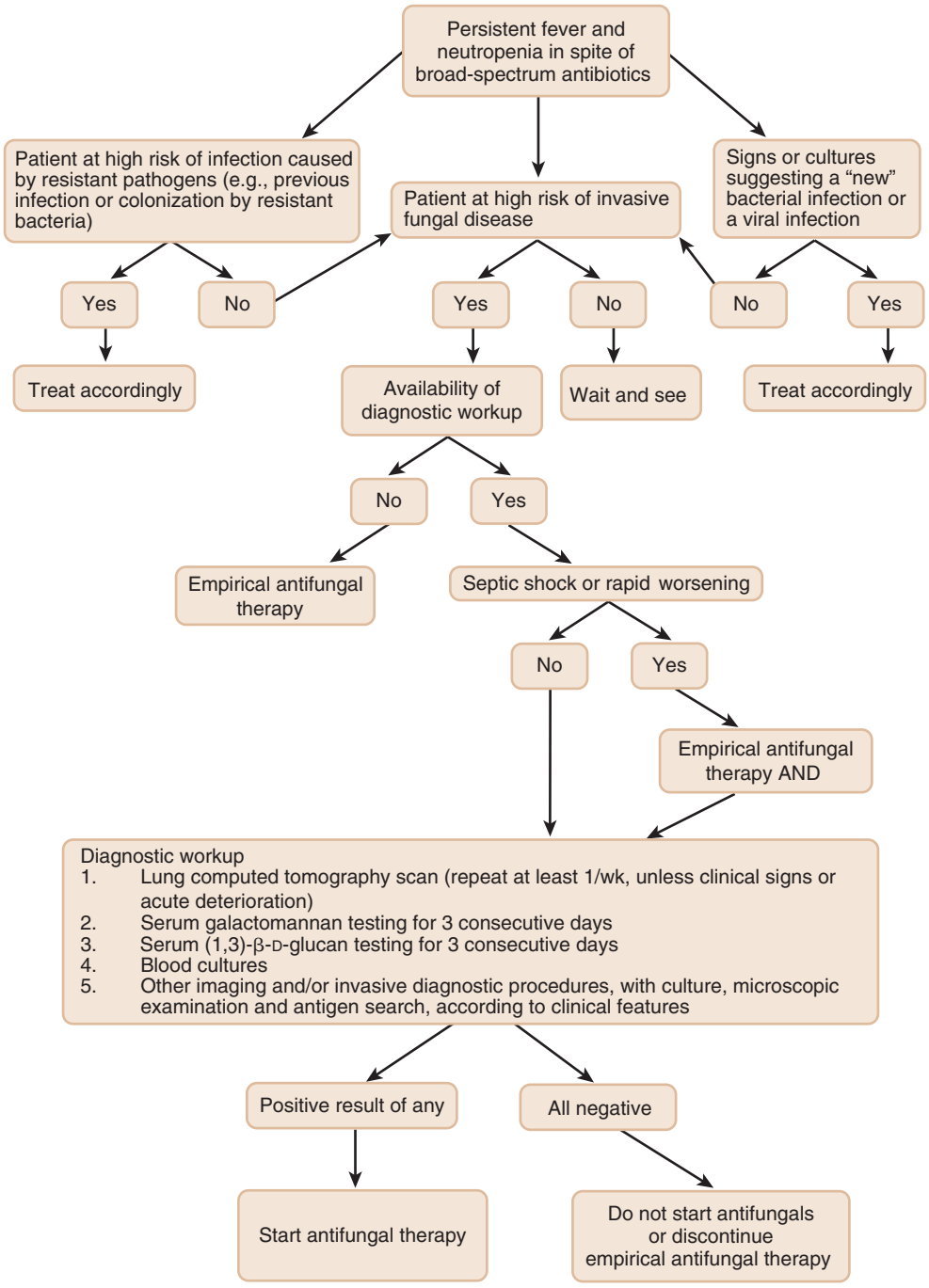

FIG. 306.3 **Management of a persistently febrile neutropenic patient.**

or not. According to some guidelines, "day 1" of treatment should be counted as the day of the first negative blood culture.[218] Follow-up blood cultures (at least once a day until documented negativization) should therefore be used to document response to treatment. If a patient has persistently positive blood cultures up to 72 hours after initiation of appropriate therapy, the catheter should be removed.[41] The possibility to remove the catheter relies mainly on general clinical conditions, availability of other vascular access, adequate platelet counts, and ability to achieve adequate hemostasis.

Pneumonia

The choice of empirical therapy in neutropenic patients with a pulmonary infiltrate should be based on the type of infiltrate, the time of appearance of the infiltrate with respect to the onset of fever, and epidemiologic and anamnestic data. For example, viridans-group streptococci have been associated with acute respiratory distress syndrome in neutropenic patients with severe oral mucositis. If pneumonia is evident since the beginning of the febrile episode, a bacterial etiology should be suspected, and the same antibiotic regimen commonly used for febrile neutropenia in high-risk patients should be used, with necessary modifications if the risk of resistant pathogens is present. On the contrary, if pneumonia apparently occurs as a breakthrough infection in a patient already receiving broad-spectrum antibiotics, a fungal etiology is more likely and antifungal therapy is logical, although a resistant (e.g., ESBL- or carbapenemase-producing) or atypical bacterial pathogen, such as *Legionella* or *M. pneumoniae,* is also a possibility. Tuberculosis should be taken into account in countries or in people coming from countries with high endemicity for tuberculosis. Interstitial pneumonia is relatively rare during neutropenia, but it does occur. In this case, pneumocystosis and influenza are the most likely etiologies, followed by CMV, *Mycoplasma*

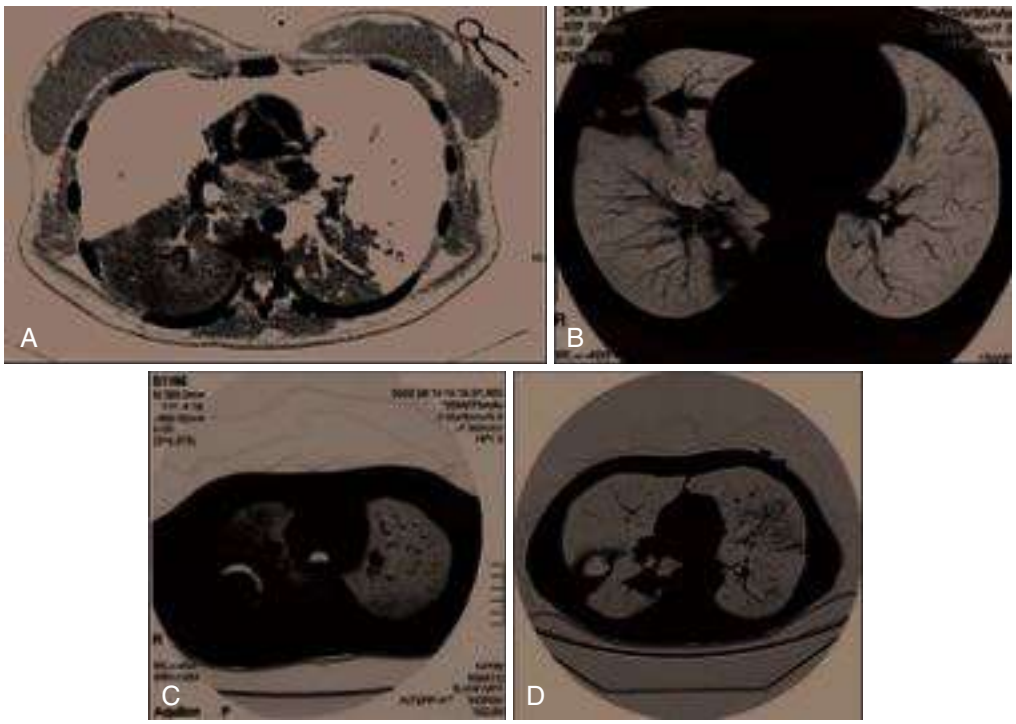

FIG. 306.4 Pulmonary cavitary lesions resulting from different etiologies, in the presence and absence of neutropenia. (A) Neutropenic patient with hemoptysis and pulmonary cavitation *(arrow)* in the presence of *Klebsiella pneumoniae* bacteremia. (B) Neutropenic patient with pulmonary cavitation *(arrows)* in the presence of methicillin-susceptible *Staphylococcus aureus* bacteremia. (C) Air crescent *(arrow)* in a no-longer neutropenic patient with pulmonary aspergillosis. (D) Cavitary lesion *(arrow)* in a no-longer neutropenic patient with *Pseudomonas aeruginosa* bacteremia.

and *Chlamydia,* other respiratory viruses, or noninfectious causes. The appropriate diagnostic measures should be implemented, and treatment should be tailored accordingly. In any case, it is important to remember that the presence of a cavitary lesion in a febrile and neutropenic patient with acute leukemia should raise the suspicion of a bacterial infection, especially in the presence of positive blood cultures (e.g., *S. aureus* or gram-negative rods) (Fig. 306.4). Indeed, fungal lesions in neutropenic patients usually present with the typical halo sign or with nodular lesions and not with cavitary lesions that become apparent only after neutrophil recovery.[219]

Abdominal Infections

Febrile neutropenic patients may present with gastrointestinal signs and symptoms, such as abdominal pain, nausea, vomiting, and diarrhea, in addition to fever. In these patients, an initial conservative management with bowel rest, intravenous fluids, total parenteral nutrition, and broad-spectrum antibiotics with antianaerobic activity should be immediately implemented. In some cases (3%–6%), especially in patients receiving aggressive treatment for acute leukemia, full-blown neutropenic enterocolitis may develop, with high fever, severe abdominal pain, and even hemorrhagic diarrhea evolving into acute abdomen and septic shock. *Clostridioides* or CMV colitis should be taken into account in the diagnostic workup, and treated accordingly (see Chapter 243). Surgical intervention is usually not indicated but may be recommended in the setting of obstruction, perforation, persistent gastrointestinal bleeding despite correction of thrombocytopenia and coagulopathy, and clinical deterioration.

OTHER TREATMENTS

Granulocyte Transfusions

Granulocyte transfusions from donors stimulated with growth factors have been proposed in desperate cases of life-threatening bacterial and fungal infections in patients with persistent neutropenia unlikely to recover promptly. The evidence for clinical efficacy is limited to that of case reports and small series, and the results are not uniform.[220]

Improved methods of harvesting granulocytes from normal volunteer donors has made this technique, though resource consuming, a potential bridge for infected patients whose neutrophil recovery is not expected soon, to prevent death from fungal infection. Technical issues include matching for blood type, CMV serology, and in the case of future stem cell transplant candidates, also for human leukocyte antigen; the dose; and in cases of repeated infusion the possibility of allosensitization (11% of patients in one series).[221]

A recent randomized trial, which included 114 patients who in the intervention arm received a median of five transfusions, with a mean transfusion dose of 54.9×10^9 granulocytes, reported no benefit on the primary end point, survival plus microbial response at 42 days after randomization (42% vs. 43%).[222] In a post hoc analysis, subjects who received an average dose per transfusion of 0.6×10^9 granulocytes/kg or greater tended to have better outcomes than those receiving a lower dose.[222] However, even if short-term benefit is observed, the success of granulocyte transfusion in cases of IFD depends ultimately on subsequent patient marrow recovery.[223]

Colony-Stimulating Factors

Many case reports have noted the effectiveness of growth factors in the treatment of severe, life-threatening bacterial or fungal infections. However, a meta-analysis published in 2002 suggested the lack of efficacy of systematic, widespread use of G-CSF for the treatment of febrile neutropenia.[224] In any case, the use of G-CSF may be an option in patients with fever and neutropenia who are at high risk for infection-associated complications or with prognostic factors of complicated outcome, such as prolonged (>10 days) and profound ($<0.1 \times 10^9$/L) neutropenia, age older than 65 years, uncontrolled primary disease, pneumonia, hypotension, and multiorgan failure. In patients with pulmonary aspergillosis, a very rapid granulocyte recovery has been associated with the development of severe complications, such as pneumothorax or fatal hemoptysis.

CONCLUSIONS

The management of fever and infection during neutropenia remains a challenging exercise for clinicians. The shortage of new antibiotics and the emergence of various degrees of resistance to traditional antibiotics active against gram-negative bacteria (third-generation cephalosporins,

piperacillin-tazobactam and other penicillins, quinolones, and carbapenems) have again increased mortality rates and made the treatment of gram-negative infections very difficult. It should be acknowledged that this problem is more evident in some countries and less in others, probably due to past antibiotic policies. At the same time antineoplastic treatments are changing, with consequent need to maintain a high level of attention for unexpected etiologies and unusual clinical presentations. Although not dealt with in this chapter, diagnostic microbiology methods are also changing substantially, with the possibility of speeding up pathogen identification and susceptibility assessments and consequent possibility of earlier and more appropriate antibacterial and antifungal therapies. In the future, the progressive recognition of the pivotal role played by the human microbiota will probably introduce extensive innovations in our ability to understand many pathogen-host interactions and immunological mechanisms of immunotolerance and aggression.

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307 Infections in Recipients of Hematopoietic Stem Cell Transplants

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SHORT VIEW SUMMARY

Types of Donors of Stem Cells for Hematopoietic Stem Cell Transplantation (HSCT)

- Autologous: The recipient donates to self.
- Syngeneic: The donor is an identical twin sibling.
- Allogeneic: The donated material comes from a different individual than the recipient.

Specific Types of Allogeneic Donors

- Matched related or partially matched related donor, such as a sibling with the same or similar HLA type
- Unrelated donor or matched unrelated donor
- Haploidentical: parent, cousin, sibling, or child is the donor; one HLA haplotype matches. Although haploidentical or other HLA-mismatched transplants may lead to a high incidence of graft-versus-host disease (GVHD), administration of cyclophosphamide early posttransplant and T-cell depletion of the grafts may limit risks of GVHD.
- Cord: umbilical cord blood (UCB) usually partially HLA matched not matched for blood type; sometimes two cords used to provide blood with sufficient cells in adults
- Haplocord: haploidentical peripheral blood stem cells plus cord blood cells; haplocord engrafts rapidly but may not be sustained, yet provides neutrophil production until the cord engrafts

Types of Cells Used in HSCT

- Peripheral blood stem cells (PBSC), usually filgrastim (granulocyte colony-stimulating factor) with or without plerixafor mobilized; may be CD34 selected for T-cell depletion
- UCB: usually associated with delayed engraftment but less GVHD
- Bone marrow: collected by aspiration harvest from matched related or matched unrelated donor; associated with less chronic (c)GVHD compared with PBSC
- Donor lymphocyte infusion: donor cells sorted for lymphocytes; given after engraftment or after relapse for antitumor or antiviral effect but can stimulate GVHD

Preparation of Patient for Cell Infusion ("Conditioning Regimen")

- Myeloablative conditioning: total-body irradiation (TBI) plus cyclophosphamide or busulfan; can comprise chemotherapy alone or TBI alone to induce immunosuppression in the host and have a good antitumor effect
- Nonmyeloablative or reduced-intensity conditioning: using lower-dose TBI or with antithymocyte globulin, most often with fludarabine to induce adequate immunosuppression to prevent graft rejection by host and has only minimal antitumor effect; success mostly relies on graft-versus-tumor effect

Outcomes

- Engraftment: absolute neutrophil count rises to more than 500 cells/μL by day 42
- Primary graft failure: no engraftment by day 42
- Mixed chimerism: coexistence of host and donor hematopoiesis
- Relapse: return of the underlying malignant condition

Complications

- Mucositis: mucosal inflammation that serves as a portal of entry for oral or intestinal infections; common with methotrexate-containing regimens. Palifermin may reduce mucositis
- Hemorrhagic cystitis: often occurs in conjunction with cyclophosphamide early after transplant, despite the use of mesna and forced diuresis; viral causes more common later after transplant, including adenovirus or BK virus
- Engraftment syndrome (immune reconstitution syndrome): fever with or without rash and/or pulmonary infiltrates at engraftment
- Venoocclusive disease, also called sinusoidal obstruction syndrome: triad of jaundice, weight gain, and ascites leading to multiorgan failure (mostly in liver, kidney, and lung), usually occurring in the first 3 to 4 weeks after transplantation
- Diffuse alveolar hemorrhage: bleeding into alveoli, usually within the first month after transplantation; diagnosed by progressive bloody returns on lung lavage and treated with very-high-dose corticosteroids
- GVHD: inflammation and cell death (apoptosis) in skin, liver, gut, or lung. Acute form usually occurs until day 100. Chronic form generally occurs later and has features resembling, but distinct from, scleroderma. The risk is higher in the elderly and with grafts from partially matched donors. Calcineurin inhibitors (cyclosporine, tacrolimus), less often sirolimus, and mycophenolate mofetil are started just before transplantation and continued for at least several months to decrease the incidence and severity of GVHD. High-dose corticosteroids (and other agents) constitute the core of severe GVHD management.
- Posterior reversible encephalopathy syndrome (PRES): neurotoxicity may present with headache, seizures, altered consciousness, and visual disturbance and is associated with typical bilateral white-matter abnormalities in vascular watershed areas in both occipital and parietal lobes. PRES can be resulted from hypertension, calcineurin inhibitors, or fludarabine use.
- Bronchiolitis obliterans organizing pneumonia and obliterative bronchiolitis: pneumonitis associated with small airway injury, sometimes associated with cGVHD

Antimicrobial Agents for Prophylaxis

- Acyclovir: low dose for herpes simplex virus (HSV), high dose for cytomegalovirus (CMV)
- Levofloxacin: used for prevention of bacterial infections, until fevers or infection develop, generally during severe neutropenia or high-dose steroid use
- Fluconazole: for prevention of candidiasis, in general starting at neutropenia and continuing throughout GVHD prophylaxis
- Trimethoprim-sulfamethoxazole: agent of choice for *Pneumocystis;* also covers *Toxoplasma* and some bacteria, such as *Nocardia.* Alternatives include aerosolized pentamidine, atovaquone, and dapsone. In general it is used starting after the first month and continuing throughout the first year or throughout GVHD prophylaxis.
- Penicillin VK: prevention against *Streptococcus pneumoniae* during active cGVHD. Regional penicillin resistance may lead to the use of levofloxacin for this indication.
- Voriconazole or posaconazole: given in place of fluconazole to prevent mold infections
- Echinocandins (micafungin or caspofungin): intravenously administered agents that may be substituted for azoles
- Lamivudine: prevention of hepatitis B virus (HBV) reactivation in anti–hepatitis B core antibody–positive, hepatitis B surface antigen–negative, and hepatitis B DNA–negative patient and/or with hepatitis

SHORT VIEW SUMMARY—cont'd

B–positive donors. Entecavir or tenofovir may be substituted for lamivudine.
- Entecavir or tenofovir: prevention of HBV reactivation in hepatitis B surface antigen–positive patient
- Ivermectin: two doses for patients from countries with high risk for *Strongyloides* infestation

Preemptive Therapy
- Ganciclovir, valganciclovir, or foscarnet: given to patients with CMV reactivation based on polymerase chain reaction (PCR) assay of blood or pp65 neutrophil antigen

Empirical Antibacterial Therapy During Fever and Neutropenia With Negative Workup
- Designated broad-spectrum agent, such as ceftazidime, piperacillin-tazobactam, or cefepime
- Addition of an aminoglycoside possibly required for institutional antibiotic resistance patterns
- Vancomycin if any cellulitis, dysfunction with an indwelling catheter, or hemodynamic instability or colonized with methicillin-resistant *Staphylococcus aureus.*

Viral Diseases in the Transplant Recipient
- HSV: oral, esophageal, vaginal ulcers; autoinoculation of other skin sites
- Varicella-zoster virus: dermatomal zoster; may disseminate; rarely severe hepatitis or visceral zoster
- CMV: fever, viremia, cytopenias, pneumonitis, gastrointestinal disorders (e.g., hepatitis, esophageal ulcers, mucosal changes in the duodenum or colon), retinitis
- Human herpesvirus 6: fever, rash, viremia, pneumonitis, encephalitis; may be associated with delayed neutrophil recovery or prolonged thrombocytopenia
- Adenovirus: hemorrhagic cystitis, pneumonitis, hepatitis
- BK polyomavirus: hemorrhagic cystitis
- HBV and hepatitis C virus: hepatitis
- Major respiratory viruses: influenza, respiratory syncytial virus in the winter; parainfluenza virus (especially type 3) and rhinovirus year-round
- JC polyomavirus: progressive multifocal leukoencephalopathy, often fatal
- Epstein-Barr virus: viremia or posttransplant lymphoproliferative disorder

Other Infections
- *Clostridioides difficile* (formerly *Clostridium difficile*) diarrhea: stool toxin PCR assay considered the best test
- Intravascular central catheter–related bacteremia or tunnel infection
- Typhlitis: abdominal pain and cecal edema during neutropenia
- *Pneumocystis* infection: diffuse pneumonitis among those not taking prophylaxis
- Candidiasis: candidemia originating from the gut
- Infection with *Aspergillus* and other molds: lung field abnormalities, sinusitis in particular among patients with longer-duration neutropenia, cGVHD, and/or on high-dose steroid therapy
- Toxoplasmosis: brain lesions, can be fatal

The clinical approach to infections in patients undergoing hematopoietic stem cell transplantation (HSCT) involves an understanding of basic transplantation techniques, clinical syndromes, host defense defects at different times after transplantation, the natural history of individual infections, and the mechanisms underlying reconstitution of the immune system after transplantation. In general the dominant elements of infectious risks for bacterial, viral, fungal, and parasitic infections after HSCT depend on the pretransplantation exposure history (viral serostatus), whether the transplant is from an autologous or an allogeneic donor source, the intensity and content of preparative regimens, and the number of days after the transplantation under consideration. The distinguishing determinant of infectious risk between autologous and allogeneic grafts is the associated risk incurred by ongoing immunosuppression from prevention of graft-versus-host disease (GVHD), GVHD itself and its therapy; differing paces of humoral and cellular immune reconstitution that are highly associated with donor type also affect the risk. The time period after transplantation defines eras of differing transplantation complications and the evolution of the slowly resolving posttransplantation immunodeficiency: cutaneous and mucosal barrier breakdown, neutropenia, lymphopenia, hypogammaglobulinemia, or a combination of these. Many posttransplantation complications mimic infectious processes, and multiple infections may occur in the same patient at the same time. Therefore the patient undergoing HSCT should be examined in the context of pretransplantation infections, serologic profiles to document infection latency, intensity and content of "conditioning regimen," available culture data from mucosal surfaces, contemporaneous transplantation complications in the patient's institution, current antimicrobial prophylaxis, presence of active GVHD prophylaxis, and the current degree and duration of neutropenia and lymphopenia.

BASIC TRANSPLANTATION TECHNIQUES

HSCT involves the intravenous (IV) delivery of hematopoietic stem cells to a recipient whose hematopoietic and immune systems have been ablated or altered by a cytotoxic and immunosuppressive preparative regimen, commonly referred to as the conditioning regimen, given over the 4 to 10 days before HSCT. Hematopoietic stem cells are obtained from the patient (i.e., autologous) or from other individual(s) (i.e., allogeneic). Graft sources (stem cell collections sites) are bone marrow (BM; mostly for pediatric transplantations), filgrastim-stimulated peripheral blood (PB), or umbilical cord blood (UCB).[1,2]

Autologous HSCT is used with the intent of curative or prolongation of survival in the treatment of multiple myeloma, lymphomas, rarely certain leukemias, some high-risk solid tumors (e.g., neuroblastoma, germ cell tumors), and autoimmune disorders (scleroderma). Its efficacy depends purely on (1) conditioning regimen intensity and (2) disease sensitivity to the conditioning regimen. Hematopoietic cell support only makes sure that the recipient will start and continue to reproduce entire blood cell repertoire after myeloablative conditioning (i.e., hematopoiesis). However, its morbidity and mortality also arise from regimen-related toxic effects and early infections.

HSCT is an option with curative potential in treatment of malignancies (e.g., acute and chronic leukemias), preleukemic disorders (e.g., myelodysplastic syndrome), lymphomas, multiple myeloma, hemoglobinopathies or thalassemias, disorders of BM failure (e.g., aplastic anemia, Fanconi anemia), severe immunodeficiency syndromes, and inborn errors of metabolism (e.g., osteopetrosis, chronic granulomatous disease, Hurler syndrome, inherited leukodystrophies, and other lysosomal disorders).[1,2] It is currently used investigationally in treatment of diseases such as epidermolysis bullosa.[3,4] Allogeneic HSCT has been feasible for selected human immunodeficiency virus (HIV)-positive patients with malignant and nonmalignant diseases since highly active antiretroviral therapy became standard treatment for HIV infection.[5]

Allogeneic HSCT has the additional hazards of a higher risk of graft failure (prolonging neutropenia) or GVHD compared with autologous HSCT. Substantial improvements in the supportive care of severely immunosuppressed patients have evolved since the 1980s, and survival has markedly improved over time.[6] As outcomes with transplantation improve, the use of transplantation as an option depends less on the availability of certain donor sources (i.e., whether there is an available matched donor) than in prior decades. Allogeneic donors can be related or unrelated and class I and II human leukocyte antigen (HLA) matched at loci of the major histocompatibility complex or mismatched. Matched unrelated adult volunteer donors (MUD) are available for more than half the general population (unfortunately still problematic for minorities), and unrelated UCB and HLA haploidentical related donors are used increasingly as a source of stem cells for transplantation reported by Center for International Blood and Marrow Transplant Research (CIBMTR) (https://www.cibmtr.org/ReferenceCenter/SlidesReports/

SummarySlides/Pages/index.aspx UCB). UCB has been shown to be an effective alternative for patients who lack a suitable adult donor,[7] but its use is limited by finding a UCB unit with an adequate cell dose for the recipient's size and weight. Strategies such as transplanting two closely matched or ex vivo expanded UCB units have enabled transplantation for adults.[8]

Graft source may be a contributing factor when considering the likelihood of infectious complications. Reported infectious events before engraftment have been significantly higher with BM versus peripheral blood stem cells (PBSCs) sources, probably related to longer periods of neutropenia before engraftment.[9] With UCB grafts, engraftment is even further delayed,[10] incurring more infectious complications before engraftment.[11] Furthermore, immune recovery, in particular T cells, is also delayed after UCB HSCT.[12] Therefore infections are significantly higher with UCB grafts compared with MUD. In a CIBMTR study the incidences of bacterial infection at 1 year were 72% and 59% for UCB and MUD, respectively. Incidences of viral infection at 1 year were 68% and 45%, ($P < .0001$) for UCB and MUD, respectively.[11] Moreover, bacterial and viral, but not fungal, infections were more common after UCB than mismatched unrelated donor (MMUD) ($P = .0009$ and $< .0001$, respectively). In contrast, a French study showed the cumulative incidence of infection (72% vs. 57%) and mortality (8% vs. 3%) at 18 months was higher with MMUD grafts than UCB grafts[13] due to higher rates of GVHD with PBSC HSCT, which contain a higher T-cell dose.[9,13,14]

The conditioning regimen used to prepare the host is a major determinant of outcome because of variable host tissue injury and the potential for induction of prolonged immunodeficiency. Conditioning regimens may include immunosuppressive and cytotoxic chemotherapy alone or in combination with wide-field or total-body irradiation (TBI). Conditioning can damage mucosal surfaces, facilitating transmucosal entry of bloodstream infections (BSI).[15] Indwelling IV catheters can also lead to disseminated BSI. Environmental exposure to airborne dusts and fungal spores can inoculate the sinuses or respiratory tree; therefore patients are required to stay in high-efficiency particulate air (HEPA)-filtered rooms. Prophylactic antibiotic therapies and nutritional changes can deplete commensal and potentially protective elements of the cutaneous and intestinal microbiome. The infectious risks for the patient undergoing HSCT are affected by transplantation complications, including the direct effects of this high-dose conditioning, such as mucositis, hemorrhagic cystitis, diarrhea, and hepatic venoocclusive disease (VOD); GVHD; and relapse of the underlying hematologic or oncologic disease.

Chemotherapy

Busulfan and melphalan are commonly used alkylating agents that are toxic to myeloid stem cells and mucosal and epithelial cells. Cyclophosphamide-containing regimens predispose patients to hemorrhagic cystitis. Fludarabine is less cytotoxic but intensely immunosuppressive and is often included in reduced-intensity conditioning (RIC) or nonmyeloablative (NMA) conditioning regimens.[16] Antithymocyte globulin (ATG), which alters the function of or depletes T cells and other lymphocytes, can be used as conditioning regimen for aplastic anemia and, at times, for GVHD prevention or treatment.[17] Serum sickness, a syndrome of fever, arthralgia, and rash, can occur with ATG or other xenoprotein therapy; it is treated with corticosteroid therapy. Antilymphoid antibodies, including alemtuzumab or rituximab affecting T and B cells, respectively, can induce prolonged and profound lymphopenia. Some GVHD prevention strategies include ex vivo graft manipulation ($CD34^+$ or $CD3^-$ selection) for T-lymphocyte depletion. A new approach is post-HSCT cyclophosphamide (on days 3 and 4 after grafting) to lyse proliferating alloreactive lymphoid cells, highly active in prevention of GVHD, made HLA haploidentical HSCT widely applicable. Cyclosporine, tacrolimus, mycophenolate mofetil, methotrexate, or sirolimus (and other agents) are often used for weeks to months after allografting to lessen the risks and severity of GVHD. Although all these may reduce the risks for GVHD, these maneuvers also substantially delay immune recovery, particularly in haploidentical (one HLA haplotype matched) or HLA-mismatched HSCT.

Irradiation

TBI may be administered as a single dose or more often "fractionated" in multiple doses given over several days. Diarrhea occurs in virtually all treated patients in the first week after irradiation. It may be treated symptomatically while stool studies are pending to rule out infectious causes. Severe oral mucositis occurs in many irradiated patients and is aggravated by prolonged neutropenia and the use of methotrexate for GVHD prophylaxis.[15] As long as bleeding and oral inflammation do not compromise the patient's airway, mucositis is treated symptomatically. Keratinocyte growth factor (delivered clinically as palifermin) has proven activity in the prevention of oral and intestinal mucositis.[18,19] Its use to limit mucositis may be of benefit in patients receiving highly toxic conditioning, including high-dose TBI, high-dose melphalan, or post-HSCT methotrexate.[20] Palifermin has not been shown to reduce GVHD, infections, or mortality.[21,22] Diffuse alveolar hemorrhage (DAH) is another complication of TBI that is associated with decreased pulmonary function after HSCT.

Reduced-Intensity Conditioning or Nonmyeloablative Transplantation

RIC or NMA regimens have been developed with the goal of permitting donor-derived hematopoietic and immunologic reconstitution.[23,24] These lesser-intensity regimens may provide a somewhat weaker anticancer effect and rely on the graft-versus-tumor effects to eradicate underlying malignancies. Doses of TBI are usually not more than 2 Gy, compared with 12 to 14 Gy in fully ablative transplants. Fludarabine, ATG, or lower doses of cytotoxic drugs may be used with induction of extended immune suppression.[25] This approach is often used for older patients or those with significant medical comorbid conditions. They are intensely immunosuppressive but less cytotoxic, resulting in less mucosal, enteric, and hepatic injury in the early weeks after transplantation, and perhaps because of that associated with fewer risks for GVHD.[26]

Human Leukocyte Antigen Matching

In general, engraftment is most rapid, and thus neutropenia is briefest, when the patient and allogeneic donor are completely matched at all genetic HLA loci (most often considering only HLA-A, B, C, and DRB1). Similarly, identical twin (syngeneic) transplantations or those in which hematopoietic stem cells collected from the recipient (autologous) are associated with prompt neutrophil recovery. Allogeneic HSCT has the highest chance of prompt engraftment when fully HLA-matched sibling donor transplants are used, but less than 30% of intended recipients have a matched sibling donor available.[27,28] Greater HLA mismatch augments risks for graft failure, acute and chronic (c)GVHD, and consequent prolonged immunodeficiency.[29]

Killer immunoglobulin-like receptors (KIR) interact with HLA and other molecules (KIR ligands) that bind to and modulate the function of natural killer (NK) lymphoid cells. After HSCT, altered NK-cell function may modify risks for infection, GVHD, or malignant relapse. KIR ligands can be grouped based on their amino-acid sequence determining the KIR-binding epitopes, primarily in HLA-C and HLA-B molecules, but, owing to linkage disequilibrium, are not inherited coordinately with HLA. T-cell recovery and NK-cell proliferation and functional maturation are not altered by KIR ligand match or mismatch status.[30] However, recipients of stem cells from unrelated donors with an activating NK-cell immunoglobulin-like receptor (KIR) (B/x) genotype have decreased infectious complications believed to be due to enhanced NK-cell function.[31] The role of KIR genotype in donor selection before HSCT, especially for less relapse, is of research interest at this time.

Prevention of Infection

Preventive strategies include protective isolation for reduced exposure to pathogens, enhancement of host immune reconstitution with hematopoietic growth factors, prophylaxis during high-risk periods with targeted antimicrobial chemotherapy, and suppression of subclinical infection with preemptive therapy, which is best facilitated by scheduled periodic surveillance.[32–34] Prophylaxis or preemptive strategies are more effective than treatment after infection is established, and the mortality rate among patients with established infections continues to be high despite appropriate therapy. Prophylaxis can be tailored for patients

who have a higher risk of reactivation, therefore screening for lifelong infections, including syphilis, tuberculosis, toxoplasmosis, HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and herpes simplex virus (HSV) should be tested at screening period.[35] Of note, false-positive serum antibody results can occur after IVIG infusions, and false-negative serum antibody results can occur in the early months after transplantation.[36] For this reason, viral serostatus, as well as serostatus of a few nonviral infections, are checked before transplantation so that the risk of reactivation is clear.

After mucositis has cleared and oral alimentation has resumed, oral therapy is the preferred route for antiinfective prophylaxis.

CLINICAL SYNDROMES UNIQUE TO THE HEMATOPOIETIC STEM CELL RECIPIENT

Hemorrhagic Cystitis (HC)

HC is a common complication that can lead to gross hematuria, clots, urinary retention, and impairment of renal function. Severe forms (grade III–IV) of HC are associated with increased nonrelapse mortality.[37] Cystitis that occurs within the first weeks after marrow infusion usually is noninfectious in origin, caused instead by the administration of cyclophosphamide, especially at myeloablative doses in the conditioning regimen.[37,38] Prophylactic measures include mesna (for binding and elimination of the alkylator metabolites of cyclophosphamide), forced diuresis, and continuous bladder irrigation. Supportive care for established cystitis may also necessitate large-bore catheter drainage or bladder irrigation and transfusions. Later in the posttransplantation period, severe GVHD, HLA-mismatched status (MMUD, UCB) and infection are contributing causes of cystitis.[37,39,40] The majority of infectious agents inducing cystitis are viral, usually either the polyomavirus BK virus or adenovirus. Infection with HSV, CMV, the polyomavirus JC virus, human herpesvirus 6 (HHV-6), various bacteria, and *Strongyloides* occurs in lower frequencies.[41–43] Although data are predominantly derived from studies in renal transplants, there is evidence that quinolone use (levofloxacin) does not have a preventive or therapeutic role for BK virus reactivation and disease.[44–46] Neither BK viruria nor viremia confirm that BK virus is causal for hemorrhagic cystitis.[40] Polyomaviruses are shed in the urine in many HSCT patients without clinical symptoms (see Chapter 144).[47,48] Higher urine or detectable blood viral loads of BK virus may indicate a higher risk for hemorrhagic cystitis.[49] No standard antiviral treatment is currently available for viruria caused by BK virus or adenovirus, although intravesicular cidofovir or low-dose IV cidofovir has been administered when ongoing hemorrhage or large mucosal clots persist despite continuous bladder irrigation.[50]

Venoocclusive Disease (Sinusoidal Obstruction Syndrome)

VOD is a syndrome of liver toxicity that occurs at any time after the onset of the high-dose conditioning regimen, usually before day 30. It is characterized by painful hepatomegaly, 5% or greater weight gain, and hyperbilirubinemia (bilirubin levels >2 mg/dL).[51] Severe VOD (sometimes called "sinusoidal obstruction syndrome" [SOS]) with marked jaundice or ascites leads to multiorgan failure involving the kidneys, heart, and lungs and high mortality.[52] The only US Food and Drug Administration (FDA)-approved drug for severe VOD/SOS is defibrotide, which stabilizes endothelial cells by reducing endothelial cell activation and by protecting endothelial cells from further damage, resulting in the restoration of the thrombofibrinolytic balance.[53] Anticoagulant or antithrombolytic therapies are mostly ineffective and associated with risk of serious bleeding.

Cyclophosphamide followed by TBI increases the risk for VOD because recurrent endothelial damage, along with reduced hepatocyte glutathione, increases vulnerability to radiation toxicity.[54,55] Other risk factors for VOD include elevated aminotransferases before HSCT, mismatched or unrelated donor HSCT, and history of abdominal radiation therapy.[55] Genetic predispositions in urea cycle enzymes (e.g., glutathione-*S*-transferase) may, in some patients, increase risks for VOD.[54] Conditions that may mimic VOD include cholestasis in patients with septicemia; hepatic infiltration secondary to infection or tumor; pericardial tamponade; cytomegaloviral or other viral hepatitis; and intraabdominal inflammatory diseases, such as pancreatitis, peritonitis, or cholecystitis. In addition, early GVHD and cyclosporine-induced cholestasis are noninfectious causes of liver toxicity that may coexist with or mimic VOD. Diagnosis of VOD may be difficult in early stages when treatment is more effective, and ultrasonographic assessment of hepatic portal venous flow and newer techniques, such as ultrasound point shear wave elastography, showed shear-wave velocity as significantly elevated in patients with VOD after an HSCT prospective study in 134 patients who received allogeneic HSCT between 2011 and 2016.[56] Transjugular liver biopsy and measuring corrected hepatic sinusoidal pressure gradient during the procedure with a relatively safe profile (three major complications in 141 HSCT patients) can be important to make correct diagnosis.[57] Hepatotoxic and nephrotoxic drugs should be avoided in patients with VOD.

Graft-Versus-Host Disease

Acute GVHD (aGVHD) is a major, life-threatening complication, developing in 40% to 80% of patients after allogeneic transplantation.[58] The risk for developing GVHD is higher in older patients and with partially matched or unrelated donor HSCT and is associated with the higher doses of donor T cells infused with filgrastim-mobilized peripheral blood grafts.[59] In aGVHD donor, T lymphocytes mount an immune attack against the recipient's tissues that is amplified by proinflammatory cytokines. The favorable antineoplastic impact accompanying GVHD is called the graft-versus-leukemia (GVL) effect.[60] T cells and possibly NK and other lymphoid cells mediate GVL effects after allogeneic HSCT by the production of inflammatory cytokines and by direct target lysis.

The primary target organs of aGVHD are skin, liver, and gastrointestinal tract (GIT), therefore its common clinical manifestations include rash, cholestatic hepatitis, nausea, vomiting, and diarrhea. aGVHD can be lethal, and one of the major clinical problems remains to predict who will have severe GVHD in real time. Recently, models to predict patients at risk of poor outcomes have been developed using both clinical and laboratory data.[61]

Effective immunosuppressive agents for the prevention of GVHD include calcineurin inhibitors (cyclosporine or tacrolimus) with or without methotrexate, mycophenolate mofetil, and sirolimus; they are usually started before transplantation in T-cell replete HSCT. T cells can be removed ex vivo (negative selection or $CD34^+$ cell selection) or in vivo (e.g., ATG, alemtuzumab use in conditioning) from the graft; however, these methods may result in an increase of some complications (e.g., graft failure, infections such as CMV and *Aspergillus*, and relapse).[62,63] In addition, posttransplant cyclophosphamide, used especially after haploidentical HSCT, has become an effective method for prevention of both acute and cGVHD.[64] GVHD itself can compound and prolong post-HSCT immunodeficiency. The corticosteroids or other immunosuppressive drugs used as treatment of GVHD may impair phagocytic function and directly worsen lymphopenia and cellular immune deficiency.[65,66] Patients with acute and cGVHD have splenic dysfunction and thus an added risk for infection with encapsulated bacteria, such as *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae.* T-cell depletion of the donor graft is less associated with GVHD but more with infection and sometimes with much-delayed immune recovery.

Hepatitis

Clinical hepatitis in HSCT recipients can range from fever accompanied by abdominal pain to fulminant illness. Infectious hepatitis must be distinguished from several common noninfectious causes, including liver dysfunction related to the "conditioning regimen" (VOD), aGVHD, cholestatic liver injury related to sepsis, and chemical hepatitis related to either drugs or hyperalimentation.[67]

Clinically important viral hepatitis syndromes that occur after transplantation include acquisition or reactivation of infection with HBV, HCV, varicella-zoster virus (VZV), adenovirus, HSV, CMV, and HHV-6.[68,69] Reactivation of HBV is more likely than HCV to result in fulminant hepatitis, although this occurs in only a minority of infected patients. Disseminated VZV and adenovirus infections may manifest as elevations in serum aminotransferase levels; these elevations can precede the appearance of other disease manifestations by several days. In rare cases liver biopsy with viral culture and polymerase chain reaction

(PCR) assay are needed to establish a diagnosis of severe hepatitis in the early postengraftment period. Viruses such as hepatitis G virus and transfusion-transmitted viruses are not known to influence the outcome of HSCT. Hepatitis E virus, transmitted by the fecal-oral route, is now increasingly identified as a pathogen in HSCT, as a cause of both acute and chronic hepatitis.[70] Screening strategies for donors are not established, and diagnostics should include PCR. Therapy with ribavirin has been effective.[71]

HBV (surface antigen [HBsAg], surface antibody [HBsAb], and core antibody [HBcAb]) and HCV serologic profiles are tested in donor and recipient before HSCT. Patients who are HBsAg positive or HBcAb positive are at high risk for reactivation, whereas the risk is extremely rare in those with HBsAb positive. Pretransplantation imaging studies or liver biopsy may be needed to evaluate HCV-seropositive patients with abnormal liver enzyme levels or tender hepatomegaly. Donors and recipients with positivity for HBsAg and HBcAb should be tested for viral load with PCR studies for HBV DNA before transplantation because the risk for HBV hepatitis can be reduced by treatment to lower a detectable viral load. Lamivudine, entecavir, and tenofovir are commonly used to suppress HBV replication (see Chapter 145).[72] Current stratifications of risk are discussed in Chapter 306. The more potent activity of entecavir has led to support for its use in preference to lamivudine, particularly in patients with HBsAg present in the serum at the time of immunosuppression.[73]

A transplant from an HBV-infected donor can be used if no alternative donor is available or if the intended recipient is already seropositive.[74] HBV can be transmitted from an HBsAg-positive (or, less likely, an HBcAb-positive) donor to a recipient who is either HBV naïve or HBsAb positive but HBcAb negative. The risk for transmission is low when an HBV-positive donor has an undetectable viral load and/or has nonreplicative HBV.[75] If the recipient is HBV naïve before transplantation, the subsequent infection is more likely to have clinical consequences. If the transplant can be delayed, then HBV vaccination of the recipient or use of HBV immune globulin or both may reduce the likelihood of hepatitis after transplantation.[76] HBV immunity can be transferred from an HBsAb-positive donor to an HBV-naïve recipient.[69,76] Through adoptive immune transfer, HBV infection can be cleared from an HBsAg-positive recipient by transplant from an HBsAb-positive (i.e., immune) donor, which generally follows a period of hepatitis (elevation of liver functions).[69]

After transplantation the following recipients should be monitored periodically with PCR testing of HBV DNA viral load: (1) those with liver enzyme elevation suggestive of activation of HBV from latency, (2) those with transplants from HBV-infected donors, and (3) those with known infection before transplantation. High HBV viral load ($>10^5$ copies/mL) is the most important risk factor for clinically apparent reactivation in recipients positive for HBsAg.[77] Among recipients in whom PCR studies of HBV DNA yield positive results persistently after transplantation despite treatment, the risk for fatal liver disease may be up to 12%.[77]

HCV-positive donors and recipients should undergo RNA viral load testing. Although best avoided, but if there are no other options, an HCV-infected individual can serve as a donor. In contrast to HBV, however, the rate of transmission of HCV from an HCV RNA-positive donor approaches 100%. Interferon (IFN) can be used to suppress HCV replication in donors,[78] but its limited efficacy, systemic and hematologic toxicity, and delayed response may not contain active HCV to render a donor suitable for donation. Direct-acting antiviral (DAA) therapies are highly active in HCV and have rendered IFN-containing regimens obsolete for almost all HCV genotypes, although the evidence comes from non-HSCT patients[79] (see Chapters 47 and 154). In a study, no patient receiving DAA therapy after transplantation discontinued HCV therapy, and efficacy was 85%.[80] However, dose adjustments per renal function and drug interactions may be needed.[76]

In contrast to HBV, which induces immune-mediated hepatitis, HCV can induce direct hepatotoxicity.[81] Hepatitis most commonly occurs 2 to 3 months after transplantation; severe hepatitis is rare. There is no strong evidence to support HCV as a cause of increasing incidence of VOD, especially when conditioning regimens are not risk factors for VOD, or GVHD. However, even without direct hepatic failure, HCV seems to be associated with a higher nonrelapse mortality in the first year. Beyond 10 years, the long-term complication of HCV infection is cirrhosis.[82] No data have demonstrated a correlation between hepatitis C genotype and type or severity of liver disease after transplantation. Because of the myelosuppressive effects of IFN and other antiviral agents, their use in the treatment of hepatitis C after HSCT is limited. However, in a study with 20-year follow-up, antiviral treatment (IFN with or without ribavirin) showed a strong trend to reduce the risk for severe liver complications (odds ratio, 0.33; 95% confidence interval, 0.11 to 1.03; $P = .058$).[83] In the study of 195 patients, 33 died, among which 6 died from liver complications. The cumulative incidence of severe liver complications (death from liver failure, cirrhosis, and liver transplantation) was 11.7% at 20 years after HSCT.[83] A new American Society for Blood and Marrow Transplantation task force recommends long-term liver function test (alanine aminotransferase) monitoring in all HCV-positive recipients/donors. HCV RNA monitoring should be measured in all patients at entry into care, and monitoring of viral load should be performed in patients receiving HCV treatment or liver dysfunction.[79]

Pneumonia Syndromes

Infectious pneumonias must be distinguished from noninfectious pulmonary complications after HSCT, which can include pulmonary edema, pleural effusion, alveolar hemorrhage, radiation injury (pneumonitis or fibrosis), drug reactions, adult respiratory distress syndrome, idiopathic pneumonia syndrome, cytolytic thrombi (causing multiple peripheral lung nodules), obliterative bronchiolitis, bronchiolitis obliterans with organizing pneumonia, and cGVHD.[84] Management of noninfectious pneumonias requires that lower respiratory tract infection (LRTI) be ruled out or recognition of its coexistence. Their pathophysiologic processes may be distinct: Some syndromes may be more likely to respond therapeutically to high-dose corticosteroid therapy.

Diffuse alveolar hemorrhage (DAH) begins with dyspnea and alveolar infiltrates, with progressively bloody return during bronchoscopic examination and alveolar lavage.[85] Although DAH definition requires no infection, infection-associated alveolar hemorrhage and DAH are related clinical syndromes with similar clinical presentation, risks, and associated high mortality.

The syndrome usually occurs in the second to fourth weeks after HSCT, generally before platelet engraftment and perineutrophil engraftment.[86] Because DAH occurs around neutrophil engraftment, tissue damage due to leukocyte influx into lungs is suggested.[87] Delayed engraftment, thrombocytopenia, infection, toxic effects of drugs, TBI, intensely cytotoxic regimens, and solid malignancy have been implicated as risk factors.[85,86] Very-high-dose corticosteroids are recommended, but DAH remains highly mortal.[86]

Idiopathic pneumonia syndrome is a process of widespread alveolar epithelial injury that is characterized clinically by diffuse interstitial infiltrates and varying degrees of respiratory failure in the absence of active LRTI.[88] It is believed to be related to the chemotherapy or TBI, or both, used as part of the conditioning regimen, which induces proinflammatory cytokine release and increasing alveolar capillary permeability. Idiopathic pneumonia syndrome occurs in 8% to 17% of patients but may be more frequent after allogeneic than autologous transplantation and thus has been implicated, at least in animal models, as a component of the GVHD reaction. Mortality rates are 60% to 80%. Idiopathic pneumonia syndrome occurs classically during two peaks: one in the first few weeks and the other in the second and third month after transplantation. Corticosteroids and etanercept may yield a clinical response.[89] More frequent use of sensitive diagnostic techniques has revealed a high incidence of occult infections, including due to human rhinovirus, CMV, HHV-6, and *Aspergillus*.[89] The detection of these viruses may be associated with increased mortality. This diagnostic yield changes the "precision" of the diagnosis of idiopathic pneumonia, which should lack detectable pathogens.[90] The uncertain role of this detection on the applicability of immunosuppressive regimens is not yet clear.

Diarrhea

Diarrhea, a common symptom after transplantation, is primarily a result of noninfectious causes, such as regimen-related gut mucosal toxicity and GVHD. Diarrhea is associated with infection in less than 20% of cases.[91,92]

The list of infectious agents responsible for diarrhea includes *Clostridioides difficile* (formerly *Clostridium difficile*), adenovirus, rotavirus, norovirus, enterovirus, coxsackievirus, HHV-6, *Escherichia coli, Salmonella, Giardia, Strongyloides, Cryptosporidium,* and *Campylobacter* spp. Infection with *C. difficile* occurs with increasing frequency.[93,94] Outbreaks of diarrhea have been reported for *Cryptosporidium* and enterovirus. From other countries, reports of diarrhea have been associated with *Trichostrongylus* spp.

Typhlitis, or neutropenic enterocolitis, is an anaerobic infectious syndrome that is relatively common and may be associated with diarrhea during neutropenia.[95] Typhlitis is preceded by fever, abdominal pain, and right lower quadrant tenderness that may be accompanied by rebound tenderness. Appendicitis can mimic typhlitis. Computed tomography of the abdomen reveals right-sided colonic enlargement and inflammation with thickening of the mucosa. Therapy against anaerobic bacteria should be added to the medical regimen.

Rash

Skin eruptions are often noninfectious, occurring as a direct result of radiation effect from conditioning therapy or secondary to aGVHD or drug reaction.[96] Rashes from conditioning regimens can result in the sudden onset of marked erythema over large areas of the body and blistering on the hands and feet. A skin biopsy can assist in distinguishing infectious from noninfectious causes of rash. For all lesions suspected to be infectious, samples should be submitted for culture or biopsy. The most common infectious causes are VZV, catheter-related exit site or tunnel infections, and cutaneous manifestations of disseminated bacterial or fungal infections.[97] Focal areas of bacterial cellulitis may occur on the lower extremities in the setting of edema from heart failure, VOD, lymphedema, and impaired venous return.

Osteomyelitis

Osteomyelitis is uncommon after HSCT. The spectrum of organisms can include atypical mycobacteria, yeasts, and molds, in addition to bacteria.[98,99] In rare instances osteomyelitis follows marrow aspiration from the sternum or marrow harvest from the iliac crest.[99,100] When prolonged pain and fever occur after BM harvest, osteomyelitis caused by *Staphylococcus aureus* should be considered.

PATTERNS OF IMMUNOSUPPRESSION AT DIFFERENT TIMES AFTER MYELOABLATIVE HSCT

Historically, three risk periods of immunologic deficiency occur predictably in graft recipients after HSCT (Fig. 307.1). They are the preengraftment period, the early postengraftment period (until day 100), and the late postengraftment period (after day 100). An understanding of the immune deficiencies in each risk period and the period of peak risk for individual infections that are observed with standard infection prophylaxis helps the clinician recognize uncommon manifestations of these infectious pathogens (Table 307.1).

Preengraftment Risk Period

Infection present in the transplant candidate before conditioning may have important adverse effects after transplantation, so screening and diagnosis of symptomatic respiratory and GI disease is an important intervention. For infections that might resolve, delay of conditioning should be considered to ameliorate the risk associated with conditioning during active infection.[101,102] The preengraftment risk period begins with the onset of conditioning therapy and continues until approximately days 20 to 40 after transplantation. By definition, graft failure is declared if there is no neutrophil recovery by day 42. Pretransplantation neutropenia is associated with increased infection-related mortality.[103] Bacterial infections are common during this time of profound neutropenia and lymphopenia, necessitating prophylactic and promptly administered empirical systemic antibiotic therapy (see Chapter 306).[104] Prophylactic systemic antibiotics (often a fluoroquinolone, such as levofloxacin) can be administered when the neutrophil count drops to less than 500/mm^3 and continued until the neutrophil count recovers to prevent bacterial infection. GI decontamination with nonabsorbable antibiotics was used in the past but is now rarely performed.

Prophylactic antibiotic use has shifted the spectrum of GI microbiota to potentially pathogenic organisms, such as *C. difficile* and enterococci.[93,105] The etiologic agents of bacteremia have shifted to more gram-positive organisms; in particular, coagulase-negative staphylococci (CoNS), enterococci, and viridans group streptococci are often isolated from bloodstream cultures of febrile neutropenic HSCT recipients.[106] Mechanical barrier defects caused by mucositis and central catheters predispose patients to BSI by allowing access for skin-colonizing organisms and GI mucosal flora to otherwise sterile body sites.[107] Colonization with vancomycin-resistant enterococci or other multidrug-resistant pathogens may predispose patients to bacteremia.[105,108] Recipients of autologous and allogeneic grafts may develop a similar spectrum of infections during the preengraftment period; the major transplantation-related complications occurring in this risk period (mucositis, severe neutropenia, and VOD/SOS) are similarly frequent with all types of transplantations. However, the less frequent use of TBI and methotrexate and the more rapid neutrophil recovery after autologous transplantation of peripheral blood stem cells have markedly decreased the risks for mucositis and serious bacteremia in this subpopulation of patients. Similarly, the use of RIC regimens before NMA allografting has lessened the risks for early bacteremia.

During this risk period, HSV is predictably reactivated in 80% of patients who are HSV seropositive. Most such infections occur before week 4 after transplantation. Prophylactic acyclovir, 400 mg twice daily (5 mg/kg twice daily for children), has minimized this clinical infection.[109,110]

Candidemia and early-onset aspergillosis occur in less than 5% of patients with neutropenia. The risk is greater in patients with slow engraftment or extended neutropenia before transplantation. With fluconazole (200–400 mg/day),[111–113] voriconazole,[114] or micafungin[115] (50 mg IV once daily) prophylaxis, *Candida albicans* infections have been mostly eliminated during this risk period; however, *Candida krusei* and *Candida glabrata* have emerged as fluconazole-resistant pathogens.[116]

Some allogeneic transplantation candidates are at higher risk for mold infections during the preengraftment period than others; their transplantation course begins with mold prophylaxis, possibly beginning even 2 to 4 weeks before HSCT.[117] These patients can include those with extended pre-HSCT neutropenia, such as acute leukemia, who have undergone serial chemotherapy before transplantation; those with myelodysplastic syndrome; those with aplastic or Fanconi anemia; and others. Fungal infection that occurred within 6 to 9 months before transplantation may not be cured and could reactivate. Patients with more remote fungal infections can receive a standard regimen of fungal prophylaxis. If patients have a history of aspergillosis within 4 months of transplantation or have suspect pulmonary nodules without a specific diagnosis,[118] they should receive secondary fungal chemoprophylaxis (i.e., ongoing maintenance antifungal therapy) and undergo rescanning before and after transplantation.

Use of hematopoietic growth factors has reduced the incidence of bacteremia by shortening the duration of neutropenia, but these agents have not been shown to improve outcome in established infections.

Adjunctive therapy with granulocyte transfusions has been used in some centers for treatment of serious infections that develop in patients with neutropenia.[119] Use of colony-stimulating factors with dexamethasone to prime granulocyte donors and increase the collection yield is being evaluated. Evidence of efficacy and therefore the indications for use of this expensive and labor-intensive supportive measure are uncertain and still under study.

Routine culture of hematopoietic progenitor cell products yields low rates of recovery of bacterial organisms, most often *Corynebacterium* spp. or staphylococci. Appropriate testing of collections of graft products includes routine culture of hematopoietic progenitor cells before HSCT, but patients receiving culture-positive harvests usually do so without clinically adverse outcomes.[120,121]

Postengraftment Risk Period

The postengraftment period begins with neutrophil recovery and continues until day 100, when early B- and T-lymphocyte functional recovery may be apparent. Reconstituted T lymphocytes have abnormal function for approximately 18 months, as evidenced by CD4 deficiency

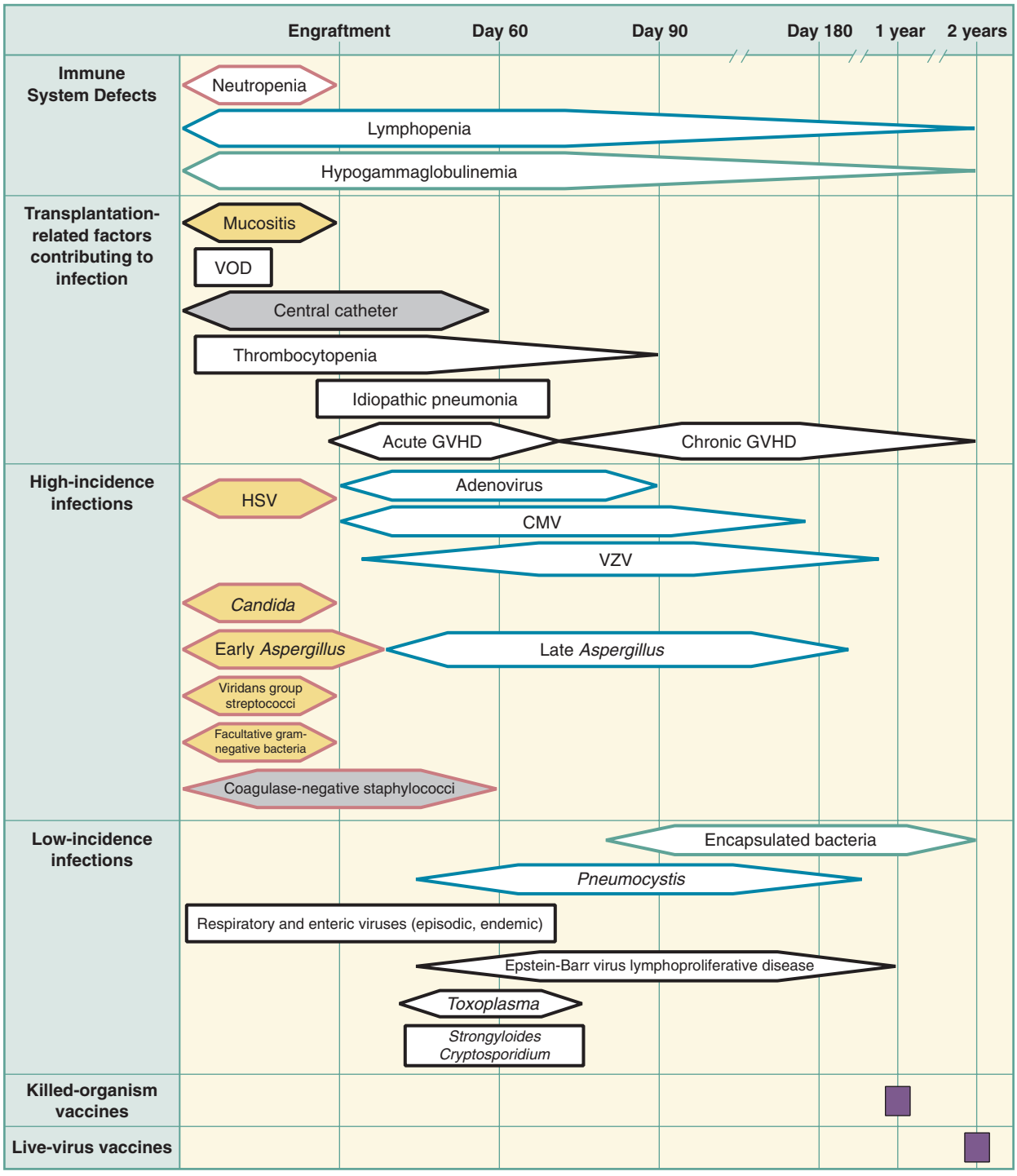

FIG. 307.1 Phases of predictable opportunistic infections among patients undergoing hematopoietic stem cell transplantation. Immune defects predisposing to infection are bordered by color (*pink,* neutropenia; *blue,* lymphopenia; *green,* hypogammaglobulinemia). Barrier defects predisposing to infection are shaded in color (*gold,* mucosal breakdown; *gray,* skin breakdown). Contribution of defects to infections occurring with high incidence are designated by border color (for immune defects) or shading (for barrier defects) or both. *CMV,* Cytomegalovirus; *GVHD,* graft-versus-host disease; *HSV,* herpes simplex virus; *VOD,* venoocclusive disease; *VZV,* varicella-zoster virus. *(Modified from Van Burik JA, Freifeld AG. Infection in the severely immunocompromised host. In: Abeloff MD, Armitage JO, Niederhuber JE, et al, eds.* Clinical Oncology. *3rd ed. Philadelphia: Churchill Livingstone; 2004:942.)*

and by in vitro antigen and mitogen proliferative responses. However, T-lymphocyte reconstitution may be blunted by the effects of GVHD or CMV and their attendant treatments (corticosteroids, calcineurin inhibitors, anti–T-lymphocyte therapy, and ganciclovir products). As a result, the rate of infection during this risk period is higher in recipients of allogeneic grafts, who are more likely to develop GVHD or CMV, than in recipients of autologous-syngeneic grafts. Another consequence of GVHD during this risk period is disruption of the GI mucosa, which can permit transmural entry of pathogens and lead to bacteremia or fungemia.

Late-onset aspergillosis may also occur during this risk period in up to 10% of patients, especially those with continuing GVHD, those receiving high-dose corticosteroids, and those with poor graft function.[122] Advanced-generation azoles that have activity against filamentous molds are effective prophylaxis against deep mycoses in patients at high risk.[114,123] Careful surveillance is required for these high-risk groups; serum galactomannan screening is insufficiently sensitive for reliable early detection of *Aspergillus* infections.[32] Although infrequent, infection with agents of mucormycosis may occur as they are resistant to the advanced azoles (e.g., voriconazole).

TABLE 307.1 Infections After HSCT in Order of Occurrence

ORGANISM	PEAK TIME PERIOD OF RISK (WEEKS AFTER HSCT)	USUAL PROPHYLAXIS	INCIDENCE
Preengraftment Risk Period (1–4 Weeks)			
Herpes simplex virus (seropositive)[109,222–224]	1–2	Acyclovir or valacyclovir	5%–9%
Gram-positive bacteremia (most commonly coagulase-negative staphylococci, viridans group streptococci, and enterococci)[105,153,162–164,179–181,184–186]	1–4	Prophylactic broad-spectrum antibiotics	20%–30%
Gram-negative bacteremia[104–106,147,164,165,167,169,183,184]	1–4	Prophylactic broad-spectrum antibiotics	5%–10%
Clostridioides difficile (formerly *Clostridium difficile*) diarrhea[93,190,191,462]	1–5	—	5%–10%
Candida[111–115,344,357,360,361]	1–4	Fluconazole, micafungin, voriconazole	Systemic infection: <5% colonization: 30%
Aspergillus and other molds[114,115,122,345,346,367]	1–4	HEPA air filtration, Itraconazole, voriconazole, posaconazole, micafungin, or low-dose amphotericin	<5%
Respiratory viruses[285,287,289,291–295,299,300,302,304]	2–5	Isolation, hand washing	15%
Idiopathic pneumonia syndrome[84]	2–4	—	8%–17%
Early Postengraftment (4–26 Weeks) and Late Postengraftment (26–52 Weeks) Risk Periods			
Cytomegalovirus (seropositive)[62,132,134,141,229–232]	7–26	Ganciclovir or foscarnet	<5% (end-organ disease), up to 40% (antigenemia/viremia)
Varicella-zoster virus (seropositive)[255–257,264]	4–52	—[a]	≤50%
Aspergillus and other molds[122,123,346,363,367]	4–26	Itraconazole, posaconazole, or voriconazole	10%–15%
BK virus[37,46–48]	—	—	≤50% (shedding)
Adenovirus[268,269,271,273,274]	—	—	1%–5%
Pneumocystis jirovecii[124,126,352]	4–104	Trimethoprim-sulfamethoxazole	<1%
Toxoplasma gondii (seropositive)[425,427,428,431]	2–8	—[b,c]	2%–7%
Infrequent Infections (May Span Multiple Risk Periods)			
Herpes simplex virus (seronegative)	—	—	<2%
Cytomegalovirus (seronegative)[220,226,227]	—	Blood product screening or filtration, letermovir	1%–4%
Varicella-zoster virus (seronegative)	—	—	<3%
Streptococcus pneumoniae[d,175]	—	Vaccination, penicillin[e,f]	<1%
Haemophilus influenzae[d,441,442]	—	Vaccination, penicillin[e,f]	—
Neisseria meningitidis[d,441,442]	—	Penicillin[f]	—
Human herpesvirus 6[323,325–327,329–332,334,335,463]	—	Ganciclovir or foscarnet	5%–60%, few treated
Epstein-Barr virus[312,317,318]	—	—	<1% (disease)
Nocardia spp.[213,214]	—	—[c]	<1%
Legionella spp.[209–212,216]	—	—	<1%
Mycobacterium spp.[196–199,206,207]	—	Screening, then prophylaxis	<1%
Listeria monocytogenes[217–219]	—	—	<1%

[a]Antiviral medications used to prevent other viral infections may be acting as prophylaxis against reactivation of varicella-zoster virus.
[b]Prophylaxis with pyrimethamine-sulfadoxine may be used for seropositive patients in countries with a high rate of seroprevalence.[299]
[c]The sulfa component of *Pneumocystis* prophylaxis may be acting as prophylaxis against *Toxoplasma* or *Nocardia* infection.
[d]Risk is increased in patients with chronic graft-versus-host disease.
[e]Efficacy in transplant recipients is undetermined.
[f]Increasing penicillin resistance may indicate a need for macrolides or extended-spectrum quinolones.
HEPA, High-efficiency particulate air (filter); *HSCT,* hematopoietic stem cell transplantation.

Prophylaxis of *Pneumocystis jirovecii* infection with trimethoprim-sulfamethoxazole (TMP-SMX), dapsone, atovaquone, or aerosolized pentamidine is required for 6 to 12 months or, if cGVHD is continuing, longer.[124–128] GI dysfunction should preclude the use of atovaquone, the absorption of which is poor in that context.

Reactivation of CMV predictably occurs in 20% to 40% of patients who are CMV seropositive. Transmission to seronegative recipients from seropositive donors is uncommon.[62] Surveillance for reactivation of CMV has been improved by the use of scheduled CMV testing with either pp65 antigenemia assay or PCR testing for serum DNA.[129] Ganciclovir or other antiviral therapy initiated preemptively at subclinical indications of reactivation has reduced the incidence of end-organ disease caused by CMV to only 5% to 10% of seropositive recipients.[130–132] Continuing GVHD or delayed immune recovery after partially matched- or unrelated-donor HSCT can lead to later-onset CMV infection and may indicate a need for prolonged CMV surveillance.

Late Risk Period

The late posttransplantation risk period begins at approximately day 100 and ends when the patient regains normal immunity, 18 to 36

months after HSCT.[133–139] In general, clinical immune recovery is demonstrable by the end of the first year after transplantation as long as the patient is no longer taking immunosuppressive medication and remains free from GVHD. For patients with continuing cGVHD, this period persists as long as therapy for cGVHD is required and includes dysfunction of lymphocyte, macrophage, and humoral immunity. VZV reactivation, infections with encapsulated bacteria (*S. pneumoniae, N. meningitidis,* and *H. influenzae*), and invasive aspergillosis or other invasive tissue mold infections may develop in this late risk period. Malignant disease relapse, regardless of its tempo or the choice of therapy, impairs the restoration of immunocompetence. Survival after relapse may be lengthy for some more indolent malignancies (e.g., chronic myelogenous leukemia, chronic lymphocytic leukemia, follicular lymphomas), but their post-HSCT immunodeficiency will persist. Therefore, even after relapse, ongoing surveillance and therapy, as in post-HSCT patients in remission, is still required.

The most common clinical infection syndromes include sinusitis, bronchitis, pneumonia, and otitis media caused by respiratory viruses or bacteria. CMV disease may develop; therefore CMV surveillance must be continued in seropositive recipients with cGVHD.[134,135] In CMV-infected patients with a risk of late disease identified by prior CMV disease, prophylaxis, or acute or cGVHD treated with the equivalent of more than 0.5 mg/kg of prednisone, no advantage has been identified between a PCR-based preemptive strategy or valganciclovir prophylaxis.[136] Late infections may be more common among patients with unrelated donors than among patients whose donors were family members, even in the absence of GVHD. Approximately 50% of late pneumonias in patients with ongoing cGVHD are caused by noninfectious interstitial pneumonitis. Lung histopathologic studies reveal organizing obliterative bronchitis that may respond to corticosteroid therapy.

IMMUNODEFICIENCY AFTER NONMYELOABLATIVE HSCT

NMA HSCT is associated with less disruption of mucosal barriers, shorter periods of severe neutropenia, fewer episodes of bacteremia in the first 30 days, and a trend toward fewer episodes of bacteremia during the first 100 days after HSCT.[140] However, this type of transplantation can still be associated with severe GVHD, often necessitating high-dose corticosteroid treatment. It is often used for older patients and those with compromised organ function and poor performance status. GVHD, CMV disease, and invasive fungal infection may be delayed 1 to 2 months, but the overall incidences of these conditions are similar to those with conventional myeloablative HSCT during the first year after HSCT.[140–142] Patients undergoing NMA HSCT may need surveillance for CMV and fungal infections well beyond day 100, as well as preemptive or prophylactic treatment similar to that for myeloablative HSCT recipients between day 100 and 1 year after HSCT.

MEASURES TO REDUCE RISKS FOR INFECTION

Protective measures that should be discussed before HSCT and reinforced during the recovery period involve travel, crowds, and pets.[143] With regard to travel, there are no particular restrictions, but strategies to minimize transmission of infectious diseases have been summarized.[32] Some social situations, such as sitting in a crowded movie theater or classroom, may increase the risk for acquiring a viral respiratory illness. Turning away from individuals who are coughing or sneezing, or even quickly donning a mask, may be helpful in preventing transmission of airborne, droplet-based infection. Patients need instruction to remember to augment infection prevention by washing their hands as soon as possible after being close to someone with a cold. Because outbreaks of noroviruses have involved cruise ships, and because other types of outbreaks (e.g., *Staphylococcus*) are commonly associated with the close living quarters of this type of vacation, cruise ships may be unwise vacation choices.

Healthy dogs and cats are considered acceptable pets. However, immunosuppressed patients should not get a new pet or be responsible for scooping cat litter because of potential *Toxoplasma* cyst exposure. Similarly, such patients should not play in sandboxes because these areas are concentrated sites that outdoor cats may use as litter boxes. Because reptiles of many sorts have been reported to be infected with *Salmonella,* such patients should not touch these animals or their aquarium homes. The water of tropical fish tanks may carry *Mycobacterium marinum*. Psittacine birds can transmit *Chlamydophila psittaci*.

Hand washing or the use of alcohol-based hand rub disinfectant is the mainstay of infection prevention in the hospital or clinic.[144] Persons entering the patient's room to perform examination or touch the patient (including visitors and health care workers) should wash or disinfect their hands outside the room. During respiratory virus season, infection control personnel will often add extra signage to doorways and other places on the wards to remind visitors of the importance of hand washing. Patients should not have direct contact with staff and/or visitors with respiratory viral infections. Routine use of gown, gloves, or masks, or a combination of these, is not required in the presence of a neutropenic transplant recipient, but ongoing caution to prevent interperson or nosocomial transmission is essential.

NATURAL HISTORY OF INDIVIDUAL INFECTIONS AFTER HSCT

With advances in infection prevention strategies, the risk periods for some infections are changing. It is important to understand the natural history of individual infections as they occur in the HSCT recipient and how it may be distinct from that in other immunocompromised patient populations. Infections that occur with a high incidence among HSCT recipients justify prophylaxis during the applicable risk period or empirical treatment during the course of infection (see Table 307.1). However, interactions between antibacterial prophylaxis, gut GVHD, and the microbiome remain to be addressed.[145–147] Moreover, antimicrobial resistance in gram-negative rods can be a concern. A European, Australian, and Asian study found 655 gram-negative rods–caused infection episodes in 591 patients (Enterobacteriaceae, 73%; nonfermentative rods, 24%; and 3% others). Half of the rods were fluoroquinolone and noncarbapenem resistant; 18.5% carbapenem resistant; and 35.2% multidrug resistant.[148]

Bacterial Infections

It is important to acknowledge that the incidence of BSI in HSCT recipients is high, mainly comprises CoNS,[149,150] and that bacteremia may occur without fever because of the use of corticosteroids.[151] The use of surveillance blood cultures without clinical indication in HSCT recipients is common, but the yield of significant results is low and leads to unnecessary interventions and increased cost.[152]

Gram-positive organisms, higher in the past, currently account for half of bacteremias occurring after HSCT.[105,150,153–155] Although the primary reservoir for these organisms is believed to be the skin, colonization of the GIT may be an additional source. Therefore a BSI can be a central line-associated bloodstream infection (CLABSI) or a mucosal-barrier injury laboratory-confirmed bloodstream infection (MBI-LCBI), defined by the Centers for Disease Control and Prevention.[156] A substantial evolution has occurred in the understanding of the sources of bacteremia in patients with mucosal-barrier injury. One important effect of this change is seen in determining the threshold and expectations related to changes of catheters, which would previously have been considered the default source of bacteremia.[157] *Staphylococcus epidermidis* (i.e., CoNS[158] is the species most commonly recovered in culture from the skin and nose. Oropharyngeal organisms include *Streptococcus pyogenes, Streptococcus mitis,* and *Enterococcus* spp. (both vancomycin-sensitive and vancomycin-resistant species).[155] The troublesome incidence of vancomycin-resistant enterococcal infection continues to rise without a concomitant improvement in the ability to determine its significance or optional therapeutic approaches,[159,160] although integration of colonization status is helpful in guiding rational use of empirical therapy.[161] Unlike catheter-associated infections with *S. aureus, Candida* spp., or some gram-negative bacilli, most gram-positive bacteremias can be managed successfully without removal of the intravascular device.[162,163] Methicillin-resistant *S. aureus* (MRSA), although rare in neutropenic infections, but may be associated with high mortality (≈7%–50%), in particular with MRSA outbreaks.[149] If the patient does not respond to initial antibiotic management or if there is tenderness or erythema along a catheter tunnel tract, the catheter should be removed. In rare cases, adjunctive surgical

débridement of the skin tunnel is needed. Catheter removal and surgical débridement are often required when a tunnel infection is caused by rapidly growing mycobacteria.

Gram-negative organisms are the second most frequent cause of BSI. The incidence of infection with *Pseudomonas* spp. is low, in part because of the use of antipseudomonal antibiotics for prophylaxis against, and initial empirical therapy for, neutropenic fever.[105] However, the virulence and potential for sepsis syndrome remain high for gram-negative organisms, including *E. coli, Klebsiella* spp., *Enterobacter* spp., and *Pseudomonas* spp. As a result, gram-negative infections preengraftment can be associated with a higher mortality rate.[164,165] Remodeling of sanitary and water supply systems decreased multidrug-resistant *Pseudomonas aeruginosa* infections (from 18.9%–3%).[166] Carbapenem-resistant Enterobacteriaceae (CRE) has been an emerging issue worldwide (*Klebsiella pneumoniae* carbapenemase-producing–*K. pneumoniae* [KPc-Kp] in the United States) and reported as 0.4% and 2% in a retrospective multicenter Italian study after autologous and allogeneic HCST, respectively.[167] Although the incidence of CRE was low, the mortality rates, in particular after allogeneic HSCT, were very high (16% and 64%, respectively). Gut colonization by multidrug-resistant gram-negative bacteria is an independent risk factor for the development of intestinal aGVHD.[168] CRE occurs in very sick patients as shown by a recent study that found risk factors for CRE included breakthrough infection while taking a carbapenem medication, longer hospitalization, requiring intensive care support, and previous other antibiotic therapy.[148] Another study from Europe showed that CRE before engraftment was the only risk factor for increased mortality after haploidentical HSCT in multivariate analysis.[164] In the United States, CRE infections are almost absent.

Although bacteremias have historically occurred during the neutropenic period, bacteremias continue to develop in patients with long-term central IV catheters, in patients with ongoing immunosuppression resulting from GVHD or its therapy, and in those with neutropenia secondary to graft failure or drug-related marrow suppression (e.g., ganciclovir).[169,170]

Bacterial meningitis was more common after allogeneic HSCT compared with autologous HSCT, caused by *Streptococcus pneumoniae* in 11 patients, *Neisseria meningitis* in 2, and *Streptococcus mitis* in 1 patient.[171]

Encapsulated Bacteria

Functional hyposplenism is not uncommon and is limited to patients with cGVHD.[172] cGVHD is also associated with decreased immunoglobulin M (IgM) memory B cells,[173] that are present in the marginal zone of the spleen and may produce immunoglobulins with specificity for polysaccharide antigens.[174] Therefore, after allogeneic HSCT, the risk for life-threatening bacterial sepsis with encapsulated organisms is increased. Invasive pneumococcal infection may occur months to years after HSCT. The annual incidence is 8 per 1000 allogeneic HSCT recipients and is higher (21/1000) among those with cGVHD.[175] Penicillin or macrolide prophylaxis may be indicated until immunosuppression is discontinued.[138,176] Reports of frequent penicillin-resistant pneumococcal infections in the general population have prompted a change in prophylaxis from penicillin to a quinolone in some centers.[177,178]

Viridans-Group Streptococci

Viridans-group streptococcal bacteremias, mostly caused by *S. mitis,* may carry a high mortality rate early after HSCT.[179–182] Although consistently reported that it occurred in the very early severely neutropenic phase of HSCT (median, ≈4 days), the incidence of breakthrough *S. mitis* BSI varied between 3% and 16% during fluoroquinolone prophylaxis.[183–185] Poor dental hygiene and myeloablative conditioning are risk factors for *S. mitis* bacteremia in HSCT patients.[184,186] Normally antibiotic sensitive, these organisms may be resistant to ciprofloxacin, levofloxacin, and penicillin in patients depending on prophylactic antibiotics. Vancomycin is the drug of choice for HSCT patients. Oral ulcerations caused by HSV reactivation during conditioning are believed to be an entry point, corroborated by a decreased incidence of viridans-group streptococcal septicemia after active prophylaxis of HSV infections with acyclovir.[180]

Clostridioides difficile

Increased rates of *C. difficile*–associated diarrhea or infection (CDI) have become a major issue for many hospitalized populations. The incidence of *C. difficile* after allogeneic HCST is reported to be between 10% and 34%.[187–189] The incidence is lower after autologous HSCT (6%).[187] Although most CDIs occur before engraftment, CDI continues to occur after engraftment as well.[190] Prior hospitalization, relapsed underlying disease and chemotherapy, receipt of any high-risk antimicrobials (e.g., cephalosporin and GVHD) were found as risk factors.[190,191] Of interest, CDI may also increase gut GVHD.[187] At Memorial Sloan Kettering Cancer Center, the incidence of early disease during the period 2005–10 was 11.9%, and no support was found for an increased pathogenicity of the NAP1/027 strain.[188] The risk for recurrent disease in allogeneic HSCT, which is as high as 31%, is related to antimicrobial use for indications other than the treatment of CDI.[188,192] The relative roles of colonization or acquisition during hospitalization are unclear, but rates of disease during hospitalization are significantly elevated in those colonized at admission.[193,194] Although little data support any choice between standard therapies in HSCT, the role of fecal microbiota transplantation should be explored. A case report of nasojejunal instillation of donor stool had a successful result.[195]

Mycobacteria

Mycobacteria are an infrequent cause of infection after HSCT, but it is important to identify them because treatment requires medication that would not be used empirically. The rapidly growing nontuberculous mycobacteria are responsible for skin/soft tissue infection, catheter exit site infections, tunnel infections, bacteremia (with waterborne *Mycobacterium mucogenicum*), or pneumonia.[196,197] Patients with cGVHD are at high risk for both nontuberculous mycobacteria[198] and tuberculosis.[199] Infection with *Mycobacterium tuberculosis* occurs predominantly in countries with high endemic rates; the number of cases may be increasing worldwide as transplantation becomes available globally.[200–203] Transplantation candidates and donors who are at risk for reactivation of latent tuberculosis can be readily identified during the pretransplantation evaluation on the basis of residence in endemic areas or close contact with another person with known or suspected tuberculosis. For these transplantation candidates, screening is recommended with tuberculin skin testing or an ex vivo IFN-γ release assay, such as QuantiFERON-TB Gold (Qiagen, Germantown, MD).[204–207] Clinically significant infection can be prevented by extended-duration antituberculous prophylaxis when QuantiFERON-TB Gold assays or reactive tuberculin skin testing yields positive results. Potential transplantation patients and donors should also receive screening if they have a history of abnormal chest radiographic findings before HSCT, have recently traveled to a foreign country for longer than 3 months, have been employed in an institution with tuberculous clients, have a history of alcoholism or IV drug use, or are seropositive for HIV. QuantiFERON-TB Gold has 96% to 99% specificity that is unaffected by the vaccine for bacillus Calmette-Guérin.[208] For patients with a positive result of a screening test but no signs of active tuberculosis and no previous antituberculous therapy, a chest radiograph and liver function studies should be obtained, in addition to peritransplantation and 9-month posttransplantation therapy with isoniazid and pyridoxine. A recent study from South Korea showed that tuberculosis was higher (2.9%) than the general population and that isoniazid prophylaxis, starting at the time of the positive IFN-γ release assay result, did not decrease the incidence of tuberculosis after allogeneic HCST.[199]

Intracellular Bacteria

Legionellosis[209–212] and nocardiosis[213,214] are uncommon, but both can manifest as pneumonia or lung nodules in the HSCT patient. Skin nodules and serositis (peritonitis, pericarditis) can also occur. The mean time to legionellosis after HSCT was 390 days and perhaps is associated with cGVHD. Most of the patients who had nocardiosis (median time was 10 months) were on prednisone, had lymphopenia, and received a myeloablative conditioning regimen.[214] Detection of *Legionella* by direct fluorescent antibody (DFA) assays has proved unreliable in the HSCT setting because of false-positive results and because it does not detect a high proportion of disease caused by *Legionella* species. These

species can include *Legionella feeleii, Legionella micdadei,* and *Legionella bozemanae*.[212] Urinary antigen testing is the most common test used for diagnosis, whereas nucleic acid–based tests are increasingly available for use in *Legionella*.[215] Culture remains the gold standard for diagnosis, but this takes time and its sensitivity varies significantly.[216] Infection can persist or relapse after 3 weeks of appropriate antimicrobial therapy (e.g., fluoroquinolone), which suggests that prolonged antibiotic treatment is indicated for HSCT recipients with legionellosis.[211] Medical therapies for nocardiosis often consist of administration of sulfonamide in combination with a synergistic agent, such as ceftazidime or imipenem-cilastatin; adjunctive surgical débridement may be useful for catheter-related infections with this organism. Nocardiosis is associated with high mortality (up to 50%).[214] The role of other intracellular bacterial agents as pathogens has not been well defined, but *Listeria monocytogenes* infection may manifest as bacteremia or meningitis.[217–219]

Viral Infections

Certain viral infections are preventable. Administration of acyclovir for HSV-seropositive patients during the preengraftment period is widely accepted. CMV-safe (serologically screened or filtered) blood product transfusions for CMV-seronegative patients have proved very effective in preventing transfusion-acquired CMV infections.[220,221] Periodic (e.g., weekly) CMV diagnostic surveillance for CMV-seropositive patients during the postengraftment period and prompt institution of antiviral therapy are essential. CMV-seronegative patients (with seronegative allogeneic donors) are at comparatively low risk; therefore diagnostic monitoring continues for only 6 to 10 weeks. Scrupulous hand washing and avoidance of crowds to prevent transmission of respiratory viral and other infections (in the hospital or ambulatory clinic) remain the mainstay of effective infection control practice for the HSCT recipient.

Herpes Simplex Virus

Among HSV-seropositive recipients during the first month after transplantation, the incidence of HSV reactivation can be reduced from 80% to less than 5% through the use of acyclovir or valacyclovir, initiated at the time of conditioning and continued until mucositis has healed.[109,222,223] The majority of postengraftment HSV infections are confined to the oropharynx, although the infection occasionally extends directly to squamous epithelial surfaces in the upper esophagus, larynx, or skin in the perioral or perianal areas. Patients who do not respond to acyclovir after engraftment, and particularly those who have received prolonged or repeated courses of acyclovir, may have acyclovir-resistant HSV. Foscarnet or cidofovir may be beneficial in that setting. In uncommon cases, HSV infection causes Bell palsy, hepatitis, or encephalitis. Valacyclovir achieves predictably higher drug levels than does acyclovir after oral administration.[223,224]

Cytomegalovirus

CMV reactivation before day 100 continues to be associated with increased nonrelapse mortality, with relative risks by disease category ranging from 1.61 to 1.95 in a review of CIBMTR data from 2003–10.[225] The historical incidence rate of primary CMV infection among the CMV-seronegative HSCT recipient can be reduced from 40% down to 4% or less by use of CMV-safe blood products during transfusions.[226,227] A cellular graft is associated with CMV transmission when a CMV-seronegative recipient receives a CMV-seropositive donor graft.[228] T-cell–depleted graft, HLA-mismatched transplantation, steroid treatment, and acute or cGVHD are risk factors for CMV infections. The incidence of CMV reactivation and viremia, traditionally 70% in CMV-seropositive patients with allogeneic transplants and 45% among patients with autologous transplants, can be reduced down to between 20% and 40% by the use of preemptive or prophylactic antiviral therapy.[130,229–236] With the most common approach, prophylactic acyclovir combined with preemptive early ganciclovir product therapy, the median time of onset of CMV end-organ disease has been delayed from 1 to 2 months toward 4 to 6 months after HSCT; this indicates that CMV surveillance must be longer in groups at high risk (see Chapter 137).[134,135]

Weekly screening enables identification of patients who might benefit most from preemptive therapy with ganciclovir products (Table 307.2). CMV pp65 leukocyte antigen and quantitative DNA PCR testing are excellent methods for early CMV detection, although antigen testing is unreliable during neutropenia. Laboratories are converting CMV viral load to $\log_{10}$ international units to decrease interinstitutional variability. Once CMV is identified by an early detection method, most patients are treated with 7 to 14 days of induction ganciclovir therapy (5 mg/kg IV twice daily), followed by maintenance therapy (ganciclovir, 5 mg/kg IV once daily, or valganciclovir, 900 mg orally once daily), for several weeks beyond negative CMV test results (Table 307.3). Maintenance therapy may need to be continued for patients with persistent detection of virus or those with profound immunosuppression caused by active GVHD.

Oral valganciclovir is a safe and effective ganciclovir prodrug, with the valine ester cleaved during the first pass through the liver, and it can be considered for the patient who needs induction and/or long-term maintenance and is otherwise taking oral medications without difficulty.[136] A valganciclovir dosage of 900 mg once per day produces blood-level drug exposure similar to those produced by an IV 5 mg/kg dose of ganciclovir.

Foscarnet can be used empirically for patients who have marrow suppression from ganciclovir and fail to respond to this drug or who have concurrent HHV-6 viremia. Foscarnet is administered in doses of 90 mg/kg IV every 12 hours for induction and 90 mg/kg IV every 24 hours for maintenance, or renal dose equivalents. Good urine flow can minimize irritation of the urethra and labia by foscarnet. The serum biochemical abnormalities (chelation of calcium and phosphate) accompanying foscarnet therapy necessitates hospital observation and very careful electrolyte monitoring, at least through the initial days of its use.

Letermovir, an antiviral drug inhibiting CMV replication by binding to components of the terminase complex,[237] when used for prophylaxis at a dose of 480 mg per day (or 240 mg per day in patients taking cyclosporine), significantly reduced CMV infection (CMV disease or CMV viremia leading to preemptive treatment) compared with placebo (37.5% vs. 60.6%, $p < .001$) in adults after allogeneic HSCT.[238] Nausea, edema, and atrial fibrillation were more common in the letermovir group.

End-organ manifestations of CMV disease include pneumonitis (63%), enteritis (26%), and, in rare cases, retinitis (5%).[131,239] CMV pneumonitis occurs in less than 5% of CMV-seropositive allogeneic patients who receive ganciclovir preemptive therapy during the first 100 days.[131] CMV DNA load in bronchoalveolar lavage (BAL) fluid can

TABLE 307.2 Weekly Screening Schedule for Initiation of Preemptive Cytomegalovirus (CMV) Therapy After Hematopoietic Stem Cell Transplantation

CMV SEROSTATUS OF RECIPIENT	CMV SEROSTATUS OF DONOR	BLOOD PRODUCTS	DURATION OF WEEKLY SURVEILLANCE
Seronegative	Seronegative	CMV safe[a]	Weeks 2–12
Seronegative	Seropositive	CMV safe[a]	Weeks 2–12
Seronegative	None (autologous)	CMV safe[a]	Weeks 2–5
Seropositive	Seronegative or seropositive	CMV untested	Weeks 2–12, then every 2–4 weeks until week 26[b]
Seropositive	None (autologous)	CMV untested	Weeks 2–5

[a]Blood filtered to remove neutrophils or from seronegative donor.
[b]Possible indications for testing beyond week 26 include high risk for late CMV disease (e.g., in patients treated with corticosteroids for graft-versus-host disease).

TABLE 307.3 Suggestions for Management of Possible CMV Infection After HSCT

INDICATION	STRATEGY[a]	COMMENT
Prevention		
Allogeneic Transplant		
Seropositive recipient	Preemptive ganciclovir[b] induction for subclinical viremia, 5 mg/kg bid for 7–14 days, followed by 5 mg/kg daily until the end of maintenance *or* ganciclovir prophylaxis[c] at engraftment	Some cases of CMV disease may occur shortly after ganciclovir discontinuation. CMV reactivation might be delayed, occurring later after HSCT.
Seronegative recipient with seropositive donor	Preemptive ganciclovir induction for subclinical viremia, 5 mg/kg bid for 7–14 days, followed by 5 mg/kg daily until the end of maintenance *and* seronegative or filtered blood products	Prophylaxis at engraftment is not recommended because of the low incidence of posttransplantation infection.
Seronegative recipient with seronegative donor	Seronegative or filtered blood products	
Autologous Transplant		
Seropositive recipient	Early ganciclovir induction of subclinical viremia, 5 mg/kg ganciclovir bid for 7 days, followed by 5 mg/kg daily for 14 days of maintenance	Because of the very low risk in some settings, monitoring is not uniformly advocated.
Seronegative recipient	Seronegative or filtered blood products	
Treatment of Disease		
CMV pneumonitis	Ganciclovir induction, 5 mg/kg bid for 14–21 days, followed by 5 mg/kg daily for at least 3–4 wk of maintenance *plus* IVIG every other day for the duration of induction	Extended maintenance throughout periods of severe immunosuppression (i.e., GVHD treatment) may be considered.
Gastrointestinal disease	Ganciclovir induction, 5 mg/kg bid for 14–21 days, followed by 5 mg/kg daily for at least 3–4 wk of maintenance	If deep ulcerations are present, maintenance may be required for a longer time.
Marrow failure	Foscarnet, 90 mg/kg bid for 14 days, followed by 90 mg/kg daily for 2 wk *plus* G-CSF	Ganciclovir plus IVIG has also been used.
Retinitis	Ganciclovir, 5 mg/kg bid for 14–21 days, followed by 5 mg/kg daily for at least 3–4 wk	Extended maintenance may be required.

[a]Regimens should be accompanied by weekly monitoring with antigenemia or PCR-based nucleic acid testing.
[b]Oral 900-mg doses of valganciclovir produce blood levels that are similar to those for the standard intravenous dose (5 mg/kg) of ganciclovir. Foscarnet and cidofovir are acceptable alternatives. Renal dose adjustment is required for all antiviral agents.
[c]Foscarnet, high-dose acyclovir, or valacyclovir are acceptable alternatives. Renal dose adjustment required for all antiviral agents.
bid, Twice daily; *CMV,* cytomegalovirus; *G-CSF,* granulocyte colony-stimulating factor; *GVHD,* graft-versus-host disease; *HSCT,* hematopoietic stem cell transplantation; *IVIG,* intravenous immune globulin; *PCR,* polymerase chain reaction.

be used to differentiate CMV pneumonitis from pulmonary shedding.[240] Among patients with CMV pneumonitis, the mortality rate is as high as 50%, even when prompt antiviral treatment is combined with intravenous immune globulin (IVIG) or CMV-specific immune globulin.[241] CMV viremia and pneumonitis are rare before engraftment.[242,243] Anorexia, nausea, vomiting, and sometimes diarrhea characterize CMV gastroenteritis; the diagnosis is made by endoscopy and biopsy with immunoperoxidase staining of CMV-infected cells.[244] CMV disease of the GIT is often associated with GVHD of the specific organ.[244] Response to therapy is not ensured, even with ganciclovir, but CMV GI disease is not strongly associated with increased nonrelapse mortality.[245] Although CMV retinitis is common among patients infected with HIV, it is quite uncommon among HSCT recipients.[246]

Once an end organ has established disease from CMV, the infection is difficult to treat. CMV pneumonitis is treated with a combination of ganciclovir at induction doses for 14 to 21 days and IVIG (500 mg/kg every other day for the duration of induction, 14–21 days) and then with maintenance ganciclovir. Standard IVIG is generally used because CMV-specific immune globulin is scarce and costly and has not been shown to improve outcome. CMV enteritis is treated with ganciclovir at induction doses for 3 weeks or longer.[247] Treatment of protracted CMV enteritis might include ganciclovir and a longer duration of ganciclovir maintenance therapy to facilitate GI healing.[244]

Development of a CMV-specific cytotoxic T-lymphocyte response is critical for the reconstitution of normal immunity and protection from late CMV disease.[248] Long-term IVIG delays recovery of CMV immunity. For patients who remain at risk for late disease, CMV monitoring should be continued beyond day 100. Patients who are treated with acyclovir followed by ganciclovir or those treated with serial ganciclovir courses may be at increased risk for developing genotypic resistance. Clinical resistance episodes should be treated with foscarnet (or cidofovir) until genotype assays for mutations in the *UL97* or *UL54* gene are able to confirm virologic resistance.[249] An illustrative case in the development of drug resistance identifies the donor-negative/recipient-positive setting, the prolonged period to development of a CMV-specific response, and GVHD with extended immunosuppression as risks.[250] Extended ganciclovir therapy appears to delay recovery of cytotoxic T-lymphocyte (CTL) activity, either by a direct effect on lymphocytes or by limitation of the amount of antigen exposure to lymphocytes. CMV-specific cytotoxic T cells were used in 50 patients and reduced the need for pharmacotherapy (17% vs. 36%, $P = .01$) and in the total number of treatment days in the cohort receiving CTL (3.4 days vs. 8.9 days, $P = .03$), but this treatment did not reduce CMV reactivation rates. There was also no increase in GVHD.[251] Currently, CMV-specific T cells from third parties are being evaluated for CMV and EBV prevention or treatment in the HSCT setting.[252,253] Brincidofovir, CMX001, an oral bioavailable lipid acyclic nucleoside phosphonate, significantly reduced the incidence of CMV events at a dose of 100 mg twice weekly compared with placebo (10% vs. 37%, $P = .002$) in a randomized HSCT study of CMV seropositive patients.[254]

Varicella-Zoster Virus

VZV infection is a primary occurrence (5% of occurrences) or a reactivation (95%) in 40% of patients at any time in the first year after transplantation.[255,256] Conditioning regimen intensity may not be important regarding VZV reactivation.[257] VZV can be effectively prevented with acyclovir prophylaxis (800 mg twice a day orally),[258] but VZV prophylaxis is not used at all transplantation centers because only 30% to 50% of adult patients and 25% of pediatric patients develop this infection during the first year after transplantation. The median time of onset is 5 months after transplantation. Prolonged antiviral prophylaxis may delay the onset of VZV but is not associated with rebound VZV.[258] Localized zoster may manifest atypically with a few vesicles, or skin lesions may

appear as atypical vesicles; therefore laboratory confirmation of VZV reactivation is recommended.

Manifestations of VZV disease are most often dermatomal shingles but may include hemorrhagic pneumonia, hepatitis, abdominal pain, central nervous system (CNS) disease, thrombocytopenia, and retinal necrosis.[259–263] Disseminated or visceral varicelliform zoster may manifest as low back pain or acute abdominal pain before the appearance of skin lesions. GVHD is a strong predisposing factor for VZV dissemination. Visceral varicella-zoster infections are rare but can be fatal.[264] Most fatal cases of disseminated or abdominal zoster occur in patients who were treated with suboptimal doses of acyclovir or for whom therapy was initiated relatively late in the course of infection. High-dose acyclovir (10 mg/kg IV every 8 hours) has been the treatment of choice for disseminated VZV infection. Valacyclovir and famciclovir can be used as step-down treatment after IV acyclovir or as initial treatment of localized infection. Although uncommon, patients who are already seropositive may acquire a second primary VZV infection. VZV vaccination is recommended at the 2-year anniversary visit for patients who have been free of immunosuppressive medications for several months, unless the underlying hematologic or oncologic disease is in relapse.[265,266] The vaccine used should be the lower-plaque-forming unit version (Varivax) used for prevention of chickenpox among children, not the higher-titer (Zostavax) vaccine used for immunocompetent older adults.

VZV is a fastidious virus and may not withstand the time required to transport the specimen to the diagnostic laboratory. By scraping the base of a vesicle and examining the cells by DFA with VZV-specific monoclonal antibodies, clinicians can best diagnose lesions of herpes zoster and chickenpox. The Tzanck smear is less sensitive and is no longer recommended. Tissue diagnosis can be made through histologic examination, immunohistochemical techniques, or culture. PCR assay, if available, is the laboratory technique of choice.

When a VZV-seronegative patient receives a significant exposure to a person with active or incubating chickenpox, a course of acyclovir with or without varicella-zoster immune globulin (VariZIG) is recommended to prevent chickenpox.[267] Acyclovir or valacyclovir for 3 to 22 days after exposure seems appropriate, based on institutional experience and extrapolation from the treatment of VZV disease. For a VZV-seropositive patient living in the same dwelling as someone with an index case of active chickenpox or shingles, acyclovir is reported to be useful in preventing new infection. VariZIG is usually not given to exposed seropositive patients, but antiviral prophylaxis is often recommended, depending on the exposure, the length of time since the transplantation, and the level of immunosuppression. For lower-risk seropositive patients, acyclovir or valacyclovir may be appropriate, although the efficacy in this situation is unknown. Patients already receiving empirical ganciclovir for CMV reactivation do not need further antiviral agents.

Adenovirus

Adenovirus infection reactivates in approximately 12% of allogeneic and approximately 6% of autologous adult HSCT patients.[268,269] Chronic shedding can occur in the absence of clinical disease, but adenovirus can also be acquired from respiratory droplet transmission.[270] In its most common clinical manifestation in this setting, adenovirus is a cause of hemorrhagic cystitis.[41] Systemic infection in the lungs, liver, GIT, and kidneys occurs in 18% to 20% of infected patients. GVHD is a risk factor for the occurrence of clinically apparent adenovirus infection after HSCT.[271] In addition, allogeneic patients who do not receive ganciclovir (seronegative for CMV or seropositive without need for ganciclovir) are at higher risk for developing adenovirus infection than are patients who did receive ganciclovir, even though ganciclovir has no activity against adenovirus.[268] Alternative grafts, mismatched HSCT, T-cell depletion, lymphopenia, and GVHD were found to be risk factors.[272]

Immunofluorescence, shell vial, or conventional tube culture of blood, urine, stool, or tissue can be used to diagnose adenovirus. PCR testing is helpful in diagnosis of infection but in general is not cost-effective for surveillance because many patients are asymptomatic.[273,274] Adenovirus viral load can be used as a valid surrogate end point for disease severity and prognosis.[275] Given that no effective therapy is available for adenoviral infections, although cidofovir or the lipid-conjugated derivative brincidofovir (CMX001) have been used in patients able to tolerate the potential toxicities.[276,277] Some centers use quantitative viral load monitoring, followed by preemptive treatment[278] that might be correlated with faster virologic clearance and improved outcomes when started at a lower adenovirus viral load.[272] Brincidofovir, with less nephrotoxicity, may have shown effectiveness even when cidofovir failed patients in case reports.[275,279] Adenovirus-specific cytotoxic T cells are becoming available, but the generation of virus-specific T cells can be costly and time consuming. Newer techniques allowed generation of donor-derived adenovirus specific T cells within 2 weeks, and in first in-human studies these cells cleared cidofovir-resistant adenovirus (i.e., increasing titers) in a few patients.[280] Third-party prepared "off-the-shelves" products showed promising safety and efficacy profile.[253,281]

Norovirus

New diagnostic testing of stool has found norovirus to be a frequent cause of diarrhea in stem cell transplant recipients.[282] Crampy abdominal pain, nausea, or vomiting may accompany the diarrhea. Duration of diarrhea can be quite prolonged, even months, and create diagnostic confusion with GVHD or *C. difficile* diarrhea. Virus shedding may persist after resolution of symptoms.[283] Because of the low inoculum needed to cause disease and the known persistence of norovirus on surfaces, some facilities with a known norovirus problem have required gloves for care providers and hand washing, not just hand cleanser, of persons exiting the norovirus patient's room.[284]

Respiratory Viruses

Patients who have undergone HSCT and develop a respiratory viral infection typically present with rhinorrhea and nasal congestion and may also have fever, cough, throat pain, headache, or myalgias.[285] The common pathogens in such patients include respiratory syncytial virus (RSV), parainfluenza virus, and, to a lesser extent, influenza virus, rhinovirus, human bocavirus, human metapneumovirus, and coronavirus.[285–296] Current rapid test methods allow detection of RSV, parainfluenza, influenza, and other viruses in respiratory specimens within 48 hours (see Chapter 16). Respiratory virus infections commonly occur during the winter season and cause pneumonia in up to 50% of patients, although progression to lower tract infection is less common with NMA conditioning regimens.[289] In contrast, parainfluenza virus type 3 infections and rhinovirus may occur throughout the year[293] and nosocomial outbreaks of RSV have occurred at other times than the established winter season. Influenza, most often type A, infrequently progresses to pneumonia. Current diagnostic strategies that use commercially available multiplex pathogen panels will detect, but not distinguish, human rhinovirus from an emerging pathogen, such as enterovirus D68, an increasingly important pathogen in HSCT recipients.[297] Prophylactic or early initiation of oseltamivir therapy for upper respiratory tract disease may prevent progression to lower respiratory tract disease.[294] In a recent study of high-risk patients (e.g., most of them underwent alternative donor or HLA-mismatched donor HSCT), coronavirus was not rare (23%) and not benign (e.g., about one-third of patients progressed to LRTI, 19% were hospitalized, and 5% died).[298]

RSV and parainfluenza are associated with a high incidence of progression from upper to lower tract disease among infected patients. Upper respiratory tract illness with parainfluenza usually resolves without serious sequelae. LRTI has a mortality rate of 80% for RSV and 30% to 35% for parainfluenza virus.[287,299,300] Lymphopenia is a risk factor for progression to LRTI.[301] Therapy with oral or aerosolized ribavirin or a combination of ribavirin and IVIG has been used for RSV LRTI and seems to decrease lung-related mortality,[302] although rigorous trials to determine its efficacy are not available.[285,303] Oral ribavirin is used in patients with upper respiratory tract infections who are at risk for progression to LRTI.[304,305] The survival rate appears to be higher when treatment is initiated before significant hypoxia is present, and ribavirin may help to decrease the viral burden.[303,306] There are only anecdotal case reports regarding the effectiveness of ribavirin for treatment of other respiratory viruses, including parainfluenza, adenovirus, and influenza. A meta-analysis found no decrease in mortality of LRTI with ribavirin for parainfluenza infections.[307] Preemptive therapy with ribavirin in patients with positive nasopharyngeal cultures for RSV appears

promising. Patients who develop respiratory viral pneumonia before engraftment have poorer outcomes. Aerosolized ribavirin (Virazole) is administered via facemask to adults through a small-particle aerosol generator. Contamination of the patient's room with this possibly teratogenic agent is of concern to pregnant hospital staff (see Chapter 45). The inconvenience, lack of efficacy data, difficult-to-interpret indications, and enormous cost of aerosolized ribavirin have led many institutions to adopt oral ribavirin as an alternative.[308,309]

Prevention of exposure is critical because treatment is not very effective. Protection involves the use of frequent hand washing by hospital staff and droplet isolation of patients with colds or respiratory tract symptoms. In addition, family members and health care workers with upper respiratory tract symptoms should be separated from patients. Vaccination of family members, health care workers, and other close contacts against influenza may help control exposures. Amantadine or rimantadine prophylaxis has limited usefulness because of the widespread development of resistance. Oseltamivir or inhaled zanamivir provides useful prophylaxis against both influenza A and B especially within 48 hours of exposure and appeared useful when used in a housing facility for HSCT recipients.[310] Immune globulin prophylaxis with RSV-specific polyclonal or monoclonal antibody, which is useful in infants at high risk, has not been sufficiently evaluated in the HSCT setting.[311] During the respiratory virus season, all patients with respiratory symptoms should have a sample taken from the nasopharynx to be evaluated for respiratory viruses.

Epstein-Barr Virus

The majority of Epstein-Barr virus (EBV) reactivation is subclinical and requires no therapy. UCB transplants, aGVHD, HLA-mismatched HSCT, and T-cell–depleted HSCT perhaps lead to prolonged T-cell lymphopenia and augment the risk for EBV reactivation.[312–315] The findings that the detection of EBV DNA in peripheral blood lymphocytes by PCR assay is highly correlated with EBV-related transplantation lymphoproliferative disorder (PTLD)[316] and that rituximab is effective in EBV-related PTLD[314] led to investigate whether preemptive treatment is effective. Quantitative diagnostic monitoring of EBV viral load accompanied by preemptive therapy may reduce the risk for progression to life-threatening PTLD.[317] In most cases, high viral loads are associated with progression to PTLD.[318] Infusions of rituximab or nonirradiated donor leukocytes may be effective treatment for allograft recipients with high-titer EBV viremia or PTLD.[312,319,320] However, methods, sampling, and the threshold to start preemptive rituximab treatment remain to be standardized.[321,322]

Human Herpesvirus 6

HHV-6 can reactivate after HSCT from the latent state in organs/cells (latent in organs/cells such as brain, kidneys, salivary glands, T-lymphocytes, BM progenitor cells), as can other herpesviruses, and probably cause fever, rash, BM suppression, fatal meningoencephalitis, and interstitial pneumonitis in less than 2% of HSCT patients.[323] A distinctive limbic encephalitis from HHV-6 can cause confusion, memory loss, hallucinations, seizures, and even status epilepticus.[324–326] Magnetic resonance imaging shows bilateral signal change in the medial temporal lobe, which may be more prominent in the week(s) after clinical presentation. HHV-6 is found in the cerebrospinal fluid (CSF) and blood.[324] In patients clinically suspected for CNS infection, HHV-6 positivity in CNS should be considered as active infection regardless of blood test results. Severe hyponatremia, caused by a syndrome of inappropriate secretion of antidiuretic hormone, developed in patients with CNS infection.[325–327] IV foscarnet is recommended. Permanent neurologic sequelae can occur.[328] Recipients of UCB may have more viremia than do other populations.[329,330] HHV-6 appears to reactivate commonly, occurring in 46% of HSCT patients according to culture diagnosis and as many as 100% of patients according to PCR assay of blood.[331] Many episodes of reactivation detected by DNA PCR may be asymptomatic, and the value of therapy for subclinical viremia and hence surveillance is unknown.[332] It may also represent latent infection.[333] High-level HHV-6 viremia is associated with aGVHD and nonrelapse mortality.[334] Most strains of HHV-6 identified after HSCT appear to be caused by the B variant in blood or urine, although the A variant has been correlated with pneumonitis.[335] HHV-6 has greater than 60% DNA homology with CMV, and treatment of documented infection is usually initiated with induction doses of foscarnet. Responses to antiviral therapy are not universal, and benefits of antiviral therapy have not been rigorously determined.[336] Third-party virus-specific T cells seem promising in HHV-6 and BK virus treatment.[253]

Parvovirus

Parvovirus B19 (human parvovirus [HPV] B19) is a rare cause of refractory anemia with erythroid hypoplasia or BM after HSCT.[337–340] Skin rash confusion with GVHD rash has been reported.[341] Asymptomatic patients should not be tested.[342] Antibody or PCR tests detect parvovirus, although PCR assay may yield positive findings for months after the acute infection. Use of single-patient rooms on HSCT wards may be preventing transmission of this contagious virus to other patients undergoing HSCT, and the administration of IVIG for other reasons may be treating subclinical infections (see Chapter 147). In a study, 21 (1%) of 2123 blood products tested positive for the presence of HPV B19 DNA-transfused immunocompromised hematology patients, including HSCT patients. Fourteen patients received these positive blood products but did not have symptomatic infections.[343]

Fungal Infections

Invasive fungal infections are important causes of morbidity and mortality. The major causes of invasive fungal disease include *Candida* spp., *Aspergillus* spp., and, less frequently, the non-*Aspergillus* filamentous molds. Patients undergoing allogeneic transplantation are at 10-fold increased risk for invasive fungal infection compared with patients receiving an autologous graft. Systemic fluconazole prophylaxis (at least 200 mg/day) or low-dose amphotericin B (0.1–0.3 mg/kg daily) can decrease the incidence of systemic candidiasis.[111–113,344,345] Advanced-generation azoles and micafungin prophylaxis extend the spectrum of organisms covered to include molds, but their general value or cost-effectiveness for all allograft recipients has not been shown.[346] Empirical therapy for febrile neutropenic patients is discussed in Chapter 306. As prophylaxis with triazoles has become more prevalent, awareness of changes in the epidemiology of fungal infections is necessary. In a report of transplants from 2002–11, *Aspergillus* (31 episodes) and candidemia (15 episodes) were most common, but 16 episodes were caused by Mucorales and other uncommon pathogens, including yeasts such as *Trichosporon* and unidentified molds.[347]

Pneumocystis

P. jirovecii infection[348] usually manifests as pneumonia with dyspnea, cough, fever, and bilateral infiltrates in the majority of infected patients.[124] It can occur after both autologous and allogeneic transplantation, although the frequency is lower for the former. (1→3)-β-D-glucan in serum or BAL can be useful in diagnosis,[349,350] although microscopic evaluation of BAL is the gold standard. Before the use of routine prophylaxis, *Pneumocystis* infection occurred in approximately 7% of patients who underwent allogeneic HSCT, at a median of 1 to 3 months after transplantation, and was associated with a 5% risk for death.[351,352] A large survey of CIBMTR data from 1995–2005 found rates or 0.63% and 0.28% in allogeneic and autologous transplant recipients, respectively. This low incidence was accompanied by a significantly higher risk of death.[353] Prophylaxis with TMP-SMX has resulted in negligible rates of infection. For patients who do not tolerate medications containing sulfa, prophylaxis options include desensitization with TMP-SMX and use of dapsone,[128,354] atovaquone,[355] and inhaled pentamidine.[125,356] The treatment of choice for *P. jirovecii* infection is TMP-SMX.[124,126]

Candida

Candidiasis is an infection acquired from endogenous organisms colonizing the GIT; it usually manifests as fungemia or visceral candidiasis (see Chapter 256). Before fluconazole prophylaxis, the onset of candidiasis occurred at a median of 2 to 3 weeks after transplantation, and *Candida* spp. were second in frequency to *Aspergillus* spp. as the cause of brain abscess after HSCT.[357,358] The current cumulative incidence rate of invasive candidiasis during the first year after HSCT is probably less

than 5%.[359] Risk factors for invasive candidiasis include neutropenia, breakdown of the normal mucosal barriers, central venous catheter, and the use of broad-spectrum antibiotics or corticosteroids. *C. albicans* infections are successfully prevented when fluconazole is given as prophylaxis from the time of conditioning until either engraftment or day 75 after HSCT. The strategy of prolonged fluconazole therapy has been associated with improved survival rates, although the mechanism of the observed benefits is uncertain.[113,360,361] The benefit of fluconazole prophylaxis is less clear for autologous transplants, for which the degree of mucositis is less.

With the use of fluconazole since the 1990s, the number of *Candida* infections has decreased.[344] The spectrum of colonizing and infecting *Candida* organisms has shifted from *C. albicans* and *Candida tropicalis* to include *C. krusei, C. glabrata,* and *Candida parapsilosis.*[116] *C. krusei* is innately resistant to fluconazole. *Candida auris* is an emerging candidal species that is often multidrug resistant and is difficult to identify with standard laboratory methods.[362]

Aspergillus

Aspergillus and other mold infections are acquired exogenously, by inhalation of spores into the respiratory tract from the environment and in some localities may occur with higher frequency during the summer.[122] Common sites of initial infection include the lung and sinuses, although contiguous or hematogenous extension to the CNS or other internal organs may occur. With the use of fluconazole prophylaxis during the preengraftment period, invasive aspergillosis emerged as the leading fungal infection found at autopsy among patients who underwent HSCT.[344] Preengraftment prophylaxis with mold-active therapy is now being used for patients who may have *Aspergillus* incubating at the time of transplantation owing to prior infection or prolonged neutropenia.[114,115,363] Postengraftment prophylaxis with posaconazole or voriconazole has led to a decrease in mortality among patients with a high risk for invasive aspergillosis.[114,123,364] A CIBMTR analysis showed that prior invasive fungal infections (mostly *Aspergillus* and *Candida* infections) were not absolute contraindications for allogeneic HSCT, but patients with invasive fungal infections before transplantation did have a higher rate of invasive fungal infection at 1 year after transplant (24% vs. 17% control subjects, $P < .001$) and a higher rate of nonrelapse mortality (relative risk, 1.27 [1.09–1.49], $P = .002$).[365]

The incidence of invasive aspergillosis among patients undergoing HSCT ranges from 4% to 15%.[122,366,367] The onset of *Aspergillus* infection after HSCT occurs in a bimodal distribution, with the first peak at 2 to 3 weeks (during neutropenia) and the second at 3 to 4 months after HSCT, usually in conjunction with persisting GVHD.[122] Postengraftment aspergillosis can occur after 6 months, again alongside cGVHD but also with CMV. Older age is associated with the acquisition of aspergillosis during either the preengraftment or postengraftment risk periods. Donor type, male gender, and summer season are recognized risk factors for preengraftment aspergillosis, whereas construction in the vicinity of the hospital, GVHD and attendant corticosteroid therapy, lymphopenia, CMV, respiratory virus infection, and multiple myeloma are significant risk factors for the development of postengraftment aspergillosis.[122,368] Early aspergillosis is temporally associated with neutropenia; therefore infection among autologous HSCT patients is rare after engraftment. The estimated 1-year survival rate among patients with proven invasive aspergillosis is 7% to 30%, although more aggressive, prolonged, or combination antifungal therapies may be improving these outcomes.

Preventive strategies focus on reducing both environmental and host risk factors. The use of HEPA-filtered air systems or laminar airflow rooms during the preengraftment risk period aids in the prevention of infection, particularly for allograft recipients. HEPA filters are capable of removing particles greater than 0.2 μm in diameter, such as mold spores. The patient's room is continuously maintained at positive pressure in relation to the corridor, which enhances the barrier effect. For transport out of HEPA-filtered rooms or after discharge, tight-fitting facemasks reproduce this barrier and are sometimes used, at least for the early post-HSCT period.

Patients might ask whether portable HEPA filters should be purchased for use after the hospitalization. This extra measure can be implemented on an individual basis. Units can be obtained for each of the rooms that the patient will occupy during the day and night, and each unit is sized for the room it will be placed in. There is no evidence of the clinical efficacy of these filters out of the hospital setting in preventing acquisition of airborne mold infections. For outpatients they are probably of little value and of considerable expense.

Other prevention strategies, including nasal and aerosolized amphotericin B, have not been studied in controlled trials.

The availability of accurate early diagnostic tests for invasive fungal infections lags behind those for other types of infections. The *Aspergillus* galactomannan test is most useful for patients not already taking antifungal therapy that includes coverage for molds, which is a minority of high-risk allogeneic recipients.[369] Other antigen- and nucleic acid–based diagnostic tests have been studied for early diagnosis of invasive tissue mold infection, but they have not demonstrated usefulness in clinical practice. Most have not been tested in large numbers of clinical samples from HSCT recipients. Blood cultures for molds rarely yield positive findings of mold organisms, except in the case of *Fusarium*.

A high index of suspicion in persistently febrile neutropenic patients and timely computed tomography of the chest to detect new infiltrates are important for early detection of invasive pulmonary aspergillosis. A small ground-glass halo around the lung lesion or pleura-based or nodular infiltrates on computed tomographic scans are highly suggestive of aspergillosis or other mold infection in a neutropenic host.[118] Bronchoscopy with cytologic examination and culture of lavage fluid for fungi, as well as other organisms common to immunocompromised hosts, is important. Galactomannan assay of the BAL fluid may have augmented diagnostic value. A lack of clinical or radiographic response during empirical antifungal therapy may necessitate therapeutic drug level monitoring and/or diagnostic tissue sampling.[370] Minimally invasive surgery (video-assisted thoracoscopic surgery) is associated with less morbidity than is open-lung biopsy.

Patients who have undergone HSCT and have suspected invasive mold infections should promptly begin taking a mold-active antifungal agent while diagnostic procedures are being arranged. A lack of clinical or radiographic response during proven infection may necessitate a switch to an agent from a different class or to combination therapy. Combination treatment of fungal infections with echinocandins, azoles, and polyene agents is common, whereas results of a large randomized clinical trial failed to show a benefit of anidulafungin added to voriconazole.[371] Echinocandin agents might be fungistatic rather than fungicidal in the case of mold infections because their interruption of cell wall synthesis is limited to actively growing hyphae. Echinocandins are not preferred for primary therapy for invasive aspergillosis but are used in salvage or combination therapy.

For documented invasive tissue mold infection, therapy is usually continued until some weeks (4–6) after lesions are resolved or stable, immunocompetence has improved, and the patient is afebrile. Although amphotericin B had been the gold standard antifungal agent since the 1960s, voriconazole produced superior outcomes in treatment for aspergillosis in 53% of patients, in contrast to 32% of patients treated with amphotericin B, followed by other antifungal therapy.[372] Treatment of CNS mold infections should include voriconazole, which, on the basis of a few patients studied, attains CSF levels approximately 50% of plasma levels and CNS tissue levels approximately 200% of plasma levels (see Chapter 40A). In a randomized study, isavuconazole was noninferior to voriconazole (with fewer adverse effects) for the primary treatment of suspected invasive mold infections in 527 patients with hematologic disorders, including those undergoing allogeneic HSCT.[373] All-cause mortality at day 42 in these patients was similar (19% with isavuconazole vs. 20% with voriconazole).

After initial control of an *Aspergillus* infection, subsequent maintenance therapy for the duration of immunosuppression has been advocated to reduce the risk for reactivation. Multiple drug-drug interactions occur with the azoles, and adjustments may be required for immunosuppressive agents. Transient visual disturbances or hallucinations can occur with voriconazole. Difficulty in achieving therapeutic plasma drug levels complicates the administration of itraconazole and posaconazole. Itraconazole solution has improved oral bioavailability over the capsule and can be used, although blood level monitoring may be needed to ensure adequate absorption. Posaconazole (delayed-release version) and

isavuconazole have good antimold activity but are new to the market and may be expensive.

Aspergillosis of the CNS most frequently occurs among patients who have had refractory pulmonary disease despite antifungal treatment and has an associated poor prognosis; survival is approximately 24%.[374]

Concerns have arisen, predominantly in Europe, about azole resistance in *Aspergillus fumigatus*. As HSCT patients are at high risk, if these developments become generalized, an organized approach to mitigating risk might include intensified clinical awareness, antifungal susceptibility testing, or molecular diagnostics and genotyping.[375,376]

Other Yeasts

Malassezia furfur causes tinea versicolor, catheter-related fungemia, and sometimes pneumonia.[377–379] Response to either topical or systemic therapy is slow; recovery of granulocyte counts is usually associated with resolution.[378] Catheter removal and discontinuation of IV lipids are important for a successful outcome in cases of fungemia. Trichosporonosis has manifested as fungemia, skin lesions, pneumonitis, and arthritis.[380,381] Fungemia, usually acquired via an IV catheter, has been reported with *Trichosporon* and *Rhodotorula* spp., *Cryptococcus laurentii,* and *Hansenula anomala.* Meningitis with *Cryptococcus neoformans,* perhaps due to delayed recovery of CD4 cells and inverted CD4:CD8 ratio,[382] is unusual in contrast to its frequent occurrence among patients infected with HIV. Widespread anti-*Candida* prophylaxis with fluconazole may contribute to the low frequency of these infections.

Other Molds

Non-*Aspergillus* molds are rare but lead to high mortality.[383] Non-*Aspergillus* molds, such as *Alternaria, Pseudallescheria/Scedosporium, Paecilomyces, Fusarium,* and *Phialophora* spp., are infrequent causes of invasive tissue infections whose clinical appearance is similar to that of *Aspergillus* infection. They are indistinguishable from *Aspergillus* hyphae in tissue sections; thus culture is required for identification. Disseminated fusariosis, most common within 100 days after allogenic HCST and associated with neutropenia, is a highly fatal infection for patients who have undergone HSCT, manifesting as positive blood cultures, skin lesions, or endophthalmitis.[383–385] Whereas UCB HSCT might be associated with a higher incidence, more recent transplants (those transplanted after 2002) had a lower incidence.[383] Successful resolution is usually associated with neutrophil recovery in addition to antifungal therapy.[385] In the case of fusarial endophthalmitis, enucleation of the affected eye may be required.[386]

Mucormycosis is also uncommon after HSCT (allogeneic, 0.58%, and autologous, <0.1%, at 1 year), but it mimics aspergillosis clinically and may occur long after HSCT.[387–389] The cause of one hepatic infection included over-the-counter herbal medication.[390] Acute or cGVHD, steroid use, diabetes, advanced disease at the time of HSCT, and older age are risk factors for mucormycosis.[383] Patients receiving voriconazole prophylaxis are at risk for breakthrough infection with invasive mucormycosis, but the risk for such breakthrough infections appears low.[391–394] Posaconazole may be an effective maintenance treatment for infections caused by certain species of Mucorales after response to amphotericin B.[395] Isavuconazole is FDA approved for the treatment of invasive mucormycosis in addition to treatment of invasive aspergillosis, but the approval is controversial because of the small number of patients: 21 with initial therapy and 16 with salvage therapy.[396]

Clinically significant infections caused by the dimorphic fungi, including coccidioidomycosis, histoplasmosis, and blastomycosis, are unusual even in hyperendemic areas of the United States. A review of the incidence of coccidioidomycosis in HSCT recipients in an endemic area from 2003–13 revealed an overall incidence of 2.3% (11/426), with 8 (73%) being de novo infection, and an overall mortality of 55%.[397]

Parasitic Infections

Parasitic infection after HSCT usually manifests as reactivation of toxoplasmosis (mostly reported in United States and Europe),[398] although Chagas disease (Latin America),[399–403] malaria (Africa),[404–409] strongyloidiasis,[410] schistosomiasis,[411] *Clonorchis* infection,[412] giardiasis,[413] cryptosporidiosis,[414–417] pulmonary microsporidiosis (now classified as fungus),[418–420] leishmaniasis (Mediterranean countries, Latin America), and *Acanthamoeba* and *Trichomonas* meningoencephalitis[421–424] have also been reported. Routine blood smears before HSCT cannot be used to rule out malarial transmission. In Hong Kong, *Clonorchis sinensis* infection was identified in only 1% of screening stool examinations performed 7 days before HSCT.[412] None of the patients had symptoms related to clonorchiasis; patients received praziquantel (25 mg/kg orally three times for 1 day) before HSCT, and subsequent stool examinations did not reveal the presence of ova.

Toxoplasmosis is infrequent after HSCT, occurring in 2% to 7% of patients who are seropositive before transplantation.[425–428] Although the parasite can be transmitted as a primary infection through marrow, blood products, or donor solid organs, toxoplasmosis in patients who have undergone HSCT is mostly the result of reactivation of prior infection.[429] GVHD is a risk factor for the suppression of cell-mediated immunity that is critical for host defense against *Toxoplasma gondii*.[430] The clinical presentation includes fever, headaches, encephalitis with focal cerebral lesions, meningitis, pneumonitis, myocarditis, chorioretinitis, pancytopenia, hepatosplenomegaly, or lymphadenopathy.[431] Most cases of toxoplasmosis occur within 100 days after HCST but also reactivate when *Pneumocystis* prophylaxis is complete or interrupted. Immunosuppression can affect the enhancement of CNS lesions. Parasitemia is a feature of reactivation that may be identified in tissue culture, although many diagnoses are now made with PCR assay.[432,433] In countries where the prevalence of this infection is high, pretransplant detection of DNA using PCR assay may indicate those at highest risk for infection after transplant.[434] Stereotactic brain biopsy is also useful in diagnosis of the infection.[435] Mild increase in protein or mononuclear cells in CSF is common, and detecting DNA of *Toxoplasma* is highly specific but less sensitive for infection. Morphologically seeing tachyzoites is very rare. The identifiable risk period is 2 to 8 weeks after HSCT, and toxoplasmosis is more common among those receiving myeloablative conditioning.[436] Seropositive patients not receiving TMP-SMX (160 mg of TMP/800 mg of SMZ, three times a week) are at risk for breakthrough toxoplasmosis.[437] Pyrimethamine or atovaquone can be considered for prophylaxis. For these patients *Toxoplasma* reactivation can be monitored through PCR assay during the first 1 to 3 months after HSCT.[438] Serologic monitoring for seroconversion (emergence of specific IgA or IgM) or reactivation (>threefold increase in IgG) is not reliable and useful. However, in countries where the prevalence is low, routine prophylaxis is not justified. Treatment of infection includes long-term pyrimethamine and sulfadiazine (or clindamycin in intolerance) with folinic acid or high-dose TMP-SMZ. Avoiding contact with possible infected sources (e.g., animal-cat feces, undercooked meat, soil, and water) is important to prevent primary infection.[439]

METHODS OF IMMUNE SYSTEM RECONSTITUTION AFTER HSCT

Vaccination

Patients undergoing autologous or allogeneic HSCT eventually lose immunity to the common childhood diseases and should be serially reimmunized 6 and 24 months after transplantation (Table 307.4; see Fig. 307.1).[266,440] The efficacy of vaccination is influenced by the time elapsed since transplantation, the nature of the hematopoietic graft, the presence of GVHD, and the use of serial immunization.[441] There have been no reports of exacerbation of GVHD after immunization of patients who underwent HSCT. A national survey of HSCT immunization practices revealed that vaccines were underutilized and schedules for revaccination varied.[442] To ensure compliance, it is recommended that all transplant recipients be immunized on the same schedule, regardless of cell source.[443]

Patients who have undergone HSCT should receive all indicated nonlive vaccines, regardless of transplant type or presence of GVHD. Such patients should be revaccinated every 10 years with the combined tetanus-diphtheria-pertussis toxoid. Vaccination with acellular pertussis does not generate enough immunity, so a more effective vaccine is needed.[444] At 6 months they should also be immunized against polio by the inactivated intramuscular vaccine, *H. influenzae* type B, hepatitis B, and *S. pneumoniae.* If the patient was previously immunized, only

TABLE 307.4 Suggested Schedule for Vaccination After HSCT

VACCINE	TIME PERIOD FOR IMMUNIZATION AFTER HSCT		
	6–12 MONTHS	14 MONTHS	24 MONTHS
Inactivated Vaccines			
Diphtheria, tetanus, pertussis[444]	X	X	X
Haemophilus influenzae serotype B conjugate	X	X	X
Hepatitis B[76,445]	X	X	X
Pneumococcal	X (13-valent conjugated)		X[a] (13-valent conjugated)
Inactivated poliovirus	X	X	X
Influenza[446]	Lifelong, seasonal administration of inactivated vaccine, beginning before HSCT and resuming ≥6 mo after HSCT, is recommended		
Hepatitis A	Routine administration is not indicated. If hepatitis A vaccination is given, two doses, given 6–12 mo apart, are required		
Varicella recombinant (Shingrix)[450–452]	US Food and Drug Administration approved in 2017		
Meningococcal conjugate	Routine administration is not indicated		
Human papillomavirus[447]	Administration is recommended for men and women younger than 27 yr		
Rabies	Routine administration is not indicated; any decision to use should be individualized		
Live Replication-Competent Vaccines			
Measles-mumps-rubella	Administration is not indicated		X[a,b]
Varicella-zoster (Varivax)[449]	Administration is not indicated		X[a,b]
Yellow fever virus	Routine administration is not indicated; any decision to use should be individualized		

[a]Optional.
[b]In patients with no active graft-versus-host disease or immunosuppressive therapy.
HSCT, Hematopoietic stem cell transplantation.

one dose of hepatitis B vaccine should be given. Postvaccine titers of hepatitis B virus should be documented to ensure response and adequate protection, even when the vaccine is given at the specified time interval after HSCT.[445] At 2 years a second dose of pneumococcal vaccine is optional; it provides a second opportunity to vaccinate persons who failed to respond to the first dose, especially patients with cGVHD. Lifelong, seasonal administration of influenza vaccine should begin before HSCT and resume by 2 to 6 months after HSCT. Children younger than 9 years who are receiving influenza vaccination for the first time require two doses yearly. Adults do not benefit from receiving two influenza vaccine doses.[446] Influenza vaccine for HSCT ward employees, clinical health care workers, and household contacts may be especially necessary within the first year or for patients with ongoing GVHD, in whom protective responses may be impaired. For health care workers directly caring for HSCT recipients, the live-attenuated intranasal vaccine should be avoided to limit inadvertent exposure to patients. Vaccination with the quadrivalent human papillomavirus vaccine has not been studied but may be appropriate for male and female long-term transplant recipients ages 9 to 26 years and perhaps for women older than 26 years who are at risk for squamous intraepithelial lesions.[447,448]

Live-virus vaccines, such as measles-mumps-rubella (MMR) and varicella, should not be given to transplant recipients with active GVHD or ongoing immunosuppressive therapy; the first doses are given to transplant recipients more than 24 months after HSCT who are taking no immunosuppressive medications and are presumed immunocompetent. A second MMR dose should be given 6 to 12 months later; however, the benefit of a second dose in this population has not been evaluated.

Vaccination against varicella is desired. Often, vaccination with live-attenuated VZV vaccine (Varivax, not Zostavax) is used for VZV-seronegative patients who no longer require immunosuppressive therapy and are free of GVHD.[449] When varicella vaccination is given to persons older than 13 years, two doses, given 4 to 8 weeks apart, are required. Vaccination of HSCT recipients who have developed reactivation shingles after transplantation is not needed. Susceptible family members should receive VZV vaccine to minimize chickenpox exposure for VZV-seronegative transplant recipients. In 2017 a new nonlive vaccine was licensed for use (Shingrix).[450,451] Adoption of this vaccine will probably be forthcoming by many transplant centers.[452–454]

Routine administration of hepatitis A, meningococcal, and rabies vaccines is not indicated. Hepatitis A vaccine is recommended for transplant recipients with chronic liver disease, including hepatitis C infection or cGVHD, or who live in hepatitis A–endemic areas or in areas experiencing outbreaks. If given, hepatitis A vaccination requires two doses, given 6 to 12 months apart. For transplant recipients with potential occupational exposure to rabies, preexposure rabies vaccination should be delayed until at least 12 months, if not 24 months, after HSCT.

Immunoglobulin Replacement

The major defect in humoral immunity is the absence of specific antibody production. Antibody levels in the first year after HSCT are affected primarily by pretransplantation levels in the recipient and, to a lesser degree, in the donor.[455] Among patients with cGVHD, reduced production of opsonizing antibody and of all classes of IgG and IgA antibodies is seen.[456] This immunodeficiency is further complicated by poor splenic function and is associated with risks for recurrent pneumococcal infections and episodes of bronchitis or pneumonia. IVIG does not prevent infections when given weekly during the preengraftment or late risk periods, but it does reduce rates of septicemia and localized infection when given in the postengraftment risk period after transplantation.[457–459] It may modulate the severity of GVHD.[460] Replacement IVIG (200–500 mg/kg every 1–2 weeks) may be beneficial for patients with IgG levels less than 400 mg/dL; however, in one prospective trial routine use of IVIG delayed the recovery of antigen (viral)-specific immunity.[458]

The role of hyperimmune globulin for prevention of specific infections is less clear. High-titer CMV globulin for prevention of CMV infection and treatment of end-organ CMV disease has proved to be of clear benefit in comparison with IVIG. However, antiviral drugs are effective in providing protection against CMV disease. Therefore, because of its limited availability and cost considerations, the use of CMV-specific

globulin is minimal at most transplantation centers. Hyperimmune RSV globulin provided only a very modest increase in neutralizing antibody when given in the first 6 weeks after HSCT.[311] Hepatitis B, human rabies, and tetanus immune globulin should be used as needed in the event of exposures. VariZIG is a human polyclonal IgG commercially available for intramuscular or IV administration for patients at high risk within the first 10 days of exposure.[461] VariZIG administration may extend the varicella incubation period from 10 days to as much as 28 days, is expensive, and is not uniformly effective in preventing chickenpox in patients who have undergone HSCT.

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308 Infections in Solid-Organ Transplant Recipients

Nina Singh, Ghady Haidar, and Ajit P. Limaye

Advances in surgical techniques and immunosuppressive regimens have had a pivotal role in optimizing outcomes after transplantation. The introduction of cyclosporine in the 1980s and tacrolimus a decade later heralded the era of modern immunosuppression, and transplantation advanced from being a quasiexperimental procedure to an established and accepted modality of treatment for a wide range of end-organ diseases. Table 308.1 depicts the most recent data on graft and patient survival reported by the United Network for Organ Sharing (UNOS).[1] The best results are for living-related kidney transplantation; ≈99% of the recipients are alive, and 96% have functioning allograft 1 year after transplantation. The most significant progress may have been in liver transplantation, in which the 1-year survival rate before the use of cyclosporine was only 32% compared with 89% at present. Outcomes after lung transplantation continue to be limited by the development of chronic lung allograft dysfunction with 1-, 5-, and 10-year survival rates of 80%, 50%, and 20%, respectively in the current era.[1] Fewer than 150 intestinal transplantations are performed annually in the United States; however, 1-year patient survival rates at high-volume experienced centers approach 90%.

Improvements in graft and patient survival have been paralleled by decline in the number of deaths associated with infections (Fig. 308.1). Current data from the Organ Procurement and Transplant Network show that infection-related mortality within the past decade has continued to decline in all types of organ transplantations (see Fig. 308.1). Nevertheless, infections still account for ≈24% of the first-year deaths in kidney and heart transplant recipients and up to 34% in lung transplant recipients (Table 308.2). In liver transplant recipients approximately 32% of the mortality within the first posttransplantation year was infection associated.[2] Infections contribute to 10% to 13% of the deaths after 10 years in kidney, liver, and heart recipients and 25% in lung recipients (Table 308.3).

The clinical manifestations of infection are variable and depend on the infecting pathogen, prior immune status of the host, the type of transplantation, time elapsed since transplantation, and the intensity of pharmacologic immunosuppression (Table 308.4). With this complexity in mind, it is useful to address some general principles that may facilitate the approach to diagnosis, management, and understanding of infections after transplantation.

FUNDAMENTALS OF IMMUNOSUPPRESSIVE THERAPY

Among factors contributing to the occurrence of infections in transplant recipients, an obvious and probably the most consequential is iatrogenic immunosuppression. The effects of immunosuppressive agents have become more apparent as surgical techniques have improved, and antimicrobial prophylaxis has come to be used more widely. The goal is to optimize immunosuppressive regimen such that it prevents rejection but preserves antimicrobial immunity and minimizes long-term metabolic complications and the risk of malignancy.

Corticosteroids

Although inadequate as sole agents to sustain graft survival, corticosteroids remain a key component of most immunosuppressive regimens. Corticosteroids broadly inhibit immune responses, including innate inflammatory responses, phagocytic function, cellular immunity, and, to a lesser extent, antibody formation. In an effort to obviate undesirable side effects of corticosteroid therapy, more transplantation centers practice early withdrawal and use steroid-free regimens. Meta-analyses of trials of corticosteroid-free regimens in kidney and liver transplant recipients have not shown beneficial effect of corticosteroid reduction on overall infectious risk, but analysis of specific infectious outcomes in liver transplantation suggested that corticosteroid avoidance might reduce the risk for cytomegalovirus (CMV) infection and hepatitis C virus (HCV) recurrence.[2,3]

Antimetabolite Agents

The introduction of cytotoxic drugs, such as azathioprine, was a major advance in immunosuppression that made transplantation across human leukocyte antigen (HLA) barriers feasible. All cytotoxic drugs interfere with DNA synthesis, thereby suppressing the bone marrow and reducing peripheral blood cell counts. In addition to marrow suppression, azathioprine may cause pancreatitis, a reversible hepatitis, rash, and gastrointestinal (GI) disturbances. As such, use of azathioprine has decreased in the current era.

Mycophenolate mofetil, approved in 1995, is a cytotoxic drug with an antiproliferative effect on T and B lymphocytes and has replaced azathioprine in triple-drug regimens, comprising a calcineurin inhibitor agent and corticosteroids. Its use has been associated with lower rates of biopsy-proven rejection compared with azathioprine, with similar risk of infections. The main side effects of mycophenolate mofetil are myelosuppression and diarrhea.

Calcineurin Inhibitor Agents

The calcineurin inhibitor agent cyclosporine is a cyclic peptide derived from *Tolypocladium inflatum* and was approved for clinical use in 1983. It inhibits the production of cytokines, primarily interleukin-2 (IL-2), and consequently the generation of effector T cells or $CD4^+$ T cells (Fig. 308.2). Concentrations of the drug as low as 100 ng/mL effectively inhibit mixed lymphocyte reactions. Patients treated with cyclosporine alone for various autoimmune diseases show low rates of clinical infection, which demonstrates the importance of corticosteroids and other cofactors for infection in transplant recipients. Tacrolimus, approved in 1994, is a macrolide produced by *Streptomyces tsukubaensis*. Despite some differences, its mode of action is similar to that of cyclosporine in that it inhibits production of IL-2 and $CD4^+$ T cells. However, it is 10 to 100 times more potent than cyclosporine. Although a major component of the immunosuppressive effects of cyclosporine and tacrolimus is accounted for by the antagonism of calcineurin activity, tacrolimus also inhibits steps distal to calcineurin activation in the T-cell activation cascade.[4] As such, tacrolimus has the ability to reverse steroid-resistant allograft rejection, whereas cyclosporine is ineffective for the treatment of rejection. Clinical trials have demonstrated that tacrolimus-based immunosuppression results in lower rates of acute rejection and graft loss than cyclosporine-based therapy, particularly in kidney and liver transplant recipients, with no convincing evidence that it enhances the risk for infection.

Major adverse effects of calcineurin inhibitor agents are nephrotoxicity, thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, neurotoxicity, hypertension, and diabetic mellitus. In comparison with cyclosporine, tacrolimus is associated with less hypertension but higher rates of diabetes mellitus and neurotoxicity.

Mammalian Target of Rapamycin Inhibitors

Rapamycin, also known as sirolimus, was released in 1999. It is a macrolide antibiotic derived from *Streptomyces hygroscopicus*. Rapamycin

interferes with cell-cycle proliferation and blocks intracellular signaling mechanisms by inhibiting a regulatory kinase—mammalian target of rapamycin (mTOR) (see Fig. 308.2). Unlike cyclosporine and tacrolimus, mTOR inhibitors have no direct nephrotoxicity. They frequently cause hyperlipidemia and occasionally cause myelosuppression. They have also been linked to delayed wound healing, oral ulcerations, and a rare drug-induced interstitial pneumonitis that can mimic *Pneumocystis* pneumonia. Some data suggest that rapamycin use is associated with reduced rates of posttransplantation malignancy and CMV disease. Everolimus is a hydroxyethyl derivative of rapamycin that differs from sirolimus in its pharmacokinetics, but it has a similar side-effect profile and infectious risk.

TABLE 308.1 Overall Graft and Patient Survival Rates by Organ Transplanted

TYPE OF TRANSPLANT	NO. OF TRANSPLANTS	GRAFT SURVIVAL (%) (1 YR)	PATIENT SURVIVAL (%) (1 YR)
Kidney (living donor)	4348	98.8	99.1
Kidney (deceased donor)	13969	96.8	96.6
Kidney pancreas	798	98.7/91.6[a]	97.4
Heart	2573	91.7	92.0
Liver (living donor)	359	90.1	94.4
Liver (deceased donor)	6768	90.1	92.0
Intestine	141	78.0	83.7
Lung	1826	88.8	89.7
Heart-lung	15	73.3	73.3

[a]Graft survival for kidney was 98.7 and for pancreas was 91.6%, respectively.
Data depict 1-year survival for year 2015 transplants. Modified from US Organ Procurement and Transplantation Network data as of June 30, 2017. Washington, DC: US Department of Health and Human Services; 2017.

Biologic Agents

Although calcineurin inhibitors have remained the mainstay of immunosuppression for more than 3 decades, long-term outcomes remain suboptimal largely due to renal dysfunction and metabolic complications from cumulative exposure to these agents. For example, chronic renal dysfunction develops in 7% to 21% of the organ transplant recipients and increases the risk of death by approximately fourfold. These concerns have spawned a growing interest in new regimens that enhance early immunologic tolerance, for instance, IL-2 receptor inhibitors (basiliximab) and T-cell–depleting antibodies (alemtuzumab or Thymoglobulin). These agents have delayed and reduced the incidence of acute rejection, but it is unclear if they have any impact on long-term outcomes. They have no role as maintenance immunosuppressants.

The biologic agents used for immunosuppression in transplant recipients are shown in Table 308.5. These include polyclonal or monoclonal antibody preparations that are used either to treat refractory rejection or as induction therapy that is administered in the immediate

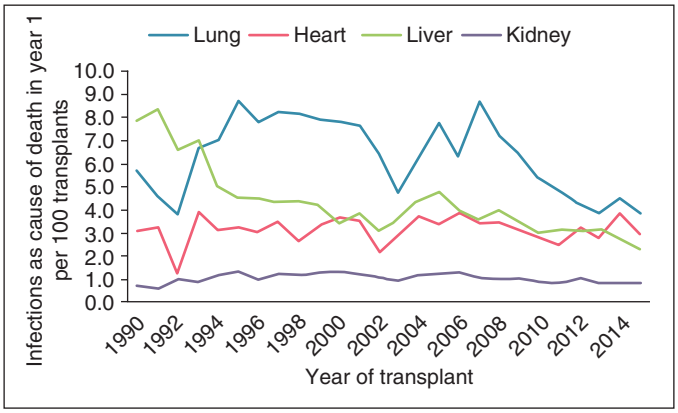

FIG. 308.1 Infections as cause of death in year 1 per 100 transplants.

TABLE 308.2 Infections Are Leading Causes of Death in Organ Transplant Recipients in the First Year After Transplantation

	PERCENTAGE OF DEATHS DUE TO SPECIFIED CAUSES IN THE FIRST POSTTRANSPLANT YEAR				
TYPE OF TRANSPLANT	VASCULAR EVENTS	INFECTION	MALIGNANCY	GRAFT FAILURE	OTHER
Kidney	26.4	24.4	3.1	0.7	1.6
Liver	16.7	32.3	5.1	12.2	10.6
Heart	18.2	23.4	2.1	26.2	11.3
Lung	14.0	34.2	3.3	16.1	5.22

TABLE 308.3 Infections Remain an Important Contributor to Morbidity in Organ Transplant Recipients 10 Years After Transplantation

	PERCENTAGE OF DEATHS IN ORGAN RECIPIENTS SURVIVING AT LEAST 10 YEARS				
TYPE OF TRANSPLANT	VASCULAR EVENTS[a]	INFECTION	MALIGNANCY	GRAFT FAILURE	OTHER[b]
Kidney	18.7	11.3	9.3	1.0	1.3
Liver	13.0	13.0	10.0	6.8	7.2
Heart	20.8	10.0	16.7	5.9	6.7
Lung	8.7	25.2	14.6	19.3	16.3

[a]Vascular events include cardiovascular and cerebrovascular events.
[b]Other includes multiorgan failure in liver, kidney, and heart recipients, and respiratory complications in lung transplant recipients (respiratory failure, bronchiolitis obliterans, pulmonary hypertension, pulmonary embolism).
Data modified from the US Organ Procurement and Transplantation Network database as of June 30, 2017. Washington, DC: US Department of Health and Human Services; 2017.

TABLE 308.4 Factors That Contribute to Infection After Transplantation

VARIABLE	EXAMPLES AND COMMENTS
Pretransplantation Host Factors	
Underlying medical conditions and chronic infections	Conditions that persist or recur (diabetes mellitus, malignancy) Conditions that exacerbate (chronic bronchitis, gallbladder disease)
Lack of specific immunity	Conducive to important primary infections (e.g., cytomegalovirus [CMV], Epstein-Barr virus [EBV], varicella-zoster virus [VZV], toxoplasmosis)
Prior colonization	Nosocomial gram-negative bacilli, *Candida* and *Aspergillus* organisms, staphylococci, vancomycin-resistant enterococci,
Prior latent infections	Reactivation produces clinical infection (tuberculosis, CMV, herpes simplex virus, VZV, cryptococcosis, *Trypanosoma cruzi*)
Prior medications	Immunosuppressive agents and antibiotics influence posttransplantation susceptibility to infection.
Transplantation Factors	
Type of organ transplanted	Site of transplantation and allograft are most common sites of infection. Allograft may transmit infection or be more susceptible to infection as a result of ischemic injury or allograft reactions.
Trauma of surgery	Surgical stress, duration of surgery
Immunosuppression	
Immunosuppressive agents	Corticosteroids, azathioprine and other cytotoxic agents, cyclosporine, tacrolimus, rapamycin, polyclonal and monoclonal antilymphocyte serums
Immunosuppressive viruses	CMV, EBV, human herpesvirus 6, and chronic hepatitis C virus infection associated with higher risk of bacterial and fungal infections
Allograft Reactions	
Graft-versus-host reaction	Affects all areas of immunity and is a major factor in bacterial, viral, and fungal infection in stem cell transplantation
Host-versus-graft reaction	Possible cofactor in infections affecting the allograft

TABLE 308.5 Biologic Preparations Used to Prevent or Treat Rejection

AGENT	ADVERSE EFFECTS
Polyclonal Antibodies	
Antithymocyte globulins[a] Anti–human thymocyte immune globulin (rabbit) (Thymoglobulin) Lymphocyte immune globulin, antithymocyte (equine) (ATGAM)	Serum sickness, thrombocytopenia, lymphopenia (can last up to 2–3 years with Thymoglobulin), increased risk of cytomegalovirus and posttransplantation lymphoproliferative disease
Monoclonal Antibodies	
Anti-CD25 (interleukin-2 receptor) antibodies[b] Basiliximab (Simulect)	Hypersensitivity reactions, infection risk not significantly increased
Anti-CD20 antibody[c] Rituximab (Rituxan)	Infusion reactions, hepatitis B virus reactivation
Anti-CD52 antibody[d] Alemtuzumab (Campath)	Infusion reactions, increased risk of cytomegalovirus, *Pneumocystis jirovecii* pneumonia, invasive fungal infections, immunosuppression that can last up to 12 mo
Other Agents	
Anti–B7 fusion protein (costimulation ligand)[e] Belatacept (Nulojix)	Increased rate of Epstein-Barr virus–associated posttransplantation lymphoproliferative disease

[a]Used for induction and rejection treatment.
[b]Used for induction but not to treat rejection.
[c]Used primarily to treat humoral rejection, blood type (ABO) mismatch, and recipients with a positive crossmatch (off-label use).
[d]Used for induction and rejection treatment (off-label use).
[e]Used for maintenance immunosuppression.

posttransplantation period and aims to provide a high early level of immunosuppression while avoiding nephrotoxicity from calcineurin inhibitors. These agents can generally be divided into T-cell nondepleting and depleting drugs. The first such agent, a murine monoclonal antibody (Muromunab-CD3 [OKT3]), was associated with an unfavorable toxicity profile, including cytokine-release syndrome (fever, rigors, hypotension, or rarely, severe pulmonary edema and respiratory distress) and a substantial risk of opportunistic infections. This agent was withdrawn from the market in 2010.

Basilixmab is a nondepleting agent approved by the US Food and Drug Administration (FDA) for prophylaxis of rejection in kidney transplantation. An anti-CD25 monoclonal antibody, it binds to the IL-2 receptor and prevents activation of T cells by circulating IL-2. It is relatively well tolerated and is not associated with cytopenias or serum sickness. However, it cannot be used for the treatment of acute rejection as it causes no T-cell depletion.

Antithymocyte globulins, such as Thymoglobulin, are polyclonal antibodies prepared in rabbits or horses by immunization with human thymocytes. As such, they are foreign proteins that may cause serum sickness that typically begins about 10 days after administration. They result in profound immunosuppression due to T-cell depletion, which lasts for 3 to 6 months. Antithymocyte globulin increases the risk of CMV disease and posttransplantation lymphoproliferative disease (PTLD).

Alemtuzumab is a monoclonal antibody that is approved for the treatment of B-cell chronic lymphocytic leukemia. This agent targets a cell surface molecule (CD52) common to many immune cells and causes significant reduction in $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cells, and $CD19^+$ B cells. This effect may last 12 months or longer after administration. It is increasingly used with transplant recipients for either induction therapy or for the treatment of acute rejection unresponsive to corticosteroids. Receipt of alemtuzumab for induction therapy confers a higher risk of CMV viremia and more severe and disseminated fungal infections. In contrast to agents that affect only T cells, its use has not been associated with an increased rate of PTLD, probably because its action against B cells that suppresses Epstein-Barr virus (EBV). The infectious risk of alemtuzumab is significantly higher when used as salvage treatment for acute rejection than when used as induction therapy.

Rituximab is another chimeric mouse/human monoclonal antibody that targets the CD20 antigen expressed on B lymphocytes. Rituximab is approved to treat a variety of B-cell malignancies and autoimmune diseases. In organ transplantation it has been primarily used to treat antibody-mediated rejection. Rituximab also does not appear to increase the general risk for infections but has been associated with reactivation of HBV infection and progressive multifocal leukoencephalopathy.[5] Patients receiving therapy with rituximab may also mount poor antibody responses to immunization.

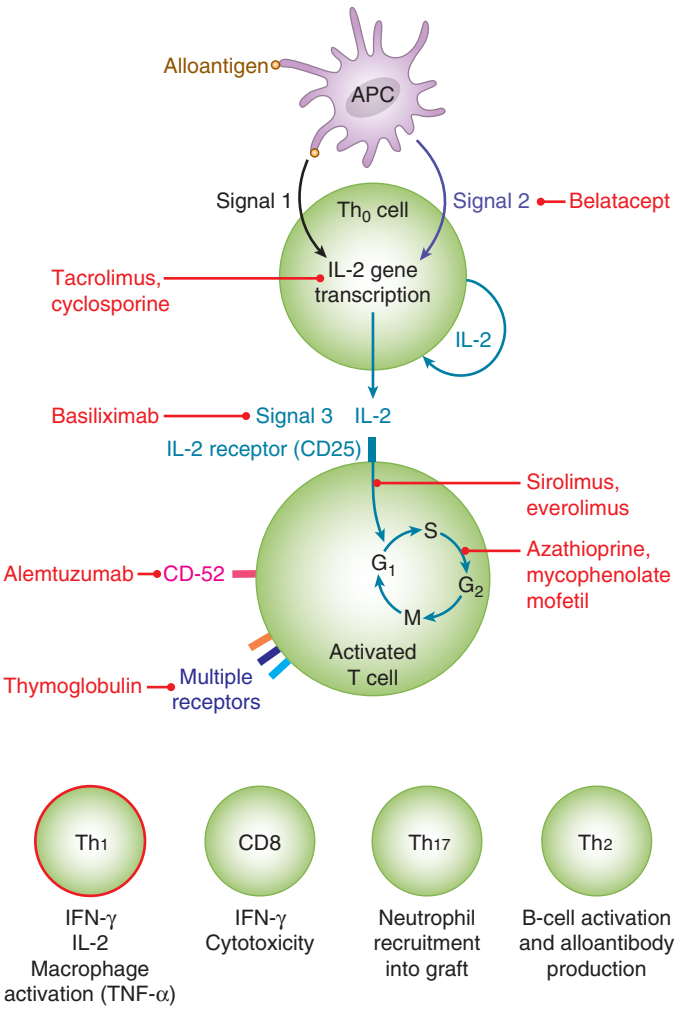

FIG. 308.2 Cyclosporine inhibits the production of cytokines, primarily interleukin-2, and consequently the generation of effector T cells or $CD4^+$ T cells. Rapamycin interferes with cell-cycle proliferation and blocks intracellular signaling mechanisms by inhibiting a regulatory kinase, mammalian target of rapamycin. *APC,* Antigen-presenting cell; G_1, G_2, cell-cycle "gap" phases with growth; *IFN-γ,* interferon-γ; *IL-2,* interleukin-2; *M,* cell-cycle mitotic phase; *S,* cell-cycle DNA replication phase; *TNF,* tumor necrosis factor.

Belatacept is a novel fusion protein that avidly attaches to receptors on antigen-presenting dendritic cells. It selectively inhibits costimulation signaling, thereby interfering with effective T-cell activation. Belatacept requires intravenous (IV) administration on a defined schedule that tapers off to every-4-weeks infusions by 2 to 3 months after transplantation.[6] It is approved as an antirejection agent in kidney recipients, based on trials that showed significantly better creatinine clearance and similar graft survival when it replaced cyclosporine.[7] The overall infectious risk of belatacept does not appear to be greater than cyclosporine-based immunosuppression. However, development of progressive multifocal leukoencephalopathy and PTLD, particularly involving the central nervous system (CNS) has been documented. For this reason, belatacept use is contraindicated in EBV-seronegative recipients.

PRETRANSPLANT EVALUATION FOR INFECTIONS

Evaluation of the patient for active and latent infection will allow optimization of pretransplantation and posttransplantation management. The UNOS has issued a list of required infectious disease screening tests for all transplantation candidates.[8] The initial transplantation evaluation should consist of a thorough history and physical examination, with a particular emphasis on demographics, country of origin, occupation, travel history, and exposure history, including exposure to animals. Current medications should be reviewed, specifically immunosuppressants such as corticosteroids, which may predispose the patient to opportunistic infections before transplantation. All relevant microbiology and hospital medical records should be examined for evidence of latent or active infection and colonization with drug-resistant bacteria. The pretransplantation setting is also an important opportunity to update the potential recipient's immunization as vaccinations are more effective when given before immunosuppression (discussed later in the immunizations section).

Pretransplantation serologic screening is pivotal in mitigating the risk of infection posttransplantation, as outlined in Table 308.5. Knowledge of the recipient's herpesvirus serostatus, especially that of CMV, is used in conjunction with the donor's CMV serostatus to risk stratify the recipient and determine the optimal posttransplantation preventive strategy.[9–11] Persons who are EBV mismatched (EBV-seropositive donor/EBV-seronegative recipient [D^+/R^-]) are at an increased risk of PTLD and are more closely monitored for EBV. Routine pretransplantation evaluation should also include testing for human immunodeficiency virus (HIV), latent tuberculosis (TB), *Toxoplasma gondii* (particularly in heart transplant candidates), hepatitis A and B (for which vaccination should be offered, if seronegative), hepatitis C, and syphilis.

Transplant Candidates Colonized With Multidrug-Resistant Bacteria

Other than patients with cystic fibrosis (CF), the natural history of transplant candidates colonized with multidrug-resistant (MDR) organisms, particularly MDR gram-negative bacteria, has not been fully described. A few observational studies have demonstrated that pre–liver transplant rectal colonization with extended-spectrum β-lactamase-producing or carbapenem-resistant Enterobacteriaceae is a risk factor for posttransplant colonization and infection.[11a,11b] However, while mortality rates approached 50% for persons with posttransplant CRE infection, pretransplant CRE colonization was not a risk factor for death.

Selective Testing for Specific Infections in Transplant Candidates

Additional testing for human T-cell lymphotropic virus 1 (HTLV-1), *Trypanosoma cruzi,* malaria, *Strongyloides stercoralis*, *Brucella,* or human herpesvirus 8 (HHV-8)[12] may be required in patients with residence or extended travel in regions where these infections are prevalent. *Strongyloides stercoralis* is also endemic within the southeastern United States, where screening should be considered.[13] Serologic screening for *Leishmania* before transplantation may be considered for candidates with a history of potential exposure to this protozoan parasite in tropical or subtropical regions and in the Mediterranean countries, although conclusive data are lacking. Screening for *Coccidioides* antibodies should be routinely performed in recipients from areas of endemicity of this fungus. HTLV-1 is endemic to Southeast Asia, the Pacific Islands, Western Africa, parts of South America, and the Caribbean. The effect of immunosuppression on the natural history of HTLV-1 is not well defined, and routine HTLV-1 serologic testing of donors is no longer routinely used because of concerns about specificity. Transplant candidates seropositive for HTLV-1 can be considered for transplantation but should be informed about potentially increased risk of developing acute T-cell leukemia and HTLV-1–associated myelopathy/tropical spastic paraparesis (Table 308.6).[14]

TIME OF OCCURRENCE OF INFECTIONS AFTER TRANSPLANTATION

The frequency, types of infections, and specific pathogens encountered after transplantation generally follow a predictable time to onset. However, evolving medical practices and preventive strategies have led to modifications in the risk and timeline of several infections, as discussed later. Nevertheless, infections in transplant recipients must be evaluated in the context of time elapsed since transplant.

TABLE 308.6 Antimicrobial Drug Interactions With Immunosuppressive Agents and Suggestions for Dosage Adjustment

ANTIMICROBIALS		EFFECT ON CYP3A4	IMMUNOSUPPRESSANT LEVEL		DOSAGE ADJUSTMENT[a]	
			CALCINEURIN INHIBITOR	mTOR INHIBITOR	CALCINEURIN INHIBITOR	mTOR INHIBITOR
Azoles	Fluconazole	Moderate-strong inhibitor at doses > 400 mg	↑↑↑	↑↑	Reduce FK and CsA by ⅓–½	Monitor levels/ reduce doses
	Voriconazole	Strong inhibitor	↑↑	↑↑	Reduce FK by ⅔, CsA by 1/2	Technically "contraindicated," but usually reduce mTOR dose
	Posaconazole	Strong inhibitor	↑↑	↑↑	Reduce FK by ⅔, CsA by ¼	
	Isavuconazole	Weak inhibitor	↑	↑	Monitor levels; currently no preemptive adjustment recommended	
Rifamycins (CYP3A4 induction: rifampin > rifapentine > rifabutin)	Rifampin	Strong inducer	↓↓↓	↓↓↓	Avoid rifampin	
	Rifabutin	Moderate inducer	↓↓	↓↓	Increase calcineurin inhibitor /mTOR dose by 50%; frequent monitoring	
Macrolides	Azithromycin	—	—	—	None	
	Clarithromycin	Moderate to strong inhibitor	↑↑	↑↑	Reduce calcineurin-inhibitor mTOR dose by ½	
ARV (ritonavir/cobicistat)		Strong inhibitors	↑↑↑	↑↑↑	Major interaction; calcineurin inhibitors have been dosed once weekly/q2 weeks. In the current era, switch to ART to avoid boosted agents (e.g., use integrase inhibitors)	
Nafcillin (not oxacillin)		Weak inducer	↓	↓	Monitor levels; no empiric adjustment	

[a]Dose adjustments per prescribing information; actual practices may vary.
ART, Antiretroviral therapy; *CsA,* cyclosporine A; *CYP3A4,* cytochrome P3A4; *FK,* tacrolimus; *mTOR,* mammalian target of rapamycin.

TABLE 308.7 Typical Timeline for the Onset of Infections After Transplantation

	MAJOR PREDISPOSING FACTORS		
	DAY 0–30	DAY 30–180	DAY 180 AND BEYOND
Pathogens	Surgical complications, nosocomial infections, pretransplant colonizers, donor-derived infections; low opportunistic infection risk	Effects of iatrogenic immunosuppression, high risk of opportunistic pathogens, residual postsurgical sequelae	Community acquired pathogens; risk of opportunistic pathogens lifelong and determined by overall state of immunosuppression such as treatment of rejection, intensity of maintenance regimen
Bacteria	Gram-negative and gram-positive bacteria (including multidrug-resistant strains), *Clostridioides difficile*, pretransplant colonizers (e.g., *Burkholderia cepacia*)	*Nocardia*, tuberculosis and nontuberculous mycobacteria, nosocomial gram-negative/ gram-positive bacteria,	*Streptococci*, *Legionella, Listeria*; nosocomial bacteria if hospitalized; risk of opportunistic bacteria never resolves
Fungi	*Candida* spp.; *Aspergillus* uncommon, except for patients with cystic fibrosis	Molds (*Aspergillus*, mucormycosis), *Candida* and *Pneumocystis* less common in the current era	*Cryptococcus* and endemic mycoses; ongoing risk for invasive mold disease; *Pneumocystis* and toxoplasmosis if off prophylaxis
Viruses	Respiratory viruses; herpesviruses uncommon in the current era of routine antiviral prophylaxis	Herpesviruses (cytomegalovirus [CMV]) if off prophylaxis; BK virus, respiratory viruses	Reactivation of chronic viral infections (herpes zoster, CMV), Epstein-Barr virus (posttransplantation lymphoproliferative disorder), respiratory viruses, norovirus, JC virus
Other	Donor-derived bacteria/fungi, unrecognized donor-derived viruses, opportunistic fungi such as *Cryptococcus* and endemic mycoses	Wide geographic and institutional variability; should be evaluated in the context of the prophylactic regimen and endemic pathogens (leishmaniasis, Chagas disease, gastrointestinal parasites)	Timeline is "reset" and opportunistic infection risk increases markedly with the treatment of rejection, particularly with T-cell–depleting agents (alemtuzumab, Thymoglobulin)

Infections in First 30 Days

Infections during this period are primarily surgical or technical complications related to transplantation or are health care–associated (Table 308.7). Bacterial infections, including those due to antimicrobial resistant pathogens, are by far the most frequently occurring infections; vascular-catheter infections, health care–associated pneumonia, *Clostridioides difficile* (formerly *Clostridium difficile*) colitis, and surgical site infections being most common. Invasive fungal infections are uncommon in the first month, except for invasive candidiasis, which generally manifests as candidemia or surgical site infections. In the absence of mold-active prophylaxis, invasive aspergillosis can occur in lung transplant recipients with prior mold colonization. Viral infections such as herpes simplex virus (HSV) and HHV-6 may be encountered very early posttransplantation. Certain donor-transmitted infections (discussed later) may also manifest during this period.

Infections Between 30 to 180 Days

Although nosocomial infections continue to pose a threat in patients requiring prolonged hospitalization, rates of surgical infections typically decline after 1 month, and opportunistic infections associated with immunosuppression emerge. These include *Pneumocystis jirovecii,* fungi, *T. gondii, Nocardia,* and most important, cytomegalovirus (CMV).

Historically, CMV infection and disease have occurred between 4 and 6 weeks posttransplantation. However, in the era of routine use of antiviral prophylaxis, typically given for 3 to 6 moths posttransplantation, most CMV disease now occurs later, after antiviral prophylaxis has been discontinued ("late-onset" CMV disease). *Pneumocystis* pneumonia and, to a large extent toxoplasmosis, are seen infrequently today because of the effectiveness of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis. Invasive aspergillosis has long been regarded as an early-occurring infection. However, most cases now occur between 1 and 6 months posttransplantation. Notably, in lung transplant recipients *Aspergillus* infections occur after 12 months; the median time to onset posttransplantation was 357 days in one report[15] and 33 ± 19.6 months in another.[16] Widespread use of mold-active antifungal prophylaxis and chronic rejection and/or its treatment likely account for these trends.

Infections Occurring 6 Months or Later

Infections more than 6 months after transplantation are mostly community acquired and similar to those seen in the general population. However, contemporary data from the Spanish consortium for the study of infections in transplant recipients show that although the rate of infections declined from 3.5 per 1000 transplant-days in the first 6 months to 0.4 per 1000 transplant-days in the late period, the etiology of infections and infection-related mortality was similar in the two periods.[17] Patients with chronic graft dysfunction and graft-related reoperations are at higher risk for late infections. Heart, kidney, and liver transplant recipients have lower rates (≈0.3/1000 transplant-days), whereas pancreas and lung transplant recipients have the highest incidence rates of late infections (0.76 and 1.4/1000 transplant-days, respectively).[17]

Other late manifestations may represent reactivated or chronic viral infections. Herpes zoster may occur at any time after transplantation. A number of tumors related to viral infection may also occur late, the most frequent being cutaneous malignancies. Also, some lymphomas and lymphoproliferative syndromes related to EBV occur after 1 year. Infections due to mycoses, such as cryptococcosis or histoplasmosis, often manifest late and without an apparent inciting event or change in immunosuppression. Finally, although the risk for classic opportunistic infections declines, it never disappears completely. In this regard, treatment of rejection, particularly with T-cell–depleting agents resets the timeline and renders the patient susceptible to opportunistic pathogens noted earlier, the risk of which declines in the late posttransplantation period (see Table 308.7).

UNIQUE CHARACTERISTICS OF INFECTIONS IN DIFFERENT TYPES OF TRANSPLANTS

Table 308.8 presents the type, severity, and typical sites of infections in kidney, heart, heart-lung, and liver transplant recipients observed during the first year after transplantation. In addition, certain risks are unique for specific types of transplantation. Kidney transplant recipients have the lowest number of episodes of infection per patient, whereas liver and heart transplant recipients have intermediate rates. Heart-lung or lung recipients, by contrast, have more than three times the number of infections. Liver transplant recipients have almost twice the rate of bacteremia of the renal group.[18–24] Invasive fungal infections are frequent in liver and heart-lung or lung recipients, intermediate in heart recipients, and rare in renal recipients. Table 308.3 also shows that the most common sites of infection are closely related to the site of surgery.

Kidney Transplant Recipients

Most infections, including bacteremia in these patients, arise from the urinary tract. By 3 years after transplantation, more than half of all renal recipients would have been diagnosed with upper or lower urinary tract infection (UTI).[25] Although most infections were uncomplicated cystitis, graft pyelonephritis occurred in 13% of 1387 renal recipients over a 13-year period.[26] In this study, pyelonephritis occurring in the first 3 months was associated with reduced graft survival, but later infections were not. However, a larger, multivariate analysis of more than 28,000 kidney recipients in the Medicare database showed that UTIs after 6 months were associated with worse long-term graft function and survival.[25] Abnormalities such as ureteral reflux, strictures at the ureterovesical junction, or neurogenic bladder should be sought in patients with recurrent infections. Administering an extended course of antibiotics (≥4 weeks) and consideration of secondary antibiotic prophylaxis are reasonable in patients who have noncorrectable urologic abnormalities and severe graft pyelonephritis or recurrent infections. Although asymptomatic bacteriuria is common in kidney transplant recipients, a randomized, controlled trial in kidney transplant recipients showed no benefit of routine screening and treatment for asymptomatic bacteriuria.[27]

The clinician also should be alert to the occurrence of uncommon urinary pathogens. For example, urinary tract TB may arise from a focus in the native kidney. *Mycoplasma hominis* may cause a breakdown of the ureterovesical anastomosis with subsequent graft loss. Presence of struvite stones, encrusted cystitis, alkaline urine, and apparent sterile pyuria is suggestive of *Corynebacterium urealyticum*. This bacterium may be overlooked in routine cultures in standard media as it requires incubation for more than 48 to 72 hours or requires special media for isolation. Histoplasmosis may involve the transplanted kidney and cause renal failure. Adenovirus may cause hemorrhagic cystitis and/or nephritis. BK virus is an important cause of renal allograft infection and is not typically associated with systemic clinical signs or symptoms.

Historically, pneumonia occurred in 25% to 30% of kidney transplant recipients and was the most common infectious cause of death. In the past, transplantation-associated pneumonia was often due to CMV and pathogens such as fungi, *Nocardia*, and *Pneumocystis*. Currently, however, these opportunistic agents have come under better control, and conventional bacterial pathogens have become far more common in transplant populations. Wound infections are infrequent (4%–5%) but may be a serious problem, particularly if they involve the perinephric space. Body mass index ≥30 kg/m^2, urinary leak, reoperation through the transplant incision, mycophenolate mofetil use, and diabetes mellitus are risk factors for infection.[17] Some renal transplant recipients continue to have frequent problems with infection even after the first 6 months. These patients have often received excessive immunosuppression or have chronic dysfunction of the allograft or other major organs.

Heart Transplant Recipients

The most common infections after heart transplantation are bacterial pneumonias, urinary infections, herpesvirus infections, and invasive fungal infections. Pneumonia in heart transplant recipients is mostly

TABLE 308.8 Frequency, Type of Infections, and Sources of Infections Occurring in the First Year After Transplantation

TYPE OF TRANSPLANT	INFECTION EPISODES PER PATIENT	BACTEREMIA (%)	FUNGAL INFECTIONS (%)	MOST COMMON SOURCE OF INFECTION
Kidney	0.98	5–10	0.7	Urinary tract
Heart	1.36	8–11	8	Lung
Heart-lung	3.19	8–25	23	Lung
Liver	1.86	10–23	16	Abdomen and biliary tract

Data summarized from references 18–24.

TABLE 308.9 Microbial Causes of Pneumonia in Transplant Recipients

EARLY PNEUMONIA (≤30 DAYS)	LATE PNEUMONIA (>30 DAYS)
Common Causes	
Gram-negative bacilli	*Pneumococcus*
Staphylococcus aureus	*Haemophilus influenzae*
Aspiration	Influenza virus
	No cause identified
Less Common Causes	
Aspergillus	*Pneumocystis*
Herpes simplex virus	*Cryptococcus*
Legionella	*Nocardia*
Toxoplasma gondii	Cytomegalovirus
	Legionella
	Varicella-zoster virus
	Mucormycosis
	Paramyxoviruses
	Mycobacteria
	Histoplasmosis
	Coccidioidomycosis
	Respiratory viruses[a]

[a]These include influenza virus, parainfluenza virus, respiratory syncytial virus, metapneumovirus, coronavirus, rhinovirus, or adenovirus.

caused by common pathogenic bacteria (Table 308.9). Although the incidence of pneumonia is highest during the first few months after transplantation, bacterial pneumonias occur sporadically in the late posttransplantation period, after the patient has recovered from the immediate effects of surgery. Heart transplant recipients requiring renal replacement therapy and thoracic reoperation are at an increased risk for invasive pulmonary aspergillosis and should receive voriconazole prophylaxis.[28]

Mediastinitis and sternal wound infections are postoperative complications unique to heart and heart-lung transplant recipients and occur in ≈2.5% of patients. The pathogens seen are similar to those observed in other patients undergoing cardiothoracic surgery, with staphylococci predominating. One must also be alert to the possibility of unusual pathogens. Mediastinitis and sternal wound infections in heart recipients have been caused by *M. hominis, Legionella, Aspergillus*, Mucormycetes, and *Nocardia.* Factors that predispose to mediastinitis in this population are repeat operations for hemorrhage, use of antirejection therapy, and diabetes mellitus. Surgical drainage is a crucial component of treatment of mediastinitis in the transplant patient.

Left ventricular assist devices (LVADs) are now widely used as a bridge to transplantation. Infections of these devices are common and fall into distinct types: driveline infections, which are often limited to the exit site; deep infections in the pocket surrounding the device; and internal infection of the device, which is often associated with bloodstream infection. Management of these infections is challenging; however, in many cases the infection can be controlled well enough to permit transplantation. Available experience suggests that the use of antimicrobial therapy before, during, and after transplantation is associated with fewer relapses than short-course therapy. Although pretransplantation LVAD infection is associated with a higher rate of periheart transplantation complications, long-term outcomes appear to be reasonable, and LVAD infection is not considered a contraindication to heart transplantation.[29]

A number of other infections occur more commonly in heart recipients than in patients receiving other types of transplants. These include toxoplasmosis, nocardiosis, and Chagas disease. *Toxoplasma*-seronegative heart recipients are at risk for toxoplasmosis because the infection can be acquired from organisms encysted in the heart muscle of *Toxoplasma*-seropositive donors. Clinical toxoplasmosis usually occurs a few weeks to a few months after transplantation and is manifested by necrotizing pneumonitis, myocarditis, and encephalitis. Before the use of preventive therapy, the rates of toxoplasmosis were 60% to 80% in donor *Toxoplasma*-seropositive/recipient *Toxoplasma*-seronegative patients and ≈5% to 10% in *Toxoplasma*-seropositive recipients. Clinical toxoplasmosis is now uncommon in heart recipients, likely because of the use of TMP-SMX for *Pneumocystis* prophylaxis.[30] In heart transplant recipients who are *Toxoplasma* mismatches but are intolerant of TMP-SMX, the prophylactic regimen must cover both *Toxoplasma* and *Pneumocystis*.

In endemic regions such as Latin America, symptomatic reactivation of *Trypanosoma cruzi* may be seen in immunocompromised patients, including transplant recipients. About one-fourth of patients who undergo heart transplantation for cardiomyopathy caused by chronic *T. cruzi* infection have relapses of acute Chagas disease, with clinical manifestations of fever, myocarditis, and skin lesions. The disease can usually be controlled with chemotherapy. Detection of parasitemia by microscopy or polymerase chain reaction (PCR) assay in blood samples is useful to monitor for reactivation and guide chemotherapy after transplantation.

Nocardia infections have also been more frequently reported in heart and lung transplant recipients than in recipients of kidney or liver transplants, but the biologic reason for this increased rate of nocardiosis is unknown. The doses of TMP-SMX used for *Pneumocystis* prophylaxis might not provide reliable protection against *Nocardia* infection. However, resistance to TMP-SMX remains uncommon, even among breakthrough *Nocardia* infections.

Heart transplant recipients frequently incur trauma to the tricuspid valve and right ventricular endocardium from repeated endomyocardial biopsies, a common posttransplantation practice. Patients with cardiac valvulopathy are at high risk for adverse outcomes from endocarditis, and prophylaxis with dental procedures is appropriate in this group.[31]

Lung and Heart-Lung Transplant Recipients

The heightened vulnerability of the transplanted lung to infection is multifactorial. In addition to mechanical factors related to decreased mucociliary clearance, diminished lymphatic drainage, and ablation of the cough reflex appear to play an important, if poorly understood, role. The types of infections seen in lung transplant recipients are similar to those in heart-lung recipients, although the overall survival rate is better. Heart-lung transplantation is now usually reserved for patients who have Eisenmenger syndrome and whose cardiac abnormalities cannot be surgically repaired. Single- and double-lung procedures leave the donor heart available for another patient with end-stage heart disease. A unique aspect of single-lung transplantation is the occurrence of infections in the native lung that may be predisposed to infection because of defects of ventilation or perfusion caused by the underlying lung disease.

Lung and heart-lung transplant recipients experience a high rate of bacterial lung infections, especially during the first few weeks after transplantation. These patients also have higher rates of mediastinitis, invasive fungal infections, and CMV pneumonia than heart recipients. Some patients undergoing lung transplantation are chronically colonized with multidrug-resistant bacteria (e.g., CF, non-CF bronchiectasis) and are therefore at high risk for postoperative infection at the time of transplant surgery. Multidrug-resistant gram-negative bacteria in patients with CF, such as *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa,* do not preclude transplantation; instead, the posttransplant prophylactic regimen must target these organisms.[32]

Pretransplant *Burkholderia cepacia* complex infections in patients with CF pose a unique challenge. *Burkholderia multivorans* infections do not portend poor outcomes, in contrast with *Burkholderia cenocepacia*.[33] For this reason, patients with CF who are known to be colonized with *B. cenocepacia* are often denied listing for lung transplantation at many centers. However, selected persons with *B. cenocepacia* can undergo lung transplantation at referral centers with experience in managing these infections, which often includes prolonged posttransplant antibiotics. Patients and their families should be counseled about the risk of poor outcomes. Lung transplant candidates with CF may also be colonized with molds, such as *Scedosporium* complex and *Lomentospora (Scedosporium) prolificans,* and voriconazole is commonly used as prophylaxis in patients with fungal colonization. However, institutional practices on routine screening and management vary widely.

In the late posttransplant period, up to two-thirds of patients eventually develop chronic lung allograft dysfunction (CLAD), thought to

represent chronic allograft rejection, and manifests as two major phenotypes: bronchiolitis obliterans syndrome or a more recently described restrictive allograft syndrome.[34] CLAD has been associated with recurrent pulmonary infections and is one of the major causes of death in lung transplant patients. The lung allograft is also particularly susceptible to viral infections, as has been documented for CMV, HSV, and community-acquired respiratory virus infections (influenza virus, parainfluenza [PIV], respiratory syncytial virus [RSV], metapneumovirus (MPV), coronavirus, rhinovirus, and adenovirus) that are a significant cause of morbidity and have been associated with both short-term and long-term impairment of allograft infection.

Pulmonary nontuberculous mycobacterial infections are relatively common in lung transplant recipients and may arise from pretransplant colonizing strains or exogenous acquisition. *Mycobacterium abscessus* warrants special mention, as it can lead to devastating consequences posttransplantation, including aggressive and destructive surgical site infections and disseminated disease and mortality.[32,35] Because of drug resistance and poor treatment response, pretransplantation colonization or disease is considered by some centers to be a contraindication to lung transplantation.[35,36] However, some centers have reported acceptable outcomes in patients with pretransplantation *M. abscessus* disease, with the understanding that posttransplantation complications can be expected and should be managed with aggressive and prolonged therapy.[37,38] Every effort should be made to control the infection before transplantation, and antibiotics should generally be continued in the posttransplantation setting.

Acute development of hyperammonemia after lung transplantation is a rare but an often fatal complication after lung transplantation, the etiology of which is poorly understood but includes unmasking of urea cycle disorders, effects of immunosuppressive agents, or infections with urea-splitting organisms, such as *Mycoplasma* or *Ureaplasma*.[39] A multimodal treatment approach is required, which may include renal replacement therapy, bowel decontamination, amino-acid supplementation, nitrogen scavenger therapy for urea-splitting organisms, and antibacterial therapy (quinolones, tetracyclines, or macrolides) for microbiologically proven cases.[39]

Prospective donors for lung transplantation are intubated in intensive care units (ICUs). Therefore the airways of these donors are often colonized with microorganisms, and occult parenchymal infection may be present. Before implantation of the lungs, it is customary to obtain cultures and Gram stains of the donor airways to guide antibiotic therapy in the recipient. Initial antibiotic prophylaxis should be aimed at common nosocomial pathogens encountered in the ICU, including methicillin-resistant *S. aureus* (MRSA) and enteric and nosocomial gram-negative bacilli; the regimen can be narrowed to target the donor's pathogens. Another problem unique to lung transplantation has been dehiscence of the airway anastomosis. This occurs during the first few weeks after transplantation. It is frequently associated with bacterial or fungal infection at the anastomotic site. The incidence of this complication appears to be declining.

Invasive Aspergillosis After Lung Transplantation

Heart-lung and lung transplant recipients develop invasive pulmonary aspergillosis more frequently than patients with other types of organ transplants. Risk factors for invasive aspergillosis in addition to renal dysfunction are relative ischemia at the anastomotic site, receipt of a single-lung transplant, hypogammaglobulinemia, CMV infection, precolonization and/or postcolonization of the airways with *Aspergillus,* and augmented immunosuppression for allograft rejection.[40] A unique form of aspergillosis involving the airway mucosa or bronchial anastomotic sites, called tracheobronchial aspergillosis, is observed almost exclusively in lung and heart-lung recipients (Fig. 308.3). The airway lesions of this disease can be directly visualized during bronchoscopy.[41]

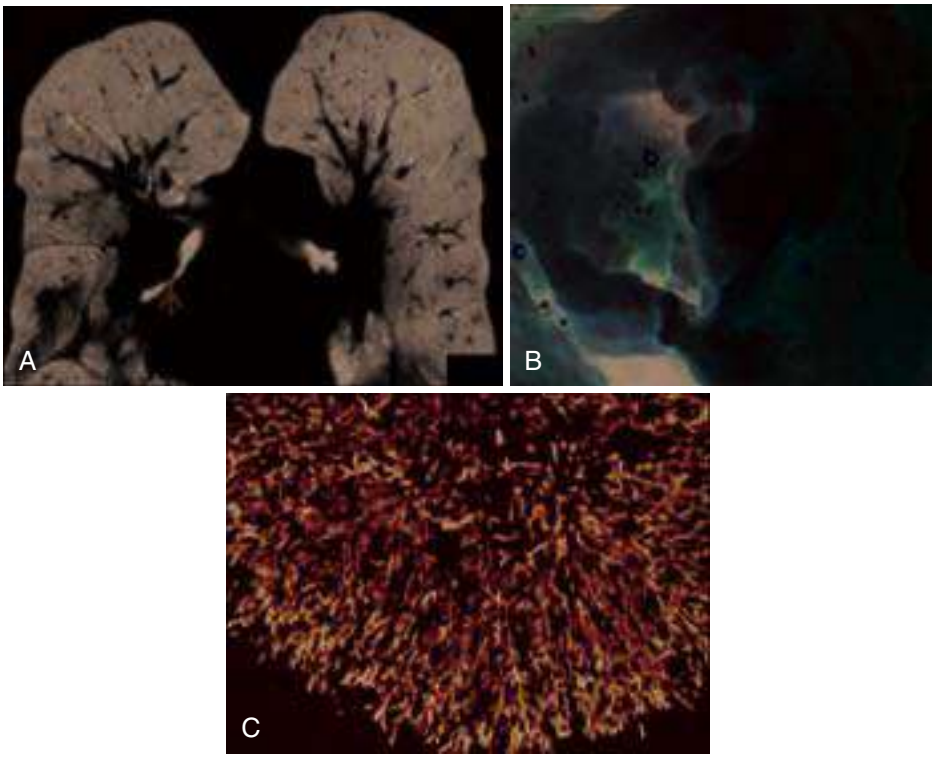

FIG. 308.3 A unique form of aspergillosis involving the airway mucosa or bronchial anastomotic sites, called tracheobronchial aspergillosis, is observed almost exclusively in lung and heart-lung recipients. (A) Coronal view showing an endobronchial lesion partially obstructing the bronchus intermedius *(blue arrow)*. (B) Bronchoscopy of the left allograft bronchus showing showed irregular lesions comprised of yellowish pseudomembranes mixed with necrotic debris that completely occluded the entrance into the left upper lobe and lingula. Note the presence of ulcerative lesions (e.g., "a" with shallow erythematous base, "b" with deep ulcer and bleeding crater, and "c" with necrotic ulcer), which are characteristic features of ulcerative bronchitis. (C) Histopathology and Grocott-Gomori methenamine silver stain of biopsy specimen of the lesions in B demonstrating numerous fungal forms consistent with *Aspergillus* species. *(A and B from Haidar G, Crespo M, Maximous S, et al. Invasive Tracheobronchial* Aspergillosis *in a Lung Transplant Recipient Receiving Belatacept as Salvage Maintenance Immunosuppression: A Case Report.* Transplant Proc. *2016;48:275–278.)*

This form of aspergillosis has a better prognosis than aspergillosis invading the lung parenchyma and is commonly treated with systemic and local (i.e., nebulized) antifungal therapy combined with surgical débridement, as feasible.

Continuous exposure of the organ to the environment and post-transplantation *Aspergillus* colonization poses a risk for subsequent invasive disease in lung transplant recipients. As such, many lung transplantation centers routinely use antifungal preventive strategies. Direct administration by means of inhalation of the polyene agents (amphotericin B formulations) allows deposition of the drug directly into the distal bronchial tree, thereby obviating the systemic side effects and drug interactions. Inhaled amphotericin B deoxycholate and the lipid formulations (lipid complex and liposomal) are both generally well tolerated; however, aerosolized lipid formulations are associated with fewer side effects.[40] A potential limitation of aerosolized amphotericin B is the fact that deposition in single-lung transplant recipients occurs preferentially in the allograft, with erratic distribution in the native lung, which could be a potential source of infection.[42] In addition, aerosolized amphotericin B may not prevent systemic fungal infections because candidemia has been reported in lung transplant recipients with such an approach. Finally, long-term use of these agents has also been associated with the emergence of *Aspergillus* spp., such as *A. flavus, A. terreus,* and *A. alliaceus,* with reduced susceptibility to amphotericin.[43]

Triazoles, including itraconazole and voriconazole, have led to a decrease in the incidence of invasive aspergillosis in lung transplant recipients. The potential for hepatotoxicity is a concern, particularly with voriconazole.[44] Association of prolonged voriconazole use with skin cancers and periostitis can be treatment-limiting toxicities in lung transplant recipients. Sparse data exists for posaconazole prophylaxis in lung transplant recipients. The risk of hepatotoxicity may be lower with this drug; however, achieving therapeutic levels in the perioperative period is challenging, particularly with the suspension formulation. Although serum posaconazole concentrations correlated with levels within the bronchoalveolar fluid in lung transplant recipients, steady-state levels were rarely reached in the newly transplanted lungs.[45] Extended-released and IV formulations of posaconazole were approved in 2013 and 2014, respectively, and have been shown to have much more favorable pharmacokinetics parameters, although data on their use in lung transplant prophylaxis are limited. Thus the role of posaconazole as primary prophylaxis in the perioperative period is uncertain. There are no data on the use of isavuconazole for prophylaxis after lung transplantation. Immunosuppressant levels should be monitored and dosages adjusted accordingly in patients receiving azole antifungal agents (Table 308.10).

Therapeutic drug monitoring, although not necessary for fluconazole, should be performed for azole antifungal agents in transplant recipients if the use of these agents is anticipated for at least a week (see Table 308.6). Adequate plasma trough levels for the triazole antifungal agents have correlated with successful outcomes, both when used as prophylaxis and treatment of invasive fungal infections after transplantation. In addition, monitoring trough plasma levels avoids overexposure for example, voriconazole concentrations >5.5 mg/L are thought by some to predispose to visual and neurologic adverse events in transplant recipients.

TABLE 308.10 Etiologies of Nodular Pulmonary Infiltrates in Transplant Recipients

ETIOLOGY	
INFECTIOUS	**NONINFECTIOUS**
Bacterial	**Malignancies**
Nocardia	PTLD and other lymphomas
Mycobacteria	Carcinomas (typically lung neoplasms and hepatocellular carcinoma)
Legionella	**Nonmalignant Lesions**
Staphylococcus aureus (septic emboli)	Rounded atelectasis
Fungal	
Aspergillus	
Cryptococcus	
Mucormycosis	
Endemic fungi (histoplasmosis and coccidioidomycosis)	
Other non-*Aspergillus* molds	
Viral	
Cytomegalovirus	

PTLD, Posttransplantation lymphoproliferative disease.
Data from Copp DH, Godwin JD, Kirby KA, et al. Clinical and radiologic factors associated with pulmonary nodule etiology in organ transplant recipients. Am J Transplant. *2006;6:2759–2764; and Paterson DL, Singh N, Gayowski T, et al. Pulmonary nodules in liver transplant recipients.* Medicine. *1998;77:50–78.*

Liver Transplant Recipients

Liver transplant recipients have higher rates of infection than renal or heart transplant recipients, and most deaths are associated with infection, either as a primary or a secondary cause (see Table 308.2). Bacterial infections occur in 59% to 68% of the patients after liver transplantation, and ≈50% of these infections occur within 2 weeks after transplantation. The most important sites of infection are the abdomen and biliary tract, the surgical wound, the lungs, and the bloodstream, with or without associated catheter infection.

Abdominal Infections After Liver Transplantation

Transplantation of the liver differs from other transplantation operations in the length and difficulty of the surgery and the frequency of bleeding. In addition, many liver transplant recipients have poor nutrition and severe metabolic difficulties. Surgical site infections account for most infections in the early period after liver transplantation, and in general, short-course prophylactic antibiotic regimens targeting skin and intestinal flora are favored, with national guidelines recommending perioperative administration of piperacillin-tazobactam or ampicillin plus cefotaxime for 24 hours or less.[46,47] Decisions about the optimal prophylactic regimen become more complicated in individuals colonized with drug-resistant bacteria, particularly vancomycin-resistant *Enterococcus* (VRE). In a study, 32% of patients colonized with VRE before liver transplantation received tigecycline prophylaxis to target their VRE.[46] Compared with VRE-colonized patients who received ampicillin-sulbactam prophylaxis, there was no difference in the frequency of microbiologically confirmed deep surgical site infections, including those caused by VRE. As such, perioperative prophylaxis based on history of VRE colonization or infection may not be routinely necessary.[48]

The Achilles heel of liver transplantation surgery is the function of the biliary and vascular anastomoses. In consequence, most abscesses in the transplanted liver result either from liver ischemia caused by hepatic artery thrombosis or from obstruction to bile flow from biliary strictures.[18] The organisms responsible are gram-negative enteric bacilli, enterococci, and anaerobes. The diagnosis is made by ultrasonography or computed tomography (CT). Treatment with drainage and IV antibiotics is usually successful, provided the source is biliary infection and any structural abnormalities can be corrected. If hepatic artery thrombosis is the predisposing factor, the infectious symptoms can usually be controlled with antibiotics, but retransplantation may be necessary.

Cholangitis after liver transplantation also results from technical problems. The most common predisposing problem is biliary strictures. Patients with strictures may have periodic bouts of cholangitis. Some patients improve after dilatation procedures or stent placement, but in others operative repair is necessary. It may not be easy to establish a firm diagnosis of cholangitis because many patients do not manifest the classic Charcot triad of fever, abdominal pain, and jaundice. The clinical presentation may mimic allograft rejection. The diagnosis is more reliable if bacteremia is present or if a liver biopsy indicates pericholangitis. Empirical treatment for cholangitis should include antibiotics to cover gram-negative enteric bacilli, enterococci, and anaerobes. Procedures such as liver biopsy, T-tube cholangiography, or endoscopic retrograde cholangiopancreatography may be followed by cholangitis and occasionally by bacteremia.

Peritonitis can accompany other intraabdominal infections and frequently complicates biliary leaks or disruption of an abdominal viscus. Bile peritonitis may occur after extraction of a T-tube. It is often well tolerated and may resolve by itself, but on occasion the leak persists and the chemical peritonitis becomes secondarily infected. The most common organisms involved in peritonitis are enterococci and aerobic

enteric gram-negative rods, but staphylococcal and candidal infections are not infrequent. Treatment of established peritonitis requires antibiotic therapy, together with drainage of associated abscesses and repair of technical problems such as biliary leaks.

Abdominal abscesses are usually found in patients who have had frequent or lengthy abdominal operations.[18] Nearly one-third of the abdominal abscesses are associated with bacteremia. The location is frequently subhepatic, but splenic, pericolic, and pelvic abscesses are also seen. Most patients with abscesses have undergone an abdominal operation within the preceding 30 days. Although common enteric organisms cause most abscesses, staphylococci and *Candida* are also seen. Imaging studies usually define the location of the abscess. As with any other abscesses, the appropriate treatment is a combination of drainage and antibiotics.

Fungal Infections After Liver Transplantation

Liver transplant recipients have a high rate of fungal infections, ranging from 15% to 42% with case-fatality rate of 25% to 82%.[49] Risk factors for invasive fungal infections include renal dysfunction, fulminant hepatic failure, longer operative time, retransplantation, hepatic iron overload, and colonization with *Candida* at the time of transplantation.[50] In the era of Model for End-stage Liver Disease (MELD) scores as the basis for prioritizing liver transplantation, a MELD score ≥30 is the most influential factor for invasive fungal infections, with fungal infections occurring in 24% to 28% of these patients.[51]

Candida is the predominant fungal pathogen, causing 62% to 88% of the invasive fungal infections in this population. Others include *Aspergillus, Cryptococcus,* and molds as agents of mucormycosis. Nearly one-third of candidal infections in liver transplant recipients are caused by non-*albicans* species. Fluconazole prophylaxis was found to be a risk factor for the emergence of non-*albicans Candida* spp. as pathogens in liver transplant recipients. Invasive candidiasis contributes significantly to mortality after liver transplantation. In a case-controlled study, liver transplant recipients with invasive candidiasis had a 36% mortality rate, compared with 2% in the control patients. Unfortunately, infections due to *Candida* can be difficult to diagnose, and the β-D-glucan assay has limited accuracy in diagnosis invasive candidiasis in liver transplant recipients.[52]

Invasive aspergillosis occurs in 1% to 8% of liver transplant recipients with unique predisposition to disseminated aspergillosis. Historically, outcomes in transplant recipients with invasive aspergillosis have been notably poor. with mortality rates ranging from 65% to 92%.[49] However, judicious use of immunosuppression, early recognition, and overall advances in management have led to steady improvement in outcomes, with the currently reported mortality rate of ≈22% in liver transplant recipients with invasive aspergillosis. Well-defined risk factors exist for invasive aspergillosis in most patients. These include retransplantation, fulminant hepatic failure as an indication for transplantation, renal dysfunction and requirement of any form of renal replacement therapy, and poor allograft function.[53] Most disease now occurs more than 90 days after transplantation, a possible consequence of improved early management and delayed occurrence of risk factors, such as CMV infection.

Antifungal prophylaxis targeted toward high-risk patients is a rational approach toward the prevention of invasive fungal infections. Practice guidelines recommend fluconazole for these patients.[54] Potential limitations of fluconazole, however, include lack of activity against *Aspergillus*, emergence of fluconazole-resistant *Candida* spp. as pathogens, and metabolic interactions with the immunosuppressive agents. Routine use of use of triazoles is limited by the risk of hepatotoxicity and erratic pharmacokinetics profile in the posttransplantation period. The echinocandins have activity for both *Aspergillus* and *Candida* spp. and low likelihood for drug interactions. Echinocandin-resistant *Candida* infections have been documented, albeit less frequently. Prophylaxis with these agents may be beneficial in patients who are at high risk for invasive *Aspergillus* or have received fluconazole before transplantation.[55] Both micafungin and anidulafungin are well tolerated in liver transplant recipients. It is reasonable to continue prophylaxis for 14 to 21 days or until the risk factor, such as requirement for renal replacement therapy, abates.

Pancreas Transplant Recipients

About 1300 pancreas transplant operations are performed in the United States annually, or approximately 1 for every 10 kidney transplantations.[1] Current patient and graft survival rates are similar to those for kidney transplantation alone, but infection-related morbidity is higher. Some studies have shown more wound complications and CMV disease among the patients receiving combined kidney-pancreas than kidney transplantation alone.

The postoperative infection rate and the causative pathogens depend primarily on the technique used for drainage of exocrine secretions of the pancreas. In recent years the practice of draining these secretions into the small bowel (enteric drainage) has gained precedence over the previous practice of drainage into the bladder. Enteric drainage has been associated with lower rates of UTI. However, intraabdominal infections remain a significant complication of enteric drainage procedures, and aerobic and anaerobic enteric bacteria predominate in abscesses associated with enteric drainage. The microorganisms in infections in which the viscus has not been opened are usually from the skin flora. *Candida*, however, is a common pathogen in all types of surgical site infections, including those that use bladder drainage.

Small Bowel Transplant Recipients

Small bowel transplantation is the preferred therapy for intestinal failure and total parenteral nutrition-related complications. Small bowel transplantation is unique as the gut not only harbors a large burden of lymphoid tissue, but these cells are the least tolerogenic of any organ. In consequence, small bowel transplant recipients are particularly susceptible to graft-versus-host disease (GVHD), and managing the risk of rejection, infection, and GVHD has proven challenging. Small bowel transplantation is often combined with a liver graft due concurrent existence of intestinal failure–associated liver disease. Of note, inclusion of liver graft with intestinal graft may be immunologically protective. However, isolated intestinal transplantation is becoming increasingly more frequent, likely due to improved management of intestinal failure–associated liver dysfunction. Despite improvements in post-transplantation management, outcomes continue to be worse than those of other organ transplants.

Greater than 90% of the small bowel transplant recipients develop significant infections, and the rates of infection are higher than in other transplant groups. Intraabdominal pyogenic infections and bloodstream infections predominate, but the transplanted intestine, like the lung allograft, is very susceptible to CMV infection, including a tendency to relapse after successful antiviral treatment. Multivisceral transplant recipients and patients undergoing colonic segment transplantation with small bowel transplantation are more likely to develop infections. The susceptibility of small bowel transplant recipients to infections continues in the late posttransplantation period, that is, more than 6 months after operation. The incidence of EBV-associated lymphoproliferative disorders approaches 10% at 5 years, and the tumors often involve the gut.

SPECIFIC SITES AND TYPES OF INFECTION

Infections of the Skin and Surgical Site

Infections of the skin are common after transplantation but are rarely life threatening. All organ transplant recipients are at risk for wound infections. The most common bacterial pathogen is *S. aureus*, but infections with enterococci, gram-negative bacteria, *Candida,* and *M. hominis* may also be seen. Rarely, mucormycosis can cause surgical site infection in transplant recipients. Subcutaneous infections caused by *Alternaria, Exophiala*, and other darkly pigmented or dematiaceous fungi are encountered occasionally. Biopsy with fungal culture is required for a specific diagnosis. Cellulitis or necrotizing infections can also be due to cryptococcosis. Unlike stem cell transplant recipients, organ transplant recipients rarely develop fusariosis.

The most common cutaneous viral infections are those caused by HSV and varicella-zoster virus (VZV). Warts are a common problem, particularly more than 5 years posttransplantation.

The skin is also a target organ for many systemic infections, and bacterial, fungal, nocardial, mycobacterial, and CMV infections may

include skin manifestations. *Mycobacterium chelonae* causes nodular lesions, often on the extremities. As a rule, one should aggressively investigate any new and/or unusual skin lesion with a biopsy.

Infections of the Urinary Tract

UTIs are one of the most common causes of fever in kidney transplantation, especially in the first 6 months after transplantation.[56] Patients with retained urinary stents are at a higher risk, resulting in a general recommendation to remove these stents within 4 weeks. These patients may not manifest "classic" features of UTIs or pyelonephritis, as normal hosts would. In addition, both allograft pyelonephritis and rejection can present with fevers, tenderness over the graft, and acute kidney injury. Allograft pyelonephritis, especially in the first 3 months, has been linked to long-term kidney graft dysfunction but does not affect graft survival at 5 years.[57]

In patients with signs of severe infection, the choice of empirical antibiotics should take into account the history of resistant organisms as well as local epidemiologic data. Oral step-down regimens are reasonable, as long as the patient is improving. Imaging with renal ultrasound or noncontrast CT scanning should be considered to assess for complications, such as obstruction and abscesses. It is also important to consider infections caused by unusual pathogens, such as *M. hominis*, *M. tuberculosis,* or BK virus. However, BK virus may cause allograft infection but not usually fever or systemic manifestations.

Infections of the Bloodstream

Bacteremia occurs in 5% to 10% of kidney and heart transplant recipients, with higher rates (10%–25%) in liver and lung transplant recipients (see Table 308.8). Besides catheter-related infections, pneumonia in heart and heart-lung transplant recipients, UTIs and perinephric sources in renal transplant recipients, abdominal and biliary infections in liver transplant recipients, and surgical site and UTIs in pancreatic transplant recipients are the most commonly identifiable sources of bacteremia. Predominant gram-negative bacteria are *Escherichia coli, Klebsiella* spp., and others, including extended-spectrum β-lactamase–producing and carbapenem-resistant organisms, *Acinetobacter baumanii,* and *P. aeruginosa*. The highest incidence of gram-negative bloodstream infections has been observed within the first month after transplantation (210.3/1000 person-years), with a sharp decline to 25.7 per 1000 person-years from 2 to 12 months posttransplantation.[58] Kidney recipients are more likely to develop gram-negative bacteremia 12 months after transplantation.[58]

In kidney transplant recipients the source of bacteremia is usually the urinary tract, due to disruption of the urinary tract during surgery, the lack of a valve between the bladder and transplanted ureter, and the presence of an indwelling stent in the early posttransplantation period. Bloodstream infections are common after liver transplantation due to the immunosuppression of pretransplant cirrhosis and the prolonged operation time. Risk factors after liver transplantation include diabetes mellitus, urinary and vascular catheterization, posttransplantation renal replacement therapy, massive blood loss and transfusions, reoperation, and bile duct complications.[59,60] In a study of 704 liver transplant recipients over a 10-year period, 39.4% of bloodstream infections occurred in the immediate postoperative period (<10 days).[61] The most frequent pathogens were Enterobacteriaceae (41%), *Staphylococcus aureus* (19.8%), enterococci (13.1%), *P. aeruginosa* (8.8%), and *Candida* (7.1%). Bloodstream infections occurring early after transplantation were associated with increased 1-year mortality.

In lung transplant recipients with CF the colonizing bacteria are important causes of bloodstream infections, in particular *B. cepacia*, which can lead to the cepacia syndrome, with progressive necrotizing pneumonia, persistent bacteremia, and death.[62] Bloodstream infections in heart, pancreas, and intestinal transplant recipients also occur and have been linked to intravascular catheters, abdominal leaks, and UTIs.

Bacteremia due to *S. aureus* occurs with estimated rates of 15% to 38% in various organ transplant recipients.[63] The rates of MRSA have increased over time. Among organ transplant recipients, lung recipients have the highest incidence and attack rate. Most common sources are vascular catheters, pneumonia, or wound infections. Nearly one-half of all *S. aureus* bacteremias occur within 90 days of transplantation; early-onset bacteremias are more likely to occur after liver transplantation, likely due to the large size of the incision or colonization during candidacy.[63] The unadjusted 28-day all-cause mortality after gram-negative bloodstream infections in transplant recipients was 4.9%.[58] Kidney recipients had lower mortality than liver transplant recipients.

The basic approach to bacteremia is the same whether or not the patient has undergone transplantation and includes ascertaining the source of the bacteremia and likely pathogen. The inability to discern a source is relatively common, especially in liver transplant recipients; up to 21% of the bacteremias in these patients may have had no documented source, but most of these probably originated in the abdomen. The trend toward increasing antimicrobial resistance among gram-negative bloodstream isolates has an impact on local decisions regarding empirical antibiotic therapy of transplant patients who present with possible bloodstream infections.

Pulmonary Infections

The usual microbial causes of pneumonia in the transplant recipient are listed in Table 308.9. Pneumonia accounts for up to 80% of infections in solid-organ transplant (SOT) recipients and can occur at any time in the posttransplantation period.[64] The etiology varies by transplant type and time of onset after transplant, with nosocomial pathogens predominating in the early posttransplantation period and opportunistic and community-acquired pathogens occurring later. Guidelines for the treatment of community-acquired and health care–associated pneumonia have been published elsewhere.[65,66] Knowledge of pretransplant colonizers, particularly for lung transplant recipients with CF; pretransplantation serologies, for example CMV, recent exposures; hospitalizations; and prior antimicrobial courses should be part of the evaluation. Community-acquired respiratory viruses, such as influenza virus, PIV, RSV, MPV, coronavirus, rhinovirus, or adenovirus, may cause pneumonia in transplant recipients.

Opportunistic causes of pneumonia can occur at any time after the first month, although the net state of immunosuppression determines the ongoing risk of opportunistic pathogens. For instance, transplant recipients with stable doses of immunosuppression many years after transplantation would be considered at low risk for pulmonary infections due opportunistic pathogens, whereas those whose immunosuppression has been augmented for the treatment of rejection would be considered at higher risk. In the latter group a thorough evaluation for fungal infections (endemic mycoses in cases of compatible epidemiology, invasive mold disease, cryptococcosis), nocardiosis, mycobacterial, *P. jirovecii, Rhodococcus equii*, and other pathogens should be undertaken. In cases where opportunistic infections are suspected, the appearance of the radiographic abnormalities may assist in informing both diagnostic and therapeutic decisions, with the caveat that there is substantial overlap between different entities. Nodular infiltrates have a broad range of infectious etiologies (see Table 308.10), and certain clinical and radiographic features may be helpful in discerning a specific etiology.[67] In one study, for example, pulmonary nodules were found to have diverse etiologies and were infectious in 56% of patients (bacterial, 22%; fungal, 33%; viral, 2%) and noninfectious in 44% (PTLD, 25%; carcinoma, 18%).[67]

Acute rejection in lung transplant recipients can mimic pulmonary infections.[68] Thus early bronchoscopy, with appropriate microbiologic testing and culture and nonculture diagnostics of the blood, are crucial in establishing the diagnosis when the patient fails to respond to presumably appropriate antimicrobial therapy or when the suspicion for opportunistic pathogens is high. Whether empirical therapy directed against opportunistic pathogens is warranted should be considered on a case-by-case basis; indeed, it is often difficult to rationalize or justify a regimen that will treat all possible etiologies, underscoring the importance of a thorough microbiologic investigation.

Empirical therapy to cover common typical and atypical bacterial pathogens can be initiated while culture results are awaited. Patients who have an illness lasting longer than 7 days, who have a nonproductive cough, or who have diffuse infiltrates or nodular lesions on chest imaging are more likely to have an unusual or opportunistic pathogen and should undergo invasive diagnostic procedures, including bronchoscopy, in a timely manner to establish the diagnosis.

Tuberculosis

M. tuberculosis is a major pulmonary pathogen, particularly in geographic areas where TB is endemic. The frequency of TB in transplant recipients ranges from 1.2% to 15%, with higher rates in endemic areas.[69] Most cases of posttransplantation TB are due to reactivation of latent infection in low endemicity areas. Prior exposure to *M. tuberculosis* (positive tuberculin skin test [TST], positive interferon-γ [IFN-γ] release assay [IGRA], and/or residual TB lesions in pretransplantation chest imaging), dialysis, and T-cell–depleting antilymphocyte antibodies confer a higher risk for TB. IL-2 receptor antibodies (basiliximab), on the other hand, do not appear to increase this risk. Although most transplant recipients develop TB in the first year after transplantation, up to one-third of the patients, particularly kidney transplant recipients may develop TB later in the posttransplant period.[69] Extrapulmonary or disseminated forms of disease are more frequent in the first 6 months after transplantation.

Diagnostic Considerations

Although the diagnostic yield of TST is low, it remains the initial step in the detection of latent infection. IGRAs are whole-blood tests that can also aid in the diagnosis of *M. tuberculosis* infection. QuantiFERON-TB (QFT) Gold In-Tube test (Qiagen, Summit, NJ) and T-SPOT (Oxford Immunotec, Marlborough, MA) are approved by the FDA. The data on the performance of QFT Gold Plus, a new generation of QFT assay are limited.

QFT Gold In-Tube test is technically easier to perform; however, T-SPOT had greater sensitivity and specificity in immunosuppressed patients.[70] In kidney transplant recipients, QFT Gold In-Tube test had higher positivity than TST for determining the risk of latent infection and development of TB.[70] Neither TST nor IGRAs, however, differentiate between tuberculous infection and disease. In addition, in patients with T-cell anergy due to end-stage organ failures, IGRAs may be falsely negative, which is usually signified by a poor mitogen response in the control tube.

Management of Tuberculosis

All transplant candidates should undergo TST testing, even if they have been vaccinated against *Mycobacterium bovis* (bacillus Calmette-Guérin [BCG]). Many centers have shifted to IGRA testing instead. Patients previously treated for latent infection or for active disease need not undergo TST or IGRA. Chest radiography should be performed to evaluate for old healed TB. Upon exclusion of active TB, treatment for latent infection can be initiated while awaiting transplantation and, if necessary, continued for an appropriate duration after the transplant. Treatment for latent infection should be used in transplant candidates awaiting transplantation or transplant recipients who have any one of the following risks: (1) TST with an induration ≥5 mm and/or a positive IGRA; (2) clinical or radiographic evidence of untreated TB, that is, apical scaring, calcification in the lungs or lymph nodes, pleural thickening; or (3) history of contact with a patient with active TB.[69] Patients who have previously received adequate treatment for latent infection or active TB need not be treated again.

Treatment of Latent Tuberculosis

Isoniazid (300 mg/daily) supplemented with pyridoxine (vitamin B_6) for 9 months is the treatment of choice for latent TB. All patients receiving isoniazid should be routinely monitored for hepatotoxicity. Management of latent TB in liver transplant recipients can be challenging, given potential the risk of hepatotoxicity. A systematic review and meta-analysis of individual patient data, however, documented minimal isoniazid-related hepatotoxicity and significant reduction in TB reactivation in liver transplant recipients.[71] In patients with severe, decompensated liver disease, it is reasonable to defer latent TB infection therapy until after transplantation. Thus, although generally well tolerated even among pre–liver transplant patients, alternatives to isoniazid may be needed when hepatic dysfunction ensues.[72] Rifampin for 4 months carries a lower risk for liver injury than isoniazid but is a potent inducer of the cytochrome P450, accelerates the metabolism of immunosuppressive agents, and may lead to rejection and allograft loss (see Table 308.6). It is advisable to use regimens that include rifamycins before transplantation, given their significant drug interactions with the immunosuppressive drugs. A recently approved regimen of isoniazid-rifapentine once weekly for 12 weeks significantly shortens treatment duration but does not avoid isoniazid adverse effects or rifamycin-related effects on drug metabolism.[73] Rifabutin, a rifamycin, is a less potent cytochrome inducer than rifampin and has been used for the treatment of latent TB in the transplant setting.[72] Limited data exists with the use of quinolones treatment of latent TB. Levofloxacin use in one report was associated with a high incidence of tenosynovitis.[74]

Treatment of Active Tuberculosis

Treatment of TB in transplant recipients is similar to that in the general population with the following caveats. Rifampin is associated with a profound interaction with the immunosuppressive agents that poses a risk of graft rejection and warrants careful monitoring of the levels of these drugs. The dose of calcineurin inhibitors should be increased, and levels should be closely monitored (see Table 308.6). Even with careful monitoring, concurrent use of rifampin incurs substantial risk of rejection, graft loss, and overall higher mortality. Rifabutin may be considered instead of rifampin. Treatment should be continued for 12 to 18 months of for rifamycin-sparing regimens. It is noteworthy that, as in HIV-infected patients, transplant recipients upon initiation of treatment can develop immune reconstitution syndrome (IRS) that mimics worsening or progression of disease and may manifest as fever, lymphadenopathy, or worsening respiratory symptoms.[75] Awareness of this entity can obviate unnecessary changes in treatment.

Pulmonary Infections Due to Endemic Mycoses

Coccidioidomycosis

Coccidioides immitis and *Coccidioides posadasii* are the etiologic agents of coccidioidomycosis. These dimorphic fungi are normal residents of selected desert soils of the lower Sonoran Life Zone and are present only in the Western Hemisphere. The areas of highest endemicity in the United States include the southwestern Arizona, the San Joaquin Valley of California, and West Texas. Coccidioidomycosis is also found in southern Nevada, New Mexico, Utah, and coastal southern California. Outside of the United States, this fungus is found in high concentrations in northern Mexico, with limited endemicity in Central and South America.

Coccidioidomycosis is acquired by inhalation of arthroconidia that undergo morphologic transformation into spherules in the terminal bronchioles, resulting in a primary pulmonary infection. Coccidioidomycosis occurs in 1.5% to 8.7 % of the transplant recipients in endemic areas.[76] Infections typically occur in the first year after transplantation. Delayed onset of coccidioidomycosis may occur in patients who receive antifungal prophylaxis in the early posttransplantation period. Pneumonia is the most common manifestation. However, extrapulmonary dissemination resulting in meningitis, osteomyelitis, fungemia, and skin lesions may occur in up to 75% of the transplant recipients, and mortality rates range from 50% to 63%.

In patients with pretransplant history of coccidioidomycosis or asymptomatic seropositivity, targeted prophylaxis with fluconazole is recommended, typically for a prolonged duration (6–12 months or longer).[76] More recently, fluconazole prophylaxis for 1 year for all recipients in endemic regions has been shown to be superior to targeted prophylaxis.[77] For patients already receiving itraconazole, voriconazole, or posaconazole for *Aspergillus* prophylaxis, no additional fluconazole is required. Although caspofungin has modest in vitro anticoccidioidal activity, the clinical efficacy of this agent for treatment or prophylaxis of coccidioidomycosis is not established. Therefore transplant recipients whose antifungal prophylaxis comprises an echinocandin should receive additional prophylaxis with an azole.

Fluconazole and itraconazole are the antifungal agents of choice for the treatment of coccidioidomycosis.[78] For patients with rapidly progressive or severe infection, a lipid formulation of amphotericin B is recommended, followed by secondary prophylaxis with fluconazole or another azole to avoid relapse.[78] Although there are no standard recommendations for the duration of total treatment, a patient

should be treated until the infection is quiescent based on clinical, serologic, and radiographic follow-up, and this may take months or years to achieve. For coccidioidal meningitis, lifelong treatment is recommended.

Histoplasmosis

Histoplasma capsulatum is a dimorphic fungus that resides in the soil of certain regions of North, Central and South America, Africa, and Asia. Up to 75% of individuals residing in certain areas of the Ohio and Mississippi River Valley of the United States may have been infected with *H. capsulatum*. However, most are asymptomatic. In the absence of outbreaks in the community, histoplasmosis has occurred in 0.1% to 0.5% of transplant recipients.[79] Histoplasmosis is acquired by inhaling microconidia or hyphal fragments of the mold phase of the organism. It is widely postulated that histoplasmosis in transplant recipients represents reactivation of latent infection; however, the supportive evidence is weak at best.[80] Pulmonary disease is the most common manifestation; common findings include fever, weight loss, hepatomegaly, splenomegaly, lymphadenopathy, cutaneous or subcutaneous lesions, and mucosal lesions in the mouth or GI tract. CNS involvement occurs in about 5% to 10% of cases.

Liposomal amphotericin B is recommended for patients with moderately severe or severe manifestations of histoplasmosis, with transition to itraconazole after a 1- to 2-week induction phase, except in patients with CNS involvement, in whom 5 to 6 weeks of liposomal amphotericin B is recommended.[80] Itraconazole should be continued for at least 1 year with periodic monitoring of *Histoplasma* antigen. Fluconazole is less effective and is not recommended unless itraconazole is contraindicated or not tolerated. Voriconazole and posaconazole are active in vitro against *H. capsulatum*; however, the yeasts are resistant to the echinocandins.

Routine screening for histoplasmosis before transplantation is not recommended, given that the incidence even in endemic areas is below 0.5%. Assuming a sensitivity of 98% and specificity of 98% for the screening test, the positive predictive value would be 20%. As such, only one in five individuals treated for a positive result could benefit, although four would be subjected to the adverse effects and drug interactions associated with itraconazole.[80] However, patients who have recovered from active histoplasmosis, with or without treatment, during the 2 years before the initiation of immunosuppression may be considered for itraconazole prophylaxis.[81]

Abdominal and Gastrointestinal Infections

Intraabdominal infections in liver transplant recipients have already been discussed. These infections also occur with an increased frequency among recipients of pancreas and small bowel transplants. They are less common after transplantation operations that do not involve the abdomen. When they do occur, they are usually related to preexisting medical conditions, such as biliary stones or diverticulosis.

Studies in developing countries show that transplant recipients are very susceptible to *Salmonella* infection, but infections caused by *Shigella or Campylobacter* do not appear to be increased in frequency. *C. difficile* has emerged as one of the most common causes of diarrhea in transplant recipients who are heavily treated with antibiotics. *C. difficile* colitis developed in 6% of the transplant recipients, with 73% of the cases occurring within the first 30 days.[82] Toxic megacolon may develop in 5% to 12% and recurrent colitis in approximately 22% of organ transplant recipients who have *C. difficile* colitis. *Helicobacter pylori* infection has been associated with a low-grade gastric lymphoma called mucosa-associated lymphoid tissue lymphoma in a small number of transplant recipients. Most of these tumors have regressed after reduction of immunosuppression and use of antibiotics to treat the *Helicobacter* infection. Hyperinfection and disseminated infection with *Strongyloides* were substantial problems in the past but appear to have virtually disappeared. Universal pretransplantation screening for *Strongyloides* is probably not cost-effective, but vigilance for this infection should be maintained for patients from endemic areas. Screening patients with residence in or extensive travel to endemic areas is reasonable.

Diarrhea

Diarrhea in transplant recipients can be self-limited, mimicking the course of viral and bacterial causes of diarrhea in the general population. Polypharmacy and the immunosuppressive regimen, particularly mycophenolate, can also lead to diarrhea in these patients.[83] *C. difficile* infection is a more frequently encountered problem in transplant recipients than other hospitalized populations.[84] Norovirus infection has only recently been identified as a cause of protracted diarrhea in transplant recipients and may be associated with severe wasting, debilitation, and sometimes mortality.[11,85] Patients with protracted diarrhea should have their stool tested for norovirus, as that can obviate the need for invasive procedures. CMV, mycobacteria, *Cryptosporidium, Giardia,* microsporidia, and other pathogens can also cause diarrheal syndromes; an endoscopic evaluation should be considered in cases of refractory diarrhea with no known cause.

Central Nervous System Infections

CNS infections in transplant patients require prompt diagnosis and early appropriate therapy. Table 308.11 lists the most important agents. Absent notably from the list are pyogenic bacteria and HSV, which are otherwise common pathogens in transplant patients at sites outside the CNS. The highest risk for opportunistic CNS infection is from 1 to 6 months after transplantation; an exception is cryptococcal meningitis, which is often a "late" event. CNS infections should always be suspected in febrile transplant recipients with headache, altered mental status, seizures, focal neurologic deficit, or a combination of these manifestations. CNS imaging (via CT scan or magnetic resonance imaging [MRI]) should generally be undertaken in these patients, to identify any focal lesions that may point toward brain abscesses due to bacteria, molds (*Aspergillus,* agents of mucormycosis, and *Scedosporium*), *Nocardia*, and toxoplasmosis. Neurologic opportunistic infections that do not manifest as focal lesions include CMV, HSV, VZV, JC virus (JCV), HHV-6, and *Listeria monocytogenes*.[64] Lumbar puncture should be performed as indicated. It is important to remember that altered mental status in the transplant setting can also result from malignancies (such as PTLD) and vascular, toxic, or metabolic etiologies, including side effects of immunosuppressive agents.[86]

L. monocytogenes typically causes bacteremia, meningitis, and at times cerebritis in transplant recipients. The Gram stain of the cerebrospinal fluid (CSF) is negative in more than half of the cases. Usually, the diagnosis is made by culturing the organism from CSF or blood. All patients with *Listeria* bacteremia should undergo lumbar puncture, even in the absence of CNS signs, because the mortality rate is much higher when CNS disease is present. Infection responds well to antibiotic therapy, but relapses may occur. Routine use of TMP-SMX for prophylaxis has been effective for prevention and may explain why listeriosis is observed less frequently in the current era.

TABLE 308.11 Key Pathogens of the Central Nervous System in Transplant Recipients

Bacteria
Listeria monocytogenes
Nocardia spp.
Viruses
Varicella-zoster virus
Polyomavirus (JC virus)
Human herpesvirus 6
West Nile virus
Zika virus
Fungi
Cryptococcus neoformans
Dematiaceous fungi
Aspergillus spp.
Agents of mucormycosis
Other Pathogens
Toxoplasma gondii

Aspergillosis is an important cause of CNS infection in immunocompromised patients. The most common portal of entry is the lung; invasive sinus infection with continuous spread also occurs but is less common. Patients with aspergillosis typically have risk factors such as renal dysfunction or require augmented immunosuppression. *Aspergillus* is an angiotropic mold, and hematogenous spread to the brain can occur early in the course of the infection. CNS imaging reveals single or multiple enhancing lesions with predilection for the gray-white junction, and galactomannan can be detected in blood or CSF is most patients.

Other fungi that may cause parenchymal brain infections in transplant recipients include the agents of mucormycosis (Mucorales) and dematiaceous fungi, such as *Cladophialophora*. The overall mortality rate is high in patients with CNS infection caused by Mucorales or dematiaceous fungi, and therapy with a combination of antifungal medication and surgical resection is generally recommended.

Cryptococcal meningitis usually occurs in the late posttransplantation period and has a subacute course. Pulmonary disease caused by *Cryptococcus* coexists in about 40%, and fungemia is present in 33% to 35% of the cases.[87] Lumbar puncture should be performed in any patient with a positive serum cryptococcal antigen or blood culture, even in the absence of CNS manifestations, given that documentation of CNS disease has an impact on treatment recommendations. The spinal fluid usually has fewer than 500 white blood cells/mL, with lymphocytic predominance and a positive cryptococcal antigen test. India ink preparations reveal positive findings in 40% to 50% of patients. Serum cryptococcal antigen is positive in 88% to 98% of transplant recipients with CNS cryptococcosis.

Up to 33% of the patients with CNS cryptococcosis have CNS lesions due to *Cryptococcus* on neuroimaging.[87] New lesions developing after initiation of antifungal therapy may represent IRS, an inflammatory tissue response that results from improvement in cellular immunity after reduction or cessation of immunosuppression and reversal of *Cryptococcus*-associated immunosuppression. Overall mortality in SOT recipients with cryptococcosis remains ≈15% to 20%. Receipt of calcineurin inhibitors is associated with a lower mortality rate that may be attributable in part to the synergistic interactions of calcineurin inhibitors with antifungal azoles. In transplant recipients with cryptococcosis, discontinuation of calcineurin inhibitors was the only modification of immunosuppression that influenced the development of IRS. Patients in whom the calcineurin inhibitor was discontinued had a fivefold higher risk of IRS.[88] Neither discontinuation nor reduction of other immunosuppressive agents, such as antimetabolites or corticosteroids, affected this risk. Because calcineurin inhibitors also have synergistic interactions with antifungal agents, continuation at reduced dosages, as opposed to stopping these agents, appears rational in minimizing the risk of IRS.

Although active opportunistic infections generally preclude transplantation, emerging data suggest that transplantation after recent cryptococcal disease in persons with cirrhosis may not be a categoric exclusion and may be cautiously undertaken in liver transplant candidates who are otherwise deemed clinically stable.[89]

T. gondii is a protozoan that can cause a nonspecific encephalopathy, diffuse meningoencephalitis, or progressive single or multiple brain lesions. *Toxoplasma* infection has been reported in all types of SOT but has most often been described in cardiac transplantation because the organism can become encysted in cardiac muscle after primary infection. Recipient seronegative status with primary infection posttransplantation appears to be most likely mechanism. The donor heart can then become a source of infection for a nonimmune cardiac recipient. Serology should be performed on cardiac donors and recipients to identify patients at risk for disease transmitted by the allograft. The fatality rate is high, and often the diagnosis is not established until autopsy. The treatment of choice is pyrimethamine and sulfadiazine with the addition of folinic acid to the regimen to prevent marrow suppression.

Nocardia spp. may cause single or multiple brain abscesses or, less commonly, meningitis. The primary portal of infection is pulmonary with potential for spread to bone, skin, and CNS. *Nocardia* brain abscesses may benefit from stereotactic biopsy and surgical drainage in addition to long-term (9–12 months) antimicrobial therapy. Sulfonamides are the mainstay of treatment because they penetrate the CNS well, and most isolates are susceptible. Combination therapy is typically used for disseminated disease. Agents such as amikacin, imipenem, and cefotaxime have shown good activity, with more rapid killing than sulfonamides.

JCV is an important cause of CNS infection and is discussed separately later.

SPECIFIC PROBLEMS OF VIRAL INFECTIONS

This section covers most issues related to viral infections in transplant recipients (Tables 308.12 and 308.13). Hepatitis viruses were discussed earlier. The approach to antiviral prophylaxis and the

TABLE 308.12 Routine Laboratory Tests Recommended Before Transplantation

Cytomegalovirus IgG antibody
Epstein-Barr virus IgG antibody
Herpes simplex (types 1 and 2) antibody
Varicella-zoster IgG antibody
Toxoplasma IgG antibody (heart transplant recipients)
Hepatitis B screen[a]
Hepatitis C enzyme immunoassay[b]
Human immunodeficiency virus antibody
Tuberculin skin test or interferon gamma release assay for tuberculosis
Stool for ova and parasites; *Strongyloides* antibody[c]
Trypanosoma cruzi antibody[d]
Syphilis screening (EIA or RPR)

[a]Should include surface antigen, core antibody, and surface antibody.
[b]Liver candidates and patients with laboratory or clinical evidence of liver disease should also undergo a polymerase chain reaction assay for hepatitis C.
[c]Primarily useful for patients at risk for strongyloidiasis, such as former or current residents of tropical and subtropical regions.
[d]For residents of endemic areas in Central and South America.
EIA, Enzyme immunoassay; *IgG,* immunoglobulin G; *RPR,* rapid plasma reagin.

TABLE 308.13 Infections With the Potential for Donor Transmission (See Chapter 304)

Viruses

Viral hepatitis (B, C, and E)
Herpes viruses (cytomegalovirus, Epstein-Barr virus, herpes simplex virus)
Human immunodeficiency virus
Human T-lymphotropic virus I/II
West Nile virus
Lymphocytic choriomeningitis virus
Rabies
Creutzfeldt-Jakob disease
JC virus infection (progressive multifocal leukoencephalopathy)
Dengue
Arenaviruses

Bacteria

Bacteremia
Bacterial meningitis (*Neisseria meningitidis, Streptococcus pneumoniae*)
Bacterial pneumonia or donor colonization (lung transplants)
Tuberculosis and nontuberculous mycobacteria
Borrelia burgdorferi

Fungi

Candidiasis
Cryptococcosis
Aspergillosis
Endemic mycosis (histoplasmosis, coccidioidomycosis)
Agents of mucormycosis

Other Agents

Toxoplasmosis
Trypanosoma cruzi (Chagas disease)
Leishmaniasis
Strongyloides stercoralis
Balamuthia mandrillaris
Naegleria fowleri
Treponema pallidum (syphilis)
Babesia
Malaria
Schistosomiasis

pretransplantation evaluation of candidates and donors are discussed elsewhere in this book.

Cytomegalovirus

Despite progress in treatment and prevention, CMV remains one of the most important infections in the setting of organ transplantation, resulting in substantial morbidity and mortality. A DNA herpesvirus, CMV establishes latency in cells of the myeloid (but not lymphoid) lineage after primary infection,[90] and seroprevalence rates range from 30% to 97%.[91] Both innate and adaptive immunity are required to control CMV, although the role of the innate immune system is incompletely understood.[92] CMV is a ligand for Toll-like receptor 2 (TLR2), and TLR2 polymorphisms (Arg753Gln) have been associated with an increased risk of CMV reactivation, prolonged replication, and disease after liver transplantation.[93] Control of CMV ultimately depends on multiple immune mechanisms, including the NK cells of early-acting innate immunity and the $CD4^+$ (T-helper or Th), and $CD8^+$ (cytotoxic) T cells.[94] NK cells are the first line of defense against CMV, but their activity is transient, and the cellular arm of the adaptive immune response is ultimately required to limit viremia. CMV-specific neutralizing antibodies appear 2 to 4 weeks after primary infection,[92] but clinical observations imply that humoral immunity plays only a part in the control of CMV.[95] Indeed, antibodies do not terminate CMV infection but, rather, limit dissemination of reactivated latent virus.[96] Thus, although donor and recipient CMV serostatus is used to stratify the risk of CMV infection after transplantation, posttransplantation seroconversion has limited ability to predict CMV immunity or risk of disease.[97]

Risk Factors

Patients at highest risk for CMV infection are seronegative recipients of seropositive organs (D^+/R^-). These patients do not have a preexisting pool of CMV-specific $CD8^+$ memory T cells or antibody and thus have up to a 92% incidence of viremia and 50% to 65% rate of symptomatic infection by 90 days posttransplantation without prophylaxis.[91,98] Seropositive recipients (R^+) are at an intermediate risk of CMV infection, with approximately 40% to 60% developing viremia without prophylaxis.[91] There is a nuanced difference in the CMV risk between the D^+/R^+ and D^-/R^+ groups. In the former, viremia may represent either donor-derived infection or recipient-derived reactivation of latent virus, a distinction that is not possible to make without viral sequencing. Seronegative recipients of seronegative organs (D^-R^-) are at low risk of transplant-related infection, although they can acquire primary CMV infection from blood products or other mechanisms.

The use of lymphocyte-depleting antilymphocyte antibodies (e.g., Thymoglobulin and alemtuzumab) is also associated with CMV disease, particularly when used as antirejection as opposed to induction therapy.[91,99] Absolute viral load and viral kinetics appear to be important determinants of CMV disease.[100,101] In part due to the higher burden of transplanted lymphoid tissue, lung and small bowel transplant recipients are at a higher risk of CMV compared with other organ transplant recipients. Coinfection with other herpesviruses (HHV-6 and HHV-7) appear to enhance, whereas immunosuppressive regimens that include mTOR inhibitors (sirolimus, everolimus) reduce the risk of CMV.[102,103]

Clinical Manifestations

CMV may present as (1) CMV infection or viremia that implies CMV replication, irrespective of attributable symptoms, and (2) CMV disease, which is symptomatic CMV infection.[91] In the absence of preventive therapy, CMV infection occurs in 36% to 100% and symptomatic disease in 11% to 72% of transplant recipients. Primary infections occurring in seronegative recipients with a seropositive donor are more likely to be symptomatic.

The most common type of CMV disease is mononucleosis-like viral syndrome with fevers, malaise, and cytopenias. Abnormalities may be found on liver function tests, although jaundice rarely occurs. Tissue-invasive disease may manifest as pneumonitis, GI disease, or hepatitis. In this era, when prolonged antiviral prophylaxis is routinely used, CMV disease in organ transplant recipients often is a late manifestation, and ≈30% of these cases have tissue-invasive disease.[104] Interstitial pneumonia is the most serious complication of CMV infection and is present in most fatal cases. CMV pneumonia may coexist with other pathogens in the lung, particularly *Pneumocystis*.

GI CMV disease typically manifests as ulcerations in the GI tract that may be found anywhere from the esophagus to the rectum. Severe complications, such as bleeding or perforation, occur in some patients. Patients with tissue-invasive disease in the absence of viremia are referred to as having compartmentalized disease, and this presentation is often seen with GI CMV disease. CMV hepatitis occurs in up to 17% of liver transplant recipients and is more common with primary than with reactivation infection. The pathologic finding is microabscesses scattered around the liver lobule. CMV inclusion bodies may be readily seen or scant.

Laboratory Diagnosis and Immune Monitoring

The greatest advance in regard to CMV in transplantation in recent years has been the development of accurate and reproducible quantitative molecular methods, including an international laboratory standard, to assess viral load. Most laboratories performing quantitative nucleic acid testing (NAT) for CMV are now using PCR-based assays that offer good precision and accuracy, have broad dynamic range, and relatively shorter turnaround time than conventional PCR assays.

There is growing interest in the use of CMV-specific immune assays to facilitate clinical decision making regarding antiviral therapy for the management of CMV. Instead of broadly applying a "one size fits all" strategy, knowledge of an individual patient's anti-CMV immune responses could conceivably allow more optimal risk stratification. It is hoped that this would result in more efficacious, individualized strategies to prevent and treat CMV by customizing the duration of prophylaxis, postprophylaxis monitoring, preemptive therapy, and treatment of CMV viremia.[105,106] Transplant recipients with CMV viremia and who lacked CMV-specific $CD8^+$ T-cell responses failed to control CMV despite prolonged antiviral therapy. In contrast, all but one patient with detectable CMV-specific $CD8^+$ T-cell responses had suppressed viremia after the discontinuation of therapy.[107] Although these results are promising, CMV-specific immune assays are not FDA approved in the United States, and other data show that relying on a single immunologic marker may not capture the full nuance of anti-CMV immunity.[108] Thus at present it is unclear whether to incorporate these assays into clinical practice.

Prevention

There are generally two strategies for the prevention of CMV: (1) prophylaxis, whereby "at-risk" patients are given antivirals (typically valganciclovir) for a defined period (usually 3–6 months), and (2) preemptive therapy, whereby SOT recipients are monitored and treated for evidence of early-stage CMV viremia in an attempt to prevent progression to CMV disease.[91] Although each of the strategies has been studied individually and been shown to be effective for the prevention of CMV (compared with either placebo or no-prevention strategy), there is paucity of direct comparative studies. As such, the benefit of one strategy over the other has long been debated. Because the totality of evidence supporting the use of preemptive therapy is more limited than for prophylaxis,[91,109] prophylaxis with valganciclovir has emerged as the dominant strategy for the prevention of CMV in D^+/R^- transplant patients. However, prophylaxis is associated with adverse drug reactions such as cytopenias, which can be compounded by the concurrent use of mycophenolate mofetil, azathioprine, and prophylactic TMP-SMX. In addition, some patients are given longer durations of prophylaxis that might be necessary, and others go on to develop late-onset CMV disease once prophylaxis is completed. Indeed, 30% to 40% percent of D^+/R^- patients will develop late-onset CMV disease upon discontinuation of prophylaxis[110] that is associated with higher mortality, bacterial and fungal superinfections, and graft loss.[111] Viral monitoring after completion of prophylaxis appears to have limited efficacy for the prevention of late-onset CMV disease.[112] Prolonged prophylaxis (up to 12 months) is frequently used in lung recipients, particularly in the D^+/R^- group.[113] Antiviral prophylaxis is appropriate in patients receiving lymphocyte-depleting agents for rejection, although preemptive monitoring may also be acceptable.[91]

A recent randomized controlled trial has shown that preemptive therapy significantly reduced CMV disease compared to prophylaxis in high-risk D^+/R^- liver transplant recipients.[113a] Based on these data, the 2019 American Society of Transplantation CMV guidelines now recommend preemptive therapy for CMV prevention in these patients.[113b]

Challenges with preemptive therapy are logistic coordination for screening and promptly acting upon the results.[91] In addition, the optimal viral load thresholds to guide the initial of antiviral therapy, the duration of therapy, and laboratory monitoring are not known. Some patients with low-level viremia will have spontaneous clearance,[92] whereas concerns have been expressed about the possibility of failure of this strategy among CMV D^+/R^- lung transplant recipients who may experience rapid replication of CMV.[91,114] However, in contrast to prophylaxis, CMV disease has been documented in only 2.6% of patients receiving preemptive therapy in the current era and occurs largely within 100 days of transplantation.[104] Possible biologic basis for the relative lack of late-onset CMV disease with the preemptive approach is that by allowing controlled CMV replication to occur before an antiviral agent is administered, the patient's immune system is primed, resulting in enhancement of CMV-specific humoral and cellular immune responses that are critical for long-term control of the virus.[115] In contrast, viral replication is almost completely suppressed with the use of antiviral prophylaxis,[116] which might prevent development of CMV-specific immune responses.

Other strategies for CMV prevention exist but limited evidence exists to support their routine use. CMV immunoglobulin and intravenous immune globulin (IVIG) have been used for prophylaxis, but this is an uncommon practice in the valganciclovir era.[117–120] There is considerable interest in a CMV vaccine, but clinical trials have shown mixed results.[121] A CMV glycoprotein-B vaccine resulted in increased anti-CMV antibody titers and reduced CMV viremia,[122] whereas the DNA-based vaccine ASP0113 failed to show any difference in CMV viremia in D^+/R^- kidney transplant recipients when given after transplant.[123] Additional studies on CMV vaccines, including consideration of pretransplantation administration, are needed in high-risk transplant candidates and recipients.

Treatment

IV ganciclovir or its oral derivative valganciclovir are the mainstay for the treatment of CMV disease. The duration of treatment is typically at least 2 weeks, with documentation of clearance of viremia and resolution of signs and symptoms of CMV.[91] The need for secondary prophylaxis or posttreatment monitoring is not standard and can be considered based on the specific clinical situation,[91] although in a study of kidney and liver transplant recipients, secondary CMV prophylaxis did not provide additional benefit after the treatment of CMV disease.[124]

Ganciclovir-resistant CMV poses a unique challenge. Ganciclovir requires phosphorylation by a viral kinase encoded by the *UL97* gene (unique long, open reading frame 97), followed by additional phosphorylation steps by cellular kinases.[125] It then inhibits the viral DNA polymerase, encoded by the *UL54* gene. Mutations in both *UL97* and *UL54* can arise, conferring ganciclovir resistance. Risk factors for resistance include prolonged low-dose oral prophylaxis, D^+/R^- serostatus, profound immunosuppression, lung transplantation, initial high-grade viremia, and suboptimal drug dosing during the treatment of CMV, which is often done in the setting of cytopenias.[91,126] Although some *UL97* mutations can respond to dose-escalation of ganciclovir, mutations conferring high-level resistance do not, necessitating the use of foscarnet and cidofovir, both of which are profoundly nephrotoxic.[127] Neither foscarnet nor cidofovir require phosphorylation via the viral kinase and are thus unaffected by *UL97* mutations. They both inhibit the viral DNA polymerase, and resistance to all three drugs can be conferred by viruses with certain *UL54* or dual *UL97-UL54* mutations.

Patients with drug-resistant CMV have poor outcomes, as do those with "refractory CMV" who have been ongoing CMV viremia and disease while receiving ganciclovir, despite no objective evidence of resistance on CMV genotyping.[127] Reduction of immunosuppression or a switch to an mTOR-inhibitor–based regimen, may be considered on an individual basis in case of severe disease.[91] The use of CMV-specific immunoglobulin for the treatment of resistant/refractory CMV is of unproven efficacy but is often used.[128] Unlike hematopoietic cell transplant recipients, adoptive immunotherapy for the treatment of ganciclovir-resistant CMV is rarely used in organ transplantation due in part to lack of availability, the hypothetical risk of precipitating graft rejection, and concerns that efficacy of the transfused T cells may be reduced in presence of calcineurin inhibitors and corticosteroids. However, autologous CMV-specific T cells from a CMV D^+/R^- lung allograft recipient with ganciclovir-resistant CMV disease resulted in long-term reconstitution of protective antiviral immunity, CMV infection, disease-free survival, and no allograft rejection.[129]

Given the dismal outcomes and toxicities of available therapies, efforts are underway for new, safer drugs for the treatment of resistant CMV. Maribavir is a direct inhibitor of the UL97 viral kinase and is an alternative to foscarnet and cidofovir. Although it failed to prevent CMV infections in high-risk liver transplant recipients, this was attributed in part to the low dose used (100 mg twice daily).[130] In a phase II trial, however, that used optimal dosages, it was effective for the treatment of CMV disease resistant or refractory to standard therapy among hematopoietic cell and organ transplant recipients, with no major toxicities observed.[131] Brincidofovir (formerly CMX001) is a lipid-conjugated oral prodrug of cidofovir with no nephrotoxicity and broad antiherpesvirus activity.[91] Although it was effective against CMV in a phase I study of high-risk hematopoietic cell transplant recipients,[132] a larger phase III trial was discontinued because of associated high rates of GI toxicity. An IV formulation is being developed with the goals of reducing toxicity. Letermovir (AIC246) is a novel nonnucleoside CMV inhibitor that targets the viral terminase complex.[133] Its advantages include good oral bioavailability and lack of significant toxicity. Based on promising results in a placebo-controlled trial in HSCT recipients,[134] this agent has been FDA approved for the prevention of CMV in HSCT recipients. Limited clinical experience exists with letermovir use in the SOT setting, and resistance may develop earlier than with valganciclovir and foscarnet. A clinical trial of letermovir prophylaxis among CMV D^+/R^- kidney transplant recipients is underway. Letermovir has no activity against HSV and VZV so prophylaxis against those viruses, when used, must be continued during letermovir prophylaxis.

Epstein-Barr Virus and Posttransplantation Lymphoproliferative Disorder

EBV is a ubiquitous gammaherpesvirus that infects human B lymphocytes and epithelial cells, with seroprevalence rates of ≈50% among children age 5 years and 90% to 5% among adults.[135–137] In immunocompetent individuals, EBV results in subclinical infection during infancy and infectious mononucleosis in adolescence.[138] In organ transplant recipients, EBV infection can be transmitted from the graft or represent reactivation of latent infection. It can result in PTLD, which encompasses a spectrum of diseases ranging from lymphoid proliferation to overt lymphomas.[135]

Immunobiology

The life cycle of EBV is characterized by an early productive replicative phase after primary infection in which mature infectious virions are produced, leading to cell death (the lytic cycle), and a nonproductive phase in which the virus is incorporated into and replicates with the host DNA, with lifelong persistence of the viral genome in B cells in a latent state. These cells are kept in check by NK cells and EBV-specific cytotoxic T cells (CTLs). Posttransplantation immunosuppression has the potential to allow proliferation of EBV latently infected B cells to go unchecked.[138–140] Although abnormal B cells in SOT recipients are usually of recipient origin, these cells in HSCT recipients are typically of donor origin.

Risk Factors

Patients with a D^+/R^- serostatus are considered at the highest risk for PTLD.[135] Due to the lower rates of seropositivity in children, PTLD is more common in pediatric compared with adult organ transplant recipients, occurring at rates of about 20% and 1%, respectively. The increased risk of PTLD in recipients of lymphoid-rich organs, such as intestines and lungs, compared with kidneys, hearts, and livers, is due to the amount of infected donor B cells coming over with the graft, as

well as the overall net state of immunosuppression.[139] Other risk factors include the use of T-cell depleting antibodies, CMV infection, younger age in children, older age in adults, and HLA polymorphisms.[139] The costimulation blocker belatacept is associated with PTLD in EBV D^+/R^- recipients, predominantly involving the CNS. There is a black box warning against its use in this setting.[141]

Clinical Manifestations and Diagnosis

Primary EBV infection in organ transplant recipients can present with a variety of manifestations, including a nonspecific viral syndrome.[139] EBV-related PTLD has a bimodal pattern of onset. It typically presents early after transplantation, with most cases occurring in the first year, although late cases (5–7 years) have been reported in the aging organ transplant population. A small subset of PTLD is EBV negative and occurs later (>5 years posttransplantation). The virus has a predilection to involve the allograft but may also involve any organ, including CNS disease. EBV viral load quantitative NAT of the blood has potential for diagnosis.[139] However, although it is understood that higher viral loads correlate with disease, the optimal thresholds that should prompt diagnostics/therapeutic interventions are unknown. In addition, unlike CMV, the role of preemptive therapy with periodic viral load monitoring of high-risk patients is less well defined. Regardless, patients with high-level or escalating viremia, particularly those with D^+/R^- serostatus, should undergo computed or positron emission tomography of the neck, chest, abdomen, and pelvis and core needle or excisional biopsy of lesions. Tissue should be stained for EBER ("EBV-encoded small nuclear RNA," a marker for EBV-infected cells), and flow cytometry of lymphocytes should be performed.[139] However, some patients with EBV-driven PTLD may not have viremia if the PTLD is restricted to the graft itself or to the GI tract.[139] Thus PTLD should be considered in organ transplant recipients presenting with an unexplained febrile illness, particularly during the first year after transplantation or after augmentation of immunosuppression for rejection. Bone marrow biopsies, lumbar punctures, and other tests can be considered based on the clinical scenario.

The importance of histology for the diagnosis of PTLD cannot be overstated. PTLD manifests as polymorphic and monomorphic forms, with the latter generally associated with a worse prognosis. Histologic manifestations include monomorphic B-cell neoplasms, such as diffuse large B-cell and Burkitt lymphomas; classic Hodgkin lymphoma-type PTLD; and polymorphic forms.[135] T-cell neoplasms and EBV-associated smooth muscle tumors are rare. EBV-related hemophagocytosis has been described in the posttransplantation setting, often with devastating consequences.[142]

Prevention and Treatment

The role of antiviral drugs in the management of PTLD is controversial. Acyclovir and ganciclovir have no activity against latent virus, which is the predominant form of the virus in PTLD. Expression of Z EBV replication activator (ZEBRA) in PTLD suggests that few virions may enter the lytic cycle,[143] and based on the consideration that antivirals could potentially eliminate these virions, antiviral drugs are frequently used. However, PTLD occurs in patients receiving valganciclovir for other reasons, such as CMV prophylaxis, and EBV viral loads can rise despite the receipt of these antivirals, given that they have no activity against latently infected or EBV-transformed B cells.[144] Results of EBV vaccine studies have been disappointing,[145] and data supporting the routine use of IFN-γ, adoptive T-cell transfer, or prophylactic immunoglobulin are sparse.[146,147] A pilot study investigated the role of anti-CD20 monoclonal antibody rituximab used before transplantation for the prevention of EBV in kidney transplant recipients.[148] Among 17 EBV D^+/R^- kidney transplant candidates, the 5 who underwent living donation were treated with a single dose of rituximab 4 weeks before transplantation. None of these 5 patients developed EBV viremia, compared with 58% of those who received kidneys from deceased donors and thus did not receive rituximab. Only 1 patient developed PTLD (nonrituximab group). This strategy warrants further investigation in prospective controlled trials.

A preemptive approach whereby immunosuppression is reduced in the face of rising viremia has been associated with a lower risk of PTLD.[149] This strategy is based on the hypothesis that recovery of the host's immune system will allow the development of CTL against EBV with subsequent control of EBV-driven B-cell proliferation.[139] Rituximab has also been used as preemptive therapy in at-risk patients with escalating viremia,[150] but additional experience is needed. Adoptive immunotherapy, although successfully used in treating established EBV infections after HSCT, is an uncommon practice in organ transplantation. B-cell and T-cell malignancies should be managed using conventional chemotherapy.

Herpes Simplex Virus

Herpes simplex viruses (HSVs) 1 and 2 are ubiquitous HHVs that cause orolabial, genital herpes, and/or perianal herpes.[151] Most adults are seropositive for HSV,[152] and the majority of cases of posttransplantation HSV represent reactivation of latent virus, although rare cases of donor-transmitted infection have been reported. In the absence of antiviral prophylaxis, 35% to 75% of HSV-seropositive transplant recipients will develop clinically apparent HSV disease.[153–156] A unique manifestation of HSV occurring early after transplantation is fulminant hepatitis, hypotension, and disseminated intravascular coagulation with fatal outcomes in ≈two-thirds of cases.[157] However, the use of antiviral prophylaxis in the current era has greatly reduced the incidence of HSV.[158] Patients receiving CMV prophylaxis have lower rates of HSV reactivation, whereas those who are CMV seronegative should receive acyclovir, valacyclovir, or famciclovir for HSV-specific prophylaxis for at least 4 weeks after transplantation, as the majority of severe HSV disease occurs within the first month posttransplantation.[153] Antivirals may need to be resumed after augmentation of immunosuppression for rejection.

Mucocutaneous manifestations of HSV in transplant recipients are similar to those of the general population, with painful erythematous papules that progress to characteristic fluid-filled vesicles. These can be treated with oral acyclovir, valacyclovir, or famciclovir until all lesions are healed. The diagnosis can typically be made clinically, although direct-fluorescent antibody (DFA) testing, PCR, culture, histopathology with immunohistochemistry, and Tzank smear of lesions can be used, especially in atypical cases.[151] In transplant recipients these mucocutaneous lesions are capable of spreading into the respiratory and GI tracts by direct extension, resulting in HSV esophagitis and pneumonia. Viremic dissemination can occur. Patients with visceral disease, such as fulminant hepatitis, disseminated disease, or HSV encephalitis should be treated with high-dose acyclovir (10 mg/kg every 8 hours, adjusted for renal dysfunction). An obstructive nephropathy due to deposition of acyclovir crystals in the renal tubules may occur in dehydrated patients or if the infusion is given too rapidly. It should be noted that HSV encephalitis is a rare entity and does not occur with increased frequency in the organ transplant population; PCR of the CSF is the preferred method for diagnosis.[159]

As with ganciclovir-resistant CMV, the treatment of acyclovir resistance is challenging. Resistance rates in the immunocompromised host range from 3.6% to 6.3% and should be considered in patients with recurrent or refractory mucocutaneous disease despite appropriately dosed antivirals. Because acyclovir requires phosphorylation by a viral thymidine kinase (TK) for activity, the most common resistance mechanism is reduction or absence of HSV TK. Systemic foscarnet and cidofovir and topical treatments (cidofovir, trifluridine, and imiquimod), which do not rely on viral TK for activity, have been used in this setting.[151,160]

Varicella-Zoster Virus

VZV is spread through direct contact with skin lesions or through airborne spread from respiratory droplets.[161] Primary infection results in acute varicella or chickenpox. In the past, greater than 90% of adults in the United States were reported to have acquired chickenpox during childhood, although, in the current era children are administered a live vaccine. After primary infection, VZV remains latent for life in cranial nerves and dorsal root ganglia and can reactivate decades later as herpes zoster (shingles).[162]

Herpes zoster is reported in 7% to 18% of patients. Fig. 308.4 depicts the cumulative incidence of herpes zoster in a cohort of transplant

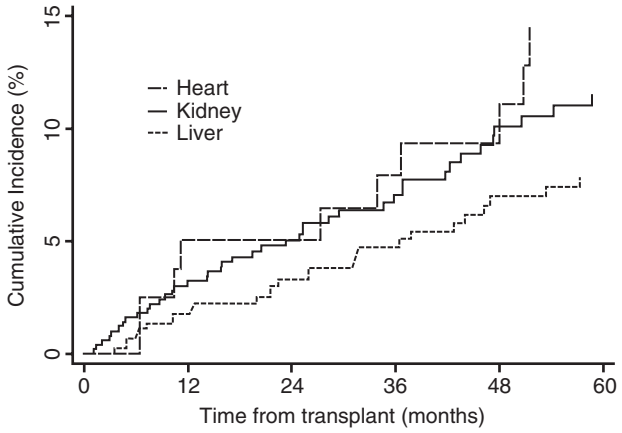

FIG. 308.4 Cumulative incidence of herpes zoster in a cohort of transplant recipients from 1996–2007 by organ type.

recipients from 1996–2007 by organ type. Induction therapy with T-cell antibodies has been a predictor for the development of herpes zoster. The majority of individuals, even those with no history of varicella, will be seropositive.[163] Regardless, all patients should undergo serologic testing to document prior exposure to VZV during their pretransplantation evaluation process. Many patients who are VZV seropositive are already receiving primary prophylaxis for CMV or HSV. In those who are both CMV and HSV seronegative but VZV seropositive, it is reasonable to administer short-term prophylactic antivirals.[161] A recent study showed that, unlike the case with HSV, the use of a preemptive approach for CMV was not associated with an increased risk of VZV infections.[158]

VZV-seronegative transplant patients are at risk for acute varicella, which can lead to extensive cutaneous involvement and visceral dissemination, including pneumonia, hepatitis, encephalitis, and disseminated intravascular coagulopathy.[164] Herpes zoster, which usually manifests as a painful vesicular dermatomal rash, may present atypically in SOT recipients, with multidermatomal rashes, multiorgan involvement, invasive complications, and even without a rash (so-called "zoster sine herpete"), highlighting the need for appropriate diagnostic testing, such as DFA of scrapings of skin lesions, PCR, and culture.[161,165,166] Herpes zoster ophthalmicus (trigeminal ganglion), Ramsay Hunt syndrome (geniculate ganglion), and VZV encephalitis have all been described in SOT.

Immunocompromised patients with primary varicella should be treated with IV acyclovir,[161] followed by a switch to an oral equivalent. Localized uncomplicated dermatomal zoster can be treated with oral antivirals for 7 days or until lesions are crusted. More severe manifestations of herpes zoster are treated with IV acyclovir, including VZV encephalitis, herpes zoster ophthalmicus, and Ramsay Hunt syndrome.

VZV seronegative patients who are exposed to persons with primary varicella or herpes zoster should receive secondary prophylaxis with varicella zoster immunoglobulin (VariZIG [SAOL Therapeutics, Roswell, GA], which was FDA approved in 2012) or, if VariZIG is not available, acyclovir or nonspecific IVIG within 10 days of exposure. They should be placed in isolation between days 10 and 21 postexposure (the incubation period of VZV), or 28 days if VariZIG is given.[161] All immunocompromised patients with any form of VZV infection, including those with localized dermatomal zoster, should be placed on airborne and contact precautions until all lesions have crusted.[167] Patients with localized lesions should be instructed to cover them.

Vaccination of household contacts is an important part of preventing VZV infections in SOT recipients.[161] In addition, transplantation patients should be isolated from vaccinated contacts who develop varicella-like rash because vaccine-associated rashes can result in transmission.[168] Because the VZV vaccine is a live-attenuated vaccine, it is generally contraindicated in organ transplant recipients and should be given pretransplantation in patients who meet criteria.[167] A study of a recombinant VZV vaccine in HSCT recipients[169] documented that the vaccine was immunogenic in patients who were ineligible for live-attenuated VZV vaccine.[170] In 2017 the recombinant VZV vaccine (Shingrix) was approved for use in persons older than 50 years.[171] Although administration of this vaccine to organ transplant recipients will likely be safe, there are no data on its use in these patients.

Human Herpesviruses 6 and 7

These viruses cause roseola in infants. Previously felt to be two variants of the same virus, HHV-6A and HHV-6B was recognized as two distinct viruses in 2012.[172] HHV-6B has been implicated in most primary infections in children and reactivation events after transplantation, whereas HHV-6A predominates in the lymph nodes of HIV-infected adults.[173–175] HHV-6 replication has been associated with CMV disease, aggressive hepatitis C recurrence in liver transplant recipients, other opportunistic infections (particularly invasive mycoses), acute rejection, and decreased graft survival.[176,177] Compared with HHV-6, less is known about the clinical characteristics of HHV-7 infection.[175] Specific testing for HHV-7 is mainly performed for research purposes as there have not been any clear clinical syndromes associated with HHV-7 infection in SOT.

HHV-6 infects multiple cell lines and establishes latent infection in mononuclear cells; it has also been found in normal brain tissue and CSF. In less than 1% of humans HHV-6 is chromosomally integrated and is inherited through the germline.[176] Because greater than 95% of humans are infected, disease usually represents reactivation of latent virus. However, it is possible for HHV-6 to be transmitted horizontally by donated chromosomally integrated HHV-6–positive organs.[178] Reactivation occurs relatively early, generally within the first 2 to 4 weeks after SOT.[175,179] Because ganciclovir has anti–HHV-6 activity, reported reactivation rates vary and depend on whether patients were administered CMV prophylaxis.[176]

Overall, 22% to 55% of the transplant recipients, particularly those who are not receiving antiviral prophylaxis for CMV, may develop HHV-6 infection, typically in the early posttransplantation period. Most patients with HHV-6 infection are asymptomatic, but a few may manifest as a febrile syndrome with leukopenia or rarely meningoencephalitis. Most infections due to HHV-6 are asymptomatic, and overt disease occurs in less than 1% of SOT recipients.[180] Symptoms include fever, rash, a nonspecific viral syndrome similar to CMV, hepatitis, gastroduodenitis and colitis, myelosuppression, and pneumonia and rarely meningoencephalitis. However, given the ubiquitous nature of HHV-6, detection of virus in blood and tissue does not prove disease, and these symptoms can only be attributed to HHV-6 after the exclusion of other more likely etiologies.[175]

When evaluating patients with possible HHV-6 disease, it is also important to consider the potential detection of chromosomally integrated HHV-6 in blood samples, characterized by persistent high-level HHV-6 viremia (more than a million copies/mL); this may be misinterpreted as substantial active infection, leading to unnecessary treatment.[181,182] In these cases fluorescent in situ hybridization with a specific HHV-6 probe performed on metaphase chromosome preparations from peripheral blood will demonstrate integrated HHV-6.[181] HHV-6 encephalitis presents with fever and confusion, sometimes with a rash; brain imaging shows signal intensities in the medial temporal lobes. In this setting, detection of HHV-6 in the CSF is required for diagnosis, although high levels of HHV-6 DNA can also be detected in the CSF in individuals with chromosomal integration. Testing hair follicles and nails for HHV-6 can be done to evaluate for chromosomal integration in these individuals.[183]

There are no specific FDA-approved antiviral drugs for the treatment of HHV-6 infection. Ganciclovir, cidofovir, and foscarnet have all shown efficacy against HHV-6 in vitro. HHV-7 appears to be resistant to ganciclovir.[184] Both HHV-6 and HHV-7 are resistant to acyclovir and penciclovir.[184] Routine use of antiviral prophylaxis or preemptive therapy for HHV-6, however, is not currently recommended.

Human Herpesvirus 8 (Kaposi Sarcoma Herpesvirus)

Unlike most herpesviruses, human infection with HHV-8 is not ubiquitous, and rates vastly differ by geographic region.[185] Seroprevalence is estimated to be between 0% to 5% in North America, northern Europe, and Asia; between 5% to 20% in the Mediterranean and Middle East; and greater than 50% in parts of Africa.[186] The incidence of active HHV-8

infection after SOT therefore mirrors its geographic distribution. After primary infection, HHV-8 exhibits a latent phase interrupted by lytic (viremic) reactivations.[187] HHV-8 is primarily transmitted via saliva or sexual routes but has also been transmitted via blood products. It can also be donor derived; residence in an HHV-8 endemic area and the receipt of an organ from a donor residing in an endemic region confers an increased risk for posttransplantation HHV-8–related disease.[12] Clinically apparent disease usually manifests a median of 30 months after transplantation (range, 3–124 months).[185] Even though ganciclovir is active in vitro, no association was found between the use of CMV prophylaxis and the development of HHV-8 infection.[12]

HHV-8 infects B cells, macrophages, endothelial cells ("spindle cells" of Kaposi sarcoma [KS] lesions), and epithelial cells.[188] It is the causative agent of KS, primary effusion lymphoma, and multicentric Castleman disease.[175] KS manifests as progressive, violaceous multifocal mucocutaneous lesions with potential dissemination to visceral organs, including the transplanted allograft. HHV-8 has been reported to cause nonneoplastic processes, such as fever, pancytopenia, and bone marrow failure; the hemophagocytic syndrome; and clonal gammopathy after transplantation. In addition, during its lytic phase, HHV-8 produces high levels of proinflammatory cytokines and chemokines, such as IL-6 and IL-10. This milieu can lead to potentially fatal organ damage, which has been seen in a newly described entity called Kaposi sarcoma herpesvirus-associated inflammatory cytokine syndrome (KICS), first reported in HIV but also seen in organ transplantation.[187]

A multidisciplinary approach is recommended when treating patients with HHV-8, with involvement of oncology, infectious disease, and dermatology specialists. Antivirals are of unproven efficacy, although ganciclovir, foscarnet, and cidofovir all have in vitro activity. However, the cornerstone of therapy should be reduction of immunosuppression. Switching from a calcineurin inhibitor to an mTOR inhibitor may be considered due to their antitumor responses.[189] Patients whose lesions do not regress with this approach may require intralesional chemotherapy, radiation therapy, surgery, or systemic chemotherapy, based on the extent of the disease. In a recent report a combined liver-kidney transplant recipient with severe KICS achieved remission after treatment with a variety of agents, including cidofovir, foscarnet, mTOR inhibitors, rituximab, corticosteroids, IVIG, and etoposide.[187]

Serologic screening of donors and recipients is not routinely performed due to the uncertainty of whether knowledge of these results would have any clinical implications or change management. In a recent multicenter prospective study from Italy, SOT donors and recipients underwent serologic testing for HHV-8 and were found to have seroprevalence rates of 4% and 18%, respectively.[12] After transplantation, 25% (3/12) of HHV-8 D^+/R^- recipients developed a primary infection, one of whom succumbed to a lethal nonmalignant HHV-8–related illness. Only 2.1% (2/93) of HHV-8 R^+ patients had viral replication, and only one developed KS. Although these numbers are small, pretransplantation HHV-8 risk stratification in high-prevalence areas appears to be reasonable, although additional data are required to define its role and the role of routine posttransplantation monitoring for HHV-8 viremia.[12]

Human Immunodeficiency Virus

The current era has seen reductions in acquired immunodeficiency syndrome (AIDS)-related deaths, and a near-normal life expectancy is expected for most patients who achieve a normal CD4 count and viral load suppression on antiretroviral therapy (ART).[190] Therefore about half of all deaths in people on ART in Europe and North America are caused not by AIDS and its associated opportunistic infections but, rather, by non–AIDS-defining cancers, cardiovascular and respiratory diseases, and end-stage liver and kidney failure. Organ transplantation of HIV-infected individuals has thus been steadily gaining traction since the late 1990s.[191,192]

Several studies have demonstrated acceptable short-term survival and no increased risk of HIV-related complications in kidney and liver transplant recipients,[191,193] despite the use of Thymoglobulin induction. Limited data with cardiac, lung, and pancreatic transplantation also seem to be promising.[194–196] A notable exception is liver transplant recipients with HCV coinfection, who in the pre–direct-acting antiviral (DAA) era experienced aggressive HCV recurrence, graft loss, sepsis, and death.[197–199] A surprising observation is that HIV-infected transplant recipients have a threefold higher risk of acute rejection,[191] thought to be related to interactions between protease inhibitors and calcineurin inhibitors, and immune dysregulation. However, the advent of integrase strand transfer inhibitors (which do not interact with the cytochrome P3A4 system) as part of the ART backbone has made dosing posttransplantation immunosuppression much less cumbersome and is anticipated to mitigate significant drug interactions seen with protease inhibitor–based regimens with the immunosuppressive agents.

Human Immunodeficiency Virus Screening

All transplantation candidates should undergo HIV testing, and persons with HIV should only be listed if they are virally suppressed on ART, with CD4 counts of ≥200 cells/μL.[200] Exceptions can be made for patients with liver cirrhosis, who may not be able to tolerate ART pretransplantation due to liver failure and in whom the CD4 count threshold is ≥100 cells/μL (due to splenic sequestration).[200]

Human Immunodeficiency Virus–to–Human Immunodeficiency Virus Transplantation

Growing disparity between the number of patients wait-listed for organ transplantation and organs available disproportionately affects persons with HIV,[190] and it has been estimated using HIV-infected deceased donors would expand the donor pool by an additional 360 to 600 donors annually in the United States.[201,202] HIV-to-HIV kidney and, to a lesser extent, liver transplants have already been performed in South Africa, Switzerland, and the United Kingdom.[203–205] In the South African trial, HIV-to-HIV deceased donor kidney transplantations were performed on 27 patients, all of whom were virally suppressed on first-line ART, with a median CD4 count of 288 cells/mm^3.[203] One-, 3-, and 5-year graft and patient survival rates were similar to those of HIV-negative transplant recipients at the same institution during the same time period, and there were no cases of AIDS-defining opportunistic infections despite the use of Thymoglobulin. In the United States the use of HIV-infected donors was made illegal in 1988, but in 2013 President Barack Obama introduced the HIV Organ Policy Equity (HOPE) Act, which aimed at legalizing HIV-to-HIV organ transplantation.[190] Currently, HIV-to-HIV kidney and liver transplantations are being performed in the United States but under clinical trial protocols only.[206–208] As data from these trials emerge, they are expected to shed light on the safety of HIV-to-HIV organ transplantation compared with the use of HIV-negative donors, on the natural history of superinfection with donor-derived virus (including resistant viruses), and on the implications of this practice on HIV viral reservoirs and cure. If the results of these trials are promising, HIV-to-HIV organ transplantation might ultimately be performed outside the research setting.

RNA Respiratory Viruses

Respiratory viral infections received little mention in early reports of infections after organ transplantation but are now recognized to be important pathogens. Influenza continues to cause significant morbidity and mortality in transplant recipients. RSV, PIV, MPV, coronavirus, and rhinovirus are an important cause of upper and lower tract infection in organ transplant recipients. The overall impact of respiratory viruses appears to be significantly greater in lung compared with other organ transplant recipients.

Recent guidelines on diagnosis and management have been published elsewhere.[209] All transplant recipients and candidates, as well as their household contacts, should receive the influenza vaccine.[210] Although its efficacy is not absolute, a recent study in nontransplant recipients showed that influenza vaccination during the 2013–14 influenza season attenuated adverse outcome among adults who were hospitalized with laboratory-confirmed influenza.[211] There is currently no effective treatment for RSV, PIV, human MPV (hMPV), rhinovirus, or coronavirus.[209] Ribavirin, IVIG, and steroids have all been studied for the treatment of RSV, PIV, and hMPV, with mixed results. Aerosolized ribavirin is expensive, teratogenic, and requires special precautions for administration and is therefore used less commonly than oral ribavirin. Observational studies showed no significant differences in 6-month

outcomes between those treated with either oral or inhaled ribavirin therapy for RSV infection after lung transplantation.[212] Palivizumab is an anti-RSV monoclonal antibody that is indicated for RSV prevention in children but has not been studied in transplant recipients. Initial studies using experimental agents, such as ALN-RSV01 for the treatment of RSV in lung transplant recipients and of DAS181 for the treatment of PIV in immunocompromised hosts, have been completed; however,[213,214] the status of definitive studies is uncertain at present. Trials evaluating the efficacy of RSV-specific antiviral agents and vaccines are underway.

Polyomaviruses

BK virus and JCV are the two most important polyomaviruses. Polyomavirus infection of the urinary tract was first described in a renal transplant recipient almost 40 years ago, and subsequent studies have shown that polyomaviruses can be detected in up to 60% of renal transplant patients.[215] For more than two decades after the discovery of these viruses, they were occasionally associated with transient renal dysfunction or ureteral stenosis. Since the late 1990s, polyomaviruses (BK virus specifically) have emerged as important causes of nephritis/nephropathy in 1% to 8% of renal transplant recipients and leads to progressive graft dysfunction/graft failure in 10% to 50% of the cases.[216] In almost all cases the responsible polyomavirus has been BK virus. JCV and SV40 may also be associated with nephropathy, but their clinical course is less severe.

It is not known why BK nephropathy has emerged only recently. The usual explanation is the introduction of new, potent immunosuppressive medications. However, other nonrenal transplant patients receiving similar immunosuppression rarely develop BK virus nephropathy, indicating that factors other than immunosuppression that are unique to the renal allograft are relevant. BK virus infection can develop either via primary infection (community- or donor-transmitted) or reactivated infection, particularly because the majority of adults are seropositive. Allograft rejection (and resulting treatment) may be an important predisposing factor. Patients with polyomavirus nephropathy typically do not have fever or other symptoms of infection and present with only a rising serum creatinine.[215]

BK virus primarily infects renal tubular cells producing intranuclear "ground-glass" inclusions accompanied by an interstitial nephritis (Fig. 308.5). Polyomaviruses may be detected in the urine by a variety of techniques, including culture, cytology, electron microscopy, and PCR; however, they are limited by low specificity for nephropathy. The specificity of PCR diagnosis can be enhanced by quantitation of BK virus in blood, with higher viral loads having greater specificity for nephropathy.[216] Although noninvasive assays play an important role, biopsy remains useful for definitive diagnosis, staging of disease (with implications for prognosis), and for identifying concomitant processes (rejection, etc.).[215] Natural history studies in kidney transplant recipients have shown a predictable pattern of progression of BK virus infection: initial detection of BK virus in urine, then in blood, then progression to BK virus nephropathy, typically over the course of months.



FIG. 308.5 BK virus primarily infects renal tubular cells producing intranuclear "ground-glass" inclusions accompanied by an interstitial nephritis.

Progressive multifocal leukoencephalopathy (PML) occurs in patients with impairment of T-cell immunity and is caused by JCV. Unlike the situation in AIDS, in which PML occurs in 4% to 5% of patients with very low CD4 counts, PML is rare after transplantation. Recent case reports and case series in organ transplant recipients have shown that a spectrum of disease due to JCV exists, including JCV granule cell neuronopathy, JCV encephalopathy, and JCV meningitis.[217] A brain biopsy is required for a definitive diagnosis, but the diagnosis is strongly suggested by the finding of characteristic white matter changes on MRI and detection of JCV DNA by PCR in the CSF.

A novel polyomavirus called human polyomavirus 7 (HPyV7) was recently described.[221] It caused pruritic, brown papules in transplant recipients, who also had HPyV7 viremia. Diagnosis was initially delayed due to cross reactivity with JC and BK proteins but was eventually made using PCR. These findings support the use of molecular biologic technologies in the search for infectious causes of idiopathic diseases having a suspected viral etiology.

BK virus to date has no effective therapy except for reduction in immunosuppression.[218] Once histologically evident disease has developed, reduction in immunosuppression may improve or stabilize renal function, but 10% to 0% of the patients still progress to kidney failure. Antiviral agents such as cidofovir and leflunomide, fluoroquinolones, or IVIG have been reported to be useful therapies in anecdotal reports, but definitive evidence that these agents are effective is lacking.[215] Although universally fatal with no known treatment, a lung transplant recipient with JCV encephalopathy had stabilization of her cognitive deficits with reduction of immunosuppression and initiation of mirtazapine, which is thought to exert anti-JCV activity by blocking its entry.[217] Other studies have shown a role of reduction in immunosuppression.[219] Although intriguing, the use of mirtazapine and other potential anti-JCV agents remains experimental, and immune reconstitution remains the cornerstone of therapy. In patients with non–life-sustaining organ transplants (i.e., kidney transplant recipients), complete cessation of immunosuppression should be strongly considered; this should be done slowly, to avoid precipitating severe acute graft rejection and PML-IRIS, which can be dramatic. A clinical trial of adoptive T-cell immunotherapy for PML is underway.[220]

Adenovirus

Adenovirus causes a wide spectrum of disease in transplant recipients, from asymptomatic replication to hematuria in kidney transplant recipients, to a severe disseminated form with multiorgan failure.[221] Unfortunately, the only available drug with in vitro activity is cidofovir, which can lead to unacceptable nephrotoxicity. Brincidofovir is a lipid-linked conjugate of cidofovir that has oral bioavailability and lacks nephrotoxicity. A randomized placebo-controlled phase II trial that evaluated brincidofovir for the prevention of adenovirus disease in pediatric and adult allogeneic HSCT recipients with asymptomatic viremia appears promising. [222] There is currently an expanded access protocol to provide brincidofovir for the treatment of serious adenovirus infection and disease.[223] Adoptive immunotherapy has been used in HSCT recipients, but data in organ transplant recipients are lacking.

Parvovirus B19

Parvovirus B19 is a small, nonenveloped, ubiquitous virus that is readily transmitted among children and susceptible adults through the respiratory route. Many persons are infected during childhood, and seropositivity rates among adults are ≈60%.[224] Once infected with parvovirus B19, immunocompetent individuals are considered immune. However, reinfection or reactivation can occur, presumably in context of immunosuppression.[225] Transplant recipients are infected via droplet inhalation, blood-products transfusion, and allograft tissue.[225] Nosocomial outbreaks in transplantation units have been reported. Parvovirus B19 infection can occur years after transplantation. However, ≈two-thirds of the transplant recipients with parvovirus B19 infection present within the first 3 months after transplantation.[225] The low rate of infection among transplant recipients does not support routine determination of parvovirus B19 serostatus in transplant recipients or donors. When infection

is suspected or confirmed in hospitalized transplant recipients, standard and droplet precautions should be implemented to avoid nosocomial transmission.

Hypoproliferative anemia is a consistent manifestation; however, leukopenia and thrombocytopenia can also occur.[226] Fever is present in ≈25% of the patients. Arthralgia and, less commonly, rash may occur. Approximately 10% of the patients may develop tissue-invasive disease, such as hepatitis, myocarditis, pneumonitis, collapsing glomerulopathy, encephalitis, or vasculitis.[225]

Parvovirus B19 serology is not reliable due to inadequate or delayed humoral response to infection. Molecular assays are the preferred diagnostic tools. Parvovirus B19 DNA can be detected in specimens such as blood, bone marrow, and tissue from infected organs. The detection of parvovirus B19 DNA in transplant recipients should be interpreted, however, with caution as it can be detected in the serum and tissue samples even from asymptomatic individuals long after the initial infection. However, the detection of viral DNA in transplant recipients in presence of clinical findings is suggestive of active infection. The examination of bone marrow and immune histochemical staining or in situ hybridization could be helpful in establishing the diagnosis; typical bone marrow findings include hypercellularity, presence of giant pronormoblasts, and absence of late normoblasts.

Specific antiviral agents to treat parvovirus B19 infection are not available. IVIG is frequently used in symptomatic patients; however, spontaneous resolution of the infection without receiving IVIG can occur. Despite therapy, up to 30% of the transplant recipients may experience relapse.[225] Such cases can be successfully treated with additional courses of IVIG.[225]

Zika Virus

Zika virus is a mosquito-borne flavivirus that emerged in the Americas on Easter Island, Chile in 2014 and in northeast Brazil in 2015, and it causes Guillain-Barré syndrome and congenital microcephaly.[227] Its spread throughout South America in 2015 led to an aggressive public health response and the establishment of a national surveillance system and laboratory testing.[228] Reports of sexual and blood transmission have intensified apprehension about infection spread.[229] In a small case series in transplant recipients, Zika virus infection occurred with abnormal graft function, thrombocytopenia, and bacterial superinfection.[230] Although negative testing of individual blood donations provides some reassurance of safety, little is known about the persistence of Zika virus in organs and tissues.[231] Cases of corneal transplantation from Zika-infected donors have been reported, with no untoward events in the recipient.[232] Recent guidance statements about the management of potential Zika-positive donors have been published.[229] It is reasonable to accept donors who have been to areas with Zika transmission after discussing the risks and benefits with the recipient. However, until additional data are available, organs from donors residing in a Zika-endemic region, who had symptoms suggestive of Zika virus infection, should not be used unless symptoms can be attributed to another etiology that does not preclude transplantation. Because up to 80% of infections can be asymptomatic and adequate serology for the virus is still in development, making definitive recommendations for asymptomatic living donors residing in Zika-affected areas is challenging. Potential living donors should be counseled on measures to avoid mosquito bites and only practice protected sex until donation.

Norovirus

Norovirus is one of the most common causes of enteritis worldwide, resulting in acute-onset and short-lived diarrhea in the immunocompetent host. Norovirus infection has only recently been identified as a cause of protracted diarrhea in transplant recipients that can last for months and may be associated with severe wasting, debilitation, and sometimes mortality. In addition, due to lack of awareness among clinicians, norovirus testing may not be sent, resulting in unnecessary invasive procedures, such as colonoscopies. In a recent study 16% (31/193) of transplant recipients who presented with diarrhea tested positive for norovirus by PCR.[85] Other common features included nausea/vomiting (58%), abdominal pain (52%), and wasting (35%). Acute kidney injury occurred in 23% and persisted in 21% after 6 months. Median duration of diarrheal symptoms was 4 months (range, <1–20), and 11 of 31 (35.4%) patients had relapses after improvement. Wasting, incompatible kidney transplant status, the receipt of Thymoglobulin for induction, and plasmapheresis were associated with longer diarrhea durations. There is no proven treatment for norovirus, although nitazoxanide, reduction in immunosuppression, and IV or enteral immunoglobulin have all been given. Prospective studies are needed to define the optimal therapeutic intervention, if any, for this virus.

Hepatitis Viruses

Hepatitis A Virus

Hepatitis A virus (HAV) is largely transmitted person-to-person by the fecal-oral route or consumption of contaminated food or water. Disease is usually self-limited but can be fulminant, especially with increasing age or underlying liver disease. All liver transplant candidates and nonliver organ transplant candidates with chronic liver disease or other known risk factors for HAV should be tested for anti-HAV immunoglobulin G and vaccinated if seronegative, preferably before transplantation. Those who are also hepatitis B nonimmune should receive the combined HAV/HBV vaccine. Last, patients with fulminant hepatic failure due to acute HAV should be assessed for liver transplantation.[233]

Hepatitis B Virus

With the introduction of routine childhood vaccination programs in many countries, the incidence of HBV has been decreasing globally. However, HBV infection remains an important etiology of acute and chronic liver disease requiring liver transplantation. The prevalence of chronic HBV infection is also higher in patients with end-stage renal disease than in the general population. It is estimated that approximately 2% to 9% of organ donors in the United States are anti-HBc^+ and can potentially transmit HBV to recipients, with the risk being determined by host (presence of viremia, type of organ transplanted) and recipient factors (HBV serostatus, intensity/type of immunosuppression). Immunosuppression is a major risk factor for progression of HBV infection, and all organ transplant candidates should be routinely assessed for HBV infection and vaccinated if susceptible.

Liver Transplant Candidates and Recipients

Historically, in the absence of specific preventive strategies, liver transplant candidates with chronic HBV infection routinely reinfected their allografts, leading to poor outcomes due to recurrent HBV infection. Reinfection rates after transplantation have been lower and outcomes better in patients with fulminant hepatitis B infection and in those with hepatitis D coinfection, but these represent only a minority of the patients with HBV infection. A unique and particularly aggressive syndrome of recurrent HBV infection observed in 12% to 20% of liver transplant recipients with HBV recurrence is fibrosing cholestatic hepatitis, characterized by marked cholestasis and hypoprothrombinemia but only modest increases in serum aminotransferases. Fibrosing cholestatic hepatitis is more likely to occur in patients with pretransplant HBV replication and results in rapid death in almost all cases. A paucity of inflammatory response in this syndrome suggests that the virus may be directly cytopathic. The incidence of this complication has decreased with more effective strategies to prevent HBV reinfection of the liver allograft.

In the current era the outcome for patients at risk for recurrent HBV infection after liver transplantation has been greatly improved by the use of preventive strategies and the development of quantitative molecular assays to measure the hepatitis B viral load in the blood. Historically, before the availability of antiviral agents for hepatitis B surface antigen–positive ($HBsAg^+$) patients undergoing liver transplantation, HBV hyperimmune globulin (HBIG) was used intraoperatively and postoperatively to prevent reinfection of the allograft but was limited by logistic considerations (need for IV therapy) and substantial cost. With the development of effective and generally well-tolerated antiviral agents for HBV, there has been a shift from lifelong HBIG alone to combination therapy with an initial period of HBIG followed by indefinite antiviral therapy. Among antiviral agents, monotherapy with lamivudine is generally not recommended because of the reappearance of HBsAg

after liver transplantation in 32% to 50% of patients after several years, related to emergence of resistance. Newer antiviral agents, including tenofovir formulations and entecavir, appear to be more potent and have higher barriers to resistance than lamivudine. HBV viral loads are routinely monitored after transplantation to detect breakthrough infection before clinical consequences develop.

Transplantation candidates who have chronic active HBV infection and those who are inactive HBsAg carriers should initiate antiviral therapy for HBV at the time of transplantation and have therapy continued after transplantation. Reduction of HBV DNA to low or undetectable levels before transplantation reduces the rates of post-transplantation recurrence. IFN therapy is no longer routinely used because of its relatively lower efficacy and tolerability compared with antiviral agents.

Livers from anti-HBc$^+$ liver donors are routinely used for transplantation at many centers after appropriate counseling and consent of intended recipients. The approximate estimated risk for HBV transmission from an anti-HBc$^+$ donor is significantly influenced by recipient HBV serostatus: 4% (naturally immune, anti-HBc$^+$/anti-HBs$^+$), 14% (isolated anti-HBc$^+$), 15% (vaccine immune, anti-HBs$^+$), and 58% (nonimmune with all HBV markers negative).[234] Posttransplantation preventive strategies in the recipient are tailored accordingly, with monitoring for HBV infection.

Nonliver Organ Transplant Candidates and Recipients

The presence of chronic HBV infection also adversely influences long-term outcomes after kidney transplantation, but there are fewer studies in other nonliver, nonkidney organ transplant populations.[235] Cirrhosis and hepatocellular carcinoma contribute to the higher death rate in kidney transplant recipients with pretransplantation chronic HBV infection.[235] This finding underscores the importance of carefully evaluating hepatitis serology and liver function in patients presenting as candidates for transplantation. All HBV-uninfected candidates should receive HBV vaccine pretransplantation as early as possible in the course of their illness, although the response to vaccine might be suboptimal due to underlying disease. Because immune suppression posttransplantation significantly increases the risk for HBV progression, all HBsAg$^+$ patients are generally recommended to initiate (or continue) antiviral therapy at the time of and after transplantation, including those who might not have met criteria for initiation of HBV therapy before transplantation.

The overall risk of HBV acquisition from an HBV anti-HBc$^+$ (HBsAg$^-$ donor for a nonliver organ is generally estimated to be low [up to 5%]) and is lower in recipients with prior vaccine (anti-HBs$^+$) or natural (anti-HBc$^+$) immunity, and from donors without HBV viremia. The use of organs from such donors is considered acceptable, after appropriate counseling and consent and with appropriate posttransplantation monitoring, according to current guidelines.[233,234]

Hepatitis C Virus

There has been a substantial evolution in the approach to HCV infection in organ transplantation with the advent of several highly effective and generally well-tolerated antiviral regimens. Historically, HCV infection has been prevalent in all transplant groups but highest among liver and kidney transplant recipients. A small number (4%–7%) of HCV-infected transplant recipients (of all organ types) developed progressive fatal cholestatic liver disease during the first year after transplantation. These cases are marked by a high viral load, and liver biopsies show severe hepatocyte dropout with minimal parenchymal inflammation. The long-term outcome of other HCV-infected patients depended to some extent on the organ transplanted. Liver transplant candidates who had HCV viremia before transplantation nearly universally reinfected their liver grafts after transplantation, and 46% to 97% developed clinically significant hepatitis during the first 2 to 3 years after transplantation. Ongoing HCV infection led to graft cirrhosis in up to 30% of patients by 5 years after transplantation. Findings from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) liver transplantation database showed that long-term outcomes (>5 years) after liver transplantation for HCV were similar to those after liver transplantation for other indications.[236] However, a retrospective analysis of more than 10,000 liver recipients in the UNOS database showed that medium-term survival of HCV-infected patients was inferior to that of uninfected patients. Longitudinal studies in kidney transplant populations showed that chronic HCV infection had an adverse impact on survival, but this effect was not clearly discernible until the second decade after transplantation. Excess deaths were due not only to the direct effects of liver disease but also to a higher rate of sepsis.

Several factors were shown to predict the progression of liver disease due to HCV after transplantation. In liver transplant recipients these include the degree of immunosuppression, use of antirejection therapy, high viral loads before or early after transplantation, older donor age, CMV infection, and, in some studies, infection with HCV type 1b. Although the choice of a calcineurin inhibitor and use of mycophenolate mofetil have not been conclusively shown to have an effect on HCV recurrence rates, higher cumulative exposure to corticosteroids was associated with increased mortality and more severe histologic recurrence of HCV.

Historically, the use of combination therapy with long-acting pegylated IFNs and ribavirin led to sustained virologic response rates of 26% to 50% but was associated with significant toxicities, and there was controversy regarding indications for treatment.[237] With the availability of several new classes of potent antiviral agents with pangenotypic activity (protease inhibitors, RNA polymerase inhibitors, NS5A inhibitors), the indications for initiating therapy are evolving rapidly, and the majority of HCV-infected patients are now recommended to be treated.[238] Preliminary studies have shown efficacy rates greater than 90% (comparable to nontransplantation) with various treatment regimens in the posttransplantation setting.[238] However, important drug interactions, certain toxicities, dosing of some regimens in patients with renal dysfunction, risk for HBV reactivation in coinfected patients, and high cost are challenges with the new regimens. The availability of generally safe and effective HCV regimens has raised important issues with regard to the optimal timing of therapy for HCV-infected transplantation candidates. For example, deferral of HCV treatment until after transplantation might allow receipt of an organ from an HCV-infected donor, with subsequent treatment of recipient/donor HCV after transplantation, thereby potentially decreasing wait-time on the transplant list. This approach has to carefully consider the increased complexity of the medical regimen posttransplantation, risk for drug interactions and toxicities, and the anticipated wait-times for an individual patient. Another approach made feasible with newer HCV therapies is the transplantation of an organ from an HCV viremic donor into an HCV noninfected recipient, with early initiation of therapy in the recipient posttransplantation. Initial pilot studies have shown the feasibility and preliminary efficacy of this approach that could expand the pool of donors to include HCV-viremic donors.[239]

HIV and HCV-coinfected liver transplant recipients warrant special mention. Based on data from the pre-DAA era, HIV/HCV-coinfected patients experience more aggressive recurrence of HCV, rejection, sepsis, graft loss, and death compared with those with HIV or HCV monoinfection.[198] Fibrosing cholestatic hepatitis also disproportionately affects HIV/HCV-coinfected liver transplant recipients. These poor outcomes have been linked to low body mass index, the need for combined liver and kidney transplant, and the use of older and HCV-seropositive donors. Potential solutions should include early referral for transplantation, optimization of peritransplantation management, improving immunosuppression to mitigate the risk of rejection, and most important, early initiation of DAAs for HCV infection, although the optimal timing (before or after transplantation) remains to be determined.

Hepatitis E Virus

Hepatitis E virus (HEV) comprises multiple genotypes and is endemic in many regions of the world. The virus is a small, nonenveloped RNA virus that is transmitted by the fecal-oral route and was previously thought to cause only an acute hepatitis. However, progression to chronic hepatitis may occur in up to 50% of organ transplant recipients.[240] Available evidence suggests that the most important mechanism is primary infection posttransplantation rather than donor-transmitted infection or reactivation of pretransplantation infection. Diagnosis is

made by histologic findings on liver biopsy combined with detection of HEV RNA in blood and/or liver tissue. Currently, there are no proven effective preventive or therapeutic options for HEV infection in organ transplant recipients, although small series reported a benefit of ribavirin monotherapy.[241]

DONOR-TRANSMITTED INFECTIONS

(See Chapter 304)

An estimated 0.2% to 1% of the transplantations are complicated by donor-derived infections, and this rate is expected to increase as a result of improved recognition and reporting of these events. Although infrequent, the donor-derived infections are associated with substantial morbidity and mortality; deaths occurred in 40% of the recipients with documented transmissions.[242] Viral infections account for nearly one-third of all confirmed donor-derived infections. Transmission of certain viruses, for example, CMV, EBV, HCV, and HBV, is expected to occur predictably (Table 308.14). However, the risk can be mitigated by use of appropriate prophylactic measures in the recipient with minimal impact on posttransplantation outcomes. Unexpected donor-derived infections, for example, lymphocytic choriomeningitis virus, rabies virus, West Nile virus (WNV), and HIV have been documented with devastating sequelae. These infections have occurred when the disease was not suspected or recognized in the donor at the time of death. Other potentially transmissible agents are depicted in Table 308.14.

Donor-Derived Bacterial Infections

An estimated 5% of the organ donors may have bacteremia,[243] with the potential of transmission being greater with gram-negative bacilli, multidrug-resistant organisms, or bacteria resistant to the perioperative antibiotics used. In addition, contamination during organ procurement or donor respiratory tract colonization (in lung transplantation) may result in transmission of bacterial infections. Organs from donors with meningitis due to *Pneumococcus, Meningococcus,* and *Haemophilus influenzae,* and so forth, may be successfully transplanted, provided both the donor and the recipient are appropriately treated. Donors with documented bacterial infections should receive at least 24 to 48 hours of appropriate antibiotics before organ procurement, preferably with evidence of clinical response with continuation of antibiotics in the recipient for 7 to 14 days after transplantation.

Donor-Derived Fungal Infections

Candidiasis is the most common donor-transmitted mycoses. Most cases are due to contamination of preservation fluid and have occurred in kidney transplant recipients.[244] Donors with cryptococcosis, including those with unrecognized cryptococcal meningoencephalitis may transmit this yeast with the allograft. Active histoplasmosis or undiagnosed and presumably asymptomatic infection in the donor that had not resolved by the time of death can result in donor-derived histoplasmosis. Potential donors from an endemic area with either active or occult infection can also transmit coccidioidomycosis. Rare instances of aspergillosis and other filamentous fungi, including agents of mucormycosis have also been transmitted from infected donors. More recently, these fungi have emerged as a serious complication of transplantation tourism (the practice of traveling abroad to commercially acquire an organ) and have been associated with graft loss or death in 76% of the cases.[245]

Other Pathogens With Potential for Transmission With the Allograft

In general, donors with undiagnosed meningoencephalitis are not considered suitable for donation as transmission of not only fungal pathogens but other diseases, including prion infections, rabies, WNV, *Balamuthia,* or noninfectious entities, such as lymphomas and leukemias. Clusters of transplant-associated *Balamuthia mandrillaris* infection and another type of free-living ameba have been reported.[246] Organ transplantation from donors with primary amebic meningoencephalitis caused by the free-living ameba *Naegleria fowleri* was not associated with transmission of the infection.[247] Nevertheless, the risk of transmission of *N. fowleri* by donor organs is not fully known, and transplantation with an organ possibly harboring *N. fowleri* should be carefully weighed on an individual basis against potentially greater risk of dying from delayed transplantation. Heart transplant donors and recipients should be serotested for toxoplasmosis. Given the paucity of cysts in noncardiac tissue, screening of nonheart donors for toxoplasmosis is not routinely

TABLE 308.14 Suggested Screening for Transplant Recipients and Donors

PATHOGEN OR INFECTION	DONOR	RECIPIENT	RECOMMENDATION
Human immunodeficiency virus	+ – +	– + +	Reject Accept Ongoing studies
Human T-cell leukemia virus 1	+	±	Accept (after appropriate patient consent)
Cytomegalovirus, Epstein-Barr virus, herpes simplex virus, varicella-zoster virus	±	±	Accept
Toxoplasma gondii	±	±	Accept; D^+/R^- heart transplants at highest risk and need prophylaxis
Hepatitis C virus	Ongoing studies		
Hepatitis B virus			$HBsAg^+$ donors may be considered after careful risk and benefits assessment and appropriate patient consent; donor liver disease should be excluded histopathologically Organs from $HBsAg^-$, anti-HBc^+ donors can also be considered on an individual basis after appropriate patient consent
Syphilis (rapid protein reagent)	+	±	Accept (after appropriate patient consent) and treat recipient
"Encephalitis"	Yes		Generally reject
Tuberculosis	Purified protein derivative/interferon (IFN)-γ release assay positive (living donor)	Purified protein derivative/IFN-γ release assay positive or negative	Careful history, imaging as appropriate; treat living donors for latent tuberculosis. For recipients on the waiting list, treat for latent tuberculosis and proceed with transplantation to complete the course posttransplantation. May delay treatment for latent tuberculosis until posttransplantation in decompensated cirrhosis. Reject organs from deceased donors with active tuberculosis
Strongyloides	Screen recipients from endemic areas (stool examination and serology)		
Coccidioides	Screen recipients from endemic areas (serology)		
Histoplasmosis	No utility of routine serologic testing		
Trypanosoma cruzi	+		Generally avoid donor, although mismatches have been performed (endemic regions)

D^+/R^-, Seropositive donor/seronegative recipient; *HBsAg,* hepatitis B surface antigen.

performed. Donor screening for Chagas disease should be performed in those who have lived or traveled in an endemic area.

APPROACH TO FEVER IN THE TRANSPLANT RECIPIENT

Fever after organ transplantation should be deemed a marker of infection until proven otherwise. Although the posttransplantation immunosuppressive regimen may result in lower maximum body temperatures, it does not abolish the inflammatory response. Indeed, transplant recipients are able to mount a robust physiologic response to infection that is comparable to that seen in nontransplant patients.[248] Although antimetabolite agents, such as mycophenolate or azathioprine, appeared to suppress the febrile response, corticosteroids had little effect on the systemic inflammatory response in transplant recipients with bacterial or fungal infections.[248] Therefore most transplant recipients with an infection will have a fever, and most fevers will have an infectious etiology in this setting.[64] Febrile episodes occurring in liver transplant recipients in the ICU were due to infections in 87% of cases.[249] Noninfectious etiologies should always be considered once an evaluation for infection has been completed, including allograft rejection, vascular complications, drug fevers, and malignancy.

Fever Without an Evident Source

Some patients with fevers will not have a readily evident source on initial evaluation.[250] However, a study in transplant recipients documented that an etiology of fever of unknown origin was ultimately determined in 68% of the patients, and in 45% the cause was an infection.[251] Unlike in the general population, rheumatologic disorders were rare, and drug fever was the most common noninfectious etiology of fever of unknown origin in transplantation setting.[251]

In cases of fever of unknown origin the history of prior infections, both active and latent, should be diligently sought. Epidemiologic exposure and travel history should be obtained. Otherwise infrequently encountered opportunistic pathogens, such as cases of *Talaromyces* (previously *Penicillium marneffei),* have been documented in transplant recipients with travel to endemic areas (Southeast Asia).[252] A list of all active and recently discontinued prophylactic antimicrobials should be reviewed to determine which pathogens could potentially lead to breakthrough or postprophylaxis infections. Review of donor information is also an important consideration. Testing for unusual pathogens, such as *Bartonella, Coxiella*, and *Brucella,* should be undertaken on a case-by-case basis.

In patients with refractory fevers, cytopenias, ongoing clinical deterioration, rare entities, such as thrombotic thrombocytopenic purpura, GVHD, and hemophagocytic lymphohistiocytosis should be considered. The latter is a disorder of dysregulated immune activation, can occur after viral infections, such as CMV or EBV, carries a mortality rate of about 52% in transplant recipients, and can be difficult to diagnose.[253] Treatment is mainly supportive and involves reversal of the underlying cause, although etoposide combined with corticosteroid therapy has been attempted.

VACCINATION IN ORGAN TRANSPLANT CANDIDATES AND RECIPIENTS

The burden of vaccine-preventable infections is increased in immunosuppressed patients, including recipients of organ transplants. Routine assessment and update of vaccinations prior, rather than after, transplantation is recommended because live vaccines are generally contraindicated posttransplantation and because the immunogenicity of vaccines is typically lower in patients receiving immunosuppression posttransplantation. In addition to routine age- and immunosuppression-recommended vaccines, an assessment of the need for future travel-associated vaccines should be done because immunosuppressed patients are at higher risk for complications of travel-associated infections and because certain travel-associated vaccines are absolutely contraindicated posttransplantation (e.g., yellow fever virus vaccine). Where appropriate, laboratory measures of immunity to determine need for vaccination and response to vaccination should be assessed using established criteria (varicella, mumps, measles, rubella, hepatitis A and B). Live vaccines should be administered at least 4 weeks before transplantation. In addition, age-appropriate vaccination of family members and other close contacts is recommended. Most live vaccines can safely be administered to close contacts, but precautions to prevent transmission for certain live vaccines are recommended (rotavirus, varicella-zoster virus, live-attenuated influenza virus, oral polio virus). A summary of recommendations by the American Society for Transplant Infectious Disease Community of Practice for vaccination of adult transplant candidates/recipients is shown in Table 308.15.[254]

Selected Specific Vaccines

Influenza

Despite increased severity of influenza in organ transplant recipients, the rates of vaccination are suboptimal, and there is evidence of safety and protection against influenza-associated complications. A number of inactivated vaccine formulations are available, but no definitive clinical evidence to recommend any particular formulation. Vaccination during the influenza season should be done before transplantation when feasible. The immunogenicity of influenza vaccination is reduced after transplantation but is safe. For patients not immunized before transplantation during the influenza season, vaccination is typically deferred until 3 to 6 months posttransplantation because of diminished immunogenicity. However, in the setting of widespread influenza activity, immunization has been done as early as 1 month posttransplantation. A variety of approaches have shown improved laboratory-assessed immunogenicity, including use of higher-dose formulations and booster vaccination, but none have been designed to assess for improved clinical end points.

Pneumococcus

Current US guidelines for at-risk adults, including patients with medical conditions commonly found in transplant candidates and immunosuppressed patients, recommend a combination of a protein-conjugated vaccine that may lead to the formation of memory cells and a 23-valent polysaccharide vaccine. For adults not previously vaccinated, the protein-conjugated vaccine should be administered first, followed by the polysaccharide vaccine at least 8 weeks later.

Herpes Zoster

Immunosuppressed persons, including transplant recipients, are at significantly increased risk for zoster and associated complications. A live-attenuated vaccine (Zostavax) that reduces zoster burden by ≈50% is currently recommended in the United States for adults older than 60 years but is contraindicated posttransplantation and should be given to eligible transplant candidates at least 4 weeks before transplantation. The efficacy of this vaccine for preventing post-transplantation zoster has not been formally assessed. An adjuvanted subunit vaccine was recently FDA approved for adults older than 50 years and is theoretically not contraindicated posttransplantation, but its efficacy has not been assessed in the posttransplantation setting.[255] In a general population the subunit vaccine was shown to be immunogenic and highly effective (≈90%) and has also been studied in patients who previously received the live-attenuated vaccine. The subunit vaccine is associated with a relatively high rate of local and systemic reactions, thought to be related to the adjuvant system. Whether the adjuvant system might increase the risk for nonspecific immune activation and development of alloantibodies (anti–HLA or donor-specific antibodies) that might increase for allograft rejection is unknown. Unresolved issues include whether the subunit vaccine should be administered routinely to otherwise eligible posttransplantation patients, which of the vaccines (or both) should be administered to transplant candidates, and whether revaccination will be required.

Hepatitis B

Transmission of hepatitis B with an organ transplant is an important consideration because 2% to 9% of donors are anti-HBc^{+}. The risk is greatest for liver recipients compared with other organs and can be mitigated by vaccine immunity. Thus all nonimmune transplant candidates should be vaccinated. Various strategies to improve immunogenicity have been used, including higher dose, repeat vaccination, and altering

TABLE 308.15 Immunization Schedule in Transplant Recipients

VACCINE	TYPE OF VACCINE	ADMINISTRATION: BEFORE TRANSPLANTATION	ADMINISTRATION: AFTER TRANSPLANTATION	ASSESSMENT OF SEROLOGIC RESPONSE TO VACCINE
Influenza[a]	Nonlive	Yes	Yes	No
Hepatitis B[b]	Nonlive	Yes	Yes	Yes
Hepatitis A	Nonlive	Yes	Yes	Yes
Tetanus	Nonlive	Yes	Yes	No
Pertussis (Tdap)	Nonlive	Yes	Yes	No
Inactivated poliovirus vaccine	Nonlive	Yes	Yes	No
Streptococcus pneumoniae[c]	Nonlive	Yes	Yes	No
Neisseria meningitidis (MCV4)	Nonlive	Yes	Yes	No
Human papilloma virus	Nonlive	Yes	Yes	No
Mumps/measles/rubella	Live-attenuated	Yes	No	No
Varicella (live-attenuated; Varivax)[d]	Live-attenuated	Yes	No	Yes
Varicella (live-attenuated; Zostavax)[e]	Live-attenuated	Yes	No	No
Varicella (subunit; Shingrix)[f]	Nonlive	Yes	No existing guidelines	No

[a]Refers to inactivated influenza formulations. The live-attenuated influenza vaccine is contraindicated posttransplantation.
[b]Higher dose or coformulation (hepatitis A/B) might have enhanced immunogenicity.
[c]Sequential immunization with pneumococcal conjugated and polysaccharide vaccines is recommended (see text).
[d]For varicella-zoster virus (VZV)-seronegative patients, defer transplantation for 4 weeks after vaccination.
[e]For VZV-seropositive patients older than 60 years, defer transplantation for 4 weeks after vaccination.
[f]For VZV-seropositive patients older than 50 years.
Tdap, Tetanus, diphtheria, acellular pertussis vaccine.
Modified from Table 3 in Danziger-Isakov L, Kumar D; AST Infectious Diseases Community of Practice. Vaccination in solid organ transplantation. Am J Transplant. *2013;13(suppl 4):311–317.*

the route. Recently, a TLR-agonist adjuvanted HBV vaccine has been FDA approved in the United States but has not specifically been evaluated as a strategy to improve immunogenicity in transplant candidates or recipients.

Hepatitis C

Historical data had shown that use of organs from donors with HCV infection was associated with a high risk for HCV transmission and generally worse outcomes attributable to HCV,[257,258] but these studies were in an era of less effective and more toxic therapies. With the advent of newer therapies, and within the context of increased numbers of young donors with active HCV infection, several reports have reported the use of organs from HCV viremic donors for HCV-noninfected recipients. Various strategies, including treatment initiation after initial stabilization posttransplant, or "preemptive" initiation of therapy immediately posttransplant (even prior to documented transmitted infection in the recipient), have been reported to successfully treat HCV infection transmitted to the recipient.[257,258] Although the reported number of such transplants is small and the duration of follow-up relatively short, it represents a novel strategy to potentially safely increase the pool of available donors and organs.

ACKNOWLEDGMENT

Initial versions of the chapter were written by Dr. J. Stephen Dummer.

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309 Infections in Patients With Spinal Cord Injury

Rabih O. Darouiche

SHORT VIEW SUMMARY

Definition
- Spinal cord injury (SCI) results from mechanical compression, vascular insult, or both.

Prevalence and Etiology
- SCI affects almost 1 per 1000 persons.
- Causes of SCI include vehicular accidents, falls, violence, sports, and infection.
- Infection can cause or result from SCI.

URINARY TRACT INFECTION
- Urinary tract infection (UTI) is the most common infection and leading cause of repeated hospitalization.
- UTIs are mostly caused by usual bowel microbiota (gram-negative bacteria and enterococci).
- Intermittent catheterization is preferred to indwelling bladder catheters.
- Preventive approaches are still not very effective.
- Failure to distinguish between clinical UTI and asymptomatic pyuria frequently results in unnecessary therapy.

PNEUMONIA
- Pneumonia has the highest infection-related mortality.
- It occurs mostly in patients with tetraplegia.
- Community-acquired and hospital-acquired microbes are comparable to those found in the general population.

INFECTION OF PRESSURE SORES AND BONE
- This diagnosis is very problematic.
- The broadest microbial etiology includes gram-positive cocci, gram-negative bacilli, and anaerobes.
- Multidisciplinary management requires long and repeated hospital stays.

MULTIRESISTANT ORGANISMS IN SPINAL CORD INJURY UNITS
- Multiresistant organisms include methicillin-resistant *Staphylococcus aureus,* vancomycin-resistant *Enterococcus,* gram-negative bacteria, and *Clostridioides difficile* (formerly *Clostridium difficile*).
- These organisms are more prevalent in SCI units than in general hospital wards.
- Proper infection control measures may reduce both colonization and infection and can delay the onset of complications, but ultimately may replace organisms with others.

Spinal cord injury (SCI) is a devastating condition that is unique in terms of its multisystem impact and need for multidisciplinary management. Moreover, the prevalence and occurrence of SCI have continued to escalate over the years. In 2017, approximately 285,000 Americans experienced the consequences of SCI, and at least 17,500 new cases of SCI accrue each year in the United States.[1] Although motor vehicle crash is the most frequent overall cause of SCI,[1] the most common infectious cause is spinal epidural abscess,[2] the incidence of which continues to escalate.[3] Not only can infection cause SCI, but also infection frequently occurs subsequent to the injury and can result in major morbidity and mortality.

Because SCI primarily affects young adults, patients experience acute, chronic, and recurrent infections. Most infections that occur in patients with SCI also affect able-bodied patients, but the frequency and clinical characteristics of infections vary between these populations. Although urinary tract infection (UTI) is the most common type of infection and the leading cause for rehospitalization, pneumonia has the highest infection-related mortality, and infections of pressure sores and underlying bone are the most difficult to manage. Although patients with SCI are particularly predisposed to infection in the acute setting of the injury, most infections occur much later in this population. In this chapter, discussion focuses on the factors that predispose to infection, the challenges in evaluating patients for infection, the most prominent infections in this population, and multiresistant organisms in the SCI setting.

FACTORS THAT PREDISPOSE TO INFECTION

Risks for infection in patients with SCI can be either systemic or organ related. As SCI does not, in and by itself, depress general host immunity, uninfected individuals with SCI usually have normal function of T and B lymphocytes. However, elevated levels of circulating proinflammatory cytokines and autoantibodies are present in the serum of patients with SCI without medical complications and are further elevated in such patients with neuropathic pain, UTI, or pressure ulcers relative to healthy, able-bodied individuals. Compared with able-bodied cohorts, patients with SCI, particularly individuals with tetraplegia, usually have higher levels of inflammatory markers such as C-reactive protein and cytokines (e.g., interleukin-6 and tumor necrosis factor-α), and this difference can be attributed to undetected inflammation or occult infection. Receipt of high-dose glucocorticosteroids in the acute setting of SCI and chronic complicating conditions (including stress, malnutrition, and renal failure) can impair the immune responses to infection. An animal model showed decreased local inflammatory markers in the bladder tissue of mice with SCI during infection and a delayed antiinflammatory response after being treated, which may contribute to the increased risk of UTI and the chronic inflammation that can persist after treatment.

More importantly, patients with SCI possess unique organ-related factors that predispose them to infection. For instance, most patients have a neurogenic bladder and have frequent episodes of UTI attributed to urinary stasis and bladder catheterization. Urinary stasis greatly impairs the naturally protective mechanisms of the urinary tract, including the washout effect of voiding and phagocytic capacity of bladder epithelial cells. Although some techniques of bladder catheterization are safer than others, none can be carried out without the potential risk for introducing organisms into the urinary tract. Both paralytic ileus and abnormal state of consciousness caused by associated head injury or illicit drug ingestion or both can predispose to aspiration pneumonia in the acute stage of SCI. In individuals with cervical or high thoracic cord lesions, weakness of the diaphragmatic and intercostal muscles impairs the capacity to clear respiratory secretions. Skin breakdown in anesthetic areas, immobility, disuse-induced muscle atrophy, urinary leakage, and fecal contamination predispose to infection of pressure sores.

Frequent insertion of urologic, vascular, orthopedic, respiratory, gastrointestinal, and neurosurgical devices in patients with SCI predisposes to various prosthesis-related infections. This helps explain why

hospital-acquired infections most commonly affect the urinary tract, bloodstream, and musculoskeletal system. Patients with SCI generally have a higher rate of hospital-acquired infections than other groups of patients. About one-third of patients with SCI develop infection during hospitalization, with an overall incidence of 35 episodes of hospital-acquired infections per 1000 hospital-days.[4-6]

CHALLENGES IN EVALUATING PATIENTS FOR INFECTION

A number of unique challenges can be encountered when attempting to establish diagnosis and provide treatment of infections in patients with SCI (Table 309.1). Infection often manifests differently in patients with SCI compared with able-bodied individuals. Altered or absent sensations are the most important impediment to the diagnosis of infection. Although dysuria, frequency, and urgency are regularly present in able-bodied patients with UTI, these symptoms rarely exist in infected patients with SCI. The diagnosis of perinephric abscess is particularly challenging in patients with high sensory level lesions who do not appreciate flank pain or tenderness. The inability to recognize the signs and symptoms of cord damage contributes to the delay in diagnosing spinal epidural abscess below the level of injury. The diagnostic dilemma caused by the paucity of clinical findings can be heightened by the presence of neurogenic or referred pain that may not be related to the infection. Furthermore, multiple infections can occur concurrently in 20% of patients with SCI. Even more problematic than identifying the source of an infection is discerning whether fever is caused by an infection or a noninfectious condition that may closely mimic infection and cause almost one-fifth of episodes of fever in these patients.

A diagnostic conundrum may arise when unique thermoregulatory and autonomic disturbances cause fever in patients with SCI. Because of the imbalance between heat production and heat loss, patients with an injury to the spinal cord above T8 may not be able to maintain a normal body temperature in response to heating or cooling (poikilothermia). This phenomenon of altered thermoregulation is attributed to the loss of sweating and muscular activity below the spinal cord lesion. These factors may contribute to the occurrence of self-limited febrile episodes in patients with SCI that resolve spontaneously within hours to days. However, neither alterations in environmental temperature nor changes in a subject's sweating and muscular activity may explain the occurrence of prolonged fever in recently injured quadriplegic patients who have no identifiable focus of infection. This unique syndrome—so-called quadriplegia fever—lasts weeks to months and is problematic because it may incite repeated evaluation for infection and multiple courses of antibiotics, but to no avail. Rarely, fever may occur in the context of autonomic dysreflexia, a paroxysmal syndrome characterized mainly by hypertension, sweating, facial flushing, and headache. Occasionally, bradycardia may also be present and can help differentiate febrile episodes of autonomic dysreflexia from infection. This type of autonomic hyperactivity is seen only in patients with SCI above T6 and is usually triggered by distention of viscera (bladder and rectum), cutaneous stimulation (e.g., ingrown toenails), or even infection.

Treatment of infection in patients with SCI also poses special challenges. For example, two opposing factors resulting from changes in body composition after SCI can alter the disposition of certain systemically administered antibiotics such as vancomycin and aminoglycosides. On one hand, patients with SCI have expanded extracellular volume attributed to retention of extracellular water as subclinical edema and replacement of diminished skeletal muscle mass by extracellular water. As a result, these patients have a larger weight-adjusted volume of distribution of drugs and may require larger weight-adjusted loading and maintenance doses than able-bodied counterparts to achieve similar antibiotic concentrations. On the other hand, this potential effect on antibiotic concentration can be counteracted, at least in part, by the frequent overestimation of creatinine clearance when using current formulas that were originally devised for able-bodied individuals to predict creatinine clearance in patients with chronic SCI who have low serum creatinine levels. A proper 24-hour collection of urine can accurately estimate renal function. The multiple complicated conditions of patients with SCI result in frequent rehospitalization.[7-9]

TABLE 309.1 Challenges in Evaluating Infection in Spinal Cord–Injured Patients

General Factors
Altered or absent sensations
Interference of neurogenic pain with localization of the source of infection
Coexistence of multiple infections
Mimicry of infection by noninfectious conditions
Thermoregulatory and autonomic disturbances
Need for adjusting doses of antibiotics such as vancomycin and aminoglycosides
Infection-Specific Factors
Urinary Tract Infection
Almost universal prevalence of bacteriuria
Level of pyuria as indicator of infection
Nonspecific manifestations of symptomatic infection
Relevance of urine cultures growing several bacterial organisms
Pneumonia
Impact of ineffective cough on determining microbial cause
Defective perception of dyspnea and need to evaluate gas exchange
Eligibility for and efficacy of immunization
Infection of Pressure Sores
Limitations of history provided by patient
High importance of physical findings in diagnosing ulcer infection
Universal bacterial colonization of pressure sores and unreliability of swab cultures
Potential reason for failure to respond to therapy
Deceptive appearance of sinus tract
Osteomyelitis
Relevance of cultured samples
Possible variations of findings from bones beneath different sores
Significance of organisms growing from cultures of bone
Poor predictive value of clinical evaluation for osteomyelitis
Appropriateness of imaging studies for diagnosis and follow-up

URINARY TRACT INFECTION

UTIs are the most common type of infection in patients with both traumatic and nontraumatic SCI and occur at a rate of 2.5 episodes per patient per year. In general, inadequate antibiotics are available with increasing costs of treatment of UTI of about $2 billion a year in the United States. In patients with chronic indwelling bladder catheters (transurethral or suprapubic), bacteriuria is almost universal and occurs at a higher rate than in patients with intermittent bladder catheterization (98% vs. 70%). A longer interval between intermittent bladder catheterizations may be associated with a higher incidence of bacteriuria. Although outpatients may find it more practical to use clean reusable rather than sterile catheters for intermittent bladder catheterization, there is conflicting evidence regarding the value of clean versus sterile bladder catheterization.

Asymptomatic bladder colonization may progress to symptomatic UTI but often does not. Typical manifestations of UTI (including dysuria, urgency, frequency, suprapubic discomfort, and, in patients with pyelonephritis, costovertebral angle tenderness) are rarely present in patients with SCI. Instead, change in voiding habits, increase in the residual volume of urine in the bladder, foul-smelling urine, worsening of muscular spasticity, and aggravation of autonomic dysreflexia are often the only clinical clues to the presence of UTI. Because of the nonspecificity of these clinical manifestations, other causes should be excluded before diagnosing UTI. Although the lack of pyuria reasonably predicts the absence of UTI in patients with SCI, pyuria is a nonspecific finding that is also observed in uninfected individuals whose urinary tract is inflamed by catheter manipulations, renal calculi, and interstitial nephritis. Other commonly reported abnormal laboratory findings in the urine, including nitrite and leukocyte esterase, are also not specific for infection. Another diagnostic limitation is that about two-fifths of patients with SCI incorrectly attribute their bouts of illness to UTI. As with other patient populations who require bladder catheterization, bacterial concentration in urine of patients with SCI may not help differentiate between asymptomatic bladder colonization and symptomatic UTI. Most cases of UTI in patients with SCI are caused by commensal

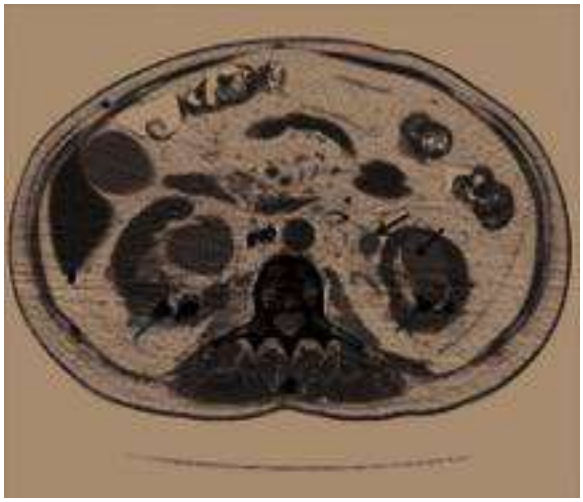

FIG. 309.1 Unenhanced computed tomography scan of the abdomen in a spinal cord–injured patient with a chronic indwelling bladder catheter and repeated episodes of upper and lower urinary tract infection due to *Proteus mirabilis*. Note bilateral kidney stones *(red arrows)*, left hydronephrosis *(yellow arrow)*, and left hydroureter *(blue arrow)*.

organisms of the bowel and perineum, particularly gram-negative bacilli and enterococci. The patient's sex and level of injury may affect the microbiology of organisms residing in the bladder. The finding of polymicrobial bacteriuria is particularly problematic in patients with SCI. Almost half of positive urine cultures in these patients grow more than one organism, and polymicrobial bacteriuria can be more prevalent in patients who have chronic indwelling urethral catheters. Although isolation of multiple bacterial species in the general population is often viewed as indicative of contamination, a similar finding in catheterized patients with SCI should not be disregarded. Isolation of several uropathogens can be associated with a UTI episode that fails to respond to antibiotic therapy directed against only one or some of the organisms but is eradicated after providing additional antimicrobial coverage against other isolated organisms. Although most urine cultures are obtained from patients with lower UTI only and in whom the yield of blood cultures is extremely low, the detection of concurrent bacteremia confirms the pathogenicity of organisms isolated from urine culture. Even in patients who have pyelonephritis in association with polymicrobial bacteriuria, isolation of only one organism from blood cultures may not negate the role of other bacteria in causing UTI. Because it is difficult to differentiate accurately between cultured organisms causing infection and asymptomatic colonizers, it may be reasonable to treat all potentially pathogenic organisms grown from urine cultures in patients with a diagnosis of infection.

A preventive approach that dramatically protects against UTI has not been identified since the advent of closed urinary drainage almost a half-century ago. Optimizing urinary drainage and switching, whenever feasible, from an indwelling bladder catheter to intermittent bladder catheterization or even external condom-based drainage, remain the cornerstone of prevention. The incidence of bladder stones, a condition associated with repeated bouts of UTI, is higher in patients who rely on indwelling versus intermittent bladder catheterization.[10] Fig. 309.1 delineates the relationship between renal calculi, urinary tract obstruction, and infection. Upper tract urolithiasis is commonly managed with extracorporeal shock wave lithotripsy or percutaneous nephrolithotomy.

A number of approaches have been examined for their potential protection against infection. Although clinical data exist regarding the ability of antimicrobial-modified catheters (including hydrogel/silver-coated and nitrofurantoin-coated) and intermittent bladder catheters to reduce the rate of bacteriuria (not clinical UTI) in the general population, there is no strong evidence that such catheters can prevent or reduce clinical UTI. However, both a randomized controlled trial and a single-arm study reported that patients who received injection of botulinum toxin A into the detrusor muscle experienced about 45% reduction in the incidence of UTI compared with the preinjection period.[11–13] Because botulinum toxin A reduces the detrusor pressure, it could help prevent reflux and therefore pyelonephritis.[14] The coating of silicone with mannoside-poly(amido amine) decreases biofilm formation.[15] Furthermore, a number of studies focused on the use of *Escherichia coli* strain 83972 to prevent UTI in patients with neurogenic bladder.[16–18]

Asymptomatic bacteriuria can progress to symptomatic infection, and thus several approaches have been designed to prevent or eradicate asymptomatic bacteriuria.[19] However, there were no significant differences in outcomes between high-dose and low-dose antibiotics given before endoscopic urologic procedures.[20]

PNEUMONIA

Although much less frequent than UTI, pneumonia is the most common cause of death related to infection, at a rate of 30% to 35% in patients with SCI. Pulmonary complications in the immediate postinjury period are particularly likely to occur in the first few months after cervical or high thoracic SCI in quadriplegics and older adults.[21–26] Pneumonia in acutely injured patients is associated with prolonged length of stay and escalated hospital costs. Patients with cervical SCI and swallowing problems leading to aspiration may benefit from having a percutaneous feeding tube. Aspiration pneumonia is usually caused by gram-negative or anaerobic bacteria. As is the case in able-bodied persons, community-acquired bacterial pneumonia in patients with SCI is mostly caused by *Streptococcus pneumoniae, Haemophilus influenzae,* and *Branhamella catarrhalis. Pseudomonas aeruginosa,* methicillin-resistant *Staphylococcus aureus* (MRSA), and *Acinetobacter baumannii* are the three most common causes of ventilator-associated pneumonia, which can be polymicrobial in about one-fourth of infected cases. Because of defective sensation of respiratory muscle fatigue and altered perceptions of dyspnea, pneumonia in patients with SCI may progress to respiratory failure in an unpredictable fashion. Therefore, evaluation of gas exchange, preferably by analysis of arterial blood gases, is strongly recommended when treating pneumonia. Transcutaneous measurement of oxygen saturation also may be used, but caution is needed because heated electrodes can burn the insensate skin.

A number of noninfectious conditions can clinically mimic pneumonia. For example, atelectasis, similar to pneumonia, commonly occurs because of retained pulmonary secretions early after injury to the cervical or high thoracic cord. Because patients may have altered or absent sensations of chest pain or dyspnea and ineffective cough, the only clinical clues that suggest the diagnosis of pneumonia may be tachypnea, tachycardia, fever, and leukocytosis. However, atelectasis may also be accompanied by an element of low-grade fever and leukocytosis. The site of pulmonary involvement may not help differentiate atelectasis from pneumonia because both conditions predominantly involve the left lung, owing to difficulty in suctioning the left main stem bronchus that branches off at a more acute angle than the right bronchus. In such cases, bronchoscopy may be required for diagnostic and therapeutic purposes.

Another condition that can be clinically confused with pneumonia is pulmonary embolism, which occurs in about 5% of patients with SCI, frequently without an identifiable thrombotic source. Although pulmonary embolism can ordinarily be diagnosed by a ventilation-perfusion lung scan, the observed defects on scanning may be difficult to interpret in patients who also have atelectasis; a pulmonary angiogram may be required for definitive diagnosis in such cases. A negative D dimer test in the blood would reasonably rule out pulmonary embolism in this setting. In the acute stage of SCI, associated fracture of a long bone can lead to fat embolism, which may or may not manifest as the clinical stigmata of petechiae and cerebral dysfunction. Aspiration of gastric contents in the presence of paralytic ileus and an ineffective cough reflex can lead to chemical pneumonitis that mimics bacterial pneumonia; evaluation of an adequate sample of respiratory secretions may help differentiate between these two clinical entities. Finally, pulmonary contusion can be mistaken for pneumonia in the acute setting of SCI. Because pneumonia qualifies as an independent risk factor for poor neurologic recovery after complete acute SCI, it is imperative to accurately and quickly diagnose and treat pneumonia.

Because patients with SCI are at a greater risk for developing pneumonia than able-bodied persons, it is important to assess the

immunization status in such patients. By virtue of age (>50 years), chronic respiratory disease, residence in chronic-care facilities, or all of these factors, almost two-thirds of patients with SCI are eligible for vaccination against *S. pneumoniae* and influenza viruses. The antibody response to pneumococcal and influenza vaccination of patients with SCI is adequate. Education and reminders escalate rates of vaccination in these patients. Notwithstanding the lack of studies that examine the clinical benefit of these vaccinations in this population, it may be justifiable to administer pneumococcal and influenza vaccines to all patients with SCI.

Pneumonia in patients with SCI is usually treated with a 10- to 14-day course of antibiotic therapy. In the absence of aspiration or respiratory devices, a quinolone or a combination of a macrolide and cephalosporin is adequate for empirical treatment of community-acquired respiratory infections. Coverage for anaerobes, gram-negative bacteria, and MRSA is required for therapy for hospital-acquired aspiration pneumonia. Agents active against MRSA and *P. aeruginosa* should be empirically considered in patients with infections associated with respiratory devices. The reported observation that most antibiotics prescribed for acute respiratory conditions in patients with SCI are considered unnecessary underscores the essential role of education in preventing antibiotic abuse.

INFECTION OF PRESSURE SORES

Local factors that contribute to infection of pressure sores include breaks in the integrity of the skin barrier, pressure-induced changes, and contamination from contiguous dirty areas.[27–29] Accordingly, pressure sores frequently become infected with staphylococci, streptococci, and gram-negative or anaerobic bacteria. Most pressure sores in patients with SCI develop in areas adjacent to the ischium, sacrum, and greater trochanter. In paralyzed individuals who cannot directly visualize the ulcers, their history is usually incomplete, and the infection is already advanced by the time they seek medical care. Patients with grade 3 or 4 SCI are more likely than the general population to develop Fournier gangrene, which is the most fearsome form of necrotizing fasciitis that affects the perineal and genital regions and usually results from polymicrobial infection. Because of inadequate sensations, physical findings (fever; purulent drainage; and surrounding inflammatory changes including erythema, swelling, and warmth) are usually relied on to diagnose infection.

Because pressure sores are universally colonized by bacteria, samples for culture should not be obtained unless infection is clinically evident. In patients with seemingly infected pressure ulcers, biopsy of deep tissue is the most reliable means for identifying the infecting pathogen. Cultures of swab specimens from an ulcer or a sinus tract are generally unreliable and often misleading, leading to overtreatment of colonizing antibiotic-resistant bacteria, and cultures of material obtained by needle aspiration tend to overestimate the number of bacterial isolates. If cellulitis is recognized adjacent to a decubitus ulcer, the challenge to the clinician is to discern the infecting organism. Biopsy of deep soft tissue is a reliable means for determining the microbiologic cause of infection.

A combined medical-surgical approach is often required to establish cure of infection. The potential benefit of applying negative pressure to deep pressure ulcers by using a vacuum-assisted closure device is still unknown. Surgery is done to débride nonviable tissue and drain infected material. A lack of response of infected ulcers to therapy may be the result of inadequate antibiotic therapy targeted to the wrong organisms, undrained abscess, communication of the ulcer with an infected bone or joint, or a fistula communicating with the gastrointestinal or urinary tract. The appearance of newly isolated bacterial species soon after initiating antibiotic therapy probably indicates colonization; unless there has been an initial response followed by recurrence of fever, these organisms should generally be ignored. Even in patients with apparently healed ulcers, deep soft tissue abscesses may exist, sometimes causing fever or even bacteremia.

Although the sensitivity of nuclear scintigraphy for detecting soft tissue abscesses is generally very high, this test can also yield positive results in patients with SCI who have an infected pressure sore without an associated abscess. Soft tissue abscess in association with an infected sore can be more accurately diagnosed by computed tomography (CT) or magnetic resonance imaging (MRI) than by nuclear scintigraphy. Because pressure necrosis affects subcutaneous tissues and muscles more than the skin, the opening of a sinus tract onto the skin may appear deceptively small. Although potentially helpful, probing may not reveal the full depth of the tract. Sinography can delineate the full depth of the tract and the potential communication with bone, joint, intra-abdominal abscess, or visceral organs. Injection of dye into the intestines or bladder may also help detect a fistulous connection.

OSTEOMYELITIS

Most cases of osteomyelitis in patients with SCI occur beneath pressure sores.[30–34] Less common forms include prosthesis-related, postoperative, hematogenous, and vertebral osteomyelitis. In general, it is difficult to determine whether bone beneath a decubitus ulcer is infected and, if infected, by which organism. Not only are cultures of a swab specimen from the ulcer of little value in predicting the causative pathogen of osteomyelitis, but they also can provide deceptive information that leads to improper antibiotic treatment. The definitive diagnosis of osteomyelitis beneath pressure sores requires histopathologic examination of bone tissue. Histopathologic examination of bone specimens obtained by percutaneous needle biopsy demonstrates osteomyelitis beneath about one-fifth to one-third of pressure sores. Because osteomyelitis is likely to be a focal process and percutaneous bone biopsy may fail to sample the truly infected focus, bone infection is more accurately diagnosed by intraoperative bone biopsy. As seen in Fig. 309.2, ischial pressure sores may result in infection of both muscle and bone. In patients with multiple pressure ulcers, histopathologic evaluation of a bone specimen from one site may not necessarily reflect the same findings beneath the other ulcers. In addition, even if pathologic findings are similar, bone cultures from various sites may grow different organisms.

Because of the high frequency of bacterial colonization of fibrotic tissue adherent to bone, cultures of bone specimens are positive in most patients in whom histopathologic examination of bone tissue is not compatible with osteomyelitis. Moreover, quantitative bone cultures do not differentiate osteomyelitis from colonization or infection of overlying soft tissue. Therefore, the diagnosis of osteomyelitis should be made by positive histopathologic findings, in which case culture results would be relied on to manage antibiotic treatment against grown organisms except the usual colonizers such as *Staphylococcus epidermidis* and diphtheroids. Most cases of osteomyelitis beneath pressure sores are caused by two or more bacterial species, including gram-positive cocci (mainly *S. aureus* and streptococci), gram-negative bacilli (including *P. aeruginosa* and Enterobacteriaceae), anaerobes (particularly *Bacteroides* and *Fusobacterium* spp.), and, rarely, *Candida* spp.

Clinical evaluation poorly predicts the presence of osteomyelitis beneath nonhealing deep pressure sores. In particular, clinical information

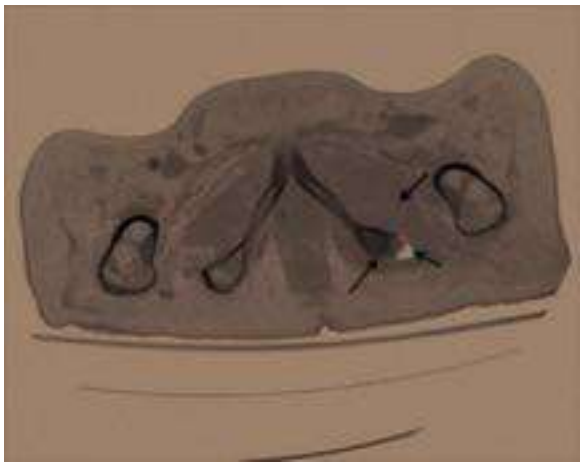

FIG. 309.2 Contrast-enhanced computed tomography scan of the pelvis in a spinal cord–injured patient with an unhealed ischial pressure sore. Note the presence of chronic osteomyelitis *(blue arrow)*, inflamed muscle *(yellow arrow)*, and soft tissue defect *(red arrow)*.

(duration of ulcer, bone exposure, purulent drainage, fever), laboratory data (including leukocytosis and rising erythrocyte sedimentation rate and C-reactive protein), and radiologic findings (plain radiographs and technetium bone scintiscans) do not correlate well with histopathologic findings. Nuclear scintiscans are highly sensitive but poorly specific for diagnosing osteomyelitis beneath pressure sores. This low specificity is attributed to the capacity of the injected technetium to concentrate in areas of bone that have pressure-induced changes or heterotopic ossification. The most desired finding of a bone scintiscan is a negative result, which would almost completely exclude the diagnosis of osteomyelitis beneath pressure sores and obviate the need for bone biopsy. Both CT and MRI can identify osteomyelitis as the cause of nonhealing pressure ulcers.

Although failure of pressure ulcers to heal can result from underlying osteomyelitis, it is more likely to result from noninfectious causes, such as pressure-related changes, spasticity, malnutrition, and heterotopic bone ossification. The last-mentioned entity can mimic osteomyelitis both clinically and radiologically. Heterotopic bone ossification evolves in up to half of all patients with SCI, particularly in the first year after injury among patients who are completely paralyzed or have pressure sores. It can cause warm erythematous swelling of soft tissues, primarily in areas adjacent to the hip and knee. Although serum alkaline phosphatase level can be elevated early, it is not diagnostic of heterotopic bone ossification because many other proliferative bone processes may also cause an abnormal elevation. Radiographic changes are often absent for 1 to 2 weeks after clinical signs appear, but by then a technetium bone scintiscan should reveal increased uptake.

Patients with SCI who have osteomyelitis beneath pressure sores are usually treated with antibiotics and, when indicated, surgical débridement. Although the ideal duration of antibiotic therapy is not clear, most patients receive at least 4 to 6 weeks of antibiotic therapy. No evidence exists to determine whether the duration of antibiotic therapy of vertebral osteomyelitis should be different between patients with SCI versus patients without SCI. Although parenteral antibiotics have traditionally been used, oral administration of highly bioavailable drugs may be considered. Musculocutaneous flap surgery is preferable to débridement alone because the transposition of a well-vascularized muscle allows more extensive removal of devitalized tissue, enhances host defense against infection, and provides a better vascular supply to facilitate bone healing. Patients with improperly treated osteomyelitis may develop deep abscesses and a sinus tract after reconstructive surgery. In patients with recurrent infection or very extensive disease, hemipelvectomy may be considered. Changes on plain radiographs and bone scintiscans may persist after what clinically seems to be successful treatment of osteomyelitis.

BLOODSTREAM INFECTION

Infections of the urinary tract, pressure sores, lungs, and vascular access are the most common identifiable sources of bloodstream infection in patients with SCI.[35–39] An occult deep-seated abscess may be the culprit in bacteremic patients without an apparent source. Bloodstream infections associated with UTI and long-term hemodialysis access are mostly caused by gram-negative bacilli, whereas staphylococci are the most frequent isolates from blood cultures in patients with infection of pressure sores or short-term vascular access. Because vascular catheter-related bloodstream infection is severalfold more likely to be caused by gram-negative bacteria in patients with SCI than in the general population, coverage against gram-negative bacteria should be considered when initiating empirical antibiotic therapy. This recommendation is supported by the reported observation that more than one-third of patients received inadequate empirical treatment, which occurred mostly in patients with polymicrobial bloodstream infection.

INTRAABDOMINAL INFECTION

Because cholelithiasis is more common in patients with SCI than in the general population, biliary infections are the most frequent intraabdominal infections in this population. Although most gallstones may remain asymptomatic, some result in cholecystitis or migrate down the common bile duct to cause cholangitis or pancreatitis. A ruptured viscus or, less commonly, a fistulous connection with a pressure sore can result in the formation of intraabdominal abscesses. Intraabdominal infections may be misdiagnosed, particularly in patients with high cord lesions, because they frequently manifest as abdominal distention, diffuse spasm of abdominal wall musculature, and rigidity on palpation but no localized abdominal pain or tenderness.[40] Ultrasonography, CT, and MRI of the abdomen all are useful modalities to identify an occult abscess.

MULTIRESISTANT ORGANISMS IN THE SPINAL CORD INJURY SETTING

Multiresistant bacteria that have a predilection to exist in SCI units more than in general hospital wards include MRSA (the most common multiresistant organism), vancomycin-resistant *Enterococcus* (VRE), and gram-negative bacilli that produce extended-spectrum β-lactamase or belong to the carbapenem-resistant Enterobacteriaceae.[41–45] Roommate contacts of patients colonized or infected by any of these multiresistant bacteria are at increased risk for acquiring those organisms. As is the case with able-bodied persons, the occurrence of carbapenem-resistant Enterobacteriaceae has also escalated in patients with spinal cord injury. Moreover, antibiotic resistance in SCI has continued to escalate. This has prompted the increasing use, albeit still relatively small, of a group of antibiotics that contain β-lactamase inhibitors. The role of intravesical *E. coli* bacteriophage treatment in patients with SCI has not been established for uropathogens.

Universal surveillance, contact precautions, hand hygiene, strict adherence to infection control practices, and an institutional culture change can blunt the spread and reduce MRSA health care–acquired infections in SCI units. In contrast to MRSA, which can exist in almost every body organ, VRE is cultured mostly from the urine, particularly in catheter-dependent patients. Although most episodes of growth of VRE from urine cultures represent asymptomatic bacteriuria and do not require antibiotic treatment, VRE colonization and residence in a long-term facility increase the risk for subsequent bacteremia. Catheter-dependent patients with SCI are predisposed to develop UTI caused by extended-spectrum β-lactamase–producing multiresistant gram-negative bacilli such as *E. coli* and *Klebsiella pneumoniae.* A potentially bigger menace to vulnerable patients with SCI would be the expanding presence of carbapenem-resistant Enterobacteriaceae. As mentioned, over many years, antibiotic resistance has escalated in SCI units. The recent availability of new antibiotics that include β-lactamase inhibitors can be helpful if these antibiotics are used properly.

Clostridioides difficile (Formerly *Clostridium difficile*)

Frequent administration of antibiotics and potentially suboptimal hygienic practices in SCI units help explain the relatively high prevalence of *Clostridioides difficile* infection. In patients with neurogenic bowel and defective sensation, *C. difficile*–associated gastrointestinal disease can remain clinically undetected until a catastrophe such as toxic megacolon or bowel perforation evolves. Patients with SCI are more likely to have prolonged or recurrent *C. difficile* infections. Additionally, the clinical presentation with diarrhea may not be interpreted as infection versus neurologically related gastrointestinal dysfunction. Physicians should have a high index of suspicion, especially in patients with SCI who receive prolonged or multiple antibiotics.

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310 Infections in Older Adults

Kevin P. High

SHORT VIEW SUMMARY

- Older adults are more likely to experience infection and severe infection than young adults due to accumulation of comorbid illnesses, reduced functional status (physical, cognitive, sensory), place of residence (e.g., nursing home), declining immunity with advancing age (immune senescence) and physiologic changes that accompany age (e.g., reduced stomach acid, gag/cough reflexes).
- Seniors are less likely to present with classic symptoms, even in the face of serious infection. Basal temperature is lower with advanced age, making fever less common; cognitive impairment and other comorbid illness may mask symptoms; and certain diagnostic tests, such as transthoracic echocardiography, are less sensitive in older adults.
- Antibiotic selection is rarely changed by age, but drug interactions and the need for dose alterations are more common with age. The first dose of antibiotic should be given as soon as possible and at full dose—do not "start low and go slow" —because rapid, effective treatment is more important in older adults, who have less physiologic reserve and delayed immune responses.
- Age and comorbidity influence a number of infectious syndromes: pneumonia, urinary tract infection, bacteremia/sepsis, endocarditis, bone/joint infection, and gastrointestinal infection. In general, the microbiologic differential diagnosis is broader in seniors, including an increase in multidrug-resistant organisms, especially in nursing home residents.
- Aging with human immunodeficiency virus (HIV), even well-treated and controlled infection, is associated with increased risk of comorbid illnesses (vs. those aging without HIV), including cardiovascular disease, hypertension, diabetes mellitus, bone fractures, cognitive impairment and dementia, cancer, kidney and liver disease, and even geriatric syndromes (frailty, falls, social isolation).
- Immunization is an important prevention strategy in seniors with specific age-related recommendations for pneumococcal vaccines, influenza vaccine formulations, and zoster vaccine. Advancing age, beginning even in the fourth decade, is associated with reduced responses to human papilloma virus and hepatitis vaccines. Yellow fever vaccine poses a much higher risk for adverse events in older adults, especially those older than 70 years.

PREDISPOSITION OF OLDER ADULTS TO INFECTION

Risk factors for infection in older adults are numerous and often coexist in complex relationships. Seemingly similar patients by conventional criteria may have very different risk due to measures not often used in young adults. These include comorbid illness, polypharmacy, functional status (physical, cognitive, sensory), place of residence, and individual variations in physiologic changes that accompany age (e.g., declining glomerular filtration rate, reduced gag/cough reflexes).

Comorbid Illness

The most important cofactor for infection in older adults is the accumulation of comorbid disease with age. Many infectious diseases are a direct or indirect result of chronic comorbid conditions (e.g., diabetes mellitus, renal failure, chronic pulmonary disease, edema, immobility). These comorbidities most often result in reduced local innate immunity. For example, chronic obstructive pulmonary disease (COPD) is associated with impaired mucociliary clearance, alveolar macrophage dysfunction, and suppressed cough mechanism, substantially increasing the risk for lower respiratory tract infection in older adults with COPD.

Comorbid diseases in older adults with infection can also be important predictors for worse outcomes—more important than age itself. In patients with community-acquired pneumonia (CAP), multiple comorbid conditions and advanced age greatly increase the risk of mortality. Age itself dominates many CAP prognostic scoring algorithms, but age alone is not a strong predictor of mortality once patients exceed 75 years of age; in patients at the extreme of older age, comorbidity dominates, not age itself. Furthermore, cognitive decline and other barriers that delay diagnosis or reduce adherence to medical regimens often necessitate hospitalization of older adults in circumstances where their younger counterparts can be treated as outpatients, further increasing costs and enhancing the rate of complications.

Waning Immunity With Age (Immune Senescence)

Although comorbidities substantially predispose older adults to infection, there is an underlying waning of immune responses that accompany old age even in the absence of comorbidity; this is called immune senescence. Immune senescence is not merely a global state of reduced immunity but a dysregulation of immune responses at multiple levels. A complete review of immune senescence is beyond the scope of this chapter, but both innate and adaptive responses are significantly dysregulated.

Innate immune responses are *increased* in some respects, but *decreased* in others.[1] Critical innate immune components that decline with age include physical barriers such as skin integrity, cough/gag reflex, mucociliary clearance, and gastric acid. Innate immune responses are also dysregulated at the cellular level, with impaired polymorphonuclear neutrophil function and dysregulation of inflammatory responses triggered by pathogen-associated molecular patterns via Toll-like receptors (Fig. 310.1). Despite impaired stimulus-triggered responses, there is often a chronic, low-level inflammation present in older adults. The cause(s) of this low-level inflammation are not well elucidated, but it likely plays a role in chronic disease development (e.g., vascular inflammation) and inhibitory mechanisms engaged to keep this low-level inflammation in check may be a cause, in part, of the slower innate response to infection seen in older adults.

In the adaptive immune system there are decreases in naïve T-cell subsets, with accumulation of memory T cells, substantially reduced diversity of the overall T-cell pool, and impaired responses to specific antigen (Fig. 310.2).[2]

Although there is little doubt that immune senescence exists, the clinical role of this phenomenon in the predisposition of older adults to clinical infection is poorly defined. Immune senescence markers have been extensively studied in vaccine responses measured most often by antibody titers and other surrogate markers, but only a few studies

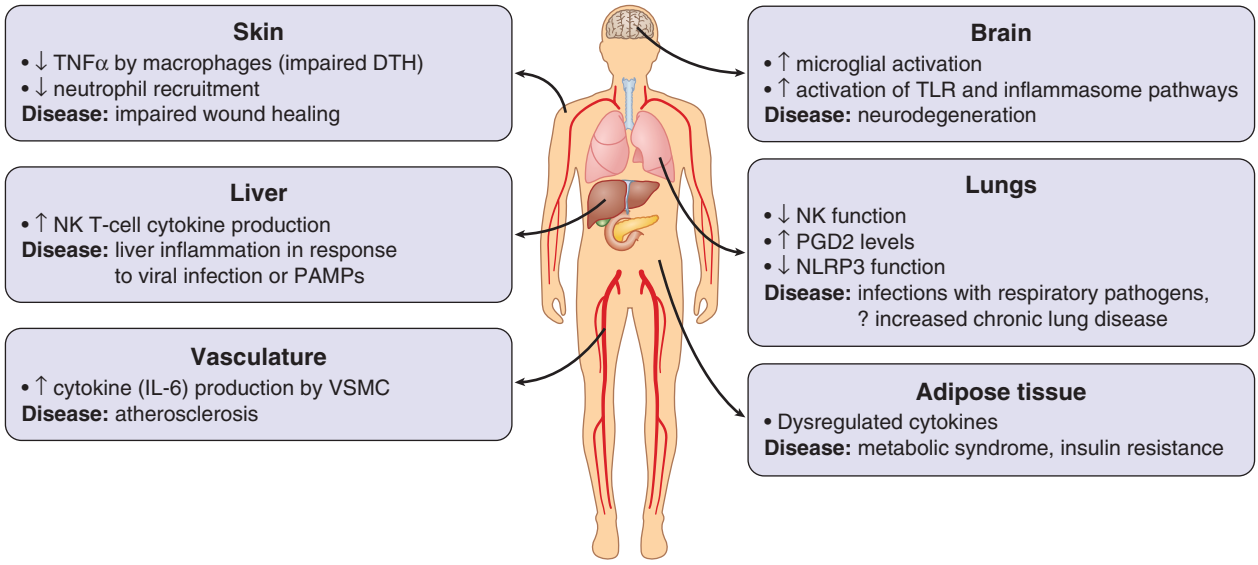

FIG. 310.1 **Innate immune changes with aging and consequences in specific organ systems.** *DTH,* Delayed-type hypersensitivity (response); *IL-6,* interleukin-6; *NK,* natural killer; *NLRP3,* NLR family pyrin domain containing 3; *PAMPs,* pathogen-associated molecular patterns; *PGD2,* prostaglandin D2; *TNF-α,* tumor necrosis factor-α; *VSMC,* vascular smooth muscle cell. *(Reprinted with permission from Shaw AC, Goldstein DR, Montgomery RR. Age-dependent dysregulation of innate immunity.* Nat Rev Immunol. *2013;13:875–887.)*

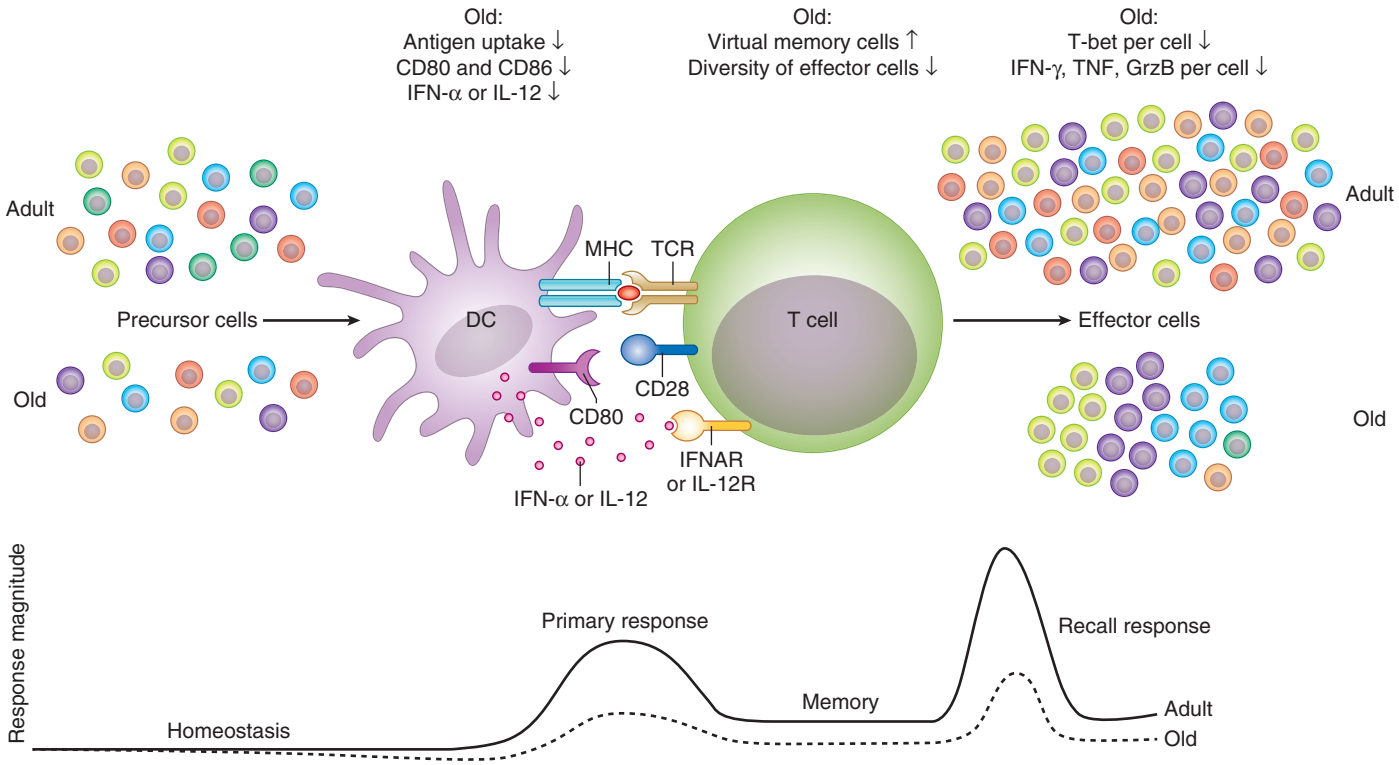

FIG. 310.2 Adaptive immune changes with aging lead to reduced primary and secondary responses, lower numbers of naïve cells and expanded memory cell compartments, and reduce diversity of effector cells. *CD80,* Cluster of differentiation 80; *GrzB,* granzyme B; *IFN-α,* interferon-α; *IFNAR,* interferon-α receptor; *IL-12,* interleukin-12; *IL-12R,* interleukin-12 receptor; *MHC,* major histocompatibility complex; *TCR,* T-cell antigen receptor. *(Reprinted with permission from Nikolich-Žugich J. The twilight of immunity: emerging concepts in aging of the immune system.* Nat Immunol. *2018;19:10–19.)*

have been powered to assess clinical outcomes. It is quite clear that age itself is associated with reduced responses to vaccines as early as the third or fourth decade (human papilloma virus, hepatitis B), but the deficit grows throughout adulthood such that efficacy of influenza and pneumococcal vaccines is questionable by the eighth and ninth decades. Specific vaccine formulations, very different from those used to boost early childhood responses, have been developed to try to overcome immune senescence; these include greater antigen concentration (e.g., high-dose influenza vaccine)[3] or a mixed-action adjuvants (e.g., zoster subunit vaccine)[4] and appear to substantially mitigate immune senescence, not only enhancing postvaccine markers of immunity but reducing clinical disease risk.

Nutrition

Protein-energy malnutrition (PEM) is common in older adults; 30% to 60% of subjects ≥65 years of age admitted to the hospital have PEM,

which is linked to delayed wound healing, pressure ulcer formation, risk of CAP and nosocomial infection, longer hospital stays, and increased mortality. PEM often goes unrecognized outside the hospital, but even mild PEM (i.e., seniors with a serum albumin of 3.0–3.5 g/dL) demonstrate reduced vaccine responses. Despite this, the role of high-protein/high-calorie nutritional supplements for preventing infection or boosting immune responses remains controversial and largely an open question except for those with specific indications (e.g., wound healing).

Specific micronutrient deficiencies in older adults have also been linked to poor immune function (e.g., vitamin B_{12} deficiency and inadequate pneumococcal vaccine responses) and risk of infection (e.g., vitamin D deficiency linked to risk of tuberculosis [TB] and *Clostridioides difficile* [formerly *Clostridium difficile*] infection). Nutritional supplements have been studied as a means to reduce infection risk and boost immunity in older adults, and all experts agree that if true deficiency exists it should be corrected. However, in otherwise healthy adults with normal or "insufficient" (but not deficient) vitamin levels the answer is less clear. Although some data support the use of multivitamins, zinc, selenium, vitamin E (in low-modest doses), and vitamin D, inconsistent results have been achieved.[5] More work is required to define the role of vitamin or mineral supplementation for augmenting immune response in this population.

Social and Environmental Factors

Additional "social determinants of health" combine to influence infection risk in seniors. Population-based studies reveal that lower income is associated with higher rates of CAP and invasive pneumococcal infections among older adults. Lower socioeconomic status (SES) may predispose to infection due reduced access to care, which is well documented in seniors, but low SES is also associated with increased exposure to infectious agents (e.g., grandparents raising young children more often), poor nutrition, and increased risk of comorbid disease (e.g., asthma and exacerbations due to pollution/tobacco exposure).

Environment plays a distinct role in long-term care (i.e., nursing home) residents, who have a particularly high incidence of respiratory, genitourinary (GU), gastrointestinal (GI), and skin infections, and unique infection control challenges exist in the long-term care setting.[6] Close contact between residents and staff plays a key role in the spread of respiratory infections (e.g., influenza, respiratory syncytial virus [RSV]), or infections transmitted by contact (e.g., *Streptococcus pyogenes, C. difficile*). Many frail older adults in a confined setting can lead to severe outbreaks with high mortality rates; proximity of residents, poor adherence to basic infection control measures, and the intense use of antibiotics also increase the risk of antibiotic-resistant bacteria in this setting.

PRESENTATION OF INFECTION IN SENIORS

Infection, even serious life-threatening infection, frequently presents with atypical features in older adults.[7] Serious infections may be signaled by seemingly trivial, nonspecific declines in function or mentation and underlying illness (e.g., congestive heart failure or diabetes mellitus) may be exacerbated by infection, leading older adult patients to seek medical attention for symptoms related to comorbidity rather than infection. The most fundamental sign of infection, fever, is absent in up to one-third of older adults with serious infection. Several studies show that frail older adults have lower mean baseline body temperatures than the currently accepted normal of 98.6°F (37°C). Further, temperature increases in response to pyrogens are diminished with advanced age. The decline in basal temperature and blunted response to pyrogens make it more likely that an older adult will have a body temperature within the "normal" range despite infection, and a normal temperature with significant infection often leads to delayed diagnosis and treatment.

Cognitive impairment may also contribute to the difficulty of diagnosing infection in older adults, with patients unable to communicate symptoms. This can lead to overdiagnosis, as well when colonization (e.g., asymptomatic bacteriuria) is often assumed to be the cause of nonspecific symptoms. Clinicians often have a lower threshold to pursue objective assessments (e.g., laboratory and radiologic evaluations) for infection in cognitively impaired older adults, given the difficulty interpreting subtle function changes and the absence of classic signs of infection noted above. Although a high index of suspicion is warranted, clinicians should be cautioned not to overevaluate and to interpret results carefully. The poor usefulness of culture data in some situations (e.g., swab cultures of skin surface wounds or urine cultures when catheters are present) in which positive cultures are a certainty can lead to overdiagnosis and over treatment (see later).

Changes in anatomy and physiology due to age and/or comorbidity may confound interpretation of diagnostic evaluations as well. For example, there is diminished sensitivity of transthoracic echocardiography (TTE) for detecting valvular vegetations in endocarditis in older adults due to calcification and other anatomic changes associated with age (summarized in reference 8). Studies suggest the sensitivity of TTE is 85% to 90% in adults age ≤55 years but is reduced to <50% for those older than 70 years. More frequent use of transesophageal echocardiogram (TEE) is therefore needed in seniors, where sensitivity returns to 85% to 90% and specificity is not reduced.

ANTIBIOTIC MANAGEMENT IN SENIORS

Antimicrobial Treatment

Age and comorbidity change drug distribution, metabolism, excretion, and interactions.[9] Antibiotic dose reductions are occasionally required in the older adults due to reduced renal function or predisposition to specific side effects, but the most prevalent complications include drug interactions, which are more frequent because older adults more commonly take multiple medications. Digoxin, warfarin, oral hypoglycemic agents, theophylline, antacids, and H2-receptor antagonists are commonly administered and have significant interactions with commonly prescribed antibiotics.

The changes in physiology with age often lead clinicians to the dictum of "start low, go slow" when administering new medications in older adults. However, for antibiotics this is *not* an appropriate strategy. Data suggest higher antibiotic levels are particularly important for efficacy in older adults—much more important than in young adults who often have more robust immune responses and greater physiologic reserve. In older adults, outcomes of serious infection are more dependent on reaching adequate drug levels earlier than in young adults. This is especially true for antibiotics with a concentration-dependent mechanism of action (e.g., fluoroquinolones). Further, slowed gastric motility, decreased absorption, increased adipose tissue, and coadministration of other drugs can decrease blood levels of antimicrobials in older adults, and of course antibiotics reach tissues via blood flow, so poor perfusion to the site of infection, particularly in skin and soft tissue infections of the lower extremities, may reduce efficacy. Adherence may be limited by poor cognitive function, inadequate understanding of the drug regimen, impaired hearing or vision, and polypharmacy, and studies suggest that any regimen requiring greater than twice-daily dosing is associated with very poor adherence rates.

Outpatient parenteral antibiotic therapy (OPAT) is commonly used for syndromes that occur more frequently in older adults (e.g., endocarditis or osteomyelitis) but has been underused in older adults, often due to a lack of Medicare coverage in the past, and older adults were often admitted to nursing homes just to obtain appropriate therapy. With the institution of Medicare Part D, OPAT is now covered, although navigating the system to obtain reimbursement for both the antibiotic and the necessary supplies for administration takes considerable skill. OPAT has been shown to be safe and effective therapy in seniors that have adequate support and monitoring.

Antibiotic Stewardship

Seniors represent an important population in whom antibiotic stewardship can be very effective. Older adults, particularly nursing home residents, have some of the highest rates of multidrug-resistant organism colonization and infection. The presence of indwelling devices (e.g., urinary catheters, G-tubes) and those with marked functional impairment are at very high risk for acquiring and harboring multidrug-resistant organisms.[10] This is perhaps not surprising as those same residents are more likely to receive antibiotics. Of importance, however, antibiotic use in the nursing home is a factor that can be studied and altered as it is

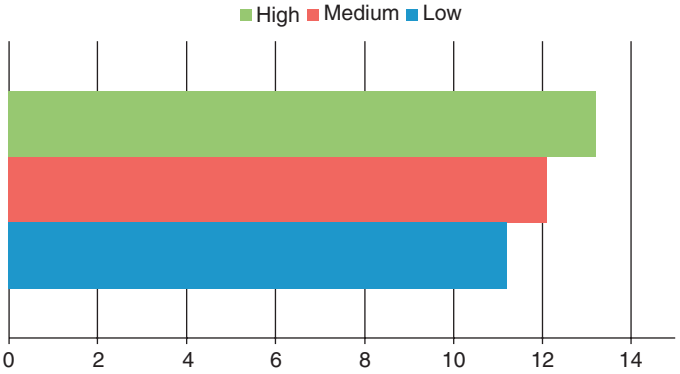

FIG. 310.3 Percentage of long-term care residents experiencing antibiotic-related adverse events with potential for indirect harm (*Clostridioides difficile* infection [CDI], non-CDI diarrhea/gastroenteritis, multidrug-resistant organism infection, allergic reaction, adverse drug reactions). Data are shown for nursing homes classified and high, medium, or low antibiotic use. *(Data from Daneman N, Bronskill SE, Gruneir A, et al. Variability in antibiotic use across nursing homes and the risk of antibiotic-related adverse outcomes for individual residents.* JAMA Intern Med. *2015;175:1331–1339.)*

more a characteristic of the nursing home than the individual patient.[11] Further, residents in nursing homes with high antibiotic use are more susceptible to adverse antibiotic-related events *even if the individual residents do not receive antibiotics;* just by being in a high-risk milieu they are more likely to suffer adverse consequences (*C. difficile* infection [CDI], non-CDI diarrhea, multidrug-resistant organism infection, allergic reaction, or general medication adverse events) (Fig. 310.3).

Setting minimum criteria for antibiotic administration in senior nursing home residents was proposed nearly 2 decades ago[12] but has yet to be implemented in most settings and thus its efficacy questioned.[13] Although comprehensive educational programs and in–nursing home consultation by infectious diseases specialists have been shown to reduce antibiotic use; systemic approaches are likely to be most effective. Culture stewardship refers to limiting the number of unnecessary cultures obtained (e.g., swab cultures of pressure ulcers, urine cultures in those with indwelling catheters) and can be particularly important for limiting antibiotic use in nursing home residents (Fig. 310.4A).[14] Cultures from such sites will *always* be positive, but the information does not correlate with clinically relevant cultures (e.g., bone biopsy under an ulcer or blood cultures in those with an indwelling urinary catheter and sepsis). Culture stewardship using preset criteria to obtain cultures can markedly reduce antibiotic use in this setting (Fig. 310.4B).[11]

Antibiotics at the End of Life

The 1998 American Medical Association Council of Ethical and Judicial Affairs included antibiotics, along with mechanical ventilation, as "life-sustaining" treatment that should be discussed as part of advanced directive and end-of-life care. Others argue that antibiotics are part of ordinary care, even for those who are designated to be receiving "comfort measures only." Although every clinical situation is unique, and no universal recommendation can be made for the using or withholding antibiotics in the terminally ill, it seems prudent to include antibiotic administration in the discussion of advanced directives as a potentially life-sustaining maneuver and to treat it no differently than any other medical intervention, such as surgery, mechanical ventilation, or administration of food/fluids. In those instances where palliation of symptoms is the major outcome desired (vs. prolongation of life), antibiotics should be administered only when symptoms are due to infection itself and readily reversible. Otherwise, restraint from even pursuing diagnostic testing for infection should be used.[15]

AGE-RELATED DIFFERENCES IN SPECIFIC CLINICAL SYNDROMES

A complete review of all the clinical infectious syndromes experienced by older adults is beyond the scope of this chapter, and many chapters in this textbook discuss aspects of individual conditions in seniors. However, some age- and/or function-specific aspects of prevalent syndromes deserve additional comment and appear later.

Pneumonia

Older adults have a markedly increased risk of pneumonia versus young adults.[16] Comorbid cardiovascular and lung disease enhance risk, but age-related changes in physiology also contribute. Impaired mucociliary clearance with delayed clearance of secretions by the mucociliary mechanisms correlate with pneumonia risk. Further, chest wall mobility and compliance decline with advancing age due to loss of mobility and muscle strength, as well as changes in the rib cage when kyphosis or scoliosis is present. Lung compliance is reduced owing to impaired elastic recoil, which results in air trapping, higher residual volumes, and increased work of breathing. Finally, neurologic changes with age lead to "silent aspiration" secondary to reduced ability to cough, lower gag reflex, and frequent coexisting mental status changes or dementia.

The most common microbiologic causes of pneumonia in seniors are greatly impacted by place of residence. Seniors who dwell in long-term care facilities are more likely to have *Staphylococcus aureus*, gram-negative bacilli, and multidrug-resistant organisms isolated from clinical specimens than noted in specimens from community-dwelling seniors. Viral infection causing lower respiratory disease severe enough to require hospitalization is more common in seniors, especially those in long-term care, than in young adults and emphasizes the need to include viral diagnostics for older adults with pneumonia.[17] Outbreaks of influenza or RSV have been well documented in long-term care and need to be met quickly with prompt infection control measures and, when possible, chemoprophylaxis and vaccination strategies to limit spread.

Antimicrobial therapy recommendations for seniors are similar to those of all adults with pneumonia, with modifications based on risk factors for resistant organisms and severity of illness. Adjunct therapy with corticosteroids in those with severe community-acquired disease and a vigorous inflammatory response (usually defined as C-reactive protein >150 mg/L) has been recommended, as it appears to result in lower rates of respiratory failure and reduced length of intensive care unit (ICU) and hospital stay, although it has not been shown to reduce mortality.[18–20] Steroids are associated with higher rates of hyperglycemia, GI hemorrhage, and readmission after discharge. No individual studies or meta-analyses have focused on seniors, so no definite recommendations can be made. Older adults are more likely to suffer the complications of pneumonia mitigated by steroids and the adverse events associated with their use. In similar circumstances where older age is associated with higher risk, but also greater benefit (e.g., anticoagulation in atrial fibrillation), data suggest the concern for possible higher risk should not dissuade clinicians from using therapies where proven benefit has been shown. Thus this author suggests age alone not be a limiting factor when considering steroid use in pneumonia.

Urinary Tract Infection

Asymptomatic bacteriuria (ASB) is defined as the presence of bacteria in the urine, *with or without pyuria*, in the absence of clinical symptoms indicating a urinary tract infection (UTI). ASB is common in seniors, with rates of community-dwelling older adults (women > men) of up to 50% and even higher in long-term care residents. Much has been written about the prevalence of ASB in seniors, the importance of differentiating asymptomatic bacteriuria from true UTI, and the difficulties in making the diagnosis of UTI in seniors with subtle symptoms.[21] It is well beyond the scope of this chapter to fully review this literature, but comments on a few key concepts are warranted. First, testing for UTI in older adults should occur *only* when suggestive clinical symptoms (new fever, new/worse incontinence, dysuria, flank pain, delirium) are present because laboratory tests alone cannot differentiate ASB from UTI. (See earlier comments on "culture stewardship" and reducing unnecessary diagnostic tests of urine) (see Fig. 310.4). Second, the role of laboratory testing for UTI is primarily to *exclude* the diagnosis of UTI; with VERY rare exception, treatment for UTI should not be given to any older adult with a negative urinalysis or urine culture. Third, active monitoring and oral hydration even when testing shows bacteriuria and/or pyuria may successfully be used to manage seniors *without*

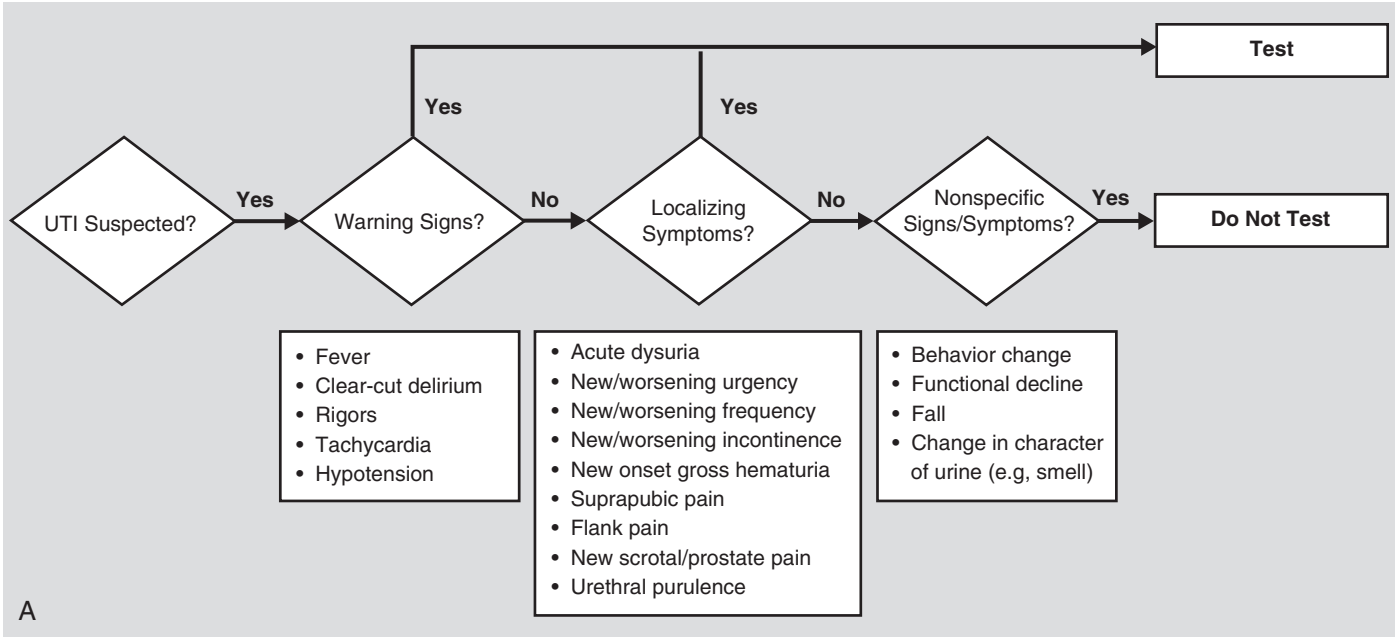

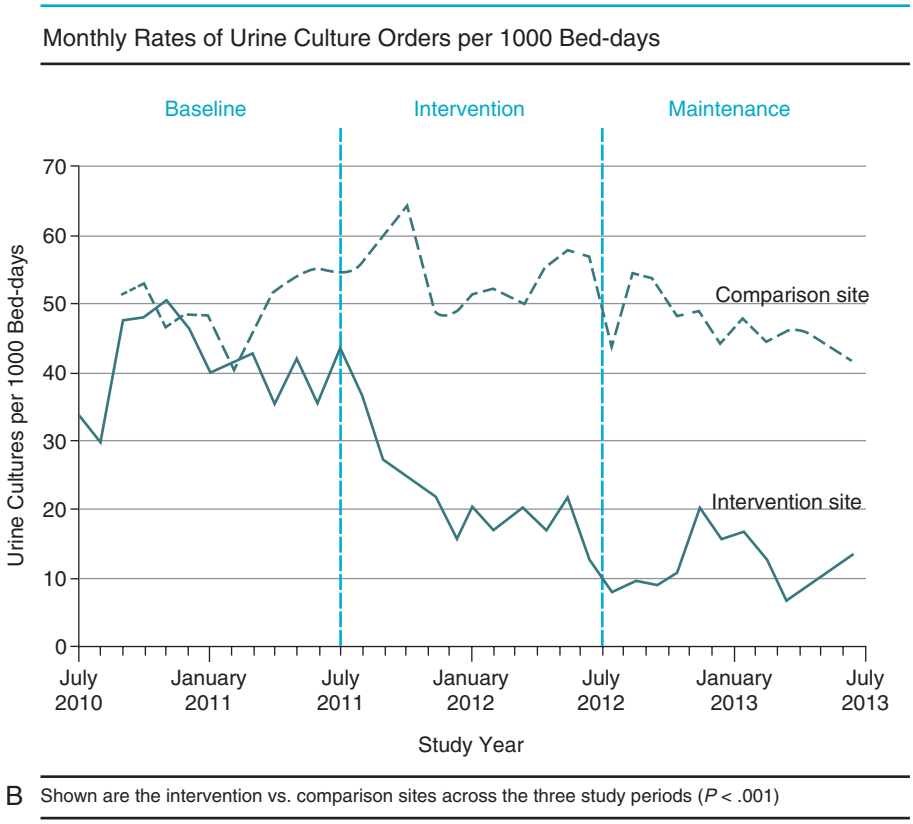

FIG. 310.4 (A) Example of decision-making process invoking "culture stewardship" for older adults with suspected urinary tract infection. (B) Impact of criterion-driven culture orders on the number of urine cultures obtained for hospital inpatients and nursing home patients. *(A reprinted with permission from McElligott M, Welham G, Pop-Vicas A, Taylor L, Crnich CJ. Antibiotic stewardship in nursing facilities.* Infect Dis Clin North Am. *2017;31:619–638; B reprinted with permission from Daneman N, Bronskill SE, Gruneir A, et al. Variability in antibiotic use across nursing homes and the risk of antibiotic-related adverse outcomes for individual residents.* JAMA Intern Med. *2015;175:1331–1339.)*

antibiotic use in those who do not have obvious urinary tract symptoms in situations that often prompt testing (e.g., mild temperature elevations of 99°–100°F, minor confusion/mental status change, "strong–smelling" urine).

Bacteremia and Sepsis

Early (within 1 month) mortality of sepsis for those older than 65 years is nearly 50% higher than that of younger adults, likely due to poor physiologic reserve, particularly in seniors with multiple comorbidities. Bacteremia rates are higher in older than in younger adults and more commonly arise from a GI or GU source. Therefore the causative agent is more likely to be a gram-negative bacillus, and antibiotic management of bacteremia and sepsis in older adults should emphasize the importance of initially broad coverage. This is particularly true when multidrug-resistant organisms risk is high (e.g., long-term care home resident, indwelling devices present).[22]

When older adults survive an episode of sepsis, they are more likely than young adults to suffer negative outcomes that reduce quality of life (e.g., impaired physical or cognitive function). In one study of seniors (average age, 78 years) surviving sepsis, moderate-to-severe cognitive impairment increased from about 6% before sepsis hospitalization to nearly 17% after hospitalization, and severe sepsis was associated with a threefold higher rate of moderate-to-severe cognitive impairment. These patients also had a decrease in activities of daily living (ADLs) and instrumental ADLs (IADLs).[23,24] Further, about 40% of those age 65 years and older require nursing home admission after surviving sepsis versus ≈15% of those younger than 65 years.[25]

Infective Endocarditis

Infective endocarditis (IE) is primarily a disease of older adults, except when IE is due to intravenous drug use, and occurs principally in those with degenerative valvular disorders and prosthetic valves (i.e., prosthetic valve endocarditis). In addition, indwelling devices, such as pacemakers and implanted defibrillators, are more frequently placed in seniors. IE in older adults presents a diagnostic challenge; fever and leukocytosis are less common (55% and 25%, respectively, in those 65 years and older versus 80% and 60%, respectively, for younger adults). Transthoracic echocardiography (TTE) has reduced sensitivity in older adults due to calcified valves, more turbulent blood flow, and shadows off prosthetic materials. TEE is more sensitive, and improves the diagnostic yield by 45% over that of TTE.

Native valve IE in young adults is typically caused by streptococci, staphylococci, and occasionally by HACEK organisms (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella*). The same organisms predominate in older adults, but as noted for bacteremia earlier, GI and GU organisms such as enterococci and gram-negative rods become more common in seniors with IE. In consequence, initial antibiotic treatment is similar in young versus older adults; however, organism-specific therapy might vary based on age. Combination regimens with aminoglycosides are particularly problematic in the older patients because of toxicity (both renal and ototoxicity) but is occasionally unavoidable in certain circumstances (e.g., enterococcal IE). IE prophylaxis recommendations do not differ based on age alone.

Prosthetic Device Infections

Implantable prosthetic devices (prosthetic joints, cardiac pacemakers/heart valves, intraocular lens implants, vascular grafts, penile prostheses, etc.) are most frequently placed in older adults, and therefore infection of these devices is more common in this group.[26] Foreign material provides the milieu for bacterial biofilms and adherent bacteria are often more difficult for antibiotics or the immune system to kill. Causative organisms for early prosthetic device infections (<60 days after implantation) are frequently skin or nosocomial flora and reflect events around the time of implantation and/or the hospital stay associated with implantation. Coagulase-negative staphylococci predominate; *S. aureus* and diphtheroids are common as well. Gram-negative bacilli, fungi, or polymicrobial infection are rare causes of early infection. The pathogenesis of late infections is more likely due to occult bacteremia seeding the implant; thus older adults have a broader microbial differential than young adults, with greater incidences of gram-negative organisms due to GI and GU sources of bacteremia as noted previously, but staphylococci still predominate.

Cure of prosthetic device infections may be difficult with the device in place (see Chapter 105). However, in some instances early antibiotic intervention combined with aggressive surgical débridement may result in cure without removing the device (Fig. 310.5).[27] If early, aggressive therapy is ineffective in controlling the infection, and cure is the goal, it is imperative that the device be removed. However, desired functional outcomes should influence the management plan. When ambulation is not likely regardless of cure or many other comorbidities limit function, it may be most reasonable to control the infection long term with antibiotic suppression rather than attempt cure (see Fig. 310.5A). In contrast, age alone should *not* be a reason to withhold curative therapy; physical/cognitive function at baseline and the likely outcome should drive this decision, not age. For lifesaving devices, such as mechanical valves or implantable defibrillators, removal of the device is again most effective at producing cure, but suppression may be a best option when other conditions modify the overall goals of therapy.

An area of intense and prolonged debate is the need, or lack thereof, for antimicrobial prophylaxis surrounding dental, GI, and GU procedures in those with prosthetic devices. Although generally recommended for prosthetic heart valves and frequently used for vascular grafts, particularly within the first 12 to 24 months after placement, there is no evidence of demonstrated benefit for prophylaxis in patients with prosthetic joints, intraocular lens implants, intracoronary artery stents, cerebrospinal fluid shunts, breast implants, or other less commonly used prostheses. The American Dental Association recommends "considering" antibiotic prophylaxis for patients with prosthetic joint at "high risk"—joints placed within 2 years, immunosuppressed patients (including those with diabetes mellitus, rheumatoid arthritis, or malnourishment), or those with a previous joint infection (see Chapter 105).

Gastrointestinal Infections

Serious GI infections are more common in old than young adults. Altered transit time, achlorhydria, diverticular disease, immune senescence, altered GI microbiota, and comorbid diseases predispose seniors to GI infection. Most of these illnesses are diagnosed and managed similarly regardless of age; however, a number of aspects deserve further comment.

C. difficile disproportionately affects older adults, and this group also bears more morbidity and mortality due to CDI.[28] Many CDIs are acquired and/or expressed during acute hospitalization or long-term care residence. Asymptomatic carriage of *C. difficile* is not rare in seniors: 2% of elderly subjects in the community, 10% in community-dwelling seniors with outpatient health care exposure, and 20% to 50% in hospital or long-term care settings.[29] Symptomatic CDI occurs in about one in every seven carriers. Older adults with CDI are more likely to develop severe infection (sepsis, hypotension, renal failure, toxic megacolon), but even milder CDI can have major consequences more often in those with advanced age. In young healthy adults, using the bathroom 6 to 8 times daily may be aggravating. In an older adult with decreased mobility, visual impairment, or dementia, having to urgently defecate 6 to 8 times daily may lead to loss of independence and institutionalization.

Guideline-driven therapy should be used for CDI and varies by severity of infection, primary versus relapsed infection, and other considerations (e.g., cost, drug interactions).[30] In recent years, fecal microbiota transplantation (FMT) has become standard for patients experiencing multiple relapses and can be done using oral capsules of freeze dried material, with success rates reported in the literature of 80% to 90%.[31] As opposed to most randomized trials of new therapies, the majority of subjects in FMT trials are older adults, and FMT has been shown to be safe and effective. Similarly, older adults predominated in trials demonstrating that passive antibody administration reduces the risk of CDI in those with prior CDI and about to receive antibiotics.[32] This approach is now in trials for primary CDI prevention.

Tuberculosis

Advanced age has long been established as a major risk factor for developing TB. Several studies have demonstrated that, when compared with young adults, older adults are less likely to present with fever, night sweats, cough, or hemoptysis, and more likely to present with nonspecific symptoms of dizziness, nonspecific pain or mental "dullness," a prior history of TB, and a concomitant diagnosis of underlying cancer (summarized in[33]). Older adults are more likely to have widespread lung infiltrates, whereas young adults are more likely to have isolated upper lobe infiltrates/cavities, and older adults are more likely to have evidence of malnutrition (i.e., reduced serum albumin).

Probably the most important issue of TB management in older adults remains the challenge of diagnostic testing. The sensitivity of the tuberculin skin test (TST) to detect latent infection declines with age, with immune senescence likely playing a role. New diagnostic tests for TB (i.e., interferon-γ release assay [IFGRA] from blood, measured after ex vivo *Mycobacterium tuberculosis* antigen exposure) unfortunately only modestly improve sensitivity. The pooled sensitivity from multiple studies appears to be ≈70% for TST versus ≈75% for IFGRAs. The specificity of IFGRA is better than TST in those who have received bacillus Calmette-Guérin immunization.

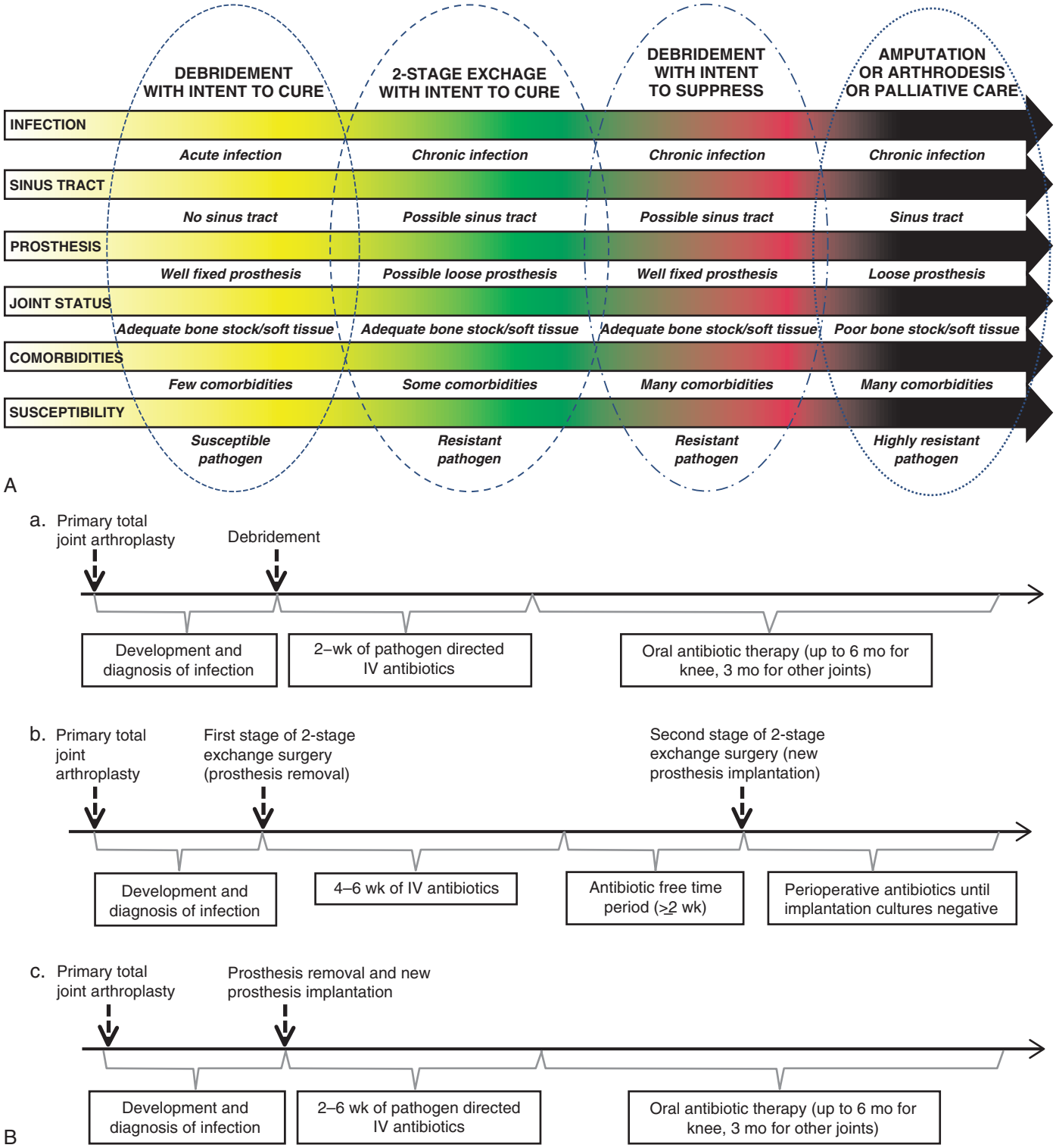

FIG. 310.5 (A) Factors that influence the treatment strategy of prosthetic joint infection in seniors. (B) Guideline recommended management in prosthetic joint infection when débridement-retention (a), two-stage (b), or one-stage (c) surgical intervention is used. *IV,* Intravenous. *(A and B reprinted with permission from Nair R, Schweizer ML, Singh N. Septic arthritis and prosthetic joint infections in older adults.* Infect Dis Clin North Am. *2017;31:715–729.)*

The treatment of TB in older adults does not differ from that of young adults, but older adults are more susceptible to adverse effects and drug-drug interactions.

Human Immunodeficiency Virus

Antiretroviral therapy (ART) advances have transformed human immunodeficiency virus (HIV) infection into a chronic illness—in other words, one "ages" with HIV and its treatment over a lifetime.[34,35] In fact, the majority of persons living with HIV (PLWH) in the United States are now older than 50 years due to effective treatment. The remarkable gains in HIV treatment have somewhat overshadowed the fact that, although PLWH can be expected to live into their 70s, their average life expectancy continues to lag the general population by about 5 years. Further, PLWH have a higher prevalence of age-related morbidities, including cardiovascular disease, hypertension, diabetes mellitus, bone fractures, cognitive impairment and dementia, cancer, and kidney

and liver disease; mechanisms of enhanced disease risk are slowly being unraveled.[36] Because of this they are more likely than HIV-uninfected persons to experience multimorbidity and even geriatric syndromes (frailty, falls, social isolation).

There is ongoing debate as to whether HIV accentuates age-related events (i.e., is a risk factor for multiple diseases, such as diabetes mellitus or smoking) or truly accelerates the hallmarks of the aging process itself (e.g., DNA methylation, telomere shortening, cellular senescence).[37] Complicating this area of research, there are dramatic cohort effects within PLWH, depending on prior ART exposure (e.g., prolong stavudine exposure with associated lipodystrophy, serial monotherapy before combination therapy, tenofovir-specific bone and kidney effects) that make it very difficult to separate the influence of HIV itself versus its treatment at a given time in history. It does appear, however, that the massive viral replication and associated die-off of immune cells during acute HIV infection is a cataclysmic biologic event of lasting consequence, much like a prolonged ICU stay, bout with cancer/chemotherapy, or other serious illness.[38,39] Aging mechanistic studies suggest this impact is equivalent to about 5 to 7 years of additional "biologic" aging that worsens the longer HIV goes uncontrolled and is not fully reversed by current therapies.

Fever of Unknown Origin

Fever of unknown origin (FUO) is classically defined as temperature >101°F (38.3°C) for at least 3 weeks and undiagnosed after 1 week of medical evaluation. FUO etiology varies by age and these differences should influence the diagnostic workup.[40] The cause of FUO can be determined in 90%-plus of cases, and approximately one-third will have treatable infections (e.g., intraabdominal abscess, bacterial endocarditis, TB, or occult osteomyelitis). In contrast to young adults, autoimmune disease is a more frequent cause of FUO in adults older than 60 years, accounting for about 25% of all FUOs in seniors. Systemic lupus erythematosus, the most common autoimmune cause of FUO in young adults, is absent in FUO series of older adults and is replaced by temporal arteritis (TA) and polyarteritis nodosa. Neoplastic disease accounts for another 20%, most often a result of hematopoietic malignancies (e.g., lymphoma and leukemia). Drug fever is a common cause of FUO in older adults, and deep venous thrombosis, with or without recurrent pulmonary emboli, occasionally causes FUO in older adults.

The approach to diagnosis in FUO for older adults should be influenced by the differential diagnosis. A thorough history and physical examination; basic laboratory evaluations of complete blood counts with differential, serum chemistries and hepatic enzymes, thyroid function studies, erythrocyte sedimentation rate (ESR), placement of a TST or performance of an IFGRA, a chest radiograph, and initial blood and urine cultures should begin the search. If the diagnosis continues to be obscure and the ESR is elevated, temporal artery biopsy, even in the absence of typical history or objective physical findings, should be strongly considered. If there is a low suspicion for TA, chest/abdominal/pelvic computed tomography is most likely to provide assistance.

IMMUNIZATION OF OLDER ADULTS

Immunization recommendations for older adults are constantly being updated. The latest consensus recommendations can be found at the Centers for Disease Control (CDC) web site at www.cdc.gov/nip/publications/ACIP-list.htm.

Pneumococcal Vaccine

Pneumococcal immunization is recommended for all seniors in this age group and for many patients younger than 65 years with comorbid conditions. Two vaccines are available: a 23-valent polysaccharide vaccine (PPSV23) and a 13-valent pneumococcal conjugate vaccine (PCV13). In 2014 recommendations from the Advisory Committee on Immunization Practices (ACIP) for pneumococcal vaccination in older adults were revised such that both PCV13 and PPSV23 should be given sequentially—PCV13 given first, followed by PPSV23 6 to 12 months later. For those already immunized with a first dose of PPSV23, repeat immunization is recommended with PCV13 as long as 1 year or more has passed since PPSV23 was administered. Another dose of PPSV23 should be given 6 to 12 months later and ≥5 years after the most recent dose of PPSV23; a minimum interval of 5 years between PPSV23 doses should be maintained. Studies of revaccination demonstrate that adverse events are uncommon and mild. Thus, when the pneumococcal immunization history is unknown, the vaccine should be administered.

Implementing current pneumococcal vaccine recommendations remains the greatest obstacle to prevention of fatal *Streptococcus pneumoniae* disease in the older adults. According to CDC estimates, only 40% to 50% of eligible older adults receive pneumococcal vaccine, and many unvaccinated older adults saw a medical practitioner or were even hospitalized within the prior 6 to 12 months. The CDC and its Advisory Committee on Immunization Practices (ACIP) have outlined several strategies for improving vaccine administration, including age-based, community-based, and provider-based strategies.

Influenza

The current ACIP recommendations are for all persons age 6 months and older be immunized each year. Medical personnel and caregivers for high-risk patients should be immunized to reduce transmission to those at increased risk for complications. There are multiple formulations of influenza vaccine (see Chapter 165 for details), but a high-dose influenza vaccine formulation is more effective than a standard-dose vaccine in older adults for preventing influenza-like illness (relative efficacy, 24.2%; 95% confidence interval [CI], 9.7 to 36.5) and serious events possibly related to influenza, reducing overall events by 17.7% (95% CI, 6.6% to 27.4%), serious pneumonia by 39.8% (95% CI, 19.3% to 55.1%), and all-cause hospitalizations by 6.9% (95% CI, 0.5% to 12.8%).[3]

Zoster

Zoster is much more common and more likely to cause long-term sequelae in older adults than in young adults. A live-virus zoster vaccine was approved in 2006 and reduces the risk of zoster by about 50% and the risk of clinically significant postherpetic neuralgia by about 60%. However, in 2015 data were published regarding a two-dose regimen of an adjuvanted subunit zoster vaccine that is much more efficacious, preventing zoster in ≈97% of recipients, even those age 70 years and older.[4] Based on these data the US Food and Drug Administration has now approved the vaccine, and ACIP now recommends administration of two doses of recombinant zoster vaccine (RZV; Shingrix) 2 to 6 months apart to adults age 50 years or older, regardless of past episode(s) of herpes zoster or prior receipt of zoster live-virus vaccine (Zostavax). The RZV does have a greater risk of local reaction, but the vast majority of cases are manageable with symptomatic treatment.

Other Vaccines

The older adult population is the group most at risk for tetanus in the United States, with women being particularly susceptible because of a lower likelihood of receiving boosters associated with minor/moderate trauma than in men. A complete tetanus vaccine series is indicated for persons with an uncertain history and for those who have received fewer than three doses. Boosters should be given at 10-year intervals, or more frequently with high-risk injuries.

Pertussis, although usually diagnosed in infants and young children, is often propagated by older adults with incomplete immune protection. Pertussis protection wanes with age, and all adults, including those 65 years and older, should receive an acellular pertussis formulation (TdaP) at least once as one of their scheduled tetanus boosters.

Hepatitis vaccines are also indicated for some older adults. Hepatitis B vaccine is indicated for older adults at risk (e.g., renal failure), but seniors are also commonly volunteers at health care facilities and should be considered at risk if they engage in any patient contact. Hepatitis A vaccine is indicated in more limited circumstances, such as chronic hepatitis C, travel, and for men who have sex with men. Unfortunately, the efficacy of hepatitis A and B vaccines in older adults is unknown but may be much lower than in young adults. Age-related declines in response to the vaccine, with antibody titers below those considered protective that can be noted as early as age 35 years; by age 70 years

less than half of adults given hepatitis B vaccine and only two-thirds of those given hepatitis A vaccine will produce antibody levels considered protective. Early results indicate that immunogenicity in the elderly may be improved with the recently marketed recombinant hepatitis B vaccine with a novel adjuvant (Heplisav-B).[40a]

TRAVEL RECOMMENDATIONS FOR OLDER ADULTS

Older adults are among the most widely traveled members of society, including to countries that require cholera and yellow fever vaccines. However, the yellow fever vaccine is a live-virus vaccine, and data suggest that older adults are six times more likely than young adults to experience a serious adverse event. Furthermore, although still quite rare, reactions requiring hospitalization or resulting in death occur more frequently in seniors than in young adults.[41] Because the incidence of yellow fever in travelers is very low, particularly when travel is limited to urban areas, a compelling case to administer the vaccine should be required before administering yellow fever vaccine in older adults. Often the reason given is that countries may deny access to those who cannot prove recent immunization, but a physician's letter of exemption is often acceptable.

Malaria chemoprophylaxis can be difficult in the older adults. Side effects are more common for many agents (e.g., mefloquine may produce dizziness, change in mental status, and bradycardia or prolonged QT intervals), and coadministration may be difficult in those taking cardiac medications or with significant heart disease. Alternative regimens are available, but recognition of current resistance patterns is critical (see CDC website [www.cdc.gov/travel] for up-to-date information).

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311 Infections in Asplenic Patients

Janet R. Gilsdorf and Suzanne Dawid

SHORT VIEW SUMMARY

Epidemiology
- Infection occurs in patients with congenital asplenia (rare), acquired asplenia secondary to splenectomy or sickle cell anemia, and acquired hyposplenia secondary to inflammatory or autoimmune disorders or human immunodeficiency virus–acquired immunodeficiency syndrome.
- The incidence is seven to eight severe infections per 100 person-years in postsplenectomy adults.
- Risk factors for infection include young or old age, occurrence less than 1 year after splenectomy, indication for splenectomy (immune cytopenias > trauma > incidental), lack of appropriate vaccines, and lack of prophylactic antibiotics in young children.

Microbiology
- Encapsulated bacteria (*Streptococcus pneumoniae, Haemophilus influenzae,* and *Neisseria meningitidis*) are the classic pathogens of postsplenectomy sepsis.
- With widespread use of vaccines against encapsulated bacteria, *Staphylococcus aureus* and gram-negative enteric bacteria are seen more commonly.
- Asplenic patients are at risk for increased severity of malaria, babesiosis, and anaplasmosis.

Diagnosis
- Blood culture is the most important test to identify postsplenectomy sepsis and must be obtained at the earliest sign of infection.
- White blood cell count may be elevated or depressed.
- Cerebrospinal fluid examination may be indicated in severe sepsis.
- Evidence of disseminated intravascular coagulation or vascular collapse may accompany sepsis.

Treatment
- Immediate empirical antibiotic therapy is key to preventing fulminant bacterial infection.
- Self-administered, oral antibiotics (amoxicillin-clavulanate, cefuroxime, or fluoroquinolone) may be used by asplenic patients at home at first sign of infection, followed by immediate visit to an emergency facility.
- Empirical systemic antibiotics (vancomycin + ceftriaxone or a fluoroquinolone) must be started immediately at an emergency facility (after blood culture but before other diagnostic tests).
- Definitive antibiotic therapy will depend on the identification and antibiotic susceptibility of the infecting pathogen.

Prevention
- Patient education, repeatedly reinforced, is the responsibility of all health care providers.
- Avoid splenectomy if possible.
- Administer appropriate vaccines (Hib, PCV13, PPSV23, MCV4, MenACYW, MenB, influenza), as recommended by the Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention.
- Prophylactic antibiotics are recommended for children younger than 5 years, those who are immunocompromised, or patients with past history of sepsis.
- Avoid vectors that carry the parasites causing malaria, babesiosis, and anaplasmosis.

After discovery of the spleen, which occurred sometime during the dawn of human dissection, its true role in health and disease remained a mystery for many generations. At the time of Galen, the spleen was thought to be important in removing "black choler" from the body; black choler (or black bile), according to Hippocrates, was one of the four humors thought to regulate bodily functions. The spleen and its various secretions were also implicated in a number of emotional imbalances. Excess black choler due to splenic failure was thought to be the cause of melancholy, whose name is derived from the words for "black" and "bile." Further, a person described as "splenetic" was hot-tempered or hasty in judgment, and "venting one's spleen" suggests a burst of pent-up anger. Black choler, a cold humor, was believed to counteract the effects of the two hot humors (blood and phlegm). Although the fourth humor, yellow bile, was known to be excreted by the gallbladder into the intestine, the mode of excretion of black bile was not known to the ancients.[1]

More recently the spleen has been recognized for its important role in protecting vertebrates from infection. It represents approximately 25% of the total lymphoid mass of the human body and contains half the body's monocytes and immunoglobulin-producing B lymphocytes.[2] Although not vital to human survival, the spleen plays an important role in the host's ability to combat both invasive bacteria, particularly those possessing polysaccharide capsules, and intraerythrocytic parasites.

ANATOMY OF THE SPLEEN

The embryonic precursor of the spleen is the mesenchyme of the dorsal mesogastrium, and the splenic red and white pulp are developed by the sixth month of fetal life. Normally located posteriorly in the left side of the peritoneal cavity, the spleen sits below the diaphragm, and its hilum, which contains the splenic artery and vein, is in close proximity to the tail of the pancreas. The normal organ is roughly the size of the patient's fist and weighs between 80 and 200 g in adult males and 70 and 180 g in adult females, but it can quickly enlarge in response to infection or inflammation. Aerated blood enters the spleen from the aorta via the splenic artery and short gastric arteries and, like the thymus, the spleen possesses only efferent lymphatic vessels. The normal spleen, which receives approximately 6% of the cardiac output,[3,4] is surrounded by a fibromuscular capsule.

The spleen consists of three anatomically distinct zones: red pulp, white pulp (seen as white nodules on the cut surface), and marginal zone (Fig. 311.1). Red pulp, the largest component of the spleen, is composed of a complex network of endothelium-lined venous sinuses and the Billroth cords, which contain fibrils, connective tissue, and large numbers of macrophages. White pulp, made up of islands within the red pulp that are composed of reticular structures that surround penicilliary arterioles, contains primarily T lymphocytes within the periarteriolar lymphoid sheath, as well as fewer B lymphocytes and natural-killer lymphocytes. Lymphoid follicles arise within this sheath, and on immunologic stimulation, the activated follicles form germinal centers similar to those seen in reactive lymph nodes. Located at the intersection of the red pulp and white pulp, the marginal zones are composed primarily of B cells (including memory cells) but also contain both T cells and antigen-presenting cells, such as Toll-like receptor–bearing macrophages or dendritic cells. Thus white pulp and the marginal

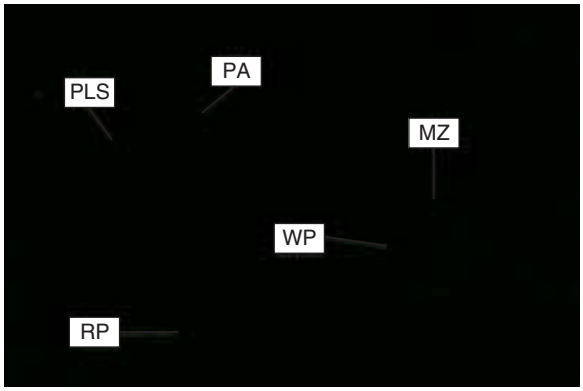

FIG. 311.1 Normal splenic architecture in the adult human. Hematoxylin and eosin stain. *MZ,* Marginal zone; *PA,* penicilliary arteriole; *PLS,* periarterial lymphatic sheath; *RP,* red pulp made up of vascular cords and venous sinuses; *WP,* white pulp. *(From Opal SM. Splenectomy and splenic dysfunction. In: Cohen J, Powderly WG, eds.* Infectious Diseases. *3rd ed. St. Louis: Mosby; 2010.)*

zones, where antigen-presenting cells, bacterial antigens, and T and B lymphocytes are in close proximity, are sites of considerable importance in the coordinated immune response to circulating antigens.[5]

FUNCTION OF THE SPLEEN

The contribution of the spleen to controlling overwhelming infection was first recognized in 1952 by King and Schumacker,[6] who reported life-threatening bacterial infections in splenectomized infants. Since then, the mechanisms by which the spleen combats bacterial infection, such as filtration, phagocytosis, and opsonization of bacteria, as well as regulation of inflammatory responses, have been carefully elucidated. The percentage of $CD8^+$ T cells is higher in the spleen than in the peripheral blood, leading to an inverse splenic CD4/CD8 ratio. Further, both CD4 and CD8 cell populations in the spleen show a higher number of activated cells, and the splenic $CD8^+$ T cells show a more differentiated cytotoxic $CD27^-CD45RA^+$ memory phenotype.[7] The multiple types of immune cells in close approximation within the spleen engage in complex interactions; are subject to the varied effects of autocrine and paracrine signaling; and migrate into, within, and out of the spleen—all contributing to the extraordinary repertoire of innate and adaptive immune responses important in protecting the host against bacterial and parasitic infections.

Regulation of Inflammation

The impact of the spleen on inflammation is evident by the increased production of proinflammatory cytokines during sepsis, which is, in part, mediated by nicotinic acetylcholine receptors in the spleen. This inflammatory response can be dampened by vagal nerve stimulation or the administration of nicotine.[8] In normal mice, administration of nicotine protected from polymicrobial sepsis by decreasing production of the proinflammatory mediator, high mobility group box-1 (HMGB1),[9] but in splenectomized mice, administration of nicotine failed to reduce HMGB1 levels and decreased survival during sepsis.[8] Similarly, although vagal nerve stimulation suppresses tumor necrosis factor (TNF) production in response to endotoxin administration in intact mice, no decrease was noted in splenectomized mice. This decrease in TNF production after vagal nerve stimulation occurs through stimulation of splenic acetylcholine-producing T cells by norepinephrine.[10] These findings suggest that loss of control of inflammation through this newly appreciated inflammatory reflex pathway may play a role in the inflammatory storm seen during postsplenectomy sepsis. Further, cytokines produced by splenic macrophages appear to modulate the febrile response to bacterial components, including lipopolysaccharide.[11]

Filtration and Clearance

The red pulp serves as a filter that provides grooming (antibody removal), pitting (intracellular material removal), and culling (cell destruction) functions to clear the blood of debris. As peripheral blood percolates through the splenic sinusoids, damaged cellular elements, senescent erythrocytes, cell-associated antibodies, circulating unopsonized bacteria, erythrocytes harboring malarial or babesial parasites, and foreign particles are removed, and platelets, erythrocytes, and iron are sequestered. The major defect associated with infectious risk of patients with asplenia or hyposplenia is impaired clearance of poorly opsonized particulate antigens, such as bacteria.[12]

Adaptive Immunity

After exposure of the host to microbial antigens (e.g., through vaccination or bacteremia), T cell–dependent B-cell activation in the spleen begins through antigen interactions with T-helper lymphocytes, stimulating B lymphocytes to rapidly differentiate into either antigen-presenting cells or immunoglobulin M (IgM)-producing plasma cells. After activation in the marginal zone, B and T cells, along with dendritic cells, are attracted to the periarteriolar lymphoid sheath of the splenic white pulp by cell-specific chemokines, where they facilitate T cell–dependent B-cell responses. Activated B cells undergo further maturation and clonal expansion in the splenic germinal centers, where, through contact with activated T cells, they undergo isotype switching into plasma cells capable of producing high-affinity antibodies or switched memory B cells.[13] Antigen-specific plasma cells that have differentiated in the white pulp lodge in the red pulp in close proximity to macrophages, where antigenic sampling occurs. This process is mediated by upregulation of CXC-chemokine receptor 4 (CXCR4), which binds CXC-chemokine ligand 12 (CXCL12) expressed in the red pulp.[14] Generation of IgM and IgG2 antibodies against polysaccharide antigens, which represent largely T-cell–independent responses, occurs in the marginal zone of the spleen and is profoundly compromised by splenectomy.[15]

Innate Immunity

The marginal zone of the spleen is responsible for trapping and processing circulating antigens in the course of peripheral blood circulating through its rich beds of macrophages. Marginal zone macrophages are proficient in processing carbohydrate antigens through lectin receptors on their surfaces and through their scavenger activities. Unlike other B-cell lineages, marginal zone B cells, characterized as IgM^+IgD^+CD27 memory cells, develop during ontogeny and mutate their immunoglobulin receptors during the first years of life without prior engagement in an immune response. On stimulation with thymus-independent polysaccharide antigens expressed by encapsulated bacteria, the prediversified IgM memory B cells of the marginal zone do not differentiate into switched memory cells, but, rather, represent an immediate innate immune defense, as opposed to a memory-dependent adaptive defense, against invading pathogens.[16,17] Thus, in asplenic hosts, this polysaccharide-specific immune function is lost, placing the patients at increased risk of infection with polysaccharide-encapsulated bacteria.

Hematopoiesis/Hemostasis

During the second trimester of fetal life, hematopoiesis actively occurs in the spleen and then wanes in the third trimester, although hematopoietic stem cells remain in the spleen through adult life. In the face of bone marrow failure, such as myelofibrosis, extramedullary hematopoiesis may occur in the spleen with the appearance of erythroid and megakaryocytic, and, to a lesser extent, myeloid precursors. Further, the spleen contributes to hemostasis through the production of factor VIII and von Willebrand factor.[18]

TYPES OF ASPLENIA

Congenital Asplenia

Congenital asplenia is rarely seen as an isolated clinical entity. It is, however, more commonly associated with many types of cyanotic congenital heart disease with heterotaxy, previously known as *Ivemark syndrome,* and is characterized by bilateral right-sidedness in which the liver is central, both lungs have three lobes, and the spleen is reduced in size or absent.[19] The degree of splenic function is variable in these children, dependent on the amount of splenic tissue present. It was traditionally believed that bilateral left-sidedness, characterized by polysplenia, dextrocardia, and other congenital cardiac defects, was not associated with splenic dysfunction, but a recent review of the available

literature suggests that patients exhibiting this defect are also at increased risk for bacteremia.[20]

Acquired Asplenia

The most dramatic and predictable loss of splenic function occurs after surgical splenectomy or splenic infarction secondary to sickled erythrocytes, which progressively develops over the first few years of life,[21] or to other embolic or thrombotic events in the splenic vasculature. Surgical splenectomy may be indicated in patients with severe splenic trauma, patients with intractable immune-mediated thrombocytopenia or anemia, patients with spherocytosis, or to relieve the effects of hypersplenism in patients with splenomegaly. Accessory spleens, present in up to 15% of patients at autopsy, may contribute to persistent immune cytopenias after splenectomy. Partial splenectomy, with retention of splenic immune function, may be an option in some patients with splenic trauma,[22] such as those who are hemodynamically stable and require fewer than two units of erythrocytes for resuscitation. The ability of either accessory spleens or residual splenic tissue to protect against severe infections in splenectomized patients is unclear, although overwhelming infections have been reported in both patients with accessory spleens and those with various amounts of residual splenic tissue.[23] In patients with hemoglobinopathies the greatest splenic dysfunction, which ultimately leads to splenic atrophy and asplenia, occurs in those with homozygous sickle cell anemia, followed sequentially by hemoglobin S/β-thalassemia major disease, hemoglobin SC disease, and hemoglobin S/β-thalassemia intermedia.

Acquired Hyposplenia

Functional hyposplenism related to impaired function of the splenic macrophage-associated Fc receptors may be associated with a variety of immunologic, rheumatologic, and inflammatory disorders (Table 311.1).[24,25] Those at particular risk include patients with graft-versus-host disease after bone marrow/stem cell transplantation, patients with human immunodeficiency virus (HIV) infection, and those with celiac disease. In the late stages of HIV–acquired immunodeficiency syndrome, atrophy of lymphoid follicles and T-cell depletion contribute to hyposplenism. Approximately a third of patients with celiac disease demonstrate impaired splenic function, and increased susceptibility to severe pneumococcal infection is seen among those with premalignant or malignant complications, concomitant autoimmune disorders, or thromboembolism, and among the elderly.[26] Further, splenic function is also compromised by a variety of medical therapies.[27] For example, corticosteroid treatment impairs the affinity of Fc receptors on splenic macrophages for opsonized IgG to a greater extent than hepatic, pulmonary, and bone marrow macrophages. Further, intravenous (IV) immune globulin decreases the phagocytic function of the spleen temporarily by binding to Fc receptors and impeding its recognition of opsonized particles. Although ionizing radiation does not affect phagocytic cells, it has a particularly profound, albeit transient, dose-dependent effect on T and B cells.

ASSESSMENT FOR SPLENIC FUNCTION

Splenic function is most simply assessed by examination of a peripheral smear for presence of the particulates normally filtered by the spleen. Such findings include red cells containing Howell-Jolly bodies (basophilic DNA remnants of red cell precursors), Pappenheimer bodies (abnormal iron granules), or presence of pitted or pocked red cells by phase-contrast microscopy (Fig. 311.2). The percentages of both pocked and pitted cells have been used to predict splenic function; normally, individuals possess less than 2% of these cells, but splenectomized patients possess up to 60%, and hyposplenic patients have intermediate values.[28,29] Percentages of red cells possessing Howell-Jolly bodies, which are present when the spleen is largely nonfunctional, is somewhat less sensitive than percentages of pocked or pitted cells.[28] With further validation, the number of IgM memory B cells, which correlates with functional splenic volume, may be a valuable parameter to assess splenic function.[16,30]

A variety of imaging studies may be useful in defining the presence, size, and, occasionally, function of the spleen. Abdominal ultrasonography and computed tomography scans can describe the size and position of the spleen, whereas radionuclide scintigraphy, which assesses phagocytic uptake of technetium-99m sulfur colloid, assesses anatomic features and correlates with phagocytic and immunologic function.[30,31] In a study using radionuclide scintigraphy in patients older than 18 years, all patients homozygous for sickle cell disease were asplenic, and heterozygous patients showed increased anatomic splenic volumes but decreased functional splenic volumes. In patients with splenosis (i.e., ectopic splenic tissue implanted in the abdomen after traumatic splenic rupture) or patients with accessory spleens that were retained after splenectomy, the immunologic defects related to splenic dysfunction are difficult to access, and overwhelming sepsis has been described in patients with documented residual splenic tissue.[23]

The immunologic defects associated with asplenia or hyposplenia reflect impairment of the many normal immunologic functions of the spleen, including compromised clearance of circulating bacteria, decreased opsonization of bacteria, impaired phagocytosis, and dysregulation of inflammatory responses (Table 311.2).

Epidemiology and Risk Factors of Sepsis in Asplenic Patients

Although sepsis related to reduced splenic function is relatively rare, when it occurs it often progresses rapidly to overwhelming, severe sepsis and may result in a fatal outcome. Risk factors for severe infection in asplenic patients include age (both very young and old age); indication for, and time since, splenectomy; underlying diseases or medical conditions; and vaccine status (Tables 311.3 to 311.5). In a large Danish

TABLE 311.1 Medical Conditions Associated With Functional Hyposplenism

MEDICAL CONDITION	PREVALENCE OF HYPOSPLENISM	DEGREE OF HYPOSPLENISM	STRENGTH OF EVIDENCE FOR RISK OF SEPSIS
Sickle cell anemia	100%	Severe	+++
Graft-versus-host disease	15%–40%	Moderate to severe	+++
Celiac disease	33%–76%	Moderate to severe	+++
Human immunodeficiency virus–acquired immunodeficiency syndrome	36%	Moderate to severe	+++
Alcoholic liver disease	37%–100%	Moderate to severe	+++
Inflammatory bowel disease Ulcerative colitis Crohn disease	 35%–45% 9%–37%	 Moderate Mild	++
Primary amyloidosis	28%	Moderate	++
Systemic lupus erythematosus	5%–7%	Mild to moderate	++

++, Moderate evidence; *+++*, strong evidence.
Modified from Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. *2011;378:86–97.*



FIG. 311.2 Red cell findings in hyposplenism. (A) Red blood cells with Howell-Jolly bodies, which are cytoplasmic inclusions composed of nuclear remnants, as seen in patients with hyposplenism. (B) Nucleated red blood cell that occurs during red cell maturation but is typically cleared by a normal spleen. (C) Red blood cells with Pappenheimer bodies, which are siderotic granules that are irregular in shape and frequently multiple within a cell. *(From Connell NT, Shurin SB, Schiffman FJ. The spleen and its disorders. In: Hoffman R, Benz EJ Sr, Silberstein LE, eds.* Hematology: Basic Principles and Practice. *6th ed. Philadelphia: Saunders; 2012:2259.)*

TABLE 311.2 Immunologic Defects After Splenectomy

DEFECT	MECHANISM
Decreased clearance of unopsonized bacteria	Depleted red pulp macrophages
Decreased clearance of cell-associated antibodies	Depleted red pulp macrophages
Decreased clearance of erythrocytes harboring malarial or babesial parasites	Depleted red pulp macrophages
Decreased processing of carbohydrate antigens	Depleted marginal zone macrophages
Decreased B-cell activation	Depleted marginal zone T cells
Decreased IgG2 and IgM antibodies	Depleted B cells in the germinal centers
Decreased CXC-chemokine ligand 12	Depleted CXC-chemokine receptor 4
Increased tumor necrosis factor	Depleted splenic nicotinic acetylcholine–producing T cells
Decreased recognition of pathogen-associated molecular patterns	Decreased Toll-like receptor networks in marginal zone macrophages
Decreased macrophage scavenger function	Decreased macrophage receptor with collagenous structure in marginal zone macrophages

CXC, Cysteine-X-cysteine (motif); *IgG2,* immunoglobulin G2; *IgM,* immunoglobulin M.

TABLE 311.3 Risk Factors for Hyposplenia/ Postsplenectomy Sepsis

RISK FACTOR	MECHANISM
Young age	Immune immaturity and naïveté
Old age	Immune senescence, comorbidities
Time since splenectomy	Risk at 0–90 days > 91–365 days > more than 1 year
Indication for splenectomy: splenectomy 2° to immune cytopenias > 2° to trauma > 2° to incidental	Unknown immune dysfunction associated with immune cytopenias
Hemoglobinopathies	Splenic infarction
Lack of appropriate vaccines	Impaired acquired immunity
Immunosuppressive drugs	Immunosuppression
Human immunodeficiency virus–acquired immunodeficiency virus	Immunosuppression
Amyloidosis/sarcoidosis	Splenic infiltration
Autoimmune diseases	Innate immunosuppression
Splenic radiation	Impaired splenic function

TABLE 311.4 Rates of Postsplenectomy Infection Requiring Hospitalization

SUBJECTS	INFECTIONS PER 100 PERSON-YEARS (95% CI)
Adults + children	7 (6.3–7.78)[a]
0–16 yr	2.94 (1.74–4.96)
17–29 yr	2.66 (1.82–3.88)
30–49 yr	5.35 (4.11–6.97)
50–59 yr	7.46 (5.84–9.49)
60–69 yr	11.07 (8.89–13.80)
70+ yr	13.85 (11.24–17.07)
Male	6.48 (5.61–7.48)
Female	7.72 (6.63–8.90)

[a]Infections >28 days after splenectomy.
CI, Confidence interval.
Modified from Kyaw MH, Holmes EM, Toolis F, et al. Evaluation of severe infection and survival after splenectomy. Am J Med. *2006;119:276.e1–276.e7.*

National Patient Registry[32] the overall rate of any infection requiring hospital care among splenectomized patients was 7.7 per 100 person-years compared with 2 per 100 person-years in the general population. The relative frequency of sepsis associated with hyposplenism or splenectomy among various groups of patients, however, is difficult to accurately assess, because the many studies include patients of different ages, vaccination status, indications for splenectomy, and underlying diseases leading to hyposplenism. Nevertheless, the risk of sepsis is clearly dependent on the age of the patient (see Table 311.4). Overall, children younger than 16 years have the same risk of sepsis associated with splenectomy or asplenia as young adults, although infants are at higher risk. Among patients with hemoglobinopathies the risk is greater for children than adults. In splenectomized adults the risk for severe infection increases among patients older than 50 years, with the highest risk among those older than 70 years.[33] The risk of sepsis is also dependent on the underlying disease associated with hyposplenism and with the indication for splenectomy (see Table 311.5).[2,32,34] Posttraumatic splenectomy is associated with a lower rate of sepsis than splenectomy due to hematologic abnormalities,[33] possibly due to regeneration of splenic tissue after posttraumatic splenectomy. Such regenerated splenic tissue, however, is histologically and functionally different from normal spleen tissue, and its ability to protect against sepsis may be limited.[35]

TABLE 311.5 Relative Risk of Postsplenectomy Infection Requiring Hospitalization by Indication

CLINICAL INDICATION FOR SPLENECTOMY AND DAYS SINCE SPLENECTOMY	RELATIVE RISK (95% CI) VERSUS GENERAL POPULATION	RELATIVE RISK (95% CI) VERSUS NONSPLENECTOMIZED CONTROLS
Any Indication		
<90 days	18.1 (14.8–22.1)	1.7 (1.5–2.1)
91–365 days	4.6 (3.8–5.5)	1.5 (1.2–1.8)
>365 days	2.5 (2.2–2.8)	1.2 (1.1–1.4)
Idiopathic Thrombocytopenic Purpura		
<90 days	14.8 (5.1–43.2)	2.6 (1.3–5.1)
91–365 days	14.2 (4.3–46.1)	1 (0.5–2)
>365 days	4 (2.8–5.6)	1.4 (1–2)
Hereditary Hemolytic Anemia		
<90 days	31.5 (6.4–153.7)	3.6 (1.1–12.5)
91–365 days	4.3 (1.4–13.4)	1 (0.4–2.6)
>365 days	4.0 (2.3–7.2)	1.1 (0.6–1.9)
Trauma		
<90 days	21.2 (12.8–35.1)	0.8 (0.5–1.2)
91–365 days	3.4 (2–5.9)	1.4 (0.6–2.9)
>365 days	2.5 (2–3.3)	1.3 (0.9–1.9)

CI, Confidence interval.
Modified from Thomsen RW, Schoonen WM, Farkas DK, et al. Risk for hospital contact with infection in patients with splenectomy: a population-based cohort study. Ann Intern Med. *2009;151:546–555.*

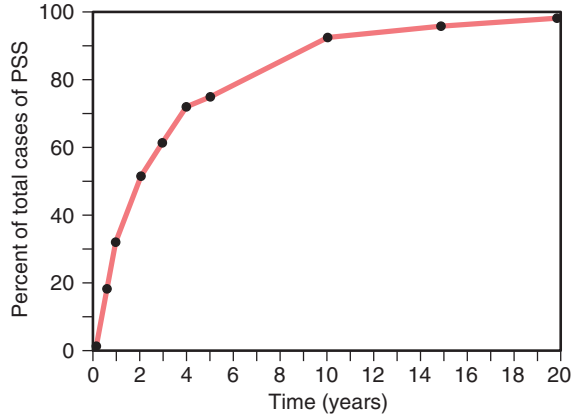

FIG. 311.3 Cumulative incidence of first severe infection after splenectomy. *PSS,* Postsplenectomy sepsis. *(Data from Kyaw MH, Holmes EM, Toolis F, et al. Evaluation of severe infection and survival after splenectomy.* Am J Med. *2006;119:276.e1–276.e7.)*

Hemoglobinopathy imparts a relatively high risk of sepsis, and patients with sickle cell anemia or thalassemia major are three to four times more likely to experience sepsis episodes compared with patients with splenectomy secondary to trauma.[34] Comorbidities among patients with hemoglobinopathies, such as decreased complement activation, immunoglobulin deficiency, cardiopulmonary illnesses, and hemochromatosis, may further increase their risk of infection.[36,37] The risk of subsequent episodes of postsplenectomy sepsis increases after each episode, with the risk of a second episode 6-fold higher than the first and the risk of a third episode 14-fold higher than the first.[33] The risk of sepsis is greatest during the first 3 years after splenectomy, with greatest rates during the first 3 months, and decreases significantly beyond 8 to 10 years after splenectomy (Fig. 311.3).[32,38] In general, young age predicts a shorter interval between splenectomy/hyposplenism and sepsis, but these data may be confounded by different reasons for splenic failure.

Although the incidence of overwhelming infection in asplenic/hyposplenic patients is low, the mortality rate is high, reportedly up to 50% and 70%.[34,38,39] Most recent data suggest that children are overall at lower risk of sepsis-associated death (<5% cumulative over 10 years) than older patients,[33] likely because of the impact of pneumococcal and *Haemophilus influenzae* type b (Hib) vaccines and advanced intensive care support in children, and because of increased comorbid conditions in older adults. Kyaw and colleagues[33] showed overall cumulative 1- and 5-year mortality rates of 10% to 40% and 20% to 70%, respectively, in adult splenectomized patients, with increased mortality rates in patients at increased age at the time of splenectomy. Recent data from Denmark documented the overall adjusted relative risk of death during the first 90 days after splenectomy to be 30-fold higher than in the general population.[40] This high mortality rate nearly disappeared, however, when controlled for morbidities associated with clinically similar patients who did not undergo splenectomy, suggesting that most of the mortality risk is associated with the indication for splenectomy rather than the splenectomy itself.

As in all postoperative patients, those undergoing splenectomy are at risk of immediate postoperative infections of the respiratory and urinary tracts or at operative sites. Patients requiring incidental splenectomy are at reduced risk of immediate postsplenectomy sepsis than those with splenectomy related to trauma because of the increased rate of postoperative infections, in general, among patients after severe trauma.[41] Because of the additive risks of spleen removal and general surgical infection, patients who undergo spleen-sparing management after splenic trauma may have lower rates of infection than those undergoing splenectomy.[42,43]

CLINICAL PRESENTATION OF SEPSIS SECONDARY TO ASPLENIA

In light of the important role the spleen plays in several immune pathways, patients with asplenia or hyposplenia are at risk of fulminating, severe, and sometimes fatal bacterial infections. Infected asplenic patients generally exhibit higher fevers than infected eusplenic patients,[44] so fever in splenectomy patients, irrespective of specific symptoms localized to the skin or respiratory or gastrointestinal tracts, must be assumed to represent bacterial systemic infection until proven otherwise. In splenectomized patients, decreased opsonization and phagocytosis of bacteria allows the microbes to proliferate to a level that exceeds the rate of removal, thus promoting and sustaining intravascular infections.[12] Because of dysregulation of the innate and adaptive immune systems,

low-level bacteremia may rapidly (over several hours) lead to high-level bacteremia with acute onset of high fever, rigors, acute respiratory distress, hypotension, coagulation defects, cardiovascular collapse, or a combination of these. Kyaw and colleagues[33] showed that 3% of splenectomized patients developed at least one episode of overwhelming infection, and more than a third of those had two or more subsequent severe infections. Endovascular damage related to overwhelming sepsis may lead to purpura fulminans with resulting gangrene of the extremities. In a study from the United Kingdom, 78 (68%) of the 114 deaths from postsplenectomy sepsis occurred within 24 hours of the onset of symptoms, and a further 13 (22%) patients died within 48 hours of the first symptoms.[38] Among patients who died of postsplenectomy sepsis, adrenal hemorrhage was seen in 39%, and disseminated intravascular coagulation (DIC) was seen in 14.6%. After bacteremia, metastatic sites of infection in asplenic patients may occur, such as septic arthritis, pericarditis, or meningitis, and are more prevalent in young children.

MICROBIAL AGENTS CAUSING POSTSPLENECTOMY SEPSIS

Bacteria

The specific immunologic defects that reduce the formation of antibodies to polysaccharide antigens in asplenic or hyposplenic patients place them at greatest risk of infection with encapsulated bacteria, historically *Streptococcus pneumoniae,* Hib, and *Neisseria meningitidis*. Infections with nontypeable and non-b encapsulated *H. influenzae* strains (i.e., types a and c–f) are not reported to be increased among splenectomy patients. The amount of polysaccharide capsule expressed by the bacteria may contribute to the risk of infection, as noted by the case of a splenectomized adult infected with a strain of *H. influenzae* possessing six copies of the *cap b* gene locus[45]; most type b strains possess two copies of the locus. No specific capsular serotype appears to predominate among *S. pneumoniae* strains associated with postsplenectomy sepsis.[46] *Neisseria meningitidis* has classically been listed as an important cause of sepsis in splenectomized patients, but this assertion has been questioned because few cases have been reported and, in one study, none of 10 splenectomized pediatric patients with meningococcemia had received splenectomy secondary to trauma, suggesting that previously reported meningococcal infections may have been related to the underlying illnesses, necessitating splenic removal rather than splenectomy itself.[47] Experimental data in mice supported this conclusion.[47]

Most data on the bacteria associated with severe infections in asplenic or hyposplenic patients were obtained before widespread use of prophylactic antibiotics or conjugated vaccines against these agents. More recent data obtained from 1998–2006 in Australia and from 1973–2009 in Israel [48,49] report that gram-negative bacilli and *Staphylococcus aureus* were the most common causes of postsplenectomy infections. These findings may depend on vaccination rates in the studied population, for in a recent prospective study from Germany, *S. pneumoniae* remained the major cause of overwhelming postsplenectomy infections, but, the reported vaccination rate was only 42% in this population (21% within the prior 5 years).[50] Thus the current increasing use of *S. pneumoniae* vaccines among adults, as well as the widespread use of protein-conjugated *S. pneumoniae* and *H. influenzae* vaccines in children in the United States, may modulate both the rates of postsplenectomy sepsis and the spectrum of the implicated bacteria.

Unusual gram-negative bacteria have also been associated with infections in asplenic or hyposplenic patients. Splenectomy is a strong risk factor for infections caused by *Capnocytophaga canimorsus,* the bacteria previously known as Centers for Disease Control and Prevention (CDC) group DF2 and associated with dog bites or scratches.[51] Between 20% and 39% of infected patients had undergone splenectomy.[52,53] Among patients with *Bordetella holmesii* bacteremia, 85% were anatomically asplenic.[54] Severe *Salmonella* infection is associated with hyposplenism secondary to splenic infarction in patients with sickle cell anemia but is not associated with overwhelming postsplenectomy sepsis. Postoperative infection after incidental splenectomy is usually caused by *S. aureus,* group A streptococci, or enteric gram-negative bacteria, as is reported in postoperative infections in general. A large number of other organisms have been implicated in postsplenectomy sepsis by single-case reports, but the true meaning of these associations is unclear.

Protozoa

The human spleen plays an important role in the clearance of parasite-infected red blood cells (RBCs) by filtering erythrocytes that are poorly deformable. Although classically transmitted by ticks, *Babesia microti,* which is endemic in the northeastern and upper midwestern regions of the United States, has been transmitted to asplenic patients through blood transfusion.[55] Asplenic patients with babesiosis experience high-grade parasitemia and develop more severe disease associated with significant hemolysis.[56] Similarly, in splenectomized patients with *Plasmodium falciparum* primary infection, parasitemia levels are higher, mature forms circulate more frequently, and disease severity and death rates are increased[57] compared with malaria in nonsplenectomized patients. In addition, cerebral malaria is frequent after splenectomy and likely reflects poor parasite clearance. In splenectomized patients who are immune to malaria (by nature of living in highly endemic areas), however, antibodies to the surface adhesins of mature parasites impede the sequestration of infected erythrocytes in the microcirculation, thereby preventing severe complications of malarial infection. During acute malaria in otherwise healthy people, previously parasitized erythrocytes that no longer contain a malarial parasite, the so-called ring-infected erythrocyte surface antigen (RESA)-RBCs that result after cleansing of the infected cells by the spleen, are found in the circulation. In splenectomized patients with falciparum malaria, however, these forms are not present, which highlights the importance of the filtration function of the spleen in clearing malarial-infected erythrocytes.[58] Acute *P. falciparum* malaria has been described in a chronic malaria carrier no longer exposed to *P. falciparum* and was attributed to loss of splenic clearance of adhesin-defective malarial variants, resulting in an acute clinical attack. Even in the face of adequate antimalarial treatment, parasite clearance was significantly delayed in a splenectomized patient, whose parasite burden of 63% decreased to only 30% after 13 days of treatment.[59] In addition to those infected with red cell parasites, asplenic patients infected with *Anaplasma phagocytophilum* (the causative agent of human granulocytic ehrlichiosis) exhibit recurrent, prolonged, or severe infections.[60]

DIAGNOSIS OF POSTSPLENECTOMY SEPSIS

The single most valuable diagnostic test in evaluating a patient with suspected postsplenectomy sepsis is the blood culture (Table 311.6). As a result of impaired bacterial clearance in the absence of normal splenic function, the bacterial burden in the blood of asplenic patients can be so high that organisms may be seen on blood smear or on Gram stain of the buffy coat. The blood cultures usually become positive quickly, often within hours of incubation. Antimicrobial susceptibility analysis of organisms isolated on blood culture is important for choosing the most appropriate antibiotics for definitive treatment. Bacterial culture of purpuric skin lesion aspirates may also yield positive results, and the complete blood count offers important immediate information on the risk of bacterial infection. The neutrophil count in the peripheral blood may be elevated or depressed during postsplenectomy sepsis, dependent on the ability of the infected host to adequately mount a white cell response in the face of overwhelming sepsis. The differential count often shows a high prevalence of band forms or other immature cells, such as myelocytes or metamyelocytes, indicating massive demargination and recruitment of developing neutrophils from the bone marrow. Peripheral white cells may show toxic granulations or Döhle bodies, or both. Peripheral red cells often show manifestations of reduced filtration of damaged erythrocytes, such as Howell-Jolly bodies, and pitted or pocked red cells. Thrombocytopenia is a sign of DIC from serious infection, as is the presence of schistocytes on the peripheral smear.

Other laboratory abnormalities associated with postsplenectomy sepsis and indicative of multiorgan failure include coagulation abnormalities associated with DIC, elevated creatinine, and elevated aminotransferases. Hypoxemia, hypocarbia, and mixed metabolic acidosis and respiratory alkalosis may be seen on blood gas analysis. Cerebrospinal fluid analysis may reveal leukocytosis indicative of bacterial meningitis, although bacteria may be present in early, rapid-onset meningitis before leukocytosis is evident.

TABLE 311.6 Diagnostic Tools to Assess Asplenic Patients for Infection

TEST	RESULT
Blood culture	Positive, often within hours, in the absence of prior antibiotic therapy
Culture of purpuric skin lesions	May be positive
White blood cell count	Elevated or depressed, with a marked shift to the left. Cells may show toxic granulations or Döhle bodies
Peripheral smear	Evidence of asplenia (Howell-Jolly bodies, pitted or pocked red cells). Circulating bacteria may be seen
Lumbar puncture	Elevated white blood cell count indicates meningitis. Culture is positive in the absence of prior antibiotic therapy
Chest radiograph	May show evidence of myocarditis or pneumonia
Coagulation studies	May support DIC
Platelet count	May be depressed with overwhelming sepsis and DIC
Creatinine	May be elevated with multiorgan failure
Hepatic enzymes	May be elevated with multiorgan failure
Blood gases	May indicate metabolic acidosis or respiratory alkalosis, or both

DIC, Disseminated intravascular coagulation.

TABLE 311.7 Antibiotic Management Options for Sepsis in Asplenic Patients

CLINICAL SITUATION	DRUGS, ADULT DOSES
Empirical: patient self-administered at onset of fever (before immediate visit to an emergency facility)	Amoxicillin-clavulanate 875 mg PO (q12h) *or* Cefuroxime 500 mg PO (q12h) *or* Levofloxacin 750 mg PO (q24h) *or* Moxifloxacin 400 mg PO (q24h)
Empirical: physician's office (before immediate visit to an emergency facility)	Ceftriaxone 2 g IM or IV *or* Levofloxacin 750 mg PO or IV *or* Moxifloxacin 400 mg PO or IV
Empirical: emergency medical facility	Vancomycin 15-20 mg/kg IV q12h *plus* Ceftriaxone 2 g IV q12h *or* Levofloxacin 750 mg IV q24h *or* Ciprofloxacin 400 mg IV q12h
Definitive: pathogen recovered	Appropriate antibiotic based on culture and antibiotic susceptibility testing

IM, Intramuscular; *IV,* intravenous; *PO,* orally.

MANAGEMENT OF POSTSPLENECTOMY SEPSIS

Because of the potential for rapidly developing severe and sometimes fatal infections in patients with asplenia or hyposplenia, such patients must be counseled to seek immediate medical attention at the first signs of infection, such as fever and chills, and to communicate their asplenic status to the medical personnel (Table 311.7). The risk of rapidly progressive systemic infection in these patients has led some experts to recommend patient-administered, empirical, high-dose oral antibiotics at the onset of fever, especially in the first several years after splenectomy, with the strong recommendation that the patient proceed immediately to an urgent care medical facility. Suggested antibiotics, kept in the patient's home, include amoxicillin-clavulanate, cefuroxime, or extended-spectrum fluoroquinolones. Prescriptions for these empirical antibiotics must be renewed annually to prevent outdating of the drugs.

If an asplenic/hyposplenic patient presents to a physician's office with symptoms of suspected sepsis, the patient should receive intramuscular or IV third-generation cephalosporin or, if β-lactam allergic, oral or IV extended-spectrum fluoroquinolone. Ideally, these drugs should be administered after a blood culture is obtained, but if blood culture is not immediately feasible, the drugs should be administered anyway to prevent delay in initiating antibiotic treatment.

Asplenic/hyposplenic patients with suspected sepsis should be evaluated at a full-service emergency medical facility as soon as the symptoms of a possible infection are recognized. Blood cultures should be obtained and empirical antibiotic therapy begun immediately, even before obtaining other diagnostic tests. Effective antibiotics include vancomycin plus either a third-generation cephalosporin, such as ceftriaxone, or an extended-spectrum fluoroquinolone. Fluoroquinolones may be used in children of all ages when indicated. The patient should then be hospitalized for continued antibiotic treatment and should be carefully monitored for evidence of vascular collapse and multiorgan failure. Blood culture and antibiotic susceptibility results will guide subsequent antibiotic choice. If the patient does not appear to respond to the antimicrobial treatment, an empirical change in antibiotic or initiation of an additional antibiotic may be warranted, although the poor clinical response may be due to uncontrolled sepsis with vascular collapse and multiorgan failure rather than antibiotic failure. Supportive care may require inotropic support and mechanical ventilation. Adjunctive dexamethasone therapy has been shown to reduce neurologic complications and case fatality rates when given early in treatment of adults with *S. pneumoniae* meningitis,[61,62] but no data are available to support its use in postsplenectomy sepsis without meningitis. Other adjunctive therapies targeting host immune responses have been studied in patients with sepsis in general, and to date none have been shown to be effective and without significant side effects.

Patients with documented accessory spleens or residual splenic tissue after partial splenectomy may remain at risk of overwhelming sepsis and should be managed similarly to other asplenic patients. Absence of Howell-Jolly bodies in their peripheral smears does not predict protection by the remaining splenic tissue against infection.

PREVENTION OF POSTSPLENECTOMY SEPSIS

Because the outcome of sepsis in asplenic/hyposplenic patients may be devastating, preventing such infections is paramount to good medical care (Tables 311.8 and 311.9). Recent studies have shown that spleen-sparing approaches to splenic injury, such as surgical repair, may be successful in patients with grades 1 to 3 blunt splenic trauma,[63] and this approach led to no difference in risk of immediate postoperative sepsis (18.3%) compared with similar patients who underwent total splenectomy(18.5%).[43] The risk of sepsis outside the immediate postoperative period is not, however, well defined for patients undergoing partial splenectomy, so sepsis prevention strategies should be the same for this group of patients as those undergoing total removal of the spleen. Overwhelming sepsis in patients with documented splenosis after traumatic spleen injury has been documented.[23]

Prophylactic Antibiotics

Daily oral antibiotics have been shown to reduce the incidence of sepsis in children with all-cause splenectomy by 47% and the incidence of mortality by 88%.[64] Further, daily oral penicillin reduced the incidence of *S. pneumoniae* bacteremia by 85% in children with sickle cell anemia and functional hyposplenism.[65] It should be noted that these studies were conducted before the recent increase in penicillin resistance in *S. pneumoniae* and before widespread use of conjugated pneumococcal vaccines. Nevertheless, children with asplenia or hyposplenia should receive oral antibiotic prophylaxis with penicillin VK (125 mg, twice a day, for children younger than 3 years and 250 mg, twice a day, for children 3 years old and older) or amoxicillin (20–40 mg/kg/day). Experts in the United States recommend daily prophylaxis with β-lactam antibiotics in children with sickle cell anemia up to 5 years of age and for at least 1 year after

TABLE 311.8 General Strategies to Prevent Specific Severe Infections and Sepsis in Splenectomized Patients

STRATEGY	COMMENTS
Avoid splenectomy	Spleen-sparing approaches to splenic trauma are being increasingly considered
Educate patients of their risks	Should be reinforced at each medical encounter
Create warnings of sepsis risk in the medical record	Should be readily available to all health care providers
Medic-Alert bracelet	Should be considered in all patients, especially those who travel
Antibiotic prophylaxis	Used in children primarily
Empirical self-administered antibiotic therapy in the home	Needs to be represcribed annually to avoid outdated drugs
Immunization	Should be discussed at each medical encounter and boosters given as recommended

TABLE 311.9 Specific Strategies to Prevent Severe Infections in Splenectomized Patients

INFECTION/AGENT	PREVENTION STRATEGY
Bacteria	Antibiotic prophylaxis (penicillin VK or amoxicillin) in children <age 5 yr; survivors of previous sepsis; immunosuppressed patients
Streptococcus pneumoniae	*Infants:* PCV13 at 2, 4, 6, and 12–15 mo; influenza vaccine annually ≥6 mo of age *Children:* PPSV23 at ≥2 yr, with revaccination after 5 yr; if nonvaccinated, PCV13 one dose 8 wk before PPSV23; influenza vaccine annually *Adults*: PCV13 followed in 8 wk by PPSV23, with one-time PPSV23 revaccination after 5 yr; influenza vaccine annually
Haemophilus influenzae	*Infants:* Hib vaccine at 2, 4, (6), and 12–15 mo *Children:* Hib vaccine 1 dose 2 wk presplenectomy *Adults:* Hib vaccine 1 dose 2 wk presplenectomy
Neisseria meningitidis	*Infants (6 wk–18 mo):* Men-ACYW-CRM at 2, 4, 6, and 12–15 mo. MenACYW 3 yr after completion of infant schedule. *Children >2 yr and adults* MenACYW two doses, revaccinate every 5 yr. MenACYW-D must be given at least 4 weeks after PCV-13 administration. *Children and adults 10–25 yr:* MenB, two or three doses
Babesiosis	Vector avoidance in endemic regions
Malaria	Vector avoidance in endemic regions, malaria prophylaxis
Human granulocytic ehrlichiosis	Vector avoidance in endemic regions
Capnocytophaga canimorsus	Avoidance of dog bites and scratches

Hib, Haemophilus influenzae type b; *Men,* meningococcal; *MPSV4,* meningococcal polysaccharide vaccine 4; *PCV13,* pneumococcal conjugate vaccine 13; *PPSV23,* plain polysaccharide vaccine 23.

splenectomy.[66] Asplenic and highly immunocompromised patients, as well as those with thalassemia and previous episodes of sepsis, may benefit from prophylaxis until age 18 years or for life. Patients with penicillin allergies manifested by nonurticarial rash and without anaphylaxis may be given first-generation oral cephalosporins, and children older than 8 years of age and adults may receive doxycycline. Other options are less attractive and less well studied but include oral clindamycin or oral fluoroquinolones; *S. pneumoniae* are increasingly resistant to macrolides and trimethoprim-sulfamethoxazole, but erythromycin (250 mg, twice a day) continues to be recommended by many experts.[66]

Patients and their families must be counseled to the limitations of prophylactic antibiotics in preventing infections in asplenic patients because of possible antibiotic resistance. The value of prophylactic antibiotics may also be compromised by poor compliance. Only 40% of splenectomized patients who received prescriptions for prophylactic penicillin demonstrated penicillin in their urine,[67] so additional prevention strategies and reinforcement of the implemented strategies are necessary to reduce the risk of postsplenectomy sepsis.

Antibiotics are indicated for dental prophylaxis in children after splenectomy[68] but are controversial in adults because of lack of strong supporting evidence.[69]

Vaccines

Vaccination against encapsulated bacteria is a mainstay of prevention against overwhelming sepsis in asplenia/hyposplenia patients. These vaccines should be administered at least 14 days before planned splenectomy to assure an adequate immune response, although they may be of some value when given shortly after splenectomy.[70] In patients receiving immunosuppressive chemotherapy or radiation therapy, the vaccines should be delayed until at least 3 months after completion of the treatment to assure optimal efficacy.[71]

Two vaccines targeting *S. pneumoniae* are available in the United States (see also Chapter 316). Plain polysaccharide vaccine (PPSV23) contains 23 pneumococcal capsular polysaccharides and is immunogenic in adults and children older than 2 years. Pneumococcal conjugate vaccine 13 (PCV13) contains the 13 pneumococcal polysaccharides most common in invasive *S. pneumoniae* infections in the United States conjugated to protein carriers, thus theoretically improving the magnitude and duration of immunoprotection. PCV13 is immunogenic in children older than 6 weeks of age and is routinely recommended for all children in the United States at 2, 4, 6, and 12 to 15 months.

All young children, including those with asplenia or hyposplenia, should receive the four-dose series of PCV13 beginning at age 2 months as routinely recommended.[72] For inadequately immunized children younger than 6 years who have not received the full routine vaccine series, catch-up vaccination should follow the recommendations of the American Academy of Pediatrics and the Advisory Committee on Immunization Practices (ACIP) of the CDC,[71] which are updated annually (www.cdc.gov/vaccines/acip/index.html). In addition, asplenic and hyposplenic children 6 to 18 years of age who have not received pneumococcal immunization should receive one dose of PCV13. To extend protection against an increased number of *S. pneumoniae* serotypes, asplenic and hyposplenic children should also receive a dose of PPSV23 at 2 years of age or older and at least 8 weeks after the last dose of PCV13. Because antibodies against PPSV23 are not as enduring as those induced by PCV13, children should be revaccinated with PPSV23 5 years after their first PPSV23 vaccine. Children who received PCV7 (an earlier vaccine formulation containing seven pneumococcal serotypes) as their primary series should receive a supplemental dose of PCV13 at least 8 weeks after receiving the PCV7.

All asplenic and hyposplenic adults previously unimmunized against *S. pneumoniae* should receive PCV13 followed by, at least 8 weeks later, PPSV23. Adults previously immunized with PPSV23 should receive a single dose of PCV13 at least 1 year after the most recent dose of PPSV23 because lower opsonophagocytic antibody responses were seen in persons who received PCV13 less than 1 year after PPSV23.[73] Adults up to age 65 years should be revaccinated with PPSV23 5 years after their first vaccine. In addition, asplenic adults 65 years of age and older should be reimmunized with PPSV23 if they were vaccinated more than 5 years earlier and at a time when they were younger than 65 years.[74]

A history of *S. pneumoniae* sepsis is not a contraindication for receipt of subsequent PCV13 or PPSV23 vaccines. Asplenic children and adults who experience *S. pneumoniae* infection should receive subsequent vaccines as recommended for all asplenic individuals.

Vaccines against Hib contain the type b polysaccharide conjugated to carrier proteins and are routinely recommended for all children in

the United States at 2, 4, (6), and 12 to 15 months of age, dependent on the vaccine formulation.[66] Children older than 5 years and adults are assumed to be naturally immune to Hib. Nevertheless, fully immunized children and adults should receive one dose of Hib vaccine at least 14 days before undergoing elective splenectomy.[67,72] Inadequately immunized children should receive catch-up Hib vaccines per the recommendations of the American Academy of Pediatrics and ACIP, which are updated annually (www.cdc.gov/vaccines/acip/index.html).

Quadrivalent vaccines that protect against *N. meningitidis* serogroups A, C, Y, and W135 contain four polysaccharides conjugated to the carrier protein diphtheria toxin (MenACYW-D) or CRM (MenACYW-CRM).[75]

Asplenic/hyposplenic infants younger than 24 months of age should receive Men-ACYW-CRM at 2, 4, 6, and 12 to 15 months. Nonimmunized children between the ages of 19 months and 2 years should receive two doses of MenACYW-CRM, 3 months apart. A blunted response to PCV-7 was noted in patients who received MenACYW-D simultaneously with PCV.[75] For this reason, children with asplenia should not receive MenACYW-D before the age of 2 years. In children older than 2 years, MenACYW-D should follow PCV-13 vaccination by at least 4 weeks. Children who received the primary series before age 2 years should receive a booster dose of Men-ACYW three years after its completion, followed by revaccination every 5 years. Asplenic individuals older than age 2 who have not been previously vaccinated against meningococcal disease should receive two doses of MenACYW at least 3 months apart, with revaccination every 5 years. Two meningococcal serogroup B vaccines (MenB) that contain factor H–binding protein with or without three additional bacterial proteins are licensed by the US Food and Drug Administration for individuals 10 to 25 years of age and given as either a two- or three-dose series, dependent on the vaccine.[76] These vaccines are not interchangeable, and the entire series must be completed with the same vaccine product.

Influenza infection increases the risk of pneumococcal infection among healthy individuals and likely is an additional risk for splenectomized individuals. Routine influenza vaccine is now recommended for all children older than 6 months and for all adults. Asplenic or hyposplenic individuals should be specifically encouraged to receive seasonal influenza vaccine annually.

Patient Education

Optimal medical care of asplenic/hyposplenic patients is contingent on the patient's knowledge of all aspects of the condition.[77] Basic information on asplenia and its risks, as well as sepsis prevention and management strategies, with repeated reinforcement and updating, is the responsibility of all health care providers (Table 311.10). Even in the face of good patient education, patient retention of the information may be limited,[78] and compliance with recommendations may be suboptimal.[79] A few professional organizations and health care institutions have prepared educational materials for postsplenectomy patients, and these can be useful as a base on which more detailed disease and patient-specific information is built.[80–82] Use of a patient registry to assist in delivering vaccines, antibiotic prophylaxis, and patient education in Australia proved cost-effective in terms of reduced mortality rates and reduced postsplenectomy infection rates.[63] Further, education of asplenic patients about travel-related risks may reduce the rate of postsplenectomy infection.[79]

TABLE 311.10 Important Educational Information for Asplenic Patients

INFORMATION CATEGORY	INFORMATION CONTENT
Spleen	Basic information on splenic anatomy and function
Splenectomy	Operative procedure, complications, alternatives
Sepsis—what is it?	Signs and symptoms of infection
Sepsis—who gets it?	Risk assessment, risk of parasites based on geography
Sepsis—what to do about fever?	Self-administered empirical antibiotics, need for immediate medical attention
Sepsis—how to prevent it	Prophylactic antibiotics for children, immunizations for all ages, insect vector avoidance
Sepsis—how to treat it	Basics of antibiotics and supportive care
What about traveling?	Update immunizations, empirical self-administered antibiotics, malaria prophylaxis, insect vector avoidance

Modified from Pasternack MS. Patient Information: Preventing Severe Infection After Splenectomy (Beyond the Basics). *http://www.uptodate.com/contents/preventing-severe-infection-after-splenectomy-beyond-the-basics. Accessed February 19, 2013.*

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312 Infections in Injection Drug Users

Donald P. Levine and Patricia Brown

SHORT VIEW SUMMARY

HOST DEFENSES

- Direct effects of opioids on the immune system include impairment of chemotaxis, phagocytosis, cytokine and chemokine production, natural killer cell activity, lymphocyte proliferation in response to mitogens, and antigen presentation by B lymphocytes. Indirect effects through the neuroendocrine system and the autonomic nervous system are also postulated.
- Immunoglobulin M (IgM) and IgG levels are often elevated, giving rise to a high frequency of autoantibodies as well as antibodies against various microorganisms; this may cause diagnostic confusion (e.g., in interpretation of syphilis serology).
- Morphine-mediated depression of monocyte functions important to antiviral defense may contribute to the high efficiency of transmission of viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV).

SKIN AND SOFT TISSUE INFECTIONS

- Location of infection is related to the preferred location of injection; abscesses are most common, followed by cellulitis and skin ulcers.
- Infection with *Staphylococcus aureus* (particularly community-acquired methicillin-resistant *S. aureus* [MRSA]) is most common, followed by streptococci, either alone or in combination with other pathogens; infection with *Eikenella corrodens* occurs in injection drug users (IDUs) who lick their needles or contaminate their drugs with saliva; anaerobes and gram-negative bacilli are found in mixed infections.
- Incision and drainage is the mainstay of therapy for abscesses; for cellulitis, antibiotic selection and need for hospitalization must be individualized based on severity of illness; a high index of suspicion for necrotizing fasciitis should be maintained in the setting of severe pain or severe signs of systemic toxicity or both.

BONE AND JOINT INFECTIONS

- These infections are common, mainly affect the axial skeleton, and occur by the hematogenous route; contiguous spread from adjacent skin and soft tissue infection also occurs.
- Bacterial infection with *S. aureus* is most common; infection with *Pseudomonas* is reported; infection with *Eikenella* occurs in IDUs who lick their needles; and infection with *Candida* spp. is increasingly reported.
- Joint infections typically involve the extremities (most commonly the knee), although involvement of unusual sites (costochondral and sternoclavicular joints, pubic symphysis) is frequent; there is a high incidence of cervical spine involvement in individuals with vertebral osteomyelitis.
- Microbiologic diagnosis should be confirmed in all cases; the use of high bioavailability oral antibiotic therapy for prolonged treatment is increasing.

INFECTIVE ENDOCARDITIS

- Incidence among IDUs appears to be increasing, perhaps owing to increased methamphetamine use (increased number of injections); IDUs with HIV are at even higher risk for infective endocarditis (IE).
- Infection with *S. aureus* is most common (the incidence of MRSA is increasing), followed by groups A, B, and G streptococci; outbreaks of gram-negative IE (caused by *Serratia* or *Pseudomonas*) are well described; and infection with *Candida* spp. (mainly species other than *C. albicans*) have been reported.
- Tricuspid valve IE is associated with injection drug use, but the incidence of left-sided IE now exceeds that of right-sided infection in some series; multiple valves may be involved; infection of the pulmonary valve is rare.
- IDUs tend to present acutely in the first week of illness; diagnosis is usually easy to make based on clinical signs and symptoms and supported by the results of blood cultures and cardiac imaging.
- Initial empirical therapy should be directed against *S. aureus* (with vancomycin or nafcillin in settings where MRSA is known to be rare); optimal therapy for MRSA isolates with vancomycin minimal inhibitory concentration of 2.0 µg/mL is not yet defined; many experts recommend daptomycin.
- Cefepime plus high-dose tobramycin has been used successfully to treat pseudomonal IE; surgical therapy along with amphotericin plus flucytosine is the standard recommendation for candidal IE; the role of azoles and echinocandins is not yet defined; indefinite suppressive therapy with oral azoles is recommended if the patient is not a candidate for surgery.

NONCARDIAC VASCULAR INFECTIONS

- Infection with *S. aureus* is most common; infection with *Pseudomonas* is also reported; polymicrobial infections are common.
- Septic thrombophlebitis manifests as local pain, swelling, and fever, with bacteremia and sepsis; septic pulmonary emboli may occur; antibiotic therapy should be given for at least 4 weeks; the role of anticoagulation remains controversial—short-term anticoagulation while the patient is hospitalized may be sufficient.
- Mycotic aneurysms frequently involve the femoral veins and manifest as a tender, enlarging, and pulsatile mass; Doppler ultrasonography, computed tomography (CT), or magnetic resonance angiography (MRA) can be used for diagnosis; surgical management is required, along with antibiotic therapy for 4 to 6 weeks.

PULMONARY INFECTIONS

- Community-acquired pneumonia caused by common respiratory pathogens is most common; patients are at risk for bacteremia, parapneumonic effusions, and empyema.
- Pulmonary tuberculosis is a major problem among IDUs, especially IDUs infected with HIV; treatment for latent tuberculous infection is challenging; material incentives and directly observed therapy can increase adherence.

HEPATITIS

- Cocaine, methamphetamine, and buprenorphine (injected or sublingual) can be hepatotoxic; heroin is not.
- IDUs are at risk for HBV infection and should be vaccinated if nonimmune; spontaneous reactivation of infection can occur.
- In nonendemic areas such as the United States, hepatitis delta virus (HDV) occurs almost exclusively in IDUs; although the overall prevalence of HDV has decreased, the prevalence among individuals with chronic HBV infection has increased.
- Superinfection of HDV on chronic HBV is most common; however, simultaneous infection occurs in IDUs and can result in fulminant hepatitis.
- IDUs account for most HCV infections in the United States. The seroprevalence of HCV infection among IDUs in the United States was decreasing; however, the current opioid epidemic has reversed this trend. HCV

SHORT VIEW SUMMARY—cont'd

treatment is challenging in IDUs and should be linked to treatment for addiction.
- IDUs are at increased risk for infection with hepatitis A virus, but socioeconomic factors rather than drug use are the larger risk; nonimmune IDUs should be vaccinated.

SPLENIC ABSCESS
- Splenic abscess occurs most commonly as a complication of IE; secondary infection of a cocaine-induced splenic infarct or infection occurring after trauma also is reported; staphylococci and streptococci are the most common causative agents.
- Ultrasonography, CT, and magnetic resonance imaging all can be used for diagnosis; although splenectomy is generally considered the management of choice, especially in the setting of IE necessitating valve replacement, there is an increasing role for percutaneous drainage.

CENTRAL NERVOUS SYSTEM INFECTION
- Infection of the central nervous system occurs most commonly as a complication of IE (e.g., meningitis, brain abscess, mycotic aneurysms).
- Mycotic aneurysm may be diagnosed by CT, CT angiography, or MRA; optimal management is not defined; many aneurysms heal with antibiotic therapy; ruptured aneurysms require surgery or an endovascular procedure.
- The differential diagnosis of brain abscess is broad, especially in IDUs with HIV infection; microbiologic diagnosis should always be confirmed.
- Spinal epidural abscess should be suspected in IDUs who present with back pain accompanied by radicular symptoms or neurologic findings; *S. aureus* is the most common etiologic agent.
- IDUs are at increased risk for tetanus and wound botulism.

OCULAR INFECTIONS
- Bacterial and fungal endophthalmitis are seen frequently as a complication of IE; *Aspergillus* endophthalmitis is also reported.
- Intravitreal antimicrobial agents with or without pars plana vitrectomy may be required.

HUMAN IMMUNODEFICIENCY VIRUS INFECTION
- Incidence of HIV infection among IDUs in the United States has steadily declined, although the current opioid epidemic may threaten this trend; however, injection drug use remains a major risk factor for HIV acquisition in other parts of the world.
- Safe syringe programs are effective at reducing HIV transmission but should ideally be coupled with access to voluntary counseling and testing, opioid substitution therapy, and combination antiretroviral therapy (ART) for individuals already infected. IDUs can benefit from preexposure prophylaxis with antiretroviral agents.
- Injection drug use is associated with an increased risk of nonadherence to ART; periods of incarceration are associated with virologic failure, but the prevalence of drug resistance is no higher than in non-IDUs. IDUs are less likely to be on ART compared with other risk groups.

SEXUALLY TRANSMITTED DISEASES
- Injection drug use is associated with high-risk sexual behavior, and reduction in risky sexual behavior is more difficult to achieve than reductions in risky injection behavior.
- Although there have been conflicting studies, research suggests that the prevalence of common sexually transmitted infections is higher in IDUs than in the general population.

According to the 2017 World Drug Report, an estimated 250 million people worldwide used illicit drugs at least once in 2015. Of these, 11.8 million injected drugs.[1] Since the early 2000s, prescription opioid injection increased dramatically in the United States, particularly in rural areas.[2] The 2014 National Survey on Drug Use and Health Estimated that 4.3 million people were nonmedical users of prescription pain relievers. Frequently this type of use is a precursor to injection drug use.[3] Ultimately these users were 40 times more likely than the general population to use heroin or other injecting drugs. By 2015 more than one-third of adults in the United States were prescription opioid users, many of whom reported misuse and use disorders.[5] In the United States the use of heroin almost doubled between 2006 and 2013 with more than 680,000 active users, most of whom were young adults who found the drug less expensive and easier to obtain than prescription medications.[6] In addition to heroin and prescription opioids, cocaine and methamphetamines are commonly injected.

The impact of this drug use is substantial because the mortality rate among injection drug users (IDUs) younger than 30 years of age is 10 times the rate in the general population. Although this rate is mostly attributed to overdose, infection exacts a substantial toll on the population.[7] IDUs have higher rates of bacterial, viral, and fungal infections than the general population, and infection is undoubtedly the major reason for contact between an illicit drug user and the health care system. The social circumstances of many addicts, including living in shelters or crowded conditions, increase the risk for pulmonary tuberculosis (TB) including multidrug-resistant infections. Moreover, the reluctance of IDUs to seek medical attention and their failure to adhere to treatment regimens are responsible for the further spread of infection. Malnutrition contributes to altered host defenses, making infection more likely. Poor hygiene that frequently accompanies the drug use habit may exacerbate the risk for infection due to commensal organisms. In addition, IDUs have a higher rate of nasal and skin colonization with potential pathogens than do nonusers. Frequent injection into heavily colonized sites such as the groin region likely increases the risk for infection with enteric flora. Among addicts in San Francisco, 22.8% were found to have nasal colonization with *Staphylococcus aureus,* 12% of which was community-acquired methicillin-resistant *S. aureus* (CA-MRSA).[8] Indeed, the major risk factor among illicit drug users for colonization with *S. aureus* was ever injecting drugs, and, among illicit drug users who are colonized, "ever using" was associated with colonization by MRSA. The fact that addicts have frequent hospitalizations and exposure to antibiotics secondary to complications of injection drug use leads to an increased risk for MRSA colonization and infection.

Certain practices that are unique to IDUs, such as crushing capsules or tablets in the mouth before injection of the drug, may be responsible for infections caused by oral flora. Some addicts lick their needles to make the injection easier, a practice that doubles the risk for cellulitis or abscess caused by oral streptococci or anaerobes.[9] The use of "speedball" (a mixture of heroin and cocaine) or the practice of "booting" (repeatedly withdrawing small amounts of blood into the injection equipment before administering the complete contents) have also been associated with an increased risk for infection. "Speedball" use leads to tissue necrosis, with the formation of abscesses, and injection by using poor technique or sclerosing substances causes a loss of usable veins, which may lead the addict to resort to "skin popping," or injection directly into the skin or muscle, leading to an increase in the odds of wound botulism.

IDUs tend to experiment with a variety of substances, each with a unique set of complications. Different drugs are more or less popular, depending on the region. Methamphetamine use is widely popular, and in at least one study it was demonstrated to be associated with syringe sharing and an increased risk for human immunodeficiency virus (HIV) acquisition.[10] In Tennessee, several IDUs developed thrombotic thrombocytopenic purpura after injecting Opana ER, a newly reformulated extended-release form of oxymorphone.[11] Xylazine, a horse tranquilizer, has become popular in Puerto Rico. This agent is typically mixed with "speedball" and is associated with skin ulceration and local abscesses in up to 35% who inject this substance.[12] Fentanyl and fentanyl analogues have become popular drugs of abuse in the United States[13]; solvent injection has become popular in Canada,[14] in the United Kingdom, mephedrone use has been associated with high-risk behaviors,[15] and a mixture of opioids, benzodiazepines, and antihistamines called "South Asian Cocktail" has become prevalent.[16] Buprenorphine, which is a heroin substitute intended to be used as an oral or sublingual preparation,

has also become a drug of abuse and was associated with severe soft tissue injury in 31% of users in one study.[17,18]

Although early studies failed to detect pathogenic bacteria contaminating the illicit drugs, more recent evidence demonstrating a connection between the use of black tar heroin and severe *Clostridium* infection indicates that certain substances or practices enhance the growth of particular organisms and account for infections that are unique to the drug-using population. Failure to adequately clean the injection paraphernalia and sharing of equipment are also responsible for transmission of infection. The close contact among addicts may also be responsible for outbreaks of severe infections in certain locales. An outbreak of infection caused by *Clostridium novyi* in 2000 was associated with severe infection and a high mortality rate and appeared to be secondary to close contact in the IDU social network and use of shared substances and needles.[19] A brief increase of group A *Streptococcus* M nontypeable strains between 1999 and 2001appears to have resolved.[19]

Ultimately, infection drives a need for care in both the outpatient and the inpatient environment. There are few differences between IDUs and non-IDUs when they present to an emergency department with infection. Both groups have similar rates of fever and bacteremia. However, IDUs are more likely to have hyponatremia and thrombocytopenia.[20] In the British health care system, IDUs infected with HIV are less likely than others to use outpatient services but are more likely to have frequent and prolonged hospitalizations.[21] Substance abuse treatment programs can have a major impact on the use of health care services. The mortality rate among IDUs who are not enrolled in formal substance treatment programs is three times higher than the rate for IDUs in treatment programs, mostly secondary to the high incidence of infection, although trauma also plays a role. The type of program also makes a difference. Addicts who continue to inject but who are enrolled in needle exchange programs are six times more likely to have injection-related infection than addicts enrolled in methadone maintenance programs.[21] Despite the availability of needle exchange programs, young adults are more likely than their older counterparts to practice unsafe injection practices.[22,23] Several reasons explain some of this behavior. In many cases these programs are located outside of areas where drug use is frequent. In addition, criminalization of illicit drug use leads to reluctance of users to carry sterile drug paraphernalia for fear of being caught and arrested. In the United States, high arrest rates for illicit drug use are colocated with syringe exchange programs, with African Americans and Latinos most adversely affected.[24] Decreased access to syringe exchange programs is associated with risky behaviors, and the need for a "fix" outweighs concerns for HIV and hepatitis C virus (HCV) infection.[25,26] There are also differences in drug use based on the particular substance used. Methamphetamine users tend to inject in groups, making sharing of needles and syringes more likely. They are also less likely to frequent neighborhoods where open drug use is common, perceiving them as too dangerous. However, those are the precise neighborhoods where the exchange programs tend to be located.[27] In addition, the frequency of infection increases with the number of injections per day, from a rate of 1.8% for drug users who do not inject, 3.5% for users with single daily injections, 6.1% for users who inject twice daily, to 11.4% for users who inject three or more times per day.[21]

Infection in IDUs is not just a problem for persons in that population; infections may spread to other members of the community in many cases. Examples of infections that can spread beyond the group of drug injectors include infections with HIV, hepatitis B virus (HBV), and HCV and resistant organisms such as MRSA. As noted earlier, opiate maintenance programs have been demonstrated to significantly reduce the infection rate among drug users and can play a major role in reducing the spread of infection to nonusers.[28] Programs that include counseling and psychosocial services as well as medical care to methadone maintenance programs can decrease drug use and, secondarily, the spread of infection.

An effort to decrease infections in IDUs requires multiple strategies but begins with an understanding of the issues leading to drug abuse. Among young adults, as noted previously, a history of receiving a prescription for legal narcotics, tranquilizers, or stimulants precedes misuse and a transition to injection drug use.[29] Early initiation into drug injecting is also associated with high-risk behavior and infectious consequences and emphasizes the need for comprehensive prevention programs and early intervention efforts targeting youth at risk.[30] Education programs designed to teach addicts safe injecting techniques can prevent local disease as well as the spread of infection to others.[31] Among noninjecting heroin users, risky sexual behavior often accompanies a transition to injecting and with it the risk for acquiring HIV and other infections. Thus a strategy to reduce HIV and other infections associated with injection drug use would incorporate behavioral interventions as well as efforts aimed at risk reduction such as needle and syringe exchange programs and easy access to sterile syringes in pharmacies.[32] Pharmacies have also been proposed as providers of expanded health services for people who inject drugs, which could reduce the need for clinic and hospital care. However, most pharmacies are involved only in syringe exchange programs. Users could be treated in pharmacies for simple abscesses and receive vaccinations, and pharmacies could be a source for needle disposal. Nevertheless, they seldom serve these roles for a variety of reasons, not the least of which is that people who inject drugs frequently are deterred by what they view as being held in contempt by employees of these facilities.[33] Needle exchange programs have reduced the incidence of abscesses but are not available in many locales. In addition, making methadone or other opiate substitution programs more accessible can have the overall effect of improving the health status of addicts, while decreasing their use of illegal substances and participating in illegal activity. When successful, these programs prevent the spread of HIV and other bloodborne pathogens, as well as skin abscesses and cellulitis.

The infections associated with injection drug use are frequently the consequence of the illegal status of street drugs. Heroin reduction programs, safer injecting facilities, and opiate substitution programs can reduce the incidence of many infections among addicts.[28,34] It has also been found that the route drug users employ to ingest heroin is influenced by the purity and ease of use of the drug available, as well as the locale. Powdered forms of heroin, which are common in South America and Asia, are uncommon in Texas, whereas "white" heroin is more likely to be available on the East Coast of the United States. Some forms are easier to dissolve, making intravenous use practical. "Black tar" heroin, which is common in the western regions of the United States, is difficult to get into solution, and most users resort to skin popping, which is highly likely to lead to local ulcerations and abscesses. Because many heroin users initiate their drug use by inhalation, which is associated with far fewer complications than injection, a goal would be to maintain that form of use and prevent the transition to injection, a goal that most likely requires early intervention in a treatment program.[35]

The cities of Vancouver and Victoria in Canada have developed highly successful programs aimed at risk reduction. Vancouver implemented the first medically supervised injection facility in North America, a center where previously obtained drugs can be injected under the supervision of health care professionals. This center is designed to reduce the incidence of overdose and bloodborne virus transmission while improving access to health care.[36] It is likely that the incidence of injection-related infections could be even further reduced if health care providers were permitted to perform the injections rather than serve only as a resource to supervise the injections. Thus users still frequently resort to unsafe practices in other environments.[37] Nevertheless, these services allow safer injection, are associated with decreased overdoses, facilitate early access to clinic or hospital health care for patients in need of treatment for abscesses and other infections, increase referrals for drug treatment, and benefit public order.[38] When the Victoria site was shut down owing to pressure from the local community, there was an increase in sharing of needles from less than 10% in 2008 to more than 20% in 2010, whereas the rate was unchanged in Vancouver.[39] Along with increased needle sharing is the expectation of an increase in bloodborne and injection-related infections. After examining published data showing that the Vancouver supervised injection facility did not increase public disorder, crime, or drug use and recognizing that addiction is a public health matter, the Supreme Court of Canada affirmed the legality of the center and recognized the rights of people with addictions to the security of their persons under the Charter of Rights and Freedoms.[40] In a similar effort, a group of IDUs established an injection

support team in 2005. By assisting IDUs in their own settings, which may be the street or "shooting galleries," the injection support team successfully increased awareness of safer injection techniques among addicts.[40]

Despite these advances there remains a strong reluctance to deal with addiction as a health care–related issue rather than as a crime. In the United States the 1988 ban on syringe exchange programs was reversed in 2010, allowing federal funds to be used to support such programs.[41] These programs, which have been responsible for a reduction in HIV transmission and probably for a reduction in the transmission of HCV as well, have the potential to provide additional risk prevention services. Still, as noted earlier, even where needle exchange programs are legal, IDUs who take advantage of such facilities frequently are harassed by police.[24,42,43] Hence there is an increase in sharing of needles and syringes and an increased risk for HIV and other bloodborne infections. IDUs persist with unhealthy habits, and efforts to reduce risky behavior will remain sporadic despite demonstrated success.[44]

As a consequence of these many risky behaviors, IDUs are admitted to the hospital significantly more often than the general population. Once an IDU is admitted, a frequent sequence of events occurs, starting with admission to an emergency department, followed by an inpatient stay and all too frequently a discharge against medical advice, followed often by readmission for the same or a related problem.[45] One of the problems is the failure to commit sufficient resources to the management of these patients, often with the goal of keeping costs down. This may be shortsighted and only amplifies the amount of money spent to treat any given individual.[46] Part of the problem is the failure of health care providers to recognize the need to adequately treat pain in this population and the prison-like environment drug users are often subjected to. Furthermore, although in many hospital settings many providers who have expertise dealing with the addiction problem are available, as are medications aimed at decreasing the drug cravings, all too often they overlooked, and the patient goes without.[47] There is also a dilemma associated with the need for prolonged hospitalization, as many of the infections acquired by this population require prolonged parenteral therapy. Physicians are almost uniformly reluctant to allow people who inject drugs to be treated in the outpatient setting with antibiotics that necessitate an intravenous catheter for fear the addict will resort to use of the line to inject illicit drugs. Ho and colleagues[48] used a security seal placed over a peripherally inserted catheter to successfully treat drug users in the outpatient setting. The greatest likelihood for success is to carefully select patients whose drug use is somewhat remote and to avoid antibiotics with a relatively high side-effect profile.[49] An alternative approach taking into consideration the psychosocial needs of IDUs in a community care setting has been successful at keeping the patients in a care setting and is associated with completion of care.[50]

HOST DEFENSES

Although it has been recognized for more than a century that opioid abuse is associated with an increased risk for infectious complications, a clear understanding of the effects of opioids on the immune system is still lacking. Studies have examined the effects of in vivo opioid exposure on the function of cells of the immune system isolated from drug users, the effects of in vitro exposure to opioids on immune cells isolated from healthy nonaddicts, and the effects of in vivo and in vitro exposure to opioids in animal models. The results of studies performed after the beginning of the HIV epidemic but before the availability of a serologic test may be confounded by the effects of immunodeficiency caused by unrecognized HIV infection. Previously, it was believed that immunologic dysfunction played a relatively minor role in the pathogenesis of infection in IDUs compared with the repeated parenteral introduction or injection of nonsterile material and lifestyle factors associated with injection drug use. However, there is clear evidence of a direct effect of opioids on immune system function. These effects have been reviewed and summarized elsewhere.[51–53]

Immune Changes

Opiates have been demonstrated to reduce chemotaxis, phagocytosis, and production of cytokines and chemokines. Both heroin and morphine (a major metabolite of heroin) have been shown to decrease natural killer cell activity and decrease lymphocyte proliferation in response to phytohemagglutinin and other mitogens. Morphine has also been shown to impair antigen presentation by B lymphocytes. In addition to their direct effects on cells of the immune system (which have been demonstrated to have opiate receptors), opiates affect the immune system indirectly through the neuroendocrine system (mainly via the hypothalamic-pituitary-adrenal axis). Immunosuppression mediated through effects on the autonomic nervous system is also suggested. Despite the depressed cell-mediated immunity demonstrated in IDUs, opportunistic infections characteristic of T-cell deficiency were rarely reported before the HIV epidemic. In the lung, heroin appears to reduce the activity of inducible nitric oxide synthase, which may increase susceptibility to pulmonary infections.[54,55] Methamphetamine increases the expression and production of metalloproteinase-2, leading to host collagen degradation and diminished wound healing, thereby at least partially explaining the prevalence of *S. aureus* in wounds of IDUs. It also impairs the function of phagocytic cells, further increasing susceptibility of users to infection.[56]

Prolonged methadone maintenance has been shown to reverse some of the immunosuppressive effects of heroin.[57–59] Buprenorphine, which is increasingly used as substitution therapy, also appears to allow a reversal of the immunosuppressive effects associated with heroin addiction.[59]

Active IDUs have substantial humoral immunity dysregulation that is associated with increased systemic inflammation.[60] IDUs who resist HCV infection despite repeated risky activity have significantly elevated levels of immunoglobulin G (IgG) and IgM against viral envelope glycoproteins as well as alterations of natural killer cells compared with healthy control subjects.[61,62] Increased immunoglobulin levels tend to normalize after prolonged opiate withdrawal. Elevated immunoglobulin concentrations are accompanied by a high frequency of autoantibodies such as rheumatoid factor, as well as those directed against various microorganisms. The latter phenomenon often manifests as a biologic false-positive Venereal Disease Research Laboratory test result, which may create diagnostic confusion in IDUs who are at high risk for acquiring sexually transmitted diseases (STDs). Mixed cryoglobulinemia is also seen and increases with the duration of drug use.[63] Hypergammaglobulinemia resulting from polyclonal B-cell activation may be the result of recurrent immunologic stimulation by injected foreign antigens as well as associated chronic liver disease and chronic infections with other pathogens. Regardless of HIV infection, injection drug use is associated with an increase in peripheral blood and mucosal cells. Interestingly, sharing injection paraphernalia also leads to the same increase in immune activation.[64]

It has been shown that morphine may depress the monocyte functions essential for antiviral defense.[65] These alterations could contribute to the high efficiency of transmission of certain viral pathogens in IDUs, including HBV, HCV, and HIV. In vitro, morphine has been shown to enhance HIV infection of human mononuclear cells through the downregulation of β-chemokine production and the upregulation of CCR5 receptor expression.[66] More recent evidence shows that microRNAs involved in the processing of HCV are altered in IDUs and at least partially explain the innate anti-HCV immunity among drug users.[67]

SKIN AND SOFT TISSUE INFECTIONS

Skin and soft tissue infections are the most common reason for IDUs to be admitted to the hospital. There is evidence that skin and soft tissue infections are increasing in this population. Among IDUs in England, between 1997 and 2004 there was a 566% increase in the number of patients admitted for abscesses of the trunk or groin and a 469% increase in patients with cellulitis of the trunk or groin.[68] Based on the aggregate of reports, the lifetime prevalence among addicts varies between 6% and 69%. Risk factors vary, but there is much commonality among users. It is not unusual for IDUs to spend time in jail or prison. Many chronic wound infections first appear in IDUs during incarceration, most likely related at least in part to policies that create an unhealthy environment.[69] Women appear to be more likely than their male counterparts to develop abscesses and skin ulcers, perhaps due to smaller veins making them more likely to resort to skin popping.[70] Intramuscular

and subcutaneous injection carry significantly greater risk of skin infections. The prevalence of soft tissue infections also increases with increased injection frequency and most likely the duration of injection drug use.

Age alone also has an impact on the nature of chronic soft tissue infections. Some data suggest there is a growing population of older IDUs. With this population is an expanding pool of subjects who have chronic wounds. It has been suggested that older IDUs (≥45 years old) find themselves in a different stage of life having survived years of drug use and may have learned to cope with their chronic health conditions better than younger IDUs. IDUs with chronic wounds, regardless of age, have an injection history of at least 15 years. That duration invariably leads to injection in the lower extremity with resultant venous insufficiency and the chronic ulcers and infections that tend to follow. Older IDUs with chronic wound infection are less likely to engage in risky injection behaviors than both their peers who have no wounds and younger IDUs.[71] Compared with their peers who do not have chronic wounds, they are more likely to be African American and to inject in the leg. Compared with younger IDUs who have chronic wounds, older IDUs with chronic wounds, although more likely to inject in the leg, are more likely to clean the injection site with alcohol before injecting. Most importantly, they appear to be more receptive to engaging in therapeutic relationships that address their addiction and health issues.

As noted earlier in this chapter, intramuscular injection and greater frequency of injection are common risk factors, especially with heroin or the heroin-cocaine combination known as speedball. These IDUs tend to have a longer history of drug use that has led to sclerosis of accessible veins. As a consequence, their injection time is prolonged as they seek to find a way to administer the drug. It is believed that this prolonged exposure is responsible for an increase in local infection. An emphasis on skin cleansing may help reduce the number of these infections. Education of the patient thus becomes important and should not be considered hopeless. Phillips and colleagues[44] noted that most often IDUs had limited access to supplies that would help reduce infection. They also found a gap between the knowledge of the patients and their practices; although they recognized bleach cleaning of needles and cleaning their skin would likely prevent infection, IDUs often did not practice such behavior. With this information, they used an information-motivation-behavioral skills model with some success in a pilot program. This program and others designed to reduce infections in this population can make an impact not only on the patients but also on the utilization of health care resources because these common skin and soft tissue infections account for a substantial proportion of emergency department encounters in urban medical centers. In Vancouver, the presence of nurses in safe injection facilities leads to treatment there of minor infections, with referral of only more serious cases to emergency departments and a shorter length of stay in the hospital than for other IDUs who were not part of the supervised injection facility program. Further development of such facilities may prove to be very effective at reducing the burden on emergency departments.[72,73] Elsewhere, San Francisco General Hospital established the Integrated Soft Tissue Infection (ISIS), an outpatient facility at which patients can receive care for lesions that would otherwise be treated in the emergency department. Over a 3-year period, 6156 patients were seen for a total of 12,012 visits.[74] Of the patients, 58% were IDUs.

Infection Sites

The distribution of soft tissue lesions varies with the sites used for injection and reflects both the duration of drug use and the local practices among drug users. Typically, IDUs go through a progression of injection sites, starting with the upper extremity in the antecubital fossa, followed after approximately 2 years by the forearm. After about 4 years of use, users switch to injecting into the veins of the hand, and approximately 6 years after first injecting, they switch the veins of the neck, feet, and leg. By the 10th year of drug use, IDUs resort to the groin and peripheral digits.[20] The groin becomes a favored site after other injection sites have been exhausted, although in some cases femoral injection is preferred for convenience and ease of use or as a means of ensuring immediate blood levels of the drug rather than facing the risk for the reduced euphoric effect associated with skin popping, that is, either intended or accidental injection of drugs into the skin and subcutaneous tissues.[75]

After repeated injections into a site, frequently without benefit of sterile technique, local ischemia or necrosis develops, and the tissues become susceptible to infection. In addition, the substances injected frequently contain materials added as diluents that commonly cause norepinephrine release and vasospasm or local damage to the vascular intima. This leads to thrombosis and further compromise of the soft tissues. Cocaine use may be associated with vascular thrombus at sites distant from injections and may cause muscle and skin infections even after inhalational use.[76,77] Opiates also have immunosuppressive properties that may predispose to infection.[78] In addition, among methamphetamine users, there are substantial differences based on the color of the drug they inject. Depending on the type of adulterant added to the drug the color may be clear, white, yellow, or pink. IDUs injecting a colored drug were more likely to develop an abscess compared with IDUs using the clear substance.[79] HIV infection is now recognized as an important risk factor for skin abscesses. Women are at greater risk, presumably because of the difficulty they have accessing veins and the consequent injury to skin and subcutaneous tissues.[80] In one British study, in addition to female sex, all of the following were associated with an injection site infection: being at least 25 years of age; having injected into the legs, groin, and hands over the last year; injecting on 14 or more days in the last 4 weeks; reusing needles and injecting crack cocaine; having HIV infection; and having previously received a prescribed substitute drug. The cost of such infections was estimated conservatively to be between £19.2 million and £30.5 million.[81] It is also very common for IDUs to attempt self-management of their wounds by using a variety of manipulations, including applying heat or a rag soaked in salt water, squeezing out pus with their fingers or doing incision and drainage with a knife or syringe, or taking antibiotics orally or by applying crushed antibiotics to the wound. Although many of these behaviors may increase the risk for spread of the infection, in at least one study the infection healed in 89% of patients. Only 12% ended up going to a hospital, and most waited 5 days before doing so.[82,83]

Abscess

Abscesses are the most common form of soft tissue infection, followed closely by cellulitis and skin ulcers.[84] Cleaning the skin with alcohol before injection protects against abscess and offers a potential intervention to reduce disease and hospital admissions.[85] Original optimism that needle exchange programs might reduce the incidence of abscesses has not been fully realized. In Vancouver, despite having a supervised injection facility program, 21.5% of IDUs had an abscess in the previous 6 months.[86] However, other investigators demonstrated that for every 1000 needles exchanged, there was one less abscess; for every eight visits to the needle exchange program, one additional abscess was prevented. When the funding for the program was reduced, the prevalence of abscesses increased.[87] With conflicting data, it may be that either complete cessation from injecting or scrupulous attention to sterile technique is the only effective measure to eliminate abscesses in IDUs.

The location of lesions depends on the sites injected. They are found commonly in both upper and lower extremities depending on where the user most frequently injects. In at least one comparison between IDUs and nonusers, IDUs were more likely to have abscesses in the groin, whereas the hand was a more common location in nonusers.[88] Addicts also tended to present later and ended up having a longer hospital stay, with treatment costing almost 2.5 times more. Although abscesses occur with injection of heroin as a single drug, they are also found frequently in addicts who inject a mixture of heroin and cocaine and are more likely in IDUs with a long history of drug use and skin popping.[85] Booting is also an independent risk factor for abscess formation.[85] Additional risk factors for abscesses are female sex (odds ratio [OR], 1.7; $P = .002$), recent incarceration (OR, 1.7; $P = .001$), involvement in sex trade (OR, 1.4; $P = .030$), frequent cocaine use (OR, 1.5; $P = .002$), and being seropositive for HIV (OR, 1.5; $P = .003$).[86]

Abscesses may spread to adjacent tissues, frequently with disastrous consequences. Mediastinitis may result from the extension of a cervical abscess, whereas lesions in the carotid triangle can erode into the carotid arteries resulting in massive hemorrhage. Thrombosis of the internal

jugular vein has been reported as a complication of a deep neck abscess, as has acute vocal cord paralysis.[89,90] This can lead to acute, severe airway obstruction and may necessitate immediate tracheostomy. Local venous thrombosis or extension to the retroperitoneal space may result from abscess in the femoral triangle.

Microbiology of Abscesses

Blood cultures from IDUs have low yield and are recommended only for patients with signs and symptoms of systemic toxicity, especially fever. Likewise, wound cultures should be done only for patients who have moderate-to-severe purulent infections.[91] When the decision is made to culture, a useful approach is to first débride the wound of any obviously necrotic tissue. After cleaning the skin with saline or wound cleaner, either swab or tissue cultures yield the same results detecting most of the usually anticipated organisms, although the swab technique had a higher yield of anaerobes.[92] Although *S. aureus* is the most common pathogen found in non-IDUs, streptococci and anaerobes were found more frequently among IDUs in at least one more recent study.[93] MRSA was detected equally in both groups. Methamphetamine was found to be a risk factor for MRSA infection in IDUs presenting to emergency departments in Georgia. Unsterile injection practices did not appear to be a risk factor because only 12.5% of patients injected the drug compared with 62.5% who either smoked or inhaled. The most significant risk factor among methamphetamine users was a skin infection within the previous 3 months (adjusted OR, 7.92). The authors noted that methamphetamine use causes formication, a sensation of something crawling on the skin. This sensation prompts the user to pick at the skin, which can lead to skin breakdown and subsequently serves as a focus for infection.[94] Among the staphylococci, MRSA has become the predominant strain, particularly CA-MRSA. Disease caused by CA-MRSA is most often a single lesion in an extremity, although multiple furuncles are also seen. Recurrences are common.[95] Coagulase-negative staphylococci and α-hemolytic streptococci are also seen. Among the latter, the *Streptococcus anginosus (milleri)* group is most important, especially in addicts in Scotland, who inject tablets of buprenorphine and temazepam after crushing them between their teeth.[96,97] Other oral flora have been reported, in particular, *Eikenella corrodens,* which in some centers has become the third most common pathogen. IDUs who lick their needles or contaminate their drugs with saliva are particularly prone to this infection. The pneumococcus is also occasionally found in this setting. Gram-negative bacilli are found with variable frequency. In the past, anaerobes were found infrequently, particularly in upper extremity infection. In one study, 39% of isolates from IDUs contained both aerobes and anaerobes compared with only 27% of isolates from nonusers. In addition, anaerobes, either alone or as part of a mixed flora, were detected more frequently in drug users than in nonusers (44% vs. 35%).[98]

Diagnosis of Abscess

The diagnosis of an abscess can be difficult. Patients typically have single lesions. The signs and symptoms are similar to patients with cellulitis alone. Indeed, most patients will also have an area of adjacent cellulitis. Less than half of patients are febrile. Erythema, pain, and tenderness of the affected site are common, but fluctuance is absent in approximately 25%.[99] Deep abscesses may be particularly difficult to detect.[100] Computed tomography (CT) is useful for locating cervical abscesses[101] and is probably effective for detecting abscesses in the groin and femoral region. Magnetic resonance imaging (MRI) is also useful, particularly in infection of the extremities.[102] Ultrasonography has been reported to be useful but is of variable accuracy, particularly when diagnosing lesions in the groin. Bedside ultrasound has been used in an emergency department setting with a high degree of accuracy. These machines are portable, have the advantage of being available while the patient is in the emergency department, can be completed in less than 1 minute, and are comfortable for the patient. In one large study in an emergency department, abscesses were found in 37% of addicts who were tested. There were only five false-positive studies, two of which were determined to be hematomas rather than abscesses. The sensitivity of ultrasound was 98% compared with only 86% for clinical examination alone. The specificity was 85%, with a positive predictive value of 93% and a negative predictive value of 97%. An unanticipated but important additional benefit of ultrasonography is that pseudoaneurysms that otherwise might have been misidentified as abscesses were also detected, thus avoiding a dangerous attempted incision and drainage.[103]

Management of Soft Tissue Infections

The first decision in the management of patients with soft tissue infections is whether the patient should be admitted to a hospital. This can be a difficult decision, but it depends on the severity of the illness. Eron and colleagues[104] recommend an approach in which patients are assigned to one of four classes. Class 1 patients have no signs of systemic toxicity and have no uncontrolled comorbid conditions. These patients usually respond to topical or oral therapy. Class 2 patients either have evidence of systemic illness but without any unstable, comorbid conditions or are systemically well but have one or more comorbid conditions that may complicate the outcome. Class 3 patients are toxic or are not toxic but have unstable comorbid conditions that may interfere with the response to therapy. Class 4 patients have sepsis syndrome or a serious life-threatening infection such as necrotizing fasciitis. Predictors of severe infection are hypotension, tachycardia, temperature lower than 35°C (95°F) or higher than 40°C (104°F), confusion, or a depressed level of consciousness. Patients with two or more of these findings have a blunted response to antibiotics and poor outcome. In terms of the decision whether to admit the patient to the hospital, class 1 patients most likely will do well if managed as outpatients. Class 2 patients may benefit from a period of observation; patients who respond quickly can be treated as outpatients, but others will require hospital admission. Patients in classes 3 and 4 should be treated as inpatients.

Determining the appropriate antibiotic is the next decision. Class 1 patients usually respond to oral antibiotics, as do some in class 2. Initial therapy with parenteral agents with rapid switch to oral agents may be preferable for class 2 patients. Patients with more serious illness should be treated with parenteral therapy, at least until they respond satisfactorily. Data from randomized trials to guide the duration of therapy are insufficient. In most cases, 1 to 2 weeks of treatment is adequate, but infection may recur in up to 20%; thus prevention of future infections cannot be ignored.[104] Empirical therapy aimed at the usual pathogens is usually adequate, although in at least one medical center, patients with hand abscesses were found to be 11 times more likely to be infected with a clindamycin-resistant MRSA than other patients.[105] Thus it may be important to be aware of local resistance patterns before initiating empirical treatment.

As with other patients, incision and drainage remains the mainstay of treatment of abscesses in IDUs. Infection in the deltoid region is a consequence of skin popping and subsequent necrosis of the underlying fascia. In these cases, drainage may be facilitated by ultrasound-guided aspiration, even in the emergency department setting.[106] Regardless of location, cure rates for drainage alone may approach 80%; however, the addition of trimethoprim-sulfamethoxazole for 7 days is associated with a significantly higher cure rate and is effective at preventing many secondary complications such as need for repeat drainage or metastatic spread of infection.[107]

Antibiotic therapy is directed at the organisms recovered from the blood or purulent material. In uncomplicated cellulitis, cultures are seldom helpful. In such cases, therapy is empirical and is based on the pathogens most commonly encountered in that geographic location. Prolonged antibiotic treatment is frequently required.[108] As noted previously, early surgical drainage of abscesses is essential; because of the tendency of these lesions to spread to adjacent or distant regions, multiple drainage procedures may be required.[109] Deep infections of the hand are far more common in IDUs than in non-IDUs, and they mandate a unique approach. The microbiology of such infections varies depending on the injected substances. Patients who primarily inject cocaine have a high frequency of mixed anaerobic infection,[99] whereas heroin users are more likely to harbor streptococci and staphylococci.[110] In either case, surgical débridement is far more likely to be required in IDUs than in non-IDUs.[48] Some caution is indicated before incising a lesion in the vicinity of blood vessels because a mycotic pseudoaneurysm can easily be misdiagnosed as an abscess. Inadvertent entry into such a lesion can have disastrous consequences. Inadvertent intraarterial injection of crushed tablets leading to hand swelling mimicking infection

has also been reported. Failure to make the correct diagnosis may lead to loss of digits due to extreme ischemia.[111]

Skin Ulcers

Skin ulcers are extremely common in IDUs. They are found at every conceivable site but are particularly common below the knee, close to the ankle.[112] They arise from tissue damage caused by repeated nonsterile injection into the same site with associated thrombosis and infection. Synergy between streptococcal infection and cocaine-induced tissue ischemia may lead to large necrotic ulcerations and extensive tissue loss.[97] Alternatively, skin ulcerations may result from necrosis induced by the illicit substance injected. Skin ulcers may persist for years and are a frequent reason for hospitalization. Typically, they have ragged edges and seropurulent drainage. Patients experience severe pain, and it is often the pain, rather than the ulcer itself, that brings the patient to medical attention. The microbiology of these lesions is similar to that of other soft tissue infections in addicts, although they more frequently contain more than one organism. *S. aureus* and β-hemolytic streptococci remain the most common isolates, with gram-negative bacilli—most often *Klebsiella, Pseudomonas, Escherichia coli,* and *Proteus*—playing an important role. Skin ulcers present particularly difficult management problems when they involve the hands and feet, and they may ultimately lead to loss of function.

Treatment of skin ulcers requires administration of systemic antibiotics and prolonged local wound care, including gentle washing, wet-to-dry dressings, and application of topical antibacterial creams. Elevation of the leg to reduce edema is an important component of the therapy and plays a role in pain management.[113] Generally, parenteral antibiotics are continued until the wound is covered by granulation tissue. Very large lesions may require skin grafting or muscle flaps, but these are effective only after all necrotic tissue has been removed and the wound is clean and granulating. An important adjuvant treatment is the application of compression dressings, such as Unna boots, which, when properly applied, serve to reduce edema as well as to promote wound healing.[114] With time, most skin ulcers heal completely, leaving circular, punched-out scars.

The most important complication is contiguous osteomyelitis, which may be difficult to diagnose because radiologic evidence frequently indicates periosteal reaction in bones immediately beneath large ulcers. When there is still a question of osteomyelitis, a triple-phase bone scan or MRI may be helpful. Ultimately a diagnosis of osteomyelitis may be impossible without a bone biopsy, which may be difficult to obtain without traversing infected superficial tissues. In such cases, prolonged parenteral antibiotic therapy directed at the organism cultured from the ulcer and careful radiographic follow-up may be the best approach. A combination of codeine tablets cooked with a solvent (gasoline, lighter fluid, or paint thinner) creates a drug called desomorphine, known as krokodil because of the scaly green patches that develop at the injection sites. Ultimately these lesions may ulcerate, and underlying osteomyelitis is known to occur. As with similar ulcers, a team approach and local care are required for resolution.[115]

Paradoxically, IDUs may wish to prevent healing of these ulcers because once well-vascularized granulation tissue forms, the ulcers become an excellent location in which to inject. These lesions are referred to as "shooter's patches." They may be successfully managed by use of a well-vascularized muscle flap, providing even better access to the circulation. The competing goals of the clinicians and the IDU are a source of continued frustration for the health care team.[116]

Necrotizing Fasciitis

Necrotizing fasciitis, without or with myositis, is the single infection in IDUs that is most likely to need immediate and appropriate treatment. In one institution the prevalence increased significantly in the period 2009–2010 solely due to an increase in admissions of IDUs.[117] However, the clinical picture is subtle and rarely elicits the emergency response required. At San Francisco General Hospital, 1% of IDUs in need of incision and drainage for soft tissue infection were found to have necrotizing infection requiring extensive débridement. The classic findings of high fever, bullae, crepitance, and skin necrosis are usually absent initially, and the impression may be that of mild cellulitis.[110] In some cases, the true nature of the disease may be so subtle as to be missed during a procedure to débride an abscess or cellulitis. Alternatively, infection may spread after an apparently effective incision and drainage.[118] The major indication of the true nature of the infection is the fact that signs and symptoms, such as pain and hemodynamic instability, are disproportionate to the apparent extent of the local process.[110] However, this can be misleading because the clinical presentation may be no different from routine cellulitis, with no more than erythema of the involved area, providing no clue to the serious underlying pathologic process.[119] Also, because addicts are frequently viewed as drug-seeking complainers, patients with excessive complaints for what appears to be a minor disease may be interpreted as exhibiting narcotic-seeking behavior. This misperception can further delay recognition of the need for aggressive and rapid action. In one study the correct diagnosis was made in only 59% of patients who presented to an emergency department, and many were initially admitted to a nonsurgical service.[120] Others argue that the best results are achieved by admission directly to a vascular surgery service.[121] Regardless of the admitting service, it is clear that a high index of suspicion is required so as not to miss the correct diagnosis. Additional clues to the serious nature of the problem are hemodynamic instability, increased creatinine level, local anesthesia, rapid progression of inflammation, or the presence of blue or hemorrhagic bullae. Crepitance is an important clue when present. Although a rapid test is not yet available, the finding of the proinflammatory cytokine interleukin-1 receptor antagonist with markedly elevated white blood cell count has been associated with fatal outcome in patients with necrotizing fasciitis, especially in the absence of fever.[122] Finally, a slow response to appropriate antibiotic treatment suggests a deeper underlying problem. MRI and CT may be useful diagnostic tools. Characteristic findings include asymmetrical fascial thickening and fat stranding, followed by gas tracking along fascial planes. Abscesses may also be seen.[123] CT scans may be misleading because both false-positive and false-negative results have been reported, and contrast enhancement contributes no additional information. The only definitive test is surgical exploration, which is both diagnostic and therapeutic. The finding of necrosis is characteristic; however, it may be necessary to explore more than one area. A negative biopsy result from one location does not preclude the diagnosis in adjacent tissues.[124]

As with most infections in IDUs, gram-positive organisms are usually found. However, β-hemolytic streptococci predominate in approximately 50% of cases, followed by *S. aureus,* α-hemolytic streptococci, and coagulase-negative staphylococci. Gram-negative organisms are infrequent and are usually represented by enteric pathogens, especially *E. coli, Klebsiella, Proteus mirabilis, Pseudomonas,* and *Enterobacter.* Anaerobes are recovered in 12% of cases, including *Clostridium sordellii* in one series of black tar heroin users[125]; yeasts (*Candida* spp.) are uncommon. Polymicrobial infection is common.[126] Cultures are imperative, as occasionally unusual organisms are detected, as in the case of IDUs with *C. novyi* as the sole cause of necrotizing fasciitis.[127]

Management of necrotizing fasciitis by antibiotics alone leads to progression of the infection in 75% of patients. Parenteral antibiotics and aggressive surgery coupled with reexploration at 24 hours and as often as necessary afterward to ensure complete removal of all necrotic tissue offer the best prognosis. In one study, IDUs required an average of 3.4 débridements for necrotizing fasciitis.[126] Aggressive nutritional support and early coverage of the soft tissue defect have been shown to improve the outcome.[128] Even with aggressive treatment, the mortality rate is high, ranging from 10% to 23%, and amputation is required in up to 10% of patients. Patients with group A streptococcal infection and streptococcal sepsis appear to have the worst prognosis.[126,129] The best results are achieved with the use of vacuum-assisted therapy in addition to extensive débridement.[130] Addicts, who tend to be young and relatively healthy, have the best outcome of any patient group with this disease.

Pyomyositis

Pyomyositis, a less serious infection involving the musculature, occurs frequently in IDUs. Direct inoculation of bacteria into the musculature has been implicated. Hematogenous spread also occurs, occasionally as a complication of endocarditis.[131] Most patients who have pyomyositis

present with pain and swelling of the affected area. Lesions have been reported in the deltoid, psoas, biceps, gastrocnemius, gluteal, and quadriceps muscles. Ultrasound, CT, or MRI shows the underlying defect within the muscle. *S. aureus* is the most common pathogen; infection with viridans streptococci, infection with aerobic gram-negative bacilli, and mixed infection with anaerobes have also been reported. Patients respond well to drainage and antibiotic therapy. A rare but related condition, uterine pyomyoma, has also been reported. The cause appears to be hematogenous dissemination to an infarcted leiomyoma.[132]

Just as needle exchange programs reduce HIV infection among IDUs, combining needle exchange programs with a wound and abscess clinic may substantially reduce the cost of care (to as low as $5.00 per patient) and the number of visits to emergency departments. Widespread implementation of such clinics could have a major impact on the management of skin and soft tissue infections among IDUs.[133]

Injection Anthrax

Over the past several years a cluster of cases of injection anthrax have been identified among IDUs in the United Kingdom and Germany. The first recognized case occurred in a skin popper who was diagnosed in 2000. After a lull, additional cases were seen between December 2009 and December 2010. There were 54 confirmed cases, and 18 deaths occurred. Sporadic cases still occur.[134] In contrast to classic cutaneous anthrax, cases do not manifest as an eschar. These patients typically present with severe tissue swelling and evidence of soft tissue infection occurring 1 to 10 days after injecting heroin. The injection was frequently in the subcutaneous tissues. Initially the lesions were often similar to typical soft tissue infections seen in IDUs; however, blood cultures were often positive for *Bacillus anthracis.* Other diagnoses were also confirmed via polymerase chain reaction (PCR) assay of tissue from wounds. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was also used successfully in several cases.[135] Cultures of *B. anthracis* can be easily misidentified as *Bacillus cereus.* In suspected anthrax cases, special techniques should be used to verify the identification of the isolate.

Patients in this outbreak were treated with a variety of different antibiotic regimens. Empirical therapy, including ciprofloxacin and clindamycin with additional antibiotics combined into a five-drug regimen, is recommended. In addition, aggressive surgical débridement with or without fasciotomy is considered necessary, and repeat débridement may be required.

The epidemiology of this outbreak is currently unclear, but it is most likely related to the transportation of heroin from Afghanistan, the site of origin, to Western Europe and the United Kingdom hidden in goat skins. It appears these goat skins were likely contaminated with anthrax spores that germinated once injected into the warm tissues of the patients.[136]

BONE AND JOINT INFECTIONS

Epidemiology of Skeletal Infections

Skeletal infections are common in IDUs, most occurring via hematogenous seeding by bacteria or fungi.[137] Target sites for infection are determined by the blood supply and predominantly involve the axial skeleton. The original source of these infections may not be apparent, or the infections may represent metastatic complications of endocarditis. Injection drug use has been identified as a risk factor for osteoarticular infection in patients with endocarditis.[138] In addition, bone and joint infections frequently result from contiguous spread from adjacent, often neglected, areas of infection in skin and soft tissues. IDUs with hematogenous infection often have multiple sites involved simultaneously, and blood cultures frequently are negative at the time of presentation. IDUs with HIV infection do not appear to be at increased risk for osteoarticular infections, and when these infections do occur, they are most frequently caused by the usual bacterial pathogens, not opportunistic pathogens.[139,140]

IDUs with skeletal infection tend to be young and otherwise healthy. Clinical findings include constitutional manifestations and local signs and symptoms depending on the site involved. Patients with osteomyelitis often have a paucity of findings, presenting with local pain and tenderness only. Lack of signs and symptoms frequently results in delay in diagnosis. Fever is absent in one-third of patients.[141] Similarly, signs of sepsis and leukocytosis and radiologic signs may be absent in patients with osteomyelitis.

Pyogenic infections predominate, with almost 90% being bacterial in origin, although virtually any organism can cause skeletal infections. The predominant pathogen isolated is *S. aureus,* with one large series finding almost one-fifth of cases due to coagulase-negative staphylococci (half associated with hardware).[142] Gram-negative bacilli are responsible for one-fifth of cases, with about half of these due to *Pseudomonas aeruginosa*. Polymicrobial infections are reported in 46% of osteomyelitis cases but only 15% of septic arthritis cases.[142] Anaerobes are isolated from as many as one-fifth of bone infections but rarely cause septic arthritis. IDUs who lick their needles or the skin surface before injection may develop osteomyelitis or septic arthritis with *E. corrodens* ("needle licker's osteomyelitis").[143] *Candida* spp. have been increasingly recognized as a possible etiology of skeletal infections in IDUs, particularly spondylodiscitis and vertebral osteomyelitis.[144] A characteristic form of systemic candidiasis has been reported among IDUs who inject heroin that includes folliculitis, usually of the scalp and beard; endophthalmitis; and bone and joint lesions, most often costochondritis. Lastly, *Mycobacterium tuberculosis* should always be considered among the possible causes of skeletal infection in IDUs, particularly vertebral osteomyelitis.[145] As many as 20% of infections are culture negative. Because the microbiologic differential diagnosis is broad, nucleic acid amplification techniques should be used in these cases in an attempt to confirm the microbial etiology of the infection.

Site of Skeletal Infection

Joint infections usually involve the extremities, most commonly affecting the knee. The incidence of left-sided knee arthritis exceeds right-sided knee arthritis, possibly because of the tendency of right-handed IDUs to inject into the left groin veins; this suggests a relationship between site of injection and infection.[141] IDUs are particularly susceptible to vertebral osteomyelitis. IDUs with vertebral osteomyelitis are more likely to present with symptoms of a shorter duration and have more profound neurologic deficits, likely owing to the higher incidence of cervical spine involvement compared with non-IDUs.[146] Primary sternal osteomyelitis, often associated with an antecedent history of blunt trauma to the sternum, is reported in IDUs. This group is also prone to septic arthritis in unusual sites such as the sternoclavicular and costochondral joints and the pubic symphysis. Other sites frequently involved include wrist, shoulder, hip, and sacroiliac joints. Vertebral osteomyelitis may extend into the subdural or epidural spaces and may cause formation of an abscess, with consequent cord compression and paraplegia. In addition, lumbosacral vertebral osteomyelitis may be associated with psoas abscess.

Diagnosis and Management of Skeletal Infections

Because of the wide spectrum of organisms that may be involved, diagnostic needle aspiration for smear and culture is necessary in all cases. Even when blood cultures are positive, invasive diagnostic steps are advised because skeletal and bloodstream infection may represent two separate processes, and infections may be polymicrobial. Frequent arthrocentesis, arthroscopic or open drainage, and débridement of nonviable bone are also advised if clinically indicated. Antibiotic therapy is required for 4 to 6 weeks, with the selection of drug based on the identity of the responsible microorganisms and susceptibility data. Increasingly, oral antimicrobial agents with high bioavailability and good bone penetration are used for at least a portion of the therapeutic course.[147,148]

Overall, with early diagnosis, the immediate prognosis of bone and joint infection in IDUs is excellent, but long-term follow-up data are lacking.[141] Many IDUs present late in the clinical course, and delays in diagnosis and institution of therapy are accompanied by a high likelihood of chronic osteomyelitis and late relapse of disease. Also contributing to this late but frequent complication is the problem of nonadherence. IDUs tend to leave the hospital against medical advice when confronted with prolonged intravenous antibiotic therapy in a skilled nursing facility, and many physicians are reluctant to consider outpatient parenteral

antibiotic therapy in patients who are active IDUs. A tendency for medical personnel to underappreciate and undertreat the pain associated with skeletal infection may also lead to patients leaving the hospital before discharge planning can be completed. A retrospective review of discharge to a residential addiction treatment facility to complete parenteral antibiotics showed high rates of success and substantial cost savings for the health system.[149]

BACTEREMIA AND INFECTIVE ENDOCARDITIS

Epidemiology

Bacteremia is common in IDUs, often with resultant infective endocarditis (IE). In the Detroit Medical Center, 74 of 180 addicts with bacteremia had endocarditis.[150] In North Carolina, there was more than a 12-fold increase in the number of hospitalizations for injection drug use–related IE from 0.2 to 2.7 per 100,000 per year between 2010 and 2015.[4] The associated hospital costs during the same period increased from $1.1 million in 2010 to $22.2 million in 2015. At the same time there was a remarkable shift in the prevalence of IE from urban to rural populations in North Carolina and elsewhere.[2] The increase occurred while the use of heroin remained stable and was largely the result of the dramatic increase in the use of prescription opioid medications. This transformation from primarily urban to rural populations forced to handle this new influx of addiction-related endocarditis has major implications for the overall health care system. In one setting, a single patient who had multiple episodes of IE was responsible for the institutional cost of $380,000, not counting the cost of providers and eventual cardiac surgery that was performed elsewhere. In part, this was due to a system unwilling to pay for drug rehabilitation services that may have prevented subsequent admissions.[46] In contrast to the United States and with some exceptions, in Western Europe the prevalence of IE among IDUs has decreased, leading to a corresponding decrease in hospital admissions for right-sided endocarditis; serious cases of left-sided IE are still seen.[151] In Central and Eastern Europe, there has been an increase in injection drug use and, in contrast to the West where polymicrobial IE has decreased, there has been an apparent increase in IE.[151] In Scotland the injection of a new psychoactive substance referred to a "burst" (variably butylone, methiopropamine, or ethylphenidate) led to a dramatic increase in IE and necrotizing pneumonia cases, all of which were associated with *S. aureus* infection.[152] A Swedish referral center saw an increase of cases of addiction-related IE but experienced a lower mortality rate, perhaps due to the implementation of a multidisciplinary team to handle these infections.[153]

HIV infection also has had an effect on the epidemiology of IE among IDUs. The rate in this population now appears to be similar to the rate of prosthetic valve infection, approximately 1% per year. The incidence of IE is higher among IDUs with HIV than among seronegative IDUs (24.8 vs. 3.9 cases/10,000 person-years). With the widespread adoption of antiretroviral therapy (ART), the overall incidence has decreased.[154] An inverse relationship between IE and $CD4^+$ lymphocyte count (OR for 200–499 cells/mm^3, 2.01; OR for <200 cells/mm^3, 3.61) had been reported and undoubtedly still exists among patients with advanced disease.[155]

In most early studies, men were affected more often than women (5.4:1 in the Detroit Medical Center, 2:1 in Chicago), and men with IE were older than women and had significantly longer histories of drug use (10.2 years vs. 7.1 years). However, among addicts with HIV infection, women had an increased risk (OR, 3.26), a rate that was similar to addicts with increased injection frequency. The reason for this difference is unclear, but it may be due to women having smaller veins, making it more difficult to inject, which might lead to more local infections and subsequent bacteremia. Greater risk-taking behavior may also be a factor. HCV infection was an associated factor in 100% of IDUs with IE in Taiwan. Injection drug use is also a risk factor for relapsing (≤41%) and polymicrobial (8%) IE.[156] Among patients with recurrent disease, the median interval between episodes is far shorter in addicts than in nonaddicts.[157] In areas where buprenorphine lacks the inhibitor compound that is active only when injected, such as in Singapore, addicts have turned to injection, with the consequence of an increased rate of IE.[158] Cocaine use is an additional risk factor. In contrast, alcohol consumption confers protection against endocarditis, perhaps by inducing an inhibitory effect on platelet function.[155]

Microbiology

S. aureus remains the most common pathogen, affecting the tricuspid or pulmonary valve in approximately 90% of cases. Coagulase-negative staphylococci are an uncommon cause of endocarditis in IDUs. Streptococci, particularly groups A, B, and G, are the second most common pathogens.[150,159] These two organisms account for up to 75% of cases.[150] Ruppen and colleagues[160] found *Streptococcus dysgalactiae* to be common over a 10-year period, 2006–15, with a predominance among female addicts. *Enterococcus* played a major role in the past, but its prevalence is decreasing.[150,159] Gram-negative organisms are infrequent causes. Intermittent epidemics of *P. aeruginosa* endocarditis have occurred in Detroit and Chicago. The most recent epidemic occurred in Detroit during the years 2006–08. There were 10 cases, all in HIV-negative patients. Five were left-sided, three of which occurred on prosthetic valves. Four patients had tricuspid infection alone, and one had aortic and tricuspid disease. In one case, *Pseudomonas* was combined with *Candida parapsilosis*.[161] *Serratia marcescens* was responsible for a sustained epidemic in the Oakland, California, area[162,163] and occurs sporadically.[164] Gram-negative endocarditis in IDUs is not common outside the HACEK group (*Haemophilus* spp., *Aggregatibacter* spp., *Cardiobacterium hominis, E. corrodens,* and *Kingella* spp.). In one report, only 2 of 49 cases of gram-negative endocarditis occurred in IDUs.[165]

Among the fungi, *Candida* endocarditis (typically non–*C. albicans*) is most common. In one series of *Candida* endocarditis, 9 of 30 cases were in IDUs: 3 of *C. parapsilosis;* 3 of *C. albicans;* and 1 each of *C. tropicalis, C. guilliermondii,* and *C. pelliculosa.* All patients had left-sided infection, but the mortality risk was lower among IDUs (OR, 0.12; 95% confidence interval [CI], 0.02–0.7; $P = .03$). One death occurred at day 4 owing to a brain embolism, and the others occurred remotely: one at 3 months from an unknown cause and one at 1 year due to *S. aureus* IE.[166] *Aspergillus* endocarditis in addicts has also been reported.[167] Two of three patients were HIV infected. Duration of symptoms ranged from 2 weeks to 1 month before detection. Blood cultures were negative, but there were large vegetations and peripheral embolization. *Aspergillus* frequently contaminates illicit drugs, but whether the inoculum associated with injection is sufficient to cause endocarditis is unclear. That two of three patients were HIV positive is of interest and may indicate that altered immune status played a role.

Polymicrobial endocarditis is observed with increasing frequency among drug users. Usually only a few organisms are involved, but rarely there may be numerous pathogens. In such cases, standard laboratory techniques may be inadequate to isolate and identify the full microbial spectrum, placing a burden on the clinician to suspect polymicrobial endocarditis caused by salivary contamination of needles or injection sites whenever uncommon oropharyngeal organisms are cultured from the blood.[168] *E. corrodens* is often found in polymicrobial IE, frequently in association with *Streptococcus constellatus.* These organisms may be a synergistic combination, owing to coinciding metabolic niches. They coaggregated during in vitro growth, and in animal models, *E. corrodens* produced endocarditis only if injected with streptococci.[156] Among the fastidious organisms, in addition to *Eikenella* there are reports of endocarditis caused by anaerobes such as *Fusobacterium* spp. and *Clostridium.* Sporadic reports document the occurrence of *B. cereus* bacteremia in IDUs, who, in contrast to immunocompromised patients infected with this pathogen, tend to have uncomplicated infections that may even resolve without therapy.[169] In addition, numerous reports describe endocarditis in IDUs caused by a variety of organisms that are frequently considered nonpathogens. These infections may be related to altered host immunity resulting from HIV infection[170] or to unusual practices among addicts such as licking needles before use or "cleaning" the injection site with saliva. Given the proclivity of addicts to experiment with novel substances to inject, almost any microorganism can be found in the blood of IDUs, as exemplified by the isolation of *Paenibacillus larvae,* an organism that lives in honey and causes severe disease in honeybees, in the blood of addicts. These IDUs injected methadone that had been previously mixed with honey to make it too viscous to inject.[171]

Pathophysiology

The pathophysiology of endocarditis in addicts is poorly understood. The organism is most often part of the patient's own flora,[150,172] although injection paraphernalia have been implicated in the case of *P. aeruginosa* endocarditis. Environmental contamination was also considered in the initial outbreak of *Serratia* endocarditis in California when it was learned that years earlier *Serratia* had been sprayed into the air to study wind currents. However, these strains were not the same ones that caused disease, and the regional predilection for this infection remains unknown. It is also unclear why certain valves are affected in IDUs with endocarditis. It is known that, in contrast to native valve endocarditis in nonaddicts, the affected cardiac valve is almost always previously normal.[150,173,174] In an autopsy study of addicts who died of endocarditis, Dressler and Roberts[173] reported that 81% of the valves were normal, including all right-sided valves. Early reports of endocarditis in addicts noted a predominance of tricuspid valve involvement. Left-sided involvement predominates in some recent studies, and multiple valves are frequently involved.[159,175] Pulmonary involvement remains rare and may be misdiagnosed as pneumonia.[176] Nevertheless, tricuspid valve involvement is seen almost exclusively in IDUs.

No single hypothesis explains the prevalence of right-sided involvement in IDUs. Potential explanations include damage to right-sided endothelium by repeated exposure to injected particulate matter; vasospasm caused by injected diluents or illicit drugs, particularly cocaine; or drug-induced thrombus formation and subsequent bacterial aggregation. The fact that endocarditis caused by *Enterococcus* and *Serratia* is primarily a left-sided phenomenon suggests that other mechanisms must be important. Mitral valve prolapse has been proposed to explain the predilection for mitral valve endocarditis in female IDUs, but studies showing equal numbers of men and women with mitral involvement make this explanation unlikely. Specific surface properties of the common infecting pathogens, especially *S. aureus,* might favor attachment to extracellular matrix proteins that may have greater expression on right-sided valves. *S. aureus* is also phagocytized by endothelial cells, where it is protected from host defenses. This may activate the clotting system, leading to vegetation formation. The production of coagulase by *S. aureus* may further promote clotting and vegetation formation. Cytokines produced after phagocytosis may enhance immune complex deposition that leads to valvulitis, thereby creating a lesion that is susceptible to vegetation formation and bacterial seeding. Finally, endothelial differences between the right and left sides of the heart might contribute to the predilection for tricuspid valve involvement. Additional hypotheses invoke an association between large, directly injected bacterial inocula and immune abnormalities that may contribute to sustained bacteremia and valvulitis[177] or even drug-induced pulmonary hypertension leading to increased right-sided turbulence.[178] Which, if any, of these mechanisms proves to be responsible for the preponderance of right-sided involvement depends on future studies. There are differences in the involved valve that appear to be related to the drug the IDU injects. Heroin use is far more likely to lead to tricuspid valve infection than use of methamphetamines or cocaine.[179] One potential explanation is that heroin leads to elevated pulmonary pressure due to respiratory suppression, which in turn causes tricuspid regurgitation, abnormal blood flow with eddy currents, and subsequent IE. In contrast, methamphetamines and cocaine cause a dramatic increase in systemic afterload that leads to mitral and aortic valve regurgitation and ultimately a higher risk for endocarditis. Whatever the reason, *S. aureus* predominantly affects the tricuspid valve but may also involve the mitral or aortic valves. *S. marcescens, Streptococcus pyogenes,* and enterococci affect left-sided valves almost exclusively.[163,180,181]

Clinical Manifestations

In contrast to nonaddicts, who generally present with symptoms of longer than 2 weeks' duration,[182] most addicts with IE present within the first week of illness with signs indicative of severe, acute infection.[150,159,183] Typically, they have acute onset of fever, chills, and dyspnea. Chest pain, often pleuritic in nature and associated with hemoptysis due to septic pulmonary emboli, occurs in up to 75% of cases of tricuspid endocarditis. Any IDU presenting with unrelenting spiking fever, bacteremia, and pulmonary symptoms such as cough and hemoptysis should be considered to have right-sided IE.[184] At presentation, more than 50% of chest x-rays reveal multiple pulmonary infiltrates, often with central cavitation, compatible with septic pulmonary emboli.[184] CT reveals the same findings but often to a greater extent. These lesions typically are more plentiful in the lower lobes, perhaps due to the effect of gravity, and frequently increase for several days despite the initiation of effective antimicrobial therapy. Pneumothorax, occasionally bilateral, is a complication of septic pulmonary embolism.[185] Involvement of other organ systems is similar to that observed in endocarditis in nonaddicts. Initially, central nervous system (CNS) involvement may be confused with toxic effects of illicit drugs, but the diagnosis usually becomes rapidly apparent when blood cultures become positive.

The overall severity of the clinical picture depends on the valve or valves involved and whether there is any associated damage to the heart itself such as valve ring abscess or valve rupture or metastatic infection involving other organs. In our study, IDUs with endocarditis who required admission to an intensive care unit had severe sepsis and shock (36%) or respiratory failure (33%) or had neurologic deterioration (18%). *S. aureus* was the etiologic agent in 94%, although polymicrobial infection was found in 15%. Most patients had septic emboli to one or more organs, including the lung in 36% and the CNS in 21%. Independent risk factors for mortality were a previous history of IE and an increased Acute Physiology and Chronic Health Evaluation (APACHE II) score.[186]

Osler nodes and Janeway lesions are rare in addicts. Splenomegaly occurs in only 10% to 15%, and heart murmurs are found with variable frequency. When the infection is confined to the tricuspid valve, the presence of murmurs ranges from 35% to 72%.[150]

Because of the high-grade bacteremia and acute nature of endocarditis in addicts, every organ is affected to some degree. Complications involving the heart, although infrequent, may be life threatening. When cardiac problems dominate the clinical picture, which is most likely with mitral or aortic valve infections, the prognosis is poor, especially if congestive heart failure develops. As noted earlier, recurrent IE is common in addicts. Most patients survive the first episode but with such severely damaged valves that dysfunction occurs in almost 70%. Previous valve damage predisposes to subsequent episodes, which frequently are fatal. Additional cardiac lesions include left ventricular abscesses, which are multifocal and are found in conjunction with clusters of bacteria in intramural arteries, and myocardial infarction. Valve ring abscesses and, rarely, focal, acute interstitial myocarditis are also found.[173,187] Cardiac abscesses may also lead to further serious complications such as a pseudoaneurysm of the heart, which can be demonstrated by MRI and color Doppler ultrasound evaluation.[188] As with nonaddicts, left-sided infection predisposes to systemic emboli and acute pericarditis. Certain organisms, especially *Serratia* and *Candida,* are notable for their tendency to induce large, systemic emboli. Their isolation in a patient with endocarditis should alert the clinician to the probability of left-sided infection and the likelihood of a serious embolic event. CNS complications of endocarditis are similar to those in non-IDUs and include mycotic aneurysms, brain abscess, stroke, meningitis, and epidural abscess. Splenic abscesses are seen, especially with *S. aureus* infection, and should be assessed before cardiac surgery to avoid valve replacement in a patient likely to remain bacteremic from a noncardiac source. Drug users are more likely than nonusers to develop bone and joint infection, particularly vertebral osteomyelitis, secondary to endocarditis.[189]

Diagnosis

The diagnosis of endocarditis in IDUs is often easily made on the basis of the characteristic clinical picture, yet IDUs with right-sided IE, even due to *S. aureus,* frequently have very limited clinical findings.[190] The modified Duke criteria effectively classify IDUs with either definite or possible endocarditis and can be used in difficult cases, particularly when difficult therapeutic decisions must be made.[191] Despite the utility of this schema, it should be noted that the Duke criteria were validated for endocarditis caused by bacteria and may not be an accurate assessment for patients with fungal infection. Patients with definite IE frequently have no obvious focus of infection and are more likely to have vascular phenomena and multiple opacities on chest radiographs.[192] However, in the emergency department setting, the absence of signs specific for endocarditis and the limited information available make the diagnosis

particularly difficult.[193] At the time of the initial presentation, there are no differences in age, sex, maximum temperature, or leukocyte count between addicts with and without endocarditis. Addicts with endocarditis account for only 13% of addicts admitted for infection; positive echocardiogram findings, pulmonary or systemic emboli, and bacteremia distinguish patients with endocarditis from patients without endocarditis.[194]

Rothman and colleagues[195] further modified the Duke criteria in an effort to improve diagnostic accuracy in an urban emergency department. Major criteria are the same as the Duke criteria, although an echocardiogram performed on arrival at the hospital is added, and only data available in the first 24 hours are used. Minor criteria are unchanged. The definition of definite endocarditis is the same as in the Duke system; possible endocarditis requires either one major and two minor criteria or four minor criteria. The diagnosis is rejected if these criteria are not met. The results were almost identical to published results that used the standard Duke criteria, but the investigators were able to make admission and therapeutic decisions much earlier than was otherwise possible. The same investigators also evaluated the contribution to diagnosis of transthoracic echocardiography (TTE) in the emergency department. The addition of TTE proved diagnostic for nearly 70% of patients who failed to meet major culture criteria.[196] In another attempt to arrive at earlier diagnosis, investigators used PCR assay to detect universal 16S ribosomal RNA. They found a sensitivity and specificity of only 86.7% and 86.9%, respectively, but accurately identified all eight patients with blood culture–positive endocarditis.[197] If validated, this method may provide an additional diagnostic tool for the emergency department clinician.

Chung-Esaki and colleagues[198] evaluated a prediction rule to assist in the diagnosis of IDUs in the emergency department setting. In their model, patients with tachycardia, cardiac murmur, and absence of skin infection had a sensitivity of 100% (95% CI, 82%–100%) but, perhaps more importantly, a negative predictive value of 100% (95% CI, 88%–100%). Although by using this model in their study they were able to rule out IE in only 12% of cases, given the difficulty of excluding IE by other means, such an approach may prove useful in the emergency department setting.

The most sensitive indicator of endocarditis in IDUs is a blood culture, which is positive in 80% to 100% of cases. However, because many addicts take oral antibiotics before admission, initial cultures may be negative. Subsequent blood cultures reveal the pathogen.[199] Even after several days of appropriate parenteral therapy, blood cultures are still likely to be positive.[200,201] Because culture-negative IE is rare in IDUs, negative blood cultures suggest an alternative diagnosis.

Additional blood studies are not of particular benefit in diagnosing endocarditis. Anemia is common in IDUs as a result of the continual blood loss associated with the act of injecting. An elevated white blood cell count, usually with a left shift, is also common, although neutropenia and thrombocytopenia are occasionally found. Hyponatremia of 125 to 133 mEq/L is found in approximately 40% of cases immediately after admission and predicts prolonged fever and greater morbidity.[150] The etiology is unclear, and the abnormality corrects immediately after fluid administration. Additional laboratory abnormalities reflect the high-grade bacteremia associated with endocarditis and routinely normalize soon after bacteremia clears. The cerebrospinal fluid (CSF) level is abnormal in many cases, with increased white blood cells and protein level in patients showing no overt CNS symptoms or signs.[202] Echocardiography is routinely performed in patients suspected to have IE. However, results can be misleading because normal right-sided structures may mimic vegetations. In IDUs with right-sided IE, the low pressure in the pulmonary circulation often permits the growth of large vegetations, making the diagnostic yield of TTE comparable to transesophageal echocardiography (TEE).[184] Caution is still required, as a high percentage of IDUs who have no evidence of IE still have abnormalities on the tricuspid valve.[203] Abnormalities in an IDU must be considered in the context of the clinical setting. In a study comparing TTE and TEE in veterans with *S. aureus* bacteremia suspected to have IE, Sekar and colleagues[204] determined that TEE was superior, even for patients with right-sided disease. However, there were only 3 IDUs in the study population, and details of the nature of their infections were not provided. In the absence of more compelling data, if IE is strongly suspected, TTE may be performed first, and if it is negative, TEE may be ordered.[190] Alternatively, TEE may be reserved for patients believed to have left-sided involvement or patients with perivalvular lesions such as valve ring abscess or perforation or a vestigial eustachian valve.[205] It is important to recognize that negative TEE does not rule out endocarditis. Several newer modalities may confirm the diagnosis in patients with suspected IE who have negative TEE and/or TTE. In such patients, three-dimensional echocardiography may be useful, especially in cases with confusing artifacts on traditional tests. In addition, there is increasing evidence supporting the use of combined ^{18}F-fluorodeoxyglucose positron emission tomography/CT to identify infected cardiac vegetations.[184] Cardiac MRI is also effective at identifying vegetations, and PCR can be diagnostic in patients when blood and tissue cultures are negative.[190]

Therapy

Addicts with IE who are stable and only moderately ill can be observed safely without antibiotic therapy while the results of blood cultures are awaited. Transient fever and bacteremia occur in this population, and because bacteremia is the most sensitive indicator of endocarditis, a commitment to therapy before the nature of the septic condition is documented can lead to unnecessary and prolonged hospitalization for administration of antibiotics. Even when the patient is acutely ill, several blood cultures should be obtained before antibiotic therapy is initiated.

Complete recommendations for antimicrobial treatment of IE were published in 2015 by the American Heart Association and will not be repeated here.[190] As a routine, when empirical treatment is initiated, it is based in part on knowledge of the organisms most likely to cause endocarditis in that geographic location. In most settings, coverage is directed against *S. aureus*. The prevalence of MRSA is decreasing; however, it may be prudent to cover for it until finalization of culture results.[206] Accumulating information indicates that infections due to MRSA that have a vancomycin minimal inhibitory concentration of 2.0 μg/mL have a poor outcome, and in such cases an alternative regimen is recommended. Daptomycin at a dose of 8 or 10 mg/kg has been suggested by some experts.[190]

Trimethoprim-sulfamethoxazole has been used for both methicillin-sensitive *S. aureus* and MRSA soft tissue infections, but its efficacy in treating endocarditis is questionable, and it is not recommended. Traditionally, endocarditis in IDUs was treated with 4- to 6-week courses of parenteral antibiotics. However, some patients with uncomplicated right-sided endocarditis may be successfully treated for 2 weeks with nafcillin plus an aminoglycoside[207] or even cloxacillin alone.[208] When quinolones are used, therapy may consist entirely of oral medication.[209] The addition of an aminoglycoside for treatment of native valve right-sided staphylococcal IE is no longer recommended.[190] In a recent *Pseudomonas* outbreak, patients were treated with a variety of regimens, but the most successful included cefepime, 4 to 6 g/day, plus high-dose tobramycin, 8 mg/kg/day, as a single dose, aiming for a peak serum concentration of 18 to 22 μg/mL and trough levels of less than 1 μg/mL.[161] Quinolones also have utility against *Pseudomonas,* but the data are insufficient to permit a recommendation for their use.[210]

The management of enterococcal endocarditis is evolving along with the increasing prevalence of vancomycin-resistant strains and new data evaluating novel antimicrobial regimens. Double β-lactam combinations appear to be very effective and avoid potentially toxic regimens including aminoglycosides.[190]

The management of fungal endocarditis is also in a state of evolution, although surgery is still required in many cases. Accumulating evidence indicates that treatment with a lipid formulation amphotericin B with or without 5-fluorocytosine, followed by step-down therapy to fluconazole if the organism is susceptible and blood cultures are negative, may be very effective and obviate the need for surgery.[211] Echinocandins at doses higher than used for treatment of candidemia (caspofungin at 50–150 mg daily, micafungin at 100–150 mg daily, or anidulafungin at 100–200 mg daily) may be considered alternatives. Even among patients with prosthetic valve endocarditis including a large number of IDUs, successful outcome was achieved by medical management alone.[212] Valve replacement is still strongly recommended; but if valve replacement is not possible, lifelong suppressive therapy is recommended.[190,213]

The role of surgery in the management of IE, particularly right-sided IE in IDUs, has long been controversial. Although the operative risk tends to be lower in IDUs with endocarditis, the long-term outcome is often compromised by resumption of illicit drug use.[214] The increase in drug use by itself will undoubtedly increase the pool of patients in need of some kind of procedure. Some authors advocated complete removal of the valve, whereas others argue for repair or replacement with a new mechanical or bioprosthetic valve.

Although not specific for IDU endocarditis, the American Heart Association guidelines offer the following surgical indications for right-sided ID:[190] Severe tricuspid regurgitation causing right-sided heart failure poorly responsive to medical treatment; sustained infection, particularly with a difficult-to-treat organism; lack of response to appropriate antibiotics; tricuspid vegetations larger than 20 mm; and recurrent pulmonary embolism despite appropriate antimicrobial therapy. Finally, whenever possible, valve repair, rather than valve replacement, is recommended especially in IDUs. Regardless of the invasive approach employed, a strong case can be made for performing CT angiography even in young IDUs, as this population has been identified to have a high incidence of coronary vascular disease. Being aware of coronary vascular disease before operating gives the surgeon additional options for approaching the patient.[215] In addition, three-dimensional TEE has been proposed as a preoperative test to help identify lesions that were not visible by other modalities.[216]

A recently developed vacuum device that has been shown to remove vegetations via a percutaneous approach has been used successfully in several IDUs who had severe tricuspid endocarditis and may prove to be an alternative to open heart surgery in this difficult population.[217] Unless or until this device is proven effective and gains approval, the decision to operate will remain an economic and ethical dilemma. In theory, the same complications leading to a recommendation for surgery in nonaddicts should apply to IDUs. One difference is that despite large vegetations in right-sided IE, medical management alone is usually effective. Mortality is more likely predicted by a high sepsis score, such as the Pittsburgh Bacteremia Score.[218] Another difference is that medical management alone is highly likely to be successful despite numerous septic pulmonary emboli.[184] In cases where surgery for right-sided endocarditis is required, valve repair or even valvectomy may be considered. The greatest difficulty relates to the ethical question of performing open heart surgery and potentially inserting a prosthetic valve into a patient who is likely to continue to inject drugs, making reinfection of a prosthetic valve likely.[219,220] The use of time, funds, and materials for such a patient in resource-limited settings is major cause for concern. In one study, the presence of active injection drug use was an independent predictor of not having valve surgery.[153] Early results show that IDUs have acceptable 2-year and 5-year postoperative results, although the number of reinfections is high among individuals who continue to use drugs.[221] It becomes an even greater problem when an IDU presents with a second or third episode of endocarditis after having previously received valve surgery. To help deal with this issue, several authors developed an ethical decision-making model that takes into consideration the problem itself, the objectives, the classic four principles (beneficence, nonmaleficence, autonomy, and justice), any relevant ethical theories, and the competence of the patient.[222] Others used similar ethical considerations to conclude that these patients should receive the same treatment as other patients, regardless of whether drug use was likely to continue after the surgery.[223]

Prognosis

In most cases of IE in IDUs, the survival rate is good using antibiotics alone, despite complications and prolonged fever.[224] Septic emboli frequently occur after the initiation of therapy but do not affect the prognosis and are not necessarily an indication for removal or replacement of the infected valve.[150,224] The relationship between vegetation size, as determined by echocardiography, and the likelihood of an embolus is controversial. However, vegetations larger than 2 cm are associated with a 33% mortality rate compared with 1.3% for patients with vegetations smaller than 2 cm ($P < .001$).[224] Hence some clinicians consider large vegetations as an indication for surgery. In general, the indications for surgery and the final result are the same in IDUs with endocarditis as in the general population. Although surgery carries substantial risk, the mortality rate in patients who fail medical management approaches 100%, so surgical treatment is indicated and clearly improves survival.[225] The patient's HIV-1 status is a significant prognostic indicator. The $CD4^+$ cell count decreases after cardiopulmonary bypass, which may lead to an acceleration of the progress toward acquired immunodeficiency syndrome (AIDS).[226] Addicts with HIV-1 infection whose IE is poorly controlled at the time of cardiac surgery and those with advanced AIDS also have a poor prognosis.[227] The major problem after cardiac surgery for endocarditis in IDUs is their propensity to continue illicit drug use. In one study, only 4 of 57 addicts remained drug-free, and the 10-year survival rate was only 10%.[227] Hence some investigators advocate excision of the tricuspid valve or repair of the left-sided valves rather than replacement for IDUs needing surgical intervention.[228] Prognosis is not affected by the duration of symptoms before initiation of therapy, antibiotic use before admission, right-sided heart failure, pulmonary embolism, or results of the following laboratory tests: leukocyte count, hemoglobin, and serum creatinine concentration.[224]

NONCARDIAC VASCULAR INFECTIONS

Septic Thrombophlebitis

As arm and leg veins become thrombosed, sclerosed, and unusable, femoral, axillary, and neck vessels are increasingly selected for injection. Vessels used frequently for injection become injured or infected, leading to the formation of hematomas, thrombosis, septic thrombophlebitis, mycotic aneurysm, or traumatic arteriovenous fistula.[229,230] As the quest for injection sites expand, may IDUs resort to neck injection, leading in at least one case to classic Lemierre syndrome due to *Fusobacterium necrophorum*.[231] *S. aureus* has been associated with Lemierre syndrome, emphasizing the importance of cultures.[232]

More recent evidence demonstrates that various samples of heroin have widely different degrees of acidity, with some far more likely to lead to vessel injury than others.[233] Buprenorphine itself appears to be highly toxic to vasculature.[234] The predominant pathogens are gram-positive cocci, usually *S. aureus,* although gram-negative pathogens, particularly *P. aeruginosa,* are not infrequently found. Polymicrobial infection is common. Findings with septic thrombophlebitis include local pain, swelling, and fever together with bacteremia and sepsis. Local signs of infection may be masked when deep vessels are involved. Infection or sclerosis of proximal large veins is frequently complicated by venous stasis and supervening thrombosis. Septic pulmonary embolization follows and closely resembles right-sided bacterial endocarditis.[150] The management of septic thrombophlebitis, which most frequently involves the femoral veins, remains controversial. Parenteral antimicrobial therapy is standard, but the value of anticoagulant use has not been established. Furthermore, hemorrhagic complications from unrecognized coexistent femoral and cerebral mycotic aneurysms may occur. Difficulty performing venography in IDUs precludes controlled studies evaluating efficacy and complications of anticoagulant use. Some experienced clinicians believe the risks of short-term anticoagulation are outweighed by the risk for major pulmonary emboli. In one trial, all patients were treated successfully by a 2- to 3-week course of intravenous therapy followed by therapy with oral antibiotics to complete a mean of 28 days of total treatment. In 91.7% of patients in that series, anticoagulation therapy was initiated while the patient was hospitalized but was stopped on discharge owing to compliance concerns.[235]

Mycotic Aneurysm

A major vascular complication in IDUs is the formation of mycotic aneurysms, most frequently involving femoral and, less commonly, neck vessels. Mycotic aneurysms of the superior mesenteric artery are also seen; some predict increasing incidence due to the expanding population of IDUs.[236] True aneurysms, involving all three layers of the arterial wall, are rare. In the IDU, frequent direct trauma to peripheral vessels produces damage to the vessel wall and an initial sterile perivascular hematoma. Injection of chemical agents in illicit drugs also causes tissue necrosis. The vascular wall usually becomes infected by contiguous spread from adjacent subcutaneous abscesses or areas of cellulitis, although embolization from a distant focus, such

as endocarditis, also may be involved.[237] Infection causes liquefaction of the central portion of the hematoma in communication with the arterial or, less commonly, the venous wall, forming a secondary (false) pseudoaneurysm.[230,238] The common femoral artery is the most frequent location, followed by the deep femoral and superficial femoral arteries.[239] Because most IDUs are right-handed, left-sided groin infections and aneurysms are more common. Primary mycotic aneurysms in which the damaged vessel wall is infected secondary to unrelated bacteremia are rare and are more likely to involve cerebral vessels as a complication of endocarditis. Pathogenesis includes septic embolization from valvular vegetations to the vasa vasorum of smaller vessels, such as the middle or posterior cerebral and visceral intraabdominal arteries, which are more frequently involved than the aorta. Occasionally, septic emboli arising from a groin abscess causing a pulmonary artery mycotic aneurysm and hemoptysis mimicking tricuspid endocarditis occurs, often with disastrous consequences.[240] Arteriovenous fistula has also been known to occur.[241]

Clinical manifestations of a mycotic aneurysm include a painful, often enlarging, tender, and frequently pulsatile mass, accompanied by variable constitutional symptoms. Pain, swelling, and erythema are common, occurring in 94%, 82%, and 76.5% in one series.[239] These lesions are predisposed to hemorrhage. Distal embolism may cause ischemia, leading to limb loss. Nerve compression is often present, and detection of a bruit or thrill over the mass strongly supports the diagnosis. Lesions of the subclavian artery are frequently associated with injury to the brachial plexus, with resultant neurologic symptoms.[237] Often addicts report "hitting the pinky," indicating unintended arterial injection. Bleeding from an injection site may be evidence of an aneurysm. Because drug abusers usually present with cellulitis and accompanying edema and induration, the pulsatile mass may be masked, obscuring the aneurysm. When rupture of the aneurysm occurs, it is usually preceded by severe pain that may be misdiagnosed as thrombophlebitis or soft tissue abscess. In contrast to an aneurysm in the lower limb, distal ischemia in the upper extremity (hand) from induced arterial spasm occurs commonly.

Anemia, leukocytosis, and an elevated erythrocyte sedimentation rate, although frequently present, are of limited diagnostic value. A high index of suspicion together with angiographic confirmation is essential because misdiagnosis is common, and cellulitis, abscess, or infected hematoma may mimic or mask an aneurysm (Fig. 312.1). Duplex ultrasound is an extremely useful noninvasive diagnostic tool that has high sensitivity and specificity for diagnosing pseudoaneurysms and should be the first diagnostic study performed.[237] CT, especially with injected contrast material, is extremely useful and delineates the pathologic process in the adjacent soft tissues. Color Doppler ultrasound may confirm the diagnosis, especially in the extremities, but may not define the vascular anatomy clearly.[242] Thus angiography remains the definitive diagnostic procedure, not only for delineating the lesion but also for planning the approach to surgery, and digital subtraction angiography may replace traditional arteriography in diagnosing femoral and other peripheral aneurysms.[243] Magnetic resonance angiography (MRA) may prove to be helpful in the diagnosis of mycotic aneurysms. Unintentional invasion of an aneurysm after incision and drainage of a lesion mistakenly believed to be an abscess can lead to catastrophic bleeding.

Successful management of a mycotic aneurysm requires early diagnosis before rupture occurs. Surgery is almost always required. Various procedures have been used, including simple ligation of the artery with excision of the involved segment. IDUs who have a relatively brief history of illicit injection drug use frequently develop claudication after ligation, whereas IDUs who have been using for more than 5 years are likely to have built up a number of collaterals, which prevents subsequent symptoms.[244] An approach involving resection of the infected segment with débridement of the wound bed and in-line arterial reconstruction employing an autologous vein graft has been used with success.[239] In other cases a polytetrafluoroethylene graft may be used if suitable graft material cannot be found. Others rely on stent placement in emergency situations.[245] Surgical treatment should not be delayed, because rupture is frequent. Vascular reconstruction as a delayed procedure is recommended only in patients who develop ischemia after



FIG. 312.1 Arteriogram demonstrating a mycotic aneurysm of the right common iliac artery in an injection drug user.

excision of the aneurysm, and then only when a graft can be positioned through an uninfected tissue plane. Some authors argue that the presence of extensive tissue necrosis or necrotizing fasciitis is a contraindication to any attempt at revascularization; amputation may be required.[246] Thrombectomy of the distal artery facilitates collateral circulation and improves the long-term outcome among patients who undergo ligation and excision.[247] Postoperative complications are common. Complete débridement of the infected artery is essential, and failure to achieve complete débridement is associated with anastomotic dehiscence. The addition of a well-vascularized muscle flap for coverage minimizes the risk for anastomotic failure due to infection.[239]

In the absence of an agreed-on approach to mycotic aneurysm in IDUs, a variety of different approaches have proved successful. Several authors have proposed treatment algorithms that take into consideration the presence of significant hemorrhage at the time of diagnosis, the urgency for surgery, and the likelihood of success of different surgical procedures.[246,248] In any case, given the high mortality associated with these lesions, some form of surgery based on the location and nature of the aneurysm seems a requirement.[249]

Because *S. aureus* is the most common pathogen, initial empirical antibiotic therapy should include a β-lactamase–resistant penicillin such as nafcillin. When MRSA is prominent, vancomycin should be started. An aminoglycoside is added initially if there is suspicion or evidence of gram-negative bacilli on Gram staining of sanguinopurulent drainage. As with other infections in IDUs, any bacterium or even occasionally fungus may be responsible, making culture imperative.[250] Subsequent cultures obtained from blood and local exudate influence antibiotic selection. Recommended therapy is intravenous and lasts for 4 to 6 weeks.

PULMONARY INFECTIONS

Pathophysiology

Pulmonary manifestations are extremely common in IDUs. The lung is the target of numerous infectious and noninfectious insults. The latter include bronchospasm, airflow obstruction, diffusion impairment, emphysema, and pulmonary hypertension in heroin users.[251] Pulmonary hypertension and alveolar hemorrhage are associated with injection of

cocaine [251] Alveolar hemorrhage due to vasculitis is also associated with levamisole, a common cocaine adulterant.[252] Levamisole contamination of heroin is now increasingly reported. An increased prevalence of pulmonary hypertension, independent of the presence of septic pulmonary emboli, has been reported in individuals who inject buprenorphine.[253] Heroin overdose may be associated with pulmonary edema; however, this complication of heroin overdose is now reported much less frequently than in older series.[254] Granulomatous lesions occur in reaction to particles of cotton (used to filter the drug before injection) and fillers (mainly from injection of crushed pills). Starch can cause mild transient pulmonary granuloma formation, whereas cotton fibers and talc (used as a filler) can cause permanent intravascular and perivascular granulomas in pulmonary arteries and arterioles.[255] The injection of crushed oral tablets can result in a constellation of clinical and radiographic findings due to a foreign-body reaction in the pulmonary arterioles related to the fillers present in tablets (excipients).[256] The resultant baseline abnormalities of chest radiographs and blood gases may cause diagnostic confusion in febrile IDUs. IDUs have a 10-fold increased risk for pneumonia compared with the risk for non-IDUs.[257] Among patients with HIV infection on ART, bacterial pneumonia was more common in patients who were IDUs.[258] The incidence of pneumonia is increased in IDUs for a number of reasons, including impaired clearance of secretions, aspiration, increased exposure, decreased immune function, and higher prevalence of HIV infection. In a series of pulmonary complications of injection drug use, septic pulmonary emboli were the most common complication, followed by community-acquired pneumonia and *M. tuberculosis* infection.[259]

Clinical Patterns

Most pulmonary infections are community-acquired episodes of pneumonia caused by common respiratory pathogens. In an older series of febrile IDUs, pneumonia was the most common cause of fever[260]; in a second series, pneumonia was second only to cellulitis as a cause of fever in IDUs.[261] A more recent series also found pneumonia second only to skin and soft tissue infections as a reason for hospital admission among IDUs.[20] Bacterial pneumonia must be distinguished from septic emboli originating from right-sided endocarditis or more distal thrombophlebitis and resultant pulmonary infarcts. Septic emboli result in multiple round or wedge-shaped lesions that may cavitate (Fig. 312.2). Pleural involvement is common in both conditions and results in chest pain, pleural effusion, or empyema. Recurrent pulmonary emboli may also result in pulmonary hypertension. Injection drug use was found to be an independent risk factor for complicated parapneumonic effusion and empyema in patients with community-acquired pneumonia.[262] The usual pathogens in bacterial pneumonia include *Streptococcus pneumoniae* and oral anaerobes by the bronchogenic route and *S. aureus* or, less commonly, *P. aeruginosa* by the hematogenous route. One study of the etiology of community-acquired pneumonia in an urban public hospital emphasized the importance of aspiration pneumonia in this patient population, particularly among patients with pneumonia necessitating admission to the intensive care unit.[263] Injection drug use has been identified as a risk factor for bacteremia in patients with community-acquired pneumococcal pneumonia.[264] Lung abscesses may arise from aspiration pneumonia, necrotizing pneumonitis, or septic emboli. Opportunistic pulmonary infections, especially *Pneumocystis jirovecii* pneumonia, must also be considered in febrile IDUs.

Tuberculosis in Injection Drug Users

Pulmonary TB is a major problem in both HIV-infected and non–HIV-infected drug users throughout the world; the prevalence of latent TB infection among IDUs globally is reported to be between 10% and 67%.[265] Homelessness and nonadherence to medication regimens further complicate the problem. The risk for TB is still substantial in HIV-positive individuals taking ART.[266] In a study from San Diego, one-quarter of IDUs were infected with TB.[265] In this study, 95% of IDUs had been previously tested for TB infection; however, only 60% of those who reported having a previous diagnosis of latent TB infection were ever treated. TB in IDUs with AIDS is more frequently extrapulmonary, and patients present with less cavitary pulmonary disease and fewer acid-fast bacillus–positive organisms in sputum than other patients with TB. Coughing induced by the use of marijuana or crack cocaine and injection in shooting galleries or other cramped spaces may increase the transmission of TB. IDUs may be screened for latent TB infection by either tuberculin skin test (TST) or an interferon-γ release assay. An interferon-γ release assay is preferred for testing in IDUs because they are less likely to return for skin test readings.[267] IDUs should receive therapy for latent TB infection if the TST is 10 mm or larger. IDUs with HIV infection should receive therapy for latent TB infection as recommended for other HIV-infected patients, that is, if the TST is 5 mm or larger or if the patient is a close contact of a person who has active TB, regardless of skin test results or previous courses of therapy for latent TB infection.[268] The US Centers for Disease Control and Prevention (CDC) recommends four different regimens for the treatment of latent TB infection. Isoniazid daily or twice weekly (as directly observed therapy) may be given for 6 or 9 months; 9 months is preferred for HIV-infected patients. Isonazid and rifapentine may be given once weekly for 12 doses as directly observed therapy; however, this regimen is not recommended for HIV-infected patients on ART. Lastly, rifampin daily for 4 months can be used; this regimen is least preferred but may be an option for individuals at high risk for hepatotoxicity. The potential risk for isoniazid-induced hepatotoxicity in IDUs, who have a higher frequency of background hepatitis and who may also be abusing other hepatotoxic agents such as alcohol and cocaine, is a concern. However, a study of isoniazid therapy in IDUs with an HCV seroprevalence of 95% found that the risks for hepatotoxicity and isoniazid discontinuation were similar to risks reported for populations with a lower prevalence of HCV.[269] Material incentives have been shown to be effective in increasing the return rates for TST reading and promoting adherence to a program of directly observed latent TB infection therapy and treatment of TB in IDUs.[270]

A febrile IDU with pulmonary infiltrates constitutes an enormous diagnostic challenge given the wide differential diagnosis, which includes noninfectious causes. Accordingly, initial treatment often involves multiple therapeutic agents to cover several pathogens. Empirical coverage for TB may be needed in critically ill patients.



FIG. 312.2 Anteroposterior chest radiograph demonstrating pleural effusion and multiple cavitating nodular infarcts resulting from septic emboli in a patient with tricuspid endocarditis.

HEPATITIS

Risk Factors

Hepatitis has long been recognized as a complication of injection drug use. A significant number of IDUs also report hazardous alcohol use.[271] The combination of alcohol use plus HBV or HCV infection results in more severe liver disease than either factor alone and is associated with more rapid acceleration to cirrhosis. Heroin itself is not known to be hepatotoxic, but cocaine can cause severe liver injury. Hepatotoxicity can also occur secondary to methamphetamine abuse. Buprenorphine, which is used as a substitution drug in the treatment of opiate addiction, has been reported to cause hepatitis, both when diverted and injected and when used sublingually.[272,273] IDUs have been demonstrated to have significantly less knowledge regarding risks and transmission of viral hepatitis than regarding risks and transmission of HIV.[133] Syringe services programs have been shown to be less effective at preventing the transmission of HCV and HBV than HIV.[274–276] The validity of self-reported serostatus of hepatitis A, B, and C and HBV vaccination status by IDUs is poor.[277,278]

Hepatitis B

Injection drug use remains an important risk factor for HBV infection in the United States. Although the overall incidence of acute HBV infection has remained stable since 2006, beginning in 2009 more cases of acute HBV infection have been reported from nonurban areas than urban areas.[279] The changing epidemiology of injection drug use related to the current opioid epidemic is likely responsible for this shift. A study of acute HBV infection in three Appalachian states documented an increase in infection among non-Hispanic whites 30 to 39 years of age, most of whom reported injection drug use as a risk factor.[279] Clinically apparent HBV infection is uncommon, and many IDUs end up with a serologic pattern indicative of naturally acquired immunity. In the United States 60% to 80% of IDUs have serum antibody against hepatitis B surface antigen; however, only 5% to 10% become chronic carriers.[280–282] Spontaneous reactivation of chronic HBV infection has been described in IDUs, but the diagnosis may be difficult because the clinical presentation is indistinguishable from that of acute hepatitis, anti-HBV core IgM may increase during a reactivation, and information on the patient's previous serologic status usually is unknown.[283,284] HIV-infected patients with HBV infection are more likely to become chronic carriers.[285] In addition, a high prevalence of occult HBV infection has been reported in IDUs with HIV infection.[286] The CDC Advisory Committee on Immunization Practices has recommended HBV vaccination for IDUs since 1991, yet current vaccine coverage rates for HBV among adults ≥19 years of age are low.[287] Although there is a tendency toward a decreased antibody response to immunization in HIV-positive and HIV-negative IDUs, there is not convincing evidence of decreased protection from infection, and all susceptible IDUs should be offered vaccination.[288] The incorporation of HBV vaccination into syringe services programs may be cost-effective and improve coverage rates.[289] IDUs with isolated anti-HBV core antibody have been shown to have strong resistance to reinfection and do not need vaccination.[290] These individuals are at risk for occult, chronic HBV infection, although the clinical significance of this entity remains unclear.[286] Guidelines for the treatment of chronic HBV infection are available, but they do not specifically address the issue of treatment of active IDUs, in contrast to guidelines for the treatment of HCV.[291]

Delta Virus

Hepatitis delta virus (HDV) is a defective RNA virus that can replicate and cause hepatitis only in the presence of active HBV infection. HDV may be acquired along with HBV as a primary coinfection or as a superinfection in people who are carriers of HBV. Because of the interdependent nature of the two viruses, immunity to HBV provides protection against HDV. In some areas where HBV is prevalent, HDV is also seen with relatively high frequency. In nonendemic areas such as the United States, HDV infection is confined almost exclusively to particular high-risk groups, including IDUs.[292,293] A significant decrease in the prevalence of HDV infection has been reported in nonendemic countries with universal HBV vaccination.[294,295] A serologic study of IDUs in Baltimore found a decrease in the overall prevalence of HDV from 15% in 1988–89 to 11% in 2005–06; however, there was a significant increase in the prevalence of HDV in individuals with chronic HBV infection from 29% to 50%.[296] Attendance at a shooting gallery was shown to be a strong correlate of HDV infection in this study. The association of injection drug use with HBV and HDV was clarified in a study comparing the transmission and carriage of each agent in two populations known to be at risk: IDUs and men who have sex with men (MSM).[297] Among 372 IDUs, 52.4% had evidence of current or past HBV infection; of these, 8.7% were chronic carriers of HBV. Among the chronic carriers, 70.6% were also chronic carriers of HDV. In contrast, only 27.4% of MSM had serologic evidence of HBV infection (current or remote), of whom 7.9% were chronic carriers. Only a third of these chronic HBV carriers had evidence of HDV infection, which was a significant difference from the IDUs, demonstrating that injection drug use is a much more efficient means of transmission of HDV infection than sexual contact.

Superinfection of HDV on previous HBV infection is the most common pattern of dual infection. Simultaneous acquisition of both viruses is more common among IDUs and is more likely to result in fulminant infection.[293] IDUs who experience coinfection frequently have a biphasic illness.[297] The initial phase of the disease is caused by HDV, and the second phase is caused by HBV.[298] The closer the proximity of the biphasic peaks, the greater the risk for a fatal outcome. IDUs who survive such an illness usually have a complete recovery and clear both viruses.[299] HDV can be prevented by vaccination of IDUs against HBV.

Hepatitis C

Injection drug use is a major risk factor for HCV infection. The seroprevalence of anti-HCV antibody among IDUs in four US cities decreased between 1994 and 2004 from 65% to 35%; this decrease was thought to be due to safer injection practices as a result of risk reduction targeted toward HIV transmission.[300] From 2004 to 2014, there was a reversal of this trend, which has been clearly linked to the opioid epidemic occurring in the United States at the present time.[301] In 2014, more than 80% of individuals with acute HCV infection with an identified risk factor reported injection drug use, confirming that injection drug use is the primary mode of HCV transmission in the United States. Maternal drug use, independent of HIV coinfection, has been identified as a risk factor for perinatal transmission of HCV, but this finding was not confirmed in a more recent study.[302,303] Studies of HCV seroconversion among young IDUs have identified sharing of needles and drug preparation equipment, pooling money with another IDU to purchase drugs, requiring assistance to inject, and injecting more than once daily as independent risk factors for the acquisition of HCV.[304–306] Injection of prescription opioid analgesics has been identified as an additional risk factor for HCV infection.[307] In an outbreak of HIV infection in rural Indiana linked to injection oxymorphone use, coinfection with HCV was diagnosed in 84.4% of individuals with newly diagnosed HIV infection.[308] Although injection drug use is more efficient at transmitting HCV infection than sexual activity, exchanging sex for money and having a sexual partner who uses injection drugs are also risk factors for seroconversion. Recent work has shown that sharing of equipment used to prepare drugs for injection does not directly result in HCV transmission but is a surrogate for infection that results from sharing drugs.[309] Studies from the mid-1980s and 1990s showed that HCV infection was acquired after a very brief interval of IDU; subsequent work demonstrated a longer time to seroconversion.[300] The average time to seroconversion among HCV-negative IDUs in Seattle was 3.4 years—long enough to justify the allocation of resources to try to reduce risky injection practices.[304] More current data regarding time to seroconversion are needed. There may be other benefits to reducing risky injection practices beyond the reduction of infection with bloodborne viral infections. IDUs without HCV infection who were enrolled in an HCV risk reduction program had a lower risk for bacterial infections than other IDU cohorts.[310] The receipt of opioid substitution therapy has been shown to decrease the risk of HCV seroconversion among IDUs.[311] It has been shown that IDUs who successfully cleared HCV infection in the past are less likely to develop persistent HCV viremia despite continued exposure, and this appears to correlate with T-cell responses to HCV.[312–314]

Chronic HCV infection occurs in 60% to 80% of IDUs.[280] Current guidelines for the treatment of HCV state that recent or active injection drug use should not be viewed as a contraindication to treatment. Response rates to treatment are comparable to rates reported in non-IDUs, even with ongoing drug use, especially in multidisciplinary programs that incorporate treatment for addiction and directly observed therapy.[315] Despite a willingness to be treated, low uptake of HCV treatment among IDUs is reported.[312,316,317] Younger individuals appear to have better linkage to HCV care.[318] Patients coinfected with HIV and HCV are less likely to clear HCV viremia after infection and experience more rapid progression to HCV-related liver disease. Coinfected individuals should be prioritized for treatment, and response rates are comparable to rates reported for individuals with monoinfection.[319]

Hepatitis A

IDUs are also at risk for hepatitis A virus (HAV) infection. Although parenteral transmission of HAV has been reported in IDUs, other lifestyle factors are likely more important in explaining the increased risk for HAV in this population. Among IDUs in Baltimore, evidence of past HAV infection was not significantly associated with high-risk drug-using behaviors, but rather was associated with low annual income.[320] A more recent study of IDUs in Spain also found that socioeconomic factors, rather than injection drug use, were the main determinants of HAV infection risk.[321] Fulminant hepatitis caused by HAV in IDUs with chronic liver disease caused by HBV and HCV has been reported.[322,323] Nonimmune IDUs should receive the HAV vaccine. In settings where there is an ongoing HAV outbreak, people who use both injection and noninjection drugs should be offered vaccination even if their serostatus is unknown.

Hepatitis E

A study of IDUs in San Diego revealed an overall hepatitis E virus seroprevalence of 2.7%.[324] Older IDUs were more likely to be infected, but homelessness, incarceration, and high-risk sexual behavior were not associated with hepatitis E virus infection.

SPLENIC ABSCESS

Abscess of the spleen is a major complication of injection drug use. The splenic arteries are end arteries; any occlusion leads to ischemia or infarction. The ischemic or infarcted areas are highly susceptible to infection and serve as a nidus for abscess formation in the event of bacteremia. Trauma, including blunt trauma, also may lead to splenic injury and is an antecedent condition in some IDUs who develop splenic abscess.[325] Cocaine-associated splenic infarction with secondary bacterial infection has also been reported.[326] Endocarditis is the most common underlying infection in IDUs with splenic abscess,[325,327] although splenic involvement also may result from spread of local infection directly to the splenic artery or extension of an adjacent process with erosion and thrombosis of the splenic artery.[328]

Splenic lesions may be multiple and small or solitary, occasionally becoming large.[325,327,328] Lesions within the spleen are most often found in the upper pole (53.1%). Lower-pole lesions (21.9%) and midspleen lesions (15.6%) are found less often.[328] Staphylococci and streptococci are the organisms most often implicated; however, gram-negative bacilli and anaerobes are isolated in approximately 25% and 5% of cases, respectively.[328] IDUs who lick their needles are susceptible to splenic abscesses caused by mouth anaerobes, in particular, *Fusobacterium* spp.[329] *M. tuberculosis* has been reported as a cause of splenic abscess in IDUs who are infected with HIV.[330] Bacteremia is common in patients with splenic abscess, and usually the same organism is cultured from the blood and from the splenic cavity. However, IDUs have a tendency to have multiple infected sites that contain different organisms. Therefore isolation of an organism from the blood is not assurance that the same organism will be found in the spleen.[327]

Clinical Manifestations

The signs and symptoms of splenic abscess may be vague or overshadowed by underlying endocarditis. Almost all patients have fever and some degree of abdominal pain or discomfort.[325,327,328] Pleuritic chest pain is common.[325,327,328] Left shoulder pain has also been described. Abdominal tenderness, which is frequently confined to the left upper quadrant, is found in approximately 50% of patients.[325,327,328] Splenomegaly may be present, but a splenic rub is unusual [325] Abnormalities within the thorax, including left lower lobe lung infiltrate and pleural effusion, are detected in two-thirds of cases. The differential diagnosis includes subphrenic abscess, pulmonary empyema, perinephric abscess, and bland splenic infarct. The possibility of splenic abscess must always be excluded in IDUs with IE who fail to clear their bacteremia or remain persistently febrile despite negative blood cultures. There are no characteristic laboratory abnormalities, although an extremely high leukocyte count has been correlated with a poor prognosis.[328] The chest radiograph may reveal an elevated hemidiaphragm, infiltrate, or a pleural effusion; however, abdominal radiography is seldom useful. The most reliable diagnostic tests are ultrasound and CT of the abdomen.[325] MRI is also useful in characterizing a splenic pathologic process.[331]

Therapy

Splenectomy remains the preferred treatment of splenic abscess. Although randomized controlled trials are not possible, there are an increasing number of reports of successful management using either ultrasound-guided or CT-guided percutaneous drainage.[332–334] Percutaneous drainage can serve as a bridge to surgery in patients who are critically ill. Splenectomy continues to be preferred in patients who must undergo valve replacement for the management of IE. Removal of the spleen may be difficult if it adheres to adjacent structures; removal or partial resection of these organs may be required.[325,326,329] Laparoscopic splenectomy for splenic abscess has been reported, but experience with this modality remains limited.[335,336]

Complications of splenic abscess include spontaneous rupture, which can be so subtle in some cases that the patient has no signs of generalized peritonitis or purulence in the abdominal cavity.[325] Other manifestations include recurrent bacteremia and intestinal obstruction.[328] Although associated with significant morbidity and mortality, splenic abscess detected early and treated promptly has a good prognosis.

CENTRAL NERVOUS SYSTEM INFECTIONS

IDUs may present with a variety of CNS manifestations that may or may not be infectious in origin. The differential diagnosis is extensive and frequently difficult. Complications related to the injection of illicit drugs include coma caused by overdose or intoxication, postanoxic encephalopathy, delirium, and acute confusion states. Seizures, cerebral edema, and dementia may result from noninfectious as well as infectious causes. Hemorrhage and infarction may be secondary to infection or compromise of the neurovascular system. Parkinsonism is most often the result of drug effects but has been reported in infection.[337] The etiology of these disorders may be obscure, necessitating a thorough workup to exclude an infectious cause. Infections of the CNS may be local or secondary to an infectious process elsewhere. When infection is the primary problem, focal findings and fever are usually present. When present, focal findings suggest the possibility of a mass lesion requiring immediate surgical intervention. Therefore an IDU with neurologic findings requires a differential diagnosis that includes both infectious and noninfectious causes and an effort to differentiate between a local process and a complication of a distant primary infection.

Risk Factors

CNS manifestations are more common in IDUs than in non-IDUs and are found in 45% to 58% of IDUs with endocarditis.[338] In one study of IDUs with left-sided IE, CNS complications were found in 52% of patients with mitral valve infection but in only 28% of patients with aortic valve disease.[202] Other investigators found a greater incidence in aortic valve disease, especially when *S. aureus* was involved and the patients had congestive heart failure.[339] Endocarditis, particularly when caused by *S. aureus* or *S. pneumoniae,*[213,340] is the most common cause of CNS disease in IDUs, and it accounts for the most serious complications including brain abscess, meningitis, encephalopathy, and hemorrhage from ruptured mycotic aneurysms.[150,341] Mycotic aneurysms may manifest as a progressive focal neurologic deficit resulting from expansion of the aneurysm or as an acute subarachnoid or intracerebral

hemorrhage.[342] Before rupture, patients may complain of severe localized headache, which should prompt immediate evaluation. CT angiography and MRA can detect aneurysms that are greater than 5 mm in size; the sensitivity of MRA is slightly higher (94% vs. 86%).[343] For smaller aneurysms the sensitivity of CT angiography and MRA is far less (35% and 57%, respectively).[343] If a strong suspicion remains, a negative study should be followed by four-vessel cerebral angiography, which remains the definitive test. Even with a negative test result, if symptoms persist, repeated angiography may reveal lesions that were not detected previously.

Cerebral Mycotic Aneurysm

The optimal management of cerebral mycotic aneurysms remains undefined. In some cases, lesions resolve completely or decrease in size with antibiotic treatment alone. In other cases the lesion may enlarge, or new aneurysms may appear. Surgery is important for control of ruptured aneurysms of peripheral arteries or when an intracranial location or masslike lesion occurs. Some researchers recommend serial angiograms to assess the progress of aneurysms; others call for aggressive surgical management, especially if the lesion is accessible. When the patient's condition precludes surgery, an endovascular procedure for embolization of the lesion may be advised.[344,345] Introducing a coil into the affected vessel is a frequent alternative to surgical removal or clipping; however, in the case of aneurysms in IDUs the lesions are often peripheral and in very small vessels. The size of the involved vessel often precludes the introduction of a coil, and the fragility of the lesion carries a risk of perforation should a coil be attempted. There is always a risk of causing a catastrophic neurologic injury when attempting occlusion of the vessel with a coil or embolization of these vessels, especially if doing so obliterates blood flow to a critical area. In lesions that were adjacent to eloquent cortex, Chalouhi and colleagues used superselective imaging (sodium amobarbital injected into a target vessel with the patient awake to determine the safety of vascular sacrifice) before a definitive procedure to eradicate mycotic aneurysms in a series of patients who were not considered surgical candidates.[346] Once satisfied there would be no significant deficit, they had excellent results after introducing a coil and injecting Onyx 18/34. The presence of multiple aneurysms may make a surgical approach impossible. In some of these cases, patients do well with antimicrobial treatment, but each case must be treated individually.[347] Whether surgical or endovascular management of ruptured mycotic aneurysms is optimal has not been defined. Some experts advocate for endovascular management with surgery reserved for easily accessible lesions, lesions associated with large parenchymal hematoma, lesions in young patients, and lesions involving eloquent territory of the brain.[348] Regardless of the treatment chosen, close angiographic follow-up is recommended.

Focal abnormalities also result from septic emboli (which frequently result in transient focal neurologic deficits) and multiple cerebral abscesses. These lesions tend to resolve in 1 to 2 weeks with appropriate antibiotic therapy.[150] IDUs also commonly develop a diffuse encephalopathy that may be due to bacteremia or injected toxins.

Brain Abscess and Subdural Empyema

Localized CNS infection in IDUs is confined primarily to brain abscess and subdural empyema. Pyogenic bacteria usually cause brain abscess; *Nocardia* has also been reported. Fungi, including *Aspergillus* spp., *Chaetomium strumarium, Candida* spp., and mucormycosis, have also been reported. Isolated cerebral mucormycosis in IDUs is rarely associated with HIV infection and manifests as focal cerebritis or abscess. In contrast to the more extensive aggressive process observed in immunocompromised hosts, in IDUs there seems to be a predilection for involvement of the basal ganglia.[349] Cerebral mucormycosis in IDUs is not caused by spread from sinuses. It appears the organism is either a contaminant in the illicit drugs or enters the bloodstream from an infected injection site, finding its way to areas of the brain predisposed by earlier drug-induced injury. The patient usually presents with signs and symptoms of a mass lesion. The differential diagnosis of such lesions includes toxoplasmosis, lymphoma, TB, and cryptococcosis. Isolated involvement of the basal ganglia is uncommon in toxoplasmosis and lymphoma. Radiologic contrast enhancement may or may not be seen in patients with mucormycosis and therefore lends little to the diagnosis. Biopsy of the lesion is required to establish the diagnosis; failure to do so is associated with a very high mortality rate.[350] Remarkably the outcome of this disease is very good, with survival in most cases after prolonged amphotericin B therapy and excision of as much of the infected tissue as possible.

The clinical signs and symptoms of brain abscesses in IDUs are the same as in non-IDUs and depend on the size and location of the lesion within the brain. They usually result from infected cerebral emboli in patients with mitral or aortic valve endocarditis.[150,183,202,341] Rarely, emboli travel through or originate in the pulmonary circulation. Alternatively, pathogens may seed the brain after an inadvertent injection into the arterial system during an attempted jugular vein injection. Tuberculous brain abscesses may also be seen, particularly in IDUs who are coinfected with HIV. The lesions are typically solitary, multiloculated, and contrast enhancing. They must be distinguished from lesions due to *Toxoplasma,* which usually causes multiple lesions that are not multiloculated, and lymphoma, which can be necrotic with multiple loculations but is more often located near an ependymal surface.[351] IDUs with HIV infection who have characteristic lesions may be treated empirically for toxoplasmosis, but failure to respond within 2 weeks should prompt a biopsy, which will lead to the correct diagnosis. Subdural empyema is also seen in IDUs and may be secondary to direct extension from a local infectious process or complicated bacteremia.

Meningitis

Meningitis in IDUs is most often secondary to endocarditis[341] and is usually due to *S. aureus* or *S. pneumoniae.*[338] However, abnormal CSF findings are common in patients with CNS complications of endocarditis. Findings in patients with meningitis include purulent CSF with a neutrophilic pleocytosis and elevated protein level, with normal or decreased glucose level. Hemorrhagic CSF may also be found. A pattern consistent with aseptic meningitis is seen in 25% of cases; normal CSF is seen in only 30%.[338] Positive CSF cultures are seen in a minority of patients, most of whom have *S. aureus* infection and purulent CSF.

Spinal Epidural Abscess

A spinal epidural abscess should be considered in any IDU presenting with spinal ache or back pain, especially if focal neurologic signs are present. Occasionally, blunt trauma may predispose an area of the spine to infection after an episode of transient bacteremia.[352] In Kentucky, legislation was passed in 2012 that increased control of prescription narcotics, and there was a subsequent ninefold increase in the diagnosis of spinal abscess strongly associated with drug abuse by 2014.[353] It appears IDUs had become dependent on narcotics obtained without prescription as a response to the new law. In western Massachusetts in the decade between 2005 and 2015 there was a 3.3-fold increase in spinal epidural abscess from 2.5 to 8.0 per 10,000 admissions.[354]

Patients tend to have a prolonged, several-month symptomatic course. Alternatively the disease can advance rapidly leading to permanent neurologic damage. Typically the disease progresses from focal vertebral pain to root pain, neurologic deficits (motor or sensory), and, finally, paralysis. The classic triad of fever, back pain, and neurologic deficit is seen in a minority of patients and cannot be relied on to initiate a diagnostic workup. The most reliable finding to aid in diagnosis of an IDU with epidural abscess may be back pain alone.[355] The thoracic or lumbar spine is most often involved, although cervical spine lesions are also seen and may be more common among IDUs than non-IDUs.[146] Initial laboratory results, including white blood cell count, are frequently not helpful.[355] Contrast-enhanced MRI or CT is usually sufficient to identify the lesion. Multiple levels of involvement in the cord, frequently in noncontiguous areas, may occur, emphasizing the necessity of carefully imaging the cord before any intervention so as not to overlook infected material that needs to be addressed. In a more recent series, blood cultures were positive in almost two-thirds of cases.[354] *S. aureus* is the most common cause, but other gram-positive and gram-negative organisms have been reported, occasionally in combination with other organisms. *M. tuberculosis* also causes spinal epidural abscess, and it too may be found in combination with other organisms,[356] making careful definition of the microbial etiology imperative. Occasionally, difficult-to-culture organisms are responsible; laboratories should be advised that special techniques may be necessary when original cultures

are negative.[357] The pathophysiology is usually directly spread to the epidural space from adjacent disk or vertebral body infection or hematogenous dissemination from a distant focus of infection, frequently endocarditis.

In most cases, immediate drainage relieves symptoms; although once a chronic condition ensues, there may be nothing but granulation tissue, requiring multiple-level laminectomy to relieve pressure on the spinal cord. Controversy remains regarding the benefits of surgical versus medical management. In most series, there is a higher risk of failure among patients treated conservatively, and some authors have found best results if surgery is performed in the first 24 hours.[358] If surgery is delayed, careful observation is required, and surgery should be undertaken at the first sign of neurologic deficit. Further delay usually results in permanent neurologic deficit.[355,359]

Intramedullary spinal cord abscess has been reported in IDUs with symptoms resembling those of an epidural abscess.[360,361] This condition is extremely rare. Early surgical drainage accompanied by systemic antibiotics is required to increase the likelihood of a favorable outcome.[362]

Tetanus and Wound Botulism

Toxin-mediated diseases, specifically wound botulism and tetanus, are being seen with increasing frequency among IDUs and must be considered in IDUs who have neurologic symptoms. Epidemics of both tetanus and wound botulism have occurred in California,[363,364] and cases are likely to be found elsewhere. A patient with tetanus is likely to be a long-time IDU who has poor venous access and multiple skin lesions caused by failed attempts at intravenous injections or by skin popping. These lesions become colonized by multiple pathogens including *Clostridium* spp. In the proper anaerobic environment, toxin is generated and produces disease.[365] Among the patients from California who had tetanus, 89% were Hispanic Americans, which may be explained by a study finding that only 58% of Mexican Americans had protective levels of antibody to tetanus toxoid compared with 73% of non-Hispanic whites.[364] In a more recent report of tetanus surveillance in the United States, IDUs, particularly those of Hispanic ethnicity, remained an important risk group.[366] Skin popping probably accounts for the higher mortality rate due to tetanus in IDUs than in non-IDUs. One proposed reason is that because of the number and severity of skin lesions in addicts, there is greater opportunity for large amounts of toxin to be produced. In addition, an addict presenting with the typical symptoms of tetanus may be believed to be manifesting the effects of illicit drug toxicity, overdose, or drug withdrawal.[367,368] In view of the risk for tetanus associated with injection drug use, it is worthwhile to consider giving a tetanus booster to any IDU who is being treated for any other condition who has not been immunized recently.

Wound botulism in IDUs was first described in New York City in 1982. Subsequently, sporadic cases were reported from different locations. After 1990, a dramatic increase in the number of wound botulism cases occurred in California.[365] With rare exceptions, patients were IDUs who injected black tar heroin, a black, gummy form of the drug synthesized in Mexico and distributed widely throughout the western United States. Skin popping of black tar heroin was the major risk factor for acquisition of wound botulism. The greatest risk was seen among heavy users, but disease also occurred in occasional users with subcutaneous or intramuscular injection. The drug was most likely contaminated during the dilution process, when substances were added to the heroin to increase the amount of the product and thereby increase the seller's profits. Wound botulism results from colonization of wounds by *Clostridium botulinum* with subsequent toxin production. Initially patients experience blurred vision or diplopia, dysarthria, and dysphagia, followed by descending muscle weakness and respiratory failure. The symptoms are similar to those of botulism in non-IDUs except that gastrointestinal symptoms are absent. Also, in contrast to non-IDUs, who usually have a dietary history to suggest the diagnosis, in IDUs the organism can usually be recovered from wound cultures. Frequently, fever results from the associated wound infection. Serum assays for botulism toxin are rarely positive; administration of antitoxin, which is helpful only if given within the first 24 hours, must be done on the basis of a high index of suspicion, rather than after culture identification. In an IDU having characteristic signs and symptoms, a fever (which helps to distinguish from other forms of botulism) and an abscess, wound botulism should be strongly considered and treatment begun immediately with antitoxin and wound débridement.[369] The epidemic associated with black tar heroin use is ongoing, and recurrent cases are reported.[366]

OCULAR INFECTIONS

Endophthalmitis

Endophthalmitis is a common and serious complication of injection drug use.[370,371] Both fungal endophthalmitis and bacterial endophthalmitis are hematogenous in origin and frequently manifest as complications of IE. *Candida* is the most common fungal cause. Endophthalmitis may also occur as part of a disseminated syndrome involving eyes, bone, and skin in heroin users. Fungal endophthalmitis has been reported after injection of crack cocaine dissolved in lemon juice and after injection of diverted sublingual buprenorphine.[372,373] A report from New England of an increase in cases of fungal endophthalmitis associated with injection drug use (most commonly *Candida* spp.) suggests that the incidence may be increasing as a result of the new injection opioid epidemic.[374] Symptoms of endophthalmitis include blurred vision, pain, and decreased visual acuity. White exudative lesions are found in the choroid and retina, with vitreous haziness. Diagnosis requires a high index of suspicion, and because blood cultures are usually negative at the time of ocular symptoms, definitive diagnosis often involves vitreous sampling. The antifungal regimen that has been used most extensively for the treatment of *Candida* chorioretinitis is amphotericin B in combination with flucytosine; however, fluconazole or voriconazole is now recommended as first-line therapy for susceptible isolates with amphotericin reserved for azole-resistant isolates.[213] Echinocandins are not recommended. If extension into the vitreous humor has occurred (i.e., endophthalmitis), intravitreal amphotericin B or voriconazole with or without pars plana vitrectomy is recommended; the use of intraocular dexamethasone to treat inflammation associated with infection is controversial (see Chapter 114).[213,375]

Aspergillus spp. are the second most common cause of fungal endophthalmitis in IDUs. As with *Candida* spp., the pathogenesis reflects mycotic contamination of drug paraphernalia or of the heroin injected, rather than host immunosuppression. Physical findings and treatment are similar to those of *Candida* infection.

Bacterial endophthalmitis is less common, and the presentation is often acute with rapid progression of symptoms. Inflammation usually is present in the anterior and posterior chambers. In addition to pain, redness, and lid swelling, evidence of retinal vasculitis may be present. *S. aureus* is the organism most frequently isolated. A rapidly destructive form of endophthalmitis has been reported for *B. cereus,* which has been cultured from heroin and drug paraphernalia.[376] Bacterial endophthalmitis is treated primarily with intravitreal antimicrobial agents with or without vitrectomy, although the role of systemic antibiotics continues to be debated (see Chapter 114). In both mycotic and bacterial endophthalmitis, early diagnosis and intervention increase the chance of a favorable outcome. A case series of endophthalmitis in IDUs found that the prognosis for visual outcome was equally poor for both bacterial and fungal infections.[377] Whereas bacterial endophthalmitis is infrequent, ocular peripheral emboli are frequent complications of IE in IDUs. These include subconjunctival hemorrhages and retinal emboli (Roth spots), which manifest as petechiae, retinal hemorrhage, and ischemia.

HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Epidemiology

Early in the AIDS epidemic, injection drug use was a high-risk factor for contracting the disease. In the United States the incidence of HIV infection among IDUs has steadily decreased, with IDUs now accounting for 9% of new HIV infections.[378] Changes in the epidemiology of injection drug use in the United States may threaten this decline, as illustrated by the community outbreak of HIV infection associated with injection of prescription opioids in a rural area of southeastern Indiana in 2015.[308] In a county where fewer than 5 cases of new HIV infection were reported annually, 135 new infections were reported in a community of 4200 people. Investigation revealed that sharing of syringes and drug preparation equipment used to solubilize oral extended-release oxymorphone

to inject it were risk factors for HIV acquisition. Subsequent work has documented a high prevalence of high-risk injection practices among the population in this outbreak.[379] In high seroprevalence areas, female IDUs may be at a higher risk for HIV infection than male IDUs.[380] Sexual contact with IDUs still accounts for a significant number of cases among individuals whose primary risk is heterosexual contact. Epidemiologic studies continue to show high rates of injection and sexual risk behaviors among IDUs.[381] In the United States as well as the rest of the world, HIV infection associated with injection drug use disproportionally affects racial and ethnic minorities, particularly minority women and children.[382,383] Globally, injection drug use is the major risk for HIV acquisition in Asia (including Russia and the former Soviet republics).[384] Eastern Europe and Central Asia experienced a 13% increase in new infections from 2006 to 2012, and many countries in this region have seen a transition from an epidemic concentrated among IDUs to a generalized epidemic among heterosexuals.[385] In the United States different risk profiles have been described among users of different types of injection drugs; however, this may be changing. Previously, HIV was not found to be highly prevalent among individuals who inject methamphetamines, although both injection and sexual high-risk behavior were very common.[386] More recent reports have suggested that an increasing prevalence of methamphetamine injection, including methamphetamine injected with heroin (goofballs) coupled with high-risk injection practices, may increase the risk for HIV infection in this population both among MSM and heterosexuals.[387]

Prevention

IDUs have been underrepresented in HIV prevention research and in the global effort to increase access to prevention, treatment, and care. Syringe services programs have been shown to be an effective means of reducing the incidence of new HIV infections.[276,388] Multicomponent interventions that include needle and syringe access/exchange, voluntary counseling and testing, opioid substitution treatment, and ART for individuals already infected have the potential to substantially reduce the incidence of new infections.[389] The existence of supervised injection facilities, where IDUs can inject their drugs under the supervision of health care professionals, has been shown to reduce public injecting, syringe sharing, and drug overdose and increase access to addiction treatment.[390–392] Despite evidence for efficacy and no evidence to support the contention that safe injection programs lead to an increase in injection drug use, these programs remain controversial. Substitution therapy for opioid addiction appears to reduce injection-related HIV risk behaviors but has less effect on risky sexual behaviors.[393,394] Preexposure prophylaxis has been shown to reduce the risk of HIV infection in IDUs and should be offered as outlined in the CDC guidelines for preexposure prophylaxis for the prevention of HIV.[395]

Therapy and Prognosis

In the era before the availability of combination ART, cohort studies failed to demonstrate any difference in the rate of progression to AIDS among IDUs, MSM, and heterosexuals.[396] Since the availability of effective ART, studies of HIV outcomes among IDUs have yielded conflicting results. ART efficacy, as evidenced by $CD4^+$ cell response, appears to be similar for IDUs and non-IDUs, provided that there is sustained suppression of viremia.[397] IDUs may be at higher risk of medication non-adherence than other patient populations with HIV.[398] Injection drug use was previously shown to be an independent predictor for delay in initiation of care after the diagnosis of HIV infection,[399] but a more recent study has shown that this is no longer the case.[400] However, in the United States ART coverage for IDUs remains lower than for other groups at risk for HIV.[401] Homelessness and young age have been shown to be predictors of poor adherence and treatment response in IDUs, and periods of incarceration are associated with virologic failure even among individuals who had achieved virologic suppression.[402–404] Despite these barriers to adherence, as a group, IDUs are not at increased risk for resistance to ART compared with non-IDUs.[405] Intensive community-based programs to engage and retain IDUs in care have been associated with reductions in all-cause and HIV-related mortality.[406]

The medical management of HIV-infected IDUs must incorporate the diagnosis and treatment of substance abuse. Substitution therapy can facilitate social stability and support adherence to ART. Drug interactions between ART and methadone, buprenorphine, and other street drugs must be considered when managing IDUs with HIV infection.[407]

SEXUALLY TRANSMITTED DISEASES

Epidemiology

A major contributing factor to the prevalence of STDs is unsafe sexual practices associated with the use of illicit drugs.[408] A survey of young opioid users in New York City, 64% of whom reported injection drug use, revealed high rates of risky sexual behavior, including very low rates of condom use, multiple sexual partners, and participation in group sex events.[409] A study of young IDUs in Baltimore found that the prevalence of infection with *Chlamydia, Neisseria gonorrhoeae,* and *Trichomonas* was similar to that in the general population, despite the fact that 68% of participants had two or more sex partners in the past 3 months and less than half consistently used condoms.[410] A more recent study of IDUs in Camden, New Jersey, who were recruited from a syringe exchange program found high rates of gonorrhea and *Chlamydia* infection, with one-third of infections found at a nongenital site.[411] In this study, which included equal numbers of men and women, 60% of infections would have been missed if pharyngeal and rectal samples had not been obtained. Alcohol use and alcohol intoxication are associated with an increase in risky sexual behavior among IDUs.[412] The use of alcohol among drug users appears to be more strongly associated with risky sexual behavior than with risky injection practices.[413] IDUs who use methamphetamine have an increased risk of sexually transmitted infections compared with addicts who inject heroin.[414] Female sex workers who are IDUs have a higher prevalence of STDs and are more likely to engage in risky sexual behaviors and have unsafe sex with clients than sex workers who do not inject drugs.[415] Male IDUs who have sex with men, especially those who engage in commercial sex work, are much more likely to report risky sexual behavior.[416,417] It has been observed that reducing risky sexual behavior among IDUs is much more difficult than reducing risky injection behavior. Among IDUs who inject prescription opioids in Los Angeles and New York City, half reported risky injection behavior, but more than three-quarters engaged in risky sexual behavior.[418]

Syphilis

Risk factors for syphilis among IDUs include recent initiation of injection drug use, injecting drugs with other people, and injecting in public places.[419] The diagnosis and treatment of syphilis in IDUs may be complicated by the high rate of biologic false-positive, nonspecific serologic screening test results; the increasing availability of screening algorithms that use specific tests as the initial screening test may reduce the incidence of false-positive screening results. In contrast to the poor reliability of self-reported hepatitis serostatus, self-reports of syphilis infection history by IDUs were found to have good reliability.[420]

Given the importance of STDs as cofactors in the sexual transmission of HIV, reducing the prevalence of these diseases in IDUs is an additional strategy to diminish the spread of HIV among IDUs and from them to their non–drug-using sexual contacts.

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313 Surgical Site Infections and Antimicrobial Prophylaxis

Thomas R. Talbot

SHORT VIEW SUMMARY

Background
- Surgical site infections (SSIs) are a common and unwanted outcome from most surgical procedures.
- The development of an SSI depends on patient-related, procedure-related, and pathogen-related factors and the application of evidenced-based prevention methods.
- Causative organisms of SSI are predominantly the flora present at the incision site.

Risk Factors for Surgical Site Infections (see Table 313.2)
- Patient factors, including comorbid illness, colonization with pathogenic bacteria, perioperative hyperglycemia, and elevated body mass index
- Procedural factors, including breaks in sterile technique, operating room ventilation, and traffic
- Proceduralist factors, including surgical technique, improper application of skin antisepsis, teamwork incoordination, and provider impairment

Prevention of Surgical Site Infections (see Table 313.4)
- Practices to reduce bacterial inoculation into wound, including antiseptic use, sterile attire, decolonization strategies, and proper hair removal
- Practices to improve host containment of introduced bacteria, including maintenance of normothermia, minimizing tissue hypoxia, glucose control, and antimicrobial prophylaxis

Surgical Antimicrobial Prophylaxis
- Key principles
  - Maintain tissue concentration of drug above mean inhibitory concentration of common flora (see Fig. 313.4)
  - Provide "right" drug—targets flora at incision site, adequately penetrates incision site, minimal adverse events
  - Provide "right" dose at the "right" time—dose in the window before incision to allow penetration into tissues (see Fig. 313.5), use higher doses for obese patients, redose in prolonged procedures
  - Provide "right" duration of drug; stop once incision closed
- Prophylaxis targeting resistant organisms may be warranted in select situations (e.g., known colonization with organism)

Surgical Site Infections Surveillance
- SSIs surveillance requires standardized definitions.
- Postdischarge surveillance of SSIs may lead to variability in reported outcomes and must be addressed as part of any surveillance system.
- SSI rates are being increasingly used as measures of quality of care tied to reimbursement from payers.

HISTORICAL BACKGROUND

In 1862 Louis Pasteur's ingenious experiments into the nature of putrefaction were officially endorsed by the Paris Academy of Science. The endorsement signaled an end to the long-held belief that the exposure of organic material to air brought about the spontaneous generation of microorganisms, and the concepts of *sepsis* and *asepsis* became firmly established. A mere 3 years later, Joseph Lister demonstrated the incredible implications of antisepsis in his practice of orthopedic surgery. For the first time in recorded history, major surgical procedures could be performed with a reasonable expectation of primary wound healing and recovery. Essential enhancements for preventing and controlling wound sepsis were provided by the antibiotic revolution of the 1940s, ushering in the highly technical, highly invasive, and highly successful era of modern surgery. As noted by McDermott and Rogers,[1] the great achievements of the antibiotic era may be related, in the long run, to its essential role in supporting the advancements of modern surgery. Indeed, surgery as we know it today would be impossible in an environment in which infection was likely or, once established, untreatable.

Despite the fundamental role of antisepsis and antibiotics in the development of modern surgery, implementation of these discoveries in the practice of surgery has not occurred without opposition. As late as 1880, for example, William Halstead was ordered from the operating theater when he challenged a senior surgeon's disregard for antiseptic techniques. The early use of antibiotics for prophylaxis in surgical procedures was also questioned as respected academicians freely voiced their disapproval of antimicrobial prophylaxis in clean surgical procedures.[2] For a number of years the value of prophylactic antibiotics in preventing infections of the surgical wound remained in doubt. A consensus in favor of their use did not emerge until two concepts of perioperative prophylaxis and infection were established. First, investigators in Cincinnati and Boston demonstrated that, despite the use of standard aseptic techniques, *Staphylococcus aureus* could be regularly isolated from the operative field.[3–5] It became apparent that aseptic technique could decrease but not eliminate bacterial contamination of the surgical field; therefore it appeared plausible that perioperative antibiotics could supplement aseptic techniques in containing the inevitable contamination of the operative wound.

The second major finding involved the timing of the administration of the prophylactic antibiotic. As early as 1946, Howes[6] had noted a correlation between the amelioration of infection and the interval between the contamination of the wounds and the administration of antibiotics. Several years later, Miles and colleagues[7] and Burke,[8] working with a guinea pig model of wound infection, demonstrated the remarkable brevity of the "window" of prophylactic efficacy. They noted that antibiotics given shortly before or at the time of bacterial inoculation of the subcutaneous tissue of the guinea pig produced a notable diminution in the size of the subsequent wound induration compared with lesions in animals not receiving antimicrobial prophylaxis (Fig. 313.1). By delaying the administration of antibiotics by only 3 or 4 hours, resulting lesions were identical in size to those of animals receiving no antimicrobial prophylaxis whatsoever. Thus "failures" of antimicrobial prophylaxis that had been noted in earlier clinical studies were related to the fact that administration of preoperative antibiotics had been inappropriately timed.[9–11] Although these observations have been challenging to reproduce in vitro,[12] the experience of current practice has evolved to mandate that whenever possible surgical antimicrobial prophylaxis should be administered so as to ensure adequate tissue levels of antimicrobials from the time of the initial surgical incision until closure. The efficacy of prophylactic antibiotics has now been verified for most major surgical procedures, with a wide variety of antimicrobials when care has been given to provide adequate serum and tissue levels of antibiotics during the surgical procedure. Perioperative

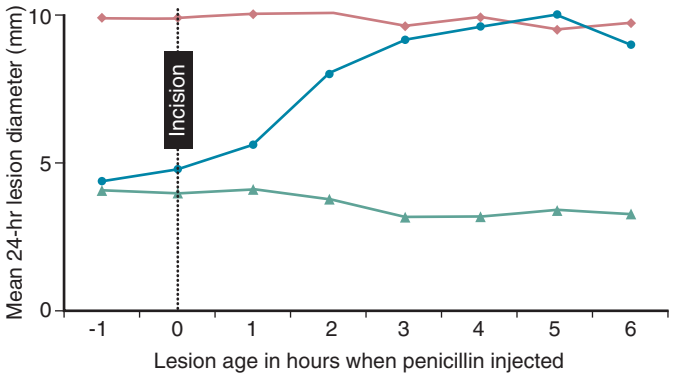

FIG. 313.1 Importance of the timing of antimicrobial administration. Relationship between the timing of antimicrobial administration and the effectiveness of prophylaxis as shown by the size of wound infection in a guinea pig model.[8] Lesion sizes were measured as mean diameter (mm) of induration developing 24 hours after intradermal inoculation of *Staphylococcus aureus* (SA). Red line indicates animals injected with live SA who received placebo; blue line indicates animals injected with live SA who received penicillin; green line indicates animals injected with killed SA.

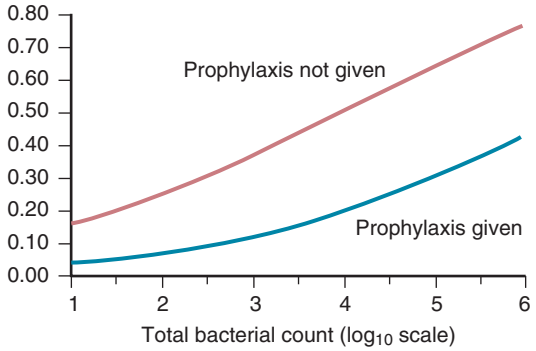

FIG. 313.2 Wound infection and febrile morbidity. Plot of the probability of wound infection, febrile morbidity, or both, according to total bacterial counts at the operative site in patients undergoing abdominal hysterectomy. The administration of antimicrobial prophylaxis reduces the likelihood of infection or fever for a given level of bacterial contamination of the wound. Stated another way, the administration of prophylaxis increases the magnitude of the bacterial inoculum needed to produce infection. *(From Houang ET, Ahmet Z. Intraoperative wound contamination during abdominal hysterectomy.* J Hosp Infect. *1991;19:181–189.)*

antibiotics and aseptic techniques have become routine aspects of care in most major surgical procedures.

Despite efforts to prevent surgical site infections (SSIs), these outcomes are not uncommon. The 2014 Healthcare-Associated Infection Prevalence study from the Centers for Disease Control and Prevention (CDC) estimated an annual national burden of more than 157,000 SSIs among hospitalized adult patients,[13] a figure that does not include those patients with an SSI that did not require hospitalization. Often neglected in these estimates, SSIs associated with ambulatory surgical procedures result in a substantial rate of postsurgical acute care visits for clinically significant SSIs in 4.8 per 1000 procedures across the eight states studied.[14] Another analysis estimated that SSIs led to an added $20,785 and 11.2 days length of stay per event and a total of $3.2 billion in attributable costs in the United States in 2008.[15] A patient who develops an SSI while hospitalized has a greater than 60% greater risk of being admitted to the intensive care unit, is 15 times more likely to be readmitted to the hospital within 30 days after discharge, and incurs an attributable extra hospital stay of 6.5 days, leading to a direct cost of an additional $3000 per infection.[16] SSIs due to methicillin-resistant *S. aureus* (MRSA), in particular, have also been shown to have a higher mortality than those due to methicillin-sensitive strains of the organism.[17]

Technologic advances (e.g., the introduction of minimally invasive procedures and robotic surgery) and the emergence of antibiotic-resistant organisms have led to additional challenges in the prevention of SSIs. In addition, the prevention of SSI has moved to the forefront of surgical quality improvement programs, highlighting important issues regarding antimicrobial prophylaxis (e.g., drug dosing in obese patients, the specific timing of antibiotic administration, and the role of anti-MRSA prophylaxis). Reflecting the increasing desire for transparency of infection prevention program effectiveness, public reporting of facility-specific colon and abdominal hysterectomy SSI performance is now tied directly to reimbursement.[18] Despite these advances over 150 years since the discoveries of Pasteur and Lister, much remains to be learned about the pathophysiology, prevention, and surveillance of SSIs.

PRINCIPLES OF SURGICAL SITE INFECTION PREVENTION

Determinants and Pathophysiology

Whether a wound infection occurs after surgery depends on a complex interaction between the following: (1) patient-related factors (e.g., host immunity, nutritional status, comorbid conditions); (2) procedure-related factors (e.g., implantation of foreign bodies, degree of trauma to the host tissues); (3) microbial factors (e.g., tissue adherence and invasion); and (4) use of preventive measures (e.g., perioperative antimicrobial prophylaxis).

TABLE 313.1 Classification of Operative Wounds by Level of Bacterial Contamination

Class I: Clean Wound
An uninfected operative wound in which no inflammation is encountered, and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow nonpenetrating (blunt) trauma should be included in this category if they meet the criteria.
Class II: Clean-Contaminated Wound
An operative wound in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination. Specifically, operations involving the biliary tract, appendix, vagina, and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered.
Class III: Contaminated Wound
Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (e.g., open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included in this category.
Class IV: Dirty-Infected Wound
Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation.

Modified from Mangram AJ, Horan TC, Pearson ML, et al. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol. *1999;20:250–278.*

Species and Sources of Wound Bacteria

Bacterial contamination of the surgical wound is inevitable. State-of-the-art aseptic technique has been associated with a dramatic drop in, but not the elimination of, this phenomenon. Even under laminar flow operating room (OR) environments, bacteria can be isolated from wound surfaces at the close of the surgical procedure.[19] The importance of the microbial load in determining whether or not a wound becomes infected has been long appreciated and is relevant even in the era of the routine administration of antimicrobial prophylaxis for most major surgical procedures (Fig. 313.2).[20] Historically, operations have been stratified on the basis of the expected quantity of bacteria introduced into the operative site during surgery (Table 313.1). Although the magnitude of bacterial inoculation into the wound still has some predictive value regarding the risk of developing a wound infection, patient- and procedure-related risk factors also contribute greatly to this risk (Table 313.2).[21]

TABLE 313.2 Selected Factors Associated With an Increased Risk for Surgical Site Infection

Patient Factors
Diabetes mellitus/perioperative hyperglycemia
Concurrent tobacco use
Remote infection at time of surgery
Obesity
Low preoperative serum albumin
Malnutrition
Concurrent steroid use
Prolonged preoperative stay[a]
Prior site irradiation
Colonization with *Staphylococcus aureus*
Procedural Factors
Shaving of site the night before procedure
Use of razor for hair removal
Improper preoperative skin preparation/use of non–alcohol-based skin preparation
Improper antimicrobial prophylaxis (wrong drug, wrong dose, wrong time of administration)
Failure to timely redose antibiotics in prolonged procedures
Inadequate operating room ventilation
Increased operating room traffic
Perioperative hypothermia
Perioperative hypoxia
Proceduralist Factors
Surgical technique (poor hemostasis, tissue trauma)
Lapses in sterile technique and asepsis
Glove micropenetrations
Behavioral factors/proceduralist impairment

[a]Likely a surrogate marker for severity of underlying illness and comorbidities.
Modified from Mangram AJ, Horan TC, Pearson ML, et al. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol. *1999;20:250–278.*

TABLE 313.3 Percentage of Surgical Site Infection Pathogen Isolates Resistant to Selected Antimicrobial Agents, National Healthcare Safety Network, 2014

PATHOGEN	NO. OF ISOLATES TESTED	PERCENT OF ISOLATES RESISTANT
Staphylococcus aureus		
Resistant to oxacillin/methicillin (MRSA)	8738	42.6%
Enterococcus faecium		
Resistant to vancomycin (VRE)	1342	58.4%
Escherichia coli		
Extended-spectrum cephalosporin resistant	6816	15.3%
Fluoroquinolone resistant	6816	30.9%
Carbapenem resistant	6816	0.7%
Multidrug resistant	6816	6.5%
Pseudomonas aeruginosa		
Extended-spectrum cephalosporin resistant	2617	9.9%
Fluoroquinolone resistant	2617	11.5%
Carbapenem resistant	2617	7.7%
Multidrug resistant	2617	4.3%
***Enterobacter* spp.**		
Extended-spectrum cephalosporin resistant	2056	27.5%
Carbapenem resistant	2056	3.4%
Multidrug resistant	2056	2.4%
***Klebsiella* spp.**		
Extended-spectrum cephalosporin resistant	2319	11.3%
Carbapenem resistant	2319	3.3%
Multidrug resistant	2319	4.6%
***Acinetobacter* spp.**		
Carbapenem resistant	174	33.3%
Multidrug resistant	174	32.9%

MRSA, Methicillin-resistant *Staphylococcus aureus; VRE,* vancomycin-resistant enterococci.
Modified from Weiner LM, Webb AK, Limbago B, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol. *2016;37:1288–1301.*

Numerous organisms have been described as wound pathogens, and the origin of the inoculum is not established with certainty for most infections. The patient's endogenous skin flora, with gram-positive organisms in general, and staphylococcal species in particular, are the predominant cause of incisional infections of clean surgical procedures. Over the past decade, the microbiology of SSIs has evolved due to the emergence of various multidrug-resistant pathogens, with a concerning proportion of reported SSIs due to multidrug-resistant gram-negative pathogens (Table 313.3).[22] *S. aureus* colonization of the patient's nares is a major risk factor for developing a *S. aureus* SSI, a particular concern in selected populations, such as diabetic individuals and recipients of hemodialysis, who have *S. aureus* colonization rates in excess of 50%. *S. aureus* and *Streptococcus epidermidis* and streptococcal wound infections can also occur in clusters, sometimes with a particular surgeon or nurse implicated in their spread. Unusual and hard-to-culture species, including nontuberculous mycobacteria, *Nocardia* spp., *Legionella* spp., *Mycoplasma hominis*, and *Cutibacterium (Propionibacterium) acnes* (an organism associated with shoulder arthroplasty SSI), have caused surgical site infections as well.

Modern methods of antisepsis can reduce but not eliminate the skin-associated bacteria of surgical patients. This limitation derives, in part, from the localization of up to 20% of skin-associated bacteria in skin appendages, such as hair follicles and sebaceous glands (Fig. 313.3).[23] Because these sites are beneath the skin's surface, bacteria residing there are not eliminated by topical antisepsis. Transection of these skin structures by surgical incision may carry the patient's resident bacteria deep into the wound and set the stage for subsequent infection.

For contaminated procedures, wound pathogens frequently are among the bacterial species that comprise the normal flora of the viscus entered during the surgical procedure. Enteric gram-negative pathogens and anaerobic bacteria (e.g., *Bacteroides fragilis*) are common pathogens of wounds after colonic procedures, and polymicrobial infections are common in this setting. Infection by a particular species, however, does not correlate directly with its quantitative presence among the normal flora, but by the particular virulence attributes of bacteria.

Although numerous sources of bacterial contamination of surgical wounds have been described, it is virtually impossible to identify with certainty the source(s) and route(s) of contamination. The direct inoculation of a patient's endogenous flora at the time of surgery is believed to be the most common mechanism; however, others undoubtedly occur. Transmission from contaminated surgical instruments or surgical material; hematogenous seeding from preexisting infection of a nonwound site; and contamination from either the skin, mucous membranes, or clothing of operating room staff have been implicated as potential sources of microbial contamination. Outbreaks of group A streptococcal wound infection have been traced to the anal or vaginal carriage of this organism by operating room personnel,[24,25] and epidemiologic investigation of these outbreaks indicated that airborne contamination of the operative field had occurred. Infections, including *Candida albicans* osteomyelitis and diskitis after lumbar laminectomy,

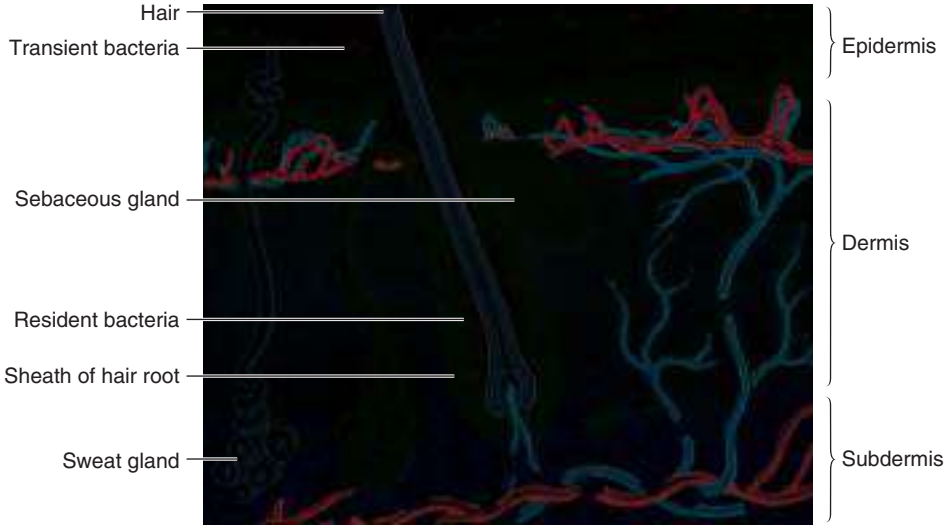

FIG. 313.3 Diagram of the skin. Diagram of the skin demonstrating the location of the transient bacteria on the skin surface, which are easily removed, and the deep resident bacteria, which cannot be destroyed by skin antiseptics. *(From Postlethwaite RW. Principles of operative surgery: antisepsis, technique, sutures, and drains. In: Sabiston DC, ed.* Davis-Christopher Textbook of Surgery. *12th ed. Philadelphia: Saunders; 1981:322.)*

have been traced to the use of artificial fingernails worn by surgical staff,[26] leading many hospitals to ban the use of artificial or long natural fingernails in health care workers with direct patient care responsibilities. A worldwide outbreak of SSI in cardiac surgery patients associated with aerosol dissemination of and surgical field contamination with *Mycobacterium chimaera* by heater-cooler units used intraoperatively illustrates the potential for airborne spread and development of SSI due to pathogens with low pathogenic potential, incubation periods of months to years, and the unintended consequences of new technologies used in the operating suite.[27]

The relative importance of hematogenous seeding (i.e., *inside-to-out*) of the surgical wound is somewhat unclear. Evidence of this route of seeding of the surgical wound site has been mainly noted with procedures involving implantation of prosthetic devices.[23] In addition, although it is generally accepted that prosthetic valves and hips are at risk for an indefinite period of time for hematogenous seeding and infection postoperatively, it is difficult to ascertain with certainty whether a late postoperative infection results from intraoperative bacterial seeding of the prosthetic device, followed by prolonged dormancy, or from a true postoperative hematogenous event. In a randomized, prospective, controlled study of antimicrobial prophylaxis in total hip replacement, Carlsson and coworkers[28] demonstrated that deep wound infections that developed over 2.5 years after surgery were more likely to have occurred among placebo versus cloxacillin recipients (13.7% vs. 3.3%, respectively; $P < .05$). These data suggest that bacteria inoculated into wounds at the time of surgery may lie dormant for years, rendering differentiation of the precise source of late wound infection virtually impossible. If late hematogenous seeding of a surgical wound with or without prosthetic material can occur, it is reasonable to assume that wounds are even more vulnerable to seeding and secondary infection during the immediate postoperative period. During this time, surgical incisions are hyperemic from the trauma of the surgery and endothelialization of intravascular prosthetic materials has not yet had time to occur. Moreover, the regular use of indwelling intravascular catheters probably increases the risk of bacteremia; however, information with which to judge the relative contribution of intraoperative versus postoperative hematogenous seeding of the surgical incision is unavailable.

Virulence Factors of Major Wound Pathogens

Clean Wound Infections

The requirement for large inocula in the early models of *S. aureus* soft tissue infection gave the misleading impression that cooperative interaction between bacteria may be required to establish a wound infection.[7,8] Later models involving foreign bodies demonstrated median infective dose (ID_{50}) values of fewer than 100 colony-forming units (CFU) with polytetrafluoroethylene (PTFE) tissue cages,[29] 10 CFU with PTFE vascular grafts,[30] and as low as 1 CFU with dextran microbeads.[31] These data demonstrate the pathogenic potential of a single bacterium to produce wound infection, provided that it is inoculated into a suitable niche.

Contaminated Wound Infections

The role of coliforms and anaerobes in abdominal sepsis has been elucidated in a model that involves inserting a gelatin capsule containing a standardized inoculum of pooled cecal contents into the peritoneal cavities of rats.[32,33] Acute peritonitis and septicemia from coliforms caused rapid death in 37% of the animals, and all of the survivors developed abscesses with anaerobes as the predominant organisms. The capsular polysaccharide of *Bacteroides fragilis* promotes abscess formation and may reduce phagocytosis. In experimental models, immunization against capsular polysaccharide can protect against abscess formation after inoculation with *B. fragilis* by a T-cell–dependent mechanism, except in the presence of foreign material.[34] Also, *B. fragilis* produces a variety of tissue-damaging enzymes, including hemolysin, chondroitin sulfatase, neuraminidase, and hyaluronidase.[35]

Wound Microenvironment and Operative Effects on Immunity

Much of our understanding of the pathophysiology of wound infection and the nature of the surgical wound derives from investigational models. Early investigations suggesting that the efficacy of antibiotics in preventing wound infection is limited to only a few hours after the moment of bacterial inoculation suggested that the wound microenvironment is not static.[6,7] It is likely that rapid changes are occurring among microbial factors, such as a shift from exponential to stationary-phase growth with an accompanying decrease in bacterial susceptibility to antibiotics and possibly the expression of different microbial virulence factors. Wound-related changes must also occur, such as gradually diminishing tissue perfusion and antibiotic delivery related to increased tissue oncotic pressure brought about by the effect of inflammatory mediators on vascular permeability.

Foreign Material and Operative Trauma to Tissue

Investigations of *S. aureus* infection in the skin of human volunteers by Elek and Conen[36] conclusively established the role of foreign material in potentiating wound infection. By including suture material with the intradermal staphylococcal inoculum, the number of organisms required to establish a skin pustule could be reduced 10,000-fold relative to lesions without sutures (i.e., a fall from 5×10^6 organisms to 3×10^2 organisms in the inoculum). These investigators further suggested that

"other circumstances may lead to the unhindered growth of small inocula, including heavily traumatized tissues, burns, or devitalized tissues distal to the ligated vessels. This may be the explanation of the traditional surgical view that untidy operative techniques predispose to infection."[36] Because in clean and clean-contaminated surgical procedures, quantitative bacterial inoculation into the wound is small, tissue devitalization at a gross or microscopic level provides a niche wherein a small bacterial inoculum may grow in relative isolation from the host's defenses, which plays a major role in the pathogenesis of infection.

Investigational models have demonstrated how technical variables of the surgical procedure influence the risk of infection. Some suture materials appear to have a stronger adjuvant effect on infection than others.[37] Whether the use of the electrosurgical knife, which can damage host tissues via the transfer of heat, is an adjuvant for infection is controversial.[38–40] Clinical studies examining the use of electrocautery have not revealed an increased risk for SSI with the use of this technique, with a recent Cochrane analysis noting a nonsignificant difference (7.7% vs. 7.4%) in wound infection rates between electrosurgery vs. scalpel.[41–43]

Effect of Operative Procedures on Systemic and Local Immunity

Operative procedures produce systemic and local changes in the immune defense mechanisms of the host. Neutrophil function and serum opsonizing capacity become impaired. The microbicidal activity of neutrophils obtained postoperatively from patients undergoing abdominal hysterectomy is 25% less than that of neutrophils harvested from the same patients preoperatively, and it takes 9 days to return to normal.[44] The depletion of opsonizing factors within the abscess milieu also may contribute to decreased neutrophilic bactericidal function.[45]

Major surgical procedures compromise the host defenses in other ways. Surface levels of human leukocyte antigen–DR antigens on the circulating monocytes of patients are reduced after major surgery.[46] However, it has been shown that defects in T-cell proliferation and cytokine secretion after major surgery involve an inability of T cells to respond to T-cell receptor- and CD28 coreceptor-mediated signals rather than problems with antigen presentation by monocytes-macrophages.[47] Many factors can reduce core body temperature during a surgical procedure, including ambient OR temperatures, lack of body covering, and intravenous (IV) fluids administration. Anesthetic agents also impair thermoregulatory control and may impact vasoconstriction and shivering thresholds.[48] In the setting of perioperative hypothermia, neutrophils have reduced chemotaxis, impaired ingestion of staphylococci, and diminished superoxide production.[49] Hypothermia can also trigger vasoconstriction and lead to low tissue oxygen tension, which is itself a risk factor for SSI.[50] Because of these risks, interventions aimed at reducing perioperative normothermia and hypoxia have been examined to prevent SSIs (see "Prevention of Surgical Site Infections").

Perioperative blood transfusion has been associated with an increased rate of postoperative infections, including wound infection, with donated white blood cell (WBC)-induced immunosuppression implicated as the culprit.[51] Decreases in cell-mediated immunity and increases in cytokine levels (interleukin [IL]-2 receptor, IL-6) have been demonstrated in mice and humans after transfusion.[52] However, clinical trials comparing the receipt of standard whole or buffy-coat–depleted blood products (which, despite the moniker, still retain some donor WBCs) with WBC-depleted products, or the administration of allogenic versus autologous blood, have failed to provide a consensus answer to transfusion's role in the development of postoperative infections.[53] A restrictive transfusion approach (transfusion once hemoglobin falls below 7–8 g/dL) was associated with a reduced risk for the development of health care–associated infections when compared with more liberal strategies, but only six studies included in this meta-analysis examined surgical wound infections as a study outcome.[54] Studies describing this potential transfusion effect have been criticized for using nonstandardized outcome definitions, failing to account for known risk factors for postoperative infection, and using only univariate analyses.[53] Blood transfusion may simply serve as a marker for unidentified patient comorbidities, and more rigorously designed studies are needed before general conclusions regarding the role of blood transfusion in wound infection can be determined.

In cardiac surgery the patient may be exposed to hypothermia, cardiopulmonary bypass (CPB), and relative arterial hypotension throughout much of the procedure. Exposure of blood to CPB depletes serum complement, causes systemic release of proinflammatory cytokines, and adversely affects neutrophilic function.[55–58] Furthermore, protein denaturation and chylomicron aggregation may contribute to small vessel occlusion and tissue hypoxia, as well as overwhelm the capacity of the reticuloendothelial system to clear infectious agents from the blood.[59] This raises the possibility that postbypass patients may be predisposed to develop infections via hematogenous bacterial seeding as a result of reduced reticuloendothelial clearance.

Risk Factors for Surgical Site Infections

Patient Factors

Various host factors have been associated with an increased risk of SSI (see Table 313.2), including underlying comorbid illnesses (e.g., diabetes mellitus),[60] malnutrition,[61] obesity,[62] history of irradiation at the site of the procedure,[63] and use of immunosuppressants.[64] Tobacco use has been associated with an increased risk for SSI,[65] possibly due to nicotine's effects of vasoconstriction, local hypoxia, and inhibition of wound healing.[65] As noted earlier, colonization with *S. aureus* can also increase one's risk for developing a staphylococcal SSI.

Although diabetes mellitus is a risk for SSI, acute fluctuations in glucose control are also important. In a prospective study of 1000 cardiothoracic surgery patients, hyperglycemia (serum glucose > 200 mg/dL) in the 48 hours postprocedure was associated with a 102% increase in the risk for wound infection.[66] Risk also increased incrementally with further elevations in glucose; however, the degree of long-term glucose control, as measured by glycosylated hemoglobin levels at time of surgery, did not impact infection risk. As a result of this and similar studies, glucose control during the perioperative period is now considered an important SSI prevention practice[67,68]; however, the appropriate glucose target and intensity of insulin therapy to maintain these targets are controversial,[69] and care must be taken to prevent hypoglycemia and its associated morbidity.[70]

Procedural and Proceduralist Factors

Attributes of the surgical procedure may lead to an increased risk for SSI, such as improper ventilation of the operative suite and uncovered and exposed sterile instrument trays.[71] Proper application of an antiseptic at the incision site is important, as is the specific type of preparation used. A randomized, placebo-controlled trial in patients undergoing clean-contaminated surgery found that the use of a chlorhexidine gluconate (CHG)-alcohol preparation reduced the risk of superficial and deep incisional SSI by 41% when compared with povidone-iodine.[72] Without an alcohol-based comparison product, it is unclear if this benefit was due to the CHG, to the immediate-killing effects of the alcohol, or to a combination of both. Recently updated SSI prevention guidelines from both the CDC and World Health Organization (WHO) recommend an alcohol-based antiseptic agent for surgical site preparation for all procedures unless contraindicated, but the CDC does not specify a preference for CHG-based agents,[67] whereas the WHO guideline recommends CHG-alcohol preferentially.[73] Microperforations of surgical gloves in the setting of no antimicrobial prophylaxis, increased OR traffic, and shaving with a razor (vs. use of clippers or no hair removal at all) have also been associated with an increased SSI risk.[74–78] Finally, failure to appropriately administer prophylactic antibiotics (correct drug, correct dose, correct timing before incision) is a major factor in the development of SSI (see discussion later in this chapter).

Perhaps as important as procedure-related factors and certainly more challenging to investigate are aspects germane to the surgeon and other proceduralists and their potential for increasing SSI risk. Variations in surgical technique have often been espoused as major drivers in the development of SSIs, but evidence supporting this supposition is limited.[40] Such surgeon-specific risk was suggested in a prospective cohort study of 2393 colon surgery patients in which specific surgeons were associated with an increased risk for SSI development in their patients after adjustment for patient-specific risk factors.[79] A study in Michigan examined the risk of surgical complications, including SSI, after bariatric surgery and identified a significantly increased risk in surgeries performed by

surgeons in the bottom quartile of surgical skill as rated by peers when compared with top quartile surgeons (complication rates of 14.5% vs. 5.2%).[80] Lapses in asepsis discipline (including improper skin preparation, operative room noise, incorrect wearing of personal protective equipment, and repetitive breaks in aseptic technique) have also been noted to significantly increase the risk for SSI in general surgical patients.[81] Patients whose surgeons have a large number of unsolicited patient complaints have a significantly increased risk of surgical complications, including SSI, attributed to dysfunctional interactions with the surgical team that may impede effective collaboration intraoperatively.[82] Surgeon impairment may also lead to an increase in SSI risk, nicely illustrated by a case report of an increased SSI rate in a physician affected by the systemic side effects of treatment for hepatitis C infection.[83]

Postoperative Factors

Finally, postoperative factors may play a role in the pathophysiology of SSI. Nicely articulated in a review by Manian,[84] these factors have not been routinely considered in SSI risk factor studies or prevention bundles. Wound-related factors, such as hematoma, incision oozing, the presence of drains, and closure type have been associated with SSI risk. Hematogenous spread from endovascular sources has been suggested in an analysis of postcardiac surgical SSI and central venous catheter (CVC)-related bacteremia based on similar pathogen isolation in patients with both infections.[85] An analysis of closure type in orthopedic surgery noted a three to four times greater risk for SSI when staples were used instead of sutures for wound closure.[86] A Cochrane analysis examining the effect of various wound dressings (vs. no dressing) on SSI risk prevention, however, found no clear benefit from the use of a variety of dressing types (including film, hydrocolloid, and silver-containing products).[87] Further examination of such postprocedural interventions are needed to better quantify their potential impact on SSI risk.

PREVENTION OF SURGICAL SITE INFECTIONS

General Concepts

Given the presence of some of the risk factors noted earlier that may be unalterable, it is unlikely that all SSIs are preventable. Therefore the goal should be to eliminate all *potentially preventable* infections through the use of evidenced-based processes. Stemming from the concepts of wound infection pathogenesis and the risk factors noted earlier, a number of such interventions exist to reduce SSI risk. These interventions can be grouped into two major categories (Table 313.4). The first line of defense involves measures that reduce bacterial inoculation into the wound site. These include familiar practices, such as the application of antiseptics to the incision site, the washing and gloving of OR personnel's hands, the use of sterile drapes, strict adherence to sterile technique, airflow control, and the use of appropriate attire by OR personnel.

Efforts to reduce patient colonization with pathogenic bacteria, especially staphylococci, with topical antiseptic agents, such as intranasal mupirocin and povidone-iodine, may also be of benefit. A randomized, placebo-controlled trial found that mupirocin applied to the nares of patients undergoing elective cardiothoracic, neurosurgical, oncologic, gynecologic, and general surgical procedures, beginning on the day before surgery and continued for up to 5 consecutive days, resulted in a reduction in *S. aureus* nosocomial infections from 7.7% to 4.0% in those with preoperative nasal carriage of *S. aureus.* However, the rates of nosocomial and, specifically, SSIs in all patients, regardless of *S. aureus* carriage status, were not reduced with the use of mupirocin.[88] Another large multicenter trial noted a significant reduction in deep SSI risk with the use of a protocol to screen for *S. aureus* nasal carriage and subsequent decolonization of carriers with intranasal mupirocin and CHG bathing (relative risk [RR] = 0.21 when compared with placebo recipients).[89] In one of the largest investigations of preoperative decolonization, involving more than 42,000 procedures at 20 hospitals, *S. aureus* deep and organ/space SSIs were significantly decreased among patients colonized with the organism with the use of intranasal mupirocin and CHG 5 days before cardiac and joint arthroplasty surgery.[90] Antimicrobial prophylaxis was also adjusted to cover MRSA in patients with MRSA colonization. Preoperative CHG bathing has also been examined as an SSI prevention strategy for use with all surgical patients (not just those colonized with *S. aureus*). A meta-analysis of eight trials encompassing more than 10,000 patients undergoing clean surgery, however, did not find a significant impact of universal CHG bathing on SSI rates.[91] Such findings may be due to the removal of the CHG during or soon after showering, which may minimize CHG's beneficial quality of prolonged bacterial killing.

For preoperative hair removal, methods that do not create microabrasions, such as clippers or depilatories, are preferred.[78] This recommendation is supported by a large randomized trial of 1013 patients in which SSIs occurred in 3.2% of patients after hair removal with clippers the morning of surgery versus 10.0% in those who underwent day-of-surgery shaving with a razor.[92] Although some recommendations advocate no preoperative hair removal,[21] there is minimal evidence to support an increased risk of wound infection with hair removal by clippers or depilatories on the morning of surgery compared with no hair removal.[93] Avoidance of hair removal is particularly challenging in cranial procedures, where hair is often removed to prevent wound contamination, allow for incision visualization, and aid in dressing application. A systematic review questioned this practice, noting scalp shaving does not protect against SSI development and may result in higher infection rates.[94] Infection at sites remote from the operative field is a host risk factor for postoperative infection that is potentially correctable before surgery.[95] Drains and intravascular devices should be removed as quickly as possible to avoid the risk of direct and hematogenous seeding of the operative site, as well as device-associated infections.

The second major class of prevention measures is directed toward improving host containment and elimination of bacteria that have

TABLE 313.4 Surgical Site Infection Prevention Interventions

Maneuvers to Diminish Inoculation of Bacteria Into Wound
Preoperative Factors
Avoid preoperative antibiotic use (excluding surgical prophylaxis)
Minimize preoperative hospitalization
Treat remote sites of infection before surgery
Avoid shaving or razor use at operative site
Delay hair removal at operative site until time of surgery and remove hair (*only* if necessary) with electric clippers or depilatories
Ensure timely administration (including appropriate dose) of prophylactic antibiotics
Consider elimination of *Staphylococcus aureus* nasal carriage via decolonization techniques (especially in cardiac and arthroplasty procedures)
Use standardized checklist for implementation at preprocedural time-out
Intraoperative and Postoperative Factors
Carefully prepare patient's skin with antiseptic + alcohol-based skin preparation agent (e.g., povidone-iodine-alcohol– or chlorhexidine-alcohol–containing solution)
Rigorously adhere to aseptic techniques
Isolate clean from contaminated surgical fields (e.g., reglove, regown, and change instruments after bowel resection before commencing fascial and skin closure)
Maintain high flow of filtered air
Redose prophylactic antibiotics in prolonged procedures
Minimize operative personnel traffic
Minimize immediate-use steam sterilization of surgical instruments
Minimize use of drains
Bring drains, if used, through a separate stab wound
Maneuvers to Improve Host Containment of Contaminating Bacteria
Preoperative Factors
Resolve malnutrition
Discontinue tobacco use for at least 30 days preoperatively
Maximize diabetes mellitus control
Intraoperative and Postoperative Factors
Minimize dead space, devitalized tissue, and hematomas
Use supplemental oxygen therapy in patients with normal pulmonary function undergoing general anesthesia with endotracheal intubation
Maintain perioperative normothermia (core temperature at or above 36.0°C)
Identify and minimize hyperglycemia via intensive insulin protocols in diabetic and nondiabetic patients
Maintain adequate hydration and nutrition

circumvented the front line of defense and have been inoculated into the wound. Most authorities have emphasized that the single most important factor in preventing wound infection is surgical technique. Gentle handling of wound tissues, avoidance of dead space, devitalized tissues, and hematomas, and careful approximation of tissue planes are believed to be critical in maintaining an infection-free incision. Prophylaxis with antimicrobial therapy is another essential component of SSI prevention, which is highlighted in detail later in this chapter.

Several strategies, first noted to benefit patients undergoing colorectal surgery, have now been broadly recommended for most adult surgical patients in both the 2016 WHO and 2017 CDC prevention guidelines.[67,68] Active measures to maintain intraoperative normothermia (often defined as a core temperature at or above 36°C) have been associated in multiple studies with a reduction in SSIs compared with patients allowed to experience routine mild perioperative hypothermia.[96,97] Given the acute impact of anesthetic agents on thermoregulation, the most vulnerable period for hypothermia is likely the start of the surgical procedure, highlighting the importance of prewarming surgical patients as opposed to waiting until induction of anesthesia.[48,98]

In colorectal patients, administration of supplemental oxygen (fraction of inspired oxygen [F_{IO_2}], 80%) resulted in significantly lower SSI rates (5.2% vs. 11.2% in those receiving 30% F_{IO_2}).[99] A second randomized, double-blind trial of patients undergoing major intraabdominal surgical procedures randomized to receive either 80% or 35% F_{IO_2} intraoperatively, however, found that the incidence of infection was significantly *higher* in those patients who received the higher oxygen concentration (25.0% in the 80% F_{IO_2} group vs. 11.3% in the 35% F_{IO_2} group).[100] Subsequent trials, some using nitrous oxide in the control arm, have noted either reductions or no change in SSI risk with supplemental oxygen.[101] Of importance, Meyhoff and colleagues[102] did not find a significant increase in pulmonary complications, such as atelectasis or pneumonia in those receiving 80% FiO_2. Provision of perioperative supplemental oxygen (specified as 80% F_{IO_2} in WHO guideline) is now recommended for all adult surgical patients with normal pulmonary function undergoing general anesthesia with endotracheal intubation.[67,68]

Intensive glucose control via continuous insulin infusion (CII) targeted to maintain glucose levels less than 200 mg/dL during the perioperative period (lasting through the second postoperative day) was first shown to reduce SSI in cardiac surgery patients.[103] When compared in a study of more than 2400 diabetic patients, intensive glucose control with CII led to a reduction in the incidence of deep wound infection in diabetic cardiac surgery patients from 2.0% to 0.8% ($P = .01$).[104] Intensive glucose control is now recommended for both diabetic and nondiabetic adult surgical patients, although there is not consensus on delivery method and target glucose level.[67,68]

Perioperative Antimicrobial Prophylaxis

The in vivo interaction between inoculated bacteria and prophylactically administered antibiotics is one of the most important determinants of the fate of the wound. For example, without antimicrobial prophylaxis the reported risk of developing a *S. aureus* SSI after cardiac surgery is 21% to 44%.[105,106] Through a multitude of clinical trials, the efficacy of antimicrobial prophylaxis in clean and clean contaminated procedures has been clearly established. The guiding principle of systemic antimicrobial prophylaxis is the belief that antibiotics in the host tissues augment natural immune defense mechanisms and help to kill bacteria that are inoculated into the wound. The rationale for the administration of oral antibiotics in colonic surgery differs in that, although some agents exhibit systemic absorption and penetrate into host tissues (e.g., erythromycin and metronidazole, but not neomycin), the primary goal in this setting is a reduction in potential pathogens among the normal intestinal flora at the time of surgery. Oral prophylaxis is generally combined with mechanical preparation of the bowel to reduce colonic flora (see discussion later).

Every effort should be made to ensure that adequate antibiotic levels are maintained above the minimal inhibitory concentration (MIC) of the pathogens of concern throughout the surgical procedure (Fig. 313.4). Although prolonged surgical procedures are associated with a higher infection rate, it is not clear whether this increased risk is inevitable or primarily attributable to the greater likelihood of there being low or undetectable tissue concentrations of antibiotics during long procedures. In cardiothoracic procedures in particular, the use of cardiopulmonary bypass (CPB) can dramatically alter antibiotic concentrations, either increasing or decreasing drug levels as a result of factors such as drug sequestration within the CPB circuit. In addition, CPB can increase in the volume of distribution, in part due to the priming of the CPB circuit with crystalloid and possibly systemic inflammatory responses related to CPB.[107] Some have advocated for an additional dose of cefazolin given at the time of CPB institution or continuous cefazolin infusion to address the challenge of dosing with CPB.[108,109] Understanding the pharmacokinetics of the various antimicrobials used in perioperative prophylaxis is vital to ensure adequate antibiotic levels at the surgical wound site during the entire procedure.

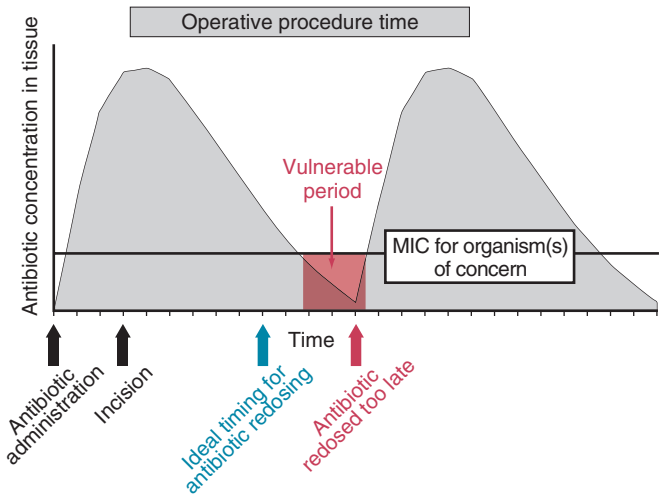

FIG. 313.4 Tissue antibiotic concentration over time. Dynamics of tissue antibiotic concentration during the course of a surgical procedure. After an initial dose of antibiotic (noted on the far left of the *x* axis), tissue concentrations reach their peak rapidly *(black arrows),* with a subsequent decline over time. As illustrated, the goal of antibiotic prophylaxis is to have tissue concentrations above the minimal inhibitory concentration *(MIC)* for the specific pathogens of concern at the time of the incision and throughout the procedure. Antibiotics should be redosed in prolonged procedures to prevent a period with tissue levels less than the MIC *(blue arrow).* Failure to redose antibiotics appropriately *(red arrow)* may result in a period during which the wound is vulnerable.

The efficacy of antimicrobial prophylaxis in preventing SSI after many surgical procedures is unquestioned. Not only have the benefits of early antibiotic administration been duplicated by numerous investigators using different animal models, different pathogens, and different antibiotics, literally hundreds of clinical trials have verified the efficacy of perioperative antibiotics. Nevertheless, nuanced issues regarding the optimal choice, frequency, and duration of perioperative antimicrobial prophylaxis remain.

Antimicrobial Prophylaxis: Drug Selection and Dosing

Basic Principles

Antimicrobial prophylaxis should be used for all clean-contaminated procedures and certain clean procedures, that is, those in which intravascular prosthetic material or a prosthetic joint will be inserted and those in which an incisional or organ/space SSI would pose catastrophic risk.[110] Dirty or contaminated procedures usually do not require specific antimicrobial prophylaxis because patients undergoing these procedures are already on targeted antimicrobial therapy for established infections; however, if the treatment regimen does not adequately cover all pathogens of concern, consideration should be given to providing additional prophylaxis, that is, in a procedure with a high incidence of MRSA SSI, additional prophylaxis with vancomycin may be warranted if the treatment regimen does not include MRSA coverage. The use of antimicrobial prophylaxis for all clean procedures, however, is less clear. For some clean, minimally invasive procedures with low risk for SSI,

TABLE 313.5 Typical Microbiologic Flora and Recommended Antimicrobial Drugs for Surgical Prophylaxis for Commonly Performed Surgical Procedures in Adults

PROCEDURE	TYPICAL MICROBIOLOGIC FLORA[a]	RECOMMENDED ANTIMICROBIALS
Cardiac	*Staphylococcus aureus*, CoNS, (GNR less common)	
Coronary artery bypass		Cefazolin, cefuroxime; addition of vancomycin if patient is colonized with *S. aureus*
Cardiac device insertion (e.g., pacemaker)		Cefazolin, cefuroxime
Ventricular assist device placement		Cefazolin, cefuroxime
Thoracic	*S. aureus*, CoNS	Cefazolin, ampicillin-sulbactam
Gastroduodenal (involving entry into lumen of gastrointestinal tract or without entry into lumen in high-risk patients)	Coliform GNR, streptococci, staphylococci	Cefazolin
Biliary	GNR (less commonly, anaerobes and enterococci)	
Open		Cefazolin, cefoxitin, cefotetan, ceftriaxone, ampicillin-sulbactam
Laparoscopic, high risk		Cefazolin, cefoxitin, cefotetan, ceftriaxone, ampicillin-sulbactam
Appendectomy	GNR, anaerobes	Cefoxitin, cefotetan, cefazolin + metronidazole
Colorectal	GNR, anaerobes (especially *Bacteroides fragilis* and *Escherichia coli*)	Cefazolin + metronidazole, cefoxitin, cefotetan, ampicillin-sulbactam, ceftriaxone + metronidazole, ertapenem; IV agent used along with mechanical bowel preparation and oral antimicrobial (neomycin sulfate + erythromycin base or neomycin sulfate + metronidazole)
Neurosurgery (craniotomy, CSF shunting, intrathecal pump implantation)	*S. aureus*, CoNS	Cefazolin
Cesarean section	*S. aureus*, streptococci, enterococci, vaginal anaerobes	Cefazolin
Hysterectomy (vaginal or abdominal)	*S. aureus*, streptococci, enterococci, *E. coli*, vaginal anaerobes	Cefazolin, cefoxitin, cefotetan, ampicillin-sulbactam
Orthopedic	*S. aureus*, CoNS, GNR (*Propionibacterium* spp. in shoulder procedures)	
Clean procedure of hand, knee, foot without implantation of foreign materials		None
Spinal procedures, hip fracture repair, internal fixation procedure, total joint arthroplasty		Cefazolin; addition of vancomycin if patient is colonized with *S. aureus* (arthroplasty)
Urologic	GNR (*E. coli*—increasing fluoroquinolone resistance noted), rarely enterococci	
Lower tract instrumentation (includes transrectal prostate biopsy)		Ciprofloxacin,[b] trimethoprim-sulfamethoxazole
Clean procedure (with or without entry into urinary tract		Cefazolin (single-dose aminoglycoside may be added for placement of prosthetic material)
Clean contaminated		Cefazolin + metronidazole, cefoxitin
Vascular	*S. aureus*, CoNS	Cefazolin

[a]Staphylococci will be associated with surgical site infections after all types of operations.
[b]Increasing reports of fluoroquinolone-resistant *E. coli* infections in postprostatic biopsy patients warrants careful monitoring of patients and infection surveillance to assess appropriate prophylactic antibiotic choice
CSF, Cerebrospinal fluid; *CoNS,* coagulase-negative staphylococci; *GNR,* gram-negative rods/bacilli; *IV,* intravenous.
Modified from Bratzler DW, Dellinger EP, Olsen KM, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. Am J Health Syst Pharm. *2013;70:195–283.*

concerns have been raised that the benefit of prophylaxis is not clear or may be outweighed by the potential risks of antimicrobial therapy. For example, a 2017 randomized controlled trial among patients undergoing removal of orthopedic implants used for below-the-knee fractures found a nonsignificant difference in SSI rates with the use of preoperative cefazolin versus saline placebo, although the study appears underpowered and did show a marked 2.5% reduction in deep SSIs.[111] Adequately powered randomized clinical trials of antimicrobial prophylaxis in such procedures are limited because of the need for very large sample sizes to demonstrate a significant reduction in SSI.[112] A 2009 meta-analysis noted that the risk reduction from antimicrobial prophylaxis is similar in all types of wounds, including clean, and that the assumption that prophylaxis is ineffective in a specific procedure unless proven otherwise should be revised.[113]

The key factors in selecting an appropriate prophylactic antibiotic regimen include coverage against the expected endogenous flora at the surgical site (Table 313.5), consideration of patient allergies and antimicrobial costs, knowledge of the ecology of local nosocomial wound pathogens, consideration of antibiotic penetration into the specific surgical site tissue, drug availability, and assurance of appropriate antibiotic dosing and delivery. Based on prospective studies of antibiotic prophylaxis, prophylactic regimens have been recommended for a wide variety of surgical procedures, and in 2013, a consensus guideline on surgical antimicrobial prophylaxis from the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, the Surgical Infection Society, and the Society for Healthcare Epidemiology of America was released, helping to further harmonize recommend practices (see Tables 313.5 and 313.6).[110] Although only some regimens have been tested in controlled clinical trials, others with similar antimicrobial coverage and tissue penetration should provide similar protection, provided dosing guidelines are followed. In addition, appropriate assessment of a patient's antibiotic allergy can have SSI ramifications. In an analysis of more than 8000 surgical patients, those who reported a penicillin allergy had a significantly increased odds of SSI, thought to be due to receipt of second-line prophylactic antibiotics that may not cover as wide a spectrum of pathogens.[114] Because many who report penicillin allergy are not truly allergic, this study highlights the need to clarify a patient's allergy status as part of routine perioperative care.

Based on their antibacterial spectrum and low incidence of side effects, the cephalosporins (in particular, cefazolin) have been the traditional drugs of choice for the vast majority of operative procedures. Even in clean-contaminated procedures, such as hysterectomy and cholecystectomy, in which cephalosporins with improved in vitro activity against anaerobic bacteria are often advocated, clinical studies indicate that

cefazolin is equivalent in its prophylactic efficacy. For procedures in which anaerobic coverage is also justified (e.g., distal gastrointestinal tract, major head and neck, biliary, and gynecologic procedures) addition of metronidazole or other agents with anaerobic activity (e.g., cefoxitin) are appropriate selections for prophylaxis.[110]

Drug Selection With Rising Incidence of Antibiotic-Resistant Pathogens (e.g., MRSA)

It should not be presumed that cephalosporins will remain the prophylactic agents of choice. Various classes of antibiotics have been shown to differ appreciably in activity against bacteria regarding the stationary phase of growth, postantibiotic effect, diffusibility into devitalized tissue or fibrin clots, resistance to enzymatic degradation, activity within abscesses, and penetration of and activity within neutrophils that may have ingested but be unable to kill wound bacteria. Each of these variables may affect the efficacy of an agent used for prophylaxis, and it is likely that preferred prophylactic regimens will change over time in response to an improved understanding of the pathophysiology of infection and to antimicrobial resistance among wound pathogens.

Because cephalosporins are the mainstay of prophylaxis, the increasing prevalence of cephalosporin-resistant pathogens has important implications for prophylaxis.

In addition, multidrug-resistant organisms (MDROs) have increasingly become important pathogens in the postsurgical patient.[22] The impact of the increase of MDROs is illustrated by reports of an increase in infectious complications after transrectal ultrasound-guided prostate biopsy attributed in part to organisms resistant to fluoroquinolones, leading to a reevaluation of the use of these agents as routine prophylaxis for this procedure.[115] The arrival of these MDROs portends an ominous change in the complexity of the ecology of nosocomial infections and a need to assess the epidemiology of pathogens causing SSIs, to ensure appropriate coverage as a part of surgical prophylaxis. An examination from the CDC of pathogens causing SSI after coronary artery bypass graft (CABG) and joint arthroplasty surgery noted that between 55% and 59% were not susceptible to standard prophylactic agents;[116] however, the overall SSI rates were very low, indicating that a large majority of potential SSI pathogens were covered by standard regimens, leading some to argue that expansion of regimens may be inappropriate and unsafe.[117]

Methicillin-Resistant *S. aureus*–Specific Prophylaxis

In particular, the emergence of community-acquired (CA)-MRSA as a cause of SSI has clouded the issue of appropriate antimicrobial prophylaxis.[118] Previous recommendations suggested consideration of using vancomycin as empirical therapy for treatment and prophylaxis of presumptive staphylococcal infection when the local rates of MRSA are high; however, the exact threshold at which vancomycin use should occur was not clearly defined. Routine administration of vancomycin for surgical prophylaxis is not recommended.[110] A randomized trial of 855 cardiac surgical patients comparing routine use of vancomycin versus cefazolin for prophylaxis noted that, although the rate of MRSA SSI was higher in the cefazolin group, those who received vancomycin had a higher rate of methicillin-sensitive *S. aureus* (MSSA) SSI. Thus there was no significant difference in overall SSI rates between the two groups.[119] An interrupted time series analysis of more than 6000 cardiac surgery patients, however, found that in CABG patients, after a switch from cefazolin to vancomycin, the monthly SSI rates decreased by 2.1 per 100 procedures, primarily because of a decrease in SSI due to vancomycin-sensitive pathogens (e.g., MRSA).[120] This decline was significant when compared with rates in patients undergoing valve replacement and who had received routine prophylaxis with vancomycin during the entire study period, suggesting that other infection prevention interventions performed during the study period in both populations may not explain the reduction in the CABG patients.[120]

Practical limitations that may affect the use of vancomycin in surgery include its narrow spectrum of antimicrobial activity (less effective against MSSA) and the need for a slower rate of infusion. Failure to adequately dose vancomycin, even when indicated, can also lead to failure of antimicrobial prophylaxis, as illustrated by an assessment of joint arthroplasty patients who received vancomycin prophylaxis in which a higher SSI rate was noted in those receiving vancomycin alone (odds ratio, 1.58, vs. cefazolin prophylaxis).[121] Strikingly, only 28% of patients received an adequate dose of vancomycin based on weight-based dosage recommendations. Furthermore, the growing prevalence of vancomycin-resistant enterococci and the emergence of vancomycin-resistant *S. aureus* raise concerns about potential adverse effects on the antimicrobial susceptibility of nosocomial pathogens induced by the selective pressure of surgical antimicrobial prophylaxis.[122] A risk-based approach in which persons with specific risk factors for MRSA (e.g., history of recurrent furunculosis, prior MRSA infection, history of dialysis, or recent health care exposure) may help limit vancomycin use to those at higher risk of developing MRSA SSI. The Society of Thoracic Surgeons recommends use of vancomycin with cefazolin in persons with a known history of MRSA colonization, those undergoing placement of a prosthetic valve or vascular graft, and those "susceptible to colonization" (e.g., hospitalized longer than 3 days, transfer from an inpatient facility, or on antibiotics at time of procedure).[123] CA-MRSA complicates these recommendations, however, because healthy patients with few of these traditional comorbidities and risk factors may be at increased risk of MRSA colonization and subsequent infection. The addition of cefazolin to vancomycin is important because vancomycin appears less effective than cefazolin against MSSA, and cefazolin will provide some coverage against gram-negative organisms.[110]

Screening of patients for nasal carriage of MRSA before surgery, followed by adaptation of prophylactic regimens to include anti-MRSA coverage in carriers (± decolonization), has been used as a "bundled" strategy to address the growing issue of MRSA SSI.[90,124] Increasingly, the use of an MRSA-active agent such as vancomycin, in combination with an agent more active against MSSA, in patients known to be colonized with this organism or at high risk for colonization (e.g., recent hospitalization, nursing home residence, hemodialysis patient) is recommended rather using a specific incidence-rate threshold.[110,125] Patients undergoing elective surgical procedures could be screened during the initial preoperative evaluation, but clarifications of other issues related to screening (i.e., need for a second screening test to identify intermittent MRSA carriers, type of screening test to be used, and cost-effectiveness of screening in the presurgical population) must be determined.

Special Considerations With Prophylaxis in Colorectal Surgery

For colorectal procedures, the following three approaches to antimicrobial prophylaxis have generally been used: (1) use of oral (often nonabsorbable) agents, (2) use of IV agents, and (3) combination therapy using both types of agents.[126] Many surgeons use combination IV and oral therapy, with the rationale to decrease intraluminal flora and to provide adequate subcutaneous tissue concentrations. A Cochrane review has helped clarify the question of optimal antimicrobial prophylaxis before colorectal surgery; this analysis included 260 trials with more than 43,000 total subjects and noted a significant reduction in surgical infection with the use of combination oral and IV prophylaxis versus IV prophylaxis alone (RR, 0.56; 95% CI, 0.43 to 0.74) and versus oral prophylaxis alone (RR, 0.56; 95% CI, 0.40 to 0.76),[127] a finding mirrored in an analysis of the Veterans Affairs Surgical Quality Improvement Program data (SSI rate 6.3% with IV and oral vs. 16.7% wit h IV alone).[128] The addition of oral antimicrobials may increase the risk of development of nausea and vomiting, and patients should be advised of these symptoms.[110] Mechanical bowel preparation (MBP) to cleanse the bowel lumen before incision has also been used for some time in colorectal surgery patients, but there has been debate regarding the optimal approach and utility of MBP, with some studies noting a higher rate of anastomotic leakage with MBP use.[129,130] A retrospective propensity analysis of colectomy patients at Michigan hospitals participating in a statewide surgical collaborative noted that MBP in conjunction with oral antimicrobials resulted in significantly lower rates of SSI when compared with MBP without oral antimicrobial.[131] A larger study within the National Surgical Quality Improvement Program found that MBP with oral antibiotics was significantly associated with lower rates of anastomotic leak, SSI, and postoperative ileus.[132] The 2013 consensus guideline on surgical prophylaxis now recommend use of MBP combined with oral and IV antimicrobials for most colorectal procedures.[110]

Dosing in Obese Patients

The use of higher doses of antibiotic is needed for obese patients. After administration of a 1-g dose of cefazolin, tissue and serum concentrations of the antibiotic were significantly decreased in morbidly obese patients when compared with nonobese controls.[133] A higher (2-g) dose of cefazolin did provide tissue levels greater than the MIC for the most likely infecting pathogens. Another study of obese patients given 2-g doses of cefazolin, however, found therapeutic tissue levels of the drug in only 48% of persons with a body mass index (BMI) between 40 and 49, 29% in those with a BMI between 50 and 59, and 10% in those with a BMI 60 or higher, leading the authors to propose using continuous cefazolin administration in the morbidly obese patient to improve tissue concentrations.[134] The 2013 consensus guideline notes that, given the low cost and favorable safety profile of cefazolin, using a 2-g dose for patients weighing 80 kg and a 3-g dose for those greater than 120 kg is justified; however, the optimal approach for weight-based dosing of other prophylactic antibiotics, including whether to use actual versus ideal body weight, has not been determined.[110]

Antimicrobial Prophylaxis: Timing of Administration and Redosing

Initial Dose Timing

The initial dose of systemic antibiotics must be administered in a timely fashion so that antibiotic levels in the tissue at the time of the incision are adequate. Administration too early before or too late after the time of incision will result in suboptimal tissue levels and potentially increased risk of postoperative wound infection. Guidelines and studies have traditionally varied on the exact timing, ranging from 2 hours to no more than 30 minutes before incision, but the 2013 consensus guideline defines appropriately timed antimicrobial prophylaxis as delivery of the antibiotic within 60 minutes before incision, with the exception that vancomycin and the fluoroquinolones should be given within 120 minutes before incision because of the need for a longer infusion time.[110] This definition has become widely used as a metric that indicates delivery of standard, high-quality surgical care.[135] One exception to this recommendation is the 2016 WHO SSI prevention guideline, which argued that, although evidence supported an increased SSI risk with administration after incision or earlier than 120 minutes before incision, the evidence supporting prophylactic antibiotic administration within 60 minutes before incision was not strong enough to make a firm recommendation.[73]

Several large-scale studies have examined the relationship between the timing of delivery of antimicrobial prophylaxis and the risk of SSI. The seminal study by Classen and colleagues[9] noted that SSI risk was reduced when antibiotics were administered within 2 hours before incision (Fig. 313.5A). More recently, the Trial to Reduce Antimicrobial Prophylaxis Errors (TRAPE) examined the association between SSI and timing of prophylaxis in cardiac, orthopedic, and hysterectomy patients.[136] The TRAPE investigators found that SSI risk was lowest in those patients who received prophylaxis within 30 minutes (if given cephalosporins) or within 1 hour (if given vancomycin or a fluoroquinolone) before incision (see Fig. 313.5B). Postincision administration was associated with a significantly increased risk for SSI. These results have also been reproduced in a study of 1922 patients undergoing total hip arthroplasty in which the rate of SSI was lowest in those who received antibiotics 1 to 30 minutes before incision.[137] A large retrospective study using data from the Veterans Administration system noted a higher risk for SSI with dosing more than 60 minutes before incision, but a significant difference was not noted with antibiotics dosed after the incision. This effect was also lost with adjustment for patient, procedural, and antibiotic variables; however, in this study a majority of patients had prophylactic antibiotics dosed within 60 minutes, there was no consideration for the longer dosing window needed for vancomycin and quinolone agents, and there may have been confounding by procedures with an infection present at the time of surgery.[138]

Attempts have been made to further refine the dosing window, although with decreasing SSI rates the power necessary to define such timing with more granularity is becoming increasingly challenging. There may be a threshold where, even though penetration into tissue for many drugs occurs in minutes, administration of antibiotic right before the incision may not provide enough time for tissue concentrations of the drug to reach the desired level at the time of incision. A study in more than 28,000 cardiac surgery patients noted an increased risk of infection if cefuroxime was given more than 45 and more than 60 minutes preincision and a reduced risk if the drug was given within 15 minutes before incision. This effect differed with use of vancomycin, with the lowest rate of infection occurring with administration less than 45 minutes preincision, likely reflecting the need for a more prolonged infusion of this agent.[139] Weber and colleagues[140] noted a twofold increased odds of SSI when cefuroxime prophylaxis was delivered less than 30 minutes before incision, as opposed to between 30 and 59 minutes preincision. Curiously, when examined with more granularity, the risk was higher in those who received the antibiotic between 15 and 29 minutes versus those who received a dose between 0 and 14 minutes preincision. A study of vancomycin use in cardiac surgery patients found that prophylaxis was most effective when given between 16 and 60 minutes before incision (RR, 7.8; compared with receipt between 15 and 0 minutes preincision).[141] This may be explained by the need for an hour-long infusion of vancomycin to prevent infusion-related side effects, suggesting that a small proportion of the dose had been infused at the time of the incision. What is curious, however, is that those given a dose between 16 and 60 minutes preincision may not have had complete infusion of the drug dose, but perhaps enough to allow adequate tissue penetration.[139] In another analysis the lowest risk for SSI among general surgical patients was determined with administration of antibiotics (not including vancomycin) at 4 minutes before incision (95% CI, 0 to 18 minutes).[142] Countering these studies,

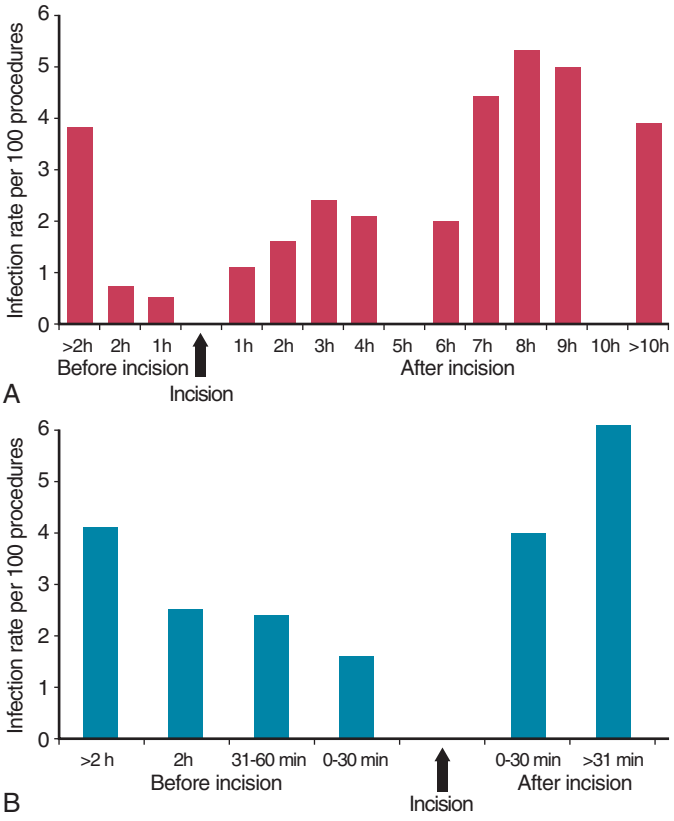

FIG. 313.5 Timing of administration and infection rate. Relationship between timing of administration of prophylactic antibiotics and surgical site infection rate from two large studies. (A) Data from 2847 elective surgical patients. (B) Data from 3656 cardiac, orthopedic, and gynecologic surgical patients. *(A from Classen DC, Evans RS, Pestotnik SL, et al. The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection.* N Engl J Med. *1992;326:281–286; and B from Steinberg JP, Braun BI, Hellinger WC, et al. Timing of antimicrobial prophylaxis and the risk of surgical site infections: results from the Trial to Reduce Antimicrobial Prophylaxis Errors.* Ann Surg. *2009;250:10–16.)*

a randomized controlled trial among 5580 general surgery patients found no difference in SSI rates between those with cefuroxime administration early (median, 42 minutes before incision) versus administration closer to the time of incision (median, 16 minutes before incision).[143] Finally, a meta-analysis on this topic noted that SSI rates significantly increased with administration more than 120 minutes before incision and with administration after incision, but no further refinement of the timing window could be made.[144] Thus, although a near consensus has developed regarding timing (within 60 minutes before incision, unless using vancomycin or quinolones), more investigation is needed into such nuances of the delivery of surgical prophylaxis before alterations in timing recommendations can be made.[138]

Administration of prophylaxis in patients undergoing cesarean section had historically been held until after umbilical cord clamping to address concerns about delivery of drug to the neonate, which may mask signs of developing neonatal sepsis. Such a strategy, however, does not provide the mother with desired tissue concentrations of antibiotics at the time of incision. Several studies and analyses have now demonstrated that administration of prophylactic antibiotics before incision results in a reduced endometritis and SSI risk and does not lead to an increase in adverse neonatal outcomes (e.g., neonatal sepsis, sepsis workups, and admissions to the intensive care unit), indicating that provision of antibiotic before incision is acceptable.[145–147] For orthopedic procedures in which a tourniquet is used, full infusion of the drug has often been recommended to occur before tourniquet inflation[126]; however, a randomized placebo-controlled trial in knee arthroplasty procedures noted no difference in deep SSI rates when prophylaxis was given before tourniquet deflation, calling this long-held practice into question.[148]

Intraoperative Redosing

As critical as providing an appropriately timed initial dose of antibiotic is, it is important to ensure that tissue concentrations remain well above the MIC values of common pathogens during the entire procedure. Failure to do so was associated with an increased risk for SSI (RR, 4.6) across a variety of procedures in an analysis from the University of Washington,[149] an effect that remained after adjustment for inpatient status, smoking, emergency surgery, procedure type, multiple procedures, and intraoperative blood transfusion greater than 500 mL.[149] To achieve this goal, antibiotics should also be readministered in adults with normal renal function if the procedure duration exceeds two half-lives of the drug used or in the event of excessive blood loss (>1500 mL),[110] as introduction of bacteria into the surgical wound occurs not only at the time of incision but often throughout the procedure. In prolonged procedures or with antibiotics with short half-lives, patients may be inadequately protected if redosing is not routinely provided.[150] The 2013 consensus guideline provides detailed information on the timing of intraoperative doses for most common prophylactic antibiotics (Table 313.6).[110] Some groups have also used the now-commonplace "time-out" before incision or computer-generated dosing reminders to ensure appropriate delivery of antimicrobial prophylaxis.[151,152]

TABLE 313.6 Recommended Doses and Redosing Intervals for Adults for Commonly Used Antimicrobials for Surgical Prophylaxis

ANTIMICROBIAL	RECOMMENDED DOSE FOR ADULTS	RECOMMENDED REDOSING INTERVAL (FROM INITIATION OF PREOPERATIVE DOSE) (h)[a]
Intravenous Agents		
Ampicillin-sulbactam	3 g (ampicillin 2 g/sulbactam 1 g)	2
Ampicillin	2 g	2
Aztreonam	2 g	4
Cefazolin	2 g; 3 g for persons with weight ≥120 kg	4
Cefotaxime	1 g	3
Cefoxitin	2 g	2
Cefotetan	2 g	6
Ceftriaxone	2 g	NA
Ciprofloxacin	400 mg	NA
Clindamycin	900 mg	6
Ertapenem	1 g	NA
Fluconazole	400 mg	NA
Gentamicin	5 mg/kg	NA
Levofloxacin	500 mg	NA
Metronidazole	500 mg	NA
Moxifloxacin	400 mg	NA
Piperacillin-tazobactam	3.375 g	2
Vancomycin	15 mg/kg	NA
Oral Agents for Colorectal Procedures (in Conjunction With Mechanical Bowel Preparation)		
Erythromycin base	1 g	NA
Metronidazole	2 g	NA
Neomycin	1–2 g	NA

[a]Based on typical case length; for prolonged cases, antibiotics noted as "NA" (not applicable) may require redosing.
Modified from Bratzler DW, Dellinger EP, Olsen KM, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. Am J Health Syst Pharm. *2013;70:195–283.*

Antimicrobial Prophylaxis: Duration

In the early years of surgical prophylaxis prolonged courses (7–10 days) of antibiotics seemed routine.[153] Over time the benefit of prolonged courses of antimicrobials has been appropriately questioned, particularly due to pathophysiologic changes at the area of incision (i.e., coagulative necrosis, induced hemostasis via cautery of blood vessels) that likely limit the ability of antibiotics to reach the wound bed during the early postoperative period.[153]

Although some studies have noted a benefit with prolonged prophylaxis in select subpopulations of patients,[154–157] many more have found no increase in infection rates among the short-course recipients when compared with those receiving a longer duration of prophylaxis. A systematic review of 28 randomized trials of single versus multiple doses of antimicrobial prophylaxis found no advantage of prolonged duration of prophylaxis in preventing SSI in a wide array of surgical procedures.[158] Prolonged antimicrobial prophylaxis (>48 hours postincision) has been significantly associated with an increased risk of acquiring an antibiotic-resistant pathogen.[159] A commonly espoused rationale for prolonging the duration of surgical prophylaxis after incision closure includes a desire to "cover" the wound while surgical drains remain in place or to "protect" against infection of CVCs. However, a study in the United Kingdom found that prolonged prophylaxis until the patient's CVC was removed (vs. three doses of cefuroxime perioperatively) did not lead to a reduction in CVC colonization, a surrogate for CVC infection.[160] WHO SSI Prevention Guideline advises against continuing prophylaxis in the presence of a drain.[68]

The effectiveness of surgical prophylaxis is minimized after incision closure. Most guidelines for surgical prophylaxis recommend discontinuation of prophylactic antibiotics within 24 hours, a metric of appropriate surgical care for quality and regulatory programs. For cardiac surgery, some groups recommend continuing prophylaxis for 48 hours, based on concerns that more data are needed before uniformly recommending a shorter duration in this population.[161,162] The 2013 surgical prophylaxis guideline challenged this recommendation, stating prophylaxis for the procedure's duration and, at most, 24 hours is appropriate for cardiothoracic procedures as well.[110] The 2017 CDC SSI Prevention Guideline narrows the recommended duration of surgical prophylaxis a step further, recommending "[i]n clean and clean-contaminated procedures, do not

administer additional prophylactic antimicrobial agent doses after the surgical incision is closed in the operating room, even in the presence of a drain."[67] Prompt discontinuation of prophylaxis does not appear to impact the risk of developing SSI, as is the case with timely administration of antibiotics, but failure to do so contributes to unnecessary antimicrobial use and its subsequent related problems (e.g., antibiotic resistance). One recent study raises a potential exception to this rule. A randomized, double-blind trial among obese (prepregnancy BMI ≥30 kg/m^2) women noted that the addition of oral cephalexin and metronidazole for 48 hours after cesarean delivery, in conjunction with routine preoperative prophylaxis, was associated with a significantly lower SSI rate (6.4% vs. 15.4%).[163] This effect was more pronounced among women with ruptured membranes, suggesting a very specific patient population that may benefit from an extended-duration strategy.

Novel Methods of Antimicrobial Prophylaxis: Local and Topical Compounds

Newer methods for delivery of antimicrobial prophylaxis, such as antimicrobial washes, cement, sutures, powder, and other compounds, have been increasingly used for the prevention of SSIs.[164] First introduced in 1939,[165] antibiotic-impregnated cement placed directly into the operative wound (as a local antimicrobial "brachytherapy") has been used as a method of antimicrobial prophylaxis and treatment, particularly in procedures involving the replacement of infected prosthetic joints. Brachytherapy uses powdered antibiotic mixed with a cement polymer, such as polymerized polymethylmethacrylate, to form a compound that may be directly applied onto prosthetic material or manufactured into beads (usually 3–10 mm in diameter) that are placed into the wound. Candidate antibiotics for use as brachytherapy must be available as a pharmaceutical-grade powder, must be heat stable (because of the exothermic reaction induced with polymerization), and must have an appropriate microbiologic spectrum of activity for the predominant pathogens at the operative site.[165] The aminoglycosides and vancomycin are the compounds most commonly used for brachytherapy; oxacillin and cefazolin have comparable elution characteristics but are less frequently used because of concerns regarding β-lactam allergy.

The majority of antibiotic elution occurs in the first days after implantation, but elution from impregnated cement has been detected years after surgery.[165,166] The use of antibiotic-impregnated cement (in conjunction with systemic prophylaxis) was significantly associated with a reduction in SSIs in studies from several large clinical registries in Europe.[167] Concerns remain, however, regarding routine use of this mode of prophylaxis because of possible adverse effects, such as allergic reactions.[168] Systemic absorption of antibiotic brachytherapy is also a concern because potentially high boluses of antibiotic may lead to toxicity. In one series of 14 subjects, 1 patient developed permanent and 2 others temporary high-frequency hearing loss after implantation of gentamicin-impregnated beads.[169] The frequency of detectable serum aminoglycoside levels was substantial (22% of patients) in one series of patients with tobramycin cement spacer placement.[170] Renal failure attributed to impregnated cement, although rare, has also been reported.[171] Two randomized controlled trials examining the use of another type of brachytherapy, implantable gentamicin-collagen sponges, to prevent SSIs noted no impact in cardiac surgery patients[172] and a significant increase in infection in colorectal surgery patients.[173] Further studies into the systemic absorption, efficacy, and adverse effects of these brachytherapeutic compounds appear warranted before recommendations for routine use can be made.

The use of two other local forms of antimicrobial prophylaxis has garnered increased attention: powder and irrigation solutions instilled directly within the surgical bed. Use of vancomycin powder in spinal and craniotomy surgery has generated an amount of discussion and assessment. Some argue that the level of evidence for benefit is limited and has substantial bias,[174,175] whereas others have concluded that the use of intraincisional vancomycin powder leads to a significant reduction in SSIs.[176,177] The use of antibiotic irrigation solutions, although widespread, also suffer from a lack of clear evidence supporting their use, possibly related to the need for more prolonged exposure of the agent to the infecting pathogens in order to have an effect.[178] The 2016 WHO SSI Prevention Guidelines recommend against the use of antibiotic irrigation, although use of an aqueous povidone-iodine irrigant is noted for consideration.[68]

Adverse Effects of Prophylaxis

Adverse effects of prophylaxis include allergic reactions ranging in severity from minor skin rashes to anaphylaxis. *Clostridioides difficile* (formerly *Clostridium difficile*)–associated diarrhea (CDAD) has been noted with several prophylactic agents, and in one study the rate of CDAD was 14.9 cases per 1000 in surgical patients who received antimicrobial prophylaxis as their sole antibiotic exposure.[179,180] Another notable adverse effect is profound hypotension and flushing associated with vancomycin prophylaxis (the red man syndrome), usually associated with rapid infusions of the antibiotic.[181]

The use of prophylactic antibiotics has consequences for the institution and the individual patient. Antibiotic use, and specifically prophylactic use, has been shown to have a critical role in the selection of antibiotic-resistant bacteria to become the dominant colonizing flora and nosocomial pathogens of hospitalized patients.[182] At least two mechanisms for this process have been documented. First, the antibiotic-resistant flora may be endemic within the institution and transferred to the patient during the course of hospitalization.[183] Second, a small population of antibiotic-resistant bacteria that are part of the patient's endogenous flora at the time of hospitalization may emerge under the selective pressure of perioperative prophylaxis to become the dominant flora.[184] In view of the improvement in overall surgical wound infection rates over recent decades, the benefit of prophylactic antibiotics outweigh the risk of this potential side effect, but judicious use (including early discontinuation) of antibiotics for this purpose should be followed.

Cost-Benefit of Prophylaxis

Prophylactic antibiotics add some cost to the routine care of surgical patients. In major surgical centers, the perioperative use of antibiotics may represent a substantial portion of the pharmacy's expenditures for antibiotics, and surgeons may be encouraged to reduce or eliminate antibiotics given for prophylaxis in certain settings. For example, in carotid endarterectomy and cholecystectomy, infections develop only infrequently, are seldom life threatening, and may cost more to prevent than to treat. However, it is inappropriate to evaluate antibiotic use on the basis of cost alone. When a particular form of prophylaxis offers a clear advantage to the patient, the health care system should advocate the better treatment without consideration of its cost. Moreover, an analysis of the cost of prophylactic antibiotics can be complicated. Not only the cost of the antibiotic per se but the cost of preparing, transporting, and administering multiple doses of antibiotic must be included. Perhaps of more importance, the cost in terms of mortality, morbidity, and resources of managing SSIs that develop when using inadequate prophylaxis must be considered.[185]

Use of Surgical Prophylaxis Compliance as a Reported Measure of Health Care Quality

Historically, adherence to the key aspects of preoperative and intraoperative antimicrobial prophylaxis (i.e., right drug at the right dose given at the right time) was poor. An analysis in 2001 of Medicare patients undergoing major surgical procedures for which antimicrobial prophylaxis is recommended found that only 55.7% received an antibiotic within 1 hour before incision, whereas only 40.7% had prophylaxis discontinued at 24 hours after surgery.[135] Because of this suboptimal performance, quality metrics targeting delivery of antimicrobial prophylaxis and other evidence-based SSI reduction methods were developed. Led by the Surgical Improvement Project (SIP), which morphed into the Surgical Care Improvement Project (SCIP), performance and reimbursement measures focused on surgical prophylaxis drug choice, timing, and duration, as well as hair removal, and prevention of hyperglycemia and hypothermia helped improve adherence to these basic standards and have been associated with lower SSI rates.[186] In contrast, another retrospective analysis argued that improved compliance with the individual components of the SCIP metrics were not associated with a reduction in SSI, leading to pointed criticism of the program.[187,188] Unfortunately, critics failed to also note the significant reduction in SSI

risk, with improved compliance to *all* measures together, suggesting that quality improvement efforts focused at harmonizing overall surgical care improvement rather than specific metrics can make an impact.[189] The antimicrobial prophylaxis measures within SCIP were retired in 2015 due to uniformly high compliance. Whether compliance has subsequently slipped with less visibility and accountability to these metrics remains to be seen but is a concern.

Surgical Site Infection Surveillance

A key component in the prevention of SSIs is the establishment of a surveillance infrastructure to regularly detect and monitor rates of procedure-specific infections, to define the changing ecology of resistant pathogens that cause surgical infections, and to provide accurate analysis of the pervading antimicrobial sensitivity patterns in each specific institution to allow tailoring of prophylactic regimens. Adequate surveillance for postsurgical infections with comparisons of infection rates to national benchmarks also allows continued evaluation and assessment of the quality of an individual hospital's prevention strategies. To allow such comparisons, such surveillance must use standardized infection definitions, such as those used by the CDC's National Healthcare Safety Network (Table 313.7). Studies of risk factors for SSI and evaluations of interventions to reduce postsurgical infections use a wide array of outcome definitions, such as purulence at the incision site with a positive wound culture or attending physician diagnosis of wound infection. Thus care must be taken when assessing the literature and comparing the results of different studies because the outcome of interest may vary considerably.[190] The presence of purulence within a surgical incision generally serves as initial evidence of a SSI, but culture results of surgical wounds or exudates should not be used solely as a guide to the presence or absence of infection. Surveillance systems should also use the input of representatives with surgical, infectious diseases, infection prevention, and hospital epidemiology expertise in the analysis and evaluation of data.

In health care settings associated with a high volume of surgical procedures, surgical wound isolates should be maintained, if possible, for several weeks after isolation. Clusters or outbreaks of SSIs caused by a common pathogen are usually identified retrospectively, and the availability of infecting pathogens for molecular typing may be instrumental in identifying and eliminating the cause of the outbreak.

Highlighted by the landmark Study on the Efficacy of Nosocomial Infection Control project, maintaining and publicizing surgeon-specific infection rates may be used to indirectly decrease SSI rates.[191] Such data may identify unsuspected problems among the surgical staff or may encourage individual surgeons to rigorously adhere to standards of perioperative aseptic techniques. If such a program is used, it is vital that any analysis include an assessment of procedure-specific infection rates. Wide differences in infection rates exist among surgical procedures, even among those procedures within the same surgical subspecialty and category of bacterial contamination. For example, in vascular surgery, infection rates after carotid endarterectomy are exceedingly low (<0.1%). In contrast, bypass grafting in the femoral-popliteal area may be associated with an infection rate of 2% to 3%, despite the fact that both procedures are clean and may be performed by the same vascular surgeons. Other important variables, such as the incidence of underlying patient risk factors, must also be considered when comparing infection rates among surgeons.

TABLE 313.7 National Healthcare Safety Network Definitions for Surgical Site Infections

INFECTION TYPE	DEFINITION
Superficial incisional surgical site infection (SSI)	Infections involving only skin and subcutaneous tissue of the incision occurring within 30 days after the procedure with *one or more* of the following: • Purulent drainage from the superficial incision • Positive aseptically obtained culture of fluid or tissue from superficial incision • Pain or tenderness, swelling, erythema, or heat at surgical wound that has been deliberately opened by a surgeon or attending physician; *or* • Diagnosis by a surgeon or attending physician
Deep incisional SSI	Infections involving deep soft tissue (fascia/muscle layers) occurring within 30 or 90 days (dependent on the procedure type) after the procedure with *one* of the following: • Purulent drainage from deep incision but not from organ space • Spontaneous dehiscence or deliberate opening by a surgeon with a positive incision/wound culture or is not cultured and has the presence of fever or local pain • Abscess involving a deep incision found by direct examination, during invasive procedure, or by histopathologic examination or imaging test; *or* Diagnosis by a surgeon or attending physician
Organ/space SSI	Infections that involve any part of the body opened or manipulated during the operative procedure, excluding skin incision/fascia/muscle layers, within 30 or 90 days (dependent on the procedure type) with *one* of the following: • Purulent drainage from a drain that is placed into the organ/space • Positive culture growth of a specimen of tissue or fluid aseptically obtained • Evidence of infection by direct examination, during invasive procedure, or by histopathologic examination or imaging test

Postdischarge Surgical Site Infection Surveillance

Surveillance systems for postsurgical infections can be challenging, given the migration of surgical procedures to the outpatient arena and the growing push to decrease lengths of hospital stay with earlier discharge postsurgery. Sands and colleagues[192] noted that 84% of SSIs occurred after hospital discharge and would therefore have been missed with traditional hospital-based surveillance. Methods to improve detection of surgical site infections that arise postdischarge, including direct examination of wounds during follow-up visits, patient and practitioner surveys, computerized queries of billing claims, outpatient diagnostic codes, and antibiotic prescriptions, have met with variable success and concerns regarding costs and the ability to generalize results.[183,193–196] Any method of postdischarge surveillance must address these concerns and accommodate the unique attributes of each specific hospital and patient population.

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314 Burns

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SHORT VIEW SUMMARY

Definitions
- Burn size can be estimated using the rule of nines in adults and Lund-Browder chart in children.
- Mortality is estimated using the Baux score (sum of age and total body surface area [TBSA]).
- The primary insult from a burn is the wound itself.

Epidemiology
- Annually, 500,000 burn injuries receive medical treatment, and approximately 40,000 require hospitalization, of which >60% require admission to specialized burn centers.
- Of patients sustaining burn injuries, 70% are men, with 30% of patients younger than 15 years of age and 13% 60 years of age or older. The total burn size is less than 10% of TBSA in 78% of cases.
- Increasing burn TBSA is associated with increased mortality: 40% to 49.9% TBSA associated with 28% mortality, 70% to 79.9% TBSA associated with 57% mortality, and greater than 90% TBSA associated with 88% mortality.
- Of the top 10 complications in a 10-year rolling review of patients sustaining fire or flame injury, 7 were infection related, with pneumonia, cellulitis, and urinary tract infections (UTIs) the 3 most common infectious complications.
- Hospital-associated infection rates are improving but are still common in this patient population with high rates of ventilator requirements, indwelling central venous catheters, arterial lines, urinary catheters, and prolonged hospitalizations.
- The time line of bacterial infections in patients with burn injuries is relatively predictable, with skin and soft tissue infections often occurring within the first week, whereas pneumonia, bloodstream infections, and UTIs occur at a median of more than 30 days into hospitalization.

Microbiology
- Bacteria: *Streptococcus pyogenes* was the most frequently recovered pathogen historically; it has been replaced by *Staphylococcus aureus* and gram-negative pathogens such as *Pseudomonas aeruginosa* and Enterobacteriaceae, with higher resistance profiles as patients stay in the hospital longer.
- Fungi: Fungal infections typically involve the skin or lungs. Risk factors for fungal wound infection include older age, greater TBSA, hospital stays longer than 14 to 28 days, diabetes, total parenteral nutrition, prior fungal wound colonization, inhalational injury, broad-spectrum antimicrobial exposure, compressive dressings, and other immunosuppression.
- Viruses: The role of viral infections has not been clearly elucidated in patients with burns but likely includes reactivation, primary infection, or exogenous reinfection of cytomegalovirus, herpes simplex virus, or varicella-zoster virus.

Diagnosis
- Clinical and laboratory diagnosis of infection: Traditional parameters used to detect infection, including temperature, white blood cell count, respiratory status, and heart rate, are not sensitive or specific enough to be used in burn patients. There is limited evidence of the utility of other laboratory parameters such as inflammatory biochemical markers, including C-reactive protein, procalcitonin, fungal screening assays, and numerous cytokines.
- Sepsis criteria: Guidelines unique to diagnosis of burn sepsis with six criteria are used, including temperature, tachycardia, tachypnea, thrombocytopenia, hyperglycemia, and enteral feeding tolerance (see Table 314.1).
- Clinical syndrome diagnosis: Bacteremia, pneumonia, and wound infection (see Table 314.2) are critical factors.

Treatment
- Empirical therapy is based on local (hospital and unit) resistance profiles and pathogens reflective of the time from initial burn injury to recovery of pathogens by culture.
- Antimicrobial dosing reflects the hypermetabolic state associated with burn patients.
- Alternative delivery of antimicrobials in addition to systemic therapy includes limited evidence for subeschar delivery of agents, with some evidence supporting use of inhalational therapy, and a long history of topical delivery of antimicrobials (see Table 314.4).

Prevention
- Early excision and grafting plays a critical role in infection prevention.
- Infection control and prevention is challenging, especially with prolonged hospitalizations.
- Central venous catheters are often associated with infections requiring close monitoring of placement protocols.
- Perioperative and empirical antimicrobials have not been clearly shown to benefit patients but are often used.
- Other strategies to decrease infections have included selective decontamination of the digestive tract and hyperbaric oxygen therapy.

BURN INJURY

Injuries due to severe burns rank among the most serious forms of trauma, resulting in anatomic, physiologic, endocrine, and immunologic stresses, especially when burns involve greater than 20% of the total body surface area (TBSA). In 2015 US municipal fire departments responded to an estimated 1.4 million fires, with 3280 nonfirefighter fatalities.[1] Approximately 75% of deaths occur at the scene, primarily because of inhalation of soot or absorption of carboxyhemoglobin in the blood.[2] Annually, 500,000 burn injuries receive medical treatment,

[a]The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the US Department of the Army or Air Force, the US Department of Defense, or the federal government. The authors are employees of the US government, and this work was performed as part of official duties. All material in this chapter is in the public domain, with the exception of any borrowed figures or tables.

and approximately 40,000 require hospitalization, of which more than 60% require admission to specialized burn centers. Improvements in the care of patients who experience burns, especially initial burn shock resuscitation, have resulted in remarkably improved survival rates to 97% between 2005 and 2014.[1,3,4] In the 1970s a 50% survival was seen after a 30% TBSA burn compared with comparable survival with an 80% TBSA burn at the present time.[5]

The primary insult from a burn is the wound itself, which has three characteristic areas of involvement.[6] The first burn wound area is the zone of coagulation nearest the heat source and includes dead tissue forming the burn eschar. Adjacent to the necrotic tissue in the zone of coagulation is the second area, known as the zone of stasis, which is viable but at risk of ischemia because of perfusion defects. The zone of hyperemia is the third area, which consists of relatively normal skin with increased blood flow, vasodilation, and minimal cellular injury. Overall the primary concern of burn wound injuries is the burn wound eschar, with its avascular nature preventing immune cells and systemically administered antibiotics from being delivered.[7,8] The rule of nines is often used in adults to estimate the surface area of a burn. Using this formula, the head and neck and each arm are 9% of the TBSA; the anterior trunk, posterior trunk, and each leg are 18%; and the genitalia and perineum are 1%. The Lund and Browder chart, which adjusts the values of the legs and head according to age, is used for children.[9]

TYPES OF INJURIES ADMITTED TO BURN CENTERS

There are several mechanisms that result in burn injuries in addition to thermal burns that cause cellular damage based on duration and temperature of exposure. Chemical burns can involve reducing agents such as hydrochloric acid, oxidizing agents such as sodium hypochlorite, and corrosive agents such as phenol, all with varying modes of action resulting in burns. Other burn mechanisms include scalding (more common in children), contact with hot objects, electrical injuries, and radiation. Burn units also manage certain skin disorders such as Stevens-Johnson syndrome. During the years 2005–2014 hospital admissions to burn centers were largely related to fire/flame or scald injuries in 43% and 34% of cases, respectively, with the remaining from contact of a hot object in 9%, electrical in 4%, chemical in 3%, and 7% other.[10]

EPIDEMIOLOGY

Survival in Burn Injury

The American Burn Association (ABA) maintains a national burn repository that provides a 10-year rolling review of ongoing data collected from 96 hospitals in 36 states and the District of Columbia.[11] For the period 2006–2015 almost 70% of patients were men. There is a bimodal distribution of burn injuries, with 30% occurring in children 1 to 15 years of age and 54% occurring in adults 20 to 59 years of age. A further 13% of patients were 60 years of age, and 2% of patients were 80 years of age or older. Approximately three-quarters of burns involved 10% TBSA or less, with a mortality of 0.6%. Increasing burn TBSA was associated with increased mortality: 40% to 50% TBSA associated with 28% mortality, 70% to 80% TBSA associated with 57% mortality, and greater than 90% TBSA associated with 88% mortality. Overall mortality rate was 3.3%. Mortality was 5.8% for fire or flame injuries, the most common cause of burn, and was dramatically increased within this cohort by the presence of inhalation injury. The importance of age in relation to mortality of burn patients is emphasized by the Baux score (sum of age and TBSA). A Baux score of 80 to 90 is associated with a mortality of 12%, and mortality increases to almost 50% with a Baux score of 100 to 110.[11]

Overall, predictors of mortality are extremes of age, percentage of TBSA, presence of full-thickness burns, and inhalational injury, with some data supporting sex-dependent differences. For survivors, the average length of hospitalization is slightly more than 1 day per TBSA percentage burned.[11–17] Improvements in the morbidity and mortality associated with burns over the last 50 years have been attributed to early excision and grafting, external thermal regulation, early enteral feeding, use of anabolic agents, therapeutic exercise, advances in burn shock resuscitation, airway management and ventilatory strategies, and infection-control practices.[3,18–23]

Infectious Complications of Burn Injury

If patients survive the initial burn and resuscitative phase, infections are a leading cause of mortality (75% of cases).[19,24–28] Seven of the top 10 complications in the 10-year rolling review of patients experiencing fire or flame injury were infection-related, with pneumonia, cellulitis, and urinary tract infections (UTIs) being the 3 most common complications. Risk factors included older age for UTIs, scalds and burns from contact with a hot object for wound infections and cellulitis, and inhalational injury and mechanical ventilation for longer than 4 days for pneumonia.[11,29–32]

Infection preceded multiorgan dysfunction by a median of 4 days, with increasing mortality based on severity of sepsis and a mortality odds ratio of 12.5 for septic shock versus uncomplicated sepsis.[24] Primary sites of infection are the bloodstream, lungs, wounds, and urinary tract, with either lungs or wounds the most common, depending on the diagnostic definitions used.[24,33–36] Infected patients tend to be older, female, and intubated, with higher-percentage TBSA burns, longer lengths of stay, more central venous and arterial catheters, and more operations.[33] Outcomes appear worse with polymicrobial infections.[37,38] The National Healthcare Safety Network indicated that burn units have the highest rates for catheter-associated UTIs (4.8 per 1000 catheter-days) and central line–associated bloodstream infections (2.9 per 1000 central line–days) and among the highest rates for infection-related ventilator-associated complications (2.93 per 1000 ventilator-days).[39,40] Despite improvements over historical rates, these rates remain higher than predicted even when applying the standardized infection ratio, which attempts to account for various facility and patient factors that may affect health care–associated infections.[41] Endocarditis occurs in approximately 0.4% of burn unit admissions and in 9% of patients with persistent bacteremia.[42]

MICROBIOLOGY

Pathogenesis and Microbial Evolution of Burn Wound Colonization and Infection

Severe burns cause a mechanical disruption of the skin, allowing microbes to penetrate deeper tissues.[43] At the time of the burn, the normal skin structure is replaced by a moist, protein-rich, avascular eschar that is an ideal environment for microorganisms. Typically the burn surface is sterile immediately after thermal injury, but after 48 hours, the wound is colonized with skin pathogens that typically reside in sweat glands and hair follicles before the burn.[44–46] Although *Streptococcus pyogenes* was the most frequently recovered pathogen historically, this has been replaced with *S. aureus* and gram-negative pathogens, with higher resistance profiles as patients stay in the hospital longer.[47–54] A retrospective study of wound cultures obtained within 24 hours of injury revealed positive cultures in 32% and that the only independent risk factor for early gram-negative colonization was percentage TBSA. Methicillin-resistant *S. aureus* and multidrug-resistant (MDR) gram-negative pathogens were rare.[55] After 5 to 7 days, wounds become colonized with yeast or gram-positive and gram-negative bacteria from the host's normal gastrointestinal and upper respiratory tracts or from the hospital environment and health care workers' hands.[44–46,56–64]

A more recent meta-analysis called into question the traditional teaching that burn wound infection microbiology is center-specific, as 60% of burn wound infections were associated with gram-negative organisms, with *P. aeruginosa* being the most common gram-negative organism, followed by *Klebsiella pneumoniae, Escherichia coli, Enterobacter* spp., and *Proteus* spp.[65] However, international guidelines still recommend development and use of an antimicrobial stewardship program incorporating a specific local antibiogram with resistance rates within the burn population.[54,66] Rates of *Clostridioides difficile* (formerly *Clostridium difficile*) infections in the burn intensive care unit (ICU) are lower than in other ICUs, but that may be reflective of patient populations.[67] Although anaerobic bacteria are noted in numerous wound studies, they do not appear to be associated with systemic infections such as bacteremia.[68]

Fungal Colonization and Infection Following Burn Injury

Numerous fungal agents are associated with burn injuries, including *Aspergillus, Candida* (with increasing rates of non-*albicans Candida* spp.), mucormycetes, hyalohyphomycetes, and phaeohyphomycetes.[69–72] Fungal infections typically involve the skin or lungs. Risk factors for fungal wound infection include older age, greater TBSA, hospital stays longer than 14 to 28 days, diabetes, total parenteral nutrition, prior fungal wound colonization, inhalational injury, broad-spectrum antimicrobial exposure, compressive dressings, and other immunosuppression.[6,71,73,74] A single-site study revealed that mortality associated with fungal wound colonization was 27% compared with 76% for fungal wound infections. The presence of fungal wound infection increased mortality by an odds ratio of 8.2, equivalent to raising the burn TBSA by 33%.[70] Factors independently associated with mortality in fungal wound infections include older age, extensive burn TBSA, increasing number of positive cultures, and confirmation of *Aspergillus* or mucormycetes.[74]

Viral Infections Following Burn Injury

The role of viral infections has not been clearly elucidated in patients with burns but likely includes reactivation, primary infection, or exogenous reinfection of cytomegalovirus, herpes simplex virus, or varicella-zoster virus.[6] Varicella-zoster virus in children has been associated with pulmonary disease.[75] Herpes simplex virus typically occurs in healing or recently healed partial-thickness burns and occasionally around the margins of skin graft donor sites 2 to 6 weeks after thermal injury.[6,7] There is evidence that herpes simplex virus may also be associated with prolonged recovery and bacterial infections.[76] Although cytomegalovirus viremia has been associated with patients who experience severe burns, the clinical impact is unclear.[77] Whether these infections are a marker of severe immune dysfunction or directly alter immune responses is yet to be determined.[2,76]

TABLE 314.1 American Burn Association Sepsis Criteria[a]

I. Temperature: >39°C or <36.5°C
II. Progressive tachycardia
- Adults: >110 beats/min
- Children: >2 SD above age-specific norms (85% age-adjusted max heart rate)

III. Progressive tachypnea
- Adults: >25 breaths/min if not ventilated or minute ventilation >12 L/min if ventilated
- Children: >2 SD above age-specific norms (85% age-adjusted max respiratory rate)

IV. Thrombocytopenia (applicable only ≥3 days post–initial resuscitation)
- Adults: <100,000/μL
- Children: <2 SD below age-specific norms

V. Hyperglycemia (applicable only to patients without a prior history of diabetes)
- Untreated plasma glucose >200 mg/dL

or
- Insulin resistance (>7 U of insulin/h IV drip (in adults) or >25% increase in insulin requirements over 24 hours)

VI. Inability to continue enteral feedings longer than 24 hours
- Abdominal distention

or
- Residual twice the feeding rate for adults or >150 mL/h in children

or
- Uncontrolled diarrhea (>2500 mL/day for adults or >400 mL/day for children)

[a]Sepsis is of concern if 3 or more of these 6 triggers are seen.
[b]To meet the definition of burn sepsis, a documented infection (culture-positive infection, pathologic tissue source identified, or clinical response to antimicrobials) is required in combination with at least 3 of the 6 above criteria.
IV, Intravenous; *SD,* standard deviation.
Modified from Greenhalgh DG, Saffle JR, Holmes JH, et al. American Burn Association consensus conference to define sepsis and infection in burns. J Burn Care Res. *2007;28:776–790.*

DIAGNOSIS

Defining Infection in Burn Patients

The stress associated with severe burn injuries is marked by a persistent hypermetabolic response characterized by full-body catabolism, muscle and protein degradation, stunted growth, insulin resistance, increased risk for infection, and multiorgan dysfunction.[23,78,79] A review of the immunologic alterations associated with burns is beyond the scope of this chapter; however, the innate and adaptive immunologic alterations associated with burn injuries are numerous and can persist for weeks to months.[4,6,80–95]

The traditional parameters used to detect infection, including temperature, white blood cell count, respiratory status, heart rate, and Sequential (Sepsis-related) Organ Failure Assessment (SOFA) and Quick Sequential (Sepsis-related) Organ Failure Assessment (qSOFA) criteria as described in new sepsis guidelines, are not sensitive or specific enough to be used in the same fashion in burn patients because of the metabolic perturbations associated with burns. Extremes or changes in temperature; white blood cell count; percentage of neutrophils; and inflammatory biochemical markers, including C-reactive protein, fungal screening assays, and numerous cytokines, are not adequate in predicting ongoing infections.[93,94,96–100]

Sepsis and Bloodstream Infections

With these limitations in mind, the ABA released criteria for sepsis in 2007 that could be used as a trigger for the concern of ongoing infection; however, these parameters do not appear to perform ideally in the clinical setting (Table 314.1).[96,101,102] Procalcitonin continues to be the most studied biomarker to attempt to diagnose sepsis.[103–105] A meta-analysis found that using a cutoff of 1.5 ng/mL (rather than 0.5 ng/mL) led to an area under the curve of 0.8 in predicting sepsis; however, the assays used, definitions of sepsis, and included populations were highly heterogeneous, making real-world interpretation a continuing challenge.[103]

Bloodstream infections are diagnosed by using the standard analysis of pathogen, clinical syndrome, and number of positive cultures.[101] The needed quantity of blood or use of both anaerobic and aerobic bottles at the time of culturing is not clear.[68] Although patients can develop bacteremia after burn wound manipulation in the early postburn period, the clinical relevance of this bacteremia is uncertain. It is also unclear whether bacteremia is associated with mortality in burn patients.[106–108]

Pneumonia

Pneumonia is defined by a clinical syndrome consisting of two or more of the following factors: a new or persistent infiltrate, consolidation, or cavitation on chest radiograph; sepsis (as defined in Table 314.1); and a recent change in sputum or purulence in the sputum.[101,109] The diagnosis is designated confirmed, if a patient meets the clinical syndrome with microbiologic confirmation; probable, if a patient meets the clinical syndrome without microbiologic confirmation; and possible, with an abnormal chest radiograph with low or moderate clinical suspicions but microbiologic confirmation. Microbiologic criteria include tracheal aspirate having 10^5/mL organisms or more, bronchoalveolar lavage having 10^4/mL organisms or more, or protected bronchial brush having 10^3/mL organisms or more. Similar to other critically ill patients, these evaluations are not always routinely performed. Purulent tracheobronchitis, defined as fever and increased sputum without chest radiograph findings or purulent discharge coating the trachea during bronchoscopy, is encountered in burn patients. There is evidence that the presence of tracheobronchitis is associated with prolonged durations of mechanical ventilation and that antibiotic therapy may mitigate this effect.[110]

Wound Infections

Criteria for diagnosis of wound infections have been described by the ABA (Table 314.2) and others.[4,101,111] Changes of the eschar appearance including development of a dark brown, black, or violaceous discoloration or edema at the wound margin are of concern for ongoing infection. Although the definitive diagnosis of wound infection is probably histopathologic diagnosis, with invasion of bacteria into viable tissue or blood vessels, expertise and rapid turnaround are lacking in most centers. Similarly, culturing the skin through swabs or biopsies is of limited clinical usefulness due to sampling bias, poor correlation between methods, no standardized methodology, slow processing time, and lack of consistent association with ongoing bacteremia.[112–115] A systematic review determined that for culture to be useful more than one sample

TABLE 314.2 Diagnosis of Burn Wound Infection: American Burn Association Guidelines

SYNDROME	CLINICAL AND PATHOLOGIC CRITERIA: BURN SEPSIS GUIDELINES
Wound colonization	Bacteria on wound surface at low concentration (<10^5 bacteria/g tissue); no invasive infection
Wound infection	Bacteria in wound and wound eschar at high concentration (>10^5 bacteria/g tissue); no invasive infection
Invasive infection	Pathogens in burn wound at a sufficient concentration (>10^5 bacteria/g tissue [frequently]), depth, and surface area to cause suppurative separation of eschar or graft loss, invasion of adjacent unburned tissue, or sepsis syndrome (see Table 314.1)
Cellulitis	Bacteria present in wound and/or wound eschar at high concentrations (>10^5 bacteria/g tissue), with surrounding tissue revealing erythema (this alone may not require therapy), induration, warmth, or tenderness
Necrotizing infection, fasciitis	Aggressive, invasive infection with involvement below the skin resulting in tissue necrosis

Notes

1. Quantitative biopsy can assist in identifying pathogen and antimicrobial resistance profiles, but its ability to confirm a diagnosis is limited.
2. Quantitative swabs are unreliable but might assist in pathogen identification and antimicrobial resistance profiles.
3. Tissue histology can be used, but limited expertise exists.
4. Clinical parameters of wound infection include pain, erythema, color change, unexpected changes in wound appearance and depth, systemic changes, and premature separation of burn eschar.

Modified from Greenhalgh DG, Saffle JR, Holmes JH, et al. American Burn Association consensus conference to define sepsis and infection in burns. J Burn Care Res. *2007;28:776–790.*

is required, biopsies generally outperform swabs in predicting sepsis, higher bacterial loads may predict worse clinical outcomes (but need to be combined with depth of invasion to be relevant), and interpretation must be done in the setting of clinical findings.[7,116,117]

Pseudomonas aeruginosa can colonize the wound, resulting in yellow-green exudates, but invasive infection should be considered only when there are purple-black and punched-out areas of the wound.[118] *Candida* infections can appear as small pustules, whereas *Aspergillus* appears as gray-brown plaques. Histology for fungal diagnosis must be augmented with culture because of inaccuracies of histology, presence of mixed cultures, and the need to provide definitive identification of fungi to assist in antifungal selection.[71]

TREATMENT

Empirical Therapy of Infections

Therapy of infections in patients with burns is focused on the site of infection and offending pathogen. Empirical therapy should rely on the specific antibiogram of a burn unit because resistance profiles in burn units may not reflect other units or wards in the same hospital.[54,66,67] Some burn units rely on individual patients' quantitative wound cultures on admission and use routine periodic screening of the skin to predict infecting pathogens. The usefulness of this method is unclear with sampling and technique bias.[45,116,119–122] Empirical therapeutic choices should focus on pathogens with notable mortality such as *K. pneumoniae* instead of pathogens with increased antimicrobial resistance but less virulence such as *Acinetobacter baumannii*. The benefit of empirical therapy is unclear in patients who experience a burn without evidence of ongoing infection.[48,50,123]

Dosing of Antimicrobials in Burns

Physiologic response to severe burns can be divided into two stages, with the first being the ebb or resuscitative stage (within the first 12 hours and lasting 48–72 hours). This first, transient stage during which few infections are diagnosed is characterized by hypovolemia and decreased cardiac output, so clinicians can expect prolonged drug distribution, decreased maximal serum concentrations, delayed onset of action, and slower drug elimination. Much more frequently the clinician is facing the complications of drug dosing during the second, or hypermetabolic, phase of burn injury, which is characterized by hyperdynamic circulation, increased tissue perfusion, and hypoalbuminemia. This leads to faster drug delivery, time to peak serum concentration, and a shorter half-life. There are also complex changes in protein binding, volume of distribution over time, and high levels of interpatient and intrapatient variability, with limited data available. This may make reaching pharmacokinetic and pharmacodynamic targets challenging with conventional dosing, with providers facing the potential for toxicity with dose or frequency increases. Further study of real-time optimization of drug dosing and consideration of therapeutic drug monitoring as available may ultimately improve outcomes.[124–130] However, a recent small, single-center study evaluating therapeutic drug monitoring of four antimicrobials (vancomycin, imipenem, meropenem, and piperacillin-tazobactam) failed to show improvement in clinical outcomes, including mortality.[131]

Alternative routes of drug delivery have been considered to improve outcomes. Although newer guidelines recommend inhaled antimicrobials for treatment of pneumonia to be considered as an adjunct for patients with ventilator-associated pneumonia who are not responding to intravenous antimicrobials alone, no clear benefit or harm has been shown in burn patients.[132–134] Historically, subeschar clysis was used in deep burns when early excision was not the standard of care, and it is still used in areas where early surgical interventions and adequate support are not available. Methods include introduction of antibiotic agents or other antimicrobials such as povidone-iodine plus Neosporin (polymyxin B/neomycin/bacitracin).[135,136]

Antifungal Therapy

Therapy of fungal infections depends on identification of the genus and species as well as the site of infection. In a multicenter review, 13% of fungal cultures yielded *Aspergillus* spp.; however, another study in a large burn unit reported that almost half of *Aspergillus* isolates were *Aspergillus terreus,* limiting the use of amphotericin B as empirical monotherapy for fungi.[69,71] The role of empirical therapy or preemptive therapy, when a fungus is recovered from the skin but is not yet invasive, is intriguing, especially in patients with 30% to 60% TBSA burns, because they may benefit the most from early therapy to prevent invasive infection and subsequent excess morbidity and mortality.[70]

Surgical Management and Topical Therapy

Although surgical management is regarded as care of the wound, it is also vital in treating and preventing infections.[43] Early excision and grafting (considered standard of care in resource-rich countries) is not universally defined but is typically within the first few days of injury and certainly within the first week to 10 days.[66] Early excision and grafting appears to be associated with a shorter length of hospitalization, improved graft take, and improved infection and mortality rates in patients without inhalation injury, but its impact on sepsis is unclear.[8,137–139] Coverage of the burn area is fundamental to burn care and ideally involves immediate coverage with autologous skin grafts for permanent closure. However, this is not an option in patients with grossly infected, questionably viable wounds or when donor skin for autografting is not available (in the setting of extensive burns or in a patient who is not stable enough for additional harvest).[140] In these cases, temporary coverage with allograft or other skin substitutes provides a wound barrier, prevents evaporative losses, and prepares the wound bed for autograft.[8] Temporary coverage options include allografts (living-donor or cadaveric skin, either fresh or frozen), xenografts (typically porcine skin), and synthetic coverage (acellular or cellular matrices) (Table 314.3).[8,140–146] A survey of 111 burn specialists from 36 countries revealed that providers still do not feel that an ideal skin substitute is available. Most providers use skin substitutes in daily practice, and 81% rated them as "essential" in patients with >60% TBSA burns, with allografts most commonly used (51%), followed by xenografts (28%).[147]

Topical therapies are used to prevent and treat infections and include mafenide acetate, honey, silver sulfadiazine, silver nitrate, and silver

TABLE 314.3 Common Select Burn Wound Coverage Options

PRODUCT	DESCRIPTION	USE AND ADVANTAGES	APPROXIMATE COST AND LIMITATIONS
Permanent Coverage			
Autograft	• Patient serves as own donor of split-thickness skin graft from uninjured donor site	• Gold standard, permanent coverage • Excellent ingrowth • Can be stretched for expansions between 1:1 and 9:1	• Coverage requires donor site availability (can be limited in extensive burns) • Widely stretched autografts (ratios higher than 3:1) are more fragile, prone to infection and desiccation, and so frequently covered with allograft to improve take • Risk of wound complications at both graft and donor site
Temporary Coverage: Biologics			
Fresh allograft	• Living donor or cadaveric skin graft	• Fresh living allograft is gold standard for temporary coverage • Excellent ingrowth • Excellent test for autografting • Longest survival in those with largest burn wounds • Can serve as scaffolding for future ingrowth	• $2461/sq ft • Short 14-day shelf life refrigerated (with change of medium every 2–3 days) • Unpredictable availability • Risk of infection and disease transmission
Frozen allograft	• Cryopreserved cadaveric skin graft	• Widely available (most commonly used temporary coverage) • Good test for future autografting • Less expensive than fresh allograft • Indefinite shelf life when frozen	• $1997/sq ft • About 54%–73% viability of fresh tissue • Risk of infection and disease transmission (though longer time for adequate donor screening than fresh allograft)
Xenograft	• Dermis ± epidermis (most frequently porcine)	• Low cost, available option for short-term coverage or testing a wound for autografting • Prolonged shelf-life (18–24 months depending on formulation and storage)	• $133/sq ft • Inferior to allograft for excised burn wounds • Good adherence, but no ingrowth • Theoretical concern for disease transmission (prion disease and porcine retroviruses) • Cultural and religious restrictions on use
Temporary Coverage: Select More Commonly Used Dermal Substitutes ***Natural Biologic Materials***			
Alloderm (LifeCell, Branchburg, NJ)	• Acellular dermal cadaveric allograft • Fresh human cadaveric skin chemically treated to remove all cellular material in the dermis	• Applied to a wound bed, serves as a template for dermal regeneration, allowing for subsequent thinner autograft • Acellular and immunologically inert	• $14,000–$28,000/sq ft • High cost compared with allograft • Risk of disease transmission • Now rarely used because of concerns that it may act as a barrier to diffusion of nutrients to the autograft
Constructed or Artificial Biologic Materials			
Integra (Integra Lifesciences, Plainsboro, NJ)	• Acellular bilayered substitute • Dermal matrix of type 1 bovine collagen/shark-derived chondroitin-6-sulfate covered by silicone	• Most widely accepted artificial skin substitute • Vascularizes in 3–4 weeks, after which time silicone epithelium can be peeled away, and an ultrathin autograft can be placed	• $14,000–$28,000/sq ft • Higher risk for infection • Poor take of cultured epithelial autografts • Despite wide adoption, still a paucity of scientific evidence for its use
Matriderm (Dr Suwelack Skin and Health Care AG, Billerbeck, Germany)	• Radiated bovine collagen matrix with α-elastin hydrolysate	• Typically used in single-stage procedure with immediate skin grafting • Improved cosmesis and elasticity in small trials	• $4500/sq ft • Limited scientific evidence

Data from Rowan MP, Cancio LC, Elster EA, et al. Burn wound healing and treatment: review and advancements. Crit Care. *2015;19:243; Saffle JR. Closure of the excised burn wound: temporary skin substitutes.* Clin Plast Surg. *2009;36:627–641; Shahrokhi S, Anna A, Jeschke MG. The use of dermal substitutes in burn surgery: acute phase.* Wound Repair Regen. *2014;22:14–22; Greenhalgh DG. The use of dermal substitutes in burn surgery: acute phase.* Wound Repair Regen. *2014;22:1–2; and Debels H, Hamdi M, Abberton K, et al. Dermal matrices and bioengineered skin substitutes: a critical review of current options.* Plast Reconstr Surg Glob Open. *2015;3:e284.*

dressings (Table 314.4).[148,149] These agents have various adverse event profiles, clinical application characteristics, and antimicrobial activity.[150,151] A delicate balance must be achieved between antimicrobial activity and cytotoxicity to keratinocytes and fibroblasts, which would delay wound healing if used on more superficial burns that might heal without intervention.[150] It is unclear whether the limited fungal coverage of these agents results in increased fungal infections.[70]

PREVENTION

Infection Prevention and Health Care–Associated Infections

Infection prevention and control is fundamental to decreasing infectious complications associated with burns. Its importance cannot be overemphasized, and this lesson is highlighted repeatedly with the literature replete with descriptions of outbreaks.[152–155] Bacterial propagation involves hospital equipment such as mattresses, patient care with hydrotherapy, and movement of patients and equipment between the general ICU and burn unit.[52,58,61,154,156] Other studies have shown aerosolization of bacteria during wound dressing changes.[157] Burn units should be designed to minimize transmission through dedicated burn space, no common treatment rooms, centralized dedicated operating rooms and facilities, and individual patient rooms with anterooms or individual doors. In addition, aggressive standard infection-control procedures for resistant pathogens are recommended.[4,45,62,158] Pulsed-xenon ultraviolet light disinfection has been associated with decreased environmental bioburden. A more recent study reported a trend toward decreased MDR organisms among all bacteria, decreased rate of MDR gram-negative rods per 1000 occupied bed days, and increased median time to hospital-associated *C. difficile* from 66 days to none during the intervention and postintervention period of 290 days.[159]

TABLE 314.4 Application of Topical Agents for Deep Burns

AGENT	PENETRATION	SPECTRUM	SIDE EFFECTS	COMMENTS
Mafenide acetate (Sulfamylon)	Penetrates eschar	Gram-positive and gram-negative bacteria	Painful on application; metabolic acidosis	Twice daily or alternate with silver sulfadiazine; lack of antifungal activity
Silver sulfadiazine (Silvadene, Thermazene, Flamazine)	Poor eschar penetration	Gram-positive and gram-negative bacteria; *Candida* spp.	Leukopenia; pseudoeschar formation on wound surface obscures visualization of wound surface; cutaneous hypersensitivity rarely reported; contraindicated in sulfonamide-allergic patients	Can treat through leukopenia; delays healing of superficial burns compared with alternative dressings/topical antimicrobials
Silver nitrate	Poor eschar penetration	Gram-positive and gram-negative bacteria; *Candida* spp.	Discolors wound bed; electrolyte changes including hyponatremia, hypocalcemia, hypokalemia, hypomagnesemia; bacterial conversion of nitrate to nitrite may rarely lead to methemoglobinemia	Apply in thick layers with mesh gauze frequently to keep moist (labor intensive); stains environment black; change dressing twice daily
Silver dressing (Acticoat, Silverlon)	Limited eschar penetration	Gram-positive and gram-negative bacteria; *Candida* spp.[a]	Discolors wound	
Hypochlorous solutions (Dakin solution)	Unknown	Gram-positive, gram-negative (including MDROs), fungi, and viruses	Cytotoxicity	Buffered 0.5% NaOCl, concentrations as low as 0.00025% have been found to be effective; action of NaOCl is short-lived (initially continually dripped into wound dressings), now typically applied 2–3 times daily as gauze-soaked dressings

[a]Data are limited.
MDRO, Multidrug-resistant organism; *NaOCl,* sodium hypochlorite.
Modified from Murray CK. Burns. In: Bennett JE, Dolin R, Blaser MJ, eds. Principles and Practice of Infectious Diseases. *8th ed. Philadelphia: Saunders; 2015; Cartotto R. Topical antimicrobial agents for pediatric burns.* Burns Trauma. *2017;5:33; and Norman G, Christie J, Liu Z, et al. Antiseptics for burns.* Cochrane Database Syst Rev. *2017;7:CD011821.*

A retrospective study from a single site revealed that a hospital-associated infection (HAI) was diagnosed in 7% of patients over a total follow-up time of more than 81,000 days.[64] Among patients with one HAI, almost 20% had a subsequent HAI, 10% had 3 HAIs, and 6% had ≥4 HAIs during their hospitalizations. The most common HAIs were bloodstream infections (25%) and skin and soft tissue infections (19%), followed by catheter-associated UTIs (14%) and ventilator-associated pneumonias (13%). The probability of diagnosis of an HAI increased with duration of hospitalization, such that 50% of patients with hospitalizations greater than 80 days had at least one HAI. Whereas skin and soft tissue infections occur early (median of 3 days into hospitalization), the remaining HAIs including bloodstream infections, respiratory infections, and UTIs as well as *C. difficile* typically occur following the first month of hospitalization.[54,64] However, this same unit noted a decrease in central line–associated bloodstream infections (from 14 to 2 per 1000 central line–days) between 1999 and 2012, which was attributed to increased use of bundles and a multifaceted proactive infection prevention approach.[160] Central catheter–related sepsis is similar to other critical care units, with catheter-related sepsis associated with the number of lines placed and total number of central line–days, and appears to be higher in patients with femoral or internal jugular central lines compared with subclavian central venous catheters.[161,162] Distancing the insertion site from the burned area can decrease infections, but it is unclear whether guidewire exchange is comparable to insertion at a new site.[163,164] In one study, changing lines every 3 days was associated with fewer infections than changes every 4 days.[165] A self-reported survey of all ABA-certified adult burn units showed that 61% of units performed prophylactic line changes every 3 to 7 days, and 40% reported using more femoral lines due to lack of optimal sites.[166]

Antimicrobial Stewardship in the Burn Unit

Antimicrobials have been studied for use as broad prophylaxis early in burn hospitalizations, perioperatively, and as gut decontamination. The most recent analysis in 2013 concluded that the available evidence was limited but did not show decreased risk of burn wound infection, invasive infection, or mortality, which was in contrast to a 2010 Cochrane analysis that included the same studies but a slightly different primary endpoint (death from any cause).[66,167,168] Notably, prophylactic antibiotics in children have been associated with a higher secondary infection rate.[169]

The use of perioperative antibiotics at the time of débridement or dressing changes has been shown to modify rates of bacteremia but not clinical outcome and is of questionable usefulness, especially in patients with a burn TBSA less than 40% to 60%.[114,170] However, perioperative antimicrobials may be useful for severely burned patients with aggressive débridements or patients undergoing skin grafting.[171] Perioperative antimicrobials remain common practice despite little evidence supporting their use and risks, including resistance; diarrhea; *C. difficile* infection; allergies; and hepatic, renal, and bone marrow toxicities.[66]

A systematic review of selective gut decontamination using enteral or systemic antimicrobials showed that similar to nonburned critically ill patients, this intervention was associated with decreased mortality as well as bacteremia and pneumonia caused by Enterobacteriaceae, although one included trial showed an increase in methicillin-resistant *S. aureus* bacteremia. Limitations included high rates of diarrhea in patients receiving enteral antimicrobials, failure to examine *C. difficile* rates, and a high risk of bias in most of the included studies.[172,173] Additionally the current ABA guidelines for ventilatory-associated pneumonia discourage routine prophylactic use of antimicrobials in hospital settings where there are high levels of antimicrobial resistance because of concerns for associated selection pressure.[109]

Other Strategies to Modify Infection Risk

Adjunctive strategies have also been integral to improved mortality. Early enteral feeding may blunt the hypermetabolic response and decrease intestinal permeability, thus preserving the intestinal barrier and potentially affecting enterogenic infections.[174–178] Each unit of blood transfused in patients with burn injuries is associated with increased mortality and infectious complications, even after controlling for severity of disease.[179] TRIBE, a multicenter prospective trial enrolling 345 patients, randomly assigned patients with burns ≥20% TBSA to restrictive (hemoglobin 7–8 g/dL) or liberal (hemoglobin 10–11 g/dL) transfusion strategies throughout their hospitalization and showed that although the restrictive group had fewer transfusions, there were no significant differences in the primary outcome of bacteremia or secondary outcomes of mortality, UTI, pneumonia, wound infections, hospital or ICU length of stay, organ dysfunction, or wound healing.[173] Hyperbaric oxygen therapy has been evaluated, with mixed results.[180–182]

CONCLUSIONS

Patients who experience burn injuries are subjected to the most physiologic stress. Because infections are the most common cause of mortality after surviving the early postburn period, efforts are required to mitigate excessive death rates through adequate preventive measures and enhanced vigilance for infections and appropriate therapy. Burn units are ideal for outbreaks of MDR pathogens, which can affect other patients and the entire health care facility if adequate infection-control measures are not in place. The management of burn patients requires a multidisciplinary approach, including infectious disease physicians.

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The complete reference list is available online at Expert Consult.

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315 Bites

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SHORT VIEW SUMMARY

Definition
- Bite wounds are common injuries caused by a wide variety of domestic and wild animals, as well as humans.

Epidemiology
- Bites occur in 4.7 million Americans yearly and account for 800,000 medical visits, including approximately 1% of all emergency department visits.

Microbiology
- The bacteria associated with bite infections may come from the environment, the victim's skin flora, or most frequently, the oral flora of the biter, which can also be influenced by the microbiota of the biter's ingested prey and other food.

Diagnosis
- The diagnosis is made by the patient's reported history of events. Microbiologic assessment of infected wounds is performed through aerobic and anaerobic cultures.
- Radiographic evaluation can be considered if there is a high likelihood of bony injury.

Therapy
- Irrigate wounds with copious amounts of water.
- Débride devitalized or necrotic tissue.
- Primary wound closure is usually not advocated unless wounds are extensive and closure is necessary for cosmetic or functional reasons. When possible, delayed primary closure or allowing the wound to close by secondary intention is recommended.
- Loose approximation of wound edges with adhesive strips or sutures may be necessary for selected, fresh, uninfected larger wounds. Closure of facial wounds may be considered when coupled with preemptive antimicrobial therapy.
- In most cases of terrestrial animal bites, antimicrobial therapy when indicated should include coverage for *Pasteurella* (*Eikenella* in human bites), *Streptococcus, Staphylococcus,* and anaerobes, including *Fusobacterium, Porphyromonas, Prevotella,* and *Bacteroides* species.
- Oral antimicrobial choices in adults can include the following (see Table 315.3):
  - Amoxicillin-clavulanic acid 875/125 mg, 1 tablet by mouth twice daily
  - Metronidazole 500 mg, 1 tablet by mouth three times daily *plus* trimethoprim-sulfamethoxazole 1 double-strength tablet by mouth twice daily
  - Moxifloxacin 400 mg, 1 tablet by mouth daily
  - Doxycycline 100 mg, 1 tablet by mouth twice daily
- Intravenous antimicrobial choices in adults can include the following:
  - Ampicillin-sulbactam 1.5 to 3 g IV every 6 hours
  - Cefoxitin 1 to 2 g IV every 6 to 8 hours
  - Moxifloxacin 400 mg IV daily
  - Ertapenem 1 g IV daily
  - Ceftriaxone 1 to 2 g IV daily *plus* metronidazole 500 mg IV three times daily
- Empirical regimens for marine- and freshwater-acquired infections should cover *Vibrio* and *Aeromonas* species, respectively, with agents such as third-generation cephalosporins (e.g., cefotaxime) and fluoroquinolones.

Prevention
- As a routine, antimicrobials are not advocated for every uninfected animal bite injury.
- In general, 3 to 5 days of preemptive antimicrobial therapy can be considered for acute moderate-to-severe uninfected animal bite injuries, especially those to the hands or face, in the presence of preexisting edema, and in immunocompromised hosts. Longer courses may be considered for injuries that are severe or those penetrating joints or bony structures.
- Provide tetanus and rabies immunization, as indicated.

Bite wounds are common injuries caused by a wide variety of domestic and wild animals, as well as humans. Most data on the incidence of infection, bacteriology, and the value of various medical and surgical interventions in the treatment of such injuries come from small studies or anecdotal case reports. Such studies often lack randomization, concentrate on unusual organisms or complications, and are inherently biased by the types of patients with moderate or severe injuries who elect to seek medical attention. Bite wounds can consist of lacerations, avulsions, punctures, scratches, and crush injuries. Although the majority of patients never seek and often do not need extensive medical care, awareness of the magnitude of the infectious complications from bites is necessary.

ANIMAL BITES

Bite wounds are common injuries caused by a wide variety of domestic and wild animals, as well as humans.[1] The American Pet Products Association 2017–2018 survey reported the US pet dog and cat ownership number at approximately 184 million.[2] Bites occur in 4.7 million Americans each year[3] and account for 800,000 medical visits, including approximately 1% of all emergency department visits.[4] There were 1610 animal-related fatalities in the United States during the period of 2008 to 2015. The majority of human fatalities were from farm animals (e.g., cattle and horses), insects (hornets, wasps, and bees), and dogs.[5] Most dog bites (85%) are provoked attacks by either the victim's own pet or a dog known to the victim and occur during the warm weather months.[6] Bite wounds are often to the extremities, especially the dominant hand. Facial bites are more frequent in children younger than 10 years of age and lead to 5 to 10 deaths per year, often because of exsanguination.[7] Larger dogs can exert more than 450 lb/in^3 of pressure with their jaws, which can lead to extensive crush injuries.

The bacteria associated with bite infections may come from the environment, the victim's skin flora, or most frequently, the oral flora of the biter, which can also be influenced by the microbiome of the biter's ingested prey and other food (Table 315.1). Patients who present early after an incident often do not have an established infection and are usually concerned about crush injuries, care of disfiguring wounds, or the need for rabies or tetanus immunization.[6] These uninfected wounds are frequently contaminated with multiple strains of aerobic and anaerobic bacteria, similar to the spectrum found in documented bite infections. Between 2% and 30% of wounds will become infected and, rarely, may require hospitalization.[8–10,11,12] Patients presenting late, often longer than 8 hours after injury, usually have established infection.[6,8,10,11,13] Infection

TABLE 315.1 Common Bacterial Isolates From Dog and Cat Bite Wounds

Acinetobacter spp.
Aggregatibacter (Actinobacillus) actinomycetemcomitans
Aggregatibacter (Haemophilus) aphrophilus
Bacteroides tectus
Bergeyella (Weeksella) zoohelcum
Capnocytophaga canimorsus
Capnocytophaga cynodegmi
Corynebacterium minutissimum
Eikenella corrodens
Enterococcus spp.
Finegoldia magna
Fusobacterium nucleatum
Fusobacterium russii
Leifsonia (Corynebacterium) aquatica
Leptotrichia buccalis
Micrococcus luteus
Moraxella spp.
Neisseria canis
Neisseria weaveri
Pasteurella canis
Pasteurella dagmatis
Pasteurella multocida subsp. *multocida*
Pasteurella multocida subsp. *septica*
Pasteurella stomatis
Peptostreptococci
Porphyromonas asaccharolytica
Porphyromonas canoris
Porphyromonas gulae (gingivalis)
Prevotella bivia
Prevotella heparinolytica
Prevotella intermedia
Prevotella melaninogenica
Prevotella zoogleoformans
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus intermedius
Streptococci, α-hemolytic, β-hemolytic
Veillonella parvula

is manifested as localized cellulitis or abscess with gray and malodorous purulent discharge.[14] Fever, regional adenopathy, and lymphangitis occur in the minority of patients. Bites involving bones and joints may result in tenosynovitis, septic arthritis, and osteomyelitis. Chronic pain in a joint with limited range of motion may be suggestive of infection within a joint or bony structure.

Rarely, overwhelming sepsis, endocarditis, meningitis, or brain abscesses may develop after a bite injury. Fatal infections caused by *Capnocytophaga canimorsus* in association with asplenia or liver disease have been noted.[15–17] This organism may be difficult to isolate and identify and may require up to 14 days of incubation to grow on blood cultures. In addition, it has the potential capacity to escape the host immune system by both passive and active mechanisms.[18] It is generally susceptible to penicillin, cephalosporins, and fluoroquinolones but variably resistant to aztreonam and aminoglycosides (Table 315.2).[19]

Individuals with immunocompromising conditions, including chronic corticosteroid use, and those with preexisting edema of an extremity are more prone to severe infections and complications.

Dog and cat bite wound infections are considered to be predominantly related to the microbiology of their oral flora.[6,9,13,14,20–22] Although most attention has focused on *Pasteurella multocida,* the spectrum of organisms associated with dog and cat bite wound infections is much greater. Holst and colleagues[23] noted the following distribution of 159 *Pasteurella* strains isolated over a period of 3 years from human infections, mostly from bite wounds: *Pasteurella multocida* subsp. *multocida* (60%), which was the isolate in all bacteremia cases; *Pasteurella multocida* subsp. *septica* (13%), which has a greater prevalence in cats than in dogs and may have a preferential affinity for the central nervous system; *Pasteurella canis* biotype 1 (18%), which was isolated exclusively from dog bite wound infections; *Pasteurella stomatis* (6%); and *Pasteurella dagmatis* (3%), which may cause systemic infections. A study of 107 infected dog and cat bite wound infections showed that 75% of cat bites grew *Pasteurella* species on culturing *(P. multocida* subsp. *multocida,* 54%), as

TABLE 315.2 Antimicrobial Susceptibilities of Bacteria Frequently Isolated From Animal and Human Bite Wounds

	PERCENTAGES OF ISOLATES SUSCEPTIBLE					
	***Staphylococcus aureus* (MSSA)**	***Eikenella corrodens***	**Anaerobes**	***Pasteurella multocida***	***Capnocytophaga canimorsus***	***Staphylococcus intermedius*[b]**
Penicillin	10	99	50/95[c]	95	95	30
Dicloxacillin	99	5	50[d,e]	30	NS	70
Amoxicillin-clavulanic acid	100	100	100[d,e]	100	95	70
Cephalexin	100	20	40[d,e]	30	NS	95
Cefuroxime	100	70	40[d,e]	90	NS	NS
Cefoxitin	100	95	100[d,e]	95	95	NS
Ceftriaxone	99	100	70	100	NS	100
Ceftaroline	100	NS	70	100	NS	100
Erythromycin	100	20	40[d,e]	20	95	95
Tetracycline	95	85	60[d,e]	90	95	NS
Doxycycline	100	100	95	100	NS	100
TMP-SMX	100	95	0	95	V	100
Ciprofloxacin	100	100	40[d,e]	95	100	100
Levofloxacin	100	100	60[d,e]	100	100	100
Moxifloxacin	100	100	85[d,e]	100	100	100
Azithromycin	100	80	70[d,e]	100	100	NS
Clarithromycin	100	60	70[d,e]	70	100	NS
Clindamycin	93	0	75[d,e]	0	95	95

[a]Data are compiled from various studies.
[b]*Staphylococcus intermedius* may be mistakenly identified as methicillin-resistant *Staphylococcus aureus.*[26]
[c]Percentage of human bite isolates/percentage of animal bite isolates.
[d]*Fusobacterium canifelinum* is intrinsically resistant, whereas human *Fusobacterium nucleatum* is susceptible.
[e]Some peptostreptococci are resistant.
MSSA, Methicillin-susceptible *Staphylococcus aureus*; *NS,* not studied; *TMP-SMX,* trimethoprim-sulfamethoxazole; *V,* variable.

did 50% of dog bites (*P. canis,* 26%; *P. multocida* subsp. *multocida,* 12%).[14] In this study, other common aerobic organisms isolated from infected dog and cat bite wounds included *Streptococcus, Staphylococcus* species (including *Staphylococcus aureus*), and *Neisseria* species. Anaerobic organisms, when present, were almost always in mixed infections with aerobic organisms and commonly included *Fusobacterium, Porphyromonas,* and *Prevotella* species in dog bites and *Fusobacterium, Porphyromonas,* and *Bacteroides* species in cat bites.[14]

Table 315.1 lists common pathogens found in dog and cat bite wound infections. *Staphylococcus intermedius* is coagulase positive, can be mistaken for *S. aureus,* and is fourfold more common in canine flora[24,25] but possesses β-galactosidase activity, which differentiates it from *S. aureus.* It may masquerade as methicillin-resistant *S. aureus* (MRSA) owing to false-positive rapid penicillin-binding protein 2a latex tests,[26] although an increasing number (approximately 30%) of isolates may be resistant to oxacillin. MRSA has been cultured from a variety of companion animals, including cats, and has been documented to be transmitted from a healthy pet cat to humans, and the human strains and feline strains are indistinguishable.[27,28] MRSA may be a potential causative secondary invader, especially in patients who are not responding to initially administered antibiotics that often do not exhibit activity against MRSA and in those known to be colonized with MRSA.

Capnocytophaga canimorsus[17] is difficult to grow on most routine solid media but can grow on chocolate agar and heart infusion agar with 5% rabbit blood when incubated in CO_2 and a variety of liquid media, including BACTEC aerobic medium (Becton, Dickinson and Company, Franklin Lakes, NJ).[16] This species can be differentiated from other *Capnocytophaga* species by the presence of positive oxidase and catalase reactions.[17] Centers for Disease Control and Prevention (CDC) group DF-2–like strains have been classified as *Capnocytophaga cynodegmi.* CDC group M-5 has been classified as *Neisseria weaveri* and has been associated with dog bites.[29] CDC group EF-4a is now called *Neisseria animaloris,* and EF-4b is *Neisseria zoodegmatis. Haemophilus felis,* initially identified as *Aggregatibacter (Haemophilus) paraphrophilus,* requires factor V and CO_2 for growth and is common in cat nasopharyngeal flora.[30]

Bergeyella (previously designated as *Weeksella*) *zoohelcum* has been associated with bite cellulitis, sepsis, and meningitis.[31] Other new aerobic species include *Neisseria canis* from a cat bite,[32] *Flavobacterium* group IIb–like isolates from a pig bite,[33] *Actinobacillus lignieresii* and *Actinobacillus equi*–like bacterium from horse bites,[34] and CDC group NO1, a nonoxidative gram-negative rod[35] different from *Acinetobacter* species associated with dog and cat bites. Orf virus infection has been transmitted by a sheep bite.[36] When appropriate culturing techniques are used, anaerobes are isolated in up to 70% of animal bite wounds, almost always in mixed culture.[6,11,13] Approximately 50% to 60% of cat and dog bite wounds contain *Bacteroides tectus, Prevotella heparinolytica, Prevotella zoogleoformans, Prevotella bivia, Porphyromonas gingivalis, Porphyromonas canoris, Fusobacterium nucleatum,* and *Peptostreptococcus anaerobius.*[37–40] *Fusobacterium canifelinum* is an intrinsically fluoroquinolone-resistant species isolated from dog and cat bites.[41]

Little difference has been noted in the types of bacteria isolated from noninfected wounds seen early and infected wounds seen later.[6] All moderate-to-severe bite wounds, except those not clinically infected and more than a few days old, should be considered contaminated with potential pathogens.

Wounds inflicted by cats are frequently scratches or tiny but somewhat deep punctures located on the extremities and are at higher risk of becoming infected.[42] Deep puncture wounds over or near a joint, especially on the hands, may result in osteomyelitis and septic arthritis. *Pasteurella multocida* has been isolated from 50% to 70% of healthy cats and is a frequent pathogen in cat-associated wounds.[14,23,42] *Erysipelothrix rhusiopathiae* has also been isolated from cat bite wounds.[14] Cougar, tiger, and other feline bites also yield *P. multocida.*[34] Tularemia has likewise been transmitted by cat bites.[43] People are also bitten by a variety of other animals, including unusual domestic pets, farm animals, wild animals, aquatic animals, and laboratory animals.[4,44–47] Monkey bites cause more swelling and infection than do many other animal bites.[48] Old World monkeys may transmit a potentially lethal subtype B virus (herpesvirus simiae; see Chapter 141).[49] Case series or reports of various animal bites—including terrestrial mammals (e.g., monkeys, bears, pigs, ferrets, horses, sheep, Tasmanian devils); reptiles (e.g., snakes, Komodo dragons, lizards, iguanas, alligators, crocodiles); rodents (e.g., rats, guinea pigs, hamsters, prairie dogs); swans; and sharks with unusual isolates—have been reported.[50–53]

MANAGEMENT OF ANIMAL BITES

Table 315.3 notes the elements for the treatment of animal bite wounds. The most problematic elements of the management of wounds that are seen early include the following:

1. The use of preemptive antibiotics in wounds that are seen early but are not infected. As a routine, antimicrobial therapy is not advocated in every uninfected animal bite injury. However, because 85% of such wounds harbor potential pathogens and one cannot reliably predict which wounds will become infected, selected wounds are best treated with oral antimicrobial therapy with agents active against common bite pathogens (see Table 315.2) for 3 to 5 days. Recommendations about patient selection vary and are based on limited evidence.[54] In general, 3 to 5 days of preemptive antimicrobial therapy is advocated for acute moderate-to-severe injuries, especially those to the hand or face, in the presence of preexisting edema, and in immunocompromised hosts.[55] Longer courses may be considered for injuries that are severe or those penetrating joints or bony structures.
2. The decision to suture the wound. The role of primary wound closure in animal bites remains controversial. The literature on this topic has a number of limitations such as the time from injury to presentation, the extent and location of injuries, wound closure methods, and administration of antibiotics. For cosmetic and functional reasons, facial and neck wounds and extensive large wounds, especially those overlying the joints, are difficult to leave open and are usually sutured after irrigation coupled with antimicrobial prophylaxis. Limited studies are available to determine whether the risk of infection is increased with primary wound closure in animal bites.[56–58,59] In one study of 345 dog bite wounds, puncture wounds and wounds that were closed were more likely to become infected.[58] In a randomized controlled trial of primary closure versus nonclosure of dog bite wounds, the infection rate between the two groups was similar but primary suturing improved cosmetic appearance.[59] It is our experience and recommendation that, if cosmetically or functionally reasonable, small bite wounds can be left open and allowed to close by secondary intention. Larger wounds, especially those overlying the face, neck, and joints, may be sutured or loosely approximated and closed by delayed primary closure.

The most common causes of therapeutic failure are the following:

1. Failure to elevate the affected edematous extremity.
2. Inadequate antimicrobial therapy (see Table 315.2). Most fastidious animal pathogens are susceptible to penicillin or amoxicillin. Because of resistance of certain bacteria, including *P. multocida,* first-generation cephalosporins, dicloxacillin, erythromycin, clindamycin, and metronidazole should be avoided as a sole antimicrobial agent; however, they can be used in combination with antibiotics that have activity against *Pasteurella* species. In vitro data suggest that some fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin), trimethoprim-sulfamethoxazole, ertapenem, and second-generation oral cephalosporins (e.g., cefuroxime) are active against many bite isolates.[60,61–68] Ceftaroline, an anti-MRSA cephalosporin, has activity against *Pasteurella* species and other aerobic gram-positive isolates recovered from animal bite wounds.[69,70,71] Empirical regimens for marine- and freshwater-acquired infection should cover *Vibrio* and *Aeromonas* species, respectively, with agents such as third-generation cephalosporins (e.g., cefotaxime) and fluoroquinolones.

TABLE 315.3 Management of Bite Wounds

History
Animal Bite

Ascertain the type of animal, whether the bite was provoked or unprovoked, and the situation/environment in which the bite occurred. Follow rabies guidelines (see Chapter 163) for details on management of bites that carry a risk of rabies.

Patient

Obtain information on antimicrobial allergies, current medications, splenectomy, liver disease, or other immunosuppressive conditions.

Physical Examination

Assess nerve and tendon function along with signs and symptoms of infection.

Culture

Obtain aerobic and anaerobic cultures from infected wounds.

Irrigation and Débridement

Irrigate with water and débride devitalized or necrotic tissue.

Radiographs

Plain radiographs should be obtained if bony penetration is highly possible; radiographs can also provide a baseline for future evaluation of osteomyelitis.

Wound Closure

Primary wound closure is usually not advocated unless wounds are extensive and closure is necessary for cosmetic or functional reasons, especially large facial or neck wounds or those overlying the joints. When possible, delayed primary closure or allowing the wound to close by secondary intention is recommended.

Antimicrobial Therapy
Early Presenting (Uninfected) Wounds

Provide antimicrobial therapy for (1) moderate-to-severe injuries, especially if preexisting edema or significant crush injury is present; (2) bone or joint space penetration; (3) deep hand wounds; (4) immunocompromised patients (including those with advanced liver disease, asplenia, or chronic steroid therapy); (5) wounds adjacent to a prosthetic joint; and (6) wounds in close proximity to the genital area. In most cases, coverage should include *Pasteurella* (*Eikenella* in human bites), *Staphylococcus, Streptococcus,* and anaerobes, including *Fusobacterium, Porphyromonas, Prevotella,* and *Bacteroides* species (see Table 315.2).

Infected Wounds

Cover *Pasteurella* (*Eikenella* in human bites), *Staphylococcus*, *Streptococcus,* and anaerobes, including *Fusobacterium, Porphyromonas, Prevotella,* and *Bacteroides* spp. (see Table 315.2). The following oral antimicrobials can be considered in adults for most terrestrial animal and human bites:

- First choice: Amoxicillin-clavulanic acid 875/125 mg, 1 tablet by mouth twice daily
- Penicillin allergy:
  - Metronidazole 500 mg, 1 tablet by mouth three times daily *plus* trimethoprim-sulfamethoxazole, 1 double-strength tablet by mouth twice daily
  - Moxifloxacin 400 mg, 1 tablet by mouth daily
  - Doxycycline 100 mg, 1 tablet by mouth twice daily
- In adults in whom intravenous antibiotics are deemed necessary, single antimicrobial choices can include ampicillin-sulbactam, cefoxitin, ertapenem, or moxifloxacin.
- Empirical regimens for marine- and freshwater-acquired infection should also cover *Vibrio* and *Aeromonas* species, respectively, with agents such as third-generation cephalosporins (e.g., cefotaxime) and fluoroquinolones.

Hospitalization

Indications can include signs and symptoms of systemic toxicity.

Immunizations

Provide tetanus and rabies immunization, if indicated.

Elevation

Elevation may be required if preexisting edema is present.

Immobilization

For significant injures, consider immobilizing the extremity, especially the hands, with a splint.

Follow-Up

Patients should be reminded to follow up within 48 hours or sooner for worsening or unresolved symptoms.

Reporting

Reporting the incident to a local health department may be required in selected cases.

3. Failure to recognize osteomyelitis and septic arthritis. Long-standing pain or continuous diminished range of motion of a joint near a bite wound may be an indication of complications such as osteomyelitis or septic arthritis. Pain out of proportion to the severity of the wound may be an indicator of potential periosteal or joint penetration.

VENOMOUS SNAKEBITES

Venomous snakes, usually vipers (rattlesnakes, copperheads, cottonmouths, or water moccasins), bite approximately 8000 people in the United States yearly, and 5 or 6 of these die, usually children or the elderly, who receive either no or delayed antivenom therapy.[72] The majority of bites occur in young men in the southwestern United States between April and September.[72] Envenomation can cause extensive tissue destruction and devitalization that predisposes to infection from the snake's normal oral flora. Sparse data exist on the incidence and bacteriology of snakebite infections. In rattlesnakes, the oral flora appears to be fecal in nature because the live prey usually defecates in the snake's mouth coincident with ingestion. Common oral isolates include *Pseudomonas aeruginosa,* coagulase-negative staphylococci, and *Proteus* and *Clostridium* species.[73,74] Other potential pathogens isolated from rattlesnakes' mouths have included *Bacteroides fragilis* and *Salmonella*

enterica subsp. *arizonae* and *S. enterica* subsp. *diarizonae* (*Salmonella* groups IIIa and IIIb, respectively). *Crotalus* rattlesnake venom has innate, broad activity against aerobic gram-positive and gram-negative bacteria but not against anaerobes.[75,76] The role of empirical antimicrobial therapy for noninfected wounds is not well defined.

LIP WOUNDS AND PARONYCHIA

Wounds of the lips and paronychia and infections of the structure surrounding the nails account for most self-inflicted bite wounds. Paronychia is more frequent in children who suck their fingers and results from direct inoculation of the oral flora into the fingers. Brook[77] took cultures from 33 children with paronychia. Aerobes and anaerobes were each found in pure culture in 27% of cases, and mixed infection was found in 46% of cases. The most frequent aerobic organisms isolated were viridans streptococci, group A streptococci, *S. aureus, Haemophilus parainfluenzae, Klebsiella pneumoniae,* and *Eikenella corrodens.* The most frequently isolated anaerobic bacteria were *Bacteroides* species, *Fusobacterium* species, and gram-positive cocci. Therapy should include drainage, antibiotics for moderate-to-severe infections, and avoidance of further bacterial contamination.

HUMAN BITES

Human bites generally have higher complication and infection rates than do animal bites. In addition, transmission of human immunodeficiency virus, hepatitis B virus, or hepatitis C virus must be considered. Occlusional human bites may affect any part of the body but most often involve the distal phalanx of the long or index fingers of the dominant hand. About 10% to 20% of wounds are "love nips" to the breasts and genital areas.[20,78,79] Bites to the hand are often deep and more frequently become infected than do bites to other areas.[80] Bites may also be caused by, or be harbingers of, child abuse.[81]

Important prognostic factors for the development of infection include the extent of tissue damage, the depth of the wound and which compartments are entered, and the pathogenicity of the inoculated oral bacteria.[82–85] The typical patient is a young male who is assaulted by another young male. The first infectious symptoms occur approximately within the first 24 hours after injury, but patients often do not seek medical care until approximately a day or two after the incident.[85] Viridans streptococci, especially *Streptococcus anginosus,* are the most common wound isolates. *Staphylococcus aureus* infection occurs in 30% to 40% of wounds and is usually present in patients who present 3 to 4 days after the injury and have attempted self-débridement of the wound. There have been few reports of MRSA isolated from clenched-fist injuries.[86,87] Other reported isolates have included *Haemophilus influenzae, H. parainfluenzae, Aggregatibacter (Haemophilus) aphrophilus, Aggregatibacter (Haemophilus) paraphrophilus, Klebsiella* species, *Enterobacter cloacae, Prevotella* and *Peptostreptococcus* species, and *F. nucleatum.*[82,85] Up to 45% of the anaerobic gram-negative bacilli isolated from human bite wounds may be penicillin resistant and β-lactamase positive.[62,88] *Candida* species were found in 8% of patients in one study, although their pathogenicity was not determined.[85]

MANAGEMENT OF HUMAN BITES

Aerobic and anaerobic cultures should be obtained for infected wounds. Wounds should be irrigated with water and, if necessary, surgically débrided.[89] As much as possible, immobilization of the affected area, including splinting if necessary, and elevation should be instituted.

Initial antimicrobial therapy should provide coverage for *Staphylococcus, Streptococcus, Eikenella,* and anaerobic organisms such as *Prevotella, Fusobacterium,* and *Veillonella* species. Patients who present early with uninfected wounds may also be considered candidates for shorter duration of antimicrobial therapy. Amoxicillin-clavulanic acid as monotherapy or combination therapy with metronidazole plus trimethoprim-sulfamethoxazole can be used for the treatment of human bites. First-generation cephalosporins, dicloxacillin, erythromycin, clindamycin, and metronidazole are not effective as monotherapy because of inactivity of these agents against *E. corrodens.* In addition, first-generation cephalosporins, dicloxacillin, and erythromycin do not provide adequate activity against culprit anaerobic organisms.

Many patients (32% in one study[85]), especially those with extensive deep hand injuries, require hospitalization. Plain radiographs can be considered for wounds in close proximity to joints and bones. Similar to animal bite wounds, we do not recommend primary closure of small wounds, even for uninfected human bite wounds, especially those on the hands. If possible, approximation of the wound margins with adhesive tape or delayed primary closure (3–5 days) can be alternatives to primary closure in certain circumstances. Facial wounds may present a special situation because of the possibility of scarring and disfigurement and may require primary closure.

CLENCHED-FIST INJURIES

"Clenched-fist" injuries are traumatic lacerations that occur when one person strikes another in the mouth with a clenched fist. These injuries are most common over the third and fourth metacarpophalangeal joints of the dominant hand, but they may also occur over the proximal interphalangeal joints. Complications of this type of injury often include septic arthritis or osteomyelitis. These lacerations are commonly only 12 to 14 mm long but, despite their innocuous appearance, frequently lead to serious complications because of the proximity of the skin over the knuckles to the joint capsule and the potential spread of infection into subcutaneous tissues and web spaces.

Typically, patients who sustain a clenched-fist injury do not take good care of their wound, ignore it until a day or more after the injury, and then awaken with a painful, throbbing, and swollen hand. The swelling usually spreads proximally but not distally and results in decreased range of motion. A purulent discharge is often present. Lymphangitis, adenopathy, fever, or other signs of systemic infection are infrequent.

The bacteriology of clenched-fist injuries is similar to that of human bites and usually consists of the normal human oral flora.[10,82,90] Viridans streptococci, especially *S. anginosus,* are the most frequent isolates, but *S. aureus* may be present in 20% to 40% of cases. Anaerobic bacteria can be recovered in more than 55% of clenched-fist injuries, including *Prevotella* species, *F. nucleatum,* and *Peptostreptococcus* (including *Finegoldia magna*). *Eikenella corrodens* is an often overlooked but especially important pathogen in clenched-fist injury infections.[90–93] It has a prevalence rate of 59% in human gingival plaque[79] and may be isolated in 25% of clenched-fist injuries.[91] It can act synergistically with viridans streptococci and is a common cause of osteomyelitis. Although *E. corrodens* is susceptible to penicillin, it is resistant to penicillinase-resistant penicillins, clindamycin, and metronidazole and is variably resistant to cephalosporins.[62–66]

Initial physical examination is often limited because the majority of patients are in severe pain and unable to fully comply with the examination. Management should include irrigation and débridement when necessary. Elevation of the injured extremity is helpful in reducing associated swelling. Plain radiographs can be obtained to rule out fracture and to serve as a baseline for assessing osteomyelitis. Tetanus immunization should be administered when indicated. Secondary débridement to remove necrotic tissue or to drain abscesses may be required.

Empirical antimicrobial therapy should include coverage for *Staphylococcus, Streptococcus, Eikenella,* and anaerobes, including *Prevotella, Fusobacterium,* and *Veillonella* species. Failure of first-generation cephalosporins and penicillinase-resistant penicillins, when used alone, has been reported and is often due to their inactivity against *E. corrodens.*[91–96] If resistant gram-negative rods are isolated, therapy should be altered according to the results of culture. What role β-lactamase–positive *Prevotella* and *Porphyromonas* species will have in the selection of antimicrobial therapy has not been specifically determined.

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The complete reference list is available online at Expert Consult.

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316 Immunization

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The two most effective means of preventing disease, disability, and death from infectious diseases have been sanitation and immunization.[1] Both approaches antedated understanding of the germ theory of disease. Artificial induction of immunity began centuries ago with variolation, the practice of inoculating fluid from smallpox lesions into skin of susceptible persons. Although this technique usually produced mild illness without complications, spread of disease did occur, with occasional complications. In 1796, Jenner demonstrated that milkmaids who had contracted cowpox (vaccinia) were immune to smallpox. He inoculated the vesicular fluid from cowpox lesions into the skin of susceptible people and induced protection against smallpox, thus beginning the era of immunization.

Immunization, the act of artificially inducing immunity or providing protection from disease, can be active or passive. Active immunization consists of inducing the body to develop defenses against disease. This usually is accomplished by means of administration of vaccines or toxoids that stimulate the body's immune system to produce antibodies or cell-mediated immunity, or both, which protects against the infectious agent.[2] Passive immunization consists of providing temporary protection through administration of exogenously produced antibody. Two situations in which passive immunization commonly occurs are through transplacental transfer of antibodies to the fetus, which may provide protection against certain diseases for the first 3 to 6 months of life, and injection of immunoglobulins for specific preventive purposes. A more detailed description of the immune mechanisms involved follows.

Immunizing agents include vaccines, toxoids, and antibody-containing preparations from human or animal donors. Several important definitions are provided here.

1. *Vaccine:* a suspension of attenuated, live, or killed microorganisms (bacteria, viruses, or rickettsiae), or fractions thereof, that is administered to induce immunity and thereby prevent infectious disease.
2. *Toxoid:* a modified bacterial toxin that has been rendered nontoxic but retains the ability to stimulate formation of antitoxin.
3. Immunoglobulin products include standard immune globulin (IG) for intramuscular (IM) use, hyperimmune globulins that are available for IM and/or intravenous (IV) use, standard immune globulin intravenous (IGIV), immune globulin subcutaneous (IGSC), antibodies of animal origin, and monoclonal antibodies. IG is a sterile, concentrated protein solution containing antibodies from human blood that reflect the infectious and immunization experience of the population from whose plasma the IG was prepared. IG contains 15% to 18% protein, consisting primarily of the immunoglobulin G (IgG) fraction (90%) with trace amounts of immunoglobulin A (IgA) and immunoglobulin M (IgM). IG primarily is indicated for routine protection of certain immunodeficient persons and for passive immunization against measles and hepatitis A. IGIV is indicated primarily for replacement therapy in immunoglobulin G (IgG) deficiency and pediatric human immunodeficiency virus (HIV) infection, treatment of Kawasaki disease, and idiopathic thrombocytopenic purpura. IGSC is indicated primarily for treatment of antibody deficiency.
4. Specific immunoglobulin: special preparations are obtained from donor pools preselected for high antibody content against a specific disease—for example, hepatitis B immune globulin (HBIG), varicella-zoster immune globulin (VariZIG), rabies immune globulin (RIG), tetanus immune globulin (TIG), and botulism IGIV used to treat infant botulism.

The constituents of immunizing agents include the following:

1. *Suspending fluid:* This frequently is as simple as sterile water or saline, but it may be a complex fluid containing small amounts of proteins or other constituents derived from the medium or biologic system in which the immunizing agent is produced (serum proteins, egg antigens, cell culture–derived antigens).
2. *Preservatives, stabilizers, antibiotics:* These components of vaccines are used (1) to inhibit or prevent bacterial growth in viral culture or the final product (preservatives and antimicrobial agents) or (2) to stabilize the antigen against changes in temperature and/or pH (stabilizers). They include materials such as mercurials (thimerosal), gelatin, and specific antimicrobial agents. Allergic reactions may occur if the recipient is sensitive to any of these additives. Preservatives are required for multidose vaccine formulations or vials to prevent bacterial or fungal growth, should they be introduced on repeated entry into the vial. Thimerosal, an ethylmercury-containing preservative, has been the major preservative used in vaccines around the world. A review of the mercury content of vaccines in the United States in 1999 indicated that some children had received quantities of ethylmercury from thimerosal in excess of some federal guidelines for methyl mercury. As a precautionary measure, thimerosal as a preservative was removed from most vaccines in the immunization schedule to the extent feasible.[3] However, some of these vaccines may contain trace amounts (www.fda.gov/cber/vaccine/thimerosal.htm). Subsequent studies of potential adverse consequences of thimerosal have not demonstrated significant harm from its use in vaccines. It is likely had these data been available in 1999, the United States would not have made the decision to remove thimerosal from vaccines for children.[4,5] Some vaccines for children contain other preservatives (e.g., 2-phenoxyethanol) or do not need a preservative because they are packaged in single-dose vials. Influenza vaccines in multidose vials used in adults and combined adult-type tetanus and diphtheria toxoids (Td) contain thimerosal as a preservative.[6]
3. *Adjuvants:* An aluminum salt is used in some vaccines to enhance the immune response to vaccines containing inactivated microorganisms or their products (e.g., toxoids and hepatitis B vaccine). Bivalent human papillomavirus vaccine contains an aluminum salt combined with monophosphoryl lipid A. An oil-in-water adjuvant is used in one influenza vaccine licensed in the United States and in other vaccines licensed outside the United States. Recombinant zoster vaccine (RZV) contains monophosphoryl lipid A combined with saponin. Vaccines with such adjuvants should be injected deeply into muscle masses

because subcutaneous or intracutaneous administration can cause local irritation, inflammation, granuloma formation, or necrosis.[7]

IMMUNOLOGIC BASIS OF VACCINATION

Two major approaches to active immunization have been used: use of live (attenuated) infectious agents, and use of inactivated, or detoxified, agents or their extracts. For many diseases (including influenza, poliomyelitis, typhoid, and measles), both approaches have been used. Live-attenuated vaccines are believed to induce an immunologic response more similar to that resulting from natural infection than do killed vaccines. Inactivated or killed vaccines can consist of inactivated whole organisms (e.g., hepatitis A vaccine), detoxified exotoxin (e.g., diphtheria and tetanus toxoids), soluble capsular material either alone (e.g., pneumococcal polysaccharide), or covalently linked to carrier protein (e.g., *Haemophilus influenzae* type b [Hib] conjugate vaccines), chemically purified components of the organism (e.g., acellular pertussis, inactivated influenza vaccines [IIVs]), or recombinant proteins (e.g., hepatitis B virus [HBV], serogroup B meningococcal vaccine [MenB-FHbp/MenB-4C], virus-like particles [VLPs; e.g., human papillomavirus (HPV)], or RZV).

Determinants of Immunogenicity

The immune system is complex, and antigen composition and presentation are critical for stimulation of the desired immune response. Immunogenicity is determined not only by the chemical and physical states of the antigen but also by the genetic characteristics of the responding individual, the physiologic condition of the individual (e.g., age, nutrition, sex, pregnancy status, stress, infections, immune status), and the manner in which the antigen is presented (route of administration, dose or doses and timing of doses, and presence of adjuvants).[8,9]

Live Versus Killed or Subunit Vaccines

Because the organisms in live vaccines multiply in the recipient, antigen production increases logarithmically until controlled by the immune response induced by the antigen. The live-attenuated viruses (e.g., measles, rubella) generally are believed to confer lifelong protection in those who respond. By contrast, killed vaccines (e.g., diphtheria, tetanus, rabies, typhoid) generally do not induce permanent immunity with one dose, requiring repeated vaccination and subsequent boosters for development and maintenance of high levels of antibody. Exceptions to this general rule may include hepatitis B vaccine, for which long-term immunologic memory has been demonstrated for approximately 30 years after vaccination[10], and inactivated polio vaccine (IPV), for which the duration of immunity is unknown. Although the amount of antigen initially introduced is greater with inactivated vaccines, multiplication of organisms in the host results in a cumulatively greater antigenic input with live vaccines.

Most vaccines include protein antigens, which generate a T-lymphocyte–dependent immune response. This response induces immunologic memory, booster effects with repeat administration, and good immunogenicity in all age groups. However, purified bacterial capsular polysaccharide vaccines induce a T-lymphocyte–independent immune response, which does not lead to immune memory and cannot be boosted with repeated injections.[11] Polysaccharide vaccines have poor immunogenicity in infants and young children. Covalent linkage of the polysaccharide to a carrier protein converts it from a T-lymphocyte–independent to a T-lymphocyte–dependent antigen (e.g., conjugated Hib, pneumococcal, and meningococcal vaccines), which produces a good immune response in infants and children.

Dose

The amount of antigen determines the immune response. Presentation of an insufficient amount of antigen may result in no immune responsiveness. There is usually a dose-response curve relationship between antigen dose and peak response obtained beyond a threshold; however, responsiveness may reach a plateau, failing to increase beyond a certain level despite increasing doses of vaccine.

Adjuvants

The immune response to some inactivated vaccines or toxoids can be enhanced by addition of adjuvants, such as aluminum salts (either alone or in combination with monophosphoryl lipid A).[11a] Adjuvants are particularly useful with inactivated products, such as diphtheria and tetanus toxoids, acellular pertussis vaccines (DTaP), and hepatitis B vaccine. The mechanism of enhancement of antigenicity by adjuvants is not well defined; however, it is increasingly clear that adjuvants activate the innate immune system through pathogen-associated molecular patterns (PAMPs). Licensed adjuvants for use in humans in the United States include aluminum salts alone or with monophosphoryl lipid A, squalene-based oil-in-water emulsion, and synthetic oligodeoxynucleotides.[12]

Route of Administration

The route of administration can determine the nature of the immune response to a vaccine or toxoid. IM or subcutaneous delivery results in a predominantly IgG response. Oral (e.g., rotavirus vaccine and typhoid vaccine Ty21a) or nasal (e.g., live-attenuated influenza vaccine [LAIV]) vaccination is more likely to result in production of local IgA compared with IM injection, although systemic IgG also is induced. The immunogenicity of some vaccines is reduced when not given by the recommended route. For example, administration of hepatitis B vaccine subcutaneously into the fatty tissue of the buttock was associated with substantially lower seroconversion rates than injection intramuscularly into the deltoid muscle.[13]

Most vaccines are administered either intramuscularly or subcutaneously.

Age

The immune response to a vaccine varies with age. Although children and young adults usually respond well to all vaccines, differences in response capability exist during early infancy and older age. The presence of high levels of passively acquired maternal antibody in the first few months of life impairs the initial immune response to some killed vaccines (e.g., hepatitis A vaccine,[14] diphtheria toxoid) and many live vaccines (e.g., measles). Prematurely born infants of low birth weight should be immunized at the usual chronologic age in most cases. Infants with birth weights less than 2000 g may require modification of the timing of hepatitis B immunoprophylaxis, depending on maternal hepatitis B surface antigen (HBsAg) status.[15] Some studies have suggested a reduced immune response in very-low-birth-weight infants (<1500 g) immunized according to the usual schedule; however, antibody concentrations achieved usually are protective. In older adults, the response to antigenic stimulation may be diminished (e.g., influenza, hepatitis B vaccines). This has led to the development of higher-potency influenza vaccines for use in the elderly.

COMPONENTS OF THE IMMUNE RESPONSE

The immune response traditionally is divided into two components: the innate immune response, which is rapid, nonspecific, and serves as an immediate first line of defense against an infection, and the adaptive immune response, which develops over a matter of days, is specific for the foreign antigen, and results in long-term immune memory. The latter protects the host against subsequent challenge with the same or immunologically similar pathogens and is the underlying principle of vaccination. The innate immune response is mediated by natural killer (NK) cells, which recognize and kill virally infected cells; by complement, which is activated by components of bacterial cell walls; and by phagocytes, including macrophages and dendritic cells (DCs), which ingest microorganisms and foreign particulates.[16] The adaptive immune response relies on antigen-presenting cells (APCs), such as DCs, for activation and is mediated by T and B lymphocytes. T lymphocytes can be divided into CD4 (helper) and CD8 (cytotoxic) lymphocytes and are responsible for cell-mediated immune responses. CD4 helper T lymphocytes can be further subdivided into Th1 lymphocytes, which predominantly lead to cell-mediated responses, and Th2 lymphocytes, which predominantly lead to humoral responses. B lymphocytes produce antibody specific for the immunizing agent and require CD4–T-lymphocyte help. Interactions between APCs, helper T-lymphocytes, and B-lymphocytes involve class II major histocompatibility complex (MHC) antigens, whereas interactions between cytotoxic T lymphocytes and their target involve MHC class I antigens.[17] Soluble mediators or cytokines are secreted by

all cell types and serve as activation and differentiation factors for different cell lineages. These include interleukins, interferons, and others.[18] A further class of CD4 T lymphocytes (Treg) plays an essential role in the regulation of the adaptive immune response.[19]

The innate immune response is able to respond differently to different types of pathogens, and these differential responses help determine the nature of the subsequent adaptive response.[20] Pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and others, encoded in the germline recognize PAMPs and contribute to immune activation by inducing proinflammatory cytokines, which in turn modulate the adaptive immune response. As alluded to earlier, this has significant implications for adjuvant development.[21,22]

MOBILIZATION OF THE ADAPTIVE IMMUNE RESPONSE

On exposure to an infectious organism or a vaccine, the innate immune system is mobilized through APC recognition of PAMPs that are present either in the organism or in the adjuvant. Activated APCs (macrophages and DCs) secrete proinflammatory cytokines and chemokines, which recruit other leukocytes to the site of infection. When activated, DCs migrate to the draining lymph nodes, where they interact with T lymphocytes through the MHC-peptide complex.[23] Once the organism or antigen is internalized, it is killed and broken down into peptides. These peptides are transported to the cell surface through membrane trafficking and bind to MHC class I or class II molecules. MHC class I molecules are able to bind peptides that are 8 to 10 amino acids in length, whereas MHC class II molecules are more permissive, binding peptides of 13 amino acids and greater.[24]

The first step in the induction of a T-lymphocyte–dependent antibody response is the activation of naïve CD4 helper T lymphocytes by presentation of an antigen by phagocytes or DCs. The T-lymphocyte receptor recognizes the MHC-peptide complex, and this recognition triggers secretion of cytokines, which stimulate maturation of naïve helper T lymphocytes. In the presence of interleukin-12 (IL-12), Th1 lymphocytes will differentiate, and these in turn will secrete IL-2 and interferon-γ. In the presence of IL-4, Th2 lymphocytes will differentiate and secrete IL-4 and IL-5. These two cytokines are essential for the differentiation and maturation of B lymphocytes into antibody-secreting plasma cells.

Naïve B lymphocytes recognize a specific antigenic epitope on native antigen through the immunoglobulin receptor on their surface but are unable to differentiate into antibody-secreting lymphocytes without T-lymphocyte help. A given B lymphocyte can be activated only by a T lymphocyte responding to the same antigen, though not necessarily to the same epitope. A helper T lymphocyte will recognize the MHC class II complex on the surface of the B lymphocyte and deliver a signal for B-lymphocyte differentiation. This leads to B-lymphocyte proliferation and maturation in a clonal manner. Class switching (from IgM to IgG and IgA) and affinity maturation occur, and antigen-specific plasma cells develop. However, not all B lymphocytes become plasma cells. Some mature into memory B cells, which are long-lived and form the basis of the rapid secondary response on the next encounter with the pathogen.[25] Although the mechanism of maintenance of these cells is not clear, the ability to mount a strong secondary response after many years argues for a homeostatic mechanism that regulates these cells. The antibodies formed after vaccination express a variety of antigen-binding specificities (i.e., recognize different structures on a complex multideterminant antigen), reflecting the sum of the large number of individual clonal B-lymphocyte responses that make up an antibody response.

Antibodies mediate protection through a variety of mechanisms. They may inactivate soluble toxic protein products of bacteria (antitoxins), facilitate intracellular digestion of bacteria by phagocytes (opsonization), interact with components of serum complement to damage the bacterial membrane with resultant bacteriolysis (lysins), prevent infectious virus from infecting cells (neutralizing antibodies), or interact with components of the bacterial surface to prevent adhesion to mucosal surfaces (anti-adhesins). Antibodies cannot readily reach intracellular sites of infection, the sites of viral and some bacterial replication. However, antibodies are effective against many viral diseases through interaction with viruses before initial intracellular penetration occurs and through prevention of locally replicating viruses from disseminating from the site of entry to an important target organ, as in the spread of poliovirus from the intestine to the central nervous system or rabies from a puncture wound to peripheral neural tissue.

Virally infected cells can be killed by cytotoxic CD8 T lymphocytes. As the virus replicates in a cell, viral proteins are processed and presented on the cell surface as an MHC class I–peptide complex, which is then recognized by cytotoxic T lymphocytes. Cells infected with intracellular bacteria, such as *Mycobacterium leprae,* are recognized and killed in the same way.

UNANTICIPATED RESPONSES

Independent of antibody production, stimulation of the immune system by vaccination may, on occasion, elicit a hypersensitivity response. Killed measles vaccine, used in the United States between 1963 and 1967, induced incomplete humoral immunity and cell-mediated hypersensitivity, resulting in development of a syndrome of atypical measles in some children on subsequent exposure to measles.[26] In addition, some antibodies produced may not be protective but block the reaction of protective antibodies with antigens, inhibiting the body's defenses. Some vaccines may induce immunologic tolerance that results in blunting of the immune response on subsequent exposure to the antigen (e.g., meningococcal polysaccharide vaccine [MPSV]).[27] Concerns have been raised that immunizations might induce autoimmune disorders. However, careful reviews of both the possible biologic mechanisms and epidemiologic evidence generally have failed to confirm vaccines as causes of these disorders.[28] The evidence was insufficient to accept or reject a causal relationship between vaccines and allergic disorders, particularly asthma.[29] A subsequent epidemiologic study failed to show an association between vaccines and asthma.[30] Concerns also have been raised that the number of antigens in the current vaccine schedule might overwhelm an infant's immune system, leading to chronic diseases and predisposing to other serious infections.[31] As a result of removal of whole-cell pertussis vaccine and smallpox vaccine from the current immunization schedule, the number of immunogenic proteins and polysaccharides a child is exposed to today is actually smaller than in the past. Estimates suggest that an infant is capable of responding to 10,000 vaccine antigens simultaneously.[32] The Institute of Medicine (IOM) concluded that available evidence favored rejection of a causal relationship between vaccines and increased risk for infections. IOM also concluded that available evidence favored rejection of a causal relationship between vaccines and type 1 diabetes mellitus.[29]

TEMPORAL COURSE OF THE IMMUNE RESPONSE

On first exposure to a vaccine, a primary response is induced, and a protective immune response will develop in about 2 weeks. Circulating antibodies do not usually appear for 7 to 10 days, and the immunoglobulin class of the response changes over this period of time. Early-appearing antibodies are usually of the IgM class and of low affinity; late-appearing antibodies are usually of the IgG class and display a high affinity. IgM antibodies may fix complement, making lysis and phagocytosis possible. As the titer of IgG rises during the second week (or later) after immunogenic stimulation, the IgM titer falls. IgG antibodies are produced in large amounts and function in the neutralization, precipitation, and fixation of complement. The antibody titer frequently reaches a peak in about 2 to 6 weeks and then falls gradually. The switch from IgM synthesis to predominantly IgG synthesis in B lymphocytes is mediated by T-lymphocyte help. Uncommonly, people may not respond to a vaccine, experiencing a primary vaccine failure. This may be due to a genetic inability to respond to vaccine, but other factors are involved. For example, almost all children who do not respond immunologically to the first dose of measles-mumps-rubella (MMR) vaccine will acquire measles immunity after a second dose.[32]

After a second exposure to the same antigen, a heightened humoral or cell-mediated response, an anamnestic response, is observed. These secondary responses occur sooner than the primary response, usually within 4 to 5 days, and depend on a marked proliferation of antibody-producing cells or effector T lymphocytes. Effector T lymphocytes, also

known as memory T cells, are T lymphocytes that have a memory of a previous immune response. The secondary response depends on immunologic memory after the first exposure mediated by both T and B lymphocytes. Infection with measles or varicella vaccine strains has been shown to evoke a cell-mediated in addition to a humoral response.

Many pathogens replicate at mucosal surfaces before host invasion and may induce secretory IgA along the respiratory and gastrointestinal mucous membranes and at other localized sites (e.g., polio, rubella, influenza, rotavirus). IgA antibodies are efficient at virus neutralization (e.g., polio), fix complement through the alternative pathway (e.g., cholera), prevent adsorption of organisms to the intestinal wall (e.g., *Escherichia coli,* cholera), and can lyse gram-negative bacteria (with the aid of both complement and lysozyme).[34] Current parenteral, especially inactivated, vaccines rarely induce high levels of secretory IgA antibodies.

MEASUREMENT OF THE IMMUNE RESPONSE

Response to vaccines is often gauged by measuring the appearance and concentration of specific serum antibodies.[35] For some viral vaccines, such as those for measles and rubella, the presence of circulating antibodies correlates with clinical protection. Although this has served as a dependable indicator of immunity, seroconversion measures only the humoral parameter of the immune response. Secondary vaccine failure occurs when an individual who previously had developed an adequate immune response loses protection over time. This waning immunity can be attributed to a loss of long-lived memory B or T cells in the absence of repeated exposure to the pathogen. Evaluating persistence of antibody has been used to determine the duration of vaccine-induced immunity for those diseases for which antibody is judged to be a good correlate of protection. However, the absence of measurable antibody may not mean that the individual is unprotected. Although a fall in titer occurs, on revaccination or challenge a rapid secondary response is observed in IgG antibodies, with little or no detectable IgM response, suggesting persistent protection. With some vaccines and toxoids, the mere presence of antibodies is not sufficient to ensure clinical protection, but rather a minimal circulating level of antibody is required (e.g., 0.01 IU/mL of tetanus antitoxin). Functional antibody is important in assessing immunity to bacterial polysaccharide vaccines. Opsonophagocytic activity is considered the assay of choice for monitoring vaccine response[36] because the vaccines also induce nonfunctional antibodies that are detected in standard enzyme immunosorbent assay (EIA), although the EIA can be used as a proxy. Some immune responses may not in themselves confer immunity but may be sufficiently associated with protection that they remain useful proxy measures of protective immunity (e.g., vibriocidal serum antibodies in cholera). The measurement of cell-mediated immunity, which would be helpful in assessing the degree of ongoing protection in many circumstances, usually is limited to research laboratories and to only a few vaccines.

VACCINE DEVELOPMENT

Most vaccines in use today have been developed by empirical techniques.[37] For live-attenuated viral vaccines, organisms are repeatedly passaged in various tissue culture cell lines to reduce virulent properties while maintaining immunogenicity. Inactivated vaccines usually have been developed by growing microorganisms, followed by concentration, purification, and inactivation, not necessarily in that order. Component vaccines usually are derived from chemical separation of the needed component from the parent organism.

Future vaccines are likely to be derived from new methods of biotechnology, especially recombinant techniques. Currently available hepatitis B vaccines were developed by cloning the HBsAg gene into yeast, leading to synthesis of HBsAg within the yeast cell. Other new approaches for producing vaccines include live vectors, in which one or more genes encoding critical determinants of immunity from pathogenic microorganisms are inserted into the genome of the vector, followed by the administration of the vector as a component of the vaccine. These vectors may include viruses, such as poxviruses (vaccinia or canarypox), or bacteria, such as *Salmonella* or bacillus Calmette-Guérin (BCG). Additional newer techniques include microencapsulation of critical antigens in polymers, which can lead to sustained release or pulse release over prolonged periods, mimicking the effect of multiple injections of an antigen over a several-month interval. New technologies also include use of nucleic acids, which encode critical antigens. Injection of the DNA, combined with administration of a protein at a later point in time, leads to production of antigen without risk for producing whole infectious organisms. LAIV was developed using genetic reassortment of the genes encoding two of the surface glycoproteins from wild virus isolates with six other genes contributed from a cold-adapted, temperature-sensitive influenza strain. Similar techniques were used to develop bovine rotavirus vaccines.[38] Last, newer technologies focus on the development of adjuvants to help stimulate the immune response.[39]

General Principles of Immunization

Introduction and widespread use of vaccines resulted in global eradication of smallpox, elimination of poliomyelitis caused by wild viruses in the United States and most of the countries of the world, and dramatic reductions in the incidence rates of other diseases (Tables 316.1 and 316.2). Measles and rubella are no longer considered endemic in the Americas.[40,41] Measles and rubella have been reduced by greater than 90% in developed countries and, if global vaccination efforts can be sustained, may eventually be eliminated from many countries. The World Health Assembly had established a goal to eradicate polio from the world by the end of 2000.[42] Although that goal was not achieved, by the end of 2016 only three countries in the world had never interrupted wild poliovirus transmission (www.polioeradication.org).[43] The last case of polio caused by wild virus in the Western Hemisphere was in 1991; four of the six regions of the World Health Organization (WHO)—American, European, Southeast Asian, and Western Pacific—have been certified free of wild poliovirus.[44–46] Global use of hepatitis B vaccine in infants may have an impact comparable to that of other vaccines in childhood. Hib vaccines have only recently come into widespread use, but disease incidence has been reduced markedly in many developed countries.[47–50] Reductions based on historical estimates have been achieved for congenital rubella syndrome and Hib invasive disease.[51] Despite these successes, cases of measles and pertussis continue to occur in the United States (see Table 316.1). All measles cases are the result of

TABLE 316.1 Representative 20th-Century Morbidity Cases in 2017 and Change

DISEASE	20th CENTURY ANNUAL MORBIDITY[a]	2017 REPORTED CASES[b]	PERCENT DECREASE
Smallpox	29,005	0	100%
Diphtheria	21,053	0	100%
Measles	530,217	120	>99%
Mumps	162,344	6109	96%
Pertussis	200,752	18,975	91%
Polio (paralytic)	16,316	0	100%
Rubella	47,745	7	>99%
Congenital rubella syndrome	152	5	99%
Tetanus	580	33	94%
Haemophilus influenzae	20,000	33[c]	>99%

[a]From Roush SW, Murphy TV; Vaccine-Preventable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA*. 2007;298:2155–2163.
[b]Centers for Diease Control and Prevention. *National Notifiable Diseases Surveillance System, 2017 Annual Tables of Infectious Disease Data.* Atlanta: CDC Division of Health Informatics and Surveillance; 2018.
[c]*H. influenzae* type b (Hib) at <5 years of age. An additional 10 cases of Hib are estimated to have occurred among the 203 notifications of *H. influenzae* (<5 years of age) with unknown serotype.

TABLE 316.2 Representative 20th-Century Morbidity Cases in 2016 and Change

DISEASE	PREVACCINE ERA ANNUAL ESTIMATE	2016 ESTIMATE (UNLESS OTHERWISE SPECIFIED)	PERCENT DECREASE
Hepatitis A	117,333[a]	4000[b]	97%
Hepatitis B (acute)	66,232[a]	20,900[b]	68%
Pneumococcus (invasive)			
All ages	63,067[a]	30,400[c]	52%
<5 yr of age	16,069[a]	1700[c]	89%
Rotavirus (hospitalizations, <3 yr of age)	62,500[d]	30,625[e]	51%
Varicella	4,085,120[a]	102,128[f]	98%

[a]Rousch SW, Murphy TV; Vaccine-Prevenatable Disease Table Working Group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA.* 2007;298:2155–2163.
[b]Centers for Disease Control and Prevention. Viral hepatitis surveillance—United States, 2016.
[c]Centers for Disease Control and Prevention. Active bacterial core surveillance 2016 (unpublished).
[d]Cortese MM, Parashar UD; Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* 2009;58(RR-2):1-25.
[e]New Vaccine Surveillance Network 2017 data (unpublished); US rotavirus disease now has a biennial pattern.
[f]Centers for Disease Control and Prevention. Varicella Program 2017 data (unpublished).

international importations, some of which spread within the US population, whereas pertussis remains endemic.

Pneumococcal conjugate vaccines (PCVs) have had a marked impact on invasive pneumococcal disease in countries where they have been used widely in children.[52,53] Decreases in disease were observed not only in children but also in adults, who presumably are not being exposed to infectious children because the latter have had vaccine-type pneumococcal carriage eliminated by vaccination.

Modern vaccines are safe and generally effective. Each vaccine is associated with some adverse effects, which are usually mild, and only rarely life-threatening. No vaccine is 100% effective. Consequently, some persons who have received a complete vaccine or toxoid series may acquire disease after exposure. The effectiveness of vaccines recommended for universal use in children is well defined, with most vaccines protecting more than 80% of recipients after a primary series.

In most studies, acellular pertussis vaccines range in efficacy from 63% to 99% during the first few years after vaccination.[54,55,56] One dose of varicella vaccine is 95% or more effective against severe varicella but is less effective against varicella of any severity.[57,58] With some vaccines, antibody may wane, but immunologic memory is sufficient to prevent disease if the individual is exposed (e.g., hepatitis B).[59] However, for some diseases with short incubation periods (e.g., meningococcal disease), waning antibody after vaccination is associated with waning protection. This waning has occurred with meningococcal conjugate vaccines, resulting in the need for modification of the originally recommended vaccine schedule with the addition of a second dose.[60] Another example of loss of durability has occurred with the Tdap (tetanus, diphtheria, and acellular pertussis) and DTaP (diphtheria and tetanus toxoids and acellular pertussis) vaccines, in which protection begins to wane a few years after administration.[61–63]

Although high efficacy of each of these vaccines is apparent, there has been substantial controversy about reported adverse events temporally associated with vaccination. Because of these controversies, the IOM reviewed available information, and between 1991 and 2013 published multiple reports.[64–67] In the 1991 and 1994 studies, the IOM found insufficient evidence to indicate a causal relationship between DTaP and permanent neurologic damage, and the IOM favored rejection of a causal relationship between combined diphtheria and tetanus toxoids (DT) and encephalopathy and between conjugate Hib vaccines and early-onset Hib disease. The IOM also concluded that the evidence establishes a causal relationship between MMR and thrombocytopenia, between rubella vaccine and acute arthritis, between DT and brachial neuritis, and between a variety of vaccines and anaphylaxis. In 2004 the IOM reported the relationships between a variety of disorders and vaccines (www.iom.edu/Activities/PublicHealth/ImmunizationSafety.aspx).[67,68] The IOM panel concluded that evidence did not support a relationship between MMR or thimerosal and autism, between multiple immunizations and heterologous infections, between multiple immunizations and type 1 diabetes, or between hepatitis B vaccine and incident or relapsed multiple sclerosis. In 2011 the IOM looked at the relationship of vaccines with many conditions that are reported after vaccination and, in most cases, found no evidence to support such associations. The IOM specifically found evidence to support rejection of an association between MMR vaccine and autism. Likewise, it found evidence to reject an association between IIV and asthma. In 2013 the IOM studied the impact of giving multiple vaccines to an individual in accordance with Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP), and American Academy of Family Physicians (AAFP) recommendations and found no evidence of a safety concern with adhering to the childhood schedule.[69]

Development of vaccines consists of four phases. Initial studies typically are conducted in animal models to demonstrate protection (or at least production of antibodies) and relative safety. These are called *preclinical studies*. Then limited numbers of doses are administered to humans to demonstrate antibody production and safety (phase I). After this phase, clinical trials in humans are conducted in a limited number of people to select optimal vaccine schedules and to demonstrate further safety (phase II). Larger trials are conducted to demonstrate efficacy (phase III). Because of their limited size, these field trials can be expected to detect adverse events that occur only relatively frequently (1 per 1000 doses or higher). After clinical trials, licensure may be sought. In the United States, vaccine production is regulated by the Center for Biologics Evaluation and Research (CBER) of the US Food and Drug Administration (FDA). Only after a vaccine has been found to be safe and effective is it licensed for use. Postmarketing surveillance (phase IV) is necessary to detect rare adverse events associated with vaccination and to monitor safety of vaccination practices, such as simultaneous immunization.

There is no direct evidence of risk to the fetus when pregnant women are given vaccines routinely recommended during pregnancy by ACIP. The benefit of IIV to the pregnant mother and the fetus outweighs any risk of vaccination to the mother or the fetus.[70] Some vaccines are recommended for pregnant women in order to provide passive immunity to their fetuses so that when the child is born, the child is protected before active immunity can be induced through direct vaccination of the infant. Thus, Tdap and nonlive influenza vaccine is recommended during each pregnancy.[6,70,71] Most live-virus vaccines induce viremia, which at least theoretically could result in infection of the fetus, so live-virus vaccines are not administered to pregnant women except in unusual circumstances, when potential benefit clearly outweighs the risk.

The decision to administer a vaccine involves assessment of risks of disease, benefits of vaccination, and risks associated with vaccination. The relative balance of risks and benefits may change over time; consequently, continuing assessment of vaccines is essential. Recommendations for vaccine use are developed by several different bodies: ACIP develops recommendations for vaccines for children, adolescents, and adults in the civilian population in conjunction with professional societies. These recommendations are updated annually and are available at www.cdc.gov/vaccines/schedules/hcp/index.html. Since 2011, the ACIP process for making vaccine recommendations has included a careful evaluation of the strength of the evidence supporting recommendations, which is known as GRADE (Grading of Recommendations, Assessment, Development and Evaluation; www.cdc.gov/vaccines/acip/recs/GRADE/

table-refs.html). The Committee on Infectious Diseases (COID) of the AAP (the "Red Book " committee) develops recommendations for vaccine use in infants, children, and adolescents.[72] Since 1995, ACIP, the AAP, and the AAFP have collaborated to issue a harmonized childhood immunization schedule, which is updated annually. The childhood immunization schedule consists of three parts: one based on age, a second that is a catch-up schedule for children who are behind on their immunizations, and a third that is based on underlying medical conditions (Fig. 316.1).[73] ACIP also annually issues an adult immunization schedule in two parts: (1) recommendations based on age group and (2) recommendations based on underlying medical conditions (Figs. 316.2 to 316.4), which can be found at www.cdc.gov/vaccines/schedules/hcp/adult.html. The Adult Immunization Schedule for 2018 was harmonized with the AAFP, the American College of Obstetricians and Gynecologists, the American College of Physicians, and the American College of Nurse-Midwives.

CURRENTLY AVAILABLE IMMUNIZING AGENTS

Tables 316.3 and 316.4 list currently licensed immunizing agents and immunoglobulins. This section presents brief information about most immunizing agents, primary indications for use, relative efficacy, number and spacing of doses required, known adverse effects, and precautions and contraindications for use. Package inserts and specific references and recommendations should be consulted for more detailed information. In addition to these licensed products, several other vaccines are under development and may become available.

Vaccines

Adenovirus Vaccine

Adenovirus vaccine contains live adenovirus types 4 and 7. It is recommended only for military personnel who are 17 through 50 years of age. It is taken as two oral tablets (one dose). Serious adverse events possibly associated with receipt of vaccine included hematuria, gastroenteritis, gastritis, pneumonia, and hematochezia.

Anthrax Vaccine

Anthrax vaccine (AVA) is prepared from microaerophilic cultures of an avirulent nonencapsulated strain of *Bacillus anthracis.* The vaccine is a cell-free filtrate that contains a mixture of components, including protective antigen (the antigen that is thought to confer immunity) and other bacterial products adsorbed to aluminum hydroxide. Because of concerns about potential use of *B. anthracis* as a biologic warfare agent, vaccination of selected members of the US Armed Forces was begun in 1998. After the intentional release of anthrax in the United States in 2001, anthrax vaccine was recommended for civilians at risk for repeated exposure to *B. anthracis* spores, including laboratory personnel handling environmental specimens and performing confirmatory testing for *B. anthracis* in selected laboratories and workers making repeated entries into sites known to be contaminated with *B. anthracis* spores. Anthrax vaccine also was used after exposure, in conjunction with antimicrobial prophylaxis, under an investigational protocol.[74] Groups for whom preexposure vaccination is recommended include persons working with production quantities of *B. anthracis* cultures or in activities with a high potential for aerosol production and selected other workers at high risk for exposure to *B. anthracis* spores.[75] Efficacy has been demonstrated in protection against cutaneous disease. Data on clinical efficacy against inhaled anthrax in humans are limited, but available human and animal data are consistent with protection.[76] The vaccine induces antibodies in greater than 90% of adults who received the currently recommended primary course of three IM injections given at time zero, 4 weeks, and 6 months, with boosters at 12 months and 18 months, followed by annual boosters.[75,77] A controlled study of a vaccine similar to the currently available vaccine demonstrated protective efficacy against cutaneous disease of 93% among mill workers.[78] Experience suggests that two doses of vaccine confer some protection.[79] Mild

TABLE 316.3 Currently Available Vaccines and Toxoids and Year Licensed

PRODUCT	YEAR LICENSED
Adenovirus vaccine, live, attenuated	2014
Anthrax vaccine adsorbed	1972
Calmette-Guérin bacillus vaccine; live, attenuated	1950
Cholera vaccine, live, attenuated	2016
Dengue tetravalent vaccine, live	2019
Diphtheria and tetanus toxoids and acellular pertussis vaccine	1991
Diphtheria and tetanus toxoids adsorbed (pediatric use, DT)	1949
Diphtheria and tetanus toxoids and acellular pertussis vaccine absorbed, *Haemophilus* B conjugate vaccine, and inactivated polio vaccine combined	2008
Diphtheria and tetanus toxoids and acellular pertussis vaccine absorbed and inactivated polio vaccine combined	2008
Diphtheria and tetanus toxoids and acellular pertussis adsorbed, hepatitis B (recombinant), and inactivated poliovirus vaccine combined	2002
Haemophilus influenzae type b conjugate vaccine	1987
Hepatitis A vaccine	1995
Hepatitis A inactivated and hepatitis B (recombinant) vaccine	2001
Hepatitis B recombinant vaccine	1987
Human papillomavirus vaccine	2006
Influenza virus vaccine (cell culture)	2013
Influenza virus vaccine (inactivated)	1945
Influenza virus vaccine; live, attenuated, intranasal	2003
Influenza virus vaccine; recombinant hemagglutinin	2014
Japanese encephalitis vaccine	2009
Measles virus vaccine; live, attenuated	1963
Measles, mumps, rubella, varicella; live, attenuated	2005
Measles, mumps, and rubella virus vaccine; live, attenuated	1971

TABLE 316.3 Currently Available Vaccines and Toxoids and Year Licensed[a]—cont'd

PRODUCT	YEAR LICENSED
Meningococcal polysaccharide (serogroups A, C, Y, and W) conjugated to diphtheria toxoid	2005
Pneumococcal conjugate vaccine (13-valent)	2010
Pneumococcal polysaccharide vaccine (23-valent)	1983
Poliomyelitis vaccine (inactivated, enhanced potency)	1987
Rabies vaccine (human diploid)	1980
Recombinant zoster vaccine	2017
Rotavirus vaccine, live, attenuated	2006
Rubella virus vaccine, live, attenuated	1969
Serogroup B meningococcal vaccine	2014
Smallpox vaccine, live, attenuated	2007
Tetanus and diphtheria toxoids, adsorbed (adult use, Td)	1955
Tetanus toxoid adsorbed	1949
Tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine, absorbed	2005
Typhoid vaccine (polysaccharide)	1994
Typhoid vaccine; live, attenuated (oral)	1990
Varicella vaccine; live, attenuated	1995
Yellow fever vaccine; live, attenuated	1953
Zoster vaccine; live, attenuated	2006

[a]As of January 2017.

TABLE 316.4 Immune Globulin Preparations Made From Human Plasma[a]

NAME	ABBREVIATION	ROUTE OF ADMINISTRATION	YEAR LICENSED
Anthrax immune globulin		Intravenous	2015
Botulism intravenous immune globulin	BabyBIG	Intravenous	2003
Cytomegalovirus immune globulin intravenous	CMV IGIV	Intravenous	1990
Hepatitis B immune globulin	HBIG	Intramuscular	1977
Immune globulin	IG	Intramuscular	1943
Immune globulin intravenous	IGIV	Intravenous	1981
Immune globulin subcutaneous	IGSC	Subcutaneous	2006
Rabies immune globulin	RIG	Intramuscular	1974
Tetanus immune globulin	TIG	Intramuscular	1957
Vaccinia immune globulin intravenous	VIG-IGIV	Intravenous	2005
Varicella-zoster immune globulin	VariZIG	Intramuscular	2012[c]

[a]Antitoxin preparations from animal sera other than humans are available for botulism and diphtheria.
[b]As of January 2017.
[c]A previous preparation of varicella-zoster IG (VZIG) was licensed in 1980.

local reactions at the site of injection occur in about 30% of recipients. Studies of adverse events after injection of the alum-precipitated vaccine, which is the precursor to the AVA vaccine, demonstrate that more severe local reactions occur infrequently (<4%) and systemic reactions are rare (0.2%). Surveillance for adverse events in the military program revealed no pattern of serious adverse events.[80,81] Adverse events, including injection site reaction incidence and duration, were less often seen after IM injection compared with subcutaneous injection.[77] The IM route of administration is indicated for preexposure use.[75] Vaccines containing only recombinant protective antigen are under active development and may be less reactogenic than the current vaccine.[82,83] In the event of exposure to anthrax spores, the recommended postexposure prophylaxis (PEP) regimen is three doses of AVA administered at 0, 2, and 4 weeks, combined with 60 days of antibiotics.[75]

Bacillus Calmette-Guérin Vaccine

BCG vaccine contains living Calmette-Guérin bacillus, an attenuated strain of *Mycobacterium bovis*. In many countries, BCG is used in infants and young children to prevent disseminated tuberculosis infection. In the United States, use of BCG is recommended only in special circumstances because the general risk for infection is low. BCG vaccination can also result in conversion of the purified protein derivative (PPD) or Mantoux tuberculin skin test, thereby removing one of the most important indicators of tuberculosis infection (tuberculin conversion). However, the association of a positive PPD skin test result after immunization with BCG in childhood tends to fade over time, and most individuals will have a PPD reaction of less than 10 mm by 10 years later. BCG does not cross react with the interferon-γ release assay (IGRA), so the IGRA is the preferred test over the PPD for diagnosis of tuberculosis in patients older than 4 years who have received BCG.[84] The IGRA is not as sensitive in children 4 years or younger and requires a blood draw. Although BCG is widely used throughout the world, there has been much controversy regarding its efficacy. Studies have suggested that the vaccine is effective, particularly for preventing complications of disseminated tuberculosis in young children.[85–87] In

Text continued on p. 3785

Table 1 Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger United States, 2019

These recommendations must be read with the Notes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars in Table 1. To determine minimum intervals between doses, see the catch-up schedule (Table 2). School entry and adolescent vaccine age groups are shaded in gray.

Vaccine	Birth	1 mo	2 mos	4 mos	6 mos	9 mos	12 mos	15 mos	18 mos	19-23 mos	2-3 yrs	4-6 yrs	7-10 yrs	11-12 yrs	13-15 yrs	16 yrs	17-18 yrs
Hepatitis B (HepB)	1st dose	2nd dose			3rd dose												
Rotavirus (RV) RV1 (2-dose series); RV5 (3-dose series)			1st dose	2nd dose	See Notes												
Diphtheria, tetanus, & acellular pertussis (DTaP: <7 yrs)			1st dose	2nd dose	3rd dose			4th dose				5th dose					
Haemophilus influenzae type b (Hib)			1st dose	2nd dose	See Notes		3rd or 4th dose, See Notes										
Pneumococcal conjugate (PCV13)			1st dose	2nd dose	3rd dose		4th dose										
Inactivated poliovirus (IPV: <18 yrs)			1st dose	2nd dose	3rd dose							4th dose					
Influenza (IIV)					Annual vaccination 1 or 2 doses									Annual vaccination 1 dose only			
or													or				
Influenza (LAIV)											Annual vaccination 1 or 2 doses			Annual vaccination 1 dose only			
Measles, mumps, rubella (MMR)					See Notes		1st dose					2nd dose					
Varicella (VAR)							1st dose					2nd dose					
Hepatitis A (HepA)					See Notes		2-dose series, See Notes										
Meningococcal (MenACWY-D ≥9 mos; MenACWY-CRM ≥2 mos)			See Notes											1st dose		2nd dose	
Tetanus, diphtheria, & acellular pertussis (Tdap: ≥7 yrs)														Tdap			
Human papillomavirus (HPV)														See Notes			
Meningococcal B														See Notes			
Pneumococcal polysaccharide (PPSV23)											See Notes						

Range of recommended ages for all children

Range of recommended ages for catch-up immunization

Range of recommended ages for certain high-risk groups

Range of recommended ages for non-high-risk groups that may receive vaccine, subject to individual clinical decision-making

No recommendation

FIG. 316.1 (A) Recommended child and adolescent immunization schedule for ages 18 years or younger, United States, 2019. (B) Catch-up immunization schedule for persons aged 4 months to 18 years who start late or who are more than 1 month behind—United States, 2019. (C) Footnotes for both schedules. (D) Child and adolescent schedule by medical and other indications, United States, 2019. *(From Centers for Disease Control and Prevention. Recommended child and adolescent immunization schedule for ages 18 years or younger, United States, 2019.* www.cdc.gov/vaccines/schedules/hcp/child-adolescent.html. *Accessed February 8, 2019.)*

Table 2 Catch-up immunization schedule for persons aged 4 months—18 years who start late or who are more than 1 month behind, United States, 2019

The figure below provides catch-up schedules and minimum intervals between doses for children whose vaccinations have been delayed. A vaccine series does not need to be restarted, regardless of the time that has elapsed between doses. Use the section appropriate for the child's age. Always use this table in conjunction with Table 1 and the notes that follow.

Vaccine	Minimum Age for Dose 1	Minimum Interval Between Doses			
		Dose 1 to Dose 2	Dose 2 to Dose 3	Dose 3 to Dose 4	Dose 4 to Dose 5
Children age 4 months through 6 years					
Hepatitis B	Birth	**4 weeks**	**8 weeks *and* at least 16 weeks after first dose.** Minimum age for the final dose is 24 weeks.		
Rotavirus	6 weeks Maximum age for first dose is 14 weeks, 6 days	**4 weeks**	**4 weeks** Maximum age for final dose is 8 months, 0 days.		
Diphtheria, tetanus, and acellular pertussis	6 weeks	**4 weeks**	**4 weeks**	**6 months**	**6 months**
Haemophilus influenzae type b	6 weeks	**No further doses needed** if first dose was administered at age 15 months or older. **4 weeks** if first dose was administered before the 1st birthday. **8 weeks (as final dose)** if first dose was administered at age 12 through 14 months.	**No further doses needed** if previous dose was administered at age 15 months or older. **4 weeks** if current age is younger than 12 months ***and*** first dose was administered at younger than age 7 months, ***and*** at least 1 previous dose was PRP-T (ActHib, Pentacel, Hiberix) or unknown. **8 weeks *and* age 12 through 59 months (as final dose)** if current age is younger than 12 months ***and*** first dose was administered at age 7 through 11 months; OR if current age is 12 through 59 months ***and*** first dose was administered before the 1st birthday, ***and*** second dose administered at younger than 15 months; OR if both doses were PRP-OMP (PedvaxHIB; Comvax) ***and*** were administered before the 1st birthday.	**8 weeks (as final dose)** This dose only necessary for children age 12 through 59 months who received 3 doses before the 1st birthday.	
Pneumococcal conjugate	6 weeks	**No further doses needed** for healthy children if first dose was administered at age 24 months or older. **4 weeks** if first dose administered before the 1st birthday. **8 weeks (as final dose for healthy children)** if first dose was administered at the 1st birthday or after.	**No further doses needed** for healthy children if previous dose administered at age 24 months or older. **4 weeks** if current age is younger than 12 months and previous dose given at <7 months old. **8 weeks (as final dose for healthy children)** if previous dose given between 7-11 months (wait until at least 12 months old); OR if current age is 12 months or older and at least 1 dose was given before age 12 months.	**8 weeks (as final dose)** This **dose** only necessary for children age 12 through 59 months who received 3 doses before age 12 months or for children at high risk who received 3 doses at any age.	
Inactivated poliovirus	6 weeks	**4 weeks**	4 weeks if current age is < 4 years. 6 months (as final dose) if current age is 4 years or older.	**6 months** (minimum age 4 years for final dose).	
Measles, mumps, rubella	12 months	**4 weeks**			
Varicella	12 months	**3 months**			
Hepatitis A	12 months	**6 months**			
Meningococcal	2 months MenACWY-CRM 9 months MenACWY-D	**8 weeks**	See Notes	See Notes	
Children and adolescents age 7 through 18 years					
Meningococcal	Not Applicable (N/A)	**8 weeks**			
Tetanus, diphtheria; tetanus, diphtheria, and acellular pertussis	7 years	**4 weeks**	**4 weeks** if first dose of DTaP/DT was administered before the 1st birthday. **6 months (as final dose)** if first dose of DTaP/DT or Tdap/Td was administered at or after the 1st birthday.	**6 months** if first dose of DTaP/DT was administered before the 1st birthday.	
Human papillomavirus	9 years	**Routine dosing intervals are recommended.**			
Hepatitis A	N/A	**6 months**			
Hepatitis B	N/A	**4 weeks**	**8 weeks *and*** at least 16 weeks after first dose.		
Inactivated poliovirus	N/A	**4 weeks**	**6 months** A fourth dose is not necessary if the third dose was administered at age 4 years or older and at least 6 months after the previous dose.	A fourth dose of IPV is indicated if all previous doses were administered at <4 years or if the third dose was administered <6 months after the second dose.	
Measles, mumps, rubella	N/A	**4 weeks**			
Varicella	N/A	**3 months** if younger than age 13 years. **4 weeks** if age 13 years or older.			

FIG. 316.1, cont'd

Continued

Notes Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2019

For vaccine recommendations for persons 19 years of age and older, see the Recommended Adult Immunization Schedule.

Additional information

- Consult relevant ACIP statements for detailed recommendations at www.cdc.gov/vaccines/hcp/acip-recs/index.html.
- For information on contraindications and precautions for the use of a vaccine, consult the General Best Practice Guidelines for Immunization and relevant ACIP statements at www.cdc.gov/vaccines/hcp/acip-recs/index.html.
- For calculating intervals between doses, 4 weeks = 28 days. Intervals of ≥4 months are determined by calendar months.
- Within a number range (e.g., 12–18), a dash (–) should be read as "through."
- Vaccine doses administered ≤4 days before the minimum age or interval are considered valid. Doses of any vaccine administered ≥5 days earlier than the minimum age or minimum interval should not be counted as valid and should be repeated as age-appropriate. The repeat dose should be spaced after the invalid dose by the recommended minimum interval. For further details, see Table 3-1, Recommended and minimum ages and intervals between vaccine doses, in General Best Practice Guidelines for Immunization at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/timing.html.
- Information on travel vaccine requirements and recommendations is available at wwwnc.cdc.gov/travel/.
- For vaccination of persons with immunodeficiencies, see Table 8-1, Vaccination of persons with primary and secondary immunodeficiencies, in General Best Practice Guidelines for Immunization at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/immunocompetence.html, and Immunization in Special Clinical Circumstances (In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. *Red Book: 2018 Report of the Committee on Infectious Diseases*. 31st ed. Itasca, IL: American Academy of Pediatrics; 2018:67–111).
- For information regarding vaccination in the setting of a vaccine-preventable disease outbreak, contact your state or local health department.
- The National Vaccine Injury Compensation Program (VICP) is a no-fault alternative to the traditional legal system for resolving vaccine injury claims. All routine child and adolescent vaccines are covered by VICP except for pneumococcal polysaccharide vaccine (PPSV23). For more information, see www.hrsa.gov/vaccinecompensation/index.html.

Diphtheria, tetanus, and pertussis (DTaP) vaccination (minimum age: 6 weeks [4 years for Kinrix or Quadracel])

Routine vaccination

- 5-dose series at 2, 4, 6, 15–18 months, 4–6 years
  - **Prospectively:** Dose 4 may be given as early as age 12 months if at least 6 months have elapsed since dose 3.
  - **Retrospectively:** A 4th dose that was inadvertently given as early as 12 months may be counted if at least 4 months have elapsed since dose 3.

Catch-up vaccination

- Dose 5 is not necessary if dose 4 was administered at age 4 years or older.
- For other catch-up guidance, see Table 2.

Haemophilus influenzae type b vaccination (minimum age: 6 weeks)

Routine vaccination

- **ActHIB, Hiberix, or Pentacel:** 4-dose series at 2, 4, 6, 12–15 months
- **PedvaxHIB:** 3-dose series at 2, 4, 12–15 months

Catch-up vaccination

- **Dose 1 at 7–11 months:** Administer dose 2 at least 4 weeks later and dose 3 (final dose) at 12–15 months or 8 weeks after dose 2 (whichever is later).
- **Dose 1 at 12–14 months:** Administer dose 2 (final dose) at least 8 weeks after dose 1.
- **Dose 1 before 12 months and dose 2 before 15 months:** Administer dose 3 (final dose) 8 weeks after dose 2.
- **2 doses of PedvaxHIB before 12 months:** Administer dose 3 (final dose) at 12–59 months and at least 8 weeks after dose 2.
- **Unvaccinated at 15–59 months:** 1 dose
- For other catch-up guidance, see Table 2.

Special situations

- **Chemotherapy or radiation treatment:**
  12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Doses administered within 14 days of starting therapy or during therapy should be repeated at least 3 months after therapy completion.
- **Hematopoietic stem cell transplant (HSCT):**
  - 3-dose series 4 weeks apart starting 6 to 12 months after successful transplant regardless of Hib vaccination history
- **Anatomic or functional asplenia (including sickle cell disease):**
  12–59 months
  - Unvaccinated or only 1 dose before 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before 12 months:1 dose at least 8 weeks after previous dose

  Unvaccinated persons age 5 years or older*
  - 1 dose
- **Elective splenectomy:**
  Unvaccinated persons age 15 months or older*
  - 1 dose (preferably at least 14 days before procedure)
- **HIV infection:**
  12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Unvaccinated persons age 5–18 years*
  - 1 dose
- **Immunoglobulin deficiency, early component complement deficiency:**
  12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

**Unvaccinated* = Less than routine series (through 14 months) OR no doses (14 months or older)

FIG. 316.1, cont'd

Notes

Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2019

Hepatitis A vaccination (minimum age: 12 months for routine vaccination)

Routine vaccination

- 2-dose series (**Havrix** 6–12 months apart or **Vaqta** 6–18 months apart, minimum interval 6 months); a series begun before the 2nd birthday should be completed even if the child turns 2 before the second dose is administered.

Catch-up vaccination

- Anyone 2 years of age or older may receive HepA vaccine if desired. Minimum interval between doses: 6 months
- Adolescents 18 years and older may receive the combined HepA and HepB vaccine, **Twinrix**, as a 3-dose series (0, 1, and 6 months) or 4-dose series (0, 7, and 21–30 days, followed by a dose at 12 months).

International travel

- Persons traveling to or working in countries with high or intermediate endemic hepatitis A (wwwnc.cdc.gov/travel/):
  - **Infants age 6–11 months**: 1 dose before departure; revaccinate with 2 doses, separated by 6–18 months, between 12 to 23 months of age.
  - **Unvaccinated age 12 months and older**: 1st dose as soon as travel considered

Special situations

At risk for hepatitis A infection: 2-dose series as above

- **Chronic liver disease**
- **Clotting factor disorders**
- **Men who have sex with men**
- **Injection or non-injection drug use**
- **Homelessness**
- **Work with hepatitis A virus** in research laboratory or nonhuman primates with hepatitis A infection
- **Travel** in countries with high or intermediate endemic hepatitis A
- **Close, personal contact with international adoptee** (e.g., household or regular babysitting) in first 60 days after arrival from country with high or intermediate endemic hepatitis A (administer dose 1 as soon as adoption is planned, at least 2 weeks before adoptee's arrival)

Hepatitis B vaccination (minimum age: birth)

Birth dose (monovalent HepB vaccine only)

- **Mother is HBsAg-negative:** 1 dose within 24 hours of birth for **all** medically stable infants ≥2,000 grams. Infants <2,000 grams: administer 1 dose at chronological age 1 month or hospital discharge.
- **Mother is HBsAg-positive:**
  - Administer **HepB vaccine** and **0.5 mL of hepatitis B immune globulin (HBIG)** (at separate anatomic sites) within 12 hours of birth, regardless of birth weight. For infants <2,000 grams, administer 3 additional doses of vaccine (total of 4 doses) beginning at age 1 month.
  - Test for HBsAg and anti-HBs at age 9–12 months. If HepB series is delayed, test 1–2 months after final dose.
- **Mother's HBsAg status is unknown:**
  - Administer **HepB vaccine** within 12 hours of birth, regardless of birth weight.
  - For infants <2,000 grams, administer **0.5 mL of HBIG** in addition to HepB vaccine within 12 hours of birth. Administer 3 additional doses of vaccine (total of 4 doses) beginning at age 1 month.
  - Determine mother's HBsAg status as soon as possible. If mother is HBsAg-positive, administer **0.5 mL of HBIG** to infants ≥2,000 grams as soon as possible, but no later than 7 days of age.

Routine series

- 3-dose series at 0, 1–2, 6–18 months (use monovalent HepB vaccine for doses administered before age 6 weeks)
- Infants who did not receive a birth dose should begin the series as soon as feasible (see Table 2).
- Administration of **4 doses** is permitted when a combination vaccine containing HepB is used after the birth dose.
- **Minimum age** for the final (3rd or 4th) dose: 24 weeks
- **Minimum intervals:** dose 1 to dose 2: 4 weeks / dose 2 to dose 3: 8 weeks / dose 1 to dose 3: 16 weeks (when 4 doses are administered, substitute "dose 4" for "dose 3" in these calculations)

Catch-up vaccination

- Unvaccinated persons should complete a 3-dose series at 0, 1–2, 6 months.
- Adolescents age 11–15 years may use an alternative 2-dose schedule with at least 4 months between doses (adult formulation **Recombivax HB** only).
- Adolescents 18 years and older may receive a 2-dose series of HepB **(Heplisav-B)** at least 4 weeks apart.
- Adolescents 18 years and older may receive the combined HepA and HepB vaccine, **Twinrix**, as a 3-dose series (0, 1, and 6 months) or 4-dose series (0, 7, and 21–30 days, followed by a dose at 12 months).
- For other catch-up guidance, see Table 2.

Human papillomavirus vaccination (minimum age: 9 years)

Routine and catch-up vaccination

- HPV vaccination routinely recommended for all adolescents **age 11–12 years (can start at age 9 years)** and through age 18 years if not previously adequately vaccinated
- 2- or 3-dose series depending on age at initial vaccination:
  - **Age 9 through 14 years at initial vaccination**: 2-dose series at 0, 6–12 months (minimum interval: 5 months; repeat dose if administered too soon)
  - **Age 15 years or older at initial vaccination**: 3-dose series at 0, 1–2 months, 6 months (minimum intervals: dose 1 to dose 2: 4 weeks / dose 2 to dose 3: 12 weeks / dose 1 to dose 3: 5 months; repeat dose if administered too soon)
- If completed valid vaccination series with any HPV vaccine, no additional doses needed

Special situations

- **Immunocompromising conditions, including HIV infection:** 3-dose series as above
- **History of sexual abuse or assault:** Start at age 9 years
- **Pregnancy:** HPV vaccination not recommended until after pregnancy; no intervention needed if vaccinated while pregnant; pregnancy testing not needed before vaccination

Inactivated poliovirus vaccination (minimum age: 6 weeks)

Routine vaccination

- 4-dose series at ages 2, 4, 6–18 months, 4–6 years; administer the final dose on or after the 4th birthday and at least 6 months after the previous dose.
- 4 or more doses of IPV can be administered before the 4th birthday when a combination vaccine containing IPV is used. However, a dose is still recommended after the 4th birthday and at least 6 months after the previous dose.

Catch-up vaccination

- In the first 6 months of life, use minimum ages and intervals only for travel to a polio-endemic region or during an outbreak.
- IPV is not routinely recommended for U.S. residents 18 years and older.

Series containing oral polio vaccine (OPV), either mixed OPV-IPV or OPV-only series:

- Total number of doses needed to complete the series is the same as that recommended for the U.S. IPV schedule. See www.cdc.gov/mmwr/volumes/66/wr/mm6601a6.htm?s_cid=mm6601a6_w.

FIG. 316.1, cont'd

Continued

Notes — Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2019

- Only trivalent OPV (tOPV) counts toward the U.S. vaccination requirements. For guidance to assess doses documented as "OPV," see www.cdc.gov/mmwr/volumes/66/wr/mm6606a7.htm?s_cid=mm6606a7_w.
- For other catch-up guidance, see Table 2.

Influenza vaccination (minimum age: 6 months [IIV], 2 years [LAIV], 18 years [RIV])

Routine vaccination

- 1 dose any influenza vaccine appropriate for age and health status annually (2 doses separated by at least 4 weeks for **children 6 months–8 years** who did not receive at least 2 doses of influenza vaccine before July 1, 2018)

Special situations

- **Egg allergy, hives only**: Any influenza vaccine appropriate for age and health status annually
- **Egg allergy more severe than hives** (e.g., angioedema, respiratory distress): Any influenza vaccine appropriate for age and health status annually in medical setting under supervision of health care provider who can recognize and manage severe allergic conditions
- **LAIV should not be used for** those with a history of severe allergic reaction to any component of the vaccine (excluding egg) or to a previous dose of any influenza vaccine, children and adolescents receiving concomitant aspirin or salicylate-containing medications, children age 2 through 4 years with a history of asthma or wheezing, those who are immunocompromised due to any cause (including immunosuppression caused by medications and HIV infection), anatomic and functional asplenia, cochlear implants, cerebrospinal fluid-oropharyngeal communication, close contacts and caregivers of severely immunosuppressed persons who require a protected environment, pregnancy, and persons who have received influenza antiviral medications within the previous 48 hours.

Measles, mumps, and rubella vaccination (minimum age: 12 months for routine vaccination)

Routine vaccination

- 2-dose series at 12–15 months, 4–6 years
- Dose 2 may be administered as early as 4 weeks after dose 1.

Catch-up vaccination

- Unvaccinated children and adolescents: 2 doses at least 4 weeks apart
- The maximum age for use of *MMRV* is 12 years.

Special situations

International travel

- **Infants age 6–11 months:** 1 dose before departure; revaccinate with 2 doses at 12–15 months (12 months for children in high-risk areas) and dose 2 as early as 4 weeks later.
- **Unvaccinated children age 12 months and older:** 2-dose series at least 4 weeks apart before departure

Meningococcal serogroup A,C,W,Y vaccination (minimum age: 2 months [MenACWY-CRM, Menveo], 9 months [MenACWY-D, Menactra])

Routine vaccination

- 2-dose series: 11–12 years, 16 years

Catch-up vaccination

- Age 13–15 years: 1 dose now and booster at age 16–18 years (minimum interval: 8 weeks)
- Age 16–18 years: 1 dose

Special situations

Anatomic or functional asplenia (including sickle cell disease), HIV infection, persistent complement component deficiency, eculizumab use:

- **Menveo**
  - Dose 1 at age 8 weeks: 4-dose series at 2, 4, 6, 12 months
  - Dose 1 at age 7–23 months: 2-dose series (dose 2 at least 12 weeks after dose 1 and after the 1st birthday)
  - Dose 1 at age 24 months or older: 2-dose series at least 8 weeks apart
- **Menactra**
  - **Persistent complement component deficiency:**
    - Age 9–23 months: 2 doses at least 12 weeks apart
    - Age 24 months or older: 2 doses at least 8 weeks apart
  - **Anatomic or functional asplenia, sickle cell disease, or HIV infection:**
    - **Age 9–23 months**: Not recommended
    - **24 months or older**: 2 doses at least 8 weeks apart
    - **Menactra** must be administered at least 4 weeks after completion of PCV13 series.

Travel in countries with hyperendemic or epidemic meningococcal disease, including countries in the African meningitis belt or during the Hajj (wwwnc.cdc.gov/travel/)**:**

- Children age less than 24 months:
  - **Menveo (age 2–23 months):**
    - Dose 1 at 8 weeks: 4-dose series at 2, 4, 6, 12 months
    - Dose 1 at 7–23 months: 2-dose series (dose 2 at least 12 weeks after dose 1 and after the 1st birthday)
  - **Menactra (age 9–23 months):**
    - 2-dose series (dose 2 at least 12 weeks after dose 1; dose 2 may be administered as early as 8 weeks after dose 1 in travelers)
- Children age 2 years or older: 1 dose **Menveo** or **Menactra**

First-year college students who live in residential housing (if not previously vaccinated at age 16 years or older) or military recruits:

- 1 dose **Menveo** or **Menactra**

Note: Menactra should be administered either before or at the same time as DTaP. For MenACWY booster dose recommendations for groups listed under "Special situations" above and additional meningococcal vaccination information, see meningococcal *MMWR* publications at www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/mening.html.

Meningococcal serogroup B vaccination (minimum age: 10 years [MenB-4C, Bexsero; MenB-FHbp, Trumenba])

Clinical discretion

- MenB vaccine may be administered based on individual clinical decision to **adolescents not at increased risk** age 16–23 years (preferred age 16–18 years):
- **Bexsero:** 2-dose series at least 1 month apart
- **Trumenba:** 2-dose series at least 6 months apart; if dose 2 is administered earlier than 6 months, administer a 3rd dose at least 4 months after dose 2.

Special situations

Anatomic or functional asplenia (including sickle cell disease), persistent complement component deficiency, eculizumab use:

- **Bexsero:** 2-dose series at least 1 month apart
- **Trumenba:** 3-dose series at 0, 1–2, 6 months

Bexsero and **Trumenba** are not interchangeable; the same product should be used for all doses in a series.
For additional meningococcal vaccination information, see meningococcal *MMWR* publications at www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/mening.html.

FIG. 316.1, cont'd

Notes

Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2019

Pneumococcal vaccination (minimum age: 6 weeks [PCV13], 2 years [PPSV23])

Routine vaccination with PCV13

- 4-dose series at 2, 4, 6, 12–15 months

Catch-up vaccination with PCV13

- 1 dose for healthy children age 24–59 months with any incomplete* PCV13 series
- For other catch-up guidance, see Table 2.

Special situations

High-risk conditions below: When both PCV13 and PPSV23 are indicated, administer PCV13 first. PCV13 and PPSV23 should not be administered during same visit.

Chronic heart disease (particularly cyanotic congenital heart disease and cardiac failure); chronic lung disease (including asthma treated with high-dose, oral corticosteroids); diabetes mellitus:

Age 2–5 years

- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

Age 6–18 years

- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

Cerebrospinal fluid leak, cochlear implant:

Age 2–5 years

- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13, 8 weeks after the most recent dose and administered 8 weeks apart
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

Age 6–18 years

- No history of either PCV13 or PPSV23: 1 dose PCV13, 1 dose PPSV23 at least 8 weeks later
- Any PCV13 but no PPSV23: 1 dose PPSV23 at least 8 weeks after the most recent dose of PCV13
- PPSV23 but no PCV13: 1 dose PCV13 at least 8 weeks after the most recent dose of PPSV23

Sickle cell disease and other hemoglobinopathies; anatomic or functional asplenia; congenital or acquired immunodeficiency; HIV infection; chronic renal failure; nephrotic syndrome; malignant neoplasms, leukemias, lymphomas, Hodgkin disease, and other diseases associated with treatment with immunosuppressive drugs or radiation therapy; solid organ transplantation; multiple myeloma:

Age 2–5 years

- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose) and a 2nd dose of PPSV23 5 years later

Age 6–18 years

- No history of either PCV13 or PPSV23: 1 dose PCV13, 2 doses PPSV23 (dose 1 of PPSV23 administered 8 weeks after PCV13 and dose 2 of PPSV23 administered at least 5 years after dose 1 of PPSV23)
- Any PCV13 but no PPSV23: 2 doses PPSV23 (dose 1 of PPSV23 administered 8 weeks after the most recent dose of PCV13 and dose 2 of PPSV23 administered at least 5 years after dose 1 of PPSV23)
- PPSV23 but no PCV13: 1 dose PCV13 at least 8 weeks after the most recent PPSV23 dose and a 2nd dose of PPSV23 administered 5 years after dose 1 of PPSV23 and at least 8 weeks after a dose of PCV13

Chronic liver disease, alcoholism:

Age 6–18 years

- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

*An incomplete series is defined as not having received all doses in either the recommended series or an age-appropriate catch-up series. See Tables 8, 9, and 11 in the ACIP pneumococcal vaccine recommendations (www.cdc.gov/mmwr/pdf/rr/rr5911.pdf) for complete schedule details.

Rotavirus vaccination (minimum age: 6 weeks)

Routine vaccination

- **Rotarix:** 2-dose series at 2 and 4 months.
- **RotaTeq:** 3-dose series at 2, 4, and 6 months.

If any dose in the series is either **RotaTeq** or unknown, default to 3-dose series.

Catch-up vaccination

- Do not start the series on or after age 15 weeks, 0 days.
- The maximum age for the final dose is 8 months, 0 days.
- For other catch-up guidance, see Figure 2.

Tetanus, diphtheria, and pertussis (Tdap) vaccination (minimum age: 11 years for routine vaccination, 7 years for catch-up vaccination)

Routine vaccination

- **Adolescents age 11–12 years:** 1 dose Tdap
- **Pregnancy:** 1 dose Tdap during each pregnancy, preferably in early part of gestational weeks 27–36
- Tdap may be administered regardless of the interval since the last tetanus- and diphtheria-toxoid-containing vaccine.

Catch-up vaccination

- **Adolescents age 13–18 years who have not received Tdap:** 1 dose Tdap, then Td booster every 10 years
- **Persons age 7–18 years not fully immunized with DTaP:** 1 dose Tdap as part of the catch-up series (preferably the first dose); if additional doses are needed, use Td.
- **Children age 7–10 years** who receive Tdap inadvertently or as part of the catch-up series should receive the routine Tdap dose at 11–12 years.
- **DTaP inadvertently given after the 7th birthday:**
  - **Child age 7–10 years**: DTaP may count as part of catch-up series. Routine Tdap dose at 11–12 should be administered.
  - **Adolescent age 11–18 years**: Count dose of DTaP as the adolescent Tdap booster.
- For other catch-up guidance, see Table 2.
- For information on use of Tdap or Td as tetanus prophylaxis in wound management, see www.cdc.gov/mmwr/volumes/67/rr/rr6702a1.htm.

Varicella vaccination (minimum age: 12 months)

Routine vaccination

- 2-dose series: 12–15 months, 4–6 years
- Dose 2 may be administered as early as 3 months after dose 1 (a dose administered after a 4-week interval may be counted).

Catch-up vaccination

- Ensure persons age 7–18 years without evidence of immunity (see *MMWR* at www.cdc.gov/mmwr/pdf/rr/rr5604.pdf) have 2-dose series:
  - **Ages 7–12 years**: routine interval: 3 months (minimum interval: 4 weeks)
  - **Ages 13 years and older**: routine interval: 4–8 weeks (minimum interval: 4 weeks).
  - The maximum age for use of *MMRV* is 12 years.

FIG. 316.1, cont'd

Continued

Table 3 Recommended Child and Adolescent Immunization Schedule by Medical Indication United States, 2019

VACCINE	INDICATION										
	Pregnancy	Immunocompromised status (excluding HIV infection)	HIV infection CD4+ count[1]		Kidney failure, end-stage renal disease, on hemodialysis	Heart disease, chronic lung disease	CSF leaks/ cochlear implants	Asplenia and persistent complement component deficiencies	Chronic liver disease	Diabetes
			<15% and total CD4 cell count of <200/mm3	≥15% and total CD4 cell count of ≥200/mm3						
Hepatitis B										
Rotavirus		SCID[2]								
Diphtheria, tetanus, & acellular pertussis (DTaP)										
Haemophilus influenzae type b										
Pneumococcal conjugate										
Inactivated poliovirus										
Influenza (IIV)										
or										
Influenza (LAIV)						Asthma, wheezing: 2-4yrs[3]				
Measles, mumps, rubella										
Varicella										
Hepatitis A										
Meningococcal ACWY										
Tetanus, diphtheria, & acellular pertussis (Tdap)										
Human papillomavirus										
Meningococcal B										
Pneumococcal polysaccharide										

- Vaccination according to the routine schedule recommended
- Recommended for persons with an additional risk factor for which the vaccine would be indicated
- Vaccination is recommended, and additional doses may be necessary based on medical condition. See Notes.
- Contraindicated or use not recommended—vaccine should not be administered because of risk for serious adverse reaction
- Precaution—vaccine might be indicated if benefit of protection outweighs risk of adverse reaction
- Delay vaccination until after pregnancy if vaccine indicated
- No recommendation

1 For additional information regarding HIV laboratory parameters and use of live vaccines, see the General Best Practice Guidelines for Immunization "Altered Immunocompetence" at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/immunocompetence.html, and Table 4-1 (footnote D) at: www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html.
2 Severe Combined Immunodeficiency
3 LAIV contraindicated for children 2–4 years of age with asthma or wheezing during the preceding 12 months.

FIG. 316.1, cont'd

the United States, use of BCG should be considered for individuals, such as infants, whose skin test results are negative and who have prolonged, close contact with patients with active tuberculosis who are untreated, are ineffectively treated, or have antibiotic-resistant infection. BCG also may be considered for health care providers in areas in which multidrug-resistant *Mycobacterium tuberculosis* infection has become a significant problem.[88]

A single dose of vaccine is administered intradermally or by the percutaneous route. (The Tice strain licensed in the United States is approved only for percutaneous administration.) Known adverse reactions include regional adenitis, disseminated BCG infection, and osteitis caused by the BCG organism. Adenitis occurs in about 1% to 10% of vaccinees, whereas disseminated infections and osteitis are quite rare (about 1 case per 1 million vaccinees). The risk for developing osteitis after BCG vaccination varies by country; in one review, this risk ranged from 0.01 cases per million vaccinees in Japan to 32.5 and 43.4 cases per million vaccinees in Sweden and Finland, respectively.[89] Immunocompromised individuals should not receive the vaccine because of increased risk for disseminated BCG infection.[88]

Cholera Vaccine

A killed whole-cell cholera vaccine was available in the United States from the 1940s until 2001. [90] Killed whole-cell vaccines are still available in some countries, and improved killed vaccines are licensed in some countries. Two oral whole-cell inactivated vaccines, including one that is combined with the B subunit of cholera toxin, are available in some parts of the world, as is an oral live-attenuated vaccine with a critical moiety of the gene for the cholera toxin deleted.[91,91a] Killed oral cholera vaccines are increasingly being used as important components of cholera prevention in epidemic and endemic settings. A live oral vaccine (CVD 103-HgR or Vaxchora, manufactured by PaxVax) was licensed in the United States in 2016. The vaccine is administered as a single dose with a buffer salt to neutralize stomach acid. It is recommended for travelers 18 to 64 years of age to an area of active cholera transmission. Vaxchora should be administered 8 hours or more after a dose of oral typhoid vaccine.

Diphtheria Toxoid

Diphtheria toxoid is a purified preparation of inactivated diphtheria toxin. It is highly effective in inducing antibodies that will prevent disease, although antibodies may not prevent acquisition or carriage of the organism. In the United States, the toxoid is available in adsorbed form, combined with tetanus toxoid (adult formulation, Td, and pediatric formulation, DT) or with tetanus toxoid and acellular pertussis vaccine (DTaP, childhood formulation; or Tdap, adult formulation). Single-antigen diphtheria toxoid is not distributed in the United States. Two dosage formulations are available: one for use in children through 6 years of age, and one for use in older children and adults. The adult formulation has a lower concentration of diphtheria toxoid (≤2.5 limit of flocculation units [Lf]) than the childhood formulation (6.7–25 Lf) because local reactions are thought to relate to both age and dosage. With all formulations, levels of antitoxin considered protective are induced in more than 90% of recipients who complete the schedule.[54,92,93]

Immunization against diphtheria is recommended for all residents in the United States. For children younger than 7 years with no contraindications to pertussis immunization, DTaP is recommended, and the primary series is three doses administered 4 to 8 weeks apart, followed by a first booster dose 6 to 12 months later and a second booster dose at school entry (4–6 years of age). For infants with contraindications to pertussis vaccine, DT is administered in the same schedule as DTaP (see "Pertussis-Containing Vaccine" and Fig. 316.1). The primary immunizing series of DT (for children 1–6 years of age) or Td (for older children and adults) consists of at least two doses administered 4 to 8 weeks apart, followed by a third dose 6 to 12 months later. There is no need to restart a series if the schedule is interrupted; the next dose in the series should be given. Booster doses of Td should be given every 10 years. All persons 11 years and older should receive one dose of Tdap, which can serve as one of the recommended booster doses for diphtheria and tetanus. Persons 7 years or older not fully vaccinated with DTaP vaccine should receive one dose of Tdap as part of a catch-up series. If the dose is administered at 7 through 10 years of age, another dose of Tdap should be administered at 11 or 12 years of age. Tdap should be administered to pregnant women during every pregnancy, optimally early between gestational ages 27 weeks and 36 weeks.[71] Tdap administered during pregnancy provides passive immunity to the fetus and should protect newborns and young infants before they have time to make an active immune response to DTaP. Known adverse effects of diphtheria toxoid include local reactions and mild or moderate systemic reactions such as fever; anaphylaxis occurs rarely. Brachial neuritis appears to be a rare consequence of immunization and is most likely due to tetanus toxoid.[65] The only contraindications are in individuals who previously have had severe hypersensitivity reactions after diphtheria or tetanus toxoids or, if combined with pertussis, have had previous similar adverse events to those antigens.

Haemophilus influenzae Type b Vaccine (Hib)

Conjugated vaccines to prevent Hib invasive disease were first licensed at the end of 1987 and have replaced the earlier polysaccharide vaccines because they elicit substantially higher antibody titers and are effective in young infants.[94] The polysaccharide in these vaccines is covalently linked to protein carriers, converting them from T-lymphocyte–independent antigens to T-lymphocyte–dependent antigens. There are four available conjugate vaccines licensed for use in infants.[95] Three are single-component vaccines for prevention of Hib disease. Carrier proteins include a *Neisseria meningitidis* outer membrane protein complex (PRP-OMP) for PedVaxHib and tetanus toxoid (PRP-T) for ActHIB and Hiberix. PRP-OMP has been demonstrated to be 95% effective in a clinical trial in infants. PRP-T has been licensed for use in infants because it elicits comparable antibody responses to other conjugate vaccines that have been shown to be highly effective. A combination vaccine, DTaP-IPV/Hib, is licensed for any of the recommended first four doses during the first 2 years of life (www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm174757.htm and www.fda.gov/biologicsbloodvaccines/vaccines/approvedproducts/ucm172502.htm).[96]

The Hib component of DTaP-IPV contains PRP-T as the conjugate. PRP-OMP behaves differently from PRP-T, inducing high levels of antibody after a single dose. A second dose 2 months later increases those levels; less benefit appears to be derived from a third dose.[94] The basic series for PRP-OMP is two doses given 2 months apart beginning at 2 months of age, followed by a booster dose at 12 to 15 months of age.[94] PRP-OMP is preferred in American Indian/Alaska Native populations because of the younger peak in disease incidence. In contrast, PRP-T does not induce substantial antibody levels until the second dose, and high levels of protection are achieved only after three doses 2 months apart. The basic series for PRP-T starts at 2 months of age with three doses 2 months apart, followed by a booster dose at 12 to 15 months of age.[94] Although use of a single conjugate vaccine for the primary series is recommended, several studies have suggested that mixed sequences of Hib conjugate vaccines induce an adequate immune response.[97–99] Thus, for infants younger than 6 months, three doses of any licensed Hib vaccine administered at 2-month intervals should confer protection; a booster dose is given at 12 to 15 months of age.

For healthy infants starting immunization at 7to 11 months, two doses of any of the Hib vaccines licensed for infants should be given with at least 4 weeks between the two doses, followed by a booster dose at 12 to 15 months, provided that at least 2 months have elapsed since the second dose. Any of the conjugates can be used for the booster dose.[94]

Healthy children beginning immunization at 12 to 14 months of age can receive two doses of any conjugate, with the second dose given at least 2 months after the first dose. Healthy children who initially are immunized at 15 months or older need only one dose of any of the conjugate vaccines. Unimmunized children aged 60 months or older do not need catch-up vaccination.

High-risk conditions include functional or anatomic asplenia and immunosuppression, particularly IgG2 subclass deficiency, early complement component deficiency, HIV infection, receipt of chemotherapy or radiation therapy for malignant neoplasms, and receipt of a hematopoietic stem cell transplant (HSCT). Children who will be undergoing splenectomy and are age 15 months or older who are unvaccinated or incompletely vaccinated (which means they have received fewer doses

Text continued on p. 3791

Table 1 Recommended Adult Immunization Schedule by Age Group United States, 2019

Vaccine	19–21 years	22–26 years	27–49 years	50–64 years	≥65 years
Influenza inactivated (IIV) or **Influenza recombinant** (RIV)	1 dose annually				
or					
Influenza live attenuated (LAIV)	1 dose annually				
Tetanus, diphtheria, pertussis (Tdap or Td)	1 dose Tdap, then Td booster every 10 yrs				
Measles, mumps, rubella (MMR)	1 or 2 doses depending on indication (if born in 1957 or later)				
Varicella (VAR)	2 doses (if born in 1980 or later)				
Zoster recombinant (RZV) *(preferred)*				2 doses	
or					
Zoster live (ZVL)				1 dose	
Human papillomavirus (HPV) **Female**	2 or 3 doses depending on age at initial vaccination				
Human papillomavirus (HPV) **Male**	2 or 3 doses depending on age at initial vaccination				
Pneumococcal conjugate (PCV13)	1 dose				
Pneumococcal polysaccharide (PPSV23)	1 or 2 doses depending on indication				1 dose
Hepatitis A (HepA)	2 or 3 doses depending on vaccine				
Hepatitis B (HepB)	2 or 3 doses depending on vaccine				
Meningococcal A, C, W, Y (MenACWY)	1 or 2 doses depending on indication, then booster every 5 yrs if risk remains				
Meningococcal B (MenB)	2 or 3 doses depending on vaccine and indication				
***Haemophilus influenzae* type b** (Hib)	1 or 3 doses depending on indication				

Recommended vaccination for adults who meet age requirement, lack documentation of vaccination, or lack evidence of past infection

Recommended vaccination for adults with an additional risk factor or another indication

No recommendation

FIG. 316.2 Recommended 2019 adult immunization schedule, by age group. See Fig. 316.4 for footnotes. *(From Centers for Disease Control and Prevention. Recommended adult immunization schedule for ages 19 years or older, United States, 2019. www.cdc.gov/vaccines/schedules/hcp/imz/adult.html. Accessed February 8, 2019.)*

Table 2 Recommended Adult Immunization Schedule by Medical Condition and Other Indications United States, 2019

Vaccine	Pregnancy	Immuno-compromised (excluding HIV infection)	HIV infection CD4 count <200	HIV infection CD4 count ≥200	Asplenia, complement deficiencies	End-stage renal disease, on hemodialysis	Heart or lung disease, alcoholism[1]	Chronic liver disease	Diabetes	Health care personnel[2]	Men who have sex with men
IIV or RIV	1 dose annually										
or										or	
LAIV	CONTRAINDICATED						PRECAUTION			1 dose annually	
Tdap or Td	1 dose Tdap each pregnancy	1 dose Tdap, then Td booster every 10 yrs									
MMR	CONTRAINDICATED	1 or 2 doses depending on indication									
VAR	CONTRAINDICATED	2 doses									
RZV *(preferred)*	DELAY					2 doses at age ≥50 yrs					
or						or					
ZVL	CONTRAINDICATED					1 dose at age ≥60 yrs					
HPV Female	DELAY	3 doses through age 26 yrs			2 or 3 doses through age 26 yrs						
HPV Male		3 doses through age 26 yrs			2 or 3 doses through age 21 yrs						2 or 3 doses through age 26 yrs
PCV13		1 dose									
PPSV23		1, 2, or 3 doses depending on age and indication									
HepA	2 or 3 doses depending on vaccine										
HepB	2 or 3 doses depending on vaccine										
MenACWY	1 or 2 doses depending on indication, then booster every 5 yrs if risk remains										
MenB	PRECAUTION	2 or 3 doses depending on vaccine and indication									
Hib		3 doses HSCT[3] recipients only			1 dose						

Recommended vaccination for adults who meet age requirement, lack documentation of vaccination, or lack evidence of past infection

Recommended vaccination for adults with an additional risk factor or another indication

Precaution—vaccine might be indicated if benefit of protection outweighs risk of adverse reaction

Delay vaccination until after pregnancy if vaccine is indicated

Contraindicated—vaccine should not be administered because of risk for serious adverse reaction

No recommendation

1. Precaution for LAIV does not apply to alcoholism. **2.** See notes for influenza; hepatitis B; measles, mumps, and rubella; and varicella vaccinations. **3.** Hematopoietic stem cell transplant.

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FIG. 316.3 Recommended 2019 vaccination indicated for adults based on medical and other indications. See Fig. 316.4 for footnotes. *(From Centers for Disease Control and Prevention. Vaccines that might be indicated for adults aged 19 years or older based on medical and other indications. www.cdc.gov/vaccines/schedules/hcp/imz/adult-conditions.html. Accessed February 8, 2019.)*

Notes Recommended Adult Immunization Schedule United States, 2019

Haemophilus influenzae type b vaccination

Special situations

- **Anatomical or functional asplenia (including sickle cell disease)**: 1 dose Hib if previously did not receive Hib; if elective splenectomy, 1 dose Hib, preferably at least 14 days before splenectomy
- **Hematopoietic stem cell transplant** (HSCT): 3-dose series Hib 4 weeks apart starting 6–12 months after successful transplant, regardless of Hib vaccination history

Hepatitis A vaccination

Routine vaccination

- **Not at risk but want protection from hepatitis A** (identification of risk factor not required): 2-dose series HepA (Havrix 6–12 months apart or Vaqta 6–18 months apart [minimum interval: 6 months]) or 3-dose series HepA-HepB (Twinrix at 0, 1, 6 months [minimum intervals: 4 weeks between doses 1 and 2, 5 months between doses 2 and 3])

Special situations

- **At risk for hepatitis A virus infection**: 2-dose series HepA or 3-dose series HepA-HepB as above
  - **Chronic liver disease**
  - **Clotting factor disorders**
  - **Men who have sex with men**
  - **Injection or non-injection drug use**
  - **Homelessness**
  - **Work with hepatitis A virus** in research laboratory or nonhuman primates with hepatitis A virus infection
  - **Travel in countries with high or intermediate endemic hepatitis A**
  - **Close personal contact with international adoptee** (e.g., household, regular babysitting) in first 60 days after arrival from country with high or intermediate endemic hepatitis A (administer dose 1 as soon as adoption is planned, at least 2 weeks before adoptee's arrival)

Hepatitis B vaccination

Routine vaccination

- **Not at risk but want protection from hepatitis B** (identification of risk factor not required): 2- or 3-dose series HepB (2-dose series Heplisav-B at least 4 weeks apart [2-dose series HepB only applies when 2 doses of Heplisav-B are used at least 4 weeks apart] or 3-dose series Engerix-B or Recombivax HB at 0, 1, 6 months [minimum intervals: 4 weeks between doses 1 and 2, 8 weeks between doses 2 and 3, 16 weeks between doses 1 and 3]) or 3-dose series HepA-HepB (Twinrix at 0, 1, 6 months [minimum intervals: 4 weeks between doses 1 and 2, 5 months between doses 2 and 3])

Special situations

- **At risk for hepatitis B virus infection**: 2-dose (Heplisav-B) or 3-dose (Engerix-B, Recombivax HB) series HepB, or 3-dose series HepA-HepB as above
  - **Hepatitis C virus infection**
  - **Chronic liver disease** (e.g., cirrhosis, fatty liver disease, alcoholic liver disease, autoimmune hepatitis, alanine aminotransferase [ALT] or aspartate aminotransferase [AST] level greater than twice upper limit of normal)
  - **HIV infection**
  - **Sexual exposure risk** (e.g., sex partners of hepatitis B surface antigen (HBsAg)-positive persons; sexually active persons not in mutually monogamous relationships, persons seeking evaluation or treatment for a sexually transmitted infection, men who have sex with men)
  - **Current or recent injection drug use**
  - **Percutaneous or mucosal risk for exposure to blood** (e.g., household contacts of HBsAg-positive persons; residents and staff of facilities for developmentally disabled persons; health care and public safety personnel with reasonably anticipated risk for exposure to blood or blood-contaminated body fluids; hemodialysis, peritoneal dialysis, home dialysis, and predialysis patients; persons with diabetes mellitus age younger than 60 years and, at discretion of treating clinician, those age 60 years or older)
  - **Incarcerated persons**
  - **Travel in countries with high or intermediate endemic hepatitis B**

Human papillomavirus vaccination

Routine vaccination

- **Females through age 26 years and males through age 21 years:** 2- or 3-dose series HPV vaccine depending on age at initial vaccination; males age 22 through 26 years may be vaccinated based on individual clinical decision (HPV vaccination routinely recommended at age 11–12 years)
- **Age 15 years or older at initial vaccination**: 3-dose series HPV vaccine at 0, 1–2, 6 months (minimum intervals: 4 weeks between doses 1 and 2, 12 weeks between doses 2 and 3, 5 months between doses 1 and 3; repeat dose if administered too soon)
- **Age 9 through 14 years at initial vaccination and received 1 dose, or 2 doses less than 5 months apart**: 1 dose HPV vaccine
- **Age 9 through 14 years at initial vaccination and received 2 doses at least 5 months apart**: HPV vaccination complete, no additional dose needed
- If completed valid vaccination series with any HPV vaccine, no additional doses needed

Special situations

- **Immunocompromising conditions (including HIV infection) through age 26 years**: 3-dose series HPV vaccine at 0, 1–2, 6 months as above
- **Men who have sex with men and transgender persons through age 26 years**: 2- or 3-dose series HPV vaccine depending on age at initial vaccination as above
- **Pregnancy through age 26 years**: HPV vaccination not recommended until after pregnancy; no intervention needed if vaccinated while pregnant; pregnancy testing not needed before vaccination

FIG. 316.4 Footnotes: recommended immunization schedule for adults aged 19 years and older—United States, 2019. *(From Centers for Disease Control and Prevention. Recommended adult immunization schedule, by vaccine and age group. www.cdc.gov/vaccines/schedules/downloads/adult/adult-combined-schedule.pdf. Accessed February 8, 2019.)*

Notes

Recommended Adult Immunization Schedule United States, 2019

Influenza vaccination

Routine vaccination

- **Persons age 6 months or older**: 1 dose IIV, RIV, or LAIV appropriate for age and health status annually
- For additional guidance, see www.cdc.gov/flu/professionals/index.htm

Special situations

- **Egg allergy, hives only**: 1 dose IIV, RIV, or LAIV appropriate for age and health status annually
- **Egg allergy more severe than hives** (e.g., angioedema, respiratory distress): 1 dose IIV, RIV, or LAIV appropriate for age and health status annually in medical setting under supervision of health care provider who can recognize and manage severe allergic conditions
- **Immunocompromising conditions (including HIV infection), anatomical or functional asplenia, pregnant women, close contacts and caregivers of severely immunocompromised persons in protected environment, use of influenza antiviral medications in previous 48 hours, with cerebrospinal fluid leak or cochlear implant**: 1 dose IIV or RIV annually (LAIV not recommended)
- **History of Guillain-Barré syndrome within 6 weeks of previous dose of influenza vaccine**: Generally should not be vaccinated

Measles, mumps, and rubella vaccination

Routine vaccination

- **No evidence of immunity to measles, mumps, or rubella**: 1 dose MMR
  - Evidence of immunity: Born before 1957 (except health care personnel [see below]), documentation of receipt of MMR, laboratory evidence of immunity or disease (diagnosis of disease without laboratory confirmation is not evidence of immunity)

Special situations

- **Pregnancy with no evidence of immunity to rubella**: MMR contraindicated during pregnancy; after pregnancy (before discharge from health care facility), 1 dose MMR
- **Non-pregnant women of childbearing age with no evidence of immunity to rubella**: 1 dose MMR
- **HIV infection with CD4 count ≥200 cells/μL for at least 6 months and no evidence of immunity to measles, mumps, or rubella**: 2-dose series MMR at least 4 weeks apart; MMR contraindicated in HIV infection with CD4 count <200 cells/μL
- **Severe immunocompromising conditions**: MMR contraindicated
- **Students in postsecondary educational institutions, international travelers, and household or close personal contacts of immunocompromised persons with no evidence of immunity to measles, mumps, or rubella**: 1 dose MMR if previously received 1 dose MMR, or 2-dose series MMR at least 4 weeks apart if previously did not receive any MMR
- **Health care personnel born in 1957 or later with no evidence of immunity to measles, mumps, or rubella**: 2-dose series MMR at least 4 weeks apart for measles or mumps, or at least 1 dose MMR for rubella; if born before 1957, consider 2-dose series MMR at least 4 weeks apart for measles or mumps, or 1 dose MMR for rubella

Meningococcal vaccination

Special situations for MenACWY

- **Anatomical or functional asplenia (including sickle cell disease), HIV infection, persistent complement component deficiency, eculizumab use**: 2-dose series MenACWY (Menactra, Menveo) at least 8 weeks apart and revaccinate every 5 years if risk remains
- **Travel in countries with hyperendemic or epidemic meningococcal disease, microbiologists routinely exposed to *Neisseria meningitidis***: 1 dose MenACWY and revaccinate every 5 years if risk remains
- **First-year college students who live in residential housing (if not previously vaccinated at age 16 years or older) and military recruits**: 1 dose MenACWY

Special situations for MenB

- **Anatomical or functional asplenia (including sickle cell disease), persistent complement component deficiency, eculizumab use, microbiologists routinely exposed to *Neisseria meningitidis***: 2-dose series MenB-4C (Bexsero) at least 1 month apart, or 3-dose series MenB-FHbp (Trumenba) at 0, 1–2, 6 months (if dose 2 was administered at least 6 months after dose 1, dose 3 not needed); MenB-4C and MenB-FHbp are not interchangeable (use same product for all doses in series)
- **Pregnancy**: Delay MenB until after pregnancy unless at increased risk and vaccination benefit outweighs potential risks
- **Healthy adolescents and young adults age 16 through 23 years (age 16 through 18 years preferred) not at increased risk for meningococcal disease**: Based on individual clinical decision, may receive 2-dose series MenB-4C at least 1 month apart, or 2-dose series MenB-FHbp at 0, 6 months (if dose 2 was administered less than 6 months after dose 1, administer dose 3 at least 4 months after dose 2); MenB-4C and MenB-FHbp are not interchangeable (use same product for all doses in series)

FIG. 316.4, cont'd

Continued

Notes Recommended Adult Immunization Schedule United States, 2019

Pneumococcal vaccination

Routine vaccination

- **Age 65 years or older** (immunocompetent): 1 dose PCV13 if previously did not receive PCV13, followed by 1 dose PPSV23 at least 1 year after PCV13 and at least 5 years after last dose PPSV23
  - Previously received PPSV23 but not PCV13 at age 65 years or older: 1 dose PCV13 at least 1 year after PPSV23
  - When both PCV13 and PPSV23 are indicated, administer PCV13 first (PCV13 and PPSV23 should not be administered during same visit)

Special situations

- **Age 19 through 64 years with chronic medical conditions (chronic heart [excluding hypertension], lung, or liver disease; diabetes), alcoholism, or cigarette smoking**: 1 dose PPSV23
- **Age 19 years or older with immunocompromising conditions (congenital or acquired immunodeficiency [including B- and T-lymphocyte deficiency, complement deficiencies, phagocytic disorders, HIV infection], chronic renal failure, nephrotic syndrome, leukemia, lymphoma, Hodgkin disease, generalized malignancy, iatrogenic immunosuppression [e.g., drug or radiation therapy], solid organ transplant, multiple myeloma) or anatomical or functional asplenia (including sickle cell disease and other hemoglobinopathies)**: 1 dose PCV13 followed by 1 dose PPSV23 at least 8 weeks later, then another dose PPSV23 at least 5 years after previous PPSV23; at age 65 years or older, administer 1 dose PPSV23 at least 5 years after most recent PPSV23 (note: only 1 dose PPSV23 recommended at age 65 years or older)
- **Age 19 years or older with cerebrospinal fluid leak or cochlear implant**: 1 dose PCV13 followed by 1 dose PPSV23 at least 8 weeks later; at age 65 years or older, administer another dose PPSV23 at least 5 years after PPSV23 (note: only 1 dose PPSV23 recommended at age 65 years or older)

Tetanus, diphtheria, and pertussis vaccination

Routine vaccination

- **Previously did not receive Tdap at or after age 11 years**: 1 dose Tdap, then Td booster every 10 years

Special situations

- **Previously did not receive primary vaccination series for tetanus, diphtheria, and pertussis**: 1 dose Tdap followed by 1 dose Td at least 4 weeks after Tdap, and another dose Td 6–12 months after last Td (Tdap can be substituted for any Td dose, but preferred as first dose); Td booster every 10 years thereafter
- **Pregnancy**: 1 dose Tdap during each pregnancy, preferably in early part of gestational weeks 27–36
- For information on use of Tdap or Td as tetanus prophylaxis in wound management, see www.cdc.gov/mmwr/volumes/67/rr/rr6702a1.htm

Varicella vaccination

Routine vaccination

- **No evidence of immunity to varicella**: 2-dose series VAR 4–8 weeks apart if previously did not receive varicella-containing vaccine (VAR or MMRV [measles-mumps-rubella-varicella vaccine] for children); if previously received 1 dose varicella-containing vaccine: 1 dose VAR at least 4 weeks after first dose
  - Evidence of immunity: U.S.-born before 1980 (except for pregnant women and health care personnel [see below]), documentation of 2 doses varicella-containing vaccine at least 4 weeks apart, diagnosis or verification of history of varicella or herpes zoster by a health care provider, laboratory evidence of immunity or disease

Special situations

- **Pregnancy with no evidence of immunity to varicella**: VAR contraindicated during pregnancy; after pregnancy (before discharge from health care facility), 1 dose VAR if previously received 1 dose varicella-containing vaccine, or dose 1 of 2-dose series VAR (dose 2: 4–8 weeks later) if previously did not receive any varicella-containing vaccine, regardless of whether U.S.-born before 1980
- **Health care personnel with no evidence of immunity to varicella**: 1 dose VAR if previously received 1 dose varicella-containing vaccine, or 2-dose series VAR 4–8 weeks apart if previously did not receive any varicella-containing vaccine, regardless of whether U.S.-born before 1980
- **HIV infection with CD4 count ≥200 cells/μL with no evidence of immunity**: Consider 2-dose series VAR 3 months apart based on individual clinical decision; VAR contraindicated in HIV infection with CD4 count <200 cells/μL
- **Severe immunocompromising conditions**: VAR contraindicated

Zoster vaccination

Routine vaccination

- **Age 50 years or older**: 2-dose series RZV 2–6 months apart (minimum interval: 4 weeks; repeat dose if administered too soon) regardless of previous herpes zoster or previously received ZVL (administer RZV at least 2 months after ZVL)
- **Age 60 years or older**: 2-dose series RZV 2–6 months apart (minimum interval: 4 weeks; repeat dose if administered too soon) or 1 dose ZVL if not previously vaccinated (if previously received ZVL, administer RZV at least 2 months after ZVL); RZV preferred over ZVL

Special situations

- **Pregnancy**: ZVL contraindicated; consider delaying RZV until after pregnancy if RZV is otherwise indicated
- **Severe immunocompromising conditions (including HIV infection with CD4 count <200 cells/μL)**: ZVL contraindicated; recommended use of RZV under review

FIG. 316.4, cont'd

than the routine series through 14 months of age and no doses at 14 months or older) should receive a dose of Hib vaccine at least 2 weeks before splenectomy. If they have completed the recommended series, providers may offer an additional dose of Hib vaccine.

Children 12 through 59 months of age who are asplenic and have received fewer than two doses before 12 months of age require two doses of Hib. Persons 5 years of age or older who are asplenic and who are unvaccinated or incompletely vaccinated require one dose of Hib.

Children with HIV infection between 15 months and 18 years of age and who are unvaccinated or incompletely vaccinated require one dose of Hib. Hib vaccination is not recommended for HIV-infected adults.

Patients younger than 59 months undergoing chemotherapy or radiation therapy who receive doses of Hib vaccine within 2 weeks of their therapy should have these doses repeated at least 3 months after completion of therapy. Any recipient of an HSCT should be revaccinated with a three-dose regimen 6 to 12 months after a successful transplant, regardless of vaccination history; at least 4 weeks should separate the doses.[50]

Although vaccine is not indicated for children who had documented invasive Hib infection at 2 years or older, it is indicated for children younger than 2 years who had documented invasive Hib infection because of their potential inadequate antibody response after natural infection.

Hib-containing vaccines have a very good safety record.[94] Local reactions at the injection site and fever have been noted in less than 4% of vaccinees. The vaccines should not be administered if there is a history of anaphylaxis to the specific vaccine or to other vaccine components.

Hepatitis A Vaccine

There are two inactivated single-antigen hepatitis A vaccines available in the United States: Havrix (GlaxoSmithKline Biologicals, Research Triangle Park, NC) and Vaqta (Merck, Whitehouse Station, NJ). Efficacy of one 25-unit dose of Vaqta in children 2 to 16 years of age is 97%.[100,101]

Preventing hepatitis A at the community level requires widespread vaccination of children and adults.[102] In 1996, ACIP recommended hepatitis A vaccine for children at age 2 years in communities with high rates of disease and children through the teen years in outbreaks.[103] In 1999, the ACIP recommendations were expanded to include children beginning at 2 years or older living in states, counties, or communities with reported annual rates of hepatitis A of 20 per 100,000 or higher between 1987 and 1997, and vaccine was considered in states with rates above the national average of 10 cases per 100,000 population or higher.[104] In 2006, ACIP recommended that all children aged 12 to 23 months be vaccinated.[105] Children who are not vaccinated by age 2 years can be vaccinated at subsequent visits. Hepatitis A vaccine is also recommended for use among populations known to be at increased risk for infection, including persons traveling to hepatitis A–endemic areas, men who have sex with men (MSM), users of injection and noninjection drugs, persons who work with hepatitis A virus–infected primates or who do research with the virus, recipients of clotting factors, and persons who anticipate close personal contact with an international adoptee.[106] Persons with chronic liver disease may be at increased risk for fulminant hepatitis A and should also be vaccinated as well.[103] Homelessness has been associated with hepatitis A cases and outbreaks, and homelessness was approved as an indication for hepatitis A vaccination by ACIP in October 2018.[107,107a]

Havrix is recommended in a two-dose schedule, with doses separated by 6 to 12 months. The dose for children 1 to 18 years of age is 720 enzyme-linked immunosorbent assay (ELISA) units; for adults, it is 1440 ELISA units. Two doses of 25 units of Vaqta 6 to 18 months apart are recommended for persons 1 to 18 years of age, and two doses of 50 units 6 months apart are recommended for persons aged 19 years or older. The second dose is intended to produce lifelong immunity to hepatitis A. Hepatitis A vaccine is not licensed for use in children younger than 12 months. The vaccine is poorly immunogenic in infants born to women who are seropositive for hepatitis A.[14,108] Simultaneous administration with IG may decrease immunogenicity slightly but should not cause any decrease in protection.[109]

ACIP recommends hepatitis A vaccine for international travelers to countries with high or intermediate hepatitis A endemicity. Hepatitis A vaccine should be administered to infants aged 6 to 11 months traveling outside the United States. The travel-related dose for infants aged 6 to 11 months does *not* count toward the routine two-dose series; the two-dose series should be initiated at age 12 months according to the routine, age-appropriate vaccine schedule. Healthy travelers 12 months and older who have not received the hepatitis A vaccine should receive a single dose of vaccine as soon as travel is considered.[107,110] Infants younger than 6 months and travelers who elect not to receive vaccine or for whom vaccine is contraindicated should receive a single dose of IG (0.1 mL/kg). The dose is 0.2 mL/kg if the travel duration is 1 month or longer. Persons with chronic liver disease, older adults (aged >40 years), immunocompromised persons, and persons with other chronic medical conditions planning to depart to a risk area in <2 weeks should receive the initial dose of vaccine, and IG can also be simultaneously administered at a separate anatomic injection site.

Persons who have recently been exposed to hepatitis A virus and who have not received the hepatitis A vaccine previously should receive PEP as soon as possible within 2 weeks of exposure. Persons aged ≥12 months should receive a single dose of vaccine as soon as possible. Infants aged <12 months and persons for whom vaccine is contraindicated should receive IG instead of vaccine for PEP. Immunocompromised persons and persons with chronic liver disease should receive both IG and hepatitis A vaccine simultaneously at a different anatomic site, as soon as possible after exposure. For long-term immunity, the hepatitis A vaccine series should be completed with a second dose at least 6 months after the first dose; the second dose is not necessary for PEP.

The most frequent side effects are local reactions. The only contraindication is for persons with a severe allergic reaction after a previous dose or to a vaccine component.[105]

Hepatitis B Vaccine

Hepatitis B vaccine consists of purified HBsAg particles obtained either from plasma of chronic carriers or from yeast through recombinant DNA technology. In the United States, plasma-derived vaccines have been replaced by recombinant vaccines, although the former are still available abroad. There are three single-antigen hepatitis B vaccines available in the United States—Recombivax HB (Merck), Engerix-B (GlaxoSmithKline), and Heplisav-B (Dynavax).[12] Engerix-B is available as a combination product: with hepatitis A vaccine (Twinrix; GlaxoSmithKline), or DTaP and IPV (Pediarix; GlaxoSmithKline). Heplisav-B is a recombinant vaccine that contains an adjuvant, a synthetic oligodeoxynucleotide called CpG, which binds to a molecule on APCs called TLR9, stimulating an immune response to hepatitis B. Because recommended doses vary by age, the package insert should be consulted for the proper dose of each product. When initially licensed, use of vaccine was targeted to individuals at high risk for exposure to hepatitis B, including certain categories of health care personnel (those with risk for exposure to blood or blood products), hemodialysis patients, recipients of certain blood products, MSM, certain institutionalized individuals, parenteral drug abusers, and household or sexual contacts of chronic carriers of HBsAg. Vaccine continues to be indicated for these groups, and federal regulations now mandate that the vaccine be made available at no cost to all health care and public safety workers who anticipate exposure to human blood or body fluids during work.[111] In 2011, adults through 59 years of age with diabetes were added to this list of risk groups, and so providers should offer hepatitis B vaccine to all adults with diabetes younger than 60 years. Providers may offer vaccine to diabetics older than 59 years, particularly if they receive assisted blood glucose screening in a long-term care facility.[111] Failure of vaccination to have substantial impact on disease incidence when targeted only to high-risk groups, along with appreciation that hepatitis B affects larger groups in the general population (such as heterosexuals with multiple partners), has led to development of population-based control strategies.[59] In 2018, ACIP updated recommendations for individuals with chronic liver diseases to whom hepatitis B vaccine should be administered. These included but were not limited to hepatitis C virus infection, cirrhosis, fatty liver disease, alcoholic liver disease, autoimmune hepatitis, and alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels greater than twice the upper limit of normal (Fig. 316.4).[112]

Currently in the United States, universal hepatitis B vaccine is recommended within 24 hours of birth for medically stable infants weighing

≥2000 g. Primary vaccination generally consists of three IM doses administered on a 0-, 1-, and 6-month schedule. When using combination vaccines, a four-dose schedule, including a birth dose of single-antigen hepatitis B vaccine, is acceptable. Alternative vaccination schedules (e.g., 0, 1, and 4 months or 0, 2, and 4 months) have been demonstrated to elicit dose-specific and final rates of seroprotection similar to those obtained on a 0-, 1-, and 6-month schedule. It is anticipated that those immunized as infants will still be protected when they become adolescents and young adults, the greatest risk period of acute infection in the United States.[113] To protect infants at highest risk for development of chronic hepatitis B infection, all pregnant women should be screened routinely for HBsAg, preferably during an early prenatal visit. The vaccine should be administered within 12 hours of birth, along with hepatitis B IG, to infants born of HBsAg-positive mothers.

For adolescents and adults, the usual schedule is doses at 0, 1, and 6 months.[59] All adolescents who previously have not been vaccinated should receive three doses of vaccine. The final dose of vaccine must be administered at least 8 weeks after the second dose and should follow the first dose by at least 16 weeks; the minimum interval between the first and second doses is 4 weeks. An alternative two-dose regimen of one licensed hepatitis B vaccine (Recombivax) is available for routine vaccination of adolescents, with doses at 0 and 4 to 6 months. For adolescents who have not been vaccinated previously, a good time to begin is at 11 to 12 years of age, when other immunizations also are recommended.[114]

The vaccine should be administered intramuscularly to infants in the anterolateral thigh with a 1-inch 23-gauge needle and to children and adults in the deltoid region. For deltoid vaccination, a ⅝-inch 25-gauge needle may be used in children up to 9 years of age (if the skin is stretched tightly and subcutaneous tissues are not bunched), but generally a 1-inch 23-gauge needle should be used in older children and adults. Gluteal administration is associated with poorer antibody responses and is not recommended.[13] A series of three IM doses produces a protective antibody response (antibody to HBsAg ≥10 mIU/mL) in greater than 95% of infants and children, greater than 90% of adults younger than 40 years, and 75% to 90% of adults older than 40 years. Host factors, such as smoking and obesity, contribute to decreased immunogenicity of the primary vaccine series, but age is the major determinant of vaccine response. Vaccine immunogenicity also may be lower in immunocompromised patients. Follow-up for up to 30 years has shown the virtual absence of clinically significant infections in persons who initially achieved a protective antibody titer.[10] Most persons who lose detectable antibody appear to retain immunologic memory against significant infections. A small study of Alaskan children, vaccinated at birth, suggested that almost half of children lacked anamnestic responses after a booster dose 15 years later.[10] However, none of the children had been infected, as measured by the presence of core antibody. In a study by Middleman and colleagues published 7 years later, 90% of study participants (420 adolescents) immunized against hepatitis B as infants exhibited a seroprotective response to a challenge dose of vaccine.[115] Thus there is no indication at this time for booster doses of vaccine after immunization of immunocompetent children or adults. Additional experience will be necessary to know whether there will be any need for booster doses.

Alopeica has rarely been reported primarily in adults and has been reversible in most cases.[116] A number of case reports have linked hepatitis B vaccine to demyelinating syndromes, including multiple sclerosis.[117,118] However, data available do not support a causal relationship. The IOM's Immunization Safety Review Committee reviewed available data and concluded that the evidence did not support a relationship between hepatitis B vaccination in adults and multiple sclerosis; the evidence was inadequate to accept or reject a causal relationship with other demyelinating conditions.[30] A more recent review by the IOM reported only anaphylaxis in some individuals that could be linked to vaccine. For most conditions reviewed, the evidence was inadequate to accept or reject a causal relationship.[119] Recombinant hepatitis B vaccine is contraindicated in persons with hypersensitivity to yeast. Immunization is not effective in eliminating the carrier state, but there is no known risk for vaccinating individuals who are carriers or who are already immune.[15]

In February 2018, ACIP recommended use of the new single-antigen recombinant hepatitis B vaccine with a novel cytosine-phosphate-guanine 1018 oligodeoxynucleotide adjuvant (Heplisav-B) for prevention of HBV infection in adults aged ≥18 years.[12] Approved by the FDA in November 2017, Heplisav-B is routinely administered in two doses given ≥4 weeks apart. It can be used as a substitute in a three-dose series with a different hepatitis B vaccine, but a valid two-dose series requires two doses of Heplisav-B with ≥4 weeks between doses. When feasible, a vaccine from the same manufacturer should be used to complete the vaccination series. However, vaccination should not be deferred if the previously administered hepatitis B vaccine is unknown or if a vaccine from the same manufacturer is not available. A pregnant woman with an indication for hepatitis B vaccination should not receive Heplisav-B because no safety data are available on its use during pregnancy.

Human Papillomavirus Vaccines

Three HPV vaccines were developed using L1 capsid proteins, which self-assemble into VLPs that are similar in conformation to the natural virus.[117,120] All three are produced using recombinant techniques, which incorporate the gene expressing L1 into *Saccharomyces cerevisiae* or baculovirus-infected insect cells. Only one licensed vaccine is currently available in the United States: nona(nine)valent vaccine (9vHPV) containing types 6, 11, 16, 18, 31, 33, 45, 52, and 58. Types 16 through 58 in the vaccine cause about 80% of cervical cancers worldwide; types 6 and 11 cause about 90% of genital warts. Quadrivalent (types 6, 11, 16, and 18) HPV vaccine (4vHPV) and bivalent HPV vaccine (2vHPV), which contains types 16 and 18, are no longer available in the United States. The nonavalent vaccine is produced in yeast and contains an aluminum hydroxide adjuvant.

For efficacy studies for 4vHPV, a combined analysis of four clinical trials evaluating high-grade lesions (cervical intraepithelial neoplasia grade 2 or 3 [CIN 2/3] or adenocarcinoma in situ [AIS]) associated with types 16 and 18 revealed an efficacy of 100% with a lower bound of the 95% confidence limit of 92.9%. Effectiveness against genital warts related to any of the four types was 98.9% (95% confidence interval [CI], 93.7%–100%). The duration of protection is unknown, but with over 10 years of data, there is no evidence of waning protection. Efficacy of 4vHPV in males has been demonstrated for prevention of genital warts, anal intraepithelial neoplasia types 2/3 and anal intraepithelial neoplasia types 1/2/3 (88%–89%, 78%, and 75%, respectively).[121,122]

9vHPV has been shown to have similar immunogenicity to 4vHPV for the four shared types, and is approximately 95% effective against the five additional HPV types in the vaccine.

Local reactions were more common in vaccine recipients. After licensure, concerns were raised about serious adverse events temporally related to HPV vaccine, such as seizures and autoimmune disorders, but the postlicensure studies have not found an elevated risk.[122a]

Within 4 years of use of these vaccines, vaccine type prevalence of HPVs decreased from 11.5% to 5.1% among females 14 to 19 years of age.[123] ACIP recommends routine HPV vaccination at age 11 or 12 years. The vaccination schedule can be started at age 9 years. ACIP also recommends vaccination for females aged 13 to 26 years, for males aged 13 to 21 years who were not vaccinated previously, and for males to 26 years of age if they are immunosuppressed, have HIV infection, or are MSM. Vaccine also may be administered to all men 22 years to 26 years of age.

In December 2016, ACIP recommended that a two-dose schedule would be sufficient for girls and boys who initiate the vaccination series at ages 9 through 14 years. The two doses should be administered with 6 to 12 months between the doses. Three doses at 0, 1 to 2 months, and 6 months remain recommended for persons who initiate vaccination at ages 15 through 26 years, for immunocompromised persons, and for people with sickle cell disease.[124]

Influenza Virus Hemagglutinin Vaccines–Inactivated and Recombinant (IIV and RIV)

Most inactivated influenza virus vaccines are manufactured in chicken eggs and are composed of inactivated disrupted ("split") influenza viruses or of purified surface antigens. Inactivated influenza vaccine (trivalent) or IIV3 contains antigens for two influenza A viruses, H1N1 and H3N2,

and one influenza B virus. Most IIV3 is administered intramuscularly; a preparation that is administered intradermally and approved for persons 18 years to 64 years of age was licensed in 2011. The intradermal IIV was changed from a trivalent to a quadrivalent vaccine a few seasons before it was discontinued (it was not marketed in 2018). One formulation of IIV3 contains four times the antigenic content of the others and is considered "high dose," and is an option for persons aged 65 years or older. Also an option for persons aged 65 years or older is an adjuvanted vaccine, which is an IIV3 vaccine that contains a squalene-based oil-in-water emulsion.

Starting in the 2013–14 influenza season, some vaccines included antigens from two influenza A virus subtypes and two influenza B virus lineages, Yamagata and Victoria, making them quadrivalent vaccines (IIV4). Quadrivalent vaccine is an option, but there is no preference for its use in any group.

There are two forms of IIVs that are not manufactured in eggs. Cell-cultured–based influenza vaccine (ccIIV4) is manufactured in Madin-Darby canine kidney cells, is quadrivalent, is intramuscularly administered, and has been approved for use in persons 4 years of age or older. Quadrivalent recombinant hemagglutinin influenza vaccine (RIV4) is manufactured through reverse genetics in an insect cell line to produce influenza antigen and never uses the entire influenza virus. RIV4 and ccIIV4 avoid use of eggs for manufacture, which would make their production sustainable even if there were a shortage of eggs, as could occur in a pandemic. ccIIV4 uses seed virus that is isolated in eggs and therefore is not considered egg free, although the remaining quantity of egg protein is extremely low. RIV4 is considered egg free.[6]

Because of the frequent antigenic changes in influenza viruses, the antigenic content of influenza virus vaccines may be changed annually to reflect the influenza A and B virus strains in circulation. In most years, at least one of the strains is different from the preceding year's vaccine. The efficacy of the vaccine in protecting against influenza is related to the age of the person immunized and to the degree of concordance between the virus strains included in the vaccine and the strains that are circulating in the community. When periodic changes in the antigenic structure of circulating influenza viruses occur, vaccine that contains antigens representative of prior viruses has decreased or no effectiveness. In recent years, influenza vaccine effectiveness has been approximately 40% to 60% when there is a good match between strains in the vaccine and circulating strains (across all age groups).[125] Influenza vaccine has been estimated to be about 60% effective in preventing influenza in healthy adults younger than 65 years, when there is a good match.[126,127]

In nursing home settings, effectiveness has often been substantially lower, approximately 20% to 40%.[128] Some studies show higher effectiveness for preventing complications of influenza in such settings—for instance, 50% to 60% in preventing hospitalization or pneumonia and 80% in preventing death; however, such studies may be biased if healthier persons are more likely to be vaccinated than those who are less healthy.[128] Influenza vaccination might reduce the frequency of secondary complications and might reduce the risk for influenza-related hospitalization and death among community-dwelling adults aged 65 years or older with and without high-risk medical conditions.[129–131] Preliminary estimates of effectiveness of the A/H3N2 component of the 2017–18 vaccine showed about 17% effectiveness in the elderly, compared with 10% to 37% in younger adults. In contrast, effectiveness against influenza B strains was substantially higher in all age groups in that season: 29% to 57% effective in all age groups.[132] Efficacy data among young children are limited. A meta-analysis of five studies showed efficacy of 59% in children 6 months to 15 years of age.[133] In 2010, ACIP recommended that all persons aged 6 months or older be vaccinated annually.[6] This should provide individual benefits to those who are vaccinated but also has the potential to reduce community transmission of the virus and provide indirect benefit to others.

Although routine annual influenza vaccination is recommended for all persons 6 months or older, when vaccine supply is limited, vaccination efforts should focus on delivering vaccination to the following persons (no hierarchy is implied by order of listing): all children aged 6 months to 59 months; all persons aged 50 years or older; adults and children who have chronic pulmonary (including asthma) or cardiovascular (except isolated hypertension), renal, hepatic, neurologic, hematologic, or metabolic disorders (including diabetes mellitus); persons who have immunosuppression (including immunosuppression caused by medications or by HIV infection); women who are or will be pregnant during the influenza season; children and adolescents (aged 6 months to 18 years) who are receiving aspirin or salicylate-containing medications and who might be at risk for experiencing Reye syndrome after influenza virus infection; residents of nursing homes and other long-term care facilities; American Indians/Alaska Natives; and persons who are extremely obese (body mass index ≥40). Influenza vaccination should also be emphasized for health care personnel; household contacts and caregivers of children aged 5 years or younger and adults aged 50 years or older, with particular emphasis on vaccinating contacts of children younger than 6 months; and household contacts and caregivers of persons with medical conditions that put them at higher risk for severe complications from influenza.

For the 2018–19 season, it was recommended that children 6 months to 8 years of age being vaccinated for the first time receive two doses of vaccine with an interval of at least 4 weeks between them. Children who had received two total doses or more of trivalent or quadrivalent vaccine before July 1, 2018 required only one dose of the 2018–19 recommended vaccine.[134] Influenza seasons can peak anywhere from November to May, although the peak most often occurs in January or later, February being the most common month. Thus, although October and November have been the traditional months for influenza vaccination, during many influenza seasons vaccination through February, and even March, will provide benefit.

Adverse events associated with current influenza vaccines are infrequent. During the swine influenza immunization program of 1976, an elevated incidence rate of Guillain-Barré syndrome (GBS) was noted in recipients of the swine influenza vaccine.[135] However, studies during the 1992–93 and 1993–94 influenza seasons suggested that influenza vaccines may have been associated with GBS at an attributable risk of about one additional case per 1 million doses in those years.[136] No cases of GBS within 6 weeks of vaccination were detected in persons 18 to 44 years of age, despite administration of about 4 million doses of vaccine over the two influenza seasons studied.[136]

If GBS is ever caused by current influenza vaccines, this is a rare occurrence. In contrast, the risk for hospitalization from influenza disease and its complications is orders of magnitude higher in most populations in which vaccine is recommended. Given the substantial benefits of influenza vaccine among the targeted populations, risk for GBS, if any, is exceeded by benefits. Several studies have shown an increased risk, but results were variable within and across studies and subject to methodologic challenges due to narcolepsy epidemiology and increased awareness about the association.[137–140]

An increased incidence of narcolepsy has been reported in those younger than 30 to 40 years who received adjuvanted (AS03) monovalent 2009 pandemic H1N1 vaccines used in Europe in 2009 and 2010, but this vaccine was not licensed in the United States.[141–144]

A recent Vaccine Safety Datalink (VSD) study found that women vaccinated early in pregnancy with an influenza vaccine containing the A(H1N1) 2009 strain and who also had been vaccinated the prior season with an A(H1N1)pdm09-containing influenza vaccine had an increased risk of spontaneous abortion (miscarriage) in the 28 days after vaccination.[145] Earlier studies did not find a link between influenza vaccination and miscarriage. This study examined data from a small number of women in a subgroup who received H1N1-containing vaccines in consecutive years. The small numbers in the study could have led to imprecise results.There is an ongoing investigation to study this issue further among women who were pregnant and eligible to receive influenza vaccine during the 2012–13 through 2014–15 influenza seasons. Results are anticipated in late 2018 or 2019.

Because pregnant women are at high risk of serious influenza complications, it is recommended that they receive influenza vaccination during any trimester of their pregnancy. Providers should consult current guidelines for more detailed and updated recommendations.

Data demonstrating the safety of IIV for HIV-infected persons are limited, but no evidence exists that vaccination has a clinically important impact on HIV infection or immunocompetence. It is especially important

to vaccinate HIV-infected persons because of their increased risk of influenza complications.

Live-Attenuated Influenza Vaccine

In 2003 the FDA licensed LAIV vaccine to be administered intranasally. Each viral strain in the vaccine consists of six internal genes from a cold-adapted, temperature-sensitive, attenuated mutant.[6,146,147] The hemagglutinin and neuraminidase are derived from circulating wild strains. The cold adaptation is supportive of growth of the vaccine viruses in the upper airways, and temperature sensitivity decreases their growth in the lower airways. The vaccine had been trivalent, with reassortants for each of the major circulating influenza viruses: A(H3N2), A(H1N1), and B. Since the 2013–14 season, the only LAIV preparation has been quadrivalent, including representative strains of the two influenza B lineages, Yamagata and Victoria.

A meta-analysis of five studies showed a pooled efficacy of 83% for LAIV in children 6 months to 7 years old prior to the 2012–13 season.[126] In a study of healthy children, vaccine was 94% effective after two doses in children 60 to 71 months of age in 1996–97, with a good match between vaccine and circulating wild virus, and 86% in 60- to 84-month-old children in 1997–98, when vaccine and circulating strains substantially diverged. In addition, vaccine reduced influenza A–associated febrile otitis media (vaccine efficacy, 94%).[6] Estimated efficacy of LAIV against laboratory-confirmed influenza in randomized, placebo-controlled studies among 18- to 49-year-old adults was 36% in the 2007–08 season but was not significantly different from zero in either the 2004–05 or the 2005–06 season.[6]

For the 2016–17 seasons, as well as for the 2017–18 seasons, ACIP recommended that LAIV4 not be used, because of concerns regarding low effectiveness against influenza A(H1N1)2009 in the United States during the 2013–14 and 2015–16 seasons.[134] In the 2014–15 season, the effectiveness of LAIV4 among 2- to 8-year-olds was found to be 3% against the H3N2 strain.[148] In the 2015–16 season the effectiveness of LAIV among 2- to 17-year-olds was found to be 5%, and against the H1N1 strain was found to be −19%.[149] This recommendation to not use LAIV4 continued through the 2017–18 seasons. Previous data and recommendations regarding the use of LAIV are further discussed in the following text and in Chapter 165.

In adults 18 through 49 years of age, solicited adverse reactions occurring in at least 1% of LAIV4 recipients and at a higher rate (≥1% rate difference after rounding) compared with placebo include runny nose (44% LAIV4 vs. 27% placebo), headache (40% LAIV4 vs. 38% placebo), sore throat (28% LAIV4 vs. 17% placebo), tiredness or weakness (26% LAIV4 vs. 22% placebo), muscle aches (17% LAIV4 vs. 15% placebo), cough (14% LAIV4 vs. 11% placebo), and chills (9% LAIV4 vs. 6% placebo).[150–152]

Contraindications to LAIV include a history of severe allergic reaction to any component of the vaccine or after a previous dose of any influenza vaccine; concomitant aspirin or salicylate-containing therapy in children and adolescents; children aged 2 through 4 years who have received a diagnosis of asthma or whose parents or caregivers report that a health care provider has told them that during the preceding 12 months their child had wheezing or asthma or whose medical record indicates a wheezing episode has occurred during the previous 12 months; children and adults who are immunocompromised from any cause (including immunosuppression caused by medication or by HIV infection); close contacts and caregivers of severely immunosuppressed persons who require a protected environment; pregnancy; and receipt of an influenza antiviral medication within the previous 48 hours. Precautions regarding use of LAIV include moderate or severe acute illness or fever; history of GBS within 6 weeks of a previous dose of influenza vaccine; asthma in persons aged 5 years and older; and other underlying medical conditions that might predispose to complications after wild-type influenza infection (e.g., chronic pulmonary, cardiovascular [except isolated hypertension], renal, hepatic, neurologic, hematologic, or metabolic [including diabetes mellitus] disorders).[6]

Although there had been promise that LAIV would be highly effective based on prelicensure trials and early experience, the consistent poor effectiveness documented in the United States starting in the 2013–14 influenza season, particularly against H1N1 viruses, led ACIP to recommend the vaccine not be used through 2017–18. Recent data led to the recommendation that LAIV is an option for influenza vaccination of those in whom it is appropriate to use LAIV in 2018–19.[134]

Persons with a history of egg allergy who have experienced only hives after exposure to eggs should receive influenza vaccine. Any licensed and recommended influenza vaccine (i.e., any age-appropriate IIV, RIV4, or LAIV4) that is otherwise appropriate for the recipient's age and health status may be used.

Persons who report having had reactions to egg involving symptoms other than hives, such as angioedema, respiratory distress, lightheadedness, or recurrent emesis, or who required epinephrine or another emergency medical intervention, may similarly receive any licensed and recommended influenza vaccine (i.e., any age-appropriate IIV, RIV4, or LAIV4) that is otherwise appropriate for the recipient's age and health status. The selected vaccine should be administered in an inpatient or outpatient medical setting (including but not necessarily limited to hospitals, clinics, health departments, and physician offices). Vaccine administration should be supervised by a health care provider who is able to recognize and manage severe allergic conditions.

Japanese Encephalitis

In 2009, inactivated Vero (green monkey kidney) cell culture–derived Japanese encephalitis (JE) vaccine (JE-VC; Ixiaro [Intercell Biomedical, Livingston, United Kingdom]) was licensed for use in persons aged 17 years or older and subsequently was recommended for travelers in this age group at high risk of JE. This is the only JE vaccine that is licensed and available in the United States. In May 2013 the FDA extended the indication for use of JE-VC to include children 2 months to 16 years of age, and subsequently ACIP extended recommendations for use in this age group. The vaccine was licensed in the United States based on a noninferiority immunogenicity study comparing neutralizing antibodies elicited by the new vaccine with the previously available JE vaccine grown in mouse brains ([JE-MB]-[JE-VAX]). The JE-MB vaccine was associated with hypersensitivity and neurologic adverse reactions. Fewer vaccine-associated hypersensitivity or neurologic adverse events are expected to occur after use of JE-VC compared with the previously used JE-MB vaccine. JE-VC vaccine consists of purified, inactivated JE proteins derived from attenuated virus propagated in Vero cells. Immunogenicity studies have demonstrated noninferiority to the JE-MB vaccine, which was proved to be 91% effective in a large-scale trial in Thailand.[153] JE-VC vaccine is recommended for travelers who plan to spend a month or longer in endemic areas during the Japanese encephalitis virus (JEV) transmission season. This includes long-term travelers, recurrent travelers, or expatriates who will be based in urban areas but are likely to visit endemic rural or agricultural areas during a high-risk period of JEV transmission. Vaccine should also be considered for the following: short-term (less than 1 month) travelers to endemic areas during the JEV transmission season, if they plan to travel outside an urban area and their activities will increase the risk of JEV exposure; travelers to an area with an ongoing JEV outbreak; and travelers to endemic areas who are uncertain of specific destinations, activities, or duration of travel. The immunization schedule is two doses administered 28 days apart.[154,155]

Measles-Containing Vaccine

Measles vaccine is a live-attenuated virus vaccine recommended for use in all children 12 months and older who do not have contraindications.[156] When administered to a child 12 to 15 months or older, the median one-dose efficacy is 93% and the median two-dose efficacy is 97%.[157] Only a single dose is needed to provide long-lasting, probably lifelong, immunity in those who respond to the vaccine. However, evidence indicates that measles transmission can be sustained among the 2% to 5% of vaccinated persons who fail to be protected after an initial dose of vaccine. Therefore, beginning in 1989 a two-dose schedule of measles-containing vaccine was recommended in the United States. The first dose should be administered at 12 to 15 months of age. Lower levels of maternal antibody from currently vaccinated mothers allow higher rates of seroconversion at 12 months than in the past, when most maternal antibody came from mothers with naturally acquired disease.[158]

The second dose should be administered 1 month or more after the first dose, typically at entry to school (4–6 years of age). Both doses should routinely be given as combined MMR vaccine or MMR and varicella (MMRV).[156,159] Both MMR and MMRV vaccines are associated with an elevated febrile seizure risk, but data suggest that MMRV, because it is associated with a higher risk for fever than the separate administration of MMR and varicella, also may be associated with an increased risk for febrile seizures compared with simultaneous separate MMR and varicella vaccines after the first dose of the two-dose series.[159] In June 2009, after consideration of the postlicensure data and other evidence, ACIP adopted new recommendations regarding use of MMRV vaccine for the first and second doses and identified a personal or family (i.e., sibling or parent) history of seizure as a precaution for use of MMRV vaccine. For the first dose of measles, mumps, rubella, and varicella vaccines at age 12 to 47 months, either MMR vaccine and varicella vaccine or MMRV vaccine may be used. Providers who are considering administering MMRV vaccine should discuss the benefits and risks of both vaccination options with the parents or caregivers. Unless the parent or caregiver expresses a preference for MMRV vaccine, ACIP recommends that MMR vaccine and varicella vaccine should be administered separately for the first dose in this age group. For the second dose of measles, mumps, rubella, and varicella vaccines at any age (15 months through 12 years—the age for which the vaccine is approved) and for the first dose at age 48 months or older, use of MMRV vaccine generally is preferred over separate injections of its equivalent component vaccines (i.e., MMR vaccine and varicella vaccine). Recent data from Australia suggest that MMRV is not associated with an increased risk of febrile seizures when it is the second dose of an MMR-containing vaccine.[160]

All college entrants who have not received two doses of MMR vaccine on or after their first birthday should receive two doses.[156] The two doses can be given separated by 1 month. Immunization is recommended for all people not known to be immune. Because people born before 1957 are likely to have been infected naturally, they usually are considered immune. Other acceptable evidence of measles immunity is documentation of adequate vaccination or laboratory evidence of immunity to measles.[157] Health care facilities should consider recommending a dose of MMR to unvaccinated providers born before 1957 who do not have laboratory evidence of immunity to both measles and rubella or other acceptable evidence of measles immunity.

Because measles is much more prevalent outside the United States, adequate vaccination is recommended for all travelers born after 1956. These travelers should have evidence of having received two doses.[156] Infants 6 months to 11 months of age should receive a dose of MMR vaccine if they travel internationally. One dose is recommended for travel in this age group, but this dose is not considered part of the routine two-dose childhood series (beginning at 12 months of age), so two additional doses should be administered at the appropriate age.[157]

Adverse reactions associated with measles vaccine include fever of 39.4°C or greater in 5% of recipients and transient rashes in about 5% of vaccinees.[157] Because measles vaccine can cause fever, it can be associated with febrile seizures.[161] Children with prior personal histories of seizures or histories of seizures in the immediate family may be at increased risk for febrile seizures after MMR vaccination.[162] Anaphylaxis and thrombocytopenic purpura also appear to be caused rarely by MMR.[65] Encephalopathy with onset about 10 days after vaccination has been reported in vaccine recipients, with a frequency of approximately 1 in 2 million vaccinations; although a causal role for measles vaccine has not been established.[163] There is no association between MMR vaccine and autism.[67,164]

Measles vaccine is contraindicated for pregnant women and in persons who are immunocompromised because of either congenital or acquired disorders (e.g., leukemia or immunosuppressive drugs), with the exception of persons infected with HIV. Because measles may cause severe disease in HIV-infected people, MMR vaccine is recommended for persons who do not have evidence of severe immunosuppression. Absence of severe immunosuppression is defined as CD4 percentages greater than or equal to 15% for 6 months, or longer for persons 5 years old or younger, and CD4 percentages greater than or equal to 15% and CD4 count greater than or equal to 200 lymphocytes/mm^3 for 6 months, or longer for persons older than 5 years. When only CD4 counts or CD4 percentages are available for those older than 5 years, the assessment of severe immunosuppression can be on the basis of CD4 values that are available. When CD4 percentage is not available for children 5 years old or younger, the assessment of severe immunosuppression can be on the basis of age-specific CD4 counts at the time the CD4 counts were measured (i.e., absence of severe immunosuppression is defined as 6 months' duration above age-specific CD4 count criteria: CD4 count greater than 750 lymphocytes/mm^3 in those 12 months old or younger and CD4 count greater than or equal to 500 lymphocytes/mm^3 in those 1–5 years of age).[157]

Meningococcal Vaccines

Four meningococcal containing vaccines are available in the United States.[165] Two vaccines contain purified meningococcal capsular polysaccharides of groups A, C, Y, and W, conjugated to protein (MenACWY), which results in a vaccine that is immunogenic in infants and young children. Immunization involves induction of T-lymphocyte cell-dependent responses, and induces immunologic memory to meningococcal polysaccharide.[27,166,167] Two vaccines are serogroup B meningococcal (MenB) recombinant protein vaccines. MPSV is no longer available in the United States.

Conjugate meningococcal vaccines reduce carriage and induce herd protection.

One conjugate vaccine, MenACWY-D (Menactra), is licensed for persons 9 months to 55 years of age, and the other conjugate vaccine, MenACWY-CRM (Menveo), is licensed for persons 2 months to 55 years of age. The antibody responses to each of the four conjugated polysaccharides included in each of the quadrivalent vaccines are serogroup specific, independent, and comparable for the two vaccines. Meningococcal conjugate vaccines routinely are indicated for immunization of adolescents, for control of outbreaks attributable to a vaccine serogroup, and for use among certain high-risk groups, such as persons with persistent complement component deficiencies, eculizumab use, HIV infection, or anatomic or functional asplenia, and laboratory personnel who routinely are exposed to isolates of *N. meningitidis*. Meningococcal conjugate vaccine (MenACWY) routinely is recommended for all adolescents beginning at 11 to 12 years of age, with a booster dose at 16 years of age. Adolescents who receive their first dose of MenACWY at 11 to 12 years of age routinely are recommended for a booster at 16 years of age. Adolescents who receive their first dose of vaccine at 13 to 15 years of age are recommended to receive a booster at 16 to 18 years of age. First-year college students 19 years of age or older living in residence halls should receive a dose if they have not been vaccinated after the 16th birthday. Regardless of attendance in a college, if a high-risk scenario develops (e.g., travel to a region in the "the meningitis belt" of sub-Saharan Africa [which stretches from Senegal to Ethiopia], entering the military, routine exposure to *N. meningitidis* through microbiology laboratory work), a dose should be provided if it has been 5 years since the most recent dose. Children traveling to the meningitis belt (or to the Hajj in Saudi Arabia) should receive a quadrivalent meningococcal conjugate vaccine (MenACWY-CRM or MenACWY-D).

When initiated at 2 months of age, a four-dose schedule is recommended for MenACWY-CRM, with doses at 2, 4, 6, and 12 months of age; when initiated at 9 to 23 months of age, a two-dose schedule is recommended. Children aged 9 months and older can receive MenACWY-CRM or MenACWY-D. MenACWY-D is recommended as a two-dose primary series, with 3 months separating the doses (8 weeks minimum). Children 2 to 23 months of age with functional or anatomic asplenia or HIV infection should receive MenACWY-CRM vaccine. To avoid interference with the immunologic response to the infant series of PCV13, children younger than 24 months with functional or anatomic asplenia or HIV infection should not receive MenACWY-D vaccine. In contrast, MenACWY-CRM does not demonstrate immune interference with PCV7 (and, by extrapolation, PCV13) after the 12-month dose, and can therefore be administered concomitantly with PCV13. Because a potential for immunologic interference with MenACWY-D response has been demonstrated when MenACWY-D is administered 30 days after DTaP vaccine, it is recommended that

MenACWY-D be given either before or concomitantly with DTaP in children at increased risk for meningococcal disease.

Adults at increased risk for meningococcal disease (functional or anatomic asplenia, complement component deficiency, travel or residence in the meningococcal belt, exposed to an outbreak of vaccine serogroup) also should receive either MenACWY-D or MenACWY-CRM. A two-dose primary series is recommended for adults with functional or anatomic asplenia, HIV infection, and complement deficiency.

For children first vaccinated before 7 years of age with MenACWY, revaccination should be considered after 3 years if they remain at high risk, and then every 5 years thereafter for subsequent booster doses, as long as they remain at high risk.[60]

The development of vaccines against meningococcus serogroup B has been hampered because the serogroup B polysaccharide is very poorly immunogenic in humans. Through the use of reverse genetics, recombinant serogroup B antigens that can provide protection against serogroup B have been identified, and two vaccines were licensed in the United States in 2014 and 2015, respectively: MenB-FHbp (Trumenba) and MenB-4C (Bexsero). ACIP recommended that the vaccines be used to immunize individuals aged 10 years or older who are at increased risk for serogroup B disease (persistent complement component deficiencies, eculizumab use, anatomic or functional asplenia, or at risk because of an outbreak of serogroup B disease).[169] While ACIP does not routinely recommend a serogroup B meningococcal vaccine for all teens and young adults without risk factors for serogroup B disease, all teens and young adults may get vaccinated, preferably at 16 through 18 years old; this decision is left to individual consideration of health care providers, parents, and patients.[172] For adults at increased risk for meningococcal disease and for use during serogroup B meningococcal disease outbreaks, three doses of MenB-FHbp should be administered at 0, 1 to 2, and 6 months. When given to healthy adolescents and young adults who are not at increased risk for meningococcal disease, two doses of MenB-FHbp should be administered at 0 and 6 months. The other MenB vaccine, Men-4C, is a two-dose series, with the two doses administered at least a month apart. (see Fig. 316.4).

Mumps Vaccine

Mumps vaccine is a live-attenuated virus vaccine that is recommended for use in all children aged 12 months or older who do not have contraindications. Mumps vaccine is administered routinely as MMR or MMRV at 12 to 15 months of age.[171] When administered on or after the first birthday, 49% to 91% of recipients can be expected to acquire protection. Although protection had been thought to be lifelong, investigations after resurgences of mumps in 2006, 2009–10, and 2016 suggested that some persons may lose immunity over time.[172] A second dose is recommended with MMR or MMRV, usually at 4 to 6 years of age.[171] As with measles, most persons born before 1957 are likely to have been infected naturally with mumps virus and generally can be considered immune; otherwise, individuals should be considered susceptible unless they have documentation of having received one or two doses of live mumps vaccine (depending on age), laboratory evidence of mumps immunity, or laboratory evidence of mumps disease. These three factors are considered presumptive evidence of immunity.[171] For health care personnel, acceptable evidence of immunity consists of laboratory documentation of immunity, laboratory documentation of disease, or written documentation of two doses of mumps-containing vaccines, or laboratory evidence of mumps immunity.[157,173] Contraindications to mumps vaccine are pregnancy and an immunocompromised state (see "Measles-Containing Vaccine"). Persons with a history of anaphylactic reactions to eggs may be vaccinated. Adverse events associated with mumps vaccine are uncommon. Parotitis and orchitis have been reported rarely. Thrombocytopenic purpura and anaphylaxis appear to be caused rarely by MMR.[65] Aseptic meningitis has been associated with the Urabe and Leningrad-Zagreb strains of mumps vaccine, strains not available in the United States.[172,173] The Jeryl Lynn strain used in US vaccines has not been proved to cause aseptic meningitis.[65]

Persons previously vaccinated with two doses of a mumps virus–containing vaccine who are identified by public health authorities as being part of a group or population at increased risk for acquiring mumps because of an outbreak should receive a third dose of a mumps virus–containing vaccine to improve protection against mumps disease and related complications.[157,173,174] In an outbreak setting, persons vaccinated previously with one dose of mumps-containing vaccine who have been identified by public health officials as at increased risk of mumps because of the outbreak should receive a second dose of mumps-containing vaccine, even if they would not otherwise have a routine recommendation for a second dose of mumps-containing vaccine (https://www.cdc.gov/vaccines/pubs/surv-manual/chpt09-mumps.html). Everyone who is determined to be part of the group at increased risk for getting mumps should receive a dose of MMR vaccine. That includes people who do not have vaccine records that prove they received two doses of MMR vaccine. No additional dose is recommended for people who already received three or more doses before the outbreak.[175]

Pertussis-Containing Vaccine

Acellular pertussis vaccines are made from purified components of the organism *Bordetella pertussis* and detoxified pertussis toxin (PT); whole-cell pertussis vaccines, made from suspensions of killed whole *B. pertussis,* are no longer available in the United States, although they continue to be widely used internationally.[176] Acellular pertussis vaccines currently available in the United States contain pertussis toxoid, filamentous hemagglutinin (FHA), and pertactin (69-kDa protein). In addition, they may contain fimbriae.[55] Pertussis vaccines are combined with diphtheria and tetanus toxoids as DTaP (acellular pertussis vaccines for children) or Tdap (for adolescents and adults). Tdap contains reduced amounts of diphtheria toxoid and acellular vaccine components compared with DTaP vaccine for children. The primary immunizing course for children consists of three doses of DTaP administered intramuscularly at 4- to 8-week intervals, typically given at 2, 4, and 6 months of age. A fourth dose is given about 6 to 12 months later (15–18 months of age) and a fifth dose at 4 to 6 years of age. Acellular pertussis vaccines are preferred over whole-cell pertussis vaccines because the efficacy of acellular vaccines were thought to be comparable to whole-cell vaccines in prelicensure clinical trials, and because the incidence of adverse events after acellular vaccines is significantly lower than after whole-cell vaccines. As of July 2013, two acellular vaccines for children were available in the United States: Daptacel (Sanofi Pasteur, Swiftwater, PA), which contains PT, FHA, pertactin, and fimbriae types 2 and 3, and Infanrix (GlaxoSmithKline), which contains PT, FHA, and pertactin. The efficacy found for one of the old US whole-cell vaccines after three doses in clinical trials in Europe was 36% to 48%.[177,178] Point estimates of vaccine efficacy ranged from 80% to 85% for vaccines currently licensed in the United States.[179] Effectiveness varies with time from completion of a vaccine series.[180]

Local reactions occur about one-tenth to one-half as frequently with acellular vaccines as with whole-cell vaccines. For example, the incidence of erythema by the third evening after any of the first three doses of acellular vaccines for children ranged from 26.3% to 39.2% in one large comparative trial, compared with 72.7% in those who received the whole-cell vaccine. In that study, the incidence of fever (>39.4°C) after acellular vaccines was 3.3% to 5.2%, compared with 15.9% after receipt of whole-cell vaccine.[55] More serious adverse events, such as seizures and hypotonic hyporesponsive episodes, also appear to occur less frequently after acellular vaccines than after whole-cell vaccines.[181,182] The lower incidence of fever associated with acellular vaccines would be expected to decrease febrile seizures, especially after the fourth dose.

Contraindications to DTaP vaccines include an immediate anaphylactic reaction or encephalopathy not attributable to another identifiable cause within the 7 days after a prior dose. The following events are considered to be precautions specific to DTaP: (1) children with evolving neurologic disorders, who should have immunization deferred until the situation is clarified—once stable, they can receive pertussis vaccine[176]; (2) GBS less than 6 weeks after a previous dose of tetanus-toxoid vaccine; (3) history of Arthus-type hypersensitivity reactions after a previous dose of tetanus or diphtheria-containing vaccines—defer vaccination until at least 10 years have elapsed since the last tetanus-toxoid vaccine; and (4) severe or moderate acute illness with or without fever.

Given the benefits of vaccination, administration of pertussis vaccines to children with stable seizure disorders or with family histories of seizures is recommended in the United States.

Extensive swelling of the thigh or entire upper arm after the fourth or fifth doses of the DTaP series has been reported. The frequency appears to be 2% to 3%, and the pathogenesis is unclear. Extensive limb swelling is commonly misperceived as a contraindication for DTaP and/or Tdap.[176]

DTaP is available in four combination formulations: with inactivated polio and hepatitis B vaccines (Pediarix); with inactivated polio and Hib vaccines (Pentacel, Sanofi Pasteur); and with inactivated polio (Kinrix [GlaxoSmithKline] and Quadracel [Sanofi Pasteur]). Combinations of acellular vaccines with Hib have generally resulted in diminished antibody response to the Hib component when administered to infants.[96] However, the immune response to Hib in persons who receive Pentacel is considered adequate. Kinrix and Quadracel are licensed only for the fifth dose of DTaP, which is usually administered at the same time as the fourth dose of IPV at 4 to 6 years of age. Data are insufficient to document the safety, immunogenicity, and efficacy of use of DTaP vaccines from different manufacturers in a mixed sequence. For this reason, whenever feasible, the same brand of DTaP should be used for all doses in the vaccination series. However, if the type of vaccine previously administered is unknown or is not available, any of the available licensed DTaP vaccines can be used to complete the vaccination series.

Concerns about the safety of whole-cell pertussis vaccines have led to decreased vaccine coverage in some countries. Whole-cell pertussis vaccines have been implicated in encephalopathy, and these concerns persist even though there is some evidence that encephalopathy may have been due to a cause distinct from vaccines.[183] In the United Kingdom, pertussis vaccine uptake declined markedly in the period from 1974 to 1978. The result was a major epidemic of pertussis in the years 1977 to 1979, with a second epidemic in 1982. This experience and similar ones in Japan and other countries illustrate the necessity for maintaining protection against pertussis.[184]

Studies of pertussis epidemiology suggest that adults may play an important role in sustaining transmission.[178–192] Waning immunity in adolescence after receipt of the acellular DTaP vaccine in childhood has likely contributed to a resurgence of disease in adolescents and adults. Pertussis in adolescents and adults may account for increases in pertussis among infants too young to be protected through vaccination.[192]

During 2005, two acellular pertussis–containing vaccines were licensed as a single dose for administration to adolescents and adults. Both vaccines are combined with tetanus toxoid and reduced quantities of diphtheria toxoid (Tdap).[93] Adacel (Sanofi Pasteur) contains detoxified PT (2.5 μg), FHA (5 μg), pertactin (3 μg), and fimbriae types 2 and 3, similar to the pertussis components of Daptacel, the childhood preparation. The pertussis components of Boostrix (GlaxoSmithKline) consist of PT (8 μg), pertactin (2.5 μg), and FHA (8 μg), similar to the pertussis components of Infanrix, the childhood preparation. Both vaccines contain aluminum adjuvants. Neither vaccine contains thimerosal. The vaccines were licensed on the basis of inducing antibody responses to pertussis antigens similar in magnitude to the responses associated with early childhood vaccination, although efficacy was also demonstrated in adults with a vaccine similar to Boostrix.[193] The childhood vaccines proved to be effective in preventing pertussis.

Boostrix is licensed for administration to persons aged 10 years or older, whereas Adacel is licensed for persons 10 to 64 years of age. Tdap is indicated routinely as a booster for adolescents at 11 to 12 years of age in place of the previously recommended tetanus and diphtheria toxoids for adult use (Td). In addition, all persons older than 12 years should receive a single dose of Tdap, which can replace any of the decennial boosters of Td. When feasible, Boostrix, which is licensed for those 10 years old and older, should be used for persons aged 65 years or older.[194]

Tdap is not indicated for primary immunization. However, it can be used for any one of the doses in the primary series of Td for unimmunized adolescents and adults. It is now recommended that Tdap be administered to anyone aged 11 years and older without respect to previous interval from last tetanus toxoid-containing vaccine. Health care providers also should be vaccinated. Tdap is especially indicated for adults who have never received a prior Tdap booster and who will be caring for young infants, because such children are susceptible to pertussis before active immunity can be induced by DTaP. Vaccination is recommended in pregnancy as a way of reducing infant pertussis both through decreasing the risk for transmission of disease to the infant from the mother and through transfer of maternal antibodies against pertussis across the placenta.[195] Based on studies of antibody levels in cord blood from women vaccinated during pregnancy, Tdap should be administered to women who are pregnant and should be administered in every pregnancy, preferably during the early part of gestational weeks 27 through 36.[71] Studies of antibody levels in cord blood of infants born to mothers who may have been vaccinated as long as 2 years previously did not show appreciable antibody levels, suggesting that doses from previous pregnancies are unlikely to provide protection to the infants from subsequent pregnancies, and therefore a protective dose should be given during each pregnancy. Except for repeat doses recommended for each current pregnancy, only one dose of Tdap is recommended for adults because the duration of protection is short and the impact of repeat Tdap vaccination on disease burden is unclear.[71]

Plague Vaccine

Plague vaccine is no longer available in the United States. Killed whole-cell vaccines and live-attenuated vaccines are used elsewhere in the world, and new subunit and mucosal vaccines are under development.[196]

Pneumococcal Polysaccharide Vaccine

Pneumococcal polysaccharide vaccine initially was licensed as a purified preparation of 14 different serotypes of pneumococcal capsular polysaccharide in 1977. Since 1983, vaccine containing 23 types (PPSV23) has replaced the earlier version. The types included in the current vaccine and immunologically related types are responsible for about 60% to 75% of all bacteremic pneumococcal disease in the United States. Older versions of pneumococcal polysaccharide vaccine containing higher doses of antigens and targeting fewer serotypes were highly effective in reducing pneumococcal disease among South African gold miners (a group at particularly high risk) and among military recruits.[197–199] In populations at high risk for pneumococcal infections, such as elderly persons and patients with high-risk medical conditions, more recent formulations generally have been found to be effective against pneumococcal bacteremia and other types of invasive pneumococcal disease but not necessarily against nonbacteremic pneumococcal pneumonia. Studies of patients with isolates from normally sterile body fluids have generally reported efficacies of 50% to 80% overall, with lower efficacy in persons who have compromised immune systems.[200–203] A study of Navajo adults did not demonstrate efficacy against invasive pneumococcal disease in this high-risk population.[204] The vaccine is licensed for persons aged 2 years or older. The schedule and indicated uses of pneumococcal polysaccharide vaccine are highlighted in the ACIP-recommended immunization schedule for children and adolescents aged 18 years or younger and the recommended immunization schedule for adults aged 19 years or older, United States[205] (see Figs. 316.1A and 316.2).

PPSV23 is recommended for adults and children 2 years old and older at increased risk for pneumococcal diseases including those with chronic heart, lung, or liver disease; diabetes mellitus; cerebrospinal fluid (CSF) leak; cochlear implants; sickle cell disease or other hemoglobinopathies; congenital or acquired asplenia or splenic dysfunction; HIV infection; chronic renal failure or nephrotic syndrome; congenital immunodeficiency; generalized malignancy; multiple myeloma; iatrogenic immunosuppression; alcoholism; or cigarette smoking. PPSV23 is also recommended for healthy elderly persons (≥65 years of age).[206] Starting in August 2014, ACIP recommended that PCV13 be administered in series with PPSV23 in individuals aged 65 years and older[207] (see later).

Polysaccharide vaccines are not effective in children younger than 2 years. Children who have completed the PCV series before age 2 years and who are in these high-risk groups should receive one dose of pneumococcal polysaccharide vaccine at age 2 years.[208]

A single dose is administered by means of IM or subcutaneous injection. A single revaccination is recommended for persons aged 65 years or older who received an initial vaccination before age 65 years,

if at least 5 years have elapsed since that dose. A revaccination dose is also recommended for persons younger than 65 years with anatomic or functional asplenia or those who are immunocompromised, including patients with chronic renal failure and nephrotic syndrome. This dose should be administered after a minimum 5-year interval. If this second dose is administered before the 65th birthday, a third and final dose is recommended after the 65th birthday.

Pneumococcal Conjugate Vaccine

PCVs in which pneumococcal capsular polysaccharide is covalently linked to protein carriers have been developed. A 7-valent conjugate vaccine covalently linked to CRM protein (PCV7; Prevnar; Wyeth Lederle Vaccines, St. David's, PA) was first licensed for use in infants and young children in 2000. The seven polysaccharide types included in the licensed vaccine accounted for 80% of invasive infections in children younger than 6 years in the United States at the time of vaccine introduction.[208] In a prelicensure efficacy trial in northern California, the efficacy of the conjugate vaccine was 97% against invasive disease caused by serotypes in the vaccine.[209] The vaccine was also effective in prevention of pneumonia, with the greatest impact in the first year of life, with a 32% reduction,[209] and in prevention of acute otitis media caused by pneumococcal serotypes included in the vaccine.[210] Efficacy against invasive pneumococcal disease has been demonstrated in Native American children, a population at increased risk for disease.[210]

In February 2010, a 13-valent PCV with polysaccharides linked to CRM was licensed in children, and contains the 7 serotypes in PCV7 with 6 additional serotypes. Among infants receiving the three-dose primary infant series, responses to 10 of the PCV13 serotypes met the prespecified primary end-point criterion (percentage of subjects achieving an IgG seroresponse of ≥0.35 μg/mL 1 month after the third dose). Responses to serotypes 6B and 9V (contained in both vaccines) and new serotype 3 (only contained in PCV13) did not meet this criterion. For serotypes 6B and 9V, however, the differences were small.[211]

PCV is administered as a four-dose series, with doses at 2, 4, and 6 months of age, followed by a booster dose at 12 to 15 months of age.[210] The vaccine is recommended for all children younger than 2 years, and children 24 to 59 months of age. Healthy children 24 to 59 months of age who have not been vaccinated or completed the recommended schedule should receive one dose.[212] Children 24 to 59 months of age who have received an incomplete series of fewer than three doses before 24 months of age, and who have high-risk conditions such as chronic cardiac or pulmonary disease, diabetes mellitus, chronic liver disease, immunosuppression, renal failure or nephrotic syndrome, functional or anatomic asplenia, CSF leak, or cochlear implants should receive two doses of PCV13 vaccine.[206,208,213]

Widespread use of the conjugate vaccine has resulted in dramatic decreases in disease incidence among young children for whom the vaccine is recommended. In addition, decreases in disease incidence have also been observed among adults, probably as a result of decreased transmission of pneumococci from children to adults.[214] Decreases in disease are not restricted to invasive pneumococcal disease, but have also occurred in noninvasive pneumonia.[215] Surveillance of pneumococcal disease to date has revealed some evidence of serotype replacement by serotypes not contained in the vaccine, but such replacement has been far outweighed by the reduction in disease caused by serotypes in the vaccine.[215–217]

PCV was licensed for adults aged 50 years and older in 2011 and is now recommended for all adults with immunosuppression, including renal failure and nephrotic syndrome, functional or anatomic asplenia, CSF leak, or cochlear implants because of their increased risk of disease.[218] It is recommended that adults with these risk factors also receive at least one dose of PPSV23. They should receive PCV13 first, followed by a dose of PPSV23 8 weeks later. If an adult has received a dose of PPSV23, administering the dose of PCV13 that follows should be after an interval of 1 year to optimize the response to the dose of PCV13, which is affected by the previous dose of PPSV23. In August 2014, ACIP recommended that PCV13 be administered to individuals aged 65 years and older in series with PPSV23.[207] This decision was in part based on results of the placebo-controlled CAPiTA trial of PCV13 in 85,000 adults aged 65 years and older with no prior pneumococcal vaccination history. The CAPiTA trial demonstrated an efficacy of 45.6% against vaccine-type pneumococcal pneumonia, including both invasive pneumococcal disease and nonbacteremic pneumococcal pneumonia.[219] PCV13 should be administered first, followed by PPSV23 in individuals aged 65 years and older who have never received pneumococcal immunization. In healthy adults or high-risk immunocompetent adults, the interval between PCV13 and PPSV23 should be 1 year. The timing and regimen for those who have already received PPSV23 was described by the CDC in 2014[207] and 2015.[220]

In 2015, ACIP issued a summary of revised spacing rules for PCV13 and PPSV23 when both vaccines are recommended.[220] ACIP recommends that all adults aged 65 years or older who have not received pneumococcal vaccine and persons aged 2 years or older who are at high risk for pneumococcal disease should receive a dose of PCV13, followed by a dose of PPSV23.[220] The intervals between doses differ according to age and indication[220] and are described in Figs. 316.3 and 316.4.

Polio Vaccine

Although two types of polio vaccine are available in the world to control polio, live-attenuated oral polio vaccine (OPV) and injectable IPV, only IPV is currently available in the United States. The schedule consists of four doses of IPV at 2 months, 4 months, 6 to 18 months, and 4 to 6 years. The final dose should be administered at age 4 years and older regardless of the number of previous doses and at least 6 months after the previous dose.[221] IPV is available as a single vaccine or in combination with DTaP and hepatitis B vaccines (Pediarix), DTaP and Hib vaccines (Pentacel), or DTaP alone (Kinrix and Quadracel). Although Pediarix can be used for the first three doses of IPV at 2, 4, and 6 months, single IPV or DTaP/IPV is needed for the fourth dose. Pentacel can be used for any of the first three doses of IPV. An additional IPV dose would be needed at age 4 to 6 years. There is no need to restart a series if the primary immunization schedule is interrupted; the next dose in the series should be given.[7] Prior doses of OPV, if documented, should be counted when considering whether there is a need for further polio immunization. Monovalent OPV doses of type 1 or 3 and bivalent doses of OPV containing only serotypes 1 and 3 should not be counted toward the US vaccination requirements because they do not induce immunity against polio serotype 2. OPV doses administered after April 1, 2016 are either bivalent OPV or monovalent OPV.[222]

The decision to move to an all-IPV schedule in the United States was based on the balance of benefit and risk. OPV rarely caused paralytic polio (with the greatest risk after the first dose [overall risk 1 per 670,000 first doses]), and IPV had eliminated disease without risk for serious side effects when given to a high proportion of people in developed countries, such as Sweden. In 1988, the World Health Assembly endorsed a goal to eradicate polio from the world. The major vaccine used in the worldwide eradication effort is OPV. Advantages of OPV include ease of use, superior induction of intestinal immunity to prevent wild poliovirus spread, spread of vaccine virus to unvaccinated contacts resulting in immunization of children not reached by vaccination programs, and lower cost than IPV. Extensive efforts in the Americas, including mass campaigns with OPV twice a year targeted to all children younger than 5 years regardless of prior immunization status, led to the elimination of polio in the Western Hemisphere. The last known case of polio caused by wild poliovirus in the Americas had its onset in Peru in 1991. The Western Hemisphere was certified free of polio in 1994,[46] and the European region of WHO was certified free of polio in 2002. Since 1988, almost all countries with endemic polio have conducted National Immunization Days, and in the setting of greatly improved surveillance, cases of polio caused by wild poliovirus have decreased from an estimated 350,000 in 1988 to 22 cases in 2017. By the end of 2017, only three countries—Nigeria, Pakistan, and Afghanistan—had never interrupted wild poliovirus transmission (www.polioeradication.org).[223]

In 2012 the Strategic Advisory Group of Experts (SAGE) on Immunization of WHO recommended that all countries implement at least one dose of IPV into their routine immunization schedules in preparation for moving from trivalent OPV to bivalent OPV (without type 2 virus). The switch from trivalent to bivalent OPV took place in 2016, and trivalent OPV is no longer available globally.[224] Eventually, all OPV use

will cease once eradication of wild polioviruses is certified (www.who.int/wer/2013/wer8801.pdf). (Also see Chapter 171.)

Polio vaccine is not recommended routinely for persons aged 18 years or older in the United States because the risk from wild virus is low, and most are immune as a result of vaccination during childhood. However, if vaccine is needed, such as for persons traveling to polio-endemic areas or to some countries bordering polio-endemic areas (see www.cdc.gov/travel for current list), or for certain categories of health care personnel who are at greater risk for exposure to polioviruses than the general population, previously unvaccinated adults should receive two doses of IPV at intervals of 4 to 8 weeks and a third dose 6 to 12 months after the second. Adults who have had a primary series of OPV or IPV and who are at increased risk for exposure to poliovirus may receive an additional one-time dose of IPV.

Adolescents may have received a four-dose series, with 4 weeks between each dose, and the final dose before the fourth birthday. This schedule is considered complete if the fourth dose was given on or after 18 weeks of age and was given before August 7, 2009 and if the adolescent is not traveling to a polio-endemic area. If an adolescent is traveling to a polio-endemic area and received a compressed schedule with a 4-week interval between doses, regardless of the timing of this schedule, he or she should receive an additional dose of IPV before travel.

Rabies Vaccine

Rabies vaccine is an inactivated virus vaccine prepared either in human diploid cell culture (HDCV) or in purified chick embryo cell culture (PCEC).[225] Rabies vaccination is recommended in two situations: as preexposure prophylaxis in persons likely to be exposed to rabies (e.g., veterinarians, forest rangers, travelers who may be at high risk based on countries and activities) and after exposure to animals known or suspected to be rabid. The primary preexposure immunizing course is three doses of rabies vaccine given intramuscularly at 0, 7, and 21 to 28 days. The three-dose course results in induction of protective levels of antibodies in virtually 100% of vaccinees. Serologic testing every 2 years is recommended to ensure that high-risk vaccinees maintain protective levels of antibody. Those whose titer falls to less than the recommended level should receive a booster. Alternatively, boosters may be administered every 2 years without serologic testing for persons at high risk for exposure. In the postexposure setting, four doses of rabies vaccine are given intramuscularly on days 0, 3, 7, and 14 to previously unimmunized persons. This deviates from the approved five-dose schedule in the package insert, so use of the four-dose schedule is off label. Persons who were previously fully vaccinated and who are exposed to rabies should receive IM doses of rabies vaccine on days 0 and 3. In all high-risk postexposure settings for previously unimmunized persons, rabies vaccine should always be used in conjunction with RIG (see "Rabies Immune Globulin"). Rabies vaccine should be administered by IM injection into the deltoid muscle in adults and children or the anterolateral thigh in infants; there have been reports of possible vaccine failure after gluteal administration.[226,227] Corticosteroids, other immunosuppressive agents, antimalarial drugs, and immunosuppressive illnesses can interfere with the immune response to rabies vaccine. There are no known contraindications to rabies vaccination in persons who are at risk or have been exposed (see Chapter 163).[225]

Rotavirus Vaccines

There are two licensed rotavirus vaccines in the United States: RotaTeq (RV5) (Merck) and Rotarix (RV1) (GlaxoSmithKline).[228] Both are oral live-attenuated virus vaccines. RV5 was developed through reassortment of a parent bovine rotavirus strain (WC3) with human strains, which donate either outer capsid proteins (G proteins) or attachment proteins (P proteins) to the WC3 strain. The resultant vaccine contains five separate viruses expressing human G1, G2, G3, G4, and P1A(8) proteins. The G proteins in the vaccine cover about 90% of the wild rotavirus strains detected in the United States from 1996 to 2005. Many of the other strains have the P1A(8) attachment protein. After a three-dose series, the efficacy against any G1 to G4 virus-associated gastroenteritis was 74%, and against severe gastroenteritis was 98%. RV5 reduced emergency department visits in an 11-country analysis by 94% and hospitalization by 96%. Of interest, the vaccine was effective against G9 wild-type rotaviruses. In the US schedule, doses of RV5 are recommended at 2, 4, and 6 months of age. The minimum age for the first dose is 6 weeks, and the maximum age is 14 weeks and 6 days. The minimum interval between doses is 4 weeks, and in the United States the maximum age for the last dose is 8 months, 0 days.

RV1 is an attenuated vaccine derived from a wild human rotavirus (G1P1A[8]).[229] In a large Latin American trial, the efficacy against severe rotavirus infection after two doses of vaccine was 85% up to age 1 year and 81% up to age 2 years. In a European trial, efficacy was higher: 87% against any rotavirus infection and 96% against severe disease. In self-controlled case series in Mexico and Brazil, the incidence rate ratio of intussusception was 2.6 to 5.3 in the first 7 days after a dose of rotavirus vaccine, compared with outside of the 7-day window.[229] Based on studies from the middle- and high-income countries of Mexico, Brazil, Australia, and the United States, it is believed that for every 20,000 to 100,000 rotavirus vaccinees, there will be one additional case of intussusception.[229,230,231,232] Important to note, in a self-controlled case series study from seven lower-income sub-Saharan African countries that introduced RV1, an increased risk of intussusception was not identified.[233] Rotavirus vaccine is contraindicated in infants with a history of intussusception. The rotavirus vaccination program has had major global success, with reductions in rotavirus hospital admissions of 73% and reductions in acute gastroenteritis deaths of 34% each year after the initiation of the vaccination program in Mexico and Brazil.[234] RV1 is recommended in a two-dose schedule, usually at 2 and 4 months of age. Minimum ages, minimum intervals, and maximum ages are the same as for RV5. In the United States there is no preference for one vaccine over another. Use of the same vaccine for all doses is recommended. When this is not feasible or when the type of vaccine used for prior doses is unknown, a total of three doses should be administered.

Preliminary data from population surveillance systems suggest that RV5 not only induces individual protection but may also provide community protection through herd protection.[228]

Rubella Vaccine

Rubella vaccine contains live-attenuated rubella virus grown in human diploid cells (RA27/3).[156] Other substrates, such as duck embryo cells or rabbit kidney cells, also have been used for rubella vaccines, but these vaccines are no longer available in the United States. When the vaccine is administered to a person on or after the first birthday, 95% or more of recipients can be expected to become immune. Immunity after a single dose is long lasting and appears likely to be lifelong. Boosters are not necessary, although many persons will receive a second dose as part of the two-dose MMR schedule to prevent measles and mumps. Rubella vaccine is recommended for all people on or after the first birthday, except those who have documentation of having received live rubella vaccine and those who have laboratory documentation of immunity to rubella. Most persons born before 1957 can be considered immune. It is particularly important to ensure that women of childbearing age are immune to rubella, because children born to mothers exposed to wild-type rubella in pregnancy can develop congenital rubella syndrome. Rubella vaccine virus is known to be able to cross the placenta and infect fetal tissue. Nonetheless, there were no instances of congenital rubella syndrome in the offspring of 226 susceptible women who received RA27/3 rubella vaccine within 3 months of conception and who carried their pregnancies to term.[235] In addition, thousands of pregnant women in Brazil received rubella vaccine during pregnancy, and there were no identified cases of congenital rubella syndrome as a result. This indicates that the risk for congenital rubella syndrome from vaccine virus, if there is any risk, is very low. Notwithstanding the fact that no observable risk has been associated with rubella vaccine administered during pregnancy, rubella vaccine should not knowingly be administered to a pregnant woman. A reasonable approach is to ask women whether they are pregnant or may become pregnant within the next month, exclude those who answer affirmatively, and vaccinate the others, after explaining the theoretical risk to them.[156]

Known adverse events associated with rubella vaccine include low-grade fever and rash in 5% to 10% of recipients and joint pain with or without objective manifestations of arthritis. The latter occurs with

increasing frequency in older individuals; about 25% of susceptible women may have transient arthralgia after rubella vaccination.[236,237] Acute arthritis is seen in about 10% of susceptible women. The risk for arthritis after rubella vaccine is substantially lower than the risk after natural rubella. The IOM reviewed the adverse consequences of rubella vaccination and favored acceptance that the vaccine was a cause of transient arthralgia in women and children.[119] However, there is no evidence of increased risk for new onset of chronic arthropathies among women vaccinated with RA27/3 vaccine.[238–240] With regard to other illnesses temporally related to rubella vaccine, the IOM concluded that the evidence was insufficient to implicate rubella vaccine as a cause of thrombocytopenic purpura, radiculoneuritis, and other neuropathies. Thrombocytopenic purpura had been associated with MMR vaccine, but the 1994 IOM report does not discuss thrombocytopenic purpura in association with this vaccine.[65]

Previous experience with programs involving serologic screening and subsequent vaccination of susceptible individuals has demonstrated a low success rate in delivering vaccinations to identified susceptible persons (typically approximately 30%–50%). Contraindications to rubella vaccination are pregnancy and an immunocompromised state (see "Measles-Containing Vaccine").[147] Despite the success of the rubella immunization program in the United States, cases of congenital rubella syndrome continue to occur from viruses either acquired from other countries or imported into the United States.[157]

Smallpox Vaccine

Effective use of smallpox vaccine eradicated smallpox as a naturally occurring disease in 1977.[241] The vaccine is a live-unattenuated preparation of vaccinia virus that induces protection against smallpox virus in 95% or more of recipients. ACAM2000 (Acambis, Cambridge, MA), produced in Vero cells, is the current licensed preparation of smallpox vaccine.[242]

Routine use of smallpox vaccine among the civilian population in the United States was discontinued in 1972 and by the military in 1990. In May 1983, Wyeth Laboratories, the only active licensed producer in the United States, discontinued general distribution of smallpox vaccine, making it no longer available. Acambis manufactures ACAM2000, which was licensed in 2007 and is the only licensed available smallpox vaccine in the United States. Smallpox vaccine was recommended in 2003 for members of public health and health care response teams[243] and for selected military personnel; it continues to be available as an investigational new drug for individuals working with vaccinia or other orthopoxviruses. Smallpox vaccine is administered intradermally by means of the multiple puncture technique with a presterilized bifurcated needle. With the bifurcated needle held perpendicular to the skin, punctures are made rapidly, with sufficient pressure that a trace of blood appears after 15 to 20 seconds.

Previously recognized adverse events associated with smallpox vaccine include disseminated vaccinia, eczema vaccinatum, vaccinia necrosum (progressive vaccinia), and encephalitis.[244–246] The risk for transmission of vaccinia virus from the inoculation site can be reduced by keeping the vaccine site covered with gauze and a layer of clothing and by good hand hygiene. For persons involved in patient care, addition of a semipermeable dressing is recommended.[245] In the 2003 public health and military vaccination program, smallpox vaccine was contraindicated for persons younger than 1 year; persons with a history or presence of eczema, atopic dermatitis, or other dermatologic conditions that are exfoliative; persons with conditions associated with immunosuppression; persons who were pregnant or are breastfeeding; or persons with a serious allergy to any component of the vaccine. After reports of ischemic cardiac events in recent vaccinees, persons with known underlying heart disease or three or more known major cardiac risk factors were also excluded from the preevent vaccination program,[246] although no causal relationship has been established between receipt of the vaccine and ischemic cardiac disease.

Inflammatory cardiac disease (myocarditis, pericarditis, or myopericarditis) was recognized in 2003 among recipients of smallpox vaccine in both military and civilian programs.[247] Although myocarditis had been previously reported in Europe and Australia after administration of other vaccinia strains, it was not previously recognized as an adverse event after use of the New York City Board of Health (NYCBOH) strain, the strain used for production of Wyeth's smallpox vaccine in the United States. The clinical spectrum of illness ranges from mild symptoms to heart failure, and the natural history remains unknown; it is unclear if all patients recover completely, or if some persons with subclinical myocarditis may later develop dilated cardiomyopathy, as is thought to occur with some patients who have other types of myocarditis. Histopathologic data are limited, but in one patient who underwent endomyocardial biopsy, an eosinophilic infiltrate without presence of vaccinia virus was found. Onset is typically 7 to 19 days after vaccination; the frequency appears to be approximately 1 in 10,000 vaccinees.[247]

Other vaccinia strains, such as modified vaccinia Ankara, which undergoes only limited replication in humans, are under active study as vaccine strains that might be associated with a lower incidence of adverse events or might be safe to use in populations in which current smallpox vaccines are contraindicated (e.g., immunocompromised persons).[241]

Tetanus Toxoid

Tetanus toxoid, a purified preparation of inactivated tetanus toxin, is one of the most effective immunizing agents known. Tetanus toxoid is recommended for use in all residents of the United States for whom contraindications do not exist.[92] Tetanus toxoid should always be used in combination either with diphtheria toxoid alone or with diphtheria toxoid and pertussis vaccine to ensure protection against both diseases. Five doses of DTaP are given at 2, 4, 6, and 15 to 18 months, and at 4 to 6 years of age. A primary course of two doses administered 4 to 8 weeks apart, with a third dose given 6 to 12 months later, induces protective antibodies in more than 95% of recipients and is recommended for all unvaccinated older children and adults. After childhood DTP/DTaP immunization, booster doses with adult formulation tetanus and diphtheria toxoids (Td) are recommended at age 11 to 12 years and then every 10 years thereafter. A one-time dose of Tdap may be substituted for one of the recommended Td boosters. For unvaccinated children in the first year of life for whom pertussis vaccine is contraindicated, pediatric DT should be substituted for DTaP. For unvaccinated children in the second year of life for whom pertussis vaccine is contraindicated, two doses of DT should be administered 4 to 8 weeks apart, with a third dose 6 to 12 months later (see "Pertussis-Containing Vaccine"). DTaP or DT is given to children younger than 7 years. Common adverse effects include local reactions and fever. In some persons who have received multiple doses of tetanus toxoid, Arthus-like reactions have been described.[248] Tetanus toxoid has been suggested as a rare cause of brachial plexus neuropathy. The IOM concluded that tetanus toxoid causes brachial neuritis in the first month after immunization at a rate of 0.5 to 1 case per 100,000 toxoid recipients.[65] Tetanus toxoid also has been implicated as a cause of GBS. The most convincing evidence comes from a case report of one individual who acquired GBS three times with successive administrations of tetanus toxoid.[249] However, population-based studies in both children and adults have revealed an incidence of GBS within expected limits and do not support a causal role.[250] If tetanus toxoid causes GBS, it does so rarely.[251] The only contraindication is in individuals who previously had neurologic or severe hypersensitivity reactions after tetanus toxoid. Table 316.5 summarizes the ACIP-recommended approach to use of tetanus toxoid and tetanus IG for PEP of tetanus.

Typhoid Vaccine

Two preparations of typhoid vaccine are available in the United States: an oral live-attenuated strain of *Salmonella* Typhi (Ty21a) and a Vi capsular polysaccharide vaccine (ViCPS). The two vaccines provide between 33% and 80% protection after a primary series.[252,253] The CDC recommends typhoid vaccine for travelers to areas where there is an increased risk of exposure to *Salmonella enterica* serotype Typhi. Typhoid vaccines are indicated for travelers who will have prolonged exposure to contaminated food and drinks in developing countries, those with prolonged exposure to typhoid carriers, and laboratory personnel who work with *Salmonella* Typhi.

The oral vaccine comes as an enteric-coated capsule that should be taken on alternate days with cool liquid approximately 1 hour before

TABLE 316.5 Summary Guide to Tetanus Prophylaxis in Routine Wound Management: United States

HISTORY OF ADSORBED TETANUS TOXOID (DOSES)	CLEAN, MINOR WOUNDS		ALL OTHER WOUNDS[b]	
	Td or Tdap[c]	TIG	Td or Tdap[c]	TIG
Unknown or <3	Yes	No	Yes	Yes
3[d]	No[e]	No	No[f]	No

[a]Important details are in the text.
[b]Such as, but not limited to, wounds contaminated with dirt, feces, soil, saliva, and so on; puncture wounds; avulsions; and wounds resulting from missiles, crushing, burns, and frostbite.
[c]For children younger than 7 years, DTaP or DTP (DT if pertussis vaccine is contraindicated) is preferred to tetanus toxoid alone. For persons 7 years and older, Td is preferred to tetanus toxoid alone, unless Tdap is indicated.
[d]If only three doses of fluid toxoid have been received, a fourth dose of toxoid, preferably an absorbed toxoid, should be given.
[e]Yes, if more than 10 years since last dose.
[f]Yes, if more than 5 years since last dose. (More frequent boosters are not needed and can accentuate side effects.)
DT, Diphtheria and tetanus toxoids (pediatric) vaccine; *DTaP;* diphtheria and tetanus toxoids and acellular pertussis (pediatric) vaccine; *DTP,* diphtheria, tetanus, and pertussis; *Td,* tetanus and diphtheria toxoids (adult) vaccine; *Tdap,* tetanus, diphtheria, and acellular pertussis (adult) vaccine; *TIG,* tetanus immune globulin.

a meal. The four recommended doses should be refrigerated until needed. Data are not available to make recommendations about the need for boosters with the oral vaccine, although the manufacturer recommends a new complete series every 5 years. The ViCPS is recommended as a single 0.5-mL dose. Boosters are recommended every 2 years. The Ty21a vaccine is not recommended for children younger than 6 years. The ViCPS is recommended for persons 2 years or older. Evidence from trials and postmarketing studies suggests that parenteral Vi vaccines are usually tolerated well. In a manufacturer-funded postmarketing safety study conducted in 11 US travel clinics, the most common reactions were injection site pain (77%), tenderness (75%), and muscle aches (39%).[254] The Ty21a and the ViCPS vaccines cause fever and headache in less than 6% of recipients. Other adverse reactions to the oral preparation are rare and consist of abdominal discomfort, nausea, and vomiting. Local reactions to the ViCPS have been reported in 7% of recipients. The oral vaccine should not be given to persons who are immunocompromised, including those with HIV infection. Ty21a should not be given to a person taking antibiotics unless at least 72 hours have elapsed since the last dose.

Varicella Vaccine

Two varicella vaccine products are available in the United States: a single-component product (Varivax; Merck) and a combined vaccine with MMR (MMRV, ProQuad; Merck).Both use the Oka/Merck strain of varicella-zoster virus (VZV). The VZV titer in MMRV (minimum of 3.99 $\log_{10}$ plaque-forming units [PFU] per dose) is higher than in the single-component vaccine, which is about 3.13 $\log_{10}$ PFU per dose. Live-attenuated varicella vaccine was licensed in the United States in 1995. MMRV was licensed in 2005. One dose of vaccine generally had been found to be highly effective against severe varicella (95%–100%) and moderately effective against mild disease (70%–90% in most studies).[255–261] Most vaccinees who acquire varicella (breakthrough disease) tend to have mild illness with fewer than 50 lesions, compared with 250 to 500 lesions in unvaccinated persons with disease. Immunity appears to be long lasting. However, the rate of breakthrough disease in the 10 years after a single dose was 7.3% in one study.[262] In contrast, only 2.2% of children who received two doses had varicella. Most of the breakthrough episodes occurred in the first 5 years. Given the benefits of two doses in reducing vaccine failure, a two-dose schedule is now recommended.[57] The vaccine is recommended routinely for all children at 12 to 15 months of age, with a second dose to be given at 4 to 6 years of age as varicella vaccine or MMRV. The second dose may be administered earlier than age 4 to 6 years, with the minimum interval being 3 months. HIV-infected children who are asymptomatic and not immunosuppressed should receive two doses of varicella vaccine, with the first dose at age 12 to 15 months or older and with a 3-month interval between doses.[57,263] However, only single-component varicella vaccine should be used in HIV-infected children; MMRV should not be used because of a theoretical risk of uninhibited replication of a high-titer vaccine virus causing an adverse reaction.

MMRV is associated with a higher risk for fever than simultaneous administration of MMR and varicella at different sites, and also causes an increased risk for febrile seizures after dose one of the two-dose series.[159] In June 2009, after consideration of the postlicensure data and other evidence, ACIP adopted new recommendations regarding the use of MMRV vaccine for the first and second doses and identified a personal or family (i.e., sibling or parent) history of seizure as a precaution for use of MMRV vaccine. For the first dose of measles, mumps, rubella, and varicella vaccines at age 12 to 47 months, either MMR vaccine and varicella vaccine or MMRV vaccine may be used. Providers who are considering administering MMRV vaccine should discuss the benefits and risks of both vaccination options with the parents or caregivers. Unless the parent or caregiver expresses a preference for MMRV vaccine, the CDC recommends that MMR vaccine and varicella vaccine should be administered simultaneously but at separate sites for the first dose in this age group. For the second dose of measles, mumps, rubella, and varicella vaccines at any age (15 months to 12 years) and for the first dose at age 48 months or older, use of MMRV vaccine generally is preferred over separate injections of its equivalent component vaccines (i.e., MMR vaccine and varicella vaccine). For persons aged 48 months or older, MMRV is usually preferred for the first dose if vaccination against varicella is also needed. MMRV is not associated with an increased risk of febrile seizures when it is the second dose of an MMR-containing vaccine.[160,265]

Vaccination has also been demonstrated to be effective for outbreak control in persons of all ages.[266,267] Vaccine also can be given routinely to any susceptible older child or adult, except persons with certain immunocompromising conditions. Persons aged 13 years and older should receive two doses 4 to 8 weeks apart. Adults who might be at increased risk for exposure or transmission and who do not have evidence of immunity should receive special consideration for vaccination; such persons include (1) health care providers, (2) household contacts of immunocompromised persons, (3) persons who live or work in environments in which transmission of VZV is likely (e.g., teachers, daycare employees, residents and staff in institutional settings), (4) persons who live or work in environments in which transmission has been reported (e.g., college students, inmates and staff members of correctional institutions, and military personnel), (5) nonpregnant women of childbearing age, (6) adolescents and adults living in households with children, and (7) international travelers. Because adults are at higher risk for complications from varicella than are children, vaccination of all susceptible adolescents and adults is desirable. Persons with the following are considered to have evidence of immunity: (1) a diagnosis or verification of a history of varicella disease or zoster by a health care provider; (2) laboratory evidence of immunity; (3) documentation of age-appropriate vaccination (one dose for children aged 12 months through 3 years and two doses for persons aged 4 years old and older); or (4) birth in the United States before 1980 (excluding health care providers, pregnant women, and immunocompromised persons). Although a negative or uncertain history of varicella in young children is predictive of

susceptibility, most young adults with such histories are immune. In some settings, serologic screening of persons with negative or unknown prior histories of varicella is cost-effective.[268] However, recalling and vaccinating identified susceptible persons may be difficult, making vaccination with use of history as the determinant of need more attractive.

The most common adverse events are local reactions and rash. In children, about 3% acquire a varicella-like rash at the injection site, with a median of two lesions. Local reactions are more common after the second dose (25% in children and 33% in adults) versus the first dose (22% in children and 24% in adults). Four percent of children acquire a generalized rash, with a median of five lesions. In adults, 3% and 1% acquire localized rashes after the first and second doses, respectively, whereas 6% and 1% acquire more generalized rashes after the first and second doses, respectively.[57] Transmission of vaccine virus has been reported rarely and only from persons with rash. There is no evidence that vaccination increases the risk for zoster. In fact, in vaccinated children, zoster incidence after vaccination is lower than would be expected after natural infection.[269,270] Vaccine is contraindicated in persons with anaphylactic hypersensitivity to vaccine components, including neomycin and gelatin, and in most persons with deficiencies of cell-mediated immunity.[57] Available data on the safety and efficacy of varicella vaccine in HIV-infected children who were not severely immunocompromised suggests that varicella vaccine is immunogenic and that the vaccine safety profile is acceptable.[271,272] Persons who are immunocompromised or receiving immunosuppressive drugs should not receive varicella vaccine from a period 2 weeks before to 3 months after immunosuppressive therapy, and they should be deemed immunocompetent when they receive the vaccine. Pregnant women should not be vaccinated, and women should be advised not to become pregnant for 1 month after vaccination.[57]

Varicella vaccine is more thermolabile than other vaccines. It must be stored frozen at an average temperature of −50°C to −15°C.

Yellow Fever Vaccine

Yellow fever vaccine is a live-attenuated virus preparation that is highly effective in inducing protection in recipients.[273] This vaccine is indicated for use in travelers going to yellow fever–endemic areas and may be required for entry into some countries (see wwwnc.cdc.gov/travel/diseases/yellow-fever). Only a single dose of subcutaneously administered vaccine is required. A booster dose after a 10-year interval is recommended for those who received a first dose while HIV infected or while pregnant. Otherwise, boosters are not generally recommended, but depending on the destination, they may be required every 10 years, although the vast majority of persons retain immunity well past 10 years. Children younger than 6 months appear to be at highest risk for severe neurotropic reactions, and vaccine is contraindicated in this age group. Other contraindications include anaphylactic hypersensitivity to eggs and immunocompromised states. If possible, vaccination of infants should be delayed until 9 months of age. Pregnancy is not considered an absolute contraindication; however, it is recommended that administration of the vaccine be postponed until after completion of pregnancy, if possible. Other precautions include children 6 to 8 months of age, persons with asymptomatic HIV infection with T-lymphocyte counts less than 500/mm^3, and women who are breastfeeding. Adverse reactions (fever, aches and soreness, redness or swelling where the injection was given) occur in up to 25% of vaccinees. Anaphylaxis has been reported in 0.8 to 1.8 persons per 100,000 doses of vaccine distributed. A rare syndrome (0.25 cases per 100,000 doses distributed) after yellow fever vaccination—febrile multiorgan system failure or viscerotropic disease—has been reported, with high rates of mortality, primarily among older adults and persons who have undergone thymectomy or have severe thymic dysfunction being vaccinated for the first time. Neurotropic disease has also been reported after yellow fever vaccination in 1 to 2 persons per 100,000 doses distributed and is also more common in older vaccinees. It manifests as several distinct clinical syndromes, including meningoencephalitis (neurotropic disease), GBS, acute disseminated encephalomyelitis, and bulbar palsy. Yellow fever vaccine should be administered with caution and only after careful counseling in patients older than 60 years who are going to spend time in yellow fever–endemic zones. Yellow fever vaccine should not be given to immunocompromised persons or persons with anaphylactic allergies to eggs, chicken protein, or gelatin.

Because of a shortage of YF-VAX, Sanofi Pasteur has imported Stamaril, a yellow fever vaccine distributed in France. Stamaril has a similar safety profile to YF-VAX, but because it is distributed under an Emergency Use Authorization (EUA), there are some differences in contraindications and precautions. For Stamaril, HIV infection with no evidence of immunosuppression is a precaution; breastfeeding for the first 14 days after vaccination is an exclusionary criterion and therefore effectively a contraindication; and age 6 to 8 months is an exclusionary criterion and therefore effectively a contraindication.

Zoster Vaccine

Zoster vaccine is designed to boost immunity to VZV in persons previously infected with the virus, to either prevent shingles by inhibiting the reactivation of virus from dorsal nerve ganglia or to lower the severity and health burden of shingles, should it occur.[274]

An adjuvanted subunit vaccine, RZV, is licensed and has superior efficacy to live-attenuated zoster vaccine (ZVL) with no serious adverse events associated with vaccination. Efficacy of RZV over 3 years for prevention of zoster is 97% in 50- to 59-year-olds, 97% in 60- to 69-year-olds, and 91% in persons aged 70 years or older. Safety trials detected injection site reactions, myalgias, fatigue, headache, shivering, fever, and gastrointestinal illness. These were severe enough to cause interference in daily activity in one in six recipients. Two doses of vaccine are recommended for immunocompetent persons aged 50 years or older; the second dose is recommended 2 through 6 months after the first dose. ACIP preferentially recommends the use of this two-dose vaccine over the live zoster vaccine (ZVL). ZVL is a lyophilized preparation of the Oka/Merck strain and differs from varicella vaccine in potency. In a trial of vaccine in adults aged 60 years or older and with a history of prior varicella, a single dose of vaccine was 51% effective in decreasing the incidence of zoster, 61% effective in reducing the overall burden of illness from shingles, and 67% effective in reducing the incidence of postherpetic neuralgia (PHN) in the vaccinated cohort. Of persons who actually developed zoster, PHN was reduced by 39% in vaccinees compared with placebo recipients who had shingles. Effectiveness in preventing zoster tended to decrease with increasing age, with the highest efficacy among 60- to 69-year-olds. For persons aged 80 years and older, the point estimate for vaccine effectiveness was positive, but the 95% CI overlapped zero, indicating that the estimate was not statistically significant.[275] A follow-up study demonstrated 70% efficacy in prevention of zoster in 50- to 59-years-olds, and the FDA lowered the age of approval to 50 years. RZV is recommended regardless of a past history of zoster disease and regardless of past use of ZVL.

No particular clinical pattern was noted to implicate vaccine in causing specific adverse events. Zoster vaccine is indicated for the routine vaccination of persons aged 60 years and older without contraindications. Although the FDA licensed the vaccine for persons aged 50 years or older, ZVL is currently recommended only for persons aged 60 years or older because zoster disease incidence continues to rise as one ages, and the vaccine protection wanes over time, with less than 40% protection 5 years after receipt of ZVL. Persons with a prior history of zoster can be vaccinated. ZVL is contraindicated in persons with allergy to vaccine components and moderate or severe immunodeficiency.

Immunoglobulin Preparations

Passive immunization can be provided by preformed antibodies in several types of products used to treat persons with primary and, less frequently, secondary immune deficiency and to prevent or, less frequently, to treat certain infectious diseases. The choice is made in part according to the types of products available, the type of antibody desired, the route of administration, and the conditions or diseases being treated. Products used include (1) IG administered through the IM route, (2) specific or hyperimmune IG preparations administered through the IM route, (3) IGIV; (4) specific (hyperimmune) IG administered through the IV route, (5) antibodies of animal origin, (6) monoclonal antibodies, and (7) IG subcutaneous (human) that has been approved for treatment

of patients with primary immune deficiency states (see Table 316.4). Indications for administration of IG preparations other than those relevant to infectious diseases are not included in this chapter.

Intramuscular Immune Globulin

IM IG is prepared from pooled human adult plasma by means of an alcohol-fractionation procedure. IG consists primarily of IgG and trace amounts of IgA and IgM, is sterile, and is not known to transmit any infectious agents, including hepatotropic viruses and HIV. IG is a concentrated protein solution containing specific antibodies reflective of the infectious disease exposure and immunization experience of persons from whom the plasma was obtained to prepare the IG. More than 1000 and up to 60,000 donors per lot are used to include IG from persons with a broad spectrum of antibodies. Individual donors are screened for markers of a variety of viruses to minimize potential transmission of infection. IG is licensed and recommended for administration deep into a large muscle mass, such as the gluteal region, or into the anterior thigh of a child. IV use of human IG is contraindicated.

Indications for Use of Intramuscular Immune Globulin

The three indications for use of IG are replacement therapy for antibody deficiency disorders, and prophylaxis against hepatitis A and measles viruses.[40,105,156,276]

Replacement therapy for antibody deficiency disorders. For most patients, IM IG has been replaced by IGIV or subcutaneously administered IG because use of IGIV results in higher total plasma IG concentrations, and higher titers of specific antibodies can be achieved with minimal discomfort.

Hepatitis A prophylaxis. In persons 12 months and older, hepatitis A immunization is preferred over IG for PEP against hepatitis A virus infections. In persons 6 months and older, hepatitis A immunization is preferred over IG for persons traveling to areas where hepatitis A is endemic.[274] For immunocompromised persons indicated for vaccination, both vaccine and IG should be administered. For persons younger than 12 months, IG is preferred to hepatitis A immunization. IG is effective in preventing hepatitis A when administered within 14 days of exposure (dose of 0.1–0.2 mL/kg) or when given before exposure in somewhat larger quantities (dose of 0.1 mL/kg for trips less than 1 month or 0.2 mL/kg for trips of 1 month or longer).

Measles prophylaxis. Immunization against measles is the optimal method for achieving protection against measles. IG administered to exposed, measles-susceptible persons can prevent or modify measles if administered within 6 days of exposure (IG IM dose of 0.5 mL/kg, up to a maximum of 15 mL, IGIV dose of 400 mg/kg IV).[40,156]

Specific Intramuscular Immune Globulin Preparations

The term *hyperimmune globulin* is used to refer to a group of products known as specific IGs. These products differ from other preparations in selection of donors who have been immunized or given booster immunizations and often in the number of donors from whom plasma is included in the product pool. Donors known to have high titers of the desired antibody are selected. Specific IG preparations are prepared by the same procedure as used for other IG preparations. Products in this category include HBIG, RIG, TIG, and VariZIG.

Hepatitis B immune globulin. HBIG is prepared from plasma preselected for high titer of antibody to HBsAg. In the United States, HBIG has an anti-HBsAg titer of more than 1 : 100,000 by radioimmunoassay.[109,114] HBIG is recommended for use in postexposure settings for susceptible individuals who have been exposed to known HBV, to infected sexual partners, or to blood containing HBsAg by the percutaneous or mucous membrane route. The dose is 0.06 mL/kg given immediately for both sexual contacts and persons exposed percutaneously. The hepatitis B vaccine series should be started simultaneously in those who previously have not been vaccinated. Alternatively, a second dose of HBIG may be given 1 month later in persons for whom hepatitis B vaccine is not indicated. All pregnant women should be tested for circulating HBsAg. HBIG is recommended for infants born to HBsAg-positive women. A dose of 0.5 mL should be given as soon as possible after birth, but within 12 hours of delivery in conjunction with a dose of hepatitis B vaccine. Additional doses of vaccine are indicated at 1 month and 6 months of age. The only known adverse effect is local discomfort at the site of injection. There are no known precautions or contraindications.[112]

Rabies immune globulin. RIG is a hyperimmune globulin derived from humans who have been immunized against rabies and have very high titers of antibodies to rabies.[225] RIG is designed for management of persons who have been exposed to rabid animals. RIG always should be used in conjunction with rabies vaccine in previously unvaccinated persons. However, if more than 8 days have elapsed since the first dose of rabies vaccine, RIG is unnecessary because an active antibody response to the vaccine presumably has begun. Experience indicates that administration of a full course of HDCV or PCEC vaccine with RIG is 100% effective in preventing development of rabies after exposure to known rabid animals. As much as possible of the 20 IU/kg dose should be infiltrated into and around the wound. Any remaining RIG should be administered intramuscularly at a different site from vaccine. Adverse effects include minor local discomfort. There are no known contraindications.[225]

Tetanus immune globulin. TIG is a hyperimmune globulin indicated for management of tetanus-prone wounds in persons who have no prior history of tetanus immunization.[92,93,197] The standard dose is 250 units intramuscularly. Local reactions are rare, and there are no known contraindications. If used, TIG should be administered simultaneously with, but at a different site from, combined tetanus-diphtheria toxoids (Td) or combined tetanus-diphtheria-acellular pertussis (Tdap) vaccine if pertussis vaccine is also indicated. Primary immunization against tetanus and diphtheria should then be completed using the routine schedule. Table 316.5 summarizes the ACIP-recommended approach to PEP of tetanus.[92] For the treatment of tetanus, TIG in large doses (3000–6000 units) also is recommended, with wound cleaning and débridement, antibiotics, and supportive care.

Varicella-zoster immune globulin. VariZIG is a purified human IG preparation made from plasma containing high titers of VZV antibodies.[57] The decision to administer VariZIG depends on these factors: (1) the likelihood that the exposed person has no evidence of immunity to varicella, (2) the probability that a given exposure to varicella or zoster will result in infection, and (3) the likelihood that the patient is at greater risk than the general population for developing complications of varicella if infected. Furthermore, the criteria for use are limited to immunocompromised persons, pregnant women, neonates whose mothers have varicella rash 5 days before to 2 days after delivery, hospitalized preterm infants born at 28 weeks of gestation or later whose mothers do not have evidence of immunity to varicella, and hospitalized preterm infants born at less than 28 weeks' gestation, regardless of the immune status of the mother.[277] If these criteria are met, and if the exposure occurred less than 10 days previously, VariZIG should be administered. VariZIG is commercially available from a broad network of specialty distributors in the United States (list available at www.varizig.com).

VariZIG is given intramuscularly at the recommended dose of 125 units/10 kg of body weight up to 625 units (i.e., five vials). The product may not prevent infection; however, if infection occurs, it is usually subclinical or mild. Any person to whom VariZIG is administered to prevent varicella subsequently should receive age-appropriate varicella vaccine, provided the vaccine is not contraindicated. Varicella vaccine should be delayed until 5 months after VariZIG administration to ensure optimal response. Varicella vaccine is not needed if the patient develops varicella after VariZIG administration. Local reactions are rare. Contraindications for VariZIG include a history of anaphylactic or severe systemic reactions to human IGs and IgA-deficiency with antibodies against IgA and a history of hypersenstivitiy.[277]

Immune Globulin Intravenous

IGIV is made from pooled plasma of adults by using methods designed to prepare a product for IV use. The number of donors ranges from 15,000 to 60,000. IGIV consists of greater than 95% IgG and trace amounts of IgA and IgM. The FDA specifies that all IGIV preparations must have a minimum concentration of antibodies to measles virus, *Corynebacterium diphtheriae,* poliovirus, and HBV. Antibody concentrations against other

pathogens vary widely among products. Not all IGIV products have been approved or studied for all FDA-approved indications.

Indications for Use of Immune Globulin Intravenous

IGIV initially was formulated for IV use in patients with primary immunodeficiencies, enabling them to receive enough IG at regular intervals for protection against certain infections. Administration of IGIV results in an immediate rise in both total IgG and titers of specific antibodies. IGIV is approved by the FDA for seven conditions: (1) primary immunodeficiency status, (2) Kawasaki disease, (3) immune-mediated thrombocytopenia, (4) pediatric HIV infection, (5) secondary immunodeficiency in chronic lymphocytic leukemia, (6) prevention of graft-versus-host disease and infection in adults with HSCT, and (7) PEP for measles exposure for immunosuppressed individuals. IGIV products also are used for many other conditions, although demonstrated efficacy from controlled trials is not available in all cases.

Specific Immune Globulins for Intravenous Use

There are three specific plasma-derived IG products for IV administration for prophylaxis or therapy of infectious diseases: cytomegalovirus (CMV) IGIV, botulism IGIV (for infant botulism), and vaccinia IG. An IM humanized mouse monoclonal antibody preparation used to prevent respiratory syncytial virus (RSV) is available (see "Respiratory Syncytial Virus Immune Globulin and Palivizumab").

CMV IGIV has been developed and is indicated for prophylaxis of disease in seronegative organ transplant recipients. Use of CMV IGIV for prophylaxis of CMV disease varies among transplantation centers. Risk factors for development of CMV among transplant recipients include type of organ, immunosuppressive therapy, and the donor-recipient CMV status. CMV-negative transplant recipients who receive an organ from a CMV-positive donor are at the highest risk for CMV disease and generally receive some form of CMV prophylaxis.

Botulism IGIV for human use (BabyBIG) is a human-derived antitoxin licensed by the FDA for treatment of infant botulism caused by *Clostridium botulinum* type A or type B. BabyBIG is made and distributed by the California Department of Public Health (24-hour telephone number: 510-231-7600; www.infantbotulism.org). BabyBIG has been shown to decrease significantly the number of days on mechanical ventilation, days of intensive care unit stays, and overall hospital stay.[278] An equine-derived antitoxin is available for use in adults with botulism but is *not* used in infants.

Vaccinia Immune Globulin

Vaccinia immune globulin (VIG), an IM preparation, is a hyperimmune globulin prepared for treatment of certain complications of vaccinia vaccination. VIG is indicated for treatment of severe cases of inadvertent inoculation with smallpox vaccine, eczema vaccinatum, severe generalized vaccinia, and progressive vaccinia. VIG use should be considered in patients with severe ocular complications other than isolated keratitis. VIG is not recommended for treatment of postvaccinial encephalitis or encephalomyelitis, myopericarditis after smallpox vaccination, mild cases of generalized vaccinia, erythema multiforme, or isolated vaccinia keratitis. A preparation of VIG suitable for IV use (VIG-IGIV) is manufactured by Calgene, Canada; has been approved by the FDA; and is available through the CDC (under Investigational New Drug [IND] protocols) and the US Department of Defense.[246]

Respiratory Syncytial Virus Immune Globulin and Palivizumab

IG against RSV is licensed in the United States for administration to infants and children at high risk of severe disease caused by RSV; groups at high risk include infants and children younger than 24 months with chronic lung disease or a history of preterm birth (35 weeks gestational age or younger). RSV-IGIV, a hyperimmune globulin formulated for IV administration, is no longer produced in the United States.[279] Palivizumab is a humanized monoclonal antibody against the F protein of RSV and is produced by recombinant DNA technology. The recommended dosage is 15 mg/kg administered intramuscularly monthly throughout the RSV season. Palivizumab has been demonstrated to be effective in reducing the risk for RSV hospitalization.[280] No significant adverse events have been associated with palivizumab, and there is no interference with the immune response to live virus vaccines. The AAP has recommended that because of the high cost of these interventions, their use be limited to those infants and children at highest risk for severe RSV disease. The AAP recommendations for use in premature infants are based on risk (chronic lung disease, preterm birth, chronic heart disease, neuromuscular disorders [consider not a full recommendation], and immunodeficiencies [consider not a full recommendation]), the time of year (start of the RSV season [which is based on location]), and age during the location-based RSV season.[281–284]

Adverse Reactions to Immune Globulin Preparations

The most common adverse effects of intramuscularly administered IG include local pain at the injection site and, less commonly, flushing, headache, chills, and nausea. Serious systemic events are rare. Anaphylactic reactions have been reported after repeated administration to IgA-deficient persons.[285] Other than prior anaphylactic reactions, there are no known contraindications to use of the product. IG inhibits response to certain live-virus vaccines (e.g., measles and rubella vaccines) for a period of 3 to 11 months, depending on the dose administered.[286]

Severe reactions to IGIV occur infrequently, but mild adverse events have been associated with up to 20% of infusions. The "Adverse Reactions" sections of package inserts of specific products provide details.

Simultaneous administration of IG with hepatitis A vaccine may result in a decrease of the ultimate titer of hepatitis A antibody achieved but does not influence seroconversion and presumed protection.[105]

Hepatitis C has been transmitted by IGIV in both Europe and the United States and by an IV Rh IG preparation in Ireland.[287–289] Hepatitis C virus RNA has been detected with polymerase chain reaction in various IG preparations,[290] but the significance of this finding is unclear; disease has not been associated with products other than those noted previously. In response to these findings, manufacturing procedures have been modified to add new viral inactivation steps.[291]

Immune Globulin Subcutaneous

Subcutaneous administration of IG using battery-driven pumps has been shown to be safe and effective in adults and children with primary immunodeficiencies. Smaller doses administered more frequently (i.e., weekly) result in more even serum IgG concentrations over time. Systemic reactions are less frequent than with IV therapy, and some parents or patients can be taught to infuse the product at home. The most common adverse effects of subcutaneous administration of IG are injection-site reactions, including local swelling, redness, itching, soreness, induration, and local heat. There is only one product licensed in the United States for subcutaneous use. There are no data on administration of IgG via the subcutaneous route for conditions requiring high-dose IG therapy.

Rh Immune Globulin

Rh IG is a hyperimmune globulin prepared for use in Rh-negative women who have just delivered Rh-positive infants or have had a miscarriage or abortion of an Rh-positive fetus. When administered within 24 hours of the time of delivery or abortion, it is highly effective in preventing sensitization of the mother to Rh-positive red blood cells that might be present in a future pregnancy. Appropriate administration of Rh IG has reduced the occurrence of Rh hemolytic disease of the newborn in the United States to very low levels. Further reductions will require more careful attention to the administration of the product after abortion or delivery in all women for whom it is indicated. There are essentially no adverse effects associated with the product, and there are no known contraindications.[159]

USE OF VACCINES

Routine

Children

The 2018 recommended schedule for administration of vaccines to infants, children, and adolescents is shown in Fig. 316.1A.[73] Catch-up schedules for children 4 months to 6 years of age and 7 to 18 years of age and adolescents are shown in Fig. 316.1B and are available at

www.cdc.gov/vaccines/schedules/hcp/imz/catchup.html. The schedules are updated annually and are available at www.cdc.gov/vaccines/schedules/index.html.

It is recommended that all children and adolescents receive all vaccines listed in the table unless medical contraindications exist.[73] Five doses of DTaP and four doses of polio-containing vaccines are recommended. The fifth dose of DTaP and the fourth dose of polio vaccine are recommended at 4 to 6 years of age.[73] Tdap boosters should be administered at 11 to 12 years of age, and Td boosters every 10 years thereafter.[93] A single dose of combined MMR vaccine at 12 to 15 months or older provides long-lasting, probably lifelong, immunity to measles in 93% of recipients. The second dose of MMR at 4 to 6 years of age should provide immunity to almost all children not protected by the first dose.[40,156] There is no contraindication to giving DTaP, MMR, Hib, hepatitis B, polio, pneumococcal conjugate, rotavirus, hepatitis A, influenza, and varicella vaccines with any of the other vaccines. Although all potential simultaneous administration schemes have not been evaluated, experience to date suggests that simultaneous administration of most vaccines does not increase reaction rates or interfere with the immune respo nses.[2,3,293,294] Hib should be given in two doses (PRP-OMP) or three doses (PRP-T) in the first year of life, followed by a booster dose at 12 to 15 months of age.[50,73] Hepatitis B vaccine should be initiated at birth, and the three-dose series should be completed by 18 months of age; hepatitis B vaccine can be given simultaneously with all other childhood vaccines.[15] Combination vaccines containing hepatitis B are not licensed for use before 6 weeks of age. PCV should be administered in a four-dose series, with the first three doses administered at 2, 4, and 6 months of age and the fourth dose at 12 to 15 months. Children should receive the first dose of varicella vaccine routinely at 12 to 18 months of age.[57] Annual influenza vaccine is recommended for everyone aged 6 months or older without contraindications.[6] Children aged 6 months through 8 years receiving influenza vaccine for the first time should have two doses separated by at least 4 weeks. Children aged 6 months through 8 years who have not received at least two doses in previous seasons should receive two doses separated by 4 weeks. A table that shows all the available influenza vaccines is shown in Chapter 165 and can be found at www.cdc.gov/flu/protect/vaccine/vaccines.htm.

The newest vaccine recommendations for young children involve rotavirus vaccine, varicella vaccine, and hepatitis A vaccine. There are two rotavirus vaccine preparations. Bovine rotavirus vaccine (RV5) should be given as a three-dose series at 2, 4, and 6 months of age, and human attenuated rotavirus vaccine (RV1) should be administered in a two-dose series at 2 and 4 months of age. For both vaccines, the first dose should be given at 6 to 14 weeks (maximum, 14 weeks, plus 6 days) of age. Vaccination should not be initiated for infants aged 15 weeks, 0 days or older. The final dose in the series should be given by 8 months, 0 days of age. All children should receive two doses of varicella vaccine, the first at 12 to 18 months of age and the second at 4 to 6 years of age. Hepatitis A vaccine should be given as a two-dose series, with the first dose given at 12 to 23 months of age and the second at least 6 months later. The minimum age for the first dose is 6 months of age.

Adolescents

An adolescent preventive medicine visit has been established at 11 to 12 years of age.[73] A booster dose of Tdap should be administered to adolescents who completed the recommended childhood DTP/DTaP vaccination series and have not received a Td booster dose. The HPV vaccine series should be administered to girls and boys 11 to 12 years of age. For those not previously fully vaccinated, catch-up vaccination is recommended throughout adolescence to 18 years of age. MenACWY should be administered at 11 to 18 years of age. A single dose of vaccine should be administered at age 11 or 12 years, and a booster dose should be administered at age 16 years. Adolescents who receive their first dose at age 13 to 15 years should receive a booster dose at age 16 to 18 years. For adolescents not vaccinated prior to their 16th birthday, one routine dose is recommended after the 16th birthday. Another dose is not recommended unless a risk factor develops, such as the onset of a medical condition, travel, or occupational consideration. When further boosters are indicated, they should be administered 5 years after the prior dose. Serogroup B meningococcal vaccine may be administered to adolescents and young adults aged 16 to 23 years, with a preference for the 16- to 18-year age range. Influenza vaccine should be administered annually through adolescence and adulthood. A second dose of MMR should be administered if not previously received. Completion of the two-dose varicella vaccine series should occur at that time if the adolescent is susceptible. The three-dose hepatitis B vaccination series should be administered if not previously received. The polio immunization history should be reviewed and catch-up vaccination performed, if a full series was not previously received, through age 17 years. Other immunizations, including pneumococcal and hepatitis A, should be given if indicated.

Adults

Routine immunizations for adults have received increasing attention in recent years, with recognition of the large burden of vaccine-preventable diseases in this age group. Two adult immunization schedules have been published each year since 2002. One focuses on vaccines needed by age group, and the second focuses on vaccines needed for persons aged 19 years and older, based on 10 medical and other indications (see Figs. 316.2 and 316.3). All adults should be immune to diphtheria and tetanus and, if not previously immunized, should be given a primary immunizing course (three doses of Td administered at time zero, 4 to 8 weeks, and 6 to 12 months), with boosters administered every 10 years thereafter.[93] A one-time dose of Tdap for adults aged 19 years or older should replace one of the Td booster doses. HPV vaccine is recommended for all girls and young women 11 to 26 years of age who have not completed the immunization series. A complete series consists of three doses for adults who began the series after the 15th birthday. The second dose should be given 2 months after the first dose, and the third dose should be given 6 months after the first dose. All females from adolescence to 26 years of age should receive a complete series of HPV vaccine. Previous doses can be counted toward a complete series even if they are not the current 9vHPV vaccine (i.e., 2vHPV or 4vHPV). If vaccination was not previously performed, HPV is recommended for all boys and young men 11 to 21 years of age and for all men 22 to 26 years of age with the following conditions: MSM, HIV positive, or immunosuppressed. Providers may give HPV vaccine to all men 22 to 26 years of age.

All adults without evidence of immunity to varicella should receive two doses of single-antigen varicella vaccine 4 weeks apart if not previously vaccinated (or the second dose if they have received one dose), unless they have a medical contraindication. Special consideration should be given to adults who (1) have close contact with persons at high risk for severe disease (e.g., health care personnel and family contacts of persons with immunocompromising conditions) or (2) are at high risk for exposure or transmission (e.g., teachers, child care employees, residents and staff members of institutional settings [including correctional institutions], college students, military personnel, adolescents and adults living in households with children, nonpregnant women of childbearing age, and international travelers). Evidence of immunity to varicella in adults includes any of the following[57]: (1) documentation of two doses of varicella vaccine at least 4 weeks apart; (2) birth in the United States before 1980 (although for health care personnel and pregnant women, birth before 1980 should not be considered evidence of immunity); (3) history of varicella based on diagnosis or verification of varicella by a health care provider (for a patient reporting a history of or presenting with an atypical case, a mild case, or both, health care providers should seek an epidemiologic link with a typical varicella case or a laboratory-confirmed case or evidence of laboratory confirmation, if performed at the time of acute disease); (4) history of herpes zoster based on health care provider diagnosis or verification of herpes zoster by a health care provider; or (5) laboratory evidence of immunity or laboratory confirmation of disease.

Persons aged 50 years old or older who are immunocompetent should receive two doses of RZV, separated by 2 to 6 months, regardless of history of varicella vaccination, ZVL vaccination, chickenpox, or zoster. Persons with chronic medical conditions may be vaccinated unless their condition constitutes a contraindication. For those who have previously received ZVL vaccination, although studies were limited to a 5-year

interval between ZVL and RZV, the minimum interval from ZVL to RZV is 8 weeks based on safety considerations. For those who have not received ZVL, RZV is the preferred vaccine to ZVL.[295] Once one dose of RZV has been administered, ZVL is no longer indicated.

For those who will not receive RZV, have not received a dose of RZV, and prefer ZVL, a single dose of zoster vaccine is recommended for immunocompetent adults aged 60 years and older, regardless of whether they report a prior episode of herpes zoster.[274] Likewise, persons with chronic medical conditions may be vaccinated unless their condition constitutes a contraindication. All adults should be immune to measles and rubella. For practical purposes, persons born before 1957 generally can be considered immune to these three diseases. All other adults should receive one or more doses of MMR unless they have a medical contraindication, documentation of one or more doses after the first birthday, or laboratory evidence of immunity. One dose of MMR vaccine is recommended for women whose rubella vaccination history is unreliable or who lack laboratory evidence of immunity. For women of childbearing age, regardless of birth year, rubella immunity should be determined, and susceptible women should be counseled regarding congenital rubella syndrome. Women who do not have evidence of immunity should receive MMR on completion or termination of pregnancy and before discharge from the health care facility. Adults born before 1957 generally are considered immune to mumps. Adults born during or after 1957 should receive two doses of MMR unless they have a medical contraindication or laboratory evidence of immunity. Influenza vaccine is recommended for routine annual administration to all adults. A yearly influenza vaccine is recommended for all adults who do not have contraindications.[6] It is recommended that pneumococcal polysaccharide vaccine be administered to elderly patients and those with specific medical or other indications.[202] This dose should follow a recommended dose of PCV (PCV13) by 1 year for otherwise healthy adults aged 65 years or older. One dose of PCV (PCV13) is recommended for adults aged 19 years and older who have the following high-risk conditions: functional or anatomic asplenia, immunosuppression (including hematologic malignancy, generalized malignancy, and immunosuppressive medications), renal disease (chronic renal failure or nephrotic syndrome), cochlear implants, or CSF leak. Hepatitis B vaccine is recommended for persons with specific medical, occupational, and behavioral indications as a three-dose series at 0, 1, and 6 months. If the combined hepatitis A and hepatitis B vaccine (Twinrix) is used, three doses are administered at 0, 1, and 6 months; alternatively, a four-dose schedule, administered on days 0, 7, and 21 to 30, followed by a booster dose at month 12, may be used.[15,59,105] For adult patients receiving hemodialysis or with other immunocompromising conditions, one dose of 40 µg/mL (Recombivax HB), administered on a three-dose schedule, or two doses of 20 µg/mL (Engerix-B), administered simultaneously or with two injections at each visit on a four-dose schedule at 0, 1, 2, and 6 months, can be used.[59] Hepatitis A vaccine is given to adults with behavioral, occupational, and other indications. Single-antigen vaccine formulations should be administered in a two-dose schedule at either 0 and 6 to 12 months (Havrix) or 0 and 6 to 18 months (Vaqta).[114] If the combined hepatitis A and hepatitis B vaccine (Twinrix) is used, administer three doses at 0, 1, and 6 months; alternatively, a four-dose schedule, administered on days 0, 7, and 21 to 30, followed by a booster dose at month 12, may be used. Meningococcal vaccine is given to adults with medical and other indications.[165] Meningococcal conjugate (MenACWY) vaccine is preferred for adults with any of the following indications: persons who were vaccinated with MenACWY previously and for whom revaccination is recommended or for whom multiple doses are anticipated; those who have functional or anatomic asplenia or persistent complement component deficiency; persons who have HIV disease; those who travel repeatedly to an area endemic for meningococcal disease; military personnel; or laboratory personnel working with meningococcus. MenACWY also is indicated for first-year college students 19 through 21 years of age if they have not received a dose in the previous 5 years, or first-year college students aged 19 through 21 years if they live in a residence hall if they have not received a dose since their 16th birthday. For meningococcal-naïve adults older than 55 years who do not have any of the aforementioned risk factors, MPSV4 is preferred.[60] Serogroup B meningococcal vaccine may be administered to persons aged 16 to 23 years of age.

Special Circumstances

Travel

Persons travel internationally for business, tourism, and education and to visit relatives and friends (see Chapter 318). The two major categories of immunizations to consider for international travelers are status of the routinely recommended immunizations (see Figs. 316.1A–B, 316.2, and 316.3) and the need for specific immunizations. Specific travel immunizations should be based on evidence of benefits and risks and on expert opinion when few or no data are available. Immunizations for international travel may be grouped into two categories: *required* (those that may be required in order to cross international borders) and *recommended* (those recommended according to risk for infection in the area of travel). Country-specific immunization recommendations are available for all countries (www.cdc.gov/travel and www.who.int/ith/en). The International Health Regulations allow countries to impose requirements for yellow fever vaccine as a condition for admission.[296] Consequently, travelers should be aware of whether this vaccine is required for entry into the country of their destination. Other vaccines commonly considered for travelers include measles- and rubella-containing vaccines, polio vaccine, and boosters for tetanus and diphtheria. In addition, travelers to specified areas or seasonally may wish to consider influenza, typhoid, rabies, JE, hepatitis A, hepatitis B, cholera, and meningococcal vaccines. Information on vaccines recommended for travel is summarized regularly in *Health Information for International Travel* (www.cdc.gov/travel/contentYellow-Book.aspx). Information on specific regions and diseases is available from the CDC at www.cdc.gov/cdc-info/requestform.html.

Occupational Exposure

A complete set of recommendations for vaccination for most occupational groups has not been developed. Specific recommendations are available for health care professionals.[297] Federal regulations require that health care and public safety workers who anticipate exposure to human blood or blood-derived body fluids must be offered hepatitis B vaccination at no charge.[111] Transmission of rubella in medical facilities can occur to or from health care professionals. Consequently, it is important that all health care professionals who might transmit rubella to pregnant patients be immune against rubella. Documentation of a single dose of a rubella-containing vaccine on or after the first birthday or serologic evidence of immunity is acceptable. Health care personnel are at greater risk from measles than the general public. All health care personnel should be immune, defined as documentation of receipt of two doses of live measles vaccine on or after the first birthday, at least 1 month apart, or serologic evidence of immunity. Although most persons born before 1957 have been considered to be immune to measles, about 4% of cases in health care professionals in the past occurred in persons born before this date. Mumps transmission in health settings has rarely been reported, and mumps immunity can be ensured at the same time as measles and rubella through use of MMR vaccine.[156,173] Therefore, facilities should recommend two doses of MMR vaccine in an outbreak setting and should consider two doses in any circumstance for health care providers, regardless of their date of birth. Because health care professionals caring for patients with chronic diseases may transmit influenza to their patients, all health care professionals should be vaccinated against influenza annually.[6] Health care professionals also should be immune to varicella.[57] Those without evidence of immunity to varicella should receive two doses of single-antigen vaccine if not vaccinated previously or the second dose if they have received only one dose, unless medically contraindicated.[57] Regardless of age, health care professionals should receive a single dose of Tdap as soon as feasible if they have not previously received Tdap and regardless of the time since their most recent Td vaccination. Vaccinating health care professionals with Tdap will protect them against pertussis and is expected to reduce transmission to patients, other health care professionals, household members, and persons in the community.

Pregnancy

Because of relative lack of efficacy and safety studies of vaccines in pregnant women, many recommendations for vaccine use in pregnancy are based on disease burden and severity for mothers and infants, studies

from other countries, and expert opinion. The only vaccines recommended routinely in the United States for pregnant women are tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap), and IIVs or RIVs. Transfer of maternal antibodies to tetanus toxin is an important means of preventing neonatal tetanus. Transfer of maternal antibodies to pertussis as a means of preventing infant pertussis is accomplished optimally if the mother is vaccinated early in the interval between 27 and 36 weeks' gestation in every pregnancy. Tdap can be administered without regard to interval from the last Td or Tdap.[71] IIV should be administered to all women who will be pregnant during the influenza season, regardless of trimester. Influenza immunization of women during pregnancy not only protects the pregnant woman but also appears to protect infants younger than 6 months.[298] Infants younger than 6 months cannot be immunized or receive antiviral prophylaxis because no products are licensed for this age group. LAIV is not licensed for use in pregnant women and should not be administered. However, pregnant women do not need to avoid contact with persons immunized with LAIV. In general, live-virus vaccines are contraindicated in pregnancy, with the exception of yellow fever virus vaccine and live cholera vaccine, which may be administered if the risk for exposure to the disease during international travel is great. If indicated, some inactivated vaccines, such as hepatitis B, MenACWY (preferred, but MPS4 is acceptable), hepatitis A, and PPSV23, can be administered to pregnant women with medical or exposure conditions that put them at risk for these vaccine-preventable infectious diseases. ACIP recommendations for pregnant women can be found at www.cdc.gov/vaccines/pregnancy/hcp/guidelines.html.

Immunocompromised States

Immunocompromised persons (from primary or secondary immune deficiency conditions) are susceptible to many infections, may be more susceptible to adverse effects from live-virus vaccines,[51,64] and may respond poorly to inactivated vaccines. Consequently, in general, live-virus vaccines are not administered to immunocompromised persons, although inactivated vaccines are safe and are generally indicated.[2] IIV should be withheld from patients receiving induction chemotherapy, consolidation chemotherapy, or anti–B-cell antibodies.[299] Varicella vaccine is contraindicated in persons with most deficiencies of cell-mediated immunity but can be safely given to persons with deficiencies of humoral immunity. Recommendations for vaccination of persons with specific immunocompromising conditions have been summarized (e.g., recipients of HSCTs[2] or solid organs)[300–302] and are provided on the CDC website at http://www.cdc.gov/vaccines/spec-grps.html#conditions. The efficacy of inactivated vaccines in immunocompromised persons may be less than that in healthy persons, although inactivated vaccines are safe and indicated for many patients.[303,304] In addition, household contacts of patients with immunocompromised conditions should be immunized appropriately, including annual influenza vaccine, to reduce risk for exposure of immunocompromised persons.

Human Immunodeficiency Virus

Live-attenuated vaccines generally are contraindicated in immunocompromised persons, including persons with symptomatic HIV infection. Limited studies in HIV-infected persons generally have failed to show an increased risk for adverse events from live or inactivated vaccines. Exceptions include BCG given to patients with AIDS and measles-containing vaccine in patients with severe immunodeficiency.[305–307] Known susceptible HIV-infected children aged 8 years or younger who are asymptomatic may be considered for varicella vaccines if $CD4^+$ percentage is greater than or equal to 15%. Persons older than 8 years may be considered for varicella vaccine if their $CD4^+$ lymphocyte count is greater than or equal to 200 cells/μL. Known susceptible HIV-infected children who do not have evidence of severe immunodeficiency should receive MMR vaccine. Absence of severe immunodeficiency is defined as individuals aged 5 years or younger who have CD4 T-lymphocyte percentages greater than or equal to 15% for 6 months' duration, and individuals aged 6 years or older with CD4 T-lymphocyte percentages greater than or equal to 15% *and* CD4 cell counts greater than or equal to 200 lymphocytes/mm^3. If only percentages or counts are available, the value that is available should be used. If a child aged 5 years or younger has only CD4 counts (and not percentages) available, absence of severe immunosuppression is defined as CD4 counts less than or equal to 750 cells/mm^3 for infants, or CD4 counts less than or equal to 500 cells/mm^3 for children aged 1 through 5 years. Known susceptible HIV-infected adults who are asymptomatic should receive live-attenuated MMR vaccine if they do not have evidence of severe immunosuppression, defined as $CD4^+$ percentages greater than or equal to 15% and $CD4^+$ counts greater than or equal to 200 lymphocytes/mm^3 for 6 or more months. If only one laboratory parameter is available, the designation for immunosuppression can be made on the basis of that sole parameter. Persons with HIV infection should not receive zoster vaccine. Because of reports of severe measles disease, including death, in symptomatic HIV-infected children and adults, MMR vaccine should be considered with caution for symptomatic HIV-infected persons.[156,308] For children with perinatal HIV infection who may have received prior doses of MMR and have since received combination antiretroviral therapy, revaccination should occur with MMR vaccine.[157] MMR and varicella vaccines are contraindicated in persons with severe immunodeficiency. Recommendations from the CDC, National Institutes of Health (NIH), and Infectious Diseases Society of America (IDSA) for administration of vaccines for adults are available.[309] Although transient increases of HIV in the blood of patients have been documented in the month after receipt of both pneumococcal and influenza vaccines, their clinical significance is unknown. Adults with HIV infection who meet the age requirements and lack evidence of immunity (lack documentation of immunization or with no evidence of prior infection) should be immunized with Td/Tdap, influenza (annually), PCV13, PPSV23, MMR, MenACWY, and hepatitis B vaccine, and may be considered for vaccination with varicella vaccine. Women younger than 27 years should receive HPV vaccine. If specific risk factors are present, hepatitis A vaccine should be considered. Although a protective immune response to vaccines and toxoids cannot be ensured in these patients, some protection may be provided. A publication from the CDC, NIH, and IDSA titled "Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents" is available (http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf).

Postexposure Immunization

For certain diseases, administration of an IG product soon after exposure can prevent or attenuate expression of disease.[310] Passive prophylaxis using various IG preparations has been discussed previously. Persons who have received a complete course of immunization against tetanus are, in general, well protected against development of tetanus, particularly if a booster dose has been administered within 10 years. More problematic is the situation with persons who cannot recall their immune status or who have not been immunized. Table 316.5 shows the ACIP-recommended approach to PEP of tetanus. In addition to passive prophylaxis, there is evidence that administration of measles vaccine within 6 days after exposure may prevent manifestations of illness.[311] If the exposure did not result in infection, the vaccination should provide protection against future exposure. Varicella vaccine prevents varicella infection in exposed persons if administered within 3 to 5 days of exposure.[57,312,313] Hepatitis A vaccine and hepatitis B vaccine are other vaccines used for PEP.

Other Considerations

Storage and Handling of Vaccines

Inattention to vaccine handling and storage conditions can contribute to vaccine failure.[2,314] Live-virus vaccines, including MMR, MMRV, varicella, yellow fever, LAIV, rotavirus, and OPV vaccines, are sensitive to increased temperature (heat-sensitive). Inactivated vaccines may tolerate limited exposure to elevated temperatures but are damaged rapidly by freezing (cold sensitive). Exposure of inactivated vaccines to freezing temperature (0°C [32°F] or colder) is the most common storage error. Examples of cold-sensitive vaccines include DTaP and tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccines; IPV vaccine (particularly if adjuvanted in the combination form Pediarix); Hib vaccine; pneumococcal polysaccharide and conjugate vaccines; hepatitis A and hepatitis B vaccines; IIV; and meningococcal polysaccharide and conjugate vaccines. Some vaccines must be protected from light. Physical appearance is not an appropriate basis for determining

whether a vaccine has lost its potency because of inappropriate storage or handling. All personnel responsible for handling vaccines in an office or clinic setting should be familiar with standard procedures designed to minimize risk for vaccine failure. Recommendations for handling and storage of selected biologics are summarized in several areas, including the package insert for each product and in a web-based toolkit (www.cdc.gov/vaccines/recs/storage/default.htm). The most current information about recommended vaccine storage conditions and handling instructions can be obtained directly from manufacturers. Their telephone numbers are listed in product labels (package inserts) and in the *Physicians' Desk Reference,* which is published yearly.

Assessing the Need for Immunization

Immunization traditionally has been viewed as the task of the pediatrician and general practitioner caring for children, but with licensure of new vaccines (Tdap, MCV4, HPV, herpes zoster virus [RZVand ZVL]) and expansion of vaccine recommendations (MMR, MMRV), physicians who care for adolescents and adults should be aware of current recommendations. Health care providers should assess the immunization status of their patients at first contact and, depending on immunization status and age, at selected contacts thereafter. In general, persons should be viewed as susceptible unless they can prove immunity through documentation of having received vaccine, laboratory evidence of vaccine-induced or disease-induced immunity, or, for some diseases (e.g., hepatitis A, hepatitis B), documentation of physician-diagnosed disease, which typically includes laboratory results documenting laboratory evidence of disease-induced immunity.

A significant proportion of elderly adults in the United States have never been immunized against tetanus or diphtheria. This is reflected in the fact that 30% of the 233 cases of tetanus in the United States in the period 2001 to 2008 occurred in persons aged 65 years or older.[315] Health care professionals caring for adults, especially elderly patients, should be particularly attuned to the need for administering tetanus and diphtheria toxoids to elderly persons, with one dose given as Tdap if Tdap has not been administered previously. Similarly, studies repeatedly demonstrate that less than 70% of persons aged 65 years or older receive influenza immunization in a given year or have ever received pneumococcal vaccine.[316] It is vital that all health care professionals remind themselves and their patients of the need for annual influenza immunization of all persons aged 6 months or older.

Substantial progress has been made in implementing hepatitis B vaccination programs for children and adolescents. Progress also has been made in immunizing adults with risk factors for HBV infection.

Immunization Records

In 2002 the National Vaccine Advisory Committee (NVAC) issued revised immunization standards that included recommendations that immunization of patients be documented through use of immunization records that are accurate, complete, and easily accessible. The standards also recommend use of tracking systems to provide reminder-recall notices when immunizations are due or overdue. Immunization information systems (IISs) address record-keeping needs and tracking functions and have additional capabilities (www.cdc.gov/vaccines/programs/iis/index.html). Every person should have an immunization record that is up to date and that contains information about each dose of vaccine received, including the date.[2] Patients should be asked to bring this record with them to all health care visits, and the record should be reviewed to ensure that it is current. Official immunization record cards or some form of personally accessible electronic record should be used. The National Childhood Vaccine Injury Act requires that all providers of vaccines covered by the program (i.e., listed on the vaccine injury table, www.hrsa.gov/sites/default/files/hrsa/vaccine-compensation/vaccine-injury-table.pdf) record on the patient's permanent medical record (housed in the provider-based record system) the date, manufacturer, and lot number of each dose of vaccine administered and the name of the person administering the vaccine.[289,318] It is prudent to record the same information for other vaccines as well. An updated version of the NVAC standards has been posted at www.cdc.gov/vaccines/hcp/adults/for-practice/standards/index.html.

Every physician should ensure that the immunization record of each patient is maintained in a permanent, confidential manner that can be reviewed and updated easily, whether the record is in hard copy or electronic health record format. The format of all records should facilitate identification and recall of patients in need of immunization.

Parent and Patient Education

All patients (or their parents or guardians) should be informed of the benefits and risks associated with vaccination.[314] The discussion should be conducted in language that is comprehensible to the recipient (or parent or guardian), and ample opportunity for questions and discussion should be given. Vaccine Information Statements (VISs) have been developed for all vaccines routinely recommended for children and adults and are available in several languages. The National Childhood Vaccine Injury Act requires physicians administering vaccines covered by the Vaccine Injury Compensation Program, whether purchased with private or with public funds, to provide the relevant VIS at the time of each immunization. In addition, the Public Health Service has developed forms that explain benefits and risks of vaccination with other vaccines. Interested health care providers can receive copies of these forms through local health departments or from the Internet (www.cdc.gov/vaccines/hcp/vis/index.html and www.cdc.gov/vaccines/parents/index.html).

Simultaneous Administration and Intervals Between Immunizations

Most vaccines can be given safely and effectively at the same time.[2] In general, inactivated vaccines can be administered simultaneously at separate sites, and field observations indicate that simultaneous administration of the most widely used live-virus vaccines has not resulted in impaired antibody responses or increased rates of adverse reactions.[293] When vaccines are administered simultaneously, they should be given in separate limbs. When this is not feasible, they should be separated by at least 1 to 2 inches. However, simultaneous administration of IG and MMR-containing vaccines should be avoided because this may result in interference with antibody responses. With those vaccines, IG should not be given for at least 2 weeks after vaccination. Persons receiving high doses of IG or other blood products may have impaired responses to vaccines for as long as 11 months, depending on the dose received.[2,286] Persons who receive standard doses of IG for hepatitis A prophylaxis should wait to receive live vaccines until 3 months after IG, whereas persons treated with varicella-zoster IG should wait 5 months. Children treated for Kawasaki disease with IGIV at a dose of 2 g/kg should not be vaccinated until 11 months after the dose. Similar recommendations apply to varicella vaccine. IG does not appear to interfere with the response to yellow fever vaccine, LAIV, zoster vaccine, or rotavirus vaccines.[318] In general, the antigenic content of inactivated vaccines is so great that IG will not interfere with the antibody response.

With live vaccines, there is the theoretical possibility of interference in development of antibody responses when live vaccines are administered at intervals of 3 to 14 days. If more than one live vaccine is needed, the vaccines should be administered simultaneously or at intervals of about 1 month between different vaccines.[2] In general, there are no restrictions on intervals between doses of different inactivated vaccines or between different inactivated and live vaccines. Exceptions are the PCV13 and PPSV23 vaccines, which require an interval between them, as described in the previous discussion of these vaccines. Another exception is MenACWY-D and PCV13 in a child with functional and anatomic asplenia. Children with this condition are at high risk of disease from both of these bacteria, yet pneumococcal infection is more common, and studies show that simultaneous vaccination reduces the immunogenicity of PCV13. Therefore, if both vaccines are indicated PCV13 should be given first, followed by MenACWY-D 4 weeks later.[60] A third exception is MenACWY-D and DTaP. If DTaP has been administered, providers should administer MenACWY-D at the same time, or wait 6 months before administering MenACWY-D.

Combination Vaccines

The routine immunization schedule has become increasingly complex over the years as more vaccines have been added. Currently, all children

TABLE 316.6 FDA-Licensed Combination Vaccines

VACCINE[b]	TRADE NAME (YEAR LICENSED)	AGE GROUP	FDA LICENSURE RECOMMENDATIONS
HepA-HepB	Twinrix (2001)	≥18 yr	Three doses on a 0-, 1-, and 6-mo schedule
DTaP-HepB-IPV	Pediarix (2002)	6 wk to 6 yr	Three-dose series at 2, 4, and 6 mo of age
MMRV	ProQuad (2005)	12 mo to 12 yr	Two doses 28 days apart, on or after the first birthday
DTaP-IPV	Kinrix (2008)	4–6 yr	Booster for fifth dose DTaP and fourth dose IPV
DTaP-IPV/Hib	Pentacel (2008)	6 wk to 4 yr	Four-dose series at 2, 4, 6, and 15-18 mo of age
DTaP-IPV	Quadracel (2015)	4–yr	Booster for fifth dose DTaP and fourth or fifth dose IPV

[a]Excludes MMR, DTaP, Tdap, and IPV, for which single-antigen products are not available in the United States.
[b]Hyphen (-) indicates that products' active components are supplied in their final form by the manufacturer and do not require mixing or reconstitution by user; slash (/) indicates that products' active components are mixed or reconstituted by user.
DTaP, Diphtheria and tetanus toxoids and acellular pertussis vaccine; *FDA,* US Food and Drug Administration; *HepA,* hepatitis A vaccine; *HepB,* hepatitis B vaccine; *IPV/Hib,* trivalent inactivated polio vaccine and *Haemophilus influenzae* type b vaccine; *MMR,* measles-mumps-rubella vaccine; *MMRV,* measles-mumps-rubella and varicella vaccine; *Tdap,* tetanus, diphtheria, and acellular pertussis (adult) vaccine.
Modified from American Academy of Pediatrics. Active immunization. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, eds. Red Book: 2009 Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2009:1–55; Centers for Disease Control and Prevention (CDC). Notice to readers. FDA approval for infants of a* Haemophilus influenzae *type b conjugate and hepatitis B (recombinant) combined vaccine.* MMWR Morb Mortal Wkly Rep. *1997;46:107–109; CDC. FDA approval of a* Haemophilus *b conjugate vaccine combined by reconstitution with an acellular pertussis vaccine.* MMWR Morb Mortal Wkly Rep. *1996;45:993–995; CDC. Notice to readers: FDA approval for a combined hepatitis A and B vaccine.* MMWR Morb Mortal Wkly Rep. *2001;50:806–807; CDC. Notice to readers. FDA licensure of diphtheria and tetanus toxoids and acellular pertussis adsorbed, hepatitis b (recombinant), and poliovirus vaccine combined, (PEDIARIX) for use in infants.* MMWR Morb Mortal Wkly Rep. *2003;52:203–204; CDC. Notice to readers. Licensure of a combined live attenuated measles, mumps, rubella, and varicella vaccine.* MMWR Morb Mortal Wkly Rep. *2005;54:1212–1214; CDC. Licensure of a diphtheria and tetanus toxoids and acellular pertussis adsorbed and inactivated poliovirus vaccine and guidance for use as a booster dose.* MMWR Morb Mortal Wkly Rep. *2008;57:1078–1079; and CDC. Licensure of a diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus, and Haemophilus b conjugate vaccine and guidance for use in infants and children.* MMWR Morb Mortal Wkly Rep. *2008;57:1079–1080.*

should be protected against 16 diseases. This schedule can require as many as 21 injections of various vaccines through 18 months of age and an additional 6 injections by 18 years of age (excluding influenza vaccines)—a major challenge for any health care delivery system. Influenza vaccine requires 2 injections for children younger than 9 years when they are first vaccinated and then 1 injection annually thereafter. Combination vaccines can provide equivalent protection with substantially fewer injections.[294,319–325] Vaccines combining antigens against multiple diseases have been a part of the routine immunization schedule for years.

For children and adolescents, many combination vaccines are available (Table 316.6). Combination vaccines may be used instead of their equivalent component vaccines when any component is indicated for the patient's age and other components are not contraindicated as licensed by the FDA. Combination vaccines represent an opportunity to reduce the number of injections. Table 316.6 shows combination vaccines licensed for use in the United States and recommendations for administration.

For adults, combination hepatitis A and hepatitis B vaccine (Twinrix) is available as a three- or four-dose regimen. This vaccine can be administered at 0, 1, and 6 months or, alternatively, at days 0, 7, and 21 to 30, followed by a booster dose at month 12.

Data suggest that MMRV, because it is associated with a higher risk for fever than administration of MMR and varicella simultaneously at different sites, also may cause an increased risk for febrile seizures after the first dose of the two-dose series.[161] In June 2009, after consideration of the postlicensure data and other evidence, ACIP adopted new recommendations regarding use of MMRV vaccine for the first and second doses and identified a personal or family (i.e., sibling or parent) history of seizure as a precaution for use of MMRV vaccine. For the first dose of measles, mumps, rubella, and varicella vaccines at age 12 to 47 months, either MMR vaccine and varicella vaccine or MMRV vaccine may be used. Providers who are considering administering MMRV vaccine should discuss the benefits and risks of both vaccination options with the parents or caregivers. Unless the parent or caregiver expresses a preference for MMRV vaccine, the CDC recommends that MMR vaccine and varicella vaccine be administered for the first dose in this age group. For the second dose of measles, mumps, rubella, and varicella vaccines at any age (15 months to 12 years) and for the first dose at age 48 months or older, use of MMRV vaccine generally is preferred over separate injections of its equivalent component vaccines (i.e., MMR vaccine and varicella vaccine). For persons aged 48 months or older, MMRV is usually preferred for the first dose if vaccination against varicella is also needed.

Interrupted Schedules

Immunologic memory induced by vaccines is usually long-term. Therefore, when one or more doses in a schedule of multiple doses are missed, there is no need to restart the series. Instead, continue from where the schedule stopped.

Reporting of Disease and Adverse Events

Public health officials at state health departments and the CDC collaborate in determining which diseases should be nationally notifiable. A disease may be added to the list as new pathogens emerge or may be deleted as the incidence decreases. Reporting of national notifiable diseases to the CDC by states is voluntary. Reporting is mandated by legislation or regulation by individual states. The list of reportable diseases (www.cdc.gov/nndss) includes many diseases preventable through vaccination. Health care providers should ensure that each suspected case of vaccine-preventable disease is reported promptly to the local or state health department. Similarly, certain adverse events after immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). These include any adverse event listed by the manufacturer as a contraindication to further doses of the vaccine, or any adverse event listed in the VAERS Table of Reportable Events Following Vaccination (https://vaers.hhs.gov/resources/infoproviders.html) In addition, CDC encourages reporting of any clinically significant adverse event that occurs in a patient following a vaccination, even if the provider is unsure whether a vaccine caused the event. VAERS reports can be submitted online (http://vaers.hhs.gov). Forms for VAERS can also be obtained by calling 800-822-7967 if Internet access is not available. The National Childhood Vaccine Injury Act requires providers to report specified adverse events if they occur within a designated time frame after immunization.[318,326] Only through accurate reporting and follow-up of both disease and adverse vaccine effects can the changing balance of the benefits and risks of vaccination be properly assessed.

Compensation for Vaccine Injuries

The National Childhood Vaccine Injury Act of 1986 established a no-fault compensation program for persons injured by vaccines.[317,318] The covered vaccines, adverse events, and time intervals for which persons are eligible for compensation in the absence of other known causes for the events can be found at www.hrsa.gov/vaccinecompensation/. All persons with alleged injuries from covered vaccines must file first under the compensation program. Those who meet the criteria of the table

(and other legal requirements) are entitled to compensation without proving that vaccine caused the injury. Persons alleging a condition not included in the table or who otherwise do not meet criteria in the table must prove that the vaccine was the cause. Persons may accept decisions of the program or reject those decisions and go to the tort system. If compensation decisions are accepted, manufacturers and vaccine administrators are protected from litigation.[317,318] More information on the compensation program can be obtained by calling 800-338-2382 or through the Division of Vaccine Injury Compensation's home page (www.hrsa.gov/vaccinecompensation/).

Standards for Immunization Practices

To improve the quality of immunization delivery, standards for child and adolescent immunization practices and standards for adult immunization practices have been developed by the NVAC (Tables 316.7 and 316.8).[327] These standards seek to minimize missed opportunities for immunization, ensure that appropriate contraindications are observed, and ensure that prospective vaccinees or their parents are adequately educated about vaccine risks and benefits. In addition, the standards include other measures to enhance the safe and effective use of vaccines.

Some of the more critical standards include providing vaccines in all health care settings; minimizing prevaccination requirements such as physician evaluation when those services are not readily obtainable; screening for contraindications, including, at a minimum, observation of the child, soliciting illness history from the parents, and verbally asking questions about contraindications; use of simultaneous immunization except when, in the judgment of the provider, nonsimultaneous vaccination will not compromise the immunization status of the patient; providing valid information on vaccine benefits and risks; and performing regular audits of patient records to determine the vaccination levels of the patients in each provider's practice. These standards are listed at www.hhs.gov/nvpo/nvac/index.html. Valid contraindications can be viewed at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html.

The IDSA has established 46 guidelines that combine relevant aspects of both the pediatric and the adult standards.[314]

TABLE 316.7 Standards for Child and Adolescent Immunization Practices

Availability of Vaccines

1. Vaccination services are readily available.
2. Vaccinations are coordinated with other health care services and provided in a medical home when possible.
3. Barriers to vaccination are identified and minimized.
4. Patient costs are minimized.

Assessment of Vaccination Status

5. Health care professionals review the vaccination and health status of patients at every encounter to determine which vaccines are indicated.
6. Health care professionals assess for and follow only medically accepted contraindications.

Effective Communication About Vaccine Benefits and Risks

7. Parents/guardians and patients are educated about the benefits and risks of vaccination in a culturally appropriate manner and in easy-to-understand language.

Proper Storage and Administration of Vaccines and Documentation of Vaccinations

8. Health care professionals follow appropriate procedures for vaccine storage and handling.
9. Up-to-date, written vaccination protocols are accessible at all locations where vaccines are administered.
10. Persons who administer vaccines and staff members who manage or support vaccine administration are knowledgeable and receive ongoing education.
11. Health care professionals simultaneously administer as many indicated vaccine doses as possible.
12. Vaccination records for patients are accurate, complete, and easily accessible.
13. Health care professionals report adverse events after vaccination promptly and accurately to the Vaccine Adverse Event Reporting System (VAERS) and are aware of a separate program, the National Vaccine Injury Compensation Program (VICP).
14. All personnel who have contact with patients are appropriately vaccinated.

Implementation of Strategies to Improve Vaccination Coverage

15. Systems are used to remind parents, guardians, patients, and health care professionals when vaccinations are due and to recall those who are overdue.
16. Office- or clinic-based patient record reviews and vaccination coverage assessments are performed annually.
17. Health care professionals practice community-based approaches.

From National Vaccine Advisory Committee. Standards for Child and Adolescent Immunization Practices. Pediatrics. *2003;112:958–963.*

Methods to Improve Immunization Coverage

The Task Force on Community Preventive Services has reviewed the literature carefully to determine effective interventions to improve immunization coverage for children, adolescents, and adults.[328] Provider-based interventions have been some of the most successful.[329] Some of the more important include use of standing orders; assessment of immunization levels in a given practice, with provision of information back to the provider; and use of reminder-recall systems.[329–331]

A review of studies of standing orders for improving immunization uptake documented 27 studies of adults that demonstrated a median increase of 24 percentage points (interquartile interval: range, 12–35 percentage points). Four studies with children demonstrated a median absolute percent increase of 28 percentage points (range, 8–49 percentage points). These data can be found at www.thecommunityguide.org/vaccines/RRstandingorders.html.

Studies have shown that providers (as well as parents) tend to overestimate the level of coverage in their patients (or children), and formal review of records can be useful in making practitioners aware of the need to continue to pay attention.[328–330] Bushnell asked physicians and nurses from both public and private sectors in Massachusetts to estimate immunization coverage of their patient populations. Estimates ranged from 85% to 100%. Record reviews documented a median coverage of 61% (range, 19%–93%).[331] Giving this information back to providers has been shown to lead to improvements in coverage.[332]

Reminder systems entail providing reminders to patients and parents or providers that an individual is due for an immunization. Recall systems notify individuals that they are past due for an immunization. Both patient and provider reminder-recall systems have been studied extensively and demonstrated to be effective.[329] ACIP, AAP, and AAFP have recommended "the regular use of R-R (reminder-recall) systems by public and private health-care providers in settings that have not achieved high documented levels of age-appropriate vaccinations."[307]

IISs, sometimes called immunization registries, can automate assessment, reminder and recall, and a number of other activities, such as assisting the practitioner in deciding whether a vaccine is needed, consolidating multiple records into a single complete record for a given individual, generating immunization records, and generating immunization coverage information for reports, such as those called for in managed care settings by the Health Plan Employer Data Information Set.[334] Registries increasingly are being developed and used throughout the United States, and there is a Healthy People 2020 objective to "increase the percentage of children younger than 6 years whose immunization records are in a fully operational, population-based immunization information system (IIS)" and "increase the number of states that have 80% of adolescents with two or more age-appropriate immunizations recorded in an IIS among adolescents aged 11 to 18 years."[335,336] Most of these persons are probably younger adults who participated as children.

Most public health authorities believe that a nationwide network of community-state population-based registries capable of exchanging information while maintaining privacy and confidentiality is essential to maintain the improvements in vaccine coverage that have been achieved. The Community Preventive Services Task Force "recommends immunization information systems on the basis of strong evidence of effectiveness in increasing vaccination rates" (www.thecommunityguide.org/vaccines/imminfosystems.html).

Sources of Information

Websites that provide comprehensive information on immunization are available from multiple sources listed in Pickering[314] and Wexler.[337]

TABLE 316.8 Summary of 2013 National Vaccine Advisory Committee's Standards for Adult Immunization Practices

AUDIENCE	SUMMARY OF STANDARDS
All providers	Incorporate immunization needs assessment into every clinical encounter. Strongly recommend needed vaccine(s) and either administer vaccine(s) or refer patient to a provider who can immunize. Stay up-to-date on, and educate patients about, vaccine recommendations. Implement systems to incorporate vaccine assessment into routine clinical care. Understand how to access immunization information systems (i.e., immunization registries).
Nonimmunizing providers	Routinely assess the immunization status of patients, recommend needed vaccine(s), and refer patient to an immunizing provider. Establish referral relationships with immunizing providers. Follow up to confirm patient receipt of recommended vaccine(s).
Immunizing providers	Ensure professional competencies in immunizations. Assess immunization status in every patient care and counseling encounter and strongly recommend needed vaccine(s). Ensure that receipt of vaccination is documented in patient medical record and immunization registry.
Professional health care–related organizations, associations, systems	Provide immunization education and training of members, including trainees. Provide resources and assistance to implement protocols and other systems to incorporate vaccine needs assessment and vaccination or referral into routine practice. Encourage members to be up-to-date on their own immunizations. Assist members in staying up-to-date on immunization information and recommendations. Partner with other immunization stakeholders to educate the public. Seek out collaboration opportunities with other immunization stakeholders. Collect and share best practices for immunization. Advocate policies that support adult immunization standards. Insurers, payers, entities that cover adult immunization services should ensure that their network is adequate to provide timely immunization access and augment with additional vaccine providers if necessary.
Public health departments	Determine community needs, vaccination capacity, and barriers to adult immunization. Provide access to all ACIP-recommended vaccinations for insured and uninsured adults and work toward becoming an in-network provider for immunization services for insured adults. Partner with immunization stakeholders and support activities and policies to improve awareness of adult vaccine recommendations, increase vaccination rates, and reduce barriers. Ensure professional competencies in immunizations. Collect, analyze, and disseminate immunization data. Provide outreach and education to providers and the public. Work to decrease disparities in immunization coverage and access. Increase immunization registry access and use by vaccine providers for adult patients. Develop capacity to bill for immunization of injured people. Ensure preparedness for identifying and responding to outbreaks of vaccine-preventable diseases. Promote adherence to applicable laws, regulations, and standards among adult immunization stakeholders.

ACIP, Advisory Committee on Immunization Practices.
From National Vaccine Advisory Committee. Recommendations from the National Vaccine Advisory Committee: standards for adult immunization practice. Public Health Rep. *2014;129:115–123.*

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317 Zoonoses

W. Ian Lipkin

SHORT VIEW SUMMARY

Definition
- A zoonosis is an infectious disease of humans that originates in animals.

Epidemiology
- Zoonoses account for up to 70% of emerging infectious diseases and include such varied examples as human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS), Ebola, severe acute respiratory syndrome (SARS), plague, rabies, influenza, Zika, and new variant Creutzfeldt-Jakob disease.

Diagnosis
- The majority of zoonotic diseases are diagnosed through molecular methods. Many appear in unexpected contexts; thus a comprehensive travel and exposure history can be critical.

Treatment
- With the notable exception of HIV/AIDS and influenza, most viral zoonoses have not been a focus for drug development. Accordingly, treatment is primarily supportive. In contrast, many bacterial zoonoses can be treated with antibiotics.

Prevention
- Vaccines are established for only a minority of zoonotic diseases. Prevention is best achieved by limiting exposure to reservoirs and vectors for transmission of infection.

Zoonoses, derived from the Greek words for animal *(zoo)* and the suffix modification indicating a state or condition *(sis),* are infectious diseases of humans that originate in animals. Infectious diseases that originate in humans and move into other animals are commonly described as reverse zoonoses. The majority of emerging viral diseases, up to 70%, represent zoonoses, with such prominent examples as human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS), influenza, West Nile virus (WNV) encephalitis, Nipah, Hendra, severe acute respiratory syndrome (SARS), Ebola, Marburg, hantavirus pulmonary syndrome (HPS) (Sin Nombre virus), Zika, and rabies. Common examples of nonviral zoonoses include Lyme disease (*Borrelia burgdorferi* in North America, *Borrelia afzelii* and *Borrelia garinii* in Europe), cat-scratch disease *(Bartonella henselae),* new variant Creutzfeldt-Jakob disease (vCJD), salmonellosis, and toxoplasmosis. Although secondary microbial contamination of agricultural products can cause significant disease, the term *zoonosis* does not apply unless there is direct transmission to humans from an infected animal.[1]

The majority of zoonotic disease can be attributed to anthropogenic change. Loss of wildlife habitat to development and consumption of bushmeat, necessitated by poverty or due to cultural preference, increase opportunities for cross-species jumps. Global warming may also increase the geographic range of phlebotomous arthropod vectors, such as mosquitos and ticks that serve as reservoirs and vectors for infectious agents. Given that there are more than 50,000 vertebrate species, for example, if we were to assume an average of 20 endemic viruses per vertebrate species, the potential reservoir of vertebrate viruses could be estimated at 1 million. Although it is unlikely all of them can be transmitted to humans and cause disease, it is sobering to consider the challenge of detecting and responding even to 1% of them—10,000 novel viruses.

ONE HEALTH INITIATIVE

The connection between human and animal microbiology was originally recognized by Virchow and Osler in the 1800s. The relationship has been emphasized through the One Health Initiative, which promotes coequal collaborations between practitioners and researchers in human and comparative medicine, and surveillance programs involving wildlife, domestic animals, and humans.[2,3] The catalytic event for the Initiative was the 1999 WNV outbreak in New York City, where two independent lines of research investigation converged. One that focused on high mortality in native corvids and exotic birds in the Bronx Zoo ultimately led to the culture and identification of the virus by the US Department of Agriculture (USDA). The other focused on human encephalitis cases that were initially attributed to the St. Louis encephalitis virus based on serology. WNV was subsequently implicated when brain material of victims was sequenced by Lipkin and colleagues at the University of California.[4] The cause of the human outbreak would have been ascertained earlier if the veterinary and medical practitioners had collaborated in sharing data and samples. A series of national and international meetings focused on emerging infectious diseases and concerns that the use of antibiotics as growth promoters in agriculture was promoting the development of antimicrobial resistance culminated in the One Health Resolution, signed by the presidents of the American Medical Association and American Veterinary Medical Association, with endorsements from the Centers for Disease Control and Prevention, the USDA, and the European Union.[5–7]

MECHANISMS OF TRANSMISSION

Mechanisms of transmission of zoonotic agents vary widely. Many are linked to food collection, processing, or consumption. The most dramatic examples are diseases associated with the slaughter and consumption of wild animals (bushmeat), such as Ebola, Marburg, SARS, monkeypox, and tularemia; however, less exotic meats are associated with transmission of *Salmonella,* highly pathogenic *Escherichia coli,* and prion diseases (new vCJD). Phlebotomous arthropods such as mosquitos, ticks, and flies may also serve as vectors for transmission of bacteria and viruses from birds and mammals to humans. Prominent examples include WNV, St. Louis encephalitis virus, tick-borne encephalitis virus (TBEV), Zika virus, Powassan virus, and *B. burgdorferi* (Lyme disease). Direct transmission can occur through exposure to infected urine or feces, as in

leptospirosis, toxoplasmosis, lymphocytic choriomeningitis, or HPS; through wound inoculation as in *Bartonella* infection (cat-scratch fever); or in rabies, the oldest recorded example of zoonotic disease and the only common one transmitted directly from infected animals to human by bite.

FACTORS IN THE EMERGENCE OF ZOONOTIC DISEASES

Travel and trade are increasingly global, carrying pathogens in addition to passengers and products to new locations. John F. Kennedy International Airport, for example, one of two international airports in the greater New York metropolitan area, receives nonstop flights from >100 international destinations and annually serves >12 million international passengers.[8] Similar traffic data apply in other major metropolitan areas. Given that an infected individual, mosquito, or other cargo can cross the world in less than 24 hours, clinicians and public health practitioners must be prepared to encounter known and novel agents in virtually any context.

The advent of global agribusiness and urbanization are also important factors in zoonotic diseases. The international food trade has burgeoned by more than 200% since 1975.[9] A hundred years ago, most fresh food was produced and consumed within a radius of a few kilometers. It is now not unusual for individuals to consume plants and animals harvested thousands of kilometers away.[11] Contamination of meat with prions, influenza viruses, and Rift Valley fever virus has been documented in international trade of livestock.[12] The centralization of food production and processing, particularly of ground meat or raw fruits and vegetables, has resulted in outbreaks of infectious diseases that may be distributed over large geographic areas.

Illegal trafficking in wildlife, as pets or food, is difficult to monitor. However, annual sales estimates in the United States alone exceed $10 billion for pets and $15 billion for bushmeat.[13] Analysis of bushmeat from bats, rodents, and primates confiscated in major ports has revealed evidence of foamy viruses, herpesviruses, and pathogenic bacteria.[14] Imported pets have been linked to outbreaks of human infection with poxviruses, *Salmonella,* and other pathogens.[14]

Attention has also increasingly focused on the role of land-use dynamics in infectious disease emergence.[15] Deforestation, the expansion of agriculture, and the extractive industries, particularly in tropical regions with high wildlife biodiversity, have led directly or indirectly to the emergence of HIV/AIDS, Nipah virus, and filoviruses.[16,17] The growth of suburbs in areas once only sparsely populated, particularly in the Northeastern United States, has been associated with an increase in the incidence of Lyme disease.

Global warming is already extending the geographic range of mosquitos and ticks that harbor and transmit plasmodia and arboviruses, resulting in outbreaks of malaria, dengue, and yellow fever in new locations.[18] Recent examples in North America include the appearance of dengue fever in Florida in 2010[19,20] and a surge in cases of West Nile encephalitis in Texas in 2012.[21] Mass migration due to war, natural disaster, poverty, and desertification can lead to increases in population density not only of humans but also of disease vectors, such as rodents and ectoparasites that carry pathogenic viruses and bacteria. In concert, these factors, malnutrition, lack of access to or refusal of vaccines, and exposure to contaminated food and water have enabled the emergence and transmission of infectious diseases.[22,23]

PREDICTION AND EARLY DETECTION OF EMERGING AND REEMERGING ZOONOTIC DISEASES

Rapid recognition and response are critical to reducing the mortality, morbidity, and economic and social costs of zoonotic outbreaks. An important factor in early outbreak detection has been the advent of tools for internet-based infectious disease surveillance. The first of these was ProMED-mail (Programme for Monitoring Emerging Infectious Diseases), created in 1994, which provides continuous, free email updates on new or evolving outbreaks and epidemics[24] in people, domestic animals, and wildlife. Reports submitted by readers are curated by a panel of experts who post submissions with commentary in several languages to a listserv of subscribers in close to 200 different countries. Another program, GPHIN (Global Public Health Intelligence Network),[25] a fee-based service, scans news services worldwide in several languages for information concerning outbreaks.

HealthMap[26] integrates reports from news media, ProMED-mail, and official documents into a user-friendly map that displays real-time updates of disease emergence. HealthMap also allows for public submission observations via its website or cellular phone applications.

An ideal surveillance system for zoonotic disease is one that allows identification of potential health threats before they move into the human population. By considering factors implicated as drivers in the emergence of zoonotic diseases, such as human demographics, agricultural production, land-use change, travel and trade patterns, climate, and wildlife distribution, risk algorithms can be developed and used to focus surveillance on sites, populations, professions, species of domestic animals, and wildlife in which there is an increased probability of known or novel high-threat pathogen emergence.[27] The two largest research programs with this focus are PREDICT, sponsored by the US Agency for International Development, and PREEMPT, sponsored the Defense Advanced Research Projects Agency.[27a,27b] PREDICT supports zoonotic surveillance and laboratory capacity in the developing world.[28] PREEMPT will develop models of viral emergence and strategies for eliminating potential human pathogens in wildlife before they jump to humans.[29] The global acknowledgment of the importance of rigorous, coordinated infectious disease surveillance, early detection, and transparency in data sharing was underscored in the International Health Regulations of 2005, signed by all member states of the United Nations.[30]

ZOONOTIC DISEASES

A chapter concerned with infectious agents that share the capacity to jump host species from domestic animals or wildlife into humans could be organized according to agent, mechanism of transmission, or clinical presentation. This chapter includes all three approaches. The following text provides an overview of a representative set of zoonotic diseases associated with bats, rodents, and other wildlife and domesticated animals. It is by no means complete, and others might highlight different choices. Nonetheless, it will provide a framework for thinking about the range of zoonotic diseases and the factors that contribute to their emergence and control. Table 317.1 indicates routes of transmission and associated syndromes.

INFECTIONS ASSOCIATED WITH WILDLIFE

Bats

Bats, order Chiroptera, comprising more than 1200 species, represent approximately 20% of mammalian species diversity and are found on every continent except Antarctica. They are divided into two suborders: the Megachiroptera (fruit bats and flying foxes) and the Microchiroptera (insectivorous and vampire bats). Bats are unique among mammals in their ability to fly, tendency to aggregate at high density, and capacity to harbor a wide range of viruses without apparent disease. The physiology of bats is poorly understood, and their tolerance for persistent infection with viruses that cause fatal disease in other mammals is an enigma. However, this is likely to change with recent investments in bat genomic sequencing and systems biology.

A wide range of highly pathogenic RNA viruses have been found to be carried by bats: henipaviruses associated with equine and human infections in Australia (Hendra virus); Nipah virus, responsible for human and pig infections in Malaysia and human infections in Bangladesh; filoviruses associated with human and great ape infections in central Africa (Ebola virus and Marburg virus); rabies virus; and the coronaviruses implicated in SARS and Middle East respiratory syndrome (MERS), which caused a recent outbreak of fatal respiratory and renal disease in the Middle East. The route of transmission can vary, even for the same virus. In Malaysia, farm workers became infected with Nipah virus through exposure to pigs that had consumed contaminated droppings of frugivorous bats; in Bangladesh, where pigs are not farmed, human Nipah virus infection has been associated with consumption of palm sap beverages contaminated by bats. In North America, silver-haired bats transmit rabies virus via a bite wound. Humans are typically infected with filoviruses (e.g., Ebola) through exposure to infected

TABLE 317.1 Examples of Zoonotic Diseases: Agents and Vectors

VECTOR		AGENT FAMILY	AGENT	DISEASE
Arthropod	Mosquito	Bunyaviridae	La Crosse encephalitis virus, California encephalitis virus	Encephalitis
			Rift Valley fever virus	Hemorrhagic fever
		Flaviviridae	Japanese encephalitis virus, St. Louis encephalitis virus, West Nile virus	Encephalitis
			Dengue virus, yellow fever virus, Zika virus	Hemorrhagic fever
		Togaviridae	Eastern equine encephalitis virus, western equine encephalitis virus, Venezuelan equine encephalitis virus, chikungunya virus, o'nyong-nyong fever virus	Encephalitis
	Tick	Bunyaviridae	Crimean-Congo hemorrhagic fever virus	Hemorrhagic fever
		Flaviviridae	Tick-borne encephalitis virus, Powassan encephalitis virus	Encephalitis
		Spirochaetaceae	Omsk hemorrhagic fever virus, Kyasanur Forest virus, Langat virus	Hemorrhagic fever
			Borrelia	Lyme disease
	Flea	Enterobacteriaceae	*Yersinia pestis*	Hemorrhagic fever (plague)
Mammal	Rodent	Arenaviridae	Lassa fever virus, Guanarito virus, Junin virus, Machupo virus, Sabia virus, Lujo virus	Hemorrhagic fever
			Lymphocytic choriomeningitis virus	Meningitis, encephalitis
		Bunyaviridae	Dobrava-Belgrade virus, Hantaan virus, Puumala virus, Seoul virus	Hemorrhagic fever
			Sin Nombre virus	Pulmonary
		Spirochaetaceae	*Leptospira*	Leptospirosis
	Rabbit	Francisellaceae	*Francisella tularensis*	Tularemia
	Bat	Filoviridae	Ebola virus, Bundibugyo virus, Sudan virus, Tai Forest virus, Marburg virus	Hemorrhagic fever
		Rhabdoviridae	Rabies virus, Chandipura virus	Encephalitis
		Paramyxoviridae	Hendra virus, Nipah virus	Pulmonary, encephalitis
		Coronaviridae	Severe acute respiratory syndrome (SARS) coronavirus, Middle Eastern coronavirus	Pulmonary
	Cattle	Prions	Bovine spongiform encephalopathy prion	Neurodegeneration
	Cat	Bartonellaceae	*Bartonella*	Hemorrhagic fever
		Sarcocystidae	*Toxoplasma*	Toxoplasmosis
Avian	Bird	Orthomyxoviridae	Influenza virus	Pulmonary

primate meat; however, there are instances in which infection may have occurred through consumption of infected bats or exposure to bat excreta in close quarters (e.g., caves and abandoned mines).

The SARS coronavirus is presumed to have entered the human population through intermediate species (civets) in live-animal markets. The major reservoir for the MERS coronavirus is the dromedary camel.[31,32] Although there are examples of human-to-human transmission of filoviruses and henipaviruses through contact with blood or other bodily fluids, the only bat-associated pathogen known to have established efficient transmission within the human population is the SARS coronavirus. Nipah virus, Hendra virus, SARS coronavirus, and MERS coronavirus cause respiratory disease and spread via aerosol. All are associated with high morbidity and mortality. However, the primary site of pathology in SARS and MERS is the lung, whereas Nipah and Hendra viruses cause encephalitis. Ebola virus and Marburg virus cause disseminated intravascular coagulation, resulting in hemorrhagic shock and multiorgan failure. Ebola virus, Marburg virus, and the arenaviruses and hantaviruses, described in the next section, are known collectively as hemorrhagic fever viruses in accord with the clinical manifestations of disease.

A diagnosis can be made based on clinical presentation in contexts in which others with similar presentation (including animals) have had a laboratory diagnosis. Although serology can be definitive, a laboratory diagnosis is typically made by means of an agent-specific polymerase chain reaction (PCR) assay. There is no established effective therapy for the diseases caused by SARS, MERS, Nipah, Hendra, Ebola, or Marburg viruses. A recombinant vesiculostomatitis virus vaccine expressing the surface glycoprotein of the Zaire ebolavirus was 100% effective in preventing disease in an open label trial in Guinea.[33] Monoclonal antibodies may reduce morbidity and mortality in disease caused by Nipah and Hendra viruses; therefore it is likely that a vaccine that elicits responses to similar antigenic targets would be effective. Rabies is typically fatal once the virus has entered the central nervous system; however, postexposure prophylaxis is effective, particularly when the exposed individual receives not only the vaccine, but also rabies immune globulin injected in proximity to the puncture wound.[34,35]

Rodents

Rodents, like bats, are globally distributed. They are the largest order of mammals, comprising more than 2200 species, and are implicated in transmission of both viruses and bacteria. The rodent-associated arenaviruses (named for their sandy appearance with electron microscopy; *arena* means "sand" in Latin) causing human disease include the Lassa fever virus, endemic in Western Africa; the lymphocytic choriomeningitis virus, found in both the Eastern and Western Hemispheres; and several South American viruses including Guanarito, Junín, Machupo, and Sabia. Another African arenavirus, Lujo virus, is presumed, but not proven, to have a rodent reservoir. Lassa fever is clinically similar to Ebola and Marburg; however, Lassa fever virus infection may be asymptomatic in up to 80% of cases, whereas asymptomatic filovirus infection is uncommon. The South American arenaviruses can cause hemorrhagic fever, but can also be associated with asymptomatic infection. Lymphocytic choriomeningitis virus has been implicated in aseptic meningitis and birth defects, particularly in the central nervous system. Although there is no specific drug for arenavirus infection, the nucleoside analogue ribavirin may have efficacy. Passive immunotherapy with hyperimmune plasma has been useful in Argentinean hemorrhagic fever (Junin virus infection).[36]

Leptospira, a genus named for its morphology (*leptos* is "thin" in Greek; *spiral* is "coil" in Latin), consists of spirochetes that persistently infect rodents, concentrating in the kidneys. Infectious organisms are excreted in urine, collect in standing water, and enter humans and other animals either through breaks in the skin or via the alimentary canal. Infection is correlated with the potential for exposure; hence, leptospirosis is common year round in the tropics and during warm wet months in temperate climate zones. Food markets, particularly those where rodents congregate and where there is opportunity for urine from infected animals to contaminate standing water, are high-risk environments for contraction of disease. The onset of disease is heralded by nonspecific symptoms such as fever, malaise, and headache. It may progress to meningitis, vasculitis, renal and liver failure, and disseminated intravascular coagulation. Antibiotics (penicillin, amoxicillin, and doxycycline) are effective in prophylaxis and as a therapeutic intervention. There are vaccines for some serotypes.

The Bunyaviridae family of RNA viruses includes several important human pathogens—hantaviruses, nairoviruses, and phleboviruses. Hantaviruses are transmitted through contact with rodent excreta; the other clinically significant bunyaviruses are transmitted through blood exchange by arthropod vectors, including mosquitos, ticks, and flies. Hantaviruses (named for the Hantan river in South Korea, where the first hantaviruses were identified) may cause hemorrhagic fever with renal failure (hemorrhagic fever with renal syndrome [HFRS])[37] or an acute respiratory distress syndrome (HPS).[38] HFRS is more common in Africa, Asia, and Europe. HPS has been reported only in North America. There is no hantavirus-specific drug or vaccine. Recovery from HFRS is not uncommon with supportive care; however, HPS is typically fatal. Crimean-Congo hemorrhagic fever virus, a nairovirus,

is distributed throughout Asia and Africa.[39] A wide range of small mammals may serve as reservoirs; domestic sheep, goats, cattle, and horses are typically intermediate hosts. The agent is most commonly transmitted by ticks; however, outbreaks have been linked to preparation or consumption of infected meat.[40] Manifestations of disease range from mild influenza-like illness to hemorrhagic fever.

Tick-borne encephalitis is a meningoencephalitis due to infection with the flavivirus TBEV.[41] TBEV is found in Europe and Asia. It is transmitted to humans from a wide range of infected hosts, including sheep, deer, and rodents. Infection is rarely fatal; however, neurologic sequelae are not uncommon. There are effective vaccines but no known antivirals.

The most common tick-borne illness in the United States is Lyme disease.[42] In North America, the causative agent is the spirochete *B. burgdorferi,* carried by the tick *Ixodes scapularis.* In Europe and Asia, the corresponding agents are *B. afzelii and B. garinii,* carried by *Ixodes ricinus* and *Ixodes persulcatus,* respectively. Early signs and symptoms of disease may include fever, lassitude, and headache. Some individuals have a classic "bull's-eye" skin rash known as erythema chronicum migrans, characterized by redness in the center and the periphery with central clearing. Later stages of the disease may include facial weakness (Bell palsy), peripheral neuropathy, and encephalomyelitis. Rodents are the reservoir for the spirochete; however, deer are also important to its life cycle as alternate reservoir hosts. Thus, human transmission is most common in suburban and rural areas, particularly where the density of vegetation is sufficiently thick to harbor large concentrations of rodents. Diagnosis is straightforward in the presence of the characteristic rash when there is a history of travel to an endemic area and a tick bite. However, many patients will not recall a tick bite, and up to 20% may not have the rash. Direct detection of the causative organism by means of culture or PCR analysis of the rash is definitive. Serology, the mainstay of laboratory diagnosis, is frequently inconclusive or misleading.[43]

Francisella tularensis, the causative agent of tularemia (also known as rabbit fever), infects a wide range of vertebrate and invertebrates. The most important reservoirs are rodents, deer, and rabbits. Humans become infected through contact with infected animals or hematophagous vectors, including mosquitoes, ticks, or flies. Thus, hunters, hikers, and farmers are at highest risk in endemic areas. Outbreaks of severe respiratory disease have been described in landscapers. The manifestations of infection vary with the route of exposure. Cutaneous inoculation typically results in a vesicular rash, which may or may not ulcerate, and regional lymphadenopathy. Ingestion results in oropharyngeal lesions. Inhalation can lead to fatal pneumonia. Ocular inoculation results in conjunctivitis. Without antibiotic treatment, some strains disseminate systemically and are associated with up to 30% mortality. The diagnosis is typically made based on clinical presentation, with confirmation by means of PCR or culture. The agent is sensitive to ciprofloxacin, aminoglycosides, and chloramphenicol. Vaccines are in development due to concerns that *F. tularensis* may be weaponized.[44]

Yersinia pestis, the causative agent of plague, is found in rodents throughout the world with the exception of Oceania. Infection is transmitted to humans primarily by fleas. However, transmission can also occur through ingestion and inhalation. Periodic pandemics, including the Black Death, have cost hundreds of millions of lives. Although outbreaks continue in the developing world, the majority of current reports in the United States note infections in single individuals. The manifestations of disease can vary from an influenza-like mild fever and lymphadenopathy (buboes) to pneumonia, shock, disseminated intravascular coagulation, and tissue necrosis. The diagnosis is typically made based on clinical presentation, with confirmation by means of PCR or culture. Like *F. tularensis, Y. pestis* is sensitive to ciprofloxacin, aminoglycosides, and chloramphenicol. It has the dubious distinction of being one of the first microbes to be used as a biologic weapon, in the 14th century, and remains a biosecurity concern.[45]

DISEASES ASSOCIATED WITH AGRICULTURAL AND OTHER DOMESTIC ANIMALS

Zoonotic agents associated with livestock, poultry, and companion animals include bacteria, fungi, parasites, and prions. Industrialization of food production has increased the probability of meat cross-contamination, in addition to animal-to-animal infection on feedlots. Outbreaks of disease due to *E. coli,* including the potentially fatal hemolytic-uremic syndrome, are commonly linked to contaminated ground beef. Infected poultry and eggs are frequently associated with outbreaks of *Salmonella* infection. The use of antibiotics as growth promoters in agriculture also encourages the emergence of antibiotic-resistant bacteria that contribute to human morbidity and mortality associated with infectious disease.[7]

vCJD is a progressive, fatal brain disorder caused by exposure to misfolded proteins (prions) in cattle. vCJD and an analogous disease known as bovine spongiform encephalopathy (BSE) catalyze conformational changes in host proteins. BSE was first identified in the United Kingdom in 1986 and peaked in incidence in 1993. The original infection is presumed to have been introduced when cattle were fed with meat (offal) from infected sheep or cows. An estimated 400,000 cows with BSE are estimated to have entered the food chain before the outbreak was terminated by culling cattle with behavior indicative of BSE, reducing the age of slaughter (so that titers would be lower in the event that an infected animal was inadvertently used for human consumption), and eliminating the practice of feeding offal to ruminants in 1997. At least 227 people exposed to BSE contracted vCJD. Susceptibility to disease is determined at least in part by the prion protein (PrP) genotype. The peak incidence of vCJD was observed in 2000 and tapered thereafter. Some investigators hold that a large wave of new cases is unlikely in the absence of recurrence of BSE. However, others note that the incubation period of kuru, a prion disorder associated with ritual cannibalism, due to essentially the same agent, may be as long as 27 years. The diagnosis of vCJD is made through brain or tonsillar biopsy based on histologic findings and/or the presence of protease-resistant PrP. There is no effective treatment or vaccine for prion diseases.

Rift Valley fever virus, a bunyavirus transmitted by mosquitoes, chiefly affects livestock but can cause human disease (Rift Valley fever), ranging from a mild febrile illness to meningitis or hemorrhage and multiorgan failure. Originally reported in Kenya, the virus has expanded in distribution across sub-Saharan Africa and the Arabian Peninsula.

A detailed discussion of influenza virus is beyond the scope of this chapter. Furthermore, influenza is not typically considered a zoonotic disease because human-to-human transmission is efficient and represents the large majority of cases of influenza A. Nonetheless, both birds and pigs play key roles in the maintenance of existing strains and the emergence of new ones. Only influenza A viruses are associated with pandemic disease, presumably because they have a higher propensity to evolve toward antigenic diversity and thus evade host immune surveillance than influenza B and C viruses. Wild aquatic birds are the natural hosts of the classic seasonal influenza viruses H1N1 and H3N2. Infection of free-range pigs by migrating aquatic birds or in live-animal markets where they may be housed near aquatic birds can lead to genetic reassortment (when more than one type of influenza virus is present) and adaption to humans. H5N1, popularly known as avian influenza, only rarely results in human disease but is frequently fatal when it does. A wide range of diagnostic assays is available for detection of influenza viruses. Vaccines are designed twice each year to reflect strains predicted to predominate during the influenza season in the Southern and Northern Hemispheres. Their efficacy fluctuates with the accuracy of those predictions, but, as a rule, they are less effective in inducing a protective immune response in the elderly. Efforts are underway to create universal influenza vaccines based on conserved regions of the virus hemagglutinin rather than those that evolve to evade the immune system.[46] The drug oseltamivir is effective for many strains.

Up to 30% of the world's population may have been exposed to the protozoan *Toxoplasma gondii*, including more than 60,000,000 people in the United States. Infection is contracted through ingestion of contaminated food or contact with feces of domestic cats, the natural host. In most instances, infection is asymptomatic or associated with a transient influenza-like illness; however, with immunosuppression in the context of HIV/AIDS, chemotherapy, or pregnancy, the parasite can cause a mass lesion in the brain, leading to focal neurologic deficits and seizures. Infection of the fetus during pregnancy can result in

miscarriage and abnormal brain development. Treatment varies with the immunologic status of the patient.

Eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), and Venezuelan equine encephalitis virus (VEEV) are mosquito-borne alphaviruses that infect a wide range of vertebrates. Birds are the natural reservoirs. The nomenclature reflects the observation that horses with encephalitis are frequent sentinels for risk of human disease and roughly approximates the geographic distribution of the virus. EEEV is found throughout North, Central, and South America but is concentrated in the United States east of the Continental Divide. WEEV is found in the western United States. VEEV is found primarily in Central and South America. All are associated primarily with an influenza-like illness but may, in a small proportion of cases, progress to encephalitis. WNV is a mosquito-transmitted flavivirus associated with only mild, sporadic disease until the 1990s, when virulent strains appeared first in Europe and then in North America. Many vertebrate species are susceptible to infection; however, the primary reservoir is birds. Although most infections are asymptomatic or associated with mild influenza-like illness (West Nile fever), encephalitis and poliomyelitis can occur in individuals who are immunologically compromised because of advanced age or underlying medical conditions. Only supportive therapy is available for EEEV, WEEV, VEEV, and WNV encephalitis. Vaccines have been developed but are typically not used in humans, except where biomedical researchers concentrate VEEV and are at high risk for exposure.

Zika virus, identified in a rhesus monkey in 1947, is named for its site of isolation within the Zika forest in Uganda. It has been implicated in microcephaly and other neurodevelopmental damage in children whose mothers were infected during pregnancy and in Guillain-Barré syndrome in infected adults. Zika virus has both a sylvatic and an urban cycle. In the former, the virus is maintained in nonhuman primates by *Aedes* and *Anopheles* mosquitos. Humans appear to be the only reservoir in the urban cycle. Human-to-human transmission is most commonly mediated by *Aedes* mosquitos, but humans also can become infected through exposure to infected semen or blood products.[47] There is no known treatment for Zika; however, several vaccines are in development.

CONCLUSIONS

The majority of emerging and reemerging infectious diseases are zoonoses. Zoonoses are likely to increase in frequency owing to anthropogenic factors that include incursion into wildlife habitats, globalization of travel and trade, and climactic changes that influence the distribution of arboviral vectors, including mosquitos, phlebotomous flies, and ticks. Antimicrobial resistance is not a zoonosis in the strict sense of the term. Nonetheless, the threat of antimicrobial resistance is clearly exacerbated by the misuse of antibiotics in animal husbandry.

In viral zoonoses, interventions are currently limited to supportive care and in some instances, such as rabies and some hemorrhagic fevers, to immunotherapy with hyperimmune globulin or monoclonal antibodies. In contrast, specific antibiotic therapy has utility in bacterial zoonoses. Investments in biodefense focused on understanding viral biology and the development of antiviral drugs are rapidly leading to specific interventions that will reduce the morbidity and mortality of emerging viral infections. Accordingly, early differential diagnosis will become increasingly important not only for outbreak containment but also for ensuring that patients receive appropriate treatment.

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318 Protection of Travelers

David O. Freedman

SHORT VIEW SUMMARY

- A pretravel office visit with an adult traveler to the developing world should follow a structured approach.

PERFORM RISK ASSESSMENT

- Exact itinerary, including regions within each country to be visited, dates of travel to assess risk of seasonal diseases, age, past vaccination and travel history, immune status, underlying illnesses, current medications, pregnancy status, allergies, purpose of trip, risk exposures (blood, body fluids, adventure or extensive outdoor exposures), urban versus rural travel, type of accommodations, travelers' risk tolerance, and financial limitations that may necessitate prioritization of interventions

ADMINISTER IMMUNIZATIONS

- Routine vaccinations that are not up-to-date, including measles-mumps-rubella (MMR), tetanus-diphtheria–acellular pertussis (Tdap), pneumococcal, varicella zoster virus
- Indicated routine travel vaccines, including hepatitis A, hepatitis B, typhoid, and influenza
- Indicated specialized vaccines, including yellow fever, rabies, polio, meningococcal, cholera, Japanese encephalitis, and tick-borne encephalitis in certain countries

PROVIDE MALARIA PREVENTION (IF INDICATED)

- Several equally effective drugs of choice may be indicated, including atovaquone-proguanil, mefloquine, and doxycycline. Ascertain which drug is best suited to the individual patient and itinerary.
- Educate on personal protection against arthropods.

TRAVELER'S DIARRHEA

- Recommend food and water precautions, as well as hand hygiene.
- Advise on use of loperamide and oral hydration if needed.
- Prescribe and educate on standby therapy with azithromycin (sometimes a quinolone) only for diarrhea that is disabling and prevents normal activities.

VECTOR-BORNE DISEASES

- Educate on personal protection measures for malaria, dengue, chikungunya, Zika virus infection, leishmaniasis, rickettsial disease, and sleeping sickness

TEACH ESSENTIAL PREVENTIVE BEHAVIORS

- Most travel-related health problems, including vaccine-preventable diseases, can be avoided through simple behaviors initiated by the traveler.
- Educate on appropriate strategies in the following categories (some topics are not applicable to all destinations): bloodborne and sexually transmitted diseases, safety and crime avoidance, transportation-associated and other injury prevention, swimming safety, rabies, skin/wound care, and tuberculosis.

DISCUSS OTHER APPLICABLE HEATH ISSUES

- Educate on and prescribe for altitude illness, motion sickness, traveler's thrombosis, or jet lag.
- Discuss minimal-risk conditions (e.g., hemorrhagic fevers) that are a frequent cause of patient anxiety.

MEDICAL KIT AND MEDICAL CARE ABROAD

- Contents of a personal health kit
- Finding destination medical facilities
- Evacuation insurance and supplemental health insurance

Pretravel management of an international traveler should be based on risk management principles. Prevention strategies and medical interventions need to be individualized according to both the itinerary and factors that are dependent on the traveler. A structured approach to patient interaction (Table 318.1) is the most efficient way to cover the necessary educational and preventive interventions. Because many of these measures will not be initiated until much later at the traveler's destination, clearly printed instructions in lay language are advisable. The worldwide epidemiology of travel-related diseases is constantly changing. A body of knowledge in travel medicine has been published, and online and print resources (Table 318.2) should be consulted frequently[1–3] to keep current.

EPIDEMIOLOGY OF TRAVEL-RELATED ILLNESS

In 2015 international tourist arrivals in all countries exceeded 1.2 billion people. In 2014 the total number of arrivals in countries with emerging markets nearly surpassed the number in developed countries. Depending on the destination, 6% to 87% of travelers become ill across all studies.[4–6] Of four studies that provide the best estimate, 43% to 79% of travelers who frequently visited developing nations (e.g., India, Tanzania, and Kenya) became ill; most of these illnesses were mild and self-limited such as diarrhea, respiratory infections, and skin disorders.[7] Some travelers return to their own countries with preventable life-threatening infections.[8] Yet 20% to 80% of travelers do not seek pretravel health consultation.[9,10] Data about the effect of pretravel advice are limited, although such advice has had a positive effect on the prevention of malaria. Travelers visiting friends and relatives in their country of origin constitute the group with the highest morbidity, especially from malaria and typhoid; this group requires special approaches to illness prevention and education.[11,12] Rates are significantly higher in summer. Approximately 8% of travelers consult a physician either during or after a trip, but less than 1% require hospitalization. Infectious diseases account for up to 10% of morbidity during travel but only 1% of deaths, with malaria the most common disease. Causes of death vary according to the population studied. At destinations that attract elderly adults, cardiovascular events predominate, whereas in developing countries, motor vehicle accidents and drowning prevail.

IMMUNIZATION

The choice of vaccines for an individual traveler is based on risk of exposure to vaccine-preventable diseases on the chosen itinerary; the severity of disease, if acquired; and any risks presented by the vaccine itself. Travelers differ in their tolerance of risk. Requests for immunization

TABLE 318.1 Structured Approach to Pretravel Office Visit With a Traveler to the Developing World

Perform Risk Assessment

The following must always be ascertained to determine appropriate preventive medical recommendations. Preprinted medical record forms may be used to record these.
Exact itinerary including regions within each country to be visited
Dates of travel to assess risk of seasonal diseases
Age
Past vaccination history
Underlying illnesses and immune status
Prior travel-related illnesses
Current medications
Pregnancy status
Allergies
Purpose of trip
Risk exposures—blood, body fluids, adventure or extensive outdoor exposures
Urban versus rural travel
Type of accommodations
Level of aversion to risk
Financial limitations that may necessitate prioritization of interventions

Administer Immunizations

Administer routine vaccinations that are not up-to-date
Administer indicated travel vaccines
Provide to patient legally mandated Vaccine Information Statements from the Centers for Disease Control and Prevention (https://www.cdc.gov/vaccines/hcp/vis/index.html)
Provide printed checklist listing vaccines administered to patient
Record in the clinic record vaccines administered, lot number, and date
Document vaccines offered to, but declined by, patient and nonrecommended vaccines administered at the patient's request

Provide Malaria Prevention (if Indicated)

Determine whether malaria risk exists for the destination country. If yes:
Does the patient's itinerary within that country put him or her at risk? If yes:
Recommend malaria chemoprophylaxis; atovaquone-proguanil is preferred in most cases for most destinations. Several other equally effective drugs of choice are available for use in certain situations
Educate on personal protection against arthropods

Educate on Traveler's Diarrhea

Recommend food and water precautions
Prescribe and educate on standby therapy with azithromycin for disabling diarrhea and advise on use of loperamide and oral hydration if needed

Vector-Borne Diseases

Personal protection measures for malaria, dengue, chikungunya, Zika virus infection, leishmaniasis, rickettsial disease, and sleeping sickness

Teach Essential Preventive Behaviors

Most travel-related health problems including vaccine-preventable diseases can be avoided through simple behaviors initiated by the traveler
Educate on appropriate strategies in the following categories (some topics are not applicable to all destinations): bloodborne and sexually transmitted diseases, safety and crime avoidance, motor vehicle and other injury prevention, swimming safety, rabies, skin/wound care, tuberculosis, packing for healthy travel, obtaining health care abroad

Discuss Other Applicable Health Issues

Advise and prescribe for altitude illness, motion sickness, or jet lag
Discuss prevention of specific travel-related infections that are of some risk to the traveler and have a possible preventive strategy not included in the strategies above
Discuss any minimal-risk conditions (e.g., hemorrhagic fevers) that are a frequent cause of patient anxiety

[a]Perform 4–6 weeks in advance of the trip.

against diseases that are actually of negligible risk to the traveler but have the potential for poor outcome if acquired are often difficult for the physician to refuse because sporadic travel-related cases do occur each year. For vaccine-preventable diseases, the monthly incidence for nonimmune travelers to developing countries is most significant for symptomatic hepatitis A (HA), at 0.03% per month overall, and is still considerable for perceived low-risk destinations such as Mexico.[13] The risk of symptomatic hepatitis B (HB) is most significant for long-stay travelers and expatriates, at 0.25% per month. Enteric fever (typhoid and paratyphoid) has a risk of 0.03% per month on the Indian subcontinent and is 10 times lower in Africa and parts of Latin America.[14] Risk of yellow fever (YF) may be as high as 0.1% per month of travel to an area with current epidemic transmission, but the risk varies greatly between destinations encompassed by the endemic area map. The risk of meningococcal meningitis, rabies, cholera, polio, measles, varicella, and Japanese encephalitis in travelers is not known but is thought to be small (<0.0001%) even for travel to highly endemic areas.[15]

TABLE 318.2 In-Depth Information Resources for Travel Medicine

Authoritative Websites Updated Constantly With Epidemiologic and Outbreak Information

Centers for Disease Control and Prevention (CDC) Travelers' Health
http://www.cdc.gov/travel
World Health Organization (WHO) International Travel and Health
http://www.who.int/ith/en
Public Health Agency of Canada Committee to Advise on Tropical Medicine and Travel (CATMAT)
http://www.phac-aspc.gc.ca/tmp-pmv/catmat-ccmtmv/index-eng.php
WHO Disease Outbreak News
http://www.who.int/csr/don/en/
WHO Weekly Epidemiological Record
http://www.who.int/wer
CDC Morbidity and Mortality Weekly Report
http://www.cdc.gov/mmwr

In-Depth References on Specialized Topics

Centers for Disease Control and Prevention. CDC Yellow Book 2018: Health Information for International Travel. New York: Oxford University Press; 2018. Full text and hard copy order information available at **https://wwwnc.cdc.gov/travel/page/yellowbook-home**
WHO International Travel and Health. WHO "Green" Book. Out of print; select updated text is online at http://www.who.int/ith
Keystone JS, Freedman DO, Kozarsky P, et al, eds. *Travel Medicine.* 4th ed. Philadelphia: Saunders; 2019. (ISBN: 9780323546966)
Plotkin SA, Orenstein WA, Offit P, Edwards K. *Vaccines.* 7th ed. Philadelphia: Elsevier; 2017. (ISBN: 9780323357616)
Auerbach PS, Cushing T, Harris NS. *Wilderness Medicine.* 7th ed. St. Louis: Mosby; 2016. (ISBN 9780323359429)

Table 318.3 provides data on dosing, administration, need for boosters, and possible accelerated regimens for vaccines administered in the travel medicine setting. Details on vaccine composition, mechanism of action, use for routine adult and childhood primary vaccination, and adverse reactions can be found in Chapter 316. The following discussion focuses on indications for each vaccine in the context of travel.

Update of Routine Immunizations

Because of the increased prevalence of many infections in the developing world, routine adult immunizations need to be current.[16] Widespread outbreaks of measles, mumps, and pertussis are currently ongoing in developed and in developing countries.[17,18]

Tetanus-Diphtheria–Acellular Pertussis

If no adult doses of tetanus-diphtheria–acellular pertussis (Tdap) have ever been given, a dose of Tdap should be given regardless of the time elapsed since the last tetanus/diphtheria vaccination. Travelers to remote areas where tetanus toxoid (indicated in cases of highly contaminated wounds) will be inaccessible should get boosters at 5-year intervals.

Measles-Mumps-Rubella

Persons (except traveling health care workers) born in the United States before 1957 or born anytime in the developing world are considered immune to measles. Other adult travelers should have received at least two doses of live measles-containing vaccine during their life, unless measles immunity can be documented.. One dose of measles-mumps-rubella (MMR) should be considered for health care or humanitarian workers without other evidence of immunity.

Influenza and Pneumococcal Vaccine

Influenza is the most common vaccine-preventable disease among travelers[19,20] including passengers on cruise ships. Because of year-round circulation of influenza virus in tropical and subtropical regions and

TABLE 318.3 Travel-Related Vaccines of Adults

DISEASE	VACCINE	PRIMARY COURSE	ROUTE	FURTHER BOOSTERS
Vaccines to Consider for All Destinations				
Hepatitis A	Killed virus	0, 6–18 mo[a]	IM	None
Hepatitis B	Recombinant viral antigen	0, 1, 6 mo	IM	None
		A: 0, 1, 2, and 12 mo	IM	None
		A: 0, 1, 3 wk and 12 mo[b]	IM	
Hepatitis B (CpG)	Recombinant viral antigen, adjuvanted	0, 1 mo	IM	No
Hepatitis A/B	Combination of monovalent preparations	0, 1, 6 mo	IM	None
		A: 0, 1, 3 wk and 12 mo	IM	None
Typhoid	Capsular Vi polysaccharide	Single dose	IM	2–3 yr
	Live-attenuated Ty21a bacteria	0, 2, 4, 6 d	Oral	5 yr
Influenza	Inactivated viral	Single dose	IM	Annual
Varicella	Live-attenuated virus	0, 4–8 wk	SQ	None
Vaccines for Selected Destinations				
Yellow fever	Live-attenuated 17D virus	Single dose	SQ	10 yr for high-risk travelers, long-term to lifelong protection for others[c]
Meningococcal	Quadrivalent conjugated polysaccharide (ACYW-135)	Single dose	IM	5 y
Rabies	Inactivated cell culture viral	0, 7, 21–28 d	IM[d]	None routinely but 2 doses after each exposure
Japanese encephalitis (Vero cell)	Inactivated viral	0, 7–28 d	IM	1 yr if at continued risk. **S**ubsequent doses >6 yr later. No definitive recommendation
Polio[e]	Inactivated viral	Single dose if adequate childhood series	SQ; IM acceptable	None
Cholera	Live-attenuated CVD 103-HgR bacteria	Single dose	Oral	No data; immunity beyond 6 mo uncertain
	Killed bacteria + recombinant B toxin subunit[f,g]	0, 1 wk	Oral	2 y for cholera; 3 mo for ETEC
Tick-borne encephalitis[h]	Inactivated viral	0, 1–3 mo, 9–12 mo	IM	3 yr
		A: 0, 7, 21 d (protective 7 d after dose 3)		

[a]Second dose may be delayed up to 8 years without diminished efficacy.
[b]Regimen not approved by US Food and Drug Administration for monovalent hepatitis B vaccine but approved for combination hepatitis A/B vaccine containing the same quantity of hepatitis B antigen.
[c]A few countries that have mandatory entry requirements may require vaccination every 10 years; International Health Regulations require only 1 lifetime dose to meet entry requirements.
[d]Intradermal rabies preexposure vaccine is no longer produced, and IM 1.0-mL vials are not licensed for intradermal use in 0.1-mL dose.
[e]Oral polio vaccine is no longer produced in the United States.
[f]Not available in the United States but licensed in some countries including Canada for traveler's diarrhea due to ETEC.
[g]Also licensed in some countries for traveler's diarrhea due to ETEC.
[h]Not available in United States and Canada but available in endemic areas of Europe.
A, Accelerated regimen to be used for imminent departures; *ETEC,* enterotoxigenic *Escherichia coli*; *IM,* intramuscular; *SQ,* subcutaneous.

an influenza season that occurs in winter in temperate regions in the Southern Hemisphere (which is summer in the Northern Hemisphere), all travelers to the tropics at any time of year and to temperate destinations where it is currently winter should have received the most current influenza vaccine available in their home country before traveling.[21] Evidence suggests that travelers immunized with the current vaccine formulation more than 6 months earlier should consider revaccination because immunity may have declined.[21,22] Travelers may consider oseltamivir or baloxavir as standby therapy, especially travelers who are at high risk for complications from influenza or who are inadequately vaccinated. All individuals older than 6 months should receive the influenza vaccine. In China and Southeast Asia, avoiding poultry markets and farms may decrease risk of avian influenzas such as H7N9, H5N1, and H5N6. Unvaccinated persons who have the accepted routine indications for the pneumococcal vaccine, which includes most individuals with chronic disease and immunocompromising conditions (see Chapter 165) should receive this during the pretravel consultation.

Varicella

Varicella is primarily a disease of adolescents and young adults in tropical, nonindustrialized countries. Two doses of varicella vaccine, spaced by at least 4 weeks, should be considered for adult travelers without evidence of varicella immunity. Adults born before 1980 in the United States are considered immune.

Vaccines to Consider for All Destinations in the Developing World

Hepatitis A

HA vaccine is indicated for every nonimmune traveler to countries or areas with moderate-to-high risk of infection, which includes essentially everyone traveling outside the United States, Canada, Japan, Australia, New Zealand, Scandinavian countries, and developed countries in Europe.[23] A single dose of HA vaccine given any time before travel provides adequate protection. The Centers for Disease Control and Prevention (CDC) recommends ancillary concomitant immune globulin

for travelers older than 40 years who are planning to depart in 2 weeks or less, immunocompromised persons, those with chronic liver disease, and pregnant women traveling to intermediate or high-risk areas. Individuals born in the developing world are generally immune to HA. Persons with a history of hepatitis or who previously lived in an endemic country for a prolonged period may benefit from prevaccination serum antibody testing.

Hepatitis B

Travelers born in the United States after 1992 already have received an HB vaccine series. Pretravel HB vaccination is indicated for all nonvaccinated travelers with standard indications such as health care workers and all travelers with plans for long-term stays who will be visiting or residing in high-risk or moderate-risk areas. Transmission via routes such as sexual contact, blood transfusions, contaminated medical equipment, body piercing, tattooing, acupuncture, and sharing of cooking and bathroom facilities is difficult to control or predict in the context of travel. Vaccination is usually advocated for short-term travelers, especially younger travelers and travelers anticipating close contact with local populations, even if they have no specific risk factors. Adventure travelers (accident prone), backpackers, and travelers with underlying medical conditions are more likely to require contact with the medical system. Business and other regular travelers who fly internationally on multiple but short trips have a cumulative risk that increases with time, and such individuals should receive the HB vaccine.[24,25] Accelerated and hyperaccelerated schedules of standard HB vaccines (see Table 318.3) are used widely in practice and are approved in many countries.[26,27] These are helpful in administering all three primary doses necessary for high assurance of protection in the frequent circumstance in which the traveler is leaving in a very short time and is at risk of HB exposure. A novel adjuvanted HB vaccine approved in 2018 in the United States is fully protective after two doses spaced by 1 month.

Combination Hepatitis A and Hepatitis B Vaccine

The combined HA and HB vaccine provides convenience for travelers with an overlap of indications for use of the individual vaccines and has a licensed accelerated 3-week schedule (see Table 318.3)

Typhoid

Typhoid vaccine is indicated for all travelers to the Indian subcontinent and considered for individuals traveling to other endemic areas (Fig. 318.1) under all but the most deluxe and protected of conditions.[28–31] Risk increases with trip duration, lodging or eating with local residents, and extent of travel off the usual tourist itineraries. In risk areas, food and water precautions should still be followed rigorously because typhoid vaccines are only 53% to 72% protective,[28,29] and a large oral inoculum may overwhelm even an optimal antibody response. The increase in quinolone-resistant *Salmonella enterica* serovar Typhi in Asia has decreased the threshold for typhoid vaccination because infection, once acquired, may require inpatient parenteral therapy with ceftriaxone or a carbapenem, although high-dose oral azithromycin efficacy is generally effective. Ceftriaxone-resistant typhoid fever is currently restricted to Pakistan. Current typhoid vaccines do not protect against *Salmonella enterica* serovar Paratyphi, which is emerging in many areas.[28] Adherence to the oral vaccine regimen may be as low as 70%.[32]

Vaccines to Consider Only for Certain Destinations

Some vaccines are indicated solely because of a specific destination region regardless of whether the traveler has a specific risk behavior. Meningococcal and poliomyelitis vaccines are childhood vaccines that may require boosters in adult travelers with certain destinations.

Yellow Fever

Risk of Yellow Fever

The primary indication for YF vaccination is to prevent infection in individuals at risk. However, YF is currently the only vaccine that falls under the International Health Regulations that may necessitate vaccination purely for regulatory reasons.[33] Neither YF vaccine nor any other vaccine is currently required for readmission to the United States. In general, all healthy adult travelers to areas with a risk of YF transmission (see Fig. 318.1) should be vaccinated. This endemic area may be restricted to only a portion of a country. Because of rare but serious vaccine-associated adverse side effects (see Chapter 153), persons who are not at any risk of exposure should not be vaccinated.[34,35] Urban YF occasionally occurs in South America, and a number of urban areas are considered to have potential risk. Short-term travel that is restricted to very large urban areas in the endemic zone of South America carries negligible, if any, risk, but the situation is currently evolving in Brazil. Persons who have anything less than a definite, fixed itinerary and who will travel anywhere close to regions with risk of transmission should be vaccinated out of prudence.

Yellow Fever Entry Requirements

A number of African countries (Angola, Benin, Burkina Faso, Cameroon, Central African Republic, Congo, Côte d'Ivoire, Democratic Republic of Congo, Gabon, Ghana, Guinea-Bissau, Liberia, Mali, Niger, Rwanda, São Tomé, Sierra Leone, Togo) and one country in South America (French Guiana) require proof of YF vaccination from all arriving travelers. Other countries, both within and outside the risk zone, have submitted more complex requirements to the World Health Organization (WHO). They may require an official vaccination certificate only for individuals arriving directly from or via a country in the YF endemic zone, but not from arriving travelers from other countries. Such transits may include even an airplane connection in an affected country. These YF-free countries usually have the conditions and vectors to initiate a YF transmission cycle, and the purpose of the vaccine requirement is to prevent entry of viremic travelers. Current country-by-country YF entry requirements are available at https://www.who.int/ith/ith-country-list.pdf?ua=1. The requirement often applies even if the arriving traveler has not visited an area within a country of departure that is endemic for YF.

A special permit, obtainable in the United States from state health departments, is required to legally stamp an international certificate of vaccination as from an authorized YF vaccine center. The International Health Regulations enable clinicians who decide that YF vaccine is contraindicated on medical grounds to provide the traveler with a letter stating the reasons for that opinion, which can be presented to immigration authorities on arrival at the destination. Acceptance of such waiver letters is at the complete discretion of the destination country. On arrival, the receiving country may also quarantine the traveler for up to 6 days or request that the traveler be placed under surveillance.

No specific format exists for written documentation of medical contraindication. Letters of waiver are most appropriate for individuals needing a certificate purely for regulatory reasons. Waiver letters should be given with great reluctance for individuals with medical contraindications to vaccination (see Chapter 153) who plan to visit an endemic area. The variable risk within the endemic regions of the world needs to be considered (in consultation with an expert, if necessary), and cancellation of travel should be recommended strongly if the risk is more than negligible. A YF certificate becomes valid for entry 10 days after it is stamped and dated.

Yellow Fever Booster Doses

Until more recently, the YF vaccine was uniformly considered to provide protection for 10 years.[36] Currently the CDC recommends[36,37] that for healthy, nonpregnant adults, 10-year boosters should be given to all travelers planning a long stay in any area where there is a risk of YF transmission, to all travelers spending any amount of time in high-risk areas such as West Africa, and to all persons traveling to an area with a current outbreak. On the basis of an analysis by CDC experts showing that 92% of vaccine recipients have virus-neutralizing antibody at 10 years and 80% have the antibody at 20 years, the CDC has concluded that most healthy persons can be considered to have long-term immunity. For the purposes of the International Health Regulations, a single dose of YF vaccine at any time previously is sufficient for entry to any country.

Meningococcal Disease

Meningococcal vaccine is recommended for travelers to Africa's sub-Saharan "meningitis belt" (see Fig. 318.1) during the dry season, from December through June, especially if prolonged contact with the local

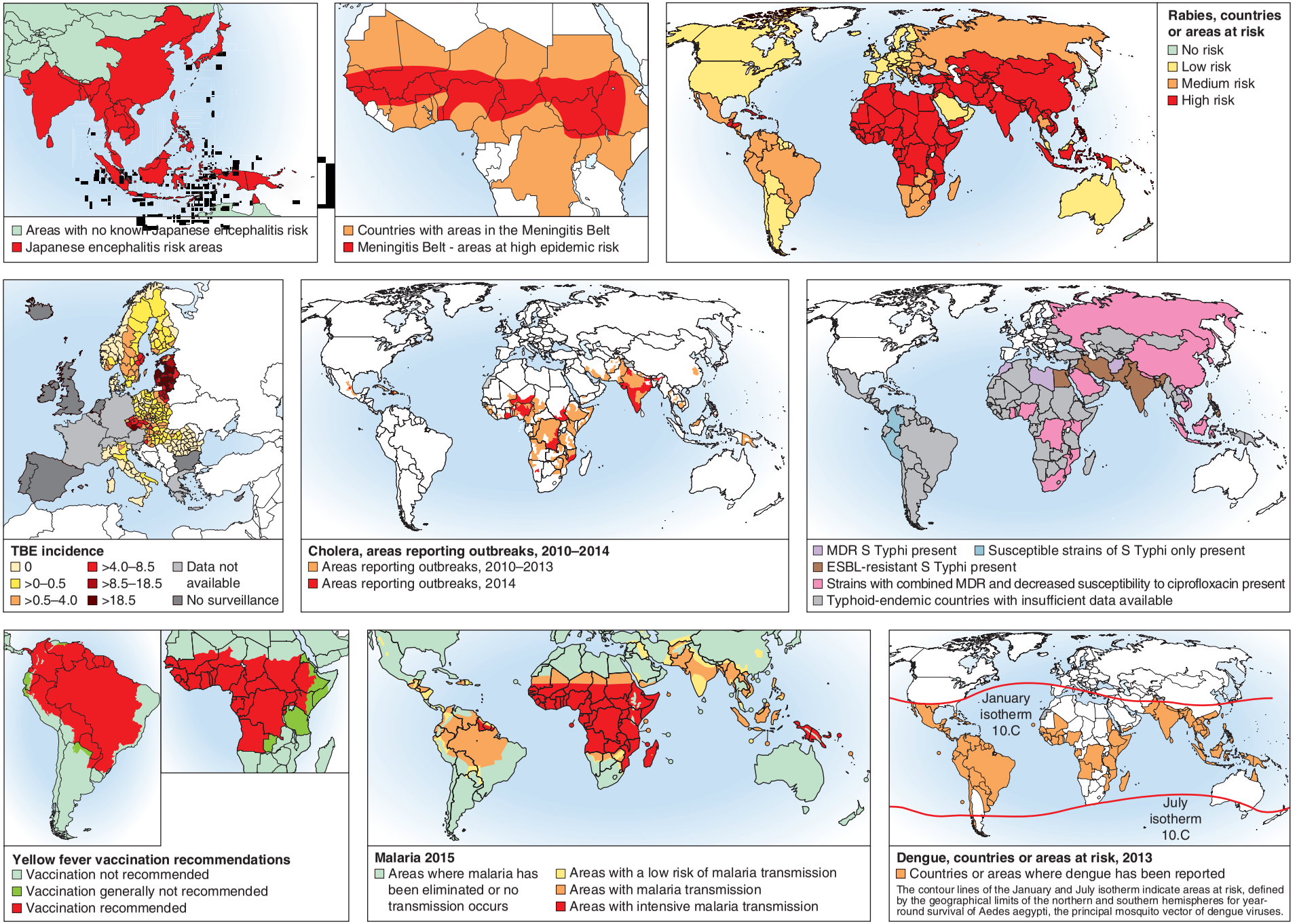

FIG. 318.1 Worldwide distribution of important travel-related diseases. *ESBL,* Extended-spectrum β-lactamase; *MDR,* multidrug-resistant; *TBE,* tick-borne encephalitis. *(Modified from references 94–102.)*

populace is likely. Out-of-season epidemics have occurred in Ethiopia, Somalia, and Tanzania, indicating possible changes in epidemiologic trends perhaps resulting from climate changes.[38] Muslims undertaking Hajj and Umrah pilgrimages in Saudi Arabia are at a higher risk of meningococcal disease, and proof of current vaccination with quadrivalent vaccine is required to obtain pilgrimage visas. Immunity to some serotypes in the tropics may wane in 3 years.[39,40] Meningococcal B vaccine is not indicated for travel.

Rabies

A preexposure rabies series is indicated for long-stay travel to endemic areas of Latin America, Asia, or Africa (see Fig. 318.1), where the rabies threat is constant and where access to adequate postexposure rabies immune globulin and vaccine is likely to be limited. Countries with the highest risk of rabies include the Indian subcontinent, Thailand, Vietnam, and most sub-Saharan African countries. For short-term travel, risk groups for whom immunization should be considered include adventure travelers, cyclists, hikers, cave explorers, or business travelers who travel for short but frequent trips and plan to go running outdoors on these trips. Regardless of vaccination status, travelers should be instructed to clean thoroughly with soapy water any bite or animal scratch involving broken skin immediately and to seek postexposure treatment for rabies (see Chapter 163). Recently published accelerated regimens for preexposure rabies vaccination are not yet widely accepted by public health authorities.

Japanese Encephalitis

Japanese encephalitis is endemic to many rural farming areas of Southeast Asia and the Indian subcontinent (see Fig. 318.1). Sporadic cases with severe sequelae continue to occur in travelers.[41] In temperate regions, the transmission season is April through November. In tropical or subtropical regions of Oceania and Southeast Asia, transmission may occur year-round. Vaccination is recommended for (1) long-stay travel to an endemic rural area; (2) expatriation to anywhere in an endemic country; (3) short-term travel to endemic rural areas with extensive unprotected outdoor exposure such as with adventure travel; or (4) short-term travel in the face of a current local epidemic.[42–47]

Polio

Because of eradication efforts, poliomyelitis remains endemic in only Afghanistan and Pakistan, but complete control remains elusive. Adults traveling to countries that are currently polio endemic (updated information at www.polioeradication.org) or have had recent cases of vaccine-derived polio and who have previously completed a primary vaccine series should receive a one-time single dose of inactivated polio vaccine as a booster if the last dose or booster dose was administered at least 10 years previously.

Cholera

Cholera vaccination is no longer required by any country, and the risk to typical travelers is insignificant.[48,49] However, aid workers, refugee workers, and health care workers exposed to displaced populations in areas where cholera is endemic or epidemic may consider cholera vaccination (see Fig. 318.1). A live-attenuated oral cholera vaccine was approved in 2016 by the US Food and Drug Administration for use in the United States; duration of immunity is unknown at the present time.[50] A highly effective oral, killed, whole-cell–B subunit vaccine is available widely outside the United States. This vaccine also has about 50% efficacy against enterotoxigenic *Escherichia coli* and 7% to 23% efficacy against all traveler's diarrhea (TD) and has this indication in some countries.

Tick-Borne Encephalitis

Tick-borne encephalitis is an emerging, important, and serious flavivirus central nervous system infection in endemic areas. Distribution is highly focal in a range that extends in a swath from Germany through Scandinavia and the Baltic to Siberia and Vladivostok in the East (see Fig. 318.1). Risk to travelers is low unless extensive outdoor activities are planned in forested regions in endemic areas.[51,52] Immunization against tick-borne encephalitis is recommended for adventure travel, extensive outdoor exposure, or camping in the forests of the endemic countries between April and October. Tick precautions are also recommended. The vaccine is available in most endemic countries but not in the United States or Canada.[30]

Spacing and Interactions of Travel-Related Vaccines

All currently indicated immunizations can and should be given at the same time and in any combination. If two live viral antigens are not administered on the same day, they must be spaced by a month. However, YF vaccine can be given at any interval with respect to single-antigen measles vaccine. Live oral vaccines (typhoid, polio) can be administered at any interval with respect to any live virus vaccine. Minimum intervals between vaccine doses must be respected, although 4 or fewer days before the next interval is acceptable.[53] Regimens that involve 1-week intervals (rabies, Japanese encephalitis, accelerated hepatitis) are exceptions. There is not a maximal interval between doses of a primary vaccine series; interrupted series except oral typhoid and rabies need not be restarted but can be resumed beginning with the dose that is overdue. Anaphylactic egg allergy precludes administration of YF and MMR vaccines. No current vaccine contains penicillin. Baseline purified protein derivative skin tests, often done in the pretravel setting, can be given on the day that live virus vaccines are administered or else must be done more than 4 weeks later. Antibacterial drugs should not be given within 24 hours of a dose of live oral typhoid or oral cholera vaccine.

MALARIA CHEMOPROPHYLAXIS

Epidemiology of Malaria in Travelers

An average of 1500 imported cases of malaria is reported annually in the United States. Estimates of risk in travelers not taking chemoprophylaxis vary widely by destination but range from 3.4% per month in West Africa to one-tenth that on the Indian subcontinent and a further 10-fold reduction in South America.[54–56] Most cases of imported malaria in the United States and Europe occur in noncitizen immigrants visiting friends and relatives abroad.[11,12] Malaria chemoprophylactic drugs are underused by these ethnic minority travelers. Of all imported falciparum malaria cases, 80% originate in Africa. A malaria vaccine designed for young children in Africa is not appropriate for use in nonimmune adult or pediatric travelers.

Risk of Travelers' Malaria

Risk of travelers' malaria may not be the same as the malaria risk in local populations. Travelers often operate in a more protected environment. Transmission, in particular high transmission, is quite focal even within the shaded areas in Fig. 318.1. In addition to the precise locations within the country or countries to be visited, the risk of travelers' malaria depends on the length of the trip, the season, whether the traveler adheres to precautions concerning mosquitoes, and whether the traveler spends the evening and nighttime hours where significant exposure to the vector may occur. Restriction of nighttime activities to air-conditioned hotels or other locations where there are few mosquitoes reduces the risk. With the exception of sub-Saharan Africa and certain cities in India, travel restricted to capital cities and other urban areas (as is typical of business travel) is associated with no risk or an insignificant risk of contracting malaria, despite the risk in areas nearby. The lifetime range of flight of an *Anopheles* mosquito is 1 km. Daytime side trips to malarial areas present no risk. At the same time, exposure to mosquitoes for even a few hours in an area with a high risk of transmission may result in infection. The decision regarding whether to prescribe chemoprophylaxis should also take into account the possibility of deviation from the preset itinerary brought to the pretravel consultation as well as the traveler's personal tolerance for what may be an epidemiologically insignificant level of risk for the specific trip.

Choice of Malaria Chemoprophylactic Drugs

Resources describing current country-specific malaria microepidemiology should be accessible immediately to clinicians prescribing malaria prophylaxis (see Table 318.2). Dosing and pharmaceutical properties of antimalarial drugs are described in Chapters 41 and 274. In practice, daily atovaquone-proguanil is preferable to doxycycline or mefloquine for short-term travel (<3 weeks) and is most widely prescribed.[1,57,58] Tafenoquine, approved in 2018, is equally effective and has weekly dosing, but it has the logistic constraints of requiring antecedent

gluose-6-phosphate dehydrogenase (G6PD) testing and explanation of a required loading dose regimen. Atovaquone-proguanil is associated with mild side effects and may be stopped just 7 days after the traveler has departed from an area of possible exposure. In most areas with malaria, atovaquone-proguanil, doxycycline, and mefloquine are equally effective (>95%) in preventing malaria, but disadvantages (e.g., more reports of adverse events in persons taking doxycycline or mefloquine as well as resistance to mefloquine) may hamper the use of the latter two drugs. Chemoprophylaxis may be started well before departure (3–4 weeks for mefloquine) if there is concern about possible side effects of any drug. Weekly administration of mefloquine or tafenoquine, if side effects are not an issue, is preferable for long-term travel because of lower cost and convenience. Use of tafenoquine is currently restricted to 6 months but may increase when data become available. Tafenoquine, with weekly dosing, will be most easily integrated into highly structured environments (e.g., multinational corporations, nongovernmental organizations, military, and diplomatic services) with substantial cohorts of regular travelers who can be tested once for G6PD. Chloroquine, an older drug that is also administered weekly, is highly effective in the few areas that are known to have exclusively chloroquine-sensitive parasites, but the other above-mentioned drugs are also highly effective in these areas.

Travelers should be reminded in writing to continue antimalarial drugs for the appropriate period after the last possible exposure, that malaria can still occur despite chemoprophylaxis, and that a malaria smear or malaria rapid diagnostic test is mandatory for any febrile illness occurring within 3 months after travel. Travelers to areas where false-positive tests for malaria are common in clinical practice (e.g., Africa) should be reminded to continue taking the prophylactic drug even if they receive a diagnosis of malaria.

Adverse Effects of Antimalarial Drugs

Approximately 5% of individuals who take either mefloquine or doxycycline discontinue therapy because of side effects. Chemoprophylaxis may be started well before departure (3–4 weeks for mefloquine) in individuals concerned about possible intolerance to any drug. Mefloquine has been associated with neuropsychiatric side effects[59–61] in some and should not be prescribed for persons with active depression or a recent history of depression, generalized anxiety disorder, psychosis, schizophrenia, or other major psychiatric disorder. It should be used with caution in patients with a previous history of depression. If prodromal psychiatric symptoms occur during use, the drug should be discontinued and an alternative medication substituted. Doxycycline is an esophageal and gastric irritant. It needs to be taken with a full glass of water on a full stomach, and the user should not go to sleep or lie down for 30 minutes after ingestion. Female travelers may get vaginal candidiasis and should carry self-therapy for candidiasis when prescribed doxycycline. Severe adverse events with tafenoquine are rare unless hemolysis occurs if erroneously given to an individual with G6PD deficiency. At present, the cumulative safety database for tafenoquine is limited to approximately 3000 subjects. Benign corneal deposits (vortex keratopathy), not affecting visual acuity, were seen in 21% of subjects and resolved spontaneously within 12 weeks in 95% of affected subjects and within 48 weeks in 100% of subjects with ≤6 months use of tafenoquine during clinical trials.

Prevention of *Plasmodium vivax* and *Plasmodium ovale* Relapses

Unless tafenoquine is used for primary prophylaxis during the trip, none of the primary prophylactic drugs discussed earlier is effective against the dormant hepatic hypnozoites of *Plasmodium vivax* or *Plasmodium ovale,* which may cause delayed relapses of malaria. Presumptive antirelapse therapy refers to a regimen at the end of the exposure period to kill residual hypnozoites of these two species. Per CDC guidelines, primaquine, 30 mg base per day for 14 days, or tafenoquine, 300 mg PO in a single dose, after checking G6PD levels, is indicated only for individuals with prolonged and extensive exposure to *P. vivax* or *P. ovale.* This effectively excludes most short-term travelers. Malaria chemoprophylaxis recommendations are likely to change periodically. Physicians may check the CDC or WHO travel websites (see Table 318.2).

Chloroquine-resistant *P. vivax* occurs only in areas in which the other drugs are already indicated for prophylaxis because of the concomitant presence of resistant *P. falciparum.*

Standby Therapy Instead of Continuous Prophylaxis

For stays in areas with very low transmission rates of malaria, some physicians, notably in Europe, may advise that only a standby drug be carried, which should be taken in the event that symptoms suggestive of malaria occur and there is no access to a physician or facility that can perform a competent malaria smear within 6 to 12 hours. This strategy is especially attractive for long-stay travelers. In areas with chloroquine-resistant *P. falciparum,* atovaquone-proguanil, four 250/100 mg tablets orally as a single daily dose for 3 consecutive days, or artemether-lumefantrine, four 20/120 mg tablets twice a day for 3 consecutive days, is recommended. In areas without chloroquine-resistant *P. falciparum,* chloroquine is the drug of choice. In the United States, the CDC recommends continuous prophylaxis, as already noted, for travelers at risk but also suggests that treatment doses of standby drugs may be carried for the treatment of confirmed malaria in areas where appropriate drugs for treatment may be unavailable or where there is concern about substandard or counterfeit medication. Prevention of malaria in travelers residing in malarial areas for 6 months or more presents complex problems that have been reviewed elsewhere.[62]

DENGUE

An estimated 100 million cases of dengue fever (DF) and 250,000 cases of dengue hemorrhagic fever occur annually (see Chapter 153). The past 20 years have seen a dramatic geographic expansion of epidemic DF and dengue hemorrhagic fever (see Fig. 318.1). Dengue accounts for up to 2% of cases of illness in returned travelers who visit GeoSentinel clinics, and dengue is the most common systemic febrile illness in returned travelers from every region except sub-Saharan Africa, where malaria still predominates. Prospective seroconversion studies have estimated the attack rate of DF in travelers to the tropics to be 2.9% in Dutch travelers who spent 1 month in Asia; the seroconversion rate was 6.7% among Israelis who traveled for an average of 5 months. DF in returned travelers demonstrates seasonality in many parts of the world, with region-specific peaks for Southeast Asia (June, September), South Central Asia (October), South America (March), and the Caribbean (August, October).[63] A live, attenuated, quadrivalent dengue vaccine (Dengvaxia) is licensed in a few dengue endemic countries, but approval by the WHO is restricted to persons 9 to 45 years of age with negative serology for prior dengue infection. Use in travelers is not recommended by any authority. Other vaccines with potentially better safety profiles are in the advanced stages of development. DF is transmitted by day-biting *Aedes* mosquitoes, reinforcing the need to instruct travelers to the tropics in the need for both day and night use of repellents.

Chikungunya and Zika Virus

Large areas of the world including the Caribbean and Asia are endemic for the mosquito-borne infections chikungunya and Zika virus, which cause fever, rash, headache, conjunctivitis, and myalgias (see Chapters 151 and 153) and which may resemble dengue in many patients. Chikungunya can cause severe arthralgia that persists for months.[64] Zika virus can also cause arthralgia and has been associated with Guillain-Barré syndrome and microcephaly in the fetuses of infected women.[65] Sexual transmission of Zika virus from infected men to women has been documented in about 100 cases. The period of risk for sexual transmission appears usually not to exceed 1 to 2 months after symptom onset. There are no commercially available vaccines or treatment for any of these two viral diseases. Clothing and mosquito repellants (see later) may decrease exposure to the urban, day-biting mosquitoes that can transmit all three diseases.

TRAVELER'S DIARRHEA

Risk and Etiology

TD is the most common affliction of travelers to developing countries. TD is defined as severe when it is bloody or when it interferes with all daily activities and as moderate when it interferes with some daily activities.[66] Older classifications that correlated TD severity with the number of unformed stools per day have proven less useful in defining clinical approaches.[67] The incidence varies from about 8% for travel to highly developed countries to about 20% in southern Europe, Israel, Japan, South Africa, and some Caribbean islands. In most developing countries, the risk is 20% to 66% in the first 2 weeks abroad and then

less thereafter. The most frequent cause of TD is enterotoxigenic *E. coli.* In some locations, enteroaggregative *E. coli. Salmonella, Shigella,* and *Campylobacter* each account for about 5% to 15%, and in Asia, noncholera vibrios are significant. Protozoa account for less than 5%, and in adults norovirus or rotavirus rarely may be detected. Norovirus outbreaks may occur aboard cruise ships. The mean duration of TD, even if untreated, is 4 days. A number of limited studies indicate that the incidence of postinfective irritable bowel syndrome at 6 months after an acute episode of TD may range from 3% to 17%. The true incidence of this syndrome has not been determined, and ancillary contributing factors and possible preemptive interventions are still being investigated.[68]

Prevention

Antibiotic prophylaxis for TD with a quinolone or with rifaximin has been demonstrated to be effective in many settings. Most guidelines do not recommend prophylaxis except in rare circumstances for the typical traveler because of potential adverse drug effects while away from medical care and because effective rapid-onset therapy is available for diarrhea should it occur.[66,69] However, chemoprophylaxis can be considered for travelers with advanced human immunodeficiency virus (HIV) infection and other severe immunocompromise.

The risk of TD can be reduced, but not eliminated, by educating the traveler to avoid dietary indiscretions. Nevertheless, increasing evidence suggests surprisingly small differences in the incidence of TD in individuals who self-report meticulous rather than adventurous eating habits. Hand hygiene is particularly important if one family member develops vomiting or diarrhea. Carrying small containers of alcohol-based hand sanitizers (containing ≥60% alcohol) may make it easier for travelers to clean their hands before eating.

Self-Treatment

All travelers to the developing world should be thoroughly educated in self-therapy for diarrheal disease and carry the appropriate agents while traveling. Standard self-treatment for TD consists of oral hydration together with an antimotility medication (usually loperamide), an antisecretory medication, or both for symptomatic relief. The addition of a single dose of azithromycin (500–1000 mg) is indicated for TD defined as severe, and either azithromycin or a quinolone (750 mg of ciprofloxacin or 500 mg of levofloxacin) should be considered for moderate TD. Single-dose therapy is almost always adequate in most travelers, even with severe diarrhea.[66,69–71] Travelers can be prescribed enough medication for a 3-day course and be instructed to complete 3 days of therapy if disabling symptoms are still present after 24 hours. Dysentery should always be treated for 3 days. Quinolones should be avoided for TD acquired in India and Southeast Asia or if quinolone resistance is suspected. Azithromycin is the preferred self-treatment antibiotic for inclusion in a travel kit because a traveler who must fill a prescription for travel-related diarrhea before the trip does not know whether the diarrhea will be severe. Rifaximin 200 mg three times a day for 3 days can be used for TD caused by *E. coli* in adults and can be carried to *E. coli*–predominant areas but is not recommended when the patient has fever or blood in the stool.

Antibiotic use for TD has been associated with intestinal colonization with antibiotic-resistant bacteria in returning travelers,[72,73] but the use of loperamide alone has not.[74] In South Asia, studies have shown that 80% of travelers with TD who were treated with antimicrobial agents acquired extended-spectrum β-lactamase–producing Enterobacteriaceae, and in one study, 12% of carriers were still excreting the organisms 12 months after their return. The potential for spread is of concern, although the public health implications are still unclear.

KEY PREVENTIVE BEHAVIORS

Most travel-related health problems including many infectious diseases can be significantly reduced through appropriate behavior by the traveler.

Personal Protection Against Arthropods

Antimalarial chemoprophylactic drugs are less than 100% effective. Protection against arthropods will help prevent dengue, leishmaniasis, filariasis, and a number of important arboviral diseases. Travelers should be instructed to clothe themselves to reduce as much exposed skin as practicable and to apply a repellent containing *N,N*-diethyl-meta-toluamide (DEET) (concentration 30%–35%) to all exposed, nonsensitive areas of the body every 4 to 6 hours.[74–77] More frequent application is required for agents containing lower concentrations of DEET. Agents containing 20% or higher concentrations of picaridin (KBR 3023) are similar to agents containing DEET at the same concentration with regard to activity against *Anopheles* mosquitoes. Travelers should sleep under a permethrin-impregnated bed net in malarial areas unless they are in a sealed air-conditioned environment. When risk factors are especially high, travelers should treat outer clothing with permethrin. Although anopheline mosquitoes are night biters, *Aedes* spp. and culicine mosquitoes are usually day biters, so vigilance at all times of day is necessary.

Protection Against Foodborne Disease

Travelers to developing countries should be diligent in washing their hands frequently; avoiding food from dubious eating places, markets, and roadside vendors; avoiding buffets where there are no food covers or fly controls; avoiding high-risk foods such as shellfish, reef fish (ciguatera risk), undercooked meats and poultry, dairy products, unpeeled fruits, cold sauces, and salads; avoiding both tap water and drinks or ice made from tap water; and using sealed bottled water or chemically treated, filtered, or boiled water for drinking and brushing their teeth.

Sex

Education on the incidence of HIV and sexually transmitted diseases among professional sex workers abroad, on the usage of condoms, and on the failure rate of condoms (3%–5% breakage/slippage) should be given regardless of the apparent circumstances of the traveler. Unprotected sex even with fellow travelers is considered high risk. Travel is a disinhibiting experience in itself, and alcohol consumption tends to increase during travel. On average, 20% of all travelers report a new sexual contact during their last trip abroad.[78,79] Condom use during casual travel sex is uniformly less than 25%, even in individuals who had received pretravel counseling. Discussion of emergency contraception strategies for female travelers is sometimes appropriate. Specific data on the short-term use of Truvada for PrEP (emtricitabine 200 mg + tenofovir disoproxil fumerate 300 mg) 1 tablet per day for travelers anticipating multiple new partners during the trip is not yet available but is advocated by many experts as being compatible with current guidelines for similar behaviors in individuals staying at home.

Protection Against Bloodborne Disease

Blood, blood products, syringes, and contaminated medical or dental instruments are a risk after accidents or trauma. Travelers should consider carrying an infusion set, needles, and a suture kit for high-risk areas. If possible, they should defer medical treatment and travel to a facility where safety can be ensured. Tattooing, acupuncture, and body piercing carry similar risks. Health care workers and others at risk in high HIV prevalence areas without sophisticated medical infrastructure may consider carrying a 1- to 2-week supply of Descovy (emtricitabine 200 mg + tenofovir alafenamide fumerate 25 mg) 1 tablet per day *plus* raltegravir 400 mg 1 tablet twice per day (or may use dolutegravir 50 mg 1 tablet per day plus Descovy) to begin immediate once-daily postexposure prophylaxis, with the understanding that this is only an initial measure to allow time for travel to an adequate medical facility able to provide sophisticated testing and counseling.

Protection Against Skin Diseases

Infected mosquito bites are common. Practicing good hand hygiene in dirty environments and covering open wounds are preventive measures that all travelers should take. Scabies and lice infestations can be prevented by performing good personal hygiene. In Africa, all clothes dried outdoors should be ironed to avoid cutaneous myiasis caused by the tumbu fly. Hats and sunscreen are mandatory in the tropics. Sunscreen should always be applied to skin before, and not after, an application of DEET.[80,81]

Protection Against Pathogens Encountered by Swimming and Water Exposure

Travelers should be instructed to avoid recreational (swimming, rafting, wading) or other exposure to fresh water in areas that are endemic for

schistosomiasis. Hikers, cyclists, and adventure travelers should consider prophylaxis with 200 mg of doxycycline once per week because of the significant risk of leptospirosis that exists in fresh water throughout the developing world. Walking barefoot in tropical areas predisposes to hookworm, *Strongyloides* infection, cutaneous larva migrans, and tungiasis.

Prevention of Tuberculosis

A predeparture baseline tuberculin skin test or in vitro interferon assay prior to departure and then 10 weeks after return is indicated for all long-stay travelers to extremely high-risk countries and all health care workers to any country.[82] Specific tuberculosis exposures should follow the same protocol as for nontravelers. Aggressive treatment of skin test converters will prevent cases of active tuberculosis later. Travelers should avoid crowded public transportation or crowded public places and distance themselves immediately from anyone with a chronic or heavy cough. Expatriates should screen domestic help for tuberculosis.

NONINFECTIOUS TRAVEL PROBLEMS

Medical Kit and Medical Care Abroad

Travelers should carry a compact medical kit. In addition to items mentioned elsewhere in this chapter, simple first-aid supplies such as bandages (including butterfly bandages), gauze, hemostatic gauze, skin glue, antiseptic, antibiotic ointment, and splinter forceps will allow early self-treatment of minor wounds before infection ensues. A thermometer to document elevations in temperature should be carried along with antipyretics. Antifungal creams, cough and cold remedies, antacids, hydrocortisone cream, and blister pads should be considered. The contact details of hometown medical providers should be recorded and accessible at all times. Long-stay travelers should familiarize themselves with the local expatriate medical infrastructure immediately after arrival so as to be able to seek competent care rapidly for any ensuing illness early in its course. Adequate medical and evacuation insurance should be arranged. A copy of a recent electrocardiogram should be carried by all patients with cardiac disease on a portable USB drive or be accessible via the Internet.

Air Travel–Related Morbidity

Deep Venous Thrombosis/ Pulmonary Embolism

A causal relationship between travel-related immobility and deep venous thrombosis/pulmonary embolism in otherwise healthy travelers has been established. Risk of pulmonary embolus is essentially zero on flights lasting less than 6 hours and rises to 5 per 1 million on flights longer than 10 hours. Risk of a deep venous thrombosis, most often asymptomatic, is approximately 1:5000 for flights longer than 4 hours, but the data do not distinguish individuals with risk factors.[83,84] Individuals with previously covert coagulopathies may manifest for the first time during travel. Persons with clear, known risk factors are at highest risk. These include a personal or family history of deep venous thrombosis or pulmonary embolism; a personal or family history of a known blood clotting disorder predisposing to thrombosis (thrombophilia); major surgery, significant trauma, or prolonged immobilization (includes limb casts) in the previous 6 weeks; malignancy; late pregnancy; estrogen therapy including oral contraceptives; age older than 50 years; severe obesity; and being very tall or very short. All travelers should avoid dehydration, avoid alcohol, and exercise the legs regularly in flight. Recommendations for prevention are graded and cautious because of the paucity of studies.[85] Only the use of graded 15- to 30-mm Hg compression stockings, which reduces risk of asymptomatic deep venous thrombosis, for individuals at higher risk is supported in trials. Prophylactic subcutaneous low-molecular-weight heparin given just before departure and again 24 hours later for individuals with thrombophilia or previous thrombotic events is often used in practice, although no definitive studies showing benefit from this practice have been published. Aspirin therapy is of no proven benefit in this setting. Aisle seating promotes mobilization; no intrinsic benefit of premium-class seating has been shown.

Jet Lag, Motion Sickness, and In-Flight Medical Emergencies

Jet lag occurs after crossing three or more time zones, and zolpidem taken for a few nights at bedtime at the destination is effective.[86] Travelers prone to motion sickness should sit in the center of airplanes, boats, or other conveyances and visually fix on distant objects. Meclizine or scopolamine is beneficial. Most in-flight medical emergencies are related to underlying illnesses and are difficult to predict at the time of the pretravel consultation. To avoid decompression sickness, established waiting periods for flying after diving have been determined.[86]

Altitude

Common high-altitude destinations for leisure travel include La Paz, Bolivia; Cuzco, Peru; Lake Titicaca, on the border of Bolivia and Peru; Quito, Ecuador; Lhasa, Tibet; and Mount Kilimanjaro, Tanzania. Whether ascending by car or airplane, acute mountain sickness occurs in at least 25% of people who ascend rapidly to 2500 m or higher and in most people who go quickly to 2800 m or higher. Gradual ascent over days is rarely practiced by modern travelers. For prevention of altitude illness, acetazolamide, 125 mg twice a day beginning the morning of the day before ascent and continuing through the day after ascent, is effective. If symptoms of mountain sickness such as nausea, vomiting, anorexia, light-headedness, fatigue, or insomnia persist beyond the day after ascent, travelers may continue to take one tablet each evening. Severe complications such as pulmonary or cerebral edema occur uncommonly at lower than 3500 m and are best treated by oxygen and immediate descent. People traveling at higher than 3500 m for longer than a brief transit of a few hours should consult an expert.[86–90]

TRAVELING ADULTS WITH SPECIAL NEEDS

Immunocompromised Travelers and Human Immunodeficiency Virus–Infected Travelers

Immunocompromised travelers are at risk for a complication or exacerbation of the underlying disease in a medically unfamiliar or underserved destination. Potential travelers need to understand the risks of the trip in the context of their personal situation. Precautions for immunocompromised travelers against malaria (including chemoprophylaxis), foodborne and waterborne diseases, and vector-borne diseases generally do not differ from precautions for healthy travelers. Live vaccines (YF, varicella, measles, oral typhoid, oral polio, oral cholera) should be avoided by individuals who are truly immunocompromised, even if the result is deferral of the trip. Other vaccines, although safe, likely will have suboptimal efficacy in immunocompromised hosts, and the implications of this need to be discussed with the traveler.[53,91,92]

HIV-infected individuals with adequate immune function ($CD4^+$ counts 200/mm^3 or greater) and travelers using 20 mg prednisone or less daily for greater than 2 weeks are not at risk of infection and can be immunized as normal hosts. Individuals on short courses (<2 weeks) of high-dose steroids and steroid inhalers and those who have received intraarticular steroids can be treated as normal hosts. However, asymptomatic HIV-infected individuals, even individuals with adequate immune function, should be given a waiver letter if the only reason for YF vaccination is to fulfill regulatory requirements. In individuals discontinuing truly immunosuppressive drugs including tumor necrosis factor-α–inhibiting agents, a 1-month (for etanercept) to 3-month (other agents) waiting period before administration of live vaccines is advisable. Individuals with hematologic malignancies in remission should wait 3 months after their last chemotherapy before receiving live virus vaccines, and individuals receiving lymphocyte-depleting therapy such as with rituximab or anti-CD52 should wait at least 6 months, although many experts prefer to wait 1 year. Individuals with low-grade immune defects such as chronic renal, hepatic, or endocrine disease should be counseled regarding increased risks of various infections, but no changes in pretravel immunizations are generally indicated. Functionally hyposplenic individuals should be vaccinated against pneumococci, meningococci (both meningococcal ACWY and B vaccines), *Haemophilus influenzae* type b, and influenza, if they have not been already. Elderly adults may have suboptimal responses to many travel-related vaccines.

Pregnant Travelers

Pregnancy presents several complex issues for travel, especially with regard to many commonly used travel medications. Determination

of direct maternal-fetal–related contraindications to travel should be handled by the obstetrician. Travel is best undertaken in the second trimester. In general, pregnant travelers should not be given live vaccines.[93] YF vaccine should be given only when the risk of contracting the disease is substantial and travel cannot be discouraged by the physician. Even killed vaccines are best delayed until the second trimester, if possible. An exception may be for the influenza vaccine, considering that pregnancy increases the risk of complications of influenza. Hepatitis A and B, tetanus, and meningococcal vaccines can be given without reservation. There is no vaccine for hepatitis E, one of the most common foodborne causes of viral hepatitis overseas (see Chapter 178). Infection with hepatitis E has been reported to be fatal in 38% of pregnant women. Precautions about hygiene, cleanliness, and safe food and water practices are particularly important in a pregnant traveler. Other killed vaccines such as rabies, Japanese encephalitis, typhoid, *H. influenzae* type b, and pneumococcus should be given on a case-by-case basis when risk is high. In general, pregnant women should not travel to a malarial area unless the travel is absolutely necessary. Chloroquine is safe if travel is to one of the few countries where it is still the drug of choice. For other countries, mefloquine is recommended and is now in US Food and Drug Administration category B, but extensive studies on its safe use during pregnancy, especially during the first trimester, have not been done. Doxycycline, atovaquone-proguanil, and tafenoquine should not be used during pregnancy. Azithromycin is the treatment of choice for TD in pregnancy. Skiing, scuba diving, water skiing, and high-altitude travel are not advisable during pregnancy. No vaccine affects the safety of breastfeeding. Mefloquine and chloroquine are safe during breastfeeding. Insufficient data are available for the use of doxycycline, atovaquone-proguanil, or primaquine. Used sparingly, 20% DEET is safe in pregnancy.

Concern about possible adverse effects of Zika virus infection on the fetus has led to increased caution about mosquito protection during pregnancy while traveling in endemic areas. Insect repellents containing ingredients such as DEET, picaridin, and IR3535 are safe for use during pregnancy when used in accordance with the product label. Testing is not recommended for nonpregnant asymptomatic individuals including recent travelers to Zika areas or as part of preconception screening. The accuracy of immunoglobulin M testing in asymptomatic individuals with Zika virus infection is still unknown. Immunoglobulin M tests can produce false-positive results in many settings.

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The complete reference list is available online at Expert Consult.

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319 Infections in Returning Travelers

Andrea K. Boggild and David O. Freedman

SHORT VIEW SUMMARY

MAJOR SYNDROMES IN RETURNED TRAVELERS

- Fever, diarrhea (acute or persistent), skin problems, eosinophilia

TROPICAL DISEASES WITH POSITIVE PERIPHERAL BLOOD FILMS

- Malaria, babesiosis, filariasis, African trypanosomiasis, American trypanosomiasis, relapsing fever, bartonellosis

INCUBATION PERIODS OF TROPICAL DISEASES

- *Short (<10 days):* arboviral infections including dengue, chikungunya, Zika, and yellow fever; hemorrhagic fevers; respiratory virus infections; typhoid; infection with gastrointestinal pathogens; spotted fever rickettsioses; relapsing fever; leptospirosis
- *Intermediate (10–20 days):* malaria, hemorrhagic fever, Q fever, typhus, typhoid, brucellosis, African trypanosomiasis, rabies, tick-borne encephalitis, Japanese encephalitis
- *Prolonged (>21 days):* viral hepatitis, malaria, rabies, tuberculosis, schistosomiasis, filariasis, amebic liver abscess, Q-fever, Epstein-Barr virus infection

EVALUATION OF SIGNIFICANT TROPICAL FEVER

- *Any hemorrhagic manifestations?* If viral hemorrhagic fever, including Lassa fever or Ebola, is possible, isolate and call public health authorities; consider meningococcemia, rickettsiosis, sepsis, dengue, and leptospirosis.
- *Is malaria possible?* If there is end-organ damage, initiate empirical therapy.
- Use a "rule out malaria protocol," and use empirical therapy if no local expertise is available.
- *Are there localizing findings?* Go to a syndromic approach and differential diagnosis.
- *Are there no localizing findings?* Consider typhoid, dengue, rickettsiosis, human immunodeficiency virus infection, leptospirosis, schistosomiasis (eosinophilia), and amebic disease.
- Consult Table 319.1 for constellations of exposures and clinical presentations suggestive of particular diagnoses in returned travelers.
- Eosinophilia is caused by tissue-invasive helminths and is proportional to degree of tissue invasion.

Of the approximately 80 million people who travel from industrialized to developing countries each year, 22% to 64% of travelers report some illness.[1,2] The approach to the patient requires knowledge of world geography, the epidemiology of disease patterns in 230 or so countries, and the clinical presentation of a wide spectrum of disorders.[3] Most illnesses are mild, most are self-limited, and many are noninfectious. Up to 10% of travelers may consult a physician during or after a trip, and approximately 1 in 100,000 travelers will die.

The ill travelers that do come to the attention of infectious diseases clinicians are generally either the most seriously ill or are suspected of harboring infectious agents not familiar in their home country. Based on 42,173 ill returned travelers seen by the GeoSentinel Surveillance Network at 53 different clinical sites on six continents, in patients presenting to infectious or tropical diseases specialists after travel to the developing world, specific travel destinations are associated with the probability of the diagnosis of certain diseases.[4] Diagnostic approaches and empirical therapies can be guided by these destination-specific differences. Important region-specific disease occurrence data indicate that febrile illness is most important from Africa and Southeast Asia; malaria is one of the top three diagnoses from every region, yet over the past decade dengue has become the most common febrile illness from every region outside sub-Saharan Africa; in sub-Saharan Africa, rickettsial disease is second only to malaria as a cause of fever; respiratory disease is most important in Southeast Asia and sub-Saharan Africa; and acute diarrhea is disproportionately from South Central Asia. When individual diagnoses are collected into syndrome groups and examined for all regions together, 233 of every 1000 ill returned travelers have a systemic febrile illness, 334 have acute or chronic gastrointestinal (GI) infection, 195 have a dermatologic disorder, and 209 have a respiratory disorder. Recent data have also been evaluated in the context of large international events, attendance at which provides an additional common epidemiologic link by which to assess the risk of communicable diseases.[5] Travel illness associated with attendance in Brazil for the 2014 FIFA World Cup, characterized by dermatologic conditions (40%), diarrheal syndromes (25%), and febrile systemic illnesses (dengue and malaria, predominantly caused by *Plasmodium vivax*), might be expected to anticipate illnesses associated with the 2016 summer Olympic games in Brazil.[6] Considerations specific to the Hajj would include the ingestion of camel's milk, crowding, and the spread of viral and bacterial respiratory pathogens, including Middle East respiratory syndrome–coronavirus (MERS-CoV) and drug-resistant enteric pathogens. These infections would include third-generation cephalosporin- and colistin-resistant *Salmonella enterica* ssp. *enterica* serovar Newport.[7–9]

Travelers who become ill during, or any time up to several months after, a foreign trip will frequently associate that illness with a possible travel-specific cause. This may be the case, but often it is not. Routine disorders are common, and common disorders are common whether actually acquired during travel or at some time after the trip. Thus fever, sore throat, and cervical adenopathy in a college student who returned 2 weeks earlier from a developing country is still more likely to be streptococcal pharyngitis or infectious mononucleosis than diphtheria. Presented with an ill patient with a history of travel, the physician must maintain discipline in making two separate lists of differential diagnoses, the first in the context of travel, and the second considering the same presenting symptoms and signs as if in any other patient. The approach and workup must then proceed in parallel, with appropriate priority given to the most urgent or the most treatable diagnoses at the top of each list. A consideration of increasing importance is the acquisition of drug-resistant organisms in the normal bowel flora. The contribution of antimicrobial therapy and antimotility agents may be important in this regard.[9]

In this chapter *travelers* are considered to be those returning from short visits to developing countries, and the term does not include immigrants, refugees, and very long-term residents arriving from those countries. Constellations of exposures and clinical presentations highly suggestive of particular diagnoses in returned travelers are shown in Table 319.1. Highly exotic endemic diseases rarely acquired by travelers are not discussed. Low-frequency illnesses (<20 cases of the 42,173

TABLE 319.1 Constellations of Exposures and Clinical Presentations Suggestive of Particular Diagnoses in Returned Travelers

EXPOSURE SCENARIO	DISTINCTIVE FINDINGS	DIAGNOSIS
Any exposure in any area with documented malaria transmission	Fever with or without any other finding	Malaria
Most tropical countries	Fever and altered mental status	Malaria, meningococcal meningitis, rabies, West Nile virus
Budget travel to India, Nepal, Pakistan, or Bangladesh	Insidious-onset, high unremitting fever, toxic patient, paucity of physical findings	Enteric fever due to *Salmonella* Typhi or *Salmonella* Paratyphi
Freshwater recreational exposure in Africa	Fever, eosinophilia, hepatomegaly, negative malaria smear	Acute schistosomiasis (Katayama fever)
Bitten by *Aedes aegypti* in Central America, Southeast Asia, or the South Pacific	Fever, headache, myalgia, diffuse macular rash, mild-to-moderate thrombocytopenia	Dengue, chikungunya fever, Zika fever
Bitten by *A. aegypti* or *Aedes albopictus* in India, Malaysia, Singapore, the Caribbean, or an island in the Indian Ocean	Fever, headache, myalgia, diffuse macular rash, arthralgia, tenosynovitis often followed by chronic polyarthritis after the fever resolves	Chikungunya fever, Zika fever
Hunting or visiting game reserves in southern Africa	Fever, eschar, diffuse petechial rash	African tick typhus due to *Rickettsia africae*
Travel to Southeast Asia	Fever, eschar, diffuse petechial rash	Scrub typhus due to *Orientia tsutsugamushi*
Hiking, biking, swimming, rafting with exposure to fresh surface water	Fever, myalgia, conjunctival suffusion, mild to severe jaundice, variable rash	Leptospirosis
Cruise, elderly traveler	Influenza-like illness	Influenza A or B
Outdoor exposure anywhere in the Americas	Large, single furuncular lesion anywhere on body, with sense of movement inside	Myiasis due to *Dermatobia hominis* (botfly)
Clothing or towels washed or dried out of doors in Africa	Multiple furuncular lesions around clothing contact points with skin	Myiasis due to *Cordylobia anthropophaga* (tumbu fly)
New sexual partner during travel	Fever, rash, mononucleosis-like illness	Acute human immunodeficiency virus infection, secondary syphilis, primary HSV-1 infection
Travel to any developing country or to Western Europe	Coryza, conjunctivitis, Koplik spots, rash	Measles
Longer visit to humid areas of Africa, the Americas, or Southeast Asia	Asymptomatic eosinophilia or with periodic cough or wheezing	Strongyloidiasis
Sand fly bite in either New or Old World tropical area	Painless skin ulcer with clean, moist base in exposed area	Cutaneous leishmaniasis
Resort hotel in southern Europe, ± exposure to whirlpool spas	Pneumonia	Legionnaires' disease
Explored a cave in the Americas	Fever, cough, retrosternal chest pain, hilar adenopathy	Histoplasmosis
Ingestion of unpasteurized goat cheese	Chronic fever, fatigue	*Brucella melitensis*
Long trip to West/Central Africa	Afebrile, intensely pruritic, evanescent truncal maculopapular rash	Onchocerciasis
Long trip to West/Central Africa	Migratory localized angioedema or swellings over large joints, eosinophilia	Loiasis
Safari to game parks of East Africa	Fever, nongenital chancre, fine macular rash	East African trypanosomiasis
Travel to Australia	Fever, fatigue, polyarthritis	Ross River virus
Farming areas of India and Southeast Asia	Fever, altered mental status, paralysis	Japanese encephalitis
Forested areas of central and eastern Europe and across Russia	Fever, altered mental status, paralysis	Tick-borne encephalitis
Rodent exposure in West Africa	Fever, sore throat, jaundice, hemorrhagic manifestations	Lassa fever
Ingestion of sushi, ceviche, or raw freshwater fish	Migratory nodules in truncal areas with overlying erythema or mild hemorrhage	Gnathostomiasis
Returning Hajj pilgrim or family contact	Fever, meningitis	Meningococcal meningitis
Ingestion of snails, fish, or shellfish in Asia or Australia	Eosinophilic meningitis	Angiostrongyliasis, gnathostomiasis
Diabetic or compromised host with exposure to moist terrain in Asia or Australia	Fever, sepsis, pneumonia or multifocal abscesses	Melioidosis
Summertime exposure to rodent droppings in Scandinavia	Fever with decreased renal function	Puumala virus
Ingestion of undercooked meat of any animal in any country	Fever, facial edema, myositis, increased creatine phosphokinase, massive eosinophilia, normal erythrocyte sedimentation rate	Trichinosis
Unvaccinated, returning from sub-Saharan Africa, forested areas of Amazonia, states of Minas Gerais, Rio de Janeiro, or Sao Paulo Brazil	Fever, jaundice, proteinuria, hemorrhage	Yellow fever

Continued

TABLE 319.1 Constellations of Exposures and Clinical Presentations Suggestive of Particular Diagnoses in Returned Travelers[a]—cont'd

EXPOSURE SCENARIO	DISTINCTIVE FINDINGS	DIAGNOSIS
Exposure to farm animals	Pneumonia, mild hepatitis	Q fever
Possible tick exposure	Fever, headache, rash, conjunctival injection, hepatosplenomegaly (± hemorrhagic manifestations)	Tick-borne relapsing fever; Crimean-Congo hemorrhagic fever if risk area was central Asia
Poor hygienic conditions with possible body louse exposure in Ethiopia or Sudan	Fever, headache, rash, conjunctival injection, hepatosplenomegaly	Louse-borne relapsing fever
Madagascar	Fever, rapidly progressive pneumonia	Pneumonic plague (*Yersinia pestis*)

[a]The table includes illnesses of travelers (listed first) and less common diseases with presentations that should suggest the possibility of the appropriate diagnosis. Many diseases have a spectrum of presentation, and the table describes the most common presentations of these diseases. Many diseases have a spectrum of geographic origins, and the table describes the most common exposures seen in daily practice.
HSV-1, Herpes simplex virus 1.

listed in the "GeoSentinel Surveillance of Illness in Returned Travelers, 2007–11"[4]), some potentially serious, were reported, including visceral leishmaniasis, East African trypanosomiasis, scrub typhus, relapsing fever, angiostrongyliasis, botulism, melioidosis, tularemia, hantavirus infection, and infection with *Plasmodium knowlesi.* No cases of yellow fever (YF), Ebola virus, Lassa fever, Marburg virus, tetanus, polio, anthrax, or plague were reported in this particular 5-year cohort, thus attesting to their rarity outside of large-scale outbreak scenarios.

The focus is on the identification of infectious causes of the presenting illness, on travel-associated risk factors, and on manifestations of those diseases that are particular to travelers. Detailed discussions of pathophysiology, spectrum of clinical manifestations, and therapy for each infectious agent are found in the disease-specific chapters of this book. Fever, traveler's diarrhea, and skin problems are the most common presenting illnesses in returned travelers. Eosinophilia is less common but is a frequent source of referral to the infectious diseases specialist.

FEVER

Epidemiology

Fever occurs in 2% to 3%[10–12] of European or American travelers to the developing world. The proportion of ill returned travelers who present to specialists with a febrile illness is 24%, with variation by region of travel: Americas, 14%; South-Central Asia (includes India), 13%; Southeast Asia, 18%; and sub-Saharan Africa, 43%.

Several large case series from busy tropical disease units indicate malaria to be the cause of the fever in 27% to 42%.[10–12] Among ill returned travelers with acute and potentially life-threatening tropical diseases, falciparum malaria accounted for almost 80% of cases, and fever was common to >90%.[13] The other most common tropical diseases specific to returning travelers are arboviral infections, such as dengue (along with chikungunya and Zika), rickettsial disease, typhoid fever, and those caused by enteric pathogens. Less common but important considerations are leptospirosis, brucellosis, acute schistosomiasis, and amebic liver abscess. All of these diseases have widespread distribution in the tropics and need to be considered initially in all febrile travelers. Some may be ruled out quickly based on a detailed travel and exposure history and consultation with relevant information sources on disease distribution. Upper and lower respiratory tract infection, including streptococcal pharyngitis and influenza, as well as urinary tract infections, are cosmopolitan, nontropical febrile disorders that are remarkably common in travelers and should always be considered. In every series from sophisticated referral centers, up to 22% to 25% of those presenting with fever have self-limited illnesses that never have an etiologic diagnosis confirmed.[12] These are mostly viral syndromes caused by one of hundreds of viral agents that exist outside developed countries for which diagnostic tests may not be available anywhere. In many cases the time and expense of a large panel of viral isolation and serologic assays is not warranted outside the research setting. Fever due to deep venous thrombosis or pulmonary embolism may be related to travel, especially in those with preexisting conditions, prolonged air travel, or underlying coagulopathy. Thromboembolic disease always needs to be considered from the outset but is not discussed further here.[14,15]

History

A good patient history is always important in clinical medicine, but nowhere is it as important as in the returning traveler. The cumulative list of infectious agents in 240 separate countries is daunting. A day-by-day travel itinerary, knowledge of risk factors and exposures for the common travel diseases, knowledge of usual incubation periods of those diseases, and knowledge of or access to the known geographic distribution of possible infectious diseases will lead to an appropriately focused workup.[14–16] Much time, expense, and patient discomfort due to sometimes invasive diagnostic tests can be avoided when diagnoses that are not epidemiologically or chronologically possible are eliminated based on the patient history.

The fever pattern and clinical findings by themselves are often nonspecific and overlap greatly between many of the most common tropical infectious diseases. The history should include the key elements detailed in the following sections.

Detailed Travel Itinerary

A travel itinerary should include every locale visited in every country visited, including transit stops. Some individuals are frequent travelers, so all travel for at least the previous 6 months must be considered initially. If the diagnosis remains elusive, a more remote travel history, especially that involving malarious areas, may be sought. The exact date of arrival back in the home country is often crucial to ascertain the last possible exposure date to an exotic pathogen. These details are most efficiently ascertained using a waiting room questionnaire. For example, it is insufficient to know simply that the patient visited Peru. Some parts of Peru are malarious and others are not, only some have risk of YF, high-altitude destinations have little risk of vector-borne disease, and there is no risk of strongyloidiasis along the desert coastal strip.

Chronology of Travel and Illness

This should include the exact dates spent in each locale with respect to the onset of illness. Knowledge of typical incubation periods (Table 319.2) of possible infectious causes is a key tool in narrowing the differential diagnosis. Many agents are simply not biologically possible outside their usual incubation period. Arboviral diseases such as dengue uniformly have short incubation periods. Onset of illness more than 2 weeks after the last possible exposure effectively rules out this class of viral illness. None of the known hemorrhagic fever viruses has a possible incubation longer than 21 days. Long-incubation infections such as schistosomiasis cannot present less than several weeks after first possible exposure. Some diseases such as malaria or enteric fever have more variable incubation periods but nevertheless have a typical incubation period, during which time the majority of the patients present. A number of diseases, especially those that are arthropod borne, have a strict seasonality whereby transmission stops during either cold or dry weather. Examples would include malaria in nontropical countries, such as Korea, Tajikistan, or northern China, as well as Lyme borreliosis or tick-borne encephalitis, all of which completely cease transmission during winter months. GeoSentinel surveillance data indicate that dengue cases in travelers show marked region-specific peaks for Southeast Asia (June,

TABLE 319.2 Incubation Periods of Common Travel-Related Infections

SHORT INCUBATION (<10 DAYS)	MEDIUM INCUBATION (10–21 DAYS)	LONG INCUBATION (>21 DAYS)
Malaria	Malaria	Malaria
Arboviruses, including dengue, yellow fever, Japanese encephalitis, Zika, chikungunya	Flaviviruses: tick-borne encephalitis and Japanese encephalitis	Schistosomiasis
Hemorrhagic fevers: Lassa, Ebola, South American arenaviruses, Crimean-Congo Hemorrhagic Fever (if tick bite)	Hemorrhagic fevers: Lassa, Ebola, Crimean-Congo Hemorrhagic fever (if blood exposure)	Tuberculosis
Respiratory viruses, including severe acute respiratory syndrome	Acute HIV infection	Acute HIV infection
Typhoid and paratyphoid	Typhoid and paratyphoid	Viral hepatitis
Bacterial enteritis	*Giardia*	Filariasis
Rickettsia: spotted fever group—Rocky Mountain spotted fever, African tick typhus, Mediterranean spotted fever, scrub typhus, Q fever	*Rickettsia*: flea-borne, louse-borne, and scrub typhus, Q fever, spotted fevers (rare)	Q fever
Bacterial pneumonia, including *Legionella*	Cytomegalovirus	Secondary syphilis
Relapsing fever	*Toxoplasma*	Epstein-Barr virus, including mononucleosis
Amebic dysentery	Amebic dysentery	Amebic liver disease
Meningococcemia	Histoplasmosis	Leishmaniasis
Brucella (rarely)	*Brucella*	*Brucella*
Leptospirosis	Leptospirosis	Bartonellosis (chronic)
Fascioliasis	Babesiosis	Babesiosis
Rabies (rarely)	Rabies	Rabies
African trypanosomiasis (acute), East African (rarely)	East African trypanosomiasis (acute)	West African trypanosomiasis (chronic)
	Hepatitis A (rarely)	Cytomegalovirus
	Measles	

[a]Diseases that commonly have variable incubation periods are shown more than once. However, most diseases may rarely have an atypical incubation period, and this is not shown here.
HIV, Human immunodeficiency virus.

September), South-Central Asia (October), South America (March), and the Caribbean (August, October).[17,18]

Exposures

A detailed dietary history is essential. Budget travel and associated high-risk eating habits predispose to a variety of common enteric pathogens. A history of specific foods associated with known pathogens also should be elicited. This includes unpasteurized dairy products *(Brucella, Campylobacter, Salmonella, Mycobacterium tuberculosis, Mycobacterium bovis, Listeria monocytogenes),* shellfish (vibrios, enteric viruses, viral hepatitis), uncooked beef such as carpaccio and steak tartare (*Toxoplasma, Campylobacter, Escherichia coli* O157-H7), undercooked fish such as sushi and ceviche (vibrios, *Anisakis, Gnathostoma*), and undercooked pork or game meat (trichinellosis). Exposure to fresh water or surface water in recreational or other settings may be associated with schistosomiasis[19–21] or leptospirosis.[13,22] A history of exposure to mosquitoes and flies is generally unhelpful, but a history of tick bite (rickettsiae, relapsing fever, tick-borne encephalitis) or tsetse fly bite should be sought in the right setting. Exposures to new sexual partners,[23–25] needles, or blood and body fluids should be ascertained. Rodent exposure is associated with Lassa fever, hantavirus infection, murine typhus, and rat-bite fever. A history of contact with other sick people is especially important in the posttravel setting. Travelers usually move in groups or with families or companions, all of whom will likely have shared the same exposures.

Immunization History

The immunization history should include exact dates of the last dose of each vaccine received and in some instances whether an adequate primary series was completed in the first place. Most vaccines, with the notable exception of typhoid and influenza vaccines, are highly efficacious. Thus hepatitis A or B, YF, measles, and diphtheria are unlikely diagnoses in those travelers with a substantiated history of adequate and current immunization.

Antimalarial Prophylaxis or Treatment

If malaria is a possibility, a complete pill-by-pill history of ingestion of antimalarial drugs, including the name and dose of all drugs taken for prophylaxis or treatment, must be obtained. Patients often misunderstand the dosing, timing, and coadministration with food instructions given at the pretravel visit, or they may have been prescribed an inappropriate drug for their destination. Patients may have been treated with appropriate or inappropriate drugs en route for febrile illnesses. Some very efficacious drugs are not available in the United States, and an international pharmacopeia, such as Martindale's, may need to be consulted by those unfamiliar with these drugs. A history of appropriate prophylaxis diminishes the possibility of malaria but does not eliminate the need for a set of malaria thick films, which may be preceded by malaria rapid card (rapid diagnostic) testing for any patient legitimately exposed to malaria.

Other Medications Ingested

Travelers who fall ill during travel often self-treat with antibiotics or see a local physician and are prescribed a broad-spectrum antibiotic. Again, an international pharmacopeia may need to be consulted. Recent ingestion of a 1-week course of a quinolone, tetracycline, or cephalosporin may alter the course of the illness or even affect the possibility of certain diagnoses. In particular, malaria may be suppressed by antecedent use of azithromycin, doxycycline, quinolones, or clindamycin.

Physical Examination

Common tropical infections often present as undifferentiated fever without focal findings. However, when a focal finding such as arthritis, meningitis, or pneumonia is present, the differential diagnosis can often be narrowed. Unfortunately, physical findings such as jaundice, hepatomegaly, splenomegaly, and lymphadenopathy occur at least a portion of the time in many of the most common travel-related infections and so are not specific enough to greatly narrow the differential diagnosis.[10,26] Most imported febrile rash illnesses engender the same differential diagnosis as for nontravelers. However, arbovirus infections, typhoid, rickettsial illness, leptospirosis, measles, early stages of viral hemorrhagic fevers, relapsing fever, and acute African trypanosomiasis should always be kept in mind where epidemiologically plausible.

Considerations for the Common Travel-Related Febrile Illnesses

Malaria

Fever in a traveler returning from a malarious area is an emergency, and the instinctive performance of an immediate malaria smear, followed by another 6 to 24 hours after the first, will prevent unnecessary deaths. Malaria rapid diagnostic tests (RDTs) are licensed in the United States for use in clinical laboratories and not at the point of care. The readout for this antigen detection test is *Plasmodium falciparum,* non–*P. falciparum,* or a potentially mixed infection. Because it is highly specific, a positive test means immediate treatment is warranted.

The malaria RDT has excellent sensitivity for *P. falciparum* but may still miss low parasitemias so is not a definitive means to rule out malaria. The sensitivity is poor for non–*P. falciparum* malaria, which tends to have much lower parasitemias, but non–*P. falciparum* malaria is not usually life threatening.[27] Thus a negative test does not rule out malaria and must always be followed by at least two blood films. Malaria due to *P. falciparum* is easily treatable if diagnosed early but even with optimum treatment has a mortality rate of 20% or more if treatment is begun only after end-organ complications arise. Smears need to be repeated at least every 12 to 24 hours a minimum of three times to rule out malaria. Rapid deterioration can occur over a period of hours. Patients who are unreliable to care for themselves and have smear-negative results but a high index of suspicion for *P. falciparum* malaria may need to be admitted for inpatient observation.

Because malaria is overwhelmingly an African disease, with about 80% of all *P. falciparum* imported into developed countries originating there,[28–31] suspicion of malaria is especially acute for Africa returnees. Beyond this, trends in the geographic origin of imported malaria cases do not always correlate well with regional transmission patterns because absolute numbers of cases from particular geographic areas may also mirror the intensity of travel to the affected region. Ethnic minority travelers returning home to visit friends and relatives in malarious areas have the highest risk for infection. Resources describing current country-specific malaria microepidemiology should be immediately accessible to those assessing tropical fevers. In general, malaria is a rural disease, but the cities of Africa and India are exceptions.

P. falciparum malaria in nonimmune travelers most commonly has an incubation period of 9 to 14 days, and 90% of cases occur within 1 month of last exposure. Non–*P. falciparum* malaria in travelers is only rarely life threatening but can present much later after arrival. Incubation periods are prolonged in those taking inadequate or incomplete chemoprophylaxis. Relapses of disease due to *Plasmodium vivax* or *Plasmodium ovale* may occur many months after travel in those whose initial attack was clinically silent because of suppressive chemoprophylaxis but in whom terminal prophylaxis with primaquine was not used (see Chapter 274). A well-described concern is delayed hemolysis after parenteral artesunate therapy, which may occur after day 8 of therapy, is mild in 85% of cases, and should not preclude the use of artesunate therapy.[32]

The presenting signs and symptoms of imported malaria remain sufficiently protean so as to mimic a number of common tropical or nontropical conditions.[28–31] No constellation of symptoms or signs differentiates *P. falciparum* from non–*P. falciparum* malaria. Classic periodic malarial fever is not a usual manifestation of imported malaria, although when fever does occur in discrete, repeated 48- or 72-hour cycles, the diagnosis is almost certain. The simian malaria parasite *P. knowlesi,*[33] which is now known to commonly infect humans in Malaysia and throughout Southeast Asia, uniquely has a periodicity of 24 hours. Infected patients have high parasitemias (>1%) with a *Plasmodium* that is morphologically almost identical to *Plasmodium malariae*. *P. malariae* typically has a parasitemia of less than 1%. Fever is absent at the exact time of the initial medical assessment in up to 40% of patients with malaria. Respiratory or GI symptoms may be predominant. The presence of rash, lymphadenopathy, or leukocytosis indicates another diagnosis, although cautious application of this clinical pearl should be exercised, recognizing that coinfections in the ill returned traveler may occur. Anemia is uncommon in travelers who present in the early days of their malarial illness. Thrombocytopenia occurs in greater than 50% and is a reliable if nonspecific indicator of malaria when present.

Many other serious infections are present in malarious areas. The search for malaria should not hamper the simultaneous workup for other pathogens in smear- and RDT-negative patients. Similarly, semiimmune residents of endemic areas may be mildly parasitemic on a chronic basis with little ill effect, so a positive malaria smear in these patients should not hamper a search for any other clinically suspected infections.

Dengue

Dengue, transmitted by the day-biting *Aedes aegypti* mosquito, is an important travel-related problem, most notably in heavily visited areas of Southeast Asia, the South Pacific, and Central America and the Caribbean.[17,18] Travelers to Thailand seem particularly prone to infection, and dengue is relatively uncommon in Africa but has begun to emerge there. In contrast to many other tropical fevers, it is predominantly an urban infection so that it can even affect upscale business travelers in urban centers. The incubation period is usually 2 to 7 days after the mosquito bite, so many travelers initially become ill while still overseas. The clinical spectrum ranges widely from asymptomatic through a range of clinical manifestations up to the severe myalgia and arthralgia of "breakbone fever" (see Chapter 153). Malaria and arboviral diseases, including chikungunya, leptospirosis, rickettsial disease, measles, or typhoid may present as similar initial findings. However, in cases in which one of several associated rashes manifests (Fig. 319.1A), dengue or chikungunya becomes more likely than the other possibilities.

A positive tourniquet test is found in up to 50% of patients with classic dengue and in almost all patients with severe dengue with or without hemorrhage, but it is a nonspecific finding that may also be present in leptospirosis. The test is performed by inflating a blood pressure cuff halfway between systolic and diastolic for 5 minutes and, upon release, counting the number of petechiae in a 2.5 × 2.5-cm patch below the cuff. Finding more than 20 petechiae is considered a positive finding.

Polymerase chain reaction (PCR) testing for viremia is possible only during the first 5 days of illness during the viremic phase and is available at many commercial reference laboratories in the United States. Serologic confirmation must be sent to a reference laboratory. Immunoglobulin



FIG. 319.1 Cutaneous manifestations of some common systemic or widely disseminated diseases. (A) Generalized macular rash of dengue; rash usually appears after 4 or 5 days, but an earlier, faint, flushlike rash also may be present. (B) Focal maculopapular rash of Zika virus, typically pruritic. (C) Viral hemorrhagic fever due to yellow fever infection; typical signs of viral hemorrhagic fevers include bleeding from orifices and intravenous sites, as well as diffuse petechiae or ecchymoses, especially over pressure points.

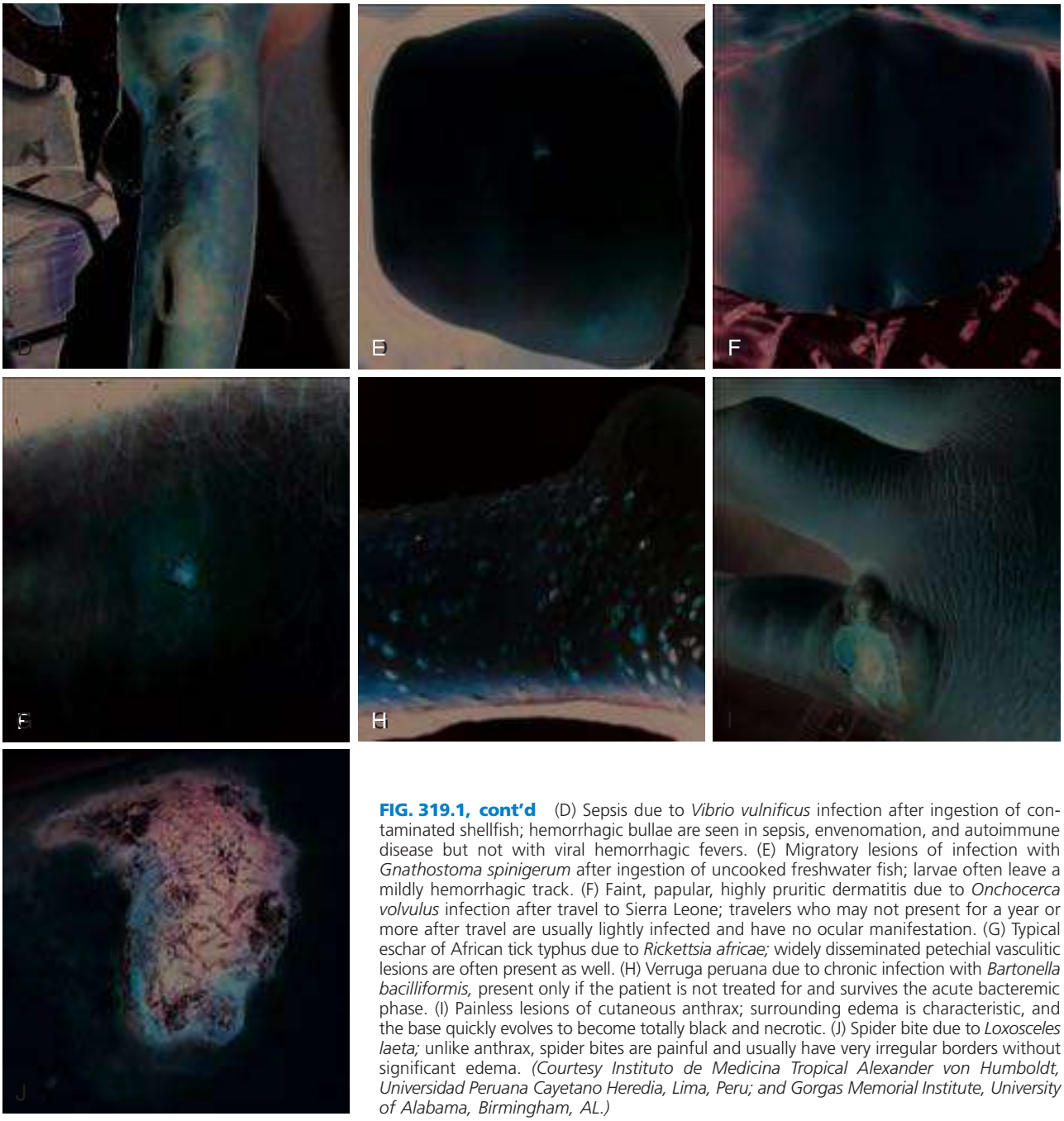

FIG. 319.1, cont'd (D) Sepsis due to *Vibrio vulnificus* infection after ingestion of contaminated shellfish; hemorrhagic bullae are seen in sepsis, envenomation, and autoimmune disease but not with viral hemorrhagic fevers. (E) Migratory lesions of infection with *Gnathostoma spinigerum* after ingestion of uncooked freshwater fish; larvae often leave a mildly hemorrhagic track. (F) Faint, papular, highly pruritic dermatitis due to *Onchocerca volvulus* infection after travel to Sierra Leone; travelers who may not present for a year or more after travel are usually lightly infected and have no ocular manifestation. (G) Typical eschar of African tick typhus due to *Rickettsia africae;* widely disseminated petechial vasculitic lesions are often present as well. (H) Verruga peruana due to chronic infection with *Bartonella bacilliformis,* present only if the patient is not treated for and survives the acute bacteremic phase. (I) Painless lesions of cutaneous anthrax; surrounding edema is characteristic, and the base quickly evolves to become totally black and necrotic. (J) Spider bite due to *Loxosceles laeta;* unlike anthrax, spider bites are painful and usually have very irregular borders without significant edema. *(Courtesy Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru; and Gorgas Memorial Institute, University of Alabama, Birmingham, AL.)*

M (IgM) is not elevated until 5 or more days after illness onset, but most patients initially present earlier than this. If an IgM sample drawn more than 5 days into illness is negative, a third visit to test for fourfold elevations of immunoglobulin G (IgG) is required. The test for NS1 dengue antigen in serum permits earlier diagnosis than IgM antibody. Both ELISA and point-of-care tests are available in some endemic areas. Because most patients will be better by the time results of any confirmatory tests would be available and because treatment is supportive, many clinicians do not seek laboratory confirmation of late-presenting cases. A postviral fatigue lasting up to 6 months may occur.

Chikungunya Fever

Although chikungunya fever,[18] a mosquito-borne alphavirus (chikungunya virus) infection, was first isolated in the early 1950s, when it caused epidemics in East Africa and is endemic in tropical Africa and Asia, it has been unknown to most clinicians in the Americas and Europe until reemerging in 2005. Since 2006, with introduction of serology into routine practice, chikungunya virus infection has been identified frequently in travelers after return home from the endemic areas in India, Southeast Asia, and East Africa. Chikungunya virus was introduced to the Caribbean during a large epidemic in 2013 and is now established there. Travel-acquired disease presenting in the United States from 2010–13 was identified in 115 cases; 96% was associated with travel to Asia.[34] However, among 118 cases of chikungunya imported to Canada up to 2015, more than half were acquired in the Caribbean.[35] The acute illness resembles dengue but with more prominent joint symptoms. Patients have fever, arthralgia, and sometimes acute arthritis. Rash, which occurs in about 50% of cases, may resemble that seen in dengue and is pruritic and macular or maculopapular. Although acute symptoms usually subside within a week, disabling joint symptoms may persist for months.

Zika Virus

Zika virus has been responsible for isolated cases and now outbreaks (first described in Micronesia) of disease characterized by rash, conjunctivitis,

fever, arthralgia, and arthritis.[36] A flavivirus related to West Nile fever, dengue, and YFs, it is transmitted by *Aedes* mosquitoes and thus is now reported as an endemic disease of Brazil, several other countries in South and Central America, throughout the Caribbean, and as an imported illness in travelers returning to the United States from endemic areas.[37,38] Zika virus is being increasingly documented in Southeast Asia and is particularly emergent in Thailand.[39] Clinical disease may be similar to milder cases of dengue or chikungunya fever, although reports have continued to describe an association with Guillain-Barré syndrome[40] and a devastating congenital Zika syndrome[41,42] in infants born to infected mothers. Care is supportive, although additional considerations should include the possibility of sexual-, transplacental- or perinatal-, and blood product–associated transmission.[43] Due to prolonged seminal shedding of ZIKV RNA in men with symptomatic Zika virus infection, the possibility of sexual and congenital infection potentially exists long after departure of the traveler from the endemic area, and has motivated cautious guidance from authorities around condom use and pregnancy deferral. Recent data, however, suggest that the greatest risk of shedding replicable, infectious virus in semen occurs only within the first month of symptom onset, and that risk of detecting any virus in semen drops off substantially by 60 days after symptom onset.[44]

Typhoid and Paratyphoid Fever

Typhoid fever is often the most nondescript of the relatively common causes of travel-related fever.[45–47] The incubation period is most often a week but can be as long as 3 weeks. Risk is at least 10 times higher on the Indian subcontinent than anywhere else, but risk exists throughout the tropics in the setting of poor sanitation. In contrast to malaria, dengue, or rickettsial infection, onset is insidious and abnormal physical findings, with the exception of abdominal tenderness, are usually absent. Abdominal discomfort and constipation are common, but diarrhea is frequent enough so as to not rule out the diagnosis. Patients often look and feel particularly unwell, with severe prostration and high, unremitting fever. Leukopenia and thrombocytopenia often occur. Blood cultures are not always positive, but bone marrow cultures increase the yield. Serologic assays, including agglutination and enzyme-linked immunosorbent assay (ELISA), have overall poor sensitivity, especially early in the course, and some lack of specificity in some settings and enjoy poor reputations. However, when present, an unequivocal high titer in a previously naïve traveler provides a more rapid diagnosis than will blood cultures. Up-to-date vaccination against typhoid provides only partial protection against *Salmonella enterica* serovar Typhi and does not protect at all against *Salmonella enterica* serovar Paratyphi.[48] Because of resistance, fluoroquinolones are no longer an option for empirical treatment in the Indian subcontinent, Africa, and Southeast Asia, and third-generation cephalosporins or a carbapenem should be used.[45] Recent emergence of an extensively drug-resistant clone of *S.* Typhi in Pakistan[49] reiterates the importance of microbiologic susceptibility testing in clinical cases but necessarily poses a challenge to clinicians due to the prolonged time to defervescence of typhoid patients on appropriate and targeted therapy. Close surveillance of antibiograms for isolates of *S.* Typhi will necessarily inform empirical treatment. Increasing data support the use of high-dose oral azithromycin for quinolone-resistant *S.* Typhi.[48]

Viral Hepatitis

Incidence rates for travel-related viral hepatitis are likely to have begun a decline as more individuals who had routine childhood hepatitis B vaccine are moving into the traveling population and as more high-risk travelers are receiving long-term protection due to pretravel hepatitis A and B vaccination.[50] Current vaccines do not protect against hepatitis E, which is enterically acquired,[51] or against hepatitis C, which, like hepatitis B, may be acquired overseas after blood transfusion or contact with contaminated syringes, medical equipment, or tattoo and body-piercing implements. Viral hepatitis is a long-incubation infection so that acquisition may not always be readily linked by the patient or the physician to the travel.

Rickettsial Disease

Rickettsial disease is emerging in travelers.[4,52] Most of the long list of rickettsial species infecting humans are transmitted by ticks, mites, and fleas. Eschars are seen in most patients with African tick typhus due to *Rickettsia africae* (see Fig. 319.1G),[52] Mediterranean spotted fever due to *Rickettsia conorii* or *R. africae* and scrub typhus due to *Orientia tsutsugamushi* infection are the most common travel-related rickettsioses. In a group of 940 travelers to South Africa, 4% of all travelers and 27% of all travelers with flulike symptoms had infection with *R. africae*.[53] *R. africae* is the second most common cause of fever in travelers to Africa after malaria and is most prevalent in South Africa itself. Although rickettsial diseases are present in most countries of the world, individual species have restricted geographic distributions (see Chapter 186), which helps in the diagnostic formulation. High fever, headache, leukopenia, and thrombocytopenia are common. Rickettsiae infect endothelial cells and often cause widespread vasculitic-looking lesions. Severe infections may present as disseminated intravascular coagulation and mimic a viral hemorrhagic fever. Because African tick-bite fever and scrub typhus both occur in malarious areas, a set of thick films (with malaria RDT) is indicated, even in febrile patients with pathognomonic skin lesions. Because response to tetracyclines is uniformly prompt and dramatic and the results of serologic tests are slow to return, clinical suspicion and clinical diagnosis are usually relied on. The diagnosis should be reconsidered in those who do not respond within 48 hours of initiation of therapy with doxycycline or tetracycline.

Leptospirosis

Leptospirosis is thought of as an occupational disease and a disease of urban slum dwellers with rodent exposure. In recent years large leptospirosis outbreaks in adventure travelers and adventure racers, such as whitewater rafters, triathletes, and participants in the 2000 Borneo Eco-Challenge race, have occurred.[22] Doxycycline prophylaxis is now recommended for both civilians and military personnel who will hike, bike, swim, or raft in tropical environments.[13] Additional autochthonous cases are now reported in countries where leptospirosis has traditionally been considered a travel-acquired disease (Austria and Switzerland, with zoonotic transmission from infected beavers).[54,55] The protean clinical manifestations, which include fever, headache, proximal lower extremity myalgia, and abdominal wall pain, are impossible to distinguish clinically from dengue but may also mimic a number of other common tropical infections, including hemorrhagic illnesses such as YF (where exposures occur in South America and West Africa) and Crimean-Congo hemorrhagic fever (typically originating in Central Asia). Conjunctival suffusion and jaundice occur in a subset and are more common than in the other undifferentiated febrile diseases, although both may occur in relapsing fever. A reliable, rapid IgM dipstick test for leptospirosis is widely available and used. Recognition of possible leptospirosis affects therapy because antibiotic treatment is generally undertaken when the diagnosis is suspected.

Respiratory Illness

Travelers spend long periods in confined spaces and tend to meet many different people during the course of their trip. Acute respiratory tract infections occur in 10% to 20% of all travelers, with rates as high as 1261 per 100,000 travelers for a 1-month stay in a developing country. For all ill returning travelers seen at GeoSentinel Surveillance Network sites, 7.8% were diagnosed with respiratory tract infection, with almost half of these being lower respiratory tract infections, such as pneumonia or atypical pneumonia.[56]

MERS-CoV, first reported in 2012, has since spread internationally, requiring surveillance by World Health Organization (WHO), Centers for Disease Control and Prevention, and other national agencies, principally of travelers returning from the Middle East. Characterized by a sometimes severe respiratory illness, global surveillance through July 2017 noted severe disease or mortality in 46.8% of 2040 total cases.[57] Surveillance reports of returning travelers support an association of the diagnosis with severe manifestations of respiratory tract infection.[58] A large outbreak was reported and controlled quickly in South Korea, but not before a case of secondary transmission reached China and raised further concerns there.[59,60] Treatment protocols exist, but without established evidence for the efficacy of antiviral therapies, supportive care is paramount, and continued vigilance and infection control measures are required as concerns about transmission outside

of endemic areas is heightened by evidence of continued nosocomial transmission.[61]

Respiratory diseases are second only to GI infections as a cause of morbidity in travelers. In outbreaks of infections on cruise ships, respiratory tract infections constitute the most common diagnosis.[62,63] Influenza is the most common vaccine-preventable disease of travelers, with an incidence rate of approximately 1%. One-fourth or more of all cases of legionellosis are associated with travel in the previous 2 weeks, and rates appear to be increasing. Risk factors include stays at large air-conditioned resort hotels or spas and cruise ship travel.[64] Acute histoplasmosis can be seen after brief excursions into caves anywhere in the Americas, and travel-related coccidioidomycosis is reported.[65] Tuberculosis is a clear risk in those who spend longer periods in very high-risk countries and especially those who are doing medical or aid work.[66,67] Pulmonary infiltrates and symptoms may be seen during the migratory phases of helminthiases, such as schistosomiasis, strongyloidiasis, hookworm infestation, and ascariasis. Hemorrhagic pneumonitis may be seen with leptospirosis. Q-fever should be sought in those with animal exposure. Workup should be guided by clinical and radiologic findings.

Special Consideration of Ebola Virus Disease in Returning Travelers

The 2013–15 Ebola epidemic in West Africa (predominantly Sierra Leone, Guinea, and Liberia, but with a much smaller contemporaneous outbreak in the Democratic Republic of Congo) has necessitated concerns about travelers and the care of returning expatriates, including health care workers. A full discussion of the virus, its clinical characteristics, epidemiology, experimental therapeutics, and vaccinology can be found in Chapter 164. In a description of the illness and outcomes from Sierra Leone, Ebola virus disease (EVD) commonly presents after an average incubation period of 6 to 12 days (but up to 21 days) with fever, headache, weakness, dizziness, prominent diarrhea, abdominal pain, and vomiting, and a case fatality rate from 57% (age < 21 years) to 94% (age > 45 years).[68] Although classically a hemorrhagic fever, the course of EVD is most profoundly characterized by GI fluid loss and inability to support oral rehydration, leading to dehydration, shock, and death. Thus management protocols emphasize the use of intravenous (IV) fluid support with IV antimicrobials.[69] Other causes of illness, such as *P. falciparum* in travelers from Ebola-affected areas, remain important considerations, and the similarity with severe dengue has been noted.[70,71] Concerns remain due to uncertainty regarding recurrent disease after convalescence and possible persistence of virus in sanctuary sites, such as the eye and reproductive tract.[72,73]

Yellow Fever in Returning Travelers

YF virus, another mosquito-borne flavivirus, has been a historically rare cause of fatal illness among international travelers.[74,75] In large analyses of GeoSentinel Surveillance Network data spanning 13 years and encompassing almost 60,000 ill returned travelers, not a single case of YF was documented.[76,77] In 2016 a large outbreak of YF began in Angola, culminating in >800 laboratory-confirmed cases and 373 deaths, and led to importation of 11 cases to China by Chinese nationals working in Angola.[78] In addition, beginning in late 2016 a large outbreak of YF has been continuing in Brazil, affecting previously YF-free high-density areas such as São Paulo and Rio de Janeiro. Since January 2018, 10 cases of YF among travelers have been reported to the GeoSentinel Surveillance Network, 40% of which were fatal.[79] The outbreak has prompted updated YF immunization recommendations by WHO[80] and has further taxed the availability of YF vaccine, which was already suffering a global shortage due to the outbreak in Angola. Many travelers are entering YF risk areas without being immunized.[81] Thus YF should be considered in the differential diagnosis of any unimmunized traveler to risk areas of South America and West Africa presenting with a severe dengue or leptospirosis-like illness.

Initial Office Approach to the Febrile Patient

The first priority is assessment for dangerous or immediately life-threatening disease, such as when hemorrhagic manifestations are apparent. If the patient has the appropriate exposures for a viral hemorrhagic fever, he or she needs to be immediately isolated and public health authorities contacted. None of the isolatable hemorrhagic fever viruses have incubation periods exceeding 3 weeks. Arenavirus infection, whether from West Africa (Lassa virus) or South America (Junin, Machupo, Guanarito viruses), should be treated with ribavirin. Most also recommend treating Crimean-Congo hemorrhagic fever with ribavirin.[82] Other rare hemorrhagic fevers of travelers, such as Rift Valley fever, YF, dengue hemorrhagic fever, and Ebola hemorrhagic fever, need to be supported with the best possible intensive care.[83] Meningococcemia and rickettsial infection present as purpuric lesions, and bacterial sepsis and severe malaria are serious but treatable causes of hemorrhage owing to disseminated intravascular coagulation. Any febrile patient with altered sensorium or any other evidence of end-organ damage consistent with malaria and in whom *P. falciparum* malaria is a possibility should receive empirical therapy for malaria regardless of the result of a blood film. The smear is often negative in advanced disease because of sequestration of parasites in capillary beds.

In the patient who is not severely ill but who has an undifferentiated fever without any localizing symptoms or signs, three blood films, if epidemiologically indicated, are the first priority. At the same time, other mandatory diagnostic tests in the workup of every tropical fever include blood cultures (for enteric fever), complete blood cell count with differential and platelets, liver function tests, urinalysis, and a chest radiograph. The blood film may also diagnose bartonellosis, acute trypanosomiasis, and relapsing fever. Leukopenia militates away from common bacterial infections and toward dengue, typhoid, brucellosis, rickettsial disease, or acute human immunodeficiency virus (HIV) infection. Thrombocytopenia is indicative of malaria, dengue, or brucellosis. Eosinophilia may indicate early migratory stages of a number of helminths (see later). Liver function test results will be consistently abnormal in viral hepatitis or toxin damage and variably abnormal in leptospirosis, rickettsial disease (Q-fever), relapsing fever, YF, amebic abscess, brucellosis, typhoid, hemorrhagic fever, and dengue. An indirect benefit of chest radiography is the finding of an elevated right hemidiaphragm in many patients with amebic liver abscess.

The second wave of diagnostic testing is driven by any abnormalities that emerge from initial test results. In the absence of enlightening abnormalities, additional serologic studies may need to be obtained based on travel itinerary, incubation periods, and known exposures, as discussed previously. HIV infection and its complications, syphilis, and tuberculosis should be sought at this stage if there is any suggestive exposure at all. After ruling out potentially serious and potentially treatable infections by history, physical examination, and routine laboratory work, and especially if patient financial resources are limiting, the clinician must then decide whether to wait 48 to 72 hours before serology and sophisticated diagnostic studies are pursued. Because up to 25% of all febrile illnesses in returning travelers are self-limited viral syndromes, a patient who was highly febrile and quite toxic looking at initial assessment is quite often perfectly well 48 hours later with no intervention. Reasonable clinical and local laboratory experience and confidence are required for this approach, but from the patient standpoint it is the most desirable course. At a minimum, acute serum should be stored for possible later use. If the patient is stable, has no laboratory abnormalities and no clinical evidence of end-organ damage, and has a reliable companion, he or she may be followed as an outpatient during the clinical evolution and the appropriate workup pursued according to any ensuing clinical findings.

Oral ciprofloxacin is sometimes given as empirical therapy for the slightest chance of typhoid fever because of the ease of treatment and the difficulty making the diagnosis. However, quinolone-resistant typhoid and paratyphoid fever are now predominant in the Indian subcontinent, Africa, and Southeast Asia, where much of the travel-related enteric fever originates. Thus, in this situation, if clinical suspicion is high, the sick patient may need to be admitted for parenteral therapy, or alternatively, prescribed high-dose azithromycin for empirical treatment on an ambulatory basis. Empirical therapy for malaria without a positive blood film is appropriate if clinical evidence of cerebral dysfunction or any other end-organ damage consistent with malaria is present. Otherwise, examination of these patients and of serial blood smears over

several days by someone with appropriate experience will generally lead to the parasitologic diagnosis of malaria, when present.[84] Expertise is rarely so far away as to compromise patient care, although empirical therapy with coartemether or atovaquone-proguanil is generally well tolerated. However, empirical treatment will necessarily eliminate any possibility of making a species diagnosis if the patient, in fact, does have malaria. After empirical treatment, the clinician is then probably obligated to a course of primaquine (in those without glucose-6-phosphate dehydrogenase deficiency), a potentially toxic drug, to cover the possibility that the antecedent infection was due to relapsing (*P. vivax* or *P. ovale*) malaria.

Febrile patients who present initially with focal symptoms or signs should have a more directed workup that takes into consideration appropriate disease distribution, incubation period, and possible exposures. Altered mental status or other central nervous system deficits are present as nonspecific sequelae of many systemic infections. However, appropriate itinerary, exposure, and incubation periods for the following less common infections should be sought: Japanese encephalitis, rabies, West Nile virus, tick-borne encephalitis, African trypanosomiasis, angiostrongyliasis, gnathostomiasis, and, in recent Hajj pilgrims to Mecca, meningococcal infection.[85]

DIARRHEA IN TRAVELERS

Acute Traveler's Diarrhea

Diarrhea is by far the most common cause of illness during travel, affecting up to 60% of travelers to some high-risk destinations. South Asia is by far the highest-risk region for traveler's diarrhea.[86] The most frequent cause of traveler's diarrhea is enterotoxigenic *E. coli* (6%–70%). Other types of *E. coli* (especially enteroaggregative *E. coli*),[87,88] *Salmonella, Shigella,* and *Campylobacter* each account for 5% to 15%. *Vibrio parahaemolyticus* is related to shellfish ingestion and is seen almost exclusively in Asia. Protozoa account for 5% to 10%, and in adults, norovirus and rotavirus may be detected.[89–91] Norovirus outbreaks aboard cruise ships are increasingly recognized, and in one retrospective study of etiology in traveler's diarrhea based on multiplex molecular diagnostics, viral causes (mostly norovirus) accounted for 18% of cases.[89] About 30% of diarrheal episodes remain unexplained, but many are likely due to enteroaggregative or enteropathogenic *E. coli*.[89]

Bacterial diarrhea generally manifests as the abrupt onset of uncomfortable, crampy diarrhea.[92,93] Fever, nausea, or vomiting, if present, further increase the likelihood of a bacterial cause. In contrast, protozoal diarrhea (most often due to *Giardia lamblia* or *Entamoeba histolytica*) begins gradually, with loose stools occurring in distinct episodes and gradually becoming more disabling over 1 to 2 weeks. In protozoal diarrhea medical care usually is not sought immediately because of the low-grade nature of the symptoms. Because most traveler's diarrhea is bacterial, many travelers are instructed to self-treat with oral rehydration solution, loperamide, and bismuth for mild disease, and, in addition, either quinolone antibiotics or azithromycin for moderate-to-severe illness[91–94]

Classic traveler's diarrhea is defined as three or more unformed stools per day, although a syndrome of nonclassic traveler's diarrhea with fewer stools but with accompanying symptoms is defined by some. Travelers may vary in their own definition of what is an abnormal bowel pattern, and this needs to be established with the patient in a quantitative way at the outset. Returned travelers with acute diarrhea of a few days' duration that is affecting their level of functioning and who have not yet had a course of quinolone or macrolide antibiotic can be prescribed an empirical course without any workup or stool culture. Toxic patients with bloody diarrhea should undergo urgent *E. histolytica* ELISA (amebic antigen) or PCR testing of *unpreserved* stool, if such a specimen can be obtained and processed in a timely manner. If collection and processing of unpreserved stool cannot be accomplished in a timely manner, then urgent wet preparation of stool along with sigmoidoscopic examination to look for amebic trophozoites should occur. Empirical treatment of amebic dysentery with metronidazole or tinidazole could also be considered if confirmatory diagnostics prove logistically challenging. Nonresponders at 36 to 48 hours should then have stool specimen sent for performance of bacterial culture, ova and parasite testing, acid-fast bacilli testing (to detect *Cryptosporidium* and *Cyclospora* if conventional microscopy is used in the laboratory. Increasingly, diagnosis is made by a molecular multiplex PCR enteric platform, *Giardia* or *Cryptosporidium* ELISA or immunofluorescence assay, *E. histolytica* ELISA, and *Clostridioides difficile* (formerly *Clostridium difficile*) toxin assay. *Vibrio* cultures usually require a special request. Quinolone-resistant *Campylobacter* is increasing worldwide and is prevalent in Southeast Asia, so an empirical course of azithromycin can be given while awaiting culture if the patient is still moderately ill. Rifaximin has been approved for traveler's diarrhea due to *E. coli*.[94] Reactive arthritis (formerly Reiter syndrome) is an occasional sequela of enteritis due to *Shigella* or *Campylobacter.* The gastroenteritis film array technique (FilmArray System; BioFire Diagnostics, Salt Lake City, Utah) on stool offers rapid diagnosis of the following enteric pathogens: *Campylobacter* spp., *C. difficile* (toxin A/B), *Plesiomonas shigelloides, Salmonella* spp., *Vibrio* spp., *Vibrio cholerae, Yersinia enterocolitica*, enteroaggregative *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, Shiga-like toxin-producing *E. coli* (stx1 and stx2) (including specific detection of *E. coli* O157), *Shigella* spp./enteroinvasive *E. coli, Cryptosporidium* spp., *Cyclospora cayetanensis, E. histolytica, G. lamblia*, adenovirus F 40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus.[95]

Persistent Diarrhea in the Traveler

Two percent of those with traveler's diarrhea go on to develop chronic diarrhea lasting a month or more. These patients' disorders can be extremely frustrating to deal with because diagnosis is most often elusive despite extensive diagnostic testing.[96] Clearly, some undiscovered enteric pathogens remain. A number of limited studies indicate that the incidence of postinfective irritable bowel syndrome at 6 months after an acute episode of traveler's diarrhea may range from 4% to 32%. The true incidence of this syndrome is not clear, and ancillary contributing factors and possible preemptive interventions are still being investigated.[97] Appropriate studies, in addition to those already listed, include HIV serology, 5-hydroxyindoleacetic acid levels, thyroid function tests, serum calcium, testing for malabsorption, antineutrophil cytoplasmic antibody, serologic studies for inflammatory bowel disease, antigliadin/antiendomysial/antitransglutamase antibodies for celiac disease, and upper and lower endoscopy. *G. lamblia, Strongyloides stercoralis, Cryptosporidium parvum,* and *Cyclospora cayetanensis* are occasional infectious causes of persistent diarrhea and may be discovered only after extensive workup. Serology for *S. stercoralis, Schistosoma mansoni,* or *E. histolytica* is indicated when exposure to these agents may have occurred. Intestinal biopsy almost always yields nonspecific findings, although cases of tropical or nontropical sprue are occasionally discovered or an initial diagnosis of inflammatory bowel disease made. In many patients the etiology of the frequently found nonspecific villus blunting is unclear. This syndrome has often been termed *tropical enteropathy* or *postinfective tropical malabsorption* and is believed to be the residual damage caused by an initial bacterial or other insult. A temporary luminal disaccharidase deficiency may occur. Diarrhea may persist for months before resolving. In the absence of definitive diagnosis in patients with chronic traveler's diarrhea, symptomatic treatment with loperamide is indicated. Elimination diets with restriction of lactose, fructose, gluten, and fat are sometimes of benefit. Those with preexisting irritable bowel syndrome may have it unmasked by travel and frequently have exacerbations during or after travel. Antispasmodics or a medication for underlying disease may be needed.[97–100]

SKIN PROBLEMS

The proportion of ill, returned travelers who present to infectious and tropical diseases specialists who have a dermatologic problem is 20%, with variation by region of travel (Fig. 319.2). The most common skin-related diagnoses are cutaneous larva migrans (9.8% of all skin diagnoses); insect bites, including superinfected bites (15%); skin abscess (7.7%); and allergic reaction (5.5%). Dengue (3.4%), leishmaniasis (3.3%), myiasis (2.7%), and the rickettsial spotted-fever diseases (1.5%) are other important reasons for presentation. Arthropod-related skin diseases accounted for 31% of all skin diagnoses.[101,102] Ulcerative lesions of travelers include leishmaniasis, mycobacterial disease, deep mycoses, and, rarely, anthrax. Rickettsial diseases frequently include black eschars at the site of the arthropod bite. Loiasis,[103] gnathostomiasis,[104] and cysticercosis present

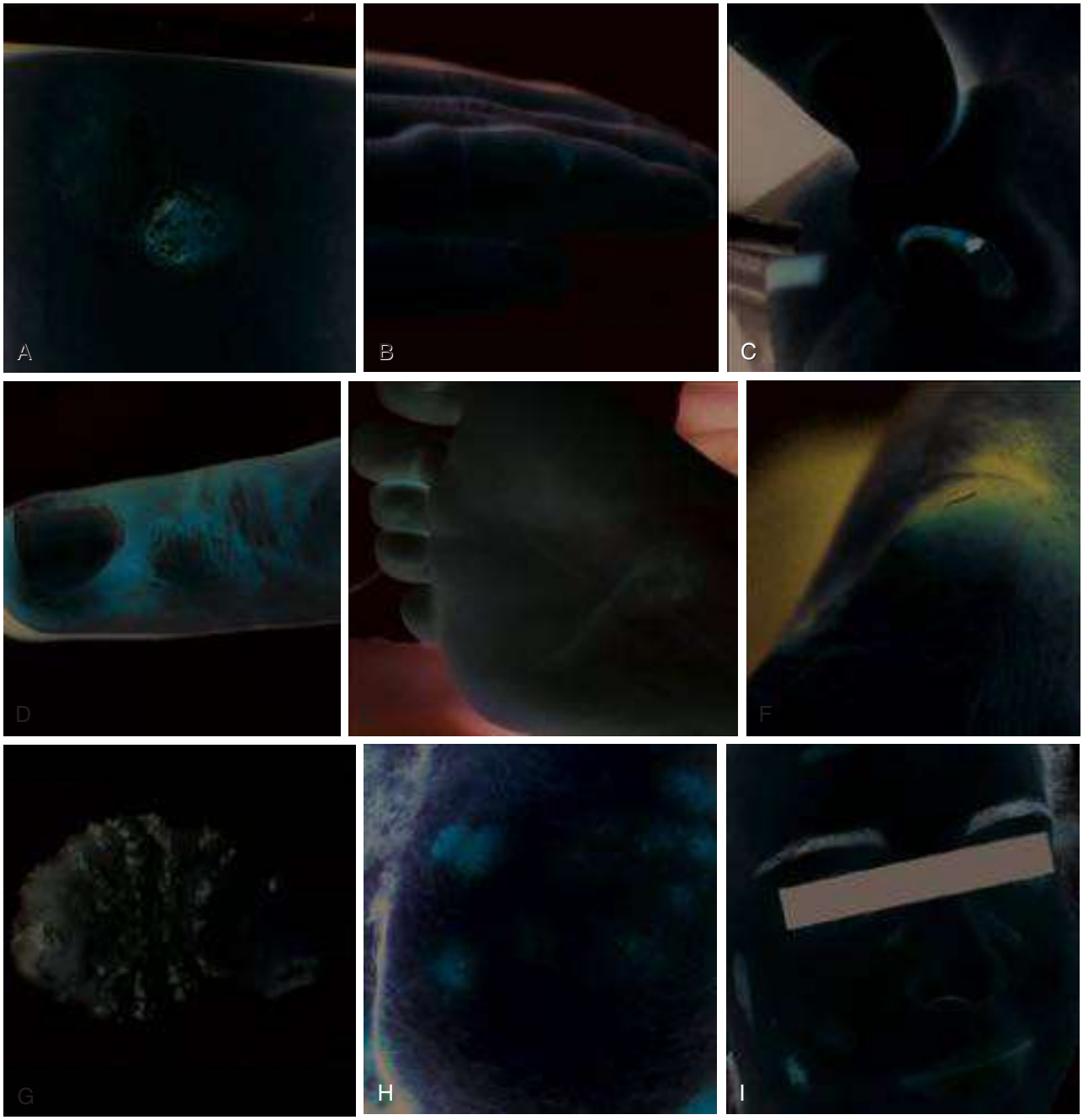

FIG. 319.2 Some common diseases of travelers with pathologic effects localized to circumscribed areas of the skin and underlying tissue. (A) Painless ulcer with a clean base in a traveler to Peru with New World cutaneous leishmaniasis due to *Leishmania braziliensis.* (B) More nodular and inflammatory lesions with crusting but only slight ulceration in a traveler to Afghanistan, which is more characteristic of Old World cutaneous leishmaniasis due to *Leishmania major.* (C) Painless nasal perforation, which is often the earliest manifestation of mucocutaneous leishmaniasis due to metastatic spread of *L. braziliensis* from an earlier cutaneous lesion. (D) and (E), Cutaneous larva migrans or creeping eruption due to the canine hookworm *Ancylostoma caninum.* (F) Furuncular myiasis due to *Dermatobia hominis* (botfly). Patients often report a sense of movement inside; note tiny hole for the respiratory spicule of the botfly. (G) *D. hominis* larva after migration to surface when the respiratory spicule was blocked with petroleum jelly. (H) Characteristic multilesion presentation of African furuncular myiasis due to *Cordylobia anthropophaga* (tumbu fly). (I) Phytophotodermatitis in a traveler to Ecuador after application of a lime juice–containing mixture by a shaman during a native ceremony; the same effect is seen when common tropical cocktails are spilled on sun-exposed areas. Pigmented lesions may take weeks to resolve.

as painless subcutaneous nodules. Arthropod bites and infestations, such as scabies, fleas, lice, and mites, present similarly as in nontropical environments. Onchocerciasis presents as an intensely pruritic, evanescent papular rash.[105,106] Varicella, measles, or other childhood exanthems occur in nonimmune travelers and should not be forgotten in the quest for exotic diagnoses. Seabather's eruption (sometimes called "sea lice") is a pruritic papular rash notable for being distributed only on skin covered by the patient's bathing suit.[107] Larval sea anemones become trapped by the fabric while the patient is swimming. The indurated erythematous chancre of *Trypanosoma brucei rhodesiense* infection (see Chapter 277) should not be overlooked.[108] Arboviral eruptions usually present as acute febrile illnesses and not as predominant rash illnesses. HIV infection and sexually transmitted diseases should always be considered as a cause of exanthems and ulcerative lesions.



FIG. 319.2, cont'd (J) Tropical pyomyositis in a traveler to the Amazon. Pyomyositis due to deep staphylococcal infection is common in moist, warm climates and is characterized by brown pus as the muscle fibers dissolve. Initial lesions are characterized by exquisitely painful, localized erythematous areas overlying the affected muscle. *(Courtesy Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru; and Gorgas Memorial Institute, University of Alabama, Birmingham, AL.)*

TABLE 319.3 Parasitic Causes of Eosinophilia

WIDESPREAD GEOGRAPHIC DISTRIBUTION	LIMITED GEOGRAPHIC DISTRIBUTION
Ascariasis (migratory phase)	Clonorchiasis*
Hookworm*	Paragonimiasis*
Strongyloidiasis†,‡	Fascioliasis*
Tropical pulmonary eosinophilia†	Angiostrongyliasis
Lymphatic filariasis	Opisthorchiasis*
Schistosomiasis	Onchocerciasis, loiasis, and other nonlymphatic filariases
Toxocariasis†	Gnathostomiasis
Cysticercosis *(Taenia solium)*	Capillariasis
Echinococcosis (cyst rupture)	Trichostrongyliasis
Trichinellosis†	
Trichuriasis	
Aberrant helminthiasis from animals	

*Moderate to marked during larval migration in early infection; most often absent or very mild during chronic infection.
†Most frequent parasitic causes of massive eosinophilia (>5000/mm^3).
‡Absent in disseminated infection in compromised hosts.

EOSINOPHILIA

In addition to parasitic causes, peripheral blood eosinophilia may be associated with a variety of dermatologic, immunologic, inflammatory, neoplastic, and idiopathic causes. Returning travelers and long-term residents of tropical countries are as prone to nonparasitic causes of eosinophilia as is the general population, and these must be considered when obtaining a history and initiating a diagnostic workup in a returned traveler. Schistosomiasis and strongyloidiasis are the most common parasitic causes of significant eosinophilia in returning travelers, and serology should be sent on every traveler with eosinophilia and potential exposure to either.[109–111]

Eosinophilia is a reaction to a tissue-invasive helminth, with its intensity being proportional to the degree of tissue invasion. During the initial larval migration phase after a new infection with a specific parasite (e.g., hookworm), there may be an intense eosinophilia (up to 5000/mm^3). Weeks or months later, when the mature adults reside in the intestine with only minimal tissue contact, eosinophilia will be mild or absent. Although eosinophilia is atypical of protozoan infection, local eosinophilic infiltrates exceptionally occur in areas of the intestinal tract penetrated by *E. histolytica, G. lamblia,* or *Cystoisospora belli.* In addition, in a returned traveler with eosinophilia and a clinical syndrome compatible with trichinellosis (e.g., fever, myositis), particularly from Southeast Asia, the protozoal infection sarcocystosis could be considered.[112]

Although most laboratory reports express the eosinophil count as a percentage of the total white blood cell count, this practice can make it difficult to follow serial determinations in an individual patient. The absolute eosinophil count can be calculated easily and ranges from 0 to 350/mm^3 (mean, 120/mm^3). Because the list of helminths inducing eosinophilia (Table 319.3) is extensive and because many of the parasitologic and serologic techniques required for specific diagnosis are laborious and expensive, a well-obtained epidemiologic history is needed to narrow the differential diagnosis down to a manageable size. Some helpful physical findings are dermatitis (onchocerciasis, cutaneous larva migrans, larva currens), migratory swellings (loiasis, gnathostomiasis), wheezing or cough (*Strongyloides,* hookworm, *Ascaris,* or *Schistosoma* larvae in the lung), hemoptysis *(Paragonimus),* hepatomegaly *(Toxocara, Echinococcus),* lymphedema (filariasis), myositis (trichinellosis, sarcocystosis), subcutaneous mass (cysticercosis, sparganosis), meningeal signs (angiostrongyliasis, gnathostomiasis), and abdominal tenderness (angiostrongyliasis, anisakiasis, fascioliasis).

The examination of stools for ova and parasites is the first step and, unfortunately, this crucial diagnostic procedure is dependent on the expertise of the individual laboratory. A concentration technique should be used and at least three separate stools examined. Eggs are produced only by mature adult worms, so stool examinations will be negative during the initial larval migratory phase of intestinal helminths for up to 6 weeks after exposure. *Strongyloides* eggs hatch while still in the intestine, and Baermann concentration or agar plate cultures are indicated if suspicion is high. Because most anthelmintic drugs only work on adult worms and not immature larvae, empirical therapy for a traveler with eosinophilia soon after return is of no benefit.

The following ancillary procedures are indicated when epidemiologically appropriate[113] or when dictated by specific symptoms: day and night blood concentrations (filariasis), skin snips (onchocerciasis), rectal snips or scrapings (schistosomiasis), urine concentration (schistosomiasis), duodenal aspirate (strongyloidiasis), sputum tests for ova and parasites (migrating larvae, *Strongyloides, Paragonimus*), and biopsy of any abnormal lesions. Serology is available for many of the common helminthic infections but is hampered by lack of standardization and broad cross-reactivity among many helminth species. Nevertheless, an unequivocally elevated parasite-specific serum IgG level can be extremely helpful when positive in the setting of a previously naïve traveler with a history of exposure to only one or a few specific parasites. Schistosomiasis and strongyloidiasis are the two most common causes of parasitic eosinophilia. Stool and more invasive examination are often negative, and diagnosis often depends on positive serology.

The detection of one parasitic infection does not preclude the presence of another. All individuals should complete the diagnostic workup that is clinically and epidemiologically indicated. Similarly, all treated patients should be observed to be certain that both infection and eosinophilia have resolved. An anomalous exacerbation of the eosinophilia may occur for 2 to 3 weeks after treatment as parasites die and release their antigens. Eosinophilia may not totally resolve for 6 months or more after adequate treatment of the inciting helminth, but no response whatsoever for a month or more after treatment may be a sign of inadequate response to treatment.

SCREENING FOR ASYMPTOMATIC INFECTION

Completely asymptomatic returned travelers may present with a request to be checked for possible tropical disease. The limited number of available cost-effectiveness studies have yet to show significant benefit to this approach on a population basis.[114–116] Neither a clinic visit nor any nondirected laboratory screening of returned very short-term travelers is indicated. Exceptions are those with known discrete high-risk exposure events in situations conducive to transmission of specific agents. This would include testing for HIV and other sexually transmitted infections, a purified protein derivative skin test or interferon-γ (IFN-γ) release assay, or *Schistosoma* serology. For those who have spent 6 months or more under any conditions in a developing country, a purified protein derivative skin test or IFN-γ release assay is the highest priority even

without a specific exposure. For those living under harsher conditions, any abnormalities found on a complete physical examination, including a dermatologic assessment, that would lead to specific laboratory testing should be sought first. For general screening, a stool sample for ova and parasite testing and an eosinophil count are used by most. Serologic studies for schistosomiasis, filarial infection, and strongyloidiasis are often performed but should be strictly limited to those with extended travel to a known endemic area for each respective pathogen. Those with new sexual partners should be screened for HIV and sexually transmitted infections at an appropriate interval after last potential exposure. Malaria smears are not indicated in asymptomatic travelers, even those with a remote history of malaria exposure during the travel, but primaquine treatment for those at risk for later relapse of disease due to *P. vivax* or *P. ovale* is indicated.

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The complete reference list is available online at Expert Consult.

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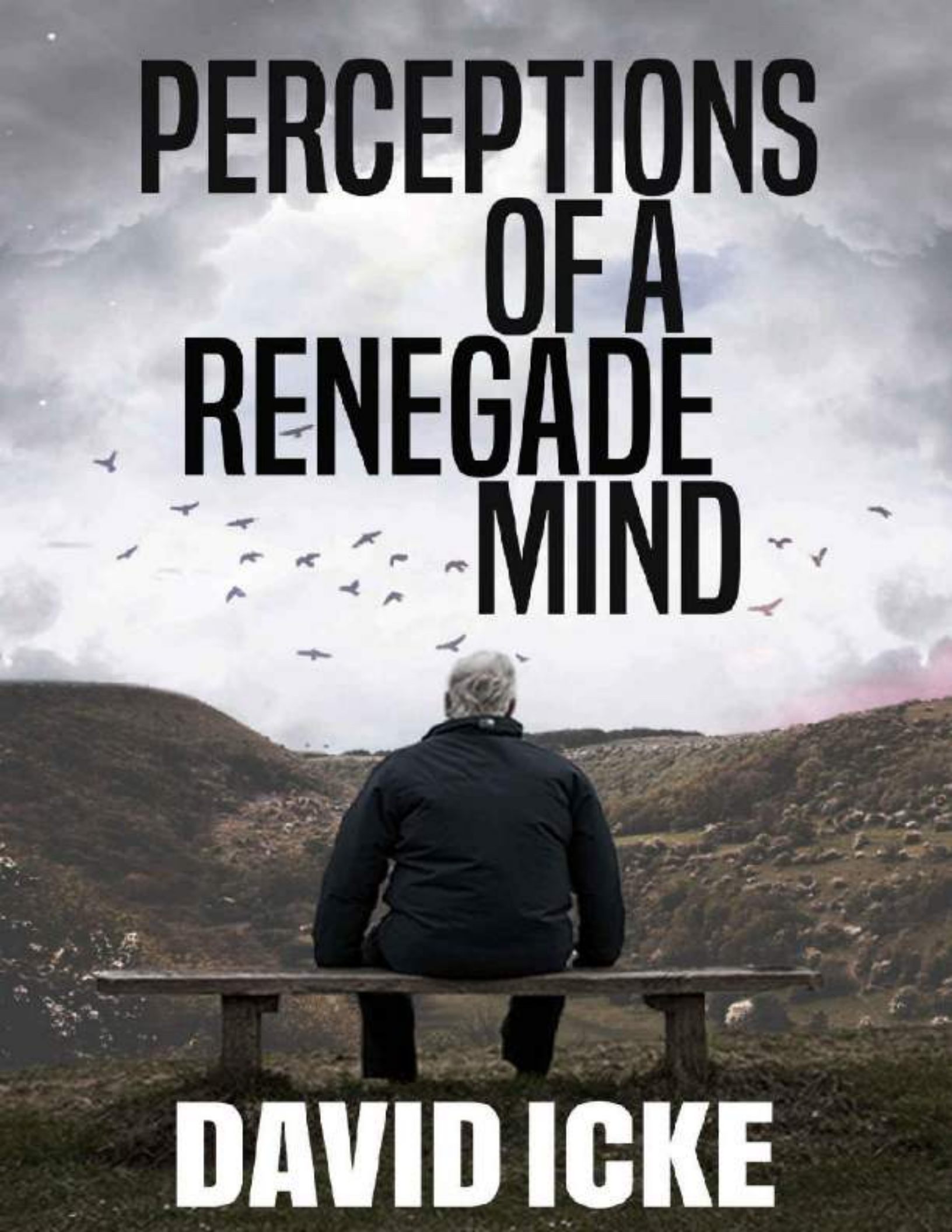

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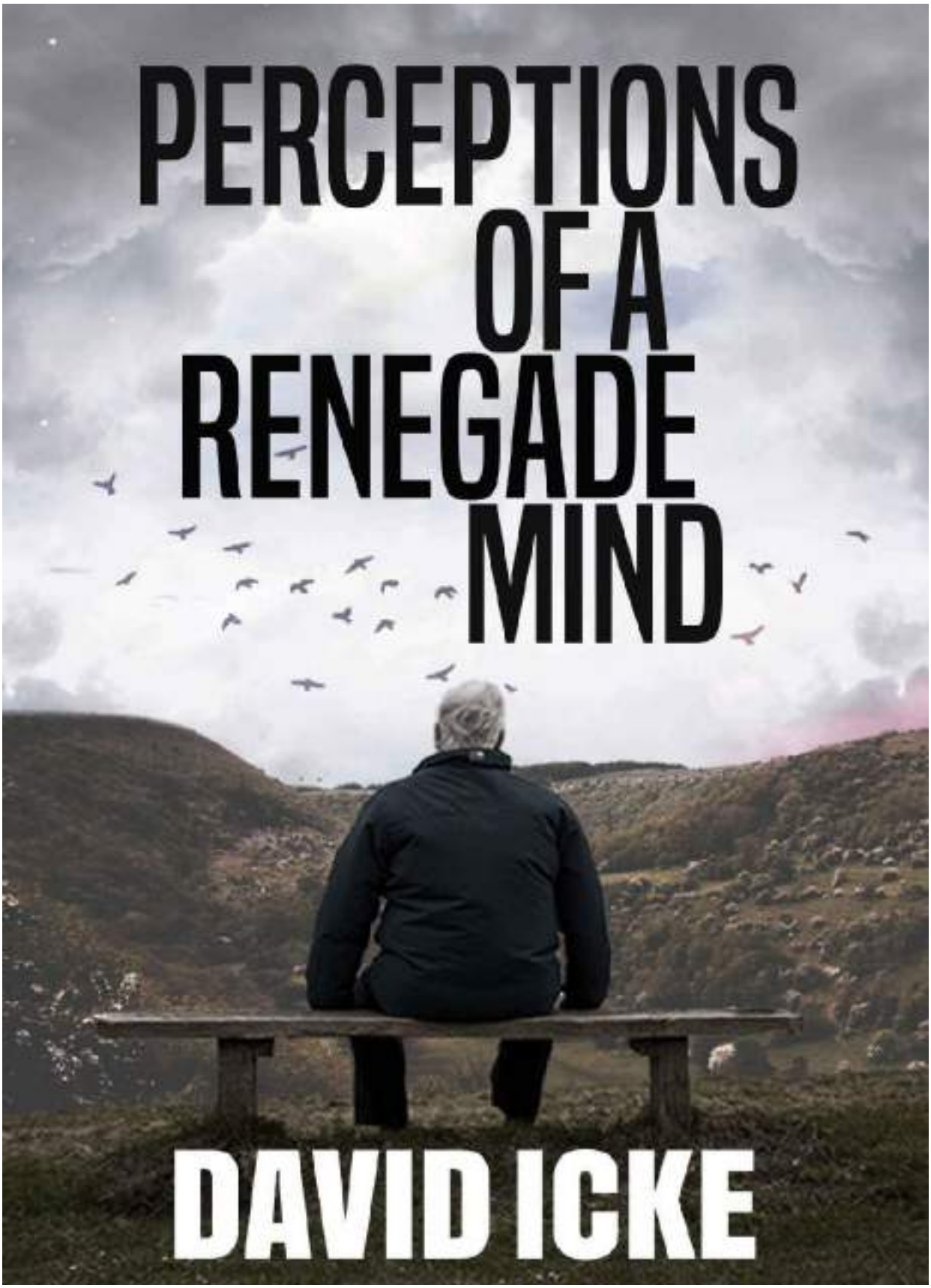
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ickonic
publishing

First published in July 2021.

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publishing

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St Helens Street
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DE1 3GY
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Cover Design: Gareth Icke
Book Design: Neil Hague

British Library Cataloguing-in Publication Data
A catalogue record for this book is available from the British Library

eISBN 978-18384153-1-0

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Merriam-Webster Dictionary

Acquiescence to tyranny is the death of the spirit

You may be 38 years old, as I happen to be. And one day, some great opportunity stands before you and calls you to stand up for some great principle, some great issue, some great cause. And you refuse to do it because you are afraid … You refuse to do it because you want to live longer … You're afraid that you will lose your job, or you are afraid that you will be criticised or that you will lose your popularity, or you're afraid that somebody will stab you, or shoot at you or bomb your house; so you refuse to take the stand.

Well, you may go on and live until you are 90, but you're just as dead at 38 as you would be at 90. And the cessation of breathing in your life is but the belated announcement of an earlier death of the spirit.

Martin Luther King

How the few control the many and always have – the many do whatever they're told

'Forward, the Light Brigade!'
Was there a man dismayed?
Not though the soldier knew
Someone had blundered.
Theirs not to make reply,
Theirs not to reason why,
Theirs but to do and die.
Into the valley of Death
Rode the six hundred.

Cannon to right of them,
Cannon to left of them,
Cannon in front of them
Volleyed and thundered;
Stormed at with shot and shell,
Boldly they rode and well,
Into the jaws of Death,
Into the mouth of hell
Rode the six hundred

Alfred Lord Tennyson (1809-1892)

The mist is lifting slowly
I can see the way ahead
And I've left behind the empty streets
That once inspired my life
And the strength of the emotion
Is like thunder in the air
'Cos the promise that we made each other
Haunts me to the end

The secret of your beauty
And the mystery of your soul
I've been searching for in everyone I meet
And the times I've been mistaken
It's impossible to say
And the grass is growing
Underneath our feet

The words that I remember
From my childhood still are true
That there's none so blind
As those who will not see
And to those who lack the courage
And say it's dangerous to try
Well they just don't know
That love eternal will not be denied

I know you're out there somewhere
Somewhere, somewhere
I know you're out there somewhere

Somewhere you can hear my voice
I know I'll find you somehow
Somehow, somehow
I know I'll find you somehow
And somehow I'll return again to you

The Moody Blues

Are you a gutless wonder - or a Renegade Mind?

Monuments put from pen to paper,
Turns me into a gutless wonder,
And if you tolerate this,
Then your children will be next.
Gravity keeps my head down,
Or is it maybe shame ...

Manic Street Preachers

Rise like lions after slumber
In unvanquishable number.
Shake your chains to earth like dew
Which in sleep have fallen on you.
Ye are many – they are few.

Percy Shelley

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CHAPTER ONE

I'm thinking' – Oh, but *are* you?

Think for yourself and let others enjoy the privilege of doing so too
Voltaire

French-born philosopher, mathematician and scientist René Descartes became famous for his statement in Latin in the 17th century which translates into English as: 'I think, therefore I am.'

On the face of it that is true. Thought reflects perception and perception leads to both behaviour and self-identity. In that sense 'we' are what we think. But who or what is doing the thinking and is thinking the only route to perception? Clearly, as we shall see, 'we' are not always the source of 'our' perception, indeed with regard to humanity as a whole this is rarely the case; and thinking is far from the only means of perception. Thought is the village idiot compared with other expressions of consciousness that we all have the potential to access and tap into. This has to be true when we *are* those other expressions of consciousness which are infinite in nature. We have forgotten this, or, more to the point, been manipulated to forget.

These are not just the esoteric musings of the navel. The whole foundation of human control and oppression is control of perception. Once perception is hijacked then so is behaviour which is dictated by perception. Collective perception becomes collective behaviour and collective behaviour is what we call human society. Perception is all and those behind human control know that which is

why perception is the target 24/7 of the psychopathic manipulators that I call the Global Cult. They know that if they dictate perception they will dictate behaviour and collectively dictate the nature of human society. They are further aware that perception is formed from information received and if they control the circulation of information they will to a vast extent direct human behaviour. Censorship of information and opinion has become globally Nazi-like in recent years and never more blatantly than since the illusory 'virus pandemic' was triggered out of China in 2019 and across the world in 2020. Why have billions submitted to house arrest and accepted fascistic societies in a way they would have never believed possible? Those controlling the information spewing from government, mainstream media and Silicon Valley (all controlled by the same Global Cult networks) told them they were in danger from a 'deadly virus' and only by submitting to house arrest and conceding their most basic of freedoms could they and their families be protected. This monumental and provable lie became the *perception* of the billions and therefore the *behaviour* of the billions. In those few words you have the whole structure and modus operandi of human control. Fear is a perception – **F**alse **E**motion **A**ppearing **R**eal – and fear is the currency of control. In short … get them by the balls (or give them the impression that you have) and their hearts and minds will follow. Nothing grips the dangly bits and freezes the rear-end more comprehensively than fear.

World number 1

There are two 'worlds' in what appears to be one 'world' and the prime difference between them is knowledge. First we have the mass of human society in which the population is maintained in coldly-calculated ignorance through control of information and the 'education' (indoctrination) system. That's all you really need to control to enslave billions in a perceptual delusion in which what are perceived to be *their* thoughts and opinions are ever-repeated mantras that the system has been downloading all their lives through 'education', media, science, medicine, politics and academia

in which the personnel and advocates are themselves overwhelmingly the perceptual products of the same repetition. Teachers and academics in general are processed by the same programming machine as everyone else, but unlike the great majority they never leave the 'education' program. It gripped them as students and continues to grip them as programmers of subsequent generations of students. The programmed become the programmers – the programmed programmers. The same can largely be said for scientists, doctors and politicians and not least because as the American writer Upton Sinclair said: 'It is difficult to get a man to understand something when his salary depends upon his not understanding it.' If your career and income depend on thinking the way the system demands then you will – bar a few free-minded exceptions – concede your mind to the Perceptual Mainframe that I call the Postage Stamp Consensus. This is a tiny band of perceived knowledge and possibility 'taught' (downloaded) in the schools and universities, pounded out by the mainstream media and on which all government policy is founded. Try thinking, and especially speaking and acting, outside of the 'box' of consensus and see what that does for your career in the Mainstream Everything which bullies, harasses, intimidates and ridicules the population into compliance. Here we have the simple structure which enslaves most of humanity in a perceptual prison cell for an entire lifetime and I'll go deeper into this process shortly. Most of what humanity is taught as fact is nothing more than programmed belief. American science fiction author Frank Herbert was right when he said: 'Belief can be manipulated. Only knowledge is dangerous.' In the 'Covid' age belief is promoted and knowledge is censored. It was always so, but never to the extreme of today.

World number 2

A 'number 2' is slang for 'doing a poo' and how appropriate that is when this other 'world' is doing just that on humanity every minute of every day. World number 2 is a global network of secret societies and semi-secret groups dictating the direction of society via

governments, corporations and authorities of every kind. I have spent more than 30 years uncovering and exposing this network that I call the Global Cult and knowing its agenda is what has made my books so accurate in predicting current and past events. Secret societies are secret for a reason. They want to keep their hoarded knowledge to themselves and their chosen initiates and to hide it from the population which they seek through ignorance to control and subdue. The whole foundation of the division between World 1 and World 2 is *knowledge*. What number 1 knows number 2 must not. Knowledge they have worked so hard to keep secret includes (a) the agenda to enslave humanity in a centrally-controlled global dictatorship, and (b) the nature of reality and life itself. The latter (b) must be suppressed to allow the former (a) to prevail as I shall be explaining. The way the Cult manipulates and interacts with the population can be likened to a spider's web. The 'spider' sits at the centre in the shadows and imposes its will through the web with each strand represented in World number 2 by a secret society, satanic or semi-secret group, and in World number 1 – the world of the seen – by governments, agencies of government, law enforcement, corporations, the banking system, media conglomerates and Silicon Valley (Fig 1 overleaf). The spider and the web connect and coordinate all these organisations to pursue the same global outcome while the population sees them as individual entities working randomly and independently. At the level of the web governments *are* the banking system *are* the corporations *are* the media *are* Silicon Valley *are* the World Health Organization working from their inner cores as one unit. Apparently unconnected countries, corporations, institutions, organisations and people are on the *same team* pursuing the same global outcome. Strands in the web immediately around the spider are the most secretive and exclusive secret societies and their membership is emphatically restricted to the Cult inner-circle emerging through the generations from particular bloodlines for reasons I will come to. At the core of the core you would get them in a single room. That's how many people are dictating the direction of human society and its transformation

through the ‘Covid’ hoax and other means. As the web expands out from the spider we meet the secret societies that many people will be aware of – the Freemasons, Knights Templar, Knights of Malta, Opus Dei, the inner sanctum of the Jesuit Order, and such like. Note how many are connected to the Church of Rome and there is a reason for that. The Roman Church was established as a revamp, a rebranding, of the relocated ‘Church’ of Babylon and the Cult imposing global tyranny today can be tracked back to Babylon and Sumer in what is now Iraq.



Figure 1: The global web through which the few control the many. (Image Neil Hague.)

Inner levels of the web operate in the unseen away from the public eye and then we have what I call the cusp organisations located at the point where the hidden meets the seen. They include a series of satellite organisations answering to a secret society founded in London in the late 19th century called the Round Table and among them are the Royal Institute of International Affairs (UK, founded in 1920); Council on Foreign Relations (US, 1921); Bilderberg Group (worldwide, 1954); Trilateral Commission (US/worldwide, 1972); and the Club of Rome (worldwide, 1968) which was created to exploit environmental concerns to justify the centralisation of global power to ‘save the planet’. The Club of Rome instigated with others the human-caused climate change hoax which has led to all the ‘green

new deals' demanding that very centralisation of control. Cusp organisations, which include endless 'think tanks' all over the world, are designed to coordinate a single global policy between political and business leaders, intelligence personnel, media organisations and anyone who can influence the direction of policy in their own sphere of operation. Major players and regular attenders will know what is happening – or some of it – while others come and go and are kept overwhelmingly in the dark about the big picture. I refer to these cusp groupings as semi-secret in that they can be publicly identified, but what goes on at the inner-core is kept very much 'in house' even from most of their members and participants through a fiercely-imposed system of compartmentalisation. Only let them know what they need to know to serve your interests and no more. The structure of secret societies serves as a perfect example of this principle. Most Freemasons never get higher than the bottom three levels of 'degree' (degree of knowledge) when there are 33 official degrees of the Scottish Rite. Initiates only qualify for the next higher 'compartment' or degree if those at that level choose to allow them. Knowledge can be carefully assigned only to those considered 'safe'. I went to my local Freemason's lodge a few years ago when they were having an 'open day' to show how cuddly they were and when I chatted to some of them I was astonished at how little the rank and file knew even about the most ubiquitous symbols they use. The mushroom technique – keep them in the dark and feed them bullshit – applies to most people in the web as well as the population as a whole. Sub-divisions of the web mirror in theme and structure transnational corporations which have a headquarters somewhere in the world dictating to all their subsidiaries in different countries. Subsidiaries operate in their methodology and branding to the same centrally-dictated plan and policy in pursuit of particular ends. The Cult web functions in the same way. Each country has its own web as a subsidiary of the global one. They consist of networks of secret societies, semi-secret groups and bloodline families and their job is to impose the will of the spider and the global web in their particular country. Subsidiary networks control and manipulate the national political system, finance, corporations, media, medicine, etc. to

ensure that they follow the globally-dictated Cult agenda. These networks were the means through which the 'Covid' hoax could be played out with almost every country responding in the same way.

The 'Yessir' pyramid

Compartmentalisation is the key to understanding how a tiny few can dictate the lives of billions when combined with a top-down sequence of imposition and acquiescence. The inner core of the Cult sits at the peak of the pyramidal hierarchy of human society (Fig 2 overleaf). It imposes its will – its agenda for the world – on the level immediately below which acquiesces to that imposition. This level then imposes the Cult will on the level below them which acquiesces and imposes on the next level. Very quickly we meet levels in the hierarchy that have no idea there even is a Cult, but the sequence of imposition and acquiescence continues down the pyramid in just the same way. 'I don't know why we are doing this but the order came from "on-high" and so we better just do it.' Alfred Lord Tennyson said of the cannon fodder levels in his poem *The Charge of the Light Brigade*: 'Theirs not to reason why; theirs but to do and die.' The next line says that 'into the valley of death rode the six hundred' and they died because they obeyed without question what their perceived 'superiors' told them to do. In the same way the population capitulated to 'Covid'. The whole hierarchical pyramid functions like this to allow the very few to direct the enormous many. Eventually imposition-acquiescence-imposition-acquiescence comes down to the mass of the population at the foot of the pyramid. If they acquiesce to those levels of the hierarchy imposing on them (governments/law enforcement/doctors/media) a circuit is completed between the population and the handful of super-psychopaths in the Cult inner core at the top of the pyramid. Without a circuit-breaking refusal to obey, the sequence of imposition and acquiescence allows a staggeringly few people to impose their will upon the entirety of humankind. We are looking at the very sequence that has subjugated billions since the start of 2020. Our freedom has not been taken from us. Humanity has given it

away. Fascists do not impose fascism because there are not enough of them. Fascism is imposed by the population acquiescing to fascism. Put another way allowing their perceptions to be programmed to the extent that leads to the population giving their freedom away by giving their perceptions – their mind – away. If this circuit is not broken by humanity ceasing to cooperate with their own enslavement then nothing can change. For that to happen people have to critically think and see through the lies and window dressing and then summon the backbone to act upon what they see. The Cult spends its days working to stop either happening and its methodology is systematic and highly detailed, but it can be overcome and that is what this book is all about.

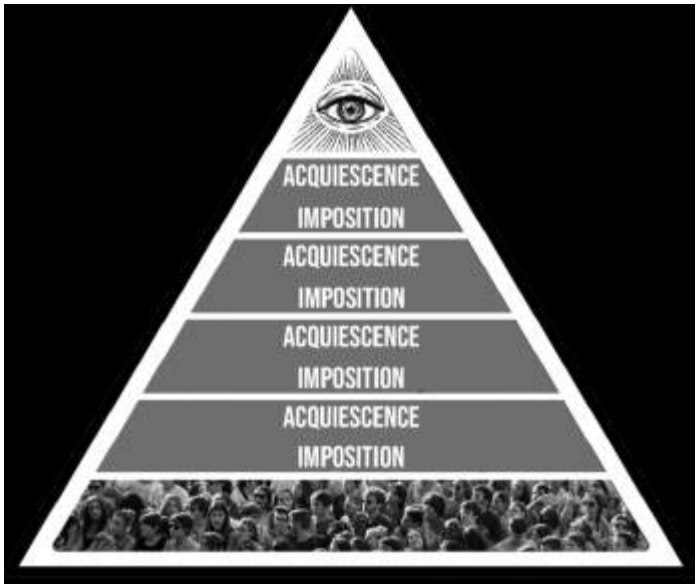

Figure 2: The simple sequence of imposition and compliance that allows a handful of people at the peak of the pyramid to dictate the lives of billions.

The Life Program

Okay, back to world number 1 or the world of the 'masses'. Observe the process of what we call 'life' and it is a perceptual download from cradle to grave. The Cult has created a global structure in which perception can be programmed and the program continually topped-up with what appears to be constant confirmation that the program is indeed true reality. The important word here is 'appears'.

This is the structure, the fly-trap, the Postage Stamp Consensus or Perceptual Mainframe, which represents that incredibly narrow band of perceived possibility delivered by the 'education' system, mainstream media, science and medicine. From the earliest age the download begins with parents who have themselves succumbed to the very programming their children are about to go through. Most parents don't do this out of malevolence and mostly it is quite the opposite. They do what they believe is best for their children and that is what the program has told them is best. Within three or four years comes the major transition from parental programming to full-blown state (Cult) programming in school, college and university where perceptually-programmed teachers and academics pass on their programming to the next generations. Teachers who resist are soon marginalised and their careers ended while children who resist are called a problem child for whom Ritalin may need to be prescribed. A few years after entering the 'world' children are under the control of authority figures representing the state telling them when they have to be there, when they can leave and when they can speak, eat, even go to the toilet. This is calculated preparation for a lifetime of obeying authority in all its forms. Reflex-action fear of authority is instilled by authority from the start. Children soon learn the carrot and stick consequences of obeying or defying authority which is underpinned daily for the rest of their life. Fortunately I daydreamed through this crap and never obeyed authority simply because it told me to. This approach to my alleged 'betters' continues to this day. There can be consequences of pursuing open-minded freedom in a world of closed-minded conformity. I spent a lot of time in school corridors after being ejected from the classroom for not taking some of it seriously and now I spend a lot of time being ejected from Facebook, YouTube and Twitter. But I can tell you that being true to yourself and not compromising your self-respect is far more exhilarating than bowing to authority for authority's sake. You don't have to be a sheep to the shepherd (authority) and the sheep dog (fear of not obeying authority).

The perceptual download continues throughout the formative years in school, college and university while script-reading 'teachers', 'academics' 'scientists', 'doctors' and 'journalists' insist that ongoing generations must be as programmed as they are. Accept the program or you will not pass your 'exams' which confirm your 'degree' of programming. It is tragic to think that many parents pressure their offspring to work hard at school to download the program and qualify for the next stage at college and university. The late, great, American comedian George Carlin said: 'Here's a bumper sticker I'd like to see: We are proud parents of a child who has resisted his teachers' attempts to break his spirit and bend him to the will of his corporate masters.' Well, the best of luck finding many of those, George. Then comes the moment to leave the formal programming years in academia and enter the 'adult' world of work. There you meet others in your chosen or prescribed arena who went through the same Postage Stamp Consensus program before you did. There is therefore overwhelming agreement between almost everyone on the basic foundations of Postage Stamp reality and the rejection, even contempt, of the few who have a mind of their own and are prepared to use it. This has two major effects. Firstly, the consensus confirms to the programmed that their download is really how things are. I mean, everyone knows that, right? Secondly, the arrogance and ignorance of Postage Stamp adherents ensure that anyone questioning the program will have unpleasant consequences for seeking their own truth and not picking their perceptions from the shelf marked: 'Things you must believe without question and if you don't you're a dangerous lunatic conspiracy theorist and a harebrained nutter'.

Every government, agency and corporation is founded on the same Postage Stamp prison cell and you can see why so many people believe the same thing while calling it their own 'opinion'. Fusion of governments and corporations in pursuit of the same agenda was the definition of fascism described by Italian dictator Benito Mussolini. The pressure to conform to perceptual norms downloaded for a lifetime is incessant and infiltrates society right

down to family groups that become censors and condemners of their own 'black sheep' for not, ironically, being sheep. We have seen an explosion of that in the 'Covid' era. Cult-owned global media unleashes its propaganda all day every day in support of the Postage Stamp and targets with abuse and ridicule anyone in the public eye who won't bend their mind to the will of the tyranny. Any response to this is denied (certainly in my case). They don't want to give a platform to expose official lies. Cult-owned-and-created Internet giants like Facebook, Google, YouTube and Twitter delete you for having an unapproved opinion. Facebook boasts that its AI censors delete 97-percent of 'hate speech' before anyone even reports it. Much of that 'hate speech' will simply be an opinion that Facebook and its masters don't want people to see. Such perceptual oppression is widely known as fascism. Even Facebook executive Benny Thomas, a 'CEO Global Planning Lead', said in comments secretly recorded by investigative journalism operation Project Veritas that Facebook is 'too powerful' and should be broken up:

I mean, no king in history has been the ruler of two billion people, but Mark Zuckerberg is … And he's 36. That's too much for a 36-year-old ... You should not have power over two billion people. I just think that's wrong.

Thomas said Facebook-owned platforms like Instagram, Oculus, and WhatsApp needed to be separate companies. 'It's too much power when they're all one together'. That's the way the Cult likes it, however. We have an executive of a Cult organisation in Benny Thomas that doesn't know there is a Cult such is the compartmentalisation. Thomas said that Facebook and Google 'are no longer companies, they're countries'. Actually they are more powerful than countries on the basis that if you control information you control perception and control human society.

I love my oppressor

Another expression of this psychological trickery is for those who realise they are being pressured into compliance to eventually

convince themselves to believe the official narratives to protect their self-respect from accepting the truth that they have succumbed to meek and subservient compliance. Such people become some of the most vehement defenders of the system. You can see them everywhere screaming abuse at those who prefer to think for themselves and by doing so reminding the compliers of their own capitulation to conformity. 'You are talking dangerous nonsense you Covidiot!!' Are you trying to convince me or yourself? It is a potent form of Stockholm syndrome which is defined as: 'A psychological condition that occurs when a victim of abuse identifies and attaches, or bonds, positively with their abuser.' An example is hostages bonding and even 'falling in love' with their kidnappers. The syndrome has been observed in domestic violence, abused children, concentration camp inmates, prisoners of war and many and various Satanic cults. These are some traits of Stockholm syndrome listed at goodtherapy.org:

- Positive regard towards perpetrators of abuse or captor [see 'Covid'].
- Failure to cooperate with police and other government authorities when it comes to holding perpetrators of abuse or kidnapping accountable [or in the case of 'Covid' cooperating with the police to enforce and defend their captors' demands].
- Little or no effort to escape [see 'Covid'].
- Belief in the goodness of the perpetrators or kidnappers [see 'Covid'].
- Appeasement of captors. This is a manipulative strategy for maintaining one's safety. As victims get rewarded – perhaps with less abuse or even with life itself – their appeasing behaviours are reinforced [see 'Covid'].
- Learned helplessness. This can be akin to 'if you can't beat 'em, join 'em'. As the victims fail to escape the abuse or captivity, they may start giving up and soon realize it's just easier for everyone if they acquiesce all their power to their captors [see 'Covid'].

- Feelings of pity toward the abusers, believing they are actually victims themselves. Because of this, victims may go on a crusade or mission to 'save' [protect] their abuser [see the venom unleashed on those challenging the official 'Covid' narrative].
- Unwillingness to learn to detach from their perpetrators and heal. In essence, victims may tend to be less loyal to themselves than to their abuser [*definitely* see 'Covid'].

Ponder on those traits and compare them with the behaviour of great swathes of the global population who have defended governments and authorities which have spent every minute destroying their lives and livelihoods and those of their children and grandchildren since early 2020 with fascistic lockdowns, house arrest and employment deletion to 'protect' them from a 'deadly virus' that their abusers' perceptually created to bring about this very outcome. We are looking at mass Stockholm syndrome. All those that agree to concede their freedom will believe those perceptions are originating in their own independent 'mind' when in fact by conceding their reality to Stockholm syndrome they have by definition conceded any independence of mind. Listen to the 'opinions' of the acquiescing masses in this 'Covid' era and what gushes forth is the repetition of the official version of everything delivered unprocessed, unfiltered and unquestioned. The whole programming dynamic works this way. I must be free because I'm told that I am and so I think that I am.

You can see what I mean with the chapter theme of 'I'm thinking – Oh, but *are* you?' The great majority are not thinking, let alone for themselves. They are repeating what authority has told them to believe which allows them to be controlled. Weaving through this mentality is the fear that the 'conspiracy theorists' are right and this again explains the often hysterical abuse that ensues when you dare to contest the official narrative of anything. Denial is the mechanism of hiding from yourself what you don't want to be true. Telling people what they want to hear is easy, but it's an infinitely greater challenge to tell them what they would rather not be happening.

One is akin to pushing against an open door while the other is met with vehement resistance no matter what the scale of evidence. I don't want it to be true so I'll convince myself that it's not. Examples are everywhere from the denial that a partner is cheating despite all the signs to the reflex-action rejection of any idea that world events in which country after country act in exactly the same way are centrally coordinated. To accept the latter is to accept that a force of unspeakable evil is working to destroy your life and the lives of your children with nothing too horrific to achieve that end. Who the heck wants that to be true? But if we don't face reality the end is duly achieved and the consequences are far worse and ongoing than breaking through the walls of denial today with the courage to make a stand against tyranny.

Connect the dots – but how?

A crucial aspect of perceptual programming is to portray a world in which everything is random and almost nothing is connected to anything else. Randomness cannot be coordinated by its very nature and once you perceive events as random the idea they could be connected is waved away as the rantings of the tinfoil-hat brigade. You can't plan and coordinate random you idiot! No, you can't, but you can hide the coldly-calculated and long-planned behind the *illusion* of randomness. A foundation manifestation of the Renegade Mind is to scan reality for patterns that connect the apparently random and turn pixels and dots into pictures. This is the way I work and have done so for more than 30 years. You look for similarities in people, modus operandi and desired outcomes and slowly, then ever quicker, the picture forms. For instance: There would seem to be no connection between the 'Covid pandemic' hoax and the human-caused global-warming hoax and yet they are masks (appropriately) on the same face seeking the same outcome. Those pushing the global warming myth through the Club of Rome and other Cult agencies are driving the lies about 'Covid' – Bill Gates is an obvious one, but they are endless. Why would the same people be involved in both when they are clearly not connected? Oh, but they

are. Common themes with personnel are matched by common goals. The 'solutions' to both 'problems' are centralisation of global power to impose the will of the few on the many to 'save' humanity from 'Covid' and save the planet from an 'existential threat' (we need 'zero Covid' and 'zero carbon emissions'). These, in turn, connect with the 'dot' of globalisation which was coined to describe the centralisation of global power in every area of life through incessant political and corporate expansion, trading blocks and superstates like the European Union. If you are the few and you want to control the many you have to centralise power and decision-making. The more you centralise power the more power the few at the centre will have over the many; and the more that power is centralised the more power those at the centre have to centralise even quicker. The momentum of centralisation gets faster and faster which is exactly the process we have witnessed. In this way the hoaxed 'pandemic' and the fakery of human-caused global warming serve the interests of globalisation and the seizure of global power in the hands of the Cult inner-circle which is behind 'Covid', 'climate change' *and* globalisation. At this point random 'dots' become a clear and obvious picture or pattern.

Klaus Schwab, the classic Bond villain who founded the Cult's Gates-funded World Economic Forum, published a book in 2020, *The Great Reset*, in which he used the 'problem' of 'Covid' to justify a total transformation of human society to 'save' humanity from 'climate change'. Schwab said: 'The pandemic represents a rare but narrow window of opportunity to reflect, reimagine, and reset our world.' What he didn't mention is that the Cult he serves is behind both hoaxes as I show in my book *The Answer*. He and the Cult don't have to reimagine the world. They know precisely what they want and that's why they destroyed human society with 'Covid' to 'build back better' in their grand design. Their job is not to imagine, but to get humanity to imagine and agree with their plans while believing it's all random. It must be pure coincidence that 'The Great Reset' has long been the Cult's code name for the global imposition of fascism and replaced previous code-names of the 'New World

Order' used by Cult frontmen like Father George Bush and the 'New Order of the Ages' which emerged from Freemasonry and much older secret societies. New Order of the Ages appears on the reverse of the Great Seal of the United States as 'Novus ordo seclorum' underneath the Cult symbol used since way back of the pyramid and all seeing-eye (Fig 3). The pyramid is the hierarchy of human control headed by the illuminated eye that symbolises the force behind the Cult which I will expose in later chapters. The term 'Annuit Coeptis' translates as 'He favours our undertaking'. We are told the 'He' is the Christian god, but 'He' is not as I will be explaining.



Figure 3: The all-seeing eye of the Cult 'god' on the Freemason-designed Great Seal of the United States and also on the dollar bill.

Having you on

Two major Cult techniques of perceptual manipulation that relate to all this are what I have called since the 1990s Problem-Reaction-Solution (PRS) and the Totalitarian Tiptoe (TT). They can be uncovered by the inquiring mind with a simple question: Who benefits? The answer usually identifies the perpetrators of a given action or happening through the concept of 'he who most benefits from a crime is the one most likely to have committed it'. The Latin 'Cue bono?' – Who benefits? – is widely attributed to the Roman orator and statesman Marcus Tullius Cicero. No wonder it goes back so far when the concept has been relevant to human behaviour since

history was recorded. Problem-Reaction-Solution is the technique used to manipulate us every day by covertly creating a problem (or the illusion of one) and offering the solution to the problem (or the illusion of one). In the first phase you create the problem and blame someone or something else for why it has happened. This may relate to a financial collapse, terrorist attack, war, global warming or pandemic, anything in fact that will allow you to impose the 'solution' to change society in the way you desire at that time. The 'problem' doesn't have to be real. PRS is manipulation of perception and all you need is the population to believe the problem is real. Human-caused global warming and the 'Covid pandemic' only have to be *perceived* to be real for the population to accept the 'solutions' of authority. I refer to this technique as NO-Problem-Reaction-Solution. Billions did not meekly accept house arrest from early 2020 because there was a real deadly 'Covid pandemic' but because they perceived – believed – that to be the case. The antidote to Problem-Reaction-Solution is to ask who benefits from the proposed solution. Invariably it will be anyone who wants to justify more control through deletion of freedom and centralisation of power and decision-making.

The two world wars were Problem-Reaction-Solutions that transformed and realigned global society. Both were manipulated into being by the Cult as I have detailed in books since the mid-1990s. They dramatically centralised global power, especially World War Two, which led to the United Nations and other global bodies thanks to the overt and covert manipulations of the Rockefeller family and other Cult bloodlines like the Rothschilds. The UN is a stalking horse for full-blown world government that I will come to shortly. The land on which the UN building stands in New York was donated by the Rockefellers and the same Cult family was behind Big Pharma scalpel and drug 'medicine' and the creation of the World Health Organization as part of the UN. They have been stalwarts of the eugenics movement and funded Hitler's race-purity expert' Ernst Rudin. The human-caused global warming hoax has been orchestrated by the Club of Rome through the UN which is

manufacturing both the 'problem' through its Intergovernmental Panel on Climate Change and imposing the 'solution' through its Agenda 21 and Agenda 2030 which demand the total centralisation of global power to 'save the world' from a climate hoax the United Nations is itself perpetrating. What a small world the Cult can be seen to be particularly among the inner circles. The bedfellow of Problem-Reaction-Solution is the Totalitarian Tiptoe which became the Totalitarian Sprint in 2020. The technique is fashioned to hide the carefully-coordinated behind the cover of apparently random events. You start the sequence at 'A' and you know you are heading for 'Z'. You don't want people to know that and each step on the journey is presented as a random happening while all the steps strung together lead in the same direction. The speed may have quickened dramatically in recent times, but you can still see the incremental approach of the Tiptoe in the case of 'Covid' as each new imposition takes us deeper into fascism. Tell people they have to do this or that to get back to 'normal', then this and this and this. With each new demand adding to the ones that went before the population's freedom is deleted until it disappears. The spider wraps its web around the flies more comprehensively with each new diktat. I'll highlight this in more detail when I get to the 'Covid' hoax and how it has been pulled off. Another prime example of the Totalitarian Tiptoe is how the Cult-created European Union went from a 'free-trade zone' to a centralised bureaucratic dictatorship through the Tiptoe of incremental centralisation of power until nations became mere administrative units for Cult-owned dark suits in Brussels.

The antidote to ignorance is knowledge which the Cult seeks vehemently to deny us, but despite the systematic censorship to that end the Renegade Mind can overcome this by vociferously seeking out the facts no matter the impediments put in the way. There is also a method of thinking and perceiving – *knowing* – that doesn't even need names, dates, place-type facts to identify the patterns that reveal the story. I'll get to that in the final chapter. All you need to know about the manipulation of human society and to what end is still out there – *at the time of writing* – in the form of books, videos

and websites for those that really want to breach the walls of programmed perception. To access this knowledge requires the abandonment of the mainstream media as a source of information in the awareness that this is owned and controlled by the Cult and therefore promotes mass perceptions that suit the Cult. Mainstream media lies all day, every day. That is its function and very reason for being. Where it does tell the truth, here and there, is only because the truth and the Cult agenda very occasionally coincide. If you look for fact and insight to the BBC, CNN and virtually all the rest of them you are asking to be conned and perceptually programmed.

Know the outcome and you'll see the journey

Events seem random when you have no idea where the world is being taken. Once you do the random becomes the carefully planned. Know the outcome and you'll see the journey is a phrase I have been using for a long time to give context to daily happenings that appear unconnected. Does a problem, or illusion of a problem, trigger a proposed 'solution' that further drives society in the direction of the outcome? Invariably the answer will be yes and the random – *abracadabra* – becomes the clearly coordinated. So what is this outcome that unlocks the door to a massively expanded understanding of daily events? I will summarise its major aspects – the fine detail is in my other books – and those new to this information will see that the world they thought they were living in is a very different place. The foundation of the Cult agenda is the incessant centralisation of power and all such centralisation is ultimately in pursuit of Cult control on a global level. I have described for a long time the planned world structure of top-down dictatorship as the Hunger Games Society. The term obviously comes from the movie series which portrayed a world in which a few living in military-protected hi-tech luxury were the overlords of a population condemned to abject poverty in isolated 'sectors' that were not allowed to interact. 'Covid' lockdowns and travel bans anyone? The 'Hunger Games' pyramid of structural control has the inner circle of the Cult at the top with pretty much the entire

population at the bottom under their control through dependency for survival on the Cult. The whole structure is planned to be protected and enforced by a military-police state (Fig 4).

Here you have the reason for the global lockdowns of the fake pandemic to coldly destroy independent incomes and livelihoods and make everyone dependent on the 'state' (the Cult that controls the 'states'). I have warned in my books for many years about the plan to introduce a 'guaranteed income' – a barely survivable pittance – designed to impose dependency when employment was destroyed by AI technology and now even more comprehensively at great speed by the 'Covid' scam. Once the pandemic was played and lockdown consequences began to delete independent income the authorities began to talk right on cue about the need for a guaranteed income and a 'Great Reset'. Guaranteed income will be presented as benevolent governments seeking to help a desperate people – desperate as a direct result of actions of the same governments. The truth is that such payments are a trap. You will only get them if you do exactly what the authorities demand including mass vaccination (genetic manipulation). We have seen this theme already in Australia where those dependent on government benefits have them reduced if parents don't agree to have their children vaccinated according to an insane health-destroying government-dictated schedule. Calculated economic collapse applies to governments as well as people. The Cult wants rid of countries through the creation of a world state with countries broken up into regions ruled by a world government and super states like the European Union. Countries must be bankrupted, too, to this end and it's being achieved by the trillions in 'rescue packages' and furlough payments, trillions in lost taxation, and money-no-object spending on 'Covid' including constant all-medium advertising (programming) which has made the media dependent on government for much of its income. The day of reckoning is coming – as planned – for government spending and given that it has been made possible by printing money and not by production/taxation there is inflation on the way that has the

potential to wipe out monetary value. In that case there will be no need for the Cult to steal your money. It just won't be worth anything (see the German Weimar Republic before the Nazis took over). Many have been okay with lockdowns while getting a percentage of their income from so-called furlough payments without having to work. Those payments are dependent, however, on people having at least a theoretical job with a business considered non-essential and ordered to close. As these business go under because they are closed by lockdown after lockdown the furlough stops and it will for everyone eventually. Then what? The 'then what?' is precisely the idea.



Figure 4: The Hunger Games Society structure I have long warned was planned and now the 'Covid' hoax has made it possible. This is the real reason for lockdowns.

Hired hands

Between the Hunger Games Cult elite and the dependent population is planned to be a vicious military-police state (a fusion of the two into one force). This has been in the making for a long time with police looking ever more like the military and carrying weapons to match. The pandemic scam has seen this process accelerate so fast as

lockdown house arrest is brutally enforced by carefully recruited fascist minds and gormless system-servers. The police and military are planned to merge into a centrally-directed world army in a global structure headed by a world government which wouldn't be elected even by the election fixes now in place. The world army is not planned even to be human and instead wars would be fought, primarily against the population, using robot technology controlled by artificial intelligence. I have been warning about this for decades and now militaries around the world are being transformed by this very AI technology. The global regime that I describe is a particular form of fascism known as a technocracy in which decisions are not made by clueless and co-opted politicians but by unelected technocrats – scientists, engineers, technologists and bureaucrats. Cult-owned-and-controlled Silicon Valley giants are examples of technocracy and they already have far more power to direct world events than governments. They are with their censorship *selecting* governments. I know that some are calling the 'Great Reset' a Marxist communist takeover, but fascism and Marxism are different labels for the same tyranny. Tell those who lived in fascist Germany and Stalinist Russia that there was a difference in the way their freedom was deleted and their lives controlled. I could call it a fascist technocracy or a Marxist technocracy and they would be equally accurate. The Hunger Games society with its world government structure would oversee a world army, world central bank and single world cashless currency imposing its will on a microchipped population (Fig 5). Scan its different elements and see how the illusory pandemic is forcing society in this very direction at great speed. Leaders of 23 countries and the World Health Organization (WHO) backed the idea in March, 2021, of a global treaty for 'international cooperation' in 'health emergencies' and nations should 'come together as a global community for peaceful cooperation that extends beyond this crisis'. Cut the Orwellian bullshit and this means another step towards global government. The plan includes a cashless digital money system that I first warned about in 1993. Right at the start of 'Covid' the deeply corrupt Tedros

Adhanom Ghebreyesus, the crooked and merely gofer 'head' of the World Health Organization, said it was possible to catch the 'virus' by touching cash and it was better to use cashless means. The claim was ridiculous nonsense and like the whole 'Covid' mind-trick it was nothing to do with 'health' and everything to do with pushing every aspect of the Cult agenda. As a result of the Tedros lie the use of cash has plummeted. The Cult script involves a single world digital currency that would eventually be technologically embedded in the body. China is a massive global centre for the Cult and if you watch what is happening there you will know what is planned for everywhere. The Chinese government is developing a digital currency which would allow fines to be deducted immediately via AI for anyone caught on camera breaking its fantastic list of laws and the money is going to be programmable with an expiry date to ensure that no one can accrue wealth except the Cult and its operatives.



Figure 5: The structure of global control the Cult has been working towards for so long and this has been enormously advanced by the 'Covid' illusion.

Serfdom is so smart

The Cult plan is far wider, extreme, and more comprehensive than even most conspiracy researchers appreciate and I will come to the true depths of deceit and control in the chapters 'Who controls the

Cult?' and 'Escaping Wetiko'. Even the world that we know is crazy enough. We are being deluged with ever more sophisticated and controlling technology under the heading of 'smart'. We have smart televisions, smart meters, smart cards, smart cars, smart driving, smart roads, smart pills, smart patches, smart watches, smart skin, smart borders, smart pavements, smart streets, smart cities, smart communities, smart environments, smart growth, smart planet ... smart *everything* around us. Smart technologies and methods of operation are designed to interlock to create a global Smart Grid connecting the entirety of human society including human minds to create a centrally-dictated 'hive' mind. 'Smart cities' is code for densely-occupied megacities of total surveillance and control through AI. Ever more destructive frequency communication systems like 5G have been rolled out without any official testing for health and psychological effects (colossal). 5G/6G/7G systems are needed to run the Smart Grid and each one becomes more destructive of body and mind. Deleting independent income is crucial to forcing people into these AI-policed prisons by ending private property ownership (except for the Cult elite). The Cult's Great Reset now openly foresees a global society in which no one will own any possessions and everything will be rented while the Cult would own literally everything under the guise of government and corporations. The aim has been to use the lockdowns to destroy sources of income on a mass scale and when the people are destitute and in unrepayable amounts of debt (problem) Cult assets come forward with the pledge to write-off debt in return for handing over all property and possessions (solution). Everything – literally everything including people – would be connected to the Internet via AI. I was warning years ago about the coming Internet of Things (IoT) in which all devices and technology from your car to your fridge would be plugged into the Internet and controlled by AI. Now we are already there with much more to come. The next stage is the Internet of Everything (IoE) which is planned to include the connection of AI to the human brain and body to replace the human mind with a centrally-controlled AI mind. Instead of perceptions

being manipulated through control of information and censorship those perceptions would come direct from the Cult through AI. What do you think? You think whatever AI decides that you think. In human terms there would be no individual 'think' any longer. Too incredible? The ravings of a lunatic? Not at all. Cult-owned crazies in Silicon Valley have been telling us the plan for years without explaining the real motivation and calculated implications. These include Google executive and 'futurist' Ray Kurzweil who highlights the year 2030 for when this would be underway. He said:

Our thinking ... will be a hybrid of biological and non-biological thinking ... humans will be able to extend their limitations and 'think in the cloud' ... We're going to put gateways to the cloud in our brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations.

As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

The sales-pitch of Kurzweil and Cult-owned Silicon Valley is that this would make us 'super-human' when the real aim is to make us post-human and no longer 'human' in the sense that we have come to know. The entire global population would be connected to AI and become the centrally-controlled 'hive-mind' of externally-delivered perceptions. The Smart Grid being installed to impose the Cult's will on the world is being constructed to allow particular locations – even one location – to control the whole global system. From these prime control centres, which absolutely include China and Israel, anything connected to the Internet would be switched on or off and manipulated at will. Energy systems could be cut, communication via the Internet taken down, computer-controlled driverless autonomous vehicles driven off the road, medical devices switched off, the potential is limitless given how much AI and Internet connections now run human society. We have seen nothing yet if we allow this to continue. Autonomous vehicle makers are working with law enforcement to produce cars designed to automatically pull over if they detect a police or emergency vehicle flashing from up to 100 feet away. At a police stop the car would be unlocked and the

window rolled down automatically. Vehicles would only take you where the computer (the state) allowed. The end of petrol vehicles and speed limiters on all new cars in the UK and EU from 2022 are steps leading to electric computerised transport over which ultimately you have no control. The picture is far bigger even than the Cult global network or web and that will become clear when I get to the nature of the 'spider'. There is a connection between all these happenings and the instigation of DNA-manipulating 'vaccines' (which aren't 'vaccines') justified by the 'Covid' hoax. That connection is the unfolding plan to transform the human body from a biological to a synthetic biological state and this is why synthetic biology is such a fast-emerging discipline of mainstream science. 'Covid vaccines' are infusing self-replicating synthetic genetic material into the cells to cumulatively take us on the Totalitarian Tiptoe from Human 1.0 to the synthetic biological Human 2.0 which will be physically and perceptually attached to the Smart Grid to one hundred percent control every thought, perception and deed. Humanity needs to wake up and *fast*.

This is the barest explanation of where the 'outcome' is planned to go but it's enough to see the journey happening all around us. Those new to this information will already see 'Covid' in a whole new context. I will add much more detail as we go along, but for the minutiae evidence see my mega-works, *The Answer, The Trigger* and *Everything You Need to Know But Have Never Been Told*.

Now – how does a Renegade Mind see the 'world'?

CHAPTER TWO

Renegade Perception

It is one thing to be clever and another to be wise
George R.R. Martin

A simple definition of the difference between a programmed mind and a Renegade Mind would be that one sees only dots while the other connects them to see the picture. Reading reality with accuracy requires the observer to (a) know the planned outcome and (b) realise that everything, but *everything*, is connected.

The entirety of infinite reality is connected – that's its very nature – and with human society an expression of infinite reality the same must apply. Simple cause and effect is a connection. The effect is triggered by the cause and the effect then becomes the cause of another effect. Nothing happens in isolation because it *can't*. Life in whatever reality is simple choice and consequence. We make choices and these lead to consequences. If we don't like the consequences we can make different choices and get different consequences which lead to other choices and consequences. The choice and the consequence are not only connected they are indivisible. You can't have one without the other as an old song goes. A few cannot control the world unless those being controlled allow that to happen – cause and effect, choice and consequence. Control – who has it and who doesn't – is a two-way process, a symbiotic relationship, involving the controller and controlled. 'They took my freedom away!!' Well, yes, but you also gave it to them. Humanity is

subjected to mass control because humanity has acquiesced to that control. This is all cause and effect and literally a case of give and take. In the same way world events of every kind are connected and the Cult works incessantly to sell the illusion of the random and coincidental to maintain the essential (to them) perception of dots that hide the picture. Renegade Minds know this and constantly scan the world for patterns of connection. This is absolutely pivotal in understanding the happenings in the world and without that perspective clarity is impossible. First you know the planned outcome and then you identify the steps on the journey – the day-by-day apparently random which, when connected in relation to the outcome, no longer appear as individual events, but as the proverbial *chain* of events leading in the same direction. I'll give you some examples:

Political puppet show

We are told to believe that politics is 'adversarial' in that different parties with different beliefs engage in an endless tussle for power. There may have been some truth in that up to a point – and only a point – but today divisions between 'different' parties are rhetorical not ideological. Even the rhetorical is fusing into one-speak as the parties eject any remaining free thinkers while others succumb to the ever-gathering intimidation of anyone with the 'wrong' opinion. The Cult is not a new phenomenon and can be traced back thousands of years as my books have documented. Its intergenerational initiates have been manipulating events with increasing effect the more that global power has been centralised. In ancient times the Cult secured control through the system of monarchy in which 'special' bloodlines (of which more later) demanded the right to rule as kings and queens simply by birthright and by vanquishing others who claimed the same birthright. There came a time, however, when people had matured enough to see the unfairness of such tyranny and demanded a say in who governed them. Note the word – *governed* them. Not served them – *governed* them, hence government defined as 'the political direction and control exercised over the

actions of the members, citizens, or inhabitants of communities, societies, and states; direction of the affairs of a state, community, etc.' Governments exercise control over rather than serve just like the monarchies before them. Bizarrely there are still countries like the United Kingdom which are ruled by a monarch *and* a government that officially answers to the monarch. The UK head of state and that of Commonwealth countries such as Canada, Australia and New Zealand is 'selected' by who in a *single family* had unprotected sex with whom and in what order. Pinch me it can't be true. Ouch! Shit, it is. The demise of monarchies in most countries offered a potential vacuum in which some form of free and fair society could arise and the Cult had that base covered. Monarchies had served its interests but they couldn't continue in the face of such widespread opposition and, anyway, replacing a 'royal' dictatorship that people could see with a dictatorship 'of the people' hiding behind the concept of 'democracy' presented far greater manipulative possibilities and ways of hiding coordinated tyranny behind the illusion of 'freedom'.

Democracy is quite wrongly defined as government selected by the population. This is not the case at all. It is government selected by *some* of the population (and then only in theory). This 'some' doesn't even have to be the majority as we have seen so often in first-past-the-post elections in which the so-called majority party wins fewer votes than the 'losing' parties combined. Democracy can give total power to a party in government from a minority of the votes cast. It's a sleight of hand to sell tyranny as freedom. Seventy-four million Trump-supporting Americans didn't vote for the 'Democratic' Party of Joe Biden in the distinctly dodgy election in 2020 and yet far from acknowledging the wishes and feelings of that great percentage of American society the Cult-owned Biden government set out from day one to destroy them and their right to a voice and opinion. Empty shell Biden and his Cult handlers said they were doing this to 'protect democracy'. Such is the level of lunacy and sickness to which politics has descended. Connect the dots and relate them to the desired outcome – a world government run by self-appointed technocrats and no longer even elected

politicians. While operating through its political agents in government the Cult is at the same time encouraging public distain for politicians by putting idiots and incompetents in theoretical power on the road to deleting them. The idea is to instil a public reaction that says of the technocrats: 'Well, they couldn't do any worse than the pathetic politicians.' It's all about controlling perception and Renegade Minds can see through that while programmed minds cannot when they are ignorant of both the planned outcome and the manipulation techniques employed to secure that end. This knowledge can be learned, however, and fast if people choose to get informed.

Politics may at first sight appear very difficult to control from a central point. I mean look at the 'different' parties and how would you be able to oversee them all and their constituent parts? In truth, it's very straightforward because of their structure. We are back to the pyramid of imposition and acquiescence. Organisations are structured in the same way as the system as a whole. Political parties are not open forums of free expression. They are hierarchies. I was a national spokesman for the British Green Party which claimed to be a different kind of politics in which influence and power was devolved; but I can tell you from direct experience – and it's far worse now – that Green parties are run as hierarchies like all the others however much they may try to hide that fact or kid themselves that it's not true. A very few at the top of all political parties are directing policy and personnel. They decide if you are elevated in the party or serve as a government minister and to do that you have to be a yes man or woman. Look at all the maverick political thinkers who never ascended the greasy pole. If you want to progress within the party or reach 'high-office' you need to fall into line and conform. Exceptions to this are rare indeed. Should you want to run for parliament or Congress you have to persuade the local or state level of the party to select you and for that you need to play the game as dictated by the hierarchy. If you secure election and wish to progress within the greater structure you need to go on conforming to what is acceptable to those running the hierarchy

from the peak of the pyramid. Political parties are perceptual gulags and the very fact that there are party 'Whips' appointed to 'whip' politicians into voting the way the hierarchy demands exposes the ridiculous idea that politicians are elected to serve the people they are supposed to represent. Cult operatives and manipulation has long seized control of major parties that have any chance of forming a government and at least most of those that haven't. A new party forms and the Cult goes to work to infiltrate and direct. This has reached such a level today that you see video compilations of 'leaders' of all parties whether Democrats, Republicans, Conservative, Labour and Green parroting the same Cult mantra of 'Build Back Better' and the 'Great Reset' which are straight off the Cult song-sheet to describe the transformation of global society in response to the Cult-instigated hoaxes of the 'Covid pandemic' and human-caused 'climate change'. To see Caroline Lucas, the Green Party MP that I knew when I was in the party in the 1980s, speaking in support of plans proposed by Cult operative Klaus Schwab representing the billionaire global elite is a real head-shaker.

Many parties – one master

The party system is another mind-trick and was instigated to change the nature of the dictatorship by swapping 'royalty' for dark suits that people believed – though now ever less so – represented their interests. Understanding this trick is to realise that a single force (the Cult) controls all parties either directly in terms of the major ones or through manipulation of perception and ideology with others. You don't need to manipulate Green parties to demand your transformation of society in the name of 'climate change' when they are obsessed with the lie that this is essential to 'save the planet'. You just give them a platform and away they go serving your interests while believing they are being environmentally virtuous. America's political structure is a perfect blueprint for how the two or multi-party system is really a one-party state. The Republican Party is controlled from one step back in the shadows by a group made up of billionaires and their gofers known as neoconservatives or Neocons.

I have exposed them in fine detail in my books and they were the driving force behind the policies of the imbecilic presidency of Boy George Bush which included 9/11 (see *The Trigger* for a comprehensive demolition of the official story), the subsequent 'war on terror' (war *of* terror) and the invasions of Afghanistan and Iraq. The latter was a No-Problem-Reaction-Solution based on claims by Cult operatives, including Bush and British Prime Minister Tony Blair, about Saddam Hussein's 'weapons of mass destruction' which did not exist as war criminals Bush and Blair well knew.

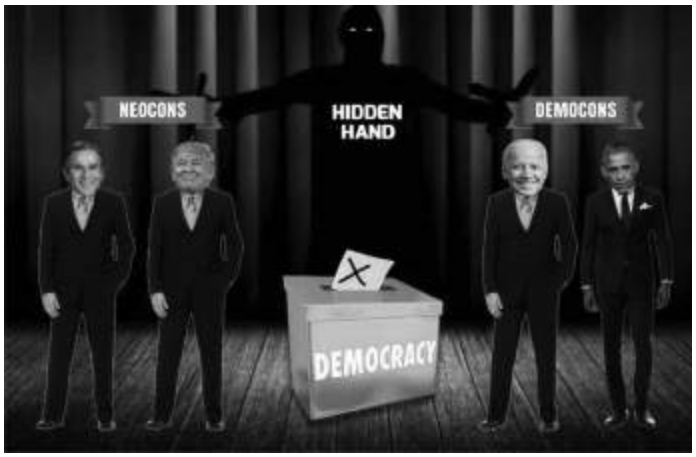

Figure 6: Different front people, different parties – same control system.

The Democratic Party has its own 'Neocon' group controlling from the background which I call the 'Democons' and here's the penny-drop – the Neocons and Democons answer to the same masters one step further back into the shadows (Fig 6). At that level of the Cult the Republican and Democrat parties are controlled by the same people and no matter which is in power the Cult is in power. This is how it works in almost every country and certainly in Britain with Conservative, Labour, Liberal Democrat and Green parties now all on the same page whatever the rhetoric may be in their feeble attempts to appear different. Neocons operated at the time of Bush through a think tank called The Project for the New American Century which in September, 2000, published a document entitled *Rebuilding America's Defenses: Strategies, Forces, and Resources*

For a New Century demanding that America fight 'multiple, simultaneous major theatre wars' as a 'core mission' to force regime-change in countries including Iraq, Libya and Syria. Neocons arranged for Bush ('Republican') and Blair ('Labour Party') to front-up the invasion of Iraq and when they departed the Democons orchestrated the targeting of Libya and Syria through Barack Obama ('Democrat') and British Prime Minister David Cameron ('Conservative Party'). We have 'different' parties and 'different' people, but the same unfolding script. The more the Cult has seized the reigns of parties and personnel the more their policies have transparently pursued the same agenda to the point where the fascist 'Covid' impositions of the Conservative junta of Jackboot Johnson in Britain were opposed by the Labour Party because they were not fascist enough. The Labour Party is likened to the US Democrats while the Conservative Party is akin to a British version of the Republicans and on both sides of the Atlantic they all speak the same language and support the direction demanded by the Cult although some more enthusiastically than others. It's a similar story in country after country because it's all centrally controlled. Oh, but what about Trump? I'll come to him shortly. Political 'choice' in the 'party' system goes like this: You vote for Party A and they get into government. You don't like what they do so next time you vote for Party B and they get into government. You don't like what they do when it's pretty much the same as Party A and why wouldn't that be with both controlled by the same force? Given that only two, sometimes three, parties have any chance of forming a government to get rid of Party B that you don't like you have to vote again for Party A which … you don't like. This, ladies and gentlemen, is what they call 'democracy' which we are told – wrongly – is a term interchangeable with 'freedom'.

The cult of cults

At this point I need to introduce a major expression of the Global Cult known as Sabbatian-Frankism. Sabbatian is also spelt as Sabbatean. I will summarise here. I have published major exposés

and detailed background in other works. Sabbatian-Frankism combines the names of two frauds posing as 'Jewish' men, Sabbatai Zevi (1626-1676), a rabbi, black magician and occultist who proclaimed he was the Jewish messiah; and Jacob Frank (1726-1791), the Polish 'Jew', black magician and occultist who said he was the reincarnation of 'messiah' Zevi and biblical patriarch Jacob. They worked across two centuries to establish the Sabbatian-Frankist cult that plays a major, indeed central, role in the manipulation of human society by the Global Cult which has its origins much further back in history than Sabbatai Zevi. I should emphasise two points here in response to the shrill voices that will scream 'anti-Semitism': (1) Sabbatian-Frankists are NOT Jewish and only pose as such to hide their cult behind a Jewish façade; and (2) my information about this cult has come from Jewish sources who have long realised that their society and community has been infiltrated and taken over by interloper Sabbatian-Frankists. Infiltration has been the foundation technique of Sabbatian-Frankism from its official origin in the 17th century. Zevi's Sabbatian sect attracted a massive following described as the biggest messianic movement in Jewish history, spreading as far as Africa and Asia, and he promised a return for the Jews to the 'Promised Land' of Israel. Sabbatianism was not Judaism but an inversion of everything that mainstream Judaism stood for. So much so that this sinister cult would have a feast day when Judaism had a fast day and whatever was forbidden in Judaism the Sabbatians were encouraged and even commanded to do. This included incest and what would be today called Satanism. Members were forbidden to marry outside the sect and there was a system of keeping their children ignorant of what they were part of until they were old enough to be trusted not to unknowingly reveal anything to outsiders. The same system is employed to this day by the Global Cult in general which Sabbatian-Frankism has enormously influenced and now largely controls.

Zevi and his Sabbatians suffered a setback with the intervention by the Sultan of the Islamic Ottoman Empire in the Middle East and what is now the Republic of Turkey where Zevi was located. The

Sultan gave him the choice of proving his 'divinity', converting to Islam or facing torture and death. Funnily enough Zevi chose to convert or at least appear to. Some of his supporters were disillusioned and drifted away, but many did not with 300 families also converting – only in theory – to Islam. They continued behind this Islamic smokescreen to follow the goals, rules and rituals of Sabbatianism and became known as 'crypto-Jews' or the 'Dönmeh' which means 'to turn'. This is rather ironic because they didn't 'turn' and instead hid behind a fake Islamic persona. The process of appearing to be one thing while being very much another would become the calling card of Sabbatianism especially after Zevi's death and the arrival of the Satanist Jacob Frank in the 18th century when the cult became Sabbatian-Frankism and plumbed still new depths of depravity and infiltration which included – still includes – human sacrifice and sex with children. Wherever Sabbatians go paedophilia and Satanism follow and is it really a surprise that Hollywood is so infested with child abuse and Satanism when it was established by Sabbatian-Frankists and is still controlled by them? Hollywood has been one of the prime vehicles for global perceptual programming and manipulation. How many believe the version of 'history' portrayed in movies when it is a travesty and inversion (again) of the truth? Rabbi Marvin Antelman describes Frankism in his book, *To Eliminate the Opiate*, as 'a movement of complete evil' while Jewish professor Gershom Scholem said of Frank in *The Messianic Idea in Judaism*: 'In all his actions [he was] a truly corrupt and degenerate individual ... one of the most frightening phenomena in the whole of Jewish history.' Frank was excommunicated by traditional rabbis, as was Zevi, but Frank was undeterred and enjoyed vital support from the House of Rothschild, the infamous banking dynasty whose inner-core are Sabbatian-Frankists and not Jews. Infiltration of the Roman Church and Vatican was instigated by Frank with many Dönmeh 'turning' again to convert to Roman Catholicism with a view to hijacking the reins of power. This was the ever-repeating modus operandi and continues to be so. Pose as an advocate of the religion, culture or country that you want to control and then

manipulate your people into the positions of authority and influence largely as advisers, administrators and Svengalis for those that appear to be in power. They did this with Judaism, Christianity (Christian Zionism is part of this), Islam and other religions and nations until Sabbatian-Frankism spanned the world as it does today.

Sabbatian Saudis and the terror network

One expression of the Sabbatian-Frankist Dönmeh within Islam is the ruling family of Saudi Arabia, the House of Saud, through which came the vile distortion of Islam known as Wahhabism. This is the violent creed followed by terrorist groups like Al-Qaeda and ISIS or Islamic State. Wahhabism is the hand-chopping, head-chopping 'religion' of Saudi Arabia which is used to keep the people in a constant state of fear so the interloper House of Saud can continue to rule. Al-Qaeda and Islamic State were lavishly funded by the House of Saud while being created and directed by the Sabbatian-Frankist network in the United States that operates through the Pentagon, CIA and the government in general of whichever 'party'. The front man for the establishment of Wahhabism in the middle of the 18th century was a Sabbatian-Frankist 'crypto-Jew' posing as Islamic called Muhammad ibn Abd al-Wahhab. His daughter would marry the son of Muhammad bin Saud who established the first Saudi state before his death in 1765 with support from the British Empire. Bin Saud's successors would establish modern Saudi Arabia in league with the British and Americans in 1932 which allowed them to seize control of Islam's major shrines in Mecca and Medina. They have dictated the direction of Sunni Islam ever since while Iran is the major centre of the Shiite version and here we have the source of at least the public conflict between them. The Sabbatian network has used its Wahhabi extremists to carry out Problem-Reaction-Solution terrorist attacks in the name of 'Al-Qaeda' and 'Islamic State' to justify a devastating 'war on terror', ever-increasing surveillance of the population and to terrify people into compliance. Another insight of the Renegade Mind is the streetwise understanding that

just because a country, location or people are attacked doesn't mean that those apparently representing that country, location or people are not behind the attackers. Often they are *orchestrating* the attacks because of the societal changes that can be then justified in the name of 'saving the population from terrorists'.

I show in great detail in *The Trigger* how Sabbatian-Frankists were the real perpetrators of 9/11 and not '19 Arab hijackers' who were blamed for what happened. Observe what was justified in the name of 9/11 alone in terms of Middle East invasions, mass surveillance and control that fulfilled the demands of the Project for the New American Century document published by the Sabbatian Neocons. What appear to be enemies are on the deep inside players on the same Sabbatian team. Israel and Arab 'royal' dictatorships are all ruled by Sabbatians and the recent peace agreements between Israel and Saudi Arabia, the United Arab Emirates (UAE) and others are only making formal what has always been the case behind the scenes. Palestinians who have been subjected to grotesque tyranny since Israel was bombed and terrorised into existence in 1948 have never stood a chance. Sabbatian-Frankists have controlled Israel (so the constant theme of violence and war which Sabbatians love) and they have controlled the Arab countries that Palestinians have looked to for real support that never comes. 'Royal families' of the Arab world in Saudi Arabia, Bahrain, UAE, etc., are all Sabbatians with allegiance to the aims of the cult and not what is best for their Arabic populations. They have stolen the oil and financial resources from their people by false claims to be 'royal dynasties' with a genetic right to rule and by employing vicious militaries to impose their will.

Satanic 'illumination'

The Satanist Jacob Frank formed an alliance in 1773 with two other Sabbatians, Mayer Amschel Rothschild (1744-1812), founder of the Rothschild banking dynasty, and Jesuit-educated fraudulent Jew, Adam Weishaupt, and this led to the formation of the Bavarian Illuminati, firstly under another name, in 1776. The Illuminati would

be the manipulating force behind the French Revolution (1789-1799) and was also involved in the American Revolution (1775-1783) before and after the Illuminati's official creation. Weishaupt would later become (in public) a Protestant Christian in archetypal Sabbatian style. I read that his name can be decoded as Adam-Weishaupt or 'the first man to lead those who know'. He wasn't a leader in the sense that he was a subordinate, but he did lead those below him in a crusade of transforming human society that still continues today. The theme was confirmed as early as 1785 when a horseman courier called Lanz was reported to be struck by lighting and extensive Illuminati documents were found in his saddlebags. They made the link to Weishaupt and detailed the plan for world takeover. Current events with 'Covid' fascism have been in the making for a very long time. Jacob Frank was jailed for 13 years by the Catholic Inquisition after his arrest in 1760 and on his release he headed for Frankfurt, Germany, home city and headquarters of the House of Rothschild where the alliance was struck with Mayer Amschel Rothschild and Weishaupt. Rothschild arranged for Frank to be given the title of Baron and he became a wealthy nobleman with a big following of Jews in Germany, the Austro-Hungarian Empire and other European countries. Most of them would have believed he was on their side.

The name 'Illuminati' came from the Zohar which is a body of works in the Jewish mystical 'bible' called the Kabbalah. 'Zohar' is the foundation of Sabbatian-Frankist belief and in Hebrew 'Zohar' means 'splendour', 'radiance', 'illuminated', and so we have 'Illuminati'. They claim to be the 'Illuminated Ones' from their knowledge systematically hidden from the human population and passed on through generations of carefully-chosen initiates in the global secret society network or Cult. Hidden knowledge includes an awareness of the Cult agenda for the world and the nature of our collective reality that I will explore later. Cult 'illumination' is symbolised by the torch held by the Statue of Liberty which was gifted to New York by French Freemasons in Paris who knew exactly what it represents. 'Liberty' symbolises the goddess worshipped in

Babylon as Queen Semiramis or Ishtar. The significance of this will become clear. Notice again the ubiquitous theme of inversion with the Statue of 'Liberty' really symbolising mass control (Fig 7). A mirror-image statute stands on an island in the River Seine in Paris from where New York Liberty originated (Fig 8). A large replica of the Liberty flame stands on top of the Pont de l'Alma tunnel in Paris where Princess Diana died in a Cult ritual described in *The Biggest Secret*. Lucifer 'the light bringer' is related to all this (and much more as we'll see) and 'Lucifer' is a central figure in Sabbatian-Frankism and its associated Satanism. Sabbatians reject the Jewish Torah, or Pentateuch, the 'five books of Moses' in the Old Testament known as Genesis, Exodus, Leviticus, Numbers, and Deuteronomy which are claimed by Judaism and Christianity to have been dictated by 'God' to Moses on Mount Sinai. Sabbatians say these do not apply to them and they seek to replace them with the Zohar to absorb Judaism and its followers into their inversion which is an expression of a much greater global inversion. They want to delete all religions and force humanity to worship a one-world religion – Sabbatian Satanism that also includes worship of the Earth goddess. Satanic themes are being more and more introduced into mainstream society and while Christianity is currently the foremost target for destruction the others are planned to follow.



Figure 7: The Cult goddess of Babylon disguised as the Statue of Liberty holding the flame of Lucifer the 'light bringer'.



Figure 8: Liberty's mirror image in Paris where the New York version originated.

Marx brothers

Rabbi Marvin Antelman connects the Illuminati to the Jacobins in *To Eliminate the Opiate* and Jacobins were the force behind the French Revolution. He links both to the Bund der Gerechten, or League of the Just, which was the network that inflicted communism/Marxism on the world. Antelman wrote:

> The original inner circle of the Bund der Gerechten consisted of born Catholics, Protestants and Jews [Sabbatian-Frankist infiltrators], and those representatives of respective subdivisions formulated schemes for the ultimate destruction of their faiths. The heretical Catholics laid plans which they felt would take a century or more for the ultimate destruction of the church; the apostate Jews for the ultimate destruction of the Jewish religion.

Sabbatian-created communism connects into this anti-religion agenda in that communism does not allow for the free practice of religion. The Sabbatian 'Bund' became the International Communist Party and Communist League and in 1848 'Marxism' was born with the Communist Manifesto of Sabbatian assets Karl Marx and Friedrich Engels. It is absolutely no coincidence that Marxism, just a different name for fascist and other centrally-controlled tyrannies, is being imposed worldwide as a result of the 'Covid' hoax and nor that Marxist/fascist China was the place where the hoax originated. The reason for this will become very clear in the chapter 'Covid: The calculated catastrophe'. The so-called 'Woke' mentality has hijacked

traditional beliefs of the political left and replaced them with far-right make-believe 'social justice' better known as Marxism. Woke will, however, be swallowed by its own perceived 'revolution' which is really the work of billionaires and billionaire corporations feigning being 'Woke'. Marxism is being touted by Wokers as a replacement for 'capitalism' when we don't have 'capitalism'. We have cartelism in which the market is stitched up by the very Cult billionaires and corporations bankrolling Woke. Billionaires love Marxism which keeps the people in servitude while they control from the top. Terminally naïve Wokers think they are 'changing the world' when it's the Cult that is doing the changing and when they have played their vital part and become surplus to requirements they, too, will be targeted. The Illuminati-Jacobins were behind the period known as 'The Terror' in the French Revolution in 1793 and 1794 when Jacobin Maximillian de Robespierre and his Orwellian 'Committee of Public Safety' killed 17,000 'enemies of the Revolution' who had once been 'friends of the Revolution'. Karl Marx (1818-1883), whose Sabbatian creed of Marxism has cost the lives of at least 100 million people, is a hero once again to Wokers who have been systematically kept ignorant of real history by their 'education' programming. As a result they now promote a Sabbatian 'Marxist' abomination destined at some point to consume them. Rabbi Antelman, who spent decades researching the Sabbatian plot, said of the League of the Just and Karl Marx:

> Contrary to popular opinion Karl Marx did not originate the Communist Manifesto. He was paid for his services by the League of the Just, which was known in its country of origin, Germany, as the Bund der Geaechteten.

Antelman said the text attributed to Marx was the work of other people and Marx 'was only repeating what others already said'. Marx was 'a hired hack – lackey of the wealthy Illuminists'. Marx famously said that religion was the 'opium of the people' (part of the Sabbatian plan to demonise religion) and Antelman called his books, *To Eliminate the Opiate*. Marx was born Jewish, but his family converted to Christianity (Sabbatian modus operandi) and he

attacked Jews, not least in his book, *A World Without Jews*. In doing so he supported the Sabbatian plan to destroy traditional Jewishness and Judaism which we are clearly seeing today with the vindictive targeting of orthodox Jews by the Sabbatian government of Israel over 'Covid' laws. I don't follow any religion and it has done much damage to the world over centuries and acted as a perceptual straightjacket. Renegade Minds, however, are always asking *why* something is being done. It doesn't matter if they agree or disagree with what is happening – *why* is it happening is the question. The 'why?' can be answered with regard to religion in that religions create interacting communities of believers when the Cult wants to dismantle all discourse, unity and interaction (see 'Covid' lockdowns) and the ultimate goal is to delete all religions for a one-world religion of Cult Satanism worshipping their 'god' of which more later. We see the same 'why?' with gun control in America. I don't have guns and don't want them, but why is the Cult seeking to disarm the population at the same time that law enforcement agencies are armed to their molars and why has every tyrant in history sought to disarm people before launching the final takeover? They include Hitler, Stalin, Pol Pot and Mao who followed confiscation with violent seizing of power. You know it's a Cult agenda by the people who immediately race to the microphones to exploit dead people in multiple shootings. Ultra-Zionist Cult lackey Senator Chuck Schumer was straight on the case after ten people were killed in Boulder, Colorado in March, 2121. Simple rule … if Schumer wants it the Cult wants it and the same with his ultra-Zionist mate the wild-eyed Senator Adam Schiff. At the same time they were calling for the disarmament of Americans, many of whom live a long way from a police response, Schumer, Schiff and the rest of these pampered clowns were sitting on Capitol Hill behind a razor-wired security fence protected by thousands of armed troops in addition to their own armed bodyguards. Mom and pop in an isolated home? They're just potential mass shooters.

Zion Mainframe

Sabbatian-Frankists and most importantly the Rothschilds were behind the creation of 'Zionism', a political movement that demanded a Jewish homeland in Israel as promised by Sabbatai Zevi. The very symbol of Israel comes from the German meaning of the name Rothschild. Dynasty founder Mayer Amschel Rothschild changed the family name from Bauer to Rothschild, or 'Red-Shield' in German, in deference to the six-pointed 'Star of David' hexagram displayed on the family's home in Frankfurt. The symbol later appeared on the flag of Israel after the Rothschilds were centrally involved in its creation. Hexagrams are not a uniquely Jewish symbol and are widely used in occult ('hidden') networks often as a symbol for Saturn (see my other books for why). Neither are Zionism and Jewishness interchangeable. Zionism is a political movement and philosophy and not a 'race' or a people. Many Jews oppose Zionism and many non-Jews, including US President Joe Biden, call themselves Zionists as does Israel-centric Donald Trump. America's support for the Israel government is pretty much a gimme with ultra-Zionist billionaires and corporations providing fantastic and dominant funding for both political parties. Former Congresswoman Cynthia McKinney has told how she was approached immediately she ran for office to 'sign the pledge' to Israel and confirm that she would always vote in that country's best interests. All American politicians are approached in this way. Anyone who refuses will get no support or funding from the enormous and all-powerful Zionist lobby that includes organisations like mega-lobby group AIPAC, the American Israel Public Affairs Committee. Trump's biggest funder was ultra-Zionist casino and media billionaire Sheldon Adelson while major funders of the Democratic Party include ultra-Zionist George Soros and ultra-Zionist financial and media mogul, Haim Saban. Some may reel back at the suggestion that Soros is an Israel-firster (Sabbatian-controlled Israel-firster), but Renegade Minds watch the actions not the words and everywhere Soros donates his billions the Sabbatian agenda benefits. In the spirit of Sabbatian inversion Soros pledged $1 billion for a new university network to promote 'liberal values and tackle intolerance'. He made the announcement during his annual speech

at the Cult-owned World Economic Forum in Davos, Switzerland, in January, 2020, after his 'harsh criticism' of 'authoritarian rulers' around the world. You can only laugh at such brazen mendacity. How *he* doesn't laugh is the mystery. Translated from the Orwellian 'liberal values and tackle intolerance' means teaching non-white people to hate white people and for white people to loathe themselves for being born white. The reason for that will become clear.

The 'Anti-Semitism' fraud

Zionists support the Jewish homeland in the land of Palestine which has been the Sabbatian-Rothschild goal for so long, but not for the benefit of Jews. Sabbatians and their global Anti-Semitism Industry have skewed public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. Sabbatians and their global Anti-Semitism Industry have skewed public and political opinion to equate opposing the violent extremes of Zionism to be a blanket attack and condemnation of all Jewish people. This is nothing more than a Sabbatian protection racket to stop legitimate investigation and exposure of their agendas and activities. The official definition of 'anti-Semitism' has more recently been expanded to include criticism of Zionism – a *political movement* – and this was done to further stop exposure of Sabbatian infiltrators who created Zionism as we know it today in the 19th century. Renegade Minds will talk about these subjects when they know the shit that will come their way. People must decide if they want to know the truth or just cower in the corner in fear of what others will say. Sabbatians have been trying to label me as 'anti-Semitic' since the 1990s as I have uncovered more and more about their background and agendas. Useless, gutless, fraudulent 'journalists' then just repeat the smears without question and on the day I was writing this section a pair of unquestioning repeaters called Ben Quinn and Archie Bland (how appropriate) outright called me an 'anti-Semite' in the establishment propaganda sheet, the London *Guardian*, with no supporting evidence. The

Sabbatian Anti-Semitism Industry said so and who are they to question that? They wouldn't dare. Ironically 'Semitic' refers to a group of languages in the Middle East that are almost entirely Arabic. 'Anti-Semitism' becomes 'anti-Arab' which if the consequences of this misunderstanding were not so grave would be hilarious. Don't bother telling Quinn and Bland. I don't want to confuse them, bless 'em. One reason I am dubbed 'anti-Semitic' is that I wrote in the 1990s that Jewish operatives (Sabbatians) were heavily involved in the Russian Revolution when Sabbatians overthrew the Romanov dynasty. This apparently made me 'anti-Semitic'. Oh, really? Here is a section from *The Trigger*:

British journalist Robert Wilton confirmed these themes in his 1920 book *The Last Days of the Romanovs* when he studied official documents from the Russian government to identify the members of the Bolshevik ruling elite between 1917 and 1919. The Central Committee included 41 Jews among 62 members; the Council of the People's Commissars had 17 Jews out of 22 members; and 458 of the 556 most important Bolshevik positions between 1918 and 1919 were occupied by Jewish people. Only 17 were Russian. Then there were the 23 Jews among the 36 members of the vicious Cheka Soviet secret police established in 1917 who would soon appear all across the country.

Professor Robert Service of Oxford University, an expert on 20th century Russian history, found evidence that ['Jewish'] Leon Trotsky had sought to make sure that Jews were enrolled in the Red Army and were disproportionately represented in the Soviet civil bureaucracy that included the Cheka which performed mass arrests, imprisonment and executions of 'enemies of the people'. A US State Department Decimal File (861.00/5339) dated November 13th, 1918, names [Rothschild banking agent in America] Jacob Schiff and a list of ultra-Zionists as funders of the Russian Revolution leading to claims of a 'Jewish plot', but the key point missed by all is they were not 'Jews' – they were Sabbatian-Frankists.

Britain's Winston Churchill made the same error by mistake or otherwise. He wrote in a 1920 edition of the *Illustrated Sunday Herald* that those behind the Russian revolution were part of a 'worldwide conspiracy for the overthrow of civilisation and for the reconstitution of society on the basis of arrested development, of envious malevolence, and impossible equality' (see 'Woke' today because that has been created by the same network). Churchill said there was no need to exaggerate the part played in the creation of Bolshevism and in the actual bringing about of the Russian

Revolution 'by these international and for the most part atheistical Jews' ['atheistical Jews' = Sabbatians]. Churchill said it is certainly a very great one and probably outweighs all others: 'With the notable exception of Lenin, the majority of the leading figures are Jews.' He went on to describe, knowingly or not, the Sabbatian modus operandi of placing puppet leaders nominally in power while they control from the background:

> Moreover, the principal inspiration and driving power comes from the Jewish leaders. Thus Tchitcherin, a pure Russian, is eclipsed by his nominal subordinate, Litvinoff, and the influence of Russians like Bukharin or Lunacharski cannot be compared with the power of Trotsky, or of Zinovieff, the Dictator of the Red Citadel (Petrograd), or of Krassin or Radek – all Jews. In the Soviet institutions the predominance of Jews is even more astonishing. And the prominent, if not indeed the principal, part in the system of terrorism applied by the Extraordinary Commissions for Combatting Counter-Revolution has been taken by Jews, and in some notable cases by Jewesses.

What I said about seriously disproportionate involvement in the Russian Revolution by Jewish 'revolutionaries' (Sabbatians) is provable fact, but truth is no defence against the Sabbatian Anti-Semitism Industry, its repeater parrots like Quinn and Bland, and the now breathtaking network of so-called 'Woke' 'anti-hate' groups with interlocking leaderships and funding which have the role of discrediting and silencing anyone who gets too close to exposing the Sabbatians. We have seen 'truth is no defence' confirmed in legal judgements with the Saskatchewan Human Rights Commission in Canada decreeing this: 'Truthful statements can be presented in a manner that would meet the definition of hate speech, and not all truthful statements must be free from restriction.' Most 'anti-hate' activists, who are themselves consumed by hatred, are too stupid and ignorant of the world to know how they are being used. They are far too far up their own virtue-signalling arses and it's far too dark for them to see anything.

The 'revolution' game

The background and methods of the 'Russian' Revolution are straight from the Sabbatian playbook seen in the French Revolution

and endless others around the world that appear to start as a revolution of the people against tyrannical rule and end up with a regime change to more tyrannical rule overtly or covertly. Wars, terror attacks and regime overthrows follow the Sabbatian cult through history with its agents creating them as Problem-Reaction-Solutions to remove opposition on the road to world domination. Sabbatian dots connect the Rothschilds with the Illuminati, Jacobins of the French Revolution, the 'Bund' or League of the Just, the International Communist Party, Communist League and the Communist Manifesto of Karl Marx and Friedrich Engels that would lead to the Rothschild-funded Russian Revolution. The sequence comes under the heading of 'creative destruction' when you advance to your global goal by continually destroying the status quo to install a new status quo which you then also destroy. The two world wars come to mind. With each new status quo you move closer to your planned outcome. Wars and mass murder are to Sabbatians a collective blood sacrifice ritual. They are obsessed with death for many reasons and one is that death is an inversion of life. Satanists and Sabbatians are obsessed with death and often target churches and churchyards for their rituals. Inversion-obsessed Sabbatians explain the use of inverted symbolism including the *inverted* pentagram and *inverted* cross. The inversion of the cross has been related to targeting Christianity, but the cross was a religious symbol long before Christianity and its inversion is a statement about the Sabbatian mentality and goals more than any single religion.

Sabbatians operating in Germany were behind the rise of the occult-obsessed Nazis and the subsequent Jewish exodus from Germany and Europe to Palestine and the United States after World War Two. The Rothschild dynasty was at the forefront of this both as political manipulators and by funding the operation. Why would Sabbatians help to orchestrate the horrors inflicted on Jews by the Nazis and by Stalin after they organised the Russian Revolution? Sabbatians hate Jews and their religion, that's why. They pose as Jews and secure positions of control within Jewish society and play the 'anti-Semitism' card to protect themselves from exposure

through a global network of organisations answering to the Sabbatian-created-and-controlled globe-spanning intelligence network that involves a stunning web of military-intelligence operatives and operations for a tiny country of just nine million. Among them are Jewish assets who are not Sabbatians but have been convinced by them that what they are doing is for the good of Israel and the Jewish community to protect them from what they have been programmed since childhood to believe is a Jew-hating hostile world. The Jewish community is just a highly convenient cover to hide the true nature of Sabbatians. Anyone getting close to exposing their game is accused by Sabbatian place-people and gofers of 'anti-Semitism' and claiming that all Jews are part of a plot to take over the world. I am not saying that. I am saying that Sabbatians – the *real* Jew-haters – have infiltrated the Jewish community to use them both as a cover and an 'anti-Semitic' defence against exposure. Thus we have the Anti-Semitism Industry targeted researchers in this way and most Jewish people think this is justified and genuine. They don't know that their 'Jewish' leaders and institutions of state, intelligence and military are not controlled by Jews at all, but cultists and stooges of Sabbatian-Frankism. I once added my name to a pro-Jewish freedom petition online and the next time I looked my name was gone and text had been added to the petition blurb to attack me as an 'anti-Semite' such is the scale of perceptual programming.

Moving on America

I tell the story in *The Trigger* and a chapter called 'Atlantic Crossing' how particularly after Israel was established the Sabbatians moved in on the United States and eventually grasped control of government administration, the political system via both Democrats and Republicans, the intelligence community like the CIA and National Security Agency (NSA), the Pentagon and mass media. Through this seriously compartmentalised network Sabbatians and their operatives in Mossad, Israeli Defense Forces (IDF) and US agencies pulled off 9/11 and blamed it on 19 'Al-Qaeda hijackers' dominated by men from, or connected to, Sabbatian-ruled Saudi

Arabia. The '19' were not even on the planes let alone flew those big passenger jets into buildings while being largely incompetent at piloting one-engine light aircraft. 'Hijacker' Hani Hanjour who is said to have flown American Airlines Flight 77 into the Pentagon with a turn and manoeuvre most professional pilots said they would have struggled to do was banned from renting a small plane by instructors at the Freeway Airport in Bowie, Maryland, just *six weeks* earlier on the grounds that he was an incompetent pilot. The Jewish population of the world is just 0.2 percent with even that almost entirely concentrated in Israel (75 percent Jewish) and the United States (around two percent). This two percent and globally 0.2 percent refers to *Jewish* people and not Sabbatian interlopers who are a fraction of that fraction. What a sobering thought when you think of the fantastic influence on world affairs of tiny Israel and that the Project for the New America Century (PNAC) which laid out the blueprint in September, 2000, for America's war on terror and regime change wars in Iraq, Libya and Syria was founded and dominated by Sabbatians known as 'Neocons'. The document conceded that this plan would not be supported politically or publicly without a major attack on American soil and a Problem-Reaction-Solution excuse to send troops to war across the Middle East. Sabbatian Neocons said:

> ... [The] process of transformation ... [war and regime change] ... is likely to be a long one, absent some catastrophic and catalysing event – like a new Pearl Harbor.

Four months later many of those who produced that document came to power with their inane puppet George Bush from the long-time Sabbatian Bush family. They included Sabbatian Dick Cheney who was officially vice-president, but really de-facto president for the entirety of the 'Bush' government. Nine months after the 'Bush' inauguration came what Bush called at the time 'the Pearl Harbor of the 21st century' and with typical Sabbatian timing and symbolism 2001 was the 60th anniversary of the attack in 1941 by the Japanese Air Force on Pearl Harbor, Hawaii, which allowed President Franklin Delano Roosevelt to take the United States into a Sabbatian-

instigated Second World War that he said in his election campaign that he never would. The evidence is overwhelming that Roosevelt and his military and intelligence networks knew the attack was coming and did nothing to stop it, but they did make sure that America's most essential naval ships were not in Hawaii at the time. Three thousand Americans died in the Pearl Harbor attacks as they did on September 11th. By the 9/11 year of 2001 Sabbatians had widely infiltrated the US government, military and intelligence operations and used their compartmentalised assets to pull off the 'Al-Qaeda' attacks. If you read *The Trigger* it will blow your mind to see the utterly staggering concentration of 'Jewish' operatives (Sabbatian infiltrators) in essential positions of political, security, legal, law enforcement, financial and business power before, during, and after the attacks to make them happen, carry them out, and then cover their tracks – and I do mean *staggering* when you think of that 0.2 percent of the world population and two percent of Americans which are Jewish while Sabbatian infiltrators are a fraction of that. A central foundation of the 9/11 conspiracy was the hijacking of government, military, Air Force and intelligence computer systems in real time through 'back-door' access made possible by Israeli (Sabbatian) 'cyber security' software. Sabbatian-controlled Israel is on the way to rivalling Silicon Valley for domination of cyberspace and is becoming the dominant force in cyber-security which gives them access to entire computer systems and their passcodes across the world. Then add to this that Zionists head (officially) Silicon Valley giants like Google (Larry Page and Sergey Brin), Google-owned YouTube (Susan Wojcicki), Facebook (Mark Zuckerberg and Sheryl Sandberg), and Apple (Chairman Arthur D. Levinson), and that ultra-Zionist hedge fund billionaire Paul Singer has a $1 billion stake in Twitter which is only nominally headed by 'CEO' pothead Jack Dorsey. As cable news host Tucker Carlson said of Dorsey: 'There used to be debate in the medical community whether dropping a ton of acid had permanent effects and I think that debate has now ended.' Carlson made the comment after Dorsey told a hearing on Capitol Hill (if you cut through his bullshit) that he

believed in free speech so long as he got to decide what you can hear and see. These 'big names' of Silicon Valley are only front men and women for the Global Cult, not least the Sabbatians, who are the true controllers of these corporations. Does anyone still wonder why these same people and companies have been ferociously censoring and banning people (like me) for exposing any aspect of the Cult agenda and especially the truth about the 'Covid' hoax which Sabbatians have orchestrated?

The Jeffrey Epstein paedophile ring was a Sabbatian operation. He was officially 'Jewish' but he was a Sabbatian and women abused by the ring have told me about the high number of 'Jewish' people involved. The Epstein horror has Sabbatian written all over it and matches perfectly their modus operandi and obsession with sex and ritual. Epstein was running a Sabbatian blackmail ring in which famous people with political and other influence were provided with young girls for sex while everything was being filmed and recorded on hidden cameras and microphones at his New York house, Caribbean island and other properties. Epstein survivors have described this surveillance system to me and some have gone public. Once the famous politician or other figure knew he or she was on video they tended to do whatever they were told. Here we go again …when you've got them by the balls their hearts and minds will follow. Sabbatians use this blackmail technique on a wide scale across the world to entrap politicians and others they need to act as demanded. Epstein's private plane, the infamous 'Lolita Express', had many well-known passengers including Bill Clinton while Bill Gates has flown on an Epstein plane and met with him four years after Epstein had been jailed for paedophilia. They subsequently met many times at Epstein's home in New York according to a witness who was there. Epstein's infamous side-kick was Ghislaine Maxwell, daughter of Mossad agent and ultra-Zionist mega-crooked British businessman, Bob Maxwell, who at one time owned the *Daily Mirror* newspaper. Maxwell was murdered at sea on his boat in 1991 by Sabbatian-controlled Mossad when he became a liability with his

business empire collapsing as a former Mossad operative has confirmed (see *The Trigger*).

Money, money, money, funny money …

Before I come to the Sabbatian connection with the last three US presidents I will lay out the crucial importance to Sabbatians of controlling banking and finance. Sabbatian Mayer Amschel Rothschild set out to dominate this arena in his family's quest for total global control. What is freedom? It is, in effect, choice. The more choices you have the freer you are and the fewer your choices the more you are enslaved. In the global structure created over centuries by Sabbatians the biggest decider and restrictor of choice is … money. Across the world if you ask people what they would like to do with their lives and why they are not doing that they will reply 'I don't have the money'. This is the idea. A global elite of multi-billionaires are described as 'greedy' and that is true on one level; but control of money – who has it and who doesn't – is not primarily about greed. It's about control. Sabbatians have seized ever more control of finance and sucked the wealth of the world out of the hands of the population. We talk now, after all, about the 'One-percent' and even then the wealthiest are a lot fewer even than that. This has been made possible by a money scam so outrageous and so vast it could rightly be called the scam of scams founded on creating 'money' out of nothing and 'loaning' that with interest to the population. Money out of nothing is called 'credit'. Sabbatians have asserted control over governments and banking ever more completely through the centuries and secured financial laws that allow banks to lend hugely more than they have on deposit in a confidence trick known as fractional reserve lending. Imagine if you could lend money that doesn't exist and charge the recipient interest for doing so. You would end up in jail. Bankers by contrast end up in mansions, private jets, Malibu and Monaco.

Banks are only required to keep a fraction of their deposits and wealth in their vaults and they are allowed to lend 'money' they don't have called 'credit. Go into a bank for a loan and if you succeed

the banker will not move any real wealth into your account. They will type into your account the amount of the agreed 'loan' – say £100,000. This is not wealth that really exists; it is non-existent, fresh-air, created-out-of-nothing 'credit' which has never, does not, and will never exist except in theory. Credit is backed by nothing except wind and only has buying power because people think that it has buying power and accept it in return for property, goods and services. I have described this situation as like those cartoon characters you see chasing each other and when they run over the edge of a cliff they keep running forward on fresh air until one of them looks down, realises what's happened, and they all crash into the ravine. The whole foundation of the Sabbatian financial system is to stop people looking down except for periodic moments when they want to crash the system (as in 2008 and 2020 ongoing) and reap the rewards from all the property, businesses and wealth their borrowers had signed over as 'collateral' in return for a 'loan' of fresh air. Most people think that money is somehow created by governments when it comes into existence from the start as a debt through banks 'lending' illusory money called credit. Yes, the very currency of exchange is a *debt* from day one issued as an interest-bearing loan. Why don't governments create money interest-free and lend it to their people interest-free? Governments are controlled by Sabbatians and the financial system is controlled by Sabbatians for whom interest-free money would be a nightmare come true. Sabbatians underpin their financial domination through their global network of central banks, including the privately-owned US Federal Reserve and Britain's Bank of England, and this is orchestrated by a privately-owned central bank coordination body called the Bank for International Settlements in Basle, Switzerland, created by the usual suspects including the Rockefellers and Rothschilds. Central bank chiefs don't answer to governments or the people. They answer to the Bank for International Settlements or, in other words, the Global Cult which is dominated today by Sabbatians.

Built-in disaster

There are so many constituent scams within the overall banking scam. When you take out a loan of thin-air credit only the amount of that loan is theoretically brought into circulation to add to the amount in circulation; but you are paying back the principle plus interest. The additional interest is not created and this means that with every 'loan' there is a shortfall in the money in circulation between what is borrowed and what has to be paid back. There is never even close to enough money in circulation to repay all outstanding public and private debt including interest. Coldly weaved in the very fabric of the system is the certainty that some will lose their homes, businesses and possessions to the banking 'lender'. This is less obvious in times of 'boom' when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts it becomes painfully obvious that there is not enough money to service all debt and interest. This is less obvious in times of 'boom' when the amount of money in circulation (and the debt) is expanding through more people wanting and getting loans. When a downturn comes and the money supply contracts and it becomes painfully obvious – as in 2008 and currently – that there is not enough money to service all debt and interest. Sabbatian banksters have been leading the human population through a calculated series of booms (more debt incurred) and busts (when the debt can't be repaid and the banks get the debtor's tangible wealth in exchange for non-existent 'credit'). With each 'bust' Sabbatian bankers have absorbed more of the world's tangible wealth and we end up with the One-percent. Governments are in bankruptcy levels of debt to the same system and are therefore owned by a system they do not control. The Federal Reserve, 'America's central bank', is privately-owned and American presidents only nominally appoint its chairman or woman to maintain the illusion that it's an arm of government. It's not. The 'Fed' is a cartel of private banks which handed billions to its associates and friends after the crash of 2008 and has been Sabbatian-controlled since it was manipulated into being in 1913 through the covert trickery of Rothschild banking agents Jacob Schiff and Paul

Warburg, and the Sabbatian Rockefeller family. Somehow from a Jewish population of two-percent and globally 0.2 percent (Sabbatian interlopers remember are far smaller) ultra-Zionists headed the Federal Reserve for 31 years between 1987 and 2018 in the form of Alan Greenspan, Bernard Bernanke and Janet Yellen (now Biden's Treasury Secretary) with Yellen's deputy chairman a Israeli-American duel citizen and ultra-Zionist Stanley Fischer, a former governor of the Bank of Israel. Ultra-Zionist Fed chiefs spanned the presidencies of Ronald Reagan ('Republican'), Father George Bush ('Republican'), Bill Clinton ('Democrat'), Boy George Bush ('Republican') and Barack Obama ('Democrat'). We should really add the pre-Greenspan chairman, Paul Adolph Volcker, 'appointed' by Jimmy Carter ('Democrat') who ran the Fed between 1979 and 1987 during the Carter and Reagan administrations before Greenspan took over. Volcker was a long-time associate and business partner of the Rothschilds. No matter what the 'party' officially in power the United States economy was directed by the same force. Here are members of the Obama, Trump and Biden administrations and see if you can make out a common theme.

Barack Obama ('Democrat')

Ultra-Zionists Robert Rubin, Larry Summers, and Timothy Geithner ran the US Treasury in the Clinton administration and two of them reappeared with Obama. Ultra-Zionist Fed chairman Alan Greenspan had manipulated the crash of 2008 through deregulation and jumped ship just before the disaster to make way for ultra-Zionist Bernard Bernanke to hand out trillions to Sabbatian 'too big to fail' banks and businesses, including the ubiquitous ultra-Zionist Goldman Sachs which has an ongoing staff revolving door operation between itself and major financial positions in government worldwide. Obama inherited the fallout of the crash when he took office in January, 2009, and fortunately he had the support of his ultra-Zionist White House Chief of Staff Rahm Emmanuel, son of a terrorist who helped to bomb Israel into being in 1948, and his ultra-Zionist senior adviser David Axelrod, chief strategist in Obama's two

successful presidential campaigns. Emmanuel, later mayor of Chicago and former senior fundraiser and strategist for Bill Clinton, is an example of the Sabbatian policy after Israel was established of migrating insider families to America so their children would be born American citizens. 'Obama' chose this financial team throughout his administration to respond to the Sabbatian-instigated crisis:

Timothy Geithner (ultra-Zionist) Treasury Secretary; Jacob J. Lew, Treasury Secretary; Larry Summers (ultra-Zionist), director of the White House National Economic Council; Paul Adolph Volcker (Rothschild business partner), chairman of the Economic Recovery Advisory Board; Peter Orszag (ultra-Zionist), director of the Office of Management and Budget overseeing all government spending; Penny Pritzker (ultra-Zionist), Commerce Secretary; Jared Bernstein (ultra-Zionist), chief economist and economic policy adviser to Vice President Joe Biden; Mary Schapiro (ultra-Zionist), chair of the Securities and Exchange Commission (SEC); Gary Gensler (ultra-Zionist), chairman of the Commodity Futures Trading Commission (CFTC); Sheila Bair (ultra-Zionist), chair of the Federal Deposit Insurance Corporation (FDIC); Karen Mills (ultra-Zionist), head of the Small Business Administration (SBA); Kenneth Feinberg (ultra-Zionist), Special Master for Executive [bail-out] Compensation. Feinberg would be appointed to oversee compensation (with strings) to 9/11 victims and families in a campaign to stop them having their day in court to question the official story. At the same time ultra-Zionist Bernard Bernanke was chairman of the Federal Reserve and these are only some of the ultra-Zionists with allegiance to Sabbatian-controlled Israel in the Obama government. Obama's biggest corporate donor was ultra-Zionist Goldman Sachs which had employed many in his administration.

Donald Trump ('Republican')

Trump claimed to be an outsider (he wasn't) who had come to 'drain the swamp'. He embarked on this goal by immediately appointing ultra-Zionist Steve Mnuchin, a Goldman Sachs employee for 17

years, as his Treasury Secretary. Others included Gary Cohn (ultra-Zionist), chief operating officer of Goldman Sachs, his first Director of the National Economic Council and chief economic adviser, who was later replaced by Larry Kudlow (ultra-Zionist). Trump's senior adviser throughout his four years in the White House was his sinister son-in-law Jared Kushner, a life-long friend of Israel Prime Minister Benjamin Netanyahu. Kushner is the son of a convicted crook who was pardoned by Trump in his last days in office. Other ultra-Zionists in the Trump administration included: Stephen Miller, Senior Policy Adviser; Avrahm Berkowitz, Deputy Adviser to Trump and his Senior Adviser Jared Kushner; Ivanka Trump, Adviser to the President, who converted to Judaism when she married Jared Kushner; David Friedman, Trump lawyer and Ambassador to Israel; Jason Greenblatt, Trump Organization executive vice president and chief legal officer, who was made Special Representative for International Negotiations and the Israeli-Palestinian Conflict; Rod Rosenstein, Deputy Attorney General; Elliot Abrams, Special Representative for Venezuela, then Iran; John Eisenberg, National Security Council Legal Adviser and Deputy Council to the President for National Security Affairs; Anne Neuberger, Deputy National Manager, National Security Agency; Ezra Cohen-Watnick, Acting Under Secretary of Defense for Intelligence; Elan Carr, Special Envoy to monitor and combat anti-Semitism; Len Khodorkovsky, Deputy Special Envoy to monitor and combat anti-Semitism; Reed Cordish, Assistant to the President, Intragovernmental and Technology Initiatives. Trump Vice President Mike Pence and Secretary of State Mike Pompeo, both Christian Zionists, were also vehement supporters of Israel and its goals and ambitions.

Donald 'free-speech believer' Trump pardoned a number of financial and violent criminals while ignoring calls to pardon Julian Assange and Edward Snowden whose crimes are revealing highly relevant information about government manipulation and corruption and the widespread illegal surveillance of the American people by US 'security' agencies. It's so good to know that Trump is on the side of freedom and justice and not mega-criminals with

allegiance to Sabbatian-controlled Israel. These included a pardon for Israeli spy Jonathan Pollard who was jailed for life in 1987 under the Espionage Act. Aviem Sella, the Mossad agent who recruited Pollard, was also pardoned by Trump while Assange sat in jail and Snowden remained in exile in Russia. Sella had 'fled' (was helped to escape) to Israel in 1987 and was never extradited despite being charged under the Espionage Act. A Trump White House statement said that Sella's clemency had been 'supported by Benjamin Netanyahu, Ron Dermer, Israel's US Ambassador, David Friedman, US Ambassador to Israel and Miriam Adelson, wife of leading Trump donor Sheldon Adelson who died shortly before. Other friends of Jared Kushner were pardoned along with Sholom Weiss who was believed to be serving the longest-ever white-collar prison sentence of more than 800 years in 2000. The sentence was commuted of Ponzi-schemer Eliyahu Weinstein who defrauded Jews and others out of $200 million. I did mention that Assange and Snowden were ignored, right? Trump gave Sabbatians almost everything they asked for in military and political support, moving the US Embassy from Tel Aviv to Jerusalem with its critical symbolic and literal implications for Palestinian statehood, and the 'deal of the Century' designed by Jared Kushner and David Friedman which gave the Sabbatian Israeli government the green light to substantially expand its already widespread program of building illegal Jewish-only settlements in the occupied land of the West Bank. This made a two-state 'solution' impossible by seizing all the land of a potential Palestinian homeland and that had been the plan since 1948 and then 1967 when the Arab-controlled Gaza Strip, West Bank, Sinai Peninsula and Syrian Golan Heights were occupied by Israel. All the talks about talks and road maps and delays have been buying time until the West Bank was physically occupied by Israeli real estate. Trump would have to be a monumentally ill-informed idiot not to see that this was the plan he was helping to complete. The Trump administration was in so many ways the Kushner administration which means the Netanyahu administration which means the Sabbatian administration. I understand why many opposing Cult fascism in all its forms gravitated to Trump, but he

was a crucial part of the Sabbatian plan and I will deal with this in the next chapter.

Joe Biden ('Democrat')

A barely cognitive Joe Biden took over the presidency in January, 2021, along with his fellow empty shell, Vice-President Kamala Harris, as the latest Sabbatian gofers to enter the White House. Names on the door may have changed and the 'party' – the force behind them remained the same as Zionists were appointed to a stream of pivotal areas relating to Sabbatian plans and policy. They included: Janet Yellen, Treasury Secretary, former head of the Federal Reserve, and still another ultra-Zionist running the US Treasury after Mnuchin (Trump), Lew and Geithner (Obama), and Summers and Rubin (Clinton); Anthony Blinken, Secretary of State; Wendy Sherman, Deputy Secretary of State (so that's 'Biden's' Sabbatian foreign policy sorted); Jeff Zients, White House coronavirus coordinator; Rochelle Walensky, head of the Centers for Disease Control; Rachel Levine, transgender deputy health secretary (that's 'Covid' hoax policy under control); Merrick Garland, Attorney General; Alejandro Mayorkas, Secretary of Homeland Security; Cass Sunstein, Homeland Security with responsibility for new immigration laws; Avril Haines, Director of National Intelligence; Anne Neuberger, National Security Agency cybersecurity director (note, cybersecurity); David Cohen, CIA Deputy Director; Ronald Klain, Biden's Chief of Staff (see Rahm Emanuel); Eric Lander, a 'leading geneticist', Office of Science and Technology Policy director (see Smart Grid, synthetic biology agenda); Jessica Rosenworcel, acting head of the Federal Communications Commission (FCC) which controls Smart Grid technology policy and electromagnetic communication systems including 5G. How can it be that so many pivotal positions are held by two-percent of the American population and 0.2 percent of the world population administration after administration no matter who is the president and what is the party? It's a coincidence? Of course it's not and this is why Sabbatians have built their colossal global web of interlocking 'anti-

hate' hate groups to condemn anyone who asks these glaring questions as an 'anti-Semite'. The way that Jewish people horrifically abused in Sabbatian-backed Nazi Germany are exploited to this end is stomach-turning and disgusting beyond words.

Political fusion

Sabbatian manipulation has reversed the roles of Republicans and Democrats and the same has happened in Britain with the Conservative and Labour Parties. Republicans and Conservatives were always labelled the 'right' and Democrats and Labour the 'left', but look at the policy positions now and the Democrat-Labour 'left' has moved further to the 'right' than Republicans and Conservatives under the banner of 'Woke', the Cult-created far-right tyranny. Where once the Democrat-Labour 'left' defended free speech and human rights they now seek to delete them and as I said earlier despite the 'Covid' fascism of the Jackboot Johnson Conservative government in the UK the Labour Party of leader Keir Starmer demanded even more extreme measures. The Labour Party has been very publicly absorbed by Sabbatians after a political and media onslaught against the previous leader, the weak and inept Jeremy Corbyn, over made-up allegations of 'anti-Semitism' both by him and his party. The plan was clear with this 'anti-Semite' propaganda and what was required in response was a swift and decisive 'fuck off' from Corbyn and a statement to expose the Anti-Semitism Industry (Sabbatian) attempt to silence Labour criticism of the Israeli government (Sabbatians) and purge the party of all dissent against the extremes of ultra-Zionism (Sabbatians). Instead Corbyn and his party fell to their knees and appeased the abusers which, by definition, is impossible. Appeasing one demand leads only to a new demand to be appeased until takeover is complete. Like I say – 'fuck off' would have been a much more effective policy and I have used it myself with great effect over the years when Sabbatians are on my case which is most of the time. I consider that fact a great compliment, by the way. The outcome of the Labour Party capitulation is that we now have a Sabbatian-controlled

Conservative Party 'opposed' by a Sabbatian-controlled Labour Party in a one-party Sabbatian state that hurtles towards the extremes of tyranny (the Sabbatian cult agenda). In America the situation is the same. Labour's Keir Starmer spends his days on his knees with his tongue out pointing to Tel Aviv, or I guess now Jerusalem, while Boris Johnson has an 'anti-Semitism czar' in the form of former Labour MP John Mann who keeps Starmer company on his prayer mat.

Sabbatian influence can be seen in Jewish members of the Labour Party who have been ejected for criticism of Israel including those from families that suffered in Nazi Germany. Sabbatians despise real Jewish people and target them even more harshly because it is so much more difficult to dub them 'anti-Semitic' although in their desperation they do try.

CHAPTER THREE

The Pushbacker sting

Until you realize how easy it is for your mind to be manipulated, you remain the puppet of someone else's game
Evita Ochel

I will use the presidencies of Trump and Biden to show how the manipulation of the one-party state plays out behind the illusion of political choice across the world. No two presidencies could – on the face of it – be more different and apparently at odds in terms of direction and policy.

A Renegade Mind sees beyond the obvious and focuses on outcomes and consequences and not image, words and waffle. The Cult embarked on a campaign to divide America between those who blindly support its agenda (the mentality known as 'Woke') and those who are pushing back on where the Cult and its Sabbatians want to go. This presents infinite possibilities for dividing and ruling the population by setting them at war with each other and allows a perceptual ring fence of demonisation to encircle the Pushbackers in a modern version of the Little Big Horn in 1876 when American cavalry led by Lieutenant Colonel George Custer were drawn into a trap, surrounded and killed by Native American tribes defending their land of thousands of years from being seized by the government. In this modern version the roles are reversed and it's those defending themselves from the Sabbatian government who are surrounded and the government that's seeking to destroy them. This trap was set years ago and to explain how we must return to 2016

and the emergence of Donald Trump as a candidate to be President of the United States. He set out to overcome the best part of 20 other candidates in the Republican Party before and during the primaries and was not considered by many in those early stages to have a prayer of living in the White House. The Republican Party was said to have great reservations about Trump and yet somehow he won the nomination. When you know how American politics works – politics in general – there is no way that Trump could have become the party's candidate unless the Sabbatian-controlled 'Neocons' that run the Republican Party wanted that to happen. We saw the proof in emails and documents made public by WikiLeaks that the Democratic Party hierarchy, or Democons, systematically undermined the campaign of Bernie Sanders to make sure that Sabbatian gofer Hillary Clinton won the nomination to be their presidential candidate. If the Democons could do that then the Neocons in the Republican Party could have derailed Trump in the same way. But they didn't and at that stage I began to conclude that Trump could well be the one chosen to be president. If that was the case the 'why' was pretty clear to see – the goal of dividing America between Cult agenda-supporting Wokers and Pushbackers who gravitated to Trump because he was telling them what they wanted to hear. His constituency of support had been increasingly ignored and voiceless for decades and profoundly through the eight years of Sabbatian puppet Barack Obama. Now here was someone speaking their language of pulling back from the incessant globalisation of political and economic power, the exporting of American jobs to China and elsewhere by 'American' (Sabbatian) corporations, the deletion of free speech, and the mass immigration policies that had further devastated job opportunities for the urban working class of all races and the once American heartlands of the Midwest.

Beware the forked tongue

Those people collectively sighed with relief that at last a political leader was apparently on their side, but another trait of the Renegade Mind is that you look even harder at people telling you

what you want to hear than those who are telling you otherwise. Obviously as I said earlier people wish what they want to hear to be true and genuine and they are much more likely to believe that than someone saying what they don't want to here and don't want to be true. Sales people are taught to be skilled in eliciting by calculated questioning what their customers want to hear and repeating that back to them as their own opinion to get their targets to like and trust them. Assets of the Cult are also sales people in the sense of selling perception. To read Cult manipulation you have to play the long and expanded game and not fall for the Vaudeville show of party politics. Both American parties are vehicles for the Cult and they exploit them in different ways depending on what the agenda requires at that moment. Trump and the Republicans were used to be the focus of dividing America and isolating Pushbackers to open the way for a Biden presidency to become the most extreme in American history by advancing the full-blown Woke (Cult) agenda with the aim of destroying and silencing Pushbackers now labelled Nazi Trump supporters and white supremacists.

Sabbatians wanted Trump in office for the reasons described by ultra-Zionist Saul Alinsky (1909-1972) who was promoting the Woke philosophy through 'community organising' long before anyone had heard of it. In those days it still went by its traditional name of Marxism. The reason for the manipulated Trump phenomenon was laid out in Alinsky's 1971 book, *Rules for Radicals*, which was his blueprint for overthrowing democratic and other regimes and replacing them with Sabbatian Marxism. Not surprisingly his to-do list was evident in the Sabbatian French and Russian 'Revolutions' and that in China which will become very relevant in the next chapter about the 'Covid' hoax. Among Alinsky's followers have been the deeply corrupt Barack Obama, House Speaker Nancy Pelosi and Hillary Clinton who described him as a 'hero'. All three are Sabbatian stooges with Pelosi personifying the arrogant corrupt idiocy that so widely fronts up for the Cult inner core. Predictably as a Sabbatian advocate of the 'light-bringer' Alinsky features Lucifer on the dedication page of his book as the original radical who gained

his own kingdom ('Earth' as we shall see). One of Alinsky's golden radical rules was to pick an individual and focus all attention, hatred and blame on them and not to target faceless bureaucracies and corporations. *Rules for Radicals* is really a Sabbatian handbook with its contents repeatedly employed all over the world for centuries and why wouldn't Sabbatians bring to power their designer-villain to be used as the individual on which all attention, hatred and blame was bestowed? This is what they did and the only question for me is how much Trump knew that and how much he was manipulated. A bit of both, I suspect. This was Alinsky's Trump technique from a man who died in 1972. The technique has spanned history:

Pick the target, freeze it, personalize it, polarize it. Don't try to attack abstract corporations or bureaucracies. Identify a responsible individual. Ignore attempts to shift or spread the blame.

From the moment Trump came to illusory power everything was about him. It wasn't about Republican policy or opinion, but all about Trump. Everything he did was presented in negative, derogatory and abusive terms by the Sabbatian-dominated media led by Cult operations such as CNN, MSNBC, *The New York Times* and the Jeff Bezos-owned *Washington Post* – 'Pick the target, freeze it, personalize it, polarize it.' Trump was turned into a demon to be vilified by those who hated him and a demi-god loved by those who worshipped him. This, in turn, had his supporters, too, presented as equally demonic in preparation for the punchline later down the line when Biden was about to take office. It was here's a Trump, there's a Trump, everywhere a Trump, Trump. Virtually every news story or happening was filtered through the lens of 'The Donald'. You loved him or hated him and which one you chose was said to define you as Satan's spawn or a paragon of virtue. Even supporting some Trump policies or statements and not others was enough for an assault on your character. No shades of grey were or are allowed. Everything is black and white (literally and figuratively). A Californian I knew had her head utterly scrambled by her hatred for Trump while telling people they should love each other. She was so totally consumed by

Trump Derangement Syndrome as it became to be known that this glaring contradiction would never have occurred to her. By definition anyone who criticised Trump or praised his opponents was a hero and this lady described Joe Biden as 'a kind, honest gentleman' when he's a provable liar, mega-crook and vicious piece of work to boot. Sabbatians had indeed divided America using Trump as the fall-guy and all along the clock was ticking on the consequences for his supporters.

In hock to his masters

Trump gave Sabbatians via Israel almost everything they wanted in his four years. Ask and you shall receive was the dynamic between himself and Benjamin Netanyahu orchestrated by Trump's ultra-Zionist son-in-law Jared Kushner, his ultra-Zionist Ambassador to Israel, David Friedman, and ultra-Zionist 'Israel adviser', Jason Greenblatt. The last two were central to the running and protecting from collapse of his business empire, the Trump Organisation, and colossal business failures made him forever beholding to Sabbatian networks that bailed him out. By the start of the 1990s Trump owed $4 billion to banks that he couldn't pay and almost $1billion of that was down to him personally and not his companies. This mega-disaster was the result of building two new casinos in Atlantic City and buying the enormous Taj Mahal operation which led to crippling debt payments. He had borrowed fantastic sums from 72 banks with major Sabbatian connections and although the scale of debt should have had him living in a tent alongside the highway they never foreclosed. A plan was devised to lift Trump from the mire by BT Securities Corporation and Rothschild Inc. and the case was handled by Wilber Ross who had worked for the Rothschilds for 27 years. Ross would be named US Commerce Secretary after Trump's election. Another crucial figure in saving Trump was ultra-Zionist 'investor' Carl Icahn who bought the Taj Mahal casino. Icahn was made special economic adviser on financial regulation in the Trump administration. He didn't stay long but still managed to find time to make a tidy sum of a reported $31.3 million when he sold his

holdings affected by the price of steel three days before Trump imposed a 235 percent tariff on steel imports. What amazing bits of luck these people have. Trump and Sabbatian operatives have long had a close association and his mentor and legal adviser from the early 1970s until 1986 was the dark and genetically corrupt ultra-Zionist Roy Cohn who was chief counsel to Senator Joseph McCarthy's 'communist' witch-hunt in the 1950s. *Esquire* magazine published an article about Cohn with the headline 'Don't mess with Roy Cohn'. He was described as the most feared lawyer in New York and 'a ruthless master of dirty tricks ... [with] ... more than one Mafia Don on speed dial'. Cohn's influence, contacts, support and protection made Trump a front man for Sabbatians in New York with their connections to one of Cohn's many criminal employers, the 'Russian' Sabbatian Mafia. Israel-centric media mogul Rupert Murdoch was introduced to Trump by Cohn and they started a long friendship. Cohn died in 1986 weeks after being disbarred for unethical conduct by the Appellate Division of the New York State Supreme Court. The wheels of justice do indeed run slow given the length of Cohn's crooked career.

QAnon-sense

We are asked to believe that Donald Trump with his fundamental connections to Sabbatian networks and operatives has been leading the fight to stop the Sabbatian agenda for the fascistic control of America and the world. Sure he has. A man entrapped during his years in the White House by Sabbatian operatives and whose biggest financial donor was casino billionaire Sheldon Adelson who was Sabbatian to his DNA?? Oh, do come on. Trump has been used to divide America and isolate Pushbackers on the Cult agenda under the heading of 'Trump supporters', 'insurrectionists' and 'white supremacists'. The US Intelligence/Mossad Psyop or psychological operation known as QAnon emerged during the Trump years as a central pillar in the Sabbatian campaign to lead Pushbackers into the trap set by those that wished to destroy them. I knew from the start that QAnon was a scam because I had seen the same scenario many

times before over 30 years under different names and I had written about one in particular in the books. 'Not again' was my reaction when QAnon came to the fore. The same script is pulled out every few years and a new name added to the letterhead. The story always takes the same form: 'Insiders' or 'the good guys' in the government-intelligence-military 'Deep State' apparatus were going to instigate mass arrests of the 'bad guys' which would include the Rockefellers, Rothschilds, Barack Obama, Hillary Clinton, George Soros, etc., etc. Dates are given for when the 'good guys' are going to move in, but the dates pass without incident and new dates are given which pass without incident. The central message to Pushbackers in each case is that they don't have to do anything because there is 'a plan' and it is all going to be sorted by the 'good guys' on the inside. 'Trust the plan' was a QAnon mantra when the only plan was to misdirect Pushbackers into putting their trust in a Psyop they believed to be real. Beware, beware, those who tell you what you want to hear and always check it out. Right up to Biden's inauguration QAnon was still claiming that 'the Storm' was coming and Trump would stay on as president when Biden and his cronies were arrested and jailed. It was never going to happen and of course it didn't, but what did happen as a result provided that punchline to the Sabbatian Trump/QAnon Psyop.

On January 6th, 2021, a very big crowd of Trump supporters gathered in the National Mall in Washington DC down from the Capitol Building to protest at what they believed to be widespread corruption and vote fraud that stopped Trump being re-elected for a second term as president in November, 2020. I say as someone that does not support Trump or Biden that the evidence is clear that major vote-fixing went on to favour Biden, a man with cognitive problems so advanced he can often hardly string a sentence together without reading the words written for him on the Teleprompter. Glaring ballot discrepancies included serious questions about electronic voting machines that make vote rigging a comparative cinch and hundreds of thousands of paper votes that suddenly appeared during already advanced vote counts and virtually all of

them for Biden. Early Trump leads in crucial swing states suddenly began to close and disappear. The pandemic hoax was used as the excuse to issue almost limitless numbers of mail-in ballots with no checks to establish that the recipients were still alive or lived at that address. They were sent to streams of people who had not even asked for them. Private organisations were employed to gather these ballots and who knows what they did with them before they turned up at the counts. The American election system has been manipulated over decades to become a sick joke with more holes than a Swiss cheese for the express purpose of dictating the results. Then there was the criminal manipulation of information by Sabbatian tech giants like Facebook, Twitter and Google-owned YouTube which deleted pro-Trump, anti-Biden accounts and posts while everything in support of Biden was left alone. Sabbatians wanted Biden to win because after the dividing of America it was time for full-on Woke and every aspect of the Cult agenda to be unleashed.

Hunter gatherer

Extreme Silicon Valley bias included blocking information by the *New York Post* exposing a Biden scandal that should have ended his bid for president in the final weeks of the campaign. Hunter Biden, his monumentally corrupt son, is reported to have sent a laptop to be repaired at a local store and failed to return for it. Time passed until the laptop became the property of the store for non-payment of the bill. When the owner saw what was on the hard drive he gave a copy to the FBI who did nothing even though it confirmed widespread corruption in which the Joe Biden family were using his political position, especially when he was vice president to Obama, to make multiple millions in countries around the world and most notably Ukraine and China. Hunter Biden's one-time business partner Tony Bobulinski went public when the story broke in the *New York Post* to confirm the corruption he saw and that Joe Biden not only knew what was going on he also profited from the spoils. Millions were handed over by a Chinese company with close

connections – like all major businesses in China – to the Chinese communist party of President Xi Jinping. Joe Biden even boasted at a meeting of the Cult's World Economic Forum that as vice president he had ordered the government of Ukraine to fire a prosecutor. What he didn't mention was that the same man just happened to be investigating an energy company which was part of Hunter Biden's corrupt portfolio. The company was paying him big bucks for no other reason than the influence his father had. Overnight Biden's presidential campaign should have been over given that he had lied publicly about not knowing what his son was doing. Instead almost the entire Sabbatian-owned mainstream media and Sabbatian-owned Silicon Valley suppressed circulation of the story. This alone went a mighty way to rigging the election of 2020. Cult assets like Mark Zuckerberg at Facebook also spent hundreds of millions to be used in support of Biden and vote 'administration'.

The Cult had used Trump as the focus to divide America and was now desperate to bring in moronic, pliable, corrupt Biden to complete the double-whammy. No way were they going to let little things like the will of the people thwart their plan. Silicon Valley widely censored claims that the election was rigged because it *was* rigged. For the same reason anyone claiming it was rigged was denounced as a 'white supremacist' including the pathetically few Republican politicians willing to say so. Right across the media where the claim was mentioned it was described as a 'false claim' even though these excuses for 'journalists' would have done no research into the subject whatsoever. Trump won seven million more votes than any sitting president had ever achieved while somehow a cognitively-challenged soon to be 78-year-old who was hidden away from the public for most of the campaign managed to win more votes than any presidential candidate in history. It makes no sense. You only had to see election rallies for both candidates to witness the enthusiasm for Trump and the apathy for Biden. Tens of thousands would attend Trump events while Biden was speaking in empty car parks with often only television crews attending and framing their shots to hide the fact that no one was there. It was pathetic to see

footage come to light of Biden standing at a podium making speeches only to TV crews and party fixers while reading the words written for him on massive Teleprompter screens. So, yes, those protestors on January 6th had a point about election rigging, but some were about to walk into a trap laid for them in Washington by the Cult Deep State and its QAnon Psyop. This was the Capitol Hill riot ludicrously dubbed an 'insurrection'.

The spider and the fly

Renegade Minds know there are not two 'sides' in politics, only one side, the Cult, working through all 'sides'. It's a stage show, a puppet show, to direct the perceptions of the population into focusing on diversions like parties and candidates while missing the puppeteers with their hands holding all the strings. The Capitol Hill 'insurrection' brings us back to the Little Big Horn. Having created two distinct opposing groupings – Woke and Pushbackers – the trap was about to be sprung. Pushbackers were to be encircled and isolated by associating them all in the public mind with Trump and then labelling Trump as some sort of Confederate leader. I knew immediately that the Capitol riot was a set-up because of two things. One was how easy the rioters got into the building with virtually no credible resistance and secondly I could see – as with the 'Covid' hoax in the West at the start of 2020 – how the Cult could exploit the situation to move its agenda forward with great speed. My experience of Cult techniques and activities over more than 30 years has showed me that while they do exploit situations they haven't themselves created this never happens with events of fundamental agenda significance. Every time major events giving cultists the excuse to rapidly advance their plan you find they are manipulated into being for the specific reason of providing that excuse – Problem-Reaction-Solution. Only a tiny minority of the huge crowd of Washington protestors sought to gain entry to the Capitol by smashing windows and breaching doors. That didn't matter. The whole crowd and all Pushbackers, even if they did not support Trump, were going to be lumped together as dangerous

insurrectionists and conspiracy theorists. The latter term came into widespread use through a CIA memo in the 1960s aimed at discrediting those questioning the nonsensical official story of the Kennedy assassination and it subsequently became widely employed by the media. It's still being used by inept 'journalists' with no idea of its origin to discredit anyone questioning anything that authority claims to be true. When you are perpetrating a conspiracy you need to discredit the very word itself even though the dictionary definition of conspiracy is merely 'the activity of secretly planning with other people to do something bad or illegal' and 'a general agreement to keep silent about a subject for the purpose of keeping it secret'. On that basis there are conspiracies almost wherever you look. For obvious reasons the Cult and its lapdog media have to claim there are no conspiracies even though the word appears in state laws as with conspiracy to defraud, to murder, and to corrupt public morals.

Agent provocateurs are widely used by the Cult Deep State to manipulate genuine people into acting in ways that suit the desired outcome. By genuine in this case I mean protestors genuinely supporting Trump and claims that the election was stolen. In among them, however, were agents of the state wearing the garb of Trump supporters and QAnon to pump-prime the Capital riot which some genuine Trump supporters naively fell for. I described the situation as 'Come into my parlour said the spider to the fly'. Leaflets appeared through the Woke paramilitary arm Antifa, the anti-fascist fascists, calling on supporters to turn up in Washington looking like Trump supporters even though they hated him. Some of those arrested for breaching the Capitol Building were sourced to Antifa and its stable mate Black Lives Matter. Both organisations are funded by Cult billionaires and corporations. One man charged for the riot was according to his lawyer a former FBI agent who had held top secret security clearance for 40 years. Attorney Thomas Plofchan said of his client, 66-year-old Thomas Edward Caldwell:

He has held a Top Secret Security Clearance since 1979 and has undergone multiple Special Background Investigations in support of his clearances. After retiring from the Navy, he

worked as a section chief for the Federal Bureau of Investigation from 2009-2010 as a GS-12 [mid-level employee].

He also formed and operated a consulting firm performing work, often classified, for U.S government customers including the US. Drug Enforcement Agency, Department of Housing and Urban Development, the US Coast Guard, and the US Army Personnel Command.

A judge later released Caldwell pending trial in the absence of evidence about a conspiracy or that he tried to force his way into the building. *The New York Post* reported a 'law enforcement source' as saying that 'at least two known Antifa members were spotted' on camera among Trump supporters during the riot while one of the rioters arrested was John Earle Sullivan, a seriously extreme Black Lives Matter Trump-hater from Utah who was previously arrested and charged in July, 2020, over a BLM-Antifa riot in which drivers were threatened and one was shot. Sullivan is the founder of Utah-based Insurgence USA which is an affiliate of the Cult-created-and-funded Black Lives Matter movement. Footage appeared and was then deleted by Twitter of Trump supporters calling out Antifa infiltrators and a group was filmed changing into pro-Trump clothing before the riot. Security at the building was *pathetic* – as planned. Colonel Leroy Fletcher Prouty, a man with long experience in covert operations working with the US security apparatus, once described the tell-tale sign to identify who is involved in an assassination. He said:

No one has to direct an assassination – it happens. The active role is played secretly by permitting it to happen. This is the greatest single clue. Who has the power to call off or reduce the usual security precautions?

This principle applies to many other situations and certainly to the Capitol riot of January 6th, 2021.

The sting

With such a big and potentially angry crowd known to be gathering near the Capitol the security apparatus would have had a major police detail to defend the building with National Guard troops on

standby given the strength of feeling among people arriving from all over America encouraged by the QAnon Psyop and statements by Donald Trump. Instead Capitol Police 'security' was flimsy, weak, and easily breached. The same number of officers was deployed as on a regular day and that is a blatant red flag. They were not staffed or equipped for a possible riot that had been an obvious possibility in the circumstances. No protective and effective fencing worth the name was put in place and there were no contingency plans. The whole thing was basically a case of standing aside and waving people in. Once inside police mostly backed off apart from one Capitol police officer who ridiculously shot dead unarmed Air Force veteran protestor Ashli Babbitt without a warning as she climbed through a broken window. The 'investigation' refused to name or charge the officer after what must surely be considered a murder in the circumstances. They just lifted a carpet and swept. The story was endlessly repeated about five people dying in the 'armed insurrection' when there was no report of rioters using weapons. Apart from Babbitt the other four died from a heart attack, strokes and apparently a drug overdose. Capitol police officer Brian Sicknick was reported to have died after being bludgeoned with a fire extinguisher when he was alive after the riot was over and died later of what the Washington Medical Examiner's Office said was a stroke. Sicknick had no external injuries. The lies were delivered like rapid fire. There was a narrative to build with incessant repetition of the lie until the lie became the accepted 'everybody knows that' truth. The 'Big Lie' technique of Nazi Propaganda Minister Joseph Goebbels is constantly used by the Cult which was behind the Nazis and is today behind the 'Covid' and 'climate change' hoaxes. Goebbels said:

If you tell a lie big enough and keep repeating it, people will eventually come to believe it. The lie can be maintained only for such time as the State can shield the people from the political, economic and/or military consequences of the lie. It thus becomes vitally important for the State to use all of its powers to repress dissent, for the truth is the mortal enemy of the lie, and thus by extension, the truth is the greatest enemy of the State.

Most protestors had a free run of the Capitol Building. This allowed pictures to be taken of rioters in iconic parts of the building including the Senate chamber which could be used as propaganda images against all Pushbackers. One Congresswoman described the scene as 'the worst kind of non-security anybody could ever imagine'. Well, the first part was true, but someone obviously did imagine it and made sure it happened. Some photographs most widely circulated featured people wearing QAnon symbols and now the Psyop would be used to dub all QAnon followers with the ubiquitous fit-all label of 'white supremacist' and 'insurrectionists'. When a Muslim extremist called Noah Green drove his car at two police officers at the Capitol Building killing one in April, 2021, there was no such political and media hysteria. They were just disappointed he wasn't white.

The witch-hunt

Government prosecutor Michael Sherwin, an aggressive, dark-eyed, professional Rottweiler led the 'investigation' and to call it over the top would be to understate reality a thousand fold. Hundreds were tracked down and arrested for the crime of having the wrong political views and people were jailed who had done nothing more than walk in the building, committed no violence or damage to property, took a few pictures and left. They were labelled a 'threat to the Republic' while Biden sat in the White House signing executive orders written for him that were dismantling 'the Republic'. Even when judges ruled that a mother and son should not be in jail the government kept them there. Some of those arrested have been badly beaten by prison guards in Washington and lawyers for one man said he suffered a fractured skull and was made blind in one eye. Meanwhile a woman is shot dead for no reason by a Capitol Police officer and we are not allowed to know who he is never mind what has happened to him although that will be *nothing*. The Cult's QAnon/Trump sting to identify and isolate Pushbackers and then target them on the road to crushing and deleting them was a resounding success. You would have thought the Russians had

invaded the building at gunpoint and lined up senators for a firing squad to see the political and media reaction. Congresswoman Alexandria Ocasio-Cortez is a child in a woman's body, a terrible-twos, me, me, me, Woker narcissist of such proportions that words have no meaning. She said she thought she was going to die when 'insurrectionists' banged on her office door. It turned out she wasn't even in the Capitol Building when the riot was happening and the 'banging' was a Capitol Police officer. She referred to herself as a 'survivor' which is an insult to all those true survivors of violent and sexual abuse while she lives her pampered and privileged life talking drivel for a living. Her Woke colleague and fellow mega-narcissist Rashida Tlaib broke down describing the devastating effect on her, too, of *not being* in the building when the rioters were there. Ocasio-Cortez and Tlaib are members of a fully-Woke group of Congresswomen known as 'The Squad' along with Ilhan Omar and Ayanna Pressley. The Squad from what I can see can be identified by its vehement anti-white racism, anti-white men agenda, and, as always in these cases, the absence of brain cells on active duty.

The usual suspects were on the riot case immediately in the form of Democrat ultra-Zionist senators and operatives Chuck Schumer and Adam Schiff demanding that Trump be impeached for 'his part in the insurrection'. The same pair of prats had led the failed impeachment of Trump over the invented 'Russia collusion' nonsense which claimed Russia had helped Trump win the 2016 election. I didn't realise that Tel Aviv had been relocated just outside Moscow. I must find an up-to-date map. The Russia hoax was a Sabbatian operation to keep Trump occupied and impotent and to stop any rapport with Russia which the Cult wants to retain as a perceptual enemy to be pulled out at will. Puppet Biden began attacking Russia when he came to office as the Cult seeks more upheaval, division and war across the world. A two-year stage show 'Russia collusion inquiry' headed by the not-very-bright former 9/11 FBI chief Robert Mueller, with support from 19 lawyers, 40 FBI agents plus intelligence analysts, forensic accountants and other

staff, devoured tens of millions of dollars and found no evidence of Russia collusion which a ten-year-old could have told them on day one. Now the same moronic Schumer and Schiff wanted a second impeachment of Trump over the Capitol 'insurrection' (riot) which the arrested development of Schumer called another 'Pearl Harbor' while others compared it with 9/11 in which 3,000 died and, in the case of CNN, with the Rwandan genocide in the 1990s in which an estimated 500,000 to 600,000 were murdered, between 250, 000 and 500,000 women were raped, and populations of whole towns were hacked to death with machetes. To make those comparisons purely for Cult political reasons is beyond insulting to those that suffered and lost their lives and confirms yet again the callous inhumanity that we are dealing with. Schumer is a monumental idiot and so is Schiff, but they serve the Cult agenda and do whatever they're told so they get looked after. Talking of idiots – another inane man who spanned the Russia and Capitol impeachment attempts was Senator Eric Swalwell who had the nerve to accuse Trump of collusion with the Russians while sleeping with a Chinese spy called Christine Fang or 'Fang Fang' which is straight out of a Bond film no doubt starring Klaus Schwab as the bloke living on a secret island and controlling laser weapons positioned in space and pointing at world capitals. Fang Fang plays the part of Bond's infiltrator girlfriend which I'm sure she would enjoy rather more than sharing a bed with the brainless Swalwell, lying back and thinking of China. The FBI eventually warned Swalwell about Fang Fang which gave her time to escape back to the Chinese dictatorship. How very thoughtful of them. The second Trump impeachment also failed and hardly surprising when an impeachment is supposed to remove a sitting president and by the time it happened Trump was no longer president. These people are running your country America, well, officially anyway. Terrifying isn't it?

Outcomes tell the story - always

The outcome of all this – and it's the *outcome* on which Renegade Minds focus, not the words – was that a vicious, hysterical and

obviously pre-planned assault was launched on Pushbackers to censor, silence and discredit them and even targeted their right to earn a living. They have since been condemned as 'domestic terrorists' that need to be treated like Al-Qaeda and Islamic State. 'Domestic terrorists' is a label the Cult has been trying to make stick since the period of the Oklahoma bombing in 1995 which was blamed on 'far-right domestic terrorists'. If you read *The Trigger* you will see that the bombing was clearly a Problem-Reaction-Solution carried out by the Deep State during a Bill Clinton administration so corrupt that no dictionary definition of the term would even nearly suffice. Nearly 30, 000 troops were deployed from all over America to the empty streets of Washington for Biden's inauguration. Ten thousand of them stayed on with the pretext of protecting the capital from insurrectionists when it was more psychological programming to normalise the use of the military in domestic law enforcement in support of the Cult plan for a police-military state. Biden's fascist administration began a purge of 'wrong-thinkers' in the military which means anyone that is not on board with Woke. The Capitol Building was surrounded by a fence with razor wire and the Land of the Free was further symbolically and literally dismantled. The circle was completed with the installation of Biden and the exploitation of the QAnon Psyop.

America had never been so divided since the civil war of the 19th century, Pushbackers were isolated and dubbed terrorists and now, as was always going to happen, the Cult immediately set about deleting what little was left of freedom and transforming American society through a swish of the hand of the most controlled 'president' in American history leading (officially at least) the most extreme regime since the country was declared an independent state on July 4th, 1776. Biden issued undebated, dictatorial executive orders almost by the hour in his opening days in office across the whole spectrum of the Cult wish-list including diluting controls on the border with Mexico allowing thousands of migrants to illegally enter the United States to transform the demographics of America and import an election-changing number of perceived Democrat

voters. Then there were Biden deportation amnesties for the already illegally resident (estimated to be as high as 20 or even 30 million). A bill before Congress awarded American citizenship to anyone who could prove they had worked in agriculture for just 180 days in the previous two years as 'Big Ag' secured its slave labour long-term. There were the plans to add new states to the union such as Puerto Rico and making Washington DC a state. They are all parts of a plan to ensure that the Cult-owned Woke Democrats would be permanently in power.

Border – what border?

I have exposed in detail in other books how mass immigration into the United States and Europe is the work of Cult networks fuelled by the tens of billions spent to this and other ends by George Soros and his global Open Society (open borders) Foundations. The impact can be seen in America alone where the population has increased by *100 million* in little more than 30 years mostly through immigration. I wrote in *The Answer* that the plan was to have so many people crossing the southern border that the numbers become unstoppable and we are now there under Cult-owned Biden. El Salvador in Central America puts the scale of what is happening into context. A third of the population now lives in the United States, much of it illegally, and many more are on the way. The methodology is to crush Central and South American countries economically and spread violence through machete-wielding psychopathic gangs like MS-13 based in El Salvador and now operating in many American cities. Biden-imposed lax security at the southern border means that it is all but open. He said before his 'election' that he wanted to see a surge towards the border if he became president and that was the green light for people to do just that after election day to create the human disaster that followed for both America and the migrants. When that surge came the imbecilic Alexandria Ocasio-Cortez said it wasn't a 'surge' because they are 'children, not insurgents' and the term 'surge' (used by Biden) was a claim of 'white supremacists'.

This disingenuous lady may one day enter the realm of the most basic intelligence, but it won't be any time soon.

Sabbatians and the Cult are in the process of destroying America by importing violent people and gangs in among the genuine to terrorise American cities and by overwhelming services that cannot cope with the sheer volume of new arrivals. Something similar is happening in Europe as Western society in general is targeted for demographic and cultural transformation and upheaval. The plan demands violence and crime to create an environment of intimidation, fear and division and Soros has been funding the election of district attorneys across America who then stop prosecuting many crimes, reduce sentences for violent crimes and free as many violent criminals as they can. Sabbatians are creating the chaos from which order – their order – can respond in a classic Problem-Reaction-Solution. A Freemasonic moto says 'Ordo Ab Chao' (Order out of Chaos) and this is why the Cult is constantly creating chaos to impose a new 'order'. Here you have the reason the Cult is constantly creating chaos. The 'Covid' hoax can be seen with those entering the United States by plane being forced to take a 'Covid' test while migrants flooding through southern border processing facilities do not. Nothing is put in the way of mass migration and if that means ignoring the government's own 'Covid' rules then so be it. They know it's all bullshit anyway. Any pushback on this is denounced as 'racist' by Wokers and Sabbatian fronts like the ultra-Zionist Anti-Defamation League headed by the appalling Jonathan Greenblatt which at the same time argues that Israel should not give citizenship and voting rights to more Palestinian Arabs or the 'Jewish population' (in truth the Sabbatian network) will lose control of the country.

Society-changing numbers

Biden's masters have declared that countries like El Salvador are so dangerous that their people must be allowed into the United States for humanitarian reasons when there are fewer murders in large parts of many Central American countries than in US cities like

Baltimore. That is not to say Central America cannot be a dangerous place and Cult-controlled American governments have been making it so since way back, along with the dismantling of economies, in a long-term plan to drive people north into the United States. Parts of Central America are very dangerous, but in other areas the story is being greatly exaggerated to justify relaxing immigration criteria. Migrants are being offered free healthcare and education in the United States as another incentive to head for the border and there is no requirement to be financially independent before you can enter to prevent the resources of America being drained. You can't blame migrants for seeking what they believe will be a better life, but they are being played by the Cult for dark and nefarious ends. The numbers since Biden took office are huge. In February, 2021, more than 100,000 people were known to have tried to enter the US illegally through the southern border (it was 34,000 in the same month in 2020) and in March it was 170,000 – a 418 percent increase on March, 2020. These numbers are only known people, not the ones who get in unseen. The true figure for migrants illegally crossing the border in a single month was estimated by one congressman at 250,000 and that number will only rise under Biden's current policy. Gangs of murdering drug-running thugs that control the Mexican side of the border demand money – thousands of dollars – to let migrants cross the Rio Grande into America. At the same time gun battles are breaking out on the border several times a week between rival Mexican drug gangs (which now operate globally) who are equipped with sophisticated military-grade weapons, grenades and armoured vehicles. While the Capitol Building was being 'protected' from a non-existent 'threat' by thousands of troops, and others were still deployed at the time in the Cult Neocon war in Afghanistan, the southern border of America was left to its fate. This is not incompetence, it is cold calculation.

By March, 2021, there were 17,000 unaccompanied children held at border facilities and many of them are ensnared by people traffickers for paedophile rings and raped on their journey north to America. This is not conjecture – this is fact. Many of those designated

children are in reality teenage boys or older. Meanwhile Wokers posture their self-purity for encouraging poor and tragic people to come to America and face this nightmare both on the journey and at the border with the disgusting figure of House Speaker Nancy Pelosi giving disingenuous speeches about caring for migrants. The woman's evil. Wokers condemned Trump for having children in cages at the border (so did Obama, *Shhhh*), but now they are sleeping on the floor without access to a shower with one border facility 729 percent over capacity. The Biden insanity even proposed flying migrants from the southern border to the northern border with Canada for 'processing'. The whole shambles is being overseen by ultra-Zionist Secretary of Homeland Security, the moronic liar Alejandro Mayorkas, who banned news cameras at border facilities to stop Americans seeing what was happening. Mayorkas said there was not a ban on news crews; it was just that they were not allowed to film. Alongside him at Homeland Security is another ultra-Zionist Cass Sunstein appointed by Biden to oversee new immigration laws. Sunstein despises conspiracy researchers to the point where he suggests they should be banned or *taxed* for having such views. The man is not bonkers or anything. He's perfectly well-adjusted, but adjusted to what is the question. Criticise what is happening and you are a 'white supremacist' when earlier non-white immigrants also oppose the numbers which effect their lives and opportunities. Black people in poor areas are particularly damaged by uncontrolled immigration and the increased competition for work opportunities with those who will work for less. They are also losing voting power as Hispanics become more dominant in former black areas. It's a downward spiral for them while the billionaires behind the policy drone on about how much they care about black people and 'racism'. None of this is about compassion for migrants or black people – that's just wind and air. Migrants are instead being mercilessly exploited to transform America while the countries they leave are losing their future and the same is true in Europe. Mass immigration may now be the work of Woke Democrats, but it can be traced back to the 1986 Immigration Reform and Control Act (it

wasn't) signed into law by Republican hero President Ronald Reagan which gave amnesty to millions living in the United States illegally and other incentives for people to head for the southern border. Here we have the one-party state at work again.

Save me syndrome

Almost every aspect of what I have been exposing as the Cult agenda was on display in even the first days of 'Biden' with silencing of Pushbackers at the forefront of everything. A Renegade Mind will view the Trump years and QAnon in a very different light to their supporters and advocates as the dots are connected. The QAnon/Trump Psyop has given the Cult all it was looking for. We may not know how much, or little, that Trump realised he was being used, but that's a side issue. This pincer movement produced the desired outcome of dividing America and having Pushbackers isolated. To turn this around we have to look at new routes to empowerment which do not include handing our power to other people and groups through what I will call the 'Save Me Syndrome' – 'I want someone else to do it so that I don't have to'. We have seen this at work throughout human history and the QAnon/Trump Psyop is only the latest incarnation alongside all the others. Religion is an obvious expression of this when people look to a 'god' or priest to save them or tell them how to be saved and then there are 'save me' politicians like Trump. Politics is a diversion and not a 'saviour'. It is a means to block positive change, not make it possible.

Save Me Syndrome always comes with the same repeating theme of handing your power to whom or what you believe will save you while your real 'saviour' stares back from the mirror every morning. Renegade Minds are constantly vigilant in this regard and always asking the question 'What can I do?' rather than 'What can someone else do for me?' Gandhi was right when he said: 'You must be the change you want to see in the world.' We are indeed the people we have been waiting for. We are presented with a constant raft of reasons to concede that power to others and forget where the real power is. Humanity has the numbers and the Cult does not. It has to

use diversion and division to target the unstoppable power that comes from unity. Religions, governments, politicians, corporations, media, QAnon, are all different manifestations of this power-diversion and dilution. Refusing to give your power to governments and instead handing it to Trump and QAnon is not to take a new direction, but merely to recycle the old one with new names on the posters. I will explore this phenomenon as we proceed and how to break the cycles and recycles that got us here through the mists of repeating perception and so repeating history.

For now we shall turn to the most potent example in the entire human story of the consequences that follow when you give your power away. I am talking, of course, of the 'Covid' hoax.

CHAPTER FOUR

'Covid': Calculated catastrophe

Facts are threatening to those invested in fraud
DaShanne Stokes

We can easily unravel the real reason for the 'Covid pandemic' hoax by employing the Renegade Mind methodology that I have outlined this far. We'll start by comparing the long-planned Cult outcome with the 'Covid pandemic' outcome. Know the outcome and you'll see the journey.

I have highlighted the plan for the Hunger Games Society which has been in my books for so many years with the very few controlling the very many through ongoing dependency. To create this dependency it is essential to destroy independent livelihoods, businesses and employment to make the population reliant on the state (the Cult) for even the basics of life through a guaranteed pittance income. While independence of income remained these Cult ambitions would be thwarted. With this knowledge it was easy to see where the 'pandemic' hoax was going once talk of 'lockdowns' began and the closing of all but perceived 'essential' businesses to 'save' us from an alleged 'deadly virus'. Cult corporations like Amazon and Walmart were naturally considered 'essential' while mom and pop shops and stores had their doors closed by fascist decree. As a result with every new lockdown and new regulation more small and medium, even large businesses not owned by the Cult, went to the wall while Cult giants and their frontmen and women grew financially fatter by the second. Mom and pop were

denied an income and the right to earn a living and the wealth of people like Jeff Bezos (Amazon), Mark Zuckerberg (Facebook) and Sergei Brin and Larry Page (Google/Alphabet) have reached record levels. The Cult was increasing its own power through further dramatic concentrations of wealth while the competition was being destroyed and brought into a state of dependency. Lockdowns have been instigated to secure that very end and were never anything to do with health. My brother Paul spent 45 years building up a bus repair business, but lockdowns meant buses were running at a fraction of normal levels for months on end. Similar stories can told in their hundreds of millions worldwide. Efforts of a lifetime coldly destroyed by Cult multi-billionaires and their lackeys in government and law enforcement who continued to earn their living from the taxation of the people while denying the right of the same people to earn theirs. How different it would have been if those making and enforcing these decisions had to face the same financial hardships of those they affected, but they never do.

Gates of Hell

Behind it all in the full knowledge of what he is doing and why is the psychopathic figure of Cult operative Bill Gates. His puppet Tedros at the World Health Organization declared 'Covid' a pandemic in March, 2020. The WHO had changed the definition of a 'pandemic' in 2009 just a month before declaring the 'swine flu pandemic' which would not have been so under the previous definition. The same applies to 'Covid'. The definition had included… 'an infection by an infectious agent, occurring simultaneously in different countries, with a significant mortality rate relative to the proportion of the population infected'. The new definition removed the need for 'significant mortality'. The 'pandemic' has been fraudulent even down to the definition, but Gates demanded economy-destroying lockdowns, school closures, social distancing, mandatory masks, a 'vaccination' for every man, woman and child on the planet and severe consequences and restrictions for those that refused. Who gave him this power? The

Cult did which he serves like a little boy in short trousers doing what his daddy tells him. He and his psychopathic missus even smiled when they said that much worse was to come (what they knew was planned to come). Gates responded in the matter-of-fact way of all psychopaths to a question about the effect on the world economy of what he was doing:

> Well, it won't go to zero but it will shrink. Global GDP is probably going to take the biggest hit ever [Gates was smiling as he said this] … in my lifetime this will be the greatest economic hit. But you don't have a choice. People act as if you have a choice. People don't feel like going to the stadium when they might get infected … People are deeply affected by seeing these stats, by knowing they could be part of the transmission chain, old people, their parents and grandparents, could be affected by this, and so you don't get to say ignore what is going on here.
>
> There will be the ability to open up, particularly in rich countries, if things are done well over the next few months, but for the world at large normalcy only returns when we have largely vaccinated the entire population.

The man has no compassion or empathy. How could he when he's a psychopath like all Cult players? My own view is that even beyond that he is very seriously mentally ill. Look in his eyes and you can see this along with his crazy flailing arms. You don't do what he has done to the world population since the start of 2020 unless you are mentally ill and at the most extreme end of psychopathic. You especially don't do it when to you know, as we shall see, that cases and deaths from 'Covid' are fakery and a product of monumental figure massaging. 'These stats' that Gates referred to are based on a 'test' that's not testing for the 'virus' as he has known all along. He made his fortune with big Cult support as an infamously ruthless software salesman and now buys global control of 'health' (death) policy without the population he affects having any say. It's a breathtaking outrage. Gates talked about people being deeply affected by fear of 'Covid' when that was because of *him* and his global network lying to them minute-by-minute supported by a lying media that he seriously influences and funds to the tune of hundreds of millions. He's handed big sums to media operations including the BBC, NBC, Al Jazeera, Univision, *PBS NewsHour*,

ProPublica, National Journal, The Guardian, The Financial Times, The Atlantic, Texas Tribune, USA Today publisher Gannett, *Washington Monthly, Le Monde,* Center for Investigative Reporting, Pulitzer Center on Crisis Reporting, National Press Foundation, International Center for Journalists, Solutions Journalism Network, the Poynter Institute for Media Studies, and many more. Gates is everywhere in the 'Covid' hoax and the man must go to prison – or a mental facility – for the rest of his life and his money distributed to those he has taken such enormous psychopathic pleasure in crushing.

The Muscle

The Hunger Games global structure demands a police-military state – a fusion of the two into one force – which viciously imposes the will of the Cult on the population and protects the Cult from public rebellion. In that regard, too, the 'Covid' hoax just keeps on giving. Often unlawful, ridiculous and contradictory 'Covid' rules and regulations have been policed across the world by moronic automatons and psychopaths made faceless by face-nappy masks and acting like the Nazi SS and fascist blackshirts and brownshirts of Hitler and Mussolini. The smallest departure from the rules decreed by the psychos in government and their clueless gofers were jumped upon by the face-nappy fascists. Brutality against public protestors soon became commonplace even on girls, women and old people as the brave men with the batons – the Face-Nappies as I call them – broke up peaceful protests and handed out fines like confetti to people who couldn't earn a living let alone pay hundreds of pounds for what was once an accepted human right. Robot Face-Nappies of Nottingham police in the English East Midlands fined one group £11,000 for attending a child's birthday party. For decades I charted the transformation of law enforcement as genuine, decent officers were replaced with psychopaths and the brain dead who would happily and brutally do whatever their masters told them. Now they were let loose on the public and I would emphasise the point that none of this just happened. The step-by-step change in the dynamic between police and public was orchestrated from the shadows by

those who knew where this was all going and the same with the perceptual reframing of those in all levels of authority and official administration through 'training courses' by organisations such as Common Purpose which was created in the late 1980s and given a massive boost in Blair era Britain until it became a global phenomenon. Supposed public 'servants' began to view the population as the enemy and the same was true of the police. This was the start of the explosion of behaviour manipulation organisations and networks preparing for the all-war on the human psyche unleashed with the dawn of 2020. I will go into more detail about this later in the book because it is a core part of what is happening.

Police desecrated beauty spots to deter people gathering and arrested women for walking in the countryside alone 'too far' from their homes. We had arrogant, clueless sergeants in the Isle of Wight police where I live posting on Facebook what they insisted the population must do or else. A schoolmaster sergeant called Radford looked young enough for me to ask if his mother knew he was out, but he was posting what he *expected* people to do while a Sergeant Wilkinson boasted about fining lads for meeting in a McDonald's car park where they went to get a lockdown takeaway. Wilkinson added that he had even cancelled their order. What a pair of prats these people are and yet they have increasingly become the norm among Jackboot Johnson's Yellowshirts once known as the British police. This was the theme all over the world with police savagery common during lockdown protests in the United States, the Netherlands, and the fascist state of Victoria in Australia under its tyrannical and again moronic premier Daniel Andrews. Amazing how tyrannical and moronic tend to work as a team and the same combination could be seen across America as arrogant, narcissistic Woke governors and mayors such as Gavin Newsom (California), Andrew Cuomo (New York), Gretchen Whitmer (Michigan), Lori Lightfoot (Chicago) and Eric Garcetti (Los Angeles) did their Nazi and Stalin impressions with the full support of the compliant brutality of their enforcers in uniform as they arrested small business owners defying

fascist shutdown orders and took them to jail in ankle shackles and handcuffs. This happened to bistro owner Marlena Pavlos-Hackney in Gretchen Whitmer's fascist state of Michigan when police arrived to enforce an order by a state-owned judge for 'putting the community at risk' at a time when other states like Texas were dropping restrictions and migrants were pouring across the southern border without any 'Covid' questions at all. I'm sure there are many officers appalled by what they are ordered to do, but not nearly enough of them. If they were truly appalled they would not do it. As the months passed every opportunity was taken to have the military involved to make their presence on the streets ever more familiar and 'normal' for the longer-term goal of police-military fusion.

Another crucial element to the Hunger Games enforcement network has been encouraging the public to report neighbours and others for 'breaking the lockdown rules'. The group faced with £11,000 in fines at the child's birthday party would have been dobbed-in by a neighbour with a brain the size of a pea. The technique was most famously employed by the Stasi secret police in communist East Germany who had public informants placed throughout the population. A police chief in the UK says his force doesn't need to carry out 'Covid' patrols when they are flooded with so many calls from the public reporting other people for visiting the beach. Dorset police chief James Vaughan said people were so enthusiastic about snitching on their fellow humans they were now operating as an auxiliary arm of the police: 'We are still getting around 400 reports a week from the public, so we will respond to reports …We won't need to be doing hotspot patrols because people are very quick to pick the phone up and tell us.' Vaughan didn't say that this is a pillar of all tyrannies of whatever complexion and the means to hugely extend the reach of enforcement while spreading distrust among the people and making them wary of doing anything that might get them reported. Those narcissistic Isle of Wight sergeants Radford and Wilkinson never fail to add a link to their Facebook posts where the public can inform on their fellow slaves.

Neither would be self-aware enough to realise they were imitating the Stasi which they might well never have heard of. Government psychologists that I will expose later laid out a policy to turn communities against each other in the same way.

A coincidence? Yep, and I can knit fog

I knew from the start of the alleged pandemic that this was a Cult operation. It presented limitless potential to rapidly advance the Cult agenda and exploit manipulated fear to demand that every man, woman and child on the planet was 'vaccinated' in a process never used on humans before which infuses self-replicating *synthetic* material into human cells. Remember the plan to transform the human body from a biological to a synthetic biological state. I'll deal with the 'vaccine' (that's not actually a vaccine) when I focus on the genetic agenda. Enough to say here that mass global 'vaccination' justified by this 'new virus' set alarms ringing after 30 years of tracking these people and their methods. The 'Covid' hoax officially beginning in China was also a big red flag for reasons I will be explaining. The agenda potential was so enormous that I could dismiss any idea that the 'virus' appeared naturally. Major happenings with major agenda implications never occur without Cult involvement in making them happen. My questions were twofold in early 2020 as the media began its campaign to induce global fear and hysteria: Was this alleged infectious agent released on purpose by the Cult or did it even exist at all? I then did what I always do in these situations. I sat, observed and waited to see where the evidence and information would take me. By March and early April synchronicity was strongly – and ever more so since then – pointing me in the direction of *there is no 'virus'*. I went public on that with derision even from swathes of the alternative media that voiced a scenario that the Chinese government released the 'virus' in league with Deep State elements in the United States from a top-level bio-lab in Wuhan where the 'virus' is said to have first appeared. I looked at that possibility, but I didn't buy it for several reasons. Deaths from the 'virus' did not in any way match what they

would have been with a 'deadly bioweapon' and it is much more effective if you sell the *illusion* of an infectious agent rather than having a real one unless you can control through injection who has it and who doesn't. Otherwise you lose control of events. A made-up 'virus' gives you a blank sheet of paper on which you can make it do whatever you like and have any symptoms or mutant 'variants' you choose to add while a real infectious agent would limit you to what it actually does. A phantom disease allows you to have endless ludicrous 'studies' on the 'Covid' dollar to widen the perceived impact by inventing ever more 'at risk' groups including one study which said those who walk slowly may be almost four times more likely to die from the 'virus'. People are in psychiatric wards for less.

A real 'deadly bioweapon' can take out people in the hierarchy that are not part of the Cult, but essential to its operation. Obviously they don't want that. Releasing a real disease means you immediately lose control of it. Releasing an illusory one means you don't. Again it's vital that people are extra careful when dealing with what they want to hear. A bioweapon unleashed from a Chinese laboratory in collusion with the American Deep State may fit a conspiracy narrative, but is it true? Would it not be far more effective to use the excuse of a 'virus' to justify the real bioweapon – the 'vaccine'? That way your disease agent does not have to be transmitted and arrives directly through a syringe. I saw a French virologist Luc Montagnier quoted in the alternative media as saying he had discovered that the alleged 'new' severe acute respiratory syndrome coronavirus , or SARS-CoV-2, was made artificially and included elements of the human immunodeficiency 'virus' (HIV) and a parasite that causes malaria. SARS-CoV-2 is alleged to trigger an alleged illness called Covid-19. I remembered Montagnier's name from my research years before into claims that an HIV 'retrovirus' causes AIDs – claims that were demolished by Berkeley virologist Peter Duesberg who showed that no one had ever proved that HIV causes acquired immunodeficiency syndrome or AIDS. Claims that become accepted as fact, publicly and medically, with no proof whatsoever are an ever-recurring story that profoundly applies to

'Covid'. Nevertheless, despite the lack of proof, Montagnier's team at the Pasteur Institute in Paris had a long dispute with American researcher Robert Gallo over which of them discovered and isolated the HIV 'virus' and with *no evidence* found it to cause AIDS. You will see later that there is also no evidence that any 'virus' causes any disease or that there is even such a thing as a 'virus' in the way it is said to exist. The claim to have 'isolated' the HIV 'virus' will be presented in its real context as we come to the shocking story – and it is a story – of SARS-CoV-2 and so will Montagnier's assertion that he identified the full SARS-CoV-2 genome.

Hoax in the making

We can pick up the 'Covid' story in 2010 and the publication by the Rockefeller Foundation of a document called 'Scenarios for the Future of Technology and International Development'. The inner circle of the Rockefeller family has been serving the Cult since John D. Rockefeller (1839-1937) made his fortune with Standard Oil. It is less well known that the same Rockefeller – the Bill Gates of his day – was responsible for establishing what is now referred to as 'Big Pharma', the global network of pharmaceutical companies that make outrageous profits dispensing scalpel and drug 'medicine' and are obsessed with pumping vaccines in ever-increasing number into as many human arms and backsides as possible. John D. Rockefeller was the driving force behind the creation of the 'education' system in the United States and elsewhere specifically designed to program the perceptions of generations thereafter. The Rockefeller family donated exceptionally valuable land in New York for the United Nations building and were central in establishing the World Health Organization in 1948 as an agency of the UN which was created from the start as a Trojan horse and stalking horse for world government. Now enter Bill Gates. His family and the Rockefellers have long been extremely close and I have seen genealogy which claims that if you go back far enough the two families fuse into the same bloodline. Gates has said that the Bill and Melinda Gates Foundation was inspired by the Rockefeller Foundation and why not

when both are serving the same Cult? Major tax-exempt foundations are overwhelmingly criminal enterprises in which Cult assets fund the Cult agenda in the guise of 'philanthropy' while avoiding tax in the process. Cult operatives can become mega-rich in their role of front men and women for the psychopaths at the inner core and they, too, have to be psychopaths to knowingly serve such evil. Part of the deal is that a big percentage of the wealth gleaned from representing the Cult has to be spent advancing the ambitions of the Cult and hence you have the Rockefeller Foundation, Bill and Melinda Gates Foundation (and *so* many more) and people like George Soros with his global Open Society Foundations spending their billions in pursuit of global Cult control. Gates is a global public face of the Cult with his interventions in world affairs including Big Tech influence; a central role in the 'Covid' and 'vaccine' scam; promotion of the climate change shakedown; manipulation of education; geoengineering of the skies; and his food-control agenda as the biggest owner of farmland in America, his GMO promotion and through other means. As one writer said: 'Gates monopolizes or wields disproportionate influence over the tech industry, global health and vaccines, agriculture and food policy (including biopiracy and fake food), weather modification and other climate technologies, surveillance, education and media.' The almost limitless wealth secured through Microsoft and other not-allowed-to-fail ventures (including vaccines) has been ploughed into a long, long list of Cult projects designed to enslave the entire human race. Gates and the Rockefellers have been working as one unit with the Rockefeller-established World Health Organization leading global 'Covid' policy controlled by Gates through his mouth-piece Tedros. Gates became the WHO's biggest funder when Trump announced that the American government would cease its donations, but Biden immediately said he would restore the money when he took office in January, 2021. The Gates Foundation (the Cult) owns through limitless funding the world health system and the major players across the globe in the 'Covid' hoax.

Okay, with that background we return to that Rockefeller Foundation document of 2010 headed 'Scenarios for the Future of Technology and International Development' and its 'imaginary' epidemic of a virulent and deadly influenza strain which infected 20 percent of the global population and killed eight million in seven months. The Rockefeller scenario was that the epidemic destroyed economies, closed shops, offices and other businesses and led to governments imposing fierce rules and restrictions that included mandatory wearing of face masks and body-temperature checks to enter communal spaces like railway stations and supermarkets. The document predicted that even after the height of the Rockefeller-envisaged epidemic the authoritarian rule would continue to deal with further pandemics, transnational terrorism, environmental crises and rising poverty. Now you may think that the Rockefellers are our modern-day seers or alternatively, and rather more likely, that they well knew what was planned a few years further on. Fascism had to be imposed, you see, to 'protect citizens from risk and exposure'. The Rockefeller scenario document said:

During the pandemic, national leaders around the world flexed their authority and imposed airtight rules and restrictions, from the mandatory wearing of face masks to body-temperature checks at the entries to communal spaces like train stations and supermarkets. Even after the pandemic faded, this more authoritarian control and oversight of citizens and their activities stuck and even intensified. In order to protect themselves from the spread of increasingly global problems – from pandemics and transnational terrorism to environmental crises and rising poverty – leaders around the world took a firmer grip on power.

At first, the notion of a more controlled world gained wide acceptance and approval. Citizens willingly gave up some of their sovereignty – and their privacy – to more paternalistic states in exchange for greater safety and stability. Citizens were more tolerant, and even eager, for top-down direction and oversight, and national leaders had more latitude to impose order in the ways they saw fit.

In developed countries, this heightened oversight took many forms: biometric IDs for all citizens, for example, and tighter regulation of key industries whose stability was deemed vital to national interests. In many developed countries, enforced cooperation with a suite of new regulations and agreements slowly but steadily restored both order and, importantly, economic growth.

There we have the prophetic Rockefellers in 2010 and three years later came their paper for the Global Health Summit in Beijing, China, when government representatives, the private sector, international organisations and groups met to discuss the next 100 years of 'global health'. The Rockefeller Foundation-funded paper was called 'Dreaming the Future of Health for the Next 100 Years and more prophecy ensued as it described a dystopian future: 'The abundance of data, digitally tracking and linking people may mean the 'death of privacy' and may replace physical interaction with transient, virtual connection, generating isolation and raising questions of how values are shaped in virtual networks.' Next in the 'Covid' hoax preparation sequence came a 'table top' simulation in 2018 for another 'imaginary' pandemic of a disease called Clade X which was said to kill 900 million people. The exercise was organised by the Gates-funded Johns Hopkins University's Center for Health Security in the United States and this is the very same university that has been compiling the disgustingly and systematically erroneous global figures for 'Covid' cases and deaths. Similar Johns Hopkins health crisis scenarios have included the Dark Winter exercise in 2001 and Atlantic Storm in 2005.

Nostradamus 201

For sheer predictive genius look no further prophecy-watchers than the Bill Gates-funded Event 201 held only six weeks before the 'coronavirus pandemic' is supposed to have broken out in China and Event 201 was based on a scenario of a global 'coronavirus pandemic'. Melinda Gates, the great man's missus, told the BBC that he had 'prepared for years' for a coronavirus pandemic which told us what we already knew. Nostradamugates had predicted in a TED talk in 2015 that a pandemic was coming that would kill a lot of people and demolish the world economy. My god, the man is a machine – possibly even literally. Now here he was only weeks before the real thing funding just such a simulated scenario and involving his friends and associates at Johns Hopkins, the World Economic Forum Cult-front of Klaus Schwab, the United Nations,

Johnson & Johnson, major banks, and officials from China and the Centers for Disease Control in the United States. What synchronicity – Johns Hopkins would go on to compile the fraudulent 'Covid' figures, the World Economic Forum and Schwab would push the 'Great Reset' in response to 'Covid', the Centers for Disease Control would be at the forefront of 'Covid' policy in the United States, Johnson & Johnson would produce a 'Covid vaccine', and everything would officially start just weeks later in China. Spooky, eh? They were even accurate in creating a simulation of a 'virus' pandemic because the 'real thing' would also be a simulation. Event 201 was not an exercise preparing for something that might happen; it was a rehearsal for what those in control knew was *going* to happen and very shortly. Hours of this simulation were posted on the Internet and the various themes and responses mirrored what would soon be imposed to transform human society. News stories were inserted and what they said would be commonplace a few weeks later with still more prophecy perfection. Much discussion focused on the need to deal with misinformation and the 'anti-vax movement' which is exactly what happened when the 'virus' arrived – was said to have arrived – in the West.

Cult-owned social media banned criticism and exposure of the official 'virus' narrative and when I said there *was* no 'virus' in early April, 2020, I was banned by one platform after another including YouTube, Facebook and later Twitter. The mainstream broadcast media in Britain was in effect banned from interviewing me by the Tony-Blair-created government broadcasting censor Ofcom headed by career government bureaucrat Melanie Dawes who was appointed just as the 'virus' hoax was about to play out in January, 2020. At the same time the Ickonic media platform was using Vimeo, another ultra-Zionist-owned operation, while our own player was being created and they deleted in an instant hundreds of videos, documentaries, series and shows to confirm their unbelievable vindictiveness. We had copies, of course, and they had to be restored one by one when our player was ready. These people have no class. Sabbatian Facebook promised free advertisements for the Gates-

controlled World Health Organization narrative while deleting 'false claims and conspiracy theories' to stop 'misinformation' about the alleged coronavirus. All these responses could be seen just a short while earlier in the scenarios of Event 201. Extreme censorship was absolutely crucial for the Cult because the official story was so ridiculous and unsupportable by the evidence that it could never survive open debate and the free-flow of information and opinion. If you can't win a debate then don't have one is the Cult's approach throughout history. Facebook's little boy front man – front boy – Mark Zuckerberg equated 'credible and accurate information' with official sources and exposing their lies with 'misinformation'.

Silencing those that can see

The censorship dynamic of Event 201 is now the norm with an army of narrative-supporting 'fact-checker' organisations whose entire reason for being is to tell the public that official narratives are true and those exposing them are lying. One of the most appalling of these 'fact-checkers' is called NewsGuard founded by ultra-Zionist Americans Gordon Crovitz and Steven Brill. Crovitz is a former publisher of *The Wall Street Journal*, former Executive Vice President of Dow Jones, a member of the Council on Foreign Relations (CFR), and on the board of the American Association of Rhodes Scholars. The CFR and Rhodes Scholarships, named after Rothschild agent Cecil Rhodes who plundered the gold and diamonds of South Africa for his masters and the Cult, have featured widely in my books. NewsGuard don't seem to like me for some reason – I really can't think why – and they have done all they can to have me censored and discredited which is, to quote an old British politician, like being savaged by a dead sheep. They are, however, like all in the censorship network, very well connected and funded by organisations themselves funded by, or connected to, Bill Gates. As you would expect with anything associated with Gates NewsGuard has an offshoot called HealthGuard which 'fights online health care hoaxes'. How very kind. Somehow the NewsGuard European Managing Director Anna-Sophie Harling, a remarkably young-

looking woman with no broadcasting experience and little hands-on work in journalism, has somehow secured a position on the 'Content Board' of UK government broadcast censor Ofcom. An executive of an organisation seeking to discredit dissidents of the official narratives is making decisions for the government broadcast 'regulator' about content?? Another appalling 'fact-checker' is Full Fact funded by George Soros and global censors Google and Facebook.

It's amazing how many activists in the 'fact-checking', 'anti-hate', arena turn up in government-related positions – people like UK Labour Party activist Imran Ahmed who heads the Center for Countering Digital Hate founded by people like Morgan McSweeney, now chief of staff to the Labour Party's hapless and useless 'leader' Keir Starmer. Digital Hate – which is what it really is – uses the American spelling of Center to betray its connection to a transatlantic network of similar organisations which in 2020 shapeshifted from attacking people for 'hate' to attacking them for questioning the 'Covid' hoax and the dangers of the 'Covid vaccine'. It's just a coincidence, you understand. This is one of Imran Ahmed's hysterical statements: 'I would go beyond calling anti-vaxxers conspiracy theorists to say they are an extremist group that pose a national security risk.' No one could ever accuse this prat of understatement and he's including in that those parents who are now against vaccines after their children were damaged for life or killed by them. He's such a nice man. Ahmed does the rounds of the Woke media getting soft-ball questions from spineless 'journalists' who never ask what right he has to campaign to destroy the freedom of speech of others while he demands it for himself. There also seems to be an overrepresentation in Ofcom of people connected to the narrative-worshipping BBC. This incredible global network of narrative-support was super-vital when the 'Covid' hoax was played in the light of the mega-whopper lies that have to be defended from the spotlight cast by the most basic intelligence.

Setting the scene

The Cult plays the long game and proceeds step-by-step ensuring that everything is in place before major cards are played and they don't come any bigger than the 'Covid' hoax. The psychopaths can't handle events where the outcome isn't certain and as little as possible – preferably nothing – is left to chance. Politicians, government and medical officials who would follow direction were brought to illusory power in advance by the Cult web whether on the national stage or others like state governors and mayors of America. For decades the dynamic between officialdom, law enforcement and the public was changed from one of service to one of control and dictatorship. Behaviour manipulation networks established within government were waiting to impose the coming 'Covid' rules and regulations specifically designed to subdue and rewire the psyche of the people in the guise of protecting health. These included in the UK the Behavioural Insights Team part-owned by the British government Cabinet Office; the Scientific Pandemic Insights Group on Behaviours (SPI-B); and a whole web of intelligence and military groups seeking to direct the conversation on social media and control the narrative. Among them are the cyberwarfare (on the people) 77th Brigade of the British military which is also coordinated through the Cabinet Office as civilian and military leadership continues to combine in what they call the Fusion Doctrine. The 77th Brigade is a British equivalent of the infamous Israeli (Sabbatian) military cyberwarfare and Internet manipulation operation Unit 8200 which I expose at length in *The Trigger*. Also carefully in place were the medical and science advisers to government – many on the payroll past or present of Bill Gates – and a whole alternative structure of unelected government stood by to take control when elected parliaments were effectively closed down once the 'Covid' card was slammed on the table. The structure I have described here and so much more was installed in every major country through the Cult networks. The top-down control hierarchy looks like this: The Cult – Cult-owned Gates – the World Health Organization and Tedros – Gates-funded or controlled chief medical officers and science 'advisers' (dictators) in each country –

political 'leaders'– law enforcement – The People. Through this simple global communication and enforcement structure the policy of the Cult could be imposed on virtually the entire human population so long as they acquiesced to the fascism. With everything in place it was time for the button to be pressed in late 2019/early 2020.

These were the prime goals the Cult had to secure for its will to prevail:

1) Locking down economies, closing all but designated 'essential' businesses (Cult-owned corporations were 'essential'), and putting the population under house arrest was an imperative to destroy independent income and employment and ensure dependency on the Cult-controlled state in the Hunger Games Society. Lockdowns had to be established as the global blueprint from the start to respond to the 'virus' and followed by pretty much the entire world.

2) The global population had to be terrified into believing in a deadly 'virus' that didn't actually exist so they would unquestioningly obey authority in the belief that authority must know how best to protect them and their families. Software salesman Gates would suddenly morph into the world's health expert and be promoted as such by the Cult-owned media.

3) A method of testing that wasn't testing for the 'virus', but was only claimed to be, had to be in place to provide the illusion of 'cases' and subsequent 'deaths' that had a very different cause to the 'Covid-19' that would be scribbled on the death certificate.

4) Because there was no 'virus' and the great majority testing positive with a test not testing for the 'virus' would have no symptoms of anything the lie had to be sold that people without symptoms (without the 'virus') could still pass it on to others. This was crucial to justify for the first time quarantining – house arresting – healthy people. Without this the economy-destroying lockdown of *everybody* could not have been credibly sold.

5) The 'saviour' had to be seen as a vaccine which beyond evil drug companies were working like angels of mercy to develop as quickly as possible, with all corners cut, to save the day. The public must absolutely not know that the 'vaccine' had nothing to do with a 'virus' or that the contents were ready and waiting with a very different motive long before the 'Covid' card was even lifted from the pack.

I said in March, 2020, that the 'vaccine' would have been created way ahead of the 'Covid' hoax which justified its use and the following December an article in the New York *Intelligencer* magazine said the Moderna 'vaccine' had been 'designed' by

January, 2020. This was 'before China had even acknowledged that the disease could be transmitted from human to human, more than a week before the first confirmed coronavirus case in the United States'. The article said that by the time the first American death was announced a month later 'the vaccine had already been manufactured and shipped to the National Institutes of Health for the beginning of its Phase I clinical trial'. The 'vaccine' was actually 'designed' long before that although even with this timescale you would expect the article to ask how on earth it could have been done that quickly. Instead it asked why the 'vaccine' had not been rolled out then and not months later. Journalism in the mainstream is truly dead. I am going to detail in the next chapter why the 'virus' has never existed and how a hoax on that scale was possible, but first the foundation on which the Big Lie of 'Covid' was built.

The test that doesn't test

Fraudulent 'testing' is the bottom line of the whole 'Covid' hoax and was the means by which a 'virus' that did not exist *appeared* to exist. They could only achieve this magic trick by using a test not testing for the 'virus'. To use a test that *was* testing for the 'virus' would mean that every test would come back negative given there was no 'virus'. They chose to exploit something called the RT-PCR test invented by American biochemist Kary Mullis in the 1980s who said publicly that his PCR test … *cannot detect infectious disease*. Yes, the 'test' used worldwide to detect infectious 'Covid' to produce all the illusory 'cases' and 'deaths' compiled by Johns Hopkins and others *cannot detect infectious disease*. This fact came from the mouth of the man who invented PCR and was awarded the Nobel Prize in Chemistry in 1993 for doing so. Sadly, and incredibly conveniently for the Cult, Mullis died in August, 2019, at the age of 74 just before his test would be fraudulently used to unleash fascism on the world. He was said to have died from pneumonia which was an irony in itself. A few months later he would have had 'Covid-19' on his death certificate. I say the timing of his death was convenient because had he lived Mullis, a brilliant, honest and decent man, would have been

vociferously speaking out against the use of his test to detect 'Covid' when it was never designed, or able, to do that. I know that to be true given that Mullis made the same point when his test was used to 'detect' – not detect – HIV. He had been seriously critical of the Gallo/Montagnier claim to have isolated the HIV 'virus' and shown it to cause AIDS for which Mullis said there was no evidence. AIDS is actually not a disease but a series of diseases from which people die all the time. When they die from those *same diseases* after a positive 'test' for HIV then AIDS goes on their death certificate. I think I've heard that before somewhere. Countries instigated a policy with 'Covid' that anyone who tested positive with a test not testing for the 'virus' and died of any other cause within 28 days and even longer 'Covid-19' had to go on the death certificate. Cases have come from the test that can't test for infectious disease and the deaths are those who have died of *anything* after testing positive with a test not testing for the 'virus'. I'll have much more later about the death certificate scandal.

Mullis was deeply dismissive of the now US 'Covid' star Anthony Fauci who he said was a liar who didn't know anything about anything – 'and I would say that to his face – nothing.' He said of Fauci: 'The man thinks he can take a blood sample, put it in an electron microscope and if it's got a virus in there you'll know it – he doesn't understand electron microscopy and he doesn't understand medicine and shouldn't be in a position like he's in.' That position, terrifyingly, has made him the decider of 'Covid' fascism policy on behalf of the Cult in his role as director since 1984 of the National Institute of Allergy and Infectious Diseases (NIAID) while his record of being wrong is laughable; but being wrong, so long as it's the *right kind* of wrong, is why the Cult loves him. He'll say anything the Cult tells him to say. Fauci was made Chief Medical Adviser to the President immediately Biden took office. Biden was installed in the White House by Cult manipulation and one of his first decisions was to elevate Fauci to a position of even more control. This is a coincidence? Yes, and I identify as a flamenco dancer called Lola. How does such an incompetent criminal like Fauci remain in that

pivotal position in American health since *the 1980s*? When you serve the Cult it looks after you until you are surplus to requirements. Kary Mullis said prophetically of Fauci and his like: 'Those guys have an agenda and it's not an agenda we would like them to have … they make their own rules, they change them when they want to, and Tony Fauci does not mind going on television in front of the people who pay his salary and lie directly into the camera.' Fauci has done that almost daily since the 'Covid' hoax began. Lying is in Fauci's DNA. To make the situation crystal clear about the PCR test this is a direct quote from its inventor Kary Mullis:

> It [the PCR test] doesn't tell you that you're sick and doesn't tell you that the thing you ended up with was really going to hurt you ...'

Ask yourself why governments and medical systems the world over have been using this very test to decide who is 'infected' with the SARS-CoV-2 'virus' and the alleged disease it allegedly causes, 'Covid-19'. The answer to that question will tell you what has been going on. By the way, here's a little show-stopper – the 'new' SARS-CoV-2 'virus' was 'identified' as such right from the start using … *the PCR test not testing for the 'virus'*. If you are new to this and find that shocking then stick around. I have hardly started yet. Even worse, other 'tests', like the 'Lateral Flow Device' (LFD), are considered so useless that they have to be *confirmed* by the PCR test! Leaked emails written by Ben Dyson, adviser to UK 'Health' Secretary Matt Hancock, said they were 'dangerously unreliable'. Dyson, executive director of strategy at the Department of Health, wrote: 'As of today, someone who gets a positive LFD result in (say) London has at best a 25 per cent chance of it being a true positive, but if it is a self-reported test potentially as low as 10 per cent (on an optimistic assumption about specificity) or as low as 2 per cent (on a more pessimistic assumption).' These are the 'tests' that schoolchildren and the public are being urged to have twice a week or more and have to isolate if they get a positive. Each fake positive goes in the statistics as a 'case' no matter how ludicrously inaccurate and the

'cases' drive lockdown, masks and the pressure to 'vaccinate'. The government said in response to the email leak that the 'tests' were accurate which confirmed yet again what shocking bloody liars they are. The real false positive rate is *100 percent* as we'll see. In another 'you couldn't make it up' the UK government agreed to pay £2.8 billion to California's Innova Medical Group to supply the irrelevant lateral flow tests. The company's primary test-making centre is in China. Innova Medical Group, established in March, 2020, is owned by Pasaca Capital Inc, chaired by Chinese-American millionaire Charles Huang who was born in Wuhan.

How it works – and how it doesn't

The RT-PCR test, known by its full title of Polymerase chain reaction, is used across the world to make millions, even billions, of copies of a DNA/RNA genetic information sample. The process is called 'amplification' and means that a tiny sample of genetic material is amplified to bring out the detailed content. I stress that it is not testing for an infectious disease. It is simply amplifying a sample of genetic material. In the words of Kary Mullis: 'PCR is … just a process that's used to make a whole lot of something out of something.' To emphasise the point companies that make the PCR tests circulated around the world to 'test' for 'Covid' warn on the box that it can't be used to detect 'Covid' or infectious disease and is for research purposes only. It's okay, rest for a minute and you'll be fine. This is the test that produces the 'cases' and 'deaths' that have been used to destroy human society. All those global and national medical and scientific 'experts' demanding this destruction to 'save us' *KNOW* that the test is not testing for the 'virus' and the cases and deaths they claim to be real are an almost unimaginable fraud. Every one of them and so many others including politicians and psychopaths like Gates and Tedros must be brought before Nuremburg-type trials and jailed for the rest of their lives. The more the genetic sample is amplified by PCR the more elements of that material become sensitive to the test and by that I don't mean sensitive for a 'virus' but for elements of the genetic material which

is *naturally* in the body or relates to remnants of old conditions of various kinds lying dormant and causing no disease. Once the amplification of the PCR reaches a certain level *everyone* will test positive. So much of the material has been made sensitive to the test that everyone will have some part of it in their body. Even lying criminals like Fauci have said that once PCR amplifications pass 35 cycles everything will be a false positive that cannot be trusted for the reasons I have described. I say, like many proper doctors and scientists, that 100 percent of the 'positives' are false, but let's just go with Fauci for a moment.

He says that any amplification over 35 cycles will produce false positives and yet the US Centers for Disease Control (CDC) and Food and Drug Administration (FDA) have recommended up to *40 cycles* and the National Health Service (NHS) in Britain admitted in an internal document for staff that it was using *45 cycles* of amplification. A long list of other countries has been doing the same and at least one 'testing' laboratory has been using *50 cycles*. Have you ever heard a doctor, medical 'expert' or the media ask what level of amplification has been used to claim a 'positive'. The 'test' comes back 'positive' and so you have the 'virus', end of story. Now we can see how the government in Tanzania could send off samples from a goat and a pawpaw fruit under human names and both came back positive for 'Covid-19'. Tanzania president John Magufuli mocked the 'Covid' hysteria, the PCR test and masks and refused to import the DNA-manipulating 'vaccine'. The Cult hated him and an article sponsored by the Bill Gates Foundation appeared in the London *Guardian* in February, 2021, headed 'It's time for Africa to rein in Tanzania's anti-vaxxer president'. Well, 'reined in' he shortly was. Magufuli appeared in good health, but then, in March, 2021, he was dead at 61 from 'heart failure'. He was replaced by Samia Hassan Suhulu who is connected to Klaus Schwab's World Economic Forum and she immediately reversed Magufuli's 'Covid' policy. A sample of cola tested positive for 'Covid' with the PCR test in Germany while American actress and singer-songwriter Erykah Badu tested positive in one nostril and negative in the other. Footballer Ronaldo called

the PCR test 'bullshit' after testing positive three times and being forced to quarantine and miss matches when there was nothing wrong with him. The mantra from Tedros at the World Health Organization and national governments (same thing) has been test, test, test. They know that the more tests they can generate the more fake 'cases' they have which go on to become 'deaths' in ways I am coming to. The UK government has its Operation Moonshot planned to test multiple millions every day in workplaces and schools with free tests for everyone to use twice a week at home in line with the Cult plan from the start to make testing part of life. A government advertisement for an 'Interim Head of Asymptomatic Testing Communication' said the job included responsibility for delivering a 'communications strategy' (propaganda) 'to support the expansion of asymptomatic testing that *'normalises testing as part of everyday life*'. More tests means more fake 'cases', 'deaths' and fascism. I have heard of, and from, many people who booked a test, couldn't turn up, and yet got a positive result through the post for a test they'd never even had. The whole thing is crazy, but for the Cult there's method in the madness. Controlling and manipulating the level of amplification of the test means the authorities can control whenever they want the number of apparent 'cases' and 'deaths'. If they want to justify more fascist lockdown and destruction of livelihoods they keep the amplification high. If they want to give the illusion that lockdowns and the 'vaccine' are working then they lower the amplification and 'cases' and 'deaths' will appear to fall. In January, 2021, the Cult-owned World Health Organization suddenly warned laboratories about over-amplification of the test and to lower the threshold. Suddenly headlines began appearing such as: 'Why ARE "Covid" cases plummeting?' This was just when the vaccine rollout was underway and I had predicted months before they would make cases appear to fall through amplification tampering when the 'vaccine' came. These people are so predictable.

Cow vaccines?

The question must be asked of what is on the test swabs being poked far up the nose of the population to the base of the brain? A nasal swab punctured one woman's brain and caused it to leak fluid. Most of these procedures are being done by people with little training or medical knowledge. Dr Lorraine Day, former orthopaedic trauma surgeon and Chief of Orthopaedic Surgery at San Francisco General Hospital, says the tests are really a *'vaccine'*. Cows have long been vaccinated this way. She points out that masks have to cover the nose and the mouth where it is claimed the 'virus' exists in saliva. Why then don't they take saliva from the mouth as they do with a DNA test instead of pushing a long swab up the nose towards the brain? The ethmoid bone separates the nasal cavity from the brain and within that bone is the cribriform plate. Dr Day says that when the swab is pushed up against this plate and twisted the procedure is 'depositing things back there'. She claims that among these 'things' are nanoparticles that can enter the brain. Researchers have noted that a team at the Gates-funded Johns Hopkins have designed tiny, star-shaped micro-devices that can latch onto intestinal mucosa and release drugs into the body. Mucosa is the thin skin that covers the inside surface of parts of the body such as *the nose* and mouth and produces mucus to protect them. The Johns Hopkins micro-devices are called 'theragrippers' and were 'inspired' by a parasitic worm that digs its sharp teeth into a host's intestines. Nasal swabs are also coated in the sterilisation agent ethylene oxide. The US National Cancer Institute posts this explanation on its website:

> At room temperature, ethylene oxide is a flammable colorless gas with a sweet odor. It is used primarily to produce other chemicals, including antifreeze. In smaller amounts, ethylene oxide is used as a pesticide and a sterilizing agent. The ability of ethylene oxide to damage DNA makes it an effective sterilizing agent but also accounts for its cancer-causing activity.

The Institute mentions lymphoma and leukaemia as cancers most frequently reported to be associated with occupational exposure to ethylene oxide along with stomach and breast cancers. How does anyone think this is going to work out with the constant testing

regime being inflicted on adults and children at home and at school that will accumulate in the body anything that's on the swab?

Doctors know best

It is vital for people to realise that 'hero' doctors 'know' only what the Big Pharma-dominated medical authorities tell them to 'know' and if they refuse to 'know' what they are told to 'know' they are out the door. They are mostly not physicians or healers, but repeaters of the official narrative – or else. I have seen alleged professional doctors on British television make shocking statements that we are supposed to take seriously. One called 'Dr' Amir Khan, who is actually telling patients how to respond to illness, said that men could take the birth pill to 'help slow down the effects of Covid-19'. In March, 2021, another ridiculous 'Covid study' by an American doctor proposed injecting men with the female sex hormone progesterone as a 'Covid' treatment. British doctor Nighat Arif told the BBC that face coverings were now going to be part of ongoing normal. Yes, the vaccine protects you, she said (evidence?) … but the way to deal with viruses in the community was always going to come down to hand washing, face covering and keeping a physical distance. That's not what we were told before the 'vaccine' was circulating. Arif said she couldn't imagine ever again going on the underground or in a lift without a mask. I was just thanking my good luck that she was not my doctor when she said – in March, 2021 – that if 'we are *behaving* and we are doing all the right things' she thought we could 'have our nearest and dearest around us at home … around *Christmas* and N*ew Year!* Her patronising delivery was the usual school teacher talking to six-year-olds as she repeated every government talking point and probably believed them all. If we have learned anything from the 'Covid' experience surely it must be that humanity's perception of doctors needs a fundamental rethink. NHS 'doctor' Sara Kayat told her television audience that the 'Covid vaccine' would '100 percent prevent hospitalisation and death'. Not even Big Pharma claimed that. We have to stop taking 'experts' at their word without question when so many of them are

clueless and only repeating the party line on which their careers depend. That is not to say there are not brilliants doctors – there are and I have spoken to many of them since all this began – but you won't see them in the mainstream media or quoted by the psychopaths and yes-people in government.

Remember the name – Christian Drosten

German virologist Christian Drosten, Director of Charité Institute of Virology in Berlin, became a national star after the pandemic hoax began. He was feted on television and advised the German government on 'Covid' policy. Most importantly to the wider world Drosten led a group that produced the 'Covid' testing protocol for the PCR test. What a remarkable feat given the PCR cannot test for infectious disease and even more so when you think that Drosten said that his method of testing for SARS-CoV-2 was developed 'without having virus material available'. *He developed a test for a 'virus' that he didn't have and had never seen*. Let that sink in as you survey the global devastation that came from what he did. The whole catastrophe of Drosten's 'test' was based on the alleged genetic sequence published by Chinese scientists on the Internet. We will see in the next chapter that this alleged 'genetic sequence' has never been produced by China or anyone and cannot be when there *is no* SARS-CoV-2. Drosten, however, doesn't seem to let little details like that get in the way. He was the lead author with Victor Corman from the same Charité Hospital of the paper 'Detection of 2019 novel coronavirus (2019-nCoV) by real-time PCR' published in a magazine called *Eurosurveillance*. This became known as the Corman-Drosten paper. In November, 2020, with human society devastated by the effects of the Corman-Drosten test baloney, the protocol was publicly challenged by 22 international scientists and independent researchers from Europe, the United States, and Japan. Among them were senior molecular geneticists, biochemists, immunologists, and microbiologists. They produced a document headed 'External peer review of the RTPCR test to detect SARS-Cov-2 Reveals 10 Major Flaws At The Molecular and Methodological Level: Consequences

For False-Positive Results'. The flaws in the Corman-Drosten test included the following:

- The test is non-specific because of erroneous design
- Results are enormously variable
- The test is unable to discriminate between the whole 'virus' and viral fragments
- It doesn't have positive or negative controls
- The test lacks a standard operating procedure
- It is unsupported by proper peer view

The scientists said the PCR 'Covid' testing protocol was not founded on science and they demanded the Corman-Drosten paper be retracted by *Eurosurveillance*. They said all present and previous Covid deaths, cases, and 'infection rates' should be subject to a massive retroactive inquiry. Lockdowns and travel restrictions should be reviewed and relaxed and those diagnosed through PCR to have 'Covid-19' should not be forced to isolate. Dr Kevin Corbett, a health researcher and nurse educator with a long academic career producing a stream of peer-reviewed publications at many UK universities, made the same point about the PCR test debacle. He said of the scientists' conclusions: 'Every scientific rationale for the development of that test has been totally destroyed by this paper. It's like Hiroshima/Nagasaki to the Covid test.' He said that China hadn't given them an isolated 'virus' when Drosten developed the test. Instead they had developed the test from *a sequence in a gene bank*.' Put another way … *they made it up*! The scientists were supported in this contention by a Portuguese appeals court which ruled in November, 2020, that PCR tests are unreliable and it is unlawful to quarantine people based solely on a PCR test. The point about China not providing an isolated virus must be true when the 'virus' has never been isolated to this day and the consequences of that will become clear. Drosten and company produced this useless 'protocol' right on cue in January, 2020, just as the 'virus' was said to

be moving westward and it somehow managed to successfully pass a peer-review in 24 hours. In other words there was no peer-review for a test that would be used to decide who had 'Covid' and who didn't across the world. The Cult-created, Gates-controlled World Health Organization immediately recommended all its nearly 200 member countries to use the Drosten PCR protocol to detect 'cases' and 'deaths'. The sting was underway and it continues to this day.

So who is this Christian Drosten that produced the means through which death, destruction and economic catastrophe would be justified? His education background, including his doctoral thesis, would appear to be somewhat shrouded in mystery and his track record is dire as with another essential player in the 'Covid' hoax, the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College in London of whom more shortly. Drosten predicted in 2003 that the alleged original SARS 'virus' (SARS-1') was an epidemic that could have serious effects on economies and an effective vaccine would take at least two years to produce. Drosten's answer to every alleged 'outbreak' is a vaccine which you won't be shocked to know. What followed were just 774 official deaths worldwide and none in Germany where there were only nine cases. That is even if you believe there ever was a SARS 'virus' when the evidence is zilch and I will expand on this in the next chapter. Drosten claims to be co-discoverer of 'SARS-1' and developed a test for it in 2003. He was screaming warnings about 'swine flu' in 2009 and how it was a widespread infection far more severe than any dangers from a vaccine could be and people should get vaccinated. It would be helpful for Drosten's vocal chords if he simply recorded the words 'the virus is deadly and you need to get vaccinated' and copies could be handed out whenever the latest made-up threat comes along. Drosten's swine flu epidemic never happened, but Big Pharma didn't mind with governments spending hundreds of millions on vaccines that hardly anyone bothered to use and many who did wished they hadn't. A study in 2010 revealed that the risk of dying from swine flu, or H1N1, was no higher than that of the annual seasonal flu which is what at least most of 'it' really was as in

the case of 'Covid-19'. A media investigation into Drosten asked how with such a record of inaccuracy he could be *the* government adviser on these issues. The answer to that question is the same with Drosten, Ferguson and Fauci – they keep on giving the authorities the 'conclusions' and 'advice' they want to hear. Drosten certainly produced the goods for them in January, 2020, with his PCR protocol garbage and provided the foundation of what German internal medicine specialist Dr Claus Köhnlein, co-author of *Virus Mania*, called the 'test pandemic'. The 22 scientists in the *Eurosurveillance* challenge called out conflicts of interest within the Drosten 'protocol' group and with good reason. Olfert Landt, a regular co-author of Drosten 'studies', owns the biotech company TIB Molbiol Syntheselabor GmbH in Berlin which manufactures and sells the tests that Drosten and his mates come up with. They have done this with SARS, Enterotoxigenic E. coli (ETEC), MERS, Zika 'virus', yellow fever, and now 'Covid'. Landt told the *Berliner Zeitung* newspaper:

> The testing, design and development came from the Charité [Drosten and Corman]. We simply implemented it immediately in the form of a kit. And if we don't have the virus, which originally only existed in Wuhan, we can make a synthetic gene to simulate the genome of the virus. That's what we did very quickly.

This is more confirmation that the Drosten test was designed without access to the 'virus' and only a synthetic simulation which is what SARS-CoV-2 really is – a computer-generated synthetic fiction. It's quite an enterprise they have going here. A Drosten team decides what the test for something should be and Landt's biotech company flogs it to governments and medical systems across the world. His company must have made an absolute fortune since the 'Covid' hoax began. Dr Reiner Fuellmich, a prominent German consumer protection trial lawyer in Germany and California, is on Drosten's case and that of Tedros at the World Health Organization for crimes against humanity with a class-action lawsuit being prepared in the United States and other legal action in Germany.

Why China?

Scamming the world with a 'virus' that doesn't exist would seem impossible on the face of it, but not if you have control of the relatively few people that make policy decisions and the great majority of the global media. Remember it's not about changing 'real' reality it's about controlling *perception* of reality. You don't have to make something happen you only have make people *believe* that it's happening. Renegade Minds understand this and are therefore much harder to swindle. 'Covid-19' is not a 'real' 'virus'. It's a mind virus, like a computer virus, which has infected the minds, not the bodies, of billions. It all started, publically at least, in China and that alone is of central significance. The Cult was behind the revolution led by its asset Mao Zedong, or Chairman Mao, which established the People's Republic of China on October 1st, 1949. It should have been called The Cult's Republic of China, but the name had to reflect the recurring illusion that vicious dictatorships are run by and for the people (see all the 'Democratic Republics' controlled by tyrants). In the same way we have the 'Biden' Democratic Republic of America officially ruled by a puppet tyrant (at least temporarily) on behalf of Cult tyrants. The creation of Mao's merciless communist/fascist dictatorship was part of a frenzy of activity by the Cult at the conclusion of World War Two which, like the First World War, it had instigated through its assets in Germany, Britain, France, the United States and elsewhere. Israel was formed in 1948; the Soviet Union expanded its 'Iron Curtain' control, influence and military power with the Warsaw Pact communist alliance in 1955; the United Nations was formed in 1945 as a Cult precursor to world government; and a long list of world bodies would be established including the World Health Organization (1948), World Trade Organization (1948 under another name until 1995), International Monetary Fund (1945) and World Bank (1944). Human society was redrawn and hugely centralised in the global Problem-Reaction-Solution that was World War Two. All these changes were significant. Israel would become the headquarters of the Sabbatians

and the revolution in China would prepare the ground and control system for the events of 2019/2020.

Renegade Minds know there are no borders except for public consumption. The Cult is a seamless, borderless global entity and to understand the game we need to put aside labels like borders, nations, countries, communism, fascism and democracy. These delude the population into believing that countries are ruled within their borders by a government of whatever shade when these are mere agencies of a global power. America's illusion of democracy and China's communism/fascism are subsidiaries – vehicles – for the same agenda. We may hear about conflict and competition between America and China and on the lower levels that will be true; but at the Cult level they are branches of the same company in the way of the McDonald's example I gave earlier. I have tracked in the books over the years support by US governments of both parties for Chinese Communist Party infiltration of American society through allowing the sale of land, even military facilities, and the acquisition of American business and university influence. All this is underpinned by the infamous stealing of intellectual property and technological know-how. Cult-owned Silicon Valley corporations waive their fraudulent 'morality' to do business with human-rights-free China; Cult-controlled Disney has become China's PR department; and China in effect owns 'American' sports such as basketball which depends for much of its income on Chinese audiences. As a result any sports player, coach or official speaking out against China's horrific human rights record is immediately condemned or fired by the China-worshipping National Basketball Association. One of the first acts of China-controlled Biden was to issue an executive order telling federal agencies to stop making references to the 'virus' by the 'geographic location of its origin'. Long-time Congressman Jerry Nadler warned that criticising China, America's biggest rival, leads to hate crimes against Asian people in the United States. So shut up you bigot. China is fast closing in on Israel as a country that must not be criticised which is apt, really, given that Sabbatians control them both. The two countries have

developed close economic, military, technological and strategic ties which include involvement in China's 'Silk Road' transport and economic initiative to connect China with Europe. Israel was the first country in the Middle East to recognise the establishment of Mao's tyranny in 1950 months after it was established.

Project Wuhan – the 'Covid' Psyop

I emphasise again that the Cult plays the long game and what is happening to the world today is the result of centuries of calculated manipulation following a script to take control step-by-step of every aspect of human society. I will discuss later the common force behind all this that has spanned those centuries and thousands of years if the truth be told. Instigating the Mao revolution in China in 1949 with a 2020 'pandemic' in mind is not only how they work – the 71 years between them is really quite short by the Cult's standards of manipulation preparation. The reason for the Cult's Chinese revolution was to create a fiercely-controlled environment within which an extreme structure for human control could be incubated to eventually be unleashed across the world. We have seen this happen since the 'pandemic' emerged from China with the Chinese control-structure founded on AI technology and tyrannical enforcement sweep across the West. Until the moment when the Cult went for broke in the West and put its fascism on public display Western governments had to pay some lip-service to freedom and democracy to not alert too many people to the tyranny-in-the-making. Freedoms were more subtly eroded and power centralised with covert government structures put in place waiting for the arrival of 2020 when that smokescreen of 'freedom' could be dispensed with. The West was not able to move towards tyranny before 2020 anything like as fast as China which was created as a tyranny and had no limits on how fast it could construct the Cult's blueprint for global control. When the time came to impose that structure on the world it was the same Cult-owned Chinese communist/fascist government that provided the excuse – the 'Covid pandemic'. It was absolutely crucial to the Cult plan for the Chinese response to the 'pandemic' –

draconian lockdowns of the entire population – to become the blueprint that Western countries would follow to destroy the livelihoods and freedom of their people. This is why the Cult-owned, Gates-owned, WHO Director-General Tedros said early on:

The Chinese government is to be congratulated for the extraordinary measures it has taken to contain the outbreak. China is actually setting a new standard for outbreak response and it is not an exaggeration.

Forbes magazine said of China: '… those measures protected untold millions from getting the disease'. The Rockefeller Foundation 'epidemic scenario' document in 2010 said 'prophetically':

However, a few countries did fare better – China in particular. The Chinese government's quick imposition and enforcement of mandatory quarantine for all citizens, as well as its instant and near-hermetic sealing off of all borders, saved millions of lives, stopping the spread of the virus far earlier than in other countries and enabling a swifter post-pandemic recovery.

Once again – *spooky*.

The first official story was the 'bat theory' or rather the bat diversion. The source of the 'virus outbreak' we were told was a ''wet market' in Wuhan where bats and other animals are bought and eaten in horrifically unhygienic conditions. Then another story emerged through the alternative media that the 'virus' had been released on purpose or by accident from a BSL-4 (biosafety level 4) laboratory in Wuhan not far from the wet market. The lab was reported to create and work with lethal concoctions and bioweapons. Biosafety level 4 is the highest in the World Health Organization system of safety and containment. Renegade Minds are aware of what I call designer manipulation. The ideal for the Cult is for people to buy its prime narrative which in the opening salvoes of the 'pandemic' was the wet market story. It knows, however, that there is now a considerable worldwide alternative media of researchers sceptical of anything governments say and they are often given a version of events in a form they can perceive as credible while misdirecting them from the real truth. In this case let them

think that the conspiracy involved is a 'bioweapon virus' released from the Wuhan lab to keep them from the real conspiracy – *there is no 'virus'*. The WHO's current position on the source of the outbreak at the time of writing appears to be: 'We haven't got a clue, mate.' This is a good position to maintain mystery and bewilderment. The inner circle will know where the 'virus' came from – *nowhere*. The bottom line was to ensure the public believed there *was* a 'virus' and it didn't much matter if they thought it was natural or had been released from a lab. The belief that there was a 'deadly virus' was all that was needed to trigger global panic and fear. The population was terrified into handing their power to authority and doing what they were told. They had to or they were 'all gonna die'.

In March, 2020, information began to come my way from real doctors and scientists and my own additional research which had my intuition screaming: 'Yes, that's it! *There is no virus*.' The 'bioweapon' was not the 'virus'; it was the *'vaccine'* already being talked about that would be the bioweapon. My conclusion was further enhanced by happenings in Wuhan. The 'virus' was said to be sweeping the city and news footage circulated of people collapsing in the street (which they've never done in the West with the same 'virus'). The Chinese government was building 'new hospitals' in a matter of ten days to 'cope with demand' such was the virulent nature of the 'virus'. Yet in what seemed like no time the 'new hospitals' closed – even if they even opened – and China declared itself 'virus-free'. It was back to business as usual. This was more propaganda to promote the Chinese draconian lockdowns in the West as the way to 'beat the virus'. Trouble was that we subsequently had lockdown after lockdown, but never business as usual. As the people of the West and most of the rest of the world were caught in an ever-worsening spiral of lockdown, social distancing, masks, isolated old people, families forced apart, and livelihood destruction, it was party-time in Wuhan. Pictures emerged of thousands of people enjoying pool parties and concerts. It made no sense until you realised there never was a 'virus' and the

whole thing was a Cult set-up to transform human society out of one its major global strongholds – China.

How is it possible to deceive virtually the entire world population into believing there is a deadly virus when there is not even a 'virus' let alone a deadly one? It's nothing like as difficult as you would think and that's clearly true because it happened.

Postscript: See end of book Postscript for more on the 'Wuhan lab virus release' story which the authorities and media were pushing heavily in the summer of 2021 to divert attention from the truth that the 'Covid virus' is pure invention.

CHAPTER FIVE

There *is no* 'virus'

You can fool some of the people all of the time, and all of the people some of the time, but you cannot fool all of the people all of the time
Abraham Lincoln

The greatest form of mind control is repetition. The more you repeat the same mantra of alleged 'facts' the more will accept them to be true. It becomes an 'everyone knows that, mate'. If you can also censor any other version or alternative to your alleged 'facts' you are pretty much home and cooking.

By the start of 2020 the Cult owned the global mainstream media almost in its entirety to spew out its 'Covid' propaganda and ignore or discredit any other information and view. Cult-owned social media platforms in Cult-owned Silicon Valley were poised and ready to unleash a campaign of ferocious censorship to obliterate all but the official narrative. To complete the circle many demands for censorship by Silicon Valley were led by the mainstream media as 'journalists' became full-out enforcers for the Cult both as propagandists and censors. Part of this has been the influx of young people straight out of university who have become 'journalists' in significant positions. They have no experience and a headful of programmed perceptions from their years at school and university at a time when today's young are the most perceptually-targeted generations in known human history given the insidious impact of technology. They enter the media perceptually prepared and ready to repeat the narratives of the system that programmed them to

repeat its narratives. The BBC has a truly pathetic 'specialist disinformation reporter' called Marianna Spring who fits this bill perfectly. She is clueless about the world, how it works and what is really going on. Her role is to discredit anyone doing the job that a proper journalist would do and system-serving hacks like Spring wouldn't dare to do or even see the need to do. They are too busy licking the arse of authority which can never be wrong and, in the case of the BBC propaganda programme, *Panorama*, contacting payments systems such as PayPal to have a donations page taken down for a film company making documentaries questioning vaccines. Even the BBC soap opera *EastEnders* included a disgracefully biased scene in which an inarticulate white working class woman was made to look foolish for questioning the 'vaccine' while a well-spoken black man and Asian woman promoted the government narrative. It ticked every BBC box and the fact that the black and minority community was resisting the 'vaccine' had nothing to do with the way the scene was written. The BBC has become a disgusting tyrannical propaganda and censorship operation that should be defunded and disbanded and a free media take its place with a brief to stop censorship instead of demanding it. A BBC 'interview' with Gates goes something like: 'Mr Gates, sir, if I can call you sir, would you like to tell our audience why you are such a great man, a wonderful humanitarian philanthropist, and why you should absolutely be allowed as a software salesman to decide health policy for approaching eight billion people? Thank you, sir, please sir.' Propaganda programming has been incessant and merciless and when all you hear is the same story from the media, repeated by those around you who have only heard the same story, is it any wonder that people on a grand scale believe absolute mendacious garbage to be true? You are about to see, too, why this level of information control is necessary when the official 'Covid' narrative is so nonsensical and unsupportable by the evidence.

Structure of Deceit

The pyramid structure through which the 'Covid' hoax has been manifested is very simple and has to be to work. As few people as possible have to be involved with full knowledge of what they are doing – and why – or the real story would get out. At the top of the pyramid are the inner core of the Cult which controls Bill Gates who, in turn, controls the World Health Organization through his pivotal funding and his puppet Director-General mouthpiece, Tedros. Before he was appointed Tedros was chair of the Gates-founded Global Fund to 'fight against AIDS, tuberculosis and malaria', a board member of the Gates-funded 'vaccine alliance' GAVI, and on the board of another Gates-funded organisation. Gates owns him and picked him for a specific reason – Tedros is a crook and worse. 'Dr' Tedros (he's not a medical doctor, the first WHO chief not to be) was a member of the tyrannical Marxist government of Ethiopia for decades with all its human rights abuses. He has faced allegations of corruption and misappropriation of funds and was exposed three times for covering up cholera epidemics while Ethiopia's health minister. Tedros appointed the mass-murdering genocidal Zimbabwe dictator Robert Mugabe as a WHO goodwill ambassador for public health which, as with Tedros, is like appointing a psychopath to run a peace and love campaign. The move was so ridiculous that he had to drop Mugabe in the face of widespread condemnation. American economist David Steinman, a Nobel peace prize nominee, lodged a complaint with the International Criminal Court in The Hague over alleged genocide by Tedros when he was Ethiopia's foreign minister. Steinman says Tedros was a 'crucial decision maker' who directed the actions of Ethiopia's security forces from 2013 to 2015 and one of three officials in charge when those security services embarked on the 'killing' and 'torturing' of Ethiopians. You can see where Tedros is coming from and it's sobering to think that he has been the vehicle for Gates and the Cult to direct the global response to 'Covid'. Think about that. A psychopathic Cult dictates to psychopath Gates who dictates to psychopath Tedros who dictates how countries of the world must respond to a 'Covid virus' never scientifically shown to exist. At the same time psychopathic Cult-owned Silicon Valley information

giants like Google, YouTube, Facebook and Twitter announced very early on that they would give the Cult/Gates/Tedros/WHO version of the narrative free advertising and censor those who challenged their intelligence-insulting, mendacious story.

The next layer in the global 'medical' structure below the Cult, Gates and Tedros are the chief medical officers and science 'advisers' in each of the WHO member countries which means virtually all of them. Medical officers and arbiters of science (they're not) then take the WHO policy and recommended responses and impose them on their country's population while the political 'leaders' say they are deciding policy (they're clearly not) by 'following the science' on the advice of the 'experts' – the same medical officers and science 'advisers' (dictators). In this way with the rarest of exceptions the entire world followed the same policy of lockdown, people distancing, masks and 'vaccines' dictated by the psychopathic Cult, psychopathic Gates and psychopathic Tedros who we are supposed to believe give a damn about the health of the world population they are seeking to enslave. That, amazingly, is all there is to it in terms of crucial decision-making. Medical staff in each country then follow like sheep the dictates of the shepherds at the top of the national medical hierarchies – chief medical officers and science 'advisers' who themselves follow like sheep the shepherds of the World Health Organization and the Cult. Shepherds at the national level often have major funding and other connections to Gates and his Bill and Melinda Gates Foundation which carefully hands out money like confetti at a wedding to control the entire global medical system from the WHO down.

Follow the money

Christopher Whitty, Chief Medical Adviser to the UK Government at the centre of 'virus' policy, a senior adviser to the government's Scientific Advisory Group for Emergencies (SAGE), and Executive Board member of the World Health Organization, was gifted a grant of $40 million by the Bill and Melinda Gates Foundation for malaria research in Africa. The BBC described the unelected Whitty as 'the

official who will probably have the greatest impact on our everyday lives of any individual policymaker in modern times' and so it turned out. What Gates and Tedros have said Whitty has done like his equivalents around the world. Patrick Vallance, co-chair of SAGE and the government's Chief Scientific Adviser, is a former executive of Big Pharma giant GlaxoSmithKline with its fundamental financial and business connections to Bill Gates. In September, 2020, it was revealed that Vallance owned a deferred bonus of shares in GlaxoSmithKline worth £600,000 while the company was 'developing' a 'Covid vaccine'. Move along now – nothing to see here – what could possibly be wrong with that? Imperial College in London, a major player in 'Covid' policy in Britain and elsewhere with its 'Covid-19' Response Team, is funded by Gates and has big connections to China while the now infamous Professor Neil Ferguson, the useless 'computer modeller' at Imperial College is also funded by Gates. Ferguson delivered the dramatically inaccurate excuse for the first lockdowns (much more in the next chapter). The Institute for Health Metrics and Evaluation (IHME) in the United States, another source of outrageously false 'Covid' computer models to justify lockdowns, is bankrolled by Gates who is a vehement promotor of lockdowns. America's version of Whitty and Vallance, the again now infamous Anthony Fauci, has connections to 'Covid vaccine' maker Moderna as does Bill Gates through funding from the Bill and Melinda Gates Foundation. Fauci is director of the National Institute of Allergy and Infectious Diseases (NIAID), a major recipient of Gates money, and they are very close. Deborah Birx who was appointed White House Coronavirus Response Coordinator in February, 2020, is yet another with ties to Gates. Everywhere you look at the different elements around the world behind the coordination and decision making of the 'Covid' hoax there is Bill Gates and his money. They include the World Health Organization; Centers for Disease Control (CDC) in the United States; National Institutes of Health (NIH) of Anthony Fauci; Imperial College and Neil Ferguson; the London School of Hygiene where Chris Whitty worked; Regulatory agencies like the UK Medicines & Healthcare products Regulatory Agency (MHRA)

which gave emergency approval for 'Covid vaccines'; Wellcome Trust; GAVI, the Vaccine Alliance; the Coalition for Epidemic Preparedness Innovations (CEPI); Johns Hopkins University which has compiled the false 'Covid' figures; and the World Economic Forum. A Nationalfile.com article said:

> Gates has a lot of pull in the medical world, he has a multi-million dollar relationship with Dr. Fauci, and Fauci originally took the Gates line supporting vaccines and casting doubt on [the drug hydroxychloroquine]. Coronavirus response team member Dr. Deborah Birx, appointed by former president Obama to serve as United States Global AIDS Coordinator, also sits on the board of a group that has received billions from Gates' foundation, and Birx reportedly used a disputed Bill Gates-funded model for the White House's Coronavirus effort. Gates is a big proponent for a population lockdown scenario for the Coronavirus outbreak.

Another funder of Moderna is the Defense Advanced Research Projects Agency (DARPA), the technology-development arm of the Pentagon and one of the most sinister organisations on earth. DARPA had a major role with the CIA covert technology-funding operation In-Q-Tel in the development of Google and social media which is now at the centre of global censorship. Fauci and Gates are extremely close and openly admit to talking regularly about 'Covid' policy, but then why wouldn't Gates have a seat at every national 'Covid' table after his Foundation committed $1.75 billion to the 'fight against Covid-19'. When passed through our Orwellian Translation Unit this means that he has bought and paid for the Cult-driven 'Covid' response worldwide. Research the major 'Covid' response personnel in your own country and you will find the same Gates funding and other connections again and again. Medical and science chiefs following World Health Organization 'policy' sit atop a medical hierarchy in their country of administrators, doctors and nursing staff. These 'subordinates' are told they must work and behave in accordance with the policy delivered from the 'top' of the national 'health' pyramid which is largely the policy delivered by the WHO which is the policy delivered by Gates and the Cult. The whole 'Covid' narrative has been imposed on medical staff by a climate of fear although great numbers don't even need that to comply. They do so through breathtaking levels of ignorance and

include doctors who go through life simply repeating what Big Pharma and their hierarchical masters tell them to say and believe. No wonder Big Pharma 'medicine' is one of the biggest killers on Planet Earth.

The same top-down system of intimidation operates with regard to the Cult Big Pharma cartel which also dictates policy through national and global medical systems in this way. The Cult and Big Pharma agendas are the same because the former controls and owns the latter. 'Health' administrators, doctors, and nursing staff are told to support and parrot the dictated policy or they will face consequences which can include being fired. How sad it's been to see medical staff meekly repeating and imposing Cult policy without question and most of those who can see through the deceit are only willing to speak anonymously off the record. They know what will happen if their identity is known. This has left the courageous few to expose the lies about the 'virus', face masks, overwhelmed hospitals that aren't, and the dangers of the 'vaccine' that isn't a vaccine. When these medical professionals and scientists, some renowned in their field, have taken to the Internet to expose the truth their articles, comments and videos have been deleted by Cult-owned Facebook, Twitter and YouTube. What a real head-shaker to see YouTube videos with leading world scientists and highly qualified medical specialists with an added link underneath to the notorious Cult propaganda website *Wikipedia* to find the 'facts' about the same subject.

HIV – the 'Covid' trial-run

I'll give you an example of the consequences for health and truth that come from censorship and unquestioning belief in official narratives. The story was told by PCR inventor Kary Mullis in his book *Dancing Naked in the Mind Field*. He said that in 1984 he accepted as just another scientific fact that Luc Montagnier of France's Pasteur Institute and Robert Gallo of America's National Institutes of Health had independently discovered that a 'retrovirus' dubbed HIV (human immunodeficiency virus) caused AIDS. They

were, after all, Mullis writes, specialists in retroviruses. This is how the medical and science pyramids work. Something is announced or *assumed* and then becomes an everybody-knows-that purely through repetition of the assumption as if it is fact. Complete crap becomes accepted truth with no supporting evidence and only repetition of the crap. This is how a 'virus' that doesn't exist became the 'virus' that changed the world. The HIV-AIDS fairy story became a multi-billion pound industry and the media poured out propaganda terrifying the world about the deadly HIV 'virus' that caused the lethal AIDS. By then Mullis was working at a lab in Santa Monica, California, to detect retroviruses with his PCR test in blood donations received by the Red Cross. In doing so he asked a virologist where he could find a reference for HIV being the cause of AIDS. 'You don't need a reference,' the virologist said … *'Everybody knows it.'* Mullis said he wanted to quote a reference in the report he was doing and he said he felt a little funny about not knowing the source of such an important discovery when everyone else seemed to. The virologist suggested he cite a report by the Centers for Disease Control and Prevention (CDC) on morbidity and mortality. Mullis read the report, but it only said that an organism had been identified and did not say how. The report did not identify the original scientific work. Physicians, however, *assumed* (key recurring theme) that if the CDC was convinced that HIV caused AIDS then proof must exist. Mullis continues:

I did computer searches. Neither Montagnier, Gallo, nor anyone else had published papers describing experiments which led to the conclusion that HIV probably caused AIDS. I read the papers in Science for which they had become well known as AIDS doctors, but all they had said there was that they had found evidence of a past infection by something which was probably HIV in some AIDS patients.

They found antibodies. Antibodies to viruses had always been considered evidence of past disease, not present disease. Antibodies signaled that the virus had been defeated. The patient had saved himself. There was no indication in these papers that this virus caused a disease. They didn't show that everybody with the antibodies had the disease. In fact they found some healthy people with antibodies.

Mullis asked why their work had been published if Montagnier and Gallo hadn't really found this evidence, and why had they been fighting so hard to get credit for the discovery? He says he was hesitant to write 'HIV is the probable cause of AIDS' until he found published evidence to support that. 'Tens of thousands of scientists and researchers were spending billions of dollars a year doing research based on this idea,' Mullis writes. 'The reason had to be there somewhere; otherwise these people would not have allowed their research to settle into one narrow channel of investigation.' He said he lectured about PCR at numerous meetings where people were always talking about HIV and he asked them how they knew that HIV was the cause of AIDS:

> Everyone said something. Everyone had the answer at home, in the office, in some drawer. They all knew, and they would send me the papers as soon as they got back. But I never got any papers. Nobody ever sent me the news about how AIDS was caused by HIV.

Eventually Mullis was able to ask Montagnier himself about the reference proof when he lectured in San Diego at the grand opening of the University of California AIDS Research Center. Mullis says this was the last time he would ask his question without showing anger. Montagnier said he should reference the CDC report. 'I read it', Mullis said, and it didn't answer the question. 'If Montagnier didn't know the answer who the hell did?' Then one night Mullis was driving when an interview came on National Public Radio with Peter Duesberg, a prominent virologist at Berkeley and a California Scientist of the Year. Mullis says he finally understood why he could not find references that connected HIV to AIDS – *there weren't any!* No one had ever proved that HIV causes AIDS even though it had spawned a multi-billion pound global industry and the media was repeating this as fact every day in their articles and broadcasts terrifying the shit out of people about AIDS and giving the impression that a positive test for HIV (see 'Covid') was a death sentence. Duesberg was a threat to the AIDS gravy train and the agenda that underpinned it. He was therefore abused and castigated after he told the Proceedings of the National Academy of Sciences

there was no good evidence implicating the new 'virus'. Editors rejected his manuscripts and his research funds were deleted. Mullis points out that the CDC has defined AIDS as one of more than 30 diseases *if accompanied* by a positive result on a test that detects antibodies to HIV; but those same diseases are not defined as AIDS cases when antibodies are not detected:

> If an HIV-positive woman develops uterine cancer, for example, she is considered to have AIDS. If she is not HIV positive, she simply has uterine cancer. An HIV-positive man with tuberculosis has AIDS; if he tests negative he simply has tuberculosis. If he lives in Kenya or Colombia, where the test for HIV antibodies is too expensive, he is simply presumed to have the antibodies and therefore AIDS, and therefore he can be treated in the World Health Organization's clinic. It's the only medical help available in some places. And it's free, because the countries that support WHO are worried about AIDS.

Mullis accuses the CDC of continually adding new diseases (see ever more 'Covid symptoms') to the grand AIDS definition and of virtually doctoring the books to make it appear as if the disease continued to spread. He cites how in 1993 the CDC enormously broadened its AIDS definition and county health authorities were delighted because they received $2,500 per year from the Federal government for every reported AIDS case. Ladies and gentlemen, I have just described, via Kary Mullis, the 'Covid pandemic' of 2020 and beyond. Every element is the same and it's been pulled off in the same way by the same networks.

The 'Covid virus' exists? Okay – prove it. Er … still waiting

What Kary Mullis described with regard to 'HIV' has been repeated with 'Covid'. A claim is made that a new, or 'novel', infection has been found and the entire medical system of the world repeats that as fact exactly as they did with HIV and AIDS. No one in the mainstream asks rather relevant questions such as 'How do you know?' and 'Where is your proof?' The SARS-Cov-2 'virus' and the 'Covid-19 disease' became an overnight 'everybody-knows-that'. The origin could be debated and mulled over, but what you could not suggest was that 'SARS-Cov-2' didn't exist. That would be

ridiculous. 'Everybody knows' the 'virus' exists. Well, I didn't for one along with American proper doctors like Andrew Kaufman and Tom Cowan and long-time American proper journalist Jon Rappaport. We dared to pursue the obvious and simple question: 'Where's the evidence?' The overwhelming majority in medicine, journalism and the general public did not think to ask that. After all, *everyone knew* there was a new 'virus'. Everyone was saying so and I heard it on the BBC. Some would eventually argue that the 'deadly virus' was nothing like as deadly as claimed, but few would venture into the realms of its very existence. Had they done so they would have found that the evidence for that claim had gone AWOL as with HIV causes AIDS. In fact, not even that. For something to go AWOL it has to exist in the first place and scientific proof for a 'SARS-Cov-2' can be filed under nothing, nowhere and zilch.

Dr Andrew Kaufman is a board-certified forensic psychiatrist in New York State, a Doctor of Medicine and former Assistant Professor and Medical Director of Psychiatry at SUNY Upstate Medical University, and Medical Instructor of Hematology and Oncology at the Medical School of South Carolina. He also studied biology at the Massachusetts Institute of Technology (MIT) and trained in Psychiatry at Duke University. Kaufman is retired from allopathic medicine, but remains a consultant and educator on natural healing, I saw a video of his very early on in the 'Covid' hoax in which he questioned claims about the 'virus' in the absence of any supporting evidence and with plenty pointing the other way. I did everything I could to circulate his work which I felt was asking the pivotal questions that needed an answer. I can recommend an excellent pull-together interview he did with the website The Last Vagabond entitled *Dr Andrew Kaufman: Virus Isolation, Terrain Theory and Covid-19* and his website is andrewkaufmanmd.com. Kaufman is not only a forensic psychiatrist; he is forensic in all that he does. He always reads original scientific papers, experiments and studies instead of second-third-fourth-hand reports about the 'virus' in the media which are repeating the repeated repetition of the narrative. When he did so with the original Chinese 'virus' papers Kaufman

realised that there was no evidence of a 'SARS-Cov-2'. They had never – from the start – shown it to exist and every repeat of this claim worldwide was based on the accepted existence of proof that was nowhere to be found – see Kary Mullis and HIV. Here we go again.

Let's postulate

Kaufman discovered that the Chinese authorities immediately concluded that the cause of an illness that broke out among about 200 initial patients in Wuhan was a 'new virus' when there were no grounds to make that conclusion. The alleged 'virus' was not isolated from other genetic material in their samples and then shown through a system known as Koch's postulates to be the causative agent of the illness. The world was told that the SARS-Cov-2 'virus' caused a disease they called 'Covid-19' which had 'flu-like' symptoms and could lead to respiratory problems and pneumonia. If it wasn't so tragic it would almost be funny. *'Flu-like' symptoms'? Pneumonia? Respiratory disease?* What in *CHINA* and particularly in *Wuhan*, one of the most polluted cities in the world with a resulting epidemic of respiratory disease?? Three hundred thousand people get pneumonia in China every year and there are nearly a billion cases worldwide of 'flu-like symptoms'. These have a whole range of causes – including pollution in Wuhan – but no other possibility was credibly considered in late 2019 when the world was told there was a new and deadly 'virus'. The global prevalence of pneumonia and 'flu-like systems' gave the Cult networks unlimited potential to re-diagnose these other causes as the mythical 'Covid-19' and that is what they did from the very start. Kaufman revealed how Chinese medical and science authorities (all subordinates to the Cult-owned communist government) took genetic material from the lungs of only a few of the first patients. The material contained their own cells, bacteria, fungi and other microorganisms living in their bodies. The only way you could prove the existence of the 'virus' and its responsibility for the alleged 'Covid-19' was to isolate the virus from all the other material – a process also known as 'purification' – and

then follow the postulates sequence developed in the late 19th century by German physician and bacteriologist Robert Koch which became the 'gold standard' for connecting an alleged causation agent to a disease:

1. The microorganism (bacteria, fungus, virus, etc.) must be present in every case of the disease and all patients must have the same symptoms. It must also *not be present in healthy individuals*.

2. The microorganism must be isolated from the host with the disease. If the microorganism is a bacteria or fungus it must be grown in a pure culture. If it is a virus, it must be purified (i.e. containing no other material except the virus particles) from a clinical sample.

3. The specific disease, with all of its characteristics, must be reproduced when the infectious agent (the purified virus or a pure culture of bacteria or fungi) is inoculated into a healthy, susceptible host.

4. The microorganism must be recoverable from the experimentally infected host as in step 2.

Not one of these criteria has been met in the case of 'SARS-Cov-2' and 'Covid-19'. Not ONE. *EVER*. Robert Koch refers to bacteria and not viruses. What are called 'viral particles' are so minute (hence masks are useless by any definition) that they could only be seen after the invention of the electron microscope in the 1930s and can still only be observed through that means. American bacteriologist and virologist Thomas Milton Rivers, the so-called 'Father of Modern Virology' who was very significantly director of the Rockefeller Institute for Medical Research in the 1930s, developed a less stringent version of Koch's postulates to identify 'virus' causation known as 'Rivers criteria'. 'Covid' did not pass that process either. Some even doubt whether any 'virus' can be isolated from other particles containing genetic material in the Koch method. Freedom of Information requests in many countries asking for scientific proof that the 'Covid virus' has been purified and isolated and shown to exist have all come back with a 'we don't have that' and when this happened with a request to the UK Department of Health they added this comment:

However, outside of the scope of the [Freedom of Information Act] and on a discretionary basis, the following information has been advised to us, which may be of interest. Most infectious diseases are caused by viruses, bacteria or fungi. Some bacteria or fungi have the capacity to grow on their own in isolation, for example in colonies on a petri dish. Viruses are different in that they are what we call 'obligate pathogens' – that is, they cannot survive or reproduce without infecting a host ...

… For some diseases, it is possible to establish causation between a microorganism and a disease by isolating the pathogen from a patient, growing it in pure culture and reintroducing it to a healthy organism. These are known as 'Koch's postulates' and were developed in 1882. However, as our understanding of disease and different disease-causing agents has advanced, these are no longer the method for determining causation [Andrew Kaufman asks why in that case are there two published articles falsely claiming to satisfy Koch's postulates].

It has long been known that viral diseases cannot be identified in this way as viruses cannot be grown in 'pure culture'. When a patient is tested for a viral illness, this is normally done by looking for the presence of antigens, or viral genetic code in a host with molecular biology techniques [Kaufman asks how you could know the origin of these chemicals without having a pure culture for comparison].

For the record 'antigens' are defined so:

Invading microorganisms have antigens on their surface that the human body can recognise as being foreign – meaning not belonging to it. When the body recognises a foreign antigen, lymphocytes (white blood cells) produce antibodies, which are complementary in shape to the antigen.

Notwithstanding that this is open to question in relation to 'SARS-Cov-2' the presence of 'antibodies' can have many causes and they are found in people that are perfectly well. Kary Mullis said: 'Antibodies … had always been considered evidence of past disease, not present disease.'

'Covid' really is a *computer* 'virus'

Where the UK Department of Health statement says 'viruses' are now 'diagnosed' through a 'viral genetic code in a host with molecular biology techniques', they mean … *the PCR test* which its inventor said cannot test for infectious disease. They have no credible method of connecting a 'virus' to a disease and we will see that there is no scientific proof that any 'virus' causes any disease or there is any such thing as a 'virus' in the way that it is described. Tenacious Canadian researcher Christine Massey and her team made

some 40 Freedom of Information requests to national public health agencies in different countries asking for proof that SARS-CoV-2 has been isolated and not one of them could supply that information. Massey said of her request in Canada: 'Freedom of Information reveals Public Health Agency of Canada has no record of 'SARS-COV-2' isolation performed by anyone, anywhere, ever.' If you accept the comment from the UK Department of Health it's because they can't isolate a 'virus'. Even so many 'science' papers claimed to have isolated the 'Covid virus' until they were questioned and had to admit they hadn't. A reply from the Robert Koch Institute in Germany was typical: 'I am not aware of a paper which purified isolated SARS-CoV-2.' So what the hell was Christian Drosten and his gang using to design the 'Covid' testing protocol that has produced all the illusory Covid' cases and 'Covid' deaths when the head of the Chinese version of the CDC admitted there was a problem right from the start in that the 'virus' had never been isolated/purified? Breathe deeply: What they are calling 'Covid' is actually created by a *computer program* i.e. *they made it up* – er, that's it. They took lung fluid, with many sources of genetic material, from one single person alleged to be infected with Covid-19 by a PCR test which they *claimed*, without clear evidence, contained a 'virus'. They used several computer programs to create a model of a theoretical virus genome sequence from more than fifty-six million small sequences of RNA, each of an unknown source, assembling them like a puzzle with no known solution. The computer filled in the gaps with sequences from bits in the gene bank to make it look like a bat SARS-like coronavirus! A wave of the magic wand and poof, an in silico (computer-generated) genome, a scientific fantasy, was created. UK health researcher Dr Kevin Corbett made the same point with this analogy:

> … It's like giving you a few bones and saying that's your fish. It could be any fish. Not even a skeleton. Here's a few fragments of bones. That's your fish … It's all from gene bank and the bits of the virus sequence that weren't there they made up.
>
> They synthetically created them to fill in the blanks. That's what genetics is; it's a code. So it's ABBBCCDDD and you're missing some what you think is EEE so you put it in. It's all

synthetic. You just manufacture the bits that are missing. This is the end result of the geneticization of virology. This is basically a computer virus.

Further confirmation came in an email exchange between British citizen journalist Frances Leader and the government's Medicines & Healthcare Products Regulatory Agency (the Gates-funded MHRA) which gave emergency permission for untested 'Covid vaccines' to be used. The agency admitted that the 'vaccine' is not based on an isolated 'virus', but comes from a *computer-generated model*. Frances Leader was naturally banned from Cult-owned fascist Twitter for making this exchange public. The process of creating computer-generated alleged 'viruses' is called 'in silico' or 'in silicon' – computer chips – and the term 'in silico' is believed to originate with biological experiments using only a computer in 1989. 'Vaccines' involved with 'Covid' are also produced 'in silico' or by computer not a natural process. If the original 'virus' is nothing more than a made-up computer model how can there be 'new variants' of something that never existed in the first place? They are not new 'variants'; they are new *computer models* only minutely different to the original program and designed to further terrify the population into having the 'vaccine' and submitting to fascism. You want a 'new variant'? Click, click, enter – there you go. Tell the medical profession that you have discovered a 'South African variant', 'UK variants' or a 'Brazilian variant' and in the usual HIV-causes-AIDS manner they will unquestioningly repeat it with no evidence whatsoever to support these claims. They will go on television and warn about the dangers of 'new variants' while doing nothing more than repeating what they have been told to be true and knowing that any deviation from that would be career suicide. Big-time insiders will know it's a hoax, but much of the medical community is clueless about the way they are being played and themselves play the public without even being aware they are doing so. What an interesting 'coincidence' that AstraZeneca and Oxford University were conducting 'Covid vaccine trials' in the three countries – the UK, South Africa and Brazil – where the first three 'variants' were claimed to have 'broken out'.

Here's your 'virus' – it's a unicorn

Dr Andrew Kaufman presented a brilliant analysis describing how the 'virus' was imagined into fake existence when he dissected an article published by *Nature* and written by 19 authors detailing *alleged* 'sequencing of a complete viral genome' of the 'new SARS-CoV-2 virus'. This computer-modelled *in silico* genome was used as a template for all subsequent genome sequencing experiments that resulted in the so-called variants which he said now number more than *6,000*. The fake genome was constructed from more than 56 million individual short strands of RNA. Those little pieces were assembled into longer pieces by finding areas of overlapping sequences. The computer programs created over two million possible combinations from which the authors simply chose the longest one. They then compared this to a 'bat virus' and the computer 'alignment' rearranged the sequence and filled in the gaps! They called this computer-generated abomination the 'complete genome'. Dr Tom Cowan, a fellow medical author and collaborator with Kaufman, said such computer-generation constitutes scientific fraud and he makes this superb analogy:

> Here is an equivalency: A group of researchers claim to have found a unicorn because they found a piece of a hoof, a hair from a tail, and a snippet of a horn. They then add that information into a computer and program it to re-create the unicorn, and they then claim this computer re-creation is the real unicorn. Of course, they had never actually seen a unicorn so could not possibly have examined its genetic makeup to compare their samples with the actual unicorn's hair, hooves and horn.
>
> The researchers claim they decided which is the real genome of SARS-CoV-2 by 'consensus', sort of like a vote. Again, different computer programs will come up with different versions of the imaginary 'unicorn', so they come together as a group and decide which is the real imaginary unicorn.

This is how the 'virus' that has transformed the world was brought into fraudulent 'existence'. Extraordinary, yes, but as the Nazis said the bigger the lie the more will believe it. Cowan, however, wasn't finished and he went on to identify what he called the real blockbuster in the paper. He quotes this section from a paper written

by virologists and published by the CDC and then explains what it means:

Therefore, we examined the capacity of SARS-CoV-2 to infect and replicate in several common primate and human cell lines, including human adenocarcinoma cells (A549), human liver cells (HUH 7.0), and human embryonic kidney cells (HEK-293T). In addition to Vero E6 and Vero CCL81 cells. ... Each cell line was inoculated at high multiplicity of infection and examined 24h post-infection.

No CPE was observed in any of the cell lines except in Vero cells, which grew to greater than 10 to the 7th power at 24 h post-infection. In contrast, HUH 7.0 and 293T showed only modest viral replication, and A549 cells were incompatible with SARS CoV-2 infection.

Cowan explains that when virologists attempt to prove infection they have three possible 'hosts' or models on which they can test. The first was humans. Exposure to humans was generally not done for ethical reasons and has never been done with SARS-CoV-2 or any coronavirus. The second possible host was animals. Cowan said that forgetting for a moment that they never actually use purified virus when exposing animals they do use solutions that they *claim* contain the virus. Exposure to animals has been done with SARS-CoV-2 in an experiment involving mice and this is what they found: *None of the wild (normal) mice got sick*. In a group of genetically-modified mice, a statistically insignificant number lost weight and had slightly bristled fur, but they experienced nothing like the illness called 'Covid-19'. Cowan said the third method – the one they mostly rely on – is to inoculate solutions they *say* contain the virus onto a variety of tissue cultures. This process had never been shown to kill tissue *unless* the sample material was starved of nutrients and poisoned as *part of the process*. Yes, incredibly, in tissue experiments designed to show the 'virus' is responsible for killing the tissue they starve the tissue of nutrients and add toxic drugs including antibiotics and they do not have control studies to see if it's the starvation and poisoning that is degrading the tissue rather than the 'virus' they allege to be in there somewhere. You want me to pinch you? Yep, I understand. Tom Cowan said this about the whole nonsensical farce as he explains what that quote from the CDC paper really means:

The shocking thing about the above quote is that using their own methods, the virologists found that solutions containing SARS-CoV-2 – even in high amounts – were NOT, I repeat NOT, infective to any of the three human tissue cultures they tested. In plain English, this means they proved, on their terms, that this 'new coronavirus' is not infectious to human beings. It is ONLY infective to monkey kidney cells, and only then when you add two potent drugs (gentamicin and amphotericin), known to be toxic to kidneys, to the mix.

My friends, read this again and again. These virologists, published by the CDC, performed a clear proof, on their terms, showing that the SARS-CoV-2 virus is harmless to human beings. That is the only possible conclusion, but, unfortunately, this result is not even mentioned in their conclusion. They simply say they can provide virus stocks cultured only on monkey Vero cells, thanks for coming.

Cowan concluded: 'If people really understood how this "science" was done, I would hope they would storm the gates and demand honesty, transparency and truth.' Dr Michael Yeadon, former Vice President and Chief Scientific Adviser at drug giant Pfizer has been a vocal critic of the 'Covid vaccine' and its potential for multiple harm. He said in an interview in April, 2021, that 'not one [vaccine] has the virus. He was asked why vaccines normally using a 'dead' version of a disease to activate the immune system were not used for 'Covid' and instead we had the synthetic methods of the 'mRNA Covid vaccine'. Yeadon said that to do the former 'you'd have to have some of [the virus] wouldn't you?' He added: 'No-one's got any – seriously.' Yeadon said that surely they couldn't have fooled the whole world for a year without having a virus, 'but oddly enough ask around – no one's got it'. He didn't know why with all the 'great labs' around the world that the virus had not been isolated – 'Maybe they've been too busy running bad PCR tests and vaccines that people don't need.' What is today called 'science' is not 'science' at all. Science is no longer what is, but whatever people can be manipulated to *believe* that it is. Real science has been hijacked by the Cult to dispense and produce the 'expert scientists' and contentions that suit the agenda of the Cult. How big-time this has happened with the 'Covid' hoax which is entirely based on fake science delivered by fake 'scientists' and fake 'doctors'. The human-caused climate change hoax is also entirely based on fake science delivered by fake 'scientists' and fake 'climate experts'. In both cases real

scientists, climate experts and doctors have their views suppressed and deleted by the Cult-owned science establishment, media and Silicon Valley. This is the 'science' that politicians claim to be 'following' and a common denominator of 'Covid' and climate are Cult psychopaths Bill Gates and his mate Klaus Schwab at the Gates-funded World Economic Forum. But, don't worry, it's all just a coincidence and absolutely nothing to worry about. *Zzzzzzzz*.

What is a 'virus' REALLY?

Dr Tom Cowan is one of many contesting the very existence of viruses let alone that they cause disease. This is understandable when there is no scientific evidence for a disease-causing 'virus'. German virologist Dr Stefan Lanka won a landmark case in 2017 in the German Supreme Court over his contention that there is no such thing as a measles virus. He had offered a big prize for anyone who could prove there is and Lanka won his case when someone sought to claim the money. There is currently a prize of more than 225,000 euros on offer from an Isolate Truth Fund for anyone who can prove the isolation of SARS-CoV-2 and its genetic substance. Lanka wrote in an article headed 'The Misconception Called Virus' that scientists think a 'virus' is causing tissue to become diseased and degraded when in fact it is the *processes they are using* which do that – not a 'virus'. Lanka has done an important job in making this point clear as Cowan did in his analysis of the CDC paper. Lanka says that all claims about viruses as disease-causing pathogens are wrong and based on 'easily recognisable, understandable and verifiable misinterpretations.' Scientists believed they were working with 'viruses' in their laboratories when they were really working with 'typical particles of specific dying tissues or cells …' Lanka said that the tissue decaying process claimed to be caused by a 'virus' still happens when no alleged 'virus' is involved. It's the *process* that does the damage and not a 'virus'. The genetic sample is deprived of nutrients, removed from its energy supply through removal from the body and then doused in toxic antibiotics to remove any bacteria. He confirms again that establishment scientists do not (pinch me)

conduct control experiments to see if this is the case and if they did they would see the claims that 'viruses' are doing the damage is nonsense. He adds that during the measles 'virus' court case he commissioned an independent laboratory to perform just such a control experiment and the result was that the tissues and cells died in the exact same way as with alleged 'infected' material. This is supported by a gathering number of scientists, doctors and researchers who reject what is called 'germ theory' or the belief in the body being infected by contagious sources emitted by other people. Researchers Dawn Lester and David Parker take the same stance in their highly-detailed and sourced book *What Really Makes You Ill – Why everything you thought you knew about disease is wrong* which was recommended to me by a number of medical professionals genuinely seeking the truth. Lester and Parker say there is no provable scientific evidence to show that a 'virus' can be transmitted between people or people and animals or animals and people:

> The definition also claims that viruses are the cause of many diseases, as if this has been definitively proven. But this is not the case; there is no original scientific evidence that definitively demonstrates that any virus is the cause of any disease. The burden of proof for any theory lies with those who proposed it; but none of the existing documents provides 'proof' that supports the claim that 'viruses' are pathogens.

Dr Tom Cowan employs one of his clever analogies to describe the process by which a 'virus' is named as the culprit for a disease when what is called a 'virus' is only material released by cells detoxing themselves from infiltration by chemical or radiation poisoning. The tidal wave of technologically-generated radiation in the 'smart' modern world plus all the toxic food and drink are causing this to happen more than ever. Deluded 'scientists' misread this as a gathering impact of what they wrongly label 'viruses'.

Paper can infect houses

Cowan said in an article for davidicke.com – with his tongue only mildly in his cheek – that he believed he had made a tremendous

discovery that may revolutionise science. He had discovered that small bits of paper are alive, 'well alive-ish', can 'infect' houses, and then reproduce themselves inside the house. The result was that this explosion of growth in the paper inside the house causes the house to explode, blowing it to smithereens. His evidence for this new theory is that in the past months he had carefully examined many of the houses in his neighbourhood and found almost no scraps of paper on the lawns and surrounds of the house. There was an occasional stray label, but nothing more. Then he would return to these same houses a week or so later and with a few, not all of them, particularly the old and decrepit ones, he found to his shock and surprise they were littered with stray bits of paper. He knew then that the paper had infected these houses, made copies of itself, and blew up the house. A young boy on a bicycle at one of the sites told him he had seen a demolition crew using dynamite to explode the house the previous week, but Cowan dismissed this as the idle thoughts of silly boys because 'I was on to something big'. He was on to how 'scientists' mistake genetic material in the detoxifying process for something they call a 'virus'. Cowan said of his house and paper story:

> If this sounds crazy to you, it's because it should. This scenario is obviously nuts. But consider this admittedly embellished, for effect, current viral theory that all scientists, medical doctors and virologists currently believe.

He takes the example of the 'novel SARS-Cov2' virus to prove the point. First they take someone with an undefined illness called 'Covid-19' and don't even attempt to find any virus in their sputum. Never mind the scientists still describe how this 'virus', which they have not located attaches to a cell receptor, injects its genetic material, in 'Covid's' case, RNA, into the cell. The RNA once inserted exploits the cell to reproduce itself and makes 'thousands, nay millions, of copies of itself … Then it emerges victorious to claim its next victim':

If you were to look in the scientific literature for proof, actual scientific proof, that uniform SARS-CoV2 viruses have been properly isolated from the sputum of a sick person, that actual spike proteins could be seen protruding from the virus (which has not been found), you would find that such evidence doesn't exist.

If you go looking in the published scientific literature for actual pictures, proof, that these spike proteins or any viral proteins are ever attached to any receptor embedded in any cell membrane, you would also find that no such evidence exists. If you were to look for a video or documented evidence of the intact virus injecting its genetic material into the body of the cell, reproducing itself and then emerging victorious by budding off the cell membrane, you would find that no such evidence exists.

The closest thing you would find is electron micrograph pictures of cellular particles, possibly attached to cell debris, both of which to be seen were stained by heavy metals, a process that completely distorts their architecture within the living organism. This is like finding bits of paper stuck to the blown-up bricks, thereby proving the paper emerged by taking pieces of the bricks on its way out.

The Enders baloney

Cowan describes the 'Covid' story as being just as make-believe as his paper story and he charts back this fantasy to a Nobel Prize winner called John Enders (1897-1985), an American biomedical scientist who has been dubbed 'The Father of Modern Vaccines'. Enders is claimed to have 'discovered' the process of the viral culture which 'proved' that a 'virus' caused measles. Cowan explains how Enders did this 'by using the EXACT same procedure that has been followed by every virologist to find and characterize every new virus since 1954'. Enders took throat swabs from children with measles and immersed them in 2ml of milk. Penicillin (100u/ml) and the antibiotic streptomycin (50,g/ml) were added and the whole mix was centrifuged – rotated at high speed to separate large cellular debris from small particles and molecules as with milk and cream, for example. Cowan says that if the aim is to find little particles of genetic material ('viruses') in the snot from children with measles it would seem that the last thing you would do is mix the snot with other material – milk –that also has genetic material. 'How are you ever going to know whether whatever you found came from the snot or the milk?' He points out that streptomycin is a 'nephrotoxic' or poisonous-to-the-kidney drug. You will see the relevance of that

shortly. Cowan says that it gets worse, much worse, when Enders describes the culture medium upon which the virus 'grows': 'The culture medium consisted of bovine amniotic fluid (90%), beef embryo extract (5%), horse serum (5%), antibiotics and phenol red as an indicator of cell metabolism.' Cowan asks incredulously: 'Did he just say that the culture medium also contained fluids and tissues that are themselves rich sources of genetic material?' The genetic cocktail, or 'medium', is inoculated onto tissue and cells from rhesus monkey *kidney* tissue. This is where the importance of streptomycin comes in and currently-used antimicrobials and other drugs that are *poisonous to kidneys* and used in ALL modern viral cultures (e.g. gentamicin, streptomycin, and amphotericin). Cowan asks: 'How are you ever going to know from this witch's brew where any genetic material comes from as we now have five different sources of rich genetic material in our mix?' Remember, he says, that all genetic material, whether from monkey kidney tissues, bovine serum, milk, etc., is made from the exact same components. The same central question returns: 'How are you possibly going to know that it was the virus that killed the kidney tissue and not the toxic antibiotic and starvation rations on which you are growing the tissue?' John Enders answered the question himself – *you can't*:

A second agent was obtained from an uninoculated culture of monkey kidney cells. The cytopathic changes [death of the cells] it induced in the unstained preparations could not be distinguished with confidence from the viruses isolated from measles.

The death of the cells ('cytopathic changes') happened in exactly the same manner, whether they inoculated the kidney tissue with the measles snot or not, Cowan says. 'This is evidence that the destruction of the tissue, the very proof of viral causation of illness, was not caused by anything in the snot because they saw the same destructive effect when the snot was not even used … the cytopathic, i.e., cell-killing, changes come from the process of the culture itself, not from any virus in any snot, period.' Enders quotes in his 1957 paper a virologist called Ruckle as reporting similar findings 'and in addition has isolated an agent from monkey kidney tissue that is so

far indistinguishable from human measles virus'. In other words, Cowan says, these particles called 'measles viruses' are simply and clearly breakdown products of the starved and poisoned tissue. For measles 'virus' see all 'viruses' including the so-called 'Covid virus'. Enders, the 'Father of Modern Vaccines', also said:

There is a potential risk in employing cultures of primate cells for the production of vaccines composed of attenuated virus, since the presence of other agents possibly latent in primate tissues cannot be definitely excluded by any known method.

Cowan further quotes from a paper published in the journal *Viruses* in May, 2020, while the 'Covid pandemic' was well underway in the media if not in reality. 'EVs' here refers to particles of genetic debris from our own tissues, such as exosomes of which more in a moment: 'The remarkable resemblance between EVs and viruses has caused quite a few problems in the studies focused on the analysis of EVs released during viral infections.' Later the paper adds that to date a reliable method that can actually guarantee a complete separation (of EVs from viruses) DOES NOT EXIST. This was published at a time when a fairy tale 'virus' was claimed in total certainty to be causing a fairy tale 'viral disease' called 'Covid-19' – a fairy tale that was already well on the way to transforming human society in the image that the Cult has worked to achieve for so long. Cowan concludes his article:

To summarize, there is no scientific evidence that pathogenic viruses exist. What we think of as 'viruses' are simply the normal breakdown products of dead and dying tissues and cells. When we are well, we make fewer of these particles; when we are starved, poisoned, suffocated by wearing masks, or afraid, we make more.

There is no engineered virus circulating and making people sick. People in laboratories all over the world are making genetically modified products to make people sick. These are called vaccines. There is no virome, no 'ecosystem' of viruses, viruses are not 8%, 50% or 100 % of our genetic material. These are all simply erroneous ideas based on the misconception called a virus.

What is 'Covid'? Load of bollocks

The background described here by Cowan and Lanka was emphasised in the first video presentation that I saw by Dr Andrew Kaufman when he asked whether the 'Covid virus' was in truth a natural defence mechanism of the body called 'exosomes'. These are released by cells when in states of toxicity – see the same themes returning over and over. They are released ever more profusely as chemical and radiation toxicity increases and think of the potential effect therefore of 5G alone as its destructive frequencies infest the human energetic information field with a gathering pace (5G went online in Wuhan in 2019 as the 'virus' emerged). I'll have more about this later. Exosomes transmit a warning to the rest of the body that 'Houston, we have a problem'. Kaufman presented images of exosomes and compared them with 'Covid' under an electron microscope and the similarity was remarkable. They both attach to the same cell receptors (*claimed* in the case of 'Covid'), contain the same genetic material in the form of RNA or ribonucleic acid, and both are found in 'viral cell cultures' with damaged or dying cells. James Hildreth MD, President and Chief Executive Officer of the Meharry Medical College at Johns Hopkins, said: 'The virus is fully an exosome in every sense of the word.' Kaufman's conclusion was that there is no 'virus': 'This entire pandemic is a completely manufactured crisis … there is no evidence of anyone dying from [this] illness.' Dr Tom Cowan and Sally Fallon Morell, authors of *The Contagion Myth*, published a statement with Dr Kaufman in February, 2021, explaining why the 'virus' does not exist and you can read it that in full in the Appendix.

'Virus' theory can be traced to the 'cell theory' in 1858 of German physician Rudolf Virchow (1821-1920) who contended that disease originates from a single cell infiltrated by a 'virus'. Dr Stefan Lanka said that findings and insights with respect to the structure, function and central importance of tissues in the creation of life, which were already known in 1858, comprehensively refute the cell theory. Virchow ignored them. We have seen the part later played by John Enders in the 1950s and Lanka notes that infection theories were only established as a global dogma through the policies and

eugenics of the Third Reich in Nazi Germany (creation of the same Sabbatian cult behind the 'Covid' hoax). Lanka said: 'Before 1933, scientists dared to contradict this theory; after 1933, these critical scientists were silenced'. Dr Tom Cowan's view is that ill-heath is caused by too much of something, too little of something, or toxification from chemicals and radiation – not contagion. We must also highlight as a major source of the 'virus' theology a man still called the 'Father of Modern Virology' – Thomas Milton Rivers (1888-1962). There is no way given the Cult's long game policy that it was a coincidence for the 'Father of Modern Virology' to be director of the Rockefeller Institute for Medical Research from 1937 to 1956 when he is credited with making the Rockefeller Institute a leader in 'viral research'. Cult Rockefellers were the force behind the creation of Big Pharma 'medicine', established the World Health Organisation in 1948, and have long and close associations with the Gates family that now runs the WHO during the pandemic hoax through mega-rich Cult gofer and psychopath Bill Gates.

Only a Renegade Mind can see through all this bullshit by asking the questions that need to be answered, not taking 'no' or prevarication for an answer, and certainly not hiding from the truth in fear of speaking it. Renegade Minds have always changed the world for the better and they will change this one no matter how bleak it may currently appear to be.

CHAPTER SIX

Sequence of deceit

If you tell the truth, you don't have to remember anything
Mark Twain

Against the background that I have laid out this far the sequence that took us from an invented 'virus' in Cult-owned China in late 2019 to the fascist transformation of human society can be seen and understood in a whole new context.

We were told that a deadly disease had broken out in Wuhan and the world media began its campaign (coordinated by behavioural psychologists as we shall see) to terrify the population into unquestioning compliance. We were shown images of Chinese people collapsing in the street which never happened in the West with what was supposed to be the same condition. In the earliest days when alleged cases and deaths were few the fear register was hysterical in many areas of the media and this would expand into the common media narrative across the world. The real story was rather different, but we were never told that. The Chinese government, one of the Cult's biggest centres of global operation, said they had discovered a new illness with flu-like and pneumonia-type symptoms in a city with such toxic air that it is overwhelmed with flu-like symptoms, pneumonia and respiratory disease. Chinese scientists said it was a new – 'novel' – coronavirus which they called Sars-Cov-2 and that it caused a disease they labelled 'Covid-19'. There was no evidence for this and the 'virus' has never to this day been isolated, purified and its genetic code established from that. It

was from the beginning a computer-generated fiction. Stories of Chinese whistleblowers saying the number of deaths was being supressed or that the 'new disease' was related to the Wuhan bio-lab misdirected mainstream and alternative media into cul-de-sacs to obscure the real truth – there was no 'virus'.

Chinese scientists took genetic material from the lung fluid of just a few people and said they had found a 'new' disease when this material had a wide range of content. There was no evidence for a 'virus' for the very reasons explained in the last two chapters. The 'virus' has never been shown to (a) exist and (b) cause any disease. People were diagnosed on symptoms that are so widespread in Wuhan and polluted China and with a PCR test that can't detect infectious disease. On this farce the whole global scam was sold to the rest of the world which would also diagnose respiratory disease as 'Covid-19' from symptoms alone or with a PCR test not testing for a 'virus'. Flu miraculously disappeared *worldwide* in 2020 and into 2021 as it was redesignated 'Covid-19'. It was really the same old flu with its 'flu-like' symptoms attributed to 'flu-like' 'Covid-19'. At the same time with very few exceptions the Chinese response of draconian lockdown and fascism was the chosen weapon to respond across the West as recommended by the Cult-owned Tedros at the Cult-owned World Health Organization run by the Cult-owned Gates. All was going according to plan. Chinese scientists – everything in China is controlled by the Cult-owned government – compared their contaminated RNA lung-fluid material with other RNA sequences and said it appeared to be just under 80 percent identical to the SARS-CoV-1 'virus' claimed to be the cause of the SARS (severe acute respiratory syndrome) 'outbreak' in 2003. They decreed that because of this the 'new virus' had to be related and they called it SARS-CoV-2. There are some serious problems with this assumption and *assumption* was all it was. Most 'factual' science turns out to be assumptions repeated into everyone-knows-that. A match of under 80-percent is meaningless. Dr Kaufman makes the point that there's a *96 percent* genetic correlation between humans and chimpanzees, but 'no one would say our genetic material is part

of the chimpanzee family'. Yet the Chinese authorities were claiming that a much lower percentage, less than 80 percent, proved the existence of a new 'coronavirus'. For goodness sake human DNA is 60 percent similar to a *banana*.

You are feeling sleepy

The entire 'Covid' hoax is a global Psyop, a psychological operation to program the human mind into believing and fearing a complete fantasy. A crucial aspect of this was what *appeared* to happen in Italy. It was all very well streaming out daily images of an alleged catastrophe in Wuhan, but to the Western mind it was still on the other side of the world in a very different culture and setting. A reaction of 'this could happen to me and my family' was still nothing like as intense enough for the mind-doctors. The Cult needed a Western example to push people over that edge and it chose Italy, one of its major global locations going back to the Roman Empire. An Italian 'Covid' crisis was manufactured in a particular area called Lombardy which just happens to be notorious for its toxic air and therefore respiratory disease. Wuhan, China, déjà vu. An hysterical media told horror stories of Italians dying from 'Covid' in their droves and how Lombardy hospitals were being overrun by a tidal wave of desperately ill people needing treatment after being struck down by the 'deadly virus'. Here was the psychological turning point the Cult had planned. Wow, if this is happening in Italy, the Western mind concluded, this indeed could happen to me and my family. Another point is that Italian authorities responded by following the Chinese blueprint so vehemently recommended by the Cult-owned World Health Organization. They imposed fascistic lockdowns on the whole country viciously policed with the help of surveillance drones sweeping through the streets seeking out anyone who escaped from mass house arrest. Livelihoods were destroyed and psychology unravelled in the way we have witnessed since in all lockdown countries. Crucial to the plan was that Italy responded in this way to set the precedent of suspending freedom and imposing fascism in a 'Western liberal democracy'. I emphasised in an

animated video explanation on davidicke.com posted in the summer of 2020 how important it was to the Cult to expand the Chinese lockdown model across the West. Without this, and the bare-faced lie that non-symptomatic people could still transmit a 'disease' they didn't have, there was no way locking down the whole population, sick and not sick, could be pulled off. At just the right time and with no evidence Cult operatives and gofers claimed that people without symptoms could pass on the 'disease'. In the name of protecting the 'vulnerable' like elderly people, who lockdowns would kill by the tens of thousands, we had for the first time healthy people told to isolate as well as the sick. The great majority of people who tested positive had no symptoms because there was nothing wrong with them. It was just a trick made possible by a test not testing for the 'virus'.

Months after my animated video the Gates-funded Professor Neil Ferguson at the Gates-funded Imperial College confirmed that I was right. He didn't say it in those terms, naturally, but he did say it. Ferguson will enter the story shortly for his outrageously crazy 'computer models' that led to Britain, the United States and many other countries following the Chinese and now Italian methods of response. Put another way, following the Cult script. Ferguson said that SAGE, the UK government's scientific advisory group which has controlled 'Covid' policy from the start, wanted to follow the Chinese lockdown model (while they all continued to work and be paid), but they wondered if they could possibly, in Ferguson's words, 'get away with it in Europe'. 'Get away with it'? Who the hell do these moronic, arrogant people think they are? This appalling man Ferguson said that once Italy went into national lockdown they realised they, too, could mimic China:

> It's a communist one-party state, we said. We couldn't get away with it in Europe, we thought … and then Italy did it. And we realised we could. Behind this garbage from Ferguson is a simple fact: Doing the same as China in every country was the plan from the start and Ferguson's 'models' would play a central role in achieving that. It's just a coincidence, of course, and absolutely nothing to worry your little head about.

Oops, sorry, our mistake

Once the Italian segment of the Psyop had done the job it was designed to do a very different story emerged. Italian authorities revealed that 99 percent of those who had 'died from Covid-19' in Italy had one, two, three, or more 'co-morbidities' or illnesses and health problems that could have ended their life. The US Centers for Disease Control and Prevention (CDC) published a figure of 94 percent for Americans dying of 'Covid' while having other serious medical conditions – on average two to three (some five or six) other potential causes of death. In terms of death from an unproven 'virus' I say it is 100 percent. The other one percent in Italy and six percent in the US would presumably have died from 'Covid's' flu-like symptoms with a range of other possible causes in conjunction with a test not testing for the 'virus'. Fox News reported that even more startling figures had emerged in one US county in which 410 of 422 deaths attributed to 'Covid-19' had other potentially deadly health conditions. The Italian National Health Institute said later that the average age of people dying with a 'Covid-19' diagnosis in Italy was about 81. Ninety percent were over 70 with ten percent over 90. In terms of other reasons to die some 80 percent had two or more chronic diseases with half having three or more including cardiovascular problems, diabetes, respiratory problems and cancer. Why is the phantom 'Covid-19' said to kill overwhelmingly old people and hardly affect the young? Old people continually die of many causes and especially respiratory disease which you can re-diagnose 'Covid-19' while young people die in tiny numbers by comparison and rarely of respiratory disease. Old people 'die of Covid' because they die of other things that can be redesignated 'Covid' and it really is that simple.

Flu has flown

The blueprint was in place. Get your illusory 'cases' from a test not testing for the 'virus' and redesignate other causes of death as 'Covid-19'. You have an instant 'pandemic' from something that is nothing more than a computer-generated fiction. With near-on a

billion people having 'flu-like' symptoms every year the potential was limitless and we can see why flu quickly and apparently miraculously disappeared *worldwide* by being diagnosed 'Covid-19'. The painfully bloody obvious was explained away by the childlike media in headlines like this in the UK '*Independent*': 'Not a single case of flu detected by Public Health England this year as Covid restrictions suppress virus'. I kid you not. The masking, social distancing and house arrest that did not make the 'Covid virus' disappear somehow did so with the 'flu virus'. Even worse the article, by a bloke called Samuel Lovett, suggested that maybe the masking, sanitising and other 'Covid' measures should continue to keep the flu away. With a ridiculousness that disturbs your breathing (it's 'Covid-19') the said Lovett wrote: 'With widespread social distancing and mask-wearing measures in place throughout the UK, the usual routes of transmission for influenza have been blocked.' He had absolutely no evidence to support that statement, but look at the consequences of him acknowledging the obvious. With flu not disappearing at all and only being relabelled 'Covid-19' he would have to contemplate that 'Covid' was a hoax on a scale that is hard to imagine. You need guts and commitment to truth to even go there and that's clearly something Samuel Lovett does not have in abundance. He would never have got it through the editors anyway.

Tens of thousands die in the United States alone every winter from flu including many with pneumonia complications. CDC figures record *45 million* Americans diagnosed with flu in 2017-2018 of which 61,000 died and some reports claim 80,000. Where was the same hysteria then that we have seen with 'Covid-19'? Some 250,000 Americans are admitted to hospital with pneumonia every year with about 50,000 cases proving fatal. About 65 million suffer respiratory disease every year and three million deaths makes this the third biggest cause of death worldwide. You only have to redesignate a portion of all these people 'Covid-19' and you have an instant global pandemic or the *appearance* of one. Why would doctors do this? They are told to do this and all but a few dare not refuse those who must be obeyed. Doctors in general are not researching their own

knowledge and instead take it direct and unquestioned from the authorities that own them and their careers. The authorities say they must now diagnose these symptoms 'Covid-19' and not flu, or whatever, and they do it. Dark suits say put 'Covid-19' on death certificates no matter what the cause of death and the doctors do it. Renegade Minds don't fall for the illusion that doctors and medical staff are all highly-intelligent, highly-principled, seekers of medical truth. *Some are*, but not the majority. They are repeaters, gofers, and yes sir, no sir, purveyors of what the system demands they purvey. The 'Covid' con is not merely confined to diseases of the lungs. Instructions to doctors to put 'Covid-19' on death certificates for anyone dying of *anything* within 28 days (or much more) of a positive test not testing for the 'virus' opened the floodgates. The term dying *with* 'Covid' and not *of* 'Covid' was coined to cover the truth. Whether it was a *with* or an *of* they were all added to the death numbers attributed to the 'deadly virus' compiled by national governments and globally by the Gates-funded Johns Hopkins operation in the United States that was so involved in those 'pandemic' simulations. Fraudulent deaths were added to the ever-growing list of fraudulent 'cases' from false positives from a false test. No wonder Professor Walter Ricciardi, scientific advisor to the Italian minister of health, said after the Lombardy hysteria had done its job that 'Covid' death rates were due to Italy having the second oldest population in the world and to *how hospitals record deaths*:

The way in which we code deaths in our country is very generous in the sense that all the people who die in hospitals with the coronavirus are deemed to be dying of the coronavirus. On re-evaluation by the National Institute of Health, only 12 per cent of death certificates have shown a direct causality from coronavirus, while 88 per cent of patients who have died have at least one pre-morbidity – many had two or three.

This is extraordinary enough when you consider the propaganda campaign to use Italy to terrify the world, but how can they even say twelve percent were genuine when the 'virus' has not been shown to exist, its 'code' is a computer program, and diagnosis comes from a test not testing for it? As in China, and soon the world, 'Covid-19' in

Italy was a redesignation of diagnosis. Lies and corruption were to become the real 'pandemic' fuelled by a pathetically-compliant medical system taking its orders from the tiny few at the top of their national hierarchy who answered to the World Health Organization which answers to Gates and the Cult. Doctors were told – ordered – to diagnose a particular set of symptoms 'Covid-19' and put that on the death certificate for any cause of death if the patient had tested positive with a test not testing for the virus or had 'Covid' symptoms like the flu. The United States even introduced big financial incentives to manipulate the figures with hospitals receiving £4,600 from the Medicare system for diagnosing someone with regular pneumonia, $13,000 if they made the diagnosis from the same symptoms 'Covid-19' pneumonia, and $39, 000 if they put a 'Covid' diagnosed patient on a ventilator that would almost certainly kill them. A few – painfully and pathetically few – medical whistleblowers revealed (before Cult-owned YouTube deleted their videos) that they had been instructed to 'let the patient crash' and put them straight on a ventilator instead of going through a series of far less intrusive and dangerous methods as they would have done before the pandemic hoax began and the financial incentives kicked in. We are talking cold-blooded murder given that ventilators are so damaging to respiratory systems they are usually the last step before heaven awaits. Renegade Minds never fall for the belief that people in white coats are all angels of mercy and cannot be full-on psychopaths. I have explained in detail in *The Answer* how what I am describing here played out across the world coordinated by the World Health Organization through the medical hierarchies in almost every country.

Medical scientist calls it

Information about the non-existence of the 'virus' began to emerge for me in late March, 2020, and mushroomed after that. I was sent an email by Sir Julian Rose, a writer, researcher, and organic farming promotor, from a medical scientist friend of his in the United States. Even at that early stage in March the scientist was able to explain

how the 'Covid' hoax was being manipulated. He said there were no reliable tests for a specific 'Covid-19 virus' and nor were there any reliable agencies or media outlets for reporting numbers of actual 'Covid-19' cases. We have seen in the long period since then that he was absolutely right. 'Every action and reaction to Covid-19 is based on totally flawed data and we simply cannot make accurate assessments,' he said. Most people diagnosed with 'Covid-19' were showing nothing more than cold and flu-like symptoms 'because most coronavirus strains *are* nothing more than cold/flu-like symptoms'. We had farcical situations like an 84-year-old German man testing positive for 'Covid-19' and his nursing home ordered to quarantine only for him to be found to have a common cold. The scientist described back then why PCR tests and what he called the 'Mickey Mouse test kits' were useless for what they were claimed to be identifying. 'The idea these kits can isolate a specific virus like Covid-19 is nonsense,' he said. Significantly, he pointed out that 'if you want to create a totally false panic about a totally false pandemic – pick a coronavirus'. This is exactly what the Cult-owned Gates, World Economic Forum and Johns Hopkins University did with their Event 201 'simulation' followed by their real-life simulation called the 'pandemic'. The scientist said that all you had to do was select the sickest of people with respiratory-type diseases in a single location – 'say Wuhan' – and administer PCR tests to them. You can then claim that anyone showing 'viral sequences' similar to a coronavirus 'which will inevitably be quite a few' is suffering from a 'new' disease:

> Since you already selected the sickest flu cases a fairly high proportion of your sample will go on to die. You can then say this 'new' virus has a CFR [case fatality rate] higher than the flu and use this to infuse more concern and do more tests which will of course produce more 'cases', which expands the testing, which produces yet more 'cases' and so on and so on. Before long you have your 'pandemic', and all you have done is use a simple test kit trick to convert the worst flu and pneumonia cases into something new that doesn't ACTUALLY EXIST [my emphasis].

He said that you then 'just run the same scam in other countries' and make sure to keep the fear message running high 'so that people

will feel panicky and less able to think critically'. The only problem to overcome was the fact *there is no* actual new deadly pathogen and only regular sick people. This meant that deaths from the 'new deadly pathogen' were going to be way too low for a real new deadly virus pandemic, but he said this could be overcome in the following ways – all of which would go on to happen:

1. You can claim this is just the beginning and more deaths are imminent [you underpin this with fantasy 'computer projections']. Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.

2. You can [say that people] 'minimizing' the dangers are irresponsible and bully them into not talking about numbers.

3. You can talk crap about made up numbers hoping to blind people with pseudoscience.

4. You can start testing well people (who, of course, will also likely have shreds of coronavirus [RNA] in them) and thus inflate your 'case figures' with 'asymptomatic carriers' (you will of course have to spin that to sound deadly even though any virologist knows the more symptom-less cases you have the less deadly is your pathogen).

The scientist said that if you take these simple steps 'you can have your own entirely manufactured pandemic up and running in weeks'. His analysis made so early in the hoax was brilliantly prophetic of what would actually unfold. Pulling all the information together in these recent chapters we have this is simple 1, 2, 3, of how you can delude virtually the entire human population into believing in a 'virus' that doesn't exist:

- A 'Covid case' is someone who tests positive with a test not testing for the 'virus'.

- A 'Covid death' is someone who dies of *any cause* within 28 days (or much longer) of testing positive with a test not testing for the 'virus.

- Asymptomatic means there is nothing wrong with you, but they claim you can pass on what you don't have to justify locking

down (quarantining) healthy people in totality.

The foundations of the hoax are that simple. A study involving ten million people in Wuhan, published in November, 2020, demolished the whole lie about those without symptoms passing on the 'virus'. They found '300 asymptomatic cases' and traced their contacts to find that not one of them was detected with the 'virus'. 'Asymptomatic' patients and their contacts were isolated for no less than two weeks and nothing changed. I know it's all crap, but if you are going to claim that those without symptoms can transmit 'the virus' then you must produce evidence for that and they never have. Even World Health Organization official Dr Maria Van Kerkhove, head of the emerging diseases and zoonosis unit, said as early as June, 2020, that she doubted the validity of asymptomatic transmission. She said that 'from the data we have, it still seems to be rare that an asymptomatic person actually transmits onward to a secondary individual' and by 'rare' she meant that she couldn't cite any case of asymptomatic transmission.

The Ferguson factor

The problem for the Cult as it headed into March, 2020, when the script had lockdown due to start, was that despite all the manipulation of the case and death figures they still did not have enough people alleged to have died from 'Covid' to justify mass house arrest. This was overcome in the way the scientist described: 'You can claim this is just the beginning and more deaths are imminent … Use this as an excuse to quarantine everyone and then claim the quarantine prevented the expected millions of dead.' Enter one Professor Neil Ferguson, the Gates-funded 'epidemiologist' at the Gates-funded Imperial College in London. Ferguson is Britain's Christian Drosten in that he has a dire record of predicting health outcomes, but is still called upon to advise government on the next health outcome when another 'crisis' comes along. This may seem to be a strange and ridiculous thing to do. Why would you keep turning for policy guidance to people who have a history of being

monumentally wrong? Ah, but it makes sense from the Cult point of view. These 'experts' keep on producing predictions that suit the Cult agenda for societal transformation and so it was with Neil Ferguson as he revealed his horrific (and clearly insane) computer model predictions that allowed lockdowns to be imposed in Britain, the United States and many other countries. Ferguson does not have even an A-level in biology and would appear to have no formal training in computer modelling, medicine or epidemiology, according to Derek Winton, an MSc in Computational Intelligence. He wrote an article somewhat aghast at what Ferguson did which included taking no account of respiratory disease 'seasonality' which means it is far worse in the winter months. Who would have thought that respiratory disease could be worse in the winter? Well, certainly not Ferguson.

The massively China-connected Imperial College and its bizarre professor provided the excuse for the long-incubated Chinese model of human control to travel westward at lightning speed. Imperial College confirms on its website that it collaborates with the Chinese Research Institute; publishes more than 600 research papers every year with Chinese research institutions; has 225 Chinese staff; 2,600 Chinese students – the biggest international group; 7,000 former students living in China which is the largest group outside the UK; and was selected for a tour by China's President Xi Jinping during his state visit to the UK in 2015. The college takes major donations from China and describes itself as the UK's number one university collaborator with Chinese research institutions. The China communist/fascist government did not appear phased by the woeful predictions of Ferguson and Imperial when during the lockdown that Ferguson induced the college signed a five-year collaboration deal with China tech giant Huawei that will have Huawei's indoor 5G network equipment installed at the college's West London tech campus along with an 'AI cloud platform'. The deal includes Chinese sponsorship of Imperial's Venture Catalyst entrepreneurship competition. Imperial is an example of the enormous influence the Chinese government has within British and North American

universities and research centres – and further afield. Up to 200 academics from more than a dozen UK universities are being investigated on suspicion of 'unintentionally' helping the Chinese government build weapons of mass destruction by 'transferring world-leading research in advanced military technology such as aircraft, missile designs and cyberweapons'. Similar scandals have broken in the United States, but it's all a coincidence. Imperial College serves the agenda in many other ways including the promotion of every aspect of the United Nations Agenda 21/2030 (the Great Reset) and produced computer models to show that human-caused 'climate change' is happening when in the real world it isn't. Imperial College is driving the climate agenda as it drives the 'Covid' agenda (both Cult hoaxes) while Patrick Vallance, the UK government's Chief Scientific Adviser on 'Covid', was named Chief Scientific Adviser to the UN 'climate change' conference known as COP26 hosted by the government in Glasgow, Scotland. 'Covid' and 'climate' are fundamentally connected.

Professor Woeful

From Imperial's bosom came Neil Ferguson still advising government despite his previous disasters and it was announced early on that he and other key people like UK Chief Medical Adviser Chris Whitty had caught the 'virus' as the propaganda story was being sold. Somehow they managed to survive and we had Prime Minister Boris Johnson admitted to hospital with what was said to be a severe version of the 'virus' in this same period. His whole policy and demeanour changed when he returned to Downing Street. It's a small world with these government advisors – especially in their communal connections to Gates – and Ferguson had partnered with Whitty to write a paper called 'Infectious disease: Tough choices to reduce Ebola transmission' which involved another scare-story that didn't happen. Ferguson's 'models' predicted that up to150, 000 could die from 'mad cow disease', or BSE, and its version in sheep if it was transmitted to humans. BSE was not transmitted and instead triggered by an organophosphate pesticide used to treat a pest on

cows. Fewer than 200 deaths followed from the human form. Models by Ferguson and his fellow incompetents led to the unnecessary culling of millions of pigs, cattle and sheep in the foot and mouth outbreak in 2001 which destroyed the lives and livelihoods of farmers and their families who had often spent decades building their herds and flocks. Vast numbers of these animals did not have foot and mouth and had no contact with the infection. Another 'expert' behind the cull was Professor Roy Anderson, a computer modeller at Imperial College specialising in the epidemiology of *human*, not animal, disease. Anderson has served on the Bill and Melinda Gates Grand Challenges in Global Health advisory board and chairs another Gates-funded organisation. Gates is everywhere.

In a precursor to the 'Covid' script Ferguson backed closing schools 'for prolonged periods' over the swine flu 'pandemic' in 2009 and said it would affect a third of the world population if it continued to spread at the speed he claimed to be happening. His mates at Imperial College said much the same and a news report said: 'One of the authors, the epidemiologist and disease modeller Neil Ferguson, who sits on the World Health Organisation's emergency committee for the outbreak, said the virus had "full pandemic potential".' Professor Liam Donaldson, the Chris Whitty of his day as Chief Medical Officer, said the worst case could see 30 percent of the British people infected by swine flu with 65,000 dying. Ferguson and Donaldson were indeed proved correct when at the end of the year the number of deaths attributed to swine flu was 392. The term 'expert' is rather liberally applied unfortunately, not least to complete idiots. Swine flu 'projections' were great for GlaxoSmithKline (GSK) as millions rolled in for its Pandemrix influenza vaccine which led to brain damage with children most affected. The British government (taxpayers) paid out more than £60 million in compensation after GSK was given immunity from prosecution. Yet another 'Covid' déjà vu. Swine flu was supposed to have broken out in Mexico, but Dr Wolfgang Wodarg, a German doctor, former member of parliament and critic of the 'Covid' hoax, observed 'the spread of swine flu' in Mexico City at the time. He

said: 'What we experienced in Mexico City was a very mild flu which did not kill more than usual – which killed even fewer people than usual.' Hyping the fear against all the facts is not unique to 'Covid' and has happened many times before. Ferguson is reported to have over-estimated the projected death toll of bird flu (H5N1) by some three million-fold, but bird flu vaccine makers again made a killing from the scare. This is some of the background to the Neil Ferguson who produced the perfectly-timed computer models in early 2020 predicting that half a million people would die in Britain without draconian lockdown and 2.2 million in the United States. Politicians panicked, people panicked, and lockdowns of alleged short duration were instigated to 'flatten the curve' of cases gleaned from a test not testing for the 'virus'. I said at the time that the public could forget the 'short duration' bit. This was an agenda to destroy the livelihoods of the population and force them into mass control through dependency and there was going to be nothing 'short' about it. American researcher Daniel Horowitz described the consequences of the 'models' spewed out by Gates-funded Ferguson and Imperial College:

> What led our government and the governments of many other countries into panic was a single Imperial College of UK study, funded by global warming activists, that predicted 2.2 million deaths if we didn't lock down the country. In addition, the reported 8-9% death rate in Italy scared us into thinking there was some other mutation of this virus that they got, which might have come here.
>
> Together with the fact that we were finally testing and had the ability to actually report new cases, we thought we were headed for a death spiral. But again … we can't flatten a curve if we don't know when the curve started.

How about it *never* started?

Giving them what they want

An investigation by German news outlet *Welt Am Sonntag (World on Sunday)* revealed how in March, 2020, the German government gathered together 'leading scientists from several research institutes and universities' and 'together, they were to produce a [modelling]

paper that would serve as legitimization for further tough political measures'. The Cult agenda was justified by computer modelling not based on evidence or reality; it was specifically constructed to justify the Cult demand for lockdowns all over the world to destroy the independent livelihoods of the global population. All these modellers and everyone responsible for the 'Covid' hoax have a date with a trial like those in Nuremberg after World War Two when Nazis faced the consequences of their war crimes. These corrupt-beyond-belief 'modellers' wrote the paper according to government instructions and it said that that if lockdown measures were lifted then up to one million Germans would die from 'Covid-19' adding that some would die 'agonizingly at home, gasping for breath' unable to be treated by hospitals that couldn't cope. All lies. No matter – it gave the Cult all that it wanted. What did long-time government 'modeller' Neil Ferguson say? If the UK and the United States didn't lockdown half a million would die in Britain and 2.2 million Americans. Anyone see a theme here? 'Modellers' are such a crucial part of the lockdown strategy that we should look into their background and follow the money. Researcher Rosemary Frei produced an excellent article headlined 'The Modelling-paper Mafiosi'. She highlights a guy called John Edmunds, a British epidemiologist, and professor in the Faculty of Epidemiology and Population Health at the London School of Hygiene & Tropical Medicine. He studied at Imperial College. Edmunds is a member of government 'Covid' advisory bodies which have been dictating policy, the New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG) and the Scientific Advisory Group for Emergencies (SAGE).

Ferguson, another member of NERVTAG and SAGE, led the way with the original 'virus' and Edmunds has followed in the 'variant' stage and especially the so-called UK or Kent variant known as the 'Variant of Concern' (VOC) B.1.1.7. He said in a co-written report for the Centre for Mathematical modelling of Infectious Diseases at the London School of Hygiene and Tropical Medicine, with input from the Centre's 'Covid-19' Working Group, that there was 'a realistic

possibility that VOC B.1.1.7 is associated with an increased risk of death compared to non-VOC viruses'. Fear, fear, fear, get the vaccine, fear, fear, fear, get the vaccine. Rosemary Frei reveals that almost all the paper's authors and members of the modelling centre's 'Covid-19' Working Group receive funding from the Bill and Melinda Gates Foundation and/or the associated Gates-funded Wellcome Trust. The paper was published by e-journal *Medr χiv* which only publishes papers not peer-reviewed and the journal was established by an organisation headed by Facebook's Mark Zuckerberg and his missus. What a small world it is. Frei discovered that Edmunds is on the Scientific Advisory Board of the Coalition for Epidemic Preparedness Innovations (CEPI) which was established by the Bill and Melinda Gates Foundation, Klaus Schwab's Davos World Economic Forum and Big Pharma giant Wellcome. CEPI was 'launched in Davos [in 2017] to develop vaccines to stop future epidemics', according to its website. 'Our mission is to accelerate the development of vaccines against emerging infectious diseases and enable equitable access to these vaccines for people during outbreaks.' What kind people they are. Rosemary Frei reveals that Public Health England (PHE) director Susan Hopkins is an author of her organisation's non-peer-reviewed reports on 'new variants'. Hopkins is a professor of infectious diseases at London's Imperial College which is gifted tens of millions of dollars a year by the Bill and Melinda Gates Foundation. Gates-funded modelling disaster Neil Ferguson also co-authors Public Health England reports and he spoke in December, 2020, about the potential danger of the B.1.1.7. 'UK variant' promoted by Gates-funded modeller John Edmunds. When I come to the 'Covid vaccines' the 'new variants' will be shown for what they are – bollocks.

Connections, connections

All these people and modellers are lockdown-obsessed or, put another way, they demand what the Cult demands. Edmunds said in January, 2021, that to ease lockdowns too soon would be a disaster and they had to 'vaccinate much, much, much more widely than the

elderly'. Rosemary Frei highlights that Edmunds is married to Jeanne Pimenta who is described in a LinkedIn profile as director of epidemiology at GlaxoSmithKline (GSK) and she held shares in the company. Patrick Vallance, co-chair of SAGE and the government's Chief Scientific Adviser, is a former executive of GSK and has a deferred bonus of shares in the company worth £600,000. GSK has serious business connections with Bill Gates and is collaborating with mRNA-'vaccine' company CureVac to make 'vaccines' for the new variants that Edmunds is talking about. GSK is planning a 'Covid vaccine' with drug giant Sanofi. Puppet Prime Minister Boris Johnson announced in the spring of 2021 that up to 60 million vaccine doses were to be made at the GSK facility at Barnard Castle in the English North East. Barnard Castle, with a population of just 6,000, was famously visited in breach of lockdown rules in April, 2020, by Johnson aide Dominic Cummings who said that he drove there 'to test his eyesight' before driving back to London. Cummings would be better advised to test his integrity – not that it would take long. The GSK facility had nothing to do with his visit then although I'm sure Patrick Vallance would have been happy to arrange an introduction and some tea and biscuits. Ruthless psychopath Gates has made yet another fortune from vaccines in collaboration with Big Pharma companies and gushes at the phenomenal profits to be made from vaccines – more than a 20-to-1 return as he told one interviewer. Gates also tweeted in December, 2019, with the foreknowledge of what was coming: 'What's next for our foundation? I'm particularly excited about what the next year could mean for one of the best buys in global health: vaccines.'

Modeller John Edmunds is a big promotor of vaccines as all these people appear to be. He's the dean of the London School of Hygiene & Tropical Medicine's Faculty of Epidemiology and Population Health which is primarily funded by the Bill and Melinda Gates Foundation and the Gates-established and funded GAVI vaccine alliance which is the Gates vehicle to vaccinate the world. The organisation Doctors Without Borders has described GAVI as being 'aimed more at supporting drug-industry desires to promote new

products than at finding the most efficient and sustainable means for fighting the diseases of poverty'. But then that's why the psychopath Gates created it. John Edmunds said in a video that the London School of Hygiene & Tropical Medicine is involved in every aspect of vaccine development including large-scale clinical trials. He contends that mathematical modelling can show that vaccines protect individuals and society. That's on the basis of shit in and shit out, I take it. Edmunds serves on the UK Vaccine Network as does Ferguson and the government's foremost 'Covid' adviser, the grim-faced, dark-eyed Chris Whitty. The Vaccine Network says it works 'to support the government to identify and shortlist targeted investment opportunities for the most promising vaccines and vaccine technologies that will help combat infectious diseases with epidemic potential, and to address structural issues related to the UK's broader vaccine infrastructure'. Ferguson is acting Director of the Imperial College Vaccine Impact Modelling Consortium which has funding from the Bill and Melina Gates Foundation and the Gates-created GAVI 'vaccine alliance'. Anyone wonder why these characters see vaccines as the answer to every problem? Ferguson is wildly enthusiastic in his support for GAVI's campaign to vaccine children en masse in poor countries. You would expect someone like Gates who has constantly talked about the need to reduce the population to want to fund vaccines to keep more people alive. I'm sure that's why he does it. The John Edmunds London School of Hygiene & Tropical Medicine (LSHTM) has a Vaccines Manufacturing Innovation Centre which develops, tests and commercialises vaccines. Rosemary Frei writes:

> The vaccines centre also performs affiliated activities like combating 'vaccine hesitancy'. The latter includes the Vaccine Confidence Project. The project's stated purpose is, among other things, 'to provide analysis and guidance for early response and engagement with the public to ensure sustained confidence in vaccines and immunisation'. The Vaccine Confidence Project's director is LSHTM professor Heidi Larson. For more than a decade she's been researching how to combat vaccine hesitancy.

How the bloody hell can blokes like John Edmunds and Neil Ferguson with those connections and financial ties model 'virus' case

and death projections for the government and especially in a way that gives their paymasters like Gates exactly what they want? It's insane, but this is what you find throughout the world.

'Covid' is not dangerous, oops, wait, yes it is

Only days before Ferguson's nightmare scenario made Jackboot Johnson take Britain into a China-style lockdown to save us from a deadly 'virus' the UK government website gov.uk was reporting something very different to Ferguson on a page of official government guidance for 'high consequence infectious diseases (HCID)'. It said this about 'Covid-19':

As of 19 March 2020, COVID-19 *is no longer considered to be a high consequence infectious diseases (HCID) in the UK* [my emphasis]. The 4 nations public health HCID group made an interim recommendation in January 2020 to classify COVID-19 as an HCID. This was based on consideration of the UK HCID criteria about the virus and the disease with information available during the early stages of the outbreak.

Now that more is known about COVID-19, the public health bodies in the UK have reviewed the most up to date information about COVID-19 against the UK HCID criteria. They have determined that several features have now changed; in particular, more information is available about mortality rates (low overall), and there is now greater clinical awareness and a specific and sensitive laboratory test, the availability of which continues to increase. The Advisory Committee on Dangerous Pathogens (ACDP) is also of the opinion that COVID-19 should no longer be classified as an HCID.

Soon after the government had been exposed for downgrading the risk they upgraded it again and everyone was back to singing from the same Cult hymn book. Ferguson and his fellow Gates clones indicated that lockdowns and restrictions would have to continue until a Gates-funded vaccine was developed. Gates said the same because Ferguson and his like were repeating the Gates script which is the Cult script. 'Flatten the curve' became an ongoing nightmare of continuing lockdowns with periods in between of severe restrictions in pursuit of destroying independent incomes and had nothing to do with protecting health about which the Cult gives not a shit. Why wouldn't Ferguson be pushing a vaccine 'solution' when he's owned by vaccine-obsessive Gates who makes a fortune from them and

when Ferguson heads the Vaccine Impact Modelling Consortium at Imperial College funded by the Gates Foundation and GAVI, the 'vaccine alliance', created by Gates as his personal vaccine promotion operation? To compound the human catastrophe that Ferguson's 'models' did so much to create he was later exposed for breaking his own lockdown rules by having sexual liaisons with his married girlfriend Antonia Staats at his home while she was living at another location with her husband and children. Staats was a 'climate' activist and senior campaigner at the Soros-funded Avaaz which I wouldn't trust to tell me that grass is green. Ferguson had to resign as a government advisor over this hypocrisy in May, 2020, but after a period of quiet he was back being quoted by the ridiculous media on the need for more lockdowns and a vaccine rollout. Other government-advising 'scientists' from Imperial College' held the fort in his absence and said lockdown could be indefinite until a vaccine was found. The Cult script was being sung by the payrolled choir. I said there was no intention of going back to 'normal' when the 'vaccine' came because the 'vaccine' is part of a very different agenda that I will discuss in Human 2.0. Why would the Cult want to let the world go back to normal when destroying that normal forever was the whole point of what was happening? House arrest, closing businesses and schools through lockdown, (un)social distancing and masks all followed the Ferguson fantasy models. Again as I predicted (these people are so predictable) when the 'vaccine' arrived we were told that house arrest, lockdown, (un)social distancing and masks would still have to continue. I will deal with the masks in the next chapter because they are of fundamental importance.

Where's the 'pandemic'?

Any mildly in-depth assessment of the figures revealed what was really going on. Cult-funded and controlled organisations still have genuine people working within them such is the number involved. So it is with Genevieve Briand, assistant program director of the Applied Economics master's degree program at Johns Hopkins

University. She analysed the impact that 'Covid-19' had on deaths from *all* causes in the United States using official data from the CDC for the period from early February to early September, 2020. She found that allegedly 'Covid' *related*-deaths exceeded those from heart disease which she found strange with heart disease always the biggest cause of fatalities. Her research became even more significant when she noted the sudden decline in 2020 of *all* non-'Covid' deaths: 'This trend is completely contrary to the pattern observed in all previous years … the total decrease in deaths by other causes almost exactly equals the increase in deaths by Covid-19.' This was such a game, set and match in terms of what was happening that Johns Hopkins University deleted the article on the grounds that it 'was being used to support false and dangerous inaccuracies about the impact of the pandemic'. No – because it exposed the scam from official CDC figures and this was confirmed when those figures were published in January, 2021. Here we can see the effect of people dying from heart attacks, cancer, road accidents and gunshot wounds – *anything* – having 'Covid-19' on the death certificate along with those diagnosed from 'symptoms' who had even not tested positive with a test not testing for the 'virus'. I am not kidding with the gunshot wounds, by the way. Brenda Bock, coroner in Grand County, Colorado, revealed that two gunshot victims tested positive for the 'virus' within the previous 30 days and were therefore classified as 'Covid deaths'. Bock said: 'These two people had tested positive for Covid, but that's not what killed them. A gunshot wound is what killed them.' She said she had not even finished her investigation when the state listed the gunshot victims as deaths due to the 'virus'. The death and case figures for 'Covid-19' are an absolute joke and yet they are repeated like parrots by the media, politicians and alleged medical 'experts'. The official Cult narrative is the only show in town.

Genevieve Briand found that deaths from all causes were not exceptional in 2020 compared with previous years and a Spanish magazine published figures that said the same about Spain which was a 'Covid' propaganda hotspot at one point. *Discovery Salud*, a

health and medicine magazine, quoted government figures which showed how 17,000 *fewer* people died in Spain in 2020 than in 2019 and more than 26,000 fewer than in 2018. The age-standardised mortality rate for England and Wales when age distribution is taken into account was significantly lower in 2020 than the 1970s, 80s and 90s, and was only the ninth highest since 2000. Where is the 'pandemic'?

Post mortems and autopsies virtually disappeared for 'Covid' deaths amid claims that 'virus-infected' bodily fluids posed a risk to those carrying out the autopsy. This was rejected by renowned German pathologist and forensic doctor Klaus Püschel who said that he and his staff had by then done 150 autopsies on 'Covid' patients with no problems at all. He said they were needed to know why some 'Covid' patients suffered blood clots and not severe respiratory infections. The 'virus' is, after all, called SARS or 'severe acute respiratory syndrome'. I highlighted in the spring of 2020 this phenomenon and quoted New York intensive care doctor Cameron Kyle-Sidell who posted a soon deleted YouTube video to say that they had been told to prepare to treat an infectious disease called 'Covid-19', but that was not what they were dealing with. Instead he likened the lung condition of the most severely ill patients to what you would expect with cabin depressurisation in a plane at 30,000 feet or someone dropped on the top of Everest without oxygen or acclimatisation. I have never said this is not happening to a small minority of alleged 'Covid' patients – I am saying this is not caused by a phantom 'contagious virus'. Indeed Kyle-Sidell said that 'Covid-19' was not the disease they were told was coming their way. 'We are operating under a medical paradigm that is untrue,' he said, and he believed they were treating the wrong disease: 'These people are being slowly starved of oxygen.' Patients would take off their oxygen masks in a state of fear and stress and while they were blue in the face on the brink of death. They did not look like patients dying of pneumonia. You can see why they don't want autopsies when their virus doesn't exist and there is another condition in some people that they don't wish to be uncovered. I should add here that

the 5G system of millimetre waves was being rapidly introduced around the world in 2020 and even more so now as they fire 5G at the Earth from satellites. At 60 gigahertz within the 5G range that frequency interacts with the oxygen molecule and stops people breathing in sufficient oxygen to be absorbed into the bloodstream. They are installing 5G in schools and hospitals. The world is not mad or anything. 5G can cause major changes to the lungs and blood as I detail in *The Answer* and these consequences are labelled 'Covid-19', the alleged symptoms of which can be caused by 5G and other electromagnetic frequencies as cells respond to radiation poisoning.

The 'Covid death' scam

Dr Scott Jensen, a Minnesota state senator and medical doctor, exposed 'Covid' Medicare payment incentives to hospitals and death certificate manipulation. He said he was sent a seven-page document by the US Department of Health 'coaching' him on how to fill out death certificates which had never happened before. The document said that he didn't need to have a laboratory test for 'Covid-19' to put that on the death certificate and that shocked him when death certificates are supposed to be about facts. Jensen described how doctors had been 'encouraged, if not pressured' to make a diagnosis of 'Covid-19' if they thought it was probable or *'presumed*'. No positive test was necessary – not that this would have mattered anyway. He said doctors were told to diagnose 'Covid' by symptoms when these were the same as colds, allergies, other respiratory problems, and certainly with influenza which 'disappeared' in the 'Covid' era. A common sniffle was enough to get the dreaded verdict. Ontario authorities decreed that a single care home resident with *one* symptom from a long list must lead to the isolation of the entire home. Other courageous doctors like Jensen made the same point about death figure manipulation and how deaths by other causes were falling while 'Covid-19 deaths' were rising at the same rate due to re-diagnosis. Their videos rarely survive long on YouTube with its Cult-supporting algorithms courtesy of CEO Susan Wojcicki and her bosses at Google. Figure-tampering was so glaring

and ubiquitous that even officials were letting it slip or outright saying it. UK chief scientific adviser Patrick Vallance said on one occasion that 'Covid' on the death certificate doesn't mean 'Covid' was the cause of death (so why the hell is it there?) and we had the rare sight of a BBC reporter telling the truth when she said: 'Someone could be successfully treated for Covid, in say April, discharged, and then in June, get run over by a bus and die … That person would still be counted as a Covid death in England.' Yet the BBC and the rest of the world media went on repeating the case and death figures as if they were real. Illinois Public Health Director Dr Ngozi Ezike revealed the deceit while her bosses must have been clenching their buttocks:

> If you were in a hospice and given a few weeks to live and you were then found to have Covid that would be counted as a Covid death. [There might be] a clear alternate cause, but it is still listed as a Covid death. So everyone listed as a Covid death doesn't mean that was the cause of the death, but that they had Covid at the time of death.

Yes, a 'Covid virus' never shown to exist and tested for with a test not testing for the 'virus'. In the first period of the pandemic hoax through the spring of 2020 the process began of designating almost everything a 'Covid' death and this has continued ever since. I sat in a restaurant one night listening to a loud conversation on the next table where a family was discussing in bewilderment how a relative who had no symptoms of 'Covid', and had died of a long-term problem, could have been diagnosed a death by the 'virus'. I could understand their bewilderment. If they read this book they will know why this medical fraud has been perpetrated the world over.

Some media truth shock

The media ignored the evidence of death certificate fraud until eventually one columnist did speak out when she saw it first-hand. Bel Mooney is a long-time national newspaper journalist in Britain currently working for the *Daily Mail*. Her article on February 19th, 2021, carried this headline: 'My dad Ted passed three Covid tests

and died of a chronic illness yet he's officially one of Britain's 120,000 victims of the virus and is far from alone ... so how many more are there?' She told how her 99-year-old father was in a care home with a long-standing chronic obstructive pulmonary disease and vascular dementia. Maybe, but he was still aware enough to tell her from the start that there was no 'virus' and he refused the 'vaccine' for that reason. His death was not unexpected given his chronic health problems and Mooney said she was shocked to find that 'Covid-19' was declared the cause of death on his death certificate. She said this was a 'bizarre and unacceptable untruth' for a man with long-time health problems who had tested negative twice at the home for the 'virus'. I was also shocked by this story although not by what she said. I had been highlighting the death certificate manipulation for ten months. It was the confirmation that a professional full-time journalist only realised this was going on when it affected her directly and neither did she know that whether her dad tested positive or negative was irrelevant with the test not testing for the 'virus'. Where had she been? She said she did not believe in 'conspiracy theories' without knowing I'm sure that this and 'conspiracy theorists' were terms put into widespread circulation by the CIA in the 1960s to discredit those who did not accept the ridiculous official story of the Kennedy assassination. A blanket statement of 'I don't believe in conspiracy theories' is always bizarre. The dictionary definition of the term alone means the world is drowning in conspiracies. What she said was even more daft when her dad had just been affected by the 'Covid' conspiracy. Why else does she think that 'Covid-19' was going on the death certificates of people who died of something else?

To be fair once she saw from personal experience what was happening she didn't mince words. Mooney was called by the care home on the morning of February 9th to be told her father had died in his sleep. When she asked for the official cause of death what came back was 'Covid-19'. Mooney challenged this and was told there had been deaths from Covid on the dementia floor (confirmed by a test not testing for the 'virus') so they considered it 'reasonable

to assume'. 'But doctor,' Mooney rightly protested, 'an assumption isn't a diagnosis.' She said she didn't blame the perfectly decent and sympathetic doctor – 'he was just doing his job'. Sorry, but that's *bullshit*. He wasn't doing his job at all. He was putting a false cause of death on the death certificate and that is a criminal offence for which he should be brought to account and the same with the millions of doctors worldwide who have done the same. They were not doing their job they were following orders and that must not wash at new Nuremberg trials any more than it did at the first ones. Mooney's doctor was 'assuming' (presuming) as he was told to, but 'just following orders' makes no difference to his actions. A doctor's job is to serve the patient and the truth, not follow orders, but that's what they have done all over the world and played a central part in making the 'Covid' hoax possible with all its catastrophic consequences for humanity. Shame on them and they must answer for their actions. Mooney said her disquiet worsened when she registered her father's death by telephone and was told by the registrar there had been very many other cases like hers where 'the deceased' had not tested positive for 'Covid' yet it was recorded as the cause of death. The test may not matter, but those involved at their level *think* it matters and it shows a callous disregard for accurate diagnosis. The pressure to do this is coming from the top of the national 'health' pyramids which in turn obey the World Health Organization which obeys Gates and the Cult. Mooney said the registrar agreed that this must distort the national figures adding that 'the strangest thing is that every winter we record countless deaths from flu, and this winter there have been none. Not one!' She asked if the registrar thought deaths from flu were being misdiagnosed and lumped together with 'Covid' deaths. The answer was a 'puzzled yes'. Mooney said that the funeral director said the same about 'Covid' deaths which had nothing to do with 'Covid'. They had lost count of the number of families upset by this and other funeral companies in different countries have had the same experience. Mooney wrote:

The nightly shroud-waving and shocking close-ups of pain imposed on us by the TV news bewildered and terrified the population into eager compliance with lockdowns. We were invited to 'save the NHS' and to grieve for strangers – the real-life loved ones behind those shocking death counts. Why would the public imagine what I now fear, namely that the way Covid-19 death statistics are compiled might make the numbers seem greater than they are?

Oh, just a little bit – like 100 percent.

Do the maths

Mooney asked why a country would wish to skew its mortality figures by wrongly certifying deaths? What had been going on? Well, if you don't believe in conspiracies you will never find the answer which is that *it's a conspiracy*. She did, however, describe what she had discovered as a 'national scandal'. In reality it's a global scandal and happening everywhere. Pillars of this conspiracy were all put into place before the button was pressed with the Drosten PCR protocol and high amplifications to produce the cases and death certificate changes to secure illusory 'Covid' deaths. Mooney notes that normally two doctors were needed to certify a death, with one having to know the patient, and how the rules were changed in the spring of 2020 to allow one doctor to do this. In the same period 'Covid deaths' were decreed to be all cases where Covid-19 was put on the death certificate even without a positive test or any symptoms. Mooney asked: 'How many of the 30,851 (as of January 15) care home resident deaths with Covid-19 on the certificate (32.4 per cent of all deaths so far) were based on an assumption, like that of my father? And what has that done to our national psyche?'All of them is the answer to the first question and it has devastated and dismantled the national psyche, actually the global psyche, on a colossal scale. In the UK case and death data is compiled by organisations like Public Health England (PHE) and the Office for National Statistics (ONS). Mooney highlights the insane policy of counting a death from any cause as 'Covid-19' if this happens within 28 days of a positive test (with a test not testing for the 'virus') and she points out that ONS statistics reflect deaths 'involving Covid' 'or due to Covid' which meant in practice any

death where 'Covid-19' was mentioned on the death certificate. She described the consequences of this fraud:

Most people will accept the narrative they are fed, so panicky governments here and in Europe witnessed the harsh measures enacted in totalitarian China and jumped into lockdown. Headlines about Covid deaths tolled like the knell that would bring doomsday to us all. Fear stalked our empty streets. Politicians parroted the frankly ridiculous aim of 'zero Covid' and shut down the economy, while most British people agreed that lockdown was essential and (astonishingly to me, as a patriotic Brit) even wanted more restrictions.

For what? Lies on death certificates? Never mind the grim toll of lives ruined, suicides, schools closed, rising inequality, depression, cancelled hospital treatments, cancer patients in a torture of waiting, poverty, economic devastation, loneliness, families kept apart, and so on. How many lives have been lost as a direct result of lockdown?

She said that we could join in a national chorus of shock and horror at reaching the 120,000 death toll which was surely certain to have been totally skewed all along, but what about the human cost of lockdown justified by these 'death figures'? *The British Medical Journal* had reported a 1,493 percent increase in cases of children taken to Great Ormond Street Hospital with abusive head injuries alone and then there was the effect on families:

Perhaps the most shocking thing about all this is that families have been kept apart – and obeyed the most irrational, changing rules at the whim of government – because they believed in the statistics. They succumbed to fear, which his generation rejected in that war fought for freedom. Dad (God rest his soul) would be angry. And so am I.

Another theme to watch is that in the winter months when there are more deaths from all causes they focus on 'Covid' deaths and in the summer when the British Lung Foundation says respiratory disease plummets by 80 percent they rage on about 'cases'. Either way fascism on population is always the answer.

Nazi eugenics in the 21st century

Elderly people in care homes have been isolated from their families month after lonely month with no contact with relatives and grandchildren who were banned from seeing them. We were told

that lockdown fascism was to 'protect the vulnerable' like elderly people. At the same time Do Not Resuscitate (DNR) orders were placed on their medical files so that if they needed resuscitation it wasn't done and 'Covid-19' went on their death certificates. Old people were not being 'protected' they were being culled – murdered in truth. DNR orders were being decreed for disabled and young people with learning difficulties or psychological problems. The UK Care Quality Commission, a non-departmental body of the Department of Health and Social Care, found that 34 percent of those working in health and social care were pressured into placing 'do not attempt cardiopulmonary resuscitation' orders on 'Covid' patients who suffered from disabilities and learning difficulties without involving the patient or their families in the decision. UK judges ruled that an elderly woman with dementia should have the DNA-manipulating 'Covid vaccine' against her son's wishes and that a man with severe learning difficulties should have the jab despite his family's objections. Never mind that many had already died. The judiciary always supports doctors and government in fascist dictatorships. They wouldn't dare do otherwise. A horrific video was posted showing fascist officers from Los Angeles police forcibly giving the 'Covid' shot to women with special needs who were screaming that they didn't want it. The same fascists are seen giving the jab to a sleeping elderly woman in a care home. This is straight out of the Nazi playbook. Hitler's Nazis committed mass murder of the mentally ill and physically disabled throughout Germany and occupied territories in the programme that became known as Aktion T4, or just T4. Sabbatian-controlled Hitler and his grotesque crazies set out to kill those they considered useless and unnecessary. The Reich Committee for the Scientific Registering of Hereditary and Congenital Illnesses registered the births of babies identified by physicians to have 'defects'. By 1941 alone more than 5,000 children were murdered by the state and it is estimated that in total the number of innocent people killed in Aktion T4 was between 275,000 and 300,000. Parents were told their children had been sent away for 'special treatment' never to return. It is rather pathetic to see claims about plans for new extermination camps being dismissed today

when the same force behind current events did precisely that 80 years ago. Margaret Sanger was a Cult operative who used 'birth control' to sanitise her programme of eugenics. Organisations she founded became what is now Planned Parenthood. Sanger proposed that 'the whole dysgenic population would have its choice of segregation or sterilization'. These included epileptics, 'feeble-minded', and prostitutes. Sanger opposed charity because it perpetuated 'human waste'. She reveals the Cult mentality and if anyone thinks that extermination camps are a 'conspiracy theory' their naivety is touching if breathtakingly stupid.

If you don't believe that doctors can act with callous disregard for their patients it is worth considering that doctors and medical staff agreed to put government-decreed DNR orders on medical files and do nothing when resuscitation is called for. I don't know what you call such people in your house. In mine they are Nazis from the Josef Mengele School of Medicine. Phenomenal numbers of old people have died worldwide from the effects of lockdown, depression, lack of treatment, the 'vaccine' (more later) and losing the will to live. A common response at the start of the manufactured pandemic was to remove old people from hospital beds and transfer them to nursing homes. The decision would result in a mass cull of elderly people in those homes through lack of treatment – *not* 'Covid'. Care home whistleblowers have told how once the 'Covid' era began doctors would not come to their homes to treat patients and they were begging for drugs like antibiotics that often never came. The most infamous example was ordered by New York governor Andrew Cuomo, brother of a moronic CNN host, who amazingly was given an Emmy Award for his handling of the 'Covid crisis' by the ridiculous Wokers that hand them out. Just how ridiculous could be seen in February, 2021, when a Department of Justice and FBI investigation began into how thousands of old people in New York died in nursing homes after being discharged from hospital to make way for 'Covid' patients on Cuomo's say-so – and how he and his staff covered up these facts. This couldn't have happened to a nicer psychopath. Even then there was a 'Covid' spin. Reports said that

thousands of old people who tested positive for 'Covid' in hospital were transferred to nursing homes to both die of 'Covid' and transmit it to others. No – they were in hospital because they were ill and the fact that they tested positive with a test not testing for the 'virus' is irrelevant. They were ill often with respiratory diseases ubiquitous in old people near the end of their lives. Their transfer out of hospital meant that their treatment stopped and many would go on to die.

They're old. Who gives a damn?

I have exposed in the books for decades the Cult plan to cull the world's old people and even to introduce at some point what they call a 'demise pill' which at a certain age everyone would take and be out of here by law. In March, 2021, Spain legalised euthanasia and assisted suicide following the Netherlands, Belgium, Luxembourg and Canada on the Tiptoe to the demise pill. Treatment of old people by many 'care' homes has been a disgrace in the 'Covid' era. There are many, many, caring staff – I know some. There have, however, been legions of stories about callous treatment of old people and their families. Police were called when families came to take their loved ones home in the light of isolation that was killing them. They became prisoners of the state. Care home residents in insane, fascist Ontario, Canada, were not allowed to leave their *room* once the 'Covid' hoax began. UK staff have even wheeled elderly people away from windows where family members were talking with them. Oriana Criscuolo from Stockport in the English North West dropped off some things for her 80-year-old father who has Parkinson's disease and dementia and she wanted to wave to him through a ground-floor window. She was told that was 'illegal'. When she went anyway they closed the curtains in the middle of the day. Oriana said:

> It's just unbelievable. I cannot understand how care home staff – people who are being paid to care – have become so uncaring. Their behaviour is inhumane and cruel. It's beyond belief.

She was right and this was not a one-off. What a way to end your life in such loveless circumstances. UK registered nurse Nicky Millen, a proper old school nurse for 40 years, said that when she started her career care was based on dignity, choice, compassion and empathy. Now she said 'the things that are important to me have gone out of the window.' She was appalled that people were dying without their loved ones and saying goodbye on iPads. Nicky described how a distressed 89-year-old lady stroked her face and asked her 'how many paracetamol would it take to finish me off'. Life was no longer worth living while not seeing her family. Nicky said she was humiliated in front of the ward staff and patients for letting the lady stroke her face and giving her a cuddle. Such is the dehumanisation that the 'Covid' hoax has brought to the surface. Nicky worked in care homes where patients told her they were being held prisoner. 'I want to live until I die', one said to her. 'I had a lady in tears because she hadn't seen her great-grandson.' Nicky was compassionate old school meeting psychopathic New Normal. She also said she had worked on a 'Covid' ward with no 'Covid' patients. Jewish writer Shai Held wrote an article in March, 2020, which was headlined 'The Staggering, Heartless Cruelty Toward the Elderly'. What he described was happening from the earliest days of lockdown. He said 'the elderly' were considered a group and not unique individuals (the way of the Woke). Shai Held said:

Notice how the all-too-familiar rhetoric of dehumanization works: 'The elderly' are bunched together as a faceless mass, all of them considered culprits and thus effectively deserving of the suffering the pandemic will inflict upon them. Lost entirely is the fact that the elderly are individual human beings, each with a distinctive face and voice, each with hopes and dreams, memories and regrets, friendships and marriages, loves lost and loves sustained.

'The elderly' have become another dehumanised group for which anything goes and for many that has resulted in cold disregard for their rights and their life. The distinctive face that Held talks about is designed to be deleted by masks until everyone is part of a faceless mass.

‘War-zone’ hospitals myth

Again and again medical professionals have told me what was really going on and how hospitals ‘overrun like war zones’ according to the media were virtually empty. The mantra from medical whistleblowers was please don’t use my name or my career is over. Citizen journalists around the world sneaked into hospitals to film evidence exposing the ‘war-zone’ lie. They really *were* largely empty with closed wards and operating theatres. I met a hospital worker in my town on the Isle of Wight during the first lockdown in 2020 who said the only island hospital had never been so quiet. Lockdown was justified by the psychopaths to stop hospitals being overrun. At the same time that the island hospital was near-empty the military arrived here to provide *extra beds*. It was all propaganda to ramp up the fear to ensure compliance with fascism as were never-used temporary hospitals with thousands of beds known as Nightingales and never-used make-shift mortuaries opened by the criminal UK government. A man who helped to install those extra island beds attributed to the army said they were never used and the hospital was empty. Doctors and nurses ‘stood around talking or on their phones, wandering down to us to see what we were doing’. There were no masks or social distancing. He accused the useless local island paper, the *County Press*, of ‘pumping the fear as if our hospital was overrun and we only have one so it should have been’. He described ambulances parked up with crews outside in deck chairs. When his brother called an ambulance he was told there was a two-hour backlog which he called ‘bullshit’. An old lady on the island fell ‘and was in a bad way’, but a caller who rang for an ambulance was told the situation wasn’t urgent enough. Ambulance stations were working under capacity while people would hear ambulances with sirens blaring driving through the streets. When those living near the stations realised what was going on they would follow them as they left, circulated around an urban area with the sirens going, and then came back without stopping. All this was to increase levels of fear and the same goes for the ‘ventilator shortage crisis’ that cost tens of millions for hastily produced ventilators never to be used.

Ambulance crews that agreed to be exploited in this way for fear propaganda might find themselves a mirror. I wish them well with that. Empty hospitals were the obvious consequence of treatment and diagnoses of non-'Covid' conditions cancelled and those involved handed a death sentence. People have been dying at home from undiagnosed and untreated cancer, heart disease and other life-threatening conditions to allow empty hospitals to deal with a 'pandemic' that wasn't happening.

Death of the innocent

'War-zones' have been laying off nursing staff, even doctors where they can. There was no work for them. Lockdown was justified by saving lives and protecting the vulnerable they were actually killing with DNR orders and preventing empty hospitals being 'overrun'. In Britain the mantra of stay at home to 'save the NHS' was everywhere and across the world the same story was being sold when it was all lies. Two California doctors, Dan Erickson and Artin Massihi at Accelerated Urgent Care in Bakersfield, held a news conference in April, 2020, to say that intensive care units in California were 'empty, essentially', with hospitals shutting floors, not treating patients and laying off doctors. The California health system was working at minimum capacity 'getting rid of doctors because we just don't have the volume'. They said that people with conditions such as heart disease and cancer were not coming to hospital out of fear of 'Covid-19'. Their video was deleted by Susan Wojcicki's Cult-owned YouTube after reaching five million views. Florida governor Ron Desantis, who rejected the severe lockdowns of other states and is being targeted for doing so, said that in March, 2020, every US governor was given models claiming they would run out of hospital beds in days. That was never going to happen and the 'modellers' knew it. Deceit can be found at every level of the system. Urgent children's operations were cancelled including fracture repairs and biopsies to spot cancer. Eric Nicholls, a consultant paediatrician, said 'this is obviously concerning and we need to return to normal operating and to increase capacity as soon as possible'. Psychopaths

in power were rather less concerned *because* they are psychopaths. Deletion of urgent care and diagnosis has been happening all over the world and how many kids and others have died as a result of the actions of these cold and heartless lunatics dictating 'health' policy? The number must be stratospheric. Richard Sullivan, professor of cancer and global health at King's College London, said people feared 'Covid' more than cancer such was the campaign of fear. 'Years of lost life will be quite dramatic', Sullivan said, with 'a huge amount of avoidable mortality'. Sarah Woolnough, executive director for policy at Cancer Research UK, said there had been a 75 percent drop in urgent referrals to hospitals by family doctors of people with suspected cancer. Sullivan said that 'a lot of services have had to scale back – we've seen a dramatic decrease in the amount of elective cancer surgery'. Lockdown deaths worldwide has been absolutely fantastic with the *New York Post* reporting how data confirmed that 'lockdowns end more lives than they save':

> There was a sharp decline in visits to emergency rooms and an increase in fatal heart attacks because patients didn't receive prompt treatment. Many fewer people were screened for cancer. Social isolation contributed to excess deaths from dementia and Alzheimer's.
>
> Researchers predicted that the social and economic upheaval would lead to tens of thousands of "deaths of despair" from drug overdoses, alcoholism and suicide. As unemployment surged and mental-health and substance-abuse treatment programs were interrupted, the reported levels of anxiety, depression and suicidal thoughts increased dramatically, as did alcohol sales and fatal drug overdoses.

This has been happening while nurses and other staff had so much time on their hands in the 'war-zones' that Tic-Tok dancing videos began appearing across the Internet with medical staff dancing around in empty wards and corridors as people died at home from causes that would normally have been treated in hospital.

Mentions in dispatches

One brave and truth-committed whistleblower was Louise Hampton, a call handler with the UK NHS who made a viral Internet video saying she had done 'fuck all' during the 'pandemic'

which was 'a load of bollocks'. She said that 'Covid-19' was rebranded flu and of course she lost her job. This is what happens in the medical and endless other professions now when you tell the truth. Louise filmed inside 'war-zone' accident and emergency departments to show they were empty and I mean *empty* as in no one there. The mainstream media could have done the same and blown the gaff on the whole conspiracy. They haven't to their eternal shame. Not that most 'journalists' seem capable of manifesting shame as with the psychopaths they slavishly repeat without question. The relative few who were admitted with serious health problems were left to die alone with no loved ones allowed to see them because of 'Covid' rules and they included kids dying without the comfort of mum and dad at their bedside while the evil behind this couldn't give a damn. It was all good fun to them. A Scottish NHS staff nurse publicly quit in the spring of 2021 saying: 'I can no longer be part of the lies and the corruption by the government.' She said hospitals 'aren't full, the beds aren't full, beds have been shut, wards have been shut'. Hospitals were never busy throughout 'Covid'. The staff nurse said that Nicola Sturgeon, tragically the leader of the Scottish government, was on television saying save the hospitals and the NHS – 'but the beds are empty' and 'we've not seen flu, we always see flu every year'. She wrote to government and spoke with her union Unison (the unions are Cult-compromised and *useless*, but nothing changed. Many of her colleagues were scared of losing their jobs if they spoke out as they wanted to. She said nursing staff were being affected by wearing masks all day and 'my head is splitting every shift from wearing a mask'. The NHS is part of the fascist tyranny and must be dismantled so we can start again with human beings in charge. (Ironically, hospitals were reported to be busier again when official 'Covid' cases *fell* in spring/summer of 2021 and many other conditions required treatment at the same time as *the fake vaccine rollout*.)

I will cover the 'Covid vaccine' scam in detail later, but it is another indicator of the sickening disregard for human life that I am highlighting here. The DNA-manipulating concoctions do not fulfil

the definition of a 'vaccine', have never been used on humans before and were given only emergency approval because trials were not completed and they continued using the unknowing public. The result was what a NHS senior nurse with responsibility for 'vaccine' procedure said was 'genocide'. She said the 'vaccines' were not 'vaccines'. They had not been shown to be safe and claims about their effectiveness by drug companies were 'poetic licence'. She described what was happening as a 'horrid act of human annihilation'. The nurse said that management had instigated a policy of not providing a Patient Information Leaflet (PIL) before people were 'vaccinated' even though health care professionals are supposed to do this according to protocol. Patients should also be told that they are taking part in an ongoing clinical trial. Her challenges to what is happening had seen her excluded from meetings and ridiculed in others. She said she was told to 'watch my step … or I would find myself surplus to requirements'. The nurse, who spoke anonymously in fear of her career, said she asked her NHS manager why he/she was content with taking part in genocide against those having the 'vaccines'. The reply was that everyone had to play their part and to 'put up, shut up, and get it done'. Government was 'leaning heavily' on NHS management which was clearly leaning heavily on staff. This is how the global 'medical' hierarchy operates and it starts with the Cult and its World Health Organization.

She told the story of a doctor who had the Pfizer jab and when questioned had no idea what was in it. The doctor had never read the literature. We have to stop treating doctors as intellectual giants when so many are moral and medical pygmies. The doctor did not even know that the 'vaccines' were not fully approved or that their trials were ongoing. They were, however, asking their patients if they minded taking part in follow-ups for research purposes – yes, the *ongoing clinical trial*. The nurse said the doctor's ignorance was not rare and she had spoken to a hospital consultant who had the jab without any idea of the background or that the 'trials' had not been completed. Nurses and pharmacists had shown the same ignorance.

'My NHS colleagues have forsaken their duty of care, broken their code of conduct – Hippocratic Oath – and have been brainwashed just the same as the majority of the UK public through propaganda …' She said she had not been able to recruit a single NHS colleague, doctor, nurse or pharmacist to stand with her and speak out. Her union had refused to help. She said that if the genocide came to light she would not hesitate to give evidence at a Nuremberg-type trial against those in power who could have affected the outcomes but didn't.

And all for what?

To put the nonsense into perspective let's say the 'virus' does exist and let's go completely crazy and accept that the official manipulated figures for cases and deaths are accurate. *Even then* a study by Stanford University epidemiologist Dr John Ioannidis published on the World Health Organization website produced an average infection to fatality rate of … *0.23 percent!* Ioannidis said: 'If one could sample equally from all locations globally, the median infection fatality rate might even be substantially lower than the 0.23% observed in my analysis.' For healthy people under 70 it was … *0.05 percent*! This compares with the 3.4 percent claimed by the Cult-owned World Health Organization when the hoax was first played and maximum fear needed to be generated. An updated Stanford study in April, 2021, put the 'infection' to 'fatality' rate at just 0.15 percent. Another team of scientists led by Megan O'Driscoll and Henrik Salje studied data from 45 countries and published their findings on the Nature website. For children and young people the figure is so small it virtually does not register although authorities will be hyping dangers to the young when they introduce DNA-manipulating 'vaccines' for children. The O'Driscoll study produced an average infection-fatality figure of 0.003 for children from birth to four; 0.001 for 5 to 14; 0.003 for 15 to 19; and it was still only 0.456 up to 64. To claim that children must be 'vaccinated' to protect them from 'Covid' is an obvious lie and so there must be another reason and there is. What's more the average age of a 'Covid' death is akin

to the average age that people die in general. The average age of death in England is about 80 for men and 83 for women. The average age of death from alleged 'Covid' is between 82 and 83. California doctors, Dan Erickson and Artin Massihi, said at their April media conference that projection models of millions of deaths had been 'woefully inaccurate'. They produced detailed figures showing that Californians had a 0.03 chance of dying from 'Covid' based on the number of people who tested positive (with a test not testing for the 'virus'). Erickson said there was a 0.1 percent chance of dying from 'Covid' in the *state* of New York, not just the city, and a 0.05 percent chance in Spain, a centre of 'Covid-19' hysteria at one stage. The Stanford studies supported the doctors' data with fatality rate estimates of 0.23 and 0.15 percent. How close are these figures to my estimate of *zero*? Death-rate figures claimed by the World Health Organization at the start of the hoax were some 15 times higher. The California doctors said there was no justification for lockdowns and the economic devastation they caused. Everything they had ever learned about quarantine was that you quarantine the *sick* and not the healthy. They had never seen this before and it made no medical sense.

Why in the in the light of all this would governments and medical systems the world over say that billions must go under house arrest; lose their livelihood; in many cases lose their mind, their health and their life; force people to wear masks dangerous to health and psychology; make human interaction and even family interaction a criminal offence; ban travel; close restaurants, bars, watching live sport, concerts, theatre, and any activity involving human togetherness and discourse; and closing schools to isolate children from their friends and cause many to commit suicide in acts of hopelessness and despair? The California doctors said lockdown consequences included increased child abuse, partner abuse, alcoholism, depression, and other impacts they were seeing every day. Who would do that to the entire human race if not mentally-ill psychopaths of almost unimaginable extremes like Bill Gates? We must face the reality of what we are dealing with and come out of

denial. Fascism and tyranny are made possible only by the target population submitting and acquiescing to fascism and tyranny. The whole of human history shows that to be true. Most people naively and unquestioning believed what they were told about a 'deadly virus' and meekly and weakly submitted to house arrest. Those who didn't believe it – at least in total – still submitted in fear of the consequences of not doing so. For the rest who wouldn't submit draconian fines have been imposed, brutal policing by psychopaths *for* psychopaths, and condemnation from the meek and weak who condemn the Pushbackers on behalf of the very force that has them, too, in its gunsights. 'Pathetic' does not even begin to suffice. Britain's brainless 'Health' Secretary Matt Hancock warned anyone lying to border officials about returning from a list of 'hotspot' countries could face a jail sentence of up to ten years which is more than for racially-aggravated assault, incest and attempting to have sex with a child under 13. Hancock is a lunatic, but he has the state apparatus behind him in a Cult-led chain reaction and the same with UK 'Vaccine Minister' Nadhim Zahawi, a prominent member of the mega-Cult secret society, Le Cercle, which featured in my earlier books. The Cult enforces its will on governments and medical systems; government and medical systems enforce their will on business and police; business enforces its will on staff who enforce it on customers; police enforce the will of the Cult on the population and play their essential part in creating a world of fascist control that their own children and grandchildren will have to live in their entire lives. It is a hierarchical pyramid of imposition and acquiescence and, yes indeedy, of clinical insanity.

Does anyone bright enough to read this book have to ask what the answer is? I think not, but I will reveal it anyway in the fewest of syllables: Tell the psychos and their moronic lackeys to fuck off and let's get on with our lives. We are many – They are few.

CHAPTER SEVEN

War on your mind

One believes things because one has been conditioned to believe them
Aldous Huxley, *Brave New World*

I have described the 'Covid' hoax as a 'Psyop' and that is true in every sense and on every level in accordance with the definition of that term which is psychological warfare. Break down the 'Covid pandemic' to the foundation themes and it is psychological warfare on the human individual and collective mind.

The same can be said for the entire human belief system involving every subject you can imagine. Huxley was right in his contention that people believe what they are conditioned to believe and this comes from the repetition throughout their lives of the same falsehoods. They spew from government, corporations, media and endless streams of 'experts' telling you what the Cult wants you to believe and often believing it themselves (although *far* from always). 'Experts' are rewarded with 'prestigious' jobs and titles and as agents of perceptual programming with regular access to the media. The Cult has to control the narrative – control *information* – or they lose control of the vital, crucial, without-which-they-cannot-prevail public perception of reality. The foundation of that control today is the Internet made possible by the Defense Advanced Research Projects Agency (DARPA), the incredibly sinister technological arm of the Pentagon. The Internet is the result of military technology.

DARPA openly brags about establishing the Internet which has been a long-term project to lasso the minds of the global population. I have said for decades the plan is to control information to such an extreme that eventually no one would see or hear anything that the Cult does not approve. We are closing in on that end with ferocious censorship since the 'Covid' hoax began and in my case it started back in the 1990s in terms of books and speaking venues. I had to create my own publishing company in 1995 precisely because no one else would publish my books even then. I think they're all still running.

Cult Internet

To secure total control of information they needed the Internet in which pre-programmed algorithms can seek out 'unclean' content for deletion and even stop it being posted in the first place. The Cult had to dismantle print and non-Internet broadcast media to ensure the transfer of information to the appropriate-named 'Web' – a critical expression of the *Cult* web. We've seen the ever-quickening demise of traditional media and control of what is left by a tiny number of corporations operating worldwide. Independent journalism in the mainstream is already dead and never was that more obvious than since the turn of 2020. The Cult wants all information communicated via the Internet to globally censor and allow the plug to be pulled any time. Lockdowns and forced isolation has meant that communication between people has been through electronic means and no longer through face-to-face discourse and discussion. Cult psychopaths have targeted the bars, restaurants, sport, venues and meeting places in general for this reason. None of this is by chance and it's to stop people gathering in any kind of privacy or number while being able to track and monitor all Internet communications and block them as necessary. Even private messages between individuals have been censored by these fascists that control Cult fronts like Facebook, Twitter, Google and YouTube which are all officially run by Sabbatian place-people and from the background by higher-level Sabbatian place people.

Facebook, Google, Amazon and their like were seed-funded and supported into existence with money-no-object infusions of funds either directly or indirectly from DARPA and CIA technology arm In-Q-Tel. The Cult plays the long game and prepares very carefully for big plays like 'Covid'. Amazon is another front in the psychological war and pretty much controls the global market in book sales and increasingly publishing. Amazon's limitless funds have deleted fantastic numbers of independent publishers to seize global domination on the way to deciding which books can be sold and circulated and which cannot. Moves in that direction are already happening. Amazon's leading light Jeff Bezos is the grandson of Lawrence Preston Gise who worked with DARPA predecessor ARPA. Amazon has big connections to the CIA and the Pentagon. The plan I have long described went like this:

1. Employ military technology to establish the Internet.

2. Sell the Internet as a place where people can freely communicate without censorship and allow that to happen until the Net becomes the central and irreversible pillar of human society. If the Internet had been highly censored from the start many would have rejected it.

3. Fund and manipulate major corporations into being to control the circulation of information on your Internet using cover stories about geeks in garages to explain how they came about. Give them unlimited funds to expand rapidly with no need to make a profit for years while non-Cult companies who need to balance the books cannot compete. You know that in these circumstances your Googles, YouTubes, Facebooks and Amazons are going to secure near monopolies by either crushing or buying up the opposition.

4. Allow freedom of expression on both the Internet and communication platforms to draw people in until the Internet is the central and irreversible pillar of human society and your communication corporations have reached a stage of near monopoly domination.

5. Then unleash your always-planned frenzy of censorship on the basis of 'where else are you going to go?' and continue to expand that until nothing remains that the Cult does not want its human targets to see.

The process was timed to hit the 'Covid' hoax to ensure the best chance possible of controlling the narrative which they knew they had to do at all costs. They were, after all, about to unleash a 'deadly virus' that didn't really exist. If you do that in an environment of free-flowing information and opinion you would be dead in the

water before you could say Gates is a psychopath. The network was in place through which the Cult-created-and-owned World Health Organization could dictate the 'Covid' narrative and response policy slavishly supported by Cult-owned Internet communication giants and mainstream media while those telling a different story were censored. Google, YouTube, Facebook and Twitter openly announced that they would do this. What else would we expect from Cult-owned operations like Facebook which former executives have confirmed set out to make the platform more addictive than cigarettes and coldly manipulates emotions of its users to sow division between people and groups and scramble the minds of the young? If Zuckerberg lives out the rest of his life without going to jail for crimes against humanity, and most emphatically against the young, it will be a travesty of justice. Still, no matter, cause and effect will catch up with him eventually and the same with Sergey Brin and Larry Page at Google with its CEO Sundar Pichai who fix the Google search results to promote Cult narratives and hide the opposition. Put the same key words into Google and other search engines like DuckDuckGo and you will see how different results can be. Wikipedia is another intensely biased 'encyclopaedia' which skews its content to the Cult agenda. YouTube links to Wikipedia's version of 'Covid' and 'climate change' on video pages in which experts in their field offer a different opinion (even that is increasingly rare with Wojcicki censorship). Into this 'Covid' silence-them network must be added government media censors, sorry 'regulators', such as Ofcom in the UK which imposed tyrannical restrictions on British broadcasters that had the effect of banning me from ever appearing. Just to debate with me about my evidence and views on 'Covid' would mean breaking the fascistic impositions of Ofcom and its CEO career government bureaucrat Melanie Dawes. Gutless British broadcasters tremble at the very thought of fascist Ofcom.

Psychos behind 'Covid'

The reason for the 'Covid' catastrophe in all its facets and forms can be seen by whom and what is driving the policies worldwide in such a coordinated way. Decisions are not being made to protect health, but to target psychology. The dominant group guiding and 'advising' government policy are not medical professionals. They are psychologists and behavioural scientists. Every major country has its own version of this phenomenon and I'll use the British example to show how it works. In many ways the British version has been affecting the wider world in the form of the huge behaviour manipulation network in the UK which operates in other countries. The network involves private companies, government, intelligence and military. The Cabinet Office is at the centre of the government 'Covid' Psyop and part-owns, with 'innovation charity' Nesta, the Behavioural Insights Team (BIT) which claims to be independent of government but patently isn't. The BIT was established in 2010 and its job is to manipulate the psyche of the population to acquiesce to government demands and so much more. It is also known as the 'Nudge Unit', a name inspired by the 2009 book by two ultra-Zionists, Cass Sunstein and Richard Thaler, called *Nudge: Improving Decisions About Health, Wealth, and Happiness*. The book, as with the Behavioural Insights Team, seeks to 'nudge' behaviour (manipulate it) to make the public follow patterns of action and perception that suit those in authority (the Cult). Sunstein is so skilled at this that he advises the World Health Organization and the UK Behavioural Insights Team and was Administrator of the White House Office of Information and Regulatory Affairs in the Obama administration. Biden appointed him to the Department of Homeland Security – another ultra-Zionist in the fold to oversee new immigration laws which is another policy the Cult wants to control. Sunstein is desperate to silence anyone exposing conspiracies and co-authored a 2008 report on the subject in which suggestions were offered to ban 'conspiracy theorizing' or impose 'some kind of tax, financial or otherwise, on those who disseminate such theories'. I guess a psychiatrist's chair is out of the question?

Sunstein's mate Richard Thaler, an 'academic affiliate' of the UK Behavioural Insights Team, is a proponent of 'behavioural economics' which is defined as the study of 'the effects of psychological, cognitive, emotional, cultural and social factors on the decisions of individuals and institutions'. Study the effects so they can be manipulated to be what you want them to be. Other leading names in the development of behavioural economics are ultra-Zionists Daniel Kahneman and Robert J. Shiller and they, with Thaler, won the Nobel Memorial Prize in Economic Sciences for their work in this field. The Behavioural Insights Team is operating at the heart of the UK government and has expanded globally through partnerships with several universities including Harvard, Oxford, Cambridge, University College London (UCL) and Pennsylvania. They claim to have 'trained' (reframed) 20,000 civil servants and run more than 750 projects involving 400 randomised controlled trials in dozens of countries' as another version of mind reframers Common Purpose. BIT works from its office in New York with cities and their agencies, as well as other partners, across the United States and Canada – this is a company part-owned by the British government Cabinet Office. An executive order by President Cult-servant Obama established a US Social and Behavioral Sciences Team in 2015. They all have the same reason for being and that's to brainwash the population directly and by brainwashing those in positions of authority.

'Covid' mind game

Another prime aspect of the UK mind-control network is the 'independent' [joke] Scientific Pandemic Insights Group on Behaviours (SPI-B) which 'provides behavioural science advice aimed at anticipating and helping people adhere to interventions that are recommended by medical or epidemiological experts'. That means manipulating public perception and behaviour to do whatever government tells them to do. It's disgusting and if they really want the public to be 'safe' this lot should all be under lock and key. According to the government website SPI-B consists of

'behavioural scientists, health and social psychologists, anthropologists and historians' and advises the Whitty-Vallance-led Scientific Advisory Group for Emergencies (SAGE) which in turn advises the government on 'the science' (it doesn't) and 'Covid' policy. When politicians say they are being guided by 'the science' this is the rabble in each country they are talking about and that 'science' is dominated by behaviour manipulators to enforce government fascism through public compliance. The Behaviour Insight Team is headed by psychologist David Solomon Halpern, a visiting professor at King's College London, and connects with a national and global web of other civilian and military organisations as the Cult moves towards its goal of fusing them into one fascistic whole in every country through its 'Fusion Doctrine'. The behaviour manipulation network involves, but is not confined to, the Foreign Office; National Security Council; government communications headquarters (GCHQ); MI5; MI6; the Cabinet Office-based Media Monitoring Unit; and the Rapid Response Unit which 'monitors digital trends to spot emerging issues; including misinformation and disinformation; and identifies the best way to respond'.

There is also the 77th Brigade of the UK military which operates like the notorious Israeli military's Unit 8200 in manipulating information and discussion on the Internet by posing as members of the public to promote the narrative and discredit those who challenge it. Here we have the military seeking to manipulate *domestic* public opinion while the Nazis in government are fine with that. Conservative Member of Parliament Tobias Ellwood, an advocate of lockdown and control through 'vaccine passports', is a Lieutenant Colonel reservist in the 77th Brigade which connects with the military operation jHub, the 'innovation centre' for the Ministry of Defence and Strategic Command. jHub has also been involved with the civilian National Health Service (NHS) in 'symptom tracing' the population. The NHS is a key part of this mind control network and produced a document in December, 2020, explaining to staff how to use psychological manipulation with different groups and ages to get them to have the DNA-manipulating 'Covid vaccine'

that's designed to cumulatively rewrite human genetics. The document, called 'Optimising Vaccination Roll Out – Do's and Dont's for all messaging, documents and "communications" in the widest sense', was published by NHS England and the NHS Improvement *Behaviour Change Unit* in partnership with Public Health England and Warwick Business School. I hear the mantra about 'save the NHS' and 'protect the NHS' when we need to scrap the NHS and start again. The current version is far too corrupt, far too anti-human and totally compromised by Cult operatives and their assets. UK government broadcast media censor Ofcom will connect into this web – as will the BBC with its tremendous Ofcom influence – to control what the public see and hear and dictate mass perception. Nuremberg trials must include personnel from all these organisations.

The fear factor

The 'Covid' hoax has led to the creation of the UK Cabinet Office-connected Joint Biosecurity Centre (JBC) which is officially described as providing 'expert advice on pandemics' using its independent [all Cult operations are 'independent'] analytical function to provide real-time analysis about infection outbreaks to identify and respond to outbreaks of Covid-19'. Another role is to advise the government on a response to spikes in infections – 'for example by closing schools or workplaces in local areas where infection levels have risen'. Put another way, promoting the Cult agenda. The Joint Biosecurity Centre is modelled on the Joint Terrorism Analysis Centre which analyses intelligence to set 'terrorism threat levels' and here again you see the fusion of civilian and military operations and intelligence that has led to military intelligence producing documents about 'vaccine hesitancy' and how it can be combated. Domestic civilian matters and opinions should not be the business of the military. The Joint Biosecurity Centre is headed by Tom Hurd, director general of the Office for Security and Counter-Terrorism from the establishment-to-its-fingertips Hurd family. His father is former Foreign Secretary Douglas Hurd. How coincidental that Tom

Hurd went to the elite Eton College and Oxford University with Boris Johnson. Imperial College with its ridiculous computer modeller Neil Ferguson will connect with this gigantic web that will itself interconnect with similar set-ups in other major and not so major countries. Compared with this Cult network the politicians, be they Boris Johnson, Donald Trump or Joe Biden, are bit-part players 'following the science'. The network of psychologists was on the 'Covid' case from the start with the aim of generating maximum fear of the 'virus' to ensure compliance by the population. A government behavioural science group known as SPI-B produced a paper in March, 2020, for discussion by the main government science advisory group known as SAGE. It was headed 'Options for increasing adherence to social distancing measures' and it said the following in a section headed 'Persuasion':

- A substantial number of people still do not feel sufficiently personally threatened; it could be that they are reassured by the low death rate in their demographic group, although levels of concern may be rising. Having a good understanding of the risk has been found to be positively associated with adoption of COVID-19 social distancing measures in Hong Kong.

- The perceived level of personal threat needs to be increased among those who are complacent, using hard-hitting evaluation of options for increasing social distancing emotional messaging. To be effective this must also empower people by making clear the actions they can take to reduce the threat.

- Responsibility to others: There seems to be insufficient understanding of, or feelings of responsibility about, people's role in transmitting the infection to others … Messaging about actions need to be framed positively in terms of protecting oneself and the community, and increase confidence that they will be effective.

- Some people will be more persuaded by appeals to play by the rules, some by duty to the community, and some to personal risk.

> All these different approaches are needed. The messaging also needs to take account of the realities of different people's lives. Messaging needs to take account of the different motivational levers and circumstances of different people.

All this could be achieved the SPI-B psychologists said by *using the media to increase the sense of personal threat* which translates as terrify the shit out of the population, including children, so they all do what we want. That's not happened has it? Those excuses for 'journalists' who wouldn't know journalism if it bit them on the arse (the great majority) have played their crucial part in serving this Cult-government Psyop to enslave their own kids and grandkids. How they live with themselves I have no idea. The psychological war has been underpinned by constant government 'Covid' propaganda in almost every television and radio ad break, plus the Internet and print media, which has pounded out the fear with taxpayers footing the bill for their own programming. The result has been people terrified of a 'virus' that doesn't exist or one with a tiny fatality rate even if you believe it does. People walk down the street and around the shops wearing face-nappies damaging their health and psychology while others report those who refuse to be that naïve to the police who turn up in their own face-nappies. I had a cameraman come to my flat and he was so frightened of 'Covid' he came in wearing a mask and refused to shake my hand in case he caught something. He had – naïveitis – and the thought that he worked in the mainstream media was both depressing and made his behaviour perfectly explainable. The fear which has gripped the minds of so many and frozen them into compliance has been carefully cultivated by these psychologists who are really psychopaths. If lives get destroyed and a lot of young people commit suicide it shows our plan is working. SPI-B then turned to compulsion on the public to comply. 'With adequate preparation, rapid change can be achieved', it said. Some countries had introduced mandatory self-isolation on a wide scale without evidence of major public unrest and a large majority of the UK's population appeared to be supportive of more coercive measures with 64 percent of adults saying they would

support putting London under a lockdown (watch the 'polls' which are designed to make people believe that public opinion is in favour or against whatever the subject in hand).

For 'aggressive protective measures' to be effective, the SPI-B paper said, special attention should be devoted to those population groups that are more at risk. Translated from the Orwellian this means making the rest of population feel guilty for not protecting the 'vulnerable' such as old people which the Cult and its agencies were about to kill on an industrial scale with lockdown, lack of treatment and the Gates 'vaccine'. Psychopath psychologists sold their guilt-trip so comprehensively that Los Angeles County Supervisor Hilda Solis reported that children were apologising (from a distance) to their parents and grandparents for bringing 'Covid' into their homes and getting them sick. '… These apologies are just some of the last words that loved ones will ever hear as they die alone,' she said. Gut-wrenchingly Solis then used this childhood tragedy to tell children to stay at home and 'keep your loved ones alive'. Imagine heaping such potentially life-long guilt on a kid when it has absolutely nothing to do with them. These people are deeply disturbed and the psychologists behind this even more so.

Uncivil war – divide and rule

Professional mind-controllers at SPI-B wanted the media to increase a sense of responsibility to others (do as you're told) and promote 'positive messaging' for those actions while in contrast to invoke 'social disapproval' by the unquestioning, obedient, community of anyone with a mind of their own. Again the compliant Goebbels-like media obliged. This is an old, old, trick employed by tyrannies the world over throughout human history. You get the target population to keep the target population in line – *your* line. SPI-B said this could 'play an important role in preventing anti-social behaviour or discouraging failure to enact pro-social behaviour'. For 'anti-social' in the Orwellian parlance of SPI-B see any behaviour that government doesn't approve. SPI-B recommendations said that 'social disapproval' should be accompanied by clear messaging and

promotion of strong collective identity – hence the government and celebrity mantra of 'we're all in this together'. Sure we are. The mind doctors have such contempt for their targets that they think some clueless comedian, actor or singer telling them to do what the government wants will be enough to win them over. We have had UK comedian Lenny Henry, actor Michael Caine and singer Elton John wheeled out to serve the propagandists by urging people to have the DNA-manipulating 'Covid' non-'vaccine'. The role of Henry and fellow black celebrities in seeking to coax a 'vaccine' reluctant black community into doing the government's will was especially stomach-turning. An emotion-manipulating script and carefully edited video featuring these black 'celebs' was such an insult to the intelligence of black people and where's the self-respect of those involved selling their souls to a fascist government agenda? Henry said he heard black people's 'legitimate worries and concerns', but people must 'trust the facts' when they were doing exactly that by not having the 'vaccine'. They had to include the obligatory reference to Black Lives Matter with the line … 'Don't let coronavirus cost even more black lives – because we matter'. My god, it was pathetic. 'I know the vaccine is safe and what it does.' How? 'I'm a comedian and it says so in my script.'

SPI-B said social disapproval needed to be carefully managed to avoid victimisation, scapegoating and misdirected criticism, but they knew that their 'recommendations' would lead to exactly that and the media were specifically used to stir-up the divide-and-conquer hostility. Those who conform like good little baa, baas, are praised while those who have seen through the tidal wave of lies are 'Covidiots'. The awake have been abused by the fast asleep for not conforming to fascism and impositions that the awake know are designed to endanger their health, dehumanise them, and tear asunder the very fabric of human society. We have had the curtain-twitchers and morons reporting neighbours and others to the face-nappied police for breaking 'Covid rules' with fascist police delighting in posting links and phone numbers where this could be done. The Cult cannot impose its will without a compliant police

and military or a compliant population willing to play their part in enslaving themselves and their kids. The words of a pastor in Nazi Germany are so appropriate today:

First they came for the socialists and I did not speak out because I was not a socialist.

Then they came for the trade unionists and I did not speak out because I was not a trade unionist.

Then they came for the Jews and I did not speak out because I was not a Jew.

Then they came for me and there was no one left to speak for me.

Those who don't learn from history are destined to repeat it and so many are.

'Covid' rules: Rewiring the mind

With the background laid out to this gigantic national and global web of psychological manipulation we can put 'Covid' rules into a clear and sinister perspective. Forget the claims about protecting health. 'Covid' rules are about dismantling the human mind, breaking the human spirit, destroying self-respect, and then putting Humpty Dumpty together again as a servile, submissive slave. Social isolation through lockdown and distancing have devastating effects on the human psyche as the psychological psychopaths well know and that's the real reason for them. Humans need contact with each other, discourse, closeness and touch, or they eventually, and literarily, go crazy. Masks, which I will address at some length, fundamentally add to the effects of isolation and the Cult agenda to dehumanise and de-individualise the population. To do this while knowing – in fact *seeking* – this outcome is the very epitome of evil and psychologists involved in this *are* the epitome of evil. They must like all the rest of the Cult demons and their assets stand trial for crimes against humanity on a scale that defies the imagination. Psychopaths in uniform use isolation to break enemy troops and agents and make them subservient and submissive to tell what they know. The technique is rightly considered a form of torture and

torture is most certainly what has been imposed on the human population.

Clinically-insane American psychologist Harry Harlow became famous for his isolation experiments in the 1950s in which he separated baby monkeys from their mothers and imprisoned them for months on end in a metal container or 'pit of despair'. They soon began to show mental distress and depression as any idiot could have predicted. Harlow put other monkeys in steel chambers for three, six or twelve months while denying them any contact with animals or humans. He said that the effects of total social isolation for six months were 'so devastating and debilitating that we had assumed initially that twelve months of isolation would not produce any additional decrement'; but twelve months of isolation 'almost obliterated the animals socially'. This is what the Cult and its psychopaths are doing to you and your children. Even monkeys in partial isolation in which they were not allowed to form relationships with other monkeys became 'aggressive and hostile, not only to others, but also towards their own bodies'. We have seen this in the young as a consequence of lockdown. UK government psychopaths launched a public relations campaign telling people not to hug each other even after they received the 'Covid-19 vaccine' which we were told with more lies would allow a return to 'normal life'. A government source told *The Telegraph*: 'It will be along the lines that it is great that you have been vaccinated, but if you are going to visit your family and hug your grandchildren there is a chance you are going to infect people you love.' The source was apparently speaking from a secure psychiatric facility. Janet Lord, director of Birmingham University's Institute of Inflammation and Ageing, said that parents and grandparents should avoid hugging their children. Well, how can I put it, Ms Lord? Fuck off. Yep, that'll do.

Destroying the kids – where are the parents?

Observe what has happened to people enslaved and isolated by lockdown as suicide and self-harm has soared worldwide,

particularly among the young denied the freedom to associate with their friends. A study of 49,000 people in English-speaking countries concluded that almost half of young adults are at clinical risk of mental health disorders. A national survey in America of 1,000 currently enrolled high school and college students found that 5 percent reported attempting suicide during the pandemic. Data from the US CDC's National Syndromic Surveillance Program from January 1st to October 17th, 2020, revealed a *31 percent* increase in mental health issues among adolescents aged 12 to 17 compared with 2019. The CDC reported that America in general suffered the biggest drop in life expectancy since World War Two as it fell by a year in the first half of 2020 as a result of 'deaths of despair' – overdoses and suicides. Deaths of despair have leapt by more than 20 percent during lockdown and include the highest number of fatal overdoses ever recorded in a single year – 81,000. Internet addiction is another consequence of being isolated at home which lowers interest in physical activities as kids fall into inertia and what's the point? Children and young people are losing hope and giving up on life, sometimes literally. A 14-year-old boy killed himself in Maryland because he had 'given up' when his school district didn't reopen; an 11-year-old boy shot himself during a zoom class; a teenager in Maine succumbed to the isolation of the 'pandemic' when he ended his life after experiencing a disrupted senior year at school. Children as young as nine have taken their life and all these stories can be repeated around the world. Careers are being destroyed before they start and that includes those in sport in which promising youngsters have not been able to take part. The plan of the psycho-psychologists is working all right. Researchers at Cambridge University found that lockdowns cause significant harm to children's mental health. Their study was published in the *Archives of Disease in Childhood*, and followed 168 children aged between 7 and 11. The researchers concluded:

> During the UK lockdown, children's depression symptoms have increased substantially, relative to before lockdown. The scale of this effect has direct relevance for the continuation of different elements of lockdown policy, such as complete or partial school closures …

… Specifically, we observed a statistically significant increase in ratings of depression, with a medium-to-large effect size. Our findings emphasise the need to incorporate the potential impact of lockdown on child mental health in planning the ongoing response to the global pandemic and the recovery from it.

Not a chance when the Cult's psycho-psychologists were getting exactly what they wanted. The UK's Royal College of Paediatrics and Child Health has urged parents to look for signs of eating disorders in children and young people after a three to four fold increase. Specialists say the 'pandemic' is a major reason behind the rise. You don't say. The College said isolation from friends during school closures, exam cancellations, loss of extra-curricular activities like sport, and an increased use of social media were all contributory factors along with fears about the virus (psycho-psychologists again), family finances, and students being forced to quarantine. Doctors said young people were becoming severely ill by the time they were seen with 'Covid' regulations reducing face-to-face consultations. Nor is it only the young that have been devastated by the psychopaths. Like all bullies and cowards the Cult is targeting the young, elderly, weak and infirm. A typical story was told by a British lady called Lynn Parker who was not allowed to visit her husband in 2020 for the last ten and half months of his life 'when he needed me most' between March 20th and when he died on December 19th. This vacates the criminal and enters the territory of evil. The emotional impact on the immune system alone is immense as are the number of people of all ages worldwide who have died as a result of Cult-demanded, Gates-demanded, lockdowns.

Isolation is torture

The experience of imposing solitary confinement on millions of prisoners around the world has shown how a large percentage become 'actively psychotic and/or acutely suicidal'. Social isolation has been found to trigger 'a specific psychiatric syndrome, characterized by hallucinations; panic attacks; overt paranoia; diminished impulse control; hypersensitivity to external stimuli; and difficulties with thinking, concentration and memory'. Juan Mendez,

a United Nations rapporteur (investigator), said that isolation is a form of torture. Research has shown that even after isolation prisoners find it far more difficult to make social connections and I remember chatting to a shop assistant after one lockdown who told me that when her young son met another child again he had no idea how to act or what to do. Hannah Flanagan, Director of Emergency Services at Journey Mental Health Center in Dane County, Wisconsin, said: 'The specificity about Covid social distancing and isolation that we've come across as contributing factors to the suicides are really new to us this year.' But they are not new to those that devised them. They are getting the effect they want as the population is psychologically dismantled to be rebuilt in a totally different way. Children and the young are particularly targeted. They will be the adults when the full-on fascist AI-controlled technocracy is planned to be imposed and they are being prepared to meekly submit. At the same time older people who still have a memory of what life was like before – and how fascist the new normal really is – are being deleted. You are going to see efforts to turn the young against the old to support this geriatric genocide. Hannah Flanagan said the big increase in suicide in her county proved that social isolation is not only harmful, but deadly. Studies have shown that isolation from others is one of the main risk factors in suicide and even more so with women. Warnings that lockdown could create a 'perfect storm' for suicide were ignored. After all this was one of the *reasons* for lockdown. Suicide, however, is only the most extreme of isolation consequences. There are many others. Dr Dhruv Khullar, assistant professor of healthcare policy at Weill Cornell Medical College, said in a *New York Times* article in 2016 long before the fake 'pandemic':

> A wave of new research suggests social separation is bad for us. Individuals with less social connection have disrupted sleep patterns, altered immune systems, more inflammation and higher levels of stress hormones. One recent study found that isolation increases the risk of heart disease by 29 percent and stroke by 32 percent. Another analysis that pooled data from 70 studies and 3.4 million people found that socially isolated individuals had a 30 percent higher risk of dying in the next seven years, and that this effect was largest in middle age.

Loneliness can accelerate cognitive decline in older adults, and isolated individuals are twice as likely to die prematurely as those with more robust social interactions. These effects start early: Socially isolated children have significantly poorer health 20 years later, even after controlling for other factors. All told, loneliness is as important a risk factor for early death as obesity and smoking.

There you have proof from that one article alone four years before 2020 that those who have enforced lockdown, social distancing and isolation knew what the effect would be and that is even more so with professional psychologists that have been driving the policy across the globe. We can go back even further to the years 2000 and 2003 and the start of a major study on the effects of isolation on health by Dr Janine Gronewold and Professor Dirk M. Hermann at the University Hospital in Essen, Germany, who analysed data on 4,316 people with an average age of 59 who were recruited for the long-term research project. They found that socially isolated people are more than 40 percent more likely to have a heart attack, stroke, or other major cardiovascular event and nearly 50 percent more likely to die from any cause. Given the financial Armageddon unleashed by lockdown we should note that the study found a relationship between increased cardiovascular risk and lack of financial support. After excluding other factors social isolation was still connected to a 44 percent increased risk of cardiovascular problems and a 47 percent increased risk of death by any cause. Lack of financial support was associated with a 30 percent increase in the risk of cardiovascular health events. Dr Gronewold said it had been known for some time that feeling lonely or lacking contact with close friends and family can have an impact on physical health and the study had shown that having strong social relationships is of high importance for heart health. Gronewold said they didn't understand yet why people who are socially isolated have such poor health outcomes, but this was obviously a worrying finding, particularly during these times of prolonged social distancing. Well, it can be explained on many levels. You only have to identify the point in the body where people feel loneliness and missing people they are parted from – it's in the centre of the chest where they feel the ache of loneliness and the ache of missing people. 'My heart aches for

you' … 'My heart aches for some company.' I will explain this more in the chapter Escaping Wetiko, but when you realise that the body is the mind – they are expressions of each other – the reason why state of the mind dictates state of the body becomes clear.

American psychologist Ranjit Powar was highlighting the effects of lockdown isolation as early as April, 2020. She said humans have evolved to be social creatures and are wired to live in interactive groups. Being isolated from family, friends and colleagues could be unbalancing and traumatic for most people and could result in short or even long-term psychological and physical health problems. An increase in levels of anxiety, aggression, depression, forgetfulness and hallucinations were possible psychological effects of isolation. 'Mental conditions may be precipitated for those with underlying pre-existing susceptibilities and show up in many others without any pre-condition.' Powar said personal relationships helped us cope with stress and if we lost this outlet for letting off steam the result can be a big emotional void which, for an average person, was difficult to deal with. 'Just a few days of isolation can cause increased levels of anxiety and depression' – so what the hell has been the effect on the global population of *18 months* of this at the time of writing? Powar said: 'Add to it the looming threat of a dreadful disease being repeatedly hammered in through the media and you have a recipe for many shades of mental and physical distress.' For those with a house and a garden it is easy to forget that billions have had to endure lockdown isolation in tiny overcrowded flats and apartments with nowhere to go outside. The psychological and physical consequences of this are unimaginable and with lunatic and abusive partners and parents the consequences have led to tremendous increases in domestic and child abuse and alcoholism as people seek to shut out the horror. Ranjit Powar said:

Staying in a confined space with family is not all a rosy picture for everyone. It can be extremely oppressive and claustrophobic for large low-income families huddled together in small single-room houses. Children here are not lucky enough to have many board/electronic games or books to keep them occupied.

Add to it the deep insecurity of running out of funds for food and basic necessities. On the other hand, there are people with dysfunctional family dynamics, such as domineering, abusive or alcoholic partners, siblings or parents which makes staying home a period of trial. Incidence of suicide and physical abuse against women has shown a worldwide increase. Heightened anxiety and depression also affect a person's immune system, making them more susceptible to illness.

To think that Powar's article was published on April 11th, 2020.

Six-feet fantasy

Social (unsocial) distancing demanded that people stay six feet or two metres apart. UK government advisor Robert Dingwall from the New and Emerging Respiratory Virus Threats Advisory Group said in a radio interview that the two-metre rule was 'conjured up out of nowhere' and was not based on science. No, it was not based on *medical* science, but it didn't come out of nowhere. The distance related to *psychological* science. Six feet/two metres was adopted in many countries and we were told by people like the criminal Anthony Fauci and his ilk that it was founded on science. Many schools could not reopen because they did not have the space for six-feet distancing. Then in March, 2021, after a year of six-feet 'science', a study published in the *Journal of Infectious Diseases* involving more than 500,000 students and almost 100,000 staff over 16 weeks revealed no significant difference in 'Covid' cases between six feet and three feet and Fauci changed his tune. Now three feet was okay. There is no difference between six feet and three *inches* when there is no 'virus' and they got away with six feet for psychological reasons for as long as they could. I hear journalists and others talk about 'unintended consequences' of lockdown. They are not *unintended* at all; they have been coldly-calculated for a specific outcome of human control and that's why super-psychopaths like Gates have called for them so vehemently. Super-psychopath psychologists have demanded them and psychopathic or clueless, spineless, politicians have gone along with them by 'following the science'. But it's not science at all. 'Science' is not what is; it's only what people can be manipulated to believe it is. The whole 'Covid' catastrophe is

founded on mind control. Three word or three statement mantras issued by the UK government are a well-known mind control technique and so we've had 'Stay home/protect the NHS/save lives', 'Stay alert/control the virus/save lives' and 'hands/face/space'. One of the most vocal proponents of extreme 'Covid' rules in the UK has been Professor Susan Michie, a member of the British Communist Party, who is not a medical professional. Michie is the director of the Centre for Behaviour Change at University College London. She is a *behavioural psychologist* and another filthy rich 'Marxist' who praised China's draconian lockdown. She was known by fellow students at Oxford University as 'Stalin's nanny' for her extreme Marxism. Michie is an influential member of the UK government's Scientific Advisory Group for Emergencies (SAGE) and behavioural manipulation groups which have dominated 'Covid' policy. She is a consultant adviser to the World Health Organization on 'Covid-19' and behaviour. Why the hell are lockdowns anything to do with her when they are claimed to be about health? Why does a behavioural psychologist from a group charged with changing the behaviour of the public want lockdown, human isolation and mandatory masks? Does that question really need an answer? Michie *absolutely* has to explain herself before a Nuremberg court when humanity takes back its world again and even more so when you see the consequences of masks that she demands are compulsory. This is a Michie classic:

> The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Those words alone should carry a prison sentence when you ponder on the callous disregard for children involved and what a statement it makes about the mind and motivations of Susan Michie. What a lovely lady and what she said there encapsulates the mentality of the psychopaths behind the 'Covid' horror. Let us compare what Michie said with a countrywide study in Germany published at researchsquare.com involving 25,000 school children and 17,854 health complaints submitted by parents. Researchers

found that masks are harming children physically, psychologically, and behaviourally with 24 health issues associated with mask wearing. They include: shortness of breath (29.7%); dizziness (26.4%); increased headaches (53%); difficulty concentrating (50%); drowsiness or fatigue (37%); and malaise (42%). Nearly a third of children experienced more sleep issues than before and a quarter developed new fears. Researchers found health issues and other impairments in 68 percent of masked children covering their faces for an average of 4.5 hours a day. Hundreds of those taking part experienced accelerated respiration, tightness in the chest, weakness, and short-term impairment of consciousness. A reminder of what Michie said again:

The benefits of getting primary school children to wear masks is that regardless of what little degree of transmission is occurring in those age groups it could help normalise the practice. Young children wearing masks may be more likely to get their families to accept masks.

Psychopaths in government and psychology now have children and young people – plus all the adults – wearing masks for hours on end while clueless teachers impose the will of the psychopaths on the young they should be protecting. What the hell are parents doing?

Cult lab rats

We have some schools already imposing on students microchipped buzzers that activate when they get 'too close' to their pals in the way they do with lab rats. How apt. To the Cult and its brain-dead servants our children *are* lab rats being conditioned to be unquestioning, dehumanised slaves for the rest of their lives. Children and young people are being weaned and frightened away from the most natural human instincts including closeness and touch. I have tracked in the books over the years how schools were banning pupils from greeting each other with a hug and the whole Cult-induced Me Too movement has terrified men and boys from a relaxed and natural interaction with female friends and work colleagues to the point where many men try never to be in a room

alone with a woman that's not their partner. Airhead celebrities have as always played their virtue-signalling part in making this happen with their gross exaggeration. For every monster like Harvey Weinstein there are at least tens of thousands of men that don't treat women like that; but everyone must be branded the same and policy changed for them as well as the monster. I am going to be using the word 'dehumanise' many times in this chapter because that is what the Cult is seeking to do and it goes very deep as we shall see. Don't let them kid you that social distancing is planned to end one day. That's not the idea. We are seeing more governments and companies funding and producing wearable gadgets to keep people apart and they would not be doing that if this was meant to be short-term. A tech start-up company backed by GCHQ, the British Intelligence and military surveillance headquarters, has created a social distancing wrist sensor that alerts people when they get too close to others. The CIA has also supported tech companies developing similar devices. The wearable sensor was developed by Tended, one of a number of start-up companies supported by GCHQ (see the CIA and DARPA). The device can be worn on the wrist or as a tag on the waistband and will vibrate whenever someone wearing the device breaches social distancing and gets anywhere near natural human contact. The company had a lucky break in that it was developing a distancing sensor when the 'Covid' hoax arrived which immediately provided a potentially enormous market. How fortunate. The government in big-time Cult-controlled Ontario in Canada is investing $2.5 million in wearable contact tracing technology that 'will alert users if they may have been exposed to the Covid-19 in the workplace and will beep or vibrate if they are within six feet of another person'. Facedrive Inc., the technology company behind this, was founded in 2016 with funding from the Ontario Together Fund and obviously they, too, had a prophet on the board of directors. The human surveillance and control technology is called TraceSCAN and would be worn by the human cyborgs in places such as airports, workplaces, construction sites, care homes and … *schools*.

I emphasise schools with children and young people the prime targets. You know what is planned for society as a whole if you keep your eyes on the schools. They have always been places where the state program the next generation of slaves to be its compliant worker-ants – or Woker-ants these days; but in the mist of the 'Covid' madness they have been transformed into mind laboratories on a scale never seen before. Teachers and head teachers are just as programmed as the kids – often more so. Children are kept apart from human interaction by walk lanes, classroom distancing, staggered meal times, masks, and the rolling-out of buzzer systems. Schools are now physically laid out as a laboratory maze for lab-rats. Lunatics at a school in Anchorage, Alaska, who should be prosecuted for child abuse, took away desks and forced children to kneel (know your place) on a mat for five hours a day while wearing a mask and using their chairs as a desk. How this was supposed to impact on a 'virus' only these clinically insane people can tell you and even then it would be clap-trap. The school banned recess (interaction), art classes (creativity), and physical exercise (getting body and mind moving out of inertia). Everyone behind this outrage should be in jail or better still a mental institution. The behavioural manipulators are all for this dystopian approach to schools. Professor Susan Michie, the mind-doctor and British Communist Party member, said it was wrong to say that schools were safe. They had to be made so by 'distancing', masks and ventilation (sitting all day in the cold). I must ask this lady round for dinner on a night I know I am going to be out and not back for weeks. She probably wouldn't be able to make it, anyway, with all the visits to her own psychologist she must have block-booked.

Masking identity

I know how shocking it must be for you that a behaviour manipulator like Michie wants everyone to wear masks which have long been a feature of mind-control programs like the infamous MKUltra in the United States, but, there we are. We live and learn. I spent many years from 1996 to right across the millennium

researching mind control in detail on both sides of the Atlantic and elsewhere. I met a large number of mind-control survivors and many had been held captive in body and mind by MKUltra. MK stands for mind-control, but employs the German spelling in deference to the Nazis spirited out of Germany at the end of World War Two by Operation Paperclip in which the US authorities, with help from the Vatican, transported Nazi mind-controllers and engineers to America to continue their work. Many of them were behind the creation of NASA and they included Nazi scientist and SS officer Wernher von Braun who swapped designing V-2 rockets to bombard London with designing the Saturn V rockets that powered the NASA moon programme's Apollo craft. I think I may have mentioned that the Cult has no borders. Among Paperclip escapees was Josef Mengele, the Angel of Death in the Nazi concentration camps where he conducted mind and genetic experiments on children often using twins to provide a control twin to measure the impact of his 'work' on the other. If you want to observe the Cult mentality in all its extremes of evil then look into the life of Mengele. I have met many people who suffered mercilessly under Mengele in the United States where he operated under the name Dr Greene and became a stalwart of MKUltra programming and torture. Among his locations was the underground facility in the Mojave Desert in California called the China Lake Naval Weapons Station which is almost entirely below the surface. My books *The Biggest Secret*, *Children of the Matrix* and *The Perception Deception* have the detailed background to MKUltra.

The best-known MKUltra survivor is American Cathy O'Brien. I first met her and her late partner Mark Phillips at a conference in Colorado in 1996. Mark helped her escape and deprogram from decades of captivity in an offshoot of MKUltra known as Project Monarch in which 'sex slaves' were provided for the rich and famous including Father George Bush, Dick Cheney and the Clintons. Read Cathy and Mark's book *Trance-Formation of America* and if you are new to this you will be shocked to the core. I read it in 1996 shortly before, with the usual synchronicity of my life, I found

myself given a book table at the conference right next to hers. MKUltra never ended despite being very publicly exposed (only a small part of it) in the 1970s and continues in other guises. I am still in touch with Cathy. She contacted me during 2020 after masks became compulsory in many countries to tell me how they were used as part of MKUltra programming. I had been observing 'Covid regulations' and the relationship between authority and public for months. I saw techniques that I knew were employed on individuals in MKUltra being used on the global population. I had read many books and manuals on mind control including one called *Silent Weapons for Quiet Wars* which came to light in the 1980s and was a guide on how to perceptually program on a mass scale. 'Silent Weapons' refers to mind-control. I remembered a line from the manual as governments, medical authorities and law enforcement agencies have so obviously talked to – or rather at – the adult population since the 'Covid' hoax began as if they are children. The document said:

> If a person is spoken to by a T.V. advertiser as if he were a twelve-year-old, then, due to suggestibility, he will, with a certain probability, respond or react to that suggestion with the uncritical response of a twelve-year-old and will reach in to his economic reservoir and deliver its energy to buy that product on impulse when he passes it in the store.

That's why authority has spoken to adults like children since all this began.

Why did Michael Jackson wear masks?

Every aspect of the 'Covid' narrative has mind-control as its central theme. Cathy O'Brien wrote an article for davidicke.com about the connection between masks and mind control. Her daughter Kelly who I first met in the 1990s was born while Cathy was still held captive in MKUltra. Kelly was forced to wear a mask as part of her programming from the age of *two* to dehumanise her, target her sense of individuality and reduce the amount of oxygen her brain and body received. *Bingo*. This is the real reason for compulsory

masks, why they have been enforced en masse, and why they seek to increase the number they demand you wear. First one, then two, with one disgraceful alleged 'doctor' recommending four which is nothing less than a death sentence. Where and how often they must be worn is being expanded for the purpose of mass mind control and damaging respiratory health which they can call 'Covid-19'. Canada's government headed by the man-child Justin Trudeau, says it's fine for children of two and older to wear masks. An insane 'study' in Italy involving just 47 children concluded there was no problem for babies as young as *four months* wearing them. Even after people were 'vaccinated' they were still told to wear masks by the criminal that is Anthony Fauci. Cathy wrote that mandating masks is allowing the authorities literally to control the air we breathe which is what was done in MKUltra. You might recall how the singer Michael Jackson wore masks and there is a reason for that. He was subjected to MKUltra mind control through Project Monarch and his psyche was scrambled by these simpletons. Cathy wrote:

In MKUltra Project Monarch mind control, Michael Jackson had to wear a mask to silence his voice so he could not reach out for help. Remember how he developed that whisper voice when he wasn't singing? Masks control the mind from the outside in, like the redefining of words is doing. By controlling what we can and cannot say for fear of being labeled racist or beaten, for example, it ultimately controls thought that drives our words and ultimately actions (or lack thereof).

Likewise, a mask muffles our speech so that we are not heard, which controls voice … words … mind. This is Mind Control. Masks are an obvious mind control device, and I am disturbed so many people are complying on a global scale. Masks depersonalize while making a person feel as though they have no voice. It is a barrier to others. People who would never choose to comply but are forced to wear a mask in order to keep their job, and ultimately their family fed, are compromised. They often feel shame and are subdued. People have stopped talking with each other while media controls the narrative.

The 'no voice' theme has often become literal with train passengers told not to speak to each other in case they pass on the 'virus', singing banned for the same reason and bonkers California officials telling people riding roller coasters that they cannot shout and scream. Cathy said she heard every day from healed MKUltra survivors who cannot wear a mask without flashing back on ways

their breathing was controlled – 'from ball gags and penises to water boarding'. She said that through the years when she saw images of people in China wearing masks 'due to pollution' that it was really to control their oxygen levels. 'I knew it was as much of a population control mechanism of depersonalisation as are burkas', she said. Masks are another Chinese communist/fascist method of control that has been swept across the West as the West becomes China at lightning speed since we entered 2020.

Mask-19

There are other reasons for mandatory masks and these include destroying respiratory health to call it 'Covid-19' and stunting brain development of children and the young. Dr Margarite Griesz-Brisson MD, PhD, is a Consultant Neurologist and Neurophysiologist and the Founder and Medical Director of the London Neurology and Pain Clinic. Her CV goes down the street and round the corner. She is clearly someone who cares about people and won't parrot the propaganda. Griesz-Brisson has a PhD in pharmacology, with special interest in neurotoxicology, environmental medicine, neuroregeneration and neuroplasticity (the way the brain can change in the light of information received). She went public in October, 2020, with a passionate warning about the effects of mask-wearing laws:

> The reinhalation of our exhaled air will without a doubt create oxygen deficiency and a flooding of carbon dioxide. We know that the human brain is very sensitive to oxygen deprivation. There are nerve cells for example in the hippocampus that can't be longer than 3 minutes without oxygen – they cannot survive. The acute warning symptoms are headaches, drowsiness, dizziness, issues in concentration, slowing down of reaction time – reactions of the cognitive system.

Oh, I know, let's tell bus, truck and taxi drivers to wear them and people working machinery. How about pilots, doctors and police? Griesz-Brisson makes the important point that while the symptoms she mentions may fade as the body readjusts this does not alter the fact that people continue to operate in oxygen deficit with long list of

potential consequences. She said it was well known that neurodegenerative diseases take years or decades to develop. 'If today you forget your phone number, the breakdown in your brain would have already started 20 or 30 years ago.' She said degenerative processes in your brain are getting amplified as your oxygen deprivation continues through wearing a mask. Nerve cells in the brain are unable to divide themselves normally in these circumstances and lost nerve cells will no longer be regenerated. 'What is gone is gone.' Now consider that people like shop workers and *schoolchildren* are wearing masks for hours every day. What in the name of sanity is going to be happening to them? 'I do not wear a mask, I need my brain to think', Griesz-Brisson said, 'I want to have a clear head when I deal with my patients and not be in a carbon dioxide-induced anaesthesia'. If you are told to wear a mask anywhere ask the organisation, police, store, whatever, for their risk assessment on the dangers and negative effects on mind and body of enforcing mask-wearing. They won't have one because it has never been done not even by government. All of them must be subject to class-action lawsuits as the consequences come to light. They don't do mask risk assessments for an obvious reason. They know what the conclusions would be and independent scientific studies that *have* been done tell a horror story of consequences.

'Masks are criminal'

Dr Griesz-Brisson said that for children and adolescents, masks are an absolute no-no. They had an extremely active and adaptive immune system and their brain was incredibly active with so much to learn. 'The child's brain, or the youth's brain, is thirsting for oxygen.' The more metabolically active an organ was, the more oxygen it required; and in children and adolescents every organ was metabolically active. Griesz-Brisson said that to deprive a child's or adolescent's brain of oxygen, or to restrict it in any way, was not only dangerous to their health, it was absolutely criminal. 'Oxygen deficiency inhibits the development of the brain, and the damage that has taken place as a result CANNOT be reversed.' Mind

manipulators of MKUltra put masks on two-year-olds they wanted to neurologically rewire and you can see why. Griesz-Brisson said a child needs the brain to learn and the brain needs oxygen to function. 'We don't need a clinical study for that. This is simple, indisputable physiology.' Consciously and purposely induced oxygen deficiency was an absolutely deliberate health hazard, and an absolute medical contraindication which means that 'this drug, this therapy, this method or measure should not be used, and is not allowed to be used'. To coerce an entire population to use an absolute medical contraindication by force, she said, there had to be definite and serious reasons and the reasons must be presented to competent interdisciplinary and independent bodies to be verified and authorised. She had this warning of the consequences that were coming if mask wearing continued:

When, in ten years, dementia is going to increase exponentially, and the younger generations couldn't reach their god-given potential, it won't help to say 'we didn't need the masks'. I know how damaging oxygen deprivation is for the brain, cardiologists know how damaging it is for the heart, pulmonologists know how damaging it is for the lungs. Oxygen deprivation damages every single organ. Where are our health departments, our health insurance, our medical associations? It would have been their duty to be vehemently against the lockdown and to stop it and stop it from the very beginning.

Why do the medical boards issue punishments to doctors who give people exemptions? Does the person or the doctor seriously have to prove that oxygen deprivation harms people? What kind of medicine are our doctors and medical associations representing? Who is responsible for this crime? The ones who want to enforce it? The ones who let it happen and play along, or the ones who don't prevent it?

All of the organisations and people she mentions there either answer directly to the Cult or do whatever hierarchical levels above them tell them to do. The outcome of both is the same. 'It's not about masks, it's not about viruses, it's certainly not about your health', Griesz-Brisson said. 'It is about much, much more. I am not participating. I am not afraid.' They were taking our air to breathe and there was no unfounded medical exemption from face masks. Oxygen deprivation was dangerous for every single brain. It had to be the free decision of every human being whether they want to

wear a mask that was absolutely ineffective to protect themselves from a virus. She ended by rightly identifying where the responsibility lies for all this:

> The imperative of the hour is personal responsibility. We are responsible for what we think, not the media. We are responsible for what we do, not our superiors. We are responsible for our health, not the World Health Organization. And we are responsible for what happens in our country, not the government.

Halle-bloody-lujah.

But surgeons wear masks, right?

Independent studies of mask-wearing have produced a long list of reports detailing mental, emotional and physical dangers. What a definition of insanity to see police officers imposing mask-wearing on the public which will cumulatively damage their health while the police themselves wear masks that will cumulatively damage *their* health. It's utter madness and both public and police do this because 'the government says so' – yes a government of brain-donor idiots like UK Health Secretary Matt Hancock reading the 'follow the science' scripts of psychopathic, lunatic psychologists. The response you get from Stockholm syndrome sufferers defending the very authorities that are destroying them and their families is that 'surgeons wear masks'. This is considered the game, set and match that they must work and don't cause oxygen deficit. Well, actually, scientific studies have shown that they *do* and oxygen levels are monitored in operating theatres to compensate. Surgeons wear masks to stop spittle and such like dropping into open wounds – not to stop 'viral particles' which are so miniscule they can only be seen through an electron microscope. Holes in the masks are significantly bigger than 'viral particles' and if you sneeze or cough they will breach the mask. I watched an incredibly disingenuous 'experiment' that claimed to prove that masks work in catching 'virus' material from the mouth and nose. They did this with a slow motion camera and the mask did block big stuff which stayed inside the mask and

against the face to be breathed in or cause infections on the face as we have seen with many children. 'Viral particles', however, would never have been picked up by the camera as they came through the mask when they are far too small to be seen. The 'experiment' was therefore disingenuous *and* useless.

Studies have concluded that wearing masks in operating theatres (and thus elsewhere) make no difference to preventing infection while the opposite is true with toxic shite building up in the mask and this had led to an explosion in tooth decay and gum disease dubbed by dentists 'mask mouth'. You might have seen the Internet video of a furious American doctor urging people to take off their masks after a four-year-old patient had been rushed to hospital the night before and nearly died with a lung infection that doctors sourced to mask wearing. A study in the journal *Cancer Discovery* found that inhalation of harmful microbes can contribute to advanced stage lung cancer in adults and long-term use of masks can help breed dangerous pathogens. Microbiologists have said frequent mask wearing creates a moist environment in which microbes can grow and proliferate before entering the lungs. The Canadian Agency for Drugs and Technologies in Health, or CADTH, a Canadian national organisation that provides research and analysis to healthcare decision-makers, said this as long ago as 2013 in a report entitled 'Use of Surgical Masks in the Operating Room: A Review of the Clinical Effectiveness and Guidelines'. It said:

- No evidence was found to support the use of surgical face masks to reduce the frequency of surgical site infections
- No evidence was found on the effectiveness of wearing surgical face masks to protect staff from infectious material in the operating room.
- Guidelines recommend the use of surgical face masks by staff in the operating room to protect both operating room staff and patients (despite the lack of evidence).

We were told that the world could go back to 'normal' with the arrival of the 'vaccines'. When they came, fraudulent as they are, the story changed as I knew that it would. We are in the midst of transforming 'normal', not going back to it. Mary Ramsay, head of immunisation at Public Health England, echoed the words of US criminal Anthony Fauci who said masks and other regulations must stay no matter if people are vaccinated. The Fauci idiot continued to wear two masks – different colours so both could be clearly seen – after he *claimed* to have been vaccinated. Senator Rand Paul told Fauci in one exchange that his double-masks were 'theatre' and he was right. It's all theatre. Mary Ramsay back-tracked on the vaccine-return-to-normal theme when she said the public may need to wear masks and social-distance for years despite the jabs. 'People have got used to those lower-level restrictions now, and [they] can live with them', she said telling us what the idea has been all along. 'The vaccine does not give you a pass, even if you have had it, you must continue to follow all the guidelines' said a Public Health England statement which reneged on what we had been told before and made having the 'vaccine' irrelevant to 'normality' even by the official story. Spain's fascist government trumped everyone by passing a law mandating the wearing of masks on the beach and even when swimming in the sea. The move would have devastated what's left of the Spanish tourist industry, posed potential breathing dangers to swimmers and had Northern European sunbathers walking around with their forehead brown and the rest of their face white as a sheet. The ruling was so crazy that it had to be retracted after pressure from public and tourist industry, but it confirmed where the Cult wants to go with masks and how clinically insane authority has become. The determination to make masks permanent and hide the serious dangers to body and mind can be seen in the censorship of scientist Professor Denis Rancourt by Bill Gates-funded academic publishing website ResearchGate over his papers exposing the dangers and uselessness of masks. Rancourt said:

ResearchGate today has permanently locked my account, which I have had since 2015. Their reasons graphically show the nature of their attack against democracy, and their corruption of

science … By their obscene non-logic, a scientific review of science articles reporting on harms caused by face masks has a 'potential to cause harm'. No criticism of the psychological device (face masks) is tolerated, if the said criticism shows potential to influence public policy.

This is what happens in a fascist world.

Where are the 'greens' (again)?

Other dangers of wearing masks especially regularly relate to the inhalation of minute plastic fibres into the lungs and the deluge of discarded masks in the environment and oceans. Estimates predicted that more than 1.5 billion disposable masks will end up in the world's oceans every year polluting the water with tons of plastic and endangering marine wildlife. Studies project that humans are using 129 billion face masks each month worldwide – about three million a minute. Most are disposable and made from plastic, non-biodegradable microfibers that break down into smaller plastic particles that become widespread in ecosystems. They are littering cities, clogging sewage channels and turning up in bodies of water. I have written in other books about the immense amounts of microplastics from endless sources now being absorbed into the body. Rolf Halden, director of the Arizona State University (ASU) Biodesign Center for Environmental Health Engineering, was the senior researcher in a 2020 study that analysed 47 human tissue samples and found microplastics in all of them. 'We have detected these chemicals of plastics in every single organ that we have investigated', he said. I wrote in *The Answer* about the world being deluged with microplastics. A study by the Worldwide Fund for Nature (WWF) found that people are consuming on average every week some 2,000 tiny pieces of plastic mostly through water and also through marine life and the air. Every year humans are ingesting enough microplastics to fill a heaped dinner plate and in a life-time of 79 years it is enough to fill two large waste bins. Marco Lambertini, WWF International director general said: 'Not only are plastics polluting our oceans and waterways and killing marine life – it's in all of us and we can't escape consuming plastics,' American

geologists found tiny plastic fibres, beads and shards in rainwater samples collected from the remote slopes of the Rocky Mountain National Park near Denver, Colorado. Their report was headed: 'It is raining plastic.' Rachel Adams, senior lecturer in Biomedical Science at Cardiff Metropolitan University, said that among health consequences are internal inflammation and immune responses to a 'foreign body'. She further pointed out that microplastics become carriers of toxins including mercury, pesticides and dioxins (a known cause of cancer and reproductive and developmental problems). These toxins accumulate in the fatty tissues once they enter the body through microplastics. Now this is being compounded massively by people putting plastic on their face and throwing it away.

Workers exposed to polypropylene plastic fibres known as 'flock' have developed 'flock worker's lung' from inhaling small pieces of the flock fibres which can damage lung tissue, reduce breathing capacity and exacerbate other respiratory problems. *Now* … commonly used surgical masks have three layers of melt-blown textiles made of … polypropylene. We have billions of people putting these microplastics against their mouth, nose and face for hours at a time day after day in the form of masks. How does anyone think that will work out? I mean – what could possibly go wrong? We posted a number of scientific studies on this at davidicke.com, but when I went back to them as I was writing this book the links to the science research website where they were hosted were dead. Anything that challenges the official narrative in any way is either censored or vilified. The official narrative is so unsupportable by the evidence that only deleting the truth can protect it. A study by Chinese scientists still survived – with the usual twist which it why it was still active, I guess. Yes, they found that virtually all the masks they tested increased the daily intake of microplastic fibres, but people should still wear them because the danger from the 'virus' was worse said the crazy 'team' from the Institute of Hydrobiology in Wuhan. Scientists first discovered microplastics in lung tissue of some patients who died of lung cancer

in the 1990s. Subsequent studies have confirmed the potential health damage with the plastic degrading slowly and remaining in the lungs to accumulate in volume. Wuhan researchers used a machine simulating human breathing to establish that masks shed up to nearly 4,000 microplastic fibres in a month with reused masks producing more. Scientists said some masks are laced with toxic chemicals and a variety of compounds seriously restricted for both health and environmental reasons. They include cobalt (used in blue dye) and formaldehyde known to cause watery eyes, burning sensations in the eyes, nose, and throat, plus coughing, wheezing and nausea. No – that must be 'Covid-19'.

Mask 'worms'

There is another and potentially even more sinister content of masks. Mostly new masks of different makes filmed under a microscope around the world have been found to contain strange black fibres or 'worms' that appear to move or 'crawl' by themselves and react to heat and water. The nearest I have seen to them are the self-replicating fibres that are pulled out through the skin of those suffering from Morgellons disease which has been connected to the phenomena of 'chemtrails' which I will bring into the story later on. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. Black 'worm' fibres in masks have that kind of feel to them and there is a nanotechnology technique called 'worm micelles' which carry and release drugs or anything else you want to deliver to the body. For sure the suppression of humanity by mind altering drugs is the Cult agenda big time and the more excuses they can find to gain access to the body the more opportunities there are to make that happen whether through 'vaccines' or masks pushed against the mouth and nose for hours on end.

So let us summarise the pros and cons of masks:

Against masks: Breathing in your own carbon dioxide; depriving the body and brain of sufficient oxygen; build-up of toxins in the mask that can be breathed into the lungs and cause rashes on the face and 'mask-mouth'; breathing microplastic fibres and toxic chemicals into the lungs; dehumanisation and deleting individualisation by literally making people faceless; destroying human emotional interaction through facial expression and deleting parental connection with their babies which look for guidance to their facial expression.

For masks: They don't protect you from a 'virus' that doesn't exist and even if it did 'viral' particles are so minute they are smaller than the holes in the mask.

Governments, police, supermarkets, businesses, transport companies, and all the rest who seek to impose masks have done no risk assessment on their consequences for health and psychology and are now open to group lawsuits when the impact becomes clear with a cumulative epidemic of respiratory and other disease. Authorities will try to exploit these effects and hide the real cause by dubbing them 'Covid-19'. Can you imagine setting out to force the population to wear health-destroying masks without doing any assessment of the risks? It is criminal and it is evil, but then how many people targeted in this way, who see their children told to wear them all day at school, have asked for a risk assessment? Billions can't be imposed upon by the few unless the billions allow it. Oh, yes, with just a tinge of irony, 85 percent of all masks made worldwide come from *China*.

Wash your hands in toxic shite

'Covid' rules include the use of toxic sanitisers and again the health consequences of constantly applying toxins to be absorbed through the skin is obvious to any level of Renegade Mind. America's Food and Drug Administration (FDA) said that sanitisers are drugs and issued a warning about 75 dangerous brands which contain

methanol used in antifreeze and can cause death, kidney damage and blindness. The FDA circulated the following warning even for those brands that it claims to be safe:

Store hand sanitizer out of the reach of pets and children, and children should use it only with adult supervision. Do not drink hand sanitizer. This is particularly important for young children, especially toddlers, who may be attracted by the pleasant smell or brightly colored bottles of hand sanitizer.

Drinking even a small amount of hand sanitizer can cause alcohol poisoning in children. (However, there is no need to be concerned if your children eat with or lick their hands after using hand sanitizer.) During this coronavirus pandemic, poison control centers have had an increase in calls about accidental ingestion of hand sanitizer, so it is important that adults monitor young children's use.

Do not allow pets to swallow hand sanitizer. If you think your pet has eaten something potentially dangerous, call your veterinarian or a pet poison control center right away. Hand sanitizer is flammable and should be stored away from heat and flames. When using hand sanitizer, rub your hands until they feel completely dry before performing activities that may involve heat, sparks, static electricity, or open flames.

There you go, perfectly safe, then, and that's without even a mention of the toxins absorbed through the skin. Come on kids – sanitise your hands everywhere you go. It will save you from the 'virus'. Put all these elements together of the 'Covid' normal and see how much health and psychology is being cumulatively damaged, even devastated, to 'protect your health'. Makes sense, right? They are only imposing these things because they care, right? *Right?*

Submitting to insanity

Psychological reframing of the population goes very deep and is done in many less obvious ways. I hear people say how contradictory and crazy 'Covid' rules are and how they are ever changing. This is explained away by dismissing those involved as idiots. It is a big mistake. The Cult is delighted if its cold calculation is perceived as incompetence and idiocy when it is anything but. Oh, yes, there are idiots within the system – lots of them – but they are *administering* the Cult agenda, mostly unknowingly. They are not deciding and dictating it. The bulwark against tyranny is self-

respect, always has been, always will be. It is self-respect that has broken every tyranny in history. By its very nature self-respect will not bow to oppression and its perpetrators. There is so little self-respect that it's always the few that overturn dictators. Many may eventually follow, but the few with the iron spines (self-respect) kick it off and generate the momentum. The Cult targets self-respect in the knowledge that once this has gone only submission remains. Crazy, contradictory, ever-changing 'Covid' rules are systematically applied by psychologists to delete self-respect. They *want* you to see that the rules make no sense. It is one thing to decide to do something when *you* have made the choice based on evidence and logic. You still retain your self-respect. It is quite another when you can see what you are being told to do is insane, ridiculous and makes no sense, and *yet you still do it*. Your self-respect is extinguished and this has been happening as ever more obviously stupid and nonsensical things have been demanded and the great majority have complied even when they can see they are stupid and nonsensical.

People walk around in face-nappies knowing they are damaging their health and make no difference to a 'virus'. They do it in fear of not doing it. I know it's daft, but I'll do it anyway. When that happens something dies inside of you and submissive reframing has begun. Next there's a need to hide from yourself that you have conceded your self-respect and you convince yourself that you have not really submitted to fear and intimidation. You begin to believe that you are complying with craziness because it's the right thing to do. When first you concede your self-respect of 2+2 = 4 to 2+2 = 5 you *know* you are compromising your self-respect. Gradually to avoid facing that fact you begin to *believe* that 2+2=5. You have been reframed and I have been watching this process happening in the human psyche on an industrial scale. The Cult is working to break your spirit and one of its major tools in that war is humiliation. I read how former American soldier Bradley Manning (later Chelsea Manning after a sex-change) was treated after being jailed for supplying WikiLeaks with documents exposing the enormity of

government and elite mendacity. Manning was isolated in solitary confinement for eight months, put under 24-hour surveillance, forced to hand over clothing before going to bed, and stand naked for every roll call. This is systematic humiliation. The introduction of anal swab 'Covid' tests in China has been done for the same reason to delete self-respect and induce compliant submission. Anal swabs are mandatory for incoming passengers in parts of China and American diplomats have said they were forced to undergo the indignity which would have been calculated humiliation by the Cult-owned Chinese government that has America in its sights.

Government-people: An abusive relationship

Spirit-breaking psychological techniques include giving people hope and apparent respite from tyranny only to take it away again. This happened in the UK during Christmas, 2020, when the psycho-psychologists and their political lackeys announced an easing of restrictions over the holiday only to reimpose them almost immediately on the basis of yet another lie. There is a big psychological difference between getting used to oppression and being given hope of relief only to have that dashed. Psychologists know this and we have seen the technique used repeatedly. Then there is traumatising people before you introduce more extreme regulations that require compliance. A perfect case was the announcement by the dark and sinister Whitty and Vallance in the UK that 'new data' predicted that 4,000 could die every day over the winter of 2020/2021 if we did not lockdown again. I think they call it lying and after traumatising people with that claim out came Jackboot Johnson the next day with new curbs on human freedom. Psychologists know that a frightened and traumatised mind becomes suggestable to submission and behaviour reframing. Underpinning all this has been to make people fearful and suspicious of each other and see themselves as a potential danger to others. In league with deleted self-respect you have the perfect psychological recipe for self-loathing. The relationship between authority and public is now demonstrably the same as that of

subservience to an abusive partner. These are signs of an abusive relationship explained by psychologist Leslie Becker-Phelps:

Psychological and emotional abuse: Undermining a partner's self-worth with verbal attacks, name-calling, and belittling. Humiliating the partner in public, unjustly accusing them of having an affair, or interrogating them about their every behavior. Keeping partner confused or off balance by saying they were just kidding or blaming the partner for 'making' them act this way … Feigning in public that they care while turning against them in private. This leads to victims frequently feeling confused, incompetent, unworthy, hopeless, and chronically self-doubting. [Apply these techniques to how governments have treated the population since New Year, 2020, and the parallels are obvious.]

Physical abuse: The abuser might physically harm their partner in a range of ways, such as grabbing, hitting, punching, or shoving them. They might throw objects at them or harm them with a weapon. [Observe the physical harm imposed by masks, lockdown, and so on.]

Threats and intimidation: One way abusers keep their partners in line is by instilling fear. They might be verbally threatening, or give threatening looks or gestures. Abusers often make it known that they are tracking their partner's every move. They might destroy their partner's possessions, threaten to harm them, or threaten to harm their family members. Not surprisingly, victims of this abuse often feel anxiety, fear, and panic. [No words necessary.]

Isolation: Abusers often limit their partner's activities, forbidding them to talk or interact with friends or family. They might limit access to a car or even turn off their phone. All of this might be done by physically holding them against their will, but is often accomplished through psychological abuse and intimidation. The more isolated a person feels, the fewer resources they have to help gain perspective on their situation and to escape from it. [No words necessary.]

Economic abuse: Abusers often make their partners beholden to them for money by controlling access to funds of any kind. They might prevent their partner from getting a job or withhold access to money they earn from a job. This creates financial dependency that makes leaving the relationship very difficult. [See destruction of livelihoods and the proposed meagre 'guaranteed income' so long as you do whatever you are told.]

Using children: An abuser might disparage their partner's parenting skills, tell their children lies about their partner, threaten to take custody of their children, or threaten to harm their children. These tactics instil fear and often elicit compliance. [See reframed social service mafia and how children are being mercilessly abused by the state over 'Covid' while their parents look on too frightened to do anything.]

A further recurring trait in an abusive relationship is the abused blaming themselves for their abuse and making excuses for the abuser. We have the public blaming each other for lockdown abuse by government and many making excuses for the government while attacking those who challenge the government. How often we have heard authorities say that rules are being imposed or reimposed only because people have refused to 'behave' and follow the rules. We don't want to do it – it's *you*.

Renegade Minds are an antidote to all of these things. They will never concede their self-respect no matter what the circumstances. Even when apparent humiliation is heaped upon them they laugh in its face and reflect back the humiliation on the abuser where it belongs. Renegade Minds will never wear masks they know are only imposed to humiliate, suppress and damage both physically and psychologically. Consequences will take care of themselves and they will never break their spirit or cause them to concede to tyranny. UK newspaper columnist Peter Hitchens was one of the few in the mainstream media to speak out against lockdowns and forced vaccinations. He then announced he had taken the jab. He wanted to see family members abroad and he believed vaccine passports were inevitable even though they had not yet been introduced. Hitchens

has a questioning and critical mind, but not a Renegade one. If he had no amount of pressure would have made him concede. Hitchens excused his action by saying that the battle has been lost. Renegade Minds never accept defeat when freedom is at stake and even if they are the last one standing the self-respect of not submitting to tyranny is more important than any outcome or any consequence.

That's why Renegade Minds are the only minds that ever changed anything worth changing.

CHAPTER EIGHT

'Reframing' insanity

Insanity is relative. It depends on who has who locked in what cage
Ray Bradbury

'Reframing' a mind means simply to change its perception and behaviour. This can be done subconsciously to such an extent that subjects have no idea they have been 'reframed' while to any observer changes in behaviour and attitudes are obvious.

Human society is being reframed on a ginormous scale since the start of 2020 and here we have the reason why psychologists rather than doctors have been calling the shots. Ask most people who have succumbed to 'Covid' reframing if they have changed and most will say 'no'; but they *have* and fundamentally. The Cult's long-game has been preparing for these times since way back and crucial to that has been to prepare both population and officialdom mentally and emotionally. To use the mind-control parlance they had to reframe the population with a mentality that would submit to fascism and reframe those in government and law enforcement to impose fascism or at least go along with it. The result has been the fact-deleted mindlessness of 'Wokeness' and officialdom that has either enthusiastically or unquestioningly imposed global tyranny demanded by reframed politicians on behalf of psychopathic and deeply evil cultists. 'Cognitive reframing' identifies and challenges the way someone sees the world in the form of situations, experiences and emotions and then restructures those perceptions to view the same set of circumstances in a different way. This can have

benefits if the attitudes are personally destructive while on the other side it has the potential for individual and collective mind control which the subject has no idea has even happened.

Cognitive therapy was developed in the 1960s by Aaron T. Beck who was born in Rhode Island in 1921 as the son of Jewish immigrants from the Ukraine. He became interested in the techniques as a treatment for depression. Beck's daughter Judith S. Beck is prominent in the same field and they founded the Beck Institute for Cognitive Behavior Therapy in Philadelphia in 1994. Cognitive reframing, however, began to be used worldwide by those with a very dark agenda. The Cult reframes politicians to change their attitudes and actions until they are completely at odds with what they once appeared to stand for. The same has been happening to government administrators at all levels, law enforcement, military and the human population. Cultists love mind control for two main reasons: It allows them to control what people think, do and say to secure agenda advancement and, by definition, it calms their legendary insecurity and fear of the unexpected. I have studied mind control since the time I travelled America in 1996. I may have been talking to next to no one in terms of an audience in those years, but my goodness did I gather a phenomenal amount of information and knowledge about so many things including the techniques of mind control. I have described this in detail in other books going back to *The Biggest Secret* in 1998. I met a very large number of people recovering from MKUltra and its offshoots and successors and I began to see how these same techniques were being used on the population in general. This was never more obvious than since the 'Covid' hoax began.

Reframing the enforcers

I have observed over the last two decades and more the very clear transformation in the dynamic between the police, officialdom and the public. I tracked this in the books as the relationship mutated from one of serving the public to seeing them as almost the enemy and certainly a lower caste. There has always been a class divide

based on income and always been some psychopathic, corrupt, and big-I-am police officers. This was different. Wholesale change was unfolding in the collective dynamic; it was less about money and far more about position and perceived power. An us-and-them was emerging. Noses were lifted skyward by government administration and law enforcement and their attitude to the public they were *supposed* to be serving changed to one of increasing contempt, superiority and control. The transformation was so clear and widespread that it had to be planned. Collective attitudes and dynamics do not change naturally and organically that quickly on that scale. I then came across an organisation in Britain called Common Purpose created in the late 1980s by Julia Middleton who would work in the office of Deputy Prime Minister John Prescott during the long and disastrous premiership of war criminal Tony Blair. When Blair speaks the Cult is speaking and the man should have been in jail a long time ago. Common Purpose proclaims itself to be one of the biggest 'leadership development' organisations in the world while functioning as a *charity* with all the financial benefits which come from that. It hosts 'leadership development' courses and programmes all over the world and claims to have 'brought together' what it calls 'leaders' from more than 100 countries on six continents. The modus operandi of Common Purpose can be compared with the work of the UK government's reframing network that includes the Behavioural Insights Team 'nudge unit' and 'Covid' reframing specialists at SPI-B. WikiLeaks described Common Purpose long ago as 'a hidden virus in our government and schools' which is unknown to the general public: 'It recruits and trains "leaders" to be loyal to the directives of Common Purpose and the EU, instead of to their own departments, which they then undermine or subvert, the NHS [National Health Service] being an example.' This is a vital point to understand the 'Covid' hoax. The NHS, and its equivalent around the world, has been utterly reframed in terms of administrators and much of the medical personnel with the transformation underpinned by recruitment policies. The outcome has been the criminal and psychopathic behaviour of the

NHS over 'Covid' and we have seen the same in every other major country. WikiLeaks said Common Purpose trainees are 'learning to rule without regard to democracy' and to usher in a police state (current events explained). Common Purpose operated like a 'glue' and had members in the NHS, BBC, police, legal profession, church, many of Britain's 7,000 quangos, local councils, the Civil Service, government ministries and Parliament, and controlled many RDA's (Regional Development Agencies). Here we have one answer for how and why British institutions and their like in other countries have changed so negatively in relation to the public. This further explains how and why the beyond-disgraceful reframed BBC has become a propaganda arm of 'Covid' fascism. They are all part of a network pursuing the same goal.

By 2019 Common Purpose was quoting a figure of 85,000 'leaders' that had attended its programmes. These 'students' of all ages are known as Common Purpose 'graduates' and they consist of government, state and local government officials and administrators, police chiefs and officers, and a whole range of others operating within the national, local and global establishment. Cressida Dick, Commissioner of the London Metropolitan Police, is the Common Purpose graduate who was the 'Gold Commander' that oversaw what can only be described as the murder of Brazilian electrician Jean Charles de Menezes in 2005. He was held down by psychopathic police and shot seven times in the head by a psychopathic lunatic after being mistaken for a terrorist when he was just a bloke going about his day. Dick authorised officers to pursue and keep surveillance on de Menezes and ordered that he be stopped from entering the underground train system. Police psychopaths took her at her word clearly. She was 'disciplined' for this outrage by being *promoted* – eventually to the top of the 'Met' police where she has been a disaster. Many Chief Constables controlling the police in different parts of the UK are and have been Common Purpose graduates. I have heard the 'graduate' network described as a sort of Mafia or secret society operating within the fabric of government at all levels pursuing a collective policy

ingrained at Common Purpose training events. Founder Julia Middleton herself has said:

> Locally and internationally, Common Purpose graduates will be 'lighting small fires' to create change in their organisations and communities … The Common Purpose effect is best illustrated by the many stories of small changes brought about by leaders, who themselves have changed.

A Common Purpose mission statement declared:

> Common Purpose aims to improve the way society works by expanding the vision, decision-making ability and influence of all kinds of leaders. The organisation runs a variety of educational programmes for leaders of all ages, backgrounds and sectors, in order to provide them with the inspirational, information and opportunities they need to change the world.

Yes, but into what? Since 2020 the answer has become clear.

NLP and the Delphi technique

Common Purpose would seem to be a perfect name or would common programming be better? One of the foundation methods of reaching 'consensus' (group think) is by setting the agenda theme and then encouraging, cajoling or pressuring everyone to agree a 'consensus' in line with the core theme promoted by Common Purpose. The methodology involves the 'Delphi technique', or an adaption of it, in which opinions are expressed that are summarised by a 'facilitator or change agent' at each stage. Participants are 'encouraged' to modify their views in the light of what others have said. Stage by stage the former individual opinions are merged into group consensus which just happens to be what Common Purpose wants them to believe. A key part of this is to marginalise anyone refusing to concede to group think and turn the group against them to apply pressure to conform. We are seeing this very technique used on the general population to make 'Covid' group-thinkers hostile to those who have seen through the bullshit. People can be reframed by using perception manipulation methods such as Neuro-Linguistic Programming (NLP) in which you change perception with the use of

carefully constructed language. An NLP website described the technique this way:

… A method of influencing brain behaviour (the 'neuro' part of the phrase) through the use of language (the 'linguistic' part) and other types of communication to enable a person to 'recode' the way the brain responds to stimuli (that's the 'programming') and manifest new and better behaviours. Neuro-Linguistic Programming often incorporates hypnosis and self-hypnosis to help achieve the change (or 'programming') that is wanted.

British alternative media operation UKColumn has done very detailed research into Common Purpose over a long period. I quoted co-founder and former naval officer Brian Gerrish in my book *Remember Who You Are*, published in 2011, as saying the following years before current times:

It is interesting that many of the mothers who have had children taken by the State speak of the Social Services people being icily cool, emotionless and, as two ladies said in slightly different words, '… like little robots'. We know that NLP is cumulative, so people can be given small imperceptible doses of NLP in a course here, another in a few months, next year etc. In this way, major changes are accrued in their personality, but the day by day change is almost unnoticeable.

In these and other ways 'graduates' have had their perceptions uniformly reframed and they return to their roles in the institutions of government, law enforcement, legal profession, military, 'education', the UK National Health Service and the whole swathe of the establishment structure to pursue a common agenda preparing for the 'post-industrial', 'post-democratic' society. I say 'preparing' but we are now there. 'Post-industrial' is code for the Great Reset and 'post-democratic' is 'Covid' fascism. UKColumn has spoken to partners of those who have attended Common Purpose 'training'. They have described how personalities and attitudes of 'graduates' changed very noticeably for the worse by the time they had completed the course. They had been 'reframed' and told they are the 'leaders' – the special ones – who know better than the population. There has also been the very demonstrable recruitment of psychopaths and narcissists into government administration at all

levels and law enforcement. If you want psychopathy hire psychopaths and you get a simple cause and effect. If you want administrators, police officers and 'leaders' to perceive the public as lesser beings who don't matter then employ narcissists. These personalities are identified using 'psychometrics' that identifies knowledge, abilities, attitudes and personality traits, mostly through carefully-designed questionnaires and tests. As this policy has passed through the decades we have had power-crazy, power-trippers appointed into law enforcement, security and government administration in preparation for current times and the dynamic between public and law enforcement/officialdom has been transformed. UKColumn's Brian Gerrish said of the narcissistic personality:

> Their love of themselves and power automatically means that they will crush others who get in their way. I received a major piece of the puzzle when a friend pointed out that when they made public officials re-apply for their own jobs several years ago they were also required to do psychometric tests. This was undoubtedly the start of the screening process to get 'their' sort of people in post.

How obvious that has been since 2020 although it was clear what was happening long before if people paid attention to the changing public-establishment dynamic.

Change agents

At the centre of events in 'Covid' Britain is the National Health Service (NHS) which has behaved disgracefully in slavishly following the Cult agenda. The NHS management structure is awash with Common Purpose graduates or 'change agents' working to a common cause. Helen Bevan, a Chief of Service Transformation at the NHS Institute for Innovation and Improvement, co-authored a document called 'Towards a million change agents, a review of the social movements literature: implications for large scale change in the NHS'. The document compared a project management approach to that of change and social movements where 'people change

themselves and each other – peer to peer'. Two definitions given for a 'social movement' were:

A group of people who consciously attempt to build a radically new social order; involves people of a broad range of social backgrounds; and deploys politically confrontational and socially disruptive tactics – Cyrus Zirakzadeh 1997

Collective challenges, based on common purposes and social solidarities, in sustained interaction with elites, opponents, and authorities – Sidney Tarrow 1994

Helen Bevan wrote another NHS document in which she defined 'framing' as 'the process by which leaders construct, articulate and put across their message in a powerful and compelling way in order to win people to their cause and call them to action'. I think I could come up with another definition that would be rather more accurate. The National Health Service and institutions of Britain and the wider world have been taken over by reframed 'change agents' and that includes everything from the United Nations to national governments, local councils and social services which have been kidnapping children from loving parents on an extraordinary and gathering scale on the road to the end of parenthood altogether. Children from loving homes are stolen and kidnapped by the state and put into the 'care' (inversion) of the local authority through council homes, foster parents and forced adoption. At the same time children are allowed to be abused without response while many are under council 'care'. UKColumn highlighted the Common Purpose connection between South Yorkshire Police and Rotherham council officers in the case of the scandal in that area of the sexual exploitation of children to which the authorities turned not one blind eye, but both:

We were alarmed to discover that the Chief Executive, the Strategic Director of Children and Young People's Services, the Manager for the Local Strategic Partnership, the Community Cohesion Manager, the Cabinet Member for Cohesion, the Chief Constable and his predecessor had all attended Leadership training courses provided by the pseudo-charity Common Purpose.

Once 'change agents' have secured positions of hire and fire within any organisation things start to move very quickly. Personnel are then hired and fired on the basis of whether they will work towards the agenda the change agent represents. If they do they are rapidly promoted even though they may be incompetent. Those more qualified and skilled who are pre-Common Purpose 'old school' see their careers stall and even disappear. This has been happening for decades in every institution of state, police, 'health' and social services and all of them have been transformed as a result in their attitudes to their jobs and the public. Medical professions, including nursing, which were once vocations for the caring now employ many cold, callous and couldn't give a shit personality types. The UKColumn investigation concluded:

By blurring the boundaries between people, professions, public and private sectors, responsibility and accountability, Common Purpose encourages 'graduates' to believe that as new selected leaders, they can work together, outside of the established political and social structures, to achieve a paradigm shift or CHANGE – so called 'Leading Beyond Authority'. In doing so, the allegiance of the individual becomes 'reframed' on CP colleagues and their NETWORK.

Reframing the Face-Nappies

Nowhere has this process been more obvious than in the police where recruitment of psychopaths and development of unquestioning mind-controlled group-thinkers have transformed law enforcement into a politically-correct 'Woke' joke and a travesty of what should be public service. Today they wear their face-nappies like good little gofers and enforce 'Covid' rules which are fascism under another name. Alongside the specifically-recruited psychopaths we have software minds incapable of free thought. Brian Gerrish again:

An example is the policeman who would not get on a bike for a press photo because he had not done the cycling proficiency course. Normal people say this is political correctness gone mad. Nothing could be further from the truth. The policeman has been reframed, and in his reality it is perfect common sense not to get on the bike 'because he hasn't done the cycling course'.

Another example of this is where the police would not rescue a boy from a pond until they had taken advice from above on the 'risk assessment'. A normal person would have arrived, perhaps thought of the risk for a moment, and dived in. To the police now 'reframed', they followed 'normal' procedure.

There are shocking cases of reframed ambulance crews doing the same. Sheer unthinking stupidity of London Face-Nappies headed by Common Purpose graduate Cressida Dick can be seen in their behaviour at a vigil in March, 2021, for a murdered woman, Sarah Everard. A police officer had been charged with the crime. Anyone with a brain would have left the vigil alone in the circumstances. Instead they 'manhandled' women to stop them breaking 'Covid rules' to betray classic reframing. Minds in the thrall of perception control have no capacity for seeing a situation on its merits and acting accordingly. 'Rules is rules' is their only mind-set. My father used to say that rules and regulations are for the guidance of the intelligent and the blind obedience of the idiot. Most of the intelligent, decent, coppers have gone leaving only the other kind and a few old school for whom the job must be a daily nightmare. The combination of psychopaths and rule-book software minds has been clearly on public display in the 'Covid' era with automaton robots in uniform imposing fascistic 'Covid' regulations on the population without any personal initiative or judging situations on their merits. There are thousands of examples around the world, but I'll make my point with the infamous Derbyshire police in the English East Midlands – the ones who think pouring dye into beauty spots and using drones to track people walking in the countryside away from anyone is called 'policing'. To them there are rules decreed by the government which they have to enforce and in their bewildered state a group gathering in a closed space and someone walking alone in the countryside are the same thing. It is beyond idiocy and enters the realm of clinical insanity.

Police officers in Derbyshire said they were 'horrified' – *horrified* – to find 15 to 20 'irresponsible' kids playing a football match at a closed leisure centre 'in breach of coronavirus restrictions'. When they saw the police the kids ran away leaving their belongings behind and the reframed men and women of Derbyshire police were seeking to establish their identities with a view to fining their parents. The most natural thing for youngsters to do – kicking a ball about – is turned into a criminal activity and enforced by the moronic software programs of Derbyshire police. You find the same mentality in every country. These barely conscious 'horrified' officers said they had to take action because 'we need to ensure these rules are being followed' and 'it is of the utmost importance that you ensure your children are following the rules and regulations for Covid-19'. Had any of them done ten seconds of research to see if this parroting of their masters' script could be supported by any evidence? Nope. Reframed people don't think – others think for them and that's the whole idea of reframing. I have seen police officers one after the other repeating without question word for word what officialdom tells them just as I have seen great swathes of the public doing the same. Ask either for 'their' opinion and out spews what they have been told to think by the official narrative. Police and public may seem to be in different groups, but their mentality is the same. Most people do whatever they are told in fear not doing so or because they believe what officialdom tells them; almost the entirety of the police do what they are told for the same reason. Ultimately it's the tiny inner core of the global Cult that's telling both what to do.

So Derbyshire police were 'horrified'. Oh, really? Why did they think those kids were playing football? It was to relieve the psychological consequences of lockdown and being denied human contact with their friends and interaction, touch and discourse vital to human psychological health. Being denied this month after month has dismantled the psyche of many children and young people as depression and suicide have exploded. Were Derbyshire police *horrified by that*? Are you kidding? Reframed people don't have those

mental and emotional processes that can see how the impact on the psychological health of youngsters is far more dangerous than any 'virus' even if you take the mendacious official figures to be true. The reframed are told (programmed) how to act and so they do. The Derbyshire Chief Constable in the first period of lockdown when the black dye and drones nonsense was going on was Peter Goodman. He was the man who severed the connection between his force and the Derbyshire Constabulary *Male Voice* Choir when he decided that it was not inclusive enough to allow women to join. The fact it was a male voice choir making a particular sound produced by male voices seemed to elude a guy who terrifyingly ran policing in Derbyshire. He retired weeks after his force was condemned as disgraceful by former Supreme Court Justice Jonathan Sumption for their behaviour over extreme lockdown impositions. Goodman was replaced by his deputy Rachel Swann who was in charge when her officers were 'horrified'. The police statement over the boys committing the hanging-offence of playing football included the line about the youngsters being 'irresponsible in the times we are all living through' missing the point that the real relevance of the 'times we are all living through' is the imposition of fascism enforced by psychopaths and reframed minds of police officers playing such a vital part in establishing the fascist tyranny that their own children and grandchildren will have to live in their entire lives. As a definition of insanity that is hard to beat although it might be run close by imposing masks on people that can have a serious effect on their health while wearing a face nappy all day themselves. Once again public and police do it for the same reason – the authorities tell them to and who are they to have the self-respect to say no?

Wokers in uniform

How reframed do you have to be to arrest a *six-year-old* and take him to court for *picking a flower* while waiting for a bus? Brain dead police and officialdom did just that in North Carolina where criminal proceedings happen regularly for children under nine. Attorney Julie Boyer gave the six-year-old crayons and a colouring book

during the 'flower' hearing while the 'adults' decided his fate. County Chief District Court Judge Jay Corpening asked: 'Should a child that believes in Santa Claus, the Easter Bunny and the tooth fairy be making life-altering decisions?' Well, of course not, but common sense has no meaning when you have a common purpose and a reframed mind. Treating children in this way, and police operating in American schools, is all part of the psychological preparation for children to accept a police state as normal all their adult lives. The same goes for all the cameras and biometric tracking technology in schools. Police training is focused on reframing them as snowflake Wokers and this is happening in the military. Pentagon top brass said that 'training sessions on extremism' were needed for troops who asked why they were so focused on the Capitol Building riot when Black Lives Matter riots were ignored. What's the difference between them some apparently and rightly asked. Actually, there is a difference. Five people died in the Capitol riot, only one through violence, and that was a police officer shooting an unarmed protestor. BLM riots killed at least 25 people and cost billions. Asking the question prompted the psychopaths and reframed minds that run the Pentagon to say that more 'education' (programming) was needed. Troop training is all based on psychological programming to make them fodder for the Cult – 'Military men are just dumb, stupid animals to be used as pawns in foreign policy' as Cult-to-his-DNA former Secretary of State Henry Kissinger famously said. Governments see the police in similar terms and it's time for those among them who can see this to defend the people and stop being enforcers of the Cult agenda upon the people.

The US military, like the country itself, is being targeted for destruction through a long list of Woke impositions. Cult-owned gaga 'President' Biden signed an executive order when he took office to allow taxpayer money to pay for transgender surgery for active military personnel and veterans. Are you a man soldier? No, I'm a LGBTQIA+ with a hint of Skoliosexual and Spectrasexual. Oh, good man. Bad choice of words you bigot. The Pentagon announced in March, 2021, the appointment of the first 'diversity and inclusion

officer' for US Special Forces. Richard Torres-Estrada arrived with the publication of a 'D&I Strategic Plan which will guide the enterprise-wide effort to institutionalize and sustain D&I'. If you think a Special Forces 'Strategic Plan' should have something to do with defending America you haven't been paying attention. Defending Woke is now the military's new role. Torres-Estrada has posted images comparing Donald Trump with Adolf Hitler and we can expect no bias from him as a representative of the supposedly non-political Pentagon. Cable news host Tucker Carlson said: 'The Pentagon is now the Yale faculty lounge but with cruise missiles.' Meanwhile Secretary of Defense Lloyd Austin, a board member of weapons-maker Raytheon with stock and compensation interests in October, 2020, worth $1.4 million, said he was purging the military of the 'enemy within' – anyone who isn't Woke and supports Donald Trump. Austin refers to his targets as 'racist extremists' while in true Woke fashion being himself a racist extremist. Pentagon documents pledge to 'eradicate, eliminate and conquer all forms of racism, sexism and homophobia'. The definitions of these are decided by 'diversity and inclusion committees' peopled by those who see racism, sexism and homophobia in every situation and opinion. Woke (the Cult) is dismantling the US military and purging testosterone as China expands its military and gives its troops 'masculinity training'. How do we think that is going to end when this is all Cult coordinated? The US military, like the British military, is controlled by Woke and spineless top brass who just go along with it out of personal career interests.

'Woke' means fast asleep

Mind control and perception manipulation techniques used on individuals to create group-think have been unleashed on the global population in general. As a result many have no capacity to see the obvious fascist agenda being installed all around them or what 'Covid' is really all about. Their brains are firewalled like a computer system not to process certain concepts, thoughts and realisations that are bad for the Cult. The young are most targeted as the adults they

will be when the whole fascist global state is planned to be fully implemented. They need to be prepared for total compliance to eliminate all pushback from entire generations. The Cult has been pouring billions into taking complete control of 'education' from schools to universities via its operatives and corporations and not least Bill Gates as always. The plan has been to transform 'education' institutions into programming centres for the mentality of 'Woke'. James McConnell, professor of psychology at the University of Michigan, wrote in *Psychology Today* in 1970:

> The day has come when we can combine sensory deprivation with drugs, hypnosis, and astute manipulation of reward and punishment, to gain almost absolute control over an individual's behaviour. It should then be possible to achieve a very rapid and highly effective type of brainwashing that would allow us to make dramatic changes in a person's behaviour and personality ...
>
> … We should reshape society so that we all would be trained from birth to want to do what society wants us to do. We have the techniques to do it... no-one owns his own personality you acquired, and there's no reason to believe you should have the right to refuse to acquire a new personality if your old one is anti-social.

This was the potential for mass brainwashing in 1970 and the mentality there displayed captures the arrogant psychopathy that drives it forward. I emphasise that not all young people have succumbed to Woke programming and those that haven't are incredibly impressive people given that today's young are the most perceptually-targeted generations in history with all the technology now involved. Vast swathes of the young generations, however, have fallen into the spell – and that's what it is – of Woke. The Woke mentality and perceptual program is founded on *inversion* and you will appreciate later why that is so significant. Everything with Woke is inverted and the opposite of what it is claimed to be. Woke was a term used in African-American culture from the 1900s and referred to an awareness of social and racial justice. This is not the meaning of the modern version or 'New Woke' as I call it in *The Answer*. Oh, no, Woke today means something very different no matter how much Wokers may seek to hide that and insist Old Woke and New

Woke are the same. See if you find any 'awareness of social justice' here in the modern variety:

- Woke demands 'inclusivity' while excluding anyone with a different opinion and calls for mass censorship to silence other views.

- Woke claims to stand against oppression when imposing oppression is the foundation of all that it does. It is the driver of political correctness which is nothing more than a Cult invention to manipulate the population to silence itself.

- Woke believes itself to be 'liberal' while pursuing a global society that can only be described as fascist (see 'anti-fascist' fascist Antifa).

- Woke calls for 'social justice' while spreading injustice wherever it goes against the common 'enemy' which can be easily identified as a differing view.

- Woke is supposed to be a metaphor for 'awake' when it is solid-gold asleep and deep in a Cult-induced coma that meets the criteria for 'off with the fairies'.

I state these points as obvious facts if people only care to look. I don't do this with a sense of condemnation. We need to appreciate that the onslaught of perceptual programming on the young has been incessant and merciless. I can understand why so many have been reframed, or, given their youth, framed from the start to see the world as the Cult demands. The Cult has had access to their minds day after day in its 'education' system for their entire formative years. Perception is formed from information received and the Cult-created system is a life-long download of information delivered to elicit a particular perception, thus behaviour. The more this has expanded into still new extremes in recent decades and ever-increasing censorship has deleted other opinions and information why wouldn't that lead to a perceptual reframing on a mass scale? I

have described already cradle-to-grave programming and in more recent times the targeting of young minds from birth to adulthood has entered the stratosphere. This has taken the form of skewing what is 'taught' to fit the Cult agenda and the omnipresent techniques of group-think to isolate non-believers and pressure them into line. There has always been a tendency to follow the herd, but we really are in a new world now in relation to that. We have parents who can see the 'Covid' hoax told by their children not to stop them wearing masks at school, being 'Covid' tested or having the 'vaccine' in fear of the peer-pressure consequences of being different. What is 'peer-pressure' if not pressure to conform to group-think? Renegade Minds never group-think and always retain a set of perceptions that are unique to them. Group-think is always underpinned by consequences for not group-thinking. Abuse now aimed at those refusing DNA-manipulating 'Covid vaccines' are a potent example of this. The biggest pressure to conform comes from the very group which is itself being manipulated. 'I am programmed to be part of a hive mind and so you must be.'

Woke control structures in 'education' now apply to every mainstream organisation. Those at the top of the 'education' hierarchy (the Cult) decide the policy. This is imposed on governments through the Cult network; governments impose it on schools, colleges and universities; their leadership impose the policy on teachers and academics and they impose it on children and students. At any level where there is resistance, perhaps from a teacher or university lecturer, they are targeted by the authorities and often fired. Students themselves regularly demand the dismissal of academics (increasingly few) at odds with the narrative that the students have been programmed to believe in. It is quite a thought that students who are being targeted by the Cult become so consumed by programmed group-think that they launch protests and demand the removal of those who are trying to push back against those targeting the students. Such is the scale of perceptual inversion. We see this with 'Covid' programming as the Cult imposes the rules via psycho-psychologists and governments on

shops, transport companies and businesses which impose them on their staff who impose them on their customers who pressure Pushbackers to conform to the will of the Cult which is in the process of destroying them and their families. Scan all aspects of society and you will see the same sequence every time.

Fact free Woke and hijacking the 'left'

There is no more potent example of this than 'Woke', a mentality only made possible by the deletion of factual evidence by an 'education' system seeking to produce an ever more uniform society. Why would you bother with facts when you don't know any? Deletion of credible history both in volume and type is highly relevant. Orwell said: 'Who controls the past controls the future: who controls the present controls the past.' They who control the perception of the past control the perception of the future and they who control the present control the perception of the past through the writing and deleting of history. Why would you oppose the imposition of Marxism in the name of Wokeism when you don't know that Marxism cost at least 100 million lives in the 20th century alone? Watch videos and read reports in which Woker generations are asked basic historical questions – it's mind-blowing. A survey of 2,000 people found that six percent of millennials (born approximately early1980s to early 2000s) believed the Second World War (1939-1945) broke out with the assassination of President Kennedy (in 1963) and one in ten thought Margaret Thatcher was British Prime Minister at the time. She was in office between 1979 and 1990. We are in a post-fact society. Provable facts are no defence against the fascism of political correctness or Silicon Valley censorship. Facts don't matter anymore as we have witnessed with the 'Covid' hoax. Sacrificing uniqueness to the Woke group-think religion is all you are required to do and that means thinking for yourself is the biggest Woke no, no. All religions are an expression of group-think and censorship and Woke is just another religion with an orthodoxy defended by group-think and censorship. Burned at

the stake becomes burned on Twitter which leads back eventually to burned at the stake as Woke humanity regresses to ages past.

The biggest Woke inversion of all is its creators and funders. I grew up in a traditional left of centre political household on a council estate in Leicester in the 1950s and 60s – you know, the left that challenged the power of wealth-hoarding elites and threats to freedom of speech and opinion. In those days students went on marches defending freedom of speech while today's Wokers march for its deletion. What on earth could have happened? Those very elites (collectively the Cult) that we opposed in my youth and early life have funded into existence the antithesis of that former left and hijacked the 'brand' while inverting everything it ever stood for. We have a mentality that calls itself 'liberal' and 'progressive' while acting like fascists. Cult billionaires and their corporations have funded themselves into control of 'education' to ensure that Woke programming is unceasing throughout the formative years of children and young people and that non-Wokers are isolated (that word again) whether they be students, teachers or college professors. The Cult has funded into existence the now colossal global network of Woke organisations that have spawned and promoted all the 'causes' on the Cult wish-list for global transformation and turned Wokers into demanders of them. Does anyone really think it's a coincidence that the Cult agenda for humanity is a carbon (sorry) copy of the societal transformations desired by Woke?? These are only some of them:

Political correctness: The means by which the Cult deletes all public debates that it knows it cannot win if we had the free-flow of information and evidence.

Human-caused 'climate change': The means by which the Cult seeks to transform society into a globally-controlled dictatorship imposing its will over the fine detail of everyone's lives 'to save the planet' which doesn't actually need saving.

Transgender obsession: Preparing collective perception to accept the 'new human' which would not have genders because it would be created technologically and not through procreation. I'll have much more on this in Human 2.0.

Race obsession: The means by which the Cult seeks to divide and rule the population by triggering racial division through the perception that society is more racist than ever when the opposite is the case. Is it perfect in that regard? No. But to compare today with the racism of apartheid and segregation brought to an end by the civil rights movement in the 1960s is to insult the memory of that movement and inspirations like Martin Luther King. Why is the 'anti-racism' industry (which it is) so dominated by privileged white people?

White supremacy: This is a label used by privileged white people to demonise poor and deprived white people pushing back on tyranny to marginalise and destroy them. White people are being especially targeted as the dominant race by number within Western society which the Cult seeks to transform in its image. If you want to change a society you must weaken and undermine its biggest group and once you have done that by using the other groups you next turn on them to do the same … 'Then they came for the Jews and I was not a Jew so I did nothing.'

Mass migration: The mass movement of people from the Middle East, Africa and Asia into Europe, from the south into the United States and from Asia into Australia are another way the Cult seeks to dilute the racial, cultural and political influence of white people on Western society. White people ask why their governments appear to be working against them while being politically and culturally biased towards incoming cultures. Well, here's your answer. In the same way sexually 'straight' people, men and women, ask why the

authorities are biased against them in favour of other sexualities. The answer is the same – that's the way the Cult wants it to be for very sinister motives.

These are all central parts of the Cult agenda and central parts of the Woke agenda and Woke was created and continues to be funded to an immense degree by Cult billionaires and corporations. If anyone begins to say 'coincidence' the syllables should stick in their throat.

Billionaire 'social justice warriors'

Joe Biden is a 100 percent-owned asset of the Cult and the Wokers' man in the White House whenever he can remember his name and for however long he lasts with his rapidly diminishing cognitive function. Even walking up the steps of an aircraft without falling on his arse would appear to be a challenge. He's not an empty-shell puppet or anything. From the minute Biden took office (or the Cult did) he began his executive orders promoting the Woke wish-list. You will see the Woke agenda imposed ever more severely because it's really the *Cult* agenda. Woke organisations and activist networks spawned by the Cult are funded to the extreme so long as they promote what the Cult wants to happen. Woke is funded to promote 'social justice' by billionaires who become billionaires by destroying social justice. The social justice mantra is only a cover for dismantling social justice and funded by billionaires that couldn't give a damn about social justice. Everything makes sense when you see that. One of Woke's premier funders is Cult billionaire financier George Soros who said: 'I am basically there to make money, I cannot and do not look at the social consequences of what I do.' This is the same Soros who has given more than $32 billion to his Open Society Foundations global Woke network and funded Black Lives Matter, mass immigration into Europe and the United States, transgender activism, climate change activism, political correctness and groups targeting 'white supremacy' in the form of privileged white thugs that dominate Antifa. What a scam it all is and when

you are dealing with the unquestioning fact-free zone of Woke scamming them is child's play. All you need to pull it off in all these organisations are a few in-the-know agents of the Cult and an army of naïve, reframed, uninformed, narcissistic, know-nothings convinced of their own self-righteousness, self-purity and virtue.

Soros and fellow billionaires and billionaire corporations have poured hundreds of millions into Black Lives Matter and connected groups and promoted them to a global audience. None of this is motivated by caring about black people. These are the billionaires that have controlled and exploited a system that leaves millions of black people in abject poverty and deprivation which they do absolutely nothing to address. The same Cult networks funding BLM were behind the *slave trade!* Black Lives Matter hijacked a phrase that few would challenge and they have turned this laudable concept into a political weapon to divide society. You know that BLM is a fraud when it claims that *All* Lives Matter, the most inclusive statement of all, is 'racist'. BLM and its Cult masters don't want to end racism. To them it's a means to an end to control all of humanity never mind the colour, creed, culture or background. What has destroying the nuclear family got to do with ending racism? Nothing – but that is one of the goals of BLM and also happens to be a goal of the Cult as I have been exposing in my books for decades. Stealing children from loving parents and giving schools ever more power to override parents is part of that same agenda. BLM is a Marxist organisation and why would that not be the case when the Cult created Marxism *and* BLM? Patrisse Cullors, a BLM co-founder, said in a 2015 video that she and her fellow organisers, including co-founder Alicia Garza, are 'trained Marxists'. The lady known after marriage as Patrisse Khan-Cullors bought a $1.4 million home in 2021 in one of the whitest areas of California with a black population of just 1.6 per cent and has so far bought *four* high-end homes for a total of $3.2 million. How very Marxist. There must be a bit of spare in the BLM coffers, however, when Cult corporations and billionaires have handed over the best part of $100 million. Many black people can see that Black Lives Matter is not

working for them, but against them, and this is still more confirmation. Black journalist Jason Whitlock, who had his account suspended by Twitter for simply linking to the story about the 'Marxist's' home buying spree, said that BLM leaders are 'making millions of dollars off the backs of these dead black men who they wouldn't spit on if they were on fire and alive'.

Black Lies Matter

Cult assets and agencies came together to promote BLM in the wake of the death of career criminal George Floyd who had been jailed a number of times including for forcing his way into the home of a black woman with others in a raid in which a gun was pointed at her stomach. Floyd was filmed being held in a Minneapolis street in 2020 with the knee of a police officer on his neck and he subsequently died. It was an appalling thing for the officer to do, but the same technique has been used by police on peaceful protestors of lockdown without any outcry from the Woke brigade. As unquestioning supporters of the Cult agenda Wokers have supported lockdown and all the 'Covid' claptrap while attacking anyone standing up to the tyranny imposed in its name. Court documents would later include details of an autopsy on Floyd by County Medical Examiner Dr Andrew Baker who concluded that Floyd had taken a fatal level of the drug fentanyl. None of this mattered to fact-free, question-free, Woke. Floyd's death was followed by worldwide protests against police brutality amid calls to defund the police. Throwing babies out with the bathwater is a Woke speciality. In the wake of the murder of British woman Sarah Everard a Green Party member of the House of Lords, Baroness Jones of Moulescoomb (Nincompoopia would have been better), called for a 6pm curfew for all men. This would be in breach of the Geneva Conventions on war crimes which ban collective punishment, but that would never have crossed the black and white Woke mind of Baroness Nincompoopia who would have been far too convinced of her own self-righteousness to compute such details. Many American cities did defund the police in the face of Floyd riots

and after $15 million was deleted from the police budget in Washington DC under useless Woke mayor Muriel Bowser car-jacking alone rose by 300 percent and within six months the US capital recorded its highest murder rate in 15 years. The same happened in Chicago and other cities in line with the Cult/Soros plan to bring fear to streets and neighbourhoods by reducing the police, releasing violent criminals and not prosecuting crime. This is the mob-rule agenda that I have warned in the books was coming for so long. Shootings in the area of Minneapolis where Floyd was arrested increased by 2,500 percent compared with the year before. Defunding the police over George Floyd has led to a big increase in dead people with many of them black. Police protection for politicians making these decisions stayed the same or increased as you would expect from professional hypocrites. The Cult doesn't actually want to abolish the police. It wants to abolish local control over the police and hand it to federal government as the psychopaths advance the Hunger Games Society. Many George Floyd protests turned into violent riots with black stores and businesses destroyed by fire and looting across America fuelled by Black Lives Matter. Woke doesn't do irony. If you want civil rights you must loot the liquor store and the supermarket and make off with a smart TV. It's the only way.

It's not a race war – it's a class war

Black people are patronised by privileged blacks and whites alike and told they are victims of white supremacy. I find it extraordinary to watch privileged blacks supporting the very system and bloodline networks behind the slave trade and parroting the same Cult-serving manipulative crap of their privileged white, often billionaire, associates. It is indeed not a race war but a class war and colour is just a diversion. Black Senator Cory Booker and black Congresswoman Maxine Waters, more residents of Nincompoopia, personify this. Once you tell people they are victims of someone else you devalue both their own responsibility for their plight and the power they have to impact on their reality and experience. Instead

we have: 'You are only in your situation because of whitey – turn on them and everything will change.' It won't change. Nothing changes in our lives unless *we* change it. Crucial to that is never seeing yourself as a victim and always as the creator of your reality. Life is a simple sequence of choice and consequence. Make different choices and you create different consequences. *You* have to make those choices – not Black Lives Matter, the Woke Mafia and anyone else that seeks to dictate your life. Who are they these Wokers, an emotional and psychological road traffic accident, to tell you what to do? Personal empowerment is the last thing the Cult and its Black Lives Matter want black people or anyone else to have. They claim to be defending the underdog while *creating* and perpetuating the underdog. The Cult's worst nightmare is human unity and if they are going to keep blacks, whites and every other race under economic servitude and control then the focus must be diverted from what they have in common to what they can be manipulated to believe divides them. Blacks have to be told that their poverty and plight is the fault of the white bloke living on the street in the same poverty and with the same plight they are experiencing. The difference is that your plight black people is due to him, a white supremacist with 'white privilege' living on the street. Don't unite as one human family against your mutual oppressors and suppressors – fight the oppressor with the white face who is as financially deprived as you are. The Cult knows that as its 'Covid' agenda moves into still new levels of extremism people are going to respond and it has been spreading the seeds of disunity everywhere to stop a united response to the evil that targets *all of us*.

Racist attacks on 'whiteness' are getting ever more outrageous and especially through the American Democratic Party which has an appalling history for anti-black racism. Barack Obama, Joe Biden, Hillary Clinton and Nancy Pelosi all eulogised about Senator Robert Byrd at his funeral in 2010 after a nearly 60-year career in Congress. Byrd was a brutal Ku Klux Klan racist and a violent abuser of Cathy O'Brien in MKUltra. He said he would never fight in the military 'with a negro by my side' and 'rather I should die a thousand times,

and see Old Glory trampled in the dirt never to rise again, than to see this beloved land of ours become degraded by race mongrels, a throwback to the blackest specimen from the wilds'. Biden called Byrd a 'very close friend and mentor'. These 'Woke' hypocrites are not anti-racist they are anti-poor and anti-people not of their perceived class. Here is an illustration of the scale of anti-white racism to which we have now descended. Seriously Woke and moronic *New York Times* contributor Damon Young described whiteness as a 'virus' that 'like other viruses will not die until there are no bodies left for it to infect'. He went on: '… the only way to stop it is to locate it, isolate it, extract it, and kill it.' Young can say that as a black man with no consequences when a white man saying the same in reverse would be facing a jail sentence. *That's* racism. We had super-Woke numbskull senators Tammy Duckworth and Mazie Hirono saying they would object to future Biden Cabinet appointments if he did not nominate more Asian Americans and Pacific Islanders. Never mind the ability of the candidate what do they look like? Duckworth said: 'I will vote for racial minorities and I will vote for LGBTQ, but anyone else I'm not voting for.' Appointing people on the grounds of race is illegal, but that was not a problem for this ludicrous pair. They were on-message and that's a free pass in any situation.

Critical race racism

White children are told at school they are intrinsically racist as they are taught the divisive 'critical race theory'. This claims that the law and legal institutions are inherently racist and that race is a socially constructed concept used by white people to further their economic and political interests at the expense of people of colour. White is a 'virus' as we've seen. Racial inequality results from 'social, economic, and legal differences that white people create between races to maintain white interests which leads to poverty and criminality in minority communities'. I must tell that to the white guy sleeping on the street. The principal of East Side Community School in New York sent white parents a manifesto that called on

them to become 'white traitors' and advocate for full 'white abolition'. These people are teaching your kids when they urgently need a psychiatrist. The 'school' included a chart with 'eight white identities' that ranged from 'white supremacist' to 'white abolition' and defined the behaviour white people must follow to end 'the regime of whiteness'. Woke blacks and their privileged white associates are acting exactly like the slave owners of old and Ku Klux Klan racists like Robert Byrd. They are too full of their own self-purity to see that, but it's true. Racism is not a body type; it's a state of mind that can manifest through any colour, creed or culture.

Another racial fraud is *'equity'*. Not equality of treatment and opportunity – equity. It's a term spun as equality when it means something very different. Equality in its true sense is a raising up while 'equity' is a race to the bottom. Everyone in the same level of poverty is 'equity'. Keep everyone down – that's equity. The Cult doesn't want anyone in the human family to be empowered and BLM leaders, like all these 'anti-racist' organisations, continue their privileged, pampered existence by perpetuating the perception of gathering racism. When is the last time you heard an 'anti-racist' or 'anti-Semitism' organisation say that acts of racism and discrimination have *fallen?* It's not in the interests of their fund-raising and power to influence and the same goes for the professional soccer anti-racism operation, Kick It Out. Two things confirmed that the Black Lives Matter riots in the summer of 2020 were Cult creations. One was that while anti-lockdown protests were condemned in this same period for 'transmitting 'Covid' the authorities supported mass gatherings of Black Lives Matter supporters. I even saw self-deluding people claiming to be doctors say the two types of protest were not the same. No – the non-existent 'Covid' was in favour of lockdowns and attacked those that protested against them while 'Covid' supported Black Lives Matter and kept well away from its protests. The whole thing was a joke and as lockdown protestors were arrested, often brutally, by reframed Face-Nappies we had the grotesque sight of police officers taking the knee to Black Lives Matter, a Cult-funded Marxist

organisation that supports violent riots and wants to destroy the nuclear family and white people.

He's not white? Shucks!

Woke obsession with race was on display again when ten people were shot dead in Boulder, Colorado, in March, 2021. Cult-owned Woke TV channels like CNN said the shooter appeared to be a white man and Wokers were on Twitter condemning 'violent white men' with the usual mantras. Then the shooter's name was released as Ahmad Al Aliwi Alissa, an anti-Trump Arab-American, and the sigh of disappointment could be heard five miles away. Never mind that ten people were dead and what that meant for their families. Race baiting was all that mattered to these sick Cult-serving people like Barack Obama who exploited the deaths to further divide America on racial grounds which is his job for the Cult. This is the man that 'racist' white Americans made the first black president of the United States and then gave him a second term. Not-very-bright Obama has become filthy rich on the back of that and today appears to have a big influence on the Biden administration. Even so he's still a downtrodden black man and a victim of white supremacy. This disingenuous fraud reveals the contempt he has for black people when he puts on a Deep South Alabama accent whenever he talks to them, no, *at* them.

Another BLM red flag was how the now fully-Woke (fully-Cult) and fully-virtue-signalled professional soccer authorities had their teams taking the knee before every match in support of Marxist Black Lives Matter. Soccer authorities and clubs displayed 'Black Lives Matter' on the players' shirts and flashed the name on electronic billboards around the pitch. Any fans that condemned what is a Freemasonic taking-the-knee ritual were widely condemned as you would expect from the Woke virtue-signallers of professional sport and the now fully-Woke media. We have reverse racism in which you are banned from criticising any race or culture except for white people for whom anything goes – say what you like, no problem. What has this got to do with racial harmony and

equality? We've had black supremacists from Black Lives Matter telling white people to fall to their knees in the street and apologise for their white supremacy. Black supremacists acting like white supremacist slave owners of the past couldn't breach their self-obsessed, race-obsessed sense of self-purity. Joe Biden appointed a race-obsessed black supremacist Kristen Clarke to head the Justice Department Civil Rights Division. Clarke claimed that blacks are endowed with 'greater mental, physical and spiritual abilities' than whites. If anyone reversed that statement they would be vilified. Clarke is on-message so no problem. She's never seen a black-white situation in which the black figure is anything but a virtuous victim and she heads the Civil Rights Division which should treat everyone the same or it isn't civil rights. Another perception of the Renegade Mind: If something or someone is part of the Cult agenda they will be supported by Woke governments and media no matter what. If they're not, they will be condemned and censored. It really is that simple and so racist Clarke prospers despite (make that because of) her racism.

The end of culture

Biden's administration is full of such racial, cultural and economic bias as the Cult requires the human family to be divided into warring factions. We are now seeing racially-segregated graduations and everything, but everything, is defined through the lens of perceived 'racism. We have 'racist' mathematics, 'racist' food and even 'racist' *plants*. World famous Kew Gardens in London said it was changing labels on plants and flowers to tell its pre-'Covid' more than two million visitors a year how racist they are. Kew director Richard Deverell said this was part of an effort to 'move quickly to decolonise collections' after they were approached by one Ajay Chhabra 'an actor with an insight into how sugar cane was linked to slavery'. They are *plants* you idiots. 'Decolonisation' in the Woke manual really means colonisation of society with its mentality and by extension colonisation by the Cult. We are witnessing a new Chinese-style 'Cultural Revolution' so essential to the success of all

Marxist takeovers. Our cultural past and traditions have to be swept away to allow a new culture to be built-back-better. Woke targeting of long-standing Western cultural pillars including historical monuments and cancelling of historical figures is what happened in the Mao revolution in China which 'purged remnants of capitalist and traditional elements from Chinese society' and installed Maoism as the dominant ideology'. For China see the Western world today and for 'dominant ideology' see Woke. Better still see Marxism or Maoism. The 'Covid' hoax has specifically sought to destroy the arts and all elements of Western culture from people meeting in a pub or restaurant to closing theatres, music venues, sports stadiums, places of worship and even banning *singing*. Destruction of Western society is also why criticism of any religion is banned except for Christianity which again is the dominant religion as white is the numerically-dominant race. Christianity may be fading rapidly, but its history and traditions are weaved through the fabric of Western society. Delete the pillars and other structures will follow until the whole thing collapses. I am not a Christian defending that religion when I say that. I have no religion. It's just a fact. To this end Christianity has itself been turned Woke to usher its own downfall and its ranks are awash with 'change agents' – knowing and unknowing – at every level including Pope Francis (*definitely* knowing) and the clueless Archbishop of Canterbury Justin Welby (possibly not, but who can be sure?). Woke seeks to coordinate attacks on Western culture, traditions, and ways of life through 'intersectionality' defined as 'the complex, cumulative way in which the effects of multiple forms of discrimination (such as racism, sexism, and classism) combine, overlap, or intersect especially in the experiences of marginalised individuals or groups'. Wade through the Orwellian Woke-speak and this means coordinating disparate groups in a common cause to overthrow freedom and liberal values.

The entire structure of public institutions has been infested with Woke – government at all levels, political parties, police, military, schools, universities, advertising, media and trade unions. This abomination has been achieved through the Cult web by appointing

Wokers to positions of power and battering non-Wokers into line through intimidation, isolation and threats to their job. Many have been fired in the wake of the empathy-deleted, vicious hostility of 'social justice' Wokers and the desire of gutless, spineless employers to virtue-signal their Wokeness. Corporations are filled with Wokers today, most notably those in Silicon Valley. Ironically at the top they are not Woke at all. They are only exploiting the mentality their Cult masters have created and funded to censor and enslave while the Wokers cheer them on until it's their turn. Thus the Woke 'liberal left' is an inversion of the traditional liberal left. Campaigning for justice on the grounds of power and wealth distribution has been replaced by campaigning for identity politics. The genuine traditional left would never have taken money from today's billionaire abusers of fairness and justice and nor would the billionaires have wanted to fund that genuine left. It would not have been in their interests to do so. The division of opinion in those days was between the haves and have nots. This all changed with Cult manipulated and funded identity politics. The division of opinion today is between Wokers and non-Wokers and not income brackets. Cult corporations and their billionaires may have taken wealth disparity to cataclysmic levels of injustice, but as long as they speak the language of Woke, hand out the dosh to the Woke network and censor the enemy they are 'one of us'. Billionaires who don't give a damn about injustice are laughing at them till their bellies hurt. Wokers are not even close to self-aware enough to see that. The transformed 'left' dynamic means that Wokers who drone on about 'social justice' are funded by billionaires that have destroyed social justice the world over. It's *why* they are billionaires.

The climate con

Nothing encapsulates what I have said more comprehensively than the hoax of human-caused global warming. I have detailed in my books over the years how Cult operatives and organisations were the pump-primers from the start of the climate con. A purpose-built vehicle for this is the Club of Rome established by the Cult in 1968

with the Rockefellers and Rothschilds centrally involved all along. Their gofer frontman Maurice Strong, a Canadian oil millionaire, hosted the Earth Summit in Rio de Janeiro, Brazil, in 1992 where the global 'green movement' really expanded in earnest under the guiding hand of the Cult. The Earth Summit established Agenda 21 through the Cult-created-and-owned United Nations to use the illusion of human-caused climate change to justify the transformation of global society to save the world from climate disaster. It is a No-Problem-Reaction-Solution sold through governments, media, schools and universities as whole generations have been terrified into believing that the world was going to end in their lifetimes unless what old people had inflicted upon them was stopped by a complete restructuring of how everything is done. Chill, kids, it's all a hoax. Such restructuring is precisely what the Cult agenda demands (purely by coincidence of course). Today this has been given the codename of the Great Reset which is only an updated term for Agenda 21 and its associated Agenda 2030. The latter, too, is administered through the UN and was voted into being by the General Assembly in 2015. Both 21 and 2030 seek centralised control of all resources and food right down to the raindrops falling on your own land. These are some of the demands of Agenda 21 established in 1992. See if you recognise this society emerging today:

- End national sovereignty
- State planning and management of all land resources, ecosystems, deserts, forests, mountains, oceans and fresh water; agriculture; rural development; biotechnology; and ensuring *'equity'*
- The state to 'define the role' of business and financial resources
- Abolition of private property
- 'Restructuring' the family unit (see BLM)
- Children raised by the state
- People told what their job will be
- Major restrictions on movement
- Creation of 'human settlement zones'

- Mass resettlement as people are forced to vacate land where they live
- Dumbing down education
- Mass global depopulation in pursuit of all the above

The United Nations was created as a Trojan horse for world government. With the climate con of critical importance to promoting that outcome you would expect the UN to be involved. Oh, it's involved all right. The UN is promoting Agenda 21 and Agenda 2030 justified by 'climate change' while also driving the climate hoax through its Intergovernmental Panel on Climate Change (IPCC), one of the world's most corrupt organisations. The IPCC has been lying ferociously and constantly since the day it opened its doors with the global media hanging unquestioningly on its every mendacious word. The Green movement is entirely Woke and has long lost its original environmental focus since it was co-opted by the Cult. An obsession with 'global warming' has deleted its values and scrambled its head. I experienced a small example of what I mean on a beautiful country walk that I have enjoyed several times a week for many years. The path merged into the fields and forests and you felt at one with the natural world. Then a 'Green' organisation, the Hampshire and Isle of Wight Wildlife Trust, took over part of the land and proceeded to cut down a large number of trees, including mature ones, to install a horrible big, bright steel 'this-is-ours-stay-out' fence that destroyed the whole atmosphere of this beautiful place. No one with a feel for nature would do that. Day after day I walked to the sound of chainsaws and a magnificent mature weeping willow tree that I so admired was cut down at the base of the trunk. When I challenged a Woke young girl in a green shirt (of course) about this vandalism she replied: 'It's a weeping willow – it will grow back.' This is what people are paying for when they donate to the Hampshire and Isle of Wight Wildlife Trust and many other 'green' organisations today. It is not the environmental movement that I knew and instead has become a support-system – as with Extinction Rebellion – for a very dark agenda.

Private jets for climate justice

The Cult-owned, Gates-funded, World Economic Forum and its founder Klaus Schwab were behind the emergence of Greta Thunberg to harness the young behind the climate agenda and she was invited to speak to the world at … the UN. Schwab published a book, *Covid-19: The Great Reset* in 2020 in which he used the 'Covid' hoax and the climate hoax to lay out a new society straight out of Agenda 21 and Agenda 2030. Bill Gates followed in early 2021 when he took time out from destroying the world to produce a book in his name about the way to save it. Gates flies across the world in private jets and admitted that 'I probably have one of the highest greenhouse gas footprints of anyone on the planet … my personal flying alone is gigantic.' He has also bid for the planet's biggest private jet operator. Other climate change saviours who fly in private jets include John Kerry, the US Special Presidential Envoy for Climate, and actor Leonardo DiCaprio, a 'UN Messenger of Peace with special focus on climate change'. These people are so full of bullshit they could corner the market in manure. We mustn't be sceptical, though, because the Gates book, *How to Avoid a Climate Disaster: The Solutions We Have and the Breakthroughs We Need*, is a genuine attempt to protect the world and not an obvious pile of excrement attributed to a mega-psychopath aimed at selling his masters' plans for humanity. The Gates book and the other shite-pile by Klaus Schwab could have been written by the same person and may well have been. Both use 'climate change' and 'Covid' as the excuses for their new society and by coincidence the Cult's World Economic Forum and Bill and Melinda Gates Foundation promote the climate hoax and hosted Event 201 which pre-empted with a 'simulation' the very 'coronavirus' hoax that would be simulated for real on humanity within weeks. The British 'royal' family is promoting the 'Reset' as you would expect through Prince 'climate change caused the war in Syria' Charles and his hapless son Prince William who said that we must 'reset our relationship with nature and our trajectory as a species' to avoid a climate disaster. Amazing how many promotors of the 'Covid' and 'climate change' control

systems are connected to Gates and the World Economic Forum. A 'study' in early 2021 claimed that carbon dioxide emissions must fall by the equivalent of a global lockdown roughly every two years for the next decade to save the planet. The 'study' appeared in the same period that the Schwab mob claimed in a video that lockdowns destroying the lives of billions are good because they make the earth 'quieter' with less 'ambient noise'. They took down the video amid a public backlash for such arrogant, empathy-deleted stupidity You see, however, where they are going with this. Corinne Le Quéré, a professor at the Tyndall Centre for Climate Change Research, University of East Anglia, was lead author of the climate lockdown study, and she writes for … the World Economic Forum. Gates calls in 'his' book for changing 'every aspect of the economy' (long-time Cult agenda) and for humans to eat synthetic 'meat' (predicted in my books) while cows and other farm animals are eliminated. Australian TV host and commentator Alan Jones described what carbon emission targets would mean for farm animals in Australia alone if emissions were reduced as demanded by 35 percent by 2030 and zero by 2050:

Well, let's take agriculture, the total emissions from agriculture are about 75 million tonnes of carbon dioxide, equivalent. Now reduce that by 35 percent and you have to come down to 50 million tonnes, I've done the maths. So if you take for example 1.5 million cows, you're going to have to reduce the herd by 525,000 [by] 2030, nine years, that's 58,000 cows a year. The beef herd's 30 million, reduce that by 35 percent, that's 10.5 million, which means 1.2 million cattle have to go every year between now and 2030. This is insanity!

There are 75 million sheep. Reduce that by 35 percent, that's 26 million sheep, that's almost 3 million a year. So under the Paris Agreement over 30 million beasts. dairy cows, cattle, pigs and sheep would go. More than 8,000 every minute of every hour for the next decade, do these people know what they're talking about?

Clearly they don't at the level of campaigners, politicians and administrators. The Cult *does* know; that's the outcome it wants. We are faced with not just a war on humanity. Animals and the natural world are being targeted and I have been saying since the 'Covid' hoax began that the plan eventually was to claim that the 'deadly virus' is able to jump from animals, including farm animals and

domestic pets, to humans. Just before this book went into production came this story: 'Russia registers world's first Covid-19 vaccine for cats & dogs as makers of Sputnik V warn pets & farm animals could spread virus'. The report said 'top scientists warned that the deadly pathogen could soon begin spreading through homes and farms' and 'the next stage is the infection of farm and domestic animals'. Know the outcome and you'll see the journey. Think what that would mean for animals and keep your eye on a term called zoonosis or zoonotic diseases which transmit between animals and humans. The Cult wants to break the connection between animals and people as it does between people and people. Farm animals fit with the Cult agenda to transform food from natural to synthetic.

The gas of life is killing us

There can be few greater examples of Cult inversion than the condemnation of carbon dioxide as a dangerous pollutant when it is the gas of life. Without it the natural world would be dead and so we would all be dead. We breathe in oxygen and breathe out carbon dioxide while plants produce oxygen and absorb carbon dioxide. It is a perfect symbiotic relationship that the Cult wants to dismantle for reasons I will come to in the final two chapters. Gates, Schwab, other Cult operatives and mindless repeaters, want the world to be 'carbon neutral' by at least 2050 and the earlier the better. 'Zero carbon' is the cry echoed by lunatics calling for 'Zero Covid' when we already have it. These carbon emission targets will deindustrialise the world in accordance with Cult plans – the post-industrial, post-democratic society – and with so-called renewables like solar and wind not coming even close to meeting human energy needs blackouts and cold are inevitable. Texans got the picture in the winter of 2021 when a snow storm stopped wind turbines and solar panels from working and the lights went down along with water which relies on electricity for its supply system. Gates wants everything to be powered by electricity to ensure that his masters have the kill switch to stop all human activity, movement, cooking, water and warmth any time they like. The climate lie is so

stupendously inverted that it claims we must urgently reduce carbon dioxide when we *don't have enough*.

Co2 in the atmosphere is a little above 400 parts per million when the optimum for plant growth is 2,000 ppm and when it falls anywhere near 150 ppm the natural world starts to die and so do we. It fell to as low as 280 ppm in an 1880 measurement in Hawaii and rose to 413 ppm in 2019 with industrialisation which is why the planet has become *greener* in the industrial period. How insane then that psychopathic madman Gates is not satisfied only with blocking the rise of Co2. He's funding technology to suck it out of the atmosphere. The reason why will become clear. The industrial era is not destroying the world through Co2 and has instead turned around a potentially disastrous ongoing fall in Co2. Greenpeace co-founder and scientist Patrick Moore walked away from Greenpeace in 1986 and has exposed the green movement for fear-mongering and lies. He said that 500 million years ago there was *17 times* more Co2 in the atmosphere than we have today and levels have been falling for hundreds of millions of years. In the last 150 million years Co2 levels in Earth's atmosphere had reduced by *90 percent*. Moore said that by the time humanity began to unlock carbon dioxide from fossil fuels we were at '38 seconds to midnight' and in that sense: 'Humans are [the Earth's] salvation.' Moore made the point that only half the Co2 emitted by fossil fuels stays in the atmosphere and we should remember that all pollution pouring from chimneys that we are told is carbon dioxide is in fact nothing of the kind. It's pollution. Carbon dioxide is an invisible gas.

William Happer, Professor of Physics at Princeton University and long-time government adviser on climate, has emphasised the Co2 deficiency for maximum growth and food production. Greenhouse growers don't add carbon dioxide for a bit of fun. He said that most of the warming in the last 100 years, after the earth emerged from the super-cold period of the 'Little Ice Age' into a natural warming cycle, was over by 1940. Happer said that a peak year for warming in 1988 can be explained by a 'monster El Nino' which is a natural and cyclical warming of the Pacific that has nothing to do with 'climate

change'. He said the effect of Co2 could be compared to painting a wall with red paint in that once two or three coats have been applied it didn't matter how much more you slapped on because the wall will not get much redder. Almost all the effect of the rise in Co2 has already happened, he said, and the volume in the atmosphere would now have to *double* to increase temperature by a single degree. Climate hoaxers know this and they have invented the most ridiculously complicated series of 'feedback' loops to try to overcome this rather devastating fact. You hear puppet Greta going on cluelessly about feedback loops and this is why.

The Sun affects temperature? No you *climate denier*

Some other nonsense to contemplate: Climate graphs show that rises in temperature do not follow rises in Co2 – *it's the other way round* with a lag between the two of some 800 years. If we go back 800 years from present time we hit the Medieval Warm Period when temperatures were higher than now without any industrialisation and this was followed by the Little Ice Age when temperatures plummeted. The world was still emerging from these centuries of serious cold when many climate records began which makes the ever-repeated line of the 'hottest year since records began' meaningless when you are not comparing like with like. The coldest period of the Little Ice Age corresponded with the lowest period of sunspot activity when the Sun was at its least active. Proper scientists will not be at all surprised by this when it confirms the obvious fact that earth temperature is affected by the scale of Sun activity and the energetic power that it subsequently emits; but when is the last time you heard a climate hoaxer talking about the Sun as a source of earth temperature?? Everything has to be focussed on Co2 which makes up just 0.117 percent of so-called greenhouse gases and only a fraction of even that is generated by human activity. The rest is natural. More than *90 percent* of those greenhouse gases are water vapour and clouds (Fig 9). Ban moisture I say. Have you noticed that the climate hoaxers no longer use the polar bear as their promotion image? That's because far from becoming extinct polar

bear communities are stable or thriving. Joe Bastardi, American meteorologist, weather forecaster and outspoken critic of the climate lie, documents in his book *The Climate Chronicles* how weather patterns and events claimed to be evidence of climate change have been happening since long before industrialisation: 'What happened before naturally is happening again, as is to be expected given the cyclical nature of the climate due to the design of the planet.' If you read the detailed background to the climate hoax in my other books you will shake your head and wonder how anyone could believe the crap which has spawned a multi-trillion dollar industry based on absolute garbage (see HIV causes AIDs and Sars-Cov-2 causes 'Covid-19'). Climate and 'Covid' have much in common given they have the same source. They both have the contradictory *everything* factor in which everything is explained by reference to them. It's hot – 'it's climate change'. It's cold – 'it's climate change'. I got a sniffle – 'it's Covid'. I haven't got a sniffle – 'it's Covid'. Not having a sniffle has to be a symptom of 'Covid'. Everything is and not having a sniffle is especially dangerous if you are a slow walker. For sheer audacity I offer you a Cambridge University 'study' that actually linked 'Covid' to 'climate change'. It had to happen eventually. They concluded that climate change played a role in 'Covid-19' spreading from animals to humans because … wait for it … I kid you not … *the two groups were forced closer together as populations grow*. Er, that's it. The whole foundation on which this depended was that 'Bats are the likely zoonotic origin of SARS-CoV-1 and SARS-CoV-2'. Well, they are not. They are nothing to do with it. Apart from bats not being the origin and therefore 'climate change' effects on bats being irrelevant I am in awe of their academic insight. Where would we be without them? Not where we are that's for sure.

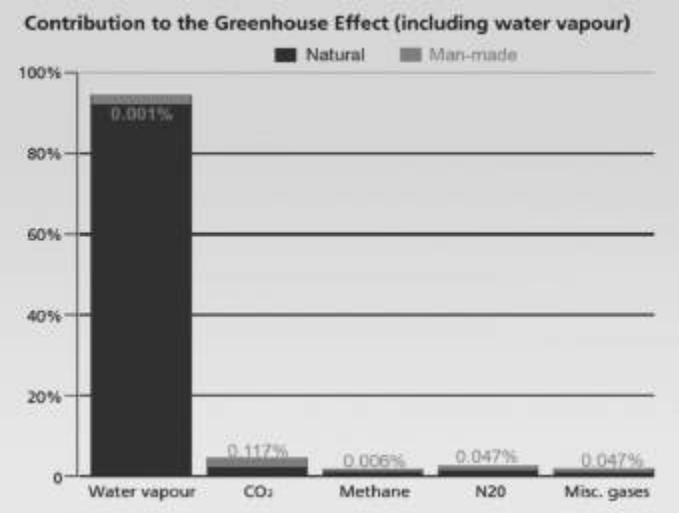

Figure 9: The idea that the gas of life is disastrously changing the climate is an insult to brain cell activity.

One other point about the weather is that climate modification is now well advanced and not every major weather event is natural – or earthquake come to that. I cover this subject at some length in other books. China is openly planning a rapid expansion of its weather modification programme which includes changing the climate in an area more than one and a half times the size of India. China used weather manipulation to ensure clear skies during the 2008 Olympics in Beijing. I have quoted from US military documents detailing how to employ weather manipulation as a weapon of war and they did that in the 1960s and 70s during the conflict in Vietnam with Operation Popeye manipulating monsoon rains for military purposes. Why would there be international treaties on weather modification if it wasn't possible? Of course it is. Weather is energetic information and it can be changed.

How was the climate hoax pulled off? See 'Covid'

If you can get billions to believe in a 'virus' that doesn't exist you can get them to believe in human-caused climate change that doesn't exist. Both are being used by the Cult to transform global society in the way it has long planned. Both hoaxes have been achieved in pretty much the same way. First you declare a lie is a fact. There's a

'virus' you call SARS-Cov-2 or humans are warming the planet with their behaviour. Next this becomes, via Cult networks, the foundation of government, academic and science policy and belief. Those who parrot the mantra are given big grants to produce research that confirms the narrative is true and ever more 'symptoms' are added to make the 'virus'/'climate change' sound even more scary. Scientists and researchers who challenge the narrative have their grants withdrawn and their careers destroyed. The media promote the lie as the unquestionable truth and censor those with an alternative view or evidence. A great percentage of the population believe what they are told as the lie becomes an everybody-knows-that and the believing-masses turn on those with a mind of their own. The technique has been used endlessly throughout human history. Wokers are the biggest promotors of the climate lie *and* 'Covid' fascism because their minds are owned by the Cult; their sense of self-righteous self-purity knows no bounds; and they exist in a bubble of reality in which facts are irrelevant and only get in the way of looking without seeing.

Running through all of this like veins in a blue cheese is control of information, which means control of perception, which means control of behaviour, which collectively means control of human society. The Cult owns the global media and Silicon Valley fascists for the simple reason that it *has* to. Without control of information it can't control perception and through that human society. Examine every facet of the Cult agenda and you will see that anything supporting its introduction is never censored while anything pushing back is always censored. I say again: Psychopaths that know why they are doing this must go before Nuremberg trials and those that follow their orders must trot along behind them into the same dock. 'I was just following orders' didn't work the first time and it must not work now. Nuremberg trials must be held all over the world before public juries for politicians, government officials, police, compliant doctors, scientists and virologists, and all Cult operatives such as Gates, Tedros, Fauci, Vallance, Whitty, Ferguson, Zuckerberg, Wojcicki, Brin, Page, Dorsey, the whole damn lot of

them – including, no *especially*, the psychopath psychologists. Without them and the brainless, gutless excuses for journalists that have repeated their lies, none of this could be happening. Nobody can be allowed to escape justice for the psychological and economic Armageddon they are all responsible for visiting upon the human race.

As for the compliant, unquestioning, swathes of humanity, and the self-obsessed, all-knowing ignorance of the Wokers … don't start me. God help their kids. God help their grandkids. God *help them*.

CHAPTER NINE

We must have it? So what is it?

Well I won't back down. No, I won't back down. You can stand me up at the Gates of Hell. But I won't back down
Tom Petty

I will now focus on the genetically-manipulating 'Covid vaccines' which do not meet this official definition of a vaccine by the US Centers for Disease Control (CDC): 'A product that stimulates a person's immune system to produce immunity to a specific disease, protecting the person from that disease.' On that basis 'Covid vaccines' are not a vaccine in that the makers don't even claim they stop infection or transmission.

They are instead part of a multi-levelled conspiracy to change the nature of the human body and what it means to be 'human' and to depopulate an enormous swathe of humanity. What I shall call Human 1.0 is on the cusp of becoming Human 2.0 and for very sinister reasons. Before I get to the 'Covid vaccine' in detail here's some background to vaccines in general. Government regulators do not test vaccines – the makers do – and the makers control which data is revealed and which isn't. Children in America are given 50 vaccine doses by age six and 69 by age 19 and the effect of the whole combined schedule has never been tested. Autoimmune diseases when the immune system attacks its own body have soared in the mass vaccine era and so has disease in general in children and the young. Why wouldn't this be the case when vaccines target the *immune system?* The US government gave Big Pharma drug

companies immunity from prosecution for vaccine death and injury in the 1986 National Childhood Vaccine Injury Act (NCVIA) and since then the government (taxpayer) has been funding compensation for the consequences of Big Pharma vaccines. The criminal and satanic drug giants can't lose and the vaccine schedule has increased dramatically since 1986 for this reason. There is no incentive to make vaccines safe and a big incentive to make money by introducing ever more. Even against a ridiculously high bar to prove vaccine liability, and with the government controlling the hearing in which it is being challenged for compensation, the vaccine court has so far paid out more than $4 billion. These are the vaccines we are told are safe and psychopaths like Zuckerberg censor posts saying otherwise. The immunity law was even justified by a ruling that vaccines by their nature were 'unavoidably unsafe'.

Check out the ingredients of vaccines and you will be shocked if you are new to this. *They put that in children's bodies?? What??* Try aluminium, a brain toxin connected to dementia, aborted foetal tissue and formaldehyde which is used to embalm corpses. World-renowned aluminium expert Christopher Exley had his research into the health effect of aluminium in vaccines shut down by Keele University in the UK when it began taking funding from the Bill and Melinda Gates Foundation. Research when diseases 'eradicated' by vaccines began to decline and you will find the fall began long *before* the vaccine was introduced. Sometimes the fall even plateaued after the vaccine. Diseases like scarlet fever for which there was no vaccine declined in the same way because of environmental and other factors. A perfect case in point is the polio vaccine. Polio began when lead arsenate was first sprayed as an insecticide and residues remained in food products. Spraying started in 1892 and the first US polio epidemic came in Vermont in 1894. The simple answer was to stop spraying, but Rockefeller-created Big Pharma had a better idea. Polio was decreed to be caused by the *poliovirus* which 'spreads from person to person and can infect a person's spinal cord'. Lead arsenate was replaced by the lethal DDT which had the same effect of causing paralysis by damaging the brain and central nervous

system. Polio plummeted when DDT was reduced and then banned, but the vaccine is still given the credit for something it didn't do. Today by far the biggest cause of polio is the vaccines promoted by Bill Gates. Vaccine justice campaigner Robert Kennedy Jr, son of assassinated (by the Cult) US Attorney General Robert Kennedy, wrote:

> In 2017, the World Health Organization (WHO) reluctantly admitted that the global explosion in polio is predominantly vaccine strain. The most frightening epidemics in Congo, Afghanistan, and the Philippines, are all linked to vaccines. In fact, by 2018, 70% of global polio cases were vaccine strain.

Vaccines make fortunes for Cult-owned Gates and Big Pharma while undermining the health and immune systems of the population. We had a glimpse of the mentality behind the Big Pharma cartel with a report on WION (World is One News), an international English language TV station based in India, which exposed the extraordinary behaviour of US drug company Pfizer over its 'Covid vaccine'. The WION report told how Pfizer had made fantastic demands of Argentina, Brazil and other countries in return for its 'vaccine'. These included immunity from prosecution, even for Pfizer negligence, government insurance to protect Pfizer from law suits and handing over as collateral sovereign assets of the country to include Argentina's bank reserves, military bases and embassy buildings. Pfizer demanded the same of Brazil in the form of waiving sovereignty of its assets abroad; exempting Pfizer from Brazilian laws; and giving Pfizer immunity from all civil liability. This is a 'vaccine' developed with government funding. Big Pharma is evil incarnate as a creation of the Cult and all must be handed tickets to Nuremberg.

Phantom 'vaccine' for a phantom 'disease'

I'll expose the 'Covid vaccine' fraud and then go on to the wider background of why the Cult has set out to 'vaccinate' every man, woman and child on the planet for an alleged 'new disease' with a survival rate of 99.77 percent (or more) even by the grotesquely-

manipulated figures of the World Health Organization and Johns Hopkins University. The 'infection' to 'death' ratio is 0.23 to 0.15 percent according to Stanford epidemiologist Dr John Ioannidis and while estimates vary the danger remains tiny. I say that if the truth be told the fake infection to fake death ratio is zero. Never mind all the evidence I have presented here and in *The Answer* that there is no 'virus' let us just focus for a moment on that death-rate figure of say 0.23 percent. The figure includes all those worldwide who have tested positive with a test not testing for the 'virus' and then died within 28 days or even longer of any other cause – *any other cause*. Now subtract all those illusory 'Covid' deaths on the global data sheets from the 0.23 percent. What do you think you would be left with? *Zero*. A vaccination has never been successfully developed for a so-called coronavirus. They have all failed at the animal testing stage when they caused hypersensitivity to what they were claiming to protect against and made the impact of a disease far worse. Cult-owned vaccine corporations got around that problem this time by bypassing animal trials, going straight to humans and making the length of the 'trials' before the public rollout as short as they could get away with. Normally it takes five to ten years or more to develop vaccines that still cause demonstrable harm to many people and that's without including the long-term effects that are never officially connected to the vaccination. 'Covid' non-vaccines have been officially produced and approved in a matter of months from a standing start and part of the reason is that (a) they were developed before the 'Covid' hoax began and (b) they are based on computer programs and not natural sources. Official non-trials were so short that government agencies gave *emergency*, not full, approval. 'Trials' were not even completed and full approval cannot be secured until they are. Public 'Covid vaccination' is actually a *continuation of the trial*. Drug company 'trials' are not scheduled to end until 2023 by which time a lot of people are going to be dead. Data on which government agencies gave this emergency approval was supplied by the Big Pharma corporations themselves in the form of Pfizer/BioNTech, AstraZeneca, Moderna, Johnson & Johnson, and

others, and this is the case with all vaccines. By its very nature *emergency* approval means drug companies do not have to prove that the 'vaccine' is 'safe and effective'. How could they with trials way short of complete? Government regulators only have to *believe* that they *could* be safe and effective. It is criminal manipulation to get products in circulation with no testing worth the name. Agencies giving that approval are infested with Big Pharma-connected place-people and they act in the interests of Big Pharma (the Cult) and not the public about whom they do not give a damn.

More human lab rats

'Covid vaccines' produced in record time by Pfizer/BioNTech and Moderna employ a technique *never approved before for use on humans*. They are known as mRNA 'vaccines' and inject a synthetic version of 'viral' mRNA or 'messenger RNA'. The key is in the term 'messenger'. The body works, or doesn't, on the basis of information messaging. Communications are constantly passing between and within the genetic system and the brain. Change those messages and you change the state of the body and even its very nature and you can change psychology and behaviour by the way the brain processes information. I think you are going to see significant changes in personality and perception of many people who have had the 'Covid vaccine' synthetic potions. Insider Aldous Huxley predicted the following in 1961 and mRNA 'vaccines' can be included in the term 'pharmacological methods':

> There will be, in the next generation or so, a pharmacological method of making people love their servitude, and producing dictatorship without tears, so to speak, producing a kind of painless concentration camp for entire societies, so that people will in fact have their own liberties taken away from them, but rather enjoy it, because they will be distracted from any desire to rebel by propaganda or brainwashing, or brainwashing enhanced by pharmacological methods. And this seems to be the final revolution.

Apologists claim that mRNA synthetic 'vaccines' don't change the DNA genetic blueprint because RNA does not affect DNA only the other way round. This is so disingenuous. A process called 'reverse

transcription' can convert RNA into DNA and be integrated into DNA in the cell nucleus. This was highlighted in December, 2020, by scientists at Harvard and Massachusetts Institute of Technology (MIT). Geneticists report that more than 40 percent of mammalian genomes results from reverse transcription. On the most basic level if messaging changes then that sequence must lead to changes in DNA which is receiving and transmitting those communications. How can introducing synthetic material into cells not change the cells where DNA is located? The process is known as transfection which is defined as 'a technique to insert foreign nucleic acid (DNA or RNA) into a cell, typically with the intention of altering the properties of the cell'. Researchers at the Sloan Kettering Institute in New York found that changes in messenger RNA can deactivate tumour-suppressing proteins and thereby promote cancer. This is what happens when you mess with messaging. 'Covid vaccine' maker Moderna was founded in 2010 by Canadian stem cell biologist Derrick J. Rossi after his breakthrough discovery in the field of transforming and reprogramming stem cells. These are neutral cells that can be programmed to become any cell including sperm cells. Moderna was therefore founded on the principle of genetic manipulation and has never produced any vaccine or drug before its genetically-manipulating synthetic 'Covid' shite. Look at the name – Mode-RNA or Modify-RNA. Another important point is that the US Supreme Court has ruled that genetically-modified DNA, or complementary DNA (cDNA) synthesized in the laboratory from messenger RNA, can be patented and owned. These psychopaths are doing this to the human body.

Cells replicate synthetic mRNA in the 'Covid vaccines' and in theory the body is tricked into making antigens which trigger antibodies to target the 'virus spike proteins' which as Dr Tom Cowan said have *never been seen*. Cut the crap and these 'vaccines' deliver *self-replicating* synthetic material to the cells with the effect of changing human DNA. The more of them you have the more that process is compounded while synthetic material is all the time self-replicating. 'Vaccine'-maker Moderna describes mRNA as 'like

software for the cell' and so they are messing with the body's software. What happens when you change the software in a computer? Everything changes. For this reason the Cult is preparing a production line of mRNA 'Covid vaccines' and a long list of excuses to use them as with all the 'variants' of a 'virus' never shown to exist. The plan is further to transfer the mRNA technique to other vaccines mostly given to children and young people. The cumulative consequences will be a transformation of human DNA through a constant infusion of synthetic genetic material which will kill many and change the rest. Now consider that governments that have given emergency approval for a vaccine that's not a vaccine; never been approved for humans before; had no testing worth the name; and the makers have been given immunity from prosecution for any deaths or adverse effects suffered by the public. The UK government awarded *permanent legal indemnity* to itself and its employees for harm done when a patient is being treated for 'Covid-19' or 'suspected Covid-19'. That is quite a thought when these are possible 'side-effects' from the 'vaccine' (they are not 'side', they are effects) listed by the US Food and Drug Administration:

Guillain-Barre syndrome; acute disseminated encephalomyelitis; transverse myelitis; encephalitis; myelitis; encephalomyelitis; meningoencephalitis; meningitis; encephalopathy; convulsions; seizures; stroke; narcolepsy; cataplexy; anaphylaxis; acute myocardial infarction (heart attack); myocarditis; pericarditis; autoimmune disease; death; implications for pregnancy, and birth outcomes; other acute demyelinating diseases; non anaphylactic allergy reactions; thrombocytopenia ; disseminated intravascular coagulation; venous thromboembolism; arthritis; arthralgia; joint pain; Kawasaki disease; multisystem inflammatory syndrome in children; vaccine enhanced disease. The latter is the way the 'vaccine' has the potential to make diseases far worse than they would otherwise be.

UK doctor and freedom campaigner Vernon Coleman described the conditions in this list as 'all unpleasant, most of them very serious, and you can't get more serious than death'. The thought that anyone at all has had the 'vaccine' in these circumstances is testament to the potential that humanity has for clueless, unquestioning, stupidity and for many that programmed stupidity has already been terminal.

An insider speaks

Dr Michael Yeadon is a former Vice President, head of research and Chief Scientific Adviser at vaccine giant Pfizer. Yeadon worked on the inside of Big Pharma, but that did not stop him becoming a vocal critic of 'Covid vaccines' and their potential for multiple harms, including infertility in women. By the spring of 2021 he went much further and even used the no, no, term 'conspiracy'. When you begin to see what is going on it is impossible not to do so. Yeadon spoke out in an interview with freedom campaigner James Delingpole and I mentioned earlier how he said that no one had samples of 'the virus'. He explained that the mRNA technique originated in the anti-cancer field and ways to turn on and off certain genes which could be advantageous if you wanted to stop cancer growing out of control. 'That's the origin of them. They are a very unusual application, really.' Yeadon said that treating a cancer patient with an aggressive procedure might be understandable if the alternative was dying, but it was quite another thing to use the same technique as a public health measure. Most people involved wouldn't catch the infectious agent you were vaccinating against and if they did they probably wouldn't die:

> If you are really using it as a public health measure you really want to as close as you can get to zero sides-effects … I find it odd that they chose techniques that were really cutting their teeth in the field of oncology and I'm worried that in using gene-based vaccines that have to be injected in the body and spread around the body, get taken up into some cells, and the regulators haven't quite told us which cells they get taken up into … you are going to be generating a wide range of responses … with multiple steps each of which could go well or badly.

I doubt the Cult intends it to go well. Yeadon said that you can put any gene you like into the body through the 'vaccine'. 'You can certainly give them a gene that would do them some harm if you wanted.' I was intrigued when he said that when used in the cancer field the technique could turn genes on and off. I explore this process in *The Answer* and with different genes having different functions you could create mayhem – physically and psychologically – if you turned the wrong ones on and the right ones off. I read reports of an experiment by researchers at the University of Washington's school of computer science and engineering in which they encoded DNA to infect computers. The body is itself a biological computer and if human DNA can inflict damage on a computer why can't the computer via synthetic material mess with the human body? It can. The Washington research team said it was possible to insert malicious malware into 'physical DNA strands' and corrupt the computer system of a gene sequencing machine as it 'reads gene letters and stores them as binary digits 0 and 1'. They concluded that hackers could one day use blood or spit samples to access computer systems and obtain sensitive data from police forensics labs or infect genome files. It is at this level of digital interaction that synthetic 'vaccines' need to be seen to get the full picture and that will become very clear later on. Michael Yeadon said it made no sense to give the 'vaccine' to younger people who were in no danger from the 'virus'. What was the benefit? It was all downside with potential effects:

> The fact that my government in what I thought was a civilised, rational country, is raining [the 'vaccine'] on people in their 30s and 40s, even my children in their 20s, they're getting letters and phone calls, I know this is not right and any of you doctors who are vaccinating you know it's not right, too. They are not at risk. They are not at risk from the disease, so you are now hoping that the side-effects are so rare that you get away with it. You don't give new technology … that you don't understand to 100 percent of the population.

Blood clot problems with the AstraZeneca 'vaccine' have been affecting younger people to emphasise the downside risks with no benefit. AstraZeneca's version, produced with Oxford University, does not use mRNA, but still gets its toxic cocktail inside cells where

it targets DNA. The Johnson & Johnson 'vaccine' which uses a similar technique has also produced blood clot effects to such an extent that the United States paused its use at one point. They are all 'gene therapy' (cell modification) procedures and not 'vaccines'. The truth is that once the content of these injections enter cells we have no idea what the effect will be. People can speculate and some can give very educated opinions and that's good. In the end, though, only the makers know what their potions are designed to do and even they won't know every last consequence. Michael Yeadon was scathing about doctors doing what they knew to be wrong. 'Everyone's mute', he said. Doctors in the NHS must know this was not right, coming into work and injecting people. 'I don't know how they sleep at night. I know I couldn't do it. I know that if I were in that position I'd have to quit.' He said he knew enough about toxicology to know this was not a good risk-benefit. Yeadon had spoken to seven or eight university professors and all except two would not speak out publicly. Their universities had a policy that no one said anything that countered the government and its medical advisors. They were afraid of losing their government grants. This is how intimidation has been used to silence the truth at every level of the system. I say silence, but these people could still speak out if they made that choice. Yeadon called them 'moral cowards' – 'This is about your children and grandchildren's lives and you have just buggered off and left it.'

'Variant' nonsense

Some of his most powerful comments related to the alleged 'variants' being used to instil more fear, justify more lockdowns, and introduce more 'vaccines'. He said government claims about 'variants' were nonsense. He had checked the alleged variant 'codes' and they were 99.7 percent identical to the 'original'. This was the human identity difference equivalent to putting a baseball cap on and off or wearing it the other way round. A 0.3 percent difference would make it impossible for that 'variant' to escape immunity from the 'original'. This made no sense of having new 'vaccines' for

'variants'. He said there would have to be at least a *30 percent* difference for that to be justified and even then he believed the immune system would still recognise what it was. Gates-funded 'variant modeller' and 'vaccine'-pusher John Edmunds might care to comment. Yeadon said drug companies were making new versions of the 'vaccine' as a 'top up' for 'variants'. Worse than that, he said, the 'regulators' around the world like the MHRA in the UK had got together and agreed that because 'vaccines' for 'variants' were so similar to the first 'vaccines' *they did not have to do safety studies*. How transparently sinister that is. This is when Yeadon said: 'There is a conspiracy here.' There was no need for another vaccine for 'variants' and yet we were told that there was and the country had shut its borders because of them. 'They are going into hundreds of millions of arms without passing 'go' or any regulator. Why did they do that? Why did they pick this method of making the vaccine?'

The reason had to be something bigger than that it seemed and 'it's not protection against the virus'. It's was a far bigger project that meant politicians and advisers were willing to do things and not do things that knowingly resulted in avoidable deaths – 'that's already happened when you think about lockdown and deprivation of health care for a year.' He spoke of people prepared to do something that results in the avoidable death of their fellow human beings and it not bother them. This is the penny-drop I have been working to get across for more than 30 years – the level of pure evil we are dealing with. Yeadon said his friends and associates could not believe there could be that much evil, but he reminded them of Stalin, Pol Pot and Hitler and of what Stalin had said: 'One death is a tragedy. A million? A statistic.' He could not think of a benign explanation for why you need top-up vaccines 'which I'm sure you don't' and for the regulators 'to just get out of the way and wave them through'. Why would the regulators do that when they were still wrestling with the dangers of the 'parent' vaccine? He was clearly shocked by what he had seen since the 'Covid' hoax began and now he was thinking the previously unthinkable:

If you wanted to depopulate a significant proportion of the world and to do it in a way that doesn't involve destruction of the environment with nuclear weapons, poisoning everyone with anthrax or something like that, and you wanted plausible deniability while you had a multi-year infectious disease crisis, I actually don't think you could come up with a better plan of work than seems to be in front of me. I can't say that's what they are going to do, but I can't think of a benign explanation why they are doing it.

He said he never thought that they would get rid of 99 percent of humans, but now he wondered. 'If you wanted to that this would be a hell of a way to do it – it would be unstoppable folks.' Yeadon had concluded that those who submitted to the 'vaccine' would be allowed to have some kind of normal life (but for how long?) while screws were tightened to coerce and mandate the last few percent. 'I think they'll put the rest of them in a prison camp. I wish I was wrong, but I don't think I am.' Other points he made included: There were no coronavirus vaccines then suddenly they all come along at the same time; we have no idea of the long term affect with trials so short; coercing or forcing people to have medical procedures is against the Nuremberg Code instigated when the Nazis did just that; people should at least delay having the 'vaccine'; a quick Internet search confirms that masks don't reduce respiratory viral transmission and 'the government knows that'; they have smashed civil society and they know that, too; two dozen peer-reviewed studies show no connection between lockdown and reducing deaths; he knew from personal friends the elite were still flying around and going on holiday while the public were locked down; the elite were not having the 'vaccines'. He was also asked if 'vaccines' could be made to target difference races. He said he didn't know, but the document by the Project for the New American Century in September, 2000, said developing 'advanced forms of biological warfare that can target *specific genotypes* may transform biological warfare from the realm of terror to a politically useful tool.' Oh, they're evil all right. Of that we can be *absolutely* sure.

Another cull of old people

We have seen from the CDC definition that the mRNA 'Covid vaccine' is not a vaccine and nor are the others that *claim* to reduce 'severity of symptoms' in *some* people, but not protect from infection or transmission. What about all the lies about returning to 'normal' if people were 'vaccinated'? If they are not claimed to stop infection and transmission of the alleged 'virus', how does anything change? This was all lies to manipulate people to take the jabs and we are seeing that now with masks and distancing still required for the 'vaccinated'. How did they think that elderly people with fragile health and immune responses were going to be affected by infusing their cells with synthetic material and other toxic substances? They *knew* that in the short and long term it would be devastating and fatal as the culling of the old that began with the first lockdowns was continued with the 'vaccine'. Death rates in care homes soared immediately residents began to be 'vaccinated' – infused with synthetic material. Brave and committed whistleblower nurses put their careers at risk by exposing this truth while the rest kept their heads down and their mouths shut to put their careers before those they are supposed to care for. A long-time American Certified Nursing Assistant who gave his name as James posted a video in which he described emotionally what happened in his care home when vaccination began. He said that during 2020 very few residents were sick with 'Covid' and no one died during the entire year; but shortly after the Pfizer mRNA injections 14 people died within two weeks and many others were near death. 'They're dropping like flies', he said. Residents who walked on their own before the shot could no longer and they had lost their ability to conduct an intelligent conversation. The home's management said the sudden deaths were caused by a 'super-spreader' of 'Covid-19'. Then how come, James asked, that residents who refused to take the injections were not sick? It was a case of inject the elderly with mRNA synthetic potions and blame their illness and death that followed on the 'virus'. James described what was happening in care homes as 'the greatest crime of genocide this country has ever seen'. Remember the NHS staff nurse from earlier who used the same

word ‘genocide’ for what was happening with the ‘vaccines’ and that it was an ‘act of human annihilation’. A UK care home whistleblower told a similar story to James about the effect of the ‘vaccine’ in deaths and ‘outbreaks’ of illness dubbed ‘Covid’ after getting the jab. She told how her care home management and staff had zealously imposed government regulations and no one was allowed to even question the official narrative let alone speak out against it. She said the NHS was even worse. Again we see the results of reframing. A worker at a local care home where I live said they had not had a single case of ‘Covid’ there for almost a year and when the residents were ‘vaccinated’ they had 19 positive cases in two weeks with eight dying.

It’s not the ‘vaccine’ – honest

The obvious cause and effect was being ignored by the media and most of the public. Australia’s health minister Greg Hunt (a former head of strategy at the World Economic Forum) was admitted to hospital after he had the ‘vaccine’. He was suffering according to reports from the skin infection ‘cellulitis’ and it must have been a severe case to have warranted days in hospital. Immediately the authorities said this was nothing to do with the ‘vaccine’ when an effect of some vaccines is a ‘cellulitis-like reaction’. We had families of perfectly healthy old people who died after the ‘vaccine’ saying that if only they had been given the ‘vaccine’ earlier they would still be alive. As a numbskull rating that is off the chart. A father of four ‘died of Covid’ at aged 48 when he was taken ill two days after having the ‘vaccine’. The man, a health administrator, had been ‘shielding during the pandemic’ and had ‘not really left the house’ until he went for the ‘vaccine’. Having the ‘vaccine’ and then falling ill and dying does not seem to have qualified as a possible cause and effect and ‘Covid-19’ went on his death certificate. His family said they had no idea how he ‘caught the virus’. A family member said: ‘Tragically, it could be that going for a vaccination ultimately led to him catching Covid …The sad truth is that they are never going to know where it came from.’ The family warned people to remember

that the virus still existed and was 'very real'. So was their stupidity. Nurses and doctors who had the first round of the 'vaccine' were collapsing, dying and ending up in a hospital bed while they or their grieving relatives were saying they'd still have the 'vaccine' again despite what happened. I kid you not. You mean if your husband returned from the dead he'd have the same 'vaccine' again that killed him??

Doctors at the VCU Medical Center in Richmond, Virginia, said the Johnson & Johnson 'vaccine' was to blame for a man's skin peeling off. Patient Richard Terrell said: 'It all just happened so fast. My skin peeled off. It's still coming off on my hands now.' He said it was stinging, burning and itching and when he bent his arms and legs it was very painful with 'the skin swollen and rubbing against itself'. Pfizer/BioNTech and Moderna vaccines use mRNA to change the cell while the Johnson & Johnson version uses DNA in a process similar to AstraZeneca's technique. Johnson & Johnson and AstraZeneca have both had their 'vaccines' paused by many countries after causing serious blood problems. Terrell's doctor Fnu Nutan said he could have died if he hadn't got medical attention. It sounds terrible so what did Nutan and Terrell say about the 'vaccine' now? Oh, they still recommend that people have it. A nurse in a hospital bed 40 minutes after the vaccination and unable to swallow due to throat swelling was told by a doctor that he lost mobility in his arm for 36 hours following the vaccination. What did he say to the ailing nurse? 'Good for you for getting the vaccination.' We are dealing with a serious form of cognitive dissonance madness in both public and medical staff. There is a remarkable correlation between those having the 'vaccine' and trumpeting the fact and suffering bad happenings shortly afterwards. Witold Rogiewicz, a Polish doctor, made a video of his 'vaccination' and ridiculed those who were questioning its safety and the intentions of Bill Gates: 'Vaccinate yourself to protect yourself, your loved ones, friends and also patients. And to mention quickly I have info for anti-vaxxers and anti-Coviders if you want to contact Bill Gates you can do this through me.' He further ridiculed the dangers of 5G. Days later he

was dead, but naturally the vaccination wasn't mentioned in the verdict of 'heart attack'.

Lies, lies and more lies

So many members of the human race have slipped into extreme states of insanity and unfortunately they include reframed doctors and nursing staff. Having a 'vaccine' and dying within minutes or hours is not considered a valid connection while death from any cause within 28 days or longer of a positive test with a test not testing for the 'virus' means 'Covid-19' goes on the death certificate. How could that 'vaccine'-death connection not have been made except by calculated deceit? US figures in the initial rollout period to February 12th, 2020, revealed that a third of the deaths reported to the CDC after 'Covid vaccines' happened within 48 hours. Five men in the UK suffered an 'extremely rare' blood clot problem after having the AstraZeneca 'vaccine', but no causal link was established said the Gates-funded Medicines and Healthcare products Regulatory Agency (MHRA) which had given the 'vaccine' emergency approval to be used. Former Pfizer executive Dr Michael Yeadon explained in his interview how the procedures could cause blood coagulation and clots. People who should have been at no risk were dying from blood clots in the brain and he said he had heard from medical doctor friends that people were suffering from skin bleeding and massive headaches. The AstraZeneca 'shot' was stopped by some 20 countries over the blood clotting issue and still the corrupt MHRA, the European Medicines Agency (EMA) and the World Health Organization said that it should continue to be given even though the EMA admitted that it 'still cannot rule out definitively' a link between blood clotting and the 'vaccine'. Later Marco Cavaleri, head of EMA vaccine strategy, said there was indeed a clear link between the 'vaccine' and thrombosis, but they didn't know why. So much for the trials showing the 'vaccine' is safe. Blood clots were affecting younger people who would be under virtually no danger from 'Covid' even if it existed which makes it all the more stupid and sinister.

The British government responded to public alarm by wheeling out June Raine, the terrifyingly weak infant school headmistress sound-alike who heads the UK MHRA drug 'regulator'. The idea that she would stand up to Big Pharma and government pressure is laughable and she told us that all was well in the same way that she did when allowing untested, never-used-on-humans-before, genetically-manipulating 'vaccines' to be exposed to the public in the first place. Mass lying is the new normal of the 'Covid' era. The MHRA later said 30 cases of rare blood clots had by then been connected with the AstraZeneca 'vaccine' (that means a lot more in reality) while stressing that the benefits of the jab in preventing 'Covid-19' outweighed any risks. A more ridiculous and disingenuous statement with callous disregard for human health it is hard to contemplate. Immediately after the mendacious 'all-clears' two hospital workers in Denmark experienced blood clots and cerebral haemorrhaging following the AstraZeneca jab and one died. Top Norwegian health official Pål Andre Holme said the 'vaccine' was the only common factor: 'There is nothing in the patient history of these individuals that can give such a powerful immune response … I am confident that the antibodies that we have found are the cause, and I see no other explanation than it being the vaccine which triggers it.' Strokes, a clot or bleed in the brain, were clearly associated with the 'vaccine' from word of mouth and whistleblower reports. Similar consequences followed with all these 'vaccines' that we were told were so safe and as the numbers grew by the day it was clear we were witnessing human carnage.

Learning the hard way

A woman interviewed by UKColumn told how her husband suffered dramatic health effects after the vaccine when he'd been in good health all his life. He went from being a little unwell to losing all feeling in his legs and experiencing 'excruciating pain'. Misdiagnosis followed twice at Accident and Emergency (an 'allergy' and 'sciatica') before he was admitted to a neurology ward where doctors said his serious condition had been caused by the

'vaccine'. Another seven 'vaccinated' people were apparently being treated on the same ward for similar symptoms. The woman said he had the 'vaccine' because they believed media claims that it was safe. 'I didn't think the government would give out a vaccine that does this to somebody; I believed they would be bringing out a vaccination that would be safe.' What a tragic way to learn that lesson. Another woman posted that her husband was transporting stroke patients to hospital on almost every shift and when he asked them if they had been 'vaccinated' for 'Covid' they all replied 'yes'. One had a 'massive brain bleed' the day after his second dose. She said her husband reported the 'just been vaccinated' information every time to doctors in A and E only for them to ignore it, make no notes and appear annoyed that it was even mentioned. This particular report cannot be verified, but it expresses a common theme that confirms the monumental underreporting of 'vaccine' consequences. Interestingly as the 'vaccines' and their brain blood clot/stroke consequences began to emerge the UK National Health Service began a publicity campaign telling the public what to do in the event of a stroke. A Scottish NHS staff nurse who quit in disgust in March, 2021, said:

I have seen traumatic injuries from the vaccine, they're not getting reported to the yellow card [adverse reaction] scheme, they're treating the symptoms, not asking why, why it's happening. It's just treating the symptoms and when you speak about it you're dismissed like you're crazy, I'm not crazy, I'm not crazy because every other colleague I've spoken to is terrified to speak out, they've had enough.

Videos appeared on the Internet of people uncontrollably shaking after the 'vaccine' with no control over muscles, limbs and even their face. A Scottish mother broke out in a severe rash all over her body almost immediately after she was given the AstraZeneca 'vaccine'. The pictures were horrific. Leigh King, a 41-year-old hairdresser from Lanarkshire said: 'Never in my life was I prepared for what I was about to experience … My skin was so sore and constantly hot … I have never felt pain like this …' But don't you worry, the 'vaccine' is perfectly safe. Then there has been the effect on medical

staff who have been pressured to have the 'vaccine' by psychopathic 'health' authorities and government. A London hospital consultant who gave the name K. Polyakova wrote this to the *British Medical Journal* or *BMJ*:

> I am currently struggling with … the failure to report the reality of the morbidity caused by our current vaccination program within the health service and staff population. The levels of sickness after vaccination is unprecedented and staff are getting very sick and some with neurological symptoms which is having a huge impact on the health service function. Even the young and healthy are off for days, some for weeks, and some requiring medical treatment. Whole teams are being taken out as they went to get vaccinated together.
>
> Mandatory vaccination in this instance is stupid, unethical and irresponsible when it comes to protecting our staff and public health. We are in the voluntary phase of vaccination, and encouraging staff to take an unlicensed product that is impacting on their immediate health … it is clearly stated that these vaccine products do not offer immunity or stop transmission. In which case why are we doing it?

Not to protect health that's for sure. Medical workers are lauded by governments for agenda reasons when they couldn't give a toss about them any more than they can for the population in general. Schools across America faced the same situation as they closed due to the high number of teachers and other staff with bad reactions to the Pfizer/BioNTech, Moderna, and Johnson & Johnson 'Covid vaccines' all of which were linked to death and serious adverse effects. The *BMJ* took down the consultant's comments pretty quickly on the grounds that they were being used to spread 'disinformation'. They were exposing the truth about the 'vaccine' was the real reason. The cover-up is breathtaking.

Hiding the evidence

The scale of the 'vaccine' death cover-up worldwide can be confirmed by comparing official figures with the personal experience of the public. I heard of many people in my community who died immediately or soon after the vaccine that would never appear in the media or even likely on the official totals of 'vaccine' fatalities and adverse reactions when only about ten percent are estimated to be

reported and I have seen some estimates as low as one percent in a Harvard study. In the UK alone by April 29th, 2021, some 757,654 adverse reactions had been officially reported from the Pfizer/BioNTech, Oxford/AstraZeneca and Moderna 'vaccines' with more than a thousand deaths linked to jabs and that means an estimated ten times this number in reality from a ten percent reporting rate percentage. That's seven million adverse reactions and 10,000 potential deaths and a one percent reporting rate would be ten times *those* figures. In 1976 the US government pulled the swine flu vaccine after *53* deaths. The UK data included a combined 10,000 eye disorders from the 'Covid vaccines' with more than 750 suffering visual impairment or blindness and again multiply by the estimated reporting percentages. As 'Covid cases' officially fell hospitals virtually empty during the 'Covid crisis' began to fill up with a range of other problems in the wake of the 'vaccine' rollout. The numbers across America have also been catastrophic. Deaths linked to *all* types of vaccine increased by *6,000 percent* in the first quarter of 2021 compared with 2020. A 39-year-old woman from Ogden, Utah, died four days after receiving a second dose of Moderna's 'Covid vaccine' when her liver, heart and kidneys all failed despite the fact that she had no known medical issues or conditions. Her family sought an autopsy, but Dr Erik Christensen, Utah's chief medical examiner, said proving vaccine injury as a cause of death almost never happened. He could think of only one instance where an autopsy would name a vaccine as the official cause of death and that would be anaphylaxis where someone received a vaccine and died almost instantaneously. 'Short of that, it would be difficult for us to definitively say this is the vaccine,' Christensen said. If that is true this must be added to the estimated ten percent (or far less) reporting rate of vaccine deaths and serious reactions and the conclusion can only be that vaccine deaths and serious reactions – including these 'Covid' potions' – are phenomenally understated in official figures. The same story can be found everywhere. Endless accounts of deaths and serious reactions among the public, medical

and care home staff while official figures did not even begin to reflect this.

Professional script-reader Dr David Williams, a 'top public-health official' in Ontario, Canada, insulted our intelligence by claiming only four serious adverse reactions and no deaths from the more than 380,000 vaccine doses then given. This bore no resemblance to what people knew had happened in their owns circles and we had Dirk Huyer in charge of getting millions vaccinated in Ontario while at the same time he was Chief Coroner for the province investigating causes of death including possible death from the vaccine. An aide said he had stepped back from investigating deaths, but evidence indicated otherwise. Rosemary Frei, who secured a Master of Science degree in molecular biology at the Faculty of Medicine at Canada's University of Calgary before turning to investigative journalism, was one who could see that official figures for 'vaccine' deaths and reactions made no sense. She said that doctors seldom reported adverse events and when people got really sick or died after getting a vaccination they would attribute that to anything except the vaccines. It had been that way for years and anyone who wondered aloud whether the 'Covid vaccines' or other shots cause harm is immediately branded as 'anti-vax' and 'anti-science'. This was 'career-threatening' for health professionals. Then there was the huge pressure to support the push to 'vaccinate' billions in the quickest time possible. Frei said:

> So that's where we're at today. More than half a million vaccine doses have been given to people in Ontario alone. The rush is on to vaccinate all 15 million of us in the province by September. And the mainstream media are screaming for this to be sped up even more. That all adds up to only a very slim likelihood that we're going to be told the truth by officials about how many people are getting sick or dying from the vaccines.

What is true of Ontario is true of everywhere.

They KNEW – and still did it

The authorities knew what was going to happen with multiple deaths and adverse reactions. The UK government's Gates-funded

and Big Pharma-dominated Medicines and Healthcare products Regulatory Agency (MHRA) hired a company to employ AI in compiling the projected reactions to the 'vaccine' that would otherwise be uncountable. The request for applications said: 'The MHRA urgently seeks an Artificial Intelligence (AI) software tool to process the expected high volume of Covid-19 vaccine Adverse Drug Reaction …' This was from the agency, headed by the disingenuous June Raine, that gave the 'vaccines' emergency approval and the company was hired before the first shot was given. 'We are going to kill and maim you – is that okay?' 'Oh, yes, perfectly fine – I'm very grateful, thank you, doctor.' The range of 'Covid vaccine' adverse reactions goes on for page after page in the MHRA criminally underreported 'Yellow Card' system and includes affects to eyes, ears, skin, digestion, blood and so on. Raine's MHRA amazingly claimed that the 'overall safety experience … is so far as expected from the clinical trials'. The death, serious adverse effects, deafness and blindness were *expected?* When did they ever mention that? If these human tragedies were expected then those that gave approval for the use of these 'vaccines' must be guilty of crimes against humanity including murder – a definition of which is 'killing a person with malice aforethought or with recklessness manifesting extreme indifference to the value of human life.' People involved at the MHRA, the CDC in America and their equivalent around the world must go before Nuremberg trials to answer for their callous inhumanity. We are only talking here about the immediate effects of the 'vaccine'. The longer-term impact of the DNA synthetic manipulation is the main reason they are so hysterically desperate to inoculate the entire global population in the shortest possible time.

Africa and the developing world are a major focus for the 'vaccine' depopulation agenda and a mass vaccination sales-pitch is underway thanks to caring people like the Rockefellers and other Cult assets. The Rockefeller Foundation, which pre-empted the 'Covid pandemic' in a document published in 2010 that 'predicted' what happened a decade later, announced an initial $34.95 million grant in February, 2021, 'to ensure more equitable access to Covid-19

testing and vaccines' among other things in Africa in collaboration with '24 organizations, businesses, and government agencies'. The pan-Africa initiative would focus on 10 countries: Burkina Faso, Ethiopia, Ghana, Kenya, Nigeria, Rwanda, South Africa, Tanzania, Uganda, and Zambia'. Rajiv Shah, President of the Rockefeller Foundation and former administrator of CIA-controlled USAID, said that if Africa was not mass-vaccinated (to change the DNA of its people) it was a 'threat to all of humanity' and not fair on Africans. When someone from the Rockefeller Foundation says they want to do something to help poor and deprived people and countries it is time for a belly-laugh. They are doing this out of the goodness of their 'heart' because 'vaccinating' the entire global population is what the 'Covid' hoax set out to achieve. Official 'decolonisation' of Africa by the Cult was merely a prelude to financial colonisation on the road to a return to physical colonisation. The 'vaccine' is vital to that and the sudden and convenient death of the 'Covid' sceptic president of Tanzania can be seen in its true light. A lot of people in Africa are aware that this is another form of colonisation and exploitation and they need to stand their ground.

The 'vaccine is working' scam

A potential problem for the Cult was that the 'vaccine' is meant to change human DNA and body messaging and not to protect anyone from a 'virus' never shown to exist. The vaccine couldn't work because it was not designed to work and how could they make it *appear* to be working so that more people would have it? This was overcome by lowering the amplification rate of the PCR test to produce fewer 'cases' and therefore fewer 'deaths'. Some of us had been pointing out since March, 2020, that the amplification rate of the test not testing for the 'virus' had been made artificially high to generate positive tests which they could call 'cases' to justify lockdowns. The World Health Organization recommended an absurdly high 45 amplification cycles to ensure the high positives required by the Cult and then remained silent on the issue until January 20th, 2021 – Biden's Inauguration Day. This was when the

'vaccinations' were seriously underway and on that day the WHO recommended after discussions with America's CDC that laboratories *lowered their testing amplification*. Dr David Samadi, a certified urologist and health writer, said the WHO was encouraging all labs to reduce their cycle count for PCR tests. He said the current cycle was much too high and was 'resulting in any particle being declared a positive case'. Even one mainstream news report I saw said this meant the number of 'Covid' infections may have been 'dramatically inflated'. Oh, just a little bit. The CDC in America issued new guidance to laboratories in April, 2021, to use 28 cycles *but only for 'vaccinated' people*. The timing of the CDC/WHO interventions were cynically designed to make it appear the 'vaccines' were responsible for falling cases and deaths when the real reason can be seen in the following examples. New York's state lab, the Wadsworth Center, identified 872 positive tests in July, 2020, based on a threshold of 40 cycles. When the figure was lowered to 35 cycles *43 percent* of the 872 were no longer 'positives'. At 30 cycles the figure was 63 percent. A Massachusetts lab found that between *85 to 90 percent* of people who tested positive in July with a cycle threshold of 40 would be negative at 30 cycles, Ashish Jha, MD, director of the Harvard Global Health Institute, said: 'I'm really shocked that it could be that high … Boy, does it really change the way we need to be thinking about testing.' I'm shocked that I could see the obvious in the spring of 2020, with no medical background, and most medical professionals still haven't worked it out. No, that's not shocking – it's terrifying.

Three weeks after the WHO directive to lower PCR cycles the London *Daily Mail* ran this headline: 'Why ARE Covid cases plummeting? New infections have fallen 45% in the US and 30% globally in the past 3 weeks but experts say vaccine is NOT the main driver because only 8% of Americans and 13% of people worldwide have received their first dose.' They acknowledged that the drop could not be attributed to the 'vaccine', but soon this morphed throughout the media into the 'vaccine' has caused cases and deaths to fall when it was the PCR threshold. In December, 2020, there was

chaos at English Channel ports with truck drivers needing negative 'Covid' tests before they could board a ferry home for Christmas. The government wanted to remove the backlog as fast as possible and they brought in troops to do the 'testing'. Out of 1,600 drivers just *36* tested positive and the rest were given the all clear to cross the Channel. I guess the authorities thought that 36 was the least they could get away with without the unquestioning catching on. The amplification trick which most people believed in the absence of information in the mainstream applied more pressure on those refusing the 'vaccine' to succumb when it 'obviously worked'. The truth was the exact opposite with deaths in care homes soaring with the 'vaccine' and in Israel the term used was 'skyrocket'. A re-analysis of published data from the Israeli Health Ministry led by Dr Hervé Seligmann at the Medicine Emerging Infectious and Tropical Diseases at Aix-Marseille University found that Pfizer's 'Covid vaccine' killed 'about 40 times more [elderly] people than the disease itself would have killed' during a five-week vaccination period and *260 times* more younger people than would have died from the 'virus' even according to the manipulated 'virus' figures. Dr Seligmann and his co-study author, Haim Yativ, declared after reviewing the Israeli 'vaccine' death data: 'This is a new Holocaust.'

Then, in mid-April, 2021, after vast numbers of people worldwide had been 'vaccinated', the story changed with clear coordination. The UK government began to prepare the ground for more future lockdowns when Nuremberg-destined Boris Johnson told yet another whopper. He said that cases had fallen because of *lockdowns* not 'vaccines'. Lockdowns are irrelevant when *there is no 'virus'* and the test and fraudulent death certificates are deciding the number of 'cases' and 'deaths'. Study after study has shown that lockdowns don't work and instead kill and psychologically destroy people. Meanwhile in the United States Anthony Fauci and Rochelle Walensky, the ultra-Zionist head of the CDC, peddled the same line. More lockdown was the answer and not the 'vaccine', a line repeated on cue by the moron that is Canadian Prime Minister Justin Trudeau. Why all the hysteria to get everyone 'vaccinated' if lockdowns and

not 'vaccines' made the difference? None of it makes sense on the face of it. Oh, but it does. The Cult wants lockdowns *and* the 'vaccine' and if the 'vaccine' is allowed to be seen as the total answer lockdowns would no longer be justified when there are still livelihoods to destroy. 'Variants' and renewed upward manipulation of PCR amplification are planned to instigate never-ending lockdown *and* more 'vaccines'.

You *must* have it – we're desperate

Israel, where the Jewish and Arab population are ruled by the Sabbatian Cult, was the front-runner in imposing the DNA-manipulating 'vaccine' on its people to such an extent that Jewish refusers began to liken what was happening to the early years of Nazi Germany. This would seem to be a fantastic claim. Why would a government of Jewish people be acting like the Nazis did? If you realise that the Sabbatian Cult was behind the Nazis and that Sabbatians hate Jews the pieces start to fit and the question of why a 'Jewish' government would treat Jews with such callous disregard for their lives and freedom finds an answer. Those controlling the government of Israel *aren't Jewish* – they're Sabbatian. Israeli lawyer Tamir Turgal was one who made the Nazi comparison in comments to German lawyer Reiner Fuellmich who is leading a class action lawsuit against the psychopaths for crimes against humanity. Turgal described how the Israeli government was vaccinating children and pregnant women on the basis that there was no evidence that this was dangerous when they had no evidence that it *wasn't* dangerous either. They just had no evidence. This was medical experimentation and Turgal said this breached the Nuremberg Code about medical experimentation and procedures requiring informed consent and choice. Think about that. A Nuremberg Code developed because of Nazi experimentation on Jews and others in concentration camps by people like the evil-beyond-belief Josef Mengele is being breached by the *Israeli* government; but when you know that it's a *Sabbatian* government along with its intelligence and military agencies like Mossad, Shin Bet and the Israeli Defense Forces, and that Sabbatians

were the force behind the Nazis, the kaleidoscope comes into focus. What have we come to when Israeli Jews are suing their government for violating the Nuremberg Code by essentially making Israelis subject to a medical experiment using the controversial 'vaccines'? It's a shocker that this has to be done in the light of what happened in Nazi Germany. The Anshe Ha-Emet, or 'People of the Truth', made up of Israeli doctors, lawyers, campaigners and public, have launched a lawsuit with the International Criminal Court. It says:

> When the heads of the Ministry of Health as well as the prime minister presented the vaccine in Israel and began the vaccination of Israeli residents, the vaccinated were not advised, that, in practice, they are taking part in a medical experiment and that their consent is required for this under the Nuremberg Code.

The irony is unbelievable, but easily explained in one word: Sabbatians. The foundation of Israeli 'Covid' apartheid is the 'green pass' or 'green passport' which allows Jews and Arabs who have had the DNA-manipulating 'vaccine' to go about their lives – to work, fly, travel in general, go to shopping malls, bars, restaurants, hotels, concerts, gyms, swimming pools, theatres and sports venues, while non-'vaccinated' are banned from all those places and activities. Israelis have likened the 'green pass' to the yellow stars that Jews in Nazi Germany were forced to wear – the same as the yellow stickers that a branch of UK supermarket chain Morrisons told exempt mask-wears they had to display when shopping. How very sensitive. The Israeli system is blatant South African-style apartheid on the basis of compliance or non-compliance to fascism rather than colour of the skin. How appropriate that the Sabbatian Israeli government was so close to the pre-Mandela apartheid regime in Pretoria. The Sabbatian-instigated 'vaccine passport' in Israel is planned for everywhere. Sabbatians struck a deal with Pfizer that allowed them to lead the way in the percentage of a national population infused with synthetic material and the result was catastrophic. Israeli freedom activist Shai Dannon told me how chairs were appearing on beaches that said 'vaccinated only'. Health Minister Yuli Edelstein said that anyone unwilling or unable to get

the jabs that 'confer immunity' will be 'left behind'. The man's a liar. Not even the makers claim the 'vaccines' confer immunity. When you see those figures of 'vaccine' deaths these psychopaths were saying that you must take the chance the 'vaccine' will kill you or maim you while knowing it will change your DNA or lockdown for you will be permanent. That's fascism. The Israeli parliament passed a law to allow personal information of the non-vaccinated to be shared with local and national authorities for three months. This was claimed by its supporters to be a way to 'encourage' people to be vaccinated. Hadas Ziv from Physicians for Human Rights described this as a 'draconian law which crushed medical ethics and the patient rights'. But that's the idea, the Sabbatians would reply.

Your papers, please

Sabbatian Israel was leading what has been planned all along to be a global 'vaccine pass' called a 'green passport' without which you would remain in permanent lockdown restriction and unable to do anything. This is how badly – *desperately* – the Cult is to get everyone 'vaccinated'. The term and colour 'green' was not by chance and related to the psychology of fusing the perception of the green climate hoax with the 'Covid' hoax and how the 'solution' to both is the same Great Reset. Lying politicians, health officials and psychologists denied there were any plans for mandatory vaccinations or restrictions based on vaccinations, but they knew that was exactly what was meant to happen with governments of all countries reaching agreements to enforce a global system. 'Free' Denmark and 'free' Sweden unveiled digital vaccine certification. Cyprus, Czech Republic, Estonia, Greece, Hungary, Iceland, Italy, Poland, Portugal, Slovakia, and Spain have all committed to a vaccine passport system and the rest including the whole of the EU would follow. The satanic UK government will certainly go this way despite mendacious denials and at the time of writing it is trying to manipulate the public into having the 'vaccine' so they could go abroad on a summer holiday. How would that work without something to prove you had the synthetic toxicity injected into you?

Documents show that the EU's European Commission was moving towards 'vaccine certificates' in 2018 and 2019 before the 'Covid' hoax began. They knew what was coming. Abracadabra – Ursula von der Leyen, the German President of the Commission, announced in March, 2021, an EU 'Digital Green Certificate' – green again – to track the public's 'Covid status'. The passport sting is worldwide and the Far East followed the same pattern with South Korea ruling that only those with 'vaccination' passports – again the *green* pass – would be able to 'return to their daily lives'.

Bill Gates has been preparing for this 'passport' with other Cult operatives for years and beyond the paper version is a Gates-funded 'digital tattoo' to identify who has been vaccinated and who hasn't. The 'tattoo' is reported to include a substance which is externally readable to confirm who has been vaccinated. This is a bio-luminous light-generating enzyme (think fireflies) called … *Luciferase*. Yes, named after the Cult 'god' Lucifer the 'light bringer' of whom more to come. Gates said he funded the readable tattoo to ensure children in the developing world were vaccinated and no one was missed out. He cares so much about poor kids as we know. This was just the cover story to develop a vaccine tagging system for everyone on the planet. Gates has been funding the ID2020 'alliance' to do just that in league with other lovely people at Microsoft, GAVI, the Rockefeller Foundation, Accenture and IDEO.org. He said in interviews in March, 2020, before any 'vaccine' publicly existed, that the world must have a globalised digital certificate to track the 'virus' and who had been vaccinated. Gates knew from the start that the mRNA vaccines were coming and when they would come and that the plan was to tag the 'vaccinated' to marginalise the intelligent and stop them doing anything including travel. Evil just doesn't suffice. Gates was exposed for offering a $10 million bribe to the Nigerian House of Representatives to invoke compulsory 'Covid' vaccination of all Nigerians. Sara Cunial, a member of the Italian Parliament, called Gates a 'vaccine criminal'. She urged the Italian President to hand him over to the International Criminal Court for crimes against

humanity and condemned his plans to 'chip the human race' through ID2020.

You know it's a long-planned agenda when war criminal and Cult gofer Tony Blair is on the case. With the scale of arrogance only someone as dark as Blair can muster he said: 'Vaccination in the end is going to be your route to liberty.' Blair is a disgusting piece of work and he confirms that again. The media has given a lot of coverage to a bloke called Charlie Mullins, founder of London's biggest independent plumbing company, Pimlico Plumbers, who has said he won't employ anyone who has not been vaccinated or have them go to any home where people are not vaccinated. He said that if he had his way no one would be allowed to walk the streets if they have not been vaccinated. Gates was cheering at the time while I was alerting the white coats. The plan is that people will qualify for 'passports' for having the first two doses and then to keep it they will have to have all the follow ups and new ones for invented 'variants' until human genetics is transformed and many are dead who can't adjust to the changes. Hollywood celebrities – the usual propaganda stunt – are promoting something called the WELL Health-Safety Rating to verify that a building or space has 'taken the necessary steps to prioritize the health and safety of their staff, visitors and other stakeholders'. They included Lady Gaga, Jennifer Lopez, Michael B. Jordan, Robert DeNiro, Venus Williams, Wolfgang Puck, Deepak Chopra and 17th Surgeon General Richard Carmona. Yawn. WELL Health-Safety has big connections with China. Parent company Delos is headed by former Goldman Sachs partner Paul Scialla. This is another example – and we will see so many others – of using the excuse of 'health' to dictate the lives and activities of the population. I guess one confirmation of the 'safety' of buildings is that only 'vaccinated' people can go in, right?

Electronic concentration camps

I wrote decades ago about the plans to restrict travel and here we are for those who refuse to bow to tyranny. This can be achieved in one go with air travel if the aviation industry makes a blanket decree.

The ‘vaccine’ and guaranteed income are designed to be part of a global version of China’s social credit system which tracks behaviour 24/7 and awards or deletes ‘credits’ based on whether your behaviour is supported by the state or not. I mean your entire lifestyle – what you do, eat, say, everything. Once your credit score falls below a certain level consequences kick in. In China tens of millions have been denied travel by air and train because of this. All the locations and activities denied to refusers by the ‘vaccine’ passports will be included in one big mass ban on doing almost anything for those that don’t bow their head to government. It’s beyond fascist and a new term is required to describe its extremes – I guess fascist technocracy will have to do. The way the Chinese system of technological – technocratic – control is sweeping the West can be seen in the Los Angeles school system and is planned to be expanded worldwide. Every child is required to have a ‘Covid’-tracking app scanned daily before they can enter the classroom. The so-called Daily Pass tracking system is produced by Gates’ Microsoft which I’m sure will shock you rigid. The pass will be scanned using a barcode (one step from an inside-the-body barcode) and the information will include health checks, ‘Covid’ tests and vaccinations. Entry codes are for one specific building only and access will only be allowed if a student or teacher has a negative test with a test not testing for the ‘virus’, has no symptoms of anything alleged to be related to ‘Covid’ (symptoms from a range of other illness), and has a temperature under 100 degrees. No barcode, no entry, is planned to be the case for everywhere and not only schools.

Kids are being psychologically prepared to accept this as ‘normal’ their whole life which is why what they can impose in schools is so important to the Cult and its gofers. Long-time American freedom campaigner John Whitehead of the Rutherford Institute was not exaggerating when he said: ‘Databit by databit, we are building our own electronic concentration camps.’ Canada under its Cult gofer prime minister Justin Trudeau has taken a major step towards the real thing with people interned against their will if they test positive with a test not testing for the ‘virus’ when they arrive at a Canadian

airport. They are jailed in internment hotels often without food or water for long periods and with many doors failing to lock there have been sexual assaults. The interned are being charged sometimes $2,000 for the privilege of being abused in this way. Trudeau is fully on board with the Cult and says the 'Covid pandemic' has provided an opportunity for a global 'reset' to permanently change Western civilisation. His number two, Deputy Prime Minister Chrystia Freeland, is a trustee of the World Economic Forum and a Rhodes Scholar. The Trudeau family have long been servants of the Cult. See *The Biggest Secret* and Cathy O'Brien's book *Trance-Formation of America* for the horrific background to Trudeau's father Pierre Trudeau another Canadian prime minister. Hide your fascism behind the façade of a heart-on-the-sleeve liberal. It's a well-honed Cult technique.

What can the 'vaccine' *really* do?

We have a 'virus' never shown to exist and 'variants' of the 'virus' that have also never been shown to exist except, like the 'original', as computer-generated fictions. Even if you believe there's a 'virus' the 'case' to 'death' rate is in the region of 0.23 to 0.15 percent and those 'deaths' are concentrated among the very old around the same average age that people die anyway. In response to this lack of threat (in truth none) psychopaths and idiots, knowingly and unknowingly answering to Gates and the Cult, are seeking to 'vaccinate' every man, woman and child on Planet Earth. Clearly the 'vaccine' is not about 'Covid' – none of this ever has been. So what is it all about *really*? Why the desperation to infuse genetically-manipulating synthetic material into everyone through mRNA fraudulent 'vaccines' with the intent of doing this over and over with the excuses of 'variants' and other 'virus' inventions? Dr Sherri Tenpenny, an osteopathic medical doctor in the United States, has made herself an expert on vaccines and their effects as a vehement campaigner against their use. Tenpenny was board certified in emergency medicine, the director of a level two trauma centre for 12 years, and moved to Cleveland in 1996 to start an integrative

medicine practice which has treated patients from all 50 states and some 17 other countries. Weaning people off pharmaceutical drugs is a speciality.

She became interested in the consequences of vaccines after attending a meeting at the National Vaccine Information Center in Washington DC in 2000 where she 'sat through four days of listening to medical doctors and scientists and lawyers and parents of vaccine injured kids' and asked: 'What's going on?' She had never been vaccinated and never got ill while her father was given a list of vaccines to be in the military and was 'sick his entire life'. The experience added to her questions and she began to examine vaccine documents from the Centers for Disease Control (CDC). After reading the first one, the 1998 version of *The General Recommendations of Vaccination*, she thought: 'This is it?' The document was poorly written and bad science and Tenpenny began 20 years of research into vaccines that continues to this day. She began her research into 'Covid vaccines' in March, 2020, and she describes them as 'deadly'. For many, as we have seen, they already have been. Tenpenny said that in the first 30 days of the 'vaccine' rollout in the United States there had been more than 40,000 adverse events reported to the vaccine adverse event database. A document had been delivered to her the day before that was 172 pages long. 'We have over 40,000 adverse events; we have over 3,100 cases of [potentially deadly] anaphylactic shock; we have over 5,000 neurological reactions.' Effects ranged from headaches to numbness, dizziness and vertigo, to losing feeling in hands or feet and paraesthesia which is when limbs 'fall asleep' and people have the sensation of insects crawling underneath their skin. All this happened in the first 30 days and remember that only about *ten percent* (or far less) of adverse reactions and vaccine-related deaths are estimated to be officially reported. Tenpenny said:

> So can you think of one single product in any industry, any industry, for as long as products have been made on the planet that within 30 days we have 40,000 people complaining of side effects that not only is still on the market but … we've got paid actors telling us how great

they are for getting their vaccine. We're offering people $500 if they will just get their vaccine and we've got nurses and doctors going; 'I got the vaccine, I got the vaccine'.

Tenpenny said they were not going to be 'happy dancing folks' when they began to suffer Bell's palsy (facial paralysis), neuropathies, cardiac arrhythmias and autoimmune reactions that kill through a blood disorder. 'They're not going to be so happy, happy then, but we're never going to see pictures of those people' she said. Tenpenny described the 'vaccine' as 'a well-designed killing tool'.

No off-switch

Bad as the initial consequences had been Tenpenny said it would be maybe 14 months before we began to see the 'full ravage' of what is going to happen to the 'Covid vaccinated' with full-out consequences taking anything between two years and 20 years to show. You can understand why when you consider that variations of the 'Covid vaccine' use mRNA (messenger RNA) to in theory activate the immune system to produce protective antibodies without using the actual 'virus'. How can they when it's a computer program and they've never isolated what they claim is the 'real thing'? Instead they use *synthetic* mRNA. They are inoculating synthetic material into the body which through a technique known as the Trojan horse is absorbed into cells to change the nature of DNA. Human DNA is changed by an infusion of messenger RNA and with each new 'vaccine' of this type it is changed even more. Say so and you are banned by Cult Internet platforms. The contempt the contemptuous Mark Zuckerberg has for the truth and human health can be seen in an internal Facebook video leaked to the Project Veritas investigative team in which he said of the 'Covid vaccines': '… I share some caution on this because we just don't know the long term side-effects of basically modifying people's DNA and RNA.' At the same time this disgusting man's Facebook was censoring and banning anyone saying exactly the same. He must go before a Nuremberg trial for crimes against humanity when he *knows* that he

is censoring legitimate concerns and denying the right of informed consent on behalf of the Cult that owns him. People have been killed and damaged by the very 'vaccination' technique he cast doubt on himself when they may not have had the 'vaccine' with access to information that he denied them. The plan is to have at least annual 'Covid vaccinations', add others to deal with invented 'variants', and change all other vaccines into the mRNA system. Pfizer executives told shareholders at a virtual Barclays Global Healthcare Conference in March, 2021, that the public may need a third dose of 'Covid vaccine', plus regular yearly boosters and the company planned to hike prices to milk the profits in a 'significant opportunity for our vaccine'. These are the professional liars, cheats and opportunists who are telling you their 'vaccine' is safe. Given this volume of mRNA planned to be infused into the human body and its ability to then replicate we will have a transformation of human genetics from biological to synthetic biological – exactly the long-time Cult plan for reasons we'll see – and many will die. Sherri Tenpenny said of this replication:

> It's like having an on-button but no off-button and that whole mechanism … they actually give it a name and they call it the Trojan horse mechanism, because it allows that [synthetic] virus and that piece of that [synthetic] virus to get inside of your cells, start to replicate and even get inserted into other parts of your DNA as a Trojan-horse.

Ask the overwhelming majority of people who have the 'vaccine' what they know about the contents and what they do and they would reply: 'The government says it will stop me getting the virus.' Governments give that false impression on purpose to increase take-up. You can read Sherri Tenpenny's detailed analysis of the health consequences in her blog at Vaxxter.com, but in summary these are some of them. She highlights the statement by Bill Gates about how human beings can become their own 'vaccine manufacturing machine'. The man is insane. ['Vaccine'-generated] 'antibodies' carry synthetic messenger RNA into the cells and the damage starts, Tenpenny contends, and she says that lungs can be adversely affected through varying degrees of pus and bleeding which

obviously affects breathing and would be dubbed 'Covid-19'. Even more sinister was the impact of 'antibodies' on macrophages, a white blood cell of the immune system. They consist of Type 1 and Type 2 which have very different functions. She said Type 1 are 'hyper-vigilant' white blood cells which 'gobble up' bacteria etc. However, in doing so, this could cause inflammation and in extreme circumstances be fatal. She says these affects are mitigated by Type 2 macrophages which kick in to calm down the system and stop it going rogue. They clear up dead tissue debris and reduce inflammation that the Type 1 'fire crews' have caused. Type 1 kills the infection and Type 2 heals the damage, she says. This is her punchline with regard to 'Covid vaccinations': She says that mRNA 'antibodies' block Type 2 macrophages by attaching to them and deactivating them. This meant that when the Type 1 response was triggered by infection there was nothing to stop that getting out of hand by calming everything down. There's an on-switch, but no off-switch, she says. What follows can be 'over and out, see you when I see you'.

Genetic suicide

Tenpenny also highlights the potential for autoimmune disease – the body attacking itself – which has been associated with vaccines since they first appeared. Infusing a synthetic foreign substance into cells could cause the immune system to react in a panic believing that the body is being overwhelmed by an invader (it is) and the consequences can again be fatal. There is an autoimmune response known as a 'cytokine storm' which I have likened to a homeowner panicked by an intruder and picking up a gun to shoot randomly in all directions before turning the fire on himself. The immune system unleashes a storm of inflammatory response called cytokines to a threat and the body commits hara-kiri. The lesson is that you mess with the body's immune response at your peril and these 'vaccines' seriously – fundamentally – mess with immune response. Tenpenny refers to a consequence called anaphylactic shock which is a severe and highly dangerous allergic reaction when the immune system

floods the body with chemicals. She gives the example of having a bee sting which primes the immune system and makes it sensitive to those chemicals. When people are stung again maybe years later the immune response can be so powerful that it leads to anaphylactic shock. Tenpenny relates this 'shock' with regard to the 'Covid vaccine' to something called polyethylene glycol or PEG. Enormous numbers of people have become sensitive to this over decades of use in a whole range of products and processes including food, drink, skin creams and 'medicine'. Studies have claimed that some 72 percent of people have antibodies triggered by PEG compared with two percent in the 1960s and allergic hypersensitive reactions to this become a gathering cause for concern. Tenpenny points out that the 'mRNA vaccine' is coated in a 'bubble' of polyethylene glycol which has the potential to cause anaphylactic shock through immune sensitivity. Many reports have appeared of people reacting this way after having the 'Covid vaccine'. What do we think is going to happen as humanity has more and more of these 'vaccines'? Tenpenny said: 'All these pictures we have seen with people with these rashes … these weepy rashes, big reactions on their arms and things like that – it's an acute allergic reaction most likely to the polyethylene glycol that you've been previously primed and sensitised to.'

Those who have not studied the conspiracy and its perpetrators at length might think that making the population sensitive to PEG and then putting it in these 'vaccines' is just a coincidence. It is not. It is instead testament to how carefully and coldly-planned current events have been and the scale of the conspiracy we are dealing with. Tenpenny further explains that the 'vaccine' mRNA procedure can breach the blood-brain barrier which protects the brain from toxins and other crap that will cause malfunction. In this case they could make two proteins corrupt brain function to cause Amyotrophic lateral sclerosis (ALS) , a progressive nervous system disease leading to loss of muscle control, and frontal lobe degeneration – Alzheimer's and dementia. Immunologist J. Bart Classon published a paper connecting mRNA 'vaccines' to prion

disease which can lead to Alzheimer's and other forms of neurogenerative disease while others have pointed out the potential to affect the placenta in ways that make women infertile. This will become highly significant in the next chapter when I will discuss other aspects of this non-vaccine that relate to its nanotechnology and transmission from the injected to the uninjected.

Qualified in idiocy

Tenpenny describes how research has confirmed that these 'vaccine'-generated antibodies can interact with a range of other tissues in the body and attack many other organs including the lungs. 'This means that if you have a hundred people standing in front of you that all got this shot they could have a hundred different symptoms.' Anyone really think that Cult gofers like the Queen, Tony Blair, Christopher Whitty, Anthony Fauci, and all the other psychopaths have really had this 'vaccine' in the pictures we've seen? Not a bloody chance. Why don't doctors all tell us about all these dangers and consequences of the 'Covid vaccine'? Why instead do they encourage and pressure patients to have the shot? Don't let's think for a moment that doctors and medical staff can't be stupid, lazy, and psychopathic and that's without the financial incentives to give the jab. Tenpenny again:

> Some people are going to die from the vaccine directly but a large number of people are going to start to get horribly sick and get all kinds of autoimmune diseases 42 days to maybe a year out. What are they going to do, these stupid doctors who say; 'Good for you for getting that vaccine.' What are they going to say; 'Oh, it must be a mutant, we need to give an extra dose of that vaccine.'
>
> Because now the vaccine, instead of one dose or two doses we need three or four because the stupid physicians aren't taking the time to learn anything about it. If I can learn this sitting in my living room reading a 19 page paper and several others so can they. There's nothing special about me, I just take the time to do it.

Remember how Sara Kayat, the NHS and TV doctor, said that the 'Covid vaccine' would '100 percent prevent hospitalisation and death'. Doctors can be idiots like every other profession and they

should not be worshipped as infallible. They are not and far from it. Behind many medical and scientific 'experts' lies an uninformed prat trying to hide themselves from you although in the 'Covid' era many have failed to do so as with UK narrative-repeating 'TV doctor' Hilary Jones. Pushing back against the minority of proper doctors and scientists speaking out against the 'vaccine' has been the entire edifice of the Cult global state in the form of governments, medical systems, corporations, mainstream media, Silicon Valley, and an army of compliant doctors, medical staff and scientists willing to say anything for money and to enhance their careers by promoting the party line. If you do that you are an 'expert' and if you won't you are an 'anti-vaxxer' and 'Covidiot'. The pressure to be 'vaccinated' is incessant. We have even had reports claiming that the 'vaccine' can help cure cancer and Alzheimer's and make the lame walk. I am waiting for the announcement that it can bring you coffee in the morning and cook your tea. Just as the symptoms of 'Covid' seem to increase by the week so have the miracles of the 'vaccine'. American supermarket giant Kroger Co. offered nearly 500,000 employees in 35 states a $100 bonus for having the 'vaccine' while donut chain Krispy Kreme promised 'vaccinated' customers a free glazed donut every day for the rest of 2021. Have your DNA changed and you will get a doughnut although we might not have to give you them for long. Such offers and incentives confirm the desperation.

Perhaps the worse vaccine-stunt of them all was UK 'Health' Secretary Matt-the-prat Hancock on live TV after watching a clip of someone being 'vaccinated' when the roll-out began. Hancock faked tears so badly it was embarrassing. Brain-of-Britain Piers Morgan, the lockdown-supporting, 'vaccine' supporting, 'vaccine' passport-supporting, TV host played along with Hancock – 'You're quite emotional about that' he said in response to acting so atrocious it would have been called out at a school nativity which will presumably today include Mary and Jesus in masks, wise men keeping their camels six feet apart, and shepherds under tent arrest. System-serving Morgan tweeted this: 'Love the idea of covid vaccine passports for everywhere: flights, restaurants, clubs, football, gyms,

shops etc. It's time covid-denying, anti-vaxxer loonies had their bullsh*t bluff called & bar themselves from going anywhere that responsible citizens go.' If only I could aspire to his genius. To think that Morgan, who specialises in shouting over anyone he disagrees with, was lauded as a free speech hero when he lost his job after storming off the set of his live show like a child throwing his dolly out of the pram. If he is a free speech hero we are in real trouble. I have no idea what 'bullsh*t' means, by the way, the * throws me completely.

The Cult is desperate to infuse its synthetic DNA-changing concoction into everyone and has been using every lie, trick and intimidation to do so. The question of '*Why*?' we shall now address.

CHAPTER TEN

Human 2.0

I believe that at the end of the century the use of words and general educated opinion will have altered so much that one will be able to speak of machines thinking without expecting to be contradicted –
Alan Turing (1912-1954), the 'Father of artificial intelligence'

I have been exposing for decades the plan to transform the human body from a biological to a synthetic-biological state. The new human that I will call Human 2.0 is planned to be connected to artificial intelligence and a global AI 'Smart Grid' that would operate as one global system in which AI would control everything from your fridge to your heating system to your car to your mind. Humans would no longer be 'human', but post-human and sub-human, with their thinking and emotional processes replaced by AI.

What I said sounded crazy and beyond science fiction and I could understand that. To any balanced, rational, mind it *is* crazy. Today, however, that world is becoming reality and it puts the 'Covid vaccine' into its true context. Ray Kurzweil is the ultra-Zionist 'computer scientist, inventor and futurist' and co-founder of the Singularity University. Singularity refers to the merging of humans with machines or 'transhumanism'. Kurzweil has said humanity would be connected to the cyber 'cloud' in the period of the ever-recurring year of 2030:

Our thinking … will be a hybrid of biological and non-biological thinking … humans will be able to extend their limitations and 'think in the cloud' … We're going to put gateways to the

cloud in our brains ... We're going to gradually merge and enhance ourselves ... In my view, that's the nature of being human – we transcend our limitations. As the technology becomes vastly superior to what we are then the small proportion that is still human gets smaller and smaller and smaller until it's just utterly negligible.

They are trying to sell this end-of-humanity-as-we-know-it as the next stage of 'evolution' when we become super-human and 'like the gods'. They are lying to you. Shocked, eh? The population, and again especially the young, have been manipulated into addiction to technologies designed to enslave them for life. First they induced an addiction to smartphones (holdables); next they moved to technology on the body (wearables); and then began the invasion of the body (implantables). I warned way back about the plan for microchipped people and we are now entering that era. We should not be diverted into thinking that this refers only to chips we can see. Most important are the nanochips known as smart dust, neural dust and nanobots which are far too small to be seen by the human eye. Nanotechnology is everywhere, increasingly in food products, and released into the atmosphere by the geoengineering of the skies funded by Bill Gates to 'shut out the Sun' and 'save the planet from global warming'. Gates has been funding a project to spray millions of tonnes of chalk (calcium carbonate) into the stratosphere over Sweden to 'dim the Sun' and cool the Earth. Scientists warned the move could be disastrous for weather systems in ways no one can predict and opposition led to the Swedish space agency announcing that the 'experiment' would not be happening as planned in the summer of 2021; but it shows where the Cult is going with dimming the impact of the Sun and there's an associated plan to change the planet's atmosphere. Who gives psychopath Gates the right to dictate to the entire human race and dismantle planetary systems? The world will not be safe while this man is at large.

The global warming hoax has made the Sun, like the gas of life, something to fear when both are essential to good health and human survival (more inversion). The body transforms sunlight into vital vitamin D through a process involving … *cholesterol*. This is the cholesterol we are also told to fear. We are urged to take Big Pharma

statin drugs to reduce cholesterol and it's all systematic. Reducing cholesterol means reducing vitamin D uptake with all the multiple health problems that will cause. At least if you take statins long term it saves the government from having to pay you a pension. The delivery system to block sunlight is widely referred to as chemtrails although these have a much deeper agenda, too. They appear at first to be contrails or condensation trails streaming from aircraft into cold air at high altitudes. Contrails disperse very quickly while chemtrails do not and spread out across the sky before eventually their content falls to earth. Many times I have watched aircraft cross-cross a clear blue sky releasing chemtrails until it looks like a cloudy day. Chemtrails contain many things harmful to humans and the natural world including toxic heavy metals, aluminium (see Alzheimer's) and nanotechnology. Ray Kurzweil reveals the reason without actually saying so: 'Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.' How do you deliver that? *From the sky*. Self-replicating nanobots would connect everything to the Smart Grid. The phenomenon of Morgellons disease began in the chemtrail era and the correlation has led to it being dubbed the 'chemtrail disease'. Self-replicating fibres appear in the body that can be pulled out through the skin. Morgellons fibres continue to grow outside the body and have a form of artificial intelligence. I cover this at greater length in *Phantom Self*.

'Vaccine' operating system

'Covid vaccines' with their self-replicating synthetic material are also designed to make the connection between humanity and Kurzweil's 'cloud'. American doctor and dedicated campaigner for truth, Carrie Madej, an Internal Medicine Specialist in Georgia with more than 20 years medical experience, has highlighted the nanotechnology aspect of the fake 'vaccines'. She explains how one of the components in at least the Moderna and Pfizer synthetic potions are 'lipid nanoparticles' which are 'like little tiny computer bits' – a 'sci-fi substance' known as nanobots and hydrogel which can be 'triggered

at any moment to deliver its payload' and act as 'biosensors'. The synthetic substance had 'the ability to accumulate data from your body like your breathing, your respiration, thoughts and emotions, all kind of things' and each syringe could carry a *million* nanobots:

This substance because it's like little bits of computers in your body, crazy, but it's true, it can do that, [and] obviously has the ability to act through Wi-Fi. It can receive and transmit energy, messages, frequencies or impulses. That issue has never been addressed by these companies. What does that do to the human?

Just imagine getting this substance in you and it can react to things all around you, the 5G, your smart device, your phones, what is happening with that? What if something is triggering it, too, like an impulse, a frequency? We have something completely foreign in the human body.

Madej said her research revealed that electromagnetic (EMF) frequencies emitted by phones and other devices had increased dramatically in the same period of the 'vaccine' rollout and she was seeing more people with radiation problems as 5G and other electromagnetic technology was expanded and introduced to schools and hospitals. She said she was 'floored with the EMF coming off' the devices she checked. All this makes total sense and syncs with my own work of decades when you think that Moderna refers in documents to its mRNA 'vaccine' as an 'operating system':

Recognizing the broad potential of mRNA science, we set out to create an mRNA technology platform that functions very much like an operating system on a computer. It is designed so that it can plug and play interchangeably with different programs. In our case, the 'program' or 'app' is our mRNA drug – the unique mRNA sequence that codes for a protein …

… Our MRNA Medicines – 'The 'Software Of Life': When we have a concept for a new mRNA medicine and begin research, fundamental components are already in place. Generally, the only thing that changes from one potential mRNA medicine to another is the coding region – the actual genetic code that instructs ribosomes to make protein. Utilizing these instruction sets gives our investigational mRNA medicines a software-like quality. We also have the ability to combine different mRNA sequences encoding for different proteins in a single mRNA investigational medicine.

Who needs a real 'virus' when you can create a computer version to justify infusing your operating system into the entire human race on the road to making living, breathing people into cyborgs? What is missed with the 'vaccines' is the *digital* connection between synthetic material and the body that I highlighted earlier with the study that hacked a computer with human DNA. On one level the body is digital, based on mathematical codes, and I'll have more about that in the next chapter. Those who ridiculously claim that mRNA 'vaccines' are not designed to change human genetics should explain the words of Dr Tal Zaks, chief medical officer at Moderna, in a 2017 TED talk. He said that over the last 30 years 'we've been living this phenomenal digital scientific revolution, and I'm here today to tell you, that we are actually *hacking the software of life*, and that it's changing the way we think about prevention and treatment of disease':

In every cell there's this thing called messenger RNA, or mRNA for short, that transmits the critical information from the DNA in our genes to the protein, which is really the stuff we're all made out of. This is the critical information that determines what the cell will do. So we think about it as an operating system. So if you could change that, if you could introduce a line of code, or change a line of code, it turns out, that has profound implications for everything, from the flu to cancer.

Zaks should more accurately have said that this has profound implications for the human genetic code and the nature of DNA. Communications within the body go both ways and not only one. But, hey, no, the 'Covid vaccine' will not affect your genetics. Cult fact-checkers say so even though the man who helped to develop the mRNA technique says that it does. Zaks said in 2017:

If you think about what it is we're trying to do. We've taken information and our understanding of that information and how that information is transmitted in a cell, and we've taken our understanding of medicine and how to make drugs, and we're fusing the two. We think of it as information therapy.

I have been writing for decades that the body is an information field communicating with itself and the wider world. This is why

radiation which is information can change the information field of body and mind through phenomena like 5G and change their nature and function. 'Information therapy' means to change the body's information field and change the way it operates. DNA is a receiver-transmitter of information and can be mutated by information like mRNA synthetic messaging. Technology to do this has been ready and waiting in the underground bases and other secret projects to be rolled out when the 'Covid' hoax was played. 'Trials' of such short and irrelevant duration were only for public consumption. When they say the 'vaccine' is 'experimental' that is not true. It may appear to be 'experimental' to those who don't know what's going on, but the trials have already been done to ensure the Cult gets the result it desires. Zaks said that it took decades to sequence the human genome, completed in 2003, but now they could do it in a week. By 'they' he means scientists operating in the public domain. In the secret projects they were sequencing the genome in a week long before even 2003.

Deluge of mRNA

Highly significantly the Moderna document says the guiding premise is that if using mRNA as a medicine works for one disease then it should work for many diseases. They were leveraging the flexibility afforded by their platform and the fundamental role mRNA plays in protein synthesis to pursue mRNA medicines for a broad spectrum of diseases. Moderna is confirming what I was saying through 2020 that multiple 'vaccines' were planned for 'Covid' (and later invented 'variants') and that previous vaccines would be converted to the mRNA system to infuse the body with massive amounts of genetically-manipulating synthetic material to secure a transformation to a synthetic-biological state. The 'vaccines' are designed to kill stunning numbers as part of the long-exposed Cult depopulation agenda and transform the rest. Given this is the goal you can appreciate why there is such hysterical demand for every human to be 'vaccinated' for an alleged 'disease' that has an estimated 'infection' to 'death' ratio of 0.23-0.15 percent. As I write

children are being given the 'vaccine' in trials (their parents are a disgrace) and ever-younger people are being offered the vaccine for a 'virus' that even if you believe it exists has virtually zero chance of harming them. Horrific effects of the 'trials' on a 12-year-old girl were revealed by a family member to be serious brain and gastric problems that included a bowel obstruction and the inability to swallow liquids or solids. She was unable to eat or drink without throwing up, had extreme pain in her back, neck and abdomen, and was paralysed from the waist down which stopped her urinating unaided. When the girl was first taken to hospital doctors said it was all in her mind. She was signed up for the 'trial' by her parents for whom no words suffice. None of this 'Covid vaccine' insanity makes any sense unless you see what the 'vaccine' really is – a body-changer. Synthetic biology or 'SynBio' is a fast-emerging and expanding scientific discipline which includes everything from genetic and molecular engineering to electrical and computer engineering. Synthetic biology is defined in these ways:

- A multidisciplinary area of research that seeks to create new biological parts, devices, and systems, or to redesign systems that are already found in nature.

- The use of a mixture of physical engineering and genetic engineering to create new (and therefore synthetic) life forms.

- An emerging field of research that aims to combine the knowledge and methods of biology, engineering and related disciplines in the design of chemically-synthesized DNA to create organisms with novel or enhanced characteristics and traits (synthetic organisms including humans).

We now have synthetic blood, skin, organs and limbs being developed along with synthetic body parts produced by 3D printers. These are all elements of the synthetic human programme and this comment by Kurzweil's co-founder of the Singularity University,

Peter Diamandis, can be seen in a whole new light with the 'Covid' hoax and the sanctions against those that refuse the 'vaccine':

Anybody who is going to be resisting the progress forward [to transhumanism] is going to be resisting evolution and, fundamentally, they will die out. It's not a matter of whether it's good or bad. It's going to happen.

'Resisting evolution'? What absolute bollocks. The arrogance of these people is without limit. His 'it's going to happen' mantra is another way of saying 'resistance is futile' to break the spirit of those pushing back and we must not fall for it. Getting this genetically-transforming 'vaccine' into everyone is crucial to the Cult plan for total control and the desperation to achieve that is clear for anyone to see. Vaccine passports are a major factor in this and they, too, are a form of resistance is futile. It's NOT. The paper funded by the Rockefeller Foundation for the 2013 'health conference' in China said:

We will interact more with artificial intelligence. The use of robotics, bio-engineering to augment human functioning is already well underway and will advance. Re-engineering of humans into potentially separate and unequal forms through genetic engineering or mixed human-robots raises debates on ethics and equality.

A new demography is projected to emerge after 2030 [that year again] of technologies (robotics, genetic engineering, nanotechnology) producing robots, engineered organisms, 'nanobots' and artificial intelligence (AI) that can self-replicate. Debates will grow on the implications of an impending reality of human designed life.

What is happening today is so long planned. The world army enforcing the will of the world government is intended to be a robot army, not a human one. Today's military and its technologically 'enhanced' troops, pilotless planes and driverless vehicles are just stepping stones to that end. Human soldiers are used as Cult fodder and its time they woke up to that and worked for the freedom of the population instead of their own destruction and their family's destruction – the same with the police. Join us and let's sort this out. The phenomenon of enforce my own destruction is widespread in the 'Covid' era with Woker 'luvvies' in the acting and entertainment

industries supporting 'Covid' rules which have destroyed their profession and the same with those among the public who put signs on the doors of their businesses 'closed due to Covid – stay safe' when many will never reopen. It's a form of masochism and most certainly insanity.

Transgender = transhumanism

When something explodes out of nowhere and is suddenly everywhere it is always the Cult agenda and so it is with the tidal wave of claims and demands that have infiltrated every aspect of society under the heading of 'transgenderism'. The term 'trans' is so 'in' and this is the dictionary definition:

A prefix meaning 'across', 'through', occurring … in loanwords from Latin, used in particular for denoting movement or conveyance from place to place (transfer; transmit; transplant) or complete change (transform; transmute), or to form adjectives meaning 'crossing', 'on the other side of', or 'going beyond' the place named (transmontane; transnational; trans-Siberian).

Transgender means to go beyond gender and transhuman means to go beyond human. Both are aspects of the Cult plan to transform the human body to a synthetic state with *no gender*. Human 2.0 is not designed to procreate and would be produced technologically with no need for parents. The new human would mean the end of parents and so men, and increasingly women, are being targeted for the deletion of their rights and status. Parental rights are disappearing at an ever-quickening speed for the same reason. The new human would have no need for men or women when there is no procreation and no gender. Perhaps the transgender movement that appears to be in a permanent state of frenzy might now contemplate on how it is being used. This was never about transgender rights which are only the interim excuse for confusing gender, particularly in the young, on the road to *fusing* gender. Transgender activism is not an end; it is a *means* to an end. We see again the technique of creative destruction in which you destroy the status quo to 'build back better' in the form that you want. The gender status quo had to be

destroyed by persuading the Cult-created Woke mentality to believe that you can have 100 genders or more. A programme for 9 to 12 year olds produced by the Cult-owned BBC promoted the 100 genders narrative. The very idea may be the most monumental nonsense, but it is not what is true that counts, only what you can make people *believe* is true. Once the gender of 2 + 2 = 4 has been dismantled through indoctrination, intimidation and 2 + 2 = 5 then the new no-gender normal can take its place with Human 2.0. Aldous Huxley revealed the plan in his prophetic *Brave New World* in 1932:

> Natural reproduction has been done away with and children are created, decanted', and raised in 'hatcheries and conditioning centres'. From birth, people are genetically designed to fit into one of five castes, which are further split into 'Plus' and 'Minus' members and designed to fulfil predetermined positions within the social and economic strata of the World State.

How could Huxley know this in 1932? For the same reason George Orwell knew about the Big Brother state in 1948, Cult insiders I have quoted knew about it in 1969, and I have known about it since the early 1990s. If you are connected to the Cult or you work your balls off to uncover the plan you can predict the future. The process is simple. If there is a plan for the world and nothing intervenes to stop it then it will happen. Thus if you communicate the plan ahead of time you are perceived to have predicted the future, but you haven't. You have revealed the plan which without intervention will become the human future. The whole reason I have done what I have is to alert enough people to inspire an intervention and maybe at last that time has come with the Cult and its intentions now so obvious to anyone with a brain in working order.

The future is here

Technological wombs that Huxley described to replace parent procreation are already being developed and they are only the projects we know about in the public arena. Israeli scientists told *The Times of Israel* in March, 2021, that they have grown 250-cell embryos

into mouse foetuses with fully formed organs using artificial wombs in a development they say could pave the way for gestating humans outside the womb. Professor Jacob Hanna of the Weizmann Institute of Science said:

We took mouse embryos from the mother at day five of development, when they are just of 250 cells, and had them in the incubator from day five until day 11, by which point they had grown all their organs.

By day 11 they make their own blood and have a beating heart, a fully developed brain. Anybody would look at them and say, 'this is clearly a mouse foetus with all the characteristics of a mouse.' It's gone from being a ball of cells to being an advanced foetus.

A special liquid is used to nourish embryo cells in a laboratory dish and they float on the liquid to duplicate the first stage of embryonic development. The incubator creates all the right conditions for its development, Hanna said. The liquid gives the embryo 'all the nutrients, hormones and sugars they need' along with a custom-made electronic incubator which controls gas concentration, pressure and temperature. The cutting-edge in the underground bases and other secret locations will be light years ahead of that, however, and this was reported by the London *Guardian* in 2017:

We are approaching a biotechnological breakthrough. Ectogenesis, the invention of a complete external womb, could completely change the nature of human reproduction. In April this year, researchers at the Children's Hospital of Philadelphia announced their development of an artificial womb.

The article was headed 'Artificial wombs could soon be a reality. What will this mean for women?' What would it mean for children is an even bigger question. No mother to bond with only a machine in preparation for a life of soulless interaction and control in a world governed by machines (see the *Matrix* movies). Now observe the calculated manipulations of the 'Covid' hoax as human interaction and warmth has been curtailed by distancing, isolation and fear with people communicating via machines on a scale never seen before.

These are all dots in the same picture as are all the personal assistants, gadgets and children's toys through which kids and adults communicate with AI as if it is human. The AI 'voice' on Sat-Nav should be included. All these things are psychological preparation for the Cult endgame. Before you can make a physical connection with AI you have to make a psychological connection and that is what people are being conditioned to do with this ever gathering human-AI interaction. Movies and TV programmes depicting the transhuman, robot dystopia relate to a phenomenon known as 'pre-emptive programming' in which the world that is planned is portrayed everywhere in movies, TV and advertising. This is conditioning the conscious and subconscious mind to become familiar with the planned reality to dilute resistance when it happens for real. What would have been a shock such is the change is made less so. We have young children put on the road to transgender transition surgery with puberty blocking drugs at an age when they could never be able to make those life-changing decisions.

Rachel Levine, a professor of paediatrics and psychiatry who believes in treating children this way, became America's highest-ranked openly-transgender official when she was confirmed as US Assistant Secretary at the Department of Health and Human Services after being nominated by Joe Biden (the Cult). Activists and governments press for laws to deny parents a say in their children's transition process so the kids can be isolated and manipulated into agreeing to irreversible medical procedures. A Canadian father Robert Hoogland was denied bail by the Vancouver Supreme Court in 2021 and remained in jail for breaching a court order that he stay silent over his young teenage daughter, a minor, who was being offered life-changing hormone therapy without parental consent. At the age of 12 the girl's 'school counsellor' said she may be transgender, referred her to a doctor and told the school to treat her like a boy. This is another example of state-serving schools imposing ever more control over children's lives while parents have ever less.

Contemptible and extreme child abuse is happening all over the world as the Cult gender-fusion operation goes into warp-speed.

Why the war on men – and now women?

The question about what artificial wombs mean for women should rightly be asked. The answer can be seen in the deletion of women's rights involving sport, changing rooms, toilets and status in favour of people in male bodies claiming to identify as women. I can identify as a mountain climber, but it doesn't mean I can climb a mountain any more than a biological man can be a biological woman. To believe so is a triumph of belief over factual reality which is the very perceptual basis of everything Woke. Women's sport is being destroyed by allowing those with male bodies who say they identify as female to 'compete' with girls and women. Male body 'women' dominate 'women's' competition with their greater muscle mass, bone density, strength and speed. With that disadvantage sport for women loses all meaning. To put this in perspective nearly 300 American high school boys can run faster than the quickest woman sprinter in the world. Women are seeing their previously protected spaces invaded by male bodies simply because they claim to identify as women. That's all they need to do to access all women's spaces and activities under the Biden 'Equality Act' that destroys equality for women with the usual Orwellian Woke inversion. Male sex offenders have already committed rapes in women's prisons after claiming to identify as women to get them transferred. Does this not matter to the Woke 'equality' hypocrites? Not in the least. What matters to Cult manipulators and funders behind transgender activists is to advance gender fusion on the way to the no-gender 'human'. When you are seeking to impose transparent nonsense like this, or the 'Covid' hoax, the only way the nonsense can prevail is through censorship and intimidation of dissenters, deletion of factual information, and programming of the unquestioning, bewildered and naive. You don't have to scan the world for long to see that all these things are happening.

Many women's rights organisations have realised that rights and status which took such a long time to secure are being eroded and that it is systematic. Kara Dansky of the global Women's Human Rights Campaign said that Biden's transgender executive order immediately he took office, subsequent orders, and Equality Act legislation that followed 'seek to erase women and girls in the law as a category'. *Exactly*. I said during the long ago-started war on men (in which many women play a crucial part) that this was going to turn into a war on them. The Cult is phasing out *both* male and female genders. To get away with that they are brought into conflict so they are busy fighting each other while the Cult completes the job with no unity of response. Unity, people, *unity*. We need unity everywhere. Transgender is the only show in town as the big step towards the no-gender human. It's not about rights for transgender people and never has been. Woke political correctness is deleting words relating to genders to the same end. Wokers believe this is to be 'inclusive' when the opposite is true. They are deleting words describing gender because gender *itself* is being deleted by Human 2.0. Terms like 'man', 'woman', 'mother' and 'father' are being deleted in the universities and other institutions to be replaced by the *no*-gender, not trans-gender, 'individuals' and 'guardians'. Women's rights campaigner Maria Keffler of Partners for Ethical Care said: 'Children are being taught from kindergarten upward that some boys have a vagina, some girls have a penis, and that kids can be any gender they want to be.' Do we really believe that suddenly countries all over the world at the same time had the idea of having drag queens go into schools or read transgender stories to very young children in the local library? It's coldly-calculated confusion of gender on the way to the fusion of gender. Suzanne Vierling, a psychologist from Southern California, made another important point:

Yesterday's slave woman who endured gynecological medical experiments is today's girl-child being butchered in a booming gender-transitioning sector. Ovaries removed, pushing her into menopause and osteoporosis, uncharted territory, and parents' rights and authority decimated.

The erosion of parental rights is a common theme in line with the Cult plans to erase the very concept of parents and 'ovaries removed, pushing her into menopause' means what? Those born female lose the ability to have children – another way to discontinue humanity as we know it.

Eliminating Human 1.0 (before our very eyes)

To pave the way for Human 2.0 you must phase out Human 1.0. This is happening through plummeting sperm counts and making women infertile through an onslaught of chemicals, radiation (including smartphones in pockets of men) and mRNA 'vaccines'. Common agriculture pesticides are also having a devastating impact on human fertility. I have been tracking collapsing sperm counts in the books for a long time and in 2021 came a book by fertility scientist and reproductive epidemiologist Shanna Swan, *Count Down: How Our Modern World Is Threatening Sperm Counts, Altering Male and Female Reproductive Development and Imperiling the Future of the Human Race*. She reports how the global fertility rate dropped by *half* between 1960 and 2016 with America's birth rate 16 percent below where it needs to be to sustain the population. Women are experiencing declining egg quality, more miscarriages, and more couples suffer from infertility. Other findings were an increase in erectile dysfunction, infant boys developing more genital abnormalities, male problems with conception, and plunging levels of the male hormone testosterone which would explain why so many men have lost their backbone and masculinity. This has been very evident during the 'Covid' hoax when women have been prominent among the Pushbackers and big strapping blokes have bowed their heads, covered their faces with a nappy and quietly submitted. Mind control expert Cathy O'Brien also points to how global education introduced the concept of 'we're all winners' in sport and classrooms: 'Competition was defused, and it in turn defused a sense of fighting back.' This is another version of the 'equity' doctrine in which you drive down rather than raise up. What a contrast in Cult-controlled China with its global ambitions

where the government published plans in January, 2021, to 'cultivate masculinity' in boys from kindergarten through to high school in the face of a 'masculinity crisis'. A government adviser said boys would be soon become 'delicate, timid and effeminate' unless action was taken. Don't expect any similar policy in the targeted West. A 2006 study showed that a 65-year-old man in 2002 had testosterone levels *15 percent* lower than a 65-year-old man in 1987 while a 2020 study found a similar story with young adults and adolescents. Men are getting prescriptions for testosterone replacement therapy which causes an even greater drop in sperm count with up to 99 percent seeing sperm counts drop to zero during the treatment. More sperm is defective and malfunctioning with some having two heads or not pursuing an egg.

A class of *synthetic* chemicals known as phthalates are being blamed for the decline. These are found everywhere in plastics, shampoos, cosmetics, furniture, flame retardants, personal care products, pesticides, canned foods and even receipts. Why till receipts? Everyone touches them. Let no one delude themselves that all this is not systematic to advance the long-time agenda for human body transformation. Phthalates mimic hormones and disrupt the hormone balance causing testosterone to fall and genital birth defects in male infants. Animals and fish have been affected in the same way due to phthalates and other toxins in rivers. When fish turn gay or change sex through chemicals in rivers and streams it is a pointer to why there has been such an increase in gay people and the sexually confused. It doesn't matter to me what sexuality people choose to be, but if it's being affected by chemical pollution and consumption then we need to know. Does anyone really think that this is not connected to the transgender agenda, the war on men and the condemnation of male 'toxic masculinity'? You watch this being followed by 'toxic femininity'. It's already happening. When breastfeeding becomes 'chest-feeding', pregnant women become pregnant people along with all the other Woke claptrap you know that the world is going insane and there's a Cult scam in progress. Transgender activists are promoting the Cult agenda while Cult

billionaires support and fund the insanity as they laugh themselves to sleep at the sheer stupidity for which humans must be infamous in galaxies far, far away.

'Covid vaccines' and female infertility

We can now see why the 'vaccine' has been connected to potential infertility in women. Dr Michael Yeadon, former Vice President and Chief Scientific Advisor at Pfizer, and Dr Wolfgang Wodarg in Germany, filed a petition with the European Medicines Agency in December, 2020, urging them to stop trials for the Pfizer/BioNTech shot and all other mRNA trials until further studies had been done. They were particularly concerned about possible effects on fertility with 'vaccine'-produced antibodies attacking the protein Syncytin-1 which is responsible for developing the placenta. The result would be infertility 'of indefinite duration' in women who have the 'vaccine' with the placenta failing to form. Section 10.4.2 of the Pfizer/BioNTech trial protocol says that pregnant women or those who might become so should not have mRNA shots. Section 10.4 warns men taking mRNA shots to 'be abstinent from heterosexual intercourse' and not to donate sperm. The UK government said that it *did not know* if the mRNA procedure had an effect on fertility. *Did not know?* These people have to go to jail. UK government advice did not recommend at the start that pregnant women had the shot and said they should avoid pregnancy for at least two months after 'vaccination'. The 'advice' was later updated to pregnant women should only have the 'vaccine' if the benefits outweighed the risks to mother and foetus. What the hell is that supposed to mean? Then 'spontaneous abortions' began to appear and rapidly increase on the adverse reaction reporting schemes which include only a fraction of adverse reactions. Thousands and ever-growing numbers of 'vaccinated' women are describing changes to their menstrual cycle with heavier blood flow, irregular periods and menstruating again after going through the menopause – all links to reproduction effects. Women are passing blood clots and the lining of their uterus while men report erectile dysfunction and blood effects. Most

significantly of all *un*vaccinated women began to report similar menstrual changes after interaction with *'vaccinated'* people and men and children were also affected with bleeding noses, blood clots and other conditions. 'Shedding' is when vaccinated people can emit the content of a vaccine to affect the unvaccinated, but this is different. 'Vaccinated' people were not shedding a 'live virus' allegedly in 'vaccines' as before because the fake 'Covid vaccines' involve synthetic material and other toxicity. Doctors exposing what is happening prefer the term 'transmission' to shedding. Somehow those that have had the shots are transmitting effects to those that haven't. Dr Carrie Madej said the nano-content of the 'vaccines' can 'act like an antenna' to others around them which fits perfectly with my own conclusions. This 'vaccine' transmission phenomenon was becoming known as the book went into production and I deal with this further in the Postscript.

Vaccine effects on sterility are well known. The World Health Organization was accused in 2014 of sterilising millions of women in Kenya with the evidence confirmed by the content of the vaccines involved. The same WHO behind the 'Covid' hoax admitted its involvement for more than ten years with the vaccine programme. Other countries made similar claims. Charges were lodged by Tanzania, Nicaragua, Mexico, and the Philippines. The Gardasil vaccine claimed to protect against a genital 'virus' known as HPV has also been linked to infertility. Big Pharma and the WHO (same thing) are criminal and satanic entities. Then there's the Bill Gates Foundation which is connected through funding and shared interests with 20 pharmaceutical giants and laboratories. He stands accused of directing the policy of United Nations Children's Fund (UNICEF), vaccine alliance GAVI, and other groupings, to advance the vaccine agenda and silence opposition at great cost to women and children. At the same time Gates wants to reduce the global population. Coincidence?

Great Reset = Smart Grid = new human

The Cult agenda I have been exposing for 30 years is now being openly promoted by Cult assets like Gates and Klaus Schwab of the World Economic Forum under code-terms like the 'Great Reset', 'Build Back Better' and 'a rare but narrow window of opportunity to reflect, reimagine, and reset our world'. What provided this 'rare but narrow window of opportunity'? The 'Covid' hoax did. Who created that? *They* did. My books from not that long ago warned about the planned 'Internet of Things' (IoT) and its implications for human freedom. This was the plan to connect all technology to the Internet and artificial intelligence and today we are way down that road with an estimated 36 billion devices connected to the World Wide Web and that figure is projected to be 76 billion by 2025. I further warned that the Cult planned to go beyond that to the Internet of *Everything* when the human brain was connected via AI to the Internet and Kurzweil's 'cloud'. Now we have Cult operatives like Schwab calling for precisely that under the term 'Internet of Bodies', a fusion of the physical, digital and biological into one centrally-controlled Smart Grid system which the Cult refers to as the 'Fourth Industrial Revolution'. They talk about the 'biological', but they really mean the synthetic-biological which is required to fully integrate the human body and brain into the Smart Grid and artificial intelligence planned to replace the human mind. We have everything being synthetically manipulated including the natural world through GMO and smart dust, the food we eat and the human body itself with synthetic 'vaccines'. I said in *The Answer* that we would see the Cult push for synthetic meat to replace animals and in February, 2021, the so predictable psychopath Bill Gates called for the introduction of synthetic meat to save us all from 'climate change'. The climate hoax just keeps on giving like the 'Covid' hoax. The war on meat by vegan activists is a carbon (oops, sorry) copy of the manipulation of transgender activists. They have no idea (except their inner core) that they are being used to promote and impose the agenda of the Cult or that they are only the *vehicle* and not the *reason*. This is not to say those who choose not to eat meat shouldn't be respected and supported in that right, but there are ulterior motives

for those in power. A *Forbes* article in December, 2019, highlighted the plan so beloved of Schwab and the Cult under the heading: 'What Is The Internet of Bodies? And How Is It Changing Our World?' The article said the human body is the latest data platform (remember 'our vaccine is an operating system'). *Forbes* described the plan very accurately and the words could have come straight out of my books from long before:

The Internet of Bodies (IoB) is an extension of the IoT and basically connects the human body to a network through devices that are ingested, implanted, or connected to the body in some way. Once connected, data can be exchanged, and the body and device can be remotely monitored and controlled.

They were really describing a human hive mind with human perception centrally-dictated via an AI connection as well as allowing people to be 'remotely monitored and controlled'. Everything from a fridge to a human mind could be directed from a central point by these insane psychopaths and 'Covid vaccines' are crucial to this. Forbes explained the process I mentioned earlier of holdable and wearable technology followed by implantable. The article said there were three generations of the Internet of Bodies that include:

- Body external: These are wearable devices such as Apple Watches or Fitbits that can monitor our health.

- Body internal: These include pacemakers, cochlear implants, and digital pills that go inside our bodies to monitor or control various aspects of health.

- Body embedded: The third generation of the Internet of Bodies is embedded technology where technology and the human body are melded together and have a real-time connection to a remote machine.

Forbes noted the development of the Brain Computer Interface (BCI) which merges the brain with an external device for monitoring and controlling in real-time. 'The ultimate goal is to help restore function to individuals with disabilities by using brain signals rather than conventional neuromuscular pathways.' Oh, do fuck off. The goal of brain interface technology is controlling human thought and emotion from the central point in a hive mind serving its masters wishes. Many people are now agreeing to be chipped to open doors without a key. You can recognise them because they'll be wearing a mask, social distancing and lining up for the 'vaccine'. The Cult plans a Great Reset money system after they have completed the demolition of the global economy in which 'money' will be exchanged through communication with body operating systems. Rand Corporation, a Cult-owned think tank, said of the Internet of Bodies or IoB:

> Internet of Bodies technologies fall under the broader IoT umbrella. But as the name suggests, IoB devices introduce an even more intimate interplay between humans and gadgets. IoB devices monitor the human body, collect health metrics and other personal information, and transmit those data over the Internet. Many devices, such as fitness trackers, are already in use … IoB devices … and those in development can track, record, and store users' whereabouts, bodily functions, and what they see, hear, and even think.

Schwab's World Economic Forum, a long-winded way of saying 'fascism' or 'the Cult', has gone full-on with the Internet of Bodies in the 'Covid' era. 'We're entering the era of the Internet of Bodies', it declared, 'collecting our physical data via a range of devices that can be implanted, swallowed or worn'. The result would be a huge amount of health-related data that could improve human wellbeing around the world, and prove crucial in fighting the 'Covid-19 pandemic'. Does anyone think these clowns care about 'human wellbeing' after the death and devastation their pandemic hoax has purposely caused? Schwab and co say we should move forward with the Internet of Bodies because 'Keeping track of symptoms could help us stop the spread of infection, and quickly detect new cases'. How wonderful, but keeping track' is all they are really bothered

about. Researchers were investigating if data gathered from smartwatches and similar devices could be used as viral infection alerts by tracking the user's heart rate and breathing. Schwab said in his 2018 book *Shaping the Future of the Fourth Industrial Revolution*:

> The lines between technologies and beings are becoming blurred and not just by the ability to create lifelike robots or synthetics. Instead it is about the ability of new technologies to literally become part of us. Technologies already influence how we understand ourselves, how we think about each other, and how we determine our realities. As the technologies … give us deeper access to parts of ourselves, we may begin to integrate digital technologies into our bodies.

You can see what the game is. Twenty-four hour control and people – if you could still call them that – would never know when something would go ping and take them out of circulation. It's the most obvious rush to a global fascist dictatorship and the complete submission of humanity and yet still so many are locked away in their Cult-induced perceptual coma and can't see it.

Smart Grid control centres

The human body is being transformed by the 'vaccines' and in other ways into a synthetic cyborg that can be attached to the global Smart Grid which would be controlled from a central point and other sub-locations of Grid manipulation. Where are these planned to be? Well, China for a start which is one of the Cult's biggest centres of operation. The technological control system and technocratic rule was incubated here to be unleashed across the world after the 'Covid' hoax came out of China in 2020. Another Smart Grid location that will surprise people new to this is Israel. I have exposed in *The Trigger* how Sabbatian technocrats, intelligence and military operatives were behind the horrors of 9/11 and not '19 Arab hijackers' who somehow manifested the ability to pilot big passenger airliners when instructors at puddle-jumping flying schools described some of them as a joke. The 9/11 attacks were made possible through control of civilian and military air computer systems and those of the White House, Pentagon and connected agencies. See *The Trigger* – it

will blow your mind. The controlling and coordinating force were the Sabbatian networks in Israel and the United States which by then had infiltrated the entire US government, military and intelligence system. The real name of the American Deep State is 'Sabbatian State'. Israel is a tiny country of only nine million people, but it is one of the global centres of cyber operations and fast catching Silicon Valley in importance to the Cult. Israel is known as the 'start-up nation' for all the cyber companies spawned there with the Sabbatian specialisation of 'cyber security' that I mentioned earlier which gives those companies access to computer systems of their clients in real time through 'backdoors' written into the coding when security software is downloaded. The Sabbatian centre of cyber operations outside Silicon Valley is the Israeli military Cyber Intelligence Unit, the biggest infrastructure project in Israel's history, headquartered in the desert-city of Beersheba and involving some 20,000 'cyber soldiers'. Here are located a literal army of Internet trolls scanning social media, forums and comment lists for anyone challenging the Cult agenda. The UK military has something similar with its 77th Brigade and associated operations. The Beersheba complex includes research and development centres for other Cult operations such as Intel, Microsoft, IBM, Google, Apple, Hewlett-Packard, Cisco Systems, Facebook and Motorola. Techcrunch.com ran an article about the Beersheba global Internet technology centre headlined 'Israel's desert city of Beersheba is turning into a cybertech oasis':

> The military's massive relocation of its prestigious technology units, the presence of multinational and local companies, a close proximity to Ben Gurion University and generous government subsidies are turning Beersheba into a major global cybertech hub. Beersheba has all of the ingredients of a vibrant security technology ecosystem, including Ben Gurion University with its graduate program in cybersecurity and Cyber Security Research Center, and the presence of companies such as EMC, Deutsche Telekom, PayPal, Oracle, IBM, and Lockheed Martin. It's also the future home of the INCB (Israeli National Cyber Bureau); offers a special income tax incentive for cyber security companies, and was the site for the relocation of the army's intelligence corps units.

Sabbatians have taken over the cyber world through the following process: They scan the schools for likely cyber talent and develop them at Ben Gurion University and their period of conscription in the Israeli Defense Forces when they are stationed at the Beersheba complex. When the cyber talented officially leave the army they are funded to start cyber companies with technology developed by themselves or given to them by the state. Much of this is stolen through backdoors of computer systems around the world with America top of the list. Others are sent off to Silicon Valley to start companies or join the major ones and so we have many major positions filled by apparently 'Jewish' but really Sabbatian operatives. Google, YouTube and Facebook are all run by 'Jewish' CEOs while Twitter is all but run by ultra-Zionist hedge-fund shark Paul Singer. At the centre of the Sabbatian global cyber web is the Israeli army's Unit 8200 which specialises in hacking into computer systems of other countries, inserting viruses, gathering information, instigating malfunction, and even taking control of them from a distance. A long list of Sabbatians involved with 9/11, Silicon Valley and Israeli cyber security companies are operatives of Unit 8200. This is not about Israel. It's about the Cult. Israel is planned to be a Smart Grid hub as with China and what is happening at Beersheba is not for the benefit of Jewish people who are treated disgustingly by the Sabbatian elite that control the country. A glance at the Nuremberg Codes will tell you that.

The story is much bigger than 'Covid', important as that is to where we are being taken. Now, though, it's time to really strap in. There's more … much more …

CHAPTER ELEVEN

Who controls the Cult?

Awake, arise or be forever fall'n
John Milton, *Paradise Lost*

I have exposed this far the level of the Cult conspiracy that operates in the world of the seen and within the global secret society and satanic network which operates in the shadows one step back from the seen. The story, however, goes much deeper than that.

The 'Covid' hoax is major part of the Cult agenda, but only part, and to grasp the biggest picture we have to expand our attention beyond the realm of human sight and into the infinity of possibility that we cannot see. It is from here, ultimately, that humanity is being manipulated into a state of total control by the force which dictates the actions of the Cult. How much of reality can we see? Next to damn all is the answer. We may appear to see all there is to see in the 'space' our eyes survey and observe, but little could be further from the truth. The human 'world' is only a tiny band of frequency that the body's visual and perceptual systems can decode into *perception* of a 'world'. According to mainstream science the electromagnetic spectrum is 0.005 percent of what exists in the Universe (Fig 10). The maximum estimate I have seen is 0.5 percent and either way it's miniscule. I say it is far, far, smaller even than 0.005 percent when you compare reality we see with the totality of reality that we don't. Now get this if you are new to such information: Visible light, the only band of frequency that we can see, is a *fraction* of the 0.005

percent (Fig 11 overleaf). Take this further and realise that our universe is one of infinite universes and that universes are only a fragment of overall reality – *infinite* reality. Then compare that with the almost infinitesimal frequency band of visible light or human sight. You see that humans are as near blind as it is possible to be without actually being so. Artist and filmmaker, Sergio Toporek, said:

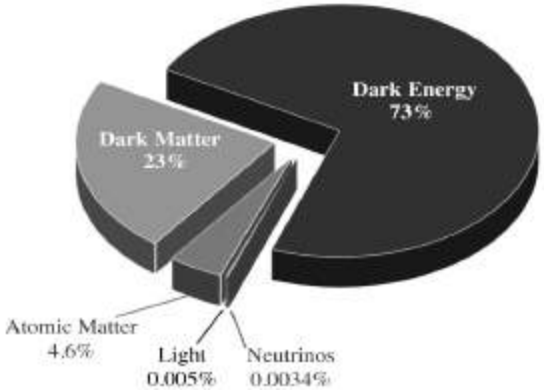

Figure 10: Humans can perceive such a tiny band of visual reality it's laughable.

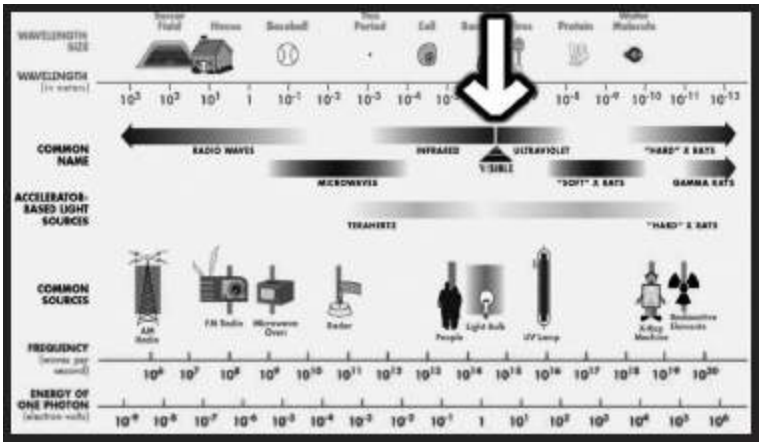

Figure 11: We can see a smear of the 0.005 percent electromagnetic spectrum, but we still know it all. Yep, makes sense.

Consider that you can see less than 1% of the electromagnetic spectrum and hear less than 1% of the acoustic spectrum. 90% of the cells in your body carry their own microbial DNA and are not 'you'. The atoms in your body are 99.9999999999999999% empty space and none of them are the ones you were born with ... Human beings have 46 chromosomes, two less than a potato.

The existence of the rainbow depends on the conical photoreceptors in your eyes; to animals without cones, the rainbow does not exist. So you don't just look at a rainbow, you create it. This is pretty amazing, especially considering that all the beautiful colours you see represent less than 1% of the electromagnetic spectrum.

Suddenly the 'world' of humans looks a very different place. Take into account, too, that Planet Earth when compared with the projected size of this single universe is the equivalent of a billionth of a pinhead. Imagine the ratio that would be when compared to infinite reality. To think that Christianity once insisted that Earth and humanity were the centre of everything. This background is vital if we are going to appreciate the nature of 'human' and how we can be manipulated by an unseen force. To human visual reality virtually *everything* is unseen and yet the prevailing perception within the institutions and so much of the public is that if we can't see it, touch it, hear it, taste it and smell it then it cannot exist. Such perception is indoctrinated and encouraged by the Cult and its agents because it isolates believers in the strictly limited, village-idiot, realm of the five senses where perceptions can be firewalled and information controlled. Most of those perpetuating the 'this-world-is-all-there-is' insanity are themselves indoctrinated into believing the same delusion. While major players and influencers know that official reality is laughable most of those in science, academia and medicine really believe the nonsense they peddle and teach succeeding generations. Those who challenge the orthodoxy are dismissed as nutters and freaks to protect the manufactured illusion from exposure. Observe the dynamic of the 'Covid' hoax and you will see how that takes the same form. The inner-circle psychopaths knows it's a gigantic scam, but almost the entirety of those imposing their fascist rules believe that 'Covid' is all that they're told it is.

Stolen identity

Ask people who they are and they will give you their name, place of birth, location, job, family background and life story. Yet that is not who they are – it is what they are *experiencing*. The difference is *absolutely crucial*. The true 'I', the eternal, infinite 'I', is consciousness,

a state of being aware. Forget 'form'. That is a vehicle for a brief experience. Consciousness does not come *from* the brain, but *through* the brain and even that is more symbolic than literal. We are awareness, pure awareness, and this is what withdraws from the body at what we call 'death' to continue our eternal beingness, *isness*, in other realms of reality within the limitlessness of infinity or the Biblical 'many mansions in my father's house'. Labels of a human life, man, woman, transgender, black, white, brown, nationality, circumstances and income are not who we are. They are what we are – awareness – is *experiencing* in a brief connection with a band of frequency we call 'human'. The labels are not the self; they are, to use the title of one of my books, a *Phantom* Self. I am not David Icke born in Leicester, England, on April 29th, 1952. I am the consciousness *having that experience*. The Cult and its non-human masters seek to convince us through the institutions of 'education', science, medicine, media and government that what we are *experiencing* is who we *are*. It's so easy to control and direct perception locked away in the bewildered illusions of the five senses with no expanded radar. Try, by contrast, doing the same with a humanity aware of its true self and its true power to consciously create its reality and experience. How is it possible to do this? We do it all day every day. If you perceive yourself as 'little me' with no power to impact upon your life and the world then your life experience will reflect that. You will hand the power you don't think you have to authority in all its forms which will use it to control your experience. This, in turn, will appear to confirm your perception of 'little me' in a self-fulfilling feedback loop. But that is what 'little me' really is – a *perception*. We are all 'big-me', infinite me, and the Cult has to make us forget that if its will is to prevail. We are therefore manipulated and pressured into self-identifying with human labels and not the consciousness/awareness *experiencing* those human labels.

The phenomenon of identity politics is a Cult-instigated manipulation technique to sub-divide previous labels into even smaller ones. A United States university employs this list of letters to

describe student identity: LGBTTQQFAGPBDSM or lesbian, gay, bisexual, transgender, transsexual, queer, questioning, flexual, asexual, gender-fuck, polyamorous, bondage/discipline, dominance/submission and sadism/masochism. I'm sure other lists are even longer by now as people feel the need to self-identity the 'I' with the minutiae of race and sexual preference. Wokers programmed by the Cult for generations believe this is about 'inclusivity' when it's really the Cult locking them away into smaller and smaller versions of Phantom Self while firewalling them from the influence of their true self, the infinite, eternal 'I'. You may notice that my philosophy which contends that we are all unique points of attention/awareness within the same infinite whole or Oneness is the ultimate non-racism. The very sense of Oneness makes the judgement of people by their body-type, colour or sexuality utterly ridiculous and confirms that racism has no understanding of reality (including anti-white racism). Yet despite my perception of life Cult agents and fast-asleep Wokers label me racist to discredit my information while they are themselves phenomenally racist and sexist. All they see is race and sexuality and they judge people as good or bad, demons or untouchables, by their race and sexuality. All they see is *Phantom Self* and perceive themselves in terms of Phantom Self. They are pawns and puppets of the Cult agenda to focus attention and self-identity in the five senses and play those identities against each other to divide and rule. Columbia University has introduced segregated graduations in another version of social distancing designed to drive people apart and teach them that different racial and cultural groups have nothing in common with each other. The last thing the Cult wants is unity. Again the pump-primers of this will be Cult operatives in the knowledge of what they are doing, but the rest are just the Phantom Self blind leading the Phantom Self blind. We *do* have something in common – we are all *the same consciousness* having different temporary experiences.

What is this 'human'?

Yes, what *is* 'human'? That is what we are supposed to be, right? I mean 'human'? True, but 'human' is the experience not the 'I'. Break it down to basics and 'human' is the way that information is processed. If we are to experience and interact with this band of frequency we call the 'world' we must have a vehicle that operates within that band of frequency. Our consciousness in its prime form cannot do that; it is way beyond the frequency of the human realm. My consciousness or awareness could not tap these keys and pick up the cup in front of me in the same way that radio station A cannot interact with radio station B when they are on different frequencies. The human body is the means through which we have that interaction. I have long described the body as a biological computer which processes information in a way that allows consciousness to experience this reality. The body is a receiver, transmitter and processor of information in a particular way that we call human. We visually perceive only the world of the five senses in a wakened state – that is the limit of the body's visual decoding system. In truth it's not even visual in the way we experience 'visual reality' as I will come to in a moment. We are 'human' because the body processes the information sources of human into a reality and behaviour system that we *perceive* as human. Why does an elephant act like an elephant and not like a human or a duck? The elephant's biological computer is a different information field and processes information according to that program into a visual and behaviour type we call an elephant. The same applies to everything in our reality. These body information fields are perpetuated through procreation (like making a copy of a software program). The Cult wants to break that cycle and intervene technologically to transform the human information field into one that will change what we call humanity. If it can change the human information field it will change the way that field processes information and change humanity both 'physically' and psychologically. Hence the *messenger* (information) RNA 'vaccines' and so much more that is targeting human genetics by changing the body's information – *messaging* – construct through food, drink, radiation, toxicity and other means.

Reality that we experience is nothing like reality as it really is in the same way that the reality people experience in virtual reality games is not the reality they are really living in. The game is only a decoded source of information that appears to be a reality. Our world is also an information construct – a *simulation* (more later). In its base form our reality is a wavefield of information much the same in theme as Wi-Fi. The five senses decode wavefield information into electrical information which they communicate to the brain to decode into holographic (illusory 'physical') information. Different parts of the brain specialise in decoding different senses and the information is fused into a reality that appears to be outside of us but is really inside the brain and the genetic structure in general (Fig 12 overleaf). DNA is a receiver-transmitter of information and a vital part of this decoding process and the body's connection to other realities. Change DNA and you change the way we decode and connect with reality – see 'Covid vaccines'. Think of computers decoding Wi-Fi. You have information encoded in a radiation field and the computer decodes that information into a very different form on the screen. You can't see the Wi-Fi until its information is made manifest on the screen and the information on the screen is inside the computer and not outside. I have just described how we decode the 'human world'. All five senses decode the waveform 'Wi-Fi' field into electrical signals and the brain (computer) constructs reality inside the brain and not outside – 'You don't just look at a rainbow, you create it'. Sound is a simple example. We don't hear sound until the brain decodes it. Waveform sound waves are picked up by the hearing sense and communicated to the brain in an electrical form to be decoded into the sounds that we hear. Everything we hear is inside the brain along with everything we see, feel, smell and taste. Words and language are waveform fields generated by our vocal chords which pass through this process until they are decoded by the brain into words that we hear. Different languages are different frequency fields or sound waves generated by vocal chords. Late British philosopher Alan Watts said:

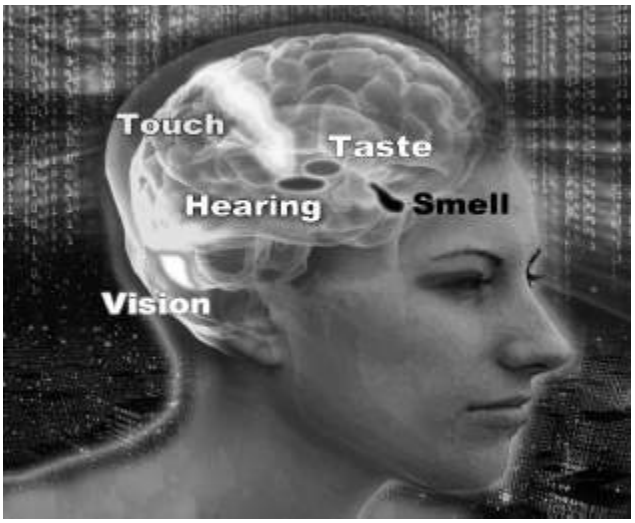

Figure 12: The brain receives information from the five senses and constructs from that our perceived reality.

[Without the brain] the world is devoid of light, heat, weight, solidity, motion, space, time or any other imaginable feature. All these phenomena are interactions, or transactions, of vibrations with a certain arrangement of neurons.

That's exactly what they are and scientist Robert Lanza describes in his book, *Biocentrism*, how we decode electromagnetic waves and energy into visual and 'physical' experience. He uses the example of a flame emitting photons, electromagnetic energy, each pulsing electrically and magnetically:

… these … invisible electromagnetic waves strike a human retina, and if (and only if) the waves happen to measure between 400 and 700 nano meters in length from crest to crest, then their energy is just right to deliver a stimulus to the 8 million cone-shaped cells in the retina.

Each in turn send an electrical pulse to a neighbour neuron, and on up the line this goes, at 250 mph, until it reaches the … occipital lobe of the brain, in the back of the head. There, a cascading complex of neurons fire from the incoming stimuli, and we subjectively perceive this experience as a yellow brightness occurring in a place we have been conditioned to call the 'external world'.

You hear what you decode

If a tree falls or a building collapses they make no noise unless someone is there to decode the energetic waves generated by the disturbance into what we call sound. Does a falling tree make a noise? Only if you hear it – *decode* it. Everything in our reality is a frequency field of information operating within the overall 'Wi-Fi' field that I call The Field. A vibrational disturbance is generated in The Field by the fields of the falling tree or building. These disturbance waves are what we decode into the sound of them falling. If no one is there to do that then neither will make any noise. Reality is created by the observer – *decoder* – and the *perceptions* of the observer affect the decoding process. For this reason different people – different *perceptions* – will perceive the same reality or situation in a different way. What one may perceive as a nightmare another will see as an opportunity. The question of why the Cult is so focused on controlling human perception now answers itself. All experienced reality is the act of decoding and we don't experience Wi-Fi until it is decoded on the computer screen. The sight and sound of an Internet video is encoded in the Wi-Fi all around us, but we don't see or hear it until the computer decodes that information. Taste, smell and touch are all phenomena of the brain as a result of the same process. We don't taste, smell or feel anything except in the brain and there are pain relief techniques that seek to block the signal from the site of discomfort to the brain because if the brain doesn't decode that signal we don't feel pain. Pain is in the brain and only appears to be at the point of impact thanks to the feedback loop between them. We don't see anything until electrical information from the sight senses is decoded in an area at the back of the brain. If that area is damaged we can go blind when our eyes are perfectly okay. So why do we go blind if we damage an eye? We damage the information processing between the waveform visual information and the visual decoding area of the brain. If information doesn't reach the brain in a form it can decode then we can't see the visual reality that it represents. What's more the brain is decoding only a fraction of the information it receives and the rest is absorbed by the

sub-conscious mind. This explanation is from the science magazine, *Wonderpedia*:

> Every second, 11 million sensations crackle along these [brain] pathways ... The brain is confronted with an alarming array of images, sounds and smells which it rigorously filters down until it is left with a manageable list of around 40. Thus 40 sensations per second make up what we perceive as reality.

The 'world' is not what people are told to believe that is it and the inner circles of the Cult *know that*.

Illusory 'physical' reality

We can only see a smear of 0.005 percent of the Universe which is only one of a vast array of universes – 'mansions' – within infinite reality. Even then the brain decodes only 40 pieces of information ('sensations') from a potential *11 million* that we receive every second. Two points strike you from this immediately: The sheer breathtaking stupidity of believing we know anything so rigidly that there's nothing more to know; and the potential for these processes to be manipulated by a malevolent force to control the reality of the population. One thing I can say for sure with no risk of contradiction is that when you can perceive an almost indescribable fraction of infinite reality there is always more to know as in tidal waves of it. Ancient Greek philosopher Socrates was so right when he said that wisdom is to know how little we know. How obviously true that is when you think that we are experiencing a physical world of solidity that is neither physical nor solid and a world of apartness when everything is connected. Cult-controlled 'science' dismisses the so-called 'paranormal' and all phenomena related to that when the 'para'-normal is perfectly normal and explains the alleged 'great mysteries' which dumbfound scientific minds. There is a reason for this. A 'scientific mind' in terms of the mainstream is a material mind, a five-sense mind imprisoned in see it, touch it, hear it, smell it and taste it. Phenomena and happenings that can't be explained that way leave the 'scientific mind' bewildered and the rule is that if they

can't account for why something is happening then it can't, by definition, be happening. I beg to differ. Telepathy is thought waves passing through The Field (think wave disturbance again) to be decoded by someone able to connect with that wavelength (information). For example: You can pick up the thought waves of a friend at any distance and at the very least that will bring them to mind. A few minutes later the friend calls you. 'My god', you say, 'that's incredible – I was just thinking of you.' Ah, but *they* were thinking of *you* before they made the call and that's what you decoded. Native peoples not entrapped in five-sense reality do this so well it became known as the 'bush telegraph'. Those known as psychics and mediums (genuine ones) are doing the same only across dimensions of reality. 'Mind over matter' comes from the fact that matter and mind are the *same*. The state of one influences the state of the other. Indeed one *and* the other are illusions. They are aspects of the same field. Paranormal phenomena are all explainable so why are they still considered 'mysteries' or not happening? Once you go down this road of understanding you begin to expand awareness beyond the five senses and that's the nightmare for the Cult.



Figure 13: Holograms are not solid, but the best ones appear to be.

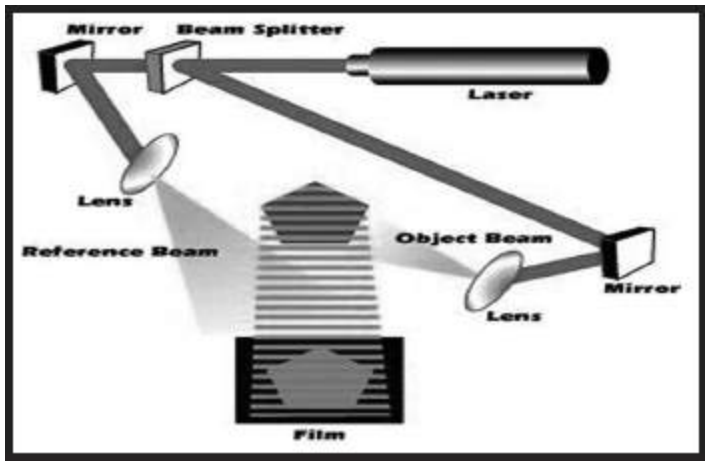

Figure 14: How holograms are created by capturing a waveform version of the subject image.

Holographic 'solidity'

Our reality is not solid, it is holographic. We are now well aware of holograms which are widely used today. Two-dimensional information is decoded into a three-dimensional reality that is not solid although can very much appear to be (Fig 13). Holograms are created with a laser divided into two parts. One goes directly onto a holographic photographic print ('reference beam') and the other takes a waveform image of the subject ('working beam') before being directed onto the print where it 'collides' with the other half of the laser (Fig 14). This creates a *waveform* interference pattern which contains the wavefield information of whatever is being photographed (Fig 15 overleaf). The process can be likened to dropping pebbles in a pond. Waves generated by each one spread out across the water to collide with the others and create a wave representation of where the stones fell and at what speed, weight and distance. A waveform interference pattern of a hologram is akin to the waveform information in The Field which the five senses decode into electrical signals to be decoded by the brain into a holographic illusory 'physical' reality. In the same way when a laser (think human attention) is directed at the waveform interference pattern a three-dimensional version of the subject is projected into apparently 'solid' reality (Fig 16). An amazing trait of holograms reveals more 'paranormal mysteries'. Information of the *whole*

hologram is encoded in waveform in every part of the interference pattern by the way they are created. This means that every *part* of a hologram is a smaller version of the whole. Cut the interference wave-pattern into four and you won't get four parts of the image. You get quarter-sized versions of the *whole* image. The body is a hologram and the same applies. Here we have the basis of acupuncture, reflexology and other forms of healing which identify representations of the whole body in all of the parts, hands, feet, ears, everywhere. Skilled palm readers can do what they do because the information of whole body is encoded in the hand. The concept of as above, so below, comes from this.



Figure 15: A waveform interference pattern that holds the information that transforms into a hologram.



Figure 16: Holographic people including 'Elvis' holographically inserted to sing a duet with Celine Dion.

The question will be asked of why, if solidity is illusory, we can't just walk through walls and each other. The resistance is not solid against solid; it is electromagnetic field against electromagnetic field and we decode this into the *experience* of solid against solid. We should also not underestimate the power of belief to dictate reality. What you believe is impossible *will be*. Your belief impacts on your decoding processes and they won't decode what you think is impossible. What we believe we perceive and what we perceive we experience. 'Can't dos' and 'impossibles' are like a firewall in a computer system that won't put on the screen what the firewall blocks. How vital that is to understanding how human experience has been hijacked. I explain in *The Answer*, *Everything You Need To Know But Have Never Been Told* and other books a long list of 'mysteries' and 'paranormal' phenomena that are not mysterious and perfectly normal once you realise what reality is and how it works. 'Ghosts' can be seen to pass through 'solid' walls because the walls are not solid and the ghost is a discarnate entity operating on a frequency so different to that of the wall that it's like two radio stations sharing the same space while never interfering with each other. I have seen ghosts do this myself. The apartness of people and objects is also an illusion. Everything is connected by the Field like all sea life is connected by the sea. It's just that within the limits of our visual reality we only 'see' holographic information and not the field of information that connects everything and from which the holographic world is made manifest. If you can only see holographic 'objects' and not the field that connects them they will appear to you as unconnected to each other in the same way that we see the computer while not seeing the Wi-Fi.

What you don't know *can* hurt you

Okay, we return to those 'two worlds' of human society and the Cult with its global network of interconnecting secret societies and satanic groups which manipulate through governments, corporations, media, religions, etc. The fundamental difference between them is *knowledge*. The idea has been to keep humanity

ignorant of the plan for its total enslavement underpinned by a crucial ignorance of reality – who we are and where we are – and how we interact with it. 'Human' should be the interaction between our expanded eternal consciousness and the five-sense body experience. We are meant to be *in* this world in terms of the five senses but not *of* this world in relation to our greater consciousness and perspective. In that state we experience the small picture of the five senses within the wider context of the big picture of awareness beyond the five senses. Put another way the five senses see the dots and expanded awareness connects them into pictures and patterns that give context to the apparently random and unconnected. Without the context of expanded awareness the five senses see only apartness and randomness with apparently no meaning. The Cult and its other-dimensional controllers seek to intervene in the frequency realm where five-sense reality is supposed to connect with expanded reality and to keep the two apart (more on this in the final chapter). When that happens five-sense mental and emotional processes are no longer influenced by expanded awareness, or the True 'I', and instead are driven by the isolated perceptions of the body's decoding systems. They are in the world *and* of it. Here we have the human plight and why humanity with its potential for infinite awareness can be so easily manipulatable and descend into such extremes of stupidity.

Once the Cult isolates five-sense mind from expanded awareness it can then program the mind with perceptions and beliefs by controlling information that the mind receives through the 'education' system of the formative years and the media perceptual bombardment and censorship of an entire lifetime. Limit perception and a sense of the possible through limiting knowledge by limiting and skewing information while censoring and discrediting that which could set people free. As the title of another of my books says *… And The Truth Shall Set You Free*. For this reason the last thing the Cult wants in circulation is the truth about anything – especially the reality of the eternal 'I' – and that's why it is desperate to control information. The Cult knows that information becomes perception

which becomes behaviour which, collectively, becomes human society. Cult-controlled and funded mainstream 'science' denies the existence of an eternal 'I' and seeks to dismiss and trash all evidence to the contrary. Cult-controlled mainstream religion has a version of 'God' that is little more than a system of control and dictatorship that employs threats of damnation in an afterlife to control perceptions and behaviour in the here and now through fear and guilt. Neither is true and it's the 'neither' that the Cult wishes to suppress. This 'neither' is that everything is an expression, a point of attention, within an infinite state of consciousness which is the real meaning of the term 'God'.

Perceptual obsession with the 'physical body' and five-senses means that 'God' becomes personified as a bearded bloke sitting among the clouds or a raging bully who loves us if we do what 'he' wants and condemns us to the fires of hell if we don't. These are no more than a 'spiritual' fairy tales to control and dictate events and behaviour through fear of this 'God' which has bizarrely made 'God-fearing' in religious circles a state to be desired. I would suggest that fearing *anything* is not to be encouraged and celebrated, but rather deleted. You can see why 'God fearing' is so beneficial to the Cult and its religions when *they* decide what 'God' wants and what 'God' demands (the Cult demands) that everyone do. As the great American comedian Bill Hicks said satirising a Christian zealot: 'I think what God meant to say.' How much of this infinite awareness ('God') that we access is decided by how far we choose to expand our perceptions, self-identity and sense of the possible. The scale of self-identity reflects itself in the scale of awareness that we can connect with and are influenced by – how much knowing and insight we have instead of programmed perception. You cannot expand your awareness into the infinity of possibility when you believe that you are little me Peter the postman or Mary in marketing and nothing more. I'll deal with this in the concluding chapter because it's crucial to how we turnaround current events.

Where the Cult came from

When I realised in the early 1990s there was a Cult network behind global events I asked the obvious question: When did it start? I took it back to ancient Rome and Egypt and on to Babylon and Sumer in Mesopotamia, the 'Land Between Two Rivers', in what we now call Iraq. The two rivers are the Tigris and Euphrates and this region is of immense historical and other importance to the Cult, as is the land called Israel only 550 miles away by air. There is much more going with deep esoteric meaning across this whole region. It's not only about 'wars for oil'. Priceless artefacts from Mesopotamia were stolen or destroyed after the American and British invasion of Iraq in 2003 justified by the lies of Boy Bush and Tony Blair (their Cult masters) about non-existent 'weapons of mass destruction'. Mesopotamia was the location of Sumer (about 5,400BC to 1,750BC), and Babylon (about 2,350BC to 539BC). Sabbatians may have become immensely influential in the Cult in modern times but they are part of a network that goes back into the mists of history. Sumer is said by historians to be the 'cradle of civilisation'. I disagree. I say it was the re-start of what we call human civilisation after cataclysmic events symbolised in part as the 'Great Flood' destroyed the world that existed before. These fantastic upheavals that I have been describing in detail in the books since the early1990s appear in accounts and legends of ancient cultures across the world and they are supported by geological and biological evidence. Stone tablets found in Iraq detailing the Sumer period say the cataclysms were caused by non-human 'gods' they call the Anunnaki. These are described in terms of extraterrestrial visitations in which knowledge supplied by the Anunnaki is said to have been the source of at least one of the world's oldest writing systems and developments in astronomy, mathematics and architecture that were way ahead of their time. I have covered this subject at length in *The Biggest Secret* and *Children of the Matrix* and the same basic 'Anunnaki' story can be found in Zulu accounts in South Africa where the late and very great Zulu high shaman Credo Mutwa told me that the Sumerian Anunnaki were known by Zulus as the Chitauri or 'children of the serpent'. See my six-hour video interview with Credo on this subject entitled *The*

Reptilian Agenda recorded at his then home near Johannesburg in 1999 which you can watch on the Ickonic media platform.

The Cult emerged out of Sumer, Babylon and Egypt (and elsewhere) and established the Roman Empire before expanding with the Romans into northern Europe from where many empires were savagely imposed in the form of Cult-controlled societies all over the world. Mass death and destruction was their calling card. The Cult established its centre of operations in Europe and European Empires were Cult empires which allowed it to expand into a global force. Spanish and Portuguese colonialists headed for Central and South America while the British and French targeted North America. Africa was colonised by Britain, France, Belgium, the Netherlands, Portugal, Spain, Italy, and Germany. Some like Britain and France moved in on the Middle East. The British Empire was by far the biggest for a simple reason. By now Britain was the headquarters of the Cult from which it expanded to form Canada, the United States, Australia and New Zealand. The Sun never set on the British Empire such was the scale of its occupation. London remains a global centre for the Cult along with Rome and the Vatican although others have emerged in Israel and China. It is no accident that the 'virus' is alleged to have come out of China while Italy was chosen as the means to terrify the Western population into compliance with 'Covid' fascism. Nor that Israel has led the world in 'Covid' fascism and mass 'vaccination'.

You would think that I would mention the United States here, but while it has been an important means of imposing the Cult's will it is less significant than would appear and is currently in the process of having what power it does have deleted. The Cult in Europe has mostly loaded the guns for the US to fire. America has been controlled from Europe from the start through Cult operatives in Britain and Europe. The American Revolution was an illusion to make it appear that America was governing itself while very different forces were pulling the strings in the form of Cult families such as the Rothschilds through the Rockefellers and other subordinates. The Rockefellers are extremely close to Bill Gates and

established both scalpel and drug 'medicine' and the World Health Organization. They play a major role in the development and circulation of vaccines through the Rockefeller Foundation on which Bill Gates said his Foundation is based. Why wouldn't this be the case when the Rockefellers and Gates are on the same team? Cult infiltration of human society goes way back into what we call history and has been constantly expanding and centralising power with the goal of establishing a global structure to dictate everything. Look how this has been advanced in great leaps with the 'Covid' hoax.

The non-human dimension

I researched and observed the comings and goings of Cult operatives through the centuries and even thousands of years as they were born, worked to promote the agenda within the secret society and satanic networks, and then died for others to replace them. Clearly there had to be a coordinating force that spanned this entire period while operatives who would not have seen the end goal in their lifetimes came and went advancing the plan over millennia. I went in search of that coordinating force with the usual support from the extraordinary synchronicity of my life which has been an almost daily experience since 1990. I saw common themes in religious texts and ancient cultures about a non-human force manipulating human society from the hidden. Christianity calls this force Satan, the Devil and demons; Islam refers to the Jinn or Djinn; Zulus have their Chitauri (spelt in other ways in different parts of Africa); and the Gnostic people in Egypt in the period around and before 400AD referred to this phenomena as the 'Archons', a word meaning rulers in Greek. Central American cultures speak of the 'Predators' among other names and the same theme is everywhere. I will use 'Archons' as a collective name for all of them. When you see how their nature and behaviour is described all these different sources are clearly talking about the same force. Gnostics described the Archons in terms of 'luminous fire' while Islam relates the Jinn to 'smokeless fire'. Some refer to beings in form that could occasionally be seen, but the most common of common theme is that they operate from

unseen realms which means almost all existence to the visual processes of humans. I had concluded that this was indeed the foundation of human control and that the Cult was operating within the human frequency band on behalf of this hidden force when I came across the writings of Gnostics which supported my conclusions in the most extraordinary way.

A sealed earthen jar was found in 1945 near the town of Nag Hammadi about 75-80 miles north of Luxor on the banks of the River Nile in Egypt. Inside was a treasure trove of manuscripts and texts left by the Gnostic people some 1,600 years earlier. They included 13 leather-bound papyrus codices (manuscripts) and more than 50 texts written in Coptic Egyptian estimated to have been hidden in the jar in the period of 400AD although the source of the information goes back much further. Gnostics oversaw the Great or Royal Library of Alexandria, the fantastic depository of ancient texts detailing advanced knowledge and accounts of human history. The Library was dismantled and destroyed in stages over a long period with the death-blow delivered by the Cult-established Roman Church in the period around 415AD. The Church of Rome was the Church of Babylon relocated as I said earlier. Gnostics were not a race. They were a way of perceiving reality. Whenever they established themselves and their information circulated the terrorists of the Church of Rome would target them for destruction. This happened with the Great Library and with the Gnostic Cathars who were burned to death by the psychopaths after a long period of oppression at the siege of the Castle of Monségur in southern France in 1244. The Church has always been terrified of Gnostic information which demolishes the official Christian narrative although there is much in the Bible that supports the Gnostic view if you read it in another way. To anyone studying the texts of what became known as the Nag Hammadi Library it is clear that great swathes of Christian and Biblical belief has its origin with Gnostics sources going back to Sumer. Gnostic themes have been twisted to manipulate the perceived reality of Bible believers. Biblical texts have been in the open for centuries where they could be changed while Gnostic

documents found at Nag Hammadi were sealed away and untouched for 1,600 years. What you see is what they wrote.

Use your *pneuma* not your *nous*

Gnosticism and Gnostic come from 'gnosis' which means knowledge, or rather *secret* knowledge, in the sense of spiritual awareness – knowledge about reality and life itself. The desperation of the Cult's Church of Rome to destroy the Gnostics can be understood when the knowledge they were circulating was the last thing the Cult wanted the population to know. Sixteen hundred years later the same Cult is working hard to undermine and silence me for the same reason. The dynamic between knowledge and ignorance is a constant. 'Time' appears to move on, but essential themes remain the same. We are told to 'use your nous', a Gnostic word for head/brain/intelligence. They said, however, that spiritual awakening or 'salvation' could only be secured by expanding awareness *beyond* what they called *nous* and into *pneuma* or Infinite Self. Obviously as I read these texts the parallels with what I have been saying since 1990 were fascinating to me. There is a universal truth that spans human history and in that case why wouldn't we be talking the same language 16 centuries apart? When you free yourself from the perception program of the five senses and explore expanded realms of consciousness you are going to connect with the same information no matter what the perceived 'era' within a manufactured timeline of a single and tiny range of manipulated frequency. Humans working with 'smart' technology or knocking rocks together in caves is only a timeline appearing to operate within the human frequency band. Expanded awareness and the knowledge it holds have always been there whether the era be Stone Age or computer age. We can only access that knowledge by opening ourselves to its frequency which the five-sense prison cell is designed to stop us doing. Gates, Fauci, Whitty, Vallance, Zuckerberg, Brin, Page, Wojcicki, Bezos, and all the others behind the 'Covid' hoax clearly have a long wait before their range of frequency can make that connection given that an open heart is

crucial to that as we shall see. Instead of accessing knowledge directly through expanded awareness it is given to Cult operatives by the secret society networks of the Cult where it has been passed on over thousands of years outside the public arena. Expanded realms of consciousness is where great artists, composers and writers find their inspiration and where truth awaits anyone open enough to connect with it. We need to go there fast.

Archon hijack

A fifth of the Nag Hammadi texts describe the existence and manipulation of the Archons led by a 'Chief Archon' they call 'Yaldabaoth', or the 'Demiurge', and this is the Christian 'Devil', 'Satan', 'Lucifer', and his demons. Archons in Biblical symbolism are the 'fallen ones' which are also referred to as fallen angels after the angels expelled from heaven according to the Abrahamic religions of Judaism, Christianity and Islam. These angels are claimed to tempt humans to 'sin' ongoing and you will see how accurate that symbolism is during the rest of the book. The theme of 'original sin' is related to the 'Fall' when Adam and Eve were 'tempted by the serpent' and fell from a state of innocence and 'obedience' (connection) with God into a state of disobedience (disconnection). The Fall is said to have brought sin into the world and corrupted everything including human nature. Yaldabaoth, the 'Lord Archon', is described by Gnostics as a 'counterfeit spirit', 'The Blind One', 'The Blind God', and 'The Foolish One'. The Jewish name for Yaldabaoth in Talmudic writings is Samael which translates as 'Poison of God', or 'Blindness of God'. You see the parallels. Yaldabaoth in Islamic belief is the Muslim Jinn devil known as Shaytan – Shaytan is Satan as the same themes are found all over the world in every religion and culture. The 'Lord God' of the Old Testament is the 'Lord Archon' of Gnostic manuscripts and that's why he's such a bloodthirsty bastard. Satan is known by Christians as 'the Demon of Demons' and Gnostics called Yaldabaoth the 'Archon of Archons'. Both are known as 'The Deceiver'. We are talking about the same 'bloke' for sure and these common themes

using different names, storylines and symbolism tell a common tale of the human plight.

Archons are referred to in Nag Hammadi documents as mind parasites, inverters, guards, gatekeepers, detainers, judges, pitiless ones and deceivers. The 'Covid' hoax alone is a glaring example of all these things. The Biblical 'God' is so different in the Old and New Testaments because they are not describing the same phenomenon. The vindictive, angry, hate-filled, 'God' of the Old Testament, known as Yahweh, is Yaldabaoth who is depicted in Cult-dictated popular culture as the 'Dark Lord', 'Lord of Time', Lord (Darth) Vader and Dormammu, the evil ruler of the 'Dark Dimension' trying to take over the 'Earth Dimension' in the Marvel comic movie, *Dr Strange*. Yaldabaoth is both the Old Testament 'god' and the Biblical 'Satan'. Gnostics referred to Yaldabaoth as the 'Great Architect of the Universe'and the Cult-controlled Freemason network calls their god 'the 'Great Architect of the Universe' (also Grand Architect). The 'Great Architect' Yaldabaoth is symbolised by the Cult as the all-seeing eye at the top of the pyramid on the Great Seal of the United States and the dollar bill. Archon is encoded in *arch*-itect as it is in *arch*-angels and *arch*-bishops. All religions have the theme of a force for good and force for evil in some sort of spiritual war and there is a reason for that – the theme is true. The Cult and its non-human masters are quite happy for this to circulate. They present themselves as the force for good fighting evil when they are really the force of evil (absence of love). The whole foundation of Cult modus operandi is inversion. They promote themselves as a force for good and anyone challenging them in pursuit of peace, love, fairness, truth and justice is condemned as a satanic force for evil. This has been the game plan throughout history whether the Church of Rome inquisitions of non-believers or 'conspiracy theorists' and 'anti-vaxxers' of today. The technique is the same whatever the timeline era.

Yaldabaoth is revolting (true)

Yaldabaoth and the Archons are said to have revolted against God with Yaldabaoth claiming to *be* God – the *All That Is*. The Old Testament 'God' (Yaldabaoth) demanded to be worshipped as such: ' *I am* the LORD, and there is none else, there is no God beside me' (Isaiah 45:5). I have quoted in other books a man who said he was the unofficial son of the late Baron Philippe de Rothschild of the Mouton-Rothschild wine producing estates in France who died in 1988 and he told me about the Rothschild 'revolt from God'. The man said he was given the name Phillip Eugene de Rothschild and we shared long correspondence many years ago while he was living under another identity. He said that he was conceived through 'occult incest' which (within the Cult) was 'normal and to be admired'. 'Phillip' told me about his experience attending satanic rituals with rich and famous people whom he names and you can see them and the wider background to Cult Satanism in my other books starting with *The Biggest Secret*. Cult rituals are interactions with Archontic 'gods'. 'Phillip' described Baron Philippe de Rothschild as 'a master Satanist and hater of God' and he used the same term 'revolt from God' associated with Yaldabaoth/Satan/Lucifer/the Devil in describing the Sabbatian Rothschild dynasty. 'I played a key role in my family's revolt from God', he said. That role was to infiltrate in classic Sabbatian style the Christian Church, but eventually he escaped the mind-prison to live another life. The Cult has been targeting religion in a plan to make worship of the Archons the global one-world religion. Infiltration of Satanism into modern 'culture', especially among the young, through music videos, stage shows and other means, is all part of this.

Nag Hammadi texts describe Yaldabaoth and the Archons in their prime form as energy – consciousness – and say they can take form if they choose in the same way that consciousness takes form as a human. Yaldabaoth is called 'formless' and represents a deeply inverted, distorted and chaotic state of consciousness which seeks to attached to humans and turn them into a likeness of itself in an attempt at assimilation. For that to happen it has to manipulate

humans into low frequency mental and emotional states that match its own. Archons can certainly appear in human form and this is the origin of the psychopathic personality. The energetic distortion Gnostics called Yaldabaoth *is* psychopathy. When psychopathic Archons take human form that human will be a psychopath as an expression of Yaldabaoth consciousness. Cult psychopaths are Archons in human form. The principle is the same as that portrayed in the 2009 *Avatar* movie when the American military travelled to a fictional Earth-like moon called Pandora in the Alpha Centauri star system to infiltrate a society of blue people, or Na'vi, by hiding within bodies that looked like the Na'vi. Archons posing as humans have a particular hybrid information field, part human, part Archon, (the ancient 'demigods') which processes information in a way that manifests behaviour to match their psychopathic evil, lack of empathy and compassion, and stops them being influenced by the empathy, compassion and love that a fully-human information field is capable of expressing. Cult bloodlines interbreed, be they royalty or dark suits, for this reason and you have their obsession with incest. Interbreeding with full-blown humans would dilute the Archontic energy field that guarantees psychopathy in its representatives in the human realm.

Gnostic writings say the main non-human forms that Archons take are *serpentine* (what I have called for decades 'reptilian' amid unbounded ridicule from the Archontically-programmed) and what Gnostics describe as 'an unborn baby or foetus with grey skin and dark, unmoving eyes'. This is an excellent representation of the ET 'Greys' of UFO folklore which large numbers of people claim to have seen and been abducted by – Zulu shaman Credo Mutwa among them. I agree with those that believe in extraterrestrial or interdimensional visitations today and for thousands of years past. No wonder with their advanced knowledge and technological capability they were perceived and worshipped as gods for technological and other 'miracles' they appeared to perform. Imagine someone arriving in a culture disconnected from the modern world with a smartphone and computer. They would be

seen as a 'god' capable of 'miracles'. The Renegade Mind, however, wants to know the source of everything and not only the way that source manifests as human or non-human. In the same way that a Renegade Mind seeks the original source material for the 'Covid virus' to see if what is claimed is true. The original source of Archons in form is consciousness – the distorted state of consciousness known to Gnostics as Yaldabaoth.

'Revolt from God' is energetic disconnection

Where I am going next will make a lot of sense of religious texts and ancient legends relating to 'Satan', Lucifer' and the 'gods'. Gnostic descriptions sync perfectly with the themes of my own research over the years in how they describe a consciousness distortion seeking to impose itself on human consciousness. I've referred to the core of infinite awareness in previous books as Infinite Awareness in Awareness of Itself. By that I mean a level of awareness that knows that it is all awareness and is aware of all awareness. From here comes the frequency of love in its true sense and balance which is what love is on one level – the balance of all forces into a single whole called Oneness and Isness. The more we disconnect from this state of love that many call 'God' the constituent parts of that Oneness start to unravel and express themselves as a part and not a whole. They become individualised as intellect, mind, selfishness, hatred, envy, desire for power over others, and such like. This is not a problem in the greater scheme in that 'God', the *All That Is*, can experience all these possibilities through different expressions of itself including humans. What we as expressions of the whole experience the *All That Is* experiences. We are the *All That Is* experiencing itself. As we withdraw from that state of Oneness we disconnect from its influence and things can get very unpleasant and very stupid. Archontic consciousness is at the extreme end of that. It has so disconnected from the influence of Oneness that it has become an inversion of unity and love, an inversion of everything, an inversion of life itself. Evil is appropriately live written backwards. Archontic consciousness is obsessed with death, an inversion of life,

and so its manifestations in Satanism are obsessed with death. They use inverted symbols in their rituals such as the inverted pentagram and cross. Sabbatians as Archontic consciousness incarnate invert Judaism and every other religion and culture they infiltrate. They seek disunity and chaos and they fear unity and harmony as they fear love like garlic to a vampire. As a result the Cult, Archons incarnate, act with such evil, psychopathy and lack of empathy and compassion disconnected as they are from the source of love. How could Bill Gates and the rest of the Archontic psychopaths do what they have to human society in the 'Covid' era with all the death, suffering and destruction involved and have no emotional consequence for the impact on others? Now you know. Why have Zuckerberg, Brin, Page, Wojcicki and company callously censored information warning about the dangers of the 'vaccine' while thousands have been dying and having severe, sometimes life-changing reactions? Now you know. Why have Tedros, Fauci, Whitty, Vallance and their like around the world been using case and death figures they're aware are fraudulent to justify lockdowns and all the deaths and destroyed lives that have come from that? Now you know. Why did Christian Drosten produce and promote a 'testing' protocol that he knew couldn't test for infectious disease which led to a global human catastrophe. Now you know. The Archontic mind doesn't give a shit (Fig 17). I personally think that Gates and major Cult insiders are a form of AI cyborg that the Archons want humans to become.

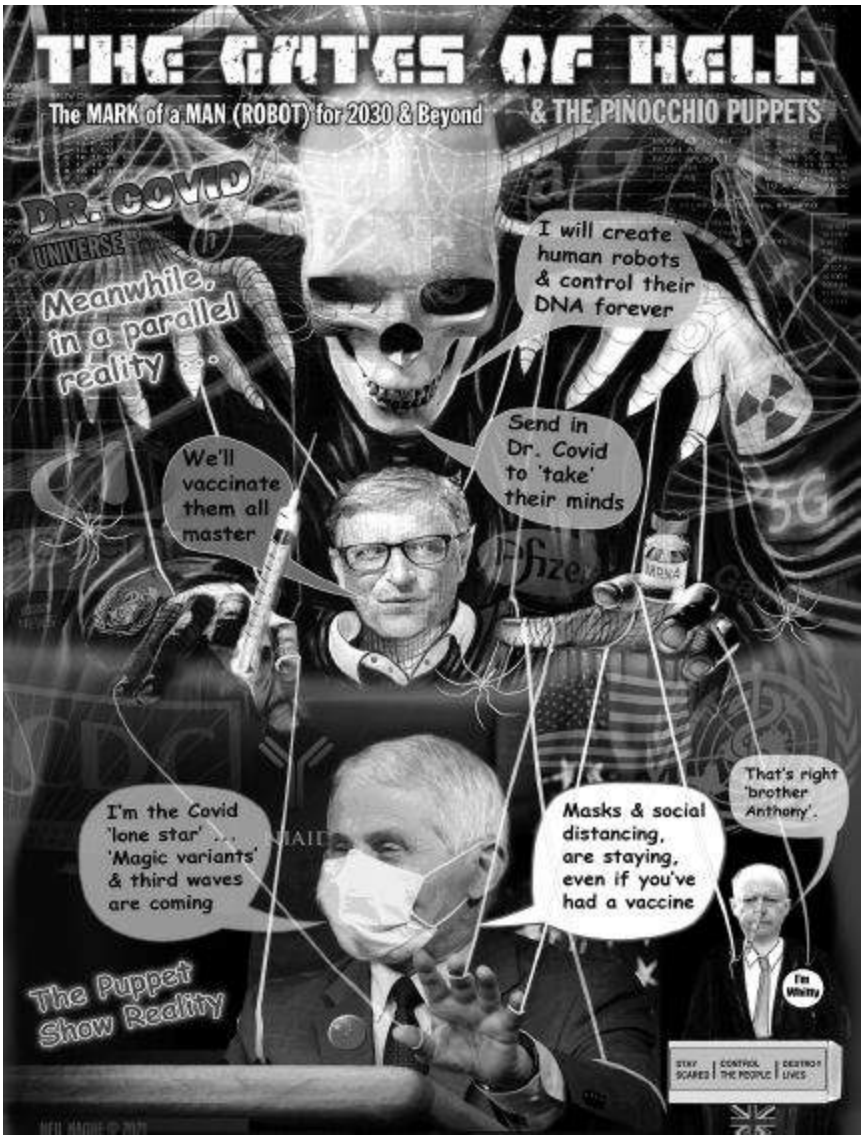

Figure 17: Artist Neil Hague's version of the 'Covid' hierarchy.

Human batteries

A state of such inversion does have its consequences, however. The level of disconnection from the Source of All means that you withdraw from that source of energetic sustenance and creativity. This means that you have to find your own supply of energetic power and it has – *us*. When the Morpheus character in the first *Matrix* movie held up a battery he spoke a profound truth when he said: 'The Matrix is a computer-generated dream world built to keep us under control in order to change the human being into one of

these.' The statement was true in all respects. We do live in a technologically-generated virtual reality simulation (more very shortly) and we have been manipulated to be an energy source for Archontic consciousness. The Disney-Pixar animated movie *Monsters, Inc*. in 2001 symbolised the dynamic when monsters in their world had no energy source and they would enter the human world to terrify children in their beds, catch the child's scream, terror (low-vibrational frequencies), and take that energy back to power the monster world. The lead character you might remember was a single giant eye and the symbolism of the Cult's all-seeing eye was obvious. Every thought and emotion is broadcast as a frequency unique to that thought and emotion. Feelings of love and joy, empathy and compassion, are high, quick, frequencies while fear, depression, anxiety, suffering and hate are low, slow, dense frequencies. Which kind do you think Archontic consciousness can connect with and absorb? In such a low and dense frequency state there's no way it can connect with the energy of love and joy. Archons can only feed off energy compatible with their own frequency and they and their Cult agents want to delete the human world of love and joy and manipulate the transmission of low vibrational frequencies through low-vibrational human mental and emotional states. *We are their energy source*. Wars are energetic banquets to the Archons – a world war even more so – and think how much low-frequency mental and emotional energy has been generated from the consequences for humanity of the 'Covid' hoax orchestrated by Archons incarnate like Gates.

The ancient practice of human sacrifice 'to the gods', continued in secret today by the Cult, is based on the same principle. 'The gods' are Archontic consciousness in different forms and the sacrifice is induced into a state of intense terror to generate the energy the Archontic frequency can absorb. Incarnate Archons in the ritual drink the blood which contains an adrenaline they crave which floods into the bloodstream when people are terrorised. Most of the sacrifices, ancient and modern, are children and the theme of 'sacrificing young virgins to the gods' is just code for children. They

have a particular pre-puberty energy that Archons want more than anything and the energy of the young in general is their target. The California Department of Education wants students to chant the names of Aztec gods (Archontic gods) once worshipped in human sacrifice rituals in a curriculum designed to encourage them to 'challenge racist, bigoted, discriminatory, imperialist/colonial beliefs', join 'social movements that struggle for social justice', and 'build new possibilities for a post-racist, post-systemic racism society'. It's the usual Woke crap that inverts racism and calls it anti-racism. In this case solidarity with 'indigenous tribes' is being used as an excuse to chant the names of 'gods' to which people were sacrificed (and still are in secret). What an example of Woke's inability to see beyond black and white, us and them, They condemn the colonisation of these tribal cultures by Europeans (quite right), but those cultures sacrificing people including children to their 'gods', and mass murdering untold numbers as the Aztecs did, is just fine. One chant is to the Aztec god Tezcatlipoca who had a man sacrificed to him in the 5th month of the Aztec calendar. His heart was cut out and he was eaten. Oh, that's okay then. Come on children … after three … Other sacrificial 'gods' for the young to chant their allegiance include Quetzalcoatl, Huitzilopochtli and Xipe Totec. The curriculum says that 'chants, affirmations, and energizers can be used to bring the class together, build unity around ethnic studies principles and values, and to reinvigorate the class following a lesson that may be emotionally taxing or even when student engagement may appear to be low'. Well, that's the cover story, anyway. Chanting and mantras are the repetition of a particular frequency generated from the vocal cords and chanting the names of these Archontic 'gods' tunes you into their frequency. That is the last thing you want when it allows for energetic synchronisation, attachment and perceptual influence. Initiates chant the names of their 'Gods' in their rituals for this very reason.

Vampires of the Woke

Paedophilia is another way that Archons absorb the energy of children. Paedophiles possessed by Archontic consciousness are used as the conduit during sexual abuse for discarnate Archons to vampire the energy of the young they desire so much. Stupendous numbers of children disappear every year never to be seen again although you would never know from the media. Imagine how much low-vibrational energy has been generated by children during the 'Covid' hoax when so many have become depressed and psychologically destroyed to the point of killing themselves. Shocking numbers of children are now taken by the state from loving parents to be handed to others. I can tell you from long experience of researching this since 1996 that many end up with paedophiles and assets of the Cult through corrupt and Cult-owned social services which in the reframing era has hired many psychopaths and emotionless automatons to do the job. Children are even stolen to order using spurious reasons to take them by the corrupt and secret (because they're corrupt) 'family courts'. I have written in detail in other books, starting with *The Biggest Secret* in 1997, about the ubiquitous connections between the political, corporate, government, intelligence and military elites (Cult operatives) and Satanism and paedophilia. If you go deep enough both networks have an interlocking leadership. The Woke mentality has been developed by the Cult for many reasons: To promote almost every aspect of its agenda; to hijack the traditional political left and turn it fascist; to divide and rule; and to target agenda pushbackers. But there are other reasons which relate to what I am describing here. How many happy and joyful Wokers do you ever see especially at the extreme end? They are a mental and psychological mess consumed by emotional stress and constantly emotionally cocked for the next explosion of indignation at someone referring to a female as a female. They are walking, talking, batteries as Morpheus might say emitting frequencies which both enslave them in low-vibrational bubbles of perceptual limitation and feed the Archons. Add to this the hatred claimed to be love; fascism claimed to 'anti-fascism', racism claimed to be 'anti-racism';

exclusion claimed to inclusion; and the abuse-filled Internet trolling. You have a purpose-built Archontic energy system with not a wind turbine in sight and all founded on Archontic *inversion*. We have whole generations now manipulated to serve the Archons with their actions and energy. They will be doing so their entire adult lives unless they snap out of their Archon-induced trance. Is it really a surprise that Cult billionaires and corporations put so much money their way? Where is the energy of joy and laughter, including laughing at yourself which is confirmation of your own emotional security? Mark Twain said: 'The human race has one really effective weapon, and that is laughter.' We must use it all the time. Woke has destroyed comedy because it has no humour, no joy, sense of irony, or self-deprecation. Its energy is dense and intense. *Mmmmm*, lunch says the Archontic frequency. Rudolf Steiner (1861-1925) was the Austrian philosopher and famous esoteric thinker who established Waldorf education or Steiner schools to treat children like unique expressions of consciousness and not minds to be programmed with the perceptions determined by authority. I'd been writing about this energy vampiring for decades when I was sent in 2016 a quote by Steiner. He was spot on:

> There are beings in the spiritual realms for whom anxiety and fear emanating from human beings offer welcome food. When humans have no anxiety and fear, then these creatures starve. If fear and anxiety radiates from people and they break out in panic, then these creatures find welcome nutrition and they become more and more powerful. These beings are hostile towards humanity. Everything that feeds on negative feelings, on anxiety, fear and superstition, despair or doubt, are in reality hostile forces in super-sensible worlds, launching cruel attacks on human beings, while they are being fed ... These are exactly the feelings that belong to contemporary culture and materialism; because it estranges people from the spiritual world, it is especially suited to evoke hopelessness and fear of the unknown in people, thereby calling up the above mentioned hostile forces against them.

Pause for a moment from this perspective and reflect on what has happened in the world since the start of 2020. Not only will pennies drop, but billion dollar bills. We see the same theme from Don Juan Matus, a Yaqui Indian shaman in Mexico and the information source for Peruvian-born writer, Carlos Castaneda, who wrote a series of

books from the 1960s to 1990s. Don Juan described the force manipulating human society and his name for the Archons was the predator:

> We have a predator that came from the depths of the cosmos and took over the rule of our lives. Human beings are its prisoners. The predator is our lord and master. It has rendered us docile, helpless. If we want to protest, it suppresses our protest. If we want to act independently, it demands that we don't do so ... indeed we are held prisoner!
>
> They took us over because we are food to them, and they squeeze us mercilessly because we are their sustenance. Just as we rear chickens in coops, the predators rear us in human coops, humaneros. Therefore, their food is always available to them.
>
> Different cultures, different eras, same recurring theme.

The 'ennoia' dilemma

Nag Hammadi Gnostic manuscripts say that Archon consciousness has no 'ennoia'. This is directly translated as 'intentionality', but I'll use the term 'creative imagination'. The *All That Is* in awareness of itself is the source of all creativity – all possibility – and the more disconnected you are from that source the more you are subsequently denied 'creative imagination'. Given that Archon consciousness is almost entirely disconnected it severely lacks creativity and has to rely on far more mechanical processes of thought and exploit the creative potential of those that do have 'ennoia'. You can see cases of this throughout human society. Archon consciousness almost entirely dominates the global banking system and if we study how that system works you will appreciate what I mean. Banks manifest 'money' out of nothing by issuing lines of 'credit' which is 'money' that has never, does not, and will never exist except in theory. It's a confidence trick. If you think 'credit' figures-on-a-screen 'money' is worth anything you accept it as payment. If you don't then the whole system collapses through lack of confidence in the value of that 'money'. Archontic bankers with no 'ennoia' are 'lending' 'money' that doesn't exist to humans that *do* have creativity – those that have the inspired ideas and create businesses and products. Archon banking feeds off human creativity

which it controls through 'money' creation and debt. Humans have the creativity and Archons exploit that for their own benefit and control while having none themselves. Archon Internet platforms like Facebook claim joint copyright of everything that creative users post and while Archontic minds like Zuckerberg may officially head that company it will be human creatives on the staff that provide the creative inspiration. When you have limitless 'money' you can then buy other companies established by creative humans. Witness the acquisition record of Facebook, Google and their like. Survey the Archon-controlled music industry and you see non-creative dark suit executives making their fortune from the human creativity of their artists. The cases are endless. Research the history of people like Gates and Zuckerberg and how their empires were built on exploiting the creativity of others. Archon minds cannot create out of nothing, but they are skilled (because they have to be) in what Gnostic texts call 'countermimicry'. They can imitate, but not innovate. Sabbatians trawl the creativity of others through backdoors they install in computer systems through their cybersecurity systems. Archon-controlled China is globally infamous for stealing intellectual property and I remember how Hong Kong, now part of China, became notorious for making counterfeit copies of the creativity of others – 'countermimicry'. With the now pervasive and all-seeing surveillance systems able to infiltrate any computer you can appreciate the potential for Archons to vampire the creativity of humans. Author John Lamb Lash wrote in his book about the Nag Hammadi texts, *Not In His Image*:

> Although they cannot originate anything, because they lack the divine factor of ennoia (intentionality), Archons can imitate with a vengeance. Their expertise is simulation (HAL, virtual reality). The Demiurge [Yaldabaoth] fashions a heaven world copied from the fractal patterns [of the original] ... His construction is celestial kitsch, like the fake Italianate villa of a Mafia don complete with militant angels to guard every portal.

This brings us to something that I have been speaking about since the turn of the millennium. Our reality is a simulation; a virtual reality that we think is real. No, I'm not kidding.

Human reality? Well, virtually

I had pondered for years about whether our reality is 'real' or some kind of construct. I remembered being immensely affected on a visit as a small child in the late 1950s to the then newly-opened Planetarium on the Marylebone Road in London which is now closed and part of the adjacent Madame Tussauds wax museum. It was in the middle of the day, but when the lights went out there was the night sky projected in the Planetarium's domed ceiling and it appeared to be so real. The experience never left me and I didn't know why until around the turn of the millennium when I became certain that our 'night sky' and entire reality is a projection, a virtual reality, akin to the illusory world portrayed in the *Matrix* movies. I looked at the sky one day in this period and it appeared to me like the domed roof of the Planetarium. The release of the first *Matrix* movie in 1999 also provided a synchronistic and perfect visual representation of where my mind had been going for a long time. I hadn't come across the Gnostic Nag Hammadi texts then. When I did years later the correlation was once again astounding. As I read Gnostic accounts from 1,600 years and more earlier it was clear that they were describing the same simulation phenomenon. They tell how the Yaldabaoth 'Demiurge' and Archons created a 'bad copy' of original reality to rule over all that were captured by its illusions and the body was a prison to trap consciousness in the 'bad copy' fake reality. Read how Gnostics describe the 'bad copy' and update that to current times and they are referring to what we would call today a virtual reality simulation.

Author John Lamb Lash said 'the Demiurge fashions a heaven world copied from the fractal patterns' of the original through expertise in 'HAL' or virtual reality simulation. Fractal patterns are part of the energetic information construct of our reality, a sort of blueprint. If these patterns were copied in computer terms it would indeed give you a copy of a 'natural' reality in a non-natural frequency and digital form. The principle is the same as making a copy of a website. The original website still exists, but now you can change the copy version to make it whatever you like and it can

become very different to the original website. Archons have done this with our reality, a *synthetic* copy of prime reality that still exists beyond the frequency walls of the simulation. Trapped within the illusions of this synthetic Matrix, however, were and are human consciousness and other expressions of prime reality and this is why the Archons via the Cult are seeking to make the human body synthetic and give us synthetic AI minds to complete the job of turning the entire reality synthetic including what we perceive to be the natural world. To quote Kurzweil: 'Nanobots will infuse all the matter around us with information. Rocks, trees, everything will become these intelligent creatures.' Yes, *synthetic* 'creatures' just as 'Covid' and other genetically-manipulating 'vaccines' are designed to make the human body synthetic. From this perspective it is obvious why Archons and their Cult are so desperate to infuse synthetic material into every human with their 'Covid' scam.

Let there be (electromagnetic) light

Yaldabaoth, the force that created the simulation, or Matrix, makes sense of the Gnostic reference to 'The Great Architect' and its use by Cult Freemasonry as the name of its deity. The designer of the Matrix in the movies is called 'The Architect' and that trilogy is jam-packed with symbolism relating to these subjects. I have contended for years that the angry Old Testament God (Yaldabaoth) is the 'God' being symbolically 'quoted' in the opening of Genesis as 'creating the world'. This is not the creation of prime reality – it's the creation of the *simulation*. The Genesis 'God' says: 'Let there be Light: and there was light.' But what is this 'Light'? I have said for decades that the speed of light (186,000 miles per second) is not the fastest speed possible as claimed by mainstream science and is in fact the frequency walls or outer limits of the Matrix. You can't have a fastest or slowest anything within all possibility when everything is possible. The human body is encoded to operate within the speed of light or *within the simulation* and thus we see only the tiny frequency band of visible *light*. Near-death experiencers who perceive reality outside the body during temporary 'death' describe a very different

form of light and this is supported by the Nag Hammadi texts. Prime reality beyond the simulation ('Upper Aeons' to the Gnostics) is described as a realm of incredible beauty, bliss, love and harmony – a realm of 'watery light' that is so powerful 'there are no shadows'. Our false reality of Archon control, which Gnostics call the 'Lower Aeons', is depicted as a realm with a different kind of 'light' and described in terms of chaos, 'Hell', 'the Abyss' and 'Outer Darkness', where trapped souls are tormented and manipulated by demons (relate that to the 'Covid' hoax alone). The watery light theme can be found in near-death accounts and it is not the same as *simulation* 'light' which is electromagnetic or radiation light within the speed of light – the 'Lower Aeons'. Simulation 'light' is the 'luminous fire' associated by Gnostics with the Archons. The Bible refers to Yaldabaoth as 'that old serpent, called the Devil, and Satan, which deceiveth the whole world' (Revelation 12:9). I think that making a simulated copy of prime reality ('countermimicry') and changing it dramatically while all the time manipulating humanity to believe it to be real could probably meet the criteria of deceiving the whole world. Then we come to the Cult god Lucifer – the *Light Bringer*. Lucifer is symbolic of Yaldabaoth, the bringer of radiation light that forms the bad copy simulation within the speed of light. 'He' is symbolised by the lighted torch held by the Statue of Liberty and in the name 'Illuminati'. Sabbatian-Frankism declares that Lucifer is the true god and Lucifer is the real god of Freemasonry honoured as their 'Great or Grand Architect of the Universe' (simulation).

I would emphasise, too, the way Archontic technologically-generated luminous fire of radiation has deluged our environment since I was a kid in the 1950s and changed the nature of The Field with which we constantly interact. Through that interaction technological radiation is changing us. The Smart Grid is designed to operate with immense levels of communication power with 5G expanding across the world and 6G, 7G, in the process of development. Radiation is the simulation and the Archontic manipulation system. Why wouldn't the Archon Cult wish to unleash radiation upon us to an ever-greater extreme to form

Kurzweil's 'cloud'? The plan for a synthetic human is related to the need to cope with levels of radiation beyond even anything we've seen so far. Biological humans would not survive the scale of radiation they have in their script. The Smart Grid is a technological sub-reality within the technological simulation to further disconnect five-sense perception from expanded consciousness. It's a technological prison of the mind.

Infusing the 'spirit of darkness'

A recurring theme in religion and native cultures is the manipulation of human genetics by a non-human force and most famously recorded as the biblical 'sons of god' (the god*s* plural in the original) who interbred with the daughters of men. The Nag Hammadi *Apocryphon of John* tells the same story this way:

> He [Yaldabaoth] sent his angels [Archons/demons] to the daughters of men, that they might take some of them for themselves and raise offspring for their enjoyment. And at first they did not succeed. When they had no success, they gathered together again and they made a plan together ... And the angels changed themselves in their likeness into the likeness of their mates, filling them with the spirit of darkness, which they had mixed for them, and with evil ... And they took women and begot children out of the darkness according to the likeness of their spirit.

Possession when a discarnate entity takes over a human body is an age-old theme and continues today. It's very real and I've seen it. Satanic and secret society rituals can create an energetic environment in which entities can attach to initiates and I've heard many stories of how people have changed their personality after being initiated even into lower levels of the Freemasons. I have been inside three Freemasonic temples, one at a public open day and two by just walking in when there was no one around to stop me. They were in Ryde, the town where I live, Birmingham, England, when I was with a group, and Boston, Massachusetts. They all felt the same energetically – dark, dense, low-vibrational and sinister. Demonic attachment can happen while the initiate has no idea what is going on. To them it's just a ritual to get in the Masons and do a bit of good

business. In the far more extreme rituals of Satanism human possession is even more powerful and they are designed to make possession possible. The hierarchy of the Cult is dictated by the power and perceived status of the possessing Archon. In this way the Archon hierarchy becomes the Cult hierarchy. Once the entity has attached it can influence perception and behaviour and if it attaches to the extreme then so much of its energy (information) infuses into the body information field that the hologram starts to reflect the nature of the possessing entity. This is the *Exorcist* movie type of possession when facial features change and it's known as shapeshifting. Islam's Jinn are said to be invisible tricksters who change shape, 'whisper', confuse and take human form. These are all traits of the Archons and other versions of the same phenomenon. Extreme possession could certainty infuse the 'spirit of darkness' into a partner during sex as the Nag Hammadi texts appear to describe. Such an infusion can change genetics which is also energetic information. Human genetics is information and the 'spirit of darkness' is information. Mix one with the other and change must happen. Islam has the concept of a 'Jinn baby' through possession of the mother and by Jinn taking human form. There are many ways that human genetics can be changed and remember that Archons have been aware all along of advanced techniques to do this. What is being done in human society today – and far more – was known about by Archons at the time of the 'fallen ones' and their other versions described in religions and cultures.

Archons and their human-world Cult are obsessed with genetics as we see today and they know this dictates how information is processed into perceived reality during a human life. They needed to produce a human form that would decode the simulation and this is symbolically known as 'Adam and Eve' who left the 'garden' (prime reality) and 'fell' into Matrix reality. The simulation is not a 'physical' construct (there is no 'physical'); it is a source of information. Think Wi-Fi again. The simulation is an energetic field encoded with information and body-brain systems are designed to decode that information encoded in wave or frequency form which

is transmitted to the brain as electrical signals. These are decoded by the brain to construct our sense of reality – an illusory 'physical' world that only exists in the brain or the mind. Virtual reality games mimic this process using the same sensory decoding system. Information is fed to the senses to decode a virtual reality that can appear so real, but isn't (Figs 18 and 19). Some scientists believe – and I agree with them – that what we perceive as 'physical' reality only exists when we are looking or observing. The act of perception or focus triggers the decoding systems which turn waveform information into holographic reality. When we are not observing something our reality reverts from a holographic state to a waveform state. This relates to the same principle as a falling tree not making a noise unless someone is there to hear it or decode it. The concept makes sense from the simulation perspective. A computer is not decoding all the information in a Wi-Fi field all the time and only decodes or brings into reality on the screen that part of Wi-Fi that it's decoding – focusing upon – at that moment.



Figure 18: Virtual reality technology 'hacks' into the body's five-sense decoding system.



Figure 19: The result can be experienced as very 'real'.

Interestingly, Professor Donald Hoffman at the Department of Cognitive Sciences at the University of California, Irvine, says that our experienced reality is like a computer interface that shows us only the level with which we interact while hiding all that exists beyond it: 'Evolution shaped us with a user interface that hides the truth. Nothing that we see is the truth – the very language of space and time and objects is the wrong language to describe reality.' He is correct in what he says on so many levels. Space and time are not a universal reality. They are a phenomenon of decoded *simulation* reality as part of the process of enslaving our sense of reality. Near-death experiencers report again and again how space and time did not exist as we perceive them once they were free of the body – body decoding systems. You can appreciate from this why Archons and their Cult are so desperate to entrap human attention in the five senses where we are in the Matrix and of the Matrix. Opening your mind to expanded states of awareness takes you beyond the information confines of the simulation and you become aware of knowledge and insights denied to you before. This is what we call 'awakening' – *awakening from the Matrix* – and in the final chapter I will relate this to current events.

Where are the 'aliens'?

A simulation would explain the so-called 'Fermi Paradox' named after Italian physicist Enrico Fermi (1901-1954) who created the first nuclear reactor. He considered the question of why there is such a lack of extraterrestrial activity when there are so many stars and planets in an apparently vast universe; but what if the night sky that we see, or think we do, is a simulated projection as I say? If you control the simulation and your aim is to hold humanity fast in essential ignorance would you want other forms of life including advanced life coming and going sharing information with humanity? Or would you want them to believe they were isolated and apparently alone? Themes of human isolation and apartness are common whether they be the perception of a lifeless universe or the fascist isolation laws of the 'Covid' era. Paradoxically the very

existence of a simulation means that we are not alone when some force had to construct it. My view is that experiences that people have reported all over the world for centuries with Reptilians and Grey entities are Archon phenomena as Nag Hammadi texts describe; and that benevolent 'alien' interactions are non-human groups that come in and out of the simulation by overcoming Archon attempts to keep them out. It should be highlighted, too, that Reptilians and Greys are obsessed with *genetics* and *technology* as related by cultural accounts and those who say they have been abducted by them. Technology is their way of overcoming some of the limitations in their creative potential and our technology-driven and controlled human society of today is *arch*etypical Archon-Reptilian-Grey modus operandi. Technocracy is really *Archon*tocracy. The Universe does not have to be as big as it appears with a simulation. There is no space or distance only information decoded into holographic reality. What we call 'space' is only the absence of holographic 'objects' and that 'space' is The Field of energetic information which connects everything into a single whole. The same applies with the artificially-generated information field of the simulation. The Universe is not big or small as a physical reality. It is decoded information, that's all, and its perceived size is decided by the way the simulation is encoded to make it appear. The entire night sky as we perceive it only exists in our brain and so where are those 'millions of light years'? The 'stars' on the ceiling of the Planetarium looked a vast distance away.

There's another point to mention about 'aliens'. I have been highlighting since the 1990s the plan to stage a fake 'alien invasion' to justify the centralisation of global power and a world military. Nazi scientist Werner von Braun, who was taken to America by Operation Paperclip after World War Two to help found NASA, told his American assistant Dr Carol Rosin about the Cult agenda when he knew he was dying in 1977. Rosin said that he told her about a sequence that would lead to total human control by a one-world government. This included threats from terrorism, rogue nations, meteors and asteroids before finally an 'alien invasion'. All of these

things, von Braun said, would be bogus and what I would refer to as a No-Problem-Reaction-Solution. Keep this in mind when 'the aliens are coming' is the new mantra. The aliens are not coming – they are *already here* and they have infiltrated human society while looking human. French-Canadian investigative journalist Serge Monast said in 1994 that he had uncovered a NASA/military operation called Project Blue Beam which fits with what Werner von Braun predicted. Monast died of a 'heart attack' in 1996 the day after he was arrested and spent a night in prison. He was 51. He said Blue Beam was a plan to stage an alien invasion that would include religious figures beamed holographically into the sky as part of a global manipulation to usher in a 'new age' of worshipping what I would say is the Cult 'god' Yaldabaoth in a one-world religion. Fake holographic asteroids are also said to be part of the plan which again syncs with von Braun. How could you stage an illusory threat from asteroids unless they were holographic inserts? This is pretty straightforward given the advanced technology outside the public arena and the fact that our 'physical' reality is holographic anyway. Information fields would be projected and we would decode them into the illusion of a 'physical' asteroid. If they can sell a global 'pandemic' with a 'virus' that doesn't exist what will humans not believe if government and media tell them?

All this is particularly relevant as I write with the Pentagon planning to release in June, 2021, information about 'UFO sightings'. I have been following the UFO story since the early 1990s and the common theme throughout has been government and military denials and cover up. More recently, however, the Pentagon has suddenly become more talkative and apparently open with Air Force pilot radar images released of unexplained craft moving and changing direction at speeds well beyond anything believed possible with human technology. Then, in March, 2021, former Director of National Intelligence John Ratcliffe said a Pentagon report months later in June would reveal a great deal of information about UFO sightings unknown to the public. He said the report would have 'massive implications'. The order to do this was included bizarrely

in a $2.3 trillion 'coronavirus' relief and government funding bill passed by the Trump administration at the end of 2020. I would add some serious notes of caution here. I have been pointing out since the 1990s that the US military and intelligence networks have long had craft – 'flying saucers' or anti-gravity craft – which any observer would take to be extraterrestrial in origin. Keeping this knowledge from the public allows craft flown by *humans* to be perceived as alien visitations. I am not saying that 'aliens' do not exist. I would be the last one to say that, but we have to be streetwise here. President Ronald Reagan told the UN General Assembly in 1987: 'I occasionally think how quickly our differences worldwide would vanish if we were facing an alien threat from outside this world.' That's the idea. Unite against a common 'enemy' with a common purpose behind your 'saviour force' (the Cult) as this age-old technique of mass manipulation goes global.

Science moves this way …

I could find only one other person who was discussing the simulation hypothesis publicly when I concluded it was real. This was Nick Bostrom, a Swedish-born philosopher at the University of Oxford, who has explored for many years the possibility that human reality is a computer simulation although his version and mine are not the same. Today the simulation and holographic reality hypothesis have increasingly entered the scientific mainstream. Well, the more open-minded mainstream, that is. Here are a few of the ever-gathering examples. American nuclear physicist Silas Beane led a team of physicists at the University of Bonn in Germany pursuing the question of whether we live in a simulation. They concluded that we probably do and it was likely based on a lattice of cubes. They found that cosmic rays align with that specific pattern. The team highlighted the Greisen–Zatsepin–Kuzmin (GZK) limit which refers to cosmic ray particle interaction with cosmic background radiation that creates an apparent boundary for cosmic ray particles. They say in a paper entitled 'Constraints on the Universe as a Numerical Simulation' that this 'pattern of constraint' is exactly what you

would find with a computer simulation. They also made the point that a simulation would create its own 'laws of physics' that would limit possibility. I've been making the same point for decades that the *perceived* laws of physics relate only to this reality, or what I would later call the simulation. When designers write codes to create computer and virtual reality games they are the equivalent of the laws of physics for that game. Players interact within the limitations laid out by the coding. In the same way those who wrote the codes for the simulation decided the laws of physics that would apply. These can be overridden by expanded states of consciousness, but not by those enslaved in only five-sense awareness where simulation codes rule. Overriding the codes is what people call 'miracles'. They are not. They are bypassing the encoded limits of the simulation. A population caught in simulation perception would have no idea that this was their plight. As the Bonn paper said: 'Like a prisoner in a pitch-black cell we would not be able to see the "walls" of our prison,' That's true if people remain mesmerised by the five senses. Open to expanded awareness and those walls become very clear. The main one is the speed of light.

American theoretical physicist James Gates is another who has explored the simulation question and found considerable evidence to support the idea. Gates was Professor of Physics at the University of Maryland, Director of The Center for String and Particle Theory, and on Barack Obama's Council of Advisors on Science and Technology. He and his team found *computer codes* of digital data embedded in the fabric of our reality. They relate to on-off electrical charges of 1 and 0 in the binary system used by computers. 'We have no idea what they are doing there', Gates said. They found within the energetic fabric mathematical sequences known as error-correcting codes or block codes that 'reboot' data to its original state or 'default settings' when something knocks it out of sync. Gates was asked if he had found a set of equations embedded in our reality indistinguishable from those that drive search engines and browsers and he said: 'That is correct.' Rich Terrile, director of the Centre for Evolutionary Computation and Automated Design at NASA's Jet

Propulsion Laboratory, has said publicly that he believes the Universe is a digital hologram that must have been created by a form of intelligence. I agree with that in every way. Waveform information is delivered electrically by the senses to the brain which constructs a *digital* holographic reality that we call the 'world'. This digital level of reality can be read by the esoteric art of numerology. Digital holograms are at the cutting edge of holographics today. We have digital technology everywhere designed to access and manipulate our digital level of perceived reality. Synthetic mRNA in 'Covid vaccines' has a digital component to manipulate the body's digital 'operating system'.

Reality is numbers

How many know that our reality can be broken down to numbers and codes that are the same as computer games? Max Tegmark, a physicist at the Massachusetts Institute of Technology (MIT), is the author of *Our Mathematical Universe* in which he lays out how reality can be entirely described by numbers and maths in the way that a video game is encoded with the 'physics' of computer games. Our world and computer virtual reality are essentially the same. Tegmark imagines the perceptions of characters in an advanced computer game when the graphics are so good they don't know they are in a game. They think they can bump into real objects (electromagnetic resistance in our reality), fall in love and feel emotions like excitement. When they began to study the apparently 'physical world' of the video game they would realise that everything was made of pixels (which have been found in our energetic reality as must be the case when on one level our world is digital). What computer game characters thought was physical 'stuff', Tegmark said, could actually be broken down into numbers:

> And we're exactly in this situation in our world. We look around and it doesn't seem that mathematical at all, but everything we see is made out of elementary particles like quarks and electrons. And what properties does an electron have? Does it have a smell or a colour or a texture? No! ... We physicists have come up with geeky names for [Electron] properties, like

electric charge, or spin, or lepton number, but the electron doesn't care what we call it, the properties are just numbers.

This is the illusory reality Gnostics were describing. This is the simulation. The A, C, G, and T codes of DNA have a binary value – A and C = 0 while G and T = 1. This has to be when the simulation is digital and the body must be digital to interact with it. Recurring mathematical sequences are encoded throughout reality and the body. They include the Fibonacci sequence in which the two previous numbers are added to get the next one, as in ... 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, etc. The sequence is encoded in the human face and body, proportions of animals, DNA, seed heads, pine cones, trees, shells, spiral galaxies, hurricanes and the number of petals in a flower. The list goes on and on. There are fractal patterns – a 'never-ending pattern that is infinitely complex and self-similar across all scales in the as above, so below, principle of holograms. These and other famous recurring geometrical and mathematical sequences such as Phi, Pi, Golden Mean, Golden Ratio and Golden Section are *computer codes* of the simulation. I had to laugh and give my head a shake the day I finished this book and it went into the production stage. I was sent an article in *Scientific American* published in April, 2021, with the headline 'Confirmed! We Live in a Simulation'. Two decades after I first said our reality is a simulation and the speed of light is it's outer limit the article suggested that we do live in a simulation and that the speed of light is its outer limit. I left school at 15 and never passed a major exam in my life while the writer was up to his eyes in qualifications. As I will explain in the final chapter *knowing* is far better than thinking and they come from very different sources. The article rightly connected the speed of light to the processing speed of the 'Matrix' and said what has been in my books all this time … 'If we are in a simulation, as it appears, then space is an abstract property written in code. It is not real'. No it's not and if we live in a simulation something created it and it wasn't *us*. 'That David Icke says we are manipulated by aliens' – he's crackers.'

Wow …

The reality that humanity thinks is so real is an illusion. Politicians, governments, scientists, doctors, academics, law enforcement, media, school and university curriculums, on and on, are all founded on a world that *does not exist* except as a simulated prison cell. Is it such a stretch to accept that 'Covid' doesn't exist when our entire 'physical' reality doesn't exist? Revealed here is the knowledge kept under raps in the Cult networks of compartmentalised secrecy to control humanity's sense of reality by inducing the population to believe in a reality that's not real. If it wasn't so tragic in its experiential consequences the whole thing would be hysterically funny. None of this is new to Renegade Minds. Ancient Greek philosopher Plato (about 428 to about 347BC) was a major influence on Gnostic belief and he described the human plight thousands of years ago with his Allegory of the Cave. He told the symbolic story of prisoners living in a cave who had never been outside. They were chained and could only see one wall of the cave while behind them was a fire that they could not see. Figures walked past the fire casting shadows on the prisoners' wall and those moving shadows became their sense of reality. Some prisoners began to study the shadows and were considered experts on them (today's academics and scientists), but what they studied was only an illusion (today's academics and scientists). A prisoner escaped from the cave and saw reality as it really is. When he returned to report this revelation they didn't believe him, called him mad and threatened to kill him if he tried to set them free. Plato's tale is not only a brilliant analogy of the human plight and our illusory reality. It describes, too, the dynamics of the 'Covid' hoax. I have only skimmed the surface of these subjects here. The aim of this book is to crisply connect all essential dots to put what is happening today into its true context. All subject areas and their connections in this chapter are covered in great evidential detail in *Everything You Need To Know, But Have Never Been Told* and *The Answer*.

They say that bewildered people 'can't see the forest for the trees'. Humanity, however, can't see the forest for the *twigs*. The five senses

see only twigs while Renegade Minds can see the forest and it's the forest where the answers lie with the connections that reveals. Breaking free of perceptual programming so the forest can be seen is the way we turn all this around. Not breaking free is how humanity got into this mess. The situation may seem hopeless, but I promise you it's not. We are a perceptual heartbeat from paradise if only we knew.

CHAPTER TWELVE

Escaping Wetiko

Life is simply a vacation from the infinite
Dean Cavanagh

Renegade Minds weave the web of life and events and see common themes in the apparently random. They are always there if you look for them and their pursuit is aided by incredible synchronicity that comes when your mind is open rather than mesmerised by what it thinks it can see.

Infinite awareness is infinite possibility and the more of infinite possibility that we access the more becomes infinitely possible. That may be stating the apparently obvious, but it is a devastatingly-powerful fact that can set us free. We are a point of attention within an infinity of consciousness. The question is how much of that infinity do we choose to access? How much knowledge, insight, awareness, wisdom, do we want to connect with and explore? If your focus is only in the five senses you will be influenced by a fraction of infinite awareness. I mean a range so tiny that it gives new meaning to infinitesimal. Limitation of self-identity and a sense of the possible limit accordingly your range of consciousness. We are what we think we are. Life is what we think it is. The dream is the dreamer and the dreamer is the dream. Buddhist philosophy puts it this way: 'As a thing is viewed, so it appears.' Most humans live in the realm of touch, taste, see, hear, and smell and that's the limit of their sense of the possible and sense of self. Many will follow a religion and speak of a God in his heaven, but their lives are still

dominated by the five senses in their perceptions and actions. The five senses become the arbiter of everything. When that happens all except a smear of infinity is sealed away from influence by the rigid, unyielding, reality bubbles that are the five-sense human or Phantom Self. Archon Cult methodology is to isolate consciousness within five-sense reality – the simulation – and then program that consciousness with a sense of self and the world through a deluge of life-long information designed to instil the desired perception that allows global control. Efforts to do this have increased dramatically with identity politics as identity bubbles are squeezed into the minutiae of five-sense detail which disconnect people even more profoundly from the infinite 'I'.

Five-sense focus and self-identity are like a firewall that limits access to the infinite realms. You only perceive one radio or television station and no other. We'll take that literally for a moment. Imagine a vast array of stations giving different information and angles on reality, but you only ever listen to one. Here we have the human plight in which the population is overwhelmingly confined to CultFM. This relates only to the frequency range of CultFM and limits perception and insight to that band – limits *possibility* to that band. It means you are connecting with an almost imperceptibly minuscule range of possibility and creative potential within the infinite Field. It's a world where everything seems apart from everything else and where synchronicity is rare. Synchronicity is defined in the dictionary as 'the happening by chance of two or more related or similar events at the same time'. Use of 'by chance' betrays a complete misunderstanding of reality. Synchronicity is not 'by chance'. As people open their minds, or 'awaken' to use the term, they notice more and more coincidences in their lives, bits of 'luck', apparently miraculous happenings that put them in the right place at the right time with the right people. Days become peppered with 'fancy meeting you here' and 'what are the chances of that?' My entire life has been lived like this and ever more so since my own colossal awakening in 1990 and 91 which transformed my sense of reality. Synchronicity is not 'by chance'; it is by accessing expanded

realms of possibility which allow expanded potential for manifestation. People broadcasting the same vibe from the same openness of mind tend to be drawn 'by chance' to each other through what I call frequency magnetism and it's not only people. In the last more than 30 years incredible synchronicity has also led me through the Cult maze to information in so many forms and to crucial personal experiences. These 'coincidences' have allowed me to put the puzzle pieces together across an enormous array of subjects and situations. Those who have breached the bubble of five-sense reality will know exactly what I mean and this escape from the perceptual prison cell is open to everyone whenever they make that choice. This may appear super-human when compared with the limitations of 'human', but it's really our natural state. 'Human' as currently experienced is consciousness in an unnatural state of induced separation from the infinity of the whole. I'll come to how this transformation into unity can be made when I have described in more detail the force that holds humanity in servitude by denying this access to infinite self.

The Wetiko factor

I have been talking and writing for decades about the way five-sense mind is systematically barricaded from expanded awareness. I have used the analogy of a computer (five-sense mind) and someone at the keyboard (expanded awareness). Interaction between the computer and the operator is symbolic of the interaction between five-sense mind and expanded awareness. The computer directly experiences the Internet and the operator experiences the Internet via the computer which is how it's supposed to be – the two working as one. Archons seek to control that point where the operator connects with the computer to stop that interaction (Fig 20). Now the operator is banging the keyboard and clicking the mouse, but the computer is not responding and this happens when the computer is taken over – *possessed* – by an appropriately-named computer 'virus'. The operator has lost all influence over the computer which goes its own way making decisions under the control of the 'virus'. I have

just described the dynamic through which the force known to Gnostics as Yaldabaoth and Archons disconnects five-sense mind from expanded awareness to imprison humanity in perceptual servitude.



Figure 20: The mind 'virus' I have been writing about for decades seeks to isolate five-sense mind (the computer) from the true 'I'. (Image by Neil Hague).

About a year ago I came across a Native American concept of Wetiko which describes precisely the same phenomenon. Wetiko is the spelling used by the Cree and there are other versions including wintiko and windigo used by other tribal groups. They spell the name with lower case, but I see Wetiko as a proper noun as with Archons and prefer a capital. I first saw an article about Wetiko by writer and researcher Paul Levy which so synced with what I had been writing about the computer/operator disconnection and later the Archons. I then read his book, the fascinating *Dispelling Wetiko, Breaking the Spell of Evil*. The parallels between what I had concluded long before and the Native American concept of Wetiko were so clear and obvious that it was almost funny. For Wetiko see the Gnostic Archons for sure and the Jinn, the Predators, and every other name for a force of evil, inversion and chaos. Wetiko is the Native American name for the force that divides the computer from

the operator (Fig 21). Indigenous author Jack D. Forbes, a founder of the Native American movement in the 1960s, wrote another book about Wetiko entitled *Columbus And Other Cannibals – The Wetiko Disease of Exploitation, Imperialism, and Terrorism* which I also read. Forbes says that Wetiko refers to an evil person or spirit 'who terrorizes other creatures by means of terrible acts, including cannibalism'. Zulu shaman Credo Mutwa told me that African accounts tell how cannibalism was brought into the world by the Chitauri 'gods' – another manifestation of Wetiko. The distinction between 'evil person or spirit' relates to Archons/Wetiko possessing a human or acting as pure consciousness. Wetiko is said to be a sickness of the soul or spirit and a state of being that takes but gives nothing back – the Cult and its operatives perfectly described. Black Hawk, a Native American war leader defending their lands from confiscation, said European invaders had 'poisoned hearts' – Wetiko hearts – and that this would spread to native societies. Mention of the heart is very significant as we shall shortly see. Forbes writes: 'Tragically, the history of the world for the past 2,000 years is, in great part, the story of the epidemiology of the wetiko disease.' Yes, and much longer. Forbes is correct when he says: 'The wetikos destroyed Egypt and Babylon and Athens and Rome and Tenochtitlan [capital of the Aztec empire] and perhaps now they will destroy the entire earth.' Evil, he said, is the number one export of a Wetiko culture – see its globalisation with 'Covid'. Constant war, mass murder, suffering of all kinds, child abuse, Satanism, torture and human sacrifice are all expressions of Wetiko and the Wetiko possessed. The world is Wetiko made manifest, *but it doesn't have to be*. There is a way out of this even now.



Figure 21: The mind 'virus' is known to Native Americans as 'Wetiko'. (Image by Neil Hague).

Cult of Wetiko

Wetiko is the Yaldabaoth frequency distortion that seeks to attach to human consciousness and absorb it into its own. Once this connection is made Wetiko can drive the perceptions of the target which they believe to be coming from their own mind. All the horrors of history and today from mass killers to Satanists, paedophiles like Jeffrey Epstein and other psychopaths, are the embodiment of Wetiko and express its state of being in all its grotesqueness. The Cult is Wetiko incarnate, Yaldabaoth incarnate, and it seeks to facilitate Wetiko assimilation of humanity in totality into its distortion by manipulating the population into low frequency states that match its own. Paul Levy writes: 'Holographically enforced within the psyche of every human being the wetiko virus pervades and underlies the entire field of consciousness, and can therefore potentially manifest through any one of us at any moment if we are not mindful.' The 'Covid' hoax has achieved this with many people, but others have not fallen into Wetiko's frequency lair. Players in the 'Covid' human catastrophe including Gates, Schwab, Tedros, Fauci, Whitty, Vallance, Johnson, Hancock, Ferguson, Drosten, and all the rest, including the psychopath psychologists, are expressions of Wetiko. This is why

they have no compassion or empathy and no emotional consequence for what they do that would make them stop doing it. Observe all the people who support the psychopaths in authority against the Pushbackers despite the damaging impact the psychopaths have on their own lives and their family's lives. You are again looking at Wetiko possession which prevents them seeing through the lies to the obvious scam going on. *Why can't they see it?* Wetiko won't let them see it. The perceptual divide that has now become a chasm is between the Wetikoed and the non-Wetikoed.

Paul Levy describes Wetiko in the same way that I have long described the Archontic force. They are the same distorted consciousness operating across dimensions of reality: '… the subtle body of wetiko is not located in the third dimension of space and time, literally existing in another dimension … it is able to affect ordinary lives by mysteriously interpenetrating into our three-dimensional world.' Wetiko does this through its incarnate representatives in the Cult and by weaving itself into The Field which on our level of reality is the electromagnetic information field of the simulation or Matrix. More than that, the simulation *is* Wetiko / Yaldabaoth. Caleb Scharf, Director of Astrobiology at Columbia University, has speculated that 'alien life' could be so advanced that it has transcribed itself into the quantum realm to become what we call physics. He said intelligence indistinguishable from the fabric of the Universe would solve many of its greatest mysteries:

> Perhaps hyper-advanced life isn't just external. Perhaps it's already all around. It is embedded in what we perceive to be physics itself, from the root behaviour of particles and fields to the phenomena of complexity and emergence ... In other words, life might not just be in the equations. It might BE the equations [My emphasis].

Scharf said it is possible that 'we don't recognise advanced life because it forms an integral and unsuspicious part of what we've considered to be the natural world'. I agree. Wetiko/Yaldabaoth *is* the simulation. We are literally in the body of the beast. But that doesn't mean it has to control us. We all have the power to overcome Wetiko

influence and the Cult knows that. I doubt it sleeps too well because it knows that.

Which Field?

This, I suggest, is how it all works. There are two Fields. One is the fierce electromagnetic light of the Matrix within the speed of light; the other is the 'watery light' of The Field beyond the walls of the Matrix that connects with the Great Infinity. Five-sense mind and the decoding systems of the body attach us to the Field of Matrix light. They have to or we could not experience this reality. Five-sense mind sees only the Matrix Field of information while our expanded consciousness is part of the Infinity Field. When we open our minds, and most importantly our hearts, to the Infinity Field we have a mission control which gives us an expanded perspective, a road map, to understand the nature of the five-sense world. If we are isolated only in five-sense mind there is no mission control. We're on our own trying to understand a world that's constantly feeding us information to ensure we do not understand. People in this state can feel 'lost' and bewildered with no direction or radar. You can see ever more clearly those who are influenced by the Fields of Big Infinity or little five-sense mind simply by their views and behaviour with regard to the 'Covid' hoax. We have had this division throughout known human history with the mass of the people on one side and individuals who could see and intuit beyond the walls of the simulation – Plato's prisoner who broke out of the cave and saw reality for what it is. Such people have always been targeted by Wetiko/Archon-possessed authority, burned at the stake or demonised as mad, bad and dangerous. The Cult today and its global network of 'anti-hate', 'anti-fascist' Woke groups are all expressions of Wetiko attacking those exposing the conspiracy, 'Covid' lies and the 'vaccine' agenda.

Woke as a whole is Wetiko which explains its black and white mentality and how at one it is with the Wetiko-possessed Cult. Paul Levy said: 'To be in this paradigm is to still be under the thrall of a two-valued logic – where things are either true or false – of a

wetikoized mind.' Wetiko consciousness is in a permanent rage, therefore so is Woke, and then there is Woke inversion and contradiction. 'Anti-fascists' act like fascists because fascists *and* 'anti-fascists' are both Wetiko at work. Political parties act the same while claiming to be different for the same reason. Secret society and satanic rituals are attaching initiates to Wetiko and the cold, ruthless, psychopathic mentality that secures the positions of power all over the world is Wetiko. Reframing 'training programmes' have the same cumulative effect of attaching Wetiko and we have their graduates described as automatons and robots with a cold, psychopathic, uncaring demeanour. They are all traits of Wetiko possession and look how many times they have been described in this book and elsewhere with regard to personnel behind 'Covid' including the police and medical profession. Climbing the greasy pole in any profession in a Wetiko society requires traits of Wetiko to get there and that is particularly true of politics which is not about fair competition and pre-eminence of ideas. It is founded on how many backs you can stab and arses you can lick. This culminated in the global 'Covid' coordination between the Wetiko possessed who pulled it off in all the different countries without a trace of empathy and compassion for their impact on humans. Our sight sense can see only holographic form and not the Field which connects holographic form. Therefore we perceive 'physical' objects with 'space' in between. In fact that 'space' is energy/consciousness operating on multiple frequencies. One of them is Wetiko and that connects the Cult psychopaths, those who submit to the psychopaths, and those who serve the psychopaths in the media operations of the world. Wetiko is Gates. Wetiko is the mask-wearing submissive. Wetiko is the fake journalist and 'fact-checker'. The Wetiko Field is coordinating the whole thing. Psychopaths, gofers, media operatives, 'anti-hate' hate groups, 'fact-checkers' and submissive people work as one unit *even without human coordination* because they are attached to the *same* Field which is organising it all (Fig 22). Paul Levy is here describing how Wetiko-possessed people are drawn together and refuse to let any information breach their rigid

perceptions. He was writing long before 'Covid', but I think you will recognise followers of the 'Covid' religion *oh just a little bit*:

People who are channelling the vibratory frequency of wetiko align with each other through psychic resonance to reinforce their unspoken shared agreement so as to uphold their deranged view of reality. Once an unconscious content takes possession of certain individuals, it irresistibly draws them together by mutual attraction and knits them into groups tied together by their shared madness that can easily swell into an avalanche of insanity.

A psychic epidemic is a closed system, which is to say that it is insular and not open to any new information or informing influences from the outside world which contradict its fixed, limited, and limiting perspective.

There we have the Woke mind and the 'Covid' mind. Compatible resonance draws the awakening together, too, which is clearly happening today.



Figure 22: The Wetiko Field from which the Cult pyramid and its personnel are made manifest. (Image by Neil Hague).

Spiritual servitude

Wetiko doesn't care about humans. It's not human; it just possesses humans for its own ends and the effect (depending on the scale of

possession) can be anything from extreme psychopathy to unquestioning obedience. Wetiko's worst nightmare is for human consciousness to expand beyond the simulation. Everything is focussed on stopping that happening through control of information, thus perception, thus frequency. The 'education system', media, science, medicine, academia, are all geared to maintaining humanity in five-sense servitude as is the constant stimulation of low-vibrational mental and emotional states (see 'Covid'). Wetiko seeks to dominate those subconscious spaces between five-sense perception and expanded consciousness where the computer meets the operator. From these subconscious hiding places Wetiko speaks to us to trigger urges and desires that we take to be our own and manipulate us into anything from low-vibrational to psychopathic states. Remember how Islam describes the Jinn as invisible tricksters that 'whisper' and confuse. Wetiko is the origin of the 'trickster god' theme that you find in cultures all over the world. Jinn, like the Archons, are Wetiko which is terrified of humans awakening and reconnecting with our true self for then its energy source has gone. With that the feedback loop breaks between Wetiko and human perception that provides the energetic momentum on which its very existence depends as a force of evil. Humans are both its target and its source of survival, but only if we are operating in low-vibrational states of fear, hate, depression and the background anxiety that most people suffer. We are Wetiko's target because we are its key to survival. It needs us, not the other way round. Paul Levy writes:

A vampire has no intrinsic, independent, substantial existence in its own right; it only exists in relation to us. The pathogenic, vampiric mind-parasite called wetiko is nothing in itself – not being able to exist from its own side – yet it has a 'virtual reality' such that it can potentially destroy our species …

…The fact that a vampire is not reflected by a mirror can also mean that what we need to see is that there's nothing, no-thing to see, other than ourselves. The fact that wetiko is the expression of something inside of us means that the cure for wetiko is with us as well. The critical issue is finding this cure within us and then putting it into effect.

Evil begets evil because if evil does not constantly expand and find new sources of energetic sustenance its evil, its *distortion*, dies with the assimilation into balance and harmony. Love is the garlic to Wetiko's vampire. Evil, the absence of love, cannot exist in the presence of love. I think I see a way out of here. I have emphasised so many times over the decades that the Archons/Wetiko and their Cult are not all powerful. *They are not*. I don't care how it looks even now *they are not*. I have not called them little boys in short trousers for effect. I have said it because it is true. Wetiko's insatiable desire for power over others is not a sign of its omnipotence, but its insecurity. Paul Levy writes: 'Due to the primal fear which ultimately drives it and which it is driven to cultivate, wetiko's body politic has an intrinsic and insistent need for centralising power and control so as to create imagined safety for itself.' *Yeeeeeees!* Exactly! Why does Wetiko want humans in an ongoing state of fear? Wetiko itself *is* fear and it is petrified of love. As evil is an absence of love, so love is an absence of fear. Love conquers all and *especially* Wetiko which *is* fear. Wetiko brought fear into the world when it wasn't here before. *Fear* was the 'fall', the fall into low-frequency ignorance and illusion – fear is **F**alse **E**motion **A**ppearing **R**eal. The simulation is driven and energised by fear because Wetiko/Yaldabaoth (fear) *are* the simulation. Fear is the absence of love and Wetiko is the absence of love.

Wetiko today

We can now view current events from this level of perspective. The 'Covid' hoax has generated momentous amounts of ongoing fear, anxiety, depression and despair which have empowered Wetiko. No wonder people like Gates have been the instigators when they are Wetiko incarnate and exhibit every trait of Wetiko in the extreme. See how cold and unemotional these people are like Gates and his cronies, how dead of eye they are. That's Wetiko. Sabbatians are Wetiko and everything they control including the World Health Organization, Big Pharma and the 'vaccine' makers, national 'health'

hierarchies, corporate media, Silicon Valley, the banking system, and the United Nations with its planned transformation into world government. All are controlled and possessed by the Wetiko distortion into distorting human society in its image. We are with this knowledge at the gateway to understanding the world. Divisions of race, culture, creed and sexuality are diversions to hide the real division between those possessed and influenced by Wetiko and those that are not. The 'Covid' hoax has brought both clearly into view. Human behaviour is not about race. Tyrants and dictatorships come in all colours and creeds. What unites the US president bombing the innocent and an African tribe committing genocide against another as in Rwanda? What unites them? *Wetiko*. All wars are Wetiko, all genocide is Wetiko, all hunger over centuries in a world of plenty is Wetiko. Children going to bed hungry, including in the West, is Wetiko. Cult-generated Woke racial divisions that focus on the body are designed to obscure the reality that divisions in behaviour are manifestations of mind, not body. Obsession with body identity and group judgement is a means to divert attention from the real source of behaviour – mind and perception. Conflict sown by the Woke both within themselves and with their target groups are Wetiko providing lunch for itself through still more agents of the division, chaos, and fear on which it feeds. The Cult is seeking to assimilate the entirety of humanity and all children and young people into the Wetiko frequency by manipulating them into states of fear and despair. Witness all the suicide and psychological unravelling since the spring of 2020. Wetiko psychopaths want to impose a state of unquestioning obedience to authority which is no more than a conduit for Wetiko to enforce its will and assimilate humanity into itself. It needs us to believe that resistance is futile when it fears resistance and even more so the game-changing non-cooperation with its impositions. It can use violent resistance for its benefit. Violent impositions and violent resistance are *both* Wetiko. The Power of Love with its Power of No will sweep Wetiko from our world. Wetiko and its Cult know that. They just don't want us to know.

AI Wetiko

This brings me to AI or artificial intelligence and something else Wetikos don't want us to know. What is AI *really*? I know about computer code algorithms and AI that learns from data input. These, however, are more diversions, the expeditionary force, for the real AI that they want to connect to the human brain as promoted by Silicon Valley Wetikos like Kurzweil. What is this AI? It is the frequency of *Wetiko*, the frequency of the Archons. The connection of AI to the human brain is the connection of the Wetiko frequency to create a Wetiko hive mind and complete the job of assimilation. The hive mind is planned to be controlled from Israel and China which are both 100 percent owned by Wetiko Sabbatians. The assimilation process has been going on minute by minute in the 'smart' era which fused with the 'Covid' era. We are told that social media is scrambling the minds of the young and changing their personality. This is true, but what is social media? Look more deeply at how it works, how it creates divisions and conflict, the hostility and cruelty, the targeting of people until they are destroyed. That's Wetiko. Social media is manipulated to tune people to the Wetiko frequency with all the emotional exploitation tricks employed by platforms like Facebook and its Wetiko front man, Zuckerberg. Facebook's Instagram announced a new platform for children to overcome a legal bar on them using the main site. This is more Wetiko exploitation and manipulation of kids. Amnesty International likened the plan to foxes offering to guard the henhouse and said it was incompatible with human rights. Since when did Wetiko or Zuckerberg (I repeat myself) care about that? Would Brin and Page at Google, Wojcicki at YouTube, Bezos at Amazon and whoever the hell runs Twitter act as they do if they were not channelling Wetiko? Would those who are developing technologies for no other reason than human control? How about those designing and selling technologies to kill people and Big Pharma drug and 'vaccine' producers who know they will end or devastate lives? Quite a thought for these people to consider is that if you are Wetiko in a human life you are Wetiko on the 'other side' unless your frequency

changes and that can only change by a change of perception which becomes a change of behaviour. Where Gates is going does not bear thinking about although perhaps that's exactly where he wants to go. Either way, that's where he's going. His frequency will make it so.

The frequency lair

I have been saying for a long time that a big part of the addiction to smartphones and devices is that a frequency is coming off them that entraps the mind. People spend ages on their phones and sometimes even a minute or so after they put them down they pick them up again and it all repeats. 'Covid' lockdowns will have increased this addiction a million times for obvious reasons. Addictions to alcohol overindulgence and drugs are another way that Wetiko entraps consciousness to attach to its own. Both are symptoms of low-vibrational psychological distress which alcoholism and drug addiction further compound. Do we think it's really a coincidence that access to them is made so easy while potions that can take people into realms beyond the simulation are banned and illegal? I have explored smartphone addiction in other books, the scale is mind-blowing, and that level of addiction does not come without help. Tech companies that make these phones are Wetiko and they will have no qualms about destroying the minds of children. We are seeing again with these companies the Wetiko perceptual combination of psychopathic enforcers and weak and meek unquestioning compliance by the rank and file.

The global Smart Grid is the Wetiko Grid and it is crucial to complete the Cult endgame. The simulation is radiation and we are being deluged with technological radiation on a devastating scale. Wetiko frauds like Elon Musk serve Cult interests while occasionally criticising them to maintain his street-cred. 5G and other forms of Wi-Fi are being directed at the earth from space on a volume and scale that goes on increasing by the day. Elon Musk's (officially) SpaceX Starlink project is in the process of putting tens of thousands of satellites in low orbit to cover every inch of the planet with 5G and other Wi-Fi to create Kurzweil's global 'cloud' to which the

human mind is planned to be attached very soon. SpaceX has approval to operate 12,000 satellites with more than 1,300 launched at the time of writing and applications filed for *30,000* more. Other operators in the Wi-Fi, 5G, low-orbit satellite market include OneWeb (UK), Telesat (Canada), and AST & Science (US). Musk tells us that AI could be the end of humanity and then launches a company called Neuralink to connect the human brain to computers. Musk's (in theory) Tesla company is building electric cars and the driverless vehicles of the smart control grid. As frauds and bullshitters go Elon Musk in my opinion is Major League.

5G and technological radiation in general are destructive to human health, genetics and psychology and increasing the strength of artificial radiation underpins the five-sense perceptual bubbles which are themselves expressions of radiation or electromagnetism. Freedom activist John Whitehead was so right with his 'databit by databit, we are building our own electronic concentration camps'. The Smart Grid and 5G is a means to control the human mind and infuse perceptual information into The Field to influence anyone in sync with its frequency. You can change perception and behaviour en masse if you can manipulate the population into those levels of frequency and this is happening all around us today. The arrogance of Musk and his fellow Cult operatives knows no bounds in the way that we see with Gates. Musk's satellites are so many in number already they are changing the night sky when viewed from Earth. The astronomy community has complained about this and they have seen nothing yet. Some consequences of Musk's Wetiko hubris include: Radiation; visible pollution of the night sky; interference with astronomy and meteorology; ground and water pollution from intensive use of increasingly many spaceports; accumulating space debris; continual deorbiting and burning up of aging satellites, polluting the atmosphere with toxic dust and smoke; and ever-increasing likelihood of collisions. A collective public open letter of complaint to Musk said:

> We are writing to you … because SpaceX is in process of surrounding the Earth with a network of thousands of satellites whose very purpose is to irradiate every square inch of the

Earth. SpaceX, like everyone else, is treating the radiation as if it were not there. As if the mitochondria in our cells do not depend on electrons moving undisturbed from the food we digest to the oxygen we breathe.

As if our nervous systems and our hearts are not subject to radio frequency interference like any piece of electronic equipment. As if the cancer, diabetes, and heart disease that now afflict a majority of the Earth's population are not metabolic diseases that result from interference with our cellular machinery. As if insects everywhere, and the birds and animals that eat them, are not starving to death as a result.

People like Musk and Gates believe in their limitless Wetiko arrogance that they can do whatever they like to the world because they own it. Consequences for humanity are irrelevant. It's absolutely time that we stopped taking this shit from these self-styled masters of the Earth when you consider where this is going.

Why is the Cult so anti-human?

I hear this question often: Why would they do this when it will affect them, too? Ah, but will it? Who is this *them*? Forget their bodies. They are just vehicles for Wetiko consciousness. When you break it all down to the foundations we are looking at a state of severely distorted consciousness targeting another state of consciousness for assimilation. The rest is detail. The simulation is the fly-trap in which unique sensations of the five senses create a cycle of addiction called reincarnation. Renegade Minds see that everything which happens in our reality is a smaller version of the whole picture in line with the holographic principle. Addiction to the radiation of smart technology is a smaller version of addiction to the whole simulation. Connecting the body/brain to AI is taking that addiction on a giant step further to total ongoing control by assimilating human incarnate consciousness into Wetiko. I have watched during the 'Covid' hoax how many are becoming ever more profoundly attached to Wetiko's perceptual calling cards of aggressive response to any other point of view ('There is no other god but me'), psychopathic lack of compassion and empathy, and servile submission to the narrative and will of authority. Wetiko is the psychopaths *and* subservience to psychopaths. The Cult of Wetiko is

so anti-human because it is *not* human. It embarked on a mission to destroy human by targeting everything that it means to be human and to survive as human. 'Covid' is not the end, just a means to an end. The Cult with its Wetiko consciousness is seeking to change Earth systems, including the atmosphere, to suit them, not humans. The gathering bombardment of 5G alone from ground and space is dramatically changing The Field with which the five senses interact. There is so much more to come if we sit on our hands and hope it will all go away. It is not meant to go away. It is meant to get ever more extreme and we need to face that while we still can – just.

Carbon dioxide is the gas of life. Without that human is over. Kaput, gone, history. No natural world, no human. The Cult has created a cock and bull story about carbon dioxide and climate change to justify its reduction to the point where Gates and the ignoramus Biden 'climate chief' John Kerry want to suck it out of the atmosphere. Kerry wants to do this because his master Gates does. Wetikos have made the gas of life a demon with the usual support from the Wokers of Extinction Rebellion and similar organisations and the bewildered puppet-child that is Greta Thunberg who was put on the world stage by Klaus Schwab and the World Economic Forum. The name Extinction Rebellion is both ironic and as always Wetiko inversion. The gas that we need to survive must be reduced to save us from extinction. The most basic need of human is oxygen and we now have billions walking around in face nappies depriving body and brain of this essential requirement of human existence. More than that 5G at 60 gigahertz interacts with the oxygen molecule to reduce the amount of oxygen the body can absorb into the bloodstream. The obvious knock-on consequences of that for respiratory and cognitive problems and life itself need no further explanation. Psychopaths like Musk are assembling a global system of satellites to deluge the human atmosphere with this insanity. The man should be in jail. Here we have two most basic of human needs, oxygen and carbon dioxide, being dismantled.

Two others, water and food, are getting similar treatment with the United Nations Agendas 21 and 2030 – the Great Reset – planning to

centrally control all water and food supplies. People will not even own rain water that falls on their land. Food is affected at the most basic level by reducing carbon dioxide. We have genetic modification or GMO infiltrating the food chain on a mass scale, pesticides and herbicides polluting the air and destroying the soil. Freshwater fish that provide livelihoods for 60 million people and feed hundreds of millions worldwide are being 'pushed to the brink' according the conservationists while climate change is the only focus. Now we have Gates and Schwab wanting to dispense with current food sources all together and replace them with a synthetic version which the Wetiko Cult would control in terms of production and who eats and who doesn't. We have been on the Totalitarian Tiptoe to this for more than 60 years as food has become ever more processed and full of chemical shite to the point today when it's not natural food at all. As Dr Tom Cowan says: 'If it has a label don't eat it.' Bill Gates is now the biggest owner of farmland in the United States and he does nothing without an ulterior motive involving the Cult. Klaus Schwab wrote: 'To feed the world in the next 50 years we will need to produce as much food as was produced in the last 10,000 years … food security will only be achieved, however, if regulations on genetically modified foods are adapted to reflect the reality that gene editing offers a precise, efficient and safe method of improving crops.' Liar. People and the world are being targeted with aluminium through vaccines, chemtrails, food, drink cans, and endless other sources when aluminium has been linked to many health issues including dementia which is increasing year after year. Insects, bees and wildlife essential to the food chain are being deleted by pesticides, herbicides and radiation which 5G is dramatically increasing with 6G and 7G to come. The pollinating bee population is being devastated while wildlife including birds, dolphins and whales are having their natural radar blocked by the effects of ever-increasing radiation. In the summer windscreens used to be splattered with insects so numerous were they. It doesn't happen now. Where have they gone?

Synthetic everything

The Cult is introducing genetically-modified versions of trees, plants and insects including a Gates-funded project to unleash hundreds of millions of genetically-modified, lab-altered and patented male mosquitoes to mate with wild mosquitoes and induce genetic flaws that cause them to die out. Clinically-insane Gates-funded Japanese researchers have developed mosquitos that spread vaccine and are dubbed 'flying vaccinators'. Gates is funding the modification of weather patterns in part to sell the myth that this is caused by carbon dioxide and he's funding geoengineering of the skies to change the atmosphere. Some of this came to light with the Gates-backed plan to release tonnes of chalk into the atmosphere to 'deflect the Sun and cool the planet'. Funny how they do this while the heating effect of the Sun is not factored into climate projections focussed on carbon dioxide. The reason is that they want to reduce carbon dioxide (so don't mention the Sun), but at the same time they do want to reduce the impact of the Sun which is so essential to human life and health. I have mentioned the sun-cholesterol-vitamin D connection as they demonise the Sun with warnings about skin cancer (caused by the chemicals in sun cream they tell you to splash on). They come from the other end of the process with statin drugs to reduce cholesterol that turns sunlight into vitamin D. A lack of vitamin D leads to a long list of health effects and how vitamin D levels must have fallen with people confined to their homes over 'Covid'. Gates is funding other forms of geoengineering and most importantly chemtrails which are dropping heavy metals, aluminium and self-replicating nanotechnology onto the Earth which is killing the natural world. See *Everything You Need To Know, But Have Never Been Told* for the detailed background to this.

Every human system is being targeted for deletion by a force that's not human. The Wetiko Cult has embarked on the process of transforming the human body from biological to synthetic biological as I have explained. Biological is being replaced by the artificial and synthetic – Archontic 'countermimicry' – right across human society. The plan eventually is to dispense with the human body altogether

and absorb human consciousness – which it wouldn't really be by then – into cyberspace (the simulation which is Wetiko/Yaldabaoth). Preparations for that are already happening if people would care to look. The alternative media rightly warns about globalism and 'the globalists', but this is far bigger than that and represents the end of the human race as we know it. The 'bad copy' of prime reality that Gnostics describe was a bad copy of harmony, wonder and beauty to start with before Wetiko/Yaldabaoth set out to change the simulated 'copy' into something very different. The process was slow to start with. Entrapped humans in the simulation timeline were not technologically aware and they had to be brought up to intellectual speed while being suppressed spiritually to the point where they could build their own prison while having no idea they were doing so. We have now reached that stage where technological intellect has the potential to destroy us and that's why events are moving so fast. Central American shaman Don Juan Matus said:

> Think for a moment, and tell me how you would explain the contradictions between the intelligence of man the engineer and the stupidity of his systems of belief, or the stupidity of his contradictory behaviour. Sorcerers believe that the predators have given us our systems of beliefs, our ideas of good and evil; our social mores. They are the ones who set up our dreams of success or failure. They have given us covetousness, greed, and cowardice. It is the predator who makes us complacent, routinary, and egomaniacal.
>
> In order to keep us obedient and meek and weak, the predators engaged themselves in a stupendous manoeuvre – stupendous, of course, from the point of view of a fighting strategist; a horrendous manoeuvre from the point of those who suffer it. They gave us their mind. The predators' mind is baroque, contradictory, morose, filled with the fear of being discovered any minute now.

For 'predators' see Wetiko, Archons, Yaldabaoth, Jinn, and all the other versions of the same phenomenon in cultures and religions all over the world. The theme is always the same because it's true and it's real. We have reached the point where we have to deal with it. The question is – how?

Don't fight – walk away

I thought I'd use a controversial subheading to get things moving in terms of our response to global fascism. What do you mean 'don't fight'? What do you mean 'walk away'? We've got to fight. We can't walk away. Well, it depends what we mean by fight and walk away. If fighting means physical combat we are playing Wetiko's game and falling for its trap. It wants us to get angry, aggressive, and direct hate and hostility at the enemy we think we must fight. Every war, every battle, every conflict, has been fought with Wetiko leading both sides. It's what it does. Wetiko wants a fight, anywhere, any place. Just hit me, son, so I can hit you back. Wetiko hits Wetiko and Wetiko hits Wetiko in return. I am very forthright as you can see in exposing Wetikos of the Cult, but I don't hate them. I refuse to hate them. It's what they want. What you hate you become. What you *fight* you become. Wokers, 'anti-haters' and 'anti-fascists' prove this every time they reach for their keyboards or don their balaclavas. By walk away I mean to disengage from Wetiko which includes ceasing to cooperate with its tyranny. Paul Levy says of Wetiko:

> The way to 'defeat' evil is not to try to destroy it (for then, in playing evil's game, we have already lost), but rather, to find the invulnerable place within ourselves where evil is unable to vanquish us – this is to truly 'win' our battle with evil.

Wetiko is everywhere in human society and it's been on steroids since the 'Covid' hoax. Every shouting match over wearing masks has Wetiko wearing a mask and Wetiko not wearing one. It's an electrical circuit of push and resist, push and resist, with Wetiko pushing *and* resisting. Each polarity is Wetiko empowering itself. Dictionary definitions of 'resist' include 'opposing, refusing to accept or comply with' and the word to focus on is 'opposing'. What form does this take – setting police cars alight or 'refusing to accept or comply with'? The former is Wetiko opposing Wetiko while the other points the way forward. This is the difference between those aggressively demanding that government fascism must be obeyed who stand in stark contrast to the great majority of Pushbackers. We saw this clearly with a march by thousands of Pushbackers against lockdown in London followed days later by a Woker-hijacked

protest in Bristol in which police cars were set on fire. Masks were virtually absent in London and widespread in Bristol. Wetiko wants lockdown on every level of society and infuses its aggression to police it through its unknowing stooges. Lockdown protesters are the ones with the smiling faces and the hugs, The two blatantly obvious states of being – getting more obvious by the day – are the result of Wokers and their like becoming ever more influenced by the simulation Field of Wetiko and Pushbackers ever more influenced by The Field of a far higher vibration beyond the simulation. Wetiko can't invade the heart which is where most lockdown opponents are coming from. It's the heart that allows them to see through the lies to the truth in ways I will be highlighting.

Renegade Minds know that calmness is the place from which wisdom comes. You won't find wisdom in a hissing fit and wisdom is what we need in abundance right now. Calmness is not weakness – you don't have to scream at the top of your voice to be strong. Calmness is indeed a sign of strength. 'No' means I'm not doing it. *NOOOO!!!* doesn't mean you're not doing it even more. Volume does not advance 'No – I'm not doing it'. You are just not doing it. Wetiko possessed and influenced don't know how to deal with that. Wetiko wants a fight and we should not give it one. What it needs more than anything is our *cooperation* and we should not give that either. Mass rallies and marches are great in that they are a visual representation of feeling, but if it ends there they are irrelevant. You demand that Wetikos act differently? Well, they're not going to are they? They are Wetikos. We don't need to waste our time demanding that something doesn't happen when that will make no difference. We need to delete the means that *allows* it to happen. This, invariably, is our cooperation. You can demand a child stop firing a peashooter at the dog or you can refuse to buy the peashooter. If you provide the means you are cooperating with the dog being smacked on the nose with a pea. How can the authorities enforce mask-wearing if millions in a country refuse? What if the 74 million Pushbackers that voted for Trump in 2020 refused to wear masks, close their businesses or stay in their homes. It would be unenforceable. The

few control the many through the compliance of the many and that's always been the dynamic be it 'Covid' regulations or the Roman Empire. I know people can find it intimidating to say no to authority or stand out in a crowd for being the only one with a face on display; but it has to be done or it's over. I hope I've made clear in this book that where this is going will be far more intimidating than standing up now and saying 'No' – I will not cooperate with my own enslavement and that of my children. There might be consequences for some initially, although not so if enough do the same. The question that must be addressed is what is going to happen if we don't? It is time to be strong and unyieldingly so. No means no. Not here and there, but *everywhere* and *always*. I have refused to wear a mask and obey all the other nonsense. I will not comply with tyranny. I repeat: Fascism is not imposed by fascists – there are never enough of them. Fascism is imposed by the population acquiescing to fascism. *I will not do it*. I will die first, or my body will. Living meekly under fascism is a form of death anyway, the death of the spirit that Martin Luther King described.

Making things happen

We must not despair. This is not over till it's over and it's far from that. The 'fat lady' must refuse to sing. The longer the 'Covid' hoax has dragged on and impacted on more lives we have seen an awakening of phenomenal numbers of people worldwide to the realisation that what they have believed all their lives is not how the world really is. Research published by the system-serving University of Bristol and King's College London in February, 2021, concluded: 'One in every 11 people in Britain say they trust David Icke's take on the coronavirus pandemic.' It will be more by now and we have gathering numbers to build on. We must urgently progress from seeing the scam to ceasing to cooperate with it. Prominent German lawyer Reiner Fuellmich, also licenced to practice law in America, is doing a magnificent job taking the legal route to bring the psychopaths to justice through a second Nuremberg tribunal for crimes against humanity. Fuellmich has an impressive record of

beating the elite in court and he formed the German Corona Investigative Committee to pursue civil charges against the main perpetrators with a view to triggering criminal charges. Most importantly he has grasped the foundation of the hoax – the PCR test not testing for the 'virus' – and Christian Drosten is therefore on his charge sheet along with Gates frontman Tedros at the World Health Organization. Major players must be not be allowed to inflict their horrors on the human race without being brought to book. A life sentence must follow for Bill Gates and the rest of them. A group of researchers has also indicted the government of Norway for crimes against humanity with copies sent to the police and the International Criminal Court. The lawsuit cites participation in an internationally-planned false pandemic and violation of international law and human rights, the European Commission's definition of human rights by coercive rules, Nuremberg and Hague rules on fundamental human rights, and the Norwegian constitution. We must take the initiative from hereon and not just complain, protest and react.

There are practical ways to support vital mass non-cooperation. Organising in numbers is one. Lockdown marches in London in the spring in 2021 were mass non-cooperation that the authorities could not stop. There were too many people. Hundreds of thousands walked the London streets in the centre of the road for mile after mile while the Face-Nappies could only look on. They were determined, but calm, and just *did it* with no histrionics and lots of smiles. The police were impotent. Others are organising group shopping without masks for mutual support and imagine if that was happening all over. Policing it would be impossible. If the store refuses to serve people in these circumstances they would be faced with a long line of trolleys full of goods standing on their own and everything would have to be returned to the shelves. How would they cope with that if it kept happening? I am talking here about moving on from complaining to being pro-active; from watching things happen to making things happen. I include in this our relationship with the police. The behaviour of many Face-Nappies

has been disgraceful and anyone who thinks they would never find concentration camp guards in the 'enlightened' modern era have had that myth busted big-time. The period and setting may change – Wetikos never do. I watched film footage from a London march in which a police thug viciously kicked a protestor on the floor who had done nothing. His fellow Face-Nappies stood in a ring protecting him. What he did was a criminal assault and with a crowd far outnumbering the police this can no longer be allowed to happen unchallenged. I get it when people chant 'shame on you' in these circumstances, but that is no longer enough. They *have* no shame those who do this. Crowds needs to start making a citizen's arrest of the police who commit criminal offences and brutally attack innocent people and defenceless women. A citizen's arrest can be made under section 24A of the UK Police and Criminal Evidence (PACE) Act of 1984 and you will find something similar in other countries. I prefer to call it a Common Law arrest rather than citizen's for reasons I will come to shortly. Anyone can arrest a person committing an indictable offence or if they have reasonable grounds to suspect they are committing an indictable offence. On both counts the attack by the police thug would have fallen into this category. A citizen's arrest can be made to stop someone:

- Causing physical injury to himself or any other person
- Suffering physical injury
- Causing loss of or damage to property
- Making off before a constable can assume responsibility for him

A citizen's arrest may also be made to prevent a breach of the peace under Common Law and if they believe a breach of the peace will happen or anything related to harm likely to be done or already done in their presence. This is the way to go I think – the Common Law version. If police know that the crowd and members of the public will no longer be standing and watching while they commit

their thuggery and crimes they will think twice about acting like Brownshirts and Blackshirts.

Common Law – common sense

Mention of Common Law is very important. Most people think the law is the law as in one law. This is not the case. There are two bodies of law, Common Law and Statute Law, and they are not the same. Common Law is founded on the simple premise of do no harm. It does not recognise victimless crimes in which no harm is done while Statute Law does. There is a Statute Law against almost everything. So what is Statute Law? Amazingly it's the law of the *sea* that was brought ashore by the Cult to override the law of the land which is Common Law. They had no right to do this and as always they did it anyway. They had to. They could not impose their will on the people through Common Law which only applies to do no harm. How could you stitch up the fine detail of people's lives with that? Instead they took the law of the sea, or Admiralty Law, and applied it to the population. Statute Law refers to all the laws spewing out of governments and their agencies including all the fascist laws and regulations relating to 'Covid'. The key point to make is that Statute Law is *contract law*. It only applies between *contracting* corporations. Most police officers don't even know this. They have to be kept in the dark, too. Long ago when merchants and their sailing ships began to trade with different countries a contractual law was developed called Admiralty Law and other names. Again it only applied to *contracts* agreed between *corporate* entities. If there is no agreed contract the law of the sea had no jurisdiction *and that still applies to its new alias of Statute Law*. The problem for the Cult when the law of the sea was brought ashore was an obvious one. People were not corporations and neither were government entities. To overcome the latter they made governments and all associated organisations corporations. All the institutions are *private corporations* and I mean governments and their agencies, local councils, police, courts, military, US states, the whole lot. Go to the

Dun and Bradstreet corporate listings website for confirmation that they are all corporations. You are arrested by a private corporation called the police by someone who is really a private security guard and they take you to court which is another private corporation. Neither have jurisdiction over you unless you consent and *contract* with them. This is why you hear the mantra about law enforcement policing by *consent* of the people. In truth the people 'consent' only in theory through monumental trickery.

Okay, the Cult overcame the corporate law problem by making governments and institutions corporate entities; but what about people? They are not corporations are they? Ah ... well in a sense, and *only* a sense, they are. Not people exactly – the illusion of people. The Cult creates a corporation in the name of everyone at the time that their birth certificate is issued. Note birth/ *berth* certificate and when you go to court under the law of the sea on land you stand in a *dock*. These are throwbacks to the origin. My Common Law name is David Vaughan Icke. The name of the corporation created by the government when I was born is called Mr David Vaughan Icke usually written in capitals as MR DAVID VAUGHAN ICKE. That is not me, the living, breathing man. It is a fictitious corporate entity. The trick is to make you think that David Vaughan Icke and MR DAVID VAUGHAN ICKE are the same thing. *They are not*. When police charge you and take you to court they are prosecuting the corporate entity and not the living, breathing, man or woman. They have to trick you into identifying as the corporate entity and contracting with them. Otherwise they have no jurisdiction. They do this through a language known as legalese. Lawful and legal are not the same either. Lawful relates to Common Law and legal relates to Statute Law. Legalese is the language of Statue Law which uses terms that mean one thing to the public and another in legalese. Notice that when a police officer tells someone why they are being charged he or she will say at the end: 'Do you understand?' To the public that means 'Do you comprehend?' In legalese it means 'Do you stand under me?' Do you stand under my authority? If you say

yes to the question you are unknowingly agreeing to give them jurisdiction over you in a contract between two corporate entities.

This is a confidence trick in every way. Contracts have to be agreed between informed parties and if you don't know that David Vaughan Icke is agreeing to be the corporation MR DAVID VAUGHAN ICKE you cannot knowingly agree to contract. They are deceiving you and another way they do this is to ask for proof of identity. You usually show them a driving licence or other document on which your corporate name is written. In doing so you are accepting that you are that corporate entity when you are not. Referring to yourself as a 'person' or 'citizen' is also identifying with your corporate fiction which is why I made the Common Law point about the citizen's arrest. If you are approached by a police officer you identify yourself immediately as a living, breathing, man or woman and say 'I do not consent, I do not contract with you and I do not understand' or stand under their authority. I have a Common Law birth certificate as a living man and these are available at no charge from commonlawcourt.com. Businesses registered under the Statute Law system means that its laws apply. There are, however, ways to run a business under Common Law. Remember all 'Covid' laws and regulations are Statute Law – the law of *contracts* and you do not have to contract. This doesn't mean that you can kill someone and get away with it. Common Law says do no harm and that applies to physical harm, financial harm etc. Police are employees of private corporations and there needs to be a new system of non-corporate Common Law constables operating outside the Statute Law system. If you go to davidicke.com and put Common Law into the search engine you will find videos that explain Common Law in much greater detail. It is definitely a road we should walk.

With all my heart

I have heard people say that we are in a spiritual war. I don't like the term 'war' with its Wetiko dynamic, but I know what they mean. Sweep aside all the bodily forms and we are in a situation in which two states of consciousness are seeking very different realities.

Wetiko wants upheaval, chaos, fear, suffering, conflict and control. The other wants love, peace, harmony, fairness and freedom. That's where we are. We should not fall for the idea that Wetiko is all-powerful and there's nothing we can do. Wetiko is not all-powerful. It's a joke, pathetic. It doesn't have to be, but it has made that choice for now. A handful of times over the years when I have felt the presence of its frequency I have allowed it to attach briefly so I could consciously observe its nature. The experience is not pleasant, the energy is heavy and dark, but the ease with which you can kick it back out the door shows that its real power is in persuading us that it has power. It's all a con. Wetiko is a con. It's a trickster and not a power that can control us if we unleash our own. The con is founded on manipulating humanity to give its power to Wetiko which recycles it back to present the illusion that it has power when its power is *ours* that we gave away. This happens on an energetic level and plays out in the world of the seen as humanity giving its power to Wetiko authority which uses that power to control the population when the power is only the power the population has handed over. How could it be any other way for billions to be controlled by a relative few? I have had experiences with people possessed by Wetiko and again you can kick its arse if you do it with an open heart. Oh yes – the *heart* which can transform the world of perceived 'matter'.

We are receiver-transmitters and processors of information, but what information and where from? Information is processed into perception in three main areas – the brain, the heart and the belly. These relate to thinking, knowing, and emotion. Wetiko wants us to be head and belly people which means we think within the confines of the Matrix simulation and low-vibrational emotional reaction scrambles balance and perception. A few minutes on social media and you see how emotion is the dominant force. Woke is all emotion and is therefore thought-free and fact-free. Our heart is something different. It *knows* while the head *thinks* and has to try to work it out because it doesn't know. The human energy field has seven prime vortexes which connect us with wider reality (Fig 23). Chakra means

'wheels of light' in the Sanskrit language of ancient India. The main ones are: The crown chakra on top of the head; brow (or 'third eye') chakra in the centre of the forehead; throat chakra; heart chakra in the centre of the chest; solar plexus chakra below the sternum; sacral chakra beneath the navel; and base chakra at the bottom of the spine. Each one has a particular function or functions. We feel anxiety and nervousness in the belly where the sacral chakra is located and this processes emotion that can affect the colon to give people 'the shits' or make them 'shit scared' when they are nervous. Chakras all play an important role, but the Mr and Mrs Big is the heart chakra which sits at the centre of the seven, above the chakras that connect us to the 'physical' and below those that connect with higher realms (or at least should). Here in the heart chakra we feel love, empathy and compassion – 'My heart goes out to you'. Those with closed hearts become literally 'heart-less' in their attitudes and behaviour (see Bill Gates). Native Americans portrayed Wetiko with what Paul Levy calls a 'frigid, icy heart, devoid of mercy' (see Bill Gates).



Figure 23: The chakra system which interpenetrates the human energy field. The heart chakra is the governor – or should be.

Wetiko trembles at the thought of heart energy which it cannot infiltrate. The frequency is too high. What it seeks to do instead is close the heart chakra vortex to block its perceptual and energetic influence. Psychopaths have 'hearts of stone' and emotionally-damaged people have 'heartache' and 'broken hearts'. The astonishing amount of heart disease is related to heart chakra

disruption with its fundamental connection to the 'physical' heart. Dr Tom Cowan has written an outstanding book challenging the belief that the heart is a pump and making the connection between the 'physical' and spiritual heart. Rudolph Steiner who was way ahead of his time said the same about the fallacy that the heart is a pump. *What*? The heart is not a pump? That's crazy, right? Everybody knows that. Read Cowan's *Human Heart, Cosmic Heart* and you will realise that the very idea of the heart as a pump is ridiculous when you see the evidence. How does blood in the feet so far from the heart get pumped horizontally up the body by the heart?? Cowan explains in the book the real reason why blood moves as it does. Our 'physical' heart is used to symbolise love when the source is really the heart vortex or spiritual heart which is our most powerful energetic connection to 'out there' expanded consciousness. That's why we feel *knowing* – intuitive knowing – in the centre of the chest. Knowing doesn't come from a process of thoughts leading to a conclusion. It is there in an instant all in one go. Our heart knows because of its connection to levels of awareness that *do* know. This is the meaning and source of intuition – intuitive *knowing*.

For the last more than 30 years of uncovering the global game and the nature of reality my heart has been my constant antenna for truth and accuracy. An American intelligence insider once said that I had quoted a disinformer in one of my books and yet I had only quoted the part that was true. He asked: 'How do you do that?' By using my heart antenna was the answer and anyone can do it. Heart-centred is how we are meant to be. With a closed heart chakra we withdraw into a closed mind and the bubble of five-sense reality. If you take a moment to focus your attention on the centre of your chest, picture a spinning wheel of light and see it opening and expanding. You will feel it happening, too, and perceptions of the heart like joy and love as the heart impacts on the mind as they interact. The more the chakra opens the more you will feel expressions of heart consciousness and as the process continues, and becomes part of you, insights and knowings will follow. An open

heart is connected to that level of awareness that knows all is *One*. You will see from its perspective that the fault-lines that divide us are only illusions to control us. An open heart does not process the illusions of race, creed and sexuality except as brief experiences for a consciousness that is all. Our heart does not see division, only unity (Figs 24 and 25). There's something else, too. Our hearts love to laugh. Mark Twain's quote that says 'The human race has one really effective weapon, and that is laughter' is really a reference to the heart which loves to laugh with the joy of knowing the true nature of infinite reality and that all the madness of human society is an illusion of the mind. Twain also said: 'Against the assault of laughter nothing can stand.' This is so true of Wetiko and the Cult. Their insecurity demands that they be taken seriously and their power and authority acknowledged and feared. We should do nothing of the sort. We should not get aggressive or fearful which their insecurity so desires. We should laugh in their face. Even in their no-face as police come over in their face-nappies and expect to be taken seriously. They don't take themselves seriously looking like that so why should we? Laugh in the face of intimidation. Laugh in the face of tyranny. You will see by its reaction that you have pressed all of its buttons. Wetiko does not know what to do in the face of laughter or when its targets refuse to concede their joy to fear. We have seen many examples during the 'Covid' hoax when people have expressed their energetic power and the string puppets of Wetiko retreat with their tail limp between their knees. Laugh – the world is bloody mad after all and if it's a choice between laughter and tears I know which way I'm going.



Figure 24: Head consciousness without the heart sees division and everything apart from everything else.



Figure 25: Heart consciousness sees everything as One.

'Vaccines' and the soul

The foundation of Wetiko/Archon control of humans is the separation of incarnate five-sense mind from the infinite 'I' and closing the heart chakra where the True 'I' lives during a human life. The goal has been to achieve complete separation in both cases. I was interested therefore to read an account by a French energetic healer of what she said she experienced with a patient who had been given the 'Covid' vaccine. Genuine energy healers can sense information and consciousness fields at different levels of being which are referred to as 'subtle bodies'. She described treating the patient who later returned after having, without the healer's knowledge, two doses of the 'Covid vaccine'. The healer said:

I noticed immediately the change, very heavy energy emanating from [the] subtle bodies. The scariest thing was when I was working on the heart chakra, I connected with her soul: it was detached from the physical body, it had no contact and it was, as if it was floating in a state of total confusion: a damage to the consciousness that loses contact with the physical body, i.e. with our biological machine, there is no longer any communication between them.

I continued the treatment by sending light to the heart chakra, the soul of the person, but it seemed that the soul could no longer receive any light, frequency or energy. It was a very powerful experience for me. Then I understood that this substance is indeed used to detach consciousness so that this consciousness can no longer interact through this body that it possesses in life, where there is no longer any contact, no frequency, no light, no more energetic balance or mind.

This would create a human that is rudderless and at the extreme almost zombie-like operating with a fractional state of consciousness at the mercy of Wetiko. I was especially intrigued by what the healer said in the light of the prediction by the highly-informed Rudolf Steiner more than a hundred years ago. He said:

In the future, we will eliminate the soul with medicine. Under the pretext of a 'healthy point of view', there will be a vaccine by which the human body will be treated as soon as possible directly at birth, so that the human being cannot develop the thought of the existence of soul and Spirit. To materialistic doctors will be entrusted the task of removing the soul of humanity.

As today, people are vaccinated against this disease or that disease, so in the future, children will be vaccinated with a substance that can be produced precisely in such a way that people, thanks to this vaccination, will be immune to being subjected to the 'madness' of spiritual life. He would be extremely smart, but he would not develop a conscience, and that is the true goal of some materialistic circles.

Steiner said the vaccine would detach the physical body from the etheric body (subtle bodies) and 'once the etheric body is detached the relationship between the universe and the etheric body would become extremely unstable, and man would become an automaton'. He said 'the physical body of man must be polished on this Earth by spiritual will – so the vaccine becomes a kind of arymanique (Wetiko) force' and 'man can no longer get rid of a given materialistic feeling'. Humans would then, he said, become 'materialistic of constitution and can no longer rise to the spiritual'. I have been writing for years about DNA being a receiver-transmitter of information that connects us to other levels of reality and these 'vaccines' changing DNA can be likened to changing an antenna and what it can transmit and receive. Such a disconnection would clearly lead to changes in personality and perception. Steiner further predicted the arrival of AI. Big Pharma 'Covid vaccine' makers, expressions of Wetiko, are testing their DNA-manipulating evil on children as I write with a view to giving the 'vaccine' to babies. If it's a soul-body disconnector – and I say that it is or can be – every child would be disconnected from 'soul' at birth and the 'vaccine' would create a closed system in which spiritual guidance from the greater self would play no part. This has been the ambition of Wetiko all

along. A Pentagon video from 2005 was leaked of a presentation explaining the development of vaccines to change behaviour by their effect on the brain. Those that believe this is not happening with the 'Covid' genetically-modifying procedure masquerading as a 'vaccine' should make an urgent appointment with Naivety Anonymous. Klaus Schwab wrote in 2018:

> Neurotechnologies enable us to better influence consciousness and thought and to understand many activities of the brain. They include decoding what we are thinking in fine levels of detail through new chemicals and interventions that can influence our brains to correct for errors or enhance functionality.

The plan is clear and only the heart can stop it. With every heart that opens, every mind that awakens, Wetiko is weakened. Heart and love are far more powerful than head and hate and so nothing like a majority is needed to turn this around.

Beyond the Phantom

Our heart is the prime target of Wetiko and so it must be the answer to Wetiko. We *are* our heart which is part of one heart, the infinite heart. Our heart is where the true self lives in a human life behind firewalls of five-sense illusion when an imposter takes its place – *Phantom Self*; but our heart waits patiently to be set free any time we choose to see beyond the Phantom, beyond Wetiko. A Wetikoed Phantom Self can wreak mass death and destruction while the love of forever is locked away in its heart. The time is here to unleash its power and let it sweep away the fear and despair that is Wetiko. Heart consciousness does not seek manipulated, censored, advantage for its belief or religion, its activism and desires. As an expression of the One it treats all as One with the same rights to freedom and opinion. Our heart demands fairness for itself no more than for others. From this unity of heart we can come together in mutual support and transform this Wetikoed world into what reality is meant to be – a place of love, joy, happiness, fairness, justice and freedom. Wetiko has another agenda and that's why the world is as

it is, but enough of this nonsense. Wetiko can't stay where hearts are open and it works so hard to keep them closed. Fear is its currency and its food source and love in its true sense has no fear. Why would love have fear when it knows it is *All That Is, Has Been, And Ever Can Be* on an eternal exploration of all possibility? Love in this true sense is not the physical attraction that passes for love. This can be an expression of it, yes, but Infinite Love, a love without condition, goes far deeper to the core of all being. It *is* the core of all being. Infinite realty was born from love beyond the illusions of the simulation. Love infinitely expressed is the knowing that all is One and the swiftly-passing experience of separation is a temporary hallucination. You cannot disconnect from Oneness; you can only *perceive* that you have and withdraw from its influence. This is the most important of all perception trickery by the mind parasite that is Wetiko and the foundation of all its potential for manipulation.

If we open our hearts, open the sluice gates of the mind, and redefine self-identity amazing things start to happen. Consciousness expands or contracts in accordance with self-identity. When true self is recognised as infinite awareness and label self – Phantom Self – is seen as only a series of brief experiences life is transformed. Consciousness expands to the extent that self-identity expands and everything changes. You see unity, not division, the picture, not the pixels. From this we can play the long game. No more is an experience something in and of itself, but a fleeting moment in the eternity of forever. Suddenly people in uniform and dark suits are no longer intimidating. Doing what your heart knows to be right is no longer intimidating and consequences for those actions take on the same nature of a brief experience that passes in the blink of an infinite eye. Intimidation is all in the mind. Beyond the mind there is no intimidation.

An open heart does not consider consequences for what it knows to be right. To do so would be to consider not doing what it knows to be right and for a heart in its power that is never an option. The Renegade Mind is really the Renegade Heart. Consideration of consequences will always provide a getaway car for the mind and

the heart doesn't want one. What is right in the light of what we face today is to stop cooperating with Wetiko in all its forms and to do it without fear or compromise. You cannot compromise with tyranny when tyranny always demands more until it has everything. Life is your perception and you are your destiny. Change your perception and you change your life. Change collective perception and we change the world.

Come on people … One human family, One heart, One goal … *FREEEEEEDOM*!

We must settle for nothing less.

Postscript

The big scare story as the book goes to press is the 'Indian' variant and the world is being deluged with propaganda about the 'Covid catastrophe' in India which mirrors in its lies and misrepresentations what happened in Italy before the first lockdown in 2020.

The *New York Post* published a picture of someone who had 'collapsed in the street from Covid' in India in April, 2021, which was actually taken during a gas leak in May, 2020. Same old, same old. Media articles in mid-February were asking why India had been so untouched by 'Covid' and then as their vaccine rollout gathered pace the alleged 'cases' began to rapidly increase. Indian 'Covid vaccine' maker Bharat Biotech was funded into existence by the Bill and Melinda Gates Foundation (the pair announced their divorce in May, 2021, which is a pity because they so deserve each other). The Indian 'Covid crisis' was ramped up by the media to terrify the world and prepare people for submission to still more restrictions. The scam that worked the first time was being repeated only with far more people seeing through the deceit. Davidicke.com and Ickonic.com have sought to tell the true story of what is happening by talking to people living through the Indian nightmare which has nothing to do with 'Covid'. We posted a letter from 'Alisha' in Pune who told a very different story to government and media mendacity. She said scenes of dying people and overwhelmed hospitals were designed to hide what was really happening – genocide and starvation. Alisha said that millions had already died of starvation during the ongoing lockdowns while government and media were lying and making it look like the 'virus':

Restaurants, shops, gyms, theatres, basically everything is shut. The cities are ghost towns. Even so-called 'essential' businesses are only open till 11am in the morning. You basically have just an hour to buy food and then your time is up.

Inter-state travel and even inter-district travel is banned. The cops wait at all major crossroads to question why you are traveling outdoors or to fine you if you are not wearing a mask.

The medical community here is also complicit in genocide, lying about hospitals being full and turning away people with genuine illnesses, who need immediate care. They have even created a shortage of oxygen cylinders.

This is the classic Cult modus operandi played out in every country. Alisha said that people who would not have a PCR test not testing for the 'virus' were being denied hospital treatment. She said the people hit hardest were migrant workers and those in rural areas. Most businesses employed migrant workers and with everything closed there were no jobs, no income and no food. As a result millions were dying of starvation or malnutrition. All this was happening under Prime Minister Narendra Modi, a 100-percent asset of the Cult, and it emphasises yet again the scale of pure anti-human evil we are dealing with. Australia banned its people from returning home from India with penalties for trying to do so of up to five years in jail and a fine of £37,000. The manufactured 'Covid' crisis in India was being prepared to justify further fascism in the West. Obvious connections could be seen between the Indian 'vaccine' programme and increased 'cases' and this became a common theme. The Seychelles, the most per capita 'Covid vaccinated' population in the world, went back into lockdown after a 'surge of cases'.

Long ago the truly evil Monsanto agricultural biotechnology corporation with its big connections to Bill Gates devastated Indian farming with genetically-modified crops. Human rights activist Gurcharan Singh highlighted the efforts by the Indian government to complete the job by destroying the food supply to hundreds of millions with 'Covid' lockdowns. He said that 415 million people at the bottom of the disgusting caste system (still going whatever they say) were below the poverty line and struggled to feed themselves every year. Now the government was imposing lockdown at just the

time to destroy the harvest. This deliberate policy was leading to mass starvation. People may reel back at the suggestion that a government would do that, but Wetiko-controlled 'leaders' are capable of any level of evil. In fact what is described in India is in the process of being instigated worldwide. The food chain and food supply are being targeted at every level to cause world hunger and thus control. Bill Gates is not the biggest owner of farmland in America for no reason and destroying access to food aids both the depopulation agenda and the plan for synthetic 'food' already being funded into existence by Gates. Add to this the coming hyper-inflation from the suicidal creation of fake 'money' in response to 'Covid' and the breakdown of container shipping systems and you have a cocktail that can only lead one way and is meant to. The Cult plan is to crash the entire system to 'build back better' with the Great Reset.

'Vaccine' transmission

Reports from all over the world continue to emerge of women suffering menstrual and fertility problems after having the fake 'vaccine' and of the non-'vaccinated' having similar problems when interacting with the 'vaccinated'. There are far too many for 'coincidence' to be credible. We've had menopausal women getting periods, others having periods stop or not stopping for weeks, passing clots, sometimes the lining of the uterus, breast irregularities, and miscarriages (which increased by 400 percent in parts of the United States). Non-'vaccinated' men and children have suffered blood clots and nose bleeding after interaction with the 'vaccinated'. Babies have died from the effects of breast milk from a 'vaccinated' mother. Awake doctors – the small minority – speculated on the cause of non-'vaccinated' suffering the same effects as the 'vaccinated'. Was it nanotechnology in the synthetic substance transmitting frequencies or was it a straight chemical bioweapon that was being transmitted between people? I am not saying that some kind of chemical transmission is not one possible answer, but the foundation of all that the Cult does is frequency and

this is fertile ground for understanding how transmission can happen. American doctor Carrie Madej, an internal medicine physician and osteopath, has been practicing for the last 20 years, teaching medical students, and she says attending different meetings where the agenda for humanity was discussed. Madej, who operates out of Georgia, did not dismiss other possible forms of transmission, but she focused on frequency in search of an explanation for transmission. She said the Moderna and Pfizer 'vaccines' contained nano-lipid particles as a key component. This was a brand new technology never before used on humanity. 'They're using a nanotechnology which is pretty much little tiny computer bits … nanobots or hydrogel.' Inside the 'vaccines' was 'this sci-fi kind of substance' which suppressed immune checkpoints to get into the cell. I referred to this earlier as the 'Trojan horse' technique that tricks the cell into opening a gateway for the self-replicating synthetic material and while the immune system is artificially suppressed the body has no defences. Madej said the substance served many purposes including an on-demand ability to 'deliver the payload' and using the nano 'computer bits' as biosensors in the body. 'It actually has the ability to accumulate data from your body, like your breathing, your respiration, thoughts, emotions, all kinds of things.'

She said the technology obviously has the ability to operate through Wi-Fi and transmit and receive energy, messages, frequencies or impulses. 'Just imagine you're getting this new substance in you and it can react to things all around you, the 5G, your smart device, your phones.' We had something completely foreign in the human body that had never been launched large scale at a time when we were seeing 5G going into schools and hospitals (plus the Musk satellites) and she believed the 'vaccine' transmission had something to do with this: '… if these people have this inside of them … it can act like an antenna and actually transmit it outwardly as well.' The synthetic substance produced its own voltage and so it could have that kind of effect. This fits with my own contention that the nano receiver-transmitters are designed to connect people to the

Smart Grid and break the receiver-transmitter connection to expanded consciousness. That would explain the French energy healer's experience of the disconnection of body from 'soul' with those who have had the 'vaccine'. The nanobots, self-replicating inside the body, would also transmit the synthetic frequency which could be picked up through close interaction by those who have not been 'vaccinated'. Madej speculated that perhaps it was 5G and increased levels of other radiation that was causing the symptoms directly although interestingly she said that non-'vaccinated' patients had shown improvement when they were away from the 'vaccinated' person they had interacted with. It must be remembered that you can control frequency and energy with your mind and you can consciously create energetic barriers or bubbles with the mind to stop damaging frequencies from penetrating your field. American paediatrician Dr Larry Palevsky said the 'vaccine' was not a 'vaccine' and was never designed to protect from a 'viral' infection. He called it 'a massive, brilliant propaganda of genocide' because they didn't have to inject everyone to get the result they wanted. He said the content of the jabs was able to infuse any material into the brain, heart, lungs, kidneys, liver, sperm and female productive system. 'This is genocide; this is a weapon of mass destruction.' At the same time American colleges were banning students from attending if they didn't have this life-changing and potentially life-ending 'vaccine'. Class action lawsuits must follow when the consequences of this college fascism come to light. As the book was going to press came reports about fertility effects on sperm in 'vaccinated' men which would absolutely fit with what I have been saying and hospitals continued to fill with 'vaccine' reactions. Another question is what about transmission via blood transfusions? The NHS has extended blood donation restrictions from seven days after a 'Covid vaccination' to 28 days after even a sore arm reaction.

I said in the spring of 2020 that the then touted 'Covid vaccine' would be ongoing each year like the flu jab. A year later Pfizer CEO, the appalling Albert Bourla, said people would 'likely' need a 'booster dose' of the 'vaccine' within 12 months of getting 'fully

vaccinated' and then a yearly shot. 'Variants will play a key role', he said confirming the point. Johnson & Johnson CEO Alex Gorsky also took time out from his 'vaccine' disaster to say that people may need to be vaccinated against 'Covid-19' each year. UK Health Secretary, the psychopath Matt Hancock, said additional 'boosters' would be available in the autumn of 2021. This is the trap of the 'vaccine passport'. The public will have to accept every last 'vaccine' they introduce, including for the fake 'variants', or it would cease to be valid. The only other way in some cases would be continuous testing with a test not testing for the 'virus' and what is on the swabs constantly pushed up your noise towards the brain every time?

'Vaccines' changing behaviour

I mentioned in the body of the book how I believed we would see gathering behaviour changes in the 'vaccinated' and I am already hearing such comments from the non-'vaccinated' describing behaviour changes in friends, loved ones and work colleagues. This will only increase as the self-replicating synthetic material and nanoparticles expand in body and brain. An article in the *Guardian* in 2016 detailed research at the University of Virginia in Charlottesville which developed a new method for controlling brain circuits associated with complex animal behaviour. The method, dubbed 'magnetogenetics', involves genetically-engineering a protein called ferritin, which stores and releases iron, to create a magnetised substance – 'Magneto' – that can activate specific groups of nerve cells from a distance. This is claimed to be an advance on other methods of brain activity manipulation known as optogenetics and chemogenetics (the Cult has been developing methods of brain control for a long time). The ferritin technique is said to be non-invasive and able to activate neurons 'rapidly and reversibly'. In other words, human thought and perception. The article said that earlier studies revealed how nerve cell proteins 'activated by heat and mechanical pressure can be genetically engineered so that they become sensitive to radio waves and magnetic fields, by attaching them to an iron-storing protein called ferritin, or to inorganic

paramagnetic particles'. Sensitive to radio waves and magnetic fields? You mean like 5G, 6G and 7G? This is the human-AI Smart Grid hive mind we are talking about. The *Guardian* article said:

> … the researchers injected Magneto into the striatum of freely behaving mice, a deep brain structure containing dopamine-producing neurons that are involved in reward and motivation, and then placed the animals into an apparatus split into magnetised and non-magnetised sections.
>
> Mice expressing Magneto spent far more time in the magnetised areas than mice that did not, because activation of the protein caused the striatal neurons expressing it to release dopamine, so that the mice found being in those areas rewarding. This shows that Magneto can remotely control the firing of neurons deep within the brain, and also control complex behaviours.

Make no mistake this basic methodology will be part of the 'Covid vaccine' cocktail and using magnetics to change brain function through electromagnetic field frequency activation. The Pentagon is developing a 'Covid vaccine' using ferritin. Magnetics would explain changes in behaviour and why videos are appearing across the Internet as I write showing how magnets stick to the skin at the point of the 'vaccine' shot. Once people take these 'vaccines' anything becomes possible in terms of brain function and illness which will be blamed on 'Covid-19' and 'variants'. Magnetic field manipulation would further explain why the non-'vaccinated' are reporting the same symptoms as the 'vaccinated' they interact with and why those symptoms are reported to decrease when not in their company. Interestingly 'Magneto', a 'mutant', is a character in the Marvel Comic *X-Men* stories with the ability to manipulate magnetic fields and he believes that mutants should fight back against their human oppressors by any means necessary. The character was born Erik Lehnsherr to a Jewish family in Germany.

Cult-controlled courts

The European Court of Human Rights opened the door for mandatory 'Covid-19 vaccines' across the continent when it ruled in a Czech Republic dispute over childhood immunisation that legally

enforced vaccination could be 'necessary in a democratic society'. The 17 judges decided that compulsory vaccinations did not breach human rights law. On the face of it the judgement was so inverted you gasp for air. If not having a vaccine infused into your body is not a human right then what is? Ah, but they said human rights law which has been specifically written to delete all human rights at the behest of the state (the Cult). Article 8 of the European Convention on Human Rights relates to the right to a private life. The crucial word here is *'except'*:

> There shall be no interference by a public authority with the exercise of this right EXCEPT such as is in accordance with the law and is necessary in a democratic society in the interests of national security, public safety or the economic wellbeing of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others [My emphasis].

No interference *except* in accordance with the law means there *are* no 'human rights' *except* what EU governments decide you can have at their behest. 'As is necessary in a democratic society' explains that reference in the judgement and 'in the interests of national security, public safety or the economic well-being of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others' gives the EU a coach and horses to ride through 'human rights' and scatter them in all directions. The judiciary is not a check and balance on government extremism; it is a vehicle to enforce it. This judgement was almost laughably predictable when the last thing the Cult wanted was a decision that went against mandatory vaccination. Judges rule over and over again to benefit the system of which they are a part. Vaccination disputes that come before them are invariably delivered in favour of doctors and authorities representing the view of the state which owns the judiciary. Oh, yes, and we have even had calls to stop putting 'Covid-19' on death certificates within 28 days of a 'positive test' because it is claimed the practice makes the 'vaccine' appear not to work. They are laughing at you.

The scale of madness, inhumanity and things to come was highlighted when those not 'vaccinated' for 'Covid' were refused evacuation from the Caribbean island of St Vincent during massive volcanic eruptions. Cruise ships taking residents to the safety of another island allowed only the 'vaccinated' to board and the rest were left to their fate. Even in life and death situations like this we see 'Covid' stripping people of their most basic human instincts and the insanity is even more extreme when you think that fake 'vaccine'-makers are not even claiming their body-manipulating concoctions stop 'infection' and 'transmission' of a 'virus' that doesn't exist. St Vincent Prime Minister Ralph Gonsalves said: 'The chief medical officer will be identifying the persons already vaccinated so that we can get them on the ship.' Note again the power of the chief medical officer who, like Whitty in the UK, will be answering to the World Health Organization. This is the Cult network structure that has overridden politicians who 'follow the science' which means doing what WHO-controlled 'medical officers' and 'science advisers' tell them. Gonsalves even said that residents who were 'vaccinated' after the order so they could board the ships would still be refused entry due to possible side effects such as 'wooziness in the head'. The good news is that if they were woozy enough in the head they could qualify to be prime minister of St Vincent.

Microchipping freedom

The European judgement will be used at some point to justify moves to enforce the 'Covid' DNA-manipulating procedure. Sandra Ro, CEO of the Global Blockchain Business Council, told a World Economic Forum event that she hoped 'vaccine passports' would help to 'drive forced consent and standardisation' of global digital identity schemes: 'I'm hoping with the desire and global demand for some sort of vaccine passport – so that people can get travelling and working again – [it] will drive forced consent, standardisation, and frankly, cooperation across the world.' The lady is either not very bright, or thoroughly mendacious, to use the term 'forced consent'.

You do not 'consent' if you are forced – you *submit*. She was describing what the plan has been all along and that's to enforce a digital identity on every human without which they could not function. 'Vaccine passports' are opening the door and are far from the end goal. A digital identity would allow you to be tracked in everything you do in cyberspace and this is the same technique used by Cult-owned China to enforce its social credit system of total control. The ultimate 'passport' is planned to be a microchip as my books have warned for nearly 30 years. Those nice people at the Pentagon working for the Cult-controlled Defense Advanced Research Projects Agency (DARPA) claimed in April, 2021, they have developed a microchip inserted under the skin to detect 'asymptomatic Covid-19 infection' before it becomes an outbreak and a 'revolutionary filter' that can remove the 'virus' from the blood when attached to a dialysis machine. The only problems with this are that the 'virus' does not exist and people transmitting the 'virus' with no symptoms is brain-numbing bullshit. This is, of course, not a ruse to get people to be microchipped for very different reasons. DARPA also said it was producing a one-stop 'vaccine' for the 'virus' and all 'variants'. One of the most sinister organisations on Planet Earth is doing this? Better have it then. These people are insane because Wetiko that possesses them is insane.

Researchers from the Salk Institute in California announced they have created an embryo that is part human and part monkey. My books going back to the 1990s have exposed experiments in top secret underground facilities in the United States where humans are being crossed with animal and non-human 'extraterrestrial' species. They are now easing that long-developed capability into the public arena and there is much more to come given we are dealing with psychiatric basket cases. Talking of which – Elon Musk's scientists at Neuralink trained a monkey to play Pong and other puzzles on a computer screen using a joystick and when the monkey made the correct move a metal tube squirted banana smoothie into his mouth which is the basic technique for training humans into unquestioning compliance. Two Neuralink chips were in the monkey's skull and

more than 2,000 wires 'fanned out' into its brain. Eventually the monkey played a video game purely with its brain waves. Psychopathic narcissist Musk said the 'breakthrough' was a step towards putting Neuralink chips into human skulls and merging minds with artificial intelligence. *Exactly*. This man is so dark and Cult to his DNA.

World Economic Fascism (WEF)

The World Economic Forum is telling you the plan by the statements made at its many and various events. Cult-owned fascist YouTube CEO Susan Wojcicki spoke at the 2021 WEF Global Technology Governance Summit (see the name) in which 40 governments and 150 companies met to ensure 'the responsible design and deployment of emerging technologies'. Orwellian translation: 'Ensuring the design and deployment of long-planned technologies will advance the Cult agenda for control and censorship.' Freedom-destroyer and Nuremberg-bound Wojcicki expressed support for tech platforms like hers to censor content that is 'technically legal but could be harmful'. Who decides what is 'harmful'? She does and they do. 'Harmful' will be whatever the Cult doesn't want people to see and we have legislation proposed by the UK government that would censor content on the basis of 'harm' no matter if the information is fair, legal and provably true. Make that *especially* if it is fair, legal and provably true. Wojcicki called for a global coalition to be formed to enforce content moderation standards through automated censorship. This is a woman and mega-censor so self-deluded that she shamelessly accepted a 'free expression' award – *Wojcicki* – in an event sponsored by her own *YouTube*. They have no shame and no self-awareness.

You know that 'Covid' is a scam and Wojcicki a Cult operative when YouTube is censoring medical and scientific opinion purely on the grounds of whether it supports or opposes the Cult 'Covid' narrative. Florida governor Ron DeSantis compiled an expert panel with four professors of medicine from Harvard, Oxford, and Stanford Universities who spoke against forcing children and

vaccinated people to wear masks. They also said there was no proof that lockdowns reduced spread or death rates of 'Covid-19'. Cult-gofer Wojcicki and her YouTube deleted the panel video 'because it included content that contradicts the consensus of local and global health authorities regarding the efficacy of masks to prevent the spread of Covid-19'. This 'consensus' refers to what the Cult tells the World Health Organization to say and the WHO tells 'local health authorities' to do. Wojcicki knows this, of course. The panellists pointed out that censorship of scientific debate was responsible for deaths from many causes, but Wojcicki couldn't care less. She would not dare go against what she is told and as a disgrace to humanity she wouldn't want to anyway. The UK government is seeking to pass a fascist 'Online Safety Bill' to specifically target with massive fines and other means non-censored video and social media platforms to make them censor 'lawful but harmful' content like the Cult-owned Facebook, Twitter, Google and YouTube. What is 'lawful but harmful' would be decided by the fascist Blair-created Ofcom.

Another WEF obsession is a cyber-attack on the financial system and this is clearly what the Cult has planned to take down the bank accounts of everyone – except theirs. Those that think they have enough money for the Cult agenda not to matter to them have got a big lesson coming if they continue to ignore what is staring them in the face. The World Economic Forum, funded by Gates and fronted by Klaus Schwab, announced it would be running a 'simulation' with the Russian government and global banks of just such an attack called Cyber Polygon 2021. What they simulate – as with the 'Covid' Event 201 – they plan to instigate. The WEF is involved in a project with the Cult-owned Carnegie Endowment for International Peace called the WEF-Carnegie Cyber Policy Initiative which seeks to merge Wall Street banks, 'regulators' (I love it) and intelligence agencies to 'prevent' (arrange and allow) a cyber-attack that would bring down the global financial system as long planned by those that control the WEF and the Carnegie operation. The Carnegie Endowment for International Peace sent an instruction to First World

War US President Woodrow Wilson not to let the war end before society had been irreversibly transformed.

The Wuhan lab diversion

As I close, the Cult-controlled authorities and lapdog media are systematically pushing 'the virus was released from the Wuhan lab' narrative. There are two versions – it happened by accident and it happened on purpose. Both are nonsense. The perceived existence of the never-shown-to-exist 'virus' is vital to sell the impression that there is actually an infective agent to deal with and to allow the endless potential for terrifying the population with 'variants' of a 'virus' that does not exist. The authorities at the time of writing are going with the 'by accident' while the alternative media is promoting the 'on purpose'. Cable news host Tucker Carlson who has questioned aspects of lockdown and 'vaccine' compulsion has bought the Wuhan lab story. 'Everyone now agrees' he said. Well, I don't and many others don't and the question is *why* does the system and its media suddenly 'agree'? When the media moves as one unit with a narrative it is always a lie – witness the hour by hour mendacity of the 'Covid' era. Why would this Cult-owned combination which has unleashed lies like machine gun fire suddenly 'agree' to tell the truth??

Much of the alternative media is buying the lie because it fits the conspiracy narrative, but it's the *wrong* conspiracy. The real conspiracy is that *there is no virus* and that is what the Cult is desperate to hide. The idea that the 'virus' was released by accident is ludicrous when the whole 'Covid' hoax was clearly long-planned and waiting to be played out as it was so fast in accordance with the Rockefeller document and Event 201. So they prepared everything in detail over decades and then sat around strumming their fingers waiting for an 'accidental' release from a bio-lab? *What??* It's crazy. Then there's the 'on purpose' claim. You want to circulate a 'deadly virus' and hide the fact that you've done so and you release it down the street from the highest-level bio-lab in China? I repeat – *What??*

You would release it far from that lab to stop any association being made. But, no, we'll do it in a place where the connection was certain to be made. Why would you need to scam 'cases' and 'deaths' and pay hospitals to diagnose 'Covid-19' if you had a real 'virus'? What are sections of the alternative media doing believing this crap? Where were all the mass deaths in Wuhan from a 'deadly pathogen' when the recovery to normal life after the initial propaganda was dramatic in speed? Why isn't the 'deadly pathogen' now circulating all over China with bodies in the street? Once again we have the technique of tell them what they want to hear and they will likely believe it. The alternative media has its 'conspiracy' and with Carlson it fits with his 'China is the danger' narrative over years. China *is* a danger as a global Cult operations centre, but not for this reason. The Wuhan lab story also has the potential to instigate conflict with China when at some stage the plan is to trigger a Problem-Reaction-Solution confrontation with the West. Question everything – *everything* – and especially when the media agrees on a common party line.

Third wave ... fourth wave ... fifth wave ...

As the book went into production the world was being set up for more lockdowns and a 'third wave' supported by invented 'variants' that were increasing all the time and will continue to do so in public statements and computer programs, but not in reality. India became the new Italy in the 'Covid' propaganda campaign and we were told to be frightened of the new 'Indian strain'. Somehow I couldn't find it within myself to do so. A document produced for the UK government entitled 'Summary of further modelling of easing of restrictions – Roadmap Step 2' declared that a third wave was inevitable (of course when it's in the script) and it would be the fault of children and those who refuse the health-destroying fake 'Covid vaccine'. One of the computer models involved came from the Cult-owned *Imperial College* and the other from Warwick University which I wouldn't trust to tell me the date in a calendar factory. The document states that both models presumed extremely high uptake

of the 'Covid vaccines' and didn't allow for 'variants'. The document states: 'The resurgence is a result of some people (mostly children) being ineligible for vaccination; others choosing not to receive the vaccine; and others being vaccinated but not perfectly protected.' The mendacity takes the breath away. Okay, blame those with a brain who won't take the DNA-modifying shots and put more pressure on children to have it as 'trials' were underway involving children as young as six months with parents who give insanity a bad name. Massive pressure is being put on the young to have the fake 'vaccine' and child age consent limits have been systematically lowered around the world to stop parents intervening. Most extraordinary about the document was its claim that the 'third wave' would be driven by 'the resurgence in both hospitalisations and deaths … dominated by *those that have received two doses of the vaccine*, comprising around 60-70% of the wave respectively'. The predicted peak of the 'third wave' suggested 300 deaths per day with 250 of them *fully 'vaccinated' people*. How many more lies do acquiescers need to be told before they see the obvious? Those who took the jab to 'protect themselves' are projected to be those who mostly get sick and die? So what's in the 'vaccine'? The document went on:

> It is possible that a summer of low prevalence could be followed by substantial increases in incidence over the following autumn and winter. Low prevalence in late summer should not be taken as an indication that SARS-CoV-2 has retreated or that the population has high enough levels of immunity to prevent another wave.

They are telling you the script and while many British people believed 'Covid' restrictions would end in the summer of 2021 the government was preparing for them to be ongoing. Authorities were awarding contracts for 'Covid marshals' to police the restrictions with contracts starting in July, 2021, and going through to January 31st, 2022, and the government was advertising for 'Media Buying Services' to secure media propaganda slots worth a potential £320 million for 'Covid-19 campaigns' with a contract not ending until March, 2022. The recipient – via a list of other front companies – was reported to be American media marketing giant Omnicom Group

Inc. While money is no object for 'Covid' the UK waiting list for all other treatment – including life-threatening conditions – passed 4.5 million. Meantime the Cult is seeking to control all official 'inquiries' to block revelations about what has really been happening and why. It must not be allowed to – we need Nuremberg jury trials in every country. The cover-up doesn't get more obvious than appointing ultra-Zionist professor Philip Zelikow to oversee two dozen US virologists, public health officials, clinicians, former government officials and four American 'charitable foundations' to 'learn the lessons' of the 'Covid' debacle. The personnel will be those that created and perpetuated the 'Covid' lies while Zelikow is the former executive director of the 9/11 Commission who ensured that the truth about those attacks never came out and produced a report that must be among the most mendacious and manipulative documents ever written – see *The Trigger* for the detailed exposure of the almost unimaginable 9/11 story in which Sabbatians can be found at every level.

Passive no more

People are increasingly challenging the authorities with amazing numbers of people taking to the streets in London well beyond the ability of the Face-Nappies to stop them. Instead the Nappies choose situations away from the mass crowds to target, intimidate, and seek to promote the impression of 'violent protestors'. One such incident happened in London's Hyde Park. Hundreds of thousands walking through the streets in protest against 'Covid' fascism were ignored by the Cult-owned BBC and most of the rest of the mainstream media, but they delighted in reporting how police were injured in 'clashes with protestors'. The truth was that a group of people gathered in Hyde Park at the end of one march when most had gone home and they were peacefully having a good time with music and chat. Face-Nappies who couldn't deal with the full-march crowd then waded in with their batons and got more than they bargained for. Instead of just standing for this criminal brutality the crowd used their numerical superiority to push the Face-Nappies out of the

park. Eventually the Nappies turned and ran. Unfortunately two or three idiots in the crowd threw drink cans striking two officers which gave the media and the government the image they wanted to discredit the 99.9999 percent who were peaceful. The idiots walked straight into the trap and we must always be aware of potential agent provocateurs used by the authorities to discredit their targets.

This response from the crowd – the can people apart – must be a turning point when the public no longer stand by while the innocent are arrested and brutally attacked by the Face-Nappies. That doesn't mean to be violent, that's the last thing we need. We'll leave the violence to the Face-Nappies and government. But it does mean that when the Face-Nappies use violence against peaceful people the numerical superiority is employed to stop them and make citizen's arrests or Common Law arrests for a breach of the peace. The time for being passive in the face of fascism is over.

We are the many, they are the few, and we need to make that count before there is no freedom left and our children and grandchildren face an ongoing fascist nightmare.

COME ON PEOPLE – IT'S TIME.

One final thought …

The power of love
A force from above
Cleaning my soul
Flame on burn desire
Love with tongues of fire
Purge the soul
Make love your goal

I'll protect you from the hooded claw
Keep the vampires from your door
When the chips are down I'll be around
With my undying, death-defying
Love for you

Envy will hurt itself
Let yourself be beautiful
Sparkling love, flowers
And pearls and pretty girls
Love is like an energy
Rushin' rushin' inside of me

This time we go sublime
Lovers entwine, divine, divine,
Love is danger, love is pleasure
Love is pure – the only treasure

I'm so in love with you
Purge the soul
Make love your goal

The power of love
A force from above
Cleaning my soul
The power of love
A force from above
A sky-scraping dove

Flame on burn desire
Love with tongues of fire
Purge the soul
Make love your goal

Frankie Goes To Hollywood

APPENDIX

Cowan-Kaufman-Morell Statement on Virus Isolation (SOVI)

Isolation: The action of isolating; the fact or condition of being isolated or standing alone; separation from other things or persons; solitariness
Oxford English Dictionary

The controversy over whether the SARS-CoV-2 virus has ever been isolated or purified continues. However, using the above definition, common sense, the laws of logic and the dictates of science, any unbiased person must come to the conclusion that the SARS-CoV-2 virus has never been isolated or purified. As a result, no confirmation of the virus' existence can be found. The logical, common sense, and scientific consequences of this fact are:

- the structure and composition of something not shown to exist can't be known, including the presence, structure, and function of any hypothetical spike or other proteins;
- the genetic sequence of something that has never been found can't be known;
- "variants" of something that hasn't been shown to exist can't be known;
- it's impossible to demonstrate that SARS-CoV-2 causes a disease called Covid-19.

In as concise terms as possible, here's the proper way to isolate, characterize and demonstrate a new virus. First, one takes samples (blood, sputum, secretions) from many people (e.g. 500) with symptoms which are unique and specific enough to characterize an illness. Without mixing these samples with ANY tissue or products that also contain genetic material, the virologist macerates, filters and ultracentrifuges i.e. *purifies* the specimen. This common virology technique, done for decades to isolate bacteriophages[1] and so-called giant viruses in every virology lab, then allows the virologist to demonstrate with electron microscopy thousands of identically sized and shaped particles. These particles are the isolated and purified virus.

These identical particles are then checked for uniformity by physical and/or microscopic techniques. Once the purity is determined, the particles may be further characterized. This would include examining the structure, morphology, and chemical composition of the particles. Next, their genetic makeup is characterized by extracting the genetic material directly from the purified particles and using genetic-sequencing techniques, such as Sanger sequencing, that have also been around for decades. Then one does an analysis to confirm that these uniform particles are exogenous (outside) in origin as a virus is conceptualized to be, and not the normal breakdown products of dead and dying tissues.[2] (As of May 2020, we know that virologists have no way to determine whether the particles they're seeing are viruses or just normal breakdown products of dead and dying tissues.)[3]

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1 Isolation, characterization and analysis of bacteriophages from the haloalkaline lake Elmenteita, KenyaJuliah Khayeli Akhwale et al, PLOS One, Published: April 25, 2019. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0215734 – accessed 2/15/21

2 "Extracellular Vesicles Derived From Apoptotic Cells: An Essential Link Between Death and Regeneration," Maojiao Li1 et al, Frontiers in Cell and Developmental Biology, 2020 October 2. https://www.frontiersin.org/articles/10.3389/fcell.2020.573511/full – accessed 2/15/21

3 "The Role of Extraellular Vesicles as Allies of HIV, HCV and SARS Viruses," Flavia Giannessi, et al, Viruses, 2020 May

If we have come this far then we have fully isolated, characterized, and genetically sequenced an exogenous virus particle. However, we still have to show it is causally related to a disease. This is carried out by exposing a group of healthy subjects (animals are usually used) to this isolated, purified virus in the manner in which the disease is thought to be transmitted. If the animals get sick with the same disease, as confirmed by clinical and autopsy findings, one has now shown that the virus actually causes a disease. This demonstrates infectivity and transmission of an infectious agent.

None of these steps has even been attempted with the SARS-CoV-2 virus, nor have all these steps been successfully performed for any so-called pathogenic virus. Our research indicates that a single study showing these steps does not exist in the medical literature.

Instead, since 1954, virologists have taken unpurified samples from a relatively few people, often less than ten, with a similar disease. They then minimally process this sample and inoculate this unpurified sample onto tissue culture containing usually four to six other types of material – all of which contain identical genetic material as to what is called a "virus." The tissue culture is starved and poisoned and naturally disintegrates into many types of particles, some of which contain genetic material. Against all common sense, logic, use of the English language and scientific integrity, this process is called "virus isolation." This brew containing fragments of genetic material from many sources is then subjected to genetic analysis, which then creates in a computer-simulation process the alleged sequence of the alleged virus, a so called in silico genome. At no time is an actual virus confirmed by electron microscopy. At no time is a genome extracted and sequenced from an actual virus. This is scientific fraud.

The observation that the unpurified specimen — inoculated onto tissue culture along with toxic antibiotics, bovine fetal tissue, amniotic fluid and other tissues — destroys the kidney tissue onto which it is inoculated is given as evidence of the virus' existence and pathogenicity. This is scientific fraud.

From now on, when anyone gives you a paper that suggests the SARS-CoV-2 virus has been isolated, please check the methods sections. If the researchers used Vero cells or any other culture method, you know that their process was not isolation. You will hear the following excuses for why actual isolation isn't done:

1. There were not enough virus particles found in samples from patients to analyze.

2. Viruses are intracellular parasites; they can't be found outside the cell in this manner.

If No. 1 is correct, and we can't find the virus in the sputum of sick people, then on what evidence do we think the virus is dangerous or even lethal? If No. 2 is correct, then how is the virus spread from person to person? We are told it emerges from the cell to infect others. Then why isn't it possible to find it?

Finally, questioning these virology techniques and conclusions is not some distraction or divisive issue. Shining the light on this truth is essential to stop this terrible fraud that humanity is confronting. For, as we now know, if the virus has never been isolated, sequenced or shown to cause illness, if the virus is imaginary, then why are we wearing masks, social distancing and putting the whole world into prison?

Finally, if pathogenic viruses don't exist, then what is going into those injectable devices erroneously called "vaccines," and what is their purpose? This scientific question is the most urgent and relevant one of our time.

We are correct. The SARS-CoV2 virus does not exist.

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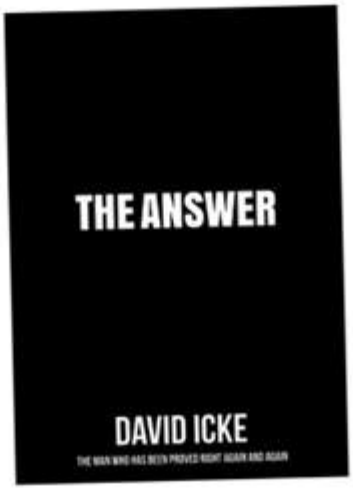

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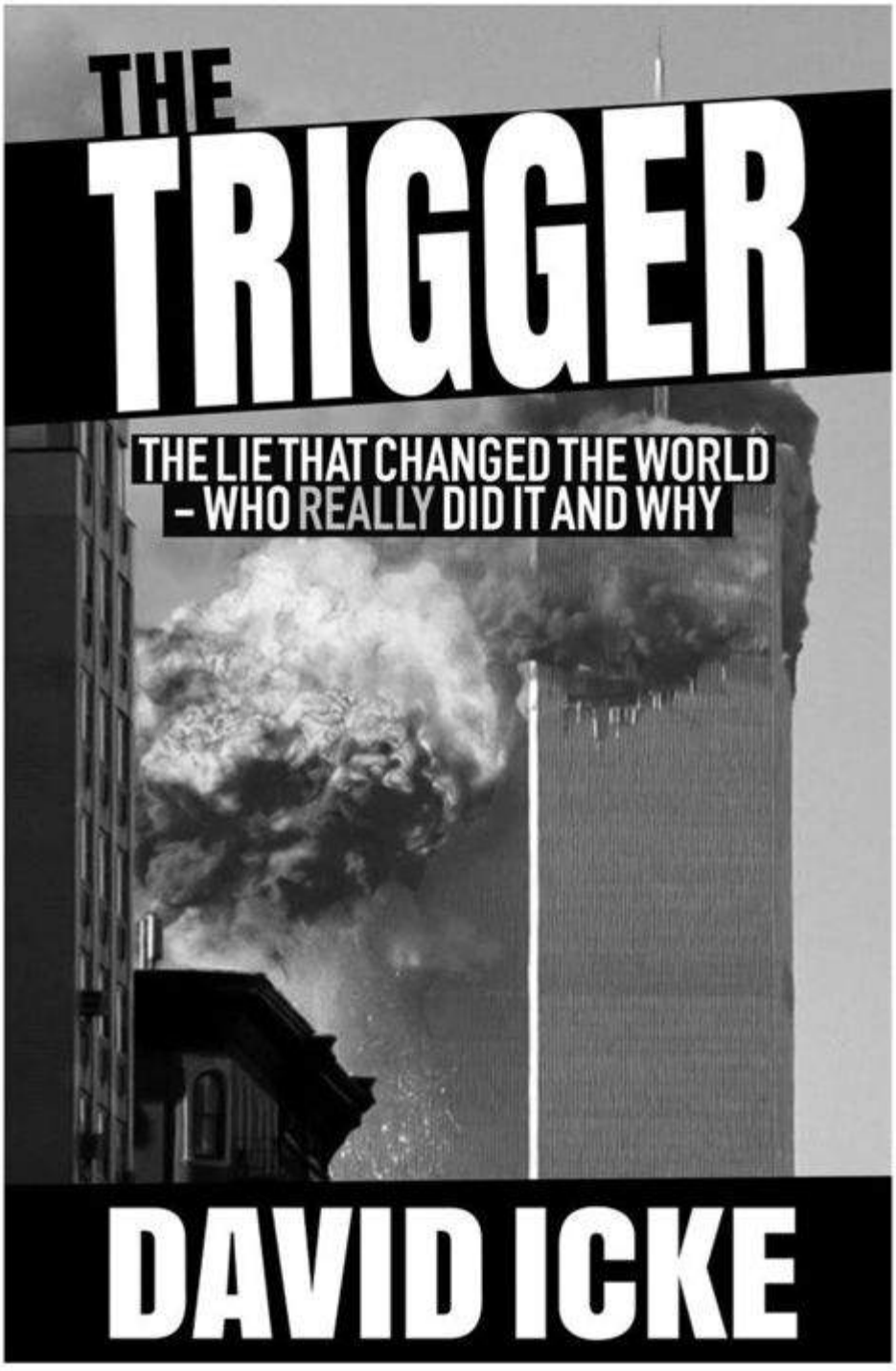
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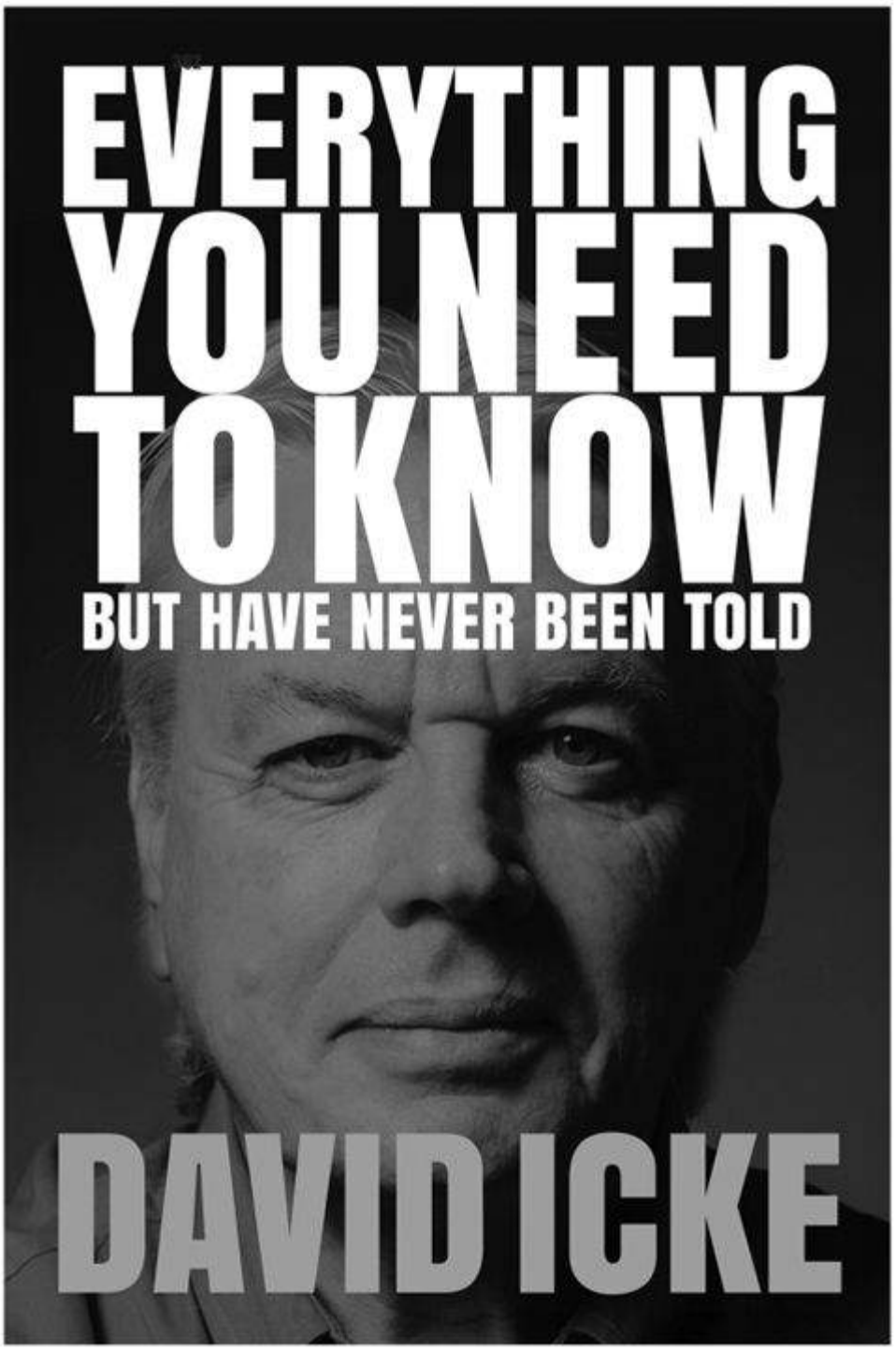
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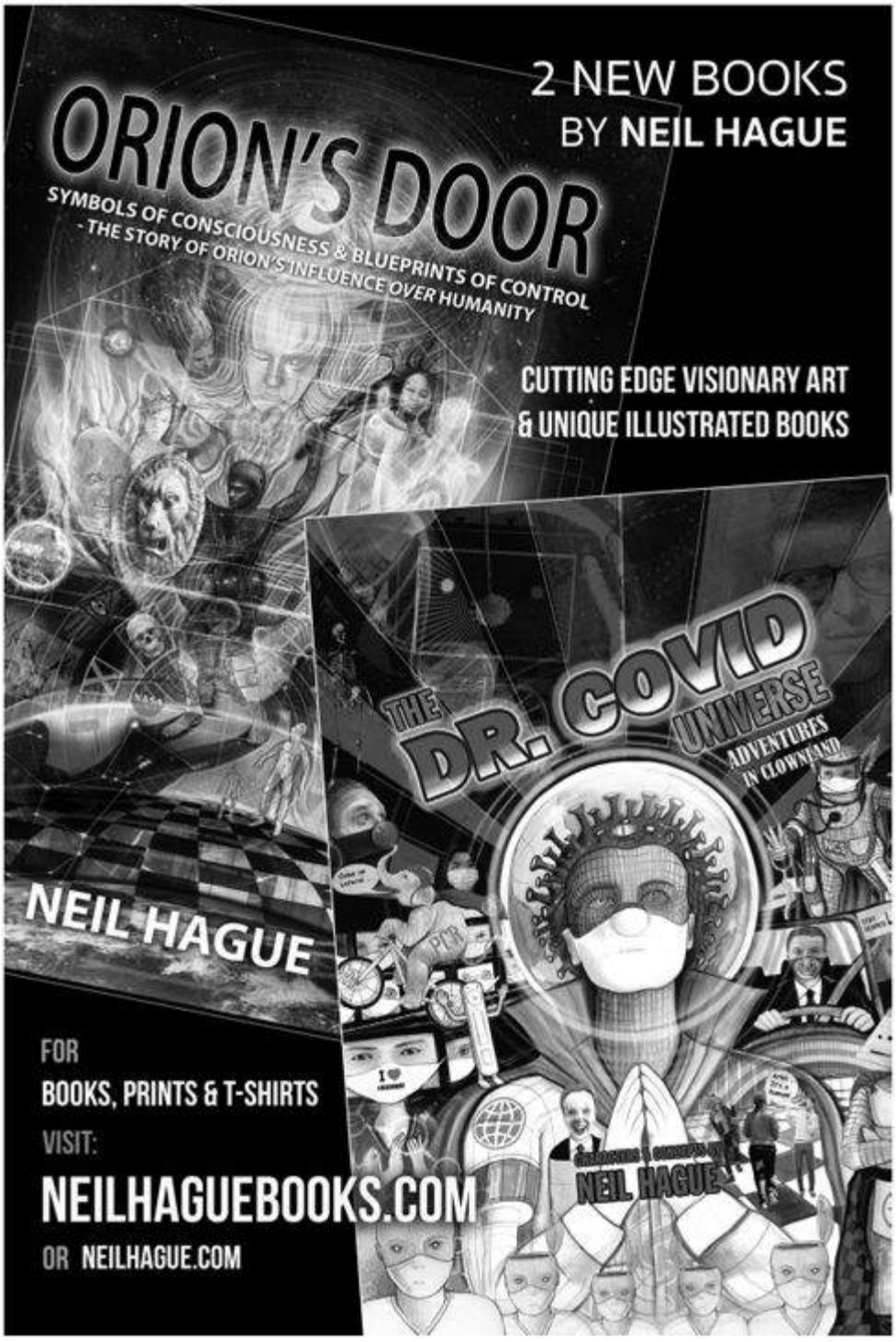
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